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Canadian Intellectual Property Office

CA 2800109 C 2020/06/30

(11)(21) 2 800 109

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(86) **Date de dépôt PCT/PCT Filing Date:** 2011/05/19
(87) **Date publication PCT/PCT Publication Date:** 2011/11/24
(45) **Date de délivrance/Issue Date:** 2020/06/30
(85) **Entrée phase nationale/National Entry:** 2012/11/20
(86) **N° demande PCT/PCT Application No.:** US 2011/037238
(87) **N° publication PCT/PCT Publication No.:** 2011/146768
(30) **Priorités/Priorities:** 2010/05/20 (US61/346,813); 2011/02/22 (US61/445,488)

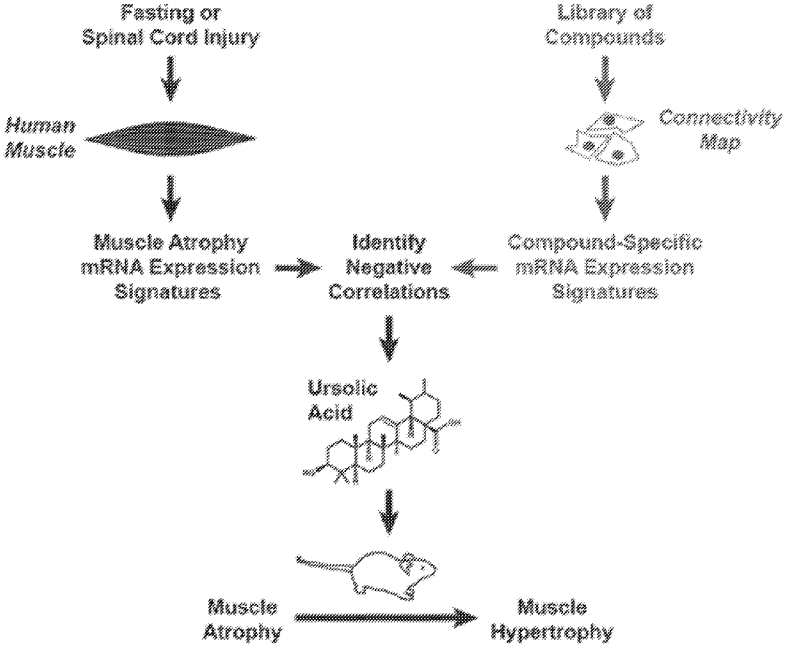
(51) **Cl.Int./Int.Cl.** ***A61K 31/56*** (2006.01), ***A61P 21/06*** (2006.01)

(72) **Inventeurs/Inventors:**
ADAMS, CHRISTOPHER M., US;
KUNKEL, STEVEN D., US;
SUNEJA, MANISH, US;
WELSH, MICHAEL, US

(73) **Propriétaires/Owners:**
UNIVERSITY OF IOWA RESEARCH FOUNDATION, US;
THE UNITED STATES GOVERNMENT AS REPRESENTED BY THE DEPARTMENT OF VETERANS AFFAIRS, US

(74) **Agent:** GOWLING WLG (CANADA) LLP

(54) **Titre : PROCEDES D'INHIBITION DE L'ATROPHIE MUSCULAIRE**
(54) **Title: METHODS FOR INHIBITING MUSCLE ATROPHY**



(57) **Abrégé/Abstract:**
In one aspect, the invention relates methods for inhibiting or preventing muscle atrophy or increasing muscle mass by providing to a subject in need thereof an effective amount of ursolic acid, a derivative thereof, or an analog of the ursane scaffold. This abstract is intended as a scanning tool for purposes of searching in the particular art and is not intended to be limiting of the present invention.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 November 2011 (24.11.2011)

PCT

(10) International Publication Number
WO 2011/146768 A1

(51) International Patent Classification:
A01N 43/04 (2006.01) *A61K 31/70* (2006.01)

(21) International Application Number:
PCT/US2011/037238

(22) International Filing Date:
19 May 2011 (19.05.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/346,813 20 May 2010 (20.05.2010) US
61/445,488 22 February 2011 (22.02.2011) US

(71) Applicant *(for all designated States except US)*: **UNIVERSITY OF IOWA RESEARCH FOUNDATION** [US/US]; Iowa Centers for Enterprise, 2660 University Capitol Centre, Iowa City, IA 52242-5500 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ADAMS, Christopher, M.** [US/US]; 726 N. Van Buren, Iowa City, IA 52245 (US). **KUNKEL, Steven, D.** [US/US]; 1406 Oaklawn Avenue, Iowa City, IA 52245 (US). **SUNEJA, Manish** [US/US]; 2348 Belmont Drive, Coralville, IA 52241 (US). **WELSH, Michael** [US/US]; 3460 5060th Street, Riverside, IA 52327 (US).

(74) Agents: **SHORTELL, D., Brian** et al.; Ballard Spahr LLP, 999 Peachtree Street, Suite 1000, Atlanta, GA 30309 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: METHODS FOR INHIBITING MUSCLE ATROPHY

(57) Abstract: In one aspect, the invention relates methods for inhibiting or preventing muscle atrophy or increasing muscle mass by providing to a subject in need thereof an effective amount of ursolic acid, a derivative thereof, or an analog of the ursane scaffold. This abstract is intended as a scanning tool for purposes of searching in the particular art and is not intended to be limiting of the present invention.

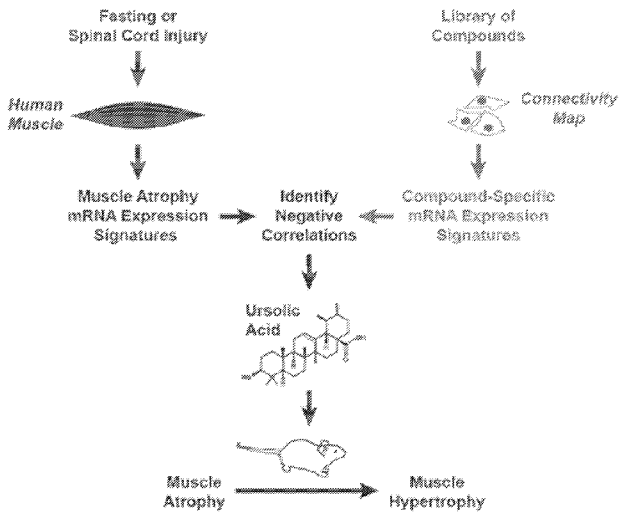


Figure 1

WO 2011/146768 A1

WO 2011/146768 A1

Published:

— *with international search report (Art. 21(3))*

METHODS FOR INHIBITING MUSCLE ATROPHY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of United States Applications No. 61/346,813, filed on May 20, 2010, and No. 61/445,488, filed on February 22, 2011.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under grant VA Career Development Award-2 to Christopher M. Adams, and support from a VA Research Enhancement Award Program to Steven D. Kunkel. The United States government has certain rights in the invention.

BACKGROUND

[0003] Skeletal muscle atrophy is characteristic of starvation and a common effect of aging. It is also a nearly universal consequence of severe human illnesses, including cancer, chronic renal failure, congestive heart failure, chronic respiratory disease, insulin deficiency, acute critical illness, chronic infections such as HIV/AIDS, muscle denervation, and many other medical and surgical conditions that limit muscle use. However, medical therapies to prevent or reverse skeletal muscle atrophy in human patients do not exist. As a result, millions of individuals suffer sequelae of muscle atrophy, including weakness, falls, fractures, opportunistic respiratory infections, and loss of independence. The burden that skeletal muscle atrophy places on individuals, their families, and society in general, is tremendous.

[0004] The pathogenesis of skeletal muscle atrophy is not well understood. Nevertheless, important advances have been made. For example, it has been been described previously that insulin/IGF1 signaling promotes muscle hypertrophy and inhibits muscle atrophy, but is reduced by atrophy-inducing stresses such as fasting or muscle denervation (Bodine SC, *et al.* (2001) *Nat Cell Biol* 3(11):1014-1019; Sandri M, *et al.* (2004) *Cell* 117(3):399-412l; Stitt TN, *et al.* (2004) *Mol Cell* 14(3):395-403; Hu Z, *et al.* (2009) *The Journal of clinical investigation* 119(10):3059-3069; Dobrowolny G, *et al.* (2005) *The Journal of cell biology* 168(2):193-199; Kandarian SC & Jackman RW (2006) *Muscle & nerve* 33(2):155-165;

Hirose M, *et al.* (2001) *Metabolism: clinical and experimental* 50(2):216-222; Pallafacchina G, *et al.* (2002) *Proceedings of the National Academy of Sciences of the United States of America* 99(14):9213-9218). The hypertrophic and anti-atrophic effects of insulin/IGF1 signaling are mediated at least in part through increased activity of phosphoinositide 3-kinase (PI3K) and its downstream effectors, including Akt and mammalian target of rapamycin complex 1 (mTORCl) Sandri M (2008) *Physiology (Bethesda)* 23:160-170; Glass DJ (2005) *The international journal of biochemistry & cell biology* 37(10):1974-1984).

[0005] Another important advance came from microarray studies of atrophying rodent muscle (Lecker SH, *et al.* (2004) *Faseb J* 18(1):39-51; Sacheck JM, *et al.* (2007) *Faseb J* 21(1):140-155; Jagoe RT, *et al. Faseb J* 16(13):1697-1712). Those studies showed that several seemingly disparate atrophy-inducing stresses (including fasting, muscle denervation and severe systemic illness) generated many common changes in skeletal muscle mRNA expression. Some of those atrophy-associated changes promote muscle atrophy in mice; these include induction of the mRNAs encoding atroginI/MAFbx and MuRF1 (two E3 ubiquitin ligases that catalyze proteolytic events), and repression of the mRNA encoding PGC-l α (a transcriptional co-activator that inhibits muscle atrophy) (Sandri M, *et al.* (2006) *Proceedings of the National Academy of Sciences of the United States of America* 103(44):16260-16265; Wenz T, *et al. Proceedings of the National Academy of Sciences of the United States of America* 106(48):20405-20410; Bodine SC, *et al.* (2001) *Science (New York, N.Y* 294(5547):1704-1708; Lagirand-Cantaloube J, *et al.* (2008) *The EMBO journal* 27(8):1266-1276; Cohen S, *et al.* (2009) *The Journal of cell biology* 185(6):1083-1095; Adams V, *et al.* (2008) *Journal of molecular biology* 384(1):48-59). However, the roles of many other mRNAs that are increased or decreased in atrophying rodent muscle are not yet defined. Data on the mechanisms of human muscle atrophy are even more limited, although atrogin-l and MuRF1 are likely to be involved (Leger B, *et al.* (2006) *Faseb J* 20(3):583-585; Doucet M, *et al.* (2007) *American journal of respiratory and critical care medicine* 176(3):261-269; Levine S, *et al.* (2008) *The New England journal of medicine* 358(13):1327-1335).

[0006] Despite advances in understanding the physiology and pathophysiology of muscle atrophy, there is still a scarcity of compounds that are both potent, efficacious, and selective modulators of muscle growth and also effective in the treatment of muscle atrophy associated and diseases in which the muscle atrophy or the need to increase muscle mass is involved.

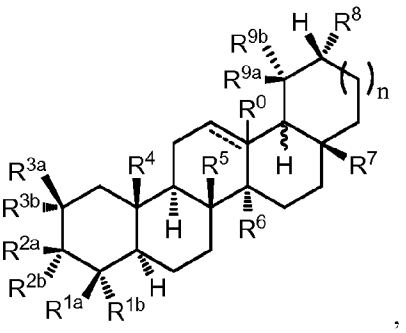
These needs and other needs are satisfied by the present invention.

SUMMARY

[0007] In accordance with the purpose(s) of the invention, as embodied and broadly described herein, the invention, in one aspect, relates to compounds useful in methods to inhibit muscle atrophy and to increase muscle mass by providing to a subject in need thereof an effective amount of ursolic acid or a derivative thereof, and pharmaceutical compositions comprising compounds used in the methods.

[0008] In further aspects, the the purpose(s) of the invention, as embodied and broadly described herein, the invention, in one aspect, relates to compounds useful in methods to modulate muscle growth, methods to inhibit muscle atrophy and to increase muscle mass, methods to induce skeletal muscle hypertrophy, methods to enhance tissue growth, and pharmaceutical compositions comprising compounds used in the methods.

[0009] Disclosed are methods for preventing or treating muscle atrophy in an animal, the method comprising administering to the animal a compound of the formula:



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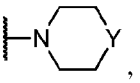
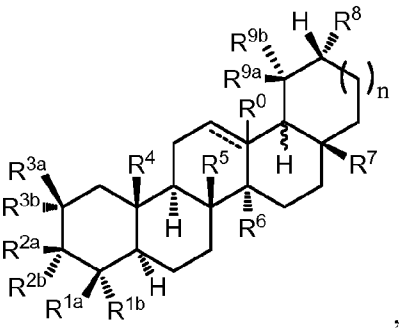
wherein each ----- is an optional covalent bond, and R^0 is optionally present; wherein n is 0 or 1; wherein R^0, when present, is hydrogen; wherein R^{1a} is selected from C1-C6 alkyl and –C(O)ZR^{10}; wherein R^{1b} is selected from C1-C6 alkyl; or wherein R^{1a} and R^{1b} are covalently bonded and, along with the intermediate carbon, together comprise optionally substituted C3-C5 cycloalkyl or optionally substituted C2-C5 heterocycloalkyl; wherein R^{2a} and R^{2b} are independently selected from hydrogen and –OR^{11}, provided that at least one of R^{2a} and R^{2b} is –OR^{11}; or wherein R^{2a} and R^{2b} together comprise =O; wherein each of R^{3a} and R^{3b} is independently selected from hydrogen, hydroxyl, C1-C6 alkyl, and C1-C6 alkoxyl, provided that R^{3a} and R^{3b} are not simultaneously hydroxyl; or wherein R^{3a} and R^{3b} are covalently

bonded and, along with the intermediate carbon, together comprise optionally substituted C3-C5 cycloalkyl or optionally substituted C2-C5 heterocycloalkyl; wherein each of R^4, R^5, and R^6 is independently selected from C1-C6 alkyl; wherein R^7 is selected from C1-C6 alkyl, $-CH_2OR^{12}$, and $-C(O)ZR^{12}$; wherein R^8 is selected from hydrogen and C1-C6 alkyl; wherein each of R^{9a} and R^{9b} is independently selected from hydrogen and C1-C6 alkyl, provided that R^{9a} and R^{9b} are not simultaneously hydrogen; or wherein R^{9a} and R^{9b} are covalently bonded and, along with the intermediate carbon, together comprise optionally substituted C3-C5 cycloalkyl or optionally substituted C2-C5 heterocycloalkyl; wherein R^{10} is selected from hydrogen and C1-C6 alkyl; wherein each R^{11} is independently selected from hydrogen, C1-C6 alkyl, C1-C5 heteroalkyl, C3-C6 cycloalkyl, C4-C6 heterocycloalkyl, phenyl, heteroaryl, and $-C(O)R^{14}$; wherein R^{11}, where permitted, is substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; wherein R^{12} is selected from hydrogen and optionally substituted organic residue having from 1 to 20 carbons; wherein Z is selected from –O– and $-NR^{13}-$; wherein R^{13} is selected from hydrogen and C1-C4 alkyl; or, wherein Z is N, R^{12} and R^{13} are covalently bonded and $-NR^{12}R^{13}$ comprises a moiety of the formula:



wherein Y is selected from –O–, –S–, –SO–, $-SO_2-$, –NH–, $-NCH_3-$; and wherein R^{14} is C1-C6 alkyl and substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, in an amount effective to prevent or treat muscle atrophy in the animal, wherein the amount is greater than 1000 per day when the compound is ursolic acid, boswellic acid, corosolic acid, betulinic acid, or UA0713.

[0010] Also disclosed are methods for increasing muscle mass and/or muscular strength in an animal, the method comprising administering to the animal a compound of the formula:



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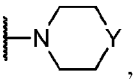
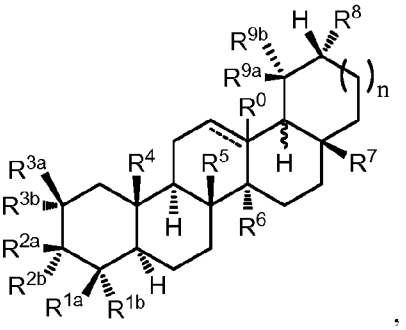
wherein each ----- is an optional covalent bond, and R^0 is optionally present; wherein n is 0 or 1; wherein R^0, when present, is hydrogen; wherein R^{1a} is selected from C1-C6 alkyl and –C(O)ZR^{10}; wherein R^{1b} is selected from C1-C6 alkyl; or wherein R^{1a} and R^{1b} are covalently bonded and, along with the intermediate carbon, together comprise optionally substituted C3-C5 cycloalkyl or optionally substituted C2-C5 heterocycloalkyl; wherein R^{2a} and R^{2b} are independently selected from hydrogen and –OR^{11}, provided that at least one of R^{2a} and R^{2b} is –OR^{11}; or wherein R^{2a} and R^{2b} together comprise =O; wherein each of R^{3a} and R^{3b} is independently selected from hydrogen, hydroxyl, C1-C6 alkyl, and C1-C6 alkoxyl, provided that R^{3a} and R^{3b} are not simultaneously hydroxyl; or wherein R^{3a} and R^{3b} are covalently bonded and, along with the intermediate carbon, together comprise optionally substituted C3-C5 cycloalkyl or optionally substituted C2-C5 heterocycloalkyl; wherein each of R^4, R^5, and R^6 is independently selected from C1-C6 alkyl; wherein R^7 is selected from C1-C6 alkyl, –CH_2OR^{12}, and –C(O)ZR^{12}; wherein R^8 is selected from hydrogen and C1-C6 alkyl; wherein each of R^{9a} and R^{9b} is independently selected from hydrogen and C1-C6 alkyl, provided that R^{9a} and R^{9b} are not simultaneously hydrogen; or wherein R^{9a} and R^{9b} are covalently bonded and, along with the intermediate carbon, together comprise optionally substituted C3-C5 cycloalkyl or optionally substituted C2-C5 heterocycloalkyl; wherein R^{10} is selected from hydrogen and C1-C6 alkyl; wherein each R^{11} is independently selected from hydrogen, C1-C6 alkyl, C1-C5 heteroalkyl, C3-C6 cycloalkyl, C4-C6 heterocycloalkyl, phenyl, heteroaryl, and –C(O)R^{14}; wherein R^{11}, where permitted, is substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; wherein R^{12} is selected from hydrogen and optionally substituted organic residue having from 1 to 20 carbons; wherein Z is selected from –O– and –NR^{13}–; wherein R^{13} is selected from hydrogen and C1-C4 alkyl; or, wherein Z is N, R^{12} and R^{13} are covalently bonded and –$NR^{12}R^{13}$ comprises a moiety of the formula:



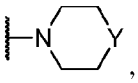
wherein Y is selected from –O–, –S–, –SO–, $–SO_2–$, –NH–, $–NCH_3–$; and wherein R^{14} is C1-C6 alkyl and substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, in an amount effective to increase muscle mass and/or muscular strength in the animal, wherein the amount is greater than about 1000 mg per day when the compound is ursolic acid, boswellic acid, corosolic acid, betulinic acid, or UA0713.

[0011] Also disclosed are methods for enhancing tissue growth *in vitro*, the method comprising administering to the tissue a compound of the formula:



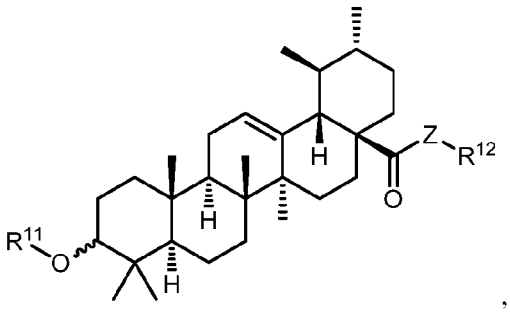
wherein each ----- is an optional covalent bond, and R^0 is optionally present; wherein n is 0 or 1; wherein R^0, when present, is hydrogen; wherein R^{1a} is selected from C1-C6 alkyl and $–C(O)ZR^{10}$; wherein R^{1b} is selected from C1-C6 alkyl; or wherein R^{1a} and R^{1b} are covalently bonded and, along with the intermediate carbon, together comprise optionally substituted C3-C5 cycloalkyl or optionally substituted C2-C5 heterocycloalkyl; wherein R^{2a} and R^{2b} are independently selected from hydrogen and $–OR^{11}$, provided that at least one of R^{2a} and R^{2b} is $–OR^{11}$; or wherein R^{2a} and R^{2b} together comprise =O; wherein each of R^{3a} and R^{3b} is independently selected from hydrogen, hydroxyl, C1-C6 alkyl, and C1-C6 alkoxyl, provided that R^{3a} and R^{3b} are not simultaneously hydroxyl; or wherein R^{3a} and R^{3b} are covalently bonded and, along with the intermediate carbon, together comprise optionally substituted C3-C5 cycloalkyl or optionally substituted C2-C5 heterocycloalkyl; wherein each of R^4, R^5, and R^6 is independently selected from C1-C6 alkyl; wherein R^7 is selected from C1-C6 alkyl, $–CH_2OR^{12}$, and $–C(O)ZR^{12}$; wherein R^8 is selected from hydrogen and C1-C6 alkyl; wherein

each of R^{9a} and R^{9b} is independently selected from hydrogen and C1-C6 alkyl, provided that R^{9a} and R^{9b} are not simultaneously hydrogen; or wherein R^{9a} and R^{9b} are covalently bonded and, along with the intermediate carbon, together comprise optionally substituted C3-C5 cycloalkyl or optionally substituted C2-C5 heterocycloalkyl; wherein R^{10} is selected from hydrogen and C1-C6 alkyl; wherein each R^{11} is independently selected from hydrogen, C1-C6 alkyl, C1-C5 heteroalkyl, C3-C6 cycloalkyl, C4-C6 heterocycloalkyl, phenyl, heteroaryl, and $-C(O)R^{14}$; wherein R^{11}, where permitted, is substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; wherein R^{12} is selected from hydrogen and optionally substituted organic residue having from 1 to 20 carbons; wherein Z is selected from –O– and $-NR^{13}-$; wherein R^{13} is selected from hydrogen and C1-C4 alkyl; or, wherein Z is N, R^{12} and R^{13} are covalently bonded and $-NR^{12}R^{13}$ comprises a moiety of the formula:



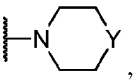
wherein Y is selected from –O–, –S–, –SO–, $-SO_2-$, –NH–, $-NCH_3-$; and wherein R^{14} is C1-C6 alkyl and substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, in an amount effective to enhance growth of the tissue.

[0012] Also disclosed are methods for enhancing muscle formation in a mammal, the method comprising administering to the mammal a compound of the formula:



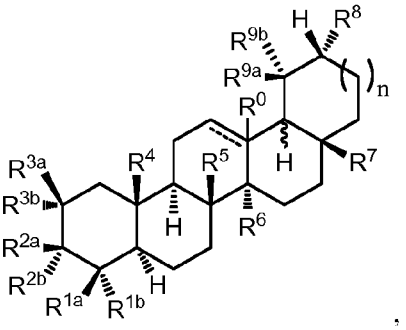
wherein R^{11} is selected from hydrogen, C1-C6 alkyl, C1-C5 heteroalkyl, C3-C6 cycloalkyl, C4-C6 heterocycloalkyl, phenyl, heteroaryl, and $-C(O)R^{14}$; wherein R^{11}, where permitted, is substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl,

ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; wherein R^{12} is selected from hydrogen and optionally substituted organic residue having from 1 to 20 carbons; wherein Z is selected from –O– and $–NR^{13}–$; wherein R^{13} is selected from hydrogen and C1-C4 alkyl; or, wherein Z is N, R^{12} and R^{13} are covalently bonded and $–NR^{12}R^{13}$ comprises a moiety of the formula:



wherein Y is selected from –O–, –S–, –SO–, $–SO_2–$, –NH–, $–NCH_3–$; and wherein R^{14} is C1-C6 alkyl and substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, in an amount greater than about 1000 mg per day and effective to enhance muscle formation in the mammal.

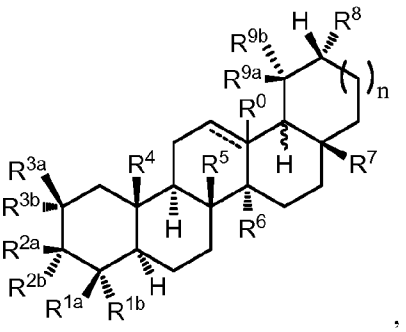
[0013] Also disclosed are methods for testing for performance enhancing use of a ursolic acid analog in an animal, the method comprising: (a) obtaining a biological test sample from the animal; and (b) measuring the amount of a compound of formula:



wherein each ----- is an optional covalent bond, and R^0 is optionally present; wherein n is 0 or 1; wherein R^0, when present, is hydrogen; wherein R^{1a} is selected from C1-C6 alkyl and $–C(O)ZR^{10}$; wherein R^{1b} is selected from C1-C6 alkyl; wherein one of R^{2a} and R^{2b} is $–OR^{11}$, and the other is hydrogen; wherein each of R^{3a} and R^{3b} is independently selected from hydrogen, hydroxyl, C1-C6 alkyl, and C1-C6 alkoxyl, provided that R^{3a} and R^{3b} are not simultaneously hydroxyl; wherein each of R^4, R^5, and R^6 is independently selected from C1-C6 alkyl; wherein R^7 is selected from C1-C6 alkyl and $–C(O)ZR^{12}$; wherein R^8 is selected from hydrogen and C1-C6 alkyl; wherein each of R^{9a} and R^{9b} is independently selected from

hydrogen and C1-C6 alkyl, provided that R^{9a} and R^{9b} are not simultaneously hydrogen; wherein R^{10} is selected from hydrogen and C1-C6 alkyl; wherein R^{11} is selected from hydrogen, C1-C6 alkyl, C1-C5 heteroalkyl, C3-C6 cycloalkyl, C4-C6 heterocycloalkyl, phenyl, heteroaryl, and $-C(O)R^{14}$; wherein R^{11}, where permitted, is substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; wherein R^{12} is selected from hydrogen and optionally substituted organic residue having from 1 to 20 carbons; wherein Z is selected from –O– and $-NR^{13}-$; wherein R^{13} is selected from hydrogen and C1-C4 alkyl; and wherein R^{14} is C1-C6 alkyl and substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, in the test sample to determine whether a superphysiological amount of the compound is present in the test sample; wherein the superphysiological amount of the compound in the test sample is indicative of performance enhancing use of the compound.

[0014] Also disclosed are pharmaceutical compositions comprising a pharmaceutically acceptable carrier and an effective amount of a compound of the formula:



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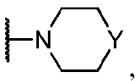
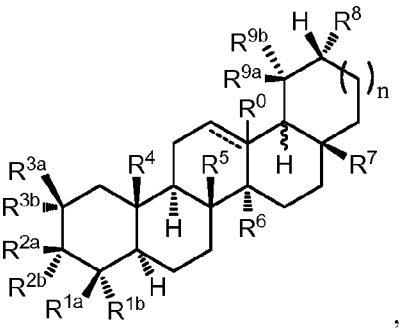
wherein each ----- is an optional covalent bond, and R^0 is optionally present; wherein n is 0 or 1; wherein R^0, when present, is hydrogen; wherein R^{1a} is selected from C1-C6 alkyl and $-C(O)ZR^{10}$; wherein R^{1b} is selected from C1-C6 alkyl; or wherein R^{1a} and R^{1b} are covalently bonded and, along with the intermediate carbon, together comprise optionally substituted C3-C5 cycloalkyl or optionally substituted C2-C5 heterocycloalkyl; wherein R^{2a} and R^{2b} are independently selected from hydrogen and $-OR^{11}$, provided that at least one of R^{2a} and R^{2b} is $-OR^{11}$; or wherein R^{2a} and R^{2b} together comprise =O; wherein each of R^{3a} and R^{3b} is

independently selected from hydrogen, hydroxyl, C1-C6 alkyl, and C1-C6 alkoxyl, provided that R^{3a} and R^{3b} are not simultaneously hydroxyl; or wherein R^{3a} and R^{3b} are covalently bonded and, along with the intermediate carbon, together comprise optionally substituted C3-C5 cycloalkyl or optionally substituted C2-C5 heterocycloalkyl; wherein each of R^4, R^5, and R^6 is independently selected from C1-C6 alkyl; wherein R^7 is selected from C1-C6 alkyl, $-CH_2OR^{12}$, and $-C(O)ZR^{12}$; wherein R^8 is selected from hydrogen and C1-C6 alkyl; wherein each of R^{9a} and R^{9b} is independently selected from hydrogen and C1-C6 alkyl, provided that R^{9a} and R^{9b} are not simultaneously hydrogen; or wherein R^{9a} and R^{9b} are covalently bonded and, along with the intermediate carbon, together comprise optionally substituted C3-C5 cycloalkyl or optionally substituted C2-C5 heterocycloalkyl; wherein R^{10} is selected from hydrogen and C1-C6 alkyl; wherein each R^{11} is independently selected from hydrogen, C1-C6 alkyl, C1-C5 heteroalkyl, C3-C6 cycloalkyl, C4-C6 heterocycloalkyl, phenyl, heteroaryl, and $-C(O)R^{14}$; wherein R^{11}, where permitted, is substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; wherein R^{12} is selected from hydrogen and optionally substituted organic residue having from 1 to 20 carbons; wherein Z is selected from –O– and $-NR^{13}-$; wherein R^{13} is selected from hydrogen and C1-C4 alkyl; or, wherein Z is N, R^{12} and R^{13} are covalently bonded and $-NR^{12}R^{13}$ comprises a moiety of the formula:

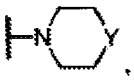


wherein Y is selected from –O–, –S–, –SO–, $-SO_2-$, –NH–, $-NCH_3-$; and wherein R^{14} is C1-C6 alkyl and substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, in an amount effective to prevent or treat muscle atrophy in the animal, wherein the amount is greater than about 1000 mg per day when the compound is ursolic acid, boswellic acid, corosolic acid, betulinic acid, or UA0713.

[0015] Also disclosed are kits comprising at least one compound having a structure represented by a formula:



wherein each ----- is an optional covalent bond, and R^0 is optionally present; wherein n is 0 or 1; wherein R^0, when present, is hydrogen; wherein R^{1a} is selected from C1-C6 alkyl and –$C(O)ZR^{10}$; wherein R^{1b} is selected from C1-C6 alkyl; or wherein R^{1a} and R^{1b} are covalently bonded and, along with the intermediate carbon, together comprise optionally substituted C3-C5 cycloalkyl or optionally substituted C2-C5 heterocycloalkyl; wherein R^{2a} and R^{2b} are independently selected from hydrogen and $–OR^{11}$, provided that at least one of R^{2a} and R^{2b} is $–OR^{11}$; or wherein R^{2a} and R^{2b} together comprise =O; wherein each of R^{3a} and R^{3b} is independently selected from hydrogen, hydroxyl, C1-C6 alkyl, and C1-C6 alkoxyl, provided that R^{3a} and R^{3b} are not simultaneously hydroxyl; or wherein R^{3a} and R^{3b} are covalently bonded and, along with the intermediate carbon, together comprise optionally substituted C3-C5 cycloalkyl or optionally substituted C2-C5 heterocycloalkyl; wherein each of R^4, R^5, and R^6 is independently selected from C1-C6 alkyl; wherein R^7 is selected from C1-C6 alkyl, –CH_2OR^{12}, and –$C(O)ZR^{12}$; wherein R^8 is selected from hydrogen and C1-C6 alkyl; wherein each of R^{9a} and R^{9b} is independently selected from hydrogen and C1-C6 alkyl, provided that R^{9a} and R^{9b} are not simultaneously hydrogen; or wherein R^{9a} and R^{9b} are covalently bonded and, along with the intermediate carbon, together comprise optionally substituted C3-C5 cycloalkyl or optionally substituted C2-C5 heterocycloalkyl; wherein R^{10} is selected from hydrogen and C1-C6 alkyl; wherein each R^{11} is independently selected from hydrogen, C1-C6 alkyl, C1-C5 heteroalkyl, C3-C6 cycloalkyl, C4-C6 heterocycloalkyl, phenyl, heteroaryl, and –$C(O)R^{14}$; wherein R^{11}, where permitted, is substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; wherein R^{12} is selected from hydrogen and optionally substituted organic residue having from 1 to 20 carbons; wherein Z is selected from –O– and –NR^{13}–; wherein R^{13} is selected from hydrogen and C1-C4 alkyl; or, wherein Z is N, R^{12} and R^{13} are covalently bonded and –$NR^{12}R^{13}$ comprises a moiety of the formula:



wherein Y is selected from —O , S , SO , SO_2 , NH . NCH_3—; and wherein R^{14} is C1-C6 alkyl and substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, and one or more of: (a) a protein supplement; (b) an anabolic agent; (c) a catabolic agent; (d) a dietary supplement; (e) at least one agent known to treat a disorder associated with muscle wasting; (f) instructions for treating a disorder associated with cholinergic activity; or (g) instructions for using the compound to increase muscle mass and/or muscular strength.

[0016] Also disclosed are methods for manufacturing a medicament associated with muscle atrophy or the need to increase muscle mass comprising combining at least one disclosed compound or at least one disclosed product with a pharmaceutically acceptable carrier or diluent.

[0017] Also disclosed are uses of a disclosed compound or a disclosed product in the manufacture of a medicament for the treatment of a disorder associated with muscle atrophy or the need to increase muscle mass.

[0017A] Further disclosed is the use of ursolic acid, or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, for increasing skeletal mass in an animal, wherein said animal is selected from the group consisting of a primate, domesticated fish, domesticated crustacean, domesticated mollusk, poultry, rabbit, dog, cat, and livestock.

[0017B] Also disclosed is the use of ursolic acid, or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, in the manufacture of a composition for increasing skeletal mass in an animal, wherein said animal is selected from the group consisting of a primate, domesticated fish, domesticated crustacean, domesticated mollusk, poultry, rabbit, dog, cat, and livestock.

[0017C] Also provided is a non-medical method of increasing skeletal muscle mass in an animal, the method comprising administering to the animal ursolic acid, or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof.

[0017D] Additionally disclosed is ursolic acid, or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, for use in increasing skeletal mass in an animal.

[0017E] Also provided is the use of ursolic acid, or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, for: reducing skeletal muscle atrophy; increasing muscular strength; or promoting muscle growth, in an animal, wherein said animal is selected from the group consisting of a primate, domesticated fish, domesticated crustacean, domesticated mollusk, poultry, rabbit, dog, cat, and livestock.

[0017F] Also disclosed is the use of ursolic acid, or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, in the manufacture of a composition for: reducing skeletal muscle atrophy; increasing muscular strength; or promoting muscle growth, in an animal, wherein said animal is selected from the group consisting of a primate, domesticated fish, domesticated crustacean, domesticated mollusk, poultry, rabbit, dog, cat, and livestock.

[0017G] Also disclosed is the use of ursolic acid, or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, in the manufacture of a composition for: reducing skeletal muscle atrophy; increasing muscular strength; or promoting muscle growth, in an animal, wherein said animal is selected from the group consisting of a primate, domesticated fish, domesticated crustacean, domesticated mollusk, poultry, rabbit, dog, cat, and livestock.

[0017H] Further disclosed is a non-medical method of: reducing skeletal muscle atrophy; increasing muscular strength; or promoting muscle growth, in an animal, the method comprising administering to the animal ursolic acid, or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof.

[0017I] Also disclosed is ursolic acid or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, for use in: reducing skeletal muscle atrophy; increasing muscular strength; or promoting muscle growth.

[0017J] Further disclosed is a non-medical method of increasing skeletal muscle mass in an animal, the method comprising use of ursolic acid, or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, for administration to the animal.

[0017K] Also disclosed is a non-medical method of: a) reducing skeletal muscle atrophy; b) increasing muscular strength; or c) promoting muscle growth, in an animal, the method comprising use of ursolic acid, or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, for administration to the animal.

[0018] While aspects of the present invention can be described and claimed in a particular statutory class, such as the system statutory class, this is for convenience only and one of skill in the art will understand that each aspect of the present invention can be described and claimed in any statutory class.

[0019] Unless otherwise expressly stated, it is in no way intended that any method or aspect set forth herein be construed as requiring that its steps be performed in a specific order. Accordingly, where a method claim does not specifically state in the claims or descriptions that the steps are to be limited to a specific order, it is no way intended that an order be inferred, in any respect. This holds for any possible non-express basis for interpretation, including matters of logic with respect to arrangement of steps or operational flow, plain meaning derived from grammatical organization or punctuation, or the number or type of aspects described in the specification.

BRIEF DESCRIPTION OF THE FIGURES

[0020] The accompanying figures, which are incorporated in and constitute a part of this specification, illustrate several aspects and together with the description serve to explain the principles of the invention.

[0021] Figure 1 shows a schematic overview of the discovery process leading to a pharmacological compound that promotes skeletal muscle growth and inhibits skeletal muscle atrophy.

[0022] Figure 2 shows representative data on the effect of fasting on skeletal muscle mRNA expression in healthy human adults.

[0023] Figure 3 shows qPCR analysis of representative fasting-responsive mRNAs from human skeletal muscle.

[0024] Figure 4 shows representative data on the identification of ursolic acid as an inhibitor of fasting-induced skeletal muscle atrophy.

[0025] Figure 5 shows representative data on the identification of ursolic acid as an inhibitor of denervation-induced muscle atrophy.

[0026] Figure 6 shows representative data on ursolic acid-mediated induction of muscle hypertrophy.

[0027] Figure 7 shows representative data on the effect of ursolic acid on mouse skeletal muscle specific tetanic force.

[0028] Figure 8 shows representative data on the effect of ursolic acid on muscle growth, atrophic gene expression, trophic gene expression, and skeletal muscle IGF-I signaling.

[0029] Figure 9 shows representative data on the effect of ursolic acid on skeletal muscle expression of IGF1 gene exons, adipose IGF1 mRNA expression, and skeletal muscle insulin signaling.

[0030] Figure 10 shows representative data on the effect of ursolic acid on adiposity.

[0031] Figure 11 shows representative data on the effect of ursolic acid on food

consumption, liver weight, kidney weight, and plasma ALT, bilirubin, and creatinine concentrations.

[0032] Figure 12 shows representative data on the effect of ursolic acid on weight gain, white adipose tissue weight, skeletal muscle weight, brown adipose tissue weight and energy expenditure in a mouse model of obesity and metabolic syndrome.

[0033] Figure 13 shows representative data on the effect of ursolic acid on obesity-related pre-diabetes, diabetes, fatty liver disease and hyperlipidemia in a mouse model of obesity and metabolic syndrome.

[0034] Figure 14 shows representative data on the effect of oleanolic acid on skeletal muscle mass.

[0035] Figure 15 shows representative data on the effect of targeted inhibition of PTP1B on skeletal muscle growth.

[0036] Figure 16 shows representative data on the effect of ursolic acid serum concentration on muscle mass and adiposity.

[0037] Additional advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or can be learned by practice of the invention.

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

DESCRIPTION

[0038] The present invention can be understood more readily by reference to the following detailed description of the invention and the Examples included therein.

[0039] Before the present compounds, compositions, articles, systems, devices, and/or methods are disclosed and described, it is to be understood that they are not limited to specific synthetic methods unless otherwise specified, or to particular reagents unless otherwise specified, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and is not intended to be

limiting. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, example methods and materials are now described.

[0040]

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided herein can be different from the actual publication dates, which can require independent confirmation.

A. Definitions

[0041] As used herein, nomenclature for compounds, including organic compounds, can be given using common names, IUPAC, IUBMB, or CAS recommendations for nomenclature. When one or more stereochemical features are present, Cahn-Ingold-Prelog rules for stereochemistry can be employed to designate stereochemical priority, *E/Z* specification, and the like. One of skill in the art can readily ascertain the structure of a compound if given a name, either by systemic reduction of the compound structure using naming conventions, or by commercially available software, such as CHEMDRAW™ (Cambridgesoft Corporation, U.S.A.).

[0042] As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a functional group," "an alkyl," or "a residue" includes mixtures of two or more such functional groups, alkyls, or residues, and the like.

[0043] Ranges can be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, a further aspect includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms a further aspect. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint. It is also understood that there are a number of values disclosed herein,

and that each value is also herein disclosed as "about" that particular value in addition to the value itself. For example, if the value "10" is disclosed, then "about 10" is also disclosed. It is also understood that each unit between two particular units are also disclosed. For example, if 10 and 15 are disclosed, then 11, 12, 13, and 14 are also disclosed.

[0044] References in the specification and concluding claims to parts by weight of a particular element or component in a composition denotes the weight relationship between the element or component and any other elements or components in the composition or article for which a part by weight is expressed. Thus, in a compound containing 2 parts by weight of component X and 5 parts by weight component Y, X and Y are present at a weight ratio of 2:5, and are present in such ratio regardless of whether additional components are contained in the compound.

[0045] A weight percent (wt. %) of a component, unless specifically stated to the contrary, is based on the total weight of the formulation or composition in which the component is included.

[0046] As used herein, the terms "optional" or "optionally" means that the subsequently described event or circumstance can or can not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not.

[0047] As used herein, "ursolic acid" refers to ursolic acid, or extracts containing ursolic acid from plants such as apples, holy basil, bilberries, cranberries, elder flower, peppermint, lavender, oregano, thyme, sage, hawthorn, bearberry or prunes.

[0048] As used herein, "ursolic acid derivatives" refers to corosolic acid, betulinic acid, hederagenin, boswellic acids, UA0713, a substituted ursolic acid analog, an ursane compound or any other pentacyclic triterpene acids that prevents muscle atrophy, reduces muscle atrophy, increases muscle mass, increases muscle strength in an animal, including in humans, increases Akt phosphorylation, increases S6K phosphorylation, or stimulates biochemical events known to precede or follow Akt phosphorylation or S6K phosphorylation. For example, and not to be limiting, biochemical events known to precede or follow Akt phosphorylation or S6K phosphorylation can be events such as insulin receptor phosphorylation, IGF-I receptor phosphorylation, insulin receptor substrate (IRS) protein

phosphorylation, phosphoinositide-3 kinase phosphorylation, phosphoinositide-3 kinase activation, phosphoinositide dependent kinase 1 activation, mammalian target of rapamycin complex 2 activation, adrenergic receptor activation, heterotrimeric G protein activation, adenylate cyclase activation, increased intracellular cyclic AMP, AMP kinase activation, protein kinase A activation, protein kinase C activation, CREB activation, mitogen activated protein kinase pathway activation, mammalian target of rapamycin complex 1 activation, 4E-BP1 phosphorylation, 4E-BP1 inactivation, GSK3β phosphorylation, GSK3 β inactivation, increased protein synthesis, increased glucose uptake, Foxo transcription factor phosphorylation, Foxo transcription factor inactivation, Cdkn1a phosphorylation, Cdkn1a inactivation, reduced *atrogin-1* mRNA, reduced *MuRF1* mRNA, increased *VEGFA* mRNA, or increased *IGF1* mRNA.

[0049] As used herein, the term "subject" refers to the target of administration, e.g. an animal. Thus the subject of the herein disclosed methods can be a vertebrate, such as a mammal, a fish, a bird, a reptile, or an amphibian. Alternatively, the subject of the herein disclosed methods can be a human, non-human primate, horse, pig, rabbit, dog, sheep, goat, cow, cat, guinea pig or rodent. The term does not denote a particular age or sex. Thus, adult and newborn subjects, as well as fetuses, whether male or female, are intended to be covered. In one aspect, the subject is a mammal. A patient refers to a subject afflicted with a disease or disorder. The term "patient" includes human and veterinary subjects. In some aspects of the disclosed methods, the subject has been diagnosed with a need for treatment of one or more muscle disorders prior to the administering step. In some aspects of the disclosed method, the subject has been diagnosed with a need for increasing muscle mass prior to the administering step. In some aspects of the disclosed method, the subject has been diagnosed with a need for increasing muscle mass prior to the administering step.

[0050] As used herein, the term "treatment" refers to the medical management of a patient with the intent to cure, ameliorate, stabilize, or prevent a disease, pathological condition, or disorder. This term includes active treatment, that is, treatment directed specifically toward the improvement of a disease, pathological condition, or disorder, and also includes causal treatment, that is, treatment directed toward removal of the cause of the associated disease, pathological condition, or disorder. In addition, this term includes palliative treatment, that is, treatment designed for the relief of symptoms rather than the curing of the disease, pathological condition, or disorder; preventative treatment, that is, treatment directed to

minimizing or partially or completely inhibiting the development of the associated disease, pathological condition, or disorder; and supportive treatment, that is, treatment employed to supplement another specific therapy directed toward the improvement of the associated disease, pathological condition, or disorder. In various aspects, the term covers any treatment of a subject, including a mammal (e.g., a human), and includes: (i) preventing the disease from occurring in a subject that can be predisposed to the disease but has not yet been diagnosed as having it; (ii) inhibiting the disease, i.e., arresting its development; or (iii) relieving the disease, i.e., causing regression of the disease. In one aspect, the subject is a mammal such as a primate, and, in a further aspect, the subject is a human. The term "subject" also includes domesticated animals (e.g., cats, dogs, etc.), livestock (e.g., cattle, horses, pigs, sheep, goats, etc.), and laboratory animals (e.g., mouse, rabbit, rat, guinea pig, fruit fly, etc.).

[0051] As used herein, the term "prevent" or "preventing" refers to precluding, averting, obviating, forestalling, stopping, or hindering something from happening, especially by advance action. It is understood that where reduce, inhibit or prevent are used herein, unless specifically indicated otherwise, the use of the other two words is also expressly disclosed.

[0052] As used herein, the term "diagnosed" means having been subjected to a physical examination by a person of skill, for example, a physician, and found to have a condition that can be diagnosed or treated by the compounds, compositions, or methods disclosed herein. For example, "diagnosed with a muscle atrophy disorder" means having been subjected to a physical examination by a person of skill, for example, a physician, and found to have a condition that can be diagnosed or treated by a compound or composition that can increase muscle mass. As a further example, "diagnosed with a need for increasing muscle mass" refers to having been subjected to a physical examination by a person of skill, for example, a physician, and found to have a condition characterized by muscle atrophy or other disease wherein increasing muscle mass would be beneficial to the subject. Such a diagnosis can be in reference to a disorder, such as muscle atrophy, and the like, as discussed herein.

[0053] As used herein, the phrase "identified to be in need of treatment for a disorder," or the like, refers to selection of a subject based upon need for treatment of the disorder. For example, a subject can be identified as having a need for treatment of a disorder (e.g., a disorder related to muscle atrophy) based upon an earlier diagnosis by a person of skill and

thereafter subjected to treatment for the disorder. It is contemplated that the identification can, in one aspect, be performed by a person different from the person making the diagnosis. It is also contemplated, in a further aspect, that the administration can be performed by one who subsequently performed the administration.

[0054] As used herein, the terms "administering" and "administration" refer to any method of providing a pharmaceutical preparation to a subject. Such methods are well known to those skilled in the art and include, but are not limited to, oral administration, transdermal administration, administration by inhalation, nasal administration, topical administration, intravaginal administration, ophthalmic administration, intraaural administration, intracerebral administration, rectal administration, sublingual administration, buccal administration, and parenteral administration, including injectable such as intravenous administration, intra-arterial administration, intramuscular administration, and subcutaneous administration. Administration can be continuous or intermittent. In various aspects, a preparation can be administered therapeutically; that is, administered to treat an existing disease or condition. In further various aspects, a preparation can be administered prophylactically; that is, administered for prevention of a disease or condition.

[0055] The term "contacting" as used herein refers to bringing a disclosed compound and a cell, target receptor, or other biological entity together in such a manner that the compound can affect the activity of the target (e.g., receptor, transcription factor, cell, etc.), either directly; i.e., by interacting with the target itself, or indirectly; i.e., by interacting with another molecule, co-factor, factor, or protein on which the activity of the target is dependent.

[0056] As used herein, the terms "effective amount" and "amount effective" refer to an amount that is sufficient to achieve the desired result or to have an effect on an undesired condition. For example, a "therapeutically effective amount" refers to an amount that is sufficient to achieve the desired therapeutic result or to have an effect on undesired symptoms, but is generally insufficient to cause adverse side affects. The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration; the route of administration; the rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental

with the specific compound employed and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of a compound at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. If desired, the effective daily dose can be divided into multiple doses for purposes of administration. Consequently, single dose compositions can contain such amounts or submultiples thereof to make up the daily dose. The dosage can be adjusted by the individual physician in the event of any contraindications. Dosage can vary, and can be administered in one or more dose administrations daily, for one or several days. Guidance can be found in the literature for appropriate dosages for given classes of pharmaceutical products. In further various aspects, a preparation can be administered in a "prophylactically effective amount"; that is, an amount effective for prevention of a disease or condition.

[0057] As used herein, "EC_{50}," is intended to refer to the concentration or dose of a substance (e.g., a compound or a drug) that is required for 50% enhancement or activation of a biological process, or component of a process, including a protein, subunit, organelle, ribonucleoprotein, etc. EC_{50} also refers to the concentration or dose of a substance that is required for 50% enhancement or activation *in vivo*, as further defined elsewhere herein. Alternatively, EC_{50} can refer to the concentration or dose of compound that provokes a response halfway between the baseline and maximum response. The response can be measusred in a *in vitro* or *in vivo* system as is convenient and appropriate for the biological response of interest. For example, the response can be measured *in vitro* using cultured muscle cells or in an *ex vivo* organ culture system with isolated muscle fibers. Alternatively, the response can be measured *in vivo* using an appropriate research model such as rodent, including mice and rats. The mouse or rat can be an inbred strain with phenotypic characteristics of interest such as obesity or diabetes. As appropriate, the response can be measured in a transgenic or knockout mouse or rat wherein the a gene or genes has been introduced or knocked-out, as appropriate, to replicate a disease process.

[0058] As used herein, "IC_{50}," is intended to refer to the concentration or dose of a substance (e.g., a compound or a drug) that is required for 50% inhibition or diminuation of a biological process, or component of a process, including a protein, subunit, organelle, ribonucleoprotein, etc. IC_{50} also refers to the concentration or dose of a substance that is required for 50% inhibition or diminuation *in vivo*, as further defined elsewhere herein. Alternatively, IC_{50} also

refers refers to the half maximal (50%) inhibitory concentration (IC) or inhibitory dose of a substance. The response can be measusred in a *in vitro* or *in vivo* system as is convenient and appropriate for the biological response of interest. For example, the response can be measured *in vitro* using cultured muscle cells or in an *ex vivo* organ culture system with isolated muscle fibers. Alternatively, the response can be measured *in vivo* using an appropriate research model such as rodent, including mice and rats. The mouse or rat can be an inbred strain with phenotypic characteristics of interest such as obesity or diabetes. As appropriate, the response can be measured in a transgenic or knockout mouse or rat wherein the a gene or genes has been introduced or knocked-out, as appropriate, to replicate a disease process.

[0059] The term "pharmaceutically acceptable" describes a material that is not biologically or otherwise undesirable, i.e., without causing an unacceptable level of undesirable biological effects or interacting in a deleterious manner.

[0060] As used herein, the term "derivative" refers to a compound having a structure derived from the structure of a parent compound (e.g., a compound disclosed herein) and whose structure is sufficiently similar to those disclosed herein and based upon that similarity, would be expected by one skilled in the art to exhibit the same or similar activities and utilities as the claimed compounds, or to induce, as a precursor, the same or similar activities and utilities as the claimed compounds. Exemplary derivatives include salts, esters, amides, salts of esters or amides, and N-oxides of a parent compound.

[0061] As used herein, the term "pharmaceutically acceptable carrier" refers to sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol and the like), carboxymethylcellulose and suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials such as lecithin, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants. These compositions can also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms can be ensured by the inclusion of

various antibacterial and antifungal agents such as paraben, chlorobutanol, phenol, sorbic acid and the like. It can also be desirable to include isotonic agents such as sugars, sodium chloride and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the inclusion of agents, such as aluminum monostearate and gelatin, which delay absorption. Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide, poly(orthoesters) and poly(anhydrides). Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues. The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable media just prior to use. Suitable inert carriers can include sugars such as lactose. Desirably, at least 95% by weight of the particles of the active ingredient have an effective particle size in the range of 0.01 to 10 micrometers.

[0062] A residue of a chemical species, as used in the specification and concluding claims, refers to the moiety that is the resulting product of the chemical species in a particular reaction scheme or subsequent formulation or chemical product, regardless of whether the moiety is actually obtained from the chemical species. Thus, an ethylene glycol residue in a polyester refers to one or more $-OCH_2CH_2O-$ units in the polyester, regardless of whether ethylene glycol was used to prepare the polyester. Similarly, a sebacic acid residue in a polyester refers to one or more $-CO(CH_2)_8CO-$ moieties in the polyester, regardless of whether the residue is obtained by reacting sebacic acid or an ester thereof to obtain the polyester.

[0063] As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, and aromatic and nonaromatic substituents of organic compounds. Illustrative substituents include, for example, those described below. The permissible substituents can be one or more and the same or different for appropriate organic compounds. For purposes of this disclosure, the heteroatoms, such as nitrogen, can have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the

heteroatoms. This disclosure is not intended to be limited in any manner by the permissible substituents of organic compounds. Also, the terms "substitution" or "substituted with" include the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, *e.g.*, a compound that does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc. It is also contemplated that, in certain aspects, unless expressly indicated to the contrary, individual substituents can be further optionally substituted (i.e., further substituted or unsubstituted).

[0064] In defining various terms, "A^1," "A^2," "A^3," and "A^4" are used herein as generic symbols to represent various specific substituents. These symbols can be any substituent, not limited to those disclosed herein, and when they are defined to be certain substituents in one instance, they can, in another instance, be defined as some other substituents.

[0065] The term "alkyl" as used herein is a branched or unbranched saturated hydrocarbon group of 1 to 24 carbon atoms, such as methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, isobutyl, *s*-butyl, *t*-butyl, *n*-pentyl, isopentyl, *s*-pentyl, neopentyl, hexyl, heptyl, octyl, nonyl, decyl, dodecyl, tetradecyl, hexadecyl, eicosyl, tetracosyl, and the like. The alkyl group can be cyclic or acyclic. The alkyl group can be branched or unbranched. The alkyl group can also be substituted or unsubstituted. For example, the alkyl group can be substituted with one or more groups including, but not limited to, alkyl, cycloalkyl, alkoxy, amino, ether, halide, hydroxy, nitro, silyl, sulfo-oxo, or thiol, as described herein. A "lower alkyl" group is an alkyl group containing from one to six (e.g., from one to four) carbon atoms.

[0066] Throughout the specification "alkyl" is generally used to refer to both unsubstituted alkyl groups and substituted alkyl groups; however, substituted alkyl groups are also specifically referred to herein by identifying the specific substituent(s) on the alkyl group. For example, the term "halogenated alkyl" or "haloalkyl" specifically refers to an alkyl group that is substituted with one or more halide, *e.g.*, fluorine, chlorine, bromine, or iodine. The term "alkoxyalkyl" specifically refers to an alkyl group that is substituted with one or more alkoxy groups, as described below. The term "alkylamino" specifically refers to an alkyl group that is substituted with one or more amino groups, as described below, and the like. When "alkyl" is used in one instance and a specific term such as "alkylalcohol" is used in another, it is not meant to imply that the term "alkyl" does not also refer to specific terms

such as "alkylalcohol" and the like.

[0067] This practice is also used for other groups described herein. That is, while a term such as "cycloalkyl" refers to both unsubstituted and substituted cycloalkyl moieties, the substituted moieties can, in addition, be specifically identified herein; for example, a particular substituted cycloalkyl can be referred to as, *e.g.*, an "alkylcycloalkyl." Similarly, a substituted alkoxy can be specifically referred to as, *e.g.*, a "halogenated alkoxy," a particular substituted alkenyl can be, *e.g.*, an "alkenylalcohol," and the like. Again, the practice of using a general term, such as "cycloalkyl," and a specific term, such as "alkylcycloalkyl," is not meant to imply that the general term does not also include the specific term.

[0068] The term "cycloalkyl" as used herein is a non-aromatic carbon-based ring composed of at least three carbon atoms. Examples of cycloalkyl groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, norbornyl, and the like. The term "heterocycloalkyl" is a type of cycloalkyl group as defined above, and is included within the meaning of the term "cycloalkyl," where at least one of the carbon atoms of the ring is replaced with a heteroatom such as, but not limited to, nitrogen, oxygen, sulfur, or phosphorus. The cycloalkyl group and heterocycloalkyl group can be substituted or unsubstituted. The cycloalkyl group and heterocycloalkyl group can be substituted with one or more groups including, but not limited to, alkyl, cycloalkyl, alkoxy, amino, ether, halide, hydroxy, nitro, silyl, sulfo-oxo, or thiol as described herein.

[0069] The term "polyalkylene group" as used herein is a group having two or more CH_2 groups linked to one another. The polyalkylene group can be represented by the formula —$(CH_2)_a$—, where "a" is an integer of from 2 to 500.

[0070] The terms "alkoxy" and "alkoxyl" as used herein to refer to an alkyl or cycloalkyl group bonded through an ether linkage; that is, an "alkoxy" group can be defined as —OA^1 where A^1 is alkyl or cycloalkyl as defined above. "Alkoxy" also includes polymers of alkoxy groups as just described; that is, an alkoxy can be a polyether such as —OA^1—OA^2 or —OA^1—$(OA^2)_a$—OA^3, where "a" is an integer of from 1 to 200 and A^1, A^2, and A^3 are alkyl and/or cycloalkyl groups.

[0071] The term "alkenyl" as used herein is a hydrocarbon group of from 2 to 24 carbon atoms with a structural formula containing at least one carbon-carbon double bond.

Asymmetric structures such as $(A^1A^2)C=C(A^3A^4)$ are intended to include both the *E* and *Z* isomers. This can be presumed in structural formulae herein wherein an asymmetric alkene is present, or it can be explicitly indicated by the bond symbol C=C. The alkenyl group can be substituted with one or more groups including, but not limited to, alkyl, cycloalkyl, alkoxy, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, heteroaryl, aldehyde, amino, carboxylic acid, ester, ether, halide, hydroxy, ketone, azide, nitro, silyl, sulfo-oxo, or thiol, as described herein.

[0072] The term "cycloalkenyl" as used herein is a non-aromatic carbon-based ring composed of at least three carbon atoms and containing at least one carbon-carbon double bound, *i.e.*, C=C. Examples of cycloalkenyl groups include, but are not limited to, cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclopentadienyl, cyclohexenyl, cyclohexadienyl, norbornenyl, and the like. The term "heterocycloalkenyl" is a type of cycloalkenyl group as defined above, and is included within the meaning of the term "cycloalkenyl," where at least one of the carbon atoms of the ring is replaced with a heteroatom such as, but not limited to, nitrogen, oxygen, sulfur, or phosphorus. The cycloalkenyl group and heterocycloalkenyl group can be substituted or unsubstituted. The cycloalkenyl group and heterocycloalkenyl group can be substituted with one or more groups including, but not limited to, alkyl, cycloalkyl, alkoxy, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, heteroaryl, aldehyde, amino, carboxylic acid, ester, ether, halide, hydroxy, ketone, azide, nitro, silyl, sulfo-oxo, or thiol as described herein.

[0073] The term "alkynyl" as used herein is a hydrocarbon group of 2 to 24 carbon atoms with a structural formula containing at least one carbon-carbon triple bond. The alkynyl group can be unsubstituted or substituted with one or more groups including, but not limited to, alkyl, cycloalkyl, alkoxy, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, heteroaryl, aldehyde, amino, carboxylic acid, ester, ether, halide, hydroxy, ketone, azide, nitro, silyl, sulfo-oxo, or thiol, as described herein.

[0074] The term "cycloalkynyl" as used herein is a non-aromatic carbon-based ring composed of at least seven carbon atoms and containing at least one carbon-carbon triple bound. Examples of cycloalkynyl groups include, but are not limited to, cycloheptynyl, cyclooctynyl, cyclononynyl, and the like. The term "heterocycloalkynyl" is a type of cycloalkenyl group as defined above, and is included within the meaning of the term "cycloalkynyl," where at least

one of the carbon atoms of the ring is replaced with a heteroatom such as, but not limited to, nitrogen, oxygen, sulfur, or phosphorus. The cycloalkynyl group and heterocycloalkynyl group can be substituted or unsubstituted. The cycloalkynyl group and heterocycloalkynyl group can be substituted with one or more groups including, but not limited to, alkyl, cycloalkyl, alkoxy, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, heteroaryl, aldehyde, amino, carboxylic acid, ester, ether, halide, hydroxy, ketone, azide, nitro, silyl, sulfo-oxo, or thiol as described herein.

[0075] The term "aryl" as used herein is a group that contains any carbon-based aromatic group including, but not limited to, benzene, naphthalene, phenyl, biphenyl, phenoxybenzene, and the like. The term "aryl" also includes "heteroaryl," which is defined as a group that contains an aromatic group that has at least one heteroatom incorporated within the ring of the aromatic group. Examples of heteroatoms include, but are not limited to, nitrogen, oxygen, sulfur, and phosphorus. Likewise, the term "non-heteroaryl," which is also included in the term "aryl," defines a group that contains an aromatic group that does not contain a heteroatom. The aryl group can be substituted or unsubstituted. The aryl group can be substituted with one or more groups including, but not limited to, alkyl, cycloalkyl, alkoxy, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, heteroaryl, aldehyde, amino, carboxylic acid, ester, ether, halide, hydroxy, ketone, azide, nitro, silyl, sulfo-oxo, or thiol as described herein. The term "biaryl" is a specific type of aryl group and is included in the definition of "aryl." Biaryl refers to two aryl groups that are bound together *via* a fused ring structure, as in naphthalene, or are attached *via* one or more carbon-carbon bonds, as in biphenyl.

[0076] The term "aldehyde" as used herein is represented by the formula —C(O)H. Throughout this specification "C(O)" is a short hand notation for a carbonyl group, *i.e.*, C=O.

[0077] The terms "amine" or "amino" as used herein are represented by the formula —NA^1A^2, where A^1 and A^2 can be, independently, hydrogen or alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group as described herein.

[0078] The term "alkylamino" as used herein is represented by the formula —NH(-alkyl) where alkyl is a described herein. Representative examples include, but are not limited to, methylamino group, ethylamino group, propylamino group, isopropylamino group, butylamino group, isobutylamino group, (sec-butyl)amino group, (tert-butyl)amino group,

pentylamino group, isopentylamino group, (tert-pentyl)amino group, hexylamino group, and the like.

[0079] The term "dialkylamino" as used herein is represented by the formula —N(-alkyl)$_2$ where alkyl is a described herein. Representative examples include, but are not limited to, dimethylamino group, diethylamino group, dipropylamino group, diisopropylamino group, dibutylamino group, diisobutylamino group, di(sec-butyl)amino group, di(tert-butyl)amino group, dipentylamino group, diisopentylamino group, di(tert-pentyl)amino group, dihexylamino group, N-ethyl-N-methylamino group, N-methyl-N-propylamino group, N-ethyl-N-propylamino group and the like.

[0080] The term "carboxylic acid" as used herein is represented by the formula —C(O)OH.

[0081] The term "ester" as used herein is represented by the formula —OC(O)A^1 or —C(O)OA^1, where A^1 can be alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group as described herein. The term "polyester" as used herein is represented by the formula —(A^{1}O(O)C-A^2-C(O)O)$_a$— or —(A^{1}O(O)C-A^2-OC(O))$_a$—, where A^1 and A^2 can be, independently, an alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group described herein and "a" is an interger from 1 to 500. "Polyester" is as the term used to describe a group that is produced by the reaction between a compound having at least two carboxylic acid groups with a compound having at least two hydroxyl groups.

[0082] The term "ether" as used herein is represented by the formula A^{1}OA^2, where A^1 and A^2 can be, independently, an alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group described herein. The term "polyether" as used herein is represented by the formula —(A^{1}O-A^{2}O)$_a$—, where A^1 and A^2 can be, independently, an alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group described herein and "a" is an integer of from 1 to 500. Examples of polyether groups include polyethylene oxide, polypropylene oxide, and polybutylene oxide.

[0083] The term "halide" as used herein refers to the halogens fluorine, chlorine, bromine, and iodine.

[0084] The term "heterocycle," as used herein refers to single and multi-cyclic aromatic or

non-aromatic ring systems in which at least one of the ring members is other than carbon. Heterocycle includes azetidine, dioxane, furan, imidazole, isothiazole, isoxazole, morpholine, oxazole, oxazole, including, 1,2,3-oxadiazole, 1,2,5-oxadiazole and 1,3,4-oxadiazole, piperazine, piperidine, pyrazine, pyrazole, pyridazine, pyridine, pyrimidine, pyrrole, pyrrolidine, tetrahydrofuran, tetrahydropyran, tetrazine, including 1,2,4,5-tetrazine, tetrazole, including 1,2,3,4-tetrazole and 1,2,4,5-tetrazole, thiadiazole, including, 1,2,3-thiadiazole, 1,2,5-thiadiazole, and 1,3,4-thiadiazole, thiazole, thiophene, triazine, including 1,3,5-triazine and 1,2,4-triazine, triazole, including, 1,2,3-triazole, 1,3,4-triazole, and the like.

[0085] The term "hydroxyl" as used herein is represented by the formula —OH.

[0086] The term "ketone" as used herein is represented by the formula $A^1C(O)A^2$, where A^1 and A^2 can be, independently, an alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group as described herein.

[0087] The term "azide" as used herein is represented by the formula $—N_3$.

[0088] The term "nitro" as used herein is represented by the formula $—NO_2$.

[0089] The term "nitrile" as used herein is represented by the formula —CN.

[0090] The term "silyl" as used herein is represented by the formula $—SiA^1A^2A^3$, where A^1, A^2, and A^3 can be, independently, hydrogen or an alkyl, cycloalkyl, alkoxy, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group as described herein.

[0091] The term "sulfo-oxo" as used herein is represented by the formulas $—S(O)A^1$, $—S(O)_2A^1$, $—OS(O)_2A^1$, or $—OS(O)_2OA^1$, where A^1 can be hydrogen or an alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group as described herein. Throughout this specification "S(O)" is a short hand notation for S=O. The term "sulfonyl" is used herein to refer to the sulfo-oxo group represented by the formula $—S(O)_2A^1$, where A^1 can be hydrogen or an alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group as described herein. The term "sulfone" as used herein is represented by the formula $A^1S(O)_2A^2$, where A^1 and A^2 can be, independently, an alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or heteroaryl group as described herein. The term "sulfoxide" as used herein is represented by the formula $A^1S(O)A^2$, where A^1 and A^2 can be, independently, an alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, cycloalkynyl, aryl, or

heteroaryl group as described herein.

[0092] The term "thiol" as used herein is represented by the formula —SH.

[0093] "R^1," "R^2," "R^3," "R^n," where n is an integer, as used herein can, independently, possess one or more of the groups listed above. For example, if R^1 is a straight chain alkyl group, one of the hydrogen atoms of the alkyl group can optionally be substituted with a hydroxyl group, an alkoxy group, an alkyl group, a halide, and the like. Depending upon the groups that are selected, a first group can be incorporated within second group or, alternatively, the first group can be pendant (*i.e.*, attached) to the second group. For example, with the phrase "an alkyl group comprising an amino group," the amino group can be incorporated within the backbone of the alkyl group. Alternatively, the amino group can be attached to the backbone of the alkyl group. The nature of the group(s) that is (are) selected will determine if the first group is embedded or attached to the second group.

[0094] As described herein, compounds of the invention may contain "optionally substituted" moieties. In general, the term "substituted," whether preceded by the term "optionally" or not, means that one or more hydrogens of the designated moiety are replaced with a suitable substituent. Unless otherwise indicated, an "optionally substituted" group may have a suitable substituent at each substitutable position of the group, and when more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. Combinations of substituents envisioned by this invention are preferably those that result in the formation of stable or chemically feasible compounds. In is also contemplated that, in certain aspects, unless expressly indicated to the contrary, individual substituents can be further optionally substituted (i.e., further substituted or unsubstituted).

[0095] The term "stable," as used herein, refers to compounds that are not substantially altered when subjected to conditions to allow for their production, detection, and, in certain aspects, their recovery, purification, and use for one or more of the purposes disclosed herein.

[0096] Suitable monovalent substituents on a substitutable carbon atom of an "optionally substituted" group are independently halogen; $-(CH_2)_{0-4}R°$; $-(CH_2)_{0-4}OR°$; $-O(CH_2)_{0-4}R°$, $-O-(CH_2)_{0-4}C(O)OR°$; $-(CH_2)_{0-4}CH(OR°)_2$; $-(CH_2)_{0-4}SR°$; $-(CH_2)_{0-4}Ph$, which may be substituted with R°; $-(CH_2)_{0-4}O(CH_2)_{0-1}Ph$ which may be substituted with R°; –CH=CHPh,

which may be substituted with R°; –$(CH_2)_{0-4}O(CH_2)_{0-1}$-pyridyl which may be substituted with R°; –NO_2; –CN; –N_3; -$(CH_2)_{0-4}N(R°)_2$; –$(CH_2)_{0-4}N(R°)C(O)R°$; –$N(R°)C(S)R°$; –$(CH_2)_{0-4}N(R°)C(O)NR°_2$; -$N(R°)C(S)NR°_2$; –$(CH_2)_{0-4}N(R°)C(O)OR°$; –$N(R°)N(R°)C(O)R°$; -$N(R°)N(R°)C(O)NR°_2$; -$N(R°)N(R°)C(O)OR°$; –$(CH_2)_{0-4}C(O)R°$; –$C(S)R°$; –$(CH_2)_{0-4}C(O)OR°$; –$(CH_2)_{0-4}C(O)SR°$; -$(CH_2)_{0-4}C(O)OSiR°_3$; –$(CH_2)_{0-4}OC(O)R°$; –$OC(O)(CH_2)_{0-4}SR$–, SC(S)SR°; –$(CH_2)_{0-4}SC(O)R°$; –$(CH_2)_{0-4}C(O)NR°_2$; –$C(S)NR°_2$; –$C(S)SR°$; –SC(S)SR°, -$(CH_2)_{0-4}OC(O)NR°_2$; -$C(O)N(OR°)R°$; –$C(O)C(O)R°$; –$C(O)CH_2C(O)R°$; –$C(NOR°)R°$; -$(CH_2)_{0-4}SSR°$; –$(CH_2)_{0-4}S(O)_2R°$; –$(CH_2)_{0-4}S(O)_2OR°$; –$(CH_2)_{0-4}OS(O)_2R°$; –$S(O)_2NR°_2$; -$(CH_2)_{0-4}S(O)R°$; -$N(R°)S(O)_2NR°_2$; –$N(R°)S(O)_2R°$; –$N(OR°)R°$; –$C(NH)NR°_2$; –$P(O)_2R°$; -$P(O)R°_2$; -$OP(O)R°_2$; –$OP(O)(OR°)_2$; $SiR°_3$; –(C_{1-4} straight or branched alkylene)O–$N(R°)_2$; or –(C_{1-4} straight or branched alkylene)C(O)O–$N(R°)_2$, wherein each R° may be substituted as defined below and is independently hydrogen, C_{1-6} aliphatic, –CH_2Ph, –$O(CH_2)_{0-1}Ph$, -CH_2-(5-6 membered heteroaryl ring), or a 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of R°, taken together with their intervening atom(s), form a 3–12–membered saturated, partially unsaturated, or aryl mono– or bicyclic ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, which may be substituted as defined below.

[0097] Suitable monovalent substituents on R° (or the ring formed by taking two independent occurrences of R° together with their intervening atoms), are independently halogen, –$(CH_2)_{0-2}R^•$, –(haloR•), –$(CH_2)_{0-2}OH$, –$(CH_2)_{0-2}OR^•$, –$(CH_2)_{0-2}CH(OR^•)_2$; -O(haloR•), –CN, –N_3, –$(CH_2)_{0-2}C(O)R^•$, –$(CH_2)_{0-2}C(O)OH$, –$(CH_2)_{0-2}C(O)OR^•$, –$(CH_2)_{0-2}SR^•$, –$(CH_2)_{0-2}SH$, –$(CH_2)_{0-2}NH_2$, –$(CH_2)_{0-2}NHR^•$, –$(CH_2)_{0-2}NR^•_2$, –NO_2, –$SiR^•_3$, –$OSiR^•_3$, -$C(O)SR^•$, –(C_{1-4} straight or branched alkylene)$C(O)OR^•$, or –$SSR^•$ wherein each R• is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently selected from C_{1-4} aliphatic, –CH_2Ph, –$O(CH_2)_{0-1}Ph$, or a 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents on a saturated carbon atom of R° include =O and =S.

[0098] Suitable divalent substituents on a saturated carbon atom of an "optionally

substituted" group include the following: =O, =S, =NNR*2, =NNHC(O)R*, =NNHC(O)OR*, =NNHS(O)2R*, =NR*, =NOR*, –O(C(R*2))2–3O–, or –S(C(R*2))2–3S–, wherein each independent occurrence of R* is selected from hydrogen, C1–6 aliphatic which may be substituted as defined below, or an unsubstituted 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents that are bound to vicinal substitutable carbons of an "optionally substituted" group include: –O(CR*2)2 3O–, wherein each independent occurrence of R* is selected from hydrogen, C1–6 aliphatic which may be substituted as defined below, or an unsubstituted 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[0099] Suitable substituents on the aliphatic group of R* include halogen, –R•, -(haloR•), -OH, –OR•, –O(haloR•), –CN, –C(O)OH, –C(O)OR•, –NH2, –NHR•, –NR•2, or –NO2, wherein each R• is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently C1–4 aliphatic, –CH2Ph, –O(CH2)0–1Ph, or a 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00100] Suitable substituents on a substitutable nitrogen of an "optionally substituted" group include –R†, –NR†2, –C(O)R†, –C(O)OR†, –C(O)C(O)R†, –C(O)CH2C(O)R†, –S(O)2R†, -S(O)2NR†2, –C(S)NR†2, –C(NH)NR†2, or –N(R†)S(O)2R†; wherein each R† is independently hydrogen, C1–6 aliphatic which may be substituted as defined below, unsubstituted –OPh, or an unsubstituted 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of R†, taken together with their intervening atom(s) form an unsubstituted 3–12–membered saturated, partially unsaturated, or aryl mono– or bicyclic ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00101] Suitable substituents on the aliphatic group of R† are independently halogen, –R•, -(haloR•), –OH, –OR•, –O(haloR•), –CN, –C(O)OH, –C(O)OR•, –NH2, –NHR•, –NR•2, or -NO2, wherein each R• is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently C1–4 aliphatic, –CH2Ph, –O(CH2)0–1Ph, or a

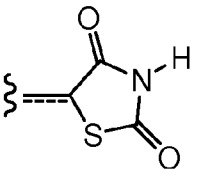
5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00102] The term "leaving group" refers to an atom (or a group of atoms) with electron withdrawing ability that can be displaced as a stable species, taking with it the bonding electrons. Examples of suitable leaving groups include halides and sulfonate esters, including, but not limited to, triflate, mesylate, tosylate, brosylate, and halides.

[00103] The terms "hydrolysable group" and "hydrolysable moiety" refer to a functional group capable of undergoing hydrolysis, e.g., under basic or acidic conditions. Examples of hydrolysable residues include, without limitatation, acid halides, activated carboxylic acids, and various protecting groups known in the art (see, for example, "Protective Groups in Organic Synthesis," T. W. Greene, P. G. M. Wuts, Wiley-Interscience, 1999).

[00104] The term "organic residue" defines a carbon containing residue, i.e., a residue comprising at least one carbon atom, and includes but is not limited to the carbon-containing groups, residues, or radicals defined hereinabove. Organic residues can contain various heteroatoms, or be bonded to another molecule through a heteroatom, including oxygen, nitrogen, sulfur, phosphorus, or the like. Examples of organic residues include but are not limited alkyl or substituted alkyls, alkoxy or substituted alkoxy, mono or di-substituted amino, amide groups, etc. Organic residues can preferably comprise 1 to 18 carbon atoms, 1 to 15, carbon atoms, 1 to 12 carbon atoms, 1 to 8 carbon atoms, 1 to 6 carbon atoms, or 1 to 4 carbon atoms. In a further aspect, an organic residue can comprise 2 to 18 carbon atoms, 2 to 15, carbon atoms, 2 to 12 carbon atoms, 2 to 8 carbon atoms, 2 to 4 carbon atoms, or 2 to 4 carbon atoms.

[00105] A very close synonym of the term "residue" is the term "radical," which as used in the specification and concluding claims, refers to a fragment, group, or substructure of a molecule described herein, regardless of how the molecule is prepared. For example, a 2,4-thiazolidinedione radical in a particular compound has the structure



regardless of whether thiazolidinedione is used to prepare the compound. In some embodiments the radical (for example an alkyl) can be further modified (i.e., substituted alkyl) by having bonded thereto one or more "substituent radicals." The number of atoms in a given radical is not critical to the present invention unless it is indicated to the contrary elsewhere herein.

[00106] "Organic radicals," as the term is defined and used herein, contain one or more carbon atoms. An organic radical can have, for example, 1-26 carbon atoms, 1-18 carbon atoms, 1-12 carbon atoms, 1-8 carbon atoms, 1-6 carbon atoms, or 1-4 carbon atoms. In a further aspect, an organic radical can have 2-26 carbon atoms, 2-18 carbon atoms, 2-12 carbon atoms, 2-8 carbon atoms, 2-6 carbon atoms, or 2-4 carbon atoms. Organic radicals often have hydrogen bound to at least some of the carbon atoms of the organic radical. One example, of an organic radical that comprises no inorganic atoms is a 5, 6, 7, 8-tetrahydro-2-naphthyl radical. In some embodiments, an organic radical can contain 1-10 inorganic heteroatoms bound thereto or therein, including halogens, oxygen, sulfur, nitrogen, phosphorus, and the like. Examples of organic radicals include but are not limited to an alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, mono-substituted amino, di-substituted amino, acyloxy, cyano, carboxy, carboalkoxy, alkylcarboxamide, substituted alkylcarboxamide, dialkylcarboxamide, substituted dialkylcarboxamide, alkylsulfonyl, alkylsulfinyl, thioalkyl, thiohaloalkyl, alkoxy, substituted alkoxy, haloalkyl, haloalkoxy, aryl, substituted aryl, heteroaryl, heterocyclic, or substituted heterocyclic radicals, wherein the terms are defined elsewhere herein. A few non-limiting examples of organic radicals that include heteroatoms include alkoxy radicals, trifluoromethoxy radicals, acetoxy radicals, dimethylamino radicals and the like.

[00107] "Inorganic radicals," as the term is defined and used herein, contain no carbon atoms and therefore comprise only atoms other than carbon. Inorganic radicals comprise bonded combinations of atoms selected from hydrogen, nitrogen, oxygen, silicon, phosphorus, sulfur, selenium, and halogens such as fluorine, chlorine, bromine, and iodine, which can be present individually or bonded together in their chemically stable combinations.

Inorganic radicals have 10 or fewer, or preferably one to six or one to four inorganic atoms as listed above bonded together. Examples of inorganic radicals include, but not limited to, amino, hydroxy, halogens, nitro, thiol, sulfate, phosphate, and like commonly known inorganic radicals. The inorganic radicals do not have bonded therein the metallic elements of the periodic table (such as the alkali metals, alkaline earth metals, transition metals, lanthanide metals, or actinide metals), although such metal ions can sometimes serve as a pharmaceutically acceptable cation for anionic inorganic radicals such as a sulfate, phosphate, or like anionic inorganic radical. Inorganic radicals do not comprise metalloids elements such as boron, aluminum, gallium, germanium, arsenic, tin, lead, or tellurium, or the noble gas elements, unless otherwise specifically indicated elsewhere herein.

[00108] Compounds described herein can contain one or more double bonds and, thus, potentially give rise to cis/trans (E/Z) isomers, as well as other conformational isomers. Unless stated to the contrary, the invention includes all such possible isomers, as well as mixtures of such isomers.

[00109] Unless stated to the contrary, a formula with chemical bonds shown only as solid lines and not as wedges or dashed lines contemplates each possible isomer, *e.g.*, each enantiomer and diastereomer, and a mixture of isomers, such as a racemic or scalemic mixture. Compounds described herein can contain one or more asymmetric centers and, thus, potentially give rise to diastereomers and optical isomers. Unless stated to the contrary, the present invention includes all such possible diastereomers as well as their racemic mixtures, their substantially pure resolved enantiomers, all possible geometric isomers, and pharmaceutically acceptable salts thereof. Mixtures of stereoisomers, as well as isolated specific stereoisomers, are also included. During the course of the synthetic procedures used to prepare such compounds, or in using racemization or epimerization procedures known to those skilled in the art, the products of such procedures can be a mixture of stereoisomers.

[00110] Many organic compounds exist in optically active forms having the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L or R and S are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes d and l or (+) and (-) are employed to designate the sign of rotation of plane-polarized light by the compound, with (-) or meaning that the compound is levorotatory. A compound prefixed with (+) or d is dextrorotatory. For a given

chemical structure, these compounds, called stereoisomers, are identical except that they are non-superimposable mirror images of one another. A specific stereoisomer can also be referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric mixture. A 50:50 mixture of enantiomers is referred to as a racemic mixture. Many of the compounds described herein can have one or more chiral centers and therefore can exist in different enantiomeric forms. If desired, a chiral carbon can be designated with an asterisk (*). When bonds to the chiral carbon are depicted as straight lines in the disclosed formulas, it is understood that both the (R) and (S) configurations of the chiral carbon, and hence both enantiomers and mixtures thereof, are embraced within the formula. As is used in the art, when it is desired to specify the absolute configuration about a chiral carbon, one of the bonds to the chiral carbon can be depicted as a wedge (bonds to atoms above the plane) and the other can be depicted as a series or wedge of short parallel lines is (bonds to atoms below the plane). The Cahn-Inglod-Prelog system can be used to assign the (R) or (S) configuration to a chiral carbon.

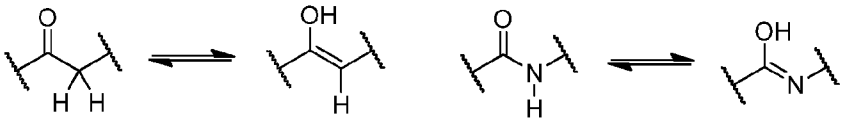
[00111] Compounds described herein comprise atoms in both their natural isotopic abundance and in non-natural abundance. The disclosed compounds can be isotopically-labelled or isotopically-substituted compounds identical to those described, but for the fact that one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number typically found in nature. Examples of isotopes that can be incorporated into compounds of the invention include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, fluorine and chlorine, such as ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{18}O, ^{17}O, ^{35}S, ^{18}F and ^{36}Cl, respectively. Compounds further comprise prodrugs thereof, and pharmaceutically acceptable salts of said compounds or of said prodrugs which contain the aforementioned isotopes and/or other isotopes of other atoms are within the scope of this invention. Certain isotopically-labelled compounds of the present invention, for example those into which radioactive isotopes such as ^{3}H and ^{14}C are incorporated, are useful in drug and/or substrate tissue distribution assays. Tritiated, i.e., ^{3}H, and carbon-14, i.e., ^{14}C, isotopes are particularly preferred for their ease of preparation and detectability. Further, substitution with heavier isotopes such as deuterium, i.e., ^{2}H, can afford certain therapeutic advantages resulting from greater metabolic stability, for example increased in vivo half-life or reduced dosage requirements and, hence, may be preferred in some circumstances. Isotopically labelled compounds of the present invention and prodrugs thereof

can generally be prepared by carrying out the procedures below, by substituting a readily available isotopically labelled reagent for a non- isotopically labelled reagent.

[00112] The compounds described in the invention can be present as a solvate. In some cases, the solvent used to prepare the solvate is an aqueous solution, and the solvate is then often referred to as a hydrate. The compounds can be present as a hydrate, which can be obtained, for example, by crystallization from a solvent or from aqueous solution. In this connection, one, two, three or any arbitrary number of solvate or water molecules can combine with the compounds according to the invention to form solvates and hydrates. Unless stated to the contrary, the invention includes all such possible solvates.

[00113] The term "co-crystal" means a physical association of two or more molecules which owe their stability through non-covalent interaction. One or more components of this molecular complex provide a stable framework in the crystalline lattice. In certain instances, the guest molecules are incorporated in the crystalline lattice as anhydrates or solvates, see e.g. "Crystal Engineering of the Composition of Pharmaceutical Phases. Do Pharmaceutical Co-crystals Represent a New Path to Improved Medicines?" Almarasson, O., et. al., The Royal Society of Chemistry, 1889-1896, 2004. Examples of co-crystals include p-toluenesulfonic acid and benzenesulfonic acid.

[00114] It is also appreciated that certain compounds described herein can be present as an equilibrium of tautomers. For example, ketones with an α-hydrogen can exist in an equilibrium of the keto form and the enol form.



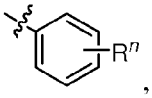
keto form enol form amide form imidic acid form

Likewise, amides with an N-hydrogen can exist in an equilibrium of the amide form and the imidic acid form. Unless stated to the contrary, the invention includes all such possible tautomers.

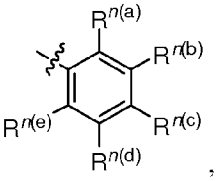
[00115] It is known that chemical substances form solids which are present in different states of order which are termed polymorphic forms or modifications. The different modifications of a polymorphic substance can differ greatly in their physical properties. The

compounds according to the invention can be present in different polymorphic forms, with it being possible for particular modifications to be metastable. Unless stated to the contrary, the invention includes all such possible polymorphic forms.

[00116] In some aspects, a structure of a compound can be represented by a formula:



which is understood to be equivalent to a formula:



wherein n is typically an integer. That is, R^n is understood to represent five independent substituents, $R^{n(a)}$, $R^{n(b)}$, $R^{n(c)}$, $R^{n(d)}$, $R^{n(e)}$. By "independent substituents," it is meant that each R substituent can be independently defined. For example, if in one instance $R^{n(a)}$ is halogen, then $R^{n(b)}$ is not necessarily halogen in that instance.

[00117] Certain materials, compounds, compositions, and components disclosed herein can be obtained commercially or readily synthesized using techniques generally known to those of skill in the art. For example, the starting materials and reagents used in preparing the disclosed compounds and compositions are either available from commercial suppliers such as Aldrich Chemical Co., (Milwaukee, Wis.), Acros Organics (Morris Plains, N.J.), Fisher Scientific (Pittsburgh, Pa.), or Sigma (St. Louis, Mo.) or are prepared by methods known to those skilled in the art following procedures set forth in references such as Fieser and Fieser's Reagents for Organic Synthesis, Volumes 1-17 (John Wiley and Sons, 1991); Rodd's Chemistry of Carbon Compounds, Volumes 1-5 and Supplementals (Elsevier Science Publishers, 1989); Organic Reactions, Volumes 1-40 (John Wiley and Sons, 1991); March's Advanced Organic Chemistry, (John Wiley and Sons, 4th Edition); and Larock's Comprehensive Organic Transformations (VCH Publishers Inc., 1989).

[00118] Unless otherwise expressly stated, it is in no way intended that any method set forth herein be construed as requiring that its steps be performed in a specific order.

Accordingly, where a method claim does not actually recite an order to be followed by its steps or it is not otherwise specifically stated in the claims or descriptions that the steps are to be limited to a specific order, it is no way intended that an order be inferred, in any respect. This holds for any possible non-express basis for interpretation, including: matters of logic with respect to arrangement of steps or operational flow; plain meaning derived from grammatical organization or punctuation; and the number or type of embodiments described in the specification.

[00119] Disclosed are the components to be used to prepare the compositions of the invention as well as the compositions themselves to be used within the methods disclosed herein. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutation of these compounds can not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a particular compound is disclosed and discussed and a number of modifications that can be made to a number of molecules including the compounds are discussed, specifically contemplated is each and every combination and permutation of the compound and the modifications that are possible unless specifically indicated to the contrary. Thus, if a class of molecules A, B, and C are disclosed as well as a class of molecules D, E, and F and an example of a combination molecule, A-D is disclosed, then even if each is not individually recited each is individually and collectively contemplated meaning combinations, A-E, A-F, B-D, B-E, B-F, C-D, C-E, and C-F are considered disclosed. Likewise, any subset or combination of these is also disclosed. Thus, for example, the sub-group of A-E, B-F, and C-E would be considered disclosed. This concept applies to all aspects of this application including, but not limited to, steps in methods of making and using the compositions of the invention. Thus, if there are a variety of additional steps that can be performed it is understood that each of these additional steps can be performed with any specific embodiment or combination of embodiments of the methods of the invention.

[00120] It is understood that the compositions disclosed herein have certain functions. Disclosed herein are certain structural requirements for performing the disclosed functions, and it is understood that there are a variety of structures that can perform the same function that are related to the disclosed structures, and that these structures will typically achieve the same result.

B. COMPOUNDS

[00121] In one aspect, the invention relates to compounds useful in methods to inhibit muscle atrophy and to increase muscle mass by providing to a subject in need thereof an effective amount of ursolic acid or a derivative thereof, and pharmaceutical compositions comprising compounds used in the methods. In a further aspect, the invention relates to compounds useful in methods to modulate muscle growth, methods to inhibit muscle atrophy and to increase muscle mass, methods to induce skeletal muscle hypertrophy, methods to enhance tissue growth, and pharmaceutical compositions comprising compounds used in the methods.

[00122] In one aspect, the compounds of the invention are useful in the treatment of muscle disorders. In a further aspect, the muscle disorder can be skeletal muscle atrophy secondary to malnutrition, muscle disuse (secondary to voluntary or involuntary bedrest), neurologic disease (including multiple sclerosis, amyotrophic lateral sclerosis, spinal muscular atrophy, critical illness neuropathy, spinal cord injury or peripheral nerve injury), orthopedic injury, casting, and other post-surgical forms of limb immobilization, chronic disease (including cancer, congestive heart failure, chronic pulmonary disease, chronic renal failure, chronic liver disease, diabetes mellitus, Cushing syndrome and chronic infections such as HIV/AIDS or tuberculosis), burns, sepsis, other illnesses requiring mechanical ventiliation, drug-induced muscle disease (such as glucorticoid-induced myopathy and statin-induced myopathy), genetic diseases that primarily affect skeletal muscle (such as muscular dystrophy and myotonic dystrophy), autoimmune diseases that affect skeletal muscle (such as polymyositis and dermatomyositis), spaceflight, or age-related sarcopenia.

[00123] It is contemplated that each disclosed derivative can be optionally further substituted. It is also contemplated that any one or more derivative can be optionally omitted from the invention. It is understood that a disclosed compound can be provided by the disclosed methods. It is also understood that the disclosed compounds can be employed in the disclosed methods of using.

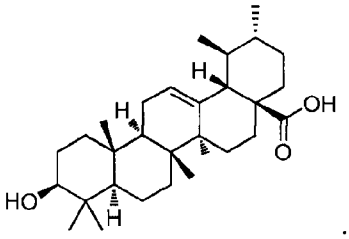
1. URSOLIC ACID DERIVATIVES

[00124] Ursolic acid is a highly water-insoluble pentacyclic triterpene acid that possesses a wide range of biological effects, including anti-cancer, anti-oxidant, anti-

inflammatory, anti-allergic, hepatoprotective, gastroprotective, hypolipidemic, hypoglycemic, lipolytic anti-obesity, anti-atherogenic and immunomodulatory effects (Liu J (1995) *Journal of ethnopharmacology* 49(2):57-68; Liu J (2005) *Journal of ethnopharmacology* 100(1-2):92-94; Wang ZH, *et al.* (2010) *European journal of pharmacology* 628(1-3):255-260; Jang SM, *et al.* (2009) *Int Immunopharmacol* 9(1):113-119). However, its effects on skeletal muscle were not known previously. At the molecular level, ursolic acid inhibits the STAT3 activation pathway, reduces matrix metalloproteinase-9 expression via the glucocorticoid receptor, inhibits protein tyrosine phosphatases, acts as an insulin mimetic, activates PPARα, inhibits NF-kB transcription factors, translocates hormone-senstive lipase to stimulate lipolysis and inhibits the hepatic polyol pathway, among many other described effects. Its effects on skeletal muscle and IGF-I signaling were not previously known.

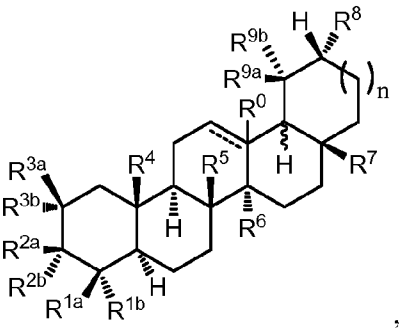
[00125] As medicine, ursolic acid is well tolerated and can be used topically and orally. Ursolic acid is present in many plants, including apples, basil, bilberries, cranberries, elder flower, peppermint, rosemary, lavender, oregano, thyme, hawthorn, prunes. Apple peels contain high quantity of ursolic acid and related compounds which are responsible for the anti-cancer activity of apple. Ursolic acid can also serve as a starting material for synthesis of more potent bioactive derivatives, such as anti-tumor agents.

[00126] Other names for ursolic acid include 3-β-hydroxy-urs-12-en-28-oic acid, urson, prunol, micromerol, urson, and malol. The structure is shown below:



[00127] Other closely related pentacyclic triterpene acids with insulin sensitizing actions include oleanolic acid (Wang *et at,* 2010), corosolic acid (Sivakumar *et at,* 2009) and UA0713 (Zhang *et at,* 2006).

[00128] In one aspect, the invention relates to compounds of the formula:



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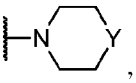
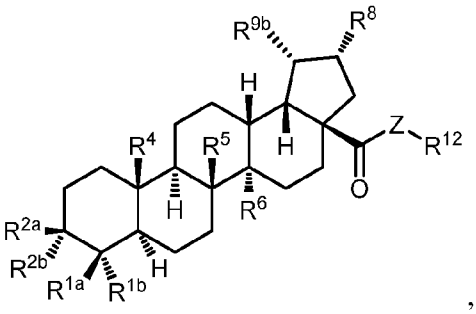
wherein each ----- is an optional covalent bond, and R^0 is optionally present; wherein n is 0 or 1; wherein R^0, when present, is hydrogen; wherein R^{1a} is selected from C1-C6 alkyl and –$C(O)ZR^{10}$; wherein R^{1b} is selected from C1-C6 alkyl, or wherein R^{1a} and R^{1b} are optionally covalently bonded and, together with the intermediate carbon, comprise an optionally substituted C3-C5 cycloalkyl or C2-C5 heterocycloalkyl; wherein one of R^{2a} and R^{2b} is –OR^{11}, and the other is hydrogen, or R^{2a} and R^{2b} together comprise =O; wherein each of R^{3a} and R^{3b} is independently selected from hydrogen, hydroxyl, C1-C6 alkyl, and C1-C6 alkoxyl, provided that R^{3a} and R^{3b} are not simultaneously hydroxyl, wherein R^{3a} and R^{3b} are optionally covalently bonded and, together with the intermediate carbon, comprise an optionally substituted C3-C5 cycloalkyl or C2-C5 heterocycloalkyl; wherein each of R^4, R^5, and R^6 is independently selected from C1-C6 alkyl; wherein R^7 is selected from C1-C6 alkyl, –CH_2OR^{12} and –$C(O)ZR^{12}$; wherein R^8 is selected from hydrogen and C1-C6 alkyl; wherein each of R^{9a} and R^{9b} is independently selected from hydrogen and C1-C6 alkyl, provided that R^{9a} and R^{9b} are not simultaneously hydrogen; or wherein R^{9a} and R^{9b} are covalently bonded and, along with the intermediate carbon, together comprise C3-C5 cycloalkyl or C2-C5 heterocycloalkyl; wherein R^{10} is selected from hydrogen and C1-C6 alkyl; wherein R^{11} is selected from hydrogen, C1-C6 alkyl, C1-C5 heteroalkyl, C3-C6 cycloalkyl, C4-C6 heterocycloalkyl, phenyl, heteroaryl, and –$C(O)R^{14}$; wherein R^{11}, where permitted, is substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; wherein R^{12} is selected from hydrogen and optionally substituted organic residue having from 1 to 20 carbons; wherein Z is selected from –O– and –NR^{13}–; wherein R^{13} is selected from hydrogen and C1-C4 alkyl; or, wherein Z is N, R^{12} and R^{13} are covalently bonded and –$NR^{12}R^{13}$ comprises a moiety of the formula:

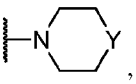


wherein Y is selected from –O–, –S–, –SO–, $–SO_2–$, –NH–, $–NCH_3–$; and wherein R^{14} is C1-C6 alkyl and substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof.

[00129] In a further aspect, the invention relates to compounds of a formula:



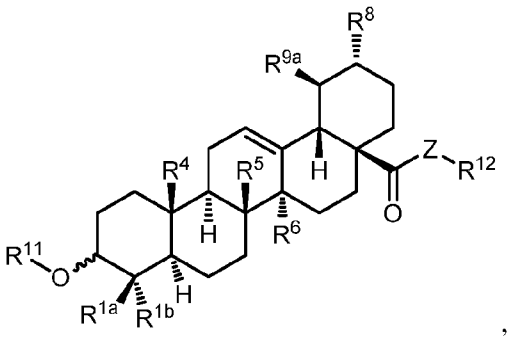
wherein each of R^{1a} and R^{1b} is C1-C6 alkyl; wherein one of R^{2a} and R^{2b} is $–OR^{11}$, and the other is hydrogen; wherein each of R^4, R^5, and R^6 is independently C1-C6 alkyl; wherein R^8 is selected from hydrogen and C1-C6 alkyl; wherein R^{9b} is C1-C6 alkyl; wherein R^{11} is selected from hydrogen, C1-C6 alkyl, C1-C5 heteroalkyl, C3-C6 cycloalkyl, C4-C6 heterocycloalkyl, phenyl, heteroaryl, and $–C(O)R^{14}$; wherein R^{11}, where permitted, is substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; wherein R^{12} is selected from hydrogen and optionally substituted organic residue having from 1 to 20 carbons; wherein Z is selected from –O– and $–NR^{13}–$; wherein R^{13} is selected from hydrogen and C1-C4 alkyl; or, wherein Z is N, R^{12} and R^{13} are covalently bonded and $–NR^{12}R^{13}$ comprises a moiety of the formula:



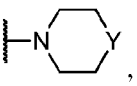
wherein Y is selected from –O–, –S–, –SO–, $–SO_2–$, –NH–, $–NCH_3–$; and wherein R^{14} is C1-C6 alkyl and substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo,

iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl.

[00130] In a further aspect, the invention relates to compounds of a formula:

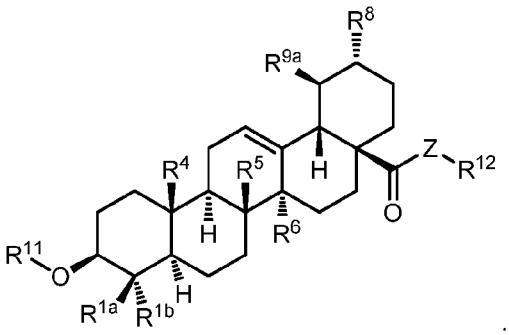


wherein R^{1a} is selected from C1-C6 alkyl and $-C(O)ZR^{10}$; wherein R^{1b} is selected from C1-C6 alkyl, or R^{1a} and R^{1b} are covalently bonded and, together with the intermediate carbon, comprise an optionally substituted 3- to 7-membered spirocycloalkyl; wherein R^8 is C1-C6 alkyl; wherein R^{9a} is C1-C6 alkyl; wherein R^{11} is selected from hydrogen, C1-C6 alkyl, C1-C5 heteroalkyl, C3-C6 cycloalkyl, C4-C6 heterocycloalkyl, phenyl, heteroaryl, and $-C(O)R^{14}$; wherein R^{11}, where permitted, is substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; wherein R^{12} is selected from hydrogen and optionally substituted organic residue having from 1 to 20 carbons; wherein Z is selected from –O– and $-NR^{13}-$; wherein R^{13} is selected from hydrogen and C1-C4 alkyl; or, wherein Z is N, R^{12} and R^{13} are covalently bonded and $-NR^{12}R^{13}$ comprises a moiety of the formula:

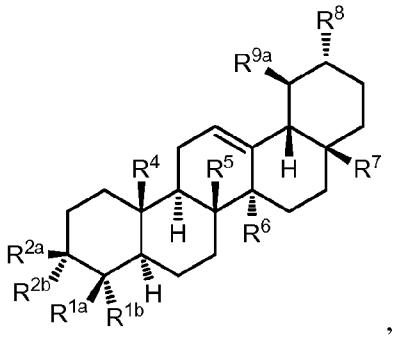


wherein Y is selected from –O–, –S–, –SO–, $-SO_2-$, –NH–, $-NCH_3-$; and wherein R^{14} is C1-C6 alkyl and substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl.

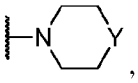
[00131] In a further aspect, the invention relates to compounds of a formula:



[00132] In a further aspect, the invention relates to compounds of a formula:



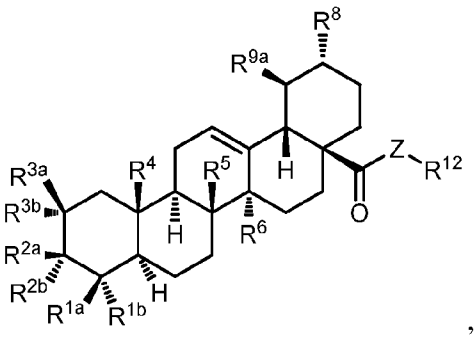
wherein R^{1a} is –C(O)ZR^{10}; wherein R^{1b} is C1-C6 alkyl; wherein one of R^{2a} and R^{2b} is –OR^{11}, and the other is hydrogen; wherein each of R^4, R^5, and R^6 is independently selected from C1-C6 alkyl; wherein R^7 is selected from C1-C6 alkyl; wherein R^8 is selected from hydrogen and C1-C6 alkyl; wherein R^{9a} is selected from hydrogen and C1-C6 alkyl; wherein Z is selected from –O– and –NR^{13}–; wherein R^{10} is selected from hydrogen and C1-C6 alkyl; wherein R^{11} is selected from hydrogen, C1-C6 alkyl, C1-C5 heteroalkyl, C3-C6 cycloalkyl, C4-C6 heterocycloalkyl, phenyl, heteroaryl, and –C(O)R^{14}; wherein R^{11}, where permitted, is substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; wherein R^{13} is selected from hydrogen and C1-C4 alkyl; or, wherein Z is N, R^{12} and R^{13} are covalently bonded and –NR^{12}R^{13} comprises a moiety of the formula:



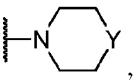
wherein Y is selected from –O–, –S–, –SO–, –SO_2–, –NH–, –NCH_3–; and wherein R^{14} is C1-C6 alkyl and substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl,

propoxyl, and butoxyl.

[00133] In a further aspect, the invention relates to compounds of a formula:

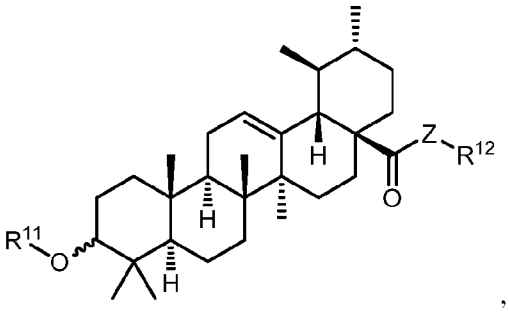


wherein each of R^{1a} and R^{1b} is independently C1-C6 alkyl; wherein one of R^{2a} and R^{2b} is –OR^{11}, and the other is hydrogen; wherein one of R^{3a} and R^{3b} is –OR^{11}, and the other is hydrogen; wherein each of R^4, R^5, and R^6 is independently selected from C1-C6 alkyl; wherein R^8 is C1-C6 alkyl; wherein R^{9a} is C1-C6 alkyl; wherein each R^{11} is independently selected from hydrogen, C1-C6 alkyl, C1-C5 heteroalkyl, C3-C6 cycloalkyl, C4-C6 heterocycloalkyl, phenyl, heteroaryl, and –$C(O)R^{14}$; wherein R^{11}, where permitted, is substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; wherein R^{12} is selected from hydrogen and optionally substituted organic residue having from 1 to 20 carbons; wherein Z is selected from –O– and –NR^{13}–; wherein R^{13} is selected from hydrogen and C1-C4 alkyl; or, wherein Z is N, R^{12} and R^{13} are covalently bonded and –$NR^{12}R^{13}$ comprises a moiety of the formula:

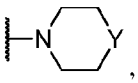


wherein Y is selected from –O–, –S–, –SO–, –SO_2–, –NH–, –NCH_3–; and wherein R^{14} is C1-C6 alkyl and substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl.

[00134] In a further aspect, the invention relates to compounds of a formula:



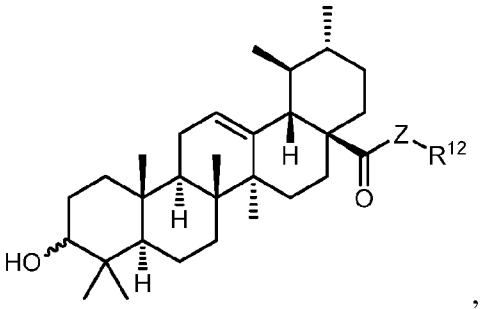
wherein R^{11} is selected from hydrogen, C1-C6 alkyl, C1-C5 heteroalkyl, C3-C6 cycloalkyl, C4-C6 heterocycloalkyl, phenyl, heteroaryl, and $-C(O)R^{14}$; wherein R^{11}, where permitted, is substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; wherein R^{12} is selected from hydrogen and optionally substituted organic residue having from 1 to 20 carbons; wherein Z is selected from –O– and $-NR^{13}-$; wherein R^{13} is selected from hydrogen and C1-C4 alkyl; or, wherein Z is N, R^{12} and R^{13} are covalently bonded and $-NR^{12}R^{13}$ comprises a moiety of the formula:



wherein Y is selected from –O–, –S–, –SO–, $-SO_2-$, –NH–, $-NCH_3-$; and wherein R^{14} is C1-C6 alkyl and substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof.

[00135] In a further aspect, the compound is administered in an amount effective to prevent or treat muscle atrophy in the animal. In a still further aspect, the compound is administered in amount is greater than about 1000 mg per day when the compound is ursolic acid, beta-boswellic acid, corosolic acid, betulinic acid, or UA0713. In a yet further aspect, the compound is administered in an amount greater than about 1000 mg per day and effective to enhance muscle formation in the mammal.

[00136] In a further aspect, the invention relates to compounds of a formula selected from:



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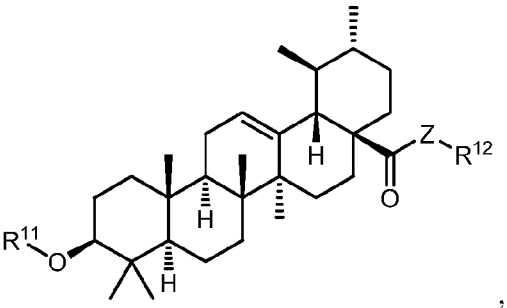

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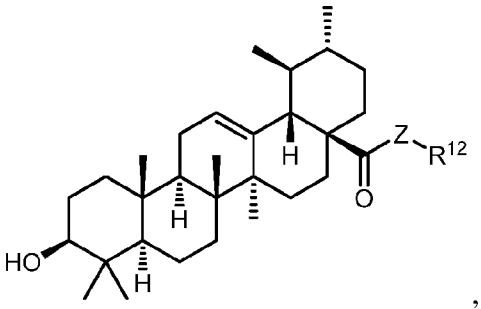

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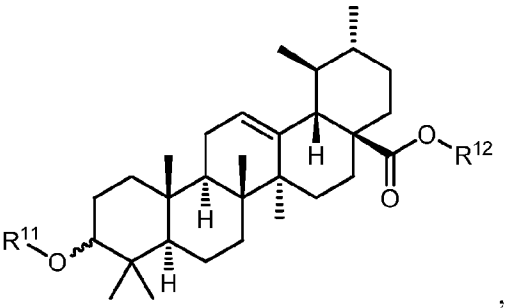

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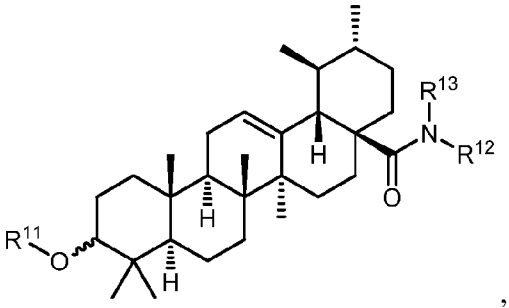
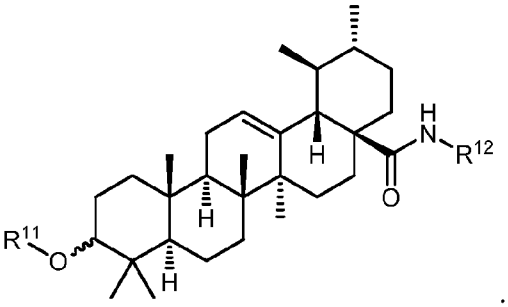


, and



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a. R^0 GROUPS AND OPTIONAL BONDS

[00137] In one aspect, an optional covalent bond can be represented by -----. Thus, in certain aspects, a particular bond is present, thereby providing a single covalent bond. In further aspects, a particular bond is present, thereby providing a double covalent bond. In further aspects, a particular bond is absent, thereby providing a double covalent bond.

[00138] In one aspect, R^0 is optionally present. That is, in certain aspects, R^0 is present. In further aspects, R^0 is absent. In a further aspect, R^0, when present, is hydrogen. It is understood that the presence and/or absence of R^0 Groups and optional bonds serve to satisfy valence of the adjacent chemical moieties.

b. R^1 Groups

[00139] In one aspect, R^{1a} is selected from C1-C6 alkyl and $-C(O)ZR^{10}$; wherein R^{1b} is selected from C1-C6 alkyl; or wherein R^{1a} and R^{1b} are covalently bonded and, along with the intermediate carbon, together comprise optionally substituted C3-C5 cycloalkyl or optionally substituted C2-C5 heterocycloalkyl. In a further aspect, R^{1a} is $-CO_2H$. In a further aspect, R^{1b} is methyl. In a further aspect, R^{1a} and R^{1b} are both methyl.

[00140] In one aspect, R^{1a} is$-C(O)ZR^{10}$. In a further aspect, R^{1a} is selected from C1-C6 alkyl, for example, methyl, ethyl, propyl, butyl, pentyl, or hexyl. In a further aspect, R^{1b} is selected from C1-C6 alkyl, for example, methyl, ethyl, propyl, butyl, pentyl, or hexyl.

[00141] In a further aspect, R^{1a} and R^{1b} are covalently bonded and, along with the intermediate carbon, together comprise optionally substituted C3-C5 cycloalkyl or optionally substituted C2-C5 heterocycloalkyl.

c. R^2 Groups

[00142] In one aspect, R^{2a} and R^{2b} are independently selected from hydrogen and $-OR^{11}$, provided that at least one of R^{2a} and R^{2b} is $-OR^{11}$; or wherein R^{2a} and R^{2b} together comprise =O. In a further aspect, R^{2a} is hydrogen, and R^{2b} is $-OR^{11}$. In a further aspect, R^{2a} is $-OR^{11}$, and R^{2b} is hydrogen. In a further aspect, R^{2a} and R^{2b} together comprise =O.

[00143] In a further aspect, R^{2a} is hydrogen. In a further aspect, R^{2a} is $-OR^{11}$; wherein R^{11} is selected from hydrogen, C1-C6 alkyl, and $-C(O)R^{14}$; wherein R^{14} is C1-C6 alkyl. In a further aspect, R^{2b} is $-OR^{11}$; wherein R^{11} is selected from hydrogen, C1-C6 alkyl, and $-C(O)R^{14}$; and wherein R^{14} is C1-C6 alkyl. In a further aspect, R^{2b} is $-OR^{11}$; wherein R^{11} is hydrogen.

[00144] In a further aspect, R^{2b} is hydrogen. In a further aspect, R^{2a} is $-OR^{11}$; wherein R^{11} is selected from hydrogen, C1-C6 alkyl, and $-C(O)R^{14}$; wherein R^{14} is C1-C6 alkyl. In a further aspect, R^{2a} is $-OR^{11}$; wherein R^{11} is hydrogen.

d. R^3 Groups

[00145] In one aspect, each of R^{3a} and R^{3b} is independently selected from hydrogen, hydroxyl, C1-C6 alkyl, and C1-C6 alkoxyl, provided that R^{3a} and R^{3b} are not simultaneously

hydroxyl; or wherein R^{3a} and R^{3b} are covalently bonded and, along with the intermediate carbon, together comprise optionally substituted C3-C5 cycloalkyl or optionally substituted C2-C5 heterocycloalkyl.

[00146] In a further aspect, R^{3a} is hydrogen. In a further aspect, R^{3b} is $-OR^{11}$; wherein R^{11} is selected from hydrogen, C1-C6 alkyl, and $-C(O)R^{14}$; wherein R^{14} is C1-C6 alkyl.

e. R^4 Groups

[00147] In one aspect, R^4 is independently selected from C1-C6 alkyl, for example, methyl, ethyl, propyl, butyl, pentyl, or hexyl. In a further aspect, R^4 is methyl. In a further aspect, R^4, R^5, and R^6 are all methyl.

f. R^5 Groups

[00148] In one aspect, R^5 is independently selected from C1-C6 alkyl, for example, methyl, ethyl, propyl, butyl, pentyl, or hexyl. In a further aspect, R^5 is methyl.

g. R^6 Groups

[00149] In one aspect, R^6 is independently selected from C1-C6 alkyl, for example, methyl, ethyl, propyl, butyl, pentyl, or hexyl. In a further aspect, R^6 is methyl.

h. R^7 Groups

[00150] In one aspect, R^7 is selected from C1-C6 alkyl, $-CH_2OR^{12}$, and $-C(O)ZR^{12}$. In a further aspect, R^7 is C1-C6 alkyl, for example, methyl, ethyl, propyl, butyl, pentyl, or hexyl. In a further aspect, R^7 is $-CH_2OR^{12}$. In a further aspect, R^7 is and $-C(O)ZR^{12}$.

i. R^8 Groups

[00151] In one aspect, R^8 is selected from hydrogen and C1-C6 alkyl. In a further aspect, R^8 is hydrogen. In a further aspect, R^8 is C1-C6 alkyl, for example, methyl, ethyl, propyl, butyl, pentyl, or hexyl.

j. R^9 Groups

[00152] In one aspect, each of R^{9a} and R^{9b} is independently selected from hydrogen and

C1-C6 alkyl, provided that R^{9a} and R^{9b} are not simultaneously hydrogen; or wherein R^{9a} and R^{9b} are covalently bonded and, along with the intermediate carbon, together comprise optionally substituted C3-C5 cycloalkyl or optionally substituted C2-C5 heterocycloalkyl.

[00153] In a further aspect, R^{9a} is hydrogen. In a further aspect, R^{9a} is C1-C6 alkyl, for example, methyl, ethyl, propyl, butyl, pentyl, or hexyl. In a further aspect, R^{9b} is hydrogen. In a further aspect, R^{9b} is C1-C6 alkyl, for example, methyl, ethyl, propyl, butyl, pentyl, or hexyl. In a further aspect, R^{9b} is selected from methyl, ethyl, vinyl, n-propyl, propen-2-yl, i-propyl, 2-propenyl, n-butyl, 1-buten-2-yl, 1-buten-3-yl, i-butyl, 1-buten-2-yl, 1-buten-3-yl, s-butyl, 2-buten-1-yl, 2-buten-2-yl, 2-buten-3-yl, and t-butyl.

[00154] In a further aspect, R^{9a} and R^{9b} are covalently bonded and, along with the intermediate carbon, together comprise optionally substituted C3-C5 cycloalkyl or optionally substituted C2-C5 heterocycloalkyl.

k. R^{10} Groups

[00155] In one aspect, R^{10} is selected from hydrogen and C1-C6 alkyl. In a further aspect, R^{10} is hydrogen. In a further aspect, R^{10} is C1-C6 alkyl, for example, methyl, ethyl, propyl, butyl, pentyl, or hexyl.

l. R^{11} Groups

[00156] In one aspect, each R^{11} is independently selected from hydrogen, C1-C6 alkyl, C1-C5 heteroalkyl, C3-C6 cycloalkyl, C4-C6 heterocycloalkyl, phenyl, heteroaryl, and –$C(O)R^{14}$; wherein R^{11}, where permitted, is substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl.

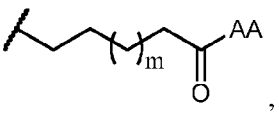
[00157] In a further aspect, R^{11} is hydrogen. In a further aspect, R^{11} is selected from C1-C6 alkyl, C1-C5 heteroalkyl, C3-C6 cycloalkyl, C4-C6 heterocycloalkyl, phenyl, heteroaryl, and –$C(O)R^{14}$. In a further aspect, R^{11} is C1-C6 alkyl. In a further aspect, R^{11} is C1-C5 heteroalkyl. In a further aspect, R^{11} is C3-C6 cycloalkyl. In a further aspect, R^{11} is C4-C6 heterocycloalkyl. In a further aspect, R^{11} is phenyl. In a further aspect, R^{11} is heteroaryl. In a further aspect, R^{11} is –$C(O)R^{14}$.

[00158] In a further aspect, R^{11} is unsubstituted. In a further aspect, R^{11}, where permitted, is substituted with 0-2 groups. In a further aspect, R^{11}, where permitted, is substituted with 1 group. In a further aspect, R^{11}, where permitted, is substituted with 2 groups.

m. R^{12} Groups

[00159] In one aspect, R^{12} is selected from hydrogen and optionally substituted organic residue having from 1 to 20 carbons. In a further aspect, R^{12} is hydrogen. In a further aspect, R^{12} is optionally substituted organic residue having from 1 to 20 carbons. In a further aspect, R^{12} is optionally substituted organic residue having from 3 to 12 carbons.

[00160] In a further aspect, R^{12} is hydrogen. In a further aspect, R^{12} is alkyl. In a further aspect, R^{12} is heteroalkyl. In a further aspect, R^{12} is cycloalkyl. In a further aspect, R^{12} is heterocycloalkyl. In a further aspect, R^{12} is aryl. In a further aspect, R^{12} is heteroaryl. In a further aspect, R^{12} is substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl. In a further aspect, R^{12} comprises a group having a formula:



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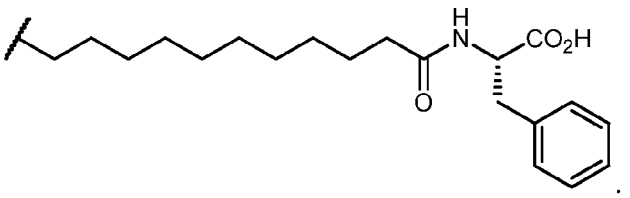
wherein m is an integer from 0 to 10 (e.g., 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10); and wherein AA represents an amino acid residue. In a further aspect, R^{12} is AA is a phenylalanine residue. In a further aspect, R^{12} comprises a group having a formula:



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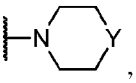
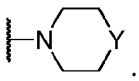
n. R^{13} Groups

[00161] In one aspect, R^{13} is selected from hydrogen and C1-C4 alkyl; or, wherein Z is N, R^{12} and R^{13} are covalently bonded and $-NR^{12}R^{13}$ comprises a moiety of the formula:



[00162] wherein Y is selected from –O–, –S–, –SO–, $–SO_2–$, –NH–, and $–NCH_3–$.

[00163] In a further aspect, R^{13} is hydrogen. In a further aspect, R^{13} is C1-C4 alkyl, for example, methyl, ethyl, propyl, or butyl. In a further aspect, Z is N, and $–NR^{12}R^{13}$ comprises a moiety of the formula:

[00164]  .

o. R^{14} Groups

[00165] In one aspect, R^{14} is C1-C6 alkyl and substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl.

[00166] In a further aspect, R^{14} is C1-C6 alkyl, for example, methyl, ethyl, propyl, butyl, pentyl, or hexyl. In a further aspect, R^{14} is unsubstituted. In a further aspect, R^{14}, where permitted, is substituted with 0-2 groups. In a further aspect, R^{14}, where permitted, is substituted with 1 group. In a further aspect, R^{14}, where permitted, is substituted with 2 groups.

p. AA Groups

[00167] In one aspect, AA represents an amino acid residue, for example, phenylalanine.

q. Y Groups

[00168] In one aspect, Y is selected from –O–, –S–, –SO–, $–SO_2–$, –NH–, and $–NCH_3–$.

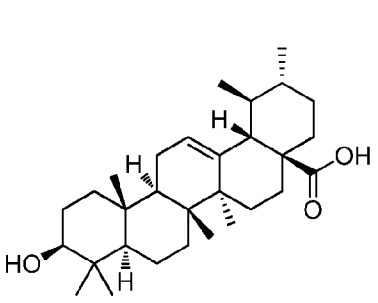
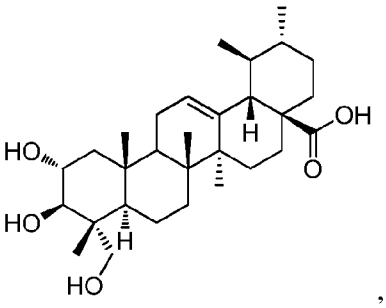
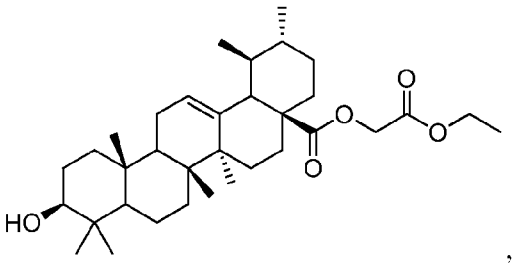
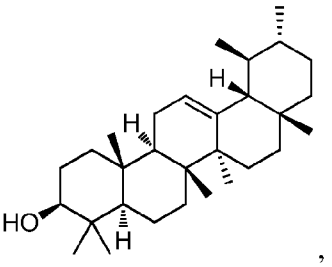
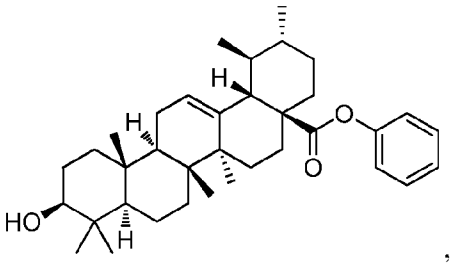
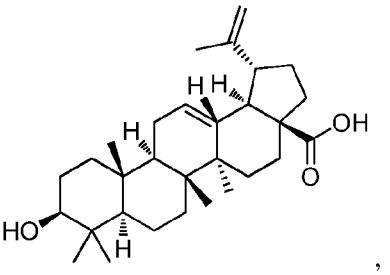
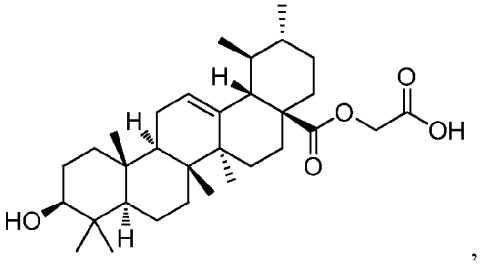
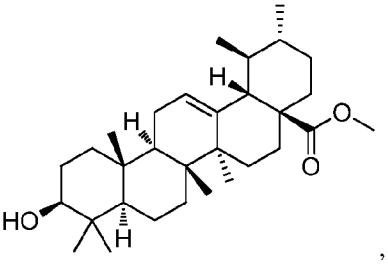
r. Z Groups

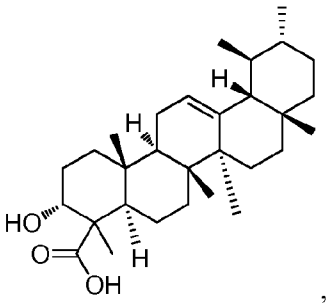
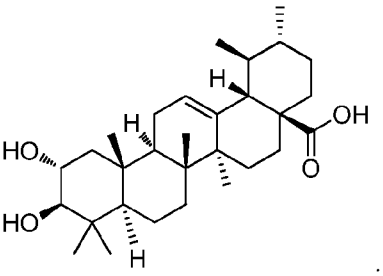
[00169] In one aspect, Z is selected from –O– and $–NR^{13}–$. In a further aspect, Z is –O–. In a further aspect, Z is$–NR^{13}–$; wherein R^{13} is hydrogen. In a further aspect, Z is$–NR^{13}–$

; wherein R^{13} is C1-C4 alkyl.

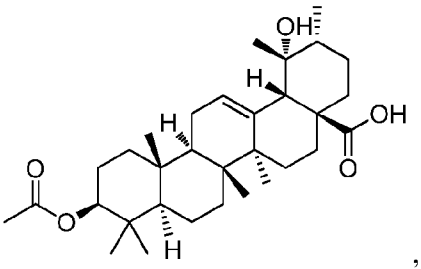
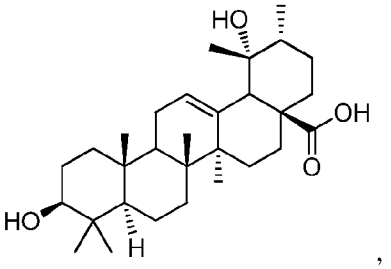
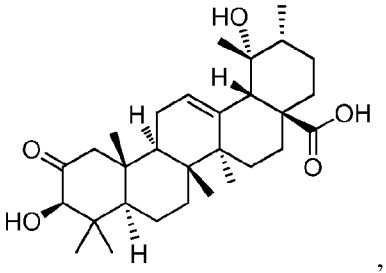
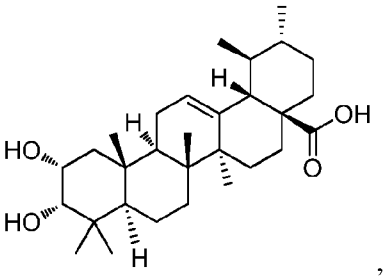
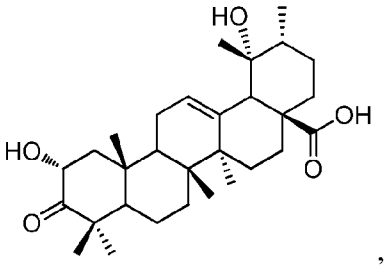
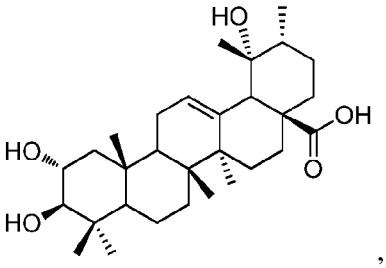
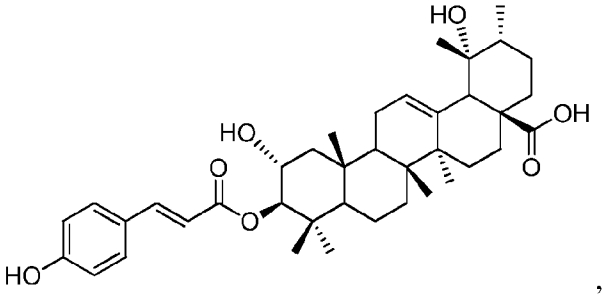
2. EXAMPLE COMPOUNDS

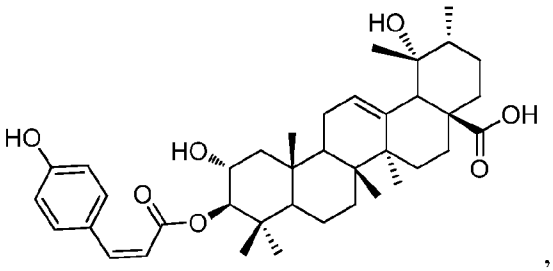
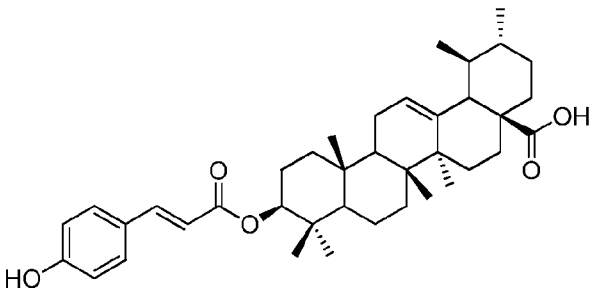
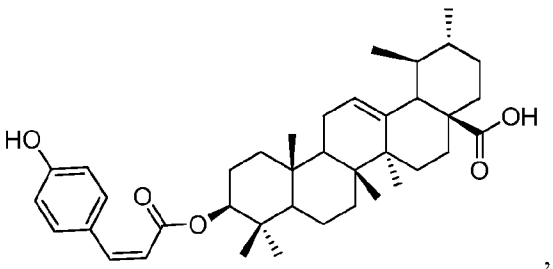
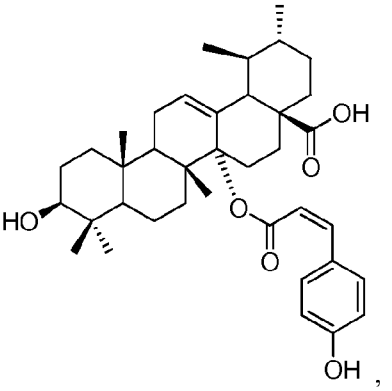
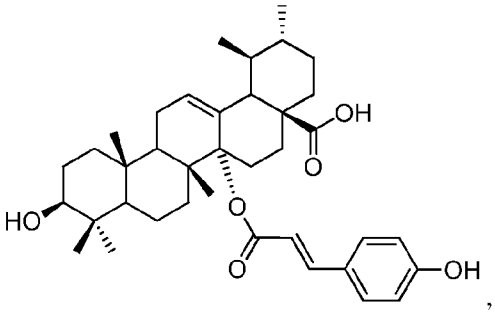
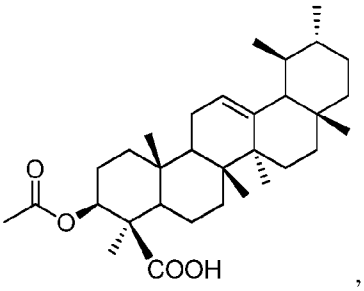
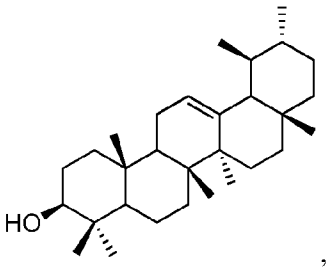
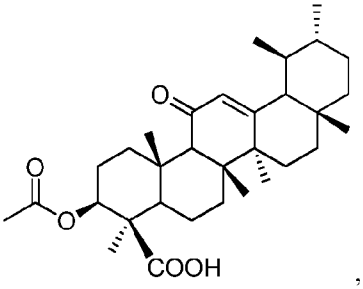
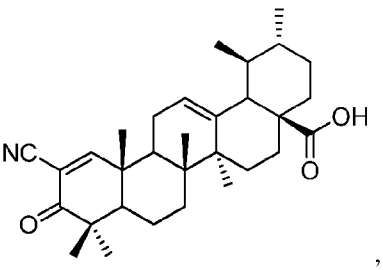
[00170] In one aspect, a compound can be present as one or more of the following structures:

[00171] In a further aspect, a compound can be present as one or more of the following structures:

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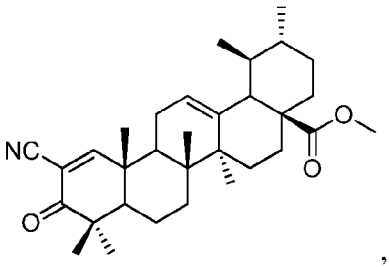
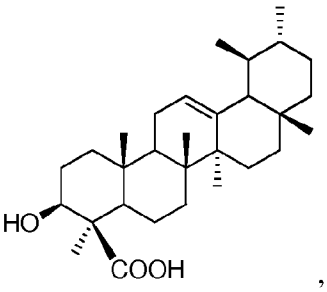
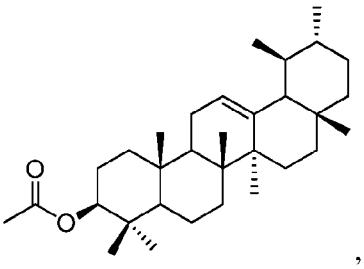
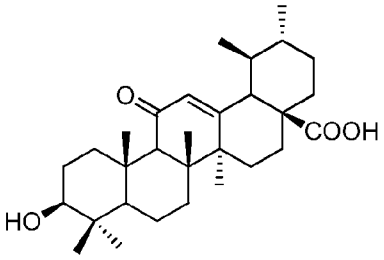
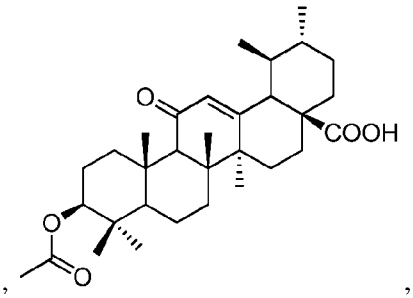
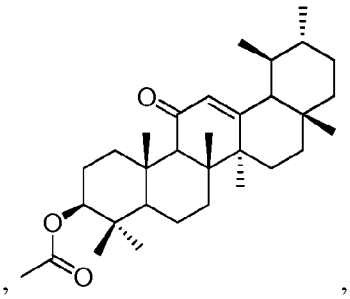
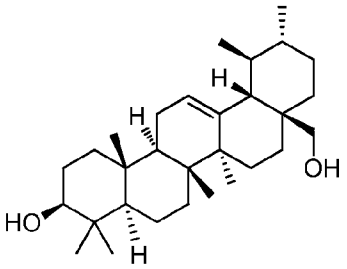
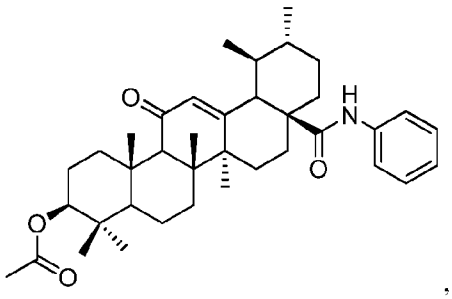
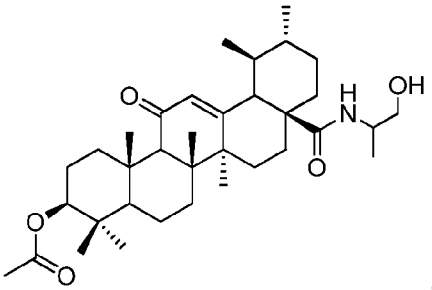
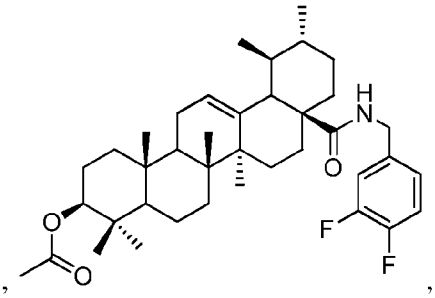
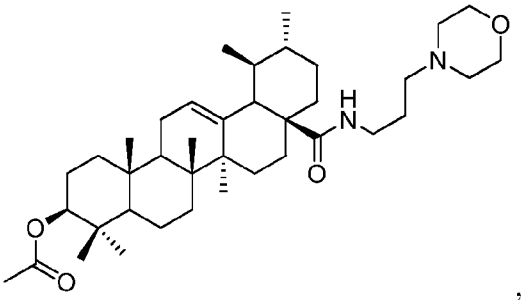
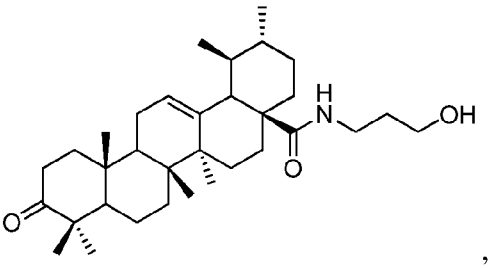
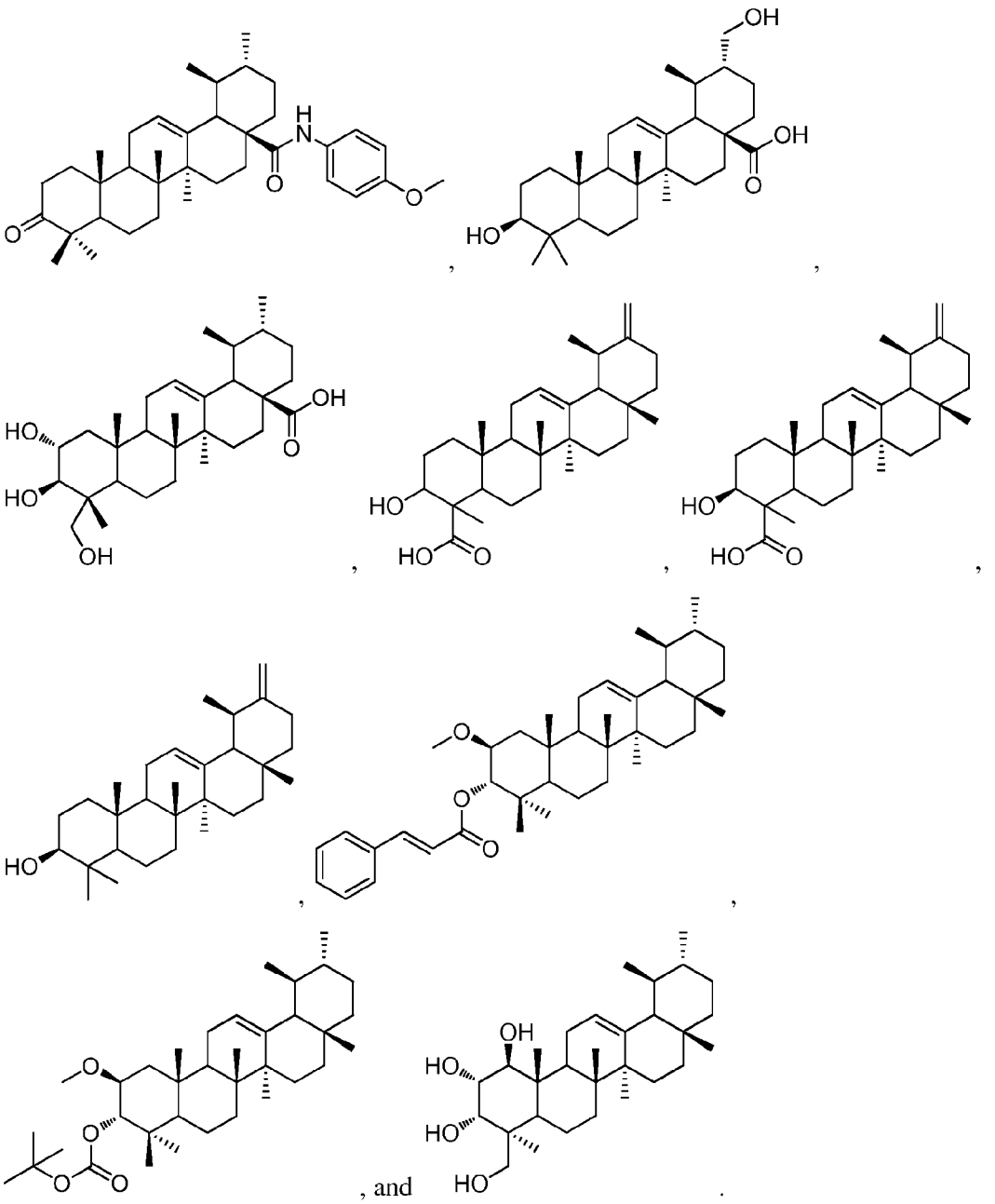















, and .

3. INHIBTION OF MUSCLE ATROPHY AND INDUCTION OF MUSCLE HYPERTROPHY

[00172] In one aspect, the disclosed compounds inhibit muscle atrophy. In a further aspect, the disclosed compounds increase muscle mass. In a still further aspect, the disclosed compounds induce muscle hypertrophy. In a yet further aspect, the disclosed compounds inhibit of muscle atrophy and increase muscle mass. In an even further aspect, the disclosed compounds inhibit of muscle atrophy and induce muscle hypertrophy. In a further aspect, the inhibition of muscle atrophy is in an animal. In an even further aspect, the increase in muscle

mass is in an animal. In a still further aspect, the animal is a mammal, In a yet further aspect, the mammal is a human. In a further aspect, the mammal is a mouse. In a yet further aspect, the mammal is a rodent.

[00173] In a further aspect, the disclosed compounds inhibit muscle atrophy when administered at an oral dose of greater than about 200 mg per day in a human. In a yet further aspect, the disclosed compounds inhibit muscle atrophy when administered at an oral dose of greater than about 300 mg per day in a human. In a still further aspect, the disclosed compounds inhibit muscle atrophy when administered at an oral dose of greater than about 400 mg per day in a human. In an even further aspect, the disclosed compounds inhibit muscle atrophy when administered at an oral dose of greater than about 500 mg per day in a human. In a further aspect, the disclosed compounds inhibit muscle atrophy when administered at an oral dose of greater than about 750 mg per day in a human. In a yet further aspect, the disclosed compounds inhibit muscle atrophy when administered at an oral dose of greater than about 1000 mg per day in a human. In a still further aspect, the disclosed compounds inhibit muscle atrophy when administered at an oral dose of greater than about mg per day in a human. In an even further aspect, the disclosed compounds inhibit muscle atrophy when administered at an oral dose of greater than about 2000 mg per day in a human.

[00174] In a further aspect, the disclosed compounds increase muscle mass when administered at an oral dose of greater than about 200 mg per day in a human. In a yet further aspect, the disclosed compounds increase muscle mass when administered at an oral dose of greater than about 300 mg per day in a human. In a still further aspect, the disclosed compounds increase muscle mass when administered at an oral dose of greater than about 400 mg per day in a human. In an even further aspect, the disclosed compounds increase muscle mass when administered at an oral dose of greater than about 500 mg per day in a human. In a further aspect, the disclosed compounds increase muscle mass when administered at an oral dose of greater than about 750 mg per day in a human. In a yet further aspect, the disclosed compounds increase muscle mass when administered at an oral dose of greater than about 1000 mg per day in a human. In a still further aspect, the disclosed compounds increase muscle mass when administered at an oral dose of greater than about mg per day in a human. In an even further aspect, the disclosed compounds increase muscle mass when administered at an oral dose of greater than about 2000 mg per day in a human.

[00175] It is contemplated that one or more compounds can optionally be omitted from the disclosed invention.

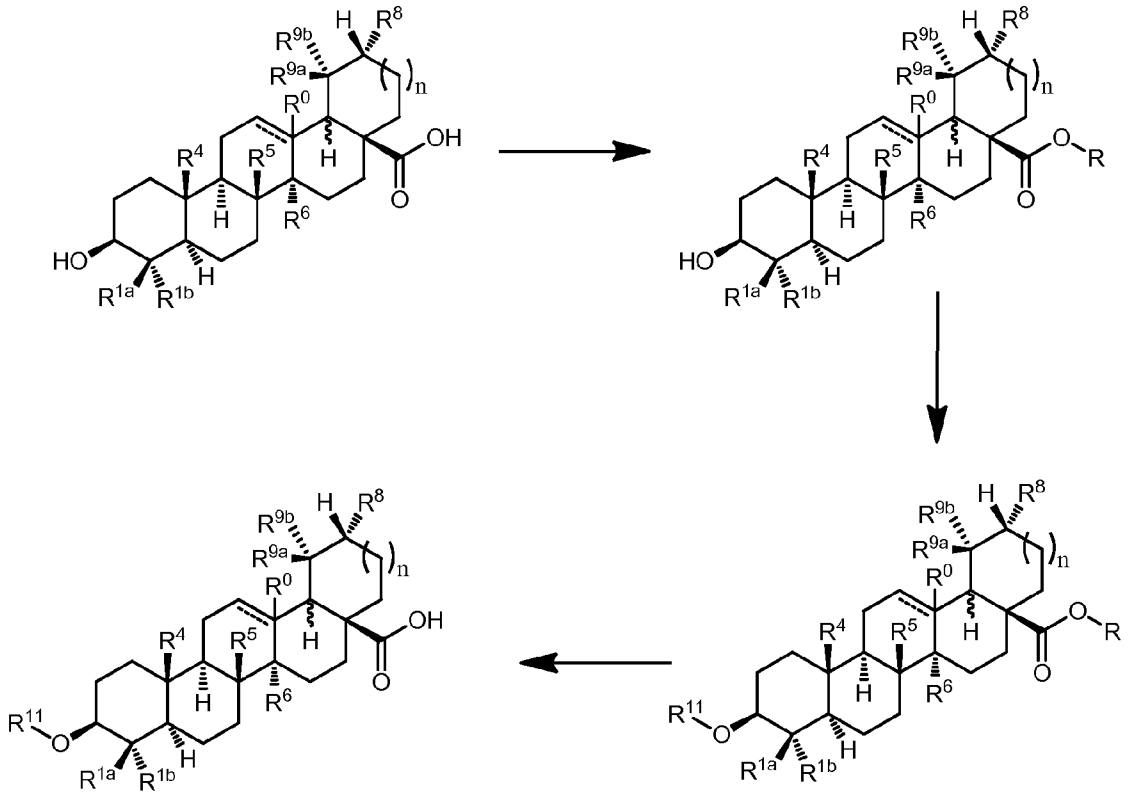
C. METHODS OF MAKING THE COMPOUNDS

[00176] In one aspect, the disclosed compounds comprise the products of the synthetic methods described herein. In a further aspect, the disclosed compounds comprise a compound produced by a synthetic method described herein. In a still further aspect, the invention comprises a pharmaceutical composition comprising a therapeutically effective amount of the product of the disclosed methods and a pharmaceutically acceptable carrier. In a still further aspect, the invention comprises a method for manufacturing a medicament comprising combining at least one compound of any of disclosed compounds or at least one product of the disclosed methods with a pharmaceutically acceptable carrier or diluent.

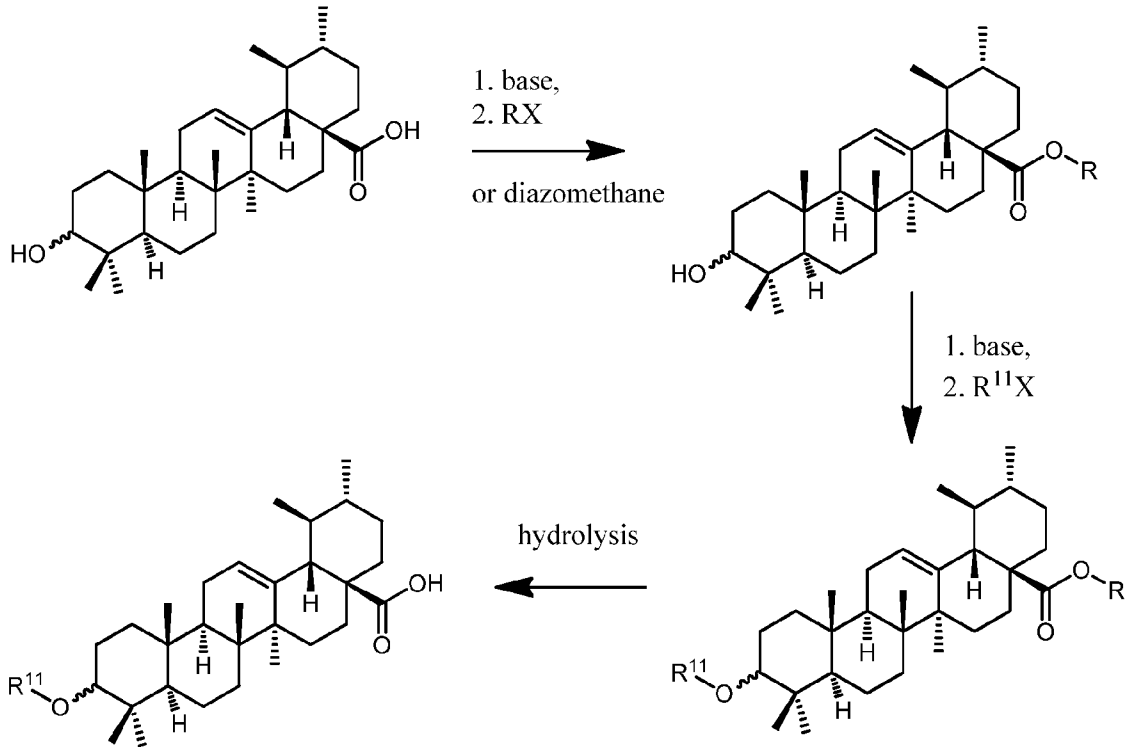
[00177] In one aspect, the invention relates to methods of making functionalized ursane compounds useful in methods of inhibiting muscle atrophy and increasing muscle mass. Such compounds can be useful in the treatment of various maladies associated with muscle wasting, useful for increasing muscle mass and/or muscle strength, as well as in enhancing muscle formation and/or muscular performance. The compounds of the invention can be prepared by employing reactions as shown in the following schemes, in addition to other standard manipulations that are known in the literature, exemplified in the experimental sections or clear to one skilled in the art. For clarity, examples having a single substituent are shown where multiple substituents are allowed under the definitions disclosed herein. The following examples are provided so that the invention might be more fully understood, are illustrative only, and should not be construed as limiting.

1. ROUTE 1: ALKYL ETHERIFICATION

[00178] In one aspect, functionalized ursane compounds of the present invention can be prepared generically as shown below.



[00179] Compounds are represented in generic form, with substituents as noted in compound descriptions elsewhere herein. A more specific example is set forth below.



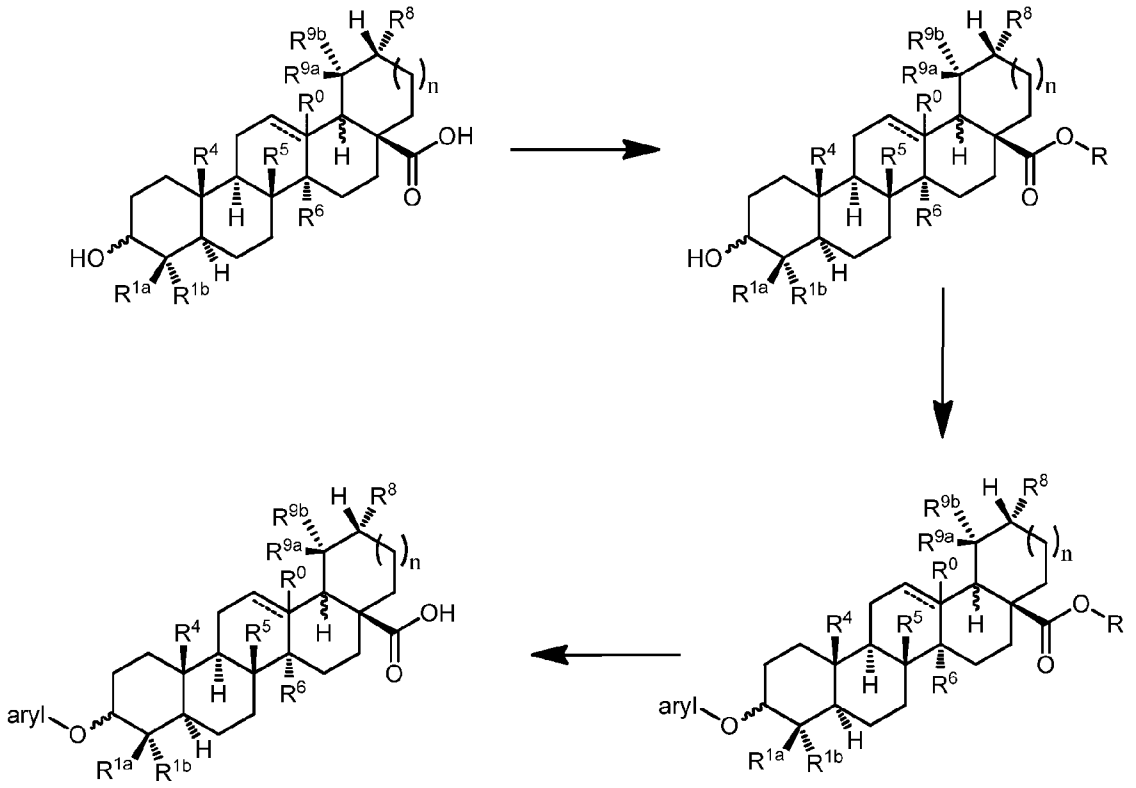
[00180] In one aspect, Route 1 step 1 begins with a free acid. In an appropriate solvent, a base (e.g., K_2CO_3, NaOH) strong enough to deprotonate the carboxylic acid, but not the alcohol, is added, and the reaction is conducted at a temperature effective and for a time effective to insure carboxylic acid deprotonation. An appropriate alkyl halide or halide equivalent is added to the reaction mixture, and the reaction is conducted at a temperature effective and for a time effective to insure alkylation of the carboxyl group. In a further aspect, an alternate Route 1 step 1 also begins with the free carboxylic acid. Diazomethane is added, and the reaction is conducted at a temperature effective and for a time effective to insure reaction.

[00181] In a further aspect, Route 1 step 2 the alkyl ester is dissolved in an appropriate dry solvent under anhydrous reaction conditions. A base is added, and the reaction is conducted at a temperature effective and for a time effective to insure deprotonation. Then, an appropriate alkyl, heteroalkyl, cycloalkyl, or heterocycloalkyl halide or halide equivalent (i.e., $R^{11}X$) is added to the reaction mixture. The reaction is conducted at a temperature effective and for a time effective insure complete reaction.

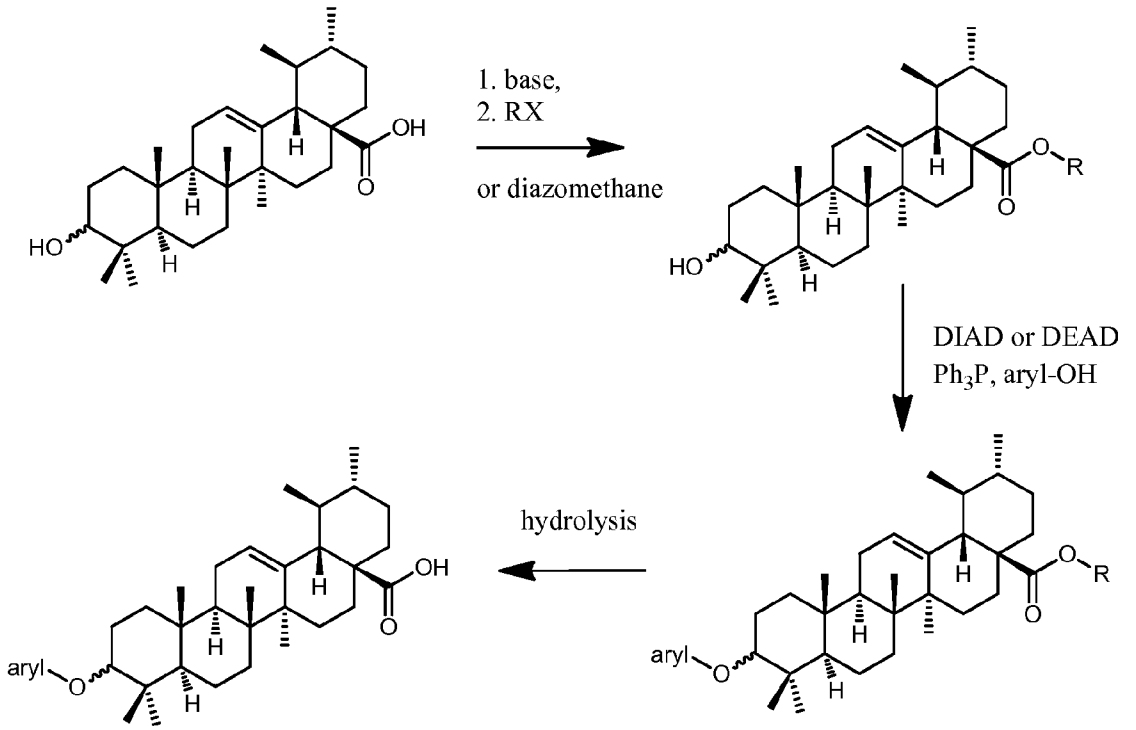
[00182] In a further aspect, in Route 1 step 3, the *O*-alkylated ursane compound alkyl ester is hydrolyzed with an appropriate base, such as LiOH, in an appropriate organic-aqueous mixed solvent system at a temperature effective and for a time effective to insure reaction. Then the reaction mixture can be acidified to a suitable pH with an appropriate aqueous acid of an sufficient concentration and at a temperature effective and for a time effective to insure reaction.

2. ROUTE 2: ARYL ETHERIFICATION

[00183] In one aspect, functionalized ursane compounds of the present invention can be prepared generically as shown below.



[00184] Compounds are represented in generic form, with substituents as noted in compound descriptions elsewhere herein. A more specific example is set forth below.

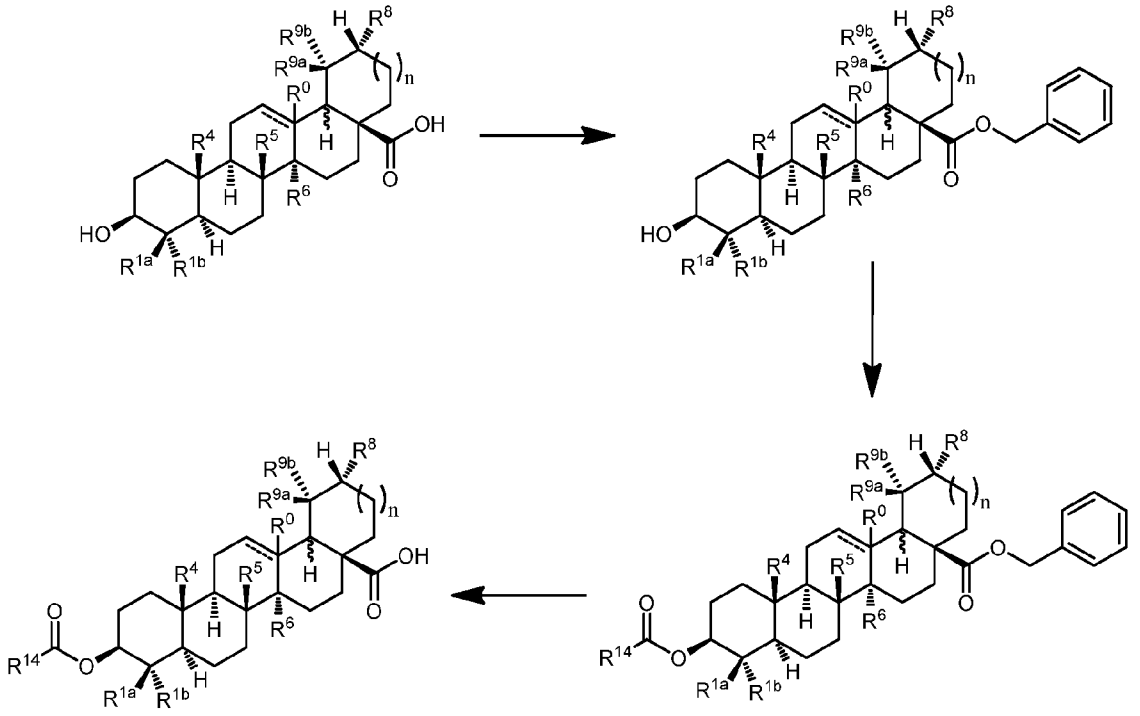


[00185] In one aspect, Route 2 step 1 begins with the ursane compound free carboxylic acid. In an appropriate solvent, a base (e.g., K_2CO_3, NaOH) strong enough to deprotonate the carboxylic acid, but not the alcohol group, is added, and the reaction is conducted at a temperature effective and for a time effective to insure deprotonation. Then, an appropriate alkyl halide or halide equivalent is added to the reaction mixture, and the reaction is conducted at a temperature effective and for a time effective to insure alkylation of the carboxyl group. In a further aspect, an alternate Route 2 step 1 begins with the ursane compound free carboxylic acid in an appropriate solvent. Diazomethane is added, and the reaction is conducted at a temperature effective and for a time effective to insure reaction.

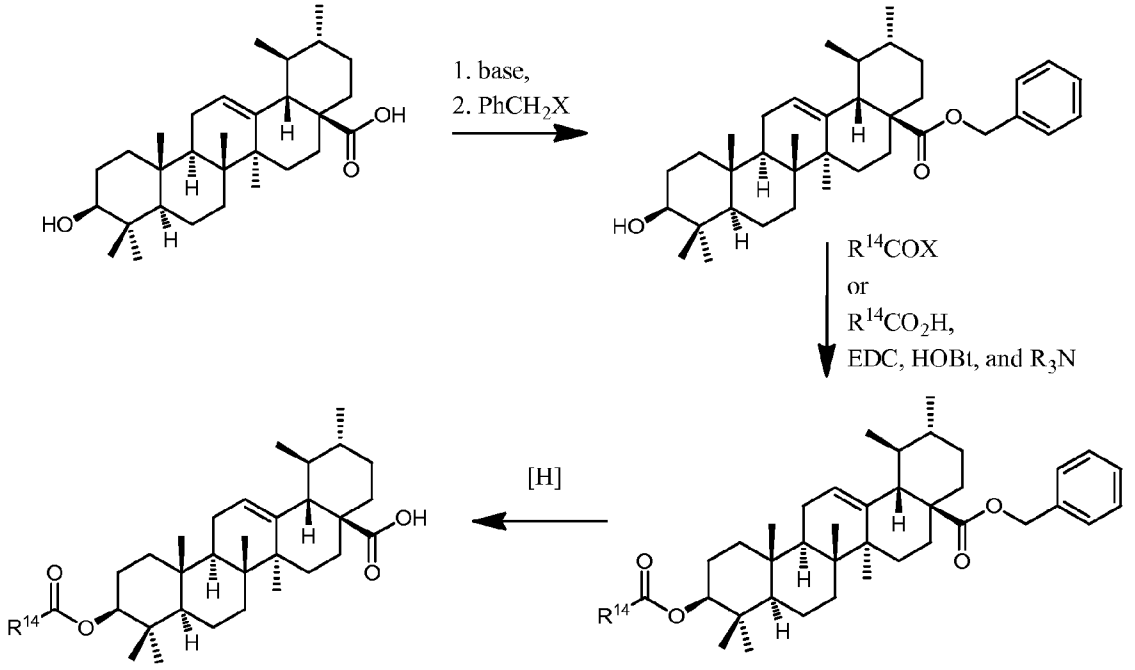
[00186] In a further apect, Route 2 step 2, the ursane compound alkyl ester is dissolved in an appropriate, dry solvent, along with phenol, an aryl alcohol, or appropriate heteroaryl alcohol, under anhydrous reaction conditions, followed by the addition of triphenylphosphine. The reaction is conducted at a effective temperature and for an effective time period. Then, an appropriate coupling agent, such as DIAD or DEAD, is added, and the reaction is conducted at a temperature effective and for a time effective to insure reaction. In a further aspect, in Route 2 step 3, the *O*-arylated or heteroarylated ursane compound alkyl ester can be treated with an appropriate base, such as LiOH, in an appropriate organic-aqueous mixed solvent system at a temperature effective and for a time effective to insure complete reaction. The reaction mixture can then be acidified to a suitable pH.

3. ROUTE 3: ACYLATION

[00187] In one aspect, functionalized ursane compounds of the present invention can be prepared generically as shown below.



[00188] Compounds are represented in generic form, with substituents as noted in compound descriptions elsewhere herein. A more specific example is set forth below.



[00189] In one aspect, Route 3 step 1 begins with the the ursane compound free carboxylic acid. In an appropriate solvent, a base (e.g., K_2CO_3, NaOH) strong enough to

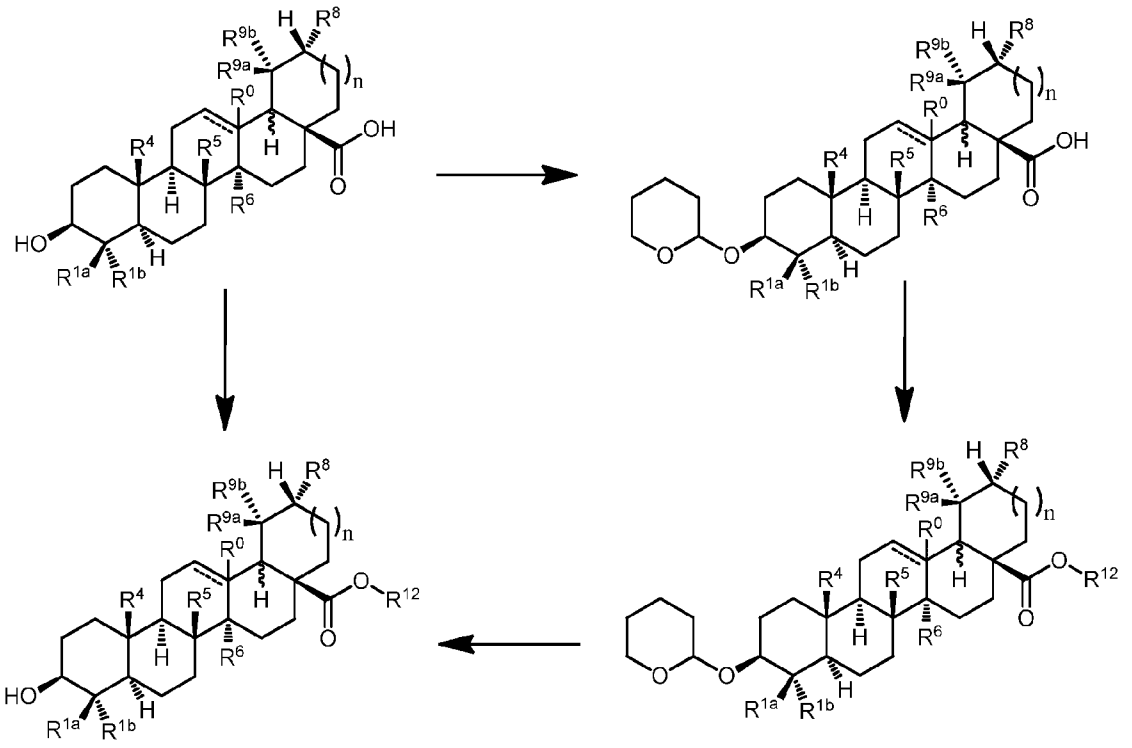
deprotonate the carboxylic acid, but not the alcohol group, is added, and the reaction is allowed to progress at a temperature effective and for a time effective to insure carboxylic acid deprotonation. Then, an appropriate benzyl halide or halide equivalent is added to the reaction mixture, and the reaction is conducted at a temperature effective and for a time effective to insure protection of the carboxyl group.

[00190] In Route 3 step 2, the ursane compound benzyl ester is dissolved in an appropriate, dry solvent under anhydrous reaction conditions, followed by the addition of an appropriate acid scavenger (weak base, e.g., K_2CO_3 or DIEA). The acyl halide (e.g., $R^{14}COX$) or equivalent acylating reagent is then added. The reaction is conducted at a temperature effective and for a time effective to insure reaction. In a further aspect, in an alternate Route 3 step 2, the the ursane compound benzyl ester and a suitable carboxylic acid (e.g., $R^{14}CO_2H$) are dissolved in an appropriate, dry solvent under anhydrous reaction conditions. Ethyl-(N',N'-dimethylamino)propylcarbodiimide hydrochloride (EDC), 1-hydroxybenzotriazole (HOBt), and a trialkylamine (R_3N) are then added, and the reaction is conducted at a temperature effective and for a time effective to insure reaction.

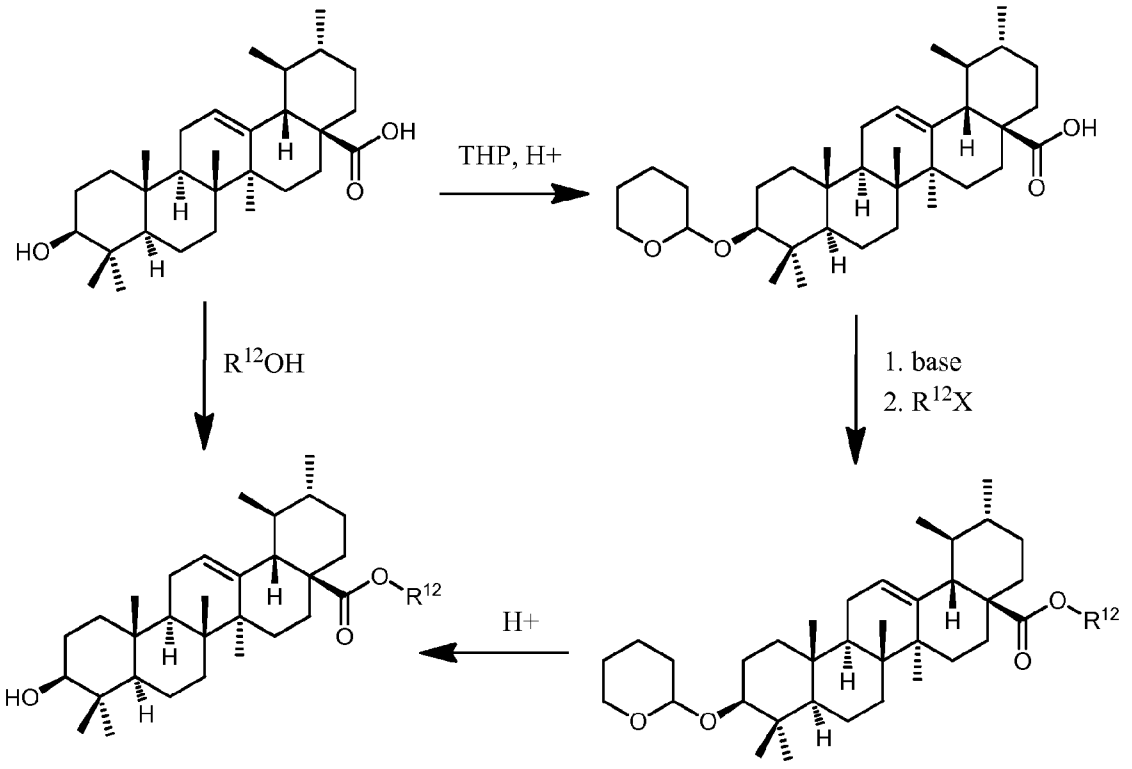
[00191] In Route 3 step 3, the acylated ursane compound benzyl ester is reduced under standard conditions (e.g., hydrogenation with hydrogen gas in the presence of a suitable palladium catalyst), thereby liberating the ursane compound free carboxlic acid.

4. ROUTE 4: ESTERIFICATION

[00192] In one aspect, functionalized ursane compounds of the present invention can be prepared generically as shown below.



[00193] Compounds are represented in generic form, with substituents as noted in compound descriptions elsewhere herein. A more specific example is set forth below.

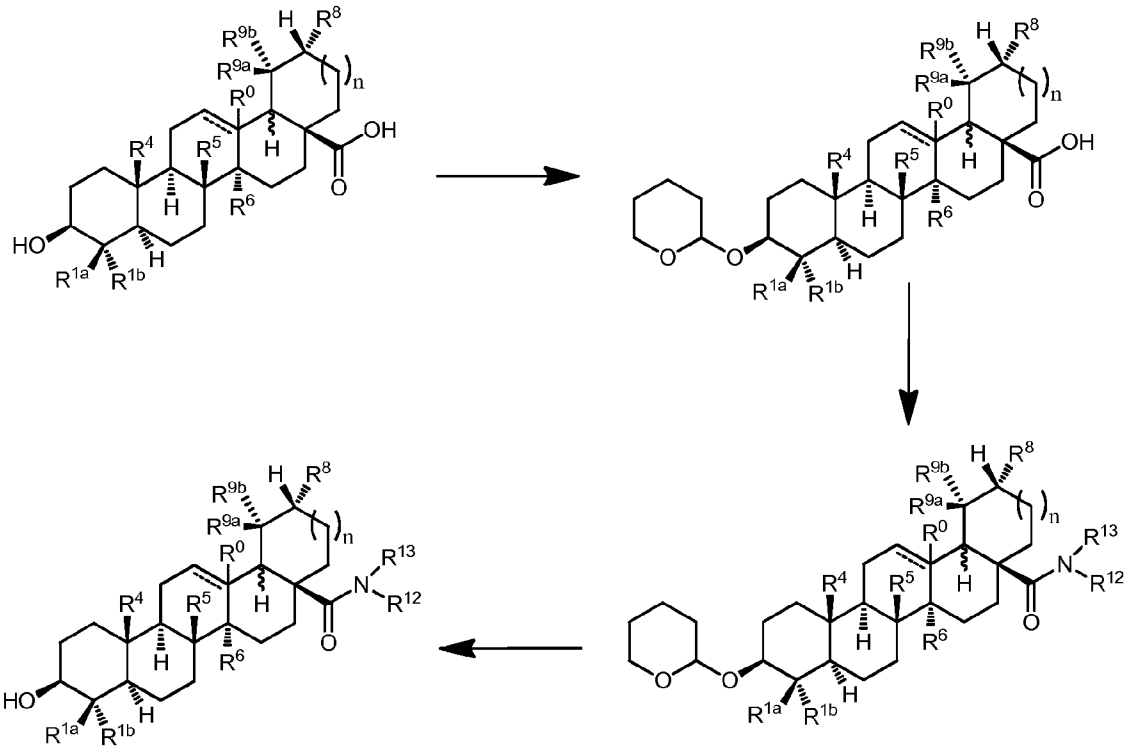


[00194] In one aspect, Route 4 step 1 begins with the ursane compound free carboxylic acid. An appropriate alcohol (e.g., $R^{12}OH$) is added, and the reaction is conducted at a temperature effective and for a time effective to time to insure reaction.

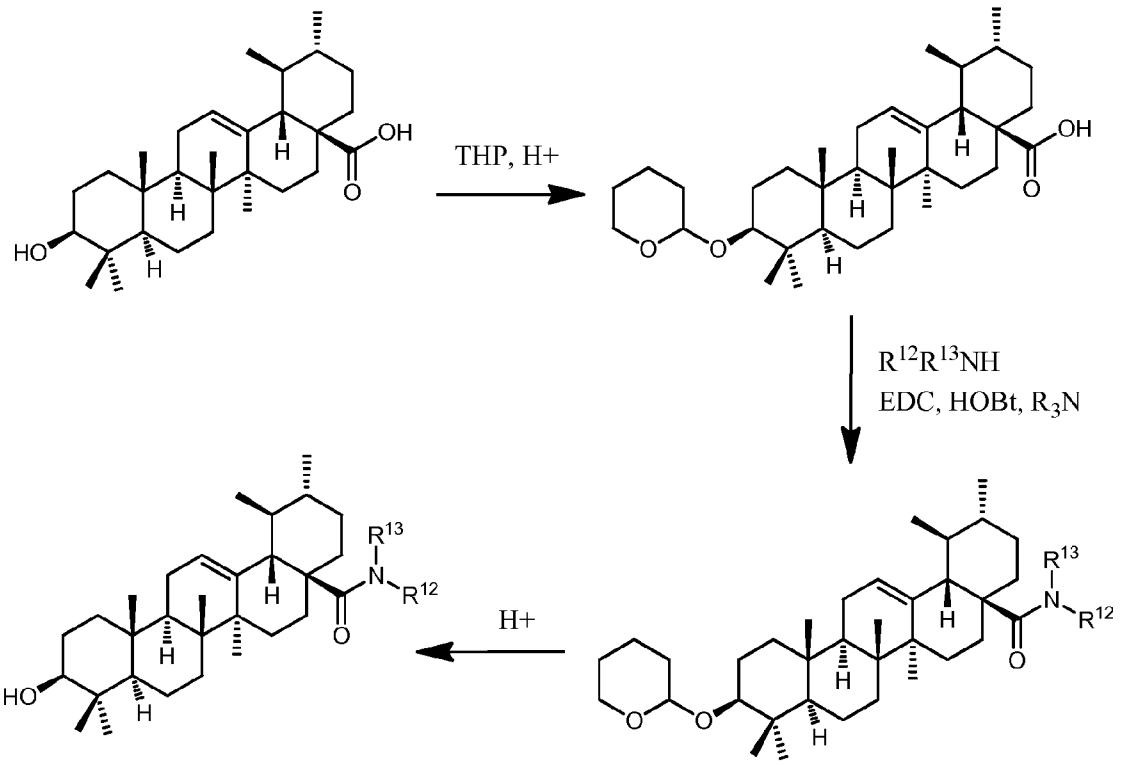
[00195] In a further aspect in an alternate syntheis, Route 4 step 1 begins with the ursane compound free carboxylic acid in a dry solvent under dry reaction conditions. Tetrahydropyran (THP) is added, along with an acid catalyst (e.g., pTsOH). The reaction is conducted at a temperature effective and for a time effective to insure protection of the hydroxyl group. A base (e.g., NaOH or NaH) is then added to the THP-protected ursane compound free carboxylic acid, in a dry solvent under anhydrous reaction conditions. The reaction is conducted at a temperature effective and for a time effective to insure carboxylic acid deprotonation. Then, an appropriate alkyl halide (i.e., $R^{12}X$) or equivalent is added to the reaction mixture, and the reaction is conducted at a temperature effective and for a time effective to insure alkylation of the carboxyl group. Route 4 step 3 begins with the THP-protected ursane compound alkyl ester in an alcohol solvent. An acid catalyst (e.g., pTsOH) is added, and the reaction is conducted at a temperature effective and for a time effective to insure deprotection.

5. ROUTE 5: AMIDE FORMATION

[00196] In one aspect, functionalized ursane compounds of the present invention can be prepared generically as shown below.



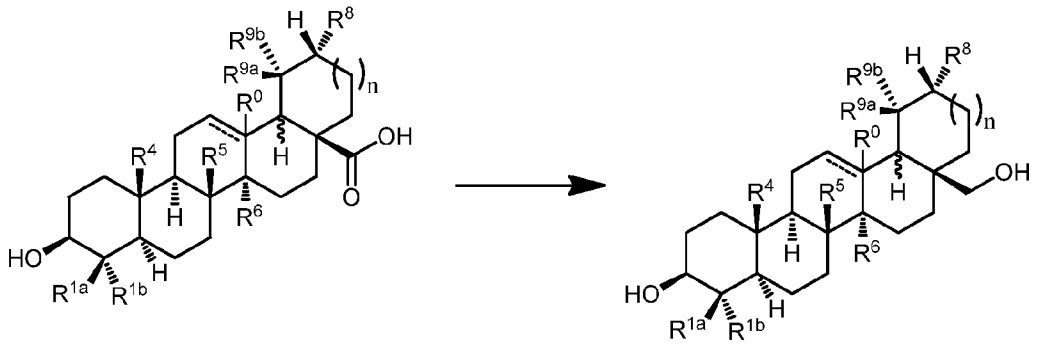
[00197] Compounds are represented in generic form, with substituents as noted in compound descriptions elsewhere herein. A more specific example is set forth below.



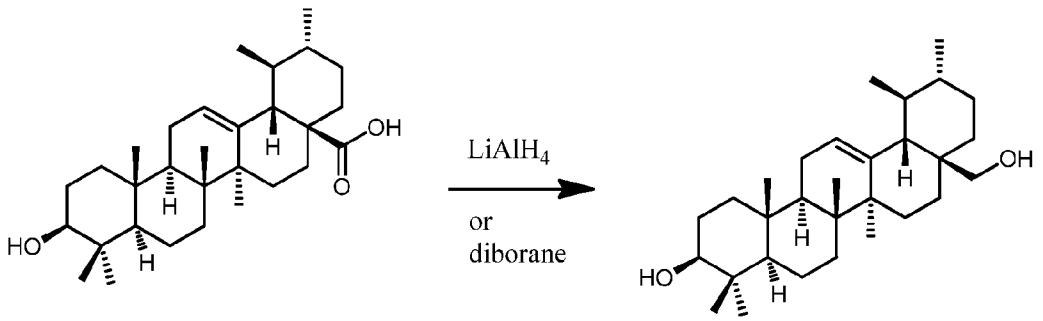
[00198] In one aspect, Route 5 step 1 begins with the ursane compound free carboxylic acid in a dry solvent. Under dry reaction conditions, tetrahydropyran (THP) and an acid catalyst (e.g., pTsOH) are added. The reaction is then conducted at a temperature effective and for a time effective to insure protection of the hydroxyl group. In Route 5 step 2, the THP-protected ursane compound free carboxylic acid is dissolved in an appropriate, dry solvent. Under anhydrous reaction conditions, a suitable amine (e.g., $R^{12}R^{13}NH$) is added, along with ethyl-(N',N'-dimethylamino)propylcarbodiimide hydrochloride (EDC), 1-hydroxybenzotriazole (HOBt), and a trialkylamine (R_3N), and the reaction is conducted at a temperature effective and for a time effective to time to insure complete reaction. In Route 5 step 3, the THP-protected ursane compound amide can then be deprotected by addition of an acid catalyst (e.g., pTsOH), and the reaction is conducted at a temperature effective and for a time effective to insure reaction.

6. ROUTE 6: REDUCTION TO ALCOHOL

[00199] In one aspect, functionalized ursane compounds of the present invention can be prepared generically as shown below.



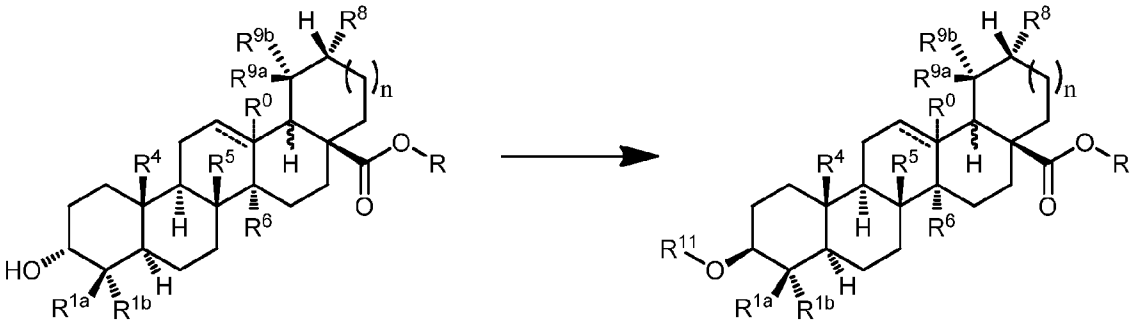
[00200] Compounds are represented in generic form, with substituents as noted in compound descriptions elsewhere herein. A more specific example is set forth below.



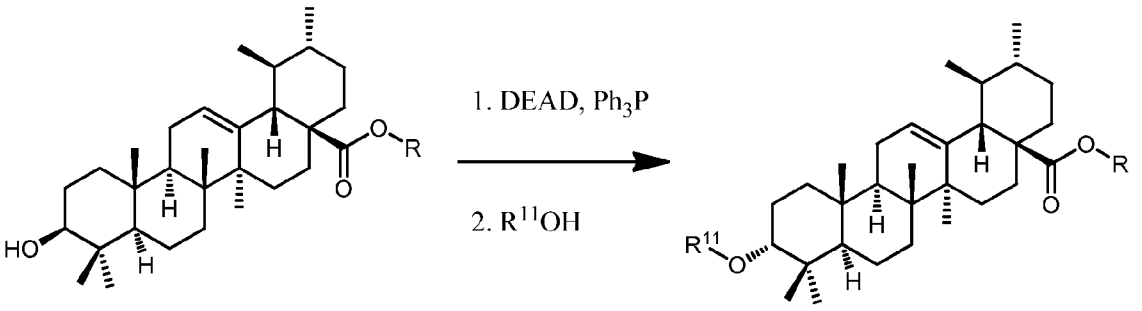
[00201] In one aspect, the ursane compound free carboxylic acid, in a dry solvent, can be reacted with lithium aluminum hydride ($LiAlH_4$) under dry reaction conditions to provide the corresponding primary alcohol. Alternatively, the ursane compound free carboxylic acid, in a dry solvent, can be reacted with diborane (B_2H_6) under dry reaction conditions to provide the corresponding primary alcohol. It is understood that protecting group chemistry, if needed, can also be used to protect sensitive remote functionality during these reaction steps.

7. ROUTE 7: HYDROXYL INVERSION

[00202] In one aspect, functionalized ursane compounds of the present invention can be prepared generically as shown below.



[00203] Compounds are represented in generic form, with substituents as noted in compound descriptions elsewhere herein. A more specific example is set forth below.



[00204] In one aspect, a hydroxyl functionality can be substituted with another group (e.g., alkoxyl, acyl, amino, etc.), while inverting the stereochemistry at the adjacent carbon, by reaction with an appropriate protic nucleophile in the presence of diethylazodicarboxylate (DEAD) and triphenylphosphine under Mitsunobu reaction conditions. While $-OR^{11}$ is shown, it is understood that additional moieties (e.g., acetoxyl, amino, etc.) can be substituted

at that position by appropriate selection of protic nucleophile (e.g., acetic acid, ammonia, etc.).

8. Plant Sources of Ursolic Acid Derivatives

[00205] Many pentacyclic acid triterpenes useful as synthetic precursors to the ursolic acid derivatives in the synthetic methods described above may be isolated and purified from a natural source such as plants or materials derived from plants. Alternatively, certain known synthetic precursors useful in the preparation of ursolic acid derivatives can often be obtained from commercial sources. Ursolic Acid is a useful known synthetic precursor to ursolic acid derivatives that can be used as a synthetic precursor to prepare certain disclosed compounds. For example, ursolic acid can be isolated from plants such as Holy Basil (*Ocimum sanctum* L.), peppermint leaves (*Mentha piperita* L.), lavender (*Lavandula augustifolia Mill.*), oregano (*Origanum vulgare* L.), thyme (*Thymus vulgaris* L.), hawthorn (*Crataegus laevigata (Poir)* DC), cherry laurel leaves (*Prunus laurocerasus* L.), loquat leaves (*Eriobotrya japonica* L.), glossy privet leaves (*Ligustrum lucidum Ait.* L.), bilberry (*Vacciunum myrtillus* L.), Devil's Claw (*Harpagophytum procumbens* DC), Elder Flowers (European var.; *Sambucus nigra* L.), and periwinkle (*Vinca minor* L.).

[00206] A variety of methods that are generally applicable to purifying ursolic acid and ursolic acid derivatives. For example, Nishimura, et al. (J. Nat. Prod. 1999, 62, 1061-1064) described the identification of 2,3-dihydroxy-24-nor-urs-4(23),12-dien-28-oic acid and 23-hydroxyursolic acid. Nishimura described procedures to isolate these compounds. Procedures described herein demonstrate these compounds will be contained in flash chromatography fraction 3 (FCF3) as described in the examples. Similar HPLC procedures described herein can be used to further purify these compounds including using a gradient with water with 0.05% TFA and acetonitrile with 0.05% TFA, mobile phase A and B respectively, with a C18 BetaMax Neutral column (250×8 mm; 5 um). The gradient may consist of 40% β isocratic for 5 min, then from approximately 40% to 70% B in 30 min. A skilled artisan would recognize the general applicability of the methods described in Nishimura et al to efficiently isolate either the ursolic acid, ursolic acid derivatives or structurally related pentacyclic acid triterpenes from various plants.

[00207] Other illustrative methods that are generally applicable to purifying ursolic

acid and ursolic acid derivatives are also known. For example, Chaturvedula, et al. (J. Nat. Prod. 2004, 67, p. 899-901) described the isolation of 3-acetoxy-2-hydroxy ursolic acid, 3-(p-coumaroyl)ursolic acid, and 2,3-diacetoxyursolic acid. Adnyana, et al. (J. Nat. Prod. 2001, 64, p. 360-363) described the isolation of 2,3,6,19-tetrahydroxyoleanolic acid, 2,3,19-trihydroxyoleanolic acid, 2,3,19,23-tetrahydroxyursolic acid, and 2,3,23-trihydroxyoleanolic acid. Ikuta, et al. (J. Nat. Prod. 2003, 66, p. 1051-1054) described the isolation of 2,3-dihydroxyurs-12-en-11-on-28-oic acid and 2,3-dihydroxy-11-methoxyurs-12-en-28-oic acid. For example, similar HPLC procedures such as those described in U.S. Patent 7,612, 045 can be used to further purify these compounds including using a gradient with water with 0.05% TFA and acetonitrile with 0.05% TFA, mobile phase A and B respectively, with a C18 BetaMax Neutral column (250×8 mm; 5 um). The gradient may consist of 40% β isocratic for 5 min, then from approximately 40% to 70% B in 30 min.

[00208] Finally, another source of the known synthetic precursors useful in the synthetic methods described above to prepare ursolic acid derivatives are commercial sources or vendors. Purified forms of corosolic acid, ursolic acid, oleanolic acid, madecassic acid, asiatic acid, pygenic acid (A, B or C), caulophyllogenin and echinocystic acid may be obtained from a commercial source. For example, ursolic acid and oleanolic acid may be purchased from Sigma-Aldrich Chemical Company (St. Louis, Mo., USA) and corosolic acid, asiatic acid, madecassic acid, pygenic acid (A, B, or C), caulophyllogenin and echinocystic acid may be purchased from Chromadex (Santa Ana, Calif., USA). The compounds obtained from commercial sources may be furthered separated and purified as needed using methods such as column chromatography, high pressure liquid chromatography (HPLC), and/or recrystallization described herein. Additional methods of isolation of precursors are described in U.S. Patent 7,612,045, U.S. Patent Application 10/355,201, and U.S. Patent Application 10/445,943.

[00209] It is further anticipated that the compounds of the invention can be obtained by direct synthesis. Direct synthesis may include either total synthesis or semi-synthesis. Exemplary synthetic methods for obtaining these compounds are described above. Additional synethetic procedures useful in the preparation of ursolic acid derivatives are described in U.S. Patent 3,903,089, U.S. Patent 7,612,045, and U.S. Patent Application 10/445,943, U.S. Patent Application 10/355,201. Further synthetic methods useful in the preparation of ursolic acid derivatives are Meng, Y., *et al.* (2010) *Molecules* 15:4033-4040; Gao, Y., *et al.* (2010)

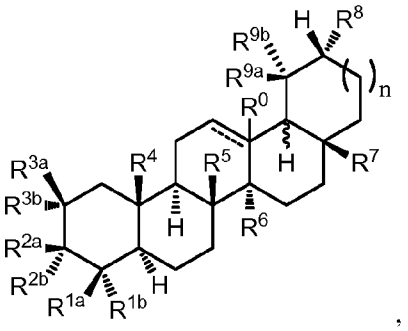
Molecules 15:4439-4449; Sporn, M.B., *et al.* (2011) *Journal of Natural Products* 74:537-545; Chadalapaka, G., *et al.* (2008) *Biorganic and Medicinal Chemistry Letters* 18(8):2633-2639; and, Sun, H., *et al.* (2006) *Botanical Studies* 47:339-368.

[00210] It is contemplated that each disclosed methods can further comprise additional steps, manipulations, and/or components. It is also contemplated that any one or more step, manipulation, and/or component can be optionally omitted from the invention. It is understood that a disclosed methods can be used to provide the disclosed compounds. It is also understood that the products of the disclosed methods can be employed in the disclosed methods of using.

D. PHARMACEUTICAL COMPOSITIONS

[00211] In one aspect, the invention relates to pharmaceutical compositions comprising the disclosed compounds. That is, a pharmaceutical composition can be provided comprising a therapeutically effective amount of at least one disclosed compound or at least one product of a disclosed method and a pharmaceutically acceptable carrier.

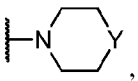
[00212] In one aspect, the invention relates to a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an effective amount of a compound having a structure represented by a formula:



,

wherein each ----- is an optional covalent bond, and R^0 is optionally present; wherein n is 0 or 1; wherein R^0, when present, is hydrogen; wherein R^{1a} is selected from C1-C6 alkyl and –C(O)ZR^{10}; wherein R^{1b} is selected from C1-C6 alkyl, or wherein R^{1a} and R^{1b} are optionally covalently bonded and, together with the intermediate carbon, comprise an optionally substituted C3-C5 cycloalkyl or C2-C5 heterocycloalkyl; wherein one of R^{2a} and R^{2b} is –OR^{11}, and the other is hydrogen, or R^{2a} and R^{2b} together comprise =O; wherein each of R^{3a}

and R^{3b} is independently selected from hydrogen, hydroxyl, C1-C6 alkyl, and C1-C6 alkoxyl, provided that R^{3a} and R^{3b} are not simultaneously hydroxyl, wherein R^{3a} and R^{3b} are optionally covalently bonded and, together with the intermediate carbon, comprise an optionally substituted C3-C5 cycloalkyl or C2-C5 heterocycloalkyl; wherein each of R^4, R^5, and R^6 is independently selected from C1-C6 alkyl; wherein R^7 is selected from C1-C6 alkyl, $-CH_2OR^{12}$ and $-C(O)ZR^{12}$; wherein R^8 is selected from hydrogen and C1-C6 alkyl; wherein each of R^{9a} and R^{9b} is independently selected from hydrogen and C1-C6 alkyl, provided that R^{9a} and R^{9b} are not simultaneously hydrogen; or wherein R^{9a} and R^{9b} are covalently bonded and, along with the intermediate carbon, together comprise C3-C5 cycloalkyl or C2-C5 heterocycloalkyl; wherein R^{10} is selected from hydrogen and C1-C6 alkyl; wherein R^{11} is selected from hydrogen, C1-C6 alkyl, C1-C5 heteroalkyl, C3-C6 cycloalkyl, C4-C6 heterocycloalkyl, phenyl, heteroaryl, and $-C(O)R^{14}$; wherein R^{11}, where permitted, is substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; wherein R^{12} is selected from hydrogen and optionally substituted organic residue having from 1 to 20 carbons; wherein Z is selected from –O– and $-NR^{13}-$; wherein R^{13} is selected from hydrogen and C1-C4 alkyl; or, wherein Z is N, R^{12} and R^{13} are covalently bonded and $-NR^{12}R^{13}$ comprises a moiety of the formula:



wherein Y is selected from –O–, –S–, –SO–, $-SO_2-$, –NH–, $-NCH_3-$; and wherein R^{14} is C1-C6 alkyl and substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, in an amount effective to prevent or treat muscle atrophy in the animal, wherein the amount is greater than about 1000 mg per day when the compound is ursolic acid, boswellic acid, corosolic acid, betulinic acid, or UA0713.

[00213] In one aspect, the animal is an animal. In a further aspect, the animal is a mammal. In a yet further aspect, the mammal is a primate. In a still further aspect, the mammal is a human. In an even further aspect, the human is a patient.

[00214] In a further aspect, the animal is a domesticated animal. In a still further

aspect, the domesticated animal is a domesticated fish, domesticated crustacean, or domesticated mollusk. In a yet further aspect, the domesticated animal is poultry. In an even further aspect, the poultry is selected from chicken, turkey, duck, and goose. In a still further aspect, the domesticated animal is livestock. In a yet further aspect, the livestock animal is selected from pig, cow, horse, goat, bison, and sheep.

[00215] In a further aspect, the effective amount is a therapeutically effective amount. In a still further aspect, the effective amount is a prophylactically effective amount. In a yet further aspect, the muscle disorder is muscle atrophy . In an even further aspect, the muscle disorder is a condition in need of increasing muscle mass. In an even further aspect, the effective amount is greater than about 1000 mg per day when the compound is ursolic acid, beta-boswellic acid, corosolic acid, betulinic acid, or UA0713.

[00216] In a further aspect, the pharmaceutical composition is administered following identification of the mammal in need of treatment of muscle atrophy. In a still further aspect, the pharmaceutical composition is administered following identification of the mammal in need of prevention of muscle atrophy. In an even further aspect, the mammal has been diagnosed with a need for treatment of muscle atrophy prior to the administering step.

[00217] In a further aspect, the compound is not ursolic acid, beta-boswellic acid, corosolic acid, betulinic acid, or UA0713. In a yet further aspect, the compound is ursolic acid, beta-boswellic acid, corosolic acid, betulinic acid, or UA0713.

[00218] In certain aspects, the disclosed pharmaceutical compositions comprise the disclosed compounds (including pharmaceutically acceptable salt(s) thereof) as an active ingredient, a pharmaceutically acceptable carrier, and, optionally, other therapeutic ingredients or adjuvants. The instant compositions include those suitable for oral, rectal, topical, and parenteral (including subcutaneous, intramuscular, and intravenous) administration, although the most suitable route in any given case will depend on the particular host, and nature and severity of the conditions for which the active ingredient is being administered. The pharmaceutical compositions can be conveniently presented in unit dosage form and prepared by any of the methods well known in the art of pharmacy.

[00219] As used herein, the term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases or acids. When the compound of

the present invention is acidic, its corresponding salt can be conveniently prepared from pharmaceutically acceptable non-toxic bases, including inorganic bases and organic bases. Salts derived from such inorganic bases include aluminum, ammonium, calcium, copper (-ic and -ous), ferric, ferrous, lithium, magnesium, manganese (-ic and -ous), potassium, sodium, zinc and the like salts. Particularly preferred are the ammonium, calcium, magnesium, potassium and sodium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, as well as cyclic amines and substituted amines such as naturally occurring and synthesized substituted amines. Other pharmaceutically acceptable organic non-toxic bases from which salts can be formed include ion exchange resins such as, for example, arginine, betaine, caffeine, choline, N,N'-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine and the like.

[00220] As used herein, the term "pharmaceutically acceptable non-toxic acids", includes inorganic acids, organic acids, and salts prepared therefrom, for example, acetic, benzenesulfonic, benzoic, camphorsulfonic, citric, ethanesulfonic, fumaric, gluconic, glutamic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, pamoic, pantothenic, phosphoric, succinic, sulfuric, tartaric, p-toluenesulfonic acid and the like. Preferred are citric, hydrobromic, hydrochloric, maleic, phosphoric, sulfuric, and tartaric acids.

[00221] In practice, the compounds of the invention, or pharmaceutically acceptable salts thereof, of this invention can be combined as the active ingredient in intimate admixture with a pharmaceutical carrier according to conventional pharmaceutical compounding techniques. The carrier can take a wide variety of forms depending on the form of preparation desired for administration, e.g., oral or parenteral (including intravenous). Thus, the pharmaceutical compositions of the present invention can be presented as discrete units suitable for oral administration such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient. Further, the compositions can be presented as a powder, as granules, as a solution, as a suspension in an aqueous liquid, as a non-aqueous

liquid, as an oil-in-water emulsion or as a water-in-oil liquid emulsion. In addition to the common dosage forms set out above, the compounds of the invention, and/or pharmaceutically acceptable salt(s) thereof, can also be administered by controlled release means and/or delivery devices. The compositions can be prepared by any of the methods of pharmacy. In general, such methods include a step of bringing into association the active ingredient with the carrier that constitutes one or more necessary ingredients. In general, the compositions are prepared by uniformly and intimately admixing the active ingredient with liquid carriers or finely divided solid carriers or both. The product can then be conveniently shaped into the desired presentation.

[00222] Thus, the pharmaceutical compositions of this invention can include a pharmaceutically acceptable carrier and a compound or a pharmaceutically acceptable salt of the compounds of the invention. The compounds of the invention, or pharmaceutically acceptable salts thereof, can also be included in pharmaceutical compositions in combination with one or more other therapeutically active compounds.

[00223] The pharmaceutical carrier employed can be, for example, a solid, liquid, or gas. Examples of solid carriers include lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, and stearic acid. Examples of liquid carriers are sugar syrup, peanut oil, olive oil, and water. Examples of gaseous carriers include carbon dioxide and nitrogen.

[00224] In preparing the compositions for oral dosage form, any convenient pharmaceutical media can be employed. For example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like can be used to form oral liquid preparations such as suspensions, elixirs and solutions; while carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents, and the like can be used to form oral solid preparations such as powders, capsules and tablets. Because of their ease of administration, tablets and capsules are the preferred oral dosage units whereby solid pharmaceutical carriers are employed. Optionally, tablets can be coated by standard aqueous or nonaqueous techniques

[00225] A tablet containing the composition of this invention can be prepared by compression or molding, optionally with one or more accessory ingredients or adjuvants.

Compressed tablets can be prepared by compressing, in a suitable machine, the active ingredient in a free-flowing form such as powder or granules, optionally mixed with a binder, lubricant, inert diluent, surface active or dispersing agent. Molded tablets can be made by molding in a suitable machine, a mixture of the powdered compound moistened with an inert liquid diluent.

[00226] The pharmaceutical compositions of the present invention comprise a compound of the invention (or pharmaceutically acceptable salts thereof) as an active ingredient, a pharmaceutically acceptable carrier, and optionally one or more additional therapeutic agents or adjuvants. The instant compositions include compositions suitable for oral, rectal, topical, and parenteral (including subcutaneous, intramuscular, and intravenous) administration, although the most suitable route in any given case will depend on the particular host, and nature and severity of the conditions for which the active ingredient is being administered. The pharmaceutical compositions can be conveniently presented in unit dosage form and prepared by any of the methods well known in the art of pharmacy.

[00227] Pharmaceutical compositions of the present invention suitable for parenteral administration can be prepared as solutions or suspensions of the active compounds in water. A suitable surfactant can be included such as, for example, hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof in oils. Further, a preservative can be included to prevent the detrimental growth of microorganisms.

[00228] Pharmaceutical compositions of the present invention suitable for injectable use include sterile aqueous solutions or dispersions. Furthermore, the compositions can be in the form of sterile powders for the extemporaneous preparation of such sterile injectable solutions or dispersions. In all cases, the final injectable form must be sterile and must be effectively fluid for easy syringability. The pharmaceutical compositions must be stable under the conditions of manufacture and storage; thus, preferably should be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol and liquid polyethylene glycol), vegetable oils, and suitable mixtures thereof.

[00229] Pharmaceutical compositions of the present invention can be in a form suitable for topical use such as, for example, an aerosol, cream, ointment, lotion, dusting powder, mouth washes, gargles, and the like. Further, the compositions can be in a form suitable for use in transdermal devices. These formulations can be prepared, utilizing a compound of the invention, or pharmaceutically acceptable salts thereof, via conventional processing methods. As an example, a cream or ointment is prepared by mixing hydrophilic material and water, together with about 5 wt% to about 10 wt% of the compound, to produce a cream or ointment having a desired consistency.

[00230] Pharmaceutical compositions of this invention can be in a form suitable for rectal administration wherein the carrier is a solid. It is preferable that the mixture forms unit dose suppositories. Suitable carriers include cocoa butter and other materials commonly used in the art. The suppositories can be conveniently formed by first admixing the composition with the softened or melted carrier(s) followed by chilling and shaping in moulds.

[00231] In addition to the aforementioned carrier ingredients, the pharmaceutical formulations described above can include, as appropriate, one or more additional carrier ingredients such as diluents, buffers, flavoring agents, binders, surface-active agents, thickeners, lubricants, preservatives (including anti-oxidants) and the like. Furthermore, other adjuvants can be included to render the formulation isotonic with the blood of the intended recipient. Compositions containing a compound of the invention, and/or pharmaceutically acceptable salts thereof, can also be prepared in powder or liquid concentrate form.

[00232] In the treatment conditions which require modulation of cellular function related to muscle growth an appropriate dosage level will generally be about 0.01 to 500 mg per kg patient body weight per day and can be administered in single or multiple doses. Preferably, the dosage level will be about 0.1 to about 250 mg/kg per day; more preferably 0.5 to 100 mg/kg per day. A suitable dosage level can be about 0.01 to 250 mg/kg per day, about 0.05 to 100 mg/kg per day, or about 0.1 to 50 mg/kg per day. Within this range the dosage can be 0.05 to 0.5, 0.5 to 5.0 or 5.0 to 50 mg/kg per day. For oral administration, the compositions are preferably provided in the from of tablets containing 1.0 to 1000 miligrams of the active ingredient, particularly 1.0, 5.0, 10, 15, 20, 25, 50, 75, 100, 150, 200, 250, 300, 400, 500, 600, 750, 800, 900 and 1000 milligrams of the active ingredient for the

symptomatic adjustment of the dosage of the patient to be treated. The compound can be administered on a regimen of 1 to 4 times per day, preferably once or twice per day. This dosing regimen can be adjusted to provide the optimal therapeutic response.

[00233] It is understood, however, that the specific dose level for any particular patient will depend upon a variety of factors. Such factors include the age, body weight, general health, sex, and diet of the patient. Other factors include the time and route of administration, rate of excretion, drug combination, and the type and severity of the particular disease undergoing therapy.

[00234] The present invention is further directed to a method for the manufacture of a medicament for modulating cellular activity related to muscle growth (*e.g.*, treatment of one or more disorders associated with muscle dysfunction or atrophy) in mammals (*e.g.*, humans) comprising combining one or more disclosed compounds, products, or compositions with a pharmaceutically acceptable carrier or diluent. Thus, in one aspect, the invention relates to a method for manufacturing a medicament comprising combining at least one disclosed compound or at least one disclosed product with a pharmaceutically acceptable carrier or diluent.

[00235] The disclosed pharmaceutical compositions can further comprise other therapeutically active compounds, which are usually applied in the treatment of the above mentioned pathological conditions.

[00236] It is understood that the disclosed compositions can be prepared from the disclosed compounds. It is also understood that the disclosed compositions can be employed in the disclosed methods of using.

E. METHODS OF USING THE COMPOUNDS AND COMPOSITIONS

1. MUSCLE ATROPHY

[00237] Muscle atrophy is defined as a decrease in the mass of the muscle; it can be a partial or complete wasting away of muscle. When a muscle atrophies, this leads to muscle weakness, since the ability to exert force is related to mass. Muscle atrophy is a co-morbidity of several common diseases, and patients who have "cachexia" in these disease settings have a poor prognosis.

[00238] Muscle atrophy can also be skeletal muscle loss or weakness caused by malnutrition, aging, muscle disuse (such as voluntary and involuntary bedrest, neurologic disease (such as multiple sclerosis, amyotrophic lateral sclerosis, spinal muscular atrophy, critical illness neuropathy, spinal cord injury, peripheral neuropathy, or peripheral nerve injury), injury to the limbs or joints, casting, other post-surgical forms of limb immobilization, or spaceflight), chronic disease (such as cancer, congestive heart failure, chronic pulmonary disease, chronic renal failure, chronic liver disease, diabetes mellitus, glucocorticoid hypersecretion, and chronic infections such as HIV/AIDS or tuberculosis), burn injuries, sepsis, other illnesses requiring mechanical ventiliation, drug-induced muscle disease (such as glucocorticoid-induced myopathy and statin-induced myopathy), genetic diseases that primarily affect skeletal muscle (such as muscular dystrophy, myotonic dystrophy and inclusion body myositis), or autoimmune diseases that affect skeletal muscle (such as polymyositis and dermatomyositis).

[00239] There are many diseases and conditions which cause muscle atrophy, including malnutrition, muscle disuse (secondary to voluntary or involuntary bedrest, neurologic disease (including multiple sclerosis, amyotrophic lateral sclerosis, spinal muscular atrophy, critical illness neuropathy, spinal cord injury or peripheral nerve injury), orthopedic injury, casting, and other post-surgical forms of limb immobilization), chronic disease (including cancer, congestive heart failure, chronic pulmonary disease, chronic renal failure, chronic liver disease, diabetes mellitus, Cushing syndrome and chronic infections such as HIV/AIDS or tuberculosis), burns, sepsis, other illnesses requiring mechanical ventilation, drug-induced muscle disease (such as glucorticoid-induced myopathy and statin-induced myopathy), genetic diseases that primarily affect skeletal muscle (such as muscular dystrophy and myotonic dystrophy), autoimmune diseases that affect skeletal muscle (such as polymyositis and dermatomyositis), spaceflight, and aging.

[00240] Muscle atrophy occurs by a change in the normal balance between protein synthesis and protein degradation. During atrophy, there is a down-regulation of protein synthesis pathways, and an activation of protein breakdown pathways. The particular protein degradation pathway which seems to be responsible for much of the muscle loss seen in a muscle undergoing atrophy is the ATP-dependent, ubiquitin/proteasome pathway. In this system, particular proteins are targeted for destruction by the ligation of at least four copies of a small peptide called ubiquitin onto a substrate protein. When a substrate is thus "poly-

ubiquitinated," it is targeted for destruction by the proteasome. Particular enzymes in the ubiquitin/proteasome pathway allow ubiquitination to be directed to some proteins but not others - specificity is gained by coupling targeted proteins to an "E3 ubiquitin ligase." Each E3 ubiquitin ligase binds to a particular set of substrates, causing their ubiquitination. For example, in skeletal muscle, the E3 ubiquitin ligases atrogin-1 and MuRF1 are known to play essential roles protein degradation and muscle atrophy.

[00241] Muscle atrophy can be opposed by the signaling pathways which induce muscle hypertrophy, or an increase in muscle size. Therefore one way in which exercise induces an increase in muscle mass is to downregulate the pathways which have the opposite effect. One important rehabilitation tool for muscle atrophy includes the use of functional electrical stimulation to stimulate the muscles which has had limited success in the rehabilitation of paraplegic patients.

[00242] Ursolic acid or ursolic acid derivatives can be used as a therapy for illness- and age-related muscle atrophy. It can be useful as a monotherapy or in combination with other strategies that have been considered, such as myostatin inhibition (Zhou, X., *et al.* (2010) Cell 142(4): 531-543). Given its capacity to reduce adiposity, fasting blood glucose and plasma lipid levels, ursolic acid or ursolic acid derivatives can also be used as a therapy for obesity, metabolic syndrome and type 2 diabetes.

[00243] The disclosed compounds can be used as single agents or in combination with one or more other drugs in the treatment, prevention, control, amelioration or reduction of risk of the aforementioned diseases, disorders and conditions for which compounds of formula I or the other drugs have utility, where the combination of drugs together are safer or more effective than either drug alone. The other drug(s) can be administered by a route and in an amount commonly used therefore, contemporaneously or sequentially with a disclosed compound. When a disclosed compound is used contemporaneously with one or more other drugs, a pharmaceutical composition in unit dosage form containing such drugs and the disclosed compound is preferred. However, the combination therapy can also be administered on overlapping schedules. It is also envisioned that the combination of one or more active ingredients and a disclosed compound will be more efficacious than either as a single agent.

[00244] Systemic administration of ursolic acid (by parenteral injection or by oral consumption) can be used to promote muscle growth and reduce muscle atrophy in all muscles, including those of the limbs and the diaphragm. Local administration of ursolic acid (by a topical route or localized injection) can be used to promote local muscle growth, as can be required following a localized injury or surgery.

[00245] In one aspect, the subject compounds can be coadministered with agents that stimulate insulin signaling, IGFl signaling and/or muscle growth including insulin, insulin analogs, insulin-like growth factor l, metformin, thiazoladinediones, sulfonylureas, meglitinides, leptin, dipeptidyl peptidase-4 inhibitors, glucagon-like peptide-l agonists, tyrosine-protein phosphatase non-receptor type 1 (PTPNl a.k.a. PTPlB) inhibitors, myostatin signaling inhibitors, clenbuterol, and androgens including testosterone and 5-dehydroepiandrosterone. The derivative can be corosolic acid, UA07l3, or other pentacyclic triterpene acids. The ursolic acid, derivative or salt thereof can be administered orally, intramuscularly, intravenously or intraarterially. The ursolic acid, derivative or salt thereof can be substantially pure. The ursolic acid, derivative or salt thereof can be administered at about l0 mg/day to l0 g/day.

[00246] In another aspect, the subject compounds can be administered in combination with agents that stimulate insulin signaling, IGFl signaling and/or muscle growth including insulin, insulin analogs, insulin-like growth factor l, metformin, thiazoladinediones, sulfonylureas, meglitinides, leptin, dipeptidyl peptidase-4 inhibitors, glucagon-like peptide-l agonists, tyrosine-protein phosphatase non-receptor type 1 (PTPNl, which is also commonly referred to as PTPlB) inhibitors, myostatin signaling inhibitors, clenbuterol, and androgens including testosterone and 5-dehydroepiandrosterone. The derivative can be corosolic acid, UA07l3, or other pentacyclic triterpene acids. The ursolic acid, derivative or salt thereof can be administered orally, intramuscularly, intravenously or intraarterially. The ursolic acid, derivative or salt thereof can be substantially pure. The ursolic acid, derivative or salt thereof can be administered at about l0 mg/day to l0 g/day.

[00247] The pharmaceutical compositions and methods of the present invention can further comprise other therapeutically active compounds as noted herein which are usually applied in the treatment of the above mentioned pathological conditions.

2. TREATMENT METHODS

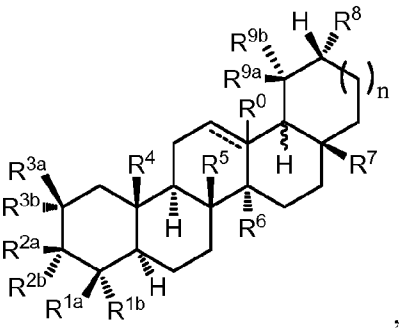
[00248] The compounds disclosed herein are useful for treating, preventing, ameliorating, controlling or reducing the risk of a variety of muscle disorders. Examples of such muscle disorders include, but are not limited to, skeletal muscle atrophy secondary to malnutrition, muscle disuse (secondary to voluntary or involuntary bedrest), neurologic disease (including multiple sclerosis, amyotrophic lateral sclerosis, spinal muscular atrophy, critical illness neuropathy, spinal cord injury or peripheral nerve injury), orthopedic injury, casting, and other post-surgical forms of limb immobilization, chronic disease (including cancer, congestive heart failure, chronic pulmonary disease, chronic renal failure, chronic liver disease, diabetes mellitus, Cushing syndrome and chronic infections such as HIV/AIDS or tuberculosis), burns, sepsis, other illnesses requiring mechanical ventiliation, drug-induced muscle disease (such as glucorticoid-induced myopathy and statin-induced myopathy), genetic diseases that primarily affect skeletal muscle (such as muscular dystrophy and myotonic dystrophy), autoimmune diseases that affect skeletal muscle (such as polymyositis and dermatomyositis), spaceflight, or age-related sarcopenia.

[00249] Thus, provided is a method for treating or preventing muscle atrophy, comprising: administering to a subject at least one disclosed compound; at least one disclosed pharmaceutical composition; and/or at least one disclosed product in a dosage and amount effective to treat the disorder in the subject.

[00250] Also provided is a method for increasing muscle mass, comprising: administering to a subject at least one disclosed compound; at least one disclosed pharmaceutical composition; and/or at least one disclosed product in a dosage and amount effective to treat the disorder in the subject.

a. PREVENTING OR TREATING MUSCLE ATROPHY

[00251] In one aspect, the invention relates to a method for preventing or treating muscle atrophy in an animal, the method comprising administering to the animal a compound of the formula:



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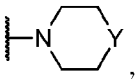
wherein each ----- is an optional covalent bond, and R^0 is optionally present; wherein n is 0 or 1; wherein R^0, when present, is hydrogen; wherein R^{1a} is selected from C1-C6 alkyl and –$C(O)ZR^{10}$; wherein R^{1b} is selected from C1-C6 alkyl, or wherein R^{1a} and R^{1b} are optionally covalently bonded and, together with the intermediate carbon, comprise an optionally substituted C3-C5 cycloalkyl or C2-C5 heterocycloalkyl; wherein one of R^{2a} and R^{2b} is –OR^{11}, and the other is hydrogen, or R^{2a} and R^{2b} together comprise =O; wherein each of R^{3a} and R^{3b} is independently selected from hydrogen, hydroxyl, C1-C6 alkyl, and C1-C6 alkoxyl, provided that R^{3a} and R^{3b} are not simultaneously hydroxyl, wherein R^{3a} and R^{3b} are optionally covalently bonded and, together with the intermediate carbon, comprise an optionally substituted C3-C5 cycloalkyl or C2-C5 heterocycloalkyl; wherein each of R^4, R^5, and R^6 is independently selected from C1-C6 alkyl; wherein R^7 is selected from C1-C6 alkyl, –CH_2OR^{12} and –$C(O)ZR^{12}$; wherein R^8 is selected from hydrogen and C1-C6 alkyl; wherein each of R^{9a} and R^{9b} is independently selected from hydrogen and C1-C6 alkyl, provided that R^{9a} and R^{9b} are not simultaneously hydrogen; or wherein R^{9a} and R^{9b} are covalently bonded and, along with the intermediate carbon, together comprise C3-C5 cycloalkyl or C2-C5 heterocycloalkyl; wherein R^{10} is selected from hydrogen and C1-C6 alkyl; wherein R^{11} is selected from hydrogen, C1-C6 alkyl, C1-C5 heteroalkyl, C3-C6 cycloalkyl, C4-C6 heterocycloalkyl, phenyl, heteroaryl, and –$C(O)R^{14}$; wherein R^{11}, where permitted, is substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; wherein R^{12} is selected from hydrogen and optionally substituted organic residue having from 1 to 20 carbons; wherein Z is selected from –O– and –NR^{13}–; wherein R^{13} is selected from hydrogen and C1-C4 alkyl; or, wherein Z is N, R^{12} and R^{13} are covalently bonded and –$NR^{12}R^{13}$ comprises a moiety of the formula:



wherein Y is selected from –O–, –S–, –SO–, $–SO_2–$, –NH–, $–NCH_3–$; and wherein R^{14} is C1-C6 alkyl and substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, in an amount effective to prevent or treat muscle atrophy in the animal, wherein the amount is greater than about 1000 mg per day when the compound is ursolic acid, beta-boswellic acid, corosolic acid, betulinic acid, or UA0713.

[00252] In a further aspect, the compound administered is a disclosed compound or a product of a disclosed method of making a compound.

[00253] In a further aspect, the animal is a mammal. In a yet further aspect, the mammal is a primate. In a still further aspect, the mammal is a human. In an even further aspect, the human is a patient.

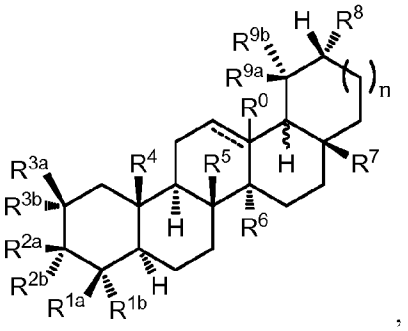
[00254] In a further aspect. the animal is a domesticated animal. In a still further aspect, the domesticated animal is a domesticated fish, domesticated crustacean, or domesticated mollusk. In a yet further aspect, the domesticated animal is poultry. In an even further aspect, the poultry is selected from chicken, turkey, duck, and goose. In a still further aspect, the domesticated animal is livestock. In a yet further aspect, the livestock animal is selected from pig, cow, horse, goat, bison, and sheep.

[00255] In a further aspect, the effective amount is a therapeutically effective amount. In a still further aspect, the effective amount is a prophylactically effective amount. In a yet further aspect, muscle atrophy is prevented by administration of the compound. In an even further aspect, muscle atrophy is treated by administration of the compound. In a still further aspect, the method further comprises the step of identifying the mammal in need of treatment of muscle atrophy. In a yet further aspect, the method further comprises the step of identifying the mammal in a need of prevention of muscle atrophy. In an even further aspect, the mammal has been diagnosed with a need for treatment of muscle atrophy prior to the administering step.

[00256] In a further aspect, the compound is not ursolic acid, beta-boswellic acid, corosolic acid, betulinic acid, or UA0713. In a still further aspect, the compound is ursolic acid, beta-boswellic acid, corosolic acid, betulinic acid, or UA0713. In yet further aspect, the compound is not administered as a foodstuff.

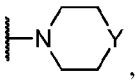
b. INCREASING MUSCLE MASS AND/OR STRENGTH

[00257] In one aspect, the invention relates to a method for increasing muscle mass and/or muscular strength in an animal, the method comprising administering to the animal a compound of the formula:



wherein each ----- is an optional covalent bond, and R^0 is optionally present; wherein n is 0 or 1; wherein R^0, when present, is hydrogen; wherein R^{1a} is selected from C1-C6 alkyl and –$C(O)ZR^{10}$; wherein R^{1b} is selected from C1-C6 alkyl, or wherein R^{1a} and R^{1b} are optionally covalently bonded and, together with the intermediate carbon, comprise an optionally substituted C3-C5 cycloalkyl or C2-C5 heterocycloalkyl; wherein one of R^{2a} and R^{2b} is –OR^{11}, and the other is hydrogen, or R^{2a} and R^{2b} together comprise =O; wherein each of R^{3a} and R^{3b} is independently selected from hydrogen, hydroxyl, C1-C6 alkyl, and C1-C6 alkoxyl, provided that R^{3a} and R^{3b} are not simultaneously hydroxyl, wherein R^{3a} and R^{3b} are optionally covalently bonded and, together with the intermediate carbon, comprise an optionally substituted C3-C5 cycloalkyl or C2-C5 heterocycloalkyl; wherein each of R^4, R^5, and R^6 is independently selected from C1-C6 alkyl; wherein R^7 is selected from C1-C6 alkyl, –CH_2OR^{12} and –$C(O)ZR^{12}$; wherein R^8 is selected from hydrogen and C1-C6 alkyl; wherein each of R^{9a} and R^{9b} is independently selected from hydrogen and C1-C6 alkyl, provided that R^{9a} and R^{9b} are not simultaneously hydrogen; or wherein R^{9a} and R^{9b} are covalently bonded and, along with the intermediate carbon, together comprise C3-C5 cycloalkyl or C2-C5 heterocycloalkyl; wherein R^{10} is selected from hydrogen and C1-C6 alkyl; wherein R^{11} is

selected from hydrogen, C1-C6 alkyl, C1-C5 heteroalkyl, C3-C6 cycloalkyl, C4-C6 heterocycloalkyl, phenyl, heteroaryl, and $-C(O)R^{14}$; wherein R^{11}, where permitted, is substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; wherein R^{12} is selected from hydrogen and optionally substituted organic residue having from 1 to 20 carbons; wherein Z is selected from –O– and $-NR^{13}-$; wherein R^{13} is selected from hydrogen and C1-C4 alkyl; or, wherein Z is N, R^{12} and R^{13} are covalently bonded and $-NR^{12}R^{13}$ comprises a moiety of the formula:



[00258] wherein Y is selected from –O–, –S–, –SO–, $-SO_2-$, –NH–, $-NCH_3-$; and wherein R^{14} is C1-C6 alkyl and substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, in an amount effective to prevent or treat muscle atrophy in the animal, wherein the amount is greater than about 1000 mg per day when the compound is ursolic acid, beta-boswellic acid, corosolic acid, betulinic acid, or UA0713. In a further aspect, the compound administered is a disclosed compound or a product of a disclosed method of making a compound.

[00259] In a further aspect, the compound administered is a disclosed compound or a product of a disclosed method of making a compound.

[00260] In a further aspect, the animal is a mammal. In a yet further aspect, the mammal is a primate. In a still further aspect, the mammal is a human. In an even further aspect, the human is a patient.

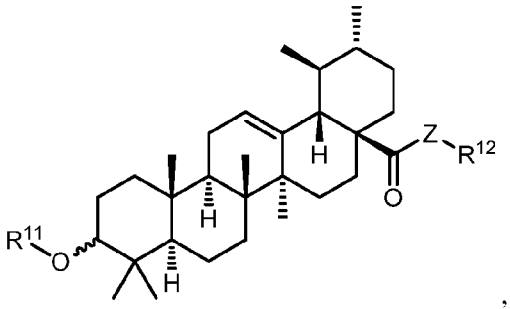
[00261] In a further aspect. the animal is a domesticated animal. In a still further aspect, the domesticated animal is a domesticated fish, domesticated crustacean, or domesticated mollusk. In a yet further aspect, the domesticated animal is poultry. In an even further aspect, the poultry is selected from chicken, turkey, duck, and goose. In a still further aspect, the domesticated animal is livestock. In a yet further aspect, the livestock animal is selected from pig, cow, horse, goat, bison, and sheep.

[00262] In a further aspect, the effective amount is a therapeutically effective amount. In a still further aspect, the effective amount is a prophylactically effective amount. In a yet further aspect, muscle atrophy is prevented by administration of the compound. In an even further aspect, muscle atrophy is treated by administration of the compound. In a still further aspect, the method further comprises the step of identifying the mammal in need of treatment of muscle atrophy. In a yet further aspect, the method further comprises the step of identifying the mammal in need of prevention of muscle atrophy. In an even further aspect, the mammal has been diagnosed with a need for treatment of muscle atrophy prior to the administering step.

[00263] In a further aspect, the compound is not ursolic acid, beta-boswellic acid, corosolic acid, betulinic acid, or UA0713. In a still further aspect, the compound is ursolic acid, beta-boswellic acid, corosolic acid, betulinic acid, or UA0713. In yet further aspect, the compound is not administered as a foodstuff.

c. Enhancing Muscle Formation

[00264] In one aspect, the invention relates to a method of enhancing muscle formation in a mammal, the method comprising administering to the mammal a compound of the formula:



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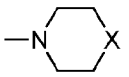
wherein R^{11} is selected from hydrogen, C1-C6 alkyl, C1-C5 heteroalkyl, C3-C6 cycloalkyl, C4-C6 heterocycloalkyl, phenyl, heteroaryl, and $-C(O)R^{14}$; wherein R^{11}, where permitted, is substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; wherein R^{12} is selected from hydrogen and optionally substituted organic residue having from 1 to 20 carbons; wherein Z is selected from –O– and $-NR^{13}-$; wherein R^{13} is selected from hydrogen and C1-C4 alkyl; or, R^{12} and R^{13}, when present, are covalently

bonded and $-NR^{12}R^{13}$ comprises a moiety represented by the formula:



wherein X is selected from O, S, SO, SO_2, NH and NCH_3; and wherein R^{14} is C1-C6 alkyl and substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, in an amount of at least about 200 mg/kg and effective to enhance muscle formation in the mammal.

[00265] In a further aspect, the compound administered is a disclosed compound or a product of a disclosed method of making a compound.

[00266] In a further aspect, the mammal is a human. In a still further aspect, the human is a patient. In a yet further aspect, administration of the compound prevents muscle atrophy in the mammal. In an even further aspect, administration of the compound treats muscle atrophy in the mammal. In a still further aspect, administration of of the compound increases muscle mass in the mammal. In a yet further aspect, administration of the compound increases muscular strength in the mammal.

[00267] In a further aspect, the compound is administered in an effective amount. In a yet further aspect, the effective amount is a therapeutically effective amount. In a still further aspect, the effective amount is a prophylactically effective amount. In a still further aspect, the method further comprises the step of identifying the mammal in need of treatment of muscle atrophy. In a yet further aspect, the method further comprises the step of identifying the mammal in need of prevention of muscle atrophy. In an even further aspect, the mammal has been diagnosed with a need for treatment of muscle atrophy prior to the administering step.

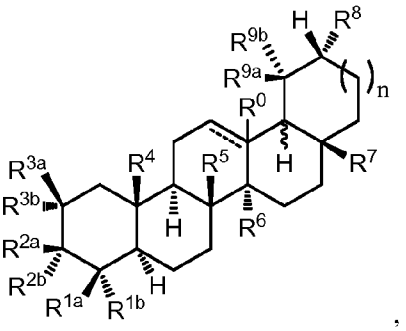
[00268] In a further aspect. the mammal is a domesticated animal. In a yet further aspect, domesticated animal is livestock. In a yet further aspect, the livestock animal is selected from pig, cow, horse, goat, bison, and sheep.

[00269] In a further aspect, the compound is not ursolic acid. In a still further aspect,

the compound is ursolic acid. In a further aspect, the compound is not ursolic acid, beta-boswellic acid, corosolic acid, betulinic acid, or UA0713. In a still further aspect, the compound is ursolic acid, beta-boswellic acid, corosolic acid, betulinic acid, or UA0713. In yet further aspect, the compound is not administered as a foodstuff.

3. ENHANCING TISSUE GROWTH *IN VITRO*

[00270] In one aspect, the invention relates to a method of enhancing tissue growth *in vitro*, the method comprising administering to the tissue a compound of the formula:



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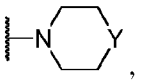
wherein each ----- is an optional covalent bond, and R^0 is optionally present; wherein n is 0 or 1; wherein R^0, when present, is hydrogen; wherein R^{1a} is selected from C1-C6 alkyl and –$C(O)ZR^{10}$; wherein R^{1b} is selected from C1-C6 alkyl, or wherein R^{1a} and R^{1b} are optionally covalently bonded and, together with the intermediate carbon, comprise an optionally substituted C3-C5 cycloalkyl or C2-C5 heterocycloalkyl; wherein one of R^{2a} and R^{2b} is –OR^{11}, and the other is hydrogen, or R^{2a} and R^{2b} together comprise =O; wherein each of R^{3a} and R^{3b} is independently selected from hydrogen, hydroxyl, C1-C6 alkyl, and C1-C6 alkoxyl, provided that R^{3a} and R^{3b} are not simultaneously hydroxyl, wherein R^{3a} and R^{3b} are optionally covalently bonded and, together with the intermediate carbon, comprise an optionally substituted C3-C5 cycloalkyl or C2-C5 heterocycloalkyl; wherein each of R^4, R^5, and R^6 is independently selected from C1-C6 alkyl; wherein R^7 is selected from C1-C6 alkyl, –CH_2OR^{12} and –$C(O)ZR^{12}$; wherein R^8 is selected from hydrogen and C1-C6 alkyl; wherein each of R^{9a} and R^{9b} is independently selected from hydrogen and C1-C6 alkyl, provided that R^{9a} and R^{9b} are not simultaneously hydrogen; or wherein R^{9a} and R^{9b} are covalently bonded and, along with the intermediate carbon, together comprise C3-C5 cycloalkyl or C2-C5 heterocycloalkyl; wherein R^{10} is selected from hydrogen and C1-C6 alkyl; wherein R^{11} is selected from hydrogen, C1-C6 alkyl, C1-C5 heteroalkyl, C3-C6 cycloalkyl, C4-C6

heterocycloalkyl, phenyl, heteroaryl, and –C(O)R^{14}; wherein R^{11}, where permitted, is substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; wherein R^{12} is selected from hydrogen and optionally substituted organic residue having from 1 to 20 carbons; wherein Z is selected from –O– and –NR^{13}–; wherein R^{13} is selected from hydrogen and C1-C4 alkyl; or, wherein Z is N, R^{12} and R^{13} are covalently bonded and –$NR^{12}R^{13}$ comprises a moiety of the formula:



wherein Y is selected from –O–, –S–, –SO–, –SO_2–, –NH–, –NCH_3–; and wherein R^{14} is C1-C6 alkyl and substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, in an amount effective to enhance growth of the tissue.

[00271] In a further aspect, the compound administered is a disclosed compound or a product of a disclosed method of making a compound.

[00272] In a further aspect, the tissue comprises animal cells. In a still further aspect, the animal cells are muscle cells. In a yet further aspect, the muscle cells are myosatellite cells. In an even further aspect, the myosatellite cells are grown on a scaffold.

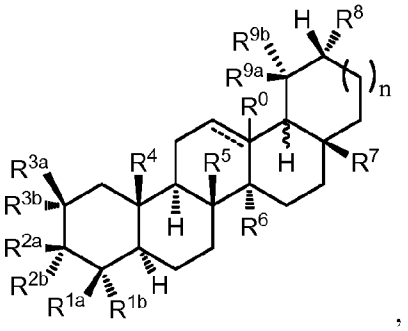
4. MANUFACTURE OF A MEDICAMENT

[00273] In one aspect, the invention relates to a method for the manufacture of a medicament for inhibiting muscle atrophy and for increasing muscle mass in a mammal comprising combining a therapeutically effective amount of a disclosed compound or product of a disclosed method with a pharmaceutically acceptable carrier or diluent.

[00274] In a further aspect, the medicament is modulates muscle growth. In a still further aspect, the medicament inhibits muscle atrophy. In a yet further aspect, the medicament increases muscle mass. In an even further aspect, the medicament induces skeletal muscle hypertrophy.

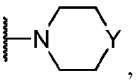
5. METHODS OF TESTING FOR PERFORMANCE ENHANCING USE

[00275] In one aspect, the invention relates to a method of testing for performance enhancing use of a ursolic acid analog in an animal, the method comprising: (a) obtaining a biological test sample from the animal; and (b) measuring the amount of a compound of formula:



wherein each ----- is an optional covalent bond, and R^0 is optionally present; wherein n is 0 or 1; wherein R^0, when present, is hydrogen; wherein R^{1a} is selected from C1-C6 alkyl and –$C(O)ZR^{10}$; wherein R^{1b} is selected from C1-C6 alkyl, or wherein R^{1a} and R^{1b} are optionally covalently bonded and, together with the intermediate carbon, comprise an optionally substituted C3-C5 cycloalkyl or C2-C5 heterocycloalkyl; wherein one of R^{2a} and R^{2b} is –OR^{11}, and the other is hydrogen, or R^{2a} and R^{2b} together comprise =O; wherein each of R^{3a} and R^{3b} is independently selected from hydrogen, hydroxyl, C1-C6 alkyl, and C1-C6 alkoxyl, provided that R^{3a} and R^{3b} are not simultaneously hydroxyl, wherein R^{3a} and R^{3b} are optionally covalently bonded and, together with the intermediate carbon, comprise an optionally substituted C3-C5 cycloalkyl or C2-C5 heterocycloalkyl; wherein each of R^4, R^5, and R^6 is independently selected from C1-C6 alkyl; wherein R^7 is selected from C1-C6 alkyl, –CH_2OR^{12} and –$C(O)ZR^{12}$; wherein R^8 is selected from hydrogen and C1-C6 alkyl; wherein each of R^{9a} and R^{9b} is independently selected from hydrogen and C1-C6 alkyl, provided that R^{9a} and R^{9b} are not simultaneously hydrogen; or wherein R^{9a} and R^{9b} are covalently bonded and, along with the intermediate carbon, together comprise C3-C5 cycloalkyl or C2-C5 heterocycloalkyl; wherein R^{10} is selected from hydrogen and C1-C6 alkyl; wherein R^{11} is selected from hydrogen, C1-C6 alkyl, C1-C5 heteroalkyl, C3-C6 cycloalkyl, C4-C6 heterocycloalkyl, phenyl, heteroaryl, and –$C(O)R^{14}$; wherein R^{11}, where permitted, is substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl,

ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; wherein R^{12} is selected from hydrogen and optionally substituted organic residue having from 1 to 20 carbons; wherein Z is selected from –O– and $–NR^{13}–$; wherein R^{13} is selected from hydrogen and C1-C4 alkyl; or, wherein Z is N, R^{12} and R^{13} are covalently bonded and $–NR^{12}R^{13}$ comprises a moiety of the formula:



wherein Y is selected from –O–, –S–, –SO–, $–SO_2–$, –NH–, $–NCH_3–$; and wherein R^{14} is C1-C6 alkyl and substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, in the test sample to determine whether a superphysiological amount of the compound is present in the biological test sample; wherein the superphysiological amount of the compound in the biological test sample is indicative of performance enhancing use of the compound.

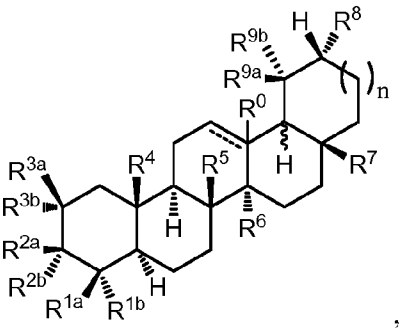
[00276] In a further aspect, the superphysiological amount is greater than the peak concentration from administration at a level of about 1000 mg per day. In a still further aspect, the superphysiological amount is the amount that results from administration of the compound at a level greater than 200 mg per day. In a still further aspect, the superphysiological amount is the amount resulting from administration of the compound at a level greater than 200 mg per day. In an even further aspect, the biological test sample is obtained about 12 hours to about 96 hours following administration of the compound.

[00277] In a further aspect, the animal is a mammal. In a yet further aspect, the animal is a domesticated animal. In a still further aspect, the mammal is a human.

[00278] In a further aspect, the biological sample is blood, urine, saliva, hair, muscle, skin, fat, or breath.

6. USE OF COMPOUNDS

[00279] In one aspect, the invention relates to the use of a compound for increasing muscle mass in a mammal, the compound having a structure represented by a formula:



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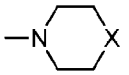
wherein each ----- is an optional covalent bond, and R^0 is optionally present; wherein n is 0 or 1; wherein R^0, when present, is hydrogen; wherein R^{1a} is selected from C1-C6 alkyl and –$C(O)ZR^{10}$; wherein R^{1b} is selected from C1-C6 alkyl, or wherein R^{1a} and R^{1b} are optionally covalently bonded and, together with the intermediate carbon, comprise an optionally substituted C3-C5 cycloalkyl or C2-C5 heterocycloalkyl; wherein one of R^{2a} and R^{2b} is –OR^{11}, and the other is hydrogen, or R^{2a} and R^{2b} together comprise =O; wherein each of R^{3a} and R^{3b} is independently selected from hydrogen, hydroxyl, C1-C6 alkyl, and C1-C6 alkoxyl, provided that R^{3a} and R^{3b} are not simultaneously hydroxyl, wherein R^{3a} and R^{3b} are optionally covalently bonded and, together with the intermediate carbon, comprise an optionally substituted C3-C5 cycloalkyl or C2-C5 heterocycloalkyl; wherein each of R^4, R^5, and R^6 is independently selected from C1-C6 alkyl; wherein R^7 is selected from C1-C6 alkyl, –CH_2OR^{12} and –$C(O)ZR^{12}$; wherein R^8 is selected from hydrogen and C1-C6 alkyl; wherein each of R^{9a} and R^{9b} is independently selected from hydrogen and C1-C6 alkyl, provided that R^{9a} and R^{9b} are not simultaneously hydrogen; or wherein R^{9a} and R^{9b} are covalently bonded and, along with the intermediate carbon, together comprise C3-C5 cycloalkyl or C2-C5 heterocycloalkyl; wherein R^{10} is selected from hydrogen and C1-C6 alkyl; wherein R^{11} is selected from hydrogen, C1-C6 alkyl, C1-C5 heteroalkyl, C3-C6 cycloalkyl, C4-C6 heterocycloalkyl, phenyl, heteroaryl, and –$C(O)R^{14}$; wherein R^{11}, where permitted, is substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; wherein R^{12} is selected from hydrogen and optionally substituted organic residue having from 1 to 20 carbons; wherein Z is selected from –O– and –NR^{13}–; wherein R^{13} is selected from hydrogen and C1-C4 alkyl; or, R^{12} and R^{13}, when present, are covalently bonded and –$NR^{12}R^{13}$ comprises a moiety represented by the formula:



wherein X is selected from O, S, SO, SO_2, NH and NCH_3; and wherein R^{14} is C1-C6 alkyl and substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof.

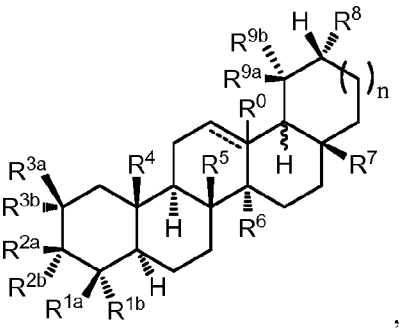
[00280] In a further aspect, a use is the treatment of a mammal. In a yet further aspect, the mammal is a human. In a still further aspect, the human is a patient. In a yet further aspect, a use is administration of the compound to a mammal to prevent muscle atrophy. In a yet further aspect, a use is administration of the compound to increase muscular strength in the mammal. In a further aspect. the mammal is a domesticated animal. In a yet further aspect, domesticated animal is livestock. In a yet further aspect, the livestock animal is selected from pig, cow, horse, goat, bison, and sheep.

[00281] In a further aspect, a use is administration of the compound in an effective amount. In a yet further aspect, the effective amount is a therapeutically effective amount. In a still further aspect, the effective amount is a prophylactically effective amount. In a still further aspect, prior to use the mammal in need of treatment of muscle atrophy is identified. In a yet further aspect, prior to use the mammal in need of prevention of muscle atrophy is identified. In an even further aspect, the mammal has been diagnosed with a need for treatment of muscle atrophy prior to the administering step.

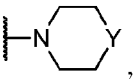
[00282] In a further aspect, the compound is not ursolic acid. In a still further aspect, the compound is ursolic acid. In a further aspect, the compound is not ursolic acid, beta-boswellic acid, corosolic acid, betulinic acid, or UA0713. In a still further aspect, the compound is ursolic acid, beta-boswellic acid, corosolic acid, betulinic acid, or UA0713. In yet further aspect, the compound is not used as a foodstuff. In an even further aspect, the compound is used in an amount is greater than about 1000 mg per day when the compound is ursolic acid, beta-boswellic acid, corosolic acid, betulinic acid, or UA0713.

7. KITS

[00283] In one aspect, the invention relates to a kit comprising at least one compound having a structure represented by a formula:



wherein each ----- is an optional covalent bond, and R^0 is optionally present; wherein n is 0 or 1; wherein R^0, when present, is hydrogen; wherein R^{1a} is selected from C1-C6 alkyl and –$C(O)ZR^{10}$; wherein R^{1b} is selected from C1-C6 alkyl; or wherein R^{1a} and R^{1b} are covalently bonded and, along with the intermediate carbon, together comprise optionally substituted C3-C5 cycloalkyl or optionally substituted C2-C5 heterocycloalkyl; wherein R^{2a} and R^{2b} are independently selected from hydrogen and $–OR^{11}$, provided that at least one of R^{2a} and R^{2b} is $–OR^{11}$; or wherein R^{2a} and R^{2b} together comprise =O; wherein each of R^{3a} and R^{3b} is independently selected from hydrogen, hydroxyl, C1-C6 alkyl, and C1-C6 alkoxyl, provided that R^{3a} and R^{3b} are not simultaneously hydroxyl; or wherein R^{3a} and R^{3b} are covalently bonded and, along with the intermediate carbon, together comprise optionally substituted C3-C5 cycloalkyl or optionally substituted C2-C5 heterocycloalkyl; wherein each of R^4, R^5, and R^6 is independently selected from C1-C6 alkyl; wherein R^7 is selected from C1-C6 alkyl, –CH_2OR^{12}, and –$C(O)ZR^{12}$; wherein R^8 is selected from hydrogen and C1-C6 alkyl; wherein each of R^{9a} and R^{9b} is independently selected from hydrogen and C1-C6 alkyl, provided that R^{9a} and R^{9b} are not simultaneously hydrogen; or wherein R^{9a} and R^{9b} are covalently bonded and, along with the intermediate carbon, together comprise optionally substituted C3-C5 cycloalkyl or optionally substituted C2-C5 heterocycloalkyl; wherein R^{10} is selected from hydrogen and C1-C6 alkyl; wherein each R^{11} is independently selected from hydrogen, C1-C6 alkyl, C1-C5 heteroalkyl, C3-C6 cycloalkyl, C4-C6 heterocycloalkyl, phenyl, heteroaryl, and –$C(O)R^{14}$; wherein R^{11}, where permitted, is substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; wherein R^{12} is selected from hydrogen and optionally substituted organic residue having from 1 to 20 carbons; wherein Z is selected from –O– and –NR^{13}–; wherein R^{13} is selected from hydrogen and C1-C4 alkyl; or, wherein Z is N, R^{12} and R^{13} are covalently bonded and –$NR^{12}R^{13}$ comprises a moiety of the formula:



wherein Y is selected from –O–, –S–, –SO–, $–SO_2–$, –NH–, $–NCH_3–$; and wherein R^{14} is C1-C6 alkyl and substituted with 0-2 groups selected from cyano, acyl, fluoro, chloro, bromo, iodo, methyl, ethyl, propyl, butyl, pentyl, hexyl, hydroxyl, acetoxyl, methoxyl, ethoxyl, propoxyl, and butoxyl; or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, and one or more of: (a) a protein supplement; (b) an anabolic agent; (c) a catabolic agent; (d) a dietary supplement; (e) at least one agent known to treat a disorder associated with muscle wasting; (f) instructions for treating a disorder associated with cholinergic activity; or (g) instructions for using the compound to increase muscle mass and/or muscular strength.

[00284] In a further aspect, the kit ocomprises a disclosed compound or a product of a disclosed method.

[00285] In a further aspect, the at least one compound and the at least one agent are co-formulated. In a still further aspect, the at least one compound and the at least one agent are co-packaged.

[00286] The kits can also comprise compounds and/or products co-packaged, co-formulated, and/or co-delivered with other components. For example, a drug manufacturer, a drug reseller, a physician, a compounding shop, or a pharmacist can provide a kit comprising a disclosed compound and/or product and another component for delivery to a patient.

[00287] It is contemplated that the disclosed kits can be used in connection with the disclosed methods of making, the disclosed methods of using, and/or the disclosed compositions.

8. NON-MEDICAL USES

[00288] Also provided are the uses of the disclosed compounds and products as pharmacological tools in the development and standardization of *in vitro* and *in vivo* test systems for the evaluation of the effects of modulators of muscle hypertrophy or inhibitors of muscle atrophy related activity in laboratory animals such as cats, dogs, rabbits, monkeys, rats and mice, as part of the search for new therapeutic agents of increase muscle mass and/or

inhibit muscle hypertrophy.

F. EXPERIMENTAL

[00289] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how the compounds, compositions, articles, devices and/or methods claimed herein are made and evaluated, and are intended to be purely exemplary of the invention and are not intended to limit the scope of what the inventors regard as their invention. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

[00290] Efforts have been made to ensure accuracy with respect to numbers (*e.g.*, amounts, temperature, etc.), but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C or is at ambient temperature, and pressure is at or near atmospheric.

[00291] Certain materials, reagents and kits were obtained from specific vendors as indicated below, and as appropriate the vendor catalog, part or other number specifying the item are indicated. Vendors indicated below are as follows: "Ambion" is Ambion, a division of Life Technologies Corporation, Austin, Texas, USA; "Applied Biosystems" is Applied Biosystems, a division of Life Technologies Corporation, Carlsbad, California, USA; "Boehringer Mannheim" is Boehringer Mannheim Corporatin, Indiapolis, Indiana, USA; "CardinalHealth" is Cardinal Health, Inc., Dublin, Ohio, USA; "Cell Signaling" is Cell Signaling Technology, Inc., Beverly, Massachussetts, USA; "Columbus Inst" is Columbus Instruments International, Columbus, Ohio, USA; "Harlan" is Harlan Laboratories, Indianapolis, Indiana, USA; "Instrumedics" is Instrumedics, Inc., Richmond, Illinois, USA; "Invitrogen" is Invitrogen Corporation, Carlsbad, California, USA; "Microm" is the Microm division (Walldorf, Germany) of Thermo Fisher Scientific Inc., Rockford, Illinois, USA; "Millipore" is Millipore Corporation, Billerica, Massachussetts, USA; a division of Merck KGaA, Darmstadt, Germany; "Ortho" is Ortho Clinical Diagnostics, Rochester, New York, USA; "Pierce" is Pierce Biotechnology, Inc., Milwaukee, Wisconsin, USA, a division of Thermo Fisher Scientific, Inc.; "R&D Systems" is R&D Systems Inc., Minneapolis,

Minnesota, USA; "Roche Diagnostics" is Roche Diagnostics Corporation, Indianapolis, Indiana, USA; "Sakura" is Sakura Finetek USA, Inc., Torrance, California, USA; "Santa Cruz" is Santa Cruz Biotechnology, Inc., Santa Cruz, California, USA; and, "Sigma" is Sigma-Aldrich Corporation, Saint Louis, Missouri, USA.

1. General Methods

a. Human Subject Protocol.

[00292] The study referred to herein was approved by the Institutional Review Board at the University of Iowa, and involved seven healthy adults who gave their informed consent before participating. One week prior to the fasting study, subjects made one visit to the Clinical Research Unit ("CRU") for anthropometric measurements, a dietary interview that established each subject's routine food intake and food preferences, and baseline determinations of blood hemoglobin ("Hb") A1c turbidimetric immunoinhibition using the BM/Hitachi 911 analyzer (Boehringer Mannheim); plasma triglycerides and plasma free T4 and TSH by electrochemiluminescence immunoassay using the Elecsys® System (Roche Diagnostics); plasma CRP by immuno-turbidimetric assay using the Roche Cobas Integra® high-sensitivity assay (Roche Diagnostics); and, plasma TNF-α levels using the Quantikine® Kit (R&D Systems). To ensure that subjects were eating their routine diet prior to the fasting study, subjects ate only meals prepared by the CRU dietician (based on the dietary interview) for 48 hours before the fasting study. The fasting study began at t = 0 hours, when subjects were admitted to the CRU and began fasting. While fasting, subjects remained in the CRU and were encouraged to maintain their routine physical activities. Water was allowed ad libitum, but caloric intake was not permitted. At about 40 hours, a percutaneous biopsy was taken from the vastus lateralis muscle using a Temno® Biopsy Needle (CardinalHealth; Cat # T1420) under ultrasound guidance. Subjects then ate a CRU-prepared mixed meal, and at t = 46 hours, a muscle biopsy was taken from the contralateral vastus lateralis muscle. Plasma glucose and insulin levels were measured at t = 36, 40, 42 and 46 hours; the Elecsys® system was used to quantitate plasma insulin. Our study protocol of humans with spinal cord injury was described previously (Adams CM, *et al.* (2011) *Muscle Nerve*. 43(1):65-75).

b. Microarray Analysis of Human Skeletal Muscle mRNA Levels.

[00293] Following harvest, skeletal muscle samples were immediately placed in RNAlater (Ambion) and stored at -80 °C until further use. Total RNA was extracted using TRIzol solution (Invitrogen), and microarray hybridizations were performed at the University of Iowa DNA Facility, as described previously (Lamb J, *et al.* (2006) *Science (New York, N.Y* 313(5795):1929-1935). The $\log_2$ hybridization signals as shown herein reflect the mean signal intensity of all exon probes specific for an individual mRNA. To determine which human skeletal muscle mRNAs were significantly altered by fasting ($P \leq 0.02$), paired t-tests were used to compare fasted and fed $\log_2$ signals. To determine which mouse skeletal muscle mRNAs were significantly altered by ursolic acid ($P \leq 0.005$), unpaired t-tests were used to compare $\log_2$ signals in mice fed control diet or diet supplemented with ursolic acid. Highly expressed mRNAs were defined as those significantly altered mRNAs that were repressed from or induced to a $\log_2$ signal > 8. These raw microarray data from humans and mice have been deposited in NCBI's Gene Expression Omnibus ("GEO") and are accessible through GEO Series accession numbers GSE28016 and GSE28017, respectively. Exon array studies of the effects of fasting on mouse skeletal muscle, and the effects of spinal cord injury on human skeletal muscle were described previously (Adams CM, *et al.* (2011) *Muscle & nerve* 43(1):65-75; Ebert SM, *et al.* (2010) *Molecular Endocrinology* 24(4):790-799).

c. Quantitative Real-Time RT-PCR (qPCR).

[00294] TRIzol-extracted mRNA was treated with DNase I using the Turbo DNA-*free* kit (Ambion). qPCR analysis of human mRNA and mouse *IGF-I* mRNA was performed using TaqMan Gene Expression Assays (Applied Biosystems). First strand cDNA was synthesized from 2 μg of RNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Part No. 4368814). The real time PCR contained, in a final volume of 20 μl, 20 ng of reverse transcribed RNA, 1 μl of 20X TaqMan Gene Expression Assay, and 10 μl of TaqMan Fast Universal PCR Master Mix (Applied Biosystems; Part No. 4352042). qPCR was carried out using a 7500 Fast Real-Time PCR System (Applied Biosystems) in 9600 emulation mode. qPCR analysis of mouse *atrogin-1* and *MuRF1* mRNA levels was performed as previously described (Ebert SM, *et al.* (2010) *Molecular Endocrinology* 24(4):790-799). All qPCR reactions were performed in triplicate and the cycle threshold (Ct)

values were averaged to give the final results. To analyze the data, the ΔCt method was used, with the level of *36B4* mRNA serving as the invariant control.

d. MOUSE PROTOCOLS.

[00295] Male C57BL/6 mice, ages 6-8 weeks, were obtained from NCI, housed in colony cages with 12h light/12h dark cycles, and used for experiments within 3 weeks of their arrival. Unless otherwise indicated, mice were maintained on standard chow (Harlan; Teklad Diet, Formula 7013, NIH-31 Modified Open Formula Mouse/Rat Sterilizable Diet). Metformin (Sigma) was dissolved in 0.9% NaCl at a concentration of 250 mg / ml. Ursolic acid (Enzo Life Sciences) was dissolved in corn oil at a concentration of 200 mg / ml (for i.p. injections); alternatively, the ursolic acid was added directly to standard chow (Harlan; Teklad Diet, Formula 7013) or standard high fat diet (Harlan; Teklad Diet, Formula TD.93075) as a customized chow. Oleanolic acid (Sigma) was dissolved in corn oil at a concentration of 200 mg / ml. Mice were fasted by removing food, but not water, for 24 hours. Fasting blood glucose levels were obtained from the tail vein with an ACCU-CHEK® Aviva glucose meter (Roche Diagnostics). Unilateral hindlimb muscle denervation was performed by transsecting the sciatic nerve under anesthesia, and was followed by administration of ursolic acid (200 mg / kg) or vehicle alone (corn oil) via i.p injection twice daily for 7 days. Forelimb grip strength was determined using a grip strength meter equipped with a triangular pull bar (Columbus Inst). Each mouse was subjected to 5 consecutive tests to obtain the peak value. Plasma IGF-I and leptin levels were measured by RIA at the Vanderbilt University Hormone Assay Core Facility. Plasma cholesterol, triglyceride, creatinine, bilirubin and ALT were measured using the VITROS® 350 Chemistry System (Ortho). All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Iowa.

e. HISTOLOGICAL ANALYSIS.

[00296] Following harvest, tissues were immediately placed in isopentane that had been chilled to -160 °C with liquid N_2. Muscles were embedded in tissue freezing medium, and 10 μm sections from the mid-belly were prepared using a Microm HM 505 E cryostat equipped with a CryoJane sectioning system (Instrumedics). Adipose tissue was fixed in 10% neutral buffered formalin, embedded in paraffin, and then 4 μm sections were prepared using

a Microm HM355 S motorized microtome (Microm). Hematoxylin and eosin stains were performed using a DRS-601 automatic slide stainer (Sakura), and examined on an Olympus IX-71 microscope equipped with a DP-70 camera. Image analysis was performed using ImageJ software (public domain, available from the National Institutes of Health, USA). Muscle fiber diameter was measured using the lesser diameter method, as described elsewhere (Dubowitz V, *et al.* (2007) *Muscle biopsy : a practical approach* (Saunders Elsevier, Philadelphia) 3rd Ed pp XIII, 611 s).

f. Analysis of IGF-I and Insulin-Mediated Protein Phosphorylation.

[00297] Mouse quadriceps muscles were snap frozen in liquid N_2, and Triton-X 100 soluble protein extracts were prepared as described previously (Ebert SM, *et al.* (2010) *Molecular endocrinology* 24(4):790-799). Mouse C2C12 myoblasts were obtained from American Type Culture Collection ("ATCC"), and maintained in Dulbecco's modified Eagle's medium (DMEM; ATCC #30-2002) containing antibiotics (100 units/ml penicillin, 100 µg/ml streptomycin sulfate) and 10% (v/v) fetal bovine serum (FBS). On day 0, myotubes were set-up in 6-well plates at a density of 2.5 X 10^5 cells / well. On day 2, differentiation into myotubes was induced by replacing 10% FBS with 2% horse serum. On day 7, myotubes were serum-starved by washing 2 times with phosphate buffered saline, and then adding fresh serum-free media. After 16 hours of serum-starvation, 10 µM ursolic acid (from a 10 mM stock prepared in DMSO), or an equal volume of DMSO, with or without 10 nM mouse IGF-I (Sigma; Cat. No. I8779) or 10 nM bovine insulin (Sigma; Cat. No. I6634) was directly added to the media. For analysis of Akt, S6K, ERK and FoxO phosphorylation, myotubes were incubated in the presence or absence of ursolic acid, IGF-I and/or insulin for 20 min, and then harvested into SDS lysis buffer (10 mM Tris-HCl, pH 7.6, 100 mM NaCl, 1% (w/v) SDS, 1 µg/ml pepstatin A, 2 µg/ml aprotonin, 10 µg/ml leupeptin, 200 µM phenylmethylsulfonyl fluoride and a 1:100 dilution of phosphatase inhibitor cocktail 3 (Sigma). An aliquot of each muscle extract or cell lysate was mixed with 0.25 volume of sample buffer (250 mM Tris-HCl, pH 6.8, 10% SDS, 25% glycerol, 0.2% (w/v) bromophenol blue, and 5% (w/v) 2-mercaptoethanol) and heated for 5 min at 95 °C, whereas a separate aliquot was used to determine protein concentration by the BCA kit (Pierce). Samples (25 µg) were subjected to 8% SDS-PAGE, then transferred to Hybond-C extra nitrocellulose

filters (Millipore). Immunoblots were performed at 4 °C for 16 h using a 1:2000 dilution of antibodies detecting total Akt, phospho-Akt(Ser473), total S6K, phospho-S6K(T421/S424), total ERK1/2, phospho-ERK(T202/Y204), FoxO3a, or phospho-FoxO1(T24)/FoxO3a(T32) (Cell Signaling). For analysis of IGF-1 receptor or insulin receptor phosphorylation, myotubes were incubated in the presence or absence of ursolic acid, IGF-I and/or insulin for 2 min, and then harvested into RIPA buffer (10 mM Tris-HCL, pH 7.4, 150 mM NaCl, 0.1% (w/v) SDS, 1% (w/v) Triton X-100, 1% Na deoxycholate, 5 mM EDTA, 1mM NaF, 1mM Na orthovanadate, 1 μg/ml pepstatin A, 2 μg/ml aprotonin, 10 μug/ml leupeptin, 200 μM phenylmethylsulfonyl fluoride, 1:100 dilution of phosphatase inhibitor cocktail 2 (Sigma) and a 1:100 dilution of phosphatase inhibitor cocktail 3 (Sigma). The protein concentration was measured using the BCA kit, after which the extract was diluted to a concentration of 1 mg/ml in RIPA buffer (final volume 500 μl). Then 2 μg anti-IGF-1 receptor β antibody (Cell Signaling) or 2 μg anti-insulin receptor β antibody (Santa Cruz) was added with 50 μl protein G plus Sepharose beads (Santa Cruz), and then the samples were rotated at 4 °C for 16 h. Immunoprecipitates were washed three times for 20 min with 1 ml RIPA buffer and then mixed with 100 μl sample buffer (50 mM Tris-HCl (pH 6.8), 2% SDS, 5% glycerol, 0.04% (w/v) bromophenol blue and 5% (w/v) 2-mercaptoethanol), then boiled for 5 min. Immunoprecipitates were subjected to 8% SDS-PAGE. For analysis of total IGF-1 receptor, phospho-insulin receptor and total insulin receptor, proteins were transferred to Hybond-C extra nitrocellulose filters (Millipore). For analysis of phospho-IGF-1 receptor, proteins were transferred to PVDF membranes (Bio-Rad). Immunoblots were performed at room temperature using a 1:2000 dilution of anti-IGF-1 receptor β antibody, 1:5000 dilution of mouse anti-phospho-tyrosine 4G10 monoclonal antibody (Millipore), a 1:2000 dilution of anti-insulin receptor β, or 1:2000 dilution of anti-phospho-insulin receptor β (Y1162/1163) (Santa Cruz).

g. PTP1B INHIBITION VIA RNA INTERFERENCE.

[00298] The plasmids *pCMV-miR-PTP1B #1* and *pCMV-miR-PTP1B #2* were generated by ligating *PTPN1*-specific oligonucleotide duplexes (Invitrogen) into the *pcDNA6.2GW/EmGFP miR* plasmid (Invitrogen), which contains a CMV promoter driving co-cistronic expression of engineered pre-miRNAs and EmGFP. *pCMV-miR-control* encodes a non-targeting pre-miRNA hairpin sequence (miR-neg control; Invitrogen) in

pcDNA6.2GW/EmGFP miR plasmid. Male C57BL/6 mice were obtained from NCI at ages 6-8 weeks, and used for experiments within 3 weeks of their arrival. Electroporation of mouse tibialis anterior muscles and isolation of skeletal muscle RNA was performed as described previously (Ebert SM, *et al.* (2010) *Molecular endocrinology* 24(4):790-799). First strand cDNA was synthesized in a 20 μl reaction that contained 2 μg of RNA, random hexamer primers and components of the High Capacity cDNA reverse transcription kit (Applied Biosystems). qPCR analysis of *PTPN1* mRNA levels was performed using a Taqman expression assay as described previously (Ebert SM, *et al.* (2010) *Molecular endocrinology* 24(4):790-799). qPCR was carried out using a 7500 Fast Real-Time PCR System (Applied Biosystems). All qPCR reactions were performed in triplicate and the cycle threshold (Ct) values were averaged to give the final results. Fold changes were determined by the ΔCt method, with level of *36B4* mRNA serving as the invariant control. Skeletal muscle sections were prepared and transfected (EmGFP-positive) muscle fibers were identified and measured as described previously (Ebert SM, *et al.* (2010) *Molecular endocrinology* 24(4):790-799).

h. Measurement of Serum Ursolic Acid Levels.

[00299] Ursolic acid is extracted from serum using a 10:1 mixture of hexane:propanol (recovery > 90%), and then conjugated via its carboxylic acid group to 2-(2,3-naphthalimino)ethyl trifluoromethanesulfonate (Invitrogen; Ne-OTf), a moiety that enhances TUV and fluorescence detection. Derivatized samples are then analyzed on a Waters Acquity UPLC equipped with a 100 X 2.1 mm C18 HSS column with 1.8 μm beads (Waters Part No. 186003533) and a TUV detector.

2. Identification of Therapeutics to Treat Muscle Atrophy

[00300] Skeletal muscle atrophy is common and debilitating condition that lacks a pharmacologic therapy. To identify and develop new therapeutic approaches to this pathophysiological condition (Figure 1), an approach using gene expression signatures to connect small molecules, genes, and disease was used. Briefly, 63 mRNAs were identified that were regulated by fasting in both human and mouse muscle, and 29 mRNAs that were regulated by both fasting and spinal cord injury in human muscle. These two unbiased mRNA expression signatures of muscle atrophy were used to query the Connectivity Map, an algorithm that allows gene signature datasets to be used to find relationships between small

molecules, genes, and disease.

[00301] Three complimentary studies to characterize global atrophy-associated changes in skeletal muscle mRNA levels in humans and mice were carried. These three studies determined the effects of: A) fasting on human skeletal muscle mRNA levels, B) spinal cord injury ("SCI") on human skeletal mRNA levels (Adams CM, *et al.* (2011) *Muscle & nerve* 43(1):65-75) and C) fasting on mouse skeletal muscle mRNA levels (Ebert SM, *et al.* (2010) *Molecular endocrinology* 24(4):790-799). In each study, exon expression arrays were used that quantitated levels of >16,000 mRNAs. Although there were many significant changes in each study, analysis focused on mRNAs whose levels were similarly altered in at least two atrophy models. Thus, by comparing the effects of fasting on human and mouse skeletal muscle, there were two sets of mRNAs identified: a) 31 mRNAs that were increased by fasting in both species, and b) 32 mRNAs that were decreased by fasting in both species. These evolutionarily conserved, fasting-regulated skeletal muscle mRNAs were termed "atrophy signature-1." Next, the effects of fasting and SCI on human skeletal muscle were determined and two sets of mRNAs were identitied: a) 18 mRNAs that were increased by fasting and SCI, and b) 17 mRNAs that were decreased by fasting and SCI. This second group of mRNAs was termed "atrophy signature-2." Almost all of the mRNAs in atrophy signatures-1 and -2 have previously uncharacterized roles in normal or atrophied skeletal muscle. It was next hypothesized that pharmacologic compounds whose effects on cellular mRNA levels were opposite to atrophy signatures-1 and -2 might inhibit skeletal muscle atrophy. To identify candidate compounds, the Connectivity Map (Lamb J, *et al.* (2006) *Science (New York, N.Y* 313(5795):1929-1935) was used to compare atrophy signatures-1 and -2 to mRNA expression signatures of > 1300 bioactive small molecules. These results identified several predicted inhibitors of human skeletal muscle atrophy, including ursolic acid. As a proof-of-concept of the utility of atrophy signatures-1 and -2 described herein, the effects of ursolic acid were assessed in mice, and surprisingly it was discovered ursolic acid inhibited muscle atrophy and promoted muscle hypertrophy.

3. EFFECTS OF FASTING ON SKELETAL MUSCLE MRNA EXPRESSION IN HUMANS.

[00302] Prolonged fasting induces muscle atrophy, but its effects on global mRNA expression in human skeletal muscle were not known heretofore. In order to determine the relationship between global mRNA expression and human skeletal muscle status, seven

healthy adult human volunteers (3 male and 4 female) with ages ranging from 25 to 69 years (mean = 46 years) were studied. The overall study design is shown in Figure 2A. The mean body mass index of these subjects (± SEM) was 25 ± 1. Their mean weight was 69.4 ± 4.8 kg. Baseline circulating levels of hemoglobin A1c (HbA1c), triglycerides (TG), thyroid-stimulating hormone (TSH), free thyroxine (free T4), C-reactive protein (CRP) and tumor necrosis factor-α (TNF-α) were within normal limits (Figure 2A). The table (Figure 2A, insert) shows baseline circulating metabolic and inflammatory markers. The graph shows plasma glucose and insulin levels (Figure 2A). Data are means ± SEM from the seven study subjects. In some cases, the error bars are too small to see. While staying in the University of Iowa Clinical Research Unit, the subjects fasted for 40 h by forgoing food but not water. The mean weight loss during the fast was 1.7 ± 0.1 kg (3 ± 0 % of the initial body weight).

[00303] After the 40 h fast, a muscle biopsy was obtained from the subjects' vastus lateralis (VL) muscle. Immediately after the muscle biopsy, the subjects ate a mixed meal. Five hours later (six hours after the first biopsy), a second muscle biopsy from their contralateral VL muscle. Thus, each subject had a muscle biopsy under fasting and nonfasting conditions. As expected, plasma glucose and insulin levels were low at the end of the 40 h fast, rose after the meal, and returned to baseline by the time of the second biopsy (Figure 2A). These data indicate comparable levels of plasma glucose and insulin at the times of the first (fasting) and second (nonfasting) muscle biopsies.

[00304] To determine the effect of fasting on skeletal muscle mRNA expression, RNA was isolated from the paired muscle biopsies and then analyzed it with exon expression arrays. Using $P \leq 0.02$ (by paired t-test) as criteria for statistical significance, it was found that 281 mRNAs were higher in the fasting state and 277 were lower (out of > 17,000 mRNAs measured; see Figure 2B). A complete list of these fasting-responsive mRNAs is shown below in Table 1 ("Change" is the mean $\log_2$ change or difference between fasting and fed states). The data in Table 1 is for all mRNAs in this study whose levels were increased or decreased by fasting ($P \leq 0.02$ by paired t-test).

[00305] Representative fasting-responsive human skeletal muscle mRNAs, and the effect of fasting on their log2 hybridization signals, as assessed by Affymetrix Human Exon 1.0 ST arrays are shown in Figure 2B. In each subject, the fasting signal was normalized to the nonfasting signal from the same subject. Data are means ± SEM from 7 subjects. $P \leq 0.02$

by paired t-test for all mRNAs shown. The complete set of 458 fasting-responsive mRNAs is shown in Table 1. Most of the differentially expressed mRNAs identified as altered by fasting surprisingly did not have previously known roles in muscle atrophy. However, fasting increased several mRNAs that encode proteins with known roles in catabolic processes such as fat oxidation, reverse cholesterol transport, thermogenesis, inhibition of protein synthesis, autophagy, ubiquitin-mediated proteolysis, glutamine transport and heme catabolism (Figure 2B). Of these, *atrogin-1*, *MuRF1* and *ZFAND5* mRNAs encode proteins known to be required for skeletal muscle atrophy in mice (Bodine SC, *et al.* (2001) *Science (New York, N.Y* 294(5547):1704-1708; Hishiya A, *et al.* (2006) *The EMBO journal* 25(3):554-564). Conversely, fasting significantly decreased several mRNAs encoding proteins with known roles in anabolic processes such as glycogen synthesis, lipid synthesis and uptake, polyamine synthesis, iron uptake, angiogenesis, and mitochondrial biogenesis (Figure 2B). Of these, *PGC-1α* mRNA encodes a protein that inhibits atrophy-associated gene expression and skeletal muscle atrophy in mice (Sandri M, *et al.* (2006) *Proceedings of the National Academy of Sciences of the United States of America* 103(44):16260-16265).

[00306] The results were further validated using qPCR to to analyze RNA from paired fed and fasted skeletal muscle biopsy samples obtained from seven healthy human subjects (see Figure 3; data are means ± SEM; * $P \leq 0.01$ by paired t-test.). In each subject, the fasting mRNA level was normalized to the nonfasting level, which was set at 1. The mRNA encoding myostatin (*MSTN*) is a control transcript whose level was not altered by fasting, as assessed by exon expression arrays. Taken together, these data established an mRNA expression signature of fasting in human skeletal muscle.

TABLE 1. FASTING-RESPONSIVE HUMAN MRNAS.

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
3062082	*PDK4*	NM_002612 // PDK4 // pyruvate dehydrogenase kinase, isozyme 4 // 7q21.3 // 5166	NM_002612	2.15	0.34	0.000
2319340	*SLC25A33*	NM_032315 // SLC25A33 // solute carrier family 25, member 33 // 1p36.22 // 84275	NM_032315	1.42	0.41	0.007

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3165957	*IFNK*	NM_020124 // IFNK // interferon, kappa // --- // 56832 /// ENST00000276943 // IF	NM_020124	0.96	0.28	0.007
3424158	*MYF6*	NM_002469 // MYF6 // myogenic factor 6 (herculin) // 12q21 // 4618 /// ENST00000	NM_002469	0.95	0.12	0.000
3422144	*LGR5*	NM_003667 // LGR5 // leucine-rich repeat-containing G protein-coupled receptor 5	NM_003667	0.88	0.12	0.000
2356115	*TXNIP*	NM_006472 // TXNIP // thioredoxin interacting protein // 1q21.1 // 10628 /// ENS	NM_006472	0.85	0.22	0.004
3233605	*PFKFB3*	NM_004566 // PFKFB3 // 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 //	NM_004566	0.84	0.18	0.002
3151607	*FBXO32*	NM_058229 // FBXO32 // F-box protein 32 // 8q24.13 // 114907 /// NM_148177 // FB	NM_058229	0.82	0.19	0.002
2745547	*GAB1*	NM_207123 // GAB1 // GRB2-associated binding protein 1 // 4q31.21 // 2549 /// NM	NM_207123	0.71	0.08	0.000
3173479	*FOXD4L3*	NM_199135 // FOXD4L3 // forkhead box D4-like 3 // 9q13 // 286380 /// NM_012184 /	NM_199135	0.68	0.25	0.017
3199500	*CER1*	NM_005454 // CER1 // cerberus 1, cysteine knot superfamily, homolog (Xenopus lae	NM_005454	0.64	0.24	0.019

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3444309	*TAS2R9*	NM_023917 // TAS2R9 // taste receptor, type 2, member 9 // 12p13 // 50835 /// EN	NM_023917	0.63	0.22	0.015
3452323	*SLC38A2*	NM_018976 // SLC38A2 // solute carrier family 38, member 2 // 12q // 54407 /// E	NM_018976	0.62	0.13	0.001
3381843	*UCP3*	NM_003356 // UCP3 // uncoupling protein 3 (mitochondrial, proton carrier) // 11q	NM_003356	0.59	0.04	0.000
3147508	*KLF10*	NM_005655 // KLF10 // Kruppel-like factor 10 // 8q22.2 // 7071 /// NM_001032282	NM_005655	0.58	0.11	0.001
3982534	*LPAR4*	NM_005296 // LPAR4 // lysophosphatidic acid receptor 4 // Xq13-q21.1 // 2846 ///	NM_005296	0.57	0.17	0.008
3384321	*RAB30*	NM_014488 // RAB30 // RAB30, member RAS oncogene family // 11q12-q14 // 27314 //	NM_014488	0.56	0.21	0.019
3256192	*C10orf116*	NM_006829 // C10orf116 // chromosome 10 open reading frame 116 // 10q23.2 // 109	NM_006829	0.55	0.19	0.013
2705690	*GHSR*	NM_198407 // GHSR // growth hormone secretagogue receptor // 3q26.31 // 2693 ///	NM_198407	0.54	0.20	0.016
3326938	*LOC100130104*	AF274942 // LOC100130104 // PNAS-17 // 11p13 // 100130104	AF274942	0.53	0.16	0.009

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
2318656	*PER3*	NM_016831 // PER3 // period homolog 3 (Drosophila) // 1p36.23 // 8863 /// ENST00	NM_016831	0.52	0.16	0.009
3209623	*ZFAND5*	NM_001102420 // ZFAND5 // zinc finger, AN1-type domain 5 // 9q13-q21 // 7763 ///	NM_001102420	0.51	0.13	0.005
3741300	*OR1D4*	NM_003552 // OR1D4 // olfactory receptor, family 1, subfamily D, member 4 // 17p	NM_003552	0.50	0.19	0.019
2899176	*HIST1H2BD*	NM_138720 // HIST1H2BD // histone cluster 1, H2bd // 6p21.3 // 3017 /// NM_02106	NM_138720	0.49	0.16	0.010
3439256	*RPS11*	ENST00000270625 // RPS11 // ribosomal protein S11 // 19q13.3 // 6205 /// BC10002	ENST00000270625	0.49	0.11	0.002
2973232	*KIAA0408*	NM_014702 // KIAA0408 // KIAA0408 // 6q22.33 // 9729 /// NM_001012279 // C6orf17	NM_014702	0.49	0.14	0.006
3291151	*RHOBTB1*	NM_014836 // RHOBTB1 // Rho-related BTB domain containing 1 // 10q21.2 // 9886 /	NM_014836	0.48	0.09	0.001
2358136	*C1orf51*	BC027999 // C1orf51 // chromosome 1 open reading frame 51 // 1q21.2 // 148523 //	BC027999	0.48	0.17	0.016
3948936		---	---	0.47	0.18	0.020
3944129	*HMOX1*	NM_002133 // HMOX1 // heme oxygenase (decycling) 1 // 22q12|22q13.1 // 3162 ///	NM_002133	0.46	0.13	0.006

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
2968652	*SESN1*	NM_014454 // SESN1 // sestrin 1 // 6q21 // 27244 /// ENST00000302071 // SESN1 //	NM_014454	0.46	0.12	0.004
2951881	*PXT1*	NM_152990 // PXT1 // peroxisomal, testis specific 1 // 6p21.31 // 222659 /// ENS	NM_152990	0.45	0.14	0.008
2819747	*POLR3G*	NM_006467 // POLR3G // polymerase (RNA) III (DNA directed) polypeptide G (32kD)	NM_006467	0.45	0.13	0.007
2957384	*GSTA2*	NM_000846 // GSTA2 // glutathione S-transferase A2 // 6p12.1 // 2939 /// NM_1536	NM_000846	0.44	0.10	0.002
4014387	*RPSA*	NM_002295 // RPSA // ribosomal protein SA // 3p22.2 // 3921 /// NM_001012321 //	NM_002295	0.44	0.16	0.018
3021158	*C7orf58*	NM_024913 // C7orf58 // chromosome 7 open reading frame 58 // 7q31.31 // 79974 /	NM_024913	0.44	0.07	0.000
2976155	*OLIG3*	NM_175747 // OLIG3 // oligodendrocyte transcription factor 3 // 6q23.3 // 167826	NM_175747	0.44	0.12	0.006
3261886	*C10orf26*	NM_017787 // C10orf26 // chromosome 10 open reading frame 26 // 10q24.32 // 5483	NM_017787	0.44	0.17	0.019
2489169		---	---	0.42	0.12	0.006

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
2790062	*TMEM154*	NM_152680 // TMEM154 // transmembrane protein 154 // 4q31.3 // 201799 /// ENST00	NM_152680	0.42	0.14	0.012
3792656	*CCDC102B*	NM_024781 // CCDC102B // coiled-coil domain containing 102B // 18q22.1 // 79839	NM_024781	0.42	0.12	0.007
3554282	*INF2*	NM_022489 // INF2 // inverted formin, FH2 and WH2 domain containing // 14q32.33	NM_022489	0.41	0.14	0.012
2614142	*NR1D2*	NM_005126 // NR1D2 // nuclear receptor subfamily 1, group D, member 2 // 3p24.2	NM_005126	0.39	0.15	0.019
3404636	*GABARAPL 1*	NM_031412 // GABARAPL1 // GABA(A) receptor-associated protein like 1 // 12p13.2	NM_031412	0.39	0.10	0.004
3063856	*tcag7.1177*	ENST00000292369 // tcag7.1177 // opposite strand transcription unit to STAG3 //	ENST00000 292369	0.39	0.09	0.003
3461981	*TSPAN8*	NM_004616 // TSPAN8 // tetraspanin 8 // 12q14.1-q21.1 // 7103 /// ENST0000039333	NM_004616	0.39	0.14	0.015
2908154	*C6orf206*	BC029519 // C6orf206 // chromosome 6 open reading frame 206 // 6p21.1 // 221421	BC029519	0.39	0.09	0.003
3415046	*FLJ33996*	AK091315 // FLJ33996 // hypothetical protein FLJ33996 // 12q13.13 // 283401 ///	AK091315	0.39	0.15	0.019

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3326400	*CAT*	NM_001752 // CAT // catalase // 11p13 // 847 /// ENST00000241052 // CAT // catal	NM_001752	0.39	0.09	0.003
2390322	*OR2M5*	NM_001004690 // OR2M5 // olfactory receptor, family 2, subfamily M, member 5 //	NM_001004690	0.38	0.12	0.011
2402536	*TRIM63*	NM_032588 // TRIM63 // tripartite motif-containing 63 // 1p34-p33 // 84676 /// E	NM_032588	0.38	0.12	0.009
2976768	*CITED2*	NM_006079 // CITED2 // Cbp/p300-interacting transactivator, with Glu/Asp-rich ca	NM_006079	0.37	0.10	0.005
3218528	*ABCA1*	NM_005502 // ABCA1 // ATP-binding cassette, sub-family A (ABC1), member 1 // 9q3	NM_005502	0.37	0.14	0.016
3377861	*DKFZp761E198*	NM_138368 // DKFZp761E198 // DKFZp761E198 protein // 11q13.1 // 91056 /// BC1091	NM_138368	0.37	0.06	0.000
2961347	*FILIP1*	NM_015687 // FILIP1 // filamin A interacting protein 1 // 6q14.1 // 27145 /// EN	NM_015687	0.37	0.10	0.005
3097580	*C8orf22*	NM_001007176 // C8orf22 // chromosome 8 open reading frame 22 // 8q11 // 492307	NM_001007176	0.37	0.08	0.002
3755655	*FBXL20*	NM_032875 // FBXL20 // F-box and leucine-rich repeat protein 20 // 17q12 // 8496	NM_032875	0.35	0.08	0.002

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3057505	*CCL26*	NM_006072 // CCL26 // chemokine (C-C motif) ligand 26 // 7q11.23 // 10344 /// EN	NM_006072	0.35	0.12	0.012
3307795	*C10orf118*	NM_018017 // C10orf118 // chromosome 10 open reading frame 118 // 10q25.3 // 550	NM_018017	0.35	0.13	0.020
3654699	*NUPR1*	NM_001042483 // NUPR1 // nuclear protein 1 // 16p11.2 // 26471 /// NM_012385 //	NM_001042483	0.35	0.10	0.007
3778252	*ANKRD12*	NM_015208 // ANKRD12 // ankyrin repeat domain 12 // 18p11.22 // 23253 /// NM_001	NM_015208	0.34	0.08	0.002
2662560	*C3orf24*	NM_173472 // C3orf24 // chromosome 3 open reading frame 24 // 3p25.3 // 115795 /	NM_173472	0.34	0.08	0.002
3896370	*RP5-1022P6.2*	NM_019593 // RP5-1022P6.2 // hypothetical protein KIAA1434 // 20p12.3 // 56261 /	NM_019593	0.34	0.10	0.007
3389566	*KBTBD3*	NM_198439 // KBTBD3 // kelch repeat and BTB (POZ) domain containing 3 // 11q22.3	NM_198439	0.34	0.08	0.003
3247818	*FAM133B*	NM_152789 // FAM133B // family with sequence similarity 133, member B // 7q21.2	NM_152789	0.34	0.11	0.010
2457988	*ZNF706*	AF275802 // ZNF706 // zinc finger protein 706 // 8q22.3 // 51123 /// BC015925 //	AF275802	0.34	0.12	0.016

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3525234	*IRS2*	NM_003749 // IRS2 // insulin receptor substrate 2 // 13q34 // 8660 /// ENST00000	NM_003749	0.34	0.09	0.004
2730281	*ODAM*	NM_017855 // ODAM // odontogenic, ameloblast asssociated // 4q13.3 // 54959 ///	NM_017855	0.34	0.12	0.016
3768969	*ABCA5*	NM_018672 // ABCA5 // ATP-binding cassette, sub-family A (ABC1), member 5 // 17q	NM_018672	0.33	0.10	0.008
3687494	*MAPK3*	NM_001040056 // MAPK3 // mitogen-activated protein kinase 3 // 16p11.2 // 5595 /	NM_001040056	0.33	0.09	0.004
3405396	*CREBL2*	NM_001310 // CREBL2 // cAMP responsive element binding protein-like 2 // 12p13 /	NM_001310	0.33	0.07	0.002
3647504	*PMM2*	NM_000303 // PMM2 // phosphomannomutase 2 // 16p13.3-p13.2 // 5373 /// ENST00000	NM_000303	0.33	0.10	0.008
3392840	*BUD13*	NM_032725 // BUD13 // BUD13 homolog (S. cerevisiae) // 11q23.3 // 84811 /// ENST	NM_032725	0.33	0.07	0.002
3453837	*TUBA1A*	NM_006009 // TUBA1A // tubulin, alpha 1a // 12q12-q14.3 // 7846 /// ENST00000301	NM_006009	0.33	0.07	0.002
2409310	*ELOVL1*	NM_022821 // ELOVL1 // elongation of very long chain fatty acids (FEN1/Elo2, SUR	NM_022821	0.32	0.09	0.005

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3837707	*ZNF114*	NM_153608 // ZNF114 // zinc finger protein 114 // 19q13.32 // 163071 /// ENST000	NM_153608	0.31	0.09	0.007
3504434	*XPO4*	NM_022459 // XPO4 // exportin 4 // 13q11 // 64328 /// ENST00000255305 // XPO4 //	NM_022459	0.31	0.10	0.009
2431877		---	---	0.31	0.11	0.017
3837836	*PSCD2*	NM_017457 // PSCD2 // pleckstrin homology, Sec7 and coiled-coil domains 2 (cytoh	NM_017457	0.31	0.05	0.000
3869396	*ZNF432*	NM_014650 // ZNF432 // zinc finger protein 432 // 19q13.33 // 9668 /// ENST00000	NM_014650	0.31	0.09	0.006
3981120	*OGT*	NM_181672 // OGT // O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-ace	NM_181672	0.31	0.10	0.013
2622607	*SLC38A3*	NM_006841 // SLC38A3 // solute carrier family 38, member 3 // 3p21.3 // 10991 //	NM_006841	0.30	0.11	0.016
3978812	*FOXR2*	NM_198451 // FOXR2 // forkhead box R2 // Xp11.21 // 139628 /// ENST00000339140 /	NM_198451	0.30	0.09	0.008
3571904	*NPC2*	NM_006432 // NPC2 // Niemann-Pick disease, type C2 // 14q24.3 // 10577 /// NM_00	NM_006432	0.30	0.10	0.011
2417945	*PTGER3*	NM_198715 // PTGER3 // prostaglandin E receptor 3 (subtype EP3) // 1p31.2 // 573	NM_198715	0.30	0.11	0.017

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3059393	*SEMA3E*	NM_012431 // SEMA3E // sema domain, immunoglobulin domain (Ig), short basic doma	NM_012431	0.30	0.09	0.009
2336456	*MGC52498*	NM_001042693 // MGC52498 // hypothetical protein MGC52498 // 1p32.3 // 348378 //	NM_001042693	0.30	0.10	0.011
3726772	*CROP*	NM_016424 // CROP // cisplatin resistance-associated overexpressed protein // 17	NM_016424	0.30	0.11	0.016
2784265	*IL2*	NM_000586 // IL2 // interleukin 2 // 4q26-q27 // 3558 /// ENST00000226730 // IL2	NM_000586	0.29	0.11	0.019
2495782	*LIPT1*	NM_145197 // LIPT1 // lipoyltransferase 1 // 2q11.2 // 51601 /// NM_145198 // LI	NM_145197	0.29	0.10	0.012
2377094	*PFKFB2*	NM_006212 // PFKFB2 // 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2 //	NM_006212	0.29	0.10	0.012
2469213	*KLF11*	NM_003597 // KLF11 // Kruppel-like factor 11 // 2p25 // 8462 /// ENST00000305883	NM_003597	0.29	0.10	0.011
3662387	*HERPUD1*	NM_014685 // HERPUD1 // homocysteine-inducible, endoplasmic reticulum stress-ind	NM_014685	0.29	0.07	0.003
3771215	*ACOX1*	NM_004035 // ACOX1 // acyl-Coenzyme A oxidase 1, palmitoyl // 17q24-q25|17q25.1	NM_004035	0.29	0.10	0.013

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3203135	*TOPORS*	NM_005802 // TOPORS // topoisomerase I binding, arginine/serine-rich // 9p21 //	NM_005802	0.28	0.11	0.018
2805482		---	---	0.28	0.09	0.008
3247757	*UBE2D1*	NM_003338 // UBE2D1 // ubiquitin-conjugating enzyme E2D 1 (UBC4/5 homolog, yeast	NM_003338	0.28	0.08	0.007
3444147	*KLRC1*	NM_002259 // KLRC1 // killer cell lectin-like receptor subfamily C, member 1 //	NM_002259	0.28	0.10	0.015
3348891	*C11orf57*	NM_018195 // C11orf57 // chromosome 11 open reading frame 57 // 11q23.1 // 55216	NM_018195	0.28	0.09	0.011
3906942	*SERINC3*	NM_006811 // SERINC3 // serine incorporator 3 // 20q13.1-q13.3 // 10955 /// NM_1	NM_006811	0.28	0.07	0.003
2930418	*UST*	NM_005715 // UST // uronyl-2-sulfotransferase // 6q25.1 // 10090 /// ENST0000036	NM_005715	0.28	0.06	0.002
3188200	*OR1L1*	NM_001005236 // OR1L1 // olfactory receptor, family 1, subfamily L, member 1 //	NM_001005236	0.28	0.09	0.011
3856075	*ZNF682*	NM_033196 // ZNF682 // zinc finger protein 682 // 19p12 // 91120 /// NM_00107734	NM_033196	0.28	0.10	0.017
3385951	*NOX4*	NM_016931 // NOX4 // NADPH oxidase 4 // 11q14.2-q21 // 50507 /// ENST00000263317	NM_016931	0.28	0.06	0.002

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3523881	*KDELC1*	NM_024089 // KDELC1 // KDEL (Lys-Asp-Glu-Leu) containing 1 // 13q33 // 79070 ///	NM_024089	0.28	0.06	0.002
2632778	*EPHA6*	NM_001080448 // EPHA6 // EPH receptor A6 // 3q11.2 // 285220 /// ENST00000389672	NM_001080448	0.28	0.09	0.010
3373272	*OR5W2*	NM_001001960 // OR5W2 // olfactory receptor, family 5, subfamily W, member 2 //	NM_001001960	0.28	0.10	0.015
4017694	*IRS4*	NM_003604 // IRS4 // insulin receptor substrate 4 // Xq22.3 // 8471 /// ENST0000	NM_003604	0.28	0.10	0.016
3545311	*KIAA1737*	NM_033426 // KIAA1737 // KIAA1737 // 14q24.3 // 85457 /// ENST00000361786 // KIA	NM_033426	0.28	0.07	0.003
3753860	*CCL5*	NM_002985 // CCL5 // chemokine (C-C motif) ligand 5 // 17q11.2-q12 // 6352 /// E	NM_002985	0.28	0.05	0.001
3617312	*SLC12A6*	NM_001042496 // SLC12A6 // solute carrier family 12 (potassium/chloride transpor	NM_001042496	0.27	0.07	0.005
3351315	*UBE4A*	NM_004788 // UBE4A // ubiquitination factor E4A (UFD2 homolog, yeast) // 11q23.3	NM_004788	0.27	0.07	0.004
3755396	*CCDC49*	NM_017748 // CCDC49 // coiled-coil domain containing 49 // 17q12 // 54883 /// EN	NM_017748	0.27	0.09	0.013

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
2870889	*C5orf13*	NM_004772 // C5orf13 // chromosome 5 open reading frame 13 // 5q22.1 // 9315 ///	NM_004772	0.27	0.09	0.010
2775259	*RASGEF1B*	NM_152545 // RASGEF1B // RasGEF domain family, member 1B // 4q21.21-q21.22 // 15	NM_152545	0.27	0.10	0.015
3165624		---	---	0.27	0.06	0.003
2771654	*CENPC1*	NM_001812 // CENPC1 // centromere protein C 1 // 4q12-q13.3 // 1060 /// ENST0000	NM_001812	0.27	0.09	0.013
3784670	*C18orf21*	NM_031446 // C18orf21 // chromosome 18 open reading frame 21 // 18q12.2 // 83608	NM_031446	0.27	0.08	0.008
2364231	*DDR2*	NM_001014796 // DDR2 // discoidin domain receptor tyrosine kinase 2 // 1q23.3 //	NM_001014796	0.26	0.10	0.018
3921442	*SH3BGR*	NM_007341 // SH3BGR // SH3 domain binding glutamic acid-rich protein // 21q22.3	NM_007341	0.26	0.08	0.007
2627368	*C3orf49*	BC015210 // C3orf49 // chromosome 3 open reading frame 49 // 3p14.1 // 132200	BC015210	0.26	0.06	0.003
3250699	*EIF4EBP2*	NM_004096 // EIF4EBP2 // eukaryotic translation initiation factor 4E binding pro	NM_004096	0.26	0.10	0.018
3237788	*PLXDC2*	NM_032812 // PLXDC2 // plexin domain containing 2 // 10p12.32-p12.31 // 84898 //	NM_032812	0.26	0.09	0.013

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3285926	*ZNF33B*	NM_006955 // ZNF33B // zinc finger protein 33B // 10q11.2 // 7582 /// ENST000003	NM_006955	0.26	0.10	0.018
3304475	*ARL3*	NM_004311 // ARL3 // ADP-ribosylation factor-like 3 // 10q23.3 // 403 /// ENST00	NM_004311	0.26	0.08	0.008
3364306	*SOX6*	NM_017508 // SOX6 // SRY (sex determining region Y)-box 6 // 11p15.3 // 55553 //	NM_017508	0.26	0.08	0.010
3185498	*SLC31A2*	NM_001860 // SLC31A2 // solute carrier family 31 (copper transporters), member 2	NM_001860	0.25	0.09	0.015
3998766	*KAL1*	NM_000216 // KAL1 // Kallmann syndrome 1 sequence // Xp22.32 // 3730 /// ENST000	NM_000216	0.25	0.07	0.006
3143266	*PSKH2*	NM_033126 // PSKH2 // protein serine kinase H2 // 8q21.2 // 85481 /// ENST000002	NM_033126	0.25	0.07	0.006
3458911	*CTDSP2*	NM_005730 // CTDSP2 // CTD (carboxy-terminal domain, RNA polymerase II, polypept	NM_005730	0.25	0.06	0.003
3195034	*PTGDS*	NM_000954 // PTGDS // prostaglandin D2 synthase 21kDa (brain) // 9q34.2-q34.3 //	NM_000954	0.25	0.08	0.010
3854066	*C19orf42*	NM_024104 // C19orf42 // chromosome 19 open reading frame 42 // 19p13.11 // 7908	NM_024104	0.25	0.08	0.010

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3819474	*ANGPTL4*	NM_139314 // ANGPTL4 // angiopoietin-like 4 // 19p13.3 // 51129 /// NM_001039667	NM_139314	0.25	0.06	0.004
3944084	*TOM1*	NM_005488 // TOM1 // target of myb1 (chicken) // 22q13.1 // 10043 /// ENST000003	NM_005488	0.25	0.07	0.006
3848243	*INSR*	NM_000208 // INSR // insulin receptor // 19p13.3-p13.2 // 3643 /// NM_001079817	NM_000208	0.24	0.09	0.014
3168415	*CLTA*	NM_007096 // CLTA // clathrin, light chain (Lca) // 9p13 // 1211 /// NM_00107667	NM_007096	0.24	0.08	0.009
2609462	*CAV3*	NM_033337 // CAV3 // caveolin 3 // 3p25 // 859 /// NM_001234 // CAV3 // caveolin	NM_033337	0.24	0.07	0.007
3393834	*C11orf60*	BC022856 // C11orf60 // chromosome 11 open reading frame 60 // 11q23.3 // 56912	BC022856	0.24	0.06	0.003
3755614	*STAC2*	NM_198993 // STAC2 // SH3 and cysteine rich domain 2 // 17q12 // 342667 /// ENST	NM_198993	0.24	0.07	0.009
3627363	*NARG2*	NM_024611 // NARG2 // NMDA receptor regulated 2 // 15q22.2 // 79664 /// NM_00101	NM_024611	0.24	0.06	0.003
3212976	*ZCCHC6*	NM_024617 // ZCCHC6 // zinc finger, CCHC domain containing 6 // 9q21 // 79670 //	NM_024617	0.24	0.08	0.014

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3275922	*PRKCQ*	NM_006257 // PRKCQ // protein kinase C, theta // 10p15 // 5588 /// ENST000002631	NM_006257	0.24	0.05	0.002
3023825	*C7orf45*	BC017587 // C7orf45 // chromosome 7 open reading frame 45 // 7q32.2 // 136263 //	BC017587	0.23	0.09	0.020
3832906	*IL29*	NM_172140 // IL29 // interleukin 29 (interferon, lambda 1) // 19q13.13 // 282618	NM_172140	0.23	0.08	0.015
3529156	*NGDN*	NM_015514 // NGDN // neuroguidin, EIF4E binding protein // 14q11.2 // 25983 ///	NM_015514	0.23	0.08	0.012
2620448	*CLEC3B*	NM_003278 // CLEC3B // C-type lectin domain family 3, member B // 3p22-p21.3 //	NM_003278	0.23	0.08	0.014
3481296	*SGCG*	NM_000231 // SGCG // sarcoglycan, gamma (35kDa dystrophin-associated glycoprotei	NM_000231	0.23	0.09	0.019
3135184	*RB1CC1*	NM_014781 // RB1CC1 // RB1-inducible coiled-coil 1 // 8q11 // 9821 /// NM_001083	NM_014781	0.23	0.07	0.008
2421843	*GBP3*	NM_018284 // GBP3 // guanylate binding protein 3 // 1p22.2 // 2635 /// ENST00000	NM_018284	0.23	0.06	0.004
3385003	*CREBZF*	NM_001039618 // CREBZF // CREB/ATF bZIP transcription factor // 11q14 // 58487 /	NM_001039618	0.23	0.09	0.020

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3610804	*IGF1R*	NM_000875 // IGF1R // insulin-like growth factor 1 receptor // 15q26.3 // 3480 /	NM_000875	0.23	0.08	0.013
3606304	*AKAP13*	NM_006738 // AKAP13 // A kinase (PRKA) anchor protein 13 // 15q24-q25 // 11214 /	NM_006738	0.23	0.04	0.000
2565579	*ANKRD39*	NM_016466 // ANKRD39 // ankyrin repeat domain 39 // 2q11.2 // 51239 /// ENST0000	NM_016466	0.23	0.05	0.003
2722151	*RBPJ*	NM_005349 // RBPJ // recombination signal binding protein for immunoglobulin kap	NM_005349	0.22	0.07	0.008
3031533	*GIMAP4*	NM_018326 // GIMAP4 // GTPase, IMAP family member 4 // 7q36.1 // 55303 /// ENST0	NM_018326	0.22	0.08	0.017
3725481	*UBE2Z*	NM_023079 // UBE2Z // ubiquitin-conjugating enzyme E2Z // 17q21.32 // 65264 ///	NM_023079	0.22	0.06	0.004
3549575	*IFI27*	NM_005532 // IFI27 // interferon, alpha-inducible protein 27 // 14q32 // 3429 //	NM_005532	0.22	0.08	0.016
3725035	*NFE2L1*	NM_003204 // NFE2L1 // nuclear factor (erythroid-derived 2)-like 1 // 17q21.3 //	NM_003204	0.22	0.07	0.011
3348748	*C11orf1*	NM_022761 // C11orf1 // chromosome 11 open reading frame 1 // 11q13-q22 // 64776	NM_022761	0.22	0.07	0.008

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3722039	*RAMP2*	NM_005854 // RAMP2 // receptor (G protein-coupled) activity modifying protein 2	NM_005854	0.22	0.05	0.003
3886704	*STK4*	NM_006282 // STK4 // serine/threonine kinase 4 // 20q11.2-q13.2 // 6789 /// ENST	NM_006282	0.22	0.07	0.012
3645901	*FLJ14154*	NM_024845 // FLJ14154 // hypothetical protein FLJ14154 // 16p13.3 // 79903 /// N	NM_024845	0.22	0.06	0.005
3367673	*MPPED2*	NM_001584 // MPPED2 // metallophosphoesterase domain containing 2 // 11p13 // 74	NM_001584	0.22	0.08	0.017
3219885	*PTPN3*	NM_002829 // PTPN3 // protein tyrosine phosphatase, non-receptor type 3 // 9q31	NM_002829	0.22	0.05	0.003
3791466		---	---	0.22	0.06	0.007
3717635	*ZNF207*	NM_001098507 // ZNF207 // zinc finger protein 207 // 17q11.2 // 7756 /// NM_0034	NM_001098507	0.22	0.08	0.015
2648141	*MBNL1*	NM_021038 // MBNL1 // muscleblind-like (Drosophila) // 3q25 // 4154 /// NM_20729	NM_021038	0.22	0.07	0.009
2436938	*PBXIP1*	NM_020524 // PBXIP1 // pre-B-cell leukemia homeobox interacting protein 1 // 1q2	NM_020524	0.21	0.05	0.002

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3299705	*PANK1*	NM_148977 // PANK1 // pantothenate kinase 1 // 10q23.31 // 53354 /// NM_148978 /	NM_148977	0.21	0.06	0.007
3628923	*FAM96A*	NM_032231 // FAM96A // family with sequence similarity 96, member A // 15q22.31	NM_032231	0.21	0.05	0.003
2353669	*CD2*	NM_001767 // CD2 // CD2 molecule // 1p13 // 914 /// ENST00000369478 // CD2 // CD	NM_001767	0.21	0.06	0.006
3474450	*PLA2G1B*	NM_000928 // PLA2G1B // phospholipase A2, group IB (pancreas) // 12q23-q24.1 //	NM_000928	0.21	0.08	0.016
3722417	*NBR1*	NM_031858 // NBR1 // neighbor of BRCA1 gene 1 // 17q21.31 // 4077 /// NM_005899	NM_031858	0.21	0.08	0.017
3234760	*CUGBP2*	NM_001025077 // CUGBP2 // CUG triplet repeat, RNA binding protein 2 // 10p13 //	NM_001025077	0.21	0.06	0.004
3627422	*RORA*	NM_134260 // RORA // RAR-related orphan receptor A // 15q21-q22 // 6095 /// NM_0	NM_134260	0.21	0.06	0.006
3382061	*XRRA1*	NM_182969 // XRRA1 // X-ray radiation resistance associated 1 // 11q13.4 // 1435	NM_182969	0.21	0.08	0.017
3015338	*STAG3*	NM_012447 // STAG3 // stromal antigen 3 // 7q22.1 // 10734 /// ENST00000317296 /	NM_012447	0.21	0.06	0.007

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
2665720	*ZNF385D*	NM_024697 // ZNF385D // zinc finger protein 385D // 3p24.3 // 79750 /// ENST0000	NM_024697	0.21	0.07	0.013
3154185	*TMEM71*	NM_144649 // TMEM71 // transmembrane protein 71 // 8q24.22 // 137835 /// ENST000	NM_144649	0.21	0.06	0.009
3789947	*NEDD4L*	NM_015277 // NEDD4L // neural precursor cell expressed, developmentally down-reg	NM_015277	0.21	0.08	0.016
2688933	*CD200R2*	ENST00000383679 // CD200R2 // CD200 cell surface glycoprotein receptor isoform 2	ENST00000 383679	0.21	0.08	0.016
3379644	*CPT1A*	NM_001876 // CPT1A // carnitine palmitoyltransferase 1A (liver) // 11q13.1-q13.2	NM_001876	0.21	0.04	0.001
3677795	*CREBBP*	NM_004380 // CREBBP // CREB binding protein (Rubinstein-Taybi syndrome) // 16p13	NM_004380	0.21	0.05	0.004
2358320	*TARS2*	NM_025150 // TARS2 // threonyl-tRNA synthetase 2, mitochondrial (putative) // 1q	NM_025150	0.21	0.06	0.007
3228373	*TSC1*	NM_000368 // TSC1 // tuberous sclerosis 1 // 9q34 // 7248 /// NM_001008567 // TS	NM_000368	0.20	0.06	0.006
3362795	*RNF141*	NM_016422 // RNF141 // ring finger protein 141 // 11p15.4 // 50862 /// ENST00000	NM_016422	0.20	0.08	0.019

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3673684	*CDT1*	NM_030928 // CDT1 // chromatin licensing and DNA replication factor 1 // 16q24.3	NM_030928	0.20	0.07	0.015
3042881	*HOXA7*	NM_006896 // HOXA7 // homeobox A7 // 7p15-p14 // 3204 /// ENST00000396347 // HOX	NM_006896	0.20	0.02	0.000
3381817	*UCP2*	NM_003355 // UCP2 // uncoupling protein 2 (mitochondrial, proton carrier) // 11q	NM_003355	0.20	0.05	0.005
3415068	*ANKRD33*	NM_182608 // ANKRD33 // ankyrin repeat domain 33 // 12q13.13 // 341405 /// ENST0	NM_182608	0.20	0.06	0.006
3633403	*SIN3A*	NM_015477 // SIN3A // SIN3 homolog A, transcription regulator (yeast) // 15q24.2	NM_015477	0.20	0.07	0.014
3380901	*NUMA1*	NM_006185 // NUMA1 // nuclear mitotic apparatus protein 1 // 11q13 // 4926 /// E	NM_006185	0.19	0.04	0.002
2598099	*BARD1*	NM_000465 // BARD1 // BRCA1 associated RING domain 1 // 2q34-q35 // 580 /// ENST	NM_000465	0.19	0.07	0.015
3139722	*NCOA2*	NM_006540 // NCOA2 // nuclear receptor coactivator 2 // 8q13.3 // 10499 /// ENST	NM_006540	0.19	0.06	0.010
3641871	*LINS1*	NM_018148 // LINS1 // lines homolog 1 (Drosophila) // 15q26.3 // 55180 /// NM_00	NM_018148	0.19	0.06	0.013

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3401217	*TULP3*	NM_003324 // TULP3 // tubby like protein 3 // 12p13.3 // 7289 /// ENST0000022824	NM_003324	0.19	0.06	0.008
3741997	*ANKFY1*	NM_016376 // ANKFY1 // ankyrin repeat and FYVE domain containing 1 // 17p13.3 //	NM_016376	0.19	0.06	0.008
2622742	*C3orf45*	BC028000 // C3orf45 // chromosome 3 open reading frame 45 // 3p21.31 // 132228 /	BC028000	0.19	0.06	0.013
3845352	*UQCR*	NM_006830 // UQCR // ubiquinol-cytochrome c reductase, 6.4kDa subunit // 19p13.3	NM_006830	0.19	0.06	0.014
3960356	*BAIAP2L2*	NM_025045 // BAIAP2L2 // BAI1-associated protein 2-like 2 // 22q13.1 // 80115 //	NM_025045	0.19	0.07	0.018
3645947	*CLUAP1*	NM_015041 // CLUAP1 // clusterin associated protein 1 // 16p13.3 // 23059 /// NM	NM_015041	0.19	0.06	0.012
3835544	*ZNF227*	NM_182490 // ZNF227 // zinc finger protein 227 // --- // 7770 /// ENST0000031304	NM_182490	0.18	0.06	0.011
3368748	*FBXO3*	NM_033406 // FBXO3 // F-box protein 3 // 11p13 // 26273 /// NM_012175 // FBXO3 /	NM_033406	0.18	0.07	0.020
3621623	*ELL3*	NM_025165 // ELL3 // elongation factor RNA polymerase II-like 3 // 15q15.3 // 80	NM_025165	0.18	0.05	0.005

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3430552	*PWP1*	NM_007062 // PWP1 // PWP1 homolog (S. cerevisiae) // 12q23.3 // 11137 /// ENST00	NM_007062	0.18	0.07	0.016
2844908	*BTNL9*	NM_152547 // BTNL9 // butyrophilin-like 9 // 5q35.3 // 153579 /// ENST0000032770	NM_152547	0.18	0.05	0.005
4021508	*ZNF280C*	NM_017666 // ZNF280C // zinc finger protein 280C // Xq25 // 55609 /// ENST000003	NM_017666	0.18	0.07	0.018
2489071	*TET3*	NM_144993 // TET3 // tet oncogene family member 3 // 2p13.1 // 200424 /// ENST00	NM_144993	0.18	0.04	0.003
2516879	*HOXD8*	NM_019558 // HOXD8 // homeobox D8 // 2q31.1 // 3234 /// ENST00000313173 // HOXD8	NM_019558	0.18	0.06	0.015
3740704	*SMYD4*	NM_052928 // SMYD4 // SET and MYND domain containing 4 // 17p13.3 // 114826 ///	NM_052928	0.18	0.06	0.012
3975467	*UTX*	NM_021140 // UTX // ubiquitously transcribed tetratricopeptide repeat, X chromos	NM_021140	0.18	0.06	0.013
3699044	*RFWD3*	NM_018124 // RFWD3 // ring finger and WD repeat domain 3 // 16q22.3 // 55159 ///	NM_018124	0.18	0.06	0.011
3473083	*MED13L*	NM_015335 // MED13L // mediator complex subunit 13-like // 12q24.21 // 23389 ///	NM_015335	0.18	0.02	0.000

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
2332711	*PPIH*	NM_006347 // PPIH // peptidylprolyl isomerase H (cyclophilin H) // 1p34.1 // 104	NM_006347	0.17	0.06	0.017
3556990	*JUB*	NM_032876 // JUB // jub, ajuba homolog (Xenopus laevis) // 14q11.2 // 84962 ///	NM_032876	0.17	0.04	0.004
2780143	*BDH2*	NM_020139 // BDH2 // 3-hydroxybutyrate dehydrogenase, type 2 // 4q24 // 56898 //	NM_020139	0.17	0.05	0.006
3899495	*C20orf12*	NM_001099407 // C20orf12 // chromosome 20 open reading frame 12 // 20p11.23 // 5	NM_001099407	0.17	0.05	0.008
3290875	*ANK3*	NM_020987 // ANK3 // ankyrin 3, node of Ranvier (ankyrin G) // 10q21 // 288 ///	NM_020987	0.17	0.03	0.001
3576014	*C14orf102*	NM_017970 // C14orf102 // chromosome 14 open reading frame 102 // 14q32.11 // 55	NM_017970	0.17	0.04	0.002
3644887	*ATP6V0C*	NM_001694 // ATP6V0C // ATPase, H+ transporting, lysosomal 16kDa, V0 subunit c /	NM_001694	0.17	0.06	0.017
2648378	*RAP2B*	NM_002886 // RAP2B // RAP2B, member of RAS oncogene family // 3q25.2 // 5912 ///	NM_002886	0.17	0.06	0.017
2362892	*ATP1A2*	NM_000702 // ATP1A2 // ATPase, Na+/K+ transporting, alpha 2 (+) polypeptide // 1	NM_000702	0.16	0.06	0.015

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
2361488	*RHBG*	NM_020407 // RHBG // Rh family, B glycoprotein // 1q21.3 // 57127 /// ENST000003	NM_020407	0.16	0.06	0.014
3415915	*PFDN5*	NM_002624 // PFDN5 // prefoldin subunit 5 // 12q12 // 5204 /// NM_145897 // PFDN	NM_002624	0.16	0.05	0.011
3433796	*PEBP1*	NM_002567 // PEBP1 // phosphatidylethanolamine binding protein 1 // 12q24.23 //	NM_002567	0.16	0.04	0.004
3788302	*SMAD4*	NM_005359 // SMAD4 // SMAD family member 4 // 18q21.1 // 4089 /// ENST0000039841	NM_005359	0.16	0.05	0.012
3436236	*ZNF664*	NM_152437 // ZNF664 // zinc finger protein 664 // 12q24.31 // 144348 /// ENST000	NM_152437	0.16	0.06	0.016
3441542	*TMEM16B*	NM_020373 // TMEM16B // transmembrane protein 16B // 12p13.3 // 57101 /// ENST00	NM_020373	0.16	0.06	0.018
3456353	*CALCOCO1*	NM_020898 // CALCOCO1 // calcium binding and coiled-coil domain 1 // 12q13.13 //	NM_020898	0.16	0.05	0.010
3888721	*PTPN1*	NM_002827 // PTPN1 // protein tyrosine phosphatase, non-receptor type 1 // 20q13	NM_002827	0.16	0.06	0.020
3138204	*CYP7B1*	NM_004820 // CYP7B1 // cytochrome P450, family 7, subfamily B, polypeptide 1 //	NM_004820	0.15	0.05	0.014

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3278401	*FRMD4A*	NM_018027 // FRMD4A // FERM domain containing 4A // 10p13 // 55691 /// ENST00000	NM_018027	0.15	0.05	0.009
3904226	*RBM39*	NM_184234 // RBM39 // RNA binding motif protein 39 // 20q11.22 // 9584 /// NM_00	NM_184234	0.15	0.05	0.015
3791850	*SERPINB13*	NM_012397 // SERPINB13 // serpin peptidase inhibitor, clade B (ovalbumin), membe	NM_012397	0.15	0.04	0.005
3665603	*CTCF*	NM_006565 // CTCF // CCCTC-binding factor (zinc finger protein) // 16q21-q22.3 /	NM_006565	0.15	0.04	0.004
3969802	*BMX*	NM_203281 // BMX // BMX non-receptor tyrosine kinase // Xp22.2 // 660 /// NM_001	NM_203281	0.15	0.05	0.016
3621276	*HISPPD2A*	NM_014659 // HISPPD2A // histidine acid phosphatase domain containing 2A // 15q1	NM_014659	0.14	0.04	0.005
2325113	*C1orf213*	NM_138479 // C1orf213 // chromosome 1 open reading frame 213 // 1p36.12 // 14889	NM_138479	0.14	0.05	0.012
3681956	*KIAA0430*	NM_014647 // KIAA0430 // KIAA0430 // 16p13.11 // 9665 /// ENST00000396368 // KIA	NM_014647	0.14	0.05	0.018
3415193	*GRASP*	NM_181711 // GRASP // GRP1 (general receptor for phosphoinositides 1)-associated	NM_181711	0.14	0.05	0.019

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3249369	*LRRTM3*	NM_178011 // LRRTM3 // leucine rich repeat transmembrane neuronal 3 // 10q21.3 /	NM_178011	0.14	0.05	0.011
3874023	*PTPRA*	NM_002836 // PTPRA // protein tyrosine phosphatase, receptor type, A // 20p13 //	NM_002836	0.14	0.04	0.004
3809621	*FECH*	NM_001012515 // FECH // ferrochelatase (protoporphyria) // 18q21.3 // 2235 /// N	NM_001012515	0.14	0.04	0.009
3351385	*MLL*	NM_005933 // MLL // myeloid/lymphoid or mixed-lineage leukemia (trithorax homolo	NM_005933	0.14	0.05	0.016
3288707	*ERCC6*	NM_000124 // ERCC6 // excision repair cross-complementing rodent repair deficien	NM_000124	0.14	0.05	0.016
3624607	*MYO5A*	NM_000259 // MYO5A // myosin VA (heavy chain 12, myoxin) // 15q21 // 4644 /// EN	NM_000259	0.14	0.04	0.006
3353859	*OR4D5*	NM_001001965 // OR4D5 // olfactory receptor, family 4, subfamily D, member 5 //	NM_001001965	0.14	0.05	0.017
2823797	*TSLP*	NM_033035 // TSLP // thymic stromal lymphopoietin // 5q22.1 // 85480 /// NM_1385	NM_033035	0.14	0.05	0.013
2414366	*PPAP2B*	NM_003713 // PPAP2B // phosphatidic acid phosphatase type 2B // 1pter-p22.1 // 8	NM_003713	0.13	0.04	0.007

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3878308	*CSRP2BP*	NM_020536 // CSRP2BP // CSRP2 binding protein // 20p11.23 // 57325 /// NM_177926	NM_020536	0.13	0.05	0.019
4025771	*CD99L2*	NM_031462 // CD99L2 // CD99 molecule-like 2 // Xq28 // 83692 /// NM_134446 // CD	NM_031462	0.13	0.04	0.007
3414776	*LETMD1*	NM_015416 // LETMD1 // LETM1 domain containing 1 // 12q13.13 // 25875 /// NM_001	NM_015416	0.13	0.05	0.014
3645253	*SRRM2*	NM_016333 // SRRM2 // serine/arginine repetitive matrix 2 // 16p13.3 // 23524 //	NM_016333	0.13	0.04	0.007
2440700	*ADAMTS4*	NM_005099 // ADAMTS4 // ADAM metallopeptidase with thrombospondin type 1 motif,	NM_005099	0.13	0.03	0.005
2609870	*BRPF1*	NM_001003694 // BRPF1 // bromodomain and PHD finger containing, 1 // 3p26-p25 //	NM_001003694	0.13	0.04	0.012
3632298	*ADPGK*	NM_031284 // ADPGK // ADP-dependent glucokinase // 15q24.1 // 83440 /// ENST0000	NM_031284	0.13	0.04	0.007
3184940	*GNG10*	NM_001017998 // GNG10 // guanine nucleotide binding protein (G protein), gamma 1	NM_001017998	0.13	0.04	0.011
3223776	*C5*	NM_001735 // C5 // complement component 5 // 9q33-q34 // 727 /// ENST00000223642	NM_001735	0.13	0.04	0.008

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3922100	*MX1*	NM_002462 // MX1 // myxovirus (influenza virus) resistance 1, interferon-inducib	NM_002462	0.12	0.04	0.015
3960478	*CSNK1E*	NM_001894 // CSNK1E // casein kinase 1, epsilon // 22q13.1 // 1454 /// NM_152221	NM_001894	0.12	0.04	0.018
3715703	*SUPT6H*	NM_003170 // SUPT6H // suppressor of Ty 6 homolog (S. cerevisiae) // 17q11.2 //	NM_003170	0.11	0.03	0.005
2322818	*PADI3*	NM_016233 // PADI3 // peptidyl arginine deiminase, type III // 1p36.13 // 51702	NM_016233	0.11	0.03	0.006
2393740	*KIAA0562*	NM_014704 // KIAA0562 // KIAA0562 // 1p36.32 // 9731 /// ENST00000378230 // KIAA	NM_014704	0.11	0.03	0.009
3784509	*ZNF271*	NM_001112663 // ZNF271 // zinc finger protein 271 // 18q12 // 10778 /// NM_00662	NM_001112663	0.11	0.04	0.020
3372253	*CUGBP1*	NM_006560 // CUGBP1 // CUG triplet repeat, RNA binding protein 1 // 11p11 // 106	NM_006560	0.11	0.04	0.011
2948259	*TRIM26*	NM_003449 // TRIM26 // tripartite motif-containing 26 // 6p21.3 // 7726 /// ENST	NM_003449	0.11	0.03	0.006
3191900	*NUP214*	NM_005085 // NUP214 // nucleoporin 214kDa // 9q34.1 // 8021 /// ENST00000359428	NM_005085	0.11	0.03	0.003

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3105581	*CA3*	NM_005181 // CA3 // carbonic anhydrase III, muscle specific // 8q13-q22 // 761 /	NM_005181	0.11	0.03	0.003
3832457	*RYR1*	NM_000540 // RYR1 // ryanodine receptor 1 (skeletal) // 19q13.1 // 6261 /// NM_0	NM_000540	0.11	0.03	0.006
3936256	*BCL2L13*	NM_015367 // BCL2L13 // BCL2-like 13 (apoptosis facilitator) // 22q11 // 23786 /	NM_015367	0.10	0.02	0.002
3599280	*PIAS1*	NM_016166 // PIAS1 // protein inhibitor of activated STAT, 1 // 15q // 8554 ///	NM_016166	0.10	0.04	0.017
3755976	*MED24*	NM_014815 // MED24 // mediator complex subunit 24 // 17q21.1 // 9862 /// NM_0010	NM_014815	0.10	0.04	0.019
3656418	*SRCAP*	NM_006662 // SRCAP // Snf2-related CREBBP activator protein // 16p11.2 // 10847	NM_006662	0.10	0.04	0.017
3943101	*DEPDC5*	NM_014662 // DEPDC5 // DEP domain containing 5 // 22q12.3 // 9681 /// NM_0010071	NM_014662	0.09	0.01	0.000
3960685	*DMC1*	NM_007068 // DMC1 // DMC1 dosage suppressor of mck1 homolog, meiosis-specific ho	NM_007068	0.09	0.03	0.013
2434776	*CDC42SE1*	NM_001038707 // CDC42SE1 // CDC42 small effector 1 // 1q21.2 // 56882 /// NM_020	NM_001038707	0.08	0.03	0.014
3438417	*SFRS8*	NM_004592 // SFRS8 // splicing factor, arginine/serine-rich 8 (suppressor-of-whi	NM_004592	0.08	0.03	0.016

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3457696	*PAN2*	NM_014871 // PAN2 // PAN2 polyA specific ribonuclease subunit homolog (S. cerevi	NM_014871	0.08	0.02	0.008
2534615	*SCLY*	NM_016510 // SCLY // selenocysteine lyase // 2q37.3 // 51540 /// ENST00000254663	NM_016510	0.08	0.02	0.004
2765865	*RELL1*	NM_001085400 // RELL1 // RELT-like 1 // 4p14 // 768211 /// NM_001085399 // RELL1	NM_001085400	0.07	0.02	0.002
3765642	*INTS2*	NM_020748 // INTS2 // integrator complex subunit 2 // 17q23.2 // 57508 /// ENST0	NM_020748	0.05	0.01	0.005
2906607	*NFYA*	NM_002505 // NFYA // nuclear transcription factor Y, alpha // 6p21.3 // 4800 ///	NM_002505	-0.07	0.02	0.011
3168102	*CREB3*	NM_006368 // CREB3 // cAMP responsive element binding protein 3 // 9pter-p22.1 /	NM_006368	-0.07	0.02	0.010
3939365	*SMARCB1*	NM_003073 // SMARCB1 // SWI/SNF related, matrix associated, actin dependent regu	NM_003073	-0.07	0.02	0.013
3415229	*NR4A1*	NM_002135 // NR4A1 // nuclear receptor subfamily 4, group A, member 1 // 12q13 /	NM_002135	-0.07	0.03	0.015
2437801	*ARHGEF2*	NM_004723 // ARHGEF2 // rho/rac guanine nucleotide exchange factor (GEF) 2 // 1q	NM_004723	-0.09	0.02	0.002

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3645565	*THOC6*	NM_024339 // THOC6 // THO complex 6 homolog (Drosophila) // 16p13.3 // 79228 ///	NM_024339	-0.10	0.04	0.018
2406766	*MRPS15*	NM_031280 // MRPS15 // mitochondrial ribosomal protein S15 // 1p35-p34.1 // 6496	NM_031280	-0.11	0.03	0.003
3553141	*KIAA0329*	NM_014844 // KIAA0329 // KIAA0329 // 14q32.31 // 9895 /// ENST00000359520 // KIA	NM_014844	-0.11	0.04	0.018
3297666	*DYDC1*	NM_138812 // DYDC1 // DPY30 domain containing 1 // 10q23.1 // 143241 /// ENST000	NM_138812	-0.11	0.02	0.000
3625674	*RFXDC2*	NM_022841 // RFXDC2 // regulatory factor X domain containing 2 // 15q21.3 // 648	NM_022841	-0.12	0.04	0.012
2926969	*PDE7B*	NM_018945 // PDE7B // phosphodiesterase 7B // 6q23-q24 // 27115 /// ENST00000308	NM_018945	-0.12	0.04	0.013
3525313	*COL4A1*	NM_001845 // COL4A1 // collagen, type IV, alpha 1 // 13q34 // 1282 /// ENST00000	NM_001845	-0.12	0.04	0.014
2438892	*FCRL5*	NM_031281 // FCRL5 // Fc receptor-like 5 // 1q21 // 83416 /// ENST00000361835 //	NM_031281	-0.12	0.04	0.009
3220846	*SUSD1*	NM_022486 // SUSD1 // sushi domain containing 1 // 9q31.3-q33.1 // 64420 /// ENS	NM_022486	-0.12	0.03	0.006

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3598430	*SLC24A1*	NM_004727 // SLC24A1 // solute carrier family 24 (sodium/potassium/ calcium excha	NM_004727	-0.12	0.05	0.019
3506431	*RNF6*	NM_005977 // RNF6 // ring finger protein (C3H2C3 type) 6 // 13q12.2 // 6049 ///	NM_005977	-0.12	0.04	0.011
3696057	*SLC12A4*	NM_005072 // SLC12A4 // solute carrier family 12 (potassium/chloride transporter	NM_005072	-0.12	0.02	0.001
2519577	*COL3A1*	NM_000090 // COL3A1 // collagen, type III, alpha 1 (Ehlers-Danlos syndrome type	NM_000090	-0.12	0.04	0.012
3734479	*TMEM104*	NM_017728 // TMEM104 // transmembrane protein 104 // 17q25.1 // 54868 /// ENST00	NM_017728	-0.13	0.04	0.015
3345157	*PIWIL4*	NM_152431 // PIWIL4 // piwi-like 4 (Drosophila) // 11q21 // 143689 /// ENST00000	NM_152431	-0.13	0.05	0.015
2949471	*NEU1*	NM_000434 // NEU1 // sialidase 1 (lysosomal sialidase) // 6p21.3 // 4758 /// ENS	NM_000434	-0.13	0.04	0.013
2599670	*CRYBA2*	NM_057093 // CRYBA2 // crystallin, beta A2 // 2q34-q36 // 1412 /// NM_005209 //	NM_057093	-0.13	0.04	0.014
3922444	*ABCG1*	NM_207628 // ABCG1 // ATP-binding cassette, sub-family G (WHITE), member 1 // 21	NM_207628	-0.13	0.03	0.003

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
2760371	*WDR1*	NM_017491 // WDR1 // WD repeat domain 1 // 4p16.1 // 9948 /// NM_005112 // WDR1	NM_017491	-0.14	0.05	0.019
2835440	*TCOF1*	NM_001008656 // TCOF1 // Treacher Collins-Franceschetti syndrome 1 // 5q32-q33.1	NM_001008656	-0.14	0.04	0.007
2451544	*MYOG*	NM_002479 // MYOG // myogenin (myogenic factor 4) // 1q31-q41 // 4656 /// ENST00	NM_002479	-0.14	0.05	0.018
3745504	*SCO1*	NM_004589 // SCO1 // SCO cytochrome oxidase deficient homolog 1 (yeast) // 17p12	NM_004589	-0.14	0.03	0.003
2835213	*PPARGC1B*	NM_133263 // PPARGC1B // peroxisome proliferator-activated receptor gamma, coact	NM_133263	-0.14	0.04	0.006
3704567	*CBFA2T3*	NM_005187 // CBFA2T3 // core-binding factor, runt domain, alpha subunit 2; trans	NM_005187	-0.14	0.05	0.020
2893562	*RREB1*	NM_002955 // RREB1 // ras responsive element binding protein 1 // 6p25 // 6239 /	NM_002955	-0.14	0.04	0.006
2672712	*SCAP*	NM_012235 // SCAP // SREBF chaperone // 3p21.31 // 22937 /// ENST00000265565 //	NM_012235	-0.14	0.04	0.009
2768197	*CORIN*	NM_006587 // CORIN // corin, serine peptidase // 4p13-p12 // 10699 /// ENST00000	NM_006587	-0.14	0.05	0.011

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
2495279	*VWA3B*	NM_144992 // VWA3B // von Willebrand factor A domain containing 3B // 2q11.2 //	NM_144992	-0.14	0.04	0.006
2903588	*PFDN6*	NM_014260 // PFDN6 // prefoldin subunit 6 // 6p21.3 // 10471 /// ENST00000399112	NM_014260	-0.14	0.05	0.014
3031383	*REPIN1*	NM_013400 // REPIN1 // replication initiator 1 // 7q36.1 // 29803 /// NM_014374	NM_013400	-0.15	0.05	0.018
3754469	*ACACA*	NM_198839 // ACACA // acetyl-Coenzyme A carboxylase alpha // 17q21 // 31 /// NM_	NM_198839	-0.15	0.05	0.010
3767480	*AXIN2*	NM_004655 // AXIN2 // axin 2 (conductin, axil) // 17q23-q24 // 8313 /// ENST0000	NM_004655	-0.15	0.05	0.013
2954506	*CRIP3*	NM_206922 // CRIP3 // cysteine-rich protein 3 // 6p21.1 // 401262 /// ENST000003	NM_206922	-0.15	0.06	0.018
3845263	*ADAMTSL5*	NM_213604 // ADAMTSL5 // ADAMTS-like 5 // 19p13.3 // 339366 /// ENST00000330475	NM_213604	-0.15	0.06	0.016
2565143	*STARD7*	NM_020151 // STARD7 // StAR-related lipid transfer (START) domain containing 7 /	NM_020151	-0.15	0.06	0.016
2321960	*PLEKHM2*	NM_015164 // PLEKHM2 // pleckstrin homology domain containing, family M (with RU	NM_015164	-0.16	0.05	0.009

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3829174	*GPATCH1*	NM_018025 // GPATCH1 // G patch domain containing 1 // 19q13.11 // 55094 /// ENS	NM_018025	-0.16	0.03	0.001
2798586	*AHRR*	NM_020731 // AHRR // aryl-hydrocarbon receptor repressor // 5p15.3 // 57491 ///	NM_020731	-0.16	0.05	0.011
2362991	*CASQ1*	NM_001231 // CASQ1 // calsequestrin 1 (fast-twitch, skeletal muscle) // 1q21 //	NM_001231	-0.16	0.06	0.015
3954525	*ZNF280B*	NM_080764 // ZNF280B // zinc finger protein 280B // 22q11.22 // 140883 /// ENST0	NM_080764	-0.16	0.04	0.005
4020991	*ACTRT1*	NM_138289 // ACTRT1 // actin-related protein T1 // Xq25 // 139741 /// ENST000003	NM_138289	-0.16	0.05	0.007
3982975	*POU3F4*	NM_000307 // POU3F4 // POU class 3 homeobox 4 // Xq21.1 // 5456 /// ENST00000373	NM_000307	-0.16	0.05	0.013
3963990	*PKDREJ*	NM_006071 // PKDREJ // polycystic kidney disease (polycystin) and REJ homolog (s	NM_006071	-0.16	0.03	0.001
2436401	*JTB*	NM_006694 // JTB // jumping translocation breakpoint // 1q21 // 10899 /// NM_002	NM_006694	-0.16	0.06	0.014
2759654	*ABLIM2*	NM_032432 // ABLIM2 // actin binding LIM protein family, member 2 // 4p16-p15 //	NM_032432	-0.16	0.05	0.007

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
2437329	*CLK2*	NM_003993 // CLK2 // CDC-like kinase 2 // 1q21 // 1196 /// NR_002711 // CLK2P //	NM_003993	-0.16	0.06	0.016
3401119	*ITFG2*	NM_018463 // ITFG2 // integrin alpha FG-GAP repeat containing 2 // 12p13.33 // 5	NM_018463	-0.16	0.04	0.004
3599709	*GLCE*	NM_015554 // GLCE // glucuronic acid epimerase // 15q23 // 26035 /// ENST0000026	NM_015554	-0.16	0.06	0.014
3882413	*C20orf114*	NM_033197 // C20orf114 // chromosome 20 open reading frame 114 // 20q11.21 // 92	NM_033197	-0.16	0.06	0.020
3712922	*C17orf39*	NM_024052 // C17orf39 // chromosome 17 open reading frame 39 // 17p11.2 // 79018	NM_024052	-0.16	0.06	0.017
2473376	*EFR3B*	BC049384 // EFR3B // EFR3 homolog B (S. cerevisiae) // 2p23.3 // 22979 /// ENST0	BC049384	-0.17	0.05	0.009
2607262	*STK25*	NM_006374 // STK25 // serine/threonine kinase 25 (STE20 homolog, yeast) // 2q37.	NM_006374	-0.17	0.06	0.015
3755580	*CACNB1*	NM_199247 // CACNB1 // calcium channel, voltage-dependent, beta 1 subunit // 17q	NM_199247	-0.17	0.06	0.013
3402150	*NTF3*	NM_001102654 // NTF3 // neurotrophin 3 // 12p13 // 4908 /// NM_002527 // NTF3 //	NM_001102654	-0.17	0.06	0.020

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3014714	*ARPC1B*	NM_005720 // ARPC1B // actin related protein 2/3 complex, subunit 1B, 41kDa // 7	NM_005720	-0.17	0.06	0.020
3723071	*DBF4B*	NM_145663 // DBF4B // DBF4 homolog B (S. cerevisiae) // 17q21.31|17q21 // 80174	NM_145663	-0.17	0.04	0.002
2371255	*SMG7*	NM_173156 // SMG7 // Smg-7 homolog, nonsense mediated mRNA decay factor (C. eleg	NM_173156	-0.17	0.06	0.014
3217487	*ALG2*	NM_033087 // ALG2 // asparagine-linked glycosylation 2 homolog (S. cerevisiae, a	NM_033087	-0.17	0.06	0.011
3352159	*LOC100130353*	AK130019 // LOC100130353 // hypothetical protein LOC100130353 // 11q23.3 // 1001	AK130019	-0.17	0.06	0.018
3401259	*TEAD4*	NM_003213 // TEAD4 // TEA domain family member 4 // 12p13.3-p13.2 // 7004 /// NM	NM_003213	-0.17	0.07	0.020
3114618	*RNF139*	NM_007218 // RNF139 // ring finger protein 139 // 8q24 // 11236 /// ENST00000303	NM_007218	-0.17	0.06	0.015
2991150	*TSPAN13*	NM_014399 // TSPAN13 // tetraspanin 13 // 7p21.1 // 27075 /// ENST00000262067 //	NM_014399	-0.18	0.05	0.006
2875193	*P4HA2*	NM_004199 // P4HA2 // procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline	NM_004199	-0.18	0.05	0.007

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
4011743	*SLC7A3*	NM_032803 // SLC7A3 // solute carrier family 7 (cationic amino acid transporter,	NM_032803	-0.18	0.06	0.009
3194015	*LCN9*	NM_001001676 // LCN9 // lipocalin 9 // 9q34.3 // 392399 /// ENST00000277526 // L	NM_001001676	-0.18	0.06	0.011
3741040	*MNT*	NM_020310 // MNT // MAX binding protein // 17p13.3 // 4335 /// ENST00000174618 /	NM_020310	-0.18	0.04	0.003
3901851	*ABHD12*	NM_001042472 // ABHD12 // abhydrolase domain containing 12 // 20p11.21 // 26090	NM_001042472	-0.18	0.05	0.004
2324919	*EPHB2*	NM_017449 // EPHB2 // EPH receptor B2 // 1p36.1-p35 // 2048 /// NM_004442 // EPH	NM_017449	-0.18	0.06	0.010
3185976	*COL27A1*	NM_032888 // COL27A1 // collagen, type XXVII, alpha 1 // 9q32 // 85301 /// ENST0	NM_032888	-0.18	0.06	0.009
2855434	*C5orf39*	NM_001014279 // C5orf39 // chromosome 5 open reading frame 39 // 5p12 // 389289	NM_001014279	-0.18	0.05	0.007
2334476	*MAST2*	NM_015112 // MAST2 // microtubule associated serine/threonine kinase 2 // 1p34.1	NM_015112	-0.18	0.02	0.000
3962734	*TTLL1*	NM_001008572 // TTLL1 // tubulin tyrosine ligase-like family, member 1 // 22q13.	NM_001008572	-0.18	0.03	0.001

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
4017538	*COL4A6*	NM_033641 // COL4A6 // collagen, type IV, alpha 6 // Xq22 // 1288 /// NM_001847	NM_033641	-0.18	0.03	0.000
3141589	*IL7*	NM_000880 // IL7 // interleukin 7 // 8q12-q13 // 3574 /// ENST00000263851 // IL7	NM_000880	-0.19	0.05	0.006
2436826	*KCNN3*	NM_002249 // KCNN3 // potassium intermediate/small conductance calcium-activated	NM_002249	-0.19	0.06	0.008
3521174	*ABCC4*	NM_005845 // ABCC4 // ATP-binding cassette, sub-family C (CFTR/MRP), member 4 //	NM_005845	-0.19	0.07	0.017
3768280	*C17orf58*	NM_181656 // C17orf58 // chromosome 17 open reading frame 58 // 17q24.2 // 28401	NM_181656	-0.19	0.07	0.017
2363784	*HSPA6*	NM_002155 // HSPA6 // heat shock 70kDa protein 6 (HSP70B') // 1q23 // 3310 /// E	NM_002155	-0.19	0.06	0.011
3928211	*GRIK1*	NM_175611 // GRIK1 // glutamate receptor, ionotropic, kainate 1 // 21q22.11 // 2	NM_175611	-0.19	0.06	0.011
2758978	*EVC2*	NM_147127 // EVC2 // Ellis van Creveld syndrome 2 (limbin) // 4p16.2-p16.1 // 13	NM_147127	-0.19	0.06	0.012
3740664	*C17orf91*	NM_032895 // C17orf91 // chromosome 17 open reading frame 91 // 17p13.3 // 84981	NM_032895	-0.19	0.07	0.015

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
2782267	*NEUROG2*	NM_024019 // NEUROG2 // neurogenin 2 // 4q25 // 63973 /// ENST00000313341 // NEU	NM_024019	-0.20	0.06	0.010
3826542	*ZNF738*	BC034499 // ZNF738 // zinc finger protein 738 // 19p12 // 148203 /// AK291002 //	BC034499	-0.20	0.05	0.003
3966000	*TYMP*	NM_001113756 // TYMP // thymidine phosphorylase // 22q13|22q13.33 // 1890 /// NM	NM_001113756	-0.20	0.05	0.003
3607447	*ABHD2*	NM_007011 // ABHD2 // abhydrolase domain containing 2 // 15q26.1 // 11057 /// NM	NM_007011	-0.20	0.05	0.005
3236448	*SUV39H2*	NM_024670 // SUV39H2 // suppressor of variegation 3-9 homolog 2 (Drosophila) //	NM_024670	-0.20	0.07	0.011
2528504	*SPEG*	NM_005876 // SPEG // SPEG complex locus // 2q35 // 10290 /// ENST00000312358 //	NM_005876	-0.20	0.06	0.009
2730746	*SLC4A4*	NM_001098484 // SLC4A4 // solute carrier family 4, sodium bicarbonate cotranspor	NM_001098484	-0.20	0.06	0.007
2544662	*DNMT3A*	NM_175629 // DNMT3A // DNA (cytosine-5-)-methyltransferase 3 alpha // 2p23 // 17	NM_175629	-0.20	0.06	0.007
2937625	*C6orf208*	BC101251 // C6orf208 // chromosome 6 open reading frame 208 // 6q27 // 80069 ///	BC101251	-0.20	0.06	0.007

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3233157	*UCN3*	NM_053049 // UCN3 // urocortin 3 (stresscopin) // 10p15.1 // 114131 /// ENST0000	NM_053049	-0.20	0.08	0.017
2548172	*FEZ2*	NM_001042548 // FEZ2 // fasciculation and elongation protein zeta 2 (zygin II) /	NM_001042548	-0.21	0.03	0.000
3877809	*OTOR*	NM_020157 // OTOR // otoraplin // 20p12.1-p11.23 // 56914 /// ENST00000246081 //	NM_020157	-0.21	0.08	0.019
3839400	*C19orf63*	NM_175063 // C19orf63 // chromosome 19 open reading frame 63 // 19q13.33 // 2843	NM_175063	-0.21	0.04	0.002
3875108	*C20orf196*	AK292708 // C20orf196 // chromosome 20 open reading frame 196 // 20p12.3 // 1498	AK292708	-0.21	0.06	0.006
2970985	*TSPYL4*	NM_021648 // TSPYL4 // TSPY-like 4 // 6q22.1 // 23270 /// ENST00000368611 // TSP	NM_021648	-0.21	0.07	0.011
3189580	*ZBTB43*	NM_014007 // ZBTB43 // zinc finger and BTB domain containing 43 // 9q33-q34 // 2	NM_014007	-0.21	0.08	0.017
3407926	*CMAS*	NM_018686 // CMAS // cytidine monophosphate N-acetylneuraminic acid synthetase /	NM_018686	-0.21	0.03	0.000
3249886	*TET1*	NM_030625 // TET1 // tet oncogene 1 // 10q21 // 80312 /// ENST00000373644 // TET	NM_030625	-0.21	0.06	0.007

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3151970	*MTSS1*	NM_014751 // MTSS1 // metastasis suppressor 1 // 8p22 // 9788 /// ENST0000032506	NM_014751	-0.21	0.07	0.009
3937183	*DGCR8*	NM_022720 // DGCR8 // DiGeorge syndrome critical region gene 8 // 22q11.2 // 544	NM_022720	-0.21	0.06	0.008
3958253	*C22orf28*	BC016707 // C22orf28 // chromosome 22 open reading frame 28 // 22q12 // 51493 //	BC016707	-0.22	0.08	0.019
3607503	*ABHD2*	NM_007011 // ABHD2 // abhydrolase domain containing 2 // 15q26.1 // 11057 /// NM	NM_007011	-0.22	0.07	0.010
2799030	*SLC6A19*	NM_001003841 // SLC6A19 // solute carrier family 6 (neutral amino acid transport	NM_001003841	-0.22	0.06	0.007
3870611	*LILRB3*	NM_001081450 // LILRB3 // leukocyte immunoglobulin-like receptor, subfamily B (w	NM_001081450	-0.22	0.08	0.016
3857811	*C19orf12*	NM_031448 // C19orf12 // chromosome 19 open reading frame 12 // 19q12 // 83636 /	NM_031448	-0.22	0.08	0.019
2500667	*FBLN7*	NM_153214 // FBLN7 // fibulin 7 // 2q13 // 129804 /// ENST00000331203 // FBLN7 /	NM_153214	-0.22	0.08	0.019
3523156	*TMTC4*	NM_032813 // TMTC4 // transmembrane and tetratricopeptide repeat containing 4 //	NM_032813	-0.22	0.07	0.010

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
2612371	*EAF1*	NM_033083 // EAF1 // ELL associated factor 1 // 3p24.3 // 85403 /// ENST00000396	NM_033083	-0.22	0.07	0.008
3988638	*LONRF3*	NM_001031855 // LONRF3 // LON peptidase N-terminal domain and ring finger 3 // X	NM_001031855	-0.23	0.08	0.012
3114240	*C8orf32*	BC008781 // C8orf32 // chromosome 8 open reading frame 32 // 8q24.13 // 55093 //	BC008781	-0.23	0.08	0.016
2460368	*TTC13*	NM_024525 // TTC13 // tetratricopeptide repeat domain 13 // 1q42.2 // 79573 ///	NM_024525	-0.23	0.08	0.014
2428425	*PPM1J*	NM_005167 // PPM1J // protein phosphatase 1J (PP2C domain containing) // 1p13.2	NM_005167	-0.23	0.06	0.003
3194986	*LCN12*	NM_178536 // LCN12 // lipocalin 12 // 9q34.3 // 286256 /// ENST00000371633 // LC	NM_178536	-0.23	0.06	0.004
3642875	*RAB11FIP3*	NM_014700 // RAB11FIP3 // RAB11 family interacting protein 3 (class II) // 16p13	NM_014700	-0.23	0.07	0.010
2532378	*CHRND*	NM_000751 // CHRND // cholinergic receptor, nicotinic, delta // 2q33-q34 // 1144	NM_000751	-0.23	0.08	0.018
2995667	*ADCYAP1R1*	NM_001118 // ADCYAP1R1 // adenylate cyclase activating polypeptide 1 (pituitary)	NM_001118	-0.23	0.05	0.002

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3390641	*ARHGAP20*	NM_020809 // ARHGAP20 // Rho GTPase activating protein 20 // 11q22.3-q23.1 // 57	NM_020809	-0.23	0.05	0.003
2830465	*MYOT*	NM_006790 // MYOT // myotilin // 5q31 // 9499 /// ENST00000239926 // MYOT // myo	NM_006790	-0.23	0.07	0.007
2452069	*PIK3C2B*	NM_002646 // PIK3C2B // phosphoinositide-3-kinase, class 2, beta polypeptide //	NM_002646	-0.23	0.02	0.000
3744127	*HES7*	NM_032580 // HES7 // hairy and enhancer of split 7 (Drosophila) // 17p13.1 // 84	NM_032580	-0.23	0.09	0.019
3327057	*FLJ14213*	NM_024841 // FLJ14213 // protor-2 // 11p13-p12 // 79899 /// ENST00000378867 // F	NM_024841	-0.23	0.07	0.007
2664332	*COLQ*	NM_005677 // COLQ // collagen-like tail subunit (single strand of homotrimer) of	NM_005677	-0.23	0.07	0.006
3829160	*C19orf40*	NM_152266 // C19orf40 // chromosome 19 open reading frame 40 // 19q13.11 // 9144	NM_152266	-0.23	0.08	0.012
3708798	*SENP3*	NM_015670 // SENP3 // SUMO1/sentrin/SMT3 specific peptidase 3 // 17p13 // 26168	NM_015670	-0.23	0.06	0.005
2358700	*MGC29891*	NM_144618 // MGC29891 // hypothetical protein MGC29891 // 1q21.2 // 126626 /// E	NM_144618	-0.23	0.09	0.019

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
2755111	*KLKB1*	NM_000892 // KLKB1 // kallikrein B, plasma (Fletcher factor) 1 // 4q34-q35 // 38	NM_000892	-0.24	0.08	0.012
2568968	*UXS1*	NM_025076 // UXS1 // UDP-glucuronate decarboxylase 1 // 2q12.2 // 80146 /// BC00	NM_025076	-0.24	0.08	0.011
2748923	*GUCY1B3*	NM_000857 // GUCY1B3 // guanylate cyclase 1, soluble, beta 3 // 4q31.3-q33 // 29	NM_000857	-0.24	0.07	0.007
3816509	*GADD45B*	NM_015675 // GADD45B // growth arrest and DNA-damage-inducible, beta // 19p13.3	NM_015675	-0.24	0.09	0.016
3376410	*SLC22A24*	BC034394 // SLC22A24 // solute carrier family 22, member 24 // 11q12.3 // 283238	BC034394	-0.24	0.07	0.007
3286393	*ZNF32*	NM_006973 // ZNF32 // zinc finger protein 32 // 10q22-q25 // 7580 /// NM_0010053	NM_006973	-0.24	0.08	0.010
2540157	*ODC1*	NM_002539 // ODC1 // ornithine decarboxylase 1 // 2p25 // 4953 /// ENST000002341	NM_002539	-0.24	0.09	0.020
2994835	*CHN2*	NM_004067 // CHN2 // chimerin (chimaerin) 2 // 7p15.3 // 1124 /// NM_001039936 /	NM_004067	-0.24	0.09	0.017
3603199	*IDH3A*	NM_005530 // IDH3A // isocitrate dehydrogenase 3 (NAD+) alpha // 15q25.1-q25.2 /	NM_005530	-0.24	0.05	0.001
3040454	*TWISTNB*	NM_001002926 // TWISTNB // TWIST neighbor // 7p15.3 // 221830 /// ENST0000022256	NM_001002926	-0.24	0.09	0.017

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
2497301	*TMEM182*	NM_144632 // TMEM182 // transmembrane protein 182 // 2q12.1 // 130827 /// ENST00	NM_144632	-0.24	0.07	0.007
3766716	*TEX2*	NM_018469 // TEX2 // testis expressed 2 // 17q23.3 // 55852 /// ENST00000258991	NM_018469	-0.25	0.07	0.007
3458819	*CYP27B1*	NM_000785 // CYP27B1 // cytochrome P450, family 27, subfamily B, polypeptide 1 /	NM_000785	-0.25	0.08	0.009
3368940	*ABTB2*	NM_145804 // ABTB2 // ankyrin repeat and BTB (POZ) domain containing 2 // 11p13	NM_145804	-0.25	0.08	0.010
3298924	*MMRN2*	NM_024756 // MMRN2 // multimerin 2 // 10q23.2 // 79812 /// ENST00000372027 // MM	NM_024756	-0.25	0.07	0.006
3529951	*KIAA1305*	NM_025081 // KIAA1305 // KIAA1305 // 14q12 // 57523 /// BC008219 // KIAA1305 //	NM_025081	-0.25	0.08	0.011
3006572	*AUTS2*	NM_015570 // AUTS2 // autism susceptibility candidate 2 // 7q11.22 // 26053 ///	NM_015570	-0.25	0.09	0.017
3025500	*BPGM*	NM_001724 // BPGM // 2,3-bisphosphoglycerate mutase // 7q31-q34 // 669 /// NM_19	NM_001724	-0.25	0.10	0.018
2494709	*CNNM4*	NM_020184 // CNNM4 // cyclin M4 // 2p12-p11.2 // 26504 /// ENST00000377075 // CN	NM_020184	-0.26	0.09	0.016

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3329983	*PTPRJ*	NM_002843 // PTPRJ // protein tyrosine phosphatase, receptor type, J // 11p11.2	NM_002843	-0.26	0.08	0.010
2769346	*LNX1*	NM_032622 // LNX1 // ligand of numb-protein X 1 // 4q12 // 84708 /// ENST0000030	NM_032622	-0.26	0.09	0.015
3867195	*FAM83E*	NM_017708 // FAM83E // family with sequence similarity 83, member E // 19q13.32-	NM_017708	-0.26	0.09	0.013
3790529	*GRP*	NM_002091 // GRP // gastrin-releasing peptide // 18q21.1-q21.32 // 2922 /// NM_0	NM_002091	-0.26	0.05	0.001
3987029	*TMEM164*	NM_032227 // TMEM164 // transmembrane protein 164 // Xq22.3 // 84187 /// ENST000	NM_032227	-0.26	0.10	0.018
3526454	*GRTP1*	NM_024719 // GRTP1 // growth hormone regulated TBC protein 1 // 13q34 // 79774 /	NM_024719	-0.26	0.09	0.015
2438344	*GPATCH4*	NM_182679 // GPATCH4 // G patch domain containing 4 // 1q22 // 54865 /// NM_0155	NM_182679	-0.26	0.07	0.006
3132927	*NKX6-3*	NM_152568 // NKX6-3 // NK6 homeobox 3 // 8p11.21 // 157848 /// ENST00000343444 /	NM_152568	-0.27	0.09	0.014
2672376	*TESSP2*	NM_182702 // TESSP2 // testis serine protease 2 // 3p21.31 // 339906 /// ENST000	NM_182702	-0.27	0.09	0.013

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
2730347	*C4orf35*	NM_033122 // C4orf35 // chromosome 4 open reading frame 35 // 4q13.3 // 85438 //	NM_033122	-0.27	0.10	0.019
3921068	*ETS2*	NM_005239 // ETS2 // v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	NM_005239	-0.27	0.03	0.000
2532894	*DGKD*	NM_152879 // DGKD // diacylglycerol kinase, delta 130kDa // 2q37.1 // 8527 /// N	NM_152879	-0.27	0.07	0.003
4018454	*AMOT*	NM_133265 // AMOT // angiomotin // Xq23 // 154796 /// NM_001113490 // AMOT // an	NM_133265	-0.27	0.09	0.012
3070507	*RNF148*	NM_198085 // RNF148 // ring finger protein 148 // 7q31.33 // 378925 /// BC029264	NM_198085	-0.27	0.10	0.017
3832256	*SPINT2*	NM_021102 // SPINT2 // serine peptidase inhibitor, Kunitz type, 2 // 19q13.1 //	NM_021102	-0.27	0.10	0.017
3371225	*CHST1*	NM_003654 // CHST1 // carbohydrate (keratan sulfate Gal-6) sulfotransferase 1 //	NM_003654	-0.27	0.07	0.005
3870494	*TFPT*	NM_013342 // TFPT // TCF3 (E2A) fusion partner (in childhood Leukemia) // 19q13	NM_013342	-0.27	0.09	0.010
3863811	*PSG9*	NM_002784 // PSG9 // pregnancy specific beta-1-glycoprotein 9 // 19q13.2 // 5678	NM_002784	-0.28	0.09	0.011

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3160175	*VLDLR*	NM_003383 // VLDLR // very low density lipoprotein receptor // 9p24 // 7436 ///	NM_003383	-0.28	0.08	0.007
2794704	*ASB5*	NM_080874 // ASB5 // ankyrin repeat and SOCS box-containing 5 // 4q34.2 // 14045	NM_080874	-0.28	0.11	0.019
3908901	*KCNB1*	NM_004975 // KCNB1 // potassium voltage-gated channel, Shab-related subfamily, m	NM_004975	-0.28	0.09	0.009
3390852	*FLJ45803*	NM_207429 // FLJ45803 // FLJ45803 protein // 11q23.1 // 399948 /// ENST000003554	NM_207429	-0.28	0.10	0.015
2600689	*EPHA4*	NM_004438 // EPHA4 // EPH receptor A4 // 2q36.1 // 2043 /// ENST00000281821 // E	NM_004438	-0.29	0.07	0.003
3469597	*NUAK1*	NM_014840 // NUAK1 // NUAK family, SNF1-like kinase, 1 // 12q23.3 // 9891 /// EN	NM_014840	-0.29	0.09	0.009
3607232	*ISG20L1*	NM_022767 // ISG20L1 // interferon stimulated exonuclease gene 20kDa-like 1 // 1	NM_022767	-0.29	0.10	0.015
2358426	*ADAMTSL4*	AK023606 // ADAMTSL4 // ADAMTS-like 4 // 1q21.2 // 54507	AK023606	-0.29	0.11	0.016
3853609	*CYP4F2*	NM_001082 // CYP4F2 // cytochrome P450, family 4, subfamily F, polypeptide 2 //	NM_001082	-0.29	0.11	0.016

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
2936971	*KIF25*	NM_030615 // KIF25 // kinesin family member 25 // 6q27 // 3834 /// NM_005355 //	NM_030615	-0.30	0.09	0.008
2997272	*EEPD1*	NM_030636 // EEPD1 // endonuclease/exonuclease/phosphatase family domain contain	NM_030636	-0.30	0.09	0.010
3961253	*RPS19BP1*	NM_194326 // RPS19BP1 // ribosomal protein S19 binding protein 1 // 22q13.1 // 9	NM_194326	-0.30	0.10	0.013
3082373	*VIPR2*	NM_003382 // VIPR2 // vasoactive intestinal peptide receptor 2 // 7q36.3 // 7434	NM_003382	-0.30	0.10	0.011
2340961	*IL12RB2*	NM_001559 // IL12RB2 // interleukin 12 receptor, beta 2 // 1p31.3-p31.2 // 3595	NM_001559	-0.30	0.08	0.005
2736462	*BMPR1B*	NM_001203 // BMPR1B // bone morphogenetic protein receptor, type IB // 4q22-q24	NM_001203	-0.30	0.08	0.004
3774504		---	---	-0.30	0.11	0.016
3395958	*OR8B4*	NM_001005196 // OR8B4 // olfactory receptor, family 8, subfamily B, member 4 //	NM_001005196	-0.30	0.11	0.018
2806231	*BXDC2*	NM_018321 // BXDC2 // brix domain containing 2 // 5p13.2 // 55299 /// ENST000003	NM_018321	-0.31	0.10	0.013
2396858	*NPPB*	NM_002521 // NPPB // natriuretic peptide precursor B // 1p36.2 // 4879 /// ENST0	NM_002521	-0.31	0.11	0.016

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3233322	*C10orf18*	NM_017782 // C10orf18 // chromosome 10 open reading frame 18 // 10p15.1 // 54906	NM_017782	-0.31	0.06	0.001
2439101	*FCRL1*	NM_052938 // FCRL1 // Fc receptor-like 1 // 1q21-q22 // 115350 /// ENST000003681	NM_052938	-0.31	0.06	0.001
2413907	*DHCR24*	NM_014762 // DHCR24 // 24-dehydrocholesterol reductase // 1p33-p31.1 // 1718 ///	NM_014762	-0.31	0.11	0.014
3231186	*C9orf37*	NM_032937 // C9orf37 // chromosome 9 open reading frame 37 // 9q34.3 // 85026 //	NM_032937	-0.31	0.09	0.008
2669955	*XIRP1*	NM_194293 // XIRP1 // xin actin-binding repeat containing 1 // 3p22.2 // 165904	NM_194293	-0.32	0.11	0.013
3345222	*AMOTL1*	NM_130847 // AMOTL1 // angiomotin like 1 // 11q14.3 // 154810 /// ENST0000031782	NM_130847	-0.32	0.11	0.012
2573326	*FLJ14816*	BC112205 // FLJ14816 // hypothetical protein FLJ14816 // 2q14.2 // 84931 /// BC1	BC112205	-0.32	0.11	0.016
3349437	*UNQ2550*	AY358815 // UNQ2550 // SFVP2550 // 11q23.1 // 100130653	AY358815	-0.32	0.09	0.005
3951117	*ACR*	NM_001097 // ACR // acrosin // 22q13-qter|22q13.33 // 49 /// ENST00000216139 //	NM_001097	-0.32	0.12	0.017
2489140		---	---	-0.32	0.07	0.002

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
2562115	*LSM3*	CR457185 // LSM3 // LSM3 homolog, U6 small nuclear RNA associated (S. cerevisiae	CR457185	-0.32	0.11	0.011
3572975	*NGB*	NM_021257 // NGB // neuroglobin // 14q24.3 // 58157 /// ENST00000298352 // NGB /	NM_021257	-0.33	0.09	0.004
2439350	*OR6N1*	NM_001005185 // OR6N1 // olfactory receptor, family 6, subfamily N, member 1 //	NM_001005185	-0.33	0.10	0.009
3590275	*CHAC1*	NM_024111 // CHAC1 // ChaC, cation transport regulator homolog 1 (E. coli) // 15	NM_024111	-0.33	0.12	0.014
2397898	*HSPB7*	NM_014424 // HSPB7 // heat shock 27kDa protein family, member 7 (cardiovascular)	NM_014424	-0.33	0.12	0.015
2364677	*PBX1*	NM_002585 // PBX1 // pre-B-cell leukemia homeobox 1 // 1q23 // 5087 /// ENST0000	NM_002585	-0.34	0.07	0.001
2474409	*DNAJC5G*	NM_173650 // DNAJC5G // DnaJ (Hsp40) homolog, subfamily C, member 5 gamma // 2p2	NM_173650	-0.34	0.09	0.004
3581373		---	---	-0.34	0.12	0.014
3508330	*HSPH1*	NM_006644 // HSPH1 // heat shock 105kDa/110kDa protein 1 // 13q12.3 // 10808 ///	NM_006644	-0.34	0.13	0.019
3751164	*DHRS13*	NM_144683 // DHRS13 // dehydrogenase/reductase (SDR family) member 13 // 17q11.2	NM_144683	-0.35	0.10	0.006

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	*P*
2908179	*VEGFA*	NM_001025366 // VEGFA // vascular endothelial growth factor A // 6p12 // 7422 //	NM_001025366	-0.35	0.13	0.016
3962448	*dJ222E13.2*	NR_002184 // dJ222E13.2 // similar to CGI-96 // 22q13.2 // 91695 /// BC073834 //	NR_002184	-0.35	0.12	0.014
3747638	*LOC201164*	BC031263 // LOC201164 // similar to CG12314 gene product // 17p11.2 // 201164 //	BC031263	-0.35	0.09	0.004
2821981	*TMEM157*	NM_198507 // TMEM157 // transmembrane protein 157 // 5q21.1 // 345757 /// ENST00	NM_198507	-0.35	0.12	0.015
3123675	*PPP1R3B*	NM_024607 // PPP1R3B // protein phosphatase 1, regulatory (inhibitor) subunit 3B	NM_024607	-0.35	0.12	0.014
2656837	*ST6GAL1*	NM_173216 // ST6GAL1 // ST6 beta-galactosamide alpha-2,6-sialyltranferase 1 // 3	NM_173216	-0.35	0.13	0.016
3746574	*PMP22*	NM_000304 // PMP22 // peripheral myelin protein 22 // 17p12-p11.2 // 5376 /// NM	NM_000304	-0.36	0.09	0.004
2771342	*EPHA5*	NM_004439 // EPHA5 // EPH receptor A5 // 4q13.1 // 2044 /// NM_182472 // EPHA5 /	NM_004439	-0.36	0.09	0.003
2888674	*MXD3*	NM_031300 // MXD3 // MAX dimerization protein 3 // 5q35.3 // 83463 /// ENST00000	NM_031300	-0.36	0.12	0.012

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
2353477	*ATP1A1*	NM_000701 // ATP1A1 // ATPase, Na+/K+ transporting, alpha 1 polypeptide // 1p21	NM_000701	-0.36	0.11	0.007
3956984	*ZMAT5*	NM_019103 // ZMAT5 // zinc finger, matrin type 5 // 22cen-q12.3 // 55954 /// NM_	NM_019103	-0.36	0.11	0.009
2551651	*ATP6V1E2*	NM_080653 // ATP6V1E2 // ATPase, H+ transporting, lysosomal 31kDa, V1 subunit E2	NM_080653	-0.37	0.13	0.017
3578069	*C14orf139*	BC008299 // C14orf139 // chromosome 14 open reading frame 139 // 14q32.13 // 796	BC008299	-0.37	0.13	0.016
2428501	*SLC16A1*	NM_003051 // SLC16A1 // solute carrier family 16, member 1 (monocarboxylic acid	NM_003051	-0.37	0.14	0.018
3061621	*TFPI2*	NM_006528 // TFPI2 // tissue factor pathway inhibitor 2 // 7q22 // 7980 /// ENST	NM_006528	-0.37	0.09	0.002
3705516	*LOC100131454*	AF229804 // LOC100131454 // similar to hCG1646635 // 17p13.3 // 100131454 /// EN	AF229804	-0.38	0.11	0.008
3306299	*XPNPEP1*	NM_020383 // XPNPEP1 // X-prolyl aminopeptidase (aminopeptidase P) 1, soluble //	NM_020383	-0.38	0.14	0.018
2763550	*PPARGC1A*	NM_013261 // PPARGC1A // peroxisome proliferator-activated receptor gamma, coact	NM_013261	-0.38	0.13	0.012

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
2769063	*USP46*	NM_022832 // USP46 // ubiquitin specific peptidase 46 // 4q12 // 64854 /// ENST0	NM_022832	-0.38	0.13	0.013
3806459	*ST8SIA5*	NM_013305 // ST8SIA5 // ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransfera	NM_013305	-0.38	0.10	0.004
3190151	*SLC25A25*	NM_001006641 // SLC25A25 // solute carrier family 25 (mitochondrial carrier; pho	NM_001006641	-0.39	0.09	0.003
2489172	*MTHFD2*	NM_001040409 // MTHFD2 // methylenetetrahydrofolate dehydrogenase (NADP+ depende	NM_001040409	-0.39	0.05	0.000
2952065	*PPIL1*	NM_016059 // PPIL1 // peptidylprolyl isomerase (cyclophilin)-like 1 // 6p21.1 //	NM_016059	-0.39	0.10	0.005
3382015	*CHRDL2*	NM_015424 // CHRDL2 // chordin-like 2 // 11q14 // 25884 /// ENST00000263671 // C	NM_015424	-0.39	0.10	0.003
2711139	*ATP13A5*	NM_198505 // ATP13A5 // ATPase type 13A5 // 3q29 // 344905 /// ENST00000342358 /	NM_198505	-0.40	0.11	0.005
2633917	*RG9MTD1*	NM_017819 // RG9MTD1 // RNA (guanine-9-) methyltransferase domain containing 1 /	NM_017819	-0.41	0.14	0.013
2974671	*C6orf192*	NM_052831 // C6orf192 // chromosome 6 open reading frame 192 // 6q22.3-q23.3 //	NM_052831	-0.41	0.15	0.018

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
2982270	*FLJ27255*	ENST00000355047 // FLJ27255 // hypothetical LOC401281 // 6q25.3 // 401281 /// AK	ENST00000355047	-0.41	0.12	0.007
2778273	*PGDS*	NM_014485 // PGDS // prostaglandin D2 synthase, hematopoietic // 4q22.3 // 27306	NM_014485	-0.41	0.08	0.001
3005332	*RCP9*	NM_014478 // RCP9 // calcitonin gene-related peptide-receptor component protein	NM_014478	-0.41	0.14	0.013
2650393	*PPM1L*	NM_139245 // PPM1L // protein phosphatase 1 (formerly 2C)-like // 3q26.1 // 1517	NM_139245	-0.42	0.12	0.006
3463056	*CSRP2*	NM_001321 // CSRP2 // cysteine and glycine-rich protein 2 // 12q21.1 // 1466 ///	NM_001321	-0.42	0.11	0.005
2459405		---	---	-0.43	0.10	0.003
2570238	*NPHP1*	NM_000272 // NPHP1 // nephronophthisis 1 (juvenile) // 2q13 // 4867 /// NM_20718	NM_000272	-0.43	0.06	0.000
2840616	*NPM1*	NM_002520 // NPM1 // nucleophosmin (nucleolar phosphoprotein B23, numatrin) // 5	NM_002520	-0.43	0.14	0.010
3601051	*NEO1*	NM_002499 // NEO1 // neogenin homolog 1 (chicken) // 15q22.3-q23 // 4756 /// ENS	NM_002499	-0.43	0.09	0.002
3936515	*TUBA8*	NM_018943 // TUBA8 // tubulin, alpha 8 // 22q11.1 // 51807 /// ENST00000330423 /	NM_018943	-0.43	0.10	0.002

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
2725013	*UCHL1*	NM_004181 // UCHL1 // ubiquitin carboxyl-terminal esterase L1 (ubiquitin thioles	NM_004181	-0.44	0.11	0.004
2380590	*TGFB2*	NM_003238 // TGFB2 // transforming growth factor, beta 2 // 1q41 // 7042 /// ENS	NM_003238	-0.44	0.16	0.017
2496382	*NPAS2*	NM_002518 // NPAS2 // neuronal PAS domain protein 2 // 2q11.2 // 4862 /// ENST00	NM_002518	-0.46	0.10	0.002
3841574	*LILRB1*	NM_006669 // LILRB1 // leukocyte immunoglobulin-like receptor, subfamily B (with	NM_006669	-0.46	0.16	0.015
3726960	*NME2*	NM_001018137 // NME2 // non-metastatic cells 2, protein (NM23B) expressed in //	NM_001018137	-0.47	0.16	0.013
2649367	*PTX3*	NM_002852 // PTX3 // pentraxin-related gene, rapidly induced by IL-1 beta // 3q2	NM_002852	-0.47	0.11	0.002
2909483	*GPR111*	NM_153839 // GPR111 // G protein-coupled receptor 111 // 6p12.3 // 222611 /// EN	NM_153839	-0.47	0.13	0.006
2881950	*SLC36A2*	NM_181776 // SLC36A2 // solute carrier family 36 (proton/amino acid symporter),	NM_181776	-0.48	0.12	0.004
3441190	*FGF6*	NM_020996 // FGF6 // fibroblast growth factor 6 // 12p13 // 2251 /// ENST0000022	NM_020996	-0.48	0.12	0.004

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3028911	*C7orf34*	NM_178829 // C7orf34 // chromosome 7 open reading frame 34 // 7q34 // 135927 ///	NM_178829	-0.49	0.18	0.019
2830861	*EGR1*	NM_001964 // EGR1 // early growth response 1 // 5q31.1 // 1958 /// ENST000002399	NM_001964	-0.49	0.19	0.020
3323891	*GAS2*	NM_177553 // GAS2 // growth arrest-specific 2 // 11p14.3-p15.2 // 2620 /// NM_00	NM_177553	-0.49	0.16	0.011
2497252	*SLC9A2*	NM_003048 // SLC9A2 // solute carrier family 9 (sodium/hydrogen exchanger), memb	NM_003048	-0.50	0.11	0.002
3018484	*GPR22*	NM_005295 // GPR22 // G protein-coupled receptor 22 // 7q22-q31.1 // 2845 /// EN	NM_005295	-0.51	0.15	0.008
2712632	*TFRC*	NM_003234 // TFRC // transferrin receptor (p90, CD71) // 3q29 // 7037 /// ENST00	NM_003234	-0.51	0.12	0.003
3214451	*NFIL3*	NM_005384 // NFIL3 // nuclear factor, interleukin 3 regulated // 9q22 // 4783 //	NM_005384	-0.53	0.14	0.004
2435981	*S100A12*	NM_005621 // S100A12 // S100 calcium binding protein A12 // 1q21 // 6283 /// ENS	NM_005621	-0.54	0.19	0.014
3320675	*RIG*	U32331 // RIG // regulated in glioma // 11p15.1 // 10530	U32331	-0.54	0.10	0.001
3290746	*SLC16A9*	NM_194298 // SLC16A9 // solute carrier family 16, member 9 (monocarboxylic acid	NM_194298	-0.54	0.15	0.006

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3055703	*NSUN5C*	NM_032158 // NSUN5C // NOL1/NOP2/Sun domain family, member 5C // 7q11.23 // 2602	NM_032158	-0.57	0.17	0.008
3265494	*TRUB1*	NM_139169 // TRUB1 // TruB pseudouridine (psi) synthase homolog 1 (E. coli) // 1	NM_139169	-0.57	0.17	0.008
3374213	*OR1S2*	NM_001004459 // OR1S2 // olfactory receptor, family 1, subfamily S, member 2 //	NM_001004459	-0.58	0.20	0.013
3318253	*OR51L1*	NM_001004755 // OR51L1 // olfactory receptor, family 51, subfamily L, member 1 /	NM_001004755	-0.59	0.18	0.009
3294280	*DNAJC9*	NM_015190 // DNAJC9 // DnaJ (Hsp40) homolog, subfamily C, member 9 // 10q22.2 //	NM_015190	-0.59	0.22	0.018
2899095	*HIST1H4A*	NM_003538 // HIST1H4A // histone cluster 1, H4a // 6p21.3 // 8359 /// ENST000003	NM_003538	-0.60	0.16	0.005
2378068	*G0S2*	NM_015714 // G0S2 // G0/G1switch 2 // 1q32.2-q41 // 50486 /// ENST00000367029 //	NM_015714	-0.63	0.22	0.016
3737677	*LOC100129503*	AF218021 // LOC100129503 // hypothetical protein LOC100129503 // 17q25.3 // 1001	AF218021	-0.64	0.19	0.007
3300115	*PPP1R3C*	NM_005398 // PPP1R3C // protein phosphatase 1, regulatory (inhibitor) subunit 3C	NM_005398	-0.69	0.26	0.020

Affymetrix ID	mRNA	Gene Assignment	Accession No.	Change (Fasting-Fed)	SEM	P
3279058	*ACBD7*	NM_001039844 // ACBD7 // acyl-Coenzyme A binding domain containing 7 // 10p13 //	NM_001039844	-0.69	0.13	0.001
4031156	*RPS4Y2*	NM_001039567 // RPS4Y2 // ribosomal protein S4, Y-linked 2 // Yq11.223 // 140032	NM_001039567	-0.71	0.17	0.003
2979246	*RAET1L*	NM_130900 // RAET1L // retinoic acid early transcript 1L // 6q25.1 // 154064 ///	NM_130900	-0.75	0.26	0.013
3321150	*ARNTL*	NM_001178 // ARNTL // aryl hydrocarbon receptor nuclear translocator-like // 11p	NM_001178	-0.80	0.20	0.004
3862873	*CYP2A6*	NM_000762 // CYP2A6 // cytochrome P450, family 2, subfamily A, polypeptide 6 //	NM_000762	-1.12	0.34	0.009

[00307]

4. IDENTIFICATION OF URSOLIC ACID AS AN INHIBITOR OF FASTING-INDUCED MUSCLE ATROPHY.

[00308] The Connectivity Map describes the effects of > 1300 bioactive small molecules on global mRNA expression in several cultured cell lines, and contains search algorithms that permit comparisons between compound-specific mRNA expression signatures and mRNA expression signatures of interest (Lamb J, *et al.* (2006) *Science (New York, N.Y* 313(5795):1929-1935). It was hypothesized herein that querying the Connectivity Map with the mRNA expression signature of fasting (atrophy signature-1) would identify inhibitors of atrophy-associated gene expression and thus, potential inhibitors of muscle atrophy. It was also reasoned herein that increasing the specificity of the query would enhance the output. To this end, as described herein, an evolutionarily conserved mRNA expression signature of fasting was discovered by comparing the effect of fasting on human skeletal muscle to the effect of a 24 h fast on mouse skeletal muscle. The mouse studies were described previously

(Ebert SM, *et al.* (2010) *Molecular endocrinology* 24(4):790-799). Altogether, 35 mRNAs that were increased by fasting and 40 mRNAs that were decreased by fasting were identified in both human and mouse skeletal muscle (Table 2; the data in column labeled "Change" show mean changes in $\log_2$ hybridization signals between fasting and fed states for the species indicated, [Mean $\log_2$ mRNA levels for fasted] minus [Mean $\log_2$ mRNA levels in unfasted]; *P*-values were determined with paired t-tests). The data shown in Table 2 includes all mRNAs whose levels were increased by fasting in human muscle ($P \leq 0.02$) and in mouse muscle ($P \leq 0.05$), and all mRNAs whose levels were decreased by fasting in human muscle ($P \leq 0.02$) and in mouse muscle ($P \leq 0.05$). Of the mRNAs shown in Table 2, 63 mRNAs were represented on the HG-U133A arrays used in the Connectivity Map (Figure 4A). These mRNAs (31 increased by fasting and 32 decreased by fasting) were used to query the Connectivity Map for candidate small molecule inhibitors of muscle atrophy.

TABLE 2. FASTING-REGULATED MRNAS COMMON TO HUMAN AND MOUSE SKELETAL MUSCLE.

mRNA	Protein	Human		Mouse	
		Mean Log2 Change		Mean Log2 Change	
		(Fasting - Fed)	*P*	(Fasting - Fed)	*P*
PDK4	pyruvate dehydrogenase kinase, isozyme 4	2.15	0.000	1.91	0.000
TXNIP	thioredoxin interacting protein	0.85	0.004	0.60	0.038
FBXO32	F-box protein 32	0.82	0.002	2.13	0.000
SLC38A2	solute carrier family 38, member 2	0.62	0.001	0.33	0.036
UCP3	uncoupling protein 3 (mitochondrial, proton carrier)	0.59	0.000	1.02	0.001
ZFAND5	zinc finger, AN1-type domain 5	0.51	0.005	0.57	0.001
HMOX1	heme oxygenase (decycling) 1	0.46	0.006	0.17	0.035
SESN1	sestrin 1	0.46	0.004	1.51	0.001
GABARAPL1	GABA(A) receptor-associated protein like 1	0.39	0.004	1.18	0.000
CAT	catalase	0.39	0.003	0.85	0.001

mRNA	Protein	Human		Mouse	
		Mean Log2 Change		Mean Log2 Change	
		(Fasting - Fed)	*P*	(Fasting - Fed)	*P*
CITED2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain	0.37	0.005	0.29	0.010
ABCA1	ATP-binding cassette, sub-family A (ABC1), member 1	0.37	0.016	0.26	0.018
FBXL20	F-box and leucine-rich repeat protein 20	0.35	0.002	0.46	0.001
XPO4	exportin 4	0.31	0.009	0.22	0.022
HERPUD 1	homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain 1	0.29	0.003	0.27	0.029
ACOX1	acyl-Coenzyme A oxidase 1, palmitoyl	0.29	0.013	0.53	0.006
NOX4	NADPH oxidase 4	0.28	0.002	0.41	0.018
UBE4A	ubiquitination factor E4A (UFD2 homolog, yeast)	0.27	0.004	1.08	0.010
INSR	insulin receptor	0.24	0.014	0.58	0.003
IGF1R	insulin-like growth factor 1 receptor	0.23	0.013	0.40	0.001
PANK1	pantothenate kinase 1	0.21	0.007	0.78	0.000
NBR1	neighbor of BRCA1 gene 1	0.21	0.017	0.39	0.009
RORA	RAR-related orphan receptor A	0.21	0.006	0.39	0.006
TMEM71	transmembrane protein 71	0.21	0.009	0.40	0.008
CPT1A	carnitine palmitoyltransferase 1A (liver)	0.21	0.001	0.21	0.020
UCP2	uncoupling protein 2 (mitochondrial, proton carrier)	0.20	0.005	0.33	0.024
TULP3	tubby like protein 3	0.19	0.008	0.22	0.008
MED13L	mediator complex subunit 13-like	0.18	0.000	0.23	0.011
CALCOCO1	calcium binding and coiled coil domain 1	0.16	0.010	0.31	0.028
MYO5A	myosin VA (heavy chain 12, myoxin)	0.14	0.006	0.36	0.012
PPAP2B	phosphatidic acid phosphatase type 2B	0.13	0.007	0.09	0.029
SRRM2	serine/arginine repetitive matrix 2	0.13	0.007	0.24	0.040

mRNA	Protein	Human		Mouse	
		Mean Log2 Change (Fasting - Fed)	*P*	Mean Log2 Change (Fasting - Fed)	*P*
ADPGK	ADP-dependent glucokinase	0.13	0.007	0.16	0.009
SUPT6H	suppressor of Ty 6 homolog (S. cerevisiae)	0.11	0.005	0.26	0.036
SFRS8	splicing factor, arginine/serine-rich 8	0.08	0.016	0.13	0.011
NFYA	nuclear transcription factor Y, alpha	-0.07	0.011	-0.31	0.045
MRPS15	mitochondrial ribosomal protein S15	-0.11	0.003	-0.25	0.001
PDE7B	phosphodiesterase 7B	-0.12	0.013	-0.51	0.011
WDR1	WD repeat domain 1	-0.14	0.019	-0.21	0.047
ACACA	acetyl-Coenzyme A carboxylase alpha	-0.15	0.010	-0.22	0.041
AXIN2	axin 2 (conductin, axil)	-0.15	0.013	-0.12	0.046
CASQ1	calsequestrin 1 (fast-twitch, skeletal muscle)	-0.16	0.015	-0.26	0.015
ZNF280B	zinc finger protein 280B	-0.16	0.005	-0.34	0.046
JTB	jumping translocation breakpoint	-0.16	0.014	-0.42	0.030
CACNB1	calcium channel, voltage-dependent, beta 1 subunit	-0.17	0.013	-0.43	0.003
ALG2	asparagine-linked glycosylation 2 homolog	-0.17	0.011	-0.39	0.019
TSPAN13	tetraspanin 13	-0.18	0.006	-0.30	0.028
P4HA2	procollagen-proline, 2-oxoglutarate 4-dioxygenase, alpha II polypeptide	-0.18	0.007	-0.12	0.012
TTLL1	tubulin tyrosine ligase-like family, member 1	-0.18	0.001	-0.29	0.043
SUV39H2	suppressor of variegation 3-9 homolog 2 (Drosophila)	-0.20	0.011	-0.26	0.014
SLC4A4	solute carrier family 4, sodium bicarbonate cotransporter, member 4	-0.20	0.007	-0.69	0.003
DNMT3A	DNA (cytosine-5-)-methyltransferase 3 alpha	-0.20	0.007	-0.48	0.000
FEZ2	fasciculation and elongation protein zeta 2 (zygin II)	-0.21	0.000	-0.50	0.019
MTSS1	metastasis suppressor 1	-0.21	0.009	-0.22	0.033
TMTC4	transmembrane and tetratricopeptide repeat containing 4	-0.22	0.010	-0.17	0.035

mRNA	Protein	Human		Mouse	
		Mean Log2 Change		Mean Log2 Change	
		(Fasting - Fed)	*P*	(Fasting - Fed)	*P*
PPM1J	protein phosphatase 1J (PP2C domain containing)	-0.23	0.003	-0.30	0.012
ARHGAP 20	Rho GTPase activating protein 20	-0.23	0.003	-0.22	0.013
ABTB2	ankyrin repeat and BTB (POZ) domain containing 2	-0.25	0.010	-0.18	0.005
CNNM4	cyclin M4	-0.26	0.016	-0.27	0.005
GRTP1	growth hormone regulated TBC protein 1	-0.26	0.015	-0.54	0.002
RNF148	ring finger protein 148	-0.27	0.017	-0.35	0.014
SPINT2	serine peptidase inhibitor, Kunitz type, 2	-0.27	0.017	-0.23	0.026
PBX1	pre-B-cell leukemia homeobox 1	-0.34	0.001	-0.22	0.000
HSPH1	heat shock 105kDa/110kDa protein 1	-0.34	0.019	-0.20	0.043
VEGFA	vascular endothelial growth factor A	-0.35	0.016	-0.26	0.002
PMP22	peripheral myelin protein 22	-0.36	0.004	-0.13	0.012
PPARGC 1A	peroxisome proliferative activated receptor, gamma, coactivator 1 alpha	-0.38	0.012	-0.39	0.030
ST8SIA5	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 5	-0.38	0.004	-0.48	0.011
PPIL1	peptidylprolyl isomerase (cyclophilin)-like 1	-0.39	0.005	-0.52	0.016
PPM1L	protein phosphatase 1 (formerly 2C)-like	-0.42	0.006	-0.46	0.000
NEO1	neogenin homolog 1 (chicken)	-0.43	0.002	-0.31	0.037
TGFB2	transforming growth factor, beta 2	-0.44	0.017	-0.30	0.003
PTX3	pentraxin-related gene, rapidly induced by IL-1 beta	-0.47	0.002	-0.48	0.000
GAS2	growth arrest-specific 2	-0.49	0.011	-0.23	0.044
TFRC	transferrin receptor (p90, CD71)	-0.51	0.003	-1.37	0.011

[00309]

[00310] The left side of Figure 4B shows the 10 Connectivity Map instances (or data sets) with the most significant positive correlations ($P < 0.004$) to the effect of fasting in

skeletal muscle. The connectivity score, represented on the y-axis, is a measure of the strength of the correlation (Lamb J, *et al.* (2006) *Science (New York, N.Y* 313(5795):1929-1935); the compound and cell-line is shown below the bar representing the Connectivity Score. Of these, 6 involved wortmannin or LY-294002 (inhibitors of phosphoinositide 3-kinase (PI3K)) or rapamycin (an inhibitor of the mammalian target of rapamycin complex 1 (mTORC1)). Since PI3K and mTORC1 mediate effects of insulin and IGF-I, and since insulin/IGF-I signaling inhibits muscle atrophy and atrophy-associated changes in skeletal muscle mRNA expression (Bodine SC, *et al.* (2001) *Nat Cell Biol* 3(11):1014-1019; Sandri M, *et al.* (2004) *Cell* 117(3):399-412), these results lent confidence that the Connectivity Map might be used to identify potential inhibitors of muscle atrophy. The right side of Figure 4B shows the 10 Connectivity Map instances with the most significant negative correlations ($P < 0.004$) to the effect of fasting in skeletal muscle. These compounds, whose effects on cultured cell lines were opposite to the effect of fasting on muscle, included metformin (an insulin-sensitizing agent widely used to treat type 2 diabetes), as well as ursolic acid. Further experiments focused on metformin and ursolic acid. To test the hypothesis that metformin and ursolic acid might reduce fasting-induced muscle atrophy, each compound was administered, or vehicle alone, via i.p. injection to C57BL/6 mice. The mice were then fasted, and after 12 hours of fasting, the mice received a second dose of the compound or vehicle. After 24 hours of fasting, the blood glucose was measured and muscles were harvested. The data shown in Figures 4C-4H are means ± SEM from 16 mice. Both metformin (250 mg / kg) and ursolic acid (200 mg / kg) significantly reduced fasting blood glucose (Figures 4C and 4D). The effects of metformin and ursolic acid on fasting-induced muscle atrophy were also examined, i.e. the effect of 24 h fast (relative to ad lib feeding) on wet weight of lower hindlimb skeletal muscle (bilateral tibialis anterior ("TA" muscle), gastrocnemius, and soleus; see Figures 4E-4G). In the absence of metformin and ursolic acid, fasting reduced muscle weight by 9 % (Figure 4E). Although metformin did not alter muscle weight in fasted mice (Figure 4F), ursolic acid increased it by 7 ± 2 % (Figure 4G). Moreover, consistent with the predicted inhibitory effect on fasting-induced gene expression described herein, ursolic acid reduced fasting levels of *atrogin-1* and *MuRF1* mRNA levels in the TA muscles of fasted mice (Figure 4H; the data shown are normalized to the levels in vehicle-treated mice, which were set at 1). In Figures 4E-4H, each data point represents one mouse and the horizontal bars denote the means. In Figures 4C-4H, *P*-values were determined using unpaired t-tests. Thus, ursolic acid, but not metformin, decreased fasting-

induced muscle atrophy.

5. URSOLIC ACID REDUCES DENERVATION-INDUCED MUSCLE ATROPHY.

[00311] The Connectivity Map was queried with a second mRNA expression signature, atrophy signature-2 (described above), to determine if this muscle atrophy signature would also correlate with ursolic acid, among other compounds. As described above, atrophy signature-2 was an mRNA expression signature identified as described herein for human skeletal muscle mRNAs that were induced or repressed by fasting and also by spinal cord injury ("SCI"). The studies of the effects of SCI on human skeletal muscle gene expression were described previously (Adams CM, *et al.* (2011) *Muscle Nerve.* 43(1):65-75). Using this approach with the muscle atrophy expression signatures described herein, there were 18 human mRNAs that were increased by fasting and SCI, and 17 human mRNAs that were decreased by fasting and SCI, and are shown in Table 3 ("Change" represents mean changes in $\log_2$ hybridization signals for pairs as indicated, e.g. fasting and fed states for column labeled "(Fasting - Fed)" or untrained and trained for the column labeled "(Untrained - Trained)"). The data in Table 3 include all mRNAs whose levels were increased by fasting ($P \leq 0.02$) and by SCI ($P \leq 0.05$), and all mRNAs whose levels were decreased by fasting ($P \leq 0.02$) and by SCI ($P \leq 0.05$). *P*-values in Table 3 were determined with paired t-tests.

TABLE 3. HUMAN SKELETAL MUSCLE MRNAS INDUCED OR REPRESSED BY FASTING AND SCI.

mRNA	Protein	EFFECT OF FASTING		EFFECT OF SCI	
		Change (Fasting – Fed)	*P*	Change (Untrained – Trained)	*P*
OR1D4	olfactory receptor, family 1, subfamily D, member 4	0.50	0.019	0.65	0.030
RHOBTB 1	Rho-related BTB domain containing 1	0.48	0.001	0.71	0.032
TSPAN8	tetraspanin 8	0.39	0.015	1.79	0.023
FLJ3399 6	hypothetical protein FLJ33996	0.39	0.019	0.68	0.020
NUPR1	nuclear protein 1	0.35	0.007	0.65	0.030
IRS2	insulin receptor substrate 2	0.34	0.004	0.21	0.035
NPC2	Niemann-Pick disease, type C2	0.30	0.011	0.39	0.042
KLF11	Kruppel-like factor 11	0.29	0.011	0.22	0.034

mRNA	Protein	EFFECT OF FASTING		EFFECT OF SCI	
		Change (Fasting – Fed)	*P*	Change (Untrained – Trained)	*P*
ZNF682	zinc finger protein 682	0.28	0.017	0.72	0.013
NOX4	NADPH oxidase 4	0.28	0.002	0.56	0.007
PLXDC2	plexin domain containing 2	0.26	0.013	0.38	0.022
CTDSP2	CTD small phosphatase 2	0.25	0.003	0.34	0.021
CAV3	caveolin 3	0.24	0.007	0.56	0.020
IGF1R	insulin-like growth factor 1 receptor	0.23	0.013	0.63	0.040
FLJ14154	hypothetical protein FLJ14154	0.22	0.005	0.30	0.021
CUGBP2	CUG triplet repeat, RNA binding protein 2	0.21	0.004	0.14	0.034
MLL	myeloid/lymphoid or mixed-lineage leukemia	0.14	0.016	0.30	0.040
SUPT6H	suppressor of Ty 6 homolog	0.11	0.005	0.19	0.024
MRPS15	mitochondrial ribosomal protein S15	-0.11	0.003	-0.33	0.001
RFXDC2	regulatory factor X domain containing 2	-0.12	0.012	-0.10	0.037
PDE7B	phosphodiesterase 7B	-0.12	0.013	-0.39	0.011
PFDN6	prefoldin subunit 6	-0.14	0.014	-0.42	0.021
ZNF280B	zinc finger protein 280B	-0.16	0.005	-0.30	0.028
TSPAN13	tetraspanin 13	-0.18	0.006	-0.56	0.023
TTLL1	tubulin tyrosine ligase-like family, member 1	-0.18	0.001	-0.37	0.020
CMAS	cytidine monophosphate N-acetylneuraminic acid synthetase	-0.21	0.000	-0.22	0.025
C8orf32	chromosome 8 open reading frame 32	-0.23	0.016	-0.11	0.049
GUCY1B3	guanylate cyclase 1, soluble, beta 3	-0.24	0.007	-0.24	0.008
ZNF32	zinc finger protein 32	-0.24	0.010	-0.21	0.030
VLDLR	very low density lipoprotein receptor	-0.28	0.007	-0.16	0.015
HSPB7	heat shock 27kDa protein family, member 7 (cardiovascular)	-0.33	0.015	-0.77	0.032
VEGFA	vascular endothelial growth factor A	-0.35	0.016	-0.43	0.020

mRNA	Protein	EFFECT OF FASTING		EFFECT OF SCI	
		Change (Fasting – Fed)	*P*	Change (Untrained – Trained)	*P*
SLC16A 1	solute carrier family 16, member 1	-0.37	0.018	-0.94	0.015
PPARGC 1A	peroxisome proliferative activated receptor, gamma, coactivator 1 alpha	-0.38	0.012	-0.74	0.001
C6orf192	chromosome 6 open reading frame 192	-0.41	0.018	-0.39	0.042

[00312]

[00313] Of the mRNAs listed in Table 3, 29 were represented on the HG-U133A arrays used in the Connectivity Map (Figure 5A), but only 10 were common to the 63 mRNAs used in the first Connectivity Map query described above for atrophy signature-1 (*IGF-IR, NOX4, SUPT6H, MRPS15, PDE7B, PGC-1a, TSPAN13, TTLL1, VEGFA* and *ZNF280B*). The mRNAs listed in Figure 5A represent human muscle atrophy signature-2: mRNAs altered by both fasting and SCI in human muscle. These mRNAs, as described above, were used to query the Connectivity Map. Inclusion criteria were: $P \leq 0.02$ in fasted human muscle (by t-test), $P \leq 0.05$ in untrained, paralyzed muscle (by t-test), and the existence of complimentary probes on HG-U133A arrays. Connectivity Map instances with the most significant positive and negative correlations to the effect of fasting and SCI in human muscle. $P < 0.005$ for all compounds are shown in Figure 5B. The results partially overlapped with the results of the first search: both search strategies identified LY-294002, wortmannin and rapamycin as predicted mimics of atrophy-inducing stress, and ursolic acid (but not metformin) as a predicted inhibitor (Figure 5B).

[00314] Because atrophy signature-2 utilized data from SCI subjects, it was hypothesized that ursolic acid might reduce denervation-induced muscle atrophy. To test this, the left hindlimb muscles a denervation-induced skeletal muscle atrophy model in mouse was used. Briefly, on day 0, the left hindlimbs of C57BL/6 mice were denervated by transsecting the left sciatic nerve. This approach allowed the right hindlimb to serve as an intra-subject control. Mice were then administered ursolic acid (200 mg/kg) or an equivalent volume of vehicle alone (corn oil) via i.p. injection twice daily for seven days. During this time, mice continued to have ad libitum access to food. On day 7, muscle tissues were harvested for

analysis, and the left (denervated) and right (innervated) hindlimb muscles in both groups (ursolic acid vs. vehicle administration) were compared. Ursolic acid significantly decreased denervation-induced muscle loss (Figure 5C). In Figure 5C, weights of the left (denervated) lower hindlimb muscles were normalized to weights of the right (innervated) lower hindlimb muscles from the same mouse. Each data point represents one mouse, and horizontal bars denote the means and the *P*-value was determined using an unpaired t-test. Histologically, this effect of ursolic acid was reflected as an increase in the size of denervated skeletal muscle fiber diameter in denervated gastrocnemius (D) and TA (E) muscles (Figures 5D and 5E, respectivel). The data shown in Figures 5D and 5E are from > 2500 muscle fibers per condition; $P < 0.0001$ by unpaired t-test. Thus, ursolic acid reduced denervation-induced muscle atrophy.

6. URSOLIC ACID INDUCES SKELETAL MUSCLE HYPERTROPHY.

[00315] The results from the denervation-induced muscle atrophy model suggested that ursolic acid reduced muscle atrophy, thus the hypothesis that ursolic acid might promote muscle hypertrophy in the absence of an atrophy-inducing stress was reasonable. Mice were provided ad lib access to either standard chow (control diet) or standard chow supplemented with 0.27% ursolic acid (ursolic acid diet) for 5 weeks before grip strength was measured and tissues were harvested. After five weeks, mice administered ursolic had increased lower hindlimb muscle weight (Figure 6A), quadriceps weight (Figure 6B), and upper forelimb muscle (triceps and biceps) weight (Figure 6C). Each data point in Figures 6A-6C represents one mouse, and horizontal bars denote the means. The effect of ursolic acid in this study on skeletal muscle fiber size distribution is shown in Figure 6D. Each distribution represents measurements of > 800 triceps muscle fibers from 7 animals (> 100 measurements / animal); $P < 0.0001$. The effect of ursolic acid on peak grip strength (normalized to body weight) is shown in Figure 6E. Each data point represents one mouse, and horizontal bars denote the means. Non-normalized grip strength data were 157 ± 9 g (control diet) and 181 ± 6 g (ursolic acid diet) ($P = 0.04$).

[00316] Moreover, dietary ursolic acid increased the specific force generated by muscles ex vivo (Figure 7). Briefly, six-week old male C57BL/6 mice were provided either standard diet or diet containing 0.27% ursolic acid for 16 weeks before being euthanized. The lower hindlimb was removed (by transsecting the upper hindlimb mid-way through the

femur), and placed in Krebs solution aerated with 95% O2 and 5% CO2. The gastrocnemius, soleus and tibialis anterior muscles, as well as the distal half of the tibia and fibula were then removed and discarded, leaving the extensor digitorum longus and peroneus muscles with their origins and insertions intact. A suture was placed through the proximal tendon and secured to the distal femur fragment. This ex vivo preparation was then mounted vertically in a water jacket bath (Aurora Scientific 1200A Intact Muscle Test System, filled with aerated Krebs solution) by attaching the suture to a servo-controlled lever (superiorly) and clamping the metatarsals (inferiorly). Passive muscle force was adjusted to a baseline of 1 g, and then muscles were stimulated with supramaximal voltage (80 V) at 100 Hz. The mean time from euthanasia to maximal force measurements was 10 min. After force measurements, muscles were removed and weighed in order to calculate specific titanic force. Maximal tetanic force and muscle weight did not differ between the two groups ($P = 0.20$ and 0.26, respectively). Data are means ± SEM from 5-6 mice per diet. P-values were determined with a t-test. Together, the data in Figures 6 and 7 provide morphological and functional evidence that ursolic acid induced skeletal muscle hypertrophy.

7. URSOLIC ACID INDUCES TROPHIC CHANGES IN SKELETAL MUSCLE GENE EXPRESSION.

[00317] The foregoing results suggested that ursolic acid might alter skeletal muscle gene expression. To test this hypothesis, an unbiased approach was used, specifically exon expression arrays were used to analyze gastrocnemius muscle mRNA expression in mice that had been fed diets lacking or containing ursolic acid for 5 weeks. Mice were provided ad lib access to either standard chow (control diet) or standard chow supplemented with 0.27% ursolic acid (ursolic acid diet) for 5 weeks before gastrocnemius muscle RNA was harvested and analyzed by Affymetrix Mouse Exon 1.0 ST arrays (n = 4 arrays per diet). Each array assessed pooled gastrocnemius RNA from two mice. Stringent criteria were used for ursolic acid-induced effects on mRNA levels ($P < 0.005$), and mRNAs with low levels of expression were disregarded (i.e. only transcripts that were increased to a mean $\log_2$ hybridization signal ≥ 8, or repressed from a mean $\log_2$ hybridization signal ≥ 8 were included). The results were that ursolic acid decreased 18 mRNAs and increased 51 mRNAs (out of > 16,000 mRNAs analyzed. The results are shown in Table 4 ("Change" is the meang $\log_2$ change or difference between mice on ursolic acid diet and control diet, i.e. [Mean $\log_2$ mRNA levels in ursolic acid diet] minus [Mean $\log_2$ mRNA levels in control diet]).

TABLE 4. MOUSE SKELETAL MUSCLE MRNAS INDUCED OR REPRESSED BY URSOLIC ACID.

mRNA	Protein	Change	*P*
Smox	spermine oxidase	0.81	0.001
Lyz2	lysozyme 2	0.71	0.001
C3	complement component 3	0.70	0.000
Tyrobp	TYRO protein tyrosine kinase binding protein	0.69	0.001
Lum	lumican	0.61	0.001
Igf1	insulin-like growth factor 1	0.56	0.005
Fmo1	flavin containing monooxygenase 1	0.47	0.000
Ostn	osteocrin	0.43	0.001
Nampt	nicotinamide phosphoribosyltransferase	0.41	0.003
H19	H19 fetal liver mRNA	0.39	0.004
Hipk2	homeodomain interacting protein kinase 2	0.38	0.002
Fbp2	fructose bisphosphatase 2	0.37	0.003
Gpx1	glutathione peroxidase 1	0.36	0.001
Sepp1	selenoprotein P, plasma, 1	0.35	0.004
Parp3	poly (ADP-ribose) polymerase family, member 3	0.32	0.001
Hspb8	heat shock protein 8	0.32	0.000
Musk	muscle, skeletal, receptor tyrosine kinase	0.31	0.004
Fhl3	four and a half LIM domains 3	0.31	0.005
Hsph1	heat shock 105kDa/110kDa protein 1	0.30	0.001
Arfgap2	ADP-ribosylation factor GTPase activating protein 2	0.30	0.001
Cd24a	CD24a antigen	0.28	0.002
Sepx1	selenoprotein X 1	0.28	0.003
Hk2	hexokinase 2	0.26	0.003
Ggct	gamma-glutamyl cyclotransferase	0.24	0.005
Trip10	thyroid hormone receptor interactor 10	0.23	0.000
Npc1	Niemann Pick type C1	0.22	0.001
Asb5	ankyrin repeat and SOCs box-containing 5	0.21	0.001
Vps29	vacuolar protein sorting 29 (S. pombe)	0.20	0.000
Ahsa2	AHA1, activator of heat shock protein ATPase homolog 2	0.18	0.001
Lsm14a	LSM14 homolog A (SCD6, S. cerevisiae)	0.18	0.004
Pdha1	pyruvate dehydrogenase E1 alpha 1	0.18	0.001
Trappc2l	trafficking protein particle complex 2-like	0.16	0.004
Ube2l3	ubiquitin-conjugating enzyme E2L 3	0.16	0.003
Ctsb	cathepsin B	0.16	0.003
D0H4S114	DNA segment, human D4S114	0.15	0.004
Psma2	proteasome (prosome, macropain) subunit, alpha type 2	0.15	0.005
Mrpl46	mitochondrial ribosomal protein L46	0.15	0.001

mRNA	Protein	Change	*P*
Eef1e1	eukaryotic translation elongation factor 1 epsilon 1	0.15	0.002
Krr1	KRR1, small subunit (SSU) processome component, homolog	0.15	0.005
Ndufaf4	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, assembly factor 4	0.14	0.005
Ndufs2	NADH dehydrogenase (ubiquinone) Fe-S protein 2	0.14	0.002
2610507 B11Rik	RIKEN cDNA 2610507B11 gene	0.14	0.000
Ssr4	signal sequence receptor, delta	0.14	0.000
Ndufs4	NADH dehydrogenase (ubiquinone) Fe-S protein 4	0.14	0.003
Sqstm1	sequestosome 1	0.12	0.001
Gfm1	G elongation factor, mitochondrial 1	0.12	0.003
2310016 M24Rik	RIKEN cDNA 2310016M24 gene	0.12	0.004
Sod2	superoxide dismutase 2, mitochondrial	0.12	0.001
Prdx5	peroxiredoxin 5	0.10	0.005
BC00400 4	cDNA sequence BC004004	0.06	0.001
Ghitm	growth hormone inducible transmembrane protein	0.05	0.005
Foxn3	forkhead box N3	-0.09	0.000
Klhl31	kelch-like 31 (Drosophila)	-0.09	0.001
Acadm	acyl-Coenzyme A dehydrogenase, medium chain	-0.11	0.001
Eif4g3	eukaryotic translation initiation factor 4 gamma, 3	-0.12	0.005
Nrap	nebulin-related anchoring protein	-0.14	0.003
Golga4	golgi autoantigen, golgin subfamily a, 4	-0.14	0.003
Paip2b	poly(A) binding protein interacting protein 2B	-0.16	0.000
Pde4dip	phosphodiesterase 4D interacting protein (myomegalin)	-0.18	0.001
Sfpq	splicing factor proline/glutamine rich	-0.18	0.005
Pnn	pinin	-0.18	0.002
D4Wsu53 e	DNA segment, Chr 4, Wayne State University 53, expressed	-0.18	0.003
Mlec	malectin	-0.19	0.003
Cacna1s	calcium channel, voltage-dependent, L type, alpha 1S	-0.22	0.001
Sfrs5	splicing factor, arginine/serine-rich 5 (SRp40, HRS)	-0.22	0.005
Nnt	nicotinamide nucleotide transhydrogenase	-0.24	0.002
Adprhl1	ADP-ribosylhydrolase like 1	-0.26	0.002
Ddit4l	DNA-damage-inducible transcript 4-like	-0.32	0.000

mRNA	Protein	Change	*P*
Fbxo32	F-box protein 32 (Atrogin-1)	-0.35	0.001

[00318]

[00319] As discussed above, *atrogin-1* and *MuRF1* are transcriptionally up-regulated by atrophy-inducing stresses (see Figure 2B and Sacheck JM, *et al.* (2007) *Faseb J* 21(1):140-155), and they are required for muscle atrophy (Bodine SC, *et al.* (2001) *Science (New York, N.Y* 294(5547):1704-1708). Moreover, in the studies of fasted mice as described herein above, ursolic acid reduced *atrogin-1* and *MuRF1* mRNAs (Figure 4H). Consistent with that finding, the arrays indicated that dietary ursolic acid reduced *atrogin-1* mRNA, which was the most highly repressed mRNA (Figure 8A). The results shown in Figure 8A represent a subset of the mRNAs from Table 4 which had the greatest increase or decrease in expression level in response to ursolic acid. Although *MuRF1* mRNA was not measured by the arrays used in these experiments, qPCR analysis confirmed that dietary ursolic acid repressed both *atrogin-1* and *MuRF1* mRNAs (Figure 8B; data are means ± SEM). Interestingly, one of the most highly up-regulated muscle mRNAs was *IGF1* (Figures 8A and 8B), which encodes insulin-like growth factor-I (IGF-I), a locally generated autocrine/paracrine hormone. *IGF1* mRNA is known to be transcriptionally induced in hypertrophic muscle (Hameed M, *et al.* (2004) *The Journal of physiology* 555(Pt 1):231-240; Adams GR & Haddad F (1996) *J Appl Physiol* 81(6):2509-2516; Gentile MA, *et al.* (2010) *Journal of molecular endocrinology* 44(1):55-73). In addition, increased skeletal muscle *IGF1* expression reduces denervation-induced muscle atrophy (Shavlakadze T, *et al.* (2005) *Neuromuscul Disord* 15(2):139-146), and stimulates muscle hypertrophy (Barton-Davis ER, *et al.* (1998) *Proceedings of the National Academy of Sciences of the United States of America* 95(26):15603-15607; Musarò A, *et al.* (2001) *Nature Genetics* 27(2):195-200). Moreover, by stimulating skeletal muscle insulin/IGF-I signaling, IGF-I represses *atrogin-1* and *MuRF* mRNAs (Sacheck JM, *et al.* (2004) *Am J Physiol Endocrinol Metab* 287(4):E591-601; Frost RA, *et al.* (2009) *J Cell Biochem* 108(5):1192-1202.), as well as *DDIT4L* mRNA (*ibid*), which, after *atrogin-1* mRNA, was the second most highly repressed mRNA in muscle from ursolic acid-treated mice (Figure 8A). Thus, 5 weeks of dietary ursolic acid altered skeletal muscle gene expression in a manner known to reduce atrophy and promote hypertrophy, and muscle-specific *IGF1* induction emerged as a likely contributing mechanism in ursolic acid-induced muscle hypertrophy. The effect of ursolic acid on plasma IGF-I levels was also determined,

which primarily reflect growth hormone-mediated hepatic IGF-I production (Yakar S, *et al.* (1999) *Proceedings of the National Academy of Sciences of the United States of America* 96(13):7324-7329). Although diets containing 0.14% or 0.27% ursolic acid increased muscle mass (described in greater detail below; Figure 10A), neither increased plasma IGF-I (Figure 8C). For the data in Figure 8C, mice were provided ad lib access to either standard chow (control diet) or standard chow supplemented with the indicated concentration of ursolic acid for 7 weeks before plasma IGF-I levels were measured. Each data point represents one mouse, and horizontal bars denote the means. *P*-values were determined by one-way ANOVA with Dunnett's post-test. Of note, exon expression arrays indicated that ursolic acid increased levels of all measured *IGF1* exons (exons 2-6; Figure 9A). The data in Figure 9A are mean exon-specific $\log_2$ hybridization signals from the arrays described in Table 2. However, ursolic acid did not alter levels of mRNAs encoding myostatin (which reduces muscle mass, for example see Lee SJ (2004) *Annu Rev Cell Dev Biol* 20:61-86), or twist or myogenin (which are induced by IGF-I during development, for example see Dupont J, *et al.* (2001) *The Journal of biological chemistry* 276(28):26699-26707; Tureckova J, *et al.* (2001) *The Journal of biological chemistry* 276(42):39264-39270). Moreover, ursolic acid did not alter the amount of *IGF1* mRNA in adipose tissue (Figure 9B). Briefly, the data shown in Figure 9B were obtained as follows: mice were provided ad lib access to either standard chow (control diet) or standard chow supplemented with 0.27% ursolic acid (ursolic acid diet) for 7 weeks before retroperitoneal adipose tissue was harvested for qPCR quantification of *IGF1* mRNA. The data shown are means ± SEM from 5 mice per group. Without wishing to be bound by a particular theory, ursolic acid-mediated *IGF1* induction may be localized to skeletal muscle.

8. URSOLIC ACID ENHANCES SKELETAL MUSCLE IGF-I SIGNALING.

[00320] Although muscle-specific *IGF1* induction is characteristic of, and contributes to, muscle hypertrophy, it may be a relatively late event that promotes hypertrophy after it has been initiated by other stimuli (Adams GR, *et al.* (1999) *J Appl Physiol* 87(5):1705-1712). Without wishing to be bound by a particular theory, it is possible that ursolic acid might have a more proximal effect on insulin/IGF-I signaling. In a previous study of non-muscle cell lines (CHO/IR and 3T3-L1 cells), ursolic acid enhanced insulin-mediated Akt activation (Jung SH, *et al.* (2007) *The Biochemical journal* 403(2):243-250). To determine whether ursolic acid might have a similar effect in skeletal muscle, the level of phosphorylated Akt

was assessed in quadriceps muscles of mice fed diets lacking or containing ursolic acid. Briefly, mice were provided ad lib access to either standard chow (control diet) or standard chow supplemented with 0.27% ursolic acid for 16 weeks. Total protein extracts from quadriceps muscles were subjected to SDS-PAGE, followed by immunoblot analysis for phosphorylated and total Akt, as indicated. A representative immunoblot is shown in Figure 8D. Immunoblot data were quantitated as follows: in each mouse, the level of phospho-Akt was normalized to the level of total Akt; these ratios were then normalized to the average phospho-Akt/total Akt ratio from control mice and the results are shown in Figure 8E (data are means ± SEM from 9 mice per diet. *P*-value was determined by unpaired t-test). The data show that in quadriceps, ursolic acid increased Akt phosphorylation by 1.8-fold.

[00321] The effect of ursolic acid on Akt activation was examined in C2C12 skeletal myotubes, a well-established in vitro model of skeletal muscle (Sandri M, *et al.* (2004) *Cell* 117(3):399-412; Stitt TN, *et al.* (2004) *Mol Cell* 14(3):395-403). Use of an *in vitro* system, such as C2C12 skeletal myotubes, circumvented potentially confounding effects from non-muscle tissues, and enabled a determination of whether IGF-I or insulin was required for ursolic acid's effect. The latter consideration was important because circulating IGF-I and insulin are always present in healthy animals. Use of an *in vitro* system also allowed testing of a clearly defined concentration of ursolic acid (10 μM, similar what was used in the Connectivity Map (8.8 μM)) for a clearly defined time of incubation (20 min). These considerations were important because the in vivo pharmacokinetic properties of ursolic acid are not yet known.

[00322] For the data shown in Figures 8F-8K, serum-starved C2C12 myotubes were treated in the absence or presence of ursolic acid (10 μM) and/or IGF-I (10 nM), as indicated. For studies of the IGF-I receptor, cells were harvested 2 min later, and protein extracts were subjected to immunoprecipitation with anti-IGF-I receptor β antibody, followed by immunoblot analysis with anti-phospho-tyrosine or anti-IGF-I receptor β antibodies to assess phospho- and total IGF-I receptor, respectively. For other studies, cells were harvested 20 min after addition of ursolic acid and/or IGF-I, and immunoblot analyses were performed using total cellular protein extracts and antibodies specific for the phosphorylated or total proteins indicated. Representative immunoblots showing effect of ursolic acid on IGF-I-mediated phosphorylation of Akt (Figure 8F), S6K (Figure 8G) and IGF-I receptor (Figure

8H). Data from immunoblots was quantitated as follows: levels in the presence of ursolic acid and IGF-I were normalized to levels in the presence of IGF-I alone, which were set at 1 and are indicated by the dashed line. The data shown in Figure 8I are means ± SEM from ≥ 3 experiments.

[00323] For the data shown in Figures 9C – 9F, serum-starved C2C12 myotubes were treated in the absence or presence of ursolic acid (10 μM), insulin (10 nM) and/or IGF-I (10 nM), as indicated. For studies of the insulin receptor, cells were harvested 2 min later, and protein extracts were subjected to immunoprecipitation with anti-insulin receptor β antibody, followed by immunoblot analysis with anti-phospho-insulin receptor β (Y1162/1163) or anti-insulin receptor β antibodies to assess phospho- and total insulin receptor, respectively. For other studies, cells were harvested 20 min after addition of ursolic acid, insulin and/or IGF-I, and immunoblot analyses were performed using total cellular protein extracts and antibodies specific for the phosphorylated or total proteins indicated.

[00324] When serum-starved myotubes were treated with ursolic acid alone, Akt phosphorylation did not increase (Figure 8F). However, in the presence of IGF-I, ursolic acid increased Akt phosphorylation by 1.9-fold (Figures 8F and 8I). Ursolic acid also increased Akt phosphorylation in the presence of insulin (Figure 9C). Thus, ursolic acid enhanced IGF-I-mediated and insulin-mediated Akt phosphorylation. The finding that ursolic acid enhanced muscle Akt activity in vivo and in vitro was consistent with the finding that ursolic acid's mRNA expression signature negatively correlated with the mRNA expression signatures of LY-294002 and wortmannin (Figures 4B and 5B), which inhibit insulin/IGF-I signaling upstream of Akt. However, ursolic acid's signature also negatively correlated with the signature of rapamycin, which inhibits insulin/IGF-I signaling downstream of Akt.

[00325] Although ursolic acid alone did not increase S6K phosphorylation (Figure 9D), it enhanced IGF-I-mediated and insulin-mediated S6K phosphorylation (Figures 8G, 8I and 9D). To further investigate the mechanism, the effect of ursolic acid on the IGF-I receptor was examined. Ursolic acid increased IGF-I receptor phsophorylation in the presence but not the absence of IGF-I (Figures 8H and 8I). Similarly, ursolic acid increased insulin receptor phosphorylation in the presence but not the absence of insulin (Figure 9E). Both of these effects were rapid, occurring within 2 minutes after the addition of ursolic acid and either IGF-I or insulin. Consistent with enhanced signaling at the level of the IGF-I and insulin

receptors, ursolic acid also enhanced IGF-I-mediated and insulin-mediated ERK phosphorylation (Figures 8J and 9F). Moreover, ursolic acid enhanced IGF-I-mediated phosphorylation (inhibition) of FoxO transcription factors, which activate transcription of *atrogin-1* and *MuRF1* mRNAs (Figure 8K; Sandri M, *et al.* (2004) *Cell* 117(3):399-412; Stitt TN, *et al.* (2004) *Mol Cell* 14(3):395-403.). Without wishing to be bound by a particular theory, ursolic acid represses atrophy-associated gene expression and promotes muscle hypertrophy by increasing activity of the IGF-I and insulin receptors.

9. URSOLIC ACID REDUCES ADIPOSITY.

[00326] Mice were provided ad lib access to standard chow supplemented with the indicated concentration (weight percent in chow, either 0.14% or 0.28% as indicated in Figure 10) of ursolic acid for 7 weeks before tissues were harvested for analysis. Data are means ± SEM from 10 mice per diet. Data for the effects of ursolic acid on weights of skeletal muscle (quadriceps + triceps), epididymal fat, retroperitoneal fat and heart are shown in Figure 10A. The *P*-values, determined by one-way ANOVA with post-test for linear trend, were < 0.001 for muscle; 0.01 and 0.04 for epididymal and retroperitoneal fat, respectively; and 0.46 for heart. The data show that 7 weeks of dietary ursolic acid increased skeletal muscle weight in a dose-dependent manner, with a peak effect at 0.14% ursolic acid. Interestingly, although ursolic acid increased muscle weight, it did not increase total body weight (Figure 10B; *P*-values were 0.71 and 0.80 for initial and final weights, respectively).

[00327] The data in Figure 10A also show that 7 weeks of dietary ursolic acid reduced the weight of epididymal and retroperitoneal fat depots, with a peak effect at 0.14%. In another study, mice were provided ad lib access to either standard chow (control diet) or standard chow supplemented with 0.27% ursolic acid (ursolic acid diet) for 5 weeks. The relationship between skeletal muscle weight (quadriceps, triceps, biceps, TA, gastrocnemius and soleus) and retroperitoneal adipose weight is shown in Figure 10C. Each data point in Figure 10C represents one mouse; $P < 0.001$ for both muscle and adipose by unpaired t-test. The data show that 5 weeks of ursolic acid administration (0.14%) also reduced adipose weight. Thus, muscle and fat weights were inversely related. Without wishing to be bound by a particular theory, ursolic acid-treated mice contain less fat because, in part, ursolic acid increases Akt activity (see Figures 8 and 9), and muscle-specific increases in Akt activity reduce adiposity as a secondary consequence of muscle hypertrophy (Lai KM, *et al.* (2004)

Molecular and cellular biology 24(21):9295-9304; Izumiya Y, *et al.* (2008) *Cell metabolism* 7(2):159-172).

[00328] Ursolic acid reduced adipose weight by reducing adipocyte size as shown by data in Figures 10D - 10F. Figure 10D shows a representative H&E stain of retroperitoneal fat for animals feed a control data or a chow with 0.27% ursolic acid as indicated. The data in Figure 10D are shown quantitatively in Figure 10E in terms of adipocyte diameter, where data point represents theaverage diameter of ≥ 125 retroperitoneal adipocytes from one mouse. The retroperitoneal adipocyte size distribution. Each distribution represents combined adipocyte measurements (> 1000 per diet) from Figure 10E.

[00329] The changes in adipocyte size were accompanied by a significant reduction in plasma leptin levels, which correlated closely with adipose weight (see Figures 10G and 10H). In Figure 10G, each data point represents one mouse, and horizontal bars denote the means. P-values were determined by t-test. In Figure 10H, each data point represents one mouse. Importantly, ursolic acid also significantly reduced plasma triglyceride (Figure 10I) and cholesterol (Figure 10J). In Figures 10I and 10J, each data point represents one mouse, and horizontal bars denote the means. P-values were determined by unpaired t-test. Although ursolic acid reduced leptin, it did not alter food intake (Figure 11A). In this study, mice were provided ad lib access to either standard chow (control diet) or standard chow supplemented with 0.27% ursolic acid (ursolic acid diet) for 4 weeks. Mice were then moved to a comprehensive animal metabolic monitoring system (CLAMS; Columbus Instruments, Columbus, OH) and provided with ad lib access to the same diets. Food consumption was measured for 48 hours. Data are means ± SEM from 6 mice per group. However, ursolic acid did not alter weights of heart (Figure 10A), liver or kidney (Figures 11B and 11C), nor did it elevate plasma markers of hepatotoxicity or nephrotoxicity (alanine aminotransferase, bilirubin and creatinine; see Figures 11D – 11F). The data in Figures 11B – 11F were obtained as follows: mice were provided ad lib access to either standard chow (control diet) or standard chow supplemented with 0.27% ursolic acid (ursolic acid diet) for 5 weeks before tissues and plasma were harvested for the indicated measurements; each data point represents one mouse, and horizontal bars denote the means. For Figure 11, *P*-values were determined with unpaired t-tests. Thus, dietary ursolic acid had two major effects: skeletal muscle hypertrophy and reduced adiposity.

10. URSOLIC ACID REDUCES WEIGHT GAIN AND WHITE ADIPOSE TISSUE.

[00330] The findings that ursolic acid increased skeletal muscle and decreased adiposity suggested that ursolic acid might increase energy expenditure, which would lead to obesity resistance. To test this, C57BL/6 mice were given ad libitum access to a high fat diet (HFD; Teklad TD.93075; 55% calories from fat) lacking or containing 0.27% ursolic acid. After 7 weeks, mice from each group were studied for three days in comprehensive lab animal monitoring systems ("CLAMS"; Columbus Instruments). In the CLAMS, mice were maintained on the same diet they had been eating since the beginning of the experiment. Following CLAMS, tissues were harvested for analysis. In high fat-fed mice, ursolic acid dramatically reduced weight gain, and this effect was apparent within one week (Figure 12A). As previously observed in mice fed ursolic acid and standard chow (Figure 6), ursolic acid increased grip strength and muscle mass (Figures 12B and 12C). Moreover, ursolic acid reduced retroperitoneal and epididymal fat (Figures 12D and 12E). Interestingly, in the scapular fat pad, which contains a mixture of white and thermogenic brown fat, ursolic acid reduced white fat (Figure 12F), but increased brown fat (Figure 12G). Importantly, increased skeletal muscle and brown adipose tissue would be predicted to increase energy expenditure. Indeed, CLAMS revealed that ursolic acid increased energy expenditure (Figure 12H), providing an explanation for how ursolic acid reduces adiposity and obesity. Remarkably, CLAMS analysis revealed that ursolic acid-treated mice consumed more food (Figure 12I), even though they gained less weight (Figure 12A). For the data shown in Figure 12A, data are means ± SEM from 12 control mice and 15 treated mice, but it should be noted that some error bars are too small to see; $P < 0.01$ at 1 wk and each subsequent time point. In Figures 12B – 12I, each data point represents one mouse and horizontal bars denote the means. *P*-values were determined with unpaired t-tests.

11. URSOLIC ACID REDUCES OBESITY-RELATED PRE-DIABETES, DIABETES, FATTY LIVER DISEASE AND HYPERCHOLESTEROLEMIA.

[00331] The study was carried out as follows: C57BL/6 mice were given ad libitum access to a high fat diet ("HFD"; Teklad TD.93075; 55% calories from fat) lacking or containing 0.27% ursolic acid. After 5 weeks, mice were fasted for 16 h before blood glucose was measured via the tail vein (Figure 13A). Normal fasting blood glucose: ≤ 100 mg/dl. (B-I) After 7 weeks, liver and plasma were harvested for analysis (Figures 13B – 13I). The data

shown in Figure 13A suggest that most mice fed HFD without ursolic acid for 6 weeks developed impaired fasting glucose (pre-diabetes) or diabetes. Importantly, this was prevented by ursolic acid (Figure 13A). In addition, mice fed HFD without ursolic acid developed fatty liver disease, as evidenced by increased liver weight (>30% increase above normal mouse liver weight of 1500 mg; Figure 13B), hepatocellular lipid accumulation (Figure 13C, H&E stain at 20X magnification; Figure 13D, lipid-staining osmium at 10X magnification), and elevated plasma liver function tests (Figure 13E, AST; 13F, ALT; 13G, alkaline phosphatase (labeled as "Alk. Phos. in figure); and, 13H, cholesterol). However, ursolic acid prevented all of these hepatic changes (Figure 13B – 13G). In addition, ursolic acid reduced obesity-related hypercholesterolemia (Figure 13H). In Figures 13A, 13B, and 13E-13H, each data point represents one mouse and horizontal bars denote the means.

12. OLEANOLIC ACID DOES NOT INCREASE SKELETAL MUSCLE MASS.

[00332] The effect of ursolic acid on skeletal muscle weight and liver weight was compared to the effects by oleanolic acid and metformin. Metformin was a compound identified from atrophy signature-1, but not atrophy signature-2. Oleanolic acid, like ursolic acid is a pentacyclic acid triterpane. This is a structurally similar compound to ursolic acid. However, the two compounds are distinct: oleanolic acid has two methyl groups at position 20, whereas ursolic acid has a single methyl group at each of positions 19 and 20 (compare Figures 14A and 14D). Both ursolic acid and oleanolic acid reduce blood glucose, adiposity and hepatic steatosis (Wang ZH, *et al.* (2010) *European journal of pharmacology* 628(1-3):255-260; Jayaprakasam B, *et al.* (2006) *J Agric Food Chem* 54(1):243-248; de Melo CL, *et al.* (2010) *Chem Biol Interact* 185(1):59-65). In addition, both ursolic acid and oleanolic acid possess a large number of cellular effects and biochemical targets, including nearly equivalent inhibition of protein tyrosine phosphatases ("PTPs"; see Zhang W, *et al.* (2006) *Biochimica et biophysica acta* 1760(10):1505-1512; Qian S, *et al.* (2010) *J Nat Prod* 73(11):1743-1750; Zhang YN, *et al.* (2008) *Bioorg Med Chem* 16(18):8697-8705). However, the effects of these compounds on skeletal muscle mass were not known.

[00333] Because some PTPs (particularly PTP1B) dephosphorylate (inactivate) the insulin receptor, PTP inhibition represented a potential mechanism to explain ursolic acid-mediated enhancement of insulin signaling. Thus, because oleanolic acid and ursolic acid inhibit PTP1B and other PTPs with similar efficacy and potency in vitro (Qian S, *et al.* (2010)

J Nat Prod 73(11):1743-1750; Zhang YN, *et al.* (2008) *Bioorg Med Chem* 16(18):8697-8705), testing oleanolic acid's effects on skeletal mass tests the potential role of PTP inhibition. It should be noted that neither ursolic acid nor oleanolic acid is known to inhibit PTPs in vivo, and neither of these compounds are known to enhance IGF-I signaling. Moreover, ursolic acid's capacity to inhibit PTPs has been disputed based on ursolic acid's failure to delay insulin receptor de-phosphorylation in cultured cells (Jung SH, *et al.* (2007) *The Biochemical journal* 403(2):243-250), and ursolic acid's capacity to act as an insulin mimetic (Jung SH, *et al.* (2007) *The Biochemical journal* 403(2):243-250). In addition, global and muscle-specific PTP1B knockout mice do not possess increased muscle mass, although they are resistant to obesity and obesity-related disorders(Delibegovic M, *et al.* (2007) *Molecular and cellular biology* 27(21):7727-7734; Klaman LD, *et al.* (2000) *Molecular and cellular biology* 20(15):5479-5489). Furthermore, ursolic acid increases pancreatic beta cell mass and serum insulin levels in vivo, perhaps via its anti-inflammatory effects (Wang ZH, *et al.* (2010) *European journal of pharmacology* 628(1-3):255-260; Jayaprakasam B, *et al.* (2006) *J Agric Food Chem* 54(1):243-248; de Melo CL, *et al.* (2010) *Chem Biol Interact* 185(1):59-65).. Importantly, inflammation is now recognized as a central pathogenic mechanism in muscle atrophy, metabolic syndrome, obesity, fatty liver disease and type 2 diabetes. Thus, the existing data suggest at least four mechanisms to explain ursolic acid's capacity to increase insulin signaling in vivo: PTP inhibition, direct stimulation of the insulin receptor, increased insulin production, and reduced inflammation. Of these four potential mechanisms, only the latter three have been demonstrated in vivo.

[00334] To compare the effects of ursolic acid and oleanolic acid on skeletal muscle and liver weight, C57BL/6 mice were administered ursolic acid (200 mg / kg), oleanolic acid (200 mg / kg), or vehicle alone (corn oil) via i.p. injection. Mice were then fasted, and after 12 hours of fasting, mice received a second dose of ursolic acid, oleanolic acid, or vehicle. After 24 hours of fasting, lower hindlimb skeletal muscles and liver were harvested and weighed. As shown previously, ursolic acid increased skeletal muscle weight (Figure 14B), but not liver weight (Figure 14C). In contrast, oleanolic acid increased liver weight (FIG. 14F), but not skeletal muscle weight (Figure 14E). Interestingly, metformin (250 mg / kg) resembled oleanolic acid in biological effect: it increased liver weight (Figure 14I), but not muscle weight (Figure 14H). Without wishing to be bound by a particular theory, ursolic acid increases skeletal muscle and inhibit muscle atrophy by a pathway that does not involve PTP

inhibition.

13. TARGETED INHIBITION OF PTP1B DOES NOT INDUCE SKELETAL MUSCLE HYPERTROPHY.

[00335] To further rule out the potential role of PTP1B inhibition in skeletal muscle hypertrophy, PTP1B expression was specifically reduced in mouse skeletal muscle by transfecting plasmid DNA constructed to express RNA interference constructs. Briefly, C57BL/6 mouse tibialis anterior muscles were transfected with 20 μg *pCMV-miR-control* (control plasmid transfected in the left TA) or either 20 μg *pCMV-miR-PTP1B #1* (encoding miR-PTP1B #1; transfected in the right TA) or 20 μg *pCMV-miR-PTP1B #2* (encoding miR-PTP1B #2; transfected in the right TA). miR-PTP1B #1 and miR-PTP1B #2 encode two distinct RNA interference (RNAi) constructs targeting distinct regions of *PTP1B* mRNA. Tissue was harvested 10 days following transfection.

[00336] Of note with regard to Figure 15A, mRNA measurements were taken from the entire TA muscle. Because electroporation transfects only a portion of muscle fibers, the data underestimate *PTP1B* knockdown in transfected muscle fibers. In Figure 15A, mRNA levels in the right TA were normalized to levels in the left TA, which were set at 1; data are means ± SEM from 3 mice. In Figure 15B, in each TA muscle, the mean diameter of > 300 transfected fibers was determined; data are means ± SEM from 3 TA muscles per condition. For both Figures 15A and 15B, *P*-values were determined with one-tailed paired t-tests.

[00337] Although both miR-PTP1B constructs reduced *PTP1B* mRNA (Figure 15A), neither increased skeletal muscle fiber diameter (Figure 15B). These data demonstrate that targeted PTP1B inhibition does not cause muscle fiber hypertrophy. Without wishing to be bound by a particular theory, ursolic acid does not increase skeletal muscle by inhibiting PTP1B.

14. URSOLIC ACID SERUM LEVELS ASSOCIATED WITH INCREASED MUSCLE MASS AND DECREASED ADIPOSITY.

[00338] To determine the dose-response relationship between dietary ursolic acid and muscle and adipose weight, C57BL/6 mice were fed standard chow containing varying amounts of ursolic acid for 7 weeks. Serum ursolic acid levels from mice were determined as

described above. As shown previously in Figure 10A, ursolic acid increased skeletal muscle weight and decreased weight of retroperitoneal and epididymal fat pads in a dose-dependent manner, but did not alter heart weight (Figure 16A; data are means ± SEM). These effects of ursolic acid were discernable at 0.035% ursolic acid and were maximal at doses ≥ 0.14% ursolic acid. Serum was collected from these same mice at the time of necropsy, and then measured random serum ursolic acid levels via ultra high performance liquid chromatography (UPLC). The data indicate that ursolic acid serum levels in the range of 0.25 – 0.5 μg / ml are sufficient to increase muscle mass and decrease adiposity (Figure 16B; data are means ± SEM). Of note, 0.5 μg / ml equals 1.1 μM ursolic acid, close to the dose used in the Connectivity Map (8.8 μM) and in the C2C12 experiments (10 μM) described above.

[00339] The data described herein indicate that ursolic acid reduced muscle atrophy and stimulated muscle hypertrophy in mice. Importantly, ursolic acid's effects on muscle were accompanied by reductions in adiposity, fasting blood glucose and plasma leptin, cholesterol and triglycerides, as well as increases in the ratio of skeletal muscle to fat, the amount of brown fat, the ratio of brown fat to white fat, and increased energy expenditure. Without wishing to be bound by a particular theory, ursolic acid reduced muscle atrophy and stimulated muscle hypertrophy by enhancing skeletal muscle IGF-I expression and IGF-I signaling, and inhibiting atrophy-associated skeletal muscle mRNA expression.

[00340] All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. It will be apparent to those skilled in the art that various modifications and variations can be made in the present invention without departing from the scope or spirit of the invention.

[00341] More specifically, certain agents which are both chemically and physiologically related can be substituted for the agents described herein while the same or similar results can be achieved.

[00342] Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein.

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THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. Use of ursolic acid, or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, for increasing skeletal muscle mass in an animal, wherein said animal is selected from the group consisting of a primate, domesticated fish, domesticated crustacean, domesticated mollusk, poultry, rabbit, dog, cat, and livestock.

2. Use of ursolic acid, or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, in the manufacture of a composition for increasing skeletal muscle mass in an animal, wherein said animal is selected from the group consisting of a primate, domesticated fish, domesticated crustacean, domesticated mollusk, poultry, rabbit, dog, cat, and livestock.

3. Use of ursolic acid, or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof for promoting muscle growth, decreasing muscle wasting, or increasing strength per unit mass of muscle in an animal.

4. The use according to any one of claims 1 to 3, wherein the ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof is formulated for administration to the animal in an amount of greater than about 200 mg per day, greater than 300 mg per day, greater than about 400 mg per day, greater than about 500 mg per day, greater than about 750 mg per day, greater than about 1000 mg per day, or greater than about 2000 mg per day.

5. The use according to any one of claims 1 to 4, wherein the animal is a primate, rabbit, dog, cat or livestock.

6. The use according to any one of claims 1 to 4, wherein the animal is a human.

7. The use according to any one of claims 1 to 4, wherein the animal is a domesticated animal.

8. The use according to any one of claims 1 to 4, wherein the animal is livestock, domesticated fish, or domesticated poultry.

9. The use according to any one of claims 1 to 8, wherein the ursolic acid is present as a pharmaceutically acceptable salt selected from salts derived from aluminum, ammonium, calcium, cupric, cuprous, ferric, ferrous, lithium, magnesium, manganese, potassium, sodium, or zinc; salts of primary, secondary, and tertiary amines; and salts derived from arginine, betaine, caffeine, choline, N,N'-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, or tromethamine.

10. Ursolic acid or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, for use in increasing skeletal muscle mass in an animal.

11. The ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof according to claim 10, wherein the ursolic acid is present as a pharmaceutically acceptable salt selected from salts derived from aluminum, ammonium, calcium, cupric, cuprous, ferric, ferrous, lithium, magnesium, manganese, potassium, sodium, or zinc; salts of primary, secondary, and tertiary amines; and salts derived from arginine, betaine, caffeine, choline, N,N'-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, or tromethamine.

12. A composition for use in increasing skeletal muscle mass in an animal, the composition comprising the ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof as defined in claim 10 or 11, wherein the ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof is present in the composition in an amount of at least 50, 75, 100, 150, 200, 250, 300, 400, 500, 600, 750, 800, 900, or 1000 mg, and a pharmaceutically acceptable carrier or excipient.

13. A composition for use in increasing skeletal muscle mass in an animal, the composition comprising the ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph

thereof as defined in claim 10 or 11 and a pharmaceutically acceptable carrier or excipient, wherein the composition is formulated for administration of 150 mg to 1000 mg of the ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof.

14. The ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof of claim 10 or 11 in an amount of about 1.0 to 1000 mg, wherein the ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof is in the form of a tablet.

15. A composition for use in increasing skeletal muscle mass in an animal, the composition comprising the ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof as defined in claim 10 or 11, wherein the ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof is present in the composition in an amount greater than about 1000 mg, and a pharmaceutically acceptable carrier or excipient.

16. A composition for use in increasing skeletal muscle mass in an animal, the composition comprising: the ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof as defined in any one of claims 10, 11, or 14; or the composition according to any one of claims 12, 13, or 15; wherein the composition comprises a diluent, a buffer, a flavoring agent, a binding agent, a surface-active agent, a thickener, a lubricant, a preservative, or a combination thereof.

17. A composition for use in increasing skeletal muscle mass in an animal, the composition comprising the ursolic acid, or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof as defined in claim 10, in an amount of at least 1.0 mg, and one or more pharmaceutically acceptable carriers, therapeutic agents, or adjuvants, wherein the composition is in oral dosage form.

18. The composition of claim 17 comprising the ursolic acid, or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, in an amount of at least 150, 200, 250, 300, 400, 500, 600, 750, 800, 900, or 1000 mg.

19. The composition of claim 17 or 18, wherein the composition is in an oral dosage form selected from powder, capsule, cachet, and tablet.

20. The composition according to any one of claims 17-19, wherein the composition is for administration of 150 mg to 1000 mg of the ursolic acid, or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof.

21. The composition of claim 17, wherein the composition comprises ursolic acid or the pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof in an amount of 100-1000 mg.

22. The composition according to any one of claims 15 to 21, wherein the composition is a pharmaceutical composition.

23. The ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof according to any one of claims 10, 11, or 14, or the composition according to any one of claims 12, 13, or 15 to 22, wherein the animal is selected from the group consisting of a primate, domesticated fish, domesticated crustacean, domesticated mollusk, poultry, rabbit, dog, cat, and livestock.

24. The ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof according to any one of claims 10, 11, or 14, or the composition according to any one of claims 12, 13, or 15 to 23, wherein the animal is a primate, rabbit, dog, cat or livestock.

25. The ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof according to any one of claims 10, 11 or 14, or the composition according to any one of claims 12, 13, or 15 to 23, wherein the animal is a human.

26. The ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof according to any one of claims 10, 11 or 14, or the composition according to any one of claims 12, 13 or 15 to 23, wherein the animal is a domesticated animal.

27. The ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof according to any one of claims 10, 11 or 14, or the composition according to any one of claims 12, 13, or 15 to 23, wherein the animal is livestock, domesticated fish, or domesticated poultry.

28. The use according to any one of claims 1 to 4, or the ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof according to any one of claims 10, 11, 14 or 23, or the composition according to any one of claims 12, 13, or 15 to 23, wherein the animal is selected from the group consisting of a dog, cat, pig, cow, horse, goat, bison, sheep, chicken, turkey, duck, goose, and domesticated fish.

29. The use according to any one of claims 1 to 4, or the ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof according to any one of claims 10, 11, 14 or 23, or the composition according to any one of claims 12, 13, or 15 to 23, wherein the animal is selected from the group consisting of a domesticated fish, poultry, pig, cow, horse, goat, sheep, dog, cat, and bison.

30. The use according to any one of claims 1 to 3, wherein the ursolic acid or pharmaceutically acceptable salt, hydrate, or solvate thereof is formulated for administration to the animal in an amount of 150 mg to 1000 mg.

31. The use according to any one of claims 1 to 3, wherein the ursolic acid or pharmaceutically acceptable salt, hydrate, or solvate thereof is formulated for administration to the animal in a composition that further comprises a preservative.

32. The use according to any one of claims 1 to 3, wherein the ursolic acid or pharmaceutically acceptable salt, hydrate, or solvate thereof is formulated for administration to the animal in an amount of greater than or equal to 100 mg per day.

33. The use according to any one of claims 1 to 3, wherein the ursolic acid or pharmaceutically acceptable salt, hydrate, or solvate thereof is formulated for administration to the animal in an amount of greater than 200 mg per day.

34. The use according to any one of claims 1 to 3, wherein the ursolic acid or pharmaceutically acceptable salt, hydrate, or solvate thereof is formulated for administration to the animal in an amount of greater than 300 mg per day.

35. The use according to any one of claims 1 to 3, wherein the ursolic acid or pharmaceutically acceptable salt, hydrate, or solvate thereof is formulated for administration to the animal in an amount of greater than 400 mg per day.

36. The use according to any one of claims 1 to 3, wherein the ursolic acid or pharmaceutically acceptable salt, hydrate, or solvate thereof is formulated for administration to the animal in an amount of greater than 500 mg per day.

37. The use according to any one of claims 1 to 3, wherein the ursolic acid or pharmaceutically acceptable salt, hydrate, or solvate thereof is formulated for administration to the animal in an amount of greater than 750 mg per day.

38. The use according to any one of claims 1 to 3, wherein the ursolic acid or pharmaceutically acceptable salt, hydrate, or solvate thereof is formulated for administration to the animal in an amount of greater than 1000 mg per day.

39. The use according to any one of claims 1-3 and 30-38, wherein the animal is selected from a human, domesticated fish, poultry, pig, cow, horse, goat, sheep, dog, cat, and bison.

40. The use according to any one of claims 1-3 and 30-38, wherein the animal is selected from a domesticated fish, poultry, pig, cow, horse, goat, sheep, dog, cat, and bison.

41. The composition according to any one of claims 15 to 21, wherein the composition is animal chow.

42. Use of ursolic acid, or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, for:

a) reducing skeletal muscle atrophy;

b) increasing muscular strength; or

c) promoting muscle growth,

in an animal, wherein said animal is selected from the group consisting of a primate, domesticated fish, domesticated crustacean, domesticated mollusk, poultry, rabbit, dog, cat, and livestock.

43. Use of ursolic acid, or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, in the manufacture of a composition for:

a) reducing skeletal muscle atrophy;

b) increasing muscular strength; or

c) promoting muscle growth,

in an animal, wherein said animal is selected from the group consisting of a primate, domesticated fish, domesticated crustacean, domesticated mollusk, poultry, rabbit, dog, cat, and livestock.

44. Use of ursolic acid, or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, for increasing muscular strength or promoting muscle growth in an animal.

45. The use according to any one of claims 42 to 44, wherein the ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof is formulated for administration to the animal in an amount of greater than about 200 mg per day, greater than 300 mg per day, greater than about 400 mg per day, greater than about 500 mg per day, greater than about 750 mg per day, greater than about 1000 mg per day, or greater than about 2000 mg per day.

46. The use according to any one of claims 42 to 45, wherein the animal is a primate, rabbit, dog, cat or livestock.

47. The use according to any one of claims 42 to 45, wherein the animal is a human.

48. The use according to any one of claims 42 to 45, wherein the animal is a domesticated animal.

49. The use according to any one of claims 42 to 45, wherein the animal is livestock, domesticated fish, or domesticated poultry.

50. The use according to any one of claims 42 to 49, wherein the ursolic acid is present as a pharmaceutically acceptable salt selected from salts derived from aluminum, ammonium, calcium, cupric, cuprous, ferric, ferrous, lithium, magnesium, manganese, potassium, sodium, or zinc; salts of primary, secondary, and tertiary amines; and salts derived from arginine, betaine, caffeine, choline, N,N'-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, or tromethamine.

51. Ursolic acid or a pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, for use in:

a) reducing skeletal muscle atrophy;

b) increasing muscular strength; or

c) promoting muscle growth.

52. The ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof according to claim 51, wherein the ursolic acid is present as a pharmaceutically acceptable salt selected from salts derived from aluminum, ammonium, calcium, cupric, cuprous, ferric, ferrous, lithium, magnesium, manganese, potassium, sodium, or zinc; salts of primary, secondary, and tertiary amines; and salts derived from arginine, betaine, caffeine, choline, N,N'-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, or tromethamine.

53. A composition for use in: a) reducing skeletal muscle atrophy; b) increasing muscular strength; or c) promoting muscle growth, the composition comprising the ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof as defined in claim 51 or 52 and a pharmaceutically acceptable carrier or excipient, wherein the ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof is present in a composition in an amount of at least 50, 75, 100, 150, 200, 250, 300, 400, 500, 600, 750, 800, 900, or 1000 mg.

54. A composition for use in: a) reducing skeletal muscle atrophy; b) increasing muscular strength; or c) promoting muscle growth, the composition comprising the ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof as defined in claim 51 or 52 and a pharmaceutically acceptable carrier or excipient, wherein the composition is formulated for administration of 150 mg to 1000 mg of the ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof.

55. The ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof of claim 51 or 52 in an amount of about 1.0 to 1000 mg, wherein the ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof is in the form of a tablet.

56. A composition for use in: a) reducing skeletal muscle atrophy; b) increasing muscular strength; or c) promoting muscle growth, the composition comprising the ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof as defined in claim 51 or 52, and a pharmaceutically acceptable carrier or excipient, wherein the ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof is present in the composition in an amount greater than about 1000 mg.

57. A composition for use in: a) reducing skeletal muscle atrophy; b) increasing muscular strength; or c) promoting muscle growth, the composition comprising the ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof as defined in any one of claims 51, 52, or 55, or the composition according to any one of claims 53, 54, or 56, wherein the composition comprises a diluent, a buffer, a flavoring agent, a binding agent, a surface-active agent, a thickener, a lubricant, a preservative, or a combination thereof.

58. A composition for use in: a) reducing skeletal muscle atrophy; b) increasing muscular strength; or c) promoting muscle growth, the composition comprising the ursolic acid, or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof as defined in claim 51, in an amount of at least 1.0 mg, and one or more pharmaceutically acceptable carriers, therapeutic agents, or adjuvant, wherein the composition is in an oral dosage form.

59. The composition of claim 58 comprising the ursolic acid, or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof, in an amount of at least 150, 200, 250, 300, 400, 500, 600, 750, 800, 900, or 1000 mg.

60. The composition of claim 58 or 59, wherein the composition is in an oral dosage form selected from powder, capsule, cachet, and tablet.

61. The composition according to any one of claims 58-60, for use in increasing skeletal muscle mass in an animal, wherein the composition is for administration of 150 mg to 1000 mg of the ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof.

62. The composition of claim 58, wherein the composition comprises ursolic acid or the pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof in an amount of 100-1000 mg.

63. The composition according to any one of claims 56 to 62, wherein the composition is a pharmaceutical composition.

64. The ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof according to any one of claims 51, 52, or 55, or the composition according to any one of claims 53, 54, or 56 to 63, wherein the animal is selected from the group consisting of a primate, domesticated fish, domesticated crustacean, domesticated mollusk, poultry, rabbit, dog, cat, and livestock.

65. The ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof according to any one of claims 51, 52, or 55, or the composition according to any one of claims 53, 54, or 56 to 64, wherein the animal is a primate, rabbit, dog, cat or livestock.

66. The ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof according to any one of claims 51, 52, or 55, or the composition according to any one of claims 53, 54, or 56 to 64, wherein the animal is a human.

67. The ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof according to any one of claims 51, 52, or 55, or the composition according to any one of claims 53, 54, or 56 to 64, wherein the animal is a domesticated animal.

68. The ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof according to any one of claims 51, 52, or 55, or the composition according to any one of claims 53, 54, or 56 to 64, wherein the animal is livestock, domesticated fish, or domesticated poultry.

69. The use according to any one of claims 42 to 45, or the ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof according to any one of claims 51, 52, 55 or 64, or the composition according to any one of claims 53, 54, or 56 to 64, wherein the animal is selected from the group consisting of a dog, cat, pig, cow, horse, goat, bison, sheep, chicken, turkey, duck, goose, and domesticated fish.

70. The use according to any one of claims 42 to 45, or the ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof according to any one of claims 51, 52, 55, or 64, or the composition according to any one of claims 53, 54, or 56 to 64, wherein the animal is selected a domesticated fish, poultry, pig, cow, horse, goat, sheep, dog, cat, and bison.

71. The use according to any one of claims 42 to 44, wherein the ursolic acid or pharmaceutically acceptable salt, hydrate, or solvate thereof is for use in increasing skeletal muscle mass in an animal and formulated for administration of 150 mg to 1000 mg of the ursolic acid or pharmaceutically acceptable salt, hydrate, solvate, or polymorph thereof to the animal.

72. The use according to any one of claims 42 to 44, wherein the ursolic acid or pharmaceutically acceptable salt, hydrate, or solvate thereof is formulated for administration to the animal in a composition that further comprises a preservative.

73. The use according to any one of claims 42 to 44, wherein the ursolic acid or pharmaceutically acceptable salt, hydrate, or solvate thereof is formulated for administration to the animal in an amount of greater than or equal to 100 mg per day.

74. The use according to any one of claims 42 to 44, wherein the ursolic acid or pharmaceutically acceptable salt, hydrate, or solvate thereof is formulated for administration to the animal in an amount of greater than 200 mg per day.

75. The use according to any one of claims 42 to 44, wherein the ursolic acid or pharmaceutically acceptable salt, hydrate, or solvate thereof is formulated for administration to the animal in an amount of greater than 300 mg per day.

76. The use according to any one of claims 42 to 44, wherein the ursolic acid or pharmaceutically acceptable salt, hydrate, or solvate thereof is formulated for administration to the animal in an amount of greater than 400 mg per day.

77. The use according to any one of claims 42 to 44, wherein the ursolic acid or pharmaceutically acceptable salt, hydrate, or solvate thereof is formulated for administration to the animal in an amount of greater than 500 mg per day.

78. The use according to any one of claims 42 to 44, wherein the ursolic acid or pharmaceutically acceptable salt, hydrate, or solvate thereof is formulated for administration to the animal in an amount of greater than 750 mg per day.

79. The use according to any one of claims 42 to 44, wherein the ursolic acid or pharmaceutically acceptable salt, hydrate, or solvate thereof is formulated for administration to the animal in an amount of greater than 1000 mg per day.

80. The use according to any one of claims 42-44 and 71-79, wherein the animal is selected from a human, domesticated fish, poultry, pig, cow, horse, goat, sheep, dog, cat, and bison.

81. The use according to any one of claims 42-44 and 71-79, wherein the animal is selected from a domesticated fish, poultry, pig, cow, horse, goat, sheep, dog, cat, and bison.

82. The composition according to any one of claims 53, 54, or 56 to 62, wherein the composition is animal chow.

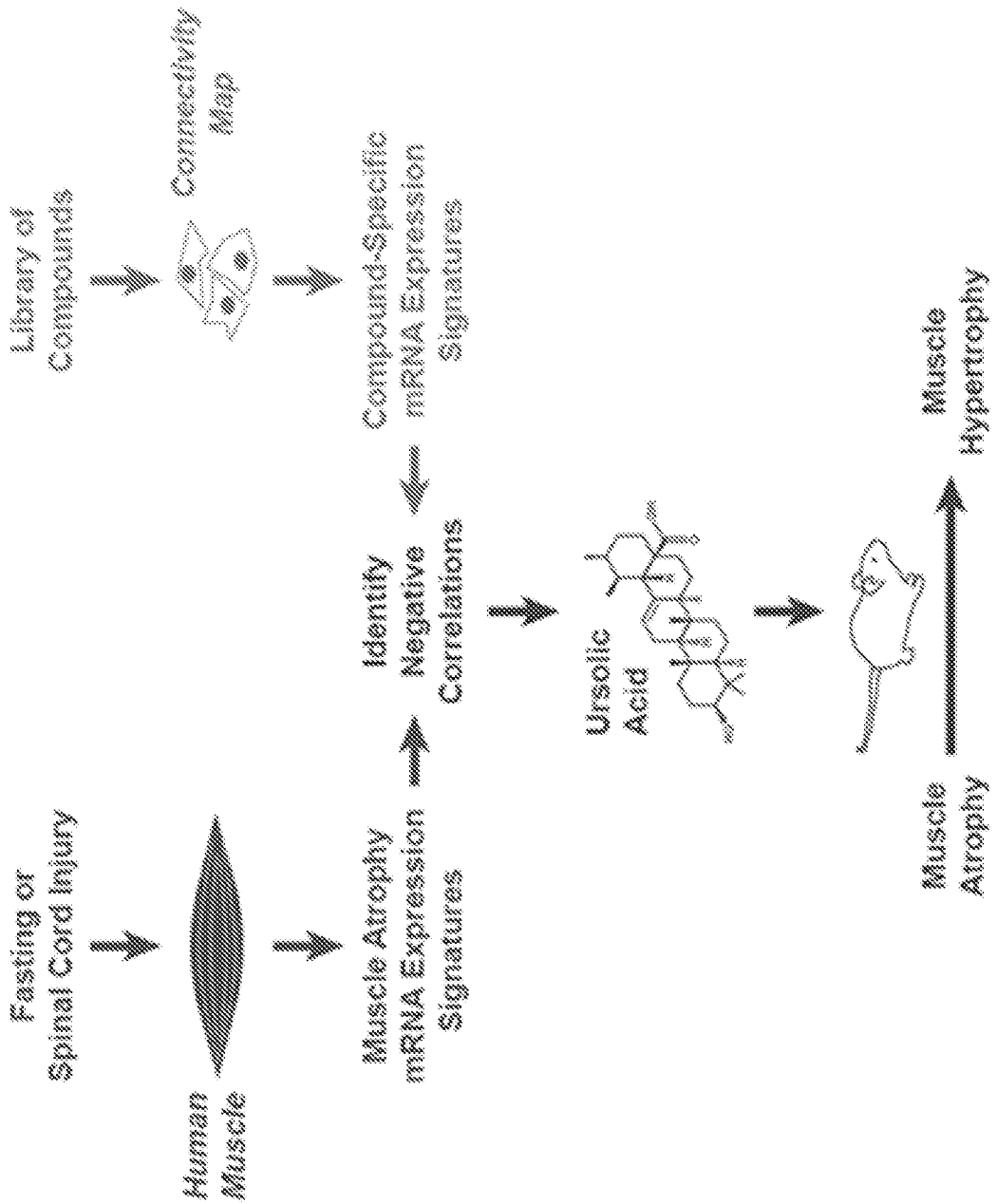

Fasting or Spinal Cord Injury
Human Muscle
Muscle Atrophy mRNA Expression Signatures
Library of Compounds
Connectivity Map
Compound-Specific mRNA Expression Signatures
Identify Negative Correlations
Ursolic Acid
Muscle Atrophy
Muscle Hypertrophy

Figure 1

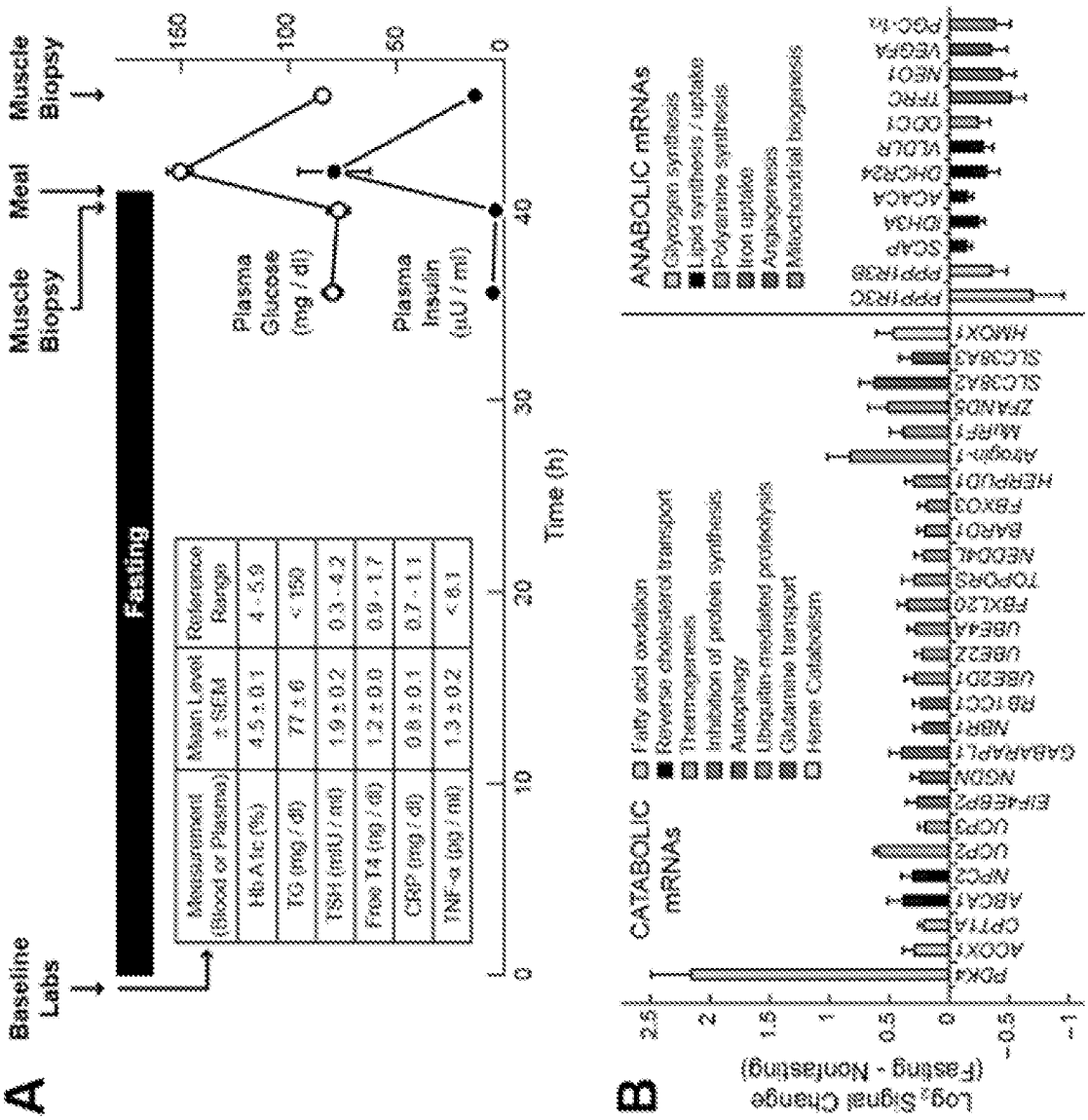

A
Baseline Labs
Muscle Biopsy
Meal
Muscle Biopsy
Fasting
Plasma Glucose (mg / dl)
Plasma Insulin (μU / ml)
Time (h)
0
10
20
30
40
60
100
150
Measurement (Blood or Plasma)
Mean Level ± SEM
Reference Range
Hb A1c (%)
4.5 ± 0.1
4 - 5.9
TG (mg / dl)
77 ± 6
< 150
TSH (mIU / ml)
1.9 ± 0.2
0.3 - 4.2
Free T4 (ng / dl)
1.2 ± 0.0
0.9 - 1.7
CRP (mg / dl)
0.8 ± 0.1
0.1 - 1.1
TNF-α (pg / ml)
1.3 ± 0.2
< 8.1
B
CATABOLIC mRNAs
Fatty acid oxidation
Reverse cholesterol transport
Thermogenesis
Inhibition of protein synthesis
Autophagy
Ubiquitin-mediated proteolysis
Glutamine transport
Heme Catabolism
ANABOLIC mRNAs
Glycogen synthesis
Lipid synthesis / uptake
Polyamine synthesis
Iron uptake
Angiogenesis
Mitochondrial biogenesis
Log2 Signal Change (Fasting - Nonfasting)
2.5
2
1.5
1
0.5
0
-0.5
-1
FOXA1
ACOX1
CPT1A
ABCA1
NPC2
UCP2
UCP3
EIF4EBP2
NCOA
GABARAPL1
NBR1
RB1CC1
UBE3D1
UBE2Z
UBE4A
FBXL20
TOPORS
NEDD4L
BARD1
FBXO3
HERPUD1
ATG9A
MLLT1
ZFAND5
SLC35A2
SLC35A3
HMOX1
PPP1R3C
PPP1R3B
SCAP
DHCR24
ACACA
DHCR24
VLDLR
CDC1
TFRC
NEO1
VEGFA
PDK1

Figure 2

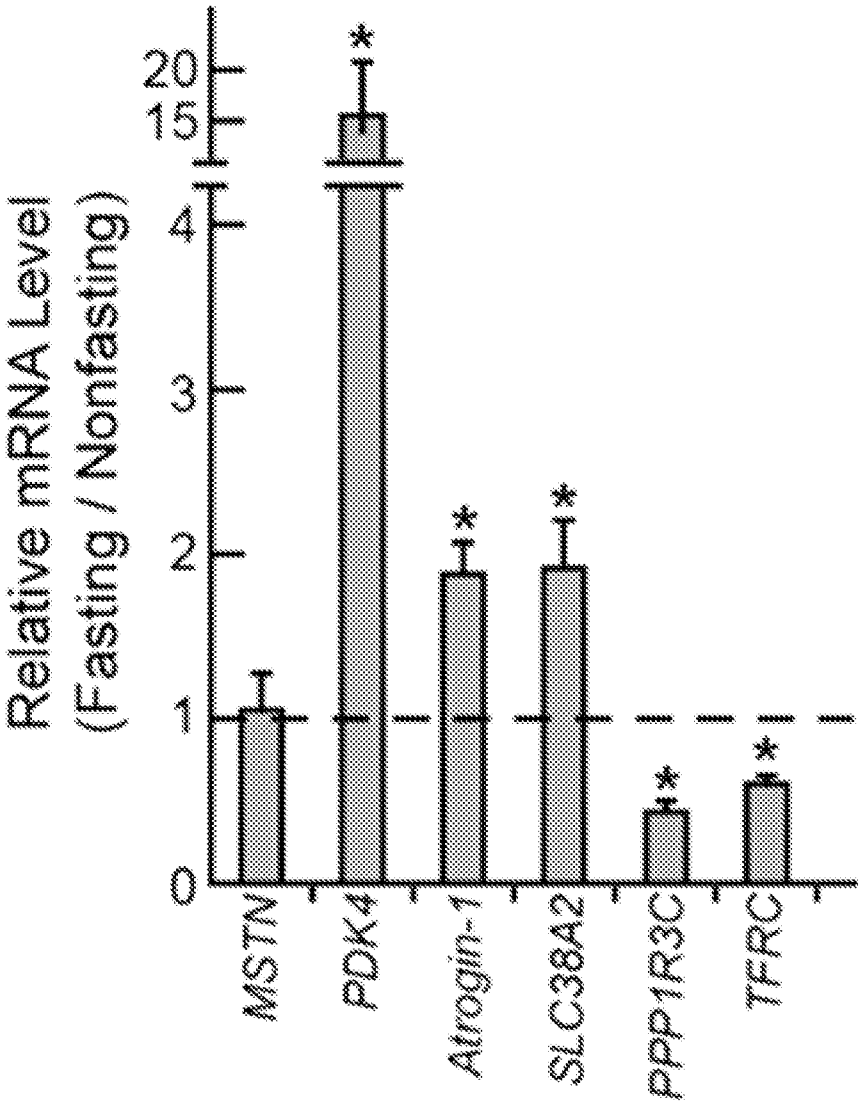

Relative mRNA Level
(Fasting / Nonfasting)
20
15
4
3
2
1
0
*
*
*
*
*
MSTN
PDK4
Atrogin-1
SLC38A2
PPP1R3C
TFRC

Figure 3

A

Conserved Effects of Fasting on Human and Mouse Skeletal Muscle	
Induced mRNAs	Repressed mRNAs
ABCA1	ACACA
ACOX1	BPGM
ADPGK	CACNB1
CALCOCO1	CASQ1
CAT	CNNM4
CITED2	DNMT3A
CPT1A	FEZ2
GABARAPL1	GAS2
HERPUD1	GRTP1
HMOX1	HSPH1
IGF1R	JTB
INSR	MRPS15
MED13L	MTSS1
MYO5A	NEO1
NBR1	NFYA
NOX4	P4HA2
PDK4	PBX1
PPAP2B	PDE7B
RORA	PMP22
SESN1	PGC-1α
SFRS8	PTX3
SLC38A2	SLC4A4
SRRM2	SPINT2
SUPT6H	ST8SIA5
TULP3	SUV39H2
TXNIP	TFRC
UBE4A	TGFB2
UCP2	TSPAN13
UCP3	TTLL1
XPO4	VEGFA
ZFAND5	WDR1
	ZNF280B

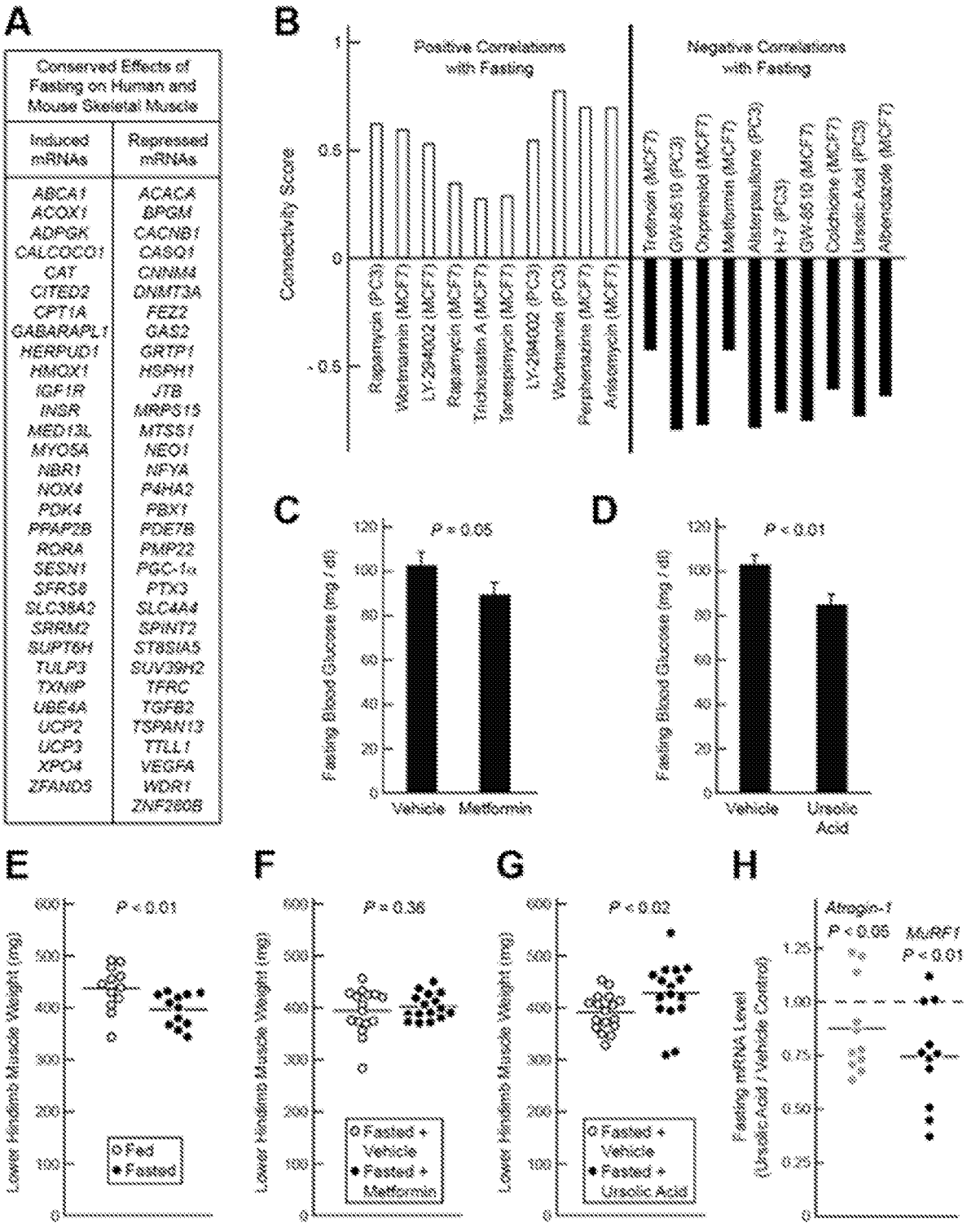


Figure 4

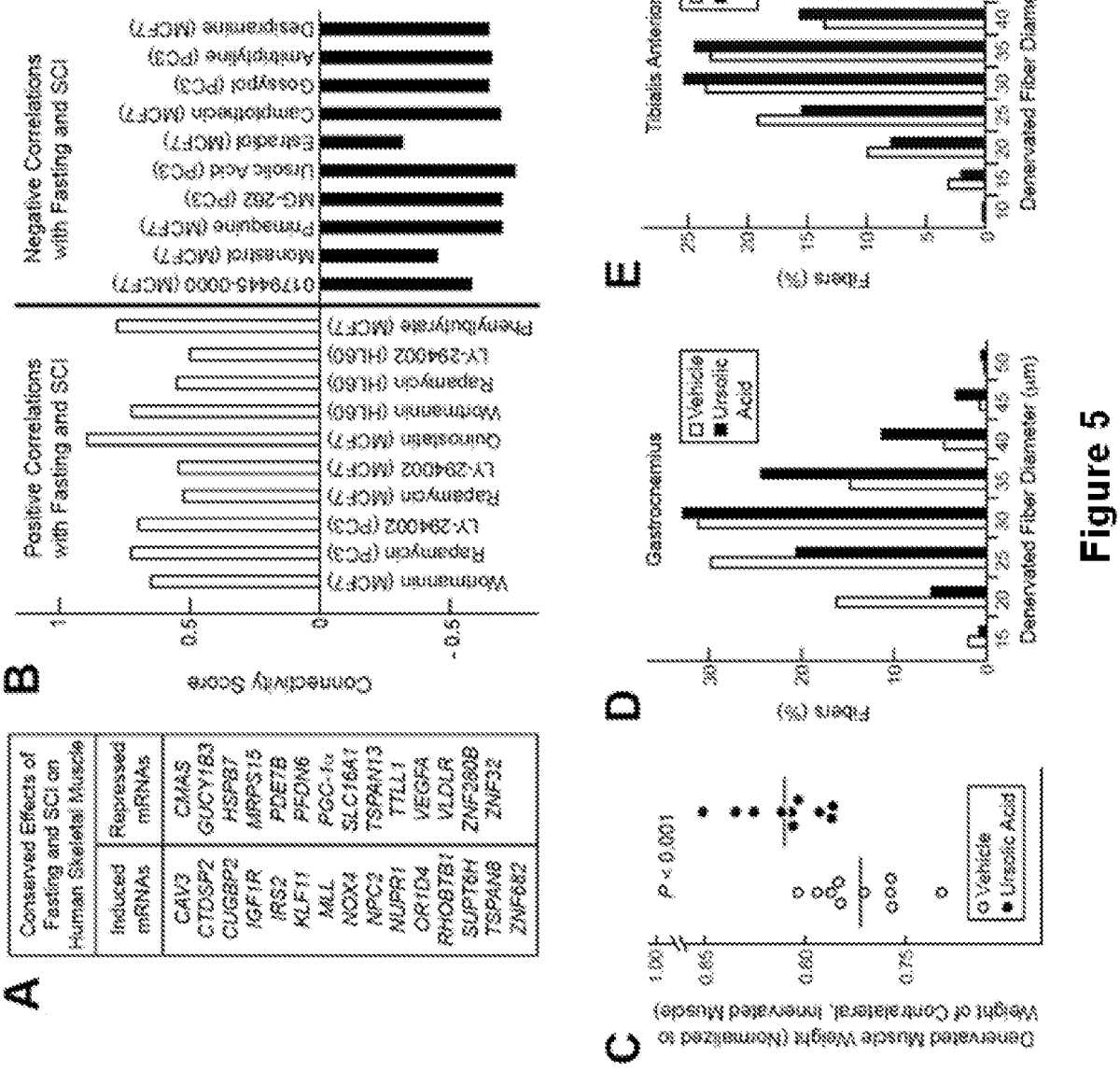

A
Conserved Effects of Fasting and SCI on Human Skeletal Muscle
Induced mRNAs
CAV2
CTDSP2
CUGBP2
IGF1R
IRS2
KLF11
MLL
NOX4
NPC2
NUPR1
OR1D4
RHOBTB1
SUPT6H
TSPAN8
ZNF282
Repressed mRNAs
CNAS
GUCY1B3
HSPB7
MRPS15
PDE7B
PFDN6
PGC-1α
SLC16A1
TSPAN13
TTLL1
VEGFA
VLDLR
ZNF280B
ZNF32
B
Positive Correlations with Fasting and SCI
Negative Correlations with Fasting and SCI
Connectivity Score
1
0.5
0
-0.5
Vorinostat (MCF7)
Rapamycin (PC3)
LY-294002 (PC3)
Rapamycin (MCF7)
LY-294002 (MCF7)
Quinostatin (MCF7)
Wortmannin (HL60)
Rapamycin (HL60)
LY-294002 (HL60)
Phenbutyrate (MCF7)
0179445-0000 (MCF7)
Monensin (MCF7)
Fmesquine (MCF7)
MG-262 (PC3)
Ursolic Acid (PC3)
Estradiol (MCF7)
Camptothecin (MCF7)
Gossypol (PC3)
Anisomycin (PC3)
Desipramine (MCF7)
C
Denervated Muscle Weight (Normalized to Weight of Contralateral, Innervated Muscle)
1.00
0.85
0.60
0.75
P < 0.001
Vehicle
Ursolic Acid
D
Gastrocnemius
Vehicle
Ursolic Acid
Fibers (%)
30
20
10
0
Denervated Fiber Diameter (µm)
10 15 20 25 30 35 40 45 50 55
E
Tibialis Anterior
Vehicle
Ursolic Acid
Fibers (%)
20
30
15
10
5
0
Denervated Fiber Diameter (µm)
10 15 20 25 30 35 40 45 50 55

Figure 5

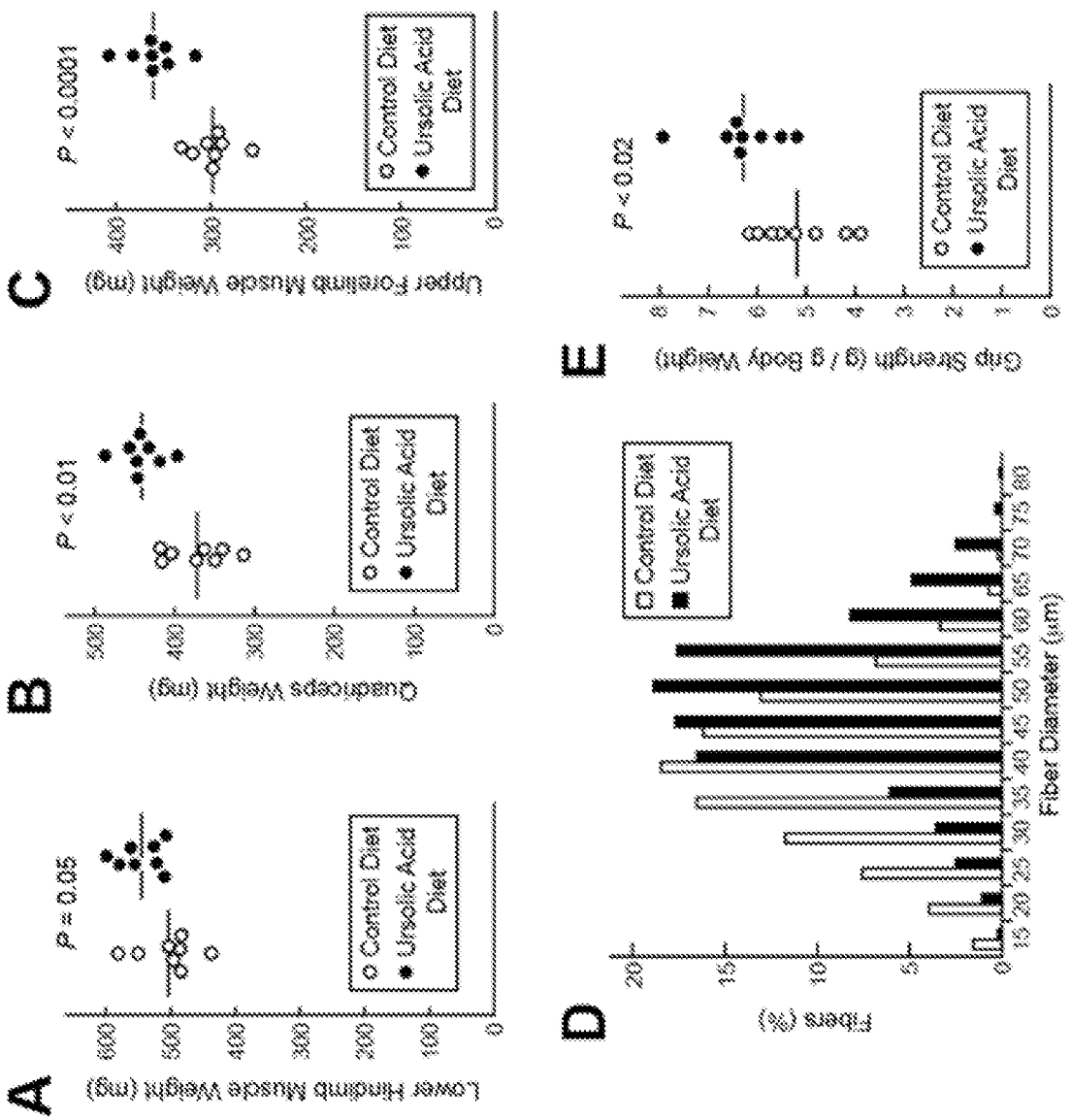

A
Lower Hindlimb Muscle Weight (mg)
P = 0.05
Control Diet
Ursolic Acid Diet
B
Quadriceps Weight (mg)
P < 0.01
Control Diet
Ursolic Acid Diet
C
Upper Forelimb Muscle Weight (mg)
P < 0.0001
Control Diet
Ursolic Acid Diet
D
Fibers (%)
Fiber Diameter (µm)
Control Diet
Ursolic Acid Diet
E
Grip Strength (g / g Body Weight)
P < 0.02
Control Diet
Ursolic Acid Diet

Figure 6

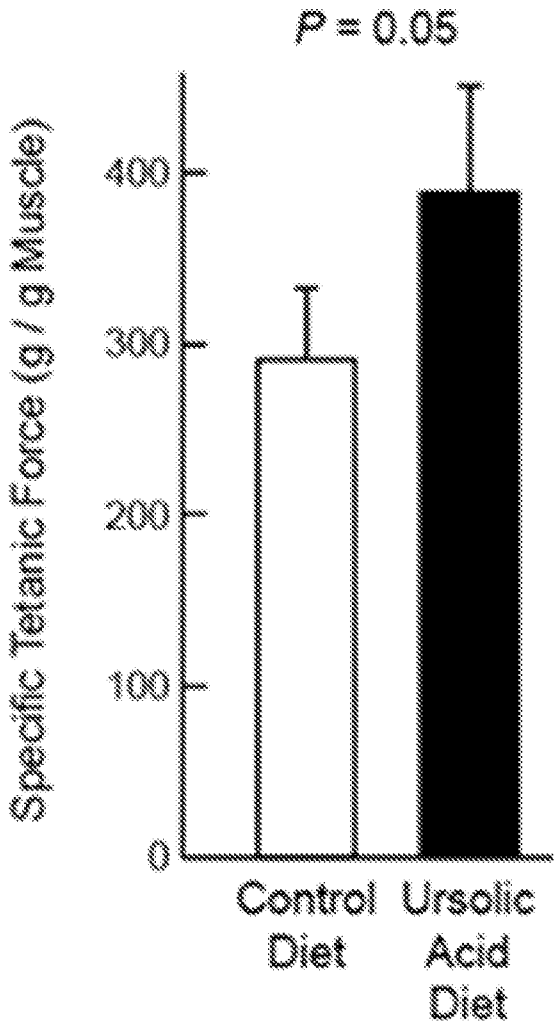

P = 0.05
Specific Tetanic Force (g / g Muscle)
400
300
200
100
0
Control Diet
Ursolic Acid Diet

Figure 7

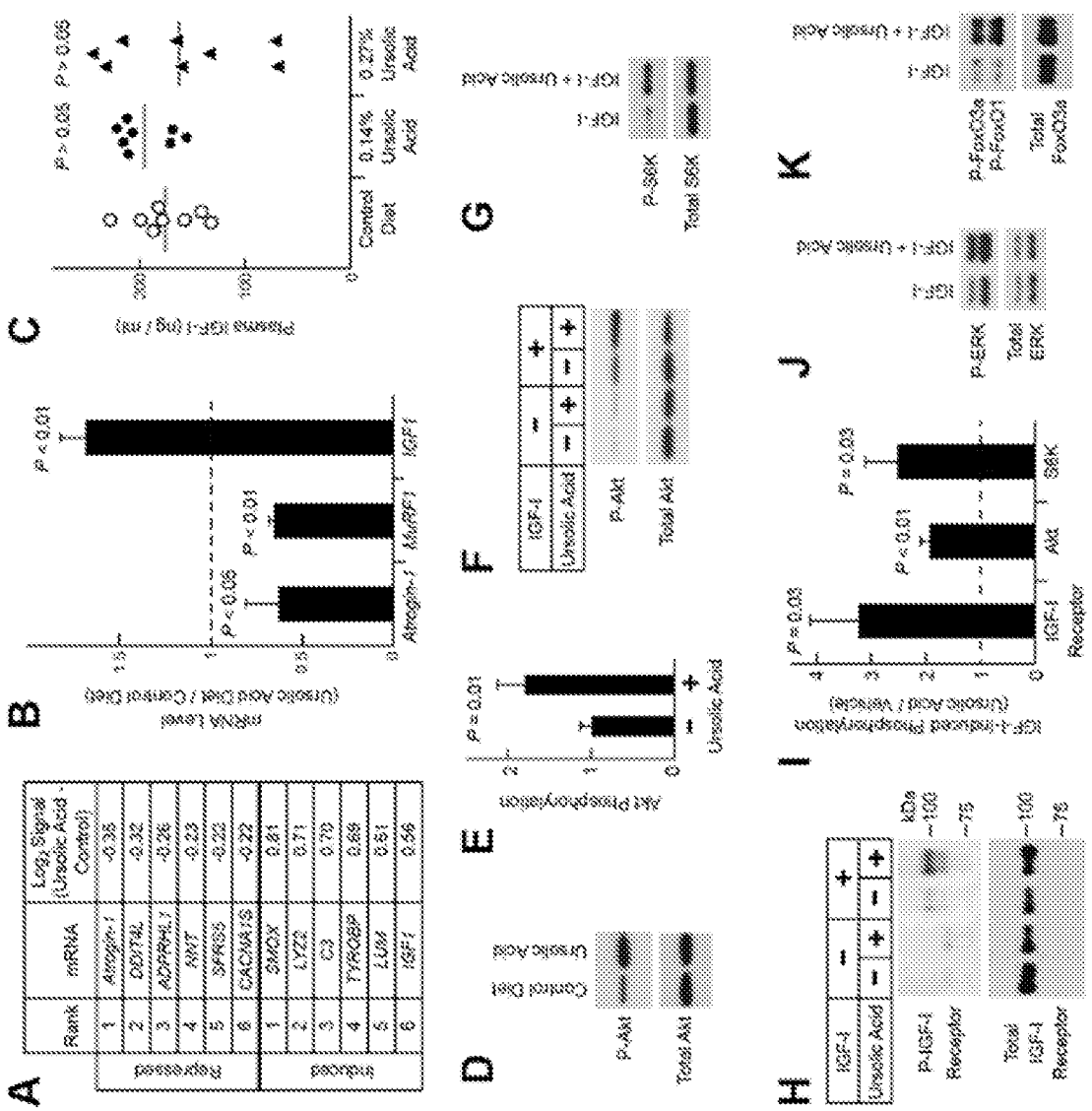

A
Repressed
Induced
Rank
mRNA
Log2 Signal (Ursolic Acid - Control)
1 Angptl-1 -0.35
2 DDIT4L -0.32
3 ADFRHL1 -0.26
4 KHT -0.23
5 SFRS5 -0.23
6 CACNA1S -0.22
1 SMOX 0.81
2 LYZ2 0.71
3 C3 0.70
4 TYROBP 0.69
5 LUM 0.61
6 IGF1 0.56
B
mRNA Level (Ursolic Acid Diet / Control Diet)
P < 0.01
P < 0.01
P < 0.05
Angptl-1
MuRF1
IGF1
C
Plasma IGF-1 (ng / ml)
P > 0.05
P > 0.05
Control Diet
0.14% Ursolic Acid
0.27% Ursolic Acid
D
Control Diet
Ursolic Acid
P-Akt
Total Akt
E
Akt Phosphorylation
P = 0.01
Ursolic Acid
F
IGF-I
Ursolic Acid
P-Akt
Total Akt
G
IGF-I
IGF-I + Ursolic Acid
P-S6K
Total S6K
H
IGF-I
Ursolic Acid
kDa
~100
~75
P-IGF-I Receptor
Total IGF-I Receptor
I
IGF-1-Induced Phosphorylation (Ursolic Acid / Vehicle)
P = 0.03
P < 0.01
P = 0.03
IGF-1 Receptor
Akt
S6K
J
IGF-I
IGF-I + Ursolic Acid
P-ERK
Total ERK
K
IGF-I
IGF-I + Ursolic Acid
P-FoxO3a
P-FoxO1
Total FoxO3a

Figure 8

A

			Log2 Hybridization Signals			
Probe ID	Probe Position	IGF1 Exon	Control	Ursolic Acid	Ursolic - Control	P
4883942	chr 10: 87322947 - 87323726	2	5.65	5.93	0.28	0.14
4714856	chr 10: 87324043 - 87324118	2	7.28	7.92	0.65	0.00
5387208	chr 10: 87327493 - 87327614	3	8.32	8.72	0.40	0.01
5261482	chr 10: 87376384 - 87376503	4	9.73	10.28	0.54	0.01
5254483	chr 10: 87378043 - 87378085	5	5.73	6.73	1.00	0.02
5298449	chr 10: 87378108 - 87378172	5	5.77	6.17	0.40	0.20
5075824	chr 10: 87378301 - 87378413	5	7.61	8.53	0.93	0.04
5467780	chr 10: 87393446 - 87393470	6	9.65	9.90	0.25	0.16

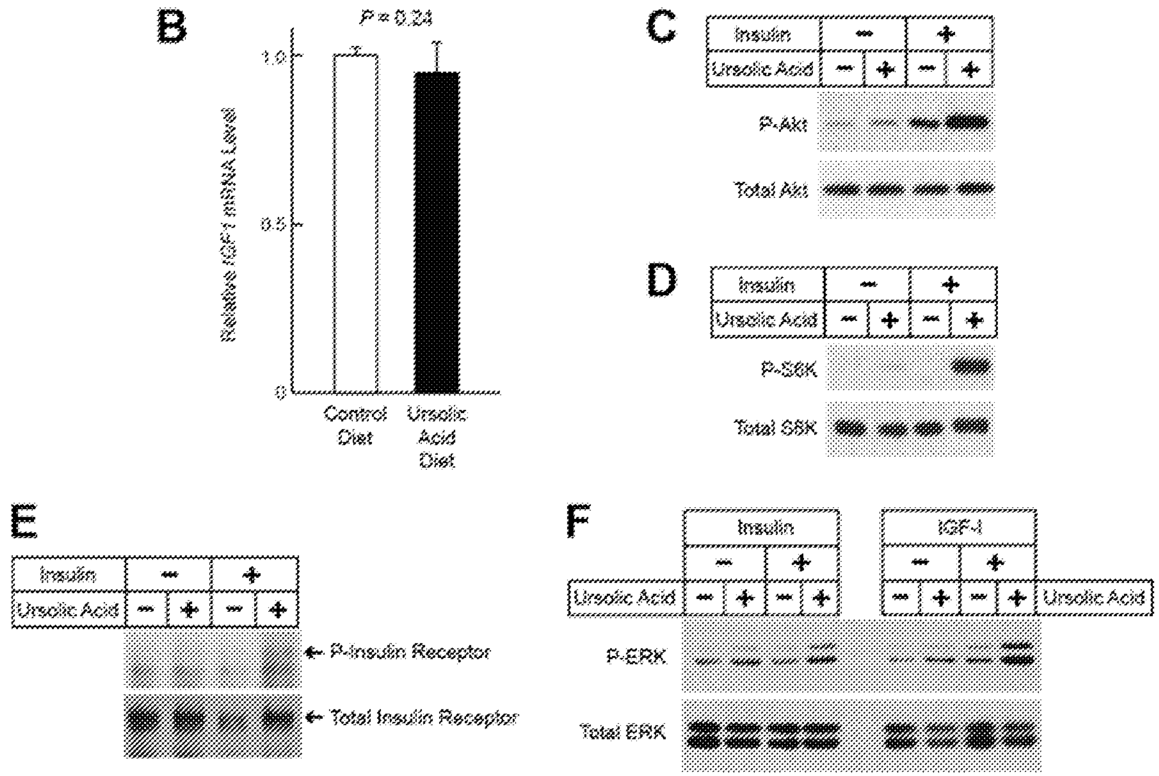


Figure 9

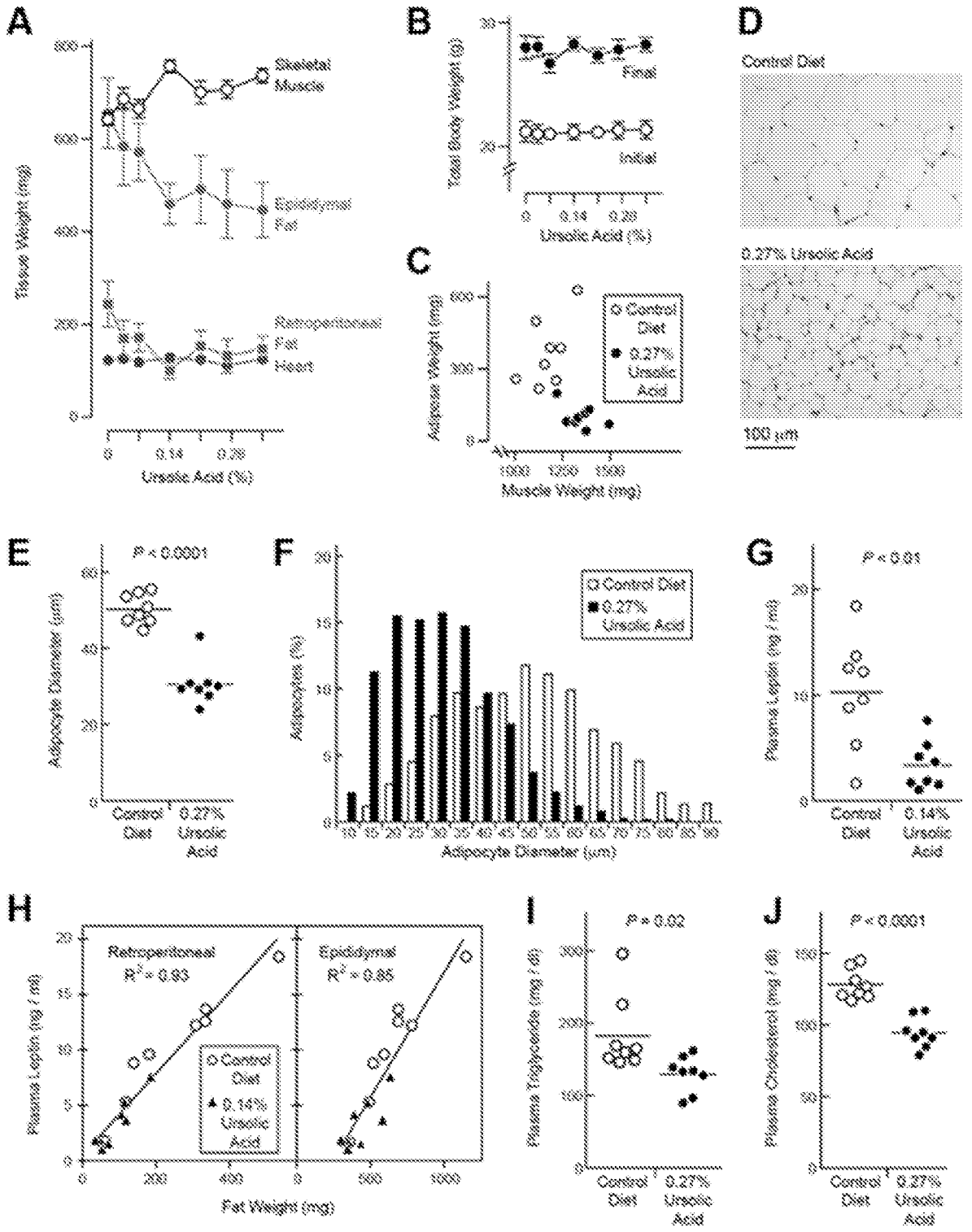

A
Tissue Weight (mg)
Skeletal Muscle
Epididymal Fat
Retroperitoneal Fat
Heart
Ursolic Acid (%)
B
Total Body Weight (g)
Final
Initial
Ursolic Acid (%)
C
Adipose Weight (mg)
Control Diet
0.27% Ursolic Acid
Muscle Weight (mg)
D
Control Diet
0.27% Ursolic Acid
100 µm
E
P < 0.0001
Adipocyte Diameter (µm)
Control Diet
0.27% Ursolic Acid
F
Adipocytes (%)
Control Diet
0.27% Ursolic Acid
Adipocyte Diameter (µm)
G
P < 0.01
Plasma Leptin (ng / ml)
Control Diet
0.14% Ursolic Acid
H
Retroperitoneal
R² = 0.93
Epididymal
R² = 0.85
Plasma Leptin (ng / ml)
Control Diet
0.14% Ursolic Acid
Fat Weight (mg)
I
P = 0.02
Plasma Triglyceride (mg / dl)
Control Diet
0.27% Ursolic Acid
J
P < 0.0001
Plasma Cholesterol (mg / dl)
Control Diet
0.27% Ursolic Acid

Figure 10

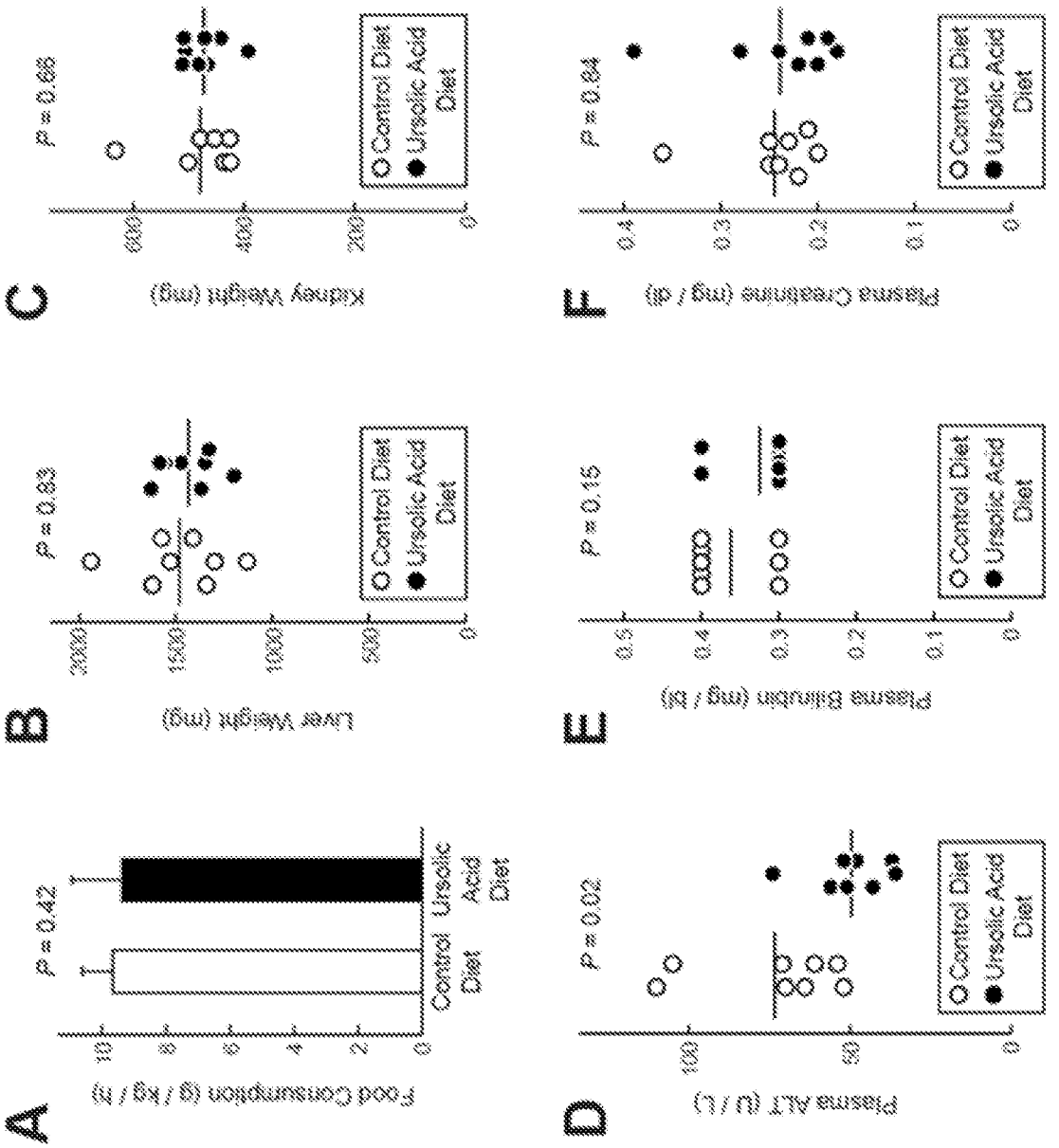

A
Food Consumption (g / kg / h)
P = 0.42
Control Diet
Ursolic Acid Diet
B
Liver Weight (mg)
P = 0.83
Control Diet
Ursolic Acid Diet
C
Kidney Weight (mg)
P = 0.66
Control Diet
Ursolic Acid Diet
D
Plasma ALT (U / L)
P = 0.02
Control Diet
Ursolic Acid Diet
E
Plasma Bilirubin (mg / dl)
P = 0.16
Control Diet
Ursolic Acid Diet
F
Plasma Creatinine (mg / dl)
P = 0.64
Control Diet
Ursolic Acid Diet

Figure 11

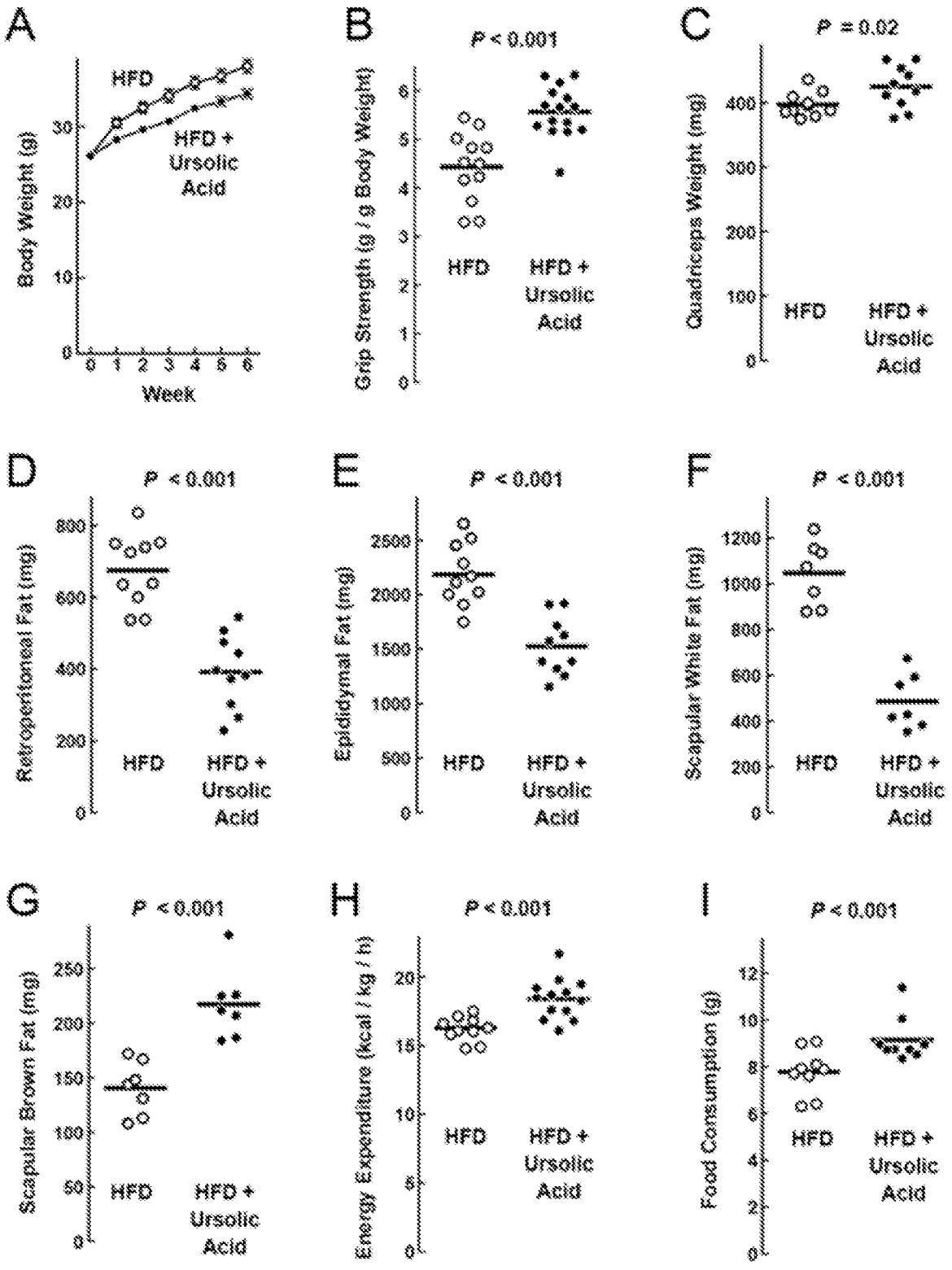

A
Body Weight (g)
HFD
HFD + Ursolic Acid
Week
B
P < 0.001
Grip Strength (g / g Body Weight)
HFD
HFD + Ursolic Acid
C
P = 0.02
Quadriceps Weight (mg)
HFD
HFD + Ursolic Acid
D
P < 0.001
Retroperitoneal Fat (mg)
HFD
HFD + Ursolic Acid
E
P < 0.001
Epididymal Fat (mg)
HFD
HFD + Ursolic Acid
F
P < 0.001
Scapular White Fat (mg)
HFD
HFD + Ursolic Acid
G
P < 0.001
Scapular Brown Fat (mg)
HFD
HFD + Ursolic Acid
H
P < 0.001
Energy Expenditure (kcal / kg / h)
HFD
HFD + Ursolic Acid
I
P < 0.001
Food Consumption (g)
HFD
HFD + Ursolic Acid

Figure 12

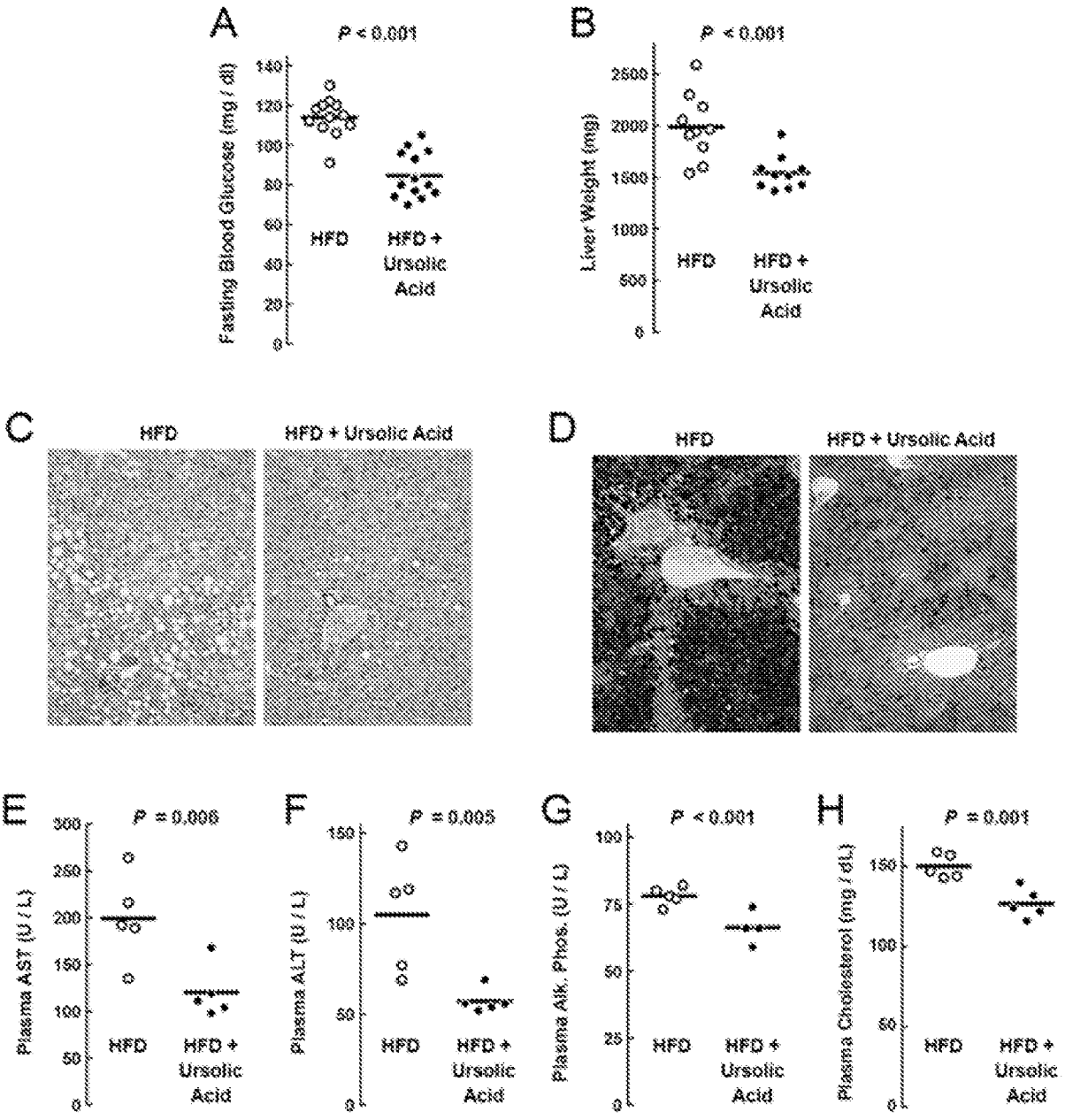

A
P < 0.001
Fasting Blood Glucose (mg / dl)
140
120
100
80
60
40
20
0
HFD
HFD + Ursolic Acid
B
P < 0.001
Liver Weight (mg)
2500
2000
1500
1000
500
0
HFD
HFD + Ursolic Acid
C
HFD
HFD + Ursolic Acid
D
HFD
HFD + Ursolic Acid
E
P = 0.006
Plasma AST (U / L)
300
250
200
150
100
50
0
HFD
HFD + Ursolic Acid
F
P = 0.005
Plasma ALT (U / L)
150
100
50
0
HFD
HFD + Ursolic Acid
G
P < 0.001
Plasma Alk. Phos. (U / L)
100
75
50
25
0
HFD
HFD + Ursolic Acid
H
P = 0.001
Plasma Cholesterol (mg / dL)
150
100
50
0
HFD
HFD + Ursolic Acid

Figure 13

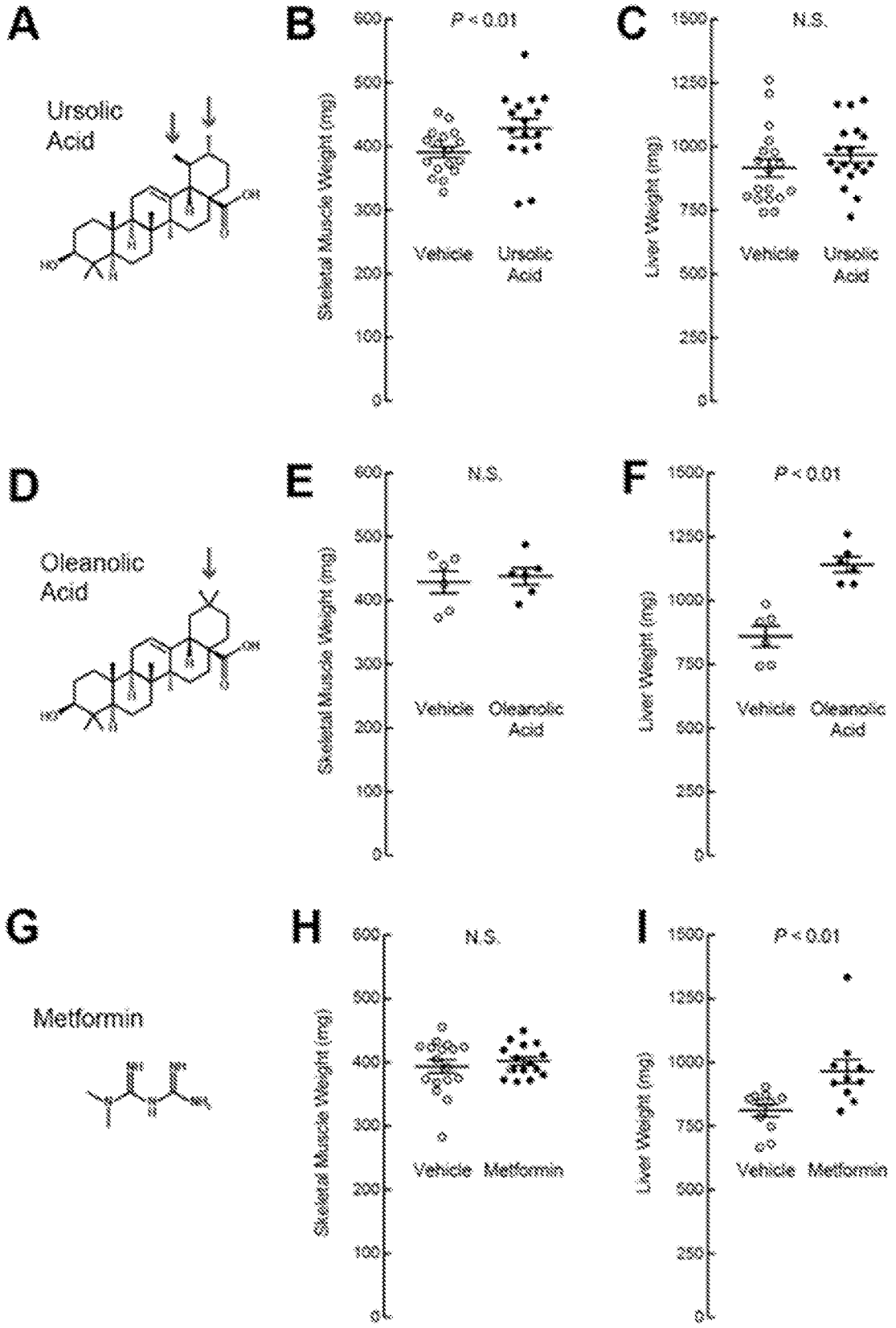

A
Ursolic Acid
B
P < 0.01
Skeletal Muscle Weight (mg)
Vehicle
Ursolic Acid
C
N.S.
Liver Weight (mg)
Vehicle
Ursolic Acid
D
Oleanolic Acid
E
N.S.
Skeletal Muscle Weight (mg)
Vehicle
Oleanolic Acid
F
P < 0.01
Liver Weight (mg)
Vehicle
Oleanolic Acid
G
Metformin
H
N.S.
Skeletal Muscle Weight (mg)
Vehicle
Metformin
I
P < 0.01
Liver Weight (mg)
Vehicle
Metformin

Figure 14

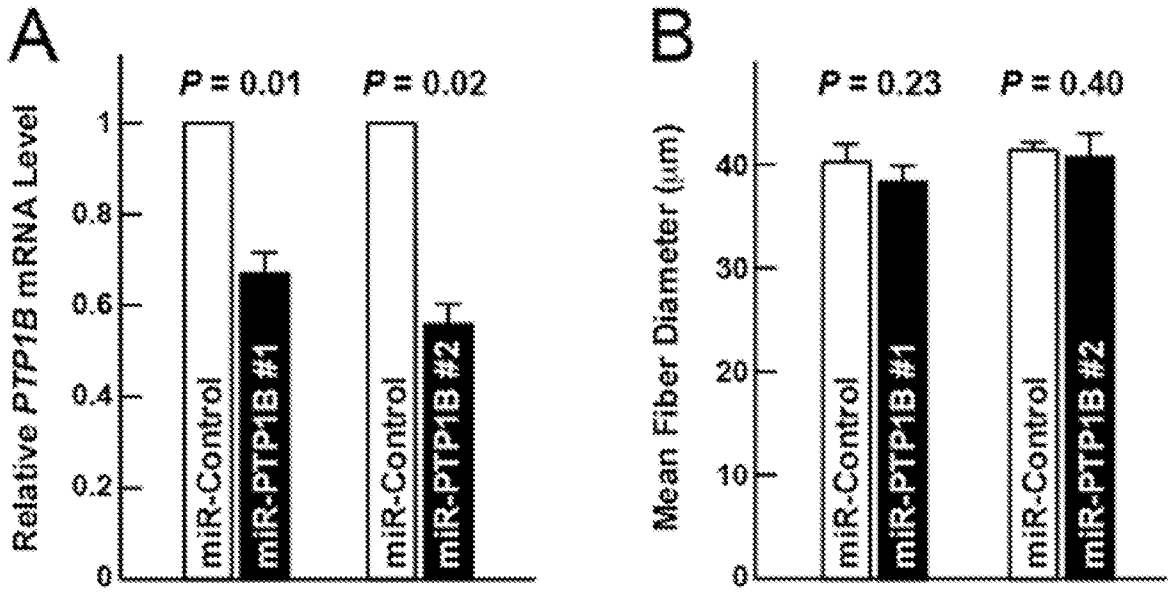


Figure 15

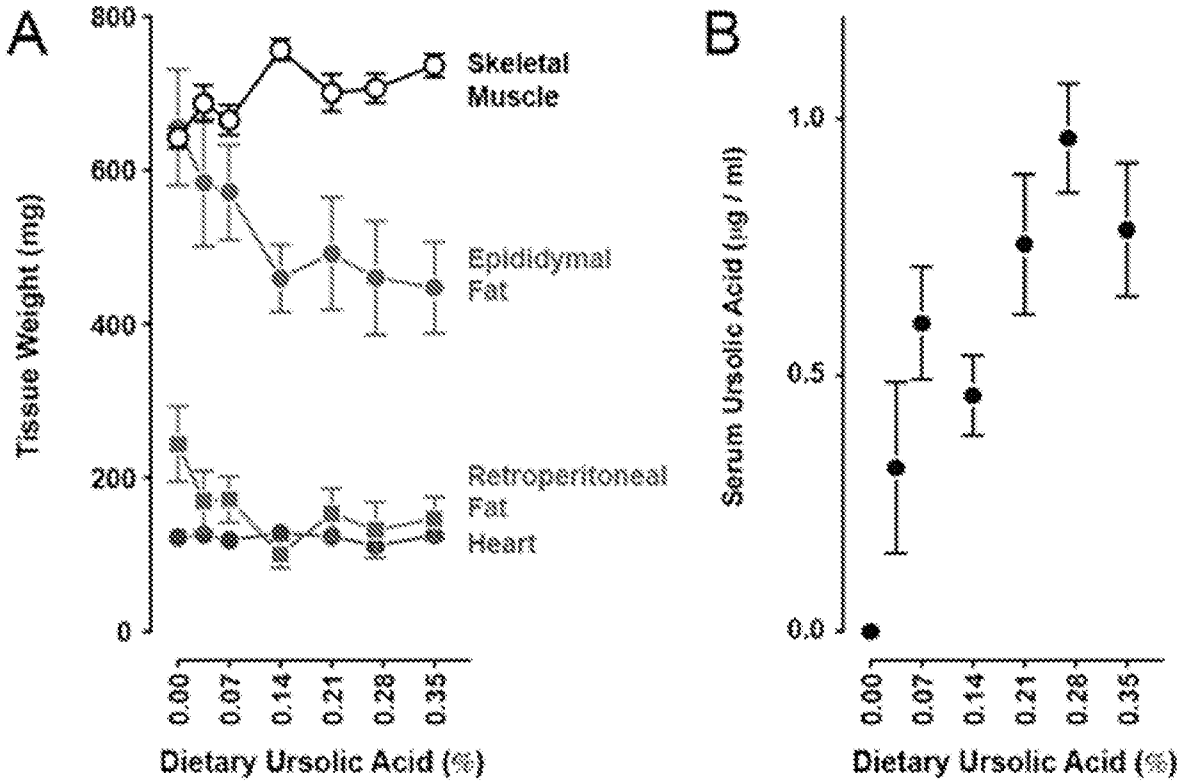
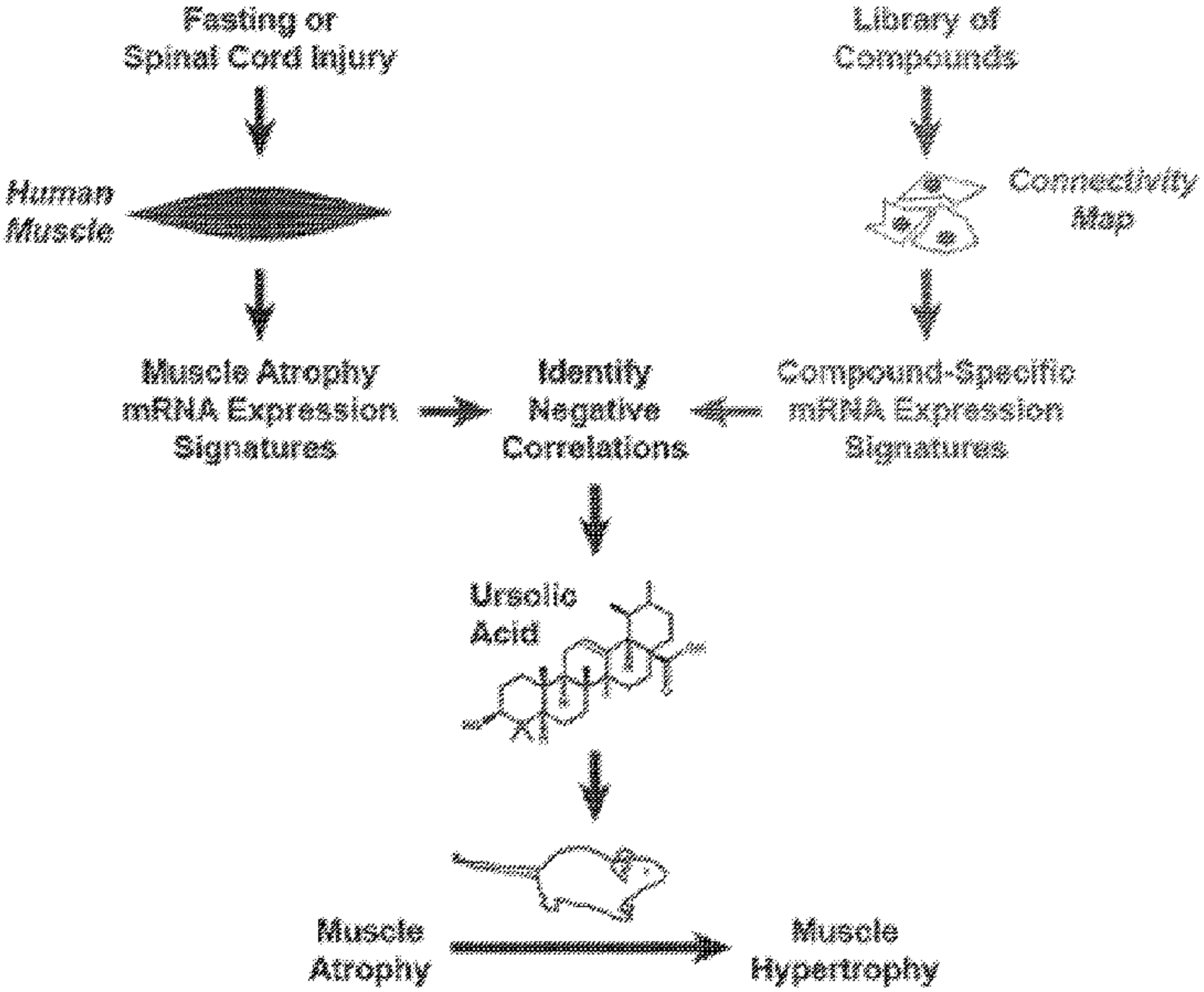


Figure 16


Fasting or
Spinal Cord Injury
Human
Muscle
Library of
Compounds
Connectivity
Map
Muscle Atrophy
mRNA Expression
Signatures
Identify
Negative
Correlations
Compound-Specific
mRNA Expression
Signatures
Ursolic
Acid
Muscle
Atrophy
Muscle
Hypertrophy