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(54) Titre : UTILISATION DE CYCLODEXTRINES DANS DES MALADIES ET DES TROUBLES IMPLIQUANT UN
DEREGLEMENT DES PHOSPHOLIPIDES
(54) Title: USE OF CYCLODEXTRINS IN DISEASES AND DISORDERS INVOLVING PHOSPHOLIPID
DYSREGULATION

(57) **Abrégé/Abstract:**

In some embodiments, the present disclosure provides certain compositions and methods that may be useful in the treatment and/or prevention of malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition, such as carcinomas, Alzheimer's and Parkinson's disease, multiple sclerosis, Paget's disease, or other aspects of aging, such as atherosclerosis or type-2 diabetes. In some such embodiments, compositions are provided that contain at least one cyclodextrin active agent, such as alpha-cyclodextrin, or an analogue or derivative thereof. In some embodiments, the composition is a clathrate of hydroxypropyl-alpha-cyclodextrin and sodium caprate.

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(54) Title: USE OF CYCLODEXTRINS IN DISEASES AND DISORDERS INVOLVING PHOSPHOLIPID DYSREGULATION

(57) Abstract: In some embodiments, the present disclosure provides certain compositions and methods that may be useful in the treatment and/or prevention of malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition, such as carcinomas, Alzheimer's and Parkinson's disease, multiple sclerosis, Paget's disease, or other aspects of aging, such as atherosclerosis or type-2 diabetes. In some such embodiments, compositions are provided that contain at least one cyclodextrin active agent, such as alpha-cyclodextrin, or an analogue or derivative thereof. In some embodiments, the composition is a clathrate of hydroxypropyl-alpha-cyclodextrin and sodium caprate.



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USE OF CYCLODEXTRINS IN DISEASES AND DISORDERS INVOLVING PHOSPHOLIPID DYSREGULATION

RELATED APPLICATIONS

This application claims the benefit of priority to U.S. Provisional Application No. 62/679,912, filed June 3, 2018, U.S. Provisional Application No. 62/643,694, filed March 15, 2018, U.S. Provisional Application No. 62/586,826, filed November 15, 2017, U.S. Provisional Application No. 62/573,658, filed October 17, 2017, and U.S. Provisional Application No. 62/565,053, filed September 28, 2017. The entire contents of the aforementioned applications are incorporated by reference in their entireties.

BACKGROUND

[001] As the human population is aging, the prevalence of age-related conditions, including, but not limited to, cancers and neurodegenerative diseases increases, yet interventions to prevent or treat these conditions are lacking or have undesirable side effects. Currently, about 5 million US individuals have Alzheimer's disease and about 1 million have Parkinson's disease. As life expectancy increases, the prevalence of neurodegenerative diseases or disorders also increases. Ten percent of people age 65 and older and 15% of people age 75 or older have Alzheimer's disease. Alzheimer's disease also disproportionately affects women, who comprise two thirds of Americans with Alzheimer's disease. Alzheimer's disease is also more likely to affect members of African American and Hispanic communities than Caucasian communities. Patients with Alzheimer's disease have few treatment options. Several recent phase 3 studies of BACE1 inhibitors have failed. Similarly, patients with Parkinson's disease initially benefit from treatment of motor symptoms (levodopa), but become non-responsive over time.

[002] Breast cancer (BCa) is the most common non-cutaneous malignancy in women. In 2016, 246,660 U.S. women were newly diagnosed, and 3,100,000 U.S. women lived with breast cancer. After lung cancer has been declining 30% since 1990, breast cancer is the second most deadly cancer, causing 40,290 deaths in 2015, 17,010 before the age of 65 (most from metastases to bone, lung, and brain). As women with a family history of breast cancer have a two-fold risk of developing breast cancer, (American Cancer Society (2015)) a substantial portion of breast cancer risk is expected to be inherited, yet the known complexes of 'cancer growth genes' PIK3CA/PTEN as well as DNA damage repair genes

BRCA1/BRCA2/PALB2 and MRE11A/RAD50/NBN/RINT1, explain only 10% of the incidence.

[003] Treatments of malignant diseases or disorders are also limited and suffer from numerous drawbacks. Treatments generally include local therapy (for instance: surgery with or without radiation in breast cancer, surgery or radiation in prostate cancer) and adjuvant systemic therapy (hormonal therapy, chemotherapy, and biologic agents) for cancer cells that may have spread. Radiation and chemotherapy often cause substantial side-effects including, but not limited to, nausea and hair loss. Hormone therapy for prostate cancer includes anti-androgens. For some hormone-receptor-positive forms of breast cancer, selective estrogen receptor modulators (SERM), such as tamoxifen and raloxifene, and aromatase inhibitors, such as exemestane and anastrozole, can interfere with disease progression. Monoclonal antibodies, such as trastuzumab and pertuzumab, are approved for the treatment of HER2 positive cancer. For patients with triple-negative breast cancer (absence of estrogen, progesterone, and the Her2/neu receptor), treatment options are limited.

[004] A common thread between neurodegenerative disorders and malignancies are lysosomal dysfunction. Lysosomal dysfunction is implicated in a diverse range of disorders and diseases, including genetic diseases. In some cases, high levels of endocytosis have been suggested as a pleiotropic factor in the etiology underlying malignant and neurodegenerative processes, including, but not limited to, a role of endocytosis in cancer, and “deranged” endocytosis in Alzheimer’s disease, where endocytic pathway abnormalities precede A β deposition and inhibition of endocytosis reduces amyloid precursor protein (“APP”) internalization and immediately lowers A β levels *in vivo*.

[005] While sufficient levels of endocytosis are necessary in early life, high levels of endocytosis may become detrimental with age. Enlarged macrophages have been identified in atherosclerosis, steatohepatitis, and cystic fibrosis, and high levels of endocytosis can increase accumulation of lipids in macrophages. Activation of the early endocytic pathway has been observed in autoimmune-diseases, such as systemic lupus erythematosus (SLE), inflammatory bowel disease (IBD), and arthritis. Attenuation of phosphoinositides (PIPs), which regulate endocytosis, restrains autoimmune disease and phosphoinositides have also been linked to several genetic diseases. Impaired phospholipid (PL) efflux increases accumulation of lipids in macrophages (M Φ s) and enlarged macrophages have been identified in atherosclerosis, steatohepatitis, and cystic fibrosis (CF). Inhibition of signaling

by the phosphoinositide-3 kinase (PI3K) has been shown to benefit inflammatory and autoimmune diseases as well as hematological malignancies. Several attempts to modulate specific phosphatases or kinases, however, have failed to result in successful therapies. Hence, in spite of strong evidence for involvement of endo-/pinocytosis in all these diseases and for involvement of phosphoinositides, effective modulators of endocytosis are lacking.

[006] Activation of the early endocytic pathway can lead to accumulation of lipids in macrophages and enlarged macrophages have been identified in atherosclerosis, steatohepatitis, and cystic fibrosis. Activation of the early endocytic pathway has been observed in autoimmune-diseases, such as systemic lupus erythematosus (SLE), inflammatory bowel disease, and arthritis. Attenuation of phosphoinositides restrains autoimmune disease. Broadly acting [cyclin-dependent kinase (CDK)] inhibitors yielded largely disappointing results, and only a small number of patients benefit from phosphoinositide 3-kinase (PI3K) inhibitors. Overall, dysregulation of phospholipids has been linked to poor quality of life with aging.

[007] Because overactive endocytosis has been linked to cancers, neurodegenerative diseases, and other pathological conditions, the same treatment that prevents metastases in cancer could also prevent accumulation of undegraded macromolecules in neurodegenerative diseases and many other age-related conditions, including formation of foam cells in atherosclerosis via macropinocytosis. Yet, effective treatments to regulate endocytosis are lacking. Therefore, there is a need for effective treatments for the symptoms of disorders and diseases that involve dysfunction involving endocytosis, phagocytosis, pinocytosis, and lysosomal pathways.

[008] The lipid-carrier molecule β -cyclodextrin (β -CD) is “generally recognized as safe” (GRAS) as food additives and the more water-soluble derivative 2-hydroxypropyl- β -cyclodextrin (HP- β -CD, HP β CD) is frequently used as an excipient to form water soluble compounds with lipophilic drugs. *In vitro* and in animal models, HP- β -CD has been shown to exert some of its effect by reducing lysosomal dysfunction (LYD) and improving autophagy ($A\Phi$), another common factor in the etiology of age-related conditions. In humans, HP- β -CD has been used for the treatment of Niemann-Pick disease type C (NPC), where its function is to reduce the aggregation of cholesterol, a lipid molecule, in lysosomes (LYs). Again, the mechanism of action is unknown. Clinical applications of HP- β -CD have been limited,

however, because depriving the ear's outer hair cells of cholesterol may cause permanent hearing loss.

[009] It has not yet been appreciated that derivatives of the smaller α -cyclodextrin (α -CD, α CD) and its derivatives, such as hydroxypropyl- α -cyclodextrin (HP- α -CD, HP α CD), which are too small to fit cholesterol, may effectively treat malignancies and neurodegenerative disorders without the undesirable side effects, such the hearing loss caused by beta-cyclodextrin.

SUMMARY

[010] Through the application of computational biostatistics and decision strategies to data from genome-wide association studies (GWAS), we have identified functionally related collections of genes and determined that endocytosis, which is controlled by phosphoinositides (PIP) comprising those generated by the PI cycle, is involved in the metastases process of certain types of cancers such as breast or prostate cancer. In this regard, we established that breast cancer risk is conferred not only by (rare) variations in DNA damage repair genes, such as BRCA1/2, but more frequently by a global dysregulation of the PI cycle and that endocytosis, which is known to be controlled by the PI cycle, is a critical component of local spread, migration, and invasion of cancer cells. See e.g., **Example 1**. Thus, in one aspect, provided herein are methods of targeting the PI cycle as a means for treating diseases or conditions causing a dysfunctional lysosomal pathway and/or elevated endocytosis, phagocytosis, or pinocytosis in a subject, in which the diseases or condition may be caused by e.g., aberrant expression of a gene or combination of genes as identified e.g., by the novel GWAS approach (see e.g., **Example 1**) described herein. Such diseases or conditions include e.g., breast cancer (BCa), Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), coronary artery disease (CAD), and Niemann-Pick type C disease (NPC). See e.g., **Examples 2, 3, 6, and 7**.

[011] Therapeutic agents known to have effects against animal models of carcinomas, neurodegenerative and several other age-related diseases are beta-cyclodextrins. These include e.g., methyl- β -cyclodextrin (M- β -CD) and HP- β -CD. See e.g., J Membr Biol. 2011 May, 241(1): 1–10; The Journal of Experimental Medicine, 209 (13), 2501-13, and the discussion set forth in **Example 5**. Traditionally, beta-cyclodextrins were believed to remove

and to extract cholesterol (Chol) from cell membranes and/or organelles. For example, the scavenging of cholesterol and/or binding directly to A β or α -synuclein was believed to be the mode of action for β -CDs in AD and PD. See The Journal of Experimental Medicine, 209 (13), 2501-13. Although β -CDs have therapeutic potential, they are ototoxic, i.e., cause damage to the ear which can result in permanent hearing loss. See J Assoc Res Otolaryngol, 16 (5), 599-611.

[012] Here, having for the first time identified the implication of phospholipid supply to the PI cycle for lysosomal function (see e.g., **Example 1**), the scavenging of cholesterol can no longer be viewed as the primary mechanism upon which cyclodextrins such as β -CDs function. See e.g., **Example 1**. Rather, our data show that cyclodextrins act by scavenging phospholipids and, thereby, regulate endocytosis, which reduces lysosomal stress and improves autophagy. For example, we have also found that α -CD restores what has been called “derailed” endocytosis in breast cancer, “deranged” endocytosis in AD and “defective” endocytosis in PD. See e.g., **Example 4**. We have also noted that α -CD is more efficient than β -CD in solubilizing phospholipids (see Example 8). Therefore, in one aspect, provided herein are methods of using α -cyclodextrins (e.g., α -CD or HP- α -CD) for treating a subject having a disease, disorder, or condition that involves impaired lysosomal function. Such diseases and disorders are described herein and include e.g., breast cancer (BCa), Alzheimer’s disease (AD), Parkinson’s disease (PD), Huntington’s disease (HD), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), coronary artery disease (CAD), and Niemann-Pick type C disease (NPC)

[013] In addition to the above findings, we have further realized that the absorption of alpha-cyclodextrins (e.g., HP- α -CD) from the intestine can be improved by the complexation with fatty acids. For example, we have demonstrated that a composition comprising a clathrate of HP- α -CD and medium chain fatty acids substantially improved absorption. Accordingly, in one aspect, provided herein are pharmaceutical compositions comprising an alpha-cyclodextrin (e.g., HP- α -CD) and a medium chain fatty acid (e.g., capric acid), as well as and their use for treating a disease or disorder that involves impaired lysosomal function and autophagy. Such diseases and disorders are described herein and include e.g., breast cancer (BCa), Alzheimer’s disease (AD), Parkinson’s disease (PD), Huntington’s disease (HD), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), coronary artery disease (CAD), and Niemann-Pick type C disease (NPC).

[014] In yet another aspect, we further identified that pharmaceutical compositions comprising an alpha-cyclodextrin (e.g., HP- α -CD) and a medium chain fatty acid (e.g., capric acid) can be used to treat diseases involving inflammation. See e.g., **Example 16**. Therefore, in one aspect, provided herein are pharmaceutical compositions comprising an alpha-cyclodextrin (e.g., HP- α -CD) and a medium chain fatty acid (e.g., capric acid) for use in treating inflammatory diseases.

[015] Additional aspects are further described below and in the Detailed Description and Examples sections of the application. The description in each of the sections of this patent application is intended to be read in conjunction with the other sections. Furthermore, the various embodiments described in each of the sections of this patent application can be combined in various different ways, and all such combinations are intended to fall within the scope of the present disclosure.

[016] In one aspect, the present disclosure provides a method of treating a malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition in a subject, the method comprising administering to the subject an effective amount of a cyclic oligosaccharide (e.g., α -cyclodextrin (α -CD)), or an analogue or derivative thereof (e.g., HP- α -CD), either alone or in combination with one or more additional active agents. In another aspect, the present disclosure provides a composition comprising α -CD, or an analogue or derivative thereof (e.g., HP- α -CD), for use in the treatment of an epithelial cancer (carcinoma) or the treatment of Parkinson's, Alzheimer's, or Huntington's disease. In one aspect, the composition comprising HP- α -CD further comprises a medium-length chain fatty acid (MCFA), e.g., fatty acids having an aliphatic tail of 6-12 carbon atoms. In one aspect, the HP- α -CD and medium-length chain fatty acid (MCFA) in the composition form a clathrate. In such clathrates, the MCFAs are guests of HP- α -CD. In one the MCFA in the composition is caprate or caprylate or is a salt thereof such as sodium caprate.

[017] In one aspect, the present disclosure provides a method of improving one or more indicators or symptoms of a malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition in a subject, the method comprising administering to a subject exhibiting one or more indicators or symptoms of a malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or

viral disease, disorder, or condition, an effective amount of α -CD, or an analogue or derivative thereof (e.g., HP- α -CD), either alone or in combination with one or more additional active agents. Suitable indicators include, but are not limited to, results of a blood test (including, but not limited to circulating tumor DNA and/or prostate-specific antigen), an x-ray evaluation, the result of a physical examination (including, but not limited to a palpable tumor), a psychiatric evaluation, or a tissue biopsy for histological evaluation and/or determination of hormone receptor status. In one aspect, a symptom or indicator is selected from the group consisting of survival, disease-free survival, distant metastasis-free survival, results of a blood test (including, but not limited to circulating tumor DNA and prostate-specific antigen), an x-ray evaluation, the result of a physical examination (including, but not limited to a palpable tumor), or a tissue biopsy for histological evaluation. In one embodiment, the “improving” comprises an increase of at least 1% in a measurement of the one or more indicators or symptoms. In another aspect, the present disclosure provides a composition comprising α -CD, or an analogue or derivative thereof (e.g., HP- α -CD), for use in the treatment of an epithelial cancer (carcinoma) or the treatment of Parkinson’s, Alzheimer’s, or Huntington’s disease. In one aspect, the composition comprising HP- α -CD further comprises a medium-length chain fatty acid (MCFA), e.g., fatty acids having an aliphatic tail of 6-12 carbon atoms. In one aspect, the HP- α -CD and medium-length chain fatty acid (MCFA) in the composition form a clathrate. In such clathrates, the MCFAs are guests of HP- α -CD. In one the MCFA in the composition is caprate or salt thereof such as sodium caprate.

[018] In some embodiments, the disclosure relates to a method of restoring the synthesis of sphingomyelin in a subject in need thereof, the method comprising administering a cyclodextrin (e.g., α -CD), or an analogue or derivative thereof (e.g., HP- α -CD), either alone or in combination with one or more additional active agents. In one aspect, the present disclosure provides a method of improving one or more indicators or symptoms of a malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition in a subject in need thereof, the method comprising administering to a subject exhibiting one or more indicators or symptoms of a malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition, an effective amount of α -CD, or a salt, analogue or derivative thereof (e.g., HP- α -CD), either alone or in combination with one or more additional active agents.

Suitable indicators include, but are not limited to, results of a blood test (including, but not limited to circulating tumor DNA and/or prostate-specific antigen), an x-ray evaluation, the result of a physical examination (including, but not limited to a palpable tumor), a psychiatric evaluation, or a tissue biopsy for histological evaluation and/or determination of hormone receptor status. In one aspect, a symptom or indicator is selected from the group consisting of survival, disease-free survival, distant metastasis-free survival, results of a blood test (including, but not limited to circulating tumor DNA and prostate-specific antigen), an x-ray evaluation, the result of a physical examination (including, but not limited to a palpable tumor), or a tissue biopsy for histological evaluation. In one embodiment, the “improving” comprises an increase of at least 1% in a measurement of the one or more indicators or symptoms.

[019] In one aspect, the present disclosure provides a method of treating and/or preventing focal segmental glomerulosclerosis (FSGS) and nephrotic swelling in a subject in need thereof the method comprising administering to the subject an effective amount of α -CD, a salt or an analogue or derivative thereof (e.g., HP- α -CD), either alone or in combination with one or more additional active agents. In some embodiments, the disclosure relates to a method of treating and/or preventing kidney damage caused by excess or dysfunctional sphingolipid catabolism in a subject in need thereof, the method comprising administering to the subject an effective amount of α -CD, or a salt, an analogue or derivative thereof (e.g., HP- α -CD), either alone or in combination with one or more additional active agents. In some embodiments, the disclosure relates to a method of restoring the synthesis of sphingomyelin in a subject in need thereof, the method comprising administering α -CD, or an analogue or derivative thereof (e.g., HP- α -CD or salt thereof), either alone or in combination with one or more additional active agents.

[020] In other embodiments the compositions described herein comprise resorption enhancers known in the art including, but not limited to bile salts (sodium glycocholate, sodium deoxycholate, sodium taurocholate, sodium fusidate, sodium glycodeoxycholate, sodium taurodihydrofusidate), surfactants (sodium lauryl sulfate, lysophosphatidylcholine (LPC), dioctyl sodium sulfosuccinate, laurenth-9, polysorbate, polyethyleneglycol-8-laurate, glyceryl monolaurate, glyceryl monocaprylate/caprate, glyceryl dicaprylate/caprate, saponin), fatty acids and derivatives (sorbitan laurate, sodium caprate, sucrose palmitate, lauroyl choline, sodium myristate, palmitoyl carnitine), glycerides (phospholipids, monohexanoin,

medium chain glycerides), chelators (ethylene diamine tetraacetate (EDTA), disodium EDTA), salicylates (salicylic acid, sodium methoxysalicylate, acetylsalicylic acid), polymers or polysaccharides (chitosan and chitosan derivatives, dextran, polyvinyl pyrrolidone (PVP), polycarbophil, sodium carboxymethylcellulose and their derivatives, inulin, pectin, chondroitin sulfate), others (azone, benzalkonium chloride, phenothiazines, nitric acid donors, menthol), newer (zonula occluden toxin, poly-l-arginines, soybean derivative glucosides, citicholine, amino acid peptides). See (Deli MA (2009) *Biochimica et Biophysica Acta (BBA) - Biomembranes* **1788**:892-910; Renukuntla J, Vadlapudi AD, et al. (2013) *Int J Pharm* **447**:75-93; Shaikh I, Derle ND, et al. (2012) *J Appl Pharmacol Sci* **2**:34-9).

In some embodiments, the cyclodextrin is administered to the subject at a dose of about 2500 mg/kg α -CD bi-weekly ($\approx$ 700 mg/kg/d), the same dose used for HP- β -cyclodextrin in two children with NPC for “over a year, with no discernable side effects” for a “targeted concentration of 0.1–1.0 mM.” (INDs 104,114 and 104,116, approval date: 2009-04-13). In other embodiments, the dose will be adjusted over time to the highest dose not causing renal adverse events or hemolysis in the patient. In some embodiments, the cyclodextrin, such as α -CD, is administered to the subject at a dose of at least about 100 mg, at least about 200 mg, at least about 500 mg, at least about 1000 mg, at least about 2000 mg, at least about 5000 mg, or at least about 10,000 mg. In some embodiments, the cyclodextrin, such as α -CD, is administered to the subject at a dose in the range of from about 1 to about 10,000 mg, from about 1 to about 7,500 mg, from about 1 to about 5,000 mg, from about 1 to about 2,500 mg, from about 1 to about 1,000 mg, from about 1 to about 500 mg, from about 1 to about 200 mg, from about 200 to about 10,000 mg, from about 200 to about 4,000 mg, from about 200 mg about 2,000 mg, about 200 to about 1,000 mg, or about 200 to about 500 mg per day. In some such embodiments, each of the dosages described above is mg/kg/day. Additional dosages that may be used are provided in the Detailed Description section of this patent application.

[021] In some embodiments, the present disclosure provides a method of treating and/or preventing Huntingdon’s disease, Alzheimer’s disease, Parkinson’s disease, multiple sclerosis, in a subject in need thereof, the method comprising administering to the subject an effective amount of α -CD, or an analogue or derivative thereof (e.g., HP- α -CD), either alone or in combination with one or more additional active agents. In some embodiments, the present disclosure provides a method of treating and/or preventing a malignant,

neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition in a subject not exhibiting disease-specific indicators or symptoms, except indicators of the subject to belong to an at-risk subpopulation. In some embodiments, the indicator will be age. In some embodiments, the indicator will be more than 30, 40, 50, or 60 years of age.

[022] In some embodiments the present disclosure provides a method of treating or preventing a malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition in a subject in need of treatment, the method comprising administering to the subject an effective amount of a drug reducing extracellular phospholipid. In some embodiment the malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition will be an epithelial cancer (carcinoma). In some embodiments the malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition will be breast cancer. In another embodiment the malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition will be Alzheimer's disease. In some embodiments the malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition will be Parkinson's disease. In some embodiments, the malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition will be Huntington's disease. In some embodiments, malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition will be non-alcoholic steatohepatitis (NASH).

[023] In some embodiments, the present disclosure provides various combinations of treatments, including pharmaceutical compositions. In some embodiments, cyclodextrins are used in combination with established pharmaceutical, radiological, or surgical interventions comprising cytotoxic interventions, receptor antagonists, monoclonal antibodies, radiation therapy, removal of tumor tissue, and the like.

[024] In one embodiment, the subject is a human. In another embodiment the subject is an adult human. In some embodiments, the subject is in need of the treatment and/or has been

identified as having a risk of developing a disease or disorder resulting from one or a plurality of cellular defects caused by lysosomal dysfunction. In some embodiments, the disclosure relates to a composition comprising an effective amount of a clathrate of HP- α -CD and a salt, such as sodium caprate. In some embodiments, the molar ratio of HP- α -CD to the fatty acid is from about 0.5 to about 5. In some embodiments, the molar ratio of HP- α -CD to the fatty acid is from about 2.0 to about 1.0. In some embodiments, the molar ratio of HP- α -CD to the fatty acid is from about 1.5 to about 1.0. In some embodiments, the molar ratio of HP- α -CD to the fatty acid is from about 1.0 to about 1.0.

[025] In some embodiments, the disclosure relates to a pharmaceutical composition comprising an effective amount of a clathrate of HP- α -CD and a salt, such as sodium caprate, wherein the composition is free or substantially free of beta-cyclodextrin and/or α -CD. In some embodiment, the disclosure relates to a method of treating any disorder disclosed herein by administering an effective amount of a pharmaceutical composition comprising (i) one or a plurality of α -cyclodextrin, derivative thereof, or a salt thereof and (ii) a fatty acid molecule, each of (i) and (ii) at an amount in weight to form a clathrate of the one or a plurality of α -cyclodextrin, derivative thereof, or a salt thereof. In some embodiments, the clathrate is from about 80 to 90% in weight of one or a plurality of α -cyclodextrin, derivative thereof, or a salt thereof, and from about 10% to about 20% in weight of the fatty acid. In some embodiments, the clathrate is from about 85% in weight of one or a plurality of α -cyclodextrin, derivative thereof, or a salt thereof, and about 15% in weight of the fatty acid. In some embodiments, the fatty acid is capric acid or a salt thereof.

[026] The disclosure relates to a method of treating and/or preventing Alzheimer's disease in a subject in need thereof, the method comprising administering an effective amount of a pharmaceutical composition comprising one or a plurality of alpha-cyclodextrins, a derivatives, salt thereof or combinations thereof to the subject. The disclosure relates to a method of treating and/or preventing Parkinson's disease in a subject in need thereof, the method comprising administering an effective amount of a pharmaceutical composition comprising one or a plurality of alpha-cyclodextrins, a derivatives, salt thereof or combinations thereof to the subject. The disclosure relates to a method of treating and/or preventing Huntington's disease in a subject in need thereof, the method comprising administering an effective amount of a pharmaceutical composition comprising one or a plurality of alpha-cyclodextrins, a derivatives, salt thereof or combinations thereof to the

subject. The disclosure relates to a method of treating and/or preventing amyotrophic lateral sclerosis (ALS) disease in a subject in need thereof, the method comprising administering an effective amount of a pharmaceutical composition comprising one or a plurality of alpha-cyclodextrins, a derivatives, salt thereof or combinations thereof to the subject. The disclosure relates to a method of treating and/or preventing multiple sclerosis (MS) disease in a subject in need thereof, the method comprising administering an effective amount of a pharmaceutical composition comprising one or a plurality of alpha-cyclodextrins, a derivatives, salt thereof or combinations thereof to the subject. In some embodiments, any of the disclosed methods, the pharmaceutical composition comprises an effective amount of a clathrate of the one or a plurality of alpha-cyclodextrins, a derivatives, salt thereof or combinations thereof, wherein the clathrate comprises a medium-length chain of fatty acid or salt thereof and the one or a plurality of alpha-cyclodextrins, a derivatives, salt thereof or combinations thereof. In some embodiments, the one or a plurality of alpha-cyclodextrins, a derivatives, salt thereof or combinations thereof and the fatty acid are in a weight/weight ratio from about 15% weight fatty acid and about 85% weight of the one or a plurality of alpha-cyclodextrins, a derivatives, salt thereof or combinations thereof in the clathrate. In some embodiments, the weight:weight ratio of fatty acid to α -cyclodextrin is sufficient for absorption into the blood.

[027] The disclosure relates to a method of treating and/or preventing inflammation in a subject in need thereof, the method comprising administering an effective amount of a pharmaceutical composition comprising one or a plurality of alpha-cyclodextrins, a derivatives, salt thereof or combinations thereof to the subject. The disclosure also relates to a method of treating and/or preventing local metastases of a breast cancer or prostate cancer characterized by dysfunctional or deficient endocytosis caused by dysfunction or deficiency of any genes identified in the disclosure related to endocytosis. The disclosure also relates to a method of treating and/or preventing local metastases of a breast cancer or prostate cancer characterized by dysfunctional or deficient lysosomal function caused by dysfunction or deficiency of any genes identified in the disclosure related to lysosomal function. In a subject in need thereof, the method comprising administering an effective amount of a pharmaceutical composition comprising one or a plurality of alpha-cyclodextrins, a derivatives, salt thereof or combinations thereof to the subject.

[028] In some embodiments, the disclosure relates to a method of reducing phospholipid levels in the blood of a subject, the method comprising administering an effective amount of a pharmaceutical composition comprising one or a plurality of alpha-cyclodextrins, a derivatives, salt thereof or combinations thereof to the subject. In some embodiments, the method of reducing phospholipid levels in the blood is accomplished without scavenging lysophospholipids. The disclosure also relates to a method of activating autophagy, the method comprising administering an effective amount of a pharmaceutical composition comprising one or a plurality of alpha-cyclodextrins, a derivatives, salt thereof or combinations thereof to the subject. In some embodiments, the method of improving or activating autophagy is accomplished without causing lysosomal dysfunction.

BRIEF DESCRIPTION OF THE DRAWINGS

[029] **FIG. 1: Cyclodextrins, Including Hydroxypropyl Cyclodextrins.** Up to $\times 3$ degrees of substitution may be realized, with numerous positional and regio-isomers possible. Substitutions for R include, but are not limited to, H (parent), methyl (including randomly methylated), butyl, 2-hydroxypropyl (HP) (shown), acetyl, succinyl, glucosyl, maltosyl, carboxymethyl ether, phosphate ester, simple polymers, or carboxymethyl. Typical cyclodextrins contain 6 (α -CD), 7 (β -CD) or 8 (γ -CD) D-glucose monomers in a ring, creating a cone shape that can accommodate guest molecules into their hydrophobic cavity.

[030] **FIG. 2: Clustering analysis of cholesterol interaction with α -CD and HP- β -CD.** Displayed are the last 25 ns of the trajectories. CD molecules are depicted using the solid surface representation method with a probe radius of 0.4 Å, density isovalue of 0.7 and grid spacing of 0.5 to visualize the binding cavity. Hydrogen atoms are omitted to enhance clarity. Modified from (Shityakov S, Salmas RE, et al. (2016) J Toxicol Sci **41**:175-84)

[031] **FIG. 3A through FIG. 3C: Specificity of Lipid Release I.** Release of **phospholipids** (3A) cholesterol, (3B), and proteins (3C) from intact (3B) or ghost (3A and 3C) erythrocytes treated with cyclodextrins. ○: α -CD; ●: β -CD; △: γ -CD. Modified from: (Ohtani Y, Irie T, et al. (1989) European Journal of Biochemistry **186**:17-22)

[032] **FIG. 4A-FIG. 4B: Specificity of Lipid Release II.** 4A) Cholesterol released from brain capillary endothelial cell (BCECs) after 2 h of incubation in the presence of various concentrations of α - and β -CDs. Results are expressed as a percentage of cholesterol released

from BCECs compared with the control. Each percentage is the mean of three different filters and representative of two series of independent experiments. **(4B)** Phosphatidylcholine (PC) (light columns) and sphingomyelin (SM, dark columns) released from BCECs after 2 h of incubation in the presence of α - and β -CDs at 0.5, 5, and 50 mM, respectively. Results are expressed as a percentage of phospholipids released from BCECs compared with the control. Each percentage is the mean of three different filters and representative of two series of independent experiments. Modified from: (Monnaert V, Tilloy S, et al. (2004) Journal of Pharmacology and Experimental Therapeutics **310**:745-51)

[033] **FIG. 5: M β CD, but not α CD protect A2E against oxidation.** The bisretinoid A2E was the first compound identified in extracts of ocular lipofuscin. Protection of A2E against oxidation was monitored by changes in the UV-visible absorption spectra of 5 μ M A2E solutions. (5A) A2E oxidative status before (●) and after (○) blue-light irradiation in the presence of indicated cyclodextrins (12 mM). (5B) A2E oxidative status at time 0 (●) and 1 d (○) after incubation at room temperature in the dark in the presence of cyclodextrins. Modified from (Nociari MM, Lehmann GL, et al. (2014) Proceedings of the National Academy of Sciences **111**:E1402-E8)

[034] **FIG. 6:** QR-Plot of ssGWAS results for CGEM. The “null” projection (horizontal) ends at the median among the endpoints of the convex projections for individual chromosomes. Genes to the right of the vertical line are above the cut-off for study-specific genome-wide significance. Regions below (to the left) of the cut-off are given for descriptive purposes only.

[035] **FIG. 7** QR-Plot of muGWAS results for CGEM. (see FIG. 6 for legend)

[036] **FIG. 8** QR-Plot of ssGWAS results for EPIC. (see FIG. 6 for legend)

[037] **FIG. 9** QR-Plot of muGWAS results for EPIC. (see FIG. 6 for legend)

[038] **FIG. 10** QR-Plot of ssGWAS results for PBCS. (see FIG. 6 for legend)

[039] **FIG. 11** QR-Plot of muGWAS results for PBCS. (see FIG. 6 for legend)

[040] **FIG. 12: Phosphatidyl-inositol (PI) cycle.** Phosphatidyl-inositol (PI) is synthesized from myo-inositol (imported by HMIT) and phosphatidic acid (PA) (via CDP-DAG), which can be synthesized from lysophosphatic acid (lyso-PA (LPA), PC, or (cytosolic)

phosphatidyl-serine PS), or salvaged from IP₃ and DAG. It can also be synthesized from 1-acyl GPI. Arrows: Within the PI cycle, phosphoinositides are phosphorylated at a 3-, 4-, or 5-position by PI-kinases (left to right) and hydrolyzed by a plethora of phosphatases (right-to-left). Genes associated with breast cancer in this GWAS are highlighted as inverted (bold: aGWS). Wide arrows in the center indicate the sequence of phosphoinositides (shown as hexagons) involved in EEC. Hexagons: PI/phosphoinositides, PM: plasma membrane, CCV: clathrin-coated vesicle, UCV: uncoated vesicle, EE: early endosome, LE: late endosome LY: lysosome. Inverted gene names indicate genes associated with phosphatidylinositol signaling and/or endocytosis. Bold arrows indicate phosphoinositides associated with endocytosis.

[041] **FIG. 13A and 13B: Known relationship of genes implicated in muGWAS with stages in the process of endocytosis (13A) and exocytosis / lysosomal function (13B).**

Boxes: genes identified in the present disclosure by stage of endo-/exocytosis: Formation of clathrin-coated vesicles (CCVs) and E3 ubiquitination, separation of inactive integrin (fast recycling) from active integrins (slow recycling), sorting between secretory, lysosomal, and (slow) recycling pathway, and lysosomal degradation. Underlined genes are known breast cancer promoters and suppressors, respectively. Clathrin-mediated endocytosis (CME) begins with co-assembly of the heterotetrameric clathrin adaptor complex AP-2 with clathrin at PI(4,5)P₂-rich plasma membrane (PM) sites. AP-2 in its open conformation recruits clathrin and additional endocytic proteins, many of which also bind to PI(4,5)P₂. Clathrin-coated pit (CCP) maturation may be accompanied by SHIP-2-mediated dephosphorylation of PI(4,5)P₂ to PI(4)P. Synthesis of PI(3,4)P₂ is required for assembly of the PX-BAR domain protein SNX9 at constricting CCPs and may occur in parallel with PI(4,5)P₂ hydrolysis to PI(4)P via synaptojanin, thereby facilitating auxilin-dependent vesicle uncoating by the clathrin-dependent recruitment and activation of PI3KC2 α , a class II PI3-kinase. PI(3,4)P₂ may finally be converted to PI(3)P en route to endosomes by the 4-phosphatases *INPP4A/B*, effectors of the endosomal GTPase Rab5. Adapted from (Posor Y, Eichhorn-Grunig M, et al. (2015) *Biochim Biophys Acta* **1851**:794-804). In the early endosome (EE), β 1 integrins are sorted. Inactive integrins undergo fast “short loop” recycling; active integrins go to the late endosome (LE) / multivesicular body (MVB) for slow “long group” recycling (RAB11), lysosomal degeneration (unless rescued by *RAB25/CLIC3*), or secretion via the trans-Golgi-network (TGN) mediated by *RAB9*. Fast recycling of epidermal growth factor receptor drives proliferation, so one would expect that the mutations identified in 13A increase influx of phospholipids into the PI cycle. Lysosomal and synaptic vesicle exocytosis share many

similarities. Endolysosome-localized phosphoinositides may regulate lysosomal trafficking (Samie MA, Xu H (2014) *J Lipid Res* **55**:995-1009) (derived, in part from Kegg pathways hsa04144 and hsa04721). Adapted from (Schmid SL, Mettlen M (2013) *Nature* **499**:161-2; Bohdanowicz M, Grinstein S (2013) *Physiol Rev* **93**:69-106; Hesketh GG, Perez-Dorado I, et al. (2014) *Dev Cell* **29**:591-606; Mosesson Y, Mills GB, et al. (2008) *Nat Rev Cancer* **8**:835-50)

[042] **FIG. 14: Endocytic mechanisms underlying tumor cell migration and invasion through tissue barriers.** The diagram presents a motile cell, the advancing lamellipodium of which moves directionally (arrow). Focal adhesions (FAs) are schematically shown, and integrin heterodimers are present at these. Cell migration necessitates polarized endocytosis and trafficking of FA complexes. Integrin internalization is controlled by dynamin, which is activated by microtubules (not shown), and protein kinases, such as FAK and protein kinase C α (PKC α). Both clathrin- and caveolin 1 (CAV1)-coated domains of the plasma membrane are involved in internalization of integrin. Once in early endosome (EE), integrins are sorted for degradation in lysosomes (LYs), recycled to the plasma membrane through a RAB4-dependent route, or transported to the perinuclear recycling compartment (PNRC). Recycling from the PNRC requires Rab11 family members, such as RAB25, and for some integrin heterodimers, also the protein kinase B (PKB) – GSK3 β (glycogen synthase kinase- β) axis, ARF6 or certain isoforms of PKC. Human tumors often aberrantly express RAB25, display a specific repertoire of growth factor-induced integrin heterodimers or present abnormally stabilized microtubules, which promote trafficking of integrins. FAK, Integrin, RAB25, and PKB have functions associated with oncogenesis and/or display aberrant expression in human tumors (modified from Mosesson Y, Mills GB, et al. (2008) *Nat Rev Cancer* **8**:835-50).

[043] **FIG. 15: EEC in Alzheimer's disease (AD).** APP is synthesized in the endoplasmic reticulum (ER), transported to the TGN, and inserted into the plasma membrane via secretory vesicles. Cell-surface APP can be internalized to endosomes from which it can either be recycled back to the cell surface or delivered to lysosomes for degradation. Within the EE, the acidic environment favors production of amyloid-beta, which can be degraded in lysosomes by cathepsins, accumulated in EEs, or released to extracellular spaces via exocytosis (modified from Chen X, Hui L, et al. (2014) *J Parkinsons Dis Alzheimers Dis* **1**).

[044] **FIG. 16: EEC in Parkinson's disease (PD).** Following endocytic entry, cargo is transported to early endosomes. From there, cargo can recycle back to the plasma membrane,

either directly or via recycling endosomes. Alternatively, cargo can be retained in the EEs, which will form LEs/MVBs, and fuse with lysosomes for degradation. In parallel, cargo are transported between EEs and the trans-Golgi network (TGN). Alterations in these processes lead to lysosomal dysfunction and accumulation of undegraded macromolecules, toxic to the cell. (adopted from (Schreij AM, Fon EA, et al. (2015) Cell Mol Life Sci))

[045] **FIG. 17: Macropinocytosis in Atherosclerosis (CAD)** Following endocytic entry, low-density lipoprotein (LDL) is transported to the lysosome for degradation. Deficiencies in the lysosomal process lead to accumulation of LDL and. macrophages turn into foam cells, which accumulate to form atherosclerotic plaques.

[046] **FIG. 18A–C: Lysosomal Dysfunction in Cancer (18A), AD/PD (18B, and CAD (18C).** Published results show overlapping genetic risk factors for lysosomal dysfunction across diseases, leading to reduced lysosomal clearance (►◄) and, in cancer, increases in recovery of integrins from the lysosome (↴)

[047] **FIG. 19: Viruses “Hijacking” Endocytosis.** Viral particles depend on clathrin-mediated endocytosis for entry into cells. CCV: clathrin-coated vesicle. Modified from Fig. 2 in (Blaising J, Polyak SJ, et al. (2014) Antiviral research **107**:84-94)

[048] **FIG. 20: M β CD in AD Mouse Cells.** M β CD (M- β -CD) caused an increase in the levels of α secretase cleavage products C83 (A) and a decrease in the levels of intracellular APPs β (A and B). APPs α levels were increased in the medium from M- β -CD-treated cells, while APPs β levels did not change (C and D). (adapted from (Cole SL, Grudzien A, et al. (2005) J Biol Chem **280**:18755-70))

[049] **Fig. 21: HP- β -CD Clearance of a-Syn.** HP- β -CD-mediated clearance of a-syn aggregates does not depend on the ability of HP- β -CD to alter cholesterol levels. H4/a-syn-GFP cells untreated or treated with HP- β -CD (1 mM) or HP- β -CD-cholesterol complex (1 mM) for 24 h. Representative images are reported. 21A) Immunofluorescence microscopy analyses of TFEB subcellular localization using a FLAG-specific anti-body. Scale bar represents 10 μ m. 21B) a-syn-GFP fluorescence microscopy analyses. Scale bar represents 20 μ m. Modified from Figure 6 in (Kilpatrick K, Zeng Y, et al. (2015) PLoS One **10**:e0120819).

[050] **FIG. 22: Cholesterol Accumulation in Mouse Brain.** Unesterified cholesterol (UC) accumulation in the brain cells of 3-week-old mice treated with different CDs. Sample fluorescence photomicrographs of dorsal neocortex from untreated stained with filipin to detect UC. Cortical layers are marked by roman numerals. Virtually, all neurons in untreated *Npc1^{-/-}* mice show positive cytoplasmic staining of UC (white spots), where as those in *Wt* mice are negative. HP- β -CD shows highly effective reduction in UC storage. HP- α -CD UC storage, albeit clearly reduced, was reported by the authors as “grossly equivalent to untreated mice”. Modified from Figure 1 in (Davidson CD, Fishman YI, et al. (2016) *Ann Clin Transl Neurol* **3**:366-80) .

[051] **FIG. 23: CDs in Lysosomal Storage Diseases.** Effect of cyclodextrins using lysotracker red staining to indicate enlarged lysosomes in ML111 fibroblasts. Wolman (LAL), TSD, Fabry (GLA), Farber (AC), MPSIIIB: mucopolysaccharidose II type B (NAGLU), NPA: Niemann-Pick type A (ASM), HBCD: HP- β -CD (Kleptose) According to the inventors, “the other cyclodextrins did not have that profound an effect”,(McKew J, Zheng WEI, et al. (2014)) It was overlooked, however, that, the effect of 6 mM α -CD (1/5 of the M- β -CD dose) was more profound than the effect of 8 mM HP- β -CD. Modified from (McKew J, Zheng WEI, et al. (2014)).

[052] **FIG. 24: Pino-/Phago-/Endocytosis:** Macropinocytosis, phagocytosis, and endocytosis are regulated in the same fashion by the PI cycle and lysosomal dysfunction in all three processing pathways causes substrates with unwanted function to be excreted (modified from FIG. 1 in (Sole-Domenech S, Cruz DL, et al. (2016) *Ageing Res Rev* **32**:89-103)).

[053] **FIG. 25: HP β CD activates autophagy.** Administration of HP- β -CD results in activation of transcription factor EB TFEB. Upon translocation from the cytoplasm to the nucleus, TFEB regulates the expression of genes involved in biogenesis and fusion of lyso- and autophago-somes. As a result, HP- β -CD administration results in enhanced clearance of the autophagy substrate ceroid lipo-pigment. The mechanism by which HP- β -CD improves TFEB activation, however, was not understood (adapted from (Song W, Wang F, et al. (2014) *J Biol Chem* **289**:10211-22))

[054] **FIG. 26: Activation of TFEB by HP β CD:** HP- β -CD has been shown to activate transcription factor EB (TFEB), yet the mechanism of action is unknown (FIG. 25). The

unexpected genetics results (Example 2) confirmed by the urinalysis results (Example 16) showed that the mechanism of action of HP- β -CD is (a) reduction of serum phospholipids, (b) reduction of PI-cycle activity, (c) reduction of endocytosis, (d) prevention of lysosomal stress, (e) prevention of MTORC1 activation, (f) continued dephosphorylation and translocation of TFEB, (g) continued activation of autophagy. Adapted from (Kim S, Choi KJ, et al. (2016) *Sci Rep* **6**:24933; Medina DL, Ballabio A (2015) *Autophagy* **11**:970-1)

[055] **FIG. 27: Phago-/Endocytosis in Multiple Sclerosis (MS).** The vicious cycle in the etiology of MS starts with microglia phagocytosing a probes of myelin from the healthy myelin sheath. If the lysosome (LY) is losing its ability to discard all of these probes, some of them are presented as antigens to T- and B-cells, which then cross the blood-brain barrier (BBB) to activate peripheral macrophages. These macrophages then infiltrate the CNS and endocytose myelin for destruction. (Modified from (Salter MW, Stevens B (2017) *Nat Med* **23**:1018-27; Luo C, Jian C, et al. (2017) *Neuropsychiatr Dis Treat* **13**:1661-7)) Equivalent mechanisms are involved in Alzheimer's disease and Rett syndrome.(Prinz M, Priller J (2014) *Nat Rev Neurosci* **15**:300-12)

[056] **Fig. 28: M β CD Restores Surface Tension.** “Minimum surface tension during dynamic cycle 20 with [Bovine Lipid Extract Surfactant] BLES containing 27 mg/ml BLES in control CBS buffer (checkers bars), 50% w/w FFA (oleic acid [, OA]) or LPC in control CBS buffer (horizontal bars) or buffer containing 40 mg/ml M- β -CD (solid black bars). (** $p \leq 0.01$, *** $p \leq 0.001$). BLES with FFA or LPC shows marked impairment, which is repaired to normal functionality in the presence of M- β -CD. Cholesterol was present at very low concentrations (~2.1%) in these experiments.” Modified from Supplementary Figure S6 in (Gunasekara L, Al-Saiedy M, et al. (2017) *Journal of cystic fibrosis : official journal of the European Cystic Fibrosis Society*)

[057] **FIG. 29: HaCaT Proliferation.** Influence of cyclodextrins on the proliferation of spontaneously immortalized aneuploid human (HaCaT) keratinocytes. Mean values after 48 h incubation, normalized to the control, of at least six independent measurements. Top: ATP-Assay, bottom: PicoGreen-Assay). W6: α -CD, W7: β -CD, W7 HP: HP- β -CD. Modified from (Hipler UC, Schonfelder U, et al. (2007) *J Biomed Mater Res A* **83**:70-9)

[058] **FIG. 30: HaCaT Caspase 3/7 Activity.** Influence of cyclodextrins on Caspase 3/7 activity of HaCaT keratinocytes. Mean values after incubation (24 h), normalized on the

control and the protein content, of at least six independent measurements. W6: α -CD, W6: β -CD, W7 HP: HP- β -CD. Modified from (Hipler UC, Schonfelder U, et al. (2007) J Biomed Mater Res A **83**:70-9)

[059] **FIG. 31: HaCaT Lactose Dehydrogenase.** Influence of cyclodextrins on lactose-dehydrogenase (LDH) of HaCaT keratinocytes. Mean values after incubation (48 h), normalized on the control and the protein content, of at least six independent measurements. W6: α -CD, W6: β -CD, W7 HP: HP- β -CD. Modified from (Hipler UC, Schonfelder U, et al. (2007) J Biomed Mater Res A **83**:70-9)

[060] **FIG. 32: Solubility Enhancements by Hydroxypropyl-cyclodextrins (HP-CD).** Enhancement of apparent solubilities of lipids by HP-cyclodextrins. Bar denoted "0" represents apparent solubilities in isotonic phosphate-buffered saline; those denoted by α , β , and γ represent the solubilities in the same saline but supplemented with the respective HP-cyclodextrins at 5% (w/w) concentration. MW: molecular weight. Modified from: (Irie T, Fukunaga K, et al. (1992) J Pharm Sci **81**:524-8), Figure 1.

[061] **FIG. 33: Nephrotoxicity of α -CD and β -CD.** The 2017 assessment by the EMA (and several other authors) of similar risks associated with parenteral administration of α -CD and β -CD in rats is based on a single study (Frank DW, Gray JE, et al. (1976) Am J Pathol **83**:367-82).

[062] **FIG. 34: Ototoxicity of CDs.** Ototoxicity of different CDs as assessed by auditory brainstem response (ABR) recordings at 12 weeks of age. Plot of hearing thresholds for individual mice reveals minute variability across mice treated with a particular CD with the exception of HP γ CD, in which hearing thresholds were more variable. Modified from Figure 5B in (Davidson CD, Fishman YI, et al. (2016) Ann Clin Transl Neurol **3**:366-80)

[063] **FIG. 35: Wound healing by cyclodextrins in breast cancer cell lines.** Dashed horizontal line indicates inhibition of wound healing in HP α CD at 1 and 4 mM respectively. ANOVA P values are shown for HP α CD v. HP β CD with MCF-7 and MDA-MB-231 as (fixed) blocks:

1 mM HP α CD v. 1 mM HP β CD, $p = .0001$

1 mM HP α CD v. 2 mM HP β CD, $p = .0252$ 4 mM HP α CD v.

4 mM HP β CD, $p = .0442$

(Modified from (Wittkowski KM, Dadurian C, et al. (2018) PLoS One **13**:e0199012)

[064] **FIG. 36: Wound Healing Assay:** Modified from Cell BioLabs Inc., Assay CBA-120

[065] **FIG. 37: Impact of PL selectivity:** (upper left corner of FIG. 12, see FIG. 12 for legends) HP- α -CD only scavenged PC (and, potentially PI), but not lysophospholipids including, but not limited to, LPC and LPA, upstream of sPLA2, which splits PC into LPC and arachidonic acid (AA).

[066] **FIG. 38a and FIG. 38b: BCa Body Weight (A) and Tumor Volume (B) by Treatment.** Individual curves and medians are shown. A: ‘†’ at the bottom and ‘+’ in the curves indicates death for unknown reason. B: Both HP- α -CD and HP- β -CD are effective (overall treatment effect Day*Treatment (D*T) < .001, ANOCA with Mice as a random factor). HP- α -CD is more effective than HP- β -CD (pairwise treatment effect Day*Treatment (D*Tla:b) = .0258, ANOCA with mice as a random factor).

[067] **FIG. 39: BCa Lung and Liver Metastases.** P-values are from pairwise comparisons of bivariate (lung/liver) data via u-statistics for multivariate data (Wittkowski KM, Lee E, et al. (2004) Stat Med **23**:1579-92)

[068] **FIG. 40: BCa Plasma Cytokines.** Individual P-values are derived from two sample t-tests $p(b)$: HP- β -CD v. Vehicle, $p(a)$: HP- α -CD v. Vehicle, $p(a:b)$: HP- α -CD v. HP- β -CD. Overall P-value for all six cytokines is calculated via u-statistics for multivariate data (Wittkowski KM, Lee E, et al. (2004) Stat Med **23**:1579-92)

[069] **FIG. 41: HTT Mice: Bodyweight.** Estimates are means $\pm$ SD, P-values for Treatment*Day interaction (P(T*D)) overall and by sex (F: female, M: male) are derived from mixed model ANOVA with mice as random factor.

[070] **FIG. 42: SOD1 Mice: Bodyweight.** Estimates are means $\pm$ SD, P-values for Treatment*Day interaction (P(T*D)) overall and by sex (F: female, M: male) are derived from mixed effects ANOVA with mice as a random factor.

[071] **FIG. 43A and 43: FPLC Standard curves for the type of FPLC columns used.** **43A)** HP- α -CD (HPaCD, 1180 Da) is confirmed to be measured mostly in Fraction 2

(nominally 1060–1267 Da). **43B)** Phosphatidylcholine (PC) is confirmed to be measured mostly in Fraction 5 (nominally 734–795 Da).

[072] **FIG. 44: HP α CD, FA mixtures (&) v. clathrate.** FPLC of morning urine 8 h after intake of different HP- α -CD preparations containing 15 g HP- α -CD each as the active substance as well as different forms of penetration enhancers. HP α CD: HP- α -CD only (n=5). HP α CD&FA: a mixture of HP α CD and 10% w/w sodium caprate and the permeation enhancing oily suspension (PE) proposed in (Tuvia S, Pelled D, et al. (2014) *Pharmaceutical Research* **31**:2010-21) (n=5). HP α CD/FA: HP- α -CD/FA clathrate (n=5). HP α CD/FA&PE (n=4): The clathrate mixed with the above penetration enhancer. Axes: as in Fig. 43. Y-axis with labels added: aCD, PC, and LPC indicate the median amount of substrate identified in the HP- α -CD fraction 2 (1060–1267 Da), the phosphatidylcholine fraction 5 (724–795 Da), and the lyso-phosphatidyl choline fractions 9 and 10 (450–534 Da). The fractions referred to on the y-axis are indicated by the same labels on the x-axis. The upward open arrows indicate the difference between the corresponding median for HP- α -CD (horizontal line) and the median for each of the four mixtures and/or clathrates, respectively, (short horizontal dotted lines).

[073] **FIG. 45: *In vivo* Evidence for Clinical Efficacy of β CDs.** Efficacy of β -CD in animal models of breast cancer, Parkinson's disease, Alzheimer's disease, CAD, systemic lupus erythematosus/ALS/MS, cystic fibrosis/IPF, NAFLD/NASH, and T2DM was consistently attributed to the ability of β -CD to scavenge cholesterol (Chol, dashed arrows), which carries the now well-known risk of cholesterol-mediated ototoxicity. The mechanism by which depletion of cholesterol should improve the various phenotypes, however, was rarely explained. Clinical results also showed phospholipid upregulation in several of these diseases. β -CDs, however, also scavenge phospholipids (phospholipids) and, thus, also downregulate the PI cycle (center), which directly benefits the various disease phenotypes (solid arrows).

[074] **FIG. 46: HP- α -CD Restores LY Function:** (update of FIG. 26). HP- β -CD has been shown to activate transcription factor EB (TFEB), yet the mechanism of action is unknown (Kilpatrick K, Zeng Y, et al. (2015) *PLoS One* **10**:e0120819; Song W, Wang F, et al. (2014) *J Biol Chem* **289**:10211-22; Sardiello M (2016) *Ann N Y Acad Sci* **1371**:3-14; Moors TE, Hoozemans JJ, et al. (2017) *Mol Neurodegener* **12**:11). The unexpected genetics results (Example 2) confirmed by the urinalysis results (Example 16) identified the mechanism. It is

not direct activation that activated the translocation of TFEB to the nucleus, but scavenging of phospholipids, which reduces autophagy and prevents the overfilling lysosome from activating MTORC1, the target of the anti-aging drugs resveratrol and rapamycin, thereby preventing translocation of TFEB from being inhibited. As a result the aggregation of various proteins in amyloid-beta and tau in Alzheimer's disease, α -synuclein in Parkinson's disease, LDL in CAD, Ataxins in spinocerebellar ataxia (SCA), myelin in MS, SOD1 in ALS, and others is prevented. Adapted from (Kim S, Choi KJ, et al. (2016) *Sci Rep* **6**:24933; Medina DL, Ballabio A (2015) *Autophagy* **11**:970-1; Sardiello M (2016) *Ann N Y Acad Sci* **1371**:3-14; Moors TE, Hoozemans JJ, et al. (2017) *Mol Neurodegener* **12**:11; Martini-Stoica H, Xu Y, et al. (2016) *Trends Neurosci* **39**:221-34)

[075] **FIG. 47: HP- α -CD Reduces Inflammation:** Effects of α -CD on arachidonic acid (AA) and known drugs with similar effects downstream of arachidonic acid. By restricting PC, rather than blocking sPLA2, the effect of α -CD is better tolerated than the effect of corticosteroids. The combination of ibuprofen and cromolyn is tested as the dry-powder inhaler ALZT-OP1 (Elmaleh D (2017)). In contrast, by downregulating all three pathways, α -CD prevents the compensatory activation seen in the not targeted pathways with ASA, NSAIDs, or LOX inhibitors. Also in contrast to α -CD, no effect on endocytosis is expected and, thus, no synergistic anti-inflammatory benefit from also improving autophagy. (modified from http://tmedweb.tulane.edu/pharmwiki/doku.php/introduction_to_eicosanoids).

[076] **FIG. 48: α -CD/Pemetrexed Compounds:** To target the compound to the neighborhood of cancer cells, which have folate-receptors, α -CD (or a derivative, such as HP- α -CD, with hydroxypropyl) can be linked, as a non-limiting example, to pemetrexed with a disulfide linker that is bound to both the α -CD and pemetrexed via an ester. The disulfide linker could be formed by either cysteine or 3-mercaptopropionic acid and 2-mercaptoethanol. The ester or disulfide links (dashed boxes) can be formed by methods well established, such as those described in (Michel D, Chitanda JM, et al. (2012) *Eur J Pharm Biopharm* **81**:548-56) and (Hunter R, Stellenboom N, et al. (2008) *Synlett* 252-4) (removing one H₂O, each to form an O from two OH), and (Butt AM, Mohd Amin MC, et al. (2015) *Int J Nanomedicine* **10**:1321-34) (removing H₂ to form an S-S from two SH).

[077] **FIG. 49A, 49B, 49C: Genetic Analysis:** Table 4 (FIGS. 49A through 49C) depicts the genetic analysis performed through IPV6/IPV1: -log₁₀(p-value) in muGWAS/ssGWAS.

DETAILED DESCRIPTION OF EMBODIMENTS

[078] The present disclosure is based, in part, on the discovery of certain disease-relevant collections of genes based on a reanalysis of three independent sets of breast cancer genetic data, which are available for analysis from the National Institutes of Health's dbGaP collection. This reanalysis differed from the earlier analyses by using a novel computational biostatistics method, which incorporates knowledge about genetics into the method itself. (Wittkowski KM, Sonakya V, et al. (2014) *Transl Psychiatry* **4**:e354) As described in detail in the Examples, this method addresses the following four points, which prior analyses of the same data using conventional bioinformatics approaches failed to consider: (i) non-additive relationships between risk alleles and incidence, (ii) cis-epistatic interaction, (iii) correlation between significance and minor allele frequency or "MAF" and (iv) non-randomization bias. It also addresses multiplicity adjustment for diplotype length, a problem arising from the use of a wide-locus approach. By addressing these points, the same strategy previously identified two novel collections of autism-specific genes now identified another novel collection of genes related to endocytosis and lysosomal function.. (Wittkowski KM, Sonakya V, et al. (2014) *Transl Psychiatry* **4**:e354)

[079] One collection of genes presented herein (FIG. 12, upper left corner) comprises eight genes (*AGPAT3/4*, *ATP8A1/BI*, *ABCA1*, *ANO4*, *LPPR1*, *DGKQ*) whose roles include providing the phosphatidyl inositol (PI) cycle with its substrate, PI. In Alzheimer's disease, "PI is one of only 10 serum lipids that can accurately predict memory loss in as much as 90% of cases, 2 years before the onset of dementia symptoms." (Waugh MG (2015) *Biochim Biophys Acta* **1851**:1066-82) The second gene cluster comprises genes directly associated with endocytosis, a process controlled by phosphoinositides (Table 4, column PI/EC). The stages include, but are not limited to, invagination and forming of CCVs and EEs (FIG. 13). Based on this discovery, the present disclosure provides, in part, a shift in the focus for cancer treatments from controlling cell growth, a process involved in many vital functions even in older subjects, to controlling spread, migration and invasion of cells, a process of relevance primarily during pre- and early postnatal development. Based on these findings, as presented in detail herein, the present disclosure provides compositions and methods for treating malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition by modulating the PI cycle and its activity. One of the surprising results in the present disclosure is that the same intervention also reduces chronic inflammation associated with aging

(“inflammaging”). Furthermore, in some embodiments, the present disclosure provides compositions and methods that may be useful for the treatment of malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition during periods, such as adulthood, where cell migration, including neuronal growth, has mostly ceased, while cell growth, such as growth of hair, skin, and the like, continues and where other cellular mechanism may decline in an age-dependent manner.

[080] In another part, the present disclosure is based on the re-evaluation of interpretations of published findings, which are commonly believed to assert that α -CD is as nephrotoxic as β -CD, although nephrotoxicity was primarily demonstrated for β -CD, which has substantially lower aqueous solubility than α -CD, and, thus, a higher risk of forming the long cytoplasmic crystals seen in the kidneys of rats. (Frank DW, Gray JE, et al. (1976) Am J Pathol **83**:367-82) For instance, lower toxicity of α -CD v. β -CD – typically at the level of the safer HP- β -CD – was shown in HaCaT cells proliferation (FIG. 29), caspase activity (FIG. 30), and lactose dehydrogenase (FIG. 31).

[081] In summary, “the [Joint Expert] Committee [on Food Additives (JECFA)] was reassured by the relatively low toxicity of this compound in animals and the fact that it was less toxic than *beta*-cyclodextrin, for which studies of human tolerance were available.” (Prakash AS, Abbott PJ (2001) WHO/JECFA Food Additive Series **48**:1030)

[082] In 2014, the CHMP suggested Permitted Daily Exposure (PDE) thresholds above which adverse effects may occur with parenteral administration of α -CD and HP- β -CD of 0.02 and 10 mg/kg/d. (Committee for Human Medicinal Products (CHMP) (2017) European Medicines Agency EMA/CHMP/333892/2013) On Oct 9, 2017, these thresholds were revised to 200 mg/kg/d each (Committee for Human Medicinal Products (CHMP) (2017) European Medicines Agency EMA/CHMP/333892/2013) and cyclodextrins were included in the Guidelines on excipients (European Medicines Agency (2017) EMA/CHMP/302620/2017/EN) (Table 1).

Table 1: Excipients and information for the package leaflet.

Name	Updated on	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Cyclodextrins e.g.: Alfadex Betadex (E 459) γ -cyclodextrin Sulfolbutyl-ether- β -cyclodextrin (SBE- β -CD) Hydroxypropyl betadex Randomly methylated β -cyclodextrin (RM- β -CD)	09/10/2017	Oral	200 mg/kg/day	Cyclodextrins may cause digestive problems such as diarrhea.	At high doses -cyclo dextrins can cause reversible diarrhoea and cecal -enlarge .ment in animals
Cyclodextrins e.g.: Alfadex Betadex (E 459) γ -cyclodextrin Sulfolbutyl-ether- β -cyclodextrin (SBE- β -CD) Hydroxypropyl betadex Randomly methylated β -cyclodextrin (RM- β -CD)	09/10/2017	Parenteral	200 mg/kg/day and use for > 2 weeks	If you have a kidney disease, talk to your doctor before you receive this medication	n children less I than years, the γ lower glomerular function may protect against renal toxicity, but can lead to higher blood levels of .cyclodextrins In patients with moderate to severe renal dysfunction accumulation of dextrins cyclo .may occur

Source: (European Medicines Agency (2017) EMA/CHMP/302620/2017/EN)

[083] In another embodiment, nephrotoxicity is further reduced by reducing the rate of delivery, the method comprising repeated doses per day, administering the drug over several hours via a peristaltic pump or administering the drug continuously via an implanted drug delivery system.

[084] Some of the main embodiments of the present disclosure are described in the above Summary section of this application, as well as in the Examples, Figures, and Claims. This

Detailed Description of Embodiments section provides additional description relating to the compositions and methods of the present disclosure, and is intended to be read in conjunction with all other sections of the present patent application, including the Summary, Examples, Figures, and Claims sections of the present application.

I. Abbreviations and Definitions

[085] The abbreviations “A β ” and “Ab” refer to amyloid beta.

[086] The abbreviations “aCD”, “ α CD”, and “ α -CD” refer to alpha-cyclodextrin.

[087] The abbreviation “AD” refers to Alzheimer’s disease.

[088] The abbreviation “AKT” refers to protein kinase B.

[089] The abbreviation “ALS” refers to amyotrophic lateral sclerosis.

[090] The abbreviation “A Φ ” refers to autophagy.

[091] The abbreviation “APP” refers to amyloid precursor protein.

[092] The abbreviations “ α -syn” and “asyn” refer to alpha-synuclein.

[093] The abbreviation “BCa” refers to breast cancer.

[094] The abbreviations “bCD”, “ β CD”, and “ β -CD” refers to beta-cyclodextrin.

[095] The abbreviation “Ca” refers to calcium.

[096] The abbreviation “CAD” refers to coronary artery disease.

[097] The abbreviation “CD” refers to cyclodextrin.

[098] The abbreviation “CDK” refers to cyclin-dependent kinase.

[099] The abbreviation “CF” refers to cystic fibrosis.

[0100] The abbreviation “CGEM” refers to Cancer Genetic Markers of Susceptibility.

[0101] The abbreviation “Chr” refers to chromosome.

- [0102] The abbreviation “dbGaP” refers to database of Genotypes and Phenotypes.
- [0103] The abbreviation “DNA” refers to deoxyribonucleic acid
- [0104] The abbreviation “EC” refers to endocytosis.
- [0105] The abbreviation “EE” refers to early endosome.
- [0106] The abbreviation “e.g.” refers to for exempli gratia (for example).
- [0107] The abbreviation “EPIC” refers to European Prospective Investigation into Cancer.
- [0108] The abbreviation “ER” refers to endoplasmic reticulum
- [0109] The abbreviation “FAK” refers to focal adhesion kinase.
- [0110] The abbreviation “Fc” refers to fragment, crystallizable.
- [0111] The abbreviation “FDA” refers to Food and Drug Administration.
- [0112] The abbreviation “FSGS” refers to focal segmental glomerulosclerosis.
- [0113] The abbreviations “gCD” and “ γ CD” refer to gamma-cyclodextrin.
- [0114] The abbreviation “GPCR” refers to G-protein coupled receptor.
- [0115] The abbreviation “GRAS” refers to generally recognized as safe.
- [0116] The abbreviation “GWAS” refers to genome-wide association study.
- [0117] The abbreviation “GRAS” refers to generally recognized as safe.
- [0118] The abbreviation “HA” refers to Hyaluronic acid
- [0119] The abbreviation “HER2/neu” refers to receptor tyrosine-protein kinase erbB-2.
- [0120] The abbreviation “HLA” refers to human leukocyte antigen.
- [0121] The abbreviation “HP” refers to hydroxypropyl.
- [0122] The abbreviations “HPaCD”, “HP- α -CD” and “HP α CD” refers to 2-hydroxypropyl-alpha-cyclodextrin.

[0123] The abbreviations “HPbCD”, “HP-β-CD” and “HPβCD” refers to hydroxypropyl-beta-cyclodextrin.

[0124] The abbreviation “IND” refers to investigational new drug.

[0125] The abbreviation “IPV” refers to inverse p-value.

[0126] The abbreviation “i.v.” refers to intravenous.

[0127] The abbreviation “LD” refers to linkage disequilibrium.

[0128] The abbreviation “LD₅₀” refers to median lethal dose.

[0129] The abbreviation “LE” refers to late endosome.

[0130] The abbreviation “LPC” refers to lysophosphatidylcholine.

[0131] The abbreviation “LY” refers to lysosome.

[0132] The abbreviation “LYD” refers to lysosomal dysfunction

[0133] The abbreviation “MAF” refers to minor allele frequency.

[0134] The abbreviation “MAP” refers to mitogen-activated protein.

[0135] “The abbreviations “M-β-CD”, “MβCD”, and “MbCD” refer to methyl-β-cyclodextrin.

[0136] The abbreviation “MΦ” refers to macrophage.

[0137] The abbreviation “MS” refers to multiple sclerosis.

[0138] The abbreviation “mTOR” refers to mechanistic target of rapamycin.

[0139] The abbreviation “muGWAS” refers to multivariate u-statistics GWAS.

[0140] The abbreviation “MVB” refers to multivesicular body.

[0141] The abbreviation “No.” refers to number.

[0142] The abbreviation “NIH” refers to National Institutes of Health.

- [0143] The abbreviation “NPC” refers to Niemann Pick disease type C.
- [0144] The abbreviation “PBCS” refers to Polish Breast Cancer Case-Control Study.
- [0145] The abbreviation “PC” refers to phosphatidylcholine”
- [0146] The abbreviation “PCa” refers to prostate cancer.
- [0147] The abbreviation “PD” refers to Parkinson’s disease.
- [0148] The abbreviation “PI” refers to phosphatidylinositol.
- [0149] The abbreviation “PI3R” refers to “phosphoinositide-3 kinase”
- [0150] The abbreviation “PIP” refers to phosphoinositide
- [0151] The abbreviation “PIP1” refers to PI(4)P.
- [0152] The abbreviation “PIP2” refers to PI(4,5)P₂.
- [0153] The abbreviation “PIP3” refers to PI(3,4,5)P₃.
- [0154] The abbreviation “PM” refers to plasma membrane”
- [0155] The abbreviation “PKB” refers to protein kinase B.
- [0156] The abbreviation “PKC” refers to protein kinase C.
- [0157] The abbreviation “PL” refers to phospholipid.
- [0158] The abbreviation “QQ” refers to “quantile-quantile”.
- [0159] The abbreviation “QR” refers to “quantile-rank”.
- [0160] The abbreviation “RTK” refers to receptor tyrosine kinase.
- [0161] The abbreviation “s.c.” refers to subcutaneous.
- [0162] The abbreviation “SLE” refers to systemic lupus erythematosus.
- [0163] The abbreviation “SNP” refers to single nucleotide polymorphism.

[0164] The abbreviation “ssGWAS” refers to single-SNP genome-wide association study.

[0165] The abbreviation “TFEB” refers to transcription factor EB.

[0166] The abbreviation “TSC” refers to tuberous sclerosis.

[0167] The abbreviation “U.S.” refers to the United States.

[0168] The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

[0169] The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Other elements may optionally be present other than the elements specifically identified by the “and/or” clause, whether related or unrelated to those elements specifically identified unless clearly indicated to the contrary. Thus, as a non-limiting example, a reference to “A and/or B,” when used in conjunction with open-ended language such as “comprising” can refer, in one embodiment, to A without B (optionally including elements other than B); in another embodiment, to B without A (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

[0170] As used herein in the specification and in the claims, “or” should be understood to have the same meaning as “and/or” as defined above. For example, when separating items in a list, “or” or “and/or” shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as “only one of” or “exactly one of,” or, when used in the claims, “consisting of,” will refer to the inclusion of exactly one element of a number or list of elements. In general, the term “or” as used herein shall only be interpreted as indicating exclusive alternatives (i.e. “one or the other but not both”) when preceded by terms of exclusivity, “either,” “one of,” “only one of,” or “exactly one of” “Consisting essentially of,” when used in the claims, shall have its ordinary meaning as used in the field of patent law.

[0171] The term “about” as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of $\pm 20\%$, $\pm 10\%$,

$\pm 5\%$, $\pm 1\%$, $\pm 0.9\%$, $\pm 0.8\%$, $\pm 0.7\%$, $\pm 0.6\%$, $\pm 0.5\%$, $\pm 0.4\%$, $\pm 0.3\%$, $\pm 0.2\%$ or $\pm 0.1\%$ from the specified value, as such variations are appropriate to perform the disclosed methods.

[0172] As used herein, the term “carrier” means a diluent, adjuvant, or excipient with which a compound is administered. Pharmaceutical carriers can be liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. The pharmaceutical carriers can also be saline, gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea, and the like. In addition, auxiliary, stabilizing, thickening, lubricating and coloring agents can be used.

[0173] As used herein, the terms “comprising” (and any form of comprising, such as “comprise”, “comprises”, and “comprised”), “having” (and any form of having, such as “have” and “has”), “including” (and any form of including, such as “includes” and “include”), or “containing” (and any form of containing, such as “contains” and “contain”), are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

[0174] As used herein, the phrase “in need thereof” means that the animal or mammal has been identified as having a need for the particular method or treatment. In some embodiments, the identification can be by any means of diagnosis. In any of the methods and treatments described herein, the animal or mammal can be in need thereof. In some embodiments, the animal or mammal is in an environment or will be traveling to an environment in which a particular disease, disorder, or condition is prevalent.

[0175] As used herein, the terms “treat,” “treating,” and “treatment” encompass a variety of activities aiming at desirable changes in clinical outcomes. For example, the term “treat”, as used herein, encompasses any detectable improvement in one or more clinical indicators or symptom of malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition – such as carcinomas, including, but not limited to, breast and prostate cancer or neurodegenerative diseases, including, but not limited to, Parkinson’s and Alzheimer’s Disease, or coronary artery disease, including, but not limited to atherosclerosis, CAD and stroke, or a digestive disorder, including, but not limited to T2DM. For example, such terms encompass alleviating, abating, ameliorating, relieving, reducing, inhibiting or slowing at least one clinical indicator or symptom, preventing additional clinical indicators or symptoms,

reducing or slowing the progression of one or more clinical indicators or symptoms, causing regression of one or more clinical indicators or symptoms, relieving a condition caused by the disease or disorder, and the like. In the case of therapeutic treatments, the methods and compositions provided herein can be used in subjects that already exhibit one or more clinical indicators or symptoms of the disease or disorder, such as malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition. In the case of malignant or neurodegenerative diseases or disorders, various clinical indicators and symptoms are known to medical practitioners and those of skill in the art.

[0176] The terms “prevent” or “preventing” as used herein encompasses stopping a disease, disorder, or symptom from starting, as well as reducing or slowing the progression or worsening of a disease or disorder. For example, “preventing” breast cancer or prostate cancer includes, but is not limited to, inhibiting the formation of cancerous cells, growth of tumors, local spread of cancerous spread, or inhibiting the metastasis of malignant growths, preventing the onset of symptoms of Alzheimer’s, Parkinson’s, Huntington’s, ALS and/or MS. In the case of prophylactic treatments, the methods and compositions provided herein can be used to prevent progression or clinical exhibiting of symptoms in subjects that do not yet exhibit any clear or detectable clinical indicators or symptoms of the disease or disorder but that are believed to be at risk of developing the disease or disorder, such as malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition.

[0177] The term “malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition” is used herein in accordance with its usual usage in the art and includes, but is not limited to malignant disorders, such as carcinomas, breast cancer, prostate cancer, malignancies of the breast, and malignancies of the prostate, as well as neurodegenerative diseases, such as Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, and “a range of disorders including brain overgrowth syndromes, [and] Charcot-Marie-Tooth neuropathies”(Waugh MG (2015) *Biochim Biophys Acta* **1851**:1066-82).

[0178] As used herein, the term “cancer” or “hyperproliferative disease” is meant to refer to those diseases and disorders characterized by hyperproliferation of cells. Examples of hyperproliferative disease includes all forms of cancer, psoriasis, neoplasia, and hyperplasia.

[0179] The terms “subject,” “individual,” and “patient” are used interchangeably herein to refer to a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, murines, simians, humans, farm animals, cows, pigs, goats, sheep, horses, dogs, sport animals, and pets. Tissues, cells and their progeny obtained *in vivo* or cultured *in vitro* are also encompassed by the definition of the term subject. The term “subject” is also used throughout the specification in some embodiments to describe an animal from which a cell sample is taken or an animal to which a disclosed cell or nucleic acid sequences have been administered. In some embodiment, the animal is a human. For treatment of those conditions which are specific for a specific subject, such as a human being, the term “patient” may be interchangeably used. In some instances in the description of the present disclosure, the term “patient” will refer to human patients suffering from a particular disease or disorder. In some embodiments, the subject may be a non-human animal from which an endothelial cell sample is isolated or provided. The term “mammal” encompasses both humans and non-humans and includes but is not limited to humans, non-human primates, canines, felines, murines, bovines, equines, caprines, and porcines.

[0180] The terms “effective amount” or “therapeutically effective amount,” as used herein, refer to an amount of an active agent as described herein that is sufficient to achieve, or contribute towards achieving, one or more desirable clinical outcomes, such as those described in the “treatment” description above. An appropriate “effective” amount in any individual case may be determined using standard techniques known in the art, such as a dose escalation study. In some embodiments, as used herein, the term “therapeutically effective amount” is meant to refer to an amount of an active agent or combination of agents effective to ameliorate, delay, or prevent the symptoms, shrink tumor size, prevent progression of cancer from non-metastatic to metastatic disease, or prolong the survival of the patient being treated for cancer or neurodegenerative disease. Determination of a therapeutically effective amount is well within the capabilities of those skilled in the art, especially in light of the detailed disclosure provided herein.

[0181] In some embodiments, cyclodextrin or derivatives thereof or pharmaceutically acceptable salts thereof can be co-administered with other therapeutics and/or part of a treatment regimen that includes radiation therapy.

[0182] The co-administration of therapeutics can be sequential in either order or simultaneous. In some embodiments cyclodextrin or derivatives thereof or pharmaceutically

acceptable salts thereof is co-administered with more than one additional therapeutic. Examples of chemotherapeutics include common cytotoxic or cytostatic drugs such as for example: methotrexate (amethopterin), doxorubicin (adrimycin), daunorubicin, cytosinarabioside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, and other nitrogen mustards (e.g. cyclophosphamide), cis-platin, vindesine (and other vinca alkaloids), mitomycin and bleomycin. Other chemotherapeutics include: purothionin (barley flour oligopeptide), macromomycin, 1,4-benzoquinone derivatives and trenimon. Anti-cancer antibodies, such as herceptin, and toxins are also examples of other additional therapeutics.

[0183] The therapeutic regimens can include sequential administration of cyclodextrin or derivatives thereof or pharmaceutically acceptable salts thereof and initiation of radiation therapy in either order or simultaneously. Those skilled in the art can readily formulate an appropriate radiotherapeutic regimen. Carlos A Perez & Luther W Brady: Principles and Practice of Radiation Oncology, 2nd Ed. JB Lippincott Co, Phila, 1992, which is incorporated herein by reference in its entirety describes radiation therapy protocols and parameters which can be used in the present disclosure.

[0184] When used in as part of the combination therapy the therapeutically effective amount of the inhibitor may be adjusted such that the amount is less than the dosage required to be effective if used without other therapeutic procedures.

[0185] In some embodiments, treatment with pharmaceutical compositions described herein is preceded by surgical intervention.

[0186] The disclosure also relates to methods of reducing the number of exosomes in a cancer cell by contacting said cancer cell with a therapeutically effective amount of a cyclodextrin.

[0187] The term “pharmaceutical composition” as used herein refers to a composition comprising at least one active agent as described herein (such as, for example, an (α -CD), and one or more other components suitable for use in pharmaceutical delivery such as carriers, stabilizers, diluents, dispersing agents, suspending agents, thickening agents, excipients, and the like. The disclosure relates to a pharmaceutical composition comprising an effective amount of any alpha-cyclodextrin molecule or derivative or salt disclosed herein and a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical composition

comprises any one or plurality of fatty acid molecules disclosed herein or a slat thereof. In some embodiments, the pharmaceutical composition is free of beta or gamma cyclodextrin.

[0188] The term “active agent” as used herein refers to a molecule that is intended to be used in the compositions and methods described herein and that is intended to be biologically active, for example for the purpose of treating malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition. The term “active agent” is intended to include molecules that either are, or can be converted to a form that is, biologically active. For example, the term “active agent” includes pro-drugs and/or molecules that are inactive or lack the intended biological activity but that can be converted to a form that is active or has the intended biological activity.

[0189] As used herein, the term “sample” refers generally to a limited quantity of something which is intended to be similar to and represent a larger amount of that thing. In the present disclosure, a sample is a collection, swab, brushing, scraping, biopsy, removed tissue, or surgical resection that is to be tested for clinical indicators of a disease or disorder. In some embodiments, samples are taken from a patient or subject that is believed to have malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition. In some embodiments, a sample believed to contain clinical indicators of a disease or disorder, such as malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition, is compared to a control sample that is known not to contain one or a plurality of clinical indicators of a disease or disorder, such as malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition. In some embodiments, a sample believed to contain a clinical indicator of a disease or disorder, such as malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition, is compared to a control sample that is known to not contain a clinical indicator of a disease or disorder, such as malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition. In some embodiments, a sample believed to contain a clinical indicator of a disease or disorder, such as malignant, neurodegenerative, vascular, metabolic,

inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition, is compared to a control sample that contains the same clinical indicators of a disease or disorder, such as malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition.

[0190] The term “scavenge” as used herein means uptake or chemically combine with and transport to including, but not limited to, the kidney for excretion.

[0191] The term "salt" refers to acidic salts formed with inorganic and/or organic acids, as well as basic salts formed with inorganic and/or organic bases. Examples of these acids and bases are well known to those of ordinary skill in the art. Such acid addition salts will normally be pharmaceutically acceptable although salts of non-pharmaceutically acceptable acids may be of utility in the preparation and purification of the compound in question. Salts of the embodiments include those formed from hydrochloric, hydrobromic, sulphuric, phosphoric, citric, tartaric, lactic, pyruvic, acetic, succinic, fumaric, maleic, methanesulphonic and benzenesulphonic acids.

[0192] In some embodiments, salts of the compositions comprising one or may be formed by reacting the free base, or a salt, enantiomer or racemate thereof, with one or more equivalents of the appropriate acid. In some embodiments, pharmaceutical acceptable salts of the present disclosure refer to amino acids having at least one basic group or at least one basic radical. In some embodiments, pharmaceutical acceptable salts of the present disclosure comprise a free amino group, a free guanidino group, a pyrazinyl radical, or a pyridyl radical that forms acid addition salts. In some embodiments, the pharmaceutical acceptable salts of the present disclosure refer to amino acids that are acid addition salts of the subject compounds with (for example) inorganic acids, such as hydrochloric acid, sulfuric acid or a phosphoric acid, or with suitable organic carboxylic or sulfonic acids, for example aliphatic mono- or di-carboxylic acids, such as trifluoroacetic acid, acetic acid, propionic acid, glycolic acid, succinic acid, maleic acid, fumaric acid, hydroxymaleic acid, malic acid, tartaric acid, citric acid or oxalic acid, or amino acids such as arginine or lysine, aromatic carboxylic acids, such as benzoic acid, 2-phenoxy-benzoic acid, 2-acetoxybenzoic acid, salicylic acid, 4-aminosalicylic acid, aromaticaliphatic carboxylic acids, such as mandelic acid or cinnamic acid, heteroaromatic carboxylic acids, such as nicotinic acid or isonicotinic acid, aliphatic sulfonic acids, such as methane-, ethane- or 2-hydroxyethane-sulfonic acid, or aromatic

sulfonic acids, for example benzene-, p-toluene- or naphthalene-sulfonic acid. When several basic groups are present mono- or poly-acid addition salts may be formed. The reaction may be carried out in a solvent or medium in which the salt is insoluble or in a solvent in which the salt is soluble, for example, water, dioxane, ethanol, tetrahydrofuran or diethyl ether, or a mixture of solvents, which may be removed *in vacuo* or by freeze drying. The reaction may also be a metathetical process or it may be carried out on an ion exchange resin. In some embodiments, the salts may be those that are physiologically tolerated by a patient. Salts according to the present disclosure may be found in their anhydrous form or as in hydrated crystalline form (i.e., complexed or crystallized with one or more molecules of water).

[0193] The term "soluble" or "water soluble" refers to solubility that is higher than 1/100,000 (mg/ml). The solubility of a substance, or solute, is the maximum mass of that substance that can be dissolved completely in a specified mass of the solvent, such as water. "Practically insoluble" or "insoluble," on the other hand, refers to an aqueous solubility that is 1/10,000 (mg/ml) or less. Water soluble or soluble substances include, for example, polyethylene glycol. In some embodiments, the polypeptide described herein may be bound by polyethylene glycol to better solubilize the composition comprising the peptide.

[0194] As used herein, percent "homology" or "sequence identity" is determined by using the stand-alone executable BLAST engine program for blasting two sequences (blZseq), which can be retrieved from the National Center for Biotechnology Information (NCBI) ftp site, using the default parameters (Tatusova TA, Madden TL (1999) FEMS microbiology letters **174**:247-50, which is incorporated herein by reference in its entirety).

[0195] The term "derivative" as applied to a phosphate containing, monophosphate, diphosphate, or triphosphate group or moiety refers to a chemical modification of such group wherein the modification may include the addition, removal, or substitution of one or more atoms of the phosphate containing, monophosphate, diphosphate, or triphosphate group or moiety. In embodiments, such a derivative is a prodrug of the phosphate containing, monophosphate, diphosphate, or triphosphate group or moiety, which is converted to the phosphate containing, monophosphate, diphosphate, or triphosphate group or moiety from the derivative following administration to a subject, patient, cell, biological sample, or following contact with a subject, patient, cell, biological sample, or protein (e.g. enzyme). In an embodiment, a triphosphate derivative is a gamma-thio triphosphate. In an embodiment, a derivative is a phosphoramidate. In embodiments, the derivative of a phosphate containing,

monophosphate, diphosphate, or triphosphate group or moiety is as described in Murakami et al. J. Med Chem., 2011, 54, 5902; Sofia et al., J. Med Chem. 2010, 53, 7202; Lam et al. ACC, 2010, 54, 3187; Chang et al., ACS Med Chem Lett., 2011, 2, 130; Furman et al., Antiviral Res., 2011, 91, 120; Vernachio et al., ACC, 2011, 55, 1843; Zhou et al, AAC, 2011, 44, 76; Reddy et al., BMCL, 2010, 20, 7376; Lam et al., J. Virol., 2011, 85, 12334; Sofia et al., J. Med. Chem., 2012, 55, 2481, Hecker et al., J. Med. Chem., 2008, 51, 2328; or Rautio et al., Nature Rev. Drug. Discov., 2008, 7, 255, all of which are incorporated herein by reference in their entirety for all purposes.

[0196] Additional definitions and abbreviations are provided elsewhere in this patent specification or are well known in the art.

Additional Description

[0197] Malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, virus, or age-related diseases, disorders, or other conditions are complex conditions in the sense that they involve many genes along common pathways. For instance, cardio-vascular diseases (CVDs), including, but not limited to, atherosclerosis, CAD, and stroke, and neurodegenerative diseases are known comorbidities of T2DM. Based in part on the findings that the phosphoinositides PI(3,4,5)P₃ and PI(3,4)P₂ are upregulated in malignant and neurodegenerative diseases (Waugh MG (2015) Biochim Biophys Acta **1851**:1066-82) and activate Akt signaling (Ooms Lisa M, Binge Lauren C, et al. (2015) Cancer cell **28**:155-69; Ma K, Cheung SM, et al. (2008) Cell Signal **20**:684-94; Majerus PW, York JD (2009) Journal of Lipid Research **50**:S249-S54; Bridges D, Saltiel AR (2015) Biochim Biophys Acta **1851**:857-66), PI signaling is widely believed to be involved in a broad range of age-related diseases. INPP4B is known as a suppressor of some cancers, but as a risk factor for others, including, but not limited to breast or prostate cancer .(Woolley JF, Dzneladze I, et al. (2015) Trends Mol Med **21**:530-2; Chew CL, Chen M, et al. (2016) Oncotarget **7**:5-6) PI3K signaling has also be implied in aging, cognitive decline, and Alzheimer's disease (O'Neill C (2013) Experimental gerontology **48**:647-53). Still, "clinical results with single-agent PI3K inhibitors have been modest to date,"(Mayer IA, Arteaga CL (2016) Annu Rev Med **67**:11-28) in part because traditional GWAS have largely failed to elucidate the precise mechanism by which PI signaling contributes to complex conditions, such as cancer and neurodegenerative disease.

[0198] From the results presented herein, all these conditions, including, but not limited to, breast and prostate cancer as well as Alzheimer's and Parkinson's disease, atherosclerosis, and type-2 diabetes are characterized by hyperactivity of the phosphatidylinositol (PI) cycle. In some aspects the present disclosure provides methods of treatment of breast and prostate cancer, cardiovascular, metabolic, and Parkinson's or Alzheimer's diseases, that comprise administering to a subject one or more therapeutically effective amounts of active agents that downregulate the PI cycle to elicit changes in endo-/exocytosis thereby causing a dampening or decrease of cellular migration and infiltration or processing of proteins, including, but not limited to, LDL, APP, tau, myelin, SOD1, HTT, and α -synuclein. Furthermore, such active agents could be, for example, drugs or compounds of drugs that are already being used safely in humans for other indications and could be repurposed for use in the treatment of malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition.

[0199] Other proposed uses are in a wide range of diseases where the same mechanisms are involved as in the above age-related diseases, including, but not limited to, neural ceroid lipofuscinoses (NCL),(Song W, Wang F, et al. (2014) J Biol Chem **289**:10211-22) Parkinson's disease, Alzheimer's disease, frontotemporal dementia (FTD), and Huntington's disease (HD), stroke,(Vecsernyes M, Fenyvesi F, et al. (2014) Arch Med Res **45**:711-29; Rivers JR, Maggo SD, et al. (2012) Neuroreport **23**:134-8), atherosclerosis (Irie T, Fukunaga K, et al. (1992) J Pharm Sci **81**:524-8; Zimmer S, Grebe A, et al. (2016) Sci Transl Med **8**:333ra50), non-alcoholic fatty liver disease (NAFLD),(Walenbergh SM, Houben T, et al. (2015) Int J Mol Sci **16**:21056-69) respiratory and pulmonary diseases, including, but not limited to, cystic fibrosis, asthma, chronic obstructive pulmonary disease (COPD) (Gunasekara L, Al-Saiedy M, et al. (2017) Journal of cystic fibrosis : official journal of the European Cystic Fibrosis Society ; Dos Santos AG, Bayiha JC, et al. (2017) Biochim Biophys Acta **1859**:1930-40; Cataldo D, Evrard B, et al. (2015) US 9034846 B2), type 2 diabetes mellitus (T2DM) and insulin resistance,(Llanos P, Contreras-Ferrat A, et al. (2015) American journal of physiology Endocrinology and metabolism **308**:E294-305) prion disease,(Prior M, Lehmann S, et al. (2007) Journal of virology **81**:11195-207) and protection from virus, fungal, or bacterial infections.(Danthi P, Chow M (2004) Journal of virology **78**:33-41; Balla T (2013) Physiological Reviews **93**:1019-137; Shishido TK, Jokela J, et al. (2015) Proc Natl Acad Sci U S A **112**:13669-74), including, but not limited to, cellular entry

of viruses (influenza, HIV-1, polio, HTLV-1, Newcastle, varicella-zoster, hepatitis B, bluetongue, and HSV).

[0200] Sphingolipids and ceramides are also involved in podocyte dysfunction (including, but not limited to Fabry's disease, Tay-Sachs disease, Alport syndrome, minimal change disease, diabetic kidney disease, focal segmental glomerulosclerosis, end-stage renal disease, kidney failure, podocytopenia, hyperhomocysteinemia, steroid-resistant nephrotic syndrome) and the pathophysiology of cancer, angiogenesis, atherosclerosis, inflammation, and insulin resistance.(Abou Daher A, El Jalkh T, et al. (2017) *Int J Mol Sci* **18**)

[0201] Phosphoinositides, which regulate endocytosis,(Posor Y, Eichhorn-Grunig M, et al. (2015) *Biochim Biophys Acta* **1851**:794-804) have been mentioned as drug targets in genetic diseases, including, but not limited to, muscular and corneal dystrophy or myotubular neuropathy, cancers, including, but not limited to, leukemias (ALL, AML, CML) and lymphomas (NHL), epilepsy, and thrombosis.(Viaud J, Mansour R, et al. (2016) *Biochimie* **125**:250-8) Neurological diseases linked to phosphoinositide dysregulation include, but are not limited to, Friedreich's ataxia, Charcot-Marie-Tooth (CMT) degenerative neuropathy, renal tubulopathies (oculocerebrorenal syndrome of Lowe, Dent's disease), Andersen-Tawil syndrome, mucopolysaccharidosis, multiple sclerosis (MS), Yunis-Varon syndrome, ALS FTL, and ciliopathies.(Waugh MG (2015) *Biochim Biophys Acta* **1851**:1066-82) Additional diseases linked to phosphoinositide dysregulation are osteoporosis, cancers (including, but not limited to, cervical cancer, leiomyosarcoma, gastric cancer, adenocarcinoma, lung cancer), cardiac hypertrophy, and autoimmune diseases (including, but not limited to, rheumatoid arthritis (RA) and systemic lupus erythematosus), and neurological diseases including, but not limited to, bipolar disorder and schizophrenia, (Falasca M (2012) *Current topics in microbiology and immunology*)

[0202] Many other diseases are known to involve loss-of-function mutations affecting the lysosome, where many of the pino-/endocytosed extracellular and autophagocytosed intracellular substrates need to be degraded.(Wang C, Telpoukhovskaia MA, et al. (2017) *Curr Opin Neurobiol* **48**:52-8) In all cases, the disease phenotype would be improved by reducing the amount of pino-/endocytosed materials that need to be degraded (MPS: mucopolysaccharidosis, MLD: metachromatic leukodystrophy):

[0203] *ABCA1*: renal disease; *ABCA2*: acoustic neuroma; *ABCB9*: acute myeloid leukemia (AML); *ACP2*: acid phosphatase deficiency, keratoconus, amyotrophic lateral sclerosis, atrichia, amebiasis; *ACP5*: spondyloenchondrodysplasia, bone giant cell tumor, hairy cell leucoma, tooth resorption; *AGA*: aspartylglucosaminuria, fucosidosis; *AP3B1*: Hermansky-Pudlak syndrome (HPS), oculocutaneous albinism, storage pool platelet disease; *AP3B2*: paraneoplastic neurologic disorders; *ARSA*: MLD, arylsulfatase a deficiency, multiple sulfatase deficiency, mucosulfatidosis; *ARSB*: MPS VI, arylsulfatase b deficiency, mucosulfatidosis; *ASAHI*: Farber lipogranulomatosis, (Kugelberg-Welander) spinal muscular atrophy, (Erdheim-Chester) lipogranulomatosis,; *ATP13A2/PARK9*: NCL 12, Kufoor-Rakeb syndrome, spastic paraplegia 78, juvenile onset Parkinson disease; *ATP6AP2* (Perrett 2015): mental retardation, Parkinsonism with spasticity, lymphocytic choriomeningitis; *ATP6V0A1*: inferior myocardial infarction; *ATP6V0A2*: wrinkly skin syndrome, cutis laxa, myelophthisic anemia; *ATP6V0A4*: renal tubular acidosis, medullary sponge kidney; *CD164*: autosomal dominant deafness, pollen allergy, prostate cancer; *CLC7*: osteopetrosis; *CLN1/PPT1*: NCL, Batten disease, Santavuori-Haltia; *CLN1/GRN*: NCL, frontotemporal dementia, primary progressive aphasia; *CLN3*: NCL, secondary corneal edema, Spielmeyer-Vogt-Sjogren-Batten disease; *CLN4/DNAJC5*: NCL, CLN4 disease; *CLN5*: NCL; *CLN5*: NCL, Finnish late infantile; *CLN6*: NCL, Kufs disease, *CLN7*: NCL, macular dystrophy, neurotic disorder, depressive neurosis; *CLN8*: Northern epilepsy, progressive with mental retardation / Turkish late infantile; *CTNS*: nephropathic cystinosis, Fanconi syndrome; *CTSA*: galactosialidosis, glycoproteinosis, gonococcal salpingitis, triosephosphate isomerase deficiency, aspartylglucosaminuria; *CTSB*: occlusion of gallbladder, ileum cancer, pancreatitis, keratolytic winter erythema, breast cancer, Alzheimer's disease; *CTSC*: Papillon-Lefevre syndrome, Haim-Munk syndrome, periodontitis, palmoplantar keratosis, actinic keratosis; *CTSD*: NCL, bone chondrosarcoma, endometrial clear cell adenocarcinoma, breast diseases, Parkinson's disease (Parnetti 2017); *CTSE*: gastric adenocarcinoma, Rosai-Dorfman disease, histiocytosis; *CTSF*: NCL, Kufs disease, akinetic mutism, coma vigilans, clonorchiasis, oriental liver fluke disease; *CTSG*: Papillon-Lefevre syndrome, cutaneous mastocytosis, suppurative periapical periodontitis, vasculitis, Wegener granulomatosis; *CTSH*: thyroid crisis, intermittent explosive disorder, narcolepsy, fibrous meningioma, amyotrophic lateral sclerosis; *CTSK*: pycnodystostosis, hypersensitivity pneumonitis, osteosclerosis, osteomyelitis, bone giant cell tumor; *CTSL*: vulva basal cell carcinoma, eccrine acrospiroma, fasciolopsiasis, tracheal cancer, rectosigmoid junction neoplasm; *CTSO*: breast cancer; *CTSS*:

mandibular cancer, non-suppurative otitis media, cercarial dermatitis, subepithelial mucinous corneal dystrophy, jaw cancer; *CTSW*: autoimmune atrophic gastritis; *CTSZ*: dacryoadenitis; *DNASE2*: calcific tendinitis, rheumatoid arthritis; *FUCA1*: fucosidosis, angiokeratoma, mucopolipidosis III, laryngotracheitis; *GAA*: glycogen storage disease II (Pompe disease), acid maltase deficiency, Danon disease, α -1,4-glucosidase deficiency; *GALC*: Krabbe disease, leukodystrophy; *GALNS*: MPS IV, chondroosteodystrophy, Scheie syndrome, Kniest dysplasia; *GBA*: Gaucher disease (GD), pseudo Gaucher disease, GBA-associated Parkinson's disease; *GLA*: Fabry disease, cramp-fasciculation syndrome, angiokeratoma, MPS VII; *GLB1*: gm1-gangliosidosis, MPS IVb; *GM2A*: GM2-gangliosidosis, Tay-Sachs disease (TSD), Sandhoff-disease, mucopolipidosis II α/β , I-cell disease; *GNPTAB*: mucopolipidosis 2, mucopolipidosis 3, MPS 3a; *GNPTG*: mucopolipidosis III, pseudo-Hurler polydystrophy, articulation disorder; *GNS*: MPS III, multiple sulfatase deficiency, mucosulfatidosis; *GUSB*: MPS VII, hydrops fetalis, necrotizing ulcerative gingivitis, choledocholithiasis; *GUSB*: Sly disease, MSP VII, hydrops fetalis, necrotizing ulcerative gingivitis, choledocholithiasis; *HEXA*: Tay-Sachs disease, gm2 gangliosidosis; *HEXB*: Sandhoff disease, mucopolipidosis IV, sphingolipidosis; *HGSNAT*: MPS IIIc, MPS IIIb, retinitis pigmentosa, Kluver-Bucy syndrome; *HYAL1*: MPS IX, bladder carcinoma, prostate cancer; *IDS*: MPS II, Hunter syndrome; *IDUA*: MPS I, Scheie syndrome, Hurler-syndrome; *IGF2R*: hepatocellular carcinoma, mucopolipidosis II, inclusion-cell disease, colorectal cancer; *LAAT1/PQLC2*: cystinosis; *LAMP1*: Salla disease, Hermansky-Pudlak syndrome, lysosomal acid phosphatase deficiency, hemophagocytic syndrome; *LAMP2*: Danon disease, cardiomyopathy, atrial standstill, glycogen storage disease II, lysosomal acid phosphatase deficiency; *LAMP3/CD63*: Hermansky-Pudlak syndrome, melanoma, Quebec platelet disorder, mast cell disease, Schwarzman phenomenon; *LAMP4/CD68/SCARD1*: (breast) granular cell tumor, follicular dendritic cell sarcoma, bacterial esophagitis, axillary lipoma; *LAPTM4A*: pain disorder; *LAPTM4B*: hepatocellular carcinoma; *LGMN*: schistosomiasis; *LIMP2/SCARB2*: progressive myoclonic epilepsy, Unverricht-Lundborg disease, hand-tooth-and-mouth disease, myoclonus; *LIPA*: Wolman disease, cholesterol ester storage disease, splenic abscess, familial hypercholesterolemia; *LITAF*: Charcot-Marie-Tooth hereditary neuropathy; *LMBRD1*: methylmalonic aciduria and homocystinuria, cblf type, hepatitis, transcobalamin II deficiency; *LYPLA3/PLA2G15*; *LYST*: Chedliak-Higashi syndrome, exfoliation syndrome, Hermansky-Pudlak syndrome, dichromatosis symmetrica; *M6PR*: MPS IIIa, mucopolipidosis II, NCL, Niemann-Pick disease; *MANB*: mannosidosis (α/β); *MCOLN1*: mucopolipidosis IV,

ataxic cerebral palsy, sphingolipidosis, strabismus, mucopolysaccharidosis; *NAGA*: Kanazaki disease, Schindler disease, neuroaxonal dystrophy, agiokeratoma; *NAGLU*: MPS III, Charcot-Marie-Tooth disease, acute pyelonephritis; *NAGPA*: familial persistent stuttering, articulation disorder, MPS IIIb, speech disorder, diabetic nephropathy; *NAPSA*: ovarian clear cell adenofibroma, Krukenberg/lung adeno/pulmonary adeno (Bishop DV (2010) PLoS One 5:e15112)/ovarian clear cell/ carcinoma, malignant fibrous mesothelioma; *NEUI*: sialidosis, hydrops fetalis, parainfluenza virus type 3; *NPC1/2*: Niemann-Pick disease C1/2; *PSAP*: atypical Gaucher disease, combined saposin deficiency, MLD, Krabbe disease; *PSEN1* (Lee 2015, Sato 2017): frontotemporal dementia, Alzheimer's disease 3, familial acne inversa, dilated cardiomyopathy; *RAB27A*: Griscelli syndrome, hemophagocytic lymphohistiocytosis, Chediak-Higashi syndrome; *RAB7* (Perrett 2015): Charcot-Marie-Tooth disease 2b, choroideremia, tabes dorsalis, NCL; *SGSH*: MPS III, Hurler-Bryer syndrome, Sanfillipo syndrome; *SLC11A1*: Buruli ulcer, tuberculosis, typhoid fever; *SLC11A2*: microcytic anemia, hemosiderosis, anemia; *SLC17A5/SIALIN*: Salla disease, sialic acid storage disorder, sialuria, fascioliasis; *SMPD1*: Niemann-Pick disease, acid sphingomyelinase deficiency, narcissistic personality disorder; *SORT1*: inclusion-cell disease, I-cell disease, myocardial infarction; *SQSTM1*: ALS; *SUMF1*: multiple sulfatase deficiency, MLD, spinocerebellar ataxia, MPS VI; *TMEM106B*: amyotrophic lateral sclerosis (ALS)/Frontotemporal lobar degeneration (FTLD).(Sullivan PM, Zhou X, et al. (2017) Lysosomes - Associated Diseases and Methods to Study Their Function 63-91) *TPPI*: NCL, spinocerebellar ataxia, Bielschowsky-Jansky disease.

[0204] Other conditions where age-related insufficient lysosomal function is involved are cardio- and cerebrovascular diseases including, but not limited to, arteriosclerosis, coronary heart disease, arrhythmia, heart failure, hypertension, orthostatic hypotension, myocardial infarction. angina pectoris. heart failure, atherosclerosis, stroke, renal artery disease or stenosis, peripheral vascular disease, chronic obstructive pulmonary disease, ("COPD"), chronic renal diseases with renal failure, and heart disease. Exemplary inflammatory diseases and conditions include arthritis, such as rheumatoid arthritis. Additional exemplary age-associated diseases and disorders include ulcers. osteopetrosis. progeria. weakness, hearing loss, and type-2 diabetes. It is to be understood that "age-related" refers to diseases frequently associated with aging, however, a given patient need not be of advanced age, but rather the subject methods and compositions may be used regardless of the patient's age.

A) Active Agents

[0205] As further described in the Examples and other sections of the present application, agents that can be used in the methods of the invention may reduce the concentration of phospholipids available to cells, including, but not limited to, neurons, macrophages, microglia, and tumor cells. In some embodiments, the agent is a cyclic oligosaccharide. In some embodiments, the cyclic oligosaccharide is an α -CD optionally modified by an alkyl group from about 1 to about 20 or more carbons in length. In some embodiments, the cyclodextrin is α -CD optionally modified by a hydroxyl-alkyl group from about 1 to about 20 or more carbons in length. In yet another embodiment, the agent is hydroxypropyl- (HP-) α -CD. In some embodiments, the agent is a clathrate of an α -CD. In some embodiments the agent is a clathrate of an α -CD with a fatty acid. A “fatty acid” as used herein means a carboxylic acid with a long aliphatic chain, which is either saturated or unsaturated. In some embodiments, the aliphatic chain is from about 3 to about 70 carbons long. In some embodiments, the aliphatic chain is from 8 to about 30 carbons long. In some embodiments the fatty acid has one or more aliphatic chains branched or unbranched with one or more substituents. In some embodiments, the fatty acid comprises from about 6 to about 22 carbon atoms in its aliphatic chain. In some embodiments, any one of the bonds between each carbon atom may be in the cis or trans configuration.

[0206] Cyclodextrins are natural compounds formed during bacterial digestion of cellulose. They are not hydrolyzed by common mammalian amylases, but can be fermented by the intestinal microflora. They are poorly resorbed in typical situations and do not interact with cells or tissues. On Dec 22, 2004, α -CD was declared GRAS by the FDA for “use in selected foods, except meat and poultry, for fiber supplementation, as a carrier or stabilizer for flavors (flavor adjuvant), as a carrier or stabilizer for colors, vitamins and fatty acids and to improve mouthfeel in beverages (GRN No. 155, updated Nov 2016 as GRN 678 to reflect an expected 90th percentile for intake of 420 mg/kg, or ~30 g/d at 70 kg). An α -CD monograph is included in the U.S. Pharmacopeia/National Formulary (USP/NF25), the European Pharmacopoeia (EP 6.0), and the Handbook of Pharmaceutical Excipients. cyclodextrins are exempted from the requirement of a tolerance under 40 CFR 180.950 when used in or on various food commodities. (FR 70 128 28780 2005-07-06). On Oct. 9, 2017, cyclodextrins were included in the Annex to the European Commission guideline on ‘Excipients in the labelling and package leaflet of medical products for human use’.

[0207] The common cyclodextrins are made up of six (α -CD), seven (β -CD), or eight (γ -CD) D-glucose molecules bound together in a toroid (truncated cone) with a lipophilic inner and a hydrophilic outer surface (FIG. 1). This combination of features makes them suitable as an expedient to solubilize lipophilic drugs. “Chemical modifications have been made to [cyclodextrins] to increase their hydrophilic activity with the hope that the improved solubility would eliminate the renal toxicity [in rats].” (Irie T, Uekama K (1997) J Pharm Sci **86**:147-62) Substitution of any of the hydroxyl groups, even by hydrophobic moieties, will result in a dramatic increase in water solubility. The main reason for the solubility enhancement is that chemical manipulation frequently transforms the crystalline cyclodextrins into amorphous mixtures of isomeric derivatives. For example, the aqueous solubility of β -cyclodextrin increases with increasing degree of methylation. The highest solubility is obtained when about two-thirds of the hydroxyl groups (i.e., 14 of 21) are methylated. HP- β -CD substantially improves water solubility over β -CD (Table 2, see also Table 8).

Table 2: Physicochemical characteristics of selected cyclodextrins

	α -CD	HP- α -CD	β -CD	HP- β -CD
Number of glucose units	6	6	7	7
Internal diameter	4.5–5.2	4.7–5.3	6.0–6.5	6.0–6.5
Solubility in water [g/l]	145	>500	18.5	>600

[0208] Common cyclodextrins obtained by the substitution of the R groups on the edge (rim) of the toroid (FIG. 1, (Brewster ME, Loftsson T (2007) Advanced Drug Delivery Reviews **59**:645-66)) include, but are not limited to, methyl (including randomly methylated) CH₃, 2-hydroxypropyl (HP): CH₂CHOHCH₃, sulfobutylether (CH₂)₄SO₃Na⁺, acetyl, succinyl, glucosyl, maltosyl, carboxymethyl ether, phosphate ester, simple polymers, or carboxymethyl. Since both the number of substitutes and their location will affect the physicochemical properties of the cyclodextrin molecules, such as their aqueous solubility and complexing abilities, each derivative listed should be regarded as a group of closely related cyclodextrin derivatives.

[0209] Low solubility in water, as with β -CD, in particular, often results in precipitation of solid cyclodextrin complexes. β -CDs, for instance, form intramolecular hydrogen bonds between secondary OH groups, which detracts from hydrogen bond formation with surrounding water molecules, resulting in low aqueous solubilities. (Loftsson T, Brewster ME (1996) J Pharm Sci **85**:1017-25)

B) Cytotoxicity/hemolysis

[0210] With parenteral delivery, “the steady-state volume of distribution for β -CD ... in rats, rabbits, dogs, and humans corresponds well with the extracellular fluid volume of each species, suggesting that no deep compartments or storage in pools are involved. [...] α - and β -[cyclodextrin] are excreted almost completely in an intact form into the urine” (Irie T, Uekama K (1997) J Pharm Sci **86**:147-62) Still cyclodextrins may cause two types of adverse events with parenteral delivery. First, they can accumulate in kidney cells, causing nephrotoxicity. Second, after the lipophilic drug is delivered, they form “a new lipid-containing compartment (or pool) in the aqueous phase into which [cellular lipid] compounds [are] extracted,”(Irie T, Uekama K (1997) J Pharm Sci **86**:147-62) which could cause hemolysis.

[0211] Phospholipids and cholesterol, the major building blocks of plasma membranes, are all lipids. Hence, when cyclodextrins are given parenterally without having their lipophilic cavity filled (or after the lipophilic drug has been delivered), they can potentially extract lipids (phospholipids and cholesterol, depending on the type of cyclodextrin) from plasma membranes. “Several cyclodextrins have been demonstrated to cause cell lysis in different types of cells, indicating that the effect is not cell-type specific [(Irie T, Uekama K (1997) J Pharm Sci **86**:147-62)]”(Stella VJ, He Q (2008) Toxicol Pathol **36**:30-42) In particular, cyclodextrins are known to induce shape changes of plasma membrane invagination on erythrocytes and, at higher concentrations, induce hemolysis of erythrocytes in the order of β -CD > α -CD > γ -cyclodextrin.(Irie T, Otagiri M, et al. (1982) J Pharmacobiodyn **5**:741-4), i.e., the hemolytic activity of α -CD is lower than that of both β -CD and HP- β -CD (Irie T, Uekama K (1997) J Pharm Sci **86**:147-62, Figure 6). In addition, β -CD, but not α -CD and HP- β -CD, induces caspase-dependent apoptotic cell death in keratinocytes by depleting plasma membrane cholesterol.(Stella VJ, He Q (2008) Toxicol Pathol **36**:30-42) HP- α -CD was found to be less cytotoxic than α -CD on heterogeneous human epithelial colorectal adenocarcinoma (Caco-2) cells.(Roka E, Ujhelyi Z, et al. (2015) Molecules **20**:20269-85; Ono N, Arima H, et al. (2001) Biol Pharm Bull **24**:395-402) From these observations, HP-cyclodextrins are have not only less nephrotoxicity, but also less cyto- and hemolytic toxicity.

[0212] HP- β -CD is used as an expedient/solvent for many lipophilic drugs, including the neurosteroid allopregnanolol. α -CD has been approved and is used as an expedient of

alprostadil (a prostaglandin) for intracavernosal injection in the treatment of erectile dysfunction.

[0213] Still, less cytotoxicity overall does not mean safe for human use. A 2010 study showed that HP- β -CD causes permanent hearing loss in cats at doses of 4–8 g/kg, (Ward S, O'Donnell P, et al. (2010) *Pediatr Res* **68**:52-6) In a recent study of 14 NPC1 patients, mid-frequency to high-frequency hearing-loss requiring hearing aids for management. (Ory DS, Ottinger EA, et al. (2017) *Lancet* **390**:1758-68) These results raised concerns for the use of β -cyclodextrin as an active ingredient (larger dose), rather than an excipient (smaller dose).

[0214] In 2014, it was observed that the reported benefit of the neurosteroid allopregnanolol in Niemann-Pick C (NPC) disease was, in fact, due to the excipient, HP- β -CD. (Vance JE, Karten B (2014) *Journal of Lipid Research* **55**:1609-21; Liu B, Turley SD, et al. (2009) *Proc Natl Acad Sci U S A* **106**:2377-82) It was demonstrated the β -cyclodextrin at therapeutic doses reduces cholesterol in cellular components, rather than extracting cholesterol from cell membranes as previously believed. In Sept 2015, a phase 2b/3 study of HP- β -CD started in patients with neurologic manifestations of NPC1 disease. (NIH (2015) *ClinicalTrials.gov* NCT02534844) The question was whether one could retain the benefit for treating NPC while avoiding risk of ototoxicity (“dead or deaf?”).

[0215] There is a positive correlation between the hemolytic activity of several cyclodextrins and their capacity to solubilize cholesterol,” (Irie T, Uekama K (1997) *J Pharm Sci* **86**:147-62) suggesting that ototoxicity might be directly related to the extraction of cholesterol from outer hair cells.

[0216] The results of the genetic study reported herein show that progression in breast cancer, including metastases, are caused, in part, by excessive conversion of serum glycerophospholipids (LPC, PC) into PI, the substrate of the PI cycle, which regulates endocytosis through phosphoinositides. Of note, LPC is also involved in surfactant inhibition in cystic fibrosis, where “LPC or FFA mediated surfactant inhibition was reversed by M- β -CD, even in the relative absence of cholesterol [likely reflecting] the capacity of M- β -CD to sequester non-steroidal lipids in addition to cholesterol.” (Gunasekara L, Al-Saiedy M, et al. (2017) *Journal of cystic fibrosis : official journal of the European Cystic Fibrosis Society*)

[0217] All cyclodextrins have the ability to scavenge phospholipids. β -CDs (seven sugars) have often been chosen over α -CDs (six sugars, FIG. 1) because they can be used as expedients for more (larger) drugs. The smaller α -CD is known to have higher specificity for the smaller phospholipids (Ohtani Y, Irie T, et al. (1989) *European Journal of Biochemistry* **186**:17-22; Vance JE, Karten B (2014) *Journal of Lipid Research* **55**:1609-21). because steroids and sterols, including, but not limited to, cholesterol, (Zarzycki PK, Ohta H, et al. (2008) *Anal Bioanal Chem* **391**:2793-801) do not fit its smaller cavity (FIG. 2). The specificity of extraction of phospholipids, cholesterol, and proteins are shown in FIG. 3 (Ohtani Y, Irie T, et al. (1989) *European Journal of Biochemistry* **186**:17-22). Among the phospholipids, α -CD is known to scavenge glycerolipids, such as PC, and sphingolipids more specifically than β -CDs (FIG. 4). In particular, α -CD is known to form complexes both around both the inositol head or the sn-2 chain of PI. (Fauvelle F, Debouzy JC, et al. (1997) *J Pharm Sci* **86**:935-43), Hence, α -CDs are expected to downregulate the substrates for the PI cycle and, thereby, downregulate endocytosis with higher specificity than β -CD. (Cai W, Du A, et al. (2013) *J Immunol* **191**:6093-100) HP- α -CD retains the preference of α -CD for phospholipids over cholesterol. (Huang Z, London E (2013) *Langmuir* **29**:14631-8) One of the surprising result in this disclosure (see Example 16) is that HP- α -CD in serum is even more specific than expected, scavenging PC, but neither lyso-phospholipids nor PS.

[0218] Ototoxicity of β -cyclodextrin has been shown to be caused by β -cyclodextrin depriving prestin of the cholesterol it needs to function in outer hair cells (Takahashi S, Homma K, et al. (2016) *Sci Rep* **6**:21973; Kamar RI, Organ-Darling LE, et al. (2012) *Biophysical Journal* **103**:1627-36). The fact that the cavity of α -CDs is too small for cholesterol explains that α -CD and its derivatives, including, but not limited to, HP- α -CD, are not ototoxic.

[0219] Long-term parenteral administration of HP- β -CD (200 mg/kg) was reported to decrease bone mineral density (BMD), which was associated with increased bone resorption, (Kantner I, Erben RG (2012) *Toxicol Pathol* **40**:742-50) while a CD-bisphosphonate conjugate, alendronate- β -CD (ALN- β -CD) was shown to be bone-anabolic. (Liu X-M, Wiswall AT, et al. (2008) *Biomaterials* **29**:1686-92)

C) Cyclodextrin specific activity

[0220] Only in part related to the different physicochemical characteristics Table 2, α -CDs, β -CDs, and γ -CD cyclodextrins have dramatically different biochemical function and, thus, different uses.

- Only γ -CDs can be hydrolyzed by pancreatic amylases and saliva and are rapidly metabolized and absorbed in the small intestine.
- β -CDs and γ -CDs can bind fats at most at a 1:1 molar ratio, but α -CD can bind fat 9-times its weight.
- Size of the cavity v. the molecule is a major determinant for the use of cyclodextrins. β -CDs (and γ -CDs, to a lesser extent), but not α -CDs can bind sterols (including cholesterol) and steroids), because these molecules are too large to fit into the smaller cavity of α -CDs.
- Methylated β -CDs have a high affinity to common constituents of cell membranes and, thus, an extreme hemolytic effect, while α -CDs have very low effect on human erythrocytes.
- The effect of the neuromuscular blocking drug rocuronium is reversed by the specifically designed γ -CD derivative sugammadex (octakis-(6-deoxy-6-S-mercaptopropionyl- γ -cyclodextrin sodium salt) to prevent residual paralysis.(Booij LH (2009) *Anaesthesia* **64 Suppl 1**:31-7)
- For cyclosporin A, a mixture of α -CD and γ -CD is used to enhance the drug solubility without causing ocular irritation.
- For intranasal insulin, DM β CD and α -CD are potent enhancers, but γ -CD, β -CD, and HP β CD are not.(Merkus FW, Verhoef JC, et al. (1991) *Pharm Res* **8**:588-92; Shao Z, Krishnamoorthy R, et al. (1992) *Pharmaceutical Research* **9**:1157-63)
- α -CD but not β -CD supplementation decreased atherosclerotic lesions in aorta in apoE-KO mice, although β -CD but not α -CD supplementation decreases intestinal lipid absorption.(Sakurai T, Sakurai A, et al. (2017) *Mol Nutr Food Res* **61**)

Only α -CD can be used for gold extraction through environmentally friendly co-precipitation.(Liu ZC, Samanta A, et al. (2016) *Journal of the American Chemical Society* **138**:11643-53)

[0221] This “in-complete” list of differences between cyclodextrin demonstrates that effects seen by one cyclodextrin cannot be extrapolated to other cyclodextrins. In fact, there are only few examples, where more than one of the cyclodextrins is used for the same application:

[0222] HP- β -CD is used, for instance,

- As the active ingredient of Febreze[®]; the smaller α -CDs would trap too few malodorous molecules.
- To remove cholesterol from whole egg, milk, the smaller α -CDs do not fit cholesterol

[0223] α -CDs are used

- In foods based on vegan coconut milk powder (UK) as a replacement for sodium caseinate
- In powdered alcohol
- In Coenzyme Q10/L-Carnitine supplements to form complexes with the essential alpha linoleic acid

[0224] Therapeutic uses also are highly specific. In one age related application (Nociari MM, Lehmann GL, et al. (2014) Proceedings of the National Academy of Sciences **111**:E1402-E8), demonstrated that HP- β -CD could bind A2E, a lipofuscin component, while α -CD could not (FIG. 5)

[0225] Rarely, if ever are several cyclodextrins used with the same type of substrates, attesting to the fact that their function is highly specific despite them comprising rings of sugars. One of the most important differences is the number of these sugars: “Cyclodextrin-Lipid Complexes: Size Matters”.(Szente L, Fenyvesi E (2017) Structural Chemistry **28**:479-92) In some embodiments, the α -CD is a hydroxypropyl, hydroxyethyl, hydroxymethyl, hydroxybutyl, hydroxypentyl- α -CD or salt or derivative thereof. In some embodiments, the pharmaceutical composition of the disclosure comprises hydroxypropyl, hydroxyethyl, hydroxymethyl, hydroxybutyl, hydroxypentyl- α -CD or salt or derivative thereof but is free of alpha-cyclodextrin or beta-cyclodextrin. In some embodiments, the pharmaceutical composition is free of beta or gamma cyclodextrin and or derivatives of salts thereof.

D) Treatment of Cancer Patients with α -CD or derivatives or salts thereof

[0226] Several embodiments of the disclosure include the use of α -CD and/or derivatives or pharmaceutically acceptable salt thereof.

[0227] Embodiments of the present disclosure are particularly useful to treat individuals who have cancer identified as having one or a plurality of cells with an abnormally high rate of endocytosis or exocytosis, or, in some embodiments, a dysfunctional PI cycle or lysosome. In some embodiments, methods for treating a subject who has cancer comprise the steps of first identifying cancer as having a high rate of endocytosis or exocytosis, or, in some embodiments, a dysregulated PI cycle, and then administering to such a subject a therapeutically effective amount of an α -CD. In some preferred embodiments, the identification of cancer as having a high rate of endocytosis or exocytosis, or, in some embodiments, a dysfunctional PI cycle, is done by PET imaging, preferably using a fluorescent tag, antibody, or other agent that identifies mutations in the amino acid sequence of Table 5b or an amino acid sequence at least about 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% homology to the amino acid sequences of Table 5b. In some embodiments, the α -CD or derivative or salt thereof is effective to scavenge phospholipid in greater than about 50% of cells in an *in vitro* migration assay at a concentration of less than 4 mM, 3 mM, 2 mM, or 1 mM. In some embodiments, the α -CD or derivative or salt thereof is effective to inhibit migration of cells in greater than about 50% of cells in an *in vitro* cell migration assay at a concentration of about 1 mM. In some embodiments, the α -CD or derivative or salt or clathrate thereof is effective to reduce cell migration in greater than 50% of cells in an *in vitro* cell migration assay at a concentration from about 0.5 to about 1.5 mM.

[0228] In some embodiments of the present disclosure, methods for treating an individual who has been identified as having cancer comprise administering to such an individual a therapeutically effective amount of the α -CD or derivative or salt thereof which is known to be effective to inhibit cell migration in greater than 50% of cells in an *in vitro* migration assay at a concentration of less than 1 mM. In some preferred embodiments, the α -CD or derivative or salt thereof is effective to inhibit cell migration in greater than 50% of cells in an *in vitro* cell migration assay at a concentration of less than about 1.5 mM. In some preferred embodiments, the α -CD or derivative or salt thereof is effective to inhibit cell migration in greater than about 50%, 60%, 70%, 80%, or 90% of cells in an *in vitro* cell migration assay at a concentration of about 1 mM. In some preferred embodiments, prior to

administration of the α -CD or derivative or salt thereof, the cancer is confirmed as being a cancer comprising one or a plurality of cells characterized by an abnormally high rate of endo- or exocytosis, or, in some embodiments, a dysfunctional PI cycle or lysosome. The preferred method of doing so is by PET imaging, polymerase chain reaction (PCR) of a sample such as a biopsy.

[0229] Methods are provided for inhibiting, even partially, metastasis of a cancer cell. The methods comprise delivering to the cancer cell an amount of α -CD or derivative or salt thereof effective to inhibit cell migration of the cell. The α -CD or derivative or salt thereof used is effective to slow migration of a cancer cell in greater than 50% of cells in an *in vitro* cell migration assay at a concentration of less than about 2 mM or about 1.5 mM. In some embodiments, the α -CD or derivative or salt thereof is effective to inhibit cell migration in greater than 50% of cells in an *in vitro* cell migration assay at a concentration of less than 1.5 mM. In some preferred embodiments, the cyclodextrin or derivative or salt thereof is effective to inhibit cell migration in greater than 50% of cells in an *in vitro* cell migration assay at a concentration of at or about less than 1.0 mM. In some embodiments, the treatment simultaneously reduces the ototoxicity of the treatment.

[0230] Embodiments of the present disclosure are particularly useful to treat patients who have cancer with cancer cells that have abnormally high rate of endo- or exocytosis, or, in some embodiments, a dysfunctional PI cycle. Such cancers include most cancers and generally exclude those cancers arising from tissues associated with lipid production such as liver cancer, and cancer involving fat cells. Cancer cells that have abnormally high rate of endocytosis or exocytosis, or, in some embodiments, a dysfunctional PI cycle, are generally limited to epithelial cell derived cancers. In some embodiments, cancer is from epithelial cells of the breast, colon, lung, or prostate. Thus, some methods of the invention relate to methods of treating a cancer patient who has cancer that have abnormally high rate of endo- or exocytosis, or, in some embodiments, a dysfunctional PI cycle, wherein such methods comprise the step of administering to such patient or subject a therapeutically effective amount of cyclodextrin. In preferred embodiments, the α -CD or derivative or salt thereof is known to be effective to slow cell migration in greater than 50% of cells in an *in vitro* cell migration assay at a concentration of less than 2 mM. In some preferred embodiments, the α -CD or derivative or salt thereof is effective to induce apoptosis in greater than about 50%, 60%, 70%, 80%, or 90% of cells in an *in vitro* cell migration at a concentration of less than

1.5 mM. Cancer cells that have abnormally high rate of endo- or exocytosis, or, in some embodiments, a dysfunctional PI cycle, generally have dysfunction of enzymes of Table 5b and/or metabolize and/or uptake high levels of phospholipids around their microenvironment. In some preferred embodiments, prior to administration of an α -CD or derivative or pharmaceutically acceptable salt thereof, the cancer is confirmed as being a cancer characterized by an abnormally high rate of endocytosis or exocytosis, or, in some embodiments, a dysfunctional PI cycle. The preferred method of doing so is by PET imaging, PCR or immunohistochemistry of a sample.

[0231] Methods are provided for preventing or inhibiting the rate of metastases of a cancer cell characterized by an abnormally high rate of endo- or exocytosis, or, in some embodiments, a dysfunctional PI cycle. The methods comprise delivering to the cancer cell an amount of an α -CD or derivative or pharmaceutically acceptable salt thereof effective to reduce cell migration of the cell. The α -CD or derivative or pharmaceutically acceptable salt thereof used is effective to inhibit or reduce the rate of cell migration in greater than about 50%, 60%, 70%, 80%, or 90% of cells in an *in vivo* cell migration assay at a concentration of less than 1.5 mM. In some preferred embodiments, the disclosed treatment herein is effective to inhibit or reduce the rate of cell migration in greater than about 50%, 60%, 70%, 80%, or 90% of cells in an *in vitro* cell migration assay at a concentration of less than 1.1 mM. In some preferred embodiments, the disclosed treatment herein is effective to inhibit or reduce the rate of cell migration in greater than about 50%, 60%, 70%, 80%, or 90% of cells in an *in vitro* cell migration assay at a concentration of less than about 1.0 mM.

[0232] In some embodiments, any of the methods disclosed herein are free of administration of a cyclodextrin that scavenges cholesterol upon administration to a subject. In some embodiments, any of the methods disclosed herein are free of administration of a beta-cyclodextrin (β -CD, β CD) or hydroxy-alkyl beta cyclodextrin.

[0233] The production of α -CDs is relatively simple and involves treatment of ordinary starch with a set of easily available enzymes. Commonly, cyclodextrin glycosyltransferase (CGTase) is employed along with α -amylase. First starch is liquefied either by heat treatment or using α -amylase, then CGTase is added for the enzymatic conversion. CGTases can synthesize all forms of cyclodextrins, thus the product of the conversion results in a mixture of the three main types of cyclic molecules, in ratios that are strictly dependent on the enzyme used: each CGTase has its own characteristic α : β : γ synthesis ratio. Purification of the

three types of cyclodextrins takes advantage of the different water solubility of the molecules: β -CD, which is very poorly water-soluble (18.5 g/l or 16.3 mM) (at 25 °C) can be easily retrieved through crystallization while the more soluble α - and γ -CDs (145 and 232 g/l respectively) are usually purified by means of chromatography techniques. As an alternative a "complexing agent" can be added during the enzymatic conversion step: such agents (usually organic solvents like toluene, acetone or ethanol) form a complex with the desired cyclodextrin which subsequently precipitates. The complex formation drives the conversion of starch towards the synthesis of the precipitated cyclodextrin, thus enriching its content in the final mixture of products. Wacker Chemie AG uses dedicated enzymes, that can produce α -, β - or γ -cyclodextrin specifically. This is very valuable especially for the food industry, as only α - and γ -cyclodextrin can be consumed without a daily intake limit.

E) Pharmaceutical Compositions and Routes of Administration

[0234] In some embodiments, the present disclosure provides compositions comprising any one or more of the active agents described herein, either alone or in combination, for example for use in treating malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition. For example, in some embodiments, the present disclosure provides compositions comprising an α -CD, or an analogue or derivative thereof, for example for use in treating breast or prostate cancer. In some embodiments, the present disclosure provides compositions comprising an α -CD, or an analogue or derivative thereof, for example for use in treating breast or prostate cancer.

[0235] Pharmaceutical compositions provided by the present disclosure include compositions wherein the active ingredient (e.g., compounds described herein, including embodiments or examples) is contained in a therapeutically effective amount, i.e., in an amount effective to achieve its intended purpose. The actual amount effective for a particular application will depend, *inter alia*, on the condition being treated. When administered in methods to treat a disease, such compositions will contain an amount of active ingredient effective to achieve the desired result, e.g., modulating the activity of a target molecule (e.g., PIP1, PIP2, PIP3), and/or reducing, eliminating, or slowing the progression of disease symptoms (e.g. symptoms of malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition such as symptoms of Alzheimer's or Parkinson's disease).

Determination of a therapeutically effective amount of a compound of the disclosure is well within the capabilities of those skilled in the art, especially in light of the detailed disclosure herein.

[0236] The pharmaceutical composition may be formulated by one having ordinary skill in the art with compositions selected depending upon the chosen mode of administration. Suitable pharmaceutical carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, A. 0501, a standard reference text in this field.

[0237] Administering the pharmaceutical composition can be effected or performed using any of the various methods known to those skilled in the art. Systemic formulations include those designed for administration by injection, e. g. subcutaneous, intravenous, intramuscular, intrathecal or intraperitoneal injection, as well as those designed for transdermal, transmucosal, oral or pulmonary administration. In some embodiments, administration of the effective amount of pharmaceutical composition disclosed herein is not limited to any particular delivery system and includes, without limitation, parenteral (including subcutaneous, intravenous (via injection or infusion), intramedullary, intraarticular, intramuscular, or intraperitoneal injection), rectal, topical, transdermal, mucosal or oral (for example, in capsules, suspensions, or tablets) administration. In some embodiments, administration to a subject in need thereof occurs in a single dose or in repeat administrations, and in any of a variety of physiologically acceptable salt forms, or with an acceptable pharmaceutical carrier or additive as part of a pharmaceutical composition. In some embodiments, any suitable and physiological acceptable salt forms or standard pharmaceutical formulation techniques, dosages, and excipients are utilized. In some embodiments, the step of administering comprises administering the composition or pharmaceutical composition intravenously, intramuscularly, topically, intradermally, intramucosally, subcutaneously, sublingually, orally, intravaginally, intracavernously, intraocularly, intranasally, intrarectally, gastrointestinally, intraductally, intrathecally, subdurally, extradurally, intraventricular, intrapulmonary, into an abscess, intraarticularly, into a bursa, subpericardially, into an axilla, intrauterine, into the pleural space, intraperitoneally, transmucosally, or transdermally.

[0238] Pharmaceutical compositions of the invention may be administered by a variety of routes including oral, buccal, sublingual, rectal, transdermal, subcutaneous, intravenous injection, intravenous infusion, intramuscular, intrathecal, intraperitoneal and intranasal.

Depending on whether intended route of delivery is oral or parenteral, the active agents can be formulated as compositions that are, for example, either injectable, topical or oral compositions. Liquid forms of compositions may include a suitable aqueous or nonaqueous vehicle with buffers, suspending and dispensing agents, colorants, flavors and other suitable ingredients known in the art. Solid forms of compositions may include, for example, binders, excipients, lubricants, coloring agents, flavoring agents and other suitable ingredients known in the art. The active agents and pharmaceutical compositions described herein may also be administered in sustained release forms or from sustained release drug delivery systems known in the art.

[0239] The pharmaceutical composition may depend on the disease or condition and on whether the administration is to prevent or to treat the disease or condition. For instance, administration for prevention of several diseases or conditions, including, but not limited to cancer, may be orally, without penetration enhancers, and at a lower dose. Administration for a subject showing symptoms of cancer, such as triple-negative node-positive breast cancer, the administration may be parenteral, regioselective, and at higher dose. Methods for targeting may include, but are not limited to combination with folate or antifolates (methotrexate, pemetrexed). Combination includes, but is not limited to esterification with or without linkers. In a preferred embodiment, the linker would be a disulfide linker (including, but not limited linkers comprising cystein, 3-mercaptopropionic acid, or 2-mercaptoethanol), linking to both the α -CD and pemetrexed via an ester. In another preferred embodiment, the α -CD/pemetrexed compound would be co-administered with folate. Administration to a subject showing symptoms of a neurodegenerative disease may be intrathecally without penetration enhancers and modifications for targeting.

[0240] In some embodiments the compositions of the present disclosure are pharmaceutical compositions comprising one or more active agents, as described herein, together with one or more conventionally employed components suitable for use in pharmaceutical delivery such as carriers, stabilizers, diluents, dispersing agents, suspending agents, thickening agents, excipients, and the like, may be placed into the form of pharmaceutical formulations. Nonlimiting examples of such formulations include solutions, creams, gels, gel emulsions, jellies, pastes, lotions, salves, sprays, ointments, powders, solid admixtures, aerosols, emulsions (e.g., water in oil or oil in water), gel aqueous solutions, aqueous solutions, suspensions, liniments, tinctures, and patches suitable for topical administration. The

pharmaceutical compositions and formulations described herein may, where appropriate, be conveniently presented in discrete unit dosage forms and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association an active agent with liquid carriers, solid matrices, semi-solid carriers, finely divided solid carriers or combinations thereof, and then, if necessary, shaping the product into the desired delivery system. Unit dosage forms of a pharmaceutical composition or formulation preferably contain a predetermined quantity of active agent and other ingredients calculated to produce a desired therapeutic effect, such as an effective amount of a therapeutically effective amount. Typical unit dosage forms include, for example, prefilled, premeasured ampules or syringes of liquid compositions, or pills, tablets, capsules or the like for solid compositions.

[0241] For parenteral administration, the cyclodextrin or derivative thereof can be, for example, formulated as a solution, suspension, emulsion or lyophilized powder in association with a pharmaceutically acceptable parenteral vehicle or pharmaceutically acceptable carrier. Examples of such vehicles or carriers are water, saline, Ringer's solution, dextrose solution, 5% human serum albumin, Ringers dextrose, dextrose and sodium chloride, lactated Ringers and fixed oils, polyethylene glycol, polyvinyl pyrrolidone, lecithin (glycerophospholipids), arachis oil or sesame oil. Liposomes and nonaqueous vehicles such as fixed oils may also be used. The vehicle or lyophilized powder may contain additives that maintain isotonicity (e. g, sodium chloride, mannitol) and chemical stability (e.g., buffers and preservatives). The formulation is sterilized by commonly used techniques. Parenteral dosage forms may be prepared using water or another sterile carrier. For example, a parenteral composition suitable for administration by injection is prepared by dissolving 1.5% by weight of active ingredient in 0.9% sodium chloride solution. Alternatively, the solution can be lyophilized and then reconstituted with a suitable solvent just prior to administration.

[0242] Pharmaceutically acceptable carriers are well known to those skilled in the art and include, but are not limited to, from about 0.01 to about 0.1 M or about 0.05 M phosphate buffer or about 0.8% saline. Intravenous carriers include fluid and nutrient replenishers, electrolyte replenishers such as those based on Ringer's dextrose, and the like. Additionally, such pharmaceutically acceptable carriers can be aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as

ethyl oleate. Aqueous carriers include water, ethanol, alcoholic/aqueous solutions, glycerol, emulsions or suspensions, including saline and buffered media.

[0243] The pharmaceutical compositions can be prepared using conventional pharmaceutical excipients and compounding techniques. Oral dosage forms may be elixirs, syrups, tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. The typical solid carrier may be an inert substance such as lactose, starch, glucose, cellulose preparations such as maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP); granulating agents; binding agents, magnesium stearate, dicalcium phosphate, mannitol and the like. A composition in the form of a capsule can be prepared using routine encapsulation procedures. For example, pellets containing the active ingredient can be prepared using standard carrier and then filled into a hard gelatin capsule; alternatively, a dispersion or suspension can be prepared using any suitable pharmaceutical carrier(s), for example, aqueous gums, celluloses, silicates or oils and the dispersion or suspension then filled into a soft gelatin capsule. Typical liquid oral excipients include ethanol, glycerol, glycerin, non-aqueous solvent, for example, polyethylene glycol, oils, or water with a suspending agent, preservative, flavoring or coloring agent and the like. All excipients may be mixed as needed with disintegrants, diluents, lubricants, and the like using conventional techniques known to those skilled in the art of preparing dosage forms. If desired, disintegrating agents may be added, such as the crosslinked polyvinylpyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. If desired, solid dosage forms may be sugarcoated or coated using standard techniques, including, but not limited to the use of chitosan, to target specific regions of the gastrointestinal tract. For oral liquid preparations such as, for example, suspensions, elixirs and solutions, suitable carriers, excipients or diluents include water, glycols, oils, alcohols, etc. Additionally, flavoring agents, preservatives, coloring agents and the like may be added.

[0244] For buccal administration, the compositions of the disclosure may take the form of tablets, lozenges, and the like formulated in conventional manner. The compounds may also be formulated in rectal or vaginal compositions such as suppositories or enemas. A typical suppository formulation comprises a binding and/or lubricating agent such as polymeric glycols, glycerides, gelatins or cocoa butter or other low melting vegetable or synthetic waxes or fats. For administration by inhalation, the compounds for use according to the present

disclosure are conveniently delivered in the form of an aerosol spray from pressurized packs or a nebulizer, with the use of a suitable propellant, e. g., di- or trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gases. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g. gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch. Even the larger β CD was seen to be suitable for inhalation.(Vecsernyes M, Fenyvesi F, et al. (2014) Arch Med Res 45:711-29)

[0245] The formulations may also be a depot preparation which can be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. In such embodiments, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

[0246] Alternatively, other pharmaceutical delivery systems may be employed. Liposomes and emulsions are well known examples of delivery vehicles that may be used. Certain organic solvents such as dimethylsulfoxide also may be employed, although usually at the cost of greater toxicity. Additionally, the compounds may be delivered using a sustained release system, such as semipermeable matrices of solid polymers containing the therapeutic agent. Various of sustained- release materials have been established and are well known by those skilled in the art. Sustained release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein stabilization may be employed.

[0247] The compounds described herein may also be formulated for parenteral administration by bolus injection or continuous infusion and may be presented in unit dose form, for instance as ampoules, vials, small volume infusions or prefilled syringes, or in multi-dose containers with an added preservative.

[0248] Preservatives and other additives can also be present, such as, for example, antimicrobials, antioxidants, chelating agents, inert gases and the like. All carriers can be mixed as needed with disintegrants, diluents, granulating agents, lubricants, binders and the like using conventional techniques known in the art.

F) Dosages

[0249] The dose of an active agent described herein may be calculated based on studies in humans or other mammals carried out to determine efficacy and/or effective amounts of the active agent (see section E, Clinical Outcomes, below). The dose amount and frequency or timing of administration may be determined by methods known in the art and may depend on factors such as pharmaceutical form of the active agent and route of administration, and patient characteristics including age, body weight or the presence of any medical conditions affecting drug metabolism.

[0250] In one embodiment, a dose may be administered as a single dose. In another embodiment, a dose may be administered as multiple doses over a period of time, for example, at specified intervals, such as, daily, bi-weekly, weekly, monthly, and the like. In another embodiment, the dose will be 700 mg/kg/d (Table 3, 2010-04-15). In another embodiment, the dose will be increased over time until early signs of renal or cytotoxicity are observed, in which case the dose level will be decreased to the previous, well-tolerated level.

[0251] In some embodiments, the dose of active agent is about at least 10 mg, at least 20 mg, at least 30 mg, at least 40 mg, at least 50 mg, at least 75 mg, at least 100 mg, at least 125 mg, at least 150 mg, at least 175 mg, at least 200 mg, at least 225 mg, at least 250 mg, at least 275 mg, at least 300 mg, at least 325 mg, at least 350 mg, at least 375 mg, at least 400 mg, at least 425 mg, at least 450 mg, at least 475 mg, at least 500 mg, at least 550 mg, at least 600 mg, at least 650 mg, at least 700 mg, at least 750 mg, at least 800 mg, at least 850 mg, at least 900 mg, at least 950 mg, at least 1000 mg, at least 1200 mg, at least 1500 mg, at least 2000 mg, at least 2500 mg, at least 3000 mg, at least 4000 mg, at least 5000 mg, at least 7500 mg, at least 10,000 mg, at least 15,000 mg, at least 20,000 mg, or at least 25,000 mg. In some such embodiments the above dosages are mg/day or mg/kg/day. In another embodiment, the dose of active agent is in the range of 1 to 10000 mg, 1 to 7500 mg, 1 to 5000 mg, 1 to 2500 mg, 1 to 1000 mg, 1 to 500 mg, 1 to 250 mg, 250 to 10000 mg, 250 to 5000 mg, 250 to 1000 mg, 250 to 500 mg, 500 to 10000 mg, 500 to 5000 mg, 500 to 1000 mg. In some embodiments, the above dosages are mg/day or mg/kg/day. In some embodiments, the dose of clathrate or α -cyclodextrin, derivative or salt thereof is from about 1 to about 20 grams per day. In some embodiments, the dose of clathrate or α -cyclodextrin, derivative or salt thereof is from about 10 to about 20 grams per day. In some embodiments, the dose of clathrate of or α -cyclodextrin, derivative or salt thereof is about 15 grams per day. In some embodiments, the

dose of clathrate of or α -cyclodextrin, derivative or salt thereof is about 15 grams per day. In some embodiments, the dose of clathrate of or α -cyclodextrin, derivative or salt thereof is about 20 grams per day. In some embodiments, the dose of clathrate of or α -cyclodextrin, derivative or salt thereof is about 25 grams per day. In some embodiments, the dose of clathrate of or α -cyclodextrin, derivative or salt thereof is about 30 grams per day. In some embodiments, the dose of clathrate of or α -cyclodextrin, derivative or salt thereof is about 35 grams per day. In some embodiments, the dose of clathrate of or α -cyclodextrin, derivative or salt thereof is about 40 grams per day. In some embodiments, the dose of clathrate of or α -cyclodextrin, derivative or salt thereof is about 45 grams per day. In some embodiments, the dose of clathrate of or α -cyclodextrin, derivative or salt thereof is about 50 grams per day. In some embodiments, the dose of clathrate of or α -cyclodextrin, derivative or salt thereof is about 55 grams per day. In some embodiments, the dose of clathrate of or α -cyclodextrin, derivative or salt thereof is about 10 grams per day. In some embodiments, the dose of clathrate of or α -cyclodextrin, derivative or salt thereof is about 15 grams per day. In some embodiments, the dose of clathrate or α -cyclodextrin, derivative or salt thereof is about 60 grams per day. In some embodiments, the dose is about 5 grams three times per day.

[0252] In some embodiments, a single dose may be administered. In another embodiment, multiple doses may be administered over a period of time, for example, at specified intervals, such as, four times per day, three times a day, twice per day, once a day, weekly, monthly, 4 times over 14 days, 2 times over 21 days, twice per month, 4 times over 21 days, 4 times per month, or 5, 6, 7, 8, 9, 10, 11, 12 or more times per month, per 21 days, per 14 days, or per week, and the like.

[0253] In one embodiment, the doses administered intravenously or intrathecally may be 5600 mg/kg per week or 400 mg per week (Table 3).

Table 3: Hempel twins dose adjustment

Date	Description
2004-01	Birth
2008-08-14	confirmed diagnosis of NPC
2009-02-22	HP- β -CD treatment plan Initial infusion: 4 d continuous i.v. of 80 mg/kg/d at a rate of 20 ml/h Next: Weekly 8 h infusions starting at 160 mg/kg/d + add'l weekly infusions Next: 320 mg/kg/d Next: 400 mg/kg/d
2009-04-13	Approval of i.v. infusion (INDs 104,114 and 104,116)
2009-07-07	Protocol extension 400 mg/kg/d administered as a weekly 8 h infusion
2009-07-16	Increased dosing frequency (twice/week) and rate of dose titration (100 mg/kg/infusion) Week 1: 500 mg/kg/d; 8 h x1 + 3-4 d 600 mg/kg/d; 8 h x1 Week 2: 700 mg/kg/d; 8 h x1 + 3-4 d 800 mg/kg/d; 8 h x1 Week 3: 900 mg/kg/d; 8 h x1 + 3-4 d 1000 mg/kg/d; 8 h x1 Initial infusion: 500 mg/kg/d over 8 h at a rate of 20 ml/h
2009-10-08	Pulmonary clinic visit 2800 mg/kg twice weekly over 8 h (800 mg/kg/d)
2010-03-07	"HP β CD Raises Hearing Threshold in ... Cats." (Ward S, O'Donnell P, et al. (2010) <i>Pediatr Res</i> 68 :52-6)
2010-04-15	Hearing unaffected despite receiving steady state IV doses of 2.5 g/kg bi-weekly for >1 yr (5 g/kg/wk $\approx$ 700 mg/kg/d)
2010-05-17	Orphan-drug designation granted
2010-08-13	Request for intrathecal delivery filed 200 mg HP- β -CD intrathecal biweekly ($\approx$ 60 mg/kg/d) (Maarup TJ, Chen AH, et al. (2015) <i>Mol Genet Metab</i> 116 :75-9)

From Hastings C (2009-02-22) Addi and Cassi Hydroxy-Propyl-Beta-Cyclodextrin Plan. Compassionate Use Clinical Study. Treatment Plan Version #2, <http://addiandcassi.com/wordpress/wp-content/uploads/2009/09/FDA-Submission-for-Addi-and-Cassi-Cyclodextrin-Treatment-Plan.pdf>

NPC: <http://www.nnpdf.org/cyclodextrin.html>

G) Subjects

[0254] The methods and compositions described herein may be used to treat or prevent malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition, in any subject in need of such treatment. In one embodiment, the subject is a human. It should be noted that, while in some embodiments the subjects to be treated are post-menopausal women, in other embodiments the methods of treatment described herein are not intended to be limited to such subjects. Rather, in some embodiments the subjects can be of any age, ranging from newborns to older adults. In some embodiments it may be desirable to treat young subjects, for example young infants, particularly where family history or genetic testing indicates that the subject is at risk for developing malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease,

disorder, or condition. Similarly, in some embodiments it may be desirable to treat much older subjects, particularly where such subjects begin to exhibit indicators or symptoms of malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition.

[0255] The methods and compositions described herein may be employed as prophylactic treatments or therapeutic treatments. For prophylactic treatments, the methods and compositions provided herein can be used preventatively in subjects that do not yet exhibit any clear or detectable clinical indicators or symptoms of malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition but that are believed to be at risk of developing malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition, such as MS, prostate cancer or breast cancer, or diseases that are characterized by dysfunctional lysosomes. A subject receiving prophylactic treatment for α -CD, for example, may not exhibit any clinical indicators or symptoms of MS, prostate cancer or breast cancer, or diseases that are characterized by dysfunctional lysosomes. In the case of therapeutic treatments, the methods and compositions provided herein can be used in subjects that already exhibit one or more clinical indicators or symptoms of the disease or disorder, such as MS, prostate cancer or breast cancer, or diseases that are characterized by dysfunctional lysosomes. A subject receiving therapeutic treatment for MS, prostate cancer or breast cancer, or diseases that are characterized by dysfunctional lysosomes, for example, may have been clinically diagnosed with MS, prostate cancer or breast cancer, or diseases that are characterized by dysfunctional lysosomes or may otherwise exhibit one or more clinical indicators or symptoms of MS, prostate cancer or breast cancer, or diseases that are characterized by dysfunctional lysosomes. In some embodiments, the disclosure relates to methods of treating cancers comprising cells that are deficient or substantially deficient in any of the genes identified in the Figures or specification, such that the limited expression or lack of expression of those genes results in lysosomal dysfunction.

[0256] In one embodiment, a subject may have been identified as being at risk of developing malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition. In one embodiment, the subject has a family history of malignant, neurodegenerative, vascular,

metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition. In one embodiment, the subject has one or more genetic risk factors associated with malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition, for example, a genetic mutation in a gene associated with PI cycle function.

H) Clinical Outcomes

[0257] In some embodiments the methods of treatment provided herein (which comprise, for example, administering to a subject an effective amount of a composition according to the present disclosure) result in, or are aimed to achieve, a detectable improvement in one or more clinical indicators or symptoms of the age-related diseases.

[0258] In the context of cancer, for instance, the clinical indicators or symptoms include, but not limited to, changes growth, migration, or invasion. In some embodiment of the present disclosure a symptom or indicator of improvement is selected from the group comprising survival, disease-free survival, distant metastasis-free survival, results of a blood test (including, but not limited to circulating tumor DNA, prostate-specific antigen, β -galactosidase/ β -hexosaminidase (Tiribuzi R, Orlacchio A, et al. (2011) J Alzheimers Dis **24**:785-97), and lysozyme and/or cathepsin S (Haldar K, Alam S (2017))), an x-ray evaluation, the result of a physical examination (including, but not limited to a palpable tumor), or a tissue biopsy for histological evaluation.

[0259] To determine the highest tolerated dose in an individual complete blood count and serum chemistry will be collected and analyzed. The serum chemistries may include, but will not be limited to evaluation of electrolytes, bicarbonate, glucose, BUN, creatinine, magnesium, phosphate, hepatic enzymes (AST and ALT), total protein, albumin, bilirubin, and alkaline phosphatase. In addition, a complete lipid panel may be obtained and shape of erythrocytes may be evaluated microscopically. Bone density may be measured to identify early signs of osteoporosis.

[0260] The compositions and methods described herein are illustrative only and not intended to be limiting. Those of skill in the art will appreciate that various combinations or modifications of the specific compositions and methods described above can be made, and all such combinations and modifications of the compositions and methods described herein may be used in carrying out the present disclosure. Furthermore, certain embodiments of the

present disclosure are further described in the following non-limiting Examples, and also in the following Claims.

[0261] All publications, including patent applications and journal articles are incorporated by reference in their entireties.

EXAMPLES

Example 1: The PI-Cycle is a Drug Target Against Metastases in Breast Cancer

[0262] The methods used to obtain the results presented in Example 1 are further described in (Wittkowski KM (2014) US 2016/0206581 A1), which is hereby incorporated by reference in its entirety.

[0263] Almost a decade after the completion of the Human Genome Project, the scientific and medical advances hoped for from genome-wide association studies (GWAS) have not yet been realized. Enlarging the sample size to tens of thousands of subjects greatly increases the duration and cost of data collection and, in a nonrandomized study, may somewhat paradoxically increase the risk of false positives. This Example describes combining a novel computational biostatistics approach with decision strategies fine-tuned to the exploratory nature of GWAS. With these methodological advances, disease-relevant functional gene clusters can now be suggested from studies of a few hundred narrowly defined cases only.

[0264] Although a history of familial breast cancer being a known risk factor of either breast and prostate cancer attests to a high degree of heritability, the genetic risk factors for breast and prostate cancer in the general population are still poorly understood. A single-SNP GWAS (ssGWAS) of breast cancer including data from 250,000 women did not result in a testable hypothesis. (Michailidou K, Lindstrom S, et al. (2017) Nature **551**:92-4) As described herein, data from three independent sub-populations of this study (available from NIH's dbGaP) were analyzed using u-statistics for genetically structured wide-locus data (muGWAS) to explore epistasis. To account for systematic, but disease-unrelated differences in (non-randomized) GWAS and for conducting multiple tests in overlapping genetic regions, a novel study-specific criterion for 'adaptive genome-wide significance' (aGWS) was applied (Wittkowski KM, Sonakya V, et al. (2014) Transl Psychiatry **4**:e354). Enrichment of the results in all three studies with genes associated with influx of phospholipids into the PI cycle and lysosome function confirms the hypothesis that an imbalance between high levels of

endocytoses and age-dependent decline of lysosome function is involved in driving metastases in breast cancer.

Subjects

[0265] The study was approved as appropriate. No human participants were involved in the research. The results described herein are based on three studies of breast cancer in the US and Europe. These studies included data from the Cancer Genetic Markers of Susceptibility (CGEM) breast cancer GWAS which included 1145 cases / 1142 controls (Hunter DJ, Kraft P, et al. (2007) *Nature Genet* **39**:870-4) (dbGaP: phs000147.v3.p1), and from two sub-studies of the nested case-control and one case-control study of estrogen receptor negative (ER⁻) breast cancer within the Breast and Prostate Cancer Cohort Consortium, both included in (Garcia-Closas M, Couch FJ, et al. (2013) *Nat Genet* **45**:392-8, 8e1-2): the European Prospective Investigation into Cancer (EPIC) of 511 ER⁻ cases and 500 controls, and the Polish Breast Cancer Case-Control Study (PBCS) of 543 ER⁻ cases (229 triple-negative) and 511 controls. (dbGaP: phs000812.v1.p1)

Methods

[0266] ssGWAS: After eliminating non-informative or low-quality SNPs, a traditional ssGWAS was performed, using u-statistics for univariate data (also known as the Mann-Whitney test (Mann HB, Whitney DR (1947) *Ann Math Stat* **18**:50-60) which is equivalent to the Wilcoxon rank-sum test (Wilcoxon F (1954) *Biometrics* **1**:80-3)). By construction, the results of this analysis are very close to those obtained with the traditional Cochran-Armitage trend test.(Armitage P (1955) *Biometrics* **11**:375-86)

[0267] Annotation: The annotation files available have proven to be inadequate to appropriately distinguish between genes (or splice variants of genes) that are too far away or close enough to be likely related to a SNP or region being implicated. On the other hand, diplotypes may span linkage-disequilibrium (LD) blocks outside of genes or their regulatory regions, in which case it is unlikely that the functional implication of the variation can be identified. Also, there may not be sufficient information available to determine the function of a gene, in which case the funding would not be useful for identifying collections of functionally related genes. Hence, the results returned from the grid/cloud infrastructure, still need to be manually reviewed to resolve ambiguities regarding the annotation.

[0268] Wide-locus approach. To overcome several of the shortcomings seen in previous applications of ssGWAS when applied to common diseases, several strategies were combined at different stages of the analysis process. Wide-loci of up-to six neighboring SNPs were aimed at as a primary outcome and the same non-parametric GWAS approach was applied based on u-statistics for structured multivariate data (Hoeffding W (1948) Ann Math Stat **19**:293-325) with genotypic structures (muGWAS) as in the previous childhood absence epilepsy (Wittkowski KM, Sonakya V, et al. (2013) Pharmacogenomics **14**:391-401) and autism (Wittkowski KM, Sonakya V, et al. (2014) Transl Psychiatry **4**:e354) studies. To avoid spurious findings, loci outside of LD blocks containing genes with known function or adjacent to their 5'- end were excluded. Loci highly influenced by a single SNP only were also excluded, unless this SNP was implicated in more than one of the studies or had been implicated in previous studies.

[0269] Information Content: In contrast to traditional regression methods, muGWAS provides an intrinsic measure of "information content", which can be used to highlight regions with high significance, but low information content as likely artifacts. In the Manhattan plot, below, highly significant results with low information content are highlighted in red and excluded (crossed out in white), unless there is other supportive evidence, such as a nearby SNP that had previously been reported as associated with breast cancer or another cancer. Some regions with low information content are dominated by a single SNP or involve diplotypes spanning LD blocks without being within a gene or its regulatory region. Diplotypes may also be excluded if moving the window by one SNPs results in a large (more than 100-fold) change in significance. Of note, these manual interventions cannot cause false positive results and current research aims at formalizing more of these rules to facilitate interpretation and avoid false negative results.

[0270] MAF-significance correlation: With any finite sample size, the significance of a u- or rank test is limited. Hence, more significant results can only be obtained for SNPs with sufficiently high MAF. ssGWAS simulations were performed with 2,500,000 permuted phenotypes, comparing two groups of equal size for various MAFs. The $1-10^{-5}$ quantile of the permutation distribution drops from the expected $s = -\log_{10} p = 5.26$ cut-off, which is routinely met for $MAF > .33$, to 4.9 (n=1000 subjects), 4.7 (n=500), and 4.5 (n=300) for a MAF of 0.05. For the 7.5 level, the bias is projected to be even larger. Due to this MAF-significance correlation, the expected diagonal in a ssGWAS QQ plot under the null

hypothesis that “no SNP is associated with the trait” (Pearson TA, Manolio TA (2008) JAMA **299**:1335-44) turns into an expected curve dropping below the diagonal towards the end. (Wittkowski KM, Sonakya V, et al. (2014) Transl Psychiatry **4**:e354)

[0271] Estimating the expected s-value ($-\log_{10}(p)$) distribution from $>10^8$ permutations to obtain stable estimates of the $1-10^{-7.5}$ quantile is neither practical, nor sufficient to avoid a biased selection of SNPs for limited tests. Due to the MAF-significance correlation, any SNP ‘significant’ when comparing observed phenotypes, is also more likely to be ‘significant’ with random phenotype permutations. (Wittkowski KM, Sonakya V, et al. (2014) Transl Psychiatry **4**:e354)

[0272] Non-randomization bias: The reason for this curvature often not being recognized is that GWAS subjects are deterministically categorized based on their outcome (e.g., non-verbal v. verbal), rather than randomly assigned to interventions (as in clinical trials). Any deterministically categorized populations, however, are expected to differ systematically in aspects related to neither the condition of interest nor common ancestry factors (which could potentially be accounted for through stratification). When the downward trend from using a limited test and the upward bias from deterministic selection are similar, the s-values may still appear to follow the diagonal, except for loci suggesting ‘true association’ (Pearson TA, Manolio TA (2008) JAMA **299**:1335-44)

[0273] Multiplicity adjustments for diplotype length: For multivariate tests of overlapping diplotypes, the estimated quantile-rank (QR) curve needs to be elevated above the diagonal throughout to account for multiple tests conducted around the same SNP. Because most of these tests are highly dependent, the elevation of the estimated QR curve compared to the estimated QQ curve (FIG. 6 – FIG. 11) is limited, but the distance is likely to vary across diseases and populations. (Wittkowski KM, Sonakya V, et al. (2014) Transl Psychiatry **4**:e354)

[0274] Projected QR curves: The diagonal of the traditional QQ-plot does not depend on any data, including the most ‘significant’ data. The s-values are expected to fit the diagonal for the most part (except for the most significant results), (Pearson TA, Manolio TA (2008) JAMA **299**:1335-44) as the vast majority of SNPs are expected not to be associated with the disease. In direct analogy, the QR curve for a multivariate test should be ‘smooth’, with upward deviations indicating ‘true association’, which could be disease-related or not. Based

on the above rationale and the simulation results mentioned above, the highest point of the projected QR curve (apex) for each chromosome can be estimated from a smooth projection of the s-values after truncating as many of the highest values as needed for the projection to have a monotone increase and, conservatively for a limited test, a non-positive second derivative. Fitting against the data also reduces the effect of population stratification (Pearson TA, Manolio TA (2008) JAMA **299**:1335-44). (For computational convenience, locally weighted polynomial regression (Cleveland WS, Devlin SJ (1988) J Am Statist Assoc **83**:596-610) was selected, as implemented in S+ (TIBCO Software Inc.) as 'loess.smooth(... degree=2, family="gaussian")')

[0275] Estimated whole genome QR apex: While chromosomes may differ with respect to their content of related and unrelated risk factors (see, e.g., the HLA region in autoimmune diseases), random errors are expected to have the same distribution across all chromosomes. Hence, the expected WG apex can be estimated as the (winsorized) median projected apex among chromosomes with the smallest deviation of s-values from the projection. (Here, ten chromosomes were selected based on the maximum norm, and the median for robustness, but the strategy to determine the optimal number, including the criteria for 'optimality', remains to be determined.) (Wittkowski KM, Sonakya V, et al. (2014) Transl Psychiatry **4**:e354)

[0276] Estimated QR curves: The estimated curve for each chromosome is then calculated as the loess projection (Cleveland WS, Devlin SJ (1988) J Am Statist Assoc **83**:596-610) of this chromosome's s-values with as many of the highest values replaced with the estimated WG apex until the curve's apex is at or below that level. Applied to the WG projection (QR plots, bottom right), this procedure yields the estimated WG curve. Simulation results demonstrate the low variance of the estimates from phenotype permutations and the similarity of their median apex with the winsorized median apex estimated from the observed s-values. (Wittkowski KM, Sonakya V, et al. (2014) Transl Psychiatry **4**:e354)

[0277] Study-specific genome-wide significance: For studies aiming to confirm individual SNPs as associated with a phenotype, the 'confirmatory' paradigm (Tukey JW (1980) American Statistician **34**:23-5) requires adjustment for multiplicity. When applied to GWAS, these adjustments are typically based on a 'customary' fixed 0.05 level, irrespective of study size or relative risk of type I over type II errors (see (Fisher RA (1956), p. 358) and (Gigerenzer G (2004) Psychol Sci **15**:286-7) for a discussion), and the assumption of 1,000,000 independent SNPs, irrespective of chip density (Pearson TA, Manolio TA (2008)

JAMA **299**:1335-44). Moving from individual SNPs to overlapping diplotypes increases the dependency of any formal multiplicity adjustment on assumptions with questionable biological validity.(Wittkowski KM, Sonakya V, et al. (2014) Transl Psychiatry **4**:e354)

[0278] As in most GWAS, however, the studies described in this Example do not aim to confirm hypotheses regarding specific SNPs. Instead, the studies described here aim at picking likely candidates from >40,000 (pseudo-) genes, whose relative importance and epistatic interactions are unknown. Since graphical procedures are particularly useful for such ‘exploratory’ studies (Tukey JW (1977)), QR plots were chosen to guide with interpretation. Exact cut-offs for deviation of s-values from the estimated curve are unknown. When “the knowledge [is] at best approximate[,] an approximate answer to the right question, which is often vague, [is far better] than an exact answer to the wrong question, which can always be made precise” (Tukey JW (1962) Ann Math Stat **33**:1-67). Hence, a heuristic approach is presented that relies on fewer unrealistic assumptions than typical attempts to quantify a particular error rate. (Wittkowski KM, Sonakya V, et al. (2014) Transl Psychiatry **4**:e354)

[0279] The expected WG curve needs to be estimated, the s-values have a complex dependency structure, and the appropriate level of significance (α) for the given sample size is unknown. Hence, a heuristic decision rule is proposed based on weak assumptions only. In the long run one would expect most s-values above the apex to be significant at any $\alpha > 0$ (consistency) and regions with the strongest association to have the highest odds at being included (unbiasedness). For a particular α , one could lower the cut-off, but to account for variance in estimating the apex, one would need to raise it. As a compromise, the estimated WG apex is proposed as a cut-off for study-specific GWS.(Wittkowski KM, Sonakya V, et al. (2014) Transl Psychiatry **4**:e354)

[0280] Quantile-rank (QR) plots: As is customary with selection procedures, p-values were used mainly for the purpose of ranking loci. As no particular hypotheses regarding specific loci were to be confirmed, the traditional approach of exploring characteristics of the ‘QQ plot’ as decision criteria was modified and formalized. For multivariate tests of overlapping diplotypes, the straight line expected in the traditional ‘QQ plot’ under the univariate WG permutation hypothesis turns into a curve because many tests are performed per SNP. Even though the number of tests performed increases substantially, the increase in s-values shown in the QR curve compared to the QQ line (FIG. 7 v. FIG. 6, FIG. 9 v. FIG. 8, FIG. 11 v. FIG.

10) is limited, because most tests are highly dependent. (Wittkowski KM, Sonakya V, et al. (2014) *Transl Psychiatry* 4:e354)

[0281] Whole-genome permutation bias: To estimate the expected distribution of s-values, one could average the results of repeated runs with random phenotype permutations. As each μ GWAS analysis may require >100,000 hours on a grid/cloud with GPU enabled nodes, however, simulations requiring >108 replications to estimate the $1-10^{-7.5}$ quantile may not be feasible. The estimate from WG permutations (including computationally efficient approximations) is affected by biases due to subjects being categorized based on their outcome (e.g., non-verbal v. verbal), rather than randomly assigned to interventions (as in clinical trials), so that the groups are expected to differ systematically in aspects related to neither the condition of interest nor common ancestry factors. With binary outcomes, significant results can also not be caused by a few 'outliers' only, so that significance is correlated with high MAF (ssGWAS) or low skewness of the scores (μ GWAS). Hence, regions with significant allelotype differences between observed phenotypes have also a larger chance to be significant among random phenotype permutations. (Wittkowski KM, Sonakya V, et al. (2014) *Transl Psychiatry* 4:e354)

[0282] Selective chromosome permutation: The proposed use of a selected chromosome permutation approach reduces this bias. While chromosomes may differ with respect to their content of disease related and unrelated risk factors, random errors are expected to have the same distribution across all chromosomes. Hence, the above biases are reduced by excluding chromosomes containing regions of high significance when determining the permutation distribution. In particular, the endpoint of the expected distribution for each chromosome can be estimated from the loess projection to the p-values after truncation to ensure a monotone increase and a non-positive second derivative. Similarly, the endpoint of the expected distribution is estimated from the median of the limited set of, e.g., ten, chromosomes with the lowest maximum deviation of the distribution of s-values from the loess projection. (Wittkowski KM, Sonakya V, et al. (2014) *Transl Psychiatry* 4:e354)

[0283] Formal QR cut-off for deviation: The estimate of the expected distribution for each chromosome is then calculated as the loess fit of the individual chromosomes' data with a sufficient number of results at the high end replaced with the expected endpoint until the curve is curtailed to that level, unless the initial loess fit already remains below this target level. The same procedure, when applied to the WG data, yields the estimation of the WG

distribution. Simulation results demonstrate the low variance of the estimates based on random permutations of the phenotypes and that their median is closely resembled by the estimate of the distribution obtained from the observed data. (Wittkowski KM, Sonakya V, et al. (2014) *Transl Psychiatry* **4**:e354)

Results

[0284] Previously known results from CGEM: Traditionally, GWAS have often identified only a small number of SNPs per study. A previous ssGWAS analysis of the CGEMS data (Hunter DJ, Kraft P, et al. (2007) *Nature Genet* **39**:870-4) had implicated two loci in trend analysis:

chr10: 124,992,475	rs10510126:	6.15	BUB3, (long EST not5 identified)
chr10: 123336180	rs1219648:	5.49	FGFR2
123341314	rs2420946	5.46	FGFR2

[0285] ssGWAS results: Single-SNP GWAS confirmed these findings at essentially equivalent levels of 6.20 and 5.57, respectively. Many other results had been dismissed in previous published analyses because p-values did not reach the traditional level of “fixed genome-wide significance” (typically, 7.5). From the ssGWAS QR plots (CGEM: FIG. 6, EPIC: FIG. 8, and PBCS: FIG. 10) many of the genes above the cut-off for study-specific genome-wide significance fit the paradigm of being involved in signaling at the membrane (GPCRs, Fc receptors, growth factor receptors, ion channels) or processes in the nucleus (cell cycle control, transcription, splicing) (see Table 4, columns Mbrn and Ncls).

[0286] muGWAS results: In muGWAS (CGEM: FIG. 7, EPIC: FIG. 9, and PBCS: FIG. 11), the proportion of genes related to membrane signaling and nuclear processes is even higher than in ssGWAS. In addition, a group of genes known to play a role in either in the PI cycle (FIG. 12) or in endocytosis (FIG. 8A) stands out.

Table 4: Genes identified by Study. Study; IPV6/IPV1: $-\log_{10}(\text{p-value})$ in muGWAS/ssGWAS; Mbrn: membrane-associated genes (GPCR, Fc-Receptor, HA, RTK, Ion channels), PI/EC: PI signaling/endocytosis, MPK: MAP kinases, Ncls: nucleus (cell cycle control, transcription, splicing). Top block of rows within each study: genes with diplotypes above aGWS in muGWAS (CGEM: 5.29, FIG. 7; EPIC: 5.71, FIG. 9; PBCS: 5.13, FIG. 11); center block of rows within each study: other genes with diplotypes among the top 41 in muGWAS; bottom block of rows within each study: genes with SNPs above the study-specific level of genome-wide significance in ssGWAS (CGEM: 4.03, FIG. 6; EPIC: 4.00, FIG. 8; PBCS: 3.84, FIG. 10); ssGWAS results for genes also implicated in muGWAS are shown next to the muGWAS results; black squares: known breast cancer genes (from <http://www.genecards.org>); Boxed gene names indicate replication of the same gene (bold, PRKCQ, MEGF11) or replication of a closely related gene belonging to the same family (*ATP8A1/ATP8B1*, *AGPAT3/AGPAT4*, *BMP7/BMPRI1B*). (See FIG. 49A through 49C).

[0287] Validation (same intragenic region): PRKCQ (chr 10), was significant by muGWAS in CGEM (mu: 6.70, ss: 3.47, " indicating study-specific genome-wide significance) and by ssGWAS in EPIC (mu: 5.26, ss: 3.87*). The same region (chr10:6,540,724-6,573,883) was implicated in both populations. In PBCS, in contrast, there was no association in this region (<2.00), consistent with the notion of different risk factors for breast cancer in different populations.

[0288] Validation (same gene): MEGF11 was implicated in both CGEM and PBCS. MEGF11 was even elevated (3.31) in EPIC. A single SNP was highly influential in either population, but it was not the same SNP (CGEM: rs189155, PBCS: rs12903880, EPIC: rs333554). All three SNPs are located in the coding region, but they are not in LD. One other SNP in *MEGF11* (rs1477798) has been implicated in colorectal cancer. (Cicek MS, Cunningham JM, et al. (2012) PLoS One 7:e38175)

[0289] Validation (similar function): In a complex disease, populations may differ with respect to the risk factors that are present in each population. In particular, the proportion of risk conferred by different genes with similar function may differ and, even if the same gene is involved, risk may be associated with different SNPs.

[0290] In ssGWAS, only one pair of functionally related genes stands out (Table 4):

BMPR1B (CGEM) – *BMP7* (EPIC)

[0291] Among muGWAS results (Table 4), there are three more pairs of functionally related genes:

ATP8B1 (CGEM) – *ATP8A1* (EPIC)

MEGF11 (CGEM, PBCS)

AGPAT4 (CGEM) – *AGPAT3* (EPIC)

[0292] Mutations in PI3K, PTEN, and SYNJ2 are known to be associated with breast cancer.

[0293] The mechanism commonly believed to be involved is the dysregulation of the AKT/TSC/mTOR growth pathway downstream of PI(3,4,5)P₃ and PI(3,4)P₂. The results of this analysis point to three additional points where the PI cycle is involved (FIG. 12):

1. PI(4,5)P₂ (*SCARB2*, *UNC13C*, *STXBP1*, *SDCBP2*, *MEGF11*, *SYT17*, *N4BP3*, *VAV3*) and
2. PI(3)P (*NLRP4*, *EEA1*, *RAB32*), as well as
3. overall activation of PI (*ATP8A1*, *ATP8B1*, *SLC5A3*, *AGPAT3*, *AGPAT4*, *ANXA4*)

[0294] With the exception of *CHMP7*, *RAPGEF4*, and *EEA1*, all these genes have previously been shown to be associated with breast cancer (<http://www.genecards.org>).

[0295] The novel finding is that breast cancer risk is conferred not only by a variety of variations in

[0296] genes involved in nuclear processes causing susceptibility for cancer and

[0297] genes involved in membrane processes providing growth signals,

[0298] as well as a few specific variations connecting the two by increasing

[0299] PI(3,4,5)P₃(loss-of-function in *PTEN*, gain-of-function in *PI3K*),

[0300] PI(3,4)P₂(gain-of-function in *SYNJ1/2* or *INPPL1*), or

[0301] PI(3)P(gain-of-function in *INPP4B*),

[0302] but by a global dysregulation of the PI cycle, including

[0303] entry of phosphatidylinositol (PI) (involving *AGPAT3*, *AGPAT4*, and *SLC5A3*), and

[0304] entry of phosphatidylserine/phosphatidylcholine (PS/PC) (involving *ATP8A1*, *ATP8B1*, and *ANXA4*),

[0305] and endocytosis as a critical component of migration and invasion. Endocytosis is known to be controlled by PI signaling, which is consistent with the genes identified in the results presented:

[0306] at the plasma membrane stage (PM, eight genes),

[0307] at the early endosome stage (EE, four genes), and

[0308] at the late endosome stage (LE, two genes).

[0309] In some embodiments, the subject has a disease or disorder that is characterized by a dysfunctional lysosomal pathway by deficient expression of any one or combination of genes disclosed above.

Discussion

[0310] The approach used here differs from traditional GWAS in both the statistical method being used and the decision strategy. To address the statistical method challenges specific to GWAS, the novel approach (a) avoids making assumptions about a particular degree of dominance, (b) draws for the fact that both SNPs neighboring a disease locus should be in LD, unless they are separated by a recombination hotspot, (c) can distinguish between SNPs belonging to the same tag sets, but differ in their order along the chromosome, (d) accounts for different disease loci within the same region having similar effects and for compound heterozygosity within the statistical method (rather than through visual inspection looking for several SNPs within a region having high significance), and (e) provides additional information ("information content") that can be used to prioritize results.

[0311] The use of a decision rule that accounts for (a) GWAS being non-randomized, (b) the aim being selecting sets of genes, knowing that some must have an effect, rather than testing

the hypothesis that no gene has an effect, at all, (c) accounting for differences in MAF in estimating the expected distribution of p-values, and (d) adjusts for tests in overlapping diplotypes being related.

[0312] The validation of μ GWAS in childhood absence epilepsy (Wittkowski KM, Sonakya V, et al. (2013) *Pharmacogenomics* **14**:391-401) demonstrated the ability of μ GWAS to identify genes modulating a known disease pathway, where traditional ssGWAS had identified a single SNP (in a pseudo-gene) only. The subsequent application to mutism in autism (Wittkowski KM, Sonakya V, et al. (2014) *Transl Psychiatry* **4**:e354) confirmed the ability of μ GWAS to identify clusters of genes related to the same biological function in two independent populations.

[0313] By using this novel decision rule alone with traditional single-SNP GWAS, the number of “significant” genes rises from none to >20 in each of the three studies considered here. The set of genes seen in ssGWAS using the novel decision rules includes half of the genes associated with the novel PIP cycling pathway. The novel non-parametric wide-locus approach then adds the other half of the genes involved in regulation and function of the PI cycle.

[0314] The PI cycle is critical for many cellular functions in eukaryotic cells, including developmental functions such as oocyte maturation, fertilization, embryogenesis, but also cell growth, cytoskeleton dynamics, membrane trafficking, and nuclear events.(Shulga YV, Myers DS, et al. (2010) *Biochemistry* **49**:312-7; Busa WB (1988) *Philos Trans R Soc Lond B Biol Sci* **320**:415-26) Hence, it is not surprising that the PI cycle is tightly controlled. In particular, PI(4,5)P₂, PI(4)P, PI(3,4)P₂, and PI(3)P are tightly regulated by both three kinases and three groups of phosphatases.(Waugh MG (2015) *Biochim Biophys Acta* **1851**:1066-82) Different subsets of phosphatases (FIG. 12, boxes) are counteracting the effects of the kinases, further reducing the impact any variation in a particular phosphatase might have on the system as a whole. Hence, a specific intervention modifying the state of this tightly regulated system might not suffice to achieve a sustained effect.

[0315] That “both PtdIns(3,4,5)P₃ and PtdIns(3,4)P₂ are likely required for a cell to achieve and sustain a malignant state”, has been formulated as the “two PIP hypothesis”(Kerr WG (2011) *Ann N Y Acad Sci* **1217**:1-17) The results presented here suggest not only that PI(3)P

is required, but shift in focus further toward to the PI cycle as a whole. This new focus has direct implications for the development of drugs.

[0316] The model of a linear (PI-PIP1-PIP2-PIP3) PI system suggested inhibition of *PI3K* as a strategy to reduce activity along the AKT/TSC/mTOR growth pathway. Only a small proportion of patients, however, benefit from interrupting this linear pathway by blocking *PI3K*. (Bosch A, Li Z, et al. (2015) *Sci Transl Med* 7:283ra51-ra51) The limited success of wortmannin and other inhibitors of *PI3K* is consistent with the ability of the PI cycle to compensate not only for natural, but also for pharmaceutical disturbance at a particular point.

[0317] High levels of PI(3,4,5)P₃, PI(3,4)P₂, and PI(3)P all are known to correlate with a negative outcome of cancers. “Altered abundance of phosphatidylinositides (PIPs) is a feature of cancer. Various PIPs mark the identity of diverse membranes in normal and malignant cells.” (Sengelaub CA, Navrazhina K, et al. (2015) *The EMBO Journal*) The model of a PI cycle tightly regulated around the PI(4,5)P₂–PI(4)P–PI(3,4)P₂–PI(3)P pathway suggests overall downregulation of PI cycle activity as a more successful strategy to correct for excessive activation involving the PI cycle than blocking individual or pairs of kinases or phosphatases.

[0318] Neither breast nor prostate cancer *per se* are lethal; it is metastases spreading to other organs that cause cancer-related death. As seen in treatments involving cytotoxic drugs, reducing cell growth, in general, often causes side-effects (nausea, loss-of-hair, ...), without necessarily reducing the risk of metastases, because growth and metastasis may be regulated by different pathways.

[0319] To metastasize, tumor cells “must develop motile and invasive phenotypes”, which is known to require endocytosis for cell migration. (Mellman I, Yarden Y (2013) *Cold Spring Harbor Perspectives in Biology* 5) As “defective vesicular trafficking of growth factor receptors, as well as unbalanced recycling of integrin- and cadherin-based adhesion complexes, has emerged in the past 5 years as a multifaceted hallmark” of cancer, correcting for “derailed endocytosis” has been suggested as a strategy to prevent cancer metastases. (Mosesson Y, Mills GB, et al. (2008) *Nat Rev Cancer* 8:835-50) “Activation of signal transduction pathways associated with endocytic trafficking (FIG. 14) is critical for tumor cell migration. Hence, selectively targeting endocytic trafficking and signaling could

potentially allow for the development of novel cancer therapeutics to prevent metastasis.”(Chew CL, Chen M, et al. (2016) *Oncotarget* **7**:5-6).

Example 2: The genetic risk factors in the PI cycle and along the endocytosis pathway are known as shared risk factors for “derailed endocytosis” in breast cancer and “defective/deranged endocytosis” Parkinson’s disease/Alzheimer’s disease.

[0320] **Alzheimer’s disease and Parkinson’s disease are known to share risk factors:** A β and α -synuclein have been hypothesized to interact,(Crews L, Tsigelny I, et al. (2009) *Neurotox Res* **16**:306-17; Tsigelny IF, Crews L, et al. (2008) *PLoS One* **3**:e3135) and “moderate association” of AD with PD was found in a meta analysis of 14 studies conducted 1986–2010,(Feldman AL, Johansson AL, et al. (2014) *Neuroepidemiology* **42**:69-80) but a meta-analysis of single-SNP summary statistics from two sets of AD and PD GWAS, each imputed to 7,815K SNPs, “resulted in no significant evidence [for SNP] loci that increase the risk of both PD and AD”.(Moskvina V, Harold D, et al. (2013) *JAMA Neurology* **70**:1268-76) Recently, having disease with Lewis bodies (DLB) diagnosed as either Parkinson’s disease or Alzheimer’s disease was identified as a potential confounder in these studies (Guerreiro R, Escott-Price V, et al. (2016) *Neurobiol Aging* **38**:214 e7-10; Bras J, Guerreiro R, et al. (2014a) *Hum Mol Genet* **23**:6139-46) and the above results suggest that lack of “significant evidence” above may have been because of ssGWAS having lower power than muGWAS for cis-epistatic effects.

[0321] **Overlapping epidemiology and etiology of breast cancer and Alzheimer’s disease/Parkinson’s disease:** breast cancer has high co-occurrence with Parkinson’s disease.(Disse M, Reich H, et al. (2016) *Dermatol Surg* **42**:141-6) Earlier reports that cancers reduce Alzheimer’s disease risk were linked to statistical models not accounting for competing risks (Hanson HA, Horn KP, et al. (2016) *J Gerontol B Psychol Sci Soc Sci*) and/or treatment effects of cancer drugs.(Malkki H (2016) *Nat Rev Neurol* **12**:126-) Another reason for limited association between breast cancer and Alzheimer’s disease may be that mutations may have opposite effects, such as gain-of-function in breast cancer (and Parkinson’s disease) and loss-of-function in Alzheimer’s disease. Overlapping genetic risk factor have already been reported. Mutations in the Parkinson’s disease gene PSEN2 were also found in breast cancer and Parkinson’s disease.(Cai R, Hao Z, et al. (2015) *Bioinformatics* **31**:1701-7) Mutations in MAPT, which encodes the Parkinson’s disease microtubule-associated protein tau, were found in breast cancer (Rouzier R, Rajan R, et al.

(2005) Proc Natl Acad Sci USA **102**:8315-20) and Parkinson's disease;(Lopez Gonzalez I, Garcia-Esparcia P, et al. (2016) Int J Mol Sci **17**:206) DJ-1 was seen as elevated,(Kawate T, Iwaya K, et al. (2015) Cancer Sci **106**:938-43) and the G₂₀₁₉S mutation in endosomal LRRK2 (Rivero-Rios P, Gomez-Suaga P, et al. (2015) Biochem Soc Trans **43**:390-5) increases risk.(Agalliu I, San Luciano M, et al. (2015) JAMA Neurol **72**:58-65) Still, "the etiology of this link continues to be elusive".(Disse M, Reich H, et al. (2016) Dermatol Surg **42**:141-6)

[0322] **Overlap of genetic risk factors for breast cancer (above and published results) and Alzheimer's disease/Parkinson's disease (published results):** The vast majority of genes related to the PI cycle and EEC genes identified in muGWAS of breast cancer (Table 4, column PI/EC) had already been identified in previous functional studies and gene expression studies of both breast cancer and Alzheimer's disease/Parkinson's disease (Table 5 and Table 6).

Table 5: PI-Cycle overlap between Breast Cancer, PD, and AD. Gene: Genes identified in breast cancer GWAS. EEC function: Known function in PI-cycle and/or EEC. KEGG: KEGG pathway (<http://www.genome.jp/kegg/pathway.html>), EC: epithelial cancer (carcinoma). ND: Neurodegenerative disease.

Gene	PI-Cycle	KEGG	EC ND References
ATP8A1	Decreasing		(Farge E, Ojcius DM, et al. (1999) <i>Am J Physiol</i> 276 :C725-33; Levano K, Sobocki T, et al. (2009) <i>Glycoconj J</i> 26 :739-48; Levano K, Punia V, et al. (2012) <i>J Neurochem</i> 120 :302-13; Andersen JP, Vestergaard AL, et al. (2016) <i>Front Physiol</i> 7 :275)
ATP8B1	extracellular PE and PS enhances endocytosis, LY, migration		BC (da Costa A, Lenze D, et al. (2012) <i>J Comp Pathol</i> 146 :143-51; Sjöblom T, Jones S, et al. (2006) <i>Science</i> 314 :268-74)
		PC	(Lee BH, Taylor MG, et al. (2013) <i>Cancer Res</i> 73 :1211-8; Sekine Y, Demosky SJ, et al. (2010) <i>Mol Cancer Res</i> 8 :1284-94; Trasino SE, Kim YS, et al. (2009) <i>Mol Cancer Ther</i> 8 :1934-45)
		PD	(Levano K, Punia V, et al. (2012) <i>J Neurochem</i> 120 :302-13)
			ATP8A2: (Zhu X, Libby RT, et al. (2012) <i>PLOS Genetics</i> 8 :e1002853)
		AD	(Soderberg M, Edlund C, et al. (1992) <i>J Neurochem</i> 59 :1646-53)
ANO4	Ca ⁺ dep. PL scramblase		ATP8B4: (Li H, Wetten S, et al. (2008) <i>Arch Neurol</i> 65 :45-53)
			(Picollo A, Malvezzi M, et al. (2015) <i>J Mol Biol</i> 427 :94-105)
		SC	(Weber GF (2015) <i>Sarcoma</i> 2015 :839182)
		AD	(Sherva R, Tripodis Y, et al. (2014) <i>Alzheimers Dement</i> 10 :45-52)
ABCA1	Regulates cellular lipid efflux; interacts with MEGF10	hsa02010	(Hamon Y, Trompier D, et al. (2006) <i>PLoS One</i> 1 :e120)
		BC	(Zhao W, Pijic S, et al. (2016) <i>Cancer Res</i> 76 :2037-49; Schimanski S, Wild PJ, et al. (2010) <i>Horm Metab Res</i> 42 :102-9)
		PC	(Lee BH, Taylor MG, et al. (2013) <i>Cancer Res</i> 73 :1211-8; Sekine Y, Demosky SJ, et al. (2010) <i>Mol Cancer Res</i> 8 :1284-94)
		PD	(Dong Y, Gou Y, et al. (2015) <i>Elife</i> 4 ; Pinho R, Guedes LC, et al. (2016) <i>PLoS One</i> 11 :e0157852; Dong X, Liu T, et al. (2016) <i>Genes & Genomics</i> 38 :243-50; Loane DJ, Washington PM, et al. (2011) <i>J Neurotrauma</i> 28 :225-36)
		AD	(Koldamova R, Fitz NF, et al. (2014) <i>Neurobiol Dis</i> 72 Pt A :13-21; Pahnke J, Langer O, et al. (2014) <i>Neurobiol Dis</i> 72 Pt A :54-60; Boehm-Cagan A, Bar R, et al. (2016) <i>PLoS One</i> 11 :e0166195; Nordestgaard LT, Tybjaerg-Hansen A, et al. (2015) <i>Alzheimers & Dementia</i> 11 :1430-8)
		HD	(Valenza M, Marullo M, et al. (2015) <i>Cell Death Differ</i> 22 :690-702)
AGPAT3	converts lyso-PI (LPI)	hsa00564	(Bradley RM, Marvyn PM, et al. (2015) <i>Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids</i> 1851 :1566-76)
AGPAT4	into phosphatidylinositol (PI)		

			BC	(Sahay D, Leblanc R, et al. (2015) <i>Oncotarget</i> 6 :20604-20; Hopkins MM, Zhang Z, et al. (2016) <i>J Clin Med</i> 5)
			PC	<i>AGPAT6</i> : (Gatto F, Miess H, et al. (2015) <i>Sci Rep</i> 5 :10738)
			PD	(Cheng D, Jenner AM, et al. (2011) <i>PLoS One</i> 6 :e17299)
			AD	(Sherva R, Baldwin CT, et al. (2011) <i>J Alzheimers Dis</i> 23 :349-59)
DGKQ	Regenerates PA from	hsa00564,		(Sakane F, Kanoh H (1997) <i>Int J Biochem Cell Biol</i> 29 :1139-43)
	diacylglycerol (DAG)	hsa04070	BC	(Filigheddu N, Cutrupi S, et al. (2007) <i>Anticancer Res</i> 27 :1489-92)
			PC	<i>AGK</i> : (Bektas M, Payne SG, et al. (2005) <i>J Cell Biol</i> 169 :801-11)
			PD	(Lill CM, Roehr JT, et al. (2012) <i>PLoS Genet</i> 8 :e1002548; Nalls MA, Pankratz N, et al. (2014) <i>Nat Genet</i> 46 :989-93)
LPPR1	complexes with		AD	(Zhu XC, Cao L, et al. (2016) <i>Mol Neurobiol</i>)
	<i>LPPR3/4/5, regulates PIS</i>			<i>LPPR4</i> : (Yu P, Agbaegbu C, et al. (2015) <i>Journal of Cell Science</i> 128 :3210-22)
	<i>(CDIPT)</i>		PD	(Moran LB, Duke DC, et al. (2006) <i>Neurogenetics</i> 7 :1-11)
SYNJ2	is recruited to the	hsa04070		(Schmid SL, Mettlen M (2013) <i>Nature</i> 499 :161-2)
	nascent		BC	(Ben-Chetrit N, Chetrit D, et al. (2015) <i>Sci Signal</i> 8 :ra7)
	clathrin coated pit		PC	(Rossi MR, Hawthorn L, et al. (2005) <i>Cancer Genet Cytogenet</i> 161 :97-103)
			PD	<i>SYNJ1</i> = <i>PARK20</i>
			AD	<i>SYNJ1</i> : (Cao J, Gaamouch FE, et al. (2017) <i>Sci Rep</i> 7 :11372)
				(Koran ME, Hohman TJ, et al. (2014) <i>J Alzheimers Dis</i> 38 :145-54)
PTENP1	PI3K/ <i>PTEN</i> and	hsa04070		<i>PTEN</i> : (Erneux C, Ghosh S, et al. (2016) <i>Curr Pharm Des</i> 22 :2309-14)
	PI(3,4,5)P3 are		BC	(Zhang H-Y, Liang F, et al. (2013) <i>Oncology Letters</i> 6 :161-8)
	involved in		PC	(Pourmand G, Ziaee AA, et al. (2007) <i>Urology journal</i> 4 :95-100)
	endocytosis/ cancer		PD	<i>PINK1</i> : (Choubey V, Cagalinec M, et al. (2014) <i>Autophagy</i> 10 :1105-19)
			AD	(Frere S, Slutsky I (2016) <i>Nat Neurosci</i> 19 :416-8)
			HD	<i>PINK1</i> : (Khalil B, El Fissi N, et al. (2015) <i>Cell Death Dis</i> 6 :e1617)

Table 5 (continued): Sequences for Table 5a Genes.

Name	Entrez	Accession			
ATP8A1	10396	AF067820			
1	mptmrrtvse	irsraegyek	tddvseksl	adqeevrtif	inqpqltkfc
	nnhvstakyn				
61	iitflprfly	sqfrraansf	flfiallqqi	pdvsptgryt	tlvp1lfila
	vaaikeiied				
121	ikrhkadv	nkkqtqvlrn	gaweivhwk	vavgeivkvt	ngehlpadli
	s1ssepqam				
181	cyietsnldg	etnlkirqgl	patsdikdvd	slmrsgrie	cespnrhlyd
	fvgnirldgh				
241	gtvplgadqi	llrgaqlrnt	qvwgdivvyt	ghdtklmqns	tspplklsv
	eritnvqili				
301	lfciliamsl	vcsvgsaiwn	rrhsgkdwy1	nlnyggasnf	glnfltfiil
	fnnlipisl1				
361	vtlevvkftq	ayfinwdldm	hyeptdtaam	artsnlnee1	gqvkyifsd
	tgtltcnvmq				
421	fkcktiagva	yghvpepedy	gcspdewqns	qfgdektfsd	ssllenlqnn
	hptapiicef				
481	ltmmavchta	vperegdkii	yqaaspdega	lvraakqlnf	vftgrtpdsv
	iidslggeer				
541	yellnvleft	sarkrmsviv	rtpsgklrly	ckgadtviyd	r1aetskyke
	itlkhleqfa				
601	teglrtlcfa	vaeisesdfq	ewravyqras	tsvqnrllkl	eesyelieln
	lqllgataie				
661	dklqdaqpet	ietlmkadik	iwiltgdkqe	tainighsck	llkknmgmiv
	inegslldgtr				
721	etlsrhcttl	gdalrkendf	aliidgktlk	yaltfgvrqy	f1dlalscka
	viccrvsp1q				
781	ksevvemvkk	qvkvvtlaig	dgandvsmiq	tahvgvgisg	neglqaanss
	dysiaqfky1				
841	knllmihgaw	nynrvskcil	ycfyknivly	ieiwfafvn	gfsgqilfer
	wciglynvmf				
901	tamppltlgi	ferscrkenm	lkypelykts	qnaldfntkv	fwvhc1nglf
	hsvilfwfpl				
961	kalqygtafg	ngktsdyll1	gnfvvtfvvi	tvcl1kaglet	sywtw1fshia
	iwgsialwvv				
1021	ffgiysslwp	aipmapdmsg	eaam1fssgv	fwmgl1fipv	as111ldvvyk
	vikrtafkt1				
1081	vdevqe1eak	sqdp1gavvl1g	ks1teraql1	knvfkknhvn	1yrses1qqn
	1lhgyafsqd				
1141	engivsqsev	iraydttkqr	pdew		
ATP8B1	5205	AF038007			
1	msterdsett	fdedsqpnde	vvpysddete	de1ddqgsav	epeqnravnre
	aeenrepfrk				
61	ectwqvkan	rkyheqphfm	ntkflcikes	kyannaikty	kynaftfipm
	nlfeqfkraa				
121	nlyflallil	qavpqistla	wytt1vp1lv	v1gvtaikdl	vddvarhkmd
	keinrtcev				
181	ikdgrfkvak	wkeiqvgdvi	r1kkndfvpa	d1111sssep	ns1cyvetae
	ldgetnlkfk				
241	msle1tdqyl	qredtlatfd	gfieecepnn	r1dkftgtlf	wrntsfp1da
	dkillrgcvi				
301	rntdfchglv	ifagadtkim	kns1gktrfkr	tkidylmnym	v1tifvvlil
	1saglaigha				
361	yweaqvgnss	wylydgeddt	psyr1gflifw	gyiiv1ntmv	pislyvsvev
	irlgqshfin				
421	wdlqmyaek	dtpakarttt	1neqlgqihy	ifsdktgtlt	qnimt1fkcc
	ingqi1gdhr				
481	dasqhn1nki	eqvdfswnty	adgk1afydh	ylieqi1sgk	epevrqfff1
	1avchtvmvd				
541	rtdgqlnyqa	aspdegalvn	aarnf1gfaf1	artqntitis	elgtertynv
	1a1ldfnsdr				
601	krmsiivrt1p	egniklyckg	adtv1yer1h	rmnptkqetq	daldifanet
	1rt1clcyke				

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2281	ttggcatcga	cgcgagctg	agcctggact	tccaccaggc	acgggaagag
	gagcctggca				
2341	agttcacaag	caggctgcac	aacaagggtg	tgtacgtgcg	ggtggggctg
	cagaagatca				
2401	gtcactctcg	gagcctgcac	aagcagatcc	ggctgcaggt	ggagcggcag
	gaggtggagc				
2461	tgccagtat	tgaaggcctc	atcttcatca	acatccccag	ctggggctcg
	ggggccgacc				
2521	tgtggggctc	cgacagcgac	accaggtttg	agaagccacg	catggacgac
	gggctgctgg				
2581	aggttgtggg	cgtgacgggc	gtcgtgcaca	tgggccaggt	ccagggtggg
	ctgcgtccg				
2641	gaatccggat	tggccagggt	tcctacttcc	gagtcacgct	cctcaaggcc
	accccggtgc				
2701	aggtggacgg	ggagccctgg	gtccaggccc	cggggcacat	gatcatctca
	gctgctggcc				
2761	ctaagggtgca	catgctgagg	aaggccaagc	agaagccgag	gagggccggg
	accaccaggg				
2821	atgcccgggc	ggatcgtgcg	cctgcccctg	agagcgatcc	taggtagggg
	tggctggggc				
2881	agcccaaggg	ctcgagccat	ctctgctccc	gccagccttg	ttttcagggtg
	gtctggaggc				
2941	agctccacgt	cacacagtgg	ctgtcatata	ttgaagttac	cttcccactg
	gaaaaaaaaat				
LPPR1	54886 AY304515				
1	gtggctcgga	ccgccgcctg	aatgtacctc	gctcccggga	gccggacggc
	ccagtagggc				

61	gcactggagg	acgctccgct	gcgggagcct	ggacagtttt	tgacggtgca
121	gtcttgctat	aatggctgt	aggaaacaac	actcaacgaa	gttattccat
181	atgggtgtgag	ttgagcttgt	catcatggct	gggacagtgc	tgcttgcccta
241	catcccgtgt	cttttcaggt	gcataatccaa	ggattcttct	gtcaggacgg
301	tttatatttg	cagggacaga	ggaagaaagc	ttcatcacc	ctctggtgct
361	ctacttcgaa	ccccaaactgc	tattatTTTT	attggtgaga	tatccatgta
421	tgacttgaca	aatccctgat	tgctcaggag	aaaacaattc	tgaccggaga
481	agacttaatg	tacttcgaag	gatcataaga	ttcacagggg	tgtttgcat
541	aagccttacc	TTTTgtaaa	cgccggacaa	gtggtcactg	ggcacttaac
601	ctattgtgtg	gcaagccaaa	ctacaccagt	gcagactgcc	aagcgcacca
661	ctggctgcca	acatttgtac	tggggacctg	gaagtgatag	aaaaggctcg
721	tttcataaaa	acgttgctct	gagcatttac	tccgccttat	atgccacgat
781	tcaacaagag	tgctgacagg	cctcaaccgg	gtctctgagt	atcggaacca
841	atgctgttac	gtttcatcct	gggcaactgca	gtggccctgt	ttctgggaat
901	ctgaaccctt	aaggaacgca	aggatctcct	tccaaaccca	agcctgagga
961	tggtactttt	tggttttccc	aaggatagaa	agccctctgg	aaaccttaag
1021	gctactgaca	ccatgaccga	agttacctga	gacgactgat	gtgtcacaag
1081	gccatacttc	ccaattctat	acttcaaaaac	acacagttgc	tcaatgtcaa
1141	ctgactgtgt	gtttatctag	ttagaagcta	atgttttgta	cattttttgt
1201	ccagtttata	gcccctgattt	TTTTTTTTTT	TTTTggtcag	ctttaatata
1261	aacaatggga	tttcttggttc	aagcgtgcat	tgaagaacca	
1321	gagatccttt	ttgttttggtg	atatttgtac	acaaattttc	ttttctcagt
1381	gagatccttt	tataacaatt	cacttttaaac	ttttattacc	acagttgctg
1441	gagatccttt	ttttaataaaa	aggcaaaactt	ttgagctgca	ggaaggacaa
1501	gagatccttt	caaagtcaat	tgtagaaaaa	aaattgtctt	caaaaagaat
1561	gagatccttt	caaattgtta	cgttcaaagt	ttaaagtgat	atattaacaa
1621	gagatccttt	gaattctgga	tttgaggagg	attgggggta	
1681	gagatccttt	tggtctctac	taaataactc	aagatctttc	tggaatgtct
1741	gagatccttt	gtcagctttt	ctccaaaaag	cagccaacat	cagcctcccc
1801	gagatccttt	atctcatatt	atatggactt	tatatgaaaa	tgaatatttt
1861	gagatccttt	ttacagaaaa	ggaatcagag	aatctacaac	atagggcccc
1921	gagatccttt	tggtttttta	ttattctaga	attttaactg	catctcattt
1981	gagatccttt	atatgtaact	cctttgattg	aaggagctct	tttgtccgta
2041	gagatccttt				

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2101	tgttttcttg	acacttccat	gttggctctt	ctcagctttt	tttgtacata		
	tttttttttt						
2161	ctaaagagaa	gaaaaagtta	tcacaaaatg	taaaaaaaga	aaaaaaaaaa	aaaaa	

Table 6: EEC overlap between breast cancer and PD/AD. (see Table 5 for legend)

Gene	EEC Function	KEGG	EC ND References
<i>ASTN2</i>	regulates trafficking of ASTN1, during early clathrin- dependent endocytosis; binds AP-2z	hsa04144	(Wilson PM, Fryer RH, et al. (2010) The Journal of Neuroscience 30 :8529-40; Solecki DJ (2012) Curr Opin Neurobiol 22 :791-8) BC (Kawauchi T (2012) Int J Mol Sci 13 :4564-90) AD (Wang KS, Tonarelli S, et al. (2015) J Neural Transm (Vienna) 122 :701-8) ID <i>ASTN1</i> : (Anazi S, Maddirevula S, et al. (2016) Mol Psychiatry) (Rainero E, Howe JD, et al. (2015) Cell Rep 10 :398-413; McCleverty CJ, Lin DC, et al. (2007) Protein Science : A Publication of the Protein Society 16 :1223-9; Burghel GJ, Lin W-Y, et al. (2013) PLoS One 8 :e83859) BC (Hall EH, Daugherty AE, et al. (2009) J Biol Chem 284 :34713-22)
<i>TNS1</i>	controls cell polarization, migration, and invasion binds α5β1 integrin during endocytosis		
<i>MEGF11</i>	In <i>C. elegans</i> , DYN-1 (<i>DNM1</i>) depends on the function of CED-1 (<i>MEGF10/11</i>)	hsa04144 hsa04721	CED-1: (Shen Q, He B, et al. (2013) Development 140 :3230-43) AD <i>MEGF10</i> : (Sherva R, Tripodis Y, et al. (2014) Alzheimers Dement 10 :45-52; Singh TD, Park SY, et al. (2010) FEBS Lett 584 :3936-42) (Baietti MF, Zhang Z, et al. (2012) Nat Cell Biol 14 :677-85; Hurley JH, Odorizzi G (2012) Nat Cell Biol 14 :654-5) BC (Yang Y, Hong Q, et al. (2013) Breast Cancer Res 15 :R50) PD (Tomlinson PR, Zheng Y, et al. (2015) Ann Clin Transl Neurol 2 :353-61) AD (Leonova EI, Galzitskaya OV (2015) Adv Exp Med Biol 855 :241-58)
<i>SDCBP2</i>	Syndecans bind PI(4,5)P2 and are involved in both endo- and exocytosis.		(Persaud A, Alberts P, et al. (2011) EMBO J 30 :3259-73; Jung S, Li C, et al. (2013) Int J Oncol 43 :1587-95) BC (Jung S, Li C, et al. (2013) Int J Oncol 43 :1587-95; Minn AJ, Gupta GP, et al. (2005) Nature 436 :518-24; Liao CJ, Chi HC, et al. (2015) Oncotarget 6 :9341-54) PD (Perrett RM, Alexopoulou Z, et al. (2015) Mol Cell Neurosci 66 :21-8)
<i>N4BP3</i>	<i>NEDD4</i> controls growth factor receptor endocytosis (<i>NEDD9</i> expression is assoc. with BC metastasis)	hsa04144	

Gene	EEC Function	KEGG	EC ND References
SYNJ2	is recruited to the nascent clathrin coated pit	hsa04070	AD (Rodrigues EM, Scudder SL, et al. (2016) J Neurosci 36 :1590-5; Salminen A, Kaarniranta K, et al. (2013) Prog Neurobiol 106-107 :33-54) BC ^{PD} see (Table 5)
NLRP4	and NLRP3 associate with BECN1, a component of the PI3K complex that mediates vesicle trafficking		(Jounai N, Kobiyama K, et al. (2011) J Immunol 186 :1646-55; Zhang Y, Sauler M, et al. (2014) The Journal of Immunology 192 :5296-304; Rohatgi RA, Shaw LM (2016) Mol Cell Oncol 3) BC (Zhiyu W, Wang N, et al. (2016) Oncotarget 7 :50766) PD (Choubey V, Caglinec M, et al. (2014) Autophagy 10 :1105-19; Wang JD, Cao YL, et al. (2015) Autophagy 11 :2057-73) AD (Antonell A, Llado A, et al. (2015) Mol Neurobiol ; Swaminathan G, Zhu W, et al. (2016) Autophagy 12 :2404-19)
PTENP1	PI3K/ PTEN and PI(3,4,5)P3 are involved in endocytosis and cancer	hsa04070	see (Table 5)
VAV3	VAV ... promote BCR endocytosis	hsa04666	(Inabe K, Ishiai M, et al. (2002) J Exp Med 195 :189-200; Malhotra S, Kovats S, et al. (2009) The Journal of Biological Chemistry 284 :36202-12) BC (Chen XIN, Chen SI, et al. (2015) Oncology Letters 9 :2143-8) PD (Moran LB, Duke DC, et al. (2006) Neurogenetics 7 :1-11) AD (Wilkinson BL, Cramer PE, et al. (2012) Neurobiology of Aging 33 :197.e21-.e32)
PDE4D*	Binds ARRB2 (fast recycling)	hsa04144	(Haddad SA, Ruiz-Narvaez EA, et al. (2016) Carcinogenesis) BC (Lin D-C, Xu L, et al. (2013) Proc Natl Acad Sci USA 110 :6109-14) PD (Yang L, Calingasan NY, et al. (2008) Exp Neurol 211 :311-4) AD (Gurney ME, D'Amato EC, et al. (2015) Neurotherapeutics 12 :49-56)
EEA1	binds to early endosomes in a Rab5 and PI(3)P dependent manner.	hsa04144	(Pfeffer SR (1999) Nat Cell Biol 1 :E145-E7) PD (Walter J, Fluhrer R, et al. (2001) J Biol Chem 276 :14634-41) AD (Armstrong A, Mattsson N, et al. (2014) Neuromolecular Med 16 :150-60)

Gene	EEC Function	KEGG	EC ND References
RAB32	RAB32/ <u>RAB38</u> interact AP-3 and with <u>LRRK2</u> (<i>PARK8</i>)	hsa05012	(Hesketh GG, Perez-Dorado I, et al. (2014) Dev Cell 29 :591-606; Waschbusch D, Michels H, et al. (2014) PLoS One 9 :e111632; Bultema JJ, Ambrosio AL, et al. (2012) J Biol Chem 287 :19550-63)
			BC (Agalliu I, San Luciano M, et al. (2015) JAMA Neurol 72 :58-65)
			PD (Fukuda M (2016) Traffic 17 :709-19)
			AD (Fukuda M (2016) Traffic 17 :709-19)
SNX32*	Sorting Nexin (late endosome), SNX-BAR retromer with other Vps17 orthologs SNX5/ <u>SNX6</u> interacts with <u>VPS35</u>	hsa04144	(Wang X, Huang T, et al. (2014) Molecular Neurodegeneration 9 :1-9; van Weering JR, Verkade P, et al. (2012) Traffic 13 :94-107; Zhang QY, Tan MS, et al. (2015) Mol Neurobiol)
			BC (Rivera J, Megias D, et al. (2010) J Cell Biochem 111 :1464-72)
			PD (Small SA, Petsko GA (2015) Nat Rev Neurosci 16 :126-32)
			AD (Reitz C (2012) Future Neurol 7 :423-31)
SCARB2	required for maintenance of endo- and lysosomes, located in limiting membranes	hsa04142	(Gonzalez A, Valeiras M, et al. (2014) Mol Genet Metab 111 :84-91)
			BC (Nishimura Y, Yoshioka K, et al. (2006) Histochem Cell Biol 126 :627-38; Nishimura Y, Itoh K, et al. (2003) Pathol Oncol Res 9 :83-95)
			PD (Alcalay RN, Levy OA, et al. (2016) NPJ Parkinsons Dis 2)
			AD (Shimizu E, Kawahara K, et al. (2008) J Immunol 181 :6503-13; Bras J, Guerreiro R, et al. (2014b) Hum Mol Genet 23 :6139-46)
GLB1	Galactosidase Beta, related to Galectin 3 (<i>LGALS3</i>)		(Ahmed H, AlSadek DM (2015) Clin Med Insights Oncol 9 :113-21)
			BC (O'Reilly EA, Gubbins L, et al. (2015) BBA Clin 3 :257-75)
			PD (van Dijk KD, Persichetti E, et al. (2013) Mov Disord 28 :747-54)
			AD (Tiribuzi R, Orlacchio A, et al. (2011) J Alzheimers Dis 24 :785-97)
RAPGEF4	GEF for RAB1A/1B/2A; involved in exocytosis through RIMS2		(Parnell E, Palmer TM, et al. (2015) Trends Pharmacol Sci 36 :203-14; Almahariq M, Tsalkova T, et al. (2013) Mol Pharmacol 83 :122-8)
			BC (Jiang HL, Sun HF, et al. (2015) Oncotarget 6 :16352-65)
			PD (Winslow AR, Chen CW, et al. (2010) J Cell Biol 190 :1023-37)

Gene	EEC Function	KEGG	EC ND References
UNC13C STXBP1 (MUNC18)	Interacting with each other and with PI(4,5)P2. Involved in docking/priming in exocytosis	hsa04721	AD (Puthiyedth N, Riveros C, et al. (2016) PLoS One 11 :e0152342; Bereczki E, Francis PT, et al. (2016) Alzheimers Dement) (Betz A, Okamoto M, et al. (1997) J Biol Chem 272 :2520-6; Martin TF (2015) Biochim Biophys Acta 1851 :785-93)
STXBP4*	Prevents interaction between <u>STX4</u> and <u>VAMP2</u> ,	hsa04130, hsa04721	BC (Fernandez-Nogueira P, Bragado P, et al. (2016) Oncotarget 7 :5313-26) PD (Keogh MJ, Daud D, et al. (2015) Neurogenetics 16 :65-7; Campbell IM, Yatsenko SA, et al. (2012) Genet Med 14 :868-76) AD (Leonova EI, Galzitskaya OV (2015) Adv Exp Med Biol 855 :241-58; Miller JA, Woltjer RL, et al. (2013) Genome Med 5 :48; Takahashi M, Iseki E, et al. (2000) J Neurol Sci 172 :63-9; Law C, Schaan Profes M, et al. (2016) J Neurosci 36 :561-76) (Zhang QY, Tan MS, et al. (2015) Mol Neurobiol)
ANXA4	Forms exocytotic complexes with <u>SYT1</u> and the <u>RAB3A</u> effector <u>RPH3A</u> .	hsa04721	BC (Antoniou AC, Beesley J, et al. (2010) Cancer Res 70 :9742-54; Day P, Riggs KA, et al. (2011) Int J Oncol 39 :863-71) PD (Diao J, Burre J, et al. (2013) Elife 2 :e00592) AD (Russell CL, Semerdjieva S, et al. (2012) PLoS One 7 :e43201) (Lizarbe MA, Barrasa JI, et al. (2013) Int J Mol Sci 14 :2652-83; Willshaw A, Grant K, et al. (2004) FEBS Lett 559 :13-21)
SYT17	"B/K protein may play a role in exocytosis"		BC (Wei B, Guo C, et al. (2015) Clin Chim Acta 447 :72-8; Yao H, Sun C, et al. (2016) Front Biosci (Landmark Ed) 21 :949-57) PD (Matigian N, Abrahamsen G, et al. (2010) Disease Models & Mechanisms 3 :785-98) AD (Kuzuya A, Zoltowska KM, et al. (2016) BMC Biol 14 :25; Tan MG, Lee C, et al. (2014) Neurochem Int 64 :29-36) HD SYT1: (Valencia A, Sapp E, et al. (2013) Journal of Huntington's disease 2 :459-75) (Chin H, Choi SH, et al. (2006) Exp Mol Med 38 :144-52; Fukuda M (2013) Madame Curie Regulated Database [Internet]) BC (Weng L, Ziliak D, et al. (2013) Annals of Oncology) AD (Gautam V, D'Avanzo C, et al. (2015) Mol Neurodegener 10 :31)

Gene	EEC Function	KEGG	EC ND References
PARK2	"Loss of parkin promotes ... endocytosis by accumulating CAV1"; <i>PARK2</i> binds AP-2 via arrestin	hsa04141	(Cha SH, Choi YR, et al. (2015) Mol Neurodegener 10 :63; Ahmed MR, Zhan X, et al. (2011) Biochemistry 50 :3749-63) BC (Wang H, Liu B, et al. (2009) J Pathol 218 :76-85) PD (Feng DD, Cai W, et al. (2015) Transl Neurodegener 4 :20; Kitada T, Asakawa S, et al. (1998) Nature 392 :605-8) AD (Martin-Maestro P, Gargini R, et al. (2016) Hum Mol Genet 25 :792-806)
DNAJC1*	ER membrane protein. <i>DNAJC</i> (Hsp40) controls release of proteins via <i>HSPA5</i> (BiP, <i>GRP78</i>); <i>DNAJC13</i> interacts with SNX-BAR	hsa04141	BC (Michailidou K, Hall P, et al. (2013) Nat Genet 45 :353-61, 61e1-2; Chen C-L, Hou W-H, et al. (2009) Journal of Cell Science 122 :1863-71) PD <i>DNAJC6/13</i> : (Seaman M, Freeman CL (2014) Commun Integr Biol 7 :e29483; Vilarino-Guell C, Rajput A, et al. (2014) Hum Mol Genet 23 :1794-801) AD (Hsu WC, Wang HK, et al. (2008) J Neural Transm (Vienna) 115 :1537-43)

*: from previous GWAS. Underlined: functionally related genes identified in the literature.

Example 3: Elevated endocytosis combined with age-related decline in lysosomal function is a common epistatic risk factor in age-related diseases

[0323] endocytosis is a known component of the etiology of many age-related diseases. In cancers (Mosesson Y, Mills GB, et al. (2008) Nat Rev Cancer **8**:835-50), Alzheimer's disease (Schreij AM, Fon EA, et al. (2015) Cell Mol Life Sci ; Wang X, Huang T, et al. (2014) Molecular Neurodegeneration **9**:1-9; Kim S, Sato Y, et al. (2016) Mol Psychiatry **21**:707-16), and PD (Rivero-Rios P, Gomez-Suaga P, et al. (2015) Biochem Soc Trans **43**:390-5), endocytosis of β 1-integrin (FIG. 14), APP (FIG. 15), and α -synuclein (SNCA, FIG. 16), respectively, are known to be critical early steps in the etiology leading to formation of plaques. The terms "derailed endocytosis" and "deranged endocytosis" have been used to characterize an important component of the etiology of cancers (Mosesson Y, Mills GB, et al. (2008) Nat Rev Cancer **8**:835-50), Alzheimer's disease (Van Dooren T, Princen K, et al.

(2014) Biomed Res Int **2014**:167024), and other “pathological conditions”.(Di Fiore PP, von Zastrow M (2014) Cold Spring Harbor Perspectives in Biology **6**) “[G]enes that influence endocytosis are overrepresented as Alzheimer’s disease risk factors [and] endocytosis-related genes are the earliest known disease-specific neuronal response in Alzheimer’s disease. They develop early in Down syndrome, a cause of early-onset Alzheimer’s disease linked to an extra copy of APP.”(Kim S, Sato Y, et al. (2016) Mol Psychiatry **21**:707-16)

[0324] “New reports implicate altered [vacuolar H⁺]-ATPase activity and lysosomal pH dysregulation in cellular aging, longevity, and adult-onset neurodegenerative diseases, including forms of [Parkinson’s disease] and [Alzheimer’s disease],” (Colacurcio DJ, Nixon RA (2016) Ageing Res Rev) but also atherosclerosis, see also FIG. 15, FIG. 16, and FIG. 17. In Parkinson’s disease, “an age-related pathological depletion of functional endosomes may increase the susceptibility to stochastic molecular defects in this same pathway, which in some individuals may trigger [a] vicious circle. [...] Disease causing mutations cluster within [the endosomal] pathway and alter receptor recycling and/or α -synuclein degradation. In turn, α -synuclein accumulation [...] exacerbates defective endosomal processing by impairing the machinery involved in the sorting or fusion of endosomes”.(Perrett RM, Alexopoulou Z, et al. (2015) Mol Cell Neurosci **66**:21-8) In Alzheimer’s disease, “accelerated endocytosis causes endocytic cargos to accumulate within enlarged [LEs] and impairs lysosomal functions. [...] Pathogenic endocytosis [...] could be modulated therapeutically at multiple possible targets.”(Kim S, Sato Y, et al. (2016) Mol Psychiatry **21**:707-16) “The underlying molecular mechanisms [in Alzheimer’s disease and Parkinson’s disease] remain poorly understood, yet dysfunction in endocytic membrane trafficking is a recurrent theme, which may explain the neurodegenerative process.”(Schreij AM, Fon EA, et al. (2015) Cell Mol Life Sci) ,

[0325] Given the failure of previous GWAS to identify functionally related collections of genes and novel insights into the etiology of common diseases, the surprising results based on the novel GWAS approach (Example 1) were consistent with previous results not only in breast cancer, but also in Alzheimer’s disease and Parkinson’s disease (Example 2), in general, and with genes involving lysosomal function, in particular (FIG. 18A and FIG. 18B for overlap in lysosomal genetic risk factors).

[0326] Endocytosis has also been implicated as playing an important role in virus infections. For instance, hepatitis B virus (HBV) has been shown to “hijacks cellular processes, in

particular the endocytic machinery” (Schroeder B, McNiven MA (2014) *Compr Physiol* **4**:1403-17) and “HBV capsid-like core particles are internalized through clathrin-mediated endocytosis.” (Cooper A, Shaul Y (2006) *J Biol Chem* **281**:16563-9) In fact, umifenovir (Arbidol), which impairs clathrin-mediated endocytosis, (Pecher EI, Borisevich V, et al. (2016) *Journal of virology* **90**:3086-92) has broad antiviral activity, including activity against hepatitis viruses (Blaising J, Polyak SJ, et al. (2014) *Antiviral research* **107**:84-94) (FIG. 19).

Example 4: β CDs exert their effects on “derailed”, “deranged”, or “defective” endocytosis not through a mechanism independent of cholesterol.

[0327] The present disclosure provides α -CD or HP- α -CD and analogues and derivatives thereof as non-limiting examples of compounds that may be useful for treating age-related conditions including but not limited to conditions involving “derailed endocytosis,” a “hallmark of cancer” (Mitra S, Cheng KW, et al. (2012) *Biochem Soc Trans* **40**:1404-8) also seen in neurodevelopmental diseases. (Rivero-Rios P, Gomez-Suaga P, et al. (2015) *Biochem Soc Trans* **43**:390-5; Van Dooren T, Princen K, et al. (2014) *Biomed Res Int* **2014**:167024). In some embodiment of the present disclosure α -CDs are used for treating age-related conditions, such as cancers (including, but not limited to, prostate and breast cancer) and neurodegenerative diseases (including, but not limited to, PD or AD). CDs lower the amount of PIPs available without directly interfering with their distribution.

[0328] In Cancer, AD, and PD, a plethora of studies have investigated the effect of β CDs *in vitro*

[0329] **Cancer:** M- β -CD suppressed invasion activity in three H7 Lewis lung cancer cell lines and highly metastatic cell lines had more β 1 integrin (Zhang Q, Furukawa K, et al. (2006) *J Biol Chem* **281**:18145-55) and breast cancer and prostate cancer cell lines were more sensitive to M- β -CD-induced cell death than their normal counterparts. (Li YC, Park MJ, et al. (2006) *Am J Pathol* **168**:1107-18; quiz 404-5) In particular, M- β -CD treatment induced a substantial decrease (40%) in activity of breast cancer resistance protein (BCRP), (Storch CH, Eehalt R, et al. (2007) *J Pharmacol Exp Ther* **323**:257-64) which transports PS (“eat me signal”) and PC analogues. (Daleke DL (2007) *J Biol Chem* **282**:821-5) In subsequent functional studies, M- β -CD inhibited spheroid migration and invasion of MDA-MB-241 and ZR751 breast cancer cells (Raghu H, Sodadasu PK, et al. (2010) *BMC Cancer* **10**:647) and also endocytosis (Palaniyandi K, Pockaj BA, et al. (2012) *Journal of cancer science &*

therapy 4:214-22) and migration (Guerra FS, Sampaio LdS, et al. (2016) Translational Medicine Communications 1:3) of MCF7 breast cancer cells. M- β -CD was more toxic for invasive than for non-invasive urothelial cancer cells,(Resnik N, Repnik U, et al. (2015) PLoS One 10:e0137878) interfered with RTK-[PIP2]-PI3K-[PIP3]-AKT signaling in HeLa cells,(Yamaguchi R, Perkins G, et al. (2015) FEBS Lett 589:4097-105) and inhibited the growth of leukemia cell lines.(Yokoo M, Kubota Y, et al. (2015) PLoS One 10:e0141946)

[0330] **AD:** M- β -CD inhibits secretion of amyloid-beta from hippocampal neurons of rats infected with recombinant Semliki Forest virus (SFV) carrying APP,(Simons M, Keller P, et al. (1998) Proc Natl Acad Sci U S A 95:6460-4) promotes the non-amylogenic α -secretase pathway,(Kojro E, Gimpl G, et al. (2001) Proc Natl Acad Sci U S A 98:5815-20) and increased activity of α -secretase while decreasing activity of β -secretase, reducing the level of cell-associated APPs β (FIG. 20) “[I]mpaired internalization of APP [...] is responsible for increased α -secretase cleavage after *acute cholesterol depletion by M β CD*”.(Cole SL, Grudzien A, et al. (2005) J Biol Chem 280:18755-70) Cell membrane cholesterol accumulation was detected in N2a cells over-expressing Swedish mutant APP (SwN2a), and the level of membrane cholesterol was reduced by HP- α -CD treatment, which dramatically lowered the levels of A β 42 in SwN2a cells, and the effects were persistent for 24 h after withdrawal. (Yao J, Ho D, et al. (2012) J Exp Med 209:2501-13)

[0331] **PD:** In HeLa cells transfected with α -synuclein, α -synuclein was shown to colocalize with the lipid raft. Incubation with 20 mM M- β -CD for 1 hr dramatically reduced this co-location of α -synuclein with the cell membrane.(Fortin DL, Troyer MD, et al. (2004) J Neurosci 24:6715-23)

[0332] The relevance of the above *in vitro* findings was confirmed by several *in vivo* studies.

[0333] **Ca:** M- β -CD had higher concentration in tumor than in other cells (except kidney and liver) and was effective in a mouse model of breast cancer,(Grosse PY, Bressolle F, et al. (1998) Br J Cancer 78:1165-9) reduced the number of lung metastases in mice implanted with H7-O Lewis lung cancer cells,(Zhang Q, Furukawa K, et al. (2006) J Biol Chem 281:18145-55) and inhibited growth of primary effusion lymphoma (PEL) in mice.(Gotoh K, Kariya R, et al. (2014) Biochem Biophys Res Commun 455:285-9) (M-) β -CDs have found to increase the effectiveness of anti-tumor drugs, such as curcumin in a lung cancer mouse model (Rocks N, Bekaert S, et al. (2012) Br J Cancer 107:1083-92) and of raloxifen in a

chemically induced tumor mouse model (Agardan NB, Degim Z, et al. (2015) AAPS PharmSciTech). HP- β -CD was necessary in triple combination treatment for tumor regression in mice implanted with renal cancer cells.(Yamaguchi R, Perkins G, et al. (2015) FEBS Lett **589**:4097-105) and prolonged survival in leukemia mouse models.(Yokoo M, Kubota Y, et al. (2015) PLoS One **10**:e0141946)

[0334] **AD:** Scavenging of cholesterol and/or binding directly to amyloid-beta or α -synuclein was also believed to be the mode of action for β -CD in Alzheimer's disease and Parkinson's disease: "HP β CD, which diminishes the pool of both cholesterol and PLs, had "neuroprotective effects [...] in a transgenic mouse model of AD [by] enhancing clearance mechanisms". Four months of subcutaneous HP β CD administration significantly improved spatial learning and memory deficits in Tg19959 mice, diminished amyloid-beta plaque deposition, and reduced tau immunoreactive dystrophic neurites (DN). Tg19959 mice are transgenic mice with 2 mutations in the amyloid precursor protein (APP) gene which have been associated with human Alzheimer's disease and beneficial effects were attributed to a reduction in cholesterol.(Yao J, Ho D, et al. (2012) J Exp Med **209**:2501-13) "Toxicity of A β 1-40/42 was reduced in rats via stereotactical injection [of β -CD] into the hippocampus,"(Jameson LP, Smith NW, et al. (2012) ACS Chem Neurosci **3**:807-19).

[0335] **PD:** "Treatment of mice with M β CD resulted in [...] reduced accumulation of α -synuclein in neuronal cell body and synapses"(Bar-On P, Rockenstein E, et al. (2006) J Neurochem **98**:1032-45) This "possibly transcriptional effect of M β CD" (Bar-On P, Rockenstein E, et al. (2006) J Neurochem **98**:1032-45) was seen as related to β -CD preventing aggregation of α -synuclein *ex vivo* (Gautam S, Karmakar S, et al. (2014) Biochemistry **53**:4081-3) via direct interaction. The results of the present invention, instead, suggest that cyclodextrins act by regulating endocytosis as a common component in the etiology, the same age-related mechanism controlled by cyclodextrins in cancer.

Clinical/epidemiological

[0336] Although Atherosclerosis and AD are comorbid,(Song Y, Stampfer MJ, et al. (2004) Ann Intern Med **141**:137-47) An analysis of the Framingham cohort also did not find an association between cholesterol levels and AD.(Tan ZS, Seshadri S, et al. (2003) Archives of internal medicine **163**:1053-7) While HP- β -CD was effective against tumors in animal models and well tolerated in most peripheral and central organ systems,(Cronin S, Lin A, et

al. (2015) J Assoc Res Otolaryngol **16**:599-611) it was shown to carry the risk of causing permanent hearing loss in mice,(Crumling MA, Liu L, et al. (2012) PLoS One **7**:e53280) cats,(Ward S, O'Donnell P, et al. (2010) Pediatr Res **68**:52-6; Vite CH, Bagel JH, et al. (2015) Sci Transl Med **7**:276ra26) and one human.(Maarup TJ, Chen AH, et al. (2015) Mol Genet Metab **116**:75-9) This ototoxicity is believed to be due to depriving prestin (SLC26A5) in outer hair cells of cholesterol.(Takahashi S, Homma K, et al. (2016) Sci Rep **6**:21973; Kamar RI, Organ-Darling LE, et al. (2012) Biophysical Journal **103**:1627-36; Yamashita T, Hakizimana P, et al. (2015) PLoS Genet **11**:e1005500)

The mechanism of HP- β -CD is independent of cholesterol

[0337] The direct action of cyclodextrins consists in extracting lipids (cholesterol and phospholipids) from membranes and serum. After parenteral application, the lipids are excreted into urine. From the mechanism of β -CD in NPC and elevated cholesterol levels seen in several cancers, including breast cancer,(Yokoo M, Kubota Y, et al. (2015) PLoS One **10**:e0141946) β -CDs are thought to reduce cancer growth by lowering cholesterol levels. In Alzheimer's disease, HP- β -CD is believed to act on hydrophobic residues of another large lipid, amyloid-beta.

[0338] Early evidence that HP- β -CD might not exert its action by directly binding top large lipids emerged from the study of exosomes, which play a key role in development of breast cancer (Lowry MC, Gallagher WM, et al. (2015) Clin Chem **61**:1457-65; Peinado H, Lavotshkin S, et al. (2011) Semin Cancer Biol **21**:139-46). Treatment of MDA-MB-231 breast cancer cells with M- β -CD inhibited the internalization of exosomes containing integrins, (Hoshino A, Costa-Silva B, et al. (2015) Nature **527**:329-35) but did so independently of cholesterol.(Koumangoye RB, Sakwe AM, et al. (2011) PLoS One **6**:e24234)

[0339] In fact, broad evidence suggests that cholesterol is not involved. Although CAD and Alzheimer's disease are comorbid,(Song Y, Stampfer MJ, et al. (2004) Ann Intern Med **141**:137-47) an analysis of the Framingham cohort did not find an association between cholesterol levels and Alzheimer's disease.(Tan ZS, Seshadri S, et al. (2003) Archives of internal medicine **163**:1053-7) In Parkinson's disease, the effect of HP- β -CD on clearance of a-syn is independent on cholesterol (Kilpatrick K, Zeng Y, et al. (2015) PLoS One **10**:e0120819) (Fig. 21).

[0340] A recent study compared surface activity of surfactant in bronchial lavage fluid (BLF) from 16 cystic fibrosis patients treated with M- β -CD and 9 healthy controls. Alveolar macrophages were enlarged as a result of lysosomal lipid overload. Since release of FFAs, such as arachidonic acid, oleic acid, LPC, and lysophosphatic acid (LPA). It was concluded that hydrolysis products of PC, LPC and FFAs, inhibited surfactant function. This dysfunction was reversed by M β CD even in the relative absence of cholesterol (Fig. 28), and M- β -CD also reduce the inflammatory effects of these products.(Gunasekara L, Al-Saiedy M, et al. (2017) Journal of cystic fibrosis : official journal of the European Cystic Fibrosis Society ; Strandvik B (2010) Prostaglandins, leukotrienes, and essential fatty acids **83**:121-9) The common involvement of AA and LPC is consistent with the known comorbidity between fibroses and CAD (Skolnik K, Levy RD, et al. (2016) Journal of cystic fibrosis : official journal of the European Cystic Fibrosis Society **15**:e70-e1)

[0341] Even in NPC, where cholesterol is seen to accumulate in late endosomes and lysosomes, the mechanism of action is uncertain and its molecular target is unknown,(Dai S, Dulcey AE, et al. (2017) Autophagy **13**:1435-51) except that HP- β -CD was shown to work in a manner that is independent of the functions of the actions of NPC1 and NPC2 on cholesterol.(Liu B, Li H, et al. (2008) J Lipid Res **49**:663-9) Moreover, mechanistic studies have shown that some of the effects of β -CDs are also seen with α -CDs, which is too small to fit cholesterol.(Shvartsman DE, Gutman O, et al. (2006) Traffic **7**:917-26) In Npc1a mice, for instance, HP- α -CD reduces cholesterol accumulation, albeit not as effectively as HP- β -CD (FIG. 22).

[0342] In US 2015/0216895 A1 (McKew J, Zheng WEI, et al. (2014)), M- β -CD was the most effective cyclodextrin against various LYD diseases (FIG. 23) and the proposed combination with 10 μ M delta-tocopherol (vitamin E) reduced the concentrations needed and, thus, reduced (but not eliminated) the risk of oto- and nephrotoxicity. While α -CD (and HP- β -CD) were less effective than M- β -CD, it was not appreciated that the fact that α -CD (which cannot fit cholesterol) was similarly effective as HP- β -CD (which can fit cholesterol) implies that the effects cannot be due to scavenging of cholesterol.

[0343] Our surprising genetics results show that the effect of β -CDs seen in age-related diseases is not mediated by cholesterol, as but by phospholipids and, thus, suggest for the first time that the less toxic α -CDs is preferable over the more toxic HP- β -CD and M- β -CD for the

treatment and prevention of age-related diseases, disorders, and conditions, and even for the treatment of LYD diseases.

Example 5: HP β CD activates formation of autophagolysosomes through a mechanism involving reduction of serum phospholipids

[0344] “Genetic variation in lysosomal genes modifies the disease course of sporadic AD”. (Whyte LS, Lau AA, et al. (2017) J Neurochem **140**:703-17). Autophagy has been linked to human oral diseases, including but not limited to “periapical lesions, periodontal diseases, and oral candidiasis”. (Tan YQ, Zhang J, et al. (2017) Autophagy **13**:225-36) “Crosstalk between [autophagy] and other cellular stresses [have been] implicated in ALS pathogenesis [with] therapeutic implications of regulating [autophagy] in ALS.” ATG16L1, a regulator of autophagy is consistently associated with inflammatory bowel disease (including, but not limited to, Crohn’s disease and ulcerative colitis). (Pugazhendhi S, Baskaran K, et al. (2017) PLoS One **12**:e0178291). Autophagy “is emerging as a core regulator of Central Nervous System (CNS) aging and neurodegeneration, [affecting diseases] including ischemia/stroke, Alzheimer’s, Parkinson’s, and Huntington’s, and multiple sclerosis, [...] involving microglial phagocytosis of apoptotic cells, amyloid-beta (A β), synaptic material, and myelin debris, and regulate the progression of age-associated neurodegenerative diseases.” (Plaza-Zabala A, Sierra-Torre V, et al. (2017) Int J Mol Sci **18**)

[0345] Activation of autophagy has been deemed desirable in Parkinson’s disease (Moors TE, Hoozemans JJ, et al. (2017) Mol Neurodegener **12**:11), Alzheimer’s disease, Huntington’s disease, and ALS (Moloudizargari M, Asghari MH, et al. (2017) Ageing Res Rev **40**:64-74), yet no effective drugs are available. “mTOR has been shown to be [a] key regulator but the detailed mechanisms are not satisfactorily solved.” (Chen Y, Yu L (2017) Traffic **18**:358-61) (FIG. 24)

[0346] The transcription factor EB (TFEB) regulates the expression of genes belonging to the Coordinated Lysosomal Expression and Regulation (CLEAR) network and of genes involved in autophagy. Overexpression of TFEB was found to decrease the accumulation of huntingtin aggregates in a rat cell model of Huntington’s disease (Settembre C, Di Malta C, et al. (2011) Science **332**:1429-33) and also to reduce neurodegeneration in *in vitro* and *in vivo* models of Parkinson’s disease by restoring lysosome levels and increasing autophagic clearance (Decressac M, Mattsson B, et al. (2013) Proc Natl Acad Sci U S A **110**:E1817-26;

Dehay B, Bove J, et al. (2010) J Neurosci **30**).”(Kilpatrick K, Zeng Y, et al. (2015) PLoS One **10**:e0120819)

[0347] Treatment of HeLa/TFEB cells with HP- β -CD up-regulated Microtubule-associated protein 1A/1B-light chain 3 (LC3), which is essential for the formation of autophagy vesicles, SQSTM1/p62 which is essential for cargo recognition, and *BECN1*, which is required for the formation of autophagosomes. In late infantile neuronal ceroid lipofuscinosis (LINCL) fibroblasts, which lack TPP1, HP- β -CD treatment resulted in clearance of ceroid lipopigment in a dose- and time-dependent fashion. HP- β -CD treatment resulted in transcriptional up-regulation of all autosomal (LC3, SQSTM1, BECN1) lysosomal genes tested (GBA, HEXA, LAMP1) through activation of TFEB. HP- β -CD treatment results in coordinated up-regulation of lysosome biogenesis and autophagy and enhanced autophagic clearance through TFEB-induced activation of the autophagy system, but blockage of downstream steps of the autophagy flux (e.g. blockage of ATG7 expression) prevents clearance of ceroid lipopigment. HP- β -CD activates the pro-survival autophagy pathway, but not apoptosis. Impairment or deregulation of autophagy is linked to the development and progression of a number of human diseases ranging from neurodegenerative diseases to cancer. The use of HP- β -CD was suggested for the treatment of diseases characterized by inefficient autophagic clearance and accumulation of storage material, although the mechanism of HP- β -CD's action remained unknown (FIG. 25) (Song W, Wang F, et al. (2014) J Biol Chem **289**:10211-22).

[0348] “HPbCD was shown to induce nuclear translocation of TFEB, upregulation of the CLEAR network, and increase in autophagic clearance (Song W, Wang F, et al. (2014) J Biol Chem **289**:10211-22). In particular, activation of TFEB in neuroglioma cells with HP- β -CD promoted clearance of α -syn aggregates (Parkinson's disease) via autophagy.(Kilpatrick K, Zeng Y, et al. (2015) PLoS One **10**:e0120819) M- β -CD also reduces α -syn aggregation [*in vitro* and *in vivo*] (Bar-On P, Rockenstein E, et al. (2006) J Neurochem **98**:1032-45);

[0349] However, the molecular mechanism underlying M- β -CD's and HP- β -CD's action on autophagy “are unclear.” (Kilpatrick K, Zeng Y, et al. (2015) PLoS One **10**:e0120819) or “still needs to be clarified”,(Sardiello M (2016) Ann N Y Acad Sci **1371**:3-14) respectively, although it is known that it is not mediated via cholesterol. “[W]e tested TFEB activation and accumulation of α -syn aggregates in cells treated with HPbCD-cholesterol inclusion complexes. ... Confocal microscopy ... revealed that [both] the extent of nuclear translocation ... of TFEB ... (Fig. 21A) [and] clearance of α -syn aggregates (Fig. 21B) [are]

independent of HPbCD [sic] ability to deplete ... cholesterol.” (Kilpatrick K, Zeng Y, et al. (2015) PLoS One **10**:e0120819)

[0350] The surprising genetic results of Example 1 provide the answer. It is already known that PI3K inhibitors, which interfere with the function of the PI cycle (FIG. 12) promote TFEB nuclear translocation.(Cheng J, Ohsaki Y, et al. (2006) Biochem Biophys Res Commun **351**:246-52) HP- β -CD reduces activity of the entire PI cycle by scavenging phospholipids, thus, promotes TFEB nuclear translation in a more consistent fashion than PI3K inhibitors (which act primarily via the PI3K/mTOR/TFEB pathway), but without causing imbalances within the PI cycle (FIG. 26).

[0351] This novel finding is supported by other evidence. Paeoniflorin is known to act by reducing serum lipids,(Yang HO, Ko WK, et al. (2004) Fitoterapia **75**:45-9) the common mechanism of action of cyclodextrins. Paeoniflorin mitigates the disease phenotype of mice with spinobulbar muscular atrophy (SBMA), where autophagy in the spinal cord is diminished. Paeoniflorin has been successfully tested in animal models of LPC induced inflammation (Li JZ, Wu JH, et al. (2013) Int J Mol Med **31**:493-7) and, like cyclodextrins of NAFLD,(Ma Z, Chu L, et al. (2017) Sci Rep **7**:44819) insulin resistance,(Ma Z, Liu H, et al. (2017) Biomed Pharmacother **90**:361-7) and atherosclerosis.(Li H, Jiao Y, et al. (2017) Inflammation **40**:2042-51)

Example 6: Elevated phagocytosis combined with lysosomal dysfunction is a known component in the etiology of multiple sclerosis (MS).

[0352] Early studies of MS in the 1970s reported “myelin-like material in lysosomes” and “synthesis of abnormal myelin by diseased glial cells” in spite of “increased lysosomal reaction”, including increased β -glycoronidase activity (Cuzner ML, Davison AN (1973) J Neurol Sci **19**:29-36) and “suggest[ed] early changes of glial cells as a basic mechanism of the disease.” It was hypothesized “that some acquired exogenous factor like virus infection is the basic cause which will trigger off the disease and the immunopathology.” (Riekkinen PJ, Rinne UK, et al. (1972) Zeitschrift fur Neurologie **203**:91-104)

[0353] In 1977, three lysosomal enzymes, N-acetyl- β -d-glucosaminidase (MGEA4, also known as *HEXC*), β -galactosidase (GLB1), and cathepsin D (CTSD) were found upregulated, the highest activity in plaque areas.” (McKeown SR, Allen IV (1977) Biochem Soc Trans

5:1416-8; (1979) *Neuropathol Appl Neurobiol* **5:405-15**) Enzyme levels in serum and CSF, in general, were not elevated in MS.(Hultberg B, Olsson JE (1978) *Acta Neurol Scand* **57:201-15;** (1979) *Acta Neurol Scand* **59:23-30**)

[0354] In 1980, it was suggested that “[macrophage]s were stimulated to increase lysosomal enzyme activity, initiated by stimulation of T-lymphocytes caused by MS-specific antigen”,(Rastogi SC, Clausen J (1980) *Clin Exp Immunol* **42:50-6**) yet no such antigens were identified, except that “a microglial component” is likely involved (Allen IV (1981) *Neuropathol Appl Neurobiol* **7:169-82**)

[0355] In 1983, a “2-step demyelination” hypothesis was proposed: (1) a toxin penetrates into myelin, and its degradation creates antigen. (2) Antigen-activated macrophages settle in and digest myelin.(Schwyzer RU, Henzi H (1983) *Med Hypotheses* **12:129-42**) yet the toxin remained elusive.(Tulpule K, Dringen R (2013) *Journal of Neurochemistry* **127:7-21**)

[0356] In 1996, still, “little is known about the source of [activated] macrophages in the early stages of plaque evolution. ... A key issue in understanding the pathogenesis of MS is the reliable identification of phagocytes capable of degrading myelin and presenting autoantigen to T cells at the onset of demyelination, [although] results indicate that microglia are the main population of phagocytes in the early staged of demyelination.” (Li H, Cuzner ML, et al. (1996) *Neuropathol Appl Neurobiol* **22:207-15**)

[0357] Aside from confirming HLA-DRB1*15:01 as a risk factor, a recent “metaanalysis” of 7125 cases (George MF, Briggs FB, et al. (2016) *Neurology Genetics* **2:e87**) did not provide any actionable insights.

[0358] Microglia have “similar transcriptome pattern” to macrophages; the function of microglia is to “scan the entire volume of the brain over the course of a few hours”. Hence, it has been proposed that dysregulation of microglial function contributes to CNS disorders, and that “targeting the mechanisms that are dysregulated may arrest or reverse neurodevelopmental and neurodegenerative disorders in which microglia play a role.”(Salter MW, Stevens B (2017) *Nat Med* **23:1018-27**) During the process of scanning the brain, microglia either targets the substrates for degradation by the lysosome or, in the case of pathogens, processes it for antigen presentation. “endocytosis, sorting, transport,

compartment acidification, and degradation ... may be altered during aging”. (Sole-Domenech S, Cruz DL, et al. (2016) Ageing Res Rev **32**:89-103)

[0359] The current invention links two recent findings in atherosclerosis and Fabry disease to the etiology of MS. Foamy macrophages, containing myelin degradation products, are abundantly found in active MS lesions.(Bogie JF, Jorissen W, et al. (2013) Acta Neuropathol Commun **1**:43) Fabry disease, a deficiency of lysosomal alpha-galactosidase A (*GLA*) is often mis-diagnosed as MS (Germain DP (2010) Orphanet journal of rare diseases **5**:30; Bottcher T, Rolfs A, et al. (2013) PLoS One **8**:e71894) and both diseases show familial comorbidity.(Cammarata G, Fatuzzo P, et al. (2015) Biomed Res Int **2015**:504784) From the better understanding of the mechanism causing foamy macrophages in atherosclerosis (intake of more LDL than lysosomes can handle, FIG. 17) and Fabry disease (lack of lysosomal galactosidase activity), the nature of the “toxin” becomes evident. The process starts, in fact, with the microglia. (Riekkinen PJ, Rinne UK, et al. (1972) Zeitschrift fur Neurologie **203**:91-104) When the microglial lysosomes cannot degrade all the myelin that is phagocytosed during scanning of the entire brain, the undegraded myelin is accidentally presented to the T-cells waiting for antigens from the microglia as “MS-specific antigen” (Rastogi SC, Clausen J (1980) Clin Exp Immunol **42**:50-6) and the T-cells then move to the circulation to activate macrophages against myelin. The activated macrophages return to the CNS where they begin to – mistakenly – endocytose myelin (FIG. 27).

[0360] As the process is the same as in Alzheimer’s disease, Parkinson’s disease, and CAD, the solution is the same: Slowing down phagocytosis of myelin by aging microglia prevents the lysosomes from “overflowing” and, thus, myelin from being presented to macrophages as “MS-specific antigen”. Slowing down endocytosis of myelin by macrophages further reduces demyelination in case that not all “MS-specific antigens” can be prevented.

[0361] In support of shared risk factors, MS and vascular diseases are comorbid. “[H]aving type-2-diabetes, hypertension, dyslipidemia or peripheral vascular disease at any point in the disease course may be associated with a greater progression in disability. ... The results of published clinical trials of statins as an intervention in MS were however conflicting.”(Tettey P, Simpson S, Jr., et al. (2014) J Neurol Sci **347**:23-33)

[0362] M- β -CD enhancing CD30 shedding has been suggested in Th1-mediated diseases, such as MS and RA.(von Tresckow B, Kallen KJ, et al. (2004) Journal of Immunology **172**:4324-31)

Example 7: Phospholipids as a drug target

[0363] The invention provides the phospholipids entering the PI cycle as the drug target (Example 1) and α -CDs as a safer version of a class of drugs (β -CDs) that have been demonstrated to be effective in models of Alzheimer's disease, Parkinson's disease, and CAD and in humans with other lysosomal function diseases (including, but not limited to, NPC).

[0364] The results presented here show that the number of combinations of genes involved in endocytosis across different patients is too large for the goal of "targeting endocytosis" to be likely achieved by targeting selected phosphatases or kinases (Table 5) or genes regulated by the PI cycle (Table 6). As discussed above, the PI cycle is designed to compensate for dysregulation of individual kinases or phosphatases. "Decreasing levels of PA" by siRNA directed toward DGKQ or PLD and use of several inhibitors (including Wortmannin) and activators aiming at "increasing intracellular levels of PIP1 and/or PIP2" were proposed based on the linear PI-PIP1-PIP2-PIP3 model and the effect of amyloid-beta on PA and phosphoinositides.(Kim TW, Di Paolo G, et al. (2012) US8288378) (US Patent 8,288,378)

[0365] Downregulating the entire PI cycle, however, could achieve the goal of reducing endocytosis. The breadth of genes involved in entry of PI and PC into the PI cycle suggests a different strategy as more effective. Reduction of serum phospholipids reduces the intracellular concentration of phosphoinositides, which are known to regulate endocytosis during ligand binding (PI(4,5)P2), pit-formation (PI(4)P), vesicle formation (PI(3,4)P2), fusion to early endosomes (PI(3)P), and sorting into cell organelles, including lysosomes (PI(3,5)P2). "Activation of signal transduction pathways associated with endocytic trafficking is critical for tumor cell migration [and disease progression in Parkinson's disease/Alzheimer's disease]. As a consequence, [...] targeting endocytic trafficking and signaling could potentially allow for the development of novel cancer therapeutics to prevent metastasis [and anti-aging therapeutics to prevent Parkinson's disease and Alzheimer's disease]." (Chew CL, Chen M, et al. (2016) Oncotarget **7**:5-6)

[0366] For instance, FAK (integrin-mediated focal adhesion kinase) is overexpressed and activated in tumors, but rarely mutated.(Alanko J, Ivaska J (2016) Trends Cell Biol **26**:391-8) FAK inhibitors have been shown to decrease tumor growth, metastasis, and angiogenesis in mice, and are in early clinical trials for non-hematologic cancers, including, but not limited to, pancreatic cancer, lung cancer, mesothelioma, and ovarian cancer, with mixed results (clinicaltrials.gov). Regulating endocytosis of integrins provides an alternative strategy for reducing the activity of FAK, either alone or in combination with immunotherapy.(Symeonides SN, Anderton SM, et al. (2017) Journal for immunotherapy of cancer **5**:17)

Example 8: α -CD is more efficient than β -CD in solubilizing phospholipids.

[0367] . In some embodiment of the present disclosure α -CDs are used for treating age-related conditions, such as cancers (including, but not limited, to breast and prostate cancer) and neurodegenerative diseases (including, but not limited to, Parkinson's disease or Alzheimer's disease). Cyclodextrins lower the amount of phosphoinositides available without directly interfering with their distribution.

[0368] Scavenging phospholipids, which regulate endocytosis more specifically (by α -CD, six sugar molecules), rather than also scavenging larger cholesterol molecules (by the larger β -CD, seven sugar molecules) avoids cholesterol-mediated side effects, including ototoxicity.(Cronin S, Lin A, et al. (2015) J Assoc Res Otolaryngol **16**:599-611). “The acryl chain of phospholipids fits tightly into the hydrophobic cavity of the smallest α -CD and more loosely into the larger inner space of β - and γ -CDs, whereas the side chain of cholesterol is preferably included in the β -CD cavity.”(Irie T, Uekama K (1997) J Pharm Sci **86**:147-62) “Cyclodextrins partially removed phospholipids ... with a potency of $\alpha > \beta \gg \gamma$. Cholesterol ... was extracted ... most effectively by [β -CD] ... , while [the effect] of [α -CD] was negligible even at hemolytic concentrations.” (Ohtani Y, Irie T, et al. (1989) European Journal of Biochemistry **186**:17-22) “ β -CD also remove proteins from erythrocyte membranes”(Irie T, Uekama K (1997) J Pharm Sci **86**:147-62) (FIG. 3).

[0369] At 10 mM, α - and β -cyclodextrin reduce transferrin endocytosis by 20% and 80%, respectively. The reduction by 20% has been interpreted as α -cyclodextrin not having “any significant effect” (Rodal SK, Skretting G, et al. (1999) Molecular Biology of the Cell **10**:961-74). The results presented here, however, show that these results are consistent with a

more physiologic 30% reduction of phospholipids, rather than an extreme 95% reduction of cholesterol (FIG. 3) (Ohtani Y, Irie T, et al. (1989) *European Journal of Biochemistry* **186**:17-22). Moreover, even increasing the concentration of α -cyclodextrin above 10 mM does not increase release of phospholipids, which might disrupt vital functions, while increasing the concentration of β -cyclodextrin results in a massive increase of protein release (FIG. 3). Hence, at the same level where β -cyclodextrin interferes with vital cell function by limiting available cholesterol, α -cyclodextrin merely reduces endocytosis to normal ranges by reducing regulatory phospholipids. (Ohtani Y, Irie T, et al. (1989) *European Journal of Biochemistry* **186**:17-22)

[0370] “PIPs are [also] involved in ... common [neurodegenerative diseases] such as [Alzheimer’s disease] that are becoming more widespread as life expectancy increases”.(Waugh MG (2015) *Biochim Biophys Acta* **1851**:1066-82)] Membrane anchored inhibitors of β -secretase have been postulated as a strategy to prevent endocytosis of **APP**.(Rajendran L, Knolker H-J, et al. (2010) *Nat Rev Drug Discov* **9**:29-42) The results presented herein provide reduction of overall endocytosis by attenuation of PI levels via α -CD as an alternative to inhibiting β -secretase (FIG. 4), which has higher affinity for neuregulin than for **APP**.(Ben Halima S, Mishra S, et al. (2016) *Cell Rep* **14**:2127-41)

[0371] Since cyclodextrins have been successfully applied both intravenously and intrathecally, different routes of administrations can be used to prevent bone and lung metastases v. glioblastoma and neurodegenerative diseases.

[0372] Materials: The degree of substitution for α -, β -, and γ -HP-CDs was 5.3, 5.1, and 5.4, respectively. In preparations of samples, 5% (w/w) NaOH was used as a solvent and catalyst in the condensation reaction of epoxide with cyclodextrin. Radioactive lipids were purchased from NEN Research Products, Boston, MA, and nonradioactive lipids were purchased from Sigma Chemical Company, St. Louis, MO. (Irie T, Fukunaga K, et al. (1992) *J Pharm Sci* **81**:524-8)

[0373] Methods: Measurement of lipids was accomplished with clinical diagnostic kits (Sigma and Wako Chemicals, Dallas, TX). Solubilities of lipids were measured after brief sonication of the suspension of an excess of radioactive sample (in phosphate-buffered isotonic saline, pH 7.4; in a closed vessel under argon atmosphere) and equilibration by shaking (for cholesterol, the period of shaking was 1 week; for cholesterol oleate, L- α -

dipalmitoylphosphatidylcholine, and triolein, the period of shaking was 3 days). Thereafter, the suspension was filtered through a membrane filter (Millex; SLGS, 0.25; OS, 0.22 μm), and the radioactivity in the filtrate was measured by liquid scintillation counting. The same process was used for sphingomyelin, except that 1 day of shaking and a clinical kit for measurement of phospholipids were used. These methods are prone to yield somewhat higher values for the solubilities of surface-active compounds in buffer, because the filtrate is enriched by the mono-layers of the surface-active compound that form at the air-buffer interface. However, these methods are suited for measurement of solubilities of lipids in HP-CD solutions. This fact was established by comparing results obtained with the filtration methods described above and those from a method that used titration of a lipid sample with a solution of HP-CD, with the end point of dissolution being determined visually. The same titration method was used to establish that cholesterol and cholesteryl methyl ether dissolve in the solutions of HP- β -CD to about the same extent. (Irie T, Fukunaga K, et al. (1992) J Pharm Sci **81**:524-8)

[0374] Results: The differential effects of α -, β -, and γ -HP-cyclodextrins on five representative pure lipids are shown in FIG. 32. HP- β -CD preferentially solubilized cholesterol, whereas phospholipids were best solubilized with HP- α -CD.

Example 9: α -CD is at least as effective as HP- β -CD in preventing lysosomal dysfunction in cell-lines of various lysosomal storage diseases.

[0375] The results of Example 9 are consistent with findings in US 2015/0216895 A1, Fig 5, (McKew J, Zheng WEI, et al. (2014)) which found that “[α CD, β CD, and γ CD] can reduce cholesterol accumulation, and MBCD [M β CD] was most potent.” In particular, the authors noted that all three CDs “can reduce cholesterol accumulation in NPC cells [and] that these CDs increased intracellular Ca^{2+} and enhanced exocytosis.”

[0376] What has not been appreciated is that efficacy of α -CD against cholesterol storage disease implies that the effect cannot be due to scavenging of cholesterol, because α -CD cannot fit cholesterol. Our novel genetics results show that the effect is instead mediated by phospholipids and, thus, explain for the first time why about 6 mM α -CD actually was more effective in reducing lysosomal storage than about 8 mM HP- β -CD (FIG. 23).

[0377] The results also provide an explanation why intravenous HP- β -CD is effective in NPC, even though neither cyclodextrins nor cholesterol can cross the blood brain barrier (BBB). The surprising findings also may end the current “battle” over intravenous v. intrathecal use of HP- β -CD. (Wadman M (2016) Science **354**:18-9) As phospholipids cross the BBB more easily, an oral (absorbed from the intestine) version of HP- α -CD may prevent the need for either implanting an intrathecal pump or administering HP- β -CD as an intravenous infusion over several hours to prevent cytoplasmic crystals from forming in the tubules of the kidney. (Frank DW, Gray JE, et al. (1976) Am J Pathol **83**:367-82)

Example 10: α -CD is less nephrotoxic than β -CD in delayed release applications.

[0378] Despite the differences in phospholipid profiles *in vitro* (Example 8), *in vivo* results are commonly interpreted as showing that α -CD is at least as nephrotoxic as β -CD:

1. “Cyclodextrins were found rather toxic and the high doses applied parenterally are definitely kidney damaging (Frank DW, Gray JE, et al. (1976) Am J Pathol **83**:367-82).” (Pitha J (1981) Life Sci **29**:307-11)
2. “The work of Frank et al. [(Frank DW, Gray JE, et al. (1976) Am J Pathol **83**:367-82)] and others [(Perrin JH, Field FP, et al. (1978) Research communications in chemical pathology and pharmacology **19**:373-6)] showing nephrotoxicity of the unmodified cyclodextrins limited further studies on the parent cyclodextrins to ... nonparenteral routes.” (Rajewski RA, Stella VJ (1996) J Pharm Sci **85**:1142-69)
3. “Early studies showing the nephrotoxicity of the parent cyclodextrins [(Frank DW, Gray JE, et al. (1976) Am J Pathol **83**:367-82)] limited administration routes ... to ... nonparenteral routes”, see p. 147 (Irie T, Uekama K (1997) J Pharm Sci **86**:147-62)
4. “[T]oxicological studies have also shown that the parent α - and β -[cyclodextrin] ... are not suitable for parenteral administration, ... [(Irie T, Uekama K (1997) J Pharm Sci **86**:147-62)]” (Del Valle EMM (2004) Process Biochemistry **39**:1033-46)
5. “The renal toxicity of α -CD and β -CD after parenteral administration [(Frank DW, Gray JE, et al. (1976) Am J Pathol **83**:367-82)] ... have been well documented [(Irie T, Uekama K (1997) J Pharm Sci **86**:147-62); Thompson 1997 (review only); Gould and Scott 2005 (β -CD only)].” See p. 31 of (Stella VJ, He Q (2008) Toxicol Pathol **36**:30-42)

6. “Two of the natural cyclodextrins [α - and β -cyclodextrin] are known to be parenterally unsafe owing to nephrotoxic effects [(Frank DW, Gray JE, et al. (1976) Am J Pathol **83**:367-82)]”(Bilensoy E, Hincal AA (2009) Expert Opin Drug Deliv **6**:1161-73)
7. “One of the hallmarks of the toxicity of CDs after parenteral administration is nephrotoxicity ... [(Frank DW, Gray JE, et al. (1976) Am J Pathol **83**:367-82)]”(Kantner I, Erben RG (2012) Toxicol Pathol **40**:742-50)
8. “When administered parenterally, [natural cyclodextrins] caused nephrotoxicity ... [(Frank DW, Gray JE, et al. (1976) Am J Pathol **83**:367-82)]”(Liu B (2012) Clinical lipidology **7**:289-301)
9. “Following administration of poorly soluble parent α - and β -CD[,] prominent microcrystals are observed in lysosomes. [(Frank DW, Gray JE, et al. (1976) Am J Pathol **83**:367-82)]”(De Schaepdrijver L, Marien D, et al. (2015) Reproductive toxicology (Elmsford, NY) **56**:87-96)

[0379] These findings are summarized in a 2017 European Medicines Agency report (Committee for Human Medicinal Products (CHMP) (2017) European Medicines Agency EMA/CHMP/333892/2013):

“Both α -CD and β -CD showed renal toxicity after parenteral administration Above 200 mg/kg/day cyclodextrin may theoretically ... cause mild renal toxicity when given parenteral[ly].”

[0380] The surprising result of a literature review regarding nephrotoxicity of cyclodextrins is that, as outlined in FIG. 33, the above statements, including the 2017 summary about the clinical safety of about the toxicity of α -CD v. β -CD, are all based on the data of the same 1976 publication (Frank DW, Gray JE, et al. (1976) Am J Pathol **83**:367-82) where the Abstract noted that “renal toxicity of ... α and β -[cyclodextrin] is manifested” and the Discussion stated: “Repeated administration of large doses of the [cyclodextrin]s resulted in numerous giant [proximal tubule] lysosomes distorted by enclosed microcrystals [in a dose-dependent manner] ... [cyclodextrin] nephrosis apparently represents irreversible injury. ... [cyclodextrin] ... concentration within the lysosomes results in the toxic injury to the cell.” Of note, neither the Abstract nor the Discussion mentioned the differences between cyclodextrins reported in the results.

[0381] Drugs can be administered parenterally to reach systemic concentration as a bolus (within seconds or minutes), e.g., intravenously (single injection), or spread out over hours, e.g., intravenously (repeated injections or infusion), subcutaneously, or intraperitoneally. The former type of parenteral application results in high peak levels, while the latter applications achieve lower and longer lasting levels. The effect of increasing the frequency of administration has been noted: “One way to avoid ... overloading [of the kidney] would be to lower the spikes of cyclodextrin concentration in blood [as r]epeated doses of cyclodextrins applied parenterally were less toxic than a single cumulative dose (Frank DW, Gray JE, et al. (1976) *Am J Pathol* **83**:367-82). ... [A] peristaltic pump was used to inject slowly the solution and thus a “spike” was spread to 6-8 hours. Using this technique ... [d]oses of 1 g/kg not only did not result in animal deaths, but did not even influence the growth of the young animals compared to controls.” (Pitha J, Szente L (1981) *Proceedings of the First International Symposium on Cyclodextrins* 457-66). In the treatment of Niemann-Pick disease (Hastings C (2010) FDA-Filing **2010**)

[0382] Consistent with the latter results, “there are no cases of kidney injury caused by cyclodextrins in humans,”(Committee for Human Medicinal Products (CHMP) (2017) European Medicines Agency EMA/CHMP/333892/2013) see also Table 3.

[0383] The surprising results of this re-evaluation of published data (Frank DW, Gray JE, et al. (1976) *Am J Pathol* **83**:367-82) is that while α -CD and β -CD had a similar LD₅₀ of 1 g/kg only with bolus i.v. injections, α -CD was less nephrotoxic than β -CD with daily s.c. injections. Only one rat died (after 2 days) among the groups given up to 7 daily doses of 1 g/kg α -CD, while all rats died within 4 days among the groups given a lower dose of 0.900 g/kg/d β -CD (see Table 7, p = .0285).”

Table 7: Toxicity of cyclodextrins in rats (compiled from Frank DW, Gray JE, et al. (1976) Am J Pathol 83:367-82)

	α -CD [g/kg]	β -CD [g/kg]
i.v. single dose (LD ₅₀)	0.788/1.008 *	0.788/1.008 *
s.c. Single dose		
Nephrosis (light microscopy),	0.100 no changes	---
	---	0.225 no changes
	---	0.450 no changes
	---	duration-dependent alterations in EM
	---	0.670 changes seen
	1.000 changes seen	1.000 changes seen
s.c. 1, 2, 3, 4, or 7 daily doses		
Nephrosis/Death	0.100 no nephrosis	---
	---	0.225 one nephrosis after 4 days
	---	0.450 severe nephrosis
	1.000 all had severe nephrosis, <u>one died</u> (after 2 days)	0.900 <u>all died</u> within 4 days

Legend:

“---“ not reported,

“*” the paper is ambiguous about which drug caused either LD₅₀,

[0384] Adding HP groups is known to reduce toxicity of native CDs (Table 8). In humans, HP- β -CD is approved (as an excipient for itraconazole) for bolus i.v. injection up to 16 g (100 mg/kg). No side effects were observed after parenteral administration of 12 g twice daily for 15 days.. The pharmacokinetics of SBE β -CD (Vfend, Pfizer, 14 g i.v.) is very similar to that of HP- β -CD (Stella VJ, He Q (2008) Toxicol Pathol 36:30-42)

Table 8: Pharmacologic and toxicologic characteristics of selected cyclodextrins

	α -CD	HP- α -CD	β -CD	HP- β -CD
Solubility in water [g/l]	145 ¹⁾	>500 ²⁾	18.5	>600 ²⁾
oral absorption in rats ¹⁾	2-3%		1-2%	≤3%
half-life (t _{1/2}) after i.v. in rats [min] ¹⁾	25		20	20
excreted after i.v. unchanged in urine	~90%		~90%	~90%
hemolytic IC ₅₀ [mM] ⁸⁾	16	>100		
whole blood TD ₅₀ [mM/L] ⁹⁾	11	360	3.6	9
i.v. toxicity, 1 g/kg daily ⁴⁾	1 died @ 2 d		all died ≤ 4 d	no signs ⁶⁾
i.v. toxicity, 500 mg/kg			kidney damage	no damage
LD ₅₀ i.v./i.p. in mice/rats [g/kg] ⁷⁾	0.5-1.0		0.8/1.0 ⁴⁾	10
BBB breakdown [mM] ⁵⁾	1	2.5	2.5	2.5
Transport across BBB [%; 2h] ⁵⁾	21.5 (0.5 mM)	16.5 (1 mM)	26.7 (1 mM)	6.6 (1 mM)

From ¹⁾(Brewster ME, Loftsson T (2007) *Advanced Drug Delivery Reviews* **59**:645-66) ²⁾(Loftsson T, Brewster ME (2010) *J Pharm Pharmacol* **62**:1607-21) ³⁾(Loftsson T, Moya-Ortega MD, et al. (2016) *J Pharm Pharmacol* **68**:544-55) ⁴⁾(Frank DW, Gray JE, et al. (1976) *Am J Pathol* **83**:367-82) ⁵⁾(Monnaert V, Tilloy S, et al. (2004) *Journal of Pharmacology and Experimental Therapeutics* **310**:745-51) ⁶⁾(Gould S, Scott RC (2005) *Food Chem Toxicol* **43**:1451-9) ⁷⁾(Riebeck WM (1990a) TNO-CIV Institute **V90**; (1990b) TNO-CIV Institute **V90**; Prinsen MK (1991) TNO-CIV Institute **V91**) ⁸⁾(Roka E, Ujhelyi Z, et al. (2015) *Molecules* **20**:20269-85) ⁹⁾(Leroy-Lechat F, Wouessidjewe D, et al. (1994) *International Journal of Pharmaceutics* **101**:97-103) ¹⁰⁾(Frijlink HW, Eissens AC, et al. (1991) *Pharm Res* **8**:9-16)

[0385] In four studies of outbred rodents (Riebeck 1990a/b/c, Prinsen 1991a, as quoted in WHO <http://www.inchem.org/documents/jecfa/jecmono/v48je10.htm>) macroscopic examination of dead and surviving animals either “did not reveal treatment-related alterations” (Riebeck 1990a/b/c) or merely “a pale renal cortex” (Prinsen 1991a).

Example 11: α -CDs are more efficient in crossing the blood-brain barrier (BBB) than β -CDs.

[0386] As phospholipids can easily cross the BBB, α -CDs would not need to cross the BBB to be effective, but it would increase their effectiveness. Published data show that α -CDs are consistently twice as efficient as β -CDs in crossing the BBB *in vitro*. After 2 h of incubation, 16.5–43.0% (vs 6.6–26.7%) were transported through the BBB (compared to 96% for caffeine or nicotine). See *Journal of Pharmacology and Experimental Therapeutics*, 310 (2), 745-51. These results were confirmed in an independent study of β CDs.(Binkowski-Machut C, Hapiot F, et al. (2006) *Bioorganic & Medicinal Chemistry Letters* **16**:1784-7)

[0387] While α -CD was more toxic, HP- α -CD, β -CD, β -CD, and HP- β -CD were similarly safe (Table 8: Pharmacologic and toxicologic characteristics of selected cyclodextrins). (Monnaert V, Tilloy S, et al. (2004) *Journal of Pharmacology and Experimental Therapeutics* **310**:745-51)

Example 12: α -CD is safer than β -CD and more effective in preventing migration of human tumor cells *in vitro*.

Introduction

[0388] Published results have shown that β -CD inhibits human MDA-MB 231 cell migration (Guerra FS, Sampaio LdS, et al. (2016) Translational Medicine Communications 1:3). This inhibition was attributed to the ability of β -CD to “deplete cholesterol”. β -CD, however, depletes also phospholipids. To determine whether inhibition of migration is caused by β -CD depleting cholesterol, as commonly assumed, or by β -CD depleting phospholipids, as implicated by the results of the present disclosure, the wound healing experiment was replicated, comparing both HP- β -CD (Sigma, 389145-5G) and HP- α -CD (Sigma, 390690-5G) v. control in both MDA-MB 231 (CRM-HTB-26, ER-) and MCF-7 (ATCC HTB-2, ER+) human breast epithelial cell lines.

Method

[0389] Cells were cultured in 24-well culture plates (Cytoselect CBA-120, 0.9 mm wound healing/gap closure migration assay) for 24 h with wound healing insert in place. Cells were then treated for 2 h with HP- β -CD, HP- α -CD, or control.

[0390] Protocol: Warm up the 24-well plate (CBA-120, Cell BioLabs Inc.) with 0.9 mm CytoSelect Wound Healing Inserts at room temperature for 10 min. Using sterile forceps, orient the desired number of inserts in the plate wells with their “wound field” aligned in the same direction. Create a cell suspension containing $0.5\text{--}1.0 \times 10^6$ cells/ml in media containing 10% fetal bovine serum (FBS). Add 500 μL of cell suspension to each well by carefully inserting the pipette tip through the open end at the top of the insert. For optimal cell dispersion, add 250 μL of cell suspension to either side of the open ends at the top of the insert. Incubate cells in a cell culture incubator for 12-24 hours. Carefully remove the insert from the well to begin the wound healing assay. Use sterile forceps to grab and lift the insert slowly from the plate well. Slowly aspirate and discard the media from the wells. Wash wells with media to remove dead cells and debris. Finally, add media to wells to keep cells hydrated. Repeat wash if wells still have debris or unattached cells. When washing is complete, add media with FBS and/or compounds to continue cell culture and wound healing process. Agents that inhibit or stimulate cell migration can be added directly to the wells. Incubate cells in a cell culture incubator (2 hours) and then wash cells with PBS and then add

fresh media without compounds. Incubate 12-24 hours before wash and fixing. Remove the fixation solution and add 400 μ L of Cell Stain Solution to each well. Allow the stain to incubate with the cells for 15 minutes at room temperature. Aspirate and discard the solution. Carefully wash each stained well 3X with deionized water. Discard washes and allow cells to dry at room temperature. Cells that migrated into the wounded area or protruded from the border of the wound were visualized and photographed under an inverted microscope. Determine the surface area of the defined wound area: Total Surface Area = 0.9mm x length. Determine the surface area of the migrated cells into the wound area: Migrated Cell Surface Area = length of cell migration (mm) x 2 x length. Percent Closure (%) = Migrated Cell Surface Area / Total Surface Area x 100 (see FIG. 36)

[0391] Cell-based scratch assay. Cells were cultured in 24-well culture plates for 24 h up to 90%—100% confluence. Scratched wound lines on the upside of cultured cells were created by 200 μ l yellow micropipette tip. The scratched cells were washed with PBS after removal of culture media. The cells were cultured for 2 h with 2 mM M β CD and after the removal of culture media cells were cultured for the next 2, 8, 12 or 24 h. All cell-based scratch assays were performed in the presence of the anti-mitotic reagent cytosine arabinoside (Sigma) at a final concentration of 10^5 M in order to inhibit cell proliferation and analyze only cell migration. The wound area was measured from the image taken with an Axiovert 100 microscope (Carl Zeiss, Germany) by Image J program (NIH, USA) at 3 different sites from each wound area of gaps. Three independent experiments were performed.

[0392] Other methods used are described in (Guerra FS, Sampaio LdS, et al. (2016) Translational Medicine Communications **1**:3; Okada SS, Kuo A, et al. (1995) J Pharmacol Exp Ther **273**:948-54), each of which are incorporated herein by reference in their entireties.

Results

[0393] Results are shown in FIG. 35. In the absence of cyclodextrins, >80% of the wound area is closed. With cyclodextrins, wound closure is inhibited in a phospholipid-dependent manner. Extraction of cholesterol does not seem to inhibit wound closure, but has been shown to cause side-effects (ototoxicity). The results are highly consistent: In all cases, migration decreased with higher concentration of either HP- α -CD or HP- β -CD and HP- α -CD had a stronger effect on migration than HP- β -CD (Inhibition at 1 mM HP- α -CD was superior to inhibition at 2 mM HP- β -CD.)

Discussion

[0394] The results in human two human tumor cancer cell lines confirm the hypothesis that the primary effect of β -CDs on cell migration is not by scavenging cholesterol, but by scavenging phospholipids. The present disclosure has demonstrated that “derailed”, “deranged”, and “defective” endocytosis in cancers, neurodegenerative diseases, and other age-related conditions is caused by genetic risk factors causing excessive influx of glycopospholipids, including PC, into the PI cycle (FIG. 12). Previous studies with β -CDs did not proceed to clinical trials because of risk of cholesterol-related ototoxicity; they can now be resumed after the elimination of cholesterol is avoided, while the phospholipid-related efficacy seen in β -CD is increased.

[0395] In addition to not carrying HP- β -CD’s risk of ototoxicity (FIG. 34), HP- α -CD has lower toxicity than HP- β -CD (Example 10) but is about twice as effective in scavenging phospholipids, in general, and even more effective in scavenging PC (Monnaert V, Tilloy S, et al. (2004) Journal of Pharmacology and Experimental Therapeutics **310**:745-51) In children with NPC (Table 3), doses of up to 1000 mg/kg/d HP- β -CD were well tolerated. With 700 mg/kg/d HP- β -CD having been proven as safe in parenteral treatment of NPC, doses up to 700 mg/kg/d HP- β -CD could now be used to continue with the human experiments that were stopped when β -CDs were shown to have cholesterol-related ototoxicity.

Example 13. HP- α -CD is more effective than HP- β -CD in reducing tumor growth, metastases, and inflammation in NSG mice infected with human MDA-MB-231 breast cancer cells.

Introduction: HP- β -CD has been shown to be effective in several animal models of breast cancer (see Example 4), but further result on HP- β -CD was halted when it was seen to cause permanent hearing loss by depriving outer hair cells in the ears of the cholesterol they need. The genetics results in Example 1 led to the novel hypothesis that HP- β -CD is acting via scavenging phospholipids, rather than cholesterol and, thus, that HP- α -CD, which also scavenges phospholipids, but is too small to fit cholesterol, is a safer alternative and the *in vitro* results in Example 12 have shown HP- α -CD to be more effective *in vitro* than HP- β -CD.

Methods: NSG mice (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ, strain #005557 (10 wk old) were obtained from Jackson Laboratories (Bar Harbor, ME), housed in isolated quarters, and fed with autoclaved water and food.

[0396] An MDA-MB-231 cell line was obtained (ATCC) and kept frozen in liquid nitrogen. Upon start of culture, cells were thawed (1.5 min in 37 °C water) and transferred to DMEM/F-12 supplemented with 5% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate, and 0.02 mM non-essential amino acids. Cells were grown to several millions in 60 mm dishes at 70–80% confluence. Before injection, cells were briefly rinsed twice in PBS followed by Versene (EDTA, Invitrogen) and placed on ice with excess phosphate-buffered saline and phenol red-free matrigel basement membrane extract to a concentration of about 15 mg/mL matrigel. 100 μ L cell mixture (20,000 cells or saline only) were injected into the lactiferous duct of the 3rd or 4th mammary glands under brief isofluorane anesthesia. Groups of 15 mice each were injected IP with 800 mg/kg of HP- α -CD, HP- β -CD per week (over four days, Mon, Tue, Thu, Fri), or saline (control) for six weeks. Size of the primary tumor was measured weekly.

[0397] Breast tissue were palpated and measured twice a week with a micro-metric analytical device scaled to 1 mm precision. Mice were weighed weekly on a digital scale (0.1 g precision).

[0398] At the end of the study period, mice were anaesthetized with isofluorane with 1 $\times$ phosphate-buffered saline (PBS) and subsequently with 4% formaldehyde in PBS. Tissue from lung and liver were removed and fixated in formalin for microscopic examination. Metastases were visualized and counted by tissue embedding in paraffin and sectioning with immunohistochemistry and histological stain (H&E) and immunostained with anti-HLA antibody. After blocking for 1 h with BSA (5%, frac V), the anti-HLA was incubated at 4 °C overnight, washed for 1 h in PBS, then incubated with biotinylated anti-mouse IgG for 1 h at room temperature. After washing with PBS, metastases were detected on the slide using Envision (Dako).

[0399] After blood collection, whole blood samples were centrifuged (at 10,000 m/s² for 15 min and then at 100,000 m/s² for 10 min), and plasma was isolated. The samples were stored at –80 °C until analysis.

[0400] Prior to analysis (30 min), samples and assay reagents were equilibrated to room temperature. The samples were run at a 1:4 dilution and quantified in duplicate using a multiplex mouse panel for quantification of IL-1 β , IL-18, IL-6, IL-8, IL-17A, and GM-CSF (Bio-Plex Pro assays, Bio-Rad Laboratories) on a Luminex Bio-Plex 200 System (Bio-Rad Laboratories).

Results: The mice grew at a typical rate (FIG. 38A) and appeared healthy, except for the two mice in the Vehicle and HP- β -CD groups each and one in the HP- α -CD group, who died ('†' at the bottom, '+' at the end of the growth curve). More growth in the Vehicle group could be due to either more tumor growth (FIG. 38B) or to the known effect of cyclodextrins to reduce the amount of fat being absorbed from the intestine and, thus, less growth. Tumors grew faster in the Vehicle group (to a median of 525 mm³) than in the cyclodextrin groups (FIG. 38B). The effect was larger for HP- α -CD (356 mm³) than for HP- β -CD (378 mm³, $p = 0.0258$).

[0401] All mice developed lung and/or liver metastases (FIG. 39), but one mouse in the Vehicle group, 3 mice in the HP- β -CD group, and 5 mice in the HP- α -CD group did not develop lung metastases. By u-statistics for multivariate data (Wittkowski KM, Lee E, et al. (2004) Stat Med **23**:1579-92), both cyclodextrins are effective in reducing metastases overall ($P < 0.001$), and the data are consistent with HP- α -CD being more effective ($P = 0.098$).

All plasma cytokines showed reduced levels under both cyclodextrins, with the exception of IL17-A under HP- β -CD (FIG. 40). At the conventional .05 level, HP- β -CD was significant in four other markers (not in IL-8), while HP- α -CD was not significant only in IL-6. A comprehensive analysis of all six markers via u-statistics for multivariate data (Wittkowski KM, Lee E, et al. (2004) Stat Med **23**:1579-92) shows HP- α -CD be more effective than HP- β -CD ($p(a:b) = 0.0417$).

Conclusion: The *in vivo* results confirm the *in vitro* results (Example 12) that HP- α -CD is more effective than HP- β -CD in preventing breast cancer tumor growth (FIG. 38, $P = 0.0258$) and metastases (FIG. 39, $P = 0.098$). Inflammation is also known as a component in the etiology of cancer. The surprising results in FIG. 40 ($P < 0.02$ for each of GM-CSF, IL-18, IL17-A, IL-4, and IL1-b) confirm the hypothesis that α -CDs also reduce inflammation by reducing the activity of sPLA2 to produce arachidonic acid, the precursor of inflammatory

COX-1, COX-2, and LOX, and that HP- α -CD, by not spending part of its lipid-carrying capacity on cholesterol, is also more effective than HP- β -CD.

Example 14. HP- α -CD reduces disease phenotypes in mouse models of ALS and HD.

[0402] Introduction: Many neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease affect higher cognitive functions and involve largely unknown, complex genetic risk factors. Hence there are few animal models that are known to represent the more relevant aspects of the disease etiology. Aggregation of amyloid-beta, tau, and alpha-synuclein are associated with Alzheimer's disease and Parkinson's disease, yet it is not known whether the aggregates are causal or merely an effect. In Alzheimer's disease, for instance, most drugs aiming at reducing the aggregation of amyloid-beta have failed. Current treatments confer symptomatic relief, but there are no treatments available to halt or slow disease progression.

[0403] The genetics result presented in Example 2 have let to novel insights on the common genetic risk factors underlying “derailed”, “deranged”, and “defective” endocytosis in metastatic and neurodegenerative diseases, which now explain the underlying mechanism for the previously observed impaired autophagy in Alzheimer's disease, Parkinson's disease, ALS, and Huntington's disease. (Guo F, Liu X, et al. (2018) Brain Pathol **28**:3-13)

[0404] In contrast to Alzheimer's disease and Parkinson's disease, where the most prevalent disease forms are “idiopathic” (of unknown genetic cause), and, thus, relevant animal models are difficult to identify, the genetic risk factors for ALS, and Huntington's disease are well understood.

[0405] Huntington's disease is caused by an expansion of exon 1 of the *HTT* gene to >40 CGA repeats (mutant *HTT*, *mHTT*). Transgenic mice with approximately 120 CAG repeat develop neuronal inclusions containing the both mHTT and ubiquitin proteins. They display a progressive neurological phenotype that, typical for a mono-genic disease closely mimics human Huntington's disease, including movement disorders and loss of body weight.

[0406] As SOD1 mutations account for 10-20% of familial ALS and 1–2% of sporadic ALS, SOD1 aggregates are routinely found in these patients, and SOD1-ALS patients have low levels of the autophagy transcription factor TFEB, mice with *sod1* mutations are a highly plausible mouse model for human ALS. SOD1 is also associated with Parkinson's disease,

Alzheimer's disease, and Down syndrome. FUS (fused in sarcoma), a major protein aggregate in affected neurons in Huntington's disease, is also associated with ALS.

[0407] Given the comorbidities, autophagy-related pathology, and genetic overlap between neurodegenerative diseases, mouse models for Huntington's disease and ALS to evaluate the activity of HP- α -CD against neurodegenerative (and other autophagy-related) diseases.

[0408] Methods: ALS mice SOD1 G93A strain (B6SJL-Tg(SOD1*G93A)1Gur/J strain #002726, 10 wk old, and HTT mice (B6CBA-Tg(HDexon1)62Gpb/3J; strain #006494 HTT: R6/2, 8 wk old), were obtained from Jackson Laboratories (Bar Harbor, ME), housed in standard housing with controlled ambient conditions and provided with water and food at libitum.

[0409] Results: Among 8 wk old HTT mice, treatment with HP- α -CD reduced decline in bodyweight over 5 wk (starting from day 7 at 9 wk of age, $P(T*D) < .0001$). The effect was more striking in male ($P(T*DIM) < .0001$) than in female mice ($P(T*DIM) = .0394$).

[0410] Untreated SOD1 mice began to lose bodyweight after 21 days of observation (at age 11 wk), while mice treated with HP- α -CD continued to grow for at least two more weeks; similar effects were seen in both sexes (FIG. 42, $p < .0001$).

[0411] Conclusion: Consistent with the known comorbidity of metastatic, neurodegenerative, and other age-related diseases and conditions, these results provide evidence that HP- α -CD is effective not only in carcinomas (Example 13), where it was shown to be more effective than HP- β -CD, but also in neurodegenerative diseases, including, but not limited to, Huntington's disease and ALS and, by extension, Parkinson's disease and Alzheimer's disease, and in other diseases, where HP- β -CD has been shown to be effective in animal models.

Example 15: A clathrate of HP α CD and sodium caprate (C-10) as an oral drug extracts phospholipids into urine and does so more efficiently than a mixture of the components.

[0412] Oral β -CD is rarely absorbed in mammals (~1%); of the smaller α -CD, 1.6% (Van Ommen B, De Bie AT, et al. (2004) Regul Toxicol Pharmacol **39 Suppl 1**:57-66) and 2.0% (Irie T, Uekama K (1997) J Pharm Sci **86**:147-62) were found in urine within 6–8 h post oral administration. In rats, instead of 1% for β -CD alone administered orally, up to 5% of β -CD were absorbed rectally when administered rectally in suppositories containing triglycerides

(Witepsol H5). Rectal absorption increased from 5% to 26% when β -CD was replaced by the more water soluble HP- β -CD. (Duchene D, Wouessidjewe D (1990) Drug Dev Ind Pharm **16**:2487-99). Absorption of oral HP- α -CD has not been studied.

[0413] Background: Published results in LDL-receptor knockout mice who were fed a “Western diet (21% milk fat)” showed that adding “2.1% of “nonabsorbable” α -CD (10% of dietary fat content)” decreased plasma phospholipids by 17.5%, (Wagner EM, Jen KL, et al. (2008) Metabolism **57**:1046-51) yet no mechanism of action was identified. From the above genetic and *in vitro* results, the reduction in plasma phospholipids seen was not (or not only) due to “saturated fat absorption” in the intestine and “excretion into the feces”, as speculated by the authors, but (also) through scavenging of phospholipids by α -CD absorbed from the intestine in the presence of milk fatty acids. A role of milk fatty acids is consistent with higher absorption of HP- β -CD in rats prior to weaning. (De Schaepdrijver L, Marien D, et al. (2015) Reproductive toxicology (Elmsford, NY) **56**:87-96)

[0414] While cyclodextrins are widely used as absorption enhancer, (Shaikh I, Derle ND, et al. (2012) J Appl Pharmacol Sci **2**:34-9) the present disclosure utilized a cyclodextrin as an active ingredient and sodium caprate as an absorption enhancer for the cyclodextrin. In particular, it utilizes the smaller α -CD, applies the HP derivatization previously shown to enhance absorption of β -CD, uses a sodium salt of a fatty acid component of triglycerides as an effective absorption enhancer for delivery in the intestine (Yoshitomi H, Nishihata T, et al. (1987) J Pharm Pharmacol **39**:887-91) The novel hypothesis was tested in a human PK/PD study.

[0415] Study design: A randomized n-of-1 dose escalation study was conducted to assess the activity of a clathrate of HP- α -CD and sodium caprate (C-10 fatty acid, capric acid, decanoic acid) in extracting phospholipids from serum into urine. Capric acid was chosen because it is known to regulate the paracellular permeability of the tight junctions in Caco-2 monolayers and in rat and human intestinal segments is more efficiently than either lauric acid (C12) or caprylic acid (C8).

[0416] Methods: Urine samples (5–10 mL) were pipetted into culture tubes and then frozen for 30 min and samples were then lyophilized for 48–72 h, sealed and stored at -80°C . Phospholipids were extracted from urine samples after reconstitution, with chloroform (CHCl_3) and methanol (3:1), and filtered through glass wool. The eluent was washed with

1 M KCl with 0.01% sodium azide. The wash was centrifuged at 3000 g for 30 min and CHCl₃ layer transferred to fresh tube and evaporated at 60 °C under nitrogen. Phospholipid extracts and standards were digested in 10 M H₂SO₄ with 0.16 Ca(NO₃)₂ and 30% H₂O₂ and heated for 25 min at 180 °C. Samples were cooled and equilibrated with H₂O/(NH₄)₆Mo₇O₂₄ and 0.65% (N₂H₅)HSO₄. These were vortexed and heated at 35 °C for 30 min. All samples were analyzed in triplicate.

[0417] Phosphate content was measured at lambda-820 using a Molecular Dynamics Lambda5 analyzer. a Waters (Milford, MA) high performance chromatography system with tandem UV-vis wavelength detector (203 nM) was employed for urinalysis and separation enhancement of interested molecules. This was coupled to a Beckman Ultra SI column using an isocratic elution profile along a mobile phase of acetonitrile-methanol-phosphoric acid solvent (100:10:1.8) at a flow rate of 1.0 mL/min at RT. The lyophilized samples were resuspended in a hexane-propanol-phenolphthalene mix (3:1:0.1) before injection.

[0418] Technical controls were formulated and injected at a final amount of 0.125 mg phosphatidylcholine (PC) and 0.6 g HP- α -CD in same resuspension buffer. The standard curves (FIG. 43) were generated using DiPalmitoyl-PC in the range of .65–32 μ M phosphate equivalence. Percentage efficiency of isolation was determined through a comparison of the extraction recovery of total phosphate from normal urine spiked with known amount of synthetic PL with a parallel PL sample digested and assayed without extraction.

[0419] The present disclosure reports the surprising results of absorption and activity of different strategies of absorption enhancement of orally administered HP- α -CD. The results shown in FIG. 44 confirm that the novel and non-obvious pharmacologic complex, a HP- α -CD/sodium-caprate clathrate to reduce endocytosis in malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition yields an intervention that is capable of reducing serum phosphatidylcholine by a clinically relevant amount. In particular, the results presented in FIG. 44 show the following findings. First, they confirm that previous observations with α -CD also apply to HP- α -CD:

[0420] **In human adults, oral HP- α -CD is not absorbed** (in a therapeutically effective amount). Among the mixtures and/or clathrates tested, HP- α -CD was the only formulation

where the median value in the HP- α -CD fraction 2 (labeled "aCD") was lower than in fraction 1.

[0421] After confirming that native HP- α -CD is not absorbed and, thus not active, the results presented in FIG. 44 show several unexpected and surprising findings:

[0422] **The proposed oral HP- α -CD/capric acid clathrate is the first oral cyclodextrin that is absorbed in human adults.** In contrast to native HP- α -CD (HPaCD), after oral application of the clathrate (HPaCD/FA, HPaCD/FA&PE), a substantial amount of HP- α -CD is found in urine after 8 h (an increase to (about 2 on the scale of arbitrary absorption units) at the fraction expected for a molecule of molecular weight of 1180 for HP- α -CD (Sigma-Aldrich 390690) (HPaCD:HPaCD/FA, fraction 2: $P_{u\text{-test}} = 0.0079$)

[0423] **An increase in HP- α -CD absorption correlates with an increase in excretion of PC.** The increase in excretion of PC into urine over native HP- α -CD ($P = .0367$) is independent of the formulation ($P = .4523$) but, as the formulations differ in HP- α -CD absorption, is low for the formulations with low absorption (HPaCD&FA, HPaCD&PE) and high for formulations with high absorption (HPaCD/FA, HPaCD/FA&PE).

[0424] **The clathrate is more effective than a mixture.** Substantially more PC is extracted with the clathrate (HPaCD/FA) than with the mixture (HPaCD&FA) ($\min(\text{fraction 5|HPaCD/FA}) = 5.78$, $\max(\text{fraction 5|HPaCD&FA}) = 5.75$, $P_{u\text{-test}} = 0.0079$) to extract a therapeutically effective amount of monomeric phospholipids present at an extremely low concentration in serum.

[0425] **A previously proposed oily suspension containing FA is not more effective than FA alone.** A comparison between the clathrate v. intake of an oily suspension comprising the same amount of HP- α -CD and C-10 and additional permeation enhancers (Tuvia S, Pelled D, et al. (2014) Pharmaceutical Research **31**:2010-21) (HPaCD&PE) showed no advantage over the mixture of HP- α -CD with the fatty acid (HPaCD&FA). A suspension of the clathrate (HPaCD/FA&PE) showed no advantage over the clathrate alone (HPaCD/FA)

[0426] **The clathrate does not extract lysophospholipids.** It is commonly believed that alpha-cyclodextrins scavenge a wide range of lipids, including lyso-phospholipids. The average median value of fractions 9 and 10 (labeled LPC) in HPaCD is higher than in all

other groups. This unexpected surprising result has broad implications (see the following Example 16).

Example 16: Selectivity of HP α CD/sodium-caprate clathrate for selected PC over PA and LPLs exerts effects similar to anti-inflammatory drugs (NSAIDs).

[0427] sPLA2 inflammatory activity is ongoing in the majority of MS patients, active or stable, regardless of treatment. In rats, EAE symptoms were attenuated by inhibition of sPLA2. (Cunningham TJ, Yao L, et al. (2006) J Neuroinflammation **3**:26) Increased sPLA2 activity is observed in the cerebrospinal fluid of Alzheimer's disease patients (Chalbot S, Zetterberg H, et al. (2011) J Alzheimers Dis **25**:505-15), and may serve as a marker of increases in permeability of the BBB (Chalbot S, Zetterberg H, et al. (2011) J Alzheimers Dis **25**:505-15). Patients with Alzheimer's disease AD and mild cognitive impairment had aberrant PLA2 activity.(Klavins K, Koal T, et al. (2015) Alzheimer's & dementia (Amsterdam, Netherlands) **1**:295-302)

[0428] .(FIG. 43A and 43B:) Excretion of the serum phospholipid PC (mW $\approx$ 780 Da) is substantially increased by the clathrate, as expected. Unexpectedly, however, PA (675) and SM (650) are not reduced and neither are lyso-phospholipids (460–550).

[0429] The unexpected high specificity of the clathrate for PC implies that the clathrate does not only reduce activity of the PI cycle, but also reduces inflammation, because PC is split by sPLA2 into LPC (which is transformed by ATX into LPA) and proinflammatory arachidonic acid (AA) FIG. 37. It is well known that “lysoPCs act as uncompetitive product inhibitors of plasma secreted PLA2 enzymes (sPLA2s)”.(Cunningham TJ, Yao L, et al. (2008) Journal of inflammation (London, England) **5**:17) Hence, scavenging primarily lysophospholipids (LPC, LPA), rather than PC could activate sPLA2 and, thus, increase inflammation (FIG. 47). Scavenging PC only, instead, reduces sPLA2 activity by increasing the LPC/PC ratio and, thus, maintaining inhibition of sPLA2 while reducing availability of its substrate.

[0430] sPLA2-IIa activates HER2 and HER3, is overexpressed in lung, breast, pancreatic, skin, liver, and prostate cancer.(Dong Z, Meller J, et al. (2014) Int J Oncol **45**:978-84) activates EGFR in MCF7 human BC cancer cells (Hernandez M, Martin R, et al. (2010) Neuro-oncology **12**:1014-23), and is a marker of metastases.

[0431] “Researchers have been considering PLA2s could be a better therapeutic target than the downstream enzymes cyclooxygenase and lipoxygenase [yet]” (Yarla NS, Bishayee A, et al. (2016) *Curr Drug Targets* **17**:1940-62) Human PLA2s are involved in eicosanoid synthesis, phagocytosis by macrophages, airway hypersensitivity, inflammation, tumorigenesis (V) and allergen-induced asthma, myocardial ischemia/reperfusion injury, AA metabolism, inflammation tumorigenesis, atherosclerosis (X). (Yarla NS, Bishayee A, et al. (2016) *Curr Drug Targets* **17**:1940-62)

[0432] The unexpected reduction of inflammatory arachidonic acid exerts effects similar to corticosteroids (sPLA2 inhibition), NSAIDs (COX inhibition), and asthma drugs (LOX and leukotriene inhibition), see FIG. 6. Hence, α -CDs could become the next “baby-aspirin.”

[0433] The best known function of PLA2s is the hydrolysis of phospholipids including PC, PE, and PS. (Mallat Z, Lambeau G, et al. (2010) *Circulation* **122**:2183-200) sPLA2 acts not only on phospholipids, but also activates integrins (α v β 3, α 4 β 1, and α 5 β 1) (Saegusa J, Akakura N, et al. (2008) *J Biol Chem* **283**:26107-15; Fujita M, Zhu K, et al. (2015) *J Biol Chem* **290**:259-71) inhibits angiogenesis. Hence the termination of the first phase 3 trial (NCT01130246) of varespladib, a pan-sPLA2 inhibitor, for lack of efficacy and possible harm (Nicholls SJ, Kastelein JJ, et al. (2014) *JAMA* **311**:252-62) could have been caused by the disruption of other pathways involving some sPLA2 or by unknown effects of varespladib on other pathways. Thus, one of the advantages of α -CDs as “starvation mimics” lies in the fact that humans have evolved to tolerate paucity of nutrients, including phosphatidylcholine (egg yolk or soy bean lecithin).

Example 17: Treatment with Phosphatidylcholine (PC, Lecithin) may worsen Alzheimer’s disease

[0434] The unexpected finding that reducing PC levels in blood improves neurodegenerative diseases *in vivo* is supported by previous findings showing that the Sandoz Clinical Assessment – Geriatric score worsens more in Patients with Alzheimer’s disease who receive dietary PC supplementation.

[0435] As choline uptake was considered a potential rate-limiting step in the biosynthesis of acetylcholine (ACh), oral lecithin (90–95% PC) was considered as a treatment. Outpatients with Alzheimer’s disease of moderate severity completed a double-blind placebo-controlled

crossover trial of lecithin. Each patient received 10 g three times daily of a placebo for 3 months. Plasma choline levels rose threefold and remained at that level throughout the lecithin administration period. A significant difference between mean baseline scores and treatment scores was found on tests of new learning ability, indicating a practice effect in these tests. However, there were no significant differences between mean placebo and lecithin scores on any of the psychological test measures. (Etienne P, Dastoor D, et al. (1981) *Neurology* **31**:1552-4)

[0436] Twenty-one Alzheimer's disease patients completed a double-blind trial on lecithin 25 g daily. Equal improvement was noted in both the lecithin and placebo groups. Serum levels of lecithin measured 12–14 h after its administration were also not increased compared with the levels in control patients. (Fisman M, Merskey H, et al. (1981) *Canadian journal of psychiatry Revue canadienne de psychiatrie* **26**:426-8)

[0437] A randomized, double blind, cross-over study in 10 patients with primary degenerative dementia (DSM-III), 65 to 85 years old, compared placebo and three lecithin doses (4, 12, and 20 g/d) for one week each. "In spite of [an] increase in plasma ... choline levels, there was no improvement in memory test performance." (Brinkman SD, Pomara N, et al. (1982) *J Clin Psychopharmacol* **2**:281-5)

[0438] A double-blind, cross-over study in 10 Alzheimer's disease patients, 54 to 78 years old, compared placebo and three lecithin doses (15.0, 22.5, 30.0 g/d) for one week each. "Lecithin did not result in any clinically significant improvement in cognitive functioning." (Dysken MW, Fovall P, et al. (1982) *Neurology* **32**:1203-4) In fact, a novel analysis of the original data of 10 outpatients and 5 inpatients showed that the "Global Impression" (SCAG-G) not only worsened after dichotomization – "they produced a pooled odds ratio estimate of 3.0 (95% confidence interval (CI) from 0.9 to 9.8, a just non-significant finding in favor of placebo" (Higgins JP, Flicker L (2003) *Cochrane Database Syst Rev* CD001015), but declined with increasing dose of PC (Table 9), although the decline was not "significant" in a reanalysis performed as part of the present disclosure ($P_{\text{ANOVA}} = .2467$)

Table 9: Increasing serum phosphatidylcholine levels worsens Alzheimer's disease. SCAG-G results (high values are better in patients with Alzheimer's disease receiving 2 weeks of lecithin at different levels. In some patients the assessment was repeated. Higher values are worse. Derived from (Harris CM (1981) PhD:140, Table XXI).

SCAG-G	Placebo	Avg	Low	Avg	Medium	Avg	High	Avg
G4	5.4	(5.40)	6.6	(6.60)	6.6	(6.60)	5.5	(5.50)
G5	5.5	(5.50)	6.5	(6.50)	6.5	(6.50)	5.6	(5.60)
G12	6.6	6.6 (6.60)	5.6	(5.60)	6.6	6.6 (6.60)	6.5	(6.50)
G15	5.5	5.5 (5.50)	5.5	(5.50)	5.5	(5.50)	5.5	5.5 (5.50)
G16	2.1	2.2 (2.15)	3.3	(3.30)	2.2	2.2 (2.20)	2.2	(2.20)
G20	4.3	(4.30)	4.4	(4.40)	4.4	(4.40)	4.4	(4.40)
G21	3.1	(3.10)	2.2	(2.20)	3.3	(3.30)	2.3	(2.30)
G22	5.5	(5.50)	5.5	(5.50)	4.6	(4.60)	5.5	(5.50)
G23	5.6	(5.60)	4.6	(4.60)	5.4	(5.40)	4.4	(4.40)
G24	2.3	(2.30)	2.3	(2.30)	2.3	(2.30)	3.2	(3.20)
G25	2.3	2.2 (2.25)	2.2	(2.20)	3.2	3.3 (3.25)	2.2	(2.20)
G26	4.4	4.3 (4.35)	3.3	4.4 (3.85)	3.4	(3.40)	4.4	(4.40)
G27	6.6	6.6 (6.60)	6.5	(6.50)	6.6	5.5 (6.05)	6.6	(6.60)
G28	6.4	(6.40)	5.5	(5.50)	4.4	(4.40)	4.4	(4.40)
G29	4.4	3.3 (3.85)	3.4	(3.40)	3.3	(3.30)	3.3	3.3 (3.30)
Avg	4.67	(4.63)	4.49	(4.53)	4.55	(4.52)	4.40	(4.40)

[0439] A long-term, randomized, double-blind, study in Alzheimer's disease patients compared 20–25 g/d lecithin v. placebo (18 and 17 subjects with complete data, respectively) for six months with at least six months of follow-up. Lecithin treatment increased plasma choline values (depending on compliance). “As a whole there were no significant differences between the lecithin and placebo groups. ... [If anything] it was the relatively poor compliers who did best.”(Little A, Levy R, et al. (1985) J Neurol Neurosurg Psychiatry **48**:736-42)

[0440] Overall, there is no evidence that lecithin (phosphatidylcholine) supplementation benefits patients with Alzheimer's disease. On the contrary, two studies provide results that are consistent with the hypothesis that PC worsens Alzheimer's disease, even when given only for a relatively short study period.

[0441] The unexpected findings of the present disclosure explain the inconsistent findings with some studies showing some improvement of dementia from dietary PC supplementation and other showing a negative effect: Reduction of overall PC may, in fact, be detrimental, but reduction of monomeric PC, which is present only “at an extremely low concentration”(Wilton DC (2005) European Journal of Lipid Science and Technology **107**:193-205), provides a benefit.

Example 18: The successful results of cyclodextrins in various animal models are the result of a synergistic effect on inflammation an autophagy

[0442] HP- β -CD had been shown to be effective in animal models for a wide range of diseases, but scavenging cholesterol, the presumed mode of action, carried the risk of

permanent hearing loss. The surprising genetic results of Example 1 and Example 2 led to the hypothesis that the mode of action of HP- β -CD was not through scavenging cholesterol, but through scavenging phospholipids (FIG. 45). Hence, HP- α -CD emerged as a safer (and more effective) alternative. The surprising results of Example 16 confirmed in FIG. 40 showed HP- α -CD to reduce systemic inflammation, in addition to improving autophagy.

[0443] In summary, α -CDs slow down several processes that are essential during early life, but can cause problems after the age of 50: (1) migration of cells during pre-natal (tissue) and early-post-natal (neuronal) development, (2) cellular uptake of nutrients during growth, and (3) innate immunity (inflammation) as a defense against pathogens not seen, yet, by the immune system. HP- α -CD achieves this by (a) reducing endocytosis and, thus, lysosomal stress (FIG. 46) and (b) inflammation, also called “inflammaging” (FIG. 47). An additional benefit is that reducing inflammation improves autophagy and, conversely, improving autophagy reduces inflammation. Hence, the effects of HP- α -CD on age-related conditions involving upregulated inflammation and downregulated autophagy are expected to be highly synergistic.

Example 19: Study design for studying Neuroprotection by alpha and beta Cyclodextrin in cell and mouse models of Alzheimer's disease

[0444] **Abstract.** There is extensive evidence that cholesterol and membrane lipids play a key role in Alzheimer disease (AD) pathogenesis. Cyclodextrins (CD) are cyclic oligosaccharide compounds and hydroxypropyl- β -CD (HP- β -CD) is widely used to bind cholesterol. Since CD exerts significant beneficial effects in Niemann-Pick type C disease, which shares neuropathological features with AD, the effects of HP- β -CD were examined in cell and mouse models of AD. Cell membrane cholesterol accumulation was detected in N2a cells over-expressing Swedish mutant APP (SwN2a), and the level of membrane cholesterol was reduced by HP- β -CD treatment. HP- β -CD dramatically lowered the levels of A β 42 in SwN2a cells, and the effects were persistent for 24 hours after withdrawal. Four months of subcutaneous HP- β -CD administration significantly improved spatial learning and memory deficits in Tg19959 mice, diminished A β plaque deposition, and reduced tau immunoreactive dystrophic neurites (DN). These are transgenic mice with 2 mutations in the APP gene which have been associated with human AD and beneficial effects were attributed to a reduction in cholesterol. Recently, a new GWAS analyses was used, which showed an overlap of phospholipid pathway genes in metastatic breast cancer with those found in AD cohorts.

HP α CD also binds phospholipids, as does HP- β -CD, but does not bind cholesterol. HP α CD was twice as effective as HP- β -CD in slowing migration of breast cancer cells. It is therefore possible that the efficacy of HP- β -CD is not due to its effects on cholesterol but rather on phospholipids. One way to distinguish these possibilities is to test the efficacy of HP α CD both in vitro and in transgenic mice with APP mutations (Tg19959). In the present proposal we intend to determine whether HP α CD is equally efficacious, or much more efficacious, without having effects on cholesterol levels or membrane cholesterol as determined using filipin staining and mass spectroscopy. We will also determine whether the effects are dose-responsive both on beta-amyloid production in the N2a cells, as well as in vivo in the Tg19959 transgenic mice. These studies will therefore provide the ground work and scientific rationale to pursue this strategy further in clinical trials in patients with AD.

[0445] **Summary of key supporting data.** Previous, the effects of HP- β -CD were examined in cell and mouse models of AD. HP- β -CD dramatically lowered the levels of A β 42 in SwN2a cells, and the effects were persistent for 24 h after withdrawal. Four months of subcutaneous HP- β -CD administration significantly improved spatial learning and memory deficits in Tg19959 mice, diminished A β plaque deposition, and reduced tau immunoreactive dystrophic neurites (DN). See (Sim I, Carini S, et al. (2012) AMIA Annu Symp Proc **2012**:856-65). HP- β -CD lowered levels of A β 42 in part by reducing β -cleavage of the APP protein, and it also up-regulated the expression of genes involved in cholesterol transport and A β clearance. This study for the first time showed neuroprotective effects of HP- β -CD in a transgenic mouse model of AD, both by reducing A β production and enhancing clearance mechanisms, which suggested that it could be a novel therapeutic strategy for disease modification in AD. Among the upregulated genes was ABCA1, a membrane transporter that exports cholesterol and phospholipids and is considered as a target of agonist peptides for the treatment of AD.(Bielicki JK (2016) Current opinion in lipidology **27**:40-6)

[0446] Recent results, (Wittkowski KM, Dadurian C, et al. (2018) PLoS One **13**:e0199012), identified several genes involved in “derailed endocytosis” of breast cancer, most of which (including ABCA1) had already been implicated in functional and gene-expression studies of AD, including

[0447] ATP8A1/ATP8B1 (Arch Neurol, 65 (1), 45-53; Internat J Mol Sci, 14 (4), 7897-922; PLOS Genetics, 8 (8), e1002853.).

[0448] ANO4 (Alzheimers Dement, 10 (1), 45-52.).

[0449] ABCA1 (PLoS One, 11 (11), e0166195; Neurobiol Dis, 72 Pt A, 13-21; Alzheimers & Dementia, 11 (12), 1430-38; Neurobiol Dis, 72 Pt A, 54-60.).

[0450] AGPAT3/AGPAT4 (J Alzheimers Dis, 23 (2), 349-59.).(Sherva R, Baldwin CT, et al. (2011) J Alzheimers Dis **23**:349-59)

[0451] DGKQ (Zhu, X. C., et al. (2016), 'Association of Parkinson's Disease GWAS-Linked Loci with Alzheimer's Disease in Han Chinese', Mol Neurobiol).

[0452] All of these genes are involved in transport and metabolism of phospholipids, while only few are involved in transport of cholesterol, suggesting that the effects seen in the above animal models of AD could be a consequence of reductions in phospholipids rather than cholesterol.

[0453] This hypothesis was supported by *in vitro* experiments in two human breast cancer cell lines, MDA-MB-231 (triple negative) and MCF-7 (estrogen receptor positive), showing 1 mM HP α CD to be more effective than 2 mM HP β CD against migration of both ER– and ER+ tumor cell migration (p = .0252). See (Wittkowski KM, Dadurian C, et al. (2018) PLoS One **13**:e0199012).

II. Aim 1 (primary): To test efficacy of equimolar HP α CD versus HP β CD (2,000 and 4,000 mg/kg) and control on spatial learning and memory deficits, in Tg19959 mice overexpressing human mutant APP

[0454] **Innovation:** HP β CD is widely believed to exert its activity, including its activity in mouse models of AD, by scavenging cholesterol. See (Yao J, Ho D, et al. (2012) J Exp Med **209**:2501-13). Our GWAS results, confirmed in *in vitro* studies, (Wittkowski KM, Dadurian C, et al. (2018) PLoS One **13**:e0199012), showed association with genes already seen in functional and expression studies as implicating derailed endocytosis” in BC (Nat Rev Cancer, 8 (11), 835-50), and “driving pathogenesis in sporadic and familial [AD]”. Biomed Res Int, 2014, 167024. Hence the effect of HP β CD seen in previous *in vitro* studies (Raghu H, Sodadasu PK, et al. (2010) BMC Cancer **10**:647) was likely true to scavenging PLs, rather than cholesterol. “Derailed/deranged signaling and associated endocytosis” in AD. (Van Dooren T, Princen K, et al. (2014) Biomed Res Int **2014**:167024) If similar results were seen

in *in vitro* models of AD, novel treatments could be sought that target PLs, rather than cholesterol.

[0455] **Significance:** The initial positive *in vivo* results on AD in mice were not followed up with clinical trials, mainly because HP β CD was shown to carry the risk of causing permanent hearing loss from depriving outer hair cells of cholesterol. See The Journal of Experimental Medicine, 209 (13), 2501-13; (Takahashi S, Homma K, et al. (2016) Sci Rep 6:21973) The recent GWAS results shift the focus from cholesterol to PLs. This, in turn, could now open novel treatment options for AD, including HP α CD, which may be more effective than HP β CD, while avoiding the risk of permanent hearing loss from scavenging cholesterol. HP α CD is also less toxic than HP β CD *in vitro*. See International Journal of Pharmaceutics, 101 (1-2), 97-103.

[0456] **Approach:** We will examine effects on cleavage of APP, expression of cholesterol and lipid transport genes, and A β clearance as well as levels of cholesterol and phospholipids. We will determine whether effects are dose-responsive by examining 2 concentrations of both HP α CD and HP β CD, and effects on lysosomes by cathepsin D immunostaining. We will perform these tests in five groups (Control, HP β CD 2g, HP β CD 4g, HP α CD 2g, HP α CD 4g) of 20 mice each.

[0457] **Pitfalls and alternatives:** Not all effects previously seen with HP β CD will be replicated with HP α CD and the differential effect will provide novel insights into the etiology. If, however, too few of the effects of HP β CD can be replicated with HP α CD, the hypothesis that the latter could become a safer treatment against AD will not be further pursued. Still, the results from Aim 2 may still provide novel insights leading to potential alternatives.

III. Aim 2: To explore the relative activity of scavenging PLs only (HP α CD) versus scavenging both PLs and cholesterol (HP β CD) on AD pathology ...Aim 2a: ... in N2a cells expressing the Swedish APP mutation (membrane cholesterol, A β production) Aim 2b: ... in Tg19959 mice overexpressing human mutant APP (A β deposition, microgliosis, amylogenesis, tau and lysosomal abnormalities, gene expression)

[0458] **Innovation:** Even if HP α CD spatial learning and memory by improving A β and tau pathology, the role of PLs v. cholesterol in the etiology of AD remains to be elucidated.

[0459] **Significance:** A single drug is unlikely to become a panacea in AD. The mechanistic studies will provide insights to design studies that could lead to more specific drugs targeting cholesterol.

[0460] **Approach:** We will aim to replicate the successful *in vitro* studies of HP β CD reducing A β production in N2a cells expressing the Swedish APP mutation, this time using HP α CD instead of HP β CD. To further elucidate the mechanism of action, we will use filipin staining to assess the effect of scavenging PLs on membrane cholesterol.

[0461] **Pitfalls and alternatives:** The role of cholesterol in AD is assumed, but not known. Hence, both positive and negative results on the effect of HP α CD on membrane cholesterol and A β production will be helpful in refining the understanding of the role of CDs in AD and provide valuable insights for the development of additional drugs.

[0462] Experimental design and methods

[0463] Based on the previous results showing 4,000 mg/kg HP β CD to be effective *in vitro* and the *in vitro* results showing HP α CD to be at least twice as effective as HP α CD in regulating EEC we will compare HP α CD_2g v. HP β CD_4g, but also HP α CD_2g v. HP β CD_2g, HP α CD_4g v. HP β CD_4g, HP α CD_4g v. HP α CD_2g, and HP α CD_4g v. HP α CD_2g. See The Journal of Experimental Medicine, 209 (13), 2501-13. As positive controls, we will replicate the previous results comparing Control v. HP β CD_4g.

[0464] To guard against regression to the mean (winner's curse) and account for multiplicity (five dependent comparisons) in Aim 1 and to ensure that negative results in the exploratory Aim 2 can be meaningfully interpreted, we will increase the sample size from 10 per group in the previous study of two groups ($\alpha = .05$, power = .80, $\delta = 1.3$) to 20 per group in this study of 5 groups ($\alpha = .01$, power = .90). See The Journal of Experimental Medicine, 209 (13), 2501-13.

[0465] **Experimental animals.** Tg19959 mice were obtained from Dr. George Carlson (McLaughlin Research Institute, Great Falls, MT). Tg19959 mice are constructed by injecting FVB $\times$ 129S6 F1 embryos with a cosmid insert containing human APP695 with two familial AD mutations (KM670/671NL and V717F), under control of the hamster PrP promoter. All experiments will be approved by the Institutional Animal Care and Use Committee at Weill-Cornell Medical College.

[0466] **Cell lines:** Mouse N2a neuroblastoma cells stably transfected with human APP695 carrying the 670/671 Swedish mutation (SwN2a) will be grown as described previously (Yao J, Hennessey T, et al. (2010) PLoS One 5:e15546).

[0467] **HP- α -CD/HP- β -CD (HP-CD) treatment in cells.** 10 mM HP-CD stock solution will be made in 1 x PBS. SwN2a and N2a cells will be treated with HP-CD (5 mM) in serum free medium at different incubation times. To measure A β levels, SwN2a cells will be recovered in serum free medium for 5 or 24 h after HP-CD treatment.

[0468] **Filipin staining and analysis.** Filipin is prepared in DMSO (50 mg/ml) and stored at -20 °C. Cells will be fixed in 4% PFA for 30 min. For filipin staining, separate permeabilization of cells is not needed since filipin itself permeabilizes the cells. The stock solution will be diluted in PBS (1:100–1:500). The cells will be incubated for 15 min at room temperature and then washed in PBS 3 $\times$ for 5 min. Anti-fading reagent (Fluoromount-G, SouthernBiotech) will be used in the mounting medium. Filipin is detected using λ_{ex} =360 nm and λ_{em} =460 nm. A fluorescence microscope connected to a CCD camera will be used, and images are taken using Metamorph (Molecular Devices). To analyze the images, we will use Image J (NIH) to apply a 70% threshold onto the images and quantify the alteration of filipin intensity of SwN2a cells with and without CD treatment. See Nat Neurosci, 9 (10), 1265-73.

[0469] **Cholesterol extraction from SwN2a cells.** Six-well plates of SwN2a cells will be treated with and without CD for 15 min. Cells will be washed 3 $\times$ with Hank's buffer and then 800 μ l of hexane/isopropanol (3/2 v/v) containing β -sitosterol (internal standard, 5 μ g of β -sitosterol per well) will be added. Lipids are extracted for 30 min under gentle shaking at room temperature. Lipid extracts will be transferred to 12 $\times$ 75 mm borosilicate glass culture tubes; and dried under Argon. The extraction will be repeated again and 50 μ l hexane will be added per tube, vortexed and then transferred into glass vials for separation and analysis on GC-Mass Spec. The levels of cholesterol/mg protein will be calculated and plotted.

[0470] **Cyclodextrin administration to mice.** Mice will be treated with HP- α -CD or HP- β -CD (HP-CD, 2000 or 4000 mg/kg) by subcutaneous injection twice weekly. HP-CD will be provided as a 20% (w/v) solution in isotonic saline with isotonic saline alone as the control. The injections of HP- CD in the mice will be started at P7, and the duration of the treatment will be four months. Twenty mice will be studied in each group.

[0471] **Morris water maze.** Spatial learning and memory will be analyzed using the Morris water maze. See Learning and Motivation, 12 (2), 239-60. The mice are handled daily, starting 1 wk prior to behavioral testing, in order to habituate them. During the acquisition period, visual cues will be arranged in the room. The hidden platform is located in the middle of the northwest (NW) quadrant. Each day, mice were placed next to and facing the wall of the basin in 4 starting positions: north, east, south, and west, corresponding to four successive trials per day. The duration of a trial will be 60 s with an inter-trial interval of 60 min. Whenever the mouse fails to reach the platform within 60 s, it is placed on the platform by the experimenter for 10 s. Latencies before reaching the platform will be recorded for 5 d and analyzed.

[0472] A probe trial will be assessed 24 h after the last trial of the acquisition period, removing the platform from the pool. Mice will be released on the north side for a single trial of 60 s, during which the time spent in the area of the platform will be measured. The velocity will also be measured.

[0473] The visible platform testing will be performed over 2 d with 4 trials per day. In this cued test, a pole will be added on the platform, and its location was changed between each trial. The duration of a trial will be 60 s with an inter-trial interval of 60 min. Latencies before reaching the platform will be recorded and averaged.

[0474] **Sample preparation from brains and cells.** Mice will be deeply anesthetized with intraperitoneal sodium pentobarbital and transcardially perfused with ice-cold saline. The brains will be removed and dissected on ice. One hemisphere will be used for histological analysis and the other hemisphere will be used for subsequent protein extraction or Trizol RNA extraction (Invitrogen). Brain tissues will be homogenized in lysis buffer containing 1% SDS + 0.5% NP-40 and protease inhibitors (Roche) for Western blot analysis. Cells will be homogenized and prepared in Trizol for RNA extraction and in RIPA buffer or 1% Triton in PBS for protein extraction. Protein concentrations will be determined by BCA protein assay (Thermo Scientific).

[0475] **Western blot analysis.** Samples with equal amount of protein will be separated by Tricine-SDS gel electrophoresis and transferred to PVDF membranes using the iBlot dry blotting system (Invitrogen). Membranes will be blocked with 5% milk/0.1% Tween20 in TBS for 1 h at room temperature, followed by incubation with primary antibodies overnight

at 4°C. Signals will be detected using HRP-conjugated secondary antibodies and enhanced chemiluminescence (Thermo Scientific). Blots will be scanned at 600 dpi and densitometry will be performed using ImageJ (NIH). We will use the following antibodies: mouse monoclonal anti-tubulin (Sigma, 1:10000), mouse anti-A β 1-16 (6E10) monoclonal antibody (Covance, 1:1000), rabbit anti-APP C-terminal antibody (Calbiochem), HRP-conjugated goat anti-mouse IgG (1:2000) and goat anti-rabbit IgG (1:3000) (KPL).

[0476] **Enzyme linked immunosorbent assay (ELISA).** A β 42 levels will be quantified using a commercial ELISA kit (Invitrogen, KHB3441). The manufacturer's protocol will be followed to measure A β levels in cell extracts, medium from SwN2a cells and brain extracts from mice. Medium from SwN2a cells will be diluted 1:1 in diluents, and brain extracts will be diluted 1:10 in diluents, and then loaded onto the plate for analysis. Each sample will be run in duplicate and the experiments will be repeated at least twice.

[0477] **Immunohistology.** The mice which will have been assessed behaviorally will be deeply anesthetized with intraperitoneal sodium pentobarbital and transcardially perfused with ice-cold saline. Brains will be post-fixed in 4% paraformaldehyde in PBS for at least 24 h. The brain tissues will be cut in 35 μ m sections, and immunostained using the avidin-biotin complex peroxidase method and visualized after DAB (diaminobenzidine) incubation for 5 min (Vector, Burlingame, CA, USA). For each animal, 5 sections will be analyzed. For amyloid deposits, brain sections will be labeled with the anti-A β 42 rabbit polyclonal antibody AB5078P (1:1000, Chemicon). For microglial activation, adjacent sections are also labeled with anti-CD-11b rat monoclonal antibody (1:100, Serotec). For phosphorylated tau, sections are labeled with AT8 antibody (1:500, Thermo Scientific). For cathepsin D, sections are labeled with RU4 antibody (generous gift from Dr. Ralph Nixon, New York University School of Medicine/Nathan Kline Institute). Using NIH Image 1.63 software (National Institutes of Health, Bethesda, MD, USA), the percentage areas occupied by AB5078P immunoreactive amyloid plaques and by CD-11b immunoreactive reactive microglia per 0.75 mm² will be calculated.

[0478] **Thioflavin-S staining.** Floating sections from Tg19959 will be washed and incubated in 1% Triton-PBS for 15 min, washed with PBS, and stained for 5 min with a solution of 0.05% thioflavin S (ThS) in 50% ethanol. Finally, sections are washed in 50% ethanol and then in water. The fluorescence of ThS is detected using λ_{ex} =488 nm with fluorescence

microscopy. The area of ThS fluorescence is determined using Image J and expressed as a fraction of total area.

[0479] **Quantitation of genes by RT-PCR.** RNA will be extracted from 4-month-old mouse brains using the Trizol protocol (Invitrogen). Total RNA (1 μ g) will then be reverse transcribed into cDNA using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, USA), with the addition of nuclease-free deionized water. Reverse transcription is performed according to the manufacturer's protocol. A total of 30 ng of cDNA is loaded into each well of the PCR plate. The cDNA is analyzed in duplicate by real-time quantitative PCR using the ABI Prism 7000 Sequence Detection System (Applied Biosystems, USA) and detected with Power SYBR Green Master Mix (Applied Biosystems, USA. Primer sequences will be obtained from previous publications. See *Biochim Biophys Acta*, 1801 (8), 831-8; *Proc Natl Acad Sci U S A*, 106 (7), 2377-82.

ABCA1 (Fwd: 5'-CGTTTCCGGGAAGTGTCTTA-3'; Rev: 5'-GCTAGAGATGACAAGGAGGATGGA-3'),

ABCA2 (Fwd: 5'-AGTGCTCAGCCTTCGTACAG-3', Rev: 5'-AGGCGCGTACAGGATTTTGG-3'),

ABCG1 (Fwd: 5'-TTTGAGGGATTTGGGTCTGAAC-3', Rev: 5'-CCCCTTTAATCGTTTTGTCTGCT-3'),

NPC1 (Fwd: 5'-GGGATGCCCCGTGCCTGCAAT-3'; Rev: 5'-CTGGCAGCTACATGGCCCCG-3'),

[0480] For each sample, the cycle number C_t to reach threshold fluorescence will be determined in duplicate for each mRNA and actin. To determine relative amounts of mRNA in Tg19959 mice v. wildtype mice, data are presented using the $2^{-\Delta\Delta C_t}$ method (Livak KJ, Schmittgen TD (2001) *Methods* **25**:402-8).

[0481] **Statistical methods.** Unpaired comparisons between groups will be performed using generalized Friedman/Kruskal-Wallis type rank-tests with Scheffè-type multiple comparisons. (Wittkowski KM (1988) *J Am Statist Assoc* **83**:1163-70, 87:258). Calculations will be performed using R (<http://www.cran.r-project.org/>) and the muStat package (<https://cran.r-project.org/package=muStat>).

[0482] The primary outcome for Aim 1 is a composite u-score comprising

- Total time spent reaching the hidden platform on day 5 and
- Total time spent in the platform area.

[0483] To guard against artifacts (outliers, skewed distributions) in the interpretation (incl. Aim 2), we will plot results as box-and-whiskers plots. See (Tukey JW (1977) Exploratory data analysis Reading, Mass.: Addison-Wesley).

Example 20: Study design for studying Neuroprotection by alpha and beta Cyclodextrin in cell and mouse models of Parkinson's disease.

[0484] The aggregation of α -synuclein (α -syn) is believed to play a critical role in the pathogenesis of disorders such as dementia with Lewy bodies and Parkinson's disease. The main objective of a previous study (Bar-On P, Rockenstein E, et al. (2006) J Neurochem **98**:1032-45) was to determine whether methyl- β -cyclodextrin (M- β -CD), interfered with α -syn accumulation in models of synucleinopathy. See J Neurochem, 98 (4), 1032-45. The authors studied the effects of M- β -CD on the accumulation of α -syn in a transfected neuronal cell line and in transgenic mice. Immunoblot analysis showed that M- β -CD reduced the level of α -syn in the membrane fraction and detergent-insoluble fraction of transfected cells. In agreement with the in vitro studies, treatment of mice with M- β -CD resulted in decreased levels of α -syn in membrane fractions and reduced accumulation of α -syn in the neuronal cell body and synapses. Taken together, these results were interpreted as suggesting that changes in cholesterol and lipid composition using M- β -CD may become a treatment of synucleinopathies. The results of the above examples, however, suggest that the effects seen were primarily due to M- β -CD, which was seen as a "cholesterol lowering agent" were, in fact, primarily due to M- β -CD scavenging phospholipids. In the current Example, the study will be replicated, this time using HP- α -CD, rather than M- β -CD.

[0485] **Effects of M- β -CD on α -syn levels in non-tg and α -syn tg mice brains.** Non-transgenic (tg) and ha-syn tg mice will be treated with M- β -CD (10 mM) for 1 week and analyzed by western blot and immunocytochemistry.

Example 21: Study design for testing the efficacy of a cyclodextrin in breast cancer

[0486] Because women after resection of breast cancer have a definitive diagnosis, clinical trials of HP- α -CD against “derailed endocytosis” should start in this population. As a non-limiting example, a seamless phase 2b/3 clinical trial for HP- α -CD for the prevention of metastases in breast cancer could be conducted in a population of women with

- triple negative breast cancer (tamoxifen and herceptin do not work well in this population) and
- axillary lymph node metastasis (patients have established the tendency to develop metastases)

[0487] The phase 2b part of the seamless design would have futility as an outcome after the first 80 patients have been seen for at least 2 yr and continue to be treated without unblinding, so that they can contribute to the primary endpoint, which would have time to distant metastasis as the outcome. As the recurrence rate is high for the first three years only data will be collected for up to 5 yrs. See J Breast Cancer, 18 (4), 371-7. Some of the later recruited patients will be administratively censored when the last patient will have been seen for two years. Gehan’s test will be used to compare treated v. placebo patients. See Biometrika, 52, 203-23.

[0488] At an effective median observation time of three years, one would expect 30% of women to have a distant recurrence and could detect a reduction in incidence by 50% (to 15%) with the standard 80% power at the 5% level with 125 subjects per group. The placebo-controlled treatment would be given on top of the standard of care (chemotherapy, radiation, ...).

As long-term parenteral administration of HP- β -CD (200 mg/kg) was reported to decrease bone mineral density (BMD) in rats (Toxicol Pathol, 40 (5), 742-50), the study should carefully monitor

- Bone density.

[0489] Human erythrocytes tolerate α -CD better than β -CD. See WHO/JECFA Food Additive Series, 48, 1030. From animal studies, which have less tolerance for cyclodextrin than humans, the dose-limiting factors are likely

- nephrotoxicity and
- hemolysis

[0490] In animal studies, α -CD did not show ototoxicity (Ann Clin Transl Neurol, 3 (5), 366-80) still, the dose-finding studies should carefully screen for

- Ototoxicity.

Example 22: Study design for testing the efficacy of a cyclodextrin in FSGS

[0491] In one aspect, the present disclosure provides a method of treating and/or preventing focal segmental glomerulosclerosis (FSGS) and/or nephrotic swelling in a subject in need thereof, the method comprising administering to the subject an effective amount of an α -CD, or an analogue or derivative thereof, either alone or in combination with one or more additional active agents. In some embodiment the composition is a clathrate of HP- α -CD and sodium caprate or caprylate.

[0492] A study will be performed in which HP- α -CD with and without clathrate will be administered orally to rats using a model set forth in Fogo, *Semin Nephrol.* 2003 Mar;23(2):161-71, which is incorporated by reference in its entirety. Other oral formulations comprising and not comprising HP- α -CD will be tested alongside HP- α -CD to measure efficacy.

[0493] Other oral formulations may include those described in WO2016105465, ORAL COMPOSITIONS FOR INSOLUBLE COMPOUNDS, also incorporated by reference in its entirety.

We expect that HP- α -CD or analogs or derivatives thereof will effectively treat and/or prevent development of FSGS in mammals as well or better than other experimental compounds without known side effects of beta-cyclodextrins.

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US 6890549 B2 2003/07/29 Artiss JD, Jen C (Priority: US 40436602 P 2002-08-19) Compositions Comprising Dietary Fat Complexer And Methods For Their Use.

A method for reducing the bioavailability of fat in a consumable fat-containing food product comprising determining the amount of fat in a consumable fat-containing food product and combining α -cyclodextrin with the consumable fat-containing food product such that the consumable fat-containing food product comprises a ratio of α -cyclodextrin to fat of about 1:20 to about 1:3 w/w wherein said α -cyclodextrin or α -cyclodextrin complexes are not removed from said food product prior to consumption.

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The present invention is also further described by the following claims.

CLAIMS

1. A composition comprising any one or plurality of cyclodextrin molecules described herein, or salt thereof, and/or a clathrate thereof.
2. A pharmaceutical composition comprising the composition of claim 1 and a pharmaceutically acceptable carrier.
3. The pharmaceutical composition of claim 2 further comprising a therapeutic compound for treatment of malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition.
4. The pharmaceutical composition of claim 2 wherein the cyclodextrin molecule comprises a chemical moiety fused or covalently bonded to the cyclodextrin molecule, salt or clathrate thereof.
5. A method of treating or preventing malignant, neurodegenerative, vascular, metabolic, inflammatory, autoimmune, pulmonary, fibrotic, hepatic, lysosomal storage, or viral disease, disorder, or condition comprising administering to a subject in need thereof a therapeutically effective amount of one or a plurality of compositions of claims 1 through 4.
6. The method of claim 5, wherein the cardiovascular disease is atherosclerosis.
7. The method of claim 5, wherein the autoimmune disease is one or a plurality of asthma, multiple sclerosis, systemic lupus erythematosus.
8. The method of claim 5, wherein the metabolic disease is chosen from one or a plurality of: fatty liver disease, NASH, cystic fibrosis, type II diabetes.
9. A method of reducing levels of serum phospholipids in a subject in need of decreased phospholipids comprising administering to a subject in need thereof a therapeutically effective amount of one or a plurality of compositions of claims 1 through 4.
10. A method of increasing absorption of a therapeutic compound in intestines of a subject in need thereof comprising administering to the subject a therapeutically effective

amount of one or a plurality of compositions of claims 1 through 4 prior to, after or simultaneously with the therapeutic compound.

11. A method of treating and/or preventing cancer, cardiovascular disease, metabolic disease, or viral infection characterized by a cell exhibiting dysfunctional lysosomes or PI cycle comprising administering to the subject in need thereof a therapeutically effective amount of one or a plurality of compositions of claims 1 through 4.

12. The method of claim 11, wherein the step of administering to the subject in need thereof a therapeutically effective amount of one or a plurality of compositions of claims 1 through 4 occurs prior to, after or simultaneously with the therapeutic compound.

13. A method of preventing or treating a lysosomal disease or disorder in a subject in need thereof comprising administering an effective amount of a cyclodextrin to the subject.

14. The method of claim 13, wherein the cyclodextrin comprises a 2-hydroxypropyl (HP)-cyclodextrin.

15. The method of claim 14, wherein the cyclodextrin comprises at least one of a HP- α -cyclodextrin and a HP- β -cyclodextrin.

16. The method of claim 14, wherein the HP-cyclodextrin is linked to a first active agent directly or using a crosslinker.

17. The method of claim 16, wherein the lysosomal disease is multiple sclerosis, and the first active agent is selected from the group consisting of interferon beta-1a, interferon beta-1b, glatiramer acetate, interferon beta-1b, peginterferon beta-1a, interferon beta-1a and daclizumab.

18. The method of claim 16, wherein the lysosomal disease is multiple sclerosis, and the first active agent is selected from the group consisting of teriflunomide, fingolimod, and dimethyl fumarate.

19. The method of claim 16, wherein the lysosomal disease is multiple sclerosis, and the first active agent is selected from the group consisting of: alemtuzumab, mitoxantrone, ocrelizumab, and natalizumab.

20. The method of any one of claims 16-19, wherein the HP-cyclodextrin further comprises at least one of a second cyclodextrins and a second active agent.
21. The method of claim 20, wherein the second active agent is different from the first active agent.
22. The method of claim 16, wherein the lysosomal disease is Paget's disease, and the active agent comprises at least one of alendronate, ibandronate, pamidronate, risedronate, and zoledronic acid.
23. The method of any one of claims 16-22, wherein the HP-cyclodextrin is coupled to a carrier.
24. The method of claim 23, wherein the carrier is a gold nanoparticle.
25. The method of claim 23, wherein the carrier is a carbohydrate.
26. A method of downregulating arachidonic acid in a subject in need thereof comprising administering to the subject an effective amount of an α -cyclodextrin or an α -cyclodextrin derivative.
27. The method of claim 26, wherein the effective amount of the α -cyclodextrin or α -cyclodextrin derivative preferentially scavenges a phospholipid, wherein the phospholipid comprises at least PC.
28. The method of claim 27, wherein the effective amount of the α -cyclodextrin or α -cyclodextrin derivative also scavenges a lysophospholipid.
29. The method of claim 26, wherein the effective amount of the α -cyclodextrin or α -cyclodextrin derivative preferentially scavenges a lysophospholipid.
30. The method of any one of claims 26-29, wherein the effective amount of the α -cyclodextrin or α -cyclodextrin derivative comprises the α -cyclodextrin derivative, and wherein the α -cyclodextrin derivative is hydroxypropyl- α -cyclodextrin.

31. A method of treating and/or preventing a disorder of arachidonic acid secretion in a subject in need thereof comprising administering to the subject an effective amount of α -cyclodextrin or an α -cyclodextrin derivative.
32. A method of downregulating arachidonic acid in a subject in need thereof comprising administering to the subject an effective amount of an α -cyclodextrin derivative of a salt thereof.
33. The method of claim 32, wherein the α -cyclodextrin derivative is a clathrate of α -cyclodextrin derivative comprising a medium-length chain fatty acid and α -cyclodextrin, HP- α -cyclodextrin or a salt thereof.
34. A method of treating and/or preventing ALS, MS, Alzheimer's, Huntington's or Parkinson's disease in a subject in need thereof comprising administering to the subject an effective amount of a pharmaceutical composition comprising α -cyclodextrin or an α -cyclodextrin derivative or salt thereof.
35. The method of claim 34, wherein the pharmaceutical composition further comprises a fatty acid.
36. The method of claim 34, wherein the pharmaceutical composition further comprises a medium-length chain fatty acid.
37. The method of claim 34, wherein the pharmaceutical composition further comprises a clathrate of medium-length chain fatty acid and the α -cyclodextrin, HP- α -cyclodextrin or a salt thereof.
38. A method of reducing phospholipid levels in a subject in need thereof, the method comprising administering to the subject an effective amount of a pharmaceutical composition comprising (i) α -cyclodextrin or an α -cyclodextrin derivative or salt thereof; (ii) a pharmaceutically acceptable carrier; and a fatty acid.
39. The method of claim 38, wherein the α -cyclodextrin or an α -cyclodextrin derivative or salt thereof; and the fatty acid are in concentration such that the α -cyclodextrin or an α -cyclodextrin derivative or salt thereof and the fatty acid form a clathrate.

(HP)-Cyclodextrins

native: $R^* = H$

HP: $R^* = H$ or $CH_2CH(OH)CH_3$

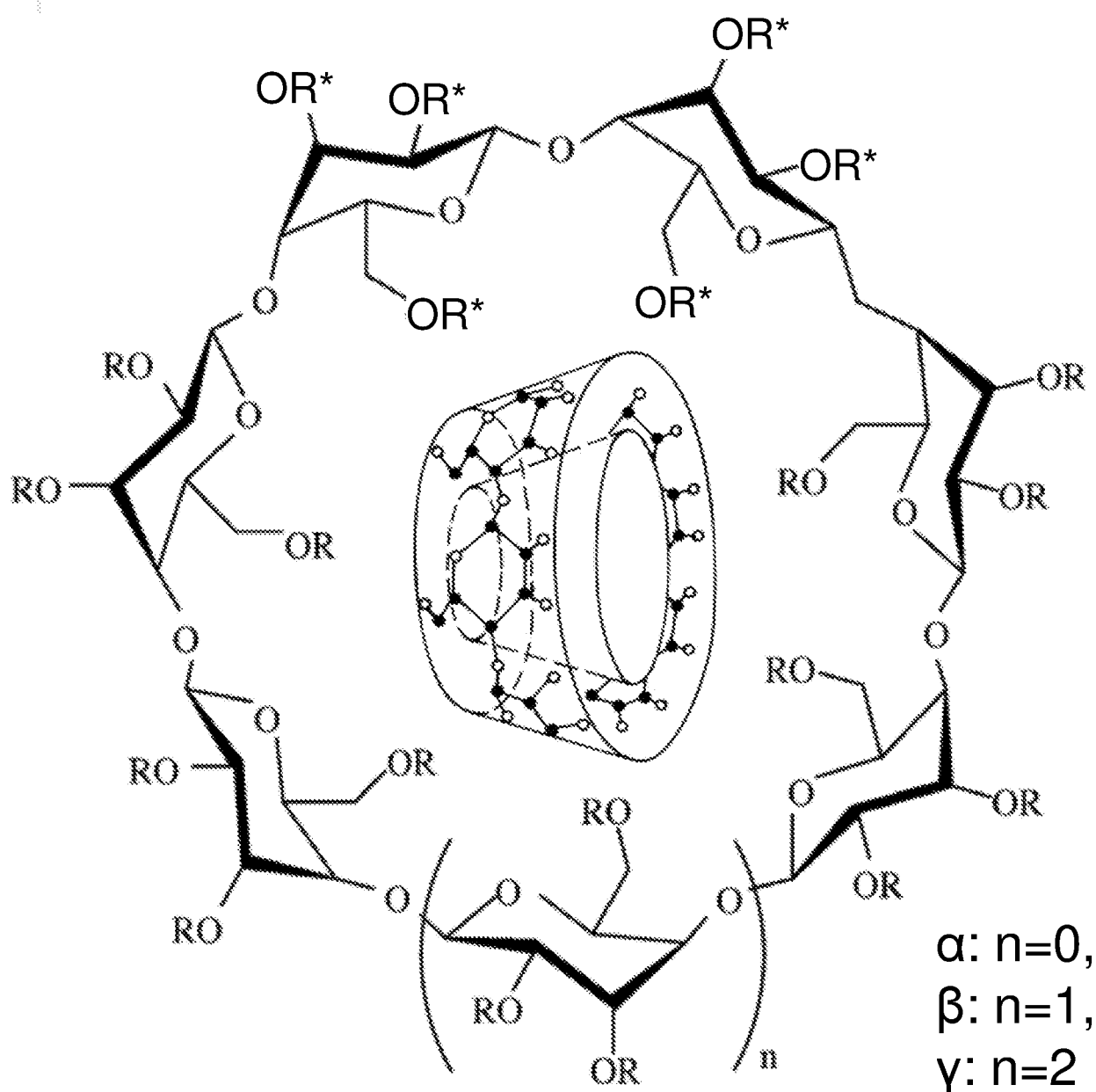
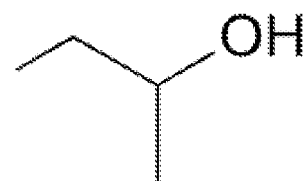


Fig. 1

Clustering Analysis

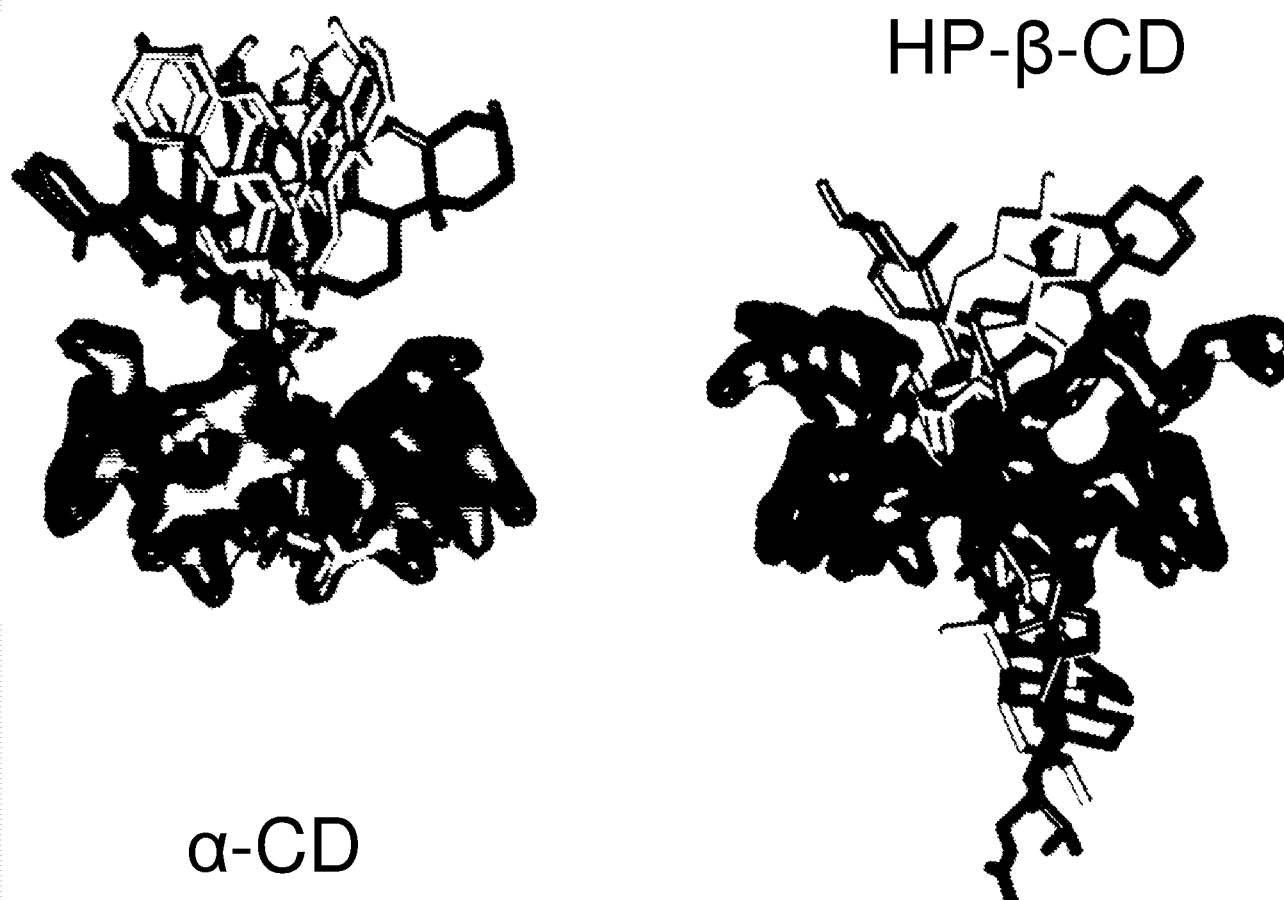


Fig. 2

CD Specificity of Lipid Release I

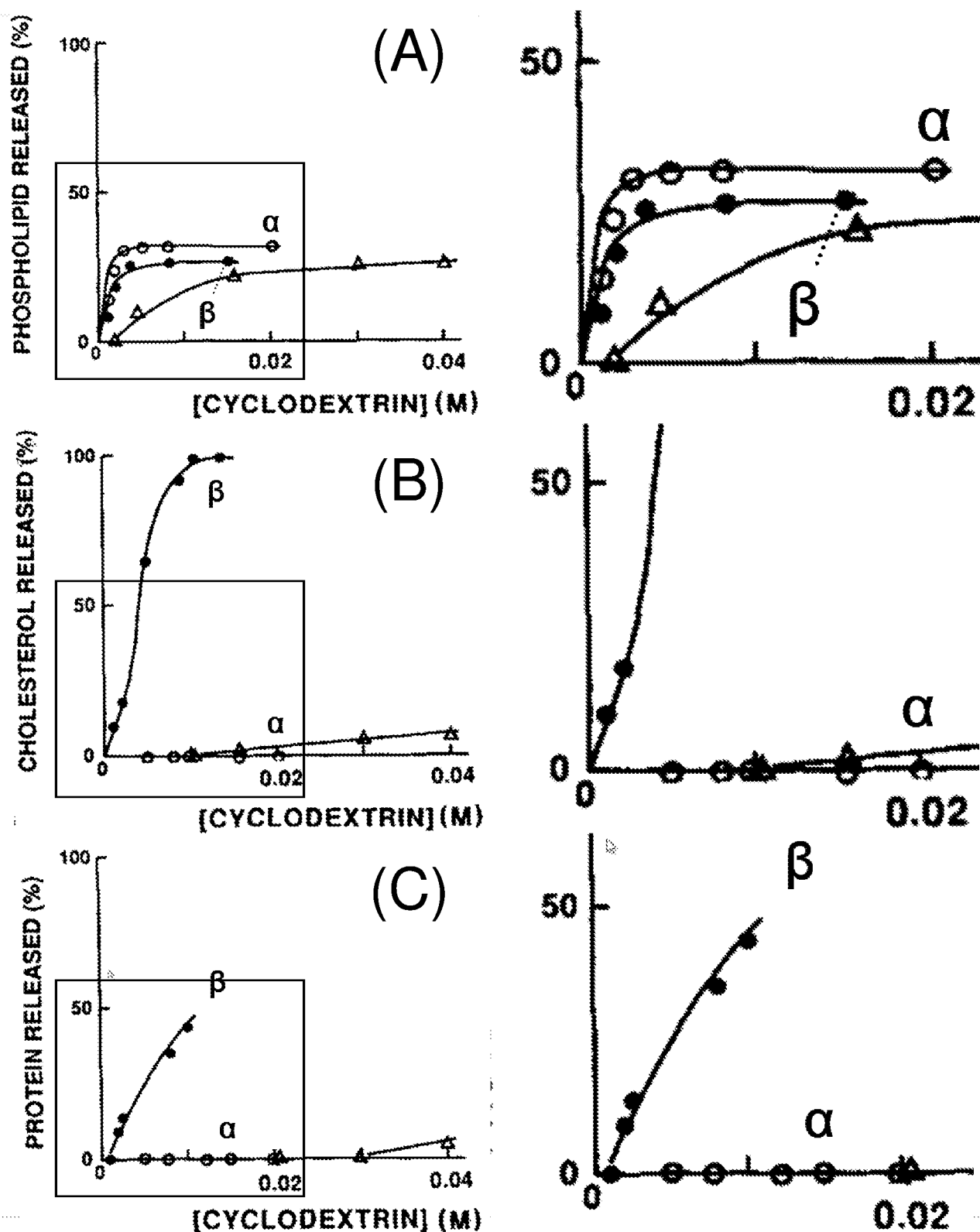


Fig. 3

CD Specificity of Lipid Release II

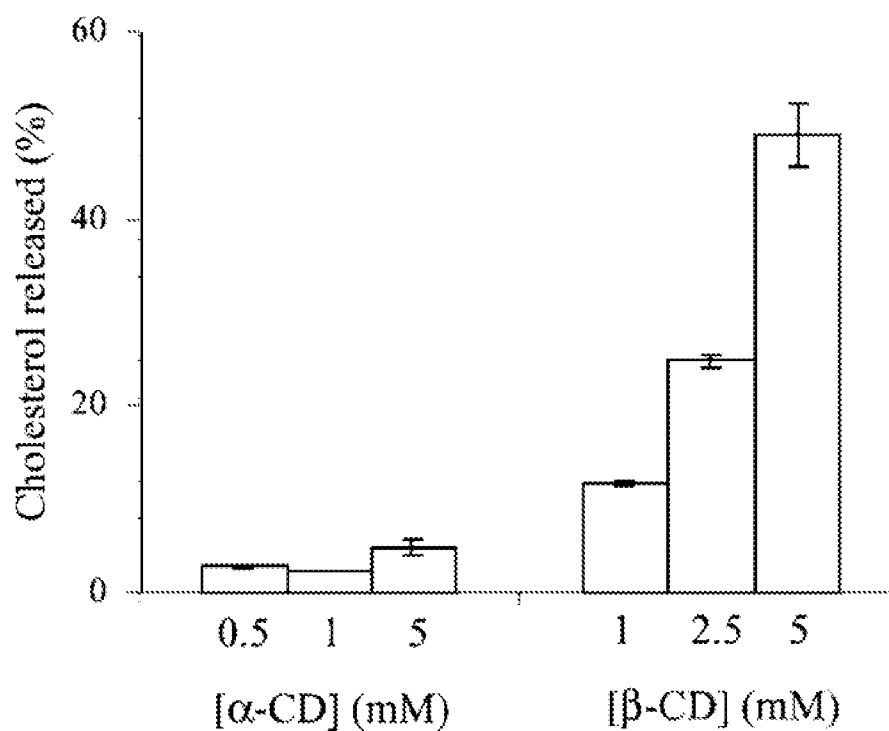


Fig. 4a

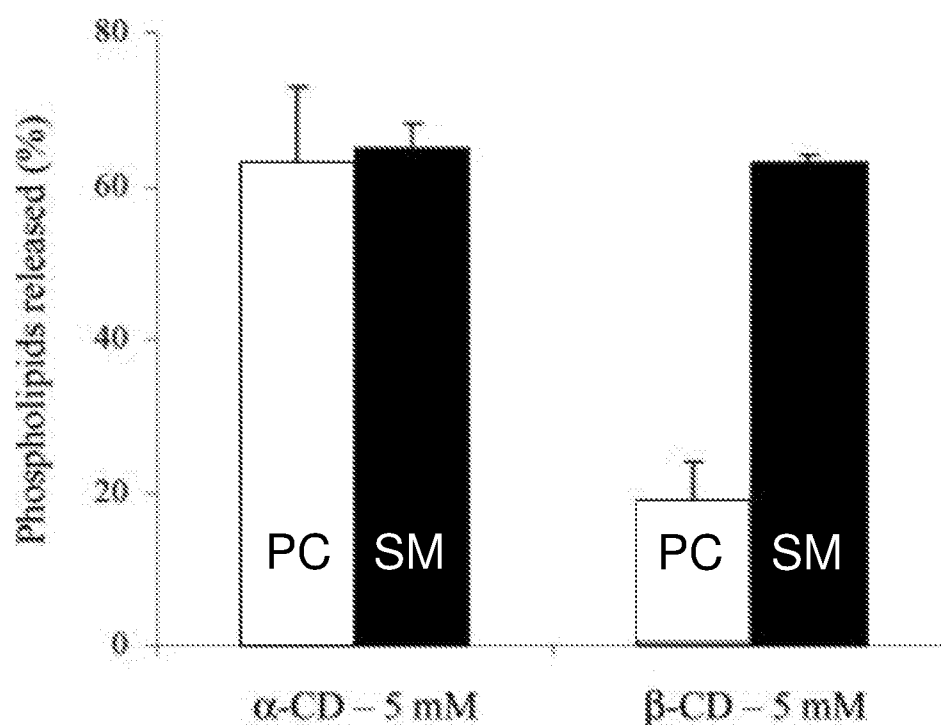


Fig. 4b

M β CD, but not α CD protect A2E

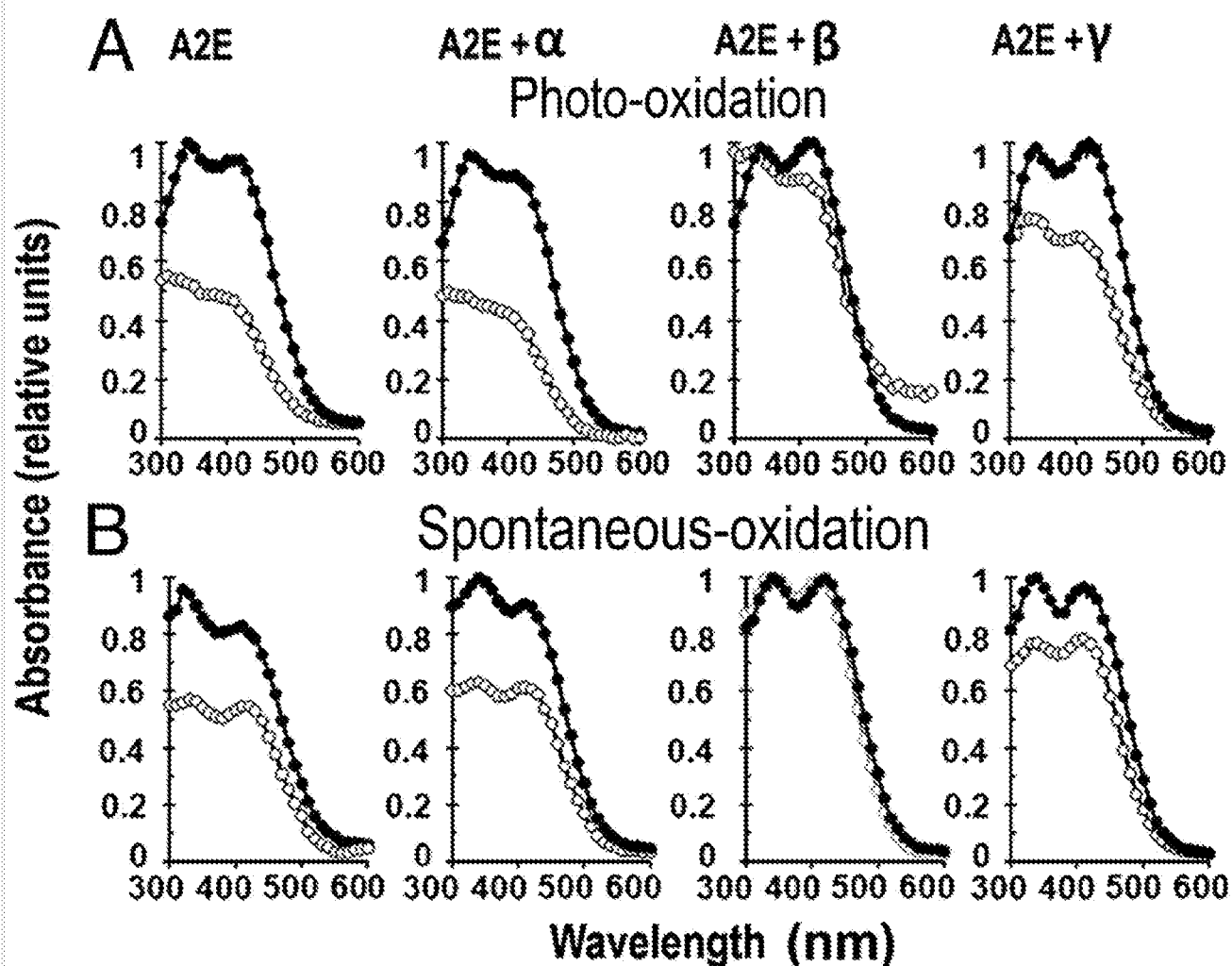


Fig. 5

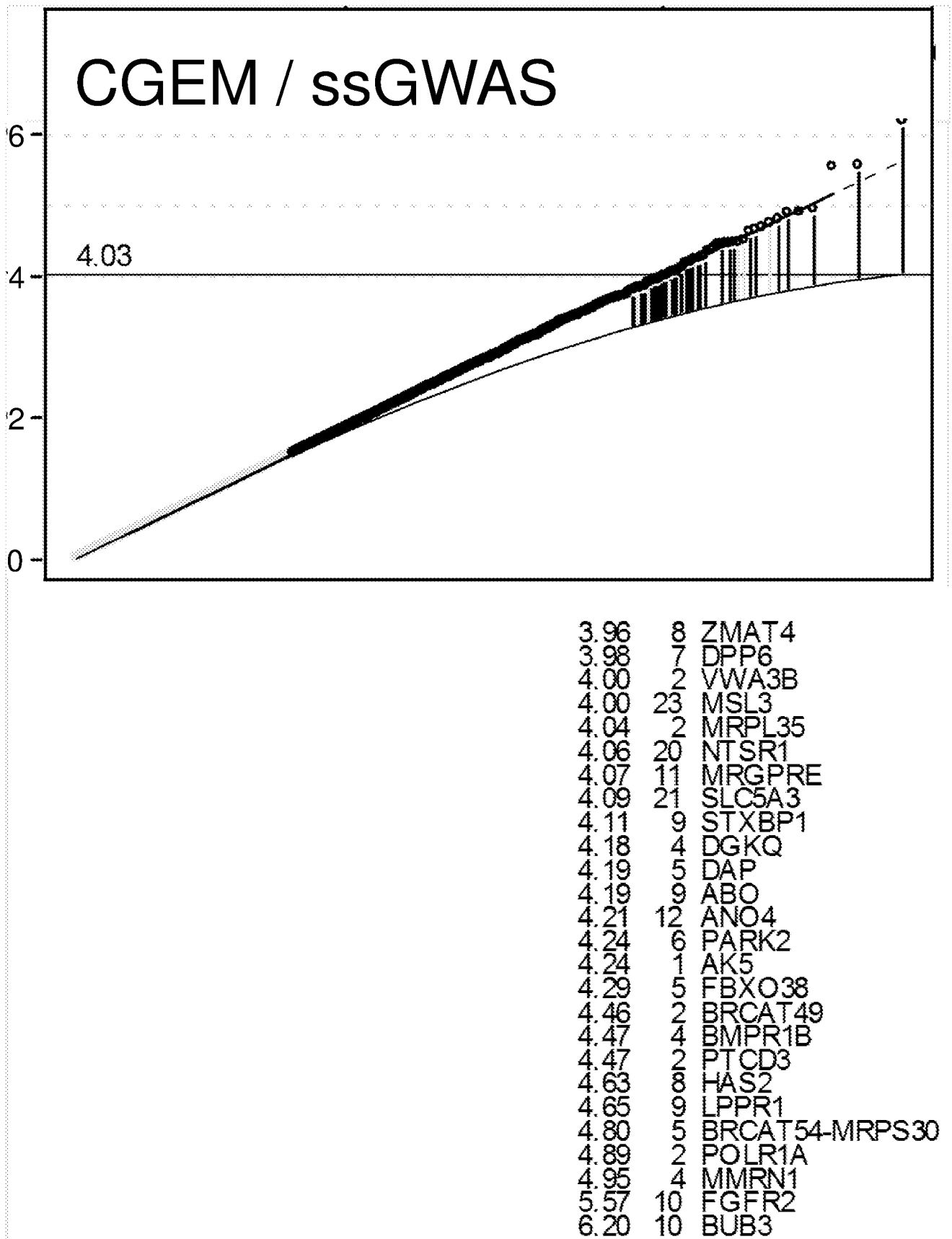


Fig. 6

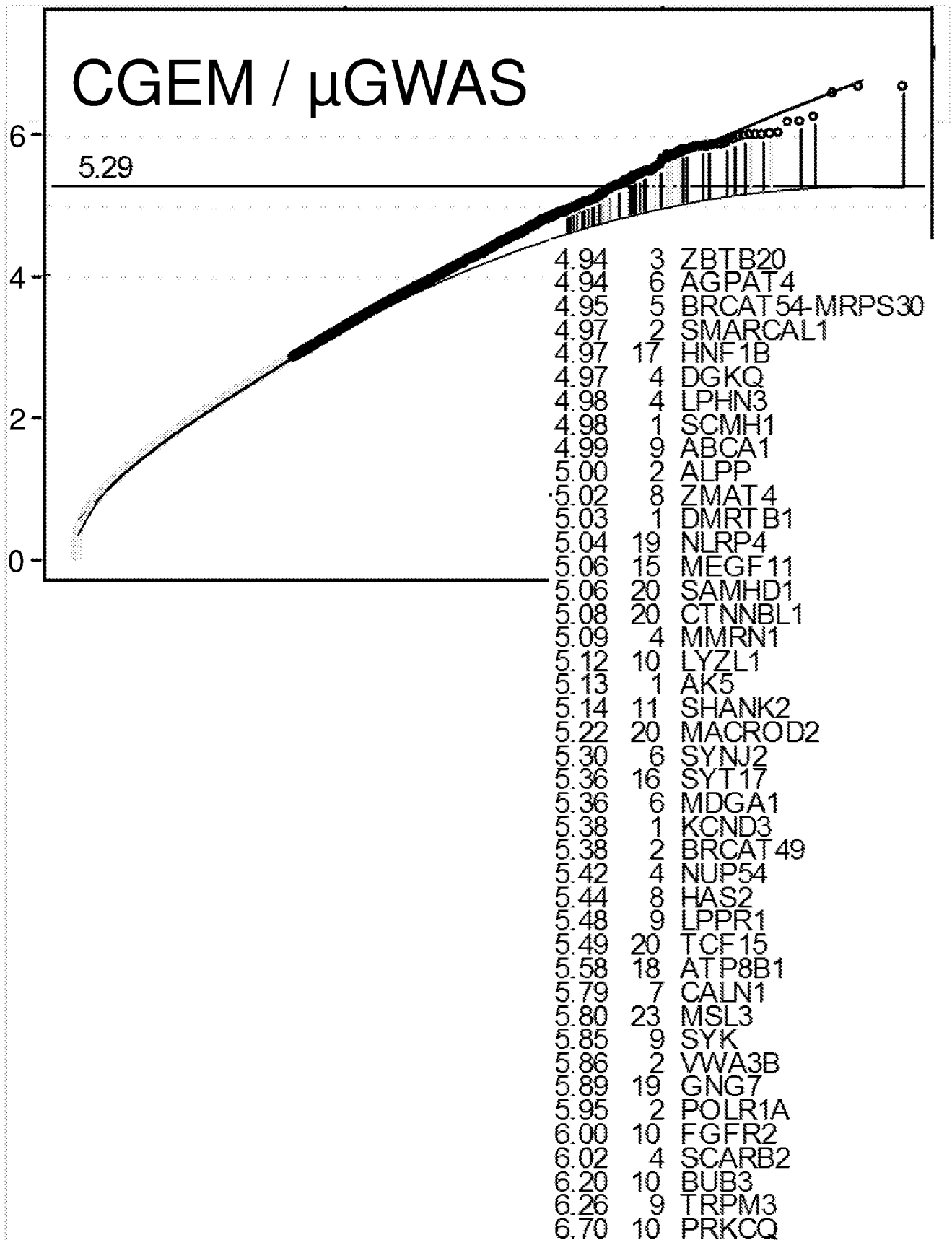


Fig. 7

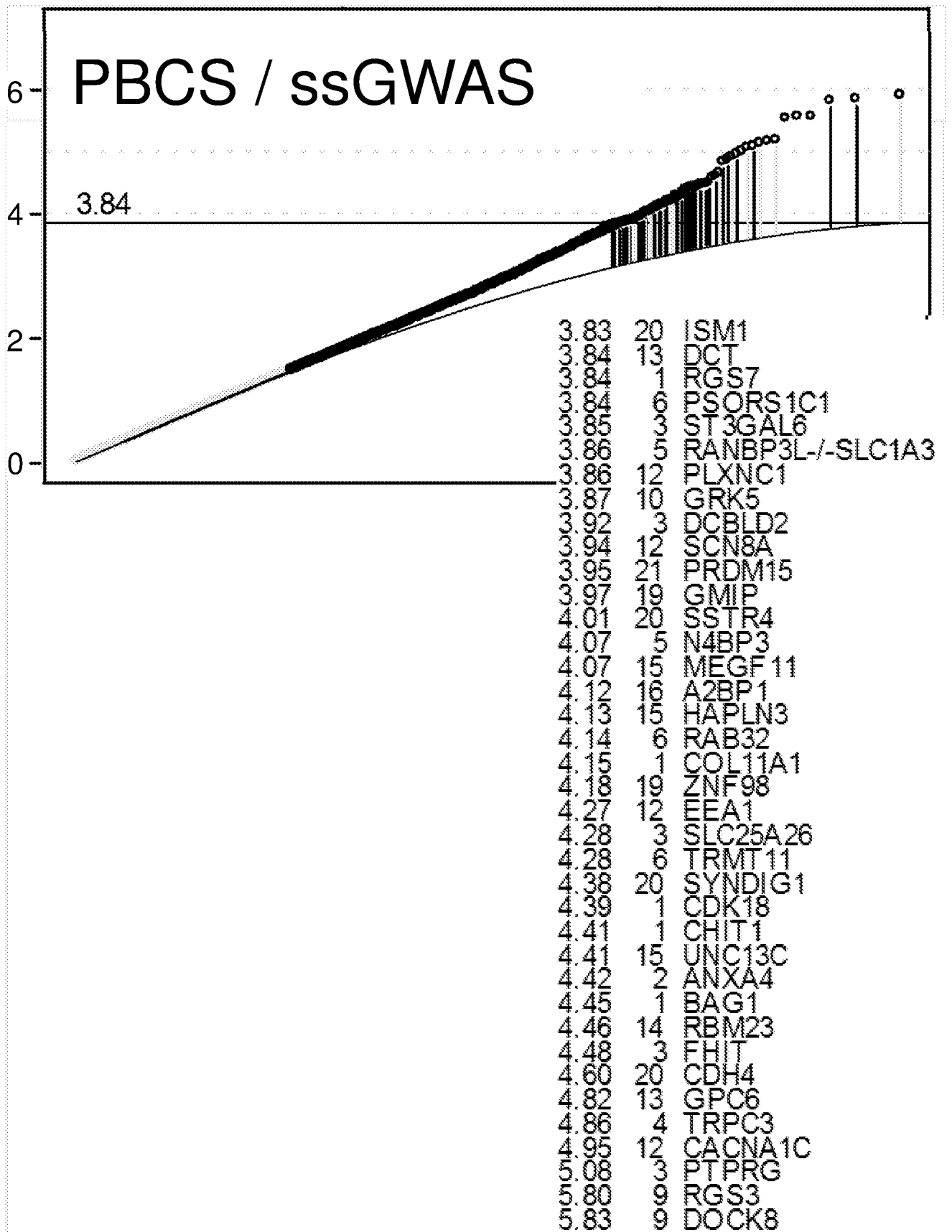


Fig. 8

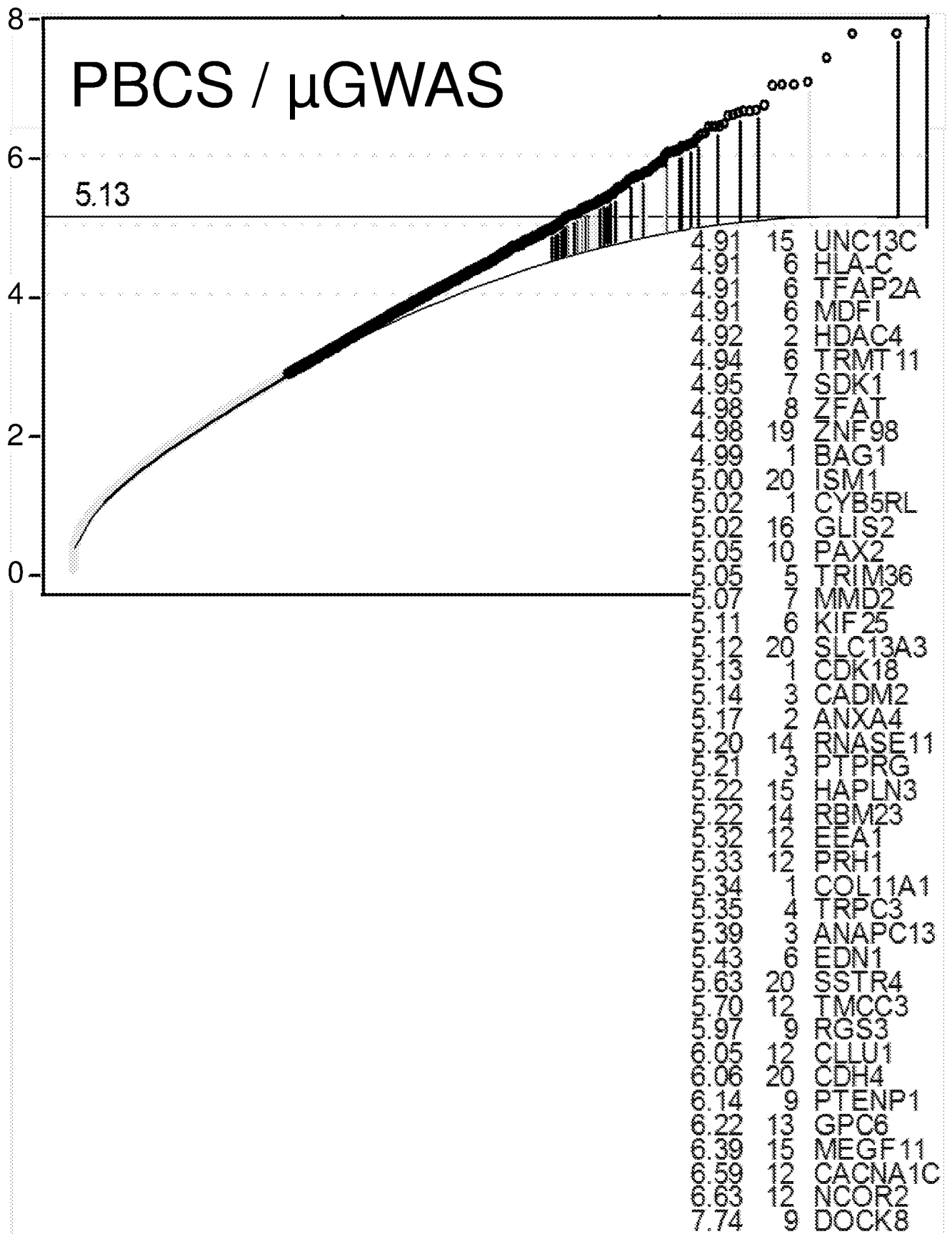


Fig. 9

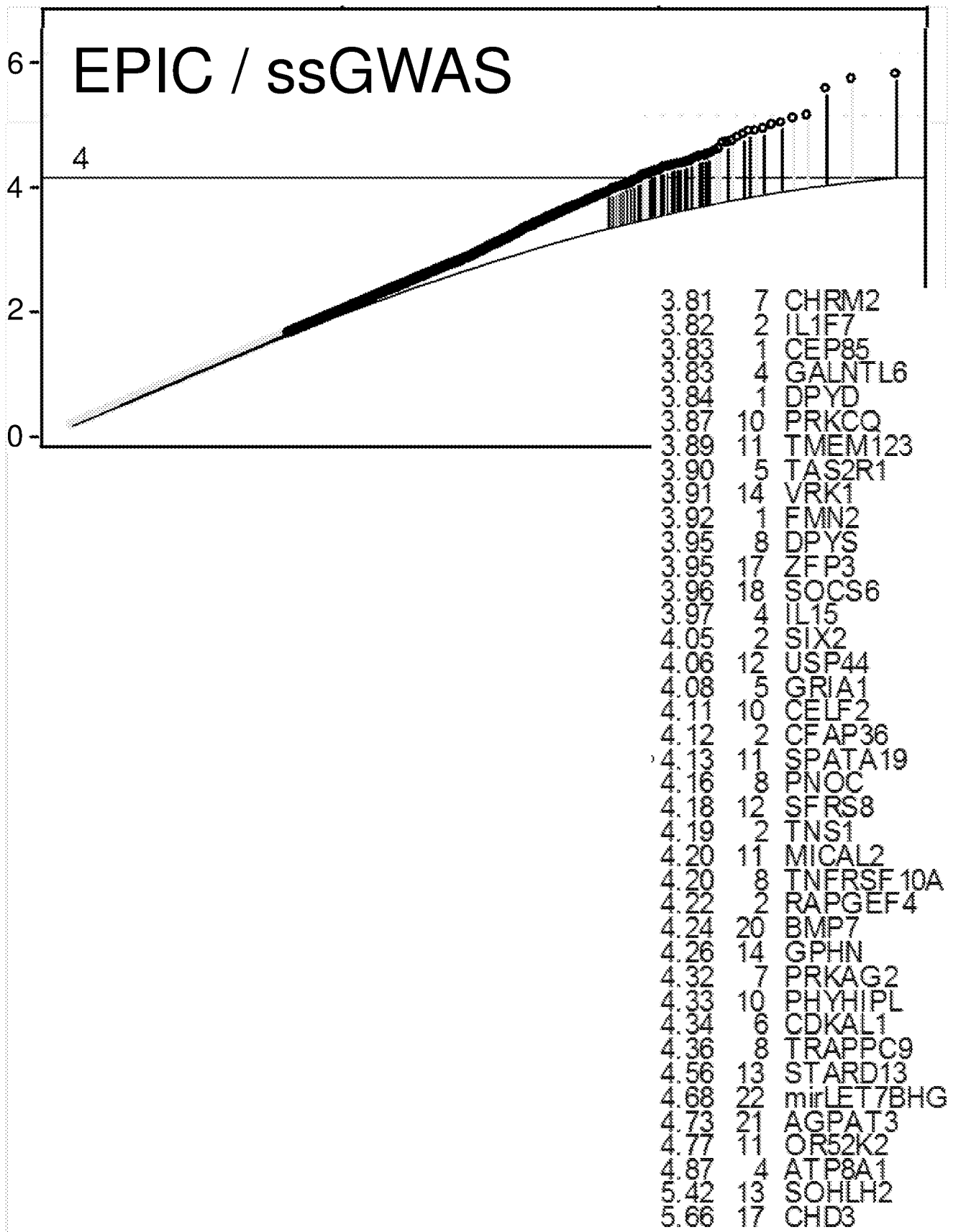


Fig. 10

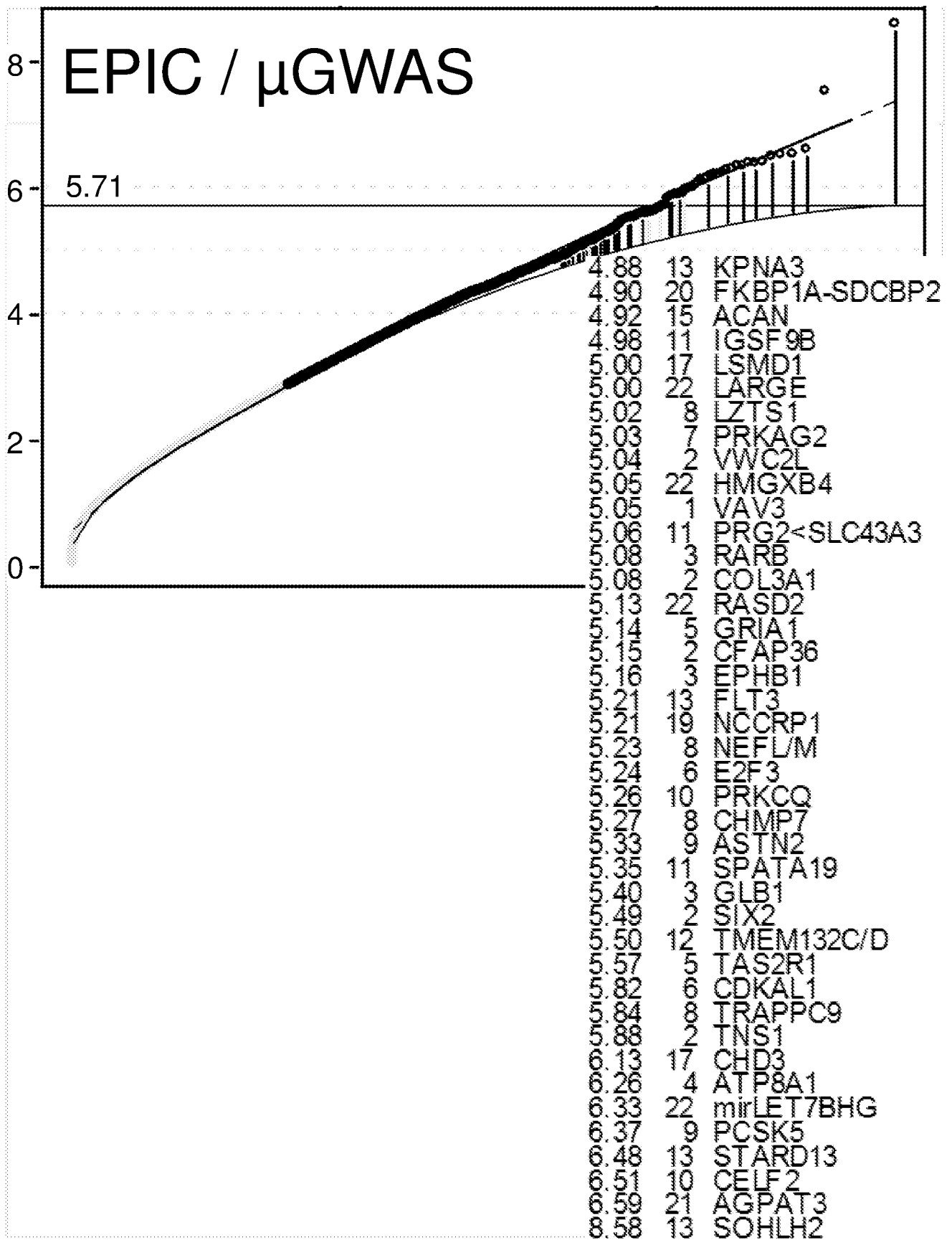


Fig. 11

PI Cycle

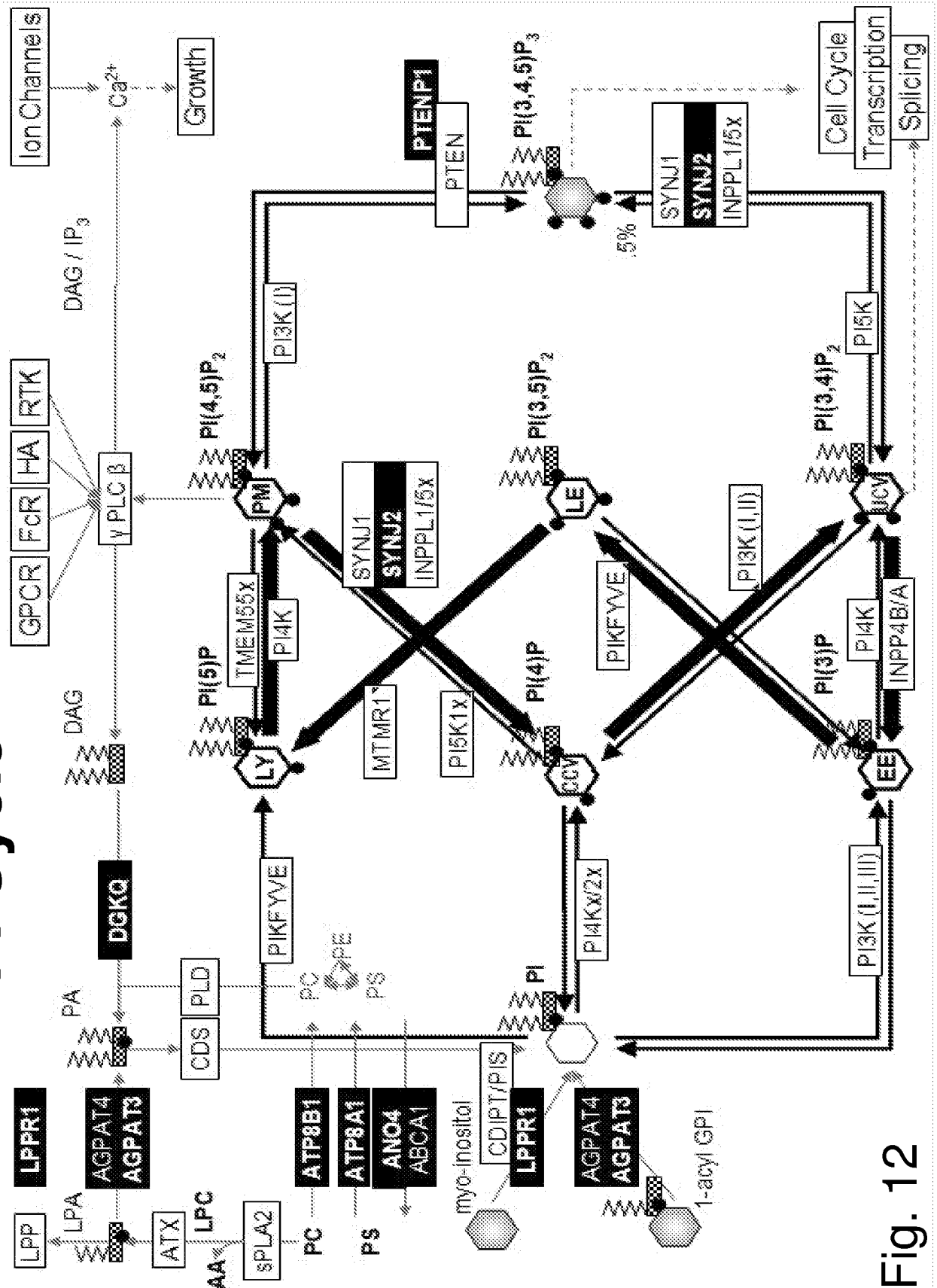


Fig. 12

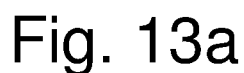


Fig. 13a

Exocytosis / Lysosomal Function

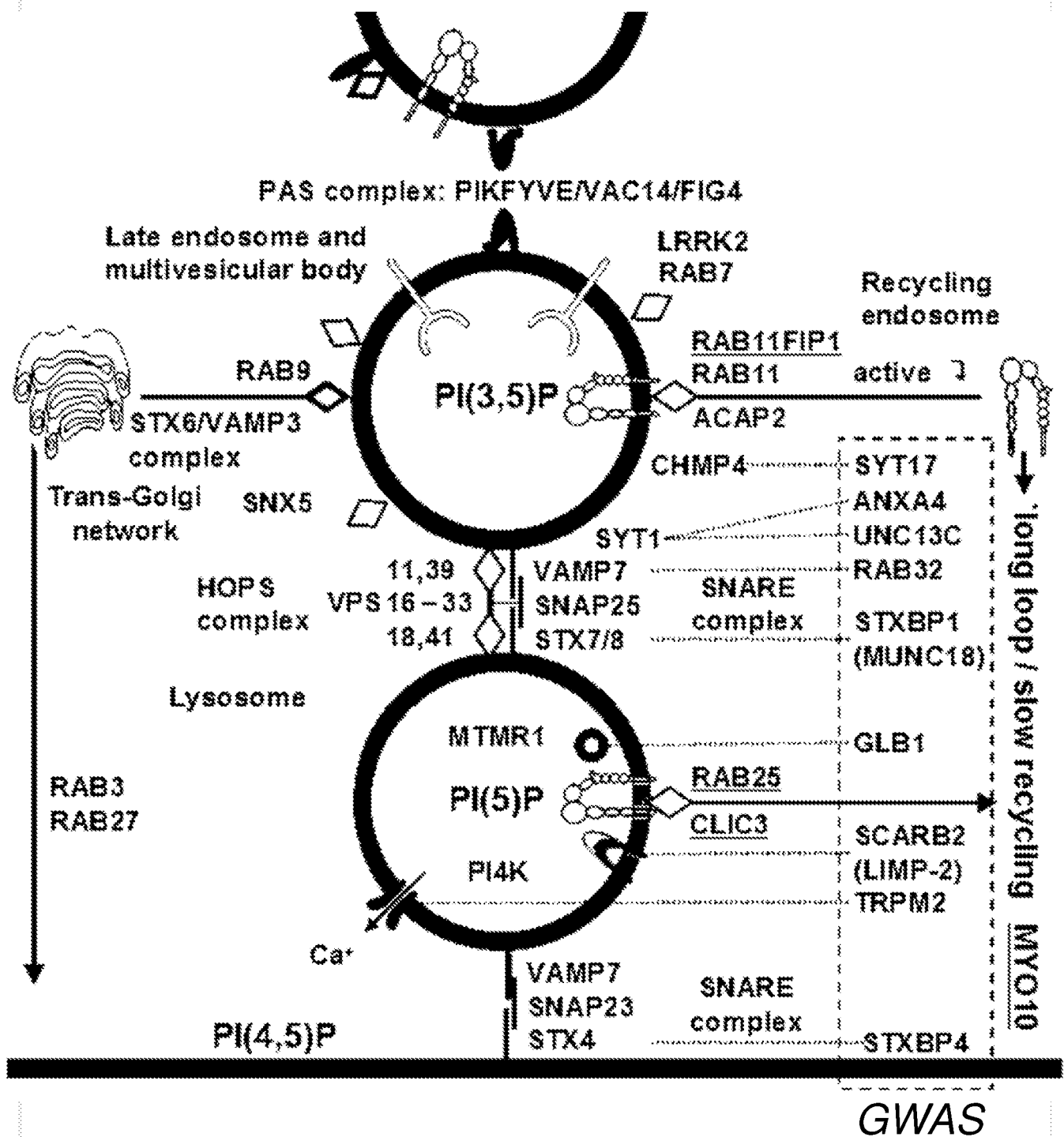


Fig. 13b

Endocytotic mechanism underlying tumor cell migration and invasion

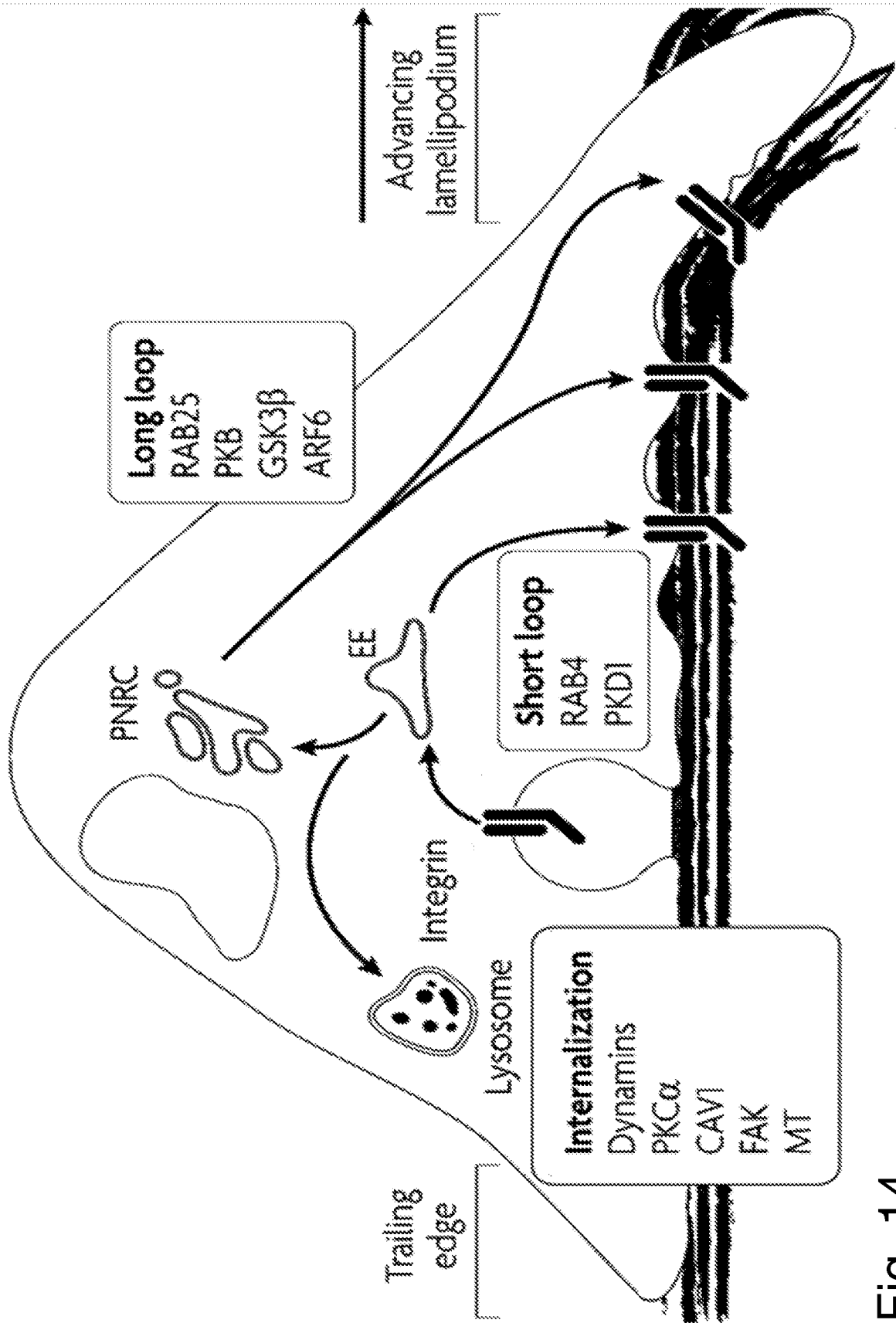


Fig. 14

Endocytosis in Alzheimer's Disease

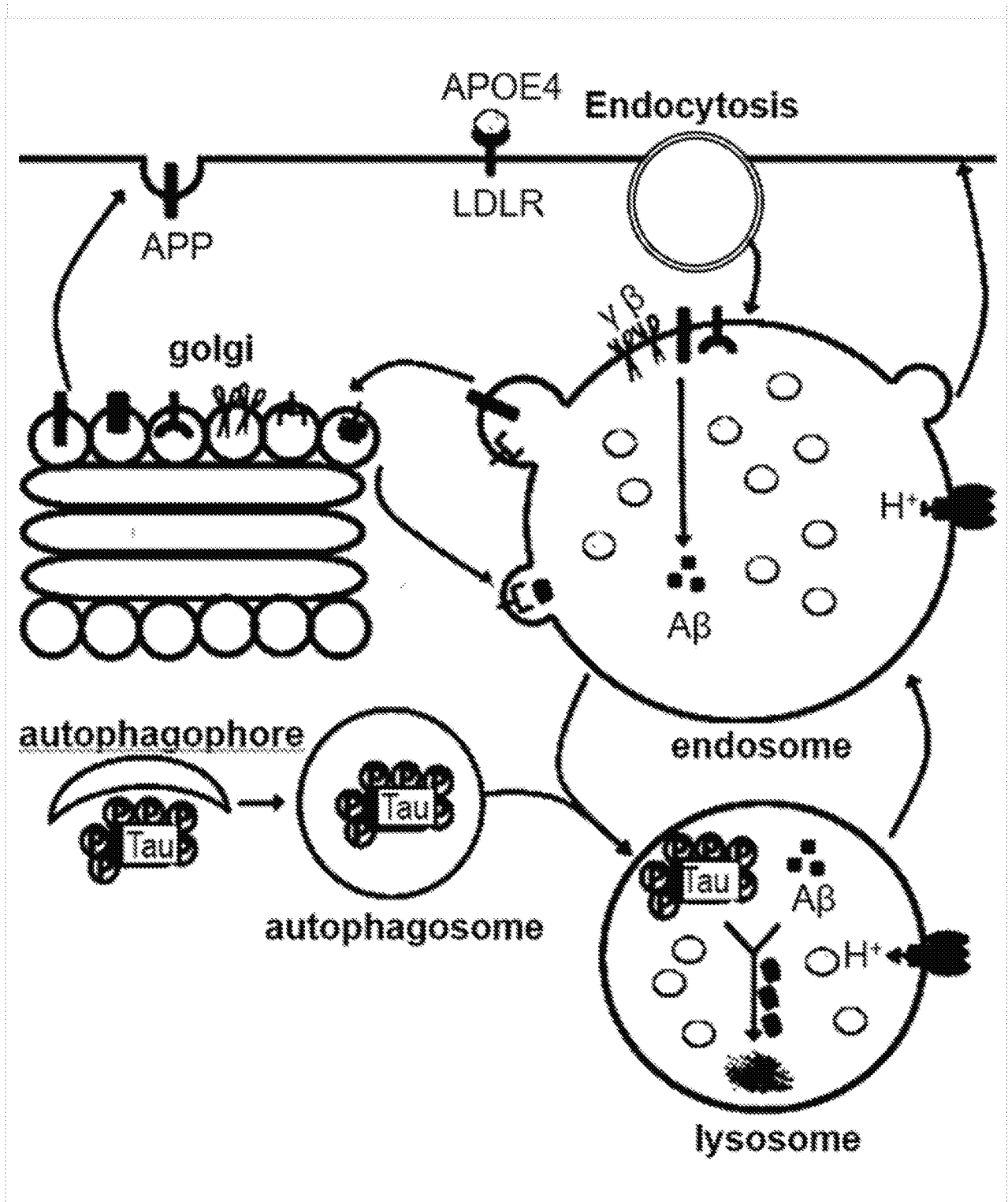


Fig. 15

Endocytosis in Parkinson's Disease

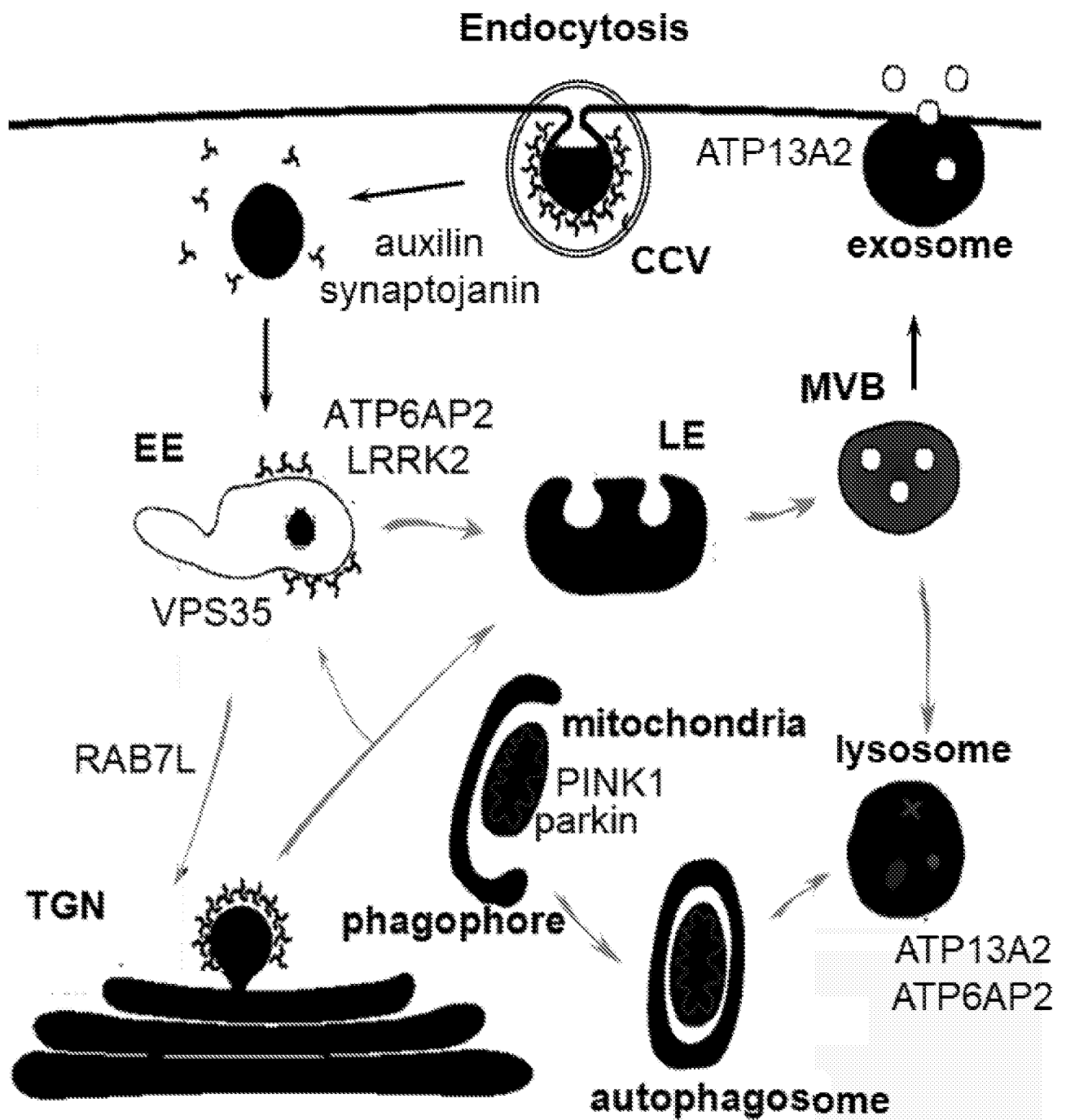


Fig. 16

Macropinocytosis in Atherosclerosis

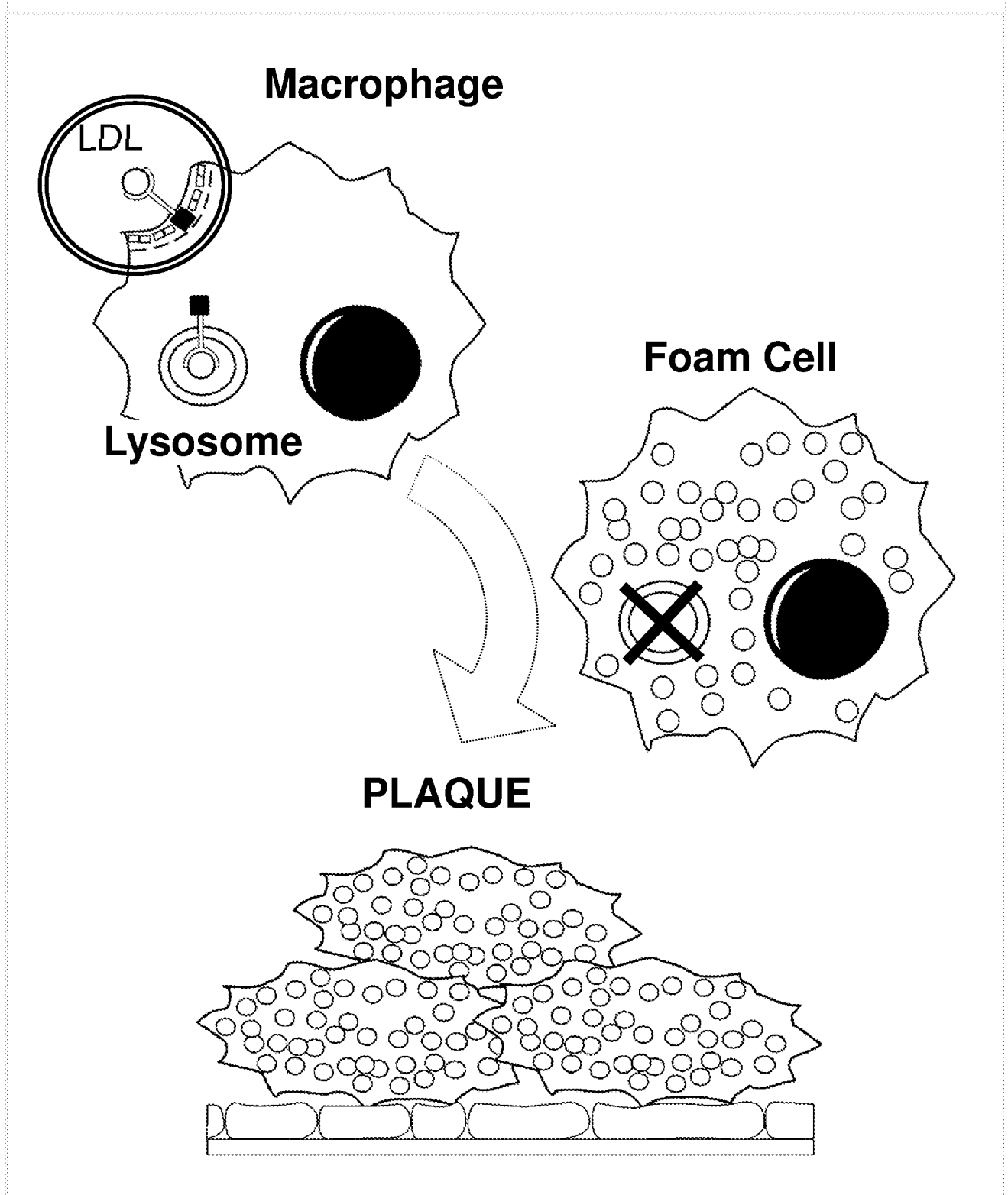


Fig. 17

Lysosomal Dysfunction in Cancer

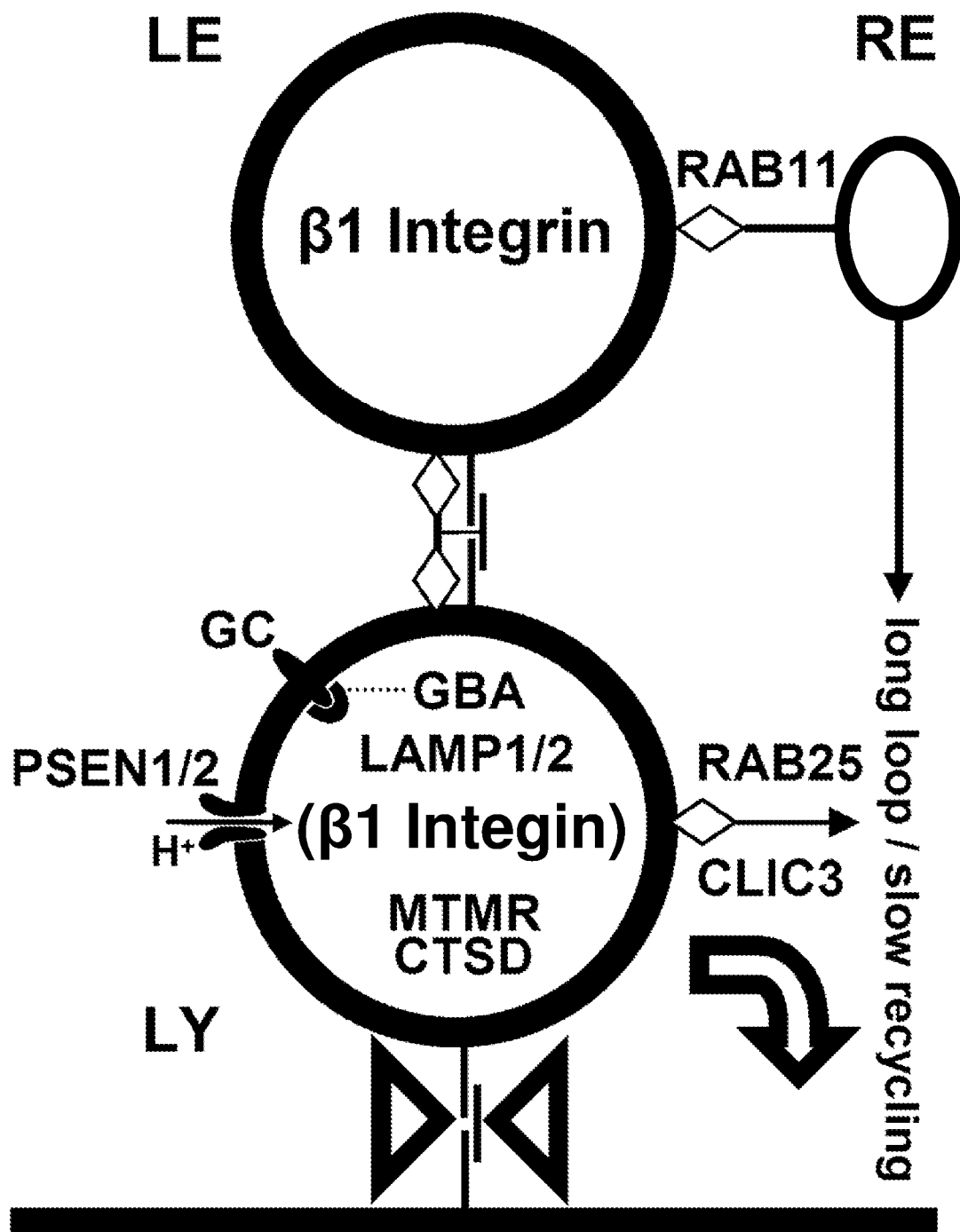


Fig. 18a

Lysosomal Dysfunction in AD/PD

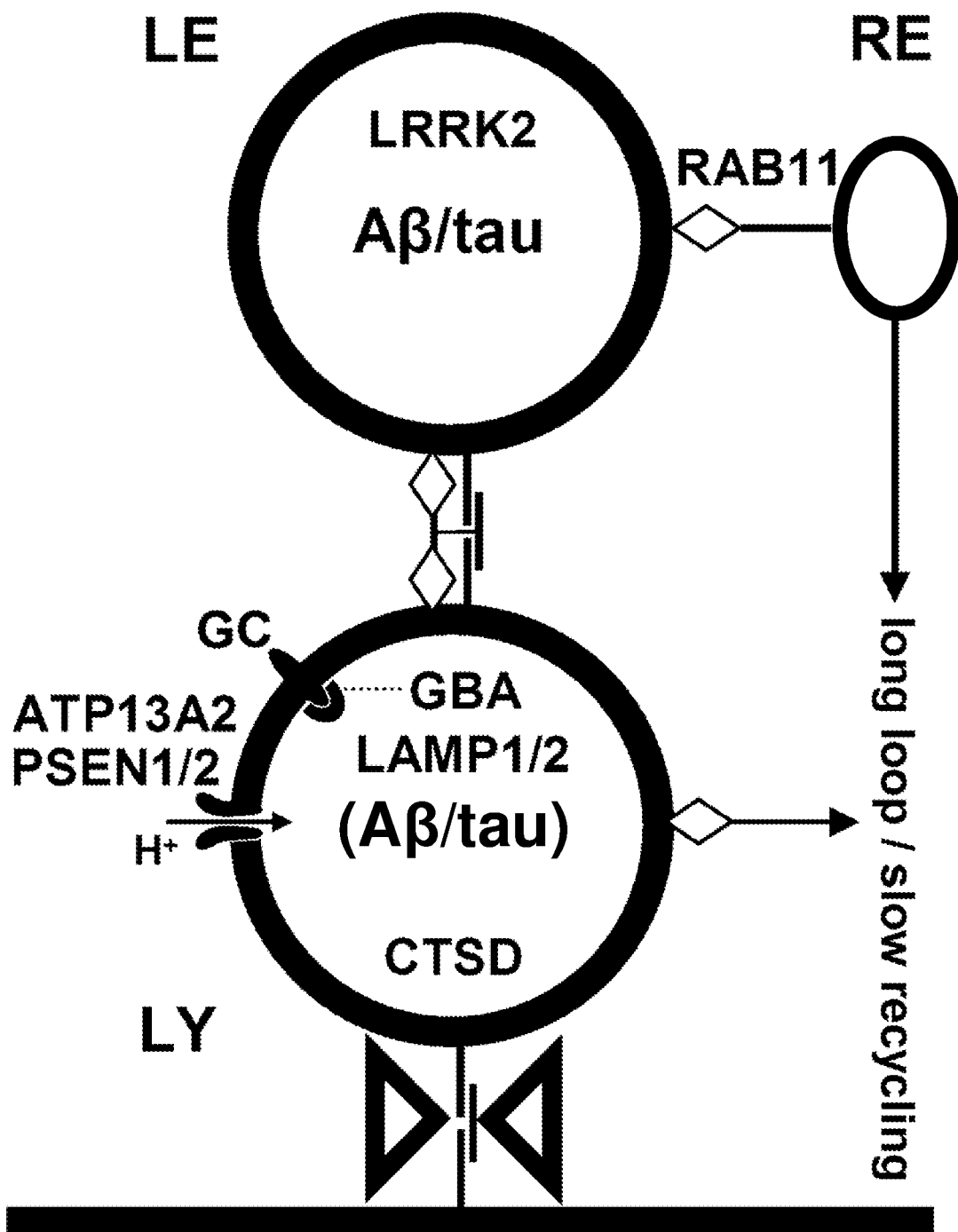


Fig. 18b

Lysosomal Dysfunction in CAD

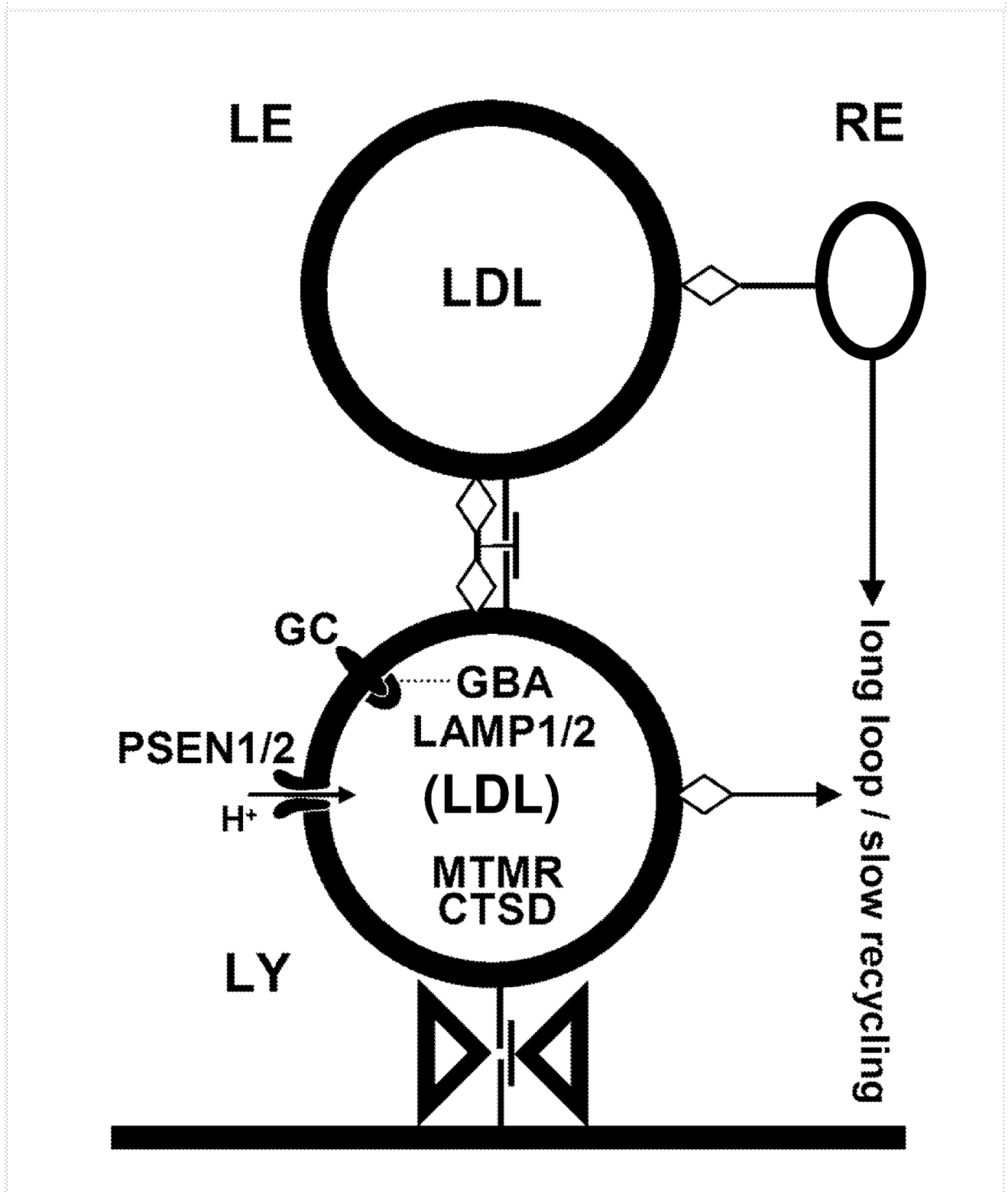


Fig. 18c

Viruses “Hijacking” Endocytosis

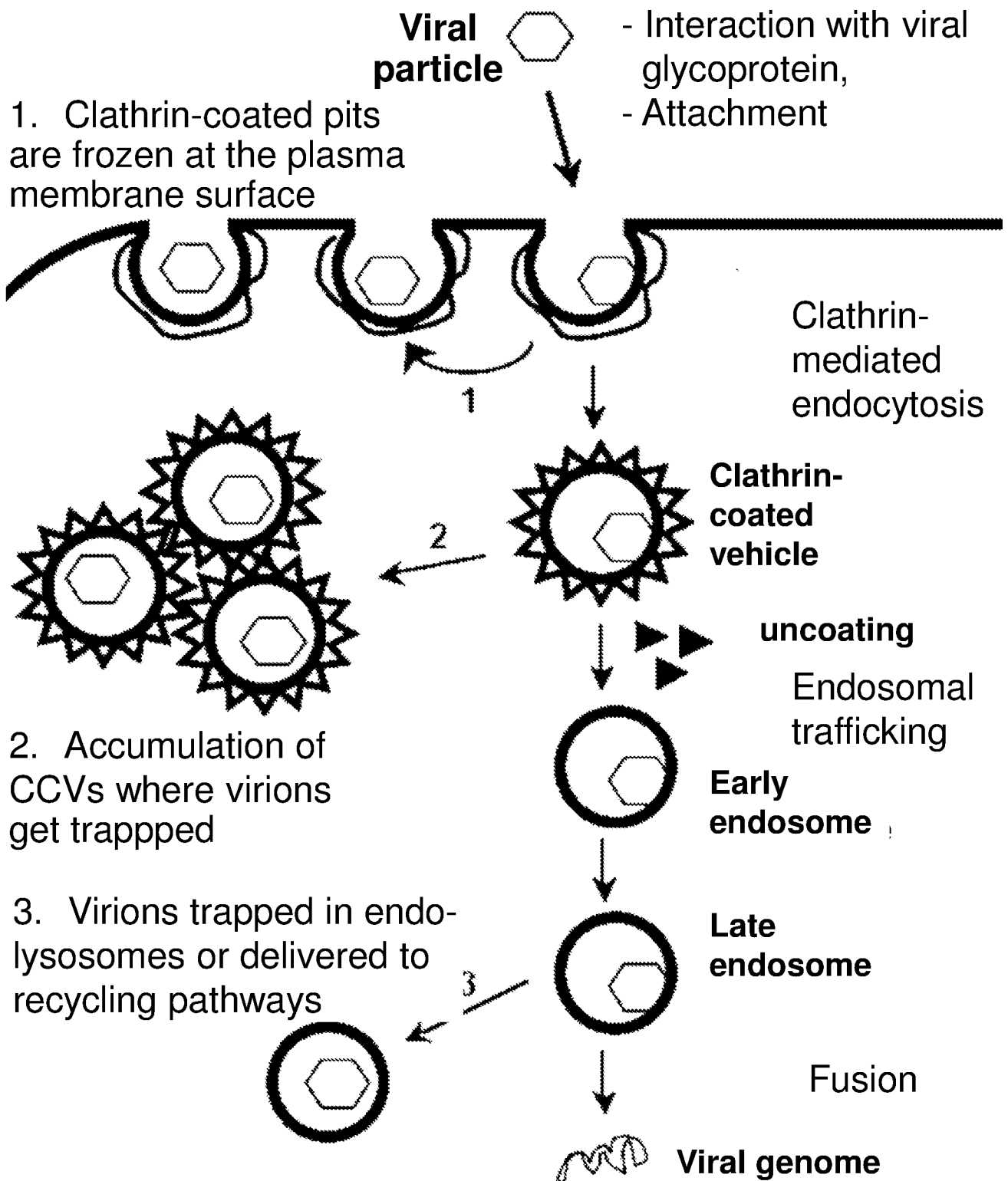


Fig. 19

M β CD in AD Mouse Cells

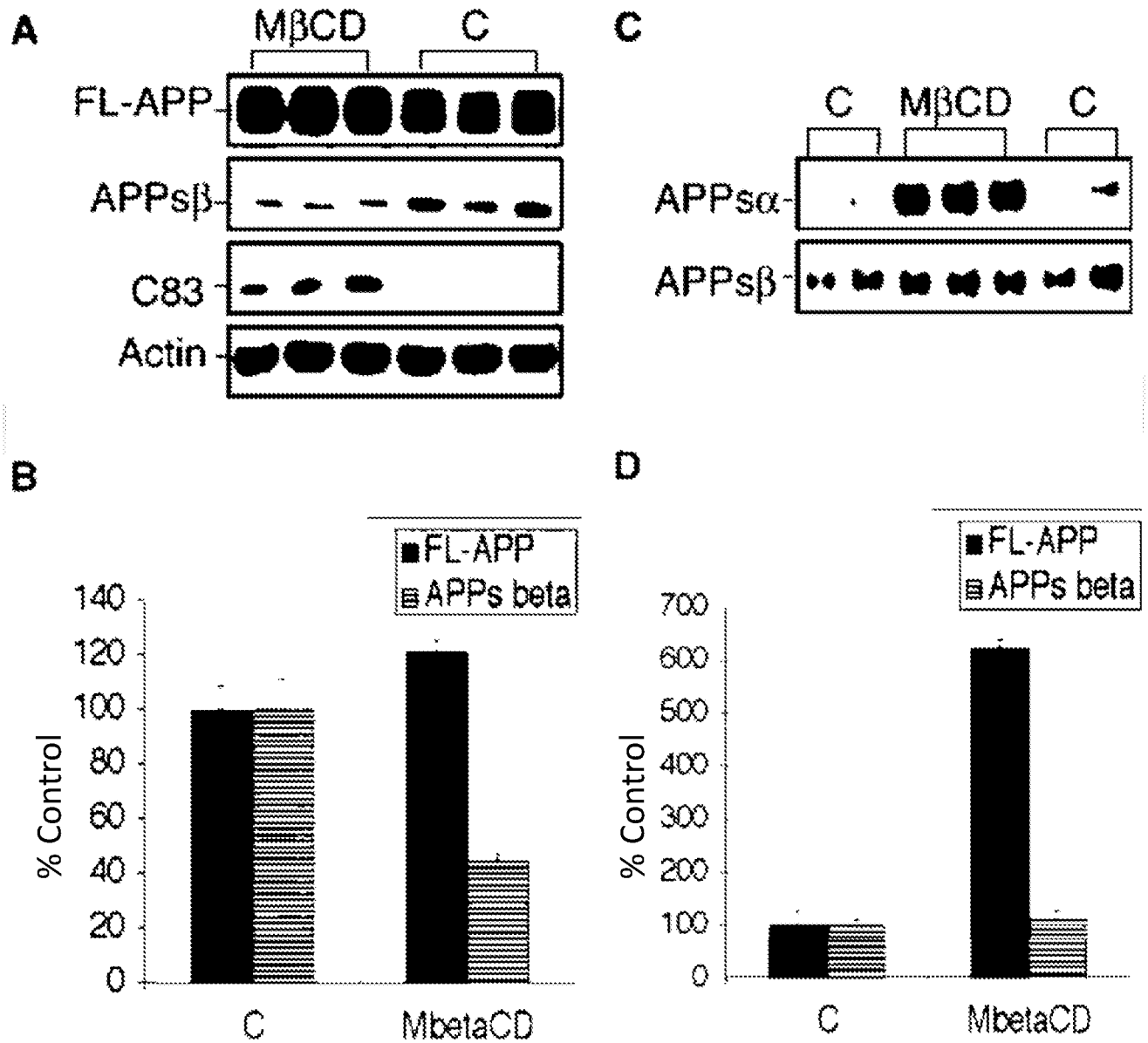


Fig. 20

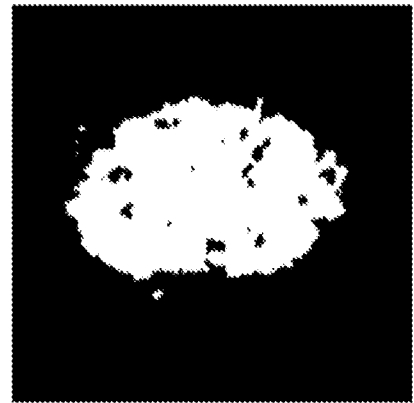
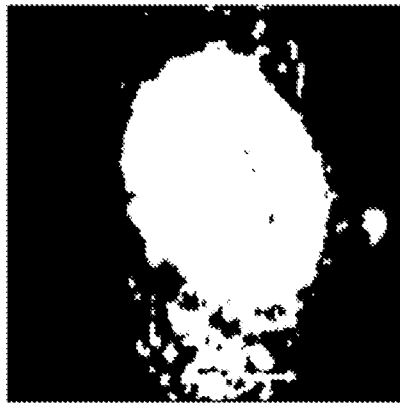
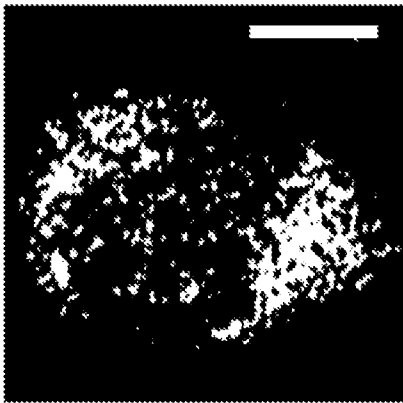
HP β CD Clearance of α -Syn

A: TFEB

Control

HP β CD

HP β CD-
cholesterol



B: α -syn-GFP

Control

HP β CD

HP β CD-
cholesterol

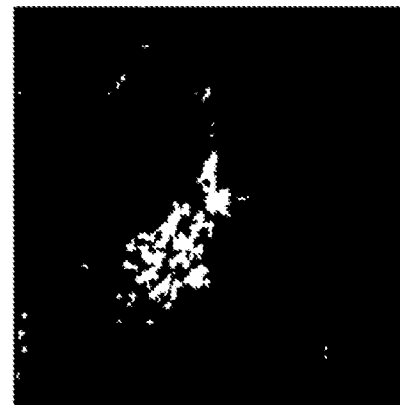
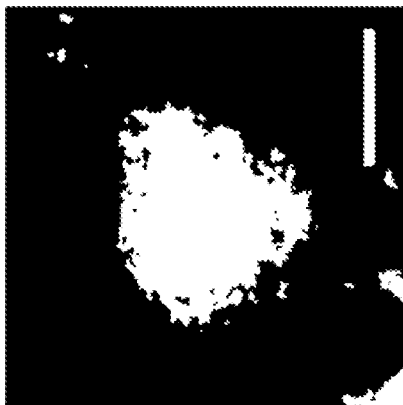


Fig. 21

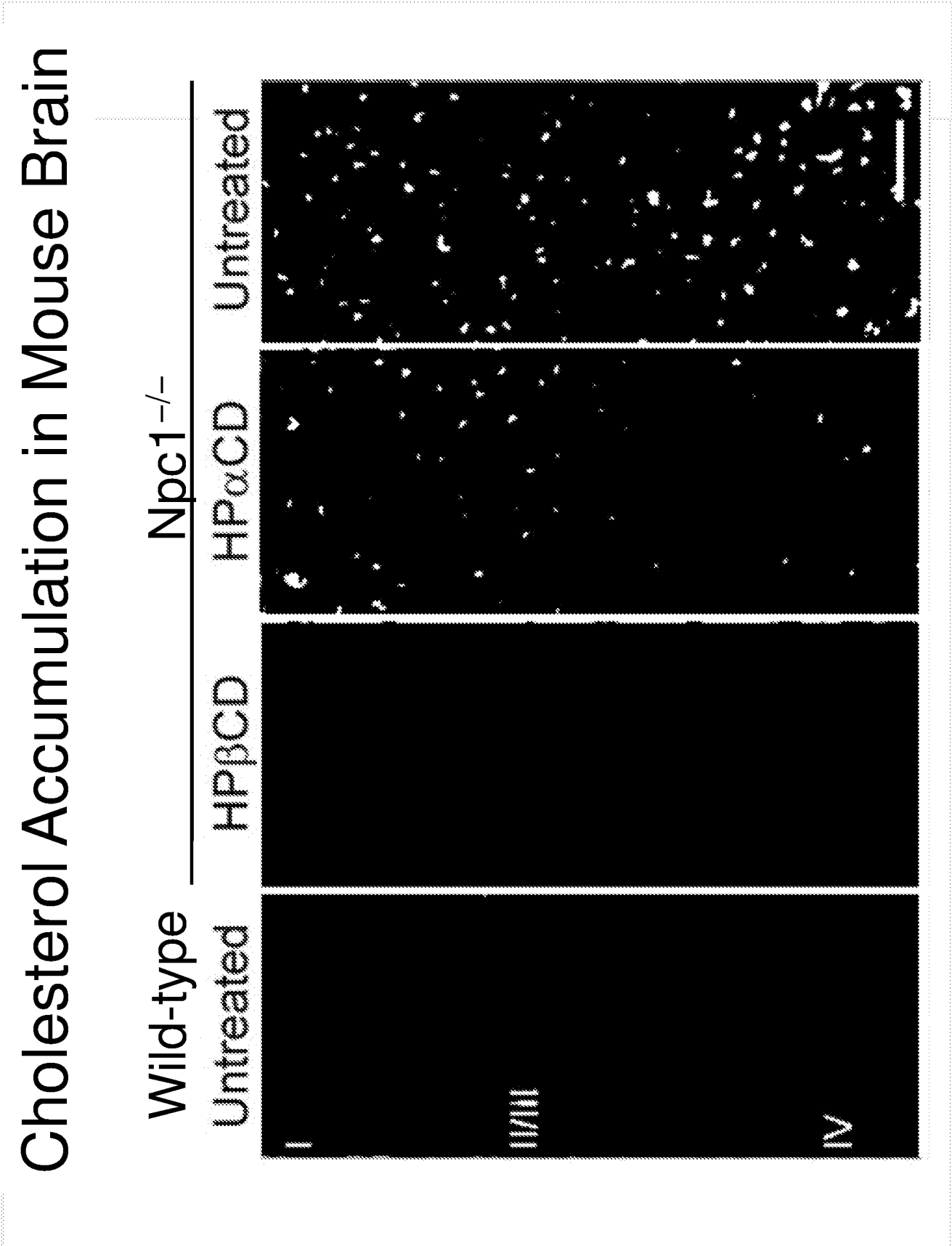


Fig. 22

CDs in Lysosomal Storage Diseases

(US 2015/0216895 A1, Fig 5)

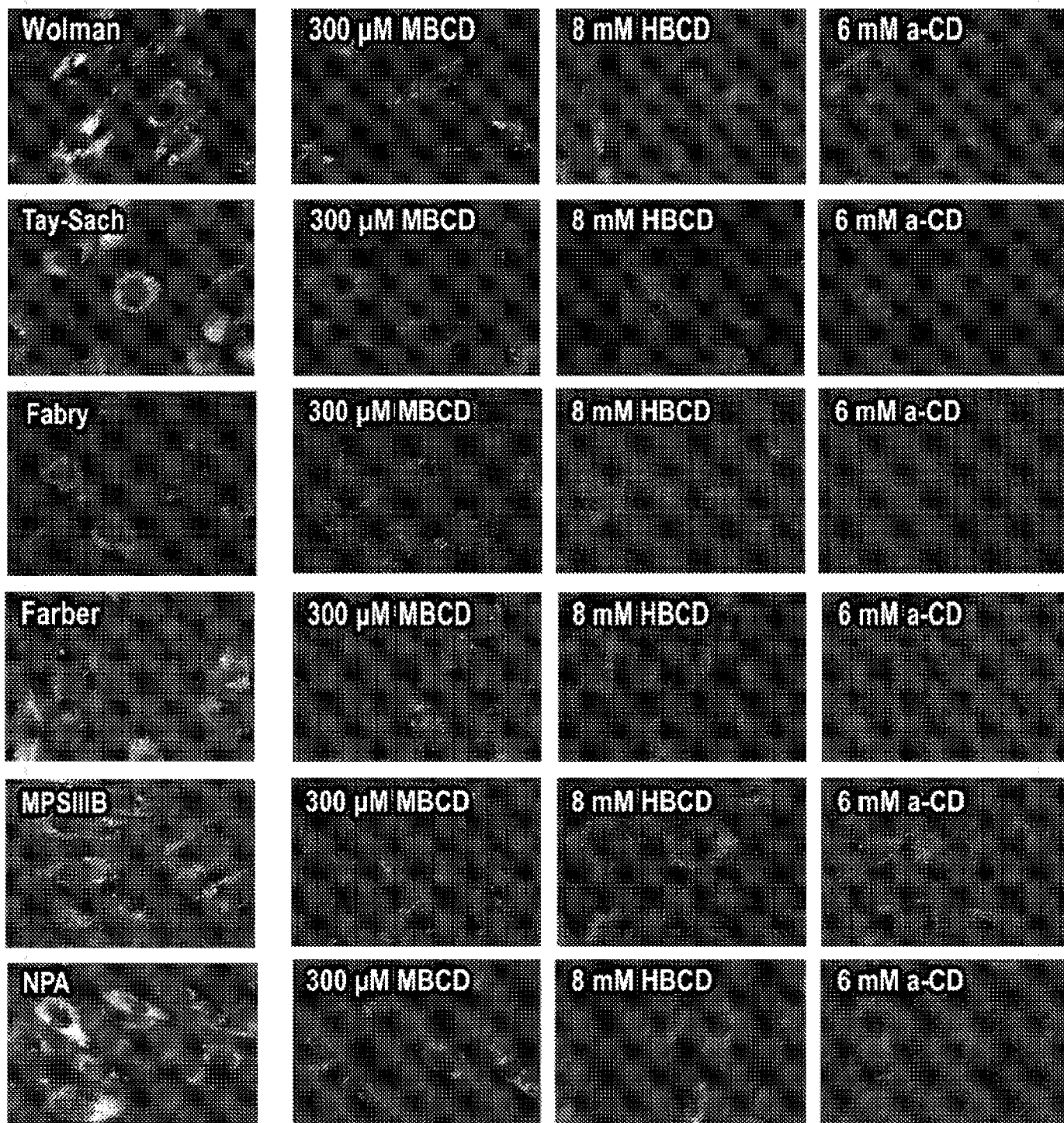


Fig. 23

Pino-/Phago-/Endocytosis

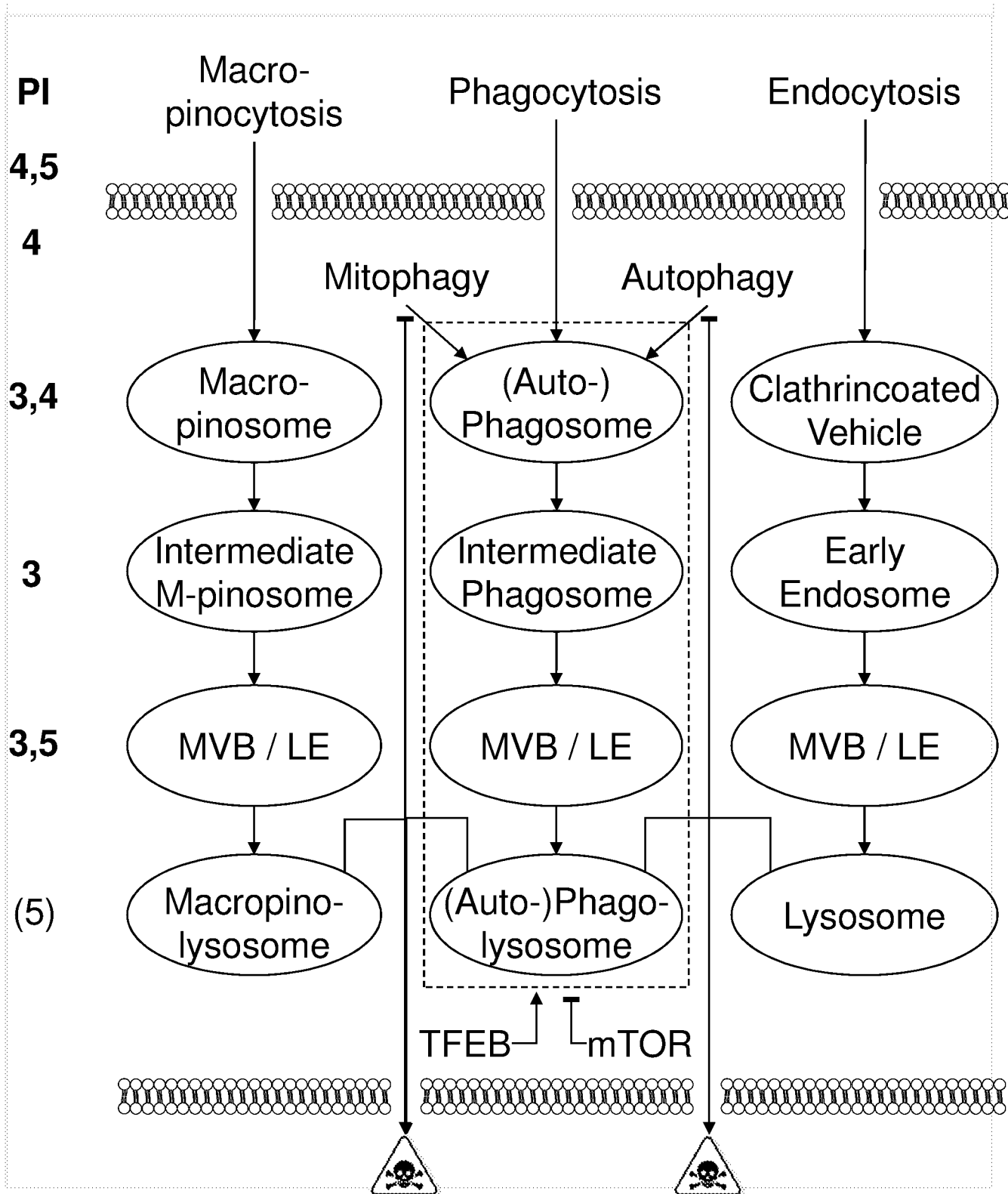


Fig. 24

HP β CD Activates Autophagy

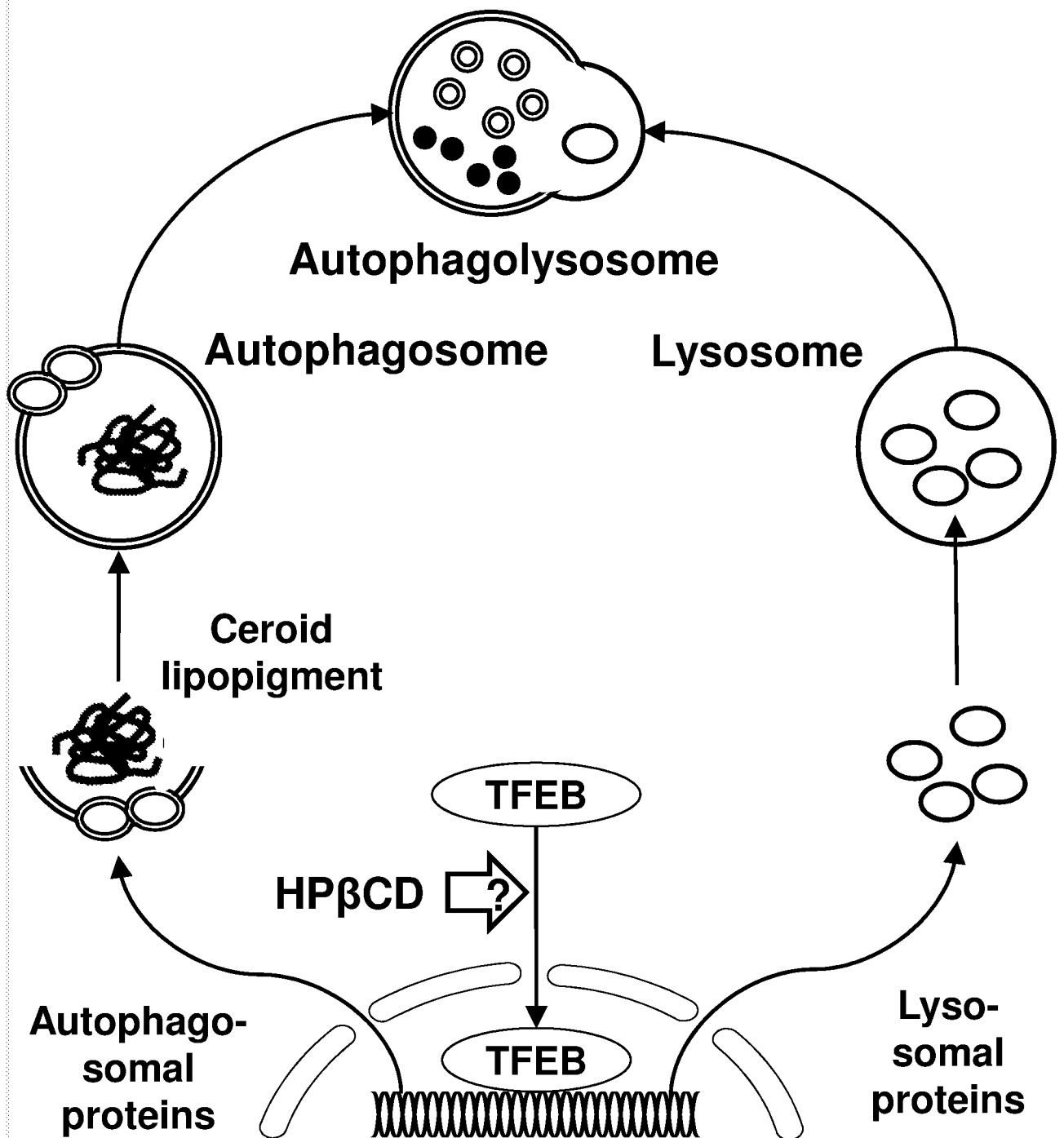


Fig. 25

HP α CD Restores LY Function

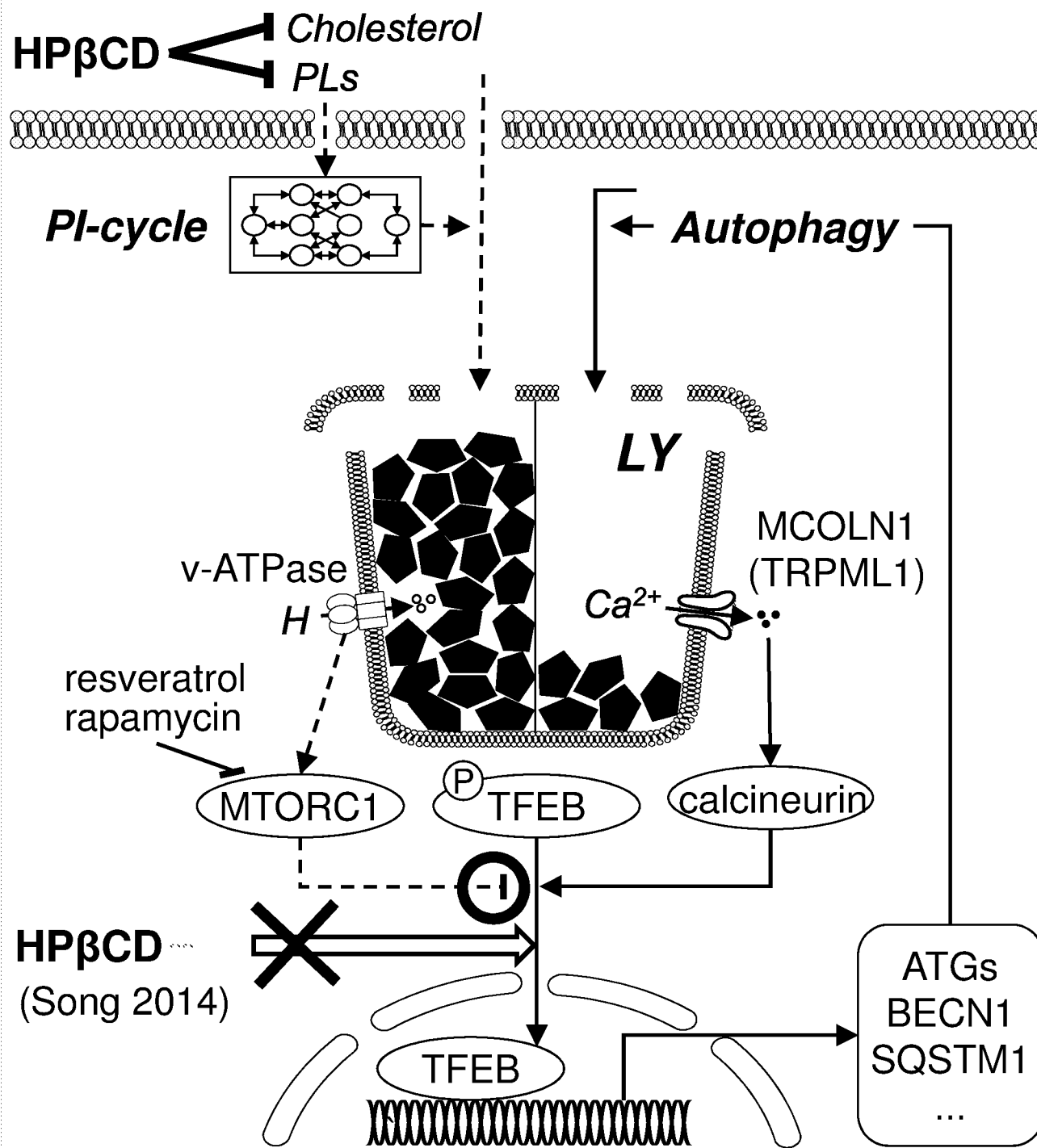


Fig. 26

Phago-/Endocytosis in MS

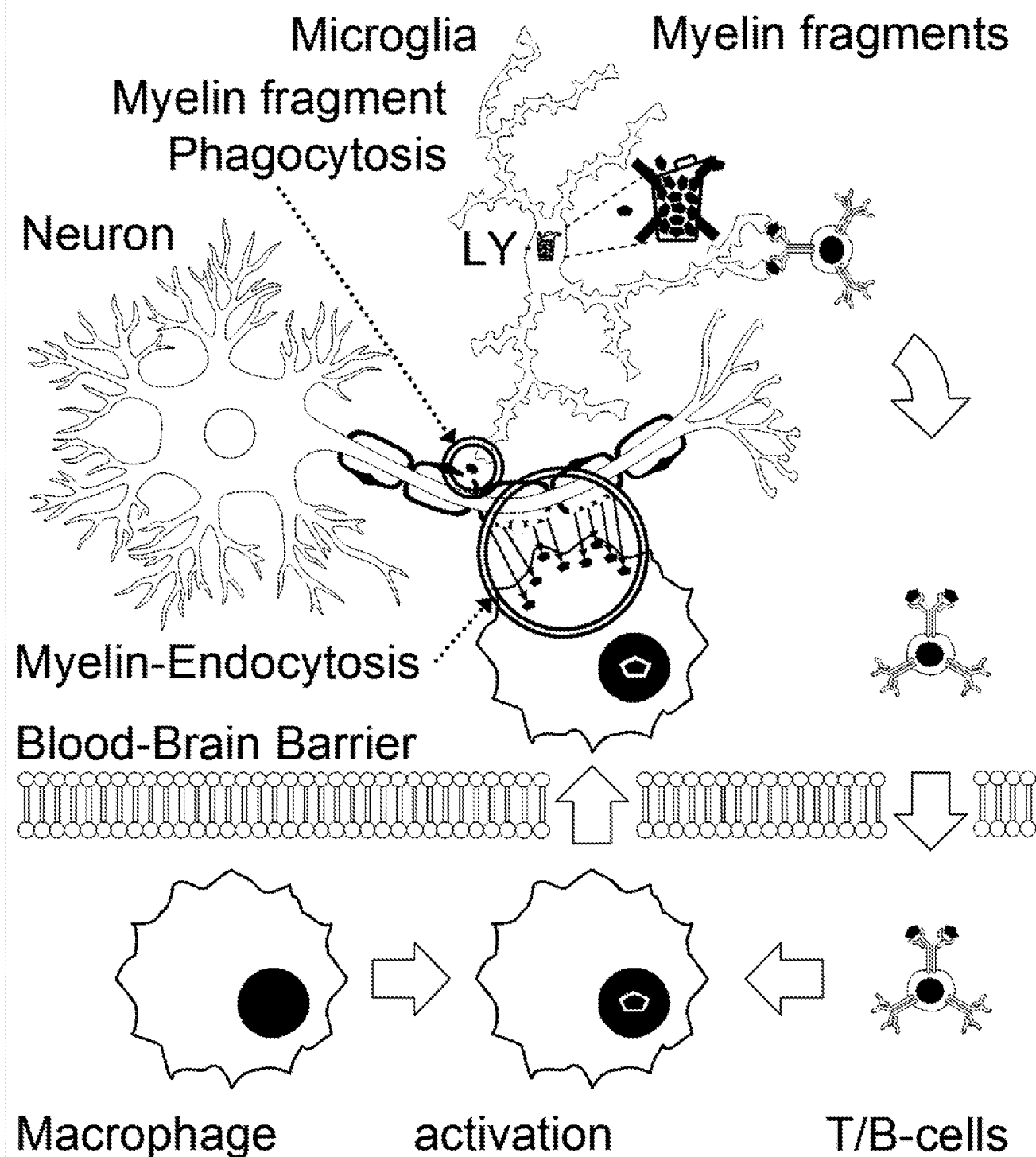


Fig. 27

M β CD Restores Surface Tension

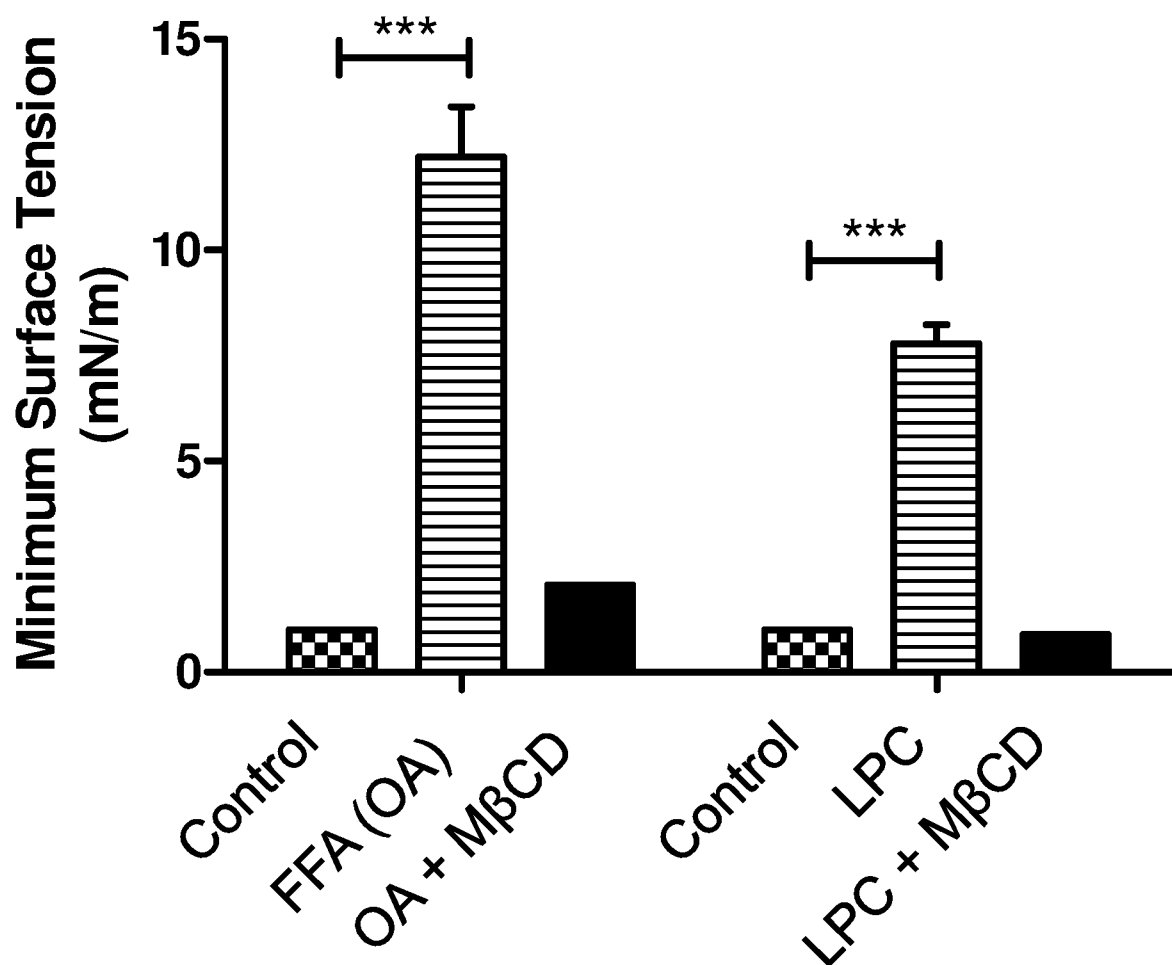


Fig. 28

HaCaT Proliferation

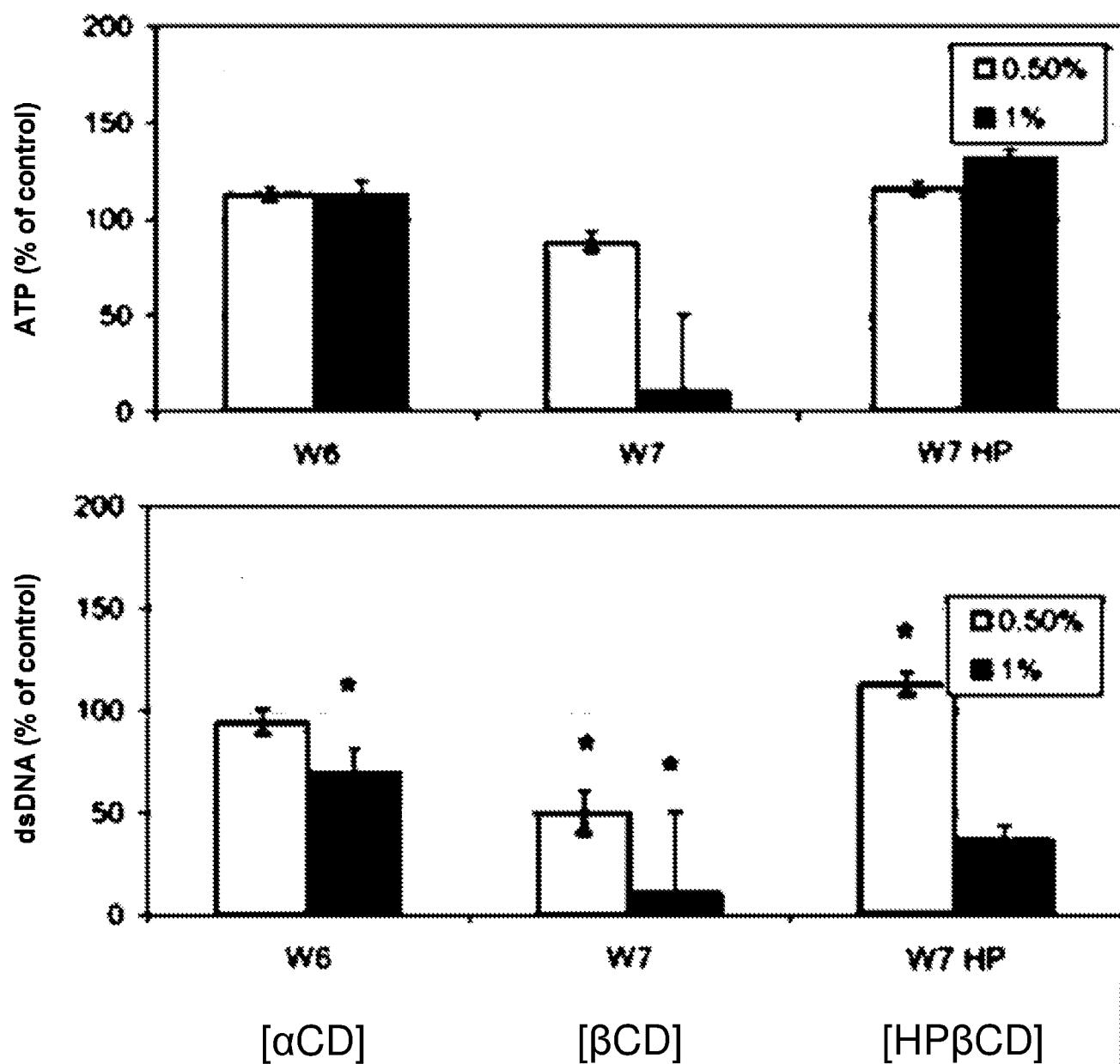


Fig. 29

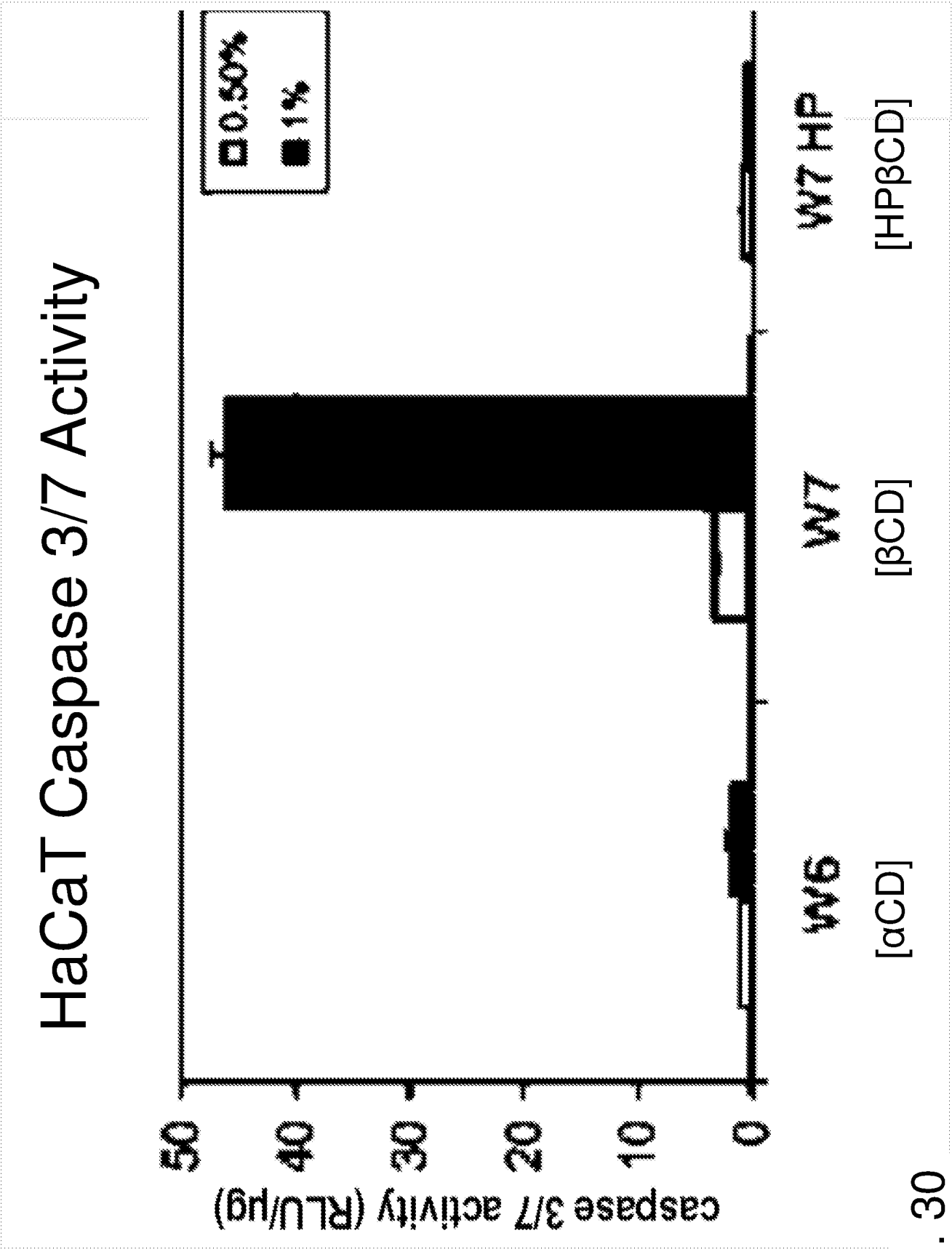
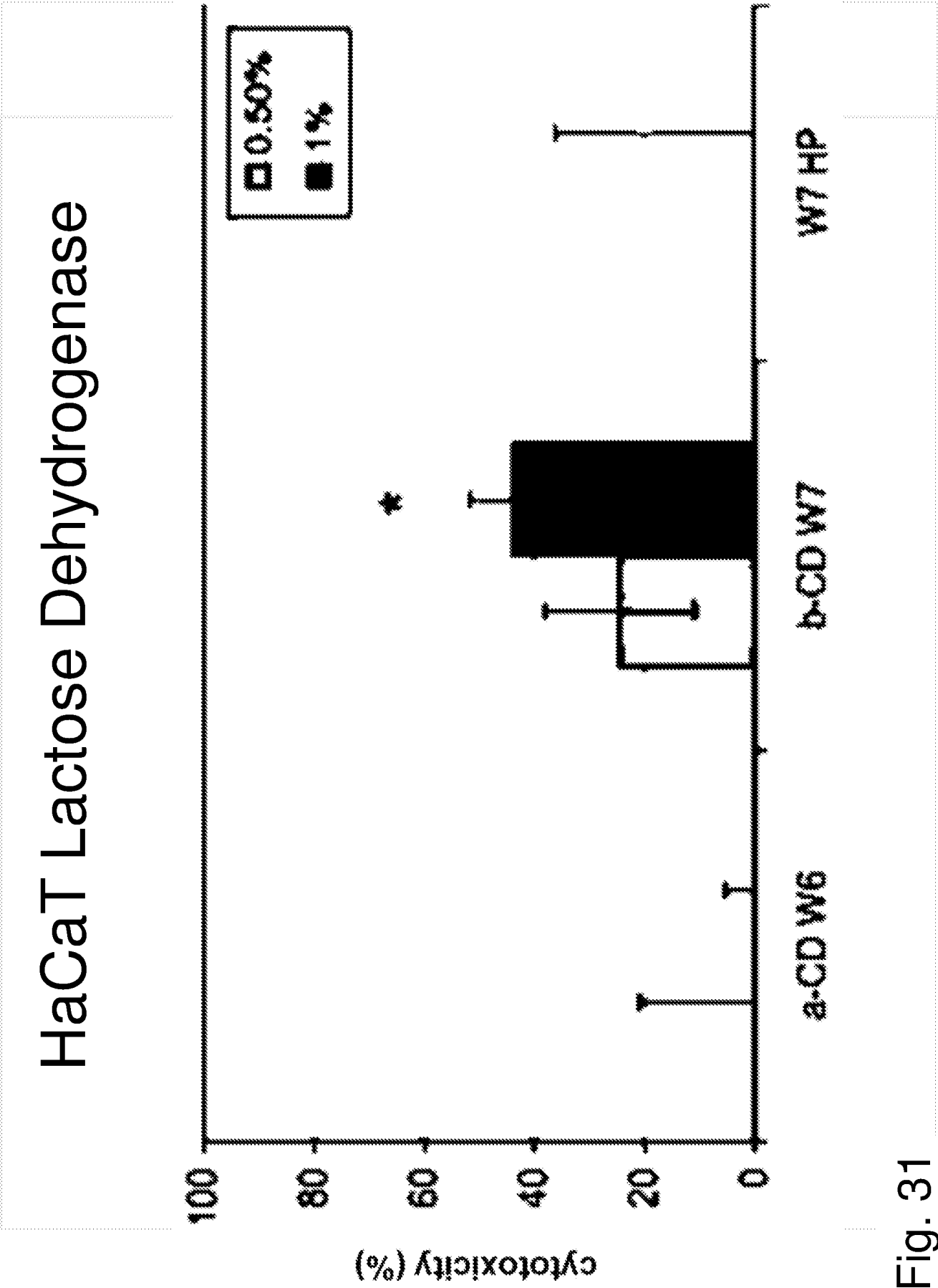


Fig. 30



Solubility Enhancement by HP-CDs

Apparent Solubility of Lipids in PBS (pH 7.3) in absence or presence of 2-hydroxypropyl cyclodextrins (5% w/w)

[$\mu\text{g/mL}$]

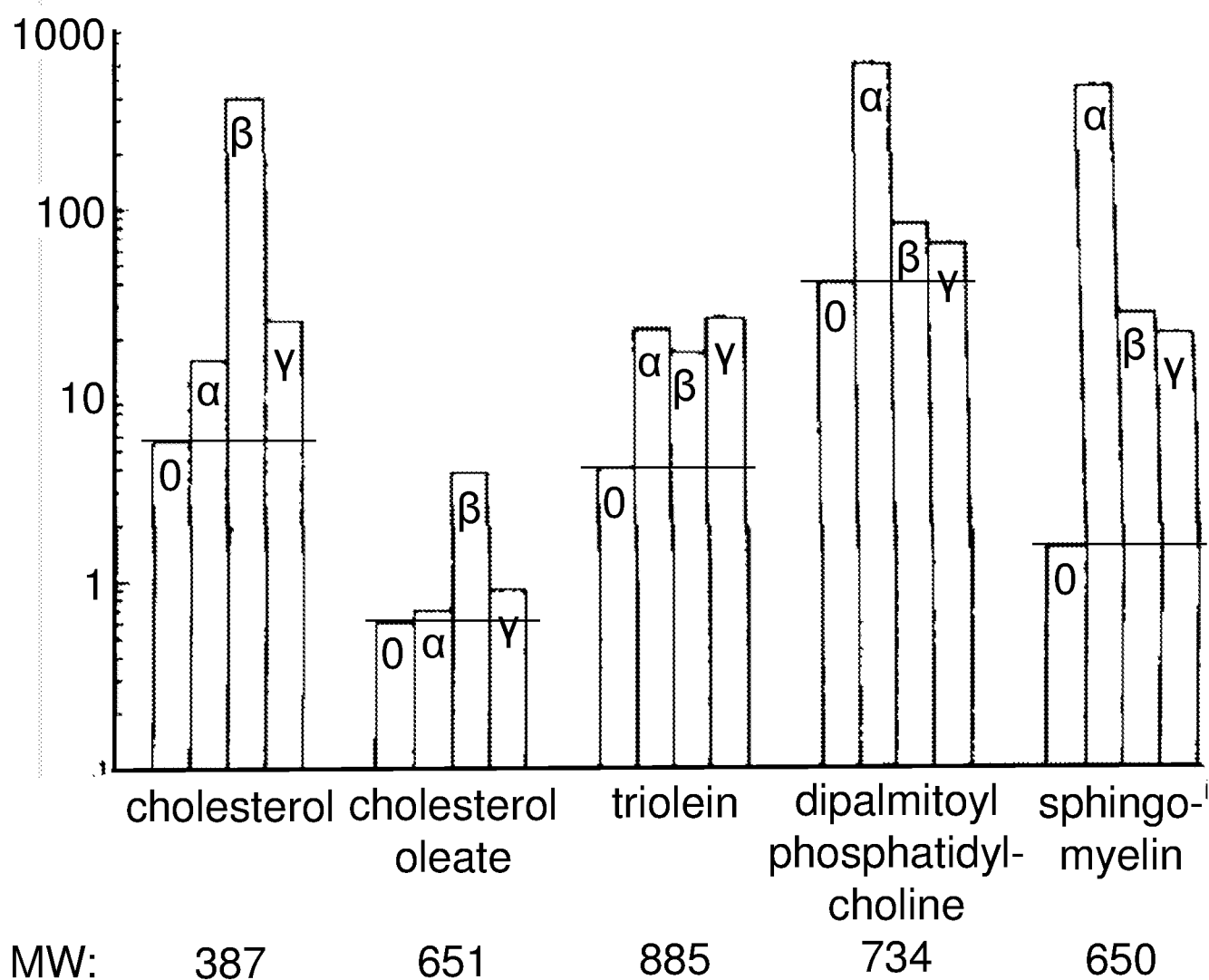


Fig. 32

Nephrotoxicity of α CD and β CD

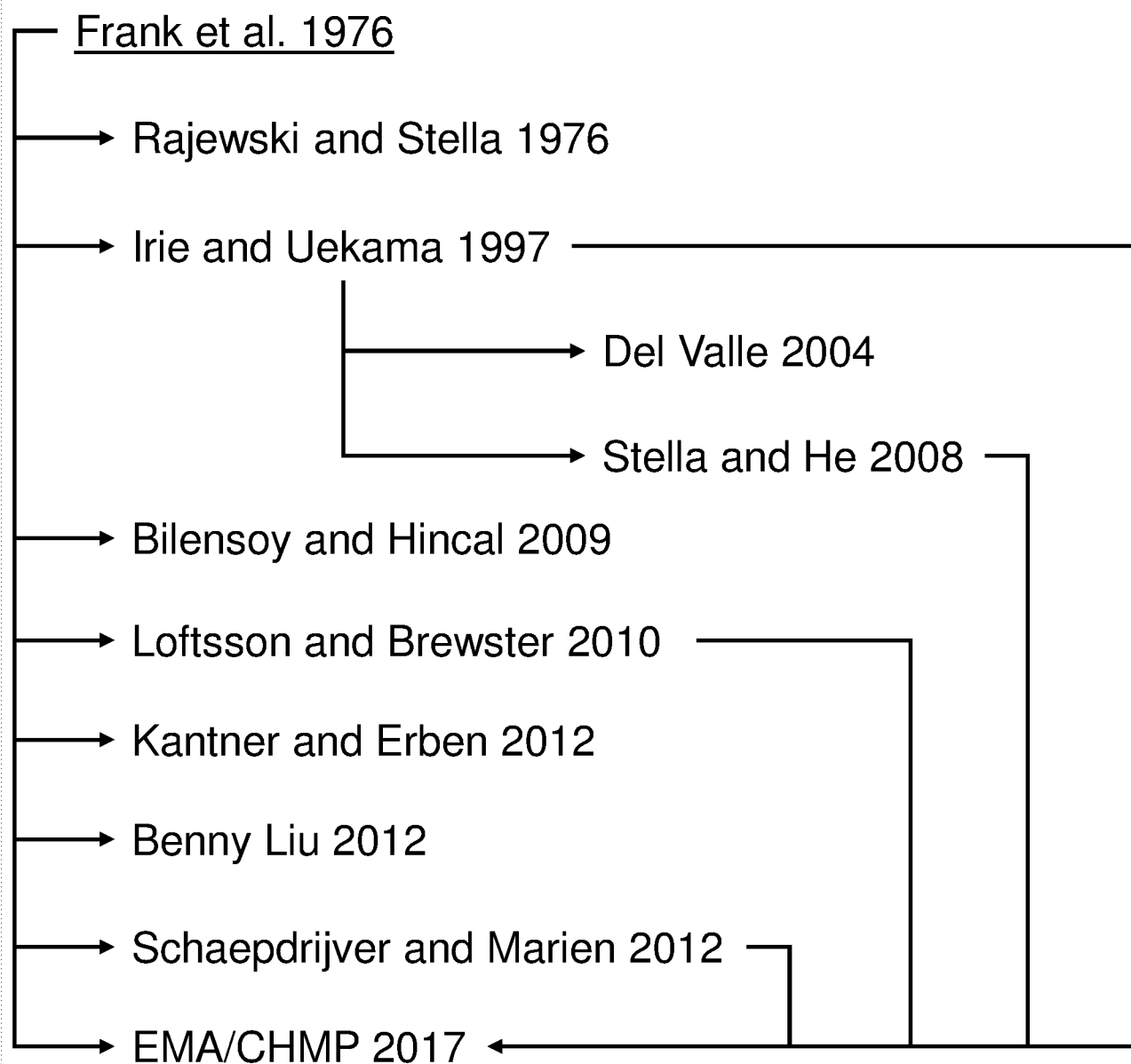


Fig. 33

Ototoxicity of CDs

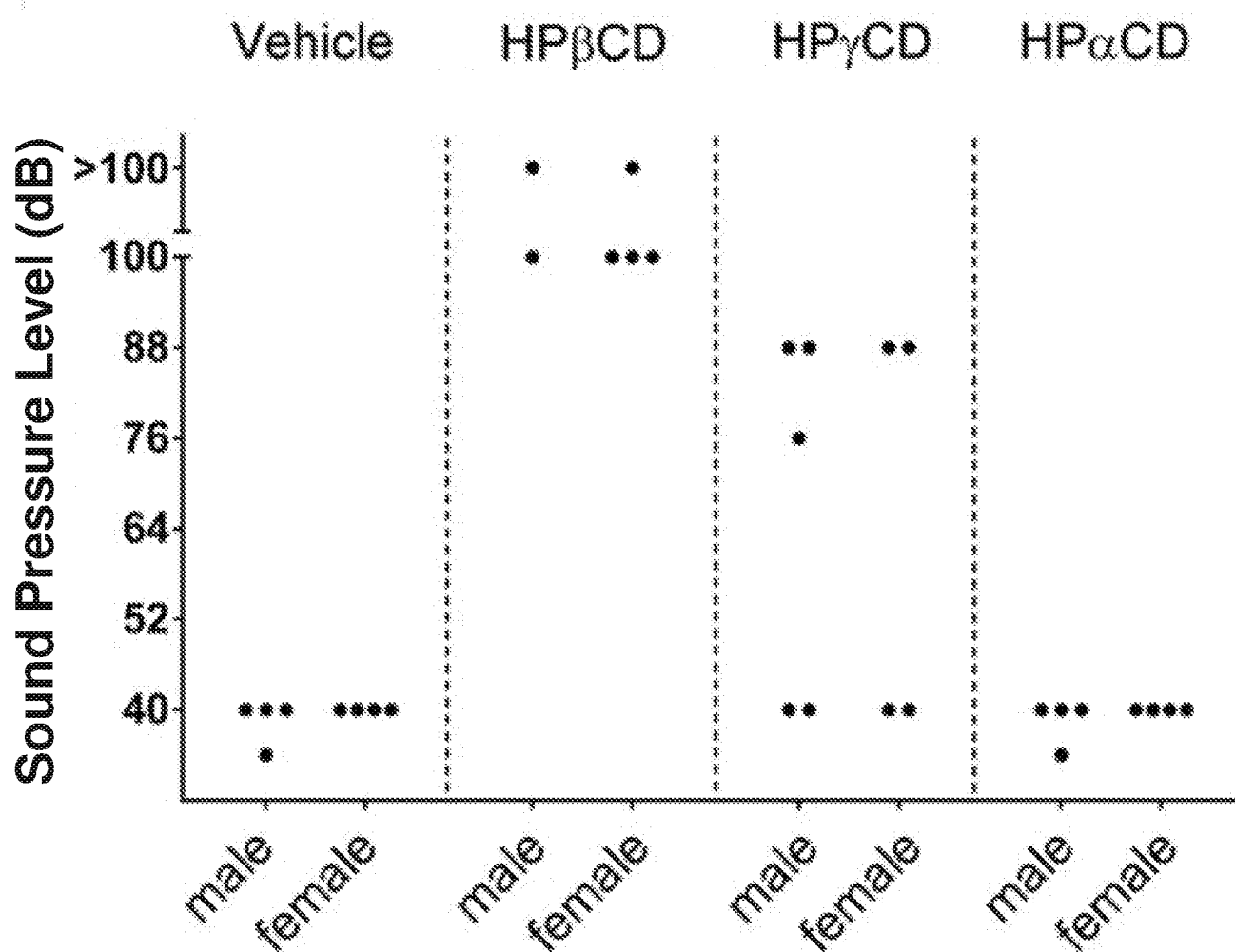


Fig. 34

Wound Healing Results

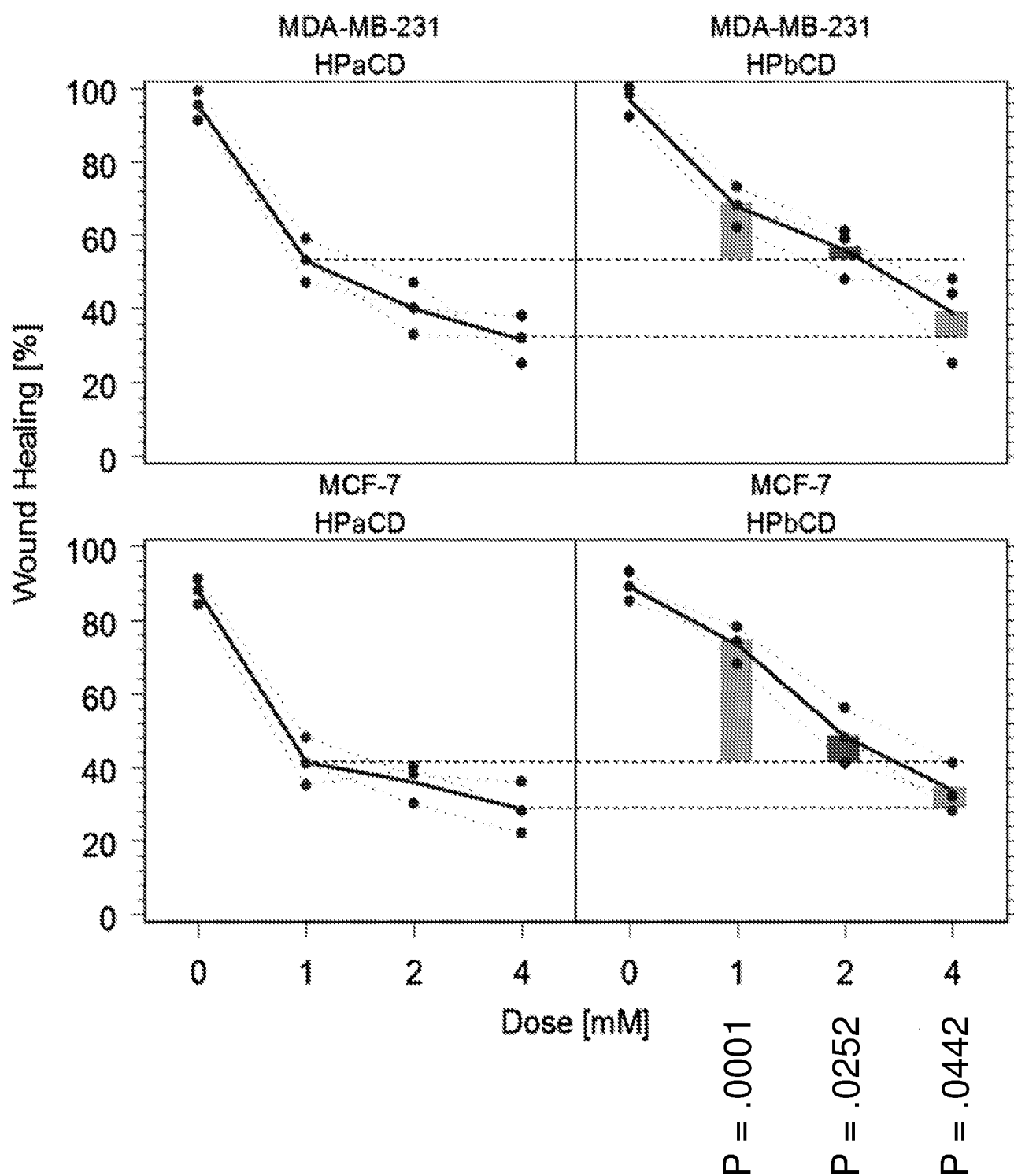


Fig. 35

Wound Healing Assay

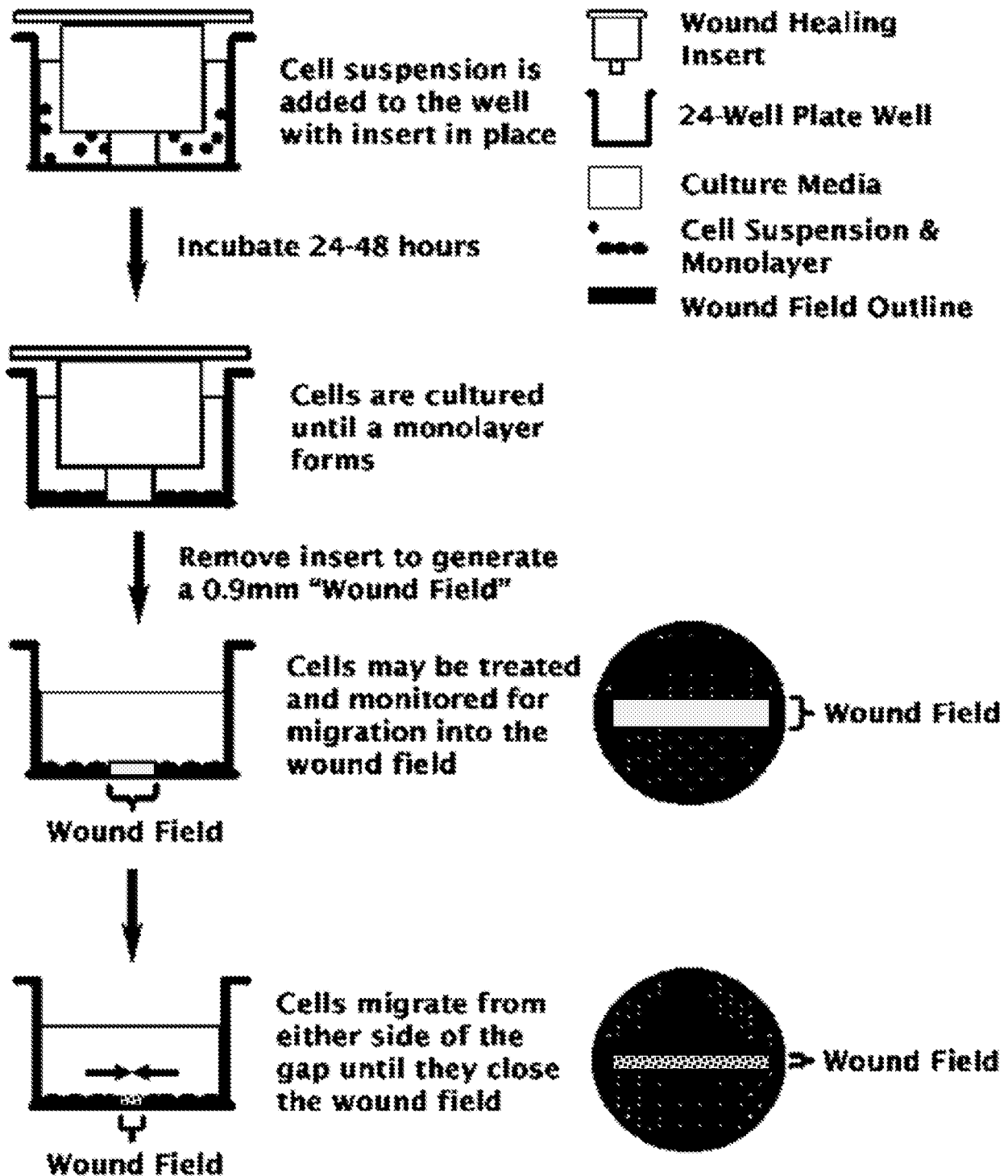


Fig. 36

Impact of PL selectivity

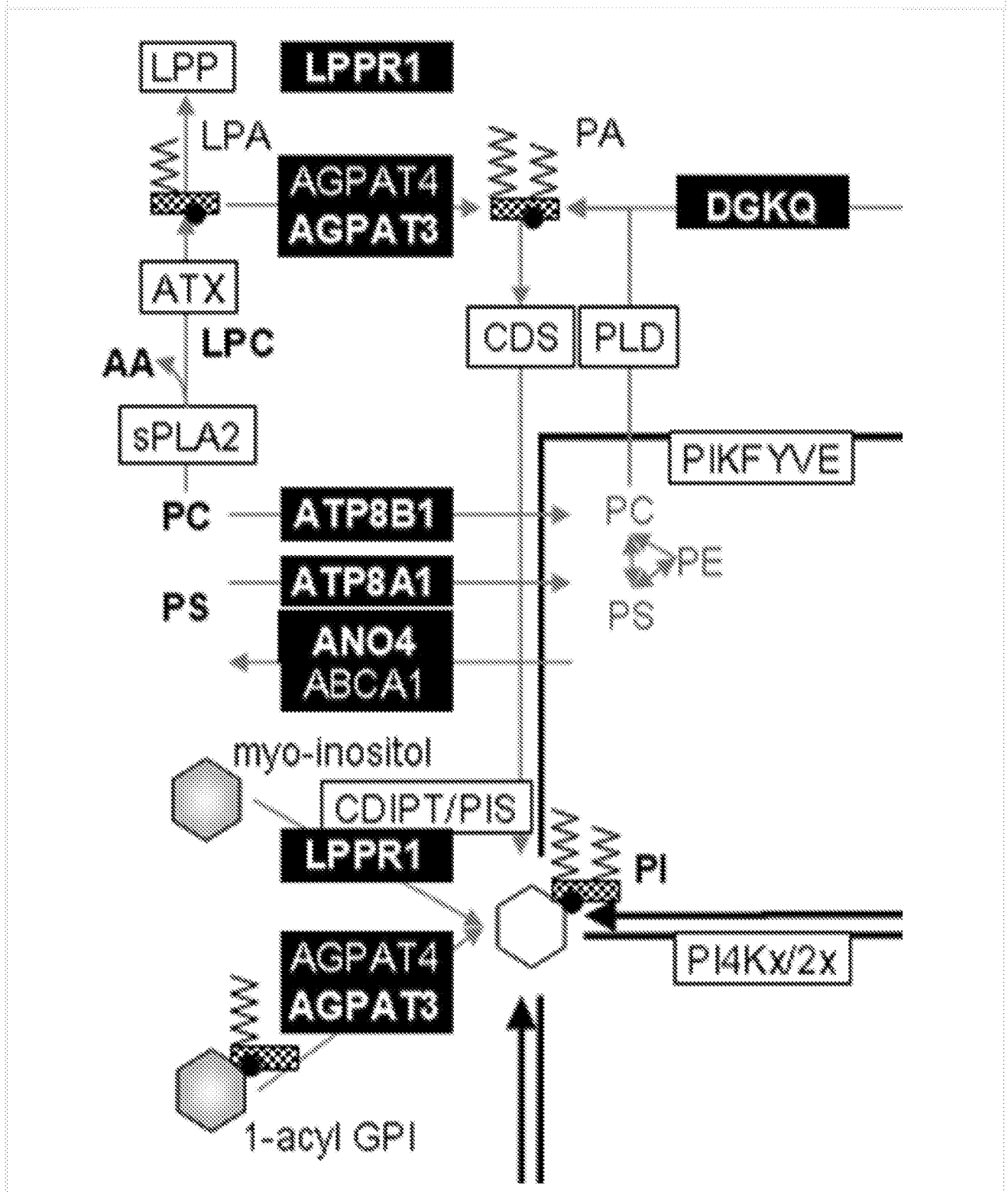


Fig. 37

BCa Mice: Bodyweight

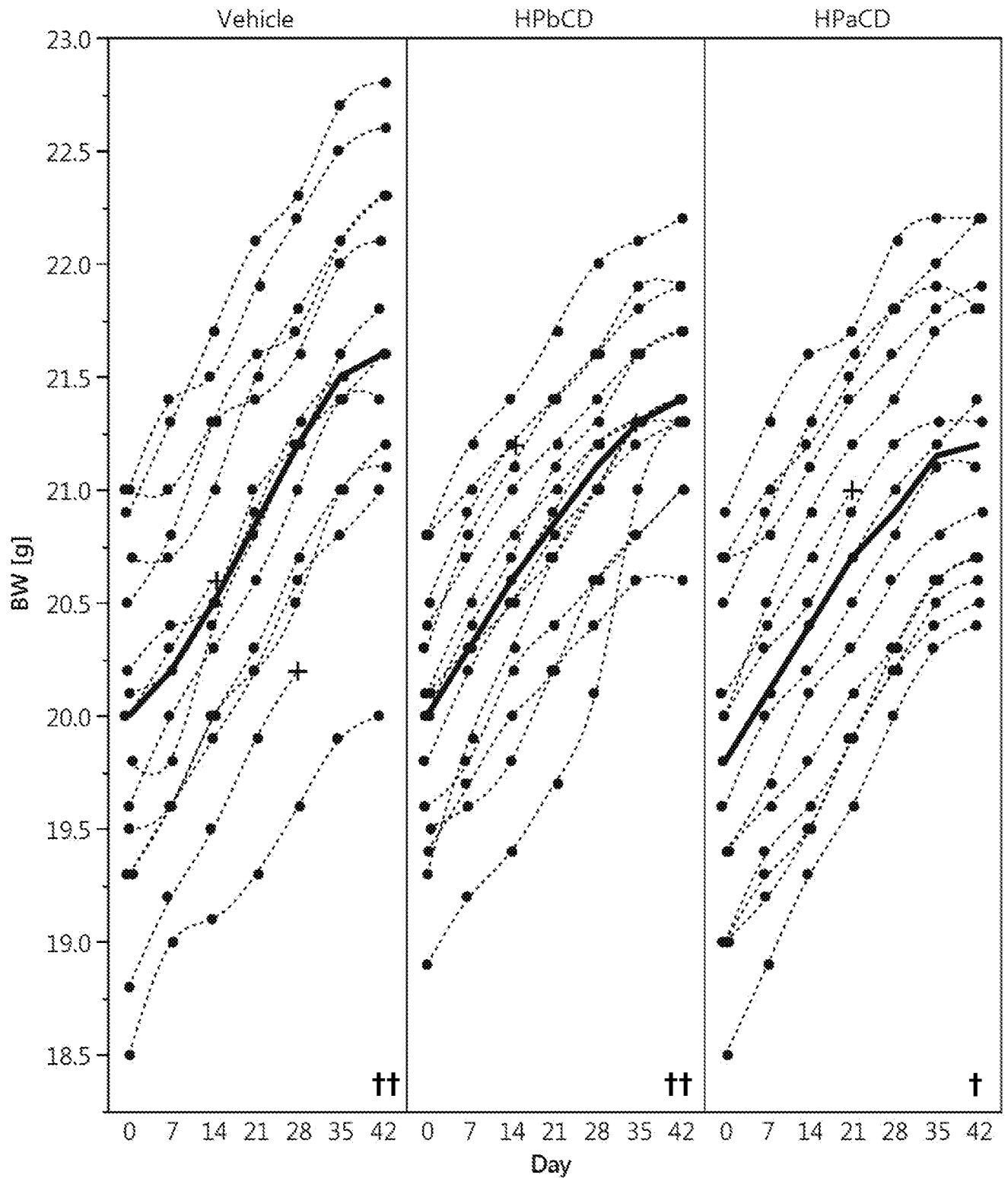


Fig. 38a

HPaCD reduces Tumor Volume

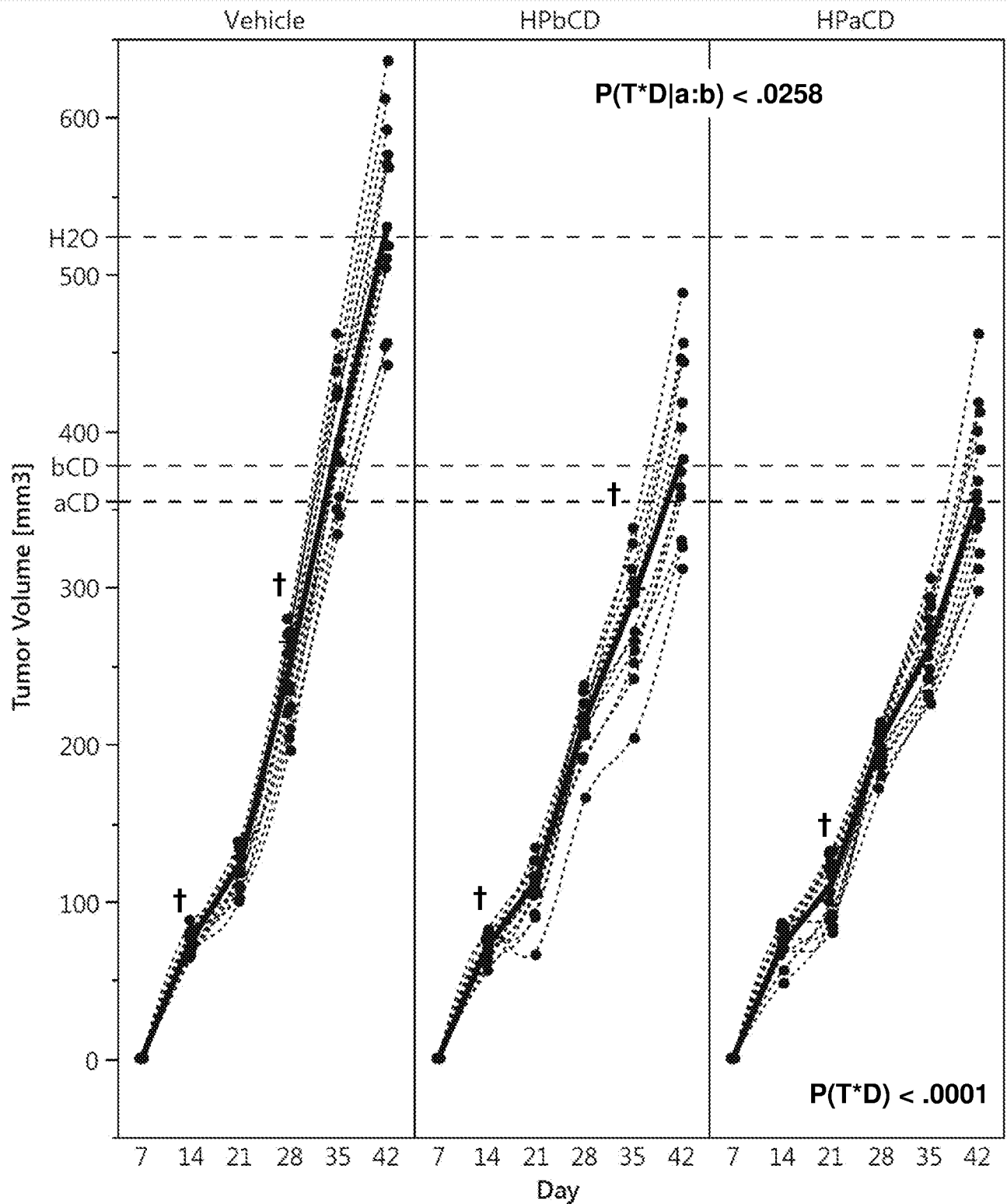


Fig. 38b

HP α CD reduces BCa Metastases

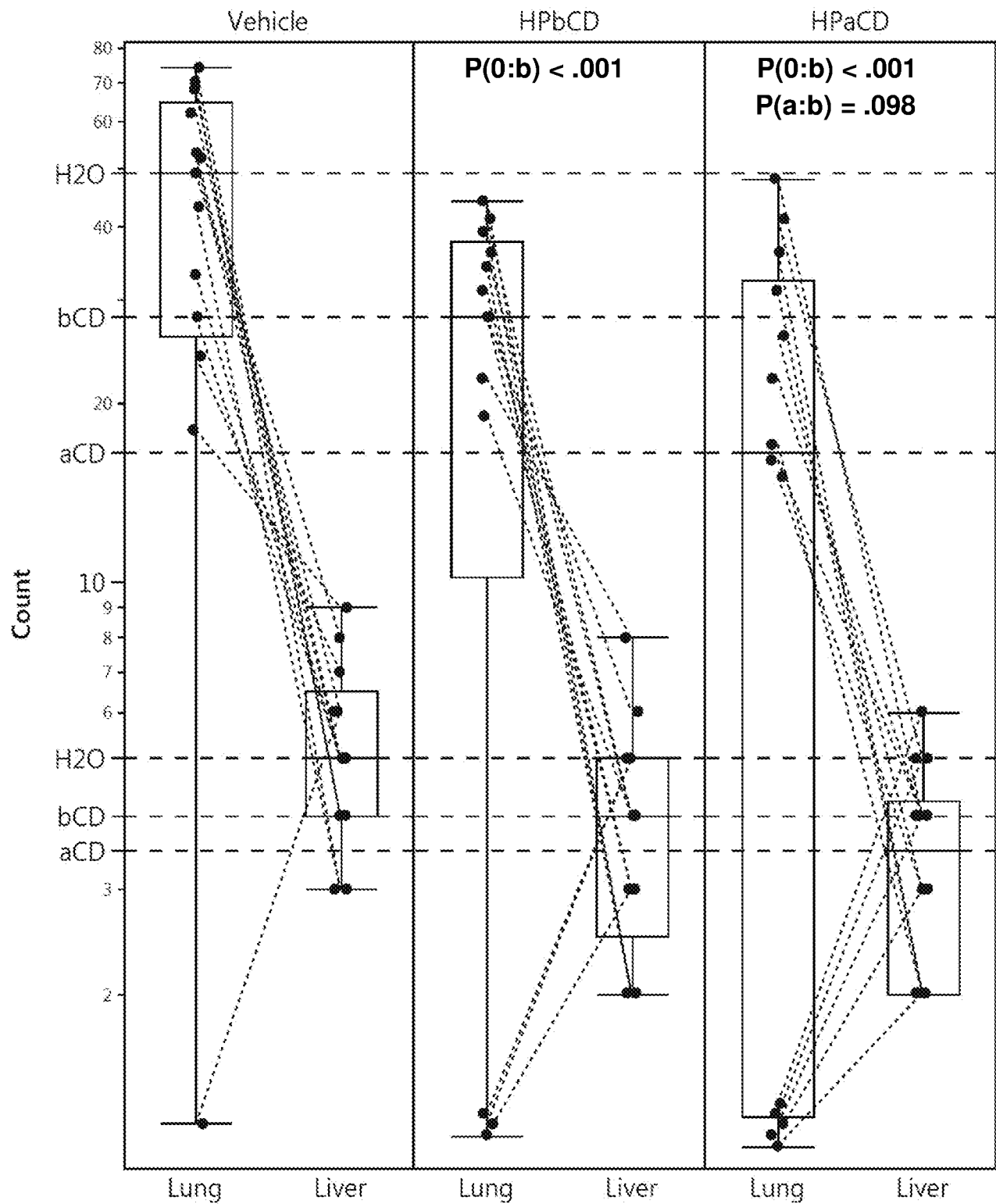


Fig. 39

HP α CD reduces Plasma Cytokines

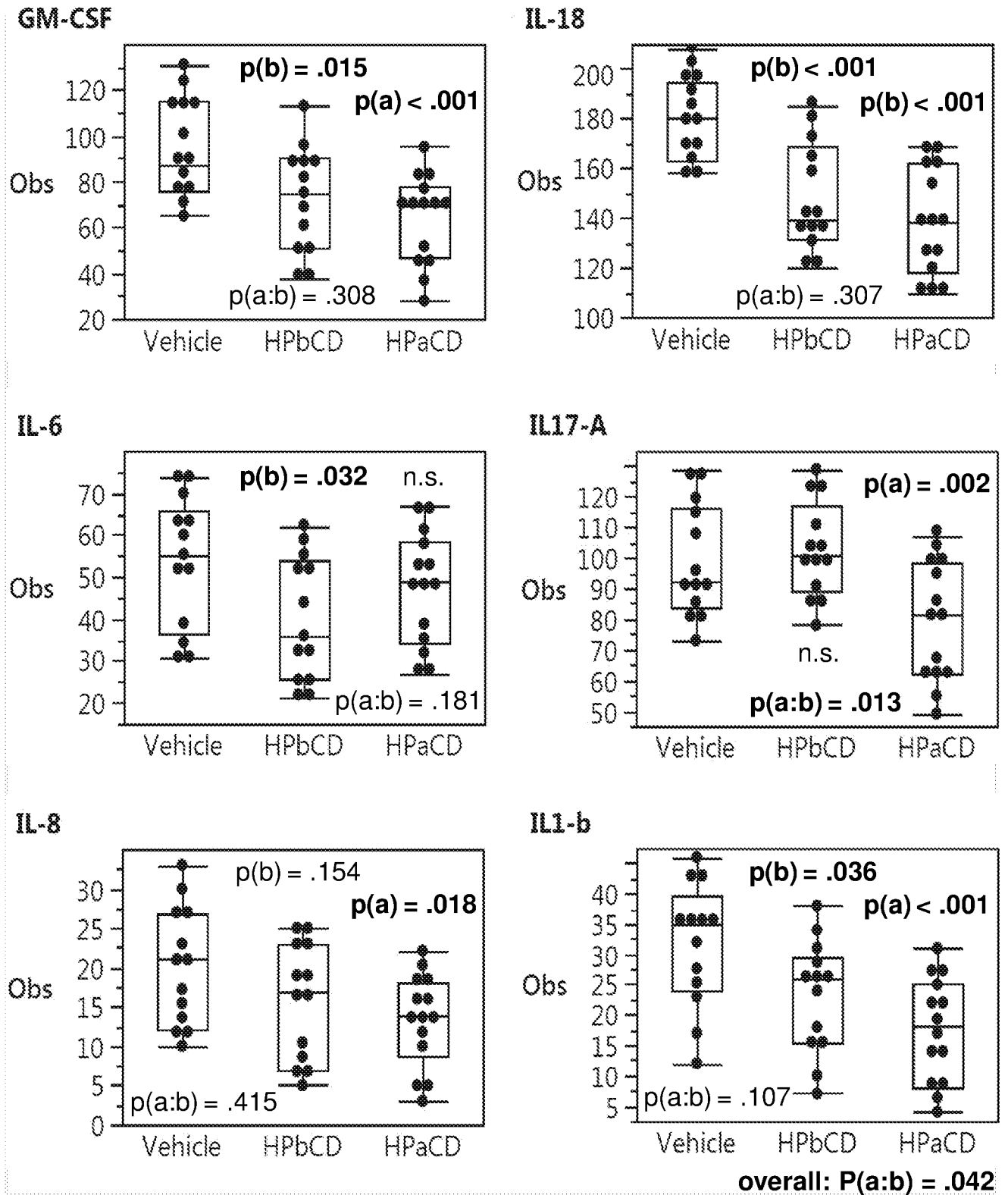
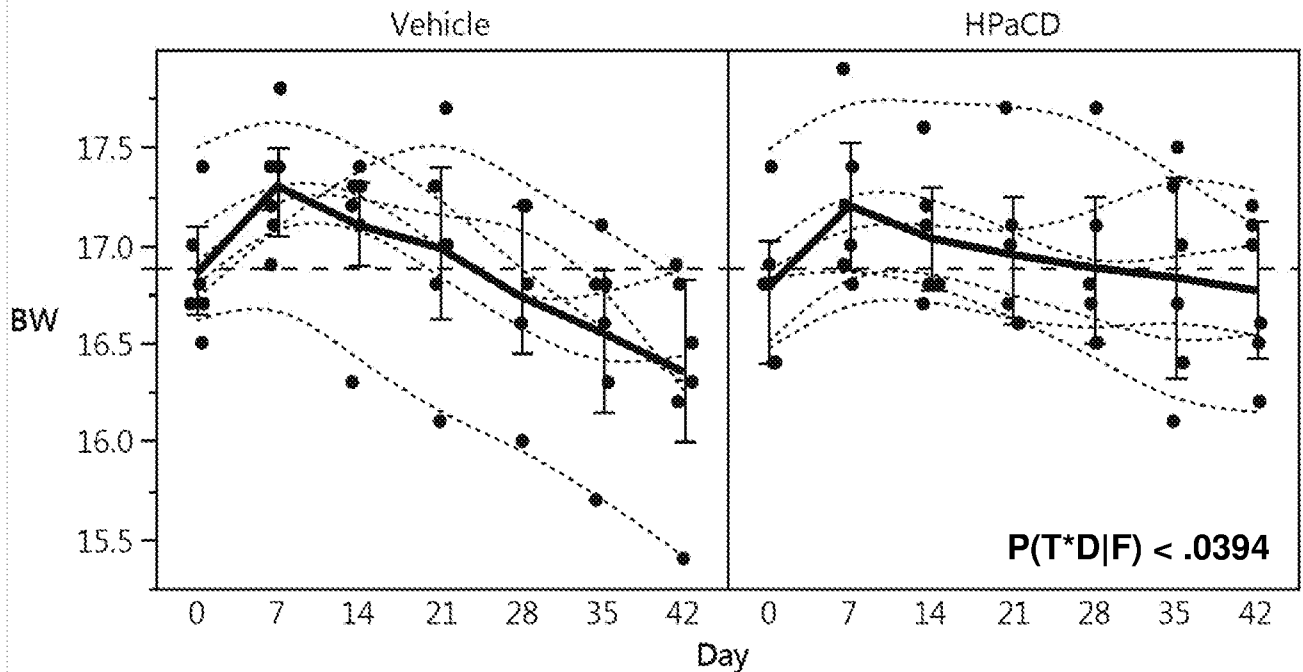


Fig. 40

HTT: HP α CD improves Bodyweight

A Sex = Female



B Sex = Male

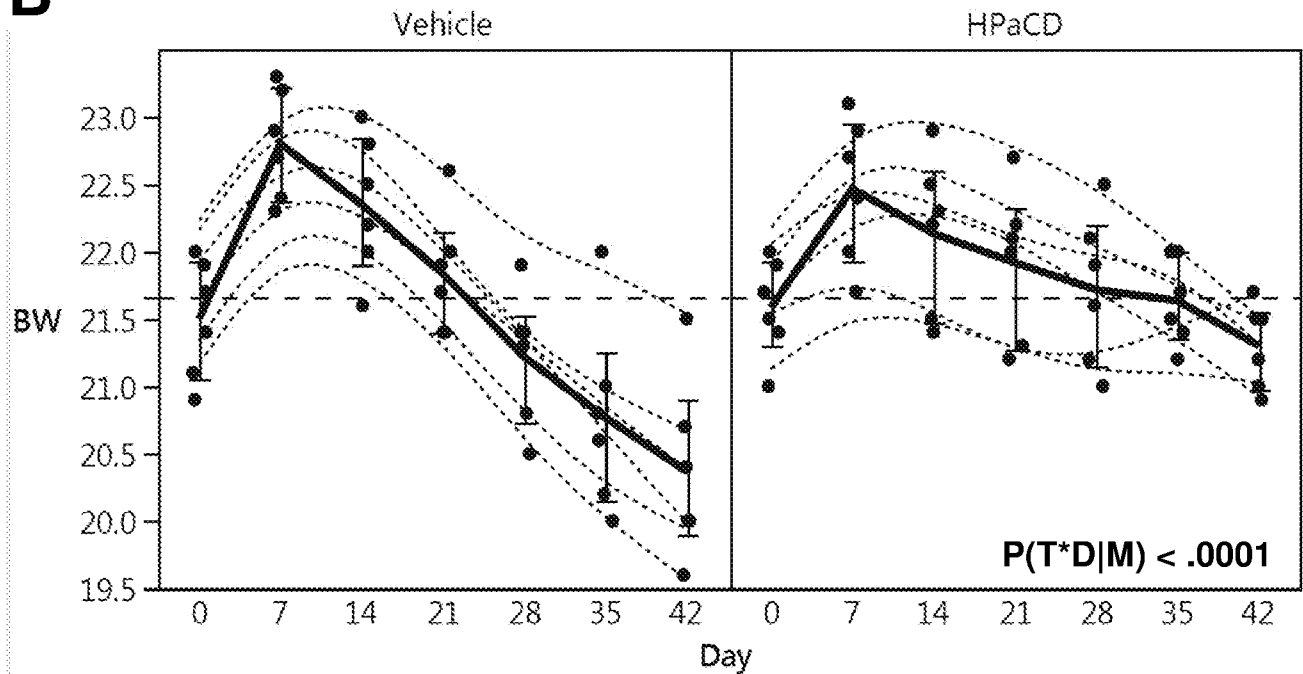
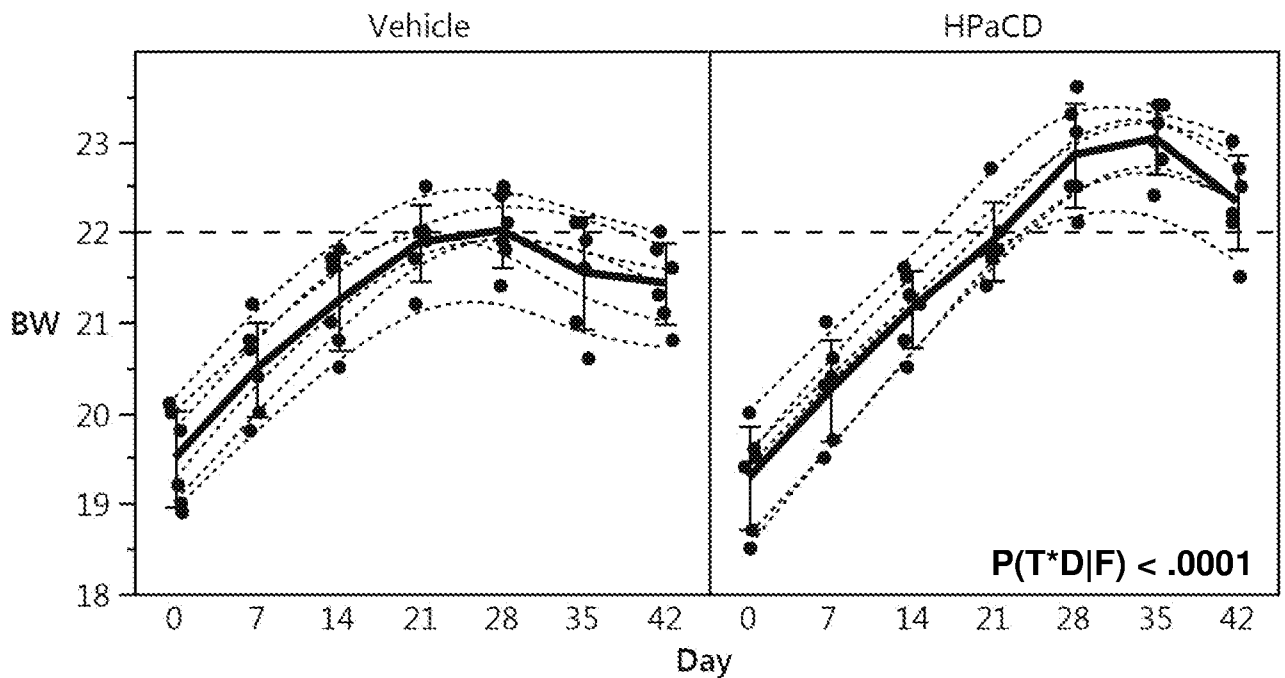


Fig. 41

SOD1: HPaCD improves Bodyweight

A Sex = Female



B Sex = Male

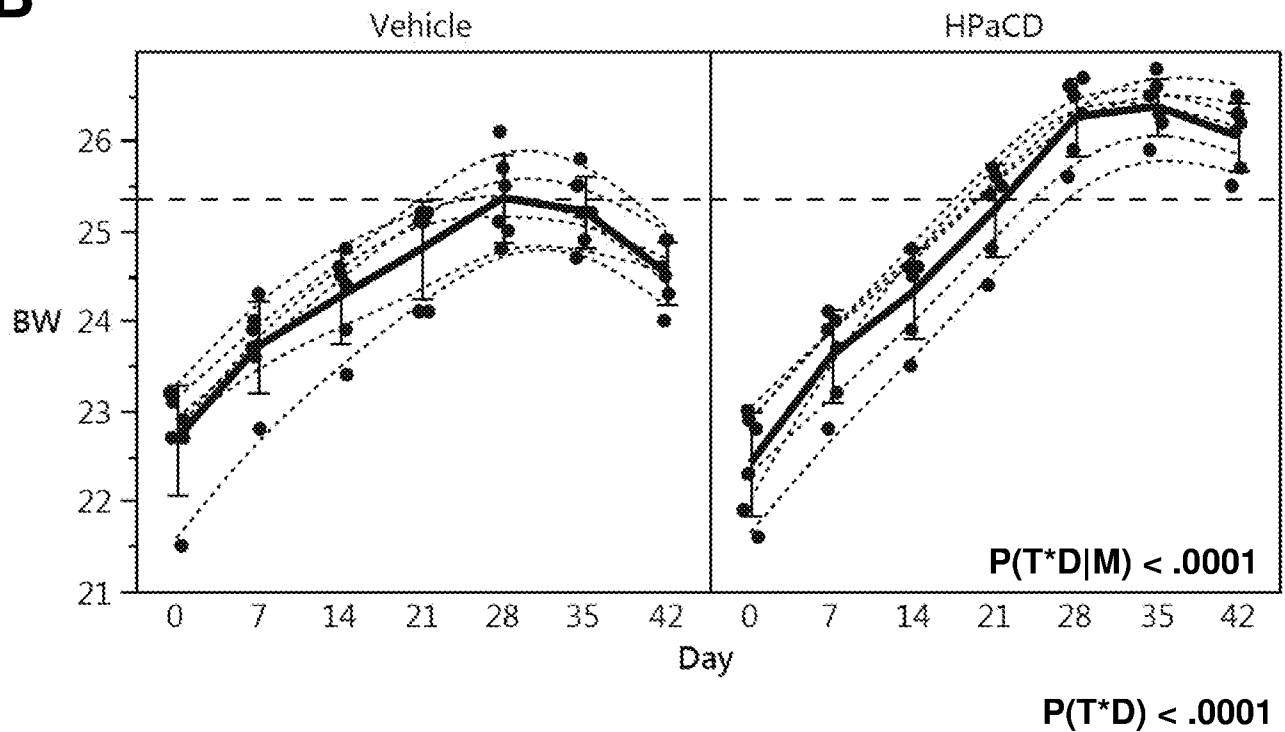


Fig. 42

FPLC Standard curves

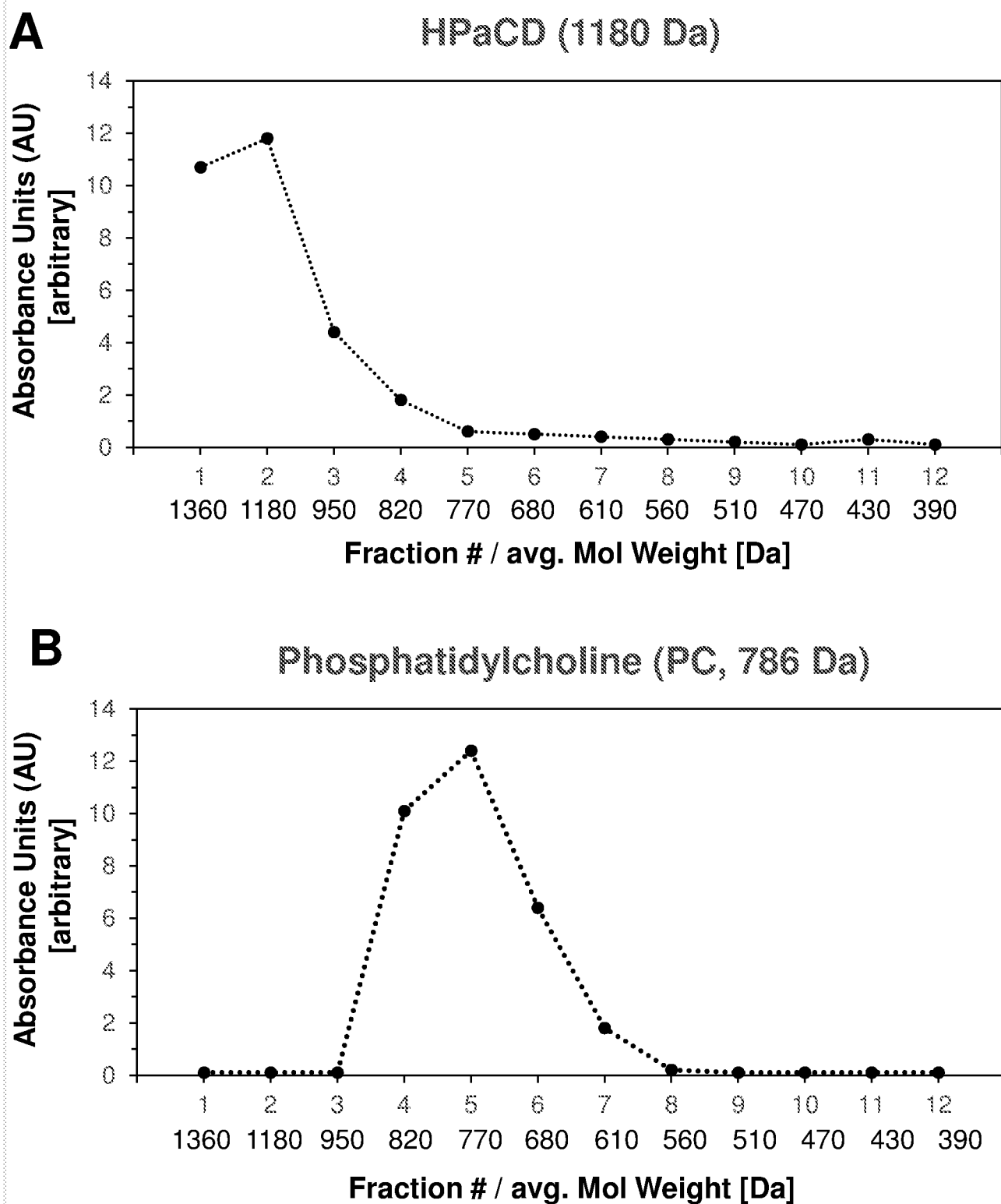


Fig. 43

HPaCD, FA mixtures (&) vs clathrate (/), PE

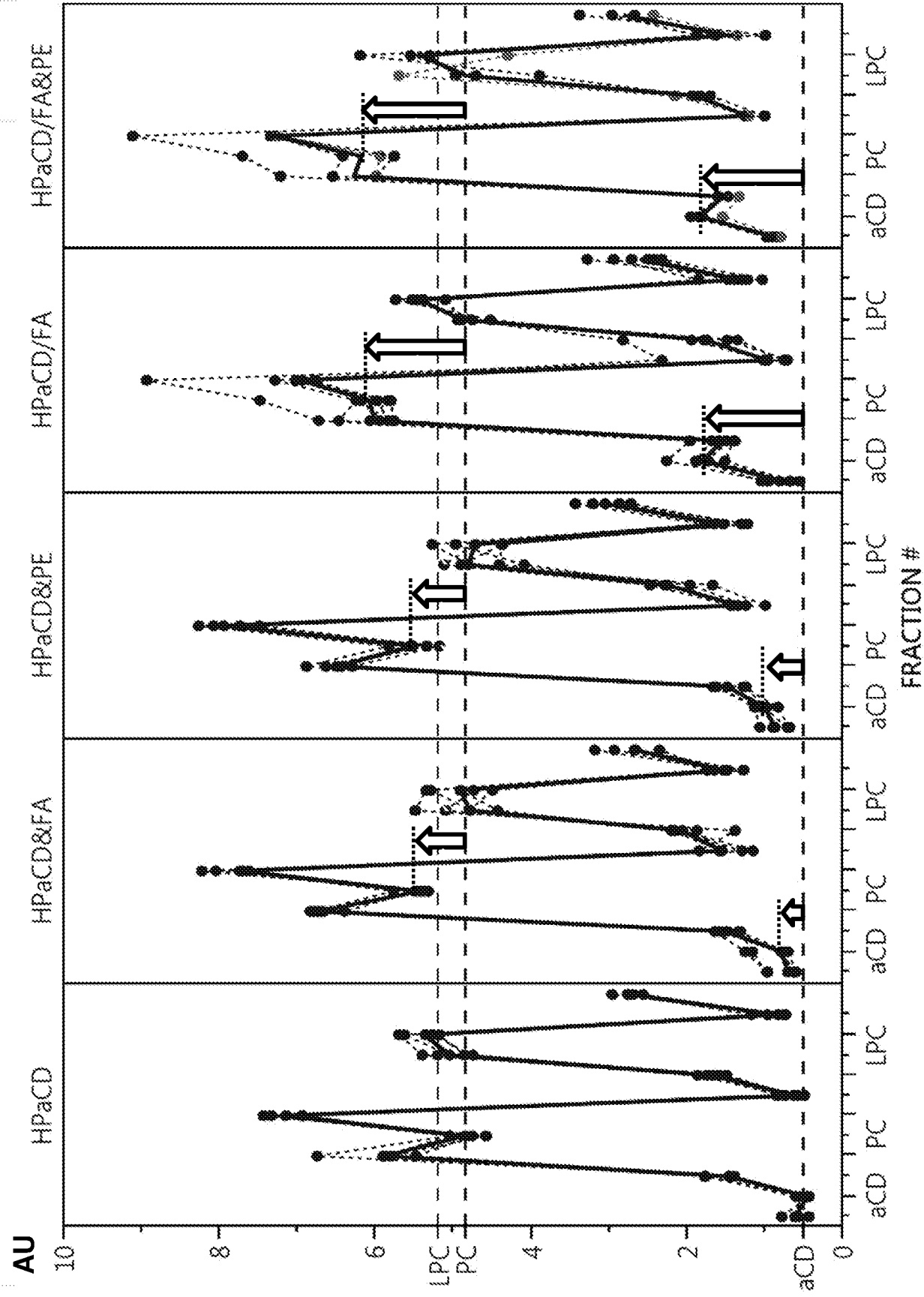
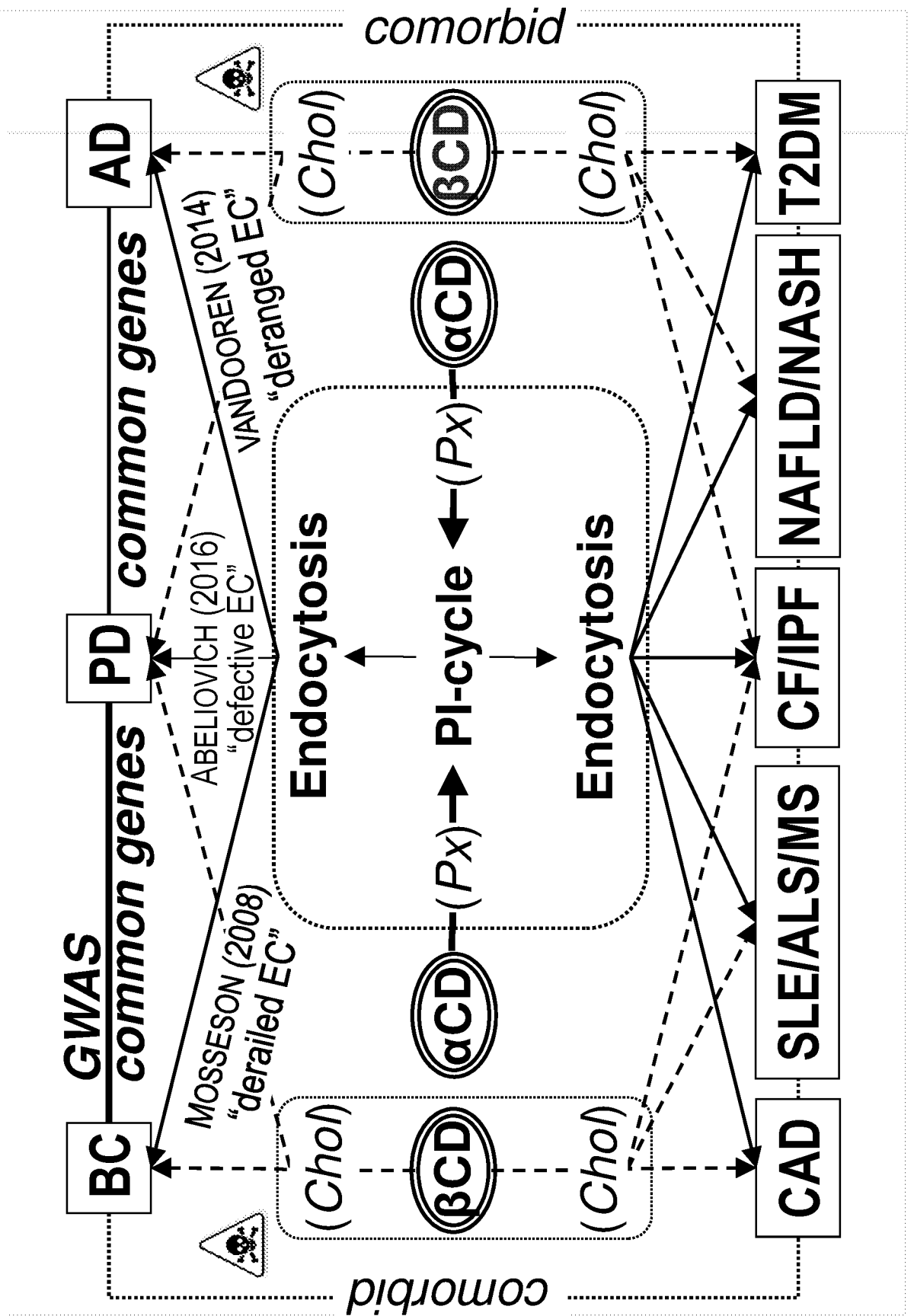


Fig. 44

Fig. 45



HP α CD Restores LY Function

Endo-/Pino-/Phagocytosis (Macro-) Autophagy

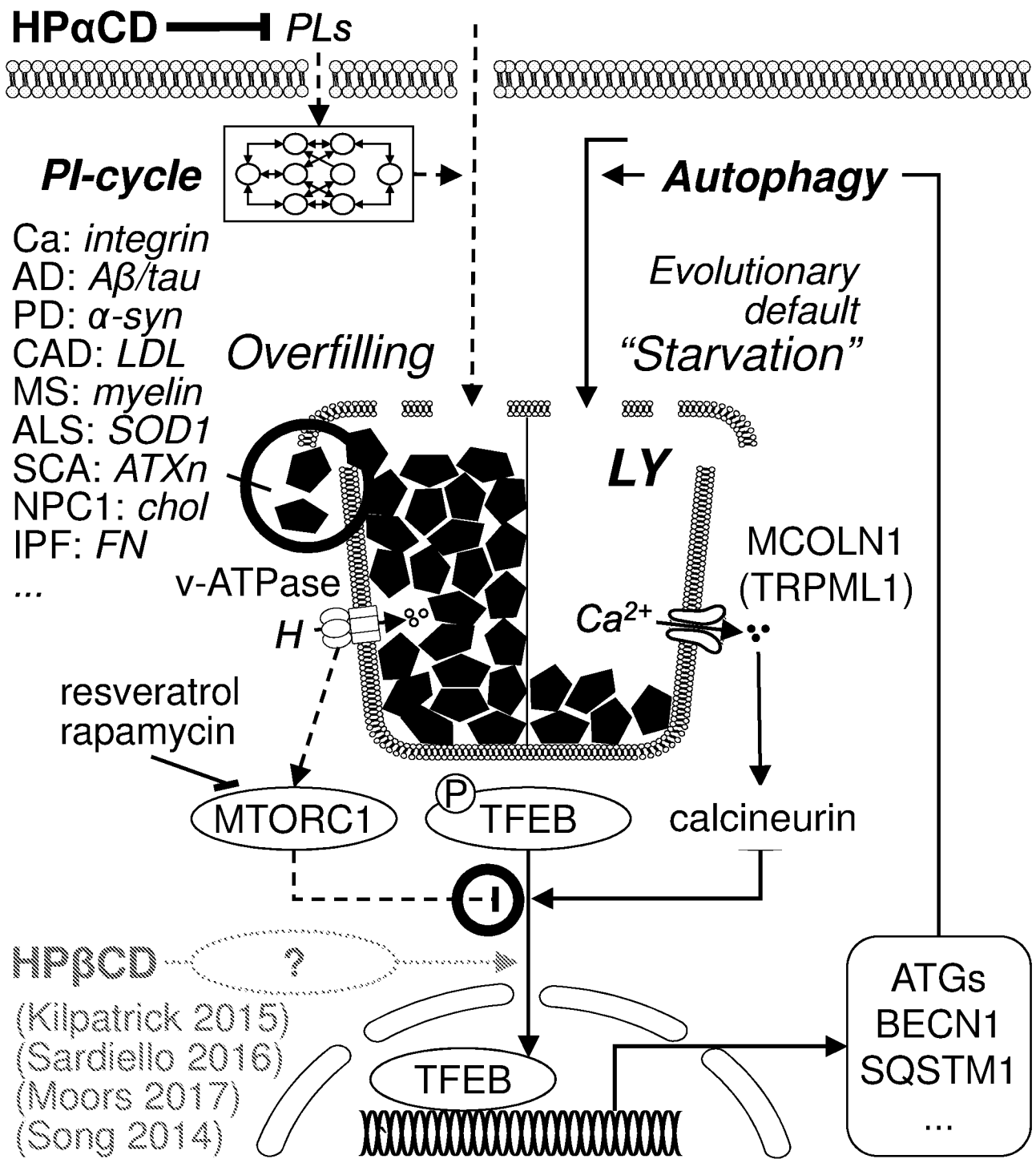
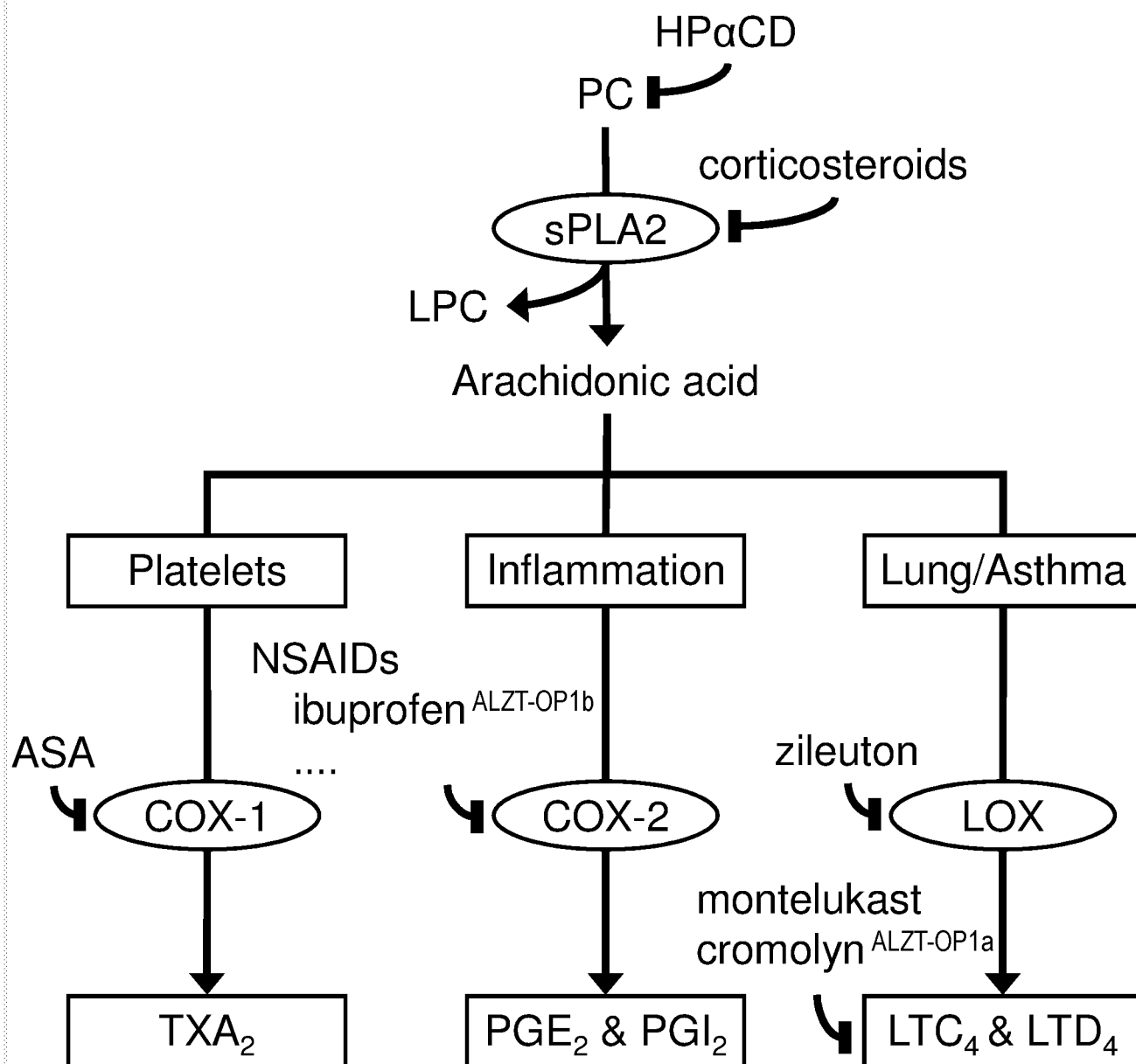


Fig. 46

HP α CD Reduces Inflammation



Therapeutic effect

- ↓vasoconstriction
- anti-inflammatory
- ↓bronchostriction
- anti-platelet
- analgesic
- anti-inflammatory

Fig. 47

α -CD/Pemetrexed Compounds

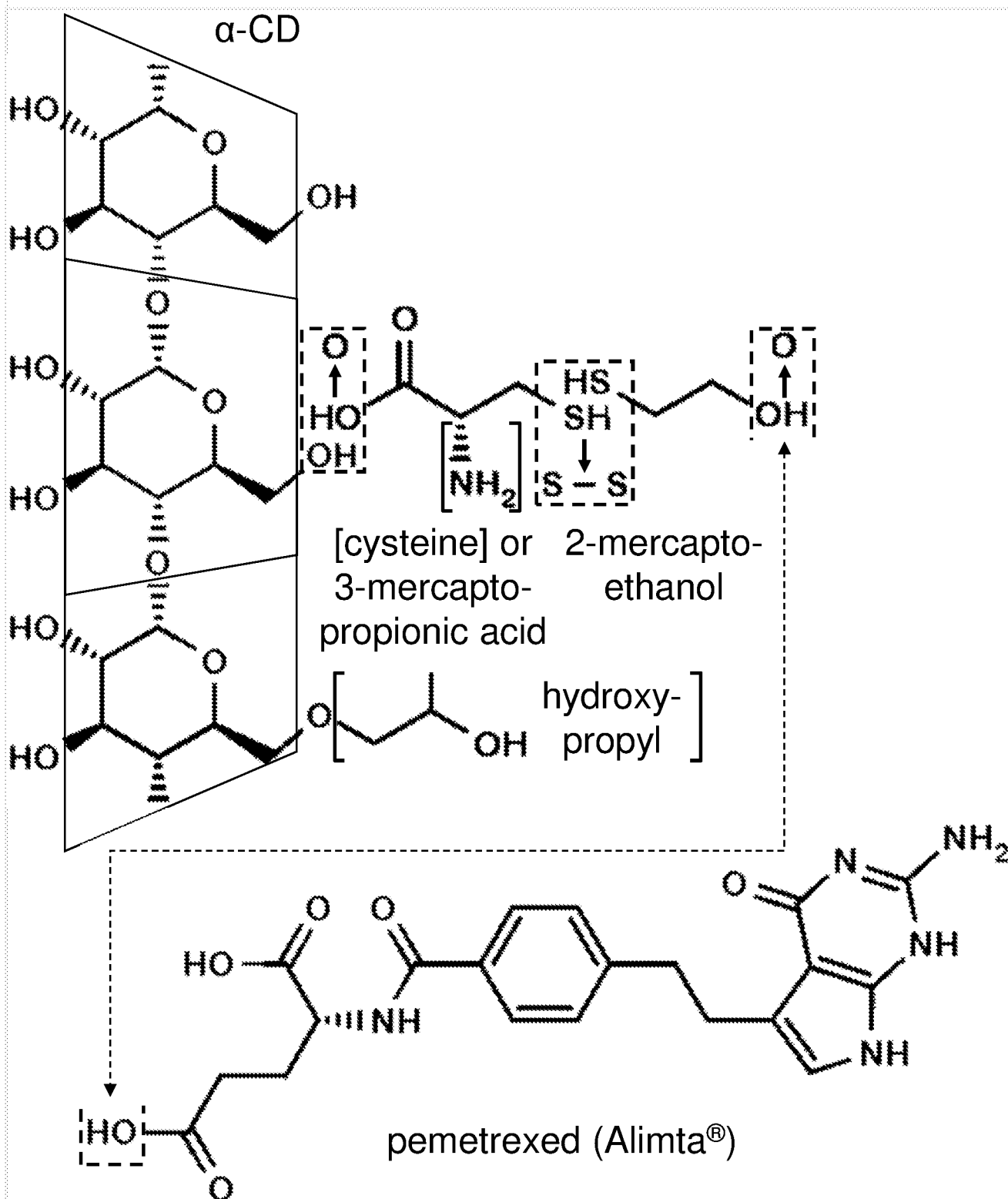


Fig. 48

Study	Gene	IPV6	IPV1	Other	Mdm	PIEC	MPK	Ncls
CGEM	PRKQ	6.70					PRKQ	
CGEM	TRPM3	6.26			TRPM3			
CGEM	BUB3	6.20	6.20					BUB3
CGEM	SCARB2	6.02				SCARB2		
CGEM	FGFR2	6.00	5.57					
CGEM	POLR1A	5.95	4.89					POLR1A
CGEM	GNQ7	5.89			GNQ7			
CGEM	VWA3B	5.86		VWA3B				
CGEM	SYK	5.85			SYK			MSL3
CGEM	MSL3	5.81						
CGEM	CALN1	5.79			CALN1			
CGEM	ATP8B1	5.79				ATP8B1		
CGEM	TCF15	5.49						TCF15
CGEM	LPPR1	5.48	4.65			LPPR1	LPPR1	
CGEM	HAS2	5.44	4.63		HAS2			NUP54
CGEM	NUP54	5.42						
CGEM	BRCAT49	5.38	4.46	BRCAT49				
CGEM	KOND3	5.38			KOND3			
CGEM	MDGA1	5.37		MDGA1				
CGEM	SYT17	5.36				SYT17		
CGEM	SYNJ2	5.30				SYNJ2		
CGEM	MACROD2	5.22						MACROD2
CGEM	SHANK2	5.14			SHANK2			
CGEM	AK5	5.13	4.24	AK5				
CGEM	LYZL1	5.12			LYZL1			
CGEM	MMRN1	5.09	4.95	MMRN1				
CGEM	CTNBL1	5.08						CTNBL1
CGEM	SAMHD1	5.06						SAMHD1
CGEM	MEGF11	5.06				MEGF11		
CGEM	NLRP4	5.04				NLRP4		
CGEM	DMRTB1	5.03						DMRTB1
CGEM	ZMAT4	5.02						ZMAT4
CGEM	ALPP	5.00		ALPP				
CGEM	ABCA1	4.99				ABCA1		
CGEM	SCMH1	4.98						SCMH1
CGEM	LPHN3	4.98			LPHN3			
CGEM	DGKQ	4.97	4.18			DGKQ		
CGEM	HNF1B	4.97						HNF1B
CGEM	SMARCA1	4.97						SMARCA1
CGEM	BRCAT54	4.95	4.81	BRCAT54				
CGEM	AGPAT4	4.94				AGPAT4		
CGEM	PTCD3	4.92	4.47	PTCD3				
CGEM	BMPR1B	4.89	4.47	BMPR1B				
CGEM	FBXO38	4.29	4.29					FBXO38
CGEM	PARK2	4.86	4.24	PARK2				
CGEM	ANO4	4.21	4.21		ANO4			
CGEM	ABO	4.24	4.19	ABO				
CGEM	DAP	4.28	4.19	DAP				
CGEM	STXB1	4.59	4.11			STXB1		
CGEM	SLC5A3	4.09	4.09			SLC5A3		
CGEM	MRGPRE	4.49	4.07		MRGPRE			
CGEM	NTSR1	4.82	4.06		NTSR1			
CGEM	MRPL35	4.04	4.04	MRPL35				

FIG. 49A

Study	Gene	IPV6	IPV1	Othr	Mbm	PIEC	MPK	Ncls
EPIC	SOHLH2	8.58	5.42	-	-	-	-	SOHLH2
EPIC	AGPAT3	6.59	4.73	-	-	AGPAT3	-	-
EPIC	CELF2	6.51	4.11	-	-	-	-	CELF2
EPIC	STARD13	6.48	4.56	-	-	-	-	-
EPIC	PCSK5	6.37	-	-	STARD13 PCSK5	-	-	-
EPIC	mirLET7BHG	6.33	4.68	-	-	-	-	-
EPIC	ATP8A1	6.26	4.87	-	-	ATP8A1	-	-
EPIC	CHD3	6.13	5.68	-	-	-	-	CHD3
EPIC	TNS1	5.88	4.19	-	TNS1	-	-	-
EPIC	TRAPPC9	5.85	4.36	-	-	-	-	-
EPIC	CDKAL1	5.82	4.34	-	-	-	-	CDKAL1
EPIC	TAS2R1	5.57	-	-	TAS2R1	-	-	-
EPIC	TMEM132C/D	5.50	-	-	TMEM132C/D	-	-	-
EPIC	SIX2	5.49	4.05	-	-	-	-	SIX2
EPIC	GLB1	5.40	-	-	GLB1	-	-	-
EPIC	SPATA19	5.35	4.13	-	SPATA19	-	-	-
EPIC	ASTN2	5.33	-	-	ASTN2	-	-	-
EPIC	CHMP7	5.27	-	-	-	CHMP7	-	-
EPIC	PRKCO	5.26	-	-	-	PRKCO	-	-
EPIC	E2F3	5.24	-	-	-	-	-	E2F3
EPIC	NEFL/M	5.23	-	-	NEFL/M	-	-	-
EPIC	NCCRP1	5.21	-	-	-	-	-	NCCRP1
EPIC	FLT3	5.21	-	-	FLT3	-	-	-
EPIC	EPHB1	5.16	-	-	EPHB1	-	-	-
EPIC	CFAP36	5.15	4.12	-	CFAP36	-	-	-
EPIC	GRIA1	5.14	4.08	-	GRIA1	-	-	-
EPIC	RASD2	5.13	-	-	RASD2	-	-	-
EPIC	COL3A1	5.08	-	-	COL3A1	-	-	-
EPIC	RARB	5.08	-	-	-	-	-	RARB
EPIC	SLC43A3	5.06	-	-	SLC43A3	-	-	-
EPIC	VAV3	5.05	-	-	VAV3	-	-	-
EPIC	HMGXB4	5.05	-	-	-	-	-	HMGXB4
EPIC	VWC2L	5.04	-	-	VWC2L	-	-	-
EPIC	PRKAG2	5.03	4.32	-	-	-	-	-
EPIC	LZTS1	5.02	-	-	-	-	-	LZTS1
EPIC	LARGE	5.00	-	-	LARGE	-	-	-
EPIC	LSMD1	5.00	-	-	LSMD1	-	-	-
EPIC	IGSF9B	4.98	-	-	IGSF9B	-	-	-
EPIC	ACAN	4.93	-	-	ACAN	-	-	-
EPIC	SDCBP2	4.90	-	-	-	-	-	-
EPIC	KPNA3	4.88	-	-	KPNA3	-	-	-
EPIC	OR52K2	4.77	4.77	-	OR52K2	-	-	-
EPIC	GPHN	4.64	4.26	-	GPHN	-	-	-
EPIC	BMP7	4.83	4.24	-	BMP7	-	-	-
EPIC	RAPGEF4	4.22	4.22	-	-	-	-	-
EPIC	TNFRSF10A	3.68	4.20	-	TNFRSF10A	-	-	-
EPIC	MICAL2	4.20	4.20	-	MICAL2	-	-	-
EPIC	SFRS8	3.90	4.18	-	-	-	-	SFRS8
EPIC	PNOC	3.57	4.15	-	PNOC	-	-	-
EPIC	USP44	4.81	4.06	-	USP44	-	-	-

FIG. 49B

Study	Gene	IPV6	IPV1	Qthr	Mbm	P/EC	MPK	Ncls
PBCS	DOCK8	7.74	5.83	-	DOCK8	-	-	-
PBCS	NCOR2	6.63	-	-	-	-	-	NCOR2
PBCS	CACNA1C	6.59	4.95	-	CACNA1C	-	-	-
PBCS	MEGF11	6.39	4.07	-	-	MEGF11	-	-
PBCS	GPC6	6.22	4.82	-	GPC6	-	-	-
PBCS	PTENP1	6.14	-	-	-	-	PTENP1	-
PBCS	CDH4	6.06	4.60	-	CDH4	-	-	-
PBCS	OLLU1	6.05	-	-	CLLU1	-	-	-
PBCS	RGS3	5.97	5.80	-	RGS3	-	-	-
PBCS	TMCC3	5.70	-	-	TMCC3	-	-	-
PBCS	SSTR4	5.63	-	-	SSTR4	-	-	-
PBCS	EDN1	5.43	-	-	EDN1	-	-	-
PBCS	ANAPC13	5.39	-	-	-	-	-	ANAPC13
PBCS	TRPC3	5.35	4.86	-	-	-	-	-
PBCS	COL11A1	5.34	4.15	-	COL11A1	-	-	-
PBCS	PRH1	5.33	-	-	PRH1	-	-	-
PBCS	EEA1	5.32	4.27	-	-	-	EEA1	-
PBCS	RBM23	5.22	4.46	-	-	-	-	RBM23
PBCS	HAPLN3	5.22	4.13	-	-	-	-	-
PBCS	PTPRG	5.21	5.08	-	HAPLN3 PTPRG	-	-	-
PBCS	RNASE11	5.21	-	-	RNASE11	-	-	-
PBCS	ANXA4	5.17	4.42	-	-	ANXA4	-	-
PBCS	CADM2	5.14	-	-	CADM2	-	-	-
PBCS	CDK18	5.13	4.39	-	-	-	-	CDK18
PBCS	SLC13A3	5.12	-	-	SLC13A3	-	-	-
PBCS	KIF25	5.11	-	-	KIF25	-	-	-
PBCS	MMD2	5.07	-	-	MMD2	-	-	-
PBCS	TRIM36	5.05	-	-	TRIM36	-	-	-
PBCS	PAX2	5.05	-	-	-	-	-	PAX2
PBCS	GLIS2	5.02	-	-	-	-	-	GLIS2
PBCS	CYB5RL	5.02	-	-	CYB5RL	-	-	-
PBCS	ISM1	5.00	3.83	-	-	-	-	ISM1
PBCS	BAG1	4.99	4.45	-	BAG1	-	-	-
PBCS	ZNF98	4.98	4.19	-	ZNF98	-	-	-
PBCS	ZFAT	4.98	-	-	-	-	-	ZFAT
PBCS	SDK1	4.95	-	-	-	-	-	-
PBCS	TRMT11	4.95	4.29	-	TRMT11	-	-	-
PBCS	HDAC4	4.92	-	-	-	-	-	HDAC4
PBCS	MDF1	4.91	-	-	-	-	-	MDF1
PBCS	TFAP2A	4.91	-	-	-	-	-	TFAP2A
PBCS	HLA-C	4.91	-	-	HLA-C	-	-	-
PBCS	FHIT	4.61	4.48	-	-	-	-	FHIT
PBCS	UNC13C	4.91	4.41	-	-	UNC13C	-	-
PBCS	CHIT1	4.42	4.41	-	CHIT1	-	-	-
PBCS	SYNDIG1	4.38	4.38	-	-	-	-	-
PBCS	SLC25A26	4.74	4.28	-	SLC25A26	-	-	-
PBCS	RAB32	4.84	4.14	-	-	-	-	-
PBCS	A2BP1	4.12	4.12	-	-	-	-	A2BP1
PBCS	N4BP3	4.59	4.07	-	-	-	-	-

FIG. 49C