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(54) Title: METHODS AND COMPOSITIONS FOR THE TREATMENT OF CANCER

(57) Abrégé/Abstract:
Oligopeptides which can be used to treat cancer are disclosed. Further disclosed are methods of treating cancer, including breast cancer, skin cancer, prostate cancer and multiple myeloma (MM). These methods include administration of a polypeptide encoded by the Mesd gene, or an oligopeptide comprising a contiguous subsequence of a Mesd polypeptide.

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(54) Title: METHODS AND COMPOSITIONS FOR THE TREATMENT OF CANCER

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METHODS AND COMPOSITIONS FOR THE TREATMENT OF CANCER

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority from U.S. Provisional Application Serial No. 60/914,902 filed April 30, 2007, which is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with Government support under grant R01-CA100520 awarded by the National Institutes of Health. The Government has certain rights in the invention.

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED IN COMPUTER READABLE FORM

The Sequence Listing, which is a part of the present disclosure, includes a computer readable form and a written sequence listing comprising nucleotide and/or amino acid sequences of the present invention. The sequence listing information recorded in computer readable form is identical to the written sequence listing. The subject matter of the Sequence Listing is incorporated herein by reference in its entirety.

INTRODUCTION

Dysregulation of the Wnt signaling pathway can result in cancer. Several components of the Wnt signaling pathway have been identified as oncogenes or tumor suppressors (showing gain-of-function or loss-of-function mutations, respectively) in human cancers. Dysregulation of Wnt signaling can cause excess proliferation of mammary progenitor cells and predisposes these cells to cancer. For example, activation of the Wnt signaling pathway in the mammary gland is sufficient to induce mammary tumorigenesis, and overexpression of *Wnt-1* can result in mammary tumorigenesis (Woodward, W.A., et al., J. Cell Sci. 118: 3585-2598, 2005; Liu, S., et al., Breast Cancer Res. 86-95, 2005; Howe, L.R. and Brown, A.M., Cancer Biol. Ther. 3: 36-41, 2004).

SUMMARY

In view of an unmet need for new treatments for cancer, the present inventors have developed oligopeptides which can prevent, slow or reverse cancer progression. These oligopeptides include full-length polypeptides encoded by the *mesd* gene, a gene identified as contributing to mesodermal development, as well as fragments thereof.

In some configurations of the present teachings, the inventors have developed oligopeptides, each of which comprises, consists essentially of, or consists of a contiguous subsequence of a polypeptide encoded by a mesd gene. An oligopeptide of these configurations can comprise a full-length mesd polypeptide or a fragment thereof. An oligopeptide of these configurations can comprise, consist essentially of, or consist of an amino acid sequence of from about 10 contiguous amino acids in length up to about 70 contiguous amino acids in length, or from about 30 contiguous amino acids in length up to about 67 contiguous amino acids in length. In various aspects, an oligopeptide can comprise, consist essentially of, or consist of an amino acid sequence selected from CADVTLEGQVYPGKGGGSKEKNQTKQEKGKKKKERDLKPRASKEDNRAGSKKEEL (SEQ ID NO: 1), CADVTLEGQVYPGKGGGSQEKNKTKQEKGKKKKEGVPKSRAKVVQEDNRAGNKREE L (SEQ ID NO: 2), CADVTLEGQVYPGKGGGSKEKNKTKQDKGKKKKEGDLKSRSSKEENRAGNKREDL (SEQ ID NO: 3), CAEVTLEGQMYPGKGGGSKEKNKTKPEKAKKKEGDPKPRASKEDNRAGSRREDL (SEQ ID NO: 4), CAEVTLEGQMYPGKGGGSKEKNKTKPEKAKKKEGDRKPRASKEDNRAGSRREDL (SEQ ID NO: 5), CADVTLEGQVYPGKGGGSKEKNKTKQDKGKKKKEGDLKSRSSKEDNRARNKREDL (SEQ ID NO: 6), CAEVTLEGQMYPGKGGGSKEKNKTKPEKGKKKEGDPKPRASKEDNRAGSRREDL (SEQ ID NO: 7), CADVTLEGQVYPGKGADGSEKGRNKTKPEKAKKKKDAEKSKSSHEDNRANQTERG (SEQ ID NO: 8), KGGGSKEKNK (SEQ ID NO: 9), KGGGSQEKNK (SEQ ID NO: 10), KGGGSKEKNQ (SEQ ID NO: 11), KGGGSKERQL (SEQ ID NO: 12), KGGGSKEKNKTKQDKGKKKKEGDLKSRSSKEENR (SEQ ID NO: 13), KGGGSKEKNKTKQDKGKKKKEGDLKSRSSKEENRAGNK (SEQ ID NO: 14,

QVYPGKGGGSKEKNKTKQDKGKKKKEGDLKSRSSKEENRAGNKREDL (SEQ ID NO: 15), KGGGSKEKNKTKPEKAKKKEGDPKPRASKEDNR (SEQ ID NO: 16), KGGGSKEKNKTKPEKAKKKEGDRKPRASKEDNR (SEQ ID NO: 17), SKEKNKTKPEKAKKKEGDPKPRASKEDNRAGSRREDL (SEQ ID NO: 18), SKEKNKTKPEKAKKKEGDRKPRASKEDNRAGSRREDL (SEQ ID NO: 19), KGGGSKEKNKTKPEKAKKKEGDPKPRASKEDNRAGSR (SEQ ID NO: 20), KGGGSKEKNKTKPEKAKKKEGDRKPRASKEDNRAGSR (SEQ ID NO: 21), QMYPGKGGGSKEKNKTKPEKAKKKEGDPKPRASKEDNRAGSRREDL (SEQ ID NO:22), EGDPKPRASKEDNRAGSR (SEQ ID NO: 23), EGDRKPRASKEDNRAGSR (SEQ ID NO: 24), TKPEKAKKKEGDPKPRAS (SEQ ID NO: 25), KGGGSKEKNKTKPEKAKKK (SEQ ID NO: 26), TKPEKAKKKEGDRKPRAS (SEQ ID NO: 27), KEDNRAGSR (SEQ ID NO: 28) and KEKNKTKPEK (SEQ ID NO: 29).

In some aspects, an oligopeptide can comprise a sequence selected from the group consisting of KGGGSKEKNKTKQDKGKKKKEGDLKSRSSKEENRAGNK (SEQ ID NO: 14), KGGGSKEKNKTKPEKAKKKEGDPKPRASKEDNRAGSR (SEQ ID NO: 20), KGGGSKEKNKTKPEKAKKKEGDRKPRASKEDNRAGSR (SEQ ID NO: 21) and QMYPGKGGGSKEKNKTKPEKAKKKEGDPKPRASKEDNRAGSRREDL (SEQ ID NO: 22).

In some aspects, an oligopeptide can consist essentially of a sequence selected from the group consisting of KGGGSKEKNKTKQDKGKKKKEGDLKSRSSKEENRAGNK (SEQ ID NO: 14) KGGGSKEKNKTKPEKAKKKEGDPKPRASKEDNRAGSR (SEQ ID NO: 20), KGGGSKEKNKTKPEKAKKKEGDRKPRASKEDNRAGSR (SEQ ID NO: 21) and QMYPGKGGGSKEKNKTKPEKAKKKEGDPKPRASKEDNRAGSRREDL (SEQ ID NO: 22).

In some aspects, an oligopeptide can consist of a sequence selected from the group consisting of KGGGSKEKNKTKQDKGKKKKEGDLKSRSSKEENRAGNK (SEQ ID NO: 14) KGGGSKEKNKTKPEKAKKKEGDPKPRASKEDNRAGSR (SEQ ID NO: 20), KGGGSKEKNKTKPEKAKKKEGDRKPRASKEDNRAGSR (SEQ ID NO: 21) and QMYPGKGGGSKEKNKTKPEKAKKKEGDPKPRASKEDNRAGSRREDL (SEQ ID NO: 22).

In some aspects, an oligopeptide of the present teachings which is at least about 10 contiguous amino acids in length up to about 70 contiguous amino acids in length can include conservative amino acid substitutions at one or more positions, as compared to an oligopeptide of a sequence set forth as SEQ ID NO: 1 through SEQ ID NO: 38. In various aspects, an oligopeptide of the present teachings can exhibit a biochemical property of antagonizing binding of a Wnt

ligand to an LRP5 receptor and/or LRP6 receptor.

Further aspects of the present teachings include a full-length mesd polypeptide which differs in one or more amino acids from those of a polypeptide presented herein by one or more conservative amino acid substitutions, in which the polypeptide antagonizes binding of a Wnt ligand to an LRP5 receptor and/or LRP6 receptor.

In related aspects, the present teachings also include oligopeptides having at least about 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79% sequence identity, at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% sequence identity, or at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with a polypeptide set forth as SEQ ID NO: 1 through SEQ ID NO: 32, and also inhibits Wnt binding an LRP5 receptor and/or LRP6 receptor. In various configurations, an oligopeptide can be a substantially pure oligopeptide or an isolated oligopeptide, including a substantially pure or isolated full-length Mesd polypeptide or a portion thereof such as an oligopeptide having a sequence set forth in SEQ ID NO: 1 through SEQ ID NO: 32, or an oligopeptide having conservative substitutions with respect to a sequence of a full-length Mesd polypeptide set forth in Table I or an oligopeptide set forth as SEQ ID NO: 1 through SEQ ID NO: 32.

Table I

Species Common Name	Sequence	SEQ ID NO:	Accession No.
Bos taurus (cow)	MAASGWARAAVIFLCACDLLLLLLLPP RAFATEGPAETPGEATPPPRKKKKDIR DYNDADMARLLEQWEKDDDIEEGDLP EHKRPSAPIDFSQIDPGKPESILKMTKK GKTLMMFVTVSGNPTEKETEEITSLWQ GSLFNANYDVQRFIVGSDRAIFMLRDG GYAWEIKDFLVSQDRCADVTLEGQVY PGKGGGSKEKNQTKQEKGKKKKERD LKPRASKEDNRAGSKKEEL	30	NM_001034469.1
Canis familiaris (dog)	MGSHVLVTRVIGAESCWRLGLHLKKD DDIEEGDLPEHKRPSAPIDFSQIDPGRPE SILKMTKKGKTLMMFVTVSGSPTEKET EEITSLWQGSLFNANYDVQRFIVGSDR AIFMLRDGSYAWEIKDFLVSQDRCAD VTLEGQVYPGKGGGSQEKNKTKQEKG KKKKEGVPKSRAAKVVQEDNRAGNK REEL	31	XM_545883.2
Homo sapien (human)	MAASRWARKAVVLLCASDLLLLLLLL PPPGSCAAEGSPGTPDESTPPPRKKKK DIRDYNDADMARLLEQWEKDDDIEEG DLPEHKRPSAPVDFSKIDPSKPESILKM TKKGKTLMMFVTVSGSPTEKETEEITS LWQGSLFNANYDVQRFIVGSDRAIFM LRDGSYAWEIKDFLVGQDRCADVTLE GQVYPGKGGGSKEKNKTKQDKGKKK KEGDLKSRSSKEENRAGNKREDL	32	NM_015154

Species Common Name	Sequence	SEQ ID NO:	Accession No.
Gallus gallus (chicken)	MAAAARWAALGLALWLCAAAHAEEP EGKRRAGPAKKKDIRDYNDADMARL LEQWEKDDDIEEGDLPEHKRPPAPIDF SKIDPGKPESILKLTKKGKTLMMFVTV SGNPTEKETEEITSLWQGSLFNANYDV QRFIVGSNRAIFMLRDGGYAWEIKDFL ISQERCADVTLEGQVYPGKGADGSEK GRNKTKPEKAKKKKDAEKSKSSHEDN RANQTERGSMTDT	33	NM_001030551
Mus musculu (house mouse	MAASRWLRAVLLFLCASDLLLLPPPN AYAADTPGEATPPPRKKKDIRDYNDA DMARLLEQWEKDDDIEEGDLPEHKRP SAPIDFSKLDPGKPESILKMTKKGKTL MMFVTVSGNPTEKETEEITSLWQGSLF NANYDVQRFIVGSDRAIFMLRDGSYA WEIKDFLVSQDRCAEVTLEGQMYPGK GGGSKEKNKTKPEKAKKKEGDRKPRA SKEDNRAGSRREDL	34	NM_023403
Pan troglodytes (chimpanzee	MAASRWARKAVVLLCASDLLLLLLLL PPPGSCAAEGSPGTPDESTPPPRKKKK DIRDYNDADMARLLEQWEKDDDIEEG DLPEHKRPSAPVDFSKIDPSKPESILKM TKKGKTLMMFVTVSGSPTEKETEEITS LWQGSLFNANYDVQRFIVGSDRAIFM LRDGSYAWEIKDFLVGQDRCADVTLE GQVYPGKGGGSKEKNKTKQDKGKKK KEGDLKSRSSKEENRAGNKREDL	35	XM_510542.1

Species Common Name	Sequence	SEQ ID NO:	Accession No.
Pongo pygmaeus (orangutan)	MAASSWARKAVVVLCASDLLLLLLLL PPPGSCAAEASPGTPDESTPPPRKKKK DIRDYNDADMARLLEQWEKDDDIEEG DLPEHKRPSAPVDFSKIDPSKPESILKM TKKGKTLMMFVTVSGSPTEKETEEITS LWQGSLFNANYDVQRFIVGSDRAIFM LRDGNYAWEIKDFLVGQDRCADVTLE GQVYPGKGGGSKEKNKTKQDKGKKK KEGDLKSRSSKEDNRARNKREDL	36	CR860539
Rattus norvegicus (Norway rat)	MAASSWLRAVLLFLCASDLLLLSPPEA YATDTPGEAITPPRKKKDIRDYNDAD MARLLEQWEKDDDIEEGDLPEHKRPS APIDFSKLDPGKPESILKMTKKGKTLM MFVTISGNPTEKETEEITSLWQGSLFNA NYDVQRFIVGSDRAIFMLRDGSYAWEI KDFLVNQDRCAEVTLEGQMYPGKGG GSKEKNKTKPEKGKKKEGDPKPRASK EDNRAGSRREDL	37	NM_001008345
Xenopus laevis (Africa clawed frog)	MGRSRSRSPERRRERRRSRSASRERER RRRERSRSRERRRSRSRSPHRRRSRSPR RHRSSSISPSRLKDRRDDDKKEPKESK GGGSKERQLAAEDLEGKTEEEIEMMK LMGFASFDSSKGKKTDGSVNAYAINV SQKRKYRQYMNRKGGFNRPLDFVA	38	BC074295

In various aspects, the sequence of a Mesd polypeptide or a portion thereof can be that of a polypeptide or a portion thereof comprising at least about 10 contiguous amino acids and encoded by a mesd gene from any animal, including, in non-limiting example, a vertebrate such as a fish, a reptile, an amphibian such as Xenopus laevis, a bird such as Gallus gallus or a mammal such as a human or rodent such as Mus musculus, provided the sequence shares at least about 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79% sequence identity, at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% sequence identity, or at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with a Mesd polypeptide or at least one sequence set forth as SEQ ID NO: 1 through SEQ ID NO: 38, and also inhibits Wnt binding to an LRP5 receptor and/or LRP6 receptor, such as an LRP5 receptor and/or LRP6 receptor expressed on a cancer cell. In various aspects, a Mesd polypeptide or oligopeptide can be at least about 20 contiguous amino acids, at least about 30 contiguous amino acids, or at least 37 contiguous amino acids in length, up to and including full length genes. In various aspects, a polypeptide or oligopeptide antagonizes binding of a Wnt ligand to an LRP5 receptor and/or LRP6 receptor.

In other aspects, the present teachings include methods of treatment of cancers in which Wnt signaling is modified. In one embodiment the method comprises administering to a subject in need of therapy, such as a human patient diagnosed with cancer, a Mesd polypeptide, a therapeutically effective amount of a Mesd polypeptide as set forth in table I or an oligopeptide which is at least about 10 contiguous amino acids in length up to about 70 contiguous amino acids in length, and comprises a sequence set forth in SEQ ID NO: 1 through SEQ ID NO: 32, or a sequence comprising at least 10 contiguous amino acids in length up to about 70 contiguous amino acids in length and sharing at least about 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79% sequence identity, at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% sequence identity, or at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with a Mesd polypeptide as set forth in Table I or at least one sequence set forth as SEQ ID NO: 1 through SEQ ID NO: 38. In related aspects, a cancer can be treated by administering to a subject a full length Mesd polypeptide, such as a polypeptide set forth in Table I, including a mammalian Mesd polypeptide such as a human or murine Mesd polypeptide.

In some configurations, a cancer can be treated by administering to a subject a polypeptide sharing at least about 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79% sequence identity, at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% sequence identity, or at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with full length

Mesd polypeptide, such as a polypeptide set forth in Table I, including a mammalian Mesd polypeptide such as a human or murine Mesd polypeptide.

In various other aspects, the inventor have developed vectors comprising a promoter operably linked to a nucleic acid sequence encoding a Mesd polypeptide, or an oligopeptide which comprises a sequence from a polypeptide encoded by the mesd gene as described herein. In various aspects, a vector can be a plasmid or a virus, and a promoter can be a eukaryotic promoter or a prokaryotic promoter. These vectors can be used to produce an oligopeptide ex vivo, e.g., by expression in a host cell or host microorganism in vitro, or can be used therapeutically, such as by administering a vector described in the present teachings, or by administering, to a subject in need of treatment, cells comprising the vector and which produce a full-length Mesd polypeptide or a Mesd oligopeptide. In such configurations, a vector can comprise, in addition to a promoter and a nucleic acid encoding an Mesd polypeptide or oligopeptide, a sequence linked to those encoding the polypeptide or oligopeptide and encodes an amino acid sequence which promotes polypeptide or oligopeptide export or secretion from a cell, such as a leader peptide sequence known to those of skill in the art.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates that Mesd binds to mature LRP6 at the cell surface with high affinity.

FIG. 2 illustrates that Mesd binds to mature LRP5 and LRP6 but not significantly to other members of the LDLR family.

FIG. 3 illustrates that the C-terminal region of Mesd is required for interaction with LRP6.

FIG. 4 illustrates that the C-terminal region of Mesd is necessary and sufficient for LRP6 binding.

FIG. 5 illustrates that the carboxy-terminal region of Mesd is required for LRP6 folding.

FIG. 6 illustrates that LRP6 is not a constitutively active endocytosis receptor.

FIG. 7 illustrates that LRP6 exhibits a limited level of Mesd degradation.

FIG. 8 illustrates that the 39 kDa specialized molecular chaperone receptor-associated protein RAP binds to LRP6 and partially competes for Mesd binding.

FIG. 9 illustrates that Mesd inhibits DKK1 binding to LRP6.

FIG. 10 illustrates that Mesd polypeptide or a Mesd oligopeptide can both bind LRP5 and inhibit binding of DKK1 to either LRP5 or LRP6.

FIG. 11 shows results from HEK293 cells stably transfected with TCF/LEF-luc reporter construct was treated without Wnt-3A or with Wnt-3A plus the indicated inhibitors (Mesd and/or Dkk1) for 16 hours at 37^0C. Wnt signaling was measured by quantifying the luciferase activity using a live cell imaging system.

FIG. 12 shows MMTV-LRP6 transgenic mouse showing mammary gland hyperplasia. MMTV-LRP6 transgenic mice were generated by overexpressing LRP6 cDNA in the mammary epithelial cells driven by the MMTV promoter. Mammary glands from littermates of either WT (panels A, C) or MMTV-LRP6 virgin mice (panels B, D) at 14 weeks of age were analyzed by whole mount staining. Note mammary hyperplasia in MMTV-LRP6 mice (panel B at low power field and D at high power field).

DETAILED DESCRIPTION

Wnt receptors are candidate oncoproteins, and mutated forms of two members of the mammalian low-density lipoprotein receptor (LDLR)-related protein (LRP) family, LRP5 and LRP6, have recently been shown to be capable of constitutively activating Wnt/β-catenin signaling in cell culture. The present findings utilize LRP5 AND LRP6-specific inhibitors such as Mesd to inhibit Wnt signaling at the cell surface.

The methods and compositions described herein utilize laboratory techniques well known to skilled artisans and can be found in laboratory manuals such as Sambrook, J., et al., Molecular Cloning: A Laboratory Manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2001; Spector, D. L. et al., Cells: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1998; Harlow, E., Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1999, and Ausubel, F. M., et al., ed., Current Protocols in Molecular Biology. For pharmaceutical compositions and methods of treatment disclosed herein, dosage forms and administration regimes can be determined using standard methods known to skilled artisans, for example as set forth in standard references such as Remington: the Science and Practice of Pharmacy (Alfonso R. Gennaro ed. 19th ed. 1995); Hardman, J.G., et al., Goodman & Gilman's The Pharmacological Basis of Therapeutics, Ninth Edition, McGraw-Hill, 1996; and Rowe, R.C., et al., Handbook of Pharmaceutical Excipients, Fourth Edition, Pharmaceutical Press, 2003.

The present inventor discloses substantially pure oligopeptides which can be used to treat cancers, including a cancer in which cancerous cells exhibit modified Wnt signaling, such as, without limitation, breast cancer, multiple myeloma (MM), prostate cancer and skin cancer. Without being limited by theory, the present inventor has found that an Mesd polypeptide can inhibit Wnt ligand binding to (LDLR)-related protein-5 (LRP5) and (LDLR)-related protein-6 (LRP6). Accordingly, Mesd polypeptide or a functional fragment thereof can be used for treating cancer and for increasing bone health, as described in US Patent application 11/557,292, filed November 7, 2006. Without being limited by theory, the inventor presumes that signaling by the low-density lipoprotein receptor (LDLR)-related protein-5 (LRP5) and (LDLR)-related protein-6 (LRP6) which are both members of the LDLR family, is subject to inhibition by an extracellular Wnt ligand. Recently, a specialized chaperone for members of the LDLR family, termed Mesd (mesoderm development) in mouse and Boca in Drosophila has been identified (Culi, J., Cell 112:

343-354, 2003; Hsieh, J.C., Cell 112: 355-367, 2003). This new chaperone was discovered due to its requirement for the folding of LRP5/LRP6, co-receptors for the Wnt/Wg signaling pathway. However, the present inventor has found that Mesd not only mediates folding of LRP5 and LRP6, it also is capable of binding mature LRP5 or LRP6 at the cell surface, and antagonizes binding of ligand such as a Wnt ligand. In addition, the present inventor has determined that the ligand-binding antagonizing activity is found in oligopeptides comprising subsequences of Mesd from the carboxy-terminal region of the Mesd polypeptide.

Oligopeptides of the present teachings comprise from about 10 contiguous amino acids up to about 70 contiguous amino acids, wherein the peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12. The sequences represent subsequences from Mesd polypeptide encoded by a mesd gene comprised by the genome of a variety of species such as, without limitation, human, mouse, dog, cow, chimpanzee, orangutan and rat. As used herein, the term "oligopeptide" refers to a molecule comprising at least two amino acids joined by peptide bonds, and the term "polypeptide" refers to a molecule comprising a full-length amino acid sequence as encoded by a gene, an mRNA or a cDNA. In various configurations, a substantially pure oligopeptide of the present teachings can comprise at least 20 or at least 30 contiguous amino acids, up to about 67 amino acids. Sequences of the present teachings are set forth herein in Table II, which present oligopeptide sequences of from 54 to 67 contiguous amino acids, and Table III, which presents oligopeptide sequences of from 10 to 46 amino acids. An oligopeptide of the present teachings can be synthesized using standard techniques well known to skilled artisans, such as, in non-limiting example Merrifield solid phase synthesis, or molecular cloning methods, including, in non-limiting example, synthesizing an oligonucleotide encoding an oligopeptide and inserting the oligonucleotide into a vector, or subcloning a portion of a cDNA into a vector using restriction enzyme digestion, ligation with a ligase, and/or polymerase chain reaction techniques. A vector comprising an oligonucleotide encoding an oligopeptide can in inserted into a cell by transfection or transformation, and expressed in the cell using methods well known to skilled artisans. Oligopeptides can be isolated and/or purified by standard techniques well known to skilled artisans.

Table II

Species Common Name	Sequence	Seq. ID No.
Bos taurus (cow)	CADVTLEGQVYPGKGGGSKEKNQTKQEKGKKKKER DLKPRASKEDNRAGSKKEEL	1
Canis familiaris (dog)	CADVTLEGQVYPGKGGGSQEKNKTKQEKGKKKKEG VPKSRAKVVQEDNRAGNKREEL	2
Homo sapiens (human)	CADVTLEGQVYPGKGGGSKEKNKTKQDKGKKKKEG DLKSRSSKEENRAGNKREDL	3
Mus musculus (house mouse)	CAEVTLEGQMYPGKGGGSKEKNKTKPEKAKKKEGD PKPRASKEDNRAGSRREDL	4
Mus musculus (house mouse)	CAEVTLEGQMYPGKGGGSKEKNKTKPEKAKKKEGD RKPRASKEDNRAGSRREDL	5
Pan troglodytes (chimpanzee)	CADVTLEGQVYPGKGGGSKEKNKTKQDKGKKKKEG DLKSRSSKEENRAGNKREDL	3
Pongo pygmaeus (orangutan)	CADVTLEGQVYPGKGGGSKEKNKTKQDKGKKKKEG DLKSRSSKEDNRARNKREDL	6
Rattus norvegicus (Norway rat)	CAEVTLEGQMYPGKGGGSKEKNKTKPEKGKKKEGD PKPRASKEDNRAGSRREDL	7
Gallus gallus (chicken)	CADVTLEGQVYPGKGADGSEKGRNKTKPEKAKKKK DAEKSKSSHEDNRANQTERG	8

Table III

Source	Sequence	Seq. ID No.
Homo sapiens (human)	KGGGSKEKNKTKQDKGKKKKEGDLKSRSSKEENR	13
Canis familiaris (dog)	KGGGSKEKNKTKQDKGKKKKEGDLKSRSSKEENR	13
Bos taurus (cow)	KGGGSKEKNKTKQDKGKKKKEGDLKSRSSKEENR	13
Xenopus laevis (African Clawed Frog)	KGGGSKEKNKTKQDKGKKKKEGDLKSRSSKEENR	13
Homo sapiens (human)	KGGGSKEKNKTKQDKGKKKKEGDLKSRSSKEENR AGNK	14
Homo sapiens (human)	QVYPGKGGGSKEKNKTKQDKGKKKKEGDLKSRSS KEENRAGNKREDL	15
Mus musculus (house mouse)	KGGGSKEKNKTKQDKGKKKKEGDLKSRSSKEENR	13
Mus musculus (house mouse)	SKEKNKTKPEKAKKKEGDPKPRASKEDNRAGSRR EDL	18
Mus musculus (house mouse)	SKEKNKTKPEKAKKKEGDRKPRASKEDNRAGSRR EDL	19
Mus musculus (house mouse)	KGGGSKEKNKTKPEKAKKKEGDPKPRASKEDNRA GSR	20

Source	Sequence	Seq. ID No.
Mus musculus (house mouse)	KGGGSKEKNKTKPEKAKKKEGDRKPRASKEDNRAGSR	21
Mus musculus (house mouse)	QMYPGKGGGSKEKNKTKPEKAKKKEGDPKPRASKEDNRAGSRREDL	22
Mus musculus (house mouse)	EGDPKPRASKEDNRAGSR	23
Mus musculus (house mouse)	EGDRKPRASKEDNRAGSR	24
Mus musculus (house mouse)	TKPEKAKKKEGDPKPRAS	25
Mus musculus (housc mousc)	KGGGSKEKNKTKPEKAKKK	26
Mus musculus (house mouse)	TKPEKAKKKEGDRKPRAS	27
Mus musculus (house mouse)	KGGGSKEKNK	9
Mus musculus (house mouse)	KEDNRAGSR	28
Mus musculus (house mouse)	KEKNKTKPEK	29

In oligopeptides of the present teachings comprising conservative substitutions, such substitutions can be from families of amino acid residues having similar side chains as have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g.,

glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Conserved substitutes for an amino acid within a native amino acid sequence can be selected from other members of the family to which the naturally occurring amino acid belongs.

A further aspect of the invention comprises polypeptides and oligopeptides which differ in one or more amino acids from those of a polypeptide or oligopeptide sequence described herein by deletion or insertion of one or more amino acids.

Accordingly, in some configurations of the present teachings, an oligopeptide can be from about 10 amino acids in length up to 67 amino acids in length. The sequence can comprise any sequence set forth in Table II or Table III, and/or can include any sequence selected from SEQ ID NO: 1 through SEQ ID NO: 38. Furthermore, a sequence of an oligopeptide can be a sequence sharing at least 70% sequence identity, at least 80% sequence identity, or at least 90% sequence identity with at least one sequence of SEQ ID NO: 1 through SEQ ID NO: 38 and has the biochemical property of antagonizing, inhibiting or blocking binding of a mature cell surface proteins LRP5 and/or LRP6 with an extracellular ligand such as a Wnt ligand, when the oligopeptide is contacted with an LRP5 and/or LRP6, such as an LRP5 and/or LRP6 comprised by a cell membrane. Furthermore, a sequence of an oligopeptide can be a sequence sharing at least 70% sequence identity, at least 80% sequence identity, or at least 90% sequence identity with at least one sequence of SEQ ID NO: 1 through SEQ ID NO: 38 and furthermore can have the biochemical property of antagonizing Wnt signaling. In various configurations, conservative substitutions can be made in oligopeptide sequences, for example substitution of a hydrophobic amino acid such as valine with a different hydrophobic amino acid such as isoleucine. Methods for identifying and selecting conservative substitutions for amino acids are well known to skilled artisans (see, e.g., Pearson, W.R., Methods Enzymol. 266: 227-258, 1996).

In other configurations of the present teachings, the inventor disclose nucleic acid vectors comprising a promoter operably linked to a nucleic acid sequence encoding an oligopeptide comprise a sequence selected from SEQ ID NO: 1 through SEQ ID NO: 38. In some aspects, the

oligopeptide or polypeptide sequence can be a sequence sharing at least about 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79% sequence identity, at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% sequence identity, or at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity with at least one of SEQ ID NO: 1 through SEQ ID NO: 38. A promoter of these configurations can be a eukaryotic promoter (i.e., a promoter which can support transcription in the environment of a eukaryotic cell such as a mammalian cell or a microbial eukaryotic cell such as a yeast cell) or a prokaryotic promoter (i.e., a promoter which can support transcription in the environment of a prokaryotic cell such as a bacterium). Non-limiting examples of a promoter which can be used in a vector of the present teachings include an actin promoter, a CUP1 promoter from a yeast metallothionein gene, and promoter-enhancer elements from the simian virus 40 (SV40) early-region or a mouse alpha 2(I)-collagen gene, and an E. coli lac operon operator/promoter. A vector can be, for example, a plasmid or a virus, such as, for example, a baculovirus or a bacteriophage. In addition, in some configurations, the present teachings encompass a cell comprising a vector as described herein. A cell comprising a vector can be a cell in which the promoter of the vector is operable, for example an E. coli cell harboring a plasmid comprising a lac operon/promoter, or an insect cell harboring a baculovirus vector.

In various configurations, the present teachings include methods of treating cancer in a subject in need of treatment. Methods of these configurations include administering to a subject a therapeutically effective amount of a Mesd polypeptide, a polypeptide sharing at least 70% sequence identity, at least 80% sequence identity, or at least 90% sequence identity with an Mesd polypeptide, or an oligopeptide comprising between 10 contiguous amino acids and about 70 contiguous amino acids, wherein the oligopeptide comprises an amino acid sequence selected from SEQ ID NO: 1 through SEQ ID NO: 38 and a sequence sharing at least 70% sequence identity, at least 80% sequence identity, or at least 90% sequence identity with at least one sequence set forth as SEQ ID NO: 1 through SEQ ID NO: 38, wherein an polypeptide or oligopeptide antagonizes binding of a Wnt ligand to an LRP5 receptor and/or LRP6 receptor. when in contact with LRP5. In some aspects, the cancer can be breast cancer and multiple myeloma (MM), prostate cancer and skin cancer, or any cancer with these characteristics. These methods can also be applied to cells or tissues in vitro or ex vivo. In addition, in some aspects, the present methods also include administering to a subject in need of treatment a vector such as described above, or cells comprising a vector, such as human cells comprising a vector comprising a eukaryotic promoter operably linked to a nucleic acid encoding an oligopeptide as described

herein. In non-limiting example, the human cells can be cells from a subject which are transformed with a vector, grown in vitro using standard cell culture techniques, and returned to the donor.

A therapeutically effective amount of a polypeptide, oligopeptide or vector of the present teachings can be determined using methods well known in the art, such as found in standard pharmaceutical texts such as Herfindal, Gourley and Hart, Williams and Wilkins, ed. Clinical Pharmacy and Therapeutics, Williams & Wilkins, 1988; Goodman, L.S. and Gilman, A., ed. The Pharmacological Basis of Therapeutics, McGraw-Hall; 2005; Kalant, H., and Roschlau, W.H.E., ed., Principles of Medical Pharmacology, Mosby, Incorporated. 1989; J.T. DiPiro, R.L. et al., ed. Pharmacotherapy: A Pathophysiologic Approach, McGraw-Hill Medical Publishing, 2005; Ascione, Principles of Scientific Literature Evaluation Critiquing Clinical Drug Trials, American Pharmacists Association, 2001; and Remington, The Science and Practice of Pharmacy, Lippincott Williams & Wilkins, 2005.

EXAMPLES

The following examples provide non-limiting illustrations of the present teachings. While some of examples may include conclusions about the way the invention may function, the inventor do not intend to be bound by those conclusions, but put them forth only as possible explanations. Unless noted by use of past tense, presentation of an example does not imply that an experiment or procedure was, or was not, conducted, or that results were, or were not actually obtained.

Example 1

This example illustrates that Mesd binds to mature LRP6 at the cell surface.

To examine whether Mesd binds with high affinity to most members of the LDLR family at the cell surface, we performed cell surface ligand binding experiments with cells stably transduced with LRP6 cDNA. Human HT1080 cells, which express undetectable levels of LRP6, were transduced with a viral vector alone (pLNCX2) or with vector containing LRP6 cDNA (Li, Y., Oncogene 23: 9129-9135, 2004) and used for ^{125}I-Mesd binding (FIG. 1A). ^{125}I-Mesd (5 nM) reached maximal binding after 2 hours incubation at 4°C with LRP6-expressing HT1080 cells (FIG. 1A). Inclusion of excess unlabeled Mesd (500 nM) completely eliminated this binding. No significant ^{125}I-Mesd binding was seen with the control cells (pLNCX2). Saturation of Mesd specific binding was seen at concentrations of >6.4 nM (FIG. 1B). Scatchard analysis of the

binding data revealed that Mesd binds LRP6 with a Kd of ~3.3 nM (FIG. 1C). This affinity of Mesd to LRP6 is comparable to that of RAP to LRP (Iadonato, S.P., Biochem. J. 296: 867-875, 1993).

As illustrated in FIG. 1, Mesd binds to mature LRP6 at the cell surface with high affinity. (A) Time course of ^{125}I-Mesd (5 nM) binding to LRP6-transduced HT1080 cells and the control cells. Assay was carried out for the indicated periods at 4°C in the absence (total) or presence of 500 nM Mesd (non-specific). (B) Saturation binding of ^{125}I-Mesd to LRP6-transduced HT1080 cells and the control cells. Assay was carried out at indicated concentrations for 3 hours at 4°C in the absence (total) or presence (non-specific) of 500 nM Mesd. (C) Scatchard plots of data in B. All values are the average of triple determinations with the s.d. indicated by error bars.

In this and all subsequent examples, the following materials and methods were used:

Materials

Human recombinant DKK1 protein and mouse recombinant Wnt3a protein were from R&D Systems. Human recombinant RAP protein was expressed in a glutathione S-transferase (GST) expression vector and isolated as described previously (Bu et al., 1993). Monoclonal anti-Myc antibody 9E10 was from Roche. Monoclonal antibody 8G1 against human LRP was from Research Diagnostics. Monoclonal anti- HA antibody has been described before (Li, Y., J. Biol. Chem. 275: 17187-17194, 2000). Polyclonal rabbit anti-LDLR was produced by immunizing rabbits with recombinant human LDLR1-294 fragment. Peroxidase-labeled antimouse antibody and ECL system were from Amersham Life Science. Plasmid pcDNA3.1C-Myc-hLRP5 containing the full-length human LRP5 cDNA and plasmid pCS-Myc-hLRP6 containing the full-length human LRP6 cDNA were from Cindy Bartels and Christof Niehrs, respectively. Carrier-free Na^{125}I was purchased from NEN Life Science Products. IODO-GEN was from Pierce. Proteins were iodinated by using the IODO-GEN method as described previously (Li, Y., J. Biol. Chem. 275: 17187-17194, 2000).

Cell lines and cell culture

LRP6-transduced HT1080 cells and the control cells have been described before (Li et al., 2004), and were cultured in DMEM medium containing 10% fetal bovine serum and 350 _g/ml G418. The LRP-null CHO cells stably transfected with human LDLR-related protein (LRP)

minireceptor mLRP4, mLRP4 tail mutant mLRP4tailess (mLRP4 without the cytoplasmic tail), human LDLR-related protein 1B (LRP1B) minireceptor mLRP1B4, human VLDLR, or human apoER2 have been described before (Li, Y., J. Biol. Chem. 275: 17187-17194, 2000; Li et al., 2001; Liu et al., 2001), and were cultured in Ham's F-12 medium containing 10% fetal bovine serum and 350 μg/ml G418. A set of genetically derived murine embryonic fibroblasts (MEF) from mouse embryos deficient for LRP and/or LDLR were obtained from Joachim Herz, University of Texas Southwestern Medical Center at Dallas (Willnow, J. Cell Sci. 107: 719-726., 1994; Narita, M., J. Biochem. 132: 743-749, 2002). These are MEF-1 (WT), MEF-2 (LRP-deficient), MEF-3 (LDLR-deficient), and MEF- 4 (LRP and LDLR-double-deficient), and are cultured in DMEM containing 10% fetal bovine serum. Culture conditions of U87, MCF- 7, and human aortic smooth muscle cells have been described before (Li, Y., FEBS Lett. 555: 346-350, 2003). HEK293 cells were from ATCC, and cultured in DMEM containing 10% fetal bovine serum.

Preparation of recombinant Mesd protein

Full-length mouse Mesd cDNA was used. The wild-type and mutant forms of mouse Mesd were generated by polymerase chain reactions, and subcloned into the expression vector pET-30a(+) (Novagen) at the EcoRI and HindIII restriction sites. The integrity of the subcloned DNA sequence was confirmed by DNA sequencing. Recombinant proteins were overexpressed from pET-30(+)Mesd in E. coli. BL21(DE3) producing a recombinant fusion protein with a polyhistidine metal-binding tail at the N-terminus, and purified with His-Bind Kits from Novagen according to the manufacturer's protocol. All the recombinant Mesd proteins lack the Mesd signal peptide.

Western blotting

To examine the expression of the LDLR family members, cells cultured in six-well plates were lysed with 0.5 ml lysis buffer (phosphate-buffered saline containing 1% Triton X-100 and 1 mM PMSF) at 4°C for 30 minutes. Equal quantities of protein were subjected to SDS-PAGE under non-reducing conditions. Following transfer to Immobilon-P membrane, successive incubations with primary antibody and horseradish peroxidase-conjugated secondary antibody were carried out for 60 minutes at room temperature. The immunoreactive proteins were then detected using the ECL system.

To examine the cytosolic β-catenin level, cells in six-well plates were treated with Mesd at various concentrations for 90 minutes at 37°C. After washing in ice-cold PBS, cells were collected and homogenized in a glass Dounce homogenizer in buffer consisting of 100 mM Tris-HCl pH 7.4, 140 mM NaCl, 2 mM DTT, 2 mM PMSF, and 1X Complete™ protease inhibitors (500 µl/well). The homogenate was centrifuged for 10 minutes at 500 g, and the supernatant was further centrifuged at 100,000 g at 4°C for 90 minutes. The resulting supernatant was designated the cytosolic fraction. The β-catenin levels were then examined by western blotting using β-catenin-specific antibody from Cell Signaling Technology. The immunoreactive proteins were detected using the ECL system. Films showing immunoreactive bands were scanned with a Kodak Digital Science DC120 Zoom Digital Camera and band intensities were analyzed with Kodak Digital Science1D Image Analysis Software.

Luciferase reporter assay

HEK293 cells were plated into six-well plates. For each well, 0.1 µg of the TOP-FLASHTCF luciferase construct (Upstate Biotechnology) was cotransfected with 0.8 µg Mesd-expressing vector, 0.8 µg Mesd mutant-expressing vector, or empty vector. A β-galactosidase-expressing vector (Promega, Madison, WI) was included as an internal control for transfection efficiency. After 48 hours, cells were lysed and both luciferase and β-galactosidase activities were determined with enzyme assay kits (Promega). The luciferase activity was determined with a luminometer using the Dual Luciferase Assay system (Promega). Luciferase activity was normalized to the activity of the β-galactosidase.

Ligand binding and degradation

Cells (2×10^5) were seeded into 12-well dishes 1 day prior to assay. Ligand-binding buffer (minimal Eagle's medium containing 0.6% BSA with a different concentration of radioligand, 0.6 ml/well) was added to cell monolayers, in the absence or the presence of 500 nM unlabeled RAP or 500 nM unlabeled Mesd, followed with incubation for 0-4 hours at 4°C. Thereafter, overlying buffer containing unbound ligand was removed, and cell monolayers were washed and lysed in low-SDS lysis buffer (62.5 mM Tris-HCl pH 6.8, 0.2% SDS, 10% v/ glycerol) and counted. The protein concentration of each cell lysate was measured in parallel dishes that did not contain the ligands.

Ligand degradation was performed using the methods as described (Li, Y., J. Biol. Chem. 275: 17187-17194, 2000). Briefly, 2 x 10^5 cells were seeded into 12-well dishes 1 day prior to assay. Pre-warmed assay buffer (minimal Eagle's medium containing 0.6% BSA with radioligand, 0.6 ml/well) was added to cell monolayers in the absence or the presence of unlabeled 500 nM RAP or 500 nM Mesd, followed by incubation for 4 hours at 37°C. Thereafter, the medium overlying the cell monolayers was removed and proteins were precipitated by addition of BSA to 10 mg/ml and trichloroacetic acid to 20%. Degradation of radioligand was defined as the appearance of radioactive fragments in the overlying medium that were soluble in 20% trichloroacetic acid. Kinetic analysis of endocytosis LRP6-transduced HT1080 cells were plated in 12-well plates at a density of 2 x 10^5 cells/well and used after overnight culture. Cells were rinsed twice in ice-cold assay buffer (minimal Eagle's medium containing 0.6% BSA), and ^{125}I-anti-HA IgG was added at 1 nM final concentration in cold assay buffer (0.5 ml/well). The binding of ^{125}Ianti- HA IgG was carried out at 4°C for 90 minutes with gentle rocking. Unbound ^{125}I-anti-HA IgG was removed by washing cell monolayers three times with cold assay buffer. Ice-cold stop/strip solution (0.2 M acetic acid, pH 2.6, 0.1 M NaCl) was added to one set of plates without warming up and kept on ice. The remaining plates were then placed in a 37°C water bath and 0.5 ml assay buffer prewarmed to 37°C was quickly added to cell monolayers to initiate internalization. After each time point, the plates were quickly placed on ice and the assay buffer was replaced with cold stop/strip solution. ^{125}I-anti-HA IgG that remained on the cell surface was stripped by incubation of cell monolayers with cold stop/strip solution for a total of 20 minutes (0.75 ml for 10 minutes, twice) and counted. Cell monolayers were then solubilized with low-SDS lysis buffer and counted. The sum of ^{125}I-anti-HA IgG that was internalized plus that remaining on the cell surface after each assay was used as the maximum potential internalization. The fraction of internalized ^{125}I-anti-HA IgG after each time point was calculated and plotted.

Cell surface DKK1 binding and immunodetection

Human DKK1 cDNA (clone MGC:868, IMAGE:3508222) was obtained from Invitrogen and subcloned into pcDNA3 (EcoRI/XbaI). To facilitate immunodetection, a c-Myc epitope was included at the C-terminus. The integrity of the subcloned DNA sequence was confirmed by DNA sequencing. Human DKK1-conditioned media were produced by transient transfection of HEK293 cells with pcDNADKK1-Myc in serum-free medium, and allowed to bind to LRP6-transduced HT1080 cells and control cells at room temperature for 60 minutes in the

absence or presence of 1 μM Mesd. Cells were then fixed in 4% paraformaldehyde, labeled with anti-Myc monoclonal antibody and detected with Alexa-488 goat anti-mouse IgG. The immunofluorescence was detected by a laser-scanning confocal microscope (Olympus Fluoview 500).

Example 2

This example illustrates that Mesd binds to mature LRP5 AND LRP6 but not significantly to other members of the LDLR family.

To determine whether Mesd binds to other members of the LDLR family, ^{125}I-Mesd binding analysis was performed with four groups of cells expressing different members of the LDLR family (FIG. 2). In the first experiment, HEK293 cells were transiently transfected with cDNAs for the LDLR, LRP5, LRP6 or empty pcDNA3 vector. In the second experiment, LRP-null Chinese hamster ovary (CHO) cells were stably transfected with LRP minireceptor mLRP4, LRP1B minireceptor mLRP1B4, apoER2, VLDLR, or empty pcDNA3 vector (Li, Y., J. Biol. Chem. 275: 17187-17194, 2000; Li, Y., J. Biol. Chem. 276: 18000-18006, 2001; Liu, C.X., J. Biol. Chem. 276:28889-28896, 2001). mLRP4 is composed of residues 3274-4525 of the full-length LRP, which includes the fourth cluster of ligand-binding repeats and the entire C-terminus of the receptor. mLRP1B4 is composed of residues 3276-4599 of the full length LRP1B, which includes the fourth cluster of ligand binding repeats and the entire C-terminus of the receptor. mLRP4 and mLRP1B4 mimic the function and trafficking of LRP and LRP1B, respectively. In the third experiment, wild-type murine embryonic fibroblasts and murine embryonic fibroblasts with genetic deficiency of LDLR, LRP, or both (Willnow, T.E., J. Cell Sci. 107: 719-726, 1994; Narita, J. Biochem. 132: 743-749, 2002) were used. In the fourth experiment, the human breast cancer cell line MCF-7, human glioblastoma cell line U87, and human aortic smooth muscle cells (SMC) were used. MCF-7 cells express LRP at an undetectable level, whereas U87 cells and SMC express abundant LRP (Li, Y., FEBS Lett. 555: 346-350, 2003). Interestingly, among the members of the LDLR family examined, only LRP5 specifically binds to Mesd, albeit at lower levels compared to LRP6 when these receptors were expressed at comparable levels (FIG. 2A). Although there is a suggestion of Mesd binding to LRP when examined in CHO and MEF cells (FIG. 2B,C), specific Mesd binding to U87 or SMC, both of which express abundant LRP, was minimal (FIG. 2D). Therefore, specific binding of Mesd to CHO and MEF cells may reflect endogenous LRP5/LRP6 in these cells.

As illustrated in FIG. 2, Mesd binds to mature LRP5 AND LRP6 but not significantly to other members of the LDLR family. (A) Binding of ^{125}I-Mesd (5 nM) to HEK293 cells transient transfected with human HA-tagged LDLR, Myc-tagged LRP5, Myc-tagged LRP6 or control vector. Lower panel, western blot analysis for the expression of the LDLR, LRP5 and LRP6. Equal amounts of cell lysate were applied for each lane. (B) Binding of ^{125}I-Mesd (5 nM) to LRP-null CHO cells stably transfected with LRP minireceptor mLRP4, LRP1B minireceptor mLRP1B4, VLDLR, apoER2 or empty pcDNA3 vector only. (C) Binding of ^{125}I-Mesd (5 nM) to wild-type murine embryonic fibroblasts (MEF-1) or MEF cell lines genetically deficient in LRP (MEF-2), LDLR (MEF-3) or both (MEF-4). Lower panel, western blot analysis of LRP and the LDLR expression in MEF cell lines. (D) Binding of ^{125}I-Mesd (5 nM) to human breast cancer and multiple myeloma (MM) cell line MCF-7, human glioblastoma cell line U87 and human aortic smooth muscle cells (SMC). Lower panel, western blot analysis of LRP expression in these cell lines. Assays were carried out for 4 hours at 4°C in the absence (total) or presence of 500 nM Mesd. Values are the means of triple determinations with the s.d. indicated by error bars.

Example 3

This example illustrates that the carboxy-terminal region of Mesd is required for LRP6 folding.

To analyze the Mesd sequences that are required for Mesd to bind to mature LRP6 at the cell surface with high affinity, sequences were compared between mouse Mesd and its homologs from different species. It was found that the first 12 amino acids of mouse Mesd are absent in the nematode worms Caenorhabditis elegans and Caenorhabditis briggsae, and that mouse Mesd, as well as human Mesd, has an extra ~30 amino acid fragment prior to the conserved endoplasmic reticulum retention signal in its C-terminus (Culi, J., Cell 112: 343-354, 2003; Hsieh, J.C., Cell 112: 355-367, 2003). We thus generated two truncated Mesd mutants lacking either the N-terminal region, MESD(12-195), or both the N-terminal and C-terminal regions, Mesd(12-155) (FIG. 3A). The ability of these mutants to bind to cell surface LRP6 was then assessed. It was found that although truncation of the N-terminal 11 amino acids of mouse Mesd had no effect on LRP6 binding, further truncation of the last 40 amino acids completely abolished LRP6 binding (FIG. 3B).

Example 4

This example illustrates that 45 amino acids of Mesd are necessary and sufficient for binding to mature LRP6.

In this example, a truncated Mesd mutant containing the last 45 amino acids of C-terminal region was generated (FIG. 3A) and its binding affinity for LRP6 was then analyzed. As shown in FIG. 4, The C-terminal region of Mesd is necessary and sufficient for LRP6 binding. (A) Binding analyses of ^{125}I-Mesd and its mutant Mesd (150-195) (5 nM) to LRP6-transduced HT1080 cells and control cells. (B) Binding analyses of ^{125}I-Mesd and its mutant Mesd (150-195) (5 nM) to LRP6-transduced HT1080 cells. Assays were carried out for 3 hours at 4°C in the absence or presence of 500 nM Mesd or its mutant. Values are the means of triple determinations with the s.d. indicated by error bars.

Example 5

This example illustrates that the carboxy-terminal region of Mesd is required for LRP6 folding.

To examine the role of this C-terminal region of Mesd on receptor folding, we generated a Mesd mutant (Mesd_C), which lacks the C-terminal region (amino acids 156-191) but retains the endoplasmic reticulum retention signal (REDL) (FIG. 3A). We next evaluated a potential role for MesdΔC on LRP6 folding. HEK293 cells were transiently transfected with cDNA for the LRP6 with cotransfection of control vector, or cDNAs for Mesd or MesdΔC. The steady-state levels of LRP6 were analyzed by western blotting with the anti-MYC antibody (FIG. 5A). As seen in the figure, two forms of the receptor, i.e. the ER form and mature form (containing complex sugar modifications), were seen for LRP6. In the presence of Mesd coexpression, but not of Mesd_C coexpression, the amount of the mature form of LRP6 was significantly increased (FIG. 5A). In FIG. 5A, HEK293 cells were transiently transfected with the indicated cDNAs. Cell lysates were analyzed by SDS-PAGE under reducing conditions and western blotted with anti-FLAG or anti-HA antibodies as indicated.

Activation of canonical Wnt signaling leads to the stabilization of β-catenin and regulation of gene transcription through transcription regulators including lymphoid-enhancing factor (LEF)-1 and T-cell factors (TCF). The TOP-FLASH luciferase reporter contains TCF-binding sites and can be directly activated by the β-catenin/TCF complex (Korinek et al., 1997). LRP6 is cell surface receptor, and only the mature receptor can reach the cell surface and modulate Wnt signaling (Cong et al., 2004). We next examined the effect of Mesd_C on Wnt signaling using the

TOP-FLASH luciferase reporter assay in HEK293 cells. As expected, Mesd coexpression, but not MesdΔC coexpression, significantly enhanced TCF/LEF transcriptional activity (FIG. 5B). In FIG. 5B, HEK293 cells were cotransfected with LRP6, MESD, MesdΔC or empty pcDNA3 vector and a TCF/LEF transcriptional activity reporter plasmid (TOP-FLASH). The luciferase activity was measured 48 hours after transfection. Values are the means of triple determinations with the s.d. indicated by error bars. Together, these results suggest that the C-terminal region of Mesd is required for LRP6 folding and its signaling function at the cell surface.

Example 6

This example illustrates that LRP6 is not a constitutively active endocytosis receptor and mediates a limited level of Mesd degradation.

Cell surface receptors that traffic between the plasma membrane and endocytic compartments contain signals within their cytoplasmic tails that allow for efficient recruitment into endocytic vesicles. In many cases (e.g. LRP and the LDLR), these signals are constitutively active and mediate continuous receptor endocytosis independently of ligand binding. To examine whether LRP6 is a constitutively active endocytosis receptor, kinetic analyses of receptor endocytosis with HT1080 cells transduced with HA-tagged LRP6 were performed. To eliminate potential effects of LRP6 ligands on its internalization, we utilized ^{125}I-anti-HA IgG for LRP6 endocytosis assays. Binding of ^{125}I-anti-HA IgG to HA-tagged LRP6 was specific, i.e. the binding of ^{125}I-anti-HA IgG to the HT1080 control cells was minimal when compared to HT1080-LRP6 cells (FIG. 6A). We used HA-tagged LRP minireceptor mLRP4 as a positive control and mLRP4tailess (mLRP4 lacking the cytoplasmic tail) as a negative control for ^{125}I-anti-HA IgG endocytosis (Li, Y., J. Biol. Chem. 275: 17187-17194, 2000).

FIG. 6 illustrates that LRP6 is not a constitutively active endocytosis receptor. (A) Anti-HA IgG binding to cell surface HA-tagged LRP6. Binding of ^{125}I-anti-HA IgG (1 nM) to LRP6-transduced HT1080 cells and the control cells was carried out for 90 minutes at 4°C. (B) LRP6 endocytosis. LRP6-transduced HT1080 cells, mLRP4-transfected CHO cells and mLRP4tailess-transfected CHO cells were incubated with 1 nM ^{125}I-anti-HA IgG at 4°C for 90 minutes, and then incubated at 37°C for the indicated times. The amount of internalized anti-HA IgG was determined. Values are the means of triple determinations with the s.d. indicated by error bars. Interestingly, we found that the endocytosis rate of LRP6 was extremely slow, and was indistinguishable from that of mLRP4tailess (FIG. 6B), indicating that LRP6 itself is unable to

initiate endocytosis. From these data, it was concluded that LRP6 is not a constitutively active endocytosis receptor and mediates a limited level of Mesd degradation.

Example 7

This example illustrates that LRP6 mediates little Mesd uptake and degradation.

LRP6-mediated Mesd uptake and degradation was investigated in the experiments illustrated in FIG. 7. (A) LRP6-mediated ^{125}I-Mesd (5 nM) degradation in LRP6-transduced HT1080 cells and control cells was carried out for 4 hours at 37°C in the absence or presence of 500 nM Mesd. (B) ^{125}I-Mesd (5 nM) binding to LRP6-transduced HT1080 cells and the control cells was carried out for 4 hours at 4°C in the absence or presence of 500 nM Mesd. Values are the means of triple determinations with the s.d. indicated by error bars. As shown in FIG. 7, HT1080 cells transduced with LRP6 exhibited ^{125}I-Mesd degradation at a level of 320 fmoles/mg cell protein after 4 hours of incubation at 37°C, whereas ^{125}I-Mesd binding following 4 hours of incubation at 4°C was detected at a level as high as 1320 fmoles/mg cell protein. These results indicate that Mesd binding to LRP6 at the cell surface does not trigger significant endocytosis, and consequently little Mesd uptake and degradation can be detected.

Example 8

This example illustrates that Mesd binding to the cell surface LRP6 does not significantly change the cytosolic β-catenin level.

β-catenin is a key molecule in the Wnt/β-catenin signaling pathway. A cytosolic pool of β-catenin interacts with DNA-binding proteins and participates in Wnt signal transduction (Hinck, L., J. Cell Biol. 125, 1327-13401994; Gottardi, C. J., J. Cell Biol. 153: 1049-1060, 2001; Klingelhofer, J., Oncogene 22, 1181-1188, 2003). To determine whether Mesd binding to cell surface LRP6 directly regulates Wnt signaling, we studied the effects of Mesd binding on cytosolic β-catenin levels in HT1080-LRP6 cells. In these experiments, LRP6-transduced HT1080 cells were treated with 0.5 to 5 nM Mesd for 2 hours at 37°C, and cytosolic β-catenin levels were examined by western blotting using an anti-β-catenin antibody. We found that there was no significant change in the cytosolic β-catenin levels upon Mesd treatment (data not shown). The results indicate that Mesd binding to cell surface LRP6 does not directly modify Wnt signaling.

Example 9

This example illustrates that RAP binds to LRP6 and partially competes for Mesd binding.

Receptor-associated protein (RAP) binds with high affinity to LRP, megalin, VLDLR and apoER2, and with a lower affinity to the LDLR (Bu, G., Int. Rev. Cytol. 209, 79-116. 2001). To determine whether RAP and Mesd bind to identical, overlapping, or different sites on the receptors, we performed binding and competition analysis of these two chaperones with HT1080 cells stably expressing LRP6. As shown in FIG. 8, to determine whether RAP also binds LRP6, we performed RAP-binding analysis with HT1080 cells stably expressing LRP6 at 4°C. Control cells, expressing vector alone, exhibited a moderate level of cell surface ^{125}I-RAP binding, probably mediated by cell surface heparan sulfate proteoglycan and endogenous receptors of the LDLR family. The presence of excess unlabeled RAP (500 nM), but not Mesd (500 nm), completely eliminated this binding (FIG. 8A). Compared to the control cells, LRP6 expressing HT1080 cells displayed ~20% increase of RAP binding, and this increase was abolished by excess unlabeled Mesd (FIG. 8A). These results suggest that RAP binds to cell surface LRP6 with a relatively low affinity.

We performed binding of 5 nM ^{125}I-Mesd (5 nM) to cell surface LRP6 in the presence of various concentrations of excess unlabeled RAP or 500 nM unlabeled Mesd (FIG. 8B). RAP inhibited ^{125}I-Mesd binding in a dose-dependent manner with ~60% inhibition achieved with 500 nM RAP, whereas the same concentration of unlabeled Mesd inhibited >90% of ^{125}I-Mesd binding (FIG. 8B). When ^{125}I-Mesd (5 nM) uptake and degradation were performed, 500 nM unlabeled Mesd completely, whereas 500 nM unlabeled RAP only partially, inhibited ^{125}I-Mesd degradation (FIG. 8C). Together, these results suggest that Mesd and RAP probably bind to different, but perhaps adjacent sites on LRP6. The lower affinity of RAP to cell surface LRP6 may also contribute to its lower efficiency in inhibition of Mesd binding.

In FIG. 8A, binding of ^{125}I-RAP (5 nM) to LRP6-transduced HT1080 cells and the control cells was carried out for 4 hours at 4°C in the absence (total) or presence of 500 nM RAP, or 500 nM Mesd. In FIG. 8B, binding of ^{125}I-Mesd (5 nM) to LRP6-transduced HT1080 cells was carried out for 2 hours at 4°C in the absence (total) or presence of various concentrations of RAP or 500 nM Mesd. (C) LRP6-mediated ^{125}I-Mesd (5 nM) degradation was carried out for 4 hours at 37°C in the absence or presence of 500 nM Mesd or 500 nM RAP. Values are the means of triple determinations with the s.d. indicated by error bars.

These experiments illustrate that RAP binds to LRP6 and partially competes for Mesd binding.

Example 10

This example illustrates that Mesd antagonizes ligand binding to LRP6 at the cell surface.

RAP is a receptor antagonist for members of the LDLR family, and is able to inhibit the binding of most known ligands of the LDLR family members. DKK1 is an LRP6-specific ligand and antagonist. To determine whether Mesd is also able to block LRP6 ligand binding, we examined cell surface DKK1 binding by immunostaining. As illustrated in FIG. 9, Myc-tagged DKK1 binds to LRP6 cells (FIG. 9B) but not to the control cells (FIG. 9A). The presence of Mesd completely blocked the binding of Myc-DKK1 to LRP6 at the cell surface (FIG. 9C). In these experiments, Serum-free conditioned medium was harvested from HEK293 cells transiently transfected with cDNA for human Myc-DKK1 and allowed to bind to LRP6-transduced HT1080 cells (B,C) and control cells (A) in the absence (A,B) or presence (C) of 1 μM Mesd. Cell-surface-bound Myc-tagged DKK proteins were fixed and detected by immunofluorescence staining with anti-Myc antibody. (D) DKK1 binding to cell surface LRP6 is inhibited by Mesd. Binding of 125I-DKK1 (5 nM) to LRP6- transduced HT1080 cells or the control cells was carried out for 3 hours at 4°C in the absence (total) or presence of 500 nM RAP or 500 nM Mesd. (E) LRP6- mediated DKK1 degradation is inhibited by Mesd. LRP6-mediated ^{125}I-Mesd (5 nM) degradation was carried out for 4 hours at 37°C in the absence or presence of 500 nM RAP or 500 nM Mesd. Values are the means of triple determinations with the s.d. indicated by error bars. Bar, 10 μm. As expected, Myc-tagged DKK1 binds to LRP6 cells (FIG. 9B) but not to the control cells (FIG. 9A). Importantly, the presence of Mesd completely blocked the binding of Myc-DKK1 to LRP6 at the cell surface (FIG. 9C).

To confirm the above results, we examined the binding and degradation of ^{125}I-DKK1. LRP6-expressing HT1080 cells exhibited significantly higher levels of ^{125}I-DKK1 binding and degradation than the control cells. The increased DKK1 binding and degradation were abolished by excess unlabeled Mesd, but not by excess unlabeled RAP (FIG. 9D,E). Together, these results indicate that Mesd can specifically block DKK1 binding to LRP6 at the cell surface.

To confirm the above results, we examined the binding and degradation of ^{125}I-DKK1. LRP6-expressing HT1080 cells exhibited significantly higher levels of ^{125}I-DKK1 binding and degradation than the control cells. The increased DKK1 binding and degradation were abolished by excess unlabeled Mesd, but not by excess unlabeled RAP (FIG. 9D,E). Together, these results indicate that Mesd can specifically block DKK1 binding to LRP6 at the cell surface.

Example 11

This example illustrates binding of Mesd polypeptide or an Mesd oligopeptide to LRP5 and LRP6.

In order to investigate the binding of Mesd polypeptide and an Mesd oligopeptide to either LRP5 or LRP6, binding assays were performed using ^{125}I-Mesd or ^{125}I-DKK1 (FIG. 10). FIG. 10A shows that both wild type Mesd and Mesd oligopeptide KGGGSKEKNKTKPEKAKKKEGDPKPRASKEDNRAGSR (SEQ ID NO: 20) can reduce binding of ^{125}I-Mesd to LRP5 up to about 10-fold, while FIG. 10C shows that both Mesd and the oligopeptide show even greater reduction of binding of ^{125}I-Mesd to LRP6. In addition, in binding assays using ^{125}I-DKK1, a small but significant reduction in binding of ^{125}I-DKK1 to LRP5 was observed when the LRP5 was contacted with either Mesd or the oligopeptide (FIG. 10B), while binding of ^{125}I-DKK1 greater than 2-fold was observed when LRP6 was the target (FIG. 10D).

These data demonstrate that both Mesd polypeptide, and the oligopeptide of sequence SEQ ID NO: 19 can both bind LRP5 and inhibit binding of DKK1 to either LRP5 or LRP6.

Example 12

This example illustrates inhibition of WNT signalling in HEK293 cells.

In this example, Wnt signaling was measured in HEK293 cells comprising TCF/LEF-Luc assays in cells were prepared as described above, and subjected to the treatments as shown in Table IV, with the results (i.e., luciferase activity) presented in FIG. 11.

Table IV

Column	Treatment
1	L cell control medium
2	Wnt3A conditioned medium
3	Wnt3A conditioned medium plus 500 nM Mesd protein
4	Wnt3A conditioned medium plus 500 nM Mesd (150-195) peptide QMYPGKGGGSKEKNKTKPEKAKKKEGDPKPRASKEDNRAGSRREDL (SEQ ID NO: 22)
5	Wnt3A conditioned medium plus 500 nM Mesd WT peptide KGGGSKEKNKTKPEKAKKKEGDRKPRASKEDNRAGSR (SEQ ID NO: 21)

6	Wnt3A conditioned medium plus 500 nM Mesd peptide KGGGSKEKNKTKPEKAKKK (SEQ ID NO: 26) (mouse Mesd-13)
7	Wnt3A conditioned medium plus 500 nM Mesd peptide EGDRKPRASKEDNRAGSR (SEQ ID NO: 24) (mouse Mesd-11)
8	Wnt3A conditioned medium plus 500 nM Mesd peptide TKPEKAKKKEGDRKPRAS (SEQ ID NO: 27) (mouse Mesd-14)
9	Wnt3A conditioned medium plus 500 nM Mesd peptide KGGGSKEKNK (SEQ ID NO: 9) (Mesd-4)
10	Wnt3A conditioned medium plus 500 nM Mesd peptide KEDNRAGSR (SEQ ID NO: 28) (mouse Mesd-15)
11	Wnt3A conditioned medium plus 500 nM Mesd peptide KEKNKTKPEK (SEQ ID NO: 29) (mouse Mesd-16)
12	Wnt3A conditioned medium plus Dkk1 protein at 10 nM
13	Wnt3A conditioned medium plus bacterially expressed Dkk1 at 10 nM
14	Wnt3A conditioned medium plus Dkk1 protein at 10 nM plus Mesd WT
15	Wnt3A conditioned medium plus Dkk1 protein at 10 nM plus Mesd-3

The data indicate that Mesd protein, Mesd (150-195) peptide, and Mesd WT peptide moderately inhibit Wnt signaling and can be used for Wnt-related cancer therapy, although Dkk1 is a more potent inhibitor. Because Mesd is also an inhibitor of Dkk1, it will have dual benefits, i.e. reducing cancer and increasing bone health.

Example 13

To generate the MMTV-LRP6 construct, human LRP6 cDNA was cloned into the MMTV-SV40-Bssk vector, between the murine mammary tumor virus long terminal repeat (MMTV-LTR) and an SV40 large T antigen intron and polyadenylation signal. Prior to injection, the construct was linearized and purified. Transgenic mice were generated in the FVB/N inbred background and transgenic founders were identified by PCR testing for the presence of the transgene. Four founders were obtained and their offsprings were characterized for the expression of LRP6, extent of Wnt signaling, and mammary gland morphology by whole mount staining. FIG. 12.

All publications, patents, patent applications and other references cited in this application are herein incorporated by reference in their entirety.

What is claimed is:

1. A method of treating cancer in a subject in need of treatment, the method comprising administering to the subject a therapeutically effective amount of a Mesd polypeptide selected from the group consisting of a full length MesD polypeptide, a polypeptide sharing at least 70% sequence identity with a Mesd polypeptide, or an oligopeptide comprising between 10 contiguous amino acids and about 70 contiguous amino acids, wherein the oligopeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, and a sequence sharing at least 70% sequence identity with at least one sequence set forth as SEQ ID NO: 1 through SEQ ID NO: 32, wherein the polypeptide or the oligopeptide antagonizes binding of a Wnt ligand to LRP5 and/or LRP6 receptor.

2. A method in accordance with claim 1, wherein the oligopeptide comprises a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23

3. A method in accordance with claim 1, wherein the oligopeptide comprises a sequence selected from the group consisting of SEQ ID NO: 15 and SEQ ID NO: 21.

4. A method in accordance with claim 1, wherein the Mesd polypeptide has an amino acid sequence selected from the group consisting of SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

5. A method in accordance with claim 1, wherein the cancer is selected from the group consisting of breast cancer, multiple myeloma (MM), prostate cancer and skin cancer.

6. A method of treating cancer in a subject in need of treatment, the method comprising administering to the subject a therapeutically effective amount of a nucleic acid vector comprising a promoter operably linked to a sequence encoding a Mesd polypeptide selected from the group consisting of a full length MesD polypeptide, a polypeptide sharing at least 70% sequence identity with an Mesd polypeptide, or an oligopeptide comprising between 10 contiguous amino acids and about 70 contiguous amino acids, wherein the oligopeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20; SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23 and a sequence sharing at least 70% sequence identity with at least one sequence set forth as SEQ ID NO: 1 through SEQ ID NO: 38, wherein the polypeptide or the oligopeptide antagonizes binding of a Wnt ligand to LRP5 receptor and/or LRP6 receptor.

7. A method in accordance with claim 6, wherein the oligopeptide comprises a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23

8. A method in accordance with claim 6, wherein the oligopeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 15 and SEQ ID NO: 21.

9. A method in accordance with claim 6, wherein the nucleic acid vector is comprised by a cell.

10. A method in accordance with claim 6, wherein the cancer is selected from the group consisting of breast cancer, multiple myeloma (MM), prostate cancer and skin cancer.

11. Use of an oligopeptide comprising from about 10 contiguous amino acids up to about 70 contiguous amino acids for the manufacture of a medicament for treatment of a cancer, wherein the oligopeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID

NO: 6, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20; SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, and a sequence sharing at least 70% sequence identity with at least one sequence set forth as SEQ ID NO: 1 through SEQ ID NO: 38, wherein the oligopeptide antagonizes binding of a Wnt ligand to LRP5 receptor and/or LRP6 receptor .

12. Use of an oligopeptide in accordance with claim 11, wherein the oligopeptide comprises a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23

13. Use of an oligopeptide in accordance with claim 11, wherein the oligopeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 15 and SEQ ID NO: 21.

14. Use of an oligopeptide in accordance with claim 11, wherein the cancer is selected from the group consisting of breast cancer and multiple myeloma (MM), prostate cancer and skin cancer.

15. Use of a polypeptide or oligopeptide for the manufacture of a medicament for treatment of cancer, wherein the polypeptide or oligopeptide comprises an amino acid sequence selected from the group consisting of a full length MesD polypeptide of Table I, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, and a sequence sharing at least 70% sequence identity with at least one sequence set forth as SEQ ID NO: 1 through SEQ ID NO: 32, wherein the polypeptide or oligopeptide antagonizes binding of a Wnt ligand to LRP5 receptor and/or LRP6 receptor.

16. Use of a polypeptide in accordance with claim 15, wherein the cancer is selected from the group consisting of breast cancer, multiple myeloma (MM), prostate cancer and skin cancer.

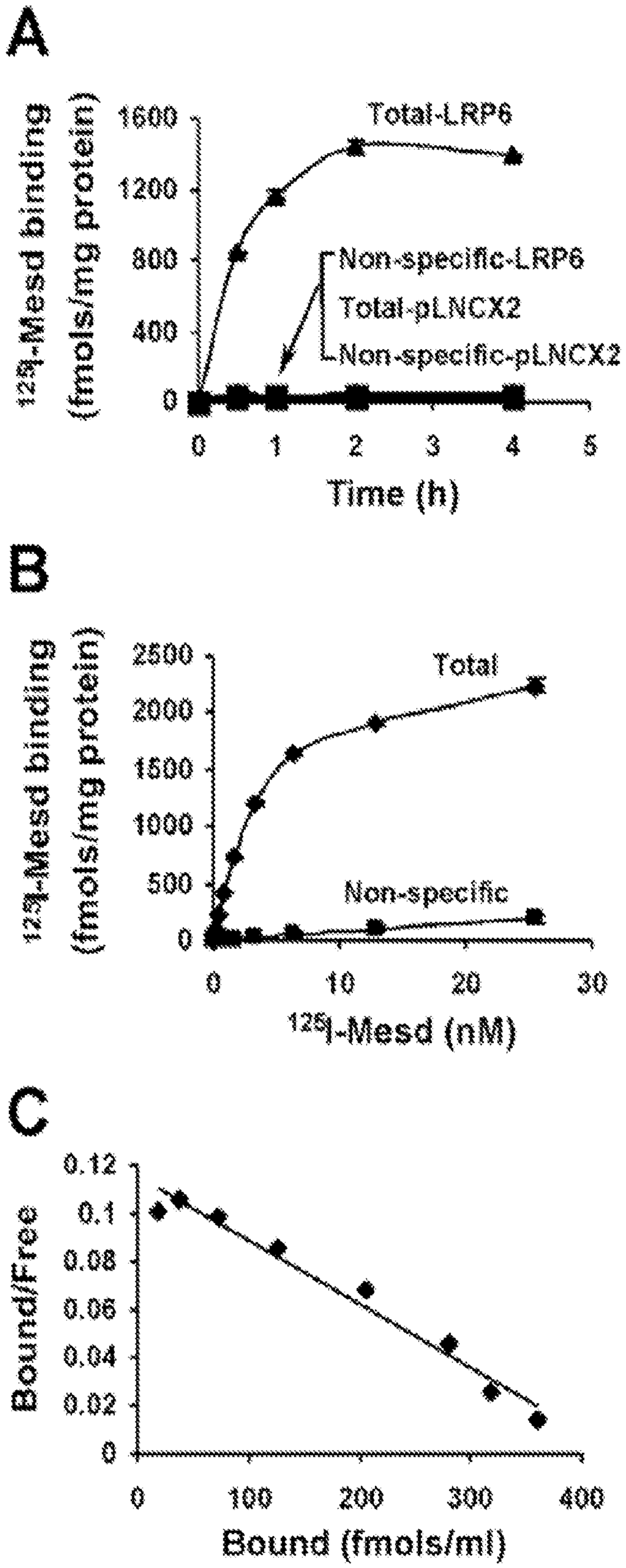

A
125I-Mesd binding (fmols/mg protein)
1600
1200
800
400
0
Total-LRP6
Non-specific-LRP6
Total-pLNCX2
Non-specific-pLNCX2
0
1
2
3
4
5
Time (h)
B
125I-Mesd binding (fmols/mg protein)
2500
2000
1500
1000
500
0
Total
Non-specific
0
10
20
30
125I-Mesd (nM)
C
Bound/Free
0.12
0.1
0.08
0.06
0.04
0.02
0
0
100
200
300
400
Bound (fmols/ml)

Figure 1

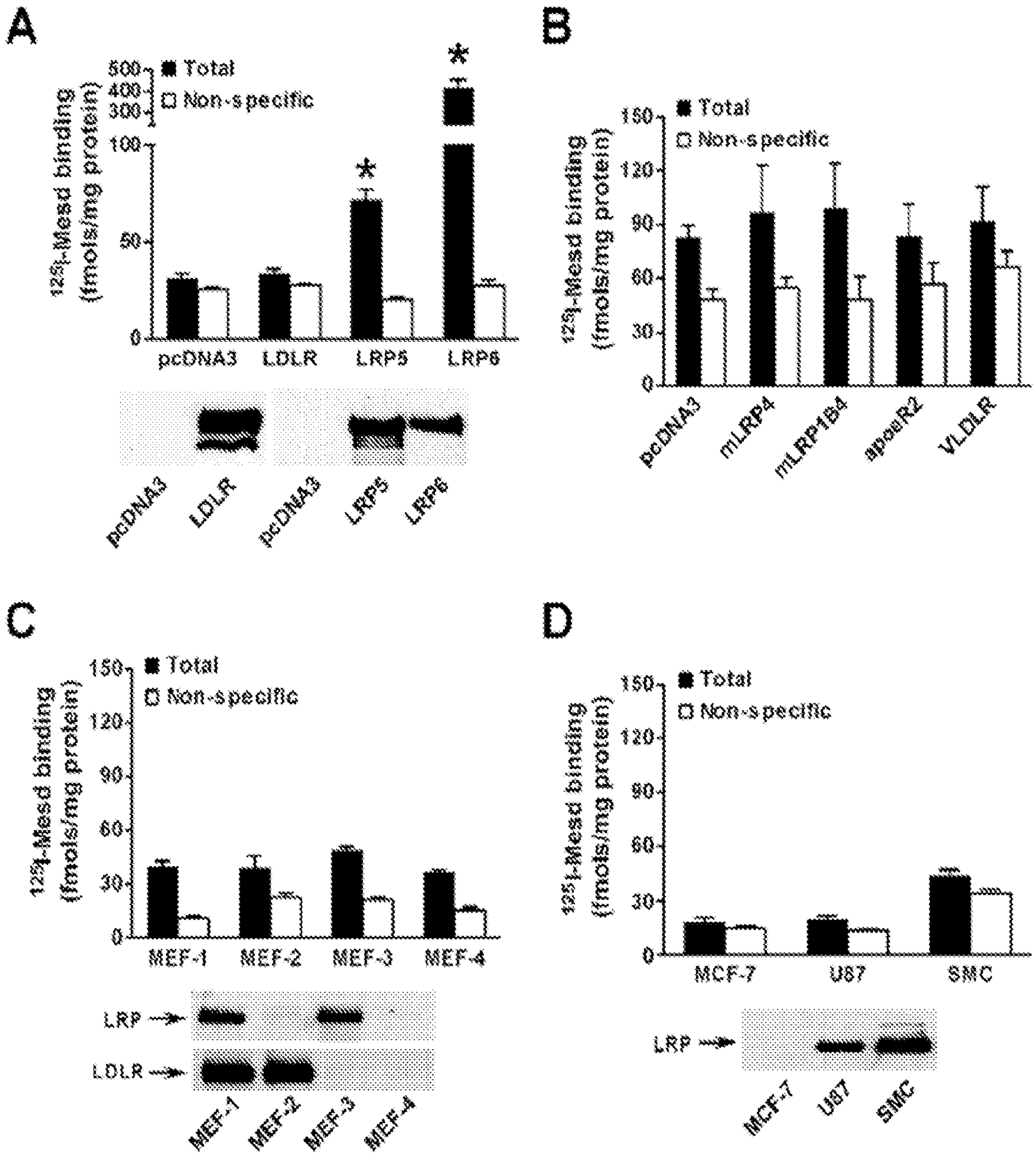

A
Total
Non-specific
125I-Mesd binding (fmols/mg protein)
500
400
300
100
50
0
pcDNA3
LDLR
LRP5
LRP6
pcDNA3
LDLR
pcDNA3
LRP5
LRP6
B
Total
Non-specific
125I-Mesd binding (fmols/mg protein)
150
120
90
60
30
0
pcDNA3
mLRP4
mLRP1b4
apoeR2
VLDLR
C
Total
Non-specific
125I-Mesd binding (fmols/mg protein)
150
120
90
60
30
0
MEF-1
MEF-2
MEF-3
MEF-4
LRP
LDLR
MEF-1
MEF-2
MEF-3
MEF-4
D
Total
Non-specific
125I-Mesd binding (fmols/mg protein)
150
120
90
60
30
0
MCF-7
U87
SMC
LRP
MCF-7
U87
SMC

Figure 2


A
Mesd (WT)
Mesd(12-195)
Mesd(12-155)
Mesd(150-195)
MesdΔC

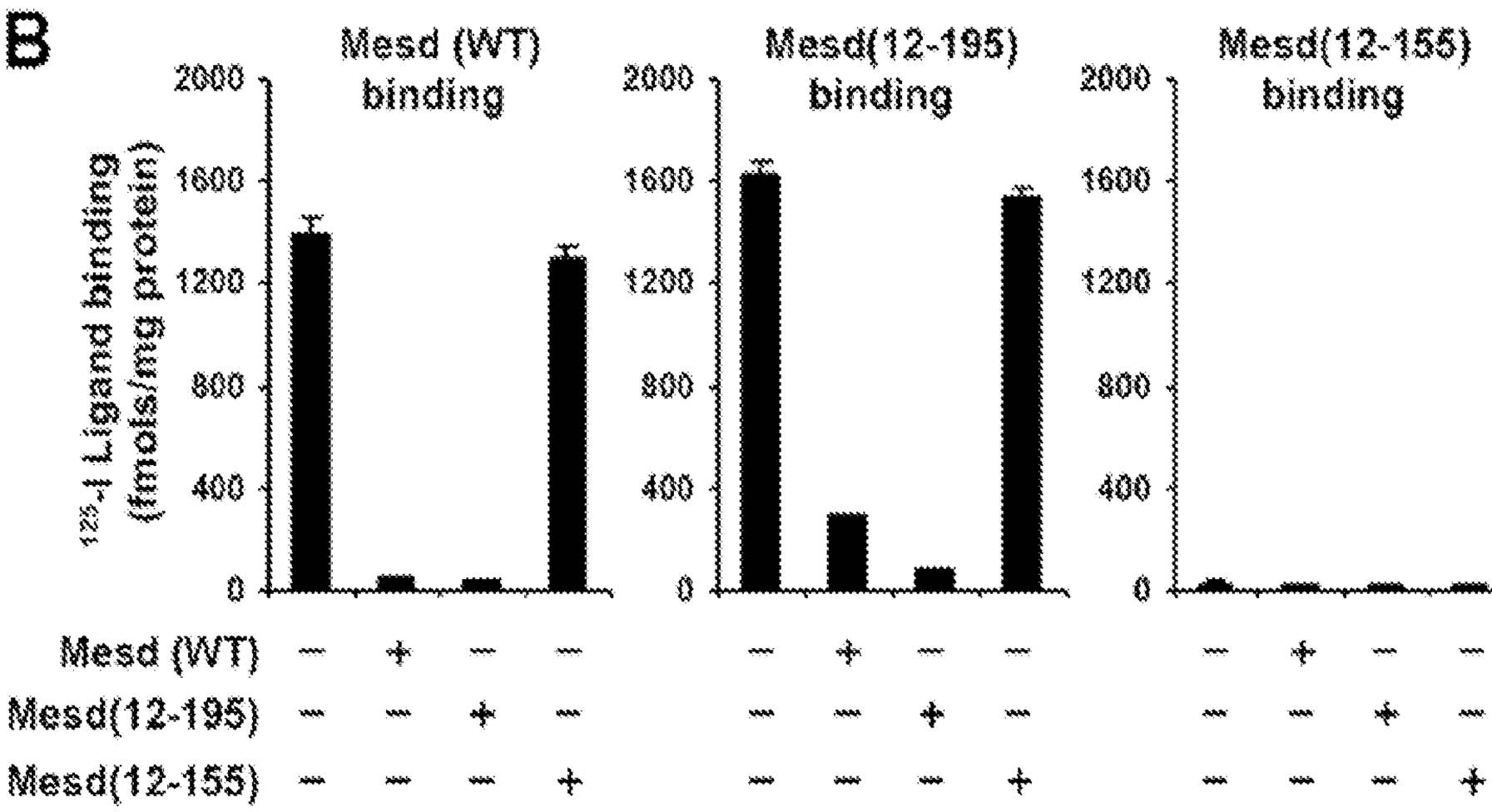

B
Mesd (WT) binding
Mesd(12-195) binding
Mesd(12-155) binding
125-I Ligand binding (fmols/mg protein)
2000
1600
1200
800
400
0
Mesd (WT) — + — — — + — — — + — —
Mesd(12-195) — — + — — — + — — — + —
Mesd(12-155) — — — + — — — + — — — +

Figure 3

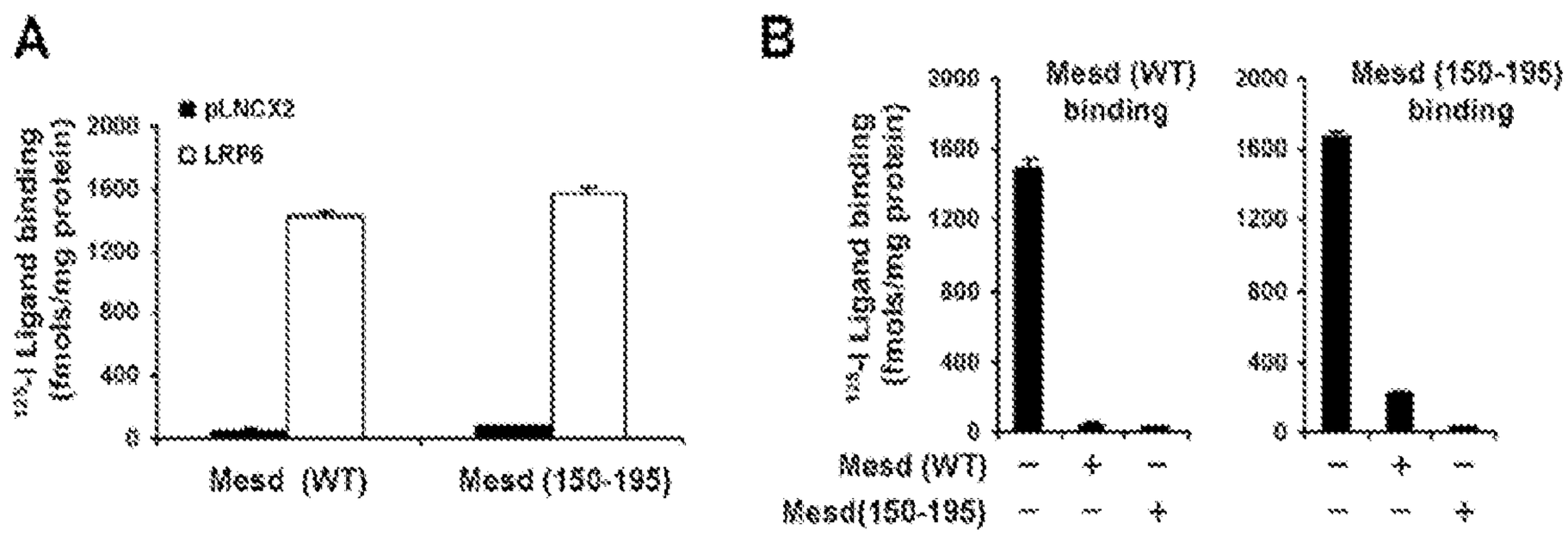


Figure 4

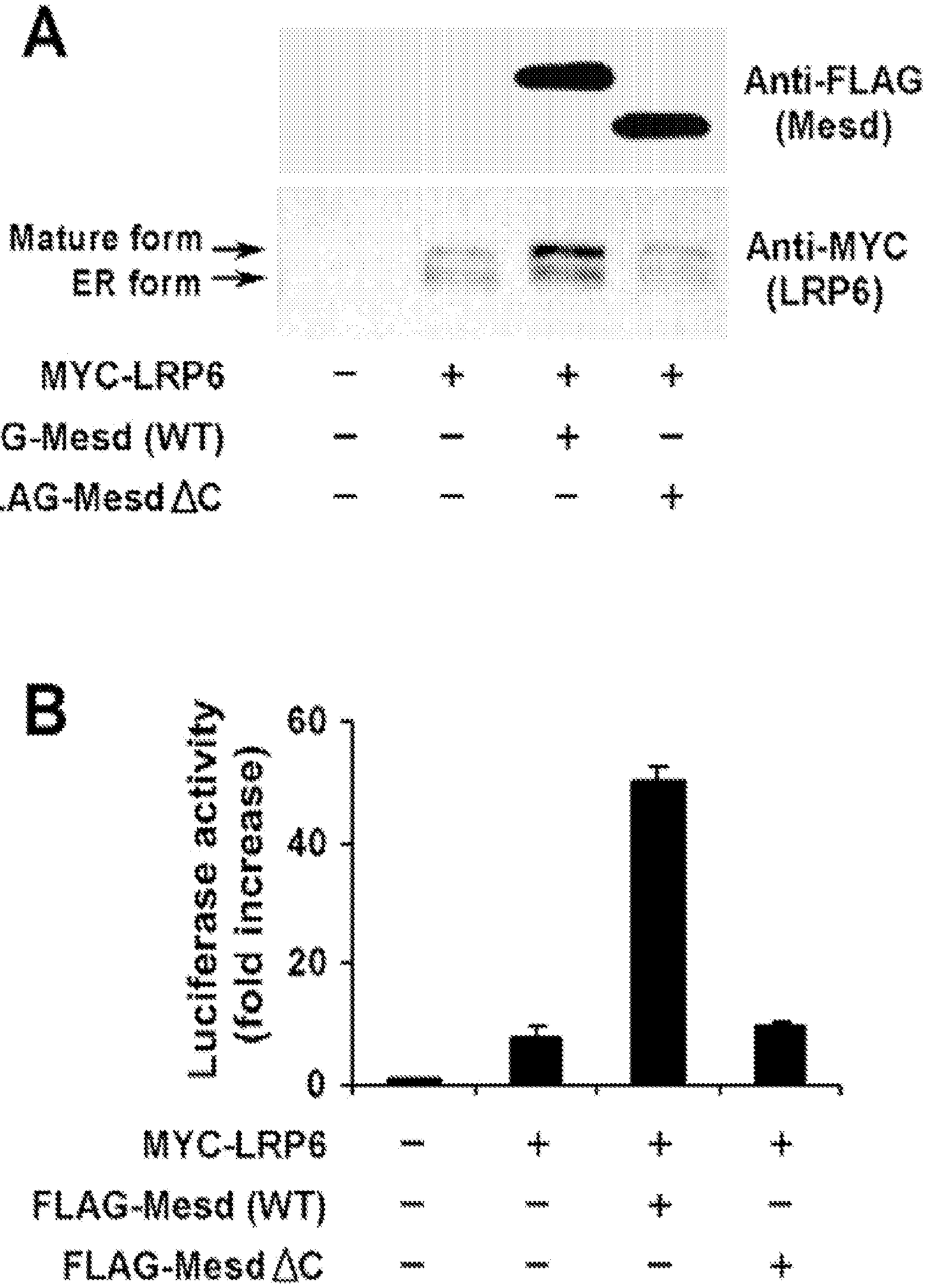

A
Anti-FLAG
(Mesd)
Mature form
ER form
Anti-MYC
(LRP6)
MYC-LRP6 – + + +
FLAG-Mesd (WT) – – + –
FLAG-Mesd ΔC – – – +
B
Luciferase activity
(fold increase)
0
20
40
60
MYC-LRP6 – + + +
FLAG-Mesd (WT) – – + –
FLAG-Mesd ΔC – – – +

Figure 5

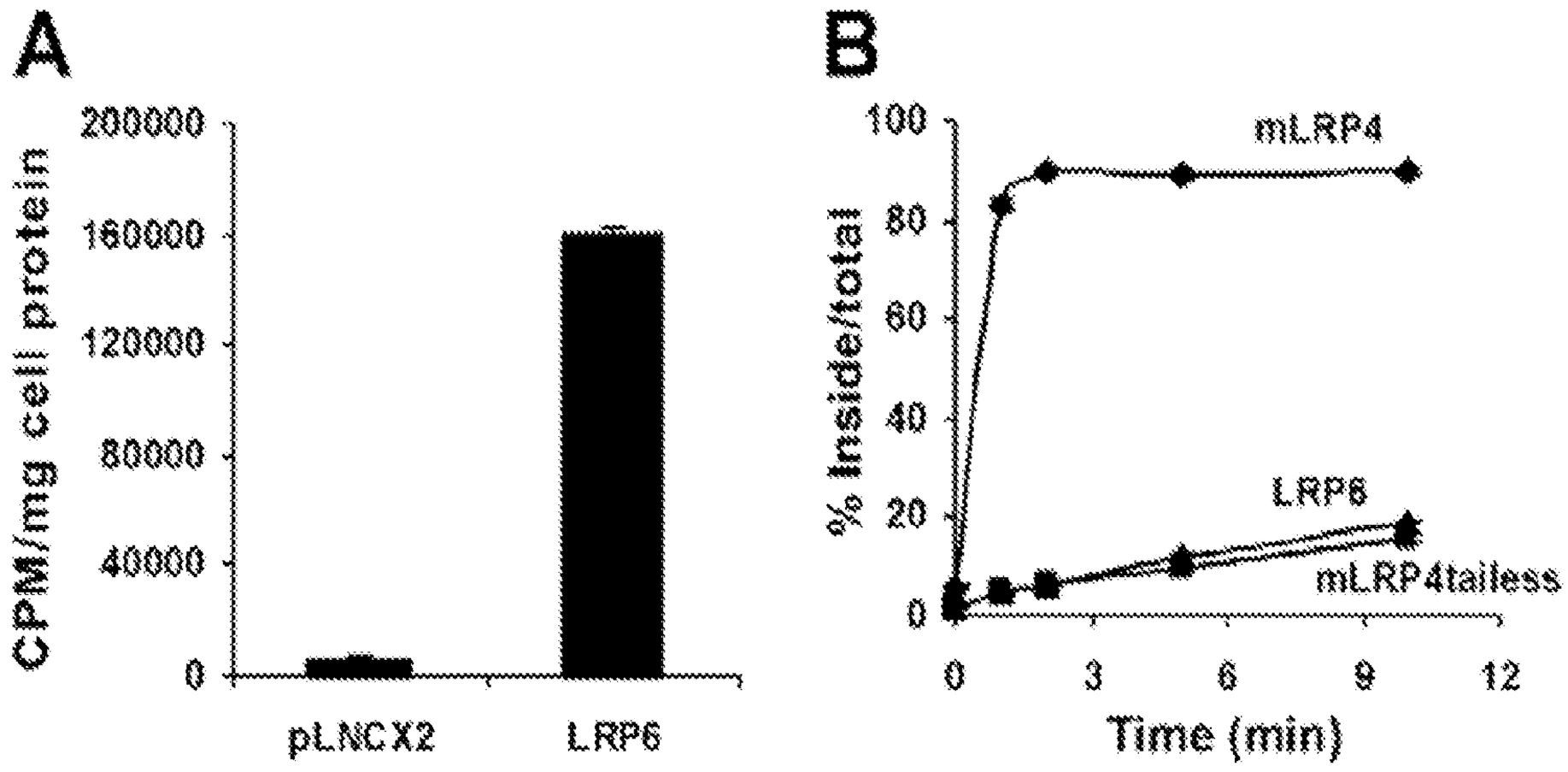


Figure 6

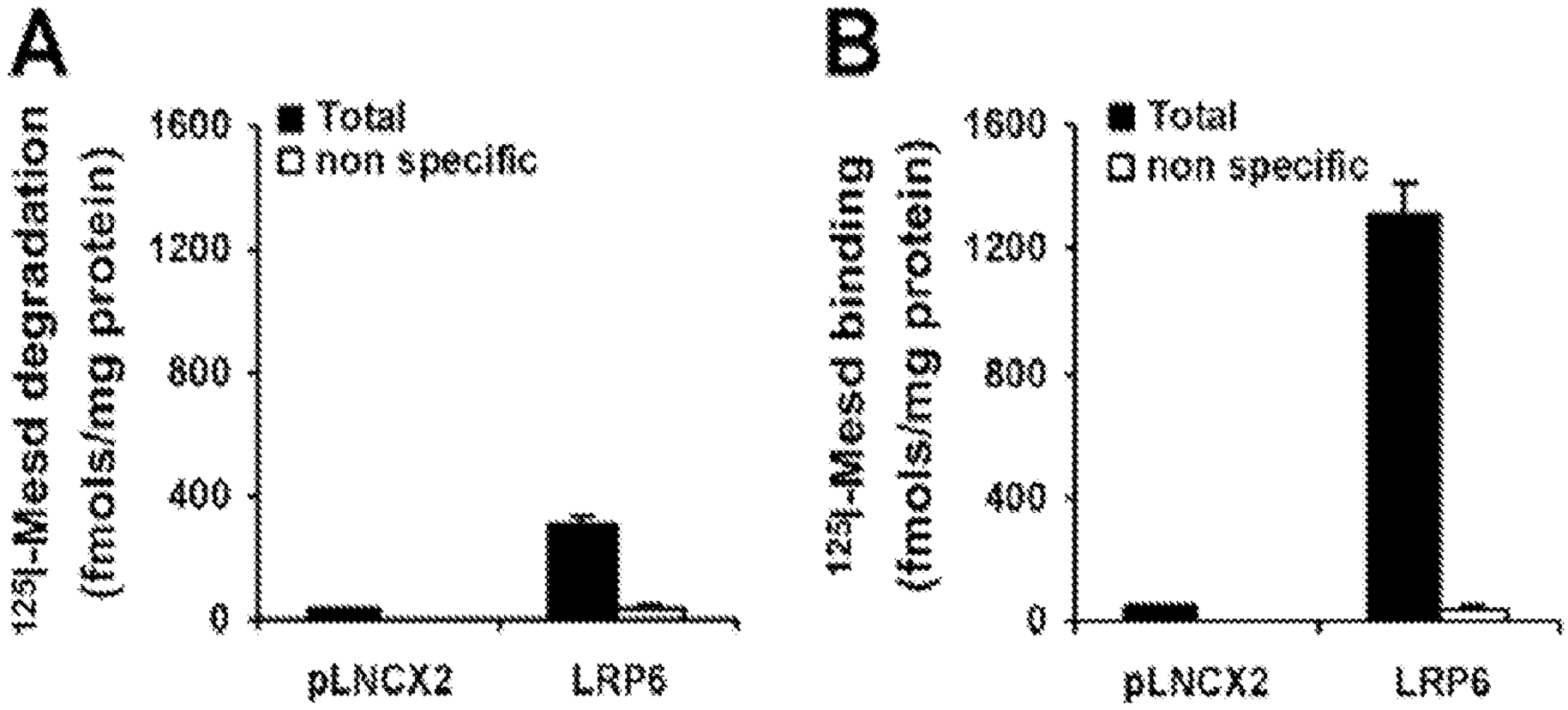


Figure 7

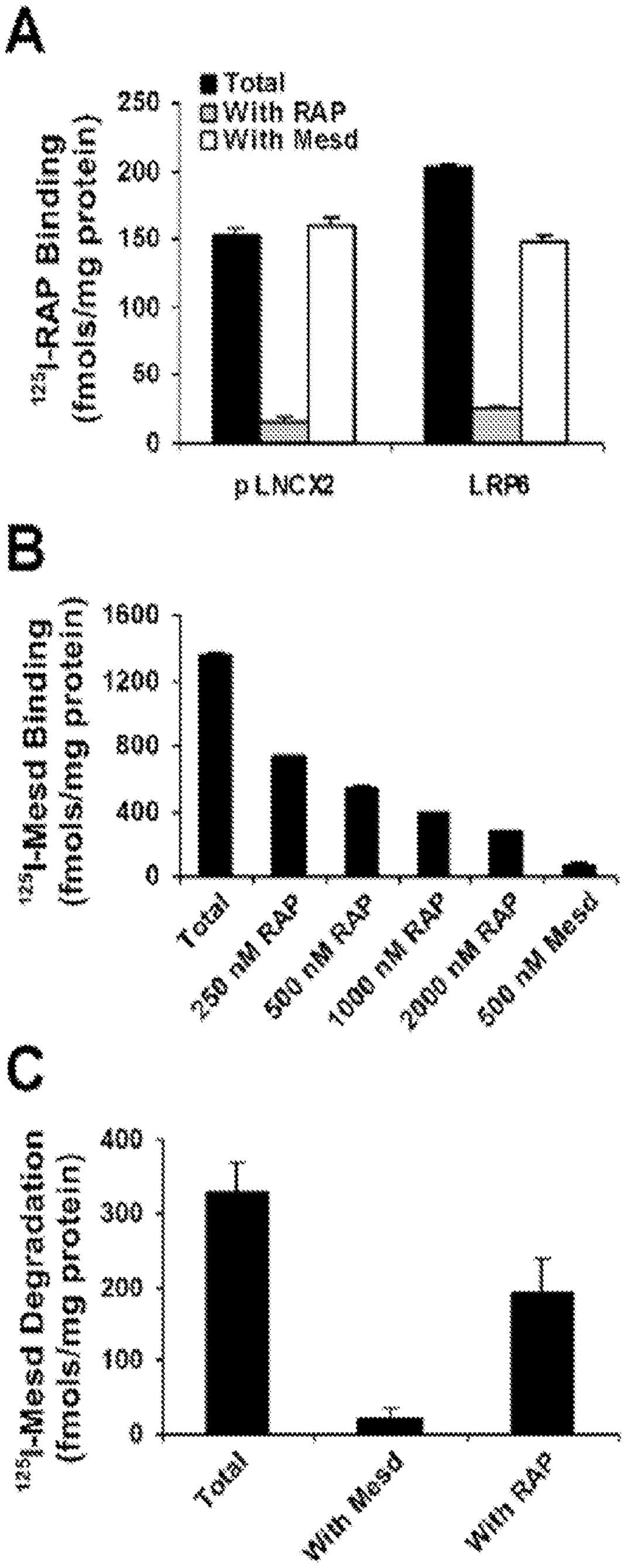
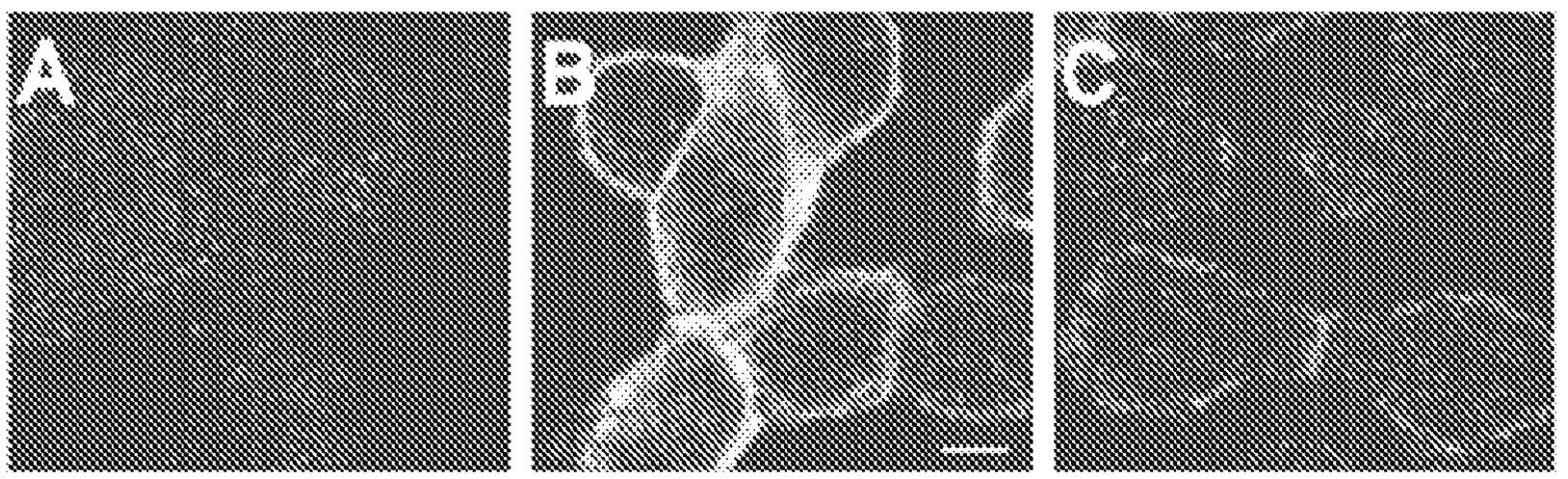
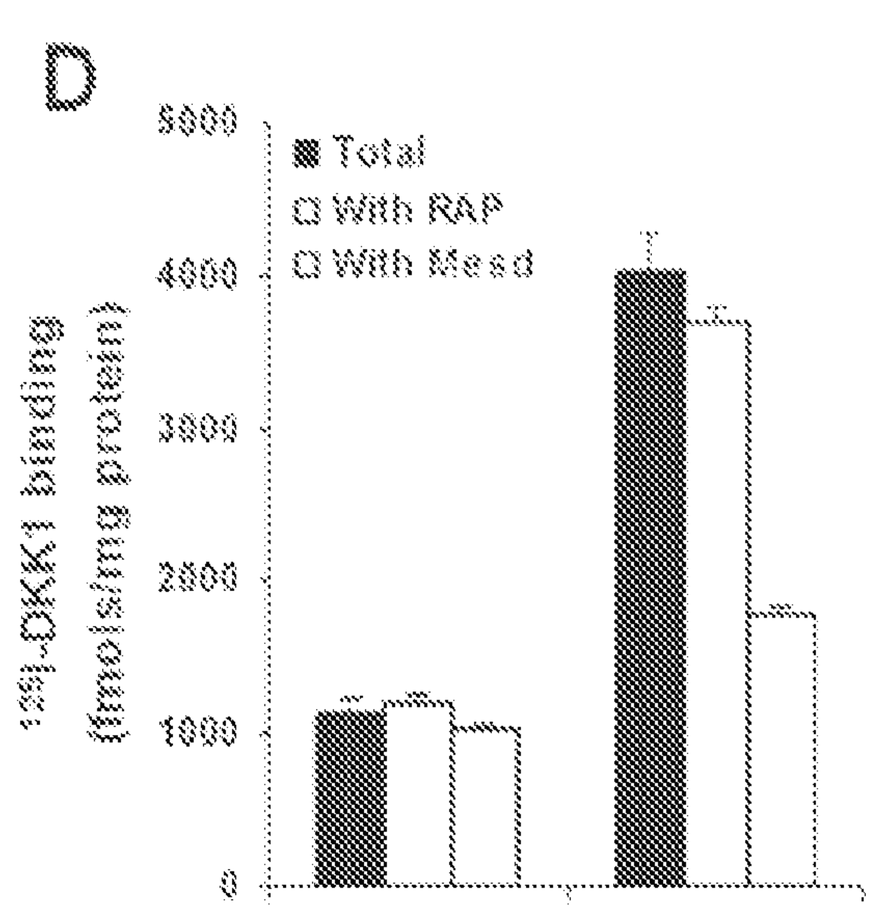

A
Total
With RAP
With Mesd
^{125}I-RAP Binding
(fmols/mg protein)
250
200
150
100
50
0
pLNCX2
LRP6
B
^{125}I-Mesd Binding
(fmols/mg protein)
1600
1200
800
400
0
Total
250 nM RAP
500 nM RAP
1000 nM RAP
2000 nM RAP
500 nM Mesd
C
^{125}I-Mesd Degradation
(fmols/mg protein)
400
300
200
100
0
Total
With Mesd
With RAP

Figure 8


A
B
C


D
125I-OKN1 binding
(fmols/mg protein)
5000
4000
3000
2000
1000
0
Total
With RAP
With Mesd
pLNCX2
LRP6

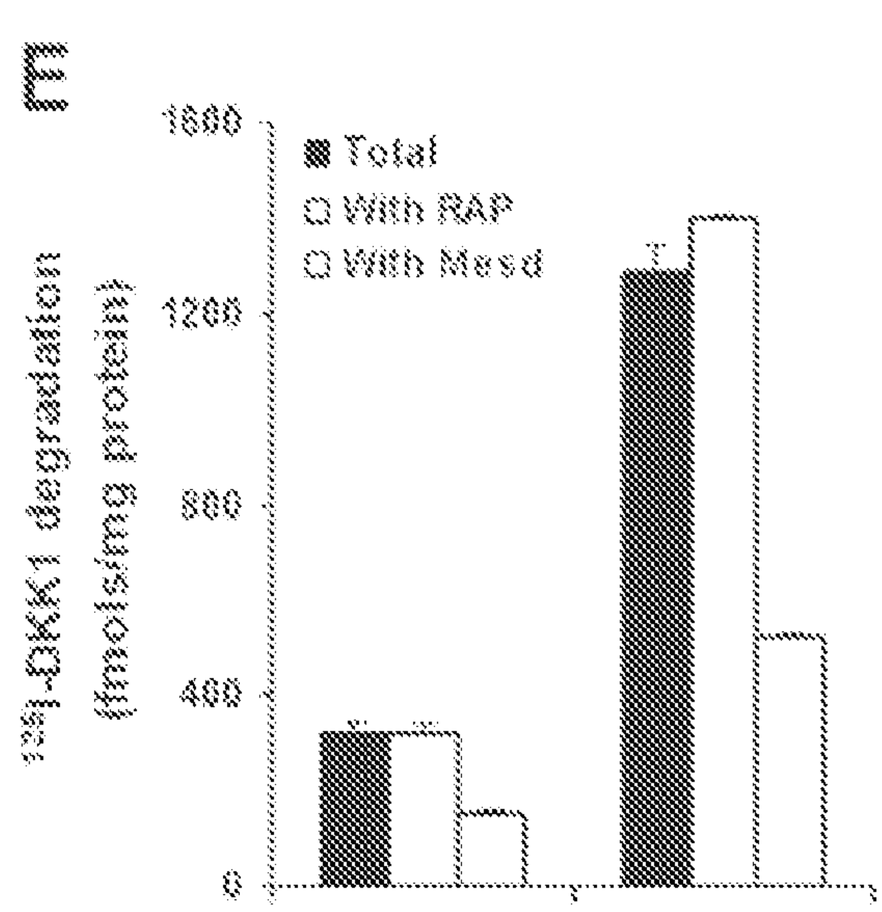

E
125I-OKN1 degradation
(fmols/mg protein)
1600
1200
800
400
0
Total
With RAP
With Mesd
pLNCX2
LRP6

Figure 9

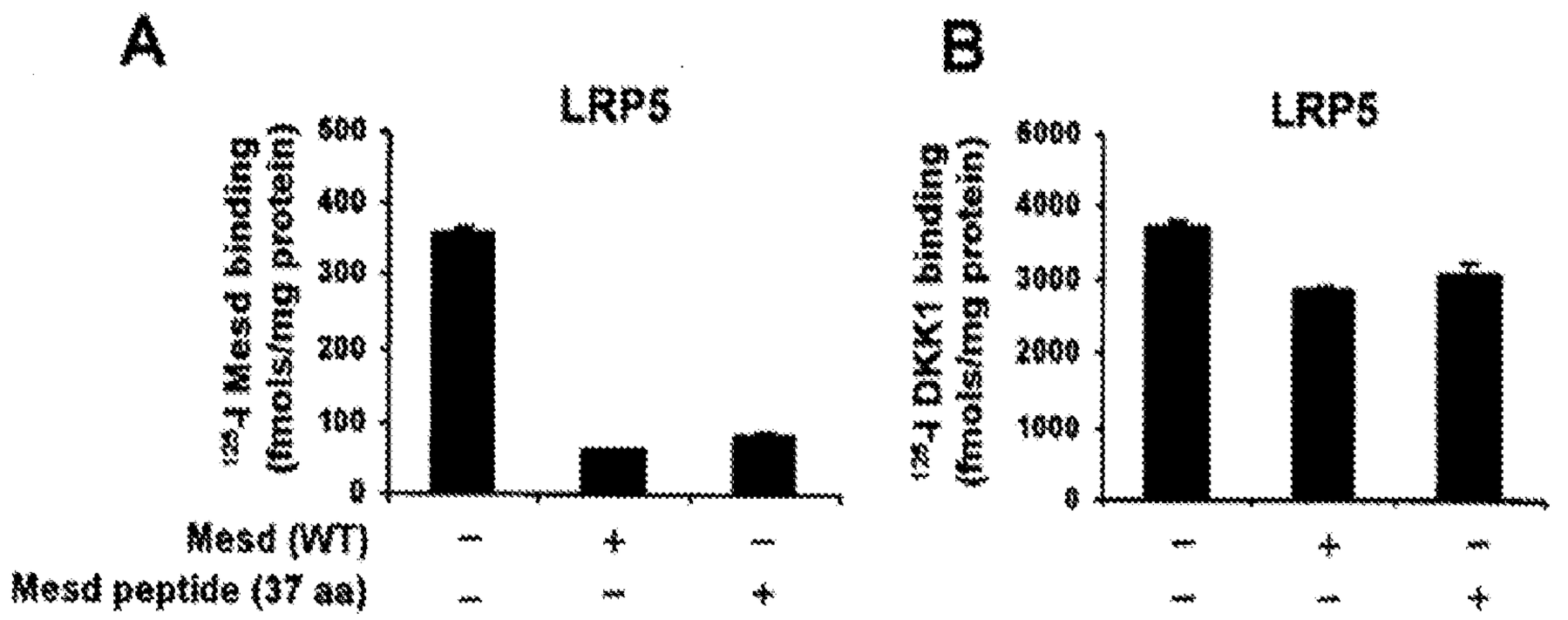

A
LRP5
^{125}I-Mesd binding (fmols/mg protein)
500
400
300
200
100
0
Mesd (WT) – + –
Mesd peptide (37 aa) – – +
B
LRP5
^{125}I-DKK1 binding (fmols/mg protein)
6000
4000
3000
2000
1000
0
– + –
– – +
C
LRP6
^{125}I-Mesd binding (fmols/mg protein)
1200
900
600
300
0
Mesd (WT) – + –
Mesd peptide (37 aa) – – +
D
LRP6
^{125}I-DKK1 binding (fmols/mg protein)
5000
4000
3000
2000
1000
0
– + –
– – +

Figure 10

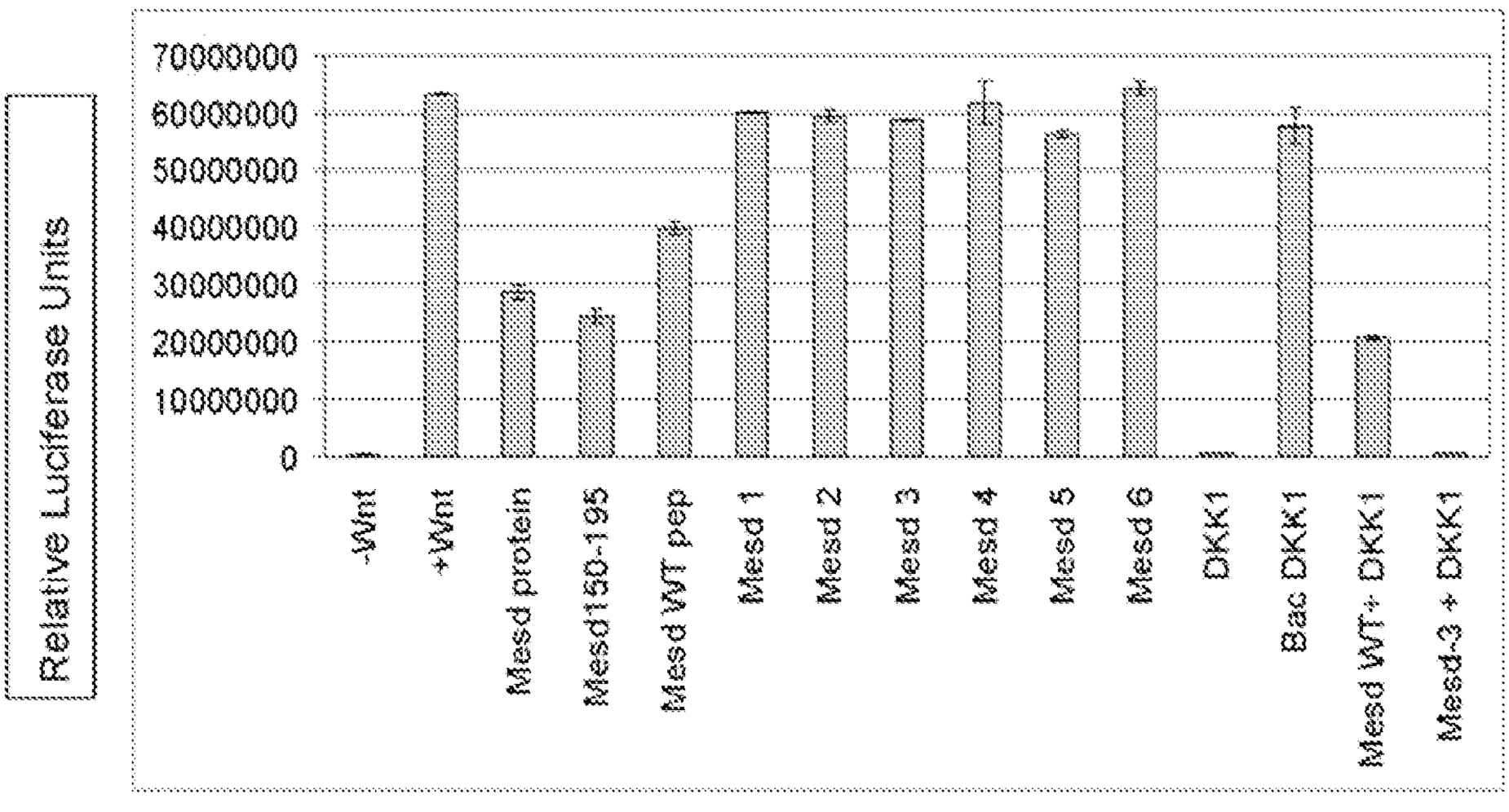


Figure 11

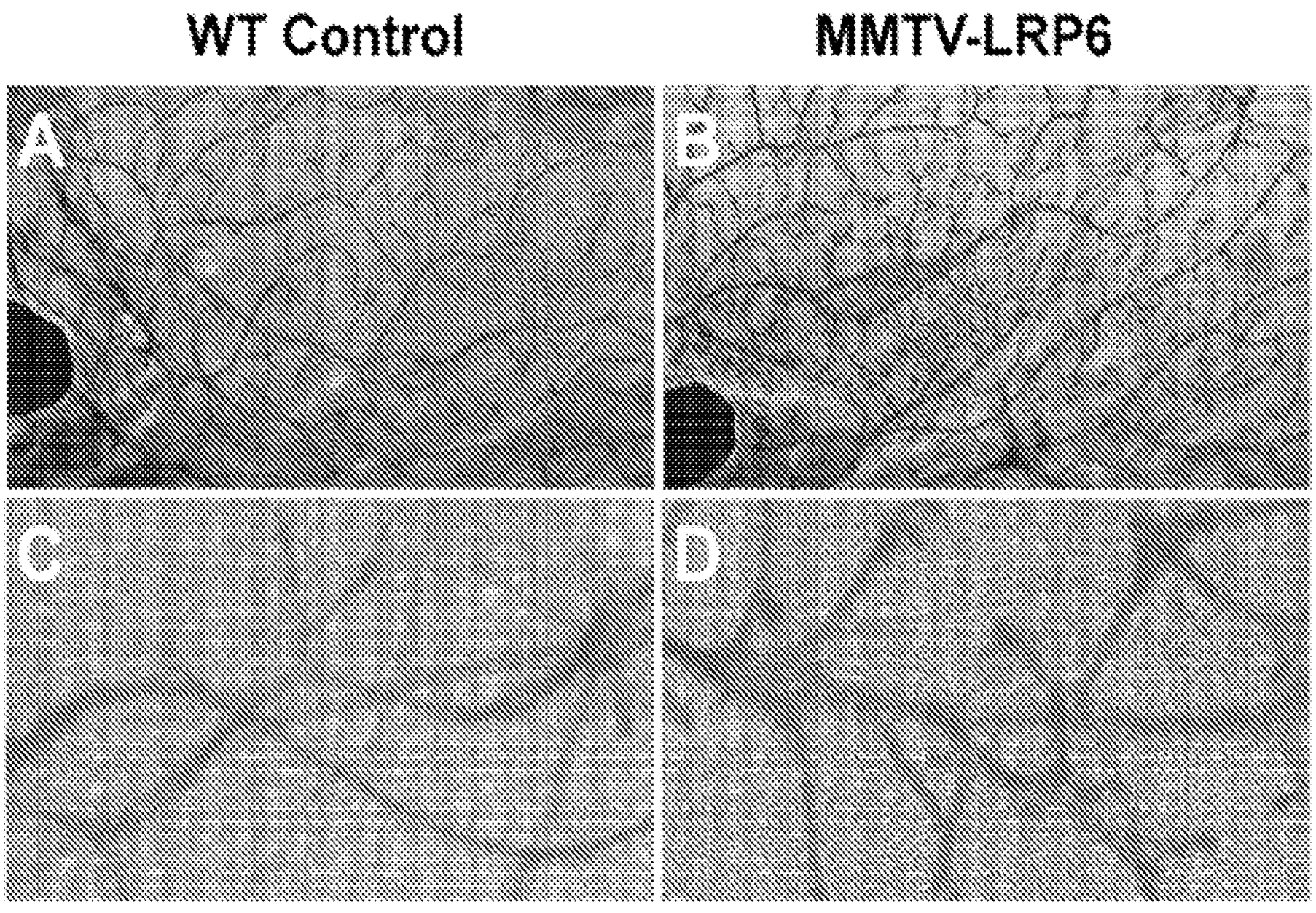


Figure 12