Supplemental data

Cloning. Human LRP6 ECD, mouse Fz8 CRD (1-155, ¹) and full-length human Dkk1 cDNA were cloned by PCR amplification from an in-house generated full-length clone using Taq polymerase. Using these cDNAs as templates for subcloning, sets of 4 PCR primers were designed to amplify the domains of interest from LRP6 and Fz8. The primers contained the coding sequences for a C-terminal His₆ tag as well as the nucleotides required for adding portions of the restriction sites BamHI and EcoRI to the resulting PCR products. Denaturation and reannealing of the resulting PCR products produced a population of DNA in which 25% of the products contained the appropriate nucleotides to represent BamHI and EcoRI overhangs at the 5' and 3' ends of the domains of interest, respectively. This DNA mixture was then ligated into a modified pAcGP67 baculovirus DNA transfer vector (BD Pharmingen) for baculovirus generation and extracellular expression in *Sf-9* or *Tni* insect cells (Expression Systems, LLC, Woodland CA.). Dkk1 was introduced by standard cloning techniques into the pVL1393 vector (BD Pharmingen) using Sma1 and BamH1 sites. The integrity of all expression constructs was confirmed by DNA sequencing.

Mass Spectrometry Analysis. 20 µL of full length LRP6 protein at a concentration of 1 mg/ml was deglycosylated with PNGaseF (New England BioLabs, 1:75 ratio), in 50mM Tris-HCl pH 7.5 for 4 h at 45 °C. The deglycosylated protein was then reduced with dithiothreitol (10 mM; 50 °C for 1 h) and alkylated with iodoacetamide (50 mM; room temperature in the dark for 30 min). Excess iodoacetamide was quenched with 3 mM dithiothreitol in the dark at room temperature for 30 min. The sample was then split into two aliquots of 10 μ L each. One sample was treated with chymotrypsin (Roche, 1:100 ratio) and the other with Glu-C (Roche, 1:100 ratio). The enzymatically digested samples were analyzed by capillary reverse phase UPLC-ESI MS/MS on a LTQ-Orbitrap XL (ThermoFisher). The samples were acidified and diluted in 0.1% trifluoroacetic acid. 1 µL (108 fmol) of each sample was directly loaded in Buffer A (0.1% trifluoroacetic acid in water) onto a 100 um x 100 nm, 1.7 um, BEH130 C18 capillary column (Waters Corp). Peptides were eluted with a 40 min gradient of 5%-90% Buffer B (0.1% formic acid in 98 % acetonitrile, 2 % water) at 1.00 µl/min generated by an ultra performance LC system (nanoAcquity UPLC, Waters Corp). Eluted peptides were introduced to the mass spectrometer by electrospray ionization through the application of a 2.0 kV potential to the PicoFrit emitter (New Objective) through a liquid junction. Mass spectral data were acquired using a method where one full MS scan in the Orbitrap (375-1600 m/z) was followed by 7 data-dependent scans. For the data dependent-scans, the top 7 most abundant ions were subjected to collision-induced dissociation in the LTQ throughout the LC gradient. In addition to the data-dependent analysis, targeted mass spectral analysis was also performed using methods consisting of one full MS scan (375-1600 m/z) in the Orbitrap at a resolution of 60,000 followed by 8 product ion scans (MS²). For the 8 targeted scan events, each mass was successively subjected to collision-induced dissociation in a cycle repeated in the LTQ throughout the LC gradient. Ions chosen for the various targeted experiments included the doubly and triply charged ions of the deamidated and non-deamidated forms of the enzymatic peptides flanking the 10 potential N-linked glycosylation sites. There were a total of 5 mass spectrometry methods created. In method 1, the targeted ions consisted of 551.31, 367.88, 550.82, and 367.55 m/z corresponding to the peptides flanking N42 enzymatically generated by Glu-C; and 669.36, 668.87, 1017.52, and 1017.03 m/z corresponding to the cleaved and singly miscleaved peptide flanking N81 as produced by the Glu-C enzyme. In method 2, the targeted ions consisted of 1078.00, 719.00, 1077.51, 718.68, 866.49, 578.00, 866.00, and 577.67 m/z corresponding to the peptides flanking N281 and N433 enzymatically generated by Glu-C. In method 3, the targeted ions consisted of 963.03, 642.35, 962.54, 642.03, 919.99, 613.66, 919.50, and 613.33 m/z corresponding to the peptides flanking N486 and N692 of LRP6 as generated by Glu-C enzyme. In method 4, the targeted ions consisted of 1006.02, 671.02, 1005.04, 670.36, 608.83, 406.22, 608.33, and 405.90 m/z corresponding to the peptides flanking N859, N865, and N1039 of LRP6 as generated by Glu-C enzyme. In method 5, the targeted ions consisted of 828.37, 552.91, 827.88, 552.25, 1157.93, 1157.60, 1245.59 and 1245.10 m/z corresponding to the chymotryptic peptides flanking N42, N81, and N926 of LRP6. Tandem mass spectral results were submitted for database searching using the Mascot program (Matrix Science) against both a subset protein database containing the sequence of interest and the Swissprot database with 30 ppm precursor ion mass and 0.8 Da MS/MS tolerance. Deamidated peptide identifications were confirmed by manual interpretation of the mass spectral data.

Cellular β -catenin Assay. On Day 1, mouse L-cells (5000 cells / 20 μ L / well) were seeded in a clear bottomed, black-walled 384-well plate and grown for 24 h at 37 °C / 5% CO₂. On Day 2, the cells were treated with 20 µL growth medium containing 2X stock of Wnt3a, Fz8 CRD, LRP6, Dkk1 or combinations of these proteins as indicated, and then incubated for 6 h at 37 °C / 5% CO₂. Cells were then fixed in 4% PFA by adding 20 µl of 12% PFA directly to the wells for 1 h at room temperature. The wells were washed three times with PBS (50 µl/well), permeabilized with PBS / 0.1% Triton X-100 (50 µl/well, three times, 2 min each), and blocked in LI-COR buffer (50 µl/well) for 2 h at room temperature (or, alternatively, overnight at 4 °C). The wells were then incubated with mouse anti-β-catenin antibody (Abcam, 1: 200 for optimal signal-tonoise ratio) in LI-COR blocking buffer for 2 h at room temperature (20 µL/well) and subsequently washed with PBS / 0.1% Tween-20 (50 µL/well, three times). Infrared anti-mouse IRDye800CW secondary antibody (1: 200) and DRAQ5 (1: 10,000) in PBS / 0.5% Tween-20 were then added (20 μ L/well). The plates were incubated for 1 h at room temperature, and the wells were washed with PBS / 0.1% Tween-20 (three times) and incubated in PBS (50 μ L/well). The plates were covered with black seals and imaged on an Odyssey infrared scanner using microplate2 settings with sensitivity of 5 in both the 700 and 800 nm wavelength channels. Data were acquired by using Odyssey software, exported and analyzed in Excel (Microsoft, Redmond, WA) or KaleidaGraph (Synergy Software). β-catenin values were background-subtracted from wells treated only with secondary antibody, and then normalized to cell numbers by dividing by the total DNA fluorescence signal to account for any fluctuations in cell number.

Supplemental Figure Legends

Figure S1. Insect cell expression and purification of LRP6 and Fz8 extracellular domains. (A) Protein domains and construct sequence boundaries for Fz8 CRD and LRP6 ECD. Molecular weight, number of cysteines and mass spectrometry analysis of each molecule is shown. To analyze the glycosylation status of LRP6 and Fz 8 CRD, they were subjected to PNGaseF treatment followed by mass spectrometry analysis. Fz8 CRD contains one glycosylation site at N49, in agreement with the reported glycosylation for Fz8 CRD isolated from mammalian cells¹. LRP6 ECD contains four N-linked glycosylation sites at positions 42 of β -propeller one, 433 of β -propeller two, 692 and 865 of β -propeller three. These four residues in LRP6 are strictly conserved in mammals (see Fig. S1E). Therefore, it seems likely that these sites will be glycosylated in mammalian cells, although it is not currently known whether glycosylation plays a role in LRP6 function. (B) Difference in LRP6 protein expression between Sf9 and Tni Pro cells using the wild type or GP67 (baculovirus coat protein) secretion signals. (C) Purity of each fraction during purification of the LRP6 E3E4 protein. Tot = total fraction, FT = flow through, W = wash, Ni = Fraction eluted from the Ni affinity column, GF = pool fraction from gel filtration. (D) SDS-PAGE analysis of purified proteins obtained after gel filtration. Lane 1 = Fz8 CRD, lane 2 = LRP6 E1E2, lane 3 = LRP6 E3E4, lane 4 = LRP6 E3E4 LDL, lane 5 = LRP6 E1E4, lane 6 =LRP6 FL ECD. (E) LRP6 primary sequence alignment. Sequences of LRP6, Hs (Homo Sapiens), Mm (Mus musculus), Gg (Gallus gallus), Xl (Xenopus Laevis), Dr (Danio rerio), Arrow Dm (Drosophila melanogaster) and LRP5 Hs (Homo sapiens) were aligned using an in-house program. Construct boundaries described in Fig S1A are indicated with red squares. Glycosylation sites identified by mass spectrometry are indicated by the blue stars.

Figure S2. Dkk1 competes with Wnt3a for LRP6 binding. Pre-binding of Dkk1 to LRP6 (red trace) inhibits the full binding of Wnt3a to LRP6 (blue trace).

Figure S3. Soluble Fz8 CRD inhibits Wnt3a-mediated β -catenin stabilization by direct binding to Wnt3a. (A) Binding assay between C-terminal Fc-Fusion of Fz8 CRD (coated on the anti-human Fc biosensor) and Wnt3a in solution ($K_D = 4$ nM). Note: We initially obtained a biotinylated form of Fz8 CRD by co-expressing a C-terminal His₆-Avi-tagged version of Fz8 CRD with biotin ligase in insect cells. Wnt3a binding to this version of Fz8 CRD immobilized on Streptavidin biosensors was not well defined. As a result, Fz8 CRD-Fc fusion was used instead in the binding assays. (B) Fz8 CRD inhibits Wnt3a-mediated β -catenin stabilization with IC_{50 =} 0.8 nM. Wnt3a concentration used is 0.6 nM.

Figure S4. Fz8 CRD behaves as a monomeric protein. (A) Comparison of Fz8 CRD gelfiltration profile (blue trace) to a Bio-Rad gel filtration standard (red trace) on a Superdex 200 16/100 column. The major peak for Fz8 CRD has a similar retention time as the 17 kDa standard, suggesting that Fz8 CRD is mainly monomeric. This is in agreement with what is known for Fz8 CRD isolated from CHO cells¹.

Figure S5. LRP6 E1E4 binds to the Wnt3a-Fz8 CRD binary complex. Graph corresponds to a detailed view of the third step (LRP6 binding step) of Figure 3B. The interaction between LRP6 E1E4 and the Fz8/Wnt3a binary complex is dependent on the concentration of E1E4.

Figure S6. Kinetics of Wnt5a and Wnt5b binding to Fz8 CRD. (A) Binding assay between C-terminal Fc-Fusion of Fz8 CRD coated on the anti-Fc biosensor tip and Wnt5a in solution ($K_D = 50$ nM). (B) Binding assay between C-terminal Fc-Fusion of Fz8 CRD coated on the anti-Fc biosensor tip and Wnt5b in solution ($K_D = 37$ nM).

Figure S7. Dkk1 competes with Wnt9b for LRP6 binding. (A) Direct binding assay between C-terminal biotinylated LRP6 E1E4 coated on the Streptavidin biosensor and Wnt9b in solution ($K_D = 11 \text{ nM}$). (B) Pre-binding of Dkk1 to LRP6 inhibits binding of Wnt9b to LRP6 (red trace). The blue trace is a control for Wnt9b binding to LRP6 in the absence of Dkk1. (C) Detailed view of the third step of the experiment described in (B).

Figure S8. Wnt3a, Wnt9b and Dkk1 binding to LRP6 E1E2 and E3E4. (A) Wnt9b binds to LRP6 E1E2; $K_D = 7 \text{ nM}$. (B) Wnt3a binds to LRP6 E3E4; $K_D = 174 \text{ nM}$. (C) Dkk1 binds to LRP6 E1E2; $K_D = 64 \text{ nM}$. (D) Dkk1 binds to LRP6 E3E4; $K_D = 21 \text{ nM}$.

Figure S9. Dkk1 utilizes a common surface to bind to LRP6 E1E2 and E3E4. Pre-binding of Dkk1 to LRP6 E3E4 prevents binding of Dkk1 to E1E2.

1. Dann, C.E. et al. Insights into Wnt binding and signalling from the structures of two Frizzled cysteine-rich domains. *Nature* **412**, 86-90 (2001).

Figure S1, Bourhis et al.



Figure S1E, Bourhis et al.

LRP6 Hs LRP6 Mm LRP6 Gg LRP6 Xi LRP6 Dr Arrow Dm LRP5 Hs	MGA MGA MAA MGA MGA MGA MGA MGA MGA MGA	LLL 24 LLL 24 6 LLL 24 LLL 24 TLL 90 LLL 36
LRP6 Hs 25 LRP6 Mm 25 LRP6 Gg 7 LRP6 XI 25 LRP6 Dr 25 Arrow Dm 91 LRP5 Hs 37	YANRRDLRLVDATNGKENATIVVGGLEDAAAVDFVFSHGLIYWSDVSEEAIKRTEFNKTESVQNVVVSGL YANRRDLRLVDATNGKENATIVVGGLEDAAAVDFVFGHGLIYWSDVSEEAIKRTEFNKTESVQNVVVSGL ATOKOTHLGHFIVDISA.WVENMVVKVCHVDAYVPKSCATEEHQNNQVDWSEGIELKVTQLHYGSNEHSGPQSNKTPYL SYANRRDLRLVDTAG.MKGNSTVVVSGLEDAAAVDFVFSRGLIYWSDVSEEAIKRIDFNKTSGSQDVVIGGL YANRRDLRLVDTAG.GRANATLVMGGLEDAAAVDFYFSRGLIYWSDVSEESIKRTLFNGSAPSGVQTTVISGL FTRNRDLRLVDAAH.GRANATLVMGGLEDAAAVDFYFSRGLIYWSDVSEESIKRTLFNGSAPSGVQTTVISGL FTRNRDLRLVDAAH.GRANATLVMGGLEDAAAVDFYSRGLIYWSDVSEESIKRTLFNGSAPSGVQTTVISGL FTRNRDLRLVDAAH.GRANATLVMGGLEDAAAVDFYSKGAVYWTDVSEEAIKRTLFNGSAPSGVQTVVSGL	LSP 97 LSP 97 LSP 88 VSP 97 ASP 99 DKP 172 VSP 110
LRP6 Hs 98 LRP6 Mm 98 LRP6 Gg 89 LRP6 XI 98 LRP6 Dr 100 Arrow Dm 173 LRP5 Hs 111	DGLACDWLGEKLYWTDSETNRIEVSNLDGSLRKVLFWQELDQPRAIALDPSSGFMYWTDWGEVPKIERAGMDGSSRFIIINS DGLACDWLGEKLYWTDSETNRIEVSNLDGSLRKVLFWQELDQPRAIALDPSSGFMYWTDWGEVPKIERAGMDGSSRFVIINT KTL	E I Y 182 E I Y 182 D I Y 137 D I Y 182 E I Y 184 H V F 259 D I Y 195
LRP6 Hs 183 LRP6 Mm 183 LRP6 Gg 138 LRP6 XI 183 LRP6 XI 183 LRP6 Dr 185 Arrow Dm 260 LRP5 Hs 196	WPNGLTLDYEEQKLYWADAKLNFIHKSNLDGTNRQAVVKGSLPHPFALTLFEDILYWTDWSTHSILACNKYTGEGLREIHSDIFSPN WPNGLTLDYGERKLYWADAKLNFIHKSNLDGTNRQAVVKGSLPHPFALTLFEDTLYWTDWNTHSILACNKYTGEGLREIHSNIFSPN WPNGLTLDYECKLYWADAKLNFIHKSNLDGSHRQAVVKGSLPHPFALTLFGDTLYWTDWNTHSILACSKYSGEDLREIHSNIFSPN WPNGLTLDYDECKLYWADAKLSFIHKANMDGSHRQAVVKGSLPHPFALTLFGDTIYWTDWNTHSILACSKYSGEDLREVDTDIFSPN WPNGLTLDYDECKLYWADAKLSFIHKANMDGSHRQAVVKGSLPHPFALTLFGDTIYWTDWNTHSILACSKYSGEDLREVDTDIFSPN WPNGLTLDYDECKLYWADAKLSFIHKANMDGSHRQTVVKGSLPHPFALTLFGDTIYWTDWNTHSILACSKYSGEDLREVDTDIFSPN WPNGLTLDYDECKLYWADAKLSFIHKANMDGSHRQTVVKGSLPHPFALTLYEDTLFWTDWNTHSILACSKYSGEDLREVDTDIFSPN WPNGLTLDYDECKLYWADAKFFIHKANMDGSSRCTVVVKGSLPHPFALTLYEDTLFWTDWNTHSILACSKYSGEDRREVDTDIFSPN WPNGLTLDYCQCLYWADAKFFIHKANNDGSSRCTVVVKGSLTHPFALTLYEDTLFWTDWRTHSINSCHKATGENSREVHSNIFSPN WPNGLTVDLGKNELIYWTDGKHHFIDVMRLDGSSRRTIVN·NLKYPFSLTFYDDRLYWTDWQRGSLNALDLQTRE·LKELIDTPKAPN	DIH 272 DIH 272 DIH 272 DIH 272 DIH 272 DIH 274 SVR 347 IDIQ 285
LRP6 Hs 273 LRP6 Mm 273 LRP6 Gg 228 LRP6 XI 273 LRP6 Dr 275 Arrow Dm 348 LRP5 Hs 286	AFSQQRQP - NATNPCGIDNGGCSHLCLMSPVKPFYQCACPTGVKLLENGKTCKDGA - TELLLLARRTDLRRISLDTPDFTDIVLQLE AFSQRQP - NATNPCGIDNGGCSHLCLMSPVKPFYQCACPTGVKLLENGKTCKDGA - TELLLLARRTDLRRISLDTPDFTDIVLQLE YFSQRQP - NATNPCGINNGGCSHLCLMSPTKPSYQCACPTGVKLLENGKTCKDGA - TELLLLARRTDLRRISLDTPDFTDIVLPLE VFSHLROP - NATNPCAAHNGGCSHLCLMSPTKPYQCACPTGVRLLENGKTCKDGA - TELLLLARRTDLRRISLDTPDFTDIVLPLE YFSHLROP - NATNPCAAHNGGCSHLCLMSPMEPFYQCACPTGVRLMEDGKKCMHGA - TELLLLARRTDLRRISLDTPDFTDIVLPLO YYSQCPMDVASPCSVRNGGCSHLCLLSPAKPYVQCACPTGVRLMEDGKKCMHGA - TELLLLARRTDLRRISLDTPDFTDIVLPLO AWDPSLQP - YEDNPCAHNNGNCSHLCLLATNSQGFSCACPTGVRLI - SANTCANGS - QEMMFIVORTO ISKISLDSPDYTIFPLPLG VLSQERQP - FFHTRCEEDNGGCSHLCLLSPSFYTCACPTGVRLI - SANTCANGS - QEMMFIVORTO ISKISLDTPDFTDIVLQVD	DIR 360 DIR 360 DIR 315 DIR 360 DIR 364 KVK 434 DIR 373
LRP6 Hs 361 LRP6 Mm 361 LRP6 Gg 316 LRP6 XI 361 LRP6 Dr 365 Arrow Dm 435 LRP5 Hs 374	HATATDYDPVEGYTYWTDDEVRAIRRSFIDGSSGSGFVVTAQTAHPDGTAVDWVARNLYWTDTGTDRTEVTRLNGTMRKILISEDLEE HATATDYDPVEGYTYWTDDEVRAIRRSFIDGSSGSGFVVTAQTAHPDGTAVDWVARNLYWTDTGTDRTEVTRLNGTMRKILISEDLEE HATATDYDPVEGYTYWTDDEVRAIRRSFIDGSSSGFVVTAQTAHPDGTAVDWVARNLYWTDTGTDRTEVTRLNGTMRKILISEDLEE HATATDYDPVEGYTYWTDDEVRAIRRSFIDGSSSGYTVTAQTAHPDGTAVDWVARNLYWTDTGTDRTEVTRLNGTMRKILISEDLEE HATATDYDPVEGYTYWTDDEVRAIRRSFIDGSSSGYTVTAQTAHPDGTAVDWVARNLYWTDTGTDRTEVTRLNGTMRKILISEDLEE HATATDYDPVEGYTYWTDDEVRAIRRSFIDGSSSGYTVTAQTAHPDGTAVDWVARNLYWTDTGTDRTEVTRLNGTMRKILISEDLEE HATATDYDPVEGYTYWTDDEVRAIRRSFIDGSSSGYTVTAQTAHPDGTAVDWVARNLYWTDTGTDRTEVTRLNGTMRKILISEDLEE HATATDYDPVEGHTYWTDDEVRAIRRSFIDGSSSGYTVTAQTAHPDGTAVDWVARNLYWTDTGTDRTEVTRLNGTMRKILISEDLEE HATATDYDPVEGHTYWTDDEVRAIRRSFIDGSSSGYTVTAQTAHPDGTAVDWVARNLYWTDTGTDRTEVTRLNGTMRKILISEDLEE HATATDYDPVEGHTYWTDDEVRAIRRSFIDGSSSGYTVTAQTAHPDGTAVDWVARNLYWTDTGTDRTEVTRLNGTMRKILISEDLEE HATATDYDPVEGHTYWTDDEVRAIRRSFIDGSSSGYTVTAQTANDGTAVDWVARNLYWTDTGTDRTEVTRLNGTMRKILISEDLEE	PRA 450 PRA 450 PRA 405 PRA 450 PRA 450 PRA 454 PRA 524 PRA 463
LRP6 Hs 540 LRP6 Mm 540 LRP6 Gg 495 LRP6 XI 540 LRP6 Dr 544 Arrow Dm 6115 LRP5 Hs 553	LLGDYYYWTDWQRRRSIERVYHKRSA - EREVIIDQLPDLMGLKATNYHRYIGSNPCAEDNGGCSHLCLYRPQGLRCACPIGLELISDMK DLLGDYYYWTDWQRRSIERVYHKRSA - EREVIIDQLPDLMGLKATSYHRIGSNPCAEDNGGCSHLCLYRPQGLRCACPIGLELINDMK SLLGDYYYWTDWQRRSIERVYHKRTG - EREVIIDQLPDLMGLKATSYHRIGSNPCAEDNGGCSHLCLYRPQGPCACPIGLELINDMK LLGDYYYWTDWQRRSIERVYHKRTG - EREVIIDQLPDLMGLKATSYHRIGSNGCAENNGGCSHLCLYRPQGPCSCACPIGLELINDMK LLGDYYYWTDWQRRSIERVYHKRTG - EREVIIDQLPDLMGLKATFYHQCAENNGGCSHLCLYRPQGPCSCACPIGLELINDMK LLGDYYYWTDWQRRSIERVYHKRTG - EREVIIDQLPDLMGLKATFYHQTACAWANGGCSSHLCLNRPRDYSCRCAPIGLELINDMK LLGDYYYWTDWQRRSIERVYHKRTG - EREVIIDQLPDLMGLKATFYHQTACAWANGGCSSHLCLNRPRDYSCRCAPIGLELINDMK LLGDYYYWTDWQRRSIERVYHKRTG - EREVIIDQLPDLMGLKATFYHQTACAWANGGCSSHLCLNRPRDYSCRCAPIGLELINDMK LLGDYYYWTDWQRRSIERVYHKRTG - EREVIIDQLPDLMGLKATFYHQTACAWANGGCSSHLCC	TCI 628 TCI 628 TCI 628 TCI 628 TCI 632 TCV 704 TCI 641
LRP6 Hs 629 LRP6 Mm 629 LRP6 Gg 584 LRP6 XI 629 LRP6 Dr 633 Arrow Dm 705 LRP5 Hs 642	VPEAFLLFSRRADIRRISLETNNNNVAIPLTGVKEASALDFDVTDNRIYWTDISLKTISRAFMNG SALEHVVEFGLDYPEGMAV VPEAFLLFSRRADIRRISLETNNNNVAIPLTGVKEASALDFDVTDNRIYWTDISLKTISRAFMNG SALEHVVEFGLDYPEGMAV VPEAFLLFSRRADIRRISLETNNNVAIPLTGVKEASALDFDVTDNRIYWTDISLKTISRAFMNG SALEHVVEFGLDYPEGMAV VPEAFLLFSRRADIRRISLETNNNVAIPLTGVKEASALDFDVTDNRIYWTDISLKTISRAFMNG SALEHVVEFGLDYPEGMAV VPEAFLLFSRRADIRRISLETNNNVAIPLTGVKEASALDFDVTDNRIYWTDISLKTISRAFMNG SALEHVVEFGLDYPEGMAV VPEAFLLFSRRADIRRISLETNNNVAIPLTGVKEASALDFDVTDNRIYWTDISLKTISRAFMNG SALEHVVEFGLDYPEGMAV VPEAFLLFSRRADIRRISLETNNNVAIPLTGVKEASALDFDVTDNRIYWTDVSLKTISRAFMNG SSLEHVVEFGLDYPEGMAV VPEAFLLFSRRADIRRISLETNNNVAIPLTGVKEASALDFDVTDNRIYWTDVSLKTISRAFMNG SSLEHVVEFGLDYPEGMAV	DWL 715 DWL 715 DWL 670 DWL 715 DWL 715 DWL 719 DWL 719 DWL 794 DWM 728
LRP6 Hs 716 LRP6 Mm 716 LRP6 Gg 671 LRP6 Xl 716 LRP6 Dr 720 Arrow Dm 795 LRP5 Hs 729	GKNLYWADTGTNRIEVSKLDGCHROVLVWKDLDSPRALALDPAEGFMYWTEWGGKPKIDRAAMDGSERTTLVPNVGRANGLTIDYAK GKNLYWADTGTNRIEVSKLDGCHROVLVWKDLDSPRALALDPAEGFMYWTEWGGKPKIDRAAMDGSERTTLVPNVGRANGLTIDYAK GKNLYWADTGTNRIEVSKLDGCHROVLVWKDLDSPRALALDPAEGFMYWTEWGGKPKIDRASMDGSERTTLVPNVGRANGLTIDYAK GKNLYWADTGTNRIEVSKLDGCHROVLVWKDLDSPRALALDPAEGFMYWTEWGGKPKIDRASMDGSERTLVPNVGRANGLTIDYAK GKNLYWADTGTNRIEVSKLDGCHROVLVWKDLDSPRALALDPAEGFMYWTEWGGKPKIDRASMDGSERTLVPNVGRANGLTIDYAK GKNLYWADTGTNRIEVSKLDGCHROVLVWKDLDSPRALALDPAEGFMYWTEWGGKPKIDRAAMDGSGRITLVPNVGRANGLTIDYAK GKNLYWADTGTNRIEVSKLDGCHROVLVWKDLDSPRALALDPAEGFMYWTEWGGKPKIDRAAMDGSGRITLVPNVGRANGLTIDYAE GKNLYWADTGTNRIEVSKLDGCHROVLVWKDLDSPRALALDPAEGFMYWTEFSDSIRAAMDGSDLOTIVVPNVGRANGLTIDYAE GKNLYWADTGTNRIEVAKLDGCHROVLVWKDLDSPRALALDPAEGFMYWTESTDSIRRAAMDGSDLOTIVVAGANAAGLTFDQET GKNLYWADTGTNRIEVAKLDGCFROVLVWRDLDNPRSLALDPTKGYIYWTEWGGKPRIVRAFMDGTNCMTLVDKVGRANDLTIDYAE	RRL 805 RRL 805 RRL 760 RRL 805 RRL 809 RRL 883 QRL 818
LRP6 Hs 806 LRP6 Mm 806 LRP6 Gg 761 LRP6 XI 806 LRP6 Dr 810 Arrow Dm 884 LRP5 Hs 819	YW - TDLDTNLIESSNMLGLNREV - IADDLPHPFGLTQYQDYIYWTDWSRRSIERANKTSGONRTIIGHLDYVMDILVFHSSRQSGW YW - TDLDTNLIESSNMLGLNREV - IADDLPHPFGLTQYQDYIYWTDWSRRSIERANKTSGONRTIIGHLDYVMDILVFHSSRQAGW YW - TDLDTNLIESSNMLGLDREI - IADDLPHPFGLTQYQDYIYWTDWSRRSIERANKTSGONRTIIGHLDYVMDILVFHSSRQAGW YW - TDLDTNLIESSNMLGLDREV - IADDLPHPFGLTQYQDYIYWTDWSRRSIERANKTSGONRTING HLDYVMDILVFHSSRQAGW YW - TDLDTNLIESSNMLGLDRVV - IADDLPHPFGLTQYQDYIYWTDWSRRSIERANKTSGONRTING HLDYVMDILVFHSSRQAGW YW - TDLDTNLIESSNMLGLDRVV - IADDLPHPFGLTQYQDYIYWTDWSRRSIERANKTSGONRTING HLDYVMDILVFHSSRQAGW YW - TDLDTNLIESSNMLGLDRVV - IADDLPHPFGLTQYQDYIYWTDWSRRSIERANKTSGONRTVIGG HLDYVMDILVFHSSRQAGW YW - TDLDTNLIESSNMLGLEREV - IADDLPHPFGLTQYQDYIYWTDWSRRSIERANKTSGONRTVIGG HLDYVMDILVFHSSRQSGW YW - TDLDTNLIESSNMLGLEREV - IADDLPHPFGLTQYQDYIYWTDWSDWSRSIERANKTSGONRTVIGG HLDYVMDILVFHSSRQSGW YW - TDLDTNNIESSNMLGQERVV - IADDLPHPFGLTQYSDYIYWTDWSDWSRSIERANKTSGONRTVIGG HLDFVMDILVFHSSRQSGW	NEC 893 NEC 848 NEC 893 NEC 893 NEC 893 NEC 893 NEC 973 NPC 973 NDC 906
LRP6 Hs 894 LRP6 Mm 894 LRP6 Gg 849 LRP6 XI 894 LRP6 Dr 898 Arrow Dm 974 LRP5 Hs 907	ASSNGH CSHLCLAVP.VGGFVCGCPAHYSLNADNRTCSAPTTFLLFSQKSAINRMVIDEQQSPDIILPIHSLRNVRAIDYDPLDKQL ASSNGH CSHLCLAVP.VGGFVCGCPAHYSLNADNRTCSAPTTFLLFSQKSAINRMVIDEQQSPDIILPIHSLRNVRAIDYDPLDKQL ASSNGH CSHLCLAVP.VGGFVCGCPAHYSLNSDNRTCSAPTTFLLFSQKNAINRMVIDEQQSPDIILPIHSLRNVRAIDYDPLDKQL ASSNGH CSHLCLAVP.VGGFVCGCPAHYSLNSDNRTCSAPTSFLLFSQKNAINRMVIDGQQSPDIILPIHSLRNVRAIDYDPLKQL ASSNGH CSHLCLAVP.VGGFVCGCPAHYSLNSDNRTCSAPSFLLFSQKNAINRMVIDGQQSPDIILPIHSLRNVRAIDYDPLKQL ASSNGH CSHLCLAVP.VGGFVCGCPAHYSLNSDNKTCSAPSFLLFSQKNAINRMVIDGQQSPDIILPIHSLRNVRAIDYDPLEKQL KUNNGGCSHLCLAVP.VGGFVCGCPAHYSLNSDNKTCSAPSFLLFSQKNAINRMVMDEQQSPDIILPIHSLRNVRAIDYDPLEKQL AASNGH CSHLCLAVP.VCSFTCGCPAHYSLNSDNKTCSAPSFLLFSQKNAINRMVMDELQSPDIILPIHSLRNVRAIDYDPLEKQL AAN NGH CSHLCLAVP.VCSFTCGCPAHYSLNSDNKTCSAPSFLFSQKSAISRMIPDDQASPDIILPIHSLRNVRAIDYDPLEKQL AAN NGH CSHLCLAVP.VCSFTCGCPAHYSLNSDNKTCSAPSFLFSQKSAISRMIPDDQASPDIILPIHSLRNVRAIDYDPLEKQL MHNNGQCGQLCLAIP.SGHRCGCASHYTLDPSSRNCSPPTTFLLFSQKSAISRMIPDDQHSPDLILPLHGLRNVKAIDYDPLDKFI	YWI 982 YWI 982 YWI 937 YWI 982 YWI 986 YWI 986 YWI 1062 YWV 994
LRP6 Hs 983 LRP6 Mm 983 LRP6 Gg 938 LRP6 Dr 983 LRP6 Dr 987 Arrow Dm 1063 LRP5 Hs 995	D S R Q N MI R K A Q E D G S O G F T V V V S S V P S Q N L E I Q P Y D L S I D I Y S R Y I Y W T C E A T N V I N V T R L D G R S V G V V L K G E Q D R P A I V V N P E K G D S R Q N S I R K A H E D G G Q G F N V V A N S V A N O N L E I Q P Y D L S I D I Y S R Y I Y W T C E A T N V I D V T R L D G R S V G V V L K G E Q D R P A I V V N P E K G D S R Q N I I R K A Q E D G S Q S L T V I S P V P N N L D M Q P Y D L S I D I Y S R Y I Y W T C E A T N V I N V T R L D G R P MG V V L K G E Q D R P R A I V V N P E K G D S R Q N I I R K A Q E D G S Q S M T I V A S T I P N Q N M D M D M D Y D L S I D I Y S R Y I Y W T C E A T N V I N V T R L D G R A I G V V L K G E Q E R P R A I L V N P E K G D S R Q N - I R R A Q E D G S Q S M T I V A S T I P N Q N M D M D Y D L S I D I Y S R I I Y W T C E A T N I N V T R L D G R A I G V V L K G E Q E R P R A I L V N P E R G D A K Q N V I R R A Q E D G N Q S V T V S G A V G G S N L G L Q P Y D L S I D I Y S R F I Y W T S E L T N V I N V T R L D G S R V G V V L K G E Q E R P R A I L V N P E R G D A K Q N V I R R A Q E D G N Q S V T V S G A V G G S N L G L Q P Y D L S I D I Y S R F I Y W T S E L T N V I N V T R T D G S R V G V V L R G E A D K P R A I A V N P E R G D A K Q N V I R R A Q E D G N Q S V U L S I D A Y S R F I Y W T S E L T N V I N V T S F L G E S V G V V L R G D R P N A A Y N A A R Y D G R Q N - I K R A K D D G T Q P F · V L T S L S Q G Q N P D R Q P H D L S I D I Y S R T L F W T C E A T N T N V H R L S G E A M G V V L R G D R D K P R A I V Y N A E R G D G R Q N - I K R A K D D G T Q P F · V L T S L S Q G Q N P D R Q P H D L S I D I Y S R T L F W T C E A T N T N V H R L S G E A M G V V L R G D R D K P R A I V Y N A E R G	YMY 1072 YMY 1072 YMY 1027 YMY 1071 YMY 1071 YMY 1076 LLF 1144 YLY 1082
LRP6 Hs 1073 LRP6 Mm 1073 LRP6 Gg 1028 LRP6 Xi 1072 LRP6 Dr 1077 Arrow Dm 1145 LRP5 Hs 1083	FTN LQERSPK I ERAALDGTEREVLFFSGLSKPIALALDSRLGKLFWADSDLRRIESSDLSGANRIVLE DSNILQPVGLTVFENWLYV FTN LQERSPKIERAALDGTEREVLFFSGLSKPIALALDSQLGKLFWADSDLRRIESSDLSGANRIVLE DSNILQPVGLTVFENWLYV FTN LQERSPKIERAALDGSEREVLFFSGLSKPIALAIDSQLGKLFWADSDLRRIESSDLSGANRIVLE DSNILQPVGLTVFENWLYV FTN LQERSPKIERAALDGSEREVLFFSGLSKPIALAIDSQLGKLFWADSDLRRIESSDLSGGHRIVLE DSNILQPVGLTVFGNYLYV FSN LQERSPKIERAALDGSEREVLFFSN LGRPVALALDNKMGKLFWADSDLRRIESSDLSGGHRIVLE DSNILQPVGLTVFGNYLYV FSN LQERSPKIERAALDGSEREVLFFSN LGRPVALALDNKMGKLFWADSDLRRIESSDLSGGHRIVLE DSNILQPVGLTVFGNFLYV FSN LQERSPKIERAALDGSEREVLFFSN LGRPVALALDNKMGKLFWADSDLRRIESSDLSGGANRIVIE DSNILQPVGLTVFGNFLYV FSN LQERSPKIERAALDGSEREVLFFSN LGRPVALALDNKMGKLFWADSDLRRIESSDLSGANRIVIE DSNILQPVGLTVFGNFLYV FSN LQERSPKIERAALDGSEREVLFFSN LGRVVALAVDNELGKLFWVDMDLRRIESSDLSGANRIVIE DSNILQPVGLTVFGNFLYV FSN LQERSPKIERAALDGSEREVLFFSN LGRVVALAVDNELGKLFWVDMDLRRIESSDLSGANRIVIE DSNILQPVGLTVFGNFLYV	IDK 1162 IDK 1162 IDR 1117 IDR 1161 IDR 1166 ID- 1229 IDR 1172
LRP6 Hs 1163 LRP6 Mm 1163 LRP6 Gg 118 LRP6 Xi 1162 LRP6 Dr 1167 Arrow Dm 1230 LRP5 Hs 1173	QQQMIEKIDMTGREGRTKVQARIAQLSDIHAVKELNLQEYRQHPCAQDNGGCSHICLVKGDGTTRCSCPMHLVLLQ.DELSCG QQQMIEKIDMTGREGRTKVQARIAQLSDIHAVKELNLQEYRQHPCAQDNGGCSHICLVKGDGTTRCSCPMHLVLLQ.DELSCG QQQMIEKIDMTGREGRTKVQARIAQLSDIHAVKELNLQEYRQHPCSQDNGGCSHICLVKGDGTTRCSCPVHLVLLQ.DELSCG QQQMIEKIDMTGREGRTKVQARIAQLSDIHAVKELNVQEYRQHPCSQDNGGCSHICIVKGDGTTRCSCPVHLVLLQ.DELSCG QQQMIEKIDMTGREGRTKVQARIAQLSDIHAVKELNVQEYRQHPCSQDNGGCSHICIVKGDGTTRCSCPVHLVLLQ.DELSCG QQQMIEKIDMTGREGRTKVQARIAQLSDIHAVKELNVQEYRQHPCSQDNGGCSHICIVKGDGTTRCSCPVHLVLLQ.DELSCG QQQMIEKIDMTGREGRTKVQARIAQLSDIHAVKELNVQUPNCYRAND QQQMIEKIDMTGREGRTKVQARIAQLSDIHAVKELNVQUPNCYRAND QQQMIERIEKICVGCVGCVCVCVCVCVCVCVCVCVCVCVCVCVCVCVCVC	E P P 1247 E P P 1247 E P P 1202 E P P 1247 E P P 1251 A F P 1317 E P P 1257
LRP6 Hs 1248 LRP6 Gg 1203 LRP6 Gg 1203 LRP6 JI 1248 LRP6 Dr 1252 Arrow Dm 1318 LRP5 Hs 1258	T CS P Q Q F T C F T G E I D C I P V A W R C D G F T E C E D H S D E L N C P V C S E S Q F Q C A S G Q C I D G A L R C N G D A N C Q D K S D E K N C E V L C L T C S P Q Q F T C F T G E I D C I P V A W R C D G F T E C E D H S D E L N C P V C S E S Q F Q C A S G Q C I D G A L R C N G D A N C Q D K S D E K N C E V L C L T C S P Q Q F T C F T G E I D C I P V A W R C D G F T E C E D H S D E L N C P V C S E S Q F Q C A S G Q C I D S A L R C N G D A N C Q D K S D E K N C E V L C L T C S P Q Q F T C F T G E I D C I P V A W R C D G F T E C E D H S D E K N C P V C S D T Q F Q C E S G Q C I D S A L R C N G E A N C Q D N S D E K N C E V L C L T C S P Q Q F T C F T G E V D C I P A E W R C D G F T E C E D H S D E K N C P V C S S D C Y C T S G Q C I D S A L R C N G E A N C Q D N S D E K N C E V L C L T C S P E Q F S C V S G E V D C I P A E W R C D G G F A E C D D S S D E R D C P V C S S R E F Q C D S R Q C V D A A L R C N G E I N C Q D R S D E K N C E V L C F A C G P D H F T C A A P V S G I S D V N K D C I P A G W R C D G G F A C A D G C P D C S A A G F P Q C A R G Q V D L R L R Q D G E A D C Q D R S D E A D C C A T G C P D C F A C A T G E V D C I P A G W R C D G G F A C A D G F P C C A S G Q S U D L R L R Q D G E A D C C A A C A C A C A C A C A C A C A	IDQ 1330 IDQ 1330 TNQ 1285 PDQ 1330 ADQ 1334 PGE 1405 PNQ 1340
LRP6 Hs 1331 LRP6 Mm 1331 LRP6 Gg 1286 LRP6 Xi 1331 LRP6 Dr 1335 Arrow Dm 1406 LRP5 Hs 1341	FRCANGQ - CIGKHKKCDHNVDCSDKSDELDCYPTEEP APQATNTVGSVIGVIVTIFVSGTVYFICQRMLCPRMKGDGETMTNDY FRCANGQ - CIGKHKKCDHNVDCSDRSDELDCYPTEEP APQATNTVGSVIGVIVTIFVSGTYYFICQRMLCPRMKGDGETMTNDY FRCASGQ - CIGKSKKCDHNLDCSDSSDEQGCYTTEEP APQPNNTIGSIIGVILTVFVVGAWYFICQRVLCPRMKGDGETMTNDY FRCSSGQ - CIGKSKCDNNDCSDSSDEQGCYTTEEP PPPSTNTIGSIIGVILTVFVVGAVYFICQRVLCPRMKGDGETMTNDY FTCSNGQ - CIGKHKRCDLSPDCSDSSDEQGCYTEEP SFAPTNTIGSIIGVILTVFVVGAVYFICQRVLCPRMKGDGETMTNDY FTCSNGQ - CIGRHKKCDNNMDCTDNSDEIGCYATEEP SFAPTNTIGSIVGVVMVLFVVGAVYFVCQRVLCPRMKGDGETMTNDY FTCSNGQ - CIGRHKKCDNNMDCTDNSDEIGCYATEEP SFAPTNTIGSIVGVVMVLFVVGAVYFVCQRVLCPRMKGDGETMTNDY FTCSNGQ - CIGRHKKCDNNMDCTDNSDEIGCYATEEP MAPATDKRAFMILIGATMITIFSIVYLQ - FCRTRIGKSRTEPKDC FQCSGQ - CIGRHKKCDNNMCTDNSDEIGCYATEEP MAPATDKRAFMILIGATMITVFVGQVVVLCPVVCQRVACANDFPHEY	VVH 1416 VVH 1416 VVH 1371 VVH 1416 VVH 1416 VVH 1410 QAT 1490 -VS 1428

Figure S2, Bourhis et al.



Figure S3, Bourhis et al.



Figure S4, Bourhis et al.



Figure S5, Bourhis et al.



Figure S6, Bourhis et al.



Time (sec)

Figure S7, Bourhis et al.



Figure S8, Bourhis et al.



Figure S9, Bourhis et al.

