

Crystal structures of the extracellular domain of LRP6 and its complex with DKK1

Zhihong Cheng¹, Travis Biechele², Zhiyi Wei^{1,3}, Seamus Morrone¹, Randall T. Moon², Ligu Wang¹, Wenqing Xu¹

¹Department of Biological Structure, ²Department of Pharmacology, Howard Hughes Medical Institute, and Institute for Stem Cell and Regenerative Medicine, University of Washington School of Medicine, Seattle, WA 98195

³Present address: Division of Life Science, Hong Kong University of Science and Technology, Hong Kong

Correspondence should be addressed to W.X. (wxu@u.washington.edu).

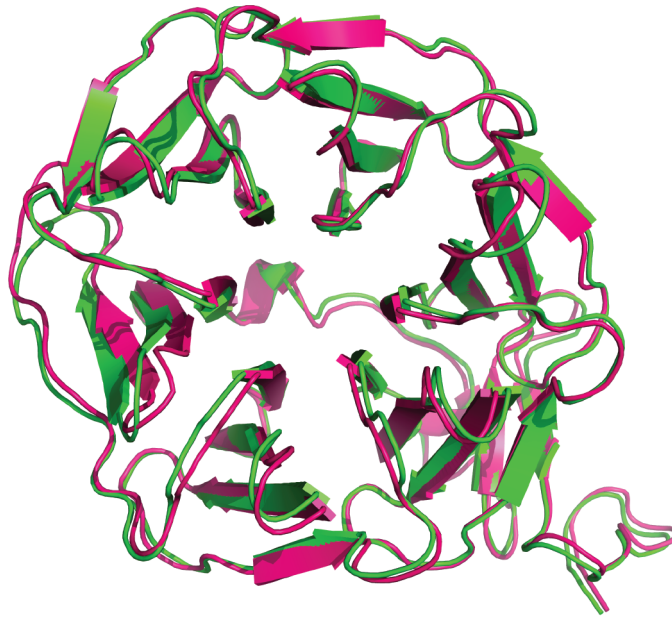
Supplementary Information

This PDF file includes:
Supplementary Figures 1 to 6

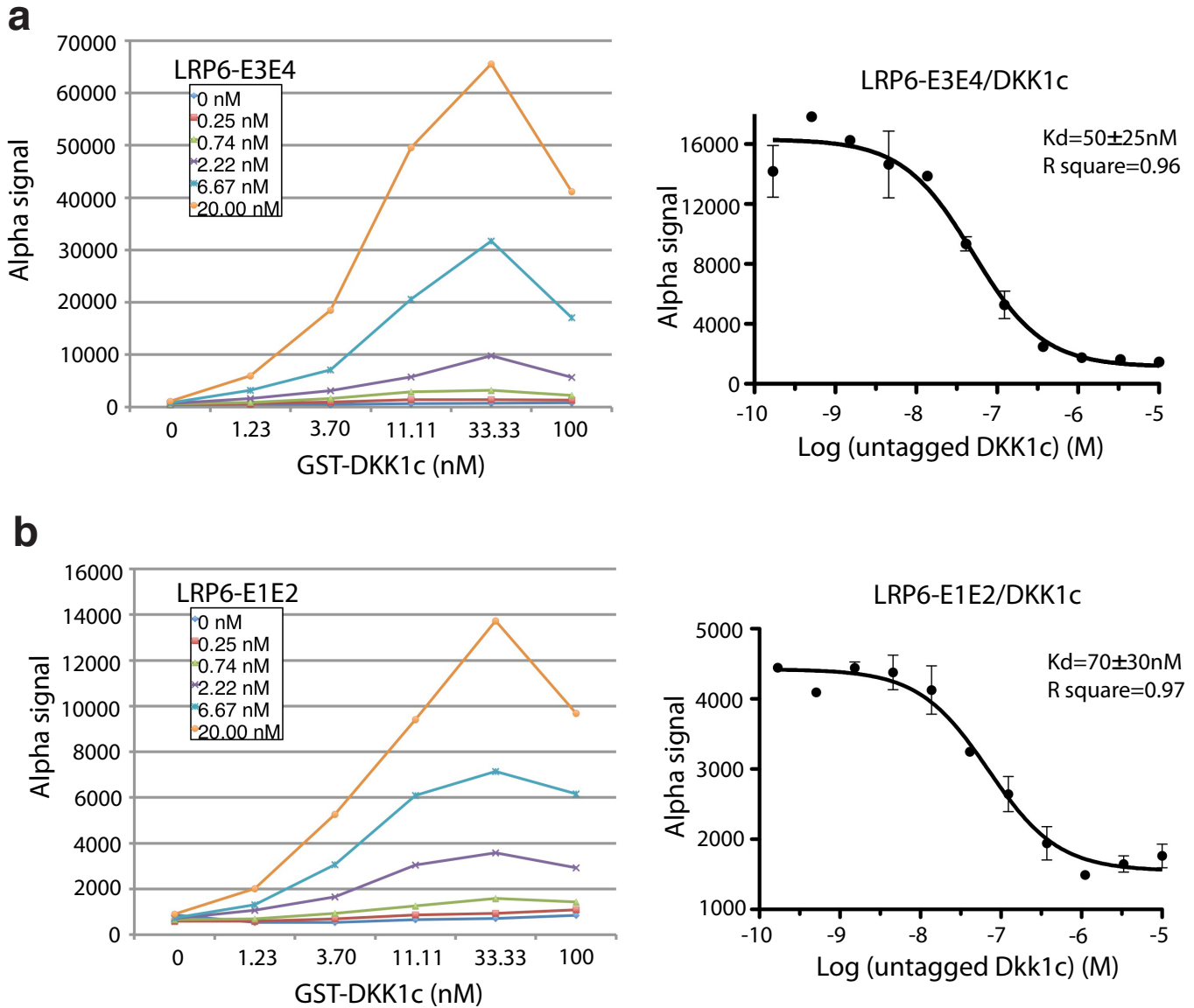
LRP5_1	SPLLLLFANRRDRLVDAGG-VKLESTIVVSGLEDAAAVDFQFSKGAVYWTVDVSEEAIKQT	90
LRP6_1	APLLLLYANRRDLRLVDATN-GKENATIVVGGLEDAAAVDFVFSHGLIYWSDVSEEAIKRT	78
LRP5_3	EAFLLVFTSRAAIHRISLET-NNNDVAIPLTGVEKASALDFDVSNNHIYWTVDVSLKTI SRA	702
LRP6_3	EAFLLFSRRADIRRI SLET-NNNNVAIPLTGVEKASALDFDVTDNRIYWTDISLKTISRA	689
LRP5_2	EEVLLLARRTDLRRISLDTDPDFTDIVLQVDDIRHAI AIDYDPLEGYVYWTDDDEVRAIRRA	400
LRP6_2	TELLLLARRTDLRRISLDTDPDFTDIVLQLEDIRHAI AIDYDPEGYIYWTDDDEVRAIRRS	387
LRP5_4	TTFLLSQKSAISRMI PDDQHSPLILPLHGLRNVKAIDYDPLDKFIYWVDGRQN- IKRA	1003
LRP6_4	TTFLLSQKSAINRMVIDEQQSPDIILPIHSLRNVRAIDYDPLDKQLYWIDSRQNMIRKA	992
	. * : : : : : : : : : : * : * : . : * * * . * : :	
LRP5_1	YLNQTGAAVQNVVISGLVS----P D GLACDWVGKKLYWTDSETNRIEVANLNGTSRKVLF	146
LRP6_1	EFNKT-ESVQNVVVSGLLS----PDGLACDWLGEKLYWTDSETNRIEVSNLDGSLRKVLF	133
LRP5_3	FMNGS--SVEHVVEFGLDY----PEGMAVDWMGKNLYWADTGTNRIEVARLDGQFRQVLV	756
LRP6_3	FMNGS--ALEHVVEFGLDY----PEGMVDWLGNLYWADTGTNRIEVSKLGDGQHRQVLV	743
LRP5_2	YLDGS--GAQTLVNT EIND----PDGIAVDWVARNLYWTDGTGTDRIEVTRLNGTMRKILI	454
LRP6_2	FIDGS--GSQFVVTAQIAH----PDGIAVDWVARNLYWTDGTGTDRIEVTRLNGTMRKILI	441
LRP5_4	KDDGTQPF-VLTSLSQGGQNPDRQPHDLSIDIYSRTLFWTCEATNTINVHRLSGEAMGVVL	1062
LRP6_4	QEDGSQGFVVVSSVPSQNL EIQPYDLSIDIYSRYIYWTCEATNVI NVTRLDGRSVGVVL	1052
	: : : : : * . : : * . . : : * : * : * * . * * : : .	
LRP5_1	WQDLQDPRAIALDPAHGYMYWTDW G ET-PRIERAGMDGSTRKII VDSDIYWPNGLTIDLE	205
LRP6_1	WQELDQPRALALDPSSGFMYWTDWGEV-PKIERAGMDGSSRFII INSEIYWPNGLTLDYE	192
LRP5_3	WRDLDNPRSLALDPTKGYIYWTEWGGK-PRIVRAFMDGTNCMTLVD-KVGRANDLTIDYA	814
LRP6_3	WKDLDSPRALALDPAEGFMYWTEWGGK-PKIDRAAMDGSERTTLVP-NVGRANGLTIDYA	801
LRP5_2	SEDLDEPRAIALHPPVGMGLMYWTDWGEN-PKIECANLDGQERRVLVNASLGPNGLALDLQ	513
LRP6_2	SEDLEEPRAIVLDPVMGYMYWTDWGEI-PKIERAALDGSDRVVLVNTSLGPNGLALDYD	500
LRP5_4	RGDRDKPRAIVVNAERGYLYFTNMQDRAAKIERAALDGTEREVLFTTGLIRPVALVVDNT	1122
LRP6_4	KGEQDRPRAIVVNPEKGYMYFTNLQERSPKIERAALDGTEREVLFFSGLSKPIALALDSR	1112
	: : * : : : . . * : * : * : . : * * * : : . : * : *	
LRP5_1	EQKLYWADAKLSFIHRANLDGFSFRQKVVEGSLTHPFALTLSGDTLYWTDWQTRS IHACNK	265
LRP6_1	EQKLYWADAKLNF I HKSNLDGTNRQAVVKGSLPHPFALTLFEDILYWTDWSTHSILACNK	252
LRP5_3	DORLYWTDLDLTNMI ESSNMLGQER-VVIADDLPHPFGLTQYSDYIYWTDWNLHSIERADK	873
LRP6_3	KRRLYWTDLDLTNLI ESSNMLGLNR-EVIADDLPHPFGLTQYQDYIYWTDWNRSSIERANK	860
LRP5_2	EGKLYWGDAKTDKIEVINVDGTRKRRTLLEDKLP HIFGFTLLGDFIYWTDWQRRS IERVHK	573
LRP6_2	EGKIYWGDAKTDKIEVMNTDGTGRRVLVEDKIP HIFGFTLLGDYVYWTDWQRRS IERVHK	560
LRP5_4	LGKLFWVDADLKRIE SCDLSGANRLTLEDANIVQPLGLTILGKHL YWIDRQQQM IERVEK	1182
LRP6_4	LGKLFWADSDLRRIE SSDLSGANRIVLEDSNILQPVGLTVFENWLYWIDKQQQM IEKIDM	1172
	: : : * * . * . : * * : . : : . . : * . : * * * . : * .	
LRP5_1	RTGGKRKEILSALYSPMDIQVLSQERQPF-FHTRCEEDNGGCSHLCLLSPSEPFYTCACP	324
LRP6_1	YTGEGLREIHSDFISPMDIHAFSQQRQPN-ATNPGCIDNGGCSHLCLMSPVKPFYQCACP	311
LRP5_3	TSGRNRTLIQGHLDVMDILVFHSSRQD--GLNDCMHNNGQCGQLCLAI P--GGHRCGCA	929
LRP6_3	TSGQNRTIIQGHLDYVMDILVFHSSRQS--GWNECASSNGHCSHLCLAVPV-GGFVCGCP	917
LRP5_2	VK-ASRDVIIDQLPDLMGLKAVNVAKVV--GTNPCADRNGGCSHLCLFFTPH--ATRCGCP	628
LRP6_2	RS-AEREVIIDQLPDLMGLKATNVHRVI--GSNP CAEENGGCSHLCLLYRPQ--GLRCACP	615
LRP5_4	TTGDKRTRIQGRVAHLTGIHAVEEVSLEEFSAHPCARDNGGCSHICIAKGD-GTPRCSCP	1241
LRP6_4	TGREGRTKVQARIAQLSDIHAVKELNLQEYRQHPCAQDNGGCSHICLVKGD-GTTRCSCP	1231
	: : : : . . * * * . : : * : * .	
LRP5_1	TGVQLQDNGRTCKAGA	340
LRP6_1	TGVKLENGKTCKDGA	327
LRP5_3	SHYTLDPSSRNC SPP-	944
LRP6_3	AHYSLNADNRTCSAP-	932
LRP5_2	IGLELLSDMKTCIVP-	646
LRP6_2	IGFELISDMKTCIVP-	630
LRP5_4	VHLVLLQNL LTCGEP	1257
LRP6_4	MHLVLLQDELSCGEP	1247
	* . . *	

Supplementary Figure 1. Sequence alignment of LRP5/6 propellers. LRP6 residues on interfaces A and B are highlighted by green and cyan background, respectively. Residues used by both interfaces are highlighted with red background. Seven observed N-glycosylation sites (three in LRP6-E1E2 and four in E3E4) are in purple font and underlined, whereas corresponding disease associated missense mutation sites in LRP5/6 are in bold red font.

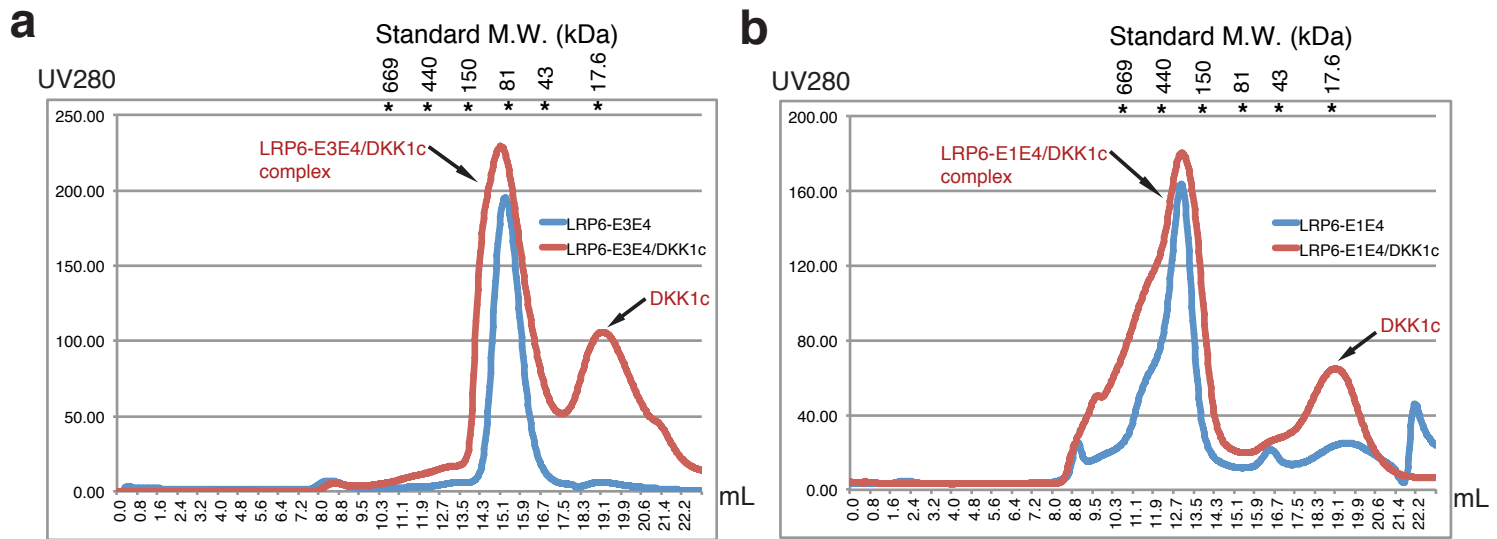
LRP6-E3 Apo vs. Dkk1c-bound LRP6-E3



Supplementary Figure 2. Structural comparison of LRP6-E3 in bound and unbound states. There is no significant conformational change on the top surface of LRP6-E3 propeller.



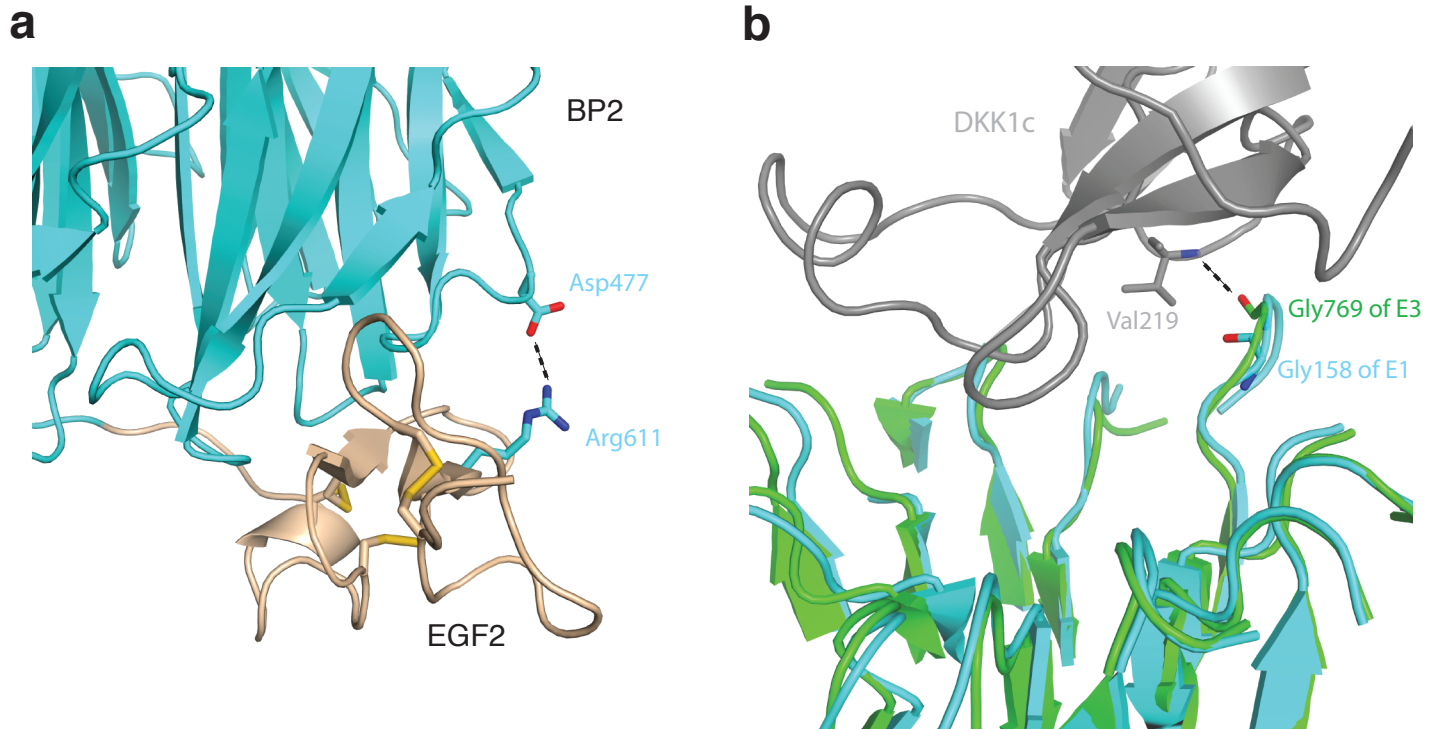
Supplementary Figure 3. Interactions between DKK1c and LRP6-E1E2/E3E4. Binding affinities between DKK1c and two LRP6 rigid blocks (E3E4 and E1E2) were measured using Alpha Technology binding assay (Perkin Elmer, MA). Streptavidin Donor beads and Glutathione Acceptor beads were used in this assay. The experimental procedure is based on the “Determining K_d with an Alpha assay” protocol from PerkinElmer. **(a) (Left):** Saturation curves of the interaction between biotinylated LRP6-E3E4 and GST-tagged DKK1c. **(Right):** Competition binding assay to determine the binding affinity K_d . **(b) (Left):** Saturation curves of the interaction between biotinylated LRP6-E1E2 and GST-tagged DKK1c. **(Right):** Competition binding assay to determine the binding affinity K_d . The error bars for each data point indicate standard deviations, whereas the “ \pm ” signs associated with K_d values indicate that K_d ranges as determined by the software GRAPHPAD PRISM.



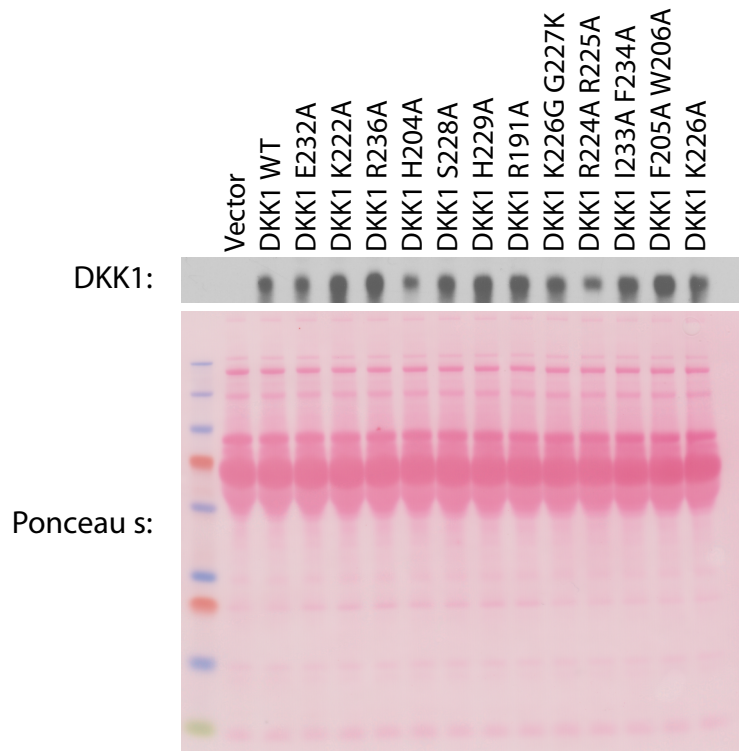
c Summary of Dynamic Light Scattering (DLS)

Protein sample	Rh (nm)	%Poly dispersity	Measured M.W. (kDa)	Predicted M.W. (kDa) (1:1)
LRP6-E3E4	3.98	19.8	82.5	70
LRP6-E3E4/DKK1c	4.14	19.2	91.02	79.5
LRP6-E1E4	8.89	38.3	581	151
LRP6-E1E4/DKK1c	12.9	65.5	1456	160.5

Supplementary Figure 4. Size Exclusion Chromatography (SEC) and Dynamic Light Scattering (DLS) analysis of LRP6-E3E4 and LRP6-E1E4 with/without DKK1c. (a) SEC of LRP6-E3E4 and its complex with DKK1c. Individually purified proteins were mixed with excess of DKK1c and incubated for 1 hour at 4°C prior to SEC. Standard molecular weight was labeled with “*” in corresponding positions. (b) SEC of LRP6-E1E4 and its complex with DKK1c. LRP6-E1E4 by itself tends to form oligomers, while DKK1c binding promotes this oligomerization to higher orders with a peak much broader than that of LRP6-E1E4 alone. (c) DLS analysis of the above samples. The derived molecular weight of LRP6-E3E4 and LRP6-E3E4/DKK1c from DLS is close to the calculated molecular weight and indicates 1:1 binding at the 1 mg/ml. The 12.5 kDa difference of LRP6-E3E4 can be accounted for by glycosylation. The deduced molecular weight of LRP6-E1E4 is much larger than its calculated molecular weight, suggesting that LRP6-E1E4 by itself tends to form oligomers. The observation of a much larger size of the LRP6-E1E4/DKK1c complex is consistent with the broadened SEC peak, suggesting DKK1c induced LRP6-E1E4 aggregation.



Supplementary Figure 5. Structural basis of pathological LRP5/6 mutations. (a) LRP6 R611C mutation. (b) LRP5 G171V mutation corresponding to LRP6 G158V.



Supplementary Figure 6. Expression of FLAG-tagged DKK1 and DKK1 mutants in conditioned media. 10 μ l of conditioned media containing the indicated FLAG-tagged wildtype (WT) or mutant DKK1 proteins was separated by SDS-PAGE and transferred to nitrocellulose. The nitrocellulose was stained with Ponceau S to show equal loading followed by western blot analysis using an anti-FLAG primary antibody. It should be noted that some of the DKK1 mutants were expressed in higher levels than WT. However all of these expressed in higher levels were found to be inactive – even with higher protein levels. Therefore all our conclusions about mutations that can abolish DKK1 activity hold true.