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

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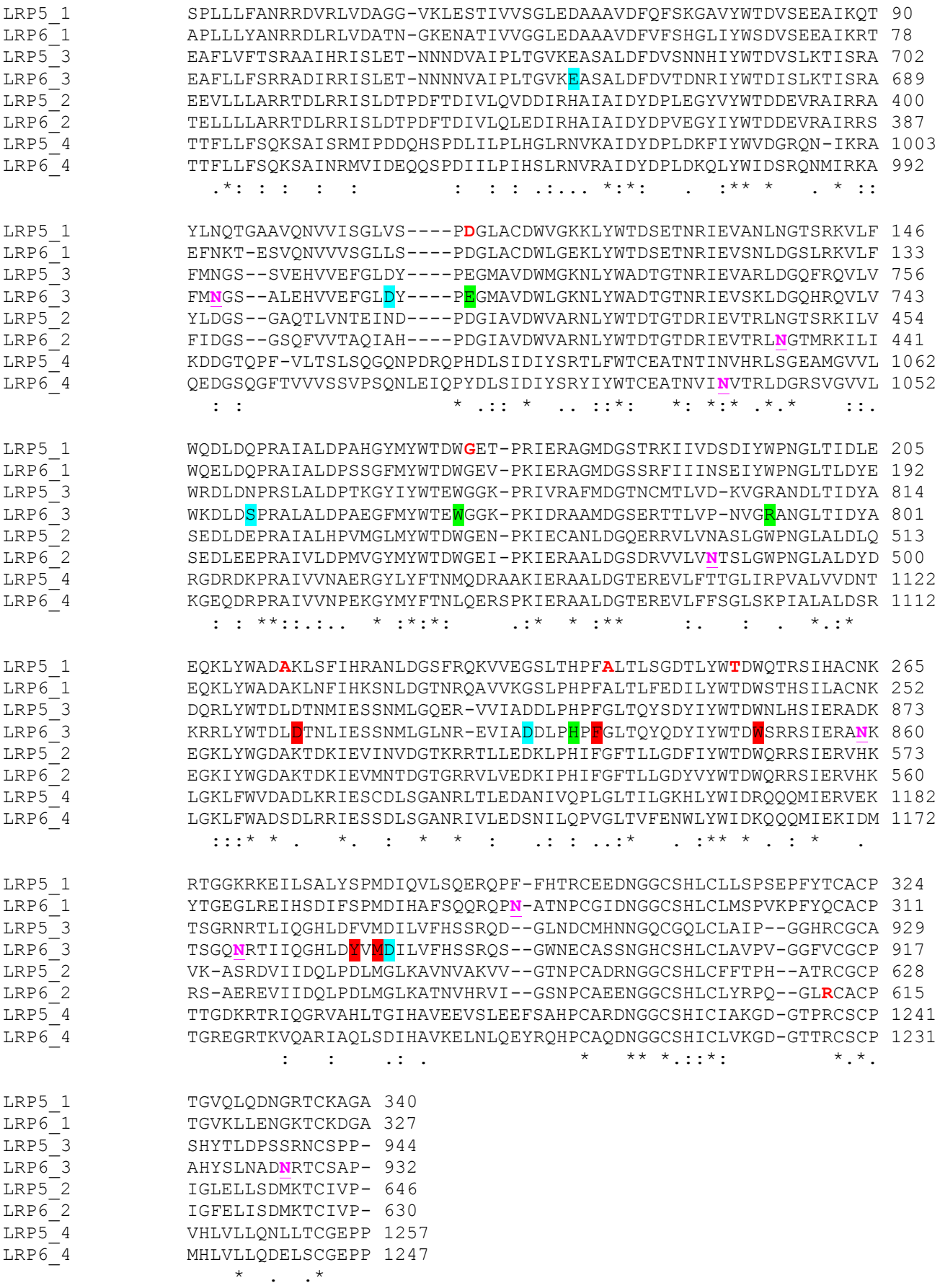
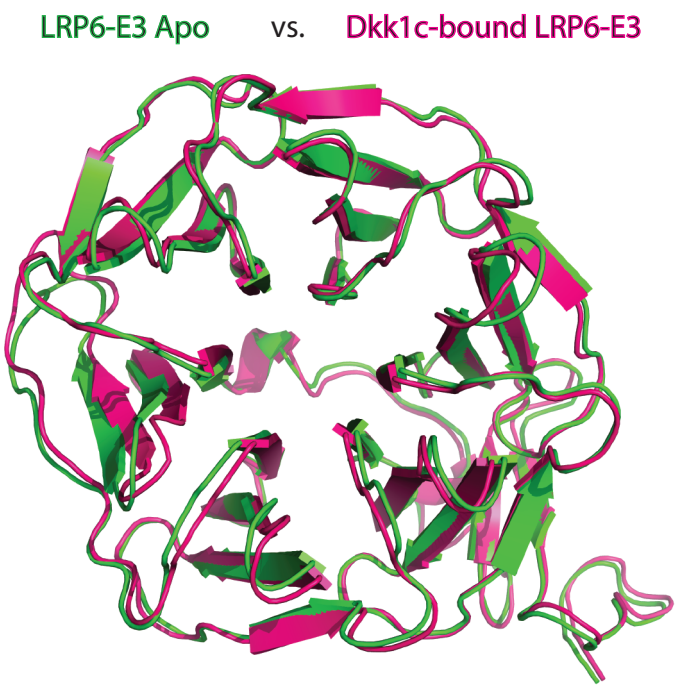
Supplementary Information

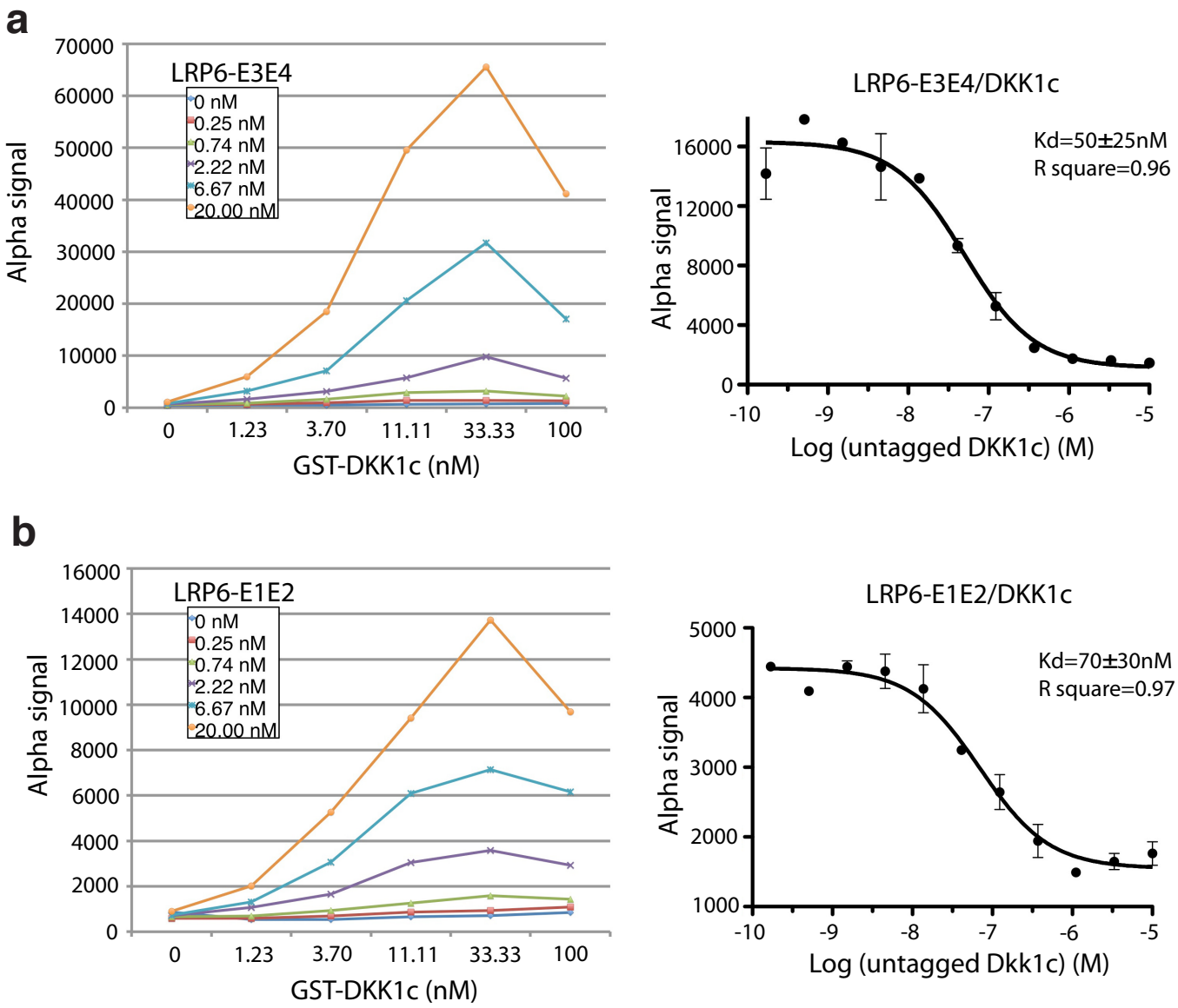
This PDF file inclues:
Supplementary Figures 1 to 6



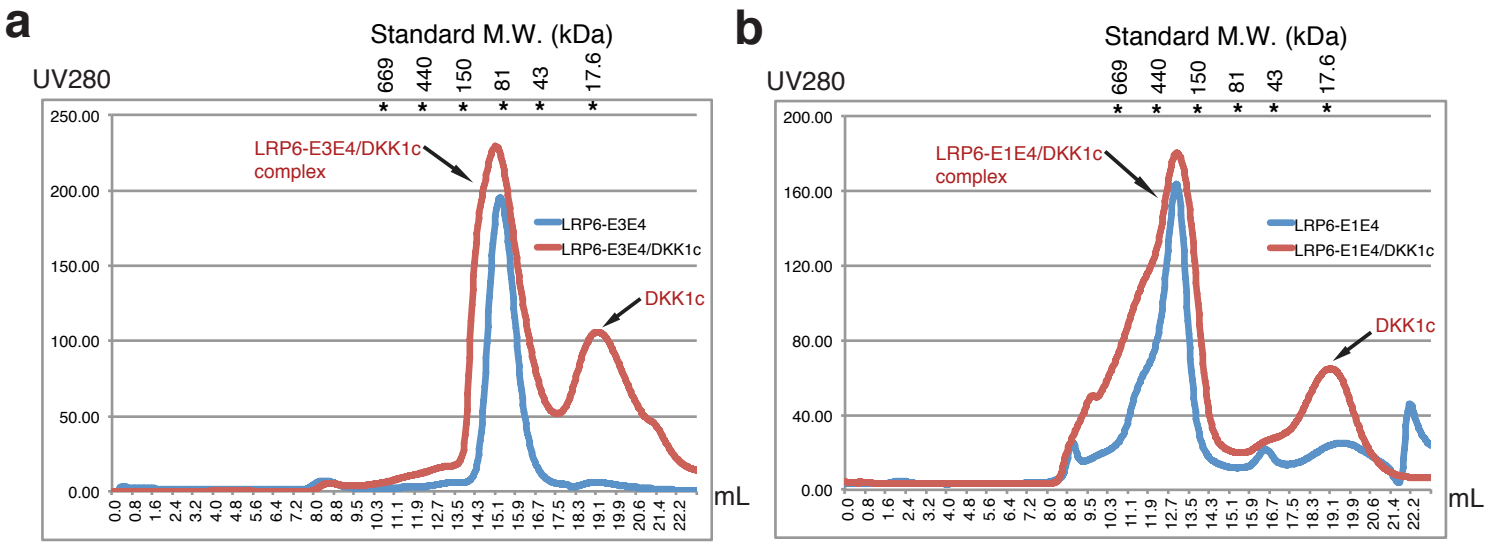
Supplementary Figure 1. Sequence alignment of LRP5/6 propellers. LRP6 residues on interfaces A and B are highlighted by green and cyan background, respectively. Residues used by both interfaces are highlighted with red background. Seven observed N-glycosylation sites (three in LRP6-E1E2 and four in E3E4)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



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 Kd 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 Kd. (b) (Left): Saturation curves of the interaction between biotinylated LRP6-E1E2 and GST-tagged DKK1c. (Right): Competition binding assay to determine the binding affinity Kd. The error bars for each data point indicate standard deviations, whereas the "±" signs associated with Kd values indicate that Kd ranges as determined by the software GRAPHPAD PRISM.

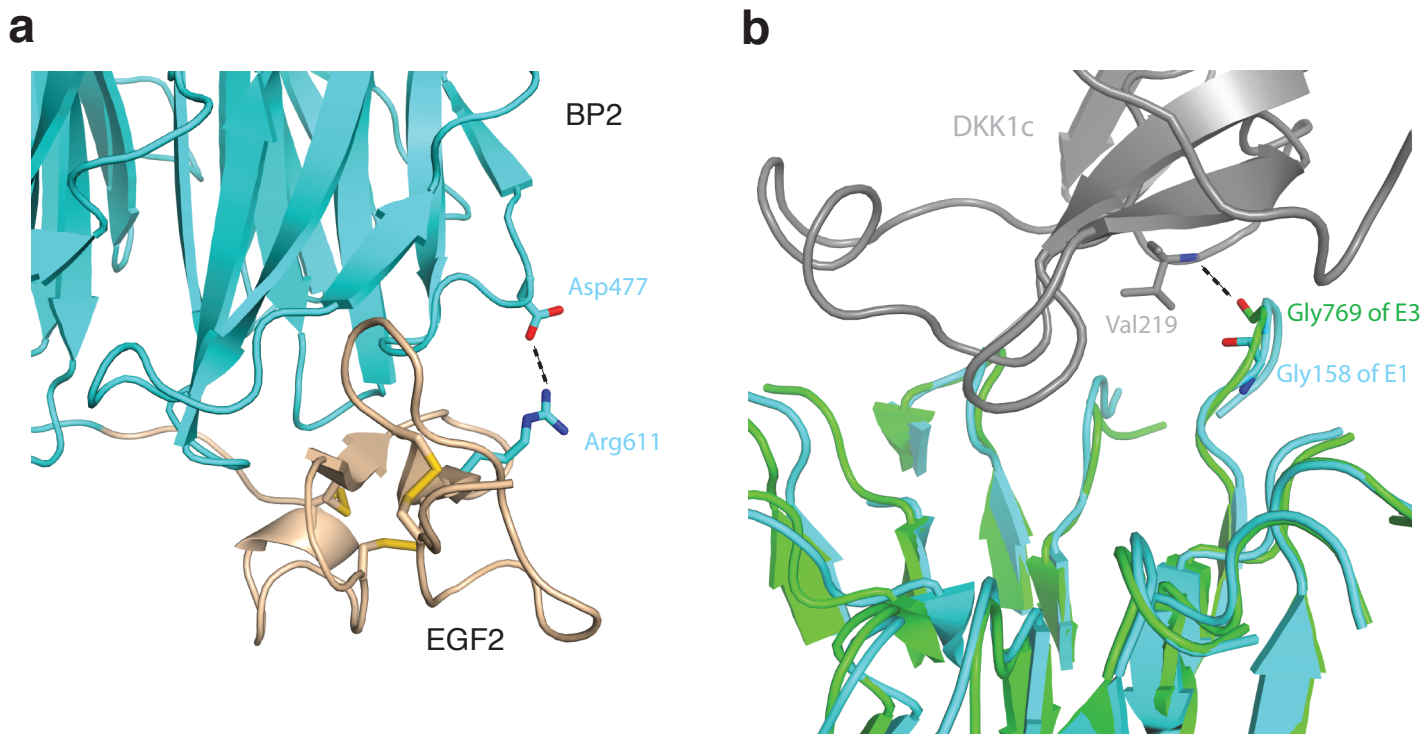


c

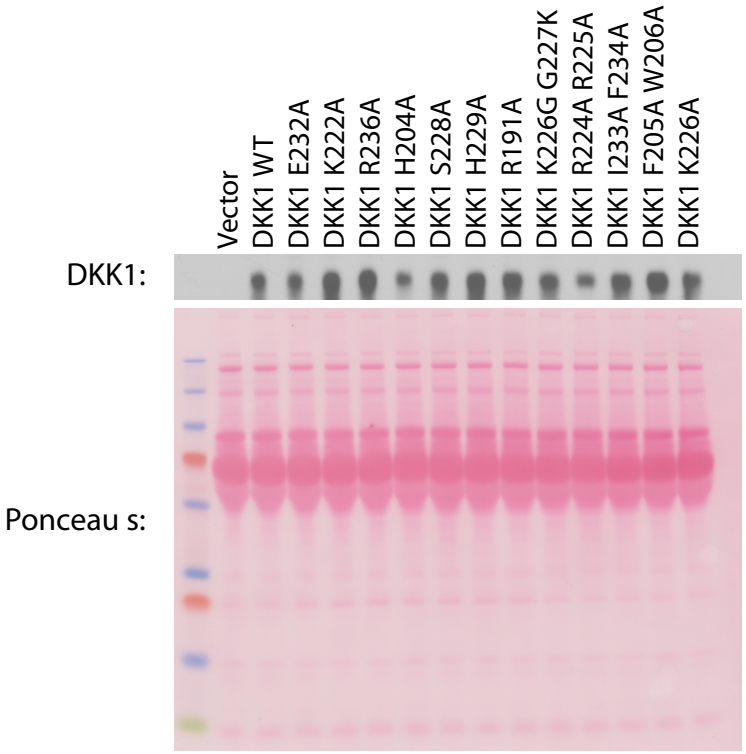
Summary of Dynamic Light Scattering (DLS)

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 µl 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.