Kim et al., http://www.jcb.org/cgi/content/full/jcb.201206096/DC1

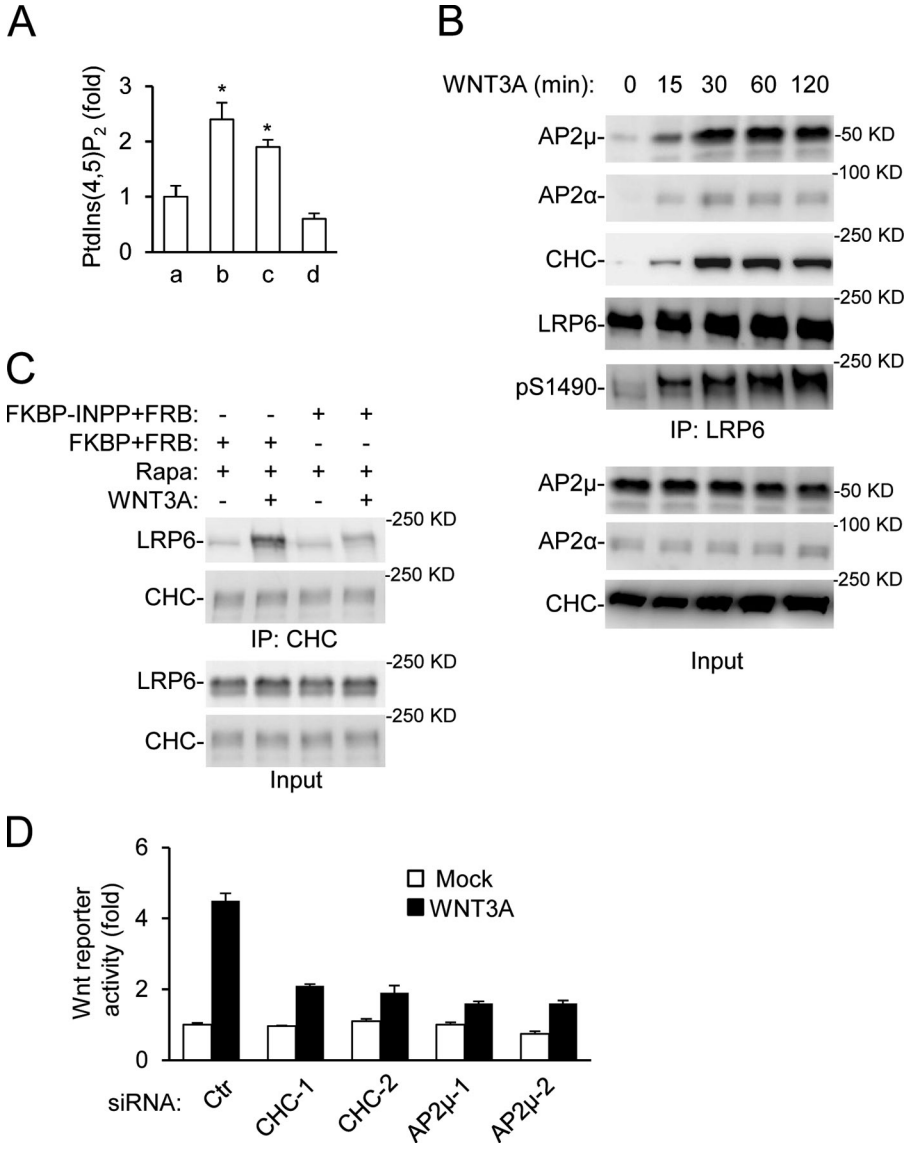

Figure S1. **PtdIns(4,5)P_2 contents and the interactions between LRP6 and CHC/AP2.** (A) PtdIns(4,5)P_2 contents in the heavy fractions (fractions 1–4) of experiments a–d in Fig. 1 C. Data are presented as means ± SD (*, $P < 0.05$ vs. a, Student's *t* test). (B) The interaction of LRP6 with CHC or AP2 is WNT3A dependent. HEK293 cells were treated with 50 ng/ml WNT3A and subjected to immunoprecipitation using a LRP6 antibody. (C) The interaction of CHC with LRP6 is PtdIns(4,5)P_2 dependent. HEK293 cells were cotransfected with FRB and FKBP or FKBP-INPP and treated with 50 ng/ml WNT3A and/or 100 nM rapamycin for 1 h. The cells were then subjected to immunoprecipitation using a CHC antibody. (D) Effect of CHC or AP2 knockdown on the Wnt reporter gene activity in HEK293 cells.

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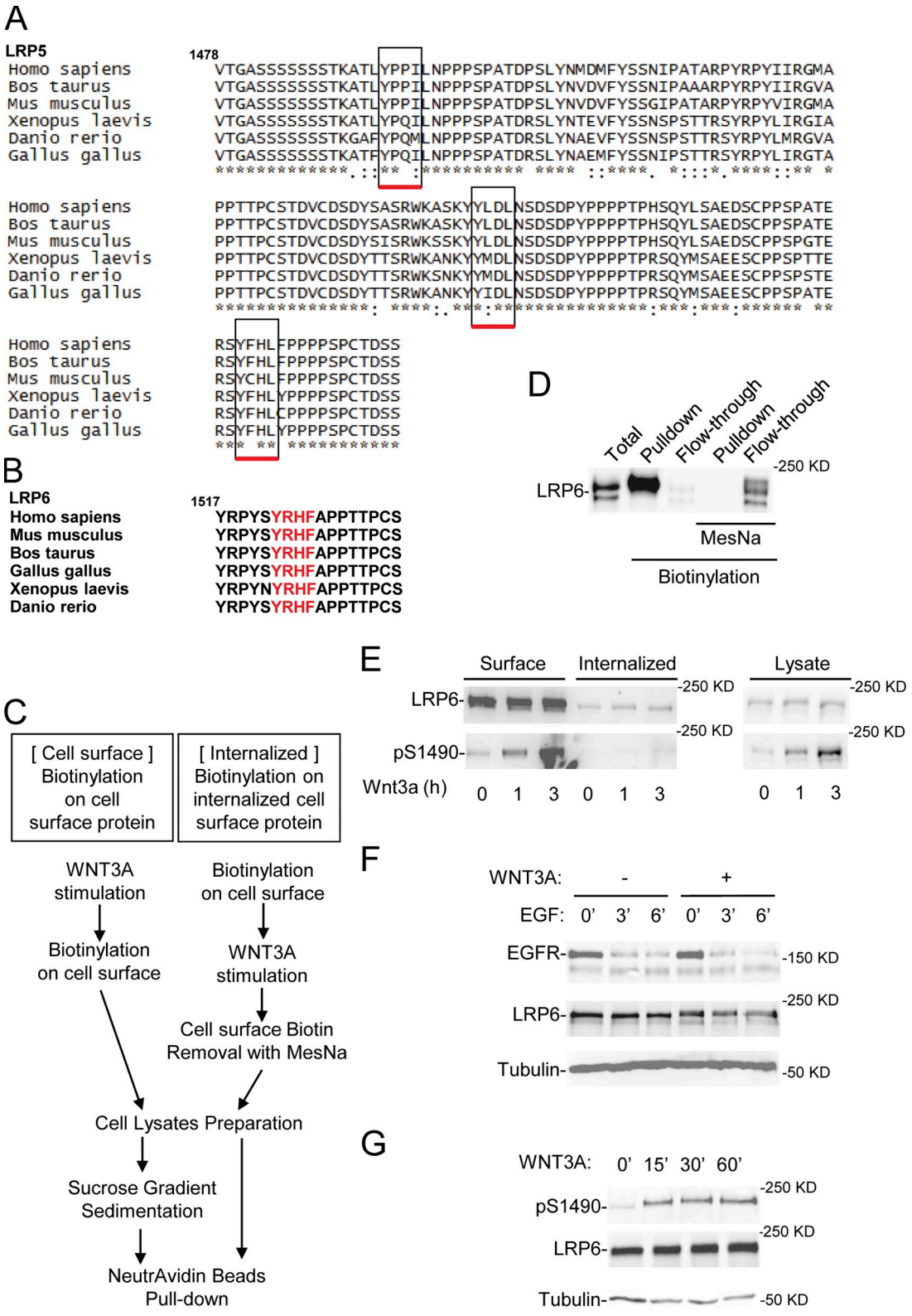

Figure S2. **LRP5/6 C-terminal amino acid sequence alignments and internalization assays.** (A and B) Alignments of the C-terminal amino acid sequences of LRP5 and 6 from different species. (C) Flow chart of detection of cell surface or internalized LRP6 using the surface protein biotinylation method. (D) MesNa efficiently removes labeled biotins. HEK293 cells were labeled with biotin and treated with or without MesNa. Cell lysates were then subjected to NeutrAvidin pull-down and Western analysis using a LRP6 antibody. (E) WNT3A does not induce significant internalization of LRP6. HEK293 cells were stimulated with 50 ng/ml Wnt for times indicated and labeled with biotins without sucrose density gradient centrifugation as described in C. After precipitation using the NeutrAvidin beads, precipitated proteins and whole-cell lysates were analyzed by Western blot. (F) EGF induces rapid internalization of its receptor. HEK293 cells were stimulated with or without 50 ng/ml WNT3A for 45 min, followed with 10 ng/ml EGF. The cells were then labeled with biotins and subjected to NeutrAvidin pull-down and Western analysis using anti-EGF receptor and anti-LRP6 antibodies. (G) WNT3A does not induce strong LRP6 internalization in MEF cells. Cells were stimulated with 50 ng/ml Wnt for times indicated and labeled with biotins, followed by NeutrAvidin pull-down and Western analysis. The tubulin levels in cell lysates are also shown.

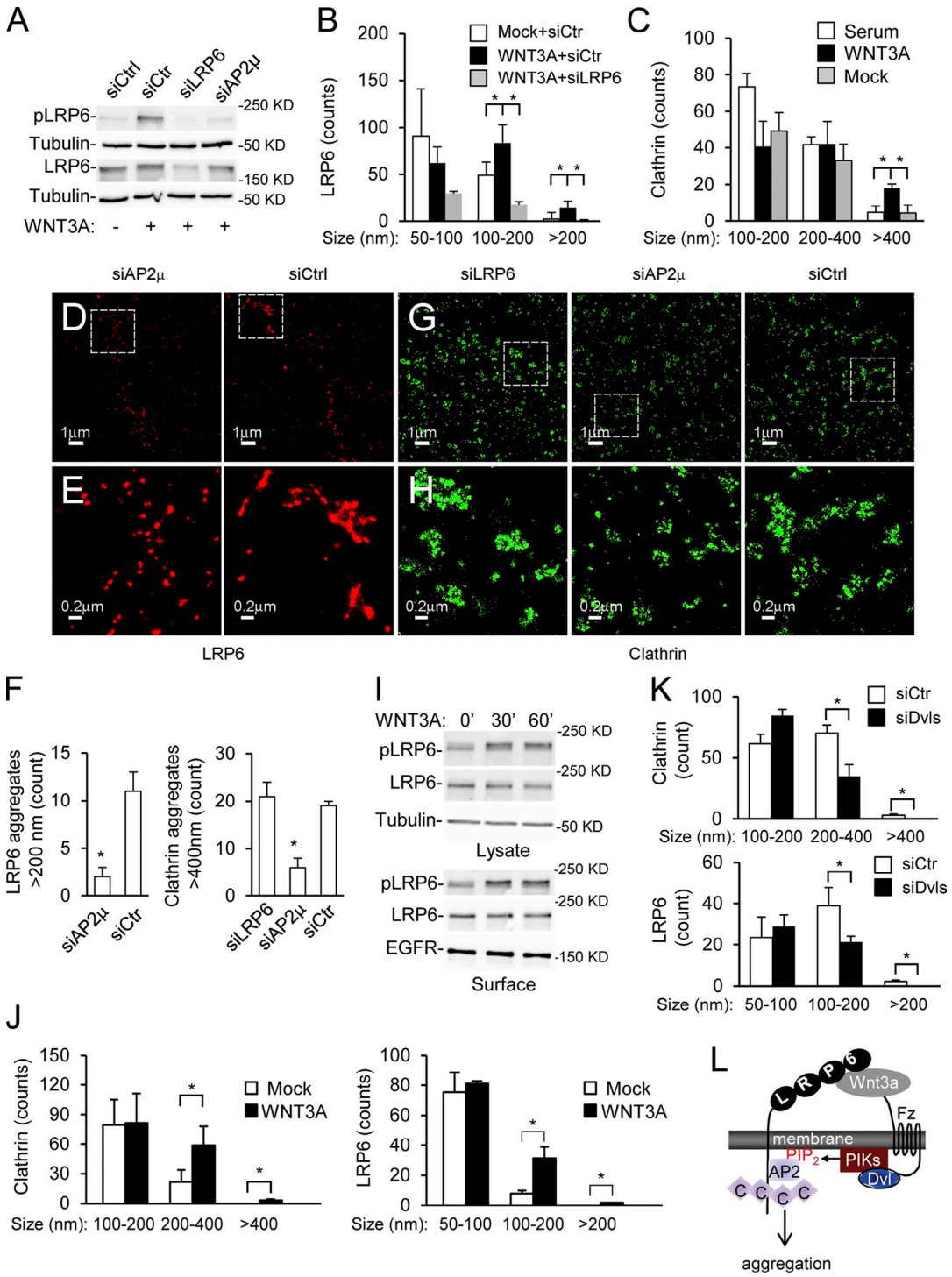

Figure S3. **STORM analysis of clathrin and LRP6 staining.** (A) Efficiency of siRNA-mediated gene silencing in MEF cells. MEFs were transfected with indicated siRNA for 72 h before Western analysis. (B and C) Quantification of LRP6 and clathrin clusters. STORM images acquired from MEFs were quantified using the DBSCAN program on three randomly selected areas per cell (mean ± SD; $n > 3$; * < 0.05, Student's *t* test). (D–H) Effect of AP2 and LRP6 knockdown on clathrin and LRP6 clustering. MEF cells were transfected with indicated siRNA for 72 h before stimulation with 50 ng/ml WNT3A for 1 h, followed by STORM. E and H are the enlargement of the boxed areas in D and G, respectively. STORM images were quantified using the DBSCAN program on three randomly selected areas per cell, and data (F) are presented as mean ± SD ($n = 3$; * < 0.05 vs. siCtr controls, Student's *t* test). (I) Effects of WNT3A on LRP6 phosphorylation and LRP6 internalization in BS-C-1 cells. The cells were stimulated with 50 ng/ml Wnt for times indicated and labeled with biotins, followed by NeutrAvidin pull-down and Western analysis. (J) Quantification of LRP6 and clathrin clusters on the top cell surfaces. The number and sizes of clusters localized at the top surfaces of BS-C-1 in 3D STORM images was quantified using the DBSCAN program (means ± SD; $n = 3$; * < 0.05, Student's *t* test). (K) Dvl knockdown reduces the number of LRP6 and clathrin clusters on the top surface of BS-C-1 cells observed by 3D STORM. BS-C-1 cells were transfected with siRNAs for DVl1-3 and 3D STORM was performed 3 d later. The number and sizes of clusters was quantified using the DBSCAN program (means ± SD; $n = 3$; * < 0.05, Student's *t* test). (L) A model for the involvement of AP2 and clathrin in the formation of LRP6 signalosomes. Wnt proteins bind to both LRP6 and Fz and stimulate the production of PtdIns(4,5)P_2 (PIP_2) via Fz, Dvl, and PtdIns kinases (PIKs). A multi-component complex, namely the LRP6 signalosome, which includes but is not limited to LRP6, AP2, and clathrin (C), is formed at least in part due to the interactions between AP2 and PtdIns(4,5)P_2 and between LRP6 and AP2.