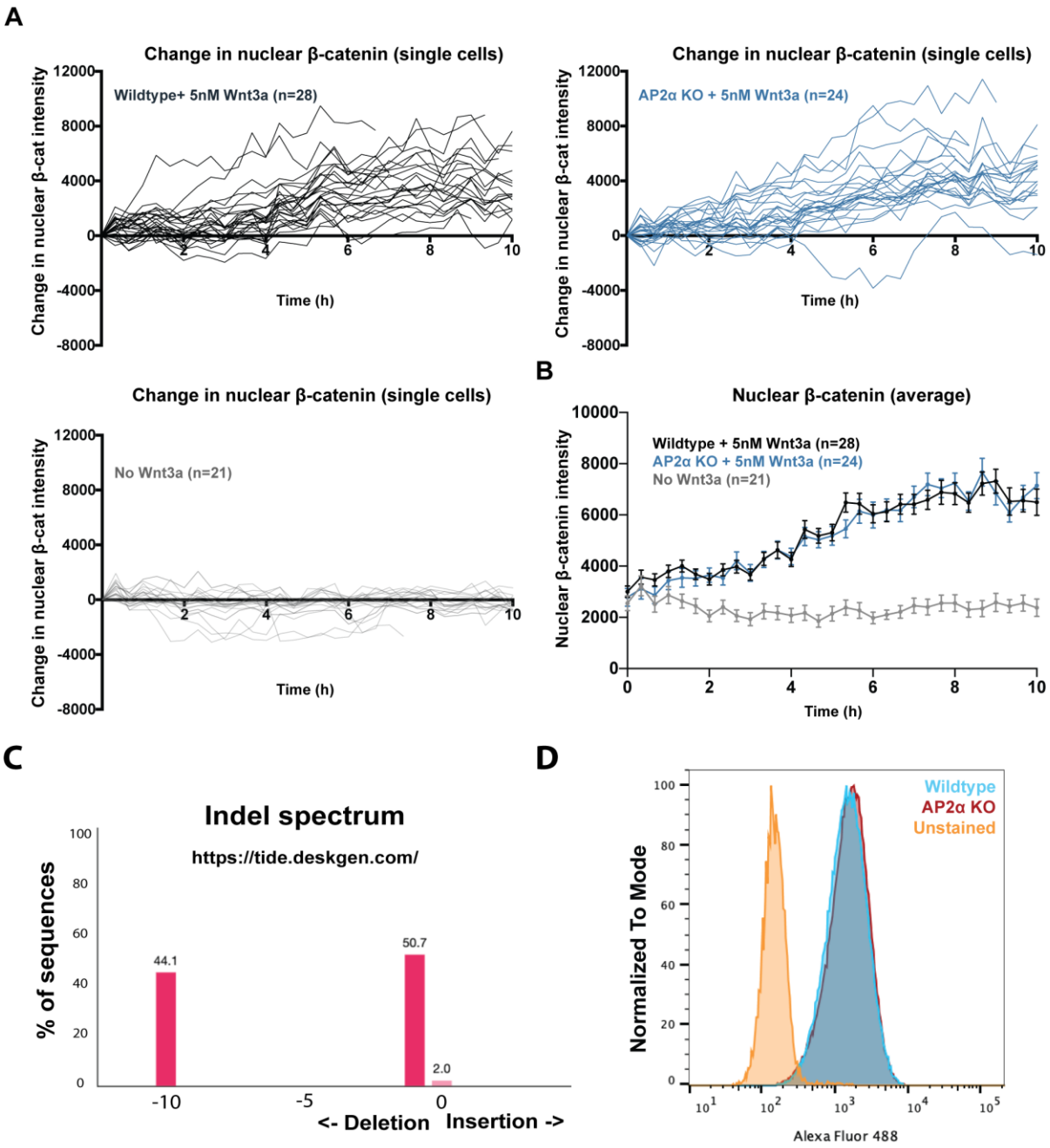
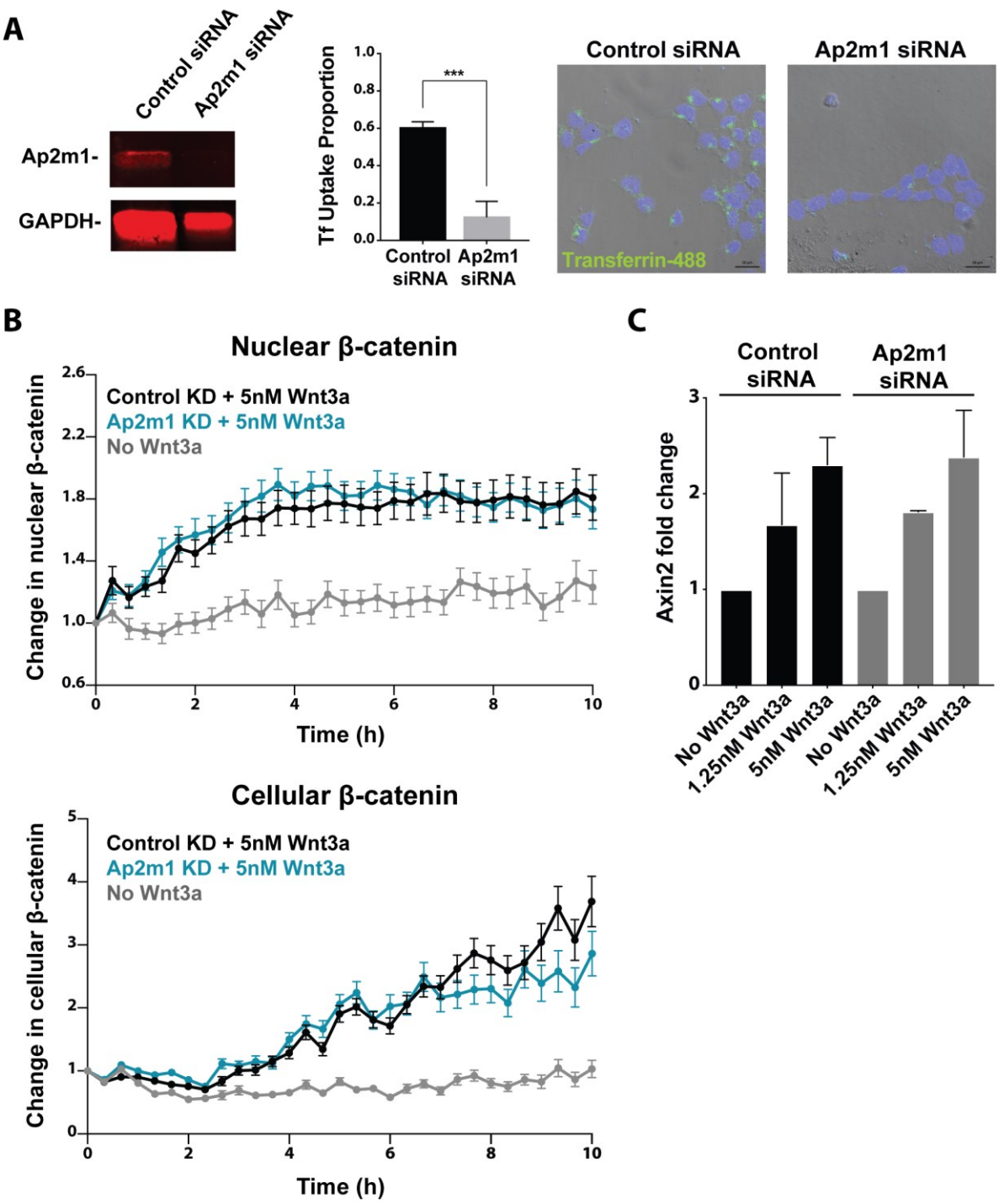
Supplemental Materials

Molecular Biology of the Cell

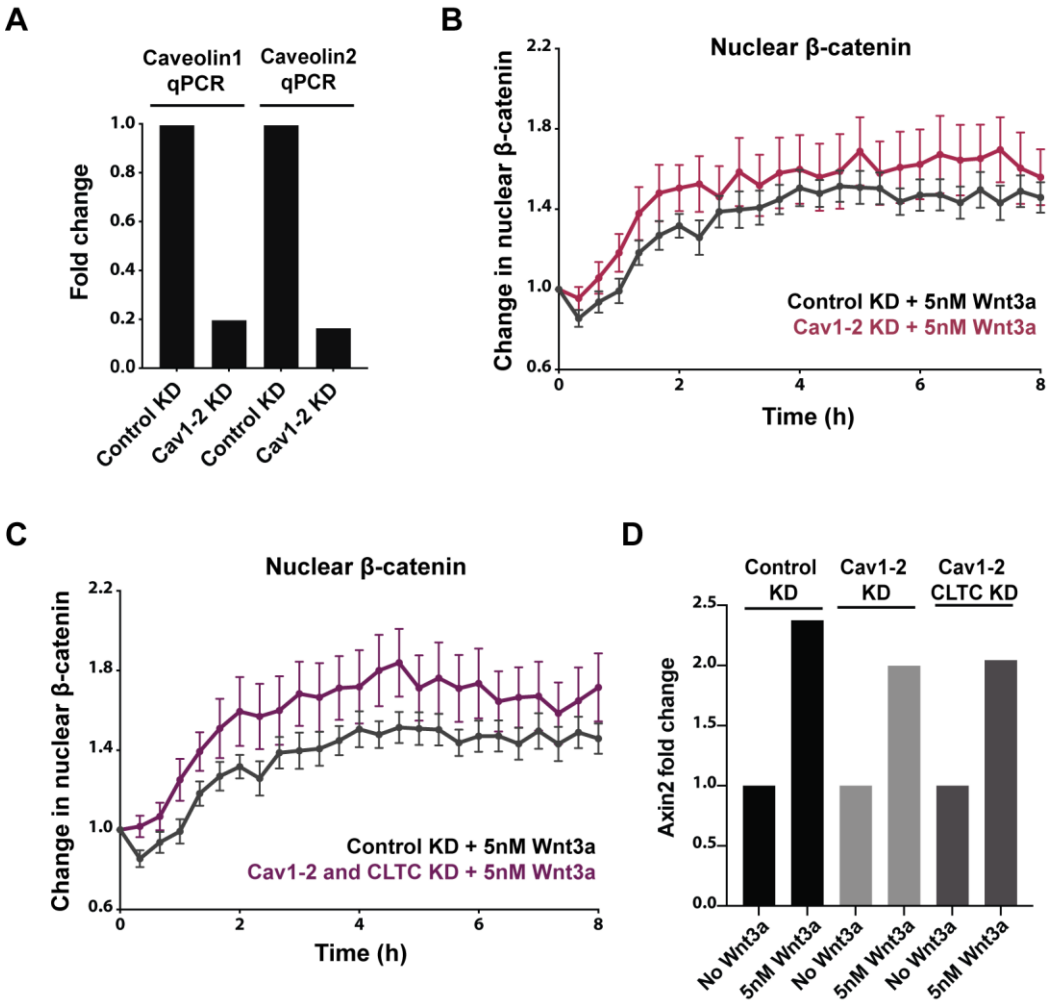
Rim et al.



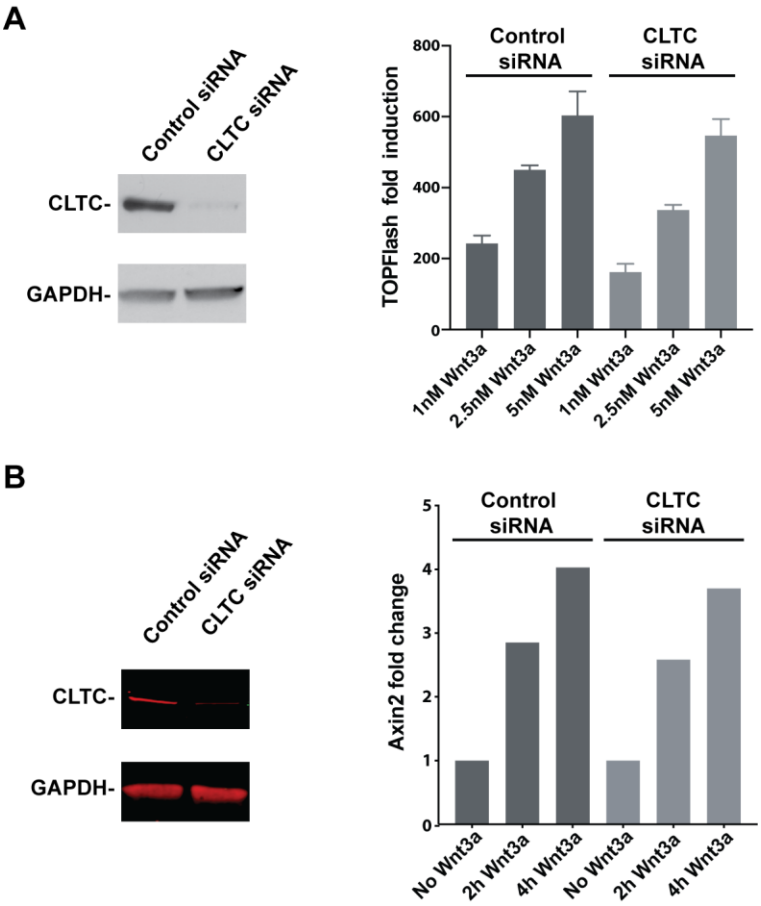
Supplemental Figure S1. Loss of AP2α does not affect absolute level changes in nuclear β-catenin and Frizzled receptor abundance on the plasma membrane. (A) Change in absolute levels of nuclear β-catenin upon stimulation with 5nM Wnt3a was quantified in wildtype and AP2α knockout mESCs. Each track represents a cell imaged over 10 hours (Wildtype, n=28; AP2α knockout, n=24; no Wnt3a, n=21). (B) Change in absolute levels of nuclear β-catenin shown in (A) was averaged. (C) Following CRISPR-mediated targeting of AP2α, a clone with frameshift deletion alleles was identified. (D) FACS analysis of mESCs stained with OMP-18R5 and detected with Alexa-488 secondary showed that loss of AP2α does not alter cell surface level of Frizzled receptors.



Supplemental Figure S2. Knockdown of AP2µ impairs Clathrin-mediated cargo uptake but does not affect Wnt signal transduction. (A) Effective siRNA-mediated knockdown of AP2µ was confirmed via Western blot. AP2µ knockdown impaired cellular uptake of fluorescently labeled Transferrin ($p < 0.001$). (B) Change in nuclear and cellular β-catenin upon stimulation with 5nM Wnt3a was quantified in mESCs subjected to control or AP2µ knockdown (Control siRNA, n=30; AP2µ siRNA, n=26; no Wnt3a, n=17). (C) Transcription of the Wnt target gene Axin2 was assayed in mESCs subjected to control or AP2µ knockdown.



Supplemental Figure S3. Double knockdown of Caveolin1 and 2 does not affect Wnt signal transduction. (A) Simultaneous knockdown of Caveolin1 and Caveolin2 was confirmed via qPCR. (B) Change in nuclear β-catenin upon stimulation with 5nM Wnt3a was quantified in mESCs subjected to control or Caveolin1-2 double knockdown (Control siRNA, n=19; Caveolin1-2 siRNA, n=14). (C) Change in nuclear β-catenin upon stimulation with 5nM Wnt3a was quantified in mESCs subjected to control or Caveolin 1-2 and CLTC triple knockdown (Control siRNA, n=19; Caveolin1-2 and CLTC siRNA, n=14). (D) Transcription of Axin2 was assayed in mESCs subjected to control, Caveolin 1-2 double, or Caveolin 1-2 and CLTC triple knockdown.



Supplemental Figure S4. Knockdown of Clathrin heavy chain does not affect Wnt signal transduction in additional cell lines. (A) Effective knockdown of CLTC in mouse L cells was confirmed via Western blot. TOPFlash luciferase activity upon stimulation with 1nM-5nM Wnt3a was measured in L cells subjected to control or CLTC knockdown. (B) Effective knockdown of CLTC in HEK293T cells was confirmed via Western blot. Transcription of Axin2 upon stimulation with 5nM Wnt3a was assayed in HEK293T cells subjected to control or CLTC knockdown.