

APC controls Wnt-induced β -catenin destruction complex recruitment in human colonocytes

Taybor W. Parker and Kristi L. Neufeld¹

Department of Molecular Biosciences, University of Kansas, Lawrence, KS

¹Corresponding author: Kristi L. Neufeld, 7049 Haworth Hall, 1200 Sunnyside Ave., University of Kansas, Lawrence, KS 66045; phone (785)864-5079; email: klneuf@ku.edu

Author Information:

Taybor W. Parker, parkertw@ku.edu, ORCID: 0000-0002-3829-7554

Kristi L. Neufeld, klneuf@ku.edu, ORCID: 0000-0003-3653-9385

Supplemental Figure Legends

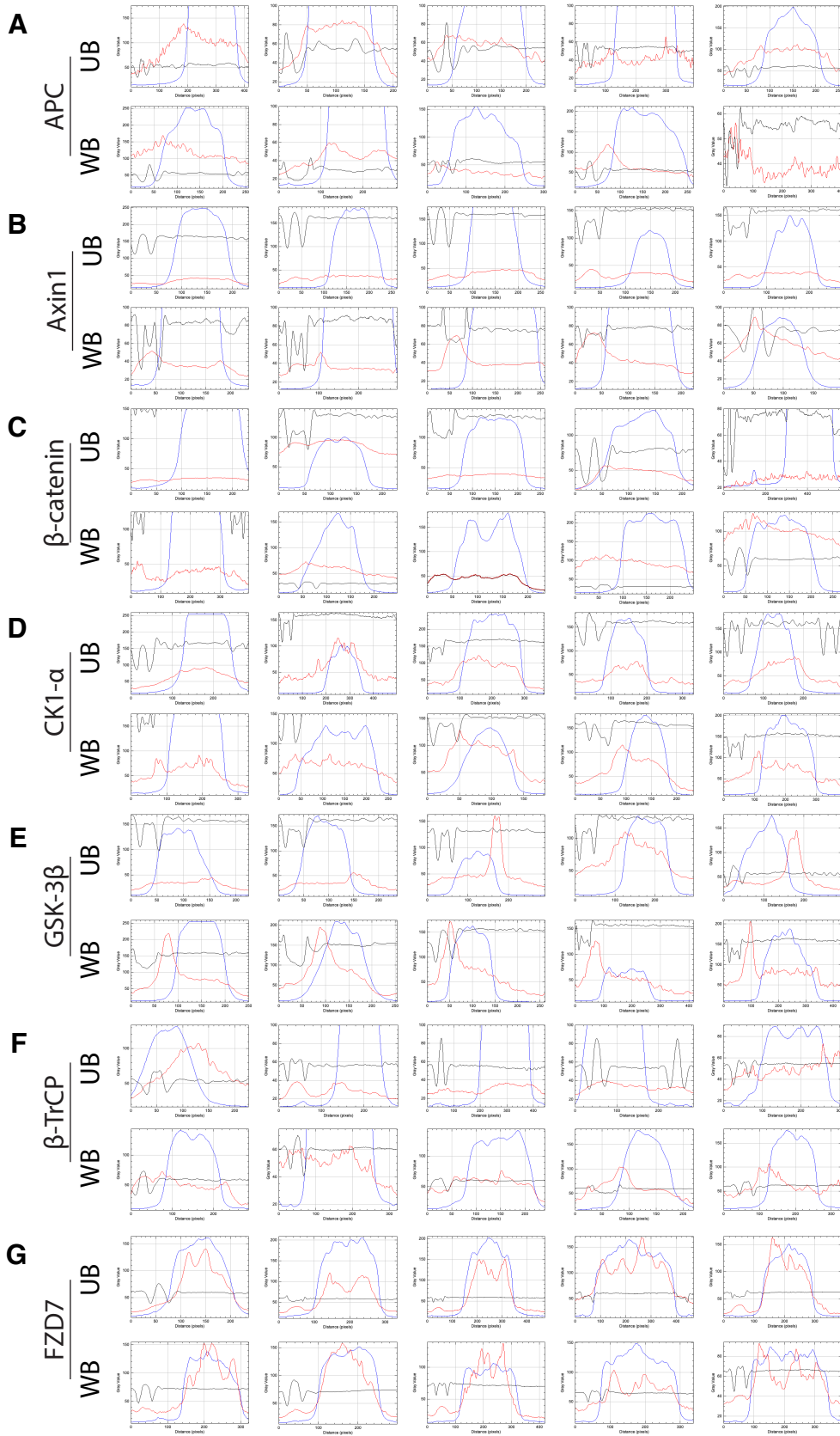
Supplemental Figure 1. Line scan analysis of signalosome and destruction complex components in RKO cells. Line scans were performed on RKO cells containing an intact Wnt signaling pathway using ImageJ/FIJI (NIH). Lines were drawn beginning at the Wnt- or Unloaded-bead and run across the cell nucleus. Line colors correspond to bright-field (black), DAPI (blue), and protein (red). If brightfield image was unavailable, the black line corresponds to DAPI. Line scans correspond to A) APC, B) Axin1, C) β -catenin, D) CK1- α , E) GSK-3 β , F) β -TrCP, and G) FZD7. Line scans were performed on five cells per condition.

Supplemental Figure 2. Line scan analysis of signalosome and destruction complex in HCT116 β m cells. Line scans were performed on HCT116 β m cells containing stabilized β -catenin through Ser45 deletion. Lines were drawn beginning at the Wnt- or Unloaded-bead and run across the cell nucleus. Line colors correspond to bright-field (black), DAPI (blue), and protein (red). If brightfield image was unavailable, the black line corresponds to DAPI. Line scans correspond to A) APC, B) Axin1, C) β -catenin, D) CK1- α , E) GSK-3 β , F) β -TrCP, and G) FZD7. Line scans were performed on five cells per condition.

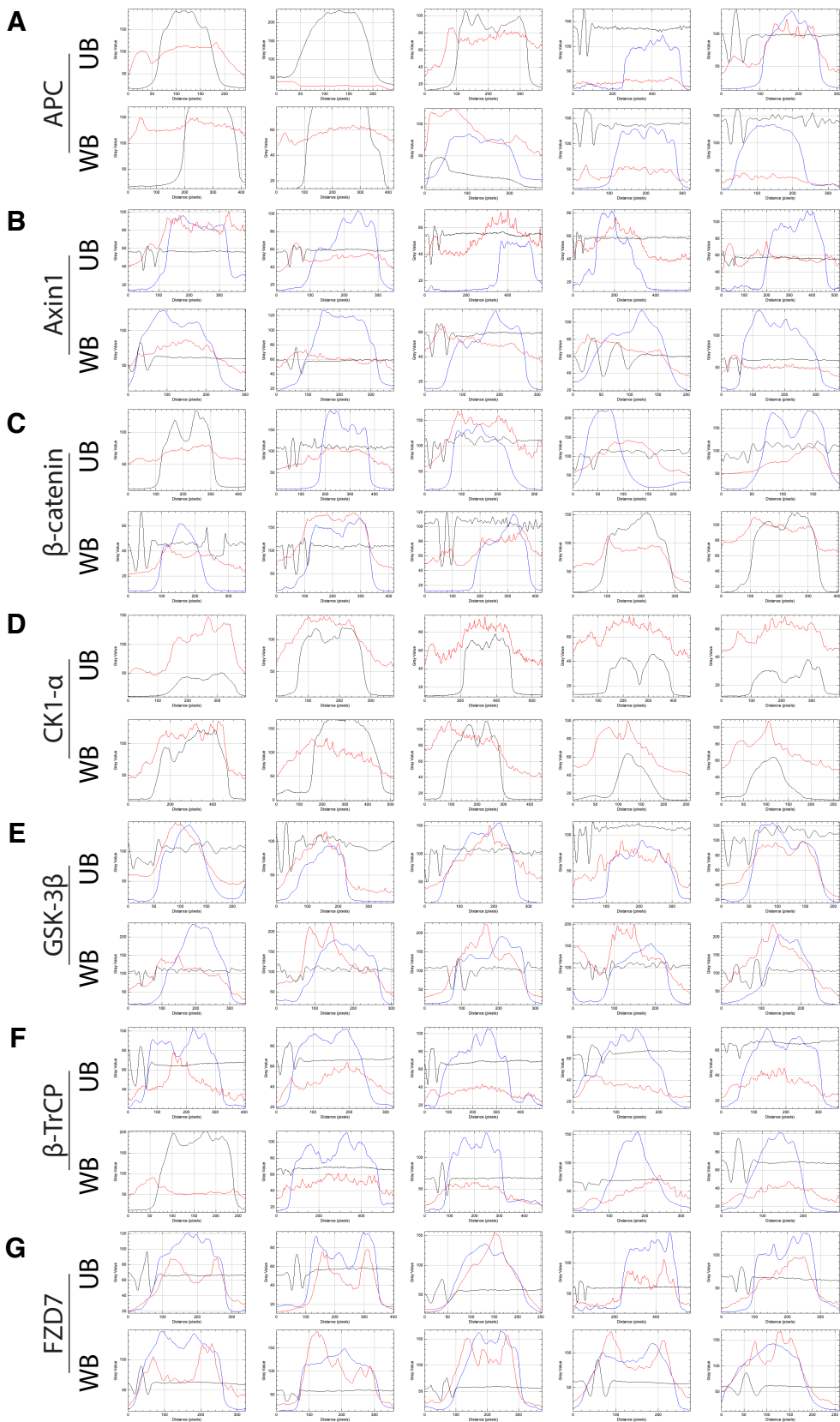
Supplemental Figure 3. Line scan analysis of signalosome and destruction complex in DLD1 cells. Line scans were performed on DLD1 cells which harbor truncated APC. Lines were drawn beginning at the Wnt- or Unloaded-bead and run across the cell nucleus. Line colors correspond to bright-field (black), DAPI (blue), and protein (red). If brightfield image was unavailable, the black line corresponds to DAPI. Line scans correspond to A) APC, B) Axin1, C) β -catenin, D) CK1- α , E) GSK-3 β , F) β -TrCP, and G) FZD7. Line scans were performed on five cells per condition.

Supplemental Figure 4. HCEC 1CT images from Figure 7. Immunofluorescent microscopy images of HCEC 1CT cells following APC, Axin1, or control siRNA treatment and Wnt-bead treatment. Images shown are individual channels of the images used in Figure 7. Immunofluorescent images of (A) APC and β -catenin, (B) Axin1, and (C) GSK-3 β following siCtl and siAPC treatment. (D) Immunofluorescent images of APC and β -catenin following siAxin1 treatment.

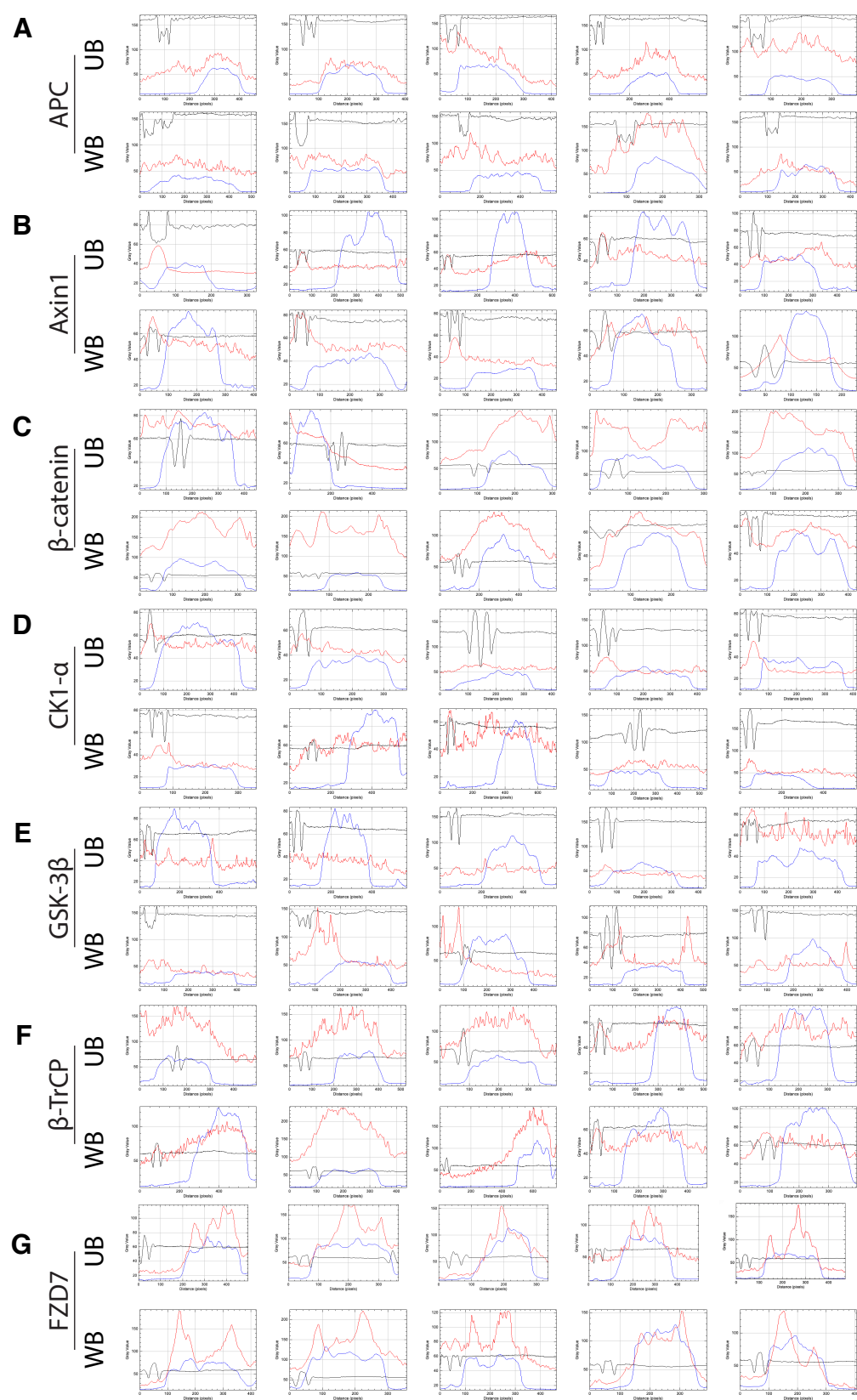
Supplemental Figure 5. **Full-length western blots.** (A) Wnt-bead “pull down” assay demonstrated in Figure 3. (B) APC knock-down shown in Figure 7. (C) Axin1 knock-down shown in Figure 7. Each individual set of images are taken from the same gel/membrane.



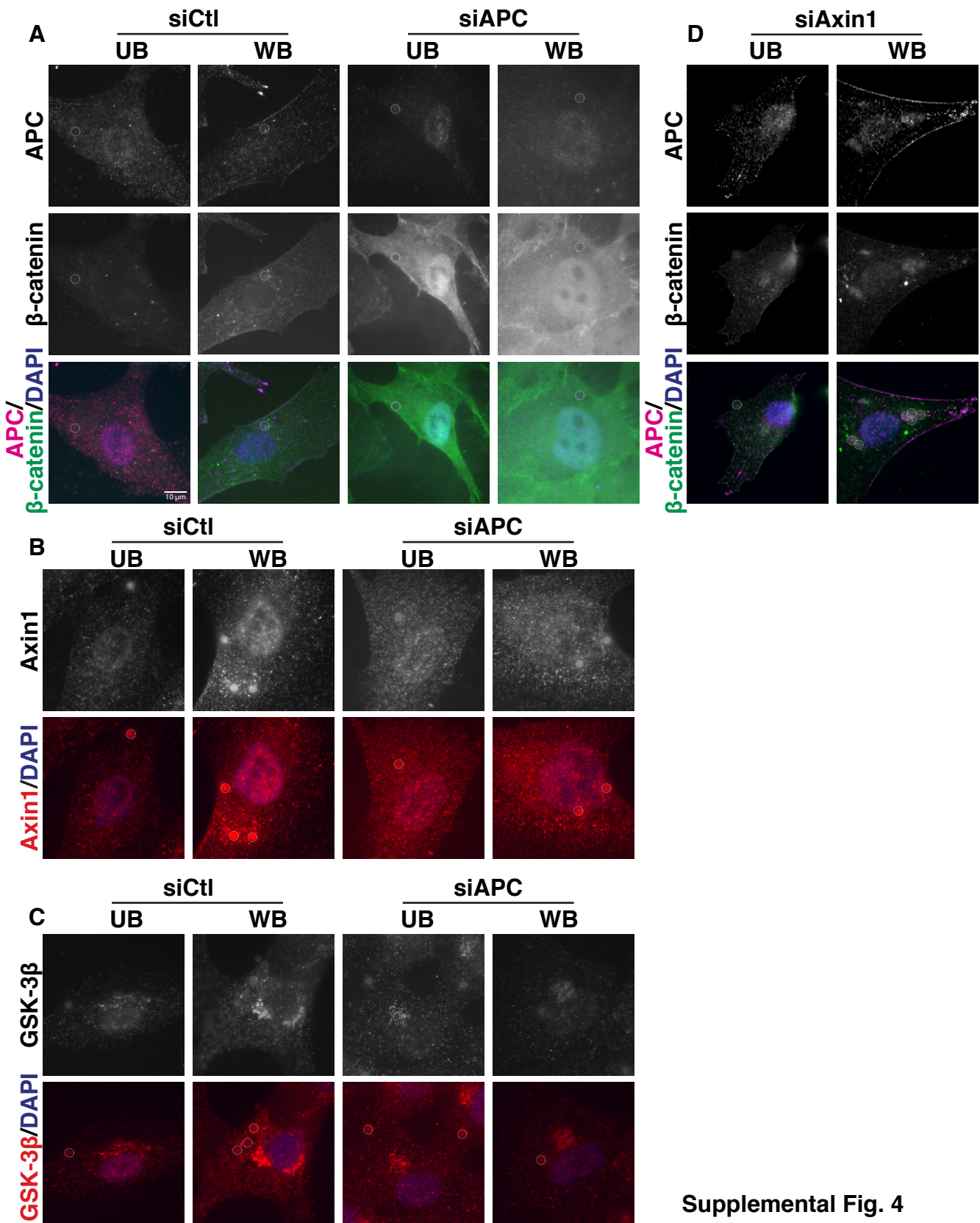
Supplemental Fig. 1

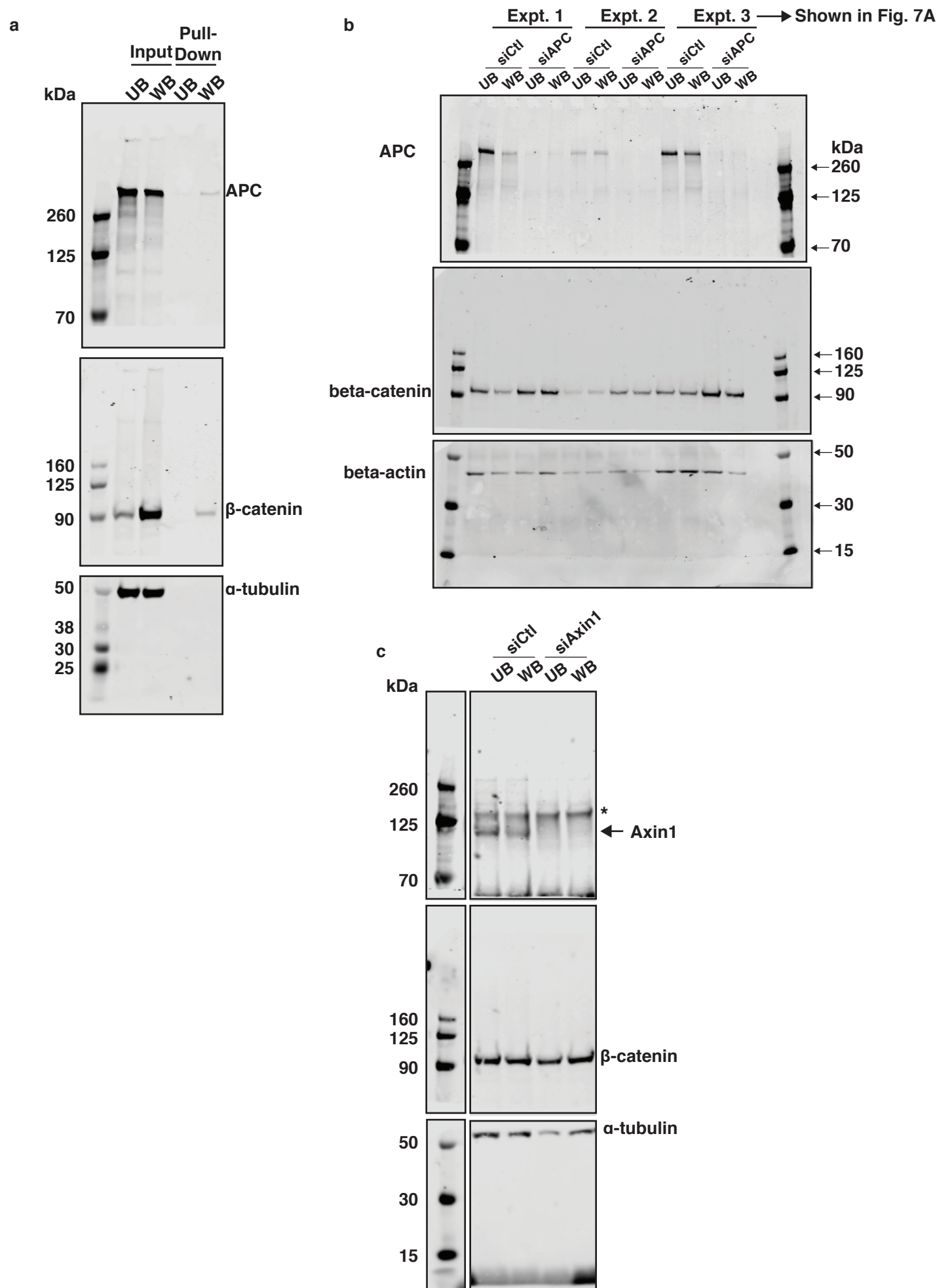


Supplemental Fig. 2



Supplemental Fig. 3





Supplemental Fig. 5