Supplemental Figure 1: Microtubule plus-ends are oriented towards sites of APC

(A) Subconfluent HUVECs fixed and stained for APC (red), α-tubulin (green), and DAPI (blue). Boxed region corresponds to enlarged image showing APC cluster within the monolayer. (B and C) Confluent HUVECs fixed and stained for APC (red), EB1 (green), and DAPI or α-tubulin (blue). Boxed regions of clusters [C] or lateral membrane [L] correspond to enlarged images. In (C) only enlarged image with clusters [C] is shown. Scale bar, 20μm.

Supplemental Figure 2: APC at lateral membranes, but not in clusters, co-localizes with VE-cadherin and requires cell-cell adhesion for localization

(A-C) Subconfluent (A) or confluent (B and C) HUVECs fixed and stained for APC (red), VE-cadherin (green), and DAPI (blue). Boxed regions of clusters [C] or lateral membrane [L] correspond to enlarged images. Scale bar, 20μm. (C) HUVEC monolayer was treated with 2mM EGTA for 1 hr before fixation. No lateral APC or VE-cadherin localization is detected. APC clusters are indicated with [C].

Supplemental Figure 3: APC is not targeted to the lateral membrane by ZO-1

(A) Confluent HUVECs stained for APC (red), ZO-1 (green), and DAPI (blue). Boxed regions of lateral membrane [L] correspond to enlarged images. Scale bar, 20μm. (B) APC immunoprecipitation from 0.5% NP40 extracts of HUVECs blotted with antibodies against APC and ZO-1; representative image from 2 independent experiments. (C) β-Catenin siRNA treated HUVECs fixed and stained for β-catenin (red), ZO-1 (green), and

DAPI (blue). Enlarged images of lateral membrane between two β-catenin depleted cells [L1] and two cells that were not depleted [L2]. Scale bar, 20μm.

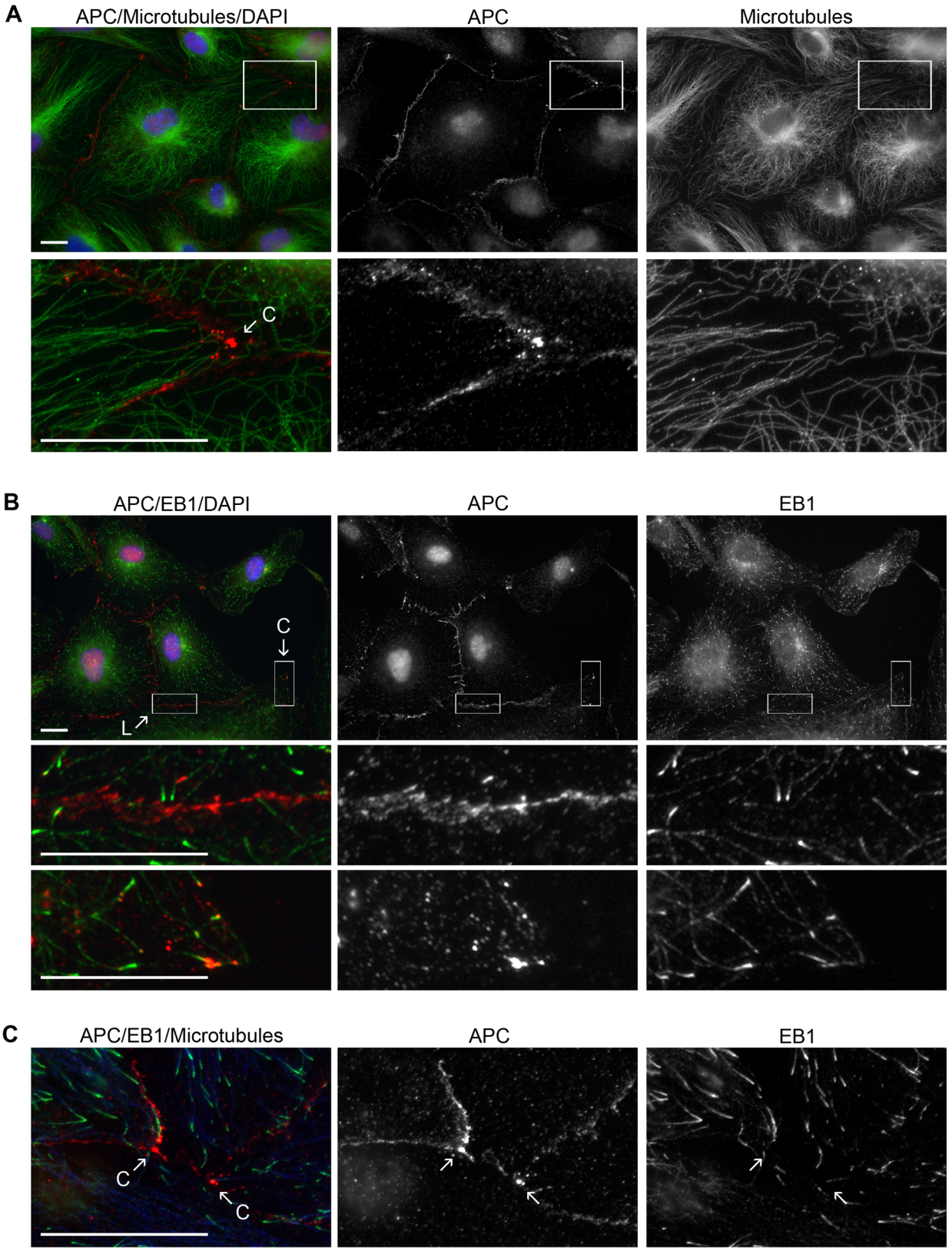
Supplemental Figure 4: Pharmacological inhibition of GSK3β/CKI does not significantly affect cell-cell barrier functions

(A) Paracellular diffusion of molecular tracer. HUVECs were plated to confluency on 0.4 μm-pore polycarbonate filters. After 24 hrs HUVECs were treated with either 20μM GSK3β inhibitor for 1 hr, 50μM CKI inhibitor for 4 hrs, or 20μM GSK3β+50μM CKI inhibitors for 4 hrs. 50μg/ml Alexa-647 Dextran 10,000 MW was to the apical compartment. After 1 hr at 37°C, the amount of A647-Dextran in the basal compartment was determined. Control filter with no HUVECs shows maximum amount of A647-Dextran that diffuses from apical to basal compartment in 1 hr. Mean values ± SEM from 3 experiments. ***; P = 0.0005 by Student's *t*-test. (B) Neutrophil transmigration assay. HUVECs were plated to confluency on 5μm-pore polycarbonate filters. After 3 days HUVECs were treated with either 20μM GSK3β inhibitor for 1 hr, 50μM CKI inhibitor for 4 hrs, or 20μM GSK3β+50μM CKI inhibitors for 4 hrs. To start migration, HUVEC media was removed and 1X10^6 differentiated HL-60 cells were added to apical chamber. HL-60 media containing 20nM fMLP was added to lower chamber. After 1 hr at 37°C, number of HL-60 cells in the basal compartment was determined using hemocytometer. Mean values ± SEM from 2 experiments.

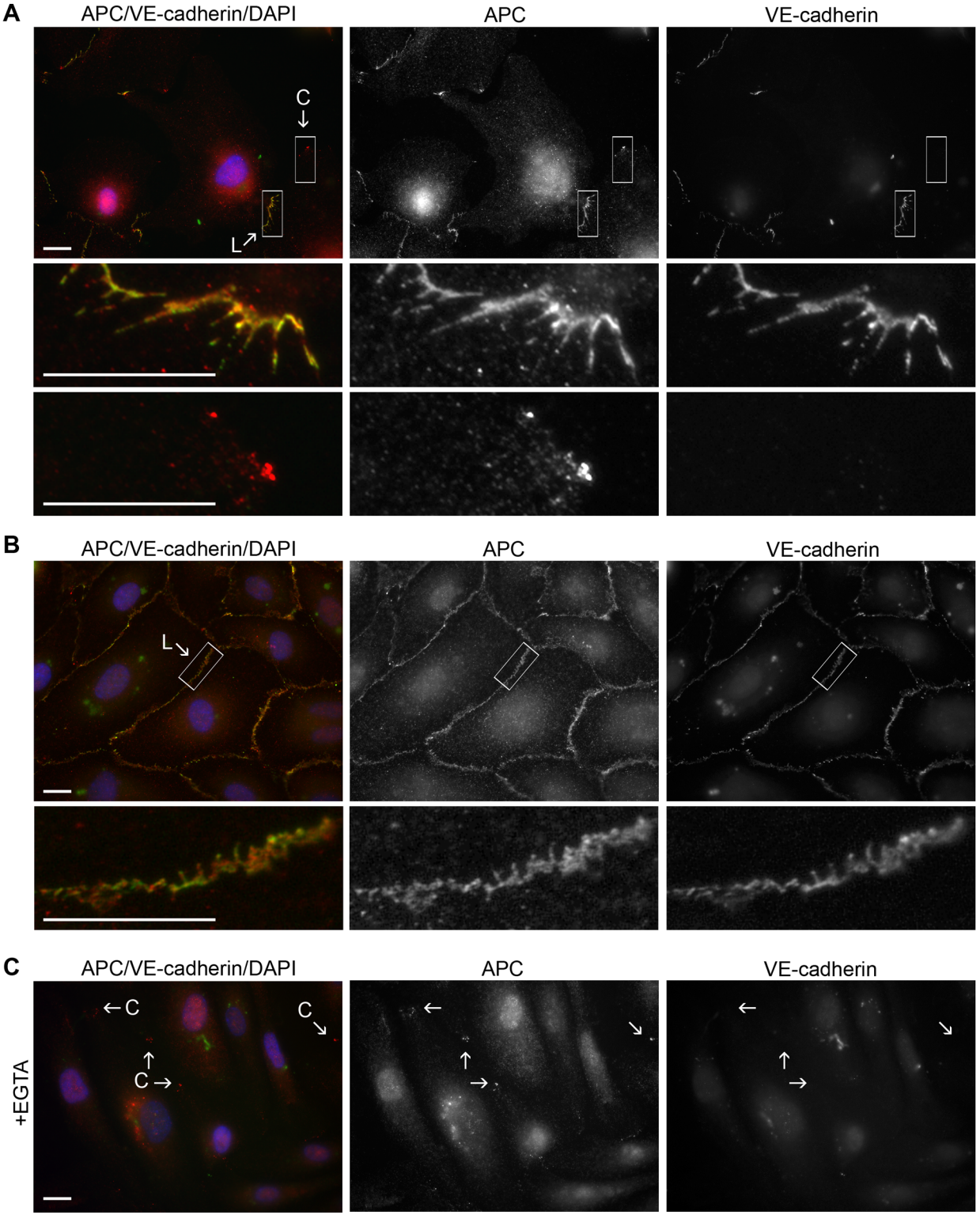
Supplemental Figure 5: APC clusters in MDCK cells are also phosphorylated by GSK3β/CKI

(A) Subconfluent MDCK cells stained for APC (red), α-tubulin (green) and E-cadherin (blue). Scale bar, 20μm. (B) APC immunoprecipitation from 0.5% NP40 extracts of MDCK cell blotted for APC, E-cadherin, α-catenin, and β-catenin. (C) MDCK cells were treated with either 20μM GSK3β+50μM CKI inhibitors for 4 hrs, 10μM MG132 for 4 hrs, or 5mM EGTA for 1 hr before preparation of SDS-whole cell lysates; blotted for APC, E-cadherin, β-catenin, GSK3β-phosphorylated β-catenin, and GAPDH. (D) Reporter luciferase assay using extracts from MDCK cells transfected with either TOPFLASH Tcf/Lef-driven luciferase reporter (gray bars) or FOPFLASH (dashed bars). These cells were either treated with indicated chemical reagents or co-transfected with GFP-ΔGSK-βcat or GFP empty vector, as described for HUVECs (see Figure 4). Mean values ± SEM from 2 independent experiments performed in triplicate. ***; $P < 0.0001$ by Student's *t*-test. (E and F) Subconfluent MDCK cells treated with 20μM GSK3β+50μM CKI inhibitors for 4 hrs, fixed, and stained for APC (red), α-tubulin (green) and DAPI (blue). Scale bar, 20μm. (F) Quantification of % MDCK cells with APC clusters. Mean values ± SEM from 3 experiments. **; $P = 0.002$ by Student's *t*-test.

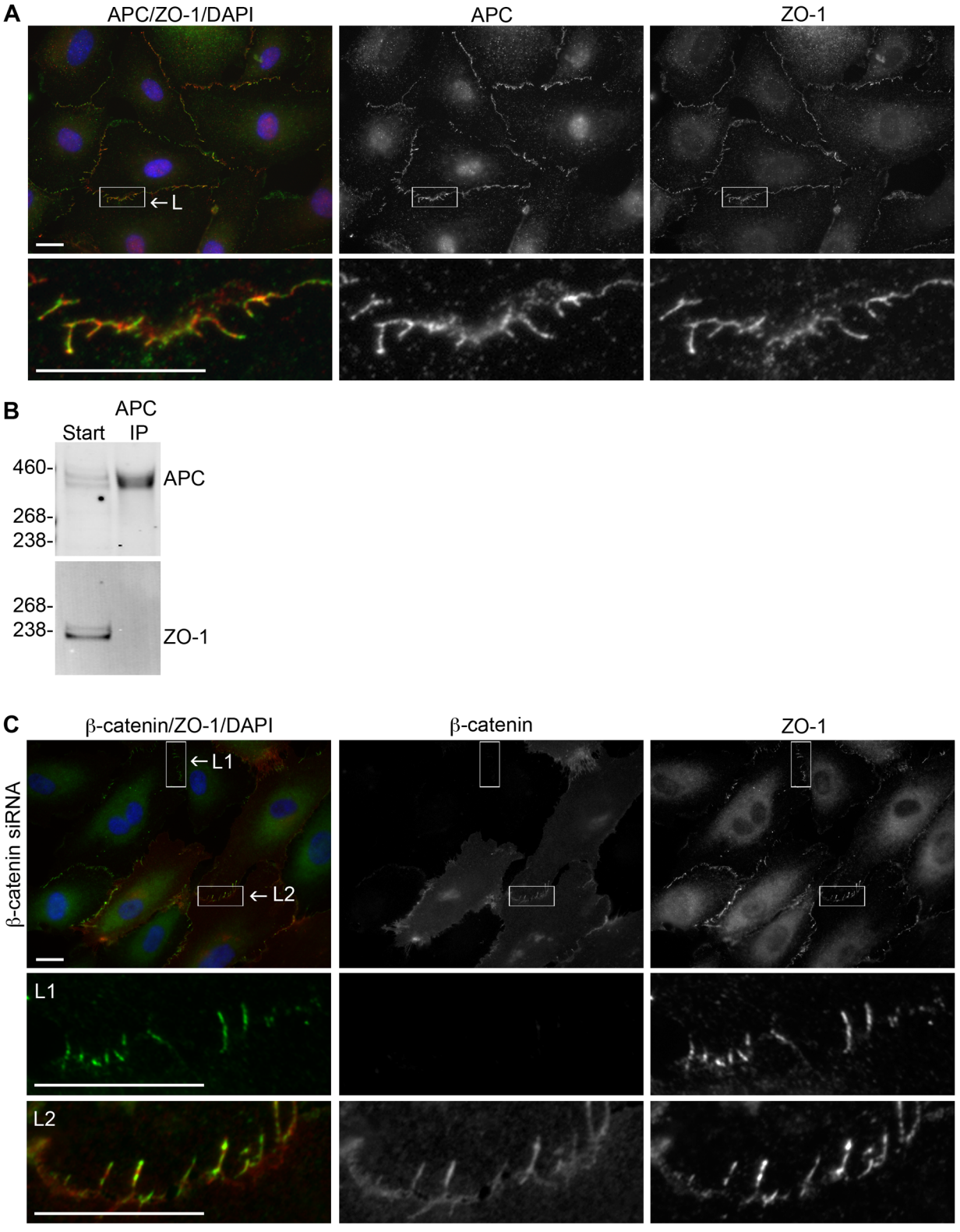
Harris_Supplemental Figure 1



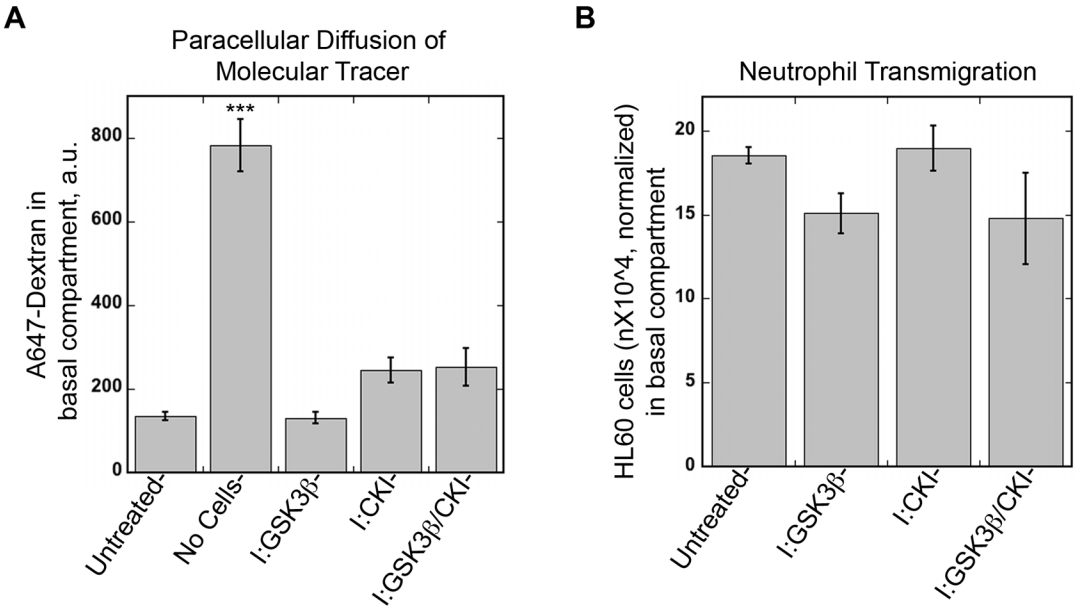
Harris_Supplemental Figure 2



Harris_Supplemental Figure 3



Harris_Supplemental Figure 4



Harris_Supplemental Figure 5