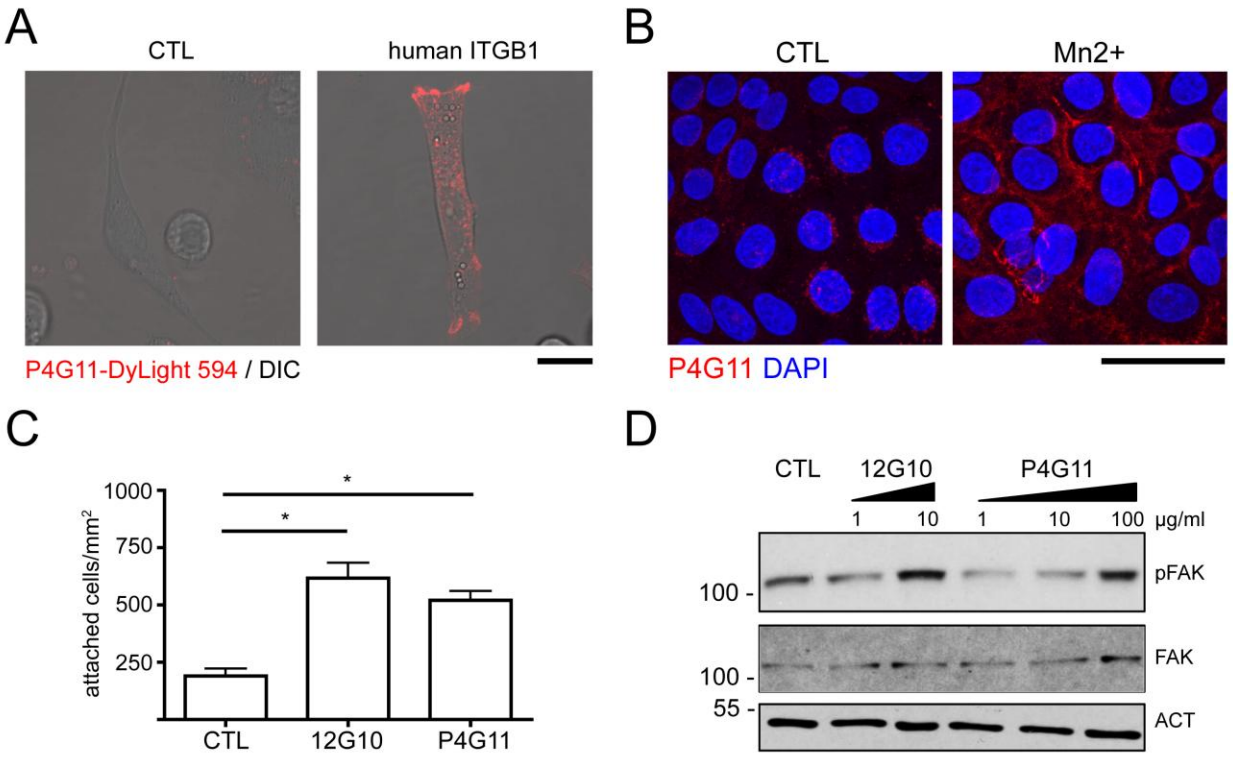
Supplemental Materials

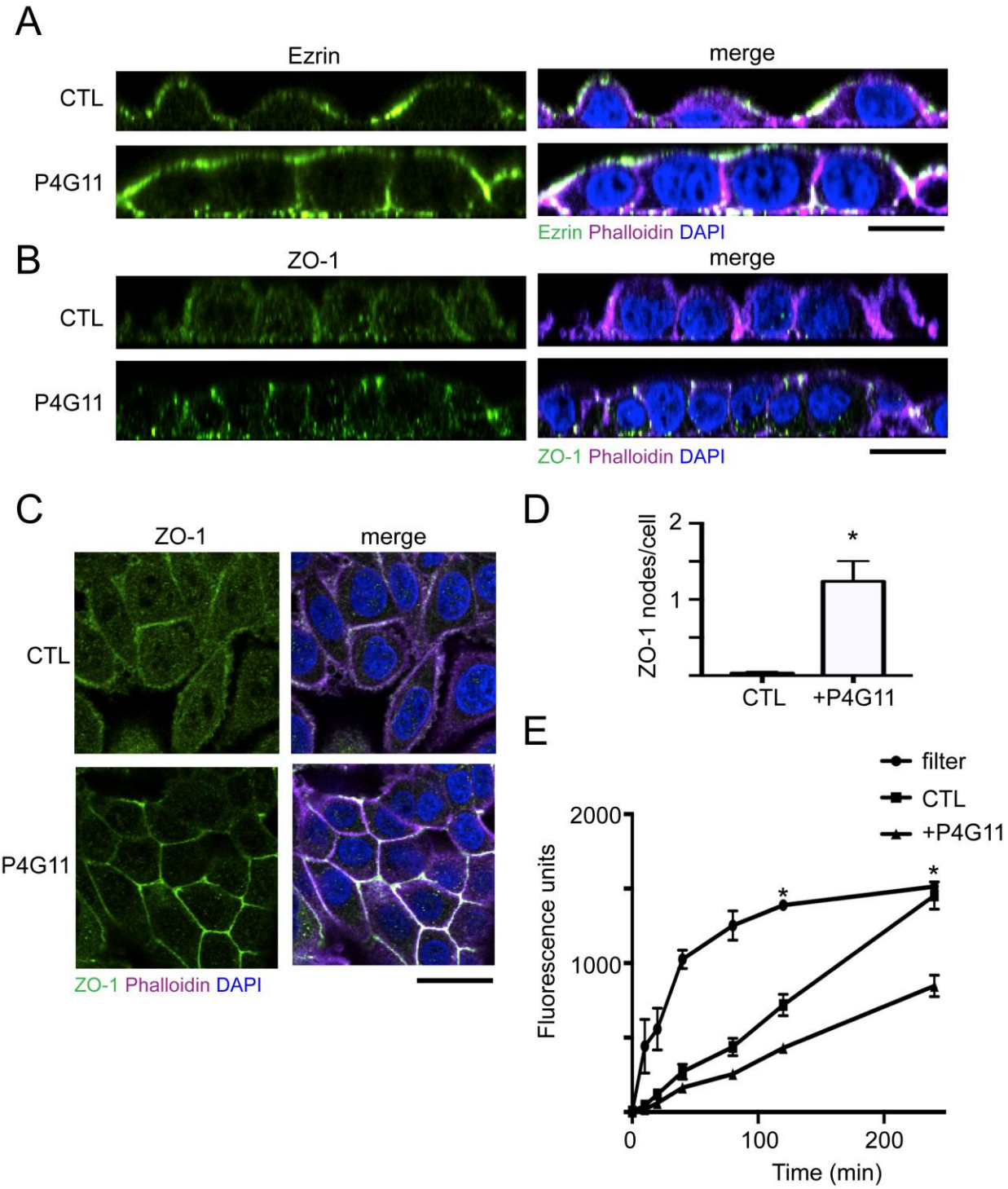
Molecular Biology of the Cell

Starchenko et al.

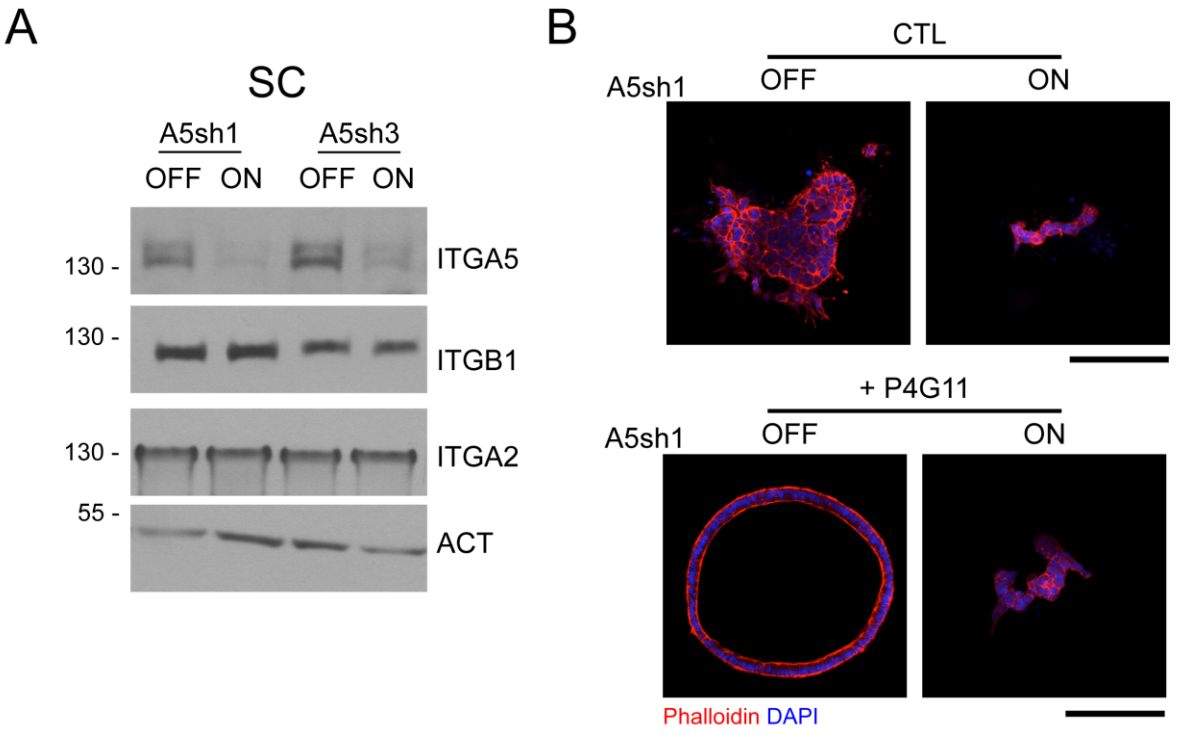
Supplemental Figures:



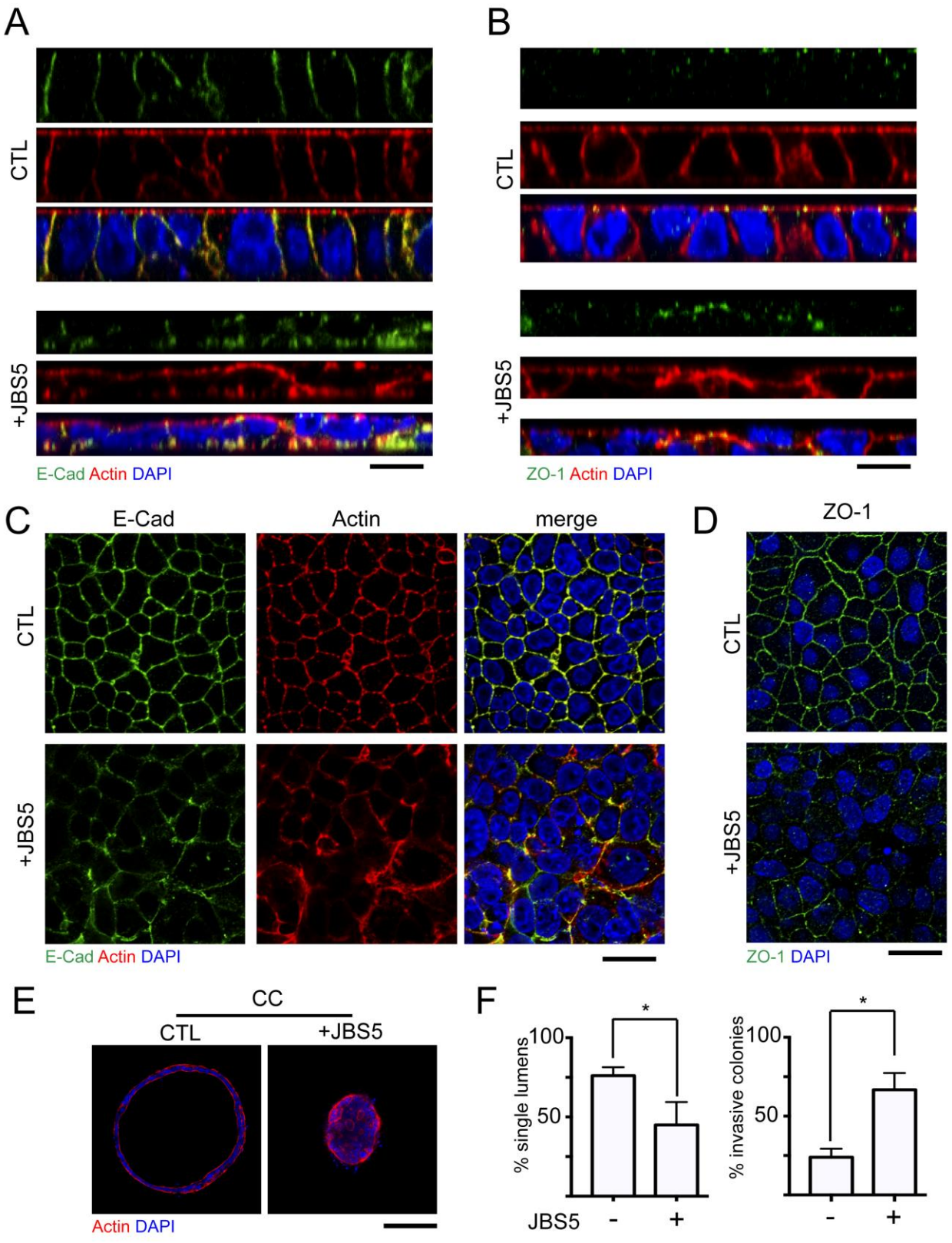
Supplemental Figure S1: P4G11 binds human integrin β1 and activates integrin signaling. (A) Representative confocal images of P4G11-594 (red) binding pattern in murine-derived cells expressing murine integrin β1 (CTL) or human integrin β1 with merged DIC (grayscale). Scale bar, 10 µm. (B) Representative confocal maximal intensity projection of SC cells grown on MMC-coated coverglass and treated with 1 mM Mn^{2+} for 5 min prior to fixation. Fixed cells were permeabilized and stained with P4G11 (red) and DAPI (blue). Scale bar, 20 µm. (C) Quantification of average number of SC cells adherent to a MMC-coated coverglass surface after 30 min in the presence of indicated mAb (mean±SEM, n = 5 fields of view from each of three separate biological replicates). (D) Levels of FAK activation in SC cells grown on MMC-coated coverglass measured by immunoblot analysis of phosphorylated Y392 after a 15 min treatment with indicated concentration of indicated mAb. Analysis was performed using antibodies against pY392 FAK, total FAK, and β-actin. Notably, changes in pFAK with P4G11 were only detected at 10-fold higher concentrations than used for 3D type-1 collagen rescue and conversion experiments. Asterisks signify statistical significance with a p-value < 0.05.


A
Ezrin
merge
CTL
P4G11
Ezrin Phalloidin DAPI
B
ZO-1
merge
CTL
P4G11
ZO-1 Phalloidin DAPI
C
ZO-1
merge
CTL
P4G11
ZO-1 Phalloidin DAPI
D
ZO-1 nodes/cell
2
1
*
CTL
+P4G11
E
filter
CTL
+P4G11
2000
1000
Fluorescence units
*
*
0
100
200
Time (min)

Supplemental Figure S2: Treatment of SW480 cells with P4G11 restores apico-basolateral polarity. SW480 cells were grown on MMC-coated coverglass for 24 hrs and treated with P4G11 for 48 hrs. Cells were fixed and analyzed by confocal microscopy. (A) X-Z plane confocal reconstruction of SW480 cells stained with anti-ezrin antibody (green), phalloidin (purple), and DAPI (blue). Scale bar, 25 μm. (B) X-Z plane confocal reconstruction of SW480 cells stained with anti-ZO-1 antibody (green), phalloidin (purple), and DAPI (blue). Scale bar, 25 μm. (C) Representative confocal cross-section through SW480 cells stained with anti-ZO-1 antibody (green), phalloidin (purple), and DAPI (blue). Scale bar, 25 μm. (D) Quantification of the number of ZO-1 nodes formed by SW480 cells with and without P4G11 treatment (mean±SEM, **N** = 5, 20x fields of view from three separate biological replicates). (E) Quantification of diffusion of 70 kDa FITC-Dextran across the Transwell filter of P4G11-treated SW480 cells over time (mean±SEM, n = 4 biological replicates). Asterisks signify a statistically significant difference at T = 120 min and T = 240 min between the CTL and P4G11-treated samples as determined by a T-test with p-values < 0.05.

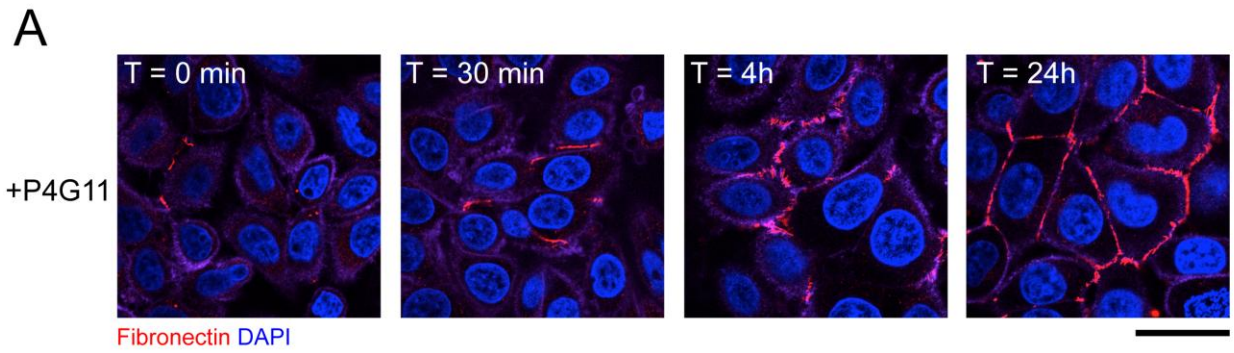


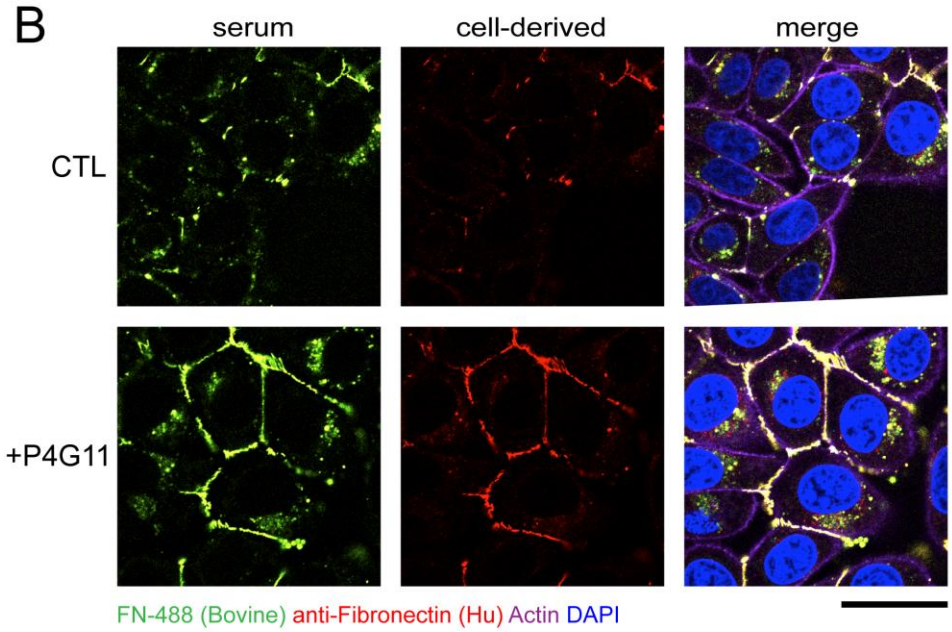
Supplemental Figure S3: Integrin α5 is necessary for P4G11-mediated rescue of epithelial junctions *in vitro*. (A) Immunoblot analysis of total levels of integrin α5, integrin β1, integrin α2 and β-actin in SC cells engineered to produce anti-integrin α5 shRNA in the presence (ON) or absence (OFF) of doxycycline. Two different shRNAs targeting different parts of the integrin α5 gene were compared to reduce likelihood of a non-specific phenotype (A5sh1 and A5sh3). (B) Representative confocal image of SCshV1 cells grown as in Figure 1A in presence (ON) or absence (ON) of anti-integrin α5 shRNA (sh1 shown) stained with phalloidin (Red) and DAPI (blue). Scale bar, 100 μm.

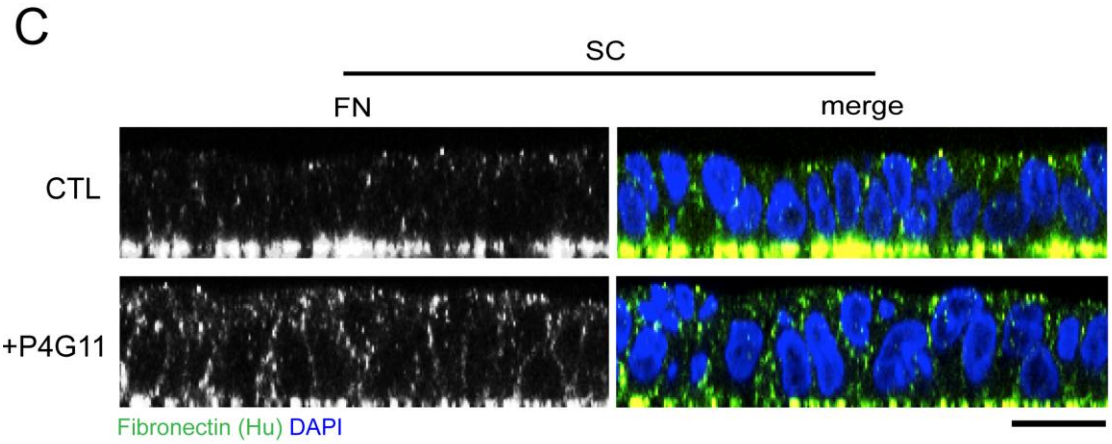


Supplemental Figure S4: Blockade on integrin α5 leads to loss of epithelial organization in 2D and 3D. (A-D) SC cells grown on MMC-coated Transwell filters and treated with integrin α5

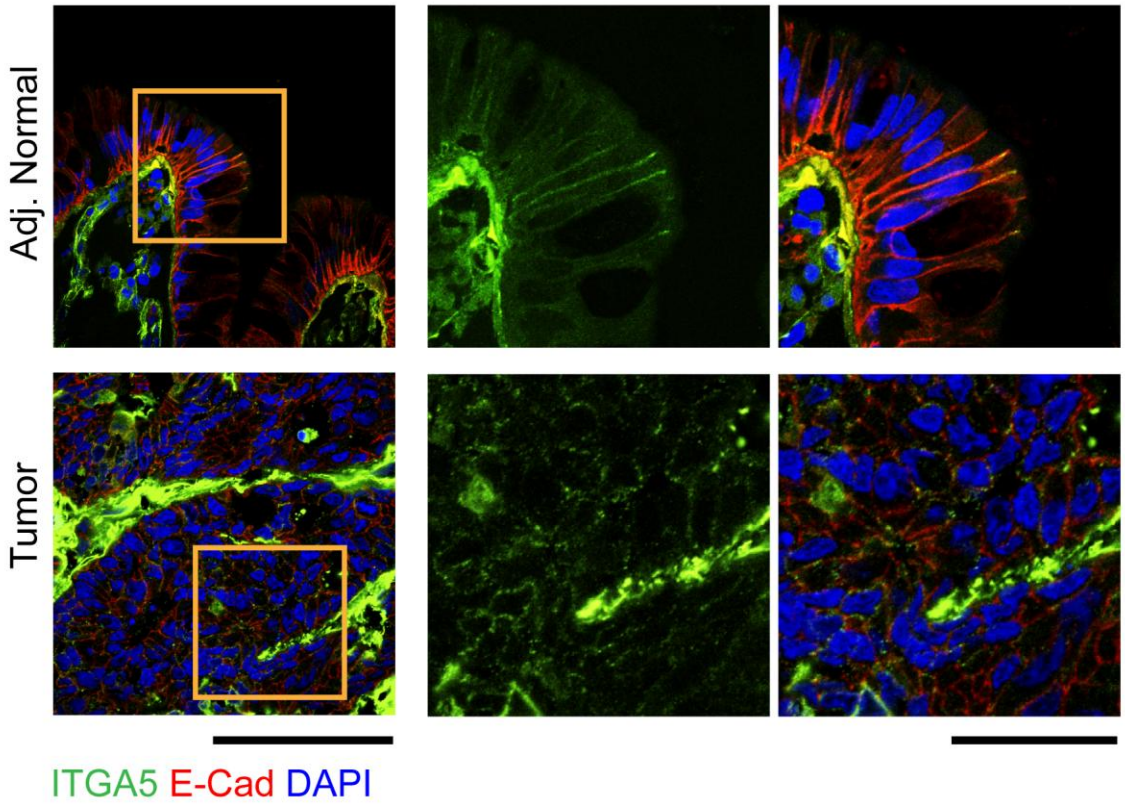
blocking antibody JBS on days 2-6 and analyzed by confocal microscopy (A) shown is a representative confocal z-reconstruction of SC stained with anti-E Cadherin antibody (green), phalloidin (red), and DAPI (blue). Scale bar, 20 μm. (B) Representative confocal z-reconstruction of SC stained with antibody against ZO-1 antibody (green), phalloidin (red) and DAPI (blue). Scale bar, 20 μm. (C) Representative confocal image of SC in (A) seen in the XY plane stained with antibody against E-cadherin (green), phalloidin (red), and DAPI (blue). Scale bar, 20 μm. (D) Representative confocal image of SC in (B) seen in the XY plane stained with antibody against ZO-1 (green), phalloidin (red), and DAPI (blue). Scale bar, 20 μm. (E) Single CC cells grown into mature colonies using a collagen 1 sandwich assay were treated with JBS5 on days 1-15 and stained with phalloidin (red) and DAPI (blue). Scale bar, 100 μm. (F) Quantification of number of CC colonies exhibiting a single central lumen or an invasive phenotype in (E) (mean±SEM; n = 3 biological replicates).


A
T = 0 min
T = 30 min
T = 4h
T = 24h
+P4G11
Fibronectin DAPI


B
serum
cell-derived
merge
CTL
+P4G11
FN-488 (Bovine) anti-Fibronectin (Hu) Actin DAPI


C
SC
FN
merge
CTL
+P4G11
Fibronectin (Hu) DAPI

Supplemental Figure S5: P4G11 treatment induces fibronectin polymerization in SW480 and SC cells. (A) A representative confocal cross-section of SW480 cells grown on MMC-coated coverglass, treated with P4G11 and fixed at different time points were stained with phalloidin (purple), DAPI (blue) and an anti-fibronectin antibody (red). Scale bar, 25 μm. (B) Representative confocal cross-section of SW480 cells grown on MMC-coated coverglass, treated with P4G11 in the presence of 488-fibronectin in the serum for 48 hrs. Cells were stained with an antibody against human fibronectin (red) and DAPI (blue) and fluorescence of serum-derived 488-fibronectin (green) can also be seen. Note that there is significantly more 488-fibronectin signal than that arising from the human fibronectin. Scale bar, 25 μm. (C) SC cells grown on filters for 5 days on MMC-coated Transwell filters were treated with P4G11 days 5-6 and stained with an antibody against fibronectin (green) and DAPI (blue). Scale bar, 20 μm.



Supplemental Figure S6: Integrin α5 is present at the lateral surface in adjacent normal colon but is absent from the surface in tumor. Representative confocal images of a tumor and an adjacent normal human colon section stained with antibody against integrin α5 (green), E-cadherin (red), and DAPI (blue) scale bar, 100 μm. High magnification view of adj. normal and tumor sections. Scale bar, 50 μm.