CD13 orients the apical-basal polarity axis necessary for lumen formation

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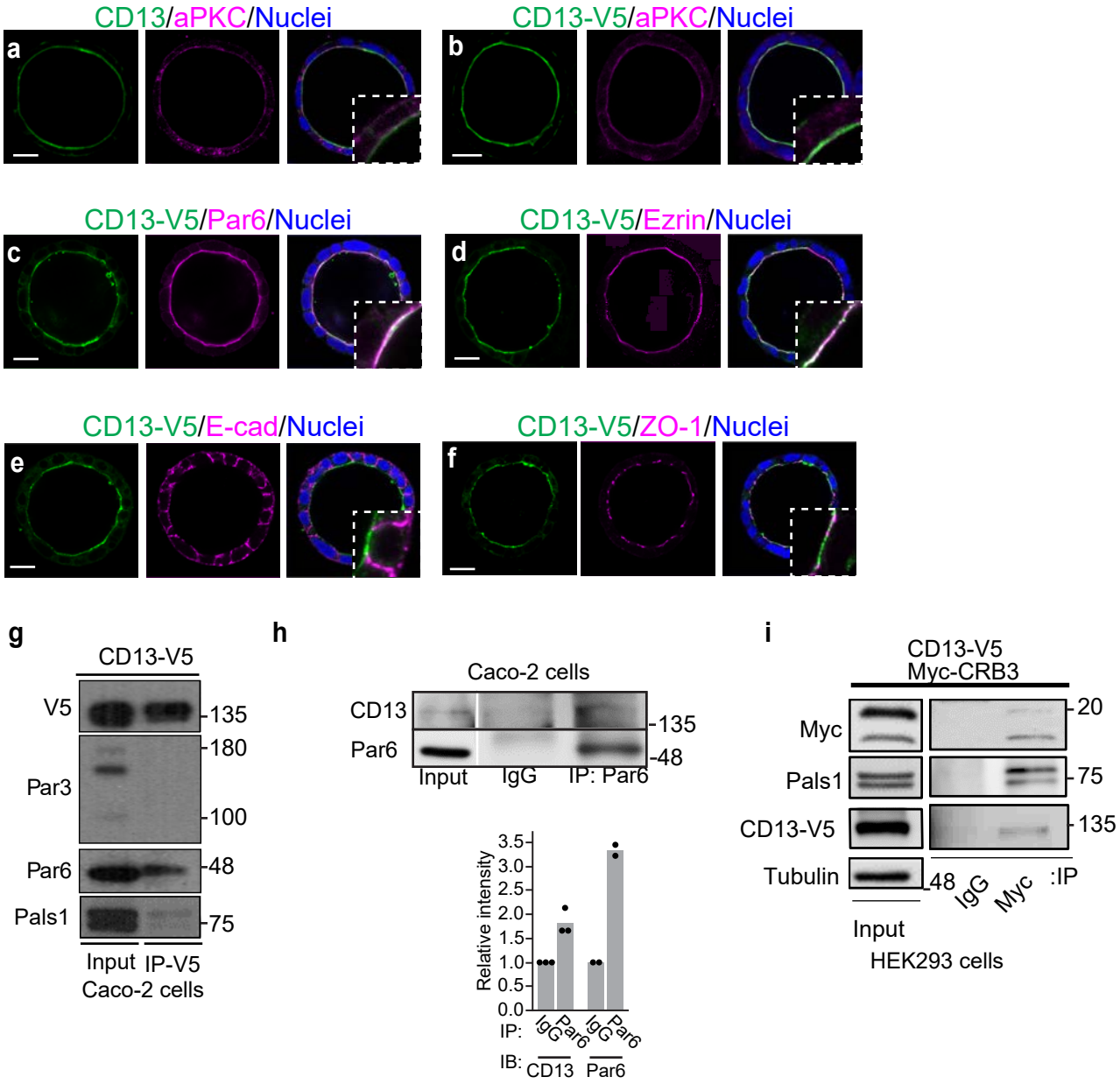
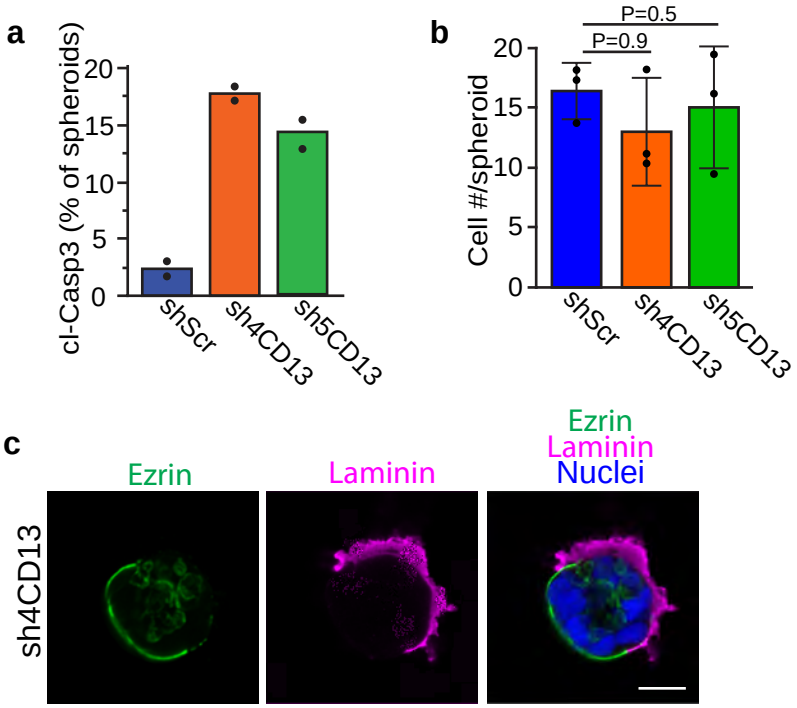
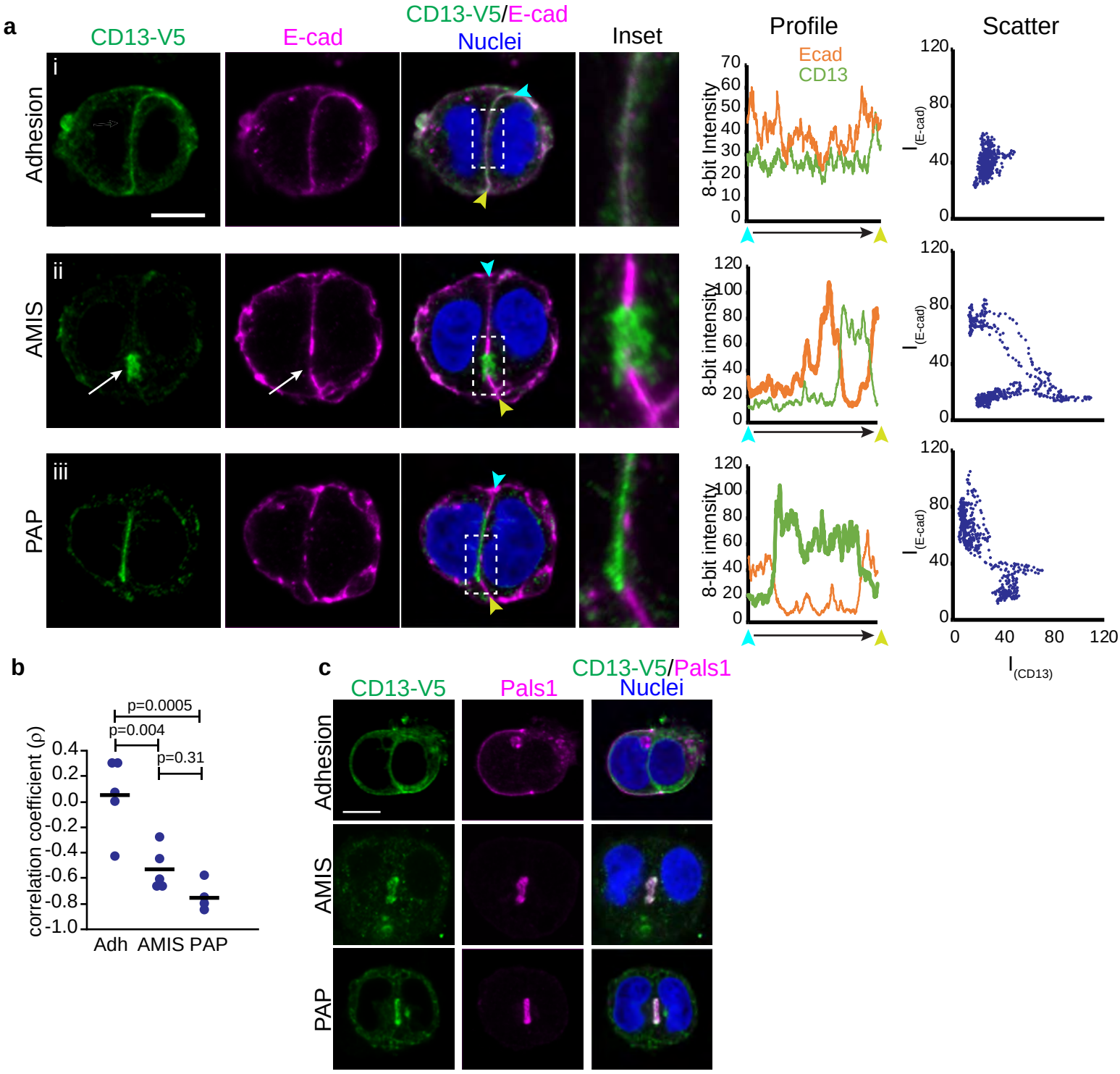
SUPPLEMENTARY INFORMATION



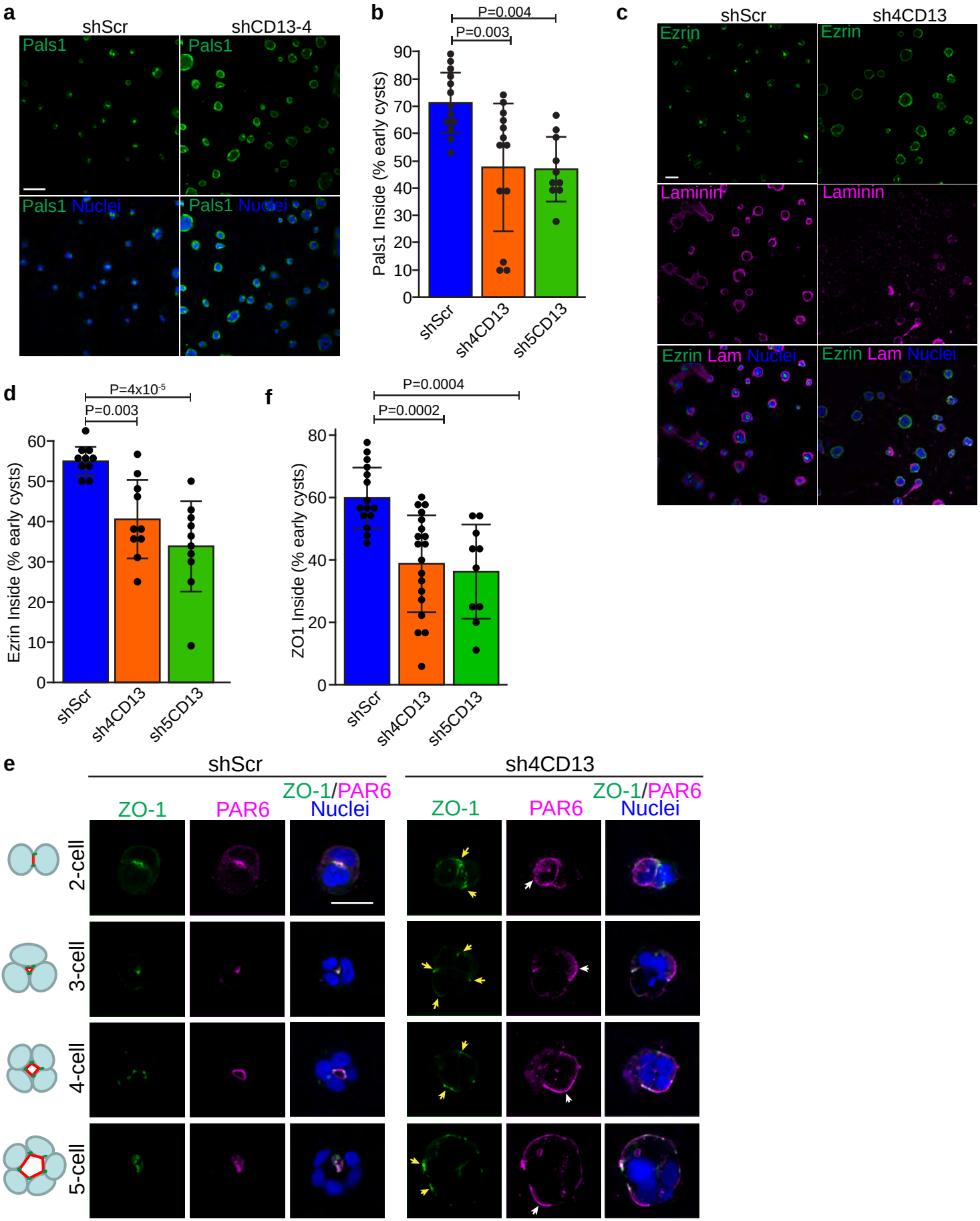
Supplemental Figure 1. **CD13 associates with apical proteins and localizes at the apical domain of 3D Caco-2 cysts. Associated with Figure 1. a** Confocal images of polarized 3D Caco-2 cysts cultured for 10 days then immunostained for endogenous CD13 (green) and apical aPKC (magenta). **b-f** Representative confocal images of polarized 3D Caco-2 cysts immunostained for V5-tagged CD13 (green) and polarity markers (magenta) aPKC (b), Par6 (**c**), Ezrin (**d**), E-cad (**e**), and ZO-1 (**f**). **g** Immunoprecipitation was performed with anti-V5 in stably expressed CD13-V5 Caco-2 cells and blotted for V5, Par3, Par6, and Pals1. **h** Immunoprecipitation was performed in Caco-2 lysates with IgG or anti-Par6 antibody and blotted for endogenous CD13 and Par6. The lower graph shows quantification of band intensities from three independent experiments. **i** Co-immunoprecipitation of CD13 and CRB3 was performed with anti-IgG or anti-myc in HEK293 cells. The presence of CD13 in immunoprecipitates was determined by western blot analysis using anti-V5. Images are representative from three independent experiments. Bars: **a-f**, 20μm.



Supplemental Figure 2. **CD13 knockdown disrupts the orientation of apical-basal polarity. Associated with Figure 2. a** Caco-2 were cultured for 10 days and the proportion of spheroids with one or more apoptotic cells (measured by cleaved Caspase) were quantified from n=7 fields of view for each condition. Individual dots represent mean values from independent experiments. **b** Quantification of the mean number of cells per spheroid +/- SEM for the indicated conditions (n=201 shScr, n=242 sh4CD13, n=208 sh5CD13 spheroids) after 10 days in culture. Dots represent mean values from independent experiments. The p-values were determined by unpaired two-tailed student's t-test. **c** Confocal images of 3D Caco-2 spheroids immunostained for Ezrin (green) and laminin (magenta). Images are representative from least three independent experiments. Bars: **c**, 30μm.

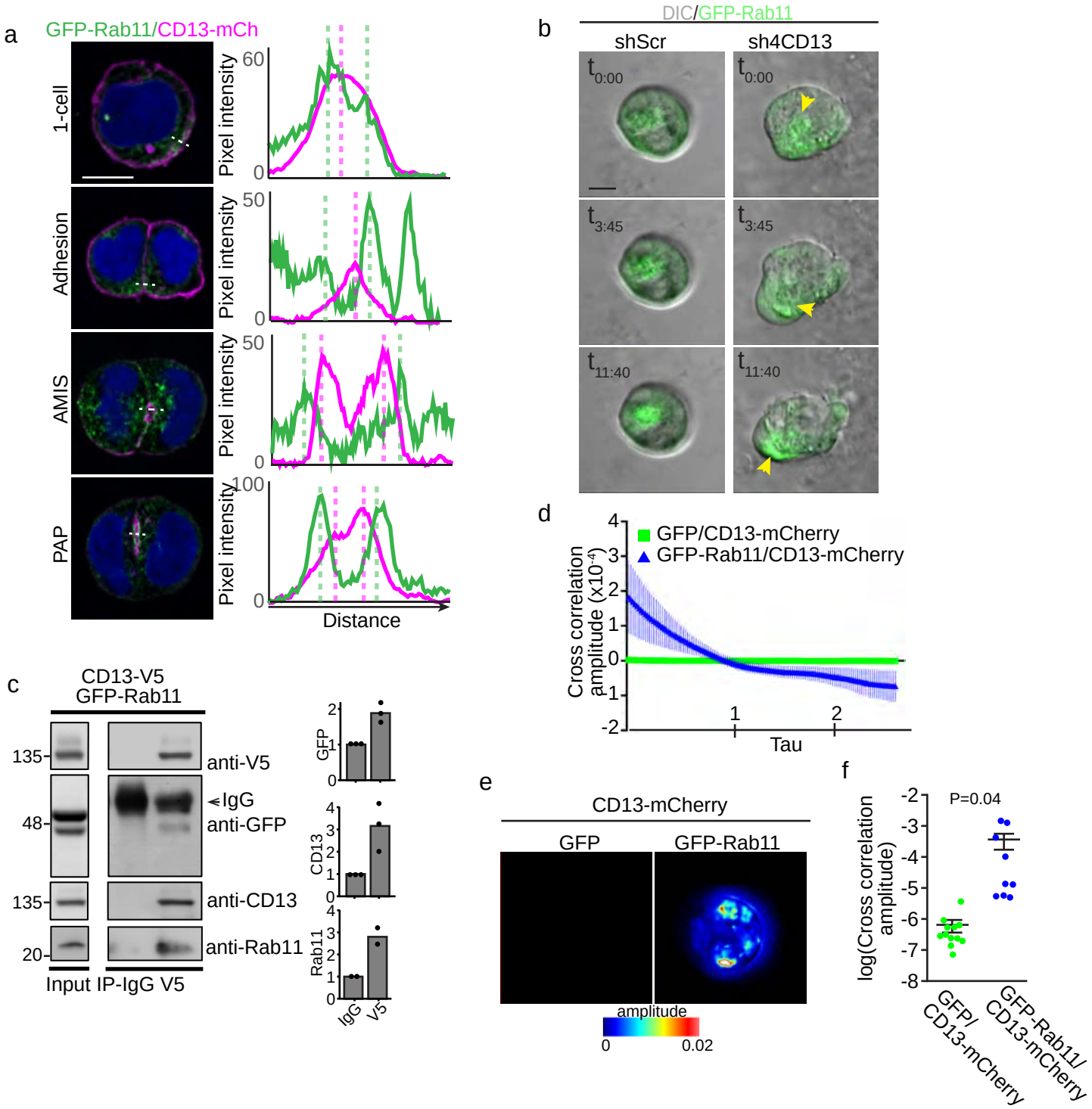


Supplemental Figure 3. **Recruitment of CD13 to focal sites is coincident with E-cadherin displacement during polarization in Caco-2 cells. Associated with Figure 3. a** Confocal images for CD13-V5 (green) and E-cad (magenta) showing the relative localization of CD13 and E-cad at adhesion, AMIS, and PAP stage in two cell structures of Caco-2 cysts. Graphic profile depicting changes in fluorescent intensity (8-bit intensity range: 0-255) from blue to yellow arrows. Adjacent scatter plots showing the relationships between CD13 and E-cad. **b** Quantification of the correlation coefficient of CD13 and E-cad at adhesion, AMIS, and PAP stage in two cell structures of 3D Caco-2 cysts. p-values were determined by unpaired two-tailed student's t-test. **c** Confocal images of 2-cell Caco-2 structures immunostained for CD13-V5 (green) and Pals1 (magenta) showing their localization during polarity initiation. Images are representative from three independent experiments (**a,c**). Bars: 10μm.

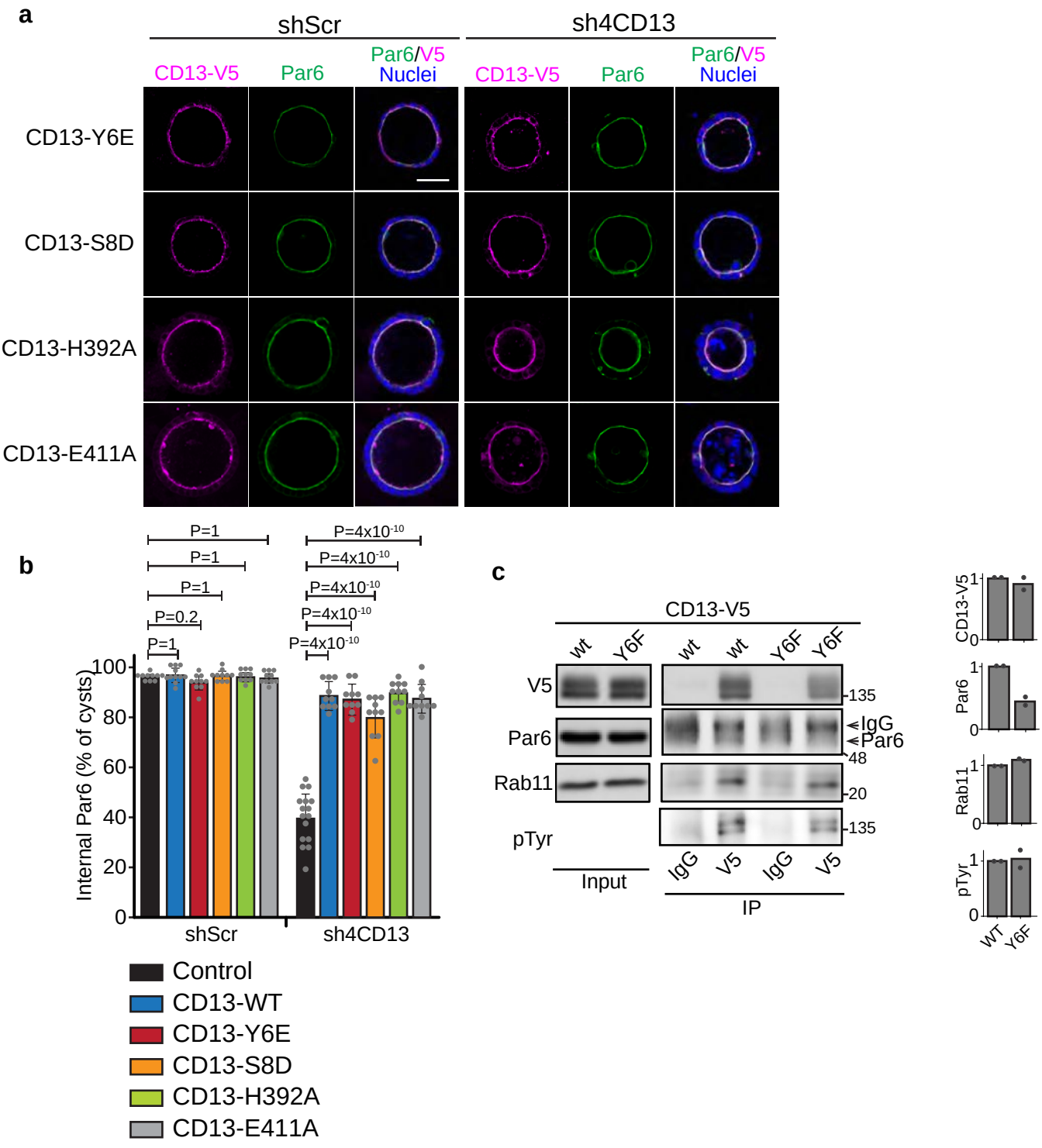


Supplementary Figure 4. Figure legend on following page.

Supplemental Figure 4. **CD13 depletion disrupts early polarization in 3D Caco-2 cysts. Associated with Figure 4. a** Low-power confocal images of control (shScr) and CD13 knock-down (sh4CD13) 3D Caco-2 cells cultured for 2 days then immunostained for Pals1 (green). **b** Quantification of the percentage of Caco2 structures with Palsl localize to the inside of shScr (n=235), sh4CD13 knock-down (n=326) and sh5CD13 knock-down (n=154) in 3D Caco-2 cysts at early stages. Dots represent means from independent experiments. **c** Confocal images of control (shScr) and CD13 knock-down (sh4CD13) 3D Caco-2 cells cultured for 2 days then immunostained for Ezrin (green) and laminin (magenta). **d** Quantification of the percentage Caco-2 structures with Ezrin localization to the inside for shScr (n=153), sh4-CD13 knock-down (n=162) and sh5-CD13 knock-down (n=147) cells. Dots represent means from independent experiments. **e** Confocal images of control (shScr) and CD13 knock-down (sh4CD13) 3D Caco-2 cysts cultured for 2 days then immunostained for tight junction marker, ZO1 (green) and Par6 (magenta). **f** Quantification of the percentage of Caco-2 structures with ZO-1 localized to the inside of shScr (n=302), sh4CD13 knock-down (n=278) and sh5CD13 knock-down (n=146) cells. Dots represent means from independent experiments. p-values were determined by unpaired two-tailed student's t-test (**b,d,f**). Images are representative from three independent experiments. Bars: **a,c**, 100μm; e, 30μm.



Supplementary Figure 5. **Knockdown CD13 disrupts Rab11-dependent early polarization in Caco-2 cells. Associated with Figure 5. a** Confocal images showing the localization of CD13-mCherry (magenta) and GFP-Rab11 (green) at 1-cell, and adhesion, AMIS, and PAP stage in 2-cell Caco-2 structures from Fig. 5A with local intensity profiles of CD13 and Rab11 (right panels). Vertical lines show adjoining but non-overlapping CD13 and Rab11 intensity peaks. **b** DIC/confocal images showing selected frames from time-lapse series of shScr and sh4CD13 3D Caco-2 structures. Images were captured every 25 min. **c** Co-immunoprecipitation of CD13-V5 and GFP-Rab11 was performed with IgG or anti-V5 in HEK293 cells. The presence of Rab11 in immunoprecipitates was determined by western blot analysis using anti-GFP and anti-Rab11. Graphs on the right show quantification of band intensities from two independent experiments. **d** Relative cross correlation amplitude profile of cells co-expressing GFP or GFP-Rab11 and CD13-mCherry fusions over time. Tau represents the time separation between two acquired images. **e** Co-binding intensity profile of Caco-2 cells co-expressing GFP (n=12) or GFP-Rab11 (n=9) with CD13-mCherry. **f** Relative cross correlation amplitude profile of cells co-expressing GFP (n=18) or GFP-Rab11 (n=17) with CD13-mCherry fusions at the first time-point. Data are presented as mean values +/- SEM. p-values were determined by unpaired two-tailed student's t-test. Images are representative from three independent experiments. Bars: 10µm.



Supplemental Figure 6. **The intracellular domain of CD13 is required to establish apical-basal polarity. Associated with Figure 7. a** Confocal images of Caco-2 cells with control (shScr) or CD13 knockdown (sh4CD13) cells expressing CD13 mutants immunostained for CD13-V5 (magenta) and Par6 (green). Images are representative from two independent experiments. **b** Quantification of the percentage of Par6 internally in wildtype (shScr, n=515; shCD13, n=292 cell structures), CD13-Y6E (shScr, n=555; shCD13, n=285 cell structures), CD13-S8D (shScr n=400; shCD13, n=337 cell structures), CD13-H392A (shScr, n=475; shCD13, n=298 cell structures), and CD13-E411A (shScr, n=513; shCD13, n=283 cell structures) for shScr and shCD13 Caco-2 cysts. Data are presented as mean values +/- SD. Comparisons of multiple means was performed by ANOVA using Tukey's post-hoc test. **c** Immunoprecipitation was performed with anti-IgG or anti-V5 in stably expressed CD13-wild type or CD13-Y6F Caco-2 cells. Immunoprecipitates were blotted for V5, Par6, Rab11 and pan-phosphotyrosine. Graphs to the right show quantification of band intensity from two independent experiments. Bars: **a**, 50μm.

Supplementary Table 1. **shRNA sequences.**

shRNA Name	Sequence
sh4CD13	CCGGCCACAGCAAGAAGCTCAACTACTCGAGTAGTTGAGCTTCTTGCTGTGGTTTTTG
sh5CD13	CCGGGTGACCATAGAGTGGTGGAATCTCGAGATTCCACCACTCTATGGTCACTTTTTG
sh1Rab11A	CCGGATCATGCTGATAGTAACATTGCTCGAGCAATGTTACTATCAGCATGATTTTTTG
sh4Rab11A	GTACCGGTATCATGCTTGTGGGCAATAACTCGAGTTATTGCCCACAAGCATGATATTTTTTG
sh1Rab35	CCGGAGACGGAAGATGCCTACAAATCTCGAGATTTGTAGGCATCTTCCGTCTTTTTTG
sh5Rab35	GTACCGGTTCACGAAATCAACCAGAACTCTCGAGAGTTCTGGTTGATTTCGTGAATTTTTTG

Fig. 1C

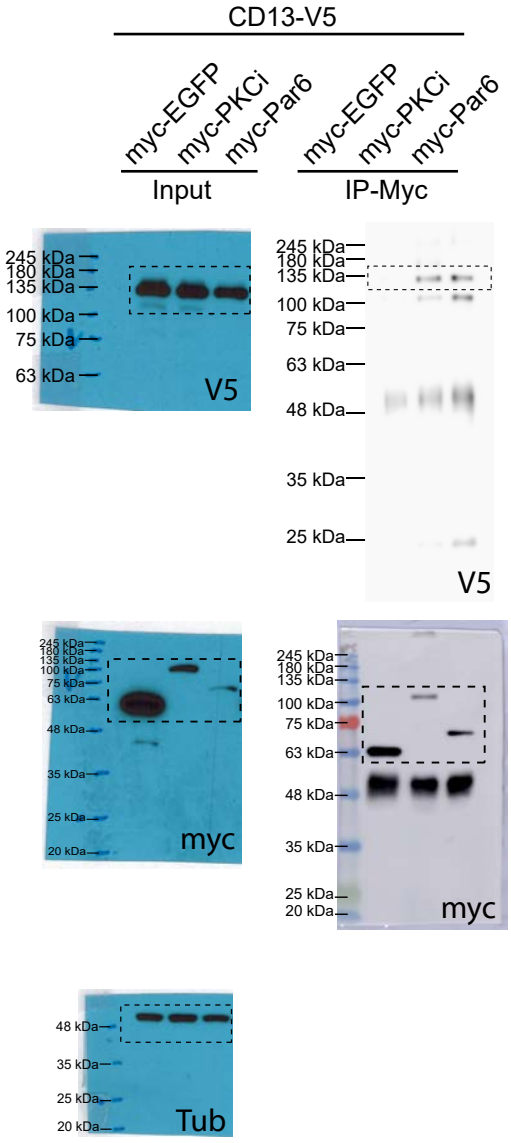


Fig. 1D

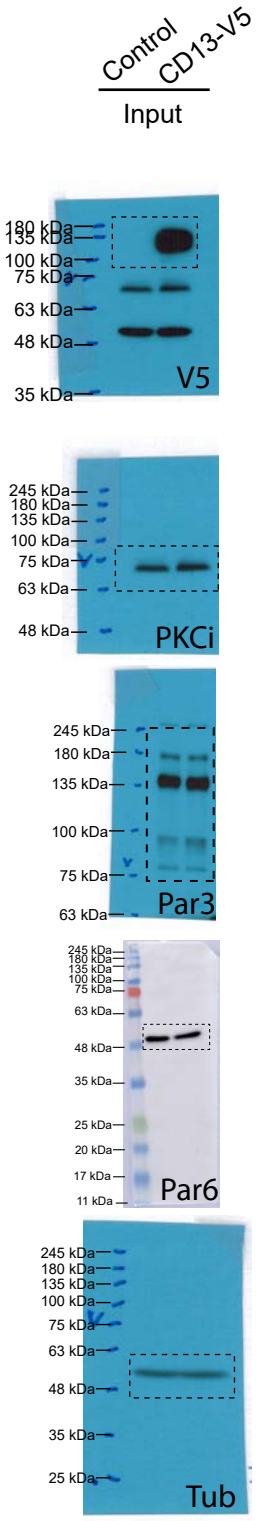


Fig. 1E

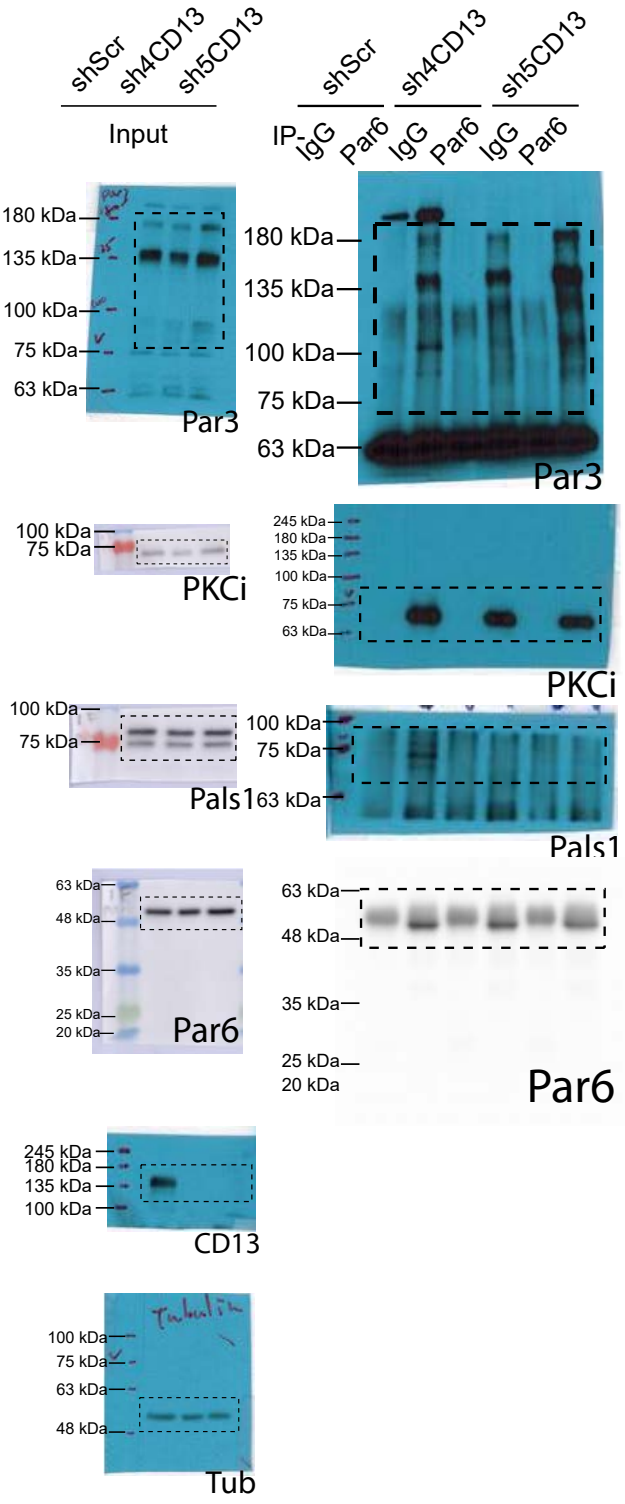
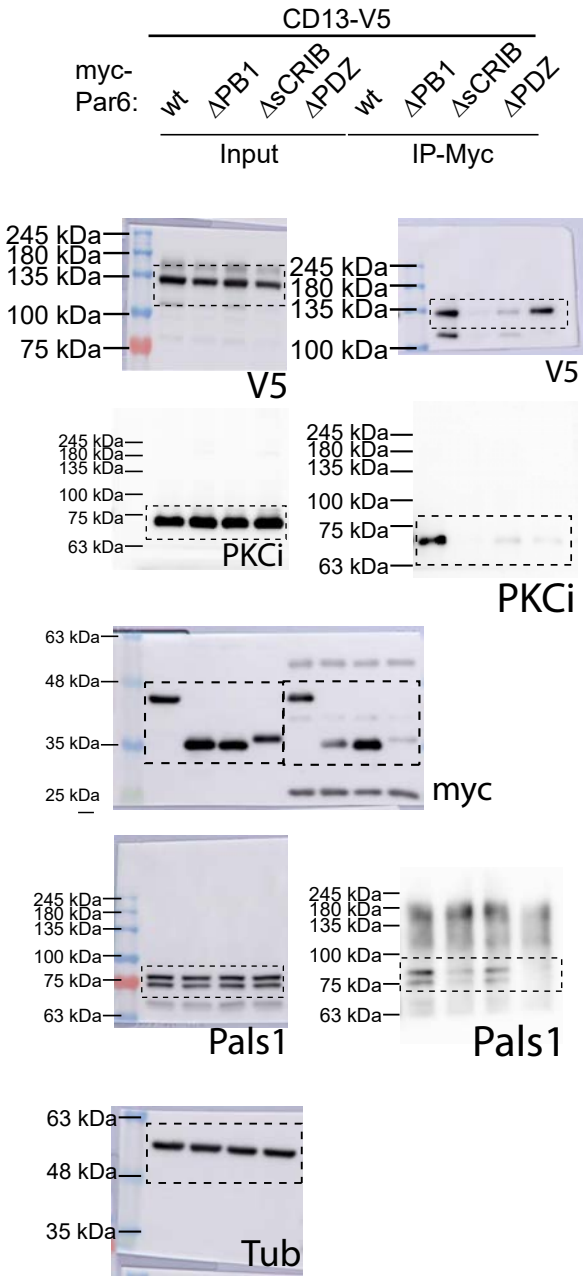


Fig. 1G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ΔPDZ
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ΔPB1
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PKCi
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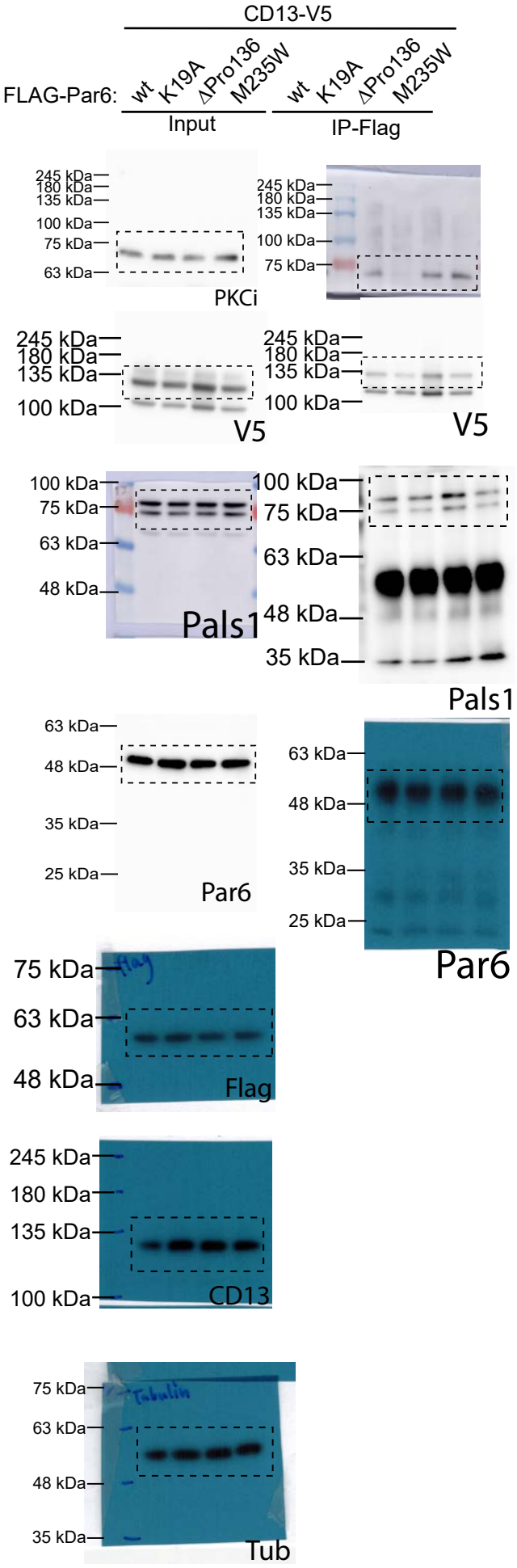
35 kDa
25 kDa
myc
Pals1
Tub

Fig. 1H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M235W
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Supplementary Figure S1

Fig. S1G

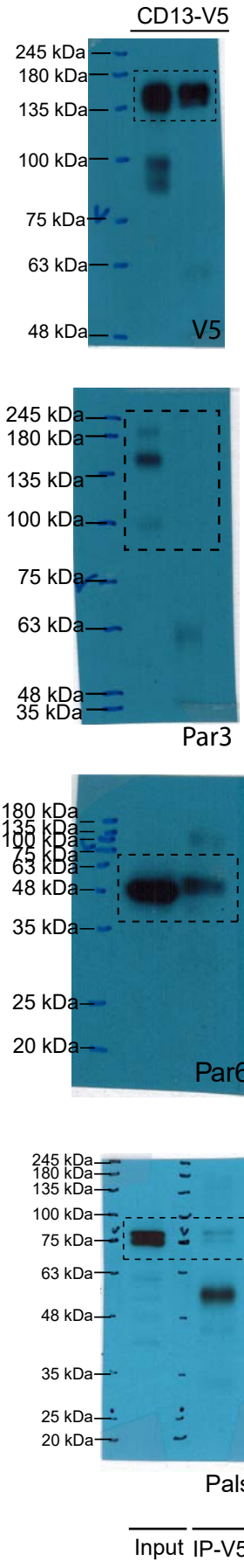


Fig. S1H

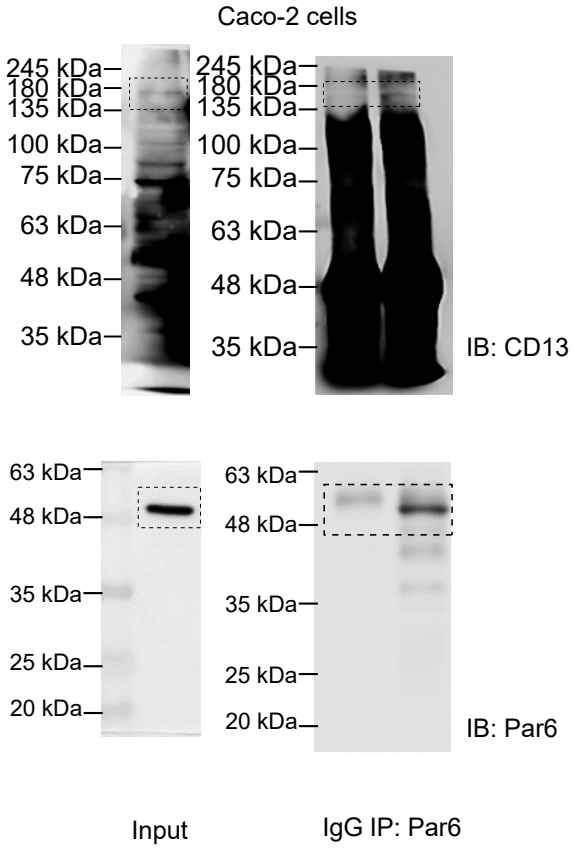
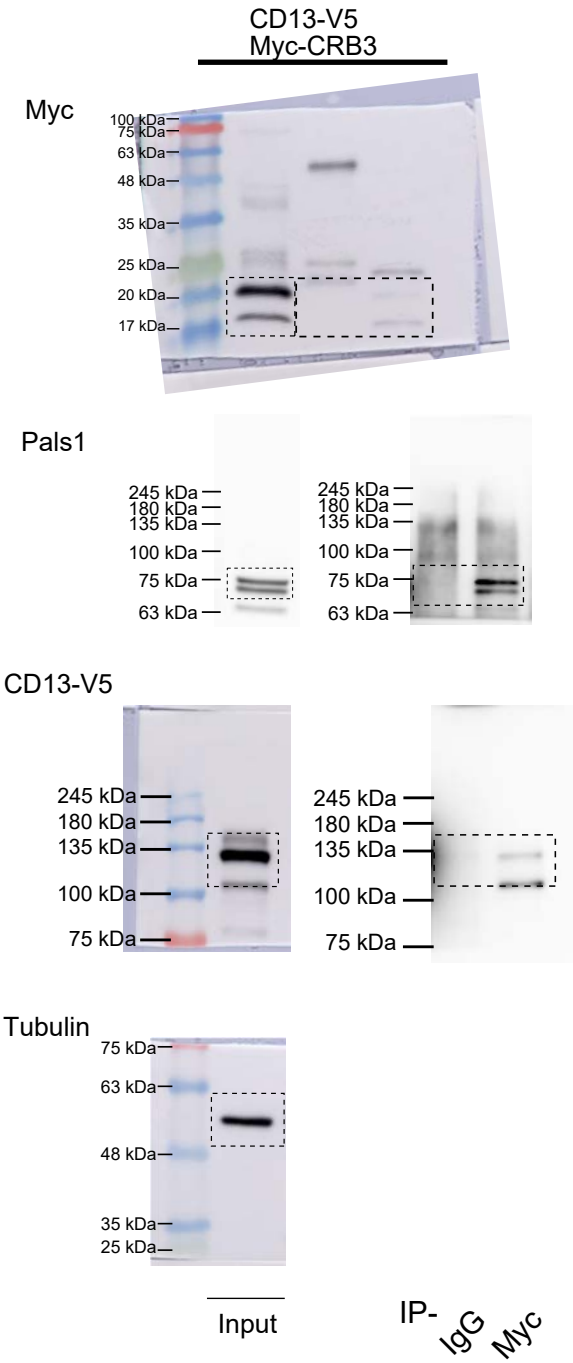
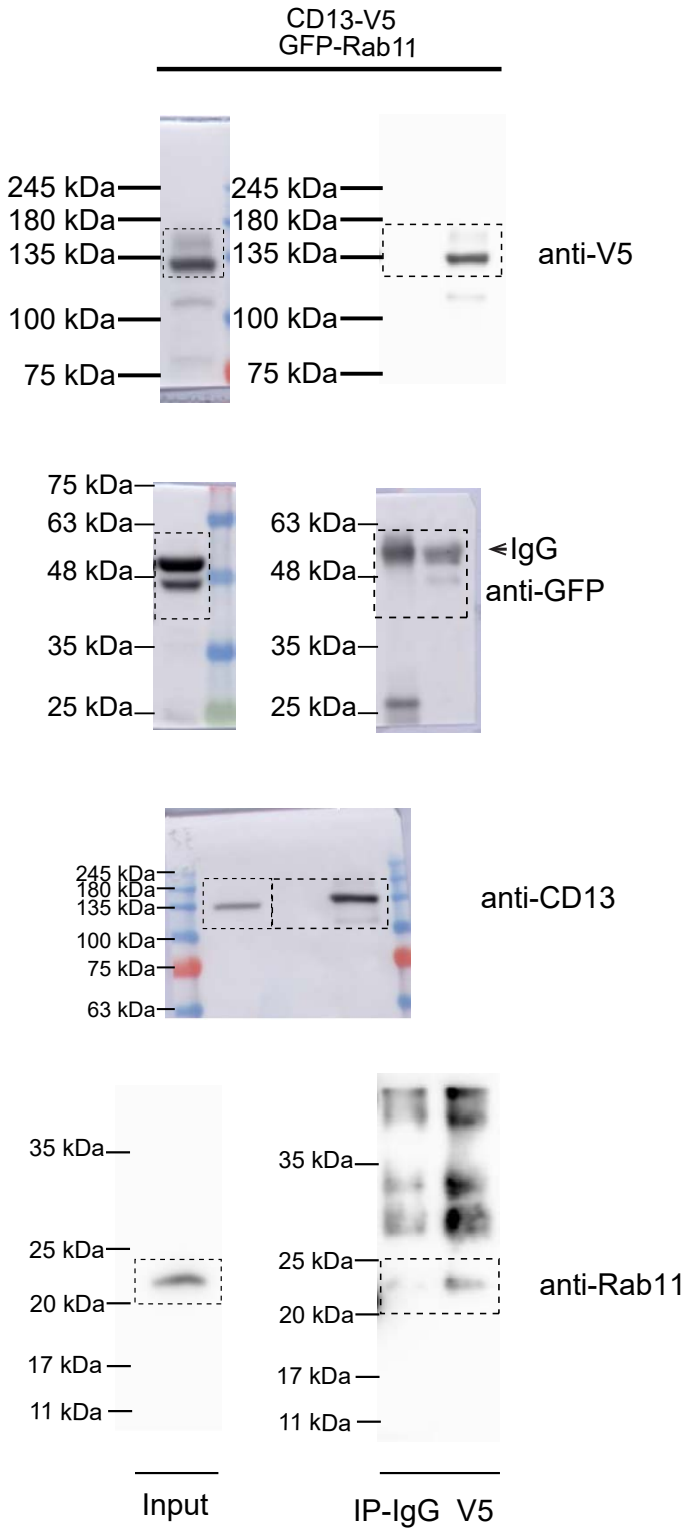


Fig. S1I



Supplementary Figure S5

Fig. S5C



Supplementary Figure S6

Fig. S6C