

Legends to supplemental figures

Supplemental Figure 1. PATJ localizes to the leading edge of migrating epithelial cells.

(A) Localization of PATJ at the leading edge of MDCKII cells during wound healing (white arrow). PATJ (green) and actin (red) were visualized 6hrs after wounding. Nuclei are shown in blue. Scale bar, 10 μ m. (B) Localization of CRB3 (upper panels, green) and GP135 (bottom panels, green) 6hrs after wounding. Actin and nuclei are shown in red and blue, respectively. Scale bar, 10 μ m.

Supplemental Figure 2. PATJ is required for the correct reorientation of the microtubule organizing center (MTOC). MTOCs were visualized 6hrs after wounding wild type (A), PATJ RNAi (B) and EGFP-PATJ rescue MDCKII cells (C). Arrows indicate the direction of migration. MTOC and nuclei are shown in red and blue, respectively. Scale bars, 20 μ m. (D) Quantification of MTOC reorientation. In wild type MDCKII cells, 65.7 +/- 1.3% of leading edge cells have the MTOC in the direction of migration whereas only 29.9 +/- 2.1% of PATJ RNAi cells show the correct orientation 6hrs post wounding, $P < 0.0001$, unpaired t test. Standard deviations are shown as error bars (n=3).

Supplemental Figure 3. Formation of lamellipodia is normal in PATJ RNAi MDCKII cells during wound healing. Cortactin (green) and PATJ (red) were visualized in wild type (A) and PATJ RNAi MDCKII cells (B) 6hr after wounding. Merge images are shown in the middle. Scale bar, 10 μ m.

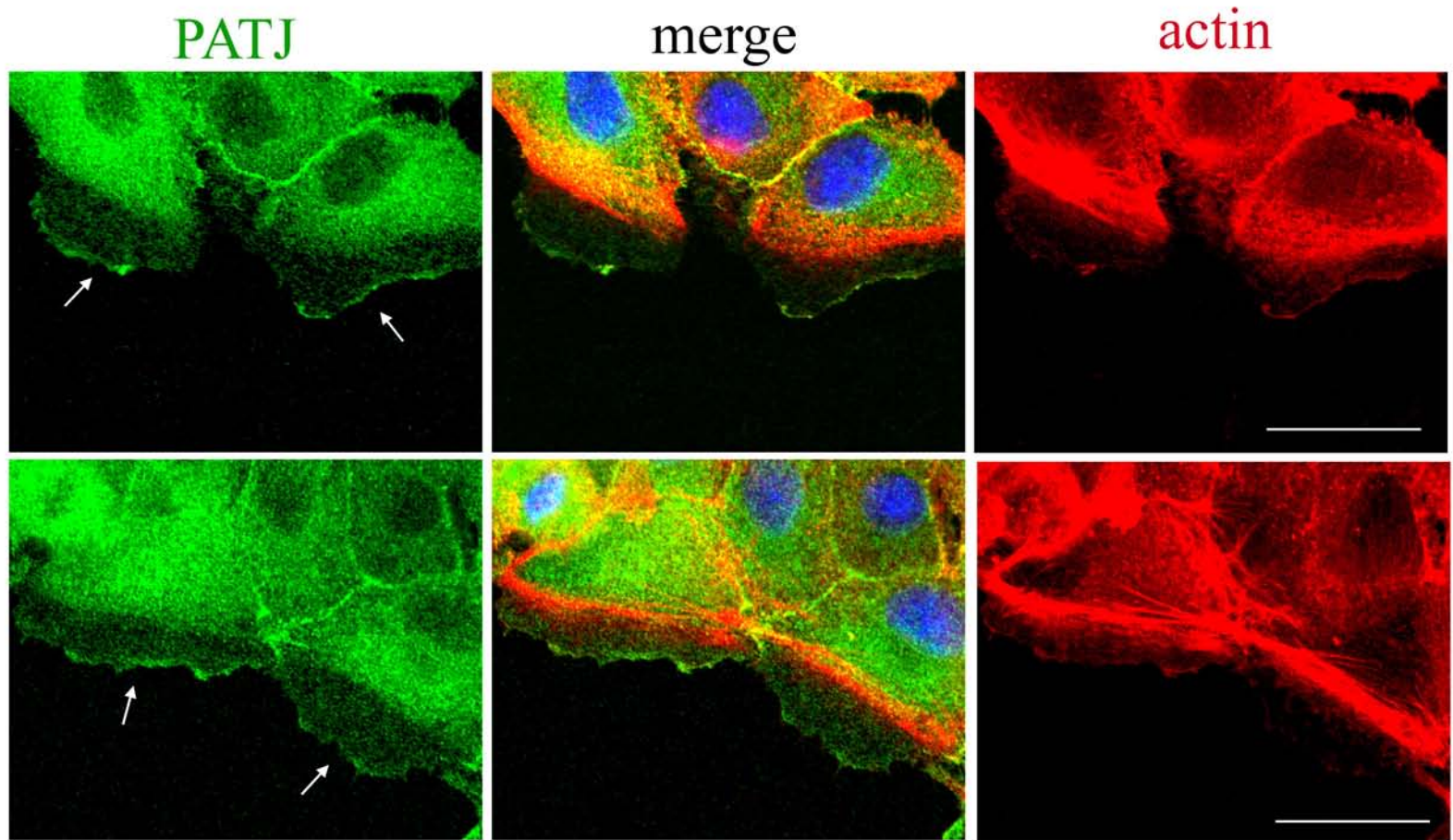
Supplemental Figure 4. (A) aPKC was immunostained 6hrs after wounding with EGFP-PATJ mutant rescue PATJ RNAi cell lines. Scale bar, 10 μ m (B) Quantification of aPKC localization during migration of EGFP-PATJ mutant rescue PATJ RNAi MDCKII cells.

Supplemental Figure 5. PALS1 is required for the migration of epithelial cells. (A) Wound closure migration assays were performed with wild type and PALS1 RNAi, MDCKII cells. Wound areas are outlined. Scale bar, 100 μ m. (B) Quantification of epithelial migration after wounding. Wild type MDCKII cells shows 100.0 \pm 0.0% wound closure, whereas PALS1 RNAi MDCKII cells exhibits 36.6 \pm 5.8% wound closure 17hrs after wounding, $P < 0.0001$, unpaired t test. Relative areas of the wound were measured in three independent experiments. Standard deviations are shown as error bars (n=3).

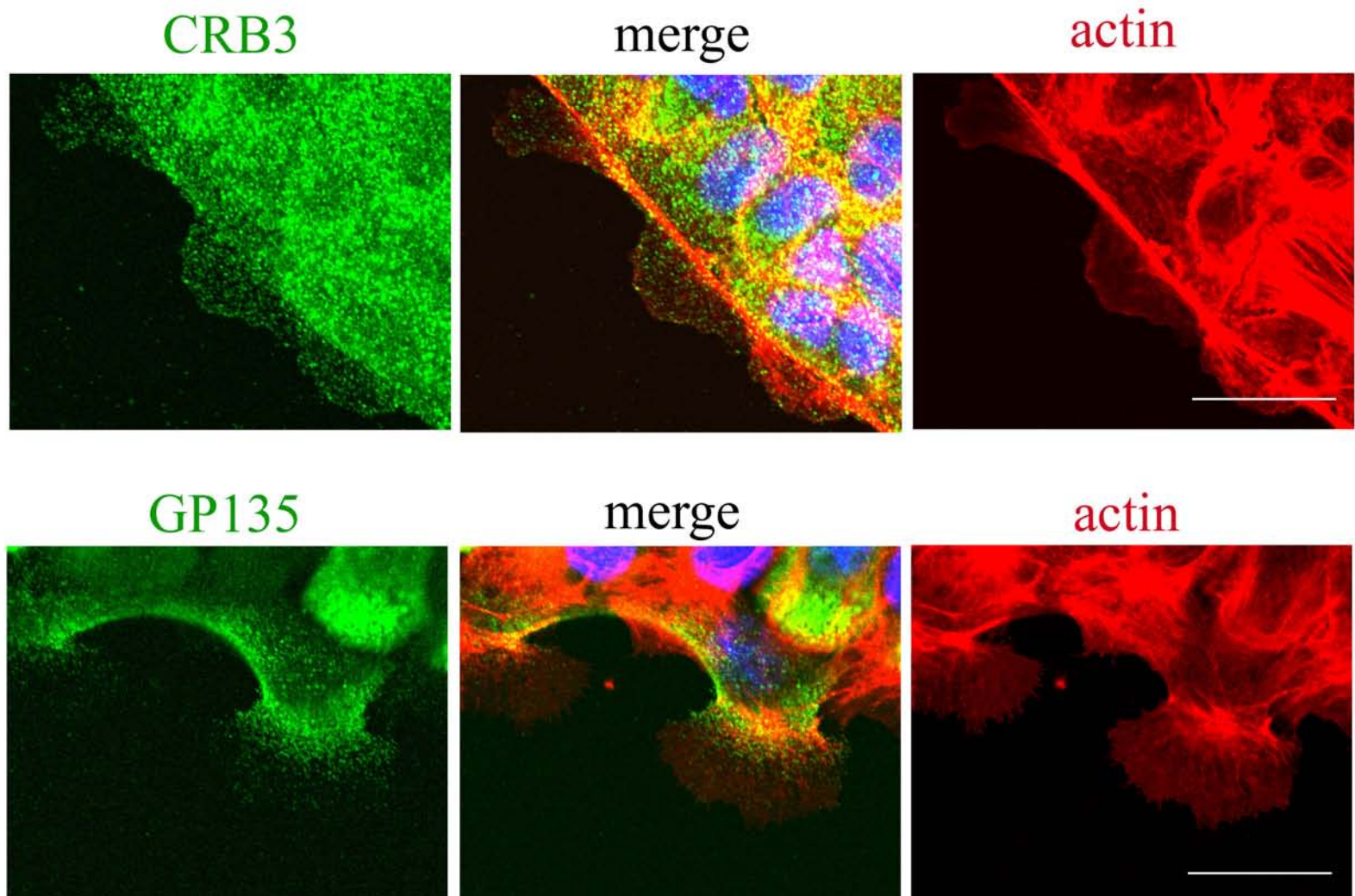
Supplemental Figure 6. CRB3 is not essential for the migration of epithelial cells. (A) Western blot was performed for PATJ, PALS1 and CRB3 in wild type and CRB3 RNAi MDCKII cells. (B) Quantification of epithelial migration after wounding. Wild type MDCKII cells shows 80.5 \pm 6.8% wound closure, and CRB3 RNAi MDCKII cells exhibits 75.3 \pm 10.4% wound closure 17hrs after wounding. Relative areas of the wound were measured in three independent experiments. Standard deviations are shown as error bars (n=3). (C and D) PATJ and actin were immunostained 6hrs after wounding in wild type (C) and CRB3 RNAi MDCKII cells (D). Scale bars, 10 μ m.

Supplemental figure1

A



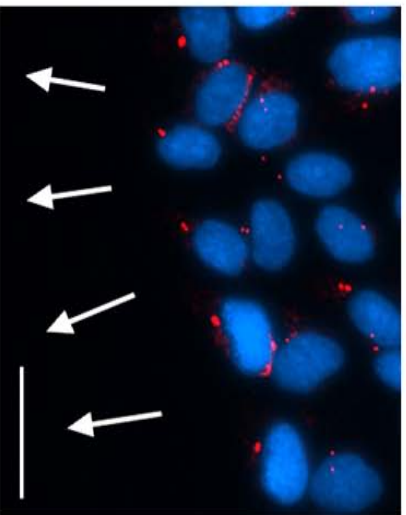
B



Supplemental figure2

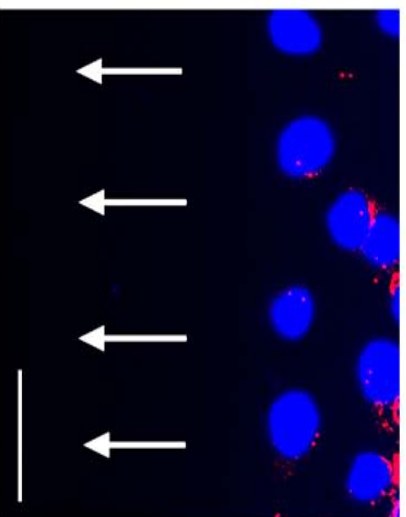
A

WT MDCKII



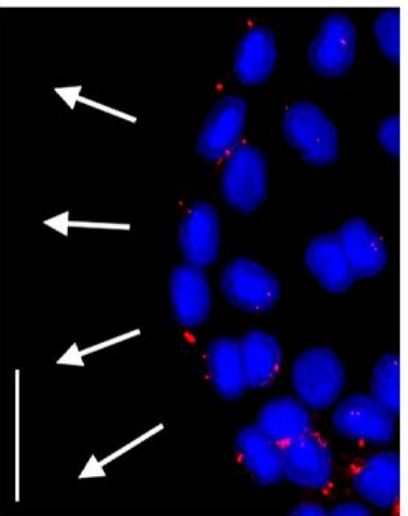
B

PATJ RNAi

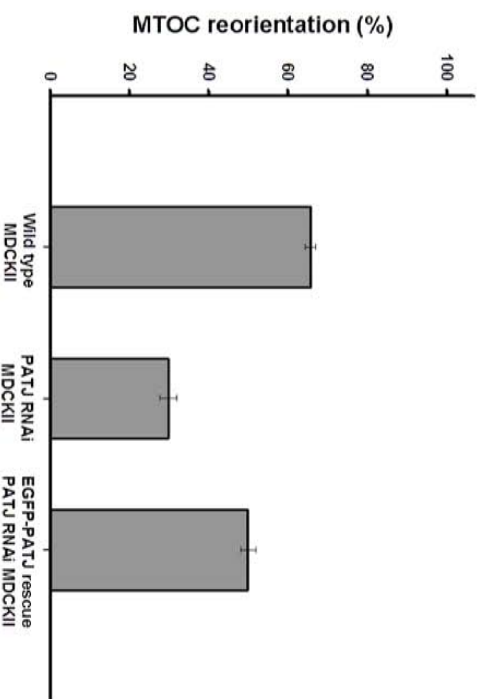


C

EGFP-PATJ rescue



D



Supplemental figure3

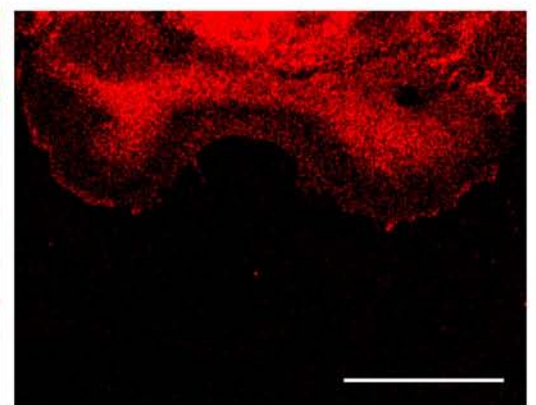
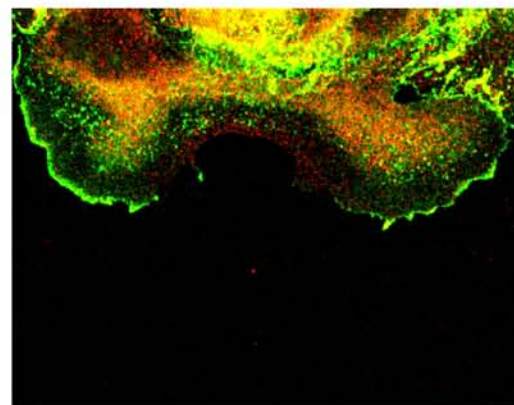
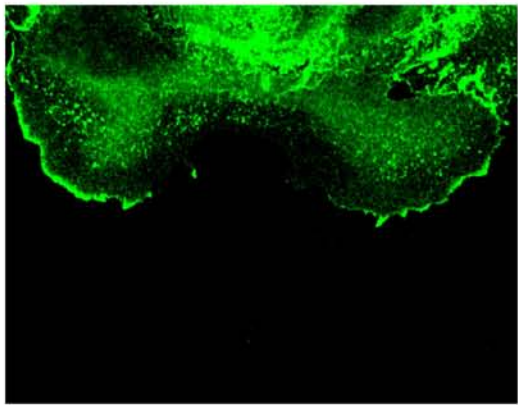
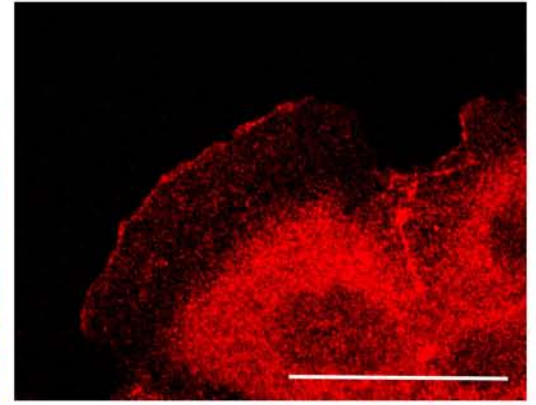
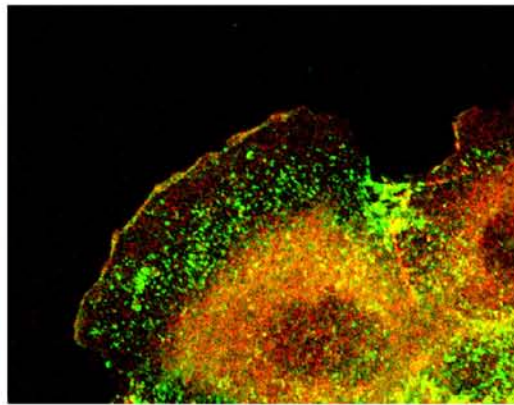
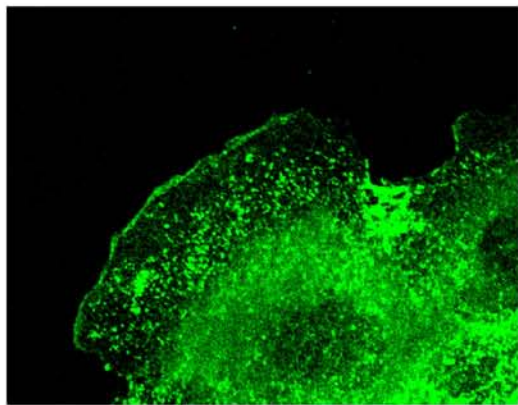
A

WT MDCKII

Cortactin

merge

PATJ



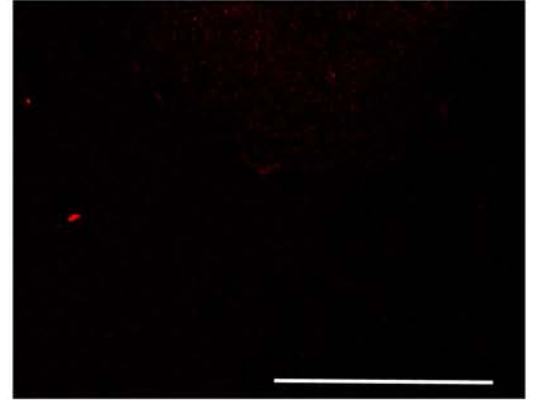
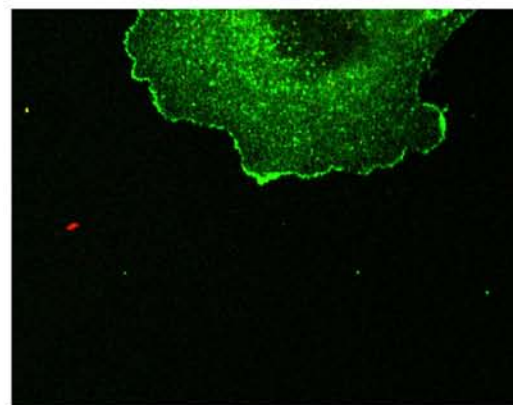
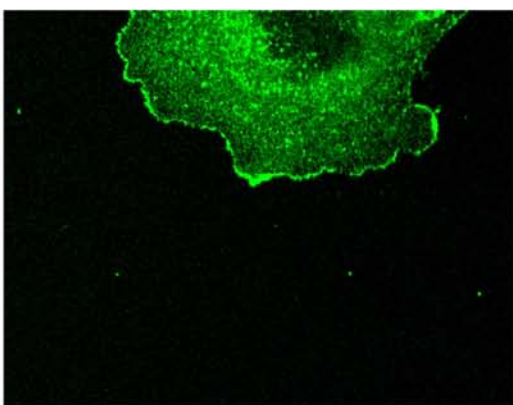
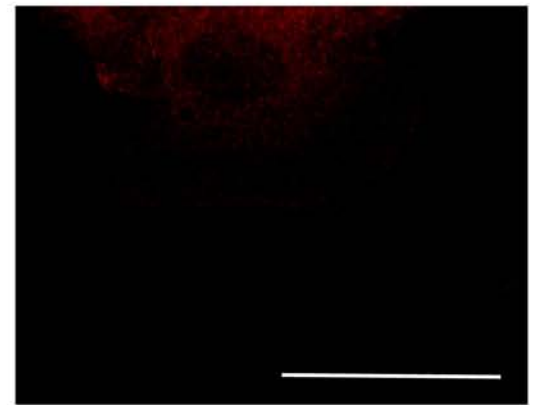
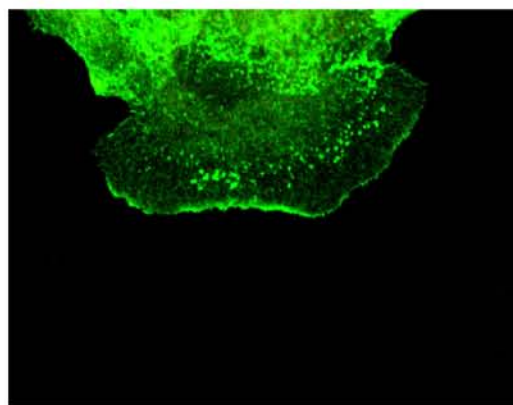
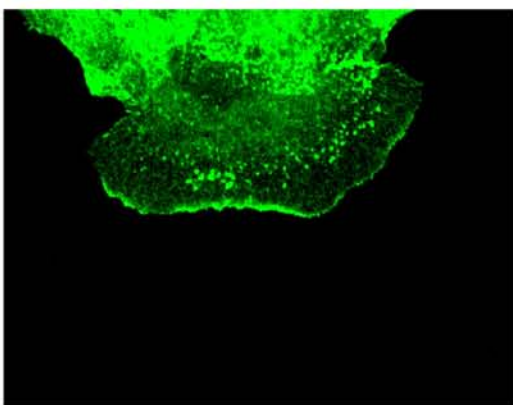
B

PATJ RNAi MDCKII

Cortactin

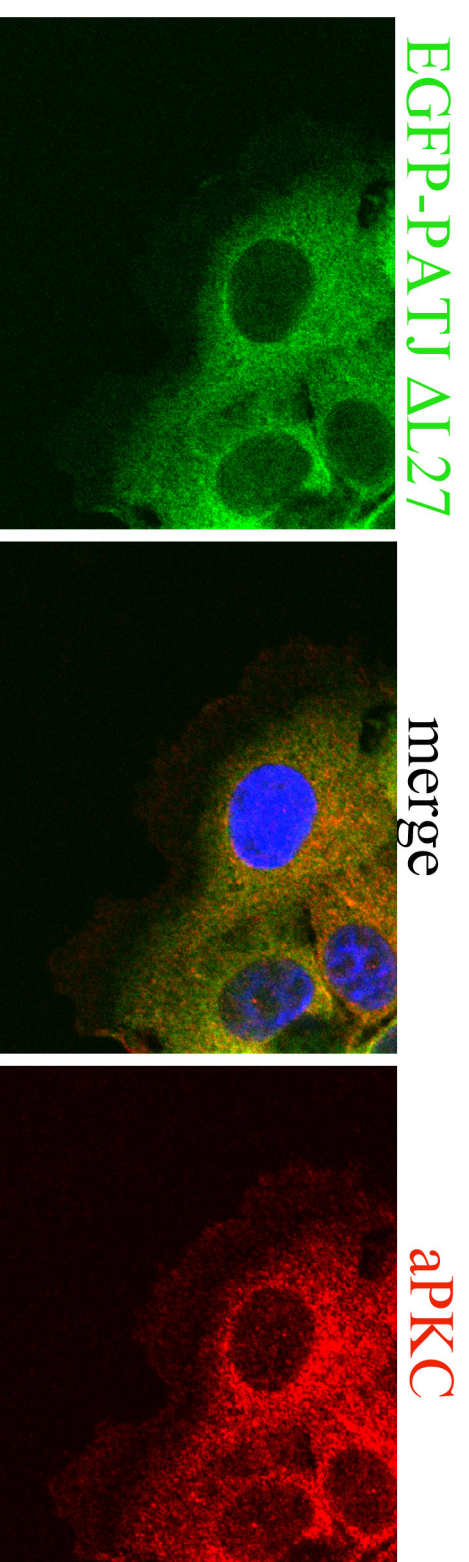
merge

PATJ

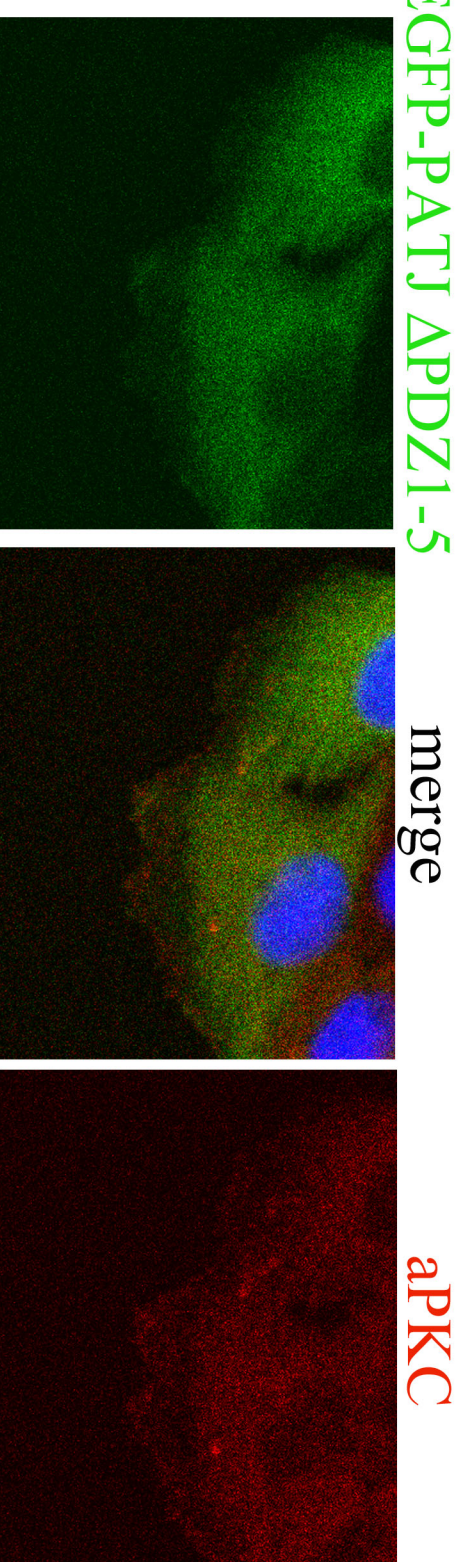


A

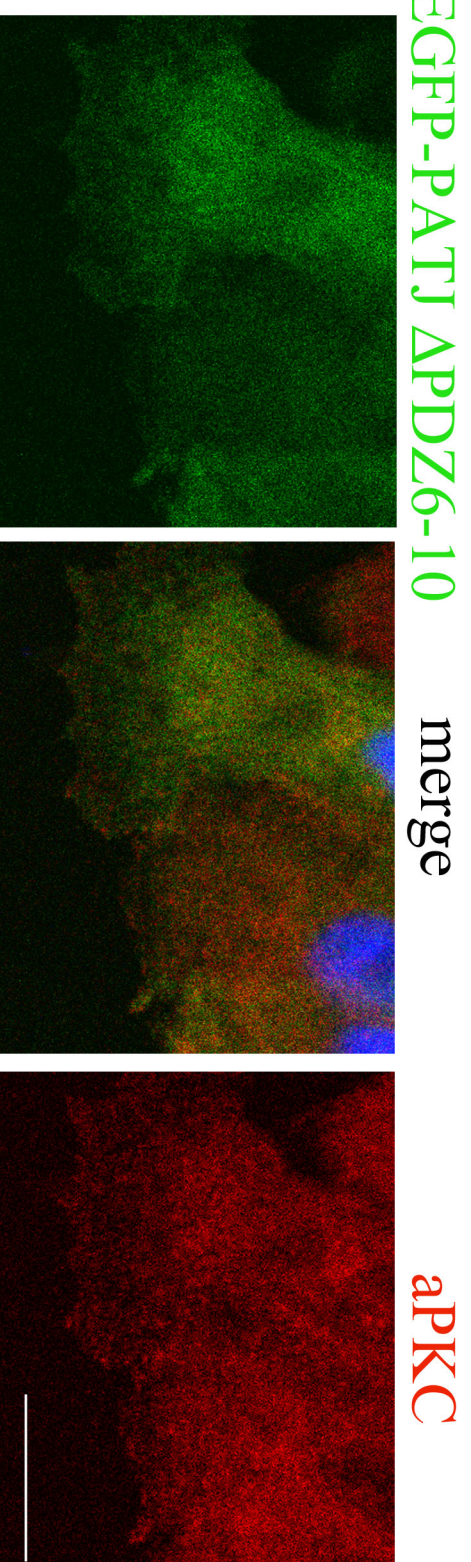
EGFP-PATJ ΔL27 rescue PATJ RNAi MDCKII



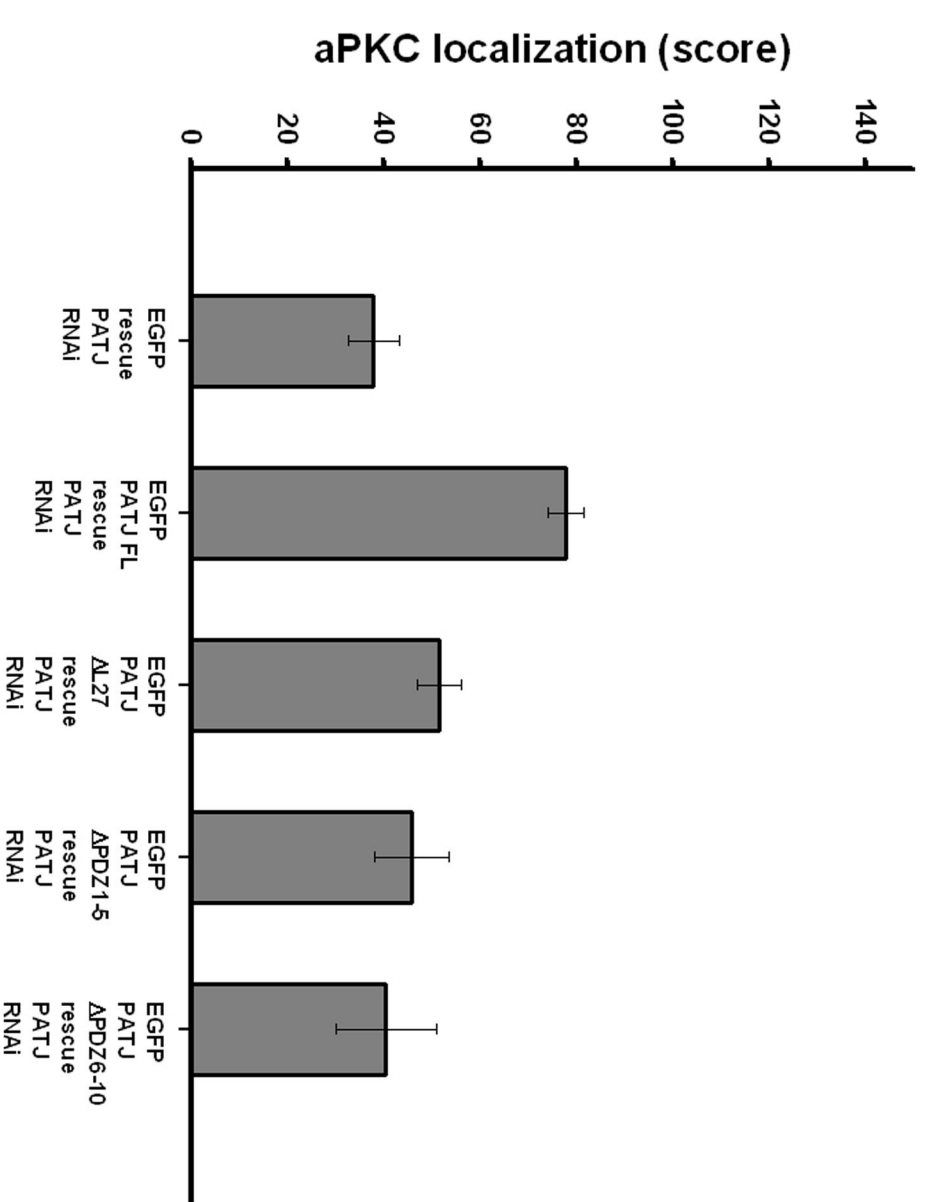
EGFP-PATJ ΔPDZ1-5 rescue PATJ RNAi MDCKII



EGFP-PATJ APDZ6-10 rescue PATJ RNAi MDCKII

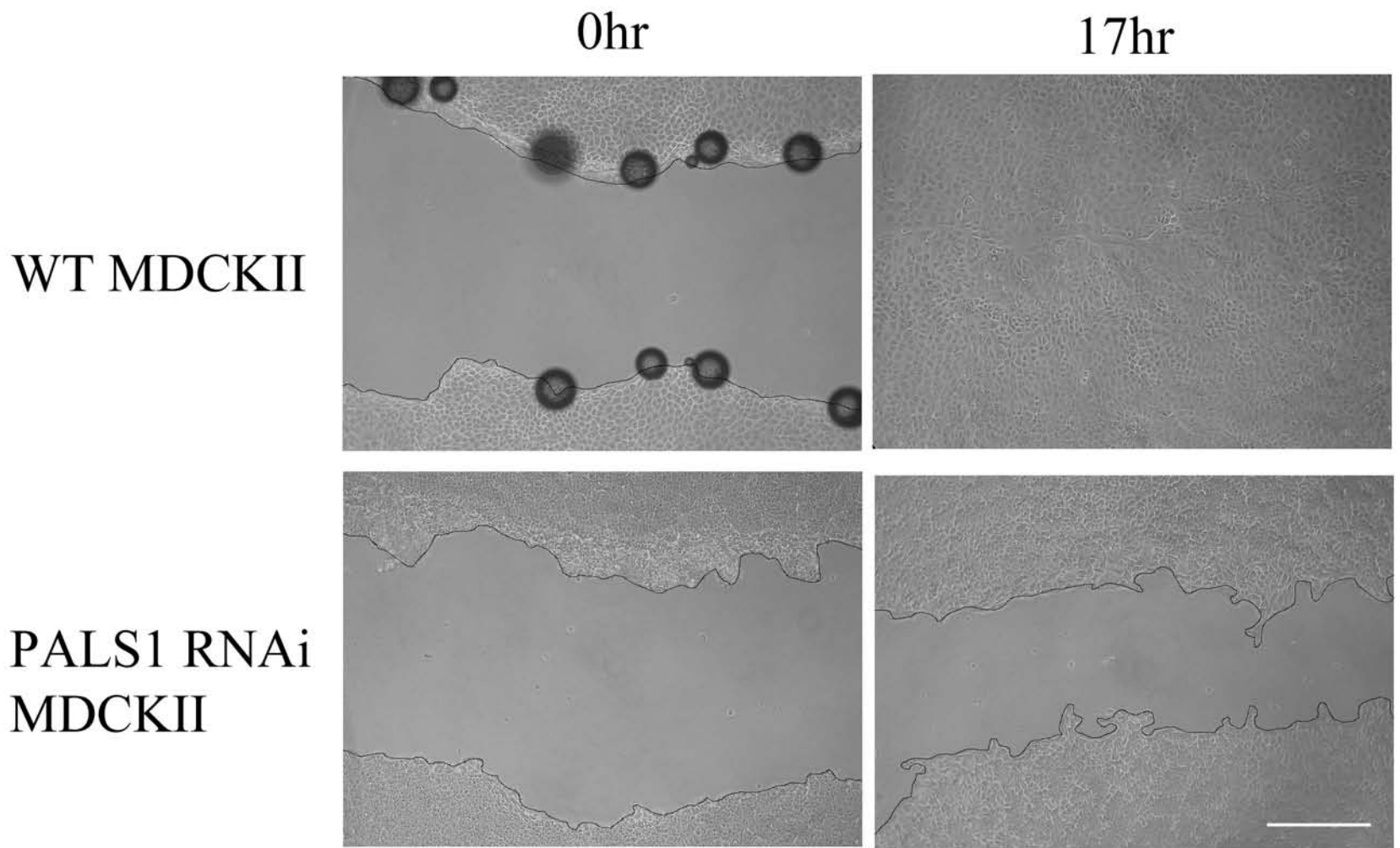


B

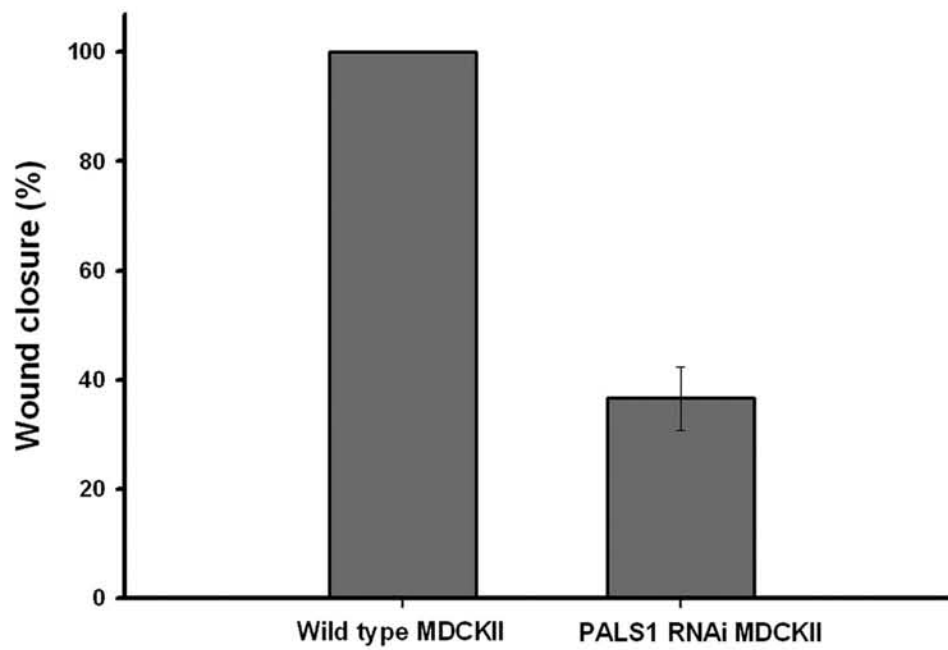


Supplemental figure 5

A

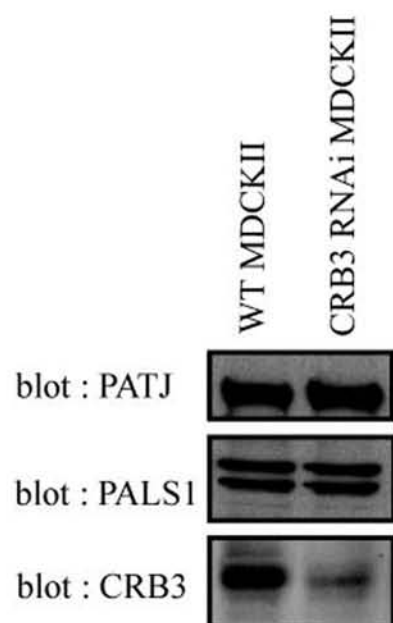


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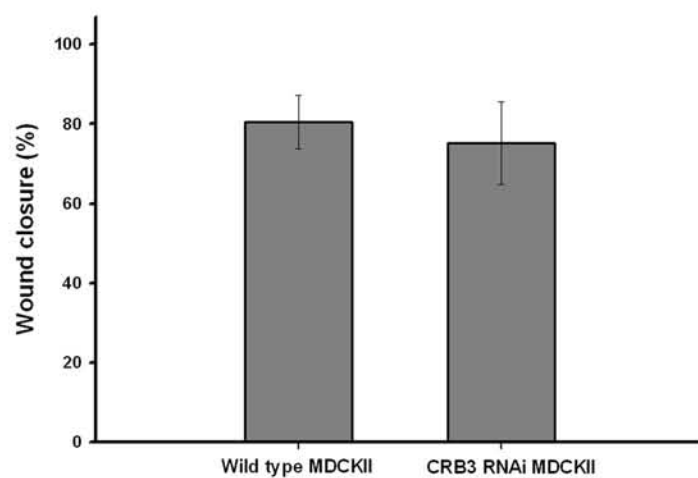


Supplemental figure6

A

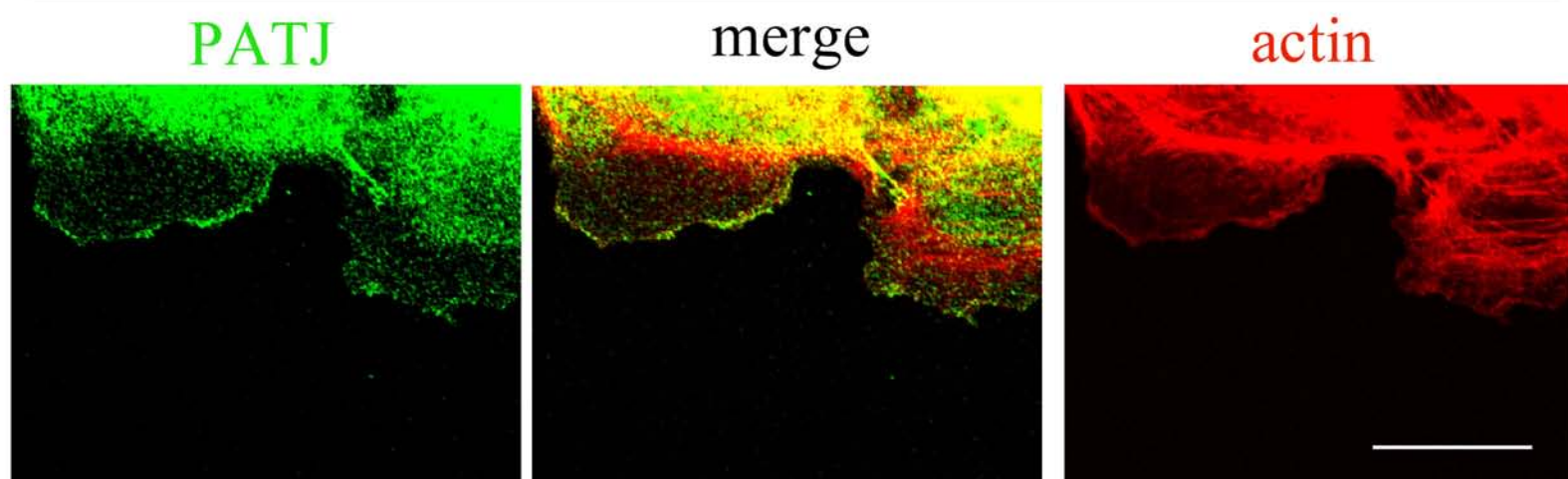


B



C

WT MDCKII



D

CRB3 RNAi MDCKII

