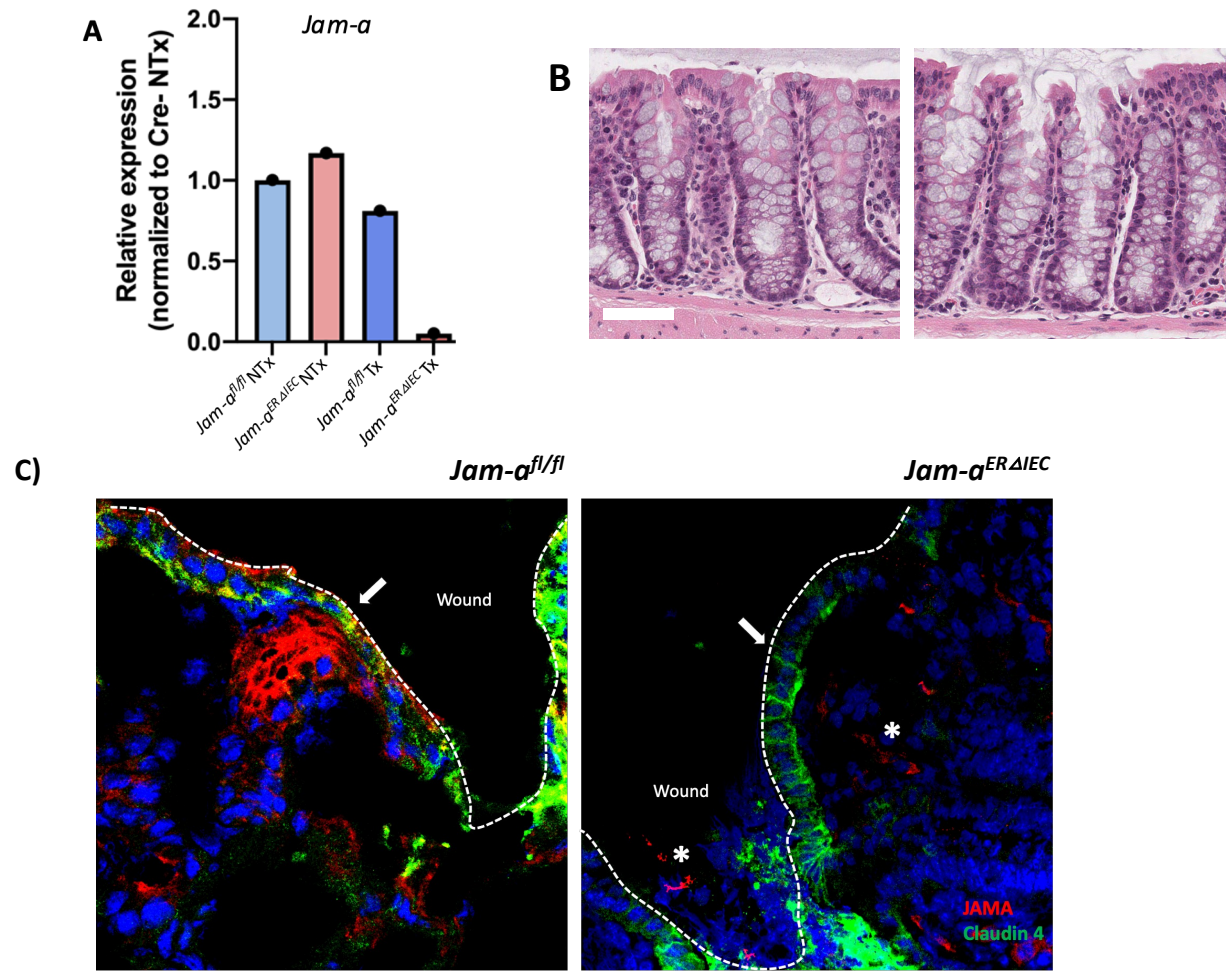
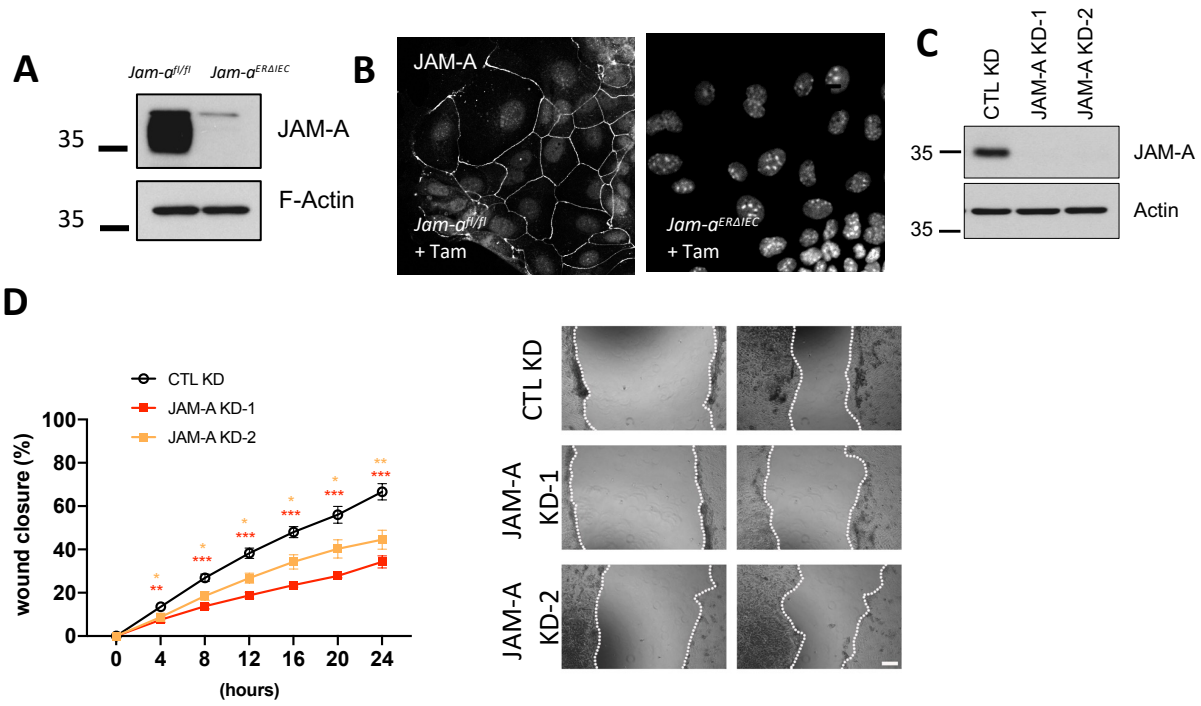
Supplemental Figure 1



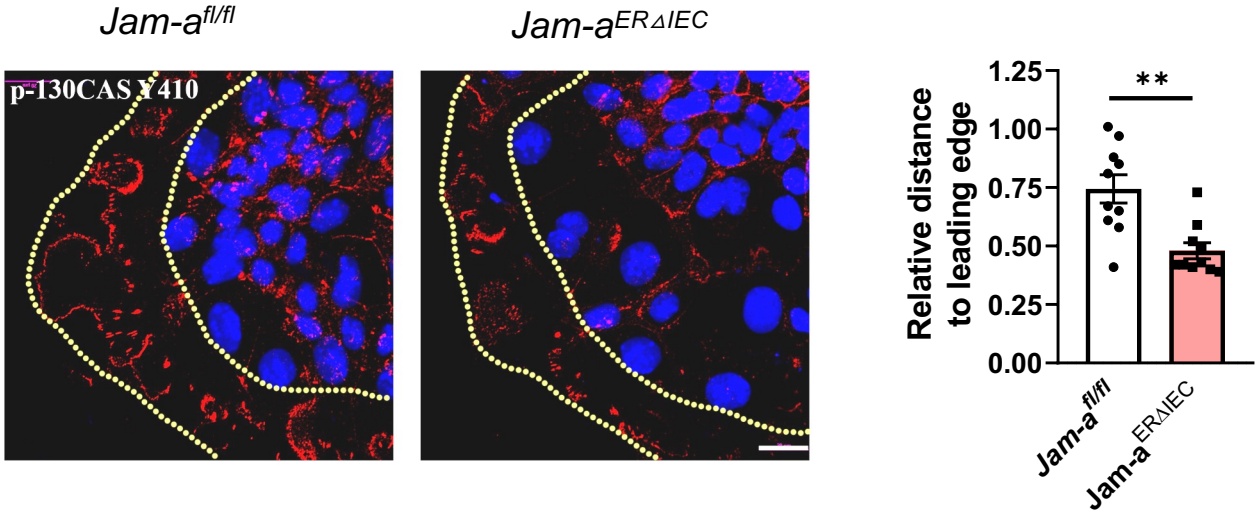
Supplementary Figure 1. Inducible epithelial JAM-A deficient mice. A) Jam-a mRNA relative expression showing tamoxifen treatment of *Jam-a*$^{ER\Delta IEC}$ mice leads to loss of JAM-A expression in IEC. **B)** Colonic mucosa of *Jam-a*$^{fl/fl}$ and *Jam-a*$^{ER\Delta IEC}$ tamoxifen-treated mice was embedded in Paraffin, sectioned and stained with Hematoxylin and Eosin for evaluation of histology and mucosal damage. Crypt architecture and mucosal composition are grossly normal following tamoxifen-induced epithelial loss of JAM-A (*Jam-a*$^{ER\Delta IEC}$ +Tam) when compared to Jam-a$^{fl/fl}$ Tx controls. C) Immunofluorescence showing JAM-A expression in *Jam-a*$^{fl/fl}$ and *Jam-a*$^{ER\Delta IEC}$. Arrows indicate epithelial JAM-A expression, asterisks show lamina propria expression. Scale bar = 100 μm.

Supplemental Figure 2



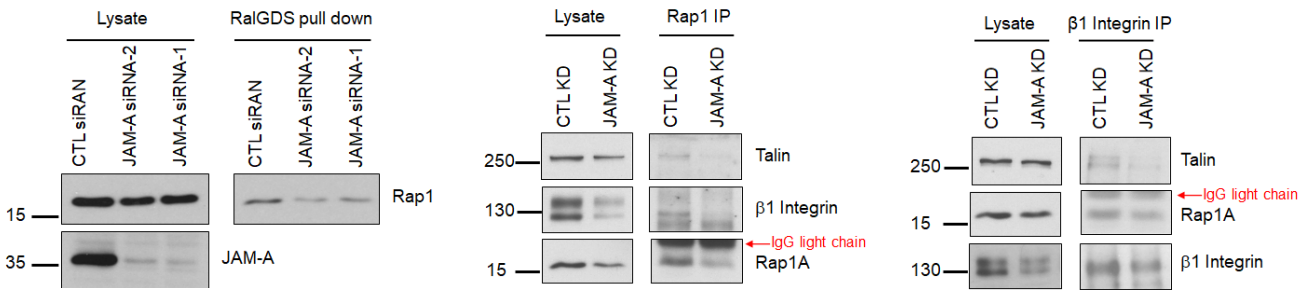
Supplementary Figure 2. In-vitro cultures of JAM-A IEC. Tamoxifen treatment of *Jam-a*$^{ER\Delta IEC}$ abolishes epithelial JAM-A expression in colonic mucosa **(A)** and in primary 2D cultures **(B)**. *Jam-a*$^{fl/fl}$ *derived* tissue and culture were used as controls. To corroborate in-vitro results from primary murine cells, JAM-A was knocked down in model human epithelial cells SKCO-15 **(C)**. Wound healing results are analogous to those obtained with murine primary cells where JAM-A silencing decreases wound repair **(D and E).** Data are representative of three independent experiments with at least 7 samples per group. Results are presented as means ± SEM. ****$p < 0.01$.

Supplemental Figure 3



Supplementary Fig 3. JAM-A deficiency results in IEC spreading defects. (Left) Spread 2d colonoids were grown on chamber slides for 48 h, fixed and stained for anti-p-p130CAS Y410 with DAPI (Blue) to visualize nuclei. Scale bar = 25 μm. (Right) Quantification of distance to leading edge in spreading 2D colonoids derived from JAM-A *Jam-a*$^{fl/fl}$ and *Jam-a*$^{ER\Delta IEC}$ mice.

Supplemental Figure 4



Supplementary Fig 4. Rap1A GTP was subjected to pull down with Rags RBD beads from lysates of spread SK CO-15 IEC. Immunoblots for Rap1A reveal less Rap1A GTP in JAM-A depleted SK CO-15 compared to control knockdown (left). Lysates from spread SK CO-15 cells after JAM-A knockdown or control knockdown were immunoprecipitated with anti-Rap1A (middle panel) or anti-β1 Integrin (right panel) antibodies followed by immunoblot for anti-talin, β1 integrin and Rap1A antibodies to evaluate composition if a functional protein complex containing Rap1A/talin/β1 integrin. Loss of JAM-A in SK CO-15 cells results in disruption of a Rap1A/talin/β1 integrin complex compared to controls.

Supplemental Table 1

Antibody name	Species	Company	Catalog Number
JAM-A	goat	R & D Systems	AF1077
JAM-A	mouse	In-house – Clone J10.4	N/A
JAM-A	rabbit	Invitrogen	36-1700
p130CAS	rabbit	Cell Signaling Technology	13846
p-p130CAS Y410	rabbit	Cell Signaling Technology	4011
Talin	mouse	Sigma-Aldrich	T3287
β1 Integrin	rabbit	Millipore	AB1952
β1 Integrin	rat	R & D system	MAB2405
Paxillin	rabbit	Abcam	Ab32048
FAK	rabbit	Cell Signaling Technology	Rap1A
p-FAK Y861	rabbit	Millipore	PS1008
Rap1A	rabbit	ThermoFisher	PA5-21457
Rap1	rabbit	Millipore	07-916
Src	rabbit	Cell Signaling Technology	2123
p-Src Y416	rabbit	Cell Signaling Technology	6843
Calnexin	rabbit	Sigma-Aldrich	C4731
β-Actin	mouse	Millipore	T6557