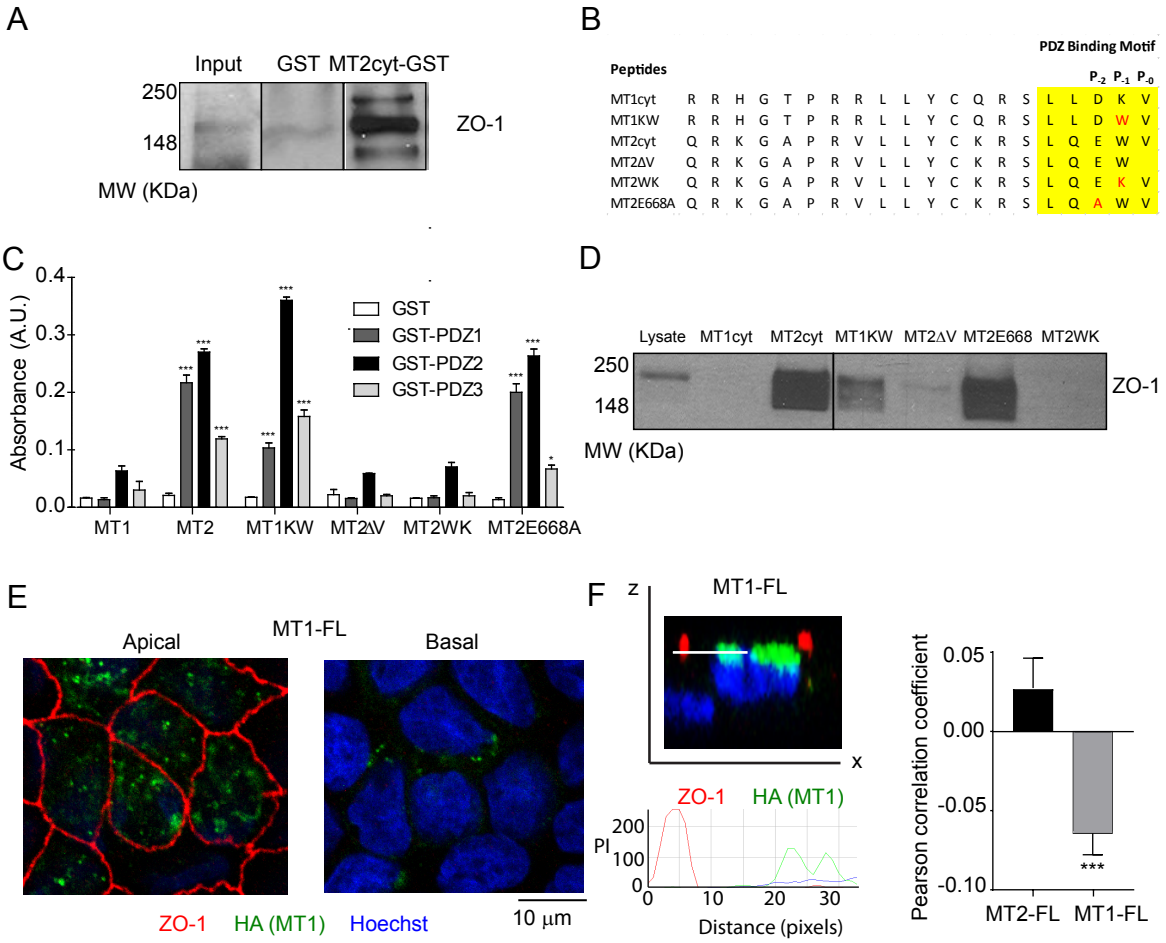

Figure S1. MT2-MMP associates with ZO-1. A, Pull-down, mass spectrometry, and precipitation assays with GST fusion protein are shown for MT2-MMP and ZO-1 interaction in HUVECs. **B,** Several MT2-MMP and MT1-MMP mutated peptides were shown. **C,** ELISA assay for analysis of interaction of the mutated peptides from B, with ZO-1 GST-PDZ domain peptides. **D**, Pull-down assay for interaction of the mutated peptides with ZO-1 is shown. Cytosolic tail (cyt), tryptophan (W), C-term valine (V), glutamic acid, Alanine (A), lysine (K). MW: molecular weight. **E**, Representative maximal projections are shown from apical and basal stacks of confocal sections from polarized MT1-MMP MDCK transfectants (MT1-FL) stained for HA (MT1-MMP, green), ZO-1 (red) and nuclei (Hoechst, blue). **F**, Orthogonal xz view from images in **E** (top) and peak intensity profile (bottom) from the line marked in the xz view (white) are shown to the left. Graph to the right shows the Pearson correlation coefficient of MT1-FL and ZO-1 compared to MT2-FL. n=20 cells in two images. *** p<0,001.

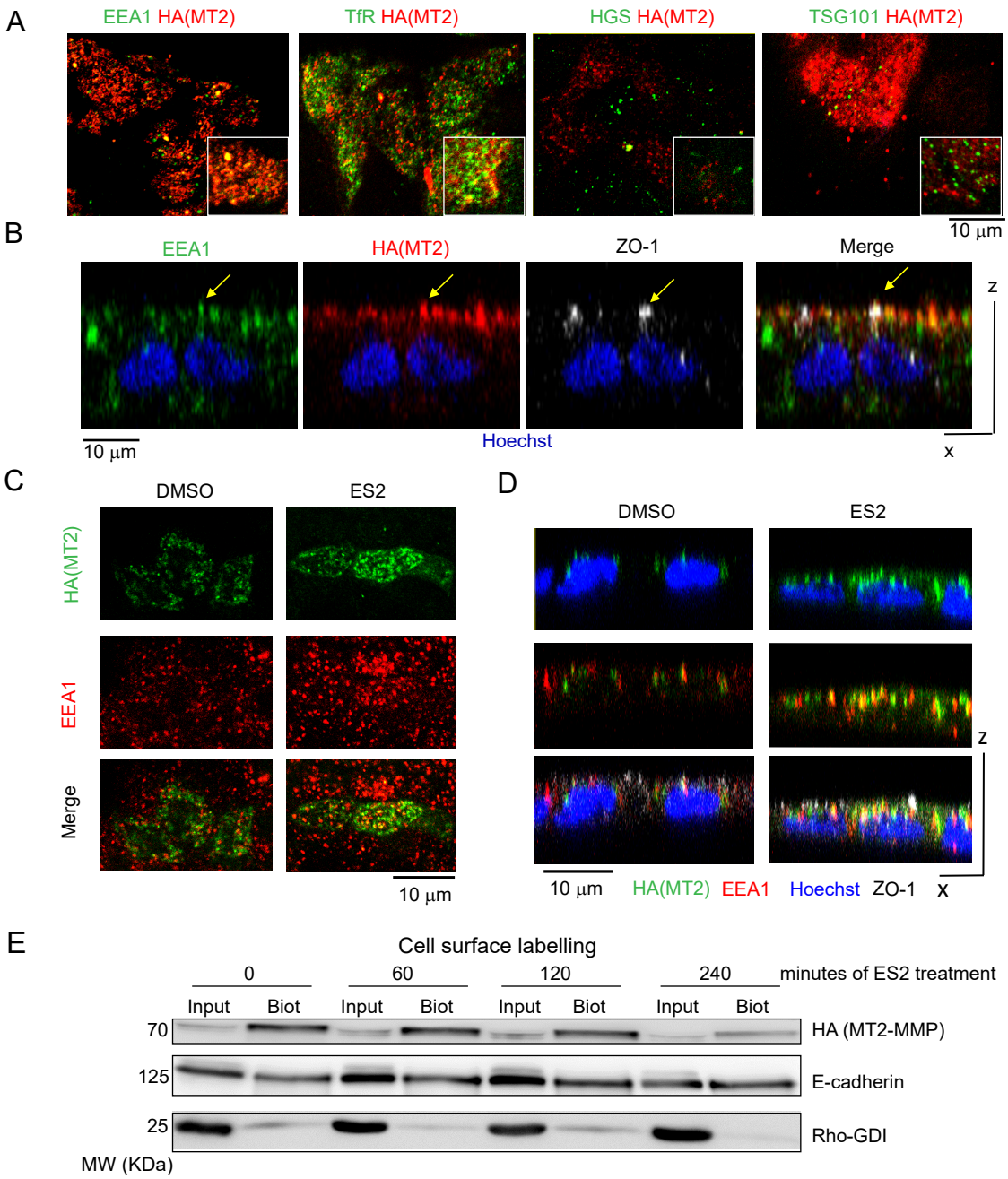

Figure S2. MT2-MMP localizes in early/recycling endosomes. A, Representative apical confocal sections from MT2-MMP MDCK transfectants stained for HA (MT2-MMP, red) and EEA1, TfR, HGS or TSG101 (green). A higher magnification is shown in the insets. **B**, Orthogonal views from MT2-MMP MDCK transfectants stained for HA (MT2-MMP, red) EEA1 (green), ZO-1 (white), and nuclei (Hoechst, blue).**C**, Representative apical confocal sections from MT2-MMP MDCK transfectants stained for HA (MT2-MMP, green) and EEA1 (red) left untreated (left) or treated with endosidin2 (ES2; 40 μM 4 hours). **D**, Orthogonal views of MT2-MMP MDCK cells treated and stained as in C and for ZO-1 (white) and nuclei (Hoechst, blue). **E**, Western blot from biotin-labeled lysates from MT2-MMP MDCKs treated with ES2 for different time points developed for HA (MT2-MMP), E-cadherin and Rho-GDI. MW: molecular weight.

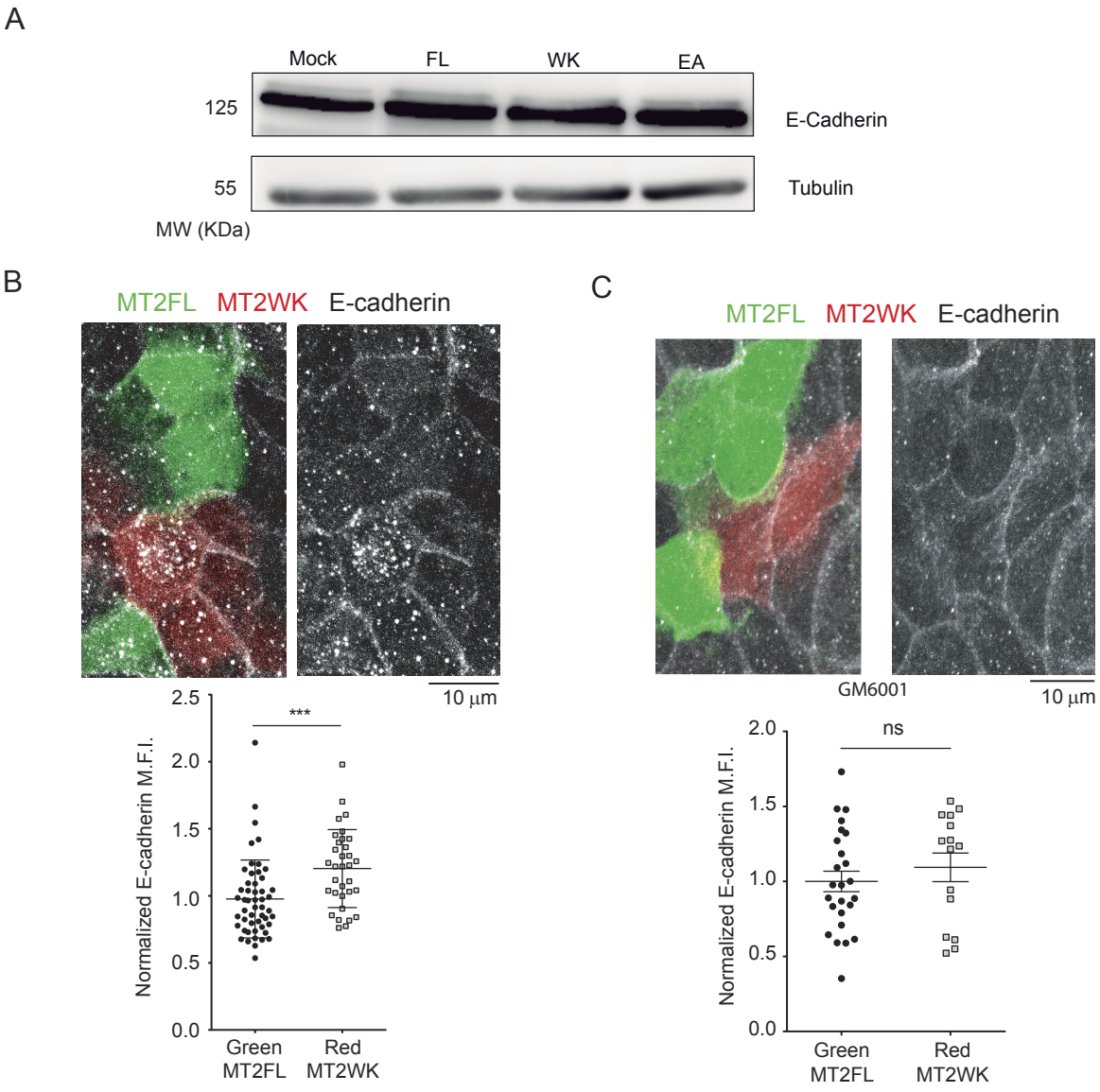

Figure S3. Impact of MT2-MMP in total and local expression of E-cadherin in MDCK cells. **A**, Western blot of E-cadherin in cell lysates from MDCK transfectants; tubulin is included as loading control. MW: molecular weight. **B**, Representative maximal projections from confocal images of polarized co-cultures of MT2FL-GFP (green) and MT2WK-mKate2 (red) MDCK cells stained for E-cadherin (white) (top). Bar shows normalized values of E-cadherin average intensity around the junctions. **C**, Same than B in which MDCK cells were treated with the metalloprotease inhibitor GM6001 (50 μM). *** p<0,001

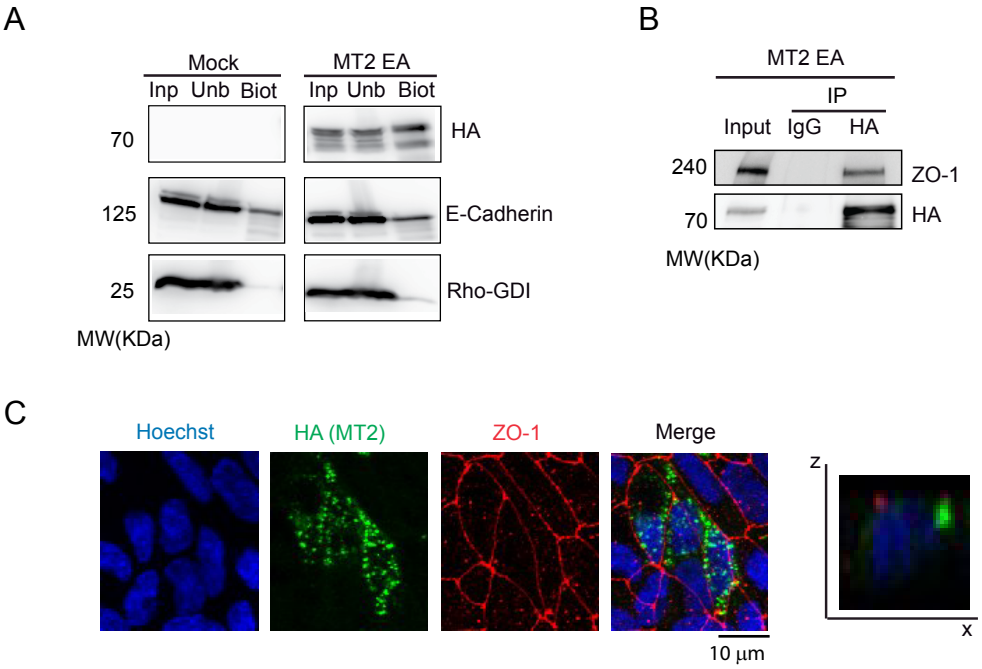

Figure S4. MT2-MMP catalytic mutant (EA) is expressed at the membrane and interacts with ZO-1. A, Western blot for HA, E-cadherin and Rho-GDI is shown of biotinylated cell lysates from Mock and MT2-MMPEA stable MDCK transfectants pulled-down with streptavidin beads; input, unbound and bound fractions are shown (Inp, Unb, Biot). **B**, Western blot is shown for ZO-1 and HA of cell lysates from MT2-MMPEA stable MDCK transfectants pulled-down with anti-HA antibody; IgG immuno-precipitates and whole lysates (Input) are also shown as controls. Mock and MT2FL corresponding controls are presented in Figure 1B. MW: molecular weight. **C**, Representative maximal projections are shown from apical stacks of confocal sections from polarized MT2EA-MDCK transfectants stained for HA (MT2-MMP, green), ZO-1 (red) and nuclei (Hoechst, blue); orthogonal xz views of 3D confocal image stack is shown to the right. Bar, 10 µm.

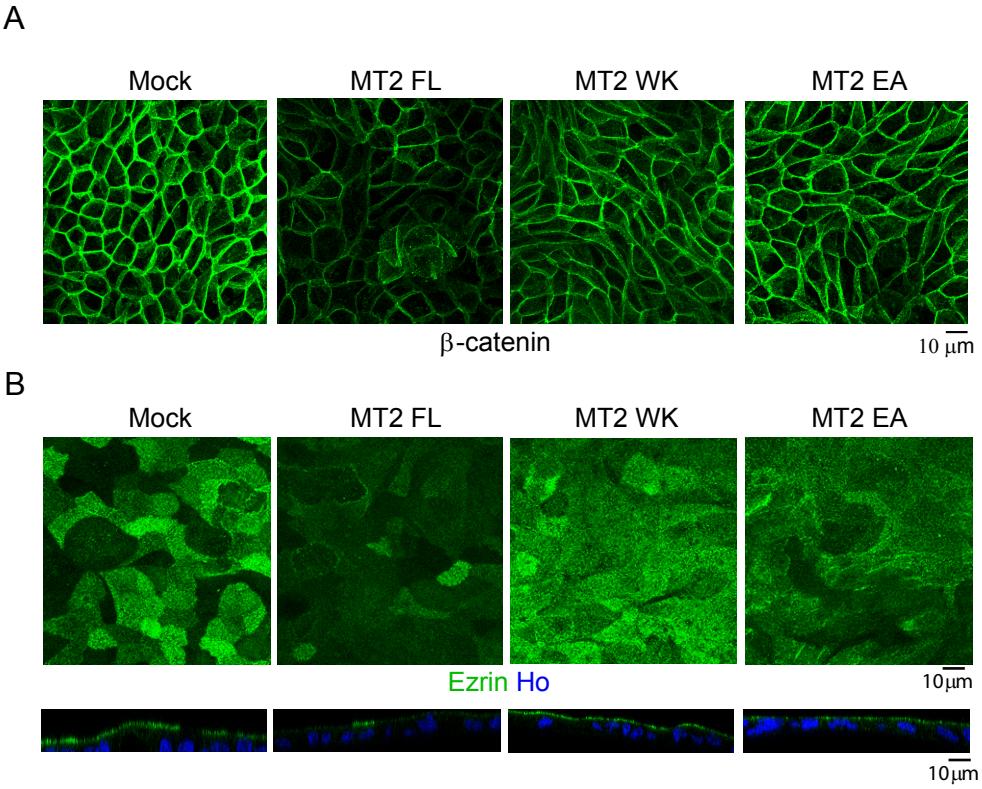

Figure S5. MT2-MMP disrupts apical E-cadherin-associated proteins and signals. **A**, Representative maximal projections are shown from confocal sections of polarized MDCK transfectants stained for β-catenin (green). Bar, 10 μm. **B**, Representative subapical projections (top) and orthogonal views (bottom) of confocal sections of polarized MDCK transfectants stained for Ezrin (green) and nuclei (Hoechst/Ho, blue). Bar, 10 μm.

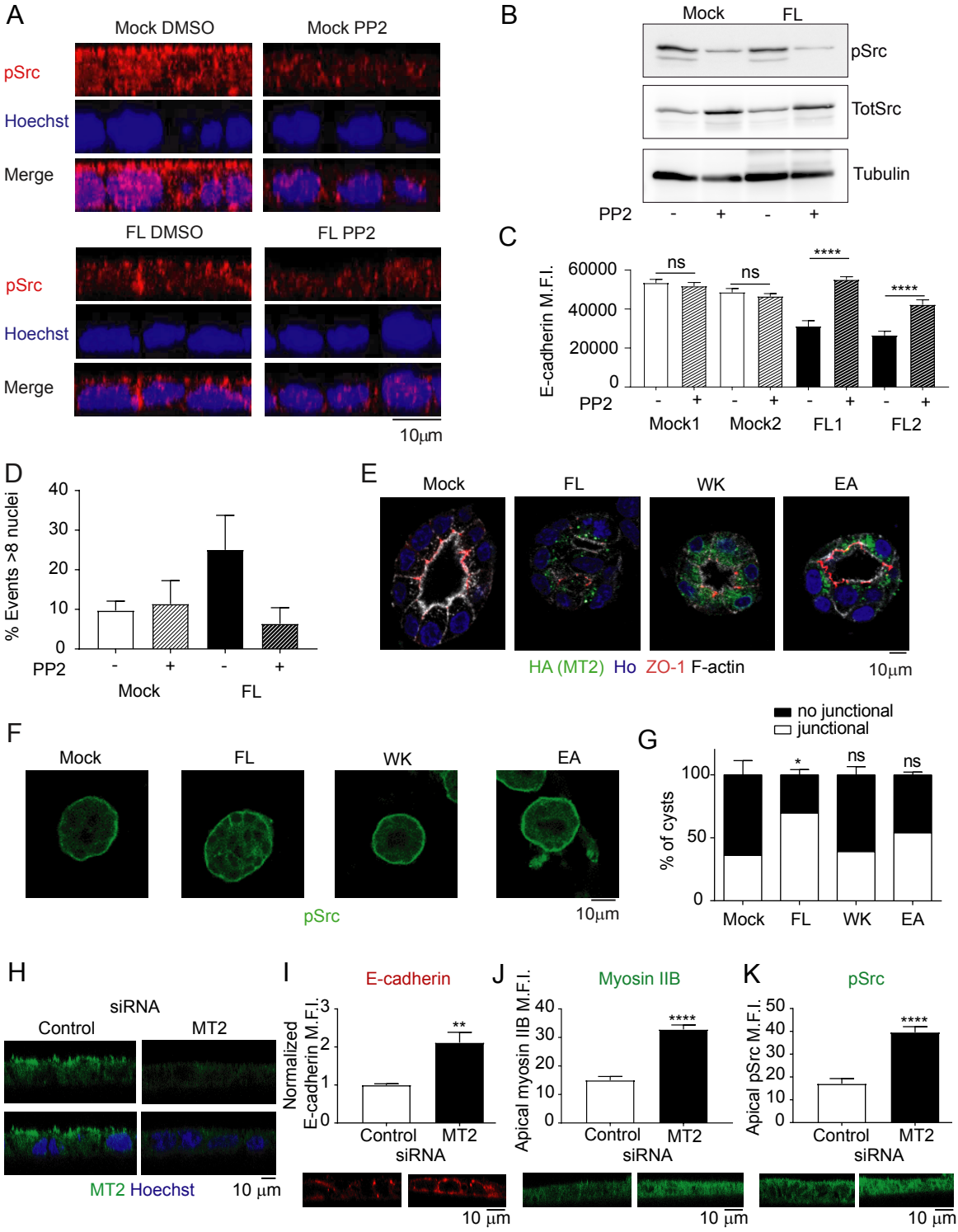

Figure S6. MT2-MMP expression changes pSrc subcellular location in MDCK cells and in Caco2 cells interfered for MT2-MMP.
A, Representative orthogonal views of polarized MDCKs treated with PP2, and stained with pSrc and Hoechst. **B**, Western Blot analysis of pSrc levels and inhibition by PP2 in MDCK transfectants. **C**, E-cadherin average mean fluorescence intensity (MFI), around the junctions formed by MDCK transfectants treated with PP2 or vehicle (DMSO); n= 4. **D**,Percentages of events with more than 8 cells in mock and MT2 FL MDCK stable transfectants after PP2 or vehicle (DMSO) treatment; n=5. **E**, Representative images of MDCK cysts embedded in matrigel stained with HA (MT2-MMP), Hoechst, ZO-1 and F-actin. **F**, Representative MDCK cysts embedded in matrigel stained with pSrc. **G**, Quantification of the percentaje of cysts with a clear junctional pSrc staining. n= 3 independent experiments with more than eighty cyst analysed. **H**, Representative orthogonal images of Caco2 cells interfered for MT2-MMP, showing MT2 staininig (green) and Hoechst (blue). **I**, E-Cadherin quantification and representative orthogonal images of Caco2 cells interfered for MT2 (n=15 images). **J**, Myosin IIB quantification and representative orthogonal images of Caco2 cells interfered for MT2 (n= 10 images). **K**, pSrc quantification and representative orthogonal images of Caco2 cells interfered for MT2 (n= 8 images). * p<0,05; ** p<0,01 ; *** p<0,001; **** p<0,0001.

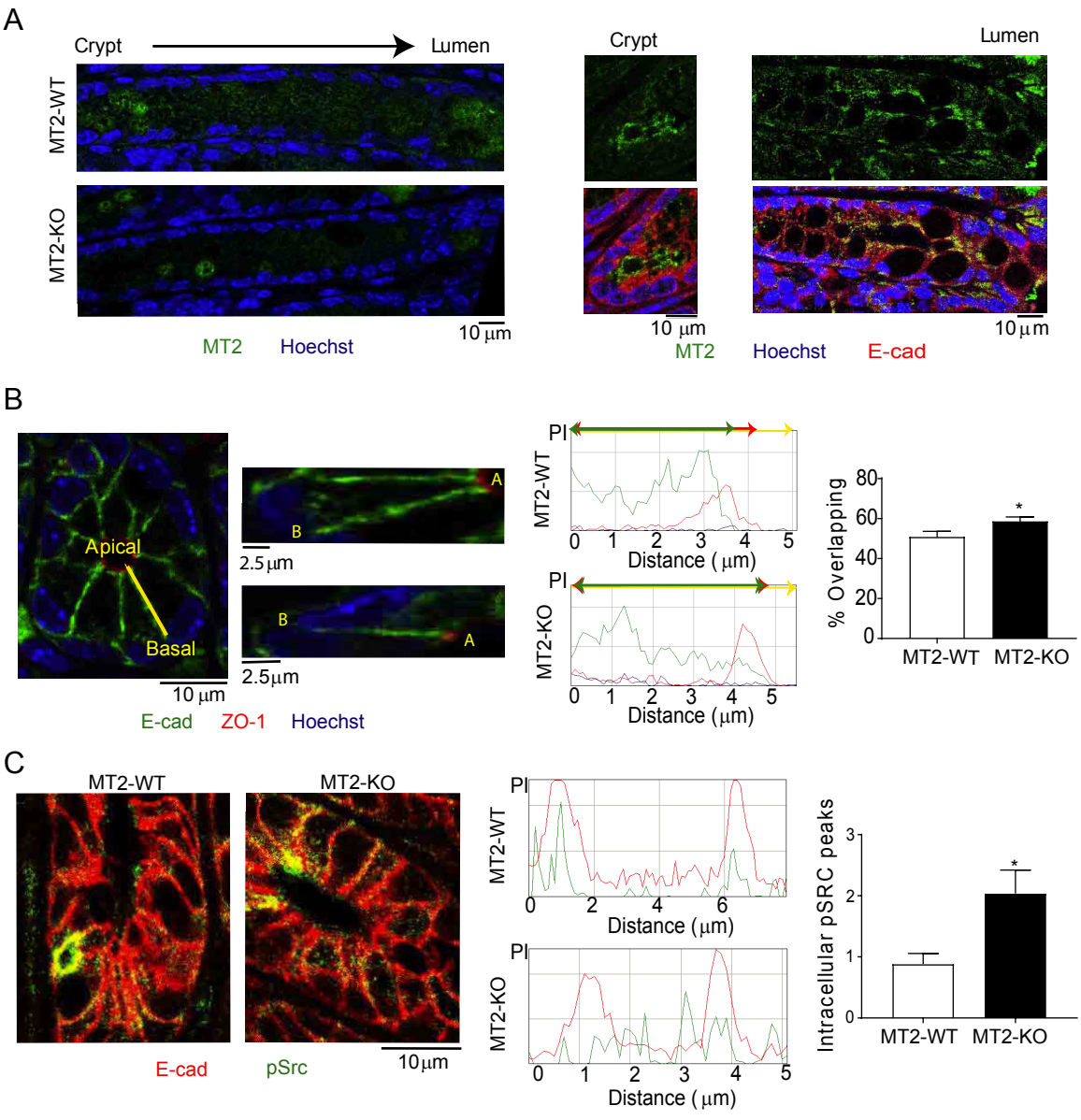

Figure S7. MT2-MMP deficiency alters mouse colon crypts. A, Representative cropped images from crypts (left) and lumen (right) of colons from wild-type (MT2-WT) and MT2-MMP-null (MT2-KO) mice stained for MT2-MMP (green), E-cadherin (red), and nuclei (Hoechst, blue); note that MT2-MMP seems to be enriched at the apical side of epithelial cells. **B**, A representative image of a colon crypt stained for E- cadherin (green), ZO-1 (red), and nuclei (Hoechst, blue) is shown to the left; the yellow arrow indicates the basal to apical junctional line selected for image analysis. Similar images from wildtype and MT2-MMP-null colons are shown (middle) with the peak intensity profiles for E-cadherin (green) and ZO-1 (red), and the junction in yellow. The bar graph on the right shows the percentage of E-cadherin/ZO-1 overlapping in junctions of epithelial cells in colons from wild-type (MT2-WT) and MT2-MMP-null (MT2-KO) mice. 10 junctions were quantitated per condition in 3 images from 2 mice per genotype. **C**, Representative images of a crypt from wild-type (MT2-WT) and MT2-MMP-null (MT2-KO) colons stained for E-cadherin (red) and pSrc (green) (left). Peak intensity profiles for pSrc (green) and ZO-1 (red) are shown in the middle. The bar graph on the right shows the quantitation of intracellular pSrc peaks per cell. 7 profiles were quantitated per condition with at least 3 cells in the profile, from 2 mice per genotype. * p<0,05.

P1 position	Residues	PWM ^ Score	Sec Struct pred	Disorder	Transmemb domain	N-mass	C-mass
20	PFPKN-LVQIK	4.93	_____EEEEE	..***.....	OOOOOOOOOO	2266.06	77961.41
59	RETGW-LKVTE	4.92	_____EEEE_	.....	OOOOOOOOOO	6608.33	73619.14
93	EDPME-IVITV	1.71	____EEEEEE	.....	OOOOOOOOOO	10406.18	69821.29
149	AIAYS-ILTQD	2.93	HHHHHHHHH_	.....	OOOOOOOOOO	16279.97	63947.50
155	LTQDP-LLPSS	1.39	HHH_____	.....	OOOOOOOOOO	16947.32	63280.15
160	LLPSS-MMFTI	4.11	_____EE	.....	OOOOOOOOOO	17444.59	62782.88
188	VPMYT-LVVQA	2.23	_____EEEEE	.....	OOOOOOOOOO	20515.09	59712.38
195	VQAAD-LQGEG	5.08	EEEEHHHHH_	.....	OOOOOOOOOO	21211.48	59015.99
241	VEIAV-LKVTD	2.72	_HHHHHHHHH	.....	OOOOOOOOOO	26074.95	54152.52
255	DTPAW-RAVYT	1.88	_____EE	.....	OOOOOOOOOO	27583.71	52643.76
257	PAWRA-VYTIL	2.38	_____EEEE	.....	OOOOOOOOOO	27810.85	52416.62
297	KQQYV-LYVTV	3.03	____HHEEEE	.....	OOOOOOOOOO	32352.09	47875.38
377	RDAAG-WLEVN	1.91	HHHHHHHHH_	.....	OOOOOOOOOO	41213.46	39014.01
378	DAAGW-LEVNP	4.22	HHHHHHHH__	.....	OOOOOOOOOO	41399.54	38827.93
409	STYEA-LIIAI	2.65	_H_____EE	.....	OOOOOOOOOO	44947.20	35280.27
445	PEPRN-MDFCQ	2.46	_____	.....	OOOOOOOOOO	48584.09	31643.38
459	PHVIN-IIDPD	3.24	_____EE	.....	OOOOOOOOOO	50235.84	29991.63
497	RESLI-LKPKK	2.80	EE_____HH	.....	OOOOOOOOOO	54410.85	25816.62
512	DYKIN-LKLTD	1.63	____HH____	.....	OOOOOOOOOO	56151.82	24075.65
546	KRTAP-YAEAG	1.32	___E_____	.....	OOOOOOOOOO	59886.62	20340.85
548	TAPYA-EAGLQ	1.42	_E_____	.....	OOOOOOOOOO	60120.72	20106.75
551	YAEAG-LQVPA	4.37	_____HH	.....	OOOOOOOOOM	60377.82	19849.65
559	PAILG-ILGGI	2.24	HHHHH__HHH	.....	OMMMMMMMMM	61169.30	19058.17
563	GILGG-ILALL	1.48	H__HHHHHHH	.....	MMMMMMMMMM	61509.50	18717.97
566	GGILA-LLILI	4.18	HHHHHHHHHH	.....	MMMMMMMMMM	61806.70	18420.77
567	GILAL-LILIL	2.74	HHHHHHHHHH	.....	MMMMMMMMMM	61919.78	18307.69
569	LALLI-LILLL	2.52	HHHHHHHHHH	.....	MMMMMMMMMM	62145.94	18081.53
676	PPYDS-LLVFD	1.41	HHH_____	.....	iiiiiiiiii	74340.98	5886.49
691	SEAAS-LSSLN	2.77	EEEEEE____	**********	iiiiiiiiii	75866.66	4360.81
721	KKLAD-MYGGG	2.81	HHHHHH___H	.....*..**	iiiiiiiiii	79385.21	842.26

Table S1. **List of potential cleavage sites for MT2-MMP (MMP15) in E-cadherin**. Predicted sites for human MT2-MMP-mediated cleavage in canine E-cadherin are shown with their cleavage scores (http://cleavpredict.sanfordburnham.org/). Candidates were further filtered according to the peptide cleavage matrix in the MEROPS database (http://merops.sanger.ac.uk/). The selected cleavage sites in E-cadherin are highlighted in yellow.