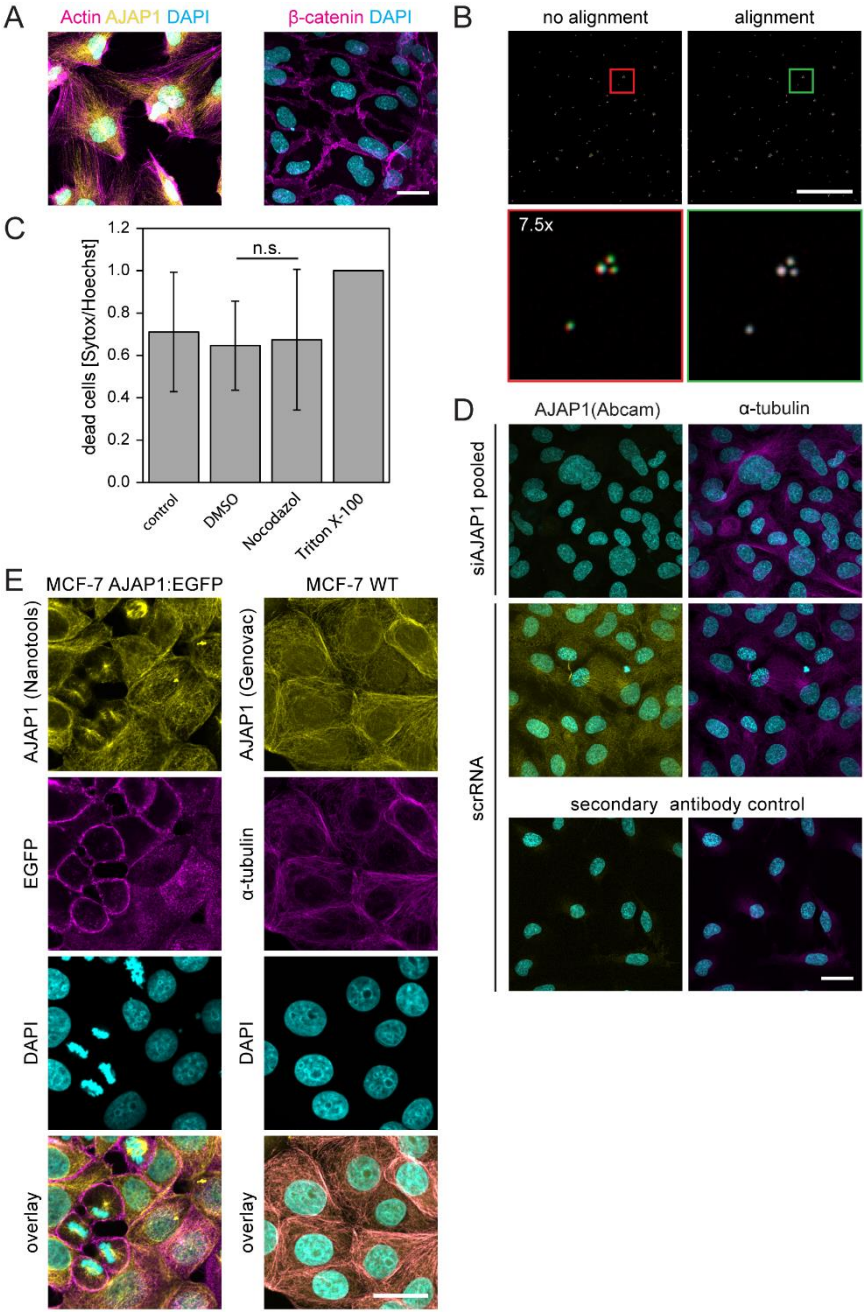
Supplemental material



Supplementary Figure 1: (A) AJAP1 does not co-localize with the actin cytoskeleton or with adherens junctions in HUVECs. Actin cytoskeleton was stained with phalloidin Alexa Fluor 488 (Life Technologies). For detecting adherens junctions HUVECs were stained for β-catenin (Santa Cruz). Microscope: Zeiss LSM 780, objective lens: Plan-Apochromat 63x/1.40 oil, scale bar: 25 µm. (B) The spatial misalignment between spectrally distinct channels is corrected with multicolor tetraspec beads (200 nm). Without channel alignment, the red and green channel show poor overlap. Channel alignment was performed using the Zeiss Zen 2012 Black software and applying the affine alignment procedure. Following channel alignment, the red and green channel overlap completely. Microscope: Zeiss Elyra, objective lens: Plan-Apochromat 63x/1.4 oil, scale bar: 10 µm. (C) Nocodazole does not affect the viability of HUVECs. HUVECs were incubated with 12.5 µM

nocodazole for 24 hours followed by performing a live-dead assay. Cells were incubated with SytoxGreen labelling the dead cells and Hoechst 33342 labelling live as well as dead cells. Fluorescence intensity of either dyes was captured with the Infinite 200 (Tecan) multiwell reader. As positive control, cells were treated with 0.1% Triton X-100. Bars show the means, error bars the SD. Statistical test: two sample t-test, n=11. (D) AJAP1 knockdown verified by immunofluorescence staining. Pooled AJAP1 siRNA or scrRNA transfected HUVECs were fixed and labeled for AJAP1 and α-tubulin. Upon downregulation, AJAP1 is not detectable while the microtubule cytoskeleton remains detectable. Microscope: Zeiss LSM780, objective lens: Plan-Apochromat 63x/1.40 oil, scale bar: 25 µm. (E) Endogenous AJAP1 appears cytoplasmic and associated with microtubules in AJAP1:EGFP overexpressing and wildtype (WT) MCF-7 cells, respectively. The Nanotools antibody does not detect the fluorescent fusion protein, which localizes to the cell circumference. Microscope: Zeiss LSM 780; objective lens: Plan-Apochromat 63x/1.40 oil, scale bar: 25 µm.

Supplementary Table 1: Anti-AJAP1 antibody epitope recognition specificity on human AJAP1 protein isoforms.

anti-AJAP1 antibody	Isoform 1	Isoform 2	Isoform 3
AAS46449C	+	+	-
AAS47449C	+	+	-
Nanotools	+	+	-
Abcam	+	+	+
Genovac F	+	+	+

Supplementary Table 2: Predicted motifs sites identified in AJAP1 by ScanProsite search.

Motif	Position (amino acid)
Protein kinase C phosphorylation site	Phosphoserine: 13-15, 65-67, 80-82, 337-339, 392-394, 403-405 Phosphothreonine: 211-213, 313-315
Aldehyde dehydrogenases glutamic acid active site	273-280
Amidation site	154-157
Casein kinase II phosphorylation site	Phosphoserine: 165-168, 251-254, 342-345, 390-393 Phosphothreonine: 176-179, 186-189, 376-379
N-myristoylation site	171-176, 248-258, 249-254, 386-391
cAMP- and cGMP-dependent protein kinase phosphorylation site	212-215, 314-317
N-glycosylation site	331-334, 351-354