Supplementary Tables

Supplementary Table S1: Primers for cloning podoplanin variants. Template: Plasmid pCMV6-mPdpn-tGFP (Origene, ref. MG201468)

Pdpn Variant	Primer	Sequence	Tm (ºC)
Delta_SP	Fw	ATGGGGACTATAGGCGTGAATGAAGATGA TATTGT	60.5
	Rv	GGGCGAGAACCTTCCAGAAATCTTCTTC	61.4
Delta_CT	Fw	ATGTGGACCGTGCCAGTGTTGTTCTG	61.1
	Rv	AACAACAATGAAGATCCCTCCGACGAAGC	61.5

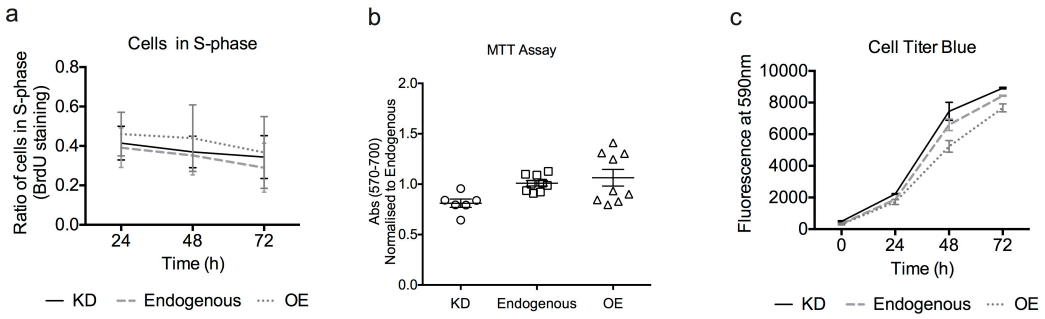
Supplementary Table S2: Primers used for cloning of Caveolin-1. Template: cDNA created from mRNA extracted from B16.F10 cells

	Primer	Sequence	Tm (ºC)
Cav1	Fw	ATGTCTGGGGGCAAATACGTAGACTCC	61.3
	Rv	TATCTCTTTCTGCGTGCTGATGCGGATG	61.4

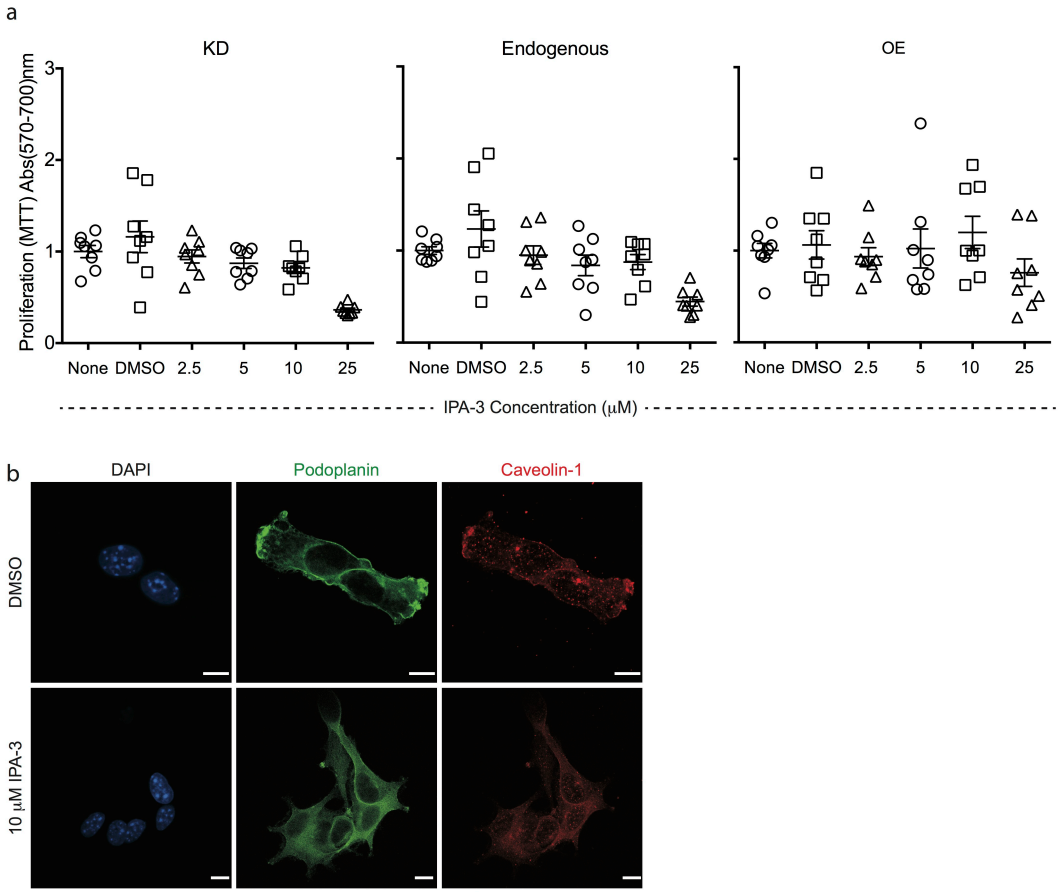
Supplementary Table S3: Antibodies used for protein detection by western blot and immunofluorescence.

Primary/Secondary antibody	Application	Dilution	Supplier
Anti-Podoplanin	WB	1:1000	R&D Systems (ref. BAF3244)
Anti-Podoplanin	Immunofluorescence	1:100	R&D Systems (ref. BAF3244)
Anti-Caveolin-1	WB	1:500	Abcam (ref. 2910)
Anti-Caveolin-1	Immunofluorescence	1:100	Abcam (ref. 2910)
Anti-Rho	WB	1:500	Pierce (ref. 1862332)
Anti-Rac 1	WB	1:500	Pierce (ref. 1862341)
Anti-Cdc42	WB	1:500	Pierce (ref. 1862345)
Anti-PAK1	WB	1:500	Cell Signaling (ref. 2602)
Anti-pPAK1	WB	1:500	Cell Signaling (ref. 9101)
Anti-pERK1/2	WB	1:500	Cell Signaling (ref. 4370)
Anti-ERK1/2	WB	1:500	Cell Signaling (ref. 9102)
Anti-Cdck5	WB	1:500	GeneTex (ref. GTX108328)
Anti-Tubulin	WB	1:2000	Sigma (ref. T6074)
Anti-Goat HRP	WB	1:1000	DakoCytomaton (ref. P0160)
Anti-Rabbit	WB	1:1000	DakoCytomaton (ref. P0399)
Anti-Mouse	WB	1:1000	DakoCytomaton (ref. P0447)

Supplementary Figures

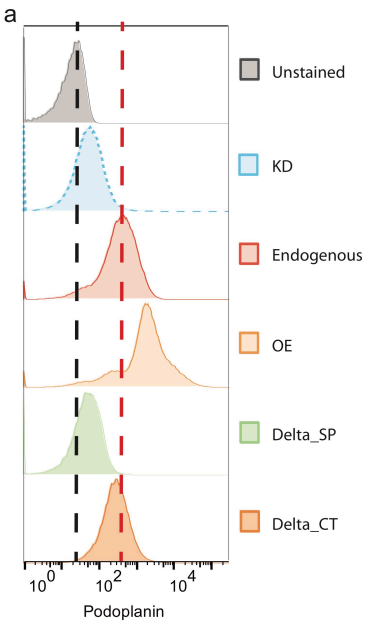


Supplementary Figure S1: a) BrdU proliferation assay for the cells with different podoplanin levels. Data from 2 independent experiments performed in triplicate. b) MTT metabolic assay for the cells with different podoplanin levels. Data from 3 independent experiments performed in triplicate. c) Cell titer blue viability assay for the cells with different podoplanin levels. Representative data from 3 independent experiments. Data presented as Mean ± SEM.



Supplementary Figure S2: a) Proliferation of cells with different levels of podoplanin levels in the presence of increasing concentrations of PAK1 inhibitor IPA-3. Higher concentrations

of IPA-3 impact the proliferative ability of cells (also measured with AnnexinV staining – data not shown). b) Immunofluorescence for podoplanin (green) and caveolin-1 (red) of cells treated with 10 μm IPA-3 or DMSO control. Nuclei counterstained with DAPI (blue).



Supplementary Figure S3: a) Flow cytometry histograms illustrating surface podoplanin expression by the different podoplanin variant cell lines. Red dashed line indicates mean podoplanin levels in endogenous cells; Black line shows unstained control.