Supplementary Materials and Methods

Flow cytometric assessment of cell proliferation

Cell cultured in EGM-2 as monolayer were stimulated with 100ng/ml Ang-1 or 200ng/ml Ang-2 for 24h. Cells were harvested by trypsin/EDTA (Sigma), rinsed with PBS+1%BSA (Sigma) and incubated with 2μg/ml propidium iodide (PI, Sigma) plus 0,1 U/L of RNAse. Stimulated and unstimulated cells were then counted in a Coulter Epics XL flow cytometer (Beckman Coulter) and data were analyzed using FCS3 express Software (De Novo Software).

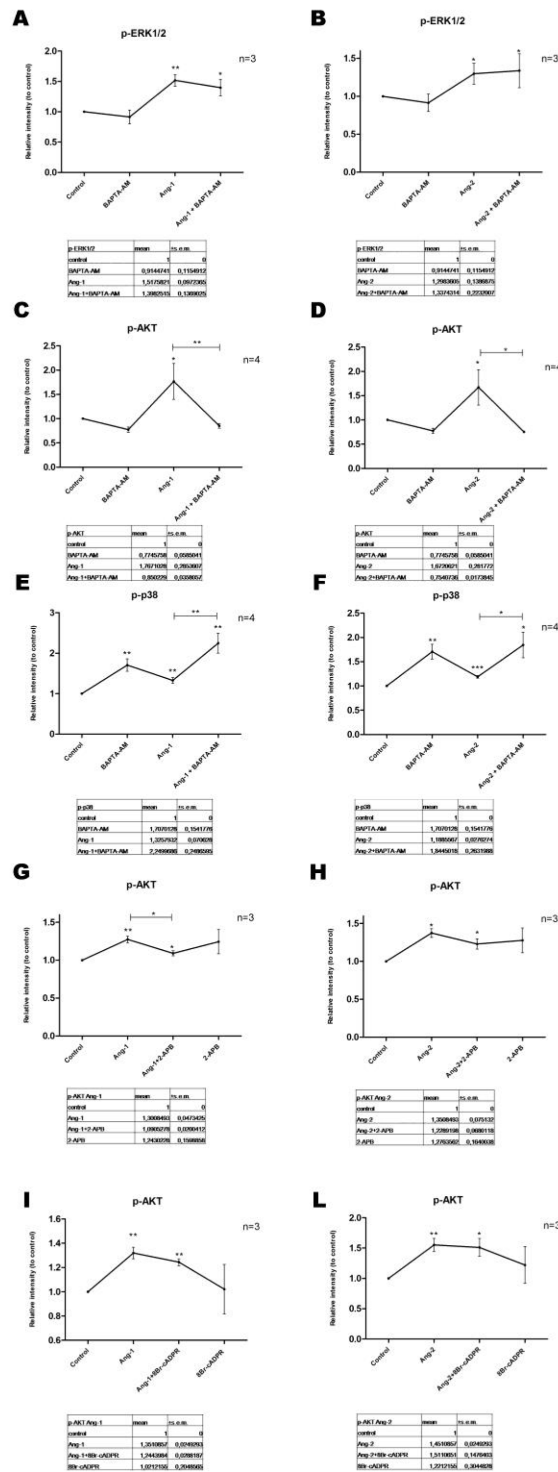
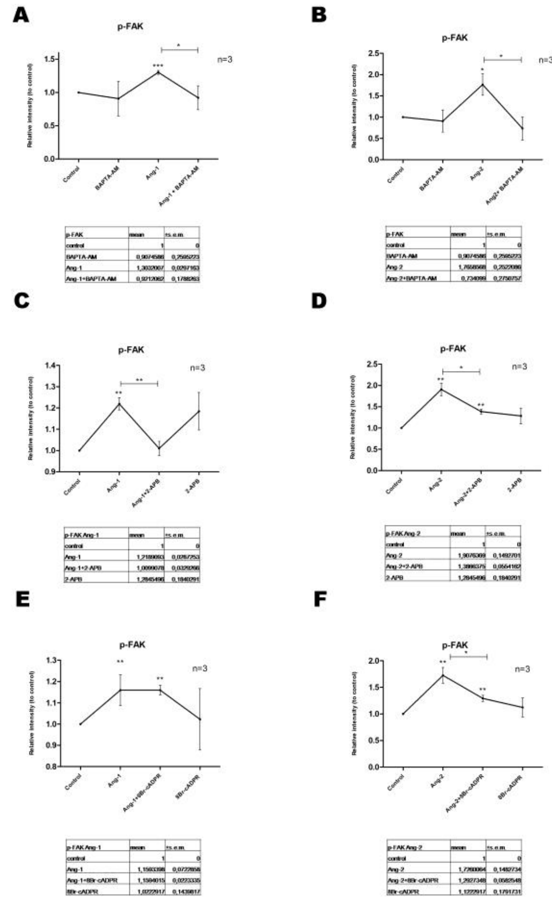


Figure S1: Quantification of calcium-dependent AKT and MAPK activation upon Ang-1/Ang-2 stimulation

Densitometric quantification of Western blots showing levels of p-ERK1/2/β actin, p-AKT/ β actin, p-p38/ β actin versus control (vehicle, set as 1) in the culture conditions detailed in the legend to Fig.5. Data are expressed as mean ± s.e.m. from at least three independent experiments. Student's *t-test* was used for statistical comparison between means. *p<0.05, **p<0.01, ***p<0.001.



p-FAK	mean	±s.e.m.
control	1	0
BAPTA-AM	0,9074586	0,2595223
Ang-1	1,3032067	0,0297163
Ang-1+BAPTA-AM	0,9212062	0,1788763

p-FAK	mean	±s.e.m.
control	1	0
BAPTA-AM	0,9074586	0,2595223
Ang-2	1,7658568	0,2522086
Ang-2+BAPTA-AM	0,734099	0,2750757

p-FAK Ang-1	mean	±s.e.m.
control	1	0
Ang-1	1,2189063	0,0267253
Ang-1+2-APB	1,0099078	0,0329266
2-APB	1,2845496	0,1840291

p-FAK Ang-2	mean	±s.e.m.
control	1	0
Ang-2	1,9076369	0,1492701
Ang-2+2-APB	1,3866375	0,0554167
2-APB	1,2845496	0,1840291

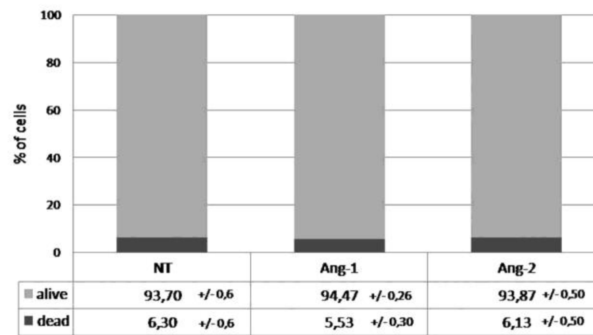
p-FAK Ang-1	mean	±s.e.m.
control	1	0
Ang-1	1,1593398	0,0727858
Ang-1+8Br-cADPR	1,1594015	0,0223335
8Br-cADPR	1,0222917	0,1439817

p-FAK Ang-2	mean	±s.e.m.
control	1	0
Ang-2	1,7260064	0,1482734
Ang-2+8Br-cADPR	1,2927348	0,0582548
8Br-cADPR	1,1222917	0,1791731

Figure S2: Quantification of calcium-dependent FAK activation upon Ang-1 and Ang-2 stimulation

Densitometric quantification of Western blots showing levels of p-FAK/β actin versus control (vehicle, set as 1) in the culture conditions detailed in the legend to Fig 6 A,B1-C2. Data are expressed as mean ± s.e.m. from at least three independent experiments. Student's *t-test* was used for statistical comparison between means. *p<0.05, **p<0.01, ***p<0.001.

Figure S3



	NT	Ang-1	Ang-2
alive	93,70 +/-0,6	94,47 +/-0,26	93,87 +/-0,50
dead	6,30 +/-0,6	5,53 +/-0,30	6,13 +/-0,50

<u>Figure S3:</u> Angs treatment fails to stimulate cell proliferation

The proliferative response of HUVECs to stimulation with 100ng/ml Ang-1 or 200ng/ml Ang-2 for 24 h, was tested by flow cytometry using propidium iodide. The percentage of live and dead cells in stimulated and control (NT) conditions are shown. Values in table represent the mean ±s.e.m. from three independent experiments.