Sup. Fig. 1. Opticin inhibits capillary morphogenesis, promotes capillary network regression and inhibits EC invasion in Matrigel™. Three different types of EC were used: BAECs (black bars), HRECs (white bars) and HUVECs (grey bars); exemplar images shown are of BAECs; the opticin concentration was 250 nM. (A) EC capillary morphogenesis was stimulated by FGF-2 in absence or presence of opticin in Matrigel™. Quantification of the network formation showed that addition of opticin to the FGF-2 stimulated cells significantly decreased the network length for BAECs, HRECs and HUVECs (*, $p<0.001$ for all tested EC types). (B) EC networks were formed in Matrigel™ under FGF-2 stimulation and after 24 h the gels were incubated in the presence or absence of opticin for a further 36 h. Analysis showed that in absence of opticin network length was maintained between 24 and 60 h under FGF-2-stimulation. However, in presence of opticin, a significant decrease in total network length was observed for BAECs, HRECs and HUVECs (*, $p<0.001$ for all tested EC types). (C) An initial vascular network was formed in Matrigel™ and then a further layer of Matrigel™ was added, with or without opticin. After 24 h, invasion of ECs was measured by counting the numbers of cells invading into the second Matrigel™ layer and the presence of opticin significantly decreased the number of invading cells for BAECs, HRECs and HUVECs (*, $p<0.001$ for all tested EC types).

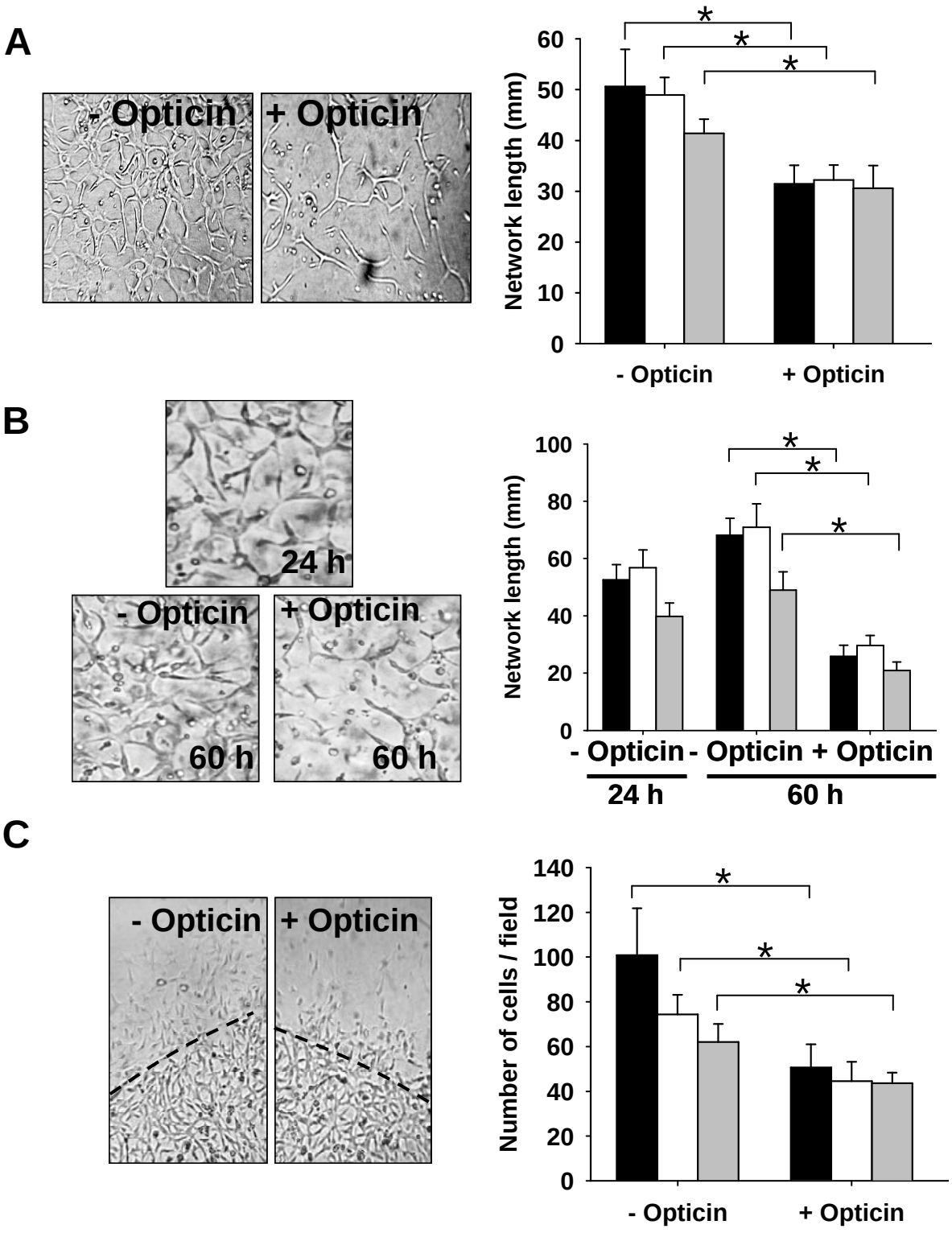
Sup. Fig. 2. Effects of opticin on endothelial cell adhesion, proliferation and apoptosis on collagen. (A) HUVECs were allowed to attach onto immobilized collagen type I in absence or presence of opticin. An antibody raised against the A-domain of the α_2 integrin subunit and a mouse IgG were used as controls. Quantification of attachment was performed by resuspending the crystal violet cell staining and reading the absorbance at 570 nm. Data were reported as a percentage of attachment by comparison with HUVECs allowed to attach to uncoated wells. Opticin did not inhibit HUVEC attachment to collagen type I, whereas the anti-α_2A-domain antibody did inhibit attachment (*, $p<0.001$; NS, $p=0.199$ and 0.847 for opticin and mouse IgG, respectively). (B) Exposure to increasing concentrations of opticin (0-1.2 µM) showed that EC proliferation including HUVECs (●), BAECs (○) and HRECs (▼) was significantly inhibited at concentrations of 0.85 µM or above (*, $p<0.001$). (C) HUVECs that had been allowed to spread on collagen type I were incubated with either opticin, staurosporin or neither for 16 h. SDS-PAGE separation of cell lysates followed by Western-blotting analysis with anti-cleaved caspase-3 antibody revealed that the cleaved activated form of caspase-3, indicative of apoptosis, was only detected in lysates from staurosporin-treated cells. Equal loading was verified by probing for actin.

Sup. Fig. 3. Opticin alters HUVEC morphogenesis on a 3D collagen matrix. Collagen type I was polymerized in the absence (A) or presence of 250 nM of opticin (B). HUVEC suspension was added on top of the collagen gels and placed under a microscope in cell culture conditions. After attachment, cell morphology was monitored by taking images every 10 min for 4 h. Images were acquired on AS MDW live cell imaging system (Leica DM IRE microscope body with motorized Z) using a 20x/0.5 HC Plan Fluotar objective. Images were then processed into a movie using ImageJ software. In absence of opticin, the HUVECs adopted an elongated morphology with only a few protrusions (two or three per cell) and generally moved persistently in the direction of these extensions. However in the presence of opticin, the cells adopted a 'fried egg' morphology with intense ruffling of the lamellipodia and disrupted motility and polarity was observed.

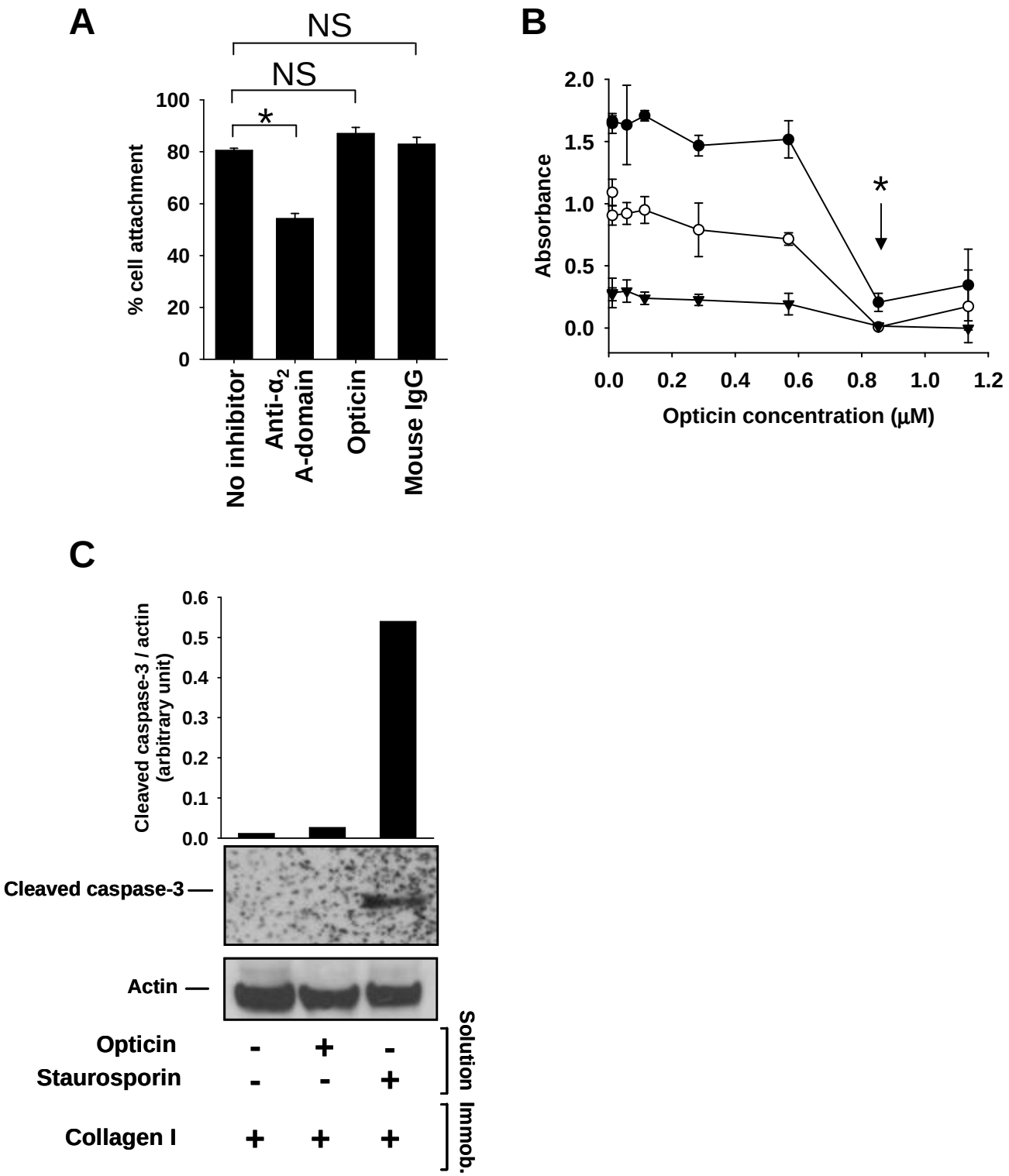
Sup. Fig. 4. Opticin disrupts the morphology of human dermal microvascular ECs (HDMECs) spread on various matrices. (A) HDMEC spreading assays were performed on immobilized collagen type I (black bars) and collagen type IV (white bars) in presence of function-blocking anti-integrin antibodies. An antibody raised against the A-domain of the α_2 integrin (anti-α_2A-domain) inhibited HDMEC spreading on both collagen types I and IV, whereas an antibody raised against the A-domain of the α_1 integrin (anti-α_1A-domain) inhibited spreading on collagen type IV, but not type I. An anti-α_2 integrin antibody that was not raised against the A-domain (anti-α_2) and a non-specific mouse IgG did not inhibit spreading on either collagen types. (B) HDMECs were allowed to spread on collagen type I, II, IV and laminin and were exposed to increasing concentrations of opticin; presented images correspond to a concentration of 250 nM. The fixed cells were fluorescently stained to visualize actin stress fibers (in red), focal adhesions (in green) and nuclei (in blue). The three different channels were merged. HDMECs spread on all studied matrices and focal adhesions and actin stress fibers were clearly visible

(top panel). However, after exposure to opticin the cells rounded up and disorganization of actin stress fibers and focal adhesions was observed (bottom panel) (scale bar=50 µm). (C) Quantification of the number of cells with an organized cytoskeleton after exposure to increasing concentrations confirmed that opticin disrupted HDMEC morphology when spread on collagen type I (—●—), collagen type II (—○—), collagen type IV (--Δ--) and laminin (--▼--) in a concentration-dependent manner.

Supplemental figure 1

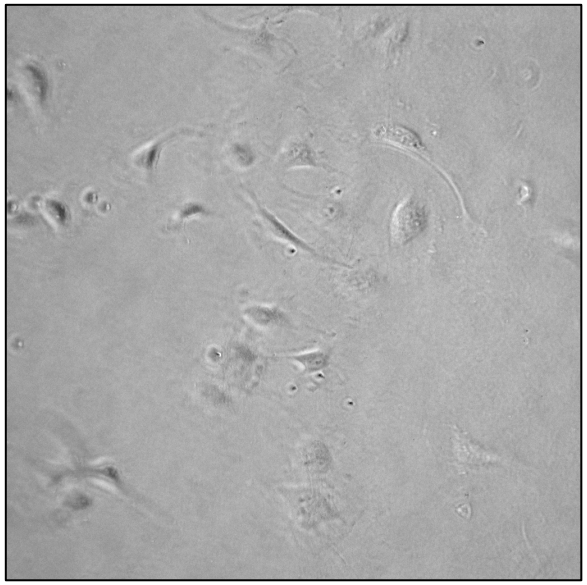


Supplemental Figure 2



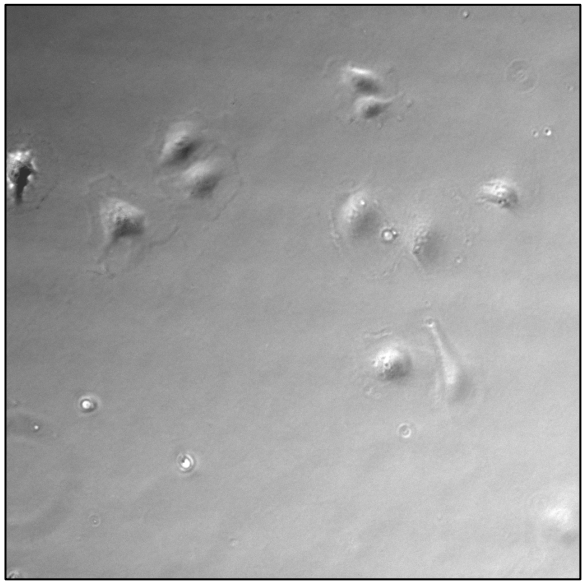
Supplemental videos

A



On collagen fibrils alone

B



On collagen fibrils with 250 nM opticin

Supplemental figure 4