Supplementary Material

The endothelial glycocalyx as a target of excess soluble fms-like tyrosine kinase-1

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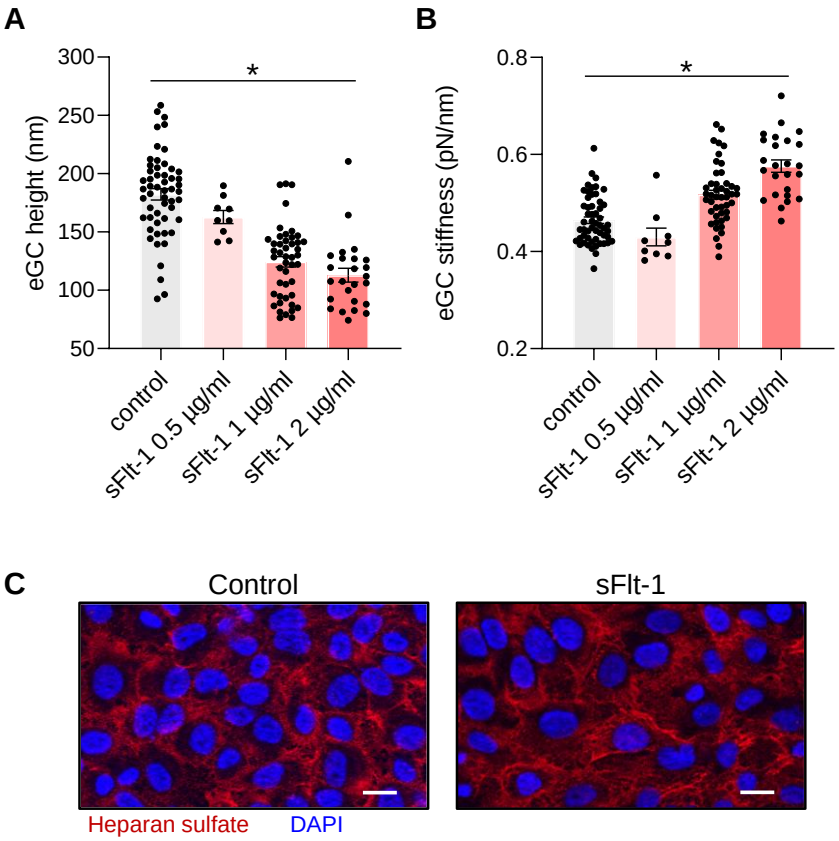

Figure S1. sFlt-1 leads to conformational changes of the eGC in a dose-dependent manner. EA.hy926 cells were cultured with increasing sFlt-1 (VEGFR1-Fc) concentrations for 24 h. Control cells were incubated with control protein (IgG-Fc, 1 µg/ml). eGC height (**A**) and stiffness (**B**) were measured by atomic force microscopy (AFM). (**C**) Representative immunofluorescence images of EA.hy926 cells treated with sFlt-1 (2 µg/ml; left panel) or control protein (2 µg/ml; right panel) and stained for anti-heparan sulfate. DAPI indicates nuclear staining. Data are given as mean ± SEM. * p <0.001 for linear trend determined by one-way ANOVA. eGC, endothelial glycocalyx

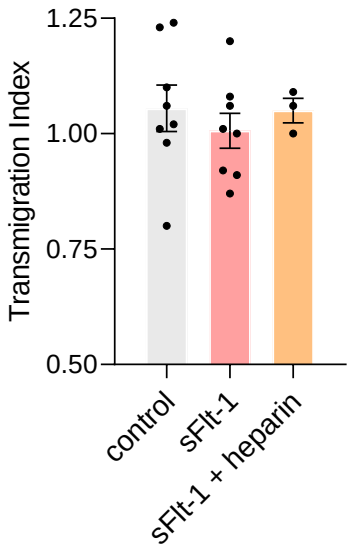

Figure S2. Monocyte transmigration across endothelial cells exposed to excess sFlt-1. HUVECs were cultured in transwell inserts (5 μm pore; 25,000 cells/insert) and treated with recombinant sFlt-1 (VEGFR1-Fc, 2 μg/ml) or control protein (IgG-Fc, 2 μg/ml) for 24h in the presence or not of heparin (10 μg/ml). Calcein-labeled THP-1 cells (400,000 cells/insert) were placed in the upper chamber and allowed to migrate across the endothelial monolayer for 90 min towards the monocyte chemoattractant protein-1 (MCP-1, 50 ng/ml) added in the lower chamber. Control wells were filled with medium only. Results are expressed as transmigration index, calculated by dividing the average count of migrated cells towards the MCP-1 gradient by the average count of migrated cells in the control wells. Data are given as mean ± SEM

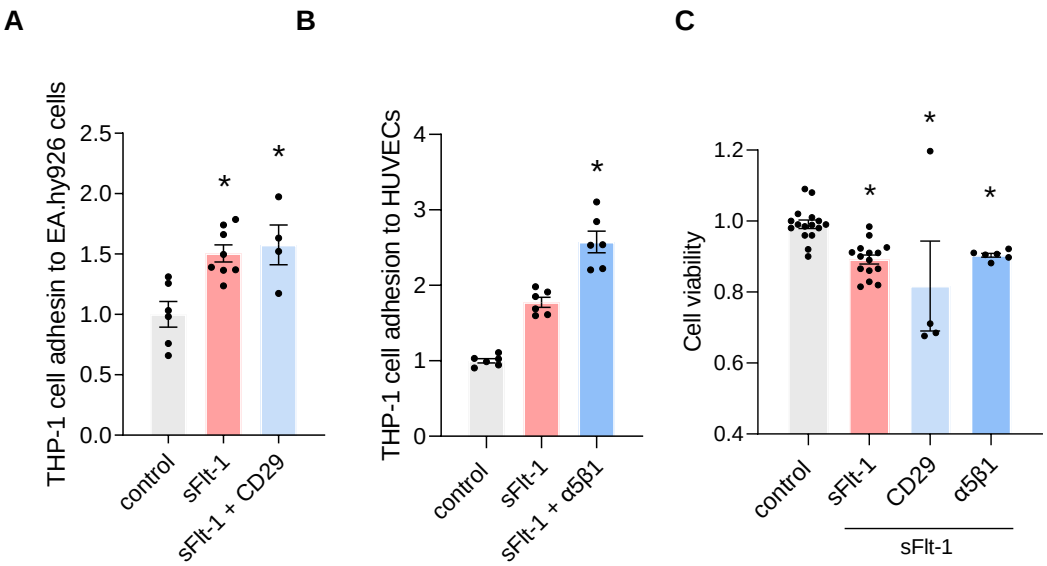

Figure S3. Endothelial cell adhesiveness to monocytes and viability under excess sFlt-1. EA.hy926 cells (**A**) or HUVECs (**B, C**) were treated with recombinant sFlt-1 (VEGFR1-Fc; 2 μg/ml) or control protein (IgG-Fc; 2 μg/ml) for 24h. As indicated, cells were also pre-treated (30 min) and co-incubated with the function-blocking antibodies to β1 and α5β1 integrins (CD29 clone Mab 13, 20 μg/ml; α5β1 clone JBS5, 10 μg/ml). (**A, B**) Monocyte adhesion to endothelial cells. (**C**) Cell viability determined using the MTT assay. The function-blocking antibodies didn't protect against sFlt-1 adverse effects. Results are expressed relative to control, and data are given as mean ± SEM. * $p < 0.05$ vs. control. Kruskal-Wallis and Dunn's multiple comparisons tests were applied