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Supplementary Materials for

The interaction of heparan sulfate proteoglycans with endothelial transglutaminase-2 limits $VEGF_{165}$-induced angiogenesis

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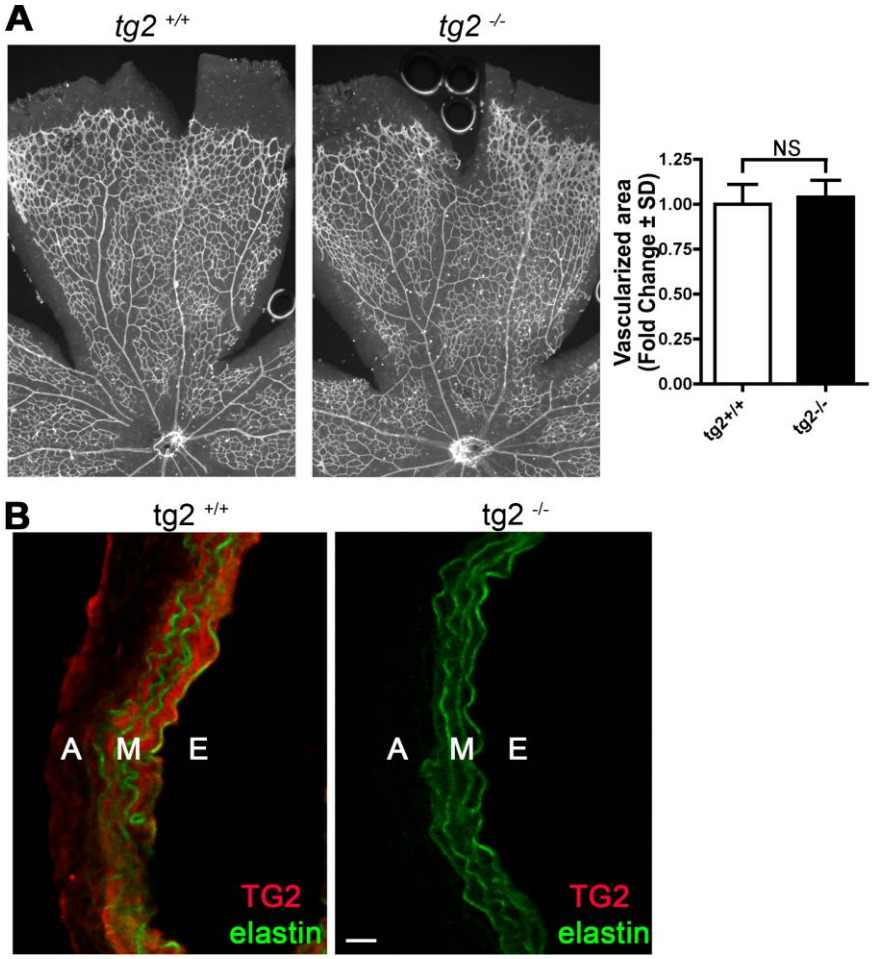

Fig. S1. Analysis of retinas and aortas from *tg2*$^{+/+}$ and *tg2*$^{-/-}$ mice. (**A**) Confocal images of retina stained with isolectin-B4 from P7 mice. The confocal images are representative of 22 *tg2*$^{+/+}$- and 20 *tg2*$^{-/-}$-retinas analyzed in 3 independent experiments. Vascular area was quantified for *tg2*$^{+/+}$ or *tg2*$^{-/-}$. Graphs represent the mean values ± SD (N=3 independent experiments, 20 to 22 retinas analyzed, Mann-Whitney *U* test). NS: no significant difference. (**B**) TG2 expression in mouse aorta. TG2 was detected by immunofluorescence in aortic sections of 9-weeks *tg2*$^{+/+}$ and *tg2*$^{-/-}$ littermates. Elastic fibers were detected by autofluorescence (A: Adventitia; M: Media; E: Endothelium). The images are representative of 3 slices obtained from 2 *tg2*$^{+/+}$- or *tg2*$^{-/-}$-aortas. Scale bar, 25 µm.

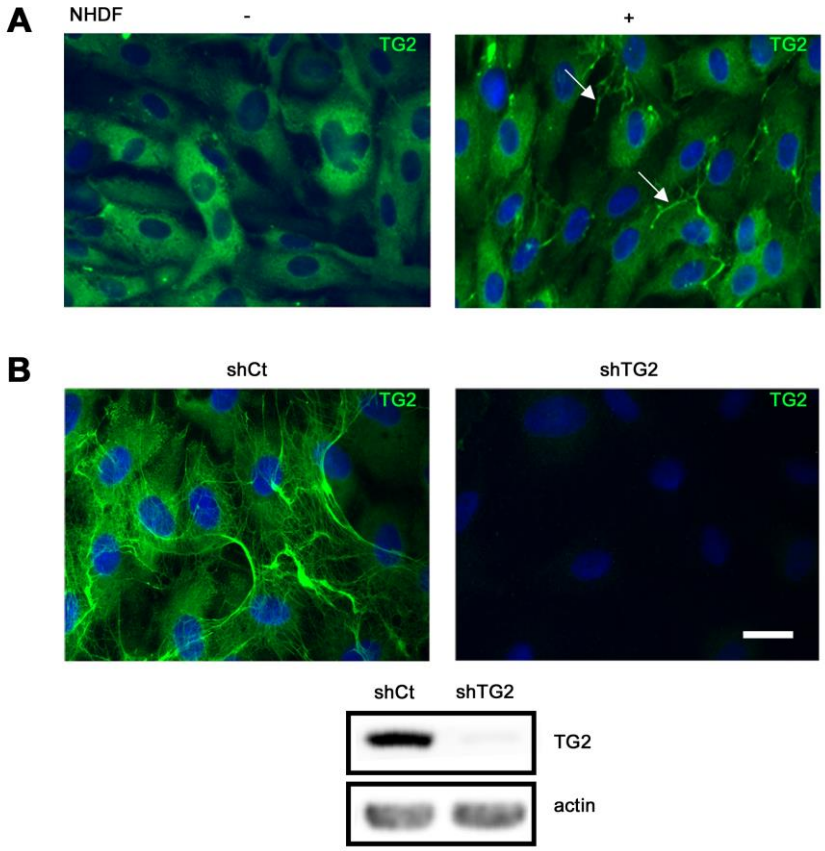

Fig. S2. TG2 accumulation in ECM and TG2 silencing in endothelial cells. (**A**) TG2 accumulation in ECM generated by HUVECs that were co-cultured with NHDFs. The confocal images are representative of 6 images obtained from 2 independent experiments. TG2 deposition in the ECM was analyzed by immunofluorescence of cells co-cultured without (-) or with (+) NHDFs for 3 days. Arrows indicate extracellular TG2 in the ECM. Nuclei were stained with DAPI. Scale bar, 20 µm. (**B**) TG2 abundance in control (shCt) or TG2-silenced (shTG2) HUVECs. TG2 silencing was analyzed by immunofluorescence (upper) and immunoblot (lower) of cell lysate of cells grown for 5 days. Actin was used as a loading control. The confocal images and immunoblots are representative of 20 or more technical replicates obtained from 10 or more independent experiments. Scale bar, 20 µm.

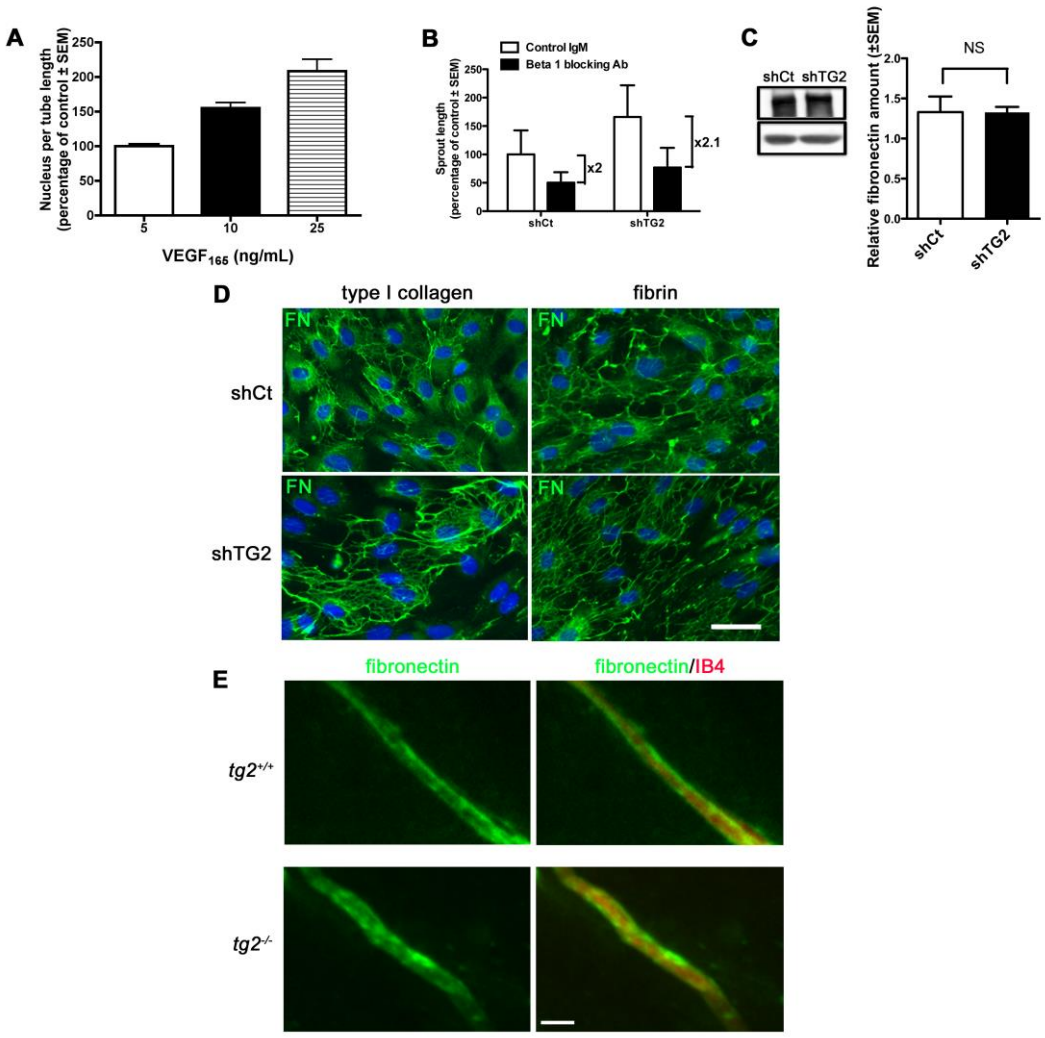

Fig. S3. Proliferation, β1 integrin-mediated sprouting, and fibronectin abundance and deposition in TG2-silenced endothelial cells. (**A**) Number of nuclei was quantified in sprouts from HUVECs in fibrin gels containing increasing concentrations of $VEGF_{165}$ and calculated as percentage of values from 5 ng/mL $VEGF_{165}$. Graph represents the mean of values ± SEM (N=2 independent experiments, 24 to 48 sprouts analyzed, Mann-Whitney *U* test). (**B**) β1 integrin-dependent sprouting. Control (shCt) or TG2-silenced (shTG2) HUVECs seeded on Cytodex beads were cultured with either 10 µg/mL control IgM (Control IgM) or 10 µg/mL β1 integrin blocking antibody (Beta1 blocking Ab). Mean of total length of sprouts per bead was normalized

to the mean of shCt treated with control IgM. Graph represents the mean of normalized values ± SEM. The fold values (x2 and x2.1) represent the ratio between control IgM and β1 blocking Ab conditions for shCt and shTG2, respectively (N=4 independent experiments, 71 to 97 sprouts analysed, Mann-Whitney *U* test). (**C**) Fibronectin protein was detected by immunoblot in total lysates of shCt and shTG2 HUVECs cultured for 4 days (top panel). Actin was used as a control (bottom panel). Immunoblot was representative of 3 independent experiments. Relative amount is the ratio of fibronectin on actin normalized to the maximal ratio. Graphs represent the mean of values ± SEM (N=3 independent experiments, Mann-Whitney *U* test). NS: no significant difference. (**D**) Fibronectin deposition was analyzed by immunofluorescence on cells cultured in 2D. Nuclei were stained with DAPI. The confocal images are representative of 4 technical replicates obtained from 5 independent experiments. Scale bar, 20 µm. (**E**) Fibronectin deposition was analyzed by immunofluorescence performed on aortic ring sprouts from *tg2*$^{+/+}$ and *tg2*$^{-/-}$ mice. Endothelial cells were stained with isolectin-B4. The images are representative of 2 to 4 technical replicates obtained from 5 *tg2*$^{+/+}$- or *tg2*$^{-/-}$-rings. Scale bar, 100 µm.

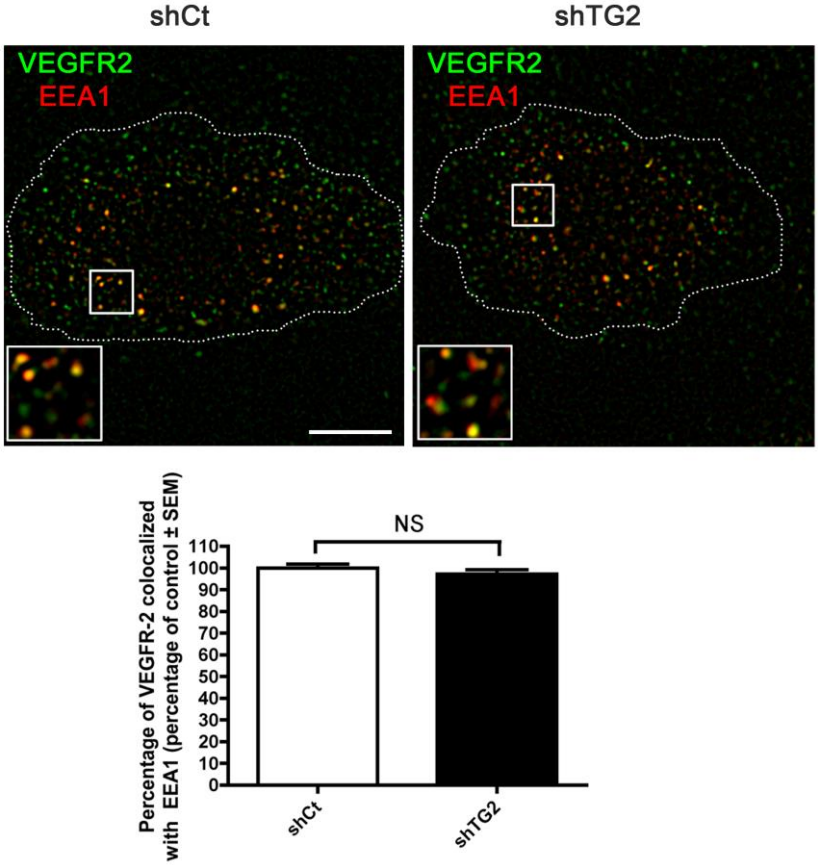

Fig. S4. VEGFR2 internalization in response to $VEGF_{165}$. Confocal images showing colocalization of VEGFR2 (green) and EEA1 (red) in endosomes in control (shCt) and TG2-silenced (shTG2) HUVECs after 5 minutes stimulation with 50 ng/ml $VEGF_{165}$ (N=3 independent experiments, 141 to 164 cells analyzed, Student's *t*-test).). NS: no significant difference. Scale bar, 20 µm.

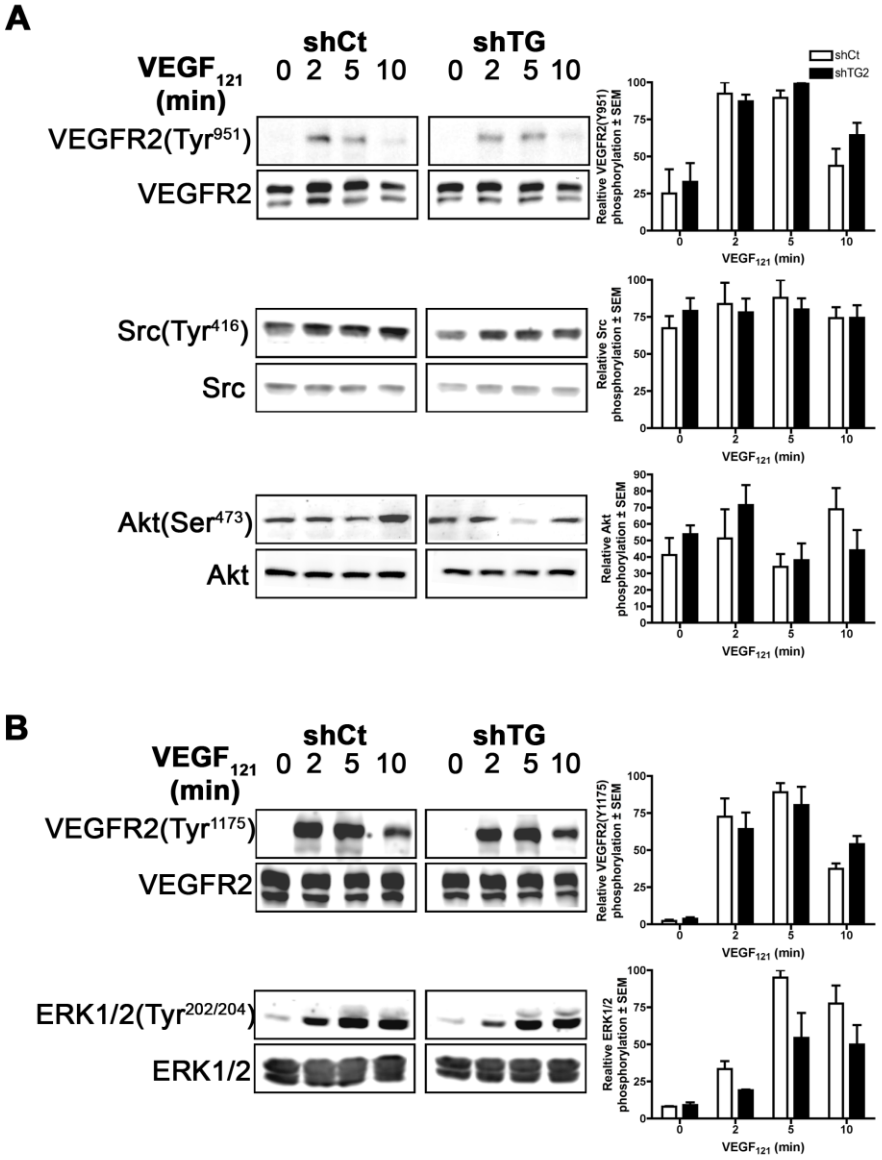

Fig. S5. TG2 silencing does not affect $VEGF_{121}$-induced signaling. Relative phosphorylation is the ratio of phosphorylated to total protein. Ratios were normalized by calculating them as percentages of the maximal ratio. Graphs represent the mean of values ± SEM. (**A**) Phosphorylation of VEGFR2 at Tyr^{951}, Src and Akt. (**B**) Phosphorylation of VEGFR2 at Tyr^{1175} and ERK1/2 (N=4 independent experiments, Welch's *t*-test).

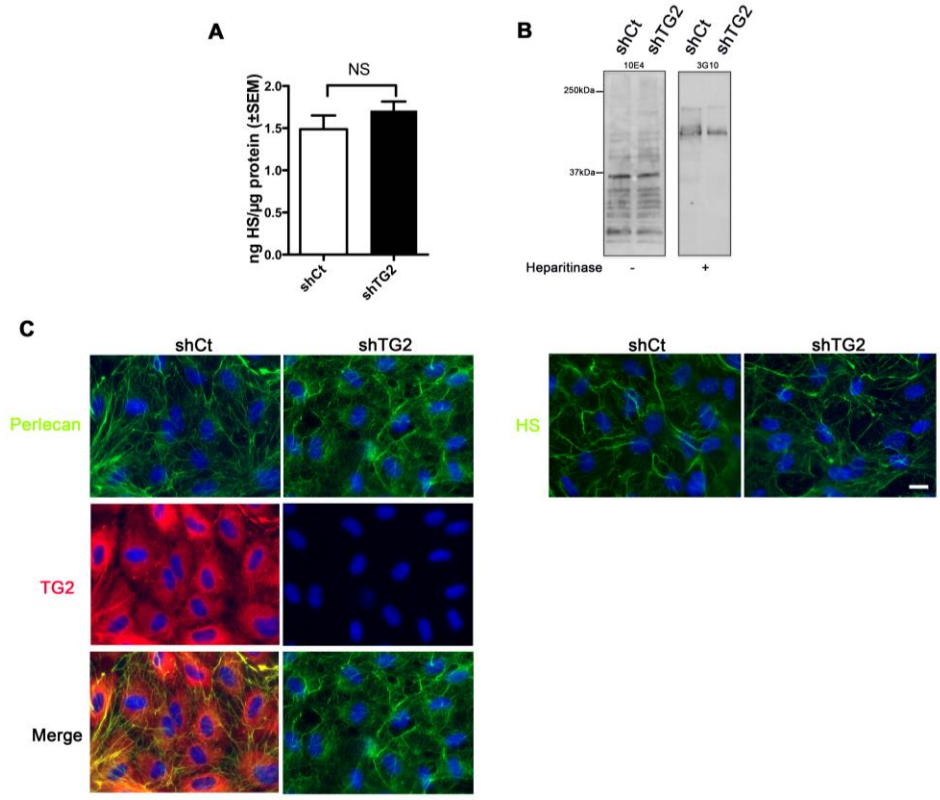

Fig. S6. HS content and deposition. (**A**) Quantification of total sulfated glycosaminoglycans in control (shCt) and TG2-silenced (shTG2) HUVECs (N=4 independent experiments, Mann-Whitney *U* test). NS: no significant difference. (**B**) Nontreated (-) and heparitinase digested (+) HSPGs from total cell lysates of control and TG2-silenced cells grown for 5 days and analyzed by immunoblotting with 10E4 and 3G10 antibodies**.** 10E4 recognizes an epitope including N-sulfated glucosamine residues present in many human HS chains. 3G10 recognizes human HS neo-epitope, namely desaturated hexuronate present at the non-reducing end of HSPGs after heparitinase digestion. Immunoblots are representative of 3 independent experiments. (**C**) Deposition of perlecan and TG2 (TG2) and colocalization (merge) or deposition of HS-bearing components (HS) were analyzed by immunofluorescence in control and TG2-silenced cells grown for 4 days. Nuclei were stained with DAPI. The confocal images are representative of 3 independent experiments. Scale bar, 20 µm.

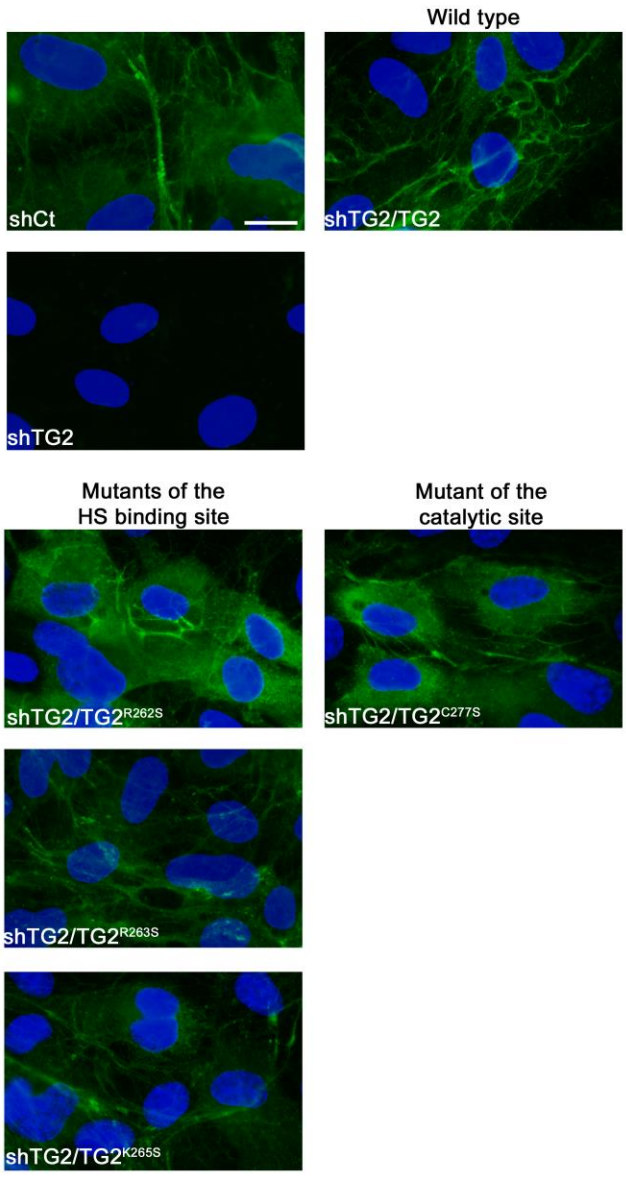

Fig. S7. Re-expression of wild-type and mutant TG2 in TG2-silenced endothelial cells. Control (shCt) and TG2-silenced (shTG2) HUVECs were shown as controls. Re-expression of wild-type TG2 (TG2) or forms of TG2 with mutations in the heparan sulfate (HS) binding site ($TG2^{R262S}$, $TG2^{R263S}$, $TG2^{K265S}$) or the catalytic site ($TG2^{C277S}$) in shTG2 HUVECs was analyzed by immunofluorescence. Nuclei were stained with Dapi. The confocal images are representative of 3 independent experiments. Scale bar, 20 µm.