
Supplementary information

**Sulfated glycans engage the Ang–Tie
pathway to regulate vascular development**

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Supplementary Information

Sulfated glycans engage the Ang/Tie pathway to regulate vascular development

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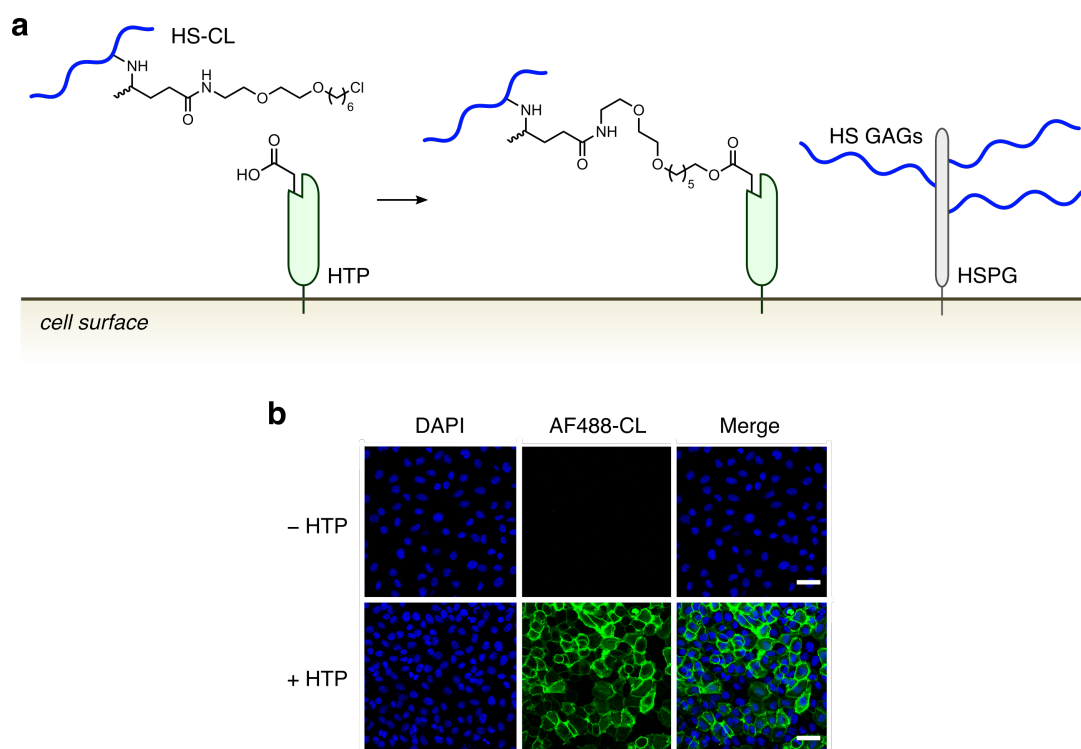
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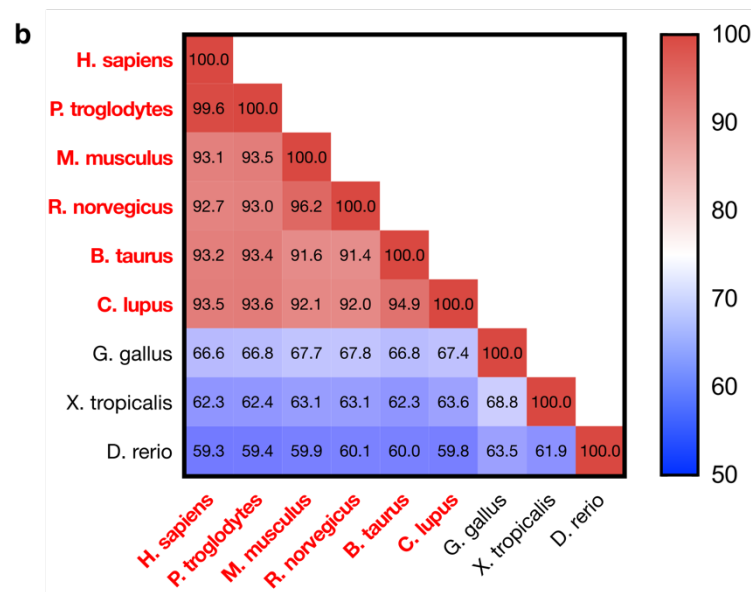
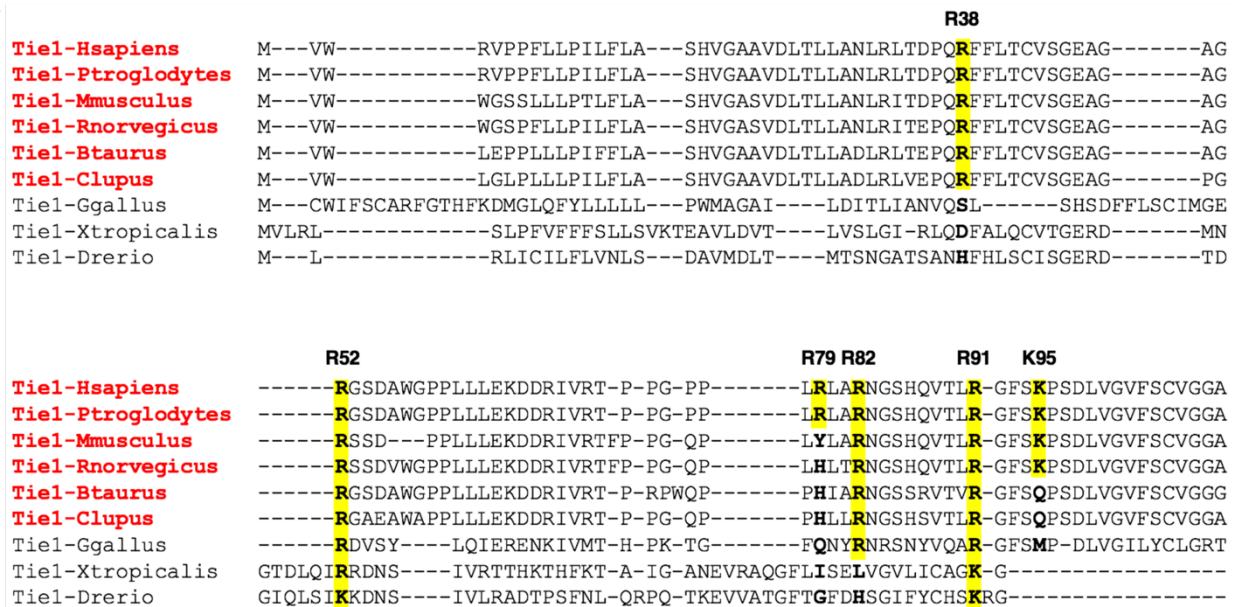
Supplementary Figure 1. HTP-mediated cell-surface engineering of endothelial cells with

HS GAGs. (a) HTP is a modified alkane dehalogenase fused to the transmembrane domain of PDGFR. A catalytic Asp residue of the transmembrane HTP construct will covalently attach to

5 biomolecules that are functionalized with the chlorohexyl linker (CL) such as HS-CL, which allows for stable cell-surface display of HS structures of interest and mimics naturally occurring HSPGs. (b) An EA.hy926 cell line stably expressing the HTP (+HTP) and the parental EA.hy926 cell line (-HTP) were treated with a cell-impermeable, CL-conjugated Alexa Fluor 488 (AF488-

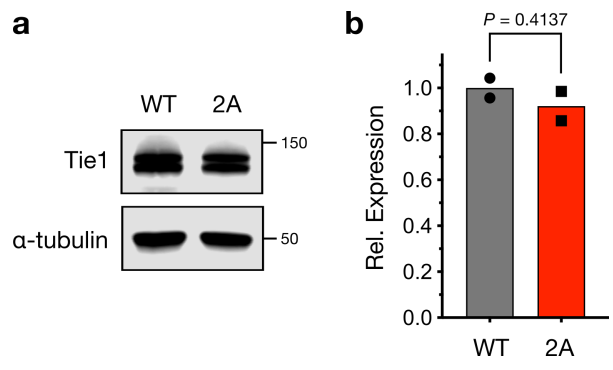
10 CL, green), and nuclei were co-stained with Hoescht 33342 (blue). The EA.hy926-HTP cell line showed robust, specific labeling by AF488-CL, confirming the expression and cell-surface trafficking of the HTP and successful labeling of the cell surface with CL-conjugated cargo.

Scale bar = 50 μ m. Images are representative of 3 biologically independent samples.



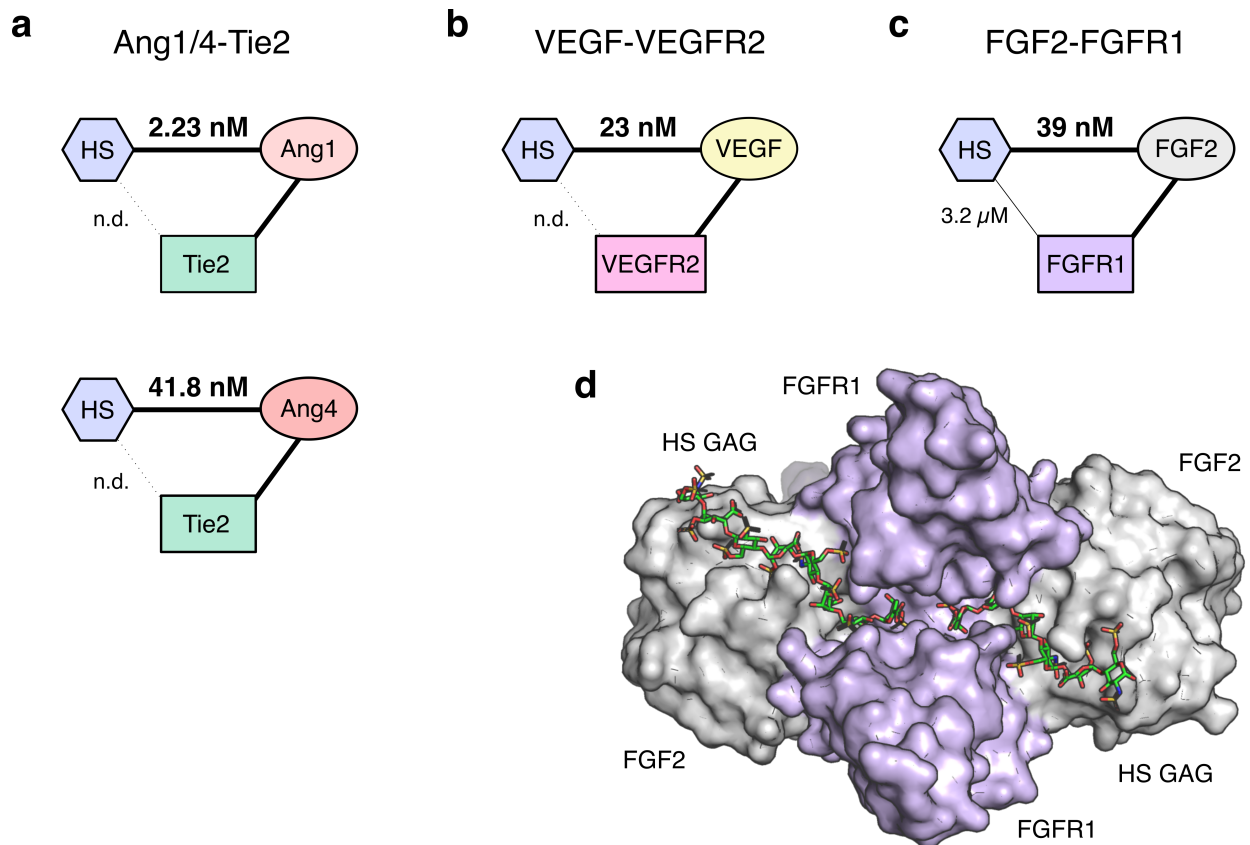
Supplementary Figure 2. Electropositive residues on Tie1 involved in GAG binding are conserved across mammals. (a) Sequence alignment and (b) percent sequence identity of Tie1 orthologs across chordates predicted by HomoloGene. Alignments were generated using MUSCLE version 3.6. Mammalian species are shown in red, predicted GAG-binding residues are shown in bold, and conserved residues are highlighted in yellow.

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Supplementary Figure 3. The 2A mutation does not affect Tie1 expression levels in cultured cells. (a) Representative Western blotting and (b) quantification of full-length Tie1-WT and Tie1-2A expression levels after transient over-expression in HEK-293T cells. Data represent mean and individual datapoints, $n = 2$, unpaired, two-tailed Student's t test.

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Supplementary Figure 4. Comparison of HS-ligand-receptor signaling complexes. The HS-

Ang1/4-Tie2 complex shares similarities to both the HS-VEGF-VEGFR2 and HS-FGF2-FGFR1 complexes. (a) HS binds with nanomolar affinity to Ang1/4 (2.23 and 41.8 nM, respectively), similar to the reported affinities of HS for (b) VEGF (23 nM)³⁷ and (c) FGF2 (39 nM).⁴⁵ No interaction between HS and Tie2 was observed by ELISA or glycan microarrays, similar to the lack of reported interaction between HS and VEGFR2³⁷ and the weak interaction (3.2 μM) reported between HS and FGFR1.⁴⁵ n.d. = not detected. (d) Crystal structure of the HS-FGF2-FGFR1 complex determined by Schlessinger *et al.*¹⁶ HS binds in a continuous groove and makes contacts with both FGF2 (light gray) and FGFR1 (purple), despite weak interactions with FGFR1.