Supplementary Information

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

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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
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-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 µm. Images are representative of 3 biologically independent samples.

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Tiel-Hsapiens	MVWSHVGAA	VDLTLLANLRLTDP	2 <mark>R</mark> FFLTCVSGEAGAG
Tie1-Ptroglodytes	MVWSHVGAA	VDLTLLANLRLTDP(2 <mark>R</mark> FFLTCVSGEAGAG
Tiel-Mmusculus	MVWSHVGAS	VDLTLLANLRITDP	2 <mark>R</mark> FFLTCVSGEAGAG
Tiel-Rnorvegicus	MVWSHVGAS	VDLTLLANLRITEP	2 <mark>R</mark> FFLTCVSGEAGAG
Tiel-Btaurus	MVWSHVGAA	VDLTLLADLRLTEP	2 <mark>R</mark> FFLTCVSGEAGAG
Tie1-Clupus	MVWSHVGAA	VDLTLLADLRLVEP	2 <mark>R</mark> FFLTCVSGEAGPG
Tie1-Ggallus	MCWIFSCARFGTHFKDMGLQFYLLLLLPWMAGA	ILDITLIANV	SLSHSDFFLSCIMGE
Tie1-Xtropicalis	MVLRLSLPFVFFFSLLSVKTEAVLDV	TLVSLGI-RL	DFALQCVTGERDMN
Tiel-Drerio	MLDAVMDL	TMTSNGATSA	NHFHLSCISGERDTD
	R52	R79 R82	R91 K95
Tiel-Hsapiens	R GSDAWGPPLLLEKDDRIVRT-P-PG-PP	LRLARNGSHQV	IL <mark>R</mark> -GFS <mark>K</mark> PSDLVGVFSCVGGA
Tie1-Ptroglodytes	R GSDAWGPPLLLEKDDRIVRT-P-PG-PP	LRLARNGSHQV	IL <mark>R</mark> -GFS <mark>K</mark> PSDLVGVFSCVGGA
Tie1-Mmusculus	R SSDPPLLLEKDDRIVRTFP-PG-QP	LYLARNGSHQV	IL <mark>R</mark> -GFS <mark>K</mark> PSDLVGVFSCVGGA
Tiel-Rnorvegicus	R SSDVWGPPLLLEKDDRIVRTFP-PG-QP	L H LT <mark>R</mark> NGSHQV	IL <mark>R</mark> -GFS <mark>K</mark> PSDLVGVFSCVGGA
Tiel-Btaurus	R GSDAWGPPLLLEKDDRIVRT-P-RPWQP	P H IA <mark>R</mark> NGSSRV	IV <mark>R</mark> -GFS Q PSDLVGVFSCVGGG
Tie1-Clupus	R GAEAWAPPLLLEKDDRIVRT-P-PG-QP	P H LL <mark>R</mark> NGSHSV	IL <mark>R</mark> -GFS Q PSDLVGVFSCVGGA
Tie1-Ggallus		FONYRNRSNYV	DA <mark>R</mark> -GESMP-DLVGILYCLGRT
Tio1-Vtropicalia	COST DOIENENRI MI HER 16		
ITEL-ACTOPICATIS	GTDLQIRRDNSIVRTTHKTHFKT-A-IG-ANEVRA	QGFL I SE L VGVLICA	AG <mark>K</mark> -G



Supplementary Figure 2. Electropositive residues on Tiel involved in GAG binding are conserved across mammals. (a) Sequence alignment and (b) percent sequence identity of Tiel 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.



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 μ M) 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.

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