

Supplementary Information

Recurrent evolution of ligand-binding domain multiplicity fine-tunes TGF β signaling in vertebrates

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Inventory:

Supplementary Figure 1: TGFBR2 LBD duplication independent of allotetraploid chromosomal evolution in *Cyprinus carpio* and *Xenopus laevis*.

Supplementary Figure 2: Genetic architecture of duplicated LBDs of TGF β receptor family members.

Supplementary Figure 3: LBD sequence identity comparison of ACVR1 and BMPR2 orthologs.

Supplementary Figure 4: Inner LBD conveys Activin A binding competence of LBD multimerized BMPR2 orthologs.

Supplementary Figure 5: Representative LSBA images of Activin A bound to gnBmpr2a variants.

Supplementary Figure 6: LBD sequence identity comparison of TGFBR2 orthologs.

Supplementary Figure 7: Sequence alignment of multimerized TGFBR2 LBDs and TGF β 1 orthologs.

Supplementary Figure 8: Evolutionary divergence of duplicated LBD domains relative to species divergence times.

Supplementary Figure 9: Functional implications of LBD multiplication in TGFBR2 orthologs of *Cyprinus carpio* and *Xenopus laevis*.

Supplementary Figure 10: *In silico* binding analysis of TGFBR2 orthologs with respective animal TGF β 1.

Supplementary Figure 11: *In silico* binding analysis of ray-finned fish TGFBR2 orthologs with human and respective animal TGF β 1.

Supplementary Figure 12: Representative LSBA images of TGF β 1 bound to dTgfr2a/b variants.

Supplementary Figure 13: Representative LSBA images of TGF β 1 bound to cTgfr2a variants.

Supplementary Figure 14: Representative LSBA images of TGF β 1 bound to xITgfr2.L variants.

Supplementary Figure 15: Representative LSBA images of TGF β 1 bound to eTGFBR2 variants.

Supplementary Figure 16: Representative LSBA images of TGF β 1 bound to gTGFBR2 variants.

Supplementary Figure 17: Linker architecture in TGFBR2 and BMPR2 variants.

Supplementary Figure 18: Representative LSBA images of TGF β 1 bound to hTGFBR2 variants.

Supplementary Figure 19: Alternative splice variants of TGFBR2 in horse and chicken.

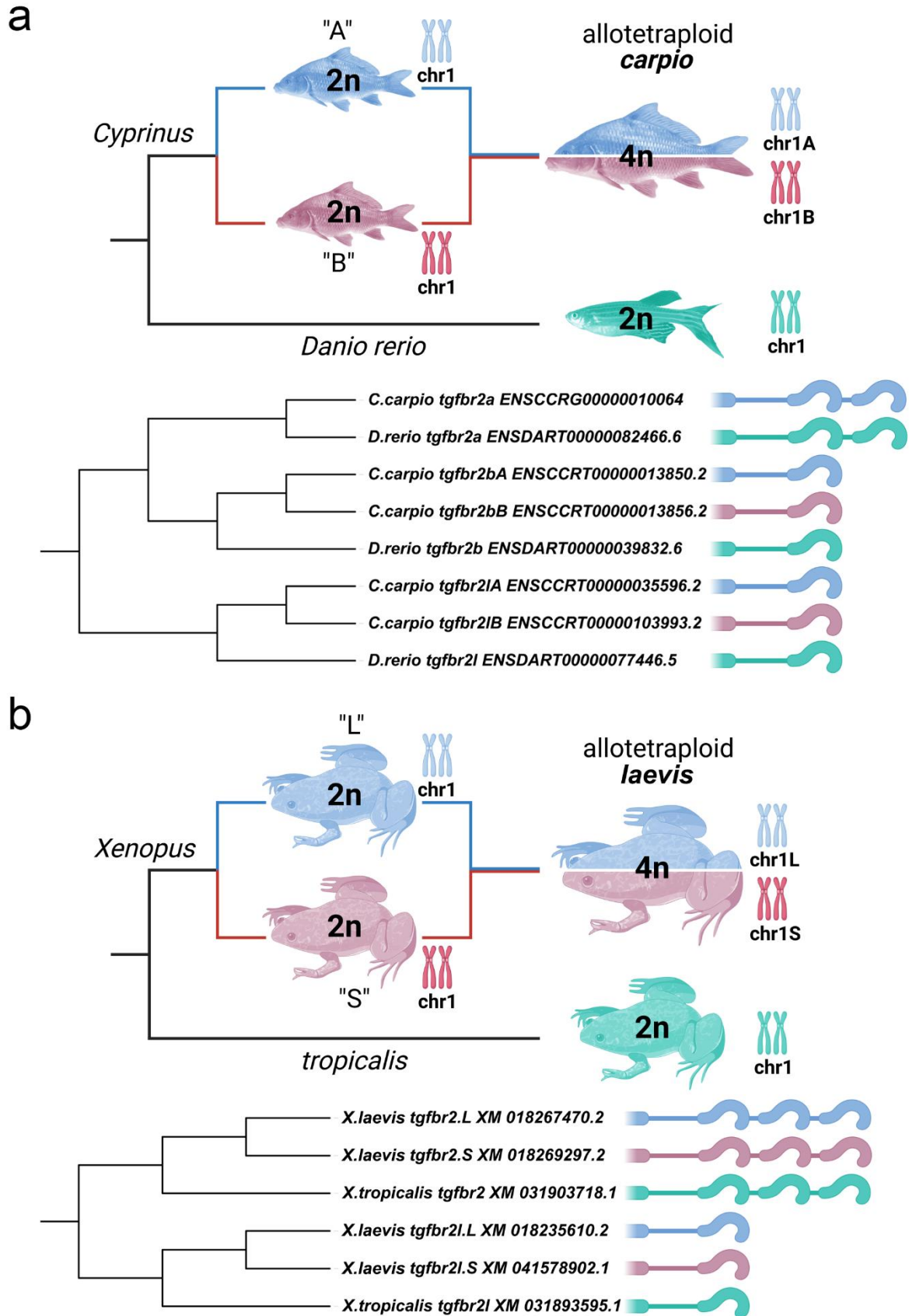
Supplementary Figure 20: Coinjected membrane bound PMT-mEGFP expression control in zebrafish embryos.

Supplementary Figure 21: Western blot expression controls of Halo-tagged receptor constructs.

Supplementary Table 1: N-terminally tagged HaloTag expression constructs

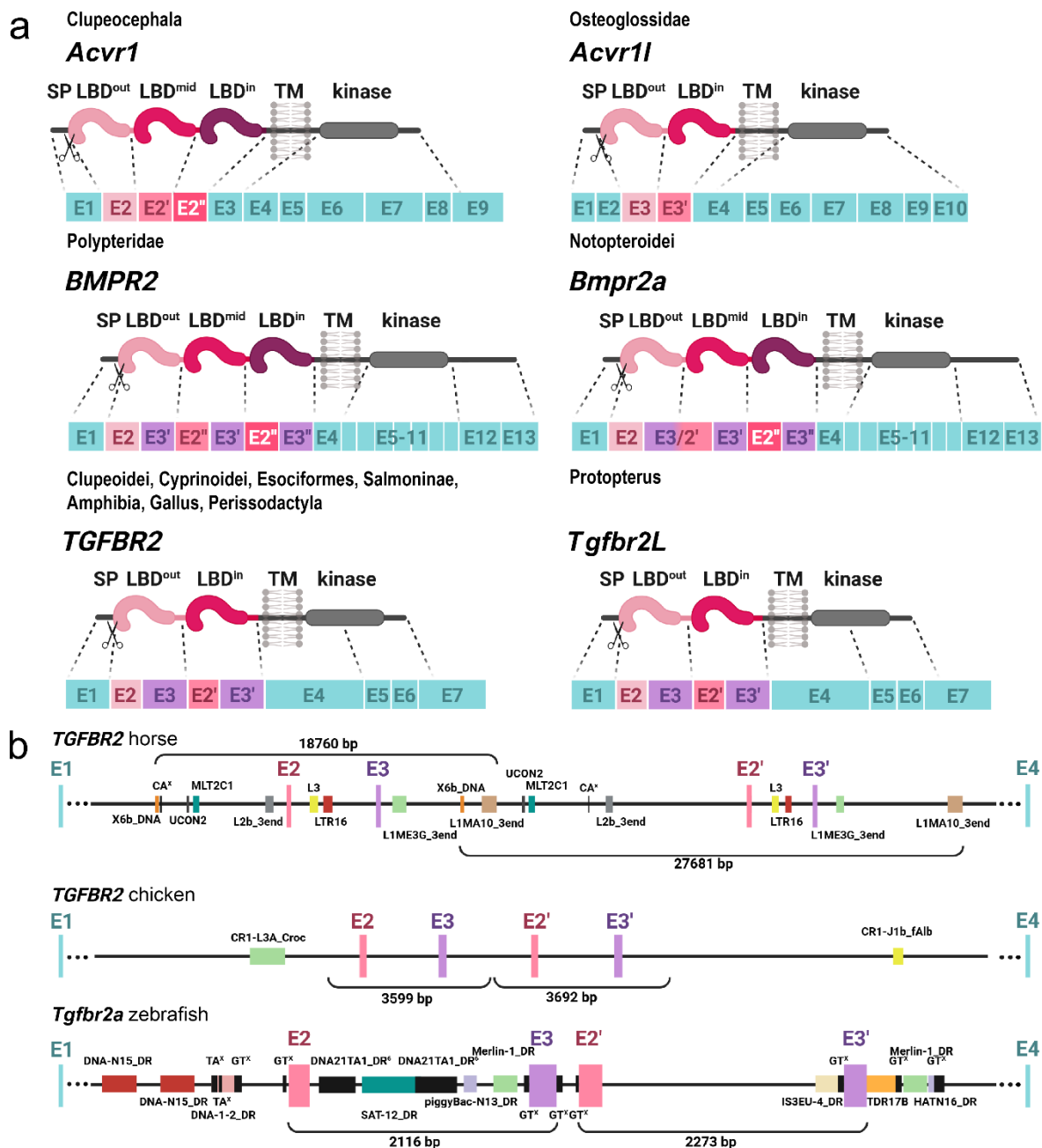
Supplementary Table 2: Oligonucleotides used for molecular cloning of LBD-Halo constructs

Supplementary Figure 22: Uncropped Western blots



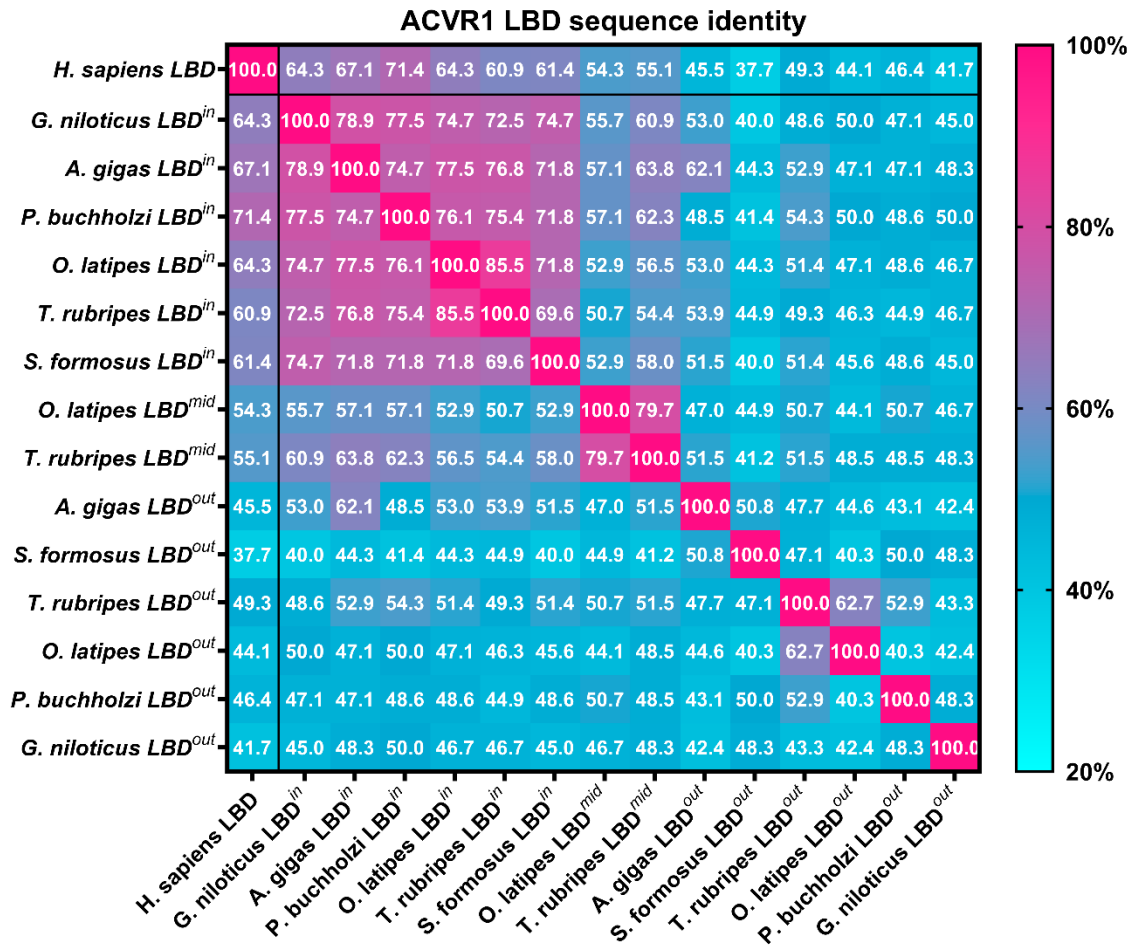
Supplementary Figure 1: TGFBR2 LBD duplication independent of allotetraploid chromosomal evolution in *Cyprinus carpio* and *Xenopus laevis*. (a, top) Phylogenetic depiction of chromosomal evolution in the *Cyprinus* lineage, illustrating the allopolyploid origin of *Cyprinus carpio* (4n) from two diploid ancestors ("A" and "B"). Each ancestor contributed a

set of chromosomes (chr1A in blue, chr1B in red) leading to a tetraploid *C. carpio* with both ancestral chromosome sets. *Danio rerio* is depicted with its diploid structure (2n), maintaining a single chromosome set (chr1). **(Bottom)** Phylogenetic tree showing the relationship of *tgfbr2a* gene variants between *C. carpio* and *D. rerio*. Structural variations in ligand-binding domains are illustrated beside each gene. **(b, top)** Phylogenetic depiction of chromosomal evolution in the *Xenopus* lineage, illustrating the allopolyploid origin of *Xenopus laevis* (4n) from two diploid ancestors ("L" and "S"). Each ancestor contributed a set of chromosomes (chr1L in blue, chr1S in red) leading to a tetraploid *X. laevis* with both ancestral chromosome sets. *Xenopus tropicalis* is depicted with its diploid structure (2n), maintaining a single chromosome set (chr1). **(Bottom)** Phylogenetic tree showing the relationship of *tgfbr2* gene variants between *X. laevis* and *X. tropicalis*. Structural variations in ligand-binding domains are illustrated beside each gene. **(a, b)** Schematics created in BioRender. Trumpp, M. (2026), <https://BioRender.com/8p64dhh>.

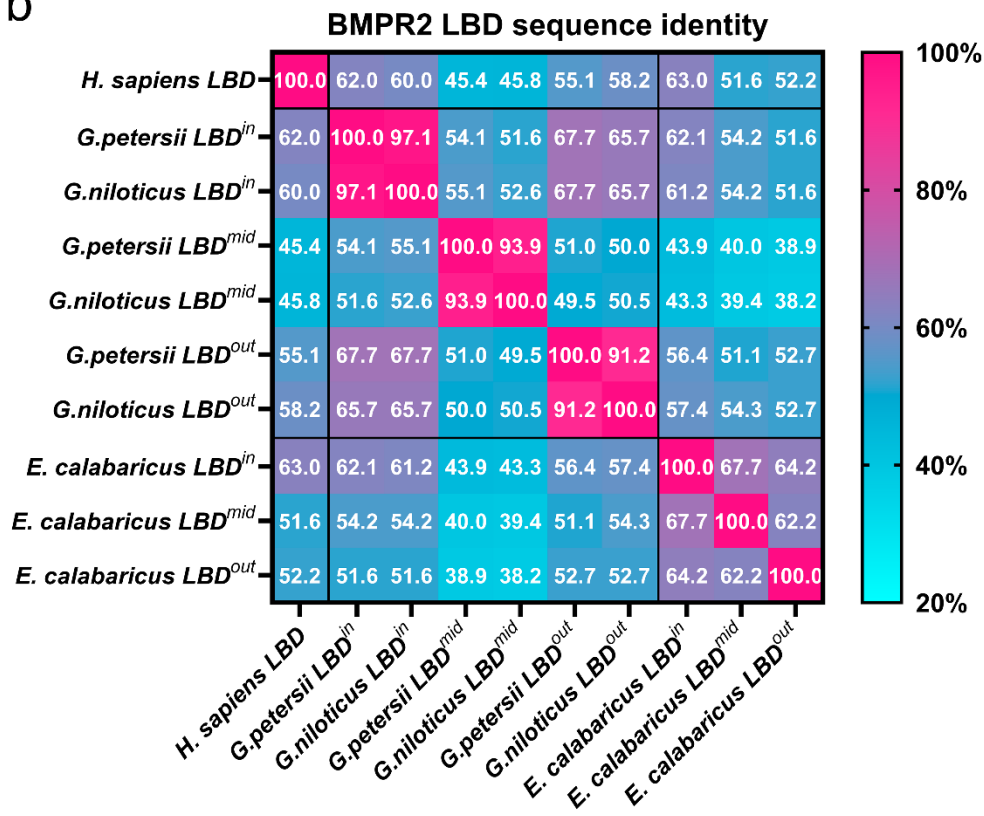


Supplementary Figure 2: Genetic architecture of duplicated LBDs of TGF β receptor family members. (a) Schematic representation of receptor gene exon composition of *Acvr1*, *Acvr1l*, *BMPR2*, *Bmpr2a*, *TGFBR2* and *TGFBR2L*. **(b)** Genomic organization of *TGFBR2* intronic sites flanking exons encoding in horse, chicken, and *Tgfbr2a* in zebrafish, including annotations size and common repetitive elements. **(a, b)** Schematics created in BioRender. Trumpp, M. (2026), <https://BioRender.com/8p64dhh>.

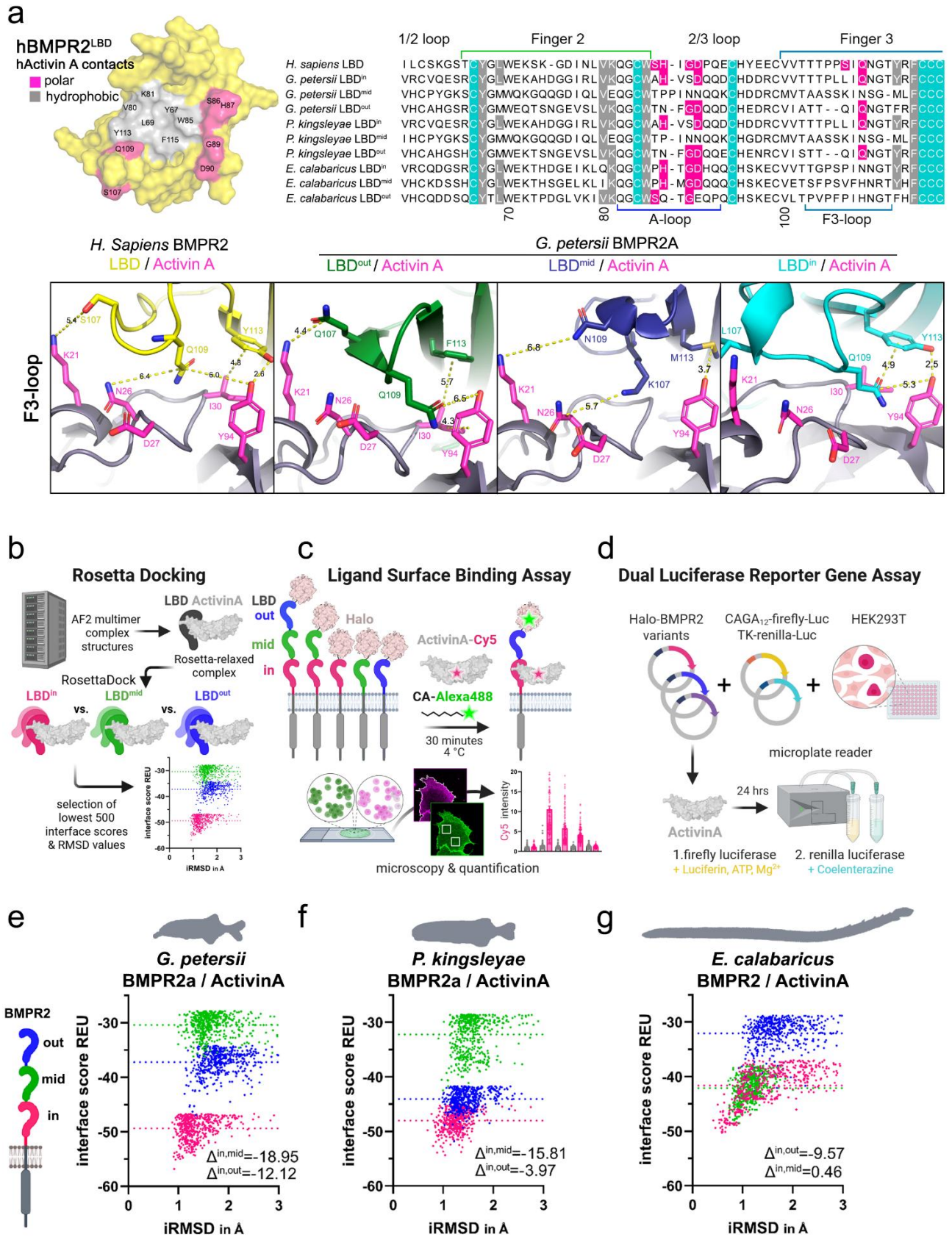
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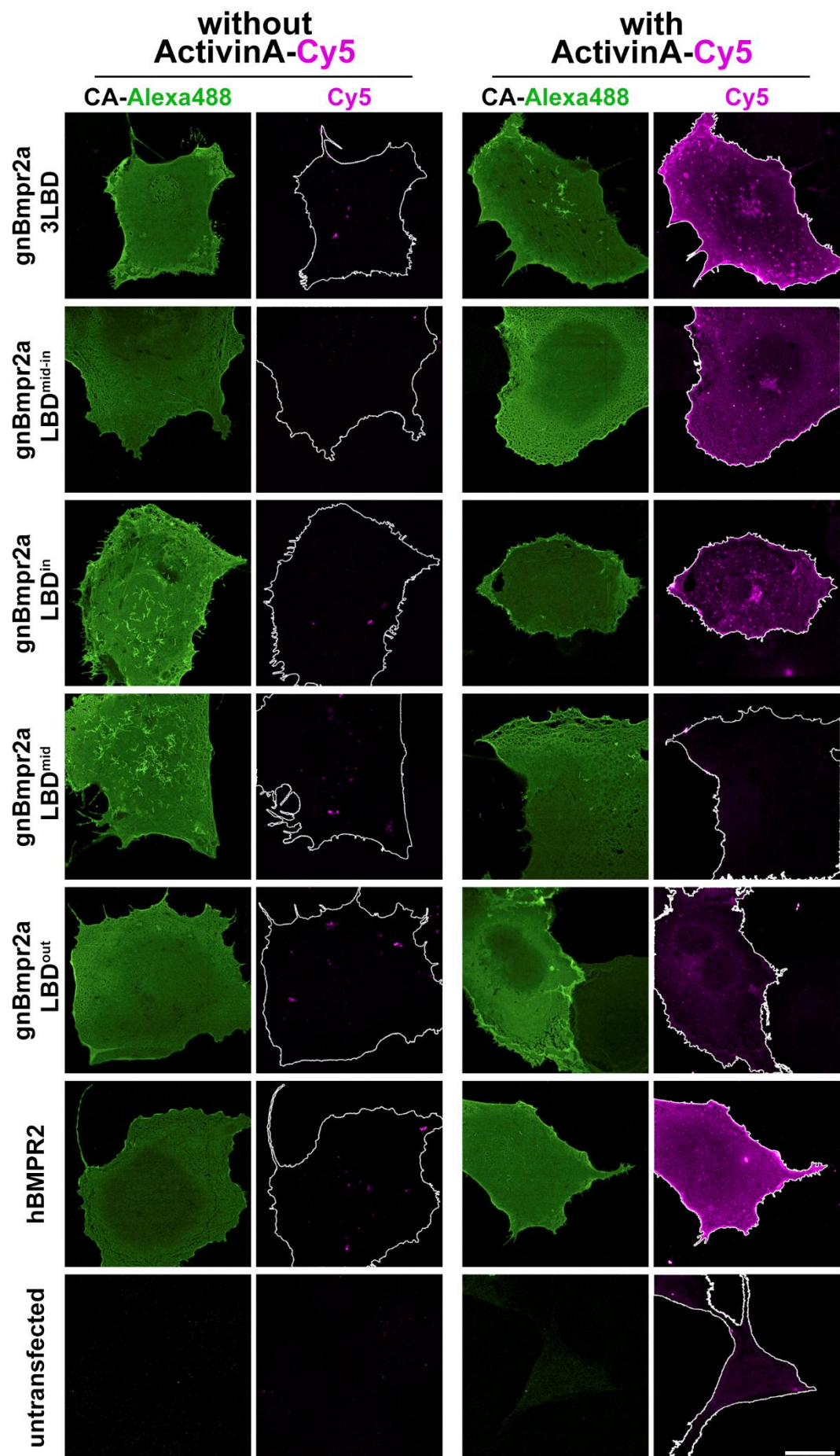


Supplementary Figure 3: LBD sequence identity comparison of ACVR1 and BMPR2 orthologs. (a) ACVR1 LBD sequence identity matrix comparing all LBD ACVR1 variants of indicated species. (b) BMPR2 LBD sequence identity matrix comparing all LBD BMPR2 variants of indicated species with each other.



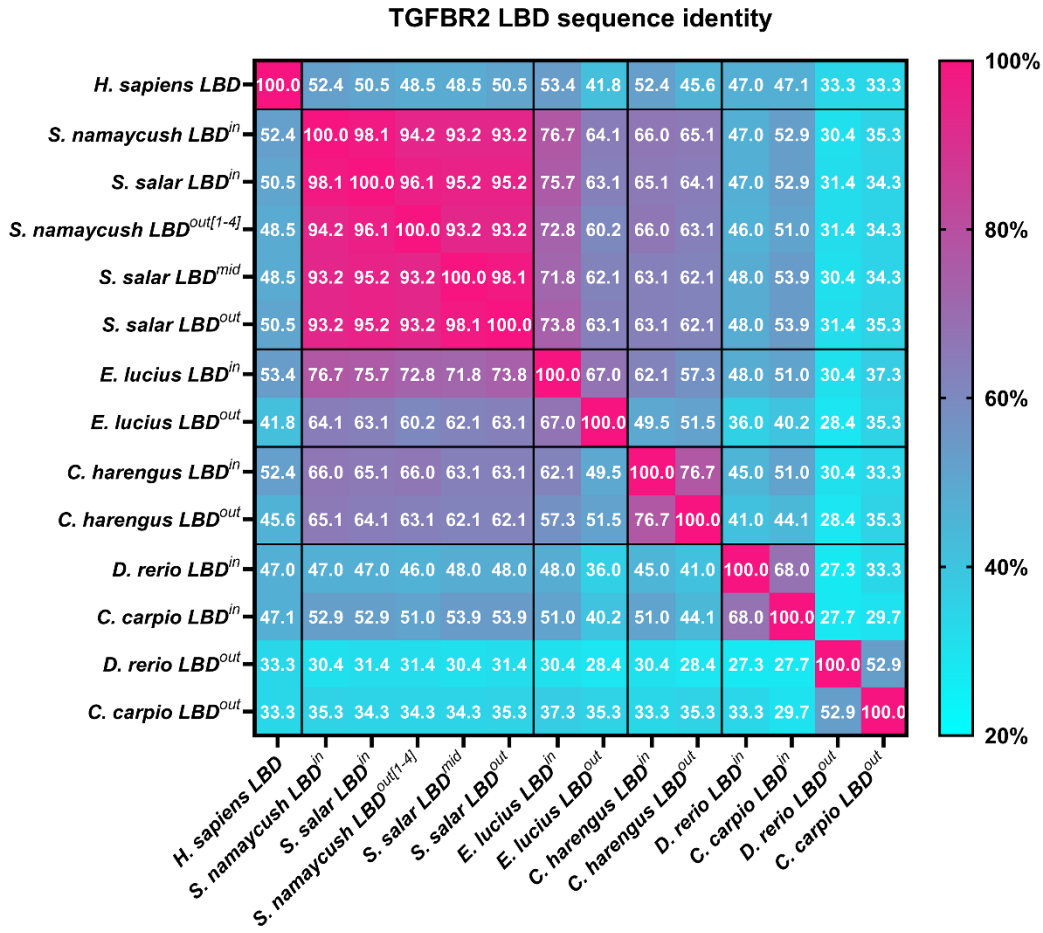
Supplementary Figure 4: Inner LBD conveys Activin A binding competence of LBD multimerized BMPR2 orthologs. (a) Molecular surface of hBMPR2 LBD showing the hActivin A interface of a rosetta-docked AlphaFold2-multimer structure prediction. Residues forming

polar or hydrophobic contacts are colored in pink and gray, respectively (**left**). Sequence alignment of BMPR2 LBDs of *Homo Sapiens*, *G. petersii*, *G. niloticus* and *E. calabaricus* LBDs with hActivin A contact residues and critical backbone cysteins (teal) highlighted; interaction sites F3-loop and A-loop are indicated with colored lines (**right**). (**a, lower**) Cartoon and stick representation of respective animal receptor LBD interaction with hActivin A (A-loop) based on AlphaFold2-multimer models docked to hActivin A using Rosetta docking protocols. PyMOL was used for image representation. (**b**) Schematic representation of *in silico* binding analysis workflow using Rosetta docking. AlphaFold (AF2 multimer) LBD-Activin A complex structures are generated, followed by initial coordinate constrained relaxation to obtain optimized H-bonds. Docking of LBDs is then performed through RosettaScripts (n=2500). Interface score (REU) versus iRMSD plots identify the top 500 models with the lowest scores, indicating different ligand binding capabilities. (**c**) Illustration of LSBA for visualization and quantification of fluorescent hActivin A binding on COS-7 cells expressing respective Halo-tagged BMPR2 receptor constructs. (**d**) Schematic representation of dual luciferase reporter gene assay used to assess Activin A signaling activity through Halo-tagged BMPR2 receptor orthologs. HEK293T cells are co-transfected with respective receptor variant, CAGA₁₂-firefly-Luc, and TK-renilla-Luc plasmids. Following Activin A treatment for 24 hours, Firefly luciferase activity (indicative of Activin A pathway activation) and Renilla luciferase activity (normalization control) are measured. *In silico* binding analysis of (**e**) *G. petersii* (**f**) *P. kingsleyae* (**g**) *E. calabaricus* Activin A interactions with its respective single Bmpr2a domains obtained via Rosetta Docking, depicted as interface score (REU) in relation to interface root mean square deviation (iRMSD) in angstroms. (**right**) Illustration of respective animal BMPR2a 3 LBD receptor domain structure as reference, highlighting the inner (pink), middle (green) and outer (blue) ligand binding domain. REU differences of out and mid LBD mean are calculated against inner LBD mean value. Source data are provided as a Source Data file. (**b–d, e**) Schematics created in BioRender. Trumpp, M. (2026), <https://BioRender.com/8p64dhh>.

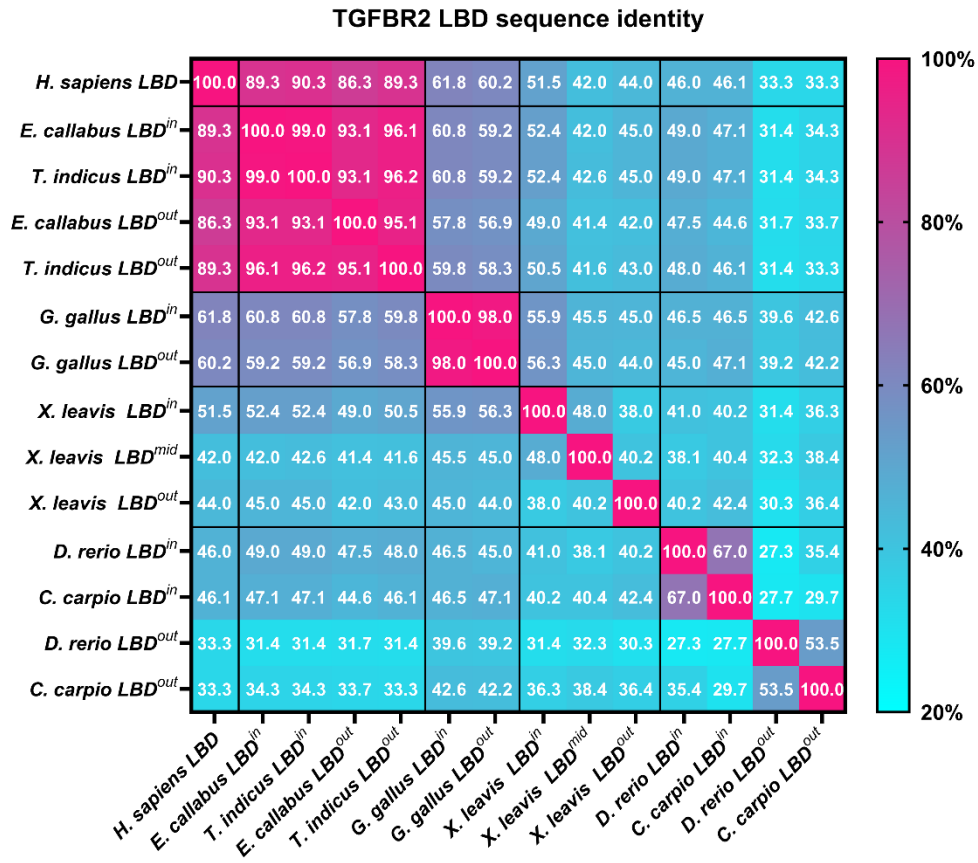


Supplementary Figure 5: Representative LSBA images of Activin A bound to gnBmpr2a variants. Representative confocal microscopy images of LSBA for unstimulated and hActivinA-Cy5 stimulated COS-7 cells expressing *Gnathonemus petersii* receptor variants (gnBmpr2a (3LBD), gnBmpr2a-LBD^{midin}, gnBmpr2a-LBDⁱⁿ, gnBmpr2a-LBD^{mid}, gnBmpr2a-LBD^{out}) or hBMPR2 or untransfected control. Scale bar $\triangleq$ 20 μ m.

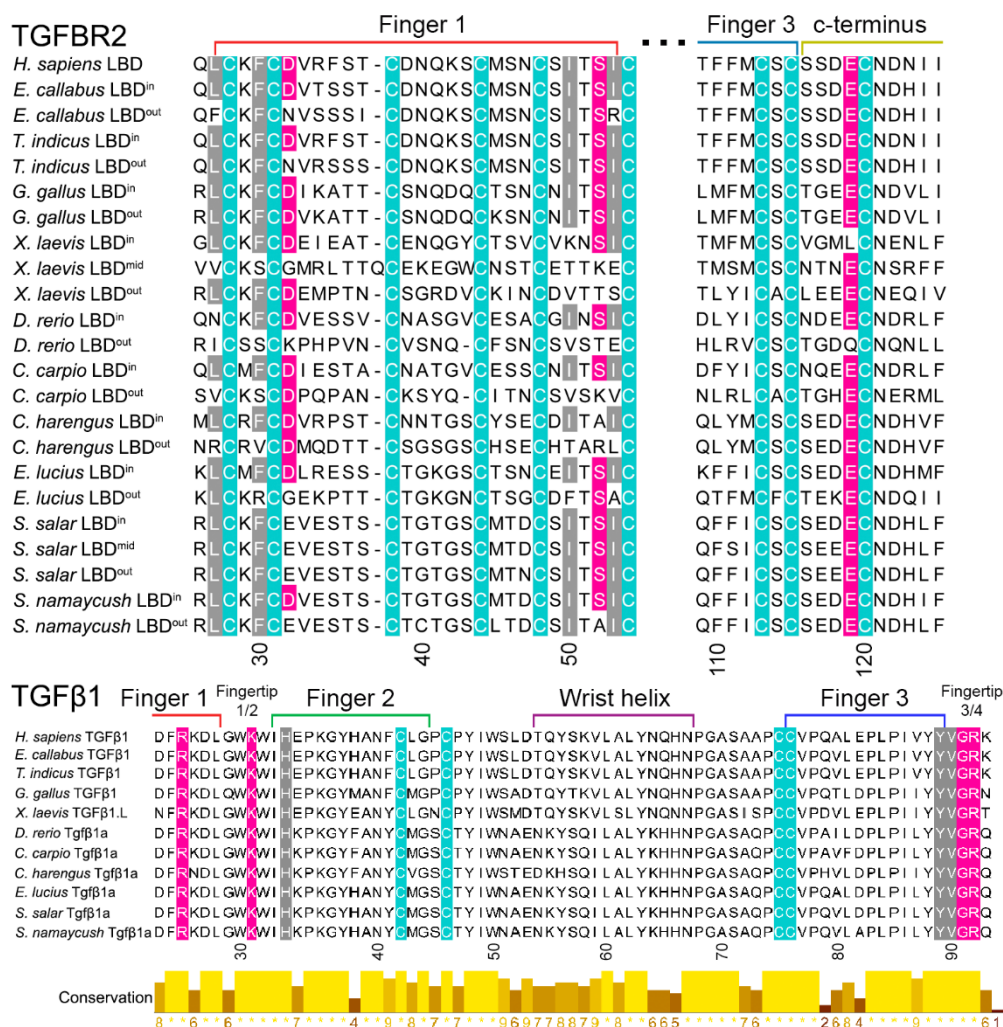
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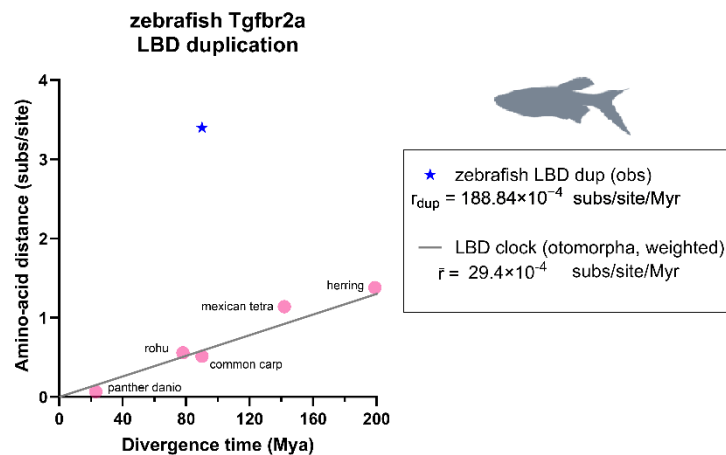
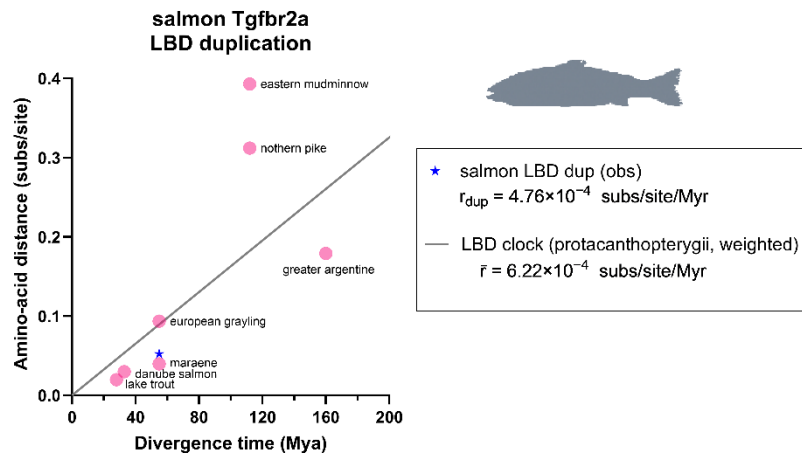
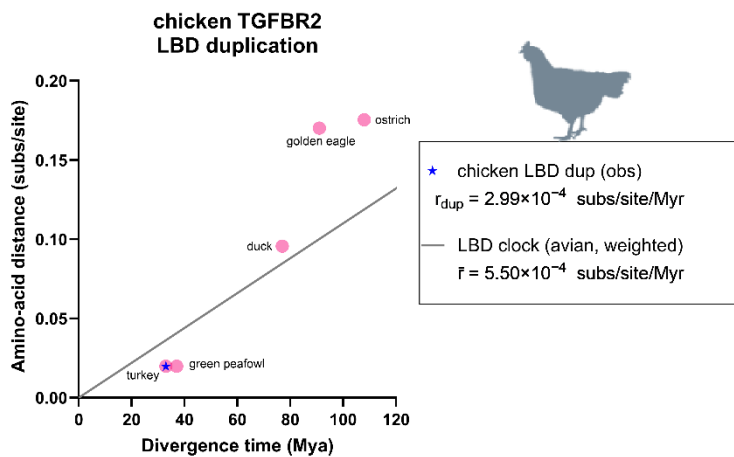
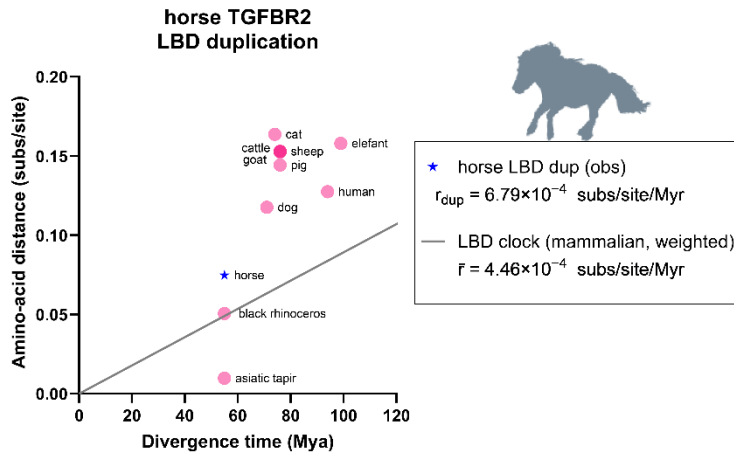
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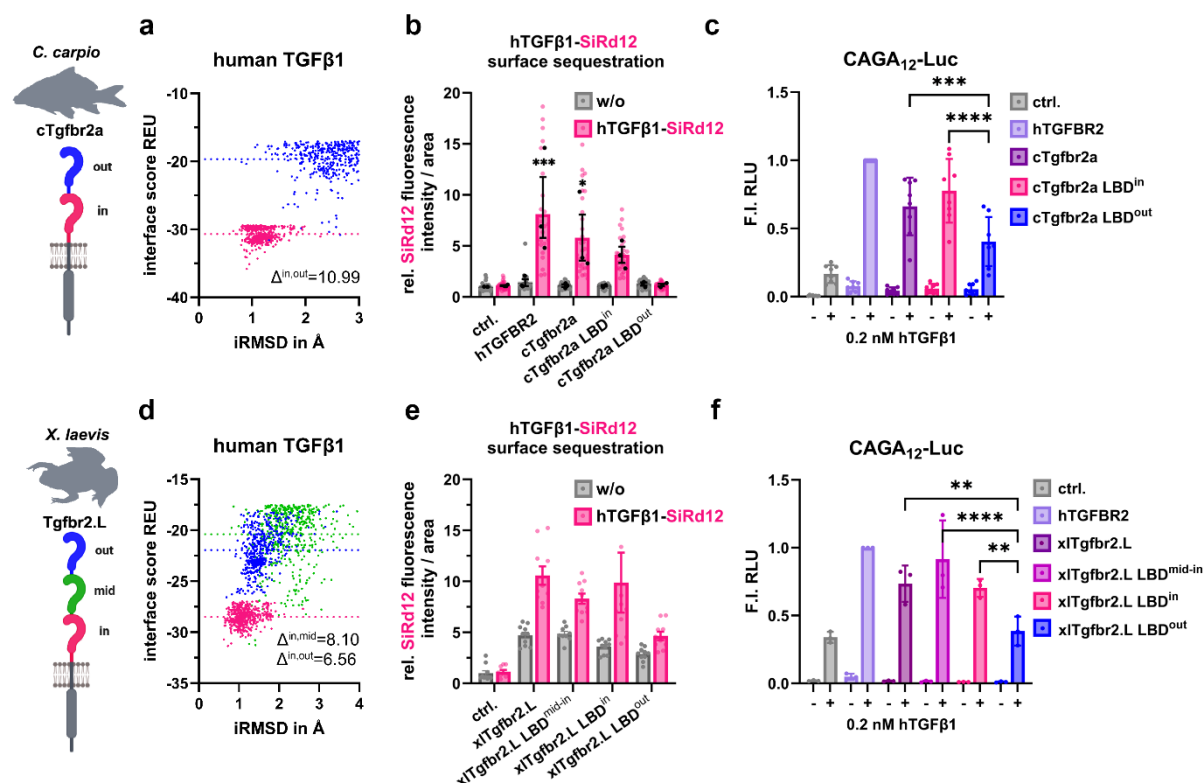
Supplementary Figure 6: LBD sequence identity comparison of TGFBR2 orthologs. (a) TGFBR2 LBD sequence identity matrix comparing all LBD TGFBR2 variants of human and indicated ray finned fish species. (b) TGFBR2 LBD sequence identity matrix comparing all LBD TGFBR2 variants of indicated jaw vertebrate as well as Cyprinoidei species.



Supplementary Figure 7: Sequence alignment of multimerized TGFBR2 LBDs and TGFβ1 orthologs. TGFBR2 LBD and TGFβ1 ligand sequence alignment of *H. sapiens*, *E. callabus*, *T. indicus*, *G. Gallus*, *X. laevis*, *D. rerio*, *C. carpio*, *C. harengus*, *E. lucius*, *S. salar*, *S. namaycush*; polar receptor:ligand interaction sites are shown in light magenta and hydrophobic interaction sites in grey and highly conserved backbone cysteins in teal. Interaction domains are indicated above.

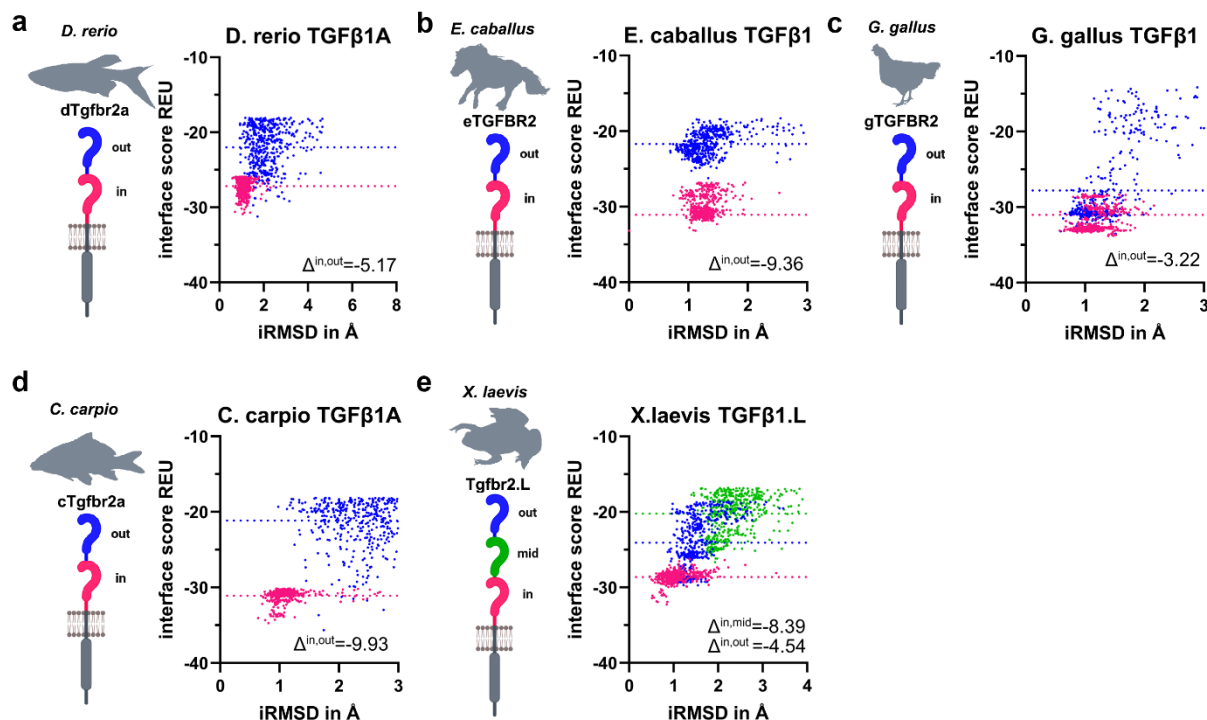


Supplementary Figure 8: Evolutionary divergence of duplicated LBD domains relative to species divergence times. Each panel shows the relationship between amino-acid distance and species divergence time for duplicated LBDs of TGFBR2/Tgfr2a in four focal species: horse, chicken, salmon, and zebrafish. Pink circles represent pairwise comparisons between the inner LBD of the focal species and the orthologous inner LBD of other species in the same clade. The gray line depicts the clade-specific molecular clock, obtained by weighted linear regression of amino-acid distance against species divergence time; the resulting substitution rate ($\bar{r}$), expressed as substitutions per site per million years, is shown in each panel. The blue star marks the observed amino-acid divergence between the two duplicated LBD copies within the focal species, and the duplication-specific evolutionary rate (r_{dup}) is reported in each panel. Source data are provided as a Source Data file.

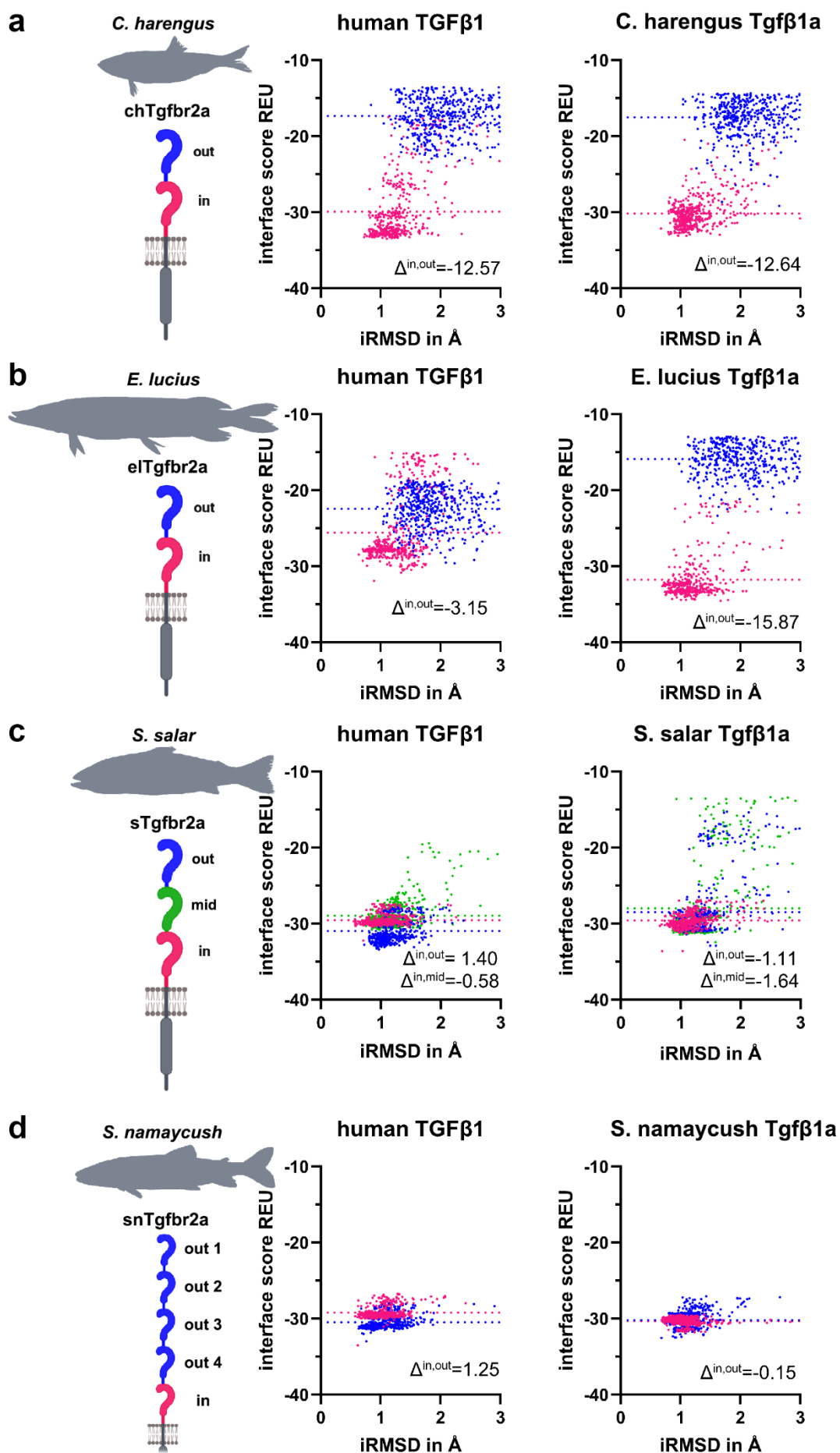


Supplementary Figure 9: Functional implications of LBD multiplication in TGFBR2 orthologs of *Cyprinus carpio* and *Xenopus laevis*. (a, d) *in silico* binding analysis via Rosetta docking of hTGFβ1 to inner (pink) and outer LBD (blue) of *C. carpio*, *X. laevis* depicted as interface score (REU) in relation to interface root mean square deviation (iRMSD) in angstroms. REU differences of in and out are calculated by respective mean REU values of each LBD. (b) hTGFβ1-SiR-d12 surface binding to *C. carpio* receptor variants, shown as relative fluorescence intensity per area ($\pm$ hTGFβ1-SiR-d12). Data are mean $\pm$ SEM. $n = 3$ independent biological replicates (independent experiments; up to 10 cells per experiment averaged). Two-way ANOVA with Dunnett's multiple comparisons test (two-sided; vs ctrl.) was used. Exact P values: hTGFBR2 vs ctrl., $p = 0.0009$; cTgfr2a vs ctrl., $p = 0.0493$; cTgfr2a LBDⁱⁿ vs ctrl., $p = 0.3244$. Error bars represent SEM; black dots indicate independent biological replicates, and grey and pink dots represent individual cells. (e) hTGFβ1-SiR-d12 surface binding to *X. laevis* receptor variants. One representative independent experiment is shown ($n = 1$; ~ 10 cells analysed); the experiment was repeated four times with similar results. Data are presented as mean $\pm$ SEM. No statistical testing was performed. Grey and pink dots represent individual cells. (c, f) pSMAD2/3-sensitive CAGA₁₂ luciferase reporter activity of *C. carpio* (c) and *X. laevis* (f) receptor variants in the presence or absence of hTGFβ1 (0.2 nM), shown as fold induction (F.I.) of relative light units (RLU) relative to hTGFBR2 (+TGFβ1). Data are mean

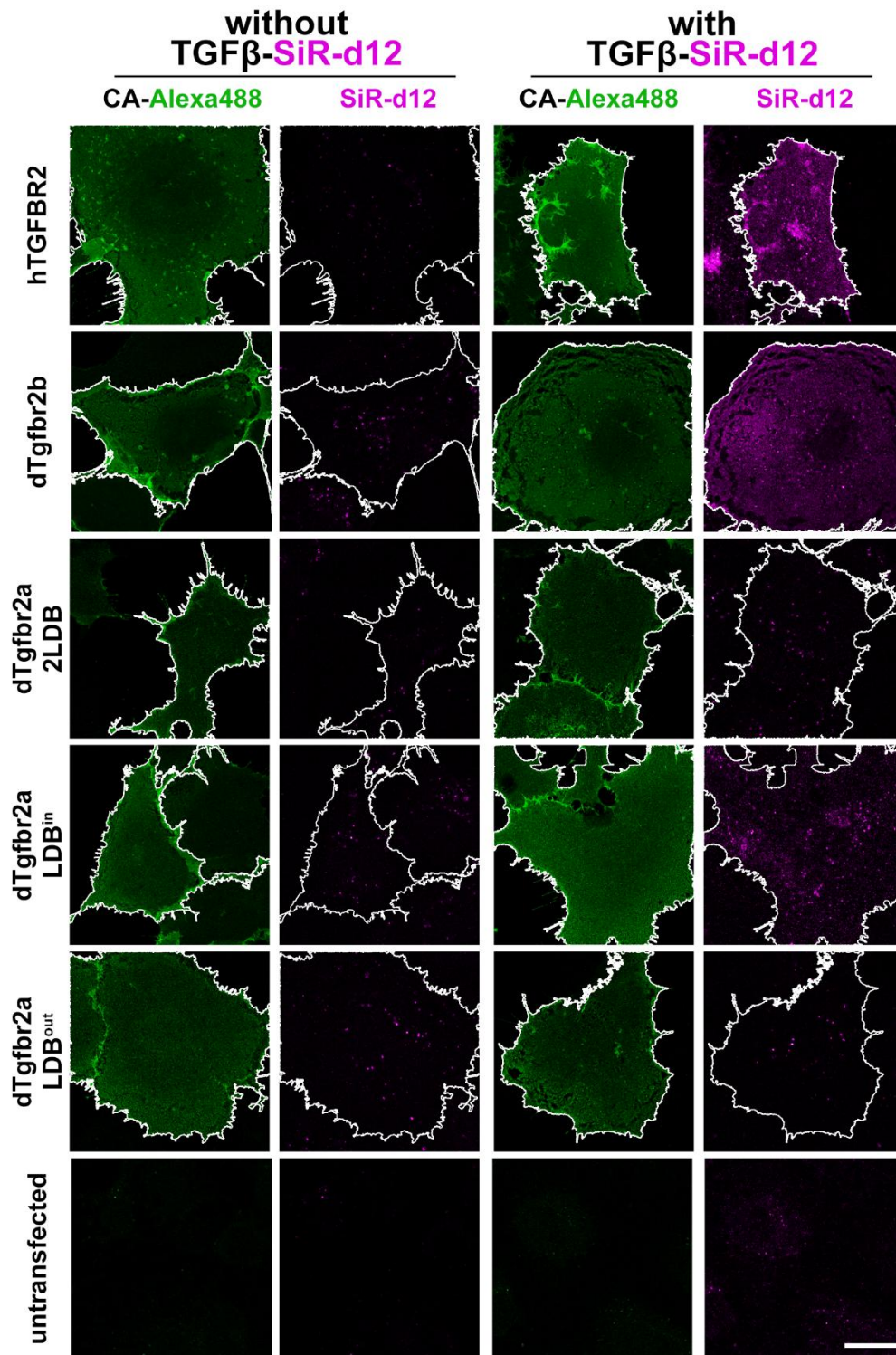
$\pm$ SD. $n = 8$ independent biological replicates (independent experiments) for (c) and $n = 3$ independent biological replicates for (f). Two-way ANOVA followed by Dunnett's multiple comparisons test (two-sided; vs LBD^{out} variants) was used. Exact P values: (c) cTGFR2 vs cTGFR2 LBD^{out}, $p = 0.0002$; cTGFR2 LBDⁱⁿ vs cTGFR2 LBD^{out}, $p < 0.0001$; (f) xITGFR2 vs xITGFR2 LBD^{out}, $p = 0.0011$; xITGFR2 LBD^{mid-in} vs xITGFR2 LBD^{out}, $p < 0.0001$; xITGFR2 LBDⁱⁿ vs xITGFR2 LBD^{out}, $p = 0.0027$. Error bars represent SD. Source data are provided as a Source Data file. (a, d) Schematics created in BioRender. Trumpp, M. (2026), <https://BioRender.com/8p64dhh>.



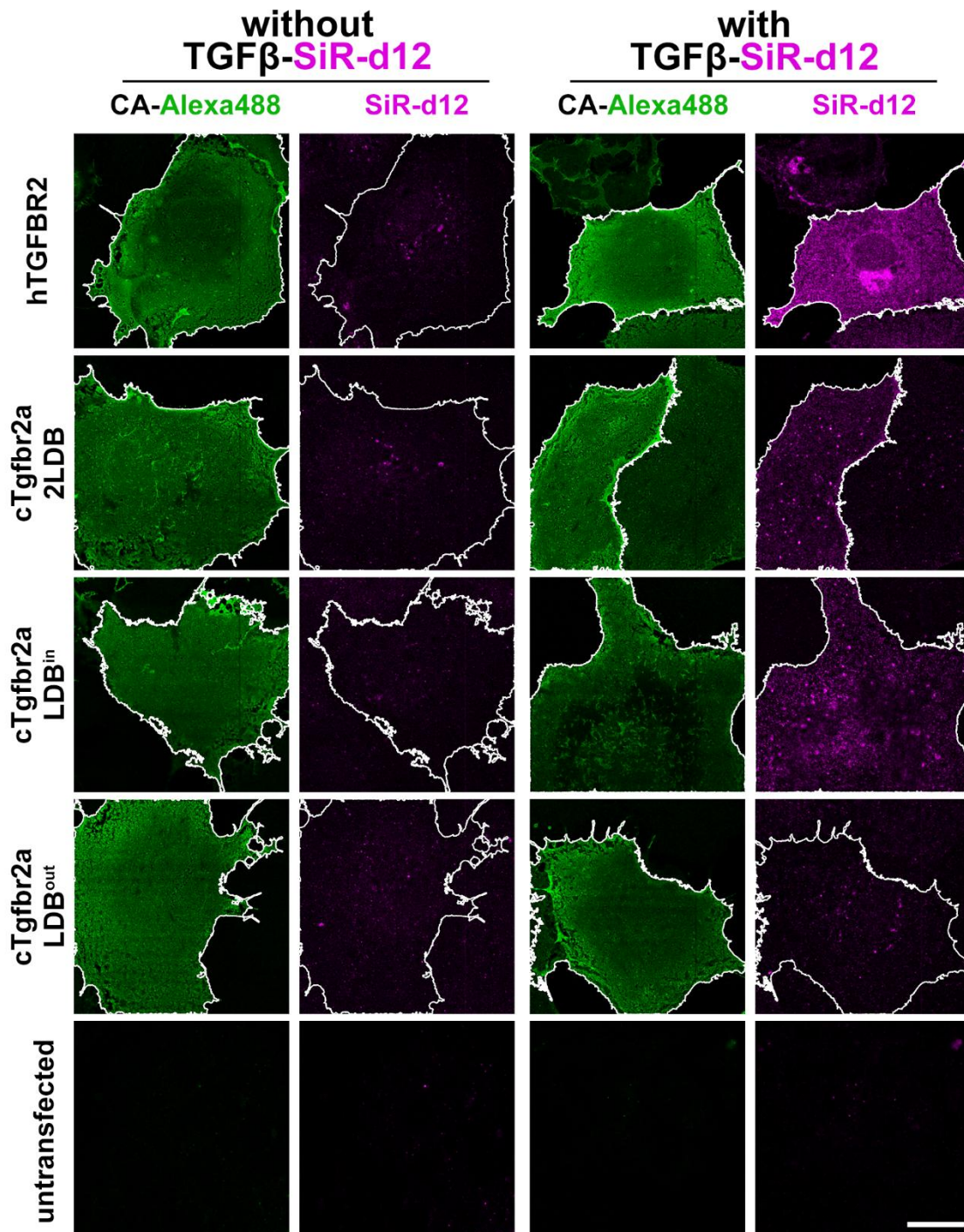
Supplementary Figure 10: *In silico* binding analysis of TGFR2 orthologs with respective animal TGFB1. *In silico* binding analysis via Rosetta docking and illustration of respective animal TGFR2 LBD receptor domain structure as color reference of (a) *Danio rerio* (b) *Equus caballus* (c) *Gallus gallus* (d) *Cyprinus carpio* (e) *Xenopus laevis* TGFβ1 interactions with its respective single TGFR2/Tgfr2a/Tgfr2.L domains, depicted as interface score (REU) in relation to interface root mean square deviation (iRMSD) in angstroms. REU differences of out and mid LBD mean are calculated against inner LBD mean value. Source data are provided as a Source Data file. (a–e) Schematics created in BioRender. Trumpp, M. (2026), <https://BioRender.com/8p64dhh>.



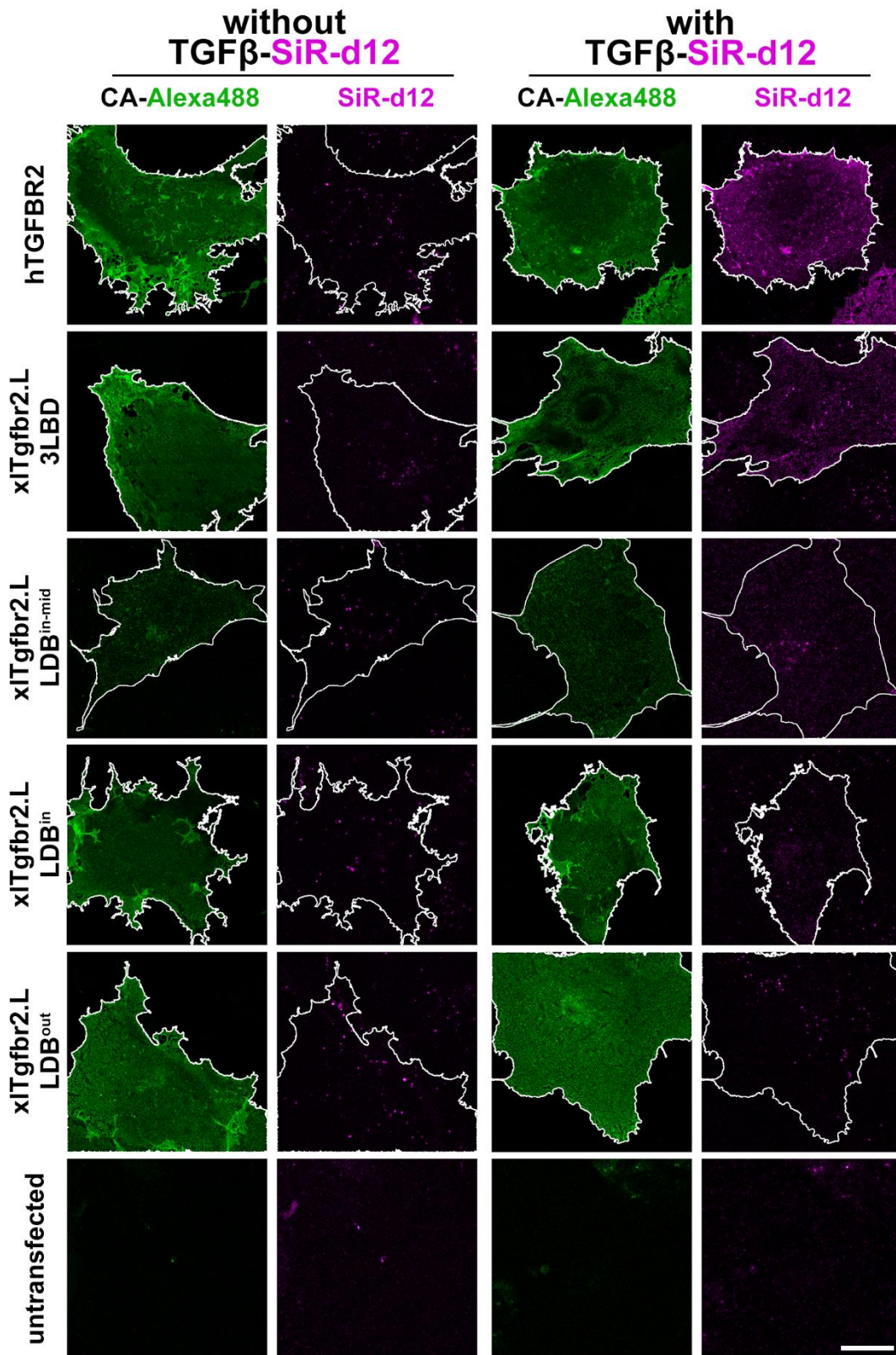
Supplementary Figure 11: *In silico* binding analysis of ray-finned fish TGFBR2 orthologs with human and respective animal TGFβ1. Illustration of respective animal TGFBR2 LBD receptor domain structure as color reference and Rosetta docking via *in silico* binding analysis of (a) *Clupea harengus* (b) *Esox lucius* (c) *Salmo salar* (d) *Salvelinus namaycush* TGFβ1 and hTGFβ1 interactions with respective single Tgfbr2a domains, depicted as interface score (REU) in relation to interface root mean square deviation (iRMSD) in angstroms. REU differences of out and mid LBD mean are calculated against inner LBD mean value. Source data are provided as a Source Data file. (a–d) Schematics created in BioRender. Trumpp, M. (2026), <https://BioRender.com/8p64dhh>.



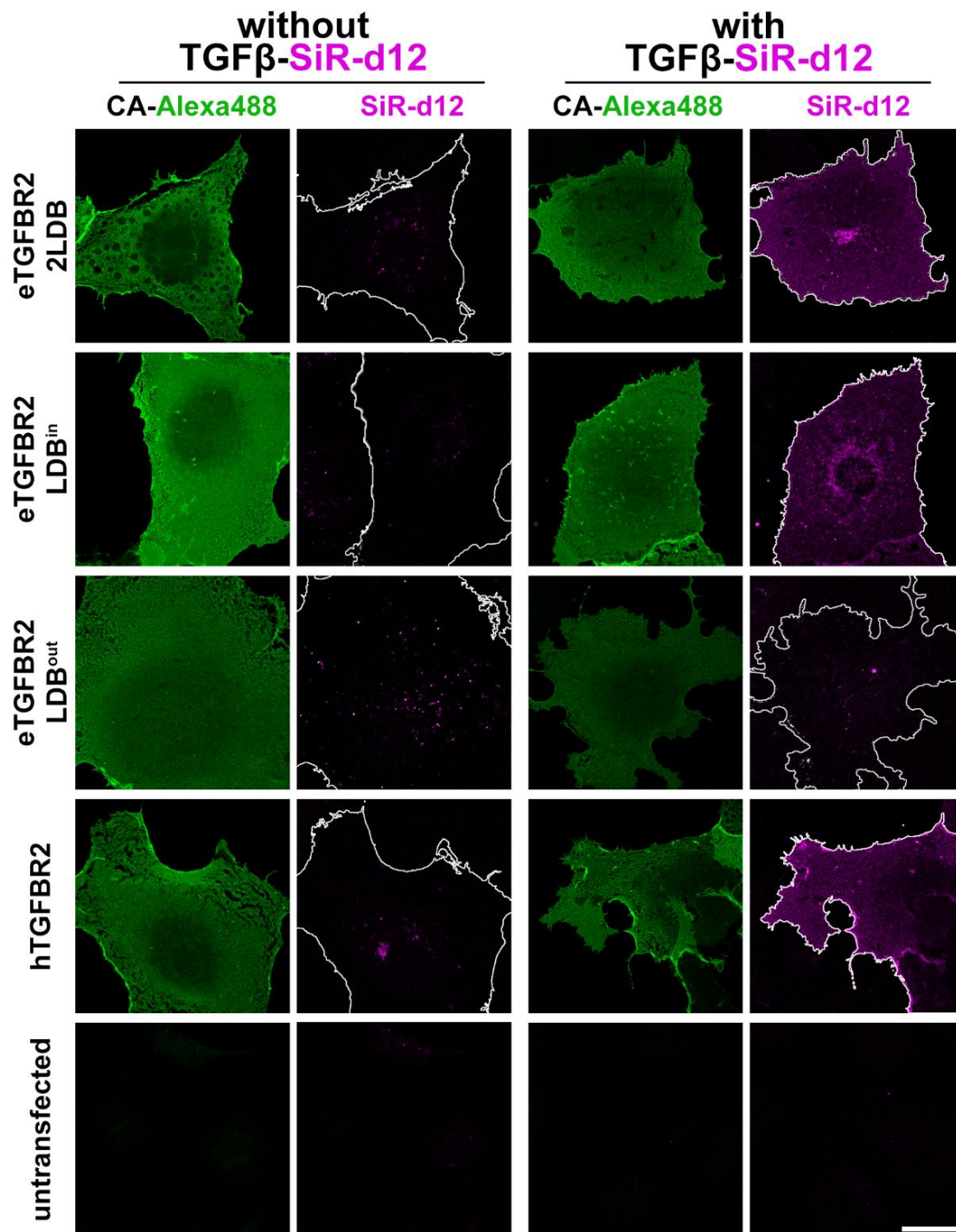
Supplementary Figure 12: Representative LSBA images of TGFβ1 bound to dTgfr2a/b variants. Representative confocal microscopy images of LSBA for unstimulated and hTGFβ1-SiR-d12 stimulated COS-7 cells expressing human BMPR2 or *danio rerio* variants (dTgfr2b, dTgfr2a (2LBD), dTgfr2a-LBDⁱⁿ, dTgfr2a-LBD^{out}) or untransfected control. Scale bar $\cong$ 20 μ m.



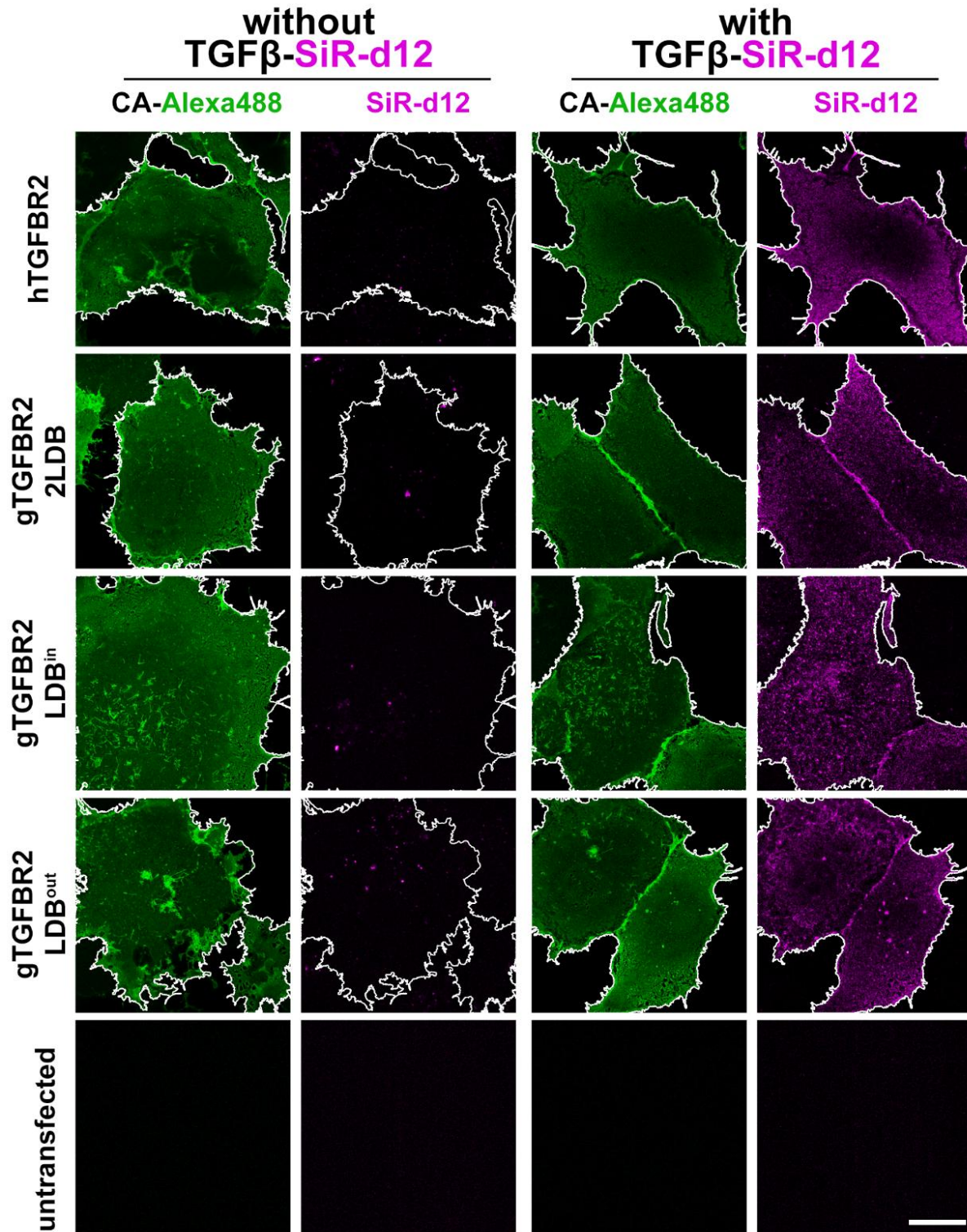
Supplementary Figure 13: Representative LSBA images of TGFβ1 bound to cTgfr2a variants. Representative confocal microscopy images of LSBA for unstimulated and hTGFβ1-SiR-d12 stimulated COS-7 cells expressing human TGFR2 or *Cyprinus carpio* variants (cTgfr2a (2LBD), cTgfr2a-LBDⁱⁿ, cTgfr2a-LBD^{out}) or untransfected control. Scale bar $\triangleq$ 20 μ m.



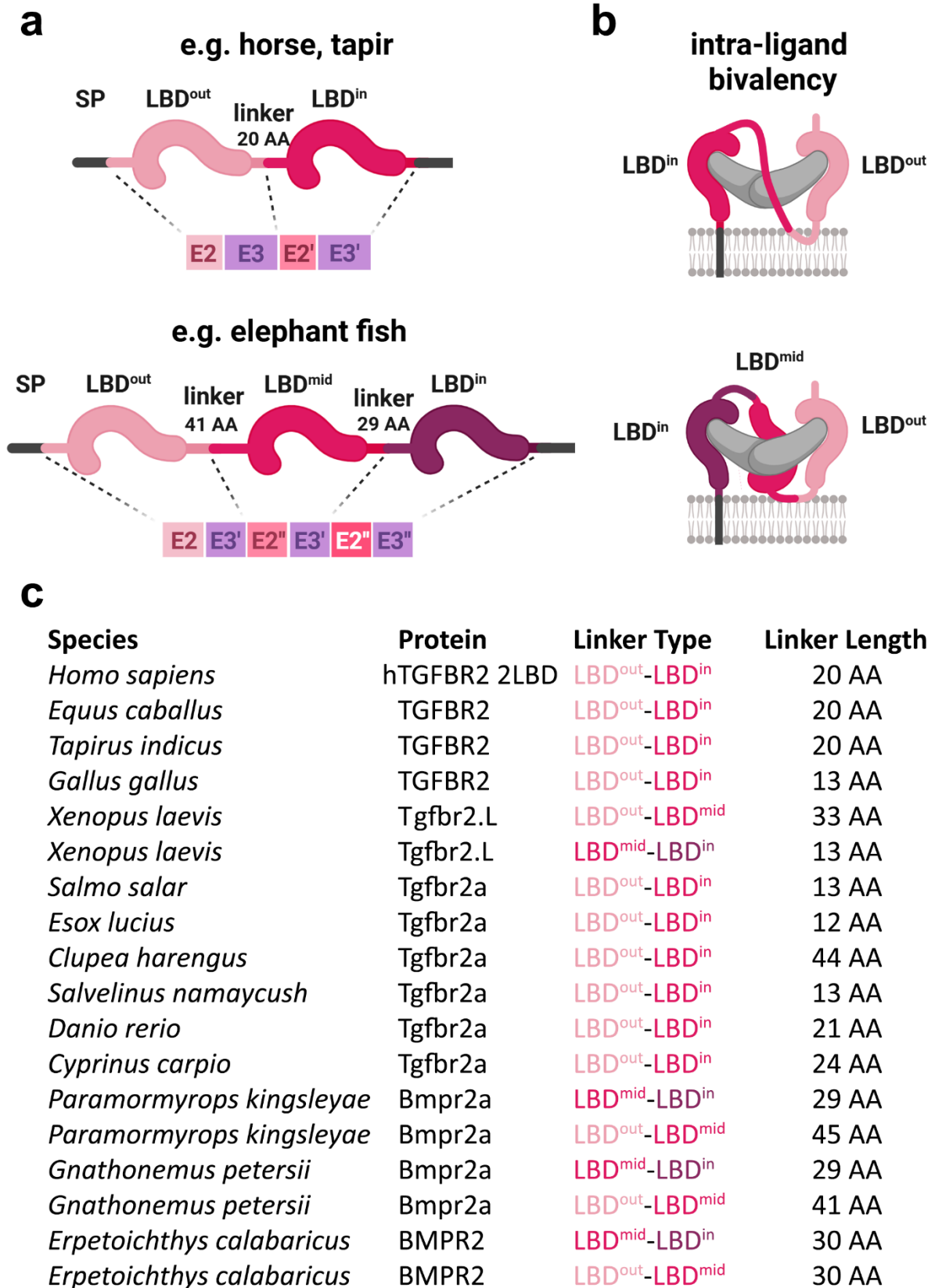
Supplementary Figure 14: Representative LSBA images of TGFβ1 bound to xITgfr2.L variants. Representative confocal microscopy images of LSBA for unstimulated and hTGFβ1-SiR-d12 stimulated COS-7 cells expressing human TGFR2 or *Xenopus laevis* variants (xITgfr2.L (3LBD), xITgfr2.L-LBD^{mid-in}, xITgfr2.L-LBDⁱⁿ, xITgfr2.L-LBD^{out}) or untransfected control. Scale bar $\cong 20 \mu\text{m}$.



Supplementary Figure 15: Representative LSBA images of TGFβ1 bound to eTGFR2 variants. Representative confocal microscopy images of LSBA for unstimulated and hTGFβ1-SiR-d12 stimulated COS-7 cells expressing *Equus caballus* variants (eTGFR2 (2LBD), eTGFR2-LBDⁱⁿ, eTGFR2-LBD^{out}) and human TGFR2 or untransfected control. Scale bar $\cong 20 \mu\text{m}$.

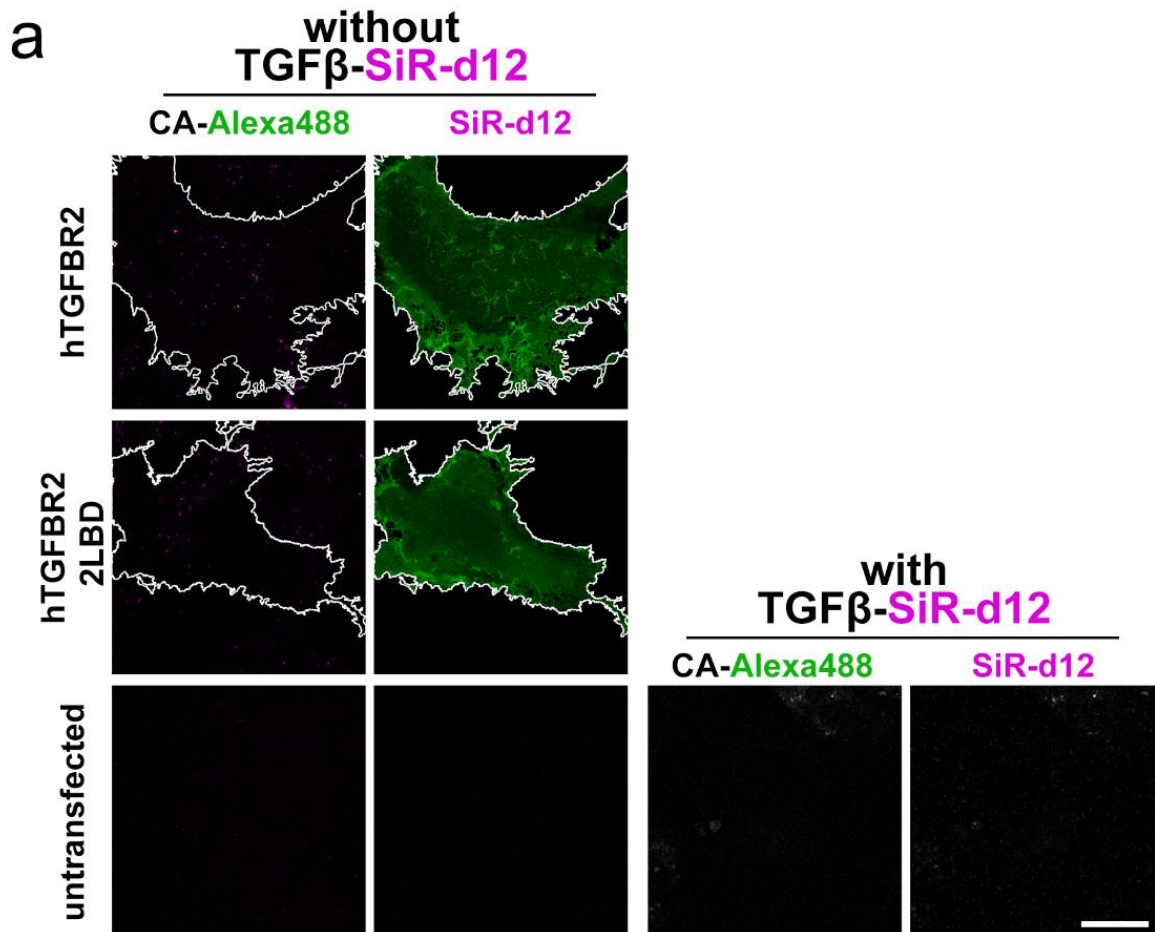


Supplementary Figure 16: Representative LSBA images of TGFβ1 bound to gTGFB2 variants. Representative confocal microscopy images of LSBA for unstimulated and hTGFβ1-SiR-d12 stimulated COS-7 cells expressing human TGFB2 and *Gallus gallus* variants (gTGFB2 (2LBD), gTGFB2-LBDⁱⁿ, gTGFB2-LBD^{out}) and human TGFB2 or untransfected control. Scale bar $\cong$ 20 μ m.

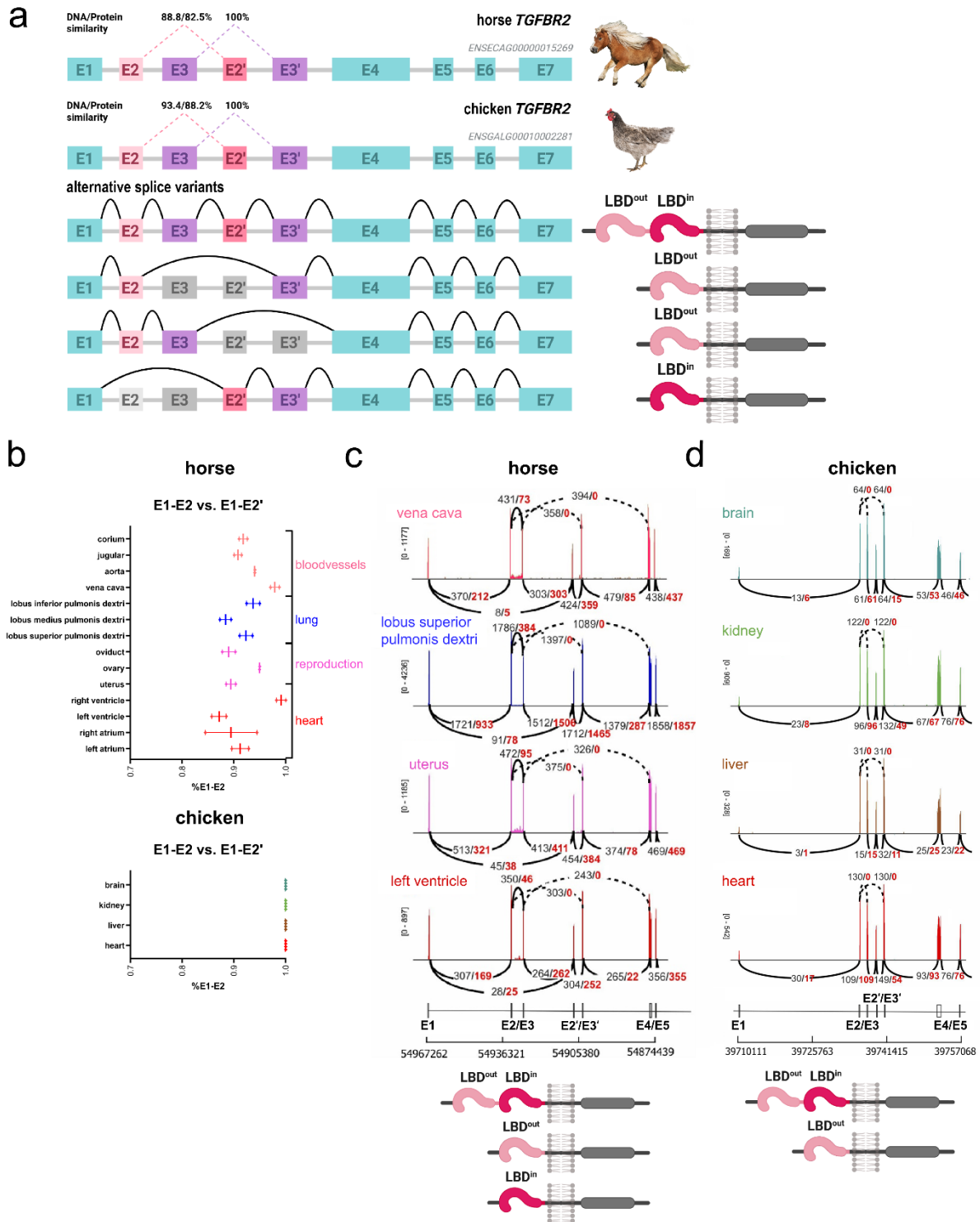


Supplementary Figure 17: Linker architecture in TGFR2 and BMFR2 variants. (a) Schematic illustration of exon architecture of receptors with duplicated (i.e. TGFR2) or triplicated (i.e. BMFR2) extracellular LBDs. Linker length is defined by the residues encoded at the junction of exons E3 and E2'. (b) Illustration of two potential modes of intra-ligand bivalency of receptors with two or three LBDs. Intra-ligand bivalency may require a sufficiently

long and flexible linker between the LBDs. The presence of a third LBD could further affect the likelihood of bivalent binding. (c) Cross-species overview of linker length variation in duplicated LBD TGFBR2 and BMPR2 variants. (a, b) Schematics created in BioRender. Trumpp, M. (2026), <https://BioRender.com/8p64dhh>.

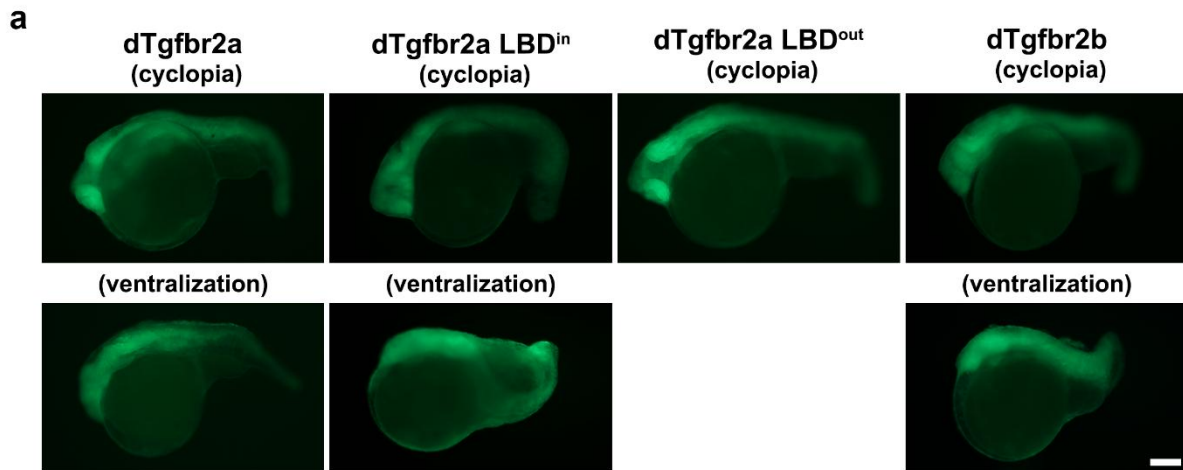


Supplementary Figure 18: Representative LSBA images of TGFβ1 bound to hTGFBFR2 variants. Representative confocal microscopy images of LSBA for unstimulated COS-7 cells expressing hTGFBFR2 or hTGFBFR2-2LBD or untransfected control and hTGFβ1-SiR-d12 stimulated untransfected cells. Scale bar $\triangleq$ 20 μ m.

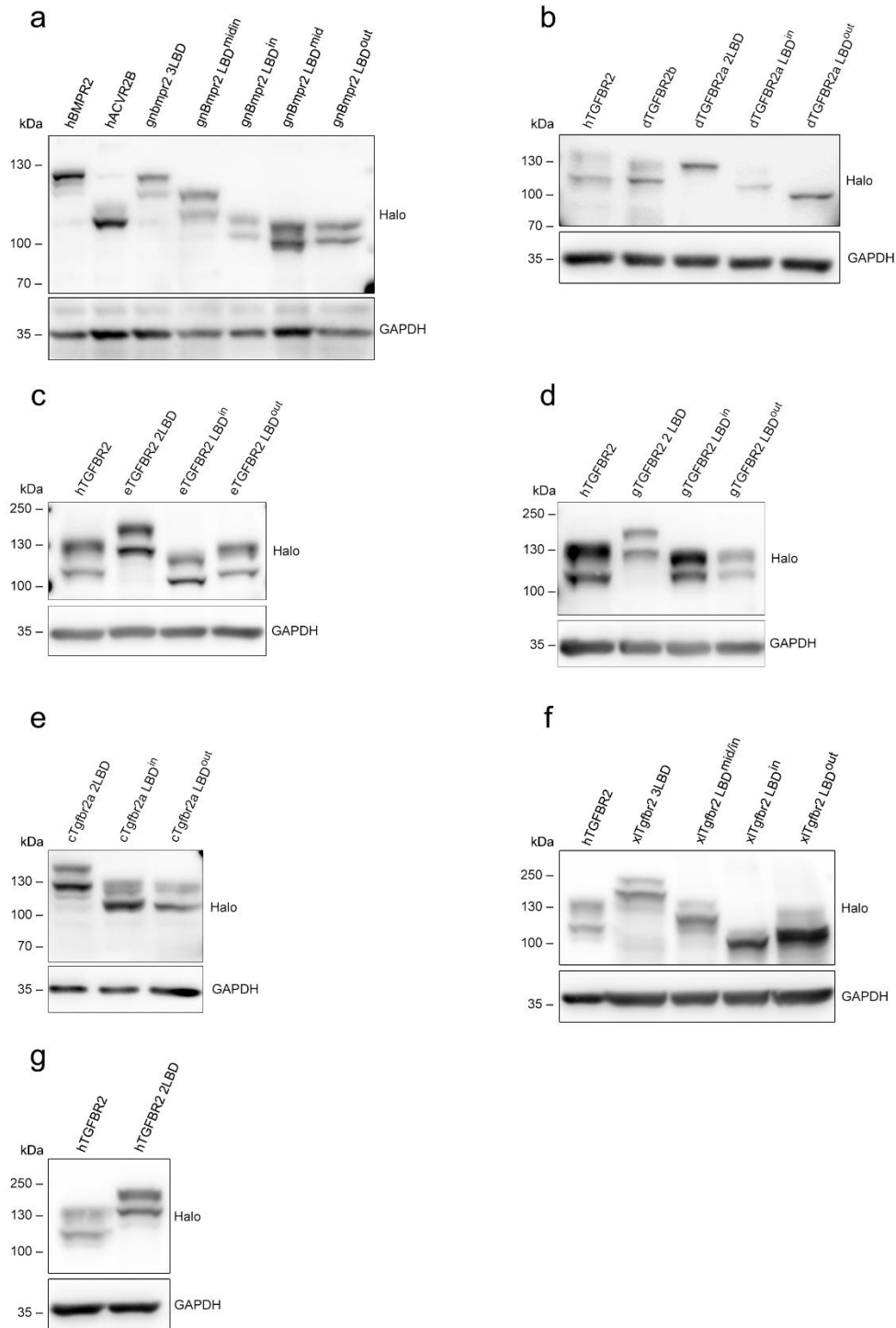


Supplementary Figure 19: Alternative splice variants of TGFBR2 in horse and chicken. (a, top) Exon organization of horse eTGFBR2 and chicken gTGFBR2 with DNA/protein similarity percentages between corresponding exons. (bottom) Illustration of alternative splice variants, showing different combinations of exons leading to 2LBD, LBD_{out}, LBD_{in} receptor variants. (b) Summary of relative usage 2a/2b usage across 14 horse tissues. Tissues are indicated on the left (reproduction, blood vessels, lung, heart). Tissues used for Sashimi plots are highlighted. (c,d) Sashimi plot of the E1 to E5 region of horse (left) and chicken (right) TGFBR2. Exon coverage is shown on the left. Junctions are indicated by lines, junctions not supported by unique reads are shown as dotted lines. Next to each junction the black number

shows all reads mapped to this junction, the red number reports unique reads only. Source data are provided as a Source Data file. (a, c–d) Schematics created in BioRender. Trumpf, M. (2026), <https://BioRender.com/8p64dhh>.



Supplementary Figure 20: Coinjected membrane bound PMT-mEGFP expression control in zebrafish embryos. (a) Expression of coinjected PMT-mEGFP allows selection of dTgfr2 overexpression embryos. Scale bar = 100 μ m.



Supplementary Figure 21: Western blot expression controls of Halo-tagged receptor constructs. Western blot analysis of HEK293T cell transiently expressing (a) *Gnathonemus petersii* receptor variants (gBmpr2a (3LBD), gBmpr2a-LBD^{midin}, gBmpr2a-LBDⁱⁿ, gBmpr2a-LBD^{mid}, gBmpr2a-LBD^{out}) or hBMPR2 and hACVR2B as size reference (b) *Danio rerio* receptor variants (dTGfbr2b, dTGfbr2a (2LBD), dTGfbr2a-LBDⁱⁿ, dTGfbr2a-LBD^{out}) or hTGfBR2 as size reference (c) *Equus caballus* variants (eTGfBR2 (2LBD), eTGfBR2-LBDⁱⁿ, eTGfBR2-LBD^{out}) and hTGfBR2 as size reference (d) *Gallus gallus* variants (gTGfBR2 (2LBD), gTGfBR2-LBDⁱⁿ, gTGfBR2-LBD^{out}) and hTGfBR2 as size reference (e) *Cyprinus carpio* variants (cTgfr2a (2LBD), cTgfr2a-LBDⁱⁿ, cTgfr2a-LBD^{out}) or hTGfBR2 as size reference (f)

Xenopus laevis variants (xITgfr2.L (3LBD), xITgfr2.L-LBD^{mid-in}, xITgfr2.L-LBDⁱⁿ, xITgfr2.L-LBD^{out}) or hTGFR2 as size reference (**g**) hTGFR2 and hTGFR2-2LBD. GAPDH was used as loading reference.

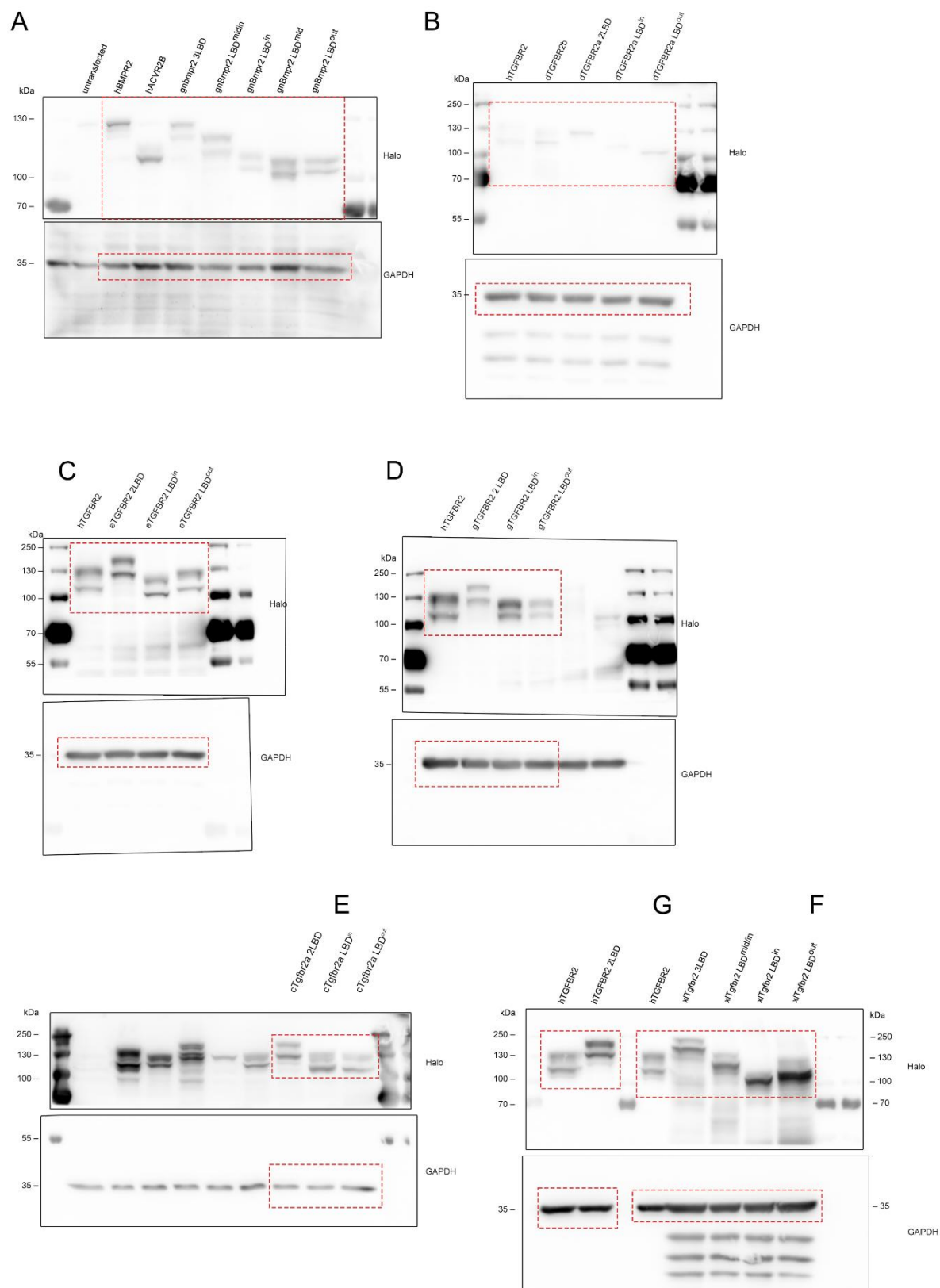
Supplementary Table 1: N-terminally tagged HaloTag expression constructs

Plasmid Name	Description	Backbone	Resistance
eTGFR2-Halo	full length eTGFR2 with N-terminal HaloTag	pcDNA3.1	Ampicillin
eTGFR2 LBD ⁱⁿ -Halo	eTGFR2 LBD ⁱⁿ variant with N-terminal HaloTag	pcDNA3.1	Ampicillin
eTGFR2 LBD ^{out} -Halo	eTGFR2 LBD ^{out} variant with N-terminal HaloTag	pcDNA3.1	Ampicillin
dTgfr2a-Halo	full length dTgfr2a with N-terminal HaloTag	pcDNA3.1	Ampicillin
dTgfr2a LBD ⁱⁿ -Halo	dTgfr2a LBD ⁱⁿ variant with N-terminal HaloTag	pcDNA3.1	Ampicillin
dTgfr2a LBD ^{out} -Halo	dTgfr2a LBD ^{out} variant with N-terminal HaloTag	pcDNA3.1	Ampicillin
dTgfr2b-Halo	dTgfr2b with N-terminal HaloTag	pcDNA3.1	Ampicillin
cTgfr2a-Halo	full length cTgfr2a with N-terminal HaloTag	pcDNA3.1	Ampicillin
cTgfr2a LBD ⁱⁿ -Halo	cTgfr2a LBD ⁱⁿ variant with N-terminal HaloTag	pcDNA3.1	Ampicillin
cTgfr2a LBD ^{out} -Halo	cTgfr2a LBD ^{out} variant with N-terminal HaloTag	pcDNA3.1	Ampicillin
gnBmpr2a-Halo	full length gnBmpr2a with N-terminal HaloTag	pcDNA3.1	Ampicillin
gnBmpr2a LBD ⁱⁿ -Halo	gnBmpr2a LBD ⁱⁿ variant with N-terminal HaloTag	pcDNA3.1	Ampicillin
gnBmpr2a LBD ^{mid-in} -Halo	gnBmpr2a LBD ^{mid-in} variant with N-terminal HaloTag	pcDNA3.1	Ampicillin
gnBmpr2a LBD ^{out} -Halo	gnBmpr2a LBD ^{out} variant with N-terminal HaloTag	pcDNA3.1	Ampicillin
gnBmpr2a LBD ^{mid} -Halo	gnBmpr2a LBD ^{mid} variant with N-terminal HaloTag	pcDNA3.1	Ampicillin
gTGFR2-Halo	full length gTGFR2 with N-terminal HaloTag	pcDNA3.1	Ampicillin
gTGFR2 LBD ⁱⁿ -Halo	gTGFR2 LBD ⁱⁿ -Halo variant with N-terminal HaloTag	pcDNA3.1	Ampicillin
gTGFR2 LBD ^{out} -Halo	gTGFR2 LBD ⁱⁿ -Halo variant with N-terminal HaloTag	pcDNA3.1	Ampicillin
xITgfr2.L – blunt end	full length xITgfr2.L in blunt end vector	pCR™-Blunt-vector	Ampicillin
xITgfr2.L	full length xITgfr2.L with N-terminal HaloTag	pcDNA3.1	Ampicillin
xITgfr2.L LBD ⁱⁿ	xITgfr2.L LBD ⁱⁿ variant with N-terminal HaloTag	pcDNA3.1	Ampicillin
xITgfr2.L LBD ^{mid-in}	xITgfr2.L LBD ^{mid-in} variant with N-terminal HaloTag	pcDNA3.1	Ampicillin
pcs2+PMT-mEGFP	Membrane bound mEGFP: Gift from Dr. Thorsten Wohland (Balasubramanian et. al, 2022, RRID: Addgene_203777), subcloned into pCS2+ backbone for capped mRNA synthesis (Le et al., 2025)	pcs2+	Ampicillin
xITgfr2.L LBD ^{out}	xITgfr2.L LBD ^{out} variant with N-terminal	pcDNA3.1	Ampicillin

Supplementary Table 2: Oligonucleotides used for molecular cloning of LBD-Halo constructs

Name	Oligonucleotide-sequence (5'-3')	
	for	rev
eTGfBR2	CATAATTTTCAGTGAAAACCCCGACTTG CTTTTAGTTATCTTTCAAG	ATAACTAAAAGCAAGTCGGGGTTTTCACTGA AAATTATGTGGTCGTTGC
eTGfBR2 LBD ⁱⁿ	GCACTGCGGCTGCTGGATCCGCAGAAA TCGGTACTGGCTTTCCATTC	ACTAAAAGCAAGTCGGGGTTTTCACTGAAAA TTATGTGGTCG
eTGfBR2 LBD ^{out}	AACCCCGACTTGCTTTTAGTTATCTTTC AAGTGACAGGGG	GGATCCAGCAGCCGCAGTGCTGACCAGCA GGATGGTCCATGG
dTgfr2a	GGTGGAGAATTCCGCATCTGCAGCTCC TGTAAGCCTCATCCAGTCAACTG	GCTCGAGCGGCCGCCTACATCTCTTGATC CCAGAATCC
dTgfr2a LBD ⁱⁿ	GGTGGAGAATTCTTGTCGAAGACAACT CCAGCAGC	GCTCGAGCGGCCGCCTACATCTCTTGATC CCAGAATCC
dTgfr2a LBD ^{out}	GATTTGAAACAATATCCAGGGGTTTCAG	CAATTTCTCCTCCTCAGTGAGTTTG
dTgfr2b	GGTGGAGAATTCCGCGGAGTCGATGGA AATAATCC	GCTCGAGCGGCCGCCTCATTTGTCGTCGCTC ACGCTCAGC
cTgfr2a	GGTGGAGAATTCAATCCACACAGTGTG TGTAAGTCATGTGACC	GCTCGAGCGGCCGCCTATTTCTCATCATTC CCAGAATCC
cTgfr2a LBD ⁱⁿ	GATAACGACACCAAGTTAGTTATATTG	ATTGAATTCTCCACCTCCACCTCCGC
cTgfr2a LBD ^{out}	GTTTCGTCTCCTCCAGGAGACTATAAG	GAGTCTGAACCCTCCTTTCGTTTC
gnBmpr2a	GGTGGAGAATTCGCCAAGCGGAAGAC CGCGTGTGC	GCTCGAGCGGCCGCCTAATTAACGGTAGGT GACACG
gnBmpr2a ⁱⁿ	GGTGGAGAATTCGCACCTAATGAAGAA AGGGAATGC	GCTCGAGCGGCCGCCTAATTAACGGTAGGT GACACG
gnBmpr2a mid-in	GGTGGAGAATTCGCCGAAAGAGACGAA TTGC	GCTCGAGCGGCCGCCTAATTAACGGTAGGT GACACG
gnBmpr2a ^{out} (Gibson)	Backbone: CCGCTGTTCCGGTCCAACATCCACTCAA CCCCTCTATCCAC LBD^{out}: GAGGTGGAGGTGGAGAATTCGCCAAG CGGAAGACCGCGTG	Backbone: GAATTCTCCACCTCCACCTCCGCCGGAAT CTCGAGCGTCG LBD^{out}: GATGTTGGACCGAACAGCGGAAAAATCTTCG GTGAAATTAAGATTAC
gnBmpr2a ^{mid} (Gibson)	Backbone: CCGCTGTTCCGGTCCAACATCCACTCAA CCCCTCTATCCAC LBD^{mid}: GAGGTGGAGGTGGAGAATTCGCCGAAA GAGACGAATTGCAATGC	Backbone: GAATTCTCCACCTCCACCTCCGCCGGAAT CTCGAGCGTCG LBD^{mid}: GATGTTGGACCGAACAGCGGAGACAGATCG GTGAAGTTTAC
gTGfBR2 & gTGfBR2 LBD ^{out}	GGTGGAGAATTCAGAAGCAAGGAAAAT GGACTGC	GCTCGAGCGGCCGCCTACTTGCGGGTGGT CACGGAGC
gTGfBR2, gTGfBR2- LBD ^{out} (Gibson)	Backbone: GGCATCCAGATGGTGTGCGAGACCCTT ATCGAGTGCTGGG Exon 6/7 Del: TGCCATTGAAGTGGACATTGTTGTTGG CAAAGGAAGGTTTGCTGAAGTG	Backbone: GGCATCCAGATGGTGTGCGAGACCCTTATC GAGTGCTGGG Exon 6/7 Del: TCGCACACCATCTGGATGCCCTGATGGTTA AGCCAGGAGCTGG
gTGfBR2 LBD ⁱⁿ	CCCCATAAGCCAGAGGAGAAAGATGAA ATTTC	CCTAAATCTGTTTGCAGAAAAAATAAG
xlTgbfr2.L – blunt end	GGTACCTCCACCAAGGGCGAATTCTGC	ACATTTCCCTTATACCTGGGTTCTAGTC
xlTgbfr2.L LBD ⁱⁿ (Gibson)	Backbone: GATGACCCATCAAAAGACATACTTGTGA AAGCTCTTCTCATC LBDⁱⁿ: GAGGTGGAGGTGGAGAATTCAGTATAA AGGATTGTGC	Backbone: GAATTCTCCACCTCCACCTCCGCCGGAAT CTCGAGCGTCGACAGCC LBDⁱⁿ: ATGTCTTTTGTATGGGTGTCATCTTTAGAAAAA ACAAGTTTTTCATTACACAGC
xlTgbfr2.L LBD ^{mid-in} (Gibson)	Backbone: GATGACCCATCAAAAGACATACTTGTGA AAGCTCTTCTCATC	Backbone: GAATTCTCCACCTCCACCTCCGCCGGAAT CTCGAGCGTCGACAGCC

	LBD^{mid-in}: CCGGCGGAGGTGGAGGTGGAGAATTC CCTGTTTCATGTACCTGTTGTATGC	LBD^{mid-in}: ATGTCTTTTGATGGGTCATCTTTAGAAAAA ACAAGTTTTCATTACACAGC
xlTgbfr2.L LBD ^{out} (Gibson)	Backbone: GATGACCCATCAAAAGACATACTTGTGA AAGCTCTTCTCATC LBD^{out}: GAGGTGGAGGTGGAGAATTCTACCTGG GTTCTAGTCGTTTATGC	Backbone: GAATTCTCCACCTCCACCTCCGCCGGAAT CTCGAGCGTCGACAGCC LBD^{out}: ATGCTTTTGATGGGTCATCATCTGTGAAGAC TATTTGTTTCATTGC



Supplementary Figure 22: (A-G) Uncropped Western blots