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Supplemental information

Recombinant production of growth factors for application in cell culture

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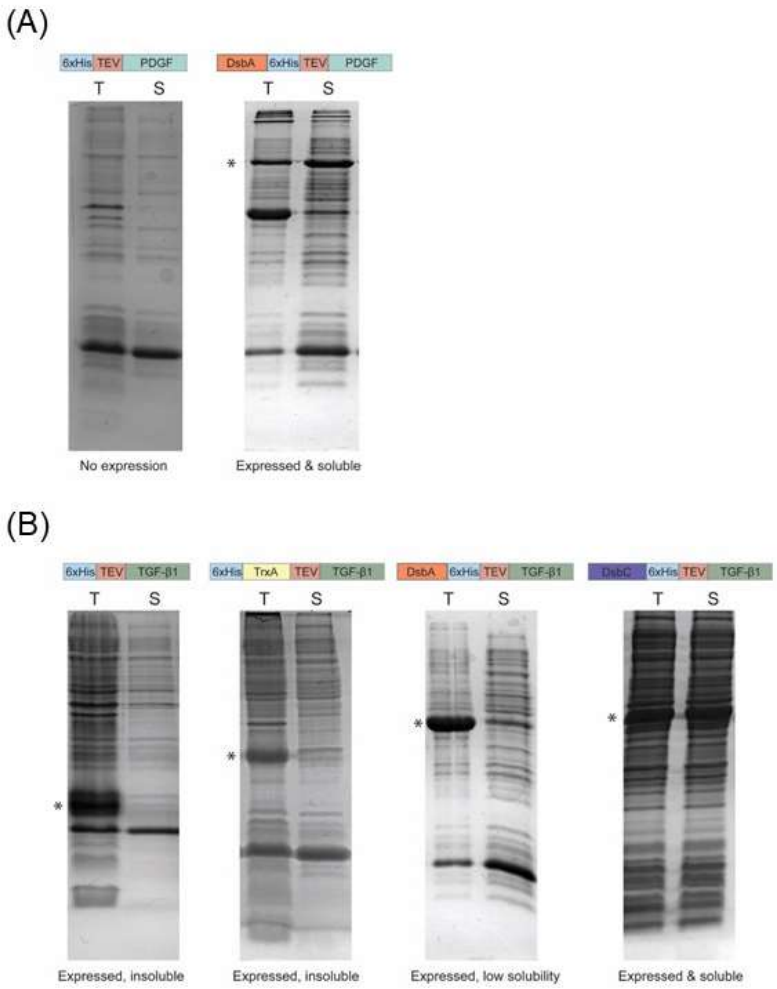
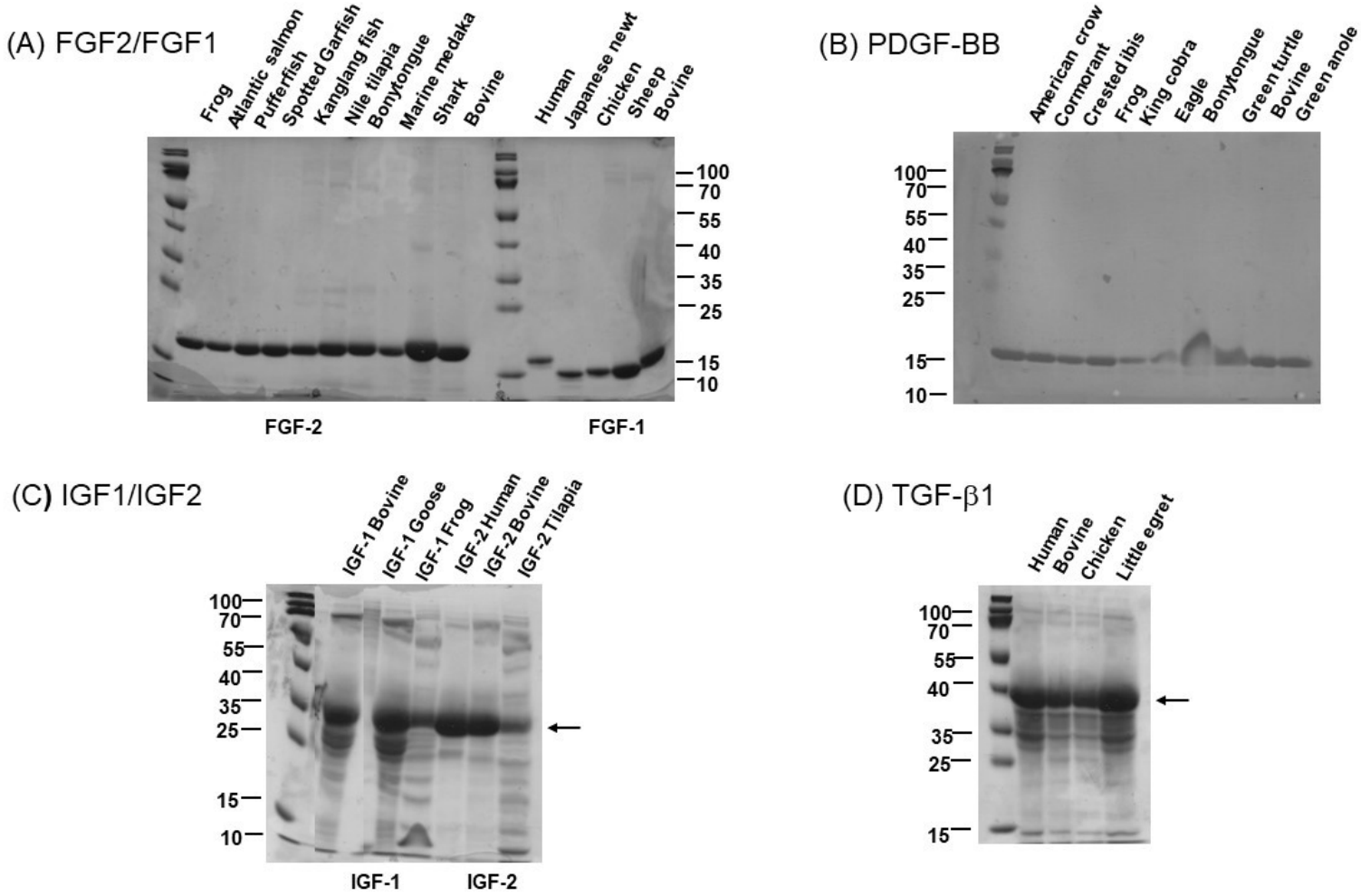
Supplemental info

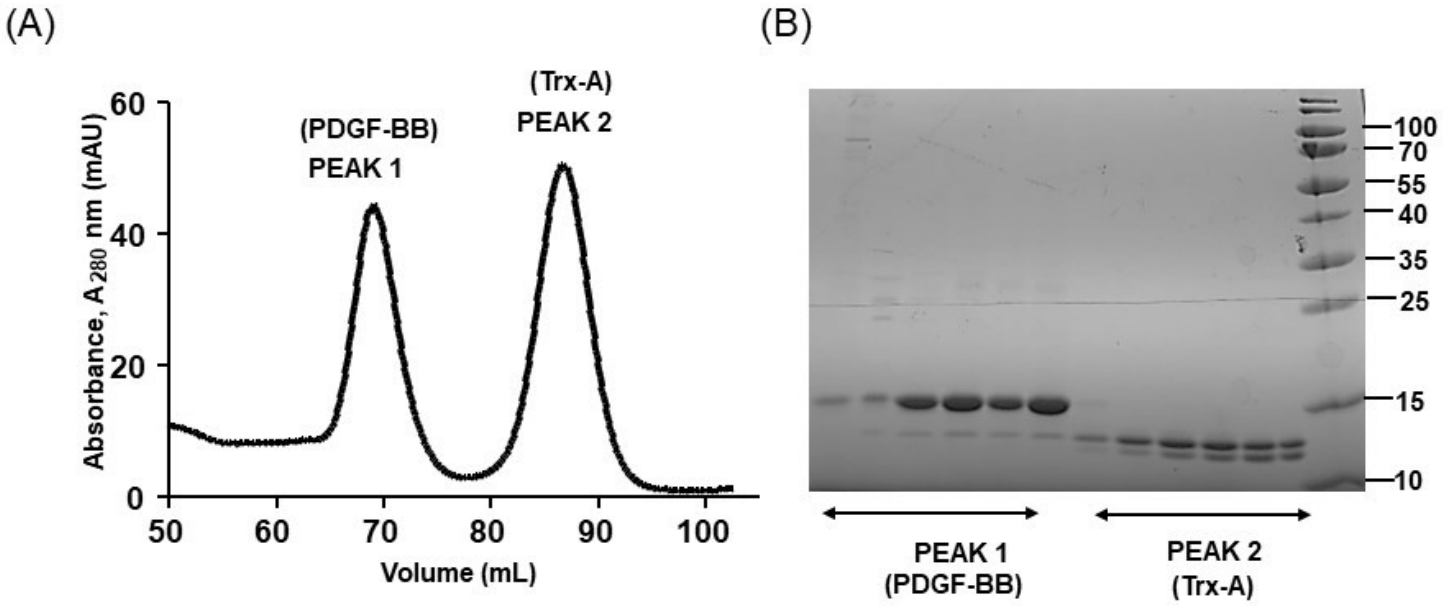
SUPPLEMENTARY FIGURES



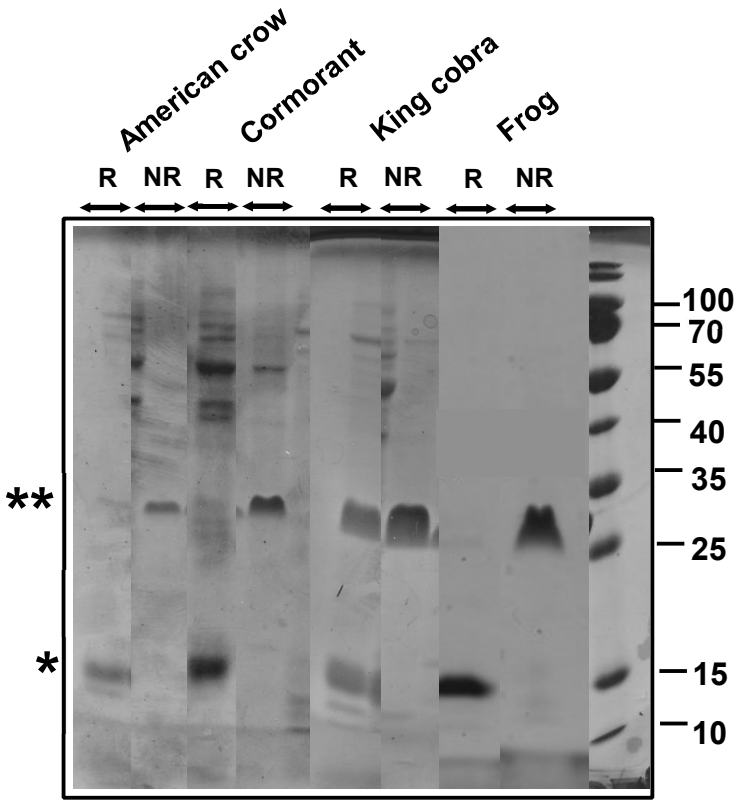
Supplemental Figure 1, related to Figure 1. Expression systems for recombinant GF production. Small-scale protein expression screening used to identify the expression vector and host strain combination capable of facilitating cytoplasmic soluble protein expression. The band corresponding to the protein of interest is marked with (*). T - total cell lysate; S - soluble fraction. (A) PDGF-B, (B) TGF-β1



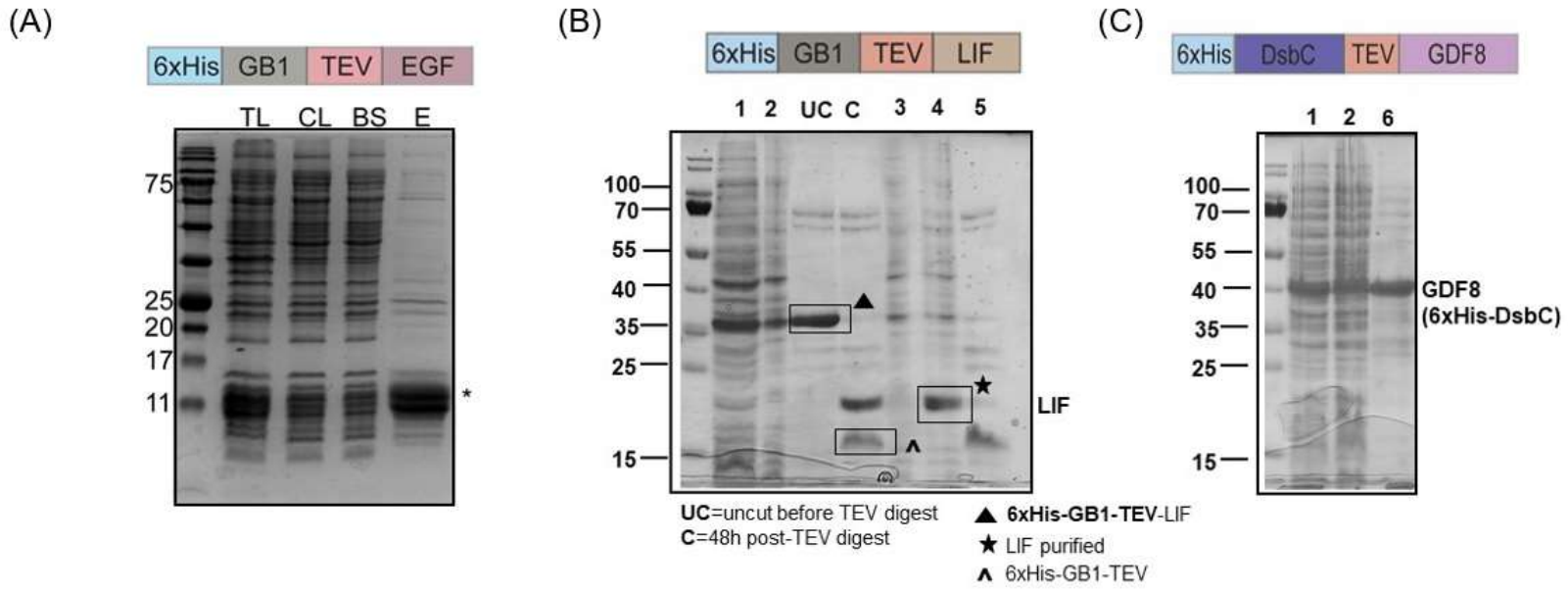
Supplementary Figure 2, related to Figure 2. Summary of a subset of GF targets recombinantly purified in this study. Analyzed on a 15% reducing SDS-PAGE gel electrophoresis **(a)** FGF2 and FGF1 orthologs, 15 kDa **(b)** PDGF-BB orthologs, 15 kDa **(c)** IGF1, IGF2 orthologs, 35 kDa and **(d)** TGF-β1 orthologs, 40 kDa



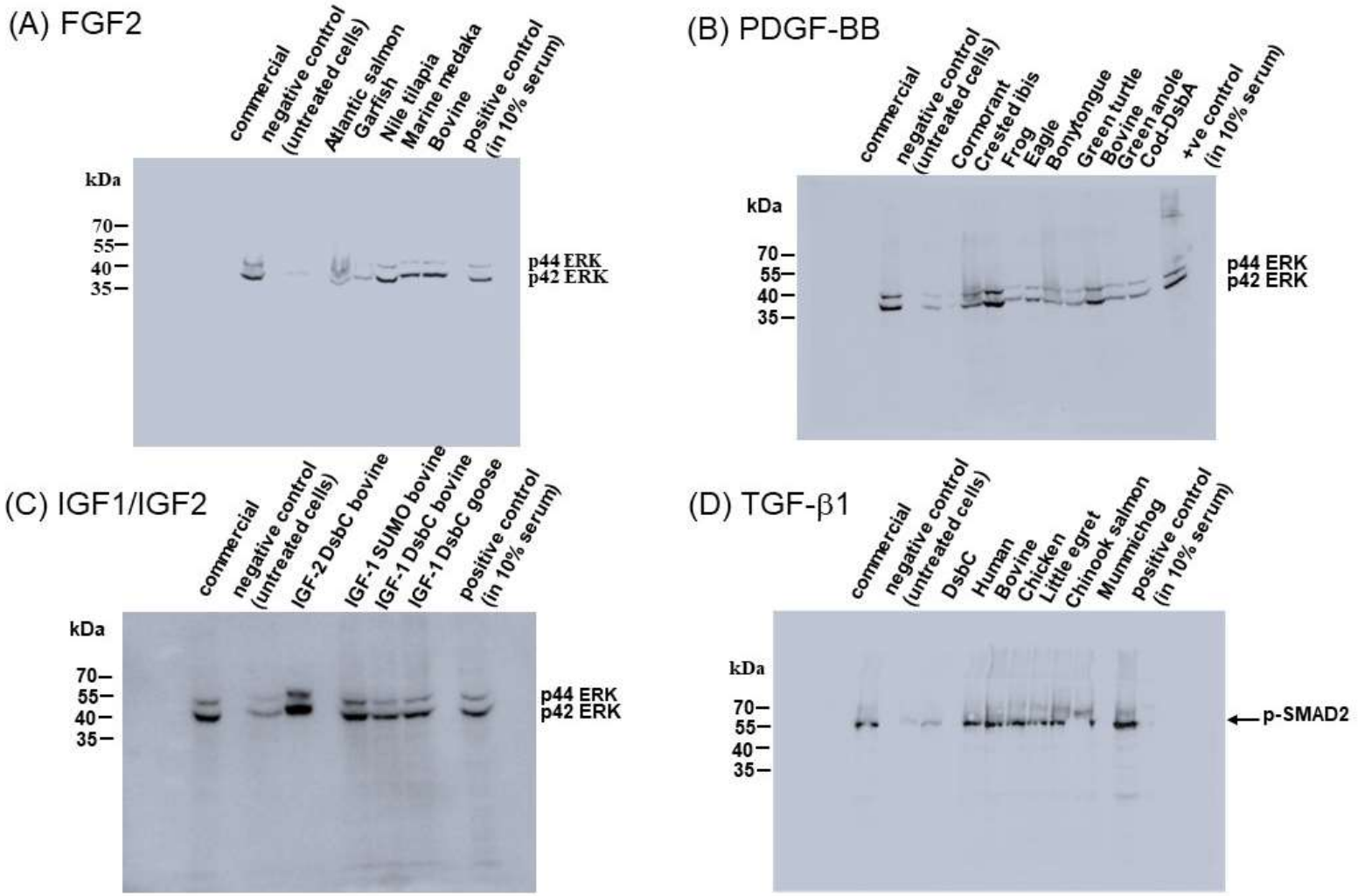
Supplementary Figure 3, related to Figure 2. **(a)**Size-exclusion chromatography Superdex 75 16/60 for separation of PDGF-BB from the TrxA tag after TEV digest. **(b)** Fractions corresponding to **PEAK 1** and **PEAK 2** were analyzed on SDS-PAGE. The fractions corresponding to PDGFBB (**PEAK 1**) was pooled and concentrated.



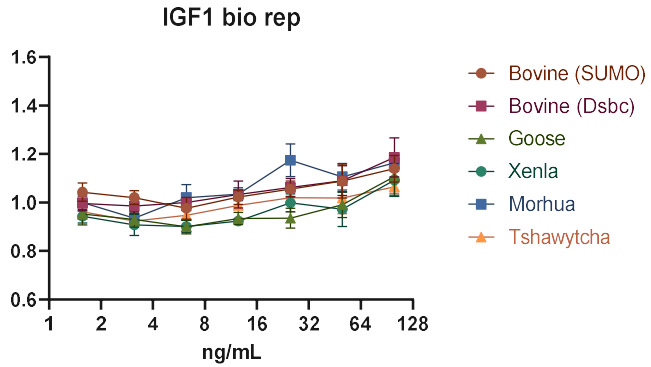
Supplementary Figure 4, related to Figure 2. Recombinantly purified PDGF-BB orthologs analyzed under reducing **(R)** and non-reducing **(NR)** conditions on a 15% gel electrophoresis. Under non-reducing conditions, all PDGF-BB orthologs dimerize marked with double asterik (**) at 25 kDa approx.

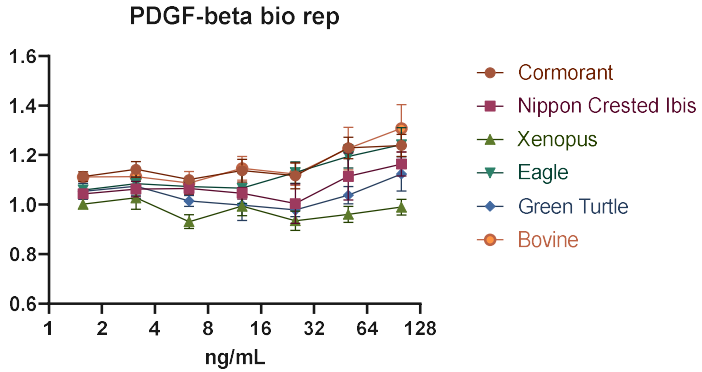


Supplementary Figure 5, related to Figure 2. (a) Scale up expression of EGF human orthologue expressed in BL21(DE3) Gold cells **(b)** Scale up expressions of LIF human ortholog expressed in BL21(DE3) Gold cells **(c)** GDF8 bovine ortholog expressed in Shuffle T7 express cells. *E.coli* cells grown in 1L terrific broth (TB), induced with 0.8 mM IPTG at OD_{600} of 1.5-2.0 units. *UC=uncut before TEV digest; C=24h post TEV digest.* After the TEV digest, LIF band can be seen at 20 kDa corresponding to the monomer LIF while the His6x-GB1-tag runs at 15 kDa, **1** – soluble fraction; **2** – supernatant post Ni-NTA batch bind; **3** – flow through after second Ni- binding post-TEV digest; **4** – 30 mM imidazole wash eluent; **5** – 250 mM imidazole wash eluent; **6** – GDF8 bovine ortholog purified post-Ni-NTA batch binding



Supplementary Figure 6, related to Figure 4. Western blot nitrocellulose membrane images of **(a)** FGF2 (p-ERK1/2) **(b)** PDGF-BB (p-ERK1/2) **(c)** IGF1/IGF2 (p-ERK1/2) **(d)** TGF-β1 (p-SMAD2)


IGF1 bio rep
1.6
1.4
1.2
1.0
0.8
0.6
1
2
4
8
16
32
64
128
ng/mL
Bovine (SUMO)
Bovine (Dsbc)
Goose
Xenla
Morhua
Tshawytcha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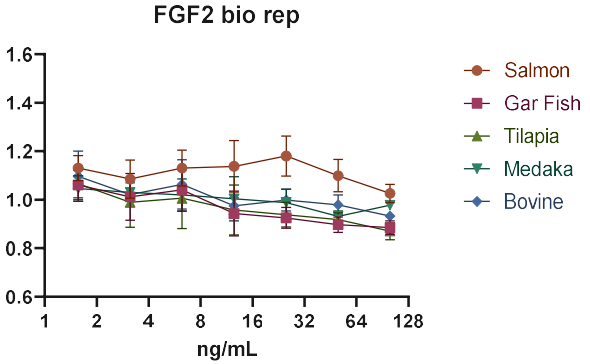
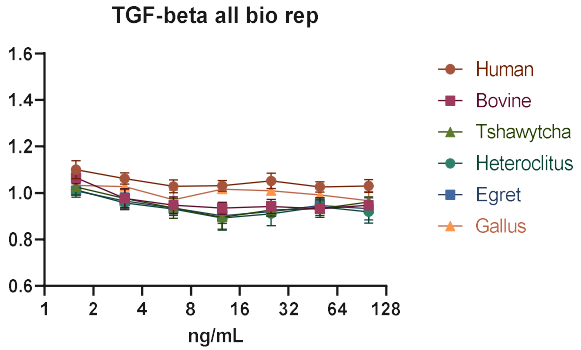
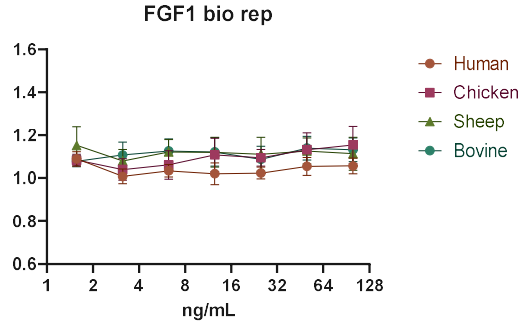
1.4
1.2
1.0
0.8
0.6
1
2
4
8
16
32
64
128
ng/mL
Cormorant
Nippon Crested Ibis
Xenopus
Eagle
Green Turtle
Bovine


FGF2 bio rep
1.6
1.4
1.2
1.0
0.8
0.6
1
2
4
8
16
32
64
128
ng/mL
Salmon
Gar Fish
Tilapia
Medaka
Bovine


TGF-beta all bio rep
1.6
1.4
1.2
1.0
0.8
0.6
1
2
4
8
16
32
64
128
ng/mL
Human
Bovine
Tshawytcha
Heteroclitus
Egret
Gallus


FGF1 bio rep
1.6
1.4
1.2
1.0
0.8
0.6
1
2
4
8
16
32
64
128
ng/mL
Human
Chicken
Sheep
Bovine

Supplementary Figure 7, related to Figure 5. Concentration Screening of Growth Factors on Bovine Satellite Cells (BSCs). Proliferation data at various concentration shown for different GF orthologs. All values are relative to 0% FBS (serum-free) control. WST-1 absorbance readings at 450 nm were recorded in duplicate wells of the three biological replicates (n = 3) for the GF samples

SUPPLEMENTARY TABLES

Supplementary Table 1. Expression constructs/tags used for soluble protein expression of growth factor targets

Growth factor family	Expression System	*E.coli* host strain
aFGF/FGF1	pMCSG53-His6-TEV	BL21 DE3 Gold
bFGF/FGF2	pMCSG53-His6-TEV	BL21 DE3 Gold
PDGF-BB	pET-Trx-His6-TEV	SHuffle T7 express
IGF1	pMCSG53-His6-DsbC-TEV*	SHuffle T7 express/BL21 DE3 Gold
IGF2	pMCSG53-His6-DsbC-TEV	SHuffle T7 express
TGF-β1	pMCSG53-His6-DsbC-TEV*	SHuffle T7 express/BL21 DE3 Gold
GDF8 (myostatin)	pMCSG53-His6-DsbC-TEV	SHuffle T7 express
LIF	pMCSG53-His6-GB1-TEV	BL21 DE3 Gold
EGF	pMCSG53-His6-GB1-TEV	BL21 DE3 Gold

*Expression was tested using His6-DsbC-TEV and DsbC-His6-TEV expression constructs. No differences were observed in yield, purity, or separation after TEV cleavage.

Supplementary Table 3. Cost analysis breakdown (COGS) for recombinant growth factor production on a "laboratory scale". *Capital costs of essential laboratory equipment (e.g., floor shakers, centrifuge, sonicator, benchtop centrifuge, autoclave, dishwashing, gel casting system, electricity etc.) not included.*

Consumable description	Quantity used	Cost (CAD)	Unit specification	Total cost (CAD)
PROTEIN PURIFICATION CONSUMABLES COSTS				
(12 L of protein grown by "shaker-flask approach"; 4L erlenmeyer flasks)				
(All cost above reflected for 12L culture purification)				
Ni-NTA resin superflow (Qiagen) NEW	3 mL total/target purified	$ 450.00	25 mL	$ 90.00
HEPES (Bioshop)	11.9 g/ L for 50 mM	$ 270.00	500 g	$ 6.43
Sodium chloride (Bioshop)	17.6 g/ L for 0.3 M	$ 50.00	10 kg	$ 0.09
Glycerol (Bioshop)	50 mL	$ 25.00	1000 mL	$ 1.25
Imidazole (Bioshop)	0.35 g/ L for 5 mM	$ 82.00	500 g	$ 0.06
(cost for making **1L buffers** for affinity purification)	2 g/ L for 30 mM			$ 0.33
	17 g/ L for 250 mM			$ 2.79
TB broth (Terrific broth culture medium)	48 g powder for 1 L	$ 60.00	500 g	$ 69.12
Vivaspin concentrator (GE Biosciences)	1 no./ per target	$ 180.00	12 nos.	$ 15.00
50 mL Falcon tubes (VWR)	15 nos.	$ 110.00	500 nos	$ 3.30
Snakeskin dialysis (30.5 cm=1 foot); ThermoFisher	30 cm	$ 235.00	35 feet (35 x 30.5 cm)	$ 6.60
Bradford reagent (BioRad)	5 mL	$ 190.00	500 mL	$ 1.90
Chromatography gravity columns (BioRad)	2 no.	$ 225.00	4 nos.	$ 112.50
Acrylamide (Bioshop)	4 mL/gel cast	$ 56.00	500 mL	$ 0.45
TEMED (Bioshop)	0.02 mL/gel cast	$ 38.00	50 mL	$ 0.02
APS (Bioshop)	0.5 mL (10%)/gel cast	$ 14.00	25 g	$ 0.56
Disodium hydrogen phosphate (Sigma)	2.88 g/2 L buffer (x2 for 4L buffer)	$ 110.00	500 g	$ 1.27
Potassium dihydrogen phosphate (EMD)	0.5 g/2 L buffer	$ 970.80	500 g	$ 1.94
Sodium chlordie (Bioshop)	16 g/2 L buffer	$ 50.00	10 kg	$ 0.16
Potassium chloride (Bioshop)	0.4 g/2 L buffer	$ 91.00	500 g	$ 0.15
(PBS buffer usage per 2 x 2L)				
Labour costs	**24h** approx. (8h x 3 days)	$ 38.00	per hour	$ 912.00
	TOTAL COST (for 12L scale up purification; 120 mg total protein yield)			**$ 1,225.90**
If protein yields	were **10 mg protein/ L**; so for 12L, **10 mg x 12 litre = 120 mg**			

CLONING CONSUMABLES COSTS (estimated for 96 targets, high throughput set up)

Consumables description	Quantity used	Cost (CAD)	Unit specification	Total cost for 96 targets (CAD)	Total cost/target (CAD)
* 440 bp for FGF-2; 340bp for TGFB1, 320 bp for PDGF-BB					
Gene synthesis/96 targets* (Twist BioSciences)	**400 bp** approx. bases/protein target	$ 0.08	base	$ 3,225.60	$ 33.60
Sequencing primers forward/96 well plate (EuroFins)	0.10/base	$ 300.00	0.10/base	$ 300.00	$ 3.13
Sequencing primers reverse/96 well plate (EuroFins)	0.10/base	$ 300.00	0.10/base	$ 300.00	$ 3.13
TCAG sequencing facility/target	$3.50 x 2 reactions	$ 3.50	per reaction	$ 672.00	$ 7.00
Miniprep 96-well plate (Qiagen)		$ 300.00	per 96 well plate	$ 300.00	$ 3.13
PCR purification 96-well plate (Qiagen)		$ 200.00	per 96 well plate	$ 200.00	$ 2.08
Miscellaneous: cloning buffers, Pfx, Taq polymerase, dNTP mix, sspI enzyme, T4 polymerase)		$ 100.00		$ 100.00	$ 1.04
Labour costs	**16h** approx. (8h x 2 days)	$ 38.00	per hour	$ 608.00	$ 6.33
				$ 5,097.60	**$ 59.43**