Supplementary Materials for

Optogenetic Fine-Tuning of *Sus scrofa* Basic Fibroblast Growth Factor Expression in *Escherichia coli*

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Table S1. Bacterial strains and plasmids used in this study.

Strains or plasmids	Relevant characteristics[a]	Source or reference
Strains		
E. coli		
DH5α	F⁻ *endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG purB20* φ80d*lacZ*Δ M15 Δ(*lacZYA-argF*)U169, hsdR17($r_K^-m_K^+$), λ^-	Lab collection
BL21 (DE3)	F⁻ *ompT gal dcm lon hsdS*$_B$($r_B^-m_B^-$) λ(DE3 [*lacI lacUV5-T7p07 ind1 sam7 nin5*]) [*malB*$^+$]$_{K-12}$(λ^S)	Lab collection
BLETF	BL21 (DE3) harboring pET28-bFGF	This study
DHELF	DH5α harboring pEL222-bFGF	This study
DHELF-V1	DH5α harboring pEL222-bFGF-V1	This study
DHELF-V2	DH5α harboring pEL222-bFGF-V2	This study
DHELF-V3	DH5α harboring pEL222-bFGF-V3	This study
DHELF-V4	DH5α harboring pEL222-bFGF-V4	This study
DHELF-V5	DH5α harboring pEL222-bFGF-V5	This study
DHELF-V6	DH5α harboring pEL222-bFGF-V6	This study
DHELF-V7	DH5α harboring pEL222-bFGF-V7	This study
DHELF-V8	DH5α harboring pEL222-bFGF-V8	This study
DHELF-V9	DH5α harboring pEL222-bFGF-V9	This study
DHELF-V10	DH5α harboring pEL222-bFGF-V10	This study
DHELF-V11	DH5α harboring pEL222-bFGF-V11	This study
DHELF-V12	DH5α harboring pEL222-bFGF-V12	This study
DHELF-V13	DH5α harboring pEL222-bFGF-V13	This study
DHELF-V14	DH5α harboring pEL222-bFGF-V14	This study
DHELF-V15	DH5α harboring pEL222-bFGF-V15	This study
DHELF-V16	DH5α harboring pEL222-bFGF-V16	This study
DHELF-V17	DH5α harboring pEL222-bFGF-V17	This study
DHELF-V18	DH5α harboring pEL222-bFGF-V18	This study
DHELF-V19	DH5α harboring pEL222-bFGF-V19	This study
DHELF-V20	DH5α harboring pEL222-bFGF-V20	This study
DHELF-Opto	DH5α harboring pEL222-bFGF-Opto	This study
Plasmids		
pET-28a (+)	Expression vector	Lab collection
pKEIR	J23118-B0034-EL222-B0015, P_{BLI}-B0034-RFP	Lab collection
pET28-bFGF	pET-28a (+) containing *bFGF* gene	This study
pEL222-bFGF	J23118-B0034-EL222-B0015, P_{BLI}-B0034-bFGF	This study
pEL222-bFGF-V1	J23112-B0034-EL222-B0015 in pEL222-bFGF	This study
pEL222-bFGF-V2	J23103-B0034-EL222-B0015 in pEL222-bFGF	This study
pEL222-bFGF-V3	J23106-B0034-EL222-B0015 in pEL222-bFGF	This study
pEL222-bFGF-V4	J23108-B0034-EL222-B0015 in pEL222-bFGF	This study
pEL222-bFGF-V5	J23102-B0034-EL222-B0015 in pEL222-bFGF	This study

pEL222-bFGF-V6	J23100-B0034-EL222-B0015 in pEL222-bFGF	This study
pEL222-bFGF-V7	A_3B_5 variant of the P_{BLI} promoter in pEL222-bFGF	This study
pEL222-bFGF-V8	A_4B_3 variant of the P_{BLI} promoter in pEL222-bFGF	This study
pEL222-bFGF-V9	P_{BLI}-B0032-bFGF in pEL222-bFGF	This study
pEL222-bFGF-V10	P_{BLI}-B0035-bFGF in pEL222-bFGF	This study
pEL222-bFGF-V11	P_{BLI}-J61100-bFGF in pEL222-bFGF	This study
pEL222-bFGF-V12	P_{BLI}-T7g10-bFGF in pEL222-bFGF	This study
pEL222-bFGF-V13	C120-25bp in pEL222-bFGF	This study
pEL222-bFGF-V14	C120-30bp in pEL222-bFGF	This study
pEL222-bFGF-V15	C120-35bp in pEL222-bFGF	This study
pEL222-bFGF-V16	C120-45bp in pEL222-bFGF	This study
pEL222-bFGF-V17	two C120 copies in pEL222-bFGF	This study
pEL222-bFGF-V18	three C120 copies in pEL222-bFGF	This study
pEL222-bFGF-V19	four C120 copies in pEL222-bFGF	This study
pEL222-bFGF-V20	five C120 copies in pEL222-bFGF	This study
pEL222-bFGF-Opto	J23106-B0034-EL222-B0015, P_{BLI} (A_4B_3 variant, $(C120\text{-}30bp)_5$)-T7g10-bFGF	This study

Table S2. Primers used in this study.

Primer	Sequence
ss-FGF-F	ATGGCTGCTGGTTCTATC
ss-FGF-R	TCAAGACTTAGCAGACATTG
28-Back-F	CACCACCACCACCACCACT
28-Back-R	GGTATATCTCCTTCTTAAAGTTAAACAAAATTATTTCTAG
28-In-F	GTTTAACTTTAAGAAGGAGATATACCATGGCTGCTGGTTCTA TC
28-In-R	AGTGGTGGTGGTGGTGGTGAGACTTAGCAGACATTGGC
KE-Back-F	AGGAATTCAGACGGTCAC
KE-Back-R	CTAGTATTTCTCCTCTTTCTCTAGACA
KE-In-F	CTAGAGAAAGAGGAGAAATACTAGATGGCTGCTGGTTCTAT C
KE-In-R	TGACCGTCTGAATTCCTTCAGTGGTGGTGGTGG
100-F	TTGACGGCTAGCTCAGTCCTAGGTACAGTGCTAGCCTAGAG AAAGAGGAG
102-F	TTGACAGCTAGCTCAGTCCTAGGTACTGTGCTAGCCTAGAGA AAGAGGAG
103-F	CTGATAGCTAGCTCAGTCCTAGGGATTATGCTAGCCTAGAGA AAGAGGAG
106-F	TTTACGGCTAGCTCAGTCCTAGGTATAGTGCTAGCCTAGAGA AAGAGGAG
108-F	CTGACAGCTAGCTCAGTCCTAGGTATAATGCTAGCCTAGAGA AAGAGGAG
112-F	CTGATAGCTAGCTCAGTCCTAGGGATTATGCTAGCCTAGAGA AAGAGGAG
EL-P-R	AAGCTTCAGGTGGCACTTTTC
Q-FGF-F	CCAGAAGACGGTGGTTCTGG
Q-FGF-R	GACCCAACTTGTATTGACCAGTTC
Q-EL-F	GGCTGACCGACAAAATCCG
Q-EL-R	GCATTTCAGCAGCACGTTC
V7-F	TTTACAAAGAAAATGGTTTGTGACTATGCTAGCCTAGAGAA AGAGGAG
V8-F	CTGACAAAGAAAATGGTTTGTTACTGTGCTAGCCTAGAGAA AGAGGAG
RBS-32-F	TCACACAGGAAAGTACTAGATGGCTGCTGGTTC
RBS-35-F	ATTAAAGAGGAGAATACTAGATGGCTGCTGGTTC
RBS-J6-F	GAAAGAGGGGACAATACTAGATGGCTGCTGGTTC
RBS-g10-F	AAGGAGATATACATTACTAGATGGGTGCTGACGAC
RBS-R	CTCTAGACATATTTATTCGACTATAAC
25-F	GGCCCCGAGGTCCAGCACCAACGCATTAGCGAAGAAAATG GTTTGTT
30-F	GGCCCCGAGGTCCAGCACCAACGCAGTCCCTTAGCGAAGA AAATGGTTTGTT

35-F	GGCCCCGAGGTCCAGCACCAACGCAGTCCCCTTTGTTAGCG AAGAAAATGGTTTGTT
45-F	GGCCCCGAGGTCCAGCACCAACGCAGTCCCCTTTGGTACGC CGACTTAGCGAAGAAAATGGTTTGTT
C120-R	TATAAACGCAGAAAGGCCC

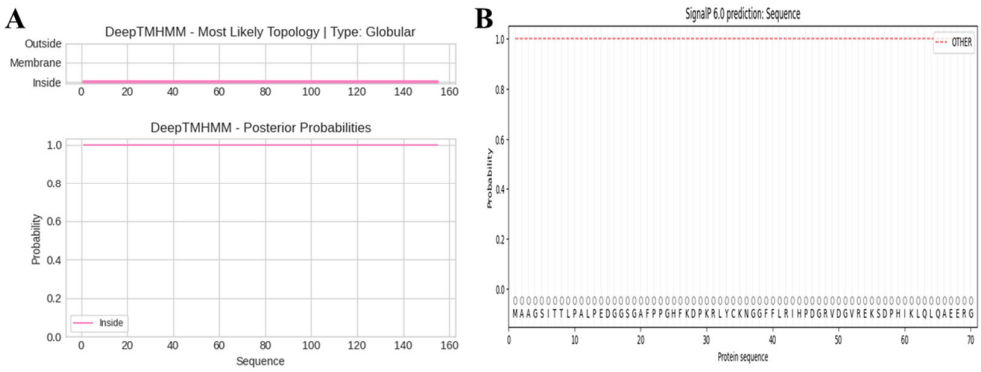
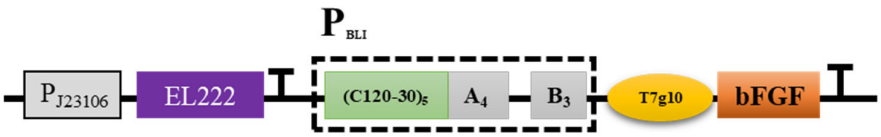


Figure S1. Structural predictions for bFGF using advanced bioinformatics tools. (A) Transmembrane topology prediction generated by the DeepTMHMM - 1.0 algorithm. (B) Signal peptide analysis performed with SignalP - 6.0.



Key sequence of pEL222-bFGF-Opto:
Single underlined = BBa_J23106
Double underlined = restriction sites
Purple = EL222 open reading frame
Green = five C120-30 copies
Bold = -30 and -10 hexamer
Yellow = T7g10 ribosome binding site
Orange = bFGF open reading frame

CACATTTCCCCGAAAAGTGCCACCTGAAGCTTTTTACGGCTAGCTCAGTCCTA
GGTATAGTGCTAGCCTAGAGAAAGAGGAGAAATACTAGATGGGTGCTGACGA
CACCCGTGTTGAAGTTCAGCCGCCGGCTCAGTGGGTTCTGGACCTGATCGAA
GCTTCTCCGATCGCTTCTGTTGTTTCTGACCCGCGTCTGGCTGACAACCCGCTG
ATCGCTATCAACCAGGCTTTCACCGACCTGACCGGTTACTCTGAAGAAGAATG
CGTTGGTCGTAACTGCCGTTTCCTGGCTGGTTCTGGTACCGAACCGTGGCTGA
CCGACAAAATCCGTCAGGGTGTTCGTGAACACAAACCGGTTCTGGTTGAAAT
CCTGAACTACAAAAAAGACGGTACCCCGTTCCGTAACGCTGTTCTGGTTGCTC
CGATCTACGACGACGACGACGAACTGCTGTACTTCCTGGGTTCTCAGGTTGAA
GTTGACGACGACCAGCCGAACATGGGTATGGCTCGTCGTGAACGTGCTGCTG
AAATGCTGCGTACCCTGTCTCCGCGTCAGCTGGAAGTTACCACCCTGGTTGCT
TCTGGTCTGCGTAACAAAGAAGTTGCTGCTCGTCTGGGTCTGTCTGAAAAAAC
CGTTAAAATGCACCGTGGTCTGGTTATGGAAAAACTGAACCTGAAAACCTCT

GCTGACCTGGTTCGTATCGCTGTTGAAGCTGGTATCTAAGGATCCAAACTCGA
GTAAGGATCTCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGG
GCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCTACTAGAGTCACACTG
GCTCACCTTCGGGTGGGCCTTTCTGCGTTTATAGGCCCCGAGGTCCAGCACCA
ACGCAGTCCCGGCCCCGAGGTCCAGCACCAACGCAGTCCCGGCCCCGAGGT
CCAGCACCAACGCAGTCCCGGCCCCGAGGTCCAGCACCAACGCAGTCCCGG
CCCCGAGGTCCAGCACCAACGCAGTCCC**CTGACA**AAGAAAATGGTTTGT**TAC**
TGTCGAATAAATATGTCTAGAGAAGGAGATATACATTACTAGATGGCTGCTGG
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AAGGGTGTTTGTGCTAACAGATACTTGGCTATGAAGGAAGACGGTAGATTGTT
GGCTTCTAAGTGTGTTACTGACGAATGTTTCTTCTTCGAAAGATTGGAATCTAA
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AGAGAACTGGTCAATACAAGTTGGGTCCAAAGACTGGTCCAGGTCAAAAGG
CTATCTTGTTCTTGCCAATGTCTGCTAAGTCTCACCACCACCACCACCACTGAA
GGAATTCAGACGGTCACAGCTTGTCTGTAAG

Figure S2. The key genetic parts of pEL222-bFGF-Opto.