Molecular cloning, sequence characterization and expression of recombinant buffalo Leukemia Inhibitory Factor (LIF) gene in COS-1 cell line

Cytogenetics and Genome Research

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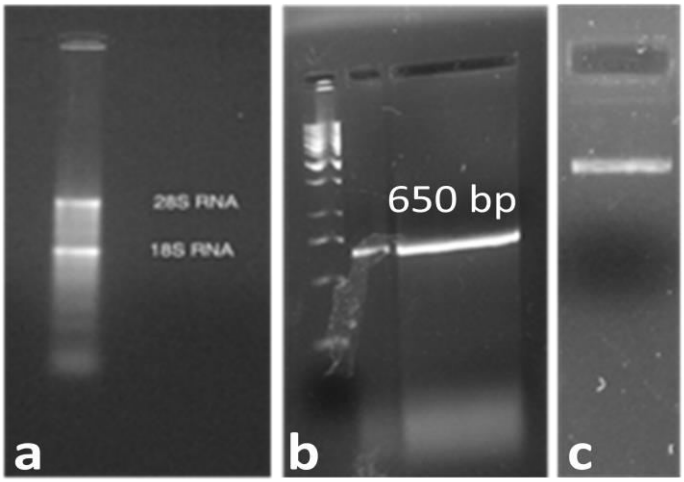

Fig. S1 PCR amplification of BuLIF and confirmation of recombinant plasmid. a) RNA isolated from cumulus oophorus cells of buffalo oocyte. b) PCR amplification of 650 bp long BuLIF. c) Purified LIF gene for cloning in an expression vector.

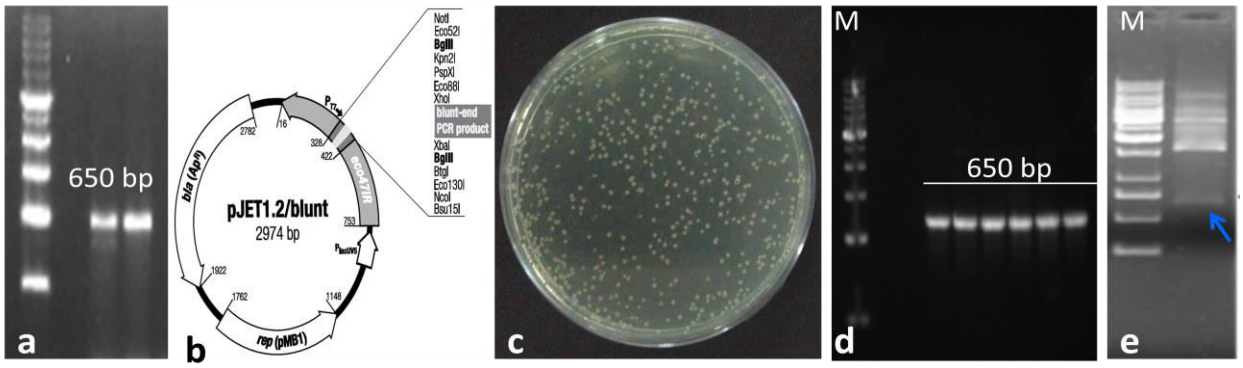

Fig. S2 Molecular cloning of Buffalo LIF gene: a) M marker and 2 PCR product of Buffalo LIF, b) pJET1.2/blunt vector c)Ampicillin resistant colonies of *E. coli* having LIF insert and d) PCR analysis for confirmation of LIF insert in ampicillin resistant *E. coli* colonies, 1 kb DNA ladder (Lane M) and PCR products of LIF amplified from the plasmids isolated from ampicillin resistant *E. coli* colonies (Lane 1 –6) e) Released LIF DNA fragment from recombinant plasmid by BglII digestion represented by blue arrow.

Forward Primer

CTCTGGAGTGCAGCCCATAATGAAGGTCTTGGCGGCAGGAGTCGTGCCCCTGCTG
CTGGTTCTCCACTGGAAACACGGGGCCGGGAGCCCCCTTCCCATCACCCCGGTCA
ACGCTACCTGTGCCACCCGCCATCCCTGTCCCAGCAACCTCATGAACCAGATCAG
AAACCAGCTGGGACAACTCAACAGCAGTGCCAACAGCCTCTTTATCCTCTATTAC
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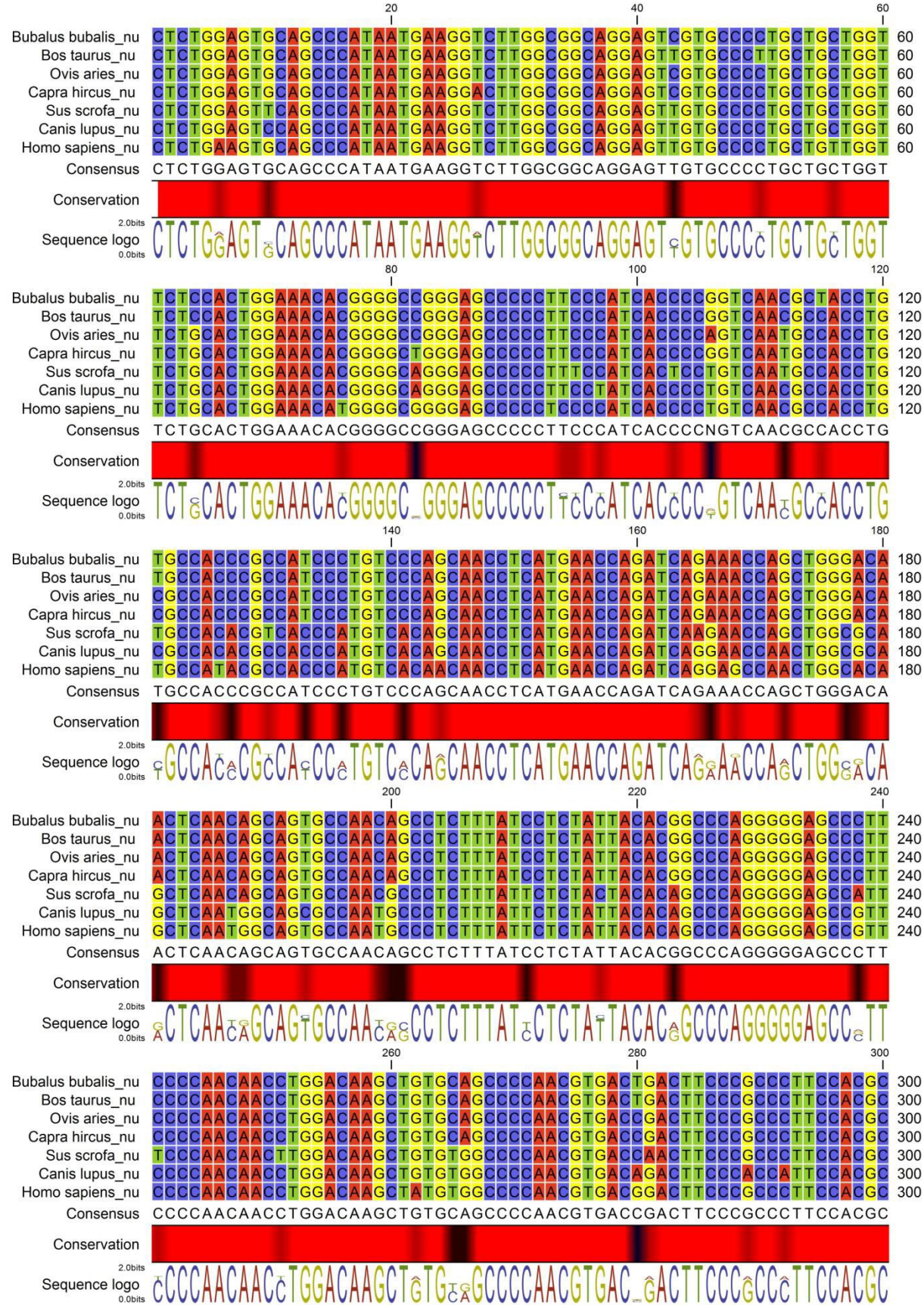
TTCCAGAAGAAGAAGCTGGGCTGTCAGCTCCTGGGGAAGTACAAGCAGGTCATCG
CCGTGCTGGCCCAGGCCTTCTAGACGGGAGGTCTTAGATAGTAGG

Reverse Primer

Fig. S3a: Nucleic acid sequence of LIF gene in buffalo. The amplified LIF gene is 650 bp with an ORF of 609 bases (Shown in blue color). Sequences underlined represents forward and reverse primers respectively (Shown in green color). Start and stop codon is represented in red color bases.

MKVLAAGVVPLLLVLHWKHGAGSPLPITPVNATCATRHPCPSNLMNQIRNQLGQLNSSA
NSLFILYYTAQGEPFPNNLDKLCSPNVTDFPPFHANGTEKARLVELYRIIAYLGASLGNITR
DQKVLNPYAHGLHSKLNTTADVLRGLLSNVLCRLCSKYHVSHVDVTYGPDTSGKDVFQK
KKLGCQLLGKYKQVIAVLAQAF

Fig. S3b: Translated amino acid sequence of LIF in buffalo. The initial 22 amino acids represent signal peptide indicated through black line bar. The mature peptide starting from 23rd amino acid (serine) is made of 180 amino acids.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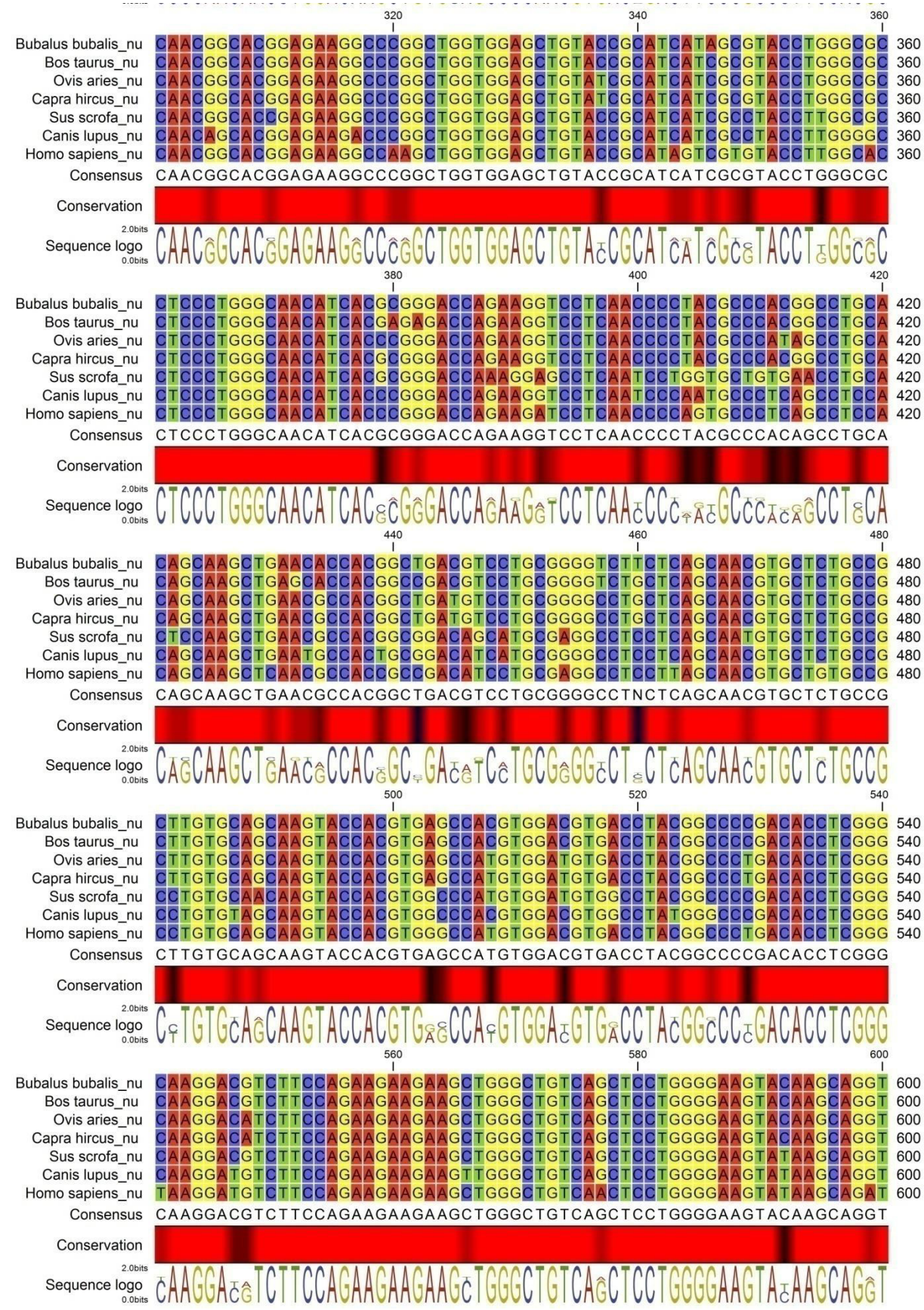
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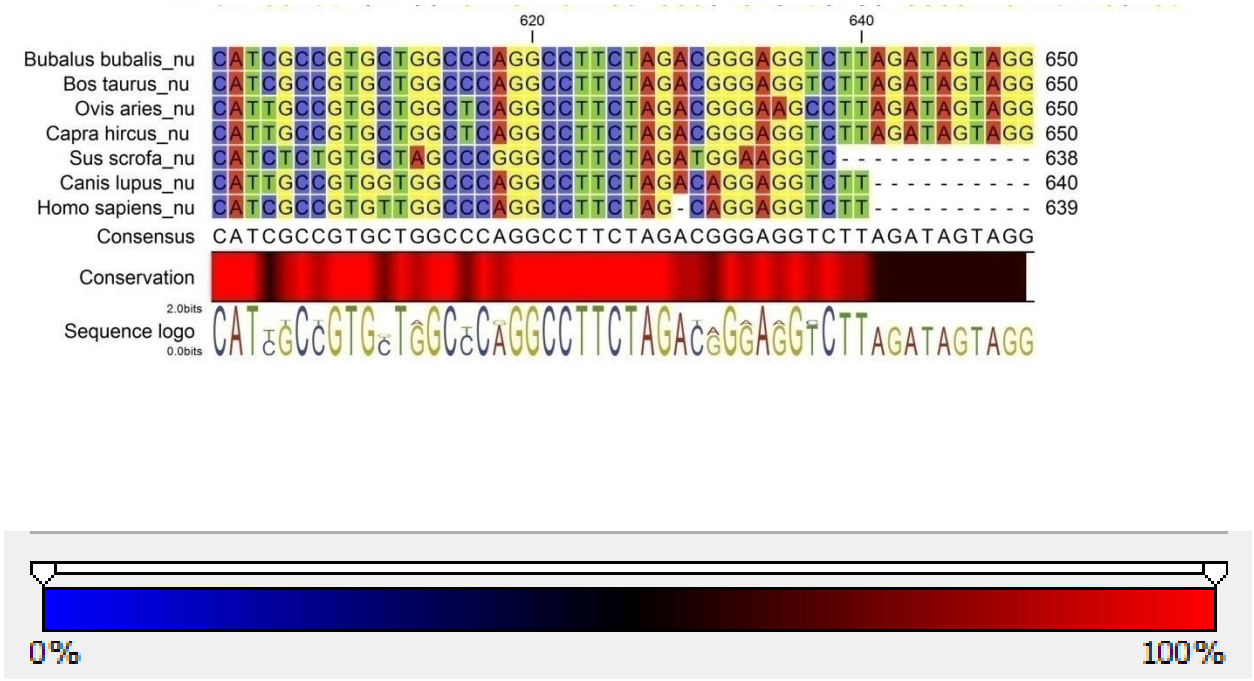

Fig. S4. Multiple Sequence alignment of Leukemia inhibitory factor gene. Conservation bar showing the conserved sequence in the form of the heat map. Dark red is a highly conserved region in sequences.

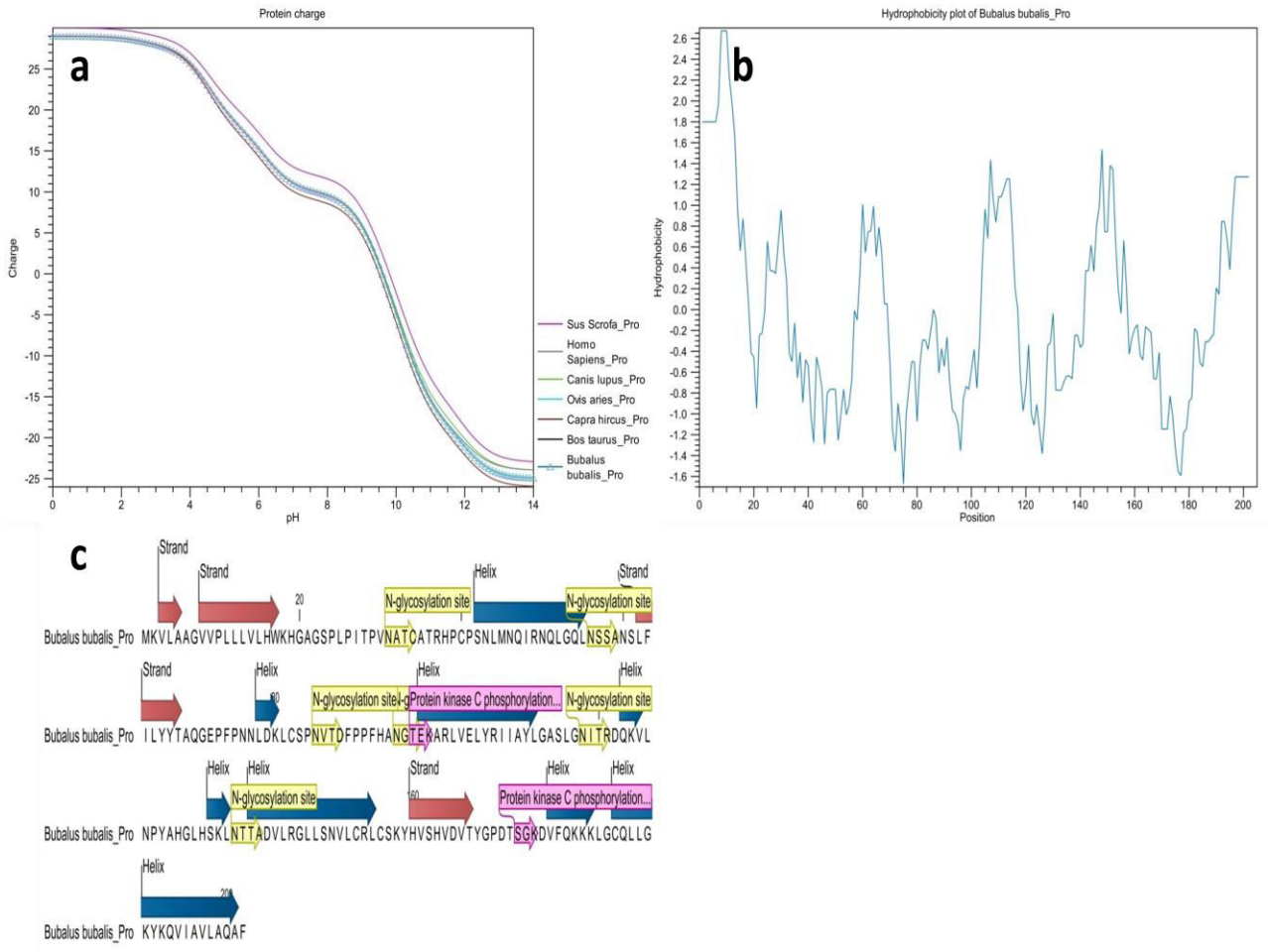

Fig. S5 a) Charge plot of all predicted and translated proteins. b) Hydrophobicity plot of predicted BuLIF. c) BuLIF representing the presence of secondary structure and theoretical sites available for post-translation modification.

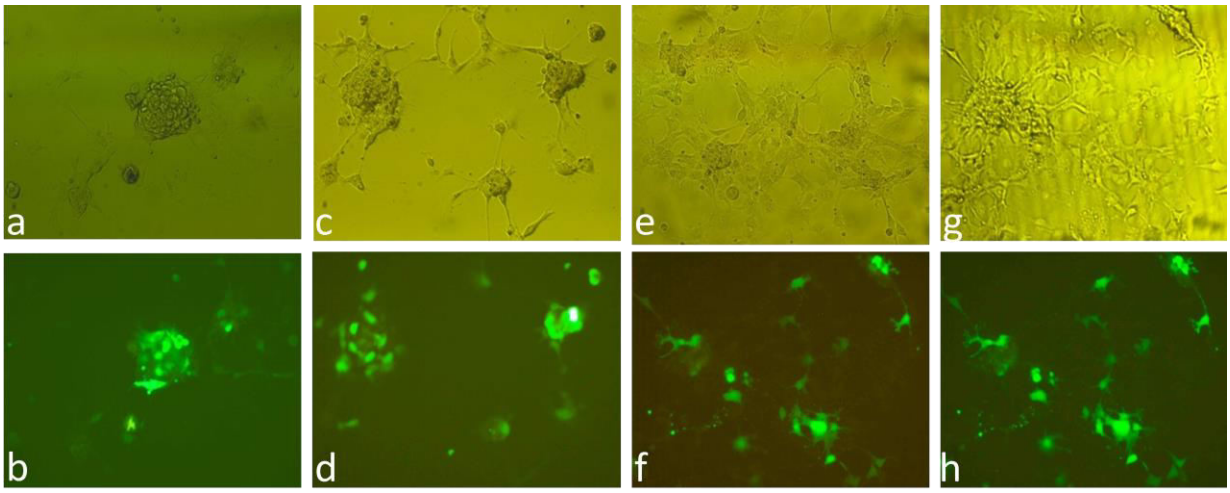

Fig. S6 Single cell colonies of stably transfected cells used for expansion and further selection of transfected cells. Transfected cell colonies were taken from different passages and cultured separately for stable selection. From left to right sequentially represents a-b 10^{th}, c-d 12^{th}, e-f 16^{th}, and g-h 20^{th} passage respectively. Transfected COS-1 cells in UV light expression GFP (b, d, f, h) and in bright light (a, c, e, g).

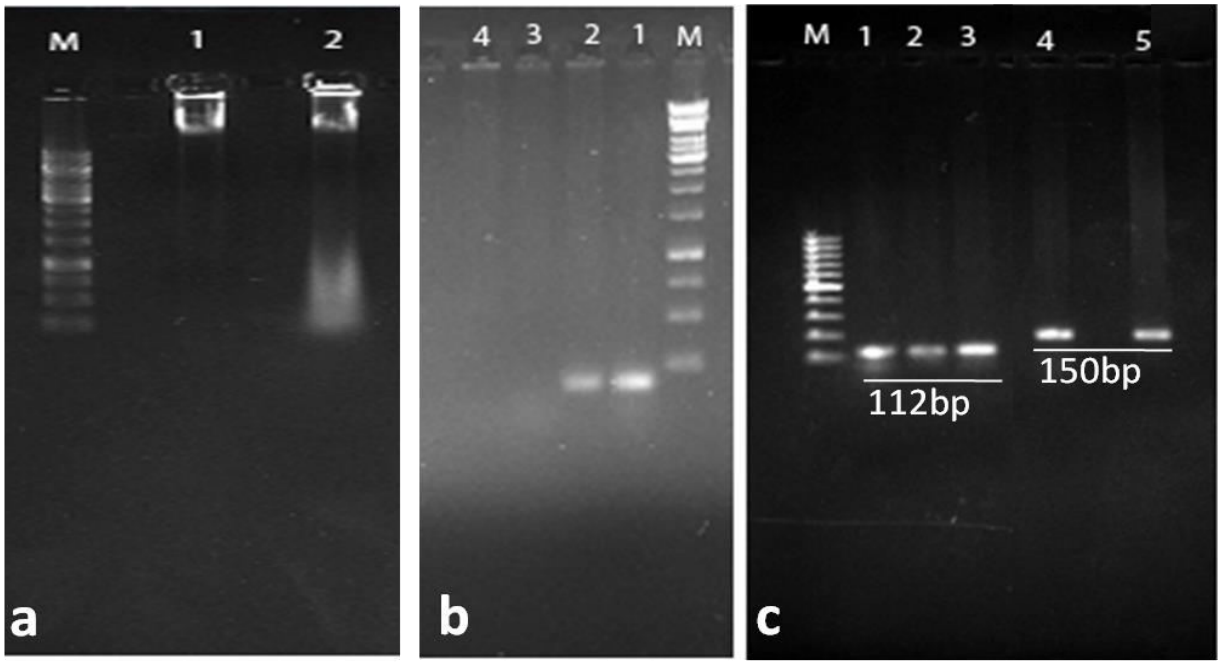

Fig. S7 Confirmation of genomic integration of BuLIF in COS-1 cells genome a) M DNA Marker, Lane-1: Genomic DNA was isolated from 50^{th} passage transfected COS-1_BuLIF cell line, Lane-2: Isolated genomic DNA from a normal COS-1 cell line. b) M DNA marker, Lane-1 and 2: PCR amplified product from transfected COS-1_BuLIF cells, Lane-3 and 4: NO PCR amplification occurred in normal COS-1 cells. c) M: DNA marker, Lane-1 to 3 PCR amplification of BuLIF sequence by genomic DNA of transfected COS-1_BuLIF cells using RT-BuLIF primers (112bp), Lane-4 and 5: PCR amplification of GFP by genomic DNA of transfected COS-1_BuLIF cells using RT-GFP primers (150bp).

a

	COS-1 (Mock)				COS-1_LIF				NTC	
	GAPDH	GAPDH	LIF	LIF	GAPDH	GAPDH	LIF	LIF	GAPDH	LIF
Ct value	13.6	13.4	24.4	23.7	15.5	15.1	19.4	19.4	—	—

b

	Control (beta actin)	Treatment	Formula
ΔC_T	10.57	4.15	$\Delta C_T = C_T$ Target gene $- C_T$ Reference gene
$\Delta\Delta C_T$	1	- 6.47	$\Delta\Delta C_T = \Delta C_T$ COS_ LIF $- \Delta C_T$ COS
Fold Change = $2^{-\Delta\Delta CT}$		88.6	

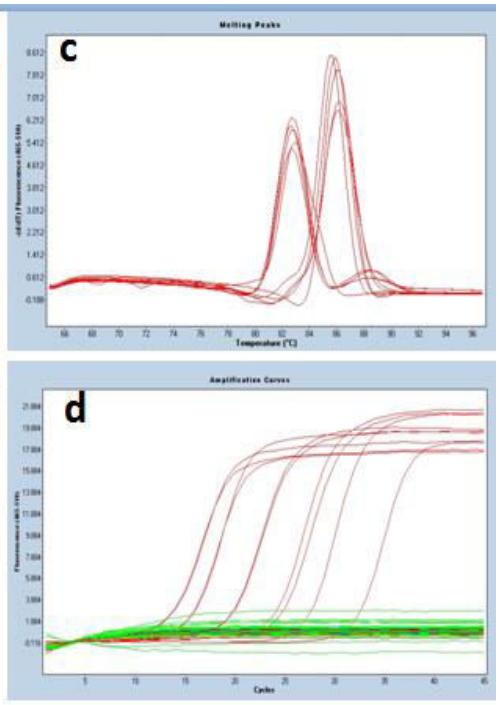

Fig. S8 a) Ct values of GAPDH and LIF gene in empty COS-1, COS1_BuLIF, and NTC. b) The calculated fold change of expression of rBuLIF transcripts by real-time quantitative qRT-PCR c) Melting curve for GAPDH and BuLIF gene. d) Amplification plot for GAPDH and BuLIF gene.

Supplementary Table 1. Position of secondary structure in BuLIF predicted protein

Start	End	Region name
3	5	beta strand
8	17	beta strand
42	55	alpha helix
62	68	beta strand
78	80	alpha helix
98	112	alpha helix
123	125	alpha helix
135	137	alpha helix
140	155	alpha helix
160	167	beta strand
177	182	alpha helix
185	201	alpha helix