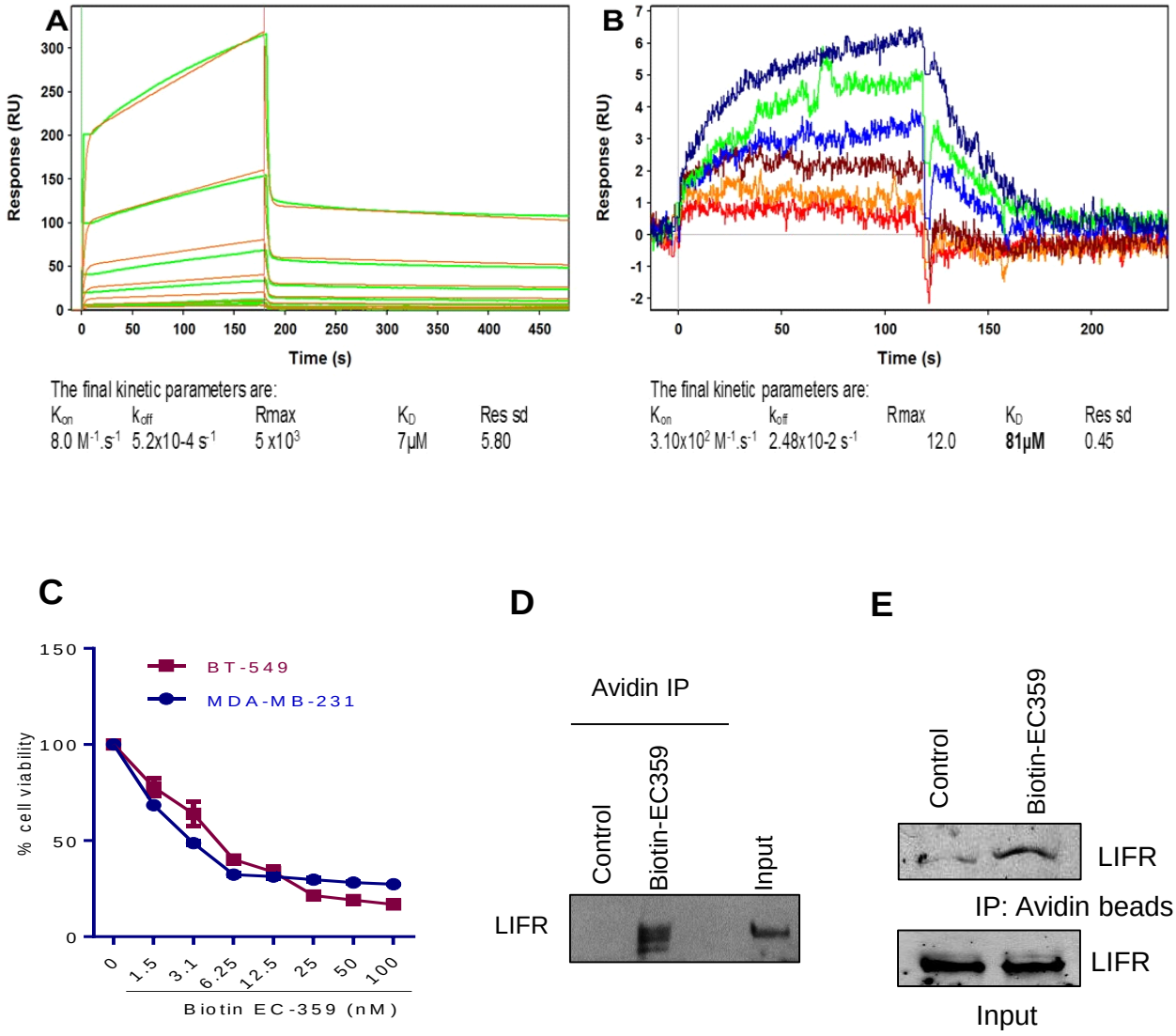
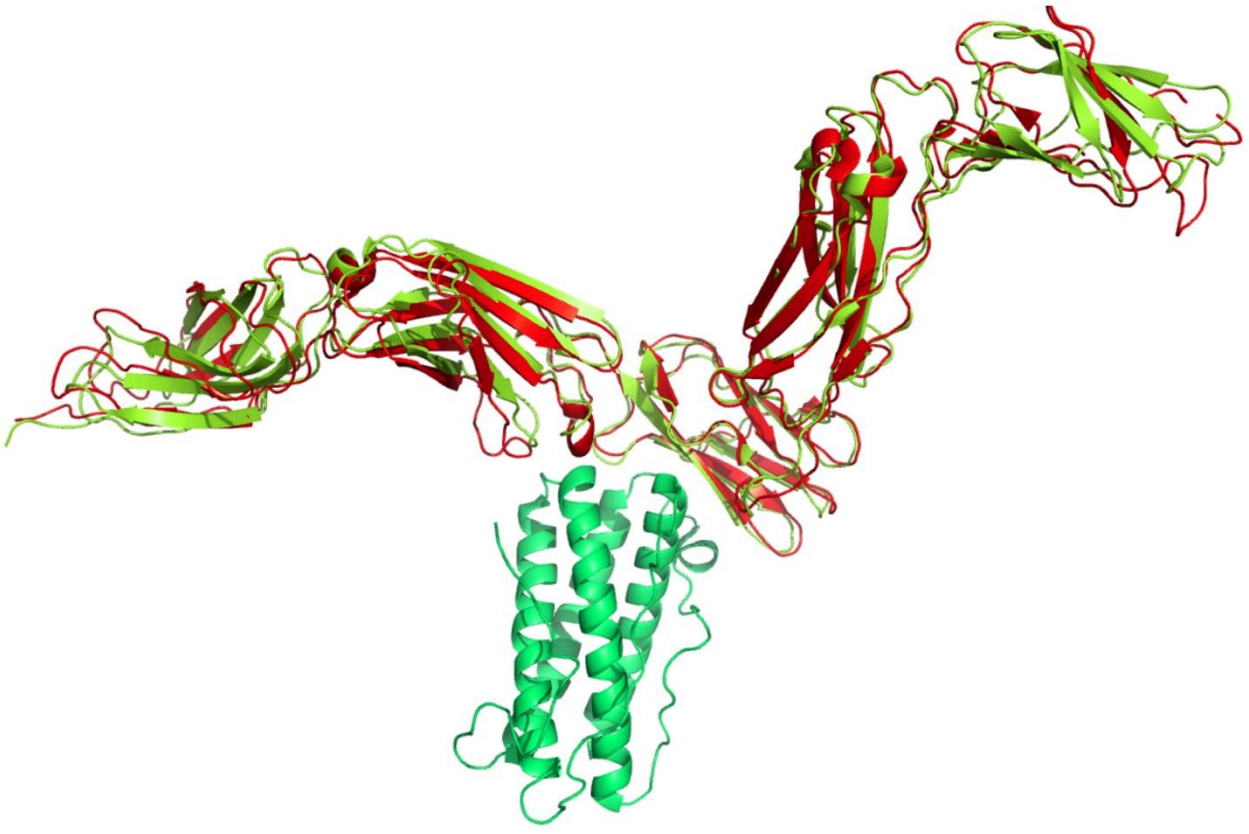
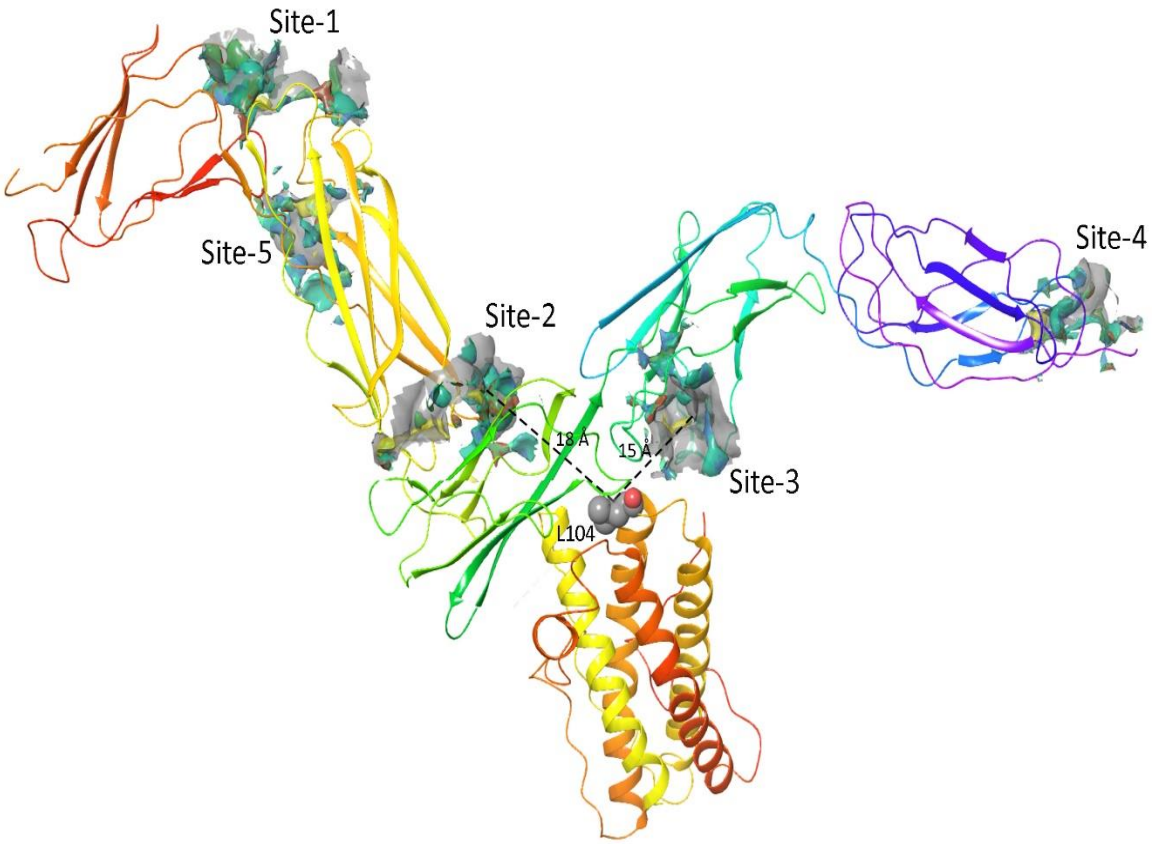


Supplementary Figure S1. SPR studies. **A**, Confirmation of functionality of LIF/LIFR interaction. **B**, Confirmation of LIFR-EC359 interaction. **C**, Activity of biotin-EC359 on cell viability was analyzed by MTT assay (n=3). Purified LIFR protein (**D**) or BT-549 total cellular lysate (**E**) was incubated with or without biotin-EC359. EC359 interaction with LIFR was confirmed by avidin IP followed by western blot analysis.

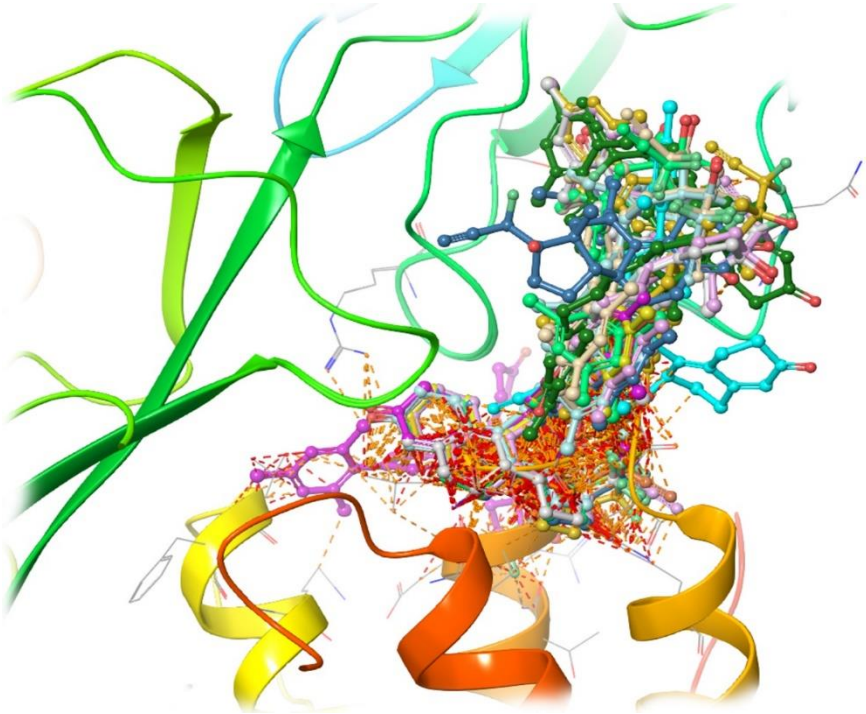


Supplementary Figure S2. Superimposition of hLIFR (pale green) onto the mLIFR (red)–hLIF (green) complex.

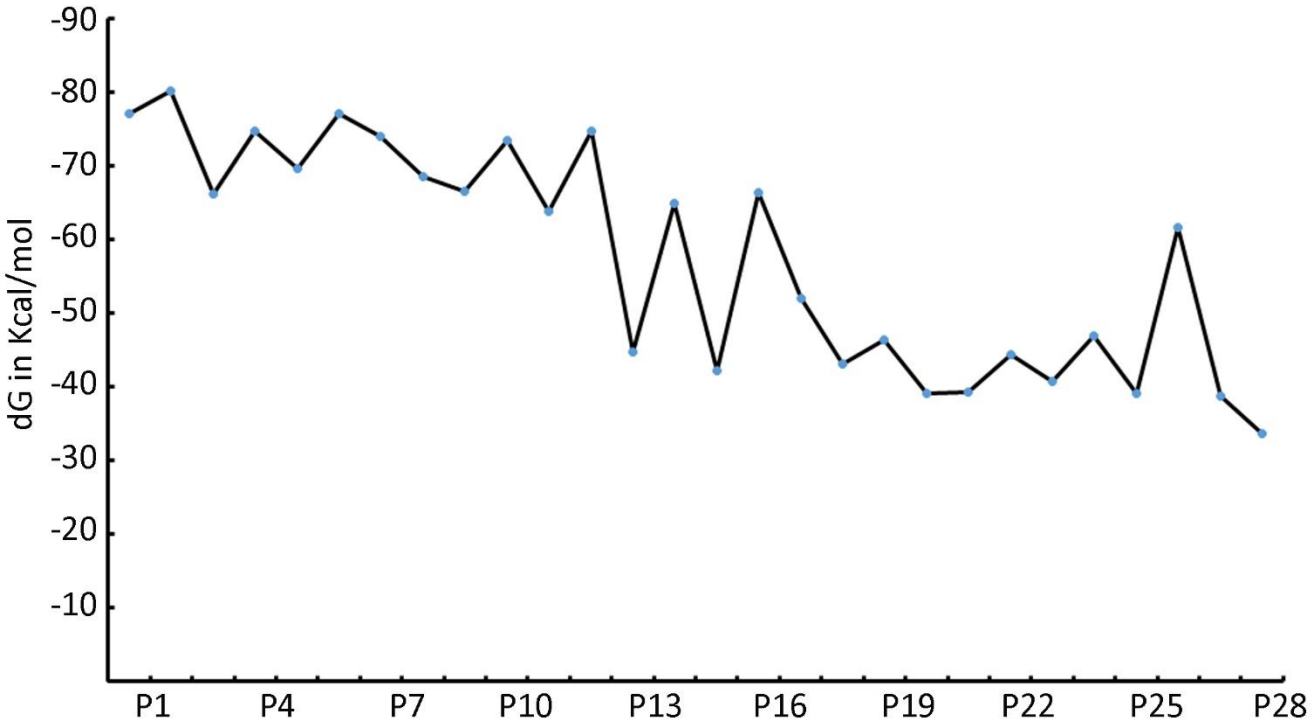
Supplementary Figure S3. Sequence alignment of human (P42702) and mouse (P42703) LIFR. The X-ray crystallographic structures of the highlighted regions (marked with blue and pink) are available in PDB.



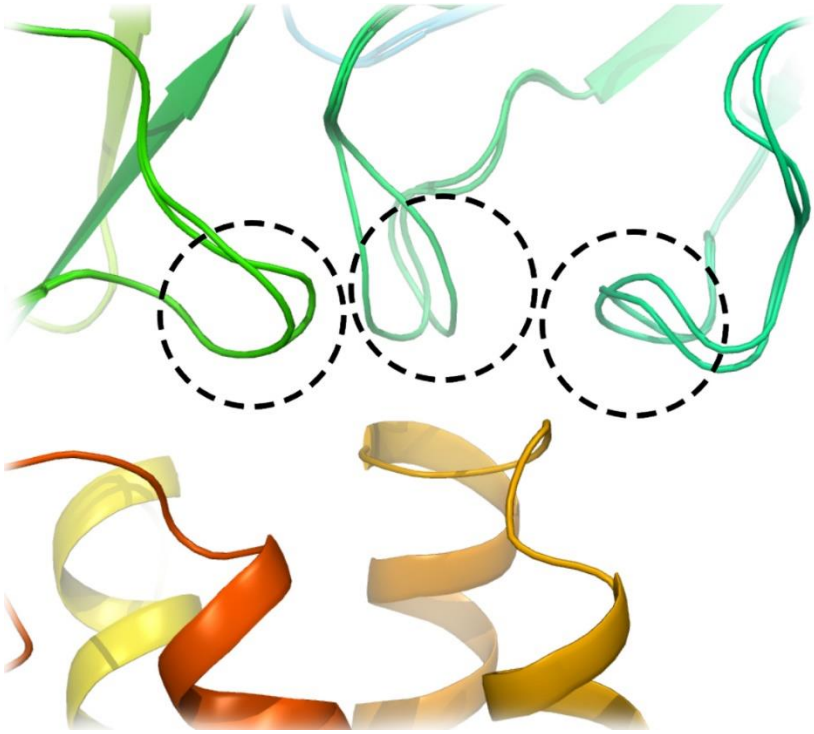
Supplementary Figure S4. Five prominent sites identified through sitemap program Schrödinger. The distances from L104 to site 2 and 3 were represented in dashed lines.



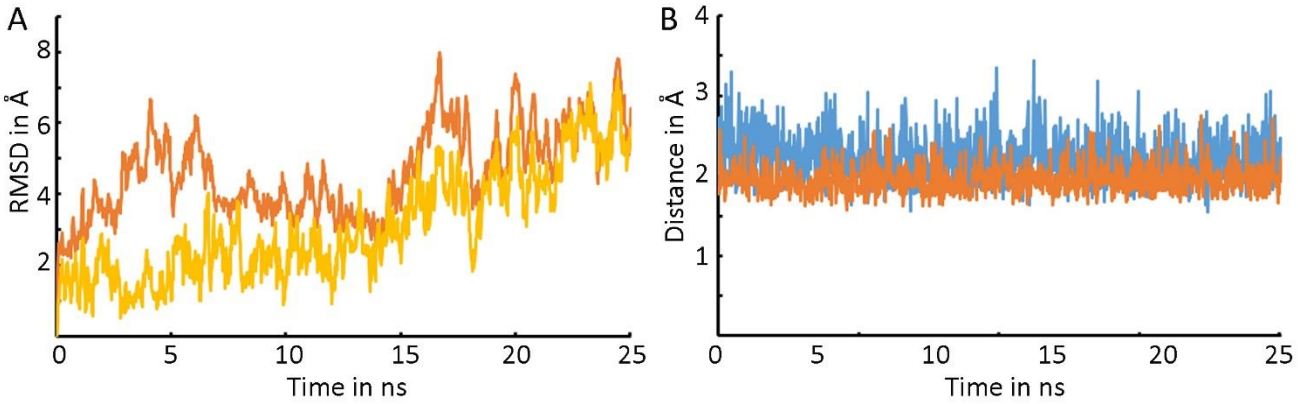
Supplementary Figure S5. Binding poses of EC359 at Site-3. Majority of the ligand poses are making steric clashes with hLIF (represented in orange color).



Supplementary Figure S6. MM-GBSA scores (represented in kcal/mol) for different poses obtained from the IFD



Supplementary FigureS 7. Ligand induced conformational changes (marked with circles) in the best scored pose. A majority of the changes occurred to the loops at the LIF binding region.

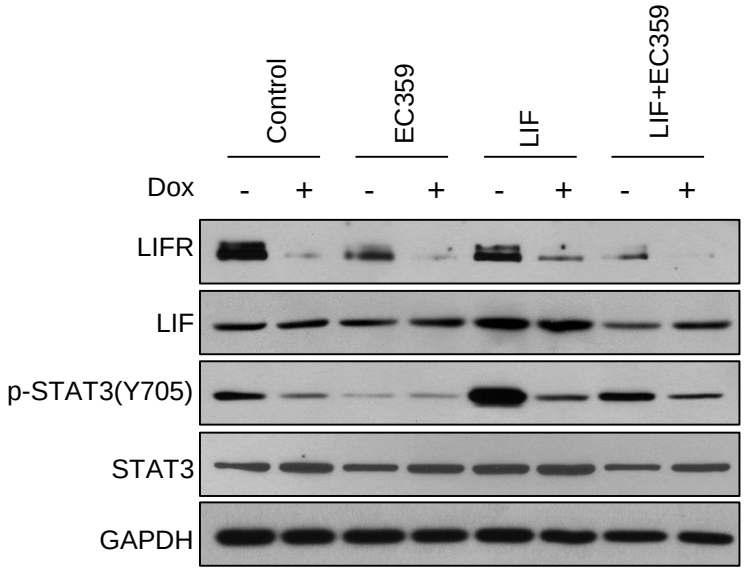


Supplementary Figure S8. A: the RMSD of protein (represented in orange color) and ligand (represented in yellow color). B: The distance of hydrogen bonds with T308 (represented in orange color) and T316 (represented in blue color).

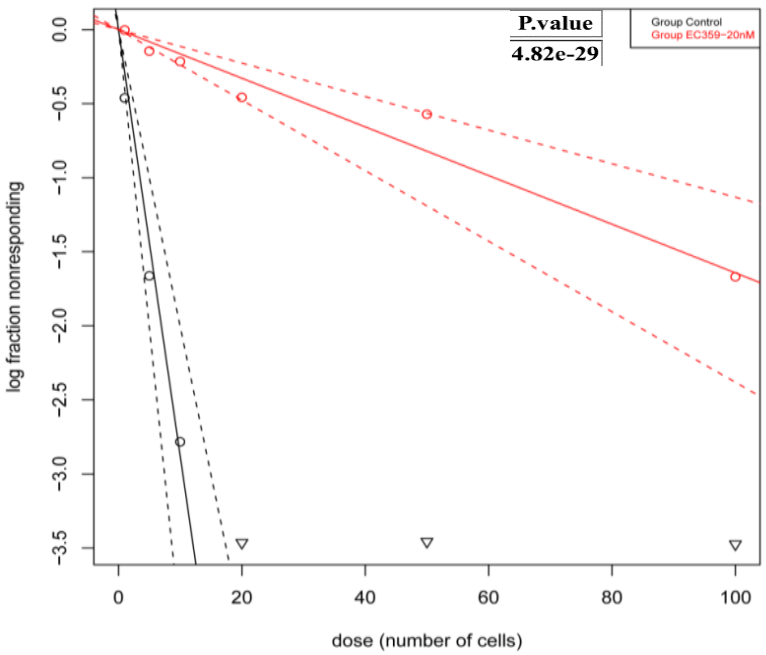
Compound	Assays	Result
EC359	Mutagenicity testing- S. typhimurium TA98, TA100, TA1535 and E.coli WP2 uvrA + E. coli WP2[pKM101] strains	No mutagenicity
EC359	Cardiotoxicity assessment (hERG): EC359 against hERG membrane using a fluorescence polarization assay	No liability
EC359	CYP inhibition: In vitro assessment of Cytochrome P450 Inhibition potential for EC359 using human liver microsomes (1A2, 2C9, 2C19, 2D6, 3A4)	2D6 inhibition
EC359	Hepatocyte stability: In vitro evaluation of EC359 compound for metabolic stability using cryopreserved human, mouse, rat and dog hepatocytes	Human & Mouse-moderate Rat & Dog - low
EC359	Microsomal stability: In vitro evaluation of EC359 compound for metabolic stability using cryopreserved human, mouse, rat and dog liver microsomes	Human & Mouse-moderate Rat & Dog- low
EC359	Single dose MTD: 10, 25, 50 and 100mg/kg	No toxicity observed
EC359	PK study in Rat & Mouse	Orally bioavailable; Mouse PK: 3.87 (iv); 1.0h (p.o) Rat PK:6h (iv); 3.0 (p.o)
EC359	Plasma protein binding (%)	Human- 99.98; Mouse- 99.63; Rat- 99.89; Dog- 99.83
EC359	Plasma stability (% remaining at 60 min)	Human-113.92; Mouse- 104.91; Rat- 108.64; Dog- 105.61
EC359	Solubility: pION, kinetic, thermodynamic	Low <10 µg/mL
EC359	Metabolite identification	Major metabolic pathway- Phase I metabolism; No glucuronide metabolites as evidenced by low CL in UDPGA
EC359	Caco2 –permeability & efflux transporter substrate activity	Low permeable; No efflux transporter substrate activity
EC359	Off-target binding study (CEREP screen)	GR and hERG were identified as off targets, however, IC 50 of binding with these receptors - up to 10uM and 30uM no GR and hERG binding respectively
EC359	LIF and LIFR binding – Thermophoresis method	Kd LIFR- 10.2nM; Kd LIF- No binding up to 5microM

Supplementary Figure S9. Pharmacological features of EC359

A



B



Confidence intervals for 1/(stem cell frequency)

Group	Lower	Estimate	Upper
Control	4.99	3.47	2.49
EC359-20nM	88.35	60.88	41.99

Supplementary Figure S10. A. Effect of dox inducible CRISPR/Cas9 mediated KO of LIFR on STAT3 signaling in the presence or absence of LIF in control or EC359 treated cells was measured using western blot analysis. GAPDH was used as a loading control. **B**. Effect of EC359 on self-renewal of cancer stem cells was determined by extreme limiting dilution assays.

Supplementary Table S1: Primer sequences used for RT-qPCR analysis

Gene name	Forward Primer (5'-3')	Reverse Primer (5'-3')
STAT1	ATCAGGCTCAGTCGGGGAATA	TGGTCTCGTGTTCTCTGTTCT
BCL2	GGTGGGGTCATGTGTGTGG	CGGTTCAGGTACTCAGTCATCC
SOX2	TGCGAGCGCTGCACAT	TCATGAGCGTCTTGGTTTTCC
CCND1	GCTGCGAAGTGGAAACCATC	CCTCCTTCTGCACACATTTGAA
C-MYC	GGCTCCTGGCAAAAGGTCA	CTGCGTAGTTGTGCTGATGT
JUNB	ACGACTCATACACAGCTACGG	GCTCGGTTTCAGGAGTTTGTAGT
TIMP1	CTTCTGCAATTCCGACCTCGT	ACGCTGGTATAAGGTGGTCTG
PTGS2	CTGGCGCTCAGCCATACAG	CGCACTTATACTGGTCAAATCCC
SOCS2	TTAAAAGAGGCACCAGAAGGAAC	AGTCGATCAGATGAACCACACT
BCL3	AACCTGCCTACACCCCTATAC	CACCACAGCAATATGGAGAGG
HIF1A	CACCACAGGACAGTACAGGAT	CGTGCTGAATAATACCACTCACA
MCL-1	GTAATAACACCAGTACGGACGG	CCACAAACCCATCCTTGGAAG
ICAM-1	ATGCCCAGACATCTGTGTCC	GGGGTCTCTATGCCCAACAA
NANOG	ACAACTGGCCGAAGAATAGCA	GGTTCCCAGTCGGGTTCAC
PIM1	GAGAAGGACCGGATTTCCGAC	CAGTCCAGGAGCCTAATGACG
LCN2	GACAACCAATTCCAGGGGAAG	GCATACATCTTTTGCGGGTCT
AKT1	AGCGACGTGGCTATTGTGAAG	GCCATCATTCTTGAGGAGGAAGT
TGFB1	CAATTCCTGGCGATACCTCAG	GCACAACTCCGGTGACATCAA
ZEB1	TTCAAACCCATAGTGGTTGCT	TGGGAGATACCAAACCAACTG
TNFRSF1A	TCACCGCTTCAGAAAACCACC	GGTCCACTGTGCAAGAAGAGA
FASL	ATTTAACAGGCAAGTCCAACTCA	GGCCACCCTTCTTATACTTCACT
GBP1	AGGAGTTCCTTCAAAGATGTGGA	GCAACTGGACCCTGTCGTT
PEG10	GAGCACCAGGGATTTCTCAGT	GGTAGTTGTGCATCAGGTAGTG
BOC	ACGGCGTGGAGAGGAATGA	GAGGGACCTCGTTCAAGTCAG
MYOGENIN	GGGGAAAACTACCTGCCTGTC	AGGCGCTCGATGTACTGGAT