SUPPLEMENTAL DATA

SUPPLEMENTAL METHODS

Liquid Chromatography/Tandem Mass Spectrometry Analysis.

Partially purified samples were mixed with 5X loading buffer, heated at 95°C for 10 minutes, and subjected to 15% SDS/PAGE until dye front was ~1 cm into the gel. The lane was removed and cut into 1-2 mm pieces and given to the Proteomics Core Facility (University of Texas Southwestern Medical Center, Dallas, Texas) for processing as follows: Samples were digested overnight with trypsin (Pierce) followed by reduction by DTT and alkylation with iodoacetamide (Sigma). The samples then underwent solid-phase extraction cleanup with an Oasis HLB μelution plate (Waters) and the resulting samples were analyzed by LC/MS/MS, using an Orbitrap Elite mass spectrometer (Thermo Electron) coupled to an Ultimate 3000 RSLC-Nano liquid chromatography system (Dionex). Samples were injected onto a 75 μm i.d., 50-cm long EasySpray column (Thermo), and eluted with a gradient from 1-28% solution B over 60 min. Solution A contained 2% (v/v) acetonitrile (ACN) and 0.1% formic acid in water, and solution B contained 80% (v/v) ACN, 10% (v/v) trifluoroethanol, and 0.1% formic acid in water. The mass spectrometer operated in positive ion mode with a source voltage of 2.4 kV and an ion transfer tube temperature of 275 °C. MS scans were acquired at 240,000 resolution in the Orbitrap and up to 14 MS/MS spectra were obtained in the ion trap for each full spectrum acquired using collisionally induced dissociation (CID) for ions with charges >1. Dynamic exclusion was set for 15 s after an ion was selected for fragmentation.

Raw MS data files were converted to a peak list format and analyzed using the central proteomics facilities pipeline (CPFP), version 2.0.3 (1, 2). Peptide identification was performed using the X!Tandem (3) and Open MS Search Algorithm (OMSSA) (4) search engines against the mouse protein database from Uniprot, with common contaminants and reversed decoy sequences appended (5). Fragment and precursor tolerances of 20 ppm and 0.5 Da were specified, and three missed cleavages were allowed. Carbamidomethylation of Cys was set as a fixed modification and oxidation of Met was set as a variable modification. Label-free quantitation of proteins across samples was performed using SINQ normalized spectral index software (6).

Immunoblot Analysis.

Tissue from mouse studies were thawed on ice and subsequently 30-50 mg of tissue was combined with 500 μl of buffer E supplemented with 1:1000 phosphatase inhibitor cocktail set I and set II (Millipore). The tissue was homogenized on ice with a hand held standard micro tissue homogenizer (ProScientific). The homogenized sample was kept on ice for 30 min, after which it was subjected to centrifugation at 4 °C for 20 min at 10,000 x g. The supernatant was collected, and for adipose tissue the upper lipid layer was avoided. Protein concentrations were measured using a NanoDrop instrument (Thermo Fisher Scientific) or a bicinchoninic acid kit (Pierce).

For cultured cell experiments after indicated treatments, medium was removed from each well of the 12-well plates, wells were washed twice with 1 ml PBS followed by the addition of 150 μl of buffer E supplemented with 1:1000 phosphatase inhibitor cocktail set I and set II. Cells were harvested by scraping and transferring to a 1.7-ml Eppendorf tube. Cells were lysed using a 22-gauge needle followed by measurement of protein concentration by bicinchroninic acid kit.

Animal tissue and cell culture extracts were mixed with 5X loading buffer, heated at 95 °C for 10 minutes, and subjected to 8% to 15% SDS/PAGE. The electrophoresed proteins were directly stained with Coomassie or transferred to nitrocellulose filters using the Bio-Rad Trans Blot Turbo system and subjected to Ponceau S stain followed by IB staining with the following primary antibodies: IgG-Actin (1:1000 dilution), IgG-LIF (1:1000 dilution), IgG-STAT1 (1:1000 dilution),

IgG-pSTAT1 (1:1000 dilution), IgG- STAT3 (1:1000 dilution), and IgG-pSTAT3 (1:1000 dilution). Bound antibodies were visualized by chemiluminescence (Super Signal Substrate; Pierce) by using a 1:5,000 dilution of donkey anti-mouse IgG (Jackson ImmunoResearch), 1:10,000 of donkey anti-rabbit IgG (Jackson ImmunoResearch) or a 1:2,000 dilution of rabbit anti-goat IgG (Sigma Aldrich) conjugated to horseradish peroxidase. Filters were exposed to Phoenix Blue X-Ray Film (F-BX810) for 1-300 s or scanned using an Odyssey FC Imager (Dual-Mode Imaging System, 2-min integration time) and analyzed using Image Studio version 5.0 (LI-COR).

1. Trudgian DC, and Mirzaei H. Cloud CPFP: a shotgun proteomics data analysis pipeline using cloud and high performance computing. *J Proteome Res.* 2012;11(12):6282-90.
2. Trudgian DC, Thomas B, McGowan SJ, Kessler BM, Salek M, and Acuto O. CPFP: a central proteomics facilities pipeline. *Bioinformatics.* 2010;26(8):1131-2.
3. Craig R, and Beavis RC. TANDEM: matching proteins with tandem mass spectra. *Bioinformatics.* 2004;20(9):1466-7.
4. Geer LY, Markey SP, Kowalak JA, Wagner L, Xu M, Maynard DM, et al. Open mass spectrometry search algorithm. *Journal of proteome research.* 2004;3(5):958-64.
5. Elias JE, and Gygi SP. Target-decoy search strategy for increased confidence in large-scale protein identifications by mass spectrometry. *Nature methods.* 2007;4(3):207-14.
6. Trudgian DC, Ridlova G, Fischer R, Mackeen MM, Ternette N, Acuto O, et al. Comparative evaluation of label-free SINQ normalized spectral index quantitation in the central proteomics facilities pipeline. *Proteomics.* 2011;11(14):2790-7.

SUPPLEMENTAL FIGURES AND TABLES

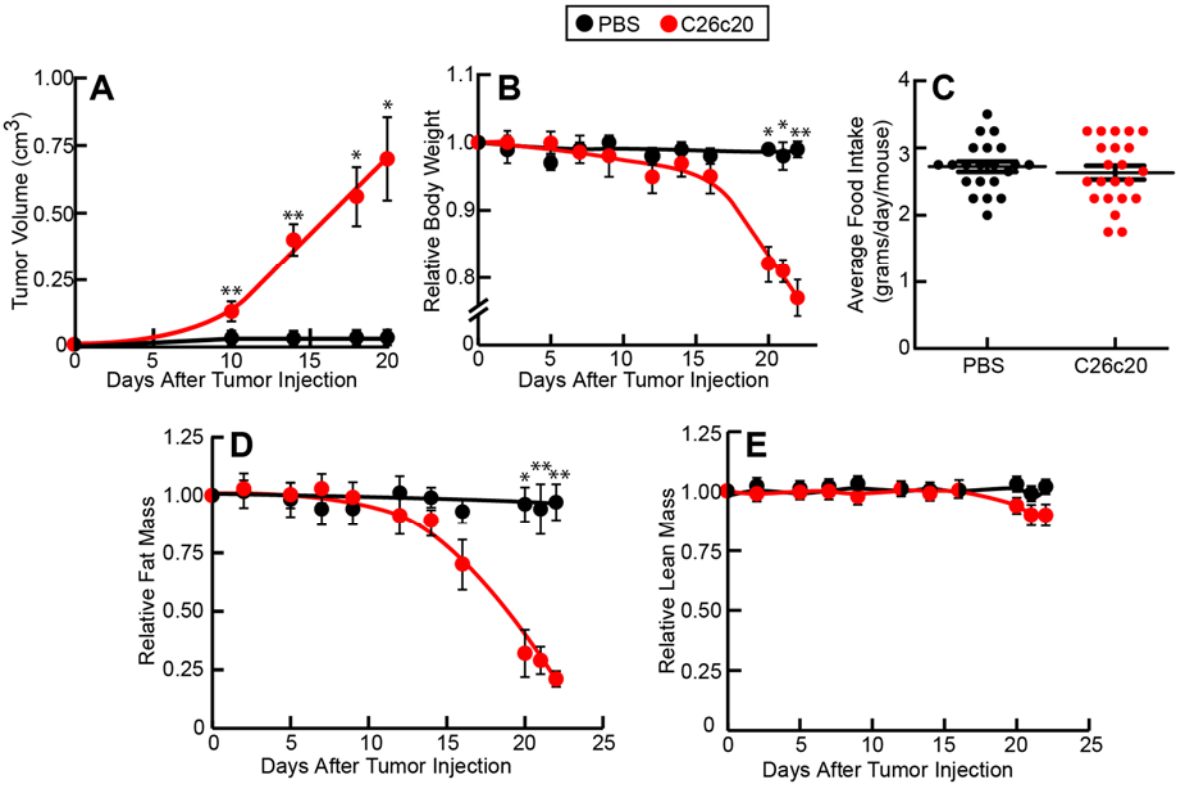

Figure S1. C26c20 Tumor Cells Induce Cachexia Phenotype In Vivo. A-E) Chow-fed Balb/c mice (11-week-old males) were housed four mice per cage and injected s.c. in right flank with 100 μl PBS in the absence or presence of $1x10^7$ C26c20 cells at day 0. Tumor volume (A) was calculated by taking half of the product of the caliper (VWR) measurements of length, width and breath at the indicated time points. Body weight (B), food intake (C), and ECHO MRI measurements of fat mass (D) and lean mass (E) were measured at 9 a.m. at the indicated time points as described in *Methods*. Body weight, fat mass, and lean mass are shown relative to the average day 0 reference value for each respective cohort. The average values for body weight (B) at day 0 were 24.3 and 25.0 g for the PBS and C26c20-injected mice, respectively. The average values for fat mass (D) at day 0 were 3.1 and 3.5 g for the PBS and C26c20 injected mice, respectively. The average values for lean mass (E) at day 0 were 18.2 and 18.7 g for the PBS and C26c20 injected mice, respectively. These results were confirmed in three independent experiments. Each value represents mean ± SEM of four mice. $^{*}p<0.05$ and $^{**}p<0.01$ based on Student's *t*-test.

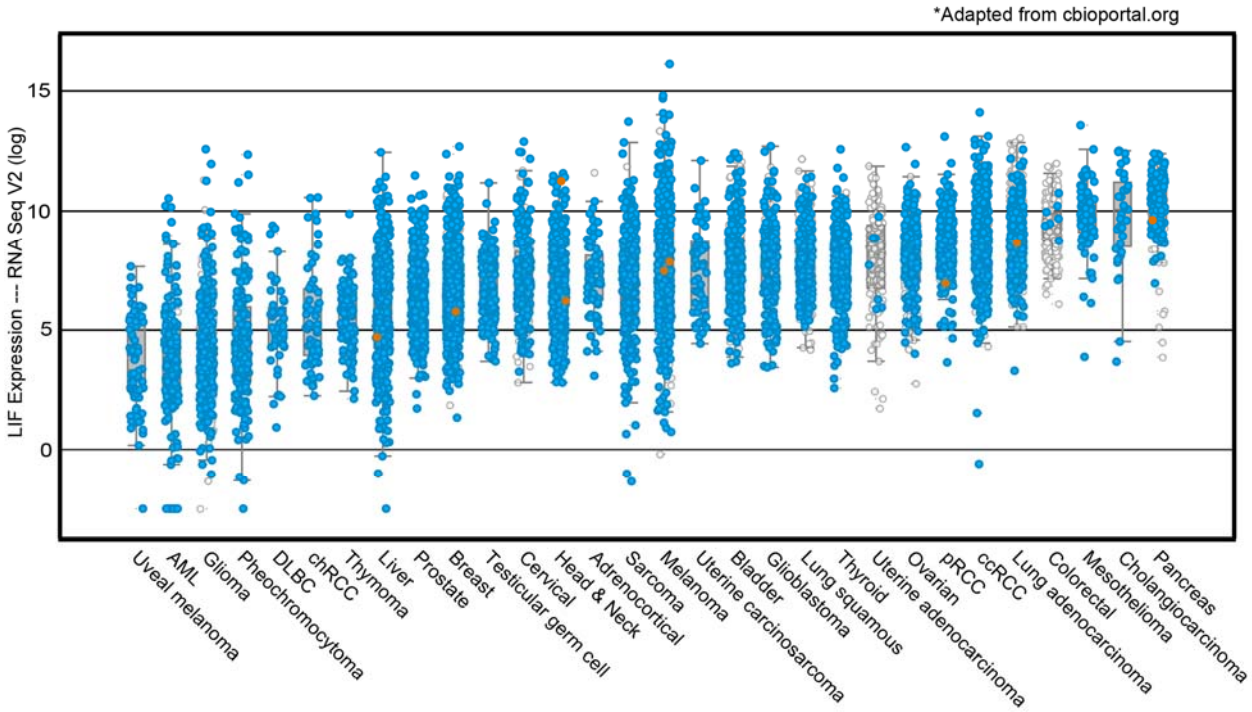

Figure S2. Relative LIF RNA Expression Among Multiple Human Cancers. Relative mRNA expression levels of LIF in different types of tumors were obtained from cbioportal.org and determined through RNA seq analysis of The Cancer Genome Atlas data. The number of samples for each cancer were as follows: adrenocortical carcinoma *n=92*, acute myeloid leukemia *n=400*, bladder cancers *n=933*, breast cancers *n=5975*, cervical cancers *n=309,* cholangiocarcinoma *n=99*, chromophobe renal cell carcinoma (chRCC) *n=132*, clear cell renal cell carcinoma (ccRCC) *n=1320*, colorectal adenocarcinoma *n=2872*, diffuse large b cell lymphoma (DLBC) *n=154*, glioblastoma n=1395, glioma *n=1713*, head & neck cancers *n=1106*, hepatocellular carcinoma and hepatocellular adenoma (Liver) *n=989*, lung adenocarcinoma *n=2048*, lung squamous cell carcinoma *n=682*, melanoma *n=879*, mesothelioma *n=109*, ovarian cancers *n=1181*, pancreas cancers *n=1023*, papillary renal cell carcinoma (pRCC) *n=293*, pheochromocytoma and paraganglioma (pheochromocytoma) *n= 184*, prostate adenocarcinoma *n=2815*, sarcoma *n=472*, testicular germ cell tumors *n=336*, thymoma *n=124*, thyroid cancers *n=1140*, uterine adenocarcinoma *n=921*, uterine carcinosarcoma *n=79*, and uveal melanoma n=80. Each tumor sample is depicted as a circle, and color signifies sequence status of LIF in that tumor sample (*white* = not sequenced, *blue* = sequenced without mutation, *orange* = sequenced with missense or frameshift mutation). Adapted from cbioportal.org.

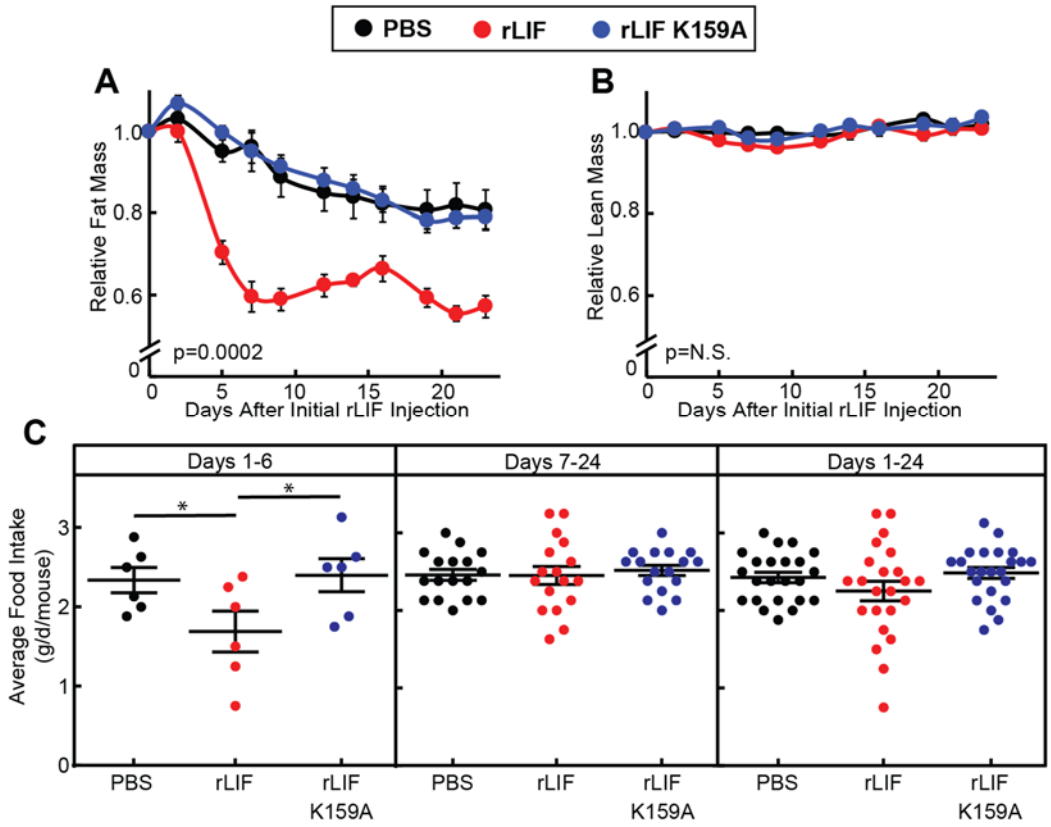

Figure S3. LIF Induces Adipose Tissue Loss with Transient Hypophagia in Wild-Type C57BL/6 Mice. A-C) LIF treatment of C57BL/6 wild-type mice. Chow-fed C57BL/6 mice (9-week-old males) were housed four mice per cage and injected i.p. with 100 μl PBS in the absence or presence of rLIF or rLIF K159A at 80 μg/kg body weight twice daily throughout the experiment. ECHO MRI measurements of fat mass (A) and lean mass (B), and food intake (C), were measured at 9 a.m. at the indicated time points. Fat mass and lean mass are shown relative to the average day 0 reference value for each respective cohort. The average values for fat mass (A) at day 0 were 2.8, 2.9 and 2.8 g for the PBS, rLIF, and rLIF K159A treated mice, respectively. The average values for lean mass (B) at day 0 were 16.5, 16.8 and 17.0 g for the PBS, rLIF, and rLIF K159A-treated mice, respectively. Each value represents mean ± SEM of eight mice. $^{*}p<0.05$ based on Student's *t*-test (C) or p-value based on use of Generalized Estimated Equation approach comparing multiple groups over time with rLIF-treated mice as the reference value (A-B). N.S. = not significant.

Table S1: Partial Purification of Adipocyte Lipolysis Activity from Cachexia-Inducing Tumor Medium.

Step	Fraction	Protein[a]	Total Activity[b]	Specific Activity	Purification	Recovery
		(mg)	*(fmol)*	*(fmol / mg)*	*(fold)*	*(%)*
1	**Cachexia Cell Medium[c]**	1,249.2	94,327	76	-	100
2	**SP-Sepharose (pH 7.5)**	2.9	15,000	5,178	69	16
3	**Superdex 200 10/300**	0.2	2,872	12,054	160	3
4	**Q-Sepharose (pH 7.5)**	0.1	2,038	19,506	258	2

[a]Protein concentration of the pooled fractions containing activity was determined as described in the *Methods*.
[b]Adipocyte lipolysis activity was determined as described in the *Methods*.
[c]The starting fraction contains at least two proteins with lipolysis activity: LIF (~20% of total activity) and unidentified protein(s) (~80% of total activity).

Table S2: Proteins Containing Signal Sequences with Peptides Identified in Purification of Cachexia-Inducing Tumor Medium.

#	PSM[a]	Accession	Symbol	Protein Description	Length (AA)	MW (Da)	Pep. Seqs[b]	% Cov.[c]
1	761	P06797	CATL1	Cathepsin L1	334	37,613	34	91
2	465	P12032	TIMP1	Metalloproteinase inhibitor 1	205	22,667	19	74
3	294	P14719-2	ILRL1	Isoform B of Interleukin-1 receptor-like 1	337	38,567	22	64
4	254	Q01149	CO1A2	Collagen alpha-2(I)	1,372	129,875	63	71
5	156	P11276	FINC	Fibronectin	2,477	273,106	47	25
6	136	Q8BND5-3	QSOX1	Isoform 3 of Sulfhydryl oxidase 1	568	63,458	38	71
7	126	Q61468	MSLN	Mesothelin	625	69,559	20	35
8	105	P20060	HEXB	Beta-hexosaminidase subunit beta	536	61,221	28	53
9	104	P09056	LIF	Leukemia inhibitory factor	203	22,327	15	52
10	100	P47880	IBP6	Insulin-like growth factor-binding protein6	238	25,384	19	70
11	95	P28798	GRN	Granulins	589	65,152	28	61
12	64	Q61398	PCOC1	Procollagen C-endopeptidase enhancer1	468	50,262	20	58
13	49	Q9R045	ANGL2	Angiopoietin-related protein 2	493	57,214	15	31
14	44	Q62181	SEM3C	Semaphorin-3C	751	85,451	18	32
15	42	P21460	CYTC	Cystatin-C	140	15,557	10	54
16	27	Q8QZR4	OAF	Out at first protein homolog	282	31,576	14	53
17	16	P08122	CO4A2	Collagen alpha-2(IV) chain	1,707	167,724	8	9
18	14	P19324	SERPH	Serpin H1	417	46,612	10	35
19	11	P47879	IBP4	Insulin-like growth factor-binding protein4	254	27,861	7	32
20	9	P08121	CO3A1	Collagen alpha-1(III) chain	1,464	139,290	5	6
21	6	Q8R0F3	SUMF1	Sulfatase-modifying factor 1	372	40,742	3	13
22	5	P06869	UROK	Urokinase-type plasminogen activator	433	48,363	3	11
23	5	P26262	KLKB1	Plasma kallikrein	638	71,516	2	3
24	5	Q91ZV3	DCBD2	Discoidin, CUB and LCCL domain protein 2	769	83,937	3	5
25	3	P11087	CO1A1	Collagen alpha-1(I) chain	1,453	118,108	2	2
26	2	Q64299	NOV	Protein NOV homolog	354	38,991	3	11

[a] Peptide Spectrum Matches (PSM)= Total number of identified peptide spectra of the respective protein.
[b] Pep. Seqs= Unique identified peptide sequences of respective protein.
[c] % Cov= Percentage of respective protein sequence matched by identified peptides.