Supporting Information

Yang et al. 10.1073/pnas.1016147107

SI Methods

Cells and Reagents. The human DLD1 colon carcinoma cell line was obtained from the American Type Culture Collection. The cells were grown in McCoy's 5A medium with L-Glutamine (HyClone). All media were supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100 μg/mL) (Gibco-BRL). Antibodies against total STAT3, Y705-phosphoryl STAT3 (pY705-STAT3), S727-phosphoryl STAT3 (pS727-STAT3), LSD1, and SET9 were from Cell Signaling Technology. In DLD1 cells, the STAT3 gene was knocked out by homologous recombination, using pAAV-Neo-loxP, as described previously (1, 2). The Y705F, S727A, K140A, K140R, R214/215A, and R414/417A mutants of STAT3 were generated from wild-type STAT3 cDNA by using the QuikChange II Site-Directed Mutagenesis Kit (Stratagene). The pLEGFP-N1 retroviral expression vector was from Clontech. Expression vectors for the above-mentioned mutants of STAT3 were generated by inserting the cDNAs into the HindIII site of the pLEGFP-N1 vector. To obtain infectious retrovirus stocks, each construct was transfected into BOSC23 packaging cells (Clontech), and supernatant media, collected after 48 h, were used to infect A4 cells on three consecutive days. A4 cells were transiently transfected with the ecotropic receptor 2 d before virus infection. Stably transduced A4 cell pools were selected in 500 μg/mL of neomycin G418 and shown to express STAT3 proteins of the expected size by Western analysis. Cells were treated with IL-6 (200 ng/mL), together with IL-6 soluble receptor (250 ng/mL) or were left untreated.

Preparation and Purification of Antibodies Against K140 Methyl STAT3 Peptides. Peptides representing human STAT3 residues 130 to 150, with and without dimethylation or monomethylation of K140, were from Chinese Peptide Inc.. The sequence of STAT3 is: Asn-His-Pro-Thr-Ala-Ala-Val-Val-Thr-Glu-Lys-Gln-Gln-Met-Leu-Glu-Gln-His-Leu-Gln-Asp. The peptides were synthesized and covalently linked to keyhole limpet hemocyanin (KLH). In the presence of adjuvant, the protein antigens were injected dorsointradermally into New Zealand rabbits. Booster immunizations were started 3 d after the priming immunization, continued on the 28th day, and then every 14 d thereafter. The antibody was produced in Lanzhou, China. All animal handling and treatments complied with local regulations and rules. Complete Freund's adjuvant was used in the first immunization and incomplete Freund's adjuvant was used during the booster immunizations. Each rabbit weighed ≈2.5 kg before the first immunization. After four immunizations, the rabbits were bled and serum was prepared. The KLH-conjugated synthetic peptides including amino acids 140 to 150 of STAT3, with and without dimethylation or monomethylation of K140, were used in ELISA experiments to measure antibody titers. When the titer from each rabbit was above 64,000, blood was collected from an ear artery, between two booster immunizations. To prevent cross reaction among the above antibodies, a negative screen was used. The fusion protein GST-Asn-His-Pro-Thr-Ala-Ala-Val-Val-Thr-Glu-Lys-Gln-Gln-Met-Leu-Glu-Gln-His-Leu-Gln-Asp (GST-tagged 21-aa wild-type STAT3) was expressed in *Escherichia coli* and purified by GST/GSH affinity chromatography. The fusion protein and the two KLH 21-aa peptides were then coupled to activated Sepharose 4B beads. The binding buffer used during purification contained 20 mM sodium phosphate, pH 7.0 and the elution buffer contained 0.1 M sodium citrate, pH 3.0. After antibody purification, the titer for anti-wild-type STAT3 was 1:100,000; the titer for anti-STAT3-K140me2 and anti-STAT3-K140me1 was 1:4,000, as evaluated by ELISAs. To control for the specificity of the antibodies, an ELISA and a dot-blot assay were used. There is no homologous sequence for the epitope used for the STAT3-K140me2 antibody among other STAT family members.

Protein Digestion and Liquid Chromatography-Tandem Mass Spectrometry. STAT3 gel pieces cut from SDS/PAGE gels were destained with 50% acetonitrile in 100 mM ammonium bicarbonate, and then 100% acetonitrile. The proteins were reduced in the gels with 20 mM DTT (DTT) at room temperature for 30 min, followed by alkylation with 50 mM iodoacetamide in 100 mM ammonium bicarbonate for 30 min in the dark. After treatment, the reagents were removed and the gel pieces were washed with 100 mM ammonium bicarbonate and then dehydrated in acetonitrile. The dried gel pieces were then rehydrated in a solution of sequencing grade, modified trypsin in 50 mM ammonium bicarbonate for digestion overnight. Tryptic peptides were extracted from the gel with 50% acetonitrile in 5% formic acid. Peptide analysis was performed using a LTQ Orbitrap XL linear ion trap mass spectrometer (Thermo Fisher Scientific) coupled with an Ultimate 3000 HPLC system (Dionex), with a linear gradient of acetonitrile from 4 to 40% in water in the presence of 0.1% formic acid over a period of 45 min. The spectra were acquired in the positive-ion mode, and automatic data-dependent methods were used, consisting of a full scan (m/z 400–2,000), and MS/MS on the five most abundant precursor ions at the collision energy of 30%. The data were submitted to the Mascot database search for initial screening of various post-translational modifications that may have occurred on STAT3. All of the modifications suggested by the initial screening were verified by manual interpretation of the MS/MS spectra.

Isolation of Cell cytoplasmic and Nuclear Fractions. These fractions were prepared by using the Sigma Nuclei Isolation Kit (Cat No: NUC201) according to the instructions in the manual. Briefly, IL-6–treated or untreated A4 cells expressing STAT3 were grown in 150-mm plates. Confluent cells were trypsinized, spun at 300 × g for 2 min and washed twice with ice-cold PBS. Cells were resuspended in 5 volumes of ice-cold buffer A (10 mM Hepes, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT, 1 mM phenylmethanesulfonyl fluoride and protease inhibitors). Cells were swollen on ice for 15 min and broken by the use of a Dounce homogenizer for 30 strokes. The lysates were spun at 1200 × g for 4 min. The supernatant is "cytoplasm," the pellet "nuclei." The nuclear fraction was lysed with Nuclei EZ Lysis buffer after washing with buffer A. Western analysis was performed by loading protein samples representing equal numbers of cells.

Luciferase Reporter Assay. A 1.0-kb DNA fragment containing the human *SOCS3* promoter (−1,000 to −1), obtained by PCR from human genomic DNA, was inserted into the pGL3-basic vector. *SOCS3* promoter-driven luciferase reporter constructs were transfected into A4 cells or A4 cells expressing STAT3 by using the Fugene 6 reagent (Roche). Cells were plated and cultured in 12-well plates to 40% confluence before transfection. Briefly, 1 μg per well luciferase plasmid plus 0.5 μg per well pCH110 (β-galactosidase plasmid for internal control) were cotransfected. Twenty-four hours later, the cells were treated with 200 ng/mL IL-6 plus 250 ng/mL IL-6 soluble receptor for another 24 h, or were untreated. The cell pellets were lysed in 200 μL of buffer (Reporter lysis buffer; Promega). Cell lysates (60 μL) were mixed with 60 μL of luciferase assay buffer (Promega) for activity measure-

ments in a luminometer. For the β-galactosidase activity assay, the luminescent β-galactosidase detection Kit II (Clontech) was used.

Immunoprecipitations. For STAT3, the EZview Red ANTI-FLAG M2 Affinity Gel system (Sigma-Aldrich) was used, as in the protocol provided by Sigma-Aldrich, with slight modifications. For immunoprecipitation of STAT3 from A4 cells expressing wild-type STAT3, cells from 10 150-mm dishes, treated with 200 ng/mL IL-6 plus 250 ng/mL IL-6 soluble receptor for 4 h or untreated, were collected and lysed in buffer (50 mM Tris·HCl, pH 8.0, 150 mM NaCl, 1% Nonidet P-40). Sepharose G beads were used to bind the proteins, which were eluted with flag peptides and mixed with 5× SDS sample loading buffer for separation in a 12% SDS/PAGE gel. The gel was treated with fixing buffer (46% methanol and 7% acetic acid) for 1 h and treated with staining buffer (46% methanol, 7% acetic acid and 0.1% Coomassie Brilliant Blue R-250) for 1 h. The gel was destained with 5% methanol and 7.5% acetic acid until the background was clear. The STAT3 bands were cut out for further analysis by MS.

Western and Northern Analyses. Cells were treated with 200 ng/mL IL-6 plus 250 ng/mL IL-6 soluble receptor for 4 or 24 h or were untreated. For Northern analyses, 20 μg of total RNA was used. Human cDNA probes for *SOCS3*, *IRF8*, *IRF9*, *FGF21*, *HSF1*, *CDCA1*, and *CD14* were cut from I.M.A.G.E. clones (Invitrogen or the American Type Culture Collection). Templates for human *GAPDH* cDNA were obtained by RT-PCR. Signals were normalized for loading by comparing the intensities of *GAPDH* mRNA on the same membranes.

EMSA. Cells were treated with 200 ng/mL IL-6 plus 250 ng/mL IL-6 soluble receptor for 4 h or were untreated. The cells were lysed in EMSA lysis buffer, supplemented with protease inhibitors. The probe was the GAS consensus sequence 5′-CGATTCCTGGAACTGCGCGG-3′ from the *SOCS3* promoter. In each binding reaction, 10^4 DPM of labeled probe was used.

Immunocytochemistry. A4 cells expressing STAT3 were grown on glass coverslips for 24 h before cytokine treatment. Cells were treated with 200 ng/mL IL-6 plus 250 ng/mL IL-6 soluble receptor for 4 or 24 h or were untreated. Cells at 25% confluency were fixed in 4% paraformaldehyde for 15 min and in absolute methanol for 5 min at room temperature and then treated with blocking buffer (1 × PBS + 0.3% Triton X-100 + 10% FBS). Signals were detected with mouse anti-human STAT3 (Cell Signaling Technology), rabbit anti-human pS727-STAT3 (Cell Signaling Technology), and rabbit anti-human STAT3-K140me2. Signals were visualized with Alexa Fluor 488 goat anti-mouse (green-fluorescent) and Alexa Fluor 494 goat anti-rabbit (red-fluorescent) secondary antibodies. Images were captured with a Zeiss Axioskop fluorescence microscope.

Histone Methyltransferase Screening. Approximately 0.1 μg of purified STAT3 protein was isolated from *E. coli* cells and incubated with 0.1 μg of recombinant enzyme and 1 μCi of methyl [^{3}H]-labeled *S*-adenosyl-methionine at 30 °C for 30 min. Reaction mixtures were divided into two parts. One was run in SDS/PAGE, followed by Gelcode (Fisher Scientific) staining. The other was analyzed by fluorography.

STAT3 Methylation and Demethylation in Vitro Assays. N-terminal His-tagged human pcDNA-SET9 and pcDNA-LSD1 recombinant proteins were expressed in A4 cells and purified by Ni-NTA agarose chromatography to >95% purity, as analyzed by SDS/PAGE with Coomassie blue staining. For the in vitro methylation assays, about 0.5 μg of SET9 and l μg of purified STAT3 were used. The protocol given by Upstate was used, with small modifications. The assay was performed with unlabeled *S*-(5′-Adenosyl)-L-methionine chloride (Sigma) instead of [^{3}H]-labeled compound. STAT3 demethylation assays were performed with 0.5 μg of LSD1 and 1 μg of STAT3 in demethylation buffer (50 mM hepes, pH 8.0, 25% glycerol). After reaction, the products were analyzed by SDS/PAGE and the signals were detected by Western blot.

Illumina Array Analyses. Total RNA was isolated by using a Qiagen RNeasy Mini Kit according the manufacturer's instructions. Total RNAs (250 ng) were reverse-transcribed into biotin-UTP labeled cRNA, by using an Illumina TotalPrep RNA Amplification kit (Ambion/Applied Biosystems). Labeled cRNAs were hybridized to the Illumina Human Ref-v3 V1 Expression BeadChip. Data were analyzed by using the Illumina BeadStudio software. Expression was normalized against the levels of GAPDH and ACTIN mRNAs in the all of the samples.

SET9 and LSD1 siRNA Transfections. A4 cells expressing wild-type STAT3 were grown in 60-mm plates to 40% confluence before transfection. Media were aspirated from the cells, which were washed twice with sterile PBS. Then, 5 mL of fresh medium was added to each plate, with 10% serum and without antibiotics. Twenty microliters of 10 μM SET9 (sc-44094; Santa Cruz) or LSD1 siRNA (sc-60970; Santa Cruz) was added to 300 μL siRNA Transfection Medium (Santa Cruz), mixed gently, and kept at room temperature for 20 min. The mixture was then added drop-wise to the plates with gentle rocking. After incubation for 24 h at 37 °C, the transfection media were removed and the cells were transfected again, following the same protocol. After another 48 h, the cells were treated with IL-6 for the times indicated or left untreated. Total RNA or protein was analyzed.

SET9 and LSD1 Mutant cDNAs. The specific siRNA sequences for SET9 (sense strand) (sc-44094; Santa Cruz) are GCCAGGGAGUUUACACUUA (nucleotides 495–512 of the RNA), CAAACUGCAUCUACGAUAU (nucleotides 1188–1205 of the RNA) and GCCAGGGUAUUAUUAUAGA (2094–2111); the first two are located in the coding sequence. In the SET9 expression plasmid, the cDNA was changed from GCCAGGGAGTTTACACTTA to GCCAaGGgGTaTAtACTTA and CAAACTGCATCTACGATAT was changed to CtAAtTGCATaTAtGATAT. The specific siRNA sequences for LSD1 (sense strand) (sc- 60970; Santa Cruz) are CAGAAGGCCTAGACATTAA (1880–1897), GGAAGGCTCTTCTAGCAAT (2755–2772) and CAAGTGCTGTGAAATAACA (2936–2953); the first is located in the coding sequence. In the LSD1 expression plasmid, the cDNA was changed from CAGAAGGCCTAGACATTAA to CAGAgGGgCTtGAtATTAA. The point mutations were made by using the QuikChange II Site-Directed Mutagenesis Kit (Stratagene).

ChIP Analyses. For ChIP analyses, 10^8 cells were cross-linked in 1% formaldehyde for 10 min before adding 0.125 M glycine to terminate the reaction. The cells were trypsinized and resuspended in 6 mL cell lysis buffer (5 mM Pipes, pH 8.0, 85 mM KCl, 0.5% Nonidet P-40, 10 μL/mL PMSF, 1 μL/mL aprotinin, and 1 μL/mL leupeptin). After incubation on ice for 10 min, nuclei were collected and resuspended in 1 mL nuclear lysis buffer (50 mM Tris·HCl, pH 8.1, 10 mM EDTA, 1% SDS) plus protease inhibitors to obtain chromatin preparations, which were then sonicated to an average length of ≈0.5 to 2 kb by using 15 pulses of 30 s each with 2-min rests at setting 5 of a Fisher Model 60 sonic dismembranator. Sonicated samples were immunoprecipitated with the indicated antibodies. The crosslinks were then reversed in 0.3 M NaCl in the presence of RNaseA (Roche), 10 mg/mL at 65 °C for 4 to 5 h. DNA fragments were purified by Qiagen DNA purification kit. The immunoprecipitated DNA was amplified by qPCR. The primer pairs used for qPCR am-

plification were as follows: SOCS3, 5′- GCGCTCAGCCTTTCTCTG-3′ and 5′- GGAGCAGGGAGTCCAAGTC-3′; CDCA1, 5′-TTCAACTCGGACCCCGAAAAC-3′ and 5′-CAAGCCCACGTCCAACCGCG-3′; CD14, 5′-ATGAGGTTCACAATCTCTTC-3′ and 5′-GACTCGGGAGCCTAAGCCTT-3′. Quantitative PCR data for occupancy of the promoters was calculated by normalizing the PCR signals from the immunoprecipitation samples to the signals obtained from the input DNA.

1. Raja SN, Grabow TS (2002) Complex regional pain syndrome I (reflex sympathetic dystrophy). *Anesthesiology* 96:1254–1260.

2. Zhang X, et al. (2008) Epitope tagging of genomic proteins for genome-wide ChIP-chip studies. *Nat Methods* 5:163–165.

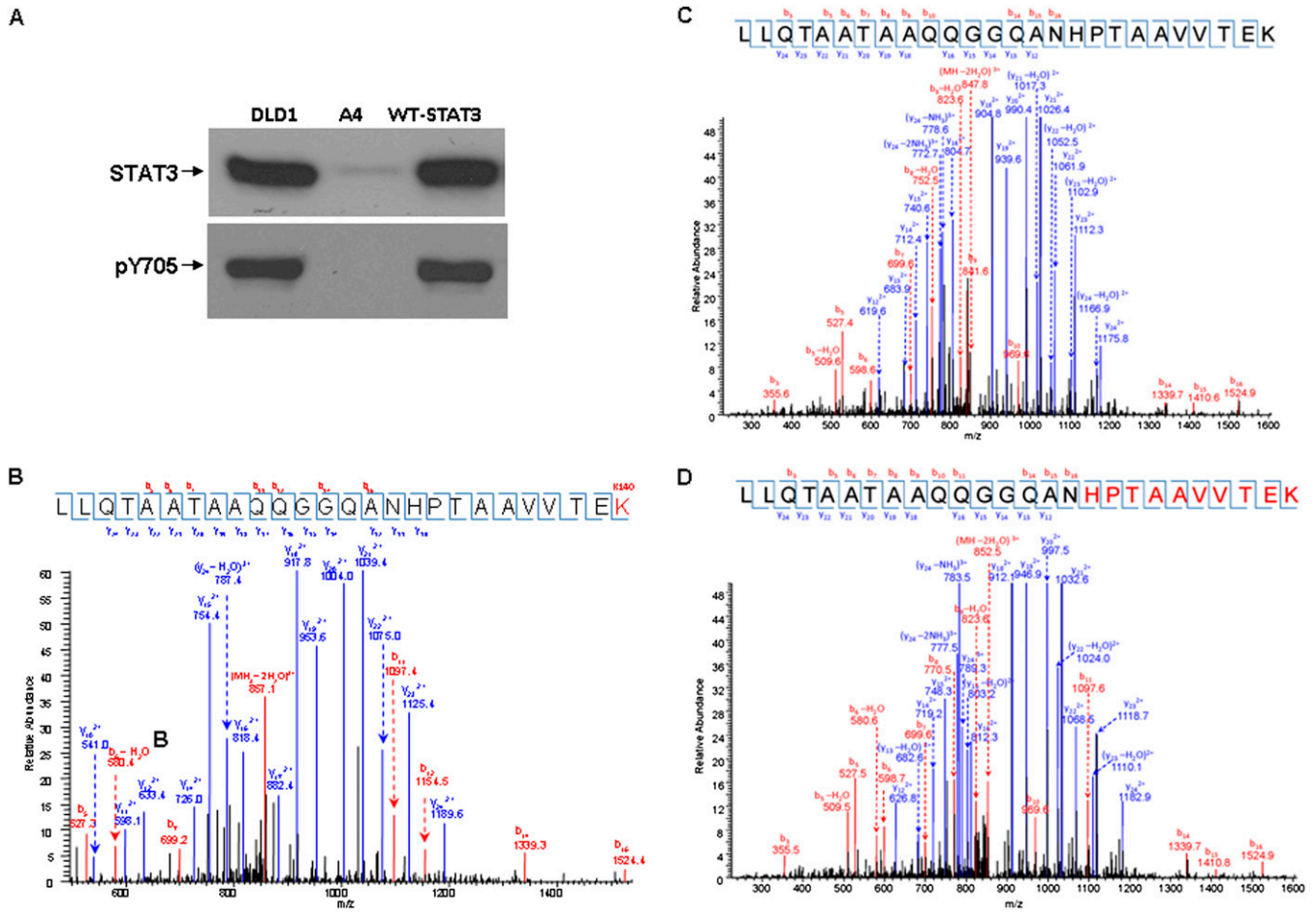

Fig. S1. MS reveals that STAT3 is methylated at K140 in response to IL-6. (*A*) Western analyses for STAT3 and Y705-phosphoryl-STAT3. STAT3-null A4 cells were infected with retroviral constructs and stable pools were selected with G418. DLD1, parental colon cancer cells; A4, STAT3 knockout cells; WT-STAT3, A4 cells expressing a normal level of wild-type STAT3. Cells were treated with IL-6 for 4 h and total cell lysates were analyzed by Western blot. (*B*) Analysis of trypsin-digested STAT3. STAT3 was immunoprecipitated from cells treated with IL-6 for 4 h, or from untreated cells. The single band of STAT3 was digested in the gel with trypsin before analysis by LC-MS/MS. The tandem mass-fragmentation spectrum represents the STAT3 peptide 115to 140 with MH+28, with triply charged precursor *m/z* 868.78 (3+) (*C*) and from monomethyl STAT3 with MH+14 (*D*), with triply charged precursor *m/z* values of 859.45 (3+) and 864.13 (3+), respectively.

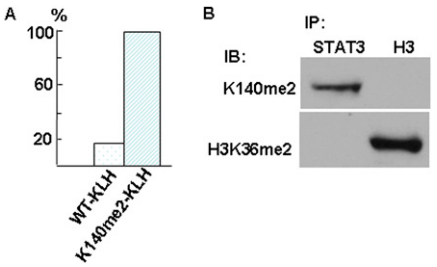

Fig. S2. Evaluation of the STAT3-K140me2 antibody. (*A*) A KLH-conjugated synthetic peptide representing amino acids 140 to 150 of STAT3, with and without dimethylation of K140, was used for ELISAs. (*B*) A4 cells expressing wild-type STAT3 were treated with IL-6 for 4 h and STAT3 and histone H3 were immunoprecipitated. The proteins were separated by SDS/PAGE and analyzed by Western blot for STAT3-K140me2 and histone3-K36me2.

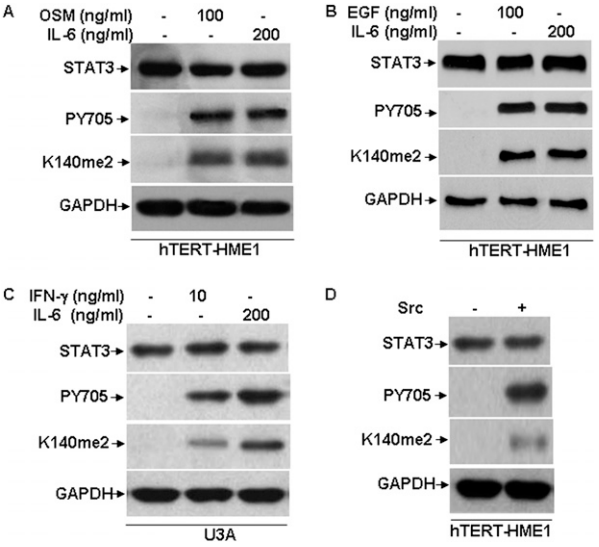

Fig. S3. Dimethylation of K140 of STAT3 in response to cytokines in different cell lines. (*A* and *B*) hTERT-HME1 or (*C*) STAT1-null U3A cells were treated with IL-6, 100 ng/mL oncostatin M (OSM), or 10 ng/mL IFN-γ for 4 h and total cell lysates were analyzed by Western blot. (*D*) hTERT-HME1 cells were transfected transiently with a pcDNA3.1-SRC construct and, 48 h later, total cell lysates were prepared and analyzed by Western blot.

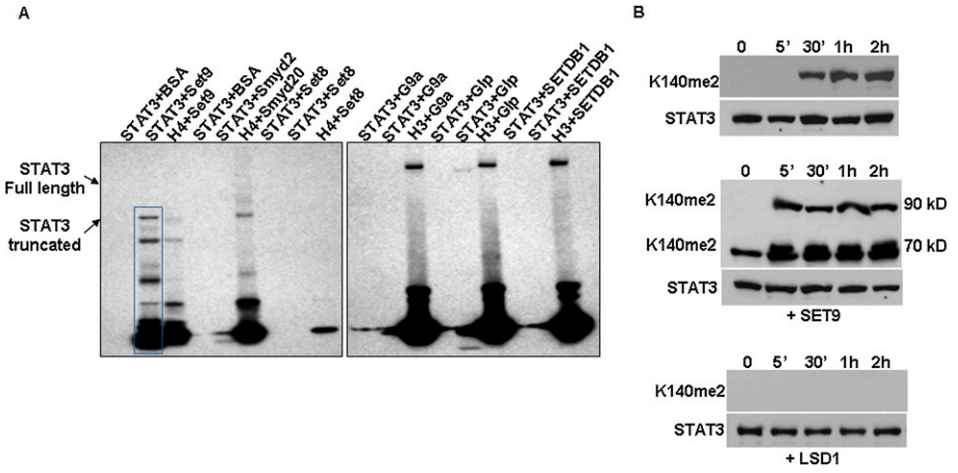

Fig. S4. In vitro STAT3 methylation and demethylation assays. (*A*) Flag-tagged human STAT3 was expressed in *E. coli* and purified by affinity chromatography. For each assay, about 0.1 μg of purified STAT3 was subjected to modification by the purified histone methyl transferases SET9, SMYD2, SET8, G9a, GLP, or SETDB1. Reaction mixtures were separated by SDS/PAGE and analyzed by fluorography. (*B*) (*Top*) A4 cells expressing wild-type STAT3 were treated with IL-6 as indicated and STAT3 was immunoprecipitated from whole-cell extracts by using anti-Flag M2 beads. Western analyses were performed to detect STAT3-K140me2 and total STAT3. (*Middle*) STAT3 was immunoprecipitated from whole-cell extracts by using anti-Flag M2 beads. STAT3 was then methylated by purified SET9 in vitro. Reaction mixtures were separated by SDS/PAGE and analyzed by Western blot. (*Bottom*) STAT3 was immunoprecipitated from whole-cell extracts by using anti-Flag M2 beads. STAT3 was then demethylated by purified LSD1 in vitro.

Table S1. Comparison of mRNAs induced by IL-6 in cells expressing wild-type or K140R STAT3

$\frac{KR4/KR0}{WT4/WT0}$ Fold change	$\frac{KR24/KR0}{WT24/WT0}$ Fold change	Gene
11.55	1.3	*FGF21*
9.93	2.37	*CDKN2A*
9.02	4.06	*KIAA1543*
6.51	2.54	*CHID1*
5.98	5.52	*TMEM184A*
5.88	7.17	*LPIN3*
5.61	0.17	*TAS2R38*
5.28	3.93	*PTPRE*
4.78	2.11	*SLC6A18*
4.78	2.87	*UNC13B*
4.55	3.24	*MFNG*
4.48	0.67	*PH-4*
4.3	1.42	*AMPH*
4.29	3.07	*TRIM38*
4.28	3.69	*SH3TC1*
4.17	1.38	*PCYOX1L*
4.05	1.63	*CES2*
3.91	2.59	*MYO3B*
3.68	1.74	*TAF6*
3.59	1.68	*BCL7A*
3.55	1.49	*RNPEPL1*
3.49	3.42	*MGC4677*
3.44	5.26	*SLC26A6*
3.44	2.4	*C14orf119*
3.43	1.21	*C9orf61*
3.41	2.95	*SPATA13*
3.36	2.24	*C7orf29*
3.34	1.64	*MAFF*
3.32	2.82	*ZNF174*
3.28	4.62	*NLRC5*
3.25	3.31	*PLD2*
3.25	5.11	*EN2*
3.24	6.15	*BAT1*
3.2	2.09	*TBC1D1OB*
3.17	1.95	*FAM26B*
3.06	1.94	*BCR*
2.84	1.3	*PRRT1*
2.84	2.52	*MYST2*
2.81	2.14	*VISA*
2.79	1.26	*S0CS3*
2.73	0.96	*TMEM38B*
2.69	2.81	*KIAA1161*
2.67	1.46	*ANXA11*
2.67	1.87	*ARFIP2*
2.65	1.56	*IFNAR2*
2.64	3.99	*MUTYH*
2.62	1.44	*AKR1A1*
2.58	3.04	*RPA4*
2.53	1.6	*HTR3A*
2.52	6.46	*ARHGEF1*
2.52	1.75	*ROR1*
2.49	2.2	*GSDML*
2.45	1.24	*IRF8*
2.4	1.11	*SLC15A3*
2.39	2.26	*IRF9*
2.39	4.31	*UBASH3A*
2.37	1.4	*CLMN*
2.36	0.76	*DSCAM*
2.35	0.85	*SFXN3*
2.34	0.82	*INHBE*
2.32	1.7	*PITPNC1*

Table S1. Cont.

$\frac{KR4/KR0}{WT4/WT0}$ Fold change	$\frac{KR24/KR0}{WT24/WT0}$ Fold change	Gene
2.3	0.78	*CNNM2*
2.25	1.71	*LAMC2*
1.81	1.33	*SAA1*
1.7	1.69	*IGFBP3*
1.27	1.28	*FOS*
1.2	1.37	*SAA1*
1.19	1.19	*IGFBP3*
1.15	2.34	*IL15RA*
1.15	1.18	*LIF*
1.03	1.01	*GCNT3*
1.02	1.15	*ID1*
1	1.04	*CDC42EP2*
1	0.83	*SAA2*
0.95	1.21	*CD14*
0.95	1.14	*CXCL1*
0.94	1.17	*CEBPD*
0.93	0.99	*TAP1*
0.93	0.8	*PPAP2B*
0.93	1	*BCL3*
0.9	1.26	*STAT3*
0.9	0.88	*STAT1*
0.88	1.13	*IER3*
0.81	0.96	*CXCL2*
0.82	0.86	*CTRL*
0.79	0.87	*SAA4*
0.77	0.86	*BCL6*
0.76	0.69	*TRIM10*
0.75	0.74	*JUNB*
0.75	0.89	*NOTCH1*
0.75	0.79	*TRIM15*
0.64	1.06	*CCDC71*
0.64	0.78	*IRF1*
0.58	0.47	*TEAD3*
0.5	0.97	*USP47*
0.5	0.71	*STARD4*
0.5	0.58	*TMPO1*
0.49	0.64	*TRUB1*
0.49	0.84	*JMJD1C*
0.49	0.86	*LOC492311*
0.48	0.46	*CDC42EP3*
0.48	0.5	*CCBL2*
0.47	1.07	*MVK*
0.47	0.68	*PFAAP5*
0.47	0.77	*ERP27*
0.46	1.01	*RALGPS1*
0.45	0.58	*HSPD1*
0.44	0.56	*ADAM9*
0.43	0.46	*MEST*
0.43	0.62	*HSF1*
0.43	0.71	*SMAD5*
0.42	0.75	*ARID2*
0.42	0.53	*MCM7*
0.41	0.64	*USP33*
0.41	0.69	*PDE5A*
0.4	0.55	*ROCK1*
0.4	0.41	*SPTBN1*
0.4	0.61	*NOMO3*
0.39	0.7	*TP53BP2*
0.38	0.33	*STEAP4*
0.38	0.5	*GRAMD4*
0.35	0.69	*ACBD5*

Table S1. Cont.

$\frac{KR4/KR0}{WT4/WT0}$ Fold change	$\frac{KR24/KR0}{WT24/WT0}$ Fold change	Gene
0.35	0.78	*QPRT*
0.33	0.41	*CASC4*
0.33	0.57	*DDX17*
0.32	0.3	*COL15A1*
0.31	0.48	*TRIM16L*
0.29	0.52	*C10orf46*