Supporting Information

Dasgupta et al. 10.1073/pnas.1503152112

SI Materials and Methods

Reagents. Human IL-6, soluble IL-6Rα, and OSM were from PeproTech. Antibodies against STAT3, tyrosine-phosphorylated STAT3, and EZH2 were from Cell Signaling Technology. Anti-GAPDH was from Santa Cruz, and anti–β-actin was from Sigma.

Constructs and Viral Transduction. pLEGFP-N1 retroviral plasmids (Clontech) harboring the K49R, K49A, K49Q, K140R, K685R, and S727A mutants of STAT3 were generated from WT-STAT3 by using the QuikChange II XL site-directed mutagenesis kit (Stratagene). To obtain infectious retroviral stocks, each construct was transfected into 293T cells along with packaging plasmid, using the Lipofectamine and Plus reagents (Invitrogen). Stable pools of each mutant cell line were obtained by infecting A4 cells with the virus collected 48 h later, followed by selection in neomycin G418 at 500 μg/mL.

Western Analyses. Cells were cultured to 80–90% confluency before treatment. The cell pellets were collected and processed as described previously (1) to obtain whole-cell lysates. Protein (20–30 μg) was loaded onto 10% SDS/PAGE gels. The separated proteins then were transferred to PVDF membranes (Millipore). After transfer the membranes were incubated overnight at 4 °C with primary antibody in 5% milk or BSA and with secondary antibody in milk for 1 h at room temperature. The membranes were developed with the Pierce ECL reagent.

Virus Production and Cell Infection to Establish Stably Transduced EZH2 Knockdown Cell Pools. To generate infectious lentivirus stocks for targeting EZH2, a lentiviral shEZH2 expression vector corresponding to the sequence GAAACAGCTGCCTTAGCTTCA was obtained from Sigma. The specificity of the shRNA clone used for targeting EZH2 has been verified and published earlier (2). A nontargeted shControl also was obtained from Sigma. 293T cells were transfected with shEZH2 or shControl, along with the packaging constructs pCMV-dR8.74 and pMD2G. Supernatant medium containing virus collected at 36–48 h was filtered and supplemented with 4 μg/mL Polybrene (Sigma-Aldrich) before being added to A4-WT-STAT3 cells that were at 50% confluency. The medium was replaced 24 h after infection, and the cells were subjected to puromycin selection at 3 μg/mL for another 24 h.

Gene-Expression Analysis. Total RNA was isolated by using the Qiagen RNeasy mini kit, according to the manufacturer's protocol. This RNA (1 μg) was used for microarray analysis on an Illumina Human Ht-12 V4 Expression BeadChip Kit. Data were analyzed by using the Illumina GenomeStudio software and normalized by the quantile method. Genes were selected according to the criteria mentioned in the respective figure legends. Heat maps were generated using the Cluster 3.0 and Treeview JavaScript software, based on $\log_2$-transformed fold changes with respect to the vector control, and were clustered with the Euclidean distance metric and average linkage method. The BioVenn web application was used to generate Venn diagrams (3).

Coimmunoprecipitations. Cells were cultured in 10-cm plates to 80–90% confluency and were treated with IL-6 or were left untreated. The cells then were lysed in coimmunoprecipitation buffer [1% Triton X-100, 50 mM Tris·HCl (pH 7.4), 150 mM NaCl]. The EZview Red ANTI-FLAG M2 Affinity Gel system (Sigma-Aldrich) was used for immunoprecipitation, following the protocol provided by Sigma-Aldrich. The gel beads were resuspended in an equal volume of 1× SDS sample loading buffer and were boiled for 5 min. The beads then were pelleted by centrifugation, and the supernatant solution was analyzed by immunoblotting.

Protein Digestion and LC-MS/MS. Flag-tagged WT-STAT3 was immunoprecipitated from A4 cells expressing normal levels of this protein, treated or not with IL-6, by using EZ-FLAG beads and EZview Red ANTI-FLAG M2 Affinity Gel (Sigma), according to the manufacturer's protocol. The immunoprecipitated Flag-STAT3 was separated on an SDS/PAGE gel and stained with Coomassie blue. The bands were cut from the gel with a punch and were washed/destained in 50% ethanol, 5% acetic acid. The gel pieces then were dehydrated in acetonitrile, dried in a SpeedVac (ThermoScientific), and digested by adding 5 μL of 10 ng/μL trypsin in 50 mM ammonium bicarbonate, with overnight incubation at room temperature. The peptides were extracted from the polyacrylamide in two 30-μL aliquots of 50% acetonitrile with 5% formic acid, were combined and concentrated to less than 10 μL in a SpeedVac, and then were resuspended in 1% acetic acid to a final volume of ∼30 μL. The LC-MS system was a Finnigan LTQ-Obitrap Elite hybrid mass spectrometer. The HPLC column was a Dionex 15 cm × 75 μm i.d. Acclaim Pepmap C18 2-μm, 100-Å reversed-phase capillary chromatography column. Five-microliter volumes of the extract were injected, and the peptides were eluted from the column in an acetonitrile/0.1% formic acid gradient at a flow rate of 0.25 μL/min. The samples were introduced into the source of the mass spectrometer on line. The nano-electrospray ion source was operated at 2.5 kV. The digest was analyzed using the data-dependent multitask capability of the instrument, acquiring full-scan mass spectra to determine peptide molecular weights and product ion spectra to determine amino acid sequence in successive instrument scans. The data were analyzed by using all collision-induced dissociation spectra collected in the experiment to search the human Reference Sequence database with the search program Mascot and more specifically against STAT3 with the program Sequest. All spectra derived from modified peptides were verified by manual interpretation. The relative abundances of the modified peptides were determined by performing a targeted analysis of specific STAT3 peptides including the modified and unmodified forms of the S727, Y705, and K49 tryptic peptides. The chromatograms for these peptides were plotted, based on known fragmentation patterns, and the peak areas of the chromatograms were used to determine the extent of modification.

Preparation and Purification of K49 Dimethyl Stat3 Polyclonal Antibody. A rabbit polyclonal antibody against a dimethylated K49 peptide was made in the Cleveland Clinic Hybridoma Core. The modified peptide ASK(49me2)ESHAC was conjugated to keyhole limpet hemocyanin, which then was injected into a rabbit four times. Terminal bleed serum was used for all experiments. The antibody was purified by using an ASK(49me2)ESHAC affinity column. Remaining antibody recognizing native STAT3 was removed by applying the affinity-purified K49me2 polyclonal antibody to a second affinity column coupled to the unmodified peptide ASKESHAC through seven cycles. The specificity of the polyclonal antibody against K49me2 STAT3 was evaluated by ELISA and Western analyses (Fig. S1), showing that the antibody binds to the peptide used for immunization but not to methylation-free STAT3 synthesized in *Escherichia coli*.

PCR and Real-Time qPCR. cDNA samples were prepared by reverse PCR from total RNAs by using the SuperScript III First-Strand Synthesis System (Invitrogen), using the random hexamer protocol. For experiments, samples either were amplified according to standard PCR procedures or were analyzed by using FastStart Universal SYBR Green Master (ROX) (Roche Diagnostics) qPCR reactions. Primers either were obtained from pga.mgh.harvard.edu/primerbank/ or were designed using AlleleID 6 software.

Primers Used for Real-Time qPCR. The following primers were used for real-time qPCR:

SOCS3: Sense 5′-GAATGTCAGCCCAGTAAGTATTG-3′;

Antisense 5′-CAGCAGTCCAGCCTCTCC-3′

SERPINA1: Sense 5′-CAAGGACACCGAGGAAGAG-3′;

Antisense 5′-TCAGGCAGGAAGAAGATGG-3′

GADD45G: Sense 5′-ATCGCACTATGACTCTGGAAG-3′;

Antisense 5′-ACTTTGGCTGACTCGTAGAC-3′

BCL3: Sense 5′-CGCCTCTCCATATTGCTGTG-3′;

Antisense 5′-GTCTGCCGTAGGTTGTTGTAG-3′

ChIP. Thirty million A4-WTSTAT3 cells stably transduced with nontargeted shRNA or shEZH2 were treated with 50 ng/mL IL-6 and 62.5 ng/mL IL-6R for 30 min or were left untreated. The cells then were crosslinked with 1% formaldehyde for 10 min at room temperature, followed by quenching for 5 min with 125 mM glycine. After cell disruption in lysis buffer (50 mM Hepes, 140 mM NaCl, 1 mM EDTA, 10% glycerol, 0.5% IPEGAL (Sigma-Aldrich), 0.25% Triton X-100), the nuclear pellets were resuspended in 1 mL shearing buffer (1% SDS, 10 mM Tris·HCl, 1 mM EDTA, 0.5 mM EGTA, 0.1% sodium deoxycholate), and the chromatin was sheared with a Misonix 3000 probe sonicator (20 cycles of 30 s each at power setting 3 with 90 s rest between each cycle). The samples then were diluted 10× for overnight immunoprecipitation at 4 °C with 5 μg rabbit IgG (sc-2027; Santa Cruz) or STAT3 antibody (sc-482 X; Santa Cruz) that was preincubated with 25 μL protein A Dynabeads (Life Technologies) for 6 h. A 1% portion of each sample was saved as input. After one washing with low-salt buffer (150 mM NaCl, 0.1% SDS, 1% Triton X-100, 20 mM Tris·HCl, 2 mM EDTA), one washing with high-salt buffer (500 mM NaCl, 0.1% SDS, 1% Triton X-100, 20 mM Tris·HCl, 2 mM EDTA), one washing with LiCl buffer (0.25 M LiCl, 1% IPEGAL, 1% sodium deoxycholate, 1 mM EDTA, 10 mM Tris·HCl), and two washings with Tris-EDTA buffer, the immunoprecipitated protein/DNA was eluted from the beads at 65 °C for 30 min with agitation in elution buffer (50 mM Tris·HCl, 10 mM EDTA, 1% SDS), and crosslinking was reversed at 65 °C for 6 h, followed by 30 min of treatment with RNase A and 1 h of treatment with proteinase K. The immunoprecipitated chromatin was purified by phenol-chloroform extraction. Two microliters of DNA from each sample was used for qPCR reactions, using primers for the human SOCS3 gene promoter (forward: 5′-AGCCTTTCTCTGCTGCGAGT-3′; reverse 5′-CCCGATTCCTGGAACTGC-3′), or negative control primers. The cycling conditions were 95 °C for 15 s, 54 °C for 30 s, and 72 °C for 30 s for a total of 40 cycles in the LightCycler 480 system (Roche).

1. Yang J, et al. (2005) Novel roles of unphosphorylated STAT3 in oncogenesis and transcriptional regulation. *Cancer Res* 65(3):939–947.
2. Yu YL, et al. (2013) Smurf2-mediated degradation of EZH2 enhances neuron differentiation and improves functional recovery after ischaemic stroke. *EMBO Mol Med* 5(4):531–547.
3. Hulsen T, de Vlieg J, Alkema W (2008) BioVenn - a web application for the comparison and visualization of biological lists using area-proportional Venn diagrams. *BMC Genomics* 9:488.

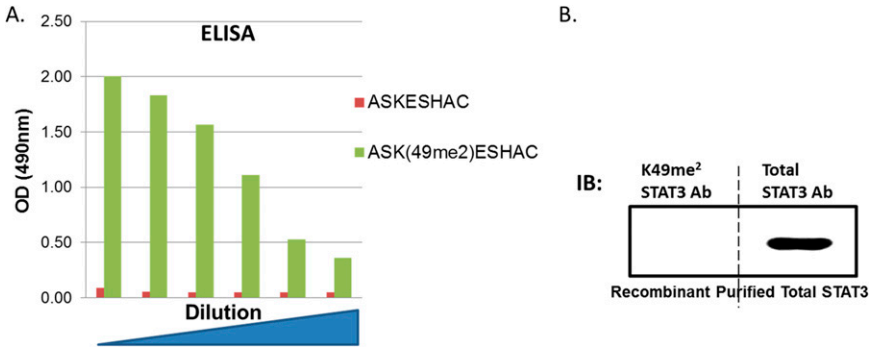

Fig. S1. Evaluation of the STAT3-K49me2 antibody. (*A*) KLH-conjugated synthetic peptides $ASK_{49}ESHAC$, with and without dimethylation of K49, were used for ELISAs. (*B*) Recombinant unmodified STAT3, purified from *E. coli*, was tested for its reactivity toward the polyclonal K49me2 antibody by Western analysis.

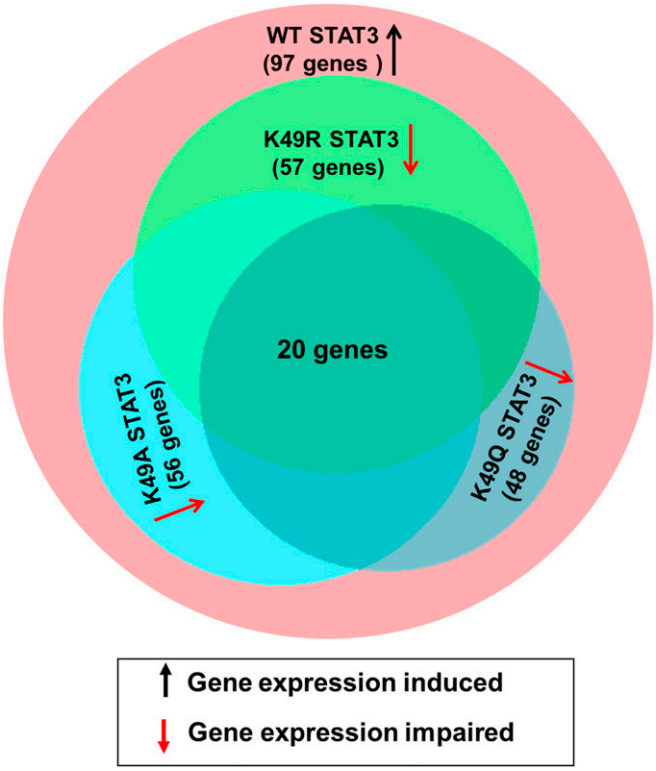

Fig. S2. Of the 97 STAT3-dependent IL-6–induced genes, the expression of 57 genes (for K49R), 56 genes (for K49A), and 48 genes (for K49Q) was impaired on K49 mutation. The expression of 20 genes was impaired by all three K49 mutations.

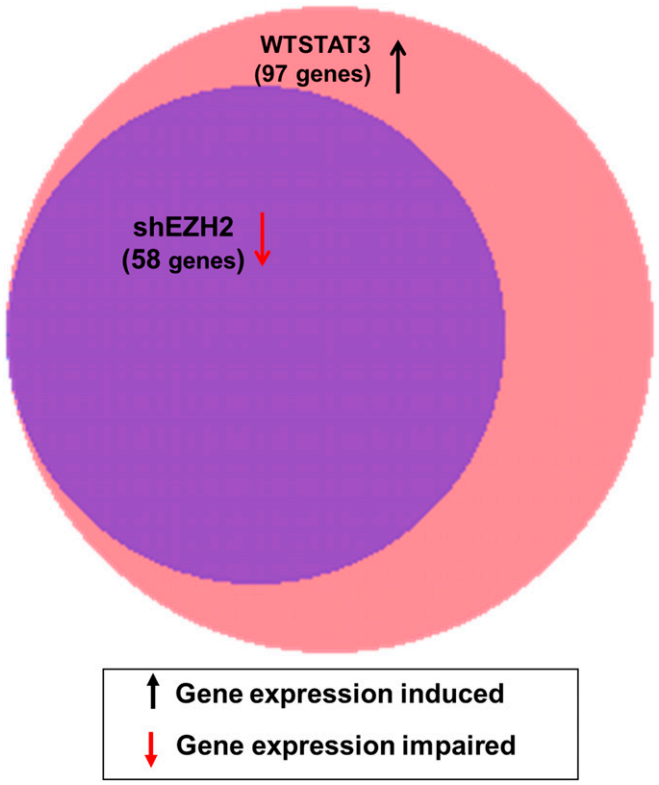

Fig. S3. EZH2 knockdown resulted in impaired expression of 58 of the 97 STAT3-dependent IL-6–induced genes.

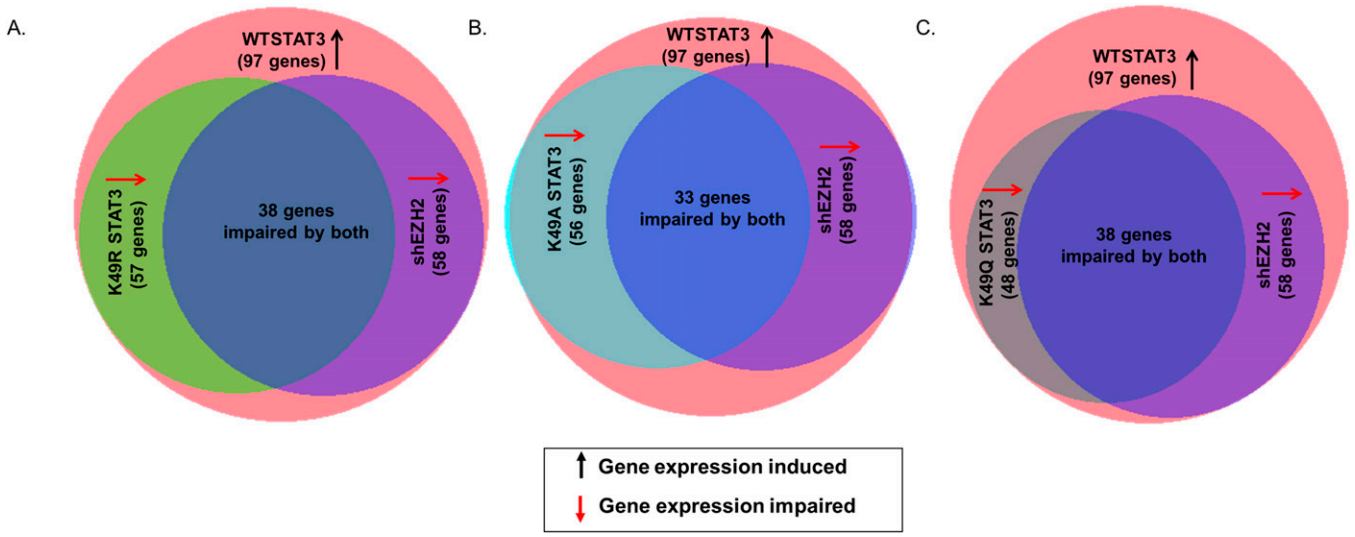

Fig. S4. Venn diagram showing that 38, 33, and 38 of the 58 genes that were impaired in WT-STAT3 EZH2 knockdown cells also were impaired by STAT3 K49 mutation to R (*A*), A (*B*), and Q (*C*).

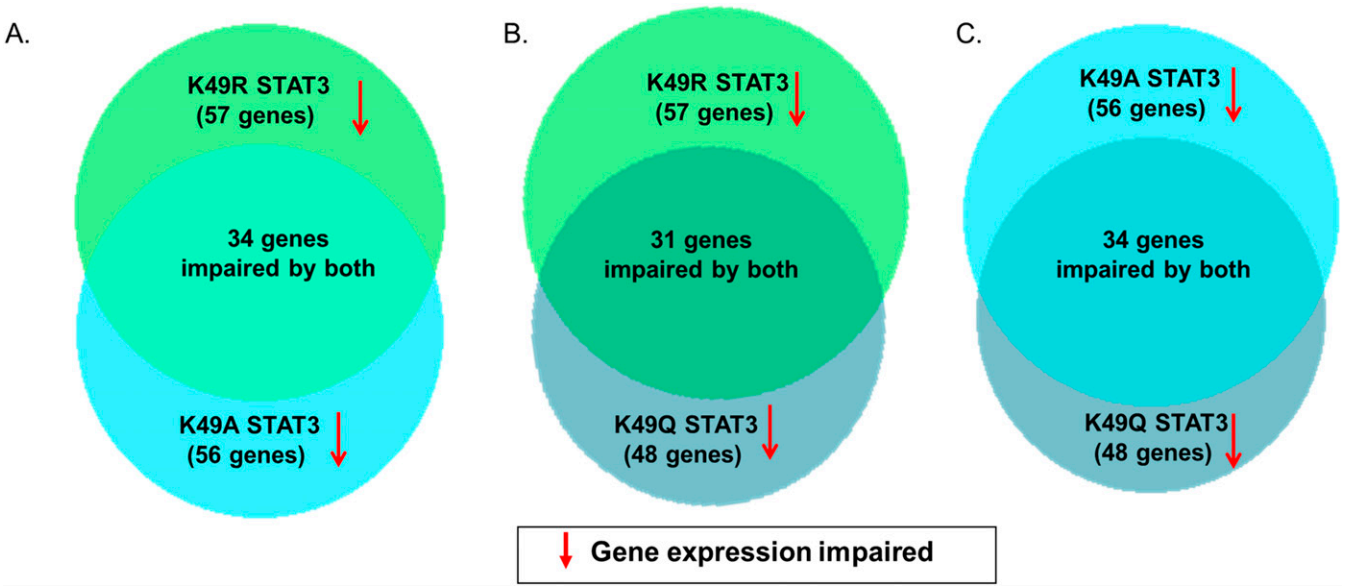

Fig. S5. (*A*) Thirty-four of the 57 genes whose expression was impaired by the K49R mutation overlapped with 34 of the 56 genes impaired by the K49A mutation. (*B*) Thirty-one of the 57 genes whose expression was impaired by the K49R mutation overlapped with 31 of the 48 genes impaired by the K49Q mutation. (*C*) Thirty-four of the 56 genes whose expression was impaired by the K49A mutation overlapped with 34 of the 48 genes impaired by the K49Q mutation.

Table S1. Complete list of genes induced twofold or more in A4-WT-STAT3 cells compared with untreated A4-WT-STAT3 cells and the A4-Vector control cells treated with IL-6

WT^+/WT^- fold change	Gene	WT^+/V^+ fold change	$WT^+/K49R^+$ fold change	$WT^+/K140R^+$ fold change	$WT^+/K685R^+$ fold change	$WT^+/S727A^+$ fold change
10.5	*PDGFD*	13.45	5.11	2.28	6.74	1.62
4.95	*GADD45G*	8.66	5.20	1.15	1.17	2.68
13.97	*SERPINA1*	8.36	9.85	1.39	1.58	2.49
4.18	*BCL3*	7.7	2.98	0.98	1.02	2.69
4.63	*SNCAIP*	7.18	6.76	1.60	1.66	2.00
2.05	*C22orf30*	4.94	3.73	3.90	4.99	1.58
2.59	*TTLL1*	4.68	2.51	2.49	3.74	1.56
2.04	*LOC648374*	4.05	4.75	3.42	2.80	2.95
2.2	*CXCL5*	3.92	1.75	1.33	1.13	1.27
2.42	*HEXIM1*	3.88	2.76	1.30	2.13	4.01
21.2	*MIR635*	3.67	9.12	2.14	1.81	1.68
9.45	*LYPD1*	3.5	3.23	1.14	1.03	1.45
2.33	*PPP2R3A*	3.34	1.26	4.08	2.19	2.10
5.56	*PCDHGB6*	3.3	3.12	2.83	14.65	2.93
10.05	*FAM135A*	3.15	1.67	0.88	1.04	1.60
2.43	*AGPAT3*	3.12	3.02	1.85	5.58	3.90
8.2	*ROR1*	3.12	2.45	4.24	3.08	3.95
9	*PCDH7*	3.06	1.10	0.89	1.13	1.50
27.39	*ARSB*	3.03	3.17	3.92	1.29	1.76
2.86	*LRG1*	3.03	1.09	1.09	1.49	1.84
2.1	*TEAD4*	2.82	1.73	0.99	1.24	1.58
4.54	*LOC647955*	2.81	3.44	2.17	2.56	3.17
2.86	*CSN1S1*	2.77	2.96	6.04	2.79	2.00
8.08	*ZNF160*	2.75	2.02	0.91	4.68	2.61
3.3	*TRIB2*	2.74	1.41	1.31	1.19	1.87
2.71	*LOC649661*	2.71	2.63	2.20	2.11	2.45
3.73	*LOC651404*	2.64	1.46	2.22	−75.00	4.23
2.02	*BHLHB2*	2.59	1.22	0.91	1.02	1.80
2.62	*EMP3*	2.51	1.70	1.13	2.50	2.00
2.19	*GCNT3*	2.51	1.02	1.70	0.89	3.16
2.24	*MGC3032*	2.46	0.99	1.17	1.45	1.36
4.25	*TRIM15*	2.44	1.51	0.83	1.05	1.43
4.08	*OR4K15*	2.43	7.02	2.19	1.36	1.73
4.44	*GPR37*	2.42	1.18	0.83	1.19	1.66
2	*LOC100131017*	2.42	2.60	1.08	1.34	2.40
2.08	*LOC642082*	2.41	1.14	1.26	0.98	1.63
2.12	*LOC390483*	2.39	2.23	2.61	2.67	1.69
2.85	*LOC644284*	2.35	1.52	1.18	1.88	1.58
2.86	*LOC653375*	2.33	2.47	1.86	1.33	1.04
15.49	*LOC100129907*	2.3	1.71	1.07	2.08	1.55
2.53	*NT5M*	2.27	36.22	2.36	1.37	1.44
4.54	*SFN*	2.25	2.04	1.09	1.07	1.59
4.75	*LOC649445*	2.23	5.09	2.75	2.33	2.00
2.01	*UBE2J2*	2.17	1.59	1.25	1.54	1.26
2.54	*HOXA2*	2.16	1.86	1.06	1.74	1.59
2.11	*FCF1*	2.13	1.45	1.58	1.72	1.29
2.11	*CHI3L1*	2.12	3.66	1.60	1.00	1.19
3.21	*TBC1D2*	2.1	1.81	1.06	1.00	1.54
2.17	*FUT2*	2.09	3.26	2.74	1.49	2.25
3.03	*LARGE*	2.08	2.00	1.28	2.08	3.47
2.84	*LOC729774*	2.06	2.17	1.39	1.55	1.41
3.41	*S100A9*	2.05	6.24	2.00	1.40	1.82
2.84	*RABL2A*	2.04	1.29	1.19	0.81	0.80
2.19	*TACSTD2*	2.04	1.33	1.22	1.01	1.76
4.62	*LOC283392*	2.03	2.60	1.19	1.59	1.54
2.65	*PTPRE*	2.02	1.14	0.96	0.89	1.35
2.06	*STOM*	2.02	1.73	1.05	1.03	2.00
2.87	*TANC1*	2.02	2.05	1.26	1.35	2.00
2.47	*CWC22*	2.01	1.45	1.10	1.41	1.06

The shaded values indicate the fold changes (≥2) by which the expressions of corresponding genes are impaired in the A4-K49R, A4-K140A, A4-K685Q, or A4-S727A STAT3-expressing cells as compared with the A4-WT STAT3-expressing cells on IL-6 treatment.

Table S2. Cross-comparison of the numbers of IL-6–induced, STAT3-dependent genes whose expression is inhibited twofold or more by each of the three K49 mutations or in response to EZH2 knockdown

Mutation				No. of genes impaired
K49R	K49A	K49Q	shEZH2	
+				57
	+			56
		+		48
			+	58
+			+	37
	+		+	33
		+	+	38
+	+			34
	+	+		31
+		+		34
+	+	+		20
+	+	+	+	15

Table S3. Complete list of genes induced twofold or more in A4-WT-STAT3 cells compared with A4-WT-STAT3 untreated cells and the A4-Vector control cells treated with IL-6

WT^+/WT^- fold change	Genes	WT^+/V^+ fold change	$WT^+/K49R^+$ fold change	$WT^+/K49A^+$ fold change	$WT^+/K49Q^+$ fold change	$WT^+/WT\text{-}shEZH2^+$ fold change
3.44	*TRIM15*	427.10	3.69	2.37	2.20	2.79
3.7	*LOC643684*	113.10	18.93	4.87	−28.15	8.79
4.6	*LOC731742*	100.77	2.87	2.58	1.47	2.18
11.25	*GPR65*	64.10	160.10	2.81	−6.71	2.37
3.44	*LOC554207*	51.48	1.58	3.14	2.29	8.32
2.74	*IFI16*	44.02	−95.07	3.42	4.30	24.93
8.6	*PRSS7*	35.65	2.24	2.11	3.20	3.11
2.73	*CCDC88B*	18.75	2.32	−4.24	−9.22	2.50
5.56	*WDR88*	8.97	7.19	−12.03	9.32	10.82
21.98	*PAH*	8.59	7.00	1.89	9.84	36.88
11.67	*UBE2V1*	8.30	15.83	10.68	20.89	26.55
2.98	*MBNL3*	7.74	2.45	5.09	2.37	4.05
2.49	*PLD3*	7.35	−23.03	−5.59	−3.68	−20.46
6.3	*ATOH7*	6.43	2.58	2.55	1.40	−5.77
2.95	*ABCA9*	6.33	1.73	1.74	1.99	1.41
4.17	*SPIB*	6.16	4.59	5.35	3.62	12.56
14.92	*LOC643395*	6.06	60.53	−9.51	1.57	−28.10
3.43	*STEAP4*	5.88	4.03	2.83	0.95	0.77
10.84	*MUC1*	5.74	1.22	1.11	1.36	1.09
6.23	*COL11A1*	5.38	8.24	3.91	−100.23	−150.40
4.08	*LOC653352*	5.37	2.10	1.74	2.77	4.29
4.66	*LOC644424*	5.28	0.81	1.75	1.95	1.52
2.37	*STXBP1*	5.04	0.97	15.14	2.12	4.27
2.5	*PCDH7*	4.63	1.05	1.48	1.04	0.83
198.4	*LOC727894*	4.52	15.35	−7.72	−15.95	2.26
2.34	*IRF5*	4.45	2.25	1.66	1.56	34.50
2.56	*NEFL*	4.41	1.42	16.43	2.33	4.32
24.38	*LOC100130134*	4.34	−125.57	−2.69	−4.23	3.50
3.36	*METRN*	4.29	2.96	−35.26	3.51	20.73
2.88	*LOC727937*	4.27	1.93	1.88	1.29	1.83
4.65	*DYRK1B*	4.20	8.09	4.36	20.07	11.12
7.95	*LOC100131436*	4.17	1.49	3.47	350.10	18.52
2.36	*TMOD2*	4.15	6.06	5.25	9.60	1.51
4.08	*LOC728832*	4.13	−4.91	2.13	3.41	2.89
28.74	*RSAD2*	4.07	1.24	2.51	2.59	3.64
6.82	*LOC729885*	4.06	2.20	2.08	2.09	13.31
40.97	*TMOD3*	3.94	3.25	4.08	2.09	3.43
2.66	*KIAA1704*	3.94	5.53	−8.65	−22.40	45.10
7.39	*C17orf81*	3.87	2.07	2.27	2.20	3.59
2.09	*LOC100131733*	3.87	−29.72	1.90	0.95	2.64
3.53	*FLJ35880*	3.84	0.74	2.72	1.11	1.18
2.18	*OSCAR*	3.77	2.05	5.03	2.65	1.94
2.49	*LRRIQ4*	3.73	3.25	2.90	1.48	1.10
5.22	*LOC653748*	3.49	6.73	−2.40	−10.79	17.04
3.93	*LOC730883*	3.28	1.79	2.62	3.50	3.42
2.58	*RETNLB*	3.27	1.46	3.31	2.98	1.90
2.3	*ARF1*	3.27	2.97	1.39	1.10	3.24
3.07	*LOC653590*	3.25	1.72	2.33	3.15	3.47
3.25	*LOC100129395*	3.22	3.27	1.49	30.33	3.46
2.34	*LOC100134444*	3.06	3.28	1.87	16.30	2.58
4.06	*LCN1*	3.00	2.60	1.96	4.07	2.23
2.59	*KCNMB2*	2.98	1.27	4.34	2.87	1.70
2.99	*SYT12*	2.96	2.93	3.09	1.42	3.11
3.4	*CEACAM1*	2.95	3.21	0.76	0.61	0.86
3.92	*LOC100129138*	2.91	5.69	4.46	1.67	2.30
7.04	*FLJ32063*	2.90	1.11	2.14	1.05	1.08
3.21	*GJB3*	2.89	11.37	56.43	4.12	3.38
2.58	*LOC642962*	2.89	1.96	3.73	3.27	3.32
2.32	*LOC728312*	2.88	6.29	3.06	3.32	8.69
2.05	*VWA5A*	2.84	1.38	2.05	1.37	1.70
2.02	*DCP1B*	2.82	2.02	3.73	6.18	3.05

Table S3. Cont.

WT^+/WT^- fold change	Genes	WT^+/V^+ fold change	$WT^+/K49R^+$ fold change	$WT^+/K49A^+$ fold change	$WT^+/K49Q^+$ fold change	$WT^+/WT\text{-}shEZH2^+$ fold change
2.34	*ARL6IP4*	2.80	1.17	1.85	2.77	2.37
2.29	*MAP1D*	2.77	1.49	1.45	1.49	2.69
2.19	*FAIM3*	2.74	2.67	5.36	1.67	1.13
2.21	*PTPN20*	2.73	1.26	2.44	2.30	−5.04
2.25	*ODF4*	2.72	4.50	26.52	3.51	2.43
8.54	*LOC389517*	2.63	3.92	3.39	−36.79	3.59
2.01	*PMS2L1*	2.63	1.67	2.80	1.28	1.44
2.06	*ODF2L*	2.63	1.66	1.24	1.72	2.57
2.6	*SPINK1*	2.61	2.08	1.94	1.03	0.70
2.52	*COLEC11*	2.60	2.17	1.10	1.57	9.04
2.57	*LOC642859*	2.59	10.10	2.34	−5.50	1.38
2.12	*RNASE10*	2.56	3.24	1.83	2.68	17.47
2.42	*LOC645558*	2.51	2.65	3.25	1.85	1.03
2.52	*RGMA*	2.46	1.88	1.67	4.25	2.01
3.12	*FAM127A*	2.40	1.57	4.41	1.17	5.82
2.01	*MON1A*	2.36	1.21	1.55	2.01	3.64
2.58	*LOC442057*	2.35	1.81	5.38	1.79	1.86
3.41	*PTPRT*	2.32	5.00	2.04	2.32	1.51
2.93	*HTR1E*	2.30	2.35	7.19	2.17	2.28
2.79	*STAT3*	2.28	1.72	1.34	1.10	1.09
2.17	*CIZ1*	2.28	1.27	9.51	1.71	13.43
2.2	*CRYGS*	2.25	1.03	1.25	1.15	1.35
2.14	*C9orf72*	2.25	2.63	2.03	1.37	1.47
3.23	*ARHGEF10L*	2.25	1.78	5.42	3.14	1.55
4.05	*SLC7A3*	2.23	1.86	1.66	1.20	1.10
2.28	*OR4M2*	2.20	2.05	1.68	1.78	1.62
2.33	*RLN2*	2.18	2.23	1.94	2.04	15.69
2.69	*LOC651186*	2.16	46.24	6.08	3.21	1.17
2.18	*SERPINA1*	2.15	1.28	2.24	1.47	1.79
2.98	*LRRC8E*	2.15	2.39	1.56	1.59	1.26
13.96	*TMEM59L*	2.13	12.38	1.98	2.43	1.91
2.46	*EGLN3*	2.13	2.22	2.35	2.01	1.49
4.01	*EPHA10*	2.11	5.12	3.49	2.81	8.94
2.12	*MNS1*	2.10	1.98	0.98	1.40	1.56
2.24	*LOC441416*	2.10	2.97	1.68	4.99	3.28
2.01	*LOC729669*	2.10	1.04	0.97	1.35	2.09

The shaded values indicate the fold changes (≥2) by which the expressions of corresponding genes are impaired in A4-K49R, A4-K140A, A4-K685Q, and A4-S727A STAT3-expressing cells as compared with the A4-WT STAT3-expressing cells on IL-6 treatment.