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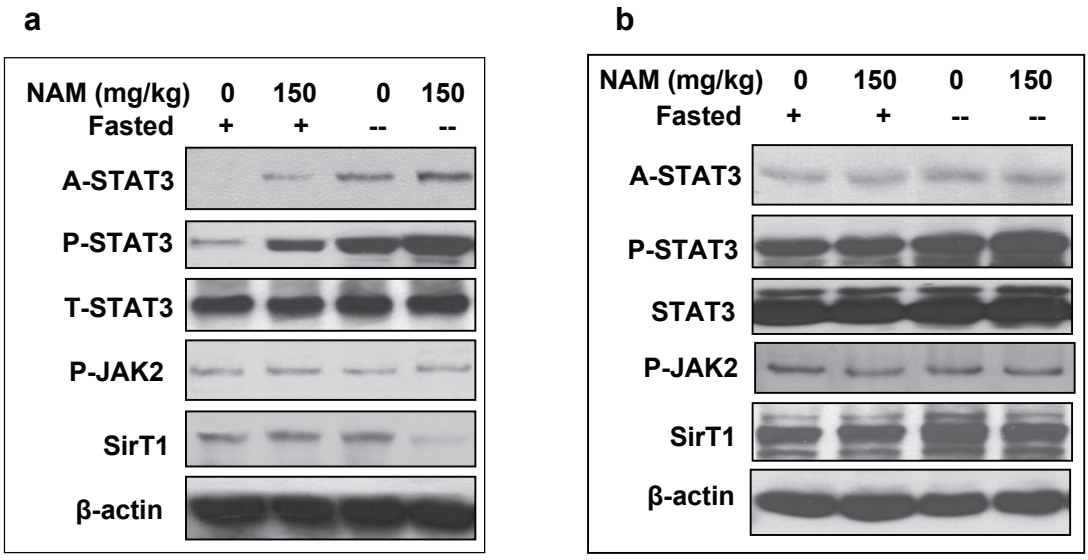


Figure S1 SirT1 inhibitor nicotinamide increased acetylation and phosphorylation of STAT3 in liver **(a)**, but not spleen **(b).** Male C57Bl/6J mice (n=5 for each group) were fed (normal chow), fasted (24 h). Mice were treated with nicotinamide at the dose of 150 mg kg^{-1} *via* i.p.

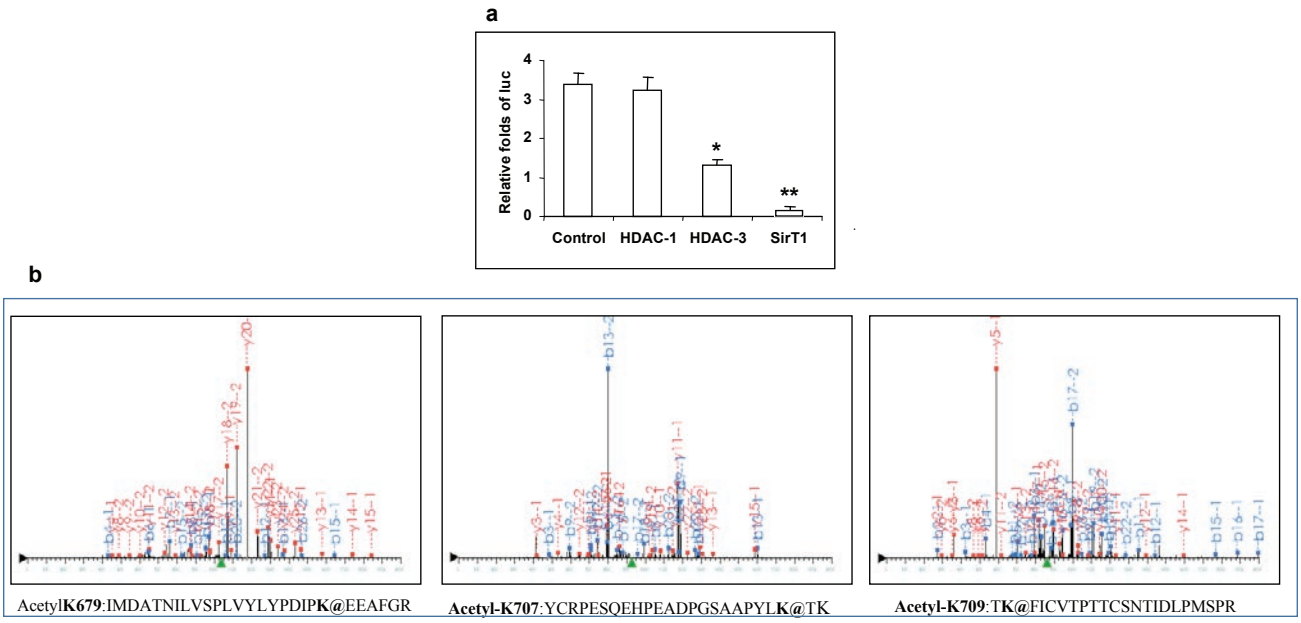


Figure S2 (a) HDAC3 at higher expression reduced the transcriptional activity STAT3. In comparison to Fig. 2j, the amount of HDAC1, HDAC3 or Sirt1 plasmids was increased from 0.05mg to 0.25mg each well (24 well-plate) in the transient cotransfection with the STAT3 specific Luciferase reporter. The result showed that the increase of HDAC3, but not HDAC1 reduced STAT3 transactivation activity. *p<0.05; **p<0.01. **(b)** Identification of three novel lysine acetylation sites in STAT3. 293T cells were cotransfected with plasmids of human P300 and cmyc-STAT3. IP products of STAT3 were subjected to a modified in-gel trypsin digestion procedure. The peptides were subjected to liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) analysis (Taplin Biological Mass Spectrometry Facility, Harvard, Boston, MT) three acetylated peptides of STAT3 indicates the sites of acetylation, which are at K679, K707 and K709.

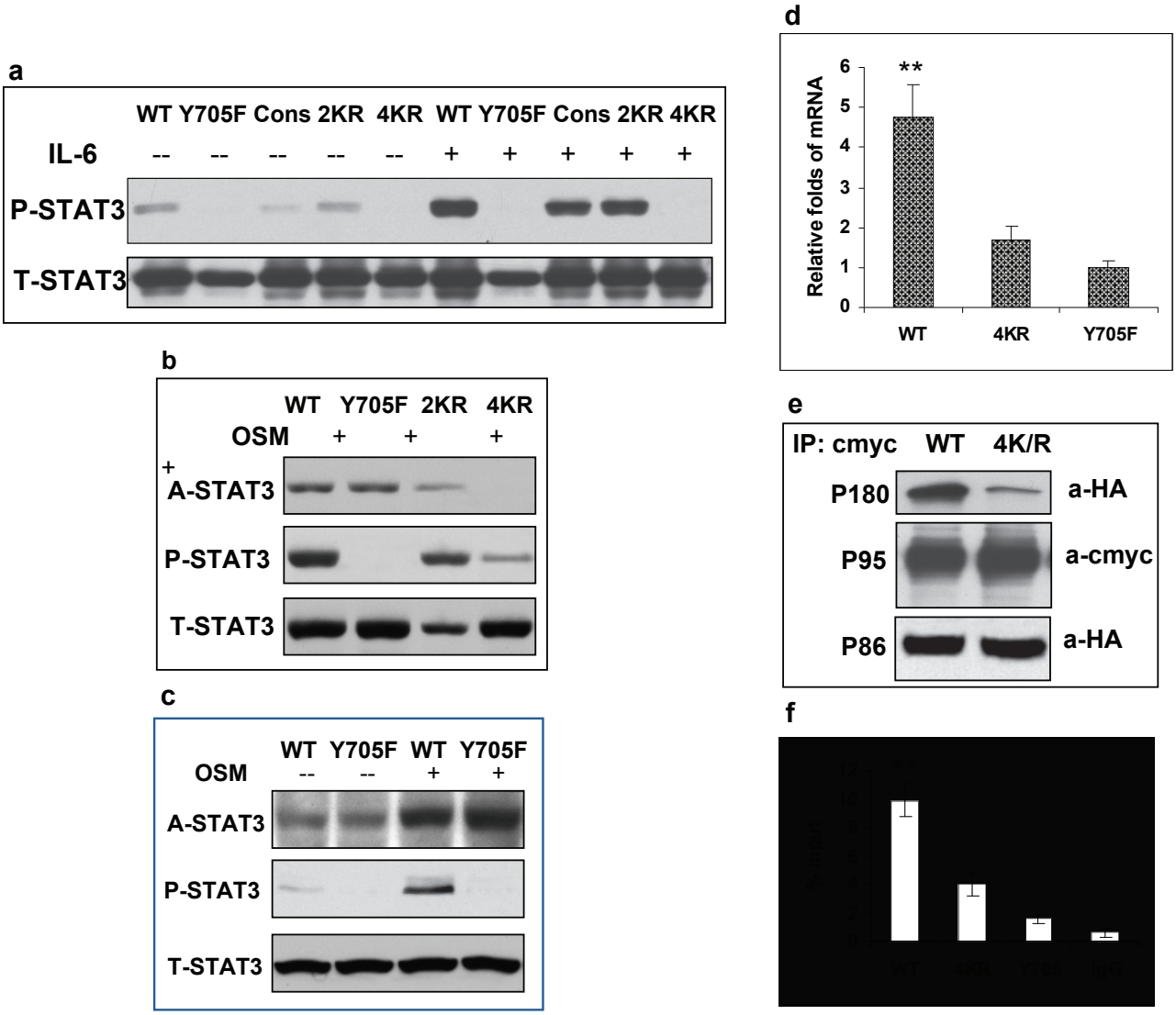


Figure S3 (a) and **(b)** K49/87R mutant (2KR) did not affect the phosphrylation of Y705 and acetylation of K685 of STAT3 in cells. **(a)** Primary hepatocytes were infected with adenovirus containing cmyc-STAT3 or its mutants. The cells were stimulated with 30ng ml^{-1} of IL-6 for 6h. (**b**) 293T cells were transfected with pcDNA3 plasmids containing cmyc-STAT3 or its mutants and stimulated with 10ng ml^{-1} OSM for 6h. **(c)** Y705F mutation did not affect STAT3 acetylation. 293T cells were transfected with pcDNA3-6myc-WT or Y705F-STAT3. Cells were stimulated with 10ng ml^{-1} OSM for 4h. **(d)** Endogenous hAGT transcripts (STAT3 target gene) were induced by WT-STAT3, but not 4K/R- or Y705F-STAT3 in HepG2 cells. HepG2 cells were transfected with STAT3 and its mutants. 12h after the transfection, IL-6 was added at 40ng ml^{-1} for 12 h. RNAs were extracted and subjected to Q-RT-PCR with strategene one step kit. **: p<0.01 hAGT, 5'-CTTCACAGAACTGGATG-3' (**f**) and 5'-GAACTCCTGGGGCTCG-3' (reverse) (product size, 241 bp). **(e)** 4K/R mutation destructed the dimerization of STAT3. 293T cells were cotransfected with either the combination of HA-WT-STAT3 and cmyc-WT-STAT3 or HA-WT-STAT3 and cmyc-4K/R-STAT3. The cell lysates were immunoprecipitated with an anti-cmyc antibody. The IP products of STAT3 were released from beads by 1.5 X loading buffer containing low levels of DTT (80mM) at 100 ºC for 90 seconds to keep the potential STAT3 dimers from dissociating. The P180 band was detected by the anti-HA antibody to reveal the dimerization of both HA-STAT3 and 6cmyc-STAT3. P95 was detected by the anti-cmyc antibody to illustrate pulled down the cmyc-STAT3s monomers. The cell lysates was directly used for Western blot analysis (the bottom panel) to detect HA-WT-STAT3 (P86) using the anti-HA antibody. **(f)** ChIP assay WT-STAT3 bound the promoter region (-3728 to -3598) of mouse pepck1, whereas the 4K/R mutant STAT3 impaired this function of STAT3. STAT3-LKO hepatocytes were reintroduced with WT-STAT3, 4K/R-STAT3, or Y705F-STAT3, respectively, by pBaBe-retrovirus and treated with IL-6 (40ng ml^{-1}, 2h) before protein-DNA cross-linking.

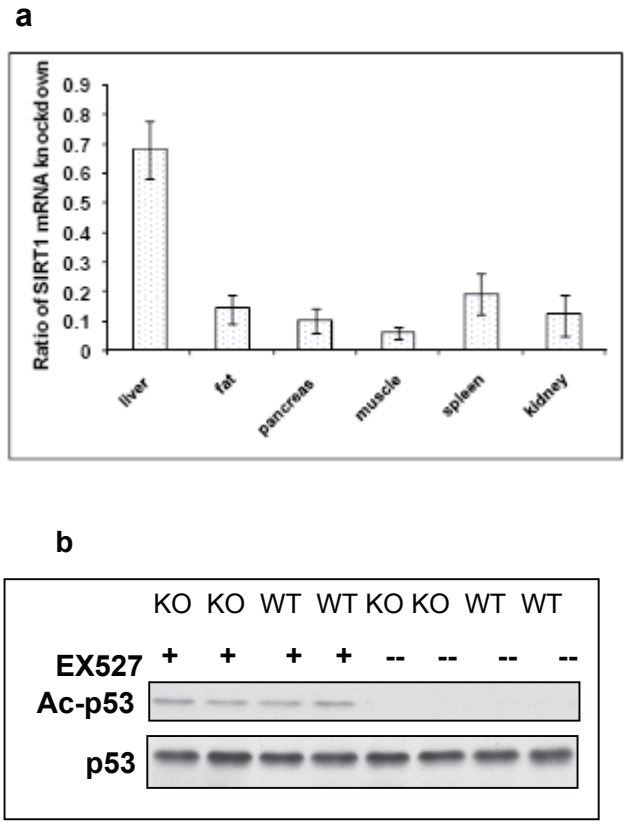


Figure S4 (a) Treatments of Nicotinamide, EX527 and ASO-Sirt1 did not affect liver function of mice. Alanine aminotransferase (ALT) is the most sensitive marker for liver cell damage. Sera were collected from animals treated with nicotinamide (150mg kg^{-1}), EX527 (10mg kg^{-1}) or ASO-Sirt1 (10mg kg^{-1} x5). **(b)** The ratios of *sirt1* mRNA knockdown in mouse different tissues. SIRT1 ASO or control ASO was administered intraperitoneally to STAT3-ff and STAT3LKO mice for 5 times with 50mg kg^{-1} within 2 weeks. RNAs were extracted and subjected to Q-RT-PCR to measure the levels of *sirt1* mRNA. Ratios of the *sirt1* mRNA knockdown = ratio of mRNA of sirt1 ASO/ Control ASO. (n=4). **(c)** Acetylated-P53 was increased by EX527 in the livers of STAT3f/f and STAT3LKO mice. Liver tissues were collected from the STAT3f/f and STAT3LKO mice (n=4) which were fasted overnight and injected with EX527 or vehicle (*i.p.* 10mg kg^{-1}). Same protocol was performed as one used in Fig.1c and Fig. 4d. The levels of Acetylated-P53 (anti-K379-P53, Cell Signaling) were measured by Western blot.

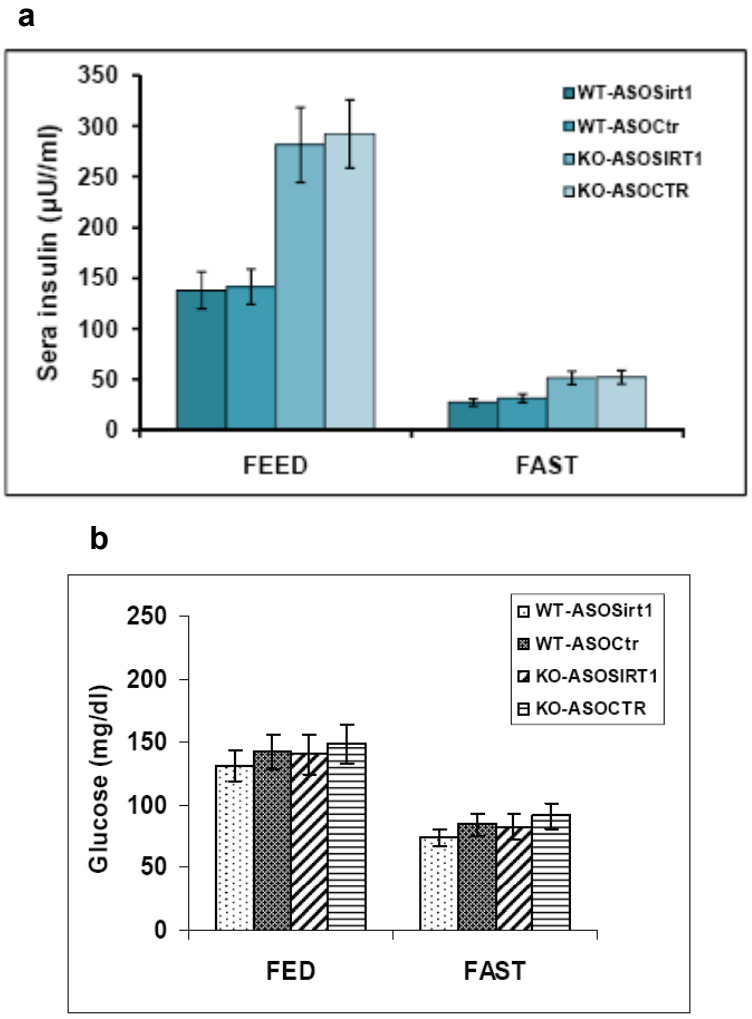


Figure S5 (a) SirT1-ASO did not affect blood insulin in mice treated with SirT1-ASO1 and fed with high fat diet. This experiment was performed at the same set as Fig. 4j. STAT3 f/f (wildtype) and STAT3 LKO mice (n=7) fed with high fat diet for 2.5 weeks days. SirT1-ASO or Control-ASO was administered intraperitoneally for 5 times at a dose of 10mg kg^{-1} within two weeks. The sera insulin was measured at feeding and overnight fasting condition. **b)** STAT3 LKO mildly impaired SirT1 knockdown induced glucose reduction in mice fed with normal chow food. This experiment was performed at the same time as Fig. 4j. STAT3 f/f (wildtype) and STAT3 LKO mice (n=7) fed with normal chow diet. SirT1-ASO or Control-ASO was administered intraperitoneally for 5 times at a dose of 10mg kg^{-1} within two weeks. The levels of plasma glucose were measured at feeding and overnight fasting condition.

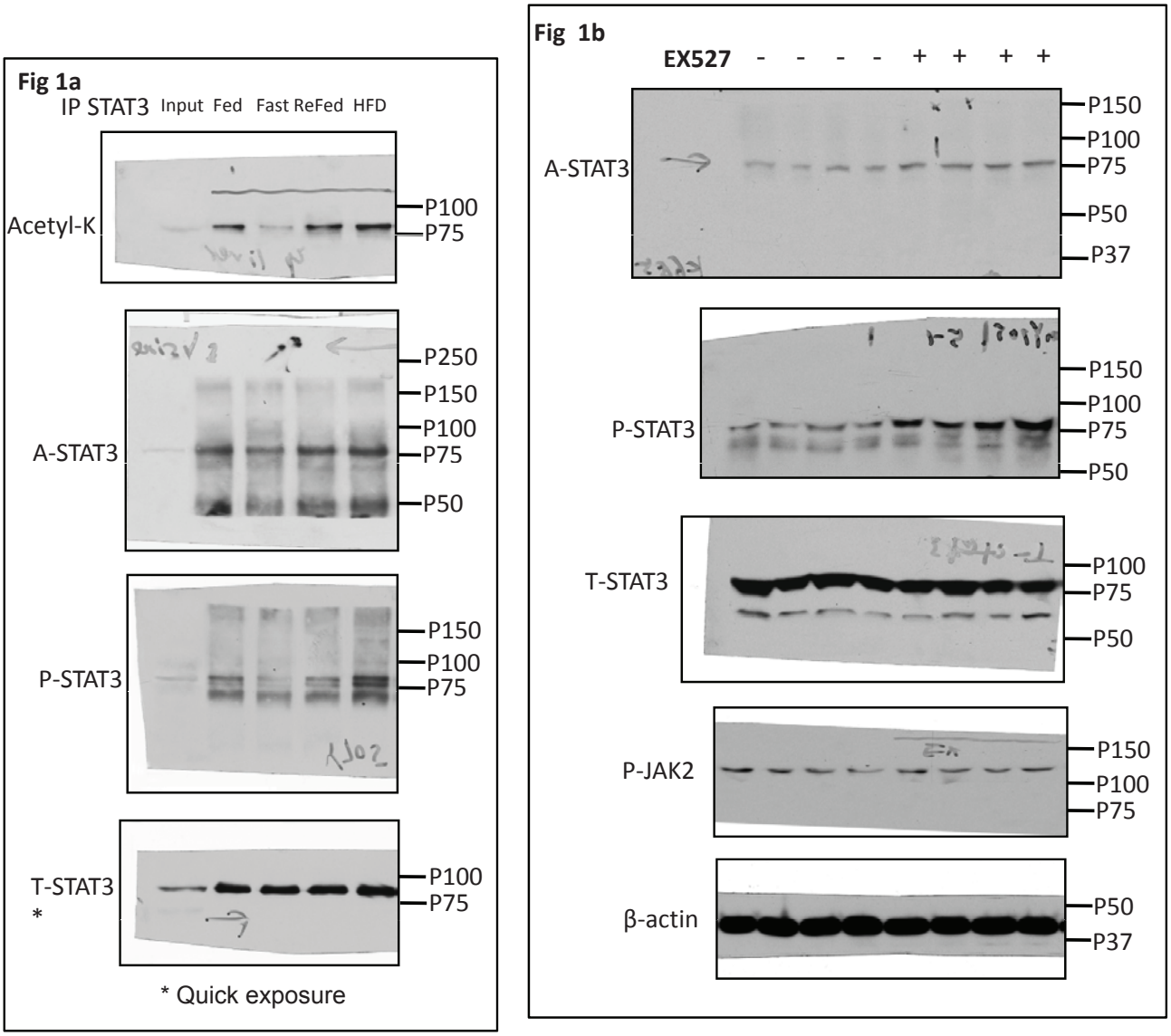
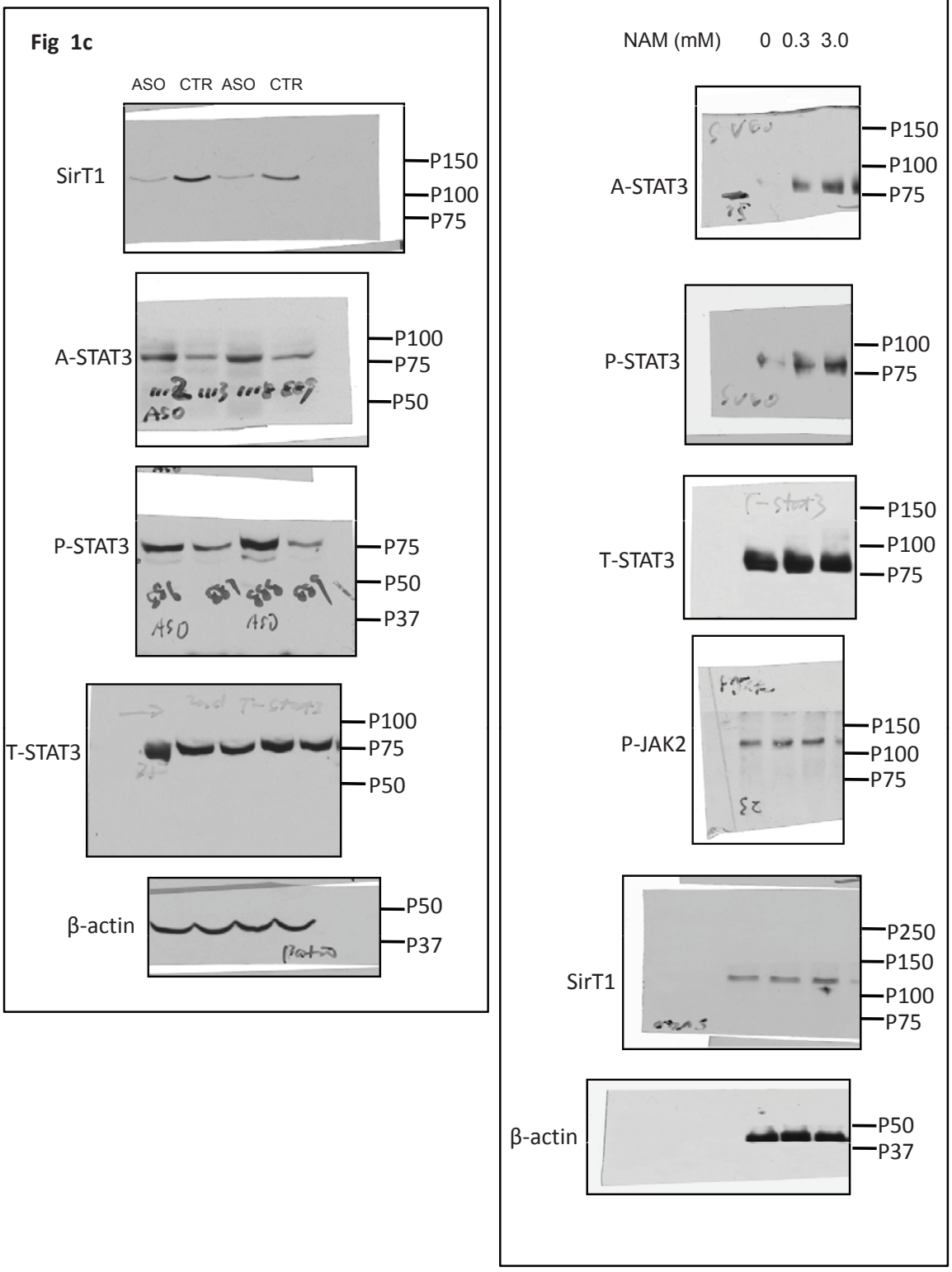


Figure S6 Full scans of western blot data



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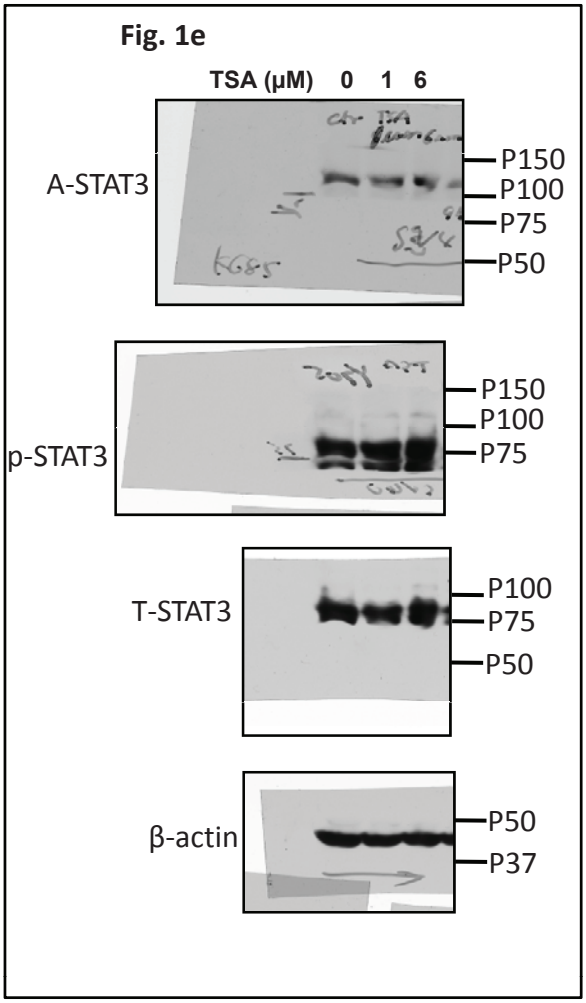

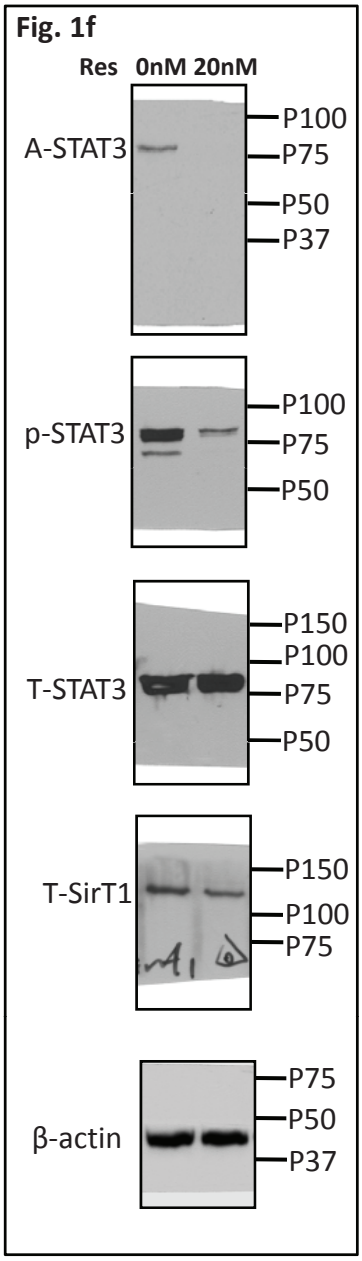

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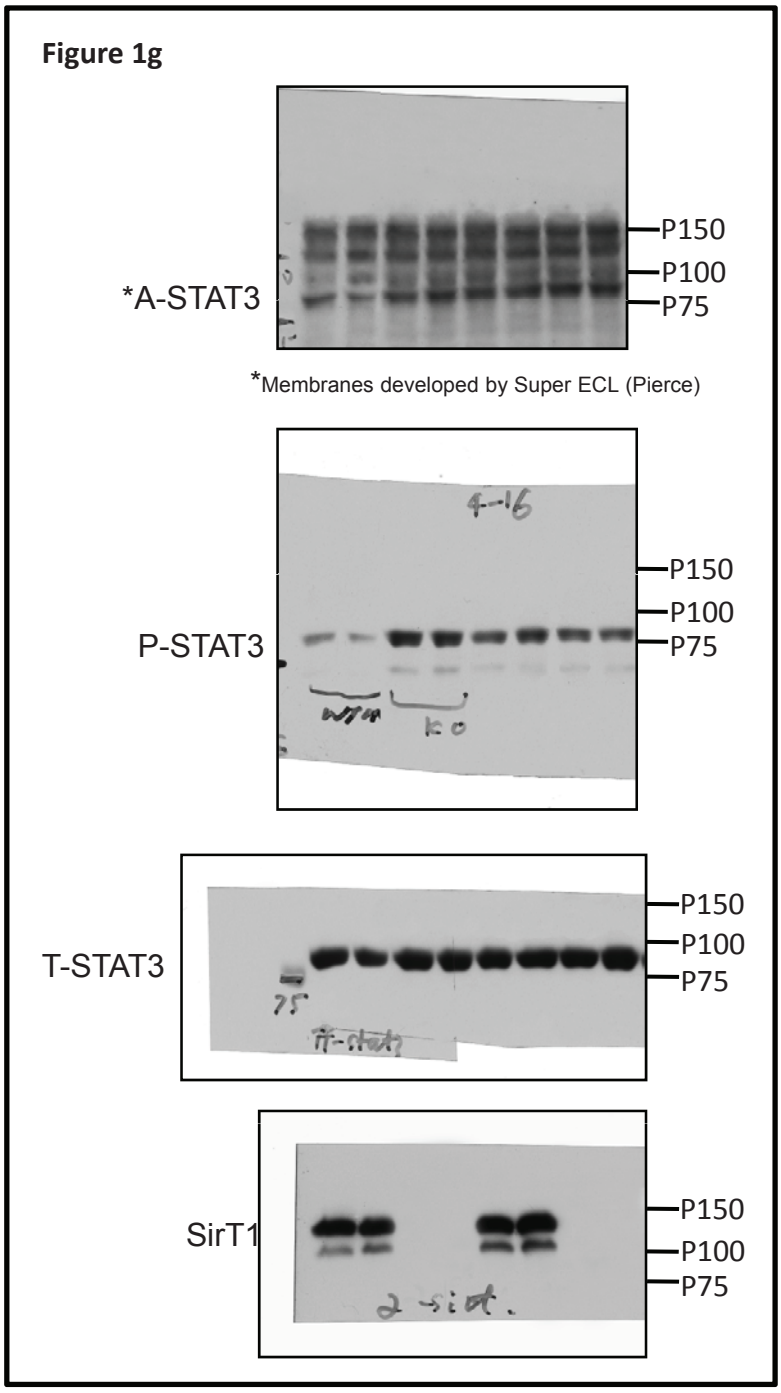

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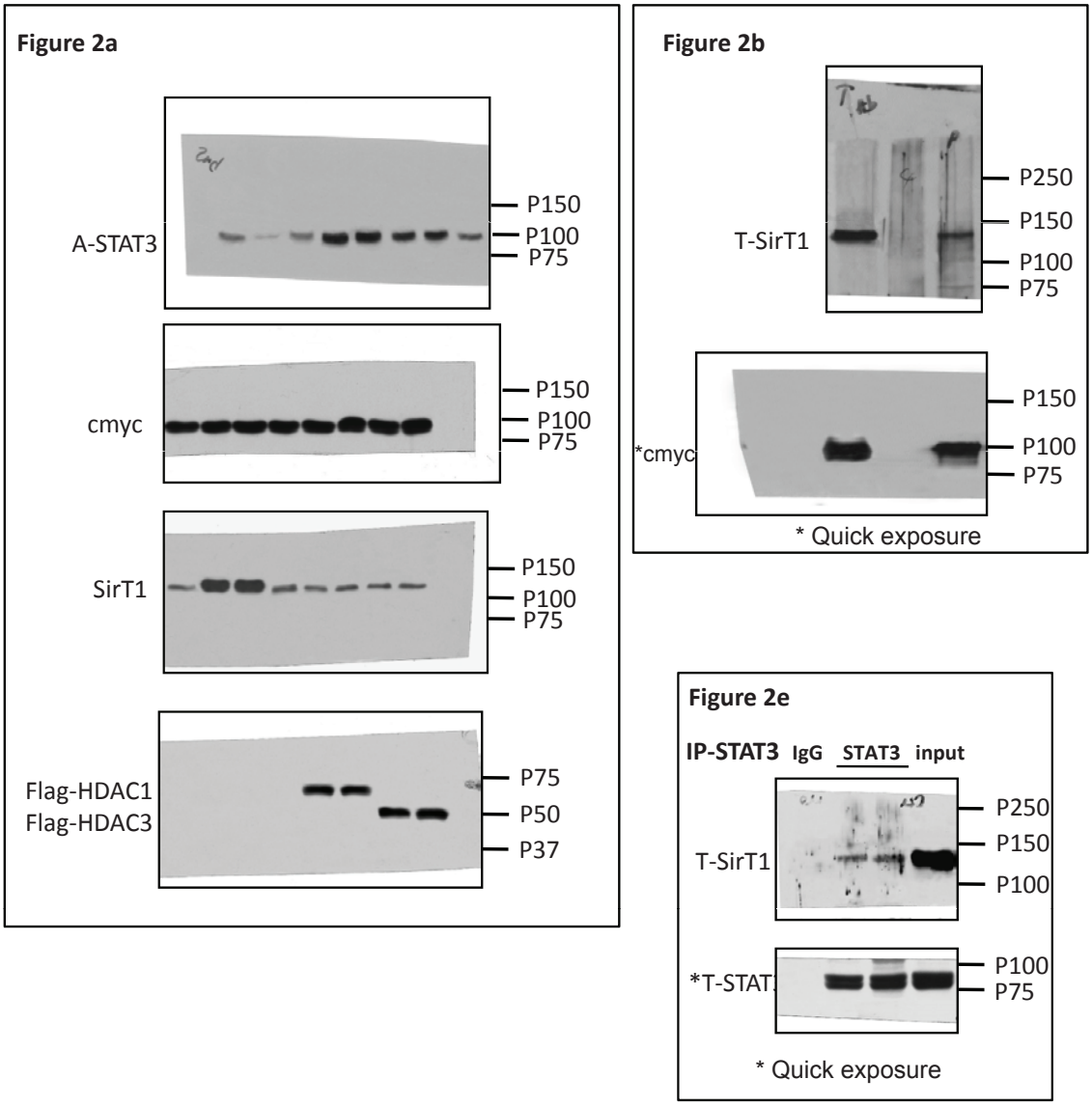

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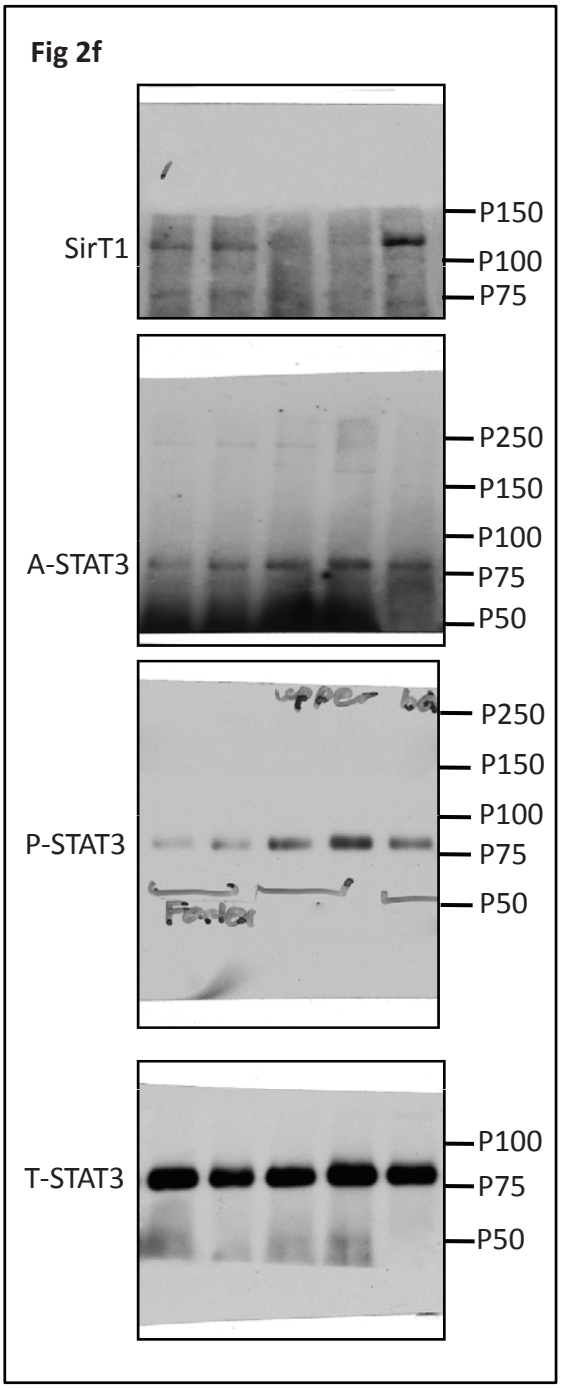

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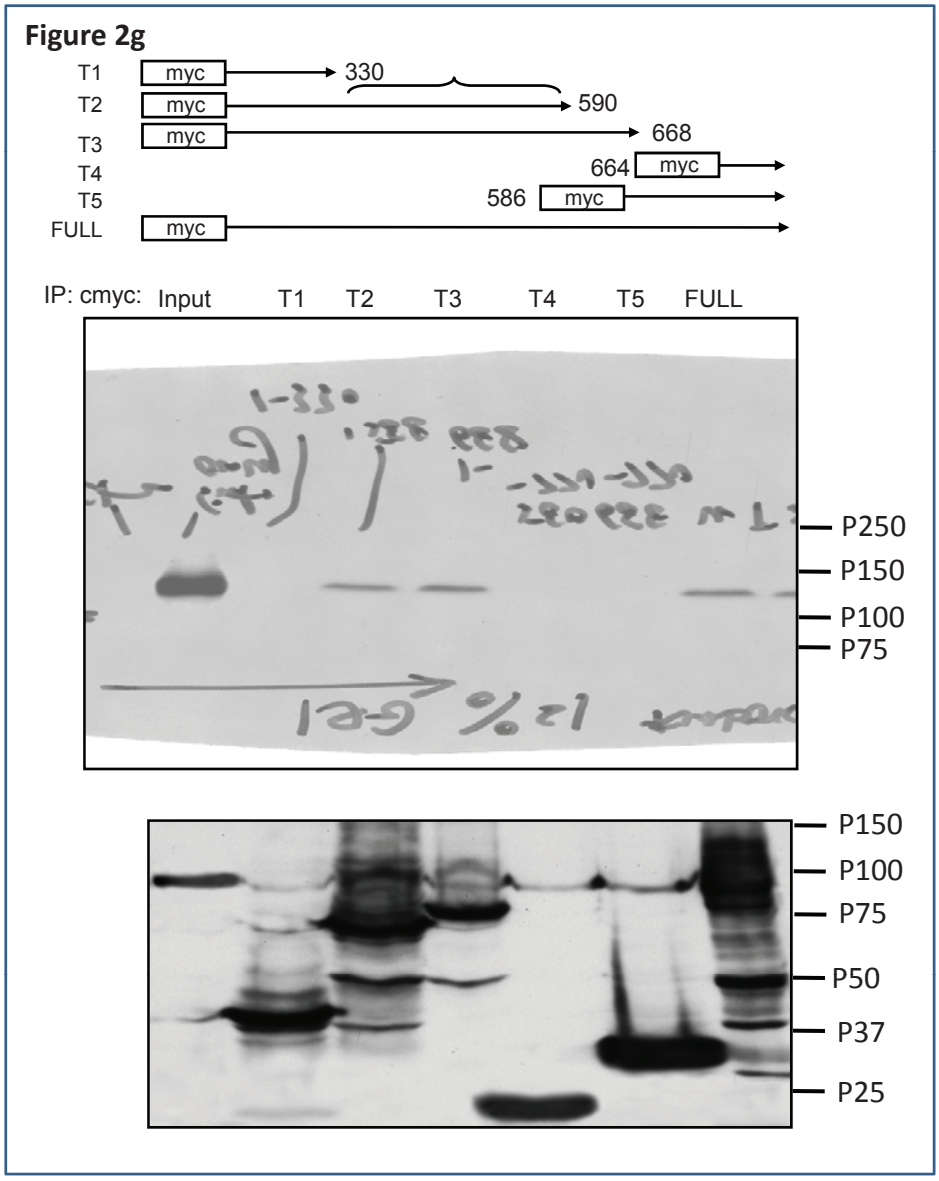

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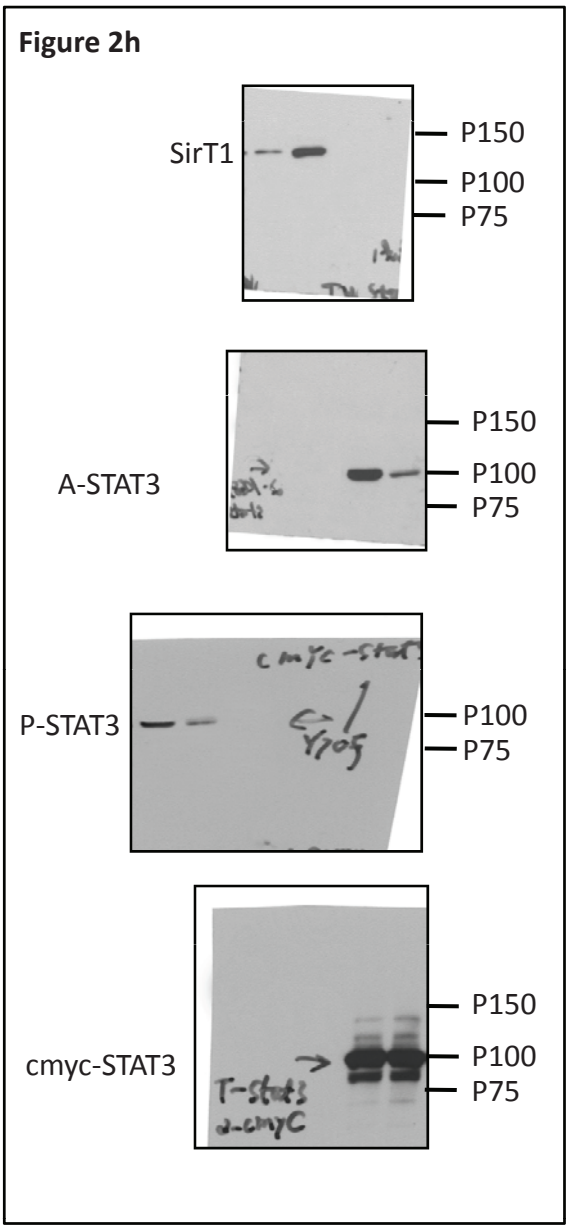

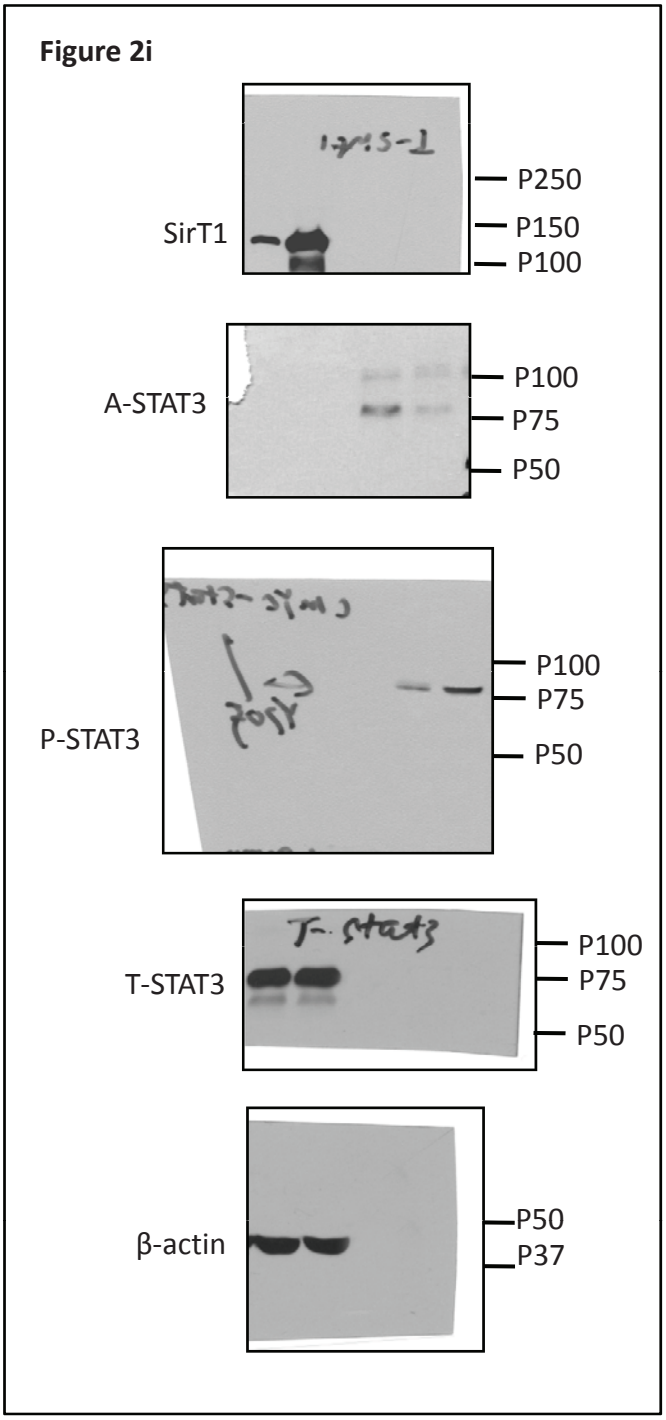

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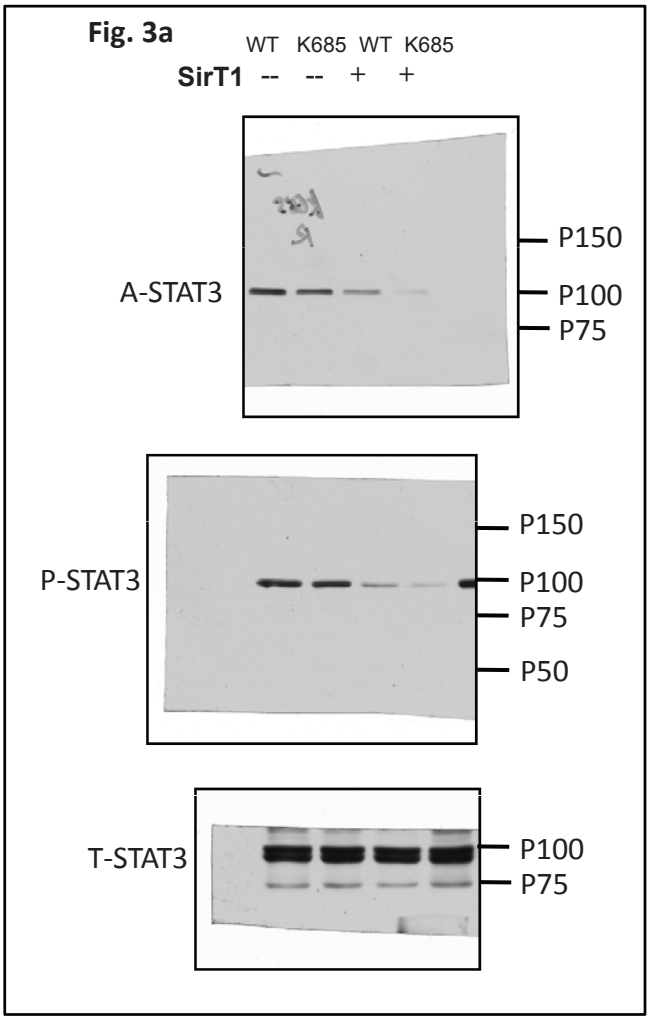

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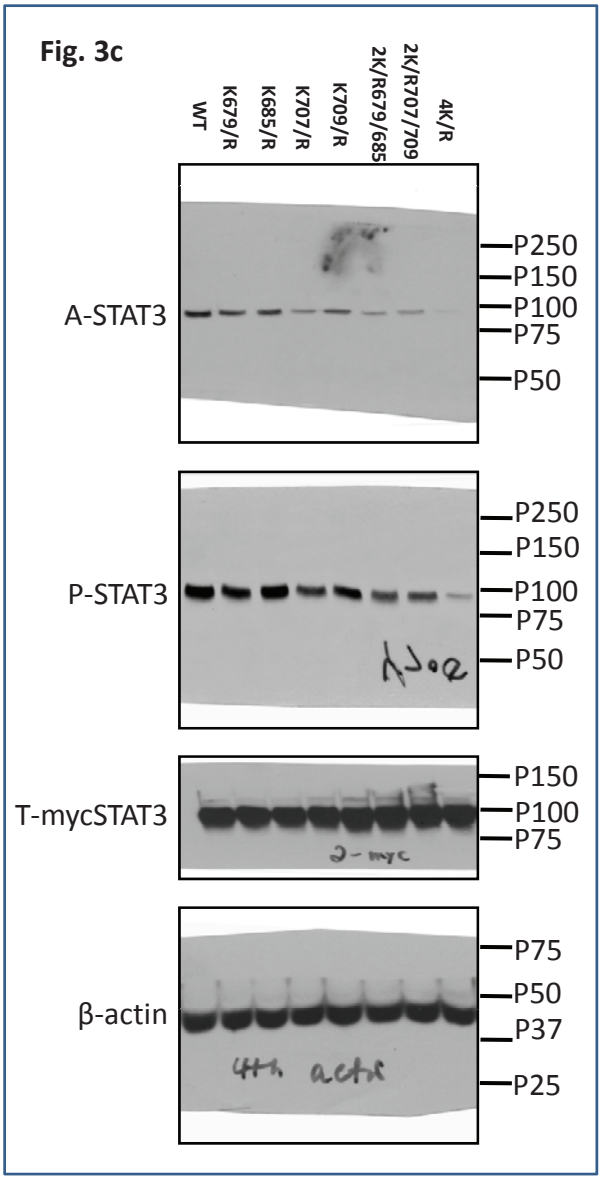

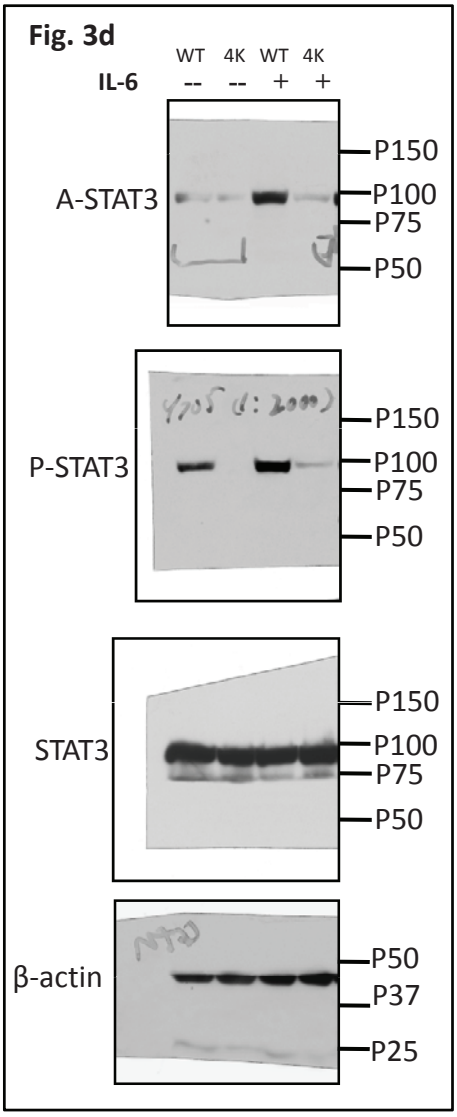

Figure S6 continued

Primer	Sequence	Annealing Temperature
F-18S	AGTCCCTGCCCTTTGTACACA	61 °C
R-18S	CGATCCGAGGGCCTCACTA	
F-tubulin	**TAGCAGAGATCACCAATGCC**	**61 °C**
R-tubulin	**GGCAGCAAGCCATGTATTTA**	
F-Cytochromsome-C	GGAGGCAAGCATAAGACTGG	61 °C
R-Cytochromsome-C	TCCATCAGGGTATCCTCTCC	
F-GK	**ACTGCGGAGATGCTCTTTGA**	**61 °C**
R-GK	**TGCCCTTGTCTATGTCTTCG**	
F-PGC1	AACAATGAGCCTGCGAACAT	61 °C
R-PGC1	CACGGCTCCATCTGTCAGT	
F-Fbpase	**GCTCTGCACCGCGATCA**	**61 °C**
R-Fbpase	**ACATTGGTTGAGCCAGCGATA**	
F-G6pase	GTGTTTGAACGTCATCTTGTG	58 °C
R-G6pase	TTAGTAGCAGGTAGAATCCAA	
F-PEPCK	**GTGGAAGGTCGAATGTGTGG**	**61 °C**
R-PEPCK	**TAAACACCCCCATCGCTAGT**	

Table S1 Primers used in Q-RT-PCR to detect the expression of gluconeogenic genes in wild type and STAT3 liver knockout mice. rRNA 18S and *tubulin* transcripts were used as internal controls in all the experiments. The primers were designed in coding regions which across introns to avoid DNA contamination. F: forward primer; R: reverse primer.

Supplementary Methods

Isolation of Primary hepatocytes. Briefly, the livers of 6-12 weeks old mice were perfused with Hanks' A then Hanks' B medium containing 0.05% collagenase (Boehringer Mannheim Biochemicals, Indianapolis, IN) and 0.8 U ml^{-1} trypsin (Sigma). During the perfusion with Hanks' B medium, the lower abdominal vena cava was intermittently occluded (15 seconds intervals) using a forceps. Livers were then excised, minced, and passed through serial nylon mesh filters, and the resultant cells were washed. The recovered cells were suspended in hepatocyte recovering medium (DMEM with 10 mg L^{-1} glucose, GIBCO), containing 10% fetal calf serum (Invitrogen), 1 ng-ml^{-1} amphotericin B (Sigma), 10 U ml^{-1} penicillin, 50 mg ml^{-1} streptomycin, 10 mg ml^{-1} gentamicin, 1 nM dexamethasone, 1 nM insulin, and plated onto collagen I (rat tail type I, BD Biosceince, Bedford, MA) coated plates or glass coverslips. Cells were incubated at 37°C for experiments.

Virus construction and infection. The STAT3 adenovirus constructs were established using AdEasy Adenoviral vector kit (Stratagene, La Jolla, CA). Briefly, various STAT3 cDNAs, including WT, Y705F, K49-87R, K697-685-707-709R etc., were cloned into the adenovirus vector, pAd-shuttle. The pShuttle-STAT3s was recombined with backbone pAdEasy-1 in BJ5183 bacteria. Adenovirus generation, amplification, and titration were done according to the guidelines of the manufacturer. PGC-1 adenovirus was a gift from Dr. Puigserver. Viral particles were purified by cesium chloride density gradient centrifugation.

Western Blot. The liver, white adipose and spleen were collected from STAT3-LKO, $STAT3^{f/f}$ and wild type C57BL/6J male mice, washed briefly with PBS and then

homogenized by sonication in RIPA lysis buffer. The homogenates were centrifuged at 4 °C at 10,000 rpm for 15 min. The supernatant was placed in a fresh tube and the protein concentration assayed by a Branford kit (Bio-Rad). Western Blots were done as previously described [37]. Rabbit Anti-phospho-(Y705)-STAT3, rabbit anti-phospho-Y1007/Y1008-JAK2, rabbit anti-JAK2, rabbit anti-Acetylated lysine, rabbit anti-acetylated 685 lysine of STAT3 antibodies were from Cell Signaling (Danvers, MA); Rabbit anti-STAT3 and mouse anti-c-myc monoclonal antibodies were from Santa Cruz (Santa Cruz, CA); rabbit anti-mouse and mouse anti-mouse monoclonal SirT1 antibody was from Upstate Biotechnology. Anti–rabbit or mouse antibodies conjugated with horseradish peroxidase (Jackson Immuno Research Labs, West Grove, PA) were used as secondary antibodies. The Western blots were visualized using chemiluminescent substrate (Pierce Chemical, Rockford, IL) The relative intensities of the signals were quantified by densitometry using image analysis software (NIH Image J).

Luciferase assays. 2-3x10^5 A2780 or 293T cells were seeded in 24-well plates in triplets (n = 3) and cotransfected with a plasmids containing STAT3 or its mutants and the luciferase reporter p4x IRF-Luc [13] or APRE−luciferase reporter by using Lipofectimane 2000 (Invitrogen). pRL-TK *Renilla* luciferase (promega, Madison, WI) reporter was used as an internal control for transfection efficiency. 24 h after transfection, cell extracts were prepared and measured for luciferase activity (Promega) using a Sirius luminometer (Berthold Technologies, Oak Ridge, TN). Protein concentrations were determined using Bio-Rad Protein Assay reagents (SmartSpectm Plus Spectrophotometer, Bio-Rad).

Chromatin Immunoprecipitation (ChIP) Assays. ChIP assays were performed using the Chromatin Immunoprecipitation (ChIP) Assay Kit and protocols (Upstate

Biotechnology, Charlottesville, VA). Briefly, 2x106 STAT3-KO-myc-WT, 4KR and Y705 cells were treated by 40 ng ml^{-1} of IL-6 for 2.5 h and then were cross-linked with 1% formaldehyde (10 min, RT), washed and pelleted by centrifugation and resuspended in 200 µl SDS lysis buffer supplemented with protease inhibitors (as described in the kit protocol). Cell lysates were sonicated to shear DNA to <0.6 kb (verified by agarose gel analysis). Sonicated lysates were centrifuged to remove debris, diluted 1:10 in dilution buffer and used for IP with desired antibodies. After immunoprecipitation, pellets were washed with 1 ml Low Salt Immune Complex Wash Buffer, High Salt Immune Complex Wash Buffer and LiCl Immune Complex Wash Buffer and TE buffer. Bead precipitates were eluted twice with fresh elution buffer (1% SDS, 0.1 M NaHCO3) and eluates were pooled and heated at 65°C for 8 h to reverse protein-DNA crosslinks. DNA was eluted into TE buffer. Q-PCR analysis (QuantiFast SYBR Green PCR kit, Qiagen, Valencia CA) was performed with 2 µl of the 70 µl DNA preparation plus the following primers: PEPCK1 (region -3728 to -3598 of the mouse PEPCK1 (NM_011044) promoter containing STAT3 binding sites), 5′- ggtttgaagtgggtgaatcc-3′ (forward primer), 5′ ggacagccagggctatacaa 3′ (reverse primer); P200 (region -226 to -24 of the mouse C/EBPδ promoter containing STAT3 binding sites, as STAT3 positive control primers), reference, 5′-GCGTGTCGGGGCCAAATCCA-3′ (forward primer), 5′TTTCTAGCCCCAGCTGACGCGC-3′ (reverse primer); P1.8k (region -1856 to -1676 of the promoter) as control, 5′-TGCTTCTATGGCATCCAG-3′ (forward primer), 5′-GAGGGGCTGTGGAATATT-3′ (reverse primer). ChIP assays performed using mouse anti-cmyc Monoclonal Antibody (sc-40, Santa Cruz), a normal mouse IgG as negative

control the blocked Protein G beads from Upstate Biotechnology. All ChIP assays were performed three times with representative results presented[38].