Supplemental Table S-1: Peripheral blood cell profiles in wild type and conditional Spry1 null mice

	#NEUT(10^3cells/ul)	#LYMPH(10^3cells/ul)	#MONO(10^3cells/ul)
Spry1 +/+	0.85 +/- 0.42	6.15 +/- 0.91	0.08 +/- 0.01
Spry1 -/-	1.12 +/- 0.10	5.17 +/- 1.80	0.07+/- 0.01

	WBC(x10^3/uL)	RBC (x10^6/uL)	Plt (x10^3/uL)	%NEUT
Spry1 +/+	7.81 +/- 1.25	9.68 +/- 0.60	1564.00 +/- 457	11.70 +/- 4.14
Spry1 -/-	6.90 +/- 1 .70	8.97 +/- 0.09	3232.67 +/- 195	15.93 +/- 1.79

	%LYMPH	%MONO	%EOS	%BASO	%LUC
Spry1 +/+	75.20 +/- 6.5	1.10 +/- 0.42	9.57 +/- 2.21	0.60 +/-0.05	2.10 +/- 0.40
Spry1 -/-	75.60 +/- 7.7	1.17+/- 0.26	5.13 +/- 6.4	0.50 +/- 0.10	1.90 +/- 0.47

	#EOS(10^3cells/ul)	#BASO(10^3cells/ul)	#LUC(10^3cells/ul)
Spry1 +/+	0.52 +/- 0.21	0.04 +/- 0.003	0.18 +/- 0.05
Spry1 -/-	0.38 +/- 0.22	0.03 +/- 0.005	0.14 +/- 0.06

Values are means +/- SE (n=3)

Supplemental Table S-2 BFUe and CFUe frequencies at steady state in control vs conditional *Spry1*-null mice

	BFUe (2×10^5)	CFUe (2×10^5)
Spry1$^{+/+}$	11 ± 3	379 ± 29
Spry1$^{-/-}$	10 ± 2	359 ± 35

Suppplemental Table S-3. Distributions of E1, E2 and E3 erythroid progenitor cells in wild-type vs. conditional Spry1-null mice at steady-state, post-phenylhydrazine (day 4), and post short-term BMT (d13.5).

E1, E2 and E3 numbers at steady-state

	wt	Spry1-/-
total cells	176784	134906
gated	155561	123888
E3	10480	14491
E2	1537	5898
E1	ND	333

E1, E2 and E3 numbers post phenylhydrazine (day 4)

	wt	Spry1-/-
total cells	71439	69080
gated	68584	66709
E3	45210	45428
E2	3074	3784
E1	1232	3229

E1, E2 and E3 numbers post BMT (day 13.5)

	wt	Spry1 -/-
total cells	125866	125753
gated	113133	119780
E3	74667	86241
E2	1349	8204
E1	1660	4072

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure S-1. May-Grumwald stained cytospins of stage E1, E2 and E3 erythroid progenitor cells. Bone marrow cell preparations were expanded in SP34ex medium for 3.5 days. CFUe-like "E1" cells, "E2" proerythroblasts, and stage "E3" erythroblasts were then isolated (by MACS and/or FACS) and used to prepare cytospins. (For additional details, please also see reference #25).

Supplemental Figure S-2. Elevated reticulocyte production at steady-state due to conditional Spry1-deletion. For n=3 pIpC-induced and n=3 control (PBS-injected mice), reticulocyte levels (upper panels) and hematocrits (lower panels) were determined (at 3-weeks post termination of pIpC dosing).

Supplemental Figure S-3. Among erythroid progenitors expanded from bone marrow of conditional Spry1-null mice, survival of stage E3 erythroblasts is modestly compromised. A] Erythroid progenitor cells from bone marrow preparations were expanded in SP34ex medium. At day 3.5, frequencies of YoPro3-positive cells among stage E1, E2 and E3 progenitors were determined. Values are means +/- SE (n=3). **B]** Representative primary data (YoPro3 staining among $Ter119^{pos}$ cells) also are illustrated.

Supplemental Figure S-4. Representative primary mass spectrometry ion-spray data for select EPO/EPOR PY-modulated signal transduction factors. Data shown are for Spry1, together with select (illustrative) known PY-modulated EPO/EPOR targets.

Supplemental Figure S-5. Western blot analysis of EPO/EPOR- induced Spry1 phosphorylation at PY53. A] UT7epo cells were stably transduced with a VSV-G packaged pMSCVneo vector encoding FLAG-epitope tagged wild-type Spry1. Exponentially growing UT7epo-Spry1(Flag) cells were washed thrice, and cultured for 20 hours in the absence of hematopoietic growth factors. Cells then were challenged with EPO (3U/mL). At 15 minutes of EPO-exposure, Igepal lysates were prepared and subjected to IP (anti-FLAG Sepharose CL4B). Elution was with a FLAG peptide. Samples in the upper panel were western blotted with an anti-PY antibody, and samples in the middle-panel were blotted with an anti-FLAG antibody. For the lower

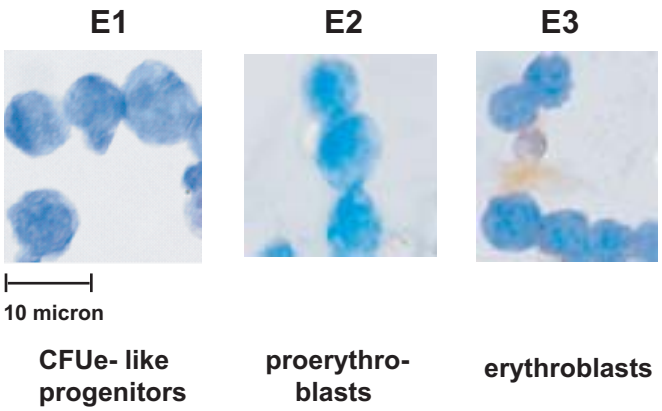
panel and blot, total cell lysates were probed with an anti-FLAG antibody. **B]** Panels include an EIC (extracted ion chromatogram at a 3.0 ppm tolerance, 723.8113 m/z, z=2) of the Spry1 phosphopeptide, GSNEY*TEGPSVVK showing both the peak area (AA) and peak height (AH) for each chromatogram. **C]** MS/MS spectra for duplicate EPO-challenged UT7epo samples. For this LCMS analysis, note the similar high-intensity signals (and y- and b- ion signals). **D]** Example MS1 spectrum for EPO-exposed UT7epo cells, and mass identification of PY-Spry1 tryptic peptide (within 1.2 ppm of theoretical mass).

Supplemental Figure S-6. Effects of ectopically expressed wild-type Spry1(Flag), and Spry1-Y53F(Flag), on UT7epo cell growth. Stably transduced UT7epo-wt-Spry1 and UT7epo-Spry1-Y53F cells were washed, and plated at 3 x 10^5 cells/mL in the presence of 0.6 U/mL EPO. At 36, 60 and 84 hours, viable cell counts were determined, and are graphed as means +/- SE (n=3).

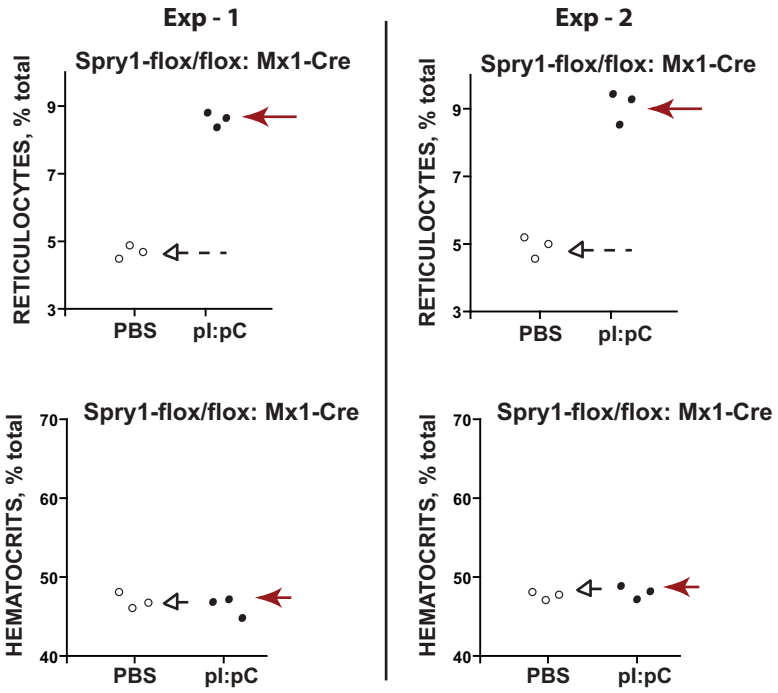
Supplemental Figure S-7. Conditional deletion of Spry1 leads to altered Stat5 activation (PY-phosphorylation) within primary bone marrow- derived proerythroblasts. Erythroid progenitor cells were expanded from bone marrow (Spry1-null, and controls) in SP34ex cultures. At day 3.5, proerythroblasts were isolated via Lin^{pos}-depletion, and $CD71^{pos}$-selection. Cells were then cultured for 5.5 hours in the absence of hematopoietic growth factors, and then EPO-challenged. At 0, 8 and 24 minutes, cells were chilled, collected and lysed. Lysates were then analyzed for levels of PY-Stat5 via western blotting. (PY-Stat1 levels also were assayed, but no significant activation was detectable).

Supplemental Figure S-8. Mx1-Cre conditional disruption of Spry1 does not result in skewing of *Spry -2, -3, or -4* expression levels. Within primary bone marrow- derived (pro)erythroblasts, *Spry* transcript levels in knock-out and control mice were assessed by quantitative RT-PCR. Values are expressed (graphed) as ratios of levels in conditional Spry1-null vs control mice.

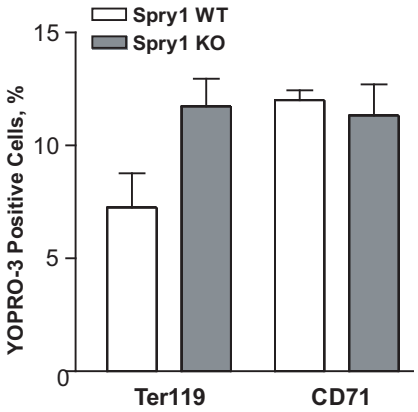
SUPPLEMENTAL FIGURE S-1

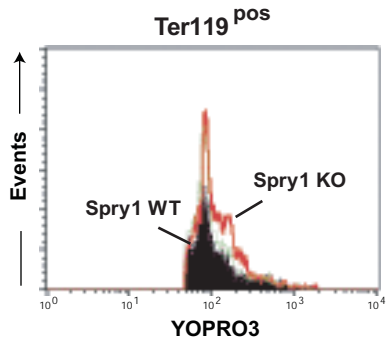


SUPPLEMENTAL FIGURE S-2



SUPPLEMENTAL FIGURE S-3

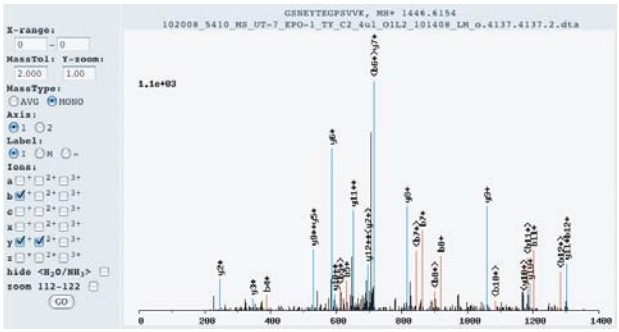




SUPPLEMENTAL FIGURE S-4

A]

SPRY1 Y53



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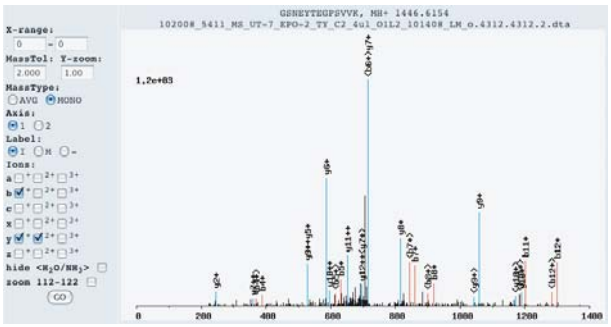
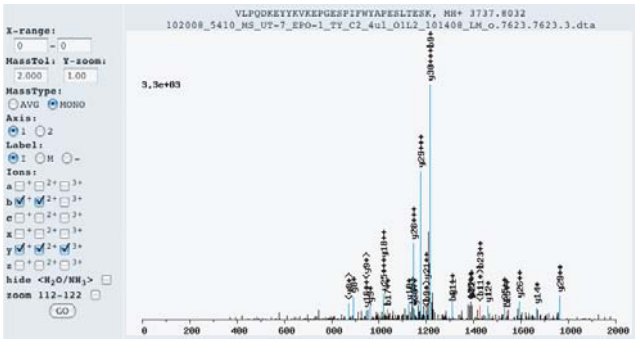
SPRY1 Y53



723.8113 m/z, z = 2, MS2 Scan: 4312, CS# 5411, Xcorr = 3.0198

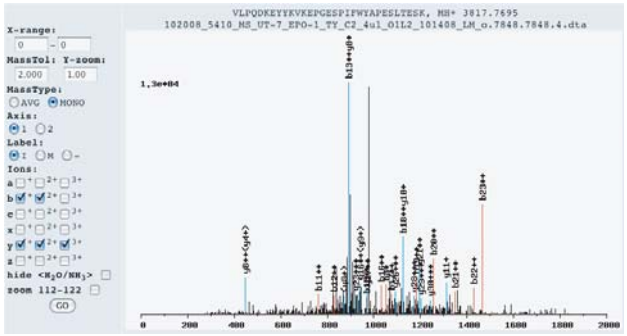
B]

JAK2 Y1008



1246.6061 m/z, z = 3, MS2 Scan: 7623, CS# 5410, Xcorr = 3.6264

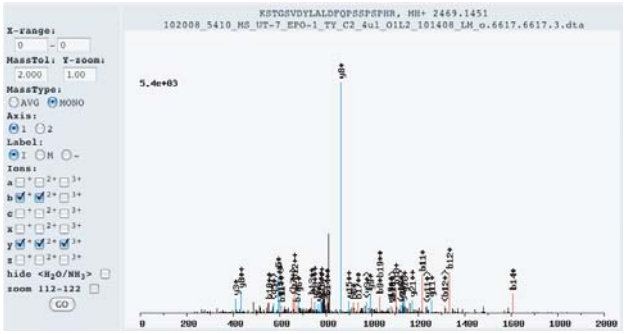
JAK2 Y1007, Y1008



955.1981 m/z, z = 4, MS2 Scan: 7848, CS# 5410, Xcorr = 4.1606

C]

GAB2 Y614



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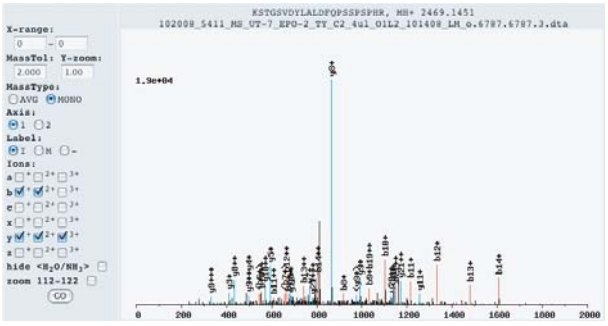
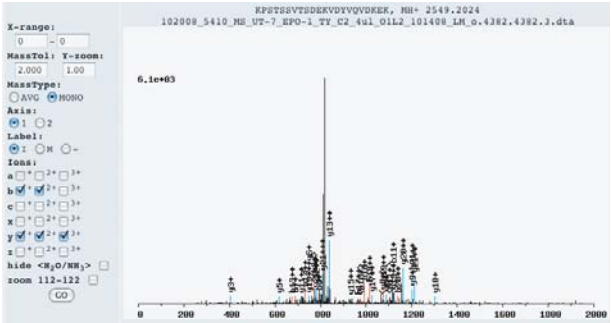
GAB2 Y614



823.7201 m/z, z = 3, MS2 Scan: 6787, CS# 5411, Xcorr = 5.2478

GAB2 Y643



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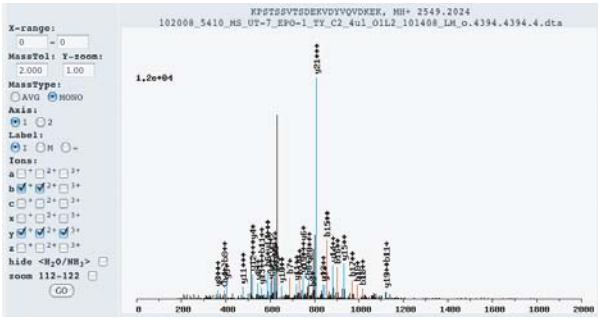
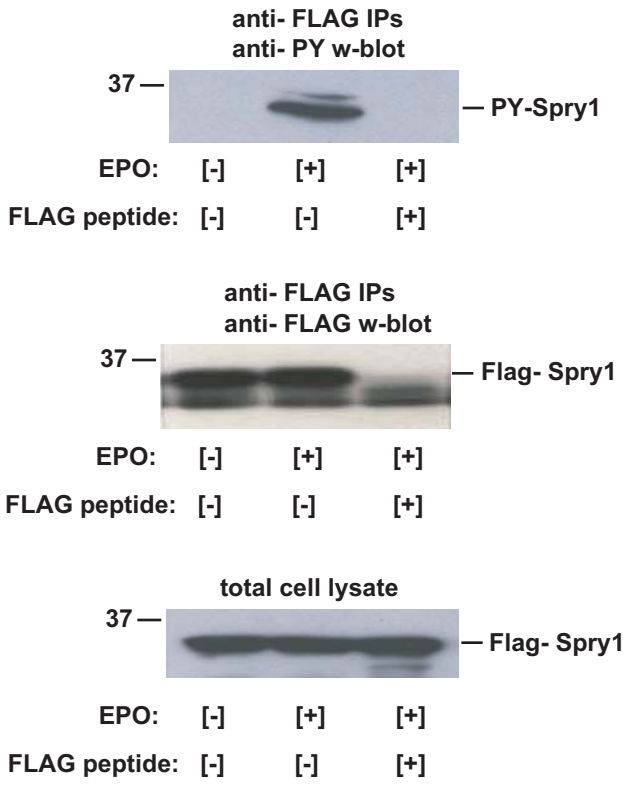
GAB2 Y643

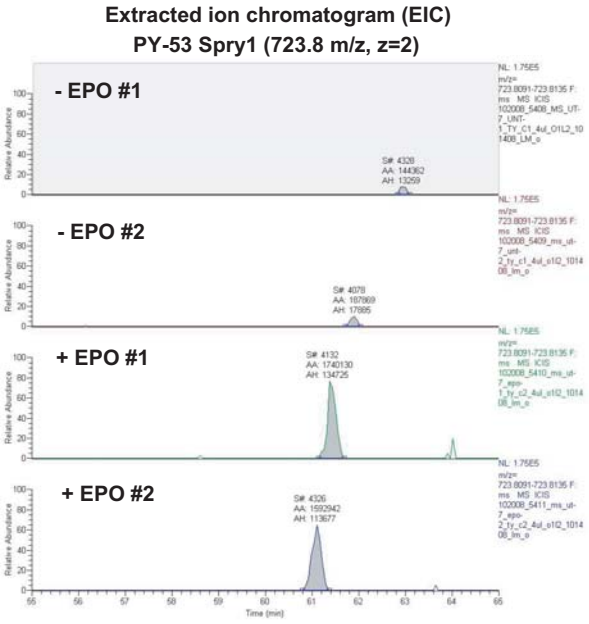


638.0563 m/z, z = 4, MS2 Scan: 4394, CS# 5410, Xcorr = 4.1097

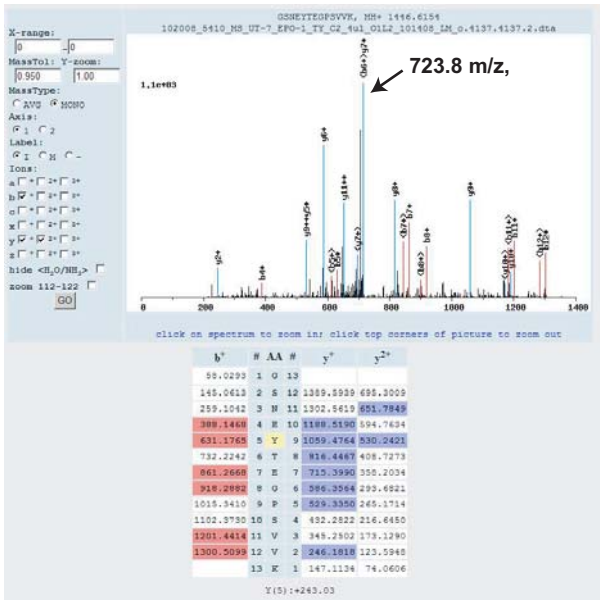
(A)

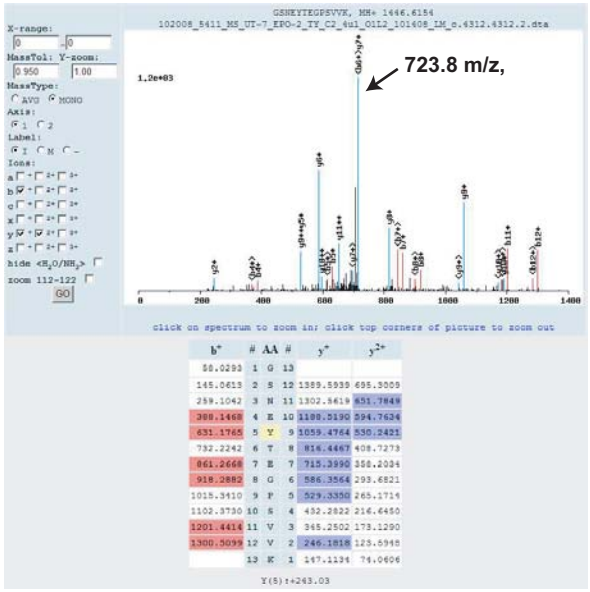


(B)

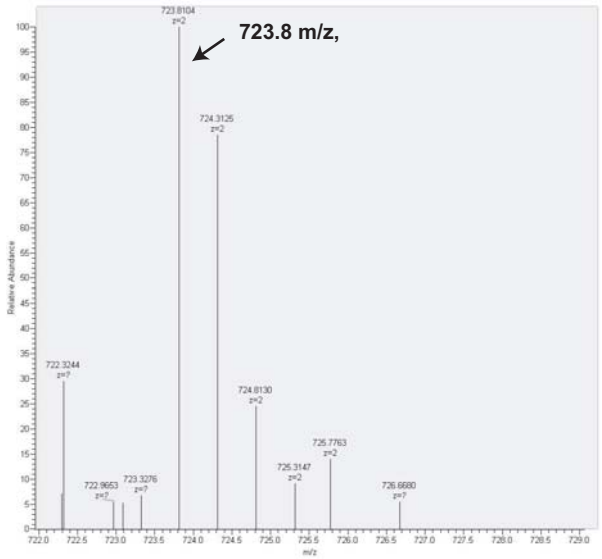


(C) MS/MS spectra

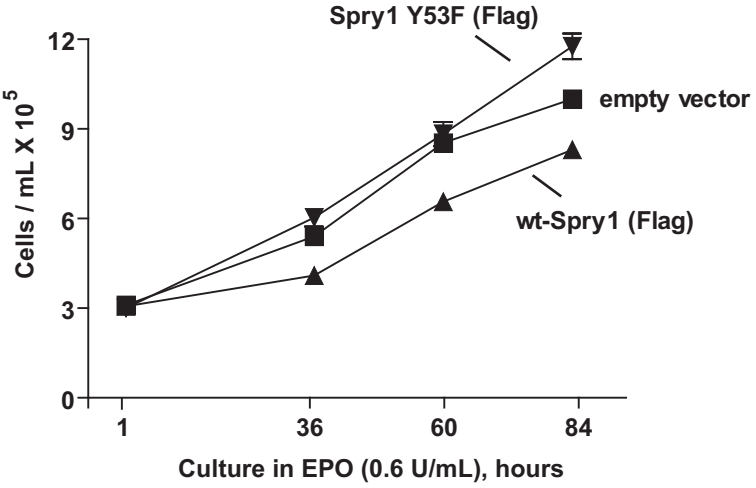




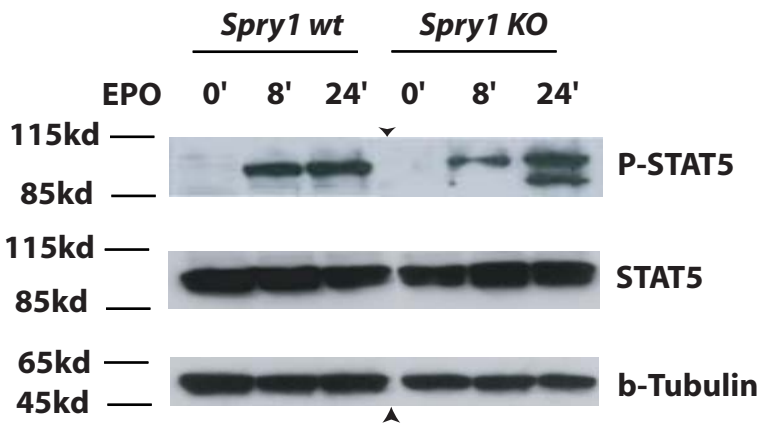
(D) MS1 spectrum



SUPPLEMENTAL FIGURE S-6



SUPPLEMENTAL FIGURE S-7



SUPPLEMENTAL FIGURE S-8