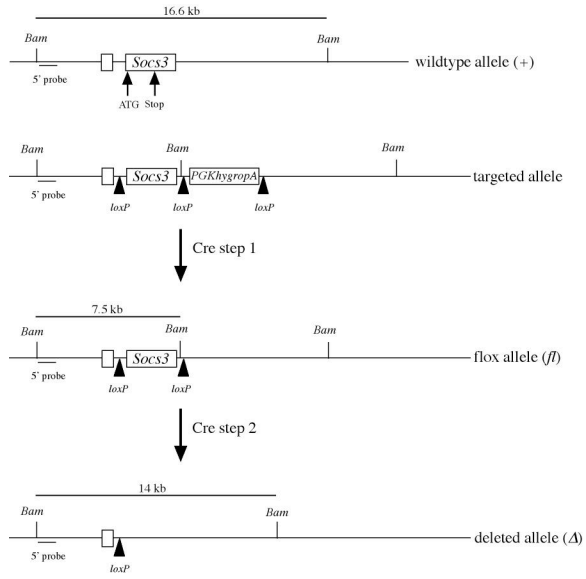
Supplemental Material

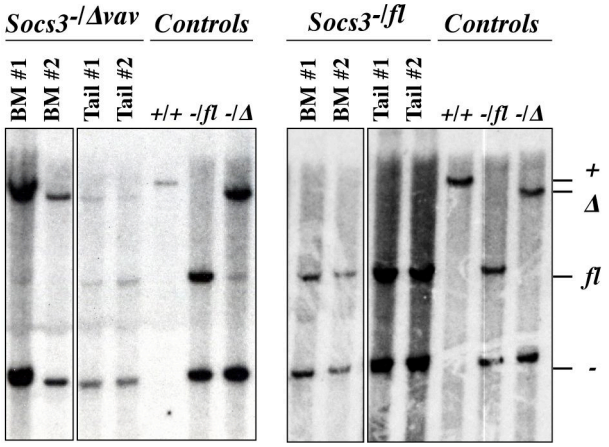
Figure. S1. Generation and characterization of the novel Socs3 floxed allele. (A) The targeted allele was generated by incorporating a hygromycin resistance (*hygro*) cassette at the 3′ end of the second exon of *Socs3* along with three *loxP* sites flanking both the second exon of *Socs3* and the *hygro* cassette. In the figure, exons are represented by boxes and the SOCS3 coding region is indicated by the ATG and stop codons. A floxed allele (*fl*) with deletion of the *hygro* cassette was generated after the transient Cre expression in targeted ES cells step. A second Cre expression step *in vivo* allowed deletion of the second exon of *Socs3* to generate a deleted allele (Δ). (B) Samples of genomic DNA from bone marrow and tails of two $Socs3^{-/\Delta vav}$ and two $Socs3^{-/fl}$ mice were digested with BamHI (*Bam*) and probed with a 5′ *Socs3* probe to distinguish the WT (+, 16.6 kb), floxed (*fl*, 7.5 kb) and deleted allele (Δ, 14 kb). This probe also hybridizes to a 5 kb fragment of the *Socs3* knockout allele (-) as previously described (Roberts et al. 2001).

In each panel, 3 control samples of the relevant genotypes are displayed. BM cells from *Socs3*$^{-/\Delta vav}$ demonstrate the highly efficient deletion of the fl allele by the *vavCre* transgene. (C) Western blot analysis of SOCS3 expression and phosphorylation of STAT3 and STAT1 in IL-6 stimulated BMM from mice bearing WT alleles (Wildtype), a deleted allele (Δ) and a knockout allele (-) (*Socs3*$^{-/\Delta vav}$), and a WT and a *fl* allele (*Socs3*$^{+/fl}$).

Figure. S2. Decay of SOCS3 protein expression in the presence of cycloheximide is independent of SOCS2. (A) As in the experiment described and presented in Fig. 4A, LN cells collected from WT and *Socs2*$^{-/-}$ mice were incubated with anti-CD3 (5μg/mL), anti-CD28 (2 μg/mL) and hIL-2 (10ng/mL) for 4 h. Following washing, cycloheximide (15μg/mL) was added to the media and the cells were incubated for a further 8 h. Throughout the experiment, cells were exposed to anti-mIL-2 (2 μg/mL). Cell lysates were collected for Western blot analyses. SOCS2 and non-specific bands (light chains) are indicated by white arrow and grey arrows, respectively. (B) Quantification of SOCS3 expression from experiments described in (A). Expression levels were normalized to the 4 h time point for each genotype and presented as relative SOCS3 expression (%).

Figure S1


16.6 kb
Bam
Bam
Socs3
wildtype allele (+)
5' probe
ATG Stop
Bam
Bam
Bam
Socs3
PGKhygropA
targeted allele
5' probe
loxP
loxP
loxP
Cre step 1
7.5 kb
Bam
Bam
Bam
Socs3
flox allele (fl)
5' probe
loxP
loxP
Cre step 2
14 kb
Bam
Bam
deleted allele (Δ)
5' probe
loxP


Socs3-/Δvav
Controls
Socs3-/fl
Controls
BM #1
BM #2
Tail #1
Tail #2
+/+ -/fl -/Δ
BM #1
BM #2
Tail #1
Tail #2
+/+ -/fl -/Δ
+
Δ
fl
-

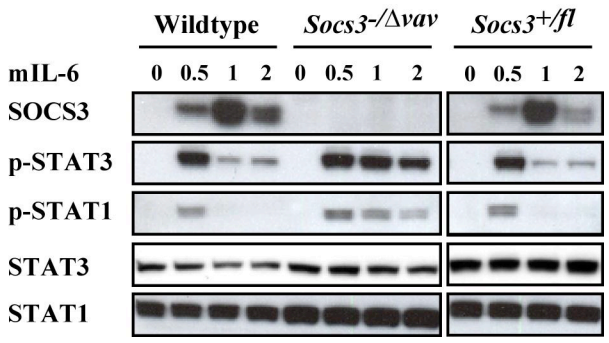
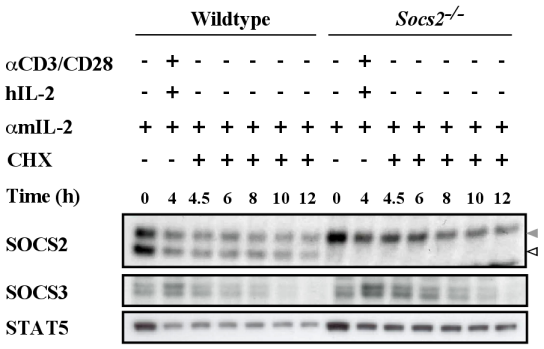

Wildtype
Socs3-/Δvav
Socs3+/fl
mIL-6
0 0.5 1 2 0 0.5 1 2 0 0.5 1 2
SOCS3
p-STAT3
p-STAT1
STAT3
STAT1

Figure S2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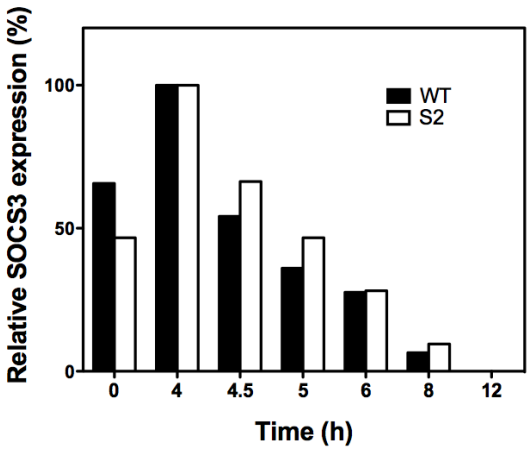
Socs2-/-
αCD3/CD28
hIL-2
αmIL-2
CHX
Time (h)
0 4 4.5 6 8 10 12 0 4 4.5 6 8 10 12
SOCS2
SOCS3
STAT5


Relative SOCS3 expression (%)
100
50
0
WT
S2
0
4
4.5
5
6
8
12
Time (h)