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Running title: SOCS2 is dispensable for HSCs and CML-like disease

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Abstract

Suppressor of cytokine signaling 2 (SOCS2) is known as a feedback inhibitor of cytokine signaling and is highly expressed in primary bone marrow cells from patients with chronic myeloid leukemia (CML). However, it has not been established whether SOCS2 is involved in CML, caused by the *BCR/ABL1* fusion gene, or important for normal hematopoietic stem cell (HSC) function. In this study, we demonstrate that although *Socs2* was found to be preferentially expressed in long term HSCs, *Socs2*-deficient HSCs were indistinguishable from wild type HSCs when challenged in competitive bone marrow transplantation experiments. Furthermore, by using a retroviral *BCR/ABL1*-induced mouse model of CML, we demonstrate that SOCS2 is dispensable for the induction and propagation of the disease, suggesting that the SOCS2-mediated feedback regulation of the JAK/STAT pathway is deficient in *BCR/ABL1*-induced CML.

Keywords: CML, BCR/ABL1, SOCS2, HSC, STAT5 phosphorylation

Introduction

Chronic myeloid leukemia (CML) arises from hematopoietic stem cells (HSC) that have acquired a reciprocal t(9;22)-translocation, creating the *BCR/ABL1* fusion gene. The P210 BCR/ABL1 fusion protein has been described to initiate signaling through several downstream pathways such as STAT5, PI3K, AKT, RAS and WNT.[1] However, only a few downstream mediators have so far been thoroughly studied *in vivo* in the context of BCR/ABL1-mediated induction of CML, using mice deficient for the specific genes. Such studies have demonstrated that interleukin 3 (*Il3*), granulocyte macrophage colony stimulating factor (*Csf2*) and *Cbl* are dispensable for a *BCR/ABL1*-induced CML-like disorder.[2, 3] In contrast, *Alox5* was reported to be a critical regulator of leukemic stem cells (LSC) in *BCR/ABL1*-induced CML.[4, 5] Moreover, cells deficient for both *Stat5a* and *Stat5b* lack the capacity to develop disease and JAK/STAT signaling has emerged as a pivotal pathway by which BCR/ABL1 elicits its transforming effects.[6-9]

The STAT5 induced suppressor of cytokine signaling 2 (SOCS2), a known feedback inhibitor of the JAK/STAT pathway, has previously been found upregulated in the advanced stages of CML and to become down regulated following imatinib treatment in both CML cell lines and primary cells.[10-15] In addition, *SOCS2* was recently identified as one of the core genes in gene expression signatures shared between normal HSC and acute myeloid leukemia (AML) LSCs, with both signatures being associated with worse clinical outcome in AML.[16] Collectively, these findings suggest that SOCS2 may be important for both normal HSC function and *BCR/ABL1*-induced leukemia. *SOCS2* is one of eight members (*CIS* and *SOCS1-7*) of the *SOCS* gene family which all have both SH2 and SOCS box domains in common.[17] This family of proteins is normally expressed upon stimulation with various cytokines and has mainly been characterized as feedback inhibitors of cytokine induced signaling.[18-20] However,

their function is more complex, as activating properties also have been suggested for SOCS2.[21-24] SOCS2 is present in several tissues and involved in the regulation of several cytokines such as GH, IGF1, IL2, IL3 and EPO, with both inhibitory and enhancing properties through other SOCS proteins and receptor-JAK complexes.[23-25] The most striking feature of *Socs2*-deficient mice is gigantism caused by a STAT5B-dependent increase of sensitivity to GH-signaling.[26, 27] However, the steady state hematopoiesis of *Socs2*-deficient mice appears normal.[26]

In this study, we found that although *Socs2* was preferentially expressed in the long-term hematopoietic stem cell (LT-HSC) population, it was dispensable for normal HSC function as determined by competitive stem cell transplantation. To clarify the role of *SOCS2* upregulation in CML, we evaluated *BCR/ABL1*-induced CML like disease from *Socs2*$^{-/-}$ versus *Socs2*$^{+/+}$ primitive bone marrow (BM) cells. Both groups developed indistinguishable CML-like disorders with similar survival curves, spleen weights, white blood cell (WBC) counts and levels of STAT5 phosphorylation. Collectively, our data demonstrate that SOCS2 is dispensable for normal HSC function and for BCR/ABL1-mediated induction of CML.

Materials and methods

Mice and genotyping

All animal experiments were approved by the local ethics committee in Lund, Sweden. Generation of the *Socs2*$^{-/-}$ mice in C57BL/6 background has been described previously.[26] The primers used for genotyping were 5´-CGAGCTCAGTCAAACAGGTAGG-3´ and 5´-GCTTTCAGATGTAGGGTGCTTTCC-3´ to detect *Socs2*, and 5´-GCAGACGATGGTGCAGGATATCC-3 and 5´-GGATCGACAGATTTGATCCAGC-3´ to detect the *β-galactosidase* gene replacing *Socs2*.[28] B6SJL mice and F1 crossings of C57BL/6xB6SJL were used as recipients and for competitor/support cells in the transplantations.

Analysis of Socs2 expression from global gene expression data

To study the gene expression pattern of *Socs2* in various subpopulations of normal mouse hematopoietic cells, the raw CEL-files for the dataset GSE14833 were downloaded from gene expression omnibus (http://www.ncbi.nlm.nih.gov/geo/). Gene expression values were normalized using GCRMA.[29] The mean expression value for the probe sets 1449109_at and 1418507_s_at was used to determine *Socs2* expression.

Flow Cytometry analysis and sorting

Fluorescence-activated cell sorting (FACS) analysis was performed with either FACS Canto II or FACS Aria (Becton Dickinson, Franklin Lakes, NJ, USA). All cells sorted for HSC analysis, were freshly extracted from *Socs*2$^{+/+}$ or *Socs*2$^{-/-}$ mice and stained with several antibodies, to identify various subpopulations. The stem cell population used for expression analysis of *Socs2* was defined as lineage negative for B220, CD3, Mac1, Gr-1, Ter.119 and, in addition, IL7Rα^{-}c-kit^{+}Sca1^{+}CD34^{-}flt3^{-}. The common myeloid progenitors (CMP) were

defined as c-kit^{+}Sca-1^{-}CD34^{+}CD16/32^{mid}. The HSCs used for transplantation were sorted as above but with the additional SLAM markers CD150^{+}CD48^{-}. Antibodies used were purchased either from Biolegend (San Diego, CA, USA) or eBioscience, (eBioscience, San Diego, CA, USA).

For multicolor analysis of lineage distribution, we used antibodies against CD19 and CD4 (eBioscience); TER.119, CD8a, CD11b, CD45.1, and CD45.2 (BioLegend); CD45R/B220, Ly-6G/Ly-6C, and Gr-1 (Becton Dickinson).

Hematopoietic stem cell transplantation

B6SJL recipient mice were lethally irradiated (900 cGy) 18h before transplantation and Ciprofloxacin was supplemented with the drinking water following transplantation. Bone marrow cells were harvested and pooled from five age and sex matched *Socs2*$^{-/-}$ and *Socs2*$^{+/+}$ donors, respectively. For each recipient, 200 lineage^{-}Sca-1^{+}c-kit^{+}CD34^{-}flt3^{-}CD150^{+}CD48^{-} cells (long-term HSC) were sorted and injected intravenously together with 300 000 C57BL/6xB6SJL competitor cells. Repopulation was calculated as (proportion CD45.2^{+} cells) / (proportion CD45.2^{+} cells + proportion CD45.1.2^{+} cells). Lineage distribution was analyzed within the donor population, determined by CD45.2 expression. Approximately 3x10^{6} bone marrow cells from each primary recipient were transplanted into two lethally irradiated secondary recipients.

P210 transduction and transplantation model

The retroviral construct MSCV-*BCR/ABL1*-IRES-*GFP* was kindly provided by Prof. Connie Eaves together with a control vector expressing only GFP (MIG).[30] Ecotropic viral particles were produced by transient transfection into 293T cells. The transductions and transplantation

experiments were performed essentially as described by Li *et.al*.[31] In brief, matched *Socs2*$^{+/+}$ and *Socs2*$^{-/-}$ donors were sacrificed and c-kit^{+} cells were isolated using midi MACS and anti-CD117 micro beads (Miltenyi biotech, Bergisch Gladbach, Germany). A total of 400 000 cells per recipient were pre-stimulated in StemSpan serum free expansion medium (Stemcell technologies, Grenoble, France) supplemented with 50 ng/mL murine SCF, 10 ng/mL IL-3, 50 ng/mL human IL-6 (Peprotech, Rocky Hill, NJ, USA), for 48 hours. Transductions were performed by preloading viral particles using Retronectin (Takara bio Inc. Otsu, Japan). Cells for transplantation were harvested approximately 18 hours after transduction, washed, and injected in bulk via the tail vein along with 100 000 freshly isolated supporting cells from whole BM. The transduction efficiency obtained was between 10-15% of the transplanted cells, as determined by FACS analysis of GFP on in vtro cultured cells, 2 days after the transduction. Recipient mice were irradiated as described above and transplanted mice received Ciprofloxacin with the drinking water throughout experiment.

Blood analysis and histopathology

Untreated mice were bled from tail veins to determine steady state blood cell counts in *Socs2*$^{-/-}$ mice, and mice transplanted with transduced cells were bled around 15 days after transplantation. Blood cell counts were analyzed on ABX Micros 60 (Horiba ABX, Montpellier, France). Femur and spleen from diseased mice were conserved in 4% formaldehyde, paraffin embedded and sectioned before staining with hematoxylin and eosin as previously described.[32]

Taqman PCR and western blot

Whole BM cells were extracted from transplanted mice after euthanasia and the BM cells were viably frozen. Freshly isolated HSCs and CMPs were sorted as described above and

cryopreserved, viable and GFP expressing cells were sorted from diseased mice (from MIG control mice all myeloid cells were sorted due to lack of GFP expression). RNA was extracted from the sorted cells using the RNeasy micro kit (Qiagen, Hilden, Germany). Gene expression was analyzed by real time quantitative reverse-transcriptase PCR (RT-QPCR) using predesigned TaqMan probes for all known members of the *Socs* gene family and murine *Actb* or *Gapdh* as endogenous controls (Applied Biosystems, Foster City, CA, USA). RT-QPCR was performed on an ABI Prism 7500 analyzer (Applied Biosystems) and ddCT values were calculated as described [33]. Western blot analysis to detect STAT5A/B proteins and STAT5 tyrosine phosphorylation was performed as described, using 150 000 sorted PI^- GFP^+Mac1^+ cells [32]. The membrane was stripped after P-STAT5 detection and relabeled to detect the STAT5 protein.

Intracellular flow cytometric analysis

Cells were harvested 48 hours after a P210 transduction was made, as described above. Fc receptors were blocked with unlabeled CD16/CD32 antibodies (Becton Dickinson). Fixation and permeabilization of cells were performed using the Cytofix/cytoperm kit (Becton Dickinson) and the cells were stained with antibodies detecting phosphorylation of STAT5 or STAT3 (Becton Dickinson).

Results

Socs2 expression is high in HSCs

To study the role of SOCS2 in normal hematopoiesis, we first investigated the gene expression pattern of *Socs2* in various hematopoietic lineages using public gene expression data. Interestingly, *Socs2* was found highly expressed in normal LT-HSC relative to other hematopoietic lineages (Fig 1a). Validations using RT-QPCR showed that the *Socs2* expression was about 30 times higher in HSCs compared to Lin^- cells and common myeloid progenitors (Fig1b), implying that SOCS2 may play a role in HSC function.

Socs2-deficient mice display normal hematopoietic stem cell function

In accordance with previous findings demonstrating that mice lacking *Socs2* have normal steady state hematopoiesis,[26] the *Socs2* deficient mice had normal peripheral blood cell counts. Although steady state hematopoiesis was unaffected in *Socs2*-deficient mice, it could still be envisioned that SOCS2, due to its role in cytokine signaling, could play an important role in regulating HSC-function under conditions when the hematopoietic system is stressed. Thus, to investigate whether loss of SOCS2 affects the function of HSCs, we performed competitive bone marrow transplantations, in which *Socs2*-deficient bone marrow cells were challenged with wild type competitor cells. At steady state, no significant difference in stem cell frequency as determined by $LSKCD150^+CD48^-$ or $LSKCD34^-flt3^-$ was found between $Socs2^{-/-}$ and $Socs2^{+/+}$ BM cells (Fig 2a). Furthermore, we observed no difference in repopulation between $Socs2^{+/+}$ and $Socs2^{-/-}$ cells in either primary or secondary recipients, suggesting that SOCS2, also under conditions of hematopoietic stress, is dispensable for both short and long term HSC function (Fig 2b and c, and Supplementary Fig 1).

SOCS2 *is dispensable for BCR/ABL1-induced CML like disease in mice*

As a regulator of JAK/STAT mediated signaling, SOCS2 has been suggested to play a role in both BCR/ABL1 and JAK2 V617F-mediated myeloid leukemogenesis.[11, 13, 34] The findings that loss of SOCS2 did not have any dramatic effects on steady state hematopoiesis or HSC function, allowed us to examine the role of BCR/ABL1-mediated CML-like disease in Socs2$^{-/-}$ cells without confounding factors attributed to effects of *Socs2*-deficiency on normal HSC function. By using the *BCR/ABL1* retroviral transduction and transplantation model previously described,[31] c-kit^{+} bone marrow cells were transduced and transplanted into lethally irradiated mice, to examine disease progression *in vivo*. About 3 weeks after transplantation, most mice receiving *BCR/ABL1* expressing cells developed CML-like symptoms similar to previous reports.[4, 31] and had to be euthanized shortly thereafter (Fig 3a). No difference in disease latency between mice receiving either *Socs2*$^{+/+}$ or *Socs*2$^{-/-}$ cells was observed. Furthermore, when peripheral blood was analyzed 14-16 days after transplantation, both *Socs2*$^{+/+}$ and *Socs2*$^{-/-}$ mice had elevated white blood cell and platelet counts compared to controls (Fig 3b and data not shown). When euthanized due to disease symptoms, mice from both groups suffered from severe splenomegaly (Fig 3c) and FACS analysis showed that most had an expansion of myeloid GFP^{+} cells in the BM (Fig 3d). To further address whether the *Socs2*$^{+/+}$ and Socs2$^{-/-}$ cells caused different leukemic phenotypes, histopathologic examinations were performed on femurs and spleens from diseased mice. In the BM, *BCR/ABL1* caused similar phenotypes in *Socs2*$^{+/+}$ and *Socs*2$^{-/-}$ mice with foci of histiocytes, enlarged sinusoids, and reduced erythropoiesis compared to the MIG control (Fig 4a and data not shown). All mice with disease had markedly enlarged spleens with infiltration of granulocytes in various maturation stages (Fig 4b and data not shown). In a number of enlarged spleens, we also found focal regions with blasts in both *Socs2*$^{+/+}$ and *Socs*$^{-/-}$ transplanted recipients (data not shown). In summary, the histopathological features were

clearly pathologic but recipient mice, transplanted with *BCR/ABL1* transduced *Socs2*$^{+/+}$ or *Socs2*$^{-/-}$ cells, were similar.

Expression analysis of all Socs family members does not suggest compensatory expression of other Socs genes

In *BCRABL1*-expressing cells from diseased mice, the *Socs2* expression was determined to be about ten-fold increased (Fig 5a). Since different SOCS proteins can interact in both enhancing and inhibitory ways and in part have overlapping functions, it is possible that compensatory upregulation of other *Socs* genes might compensate for the loss of SOCS2 in BCR/ABL1 transformed cells.[21, 35] To test this hypothesis, we sorted GFP^{+} cells from diseased mice transplanted with *Socs2*$^{+/+}$ or *Socs2*$^{-/-}$ cells, as well as fresh CMPs from Socs2$^{+/+}$ and Socs2$^{-/-}$ mice, and measured the expression of individual members of the *Socs* gene family. In this analysis, no increase in gene expression was observed throughout the *Socs* gene family in *Socs2*-deficient cells (Fig 5b and Supplementary Fig 2), suggesting that other SOCS family members do not compensate for the lack of SOCS2. SOCS2 is known to act as a feedback inhibitor of STAT5 signaling, suggesting that a *BCR/ABL1* expressing cell with functional SOCS2 should have lower STAT5 phosphorylation compared to *Socs2*$^{-/-}$ cells. However, similar levels of STAT5 phosphorylation were detected in both *Socs2*$^{+/+}$ and *Socs2*$^{-/-}$ cells from leukemic mice, indicating that the effect of BCR/ABL1 bypasses SOCS2-mediated feedback inhibition of STAT5 (Fig 5c). Because also STAT3 has been suggested to be phosphorylated by BCR/ABL1,[36] we measured STAT3 and STAT5 phosphorylation in *BCR/ABL1* transduced *Socs2*$^{+/+}$ and *Socs2*$^{-/-}$ cells by intracellular FACS. Both the *Socs2*$^{+/+}$ and *Socs2*$^{-/-}$ cells had equally increased STAT5 phosphorylation compared to MIG control, while no increase in STAT3 phosphorylation was observed (Supplementary Fig 3).

Discussion

As a modulator of the JAK/STAT signaling pathway, a pathway with a demonstrated role in CML, the strong upregulation of *SOCS2* in CML has raised the question whether SOCS2 is involved in *BCR/ABL1*-induced transformation or whether it is part of an inadequate feedback loop.[6, 11-15] SOCS2 has been suggested to play a role as feedback inhibitor in certain types of cancers, but its role may be more complex, as it is variably reported as having both enhancing and inhibitory functions in normal cytokine induced signaling.[21-24, 37] As we observed a strong upregulation of *Socs2* in mouse long-term HSC, in accordance with recent findings in human candidate HSC,[16] we first explored the role of SOCS2 in normal HSCs using competitive bone marrow transplantation experiments. Although it could be speculated that high expression of *Socs2* in LT-HSCs would serve as a brake, keeping this cell population quiescent due to insensitivity to stimulating growth factors, *Socs2*$^{-/-}$ HSCs showed equal repopulation properties both short term and long-term, as wild type HSCs. Although we cannot fully exclude that *Socs2* deficiency may have a subtle effect on HSCs, our findings strongly suggest that *Socs2* is not critical for normal HSC function.

To evaluate the potential role of SOCS2 in CML, we used the previously established retroviral *BCR/ABL1* transduction and transplantation model,[31] comparing disease manifestation derived from *Socs2*$^{+/+}$ versus *Socs2*$^{-/-}$ cells. Mice transplanted with either donor types showed similar disease onset, displayed similar symptoms such as elevated WBC counts, splenomegaly and expansion of myeloid cells, and had similar survival curves. These findings demonstrate that SOCS2 is not crucial for disease initiation and propagation in this model of CML. However, the rapid disease course and the short survival times clearly pose a potential problem as weak phenotypes may be overlooked. In an attempt to investigate if a disease model with longer disease latency could be established, we also transduced and

transplanted fewer cells. However, transplantation of fewer MIG-*BCR/ABL1* transduced cells resulted in similar disease latency, but with a reduced penetrance with only a few recipients developing a CML-like disease (data not shown). Thus, using this well established model of murine CML-like disease, it is difficult to entirely exclude subtle effects of SOCS2 in the initiation and progression of CML.

It is also well possible that functional redundancy could exist between different members of the *Socs* gene family, which could explain the lack of phenotype in this model. However, examining the expression levels of other *Socs* family members in *Socs2*-deficient cells, we did not find any compensatory upregulation of the other *Socs* family members. This indicates that functional redundancy involving other *Socs* family members is an unlikely cause of the unaltered disease course in mice receiving *BCR/ABL1*-transduced *Socs2*$^{-/-}$ bone marrow cells. However, both *CISH* and *SOCS3* have previously been reported to be upregulated in CML patients.[13] Thus, future studies combining silencing of additional *Socs* members will be needed to ultimately clarify this issue.

BCR/ABL1 is well known to activate several downstream signaling mediators such as STAT5.[6, 38] The transcription of *Socs2* is normally induced by STAT5 activation, and the SOCS2 protein typically acts as a feedback inhibitor upstream of STAT5 by targeting the interaction between JAK and cell surface receptors such as the GH receptor.[10, 27, 34, 39] In this study, we demonstrate that SOCS2 is dispensable for BCR/ABL1-mediated induction of CML, despite *Socs2* being highly upregulated by BCR/ABL1. Our finding that cells expressing *BCR/ABL1* have equal STAT5 phosphorylation independently of *Socs2*-deficiency supports a hypothesis that SOCS2 fails to act as a feedback inhibitor of the JAK2/STAT5 pathway in the context of BCR/ABL1 signaling. Because BCR/ABL1 has been shown to

activate both JAK2 and STAT5 directly,[8, 9, 36] it seems reasonable that the upstream inhibition of this pathway provided by SOCS2 would have minimal effects on BCR/ABL1 induced signaling, which is consistent with our results.

In conclusion, we have demonstrated that SOCS2 is dispensable for both normal HSC function and for CML like disease. These findings support the hypothesis that *Socs2* upregulation by BCR/ABL1 is caused by an inadequate or non-functional feedback loop induced by BCR/ABL1.

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Conflict of interests

None of the authors have any conflicting interests to declare.

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Figure legends

Figure 1. a. Comparison of *Socs2* expression in various FACS sorted hematopoietic bone marrow populations, analyzed by Affymetrix microarray. Expression data was acquired from the Gene Expression Omnibus data set GSE 14833 and normalized using the GCRMA method (see Materials and Methods). The expression values provided represent relative *Socs2* expression values for the different cell populations.
b. Validation by RT-QPCR confirmed that *Socs2* is expressed at high levels in HSCs compared to other hematopoietic cell populations. *Socs2* expression is presented as fold-change compared to the *Socs2* expression in lin^- cells, with *Gapdh* as endogenous control.

Figure 2. a. Frequency of hematopoietic stem cells (in percent) determined by FACS phenotyping of bone marrow cells from $Socs2^{+/+}$ and $Socs2^{-/-}$ mice. The LT-HSC population was defined by the markers $LSKCD34^-flt3^-$ (top panel) or by LSK and the alternative SLAM markers $CD150^+CD48^-$ (lower panel). **b and c.** Bone marrow reconstitution in peripheral blood at 4 (a) and 18 weeks (b) after competitive bone marrow transplantation. Repopulation is shown as the percentage of donor cells compared to the total amount of donor and competitor cells. Bars indicating lineage distribution show the donor cell population.

Figure 3. a. Survival of mice after transplantation of *BCR/ABL1* expressing cells. Six out of nine mice transplanted with empty MIG vector were sacrificed at day 28 to extract organs for analysis. **b.** White blood cell counts in peripheral blood, measured 14 to 16 days after transplantation. **c.** Spleen weight of leukemic mice at euthanasia. **d.** Representative FACS analysis of bone marrow cells from a mouse with *BCR/ABL1*-induced disease. The table summarizes the dominating lineage commitment of GFP^+ expressing cells in bone marrow. Gr-1 or Mac-1 was used as myeloid markers and B220 to detect B cells.

Figure 4. Histopathologic staining of bone marrow and spleen after disease onset. **a.** Hematoxylin eosin staining of bone marrow sections showing increased granulopoiesis and enlarged sinusoids in mice transplanted with both *Socs2*$^{+/+}$ and *Socs2*$^{-/-}$ cells compared to MIG. **b.** Hematoxylin eosin staining of spleen with marked pathology, including severe infiltration of hematopoietic cells at various maturation stages, after *Socs2*$^{+/+}$ and *Socs2*$^{-/-}$ transplants.

Figure 5. Gene expression analysis and western blot of STAT5 phosphorylation of bone marrow cells from diseased mice. The cells were sorted before analysis, selecting GFP^{+}Mac1^{+} leukemic *Socs2*$^{+/+}$ or *Socs2*$^{-/-}$ cells and Mac1^{+} MIG control cells. **a.** Fold difference in expression of *Socs2* in leukemic *Socs2*$^{+/+}$ cells relative to empty MIG control, with *Actb* as endogenous control. Transduced cells show increased expression *of Socs2* in *BCR/ABL1* expressing cells. **b.** Expression of individual members of the Socs gene family in *Socs2*$^{+/+}$ versus *Socs2*$^{-/-}$ cells. The expression of each *Socs* family member in *Socs2*$^{-/-}$ cells is presented as fold-change relative to *Socs* expression in the *Socs2*$^{+/+}$ cells using *Actb* as endogenous control. **c.** Western blot of the total STAT5 protein levels in sorted GFP^{+} bone marrow cells (the upper section) used as a control for the phosphorylation rate of STAT5 (the lower section).

Figure 1

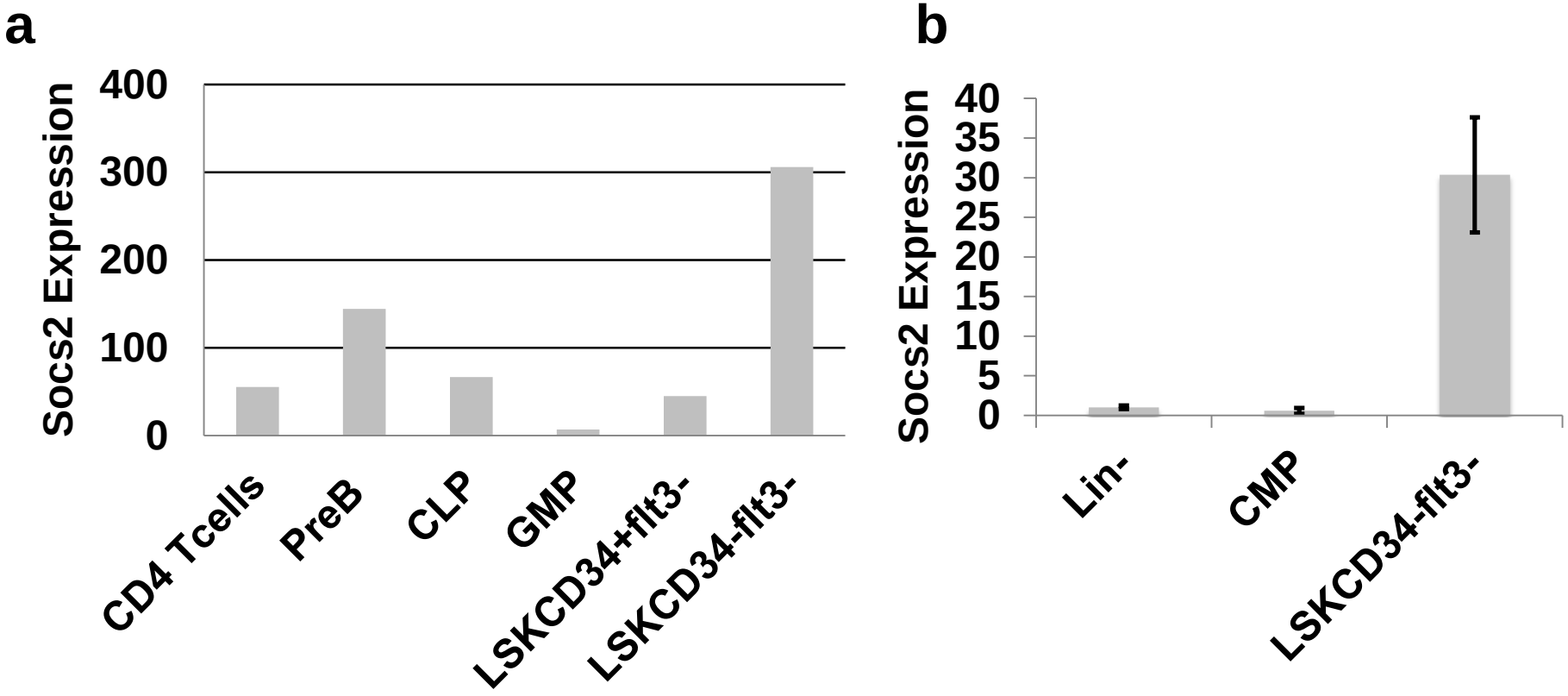
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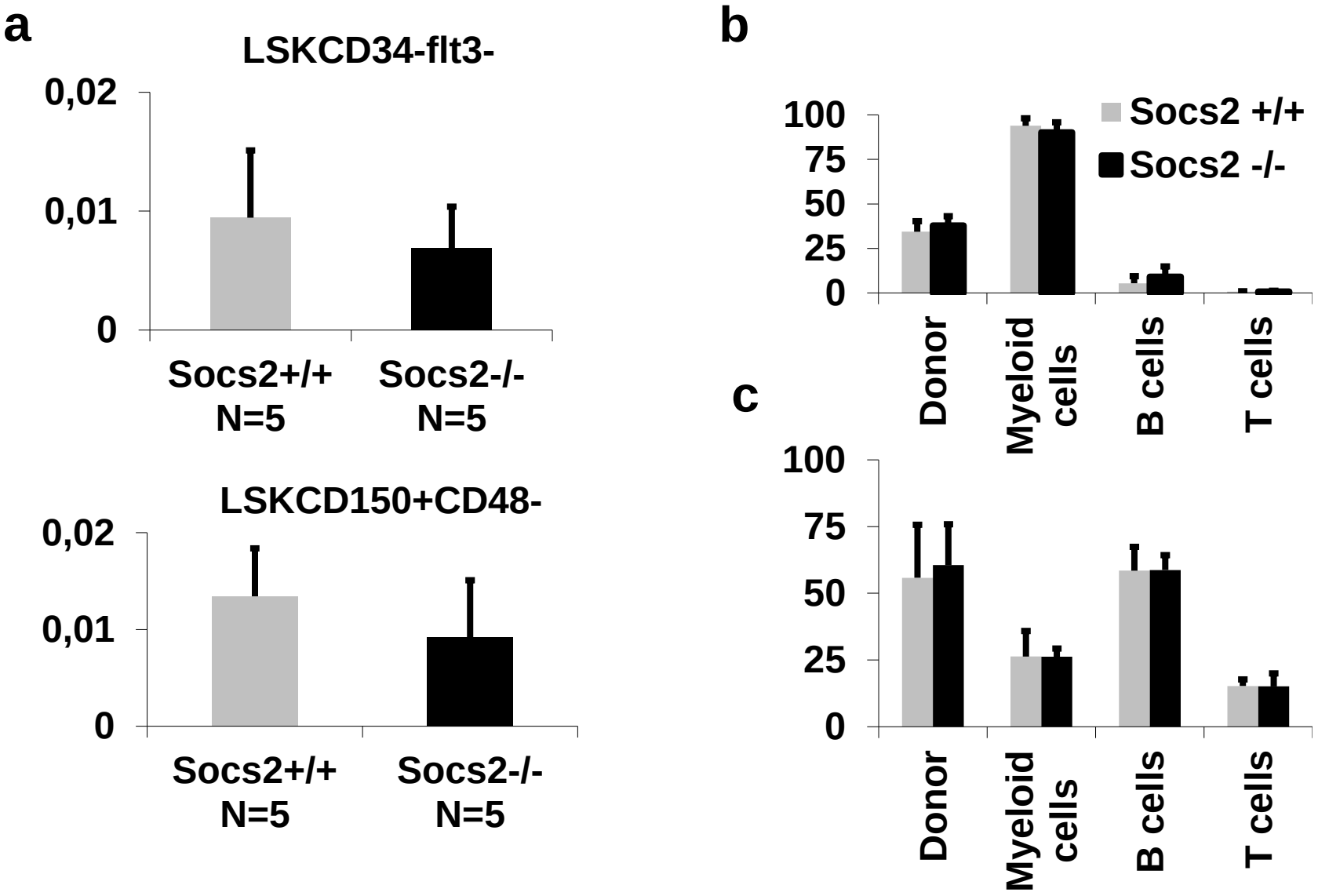
Figure 2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Socs2 -/-
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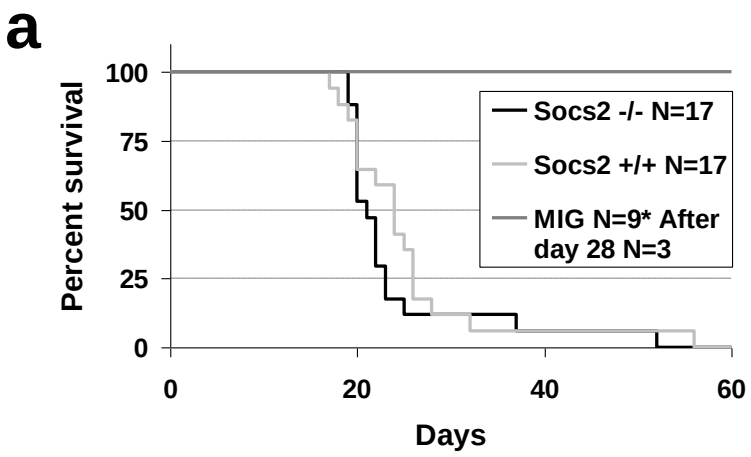
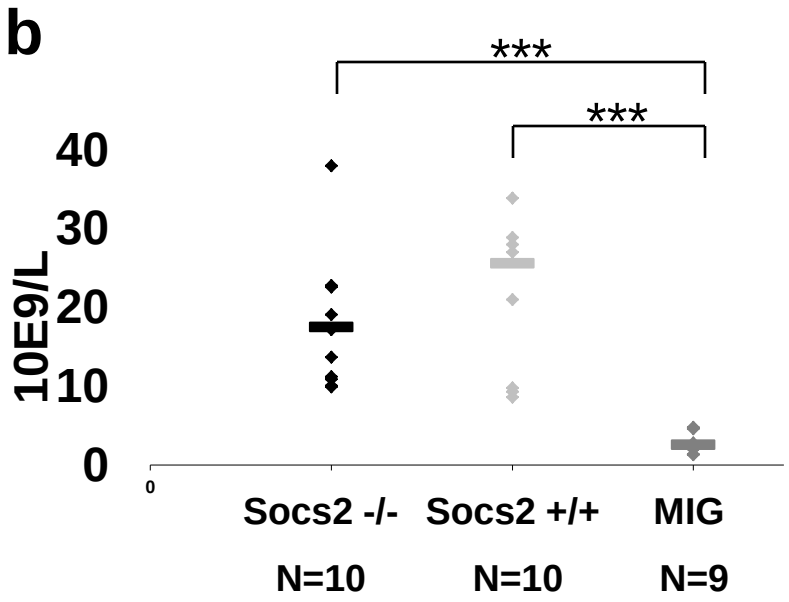
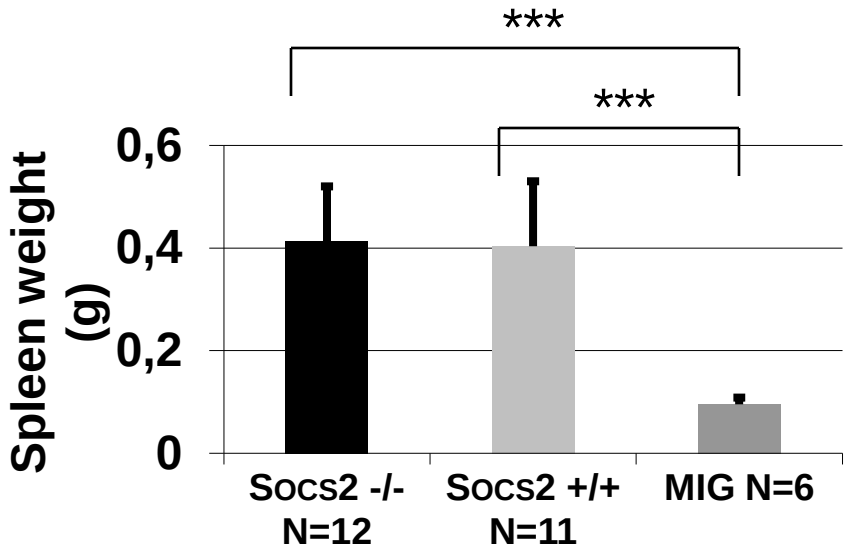
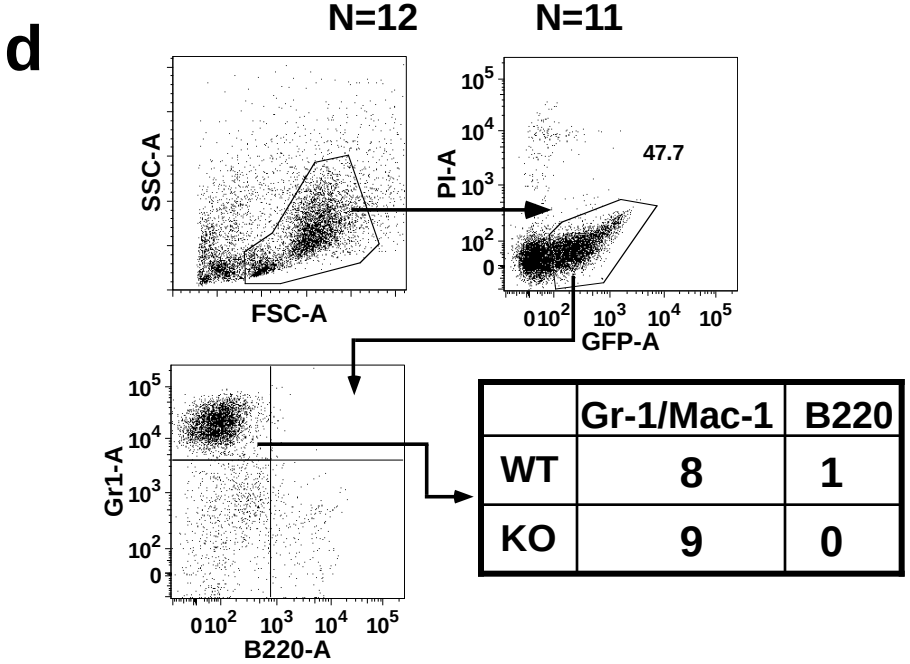
Figure 3









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WT	8	1
KO	9	0

Figure 4

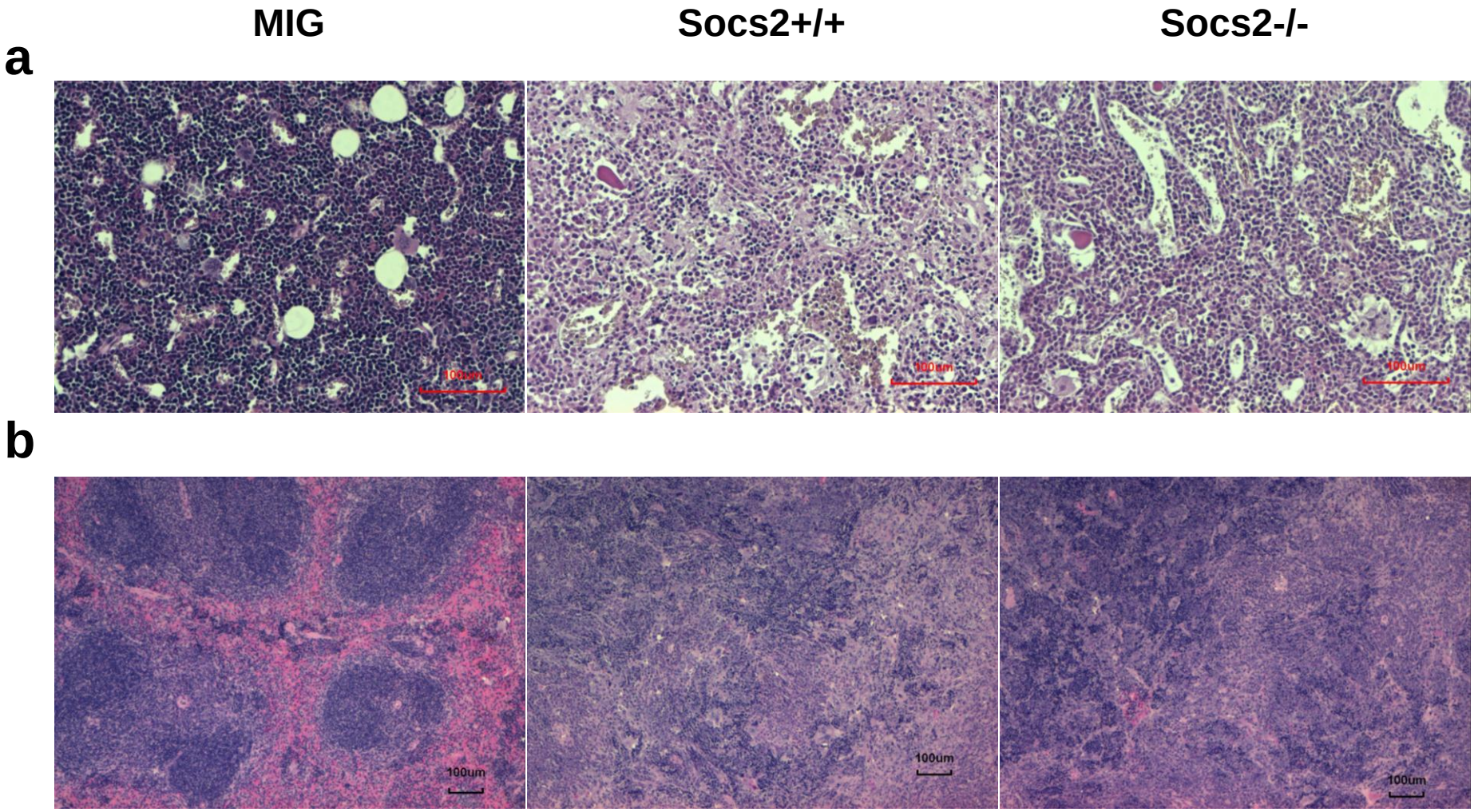
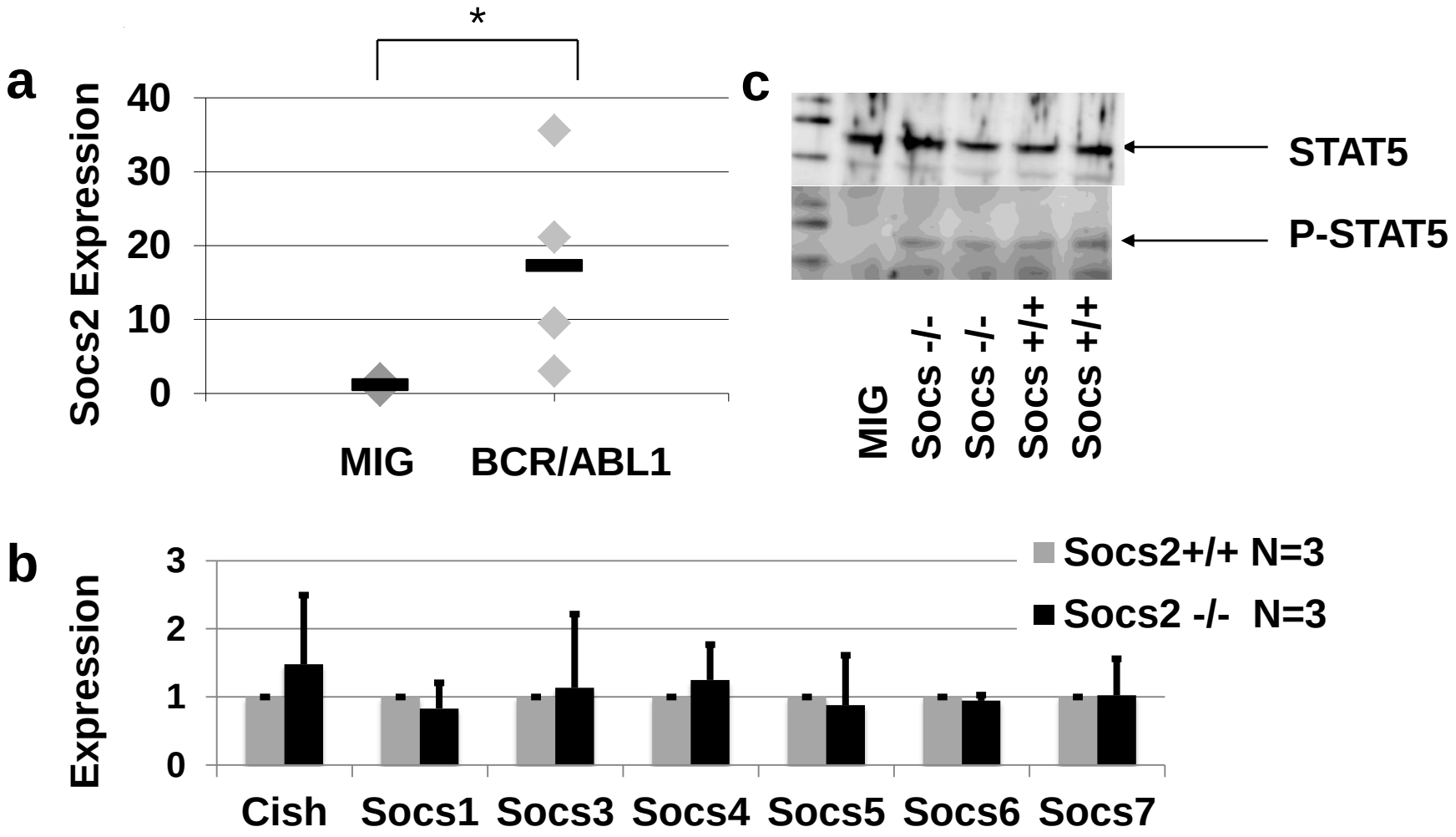


Figure 5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STAT5
P-STAT5
MIG
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Socs -/-
Socs +/+
Socs +/+
b
Expression
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2
1
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Socs2 -/- N=3
Cish
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Socs3
Socs4
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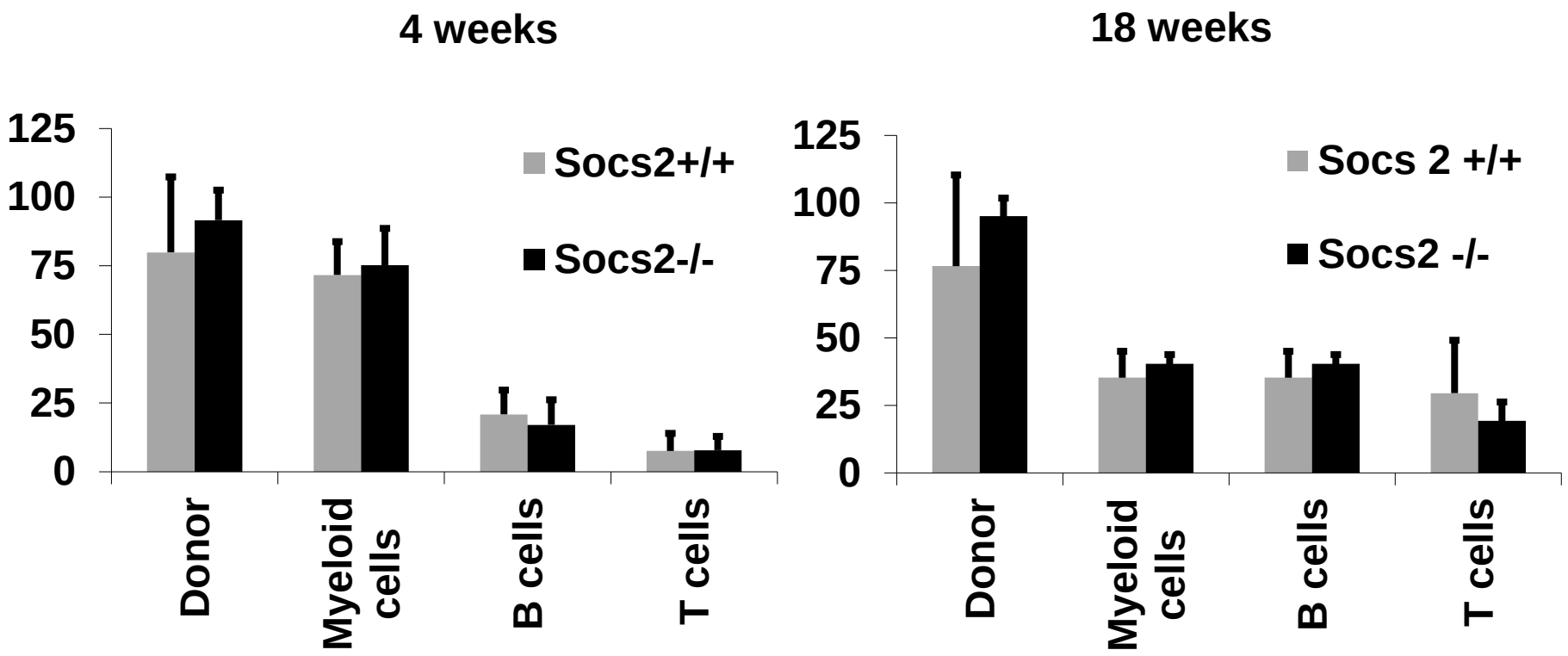
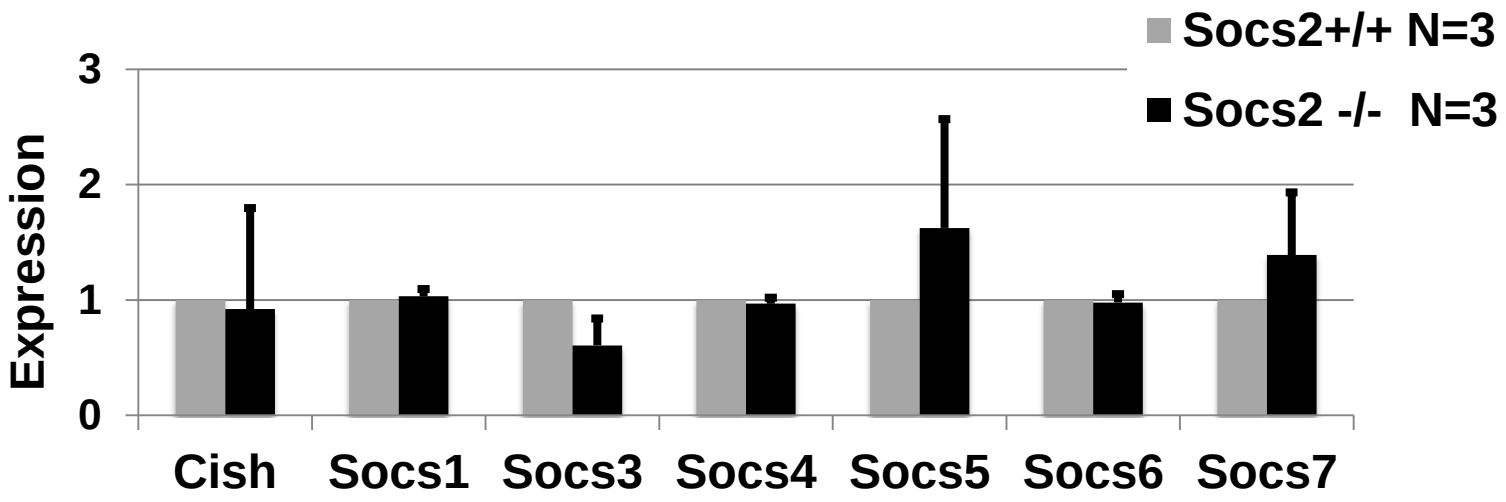
Socs6
Socs7

Supplemental figure 1



Supplemental figure 2



Supplemental figure 3