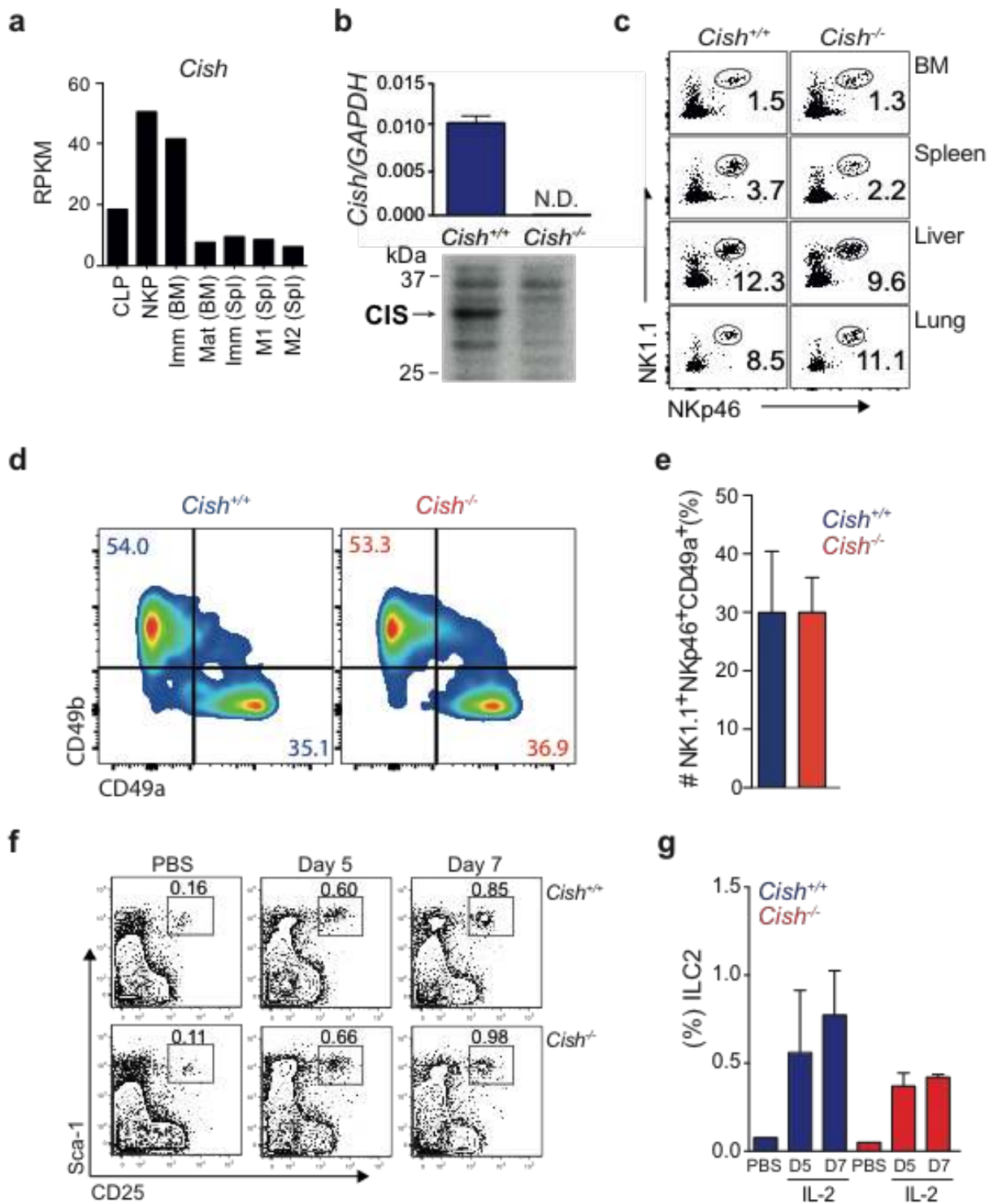


Delconte et al, Supplementary Figure 1



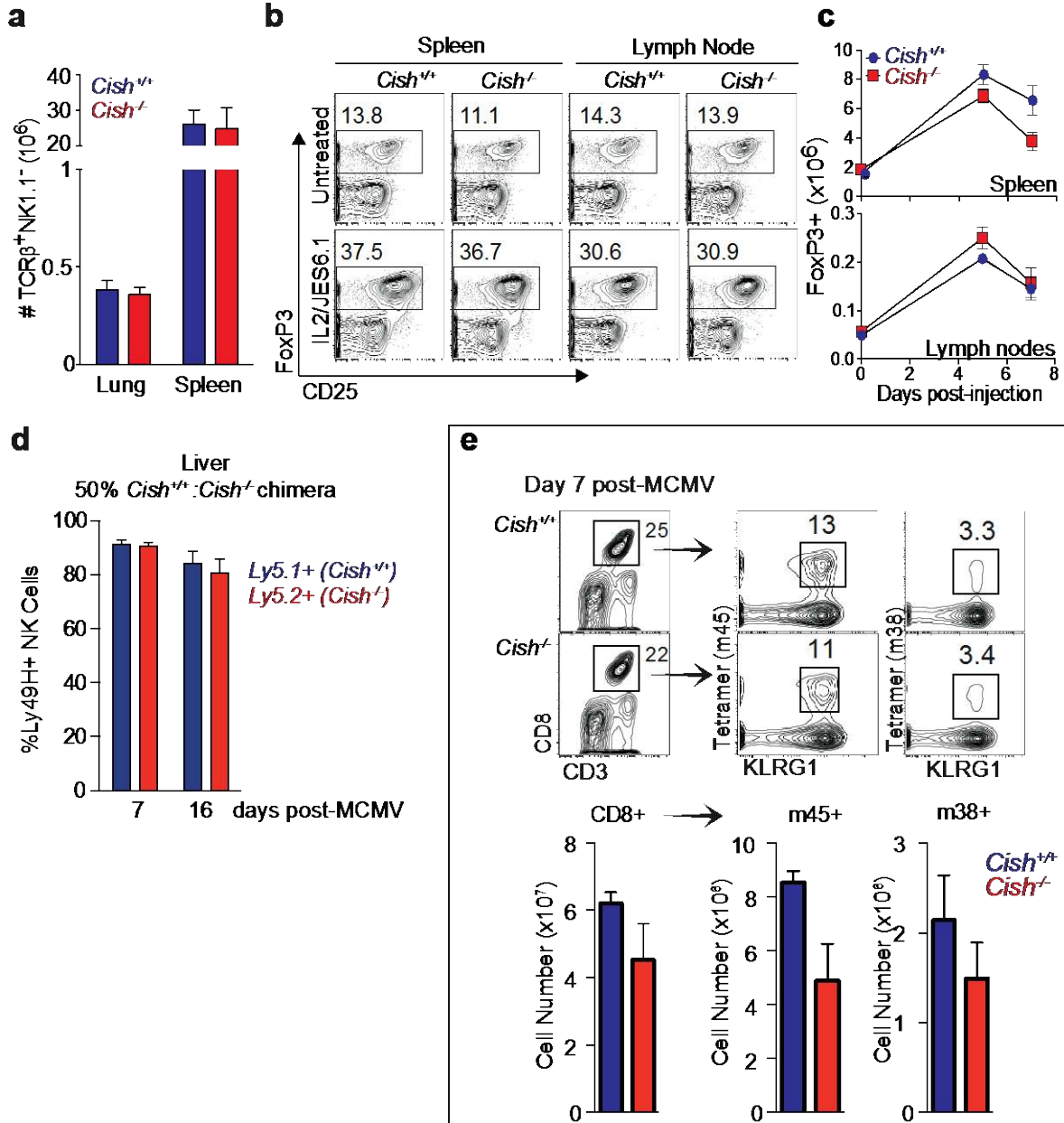
Supplementary Figure 1

Analysis of NK, ILC1 and ILC2 in *Cish*-deficient mice

(a) *Cish* expression in NK cell subsets from C57BL/6 mice. Shown as reads per kilobase of exon per million reads (RPKM). Subset definition and RNAseq data have been described previously¹⁻³. **(b)** *Cish*^{+/+} or *Cish*^{-/-} NK cells were cultured in IL-15, lysed and *Cish* mRNA analyzed by Q-PCR. Data were normalized to expression of GAPDH mRNA (upper panel). N.D.: not detected. *Cish*^{+/+} or *Cish*^{-/-} NK cells were cultured in IL-15 and the proteasomal inhibitor MG132 for 4 h prior to cell lysis and CIS protein detected in whole cell lysates by Western blotting (lower panel). **(c)** **NK cells** (NK1.1⁺NKp46⁺TCR-β⁻) were analyzed in the indicated organs from *Cish*^{+/+} and *Cish*^{-/-} mice by flow cytometry. **(d)** **ILC1** (NK1.1⁺NKp46⁺TCR-β⁻CD49a⁺CD49b⁻) in the liver of *Cish*^{+/+} and *Cish*^{-/-} were analyzed by flow cytometry and **(e)** quantified (vertical axis: % of ILC1). **(f)** **ILC2** *Cish*^{+/+} and *Cish*^{-/-} mice were treated with PBS or IL-2 complexed with anti-IL-2 antibodies (IL-2-JES6.1; expansion of CD25⁺ cells) every 2 days and were sacrificed after 5 or 7 days (D5, D7). Representative flow cytometry plots of ILC2 in the bone marrow gated on CD3/19/NK1.1/B220/Gr1 negative cells. **(g)** Frequency of ILC2 in the bone marrow following IL-2-JES6.1 treatment. **(b, e, g)** Mean ± s.e.m. n=3 biological replicates.

- 1 **Delconte, R. B. *et al.* The Helix-Loop-Helix Protein ID2 Governs NK Cell Fate by Tuning Their Sensitivity to Interleukin-15. *Immunity* 44, 103-115, doi:10.1016/j.immuni.2015.12.007 (2016).**
- 2 **Revilla, I. D. R. *et al.* The B-cell identity factor Pax5 regulates distinct transcriptional programmes in early and late B lymphopoiesis. *EMBO J* 31, 3130-3146, doi:10.1038/emboj.2012.155 (2012).**
- 3 **Holmes, M. L. *et al.* Peripheral natural killer cell maturation depends on the transcription factor Aiolos. *EMBO J* 33, 2721-2734, doi:10.15252/emboj.201487900 (2014).**

Delconte et al. Supplementary Figure 2

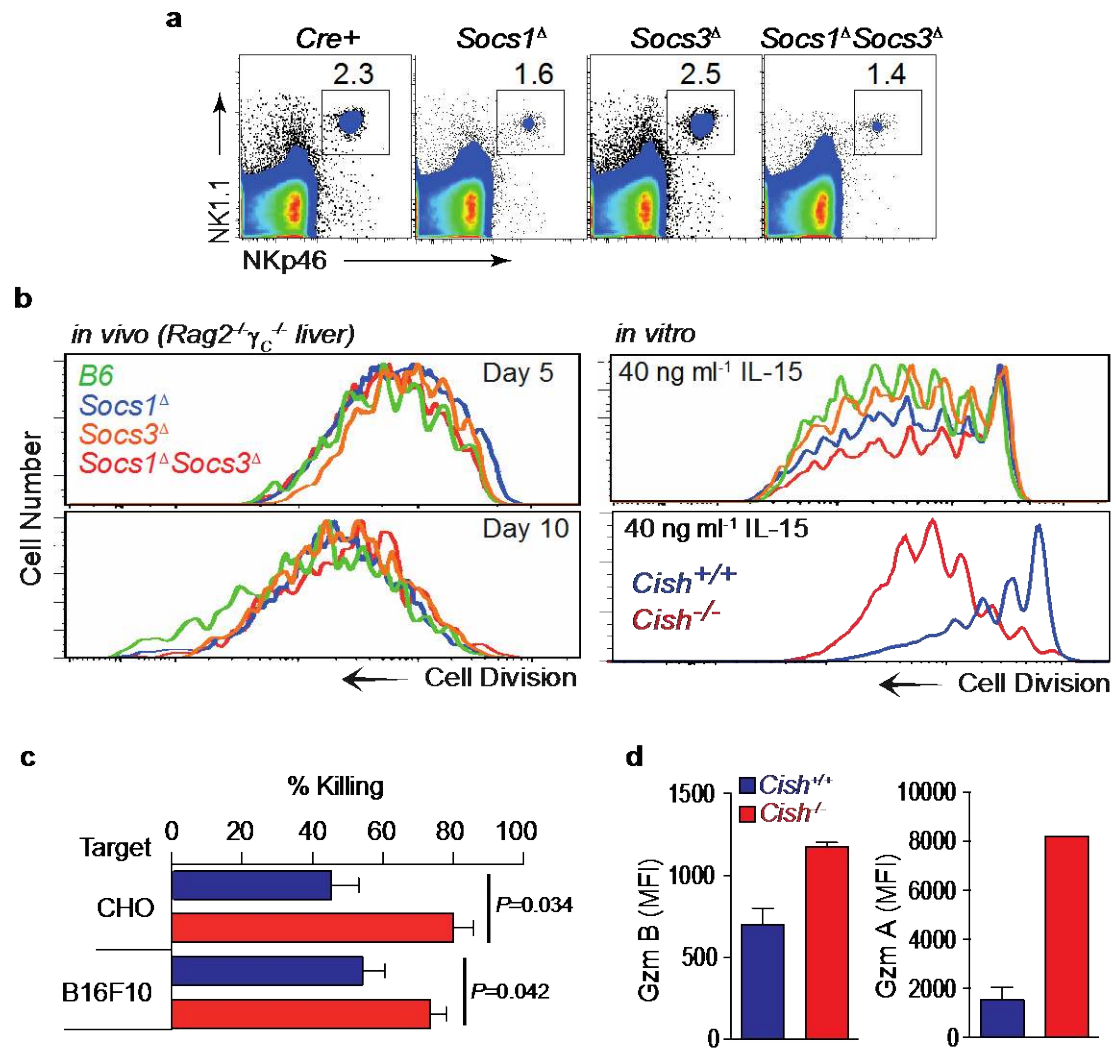


Supplementary Figure 2

Analysis of T cells, regulatory T cells and MCMV responses in *Cish*-deficient mice

(a) T cells (NK1.1⁻NKp46⁻TCR-β⁺) were analyzed in the indicated organs from *Cish*^{+/+} and *Cish*^{-/-} mice by flow cytometry. **(b) Regulatory T cells (Tregs)** Expression of FoxP3 and CD25 on CD4⁺ cells from spleen and lymph nodes of *Cish*^{+/+} and *Cish*^{-/-} mice before and 5 days after IL-2-JES6-1 treatment. Representative flow cytometry plots are shown. **(c)** Expansion and contraction of Tregs in the spleen and lymph nodes following IL-2-JES6-1 complex treatment (Mean ± S.E.M., n=1-2 mice per group). **(d)** 50:50 bone marrow chimeras (5x10⁶ Ly5.1⁺ *Cish*^{+/+} and Ly5.2⁺ *Cish*^{-/-} cells) were injected intraperitoneally with 5x10³ plaque forming units (PFU) of salivary gland-propagated virus stock of MCMV-K181-Perth and Ly49H⁺ NK cells frequency monitored by flow cytometry. (Mean ± S.E.M., n=6 mice). **(e)** Intact *Cish*^{+/+} and *Cish*^{-/-} mice were injected intraperitoneally with 5x10³ plaque forming units (PFU) of salivary gland-propagated virus stock of MCMV-K181-Perth. On day 7 spleens were analyzed for MCMV⁺ CD8⁺ T cell responses by flow cytometry. Numbers of indicated tetramer⁺ CD8⁺ T cells are shown (mean ± s.e.m., n=5 mice)

Delconte et al. Supplementary Figure 3

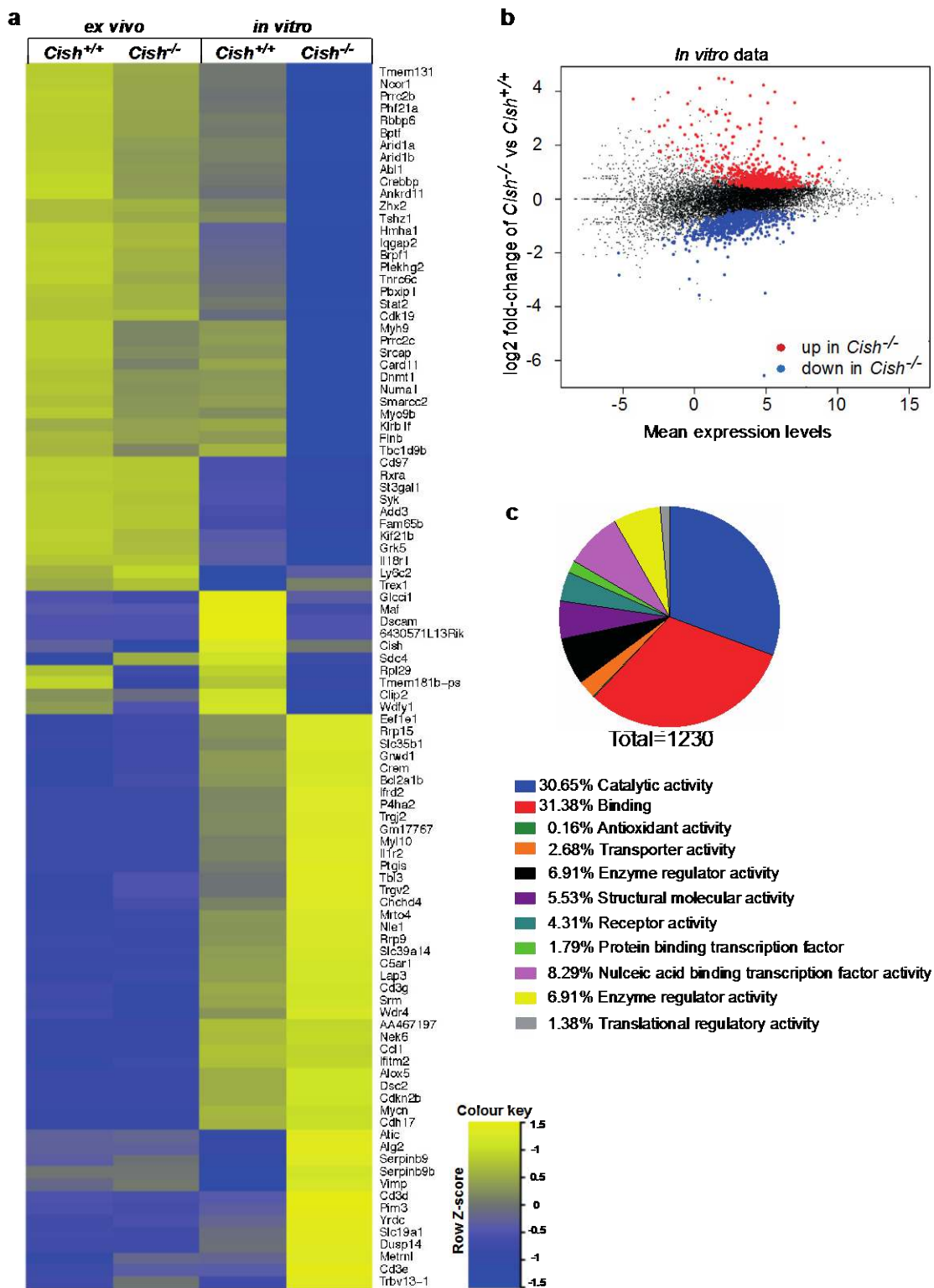


Supplementary Figure 3

Loss of *Socs1* and/or *Socs3* does not alter IL-15 responses in NK cells.

(a) *Socs3*^{+/+}*ERT2*^{Cre/+} (Cre+), *Socs1*^{-/-}*Ifn* γ ^{-/-} (*Socs1* Δ), *Socs3*^{fl/fl}*ERT2*^{Cre/+} (*Socs3* Δ), and *Socs1*^{-/-}*Ifn* γ ^{-/-}*Socs3*^{fl/fl}*ERT2*^{Cre/+} (*Socs1* Δ *Socs3* Δ) mice were treated with 4-hydroxytamoxifen (4-OHT; to induce *Socs3* deletion) by oral gavage and splenic NK cells (TCR- β ⁻NK1.1⁺NKp46⁺) analysed 14 days later by flow cytometry. Plots and values (%) are representative of 3 mice analysed for each genotype. **(b)** Splenic NK cells from mice in (a) were FACS sorted and cultured in IL-15 (50 ng ml⁻¹) for 7 days before being CFSE labelled and either i.v. transferred into alymphoid *Rag2*^{-/-} *γ C*^{-/-} recipients or cultured in IL-15 (50 ng ml⁻¹) *in vitro*. Five and ten days post-transfer, recipient livers were analysed for donor NK cells by flow cytometry. *In vitro* cultures were analysed on day 5. *Cish*^{+/+} and *Cish*^{-/-} NK cell cultures serve as a reference for differential proliferation (lower right panel). **(c) Enhanced effector function in *Cish*^{-/-} NK cells.** *Cish*^{+/+} and *Cish*^{-/-} NK cells were cultured for 7 days prior to co-culture with CHO or B16F10 target cells at a ratio of 1:1. Target cell killing at 5 h was determined by relative changes in electrical impedance using the xCELLigence system. *Cish*^{+/+} and *Cish*^{-/-} NK cells achieved maximal killing at 9:1 effector:target ratios (defined as 100% killing). **(d)** *Cish*^{+/+} and *Cish*^{-/-} mice were injected with RMA-m157 cells i.p and peritoneal NK cells analysed 18 h later for intracellular granzyme-B production by flow cytometry. Mean \pm SD of two experiments. n = 2 mice. MFI: Mean Fluorescence Index.

Delconte et al. Supplementary Figure 4

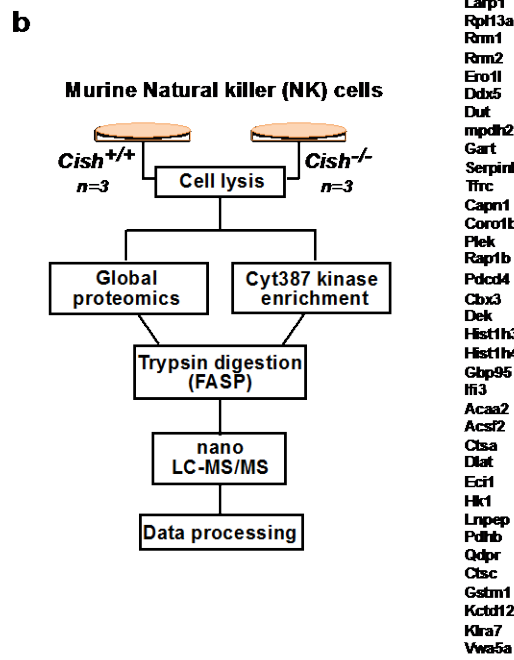
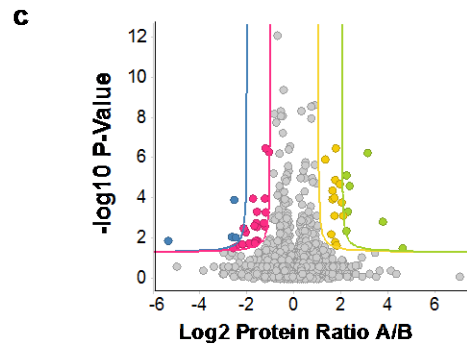
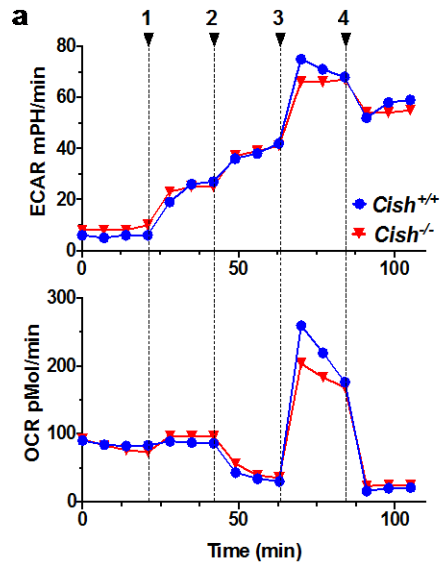


Supplementary Figure 4

Transcriptome profiling of *ex vivo* and *in vitro* cultured *Cish*^{-/-} NK cells

100bp single-ended RNAseq was performed on freshly sorted *ex vivo* *Cish*^{+/+} and *Cish*^{-/-} NK cells, and on *Cish*^{+/+} and *Cish*^{-/-} NK cells that had been cultured for 7 days in IL-15 (50 ng/ml). **(a)** Relative expression levels (Z-scores) of the top ~100 most differentially expressed genes in *Cish*^{-/-} cells are shown in the heatmap, color-coded according to the legend. Rows are scaled to have a mean of 0 and s.d. of 1. n=2 biological replicates. **(b)** Mean-difference plot of the cultured NK cell data generated in Figure 2, showing Log2-fold change versus mean expression. **(c)** Functional analysis of the 1230 differentially expressed genes observed in IL-15 cultured *Cish*^{-/-} NK cells. Gene ontology was performed using the PANTHER classification system. Major gene networks are shown as a percentage of total differentially expressed genes in *Cish*^{-/-} cells.

Delconte et al. Supplementary Figure 5

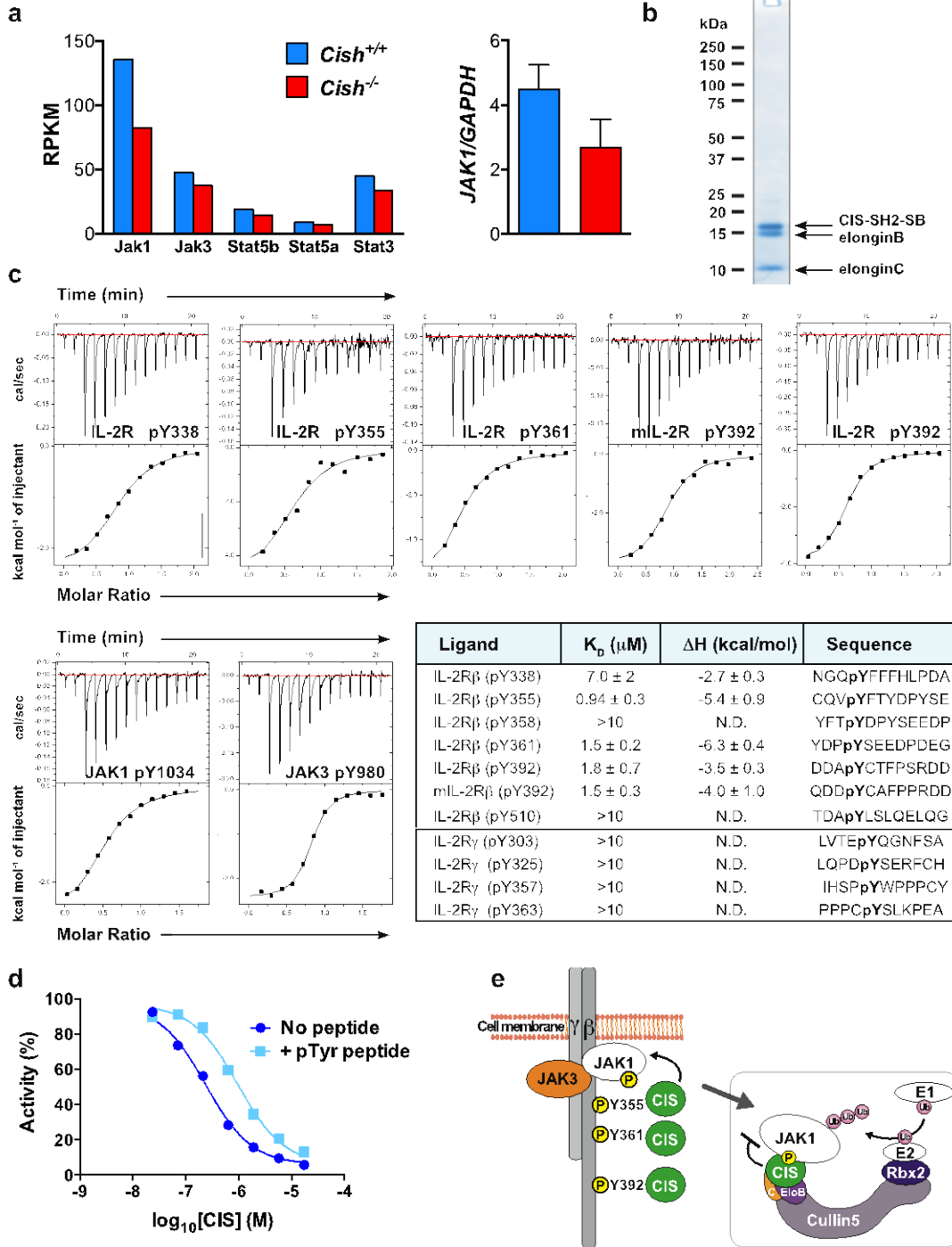


Supplementary Figure 5

Cish^{-/-} NK cells display increased JAK/STAT signaling and normal respiration and glycolysis.

(a) *Cish*^{-/-} NK cell respiration and glycolysis is unperturbed. *Cish*^{+/+} and *Cish*^{-/-} NK cells were cultured in the presence of IL-15 and the extracellular acidification rate (ACR; glycolysis) and oxygen consumption rate (OCR; mitochondrial respiration) measured using the XF Analyzer system. Glucose (1), Oligomycin (2), FCCP and pyruvate (3) and Antimycin A/Rotenone (4) were added at times indicated by the numbered arrows. **(b)** Overview of the proteomic workflow used in this study. Equal numbers of cultured NK cells derived from *Cish*^{+/+} and *Cish*^{-/-} mice were lysed and subjected to kinase enrichment using NHS-CYT-387 beads. Protein eluates from the CYT-387 resin, in addition to a portion of whole cell lysate (WCL; pre-kinase enrichment) were subjected to trypsin digestion and nanoLC-MS/MS. **(c)** Label-free quantification of global protein expression. Volcano plot showing the Log₂ protein ratios following the quantitative pipeline analysis (*Cish*^{+/+} vs *Cish*^{-/-}) from WCL. The red and yellow lines represent a 2-fold change in protein expression (log₂ ratio of 1), while blue and green lines represent a 4-fold change (log₂ ratio of 2); dots are colored accordingly and represent individual proteins. Proteins with a -log₁₀ p-value of 1.3 or greater (corresponding to a p-value of ≤ 0.05) were deemed differentially abundant. **(d)** Heat map displaying Log₂-transformed summed peptide intensities (non-imputed) for proteins with significantly differential expression in (d). Data from individual biological replicates are shown (n=3). Green to red indicates increasing expression levels. See also Extended Data **Table 2**.

Delconte et al. Supplementary Data Figure 6

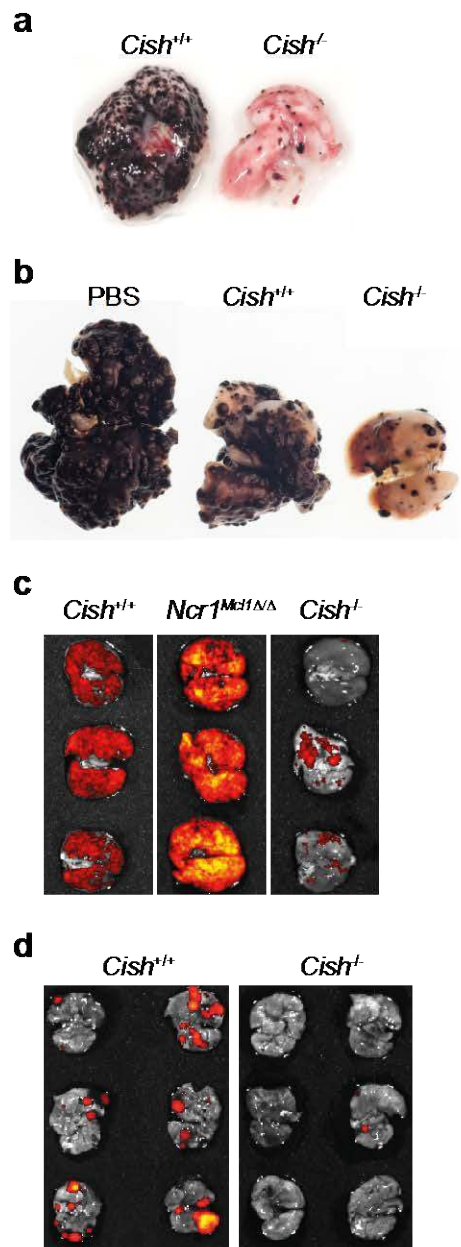


Supplementary Figure 6

CIS targets JAK and the IL-2R complex

(a) Cultured NK cells from wild-type and *Cish*^{-/-} mice were lysed, mRNA purified and analyzed by RNAseq. Mean RPKM values for duplicate samples (left panel). JAK1 mRNA levels were analyzed by Q-PCR (right panel). Mean and s.d., n=3. **(b)** 4-12% Coomassie-stained SDS-PAGE gel showing purified hCIS-SH2-SB, elongin B and elongin C complex (CIS-SH2-BC). **(c)** Isothermal calorimetry (ITC) was used to measure the affinity of hCIS-SH2-BC binding to phosphopeptides corresponding to tyrosines within the JAK1/3 kinase domain activation loops and IL-2R β and γ cytoplasmic domains. 300 μ M phosphopeptides were titrated into a 30 μ M solution of the GST-CIS-SH2-BC ternary complex. ITC titration curves and tabular view of some results (inset) showing average and range from two independent experiments. N.D.=Not detectable, p=phosphorylated. The titration curves all fitted well to a single-site model. **(d)** Kinase inhibition assays were performed with the kinase domain (JH1) of JAK1 in the presence of CIS-SH2-BC with and without excess JAK1-Y1034 phosphopeptide as a competitor. The pY1034 peptide partially reduced CIS-mediated inhibition. Data were normalised to no-CIS controls. **(e)** Diagram illustrating the *in vitro* E3 ligase ubiquitination components and proposed model for CIS-mediated inhibition of JAK activity, whereby CIS recruitment to the receptor complex promotes binding to active JAK1 and results in kinase inhibition and proteasomal degradation. eloB: elongin B; eloC: elongin C.

Delconte et al., Supplementary data Figure 7



Supplementary Figure 7

CIS-null mice resist tumor metastasis

(a) Metastatic burden in lungs of *Cish*^{+/+} and *Cish*^{-/-} mice 14 days following i.v injection of B16F10 melanoma cells (as in **Figure 7a**). (b) Metastatic burden in the lungs of NK cell-deficient (*Ncr1*^{Mcl1Δ/Δ}) mice injected i.v. with B16F10 melanoma cells and *Cish*^{+/+} or *Cish*^{-/-} NK cells or PBS (as in **Figure 7e**). (c) Metastatic burden in the lungs measured by imaging (IVIS; mCherry fluorescence) of *Cish*^{+/+}, *Cish*^{-/-} and *Ncr1*^{Mcl1Δ/Δ} (NK-null) mice 14 days following i.v injection of E0771-mCherry⁺luciferase⁺ breast cancer (as in **Figure 8a**). (d) Orthotopic E0771.LMB tumors generated as in (**Figure 8c**) were surgically removed at 400–600 mm³ and spontaneous lung metastases measured by IVIS (mCherry fluorescence) 14 days later.

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Supplementary Figure 8

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