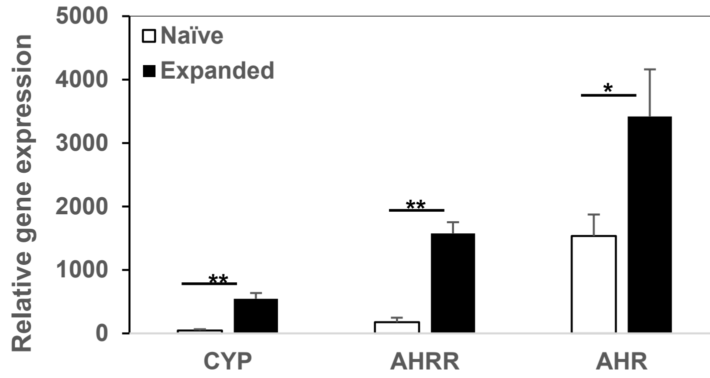
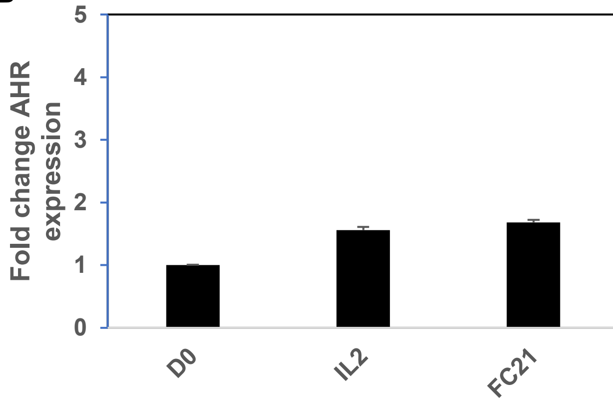
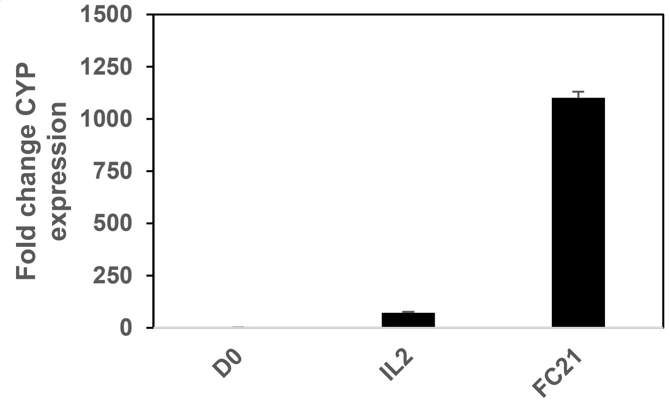
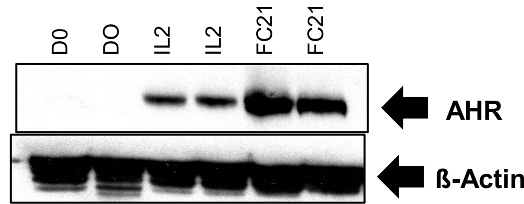
Figure S1









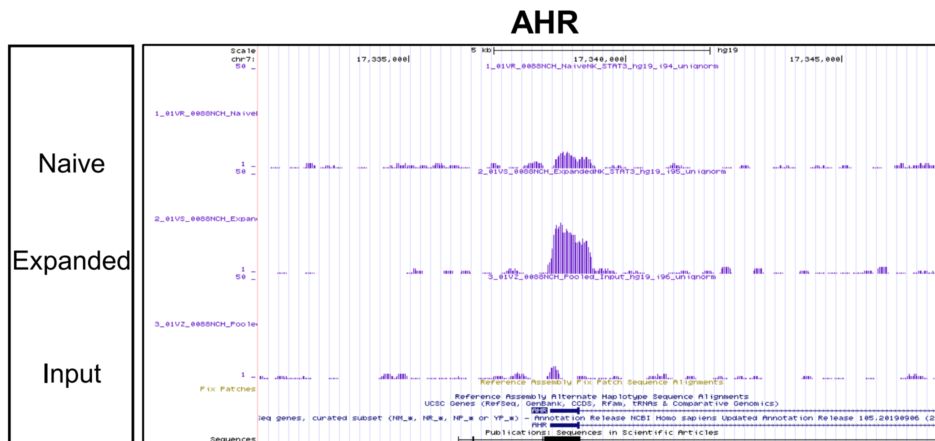


Fig S2

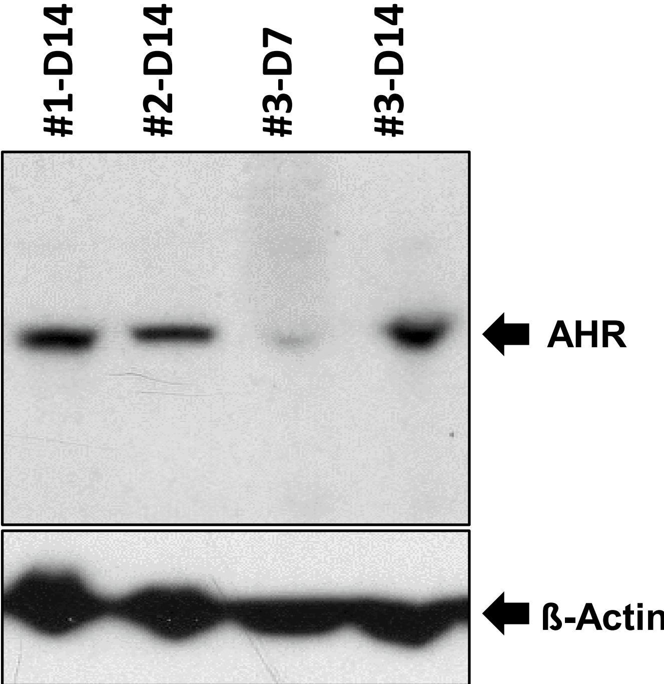


Fig S3

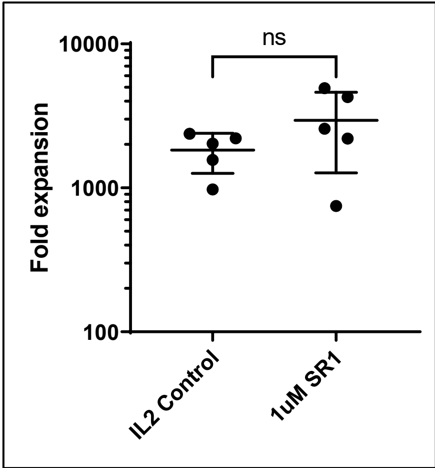
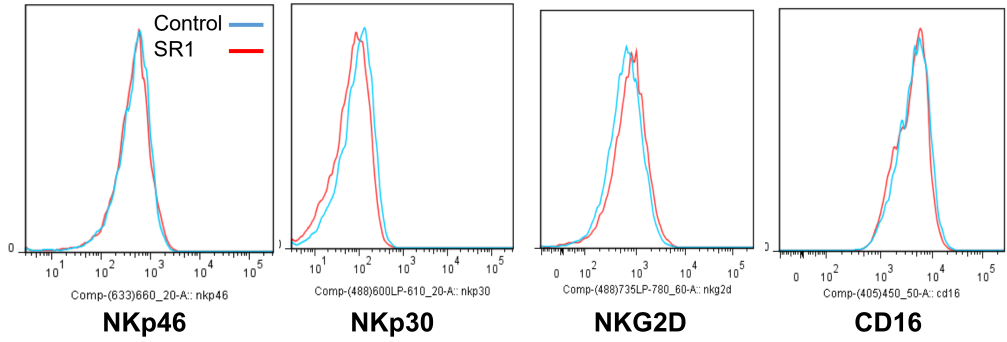


Fig S4

Day 7 NK cells



Day 14 NK cells

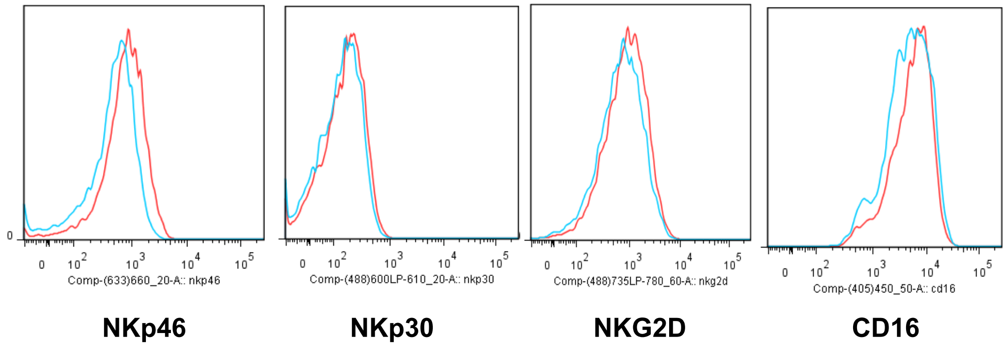
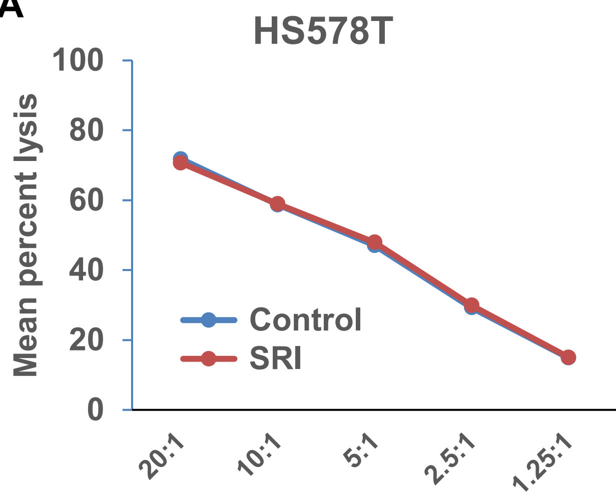
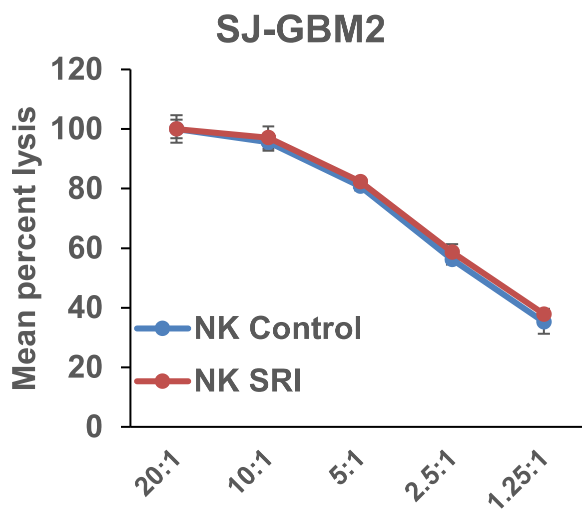


Figure S5

A





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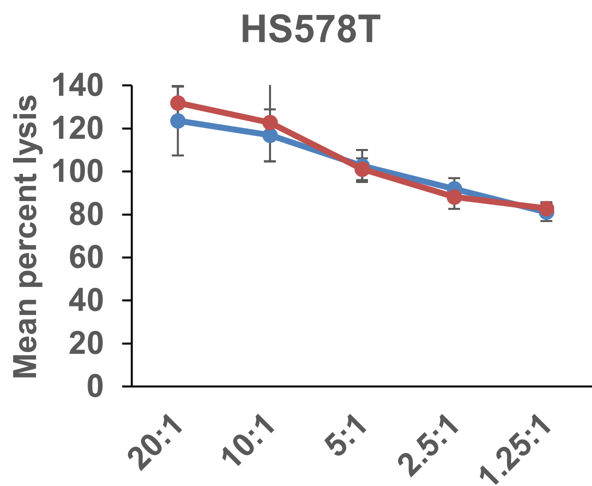


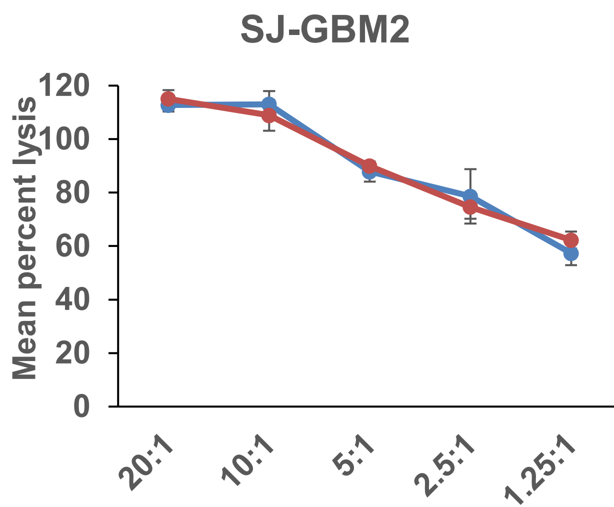


Fig S6

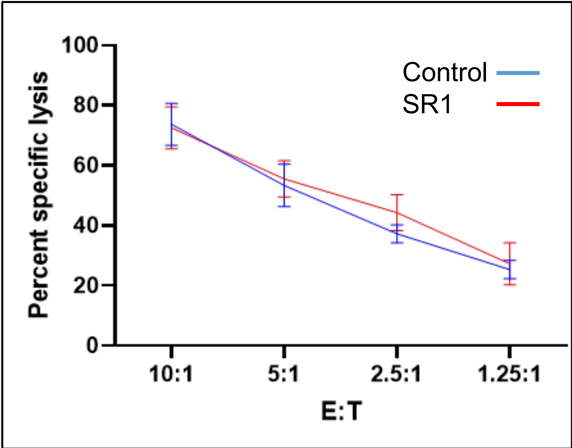
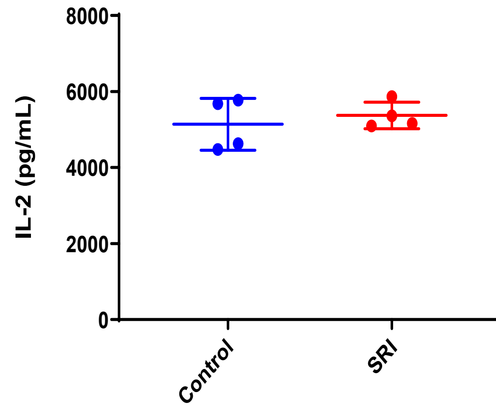


Fig S7

A



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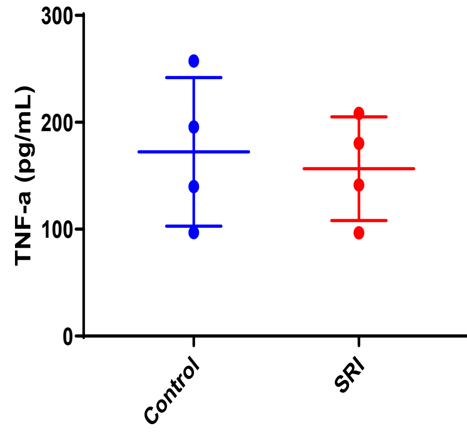

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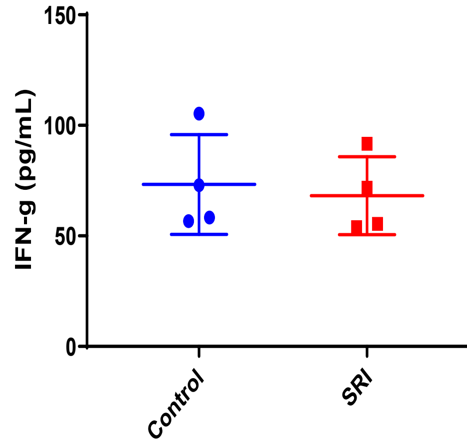

Fig S8

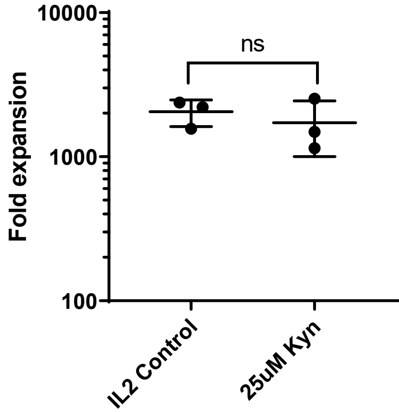

Figure S9

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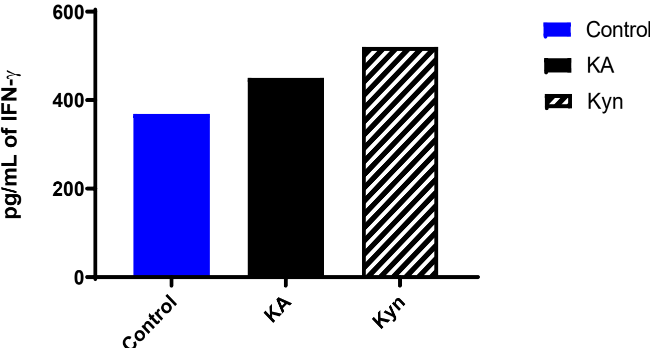

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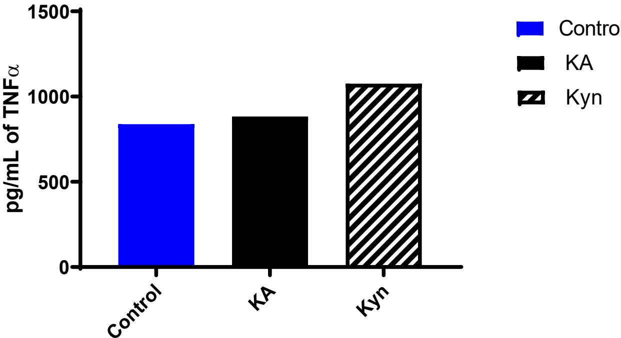

Fig S10

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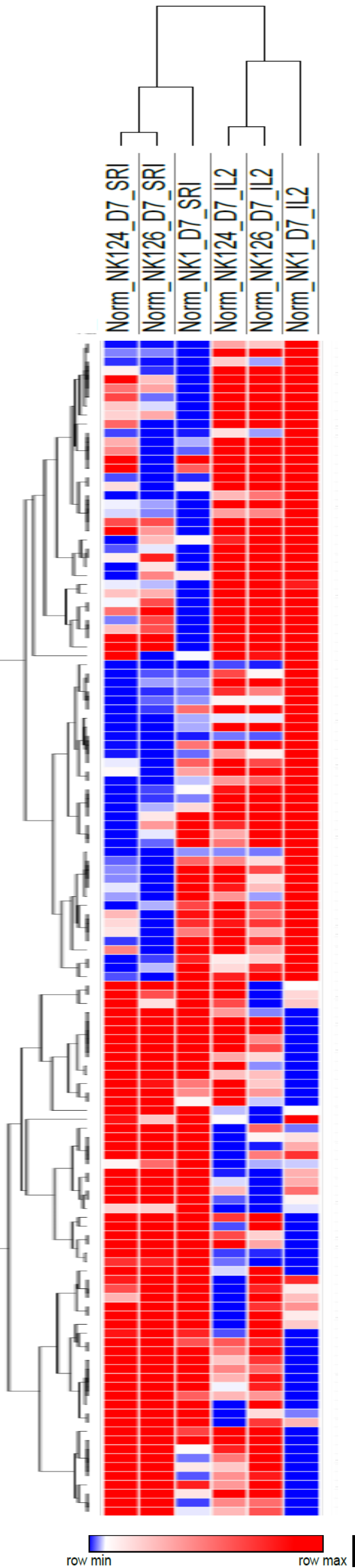

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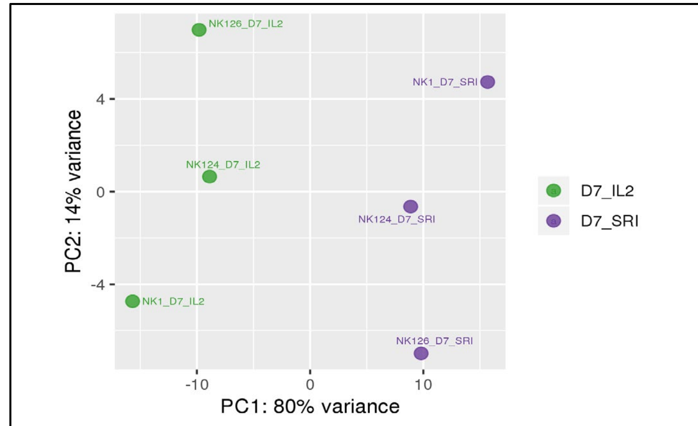

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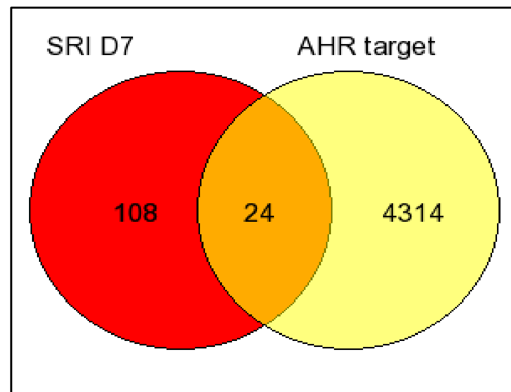


Figure S 11

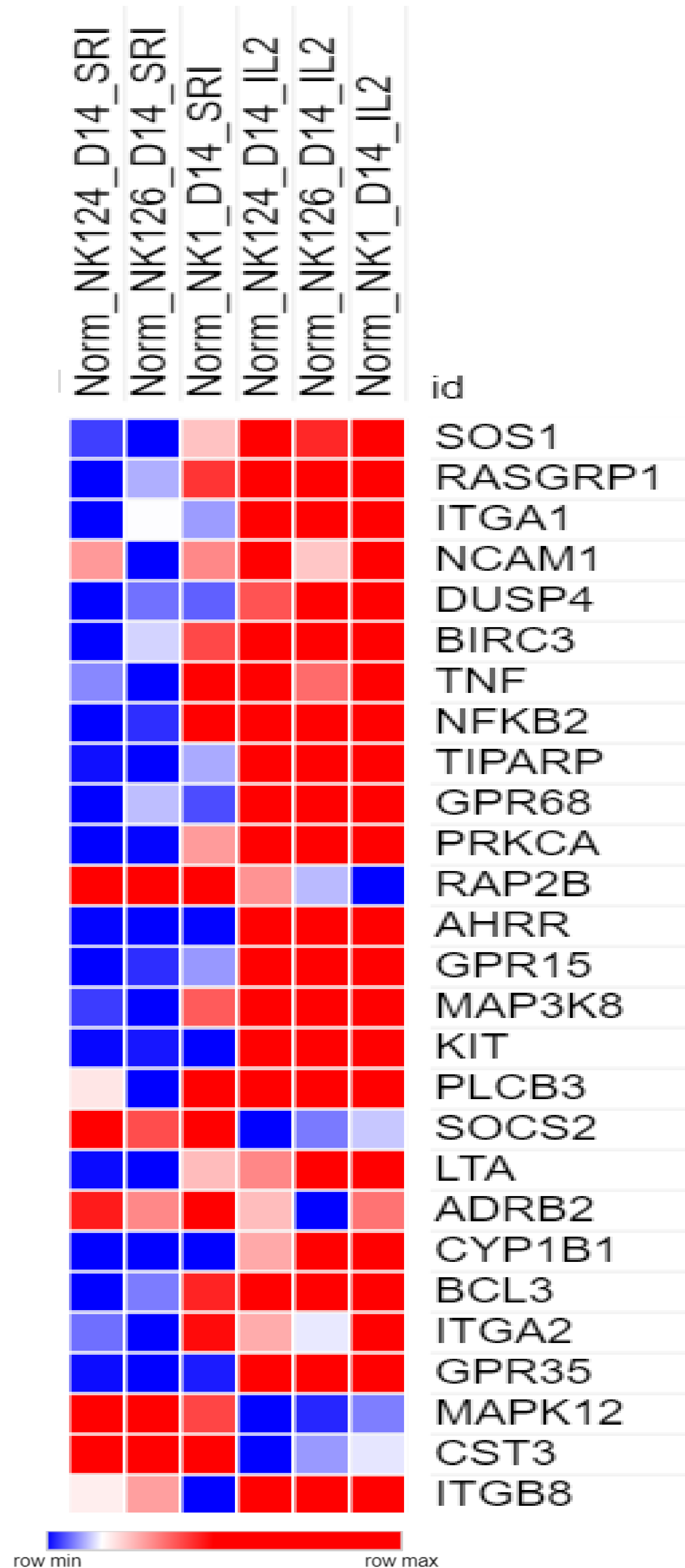


Figure S 12

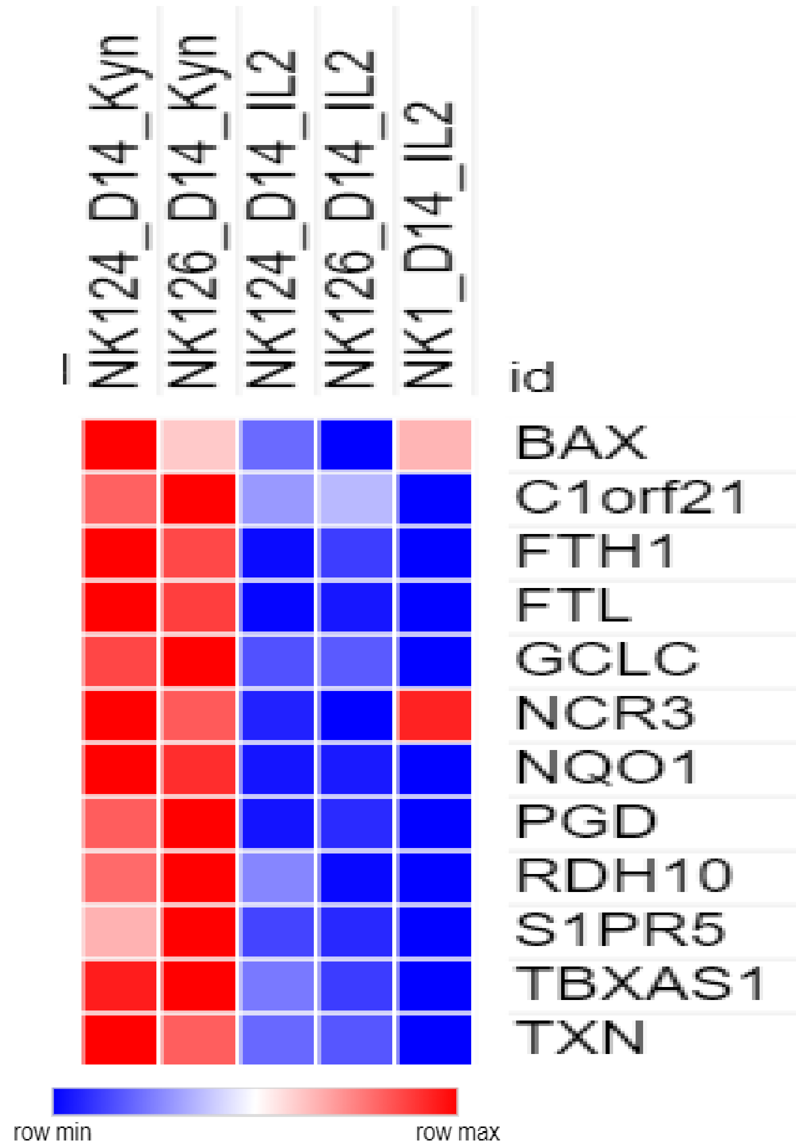
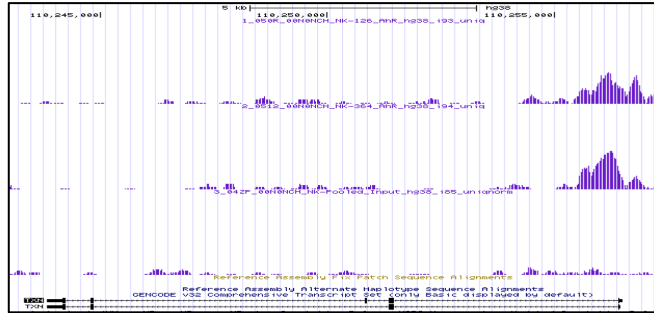
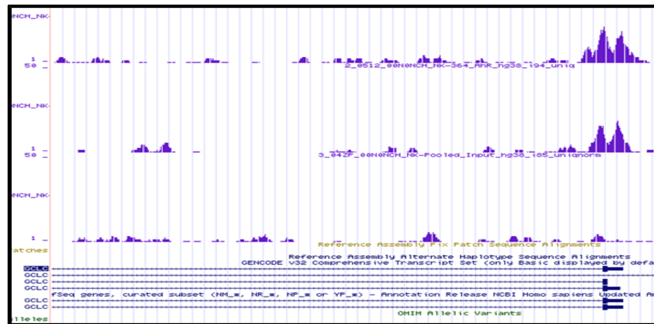


Figure S13

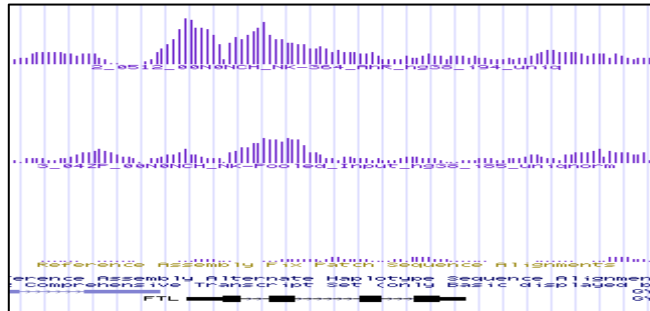
TXN



GCLC



FTL



PGD

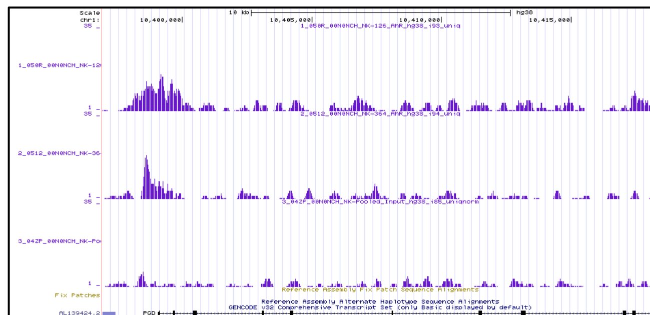
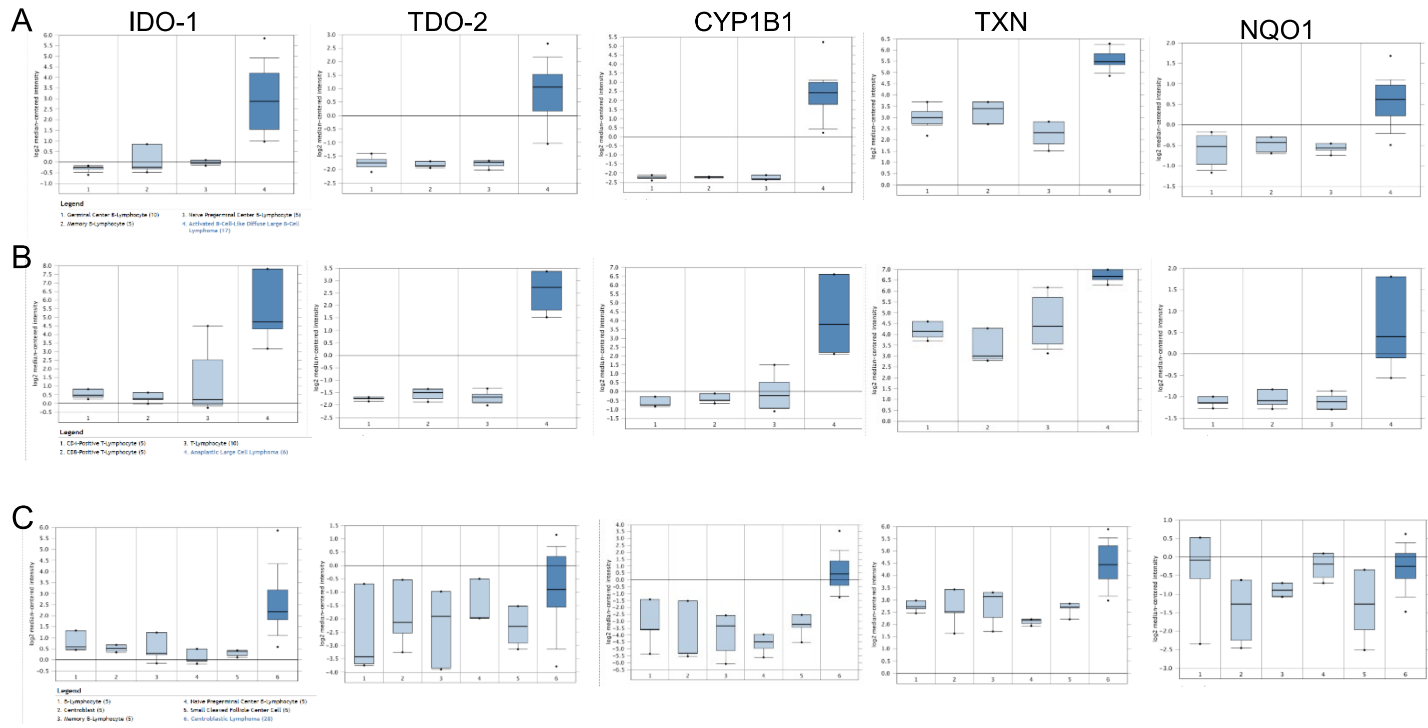


Figure S14



Supplementary Figures

S1 A. RNA seq analysis showing the expression of *AHR, AHRR* and *CYP1B1* in resting (naïve) and FC21 stimulated NK cells following two weeks of expansion. Data show mean ± SEM (n=8; **p<0.01; * p<0.05).

S1B-C. RT-PCR showing expression of AHR and CYP1B1 genes in NK cells expanded with cytokines. NK cells were expanded with cytokines IL2 (50IU/ml) without FC21 or with FC21 cells for two weeks. The value on the y-axis represents fold change in gene expression relative to day-0 (D0) NK cells.

S1D. Immunoblot showing the AHR expression in IL2 expanded NK cells. NK cells isolated from health donors were expanded using IL2 alone (50IU/ml) or FC21 (control) for two weeks.

S1E. ChIP seq showing the recruitment of STAT3 to the proximal regions of *AHR* gene in naïve and FC21 expanded NK cells. Naïve NK cells (top) and FC21 day 14 expanded NK cells (middle) and input sample (bottom).

S-2. Immunoblot showing the expression of AHR in FC21 expanded NK cells from multiple donors (#1, #2 and #3) on days 7 and 14.

S-3. NK cell proliferation was determined by counting the number of viable cells on day 14. NK cells isolated from healthy donors leukopak were expanded for a period of two weeks using FC21 feeder cells in RPMI media with IL2 (50IU/ml) and SR-1 (1um) or DMSO (control).

S4. AHR inhibition does not impact the expression of receptors on NK cells. Representative FACS histograms showing the expression of receptors on NK cells after 2-weeks of expansion with SR-1 or control (DMSO).

S5. Standard cytotoxicity assays in FC21 expanded NK cells. The cells were treated overnight with SR-1 or DMSO and tested against calcein labeled SJGBM2 or HS578T target cells.

S6. Standard cytotoxicity assays using primary NK cells (day 0) treated overnight with SR-1 against SJGBM2 cells.

S7. Cytokine levels in SR-1 expanded NK cells. NK cells expanded using FC21 were treated overnight with SR-1 or DMSO (control) were then co-cultured for 4-hours with K562 cell. The levels of cytokine in the supernatants were measured the using cytokine bead array using FACS. A. IL-2 B. Tumor necrosis factor α (TNF α) C. Interferon γ (IFN γ)

S8. NK cell proliferation determined by counting the number of viable cells at day 14. NK cells isolated from healthy donors were expanded for a period of two weeks using FC21 feeder cells in RPMI media with IL2 (50IU/ml) and kynurenine (25um) or DMSO (control).

S9. Levels of cytokines in NK cells treated overnight with Kyn (25um) or KA (25uM). NK cells were co-cultured for 4-hours with SJGBM2 cell. The levels of cytokines were measured by intercellular staining FACS. A. Interferon γ (IFN γ) B. Tumor necrosis factor α (TNF-α).

S10. A. Heatmap showing hierarchical clustering of RNA seq analysis using DEG between SR-1 and DMSO (control) in day-7 expanded NK cells. B. PCA analysis of gene expression from day 7 NK cells expanded with SR-1. Principal components. X- and Y- axis show 80% and 14% of the total variance, respectively. C. Venn diagram showing the number AHR targets genes in NK cells expanded with SR-1 as identified by AHR-ChIP seq on day 7.

S11. Heatmap showing the expression a subset of AHR target genes from D14 SR-1 expanded NK cells.

S12. Heatmap showing the expression of AHR targets gene in NK cells expanded with kynurenine on day 14.

S13. CHIP seq showing the binding of AHR to gene promoters. Thioredoxin (*TXN*), glutamate cysteine ligase (*GCLC*), ferritin light chain (*FTL*) and 6-phosphogluconate dehydrogenase (*PGD*).

S14. Tryptophan metabolites regulate the expression of AHR target genes in lymphoma patients. Expression of the genes were significantly increased ($p<0.01$) in patients with lymphoma relative to normal lymphocytes in A. Compagno dataset showing patients with diffuse large B cell

lymphoma. B. Picculuga dataset in patients with anaplastic cell lymphoma C. Basso dataset showing the patients with centroblastic lymphoma. y-axis shows the normalized expression values. Number in paratheses indicate the number of patients in each group.

Methods

Cell Lines

Cell line identity was authenticated using STR fingerprinting (Idexx Biosciences, Columbia, MO, USA) and routinely tested for mycoplasma contamination. NK cells were cultured in RPMI 1640 media (HyClone) plus Glutamax (Gibco), 10% FBS (HyClone), and 1% Penicillin/Streptomycin (Gibco). CSTX002 feeder cell line was generated by transducing K562 cells with human 4-1BBL and membrane-bound human IL-21 (FC21), as previously described [1]. HS578T cells were purchased from ATCC, the cells were cultured using DMEM high glucose media (Hyclone) with 10% FBS and 1% P/S. SJ-GBM2 cells were cultured in IMDM media (HyClone) plus 20% FBS, 1% Glutamax, and 1% P/S supplemented with 1% insulin-transferrin-selenium (ITS-Gibco #41400045).

NK Cell Isolation

NK cells were derived from peripheral blood or buffy coats of healthy volunteer donors. NK cells were isolated from whole blood or buffy coats using Rosette-Sep (StemCell Technologies, Vancouver, Canada) density-gradient centrifugation as previously described [1]. To generate enough numbers of NK cells for phenotypic and functional studies. NK cells were expanded by stimulation with FC21 feeder cells for 14–21 days as previously described [2]. Briefly, irradiated FC21 feeder cells were added to the NK cell culture conditions at a ratio of 1:2 (effector:target) on day 0 and 1:1 on days 7 and 14. All NK cells were expanded using 50 IU/mL recombinant human IL-2 alone or with the following: Stemregenin-SR-1(1uM, StemCell Technologies #72342), kynurenine (25uM, Tcoris Bioscience) or kynurenic acid (25um, Tcoris Bioscience). The media was changed every 2-3 days.

Cytotoxicity Assays

Primary or expanded NK cells were rested overnight in medium containing IL-2+DMSO (“Control”), IL-2 + SR-1 (1uM), IL-2 + KA (25uM) or Kyn (25uM). NK cells were assessed for cytotoxic function using a standard 4-hour calcein-AM release assay as previously described [1]. Briefly, tumor cells were loaded with calcein and incubated with NK cells at varying effector target ratios. IL-2 and SR-1 were left in the assay and had separate spontaneous and maximum controls. Mean percent cell lysis was determined as described previously [2].

Cytokine Assay

Nk cells were treated with SRI, Kyn or KA for overnight as described above. NK cells were co-cultured with the indicated tumor cells for 3-hours, supernatants were collected and frozen at −80°C until use. On the day of the assay, the supernatants were thawed and 50 µL of undiluted supernatant was used according to the manufacturer's instructions for the BD CBA Soluble Protein Master Kit (BD Biosciences, Cat#: 558265) and IFNγ and TNFα Flex Set (BD Biosciences, Cat: 558269, 560112) or MACSPlex Cytokine 12 Kit (Miltenyi, Cat: 130-099-169). The analytes were acquired on a BD LSR II or MACSQuant. The geometric mean for each analyte was determined in Flow Jo v. 10.1 and unknown samples were interpolated using a standard curve with $R2 \geq 0.9$ from the known standards for BD LSR II acquired samples. Analysis of MACSQuant acquired analytes was done using MACS Quantify software (version 2.8, Bergisch Gladbach, Germany). This software uses average APC median values of MACS Plex Standards and calculates the cytokine concentration in each sample.

Flow Cytometry

NK cells were labeled with a panel of fluorescently conjugated human antibodies NK cell populations: *CD56*, CD3, CD16, NKG2D, NKp30, NKp46 (Miltenyi Biotech). Cells were stained with antibodies at concentrations according to manufacturer guidelines. For cell surface staining, cells were washed with staining buffer containing phosphate buffered saline (PBS) and 2% fetal bovine serum (FBS). Cells were fixed in 1% formaldehyde and cells were analyzed on MACS Quant flow cytometer (Miltenyi Biotech). The data were analyzed using FlowJo software.

Immunoblot

Protein lysates were prepared from expanded NK cells. The cells were lysed in RIPA buffer containing protease and phosphatase inhibitors. The samples were boiled and loaded onto SDS-PAGE gel and transferred to a PVDF membrane. The membrane was probed with anti-AHR (Cell Signaling Technology-#83200) and β-actin antibodies (Cell Signaling Technology-#3700). Immunoblot analyses was performed using ECL reagent (Pierce Biotechnology) using standard protocol [3].

Real-time PCR

RNA samples were isolated from NK cells using RNeasy kit (Qiagen). Total RNA was quantified using a NanoDrop spectrophotometer and reverse transcribed into cDNA using reverse transcriptase kit (Applied Biosystems). To measure gene expression by Real-Time PCR was performed using predesigned primer/probe sets and 2x-TaqMan fast master mix with 18S as an internal control (Applied Biosystems). Gene expression was measured using BioRad CFX96 machine.

RNA sequencing

RNA samples were isolated from NK cells using RNeasy kit (Qiagen). The samples were DNase treated and concentrated using RNA concentrator kit (Zymo). On average, 112 million paired-end 151 bp RNA-Seq reads were generated for each sample (the range was 89 to 122 million). Each sample was aligned to the GRCh38 assembly of the human reference from NCBI (using version 2.6.0c of the RNA Seq aligner STAR. Transcript features were identified from version 28 of the Gencode annotations, and raw coverage counts were calculated using HT Seq. The raw RNA-Seq gene expression data was normalized, and post-alignment statistical analyses were performed using DESeq2 and custom analysis scripts written in R. Comparisons of gene expression and associated statistical analysis were made between different conditions of interest using the normalized read counts. All fold change values are expressed as test condition / control condition, where values less than one are denoted as the negative of its inverse (note that there will be no fold change values between –1 and 1, and that the fold changes of "1" and "-1" represent the same value). Transcripts were considered significantly differentially expressed using a 10% false discovery rate (DESeq2 adjusted p-value <= 0.1) [4]. The data files have been deposited in the Gene Expression Omnibus (GEO) database-GSE173220. Ingenuity pathway analysis (IPA) was performed using DEGs to determine the signaling pathways were affected in NK cell following the treatment with AHR agonist and antagonist.

Chromatin Immunoprecipitation

NK cells were fixed with 1% formaldehyde for 15 min and quenched with 0.125M glycine. Chromatin was isolated by the addition of lysis buffer, followed by disruption with a Dounce homogenizer. Lysates were sonicated and the DNA sheared to an average length of 300-500 bp.

Genomic DNA (Input) was prepared by treating aliquots of chromatin with RNase, proteinase K and heat for de-crosslinking, followed by ethanol precipitation. Pellets were resuspended and the resulting DNA was quantified using a NanoDrop spectrophotometer. Extrapolation to the original chromatin volume allowed quantitation of the total chromatin yield. An aliquot of chromatin (25 ug) was precleared with protein Agarose beads (Invitrogen). Genomic DNA regions of interest were isolated using 10ug of antibody against AHR (Enzo). Complexes were washed, eluted from the beads with SDS buffer, and subjected to RNase and proteinase K treatment. Crosslinks were reversed by incubation overnight at 65 C, and ChIP DNA was purified by phenol-chloroform extraction and ethanol precipitation.

ChIP Sequencing

Illumina sequencing libraries were prepared from the ChIP and Input DNAs by the standard consecutive enzymatic steps of end-polishing, dA-addition, and adaptor ligation. Steps were performed on an automated system (Apollo 342, Wafergen Biosystems/Takara). After a final PCR amplification step, the resulting DNA libraries were quantified and sequenced on Illumina's NextSeq 500 (75 nt reads, single end). Reads were aligned to the human genome (hg38) using the BWA algorithm. Duplicate reads were removed and only uniquely mapped reads were used for further analysis. Alignments were extended in silico at their 3'-ends to a length of 200 bp, which is the average genomic fragment length in the size-selected library and assigned to 32-nt bins along the genome. The resulting histograms (genomic "signal maps") were stored in bigWig files. Peak locations were determined using the MACS algorithm (v2.1.0) with a cutoff of p-value = 1e-7. Peaks that were on the ENCODE blacklist of known false ChIP-Seq peaks were removed. Signal maps and peak locations were used as input data [5].

Statistical Analysis

Data are expressed as mean ± s.d or ± s.e.m. Statistical differences between treatment groups were determined using an ANOVA model and Student t test. p-value < 0.05 was considered statistically significant.