Supplemental Materials

KIAA0317 Regulates Pulmonary Inflammation Through SOCS2 Degradation

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Cell Culture:

HEK293T cells:

Cells were maintained in Dulbecco's Modified Eagle Medium (ATCC) containing 4 mM L-Glutamine, 4.5 g/L D-glucose, 1 mM sodium pyruvate. This media was supplemented with 10%

fetal bovine serum (Gemini), 100 U/mL Penicillin and 100 µg/mL Streptomycin. Cells were maintained at 37°C and 95% relative humidity, under 5% CO_2.

MLE-12 and BEAS-2B cells:

Cells were maintained in HITES medium composed of a 50:50 mix of Dulbecco's medium and Ham's F12 (ATCC), containing 0.005 mg/ml Insulin, 0.01 mg/ml Transferrin, 30 nM Sodium selenite, 10 nM Hydrocortisone, 10 nM β-estradiol, 10 mM HEPES, 4.5 mM (L-glutamine (ATCC 30-2214). This media was supplemented with 10% fetal bovine serum (Gemini), 100 U/mL Penicillin and 100 µg/mL Streptomycin. Cells were maintained at 37°C and 95% relative humidity, under 5% CO_2.

Generation of KIAA0317-deficient mice using CRISPR-Cas9

KIAA0317-deficient mice were generated using CRISPR-Cas9 technology(1). In brief, a sgRNA target site in exon 13 of KIAA0317 was selected using a python script implementing the Fuzznuc algorithm (Emboss) to identify unique N_{20}NGG sequences and their associated off-targets, allowing 0-4 mismatches (2). The output was further corroborated by the Cas-OFFinder algorithm (RGEN Tools). Potential off-targets (Supplemental Table 1) with three or fewer mismatches and those without mismatch in the seed sequence (13 bp adjacent to the PAM sequence) were screened, by amplifying each off-target locus with a specific primer pair and sequencing the PCR product in founder mice. No potential off-target events were identified

The sgRNA was generated from a double strand linear DNA template by annealing a target specific primer containing a T7 promoter, the Kiaa0317 target sequence (without PAM), and part of the tracrRNA sequence with a common primer comprising the full tracrRNA sequence (Supplemental Table 2). The annealed template was subjected to a fill in reaction and the double stranded product

was amplified using the T7-19 and sgRNA-R primers (Supplemental Table 2). The resulting dsDNA template was purified with the QIAquick PCR purification kit (Qiagen) and then transcribed using the MEGAshortscript T7 kit (Invitrogen). Capped Cas9 mRNA transcripts were produced from PmeI-linearized purified pcDNA3.3topo-T7-hCas9 using the mMESSAGE mMACHINE T7 ULTRA Kit (Life Technologies). The Cas9 mRNA and the sgRNA were purified using the MEGAclear kit (Life Technologies) and eluted in nuclease-free water. Cas9 mRNA and the gRNA integrity was confirmed using a 2100 Bioanalyzer (Agilent Technologies).

C57BL/6J pronuclear-stage zygotes were obtained by natural mating of superovulated females and microinjected with sgRNA (10 ng/µl) and Cas9 mRNA (20 ng/µl) by the Gene Targeting and Transgenic Core (University of Pittsburgh). Injected zygotes were cultured overnight and transferred to pseudopregnant CD1 recipient females to obtain potential founder mice. Mice were genotyped by PCR amplification of the target locus and direct sequencing of the PCR product (Supplemental Table 3). Amplified PCR products were subsequently cloned into a sequencing vector (NEB) and sequenced to verify correct targeting. The founder mice were backcrossed to wild-type C57BL/6J (Jackson laboratories) for 2 generations and heterozygous progeny were intercrossed to generate experimental cohorts. All primers in Supplemental Table 2 and 3 were purchased from integrated DNA Technologies (IDT). Heterozygous N2 mice were intercrossed to generate experimental cohorts.

METHOD DETAILS:

Recombinant DNA Constructs— Total RNA was isolated from untreated 293FT using RNeasy Mini Kit, and reverse transcribed to cDNA using High-Capacity RNA to cDNA kits. SOCS2 and other cDNAs constructs were PCR amplified, isolated, and sequence confirmed against the respective GRCh38 reference sequences prior to PCR-cloning into pcDNA3.1D-V5-HIS vector.

Deletion mutants were generated via PCR-cloning. Point mutants of SOCS2 were generated using the QuikChange II XL kit, per the manufacturer's protocol.

In vitro *peptide-binding assays*— Biotin-labeled peptides (100 μg) were first coupled to streptavidin agarose beads for 1 hour at 25°C in binding buffer (50 mM Tris HCl pH 7.6, 150 mM NaCl, and 0.25 % v/v Triton-X-100). V5-tagged SOCS2 protein (50 μl) was in vitro synthesized using TnT translation kits for 90 minutes at 30°C. Peptide-bound resin were then incubated with in vitro–synthesized SOCS2 for 18 hours at 4°C. Following binding, resin was washed with binding buffer, and eluted in 1X Laemmli buffer at 88°C for five minutes prior to immunoblotting analysis as described above.

In vitro *kinase assays*— V5-tagged SOCS2 protein (50 μL) was in vitro synthesized using TnT translation kits for 90 minutes at 30°C. The assays were performed by combining 50 mM Tris, pH 7.6, 100 mM MgCl2, 25 mM β-Glycerolphosphate, and 5 mg/ml BSA, in a total volume of 25 μl using combinations of 0.5 mM ATP, 0.2 μCi γ-32P ATP, 500 nM of recombinant PKCA, and V5-tagged SOCS2. Reaction products were incubated at 37°C for 2 hours, resolved by SDS-PAGE, and either processed for autoradiography either by using Personal Molecular Imager (Bio-Rad Laboratories) or immunoblotting visualize substrate input.

Lentivirus construction— Lentiviral overexpression constructs were generated via PCR cloning means, and ligated into pLVX-IRES-Puro, and sequence confirmed. Silencing constructs were similarly generated, and ligated into pLKO.1 Puro. To generate lentivirus encoding plasmids, plasmid was co-transfected with Lenti-X HTX packaging plasmids (Takara Bio Inc.) into 293T cells following the manufacturer's instructions. 72 h later, virus was collected and concentrated using Lenti-X concentrator (Takara Bio Inc.). Virus was then administered to mice using above protocols.

RT-qPCR —Total RNA was isolated from treated cells, and cDNA was generated via reverse transcription following manufacturer's instructions. Quantitative PCR was performed using SYBR Green qPCR mixture as described previously (52). Fold change in message was calculated using the ΔΔCt method.

BALF Leukocyte Differential— BALF was collected from treated mice, and erythrocytes were digested in lysis buffer (150 mM NH_4Cl, 10 mM $NaHCO_3$, and 0.1 mM EDTA, pH 7.3) on ice for 30 min. Following digestion, cell count was determined by TC20 Automated cell counter. Cells were then precipitated onto glass slides using Cytospin. Immobilized cells were stained using Hema3 kit according to manufacturer's protocol. Stained cells were manually differentiated via microscopic analysis. Three fields per sample were used, and at least one hundred cells per sample were counted.

Mouse Genotyping— C57BL/6J *Kiaa0317*$^{+/+}$, $^{+/-}$, and $^{-/-}$ greater than 16 days of age were genotyped by tail clipping. Tails were immersed in ice-cold ethanol for at least 10 seconds prior to the removal of no more than 3 mm tail tissue. Post-tail analgesia was applied similarly. Tail tissue was digested in DirectPCR Lysis Reagent (Viagen) with Proteinase K (NEB, 0.4 mg/mL) at 60°C for 18 hours. Tail lysate was precipitated, and supernatant was used as template for PCR amplification using primers listed above. Genotypes were assayed by agarose gel electrophoresis and sequencing when necessary.

Single Cell RNA-sequencing— ScRNA-seq library preparation was performed using the 10X Genomics Chromium System and its associated V2 chemistry reagents per the manufacturer's

protocol. ScRNA-seq libraries were sequenced using an Illumina NextSeq-500 through the University of Pittsburgh Genomics Core Sequencing Facility. Post-processing including filtering by percent mitochondrial DNA, number of unique molecular identifiers (UMI), number of genes, and normalization of gene expression was performed using the R package Seurat v 2.3.1 and R v 3.5.(3, 4) To minimize batch effects in combining multiple samples for integrated analysis, an individual object was created for each sample, then aligned using Seurat's RunMultiCCA function.(5)

Subjects and tissue preparation

Normal control lung tissue was obtained from organ donors, after rejection of the lungs for transplant. Following explantation of the lungs, sections from the upper and lower lobe were dissected, placed in Perfadex, and arrived for processing within twenty minutes of removal from the patient. Tissue for scRNA-seq was diced then enzymatically digested in DMEM (Thermo Fisher Scientific) containing 0.7 mg/mL collagenase A (Roche) and 30 ug/mL DNAase I (Roche) for one hour while undergoing further mechanical dispersion with the gentleMACS OctoDissociator (Miltenyi Biotec). The resulting cell suspension was washed with PBS, filtered twice through a 70-micron cell strainer, and underwent RBC lysis. Cells were then resuspended in PBS containing 0.04% BSA.

Single-cell RNA library preparation and sequencing

Using the 10X Genomics Chromium System, cells were mixed with reverse transcription reagents, loaded into a Single-cell A chip, followed by 3' gel beads and partitioning oil. Cells were separated into oil micro-droplet partitions containing a cell and gel-bead scaffold for an oligonucleotide composed of oligo-DT, 10X and UMI barcodes, and reverse transcription

reagents as described.(6) Reverse Transcription was performed, the emulsion broken, and pooled fractions obtained using a recovery agent. cDNAs were amplified by 11 cycles of PCR (C1000, Bio-Rad), enzymatically sheared and DNA fragment ends were repaired, A-tailed and adaptors ligated. The library was quantified with the KAPA Universal Library Quantification Kit KK4824 (KAPA Biosystems) and evaluated for cDNA length on a bioanalyzer using a High Sensitivity DNA kit (Agilent). ScRNA-seq libraries were sequenced on an Illumina NextSeq-500 through the University of Pittsburgh Genomics Core Sequencing Facility. Raw sequencing reads were examined by quality metrics and mapped to human reference genome GRCh38 using the Cell Ranger pipeline (10X Genomics). To ensure PCR amplified transcripts were counted only once, only single UMIs were counted for gene expression level.(7) In this way, cell x UMI count matrices were generated for downstream analysis. To minimize batch effects in combining multiple samples for integrated analysis, an individual object was created for each sample then aligned using Seurat's RunMultiCCA function. Canonical correlation vectors were chosen for downstream analysis, canonical correlation analysis (CCA) subspaces. After clustering and visualization with tSNE, cell populations were identified through examination of gene markers in the associated transcriptome. Single-Cell RNA sequencing data has been uploaded to the Gene Expression Omnibus (GEO- 128169).

Mass Spectrometry Analysis— Samples were boiled at 100°C for 15 minutes in 60µL of 1.5X LDS buffer. Half of each sample was processed by SDS-PAGE using a 10% Bis-Tris NuPAGE gel (Invitrogen) with the MES buffer system. The mobility region was excised into 10 equal sized segments and in-gel digestion was performed on each using a robot (ProGest, DigiLab) with the

following protocol: Washed with 25mM ammonium bicarbonate followed by acetonitrile. Reduced with 10mM dithiothreitol at 60°C followed by alkylation with 50mM iodoacetamide at RT. Digested with sequencing grade trypsin (Promega) at 37°C for 4h. Quenched with formic acid and the supernatant was analyzed directly without further processing.

Half of each digested sample was analyzed by nano LC-MS/MS with a Waters NanoAcquity HPLC system interfaced to a ThermoFisher Q Exactive. Peptides were loaded on a trapping column and eluted over a 75μm analytical column at 350nL/min; both columns were packed with Luna C18 resin (Phenomenex). The mass spectrometer was operated in data-dependent mode, with the Orbitrap operating at 70,000 FWHM and 17,500 FWHM for MS and MS/MS respectively. The fifteen most abundant ions were selected for MS/MS. 5hrs of instrument time was used per sample.

Data were searched using a local copy of Mascot (Matrix Science) with the following parameters: Enzyme: Trypsin/P, Database: SwissProt Human (concatenated forward and reverse plus common contaminants), Fixed modification: Carbamidomethyl (C), Variable modifications: Oxidation (M), Acetyl (N-term), Pyro-Glu (N-term Q), Deamidation (N,Q), Mass values: Monoisotopic, Peptide Mass Tolerance: 10 ppm, Fragment Mass Tolerance: 0.02 Da, Max Missed Cleavages: 2, Mascot DAT files were parsed using Scaffold (Proteome Software) for validation, filtering and to create a non-redundant list per sample. Data were filtered using at 1% protein and peptide FDR and requiring at least two unique peptides per protein.

NSAF Calculation was performed by the conversion to Spectral Abundance Factor (SAF) and subsequent Normalized Spectral Abundance Factor (NSAF). This was based on the equation: NSAF = (SpC/MW)/Σ(SpC/MW)N, where SpC = Spectral Counts, MW = Protein Molecular Weight in kDa and N = Total Number of Proteins. Proteins differentially measured in sample

versus control were filtered based on, 1. Protein had at least 5 SpC in the SOCS2-GST PD sample. 2. Protein was not detected in the GST-only PD OR 3. Protein was detected with a 4-fold or more increase based on dividing NSAF values. There were 148 proteins matching these criteria, and relevant protein were listed.

Supplemental Table 1: Off Targets

Name	Sequence	MM*	PAM	Chr	Position	Strand	Location	Gene
OFF01	GAACAGGGCGAAGAGCTGGTTAG	3	NAG	chr11	69734385	-	intergenic	n/a
OFF02	GAACTGGGCGAATAGCTAACTGG	3	NGG	chr15	88402671	+	intergenic	n/a
Off03	GAAACAGGCGAAGAGCGGACTGG	3	NGG	chr19	6338926	+	exon	Men1
Off04	GAACCAGGCCAAGAGCTGCCCAG	3	NAG	chr2	180400068	+	intron	Gm6307
Off05	GAAACGGGAGAAGAGCGGACAGG	3	NGG	chr3	87087574	+	intergenic	n/a
Off06	GAACCGTGAGAAGAGCTGACTGG	2	NGG	chr5	24985989	+	exon	AK005363
Off07	CCAGGAAGCGAAGAGCTGACAGG	**	NGG	chr16	32144120	+	Exon	Nrros
Off08	ACAGTGAGCGAAGAGCTGACTAG	**	NAG	chr2	138378725	+	Intergenic	n/a
Off9	GTAGGTAGCGAAGAGCTGACGGG	**	NGG	chr17	10859954	+	Intron	Park2
Off10	TGTCTTTGCGAAGAGCTGACAGG	**	NGG	chr9	59011711	-	intron	Neo1

*MM, ** non-unique seed sequences of the sgRNA; Number of mismatches in the 20mer target sequence; Chr, chromosome; Position, coordinate of the sites (GRcM38/mm10 mouse assembly).

Supplemental Table 2: Primers used to generate dsDNA template for sgRNA generation

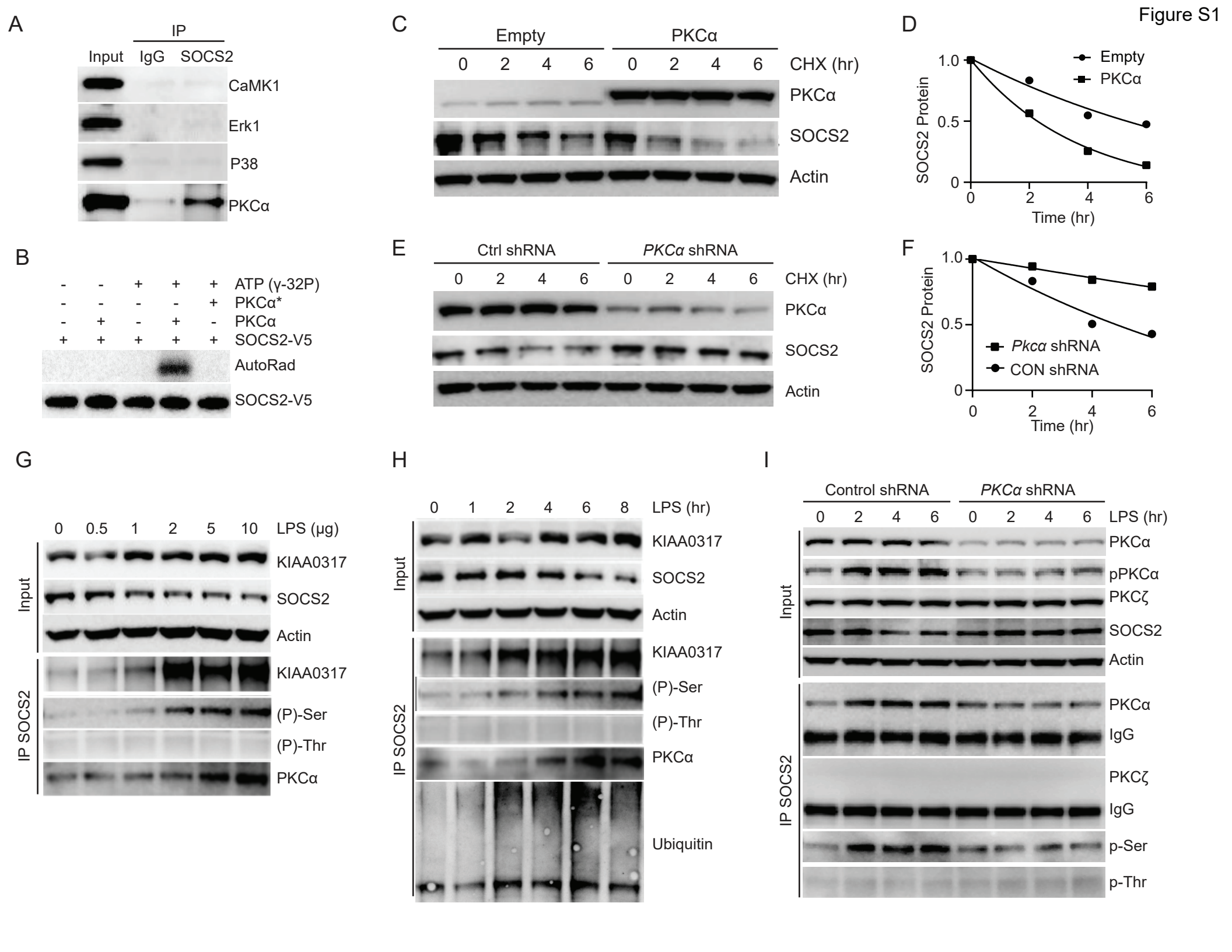
Primer	Sequence
sgRNA Specific Primer	TAATACGACTCACTATAGGgaaccgggcgaagagctgacGTTTTAGAGCTAGAAATAGCA Blue: T7 Promoter; Black: target specific sequence; Green: Partial TracRNA Sequence
sgRNA Common Primer	AAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC (complementary TracRNA sequence)
T7-19-F	TAATACGACTCACTATAGG
sgRNA-R	AAAAGCACCGACTCGGTGCC

Supplemental Table 3: Genotyping primers

Primer	Sequence
KIAA-fwd	AGGACAGGAGAATGCTACAAGTT
KIAA-rev	ACGCTCTTAGCCAAGTGCTCA

Supplemental References:

1. Pelletier S, Gingras S, and Green DR. Mouse genome engineering via CRISPR-Cas9 for study of immune function. *Immunity.* 2015;42(1):18-27.
2. Rice P, Longden I, and Bleasby A. EMBOSS: the European Molecular Biology Open Software Suite. *Trends in genetics : TIG.* 2000;16(6):276-7.
3. Macosko EZ, Basu A, Satija R, Nemesh J, Shekhar K, Goldman M, et al. Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets. *Cell.* 2015;161(5):1202-14.
4. Satija R, Farrell JA, Gennert D, Schier AF, and Regev A. Spatial reconstruction of single-cell gene expression data. *Nat Biotechnol.* 2015;33(5):495-502.
5. Butler A, Hoffman P, Smibert P, Papalexi E, and Satija R. Integrating single-cell transcriptomic data across different conditions, technologies, and species. *Nat Biotechnol.* 2018;36(5):411-20.
6. Zheng GX, Terry JM, Belgrader P, Ryvkin P, Bent ZW, Wilson R, et al. Massively parallel digital transcriptional profiling of single cells. *Nat Commun.* 2017;8:14049.
7. Islam S, Zeisel A, Joost S, La Manno G, Zajac P, Kasper M, et al. Quantitative single-cell RNA-seq with unique molecular identifiers. *Nat Methods.* 2014;11(2):163-6.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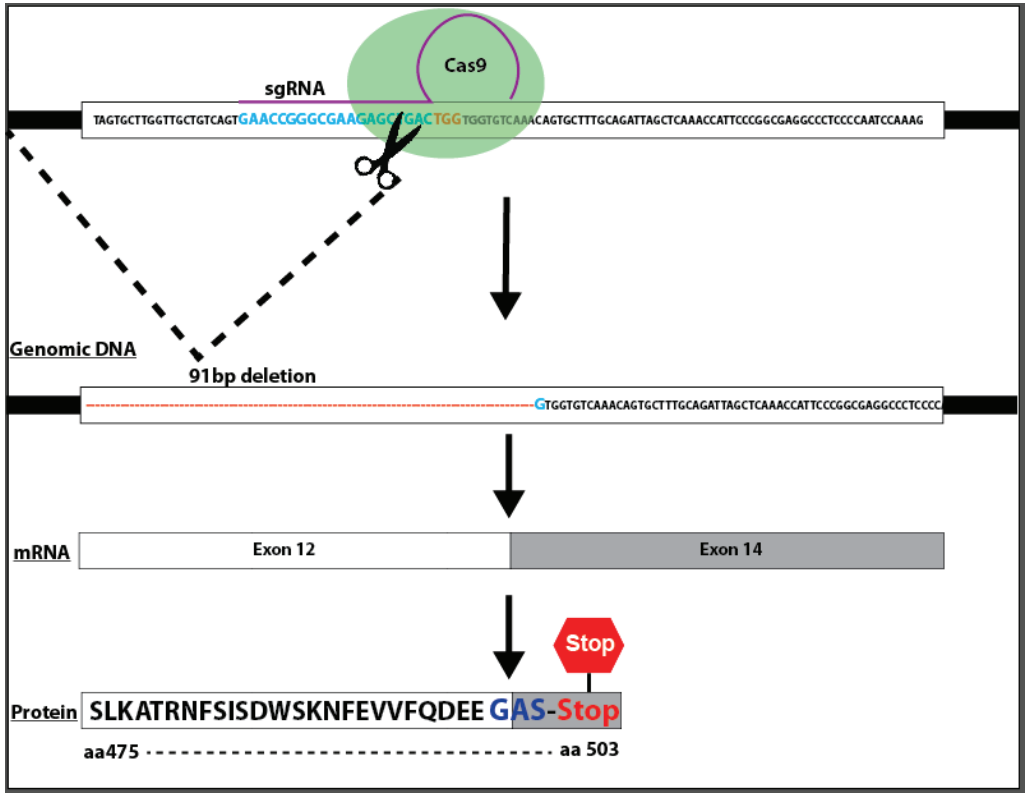
IP
Input
IgG
SOCS2
CaMK1
Erk1
P38
PKCα
B
ATP (γ-32P)
PKCα*
PKCα
SOCS2-V5
AutoRad
SOCS2-V5
C
Empty
PKCα
0 2 4 6 0 2 4 6
CHX (hr)
PKCα
SOCS2
Actin
D
SOCS2 Protein
Empty
PKCα
Time (hr)
E
Ctrl shRNA
Pkcα shRNA
0 2 4 6 0 2 4 6
CHX (hr)
PKCα
SOCS2
Actin
F
SOCS2 Protein
Pkcα shRNA
CON shRNA
Time (hr)
G
0 0.5 1 2 5 10
LPS (μg)
Input
KIAA0317
SOCS2
Actin
IP SOCS2
KIAA0317
(P)-Ser
(P)-Thr
PKCα
H
0 1 2 4 6 8
LPS (hr)
Input
KIAA0317
SOCS2
Actin
IP SOCS2
KIAA0317
(P)-Ser
(P)-Thr
PKCα
Ubiquitin
I
Control shRNA
Pkcα shRNA
0 2 4 6 0 2 4 6
LPS (hr)
Input
PKCα
pPKCα
PKCζ
SOCS2
Actin
IP SOCS2
PKCα
IgG
PKCζ
IgG
p-Ser
p-Thr

SUPPLEMENTAL FIGURE LEGENDS:

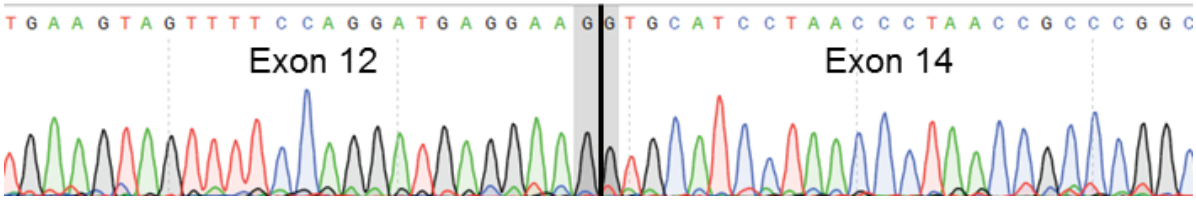
Supplementary Figure 1. SOCS2 is phosphorylated by PKCA leading to its degradation A. Endogenous SOCS2 immunoprecipitation from MLE cells and subsequent immunoblotting of several kinases (n=2). **B.** In vitro PKCA kinase assay. * indicates heat inactivated PKCA. **C.** Immunoblot analysis of MLE cells following expression of Empty or PKCA plasmids and exposure to CHX (100μg/mL) for the indicated times. **D.** SOCS2 protein densitometry (normalized to Actin) for **C.** (n=2). **E.** Immunoblot analysis of MLE cells following expression of CON shRNA or *Pkca* shRNA plasmids and exposure to CHX (100μg/mL) for the indicated times. **F.** SOCS2 protein densitometry (normalized to Actin) for **E.** (n=2). **G.** Immunoblot analysis of MLE cells exposed to LPS (8hr) for the indicated doses prior to endogenous SOCS2 immunoprecipitation. **H.** Immunoblotting of MLE cells exposed to LPS (10μg/mL) for the indicated times prior to endogenous SOCS2 immunoprecipitation. **I.** Silencing of *Pkca* and LPS time course prior to SOCS2 immunoprecipitation and immunoblot analysis in MLE cells. **A-B, G-I**. Data are representative of n=2 independent experiments.

Figure S2

A



B



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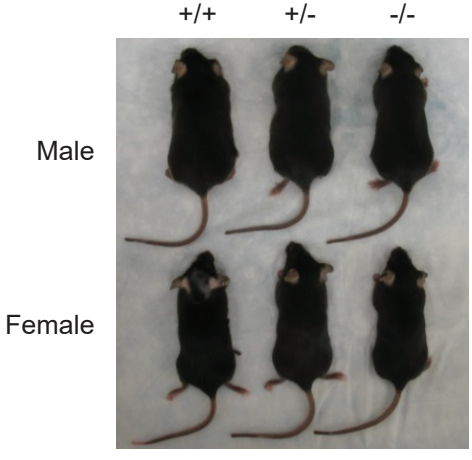
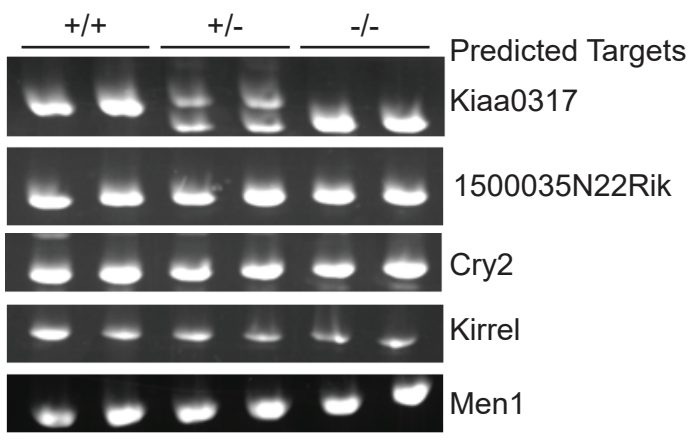
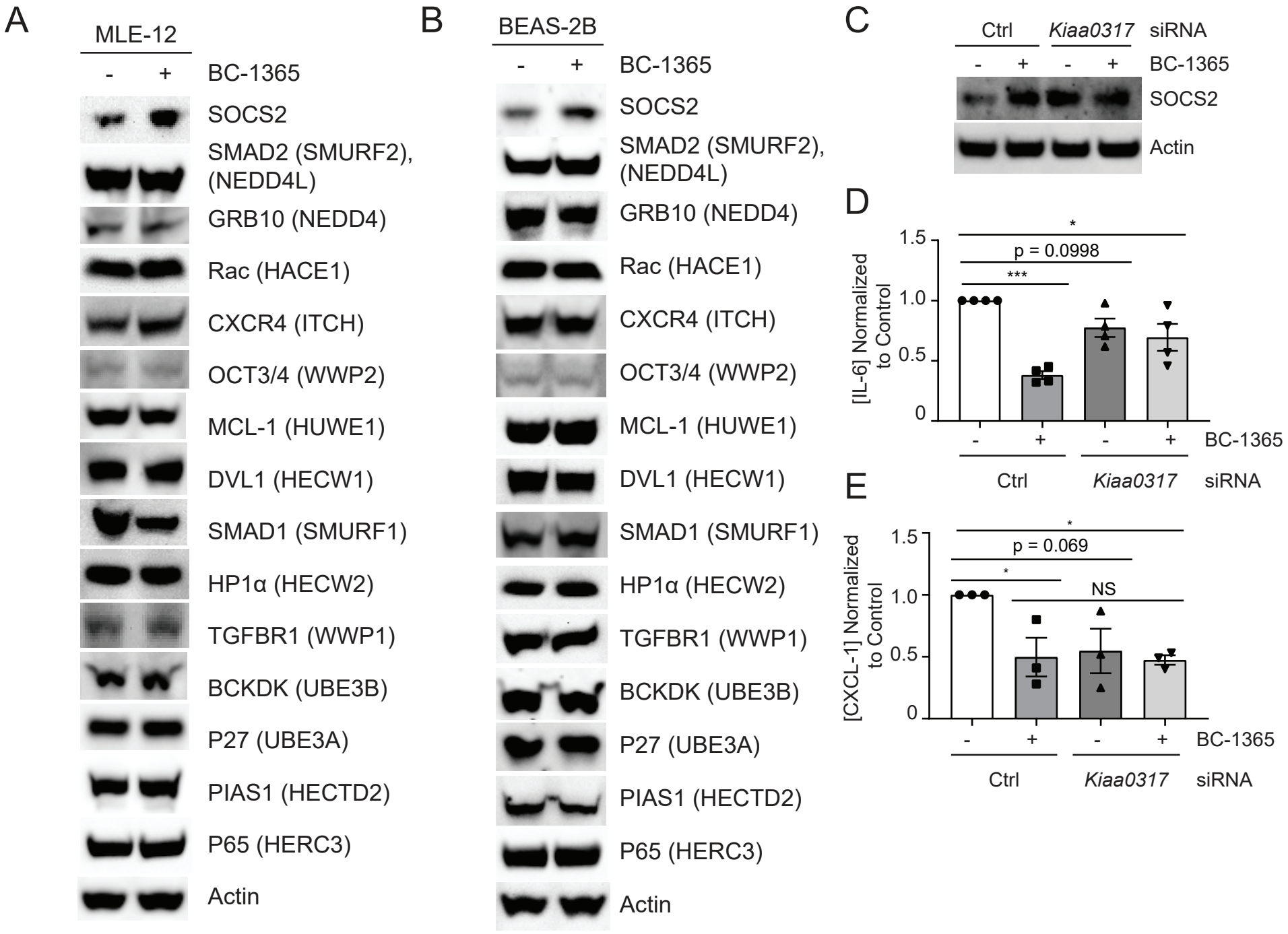


D



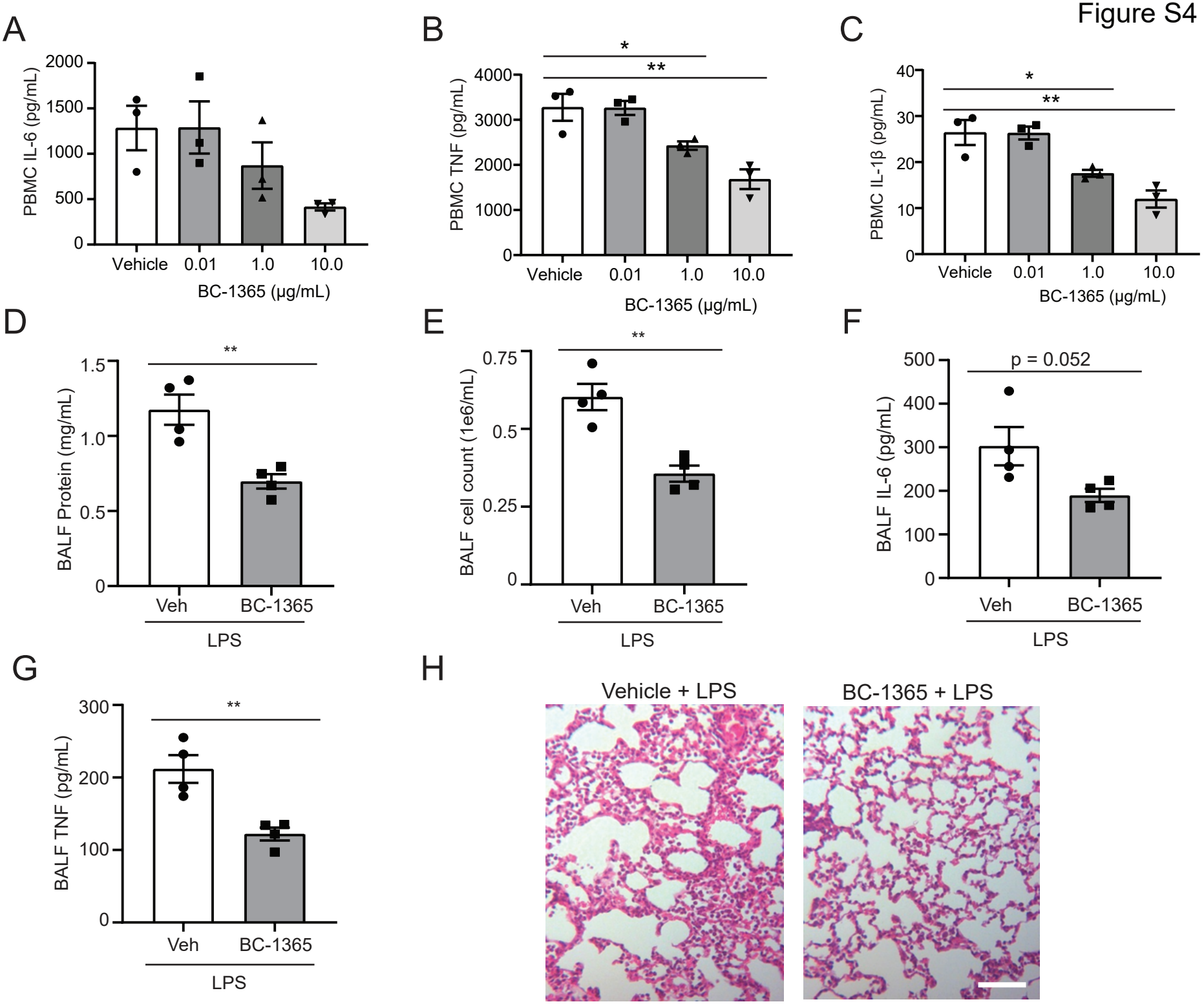
Supplementary Figure 2. Generation of *Kiaa0317*$^{-/-}$ knockout mice. A-B. CRISPR-Cas9 gene editing of C57BL6/J mice. **B.** CRISPR editing resulted in 91bp deletion in exon 13. **C.** Female and Male *Kiaa0317*$^{+/-}$ and *Kiaa0317*$^{-/-}$ mice do not show morphological differences from WT. **D.** PCR amplification and sequencing detected no off-target effects in *Kiaa0317*$^{-/-}$ mice among top predicted targets, n=2 mice per genotype.

Figure S3

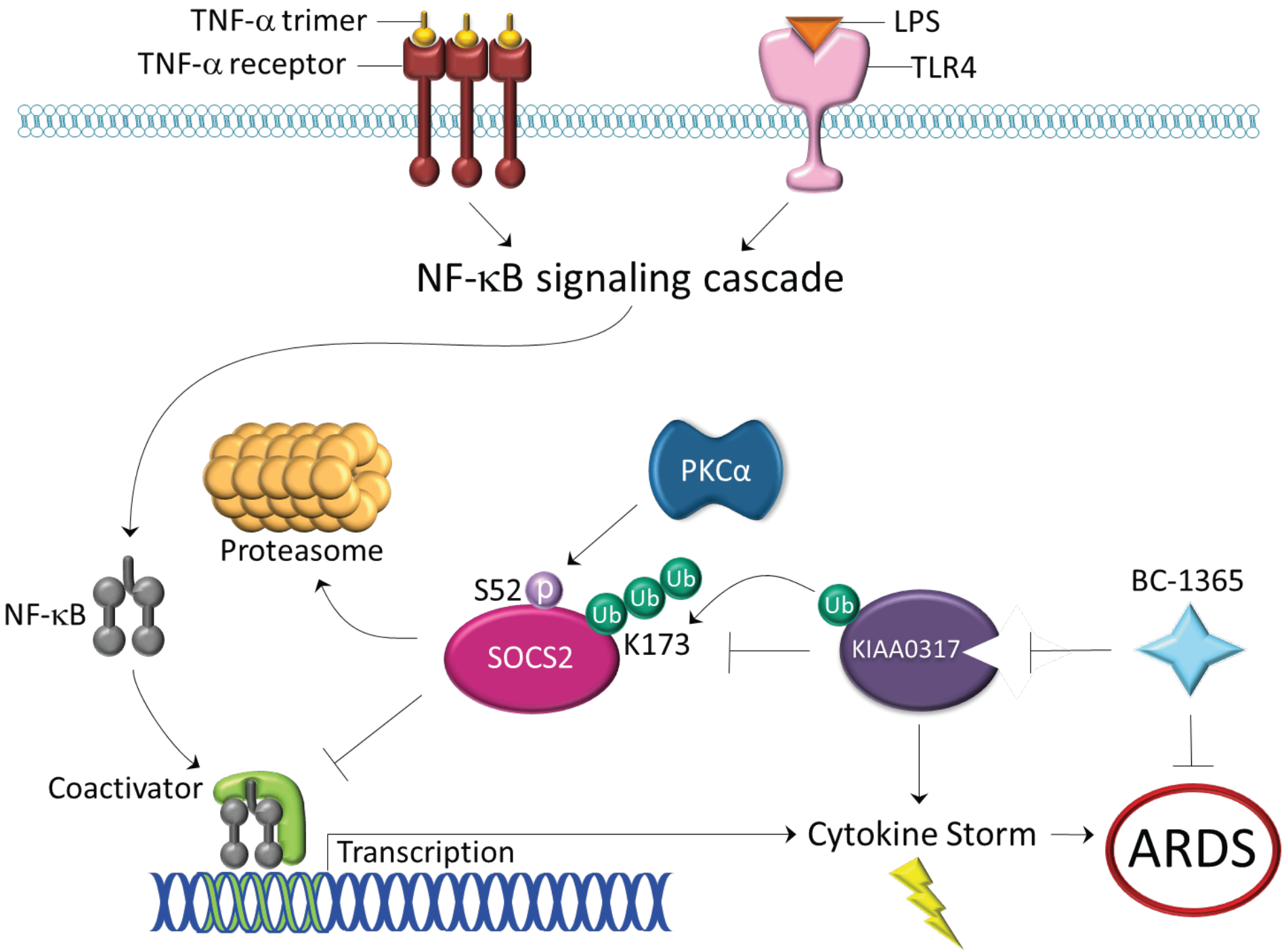

A
MLE-12
- +
BC-1365
SOCS2
SMAD2 (SMURF2), (NEDD4L)
GRB10 (NEDD4)
Rac (HACE1)
CXCR4 (ITCH)
OCT3/4 (WWP2)
MCL-1 (HUWE1)
DVL1 (HECW1)
SMAD1 (SMURF1)
HP1α (HECW2)
TGFBR1 (WWP1)
BCKDK (UBE3B)
P27 (UBE3A)
PIAS1 (HECTD2)
P65 (HERC3)
Actin
B
BEAS-2B
- +
BC-1365
SOCS2
SMAD2 (SMURF2), (NEDD4L)
GRB10 (NEDD4)
Rac (HACE1)
CXCR4 (ITCH)
OCT3/4 (WWP2)
MCL-1 (HUWE1)
DVL1 (HECW1)
SMAD1 (SMURF1)
HP1α (HECW2)
TGFBR1 (WWP1)
BCKDK (UBE3B)
P27 (UBE3A)
PIAS1 (HECTD2)
P65 (HERC3)
Actin
C
Ctrl
Kiaa0317
siRNA
- + - +
BC-1365
SOCS2
Actin
D
[IL-6] Normalized to Control
1.5
1.0
0.5
0
*
p = 0.0998
***
- + - +
BC-1365
Ctrl
Kiaa0317
siRNA
E
[CXCL-1] Normalized to Control
1.5
1.0
0.5
0
*
p = 0.069
*
NS
- + - +
BC-1365
Ctrl
Kiaa0317
siRNA

Supplementary Figure 3. BC-1365 is specific to KIAA0317. A-B. Immunoblotting Analysis of known HECT-E3 Ligase substrates from MLE-12 and BEAS-2B cells treated with BC-1365. Parentheses after substrate name indicates HECT E3 ligase shown to regulate it (e.g. GRB10 targeted by NEDD4). **C.** SOCS2 blotting from MLE treated with Control or *Kiaa0317* siRNA without or with BC-1365 treatment prior to LPS exposure. **D-E.** ELISA analysis of supernatant from cells treated in **C.** for inflammatory cytokines: **D.** IL-6 and **E.** CXCL1, data represent mean values ± SEM (n = 3-8). NS, $p>0.05$; *, $p < 0.05$; ***, $p<0.001$; compared with untreated Control, One-Way ANOVA with Tukey's Multiple Comparisons (D-E). **A-C**. Data are representative of n=2-3 independent experiments.

Figure S4


A
PBMC IL-6 (pg/mL)
2000
1500
1000
500
0
Vehicle
0.01
1.0
10.0
BC-1365 (µg/mL)
B
PBMC TNF (pg/mL)
4000
3000
2000
1000
0
*
**
Vehicle
0.01
1.0
10.0
BC-1365 (µg/mL)
C
PBMC IL-1β (pg/mL)
40
30
20
10
0
*
**
Vehicle
0.01
1.0
10.0
BC-1365 (µg/mL)
D
BALF Protein (mg/mL)
1.5
1.0
0.5
0
**
Veh
BC-1365
LPS
E
BALF cell count (1e6/mL)
0.75
0.5
0.25
0
**
Veh
BC-1365
LPS
F
BALF IL-6 (pg/mL)
500
400
300
200
100
0
p = 0.052
Veh
BC-1365
LPS
G
BALF TNF (pg/mL)
300
200
100
0
**
Veh
BC-1365
LPS
H
Vehicle + LPS
BC-1365 + LPS

Supplementary Figure 4. BC-1365 shows efficacy in cell and animal models of inflammation. **A-C.** ELISA analysis of cytokine release from LPS-treated PBMC co-treated with BC-1365 at indicated doses. Data represent mean values ± SEM (n = 3). **D-H.** C57BL/6J mice were intra-tracheally inoculated with LPS (3mg/kg) and intraperitoneal BC-1365 treatment (10mg/kg). 18h later, mice were sacrificed, and lungs were lavaged with saline and harvested. (**D-E**) Protein concentration and cell count measurements from bronchoalveolar lavage fluid (BALF). (**F-G)** BALF cytokine concentrations. **D-G.** Data represent mean values ± SEM (n = 4 mice). **J.** Histology of murine lungs following H&E staining, bar indicates 100μm. NS, $p>0.05$; *, $p < 0.05$; **, $p<0.01$; compared with Vehicle, One-Way ANOVA with Dunnett's Multiple Comparisons (A-C), or , two-tailed unpaired Student's t-test (D-G).


TNF-α trimer
TNF-α receptor
LPS
TLR4
NF-κB signaling cascade
Proteasome
PKCα
S52
p
Ub
Ub
Ub
K173
SOCS2
NF-κB
Ub
KIAA0317
BC-1365
Coactivator
Transcription
Cytokine Storm
ARDS

Supplementary Figure 5. Proposed model of action. Microbial infection or stimuli robustly trigger inflammation by decreasing anti-inflammatory proteins such as SOCS2 in cells. Specifically, during microbial infection, KIAA0317 targets SOCS2 for its ubiquitination at Lys173; this process is facilitated by PKCA phosphorylation of SOCS2 at Ser52. A small-molecule KIAA0317 inhibitor, BC-1365, lowered inflammation in the animal model of acute lung injury by antagonizing the actions of KIAA0317 on SOCS2-cytokine signaling.