**Sepsis induces interleukin 6 gp130/JAK2/STAT3, and muscle wasting in man and mice**

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**Online Data Supplement**

**Materials & Methods**

**Patient samples.**

The institutional review board of the Charité Universitätsmedizin Berlin, Germany, approved the study, and written informed consent was obtained from legal proxy (ICU patients), or the patients themselves (Charité EA2/061/06; http://www.controlled-trials.com, ISRCTN77569430). Clinical data were reported previously [1]. Here we analyzed the expression of the IL-6 target gene Suppressor of cytokine signaling 3 (*SOCS3*) and Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) in biopsy specimens obtained from the *vastus lateralis* muscle of patients at high risk to develop ICU-acquired muscle wasting and weakness. Accordingly, these patients (n=5) were critically ill, mechanically ventilated with a SOFA score ≥8 on three consecutive days within the first five days after ICU admission. Biopsy specimens were taken at day 5 and day 15 after ICU admission. Five age- and gender-matched patients undergoing elective orthopedic surgery permitted muscle biopsies and were used as controls. For more details on the experimental procedure and clinical data, please refer to [1, 2].

**Animal model of polymicrobial sepsis**

All animal procedures were performed in accordance with the guidelines of the Max-Delbrück Center for Molecular Medicine and the Charité-Universitätsmedizin Berlin, and were approved by the Landesamt für Gesundheit und Soziales (LAGeSo, Berlin, Germany) for the use of laboratory animals (permit number G 207/13). The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985), as well as the current version of German Law on the Protection of Animals. Klaus Rajewsky kindly provided conditional Il6st knockout (KO) mice. The generation and usage of the conditional *Il6st* allele was published elsewhere [3]. The Cre-loxP recombination system was used for the generation of a conditional *Il6st* allele. *Il6st*loxP/loxP mice were crossed with Cre carrying mice controlled by myocyte-specific Pax7-promotor (Pax7-Cre) (cKO, Il6stloxP/loxP; Pax7-Cre). Pax7-Cre-negative littermates were used as controls (WT, *Il6st*loxP/loxP). Genotyping was performed as recently described [4] using primer pairs shown in Table E3.

To investigate the effects of gp130 on sepsis-induced muscle atrophy, cecal ligation and puncture (CLP) surgery was performed in 12- to 16-week-old male *Il6st c*KO mice or *Il6st* wild type (WT) littermate controls. Sham mice were treated identically except for the ligation and puncture of the cecum. To investigate the effects of AG490 treatment on muscle in sepsis we used 20-week-old male C57BL/6J mice. Animals received either vehicle (10µl DMSO in 240µl normal saline) or AG490 (16 mg/kg in 250µl vehicle) 60 min prior to, and every 24 hours after surgery for 96 hours to a total of four injections.

CLP surgery was performed as recently described [4]. Briefly, mice were anesthetized with isoflurane, placed on a heating plate to assure a constant body temperature of 37°C measured by a rectal probe. After shaving and disinfection of the abdominal skin, midline laparotomy was performed, the cecum was exposed and ligated using a non-absorbable surgical suture (Ethicon 6-0, Johnson & Johnson Medical GmbH, Umkirch, Germany). A 21-gauge needle was used to puncture the cecum once, and a small amount of cecum content was extruded. The cecum was then replaced into the abdominal cavity, and the incisions of the peritoneum and skin were closed with two separate layers of surgical sutures (Ethicon 6-0, Johnson & Johnson Medical GmbH, Umkirch, Germany). Sham mice were treated identically except for the ligation and puncture of the cecum. Mice were sacrificed 24 hours and 96 hours after surgery, as indicated, and *tibialis anterior* (TA) and *gastrocnemius/plantaris* (GP) muscles were harvested for analysis. The weights of skeletal muscles were determined und tibia length was measured for reference purposes. Body weight was measured before and after the experiment. Muscle and organ weights were related to tibia length.

**RNA sequencing and statistical analyses**

For all sample types (WT sham or WT CLP, 24 hours or 96 hours after surgery) three biological replicates were sequenced. Total RNA analyses were evaluated by an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., California, USA). Library preparation of 500 ng RNA was performed using the Illumina TruSeq Stranded mRNA Kit. To check integrity, cDNA was evaluated again using an Agilent 2100 Bioanalyzer. Sequencing was performed using an Illumina HiSeq 4000 sequencer. Initial quality check of RNA-sequencing results was done by FASTQC software (v0.11.5; Babraham Bioinformatics, UK; available online at www.bioinformatics.babraham.ac.uk/projects/fastqc). Sequencing reads were mapped to the mouse whole genome (mm10) using STAR aligner (v 2.5.3a; default parameters) [5]. Read counts for each gene (Gencode vM12) were extracted from the BAM file using featureCounts software (v1.5.1) [6]. In order to avoid background signal noise, genes with less than 10 reads over all samples were excluded. Read counts from different biological groups were subjected to differential expression analysis using the DESeq2 R statistical package (v1.16.1) [7]. The transcriptome data can be found under EBI Annotare v.2.0 (Project-ID: E-MTAB-10960).

*Voronoi Treemaps* of differentially expressed genes (DEG, log2 fold change≥2, adjusted p-value<0.05) per KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway were generated as recently published [8, 9]. Plots represent overexpressed genes and pathways; the higher a gene or pathway is expressed the bigger it will be represented in the plots enabling comparison to competitive pathways or genes. To this end, we used a space proportional algorithm implemented by the Paver software (DECODON (Greifswald, Germany)) which is available through the link: https://bionic-vis.biologie.uni-greifswald.de/index.php for non-profit research or/and academic organizations. For multi-level tree maps, first, the total area is divided into polygons to represent the top-level category. Polygon areas represent copy numbers weighted by changes in gene expression. The top-level areas are then subdivided into subcategories and the procedure is continued up to the level of individual genes.

*Pathway enrichment analysis* was performed using DAVID (Database for Annotation, Visualization and Integrated Discovery) Bioinformatics Resources 6.8 (https://david.ncifcrf.gov) using the outputs BP\_ALL (all biological process terms).

*Venn Diagram* (https://bioinfogp.cnb.csic.es/tools/venny/) showing the number of genes that were increased or decreased (log2 fold change≥2, adjusted p-value<0.01) in TA compared to sham treated mice after 24 hours, 96 hours or at both time points. The DAVID functional annotation tool [10, 11] was used for gene ontology (GO) term- and KEGG-pathway analyses of each individual time point, and ten of the most enriched biological processes and pathways are shown.

**Histological analyses and measurement of myocyte cross-sectional area.**

*Tibialis anterior* and *gastrocnemius/plantaris* muscles were flash frozen in liquid nitrogen with gum tragacanth (Merck KGaA, Germany) as cryoprotectant. Histological cross sections with a thickness of 5 µm were cut using the Leica cryotome CM3050S (Leica Microsystems GmbH, Germany) and stained with metachromatic ATPase as described earlier [12, 13]. To analyze the myocyte cross sectional area (MCSA) of type II/fast-twitch fibers images were acquired with Leica CTR 6500 HS microscope and the Leica digital camera DFC 425 (Leica Microsystems GmbH, Germany). Image J software 1.51v9 software (Wayne Rasband, National Institutes of Health, USA) was used to measure 100 MSCAs per mouse and condition [2, 4, 14-16]. MCSA measurements were performed by one person who was blinded to the specific treatment.

**Measurements of plasma IL-6**

Measurements of plasma IL-6 were performed by using the Mouse ELISA Quantikine Kit for IL-6 (M6000B, R&D Systems, MN, USA) according to the manufacturers’ protocol.

**Myoblast culture, differentiation and atrophy assay.**

Cell culture experiments of murine myoblasts (C2C12 cells, American Type Culture Collection (ATCC), Manassas, VA, USA) were performed as previously described [1, 4, 16]. Differentiation of myoblasts to myotubes was induced at confluence by replacing growth medium (Dulbecco's Modified Eagle's medium (DMEM, 1 g/l glucose, Merck KGaA, Germany), 10% fetal bovine serum (FBS, Biochrom GmbH, Germany), supplemented with Penicillin and Streptomycin (Merck KGaA, Germany)), by differentiation medium ((DMEM, 1 g/l glucose; Merck KGaA, Germany; 2% FBS, Biochrom GmbH, Germany). Five days differentiated C2C12 myotubes were used for all experiments unless otherwise stated. *Atrophy assay:* myotubes were treated with indicated amounts of recombinant IL-6 (R&D Systems, MN, USA) or solvent (0.1% BSA in PBS) for 72 hours. A JAK2 inhibitor (AG490; 10µM, Sigma-Aldrich, MO, USA), and two STAT3 inhibitors (C188-9; 10µM, Merck-Milipore, Germany; S3i-201, 10µM, Selleckchem) were added 60 min prior to IL-6 treatment.

For siRNA transfection of C2C12 cells 100,000 cells per well on a six well plate were plated and differentiated as described above for four days. Cells were then incubated with serum free medium without antibiotics at 37°C in a 5% CO2 atmosphere for 24 h. 400 µl of transfection mixture per well consisting of 5 µl of siRNA (50 nM, Dharmacon / Fisher Scientific; control siRNA D-001810-10-05, Il6st siRNA, J-040007-09-0005), 10 µl of Dharmafect3 (Dharmacon/ Fisher Scientific) and 385 µl of Opti-MEM® (Thermo Fischer Scientific Inc., MA, USA) were prepared and incubated at room temperature for 20 minutes according to the manufacturers protocol. Following 48 hours of transfection cells were subjected to analysis.

Light microscopy pictures were analyzed using the Leica CTR 6500 microscope connected to the Leica DFC 360 FX digital camera. Out of 100 myotubes per condition, three diameters per myotube were measured and averaged using the ImageJ software. The person who performed these measurements was blinded to the specific treatment of the myotubes. Data are presented as frequency-distribution histograms plotting myotubes width against its frequency, or as bar graphs showing mean myotube diameter.

**RNA isolation, cDNA synthesis and quantitative real-time-PCR**.

Total RNA was isolated from C2C12 cells or the TA and GP muscles of CLP or sham operated mice using TRIzol® Reagent (Invitrogen™, Life Technologies Corporation, CA, USA) and the FastPrep-24™ instrument (MP Biomedicals GmbH) in accordance with manufacturer´s instructions and as recently reported [16, 17]. cDNA synthesis of 1 µg of RNA per sample was carried out by using the SuperScript® First-Strand Synthesis System (Invitrogen™, Life Technologies Corporation, CA, USA) in accordance with the manufacturer´s instructions. Quantitative real-time polymerase chain reaction (qPCR) was performed using *Power* SYBR® Green PCR Master Mix (Thermo Fischer Scientific Inc., MA, USA) and self-designed primers (for primer sequences see Table E4). PCR reactions were performed in a StepOnePlus™ thermocycler (Applied Biosystems) as described recently using a cDNA standard curve [2, 15, 17]. Expression of specific genes was normalized to the stably expressed reference gene glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) [2, 15, 17].

**Protein extraction and Western blot assay.**

The FastPrep-24™ instrument (MP Biomedicals GmbH) was used to homogenize muscle tissue in lysis buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 10 % glycerol, 1 % Triton X-100) supplemented with protease inhibitors (cOmplete™ protease inhibitor cocktail; Roche Diagnostics GmbH, Germany) and phosphatase inhibitor (PhosStopTM; Roche Diagnostics GmbH, Germany) using Micro Packaging Vials with 2.8 mm Precellys ceramic beads (PEQLAB Biotechnology GmbH, Germany). Lysates were cleared by centrifugation at 16.000 g for 20 min at 4 °C. Protein content in the supernatant was quantitated using Pierce® BCA reagent (Thermo Fischer Scientific Inc., MA, USA). Proteins were stored at -80°C.Western blot analysis was performed on protein samples from skeletal muscle and C2C12 cells, as previously described [12]. Briefly, proteins were separated by SDS-PAGE and transferred onto nitrocellulose or PVDF membranes (GE Healthcare, Germany). Membranes were blocked with either 5% skim milk powder or 5% bovine serum albumin (BSA) dissolved in TBS-T for 1 hour. The following primary antibodies were used: anti-STAT3 (#12640, monoclonal, mouse, 1:2000, Cell Signaling Technology Inc., MA, USA), anti–phospho STAT3 Y705 (#9145, monoclonal, mouse 1:1000, Cell Signaling Technology Inc.), anti-gp130 (Ab202850, polyclonal, rabbit 1:1000, Abcam, UK), anti-Atrogin1 (Ab92281, polyclonal, goat, Abcam, UK), anti-MuRF1 [18] (gift from T. Sommer), anti IRS-1 and anti-phospho IRS-1 S636/S639 (rabbit, 1:1000, Cell Signaling Technology Inc.), anti-Akt and anti-phospho Akt S473 (rabbit, 1:1000, Cell Signaling Technology Inc.) antibody. Equal loading was controlled with anti-GAPDH (clone 6C5, monoclonal, mouse, 1:10.000; Millipore GmbH, Germany) antibody. HRP-linked IgG horse anti-mouse, goat anti-rabbit (both 1:2000, Cell Signaling Technology Inc.) or rabbit anti-goat (1:2000, Abcam, UK) were used as secondary antibodies. Proteins were visualized with a chemiluminescence system (SuperSignal® West Pico Chemiluminescent substrate, Thermo Fischer Scientific Inc., MA, USA). Chemiluminescence detection films (GE Amersham, UK) were exposed for different durations and developed using an Agfa CP1000 developing machine (Agfa-Gevaert NV, Belgium).

**Statistical Tests**

All experiments were performed independently and at least three times using biological triplicates each. For mRNA expression and myotube diameter data from cell culture experiments, a paired t-test was used. Data on muscle weight and mRNA expression were analyzed using the Mann-Whitney U test. MCSA data were analyzed using a paired t-test. *In vitro* data are shown as mean±SD and *in vivo* data are mean±SEM. Differences were considered statistically significant at *p*≤0.05. The GraphPad Prism® 8 program (GraphPad Software, La Jolla, CA, USA), Adobe Illustrator CS6, version 16.0.0, and Photoshop CS6, version 13.0 were used to perform statistics and draw graphics, respectively. The documentation of histological staining results were performed with a Leica fluorescence microscope using a Leica camera (DFC 360 FX and DFC 425) and the LAS.AF or LAS-X software (version: 2.4.1 build 6384 and the LAS3.1 software (version 2.5.0.6735).

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**Supplementary Tables**

**Supplementary Table 1.** Primer pairs for genotyping of *Il6st* cKO and *Il6st* WT mice.

|  |  |
| --- | --- |
| **Name** | **Sequence (5’ – 3’)** |
| **Mm\_*Il6st*\_loxP forward** | TGG CTT GAG CCT CAG CTT GGC TAG |
| **Mm\_*Il6st*\_loxP reverse** | GTG AAC AGT CAC CAT GTA CTC TGT ACG C |
| **Pax7ICN forward** | GCT CTG GAT ACA CCT GAG TCT |
| **Pax7ICN forward mutant** | GGA TAG TGA AAC AGG GGC AA |
| **Pax7ICN reverse** | TCG GCC TTC TTC TAG GTT CTT GCT C |

Il6st indicates Interleukin 6 signal transducer; WT, wildtype; Pax7 Paired box protein 7; Mm, Mus musculus.

**Supplementary Table 2.** Primer pairs for quantitative real-time-PCR.

|  |  |
| --- | --- |
| **Name** | **Sequence (5’- 3’)** |
| **Hs\_*SOCS3* forward** | AGA CTT CGA TTC GGG ACC A |
| **Hs\_*SOCS3* reverse** | AAC TTG CTG TGG GTG ACC A |
| **Hs\_*GAPDH* forward** | AGC CAC ATC GCT CAG ACA C |
| **Hs\_*GAPDH* reverse** | GCC CAA TAC GAC CAA ATC C |
| **Mm\_*Socs3* forward** | GAA TTT CGC TTC GGG ACT AG |
| **Mm\_*Socs3* reverse** | AAC TTG CTG TGG GTG |
| **Mm\_*Il6st* forward** | AAA GAT GGG CCG GAA TTC |
| **Mm\_*Il6st* reverse** | CAC ACA GGC ACG ACT ATG G |
| **Mm\_*Trim63* forward** | CCT GCA GAG TGA CCA AGG A |
| **Mm\_*Trim63* reverse** | GGC GTA GAG GGT GTC AAA CT |
| **Mm\_*Fbxo32* forward** | AGT GAG GAC CGG CTA CTG TG |
| **Mm\_*Fbxo32* reverse** | GAT CAA ACG CTT GCG AAT CT |
| **Mm\_*Gapdh* forward** | ATG GTG AAG GTC GGT GTG A |
| **Mm\_*Gapdh* reverse** | AAT CTC CAC TTT GCC ACT GC |
| **Mm\_*Myh2* forward** | AAC TCC AGG CAA AAG TGA AAT C |
| **Mm\_*Myh2* reverse** | TGG ATA GAT TTG TGT TGG ATT GTT |
| **Mm\_*Myh4* forward** | GGG AAC ATG AAA TTC AAG CAA |
| **Mm\_*Myh4* reverse** | ATA GGC AGC CTT GTC AGC AA |

Trim63 indicates Tripartite motif 63; Fbxo32, F-Box only 32; SOCS3, Suppressor of cytokine signalling 3; Il6st, Interleukin 6 signal transducer ; Gapdh, glyceraldehyde-3-phosphate dehydrogenase; Myh, myosin heavy chain; Mm, Mus musculus ; Hs Homo sapiens.

**Supplementary Table 3. Body and organ weights 96 hours after CLP or sham surgery of *Il6st* WT and *Il6st* KO mice**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | *Il6st* WT  sham  (n=6) | *Il6st* WT  CLP  (n=10) | *Il6st* cKO  sham  (n=6) | *Il6st* cKO  CLP  (n=15) | *p-*value  cKO CLP vs. WT CLP |
| Survival [%] | 100.00 | 90.91 | 100.00 | 100.00 |  |
| BW pre-surgery [g/mm TL] | 2.04±0.06 | 2.00±0.05 | 2.00±0.06 | 2.00±0.03 |  |
| BW weight post-surgery [g/mm TL] | 1.92±0.14 | 1.68±0.05 | 1.94±0.06 | 1.60±0.03 |  |
| TA weight [mg/mm TL] | 3.11±0.13 | 2.41±0.07 | 2.71±0.06 | 2.34±0.03 | \* |
| GP weight [mg/mm TL] | 9.74±0.35 | 7.82±0.19 | 8.17±0.20 | 7.54±0.14 | \* |
| SOL weight [mg/mm tibia length] | 0.54±0.02 | 0.49±0.01 | 0.54±0.02 | 0.55±0.01 |  |
| EDL weight / tibia length [mg/mm TL] | 0.64±0.03 | 0.53±0.01 | 0.60±0.02 | 0.54±0.01 |  |
| TA MCSA type II fibers [µm2] | 2352±28.53 | 1726±22.33 | 1764±27.89 | 1658±20.19 |  |
| GP MCSA type I fibers [µm2] | 2759±38.35 | 1681±29.85 | 1969±35.22 | 1546±28.76 |  |
| SOL MCSA type I fibers [µm2] | 1841±28.50 | 2657±46.37 | 2170±49.86 | 2522±48.07 |  |
| SOL type I fibers [%] | 41.99±1.92 | 41.19±1.86 | 38.77±1.94 | 40.10±1.71 |  |
| SOL type II fibers [%] | 58.01±1.92 | 58.81±1.86 | 61.23±1.94 | 59.90±1.71 |  |

BW indicates body weight; CLP, cecal ligation and puncture; EDL, extensor digitorum longus; GP, gastrocnemius and plantaris; MCSA, myofiber cross sectional area; SOL, soleus; TA, tibialis anterior; TL, tibia length. Data are shown as mean±SEM\**p*≤0.05; \*\*\**p*≤0.001; n.s. = not significant.

**Supplementary Table 4: Body and organ weights 96 hours after CLP or sham surgery of AG490 and solvent treated WT mice**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Solvent | | AG490 | |
|  | **Sham**  **(n=5)** | **CLP**  **(n=15)** | **Sham**  **(n=5)** | **CLP**  **(n=15)** |
| Survival [%] | 100.00 | 90.00 | 100.00 | 92.50 |
| BW pre-surgery [g/mm TL] | 1.80±0.04 | 1.78±0.03 | 1.74±0.04 | 1.75±0.03 |
| BW post-surgery [g/mm TL] | 1.71±0.03 | 1.48±0.03 | 1.67±0.03 | 1.47±0.02 |
| TA weight [mg/mm TL] | 3.07±0.03 | 2.48±0.04 | 2.95±0.05 | 2.54±0.05 |
| GP weight [mg/mm TL] | 9.94±0.13 | 8.21±0.12 | 8.58±0.23 | 7.58±0.12 |
| SOL weight [mg/mm TL] | 0.59±0.02 | 0.51±0.01 | 0.55±0.02 | 0.48±0.01 |
| EDL weight [mg/mm TL] | 0.74±0.02 | 0.56±0.01 | 0.59±0.04 | 0.54±0.01 |
| TA MCSA type II fibers [µm2] | 2453±34.76 | 1945±28.29 | 2153±34.30 | 1967±30.09 |
| GP MCSA type II fibers [µm2] | 2574±32.40 | 1770±26.67 | 2451±35.81 | 1825±31.62 |

BW indicates body weight; CLP, cecal ligation and puncture; EDL, extensor digitorum longus; GP, gastrocnemius and plantaris; MCSA, myofiber cross sectional area; SOL, soleus; TA, tibialis anterior; TL, tibia length. Data are shown as mean±SEM\**p*≤0.05; \*\*\**p*≤0.001; n.s. = not significant.

**Supplementary Figures**

**Supplementary Figure 1. Differentially expressed genes in muscle of septic mice.** Voronoi tree map of differentially expressed genes **(**log2 fold change ≥2, adjusted p-value<0.05) in TA muscles of WT mice after 24 (left) or 96 hours (right) after CLP surgery compared to sham treated controls (n = 3 for each condition). Every tile (small polygon) represents one gene. Tiles are arranged and colored according to the hierarchical KEGG pathway maps (larger regions correspond to functional categories). The diagrams show three hierarchical KEGG pathway levels (top three panels) and the level of individual genes (bottom panel) per time point (24 hours: left panels, 96 hours: right panels). Tile sizes represent changes in gene expression.

**Supplementary Figure 2. Genes up- and downregulated in muscle of septic mice.** Significantly up (top) or down (bottom) regulated genes in TA muscle of WT mice 24 (left) or 96 hours (right) after sham or CLP surgery. Analyses for GO-terms and KEGG-pathways are shown (n = 3 for each condition). Data are p-values and presented as -log10

**Supplementary Figure 3. Decreased Genes in muscle of septic mice.** Venn Diagram showing the number of genes that were decreased (log2 fold change ≥2, adjusted p-value<0.01) in the tibialis anterior muscle of CLP-treated compared to sham treated mice after 24 (left) or 96 hours (right) or at both time points (bottom) (n = 3 for each condition). Data are p-values and presented as -log10.

**Supplementary Figure 4. Regulated genes involved in IL-6 production.** Heat map of genes contained in GO:0032635-IL-6 production that were significantly regulated (*p*<0.05) in TA muscle of WT mice 24 hours and 96 hours after CLP or sham surgery (n = 3 for each condition).

**Supplementary Figure 5. Regulated genes involved in JAK-STAT signaling.** Heat map of genes contained in GO:0007259-receptor signaling pathway via JAK-STAT that were significantly regulated (*p*<0.05) in TA muscle of WT mice 24 hours and 96 hours after CLP or sham surgery (n = 3 for each condition).

**Supplementary Figure 6. Inhibition of STAT3 attenuates IL-6 induced myotube atrophy *in vitro***. Frequency distribution histograms showing the width of differentiated C2C12 myocytes after 24 hours of treatment with solvent or IL6 after preincubation with DMSO or S3i-201 (n = 100 cells for each condition). Bar graph showing mean myotube width ± SEM. \*P <0.05 (Student’s t-test).

**Supplementary Figure 7. Deletion of *Il6st* in myocytes does not affect interleukin 6 plasma levels in septic mice.** **(A)** Quantitative RT-PCR analysis of Il6st mRNA expression in TA muscle of WT and Il6st cKO mice 96 hours after sham or CLP surgery (WT: Sham n=6, CLP: n=15; cKO: Sham: n=6; CLP: n=10). **(B)** IL-6 plasma concentrations from the same mice. Data information: Data are presented as mean ± SEM. \*\*P <0.01, \*\*\*P <0.001 (Mann-Whitney U test).

**Supplementary Figure 8. Deletion of *Il6st* in myocytes attenuates sepsis-induced *Socs3* expression and MuRF1 protein content**. **(A)** Quantitative RT-PCR analysis of Socs3 mRNA content in TA muscle of Il6st cKO and WT mice 96 hours after CLP or sham operation (WT Sham, n=6; WT CLP, n=15; KO Sham, n=6; KO CLP, n=10). Data are presented as mean ± SEM \*\*P < 0.01, \*\*\*P < 0.001 (Mann-Whitney U test). **(B)** Immunoblots with anti-Atrogin-1, anti-MuRF1 and anti-GAPDH antibodies in TA muscle of Il6st cKO and WT mice 24 hours after CLP or sham operation (n = 3 for each condition).

**Supplementary Figure 9. Inhibition of JAK2 attenuates sepsis-induced *Socs3* and *Trim63* expression and MuRF1 protein content in gastrocnemius and plantaris muscle.**

**(A)** Immunoblots with anti-pSTAT3 Y705, anti-STAT3, anti-Atrogin-1, anti-MuRF1 and anti-GAPDH antibodies in GP muscle of AG490 or solvent treated WT mice 96 hours after CLP or sham surgery (n = 3 for each condition). Bar graph showing the ratio of the relative densities of pSTAT3 Y705 and STAT3 protein contents as detected in B. B-F Quantitative RT-PCR analysis of *Socs3* **(B)***, Trim63* **(C)***, Fbxo32* **(D)***, Myh2* **(E)**and *Myh4* **(F)** mRNA expression in GP muscle of AG490 or solvent treated WT mice 96 hours after CLP or sham surgery (AG490: sham: n=5, CLP: n=15; solvent: sham: n=5, CLP: n=15). Data information: (B-F) Data are presented as mean ± SEM. \*P < 0.05, \*\*P < 0.01 (Mann-Whitney U test).