Supplemental Material

Detailed Materials and Methods:

Cell Isolation and Culture

All protocols involving collection of and experimentation with human cells and tissues were approved by the Yale University Institutional Review Board.

Isolation and culture of human ECs: Human umbilical vein ECs were isolated from de-identified umbilical cords following enzymatic digestion by collagenase. Cells used in all experiments were serially cultured at 37°C on 0.1% gelatin (Sigma)-coated tissue culture plates in Endothelial Cell Growth Medium (EGM2, Lonza) and used for experiments between passage levels 1-6.

Isolation of memory CD4+ T lymphocytes: Leukapheresis products were collected from healthy adult volunteers in the Yale blood bank and then de-identified before transfer to the laboratory. Mononuclear cells were enriched by density gradient centrifugations using Lymphocyte Separation Medium per the manufacturer's instructions and cryopreserved in 10% DMSO/90% FBS in liquid nitrogen. Thawed cells were washed in RPMI (Gibco) supplemented with 5% FBS, 1.5% L-glutamine, and 1% penicillin/streptomycin. Human CD4+ T cells were isolated from total PBMCs by positive selection with magnetic Dynabeads CD4 (Invitrogen, 11331D) per the manufacturer's protocol and released with Detachabeads (Invitrogen, 11331D). Resting memory (CD45RA-HLA-DR-) T cells were purified from total CD4+ T cells by negative selection using anti-human CD45RA antibody (Biolegend, 304102) and HLA-DR antibody (Biolegend, 307602) by magnetic separation with Dynabeads Pan Mouse IgG (Invitrogen, 11041) to remove antibody-bound cells. The final population was identified as 90-95% resting CD4+ effector memory T cells by FACS analysis.

Viral Transduction of Endothelial Cells

pLEX-MCS-ASC-GFP (made by Christian Stehlik, Addgene plasmid #73957) was used to transduce HUVECs to create stable ASC-GFP expressing ECs. The lentiviral ASC-GFP reporter vector plasmid was co-transfected with psPAX2 (Addgene, plasmid #12260) and CMV VSV-G (Addgene, plasmid #8454) packaging plasmids into human 293T cells (ATCC, Manassas, VA) using Lipofectamine 2000 (Invitrogen). Lentiviral supernatant was collected at 24h and 48h, filtered through a 0.45μM filter and with the addition of polybrene (8 μg/mL), used to transduce HUVEC for two cycles at an MOI of 10 for 8h. Puromycin selection (1 μg/mL) was subsequently carried out for 48h. To generate retroviral vector encoding mCherry-Rab5WT, mCherry-Rab5WT vector (made by Gia Voeltz, Addgene plasmid #49201) was cut at the BamH1 and Not1 sites and ligated into the retroviral expression vector pLZRSpBMN-Z (gift from Garry Nolan, Stanford University, Stanford, CA). To generate retroviral vector encoding mCherry-Rab5DN, PCR using primer sequence GCGGCCGCTCAGTTACTACAACACTGATT was used to introduce a 3' Not1 restriction site using the mCherry-Rab5DN (S34N) vector (made by Sergio Grinstein, Addgene plasmid #35139) as template and the insert was cut with BamH1 and Not1 and then ligated into pLZRSpBMN-Z. Constructs were confirmed by sequencing (Yale Keck Sequencing Facility). To generate retrovirus encoding mCherry-Rab5WT or mCherry-Rab5DN, Phoenix cells (gift from Garry Nolan, Stanford University, Stanford, CA) were transfected using Lipofectamine 2000 with the retroviral plasmids encoding mCherry-Rab5-WT and mCherry-Rab5-DN. Viral supernatants were collected and used to transduce HUVECs as described above.

Antibody and Complement Treatment of ECs

Discarded and de-identified high-titer PRA sera from transplant candidates that showed >80% reactivity to HLA class I and II antigens were collected from the Yale New Haven Hospital's tissue typing laboratory. Over the course of experimentation, twenty-four pooled preparations of PRA sera were used and at least three different PRA preparations were tested for each experiment with similar results. Where indicated, PRA sera separation into IgG(+) and IgG(-) was performed as previously described using MAbTrap kit (GE Healthcare, 17-1128-01) according to the manufacturer's instructions [1]. IgG(+) and IgG(-) fractions were serially concentrated using 3kDa Amicon-Ultra-4 Centrifugal Filter Units (Millipore-Sigma) and brought to a final volume equal to the total PRA volume prior to sera fractionation [1]. Normal human sera

and C9 deficient sera were purchased from Quidel Corporation and Sigma. Unless indicated otherwise, HUVECs were pre-treated with recombinant human IFN-γ (50ng/mL, Invitrogen, PHC4031) in EGM2 media for 48h prior to treatment with PRA to reinduce expression *in situ* levels of HLA antigen expression. Where indicated, HUVECs were treated with 1μM or 5μM MCC950 (Cayman Chemical), 20μM or 100μM Ac-YVAD-CMK (Cayman Chemical, 178603-78-6), 20μM or 100μM z-YVAD-FMK (Biovision, 1141-5), 30μM Pitstop2 (Abcam, ab120687), 80μM dynasore (Sigma, 1202867-00-2), 10μg/mL IL-1 Receptor Antagonist (Peprotech, 200-01RA), 100ng/mL LIGHT (R&D Systems, 664-LI-025) or DMSO in gelatin veronal buffer (GVB, Sigma G6514) for 30 minutes, at the indicated concentrations, prior to PRA treatment. PRA sera was added to ECs in a 1:3 dilution in GVB for the indicated times. Untreated cells were simply washed with PRA sera as time zero controls. ECs were then washed three times with PBS and GVB buffer was added. Supernatants were collected for immunoblot and ELISA analysis. To separate the effects of IFN-γ from those of complement, ECs were treated with IFN-γ as above or mock treated for 48h, after which ECs were incubated with 20μg/mL of anti-human endoglin IgG2a mouse monoclonal antibody (Santa Cruz Biotechnology, P3D1) for 1h. Unbound antibody was removed by washing with PBS (Gibco) followed by addition of normal human sera (Sigma, S1764), titrated as indicated for an additional hour. Following the specified treatments, ECs were analyzed as described below.

Immunoblot Analysis and Immunoprecipitation

ECs were washed with ice cold PBS twice and were lysed in RIPA buffer (Cell Signaling Technology, 9806). Supernatants were serially concentrated using 3kDa Amicon-Ultra-4 Centrifugal Filter Units (Millipore-Sigma) with three 30 minute spins at 2100xg at 4°C. Laemmli buffer (Bio-Rad) and 0.1M dithiothreitol (Sigma) were added to lysates and supernatants, heated at 95°C for 10 minutes, run on precast protein gels (Bio-Rad) and transferred to PVDF membrane (Bio-Rad). Membranes were blocked in 5% BSA in TBS-T. Primary antibodies were used in 1:1000 dilution with incubation overnight at 4°C. Antibodies include: Caspase-1 (Santa Cruz Biotechnology, sc-622), Cleaved IL-1β (Cell Signaling Technology, 83186S), IL-1β (Cell Signaling Technology, 12703S), NLRC4 (Cell Signaling Technology, 12421S), GSDMD (Proteintech, 20770-1-AP), STAT1 (Santa Cruz Biotechnology, sc-271661), MyD88 (Santa Cruz Biotechnology, sc-74532), TRIF (Cell Signaling Technology, 4596S), ASC (Santa Cruz Biotechnology, sc-514414), Rab5 (Cell Signaling Technology, 2143S), NIK (Cell Signaling Technology, 4994S), phospho-IKKα/β (Cell Signaling Technology, 2697T), p100/p52 (Cell Signaling Technology, 37359S), p65 (Santa Cruz Biotechnology, sc-8008), β-actin (Sigma, A1978). Of note, a recent report described that D4D8T (Cell Signaling Technology) was the only anti-NLRP3 antibody out of several tested be specific and sensitive in detecting NLRP3 by immunoblot in human samples [2]. In this study, we used both D4D8T and Cryo-2 (Adipogen, AG-20B-0014-C100), which was not tested in the report, and both showed the same sized and specific bands on immunoblot corresponding to the expected size of NLRP3 protein; the identity of the band was confirmed by specific reduction in expression following NLRP3 siRNA knockdown. Membranes were washed and incubated with the appropriate secondary antibody in a 1:10000 dilution for 1h at room temperature. Secondary antibodies used were peroxidase-AffiniPure Goat Anti-Rabbit IgG (H+L) (Jackson ImmunoResearch, 111-035-144) and Peroxidase-AffiniPure Goat Anti-Mouse IgG (H+L) (Jackson ImmunoResearch, 115-035-062). Bound HRP was visualized using SuperSignal Femto or Pico West (Pierce) and chemiluminescent X-ray films (Denville Scientific) were developed using a Konica Minolta SRX-101A film processor. Densitometry were performed and analyzed using National Institutes of Health ImageJ software. For immunoprecipitation, cells were lysed using Cell Lysis Buffer (Cell Signaling Technology, 9803). Lysates were precleared using 20μL of protein A/G-conjugated agarose beads (Thermo Scientific Pierce, 20421) and incubated with 10μL of primary antibody overnight at 4°C. Antigen-antibody was complexed to protein A/G beads over 90 minutes at 4°C. The complex bound-resin and supernatants, which were the input lysates, were collected and analyzed by Western blot.

Small interfering RNA (siRNA) knockdown of EC proteins

ECs were transfected with 20nM siRNA NLRP3 (Qiagen, Hs_CIAS1_6), AllStars Negative Control siRNA (Qiagen, SI03650318), IPAF (Qiagen, Hs_NLRC4_1), STAT1 (Dharmacon, L-003543-00-0005), MyD88 (Dharmacon, L-004769-00-0005), TICAM1 (Dharmacon, L-012833-00-0005), MAP3K14 (Dharmacon,

LQ-003580-00-0002), NFKB2 (Dharmacon, L-003918-00-0005), RELA (Dharmacon, L-003533-00-0005), control non-targeting #1 (Dharmacon, D-001810-01-05) with RNAiMax (Invitrogen), according to the manufacturers' instructions for 18h. IFN-γ (50ng/mL) was added to transfected cultures the following day for 48h unless otherwise indicated prior to treatment with PRA sera in GVB. Successful knockdown was confirmed by immunoblotting as described.

Immunofluorescence microscopy and analysis of ASC-GFP Specks

ECs were cultured on 0.1% gelatin-coated coverslips in 24-well tissue culture plates. After indicated treatments, ECs were washed twice with PBS, fixed and permeabilized with ice cold methanol for 15 minutes at 4°C and air dried for 5 min. After washing with PBS, samples were blocked with PBS containing 5% FBS and 0.01% Tween for 1h at room temperature followed by incubation with primary antibody at 4°C overnight at 1:200 dilution. Primary antibodies include NLRP3 (Adipogen, Cryo-2), RelB (Santa Cruz Biotechnology, sc-48366), p65 (Santa Cruz Biotechnology, sc-8008), NIK (Cell Signaling Technology, 4994S). After washing with PBS-T, samples were incubated for 1h at room temperature with the appropriate AlexaFluor conjugated and high-cross absorbed secondary antibodies in 5% FBS/0.01% Tween/ PBS blocking solution (1:500 dilution). Samples were washed with PBS-T and mounted using ProLong Gold Mounting Reagent with DAPI (Life Technologies). Confocal images were acquired on the Leica TCS SP5 using the LAS AF software and 63X oil objective. For the ASC-GFP speck assays, samples were mounted using ProLong Gold Mounting Reagent with DAPI (Life Technologies) after methanol fixation permeablization and PBS-T wash. Images were acquired using Volocity software and an Axiovert 200M microscopy system (Carl Zeiss MicroImaging), an epifluorescence microscope, using the 20X objective filter and the number of ASC speck+ cells were counted and averaged over three fields per sample.

Real Time Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT- PCR).

RNA was isolated from ECs using the RNeasy kit (Qiagen) according to the manufacturer's protocol. RNA was reverse transcribed to cDNA using the High-capacity cDNA Reverse Transcription kit (Applied Biosystems). cDNA was amplified using the TaqMan gene expression Master Mix, and TaqMan gene expression probes (Applied Biosystems) including IL1B (Hs01555410_m1), Il1A(Hs00174092_m1), NLRP3 (Hs00918082_m1), NLRC4 (Hs00892666_m1), GSDMD (Hs00986739_g1), CASP1 (Hs00354836_m1), HLA-DRA (Hs00219575_m1), CXCL12 (Hs03676656_mH), CCL20 (Hs00355476_m1), ICAM1 (Hs00164932_m1), SELE (Hs00174057_m1), IL6 (Hs00174131_m1), GAPDH (Hs02786624_g1). Reactions were run on a CFX96 real-time system using CFX Manager Software (Bio-Rad). Gene expressions were normalized to GAPDH.

T Cell Activation Assays

ECs were cultured on 96 well round bottom plates and pre-treated with IFN-γ for 48h. HUVEC were then treated with PRA sera or GVB control for 6h. Freshly isolated human CD4+CD45RA-HLA-DR- T cells were co-cultured with the allogeneic HUVEC at a EC:T cell ratio of 1:20 in RPMI 1640 media with 10% FBS, 2% L-glutamine, 1% penicillin. T cell proliferation was assayed by labeling T cells using Cell Trace CFSE Proliferation Kit (Invitrogen, C34554) and assessed by flow cytometry after 7 days. 100pg/mL of recombinant human IL-1β (ThermoFisher, 10139HNAE5) or vehicle control was added on day 1 of co-culture where indicated. Supernatants were collected after 24h of co-culture (72h after siRNA transfection) for cytokine secretion that was assayed by ELISA.

FACS analysis

To assess antibody and complement binding, treated ECs were suspended with trypsin versene, washed and incubated with a Fixable Viability Dye (eBioscience, 65-0865-14), anti-human IgG (ThermoFisher, A21089), anti-human C4d (Quidel, A213), anti-human C5b-9 (Dako, M077701-5) at 1:100 dilution. For experiments using anti-human endoglin antibody, anti-mouse IgG (ThermoFisher, MA1-10406) was substituted for anti-human IgG. After 7 days of EC:T cell co-culture, flow cytometry was used to assess T cell proliferation. T cells were harvested and stained for CD4 using conjugated primary antibodies (Biolegend, 317424) and a Fixable Viability Dye (eBioscience, 65-0865-14) and assessed for CFSE dilution, as an indicator of proliferation. All samples were acquired using the BD LSRII Flow Cytometer and BD FACSDiva Software and analyzed with FlowJo software.

Measurement of Cytokine Production

Supernatants collected from complement-treated ECs were analyzed for IL-1β using Human IL-1 beta/IL-1F2 DuoSet ELISA (R&D Systems, DY201-05). Freshly isolated human CD4+CD45RA-HLA-DR- T cells were co-cultured with HUVEC that had been transfected with siRNA as indicated and treated with IFN-γ for 48h. After 24h of co-culture (or equivalently 72h after siRNA knockdown), supernatants were collected and assayed by ELISA using Human IFN gamma ELISA Ready-SET-Go! (eBioscience, 88-7316-88).

Analysis of Human Artery Graft, Heart and Aorta

All protocols involving animals were approved by the Yale Institutional Animal Care and Use Committee. Sets of four non-leaky (i.e. serum IgG level < 1 μg/mL) female SCID/beige mice aged 6-8 weeks old were randomly assigned to be used as recipients for human artery grafts from the same organ donor. The allocation of treatment to each mouse was randomly assigned and the grafts were harvested by a microsurgery research team, who did not have prior knowledge of the treatments. Mice undergoing echocardiographic assessments were similarly randomly assigned to control or drug treatment groups. Prior studies from our group have not shown any effect of the sex of mouse recipients of human artery grafts on artery responses to antibody.

Human coronary arteries from the native hearts of de-identified transplant recipients of approximately 0.8 mm in diameter were interposed into the descending infrarenal aorta of female C.B-17 SCID/beige mice (Taconic Biosciences). The transplanted arteries were quiesced for 7 days. Mice were injected with vehicle (DMSO) or 10mg/kg of MCC950 (Cayman Chemical, 17510) i.p. After 2h, mice were injected with either vehicle (PBS) or 200μL of neat PRA sera i.v. Artery grafts were harvested after 24h, embedded and snap frozen in OCT and sectioned at 5-μm thickness. Sections were fixed in ice-cold acetone for 15 minutes, washed with PBS, blocked in 5% FBS in PBS-T and incubated with Ulex Europaeus Agglutinin I -Rhodamine (1:200, Vector, RL-1062), anti-C4d (1:100, Quidel, A213), poly-C9 (1:100, Dako M077701-5), Cleaved Caspase-1 (1:100, Cell-Signaling Technology, D57A2), V-CAM1 (1:50, R&D Systems, BBA22), alpha-smooth muscle actin (1:100, Sigma 14-9760-82) and IL-1β (1:100, R&D Systems, MAB601-SP) overnight at 4°C. Samples were washed with PBS and incubated with the appropriate AlexaFluor secondary (1:500) for 1h at room temperature prior to being coverslipped and mounted. Images were acquired using Volocity software and the using the 20X objective filter on the Axiovert 200M microscopy system (Carl Zeiss MicroImaging) epifluorescence microscope. At time of graft recovery, blood was collected, allowed to clot by leaving undisturbed at room temperature for 30 minutes prior to centrifuging at 2000xg for 10 minutes at 4°C to separate serum. Serum was stored at -20°C and assayed by ELISA using Human IL-1β/IL-1F2 DuoSet ELISA (R&D Systems, DY201-05).

Ultrasound imaging (Yale Translational Research Imaging Center) was conducted using the Vevo 2100 Imaging System (VisualSonics, Toronto, Canada) on mice after 24h of MCC950 or control treatment just prior to harvesting hearts and aortae. Echocardiographic assessments of mouse cardiac function were performed by an ultrasound imaging technician who was blinded to the whether the mice had received control or drug treatments. M-Mode and anatomical M-mode were used for visualization and quantification of wall motion (1000 fps resolution) for analysis of LV function. Heart, ascending and descending aorta tissues were formalin-fixed paraffin-embedded for hematoxylin and eosin staining.

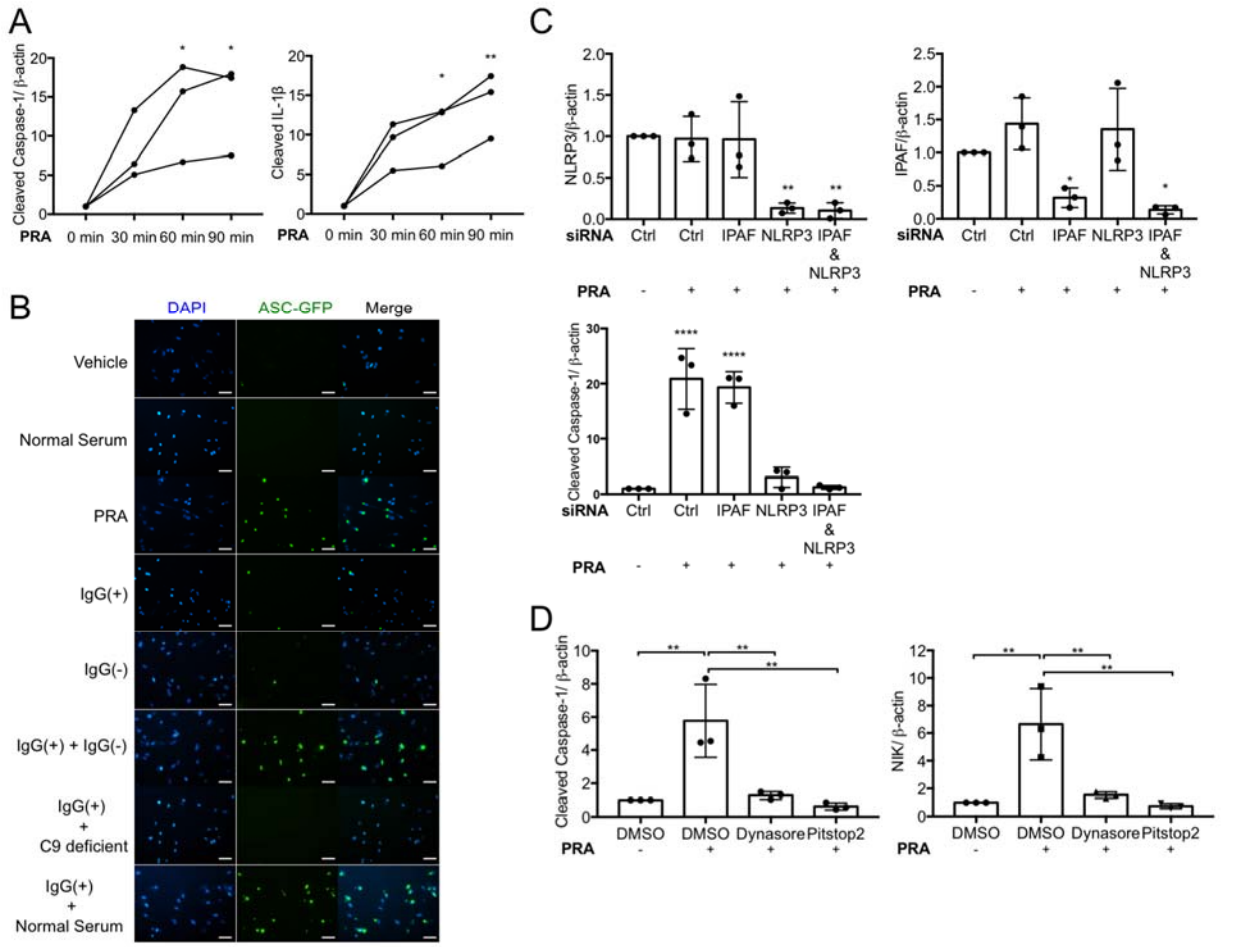
Immunofluorescence staining of paraffin-embedded patient renal biopsies

Formalin-fixed paraffin-embedded sections of de-identified patient renal biopsies taken at the time of transplant (time 0 control) or renal allograft biopsies patients with C4d deposition on peritubular capillary ECs, indicative of chronic antibody mediated rejection (CAMR) were analyzed. All biopsies were collected and sectioned at Addenbrooke's Hospital, with ethical approval by the hospital committee and diagnosis confirmed by R. Verena Broecker. Slides were deparaffinized by graded washes in xylene and ethanol and antigen retrieval was performed in sodium citrate buffer (Vector, H-3300). Using the FLICA 660 Caspase-1 Assay (Immunochemistry Technologies, 9122), FLICA-660 was added in 1:30 dilution to sections, incubated for 15 minutes at room temperature and washed with the Cell Wash Buffer. Sections were washed with PBS, permeabilized with 0.5% Triton in PBS and blocked with 10% goat serum in PBS for 1h at room temperature. Sections were stained with Ulex Europaeus Agglutinin I -Rhodamine (1:200, Vector RL-1062), C4d (1:50, Quidel, A213), Cleaved Caspase-1 (1:50, Cell Signaling Technology, D57A2). Samples were incubated with the appropriate AlexaFluor conjugated secondary antibodies in blocking solution

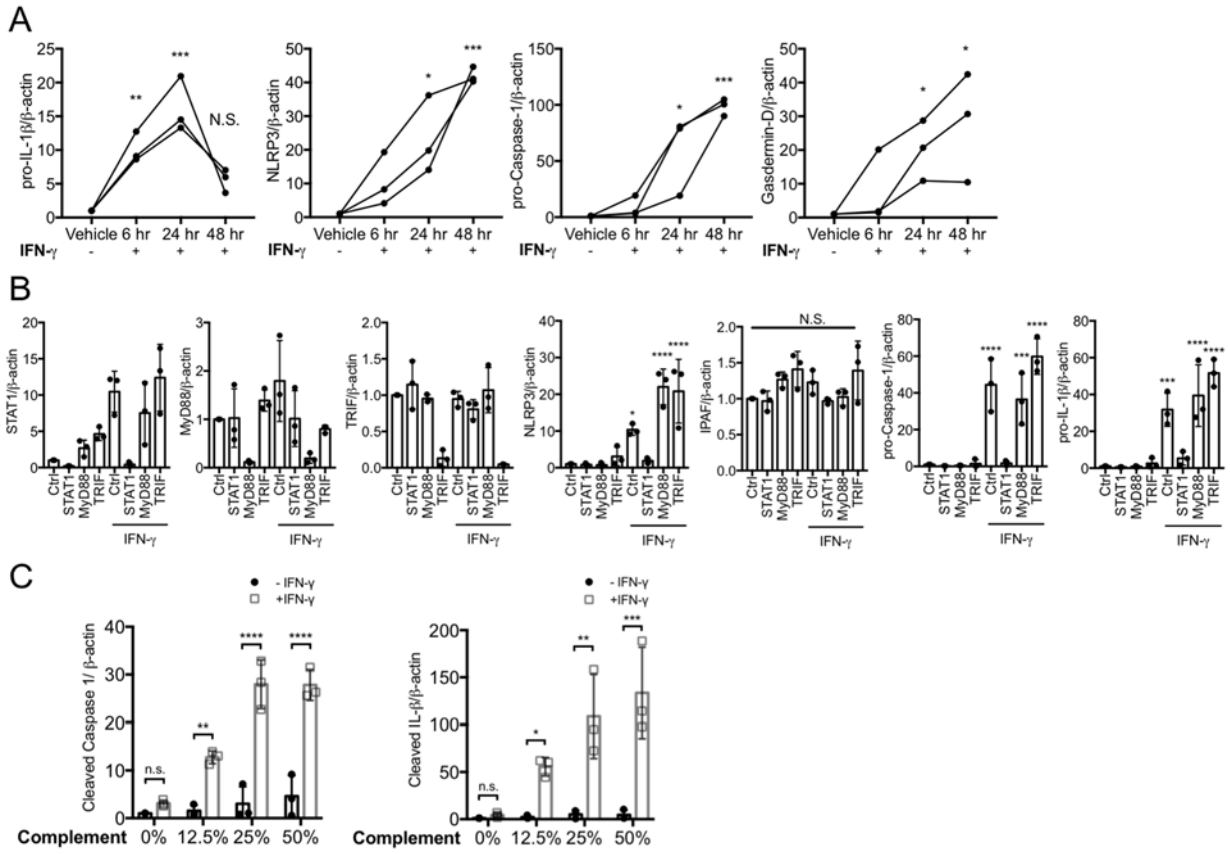
(1:500) for 1h dilution at room temperature. Control IgG controls corresponding to the same isotype of the primary antibody used including mouse IgG (ThermoFisher, MOPC21) and rabbit IgG (Abcam, ab199093) and secondary AlexaFluor antibody only controls were imaged to ensure specificity of antibody staining and to distinguish target staining above background. After coverslipped with ProLong Gold Mounting Reagent with DAPI (Life Technologies, P36935), samples were analyzed by confocal microscopy on 63X oil objective using the Leica TCS SP5. Colocalization for confocal analyses was quantified using ImageJ image analysis software (Bethesda, MD) with the Just Another Colocalisation Plugin (JACoP)

Statistics

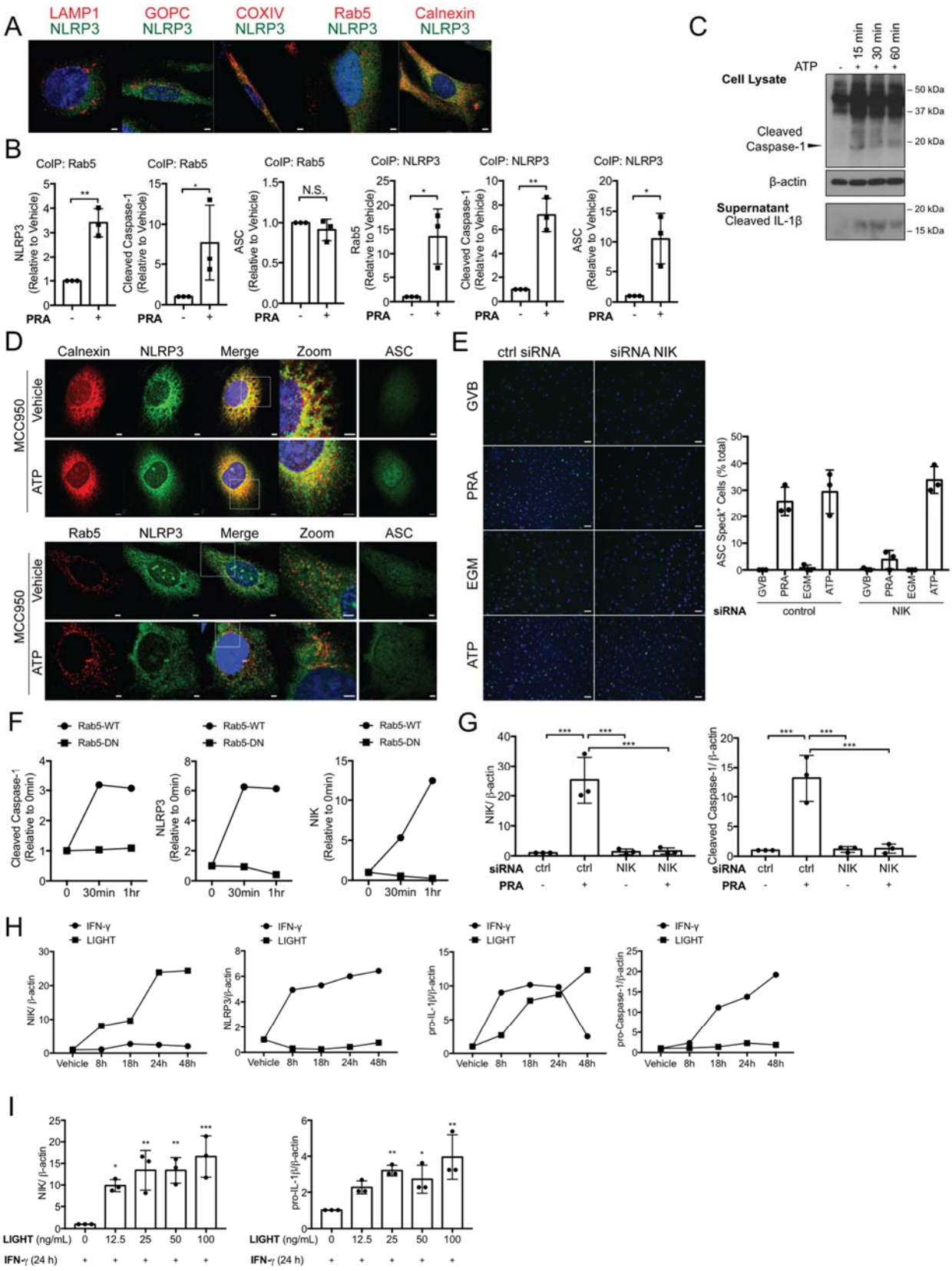
Data are expressed as mean ± SEM. Statistical analyses were performed using GraphPad Prism software. As indicated in figure legends, unpaired and paired t tests were used to make statistical comparisons between two groups. Comparisons between multiple groups were performed by one-way ANOVA with Tukey's test for post hoc analysis. In all experiments, $p < 0.05$ was considered statistically significant. Sample sizes for mouse experiments were chosen on the basis of our previous studies or publications, as well as the availability of mice and donors. These sample sizes were sufficient to establish statistical significance between samples in our experiments. At least 3 different human cell and tissue sources were used for each experiment.



Supplementary Figure I. Densitometric analyses of immunoblots from Fig. 1 and MAC induction of ASC-GFP specks. (**A**) Densitometric values of cleaved caspase-1 bands on exposed immunoblots of cell lysates from Fig. 1A were calculated and normalized to the intensity of β-actin bands. The intensity of cleaved IL-1β bands on immunoblots of culture supernatants from Fig. 1A were normalized to time 0 controls. Each line represents one experiment. (n=3, one-way ANOVA and Tukey's multiple comparisons test, SEM). (**B**) PRA sera was separated into IgG(+) and IgG(-) fractions and added to IFN-γ pre-treated ASC-GFP ECs either alone, or in combination with C9 deficient or normal serum as indicated for immunofluorescence and ASC speck formation analysis. Scale bar, 50 μm. (**C**) and (**D**) Densitometric values of the bands of proteins of interest on exposed immunoblots of cell lysates from Fig. 1, E and F, respectively, were calculated and normalized to the intensity of β-actin bands. (n=3, one-way ANOVA and Tukey's multiple comparisons test, SEM). *P<0.05, **P<0.01, ****P<0.0001.



Supplementary Figure II. Densitometric analyses of immunoblots from Fig. 2. (**A**), (**B**) and (**C**) Densitometric values of bands from proteins of interest on exposed immunoblots of cell lysates from Fig. 2, B, C and E, respectively, were calculated and normalized to the intensity of β-actin bands. The intensity of cleaved IL-1β bands on immunoblots of culture supernatants were normalized to vehicle controls. (n=3, one-way ANOVA and Tukey's multiple comparisons test, SEM). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, N.S., non-significant.


A
LAMP1 NLRP3
GOPC NLRP3
COXIV NLRP3
Rab5 NLRP3
Calnexin NLRP3
B
CoIP: Rab5
NLRP3 (Relative to Vehicle)
CoIP: Rab5
Cleaved Caspase-1 (Relative to Vehicle)
CoIP: Rab5
ASC (Relative to Vehicle)
CoIP: NLRP3
Rab5 (Relative to Vehicle)
CoIP: NLRP3
Cleaved Caspase-1 (Relative to Vehicle)
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β-actin
Supernatant
Cleaved IL-1β
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37 kDa
20 kDa
20 kDa
15 kDa
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Calnexin
NLRP3
Merge
Zoom
ASC
MCC950
Vehicle
ATP
Rab5
NLRP3
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GVB
PRA
EGM
ATP
ASC Speck+ Cells (% total)
GVB PRA EGM ATP GVB PRA EGM ATP
siRNA control NIK
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Rab5-WT
Rab5-DN
Cleaved Caspase-1 (Relative to 0min)
NLRP3 (Relative to 0min)
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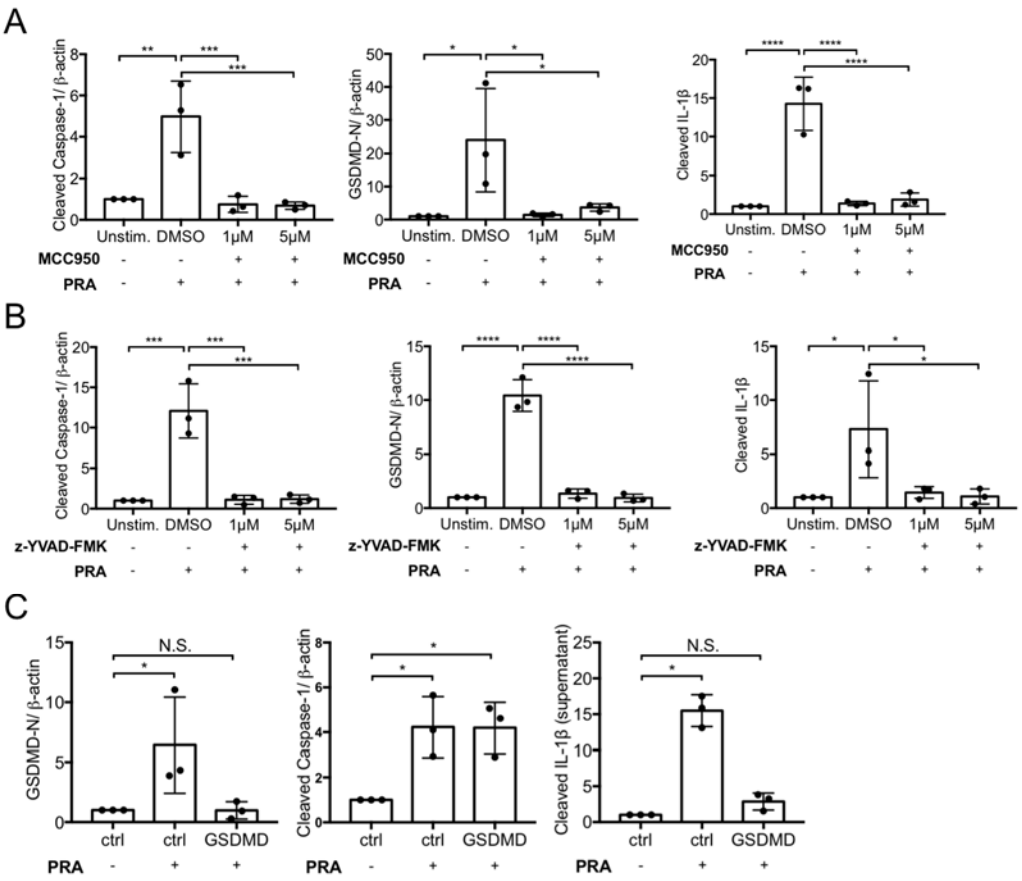
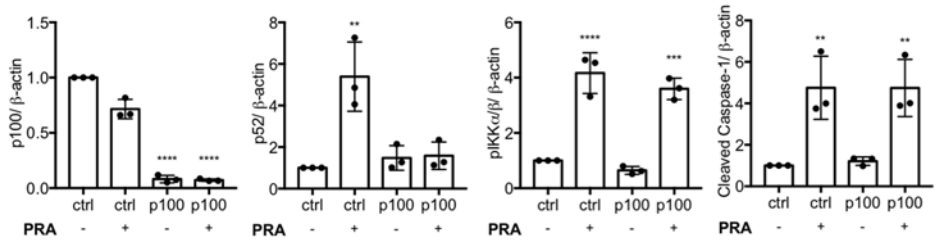
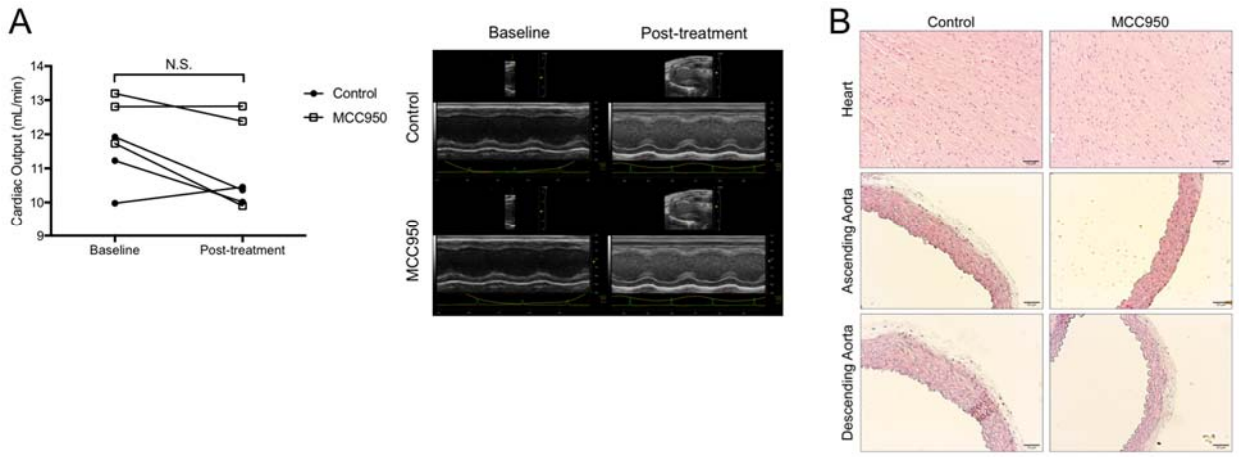
Supplementary Figure III. ATP-triggered NLRP3 inflammasome does not induce NLRP3 translocation from ER to Rab5 endosomes and does not require NIK, and densitometric analyses of immunoblots from Fig. 3. (**A**) IFN-γ-primed ASC-GFP ECs were analyzed for the colocalization of baseline NLRP3 with Rab5 endosomes, the mitochondria marker COX IV, Golgi marker GOPC, lysosomal marker LAMP1 or endoplasmic reticulum marker calnexin, using confocal microscopy. Scale bar, 5 μm. (**B**) Densitometric values of bands from proteins of interest on exposed immunoblots of co-IPs from Fig. 3, B and C, respectively, were calculated and normalized to the intensity of bands of vehicle or time 0 controls. (n=3, Student's t-test, SEM). (**D**) Confocal imaging of IFN-γ-primed ASC-GFP ECs that were pretreated with NLRP3 inhibitor MCC950 prior to ATP treatment for 30 minutes and co-staining with NLRP3 and Rab5 or calnexin. Scale bar, 5 μm. (**E**) ASC speck analysis of ASC-GFP ECs transfected with control or NIK siRNA and treated with PRA, ATP or vehicle controls. Scale bar, 50 μm. (**F**), (**G**), (**H**) and (**I**) Densitometric values of bands from proteins of interest on exposed immunoblots from Fig. 3, C, D, G and H, respectively, were calculated and normalized to the intensity of β-actin bands. (n=3, one-way ANOVA and Tukey's multiple comparisons test, SEM). *P<0.05, **P<0.01, ***P<0.001, N.S., non-significant.



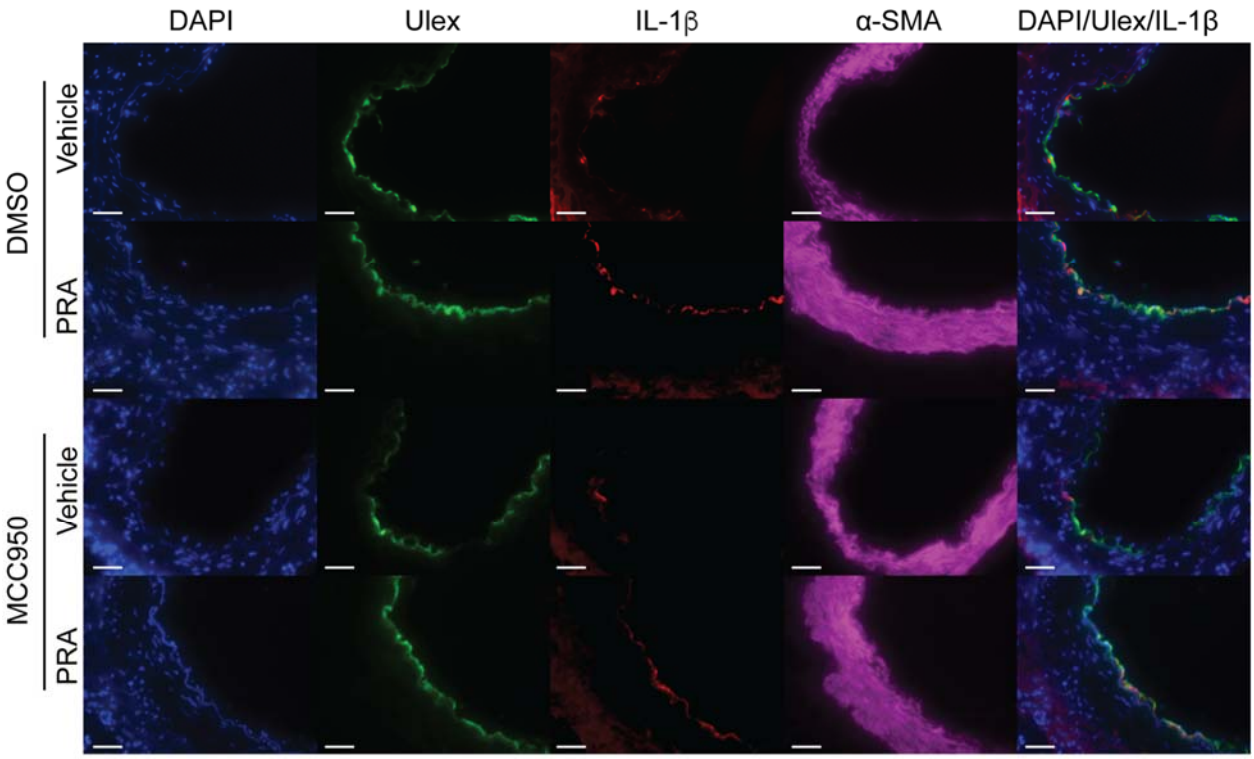
Supplementary Figure IV. Densitometric analyses of immunoblots from Fig. 4. (**A**), (**B**) and (**C**) Densitometric values of bands from proteins of interest on exposed immunoblots of cell lysates from Fig. 4, A, B, and D, respectively, were calculated and normalized to the intensity of β-actin bands. The intensity of cleaved IL-1β bands on immunoblots of culture supernatants were normalized to time 0 controls. (n=3, one-way ANOVA and Tukey's multiple comparisons test, SEM). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, N.S., non-significant.



Supplementary Figure V. Densitometric analyses of immunoblots from Fig. 5. Densitometric values of bands from proteins of interest on exposed immunoblots of cell lysates from Fig. 5E were calculated and normalized to the intensity of β-actin bands. The intensity of cleaved IL-1β bands on immunoblots of culture supernatants were normalized to vehicle controls. (n=3, one-way ANOVA and Tukey's multiple comparisons test, SEM). **P<0.01, ***P<0.001, ****P<0.0001.



Supplementary Figure VI. ***In vivo*** **MCC950 treatment does not lead to evidence of toxicity or inflammation of the heart and aorta and IL-1β is expressed by endothelial cells lining human artery grafts.** (**A**) Cardiac output values from echocardiographic assessment of SCID/bg mice at baseline and post- 24 hour treatment with MCC950 or control. (**B**) Histological assessment of hearts and aortae of SCID/bg mice harvested 24 hours after MCC950 treatment do not show evidence of inflammation as compared to those of control treated mice. Scale bar, 50 μm. (n=3, Paired t-test, SEM). N.S., non-significant.



Supplementary Figure VII. IL-1β is expressed by endothelial cells lining human artery grafts. IL-1β expression by ECs lining human artery grafts that were co-stained for Ulex, IL-1β, and alpha-smooth muscle actin (α-SMA) by immunofluorescence. Scale bar, 50 μm.

References to Expanded Materials and Methods

1. Jane-Wit D, Manes TD, Yi T, Qin L, Clark P, Kirkiles-Smith NC, Abrahimi P, Devalliere J, Moeckel G, Kulkarni S, Tellides G, Pober JS. Alloantibody and complement promote T cell-mediated cardiac allograft vasculopathy through noncanonical nuclear factor-kappaB signaling in endothelial cells. *Circulation*. 2013;128:2504-2516
2. Kosmidou C, Efstathiou NE, Hoang MV, et al. Issues with the Specificity of Immunological Reagents for NLRP3: Implications for Age-related Macular Degeneration. *Scientific reports*. 2018;8:461