ONLINE DATA SUPPLEMENT

METHODS

Treatments

For experiments using steroid ligands, cells were grown in medium supplemented with 10% (HEK293 cells and fibroblasts) or 2% (PAECs) charcoal-stripped heat inactivated FBS. SPL (Sigma, St. Louis, MO) and EPL (Tocris, Minneapolis, MN) were solubilized in ethanol or sterile DMSO (Sigma) before dilution in medium supplemented with charcoal-stripped heat inactivated FBS. Recombinant human TNFα (Peprotech, Rocky Hill, NJ) was prepared in PBS with 0.1% BSA. PMA (Calbiochem, Billerica, MA) was solubilized in sterile DMSO before further dilution. MG132 (EMD Millipore, Bellerica, MA) was solubilized in ethanol before further dilution and cells were pre-treated with 10μM for 1h prior to MR antagonist or vehicle control treatment as indicated. Working concentrations of CHX were prepared in sterile DMSO from a 100mg/mL stock solution (Sigma) and cells were treated at a final concentration of 100μg/mL as indicated. Cells in each experiment were exposed to equivalent volumes of vehicle.

DNA plasmid transfections

For total cell lysates used for Western blots, $5x10^5$ HEK293 cells were seeded in 35mm plates 48h prior to transfection. Cells were transiently transfected with 1μg of empty-vector (DDK-myc/pCMV6; Origene, Rockville, MD) plus 0.5μg of human MR-DDK-myc/pCMV6 (Origene), AR-DDK-myc/pCMV6 (Origene), GR-DDK-myc/pCMV6 (Origene), PR-DDK-myc/pCMV6 (Origene), or additional empty-vector using lipofectamine® 2000 (Life Technologies). For PXR and RXRγ, $2x10^5$ HEK293 cells/well were seeded in 12-well plates 48h prior to transfection. Cells were transiently transfected with 0.5μg of empty-vector plus 200ng of hPXR-DDK-

myc/pCMV6 (Origene), hRXRγ-DDK-myc/pCMV6 (Origene), or additional empty-vector using lipofectamine® 2000. For co-expression, cells were transiently transfected with 300ng of empty-vector plus 200ng of hPXR-DDK-myc/pCMV6 and 200ng of hRXRγ-DDK-myc/pCMV6. For XPB overexpression, cells were transiently transfected with 750ng of empty-vector (DDK-myc/pCMV6; Origene) plus 750ng of human XPB (*ERCC3*)/pCMV6-AC (Origene) or additional empty-vector. For luciferase reporter assays, $1x10^5$ HEK293 cells/well were seeded in 24-well plates 48h prior to transfection. Cells were transiently transfected as above with 100ng/well of MR, AR, GR, PR, PXR, RXRγ, PXR+RXRγ, XPB or empty-vector control plasmid along with 50ng of an internal control vector (pGL4.74/hRluc; Promega, Madison, WI). In addition, cells were simultaneously transfected with 100 ng of a destabilized luciferase reporter regulated by either NF-κB (pGL 4.32[luc2P/NF-κB-RE/Hygro]; Promega), or activator protein 1 (AP-1; pGL4.44[luc2P/AP-1-RE/Hygro]; Promega) as previously described.[1] Total amount of plasmid DNA per well was equal among experimental conditions using additional empty-vector control plasmid as necessary.

Western blotting

Cells were lysed on ice with RIPA buffer (Life Technologies) supplemented with either complete mini-protease inhibitors (Roche, Nutley, NJ) or Halt™ Protease and Phosphatase Inhibitor Cocktail (Life Technologies). Lysates were cleared by centrifugation (20,000*g* for 15min at 4°C), resolved by SDS-PAGE and transferred to a nitrocellulose membrane using the iBlot® Dry Blotting System (Life Technologies). Blots were blocked with 5% ECL Primer blocking agent (GE Healthcare, Pittsburgh, PA) and then incubated overnight at 4°C with the following antibodies against MR (1:100, rMR1-18, 1D5),[2] AR (1:250, sc-816; Santa Cruz

Biotechnology, Inc.), GR (1:500, sc-1003; Santa Cruz Biotechnology, Inc.), PR (1:200, sc-538; Santa Cruz Biotechnology, Inc.), PXR (1:100, sc-48403; Santa Cruz Biotechnology, Inc.), RXRγ (1:100, sc-365252; Santa Cruz Biotechnology, Inc.), α-tubulin (1:200, sc-5286; Santa Cruz Biotechnology, Inc.), or COX2/PTGS2 (1:1000 160107; Cayman Chemical, Ann Arbor, MI). Staining for β-actin was done at room temperature for 1h with an HRP-conjugated anti-β-actin antibody (1:50,000, A3854; Sigma). An N-terminal antibody was used for XPB detection in HEK293 cells, PAECs and fibroblasts (1:100, AF6349; R&D Systems, Minneapolis, MN). A C-terminal antibody was used for XPB detection in homogenized whole lung tissue (1:5000, sc-293X; Santa Cruz Biotechnology, Inc.). Blots were washed with 0.1% Tween-20 (Sigma) in PBS and then incubated with HRP-conjugated donkey anti-goat (705-035-147; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA), goat anti-mouse antibody (sc-2055; Santa Cruz Biotechnology, Inc.), or goat anti-rabbit (sc-2004; Santa Cruz Biotechnology, Inc. or 111-035-003; Jackson ImmunoResearch Laboratories, Inc.) for 1h at room temperature. Blots were developed with an enhanced chemiluminescence substrate (GE Healthcare) using the ChemiDoc XRS+ System (Bio-Rad Laboratories, Hercules, CA). Quantification of bands by densitometry analysis was performed using Image Lab software, version 5.0 (Bio-Rad Laboratories).

Luciferase reporter assays

The NF-κB [luc2P/NF-κB-RE] and AP-1 [luc2P/AP-1-RE] reporter constructs encode a *luc2P* (*Photinus pyralis*) gene that contains a protein destabilization sequence to better reflect induction and reduce treatment times and signal contamination by secondary effects. Cells were harvested for luciferase activity using a dual-luciferase assay kit (Promega) according to the

manufacturer's recommendations. Luciferase activity was measured using a VICTOR3 multi-label reader (PerkinElmer) and normalized to the activity of the renilla control.

NF-κB and AP-1 binding to DNA consensus sequences

HEK293 cells ($4x10^6$) were seeded onto 100mm plates and transiently transfected with an empty vector-control plasmid (DDK-myc/pCMV6) as above in order to assess binding of NF-κB (p65 and p50) and AP-1 (cFos and phosphorylated cJun) to DNA under conditions similar to the NF-κB and AP-1 luciferase reporter assays. Nuclear extracts (5μg), prepared with NE-PER Nuclear and Cytoplasmic Extraction Reagents (ThermoScientific) supplemented with Halt™ Protease and Phosphatase Inhibitor Cocktail, were used for TransAM® assays (Active Motif, Carlsbad, CA) with immobilized NF-κB or AP-1 consensus oligonucleotides. Colorimetric reactions were developed for 3-6min and read on a microplate spectrophotometer at 450nm with a reference wavelength of 655nm.

Quantitative real-time PCR

Total RNA was isolated using the RNeasy Mini Kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions, including DNase I treatment. RNA samples were then subjected to reverse transcription using the iScript cDNA Synthesis Kit (Bio-Rad; Hercules, CA). Gene expression assays were performed by quantitative real-time PCR in duplicate or triplicate using SYBR Green primers (see Table S6) and iTaq Universal SYBR Green Supermix with ROX (Bio-Rad) on the Applied Biosystems ViiA™7 cycler (Life Technologies). Reactions without reverse transcriptase were performed to rule out amplification of any residual genomic DNA. Target

gene expression was normalized to β-actin and relative gene expression was calculated using the $2^{-\Delta\Delta CT}$ method.

Immunocytochemistry

PAECs were plated at a density of $8x10^4$ cells/well on collagen-coated round glass covers (Thermo Scientific Pierce). The following day complete media was replaced with media containing charcoal-stripped serum. Cells were pre-treated with MG132 (10μM) or vehicle control for 1h followed by treatment with SPL (10μM) or vehicle control for an additional 1h and then stimulation with TNFα (5ng/mL) or vehicle control for 1h. Cells were washed 3 times with PBS, fixed and penetrated with 3.7% formaldehyde containing 0.05% triton-100 for 10min at room temperature, and then washed 3 times with PBS. Slides were blocked for 1h at room temperature with 3% BSA in PBS containing 10% normal goat serum and then stained overnight at 4°C with anti-XPB antibody (sc293X, working concentration of 2μg/ml; Santa Cruz). The following day, cells were washed again 3 times with PBS and then incubated with AlexaFluor 488 secondary antibody (Invitrogen) or Phalloidin-568 (#A12380; 3 units/mL; Invitrogen) for 1h in the dark. Cells were washed with PBS, stained with DAPI for 5min and washed again. Cells were mounted using ProLong® Gold antifade reagent (Invitrogen). Confocal images were acquired on a Zeiss LSM 710 microscope equipped with photo multiplier tubes and a 63X plan-apochromat 1.4NA oil objective. Laser Scanning Module 5 software was used for image acquisition and ImageJ was used for image processing.

Cytokine, chemokine and growth factor multiplex immunoassay

Median fluorescence intensities were collected on a Luminex-200 instrument (Luminex, Bio-Rad). A minimum of 60 beads were acquired per analyte. Low out of range values were imputed using either the lowest concentration detected in any sample or the calculated bottom of the quantifiable range, whichever was lower, both derived from standard curves for each cytokine (Bioplex v6.2 software). Likewise, high out of range values were imputed using either the highest concentration detected in any sample or the calculated top of the quantifiable range, whichever was higher, both derived from standard curves for each cytokine. For cultured PAECs, each experimental condition was performed in triplicate and four independent experiments were completed, each using different donors. Of the 27 total cytokines measured, IL-2, IL-5, and eotaxin were excluded from the subsequent analysis because concentrations of these cytokines following TNFα stimulation were all very low and below the range of detection in ≥50% of the samples at all three time points. Patient samples were run in duplicate and concentrations were determined from the standard curve using a 5-point-regression to transform mean fluorescence intensities. Of the 27 total cytokines measured, IL-1β, IL-5, and IL-15 were excluded from the subsequent analysis because serum concentrations of these cytokines were very low in all samples and below the range of detection in ≥50% of patients.

IL8 enzyme-linked immunosorbent assay (ELISA)

PAECs ($1x10^5$) were seeded onto collagen-coated 12-well plates 24h prior to treatments as indicated. Cell supernatants were aspirated and cleared by centrifugation (10,000*g* for 5min at 4°C). Concentrations of IL8 in PAEC supernatants were determined using the Quantikine®

Colorimetric Sandwich ELISA kit (R&D Systems) according to the manufacturer's recommendations.

XPB siRNA gene silencing

PAECs ($1x10^5$) were seeded in collagen-coated 6-well plates 24h prior to transfection. Cells were transfected with gene-specific siRNA targeting *ERCC3* (Dharmacon; Lafayette, CO) [siRNA#1 target sequence (Figure 4): GAUCAAGGUUAUAGCUUCA; siRNA#2 target sequence (Figure S5): CCGCGAAGAUGACAAAAUU) each at a final concentration of 10nM using DharmaFECT-1 (Dharmacon) in Opti-MEM (Invitrogen) for 6h followed by growth in EGM-2 containing 2% charcoal-stripped serum. Non-targeting siRNA (Dharmacon) (target sequence: UGGUUUACAUGUCGACUAA) was used as a control. After incubation of 48h from the time of transfection, cells were exposed to various treatments as indicated.

Chromatin immunoprecipitation assay

PAECs ($2x10^6$) were seeded in medium containing charcoal-stripped serum onto collagen-coated 15cm culture dishes 24h prior to treatment. Chromatin immunoprecipitation was performed using the EZ ChIP™ kit (EMD Millipore) according to the manufacturer's recommendations. Cells were cross-linked with 1% formaldehyde and then sonicated (Misonix) with microtip probe 4418 at a power setting of 4 and a 30% duty cycle. Sonication was performed 3 times for 10s with 50s cooling on ice between each pulse, shearing chromatin into 200-1000bp fragments. Chromatin fragments were precipitated overnight at 4°C with anti-XPB (clone S-19, sc-293, Santa Cruz Biotechnology, Inc.), anti-RNAPII (RNA pol II; clone CTD4H8, #05-623B, EMD Millipore), anti-pSer5-RNAPII (clone H14, #920401, Biolegend, San Diego, CA), or control

mouse IgG (#12-371B, Millipore). Precipitated protein/DNA complexes were eluted and crosslinking reversed for DNA purification. Independent experiments were performed using different donors. Purified DNA products were analyzed by quantitative real-time PCR using iTaq Universal SYBR Green Supermix with ROX using the Applied Biosystems ViiA^{TM}7 instrument. The *IL8* promoter region (-121 to +61) was amplified with the primer pairs 5′-GGGCCATCAGTTGCAAATC-3′ and 5′-TTCCTTCCGGTGGTTTCTTC-3′, and the *NFKBIA* promoter region (-168 to +21) was amplified with the primer pairs 5′-CTCATCGCAGGGAGTTTCT-3′ and 5′-ACTGCTGTGGGCTCTGCA-3′.[3] PCR reactions were performed in triplicate. XPB, RNAPII and pSer5-RNAPII promoter enrichment were calculated as a percentage of input DNA relative to vehicle treated, unstimulated cells.

Rat lung from the monocrotaline model of pulmonary hypertension

Samples were stored at -80ºC until further processing. Samples were thawed on ice and RIPA buffer (4µL/mg of lung tissue) containing Halt Protease and phosphatase inhibitor cocktail (Invitrogen) was added at a final concentration of 3X. Lung tissue samples were mechanically disrupted using a TissueLyser (QIAGEN) according to the manufacture's recommendations. Briefly, the samples were precooled on ice and TissueLyser disruption was performed for 30s at 30Hz followed by 10min incubation on ice. An additional 6 cycles of disruption were performed (15s at 30Hz alternating with 10min incubation on ice). Following the last cycle, the samples were incubated for 30min on ice with intermittent gentle agitation. Lysates were cleared by centrifugation (20,000*g* for 20min at 4ºC) and the supernatants were transferred to new tubes.

Statistical analysis

Dose response analyses for SPL and EPL were carried out by testing a null hypothesis that the linear regression slope equals zero except when noted. Random effects were added to the models to account for repeated measures. For quantitative real-time PCR, delta cycle thresholds rather than fold-changes were analyzed, as the latter are not normally distributed. For the analysis of secreted cytokines, chemokines and growth factors in PAECs, we tested for an interaction between each treatment and time and reported the main treatment effect when the interaction was non-significant. A multivariate analysis was performed to estimate the effects of SPL on circulating levels of inflammatory markers in a cohort of PAH patients. Cytokines (n=24) were analyzed together to increase power. Patient-level covariates examined for potential imbalances between the two groups (No SPL *versus* SPL) included age, gender, race, body mass index, diagnosis (idiopathic PAH, connective tissue disease-associated PAH versus other disease-associated PAH), New York Heart Associated-World Health Organization functional classification, 6-minute walk distance, and medication use (PDE5 inhibitors, ET-1 receptor antagonists, continuous PGI_2 infusions, warfarin, and anti-inflammatory therapies). Covariates were compared between the two groups using a Chi-squared test or Fisher's exact test for categorical variables, and t-tests for continuous variables (see Table S2). For the main analysis, only concurrent PAH-specific medications (PDE5 inhibitors, ET-1 receptor antagonists and continuous PGI_2 infusions) and patient-level covariates that showed evidence of imbalance ($P \leq 0.20$) were included in the model. To allow for different patterns for each cytokine, all two-way interaction terms involving a cytokine were included ("Full Model"). The Full Model was then simplified by removing non-significant interaction terms ("Reduced Model"). Several sensitivity analyses were performed including restriction of patient-level covariates to those that reached

statistical significance (i.e. prostacyclin infusion, hydroxychloroquine use, and obesity), propensity score adjustment and removing the 5 patients treated with hydroxychloroquine.

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2. Gomez-Sanchez CE, de Rodriguez AF, Romero DG, Estess J, Warden MP, Gomez-Sanchez MT, *et al.* Development of a panel of monoclonal antibodies against the mineralocorticoid receptor. *Endocrinology* 2006;**147**:1343-1348.
3. Nissen RM, Yamamoto KR. The glucocorticoid receptor inhibits NFkappaB by interfering with serine-2 phosphorylation of the RNA polymerase II carboxy-terminal domain. *Genes Dev* 2000;**14**:2314-2329.

FIGURE LEGENDS

Figure S1. SPL suppresses NF-κB and AP-1 promoter activity independent of PR, PXR, RXRγ, and PXR+ RXRγ expression in HEK293 cells. (A) Total cell lysate Western blots demonstrate human PR overexpression in HEK293 cells. As seen previously, in the absence of nuclear receptor overexpression, SPL dose-dependently suppressed NF-κB and AP-1 reporter activity ($P<0.0001$ for both). PR expression independent of SPL modestly suppressed NF-κB reporter activity ($P=0.01$ for a PR main effect; $P>0.3$ for an interaction with SPL). Basal AP-1 reporter activity was suppressed by PR in the absence of SPL ($P<0.0001$). SPL concentrations ≤ 5μM dose-dependently increased (*$P=0.03$), while 10μM decreased AP-1 activity (****$P<0.0001$). (B) Total cell lysate Western blots demonstrate human PXR and RXRγ overexpression in HEK293 cells. Endogenous RXRγ but not PXR was detected in these cells. Similar to MR, GR and AR, SPL dose-dependently suppressed NF-κB and AP-1 activity in the absence and presence of PXR, RXRγ and PXR+RXRγ overexpression ($P<0.0001$ the downward trend of the slopes). PXR and PXR+RXRγ overexpression, again independent of SPL ($P>0.2$ for interactions with SPL), modestly suppressed NF-κB ($P<0.04$ for both main effects), but conversely increased AP-1 signaling ($P<0.0001$ for both main effects). Twenty-four hours following transfection, cells were treated for 1h with either vehicle control or SPL followed by stimulation with either TNFα (10ng/mL; NF-κB activation) or PMA (100nM; AP-1 activation) for 5h. Luciferase activity was normalized to the renilla control. Luciferase results from five (panel A) and four (panel B) independent experiments, respectively, are presented relative to unstimulated control (mean±SE).

Figure S2. SPL suppression of NF-κB and AP-1 is independent of DNA binding or new protein synthesis, but reversed by proteasome inhibition. (A) TNFα significantly increased p65 and p50 DNA binding. However, DNA binding was unchanged across all three conditions (vehicle control, SPL and EPL) for p65 and p50. (B) PMA significantly induced cFos and cJun binding relative to control treated cells. Similar to NF-κB, AP-1 binding was unchanged across the three conditions for cFos and cJun. Vehicle control, SPL (10μM) or EPL (10μM) were added 1h prior to stimulation with TNFα (10ng/mL) or PMA (100nM). Data is presented as mean absorbance ±SE of three independent experiments. (C) In the presence of CHX (100μg/mL), TNFα stimulation resulted in super-induction of the NF-κB reporter gene (NF-κB *luc2P*; $P<0.01$ for the interaction between TNFα and CHX). However, SPL significantly and similarly suppressed NF-κB reporter activity in the absence and presence of CHX ($P=0.89$ and $P=0.76$ for the interaction of SPL and CHX in the absence and presence of TNFα, respectively). NF-κB-driven *luc2P* mRNA, as determined by quantitative real-time PCR, is presented as fold-change relative to unstimulated cells (geometric mean ± geometric SE) of four independent experiments, plotted on $\log_{10}$ scale. (D) Basal AP-1 reporter activity was higher ($P<0.0001$), while PMA-induced AP-1 activity was lower in the presence of MG132 ($P=0.0001$). Importantly, MG132 completely blocked SPL-mediated suppression of PMA-induced AP-1 reporter activation (five independent experiments). Luciferase activity (LUC) was normalized to renilla control (REN) and results are presented as the geometric mean LUC/REN ratio (x100) ± geometric SE plotted on a $\log_{10}$ scale. *, $P<0.05$; **** $P<0.0001$ (ANOVA with post-hoc tests, as indicated).

Figure S3. SPL increases proteasomal degradation of XPB but does not affect XPB (*ERCC3*) mRNA levels in HEK293 cells. (A) SPL significantly decreased XPB protein levels in

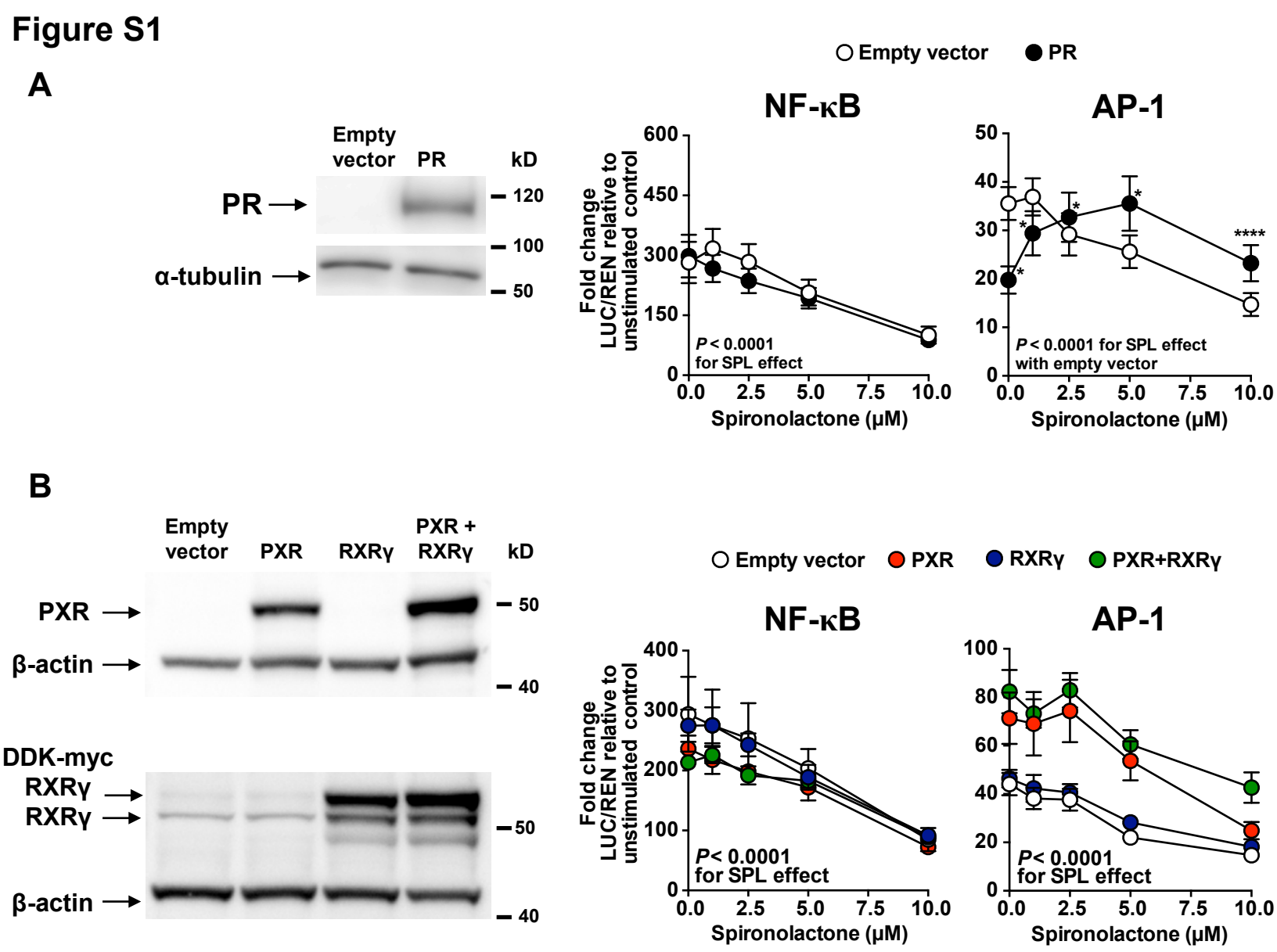
the absence and presence of MG132. However, in the presence of MG132, SPL-induced degradation of XPB was significantly decreased. In contrast to SPL, EPL had no effect on XPB protein levels in either the absence or presence of MG-132 (P=0.70 for the main effect of EPL). Total cell lysates were collected concurrent with the timing of luciferase assay experiments (Figure S2D), resolved by SDS-PAGE and immunoblotted for XPB and β-actin. Densitometric quantification of XPB protein expression relative to β-actin is presented as mean ratio ±SE of six independent experiments and a representative Western blot is shown below the graph. (B) *ERCC3* mRNA expression was not reduced by SPL treatment in either the absence (P=0.16) or presence of PMA (P=0.25). Expression of mRNA as determined by quantitative real-time PCR is presented as the fold-change relative to unstimulated cells (geometric mean ± geometric SE) of three independent experiments. *, P<0.05; **** P<0.0001 (ANOVA with post-hoc tests, as indicated).

Figure S4. Proteasome inhibition blocked the effect of SPL on PMA-induced PTGS2 and IL8 protein expression in PAECs. (A) SPL significantly decreased PMA-induced PTGS2 protein expression, whereas pretreatment with MG132 blocked SPL-mediated suppression as determined by total cell lysate Western blots. Densitometric quantification of PTGS2 protein expression relative to β-actin is presented on $\log_{10}$ scale of four independent experiments using different donors (geometric mean ratio ± geometric SE). A representative Western blot is shown below the graph. (B) Likewise, MG132 blocked SPL-mediated suppression of PMA-induced IL8 expression in PAECs as determined by ELISA of cell supernatants. PAECs were pre-treated with MG132 (10μM) for 1h followed by treatment with SPL for 1h and then stimulation with PMA (10nM) for 4h. Data are presented as the $\log_{10}$-transformed concentration of five independent

experiments using different donors (mean±SE). *, $P<0.05$; **, $P<0.01$; *** $P < 0.001$; **** $P < 0.0001$ (ANOVA with post-hoc tests, as indicated).

Figure S5. SPL in combination with XPB knockdown further suppresses TNFα target genes in PAECs. (A) Similar to Figure 4A, SPL treatment and XPB knockdown using a second siRNA both significantly reduced XPB protein levels, but the impact of SPL was significantly greater than XPB knockdown alone. Densitometric quantification of XPB protein expression relative to β-actin is presented as the geometric mean ratio ± geometric SE on $\log_{10}$ scale of four independent experiments, each with a different donor. A representative Western blot is shown below the graph. (B) SPL suppressed TNFα-induced *IL8*, *IL6*, and *CCL2*, but not *NFKBIA* (P=0.66) mRNA expression in PAECs transfected with siCTRL. Compared to SPL alone, the combination of XPB knockdown and SPL had a stronger suppressive effect on TNFα-induced *IL6*, *CCL2* and *NFKBIA*. Expression of mRNA measured by quantitative real-time PCR is presented as the fold-change relative to unstimulated cells transfected with siCTRL (geometric mean ± geometric SE) of four independent experiments, each with a different donor, plotted on $\log_{10}$ scale. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; **** $P<0.0001$ (ANOVA with post-hoc tests, as indicated).

Figure S1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P < 0.0001 for SPL effect with empty vector
Spironolactone (μM)
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RXRγ
PXR + RXRγ
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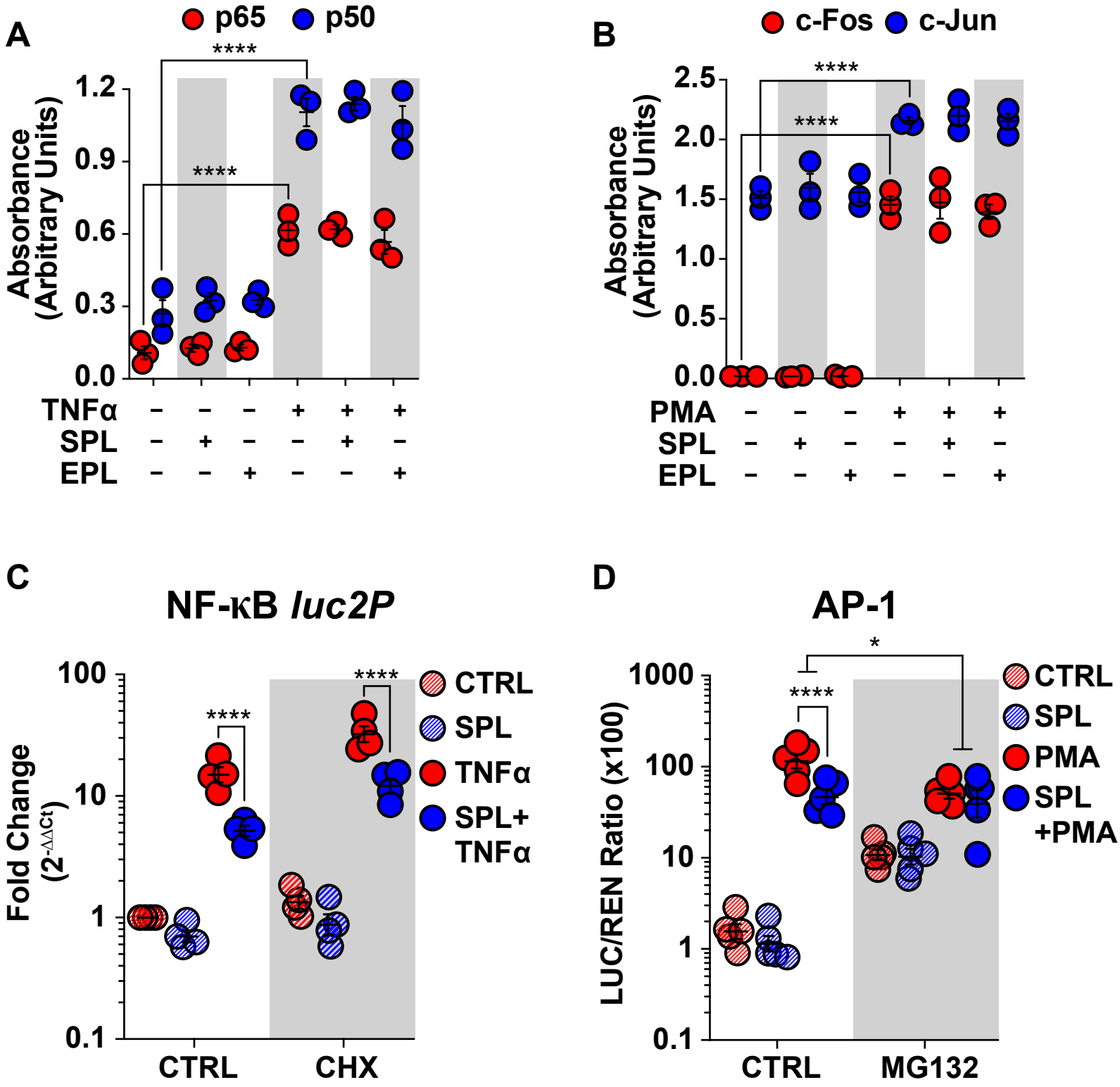
Figure S2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c-Fos
c-Jun
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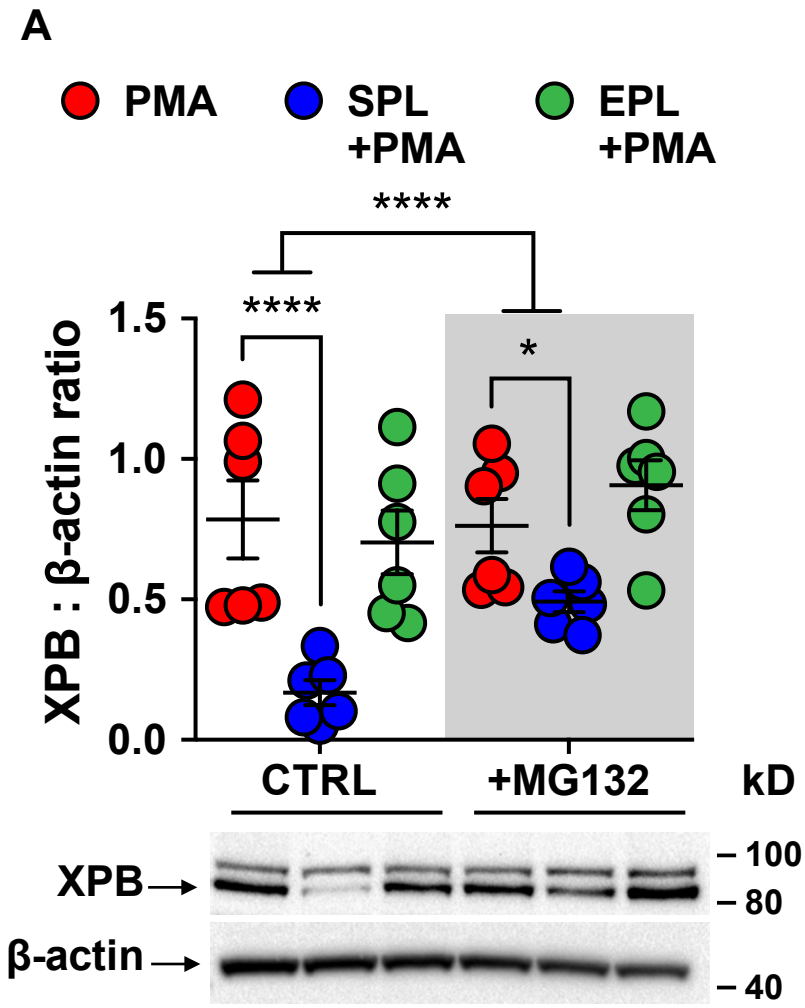
Figure S3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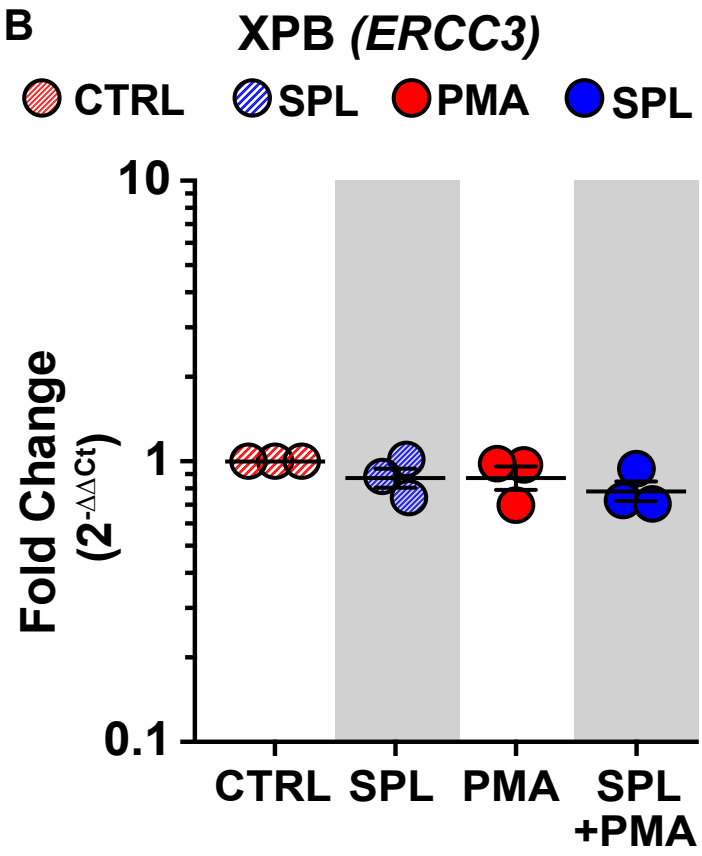
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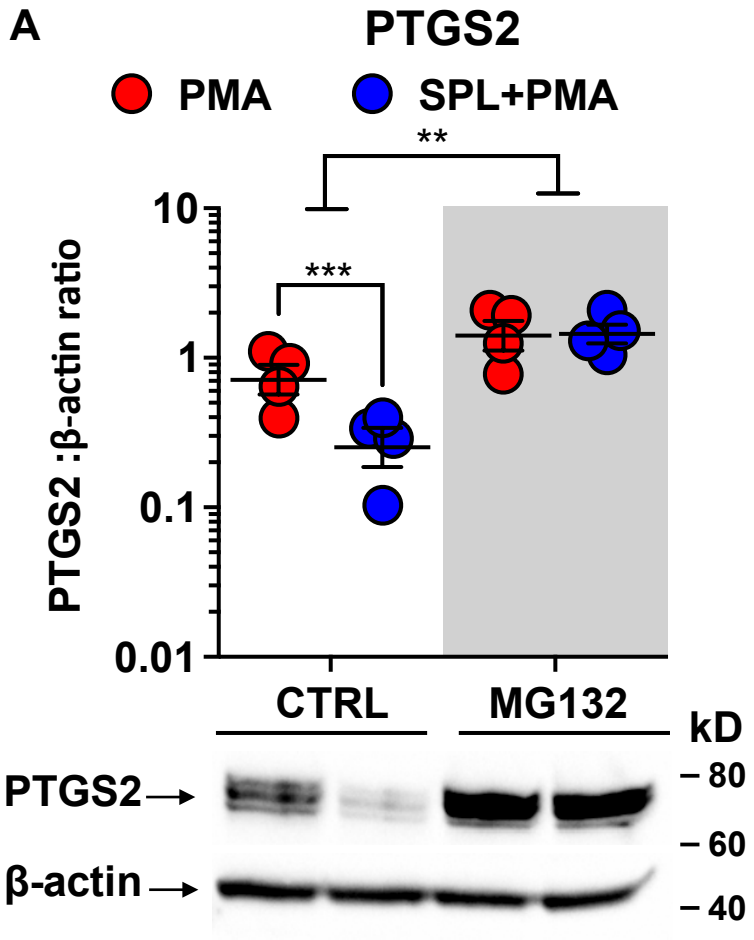
SPL +PMA

Figure S4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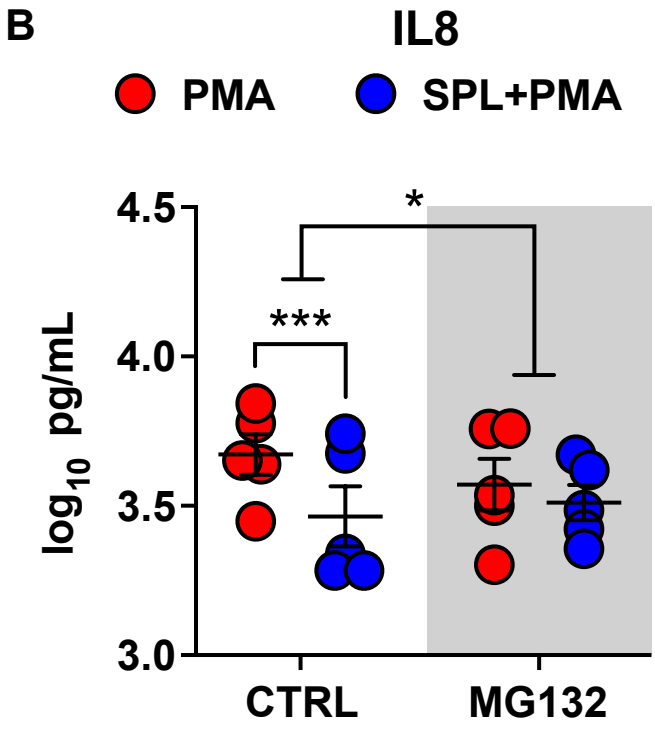
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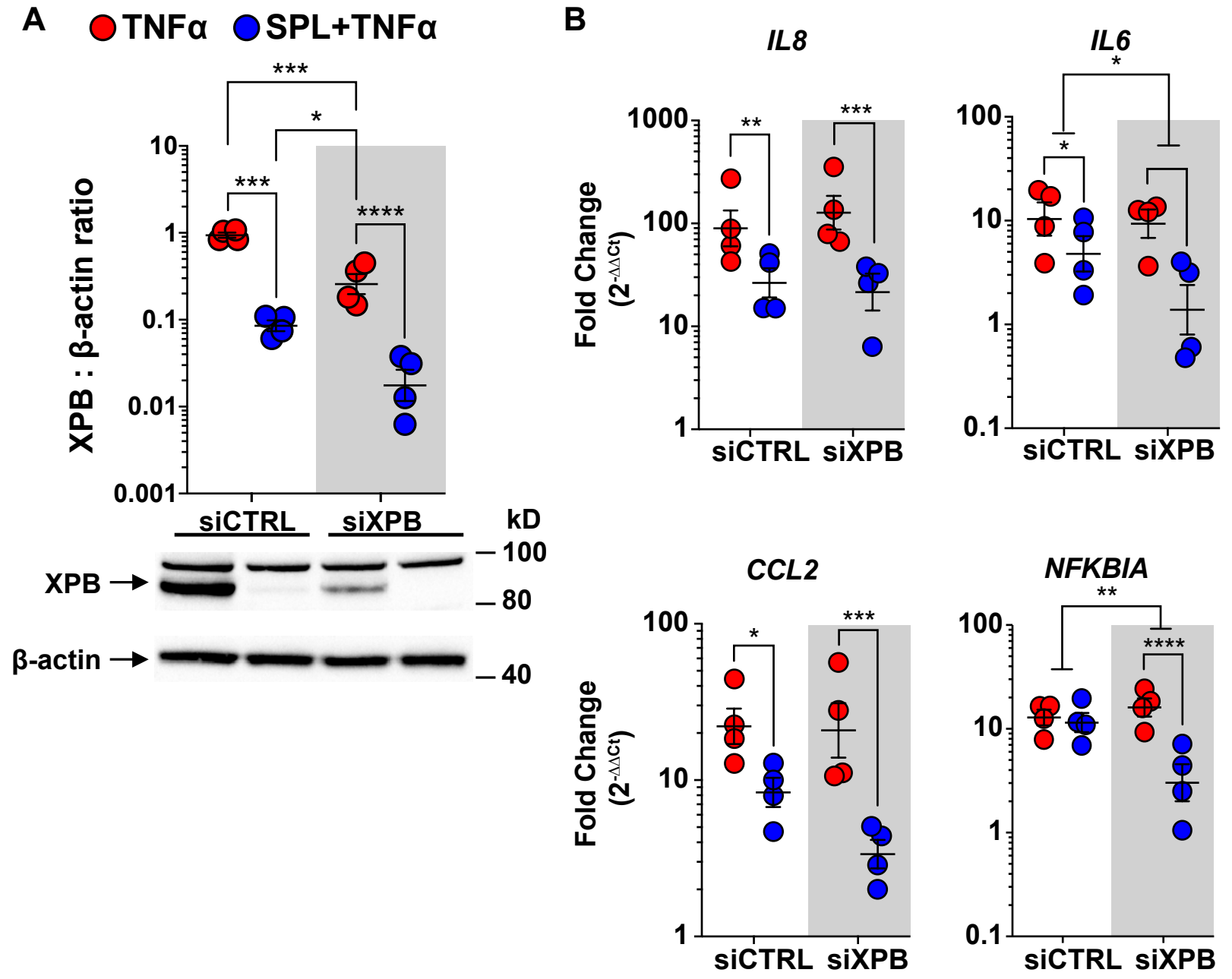
log10 pg/mL
CTRL
MG132

Figure S5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XPB : β-actin ratio
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XPB
β-actin
100
80
40
B
IL8
IL6
CCL2
NFKBIA
Fold Change (2-ΔΔCt)
siCTRL
siXPB

Table S1. Human Pulmonary Artery Endothelial Cell Donors

Donor Lot #	Age (years)	Gender	Assays	Company
225531	45	Female	Bio-Plex®	Lonza
329447	40	Male	Bio-Plex®, qRT-PCR, WB, siRNA, ChIP	Lonza
466719	57	Male	IF, WB, qRT-PCR, siRNA	
493459	60	Male	WB, ELISA, siRNA, qRT-PCR	
608199			ELISA	
4F3034	51	Female	Bio-Plex®, WB, ELISA, siRNA, ChIP	Lonza
4F3033	51	Male	IF, qRT-PCR, ELISA, siRNA, ChIP	Lonza
4F3041	52	Female	Bio-Plex®, qRT-PCR, WB, ELISA, siRNA, ChIP	Lonza
4F3028	21	Male	WB, qRT-PCR, siRNA, ChIP	Lonza

Abbreviations: qRT-PCR, quantitative real-time PCR; ChIP, chromatin immunoprecipitation; IF, immunofluorescence; WB, Western blot

Table S2. Human Fibroblasts from the National Institute of General Medical Sciences (NIGMS) Genetic Cell Repository

Donor #	Age (years)	Gender	Mutation	Clinical Description
GM13025	39	Male	F99S	Mild form of XP/CS complex
GM13027	63	Female	Not a carrier of the F99S mutation	Unaffected mother of GM13025
GM21072	10	Female	Q545X and Q739insX42	Severe form of XP/CS complex

Abbreviations: XP, Xeroderma Pigmentosum; CS, Cockayne syndrome

Table S3. The Effect of SPL and EPL on TNFα-induced Cytokine, Chemokine and Growth Factor Production in Primary Human Pulmonary Artery Endothelial Cells

	4h				8h				24h				Treatment*Time	
	Mean (pg/mL)	SE (pg/mL)	Fold-Change[a]	P-value[b]	Mean (pg/mL)	SE (pg/mL)	Fold-Change[a]	P-value[b]	Mean (pg/mL)	SE (pg/mL)	Fold-Change[a]	P-value[b]	Interaction P-value	Main Effect P-value[c]
IL1β														
CTRL	1.54	0.69	0.13	0.0003	1.35	0.57	0.12	<0.0001	1.91	0.80	0.25	0.001	0.47	<0.0001
TNFα	11.91	0.63	REF	REF	10.86	0.38	REF	REF	7.57	1.27	REF	REF	REF	REF
SPL+TNFα	12.63	1.42	1.06	0.92	7.46	0.93	0.69	0.30	5.56	1.33	0.74	0.27	0.31	0.08
EPL+TNFα	13.40	0.55	1.13	0.79	11.21	1.35	1.03	0.97	7.61	1.10	1.00	0.94	0.85	0.52
IL1ra														
CTRL	33.29	4.32	0.14	<0.0001	27.95	6.14	0.11	<0.0001	23.31	9.70	0.07	0.002	0.24	<0.0001
TNFα	239.89	20.02	REF	REF	250.61	13.42	REF	REF	352.35	52.13	REF	REF	REF	REF
SPL+TNFα	214.38	37.65	0.89	0.37	127.73	4.75	0.51	0.01	199.34	25.03	0.57	0.51	0.02	NA
EPL+TNFα	260.55	33.86	1.09	0.67	263.82	42.51	1.05	0.94	340.42	53.20	0.97	0.97	0.76	0.79
IL4														
CTRL	1.92	0.13	0.45	<0.0001	1.61	0.30	0.36	0.0001	1.37	0.28	0.22	<0.0001	0.03	NA
TNFα	4.26	0.27	REF	REF	4.50	0.36	REF	REF	6.19	0.71	REF	REF	REF	REF
SPL+TNFα	3.73	0.61	0.87	0.14	3.20	0.16	0.71	0.08	4.54	0.48	0.73	0.10	0.36	<0.0001
EPL+TNFα	4.68	0.50	1.10	0.45	4.62	0.51	1.03	0.92	5.96	0.64	0.96	0.84	0.29	0.48
IL6														
CTRL	130.07	30.84	0.30	<0.0001	143.95	38.63	0.26	<0.0001	213.07	56.07	0.07	<0.0001	0.0002	NA
TNFα	436.12	71.74	REF	REF	543.54	94.44	REF	REF	2910.71	559.39	REF	REF	REF	REF
SPL+TNFα	287.27	50.24	0.66	0.04	340.02	69.39	0.63	0.01	1607.45	421.05	0.55	0.01	0.94	0.0002
EPL+TNFα	448.40	90.42	1.03	0.95	542.84	124.32	1.00	0.73	2965.06	1358.29	1.02	0.64	0.96	0.78
IL7														
CTRL	29.06	7.31	0.86	0.01	25.50	5.71	0.79	0.001	18.27	2.12	0.58	<0.0001	0.01	NA
TNFα	33.87	6.86	REF	REF	32.39	6.00	REF	REF	31.53	4.72	REF	REF	REF	REF
SPL+TNFα	30.69	7.54	0.91	0.04	28.23	6.21	0.87	0.01	31.83	6.15	1.01	0.90	0.21	0.02
EPL+TNFα	34.36	9.11	1.01	0.71	31.87	7.23	0.98	0.49	33.34	4.47	1.06	0.28	0.54	0.97
IL8														
CTRL	673.52	82.96	0.08	<0.0001	726.39	96.44	0.03	<0.0001	1145.18	102.75	0.003	<0.0001	<0.0001	NA
TNFα	8103.17	1385.48	REF	REF	22153.98	2148.72	REF	REF	$3.80x10^5$	$1.18x10^5$	REF	REF	REF	REF
SPL+TNFα	2991.80	543.78	0.37	0.003	5709.17	973.50	0.26	<0.0001	20017.47	3897.17	0.05	<0.0001	0.001	NA
EPL+TNFα	8581.48	1876.42	1.06	0.90	22268.07	2604.62	1.01	0.99	$3.16x10^5$	$1.10x10^5$	0.83	0.54	0.87	0.77
IL9														
CTRL	5.13	1.82	0.72	0.06	4.43	1.46	0.69	0.09	3.58	1.58	0.38	0.03	0.23	0.01
TNFα	7.16	2.42	REF	REF	6.44	1.90	REF	REF	9.39	3.11	REF	REF	REF	REF
SPL+TNFα	5.55	1.93	0.78	0.14	4.39	1.49	0.68	0.09	7.29	2.60	0.78	0.17	0.49	0.03
EPL+TNFα	7.13	2.58	1.00	0.89	7.53	2.48	1.17	0.85	9.69	2.88	1.03	0.78	0.97	0.78

IL10														
CTRL	30.08	5.24	1.04	0.57	25.80	4.46	1.06	0.90	20.15	4.27	1.09	0.94	0.99	0.80
TNFα	28.82	6.11	REF	REF	24.44	0.93	REF	REF	18.44	0.80	REF	REF	REF	REF
SPL+TNFα	30.71	3.75	1.07	0.32	21.57	1.38	0.88	0.39	19.14	1.93	1.04	0.90	0.41	1.00
EPL+TNFα	32.29	8.02	1.12	0.39	26.23	5.79	1.07	0.98	20.29	0.65	1.10	0.63	0.92	0.52
IL12p70														
CTRL	107.70	46.28	0.96	0.56	87.70	39.04	1.04	0.48	30.58	4.96	0.69	0.01	0.36	0.17
TNFα	112.06	49.25	REF	REF	84.21	30.02	REF	REF	44.03	12.05	REF	REF	REF	REF
SPL+TNFα	109.13	46.40	0.97	0.81	87.19	34.94	1.04	0.94	49.90	17.56	1.13	0.51	0.76	0.71
EPL+TNFα	115.95	55.67	1.03	0.84	89.74	37.31	1.07	0.90	45.54	11.64	1.03	0.67	0.95	0.83
IL13														
CTRL	19.51	3.93	0.83	0.03	19.15	3.67	0.77	0.0001	15.19	3.18	0.61	0.0002	0.03	NA
TNFα	23.54	4.44	REF	REF	24.76	3.89	REF	REF	24.92	3.39	REF	REF	REF	REF
SPL+TNFα	20.69	4.69	0.88	0.06	21.49	3.99	0.87	0.01	23.28	4.23	0.93	0.33	0.71	0.003
EPL+TNFα	23.85	4.79	1.01	0.91	24.53	4.60	0.99	0.52	24.72	3.41	0.99	0.92	0.89	0.74
IL15														
CTRL	87.82	26.03	0.34	0.001	93.70	27.28	0.24	<0.0001	138.25	33.91	0.29	<0.0001	0.65	<0.0001
TNFα	254.60	32.17	REF	REF	386.60	7.50	REF	REF	475.46	10.76	REF	REF	REF	REF
SPL+TNFα	160.03	10.64	0.63	0.11	226.01	14.15	0.58	0.03	401.66	12.47	0.84	0.34	0.03	NA
EPL+TNFα	268.80	33.44	1.06	0.83	398.03	28.61	1.03	0.92	474.59	12.60	1.00	0.99	0.91	0.64
IL17														
CTRL	33.92	8.38	0.70	0.004	29.81	5.67	0.65	0.01	24.32	6.31	0.33	0.0003	0.01	NA
TNFα	48.75	9.18	REF	REF	45.86	7.84	REF	REF	73.98	15.56	REF	REF	REF	REF
SPL+TNFα	37.76	6.70	0.77	0.05	28.33	5.76	0.62	0.005	49.13	11.31	0.66	0.07	0.36	<0.0001
EPL+TNFα	50.50	11.03	1.04	0.91	46.50	12.31	1.01	0.56	74.16	14.13	1.00	0.95	0.88	0.83
FGFb[d]														
CTRL	1205.57	227.96	0.99	0.80	1069.70	208.74	1.00	0.59	719.08	92.00	0.92	0.12	0.73	0.33
TNFα	1219.55	240.25	REF	REF	1074.19	168.30	REF	REF	785.48	118.41	REF	REF	REF	REF
SPL+TNFα	1196.66	214.71	0.98	0.86	1020.05	166.96	0.95	0.17	747.80	127.04	0.95	0.24	0.75	0.19
EPL+TNFα	1240.64	258.33	1.02	0.71	1091.26	221.90	1.02	0.82	798.56	121.41	1.02	0.76	0.96	0.86
G-CSF														
CTRL	28.77	11.98	0.38	0.001	28.80	14.95	0.33	0.0002	40.39	27.07	0.02	<0.0001	<0.0001	NA
TNFα	74.79	14.69	REF	REF	87.39	16.39	REF	REF	1757.41	1114.60	REF	REF	REF	REF
SPL+TNFα	53.49	12.38	0.72	0.15	47.65	13.01	0.55	0.02	555.01	270.16	0.32	0.01	0.26	0.0004
EPL+TNFα	72.00	12.87	0.96	0.89	82.83	21.15	0.95	0.70	1891.70	1376.12	1.08	0.63	0.97	0.61
GM-CSF														
CTRL	716.61	31.05	0.95	0.13	724.38	10.79	0.96	0.34	694.63	44.84	0.85	0.003	0.10	0.01
TNFα	755.62	11.91	REF	REF	752.81	27.91	REF	REF	815.31	22.75	REF	REF	REF	REF
SPL+TNFα	757.46	19.16	1.00	0.96	716.78	31.26	0.95	0.20	728.84	20.34	0.89	0.02	0.11	0.03
EPL+TNFα	790.13	32.74	1.05	0.24	737.65	18.80	0.98	0.61	816.51	29.08	1.00	0.99	0.63	0.76

IFNγ														
CTRL	64.68	7.18	0.28	0.002	57.16	7.45	0.24	0.001	53.59	13.70	0.13	0.0001	0.14	<0.0001
TNFα	229.84	52.95	REF	REF	234.34	53.27	REF	REF	397.93	30.08	REF	REF	REF	REF
SPL+TNFα	233.65	13.62	1.02	0.61	160.34	29.40	0.68	0.28	212.73	47.22	0.53	0.06	0.08	NA
EPL+TNFα	264.94	38.04	11.15	0.39	261.41	41.52	1.12	0.49	350.16	46.80	0.88	0.68	0.38	0.43
IP10														
CTRL	69.90	5.63	0.51	<0.0001	68.60	8.34	0.04	<0.0001	65.44	9.83	0.01	<0.0001	<0.0001	NA
TNFα	137.32	20.72	REF	REF	1865.45	377.86	REF	REF	12384.92	1950.15	REF	REF	REF	REF
SPL+TNFα	85.20	12.89	0.62	0.001	472.83	225.60	0.25	0.001	4530.69	1144.79	0.37	0.003	0.07	NA
EPL+TNFα	148.63	23.14	1.08	0.45	1996.88	525.81	1.07	0.93	12145.34	1908.05	0.98	0.95	0.92	0.73
CCL2														
CTRL	832.88	365.41	0.18	0.0002	1019.47	454.94	0.07	<0.0001	1787.36	621.41	0.03	0.0002	0.19	<0.0001
TNFα	4596.48	819.33	REF	REF	14682.95	2223.30	REF	REF	69623.19	44386.31	REF	REF	REF	REF
SPL+TNFα	1882.95	352.34	0.41	0.03	3795.07	348.48	0.26	0.002	17998.26	1315.41	0.26	0.15	0.60	0.0001
EPL+TNFα	4640.03	763.54	1.01	0.96	15229.13	2739.96	1.04	0.95	62067.90	36854.18	0.89	0.94	0.99	1.00
MIP1α														
CTRL	2.66	0.93	0.61	0.01	2.33	0.98	0.55	0.004	2.44	0.98	0.47	0.01	0.61	<0.0001
TNFα	4.38	0.48	REF	REF	4.20	0.58	REF	REF	5.24	0.61	REF	REF	REF	REF
SPL+TNFα	3.96	0.66	0.90	0.54	3.06	0.92	0.73	0.07	4.29	0.67	0.82	0.51	0.33	0.01
EPL+TNFα	4.45	0.48	1.02	0.93	4.28	0.61	1.02	0.97	5.20	0.60	0.99	0.99	0.98	0.88
MIP1β														
CTRL	9.90	2.68	0.82	0.22	8.91	2.07	0.85	0.44	8.41	2.73	0.62	0.08	0.45	0.05
TNFα	12.10	3.38	REF	REF	10.45	2.79	REF	REF	13.64	4.61	REF	REF	REF	REF
SPL+TNFα	10.53	2.88	0.87	0.31	8.18	2.30	0.78	0.09	10.41	3.59	0.76	0.28	0.77	0.02
EPL+TNFα	12.74	3.63	1.05	0.71	11.00	3.63	1.05	0.88	12.93	4.22	0.95	0.87	0.90	0.94
PDGFbb														
CTRL	11.06	0.62	0.62	0.001	13.65	2.84	0.63	0.0001	32.65	7.73	0.40	<0.0001	0.18	<0.0001
TNFα	17.72	2.45	REF	REF	21.62	3.16	REF	REF	80.97	26.30	REF	REF	REF	REF
SPL+TNFα	16.17	2.35	0.91	0.33	16.65	2.77	0.77	0.01	76.57	26.69	0.95	0.30	0.62	0.10
EPL+TNFα	19.67	3.13	1.11	0.30	22.86	4.82	1.06	0.82	85.73	26.84	1.06	0.39	0.92	0.42
RANTES														
CTRL	40.32	7.03	0.44	0.001	39.30	9.67	0.08	<0.0001	35.46	9.61	0.01	<0.0001	<0.0001	NA
TNFα	91.73	28.00	REF	REF	500.84	182.77	REF	REF	6879.69	3357.09	REF	REF	REF	REF
SPL+TNFα	73.59	20.04	0.80	0.28	311.81	89.59	0.62	0.21	3908.36	1419.16	0.57	0.19	0.59	0.003
EPL+TNFα	98.37	33.34	1.07	0.77	502.74	173.40	1.00	0.96	5959.01	2649.30	0.87	0.73	0.75	0.88
TNFα[e]														
CTRL	11.40	1.81	0.002	<0.0001	10.36	1.86	0.003	<0.0001	11.85	2.89	0.007	<0.0001	0.01	NA
TNFα	5013.60	453.62	REF	REF	3958.44	150.66	REF	REF	1677.93	293.84	REF	REF	REF	REF
SPL+TNFα	5559.33	1059.77	1.11	0.75	2955.32	226.14	0.75	0.09	1161.16	51.73	0.69	0.28	0.19	0.07
EPL+TNFα	5674.67	172.45	1.13	0.52	4403.51	724.84	1.11	0.65	1378.20	64.04	0.82	0.59	0.33	0.84

VEGF[d]														
CTRL	1525.44	688.23	0.98	0.48	1158.37	487.95	0.96	0.04	415.64	73.01	0.64	0.001	0.16	0.06
TNFα	1562.84	703.80	REF	REF	1205.81	449.82	REF	REF	644.60	163.15	REF	REF	REF	REF
SPL+TNFα	1543.54	712.35	0.99	0.46	1209.84	479.15	1.00	0.64	717.64	232.05	1.11	0.46	0.79	0.91
EPL+TNFα	1639.60	777.17	1.05	0.52	1271.68	521.72	1.05	0.59	656.60	158.76	1.02	0.75	1.00	0.73

Abbreviations: REF, reference for fold change comparison; NA, not applicable

[a]Fold change relative to TNFα stimulation

[b]P-values were calculated based on $\log_{10}$ transformed concentrations

[c]Main effect p-values are reported when the p-value for the interaction between treatment and time is > 0.10

[d]Component of cell culture media

[e]Used to stimulate cells

Table S4. Patient Characteristics

	No SPL (N=36)	SPL (N=17)	P-value
Mean age, years (SD)	52 (15)	50 (12)	0.71
Gender, % Female (n)	92 (33)	94 (16)	1.00
Race, % Caucasian (n)	72 (26)	71 (12)	1.00
Body mass index > 30, % (n)	31 (11)	71 (12)	0.006
Diagnosis, % (n)			0.20
Idiopathic or hereditary PAH	50 (18)	41 (7)	
Systemic Sclerosis associated PAH	22 (8)	18 (3)	
Connective tissue disease-associated PAH[a]	6 (2)	29 (5)	
Congenital heart disease-associated PAH	14 (5)	12 (2)	
Other associated PAH[b]	8 (3)	0 (0)	
NYHA/WHO functional class, % (n)			1.00
I	11 (4)	12 (2)	
II	53 (19)	53 (9)	
III	31 (11)	35 (6)	
IV	6 (2)	0 (0)	
Use of phosphodiesterase 5 inhibitors, % (n)	83 (30)	94 (16)	0.41
Use of endothelin receptor antagonists, % (n)	25 (9)	47 (8)	0.11
Use of a continuous prostacyclin infusion, % (n)	31 (11)	59 (10)	0.05
Use of warfarin, % (n)	19 (7)	6 (1)	0.41
Use of immunomodulatory therapy[c], % (n)	3 (1)	24 (4)	0.03
Mean 6-minute walk distance, m (SD)	426 (129)	414 (109)	0.75
Mean 6-minute walk distance, % predicted (SD)	79 (23)	79 (22)	0.99
Mean daily dose of spironolactone, mg (SD)	-----	66 (34)	

Abbreviations: SPL, spironolactone; PAH, pulmonary arterial hypertension; NYHA/WHO, New York Heart Association/World Health Organization.

[a]In No SPL group, one patient with connective tissue disease also had portal hypertension.

[b]In No SPL group, drug-induced PAH (n=1), portal hypertension associated PAH (n=1) and hereditary hemorrhagic telangiectasia associated PAH (n=1).

[c]In No SPL group, one patient was taking hydroxychloroquine; in SPL group, all four patients were taking hydroxychloroquine

Table S5. Multivariate Analysis of Inflammatory Mediators in PAH Patients

	Eotaxin			FGFb			GM-CSF			G-CSF		
	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value
Obesity (BMI ≥ 30)	-0.018	0.10	0.85	0.087	0.10	0.36	0.23	0.10	0.017	0.20	0.10	0.034
Etiology of PAH												
IPAH/HPAH vs. other associated PAH[a]	0.11	0.12	0.34	0.19	0.12	0.11	0.18	0.12	0.14	0.11	0.12	0.35
CTD PAH vs. other associated PAH[a]	0.25	0.13	0.05	0.20	0.13	0.13	0.28	0.13	0.032	0.070	0.13	0.59
Endothelin-1 receptor antagonists	0.078	0.11	0.46	0.0061	0.11	0.95	0.12	0.11	0.28	0.20	0.11	0.056
Phosphodiesterase 5 inhibitors	0.036	0.13	0.78	-0.0070	0.13	0.96	-0.085	0.13	0.50	-0.13	0.13	0.32
Prostacyclin infusions	0.045	0.10	0.66	-0.054	0.10	0.59	-0.056	0.10	0.58	0.019	0.10	0.85
Spironolactone	-0.074	0.11	0.51	-0.073	0.11	0.52	-0.26	0.11	0.021	-0.19	0.11	0.094
Immunomodulatory therapy[b]	0.10	0.17	0.53	0.061	0.17	0.71	0.17	0.17	0.31	0.29	0.17	0.076

	IFNγ			IL10			IL12p70			IL13		
	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value
Obesity (BMI ≥ 30)	0.10	0.10	0.28	0.12	0.10	0.21	0.039	0.10	0.68	0.027	0.10	0.77
Etiology of PAH												
IPAH/HPAH vs. other associated PAH[a]	0.12	0.12	0.30	0.20	0.12	0.090	0.19	0.12	0.12	0.17	0.12	0.15
CTD PAH vs. other associated PAH[a]	0.18	0.13	0.17	0.23	0.13	0.076	0.24	0.13	0.067	0.17	0.13	0.18
Endothelin-1 receptor antagonists	-0.018	0.11	0.86	0.15	0.11	0.15	0.046	0.11	0.66	0.034	0.11	0.75
Phosphodiesterase 5 inhibitors	-0.024	0.13	0.85	-0.10	0.13	0.45	-0.16	0.13	0.21	-0.026	0.13	0.84
Prostacyclin infusions	-0.043	0.10	0.68	0.25	0.10	0.015	0.27	0.10	0.0091	0.075	0.10	0.46
Spironolactone	0.0013	0.11	0.99	-0.25	0.11	0.028	-0.14	0.11	0.23	-0.064	0.11	0.57
Immunomodulatory therapy[b]	0.013	0.17	0.94	0.22	0.17	0.19	0.061	0.17	0.71	0.079	0.17	0.63

	IL17			IL1ra			IL2			IL4		
	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value
Obesity (BMI ≥ 30)	0.17	0.10	0.072	0.11	0.10	0.26	0.18	0.10	0.060	0.037	0.10	0.70
Etiology of PAH												
IPAH/HPAH vs. other associated PAH[a]	0.21	0.12	0.076	0.20	0.12	0.090	0.24	0.12	0.042	0.094	0.12	0.43
CTD PAH vs. other associated PAH[a]	0.24	0.13	0.069	0.27	0.13	0.040	0.30	0.13	0.020	0.087	0.13	0.50
Endothelin-1 receptor antagonists	0.10	0.11	0.36	0.18	0.11	0.085	0.14	0.11	0.18	0.014	0.11	0.90
Phosphodiesterase 5 inhibitors	-0.019	0.13	0.88	0.022	0.13	0.86	0.025	0.13	0.84	-0.053	0.13	0.68
Prostacyclin infusions	-0.12	0.10	0.25	0.14	0.10	0.18	-0.054	0.10	0.59	-0.031	0.10	0.76
Spironolactone	-0.20	0.11	0.083	-0.17	0.11	0.13	-0.15	0.11	0.19	-0.044	0.11	0.70
Immunomodulatory therapy[b]	0.14	0.17	0.39	0.24	0.17	0.14	0.20	0.17	0.23	0.039	0.17	0.81

	IL6			IL7			IL8			IL9		
	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value
Obesity (BMI ≥ 30)	0.10	0.10	0.28	0.21	0.10	0.029	0.12	0.10	0.20	0.20	0.10	0.035
Etiology of PAH												
IPAH/HPAH vs. other associated PAH[a]	0.18	0.12	0.14	0.31	0.12	0.0095	0.24	0.12	0.046	0.25	0.12	0.038
CTD PAH vs. other associated PAH[a]	0.28	0.13	0.031	0.28	0.13	0.032	0.18	0.13	0.17	0.36	0.13	0.0051
Endothelin-1 receptor antagonists	0.085	0.11	0.42	0.10	0.11	0.34	0.069	0.11	0.51	0.18	0.11	0.094
Phosphodiesterase 5 inhibitors	-0.0088	0.13	0.94	-0.10	0.13	0.44	-0.077	0.13	0.55	0.011	0.13	0.93
Prostacyclin infusions	-0.0078	0.10	0.94	0.22	0.10	0.028	-0.075	0.10	0.46	-0.032	0.10	0.76
Spironolactone	-0.11	0.11	0.33	-0.28	0.11	0.015	-0.15	0.11	0.19	-0.31	0.11	0.0072
Immunomodulatory therapy[b]	0.072	0.17	0.66	0.033	0.17	0.84	0.17	0.17	0.32	0.17	0.17	0.31

	IP10			CCL2			MIP1α			MIP1β		
	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value
Obesity (BMI ≥ 30)	0.087	0.10	0.36	0.13	0.10	0.18	0.11	0.10	0.26	0.084	0.10	0.38
Etiology of PAH												
IPAH/HPAH vs. other associated PAH[a]	-0.0085	0.12	0.94	-0.021	0.12	0.86	0.21	0.12	0.076	0.0025	0.12	0.98
CTD PAH vs. other associated PAH[a]	0.25	0.13	0.050	0.10	0.13	0.43	0.19	0.13	0.14	0.080	0.13	0.54
Endothelin-1 receptor antagonists	0.10	0.11	0.34	0.13	0.11	0.24	0.026	0.11	0.80	0.034	0.11	0.75
Phosphodiesterase 5 inhibitors	-0.017	0.13	0.89	-0.032	0.13	0.80	-0.0066	0.13	0.96	-0.15	0.13	0.25
Prostacyclin infusions	-0.038	0.10	0.71	0.082	0.10	0.42	-0.13	0.10	0.20	0.079	0.10	0.44
Spironolactone	-0.11	0.11	0.34	-0.18	0.11	0.12	-0.080	0.11	0.48	-0.15	0.11	0.20
Immunomodulatory therapy[b]	-0.12	0.17	0.45	0.068	0.17	0.68	0.077	0.17	0.64	-0.088	0.17	0.59

	PDGFbb			RANTES			TNFα			VEGF		
	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value
Obesity (BMI ≥ 30)	0.11	0.10	0.27	0.14	0.10	0.15	0.11	0.10	0.25	0.063	0.10	0.51
Etiology of PAH												
IPAH/HPAH vs. other associated PAH[a]	0.20	0.12	0.10	0.16	0.12	0.18	0.22	0.12	0.071	0.19	0.12	0.12
CTD PAH vs. other associated PAH[a]	0.15	0.13	0.26	0.29	0.13	0.026	0.28	0.13	0.028	0.14	0.13	0.29
Endothelin-1 receptor antagonists	0.14	0.11	0.19	0.21	0.11	0.049	0.12	0.11	0.28	0.019	0.11	0.86
Phosphodiesterase 5 inhibitors	-0.085	0.13	0.50	-0.038	0.13	0.77	-0.0082	0.13	0.95	-0.14	0.13	0.28
Prostacyclin infusions	0.015	0.10	0.88	-0.087	0.10	0.39	0.033	0.10	0.75	0.29	0.10	0.0050
Spironolactone	-0.22	0.11	0.049	-0.19	0.11	0.10	-0.16	0.11	0.16	-0.077	0.11	0.50
Immunomodulatory therapy[b]	-0.11	0.17	0.53	0.081	0.17	0.63	0.13	0.17	0.42	0.12	0.17	0.47

[a]Congenital heart disease associated PAH (n=7), drug-induced PAH (n=1), portal hypertension associated PAH (n=1) and hereditary hemorrhagic telangiectasia associated PAH (n=1)

[b]All 5 patients were treated with hydroxychloroquine monotherapy

Table S6. Sequences for SYBR Green primers of target genes used in quantitative real-time PCR

Gene Symbol	Gene Name	Forward	Reverse
ACTB	β-Actin	5’-CCGCCGCCAGCTCACCAT-3’	5’-ACCCATGCCCACCATCACGC-3’
CCL2	Chemokine (C-C motif) ligand 2	5’-CGCCTCCAGCATGAAAAGTCT-3’	5’-ATGAAGGTGGCTGCTATGAGC-3’
PTGS2	Prostaglandin-endoperoxide synthase 2	5’-TGAATCATTCACCAGGCAAAT-3’	5’-TCTGTACTGCGGGTGGAACA-3’
ERCC3	Excision repair cross-complementation group 3	5’-GCCATTCGACTGAACAAACCC-3’	5’-TCCGGCAGATCAAACGAAGT-3’
IL8	Interleukin 8	5’-TGCAGCTCTGTGTGAAGGTGCAG-3’	5’-TGTGTTGGCGCAGTGTGGTCC-3’
IL6	Interleukin 6	5’-CCAGGAGCCCAGCTATGAAC-3’	5’-CCCAGGGAGAAGGCAACTG-3’
INHBA	Inhibin beta A subunit	5’-TTGCCGAGTCAGGAACAGC-3’	5’-GGGACTTTTAGGAAGAGCCAGAC-3’
luc2P	Luciferase reporter gene	5’-GCTCAGCAAGGAGGTAGGTG-3’	5’-TGATCAGAATGGCGCTGGTT-3’
NFKBIA	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	5’-AGCTCCGAGACTTTCGAGGA-3’	5’-CACGTGTGGCCATTGTAGTTG-3’