SUPPLEMENTAL INFORMATION

EphA2 mediates zymosan-induced inflammatory response in human monocytes independent of dectin-1

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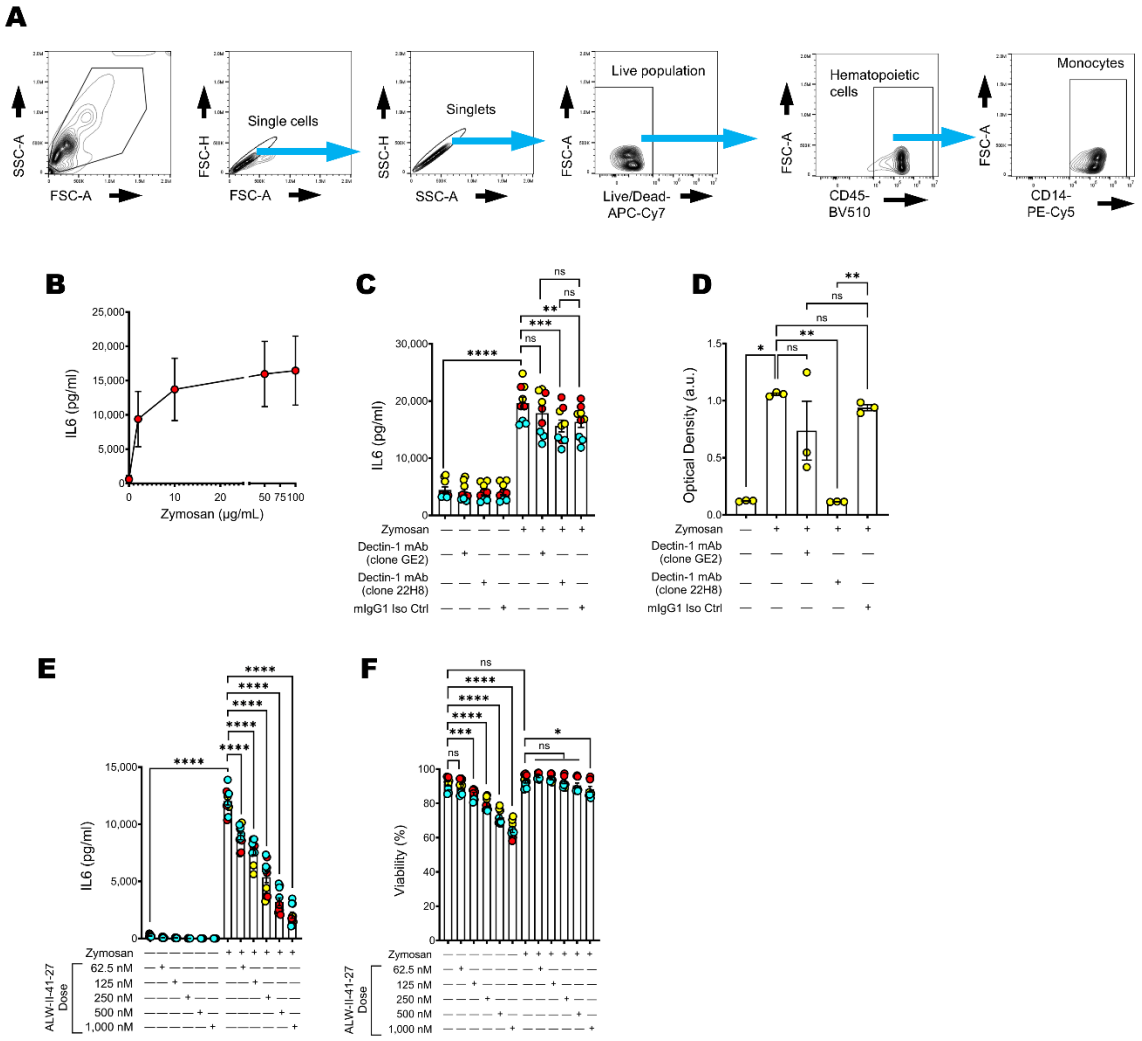
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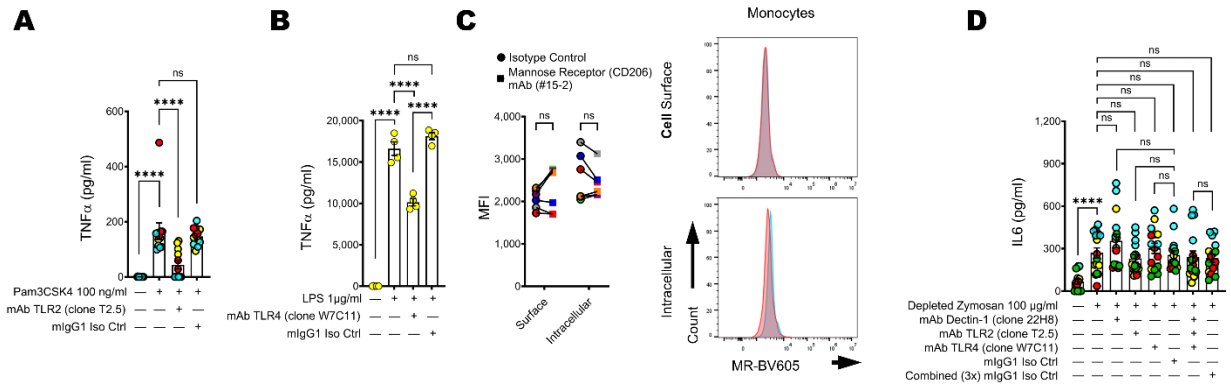
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SUPPLEMENTAL FIGURES AND FIGURE LEGENDS



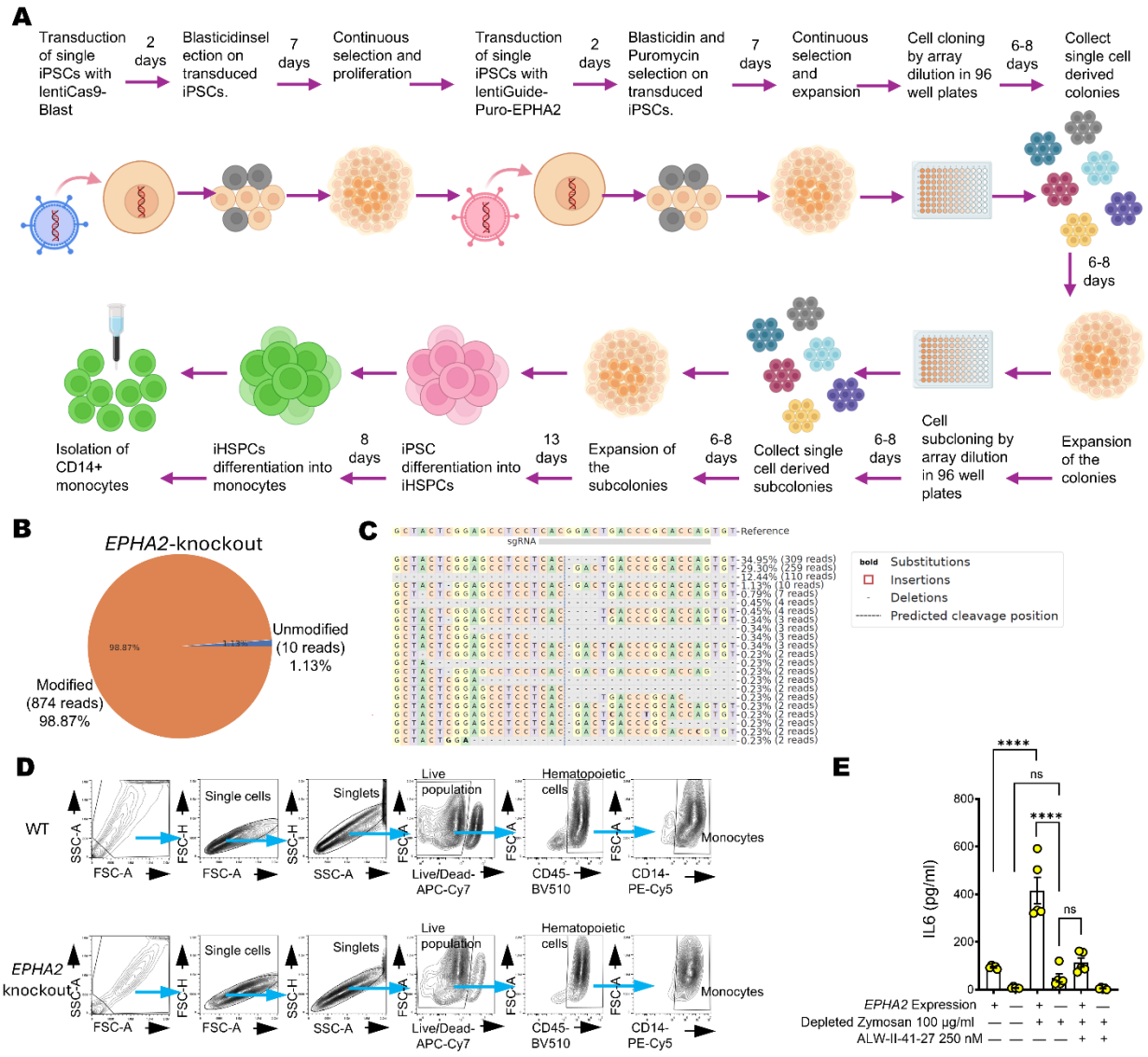
Supplemental Figure 1. EphA2 mediates zymosan-induced inflammatory response in human peripheral blood monocytes independent of Dectin-1. (**A**) Representative flow cytometry gating showing viability and purity of monocytes isolated from PBMCs by magnetic-activated cell sorting with CD14 microbeads: successive gates for single cells, singlets, live cells, CD45+ hematopoietic cells, and CD14+ monocytes. (**B**) IL6 production by monocytes stimulated with increasing concentrations of zymosan (0–100 μg/mL); 50 μg/mL was selected for subsequent experiments. Single donor, *n* = 3 replicates per dose. (**C**) IL6 production by monocytes stimulated with

zymosan (50 μg/mL), with or without anti–dectin-1 mAbs (clones GE2, 22H8) or isotype control (10 μg/mL). (**D**) SEAP reporter activity (optical density) of HEK-Blue hDectin-1a cells stimulated with zymosan, with or without anti–dectin-1 mAbs (clones GE2, 22H8) or isotype control (10 μg/mL), confirming that clone 22H8 blocks dectin-1. (**E**) Dose-dependent inhibition of zymosan-induced IL6 secretion by ALW-II-41-27. (**F**) Viability of monocytes treated with increasing doses of ALW-II-41-27 with or without zymosan. Data are mean ± SEM; each color denotes a unique donor. For multi-donor experiments (**C**, **E**, **F**; $n = 3$ donors, 3 technical replicates per donor per condition, across 3 independent experiments), significance was determined by linear mixed-effects modeling with donor as a random effect and condition as a fixed effect, with post-hoc comparisons using estimated marginal means. For the single-cell-line experiment (**D**; HEK-Blue hDectin-1a, $n = 3$ replicate wells), a fixed-effects linear model with wells as the unit of analysis was used. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$; ns, not significant.



Supplemental Figure 2. Evaluation of TLR contributions to depleted zymosan–induced inflammatory response in human monocytes. (**A**) TNFα production by monocytes stimulated with Pam3CSK4 (100 ng/mL), with or without anti-TLR2 mAb (clone T2.5) or isotype control (10 µg/mL), confirming functional TLR2 blockade. (**B**) TNFα production by monocytes stimulated with LPS (1 µg/mL), with or without anti-TLR4 mAb (clone W7C11) or isotype control (10 µg/mL), confirming functional TLR4 blockade. (**C**) Surface and intracellular mannose receptor (CD206) expression in monocytes by flow cytometry. (**D**) IL6 production in response to depleted zymosan (100 µg/mL), with or without blocking mAbs against dectin-1, TLR2, and TLR4 (clones 22H8, T2.5, W7C11) individually or combined, or isotype control (10 µg/mL each; 30 µg/mL combined). Data are mean ± SEM; each color denotes a unique donor. For multi-donor experiments (**A**: *n* = 3 donors, 4 technical replicates per donor per condition, across 3 independent experiments; **D**: *n* = 4 donors, 4 replicates per donor per condition, across 4 experiments), significance was determined by linear mixed-effects modeling with donor as a random effect and condition as a fixed effect, with post-hoc comparisons using estimated marginal means. For the single-donor experiment (**B**: *n* = 1 donor, 4 replicate wells across 1 experiment), a fixed-effects linear

model with wells as the unit of analysis was used. Pairwise comparison in **C** (n = 6 donors, 1 replicate per donor per condition, across 6 experiments) used the Wilcoxon signed-rank test. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$; ns, not significant.



Supplemental Figure 3. Generation and validation of *EPHA2*-knockout iPSC-derived monocytes. (**A**) Workflow for CRISPR-Cas editing of *EPHA2* in iPSCs and differentiation into CD14+ monocytes: sequential lentiviral transduction (lentiCas9-Blast, then lentiGuide-Puro-*EPHA2*) with antibiotic selection, single-cell cloning and subcloning by array dilution, expansion, differentiation into iPSC-derived hematopoietic stem/progenitor cells (iHSPCs), and differentiation into monocytes. (**B**) Editing efficiency in the selected *EPHA2*-knockout clone, shown as the percentage and number of

sequencing reads with modified versus unmodified alleles. (**C**) Distribution of alleles around the sgRNA cleavage site (sgRNA: CTGGTGCGGGTCAGTCCGTG). Nucleotides are colored (A, green; C, red; G, yellow; T, purple); substitutions in bold; red rectangles, insertions; horizontal dashed lines, deletions; vertical dashed line, predicted cleavage site. Amplicon sequencing was analyzed with CRISPResso2. (**D**) Representative gating showing viability and purity of CD14+ monocytes isolated by MACS (CD14 microbeads) after differentiation, for wild-type (WT) and *EphA2*-knockout lines: successive gates for single cells, singlets, live cells, CD45+ hematopoietic cells, and CD14+ monocytes. (**E**) *EPHA2*-knockout iPSC-derived monocytes show reduced IL6 production to depleted zymosan (100 µg/mL) relative to WT controls. Data are mean ± SEM. For this single iPSC-line experiment (**E**), a fixed-effects linear model with wells as the unit of analysis was used, matching the approach in **Figure 1K**. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$; ns, not significant.

SUPPLEMENTAL TABLE

Corresponding Figure	Age (yr)	Gender	Height (in)	Weight (lbs)	Race	Ethnicity
Fig. 1A–C; Supp. Fig. 1C	51	F	64	200	Caucasian	Non-Hispanic
Fig. 1A–C, J; Supp. Fig. 1C	44	F	69.6	150	Caucasian	Non-Hispanic
Fig. 1A–C; Supp. Fig. 1C	55	F	64	130	Caucasian	Non-Hispanic
Fig. 1B–D, I, J; Supp. Fig. 1E, F; Supp. Fig. 2D	54	M	74	205	Caucasian	Non-Hispanic
Fig. 1B–D; Supp. Fig. 1E, F	31	F	66	150	Caucasian	Non-Hispanic
Fig. 1B–D; Supp. Fig. 1E, F	68	F	65	170	Caucasian	Non-Hispanic
Fig. 1B, C, E, F, I; Supp. Fig. 2C, D	60	F	63	130	Caucasian	Non-Hispanic
Fig. 1E, F	27	F	63	133	Asian	Non-Hispanic
Fig. 1E–G; Supp. Fig. 2A	37	F	62	110	Latin	Hispanic
Fig. 1G	54	M	54	209	Caucasian	Non-Hispanic
Fig. 1F, G	33	F	62	105	Asian	Non-Hispanic
Fig. 1B, C, H; Supp. Fig. 2C	50	M	68	196	Caucasian	Hispanic
Fig. 1B, C, H; Supp. Fig. 2C	53	F	63	180	Caucasian	Hispanic
Fig. 1B, C, H; Supp. Fig. 2C	46	F	64	208	Caucasian	Non-Hispanic
Fig. 1B, C, I; Supp. Fig. 2C, D	40	M	64	135	Aian	Non-Hispanic
Fig. 1B, C, I; Supp. Fig. 2C, D	59	F	65	123	Caucasian	Non-Hispanic
Supp. Fig. 2A	31	M	69	155	Caucasian	Non-Hispanic
Supp. Fig. 2A	49	F	67	130	Caucasian	Non-Hispanic
Supp. Fig. 2B	44	M	74	240	Other	Non-Hispanic
Fig. 1J	59	F	68	145	Caucasian	Non-Hispanic

Supplemental Table 1. De-identified Information on Blood Donors.

SEX AS A BIOLOGICAL VARIABLE

Sex was not considered as a biological variable. The experiments were carried out according to the availability of donors.

MATERIALS AND METHODS

Isolation and Treatment of Human Monocytes

Peripheral whole blood was collected from healthy donors into EDTA-coated tubes, and mononuclear cells (PBMCs) were isolated using Lymphoprep density gradient centrifugation (StemCell, Cat. No. 18060). $CD14^+$ monocytes were then purified from the PBMC fraction using CD14 MicroBeads (Miltenyi Biotec, Cat. No. 130-050-201) according to the manufacturer's instructions. Isolated monocytes were resuspended in RPMI 1640 medium (Gibco, Cat. No. 11875093) supplemented with 10% fetal bovine serum (FBS; Gibco, Cat. No. 26140087), 2 mM L-glutamine (Gibco, Cat. No. 25030149), and 1% penicillin-streptomycin (Gibco, Cat. No. 15140148). Cells (1×10^5 monocytes/well in 75 µL) were seeded into tissue culture-treated 96-well plates (Greiner) and incubated for 1 hr at 37°C with 5% CO_2 to allow adherence. After adherence, dectin-1 blocking monoclonal antibodies (clone GE2, ThermoFisher Scientific, Cat. No. MA5-16692 or clone 22H8, InvivoGen, Cat. No. mabg-hdect), TLR2 blocking monoclonal antibodies (clone T2.5 ,InvivoGen, Cat. No. mab2-mtlr2), TLR4 blocking monoclonal antibodies (clone W7C11, InvivoGen, Cat. No. mabg-htlr4-2), an isotype control antibody (mouse IgG1, InvivoGen, Cat. No. bgal-mab9-1), all at a final concentration of 10 µg/mL, or EphA2 inhibitors (ALW-II-41-27 [Selleckchem, Cat. No. S6515], dasatinib [Selleckchem, Cat. No. S1021], bosutinib [Selleckchem, Cat. No.

S1014], or nilotinib [Selleckchem, Cat. No. S1033]) were added in 50 µL volume to the wells at indicated final concentrations. Cells were incubated with the antibodies or inhibitors for 1 additional hour at 37°C and 5% CO_2. Subsequently, 125 µL of control medium, zymosan (final concentration, 50 µg/mL; InvivoGen, Cat. No. tlrl-zyn), depleted zymosan (final concentration, 100 µg/mL; InvivoGen, Cat. No. tlrl-zyd), Pam3CSK4 (final concentration, 100 ng/mL; InvivoGen, Cat. No. tlrl-pms), or LPS (final concentration, 1 µg/mL; eBioscience, Cat. No. 00-4976-93) was added to each well. For infection assays, live *Candida albicans* (kindly provided by Dr. Georgios D. Kitsios, University of Pittsburgh, UPMC Montefiore Hospital, NW 628; 3459 Fifth Avenue, Pittsburgh, PA 15213) or *Aspergillus fumigatus* (provided by Dr. Chad Steele) were added to monocytes at an MOI of 5. Following treatment, monocytes were incubated for 20-24 hrs at 37°C and 5% CO_2. After incubation, supernatants were collected, and cytokine levels (TNFα and IL6) were quantified by ELISA using commercial kits (TNFα: ThermoFisher Scientific, Cat. No. 88-7346-88; IL6: ThermoFisher Scientific, Cat. No. 88-7066-88) per the manufacturer's protocols.

Flow Cytometry Analysis

Cells were resuspended in FACS buffer consisting of DPBS supplemented with 2.5% FBS and 2 mM EDTA and then incubated with a viability dye (Fixable Near-IR Dead Cell Stain, ThermoFisher Scientific, Cat. No. L34976), human Fc receptor blocking reagent (BioLegend, Cat. No. 422302), and True-Stain Monocyte Blocker™ (BioLegend, Cat. No. 426102) to reduce non-specific binding. After this blocking step, cells were stained with fluorochrome-conjugated antibodies against surface markers for 30 min at 4°C. Following surface staining, cells were washed three times with FACS

buffer to remove unbound antibodies and fixed in 4% paraformaldehyde (Electron Microscopy Sciences, Cat. No. 157-4). For intracellular staining, fixed cells were permeabilized using a commercial permeabilization buffer (ThermoFisher Scientific, Cat. No. GAS003) and then incubated for 20 min at 4°C with fluorochrome-conjugated antibodies targeting intracellular epitopes. After a final series of washes in FACS buffer, samples were analyzed using a CytoFLEX flow cytometer (Beckman Coulter). Data were processed and interpreted using FlowJo™ software version 10.10 (BD Life Sciences). Antibodies used in the analysis included anti-CD45 (clone 2D1, BV510, BioLegend, Cat. No. 368526), anti-CD14 (clone M5E2, APC, BioLegend, Cat. No. 301808), anti-Dectin-1 (clone GE2, FITC, Bio-Rad, Cat. No. MCA4661F) with its corresponding isotype control (FITC, Bio-Rad, Cat. No. MCA928F), anti-EphA2 (clone SHM16, PE, BioLegend, Cat. No. 356804) along with its isotype control (clone MPC-11, PE, BioLegend, Cat. No. 400312), anti-TLR2 (clone TL2.1, APC, BioLegend, Cat. No. 309719) with its corresponding isotype control (clone MOPC-173, APC, BioLegend, Cat. No. 400221), anti-TLR4 (clone HTA125, BV421, BioLegend, Cat. No. 312811) with its corresponding isotype control (clone MOPC-173, BV421, BioLegend, Cat. No. 400259), and anti-MR (clone 15-2, BV605, BioLegend, Cat. No. 321139) with its corresponding isotype control (clone MOPC-21, BV605, BioLegend, Cat. No. 400161). TLR2 and TLR4 expression analyses on monocytes are not presented.

Culture of Human Dectin-1a HEK 293 Cells and Reporter assay

HEK-Blue™ hDectin-1a cells (InvivoGen, Cat. No. hkb-hdect1a) were maintained in complete growth medium containing DMEM (4.5 g/L glucose), 10% (v/v) FBS, Pen-Strep (100 U/mL and 100 µg/mL), 100 µg/mL Normocin™, 2 mM L-glutamine, as well as

1 μg/mL puromycin and 1× HEK-Blue™ CLR Selection. The cells were used to confirm the specificity and functional blocking activity of the anti-Dectin-1 monoclonal antibodies. HEK-Blue hDectin-1a cells stably express the hDectin-1a gene. They also express genes of the dectin-1-NF-κB signaling pathway, in addition to an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene. The cell line is activated by dectin-1 ligands such as zymosan. SEAP activity was assessed with QUANTI-Blue, the alkaline phosphatase detection reagent contained in HEK-Blue™ Detection medium (InvivoGen, Cat. No. hb-det2). In brief, 50,000 cells suspended in HEK-Blue Detection medium were plated in each well of a 96-well plate and incubated for 1 hr at 37°C in 5% CO_2 with monoclonal anti-Dectin-1 blocking antibodies or isotype control (10 μg/mL final concentration). Zymosan (50 μg/mL final concentration) or control medium was subsequently added, and plates were incubated for 24 hrs at 37°C in 5% CO_2. SEAP substrate hydrolysis was then measured using a spectrophotometer at 620–655 nm.

CRISPR/Cas9 Knock-out of *EPHA2* in iPSCs

BU3 human-induced pluripotent stem cells (iPSCs) (Alias BU3-10-Cr2; normal control hiPSC; Donor: ethnicity non reported, race white, age 32, karyotype 46XY) were obtained from the stem cell bank at Boston University (gift from Dr. Finn J. Hawkins, Boston University, 72 East Concord St. Boston, MA 02118). iPSCs were maintained in mTeSR1 Plus culture media (StemCell Technologies, Cat. No.100-0276) and seeded in Vitronectin XF coated plates (Stemcell Technologies Cat. No.07180). Cas9 stable iPSCs were generated by transducing cells with lentiCas9-Blast (Addgene, 52962, a gift from Dr. Feng Zhang, Massachusetts Institute of Technology, Department of Biological

Engineering, 77 Massachusetts Avenue, Building 56-651, Cambridge, MA 02139–4307) lentivirus, which encodes Cas9 and blasticidin resistant gene. The transduction was done in 6-well plates (5x105 cells per well) using polybrene at a final concentration of 8 µg/ml. Forty-eight hours post infection, the selection antibiotic blasticidin was added to the medium at a final concentration of 5 µg/ml. The cells were in continuous selection and expansion for 7 days. The following primers were used to generate a *EPHA2*-specific lentiGuide-Puro construct (Addgene 52963, a gift from Dr. Feng Zhang): *EPHA2*_sgRNA_top: caccgCTGGTGCGGGTCAGTCCGTG, *EPHA2*_sgRNA_bottom: aaacCACGGACTGACCCGCACCAGc. The lentiGuid-Puro-*EPHA2* lentivirus was packaged and the transduction was done similarly to the Cas9-Blas lentivirus, and the selection was done by incubating the cells with both, blasticidin at 5 µg/ml and puromycin at 0.5 µg/ml. Although the transduced human iPSCs have been selected several days with the antibiotics, the iPSCs are still a heterogeneous population. Therefore, single cell derived iPSC colonies were selected by array dilution in 96-well plates. 10,000 cells were seeded into well A1 and then a 2-fold serial dilution, first vertically, then horizontally was done. Selection antibiotics were added to the culture medium, and after 2 days, wells containing single colonies were marked for pickup after an additional 4–6 days in culture. Selected colonies were transferred to 6-well plates and expanded for 6–8 days. Clones exhibiting the lower expression of the wild-type alleles was subjected to a second round of subcloning by array dilution. Following sequencing of the subclones, we selected a single subclone with no detectable wild-type alleles, indicating that the vast majority of cells were likely null for *EPHA2*.

PCR to Analyze *EPHA2* Modifications on iPSCs

Genomic DNA was isolated from subclones and amplicon sequencing (Plasmidsaurus) was performed using primers flanking the targeted sequencing. The following primer sequences (IDT) were used to generate the amplicon by PCR: *EPHA2*_FW: gccgatgagaaaacaggtcc, *EPHA2*_RV, gctctgctgcctctctctaa. PCR was performed in a 25 µL reaction containing 100ng template DNA, 0.5 µM each primer, 200 µM dNTPs (Thermo scientific, Cat. No. R0192), 1× Phusion HF buffer, Betaine 1M (Sigma-Aldrich, Cat. No.B0300-1VL), and 1X Phusion High-Fidelity DNA Polymerase (New England Biolabs, Cat. No.M0530L). Cycling conditions were 98°C for 30 sec; 39 cycles of 98°C for 10 sec, 65°C for 15 sec, and 72°C for 30 sec; followed by 72°C for 10 min. Raw data from amplicon sequencing was analyzed using CRISPResso2: Clement K, Rees H, Canver MC, Gehrke JM, Farouni R, Hsu JY, Cole MA, Liu DR, Joung JK, Bauer DE, Pinello L. CRISPResso2 provides accurate and rapid genome editing sequence analysis. Nat Biotechnol. 2019 Mar; 37(3):224-226. doi: 10.1038/s41587-019-0032-3. PubMed PMID: 30809026 (https://crispresso2.pinellolab.org/submission).

Differentiation of iPSCs into Hematopoietic Stem and Progenitor Cells (iHSPCs)

The selected subclone of iPSCs was differentiated into iHSPCs with the STEMdif Hematopoietic Kit (StemCell, Cat. No. 05310) as per manufacturer instructions. In brief, on Day -1, iPSCs were harvested and seeded as small aggregates in mTeSR Plus media onto 6-well plates coated with Matrigel (Corning, Cat. No. 354277). On Day 0 after confirming the number of adhered colonies is within 4 - 10/cm2, TeSR medium was replaced with medium A to induce the cells toward a mesoderm-like state. On Day 2, a half-medium change was performed with fresh medium A. On Day 3, medium A was replaced with medium B and half-medium changes with fresh medium B were

performed on Days 5, 7, and 10 to promote further differentiation into hematopoietic cells. By Day 12, hematopoietic progenitor cells were harvested from the culture supernatant.

Differentiation of iHSPCs into CD14+ Monocytes

On day 0, iHSPCs were resuspended in complete myeloid medium composed of StemSPan SFEM II (StemCell, Cat. No.09655), SCF (50 ng/ml; PeproTech, Cat. No. 300-07-50ug), IL3 (50 ng/ml; PeproTech, Cat. No. 200-03-50ug), FLIT3L (50 ng/ml; PeproTech, Cat. No. 300-19-50ug), TPO (5 ng/ml; R&D Systems, Cat. No. 288-tp/cf), MCSF (50 ng/ml; PeproTech, Cat. No. 300-25-50ug), GCSF (50 ng/ml; PeproTech, Cat. No. 300-23-50ug), GMCSF (25 ng/ml; PeproTech, Cat. No. 300-03-50ug), and IL6 (10 ng/ml; PeproTech, Cat. No. 200-06-50ug). Cells were seeded into 12-well plates at a density of 5×10^5cells per well in 1 mL of medium. On days 2, 4, and 6, half of the medium was replaced by removing 0.5 mL from each well. The removed medium was centrifuged at 300 × g for 5 min, after which the cell pellet was resuspended in 0.5 mL of fresh complete myeloid medium and returned to the corresponding wells. On day 8, myeloid-lineage cells were collected by transferring the culture supernatant to 15 mL conical tubes. Adherent cells were harvested by washing each well with 1 mL 1× dPBS and incubating with 0.5 mL Accutase (StemCell, Cat. No. 07920) for 5 min at 37°C. Cells were then washed with DMEM/F-12 (Gibco, Cat. No. 21331-020) and centrifuged at 300 × g for 5 min. The adherent and suspension fractions were pooled, resuspended in MACS buffer, counted, and subjected to CD14+ cell enrichment using CD14 MicroBeads (Miltenyi Biotec, Cat. No. 130-050-201) according to the manufacturer’s instructions.

iPSC-derived Human Monocytes Treated with Depleted Zymosan

Purified CD14+ iPSC-derived human monocytes were resuspended in the same complete RPMI 1640 medium used for primary monocyte culture. Cells were seeded at 5×10^4cells per well in 75 µL into tissue culture-treated 96-well plates (Greiner) and incubated for 1 h at 37°C and 5% CO2. EphA2 inhibitor ALW-II-41-27 (Selleckchem, Cat. No. S6515) was then added in 50 µL medium to achieve a final concentration of 250 nM, and cells were incubated for an additional hour at 37°C and 5% CO2. Subsequently, 125 µL of control medium or depleted zymosan (final concentration, 100 µg/mL; InvivoGen, Cat. No. tlrl-zyd) was added to each well. Monocytes were incubated for 20–24 h at 37°C and 5% CO2. After incubation, supernatants were collected, and TNFα and IL6 concentrations were measured by ELISA using commercial kits (TNFα: Thermo Fisher Scientific, Cat. No. 88-7346-88; IL6: Thermo Fisher Scientific, Cat. No. 88-7066-88) according to the manufacturer's instructions.

Statistical Analysis

TNFα, IL6, and viability data from multi-donor experiments (**Figure 1, A, D–J; Supplemental Figure 1, C, E, and F; and Supplemental Figure 2, A and D**) were analyzed using linear mixed-effects models (*lme4*, *lmerTest*) with donor modeled as a random effect and experimental condition as a fixed effect, fit by restricted maximum likelihood (REML) in R (v4.6.1). For experiments performed in a single donor (**Figure 1K; Supplemental Figure 1D; Supplemental Figure 2B; and Supplemental Figure 3E**), iPSC line, or HEK reporter cell line (optical density), an equivalent fixed-effects linear model was used, with wells as the unit of analysis. For all models, post-hoc comparisons used estimated marginal means (*emmeans*) with Kenward-Roger

degrees-of-freedom approximation where applicable; pre-specified contrasts with multivariate-t adjustment were applied depending on comparison structure, controlling family-wise error across simultaneous tests. Dose–response trends in **Figure 1, D–G; and Supplemental Figure 1E** were tested by nonparametric tests for trend and were identified as significant: **Figure 1D** (p = 0.004), **Figure 1E** (p = 0.002), **Figure 1F** (p <0.001), **Figure 1G** (p = 0.001), and **Supplemental Figure 1E** (p < 0.001). Wilcoxon signed-rank test was used for pairwise comparisons (**Figure 1, B and C; and Supplemental Figure 2C**).

Study Approval

All donors received and signed a written informed consent prior to the blood drawn. The study was approved by the Institutional Review Board at University of Pittsburgh (Study No. STUDY22120013).

Data Availability

Underlying data for the manuscript can be accessed in the “Supporting data values” XLS file; or from the corresponding author upon request.

Author Contributions

K.H.B., R.J.JV. and A.M. conceived the study; K.H.B. and R.J.JV. drafted the manuscript; C.S. provided critical feedback; R.J.J.V. conduct the experiments; J.K.A. contributed to the transduction of *EPHA2*- knockout cells; Q.V. supported iPSC-related studies; K.H.B. and S.M.N. performed the statistical analyses; and K.H.B., R.J.JV., A.M, J.K.A., and C.S. revised the manuscript.

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Competing interests: The authors declare no relevant competing interests.