SUPPLEMENTAL MATERIAL

Detailed Methods

Cell culture. The human pluripotent stem cell line HUES 9 was grown in feeder-free adherent culture in chemically defined mTeSR1 media (STEMCELL Technologies) supplemented with penicillin and streptomycin on plates pre-coated with Geltrex matrix (Invitrogen).

Macrophage differentiation protocol. Stem cells were gently dissociated with Accutase (STEMCELL Technologies), and 2 x 10^6 cells were resuspended in embryoid body culture media (DMEM, 10% knockout serum, 2 mM glutamax, penicillin/streptomycin, 0.1 mM beta-mercaptoethanol) plus 0.4 µM ROCK-inhibitor (Y-27632, Cayman Chemical). Cells were cultured in 6-well ultra low attachment plate (Costar) for 4-7 days. All large embryoid bodies were seeded into gelatin-coated 6-well plates with monocyte differentiation media (DMEM, 10% FCS, 1 mM L-Glutamine, 0.1 mM beta-mercaptoethanol, 50 ng/mL human M-CSF, 25 ng/mL human IL-3; M-CSF and IL-3 from Peprotech). Continuous monocyte production started within 15 to 20 days, and non-adherent monocytes were harvested every 4 to 5 days. Monocytes could be harvested for several months if the growth factors were replaced regularly. For maturation, monocytes were replated into uncoated plastic cell culture dishes in medium containing 100 ng/mL M-CSF.

Flow cytometry. Cells were collected in ice-cold PBS following Accutase (STEMCELL Technologies) treatment. After 1 hour of blocking with 10% FBS in the presence of human FcR blocking reagent (Milteny Biotech) cells were stained with anti-CD14 (HCD14, BioLegend). For intracellular staining, cells were fixed with 4% paraformaldehyde in PBS for 20 minutes at room temperature, and subsequently cells were permeabilized for 15 minutes with 0.25% saponin (Research Organics) in PBS. Antibody staining was performed in the presence of 0.25% saponin for 30 minutes. Anti-CD163 (GHI/61) was obtained from BioLegend and anti-CD68 (KP1) from Santa Cruz Biotechnology. Samples were recorded using a FACSCalibur platform (BD Bioscience) and analyzed using FlowJo software (Tree Star).

Cholesterol efflux assay. A cholesterol efflux assay previously described for THP-1 cells was adapted for use in stem cell-derived macrophages.[1] Macrophages were harvested, resuspended in RPMI plus M-CSF, and plated into 24-well plates at 300,000 cells/well. The cells were radioactively labeled with 6 µCi/mL [^{3}H]cholesterol (PerkinElmer) for 24 hours, followed by activation with the LXR agonist T0901317 (4 µM, Sigma) for 18 hours. Efflux assays were performed in 250 µL RPMI supplemented with apoA-I (10 µg/mL, Sigma) for 2 hours at 37°C, 5% CO_2. Supernatants and lysed cells were collected and analyzed in a scintillation counter. The efflux fraction was calculated as counts in supernatant divided by counts in supernatant + counts in cells.

Phagocytosis assay. Cells were seeded 24 hours prior to the experiment at a density of 100,000 cells per well of a 24-well plate and subsequently incubated with *Escherichia coli* (K-12 strain) BioParticles, Alexa Fluor 488 conjugates (Life Technologies) for 3 hours. Following two washes with PBS, cells were detached using 1:4 diluted Accutase, fixed with 1% PFA in PBS, and subsequently recorded using a FACSCalibur platform.

LPS stimulation, quantitative gene expression analysis, and Bio-Plex. For gene expression analyses, CD14+ monocytes were isolated from the supernatants of differentiation cultures using human CD14 magnetic MicroBeads (Miltenyi Biotec) and seeded at 100,000-250,000 cells per well of a 24-well plate. CD14+ monocytes from peripheral human blood were isolated

from leukopacs by negative selection using the RosetteSep system according to the manufacturer's instructions (STEMCELL Technologies). Cells were maturated for 5 days into macrophages in the presence of 100 ng/mL M-CSF in RPMI-10. Subsequently, cells were stimulated with 100 ng/mL LPS (*E.coli* 0111:B4, Sigma) for 18 hours. RNA isolation and subsequent analyses were performed as previously described.[2] The following probes were used to assess changes in gene expression—*IL1B*, *IL8*, and *CCL5* (TaqMan, Applied Biosystems)—using a ViiA 7 Real-Time PCR System (Applied Biosystems). Expression data was normalized against the *B2M* gene, and fold induction was calculated from $\Delta\Delta C_t$ values comparing matched unstimulated and LPS-stimulated cells. The IL-1β concentration in culture supernatants was determined using the Human Inflammatory 5-Plex Panel (Invitrogen) according to the manufacturer's instructions on the MAGPIX platform (Luminex).

CRISPR/Cas9 genome editing. HUES 9 cells were disassociated into single cells with Accutase, and 10 million cells were electroporated with 25 µg of the pCas9_GFP plasmid (http://www.addgene.org/44719/) and 25 µg of the pGuide plasmid (http://www.addgene.org/64711/), into which the protospacer sequence GTTTCCGTTACCCGACTCCT had been introduced, in a single cuvette and replated as previously described.[2,3] Cas9 was transiently expressed with this method. The cells were collected from the culture plates 48 hours after electroporation by Accutase treatment and then resuspended in PBS. Cells expressing green fluorescent protein were collected by FACS (FACSAria II, BD Biosciences) and replated on 10-cm tissue culture plates at 25,000 cells/plate to allow for recovery in growth media.

Isolation of targeted clonal cell lines. Post-FACS, the cells were allowed to recover for 7 to 10 days, after which single colonies were manually picked, dispersed, and replated individually to wells of 96-well plates. Colonies were allowed to grow to near confluence over the next 7 days, at which point they were split using Accutase and replica-plated for the creation of a working stock and a frozen stock. The working stock was grown to confluence. Genomic DNA was extracted in 96-well format from working stocks in lysis buffer [10 mM Tris (pH 7.5), 10 mM EDTA, 10 mM NaCl, 0.5% Sarcosyl] containing proteinase K at 56°C overnight in a humidified chamber. Genomic DNA was precipitated by the addition of 95% ethanol containing 75 mM NaCl for 1 hour at room temperature. The DNA was then washed two times in 70% ethanol, allowed to dry at room temperature, and then resuspended in nuclease-free water.

Genotyping at the CRISPR/Cas9 target site in *ABCA1* was then performed for each sample by PCR amplification using FastStart Taq (Roche) and a primer pair designed to yield small amplicons (~200 bp) around the target site. Amplicons were subjected to electrophoresis on 2.5% agarose gels to discriminate clones with indels, with positive clones having a band or bands visibly shifted in size from the baseline. For the positive clones, PCR amplicons were subcloned using the TOPO TA Cloning Kit (Invitrogen) and subjected to numerous sequence reads for confirmation of the presence of mutant alleles; in a similar fashion, a subset of the potentially negative clones were confirmed to be wild-type. Two clones with confirmed homozygous or compound heterozygous mutant alleles (H11 and D6) and two confirmed wild-type clones (C10 and C11) were retrieved from frozen stocks and expanded for experiments.

Off-target analysis. Genomic DNA samples harvested from all four clones used for experiments were tested for off-target mutagenesis by CRISPR/Cas9. PCR primers (see below) were designed to amplify 200-300 bp regions around 14 predicted off-target sites. Off-target site prediction was performed using the CRISPR Design tool (http://crispr.mit.edu) and the COSMID tool (http://crispr.bme.gatech.edu). Amplified DNA was analyzed by Sanger sequencing, and no insertions, deletions, or polymorphisms were observed in any of the tested sites.

Western blot analysis. Whole cell lysates of wild-type and *ABCA1*–/– macrophages were made in RIPA buffer supplemented with Protease Inhibitor Cocktail Tablets (Roche). Western blot analysis was performed using standard methods three times, with one representative blot shown. ABCA1 expression was assessed with a 1:1000 dilution of anti-ABCA1 antibody (Abcam, ab18180) and 1:5000 anti-mouse HRP secondary antibody (R&D Systems). Beta-actin expression was assessed on the same blots (1:4000 dilution, sc47778, Santa Cruz).

Statistical analyses. Normality of data was assessed with the Shapiro-Wilk test, and equality of variance was assessed using the F-test. *P* values were calculated using the Student t-test.

PCR primers for off-target analyses.

CRISPR Design tool:

Position	Locus	Forward	Reverse	Mismatches
12:57479916-57479938	*ARHGAP9*	AGAAACAGGGAGACACAAGGT	TCCCTCTGTGTTTCTCCCAC	3
2:127862610-127862632	*AMMERCL1L*	CCGTACACTTCTAGACCGCT	AGCCTTACTCCCTGCACATT	4
22:46350497-46350519	*TRMU*	TTTCTCAGCCAGGTTTCCCA	CTGGAGAAGGGGAAGTGGTG	4
5:140364618-140364640	*SLC4A9*	CCTCCCCTTCCCTACATCCT	AGGTTTCTGTGCCCTCATCA	3

COSMID tool:

Position	Locus	Forward	Reverse	Mismatches
1:77706810-77706832	Non-coding	GCTTCGTGTCAAACCGGAAA	TTGCTCTGATGTGGTGTGGG	3
1:203586363-203586385	Non-coding	GGCTCACTCAGGGGACTCAA	ACCCTCCAAAGCAGAAACGAA	3
5:140364618-140364640	*SLC4A9*	ATCTACGATGCTGTGGGCAA	CTGACCAAGGTCCCTGTTTCC	3
15:36603272-36603294	Non-coding	CACCTTTTCTCTGTGCTCCCC	TCTTCCTCTAAGAAGCCTGCCT	3
13:71703686-71703707	Non-coding	GCAGGTAAAGCTGACTGCAC	CCCCTAAGGGTGAATGCAGA	2
2:236133933-236133954	Non-coding	TTCGGTCAGCACACAGAACA	TTTGGGTTCGCCTCACCTTG	2
7:151087752-151087773	Non-coding	GTCATGAGCCTGACGGGGAT	GCCTAAGGGCCCAGAGAAAA	2
9:84155070-84155093	Non-coding	GCTTCGTGTCAAACCGGAAA	TTGCTCTGATGTGGTGTGGG	2
17:4641294-4641317	Non-coding	GAGAAAGCGAGAGGACCGAC	AAATACCGGGGAGGACATGC	2
17:6656349-6656372	Non-coding	AGAGAGAAAATGCGCACGGA	CGCTGGAGGTTTGAAATGGC	2

Supplemental References

1. Low H, Hoang A, Sviridov D. Cholesterol efflux assay. *J Vis Exp*. 2012;6:e3810.

2. Ding Q, Lee YK, Schaefer EA, et al. A TALEN genome-editing system for generating human stem cell-based disease models. *Cell Stem Cell*. 2013;12:238–251.

3. Ding Q, Regan SN, Xia Y, Oostrom LA, Cowan CA, Musunuru K. Enhanced efficiency of human pluripotent stem cell genome editing through replacing TALENs with CRISPRs. *Cell Stem Cell*. 2013;12:393–394.