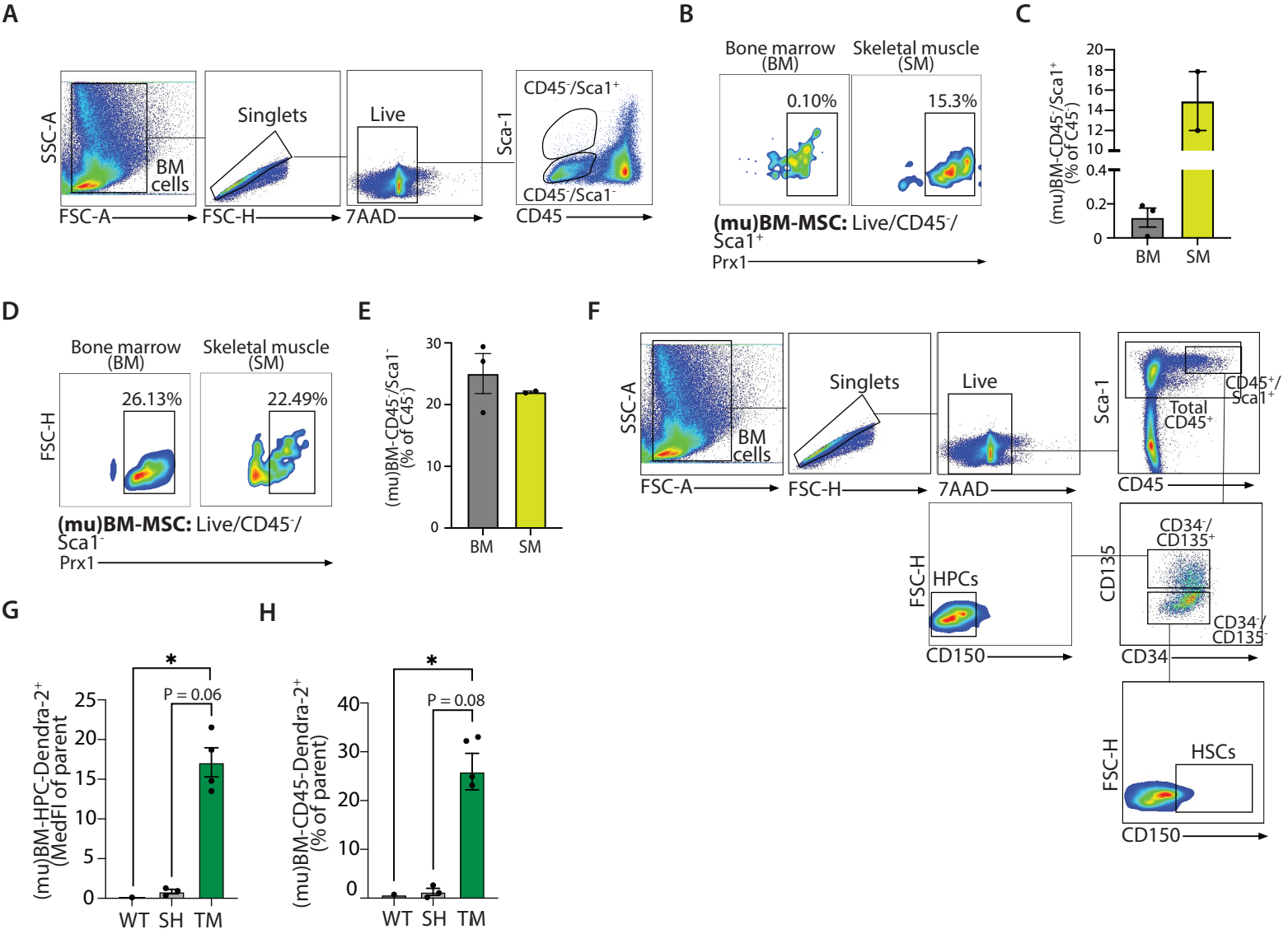

Fig. S1 | Validation of the PhAMfloxed/Prx1CreERT2 mouse model and stromal characterization. A) Gating strategy for the identification of murine bone marrow (BM) mesenchymal stromal stem (MSCs; Live/CD45$^-$/Sca-1$^+$) and progenitor cells (MPCs; Live/CD45$^-$/Sca-1$^-$). **B)** Representative flow cytometry plots showing the frequency of MSCs Prx1$^+$ cells in the BM and skeletal muscle (SM). **C)** Quantification of the frequency of Prx1$^+$ cells in BM and SM. **D)** Representative flow cytometry plots showing the frequency of MPCs Prx1$^+$ cells in the BM and SM. **E)** Quantification of the frequency of Prx1$^+$ cells within the CD45$^-$/Sca-1$^-$ stromal subset. **F)** Gating strategy for hematopoietic steam andprogenitor lineage analysis. **G)** Quantification of Dendra-2 median fluorescence intensity (MedFI) in hematopoietic progenitor cells (HPCs) across groups. **H)** Quantification of the frequency of mitochondrial transfer (Dendra-2$^+$) into the CD45$^-$ stromal compartment. Data are presented as mean ± s.e.m. n = 3–5 mice per group. P values determined by one-way ANOVA with Tukey's multiple comparisons test (*P < 0.05; **P < 0.01; ***P < 0.001).

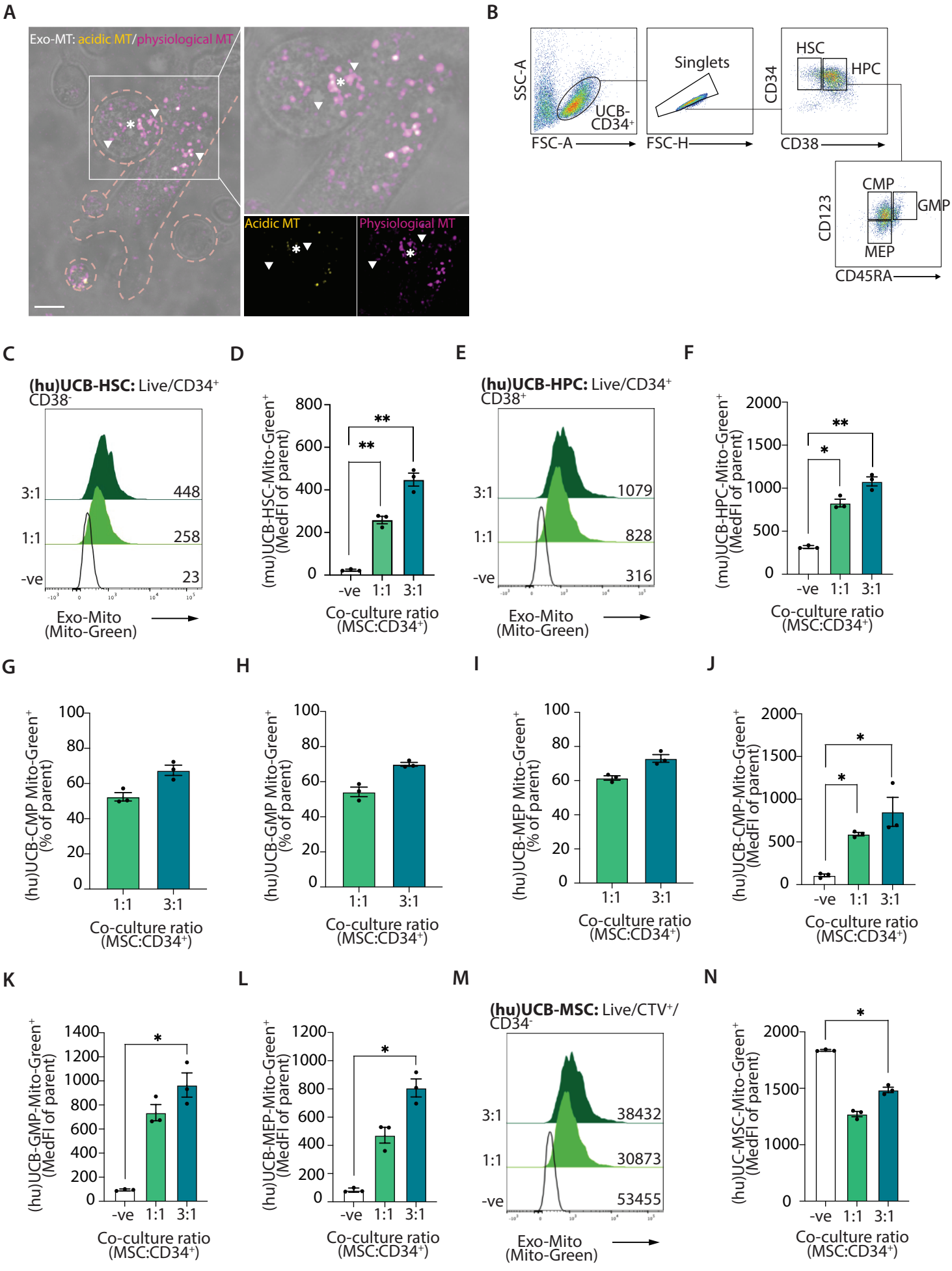
A
Exo-MT: acidic MT/physiological MT
Acidic MT
Physiological MT
B
SSC-A
UCB-CD34+
FSC-A
Singlets
FSC-H
CD34
HSC
HPC
CD38
CD123
CMP
GMP
MEP
CD45RA
C
(hu)UCB-HSC: Live/CD34+ CD38-
3:1
1:1
-ve
448
258
23
Exo-Mito (Mito-Green)
D
(mu)UCB-HSC-Mito-Green+ (MedFI of parent)
Co-culture ratio (MSC:CD34+)
E
(hu)UCB-HPC: Live/CD34+ CD38+
1079
828
316
F
(mu)UCB-HPC-Mito-Green+ (MedFI of parent)
G
(hu)UCB-CMP Mito-Green+ (% of parent)
H
(hu)UCB-GMP Mito-Green+ (% of parent)
I
(hu)UCB-MEP Mito-Green+ (% of parent)
J
(hu)UCB-CMP-Mito-Green+ (MedFI of parent)
K
(hu)UCB-GMP-Mito-Green+ (MedFI of parent)
L
(hu)UCB-MEP-Mito-Green+ (MedFI of parent)
M
(hu)UCB-MSC: Live/CTV+/ CD34-
38432
30873
53455
N
(hu)UC-MSC-Mito-Green+ (MedFI of parent)

Fig. S2 | Mitochondrial transfer dynamics in hematopoietic subpopulations. A) Representative confocal microscopy images of mt-mKeima-derived "Acidic" vs "Physiological" mitochondrial transfer in co-cultured cells. Scale bars, 10 µm. Dotted lines (pink) depict cellular morphology. **B)** Gating strategy for human hematopoietic subpopulations: Hematopoietic Stem Cells (HSC), Hematopoietic Progenitor Cells (HPC), Common Myeloid Progenitors (CMP: $CD34^{+}CD38^{+}/CD123^{+}CD45RA^{-}$), Granulocyte-Macrophage Progenitors (GMP: $CD34^{+}CD38^{+}/CD123^{+}CD45RA^{+}$), and Megakaryocyte-Erythrocyte Progenitors (MEP:$CD34^{+}CD38^{+}/CD123^{-}CD45RA^{-}$). **C-D)** Representative flow cytometry histograms **(C)** and quantification **(D)** of mitochondrial uptake (Mito-Green) MedFI in HSCs at donor-to-recipient ratios of 1:1 and 3:1. **E-F)** Representative flow cytometry histograms **(E)** and quantification **(F)** of mitochondrial uptake (Mito-Green) MedFI in HPCs at donor-to-recipient ratios of 1:1 and 3:1. **G-I)** Quantification of mitochondrial transfer mitochondrial uptake (Mito-Green) frequency and MedFI in committed progenitors: CMPs **(G)**, GMPs **(H)**, and MEPs **(I)** at donor-to-recipient ratios of 1:1 and 3:1. **J-L)** Quantification of mitochondrial transfer mitochondrial uptake (Mito-Green) MedFI in committed progenitors: CMPs **(J)**, GMPs **(K)**, and MEPs **(L)** at donor-to-recipient ratios of 1:1 and 3:1. **M-N)** Representative histograms **(M)** and quantification **(N)** of mitochondrial mass (Mito-Green MedFI) in donor MSCs after 24 h co-culture, showing a significant reduction in mitochondrial load. Data are presented as mean ± s.e.m. of n = 3 independent biological replicates. P values determined by one-way ANOVA with Dunnett's multiple comparisons test (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

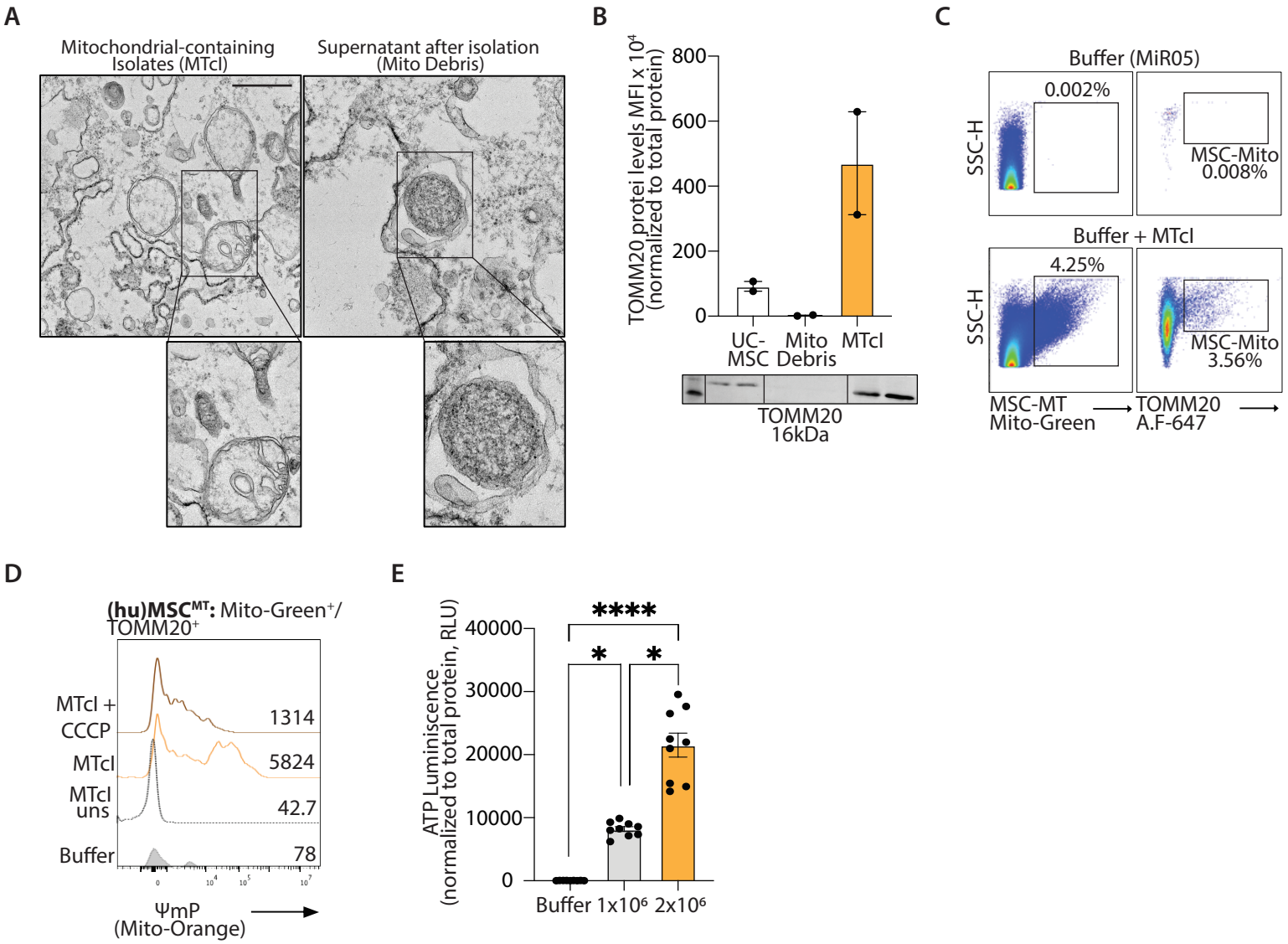

Fig. S3 | Characterization of isolated mitochondria. A) Transmission Electron Microscopy (TEM) images of mitochondrial-containing isolates (MTcl) showing preserved double-membrane structures (cristae) compared to supernatant debris. Scale bars, 500 nm. **B)** Western blot analysis of TOMM20 (mitochondrial outer membrane protein) in MSC lysates, isolates (MTcl), and debris. **C)** Flow cytometry analysis of MTcl stained with Mito-Green and anti-TOMM20. **D)** Assessment of mitochondrial membrane potential in isolates using Mito-Orange; CCCP was used as a depolarization control. **E)** ATP luminescence of mitochondrial isolates normalized to total protein content. Data are presented as mean ± s.e.m. n = 2-3 independent biological replicate. P values determined by one-way ANOVA (*P < 0.05; **P < 0.01; ***P < 0.001).

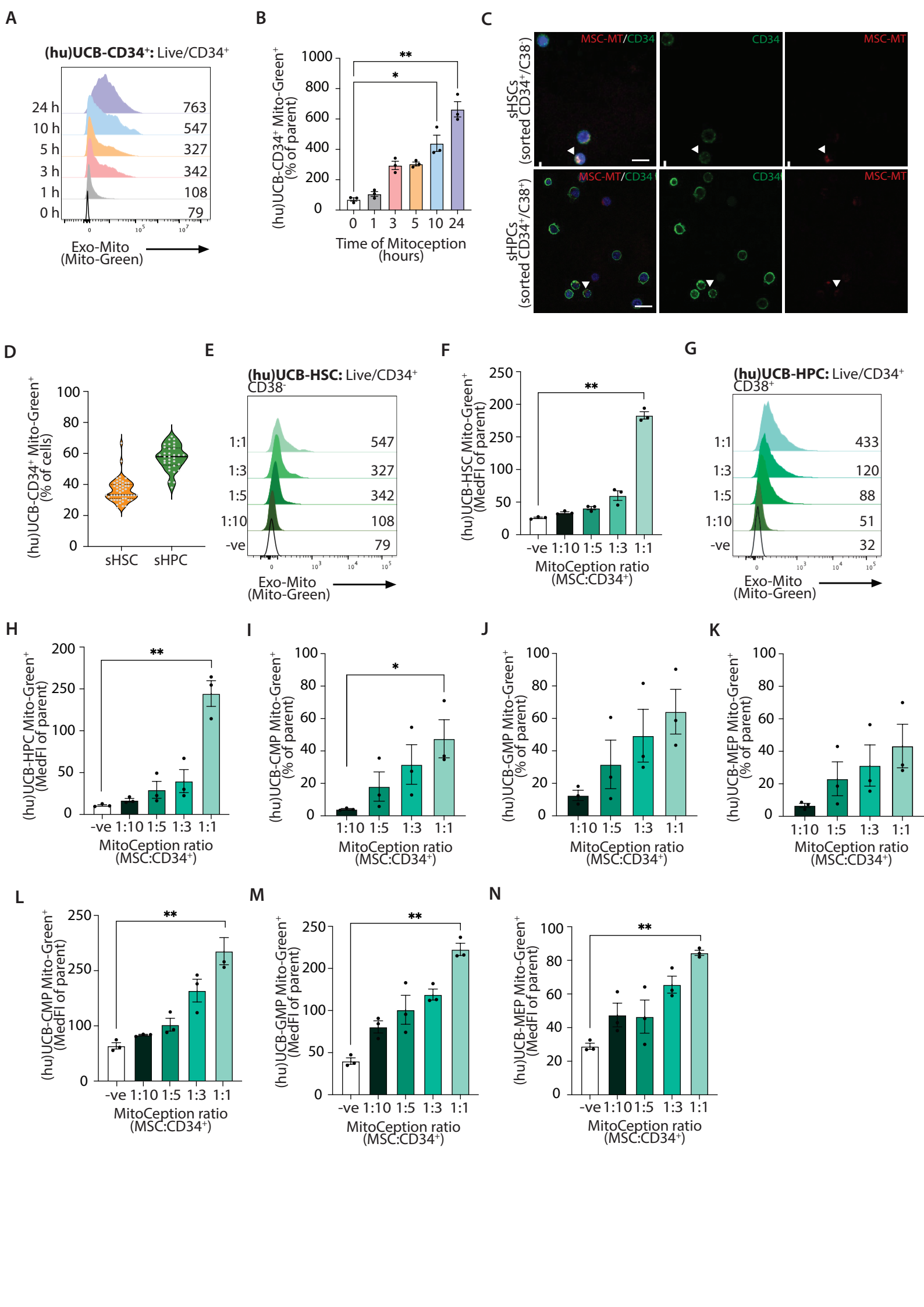
A
(hu)UCB-CD34+: Live/CD34+
24 h
10 h
5 h
3 h
1 h
0 h
763
547
327
342
108
79
Exo-Mito
(Mito-Green)
B
(hu)UCB-CD34+ Mito-Green+
(% of parent)
Time of Mitoception
(hours)
C
sHSCs
(sorted CD34+/C38-)
sHPCs
(sorted CD34+/C38+)
MSC-MT/CD34
CD34
MSC-MT
D
(hu)UCB-CD34+ Mito-Green+
(% of cells)
sHSC
sHPC
E
(hu)UCB-HSC: Live/CD34+
CD38-
1:1
1:3
1:5
1:10
-ve
547
327
342
108
79
Exo-Mito
(Mito-Green)
F
(hu)UCB-HSC Mito-Green+
(MedFI of parent)
-ve 1:10 1:5 1:3 1:1
MitoCeption ratio
(MSC:CD34+)
G
(hu)UCB-HPC: Live/CD34+
CD38+
433
120
88
51
32
H
(hu)UCB-HPC Mito-Green+
(MedFI of parent)
I
(hu)UCB-CMP Mito-Green+
(% of parent)
J
(hu)UCB-GMP Mito-Green+
(% of parent)
K
(hu)UCB-MEP Mito-Green+
(% of parent)
L
(hu)UCB-CMP Mito-Green+
(MedFI of parent)
M
(hu)UCB-GMP Mito-Green+
(MedFI of parent)
N
(hu)UCB-MEP Mito-Green+
(MedFI of parent)

Fig. S4 | Kinetics and dose-dependency of artificial mitochondrial transfer. A-B) Representative flow cytometry histograms **(A)** and quantification **(B)** of mitochondrial uptake (Mito-Green) MedFI IN $CD34^+$ cells at 0, 1, 3, 5, 10, and 24 hours post-MitoCeption. **C)** Representative confocal microscopy images of sorted HSCs (sHSC; upper panel) and HPCs (sHPC; lower panel) containing transferred mitochondria (white arrows). Scale bars, 10 µm. **D)** Violin plots showing the distribution of mitochondrial uptake in sorted sHSC vs sHPC populations. **E-F)** Representative flow cytometry histograms **(E)** and quantification **(F)** of mitochondrial uptake (Mito-Green) MedFI in HSCs using increasing ratios of mitochondrial isolates (1:10 to 1:1). **G-H)** Representative flow cytometry histograms **(G)** and quantification **(H)** of mitochondrial uptake (Mito-Green) MedFI in HPCs using increasing ratios of mitochondrial isolates (1:10 to 1:1). **I-K)** Quantification of mitochondrial transfer mitochondrial uptake (Mito-Green) frequency in committed progenitors: CMPs **(I)**, GMPs **(J)**, and MEPs **(K)** using increasing ratios of mitochondrial isolates (1:10 to 1:1). **L-N)** Quantification of mitochondrial transfer mitochondrial uptake (Mito-Green) MedFI in committed progenitors: CMPs **(L)**, GMPs **(M)**, and MEPs **(N)** using increasing ratios of mitochondrial isolates (1:10 to 1:1). Data are presented as mean ± s.e.m. of n = 3 independent biological replicates. P values determined by one-way ANOVA with Dunnett's multiple comparisons test (*P < 0.05; **P < 0.01; ***P < 0.001).

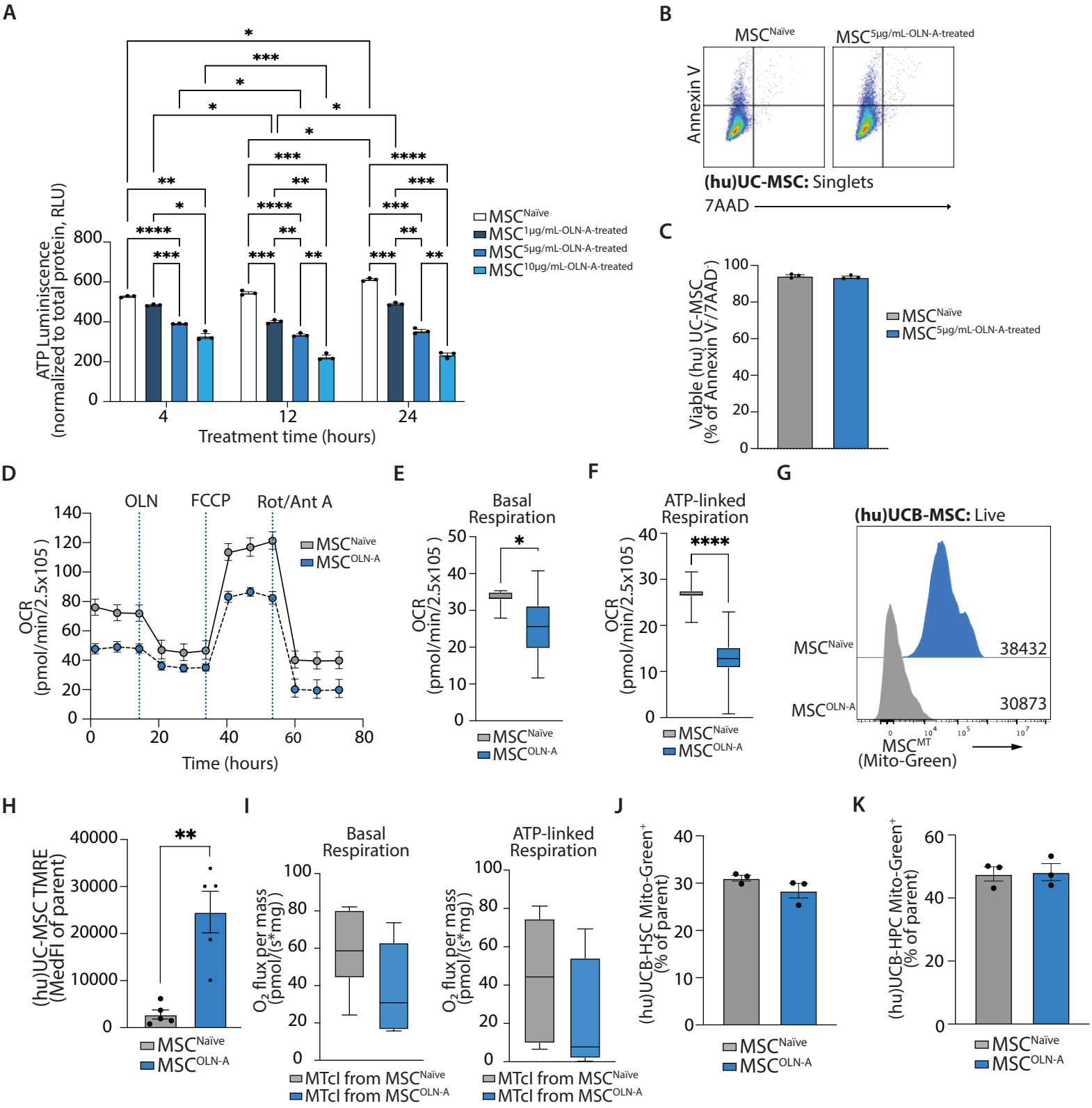

Fig. S5 | Validation of respiration-deficient mitochondria generation. A) Quantification of total ATP levels in MSCs treated with Oligomycin A (OLN-A) for 4, 12, and 24 hours. **B-C)** Representative flow cytometry plots **(B)** and quantification **(C)** of MSC viability (Annexin V$^-$/7AAD$^-$) following OLN-A treatment. **D-F)** Oxygen Consumption Rate (OCR) profiles **(D)**, basal respiration **(E)**, and ATP-linked respiration **(F)** of Naïve vs OLN-A treated MSCs. **G-H)** Representative histograms **(G)** and quantification **(H) o**f mitochondrial membrane potential (TMRE) in Naïve vs OLN-A treated MSCs. **I)** OCR analysis of basal respiration and ATP-linked respiration of isolated mitochondria (MTcl) derived from Naïve vs OLN-A treated MSCs. **J-K)** Quantification of mitochondrial uptake frequency in HSCs **(J)** and HPCs **(K)** confirming equal uptake efficiency between Naïve and OLN-A mitochondria. Data are presented as mean ± s.e.m. n = 3-4 independent biological replicates. P values determined by one-way ANOVA or Student's t-test (*P < 0.05; **P < 0.01; ***P < 0.001).

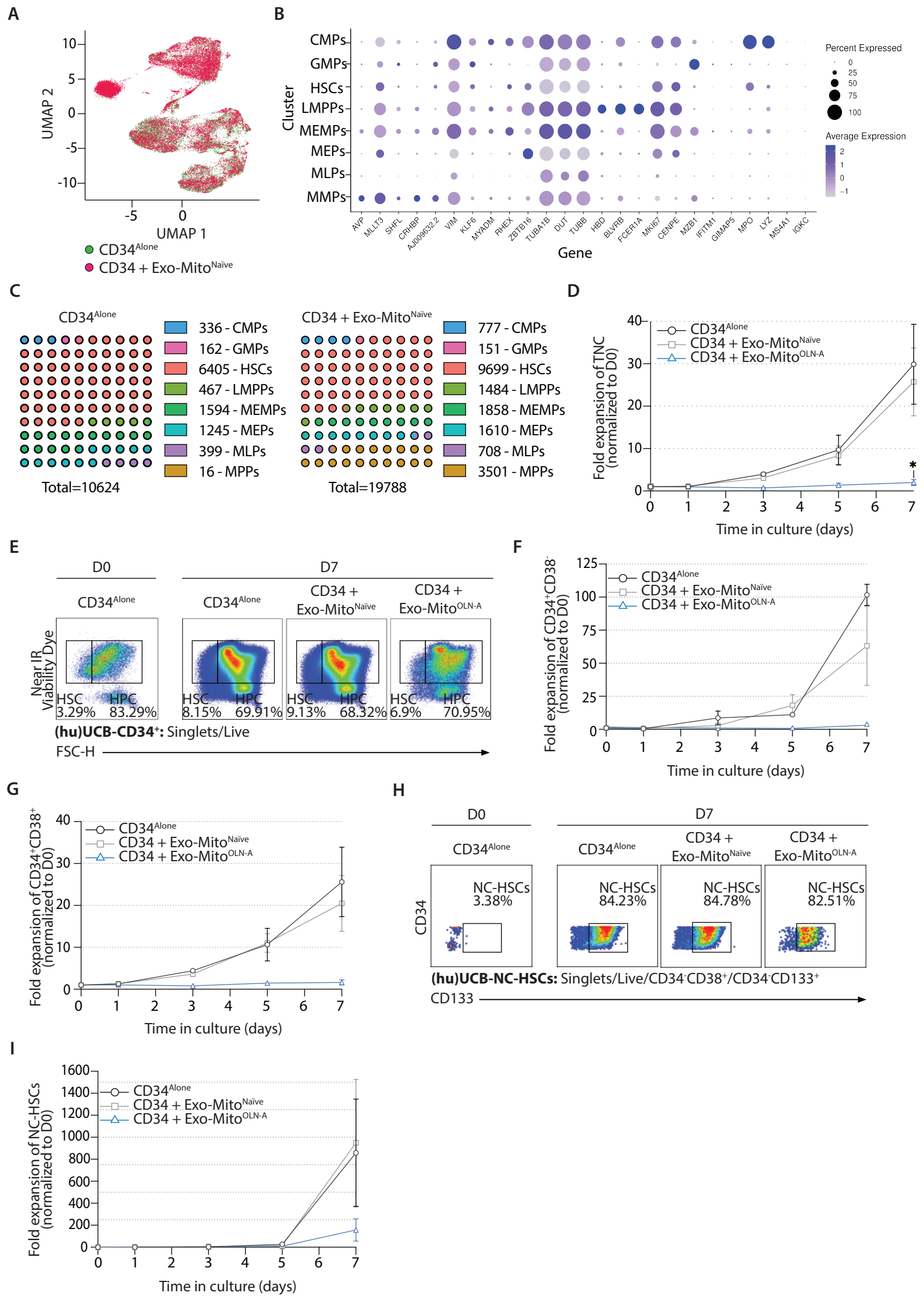
A
UMAP 2
UMAP 1
CD34Alone
CD34 + Exo-MitoNaïve
B
Cluster
CMPs
GMPs
HSCs
LMPPs
MEMPs
MEPs
MLPs
MMPs
AVP MLLT3 SHFL CRHBP AJ009632.2 VIM KLF6 MYADM RHEX ZBTB16 TUBA1B DUT TUBB HBD BLVRB FCER1A MKI67 CENPE MZB1 IFITM1 GIMAP5 MPO LYZ MS4A1 IGKC
Gene
Percent Expressed
Average Expression
C
CD34Alone
336 - CMPs
162 - GMPs
6405 - HSCs
467 - LMPPs
1594 - MEMPs
1245 - MEPs
399 - MLPs
16 - MPPs
Total=10624
CD34 + Exo-MitoNaïve
777 - CMPs
151 - GMPs
9699 - HSCs
1484 - LMPPs
1858 - MEMPs
1610 - MEPs
708 - MLPs
3501 - MPPs
Total=19788
D
Fold expansion of TNC (normalized to D0)
CD34Alone
CD34 + Exo-MitoNaïve
CD34 + Exo-MitoOLN-A
Time in culture (days)
E
D0
D7
CD34Alone
CD34Alone
CD34 + Exo-MitoNaïve
CD34 + Exo-MitoOLN-A
Near IR Viability Dye
HSC 3.29% HPC 83.29%
HSC 8.15% HPC 69.91%
HSC 9.13% HPC 68.32%
HSC 6.9% HPC 70.95%
(hu)UCB-CD34+: Singlets/Live
FSC-H
F
Fold expansion of CD34+CD38- (normalized to D0)
CD34Alone
CD34 + Exo-MitoNaïve
CD34 + Exo-MitoOLN-A
Time in culture (days)
G
Fold expansion of CD34+CD38+ (normalized to D0)
CD34Alone
CD34 + Exo-MitoNaïve
CD34 + Exo-MitoOLN-A
Time in culture (days)
H
D0
D7
CD34Alone
CD34Alone
CD34 + Exo-MitoNaïve
CD34 + Exo-MitoOLN-A
CD34
NC-HSCs 3.38%
NC-HSCs 84.23%
NC-HSCs 84.78%
NC-HSCs 82.51%
(hu)UCB-NC-HSCs: Singlets/Live/CD34-CD38+/CD34-CD133+
CD133
I
Fold expansion of NC-HSCs (normalized to D0)
CD34Alone
CD34 + Exo-MitoNaïve
CD34 + Exo-MitoOLN-A
Time in culture (days)

Fig. S6 | Mitochondrial transfer supports expansion of primitive phenotypes. A) UMAP plot of $CD34^+$ cells showing cluster distribution. **B)** Dot plot of marker gene expression used to identify single-cell clusters. **C)** Quantification of absolute cell counts per cluster derived from scRNA-seq analysis. **D)** Fold expansion of Total Nucleated Cells (TNC) over 7 days normalized to Day 0. **E-G)** Fold expansion of several hematopoietic lineages: total $CD34^+$ cells **(E)** and HSCs **(F)** and HPCs **(G)** normalized to Day 0. **H)** Representative flow cytometry plots of NC-HSCs ($CD34^+CD38^-CD133^+$) at D0 and D7. **I)** Fold expansion of NC-HSCs over 7 days normalized to Day 0.. Data are presented as mean ± s.e.m. of n = 3–4 independent biological replicates. P values determined by two-way ANOVA with Dunnett's multiple comparisons test (*P < 0.05; **P < 0.01; ***P < 0.001).

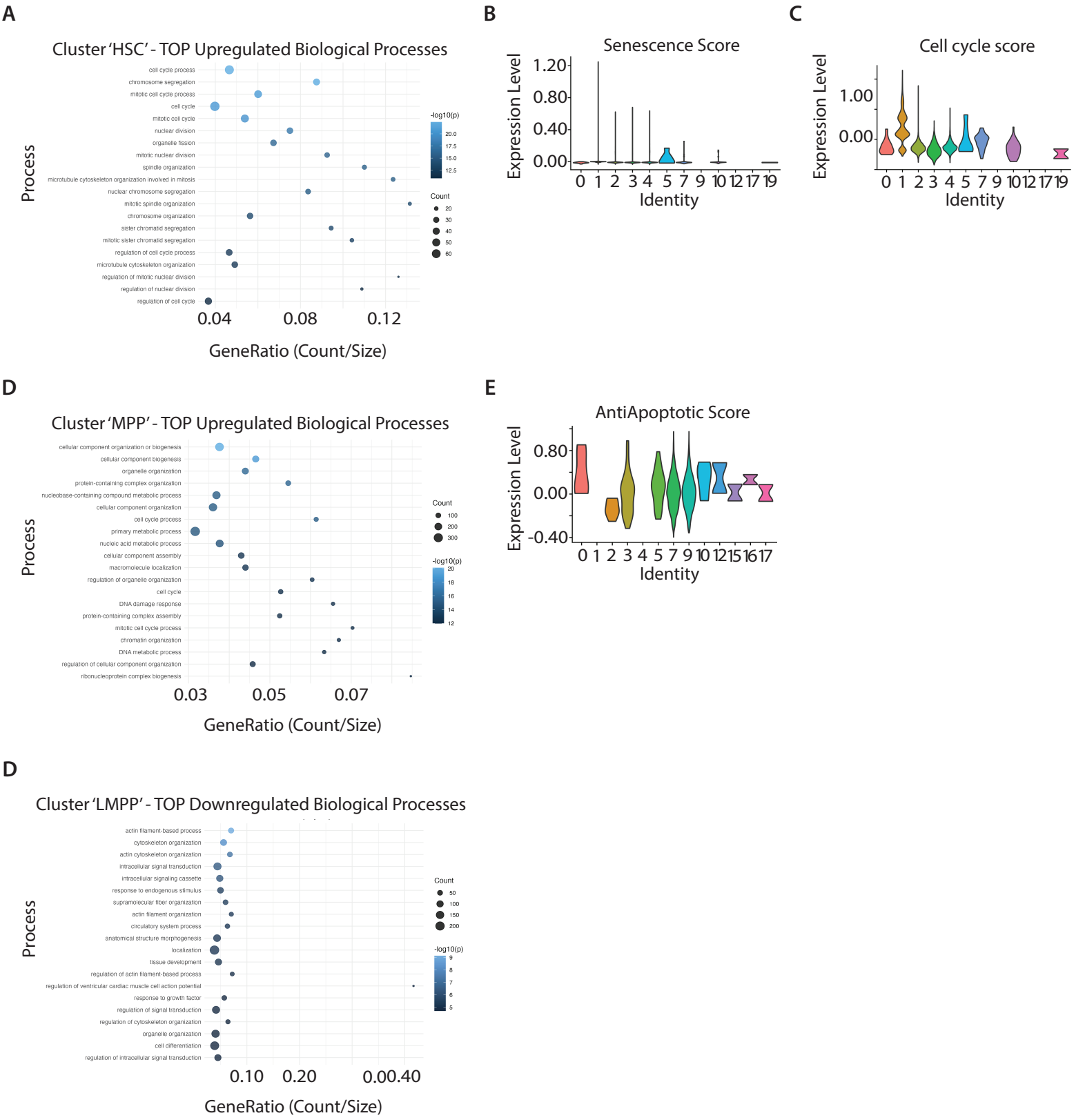

Fig. S7 | Differential gene expression and functional scoring. A) Dot plot of top upregulated GO biological processes in the HSC cluster. **B)** Violin plot of Senescence scores in the HSC clusters. **C)** Violin plot of Cell Cycle scores in the HSC clusters. **D)** Dot plot of top upregulated GO biological processes in the MPP cluster. **E)** Violin plot of Anti-Apoptotic scores in the MPP cluster.. **F)** Dot plot of top downregulated GO biological processes in the LMPP cluster. Dot size represents gene count; color intensity represents statistical significance (-log10 P value)

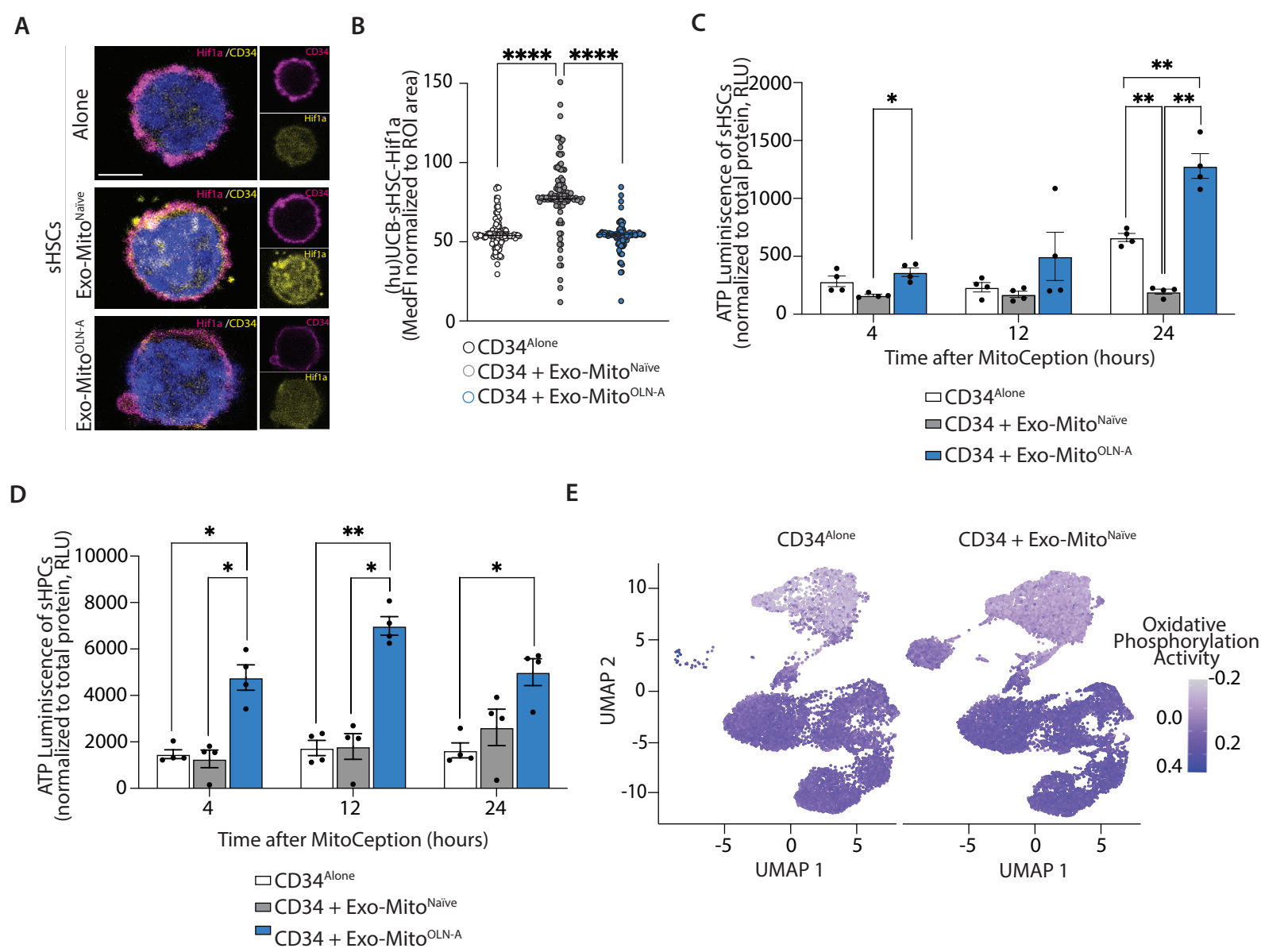

Fig. S8 | Metabolic regulation via HIF1α and ATP. A) Representative confocal images of HIF1α expression (yellow) in sorted sHSC and sHPC after mitochondrial transfer. Nuclei are stained with Hoechst (blue) and cells are stained with surface marker CD34 (magenta). Scale bars, 10 μm. **B)** Quantification of HIF1α MedFI. **C)** Quantification of total ATP levels in sorted sHSCs across conditions. **D)** Quantification of total ATP levels in sorted sHPCs. **E)** Single-cell pathway activity UMAP visualization of "Hallmark Oxidative Phosphorylation" enrichment. Data are presented as mean ± s.e.m. n = 3 independent biological replicates. P values determined by one-way ANOVA with Tukey's multiple comparisons test (*P < 0.05; **P < 0.01; ***P < 0.001).

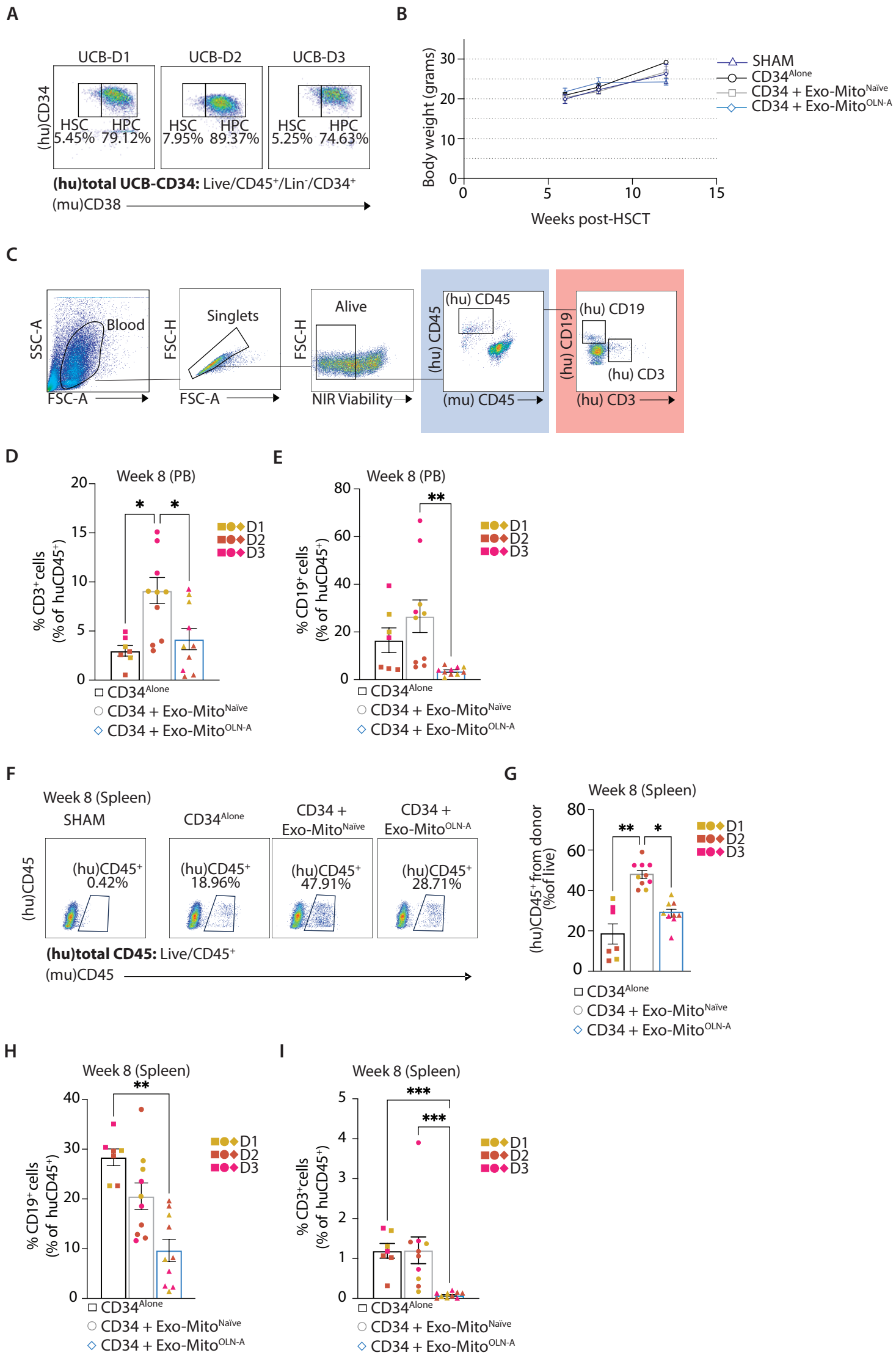
A
UCB-D1
UCB-D2
UCB-D3
(hu)CD34
HSC 5.45%
HPC 79.12%
HSC 7.95%
HPC 89.37%
HSC 5.25%
HPC 74.63%
(hu)total UCB-CD34: Live/CD45+/Lin-/CD34+
(mu)CD38
B
Body weight (grams)
Weeks post-HSCT
SHAM
CD34Alone
CD34 + Exo-MitoNaïve
CD34 + Exo-MitoOLN-A
C
SSC-A
Blood
FSC-A
FSC-H
Singlets
FSC-A
FSC-H
Alive
NIR Viability
(hu) CD45
(mu) CD45
(hu) CD19
(hu) CD3
D
Week 8 (PB)
% CD3+ cells (% of huCD45+)
D1
D2
D3
CD34Alone
CD34 + Exo-MitoNaïve
CD34 + Exo-MitoOLN-A
E
Week 8 (PB)
% CD19+ cells (% of huCD45+)
F
Week 8 (Spleen)
SHAM
CD34Alone
CD34 + Exo-MitoNaïve
CD34 + Exo-MitoOLN-A
(hu)CD45
(hu)CD45+ 0.42%
(hu)CD45+ 18.96%
(hu)CD45+ 47.91%
(hu)CD45+ 28.71%
(hu)total CD45: Live/CD45+
(mu)CD45
G
Week 8 (Spleen)
(hu)CD45+ from donor (%of live)
H
Week 8 (Spleen)
% CD19+ cells (% of huCD45+)
I
Week 8 (Spleen)
% CD3+ cells (% of huCD45+)

Fig. S9 | Extended in vivo engraftment analysis. A) Pre-transplant quality check of human UCB donors ($CD34^+$ > 5%). **B)** Body weight monitoring of NSG-SGM3 mice post-transplantation. **C)** Representative gating strategy for extramedullary organ analysis. **D-E)** Quantification of human T cells ($CD3^+$; **D)** and B cells ($CD19^+$; **E)** in peripheral blood at Week 8 post-transplantation. **F-G)** Representative flow cytometry plots **(F)** and quantification **(G)** of human chimerism ($huCD45^+$) in the spleen at Week 8 post-transplantation. **H)** Quantification of T cell ($CD3^+$) and B cell ($CD19^+$) distribution in the spleen. Data points represent independent UCB donors (D1-D3). Data are presented as mean ± s.e.m. n = 7–10 mice per group. P values determined by one-way ANOVA with Tukey's multiple comparisons test (*P < 0.05; **P < 0.01; ***P < 0.001).

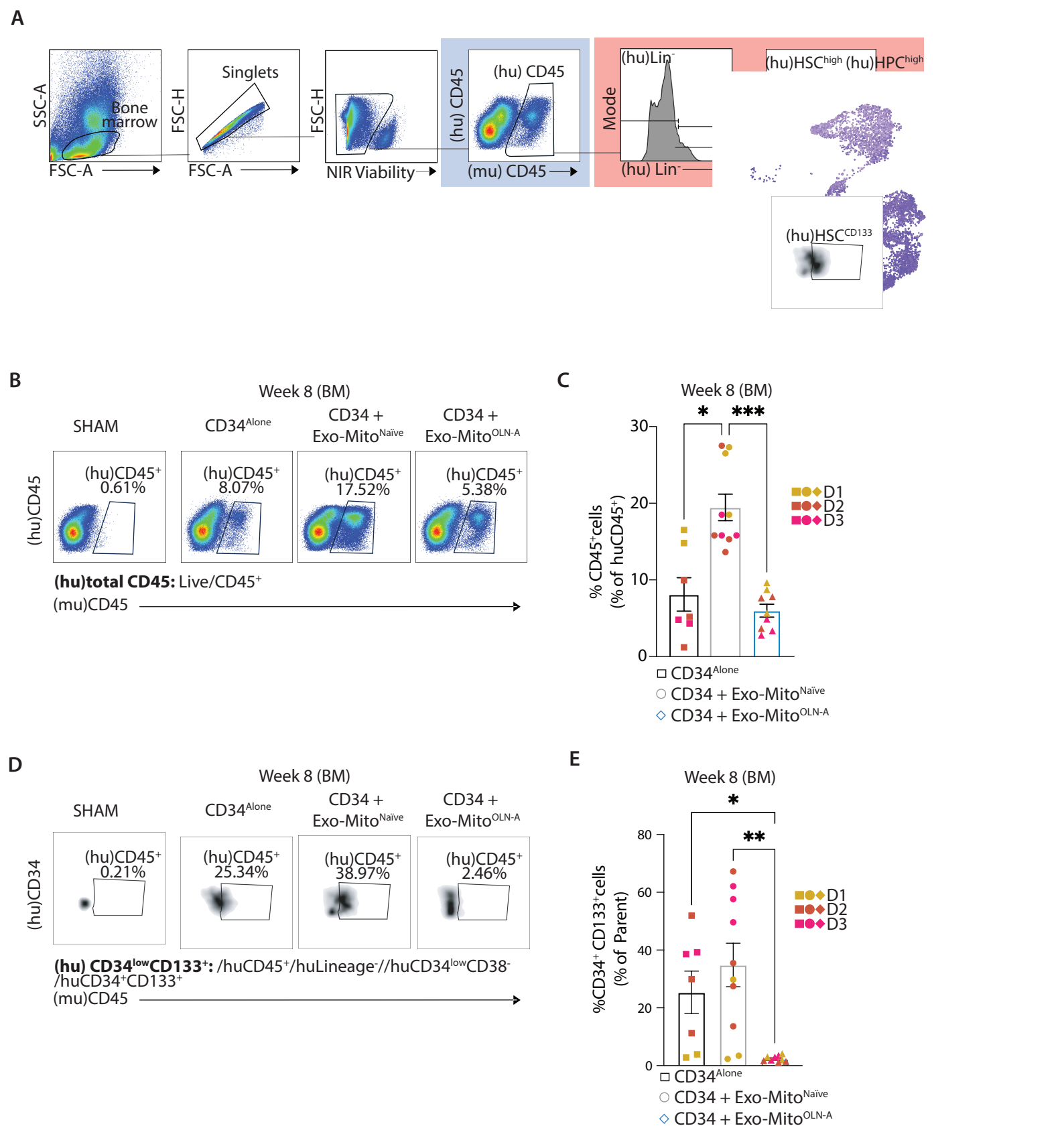

Fig. S10 | Bone marrow engraftment and stem cell preservation. A) Gating strategy for the identification of human hematopoietic populations in the murine bone marrow. **B)** Representative flow cytometry plots of human chimerism (huCD45$^+$) in the BM. **C)** Quantification of total human CD45$^+$ engraftment in the BM at Week 8. **D)** Representative plots of primitive human phenotypes (CD34^{low}CD133$^+$) in the BM. **E)** Quantification of the percentage of CD34^{low}CD133$^+$ stem cells within the human graft in the BM. Data points represent independent UCB donors (D1-D3). Data points represent independent UCB donors (D1-D3). Data are presented as mean ± s.e.m. n = 7–10 mice per group. P values determined by one-way ANOVA with Tukey's multiple comparisons test (*P < 0.05; **P < 0.01; ***P < 0.001).