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Supplemental information

Derived myeloid lineage induced pluripotent

stem as a platform to study human C-C

chemokine receptor type $5\Delta 32$ homozygotes

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Figure S1. Assessment of CD38 and CD34 expression during iPSCs towards iHSPCs differentiation, related to Figure 2. Representative graphs showing that the majority of CD34⁺ cells hold a CD38⁻ phenotype at different time points during the iPSC differentiation process towards iHSPCs.

Figure S2



Figure S2. Phenotype analysis of cells during Colony-Forming Unit (CFU) assay, related to Figure 2. Representative graphs showing the expression levels of the panhematopoietic marker CD45 (left column), the erythroid marker glycophorin (CD235, middle column), and the myeloid marker CD33 (right column) on cells analyzed at different time points during CFU assay.

Figure S3



Figure S3. Primary and iPSC-derived macrophage secretome profile, related to Figure 6. Expression levels of M1 polarization markers (A) and M2 polarization markers (B) were analyzed in primary (white bar) and iPSC-derived M0, M1, and M2 macrophages obtained from both controls (light gray bar) and CCR5 Δ 32 variant (dark gray bar). Data are expressed as pg/10⁵ cells and are presented as mean ± SEM. Statistical significance was calculated using ANOVA. *p< 0.05; **p<0.01; *** p< 0.001; **** p<0.0001.

| Primary | | iPS-derived | | | | | |
|---------|--------------|-------------|--------------|-------------|------------|--|--|
| Normal | | Normal | | CCR5D32 | | | |
| | | | iPS_ctrl_3 | | | | |
| | | iPS | iPS_ctrl_4 | | | | |
| | | | iPS_ctrl_6 | | | | |
| pmono | pmono_ctrl_1 | | imono_ctrl_1 | | | | |
| | pmono_ctrl_2 | imono | imono_ctrl_2 | | | | |
| | pmono_ctrl_7 | | imono_ctrl_5 | | | | |
| рM0 | pM0_ctrl_1 | iMO | iM0_ctrl_1 | | iM0_CCR5_1 | | |
| | pM0_ctrl_7 | | iM0_ctrl_2 | iM0_CCR5D32 | iM0_CCR5_2 | | |
| | pM0_ctrl_8 | | iM0_ctrl_3 | | iM0_CCR5_3 | | |
| | | | iM0_ctrl_4 | | | | |
| | | | iM0_ctrl_5 | | | | |
| pM1 | pM1_ctrl_1 | iM1 | iM1_ctrl_1 | iM1_CCR5D32 | iM1_CCR5_1 | | |
| | pM1_ctrl_2 | | iM1_ctrl_2 | | iM1_CCR5_2 | | |
| | pM1_ctrl_7 | | iM1_ctrl_3 | | iM1_CCR5_3 | | |
| | pM1_ctrl_8 | | iM1_ctrl_4 | | | | |
| | | | iM1_ctrl_5 | | | | |
| pM2 | pM2_ctrl_1 | iM2 | iM2_ctrl_1 | iM2_CCR5D32 | iM2_CCR5_1 | | |
| | pM2_ctrl_7 | | iM2_ctrl_2 | | iM2_CCR5_2 | | |
| | pM2_ctrl_8 | | iM2_ctrl_3 | | iM2_CCR5_3 | | |
| | | | iM2_ctrl_4 | | | | |
| | | | iM2_ctrl_5 | | | | |

 Table S1. List of samples used in RNA-seq analyses, related to Figure 4 and 5.

Table S2

| Panel | Staining | Target | Fluorochrome | FACS Filters | Clone | Company | Catalog N° | Batch |
|----------------------------|----------|--------------|--------------|--------------|-----------|-----------|------------|----------|
| iHSCs | Surface | CD34 | PE | YG582/15 | 561 | Biolegend | 343606 | B204487 |
| | Surface | CD34 | BV510 | V510/20 | 581 | Biolegend | 343528 | B311638 |
| | Surface | CD45 | APC | R670/14 | H130 | Biolegend | 304012 | B190802 |
| | Surface | CD33 | FITC | B530-30 | HIM3-4 | Biolegend | 303304 | B284834 |
| | Surface | CD38 | AF700 | R730/45 | HB-7 | Biolegend | 303516 | B303849 |
| | Surface | CD235 | PE | YG582/15 | HIR2 | Biolegend | 306604 | B203766 |
| Monocytes | Surface | CD14 | FITC | B530/30 | GCD14 | Biolegend | 325604 | B211977 |
| | Surface | CD16 | APC | R670/14 | 3G8 | Biolegend | 302012 | B212891 |
| | Surface | CD11b | PE Cy5 | B655-730 | ICRF44 | Biolegend | 301308 | B191268 |
| Macrophage polarization | Surface | CD115 | BV421 | V450/50 | 9-4D2-1E4 | BD | 565347 | 5082979 |
| | Surface | CD14 | BV570 | V575/26 | M5E2 | Biolegend | 301831 | B172009 |
| | Surface | CD16 | AF700 | R730/45 | 3G8 | Biolegend | 302026 | B181825 |
| | Surface | CD209 | PE | YG582/15 | DCN46 | BD | 551265 | 2097966 |
| | Surface | CD80 | FITC | B530/30 | L307-4 | BD | 557226 | 96060 |
| M1 Macrophage phenotype | Surface | CD86 | FITC | B530/30 | 2331 | BD | 555657 | 5606 |
| | Surface | CD215 | APC | R670/14 | JM7A4 | Biolegend | 330209 | 163590 |
| | Surface | HLA-DR | APC Cy7 | R780/60 | L243 | BD | 335831 | 4226954 |
| | Surface | CD195 (CCR5) | PE | YG582/15 | 2D7/ CCR5 | BD | 555993 | 93581 |
| | Surface | CCR7 | PE CF594 | YG610/20 | 150503 | BD | 562381 | 4085878 |
| M2 macrophage phenotype | Surface | CD206 | FITC | B530/30 | 19.2 | BD | 551135 | 87315 |
| | Surface | CD163 | PE | YG582/15 | GHI/61 | BD | 556018 | 8289 |
| | Surface | CD200R | APC | R670/14 | OX-108 | Biolegend | 329307 | B196410 |
| | Surface | CD204 | AF700 | R730/45 | 351615 | R&D | FAB2708N | F0114031 |

Table S2. FACS Antibody Information, related to Figures 2-3, and 6 and to Supplementary Figures S1 and S2.