

Supplemental Information

Figure S1 (related to Figure 1 and Supplemental File 1)

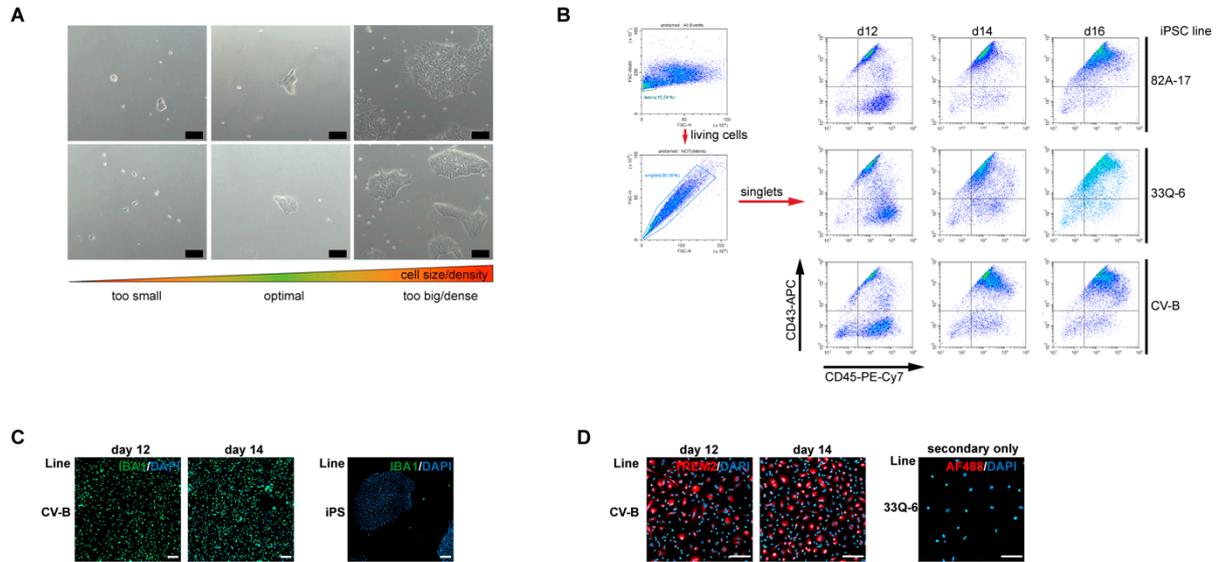


Figure S1 (related to Figure 1 and Supplemental File S1)

(A) Representative bright field pictures of iPSCs at start of HPC differentiation. Depicted are two examples of too small, optimally seeded and too big/dense iPSC colonies. Scale bar represents 100 μ m. (B) Scatterplots depicting gating strategy for FACS analysis of CD43/CD45 on HPCs. Scatterplots depicting gating of living cells and singlets (two left scatterplots). The grid on the right half shows scatterplots of CD43 fluorescence intensity (y-axis) vs. CD45 fluorescence intensity (x-axis) for HPCs differentiated from 3 different iPSC lines (82A-17, 33Q-6, CV-B) that were harvested on day 12, 14 and 16 and depicts the gates which were used to identify CD43+, CD45+ and CD43/CD45 double positive cells in subsequent analysis. (C) Fluorescence microscopy pictures depicting iMGLs from iPSC line CV-B which were matured from HPCs harvested at respective days (12 and 14) and stained for IBA1 and DAPI (two left panels). Fluorescence microscopy pictures of undifferentiated iPS cells stained for IBA1 and DAPI as negative control (right panel). Scale bar represents 100 μ m. (D) Fluorescence microscopy pictures depicting iMGLs from iPSC line CV-B which were matured from HPCs harvested at respective days (12 and 14) and stained for TREM2 (red) and DAPI (blue) (two left panels). Fluorescence microscopy picture of iMGLs differentiated from iPSC line 33Q-6 stained with Alexa-Fluor 488 labelled secondary antibody only and DAPI as negative control (right panel). Scale bar represents 100 μ m.

Figure S2 (related to Figure 3)

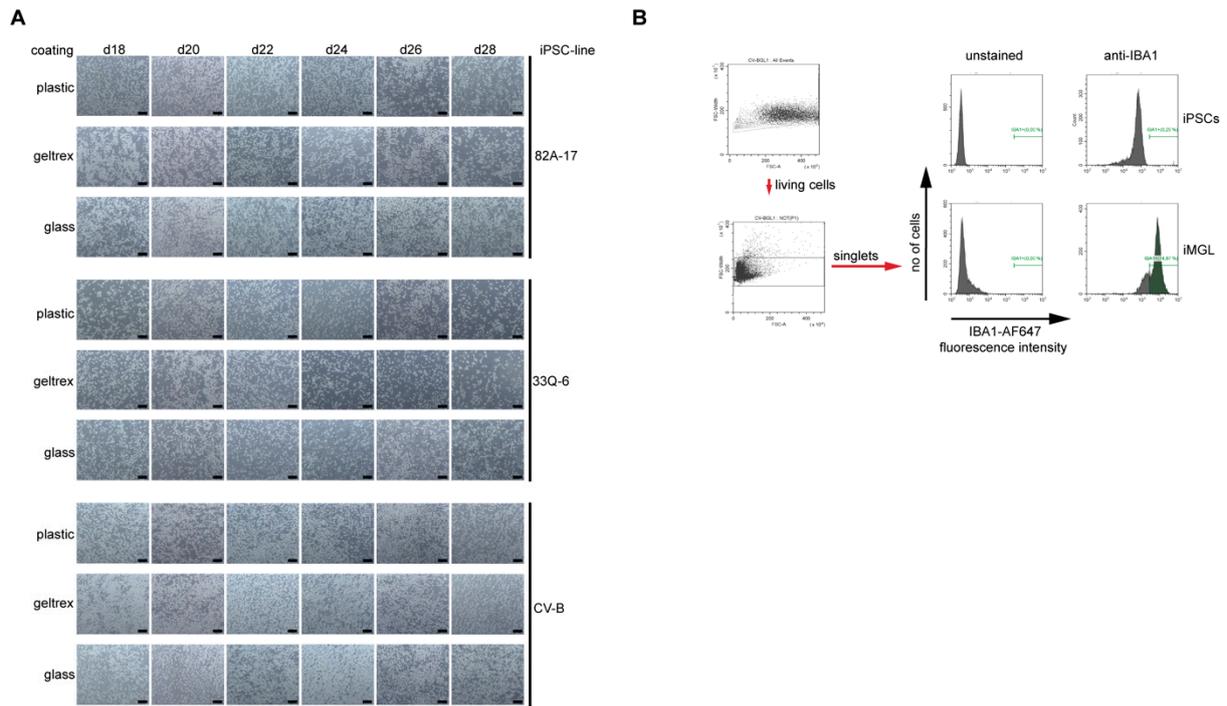


Figure S2 (related to Figure 3)

(A) Representative bright field pictures of HPCs matured into microglia on three different coatings (plastic, geltrex and glass) over the course of maturation from day 18 to day 28. Depicted are HPCs from three different iPSC lines (82A-17, 33Q-6, CV-B) that were further matured into iMGL. Scale bar represents 100 μ m. (B) Gating strategy for FACS analysis of IBA1 positivity on iMGL. Scatterplots depicting gating of living cells and singlets (two left scatterplots). The right half of the figure shows 4 histograms depicting no of cells (y-axis) vs. fluorescence intensity on unstained and anti-IBA1 stained iPSCs serving as negative control and iMGL differentiated from one iPSC line (CV-B) which were either left unstained or stained with anti-IBA1 antibody. The histograms include the gate which was used for analysis of IBA1-positive cells in subsequent analysis.

Tables

Table S1 – RNAseq intermediate files (provided as file)

Table S2 – qPCR primers

| Target mRNA | Fw primer | Rev primer |
|-------------|--|--|
| IL1B | TCCAGGGACAGGATATGGAG | CCCAAGGCCACAGGTATTT |
| IL6 | AGACAGCCACTCACCTCTTCAG | TTCTGCCAGTGCCTCTTTGCTG |
| GAPDH | GAPDH TaqMan Mastermix (Applied Biosystems #402869) | GAPDH TaqMan Mastermix (Applied Biosystems #402869) |
| HPRT | CCTGGCGTCGTGATTAGTG | TCCCATCTCCTTCATCACATC |
| B2M | GAGGCTATCCAGCGTACTCC | AATGTCCGGATGGATGAAACC |

Table S3 – Cell culture compounds

| Compound | Vendor | Catalog no. | stock |
|--|-----------------------|-------------|---|
| mTeSR Plus ES/iPS medium | Stemcell Techn. | 05825 | 1x |
| RPMI1640 + GlutaMAX | ThermoFisher | 61870036 | 1x |
| FBS, qualified and heat inactivated | ThermoFisher | 10270106 | 1x |
| Gentle Cell Dissociation Reagent | StemCell Technologies | 100-0485 | 1x |
| IL-34, human recombinant | Peprotech | 200-34 | 100µg/ml |
| GM-CSF, human recombinant | Peprotech | 300-03 | 50µg/ml |
| Penicillin/Streptomycin | ThermoFisher | 15140122 | 100x |
| STEMdiff Hematopoietic kit | StemCell Technologies | 05310 | - |
| Geltrex LDEV free Matrix, hESC qualified | ThermoFisher | A1413302 | 9.6mg/ml, use at 0.02mg/cm ² |
| DMSO, >99,5% BioScience grade | Roth | A994.1 | 1x |
| TrypLE Express | ThermoFisher | 12605010 | 1x |

Table S4- Antibodies

| Antibody | Vendor | Catalog no. | working dilution |
|---------------------------|--------------|-------------|-----------------------------|
| rabbit anti IBA1 | Wako | 019-19741 | FACS 1:50 IF 1:100-1:200 |
| goat anti TREM2 | R+D systems | AF1828 | IF 1:200 |
| donkey anti rabbit AF 647 | ThermoFisher | A-31573 | FACS 1:200 IF 1:500 |
| donkey anti rabbit AF 488 | ThermoFisher | A-21206 | FACS 1:200 IF 1:500 |
| donkey anti goat AF488 | ThermoFisher | A-11055 | IF 1:500 |
| anti CD43 APC | Biolegend | 343205 | FACS: 1:50 |
| anti CD45 PE-Cy7 | Biolegend | 103113 | FACS: 1:50 |

Table S5 – iPSC lines used in this study

| Cell line | Code | Age at donation | Gender | origin |
|------------------|------------------|------------------------|---------------|------------------------------|
| 33Q-6 | UKERi-33Q-R2-006 | 45 | female | University Hospital Erlangen |
| 82A-17 | UKERi-82A-S1-017 | 66 | female | University Hospital Erlangen |
| CV-B | CV-hiPS-B | 63 | male | Gore et al., 2011 [23] |

Files

Supplemental File S1 – Step-by-step protocol