Supplemental Information –Zweigerdt et al.

Supplemental Experimental Procedures

Calculation of viability

Culture viability [%] was calculated as

$$Viability = \frac{TCD - DCD}{TCD} \times 100$$

where TCD is the total cell density [cells/ mL] and DCD the dead cell density [cells/ mL] as determined by trypan blue staining as described in detail elsewhere [1].

Glucose and lactate analysis

Glucose and lactate concentrations from cell-free supernatant were analyzed using an YSI 2700 Select™ Biochemistry Analyzer (YSI Incorporated Life Sciences).

Specific glucose consumption rates *qGlc* [pmol/ cell/ d] for the process days without medium change and in the repeated batch processes between two feeding intervals were calculated as

$$qGlc_{t_{n+1}} = \left(\frac{Glc_{t_{n+1}} - Glc_{t_n}}{t_{n+1} - t_n}\right) \times \frac{1}{\bar{X}_{t_{n+1}}}$$

where *Glc* is the glucose concentration [pmol/ L], *t* the process time point [d], and $\bar{X}$ the mean cell concentration [cells/ L] calculated as $\bar{X}_{t_{n+1}} = \frac{X_{t_{n+1}} - X_{t_n}}{\ln(X_{t_{n+1}}) - \ln(X_{t_n})}$

In the perfused cultures the specific glucose consumption rate is calculated as

$$qGlc_{t_{n+1}} = \left[\left(\frac{Glc_{t_{n+1}} - Glc_{t_n}}{t_{n+1} - t_n}\right) + \frac{F}{V}\left(\overline{Glc}_{t_{n+1}} - Glc_f\right)\right] \times \frac{1}{\bar{X}_{t_{n+1}}}$$

where *F* is the flow rate of feed and waste stream [L/ d], *V* the culture volume [L], Glc_f the glucose concentration in the feed stream [pmol/ L] and $\overline{Glc}$ is the mean glucose concentration in the culture calculated as

$$\overline{Glc}_{t_{n+1}} = \frac{Glc_{t_{n+1}} - Glc_{t_n}}{\ln\left(Glc_{t_{n+1}}\right) - \ln\left(Glc_{t_n}\right)}$$

Specific lactate production rates *qLac* [pmol/ cell/ d] for the process days without medium change and in the repeated batch processes between two feeding intervals are calculated accordingly as

$$qLac_{t_{n+1}} = \left(\frac{Lac_{t_{n+1}} - Lac_{t_n}}{t_{n+1} - t_n}\right) \times \frac{1}{\bar{X}_{t_{n+1}}}$$

where *Lac* is the lactate concentration [pmol/ L].

In the perfused cultures the specific lactate production rate is calculated as

$$qLac_{t_{n+1}} = \left[\left(\frac{Lac_{t_{n+1}} - Lac_{t_n}}{t_{n+1} - t_n}\right) + \frac{F}{V} \times \overline{Lac}_{t_{n+1}}\right] \times \frac{1}{\bar{X}_{t_{n+1}}}$$

where $\overline{Lac}$ is the mean lactate concentration in the culture calculated as

$$\overline{Lac}_{t_{n+1}} = \frac{Lac_{t_{n+1}} - Lac_{t_n}}{\ln\left(Lac_{t_{n+1}}\right) - \ln\left(Lac_{t_n}\right)}$$

The yield coefficient of lactate from glucose *Y(qLac/qGlc)* [-] is calculated as follows

$$Y(qLac/qGlc) = \left|\frac{qLac}{qGlc}\right|$$

Amino acid analysis

Amino acid levels were determined via high-performance liquid chromatography using a precolumn derivatization method with ortho-phthaldialdehyd (OPA) utilizing a Shimadzu RF-10AxL fluorescence detector (excitation 330 nm, emission 420 nm), an Agilent 1200 pump (Agilent), a triathlon autosampler (Spark), a 3-channel degasser (Sykam), a T-1 column stove (Techlab) and a Waters Resolve C18 column (5 µm, 3.9 x 150 mm). Protein precipitation is performed at -20 °C overnight via addition of 100 µL methanol to 25 µL cell-free supernatant. After centrifugation, 30 µL protein-free samples were incubated with 50 µL OPA-reagent (27 mg OPA, 5 mL ethanol, 0.2 mL mercaptoethanol, 45 mL 0.4 M potassium borate buffer, pH 9.5) for 1.5 min and subsequently 10 µL were loaded onto the column. Chromatography was performed with a flow rate of 1 mL/ min at 30 °C utilizing a gradient of 0 % to 100 B % with elution buffer A (0.05 M sodium acetate, 0.05 M sodium dihydrogen phosphate, pH 7, 2 % tetrahydrofuran and 2 % methanol) and elution buffer B (54 % methanol).

Specific amino acid consumption or production rates *qAC* [pg/ cell/ d] for the process days without medium change and in the repeated batch processes between two feeding intervals were calculated as

$$qAC_{t_{n+1}} = \left(\frac{AC_{t_{n+1}} - AC_{t_n}}{t_{n+1} - t_n}\right) \times \frac{1}{\bar{X}_{t_{n+1}}}$$

where A*C* is the amino acid concentration [pg/ L], *t* the process time point [d], and $\bar{X}$ the mean cell concentration [cells/ L] calculated as $\bar{X}_{t_{n+1}} = \frac{X_{t_{n+1}} - X_{t_n}}{\ln(X_{t_{n+1}}) - \ln(X_{t_n})}$

In the perfused cultures the specific amino acid consumption or production rates are calculated as

$$qAC_{t_{n+1}} = \left[\left(\frac{AC_{t_{n+1}} - AC_{t_n}}{t_{n+1} - t_n}\right) + \frac{F}{V}\left(\overline{AC}_{t_{n+1}} - AC_f\right)\right] \times \frac{1}{\bar{X}_{t_{n+1}}}$$

where *F* is the flow rate of feed and waste stream [L/ d], *V* the culture volume [L], AC_f the amino concentration in the feed stream [pmol/ L] and $\overline{AC}$ is the mean amino acid concentration in the culture calculated as

$$\overline{AC}_{t_{n+1}} = \frac{AC_{t_{n+1}} - AC_{t_n}}{\ln\left(AC_{t_{n+1}}\right) - \ln(AC_{t_n})}$$

For both process conditions, positive values indicate the production and negative values the consumption of a specific amino acid, respectively.

Osmolarity

Culture osmolarity was analyzed via freezing point osmometer OSMOMAT 3000 basic (Gonotec).

Static suspension cultures

Single cells were seeded at inoculation density of 3.5×10^5 cells/ well in Costar® ultra-low attachment 6 well plates (Corning Life Sciences) in 3 mL mTeSR1 (STEMCELL Technologies) + 10 µM Y-27632 supplemented with recombinant CXCL1 and/or CXCL5 (PeproTech) if applicable. Medium was exchanged on day 2 while respective amounts of cytokines were added. Cell count was performed on day 3.

Flow cytometry

To assess expression of pluripotency associated surface markers SSEA3, SSEA4 and TRA 1-60 by flow cytometry cells were incubated with primary antibodies against SSEA4 (1:100; mouse IgG3, DSHB), SSEA3 (1:100; mouse IgM, DSHB) or TRA 1-60 (1:100; mouse IgM, Abcam) for 20 min at 4 °C. After washing, cells were incubated with corresponding secondary antibodies (1:300; Cy™5-labeled donkey anti-mouse IgG for SSEA4 and Cy™5-labeled donkey anti-mouse IgM for TRA 1-60, both Jackson Immunoresearch Laboratories) for 30 min at 4 °C. Antibodies were diluted in PBS without Ca^{2+} / Mg^{2+} with 0.5% BSA.

For intra cellular pluripotency markers OCT4 and NANOG single cell suspensions were fixed with 90 % methanol for 15 min and subsequently incubated with primary antibodies against OCT4 (1:40; mouse IgG_{2b}, Santa Cruz Biotechnology) or NANOG (1:100; rabbit IgG, Cell Signaling Technology) for 1 h at room temperature. After washing, cells were incubated with secondary antibodies (1:300 Cy™5-labeled donkey anti-mouse IgG for OCT4; 1:300 Cy™3-labeled donkey anti-rabbit IgG for NANOG; both Jackson Immunoresearch Laboratories) for 30 min at room temperature. Antibodies were diluted in PBS without Ca^{2+} / Mg^{2+} with 0.5% BSA and 1% Triton X-100.

Flow cytometry for cardiac markers sarcomeric actinin, MHC and β-MHC was performed as described previously [2, 3]. In brief, cell were fixed with 1:1 PBS without Ca^{2+} / Mg^{2+} and solution A of the Fix&Perm kit (ADG) for 15 min at room temperature and subsequently incubated with primary antibodies against sarcomeric actinin (1:80; mouse IgG1, Sigma-Aldrich), MHC (1:25; mouse IgG_{2b}, DSHB) or β-MHC (1:1,000; mouse IgG1, Sigma-Aldrich) for 45 min at room temperature. After washing, cells were incubated with secondary antibodies (1:200 Cy™5-labeled donkey anti-mouse IgG, both Jackson Immunoresearch Laboratories) for 30 min at room temperature. Antibodies were diluted in PBS without Ca^{2+} / Mg^{2+} with 0.5% BSA and 0.1% Triton X-100.

Cells were analyzed using a BD Accuri™ C6 flow cytometer (BD Bioscience) and data were further processed using FlowJo v10 software.

In vitro differentiation

For serum-based *in vitro* differentiation aggregates were transferred to 6 well ultra-low attachment plates (Corning) in differentiation medium consisting of 80 % IMDM (Invitrogen) supplemented with 20 % fetal calf serum (HyClone), 1 mM L-glutamine, 0.1 mM β-Mercaptoethanol, and 1 % nonessential amino acid stock (all Invitrogen). After 1 week, aggregates were plated on 0.1 % gelatin coated 6 well plates and further cultured for 2 weeks in differentiation medium thereby applying media change every 3 – 4 days.

Directed differentiation into cardiomyocytes was performed as described previously [2, 3]. In brief, differentiation was conducted in RPMI medium supplemented with B27 (without insulin; Life Technologies) by addition of 7.5 µM CHIR99021 for 24h followed by WNT pathway inhibition using 5 µM IWP2 (Tocris) for 48h. The medium was refreshed every two days. Cells were kept in RPMI supplemented with B27 (with insulin) from day 7 onwards. Differentiation efficiency was quantified by flow cytometry for cardiomyocyte specific markers as described above.

Generation of cryosections

Bioreactor-expanded aggregates were embedded in Tissue-Tek (Sakura-Finetek). Subsequently, 10 µm cryosections were generated with a microm cryostar HM 560 (Thermo Scientific) cryotome.

Immunocytological staining

Immunostaining was performed as described previously [4, 5]. In brief, cells were fixed with 4 % paraform aldehyde at room temperature for 10 min and 5 min, respectively. After blocking with 5 % donkey serum and 0.25 % Triton X-100 (Sigma Aldrich) in Tris-buffered saline for 20 min at room temperature, cells were incubated with respective monoclonal primary antibodies (TRA 1-60: 1:100, mouse IgM, Abcam; SSEA3: 1:100, mouse IgM, DSHB; OCT4: 1:4, mouse IgG2b, Santa Cruz Biotechnology; NANOG: 1:100, rabbit IgG, Cell Signaling Technology; Ki67: 1:50, mouse IgG1, Dako; α-Fetoprotein: 1:300, mouse IgG1, R&D Systems; β3-Tubulin: 1:400, mouse IgG2a, Upstate; sarcomeric

actinin: 1:80, mouse IgG1, Sigma-Aldrich; cardiac Troponin T: 1:200, mouse IgG1, Thermo Scientific). After washing plated aggregates or cryosection were incubated with secondary antibodies (1:300 Cy™5-labeled donkey anti-mouse IgM for TRA 1-60, SSEA3, sarcomeric actinin und cardiac Troponin T; 1:300 Alexa Fluor 488 donkey anti-mouse IgG for OCT4, Ki67, α-Fetoprotein and β3-Tubulin; 1:300 Cy™3-labeled donkey anti-rabbit IgG for NANOG; all Jackson Immunoresearch Laboratories) for 30 min at room temperature. After washing, cells were counterstained with DAPI (Sigma-Aldrich) for 15 min, and analyzed via Axio Oberserver A1 fluorescence microscope (Zeiss).*Real-time polymerase chain reaction*

Total RNA isolation using TriZol (Invitrogen) and the RNeasy Kit (Macherey-Nagel) as well as reverse transcription via RevertAid™ H Minus (Fermentas, Thermo Scientific) using random primers were performed according to manufacturer's instructions. Subsequently, quantitative real time PCR was performed as described previously [6]. In brief, the quantitative real-time PCR was performed using the Absolute qPCR SYBR Green Mix (ABgene) and a Mastercycler ep realplex instrument (Eppendorf). Reaction mixtures contained 5 µL cDNA template, 6.25 µL water, 12.5 µL mastermix and 1.25 µL of primers. Sequences and specifications of primers are given in Supplemental Table 3 in [7]. Quantitative real-time PCR conditions included enzyme activation at 95 °C for 15 min, followed by 40 cycles (95 °C for 15 sec, 60 °C for 60 sec), and a final 10 °C step. Absence of nonspecific product was controlled by melting curves. Expression levels of target genes were normalized to β-actin.

Microarray analysis

Microarray analysis was performed using a refined version of a Whole Human Genome Oligo Microarray 4x44K v2 covering roughly 26000 transcripts (design ID 054261, Agilent Technologies). Arrays were hybridized with Cy3-labeled cRNA and scanned on the Agilent Micro Array Scanner G2565CA as described previously [3]. Principal component analysis and heat map generation was performed using Qlucore Omics Explorer 3.0 (Qlucore AB). Differential expression (2-fold increase; intensity threshold 50) in repeated batch and perfusion bioreactors on day 7 as well as expression

levels of genes associated with pluripotency and early differentiation were identified and processed using the RCUTAS filter tool (Research Core Unit Transcriptomics of Hannover Medical School). Gene ontology analysis was performed using the Gorilla web tool by comparing the identified genes lists to the background gene list (http://cbl-gorilla.cs.technion.ac.il).

Data in the lower part of Figure 6B and Supplemental Figure 6A were normalized as

$$log2\ (\frac{MFI_{bioreactor}}{MFI_{2D\ preculture}})$$

where $MFI_{bioreactor}$ and $MFI_{2D\ preculture}$ are the median fluorescence intensities [AU] of bioreactor and 2D preculture samples, respectively.

Multi-Analyte Profiling (MAP)

Multi-Analyte Profiling was performed using a multiplexed bead-based sandwich immunoassay. Samples were thawed at room temperature, vortexed, spun at 18,000 x g for 1 minute for clarification and required sample volumes were removed for MAP analysis. Supernatant samples, 8-point calibrators and controls were introduced into the capture microsphere multiplexes and incubated for 1h at room temperature followed by incubation of multiplexed cocktails of biotinylated reporter antibodies for an additional hour at room temperature. Subsequently, streptavidin-phycoerythrin solution was added in excess and incubated for 1h at room temperature. After vacuum filtration and dilution of samples, calibrators and controls, analysis was performed in a Luminex 100/200 instrument (Luminex Corporation Austin, Texas, USA). The resulting data stream was interpreted using proprietary data analysis software developed at Myriad RBM (Austin, Texas, USA). Unknown values for each of the analytes were determined using 4 and 5 parameter, weighted and non-weighted curve fitting algorithms included in the data analysis package. Kit components of the bead-based multiplexed sandwich immunoassays used were kindly provided by Myriad RBM.

1. Zweigerdt R, Olmer R, Singh H, et al. Scalable expansion of human pluripotent stem cells in suspension culture. Nat Protoc 2011;6:689-700.

2. Kempf H, Kropp C, Olmer R, et al. Cardiac differentiation of human pluripotent stem cells in scalable suspension culture. Nat Protoc 2015;10:1345-1361.
3. Kempf H, Olmer R, Kropp C, et al. Controlling expansion and cardiomyogenic differentiation of human pluripotent stem cells in scalable suspension culture. Stem Cell Rep 2014.
4. Haase A, Olmer R, Schwanke K, et al. Generation of induced pluripotent stem cells from human cord blood. Cell Stem Cell 2009;5:434-441.
5. Olmer R, Lange A, Selzer S, et al. Suspension culture of human pluripotent stem cells in controlled, stirred bioreactors. Tissue Eng Part C Methods 2012;18:772-784.
6. Mauritz C, Schwanke K, Reppel M, et al. Generation of functional murine cardiac myocytes from induced pluripotent stem cells. Circulation 2008;118:507-517.
7. Olmer R, Haase A, Merkert S, et al. Long term expansion of undifferentiated human iPS and ES cells in suspension culture using a defined medium. Stem Cell Res 2010;5:51-64.

Supplemental Figures

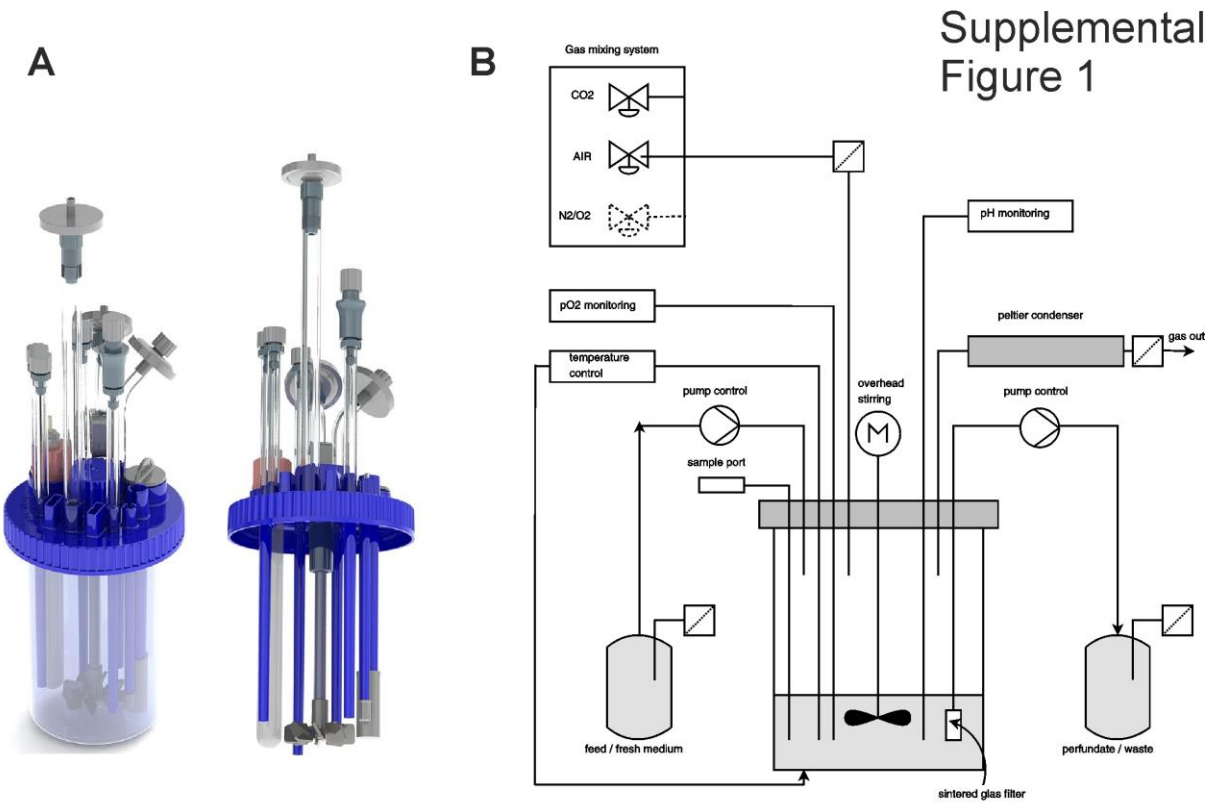
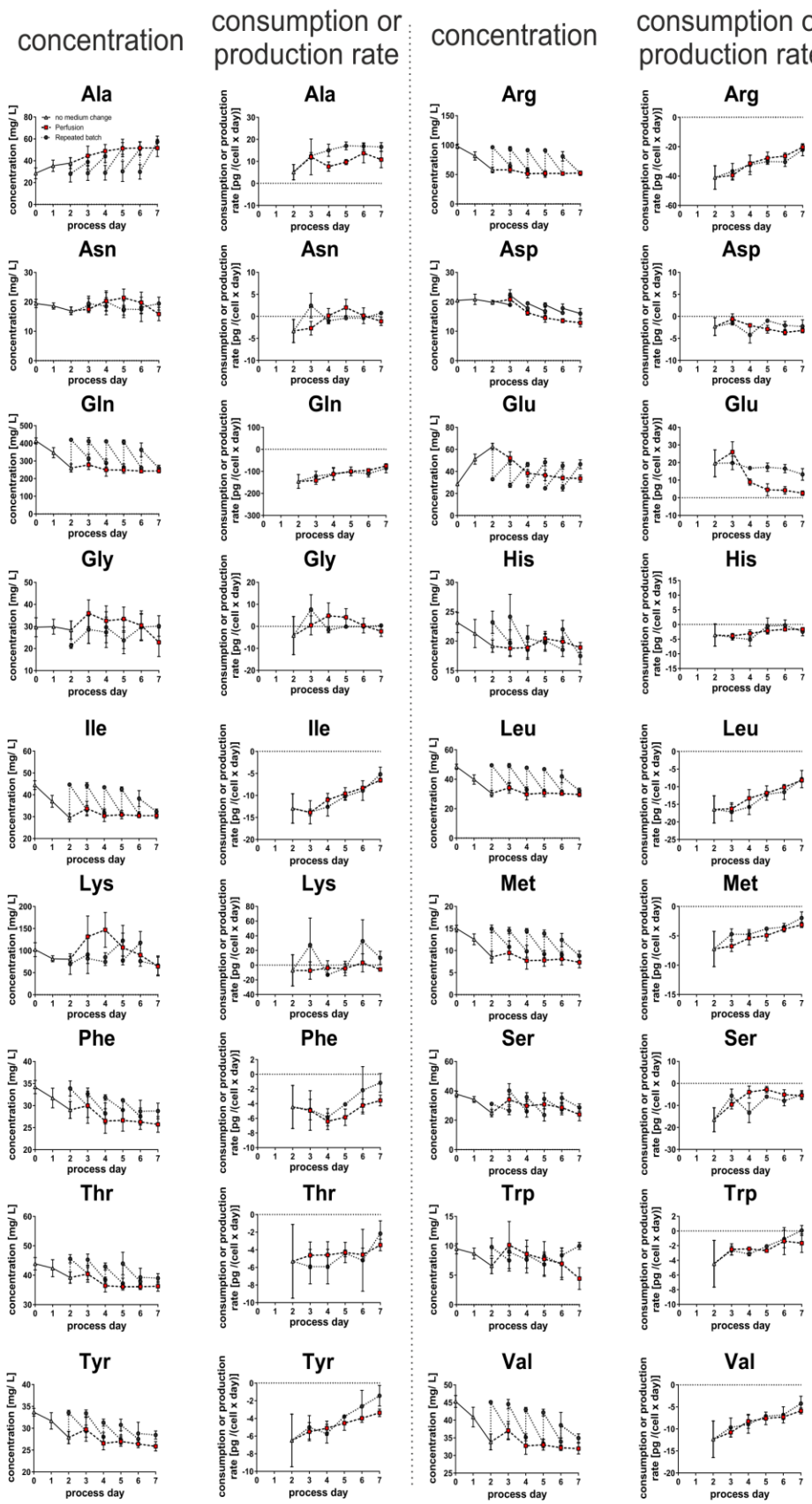


Figure S1: Bioreactor setup. BioBLU 0.3 Single-Use Vessels in 100 - 250 mL scale from Eppendorf AG equipped with an 8-blade pitched impeller optimized for expansion of human pluripotent stem cells (A). The feeding circuit for perfusion bioreactors consisted of a feed line connected to a fresh medium reservoir, a waste line connected to a waste bottle, a porous filter used as retention system placed at the outflow (waste) medium stream, and peristaltic pumps for continuous pumping. Besides the 8 blade pitched impeller the BioBLU Single-Use Vessel was equipped with temperature sensor, dissolved oxygen (DO)- and pH-electrodes, various connections for liquid addition and a sample port. Temperature control of the bioreactor as well as exhaust condensation was performed liquid-free via Peltier technology. A magnetic coupled overhead drive was used for stirring. Gassing of air and CO_2 was performed via the head space utilizing a mass flow-controlled gas mixing system (B). Aggregate diameters were determined by AxioVision LE (Zeiss), here exemplarily shown for d5 aggregates from perfusion culture (C).



Supplemental Figure 2

Figure S2: Analysis of the amino acid metabolism in repeated batch and perfusion cultures (hCBiPS2). Amino acids concentrations as well as specific amino acid consumption or production rates for repeated batch and perfusion cultures using hCBiPS2 cells. Positive rates indicate the production and negative rates the consumption of a specific amino acid, respectively. Samples were taken every 24 h of culture and additionally after medium exchange for repeated batch cultures (n=4). Due to the cell loss from d0 to d1 in our expansion processes consumption or production rates are not shown for this early process phase.

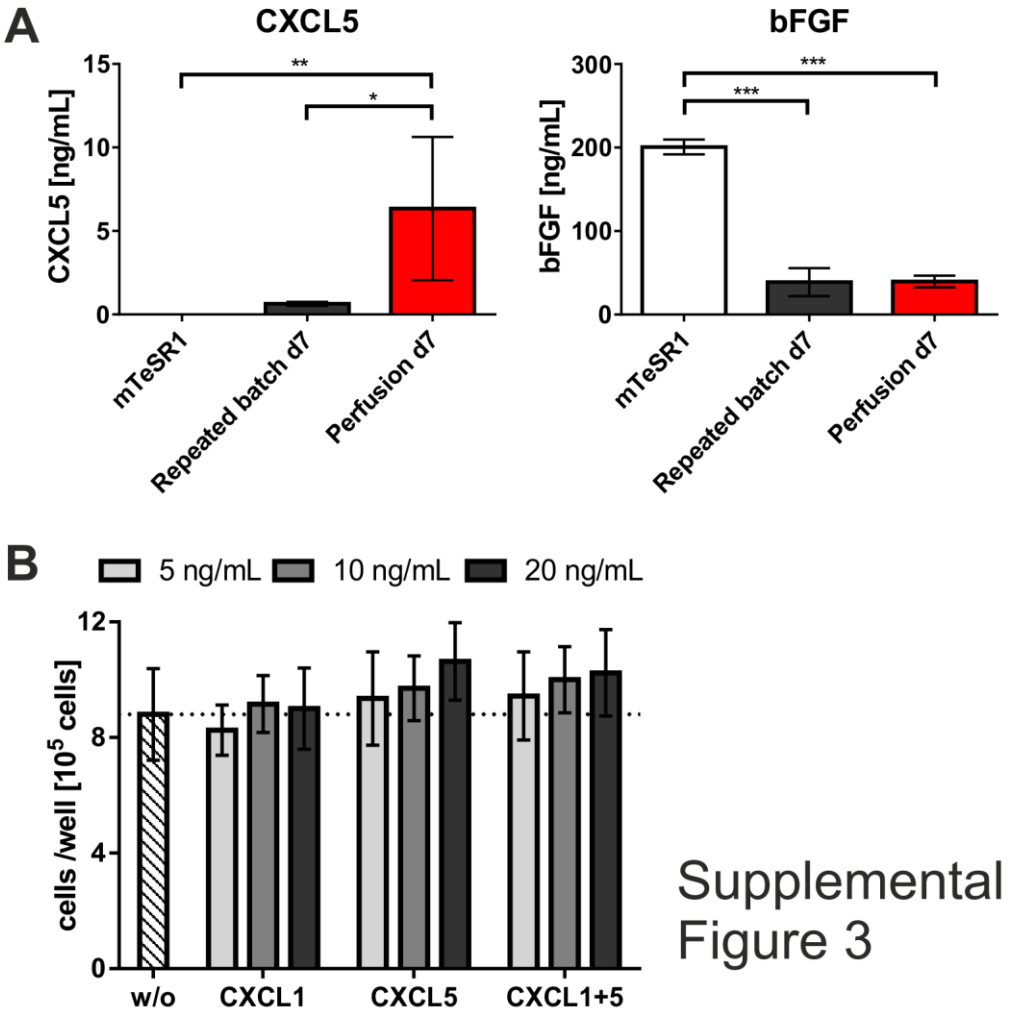


Figure S3: Growth factor and cytokine concentrations in repeated batch and perfusion cultures. Multi analyte profiling revealed an accumulation of CXCL5 in d7 perfusion cultures compared to d7 repeated batch cultures and fresh culture medium whereas no difference between the both feeding strategies could be observed for bFGF. Results are reported as mean ± SEM. Differences were considered statistically significant at *p<0.05, **p<0.01 and ***p<0.001 (A). hCBiPS2 cells were seeded as single cell suspensions in ultra-low attachment 6 well suspension culture plates ($3.5x10^5$ cells/ 3 mL/ well) in mTeSR™ 1 + 10 µM Y-27632 and without (w/o) or supplemented with 5 – 20 ng/ mL CXCL1 or CXCL5 or both. Medium was exchanged on day 2 including respective cytokines. Cell numbers per well after 3 days were determined by counting the number of viable cells after aggregate dissociation (n = 4) (B).

Supplemental Figure 4

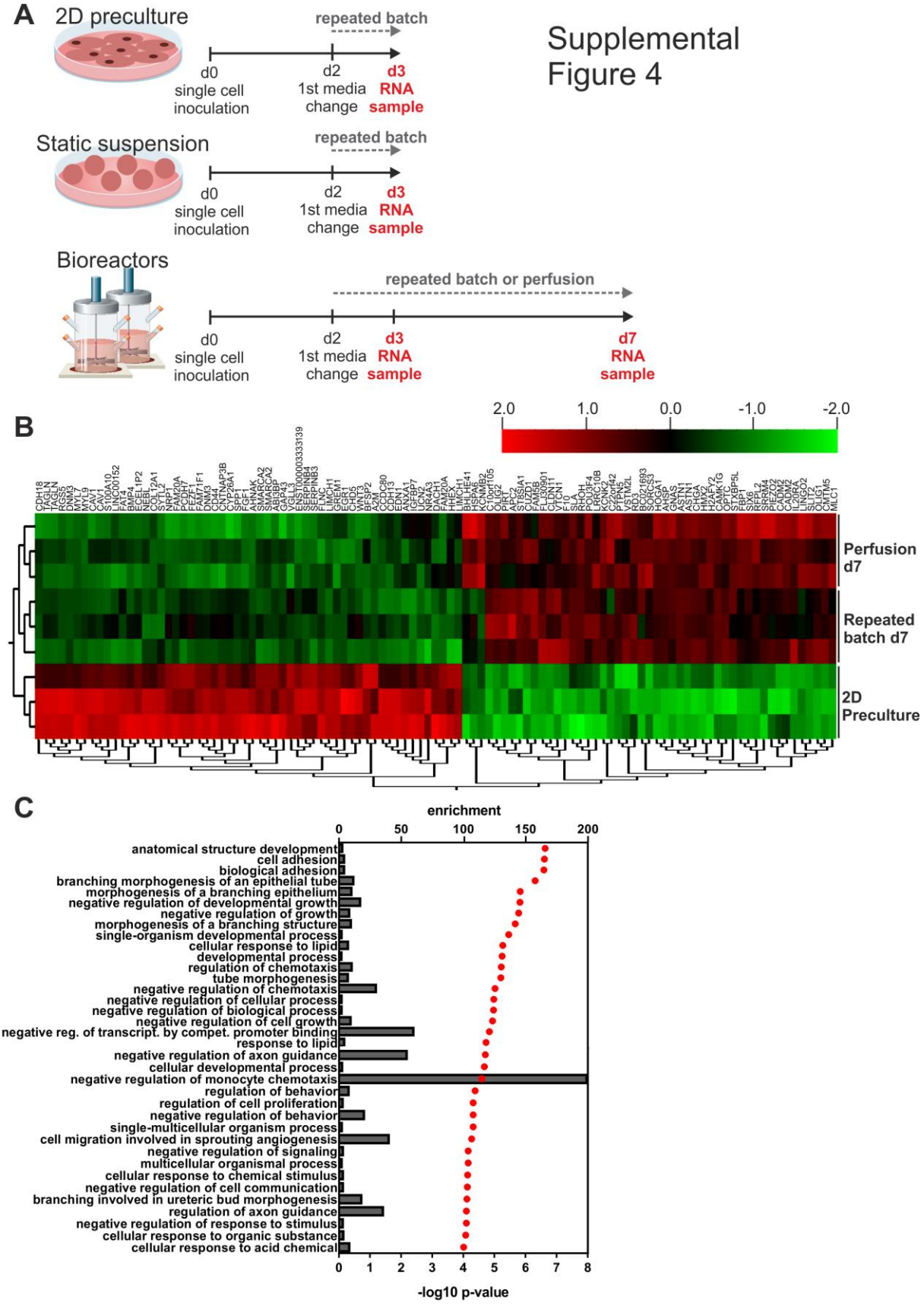
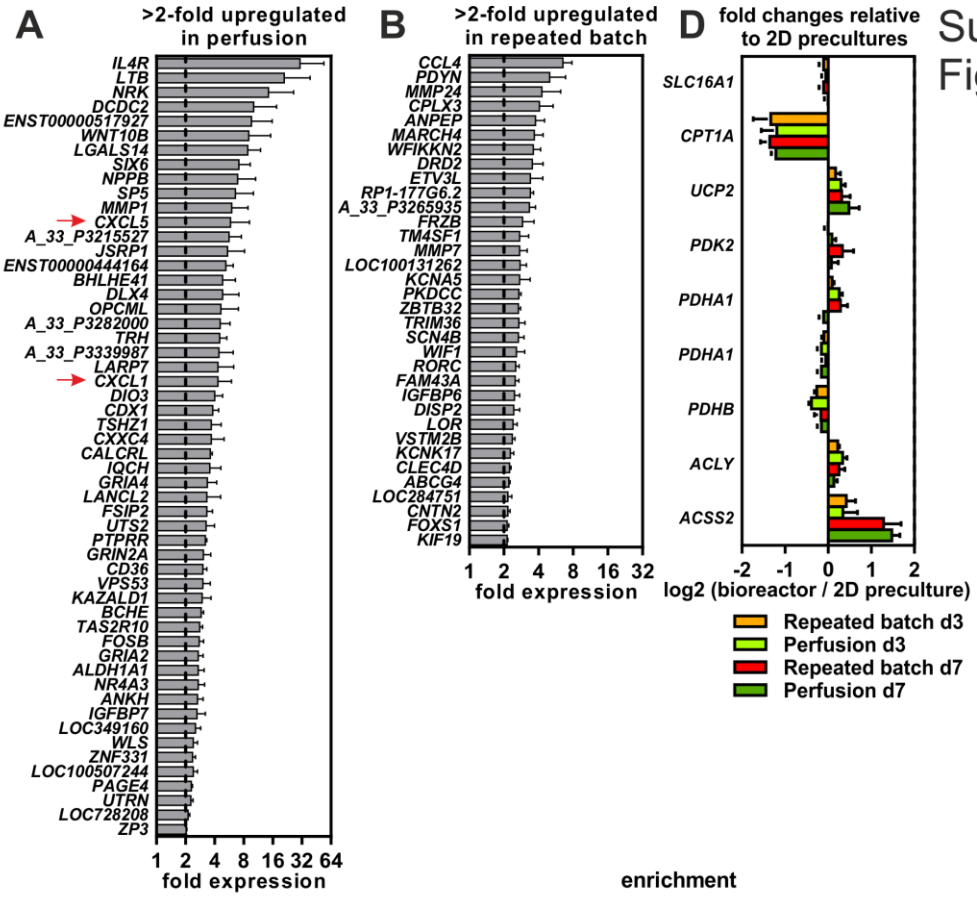


Figure S4: Microarray analysis and gene ontology enrichment analysis comparing gene expression of 2D precultures, 3D static suspension culture and 3D bioreactor cultures for hCBiPS2 cells. RNA samples for global gene expression analysis were taken from 2D feeder-free precultures and 3D static suspension cultures after 3 days as well as from 3D dynamic suspension cultures (bioreactors) after 3 and 7 days of culture (A). Heat maps displaying differentially expressed genes in d7 repeated batch and perfusion bioreactor-derived aggregates compared to 2D precultures. Genes were identified by two and multiple group comparison, respectively ($p \leq 0.01$; $\sigma/\sigma_{max} < 0.255$; fold change cut-off 2.0 in A; $p \leq 0.005$; $\sigma/\sigma_{max} < 0.28$ in B). Repeated indications of genes represent multiple probes for the same gene (B). Significant over-represented GO terms with respective enrichment (grey bars) and negative decadic logarithm of enrichment p-values (red dots) associated with >2-fold regulated genes in 2D precultures compared to d7 repeated batch- and perfusion-bioreactor cultures (C). The microarray dataset presented in this figure was deposited in Array Express with the accession number E-MTAB-3898 (http://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-3898).



Supplemental Figure 5

Figure S5: Microarray analysis comparing gene expression of 2D precultures, 3D static suspension- and 3D repeated batch and perfusion bioreactor d7-derived hCBiPS2-aggregates. Ranked diagrams display >2-fold upregulated genes in d7 perfusion and repeated batch cultures (A,B). Gene ontology enrichment analysis on >2-fold upregulated genes in perfusion compared to repeated batch d7 cultures. Negative decadic logarithm of enrichment p-values (red dots) and significant over-represented GO terms with respective enrichment (grey bars) are displayed (n=3) (C). Similar gene expression levels of SLC16A1, UCP2, PDK2, PDHA1, PDHB and ACLY in 2D precultures and bioreactor samples was detected. Only ACSS2, which is responsible for Ac-CoA formation, shows an increase for repeated batch and perfusion bioreactor-derived aggregates on day 7 compared to 2D precultures whereas CPT1A is decreased in the all bioreactor conditions compared to 2D precultures (n=3). Repeated indications of genes represent multiple probes for the same gene (D). The microarray dataset presented in this figure was deposited in Array Express with the accession number E-MTAB-3898 (http://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-3898).

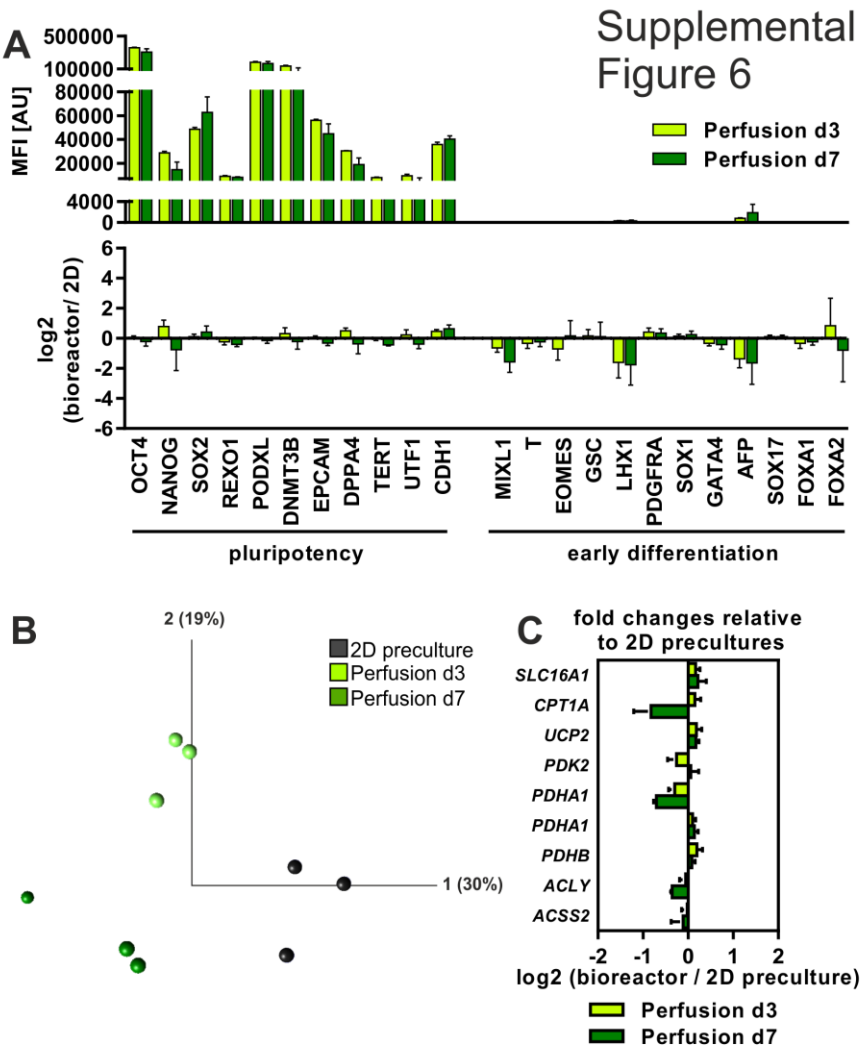

Supplemental Figure 6
A
MFI [AU]
500000
100000
80000
60000
40000
20000
4000
0
Perfusion d3
Perfusion d7
log2 (bioreactor/ 2D)
6
4
2
0
-2
-4
-6
OCT4
NANOG
SOX2
REXO1
PODXL
DNMT3B
EPCAM
DPPA4
TERT
UTF1
CDH1
MIXL1
T
EOMES
GSC
LHX1
PDGFRA
SOX1
GATA4
AFP
SOX17
FOXA1
FOXA2
pluripotency
early differentiation
B
2 (19%)
1 (30%)
2D preculture
Perfusion d3
Perfusion d7
C
fold changes relative to 2D precultures
SLC16A1
CPT1A
UCP2
PDK2
PDHA1
PDHA1
PDHB
ACLY
ACSS2
-2
-1
0
1
2
log2 (bioreactor / 2D preculture)
Perfusion d3
Perfusion d7

Figure S6: Microarray analysis comparing gene expression of 2D preculture, and perfusion bioreactor-derived aggregates for hHSC_F1285T_iPS cells. Expression levels of genes associated with pluripotency and early differentiation for perfusion-expanded hHSC_F1285T_iPS2 cells harvested at process endpoint d7 (n=3). Data are displayed as absolute processed fluorescence intensity (top) and normalized to 2D precultures (bottom) (A). Principal component analysis (PCA) of the processed microarray data. Each dot represents an independent sample of hHSC_F1285T_iPS2 cells in 2D preculture on day 3 (grey), and in perfusion cultures on day 3 (light green) or on process endpoint day 7 (dark green). Visualization comprises >100 genes with $\sigma/\sigma max<0.255$ and $p<0.001$ in a group comparison (B). A subset of genes described to be involved in metabolism-driven early differentiation processes. Expression is displayed as fold changes relative to 2D precultures. Repeated indications of genes represent multiple probes for the same gene (C). The microarray dataset presented in this figure was deposited in Array Express with the accession number E-MTAB-4149 (http://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-4149).

Table S1: Perfusion in 100 mL bioreactor scale results in ~43 % less medium consumption compared to conventional hPSC culture in 2D. Conservative assumptions (based on our experience; data not shown) suggest that seeding of $0.3x10^6$ cells/ 10 cm dish (~60 cm^2 growth area) enables the production of up to $10x10^6$ hPS cells/ dish (~30-fold expansion per passage, 7 days) thereby consuming about 50 mL medium. Thus, the exemplary production of a batch of 10^8 hPS cells requires 500 mL medium consumption in such conventional 2D monolayer culture. The table reveals that production of an equivalent cell batch of 10^8 hPSC in the perfusion bioreactor approach developed in our study requires about 43% less medium; this calculation notably includes the medium consumption required for the preculture for bioreactor inoculation. Passage duration is 7 days for all vessel types and culture formats.

Vessel type & Culture format	media consumed for preculture [mL]	cell seeding [cells/ vessel]	media consumed per passage [mL]	fold expansion per passage	cell yield [cells/ vessel]	vessels per 10^8 cells	media consumed per 10^8 cells [mL]	reduction of media consumption per 10^8 cells [%]	efficiency index [cells produced / mL]
10 cm dish 2D-monolayer	~0	$0.3x10^6$	50	~30	$0.1x10^8$	10	500	NA	$2.0x10^5$
Bioreactor 3D repeated batch	190	$50x10^6$	625	~4	$1.94x10^8$	0.52	420	15.98	$2.4x10^5$
Bioreactor 3D perfusion	190	$50x10^6$	625	~6	$2.85x10^8$	0.35	286	42.81	$3.5x10^5$