Supplementary Information

Vero cells gain renal tubule markers in low-calcium and magnesium chemically defined media

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Supplementary Table S1. List of compounds that were tested in the Plackett-Burman experiments to formulate a suspension media for Vero cells. A review of journal articles and patents for serum-free mammalian media was conducted to create a design space for media development.

Amino Acids	Metals	Vitamins	Lipids, Fatty acids and Steroids	Other
Arginine	$CaCl_2$	Ascorbic acid	Cholesterol	Glucose
Asparagine	$CoCl_2$	α-tocopherol	Arachondic Acid	Bicarbonate
Aspartate	$CuCl_2$	Choline Chloride	Myristic Acid	rEGF
Cysteine	Fe·Citrate	D-Pantothenate ½ Calcium	Linoleic Acid	IGF-1
Glutamine	$MnSO_4$	D-Biotin	Linolenic Acid	Dextran Sulfate
Isoleucine	$MgCl_2$	Folic Acid	Oleic Acid	Poloxamer 188
Leucine	$MgSO_4$	Myo-inositol	Palmitic Acid	Ethanolamine
Lysine	$NaSiO_3$	Nicotinic Acid	Stearic Acid	Adenine
Methionine	Na_2SeO_3	Niacinamide	Tween-80	Guanosine
Ornithine	$NiSO_4$	Pyridoxine	Hydrocortisone	Hypoxanthine
Serine	$(NH_4)_6Mo_7O_{24}$	Thiamine	Dexamethasone	Thymidine
Threonine	$SnCl_2$	Vitamin B_{12}		Uridine
Tryptophan	V_2O_5			Glutathione
				Putrescine

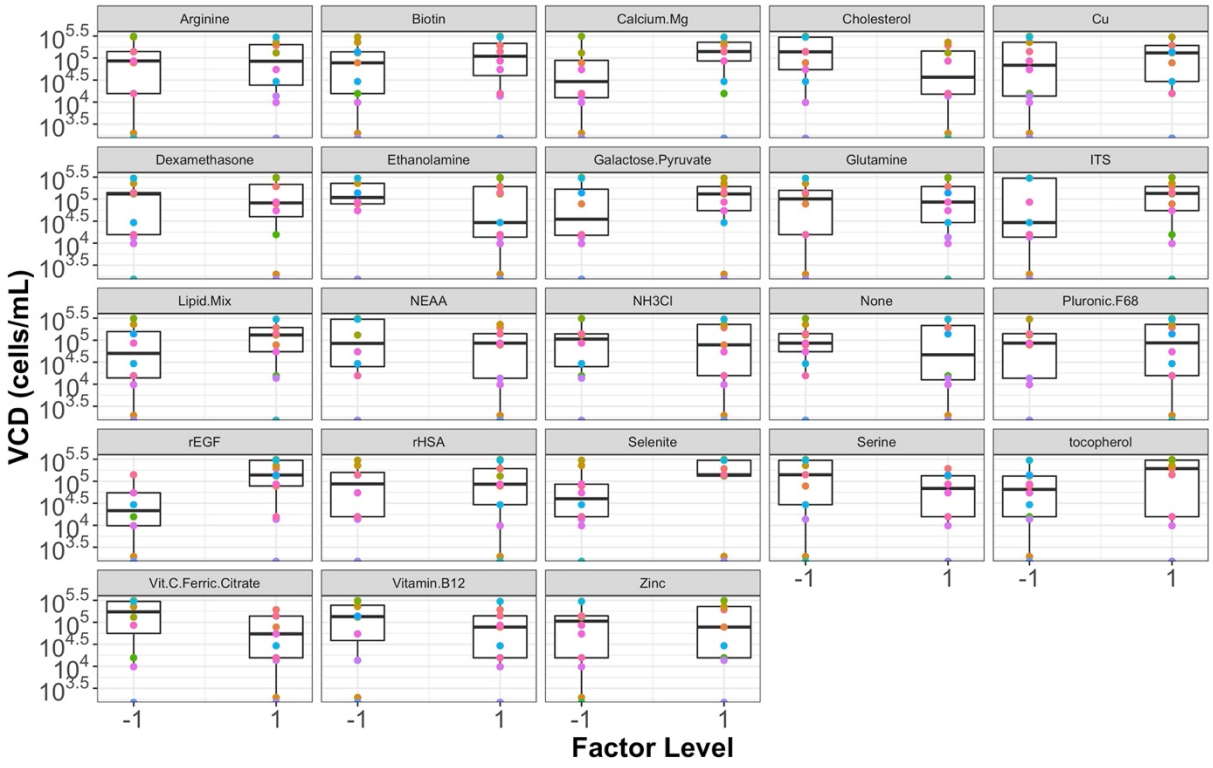
Supplementary Table S2 Summary of RNA-seq data collected of Vero cell lines in different conditions. *Con: control cells in FBS containing media. # Adh: adherent Vero cells in defined media. $ Sus: suspension Vero cells in defined media

Sample:	Con1*	Con2	Con3	Con-4	Con-5	Adh-1#	Adh-2	Adh-3	Adh-4	Sus-1$	Sus-2	Sus-3	Sus-4
Input reads (in mil)	20.6	20.5	21.6	20.4	20.7	22.1	15.0	22.0	23.5	21.4	20.9	18.6	22.8
Uniquely Aligned (in mil)	19.9	19.8	20.8	19.7	19.8	21.3	14.5	21.1	22.4	20.6	20.1	17.9	21.9
Uniquely mapped reads %	96.17%	96.24%	96.15%	96.17%	96.04%	96.17%	96.49%	96.44%	95.40%	96.03%	96.18%	96.20%	96.16%
Number of reads mapped to multiple loci	692,601	682,474	732,797	686,586	711,826	731,493	444,772	658,007	909,751	732,510	684,189	603,512	758,962
% of reads mapped to multiple loci	3.35%	3.33%	3.38%	3.35%	3.45%	3.31%	2.96%	3.00%	3.87%	3.42%	3.27%	3.25%	3.33%
Detected genes	14,656	14,670	14,723	14,610	14,462	14,809	14,433	14,782	14,775	14,650	14,633	14,539	14,711

S3	Excel Sheet	CPM values after filtering
S4-S7	Excel Sheet	DEG analysis results
S8	Excel Sheet	GSEA of GO BP gene sets
S9	Excel Sheet	GSEA of renal tubule specific gene sets
S10	Excel Sheet	GSEA of transcription factor specific gene sets

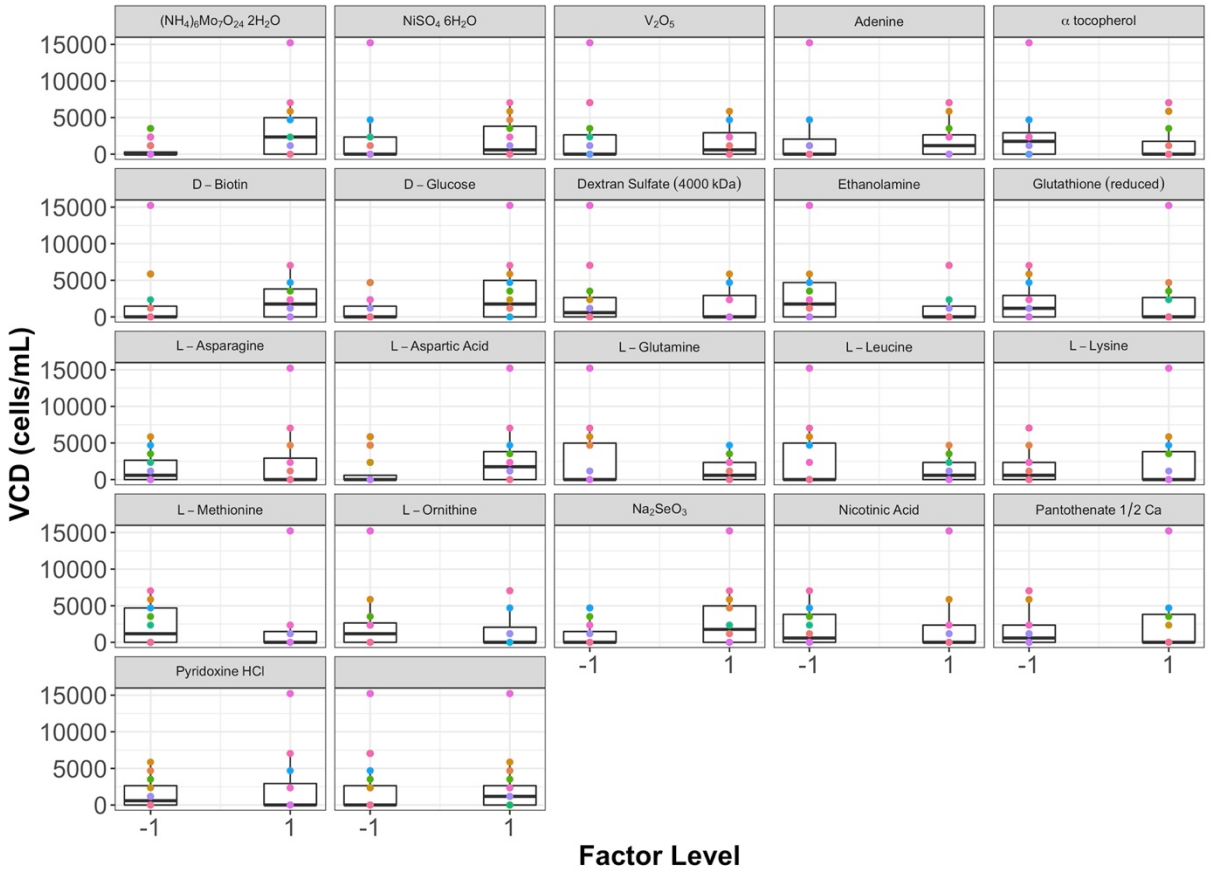
Supplementary Figures

Supplementary Figure S1. Plackett Burman screening experiment #1. The box plots that show the effect of each media component on viable cell density (VCD) for a Plackett-Burman experiment with 23 factors in 24 runs. This experiment used a basal media with low calcium and magnesium to encourage suspension growth and spiked in components at various concentrations that are listed in the table to the right.



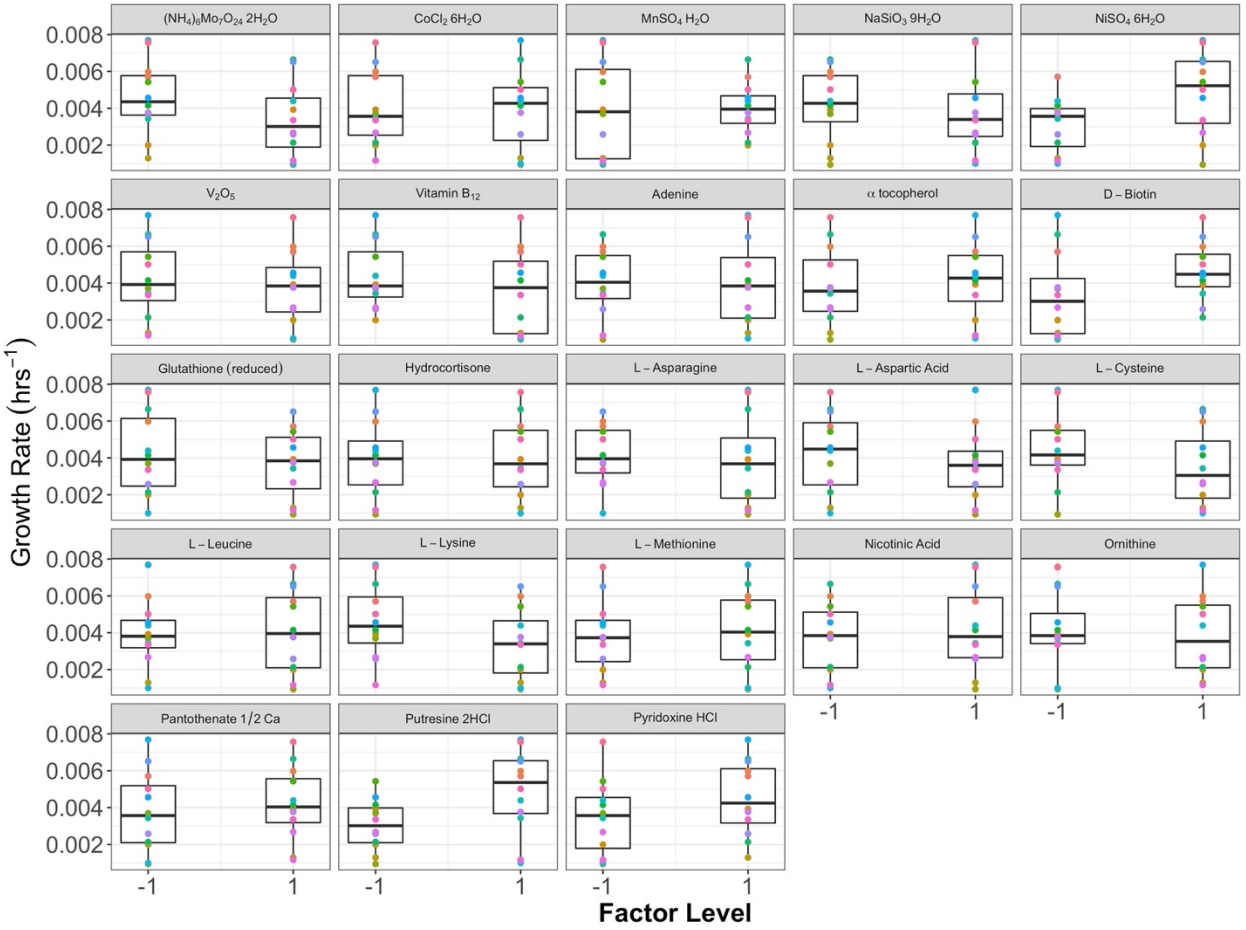
Compound		-1 (mM)	1 (mM)
L-Arginine		0.7	5.7
D- Biotin		0.00001	0.00011
Calcium + Magnesium	$CaCl_2$	0.1	1
	$MgCl_2$	0.03	0.3
	$MgSO_4$	0.18	1.8
Cholesterol Concentrate (250x)		5% (v/v)	15% (v/v)
Copper		5.2×10^{-6}	0.00052
Dexamethasone		0	0.001
Ethanolamine HCl		0	0.023
Galactose + Pyruvate	Galactose	0	5
	Pyruvate	0.5	1.5
L-Glutamine		2.5	4
ITS		0	10% (v/v)
Lipid Mix		0	10% (v/v)
NEAA		0	10% (v/v)
NH_3Cl		0	4
None		0	0
Pluronic F68		0	0.02 % (w/v)
rEGF		0	0.00002
rHSA		0	0.0025 g/mL
Selenite		0	0.0003
L-Serine		0.25	2.25
α-tocopherol acetate		1.2×10^{-5}	3.2×10^{-4}
Vitamin C + Ferric Citrate	Vitamin C	0	0.1
	Ferric Citrate	0	0.3
Vitamin B_{12}		0.0005	0.001
Zinc ($ZnCl_2$)		0.0015	0.0055

Supplementary Figure S2. Plackett Burman screening experiment #2. The box plots that show the effect of each media component on viable cell density (VCD) for a Plackett-Burman experiment with 23 factors in 24 runs. This experiment used a basal media with low calcium and magnesium to encourage suspension growth and spiked in components at various concentrations that are listed in the table to the right.



Compound	-1 (mM)	1 (mM)
D-Glucose	17.5	25
L-Glutamine	2.5	5
L-Ornithine HCl	0.3	0.65
L-Glutamic Acid	0.05	0.65
L-Leucine	0.5	2.81
L-Lysine HCl	0.5	2.74
L-Methionine	0.1	0.77
Pyridoxine HCl	0.00015	0.00079
L-Aspartic Acid	0.05	0.30
L-Asparagine H_2O	0.05	0.27
$NiSO_4$ $6H_2O$	$4.21x10^{-7}$	$9.26x10^{-7}$
$(NH_4)_6Mo_7O_{24}$ $4H_2O$	$1.00x10^{-6}$	$5.00x10^{-6}$
V_2O_5	$5.5x10^{-5}$	$7.5x10^{-6}$
Na_2SeO3	$3.00x10^{-5}$	$3.00x10^{-4}$
Nicotinic Acid	0.00406	0.00162
D-Biotin	$1.43x10^{-5}$	$5.32x10^{-5}$
α-tocopherol acetate	0.000296	0.000381
Pantothenate ½ Calcium	0.0168	0.0621
Putrescine 2HCl	0.000503	0.00621
Adenine	0	0.00127
Ethanolamine HCl	0.022	0.041
Glutathione (reduced)	0	0.00325
Dextran Sulfate (4,000 kDa)	0	0.03 g

Supplementary Figure S3. Results from Plackett-Burman experiment #3. The box plots that show the effect of each media component on growth rate for a Plackett-Burman experiment with 23 factors in 24 runs. This experiment used a basal media with low calcium and magnesium to encourage suspension growth and spiked in components at various concentrations that are listed in the table to the right.

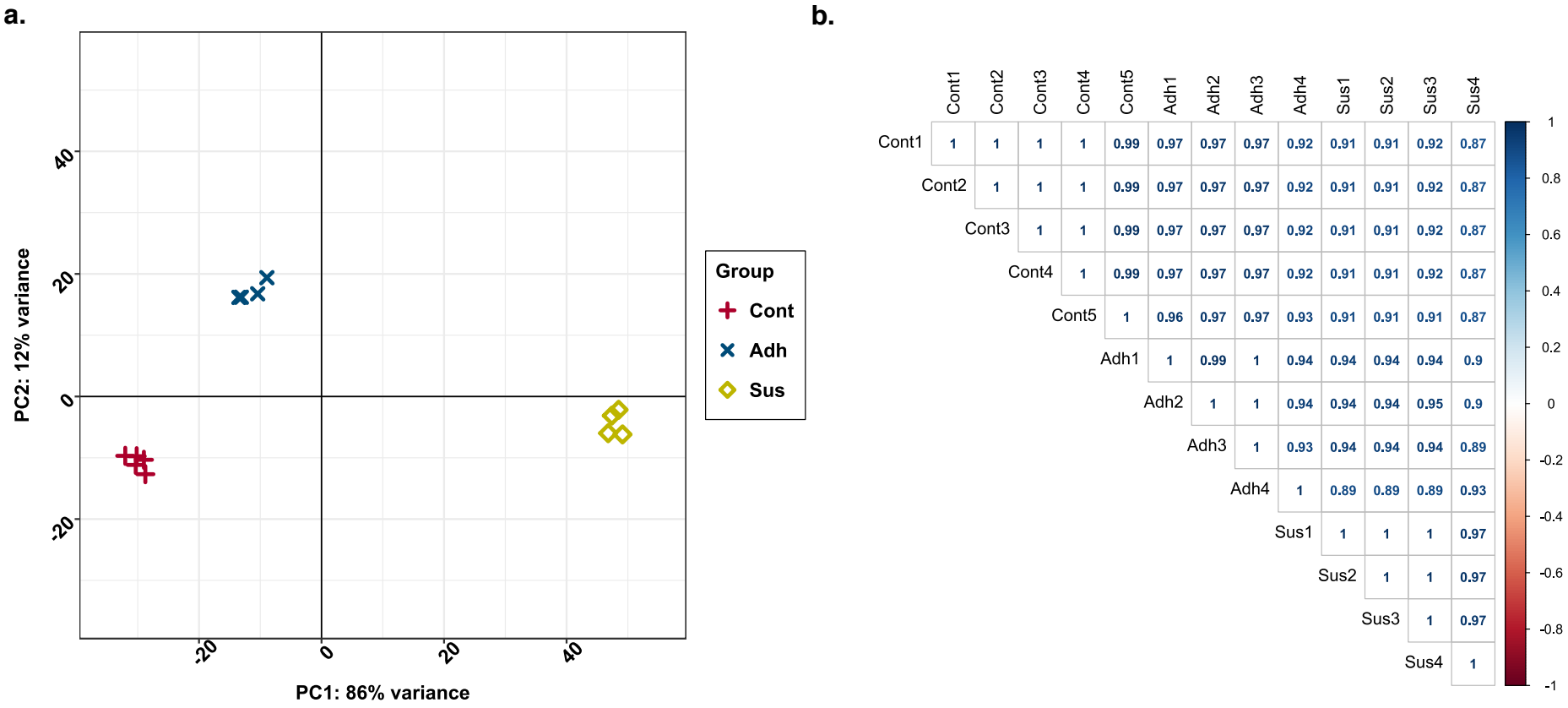


Compound	-1 (mM)	1 (mM)
$(NH_4)_6Mo_7O_{24}$ $2H_2O$	5.0 x 10^{-6}	5.5 x 10^{-6}
$CoCl_2$ $6H_2O$	4.2 x 10^{-6}	8.0 x 10^{-6}
$MnSO_4$ H_2O	0.9 x 10^{-6}	1 x 10^{-6}
$NaSiO_3$ $9H_2O$	54 x 10^{-6}	60 x 10^{-6}
$NiSO_4$ $6H_2O$	0.9 x 10^{-6}	1 x 10^{-6}
V_2O_5	5.5 x 10^{-6}	5.7 x 10^{-6}
Vitamin B_{12}	0.4 x 10^{-6}	4.0 x 10^{-6}
Adenine	1.3 x 10^{-3}	1.4 x 10^{-3}
α-tocopherol	3.8 x 10^{-4}	4.3 x 10^{-4}
D- Biotin	5.3 x 10^{-5}	5.9 x 10^{-5}
Glutathione (reduced)	3.3 x 10^{-3}	3.6 x 10^{-3}
Hydrocortisone	0	2.8 x 10^{-8}
L-Asparagine	0.27	0.53
L-Aspartic Acid	0.30	0.60
L- Cysteine	0.16	0.32
L-Leucine	0.45	0.48
L-Lysine	2.74	2.76
L-Methionine	0.12	0.17
Nicotinic Acid	4.1 x 10^{-3}	4.3 x 10^{-3}
Ornithine	0.65	0.72
Pantothenate ½ Calcium	0.046	0.051
Putrescine 2HCl	5.0 x 10^{-4}	5.2 x 10^{-4}
Pyridoxine HCl	1.5 x 10^{-4}	2.8 x 10^{-4}

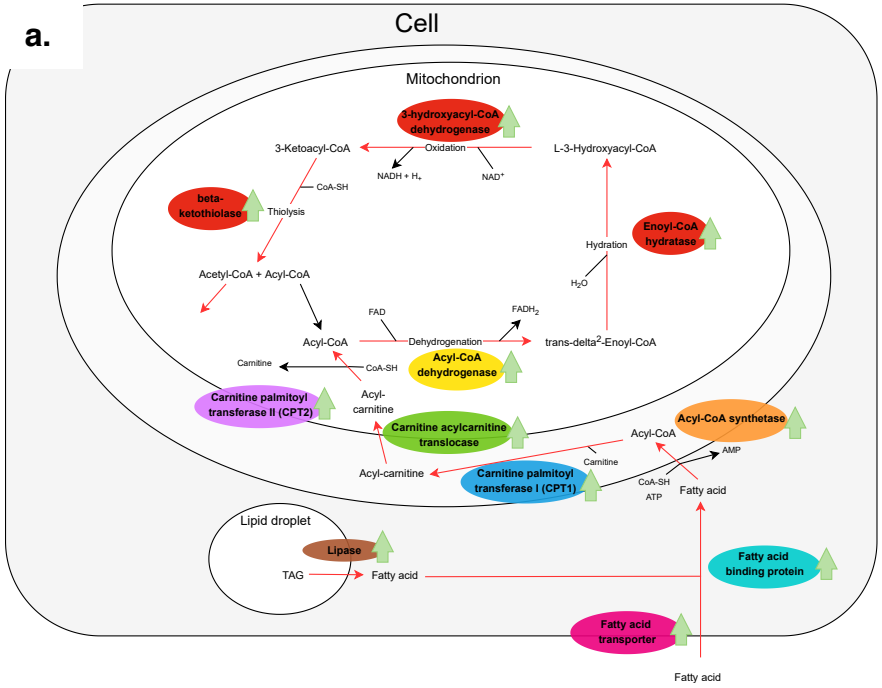
Supplementary Figure S4. Results from Plackett-Burman experiment #4. The box plots that show the effect of each media component on growth rate for a Plackett-Burman experiment with 23 factors in 24 runs. This experiment used a basal media with low calcium and magnesium to encourage suspension growth and spiked in components at various concentrations that are listed in the table to the right.

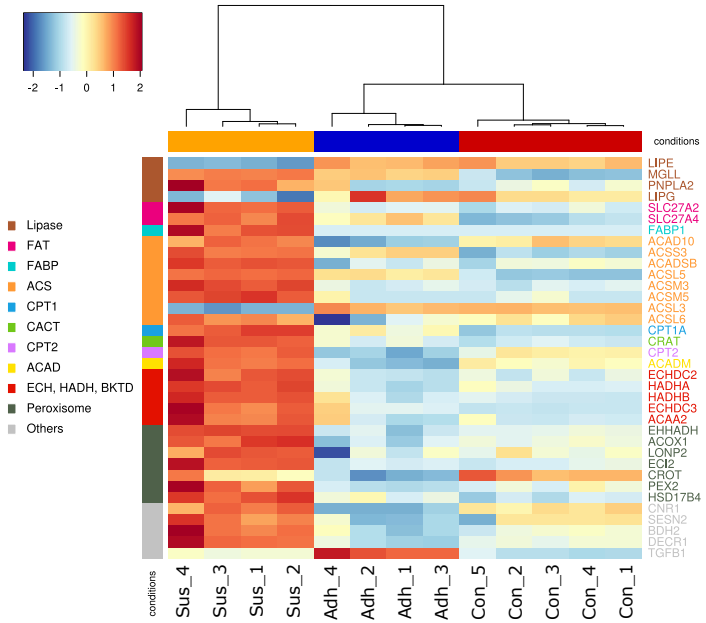
Compound		-1 (mM)	1 (mM)
$CoCl_2$ 6H_2O		4.2×10^{-6}	12×10^{-6}
$NaHCO_3$		14.3	26.2
$NiSO_4$ 6H_2O		3.7×10^{-6}	6.2×10^{-6}
$SnCl_2$ 7H_2O		0	0.45×10^{-6}
100x HT Supplement	Hypoxanthine	0.1	0.2
	Thymidine	0.016	0.032
Adenine		2×10^{-3}	5.8×10^{-3}
α-tocopherol		5.4×10^{-4}	13.6×10^{-4}
Choline Chloride		0.06	0.10
D- Biotin		1.0×10^{-4}	3.8×10^{-4}
Folic Acid		6.0×10^{-3}	10.1×10^{-3}
Guanosine		2.5×10^{-3}	3.5×10^{-3}
Insulin-like Growth Factor (IGF)		0	1×10^{-5} gg/mL
ι-inositol		0.07	0.12
L- Arginine		0.70	1.11
L-Isoleucine		0.42	1.03
L-Leucine		0.76	2.14
L-Lysine		1.4	2.73
L-Methionine		0.67	1.34
L-Threonine		0.45	1.65
L-Tryptophan		0.04	0.51
Putrescine 2HCl		6.2×10^{-4}	95×10^{-4}
Thiamine		6.4×10^{-3}	7.1×10^{-3}
Uridine		5.1×10^{-4}	51×10^{-4}

Supplementary Figure S5. Analysis of RNA-seq data variance. **a.** two-dimensional plot of principal component analysis (PCA) for all 13 samples of the 500 most variable genes. Each symbol represents one sample. Group affiliation is shown in the legend on the right side. **b.** Pearson's correlation between all samples for 3122 detected housekeeping genes. Correlation coefficients *r* values are annotated, and colour coded according to the legend on the right side of the figure.

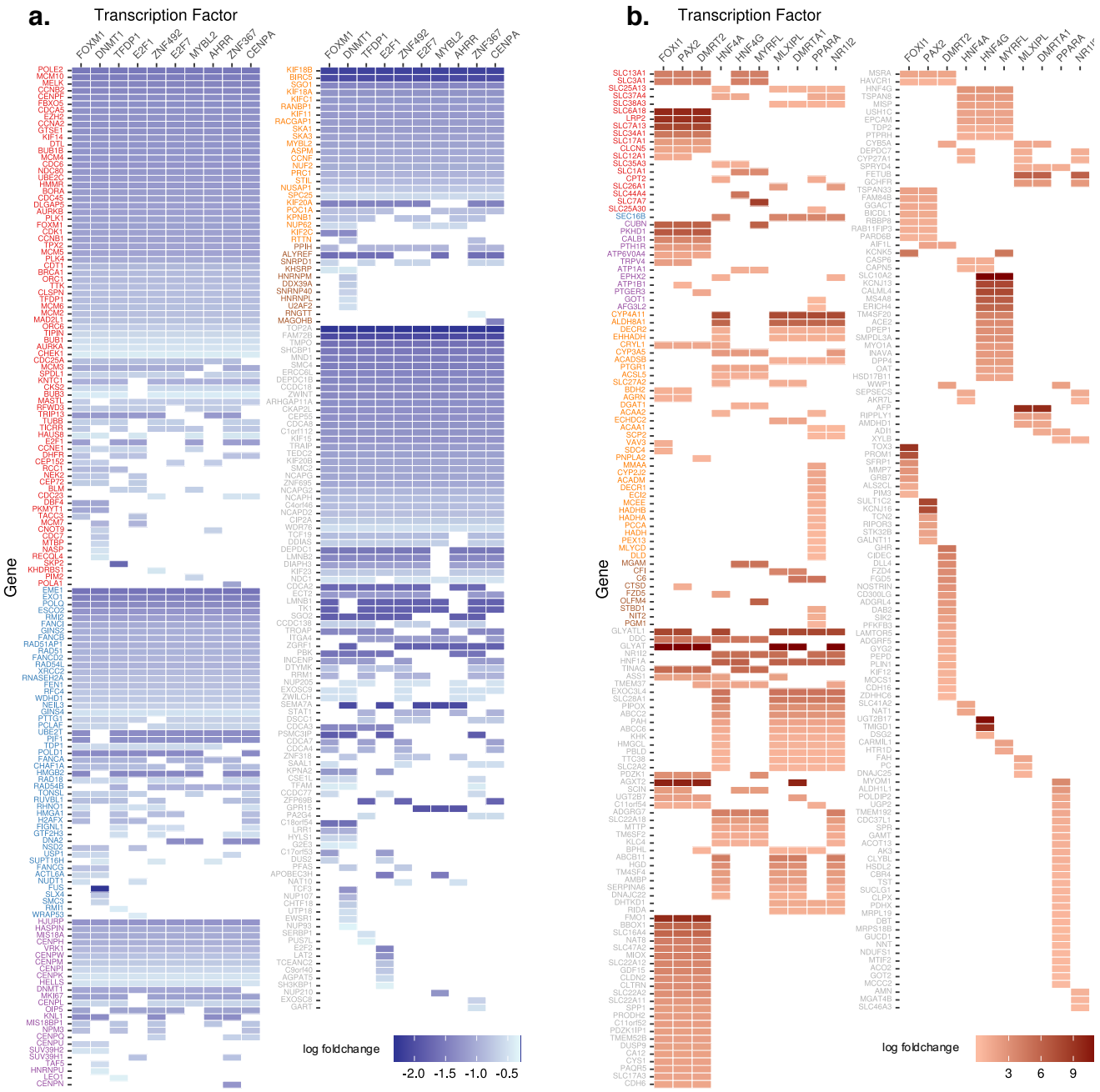


Supplementary Figure S6. Upregulation of fatty acid beta oxidation related genes (GO:0006635). **a.** Diagram of fatty acid beta oxidation with the main reactions and enzymes in mitochondria. Green arrows label significant upregulation of at least one gene in that reaction group with an FDR threshold of 0.001. Red arrows emphasize the fatty acid and derivatives flow through the process until Acetyl-CoA. **b.** heatmap of differentially regulated genes. The x-axis shows the samples, and the y-axis represents the HGNC symbols. Coloring of the samples is the same as in figure A. The coloring of the heatmap represents the standardized, normalized expression log2CPM values according to the legend at the top left side of each heatmap.





Supplementary Figure S7. Identification of directed gene expression changes caused by known transcription factors. Target gene sets were received from the GTEx dataset. The x-axis represents the regulatory transcription factor and the y-axis shows the identified target genes contributing to the leading edge of the GSEA. The color scheme of the heatmaps depicts the LFC of the identified genes for the contrast Sus vs. Adh_CDM2. **a.** target gene heatmap of the top ten transcription factors with the lowest normalized enrichment score in the GSEA. The colors of the gene labels refer to key gene sets of the identified, down-regulated clusters from Figure 2. The Gene ontology gene set names are the following: red: Cell cycle phase transition, blue: DNA repair, purple: Chromatin organization, orange: Microtubule cytoskeleton organization, brown: mRNA metabolic process. **b.** target gene heatmap of the top ten transcription factors with the highest normalized enrichment score in the GSEA. The colors of the gene labels refer to key gene sets of the identified, up-regulated clusters from Figure 2. The Gene ontology gene set names are the following: red: Anion transmembrane transport, blue: Endoplasmic reticulum to Golgi vesicle mediated transport, purple: Cellular ion homeostasis, orange: Cellular lipid metabolic process, brown: Leukocyte mediated immunity.


a.
Transcription Factor
FOXM1
DNMT1
TFDP1
E2F1
ZNF492
E2F7
MYBL2
AHRR
ZNF367
CENPA
Gene
log foldchange
-2.0
-1.5
-1.0
-0.5
b.
Transcription Factor
FOXI1
PAX2
DMRT2
HNF4A
HNF4G
MYRFL
MLXIPL
DMRTA1
PPARA
NR1I2
Gene
log foldchange
3
6
9