

## Supplementary Material

### Culture environment regulates amino acid turnover and glucose utilisation in human ES cells

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**Table S1. Primer sequences used for qPCR analysis of gene expression**

Gene	Forward primer 5'-3'	Reverse primer 5'-3'
<i>B-ACTIN</i>	CGCACCCTGGCATTGTC	TCCTCCTTGATGTCACGCAC
<i>BRACHYURY</i>	GTGCTGTCCCAGGTGGCTTACAGATG	CCTTAACAGCTCAACTCTAACTACTTG
<i>GATA4</i>	CTAGACCGTGGGTTTTGCAT	TGGGTTAAGTGCCCCTGTAG
<i>NANOG</i>	CAAAGGCAAACAACCCACTT	TCTGCTGGAGGCTGAGGT
<i>OCT4</i>	AGCGAACCAGTATCGAGAAC	TTACAGAACCACACTCGCAC
<i>SOX2</i>	ATGCACCGCTACGACGTGA	CTTTTGCACCCCTCCCATTT

**Table S2. Measured concentrations of glucose and lactate in control media**

	Expected glucose concentration (mM)	Measured glucose concentration (mM)	Expected lactate concentration (mM)	Measured lactate concentration (mM)
<b>mTeSR</b>	13.7	15.2 ± 0.4	0	0
<b>mTeSR + FCS</b>		13.4 ± 0.5		2.4 ± 0.1
<b>mTeSR + BMP4</b>	13.7	14.5 ± 0.3	0	0
<b>mTeSR + KOSR</b>		13.7 ± 0.4		0

**Table S3. Measured concentrations of amino acids in mTeSR and mTeSR supplemented with 20% Life Technologies FCS, compared to the expected concentrations from the published formulation of the medium** <sup>18</sup>

\*\* p<0.01; significance was tested for those amino acids showing an increased concentration in serum containing medium

	<b>mTeSR formulation (<math>\mu</math>M)</b>	<b>mTeSR (<math>\mu</math>M)</b>	<b>mTeSR + FCS (<math>\mu</math>M)</b>
<b>Ala</b>	137	158 $\pm$ 3.54	326 $\pm$ 4.6**
<b>Arg</b>	548	459 $\pm$ 8.5	328 $\pm$ 5.6
<b>Asn</b>	137	129 $\pm$ 2.1	103 $\pm$ 1.5
<b>Asp</b>	137	157 $\pm$ 3.4	144 $\pm$ 2.8
<b>Glu</b>	137	157 $\pm$ 2.9	301 $\pm$ 3.6**
<b>Gln</b>	2940	2362 $\pm$ 38.1	1973 $\pm$ 38
<b>Gly</b>	294	269 $\pm$ 7.9	336 $\pm$ 10.7**
<b>His</b>	118	151 $\pm$ 1.9	145 $\pm$ 2.5
<b>Ile</b>	326	318 $\pm$ 6.1	291 $\pm$ 3.5
<b>Leu</b>	354	352 $\pm$ 7.1	333 $\pm$ 3.8
<b>Lys</b>	391	358 $\pm$ 5.5	331 $\pm$ 3.2
<b>Met</b>	90.6	89 $\pm$ 1.9	76 $\pm$ 1.2
<b>Phe</b>	169	187 $\pm$ 3.5	182 $\pm$ 2.1
<b>Pro</b>	216	233 $\pm$ 2.8	237 $\pm$ 2.1
<b>Ser</b>	294	304 $\pm$ 8.9	300 $\pm$ 8.1
<b>Thr</b>	352	343 $\pm$ 6.4	301 $\pm$ 3.2
<b>Trp</b>	34.6	68 $\pm$ 1.2	71 $\pm$ 1.3
<b>Tyr</b>	168	174 $\pm$ 3.1	160 $\pm$ 1.7
<b>Val</b>	355	355 $\pm$ 6.4	355 $\pm$ 3.8

**Table S4. Measured concentrations of amino acids in mTeSR and mTeSR supplemented with 20% Life Technologies KOSR, compared to the expected concentrations from the published formulation of mTeSR<sup>18</sup> and mTeSR + KOSR**

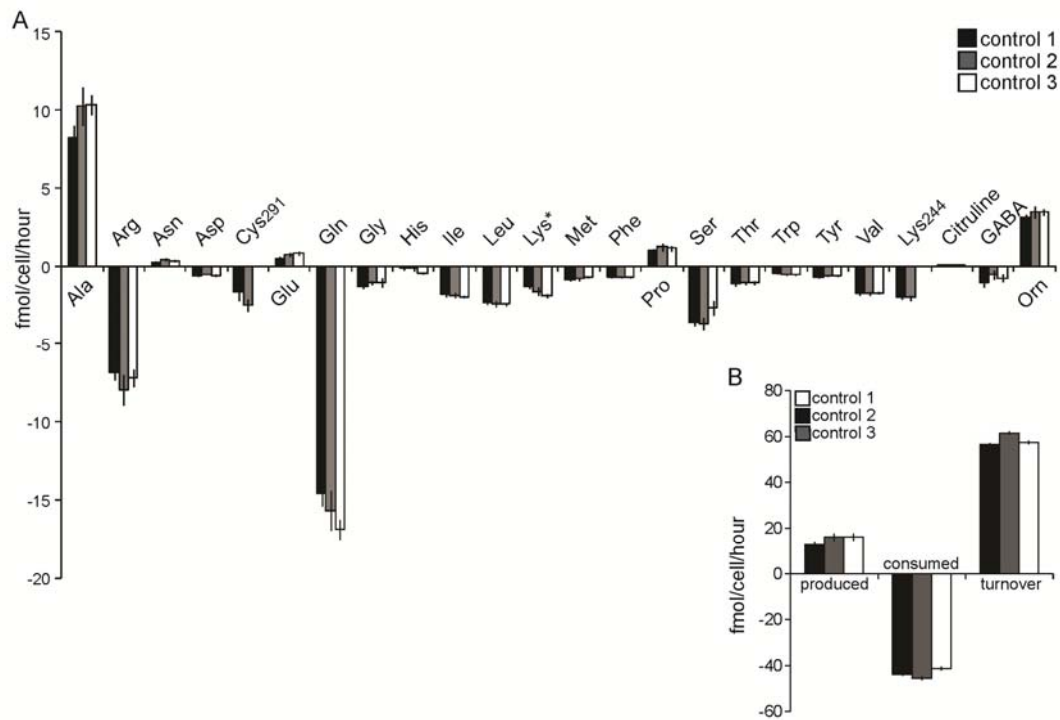
Expected concentrations for amino acids in KOSR were taken from the preferred embodiment of the supplement<sup>20</sup>

	mTeSR formulation (μM)	mTeSR (μM)	mTeSR + KOSR formulation (μM)	mTeSR + KOSR (μM)
<b>Ala</b>	137	144 ± 2.5	109.6	123 ± 4.9
<b>Arg</b>	548	581 ± 4.9	438.4	463 ± 16.1
<b>Asn</b>	137	142 ± 2.2	109.6	113 ± 2.7
<b>Asp</b>	137	139 ± 8.8	109.6	106 ± 4
<b>Glu</b>	137	138 ± 6.2	109.6	105 ± 6
<b>Gln</b>	2940	2614 ± 29.5	2352	2065 ± 46.5
<b>Gly</b>	294	296 ± 4.5	1176.4	621 ± 19.1
<b>His</b>	118	116 ± 7.6	1667	925 ± 36.3
<b>Ile</b>	326	332 ± 6.4	6512	4957 ± 118.8
<b>Leu</b>	354	358 ± 6.8	283.2	369 ± 9.4
<b>Lys</b>	391	393 ± 10.7	312.8	308 ± 8.3
<b>Met</b>	90.6	81.6 ± 4.3	465.68	172 ± 4.4
<b>Phe</b>	169	188 ± 4.1	2847.2	2129 ± 55.6
<b>Pro</b>	216	242 ± 5.9	7111.2	6445 ± 144.5
<b>Ser</b>	294	281 ± 4.7	2290.6	1635 ± 46.5
<b>Thr</b>	352	353 ± 9	5038.2	3452 ± 82
<b>Trp</b>	34.6	47 ± 2.9	812	455 ± 13
<b>Tyr</b>	168	226 ± 4.9	752	225 ± 4.9
<b>Val</b>	355	370 ± 7.4	5450	3977 ± 92.6

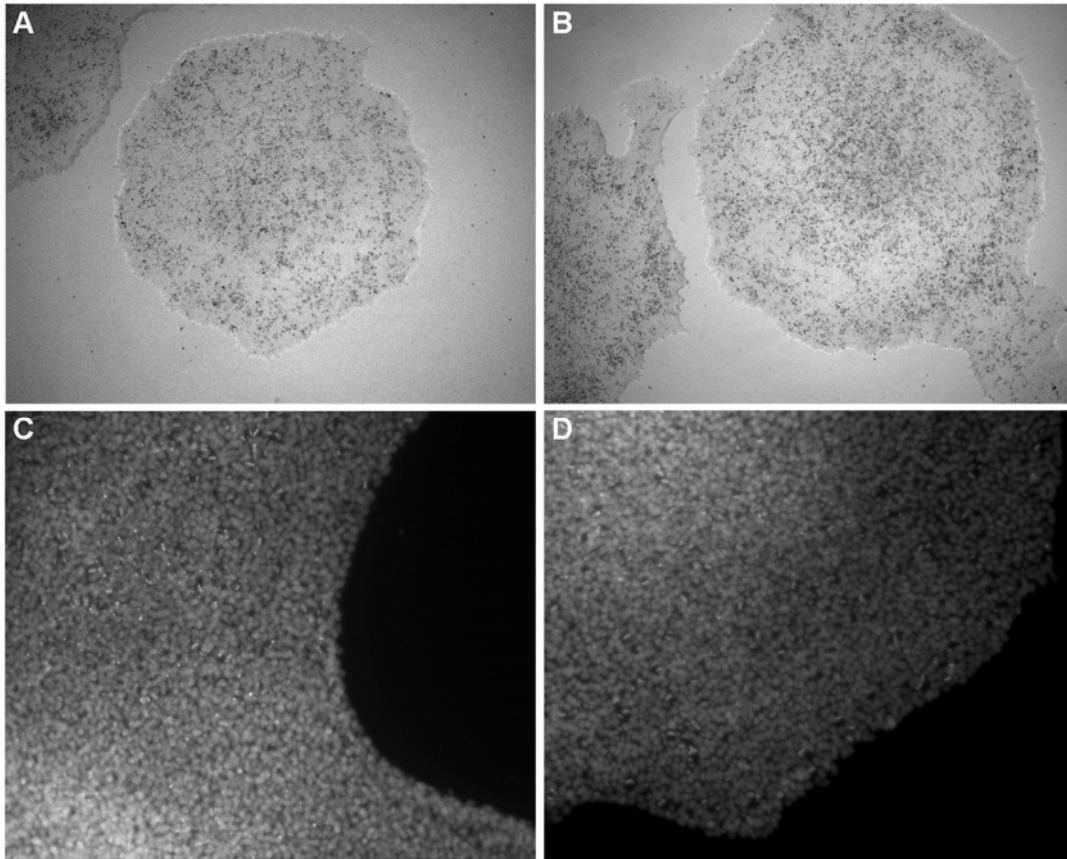
**Table S5. Proliferation of human ES cells, shown as the % of cells positive for phosphorylated histone H3, in medium conditions used, and final live cell density, estimated by Alamar Blue staining, shown as fluorescent intensity**

*P*-value determined by Student's t-test when compared to cells cultured in mTeSR. Analysis was of 20 fields from two independent replicates (proliferation rate) and 6 replicate wells (final live cell density)

	% cells in mitosis ± s.e.m.	<i>P</i> -value	Fluorescent intensity (relative units) ± s.e.m.	<i>P</i> -value
<b>mTeSR</b>	3.6 ± 0.3		2.95 ± 0.3	
<b>mTeSR + F12</b>	3.6 ± 0.3	0.86	2.76 ± 0.2	0.65



**Fig. S1.** Comparison of amino acid use in human ES cell cultures across experimental procedures. (A) Spent medium from the final 24 h of culture of human ES cells in mTeSR1 for 7 days was analysed for the presence and concentration of amino acids. Amino acid production or consumption was normalised to cell number and is expressed as fmol/cell/h.  $n = 8$  (Control 1);  $n = 6$  (Control 2);  $n = 12$  (Control 3). Error bars represent s.e.m. Data were analysed using ANOVA,  $*P < 0.05$ . (B) Total amino acid production, consumption and turnover by cells cultured in mTeSR1.



**Fig. S2.** Morphology of human ES cells cultured in mTeSR1 supplemented with 20% Hams F12 medium. Human ES cells were cultured for 3 days in mTeSR1 before being changed into mTeSR1 (A, C) or mTeSR1 supplemented with 20% Hams F12 (B, D) and maintained for a further 4 days. Images of colon morphology were captured in phase contrast at 4 $\times$  magnification (A, B) or with fluorescence microscopy after staining for DNA with DAPI (C, D; 20 $\times$  magnification).  $n = 3$ ; representative images are shown.