

**Supplementary Table S1.** List of primers, and forward and reverse sequence and length of the 26 primers used for qPCR.

Primer	Sequence	Length
GAPDH F	AAT GGT GAA GGT CGG TGT G	19
GAPDH R	GTG GAG TCA TAC TGG AAC ATG TAG	24
$\beta$ -ACTIN F	CTG AAC CCT AAG GCC AAC C	19
$\beta$ -ACTIN R	GTA CGA CCA GAG GCA TAC AG	20
IGF1 F	AGT ACA TCT CCA GTC TCC TCA G	22
IGF1 R	ATG CTC TTC AGT TCG TGT GT	20
IGF2 F	CAC TCT TCC ACG ATG CCA	18
IGF2 R	CAC GCT TCA GTT TGT CTG TTC	21
FGF2 F	GAA ACA CTC TTC TGT AAC ACA CTT	24
FGF2 R	GTC AAA CTA CAA CTC CAA GCA G	22
FGF8 F	GTA GTT GTT CTC CAG CAC GAT	21
FGF8 R	GAC AGG TCT CTA CAT CTG CAT	21
NGF F	TTG CTA TCT GTG TAC GGT TCT G	22
NGF R	GGA CGC AGC TTT CTA TAC TGG	21
SHH F	CGT AAG TCC TTC ACC AGC TTG	21
SHH R	GAA TCC AAA GCT CAC ATC CAC	21
SFRP F	CTT CTT GTC ACC GTT TTC CTT C	22
SFRP R	GAG TTG AAG TCA GAG GCC ATC	21
VEGFA F	TGG TGA CAT GGT TAA TCG GT	20
VEGFA R	AGA AAG ACA GAA CAA AGC CAG A	22
VEGFB F	GCT TCA CAG CAC TCT CCT T	19
VEGFB R	CAA GTC CGA ATG CAG ATC CTC	21
VEGFC F	CAG CGG CAT ACT TCT TCA CTA	21
VEGFC R	GAA GTT CCA CCA TCA AAC ATG C	22
NTF3 F	ACA TCA CCT TGT TCA CCT GTA	21
NTF3 R	AGT CCA CCT TTC TCT TCA TGT C	22
NTF5 F	GAA GAG GAA AAG GAG GAG AGA AC	23
NTF5 R	GAG ACT ACC TGT ATC CTA CAA AGG	24
PAX1 F	GCC CAG TCT TCC ATC TTG G	19
PAX1 R	GCA CAT TCA GTC AGC AAC ATC	21
ALDH1A1 F	ACC CAG TTC TCT TCC ATT TCC	21
ALDH1A1 R	CAT CAC TGT GTC ATC TGC TCT	21
WNT5a F	CCA GAC ACT CCA TGA CAC TTA C	22
WNT5a R	ACG CAT CCT CAT GAA CTT ACA C	22
WNT9a F	TTC CAC TCC AGC CTT TAT CAC	21
WNT9a R	GTA CAG CAG CAA GTT TGT CAA G	22
CXCL12 F	ACA GTT TGG AGT GTT GAG GAT	21
CXCL12 R	GCG CTC TGC ATC AGT GA	17
GDNF F	CGT CAT CAA ACT GGT CAG GAT	21
GDNF R	CCG CTG AAG ACC ACT CC	17
TGF $\beta$ 1 F	CCG AAT GTC TGA CGT ATT GAA GA	23
TGF $\beta$ 1 R	GCG GAC TAC TAT GCT AAA GAG G	22
TGF $\beta$ 2 F	CTG ATC ACC ACT GGC ATA TGT AG	23
TGF $\beta$ 2 R	TGT ACC TTC GTG CCG TCT A	19
TGF $\beta$ 3 F	ACT GAG GAC ACA TTG AAA CGA	21
TGF $\beta$ 3 R	GCC AAA GAG ATC CAT AAA TTC GAC	24
BDNF F	GCA ACC GAA GTA TGA AAT AAC CA	23
BDNF R	GTT TAT CAC CAG GAT CTA GCC A	22
CNTF F	AGA TAG AGC GGC TAC AGA GG	20
CNTF R	GTG AAG ACA GAA GCA AAC CAG	21

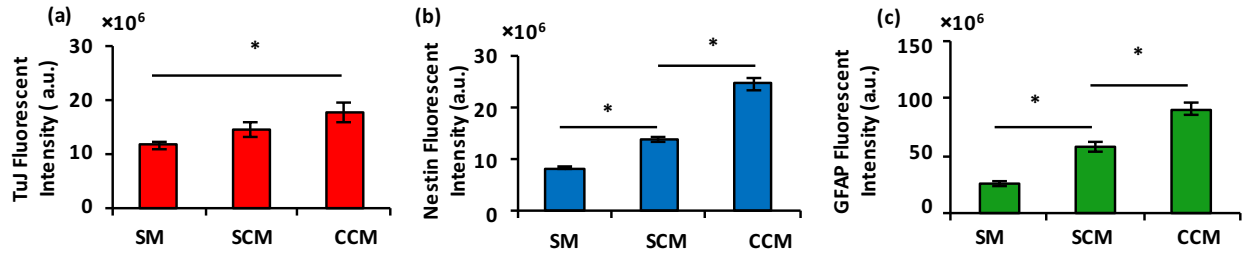
**Supplementary Table S2.** List of the reagents used, with their vendors and catalog numbers.

<b>Reagents</b>	<b>Vendor</b>	<b>Catalog #</b>
Gelatin	Sigma	G1393
Glasgow minimum essential medium (GMEM)	Life Technologies	11710-035-6
Knockout serum replacement (KSR)	Life Technologies	10828028
Glutamax	Life Technologies	35050-061
Fetal bovine serum (FBS)	Sigma	12306C
Non-essential amino acids (NEAA)	Life Technologies	11140-050
Sodium pyruvate	Life Technologies	11360-050
2-mercaptoethanol	Life Technologies	21985023
Leukemia inhibitory factor (LIF)	Millipore	ESG1107
Alpha MEM	Life Technologies	12571-048
Antibiotic-antimycotic	Life Technologies	15240-062
Mitomycin-c	Sigma	M4287
Polyethylene glycol (PEG)	Sigma	94646
Dextran (DEX)	Pharmacosmos	551005009007
Formaldehyde	Fisher Scientific	BP531-500
Donkey serum	Sigma	D9663
PBS	Sigma	D8537
B-tubulin antibody	Biologend	MRB 435P
Nestin antibody	Neuromics	CH23001-100
Glial fibrillary acidic protein (GFAP) antibody	Neuromics	CH22102
Aminomethylcoumarin (AMCA) conjugated secondary antibody	Jackson ImmunoResearch	703-155- 155
Rhodamine red conjugated secondary antibody	Jackson ImmunoResearch	711-295- 152
Alexa fluor-488 conjugated secondary antibody	Jackson ImmunoResearch	703-545-155
Mercaptoethanol ultra	Sigma	63689
RNA isolation kit	Omega Biotek	R6834-02
RNase-free DNase kit	Omega Biotek	E1091-02
Homogenizer mini columns	Omega Biotek	HCR003
Transcriptor reverse transcriptase cDNA synthesis kit	Roche	04 897 030 001

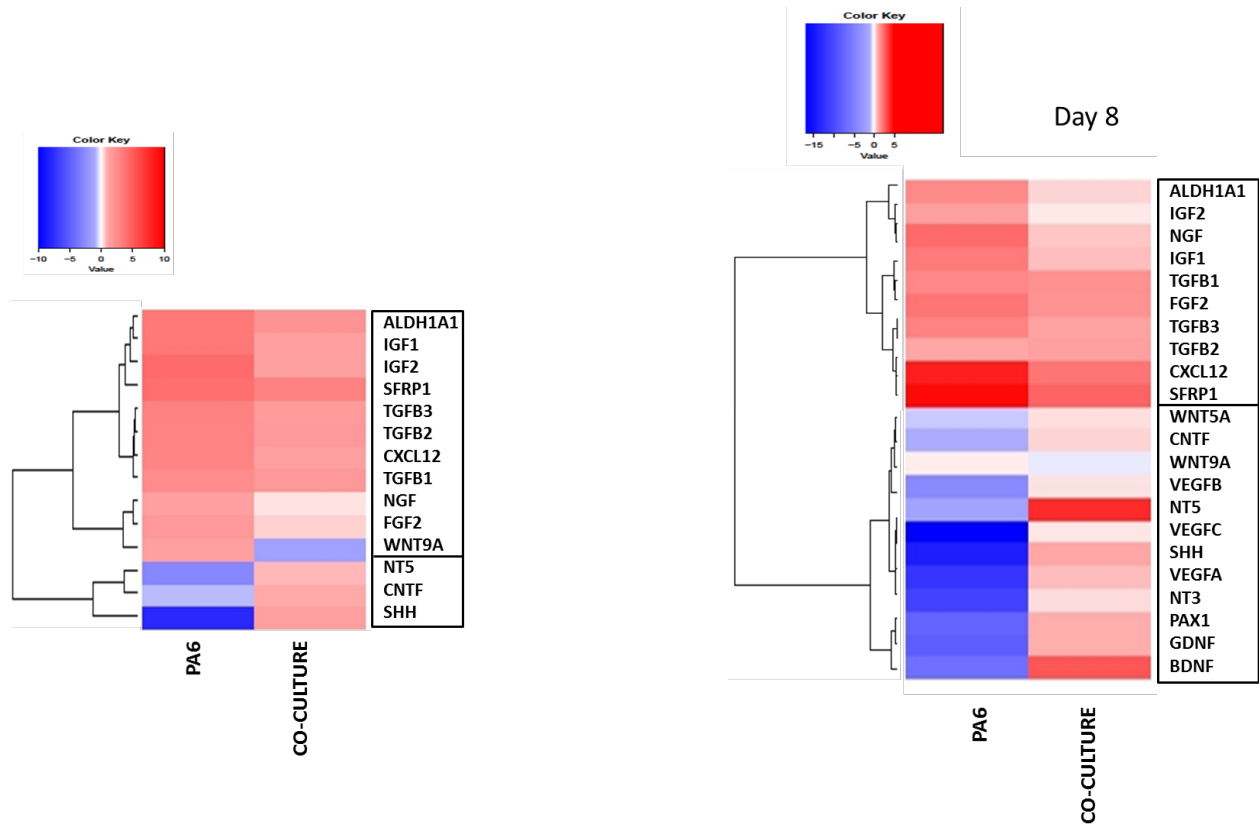
Sybr green master mix

Roche

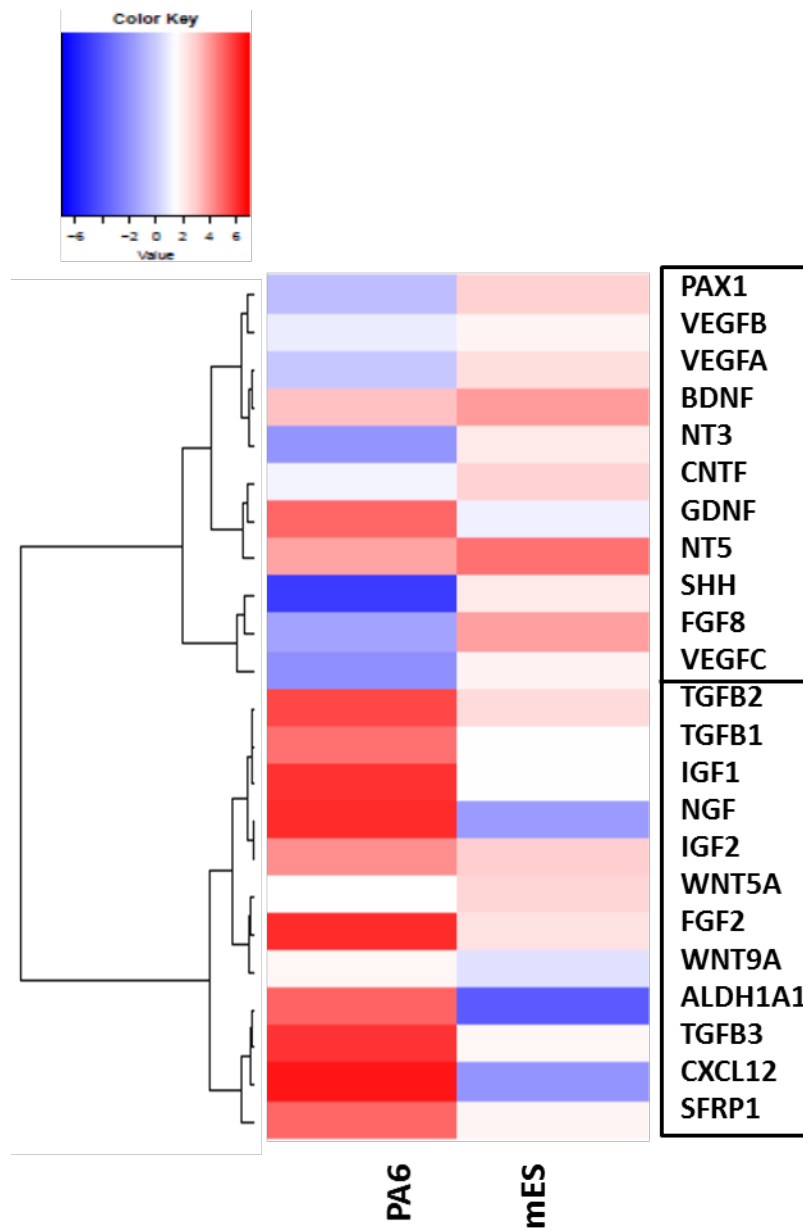
04 707 516 001



**Supplementary Figure S1.** Immunofluorescence quantification of neural cell differentiation of mESCs for (a) TuJ, (b) Nestin, and (c) GFAP. Data represent differentiation of a single mESC colony in co-culture with PA6 cells in a microwell under treatment with SM, SCM, and CCM. The number of replicates was N=20.



**Supplementary Figure S2.** Heatmap and hierarchical clustering of genes of representing specific soluble factors on (a) day 4 and (b) day 6 of cultures. Factors with fold change less than 2 are not included in the analysis.



**Supplementary Figure S3.** Heatmap and hierarchical clustering of genes representing specific soluble factors. Data are from qPCR experiments with mESCs performed on day 8 after manually separating the differentiated mES colonies from PA6 feeder layer.