

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 |
| β -ACTIN F | CTG AAC CCT AAG GCC AAC C | 19 |
| β -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 β 1 F | CCG AAT GTC TGA CGT ATT GAA GA | 23 |
| TGF β 1 R | GCG GAC TAC TAT GCT AAA GAG G | 22 |
| TGF β 2 F | CTG ATC ACC ACT GGC ATA TGT AG | 23 |
| TGF β 2 R | TGT ACC TTC GTG CCG TCT A | 19 |
| TGF β 3 F | ACT GAG GAC ACA TTG AAA CGA | 21 |
| TGF β 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 |

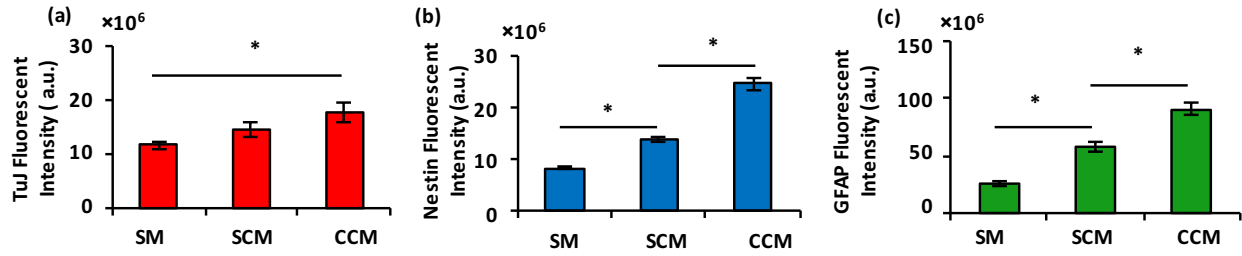
Supplementary Table S2. List of the reagents used, with their vendors and catalog numbers.

| Reagents | Vendor | Catalog # |
|--|------------------------|------------------|
| 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 |

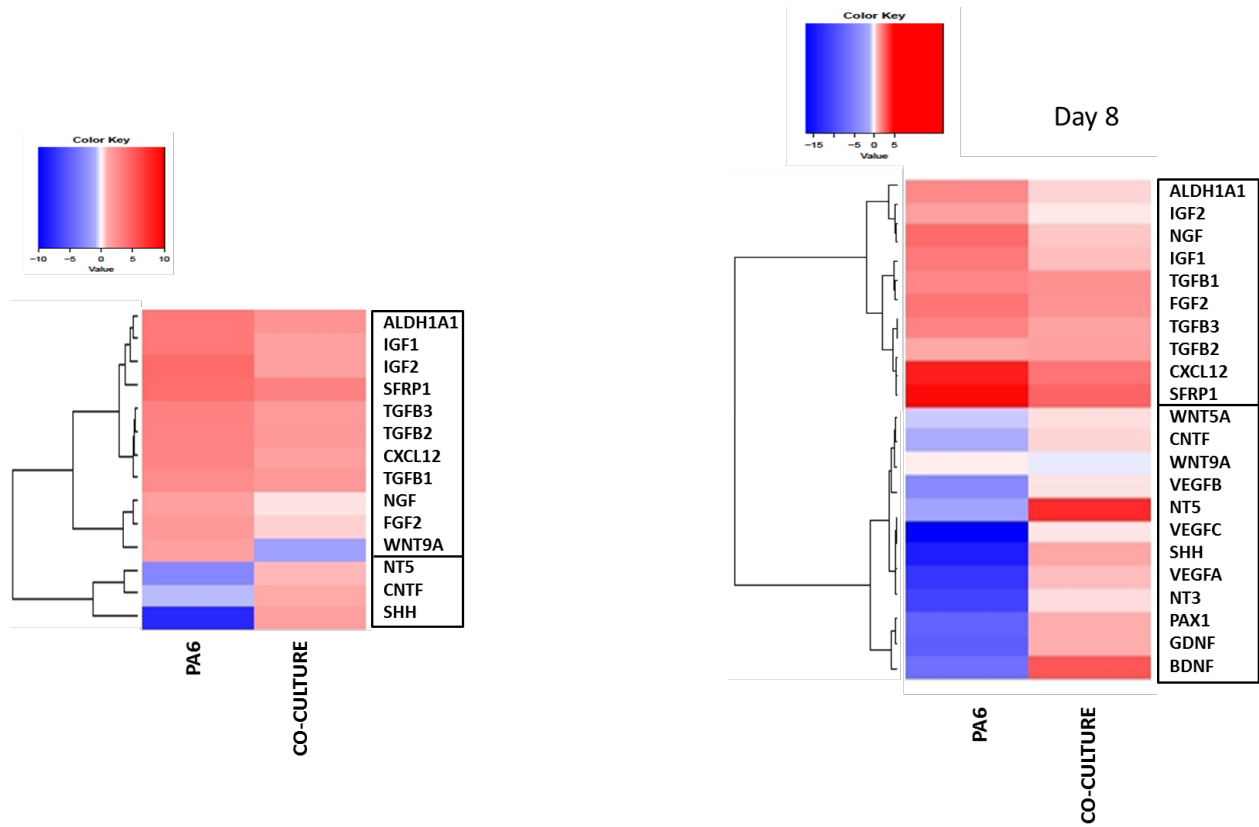
Sybr green master mix

Roche

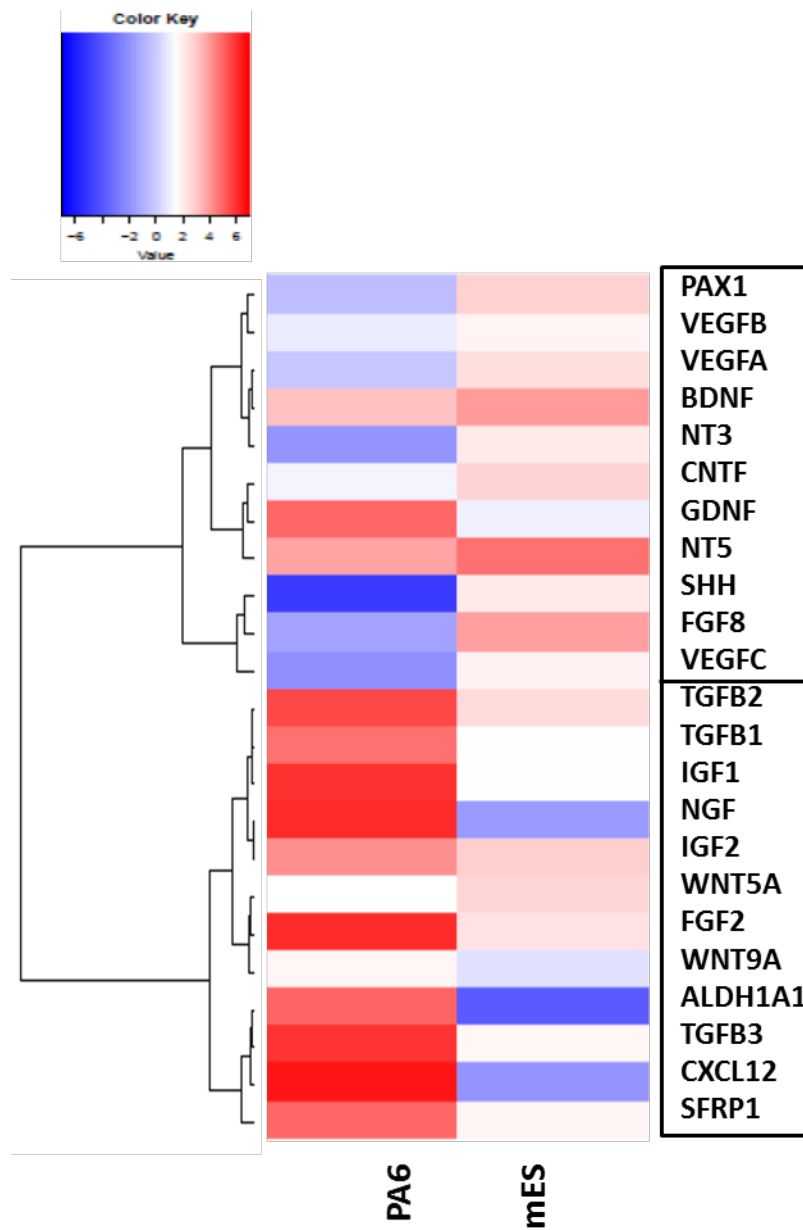
04 707 516 001



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.