



Supplementary information, Figure S5 *In vitro* differentiation of mESCs maintained in sunitinib-containing medium.

(A) qRT-PCR analysis of lineage specific genes in EBs (day 11) derived from E14 cells maintained in LIF, sunitinib (1 μ M) or 2i (3 μ M PD0325901 + 3 μ MCHIR99021) for 11 passages. All chemicals were removed during EB formation. (B) Immunostaining of endoderm (HNF-3 β), mesoderm (SMA), and ectoderm (Tuj1) markers in EBs (day12) derived from E14 cells cultured in LIF-free mES medium supplemented with 1 μ M sunitinib for 11 passages. Nuclei were stained with Hoechst 33342. (Scale bar: 10 μ m). (C and D) VEGF induced differentiation. EBs were formed in hanging drop for 2 days and then attached onto 0.1% gelatin-coated 24-well plate (10-15 EBs per well) in DMEM medium with 10% FBS. On the next day, vascular differentiation medium supplemented with 50 ng/mL mVEGF₁₆₄ were used to culture the EBs up to 14 days. The vascular markers were analyzed by qRT-PCR (C) and immunofluorescent staining (D). Data are mean \pm SEM (n=3), *p < 0.05, **p < 0.01, ***p < 0.001 vs ES cells.