Supplementary Materials and Methods

Hematopoietic cell differentiation: CM ESCs were detached from MEF feeder layers by trypsinization and cultured under feeder-free conditions for 4 days. Then cells were processed for EB formation assay on low cell-bind 12 well multidish (Nalge Nunc International KK, Tokyo, Japan) in Stemline II medium (Sigma-Aldrich, MO, USA) containing bone morphogenetic protein 4 (BMP4: 50 ng/ml; *R&D* Systems, MN, USA), vascular endothelial growth factor (VEGF: 50 ng/ml; PeproTech) on day0. Half of the medium was removed and the fresh medium was added on day2, with the final concentrations of BMP4 (50 ng/ml), VEGF (50 ng/ml), stem cell factor (SCF: 20 ng/ml; *PeproTech*), thrombopoietin (TPO: 20 ng/ml; PeproTech*)* and FMS like tyrosine kinase 3 ligand (FLT3L: 20 ng/ml; PeproTech). On day4, the medium was removed and fresh medium containing SCF (50 ng/ml), TPO (50 ng/ml) and FLT3L (50 ng/ml) was added, and half of the medium was changed with the fresh medium containing the same cytokines on day6. To examine hematopoietic potential of day8-EBs, we conducted colony-forming unit (CFU) assay. Day8-EBs were collected and dissociated by 0.25% trypsin, and resuspended in IMDM medium, and the single-cell suspension of 1×10^5 cells with 1 ml of MethoCult SF H4436 (STEMCELL Technologies, Vancouver, Canada) was plated in 35 mm culture dish (STEMCELL Technologies) and cultured at 37ºC in 5% CO_2 for 10 to 14days. Colonies were observed by optical microscopy (BZ-9000; Keyence, Japan).

Neuroepithelial cell differentiation: CM ESCs were detached from MEF feeder layers by trypsinization and cultured under feeder-free conditions for 4 days. Then cells were processed for EB formation assay on low cell-bind 12 well multidish (Nalge Nunc International KK, Tokyo, Japan) in the medium consisted of Dulbecco modified Eagle

medium (DMEM)/F12 medium (Life Technologies, NY, USA) containing 20% Knockout Serum Replacement (KSR; Life Technologies), 1 mM GlutaMAX™ (Life Technologies), 0.1 mM MEM non-essential amino acids (Life Technologies), 0.1 mM 2-mercaptoethanol (2-ME; Sigma-Aldrich, Taufkirchen, Germany). On day4, EBs were fed with the neural induction medium consisted of DMEM/F12 containing N2 supplement (Life Technologies), 0.1 mM MEM non-essential amino acids (Life Technologies) and 2 μg/ml heparin (Mochida, Japan) for another 2 days. On day6, EBs were transferred onto laminin-coated dish, and cultured in the neural induction medium. On day10, the medium was replaced to the neural induction medium supplemented with 0.1 μM retinoic acids (WAKO, Japan), and the cells were cultured for 4 days. On day14, the cells were fixed and processed for immunocytochemistry.

Hepatic cell differentiation: CM ESCs were cultured under feeder-free conditions for 4 days. Then cells were cultured in RPMI1640 (Nacalai Tesque) containing B27 supplement (Life Technologies) and 100 ng/ml activinA (PeproTech) for 3 days (day0-3). On day3, the culture medium was replaced with CM ESC medium. On day10, the medium was replaced with CM ESC medium supplemented with 20 ng/ml hepatocyte growth factor (HGF) (PeproTech), and the cells were cultured for another 7 days (day10-17). On day17, the cells were fixed and processed for immunocytochemistry.