Differentiation assay

For the all differentiation assay,

I suggest stem cells should be trypsinized and plated on 0.1% gelatin-coated plates for 1 hour to remove feeder cell.

1. Embryoid body (EB) differentiation
2. - For EB differentiation, ESCs/iPSCs were plated in 10 cm petri dish (150,000 cells/ml, Fisher #FB0875713) in ES cell medium without LIF and gently cultured on a rotator in incubator.
3. ; I set up between 2.5-3 rotator speeds on the rotator what Helab has.
4. ; When you set up rotator in the incubator, please carefully clean the rotator using 70% EtOH brfore you put it in to avoid contamination.
5. - Collect the samples every 3 other day (or depends on your purpose) and at least 4-5 time points (or depends on you purpose) for experiments (qPCR or staining etc.)

- Recommend use new petridish when you change media.

1. XEN differentiation

- ESCs/iPSCs were plated at a density of 1X104 cells/cm2 and then cultured in standard XEN medium (RPMI 1640 (Life Technologies, Cat # 11875119) supplemented with 15% FBS (Life Technologies, Cat # 16141079), 1% penicillin-streptomycin (Life Technologies, Cat # 15140163), 2 mM L-glutamine (Life Technologies, Cat # 25030164), and100 **μ**M **β**- mercaptoethanol (Sigma, Cat # M3148)).

- 24 hours after initial plating, cells were cultured for 48 hours in XEN induction medium containing standard XEN medium supplemented with 0.01 ****M retinoic acid (Sigma, Cat. # R2625), 10 ng/ml activin A (R&D Systems, Cat. # 338-AC-010), 24 ng/ml recombinant mouse FGF2 (R&D Systems, Cat. # 3139-FB-025) and 1 g/ml heparin (Sigma, Cat. # H3149).

- Subsequently, the cells were counted and passaged with a density of 1X104 cells/cm2 on feeders in standard XEN medium.

- These cells were trypsinized using Trypsin 0.25% EDTA (Life Technologies, Cat # 25200056) and passaged every two days.

- After six days of culture, cells were collected for real time PCR analyses and IF.

ESC/iPSC culture condition

Knockout DMEM (Invitrogen, catalogue no. 10829-018), 15% ES-grade fetal bovine serum

(Invitrogen, catalogue no. 16141079), 2mM **l**-glutamine (Invitrogen, catalogue no.

25030-164), 1**\_**10**􀀀**4 M MEM non-essential amino acids (Invitrogen, catalogue no.

11140-076), 1**\_**10**􀀀**4 M 2-mercaptoethanol (Sigma, catalogue no. M3148) and 1%

100**\_**penicillin and streptomycin