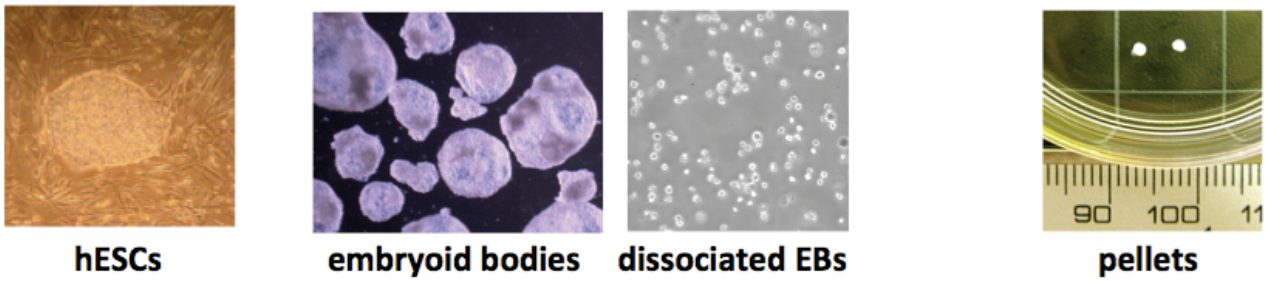
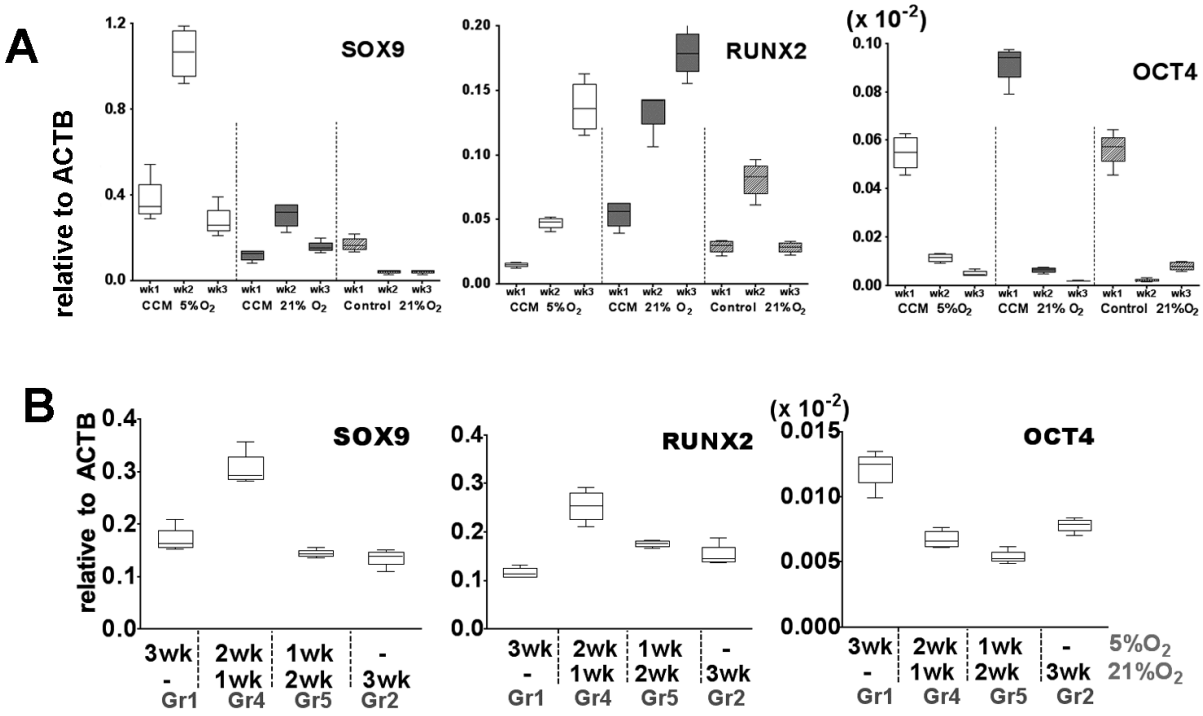


1 expansion	2 induction	3 Pellet formation
	EB1 EB2 EB3	P1 P2 P3 P4 P5 P6
Expand hESCs for down stream experiment **Consistent source of hESCs (passage 30)**	Recapitulate the differentiation of ESCs into a variety of cell types *in vitro* Study synergistic effects of morphogenetic factors and hypoxia on differentiation of hESCs toward precursor cells of chondrocytes	**Cartilage tissue development** Three-dimensional pellet culture to recapitulate • condensation of precartilagineous mesenchyme, • commitment to the chondrogenic lineage and • differentiation into chondroblasts and eventually into chondrocytes. Kawakami, Y et. al. (2006) Curr. Opin. Cell Biol

Supplemental Figure 1. The overview of study hESCs line H9 were expanded from p.28 to p.30 and kept in liquid N_2 as stock until use. Embryoid bodies were induced using hypoxia and secreted soluble factors from primary chondrocytes. After 3 weeks of induction, EBs were dissociated into single cells and formed chondrogenic pellets for 6 weeks.



Supplemental Figure 2. Gene expression in EBs cultured in conditioned medium (CCM) under hypoxic conditions. Results are presented as a boxplot. The boundary of the box closet to zero indicates 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicate 75th percentile**. (A)** Real-time PCR showed expression profiles of transcription factors and extracellular matrix over 3 weeks time course of EB induction (study 1). **(B)** EBs were cultured in CCM with different exposure periods of 5% O_2 for 3 weeks (study 2). Gene expression levels were normalized with *ACTB*.