

**Supplementary information, Fig. S2 Identification of naïve hESCs, related to Fig. 2.** a Immunostaining of general pluripotency and hypoblast markers for AIC-N hESCs. **b** Flow cytometry analysis of Tra-1-60, Tra-1-85 and SUSD2 in AIC-N hESCs. **c** G-banding karyotype analysis of four different AIC-N hESC lines. The passage (P) number for karyotyping is indicated. **d** AIC-N hESCs gave rise to teratomas including three germ lineages. **e** Heatmap of representative primed and naïve genes in hPSCs cultured in different conditions. Values represent log<sub>2</sub> (FPKM+1) scaled by gene

expression across samples. **f** Comparisons of global methylation in AIC-N1 (female), AIC-N3 (female) and CC-H9 (female, conventional) hESCs by averaging CpG methylation levels over 50-kb windows. **g** Differentiation schematic of trophoblast lineages from AIC-hESCs. **h** Representative contrast-phase and immunostaining images of different types of trophoblast cells differentiated from AIC-N hESCs. STB, syncytiotrophoblast-like cells; EVT, extravillous cytotrophoblast-like cells. Scale bars, 100  $\mu$ m.