

Supplemental Figures

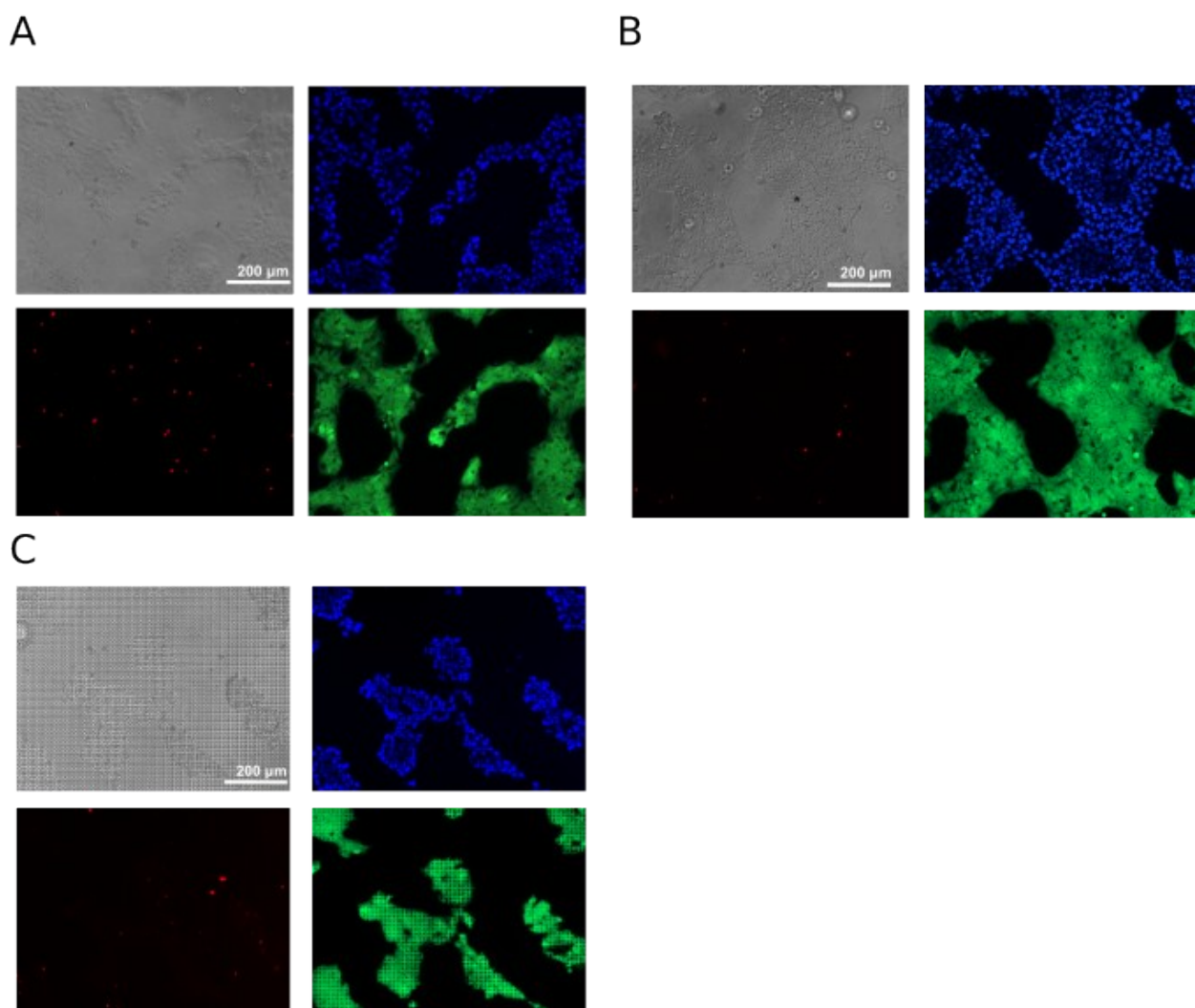


Figure 1S: LIVE/DEAD viability assay. Fluorescence analysis shows nuclei marked with hoechst (in blue), live cells marked with calcein-AM (in green) and dead cells marked with ethidium homodimer-1 (in red). A) Flat plastic substrate; B) flat PDMS substrates; C) μ P PDMS substrate. Fluorescence analysis was performed after 72 hours of culture.

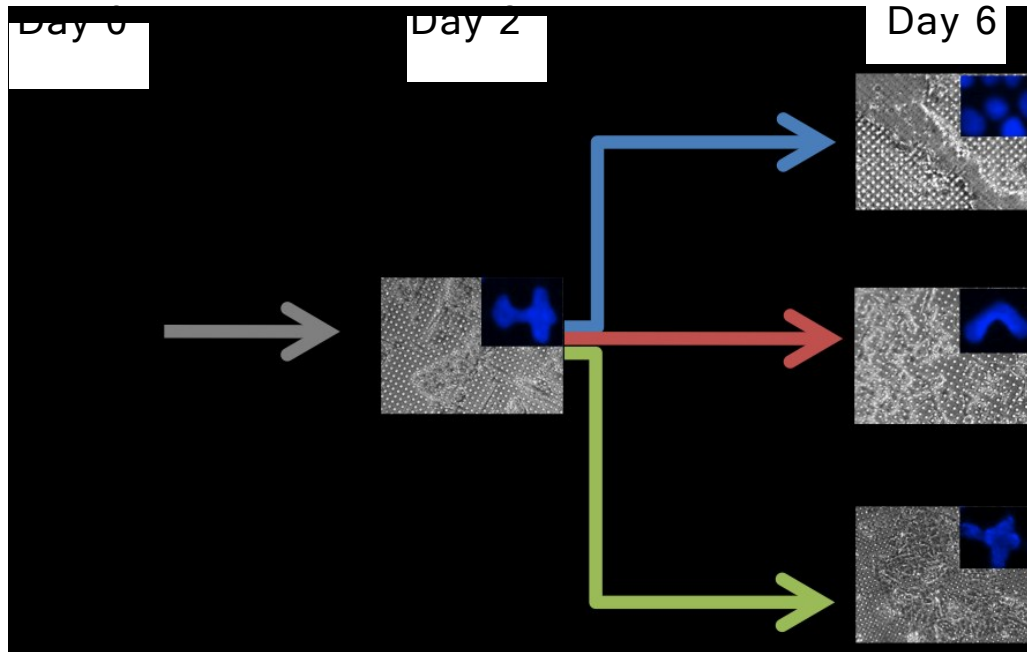


Figure 2S: Schematic experimental procedure of early germ layer commitment of hESCs on microstructured (μ P) substrates. Cell are passaged on μ P PDMS substrates and expanded for 2 days. Different replicates are then induced with specific media into 3 germ layers for 4 days. Nuclear concavity is then calculated in the three different conditions (ectoderm, mesoderm, endoderm early germ layer specification). Scale bar 100 μ m. Scale bar of the insets, 10 μ m.

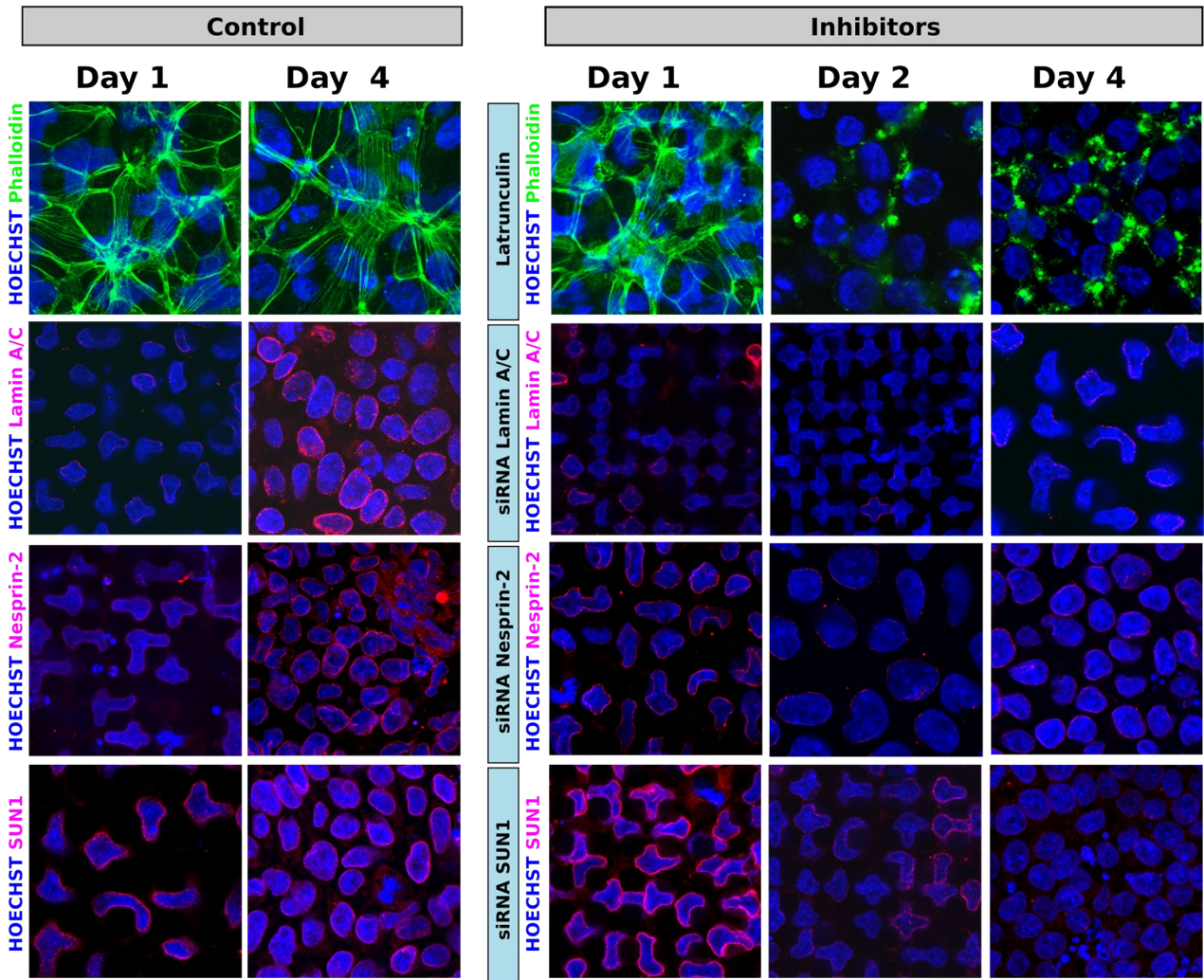


Figure 3S: Immunofluorescence analysis of actin filaments, Lamin A/C, Nesprin-2 and SUN1 during ectodermal germ layer commitment. *Left*) Control cells; *right*) cells, where the marked proteins were inhibited through chemical inhibitors or through siRNA.