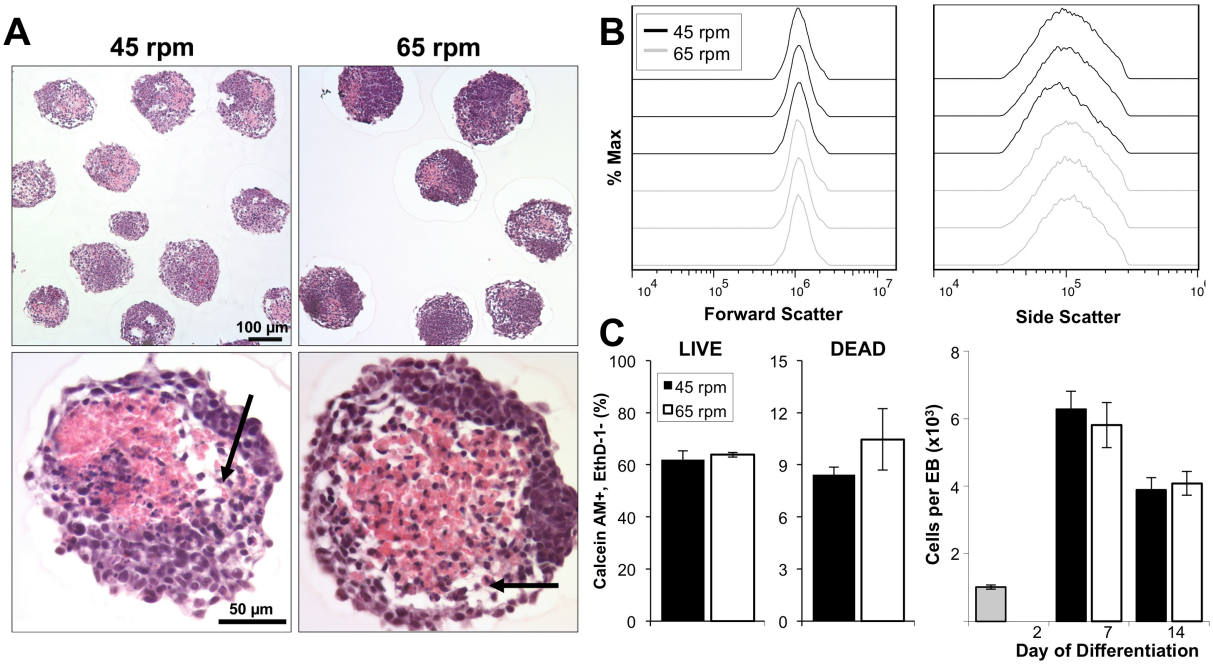
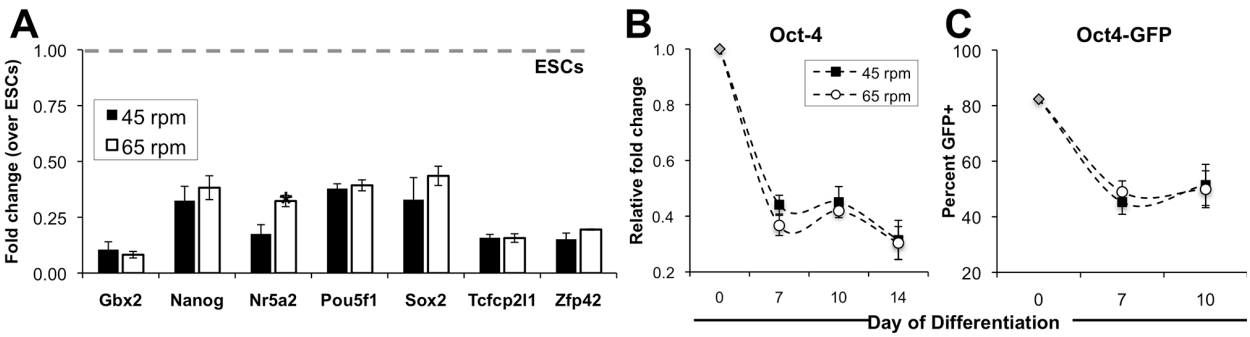
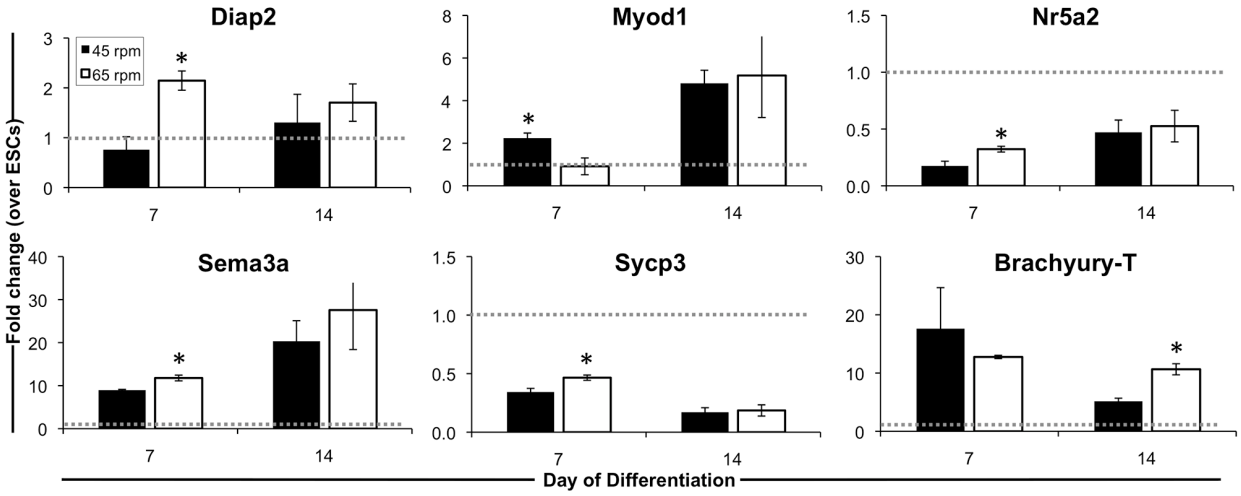


Supplemental Fig. 1. Morphological features of EBs and ESCs in hydrodynamic environments. (A) ESCs within EBs exhibited epithelial- and mesenchymal-like (arrows) morphologies; however, few distinct changes were apparent between rotary conditions (scale bars = 100 µm, 50 µm). (B) Overlays of Forward scatter (FSC) and side scatter (SSC) histograms from flow cytometry indicated similarities in cell properties between conditions. (C) viability and total cell yield per EB were not affected by hydrodynamics at the mixing speeds tested.



Supplemental Fig. 2. Pluripotency in EBs. Transcription factors related to maintaining pluripotency exhibited decreased expression compared to ESCs in (A) PCR array analysis, (B) RT-PCR for Oct-4, and (C) incidence of GFP+ cells in a cell line expressing GFP driven by the Oct-4 promoter.



Supplemental Fig. 3. Genes differentially regulated between hydrodynamic conditions. Several genes exhibited statistically significant changes in gene expression between the rotary orbital conditions, after 7 (*Diap2, Myod1, Nr5a2, Sema3a, Sycp3*) and 14 (*Brachyury-T*) days of differentiation. Differences were apparent in genes that were both increased and decreased compared to ESCs (dotted line). *P < 0.05