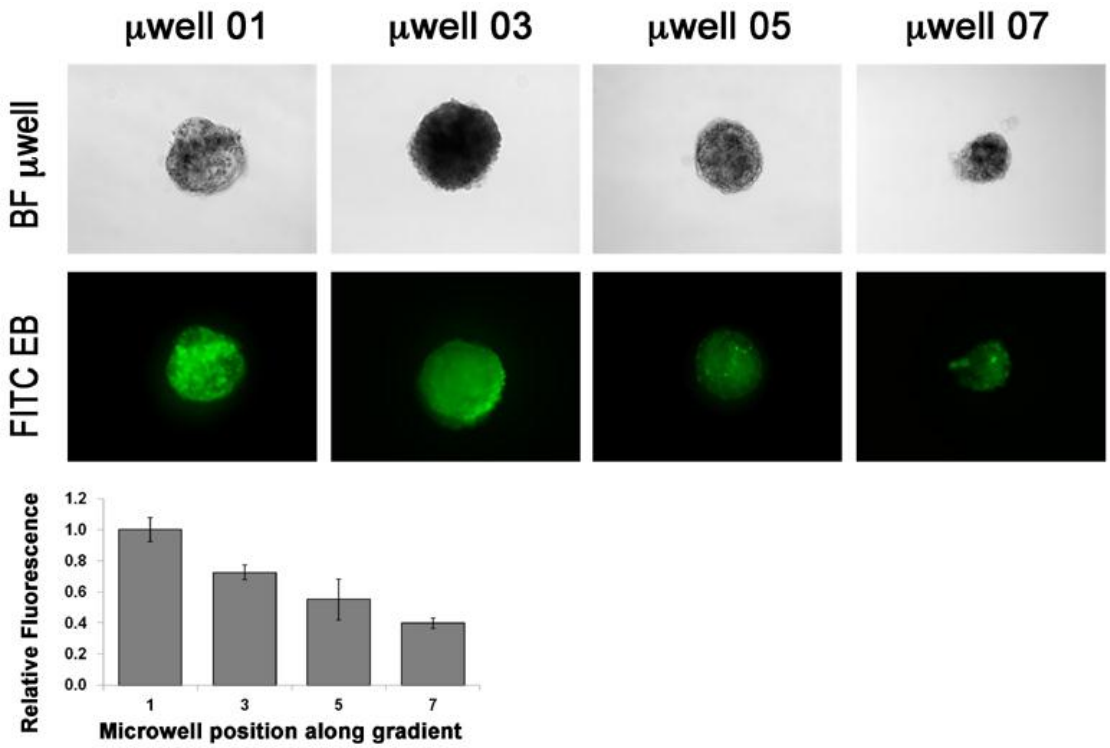
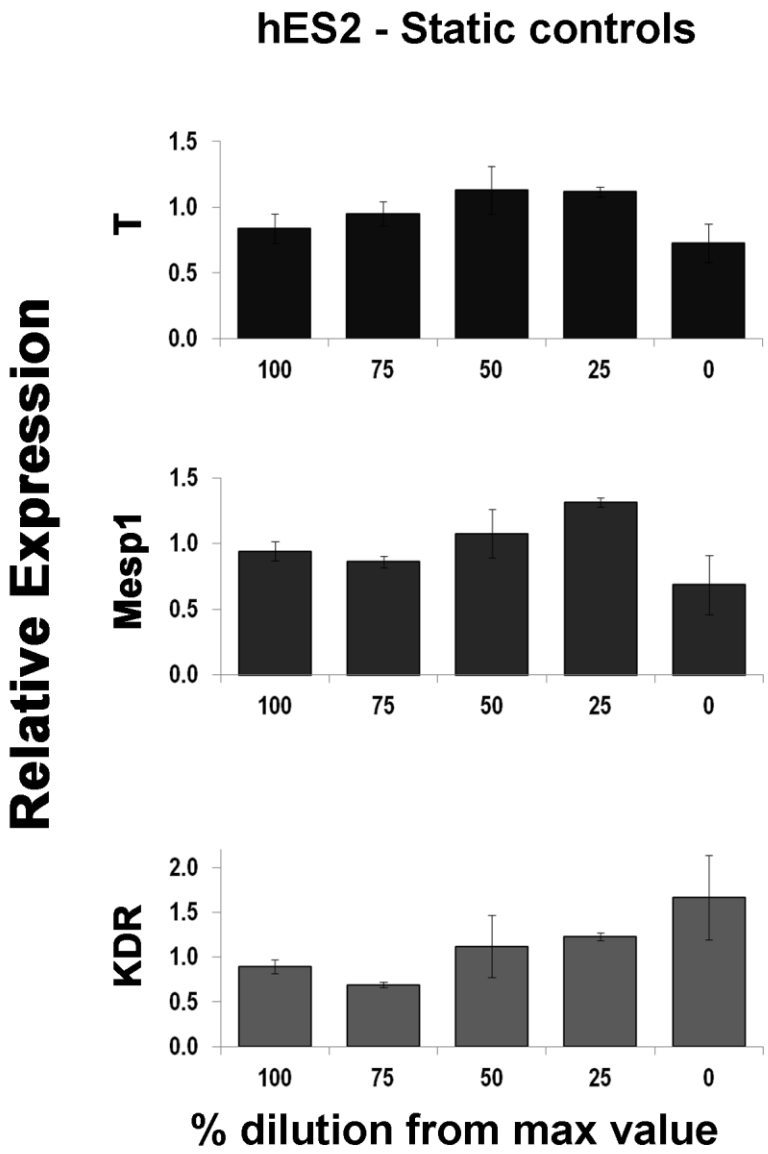
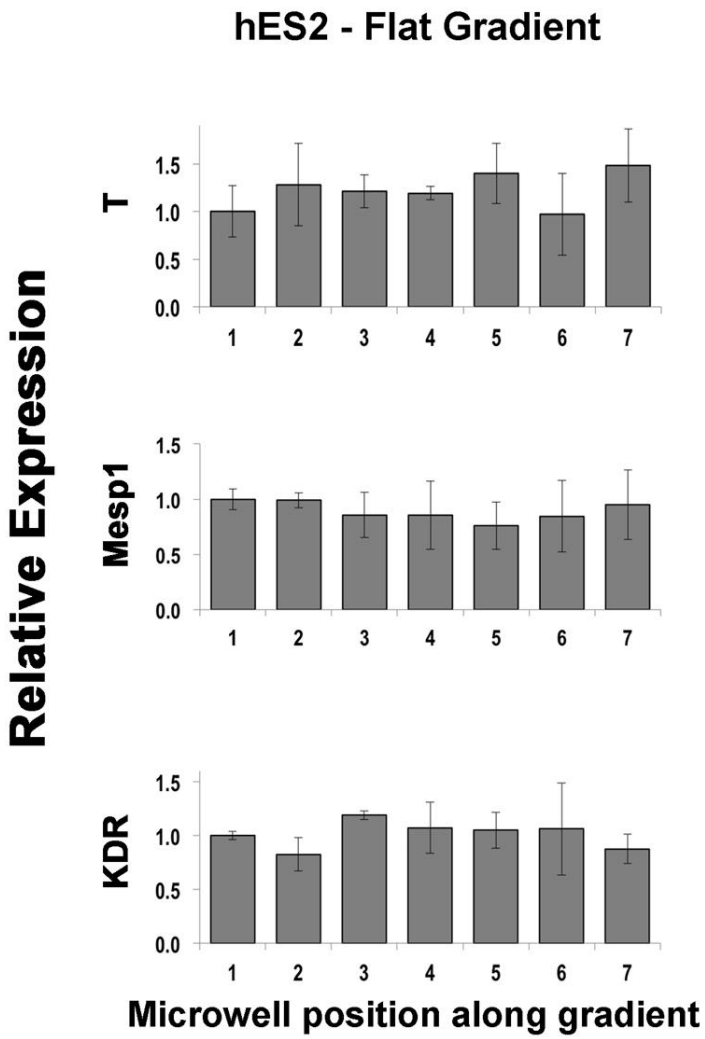


Supplementary Figure 1. Linear response of EBs exposed to a linear concentration gradient. hES2-derived EBs were exposed to a linear gradients of Calcein AM (which is easily transported through the cellular membrane, retained and enzymatically converted into fluorescent calcein by live cells) in culture medium. Representative images of EBs harvested from the microwells and the corresponding semi-quantitative measurements of fluorescence intensities are shown, to illustrate data used to validate the correct operations of our systems.



Supplementary Figure 2. Expression of mesodermal genes on hES2-derived EBs following static exposure to discrete ActivinA/BMP4 concentrations. Levels of gene expression, determined by qPCR and expressed relative to GAPDH, are shown for representative mesodermal and mesendodermal genes following exposure of hES2-derived EBs to sequential dilutions of ActivinA/BMP4 supplemented medium in statically operated microbioreactors. EBs were exposed to the cocktail of two morphogens for 24 hours between day3 and day4 of induction. The maximum concentration of 100% corresponds to 9 and 13 ng/mL of ActivinA and BMP4, respectively. 4 serial dilutions were performed, obtaining a total of 5 conditions.



Supplementary Figure 3. Expression of mesodermal genes in hES2-derived EBs following exposure to a constant concentrations of ActivinA/BMP4. Levels of gene expression, determined by qPCR and expressed relative to GAPDH, are shown for several mesodermal and mesendodermal genes following exposure of HES2 to a constant concentration of ActivinA/BMP4, established by flowing the same cocktail of cytokines in culture medium from both micro-bioreactors inlets. EBs were subjected to a stable concentration of the two morphogens for 24 hours between day3 and day4 since initiation of differentiation. Medium compositions were 9 and 13 ng/mL for ActivinA and BMP4, respectively, for both streams entering the system. Exposure to "flat gradient" of mesodermal factors resulted in a "flat" gene expression profile.