Supplemental Text

Discrepancies of fold-changes between immunofluorescent imaging and live-imaging

In figure 2C the max amplitude measured from immunofluorescent data is ~ 2.3, while the GFP amplitude is ~1.5. In most live-cell movies the max amplitude from GRP is ~1.7. All of this can be explained by two points: (1) The immunofluorescent antibody staining was imaged using secondary antibodies in the far-red spectrum where cellular-auto-fluorescence is minimal compared to the GFP spectrum and (2) GFP signal is reduced after PFA fixation. Thus, measurements in the far-red spectrum have less background signal and will show increased fold-change any time the initial signal is very low. Meanwhile, GFP signal in live-cells will always be higher than that in cells fixed with 4% PFA.

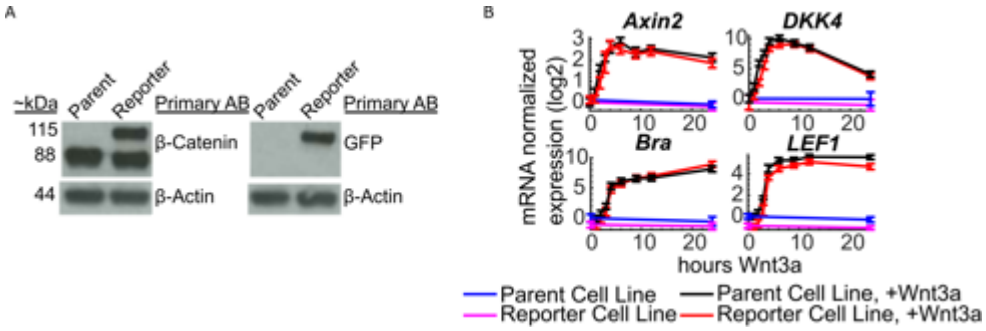
Strategies for improving measurement sensitivity

In our previous work on SMAD4 signaling (15), we employed higher resolution imaging (40X oil immersion objective, vs 20X air-gapped objective) and a more sophisticated, but more time-consuming, image quantification process where the nuclear fluorescent intensities were measured from the z-slice containing the majority of nuclear pixels. The data in supplemental figure 7 was obtained and analyzed in a similar way, and demonstrates that this strategy increases sensitivity of GFP-ß-catenin measurements in hESCs, as here the max amplitude over baseline approaches 3, versus 1.7 in our other experiments. However, in this work, our experiments required simultaneous imaging of many different experimental conditions in multi-well formats. Imaging with the oil immersion objective in this context required constant reapplication of oil throughout the experiment, largely due to the increased surface area required for the objective to travel at each time point, and proved to be very time-consuming. Therefore, in all of the live-cell experiments besides supplemental figure 7, we forgo use of the higher resolution objective in favor of an air-gapped 20X objective which was more suited to longer term imaging across multiple wells. Quantifying specific z-slices yielded very little gain in signal with the 20X objective versus quantifying a max intensity projection, and since it greatly increased computation time it was not included in our image quantification methods used in the other experiments.

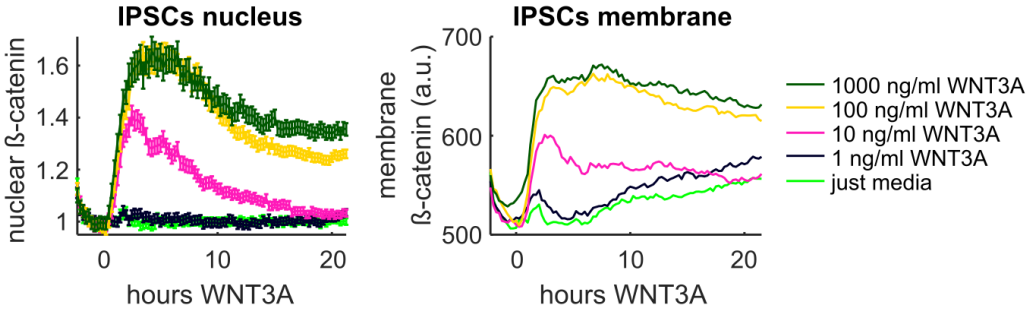
Potency of recombinant WNT3A varied across batch and manufacturer

Over the course of this study we observed substantial differences in the dose of WNT3A that saturated peak signaling when WNT3A came from a different batch, which ranged from 100ng/ml to more than 300ng/ml. In light of this, we made a bulk purchase of WNT3A from R&D systems, so that our data would be more comparable across experiments. We also obtained a small amount of WNT3A from AMSBIO (see supplemental figure 4) which appeared to be up to 100 times as potent as the WNT3A obtained from R&D systems. It is our recommendation that until manufacturers provide adequate quality control data describing the potency of each batch of WNT3A, researchers perform a dose response experiment on any WNT they obtain.

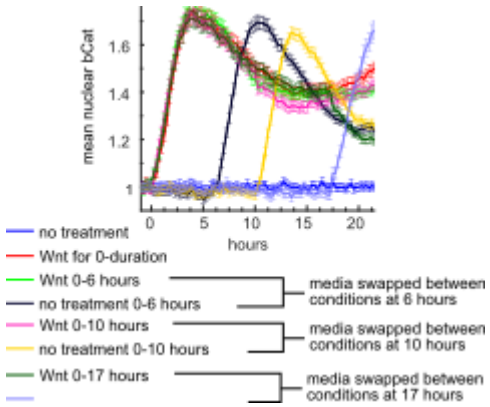
Supplemental Figures



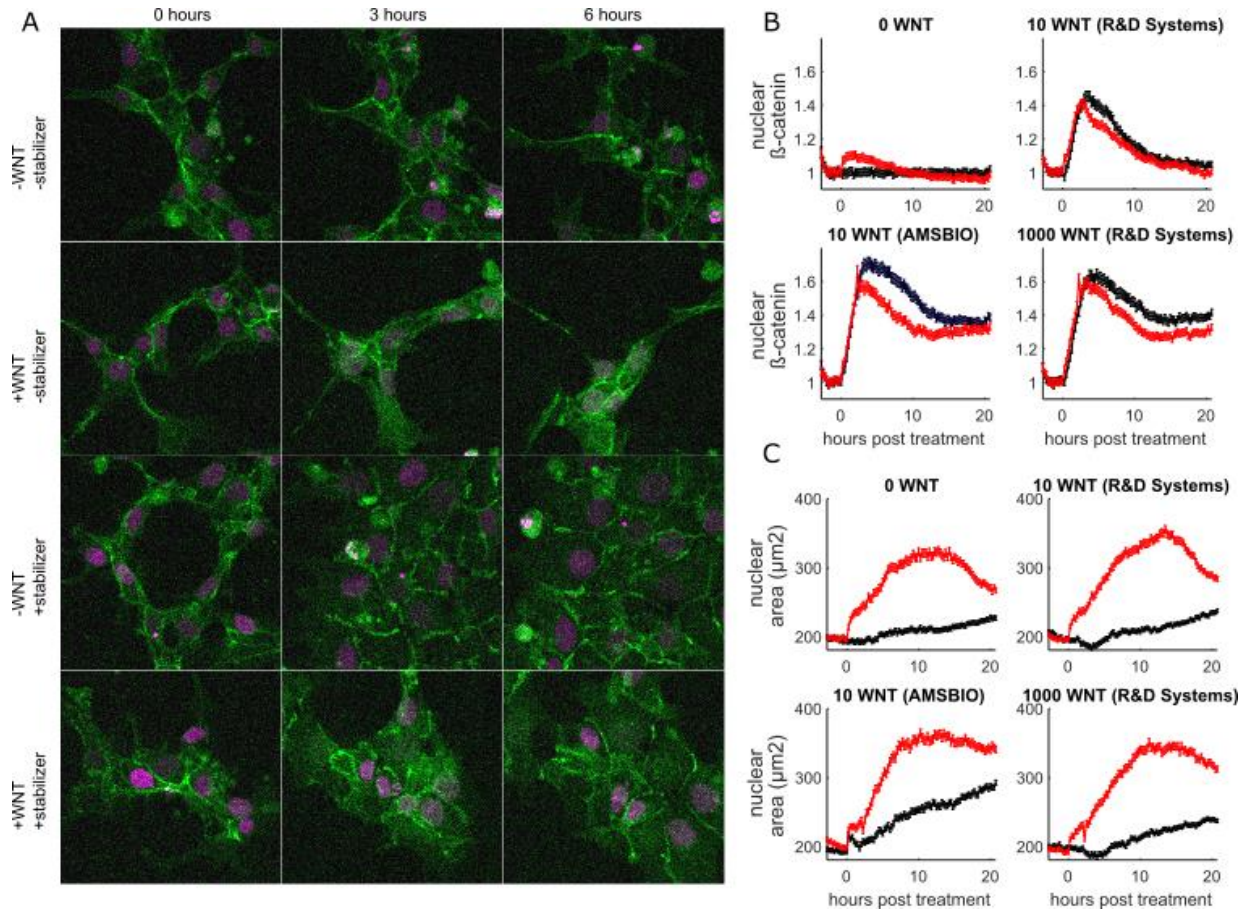
Supplemental Figure 1. Validation of CRISPR knock-in and WNT-ß-catenin target gene transcription dynamics in reporter hESCs. (A) Western-blot stained for anti-βcatenin or anti-GFP along with anti-β-Actin showing the 88 kDa β-catenin in parent and reporter cells, along with the 115 kDa GFP-βcatenin in reporter cells. (B) Induction of β-catenin target-genes measured by qRT-PCR in the parent and reporter cell line.



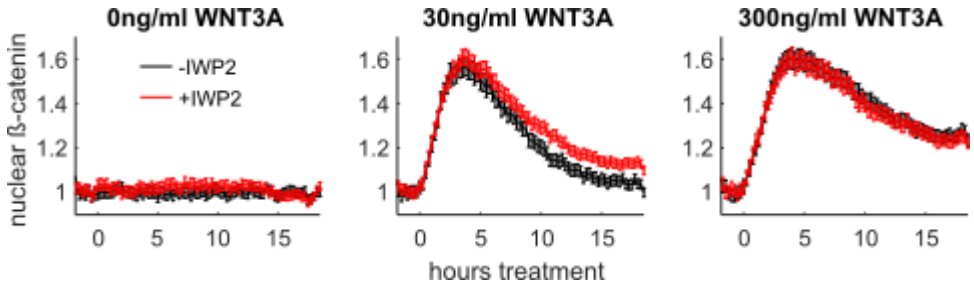
Supplemental Figure 2. IPSCs adapt to WNT. Quantification of time-lapse imaging of induced pluripotent stem cells containing GFP-labeled endogenous ß-catenin treated with indicated doses of WNT3A similar to Figure 2.



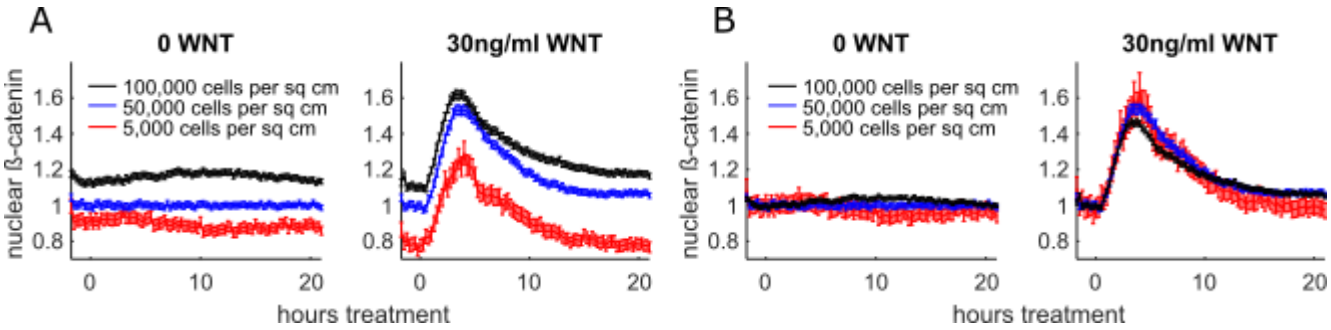
Supplemental Figure 3. Conditioned media with saturating WNT3A retains potential to activate signaling over entire period of adaptation. Quantification of time-lapse microscopy showing mean nuclear GFP-β-catenin trajectories in hESCs. Cells were treated with 1000ng/ml WNT3A and then media was swapped in between the indicated wells after 6, 10, or 17 hours.



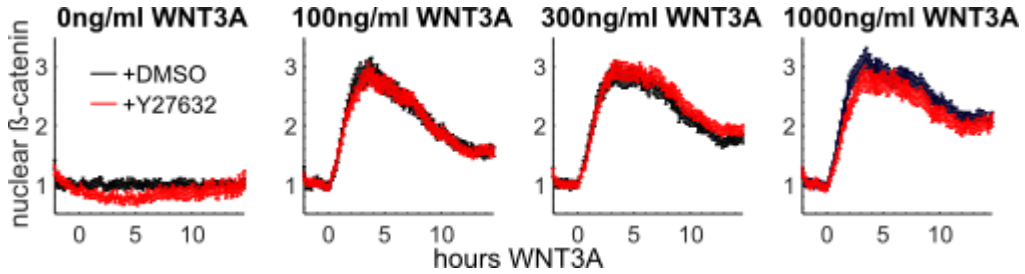
Supplemental Figure 4. WNT-stabilizer affects cell morphology but hESCs still adapt. (A) Representative images of cells treated with/without WNT with/without WNT-stabilizer reagent from AMSBIO. GFP-ß-catenin is green, RFP-H2B is magenta. (B-C) Quantification of nuclear ß-catenin (B) and nuclear area (C) from time-lapse movies of GFP-ß-catenin containing hESCs treated with (red) or without (black) WNT-stabilizer at the indicated concentrations of WNT3A from either R&D systems or AMSBIO. Recombinant WNT3A from R&D Systems is used throughout the rest of paper.



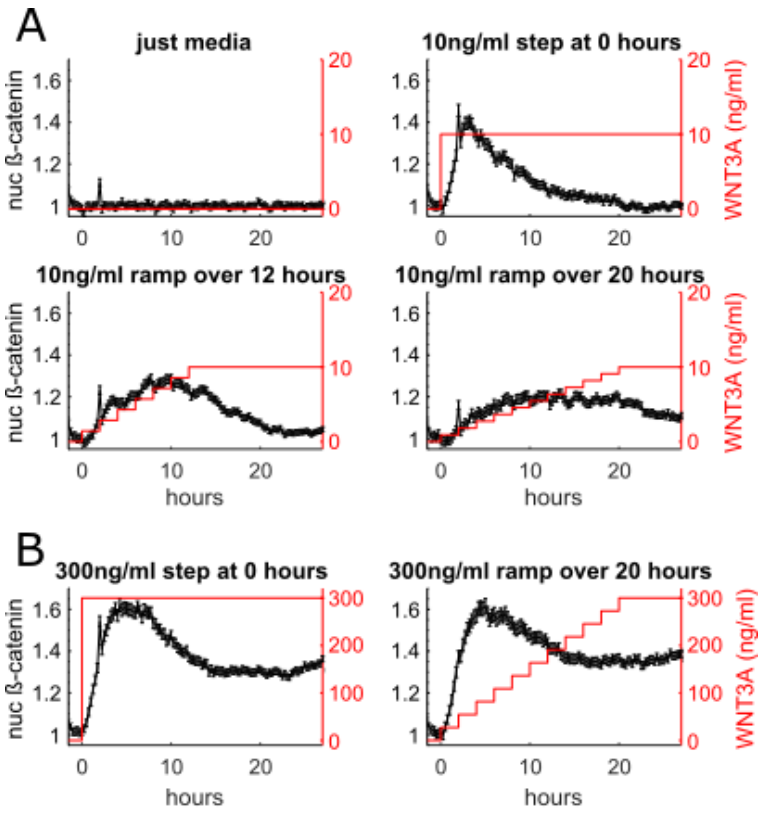
Supplemental Figure 5. Endogenous WNTs maintain a low level of signaling following adaptation at intermediate concentrations of exogenous WNT3A. Quantification of time-lapse movies of GFP-ß-catenin containing hESCs treated with WNT3A at indicated concentrations with (red) or without (black) endogenous WNT secretion inhibitor, IWP2.



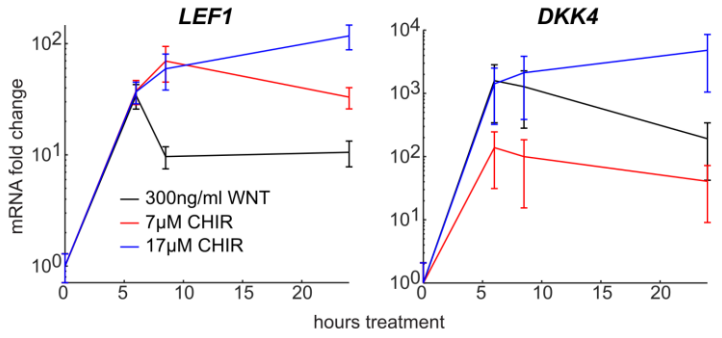
Supplemental Figure 6. Cell density affects the total amount of ß-catenin but not signaling dynamics. Quantification of nuclear GFP-ß-catenin in time-lapse movies of hESCs at indicated seeding densities treated with or without WNT3A. (A) Data is represented as ratio over 50,000 cells/cm^2 with 0 WNT. This is the standard seeding density in all other experiments. (B) Same data as in A, but data for each cell density are represented as ratio to mean signaling prior to WNT addition at that density. Error bars are SEM of cell means.



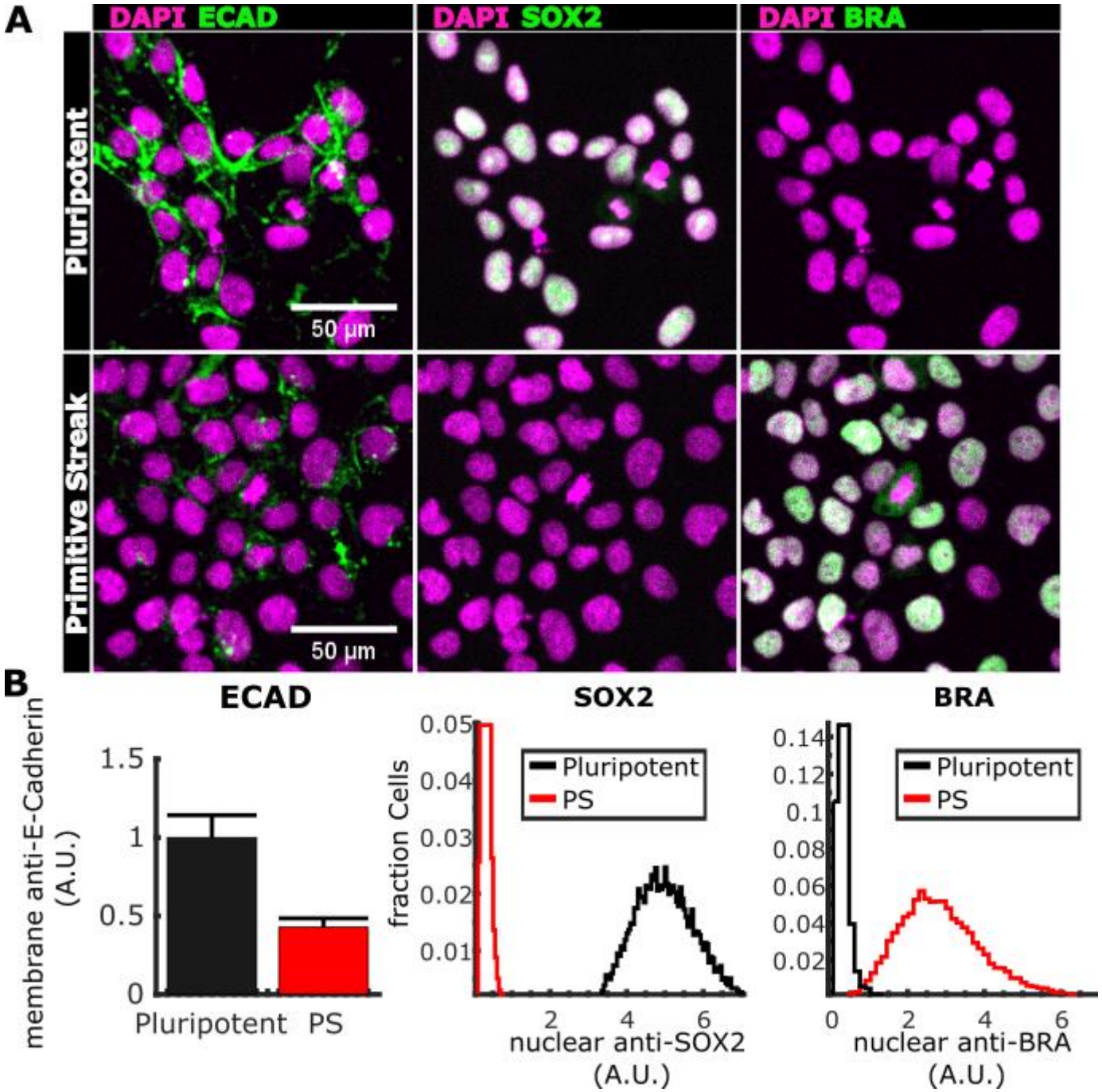
Supplemental Figure 7. RI does not affect WNT/ß-catenin signaling in hESCs. Quantification of GFP-ß-catenin in time-lapse movies of hESCs treated with indicated concentrations of WNT3A with ROCK inhibitor Y27632 (red) or DMSO (black). Error bars are SEM of cell means. In this experiment only, a strategy for increasing measurement sensitivity was used, and is responsible for the increased amplitude seen here. See supplemental discussion for a description of methods, and for why this approach was not used elsewhere.



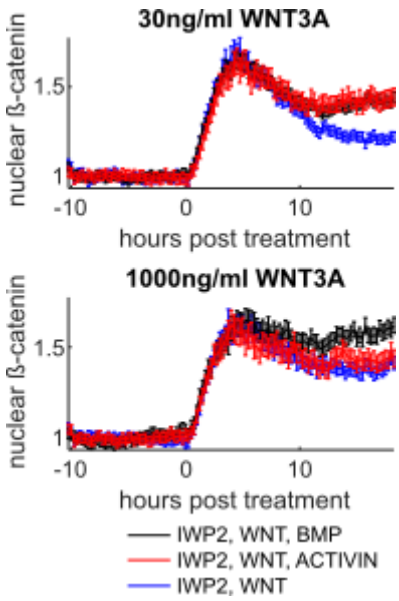
Supplemental Figure 8. Levels of nuclear ß-catenin are sensitive to ligand dynamics. Quantification of time-lapse movies of nuclear GFP-ß-catenin (black) in hESCs exposed to different ligand presentation schemes (red). Exogenous WNT3A is added all at once (step) or in smaller increments over time (ramp) where cumulative WNT3A is the same in step and ramps. Total dose of WNT was either 10 ng/ml (A) or 300 ng/ml (B).



Supplemental Figure 9. Transcript induction becomes more sustained with high CHIR99021. hESCs treated for indicated time with treatments specified in legend. mRNA induction is measured with qRT-PCR.



Supplemental Figure 10. Verification of primitive-streak markers in PS-like differentiated cells. (A) Representative images of hESCs maintained in the pluripotent state (top) or treated with primitive streak differentiating media (bottom) for 24 hours and stained for DAPI, anti-E-cadherin, anti-SOX2, and anti-Brachyury via immunocytochemistry. (B) Quantification of experiment in A. For ECAD, left, error bars represent SEM of 25 image means. For SOX2 and BRA histograms represent a minimum of 7x10^3 cells.



Supplemental Figure 11. Effect of Activin and BMP pretreatment on response to exogenous WNT3A. Quantification of nuclear ß-catenin from live cell imaging of cells treated with either Activin or BMP 10 hours (t = -10) before treatment with WNT3A (t = 0). IWP2 was maintained in the media for all conditions throughout the experiment.