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Supplemental Information

An *In Vitro* Human Segmentation Clock Model Derived from Embryonic Stem Cells

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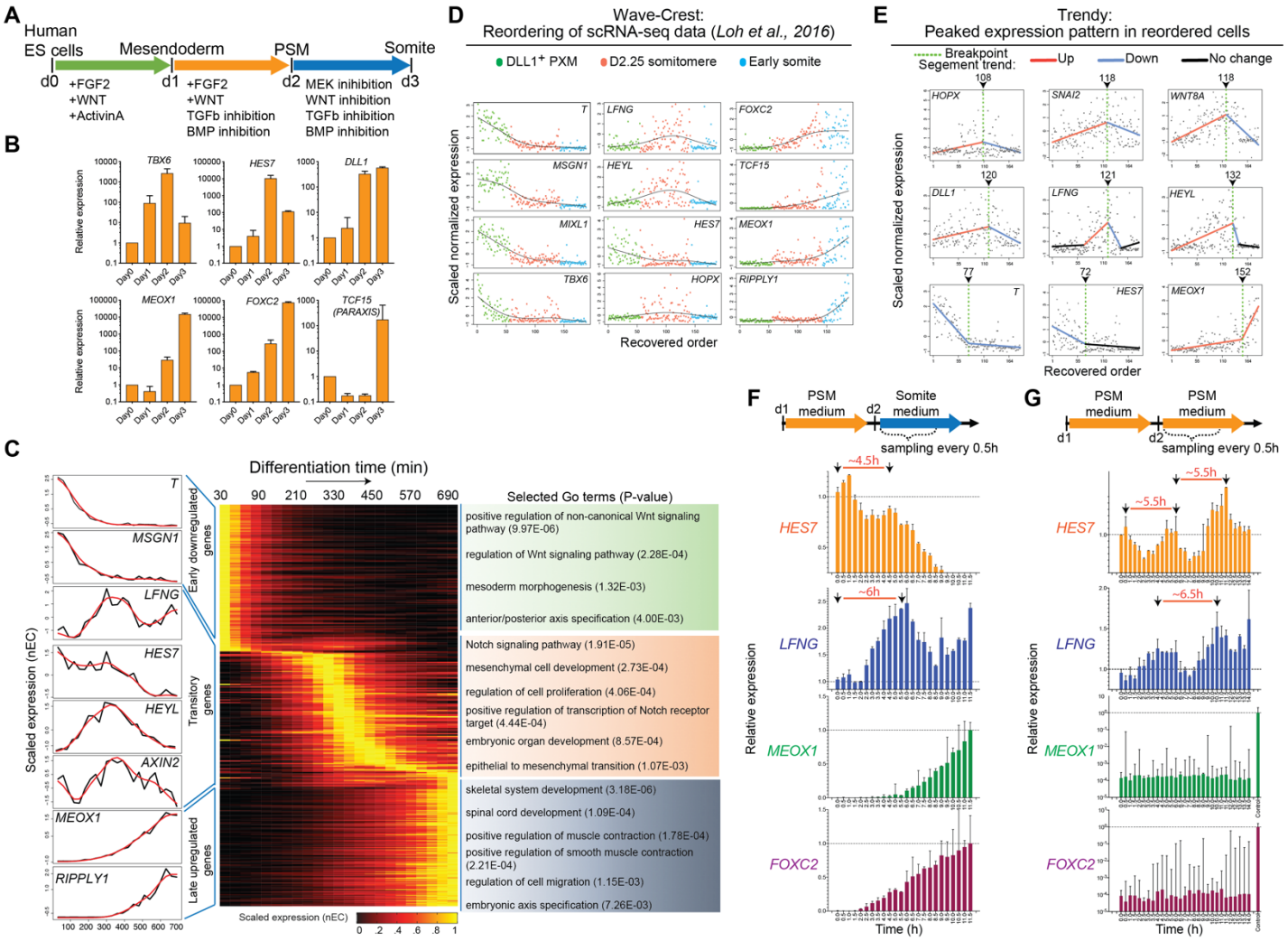
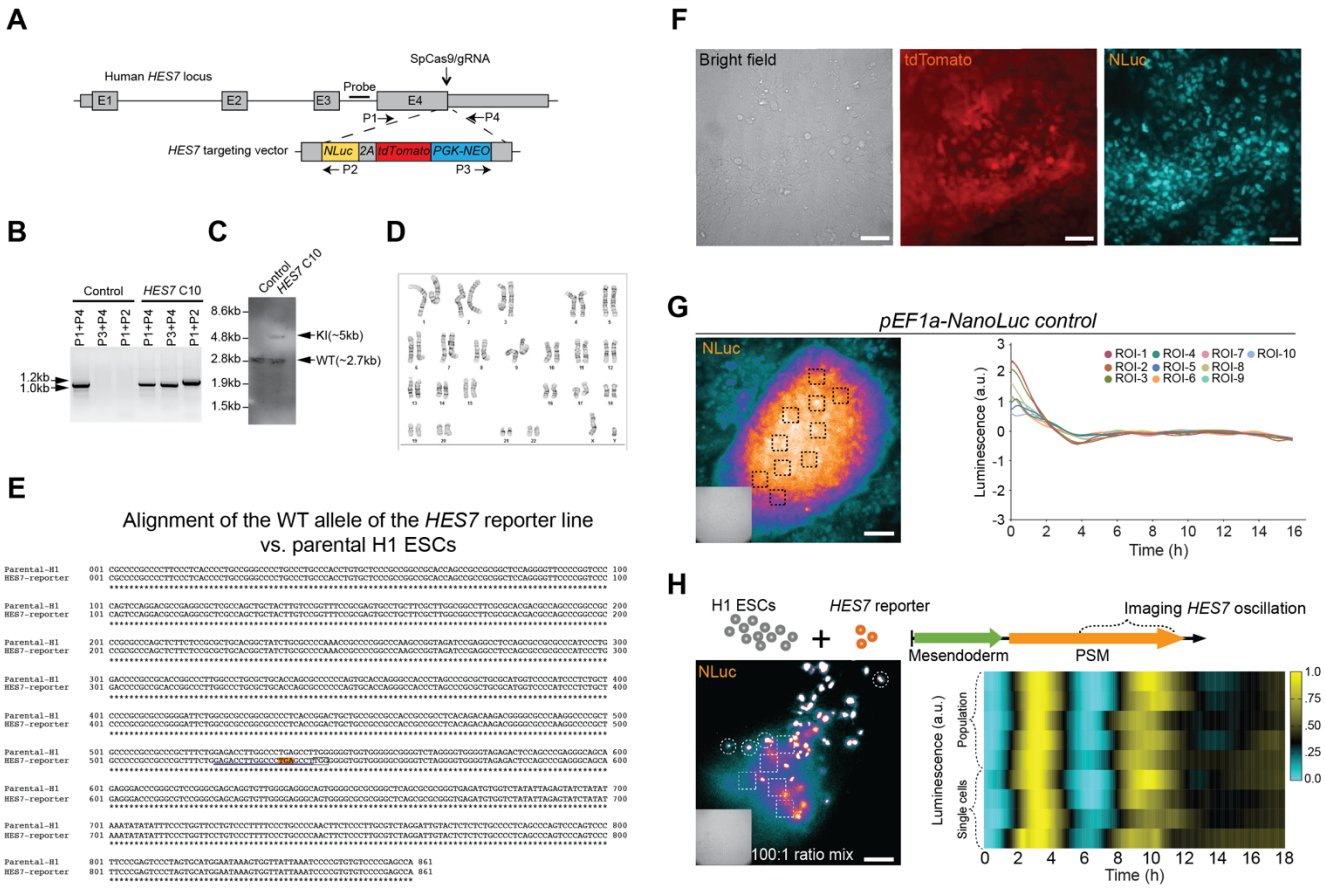
Figure S1



Supplemental Figure 1, related to Figure 1. Human ES cell differentiation to PSM and somite cell states. (A) Schematic of differentiation strategy, annotated with signaling pathway manipulations (see Methods). **(B)** qRT-PCR analysis of select markers representing ES, mesendoderm, PSM and somite state. All relative expression values were normalized to endogenous *GAPDH* expression than to Day0 samples. All data are presented as the mean ± S.D. **(C)** Left and middle panels, representative genes and a heatmap of gene expression selected from temporal RNA-seq data, shown top 100 early downregulated genes, 120 transitory genes and 162 late upregulated genes. The x-axis indicates the differentiation time as samples were collected. Expression values (normalized expect count, nECs) from replicate samples were first averaged then scaled minimum to maximum expression per gene row and smoothed. Expression value scale is indicated as a horizontal bar. Right panel, enriched top GO terms from each category indicated (see Methods). **(D)** Temporal reordering of scRNA-seq data (from Loh et al., 2016) using Wave-crest with twelve marker

genes. The x-axis shows the reordered single cells. The y-axis shows scaled gene expression values, nECs. Each cell type is color annotated from the original study. **(E)** Examples of peak/breakpoint cells identified by Trendy with the selected markers. The number, arrows and green dashed lines indicate the number of peak/breakpoint cells identified by Trendy. The fitted trend is overlaid and color annotated: red, upward trend; blue, downward trend; black, flat trend. **(F)** Upper panel, schematic of RNA-seq profiling collecting samples every 30 minutes for 12 hours, immediately after switching to somite medium. Lower panel, RT-qPCR results of *HES7*, *LFNG, MEOX1* and *FOXC2*. All expression levels were first normalized to endogenous *GAPDH*, then to the first time point (*HES7, LFNG*) or the last time point (*MEOX1, FOXC2*). Arrows indicate estimated peak-to-peak duration. All data are presented as the mean ± SD. **(G)** Upper panel, schematic of time course experiments collecting samples every 30 minutes for 14 hours, immediately after feeding cells with fresh PSM medium. Lower panel, RT-qPCR results of *HES7*, *LFNG, MEOX1* and *FOXC2*. All expression levels were first normalized to endogenous *GAPDH*, then to either the first time point (*HES7*, *LFNG*) or a control somite cell state sample (*MEOX1, FOXC2*). Because *MEOX1* and *FOXC2* are minimally expressed, a log scale is applied. Arrows indicate estimated peak-to-peak duration. All data are presented as the mean ± SD. ES, embryonic stem; PSM presomitic mesoderm.

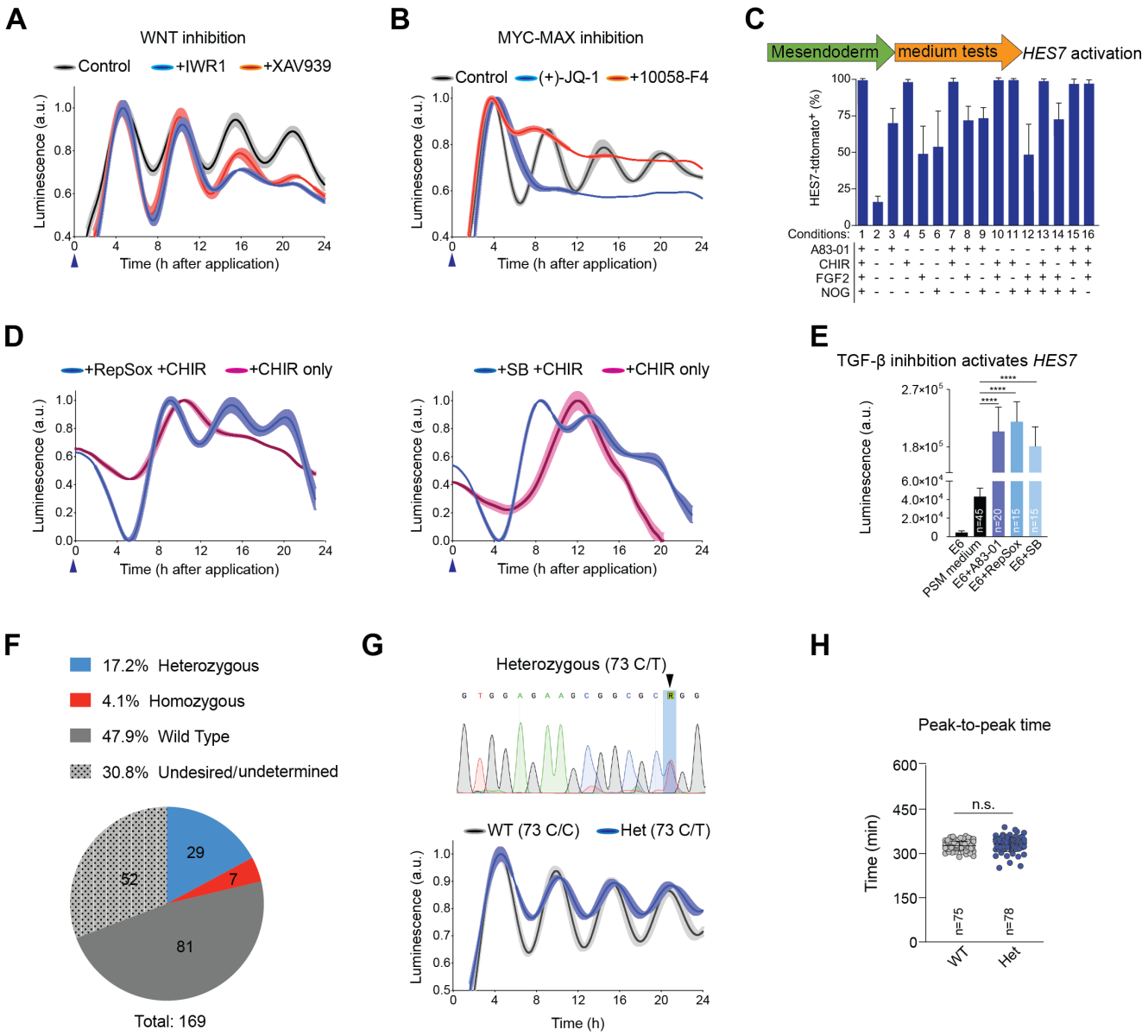
Figure S2



Supplemental Figure 2, related to Figure 2. Detection and quantification of *HES7* oscillation. (A) Gene targeting strategy of knock-in *HES7-NLuc-2A-tdTomato-PGK-NEO* cassette. The position of the CRISPR-Cas9/gRNA cut site is indicated. Gray boxes indicate the exons of *HES7*. The positions of DNA oligos used for junction PCR and Southern blotting probe are indicated. **(B)** Junctional PCR to confirm the knock-in cassette of the correct targeted clone, C10. Control: genomic DNA from parental H1 human ES cells. **(C)** Southern blotting result of correct targeted clone, C10. Control: genomic DNA from parental H1 cells. **(D)** Confirmed normal karyotype of the correctly targeted clone after expansion. **(E)** Pairwise alignment results confirmed 100% match of WT allele sequences obtained from parental H1 human ESC and *HES7*-reporter lines. WT allele specific primers were used to amplify ~861 bps surrounding the CRISPR-Cas9 cutting cite. Stop codon is highlighted in orange. The gRNA sequences is indicated by underline, PAM domain is indicated by a box. **(F)** Live cell images of *HES7* reporter line differentiated to PSM state with brightfield, tdTomato and NLuc expression. Luminescence detection channel (NLuc) is pseudo colored in cyan. All scale bars = 100 μm. **(G)** Negative control for oscillatory luminescence imaging analysis. A control H1 human ES cells carry a

NLuc transgene expression under the control of a constitutive promoter (*EF1a)*. Left panel, a representative frame of *NLuc* luminescence overlay with the locations of 10 region of interests (ROIs) indicated in black boxes. Right panel, quantification results of 10 ROIs over 16 hours of time lapse imaging. The y axis indicates the luminescence signals (scaled from minimum to maximum from representative samples, arbitrary unit [a.u.]). Insert shows the corresponding brightfield image. **(H)** Upper panel, experimental strategy for mixing *HES7* reporter line with parental H1 human ES cells for luminescence imaging analysis. Left panel, a representative frame of NLuc luminescence overlay with the locations of 5 multiple-cell ROIs (white boxes) and 5 single-cell ROIs (white circles). Right panel, the quantification results of all 10 ROIs over 18 hours of time-lapse imaging shown in heatmap. The y axis indicates the luminescence signals (scaled from minimum to maximum from representative samples, in a.u.). Insert shows the corresponding brightfield image. All scale bars = 100 μm. ES, embryonic stem.

Figure S3



Supplemental Figure 3, related to Figure 3. Characterizations of *HES7* oscillation and modeling SCDO4 with genome editing. (A) The impact of inhibiting WNT signaling on *HES7* oscillation. The control data (PSM medium, black line) is obtained from the same experiment. Small molecules applied are indicated by color. The blue arrowhead indicates the time (0h) when the luciferin substrate and small molecules were applied. All luminescence oscillation data presented is scaled from minimum to maximum signal and shown as mean ± SEM (shaded area), obtained from 4 to 5 replicates in each experiment, y axis in a.u. **(B)** The impact of inhibiting MYC-MAX transcriptional activity on *HES7* oscillation. The data are annotated the same ways as in (A). **(C)** Medium test on the *HES7* activation

measured by flow cytometry for the percentage of tdTomato-positive cells with combinations or dropouts of the small molecule inhibitors or growth factors in PSM medium. A total of 16 conditions are shown, with data collected from four independent differentiation experiments. All data are presented as the mean ± SD. **(D)** Comparing *HES7* oscillation when replacing A83-01 (TGF-β signaling inhibition) with RepSox or SB (SB 431542) in combination with CHIR (CHIR99021, WNT signaling activation) at the second day of differentiation (blue in both panels) as indicated in condition 7 in (C). No *HES7* oscillation is detected in CHIR only (magenta) condition as indicated in condition 4 in (C). All data are presented as the mean ± SEM. **(E)** Quantification of *HES7* expression level after switching to indicated medium with TGF-β inhibitors or controls. E6: base medium. Control: PSM medium. SB: SB-431542. **** p-value <0.0001, Student's t-test. Number of samples analyzed are indicated. All data are presented as the mean ± SD. **(F)** Summary statistics for clones bearing point mutations in *HES7* locus. **(G)** Upper panel, sequencing results of a representative heterozygous clone for *HES7* 73C-T mutation. The arrowhead indicates the mutated nucleotide, highlighted as R (C/T). Lower panel, representative *HES7* oscillation profile overlay the parental line (black) and a heterozygous clone (blue). All data are presented as the mean ± SEM. **(H)** Quantification of oscillation peak-to-peak time between the parental line and a representative heterozygous clone. n.s., not significant difference, Student's t-test. The number of peaks quantified are indicated.