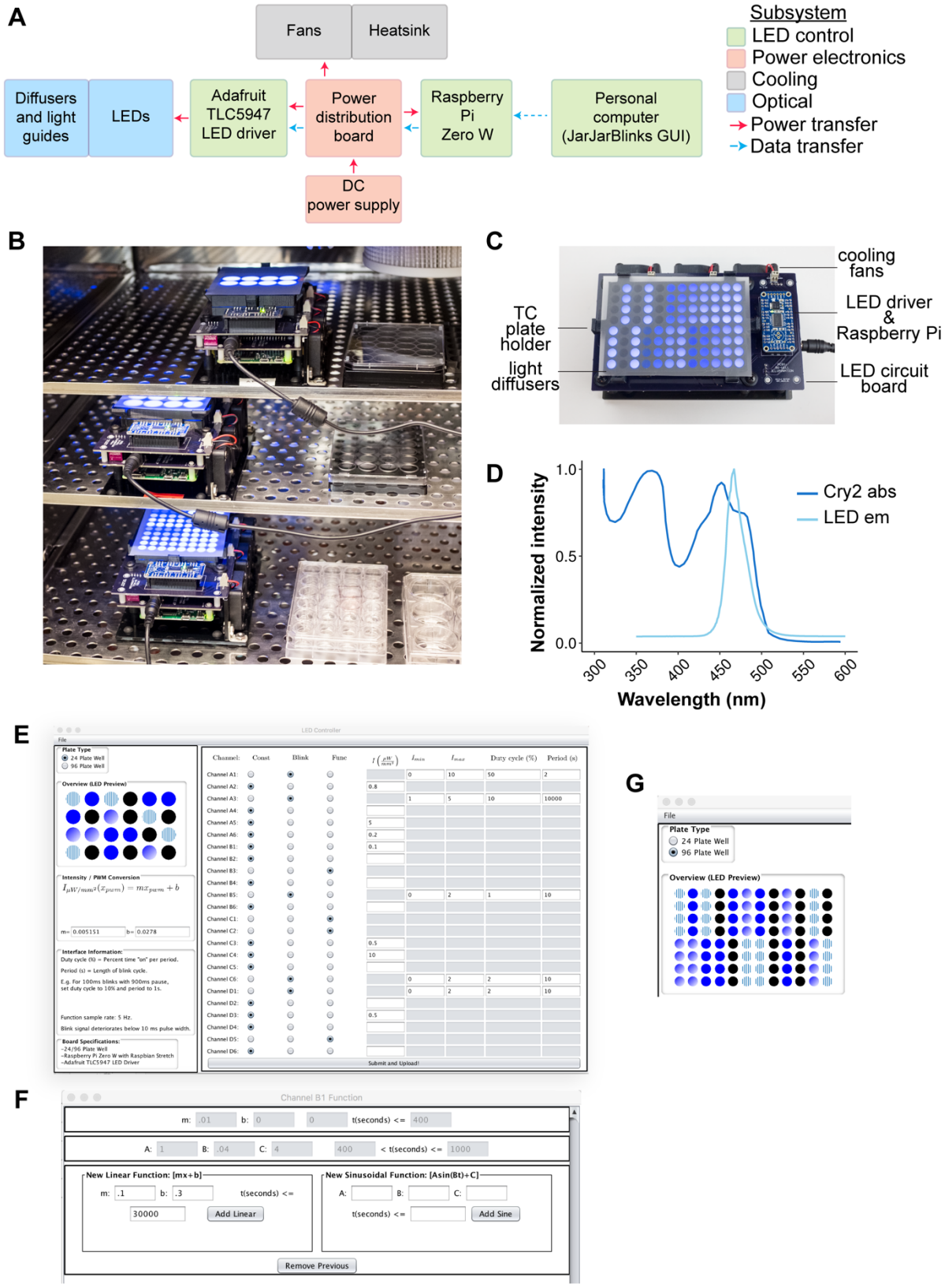
Cell Reports, Volume *31*

Supplemental Information

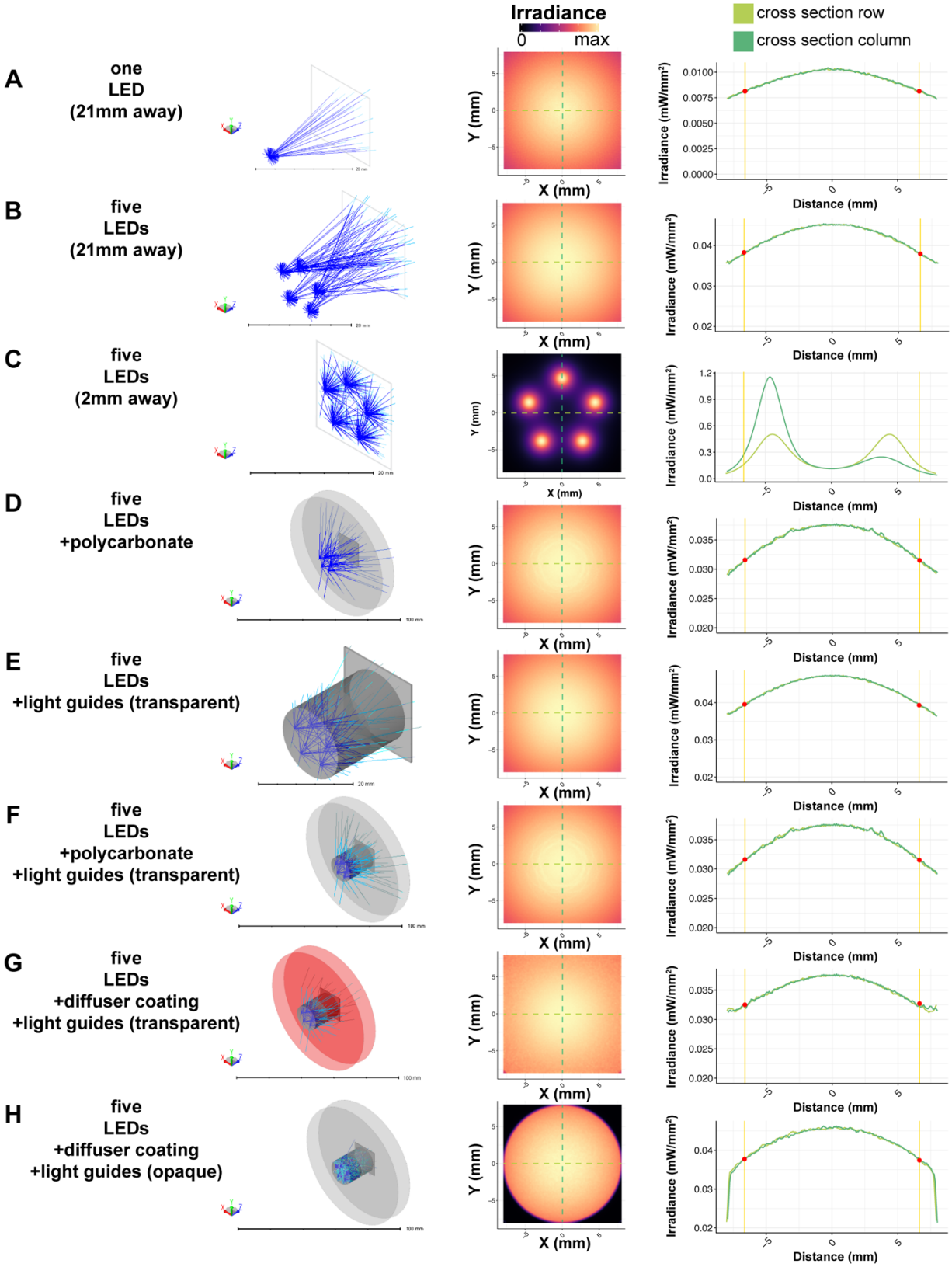
Engineered Illumination Devices for Optogenetic Control of Cellular Signaling Dynamics

Nicole A. Repina, Thomas McClave, Hunter J. Johnson, Xiaoping Bao, Ravi S. Kane, and David V. Schaffer



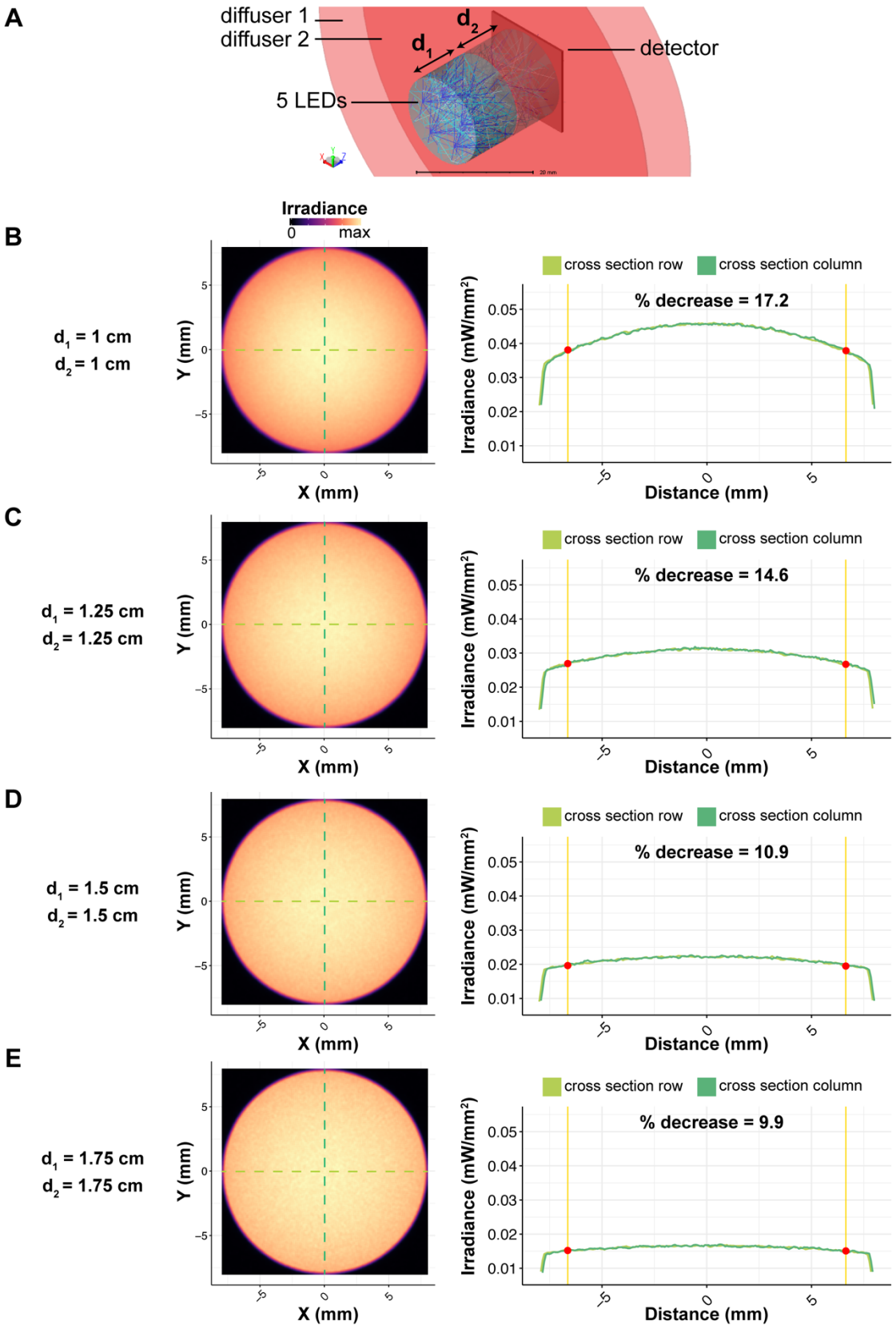
Supplementary Figure S1. Overview of 24-well and 96-well LAVA devices. Related to Figure

1. **a)** System block diagram of LAVA device. **b)** Image of 24-well and 96-well LAVA devices in TC incubator. **c)** Image of assembled 96-well LAVA board, with optical, cooling, and electronic subsystems highlighted. **d)** Emission spectrum of 470 nm blue LEDs match the absorption spectrum of Cry2. Cry2 spectrum adapted from reference (Banerjee et al. 2007). **e)** Screenshot of GUI under 24-well TC plate setting. **f)** Screenshot of dialog box for input of time-varying light patterns as piecewise functions. **g)** Screenshot of GUI under 96-well TC plate setting.



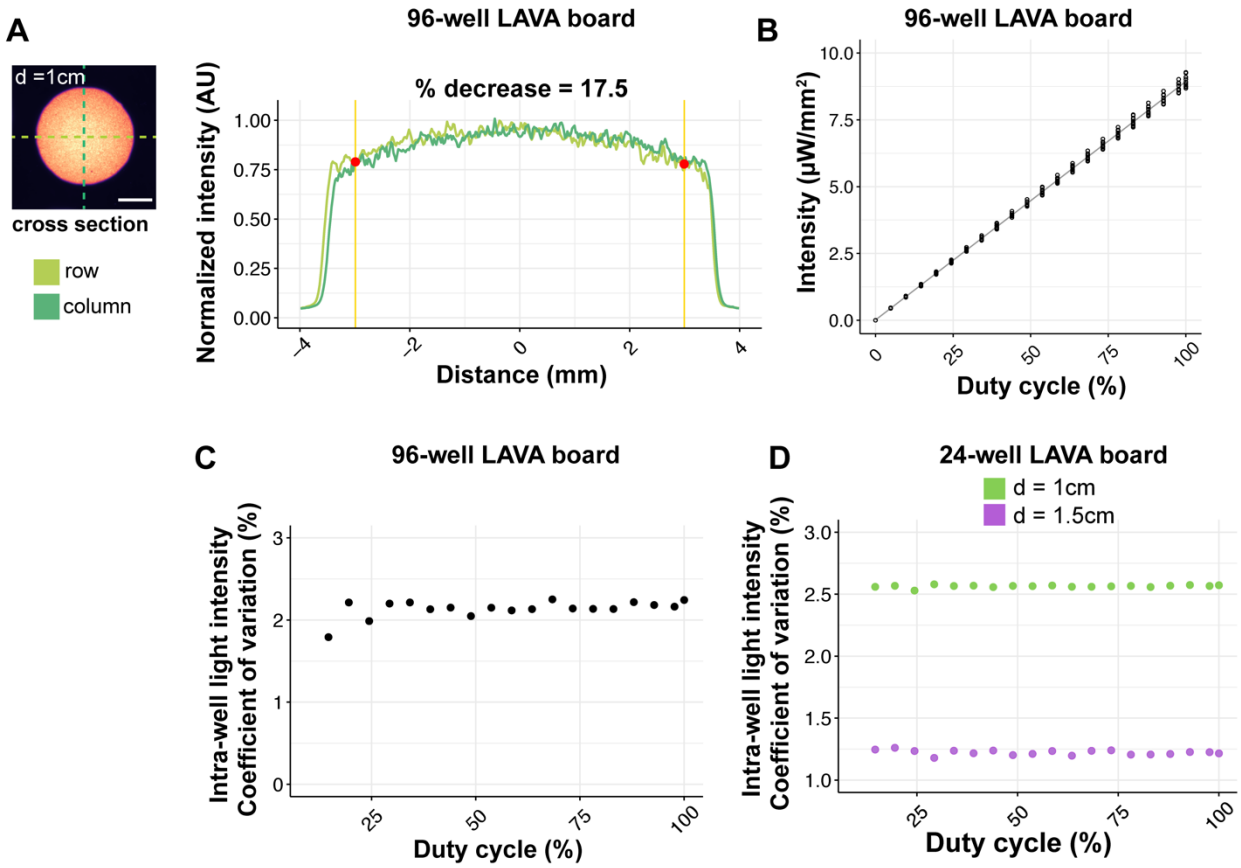
Supplementary Figure S2. In silico validation of Zemax ray tracing model. Related to Figure 2.

Schematic of LED configuration (left), modeling result at detector plane (middle), and column and row cross-sections (right) with well edge of 24-well plate indicated with red points. **a)** Single LED illuminating detector 21mm away. **b)** Five LEDs, distributed along 1cm diameter circle, illuminating detector 21mm away. **c)** Five LEDs illuminating detector 2mm away. **d)** Five LEDs illuminating detector 21mm away through two 0.01" thick sheets of polycarbonate. **e)** Five LEDs illuminating detector 21mm away through two 10mm transparent light guides. **f)** Five LEDs illuminating detector 21mm away through two 0.01" thick sheets of polycarbonate and two 10mm transparent light guides. **g)** Five LEDs illuminating detector 21mm away through two 0.01" thick sheets of polycarbonate with 80˚ diffuser coating and two 10mm transparent light guides. **h)** Five LEDs illuminating detector 21mm away through two uncoated 0.01" thick sheets of polycarbonate and two 10mm reflective light guides with Lambertian scattering.

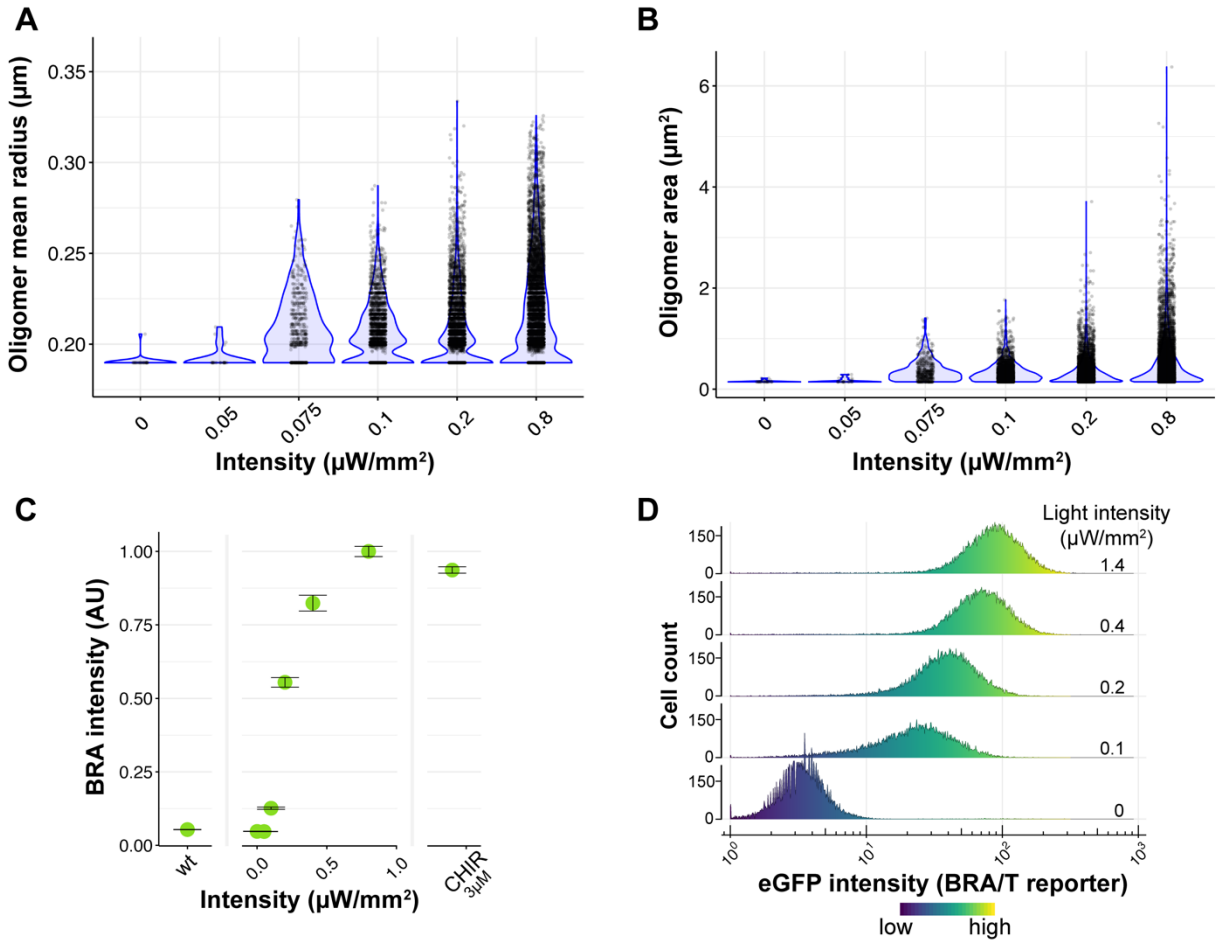


Supplementary Figure S3. Results of Zemax modeling at variable light guide thicknesses, d_1

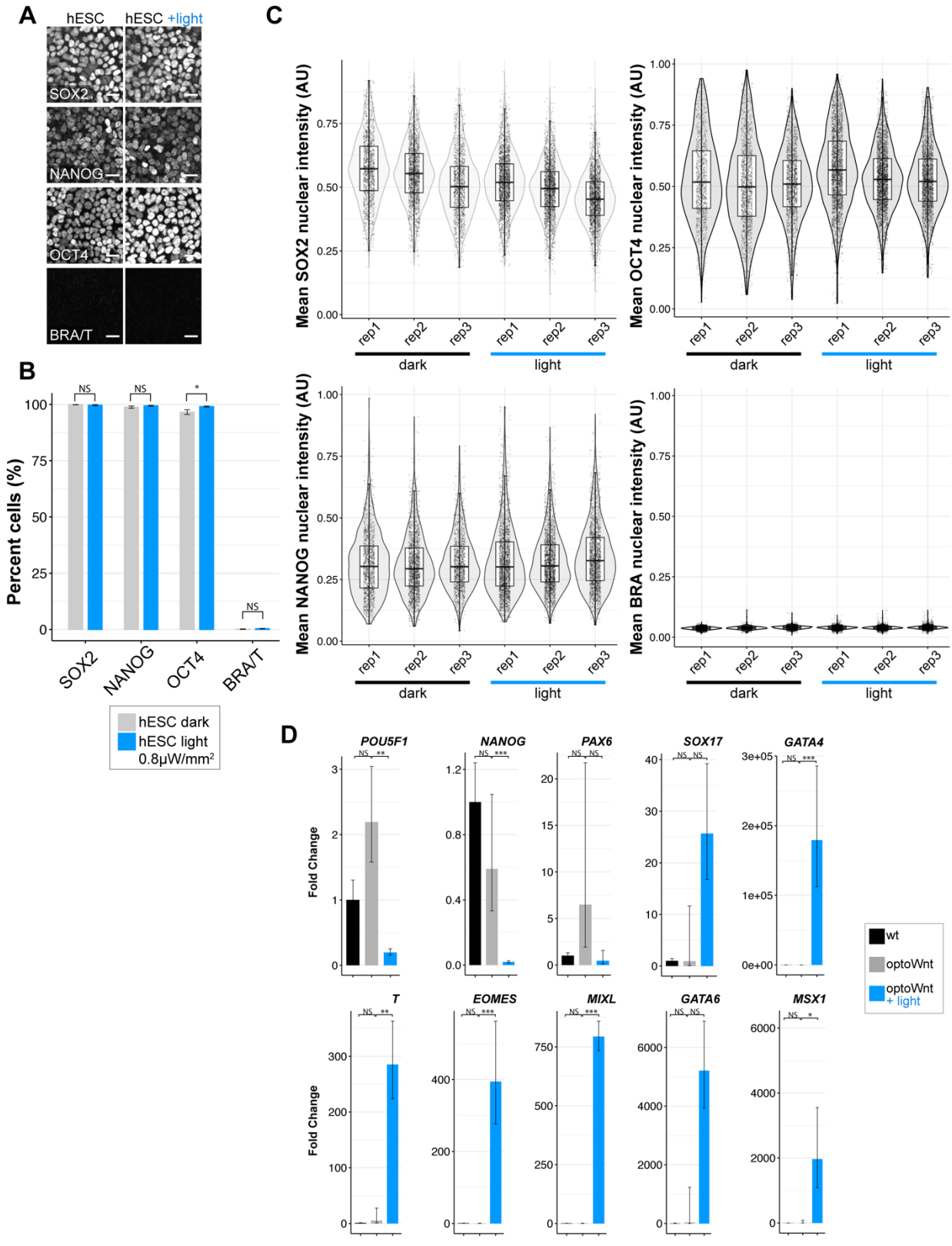
and d_2. Related to Figure 2. **a)** Schematic of modeling setup. Five LEDs illuminate detector through two 0.01" thick sheets of polycarbonate with 80˚ diffuser coating (red) and two reflective light guides with Lambertian scattering (grey cylinder). **b-e)** Modeling results at indicated values of d_1, d_2. Image at detector plane (left) and column and row cross-sections (right) with well edge of 24-well plate indicated with red points show improved illumination uniformity at expense of light intensity with increasing light guide thickness.



Supplementary Figure S4. Intensity characterization of 24-well and 96-well LAVA boards. Related to Figure 2. **a)** Brightfield images of well (left) and graph of intensity linescans along indicated cross-sections (right) characterize the intensity uniformity of the 96-well LAVA board when d = 1 cm. Percent decrease is calculated between intensity at center of well and intensity at highlighted red point, which indicates location of well edge of a 96-well culture plate. Graph shows mean normalized intensity over 2 independent wells. Scale bar 2.5 mm. **c)** Measured light intensity in response to the programmed duty cycle of the LED pulse-width modulation signal. Graph shows measured intensity from wells of a 96-well LAVA board and curve fit to a linear regression model. **d)** Coefficient of variation of light intensity between the 24 independent light channels of a 96-well LAVA device measured at different programmed intensities. **e)** Coefficient of variation of light intensity between the 24 independent light channels of a 24-well LAVA device measured at different programmed intensities. Green points correspond to optical configuration with d = 1 cm, violet points show optical configuration with d = 1.5 cm.

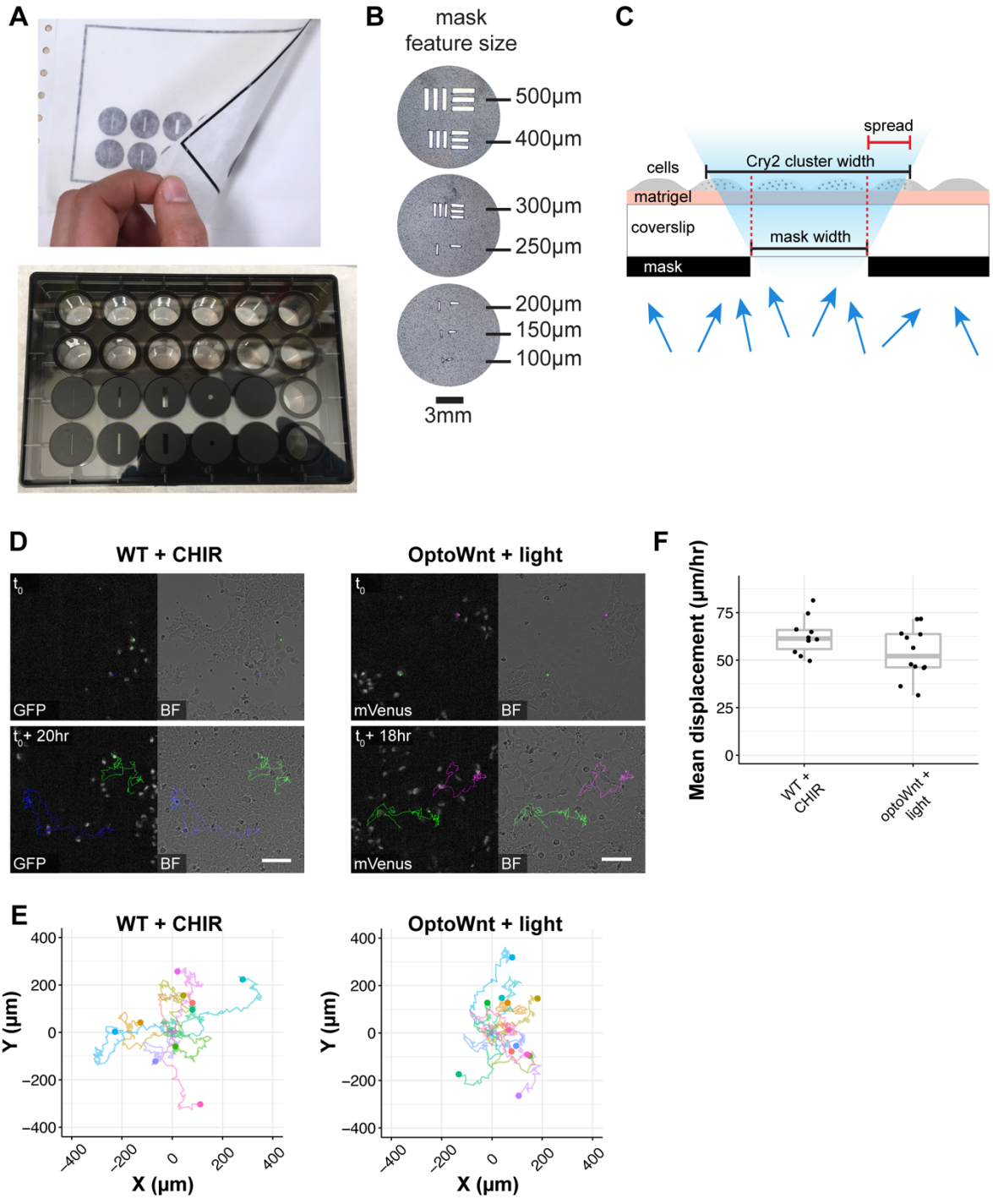


Supplementary Figure S5. LRP6 oligomer size and BRA expression in optoWnt cells is dose-responsive to light intensity. Related to Figure 4. **a-b)** Quantification of LRP6 oligomer size from immunostaining of optoWnt hESCs illuminated at indicated light intensities for 1 hr. Each point represents an LRP6 oligomer, with > 100 cells analyzed per condition. **c)** Quantification BRA immunostaining in response to increasing light intensity after 24 hr illumination or 3 μM CHIR treatment. Average BRA intensity per hESC was calculated for each biological replicate. Graph shows mean of biological replicates ± 1 s.d., n = 3 replicates. **d)** Flow cytometry histograms of optoWnt hESCs expressing eGFP reporter for BRA/T after 24 hr illumination at varying light intensities. Graph shows sum of n = 3 replicates.



54
55 **Supplementary Figure S6.** Characterization of phototoxicity and spontaneous differentiation

during continuous optogenetic stimulation of hESC cultures. Related to Figure 3. **a)** Representative fluorescence images of wild-type hESCs stained for cell fate markers SOX2, NANOG, OCT4, and BRA after 48 hrs illumination at 0.8 μW/mm^2. Scale bar 25 μm. **b)** Quantification of images shown in (a). Student's t-test (two-tail). Graph shows mean $\pm$ 1 s.d., n = 3 biological replicates. **c)** Intensity distribution of pluripotency and differentiation markers shown in (a). Graph shows mean nuclear intensity of indicated marker for each biological replicate (rep1-rep3). Each point corresponds to single cell. **d)** qPCR analysis of wild-type and optoWnt cells shows no detectable spontaneous cell differentiation in unilluminated optoWnt cells. Graphs show mean fold change $\pm$ 1 s.d., n = 3 biological replicates. ANOVA followed by Tukey test.



Supplementary Figure S7. Spatial control of illumination and single-cell migration tracking during Wnt activation. Related to Figure 6. **a)** Images of adhesive die-cut masks applied using transfer tape (top) onto 24-well TC plate (bottom). **b)** Brightfield images of die-cut mask illustrate resolution limit of die cutter. Scale bar 3 mm. **c)** Schematic of light scattering from photomask. **d)** Representative images of hESC cell migration during Wnt activation. Images at the start of migration timecourse (top) and after the indicated time interval (bottom) are displayed with an overlay of single-cell migration track. Wnt activation was achieved with CHIR treatment of wild-

type cells (3 μM**,** left) or light stimulation of optoWnt cells (right). Cell tracking was started 24 hrs after initial CHIR treatment or light stimulation (t_0 = 24 hrs). Brightfield (BF) images show the entire cell population within the field of view, with the sparse fluorescent subpopulation used for cell tracking (~10% of total cells) visualized in the fluorescence channel (GFP+ wild-type hESCs, mVenus+ optoWnt hESCs). Scale bar 100 μm. See also Supplementary Videos 2-3. **e)** Single-cell migration trajectories from timecourses displayed in (a). Each color represents individual cell trajectory (n=10 wild-type+CHIR, n=11 optoWnt+light). **f)** Quantification of mean cell displacement for trajectories shown in (e). Total cell displacement (μm) was normalized to trajectory duration (hr) for each tracked cell. Each point represents single cell.

Supplementary Table 1. Primary antibodies used for immunostaining. Related to Figures 4 - 6.

Antigen	Species	Antibody	Concentration
LRP6	Rbt	Ab134146	1:1000
BRA	Rbt	Sc20109	1:500
β-CATENIN	Rbt	Cell sig 8480	1:500
SLUG	Rbt	Cell sig 9585	1:500
OCT4	Ms	Sc5279	1:500
SOX2	Rbt	Ab97959	1:500
NANOG	Ms	Ab62734	1:500