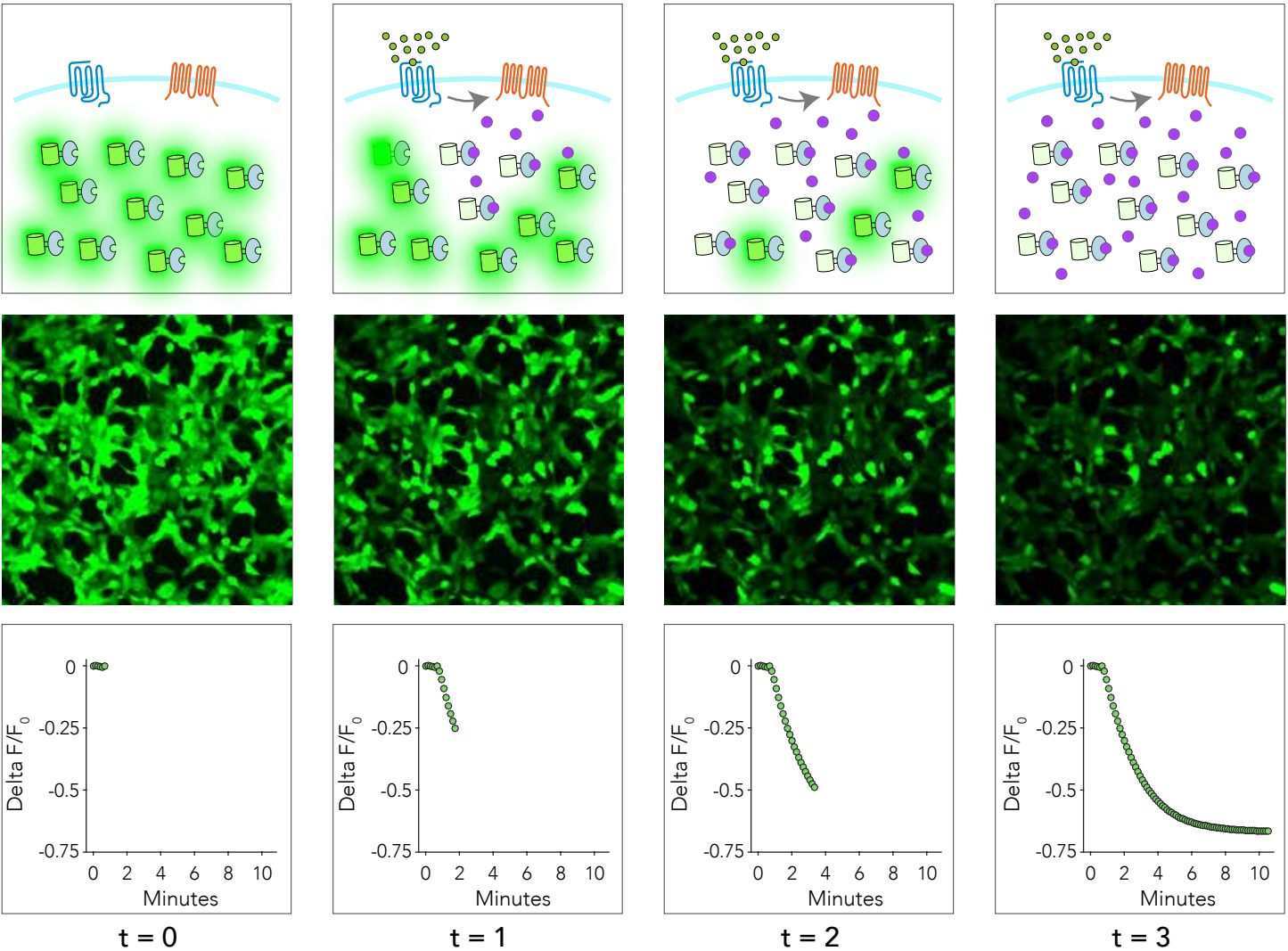
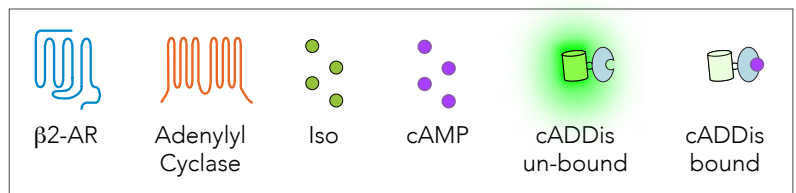




Taking a Closer Look at cAMP Production with cADDIs.



Top Row: cADDIs detects cAMP in living cells. At $t = 0$, no ligand is present, cAMP levels are low and the sensor is bright. At $t = 1$, Isoproterenol binds β 2-adrenergic receptors and adenylyl cyclase converts cellular ATP into cAMP. Fluorescence decreases as the population of cAMP bound to cADDIs increases. At $t = 3$ cAMP levels have saturated cADDIs and the fluorescence is at its lowest point.



Middle Row: HEK293 cells expressing cADDIs at the 4 depicted timepoints: Images were obtained on epi-fluorescence inverted microscope with blue LED excitation, 520/35 collection of fluorescence, and a 10x water immersion objective. Note the decrease in cell brightness from $t = 0$ to $t = 3$, indicating increased cellular cAMP concentration.

Bottom Row: Plot of fluorescence over time from the images in Middle Row. Fluorescence from cADDIs is plotted as $\Delta F/F_0$, where ΔF is equal to the absolute difference in fluorescence values between a given timepoint to the fluorescence at $t = 0$ and F_0 is equal to the fluorescence at $t = 0$.