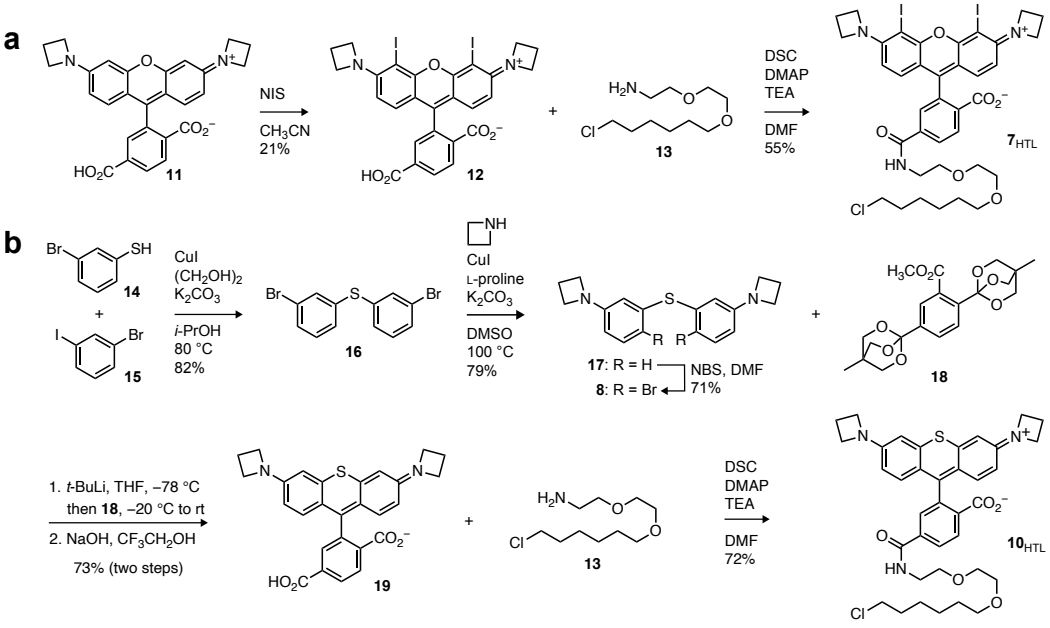


**Figure S1. Synthesis Schemes for Photosensitizer Janelia Fluor HaloTag Ligands, Related to Figure 2.** (**a**) Synthesis of $JF_{567}$–HaloTag ligand (**$7_{HTL}$**). (**b**) Synthesis of $JF_{570}$–HaloTag ligand (**$10_{HTL}$**).

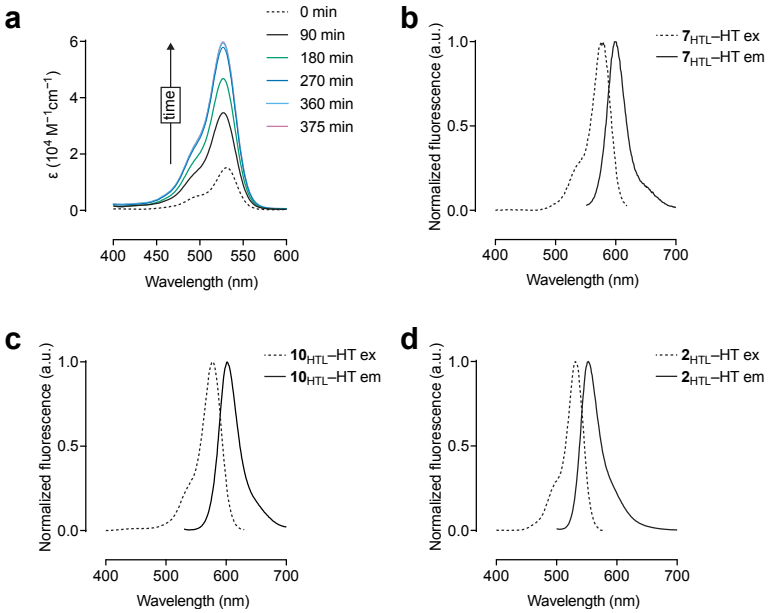


**Figure S2. Spectral Properties of Photosensitizer HaloTag Ligands, Related to Figure 2.** (**a**) Absorption spectra of $\mathbf{2_{HTL}Ac_2}$ incubated with PLE over time. (**b–d**) Normalized fluorescence excitation and emission spectra of $JF_{567}$–HaloTag ligand ($\mathbf{7_{HTL}}$; **b**), $JF_{570}$–HaloTag ligand ($\mathbf{10_{HTL}}$; **c**), and Eosin–HaloTag ligand ($\mathbf{2_{HTL}}$; **d**) bound to HaloTag protein.

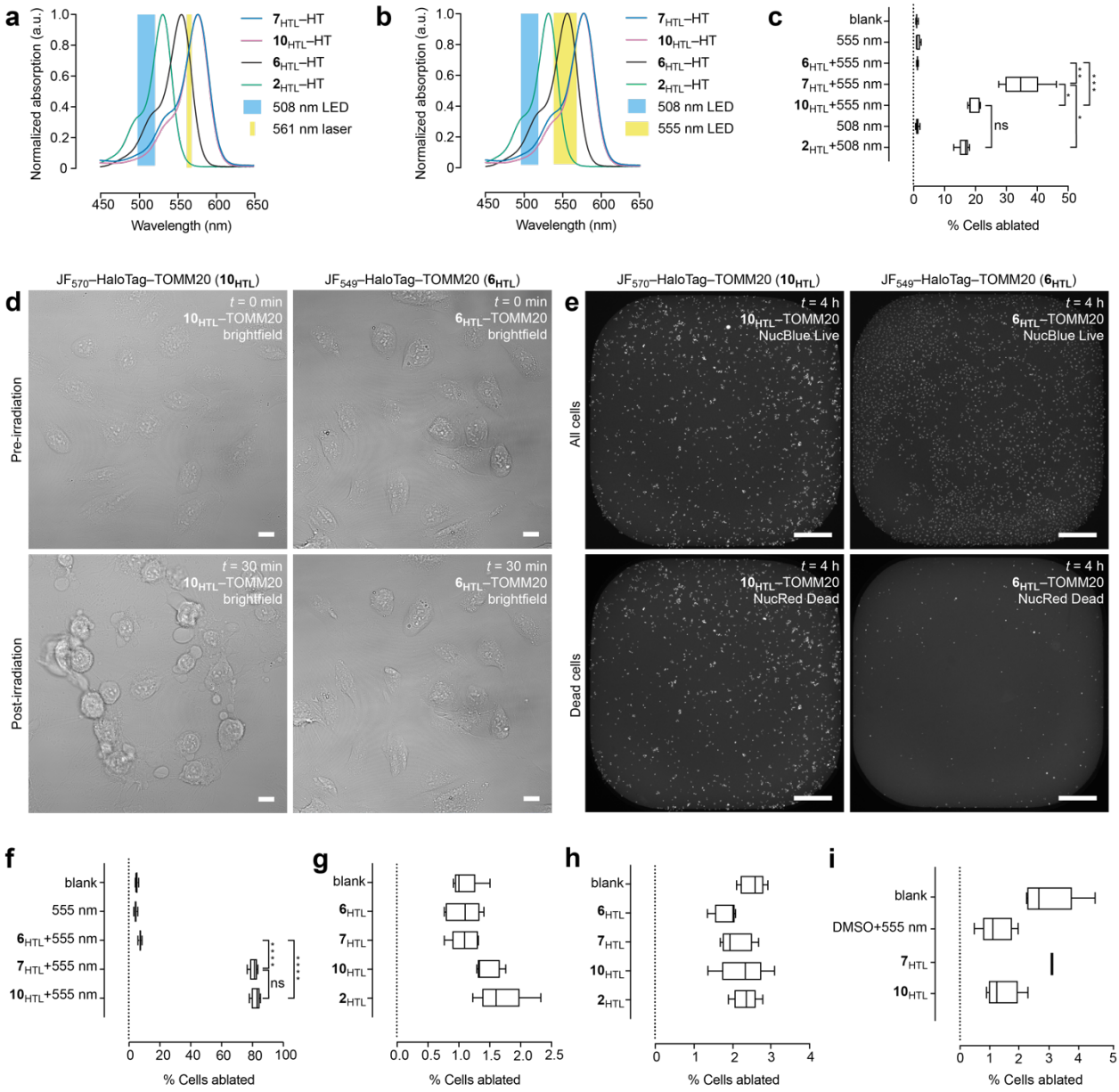


**Figure S3. Data for Cellular Experiments, Related to Figure 2.** (**a**) Normalized absorption spectra of $\mathbf{2}_{HTL}$, $\mathbf{6}_{HTL}$; $\mathbf{7}_{HTL}$, and $\mathbf{10}_{HTL}$ overlaid with light sources used for CALI experiment. (**b**) Normalized absorption spectra of $\mathbf{2}_{HTL}$, $\mathbf{6}_{HTL}$; $\mathbf{7}_{HTL}$, and $\mathbf{10}_{HTL}$ overlaid with light sources used for cell culture ablation experiment. (**c**) Comparison of cell-ablation efficacy using $\mathbf{6}_{HTL}$, $\mathbf{7}_{HTL}$, and $\mathbf{10}_{HTL}$ with 555 nm excitation light or $\mathbf{2}_{HTL}$ using 508 nm excitation light in U2OS cells stably expressing HaloTag–H2B fusion protein exposed to 55 mW/cm$^2$ excitation light for 3 min. Data pooled from two experiments and analyzed via Welch's ANOVA and Games–Howell post-hoc test ($n$ = 10 for no treatment control, otherwise $n$ = 5, 10.89 mm$^2$ microplate wells). (**d**) Enlarged representative brightfield microscopy images showing U2OS cells expressing HaloTag–TOMM20 fusion protein incubated with $\mathbf{10}_{HTL}$ (left) or $\mathbf{6}_{HTL}$ (right) before ($t$ = 0 min; top) and after ($t$ = 30 min; bottom) widefield irradiation with 560 nm-centered light; scale bars: 13 μm. (**e**) Representative data from cell-ablation experiment showing U2OS cells expressing HaloTag–TOMM20 fusion protein incubated with $\mathbf{10}_{HTL}$ (left) or $\mathbf{6}_{HTL}$ (right) after irradiation. Top panels show samples stained with NucBlue Live, which stains all cells and bottom panels show samples stained with NucRed Dead, which stains only dead cells; scale bars: 500 μm. (**f**) Comparison of cell-ablation efficacy using $\mathbf{6}_{HTL}$, $\mathbf{7}_{HTL}$, and $\mathbf{10}_{HTL}$ with 555 nm excitation light in U2OS cells stably expressing HaloTag–TOMM20 fusion protein exposed to 147 mW/cm$^2$ excitation light for 3 min, $n$ = 5. (**g**–**h**) Control experiments comparing cell-ablation in the absence of light in cells expressing HaloTag–H2B (**g**) or HaloTag–TOMM20 (**h**); $n$ = 5. (**i**) Control experiment comparing cell ablation in untransfected U2OS cells exposed to compounds or light; $n$ = 5 except for $\mathbf{7}_{HTL}$ experiment where $n$ = 2. For plots **c** and **f**–**i** center line indicates median; box limits indicate upper and lower quartiles; whiskers indicate min–max; Data analyzed via ANOVA and Tukey's HSD post-hoc test ($n$ = 5, 10.89 mm$^2$ microplate wells); statistical significance in **c** and **f** reported as follows: * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001.

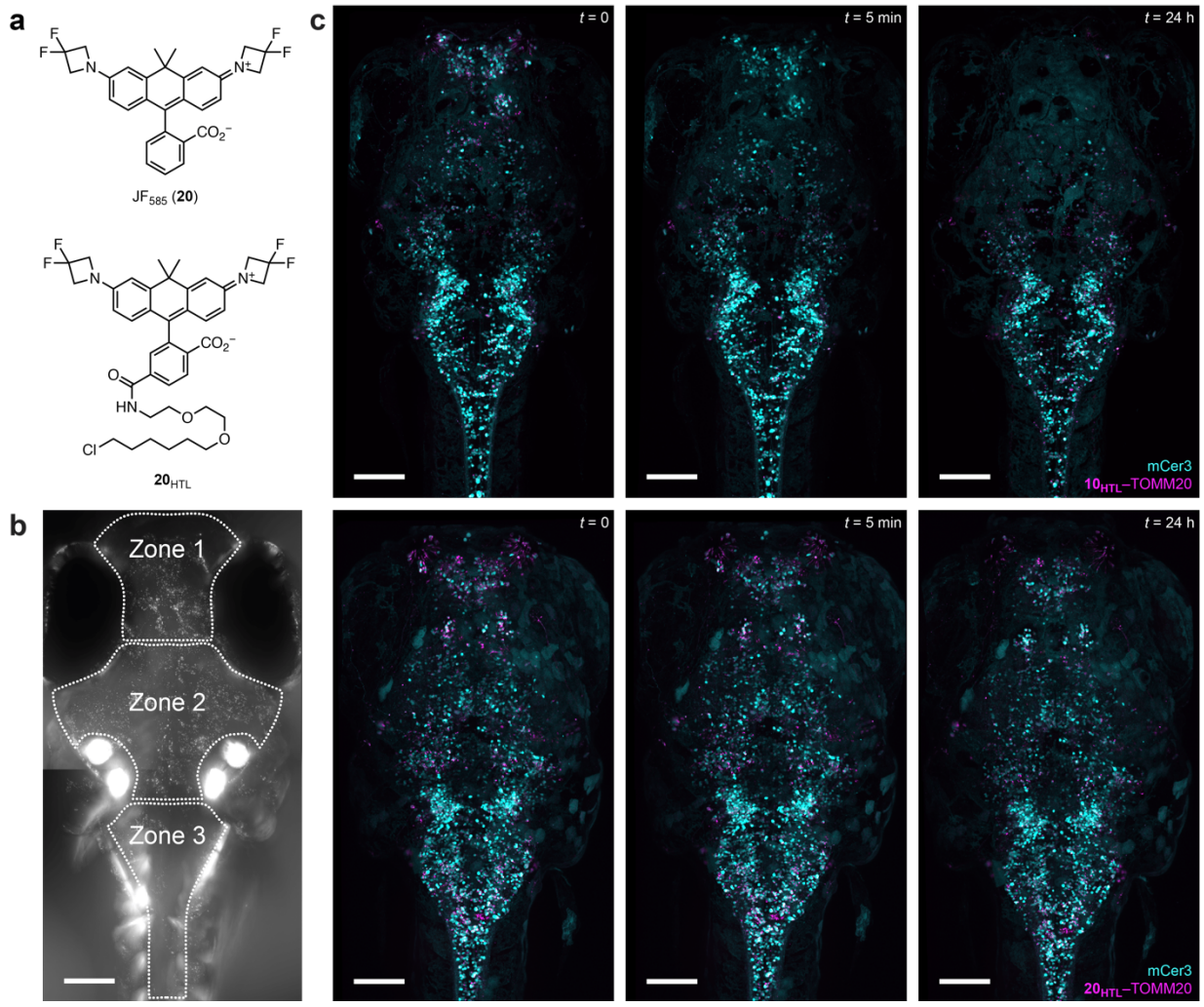


**Figure S4. Data for *in vivo* Experiments, Related to Figure 3.** (**a**). Chemical structures of $JF_{585}$ (**20**) and $JF_{585}$–HaloTag ligand ($\mathbf{20}_{HTL}$). (**b**) Brightfield microscopy image (z-projection) indicating different zones of the zebrafish brain. (**c**) Enlarged representative fluorescence microscopy images from Figure 3c showing of zebrafish brains from animals expressing HaloTag–TOMM20 fusion proteins (magenta) and mCerulean (cyan) incubated with $JF_{570}$–HaloTag ligand ($\mathbf{10}_{HTL}$, top) or $\mathbf{20}_{HTL}$ (bottom) pre-irradiation ($t$ = 0 min), immediately post-irradiation ($t$ = 5 min), and 1 day post-irradiation ($t$ = 24 h); scale bars for all images: 100 μm.