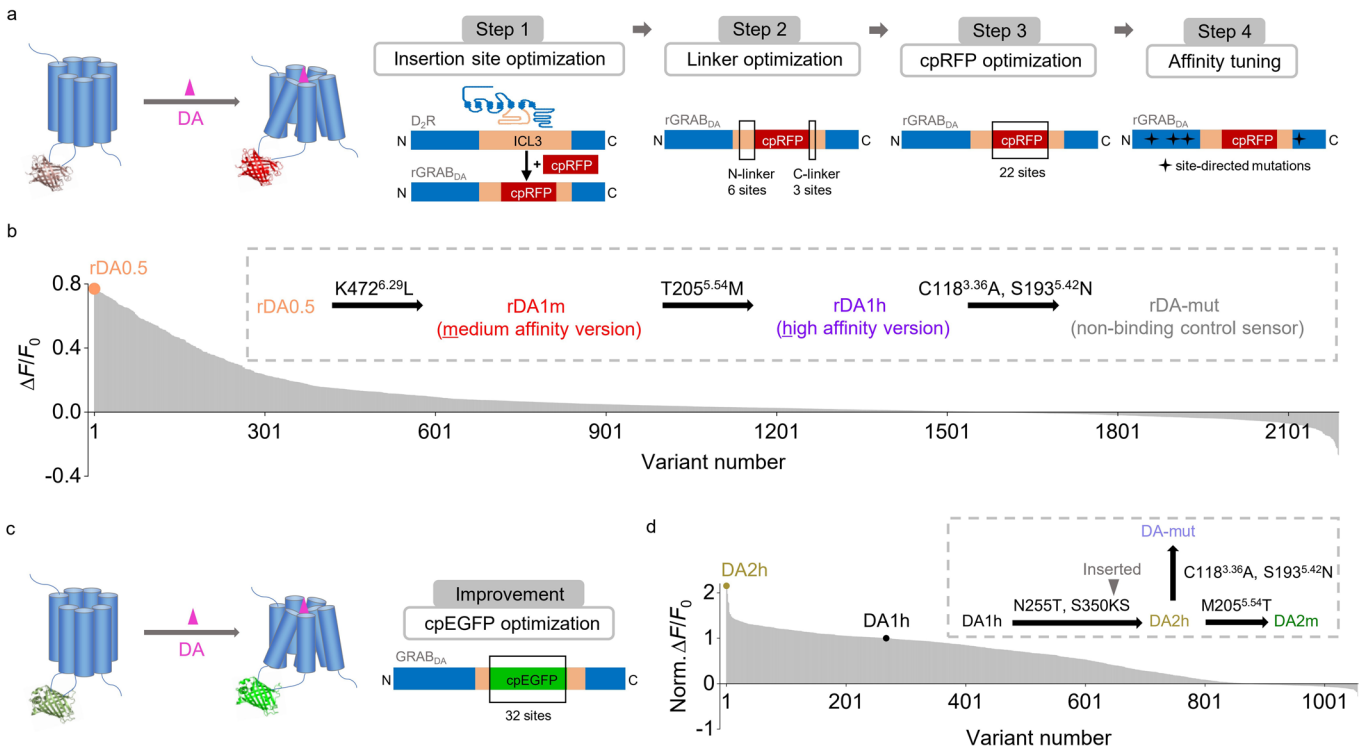
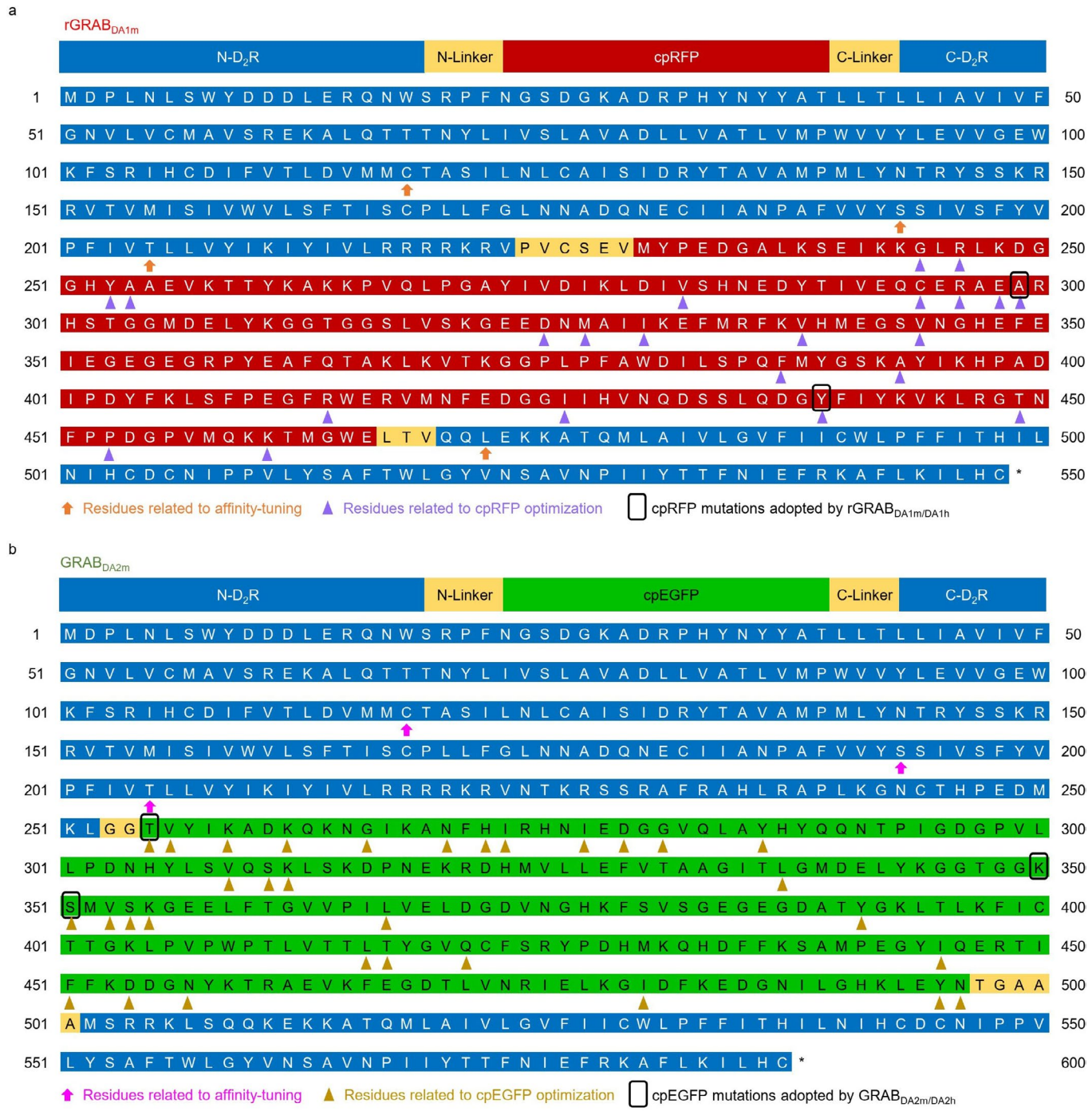
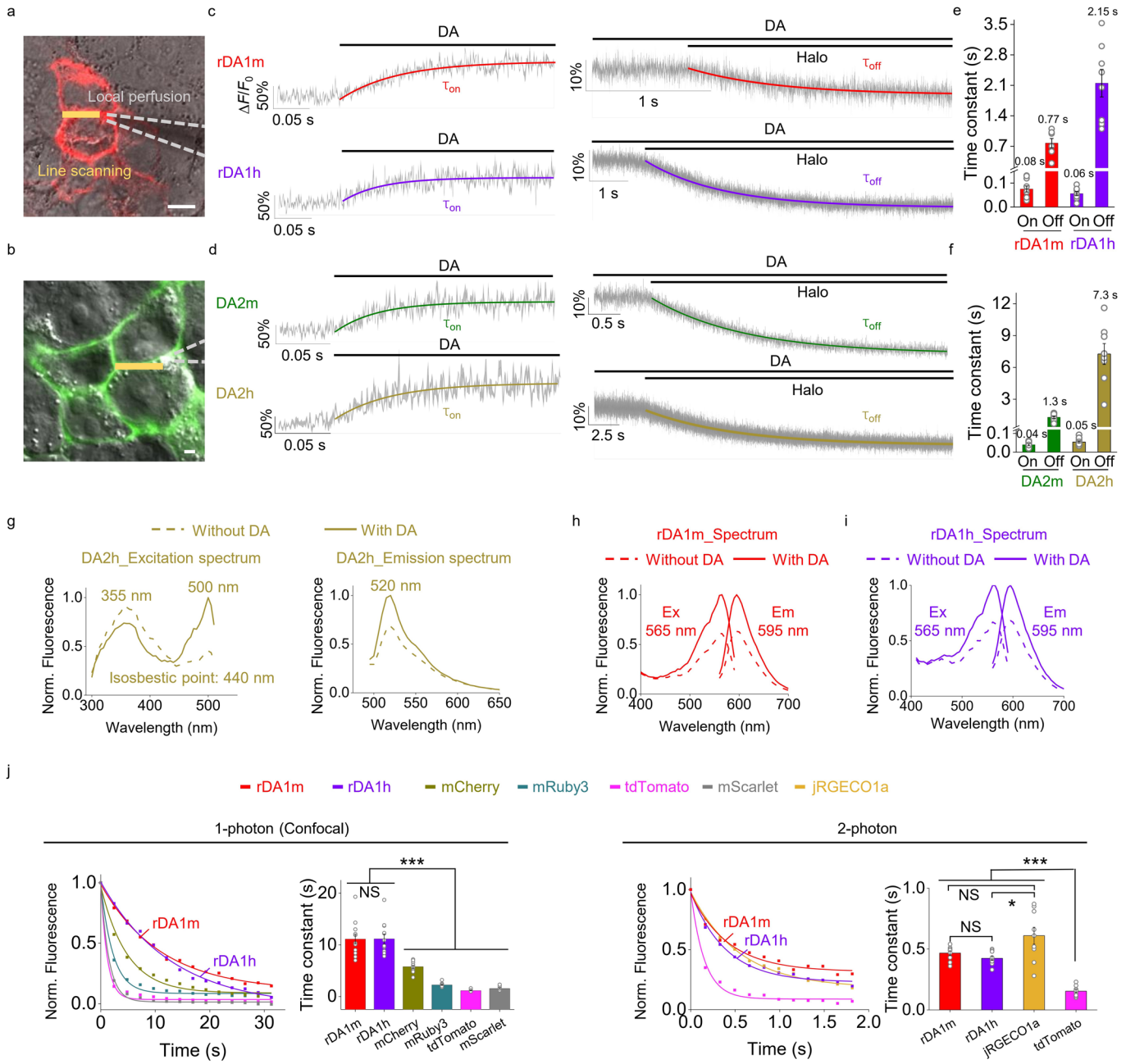


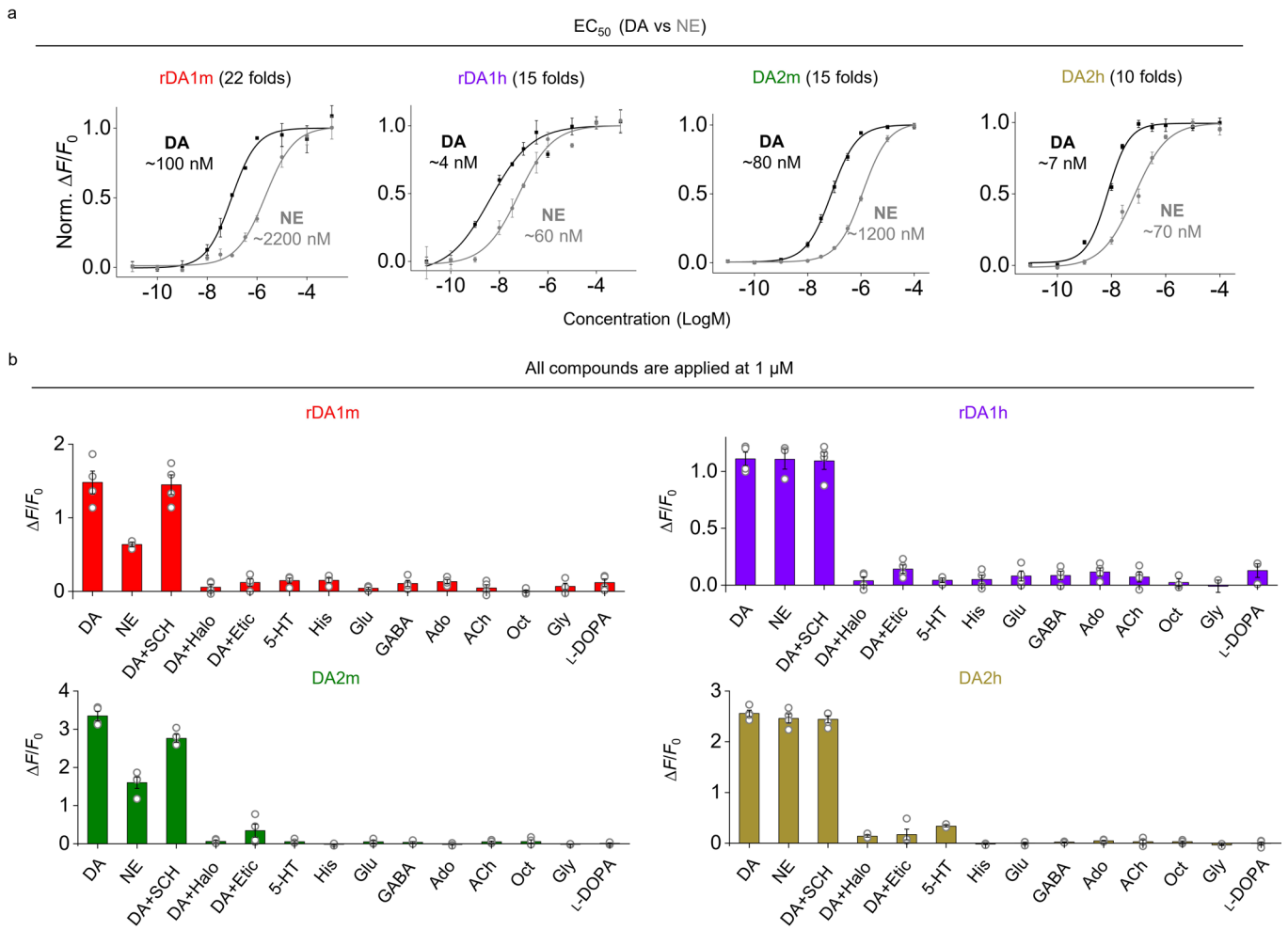
Extended Data Fig. 1 | The development of red fluorescent DA sensors and second-generation green fluorescent DA sensors. a, Schematic illustration showing the design and optimization of the red fluorescent $GRAB_{DA}$ sensors. **b**, The response to 100 μM DA measured for red fluorescent DA sensor variants during steps 1–3. The variant with the highest fluorescence change (named rDA0.5) was then sequentially mutated as shown to generate rDA1m, rDA1h, and rDA-mut. **c**, Schematic illustration showing the design and optimization of the green fluorescent $GRAB_{DA}$ sensors. **d**, Normalized $\Delta F/F_0$ in response to 100 μM DA measured for green fluorescent DA sensor variants, normalized to the first-generation DA1h sensor. DA2h was then mutated as shown to generate DA2m and DA-mut. The superscripts in the insets of **b**,**d** are based on the Ballesteros–Weinstein numbering scheme[54], indicating the mutation sites in the D_2R.



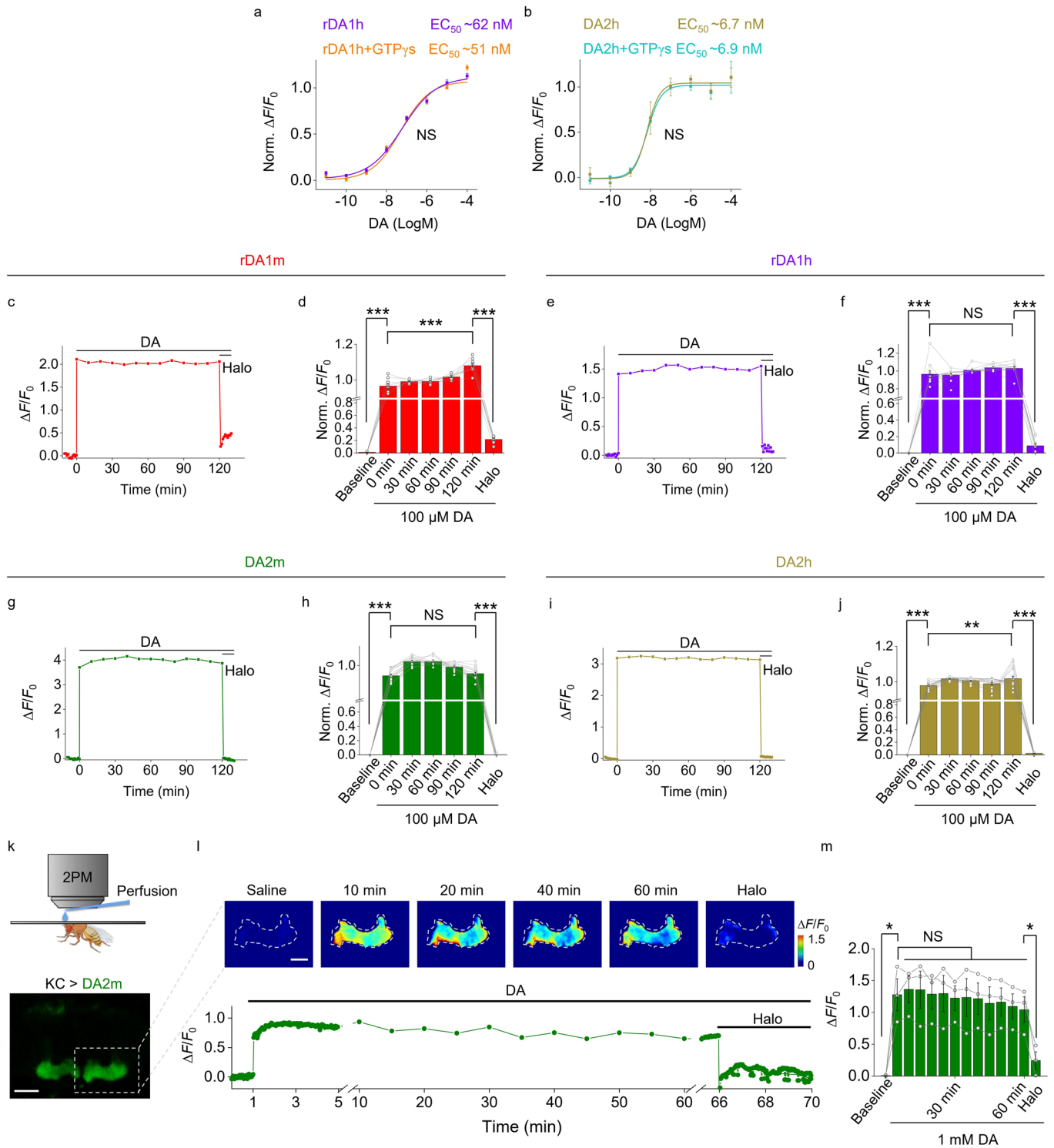
Extended Data Fig. 2 | The sequences of GRAB$_{DA}$ sensors and the residues related to affinity-tuning, cpRFP and cpEGFP optimization. a,**b**, The sequences of rGRAB$_{DA1m}$ (**a**) and GRAB$_{DA2m}$ (**b**). The residues related to affinity-tuning, cpRFP (**a**) and cpEGFP (**b**) optimization are marked.



Extended Data Fig. 3 | Characterization of the sensors in HEK293T cells. a, **b**, Schematic illustration showing the local perfusion system. Scale bars, 10 μm. **c**,**d**, Representative traces showing the response to DA (left) and subsequent addition of Halo (right). The traces were the average of 3 different regions of interest (ROIs) on the scanning line, shaded with ± s.e.m.. Each trace was fitted with a single-exponential function to determine τ_{on} (left) and τ_{off} (right). Similar results were observed for 7–10 cells. **e**,**f**, Group summary of τ_{on} and τ_{off}. $n = 10, 7, 9, 8, 10, 8, 10, 8$ cells for rDA1m (τ_{on}), rDA1m (τ_{off}), rDA1h (τ_{on}), rDA1h (τ_{off}), DA2m (τ_{on}), DA2m (τ_{off}), DA2h (τ_{on}), DA2h (τ_{off}). **g–i**, Excitation and emission spectra for the indicated sensors in the absence and presence of DA. **j**, Photostability of rDA1m and rDA1h (in the presence of 100 μM DA) and the indicated fluorescent proteins was measured using 1-photon and 2-photon microscopy. Each photobleaching curve was fitted with a single-exponential function to determine the time constant. 1-photon, $n = 12$ cells each. 2-photon, $n = 10, 10, 9, 10$ cells for rDA1m, rDA1h, jRGECO1a, tdTomato. Two-tailed Student's *t*-test was performed. 1-photon, $P = 0.9755$ between rDA1m and rDA1h; $P = 2.72 \times 10^{-5}$ between rDA1m and mCherry; $P = 7.10 \times 10^{-9}$ between rDA1m and mRuby3; $P = 7.90 \times 10^{-10}$ between rDA1m and tdTomato; $P = 1.95 \times 10^{-9}$ between rDA1m and mScarlet; $P = 1.28 \times 10^{-5}$ between rDA1h and mCherry; $P = 2.50 \times 10^{-9}$ between rDA1h and mRuby3; $P = 2.66 \times 10^{-10}$ between rDA1h and tdTomato; $P = 6.75 \times 10^{-10}$ between rDA1h and mScarlet. 2-photon, $P = 0.0963$ between rDA1m and rDA1h; $P = 0.0511$ between rDA1m and jRGECO1a; $P = 0.0139$ between rDA1h and jRGECO1a; $P = 2.82 \times 10^{-11}$ between rDA1m and tdTomato; $P = 1.71 \times 10^{-10}$ between rDA1h and tdTomato; $P = 2.96 \times 10^{-6}$ between jRGECO1a and tdTomato. Data are presented as the mean ± s.e.m. in **e**,**f**,**j** (bar graph). $^*P < 0.05$; $^{***}P < 0.001$.

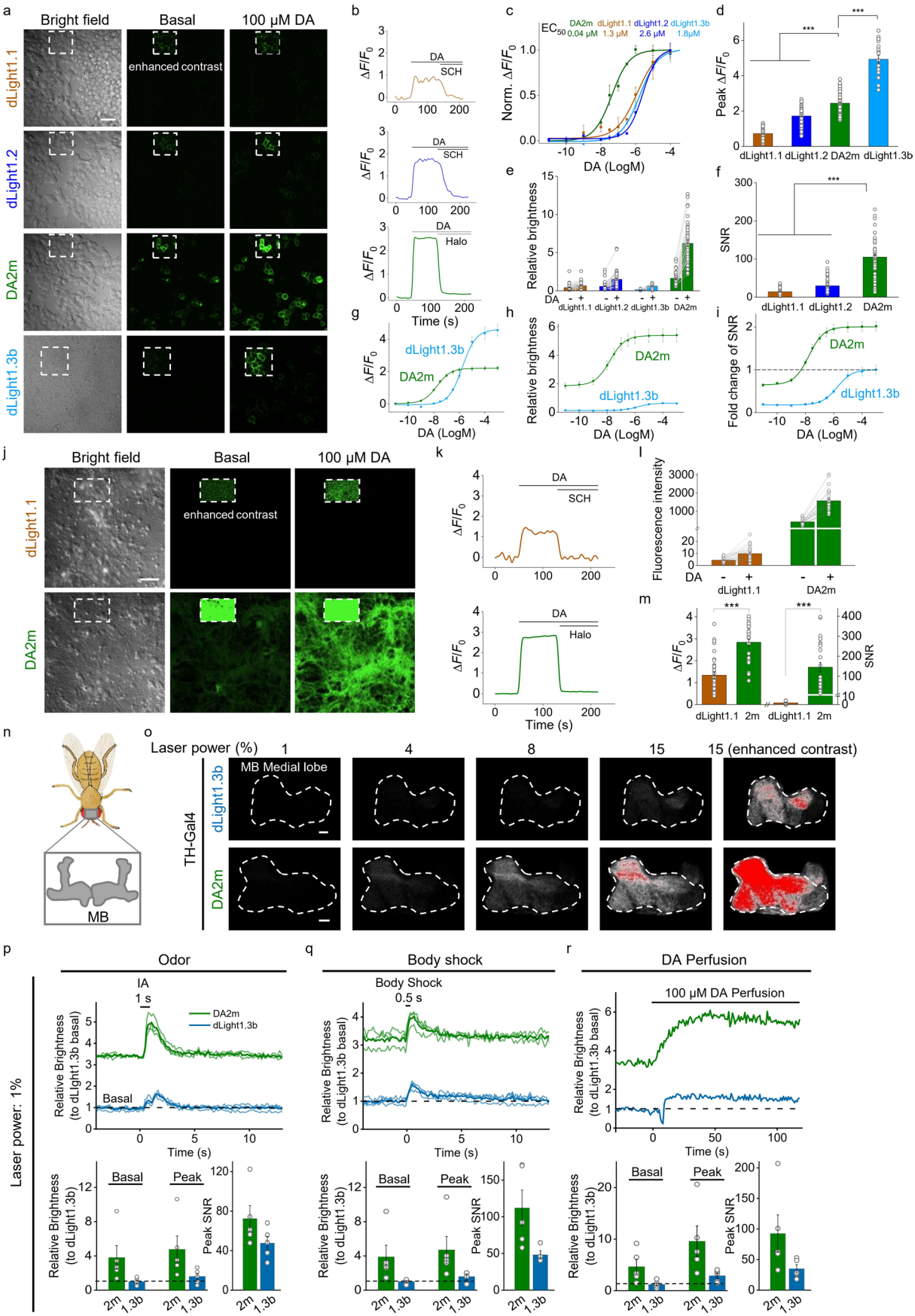


Extended Data Fig. 4 | The response of GRAB$_{DA}$ sensors to different compounds. a, The normalized dose-response curves for DA and NE in sensor-expressing HEK293T cells. $n = 3$ wells with 200–800 cells/well. **b**, The $\Delta F/F_0$ in sensor-expressing cells in response to the indicated compounds applied at 1 μM. $n = 3$ wells for rDA1h in response to NE, 5-HT, Oct, Gly and L-DOPA. $n = 4$ wells for the others. Each well contains 200–1200 cells. Data are presented as the mean ± s.e.m.. Data replotted from Fig. 2a.



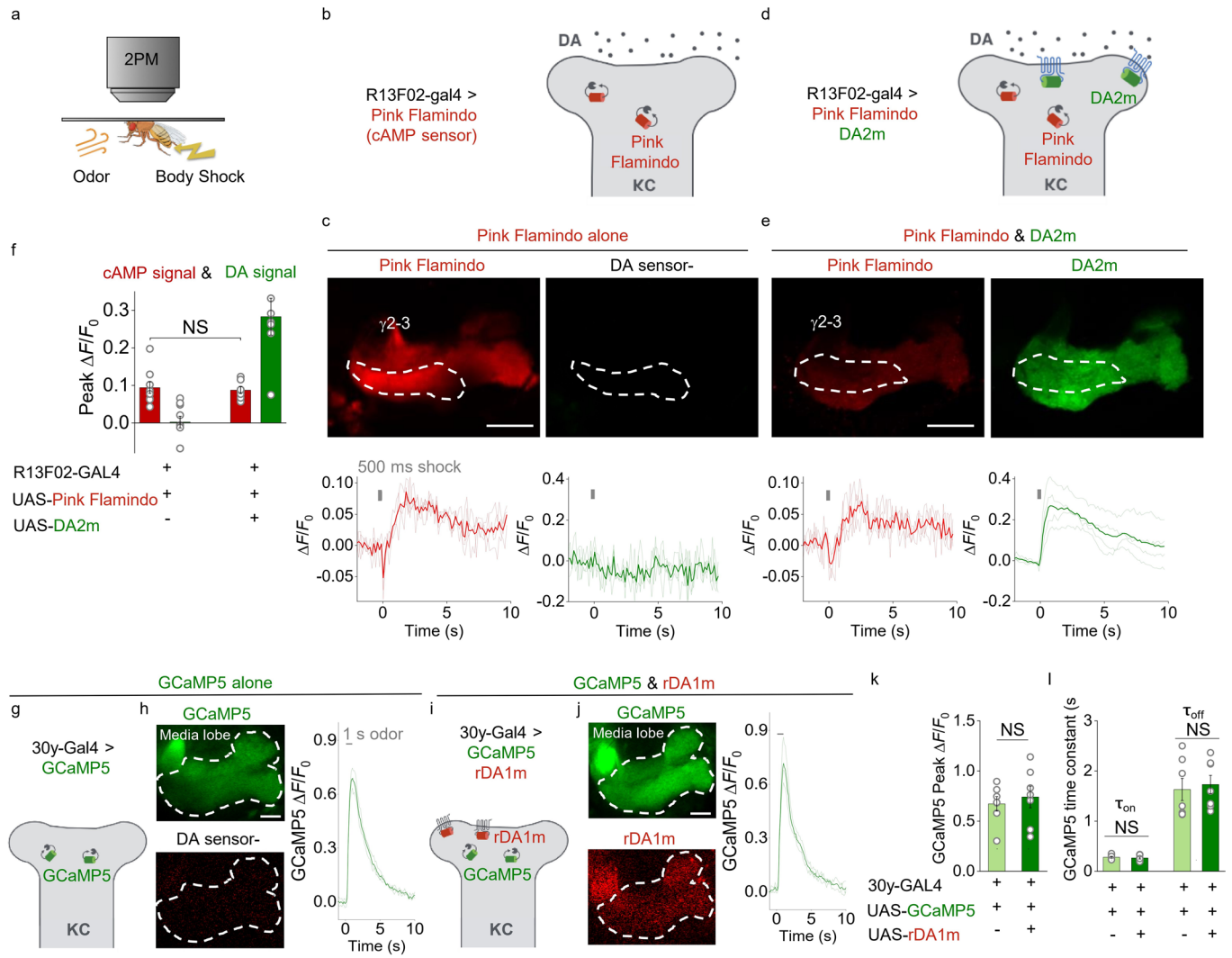
Extended Data Fig. 5 | See next page for caption.

Extended Data Fig. 5 | The minimal coupling of GRAB$_{DA}$ sensors to downstream G$_i$ pathway and β-arrestin pathway. a,**b**, Normalized $\Delta F/F_0$ in sensor-expressing cells in response to DA, with or without the pre-bathing of GTPγS. $n = 3$ wells with 500–3000 cells/well. **c**,**d**, The representative trace of $\Delta F/F_0$ (**c**) and the group summary of normalized $\Delta F/F_0$ (**d**) in rDA1m-expressing neurons during a 2-hour treatment of 100 μM DA. $n = 9$ neurons. For the group summary, the averaged $\Delta F/F_0$ of each neuron during the 2-hour DA treatment is normalized to 1. Two-tailed Student's *t*-test was performed. $P = 2.10 \times 10^{-21}$ between baseline and 0 min; $P = 2.99 \times 10^{-17}$ between 120 min and Halo; $P = 1.24 \times 10^{-5}$ between 0 min and 120 min. **e**,**f**, Similar to **c** and **d** except that rDA1h was expressed in cultured neurons. $n = 11$ neurons. Two-tailed Student's *t*-test was performed. $P = 1.87 \times 10^{-6}$ between baseline and 0 min; $P = 3.43 \times 10^{-17}$ between 120 min and Halo; $P = 0.1519$ between 0 min and 120 min. **g**,**h**, Similar to **c** and **d** except that DA2m was expressed in cultured neurons. $n = 15$ neurons. Two-tailed Student's *t*-test was performed. $P = 2.48 \times 10^{-39}$ between baseline and 0 min; $P = 7.42 \times 10^{-35}$ between 120 min and Halo; $P = 0.3322$ between 0 min and 120 min. **i**,**j**, Similar to **c** and **d** except that DA2h was expressed in cultured neurons. $n = 17$ neurons. Two-tailed Student's *t*-test was performed. $P = 1.14 \times 10^{-52}$ between baseline and 0 min; $P = 9.80 \times 10^{-38}$ between 120 min and Halo; $P = 0.0061$ between 0 min and 120 min. **k**, Top, schematic illustration depicting the in vivo perfusion experiment. Bottom, the fluorescence image of a transgenic fly expressing DA2m in MB KCs. Scale bar, 50 μm. **l**,**m**, Representative images (**l**, top), trace (**l**, bottom) and group summary (**m**) of $\Delta F/F_0$ in response to the 1-hour perfusion of 1 mM DA followed by 100 μM Halo in a transgenic fly expressing DA2m in MB KCs. $n = 3$ flies. Scale bar, 25 μm. Two-tailed Student's *t*-test was performed. $P = 0.0382$ between baseline and 10 min; $P = 0.0293$ between 60 min and Halo; $P = 0.5289$, 0.5593, 0.9559, 0.8537, 0.6346, 0.6530, 0.2760, 0.1649, 0.1547, 0.1152, 0.1044 between 5 min and 10 min, 15 min, 20 min, 25 min, 30 min, 35 min, 40 min, 45 min, 50 min, 55 min, 60 min, respectively. Data are presented as the mean ± s.e.m.. in **a**,**b**,**d**,**f**,**h**,**j**,**m**. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

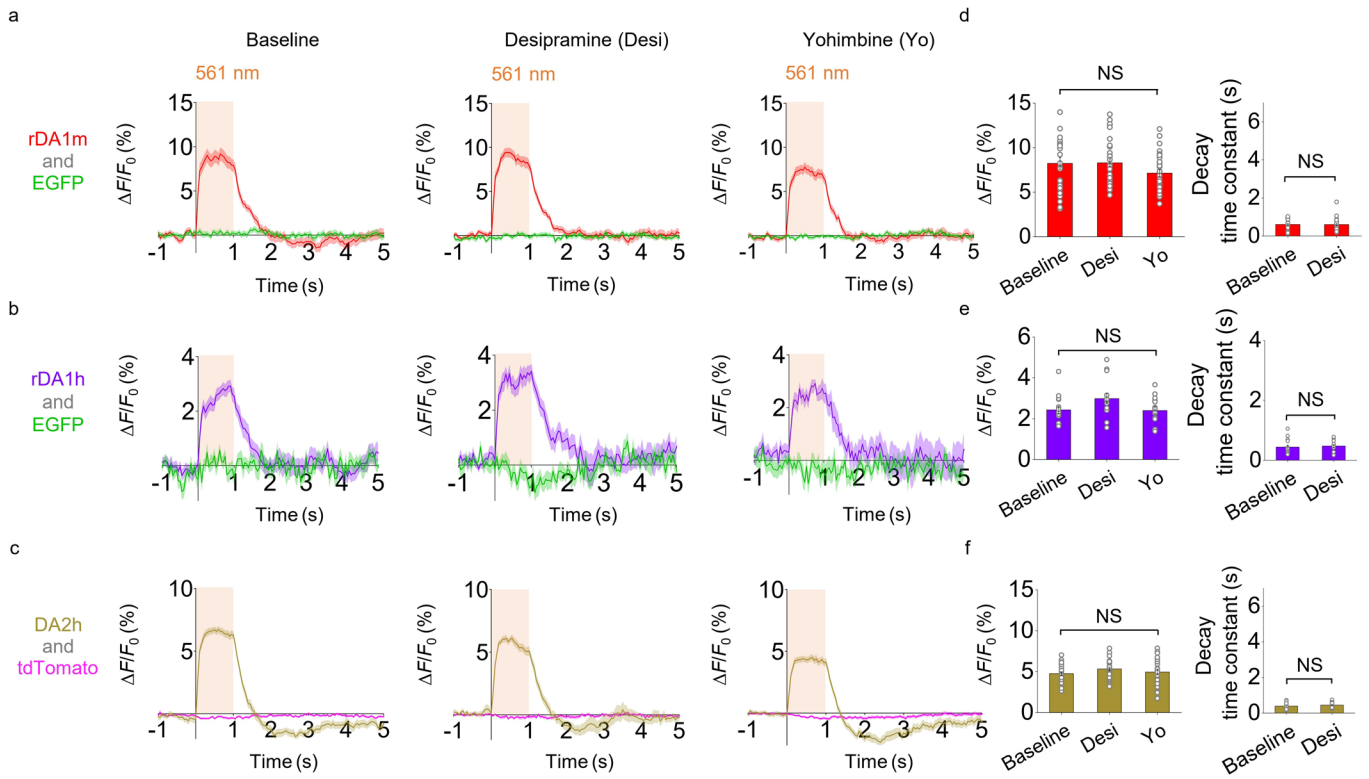


Extended Data Fig. 6 | See next page for caption.

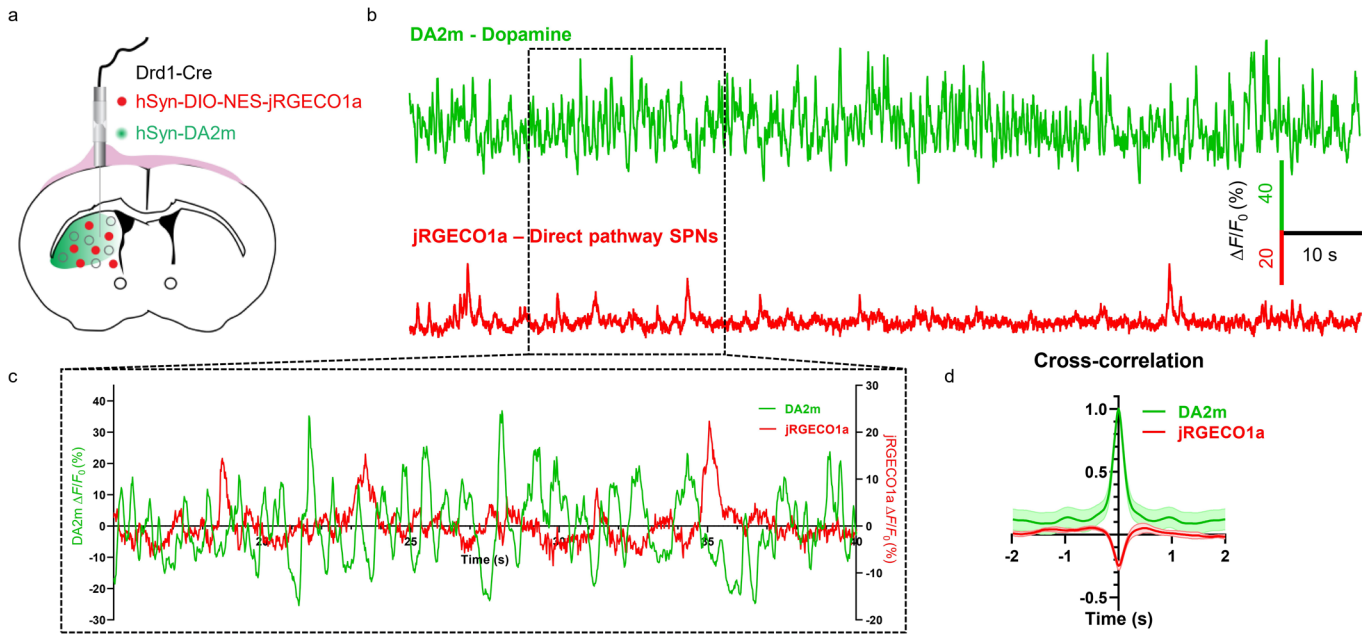
Extended Data Fig. 6 | Comparison between dLight and GRAB$_{DA}$. **a**, Representative bright-field and fluorescence images acquired before (baseline) and after application of DA in sensor-expressing HEK293T cells. Similar results were observed for more than 20 cells. Scale bar, 50 μm. **b**, Representative traces of $\Delta F/F_0$ in response to 100 μM DA followed by either 10 μM SCH or 10 μM Halo. Similar results were observed for more than 30 cells. **c**, Normalized dose-response curves. $n = 3$ wells with 100–500 cells/well. **d–f**, Group summary of the peak $\Delta F/F_0$ (**d**), relative brightness (green/red ratio, GR ratio) (**e**), and signal-to-noise ratio (SNR) (**f**) in response to 100 μM DA. **d**, $n = 73, 62, 61, 20$ cells for dLight1.1, dLight1.2, DA2m, dLight1.3b. **e**, $n = 77, 66, 20, 60$ cells for dLight1.1, dLight1.2, dLight1.3b, DA2m. **f**, $n = 74, 63, 61$ cells for dLight1.1, dLight1.2, DA2m. Two-tailed Student's *t*-test was performed. **d**, $P = 2.10 \times 10^{-48}$ between dLight1.1 and DA2m; $P = 1.31 \times 10^{-12}$ between dLight1.2 and DA2m; $P = 1.22 \times 10^{-10}$ between dLight1.3 and DA2m. **f**, $P = 4.09 \times 10^{-22}$ between dLight1.1 and DA2m; $P = 1.13 \times 10^{-33}$ between dLight1.2 and DA2m. **g–i**, Dose-response curves (**g**), relative brightness (**h**), and fold change of SNR (**i**) for dLight1.3b and DA2m. $n = 20$ cells each. **j-m**, Similar to **a-f**, except that dLight1.1 and DA2m were expressed in cultured neurons. **m**, left, $n = 30, 28$ cells for dLight1.1, DA2m. **m**, right, $n = 30$ cells each. Scale bar, 50 μm. Two-tailed Student's *t*-test was performed. **m**, left, $P = 4.43 \times 10^{-8}$; right, $P = 3.59 \times 10^{-8}$. **n**, Schematic illustration depicting the location of the *Drosophila* olfactory mushroom body (MB). **o**, Fluorescence images of the MB using 2-photon microscopy at the indicated laser power settings. Enhanced-contrast images at 15% laser power are shown. Fluorescence is shown in grayscale, with saturated pixels shown in red. Similar results were observed for 4–5 flies. Scale bars, 10 μm. **p-r**, Representative traces (top) and group summary of relative brightness during odorant application (**p**), body shock (**q**), and DA perfusion (**r**). **p**,**r**, $n = 5$ flies each. **q**, $n = 5, 4$ flies for DA2m, dLight1.3b. Average traces (bold) overlaid with single-trial traces (light) from one fly are shown for representation in **p**,**q**. Data are presented as the mean ± s.e.m. in **c**,**d**,**e**,**f**,**g**,**h**,**i**,**l**,**m**,**p**,**q**,**r**. $^{***}P < 0.001$.



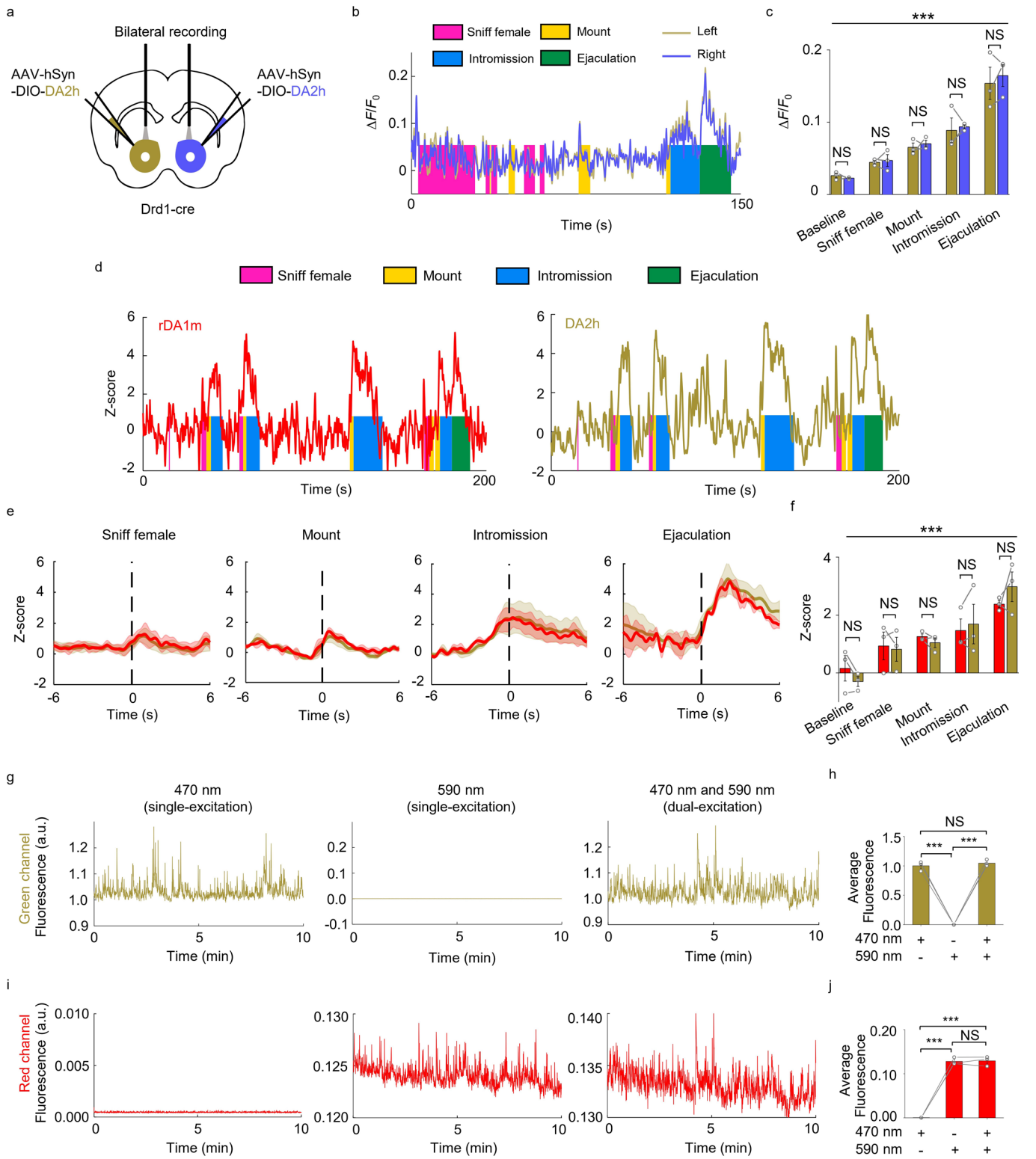
Extended Data Fig. 7 | Expressing GRAB$_{DA2m}$ or GRAB$_{rDA1m}$ sensors shows no significant effect on cAMP or calcium signaling respectively *in vivo*. **a**, Schematic illustration depicting the experimental setup. **b**–**e**, Schematic illustrations depicting the experimental strategy (**b**,**d**), representative fluorescence images and $\Delta F/F_0$ traces (**c**,**e**) in flies expressing the cAMP sensor Pink-Flamindo (**b**,**c**) or co-expressing Pink-Flamindo and DA2m (**d**,**e**) in MB KCs. The ROIs for measuring the γ2-γ3 compartments in the MB are indicated by dashed white lines. Scale bars, 25 μm. **f**, Group summary of peak $\Delta F/F_0$. $n = 9, 7$ flies for Pink Flamindo alone, Pink Flamindo & DA2m. Two-tailed Student's t-test was performed. $P = 0.7332$. **g**–**j**, Schematic illustrations depicting the experimental strategy (**g**, **i**), representative fluorescence images and $\Delta F/F_0$ traces (**h**,**j**) in flies expressing the calcium sensor GCaMP5 (**g**,**h**) or co-expressing GCaMP5 and rDA1m (**i**,**j**) in MB KCs. The ROIs for measuring the MB media lobe are indicated by dashed white lines. Similar results were observed for 7 flies. Scale bars, 25 μm. **k**,**l**, Group summary of GCaMP5 peak $\Delta F/F_0$ and time constants. $n = 7$ flies each. Two-tailed Student's *t*-test was performed. **k**, $P = 0.607$. **l**, $P = 0.601$, 0.735 for τ_{on}, τ_{off}. Average traces (bold) overlaid with single-trial traces (light) from one fly are shown for representation in **c**, **e**,**h**,**j**. Data are presented as the mean ± s.e.m. in **f**,**k**,**l**.



Extended Data Fig. 8 | Optogenetically induced nigrostriatal DA release in freely moving mice is not affected by desipramine or yohimbine. **a**–**c**, Average traces of $\Delta F/F_0$ in mice expressing rDA1m and EGFP (**a**), rDA1h and EGFP (**b**), or DA2h and tdTomato (**c**) in the dorsal striatum. Where indicated, the experiments were conducted in mice treated with either the norepinephrine transporter blocker desipramine or the α_2-adrenergic receptor antagonist yohimbine. **d**–**f**, Group summary of $\Delta F/F_0$ and τ_{off} for the experiments shown in **a**-**c**, respectively. $n = 30$ trials from 6 hemispheres of 6 mice for rDA1m. $n = 15$ trials from 3 hemispheres of 3 mice for rDA1h, $n = 25$ trials from 5 hemispheres of 4 mice for DA2h. Two-tailed Student's t-test was performed. **d**, left, $P = 0.1614$; right, $P = 0.9836$. **e**, left, $P = 0.9018$; right, $P = 0.6605$. **f**, left, $P = 0.6489$; right, $P = 0.2322$. Average traces shaded with ± s.e.m. are shown in **a**–**c**. Data are presented as the mean ± s.e.m. in **d**-**f**.



Extended Data Fig. 9 | Dual-color recording of DA dynamics and striatal neural activity using DA2m and jRGECO1a in freely moving mice. a, Schematic illustration depicting the experimental strategy. **b**, Representative traces showing the fluorescence responses of DA2m and jRGECO1a. **c**, The zoom-in traces from **b** during a 25 s recording. **d**, The cross-correlation between the fluorescence responses of DA2m and jRGECO1a during a 2 min recording. $n = 8$ hemispheres of 5 mice. Average traces shaded with ± s.e.m. are shown.



Extended Data Fig. 10 | See next page for caption.

Extended Data Fig. 10 | The DA signal in the mouse NAc during sexual behavior. a, Schematic illustration depicting the experimental strategy. **b**, **c**, Representative traces (**b**) and group summary (**c**) of $\Delta F/F_0$ measured from left and right hemispheres during the indicated stages of mating. $n=3$ mice. $F_{4,16}=80.92$, $P<10^{-6}$ for row factor and $F_{1,4}=0.1224$, $P=0.7441$ for column factor by two-way ANOVA. Bonferroni's multiple comparisons test was performed between groups, $P>0.9999$, $P>0.9999$, $P>0.9999$, $P>0.9999$, $P>0.9999$. **d**, Representative traces of the concurrent Z-score signals of rDA1m and DA2h during the indicated stages of sexual behavior. Similar results were observed for 3 mice. **e**, Average post-stimulus histograms showing the Z-score signals of rDA1m and DA2h aligned to the onset of the indicated mating events. $n=3$ mice. Average traces shaded with ± s.e.m. are shown. **f**, Group summary of the Z-scores measured for rDA1m and DA2h during the indicated mating events. $n=3$ mice. $F_{4,16}=13.02$, $P=6.6\times10^{-5}$ for row factor and $F_{1,4}=0.001$, $P=0.9797$ for column factor by two-way ANOVA. Bonferroni's multiple comparisons test was performed, $P>0.99$, $P>0.99$, $P>0.99$, $P>0.99$, $P>0.99$. **g**,**h**, The representative fluorescence signal (**g**) and group analysis (**h**) in the green channel when the excitation light is delivered at 470 nm alone (**g**, left), at 590 nm alone (**g**, center) or at 470 nm and 590 nm simultaneously (**g**, right). $n=3$ mice. $F_{2,4}=531.6$, $P=3.1\times10^{-5}$ by one-way ANOVA. Tukey's multiple comparisons test was performed between groups, $P=3.1\times10^{-5}$, $P=2.6\times10^{-5}$, $P=0.4904$. **i**,**j**, Similar to **g** and **h** except the fluorescence signal in the red channel is analyzed. $n=3$ mice. $F_{2,4}=414.2$, $P=2.3\times10^{-5}$ by one-way ANOVA. Tukey's multiple comparisons test was performed between groups, $P=4.8\times10^{-5}$, $P=4.6\times10^{-5}$, $P=0.9738$. Data are presented as the mean ± s.e.m. in **c**,**f**,**h**,**j**. ***$P<0.001$.