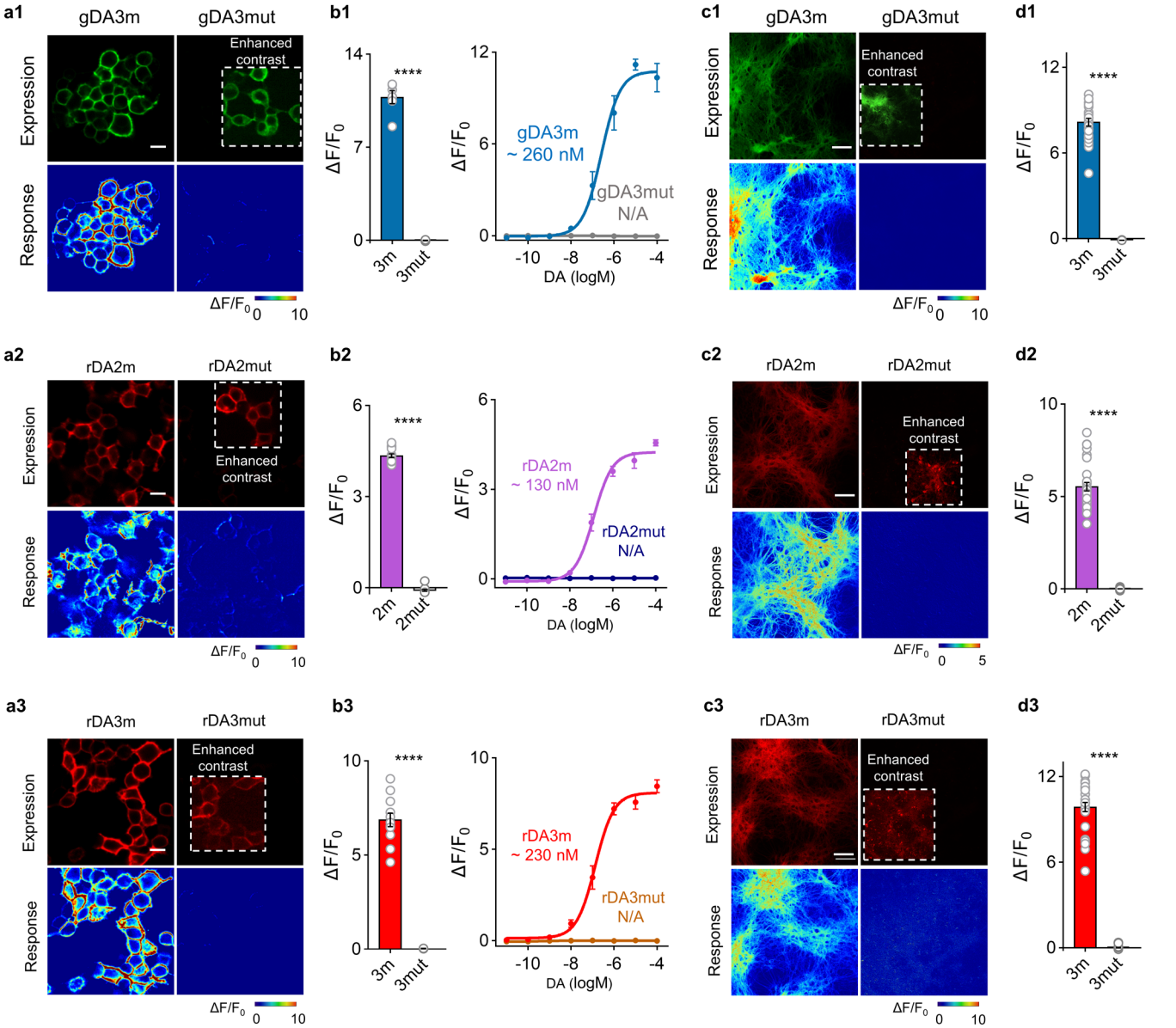
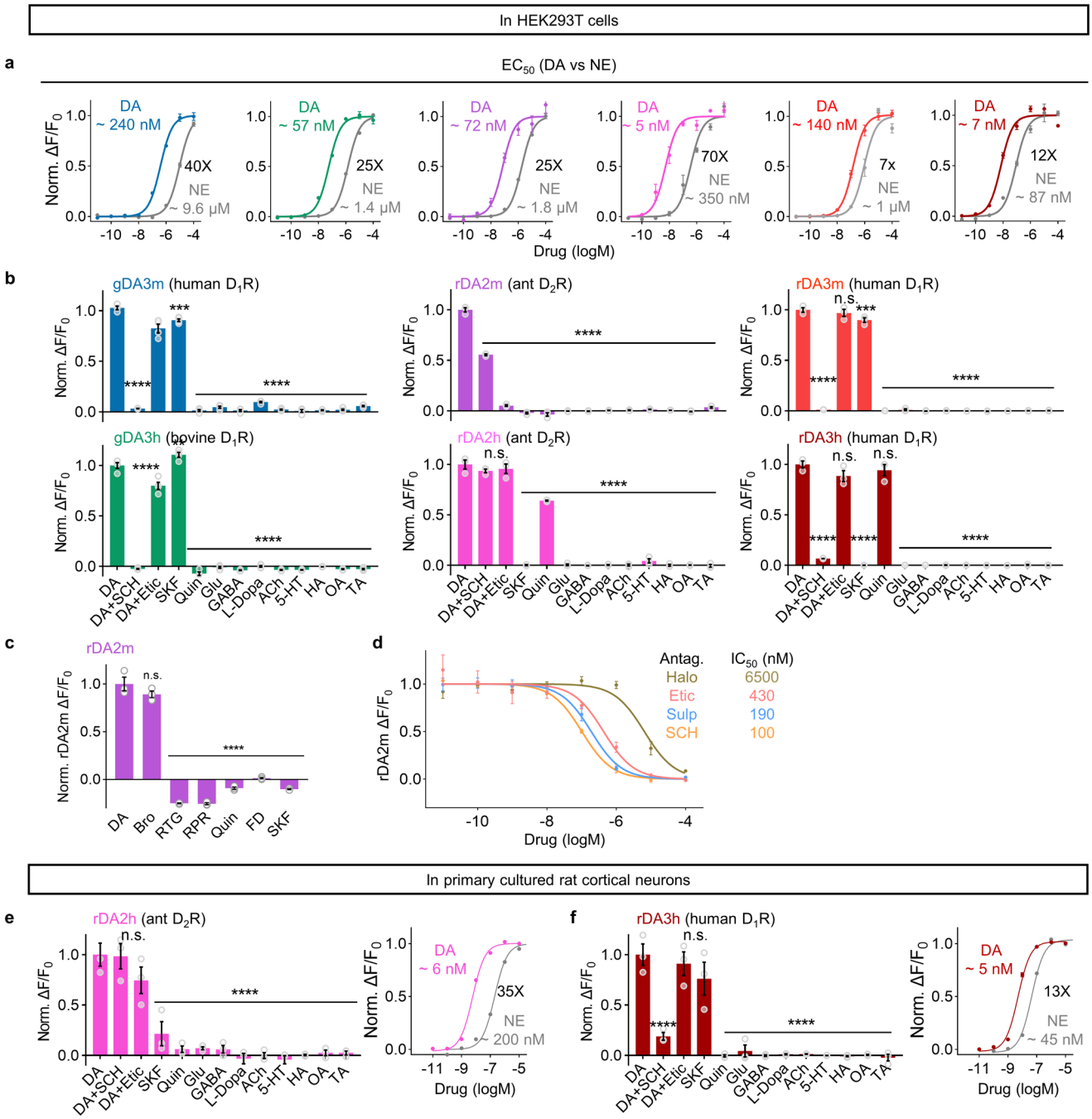
https://doi.org/10.1038/s41592-023-02100-w



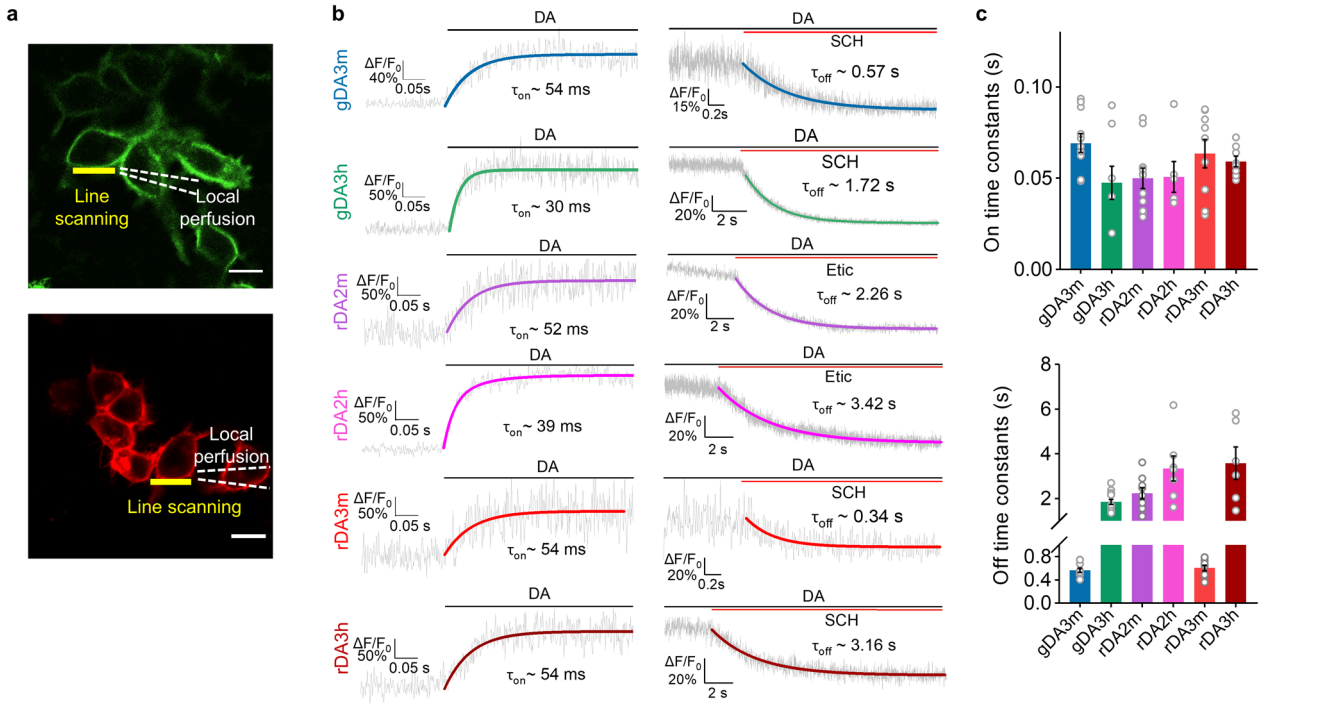
Extended Data Fig. 1 | Performance of DA-insensitive mutant sensors. **a**, Representative images showing sensor expression (top) in HEK293T cells and fluorescence response to 100 μM DA (bottom) of indicated sensor variants. Scale bar, 20 μm. **b**, Group summary of maximal $\Delta F/F_0$ in response to 100 μM DA (left) and titration DA curves (right) of indicated sensors in HEK293T cells. Left, $n = 6$, 6, 15, 15, 12, 3 wells for gDA3m, gDA3mut, rDA2m, rDA2mut, rDA3m and rDA3mut. Each well contains 400–500 cells. Two-tailed Student's t-test was performed. Right, $n = 3$ wells (with 400–500 cells per well) for each group. **c**, Representative images showing sensor expression (top) in cultured neurons and fluorescence response to 100 μM DA (bottom) of indicated sensor variants. Scale bar, 50 μm. **d**, Group summary of maximal $\Delta F/F_0$ of indicated sensors in response to 100 μM DA in cultured neurons. $n = 60$ neurons from 4 cultures for rDA2mut, n = 30/2 for others, mean±s.e.m. Two-tailed Student's t-test was performed.

https://doi.org/10.1038/s41592-023-02100-w



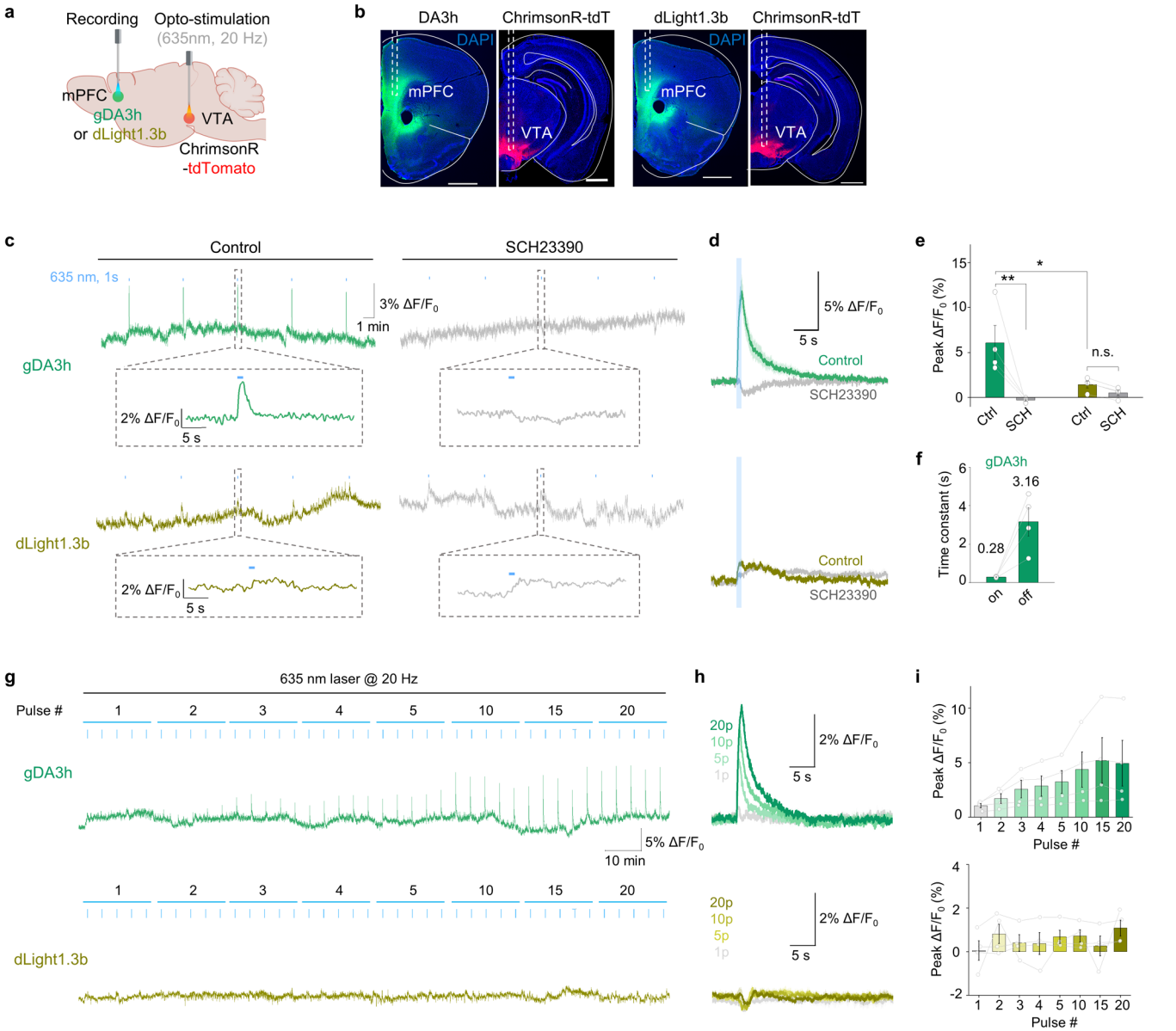
Extended Data Fig. 2 | Pharmacological profiles of GRAB$_{DA}$ sensors measured in cultured cells. a, Titration curves of indicated sensors for the response to DA or NE in HEK293T cells. $n = 3$ cells with 400–500 cells per well, mean±s.e.m. **b**, The normalized $\Delta F/F_0$ in sensor-expressing HEK293T cells in response to the indicated compounds. Antagonists were applied at 10 μM, others at 1 μM. $n = 4$ wells for gDA3m and gDA3h, $n = 3$ wells for others, mean±s.e.m. One-way Anova, post hoc Dunnett's test: gDA3m, $p = 0.0002$, 8.9×10^{-10} between DA and SFK, or DA+Etic, $p = 1.3 \times 10^{-13}$ between DA and others; gDA3h, $p = 0.0020$, 4.3×10^{-9} between DA and SFK, or DA+Etic, $p = 1.3 \times 10^{-13}$ between DA and others; rDA2m, $p = 3.3 \times 10^{-14}$ between DA and others; rDA2h, $p = 0.6059$, 0.9530 between DA and DA + SCH, or DA+Etic, $p = 3.3 \times 10^{-14}$ between DA and others; rDA3m, $p = 0.9182$, 0010 between DA and DA+Etic, or SKF, $p = 3.3 \times 10^{-14}$ between DA and others; rDA3h, $p = 0.0724$, 0.8723 between DA and DA+Etic, or Quin, $p = 3.3 \times 10^{-14}$ between DA and others. **c**, The normalized $\Delta F/F_0$ in rDA2m-expressing HEK293T cells in response to indicated DA agonists. Bromocriptine (Bro), Rotigotine (RTG), D_2R/D_1R agonists; Ropinirole (RPR), Quin, D_2R-specific agonists; Fenodopam (FD), SKF, D_1R-specific agonist. All chemicals were bath-applied in 100 μM. $n = 3$ wells, mean±s.e.m. One-way Anova, post hoc Dunnett's: $p = 0.1074$ between DA and Bro, $p = 8.0 \times 10^{-12}$ between DA and others. **d**, Titration curves of indicated dopamine receptor antagonists. The fluorescence intensity in the presence with 10 μM DA was set as F_0 and the relative fluorescence changes under indicated compound concentration were plotted. $n = 3$ wells with 400–500 cells per well, mean±s.e.m. **e**, **f**, Pharmacological specificity (left) and titration curves of indicated sensors for the response to DA or NE (right) in cultured neurons. Left, antagonists at 10 μM, others at 1 μM. $n = 3$ wells, mean±s.e.m. One-way Anova, post hoc Dunnett's test: rDA2h, $p = 0.9998$, 0.1458 between DA and DA + SCH, or DA+Etic, $p = 1.5 \times 10^{-7}$ between DA and others; rDA3h, $p = 0.9591$, 0.1309 between DA and DA+Etic, or SKF, $p = 4.0 \times 10^{-9}$ between DA and others.

https://doi.org/10.1038/s41592-023-02100-w



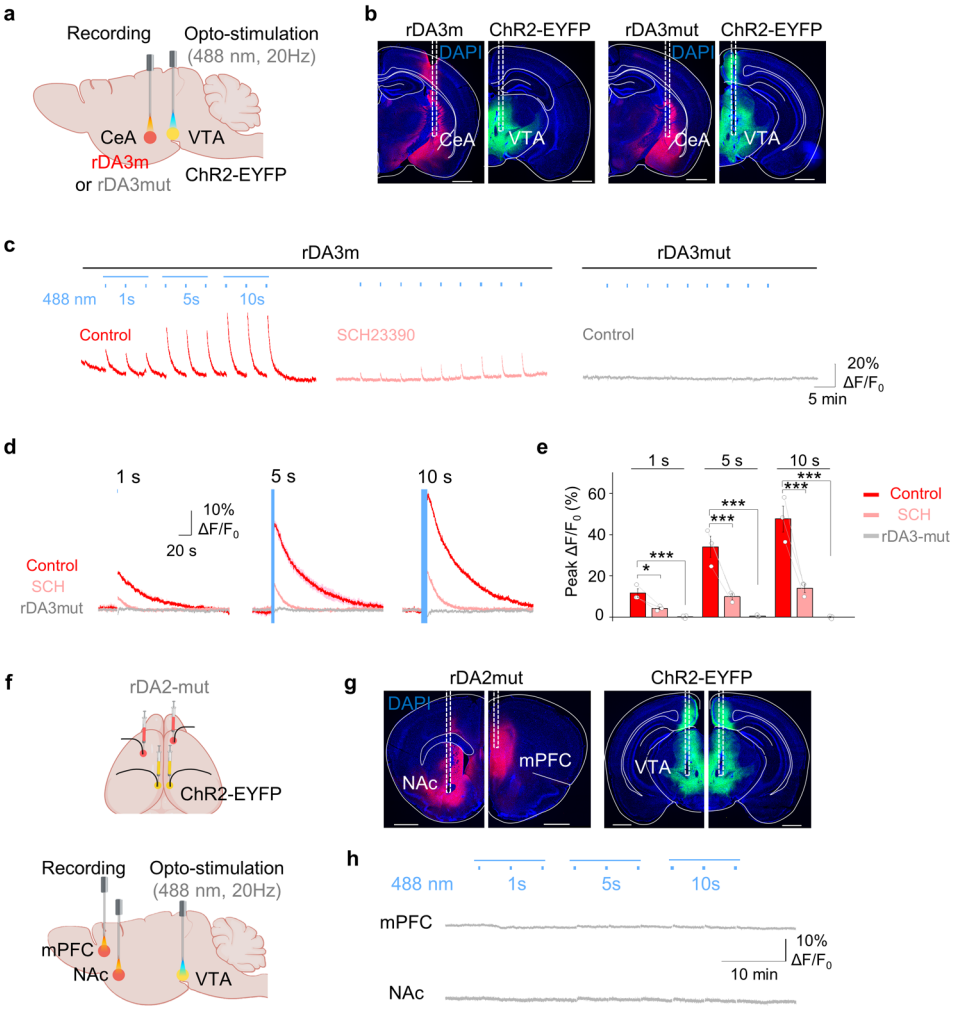
Extended Data Fig. 3 | Kinetics measurement of GRAB$_{DA}$ sensors in HEK293T cells. a, Schematic illustration showing the local perfusion system using a glass pipette containing 100 μM DA and/or receptor-specific antagonist positioned above the sensor-expressing cell. The yellow line indicates the area for line scanning. The dash lines indicate the pipette. Scale bar, 20 μm. **b**, Representative traces showing the response measured using line-scanning; when indicated, DA and receptor-specific antagonist were puffed onto the cell. The trace were the average of 3 different ROIs on the scanning line. Data are shown as mean ± SD. Each trace was fitted with a single-exponential function to determine the τ_{on} (left) and τ_{off} (right). **c**, Group summary of τ_{on} and τ_{off}. τ_{on}, $n = 11, 8, 11, 6, 9, 8$ cells for gDA3m, gDA3h, rDA2m, rDA2h, rDA3m, rDA3h; τ_{off}, $n = 10, 14, 9, 7, 10, 6$ cells for gDA3m, gDA3h, rDA2m, rDA2h, rDA3m, rDA3h, mean±s.e.m.

https://doi.org/10.1038/s41592-023-02100-w



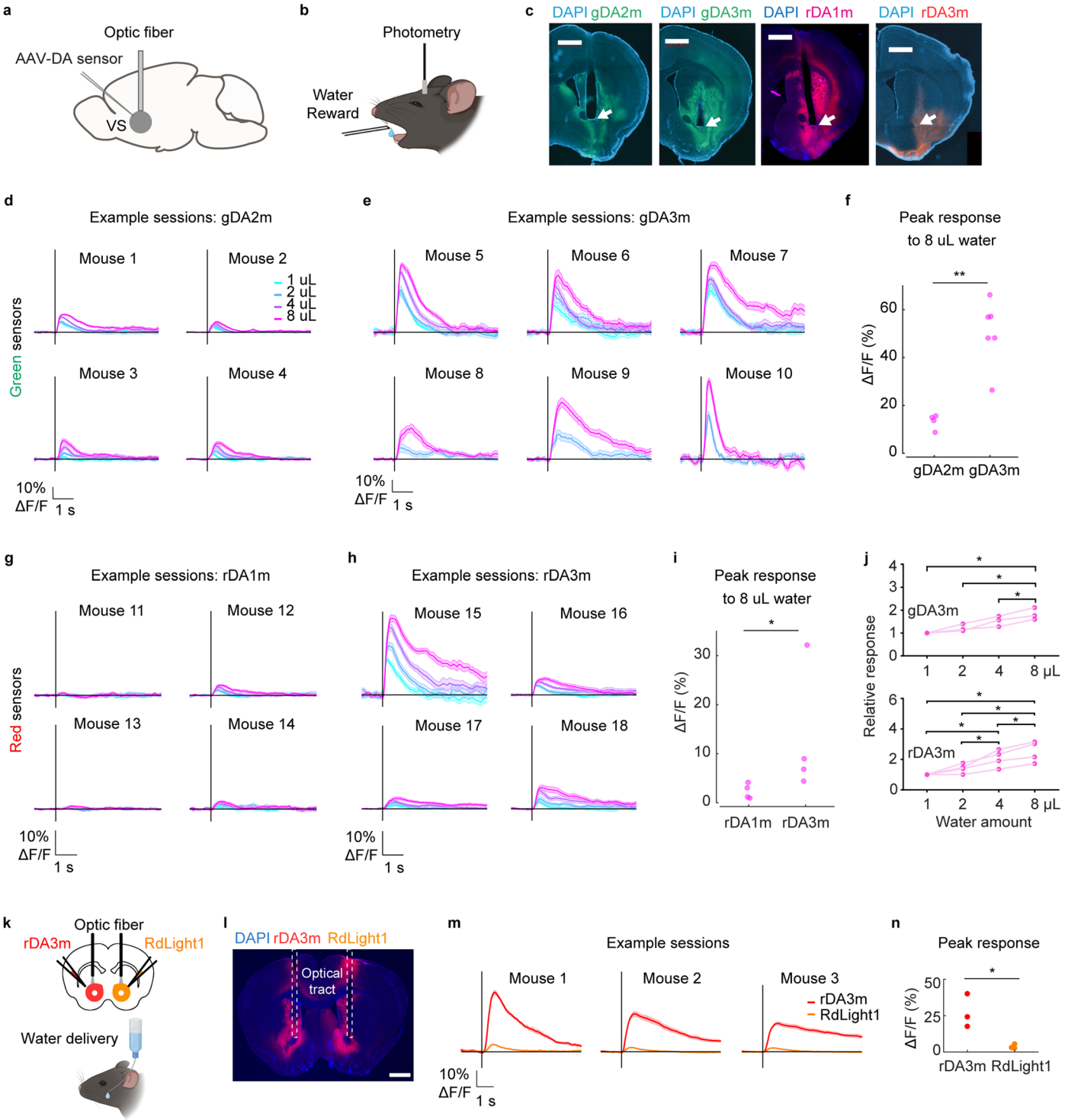
Extended Data Fig. 4 | gGRAB$_{DA3h}$ sensors report optogenetically-elicited DA release in the mouse mPFC. a, Schematic illustration depicting the experimental design for panel **b**-**i**. **b**, Histological verification of indicated sensor expression in mPFC and ChrimsonR expression in VTA. Dashed boxes indicate the location of optical tract. Scale bar, 1 mm. **c**, Representative fluorescence changes and zoom-in view (indicated by dashed box) of indicated sensors during optogenetic stimulations under control condition or in the presence of SCH-23390 (SCH). **d**, Average traces of the change in gDA3h (top) or dLight1.3b (bottom) fluorescence from a mouse. Data are shown as mean±s.d. **e**, Group summary of $\Delta F/F_0$ for the indicated sensors. $n = 4$ mice for gDA3h and dLight1.3b, respectively, mean±s.e.m. One-way ANOVA, post hoc Tukey's test was performed. **p = 0.0035 for gDA3h; n.s. p = 0.9122 for dLight1.3b; *p = 0.0295 between gDA3h and dLight1.3b. **f**, Group summary of the rise and decay time constant of the gDA3h signals in response to optogenetic stimulations. $n = 4$ mice, mean±s.e.m. **g**, **h**, Example fluorescence response (**g**) and corresponding average traces (**e**) of gDA3h (top) or dLight1.3b (bottom) to indicated optogenetic stimulation. The average traces are shown as mean±s.d. **i**, Group summary of peak $\Delta F/F_0$ of gDA3h or dLight1.3b in response to indicated optogenetic stimulation. $n = 4$ mice for gDA3h and dLight1.3b, mean±s.e.m.

https://doi.org/10.1038/s41592-023-02100-w



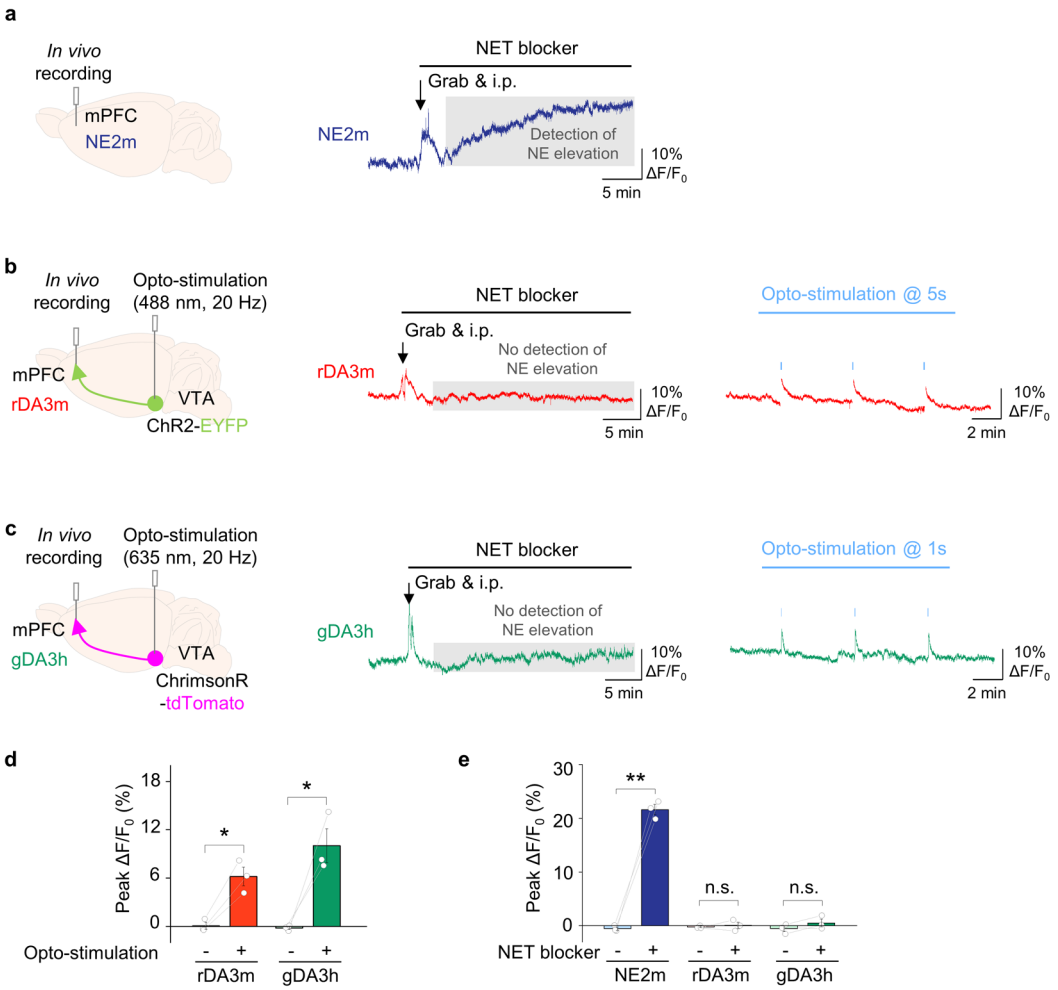
Extended Data Fig. 5 | rGRAB sensors report optogenetically-elicited DA release in multiple brain regions *in vivo*. **a**, Schematic illustration depicting the experimental design for panel **b**-**e**. **b**, Histological verification of indicated sensor expression in CeA and ChrimsonR expression in VTA. Dashed boxes indicate the location of optical tract. Scale bar, 1 mm. **c**, Representative traces of rDA3m or rDA3mut signals during optogenetic stimulations. rDA3m signals were measured before and after SCH-23390 (SCH) administration. **d**, Average traces of the change in sensor fluorescence to 1-, 5- or 10-s opto-stimulation from a mouse. Data are shown as mean ± SD. The blue shaded area indicates the application of opto-stimulation. **e**, Group summary of peak response of rDA3m or rDA3mut to indicated optogenetic stimulation. $n = 3$ mice for rDA3m and $n = 5$ for rDA3mut, mean±s.e.m. Two-tailed Student's t-test was performed. p = 0.0278, 0.0101, 0.0068 between control and SCH to 1-, 5-, 10-s opto-stimulation. p = 0.0003, 0.0001, 0.00004 between rDA3m and rDA3mut to 1-, 5-, 10-s opto-stimulation. **f**, Schematic illustration depicting the experimental design for panel **g**, **h**. **g**, Histological verification of rDA2mut expression in mPFC and NAc, and ChrimsonR expression in VTA. Dashed boxes indicate the location of optical tract. Scale bar, 1 mm. **h**, Representative traces of rDA2mut signals simultaneously recorded in the mPFC (top) and NAc (bottom) during optogenetic stimulations.

https://doi.org/10.1038/s41592-023-02100-w



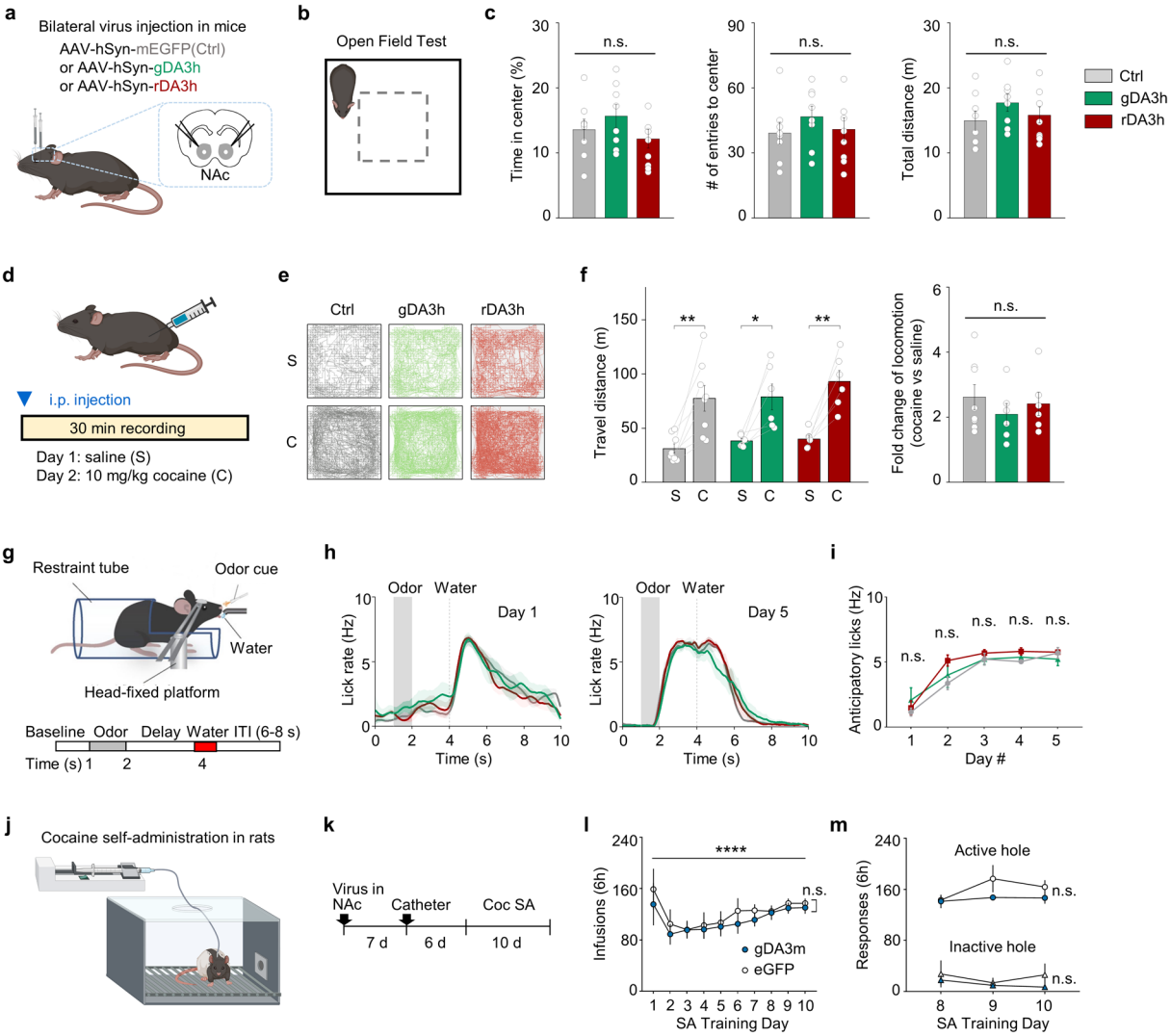
Extended Data Fig. 6 | *In vivo* comparison of the third-generation DA sensors versus previous variants in water-restricted mice receiving water rewards. **a**, Diagram of mouse surgical procedure. AAVs carrying $gGRAB_{DA2m}$, $gGRAB_{DA3m}$, $rGRAB_{DA1m}$, or $rGRAB_{DA3m}$ were injected unilaterally into NAc. An optic fiber was implanted above the injection site. **b**, Illustration of behavioral experiment. **c**, Histological verification of indicated sensor expression in NAc. White arrows indicate the location of fiber tips. Scale bar, 1 mm. **d**, Recording sessions from gDA2m mice, mean±s.e.m. Vertical black bars indicate water delivery. Colors indicate water volume. **e**, Recording sessions from gDA3m mice, mean±s.e.m. **f**, Peak response to 8 μL water for the sessions shown in **d** and **e**. n = 4 mice for gDA2m, n = 6 for gDA3m. p = 0.0095, Two-tailed Mann-Whitney U test. **g**, Recording sessions from rDA1m mice, mean±s.e.m. **h**, Recording sessions from rDA3m mice, mean±s.e.m. **i**, Peak response to 8 μL water for the sessions shown in **g** and **h**. n = 4 for rDA1m, n = 4 for rDA3m. p = 0.0286, Two-tailed Mann-Whitney U test. **j**, Group summary of sensor responses to each water amount. The response of each mouse was relative to that to 1 μL water reward. *n* = 3 mice for gDA3m, *n* = 4 mice for rDA3m. Two-tailed Student's t-test was performed between groups. For gDA3m, p = 0.032, 0.0185 and 0.0312 between 1, 2, 4 vs 8 μL, respectively; For rDA3m, p = 0.0217, 0.017 and 0.0179 between 1, 2, 4 vs 8 μL, and p = 0.032 and 0.0378 between 1, 2 vs 4 μL respectively. **k**, Schematic illustration depicting the mouse surgical procedure and the experimental design for panel **l**-**n**. **l**, Histological verification of rDA3m (left side) and RdLight1 (right side) in NAc. Dashed boxes indicate the location of optical tract. Scale bar, 1 mm. **m**, Recording sessions from 3 mice. Vertical black bars indicate water delivery. Colors indicate sensor version. **n**, Peak response of rDA3m and RdLight1 for the sessions shown in **m**. *n* = 3 mice for rDA3m and RdLight1. p = 0.0249, Two-tailed Student's t-test.

https://doi.org/10.1038/s41592-023-02100-w



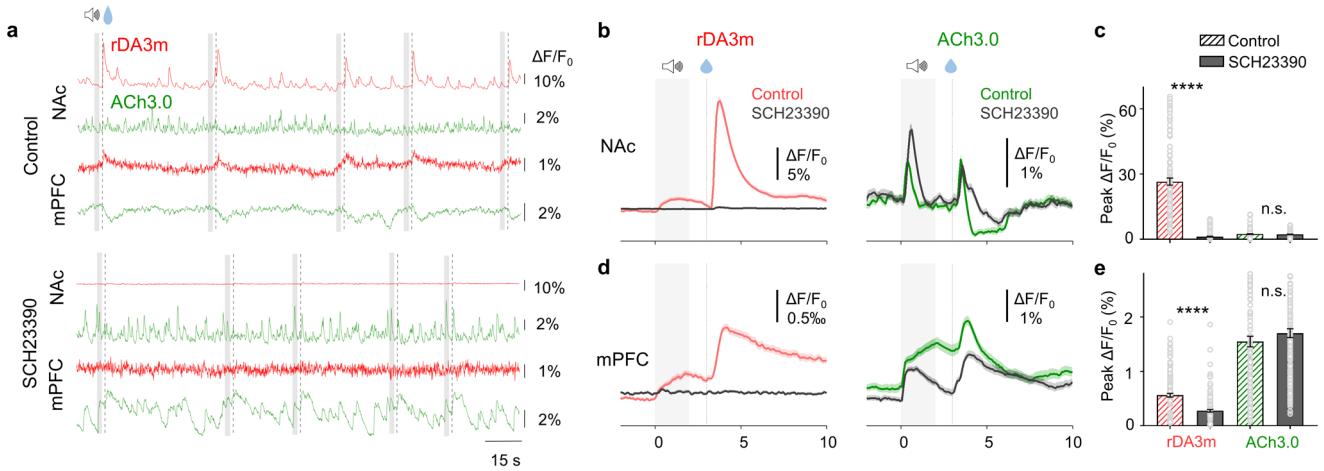
Extended Data Fig. 7 | GRAB$_{DA}$ sensors show minimal signal changes towards endogenous NE elevation. a, Schematic illustration (left) depicting the experimental design and representative fluorescence changes of NE2m towards systematic administration of NET blocker (3 mg/kg desipramine). **b**, Schematic illustration (left) depicting the experimental design and representative fluorescence changes of rDA3m towards systematic administration of NET blocker (middle) and upon optogenetic stimulations (right). **c**, Schematic illustration (left) depicting the experimental design and representative fluorescence changes (right) of gDA3h towards systematic administration of NET blocker (middle) and upon optogenetic stimulations (right). **d**, Group summary of $\Delta F/F_0$ for the indicated sensors upon opto-stimulation of VTA neurons. $n = 3$ mice for rDA3m and gDA3h, mean±s.e.m. Paired two-tailed Student's t-test was performed within group. *p = 0.0167 for rDA3m and *p = 0.0463 for gDA3h. **e**, Group summary of $\Delta F/F_0$ for the indicated sensors towards systematic administration of NET blocker. $n = 3$ mice for NE2m, rDA3m and gDA3h, respectively, mean±s.e.m. Paired two-tailed Student's t-test was performed within group. p = 0.6827, 0.2155 and 0.0012 for rDA3m, gDA3h and NE2m.

https://doi.org/10.1038/s41592-023-02100-w



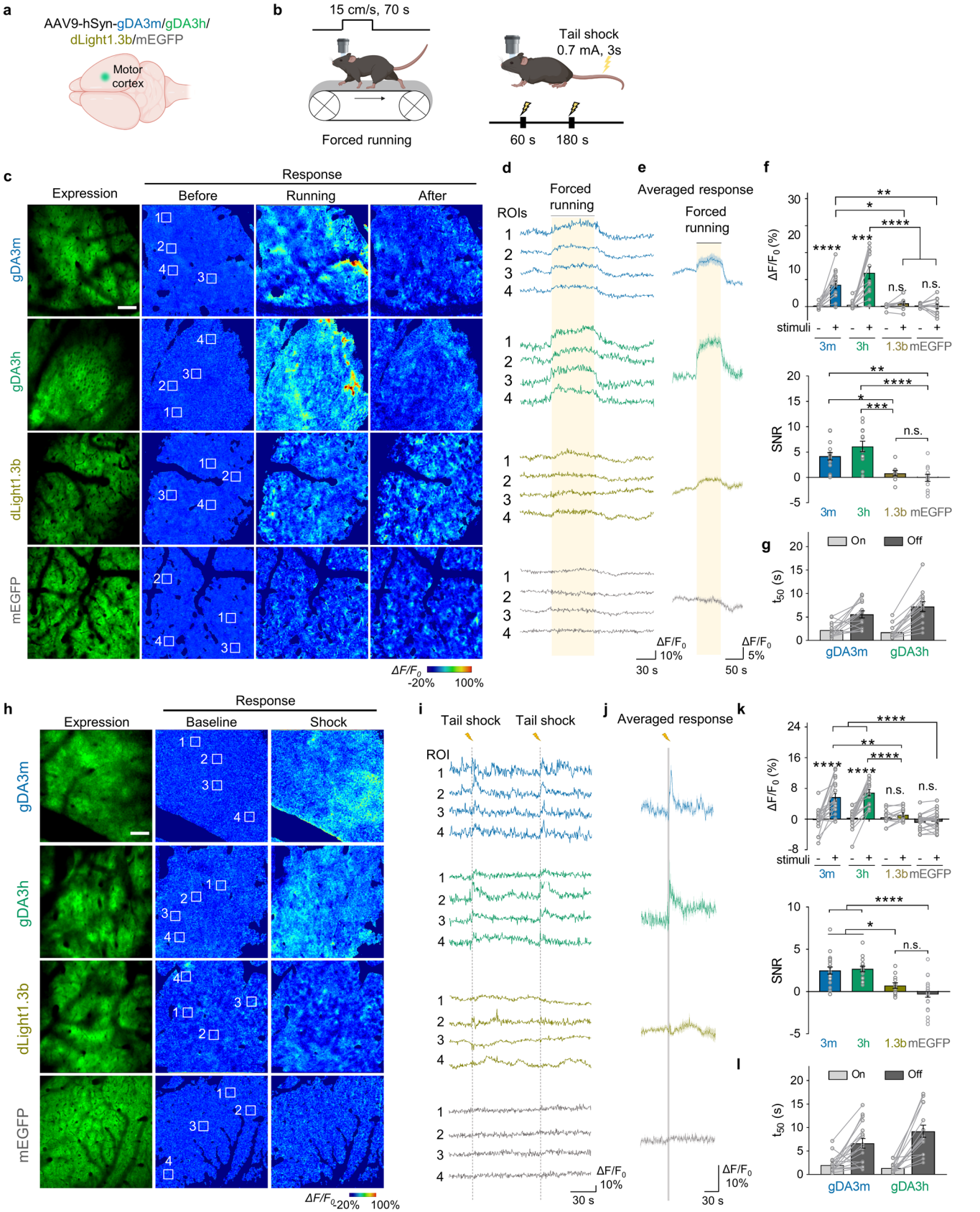
Extended Data Fig. 8 | GRAB$_{DA}$ expression in NAc has minimal effects on DA-related animal behaviors. a, Schematic representation of viral injections in the bilateral NAc. **b**. Schematic illustration showing the open field test (OFT). **c**, Quantification of behavioral parameters in the OFT. *n* = 8, 8 and 10 mice for the control, gDA3h and rDA3h group, respectively, mean±s.e.m. One-way ANOVA was performed. p = 0.3118, 0.5870 and 0.3736. **d**, Schematic illustration depicting the experimental designs for panel **e**-**f**. **e**, Representative track of control, gDA3h and rDA3h animals. **f**, Quantification of behavioral parameters during the experiments. *n* = 8, 6 and 6 mice for the control, gDA3h and rDA3h group, respectively, mean±s.e.m. Two-tailed Student's t tests were performed within groups: p = 0.0026, 0.0196 and 0.0039; One-way ANOVA was performed among groups: p = 0.6016. **g**, Schematic illustration showing the odor-reward associative learning task. **h**, Mean lick rate of Ctrl, gDA3h and rDA3h mice on day 1 and day 5 conditioning. *n* = 5 mice for each, mean±s.e.m. **i**, Quantification of anticipatory lick rate across five conditioning days. *n* = 5 mice for each, mean±s.e.m. Two-way ANOVA was performed among groups, p = 0.1076. **j**, Schematic diagram illustrating intravenous cocaine self-administration in rats. **k**, Timeline describing intravenous cocaine self-administration experiments. **l**, Cocaine infusions over 10 days of SA training did not differ between rats expressing gDA3m and eGFP virus bilaterally in the NAc core. *n* = 8 rats for each, mean±s.e.m. Two-way ANOVA mixed-effects model (Day x Virus): Day, F(9,126) = 4.50, p = 0.00004; Virus, F(1,14) = 0.35, p = 0.56; Day x Virus, F(9,126) = 0.21, p = 0.99. **m**, Nose-pokes in the active and inactive ports over the last 3 days of SA training did not differ between virus groups. Two-way ANOVA mixed-effects models (Day x Virus). *n* = 8 rats for each, mean±s.e.m. Active port: Day, F(2,28) = 3.21, p = 0.06; Virus, F(1,14) = 1.35, p = 0.27; Day x Virus, F(2,28) = 1.48, p = 0.24. Inactive port: Day, F(2, 28) = 1.97, p = 0.16; Virus, F(1,14) = 0.48, p = 0.50; Day x Virus, F(2,28) = 0.92, p = 0.41.

https://doi.org/10.1038/s41592-023-02100-w



Extended Data Fig. 9 | The signals in the mouse NAc and mPFC during Pavlovian conditioning. a, Representative fluorescence signals recorded during consecutive water trials pre (top, control) and post SCH-23390 (bottom, SCH-23390) treatment. The audio and water delivery are indicated above. **b**, Averaged traces of rDA3m (left) and ACh3.0 (right) fluorescence measured in the NAc from a mouse under control condition or in the presence of SCH-23390 in one mouse, mean±s.e.m. The grey shaded area indicates the application of audio. The dashed line indicates the delivery of water. **c**, Group summary of the peak fluorescence change of rDA3m and ACh3.0 signals in the NAc under the indicated condition. $n = 155$ trials from 3 mice for each group, mean±s.e.m. Two-tailed Student's t-test was performed between control and SCH-23390 group. p = 0.2624 for ACh3.0. **d**, **e**. same as (**b**, **c**) with simultaneously recorded rDA3m and ACh3.0 signals in the mPFC. Two-tailed Student's t-test was performed between control and SCH-23390 group. p = 0.2274 for ACh3.0.

https://doi.org/10.1038/s41592-023-02100-w



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

https://doi.org/10.1038/s41592-023-02100-w

Extended Data Fig. 10 | *In vivo* two-photon imaging of cortical DA dynamics in mice. a, **b**, Schematic illustration depicting the experimental design for panel **c**-**j**. **c**–**e**, Representative expression and pseudocolored response images (**c**), representative traces measured at the indicated ROIs (**d**), and average traces per forced running (**e**) measured in the motor cortex expressing indicated sensors. Scale bar, 100 µm. **f**, Group summary of the peak response (top) and SNR (bottom) of indicated sensors measured during forced running. n = 14/4 (14 trials from 4 mice), 13/4, 9/3 and 12/4 for gDA3m, gDA3h, dLight1.3b and mEGFP, respectively, mean±s.e.m. Paired two-tailed Student's t-test was performed for response: $p = 6 \times 10^{-5}$, 0.0002, 0.0683, 0.6275 for gDA3m, gDA3h, dLight1.3b and mEGFP. One-way ANOVA, post hoc Tukey's test was performed across groups: response, $p = 9 \times 10^{-5}$, 4×10^{-6}, 0.0214, 0.0022, 0.1611 and 0.9577 between gDA3h and dLight1.3b, gDA3h and EGFP, gDA3m and dLight1.3b, gDA3m and mEGFP, gDA3m and gDA3h, and dLight1.3b and mEGFP, respectively; SNR, $p = 9 \times 10^{-6}$, 0.0004, 0.0016, 0.0337 and 0.8812 between gDA3h and mEGFP, gDA3h and dLight1.3b, gDA3m and mEGFP, gDA3m and dLight1.3b, and dLight1.3b and mEGFP, respectively. **g**, Summary of the rise and decay t_{50} values of indicated sensors to forced running. n = 14/4 for gDA3m, n = 13/4 for gDA3h, mean±s.e.m. **h**–**j**, Same as (**c**-**e**) except mice were subjected to tail shock. **k**, Group summary of the response (top) and SNR (bottom) of indicated sensors measured upon tail shock. n = 19/4 for gDA3m, 16/4 for gDA3h, 12/3 for dLight1.3b, 26/4 for mEGFP, mean±s.e.m. Paired two-tailed Student's t-test was performed for response: $p = 3 \times 10^{-5}$, 4×10^{-7}, 0.1774 and 0.2554 for gDA3m, gDA3h, dLight1.3b and mEGFP. One-way ANOVA, post hoc Tukey's test was performed across groups: response, $p = 8 \times 10^{-5}$, 1×10^{-8}, 0.0.0013, 4×10^{-8}, 0.7169 and 0.3714 between gDA3h and dLight1.3b, gDA3h and EGFP, gDA3m and dLight1.3b, gDA3m and mEGFP, gDA3m and gDA3h, and dLight1.3b and mEGFP, respectively; SNR, $p = 1 \times 10^{-7}$, 0.0104, 1×10^{-6}, 0.0186 and 0.2607 between gDA3h and mEGFP, gDA3h and dLight1.3b, gDA3m and mEGFP, gDA3m and dLight1.3b, and dLight1.3b and mEGFP, respectively. **l**, Summary of the rise and decay t_{50} values of indicated sensors to tail shock. mEGFP data replotted from Fig. 6f. n = 18/4 for gDA3m, n = 15/4 for gDA3h, mean±s.e.m.