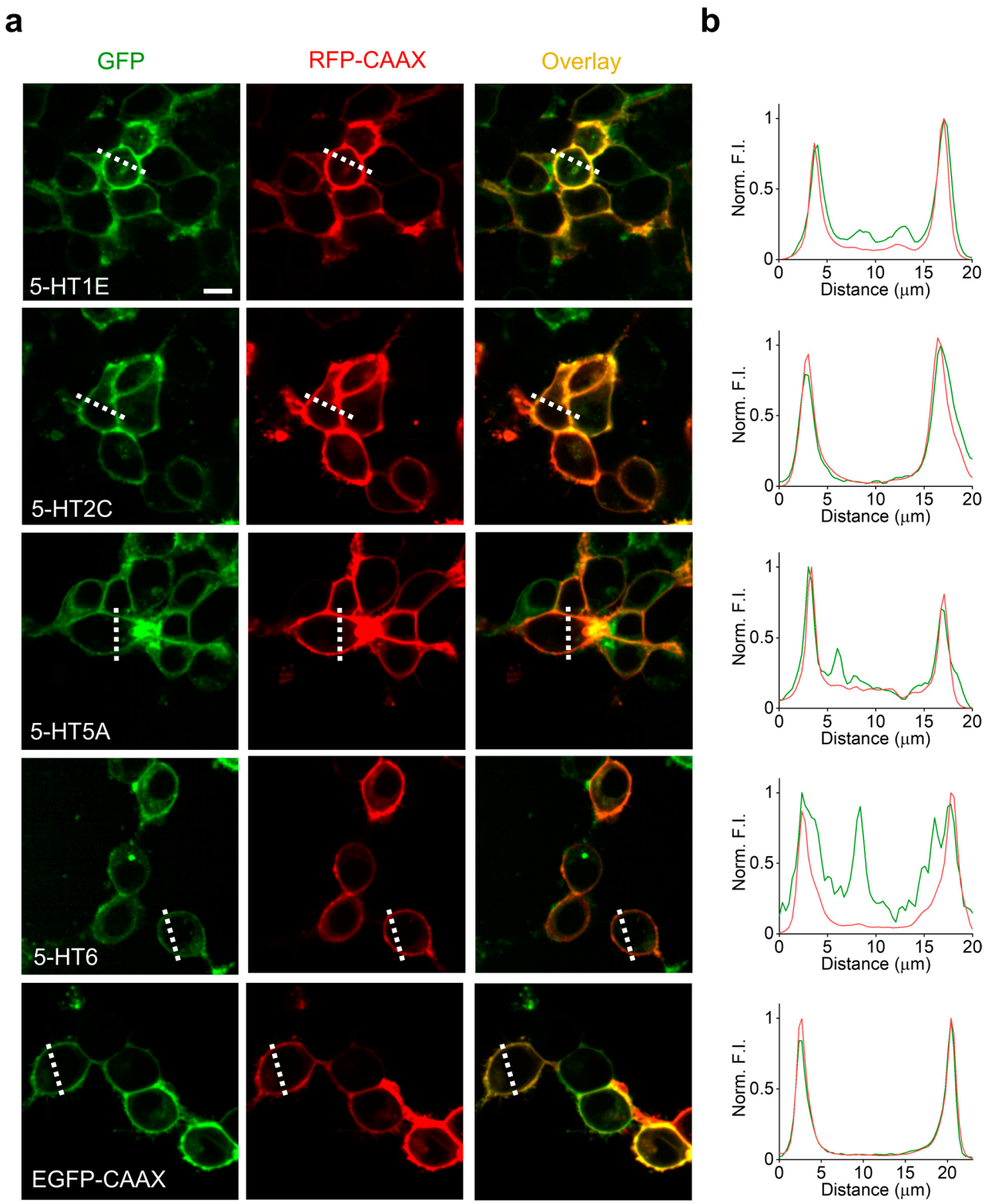
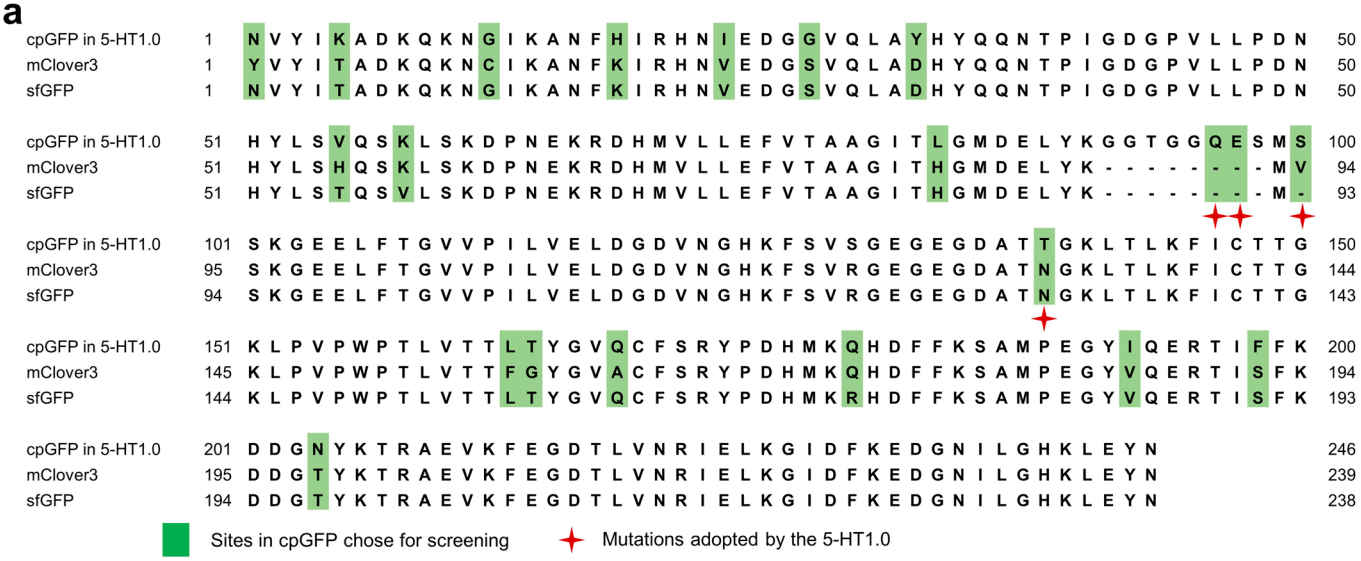


Extended Data Fig. 1 | Characterization of the membrane trafficking for 5-HT receptor-based chimeras. a, Representative fluorescence images of HEK293T cells co-expressing the indicated 5-HT receptors fused with cpGFP (green) and RFP-CAAX (red); EGFP-CAAX was used as a positive control. Similar results were observed for more than 100 cells. Scale bar, 10 μm. **b**, Normalized fluorescence intensity measured at the white dashed lines shown in (**a**) for each candidate sensor.

a



Extended Data Fig. 2 | Sequence alignment of cpGFP from 5-HT1.0 sensor, sfGFP, and mClover3. a, The sequence of cpGFP from the 5-HT1.0 sensor, sfGFP, and mClover3 are aligned. Amino acids in the cpGFP chose for optimization are labeled with light green color, and the mutations adopted by the 5-HT1.0 sensor are indicated with red stars.

a

b

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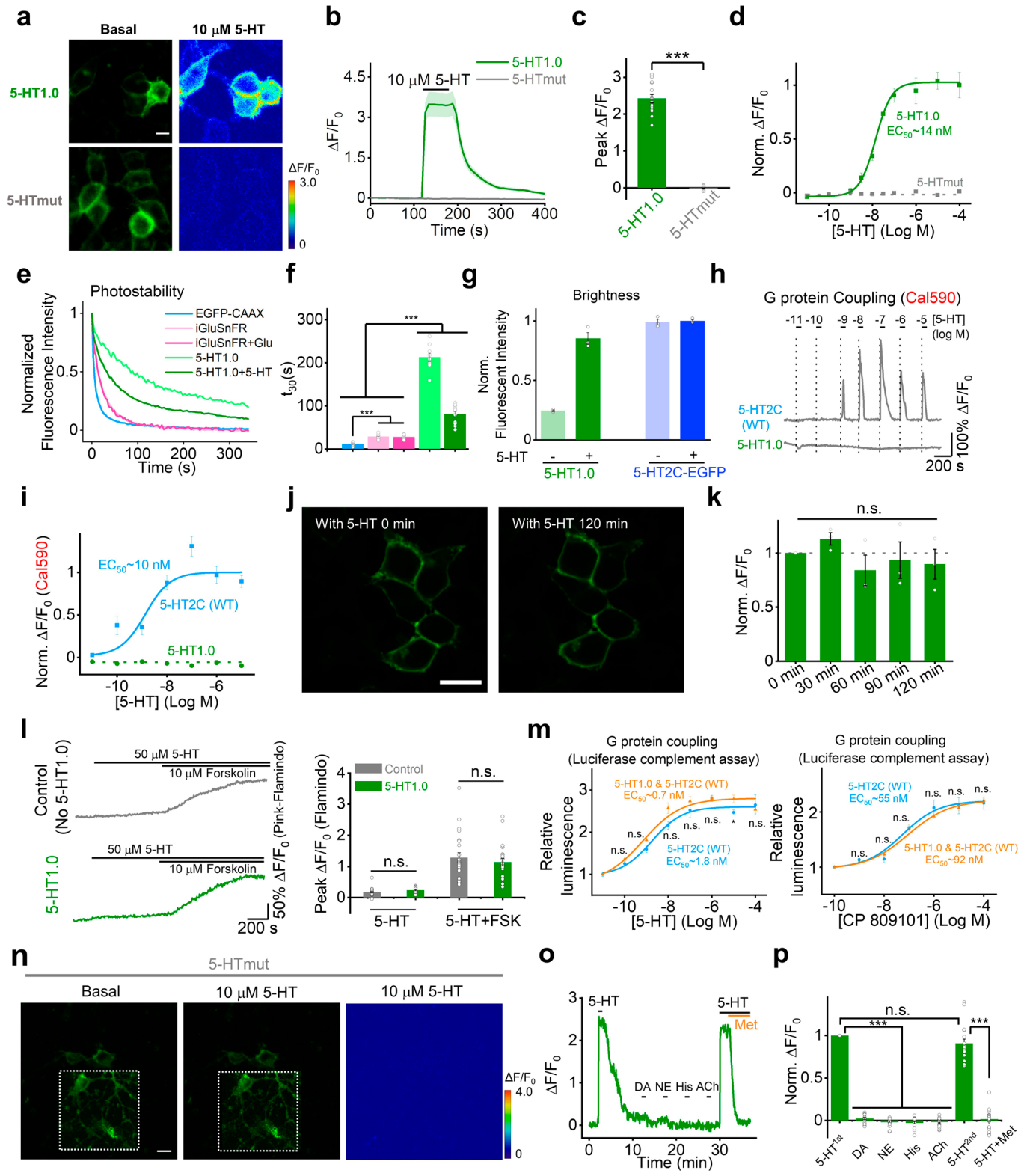
1   METDTLLLWVLLLWVPGSTGDTSLYKKVGTTGVNLRNAVHSFLVHLIGLL  50
51  VWQCDISVSPVAAIVTDIFNTSDGGRFKFPDGVQNWPALSIVIIIIMTIG 100
101 GNILVIMAVSMEKKLHNATNYFLMSLAIADMLVGLLVMPLSLLAILYDYV 150
151 WPLPRYLCPVWISLDVLFSTASIMHLCAISLDRYVAIRNPIEHSRFNSRT 200
201 KAIMKIAIVWAISIGVSVPIPVIGLRDEEKVFVNNTTCVLNDPNFVLIGS 250
251 FVAFFIPLTIMVITYCLTIYVLRRQALMFLNGNVYIKADKQKNGIKANFH 300
301 IRHNIEDGGVQLAYHYQQNTPIGDGPVLLPDNHYLSVQSKLSKDPNEKRD 350
351 HMVLLEFVTAAGITLGMDELYKGGTGGQESMSSKGEELFTGVVPILVELD 400
401 GDVNGHKFSVSGEGEGDATTGKLTLKFICTTGKLPVPWPTLVTTLTYGVQ 450
451 CFSRYPDHMKQHDFFKSAMPEGYIQERTIFFKDDGNYKTRAEVKFEGDTL 500
501 VNRIELKGIDFKEDGNILGHKLEYNGFATANNERKASKVLGIVFFVFLIM 550
551 WCPFFITNILSVLCEKSCNQKLMEKLLNVFVWIGYVCSGINPLVYTLFNK 600
601 IYRRAFSNYLRCNYKVEKKPPVRQIPRVAATALSGRELNVNIYRHTNEPV 650
651 IEKASDNEPGIEMQVENLELPVNPSSVVSERISSV*                700
```

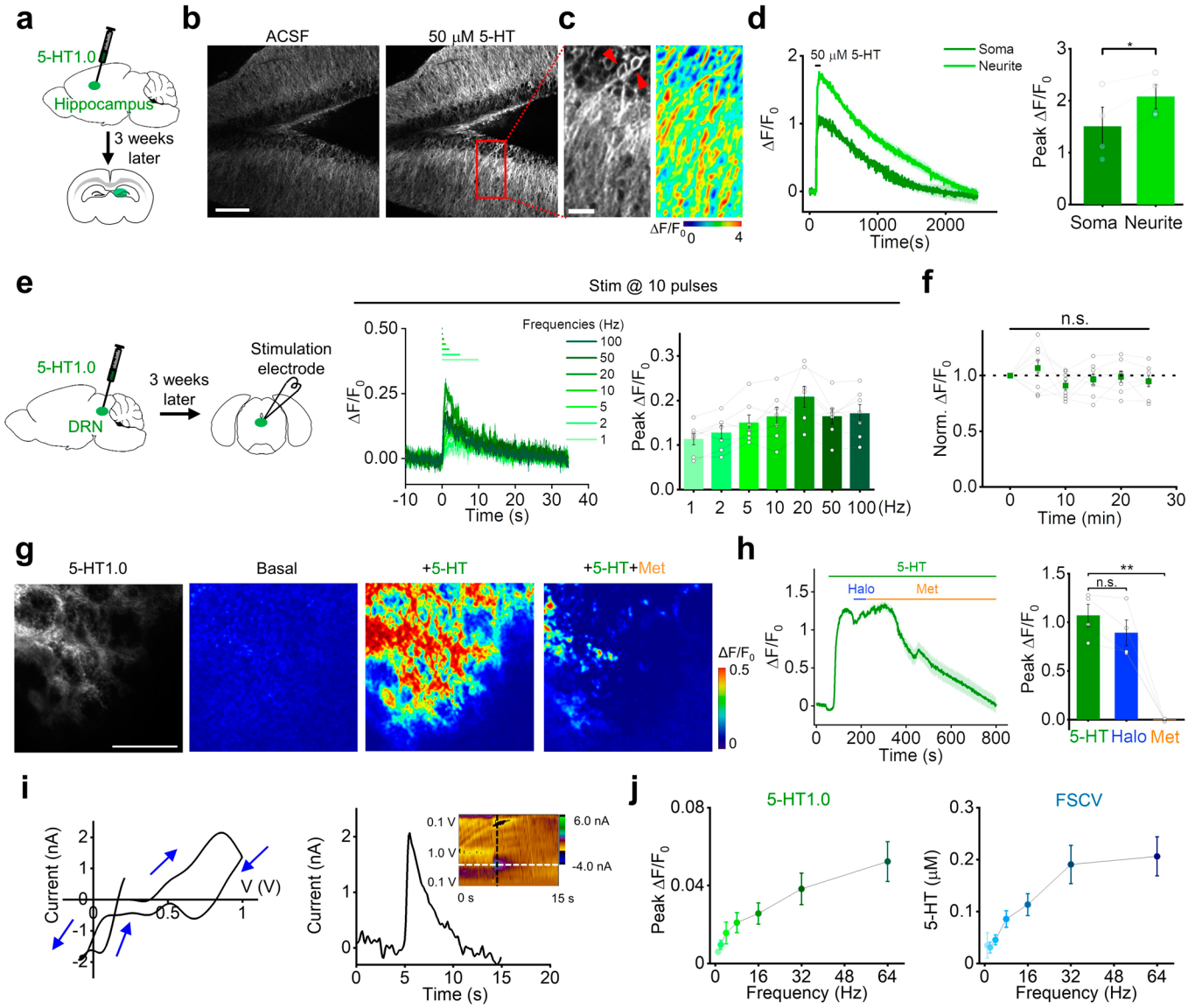
✦ Mutations adopted in cpGFP by the 5-HT1.0

Extended Data Fig. 3 | The amino acid sequence of 5-HT1.0. a, Schematic representation of the 5-HT1.0 structure. For simplicity, TM1-4, TM7, and H8 are not shown. **b**, The amino acid sequence of the 5-HT1.0 sensor after three steps of evolution. The mutated amino acids in cpGFP (cpGFP from GCaMP6s, see Chen, T.W., *et al*. 2013.) are indicated with red stars.

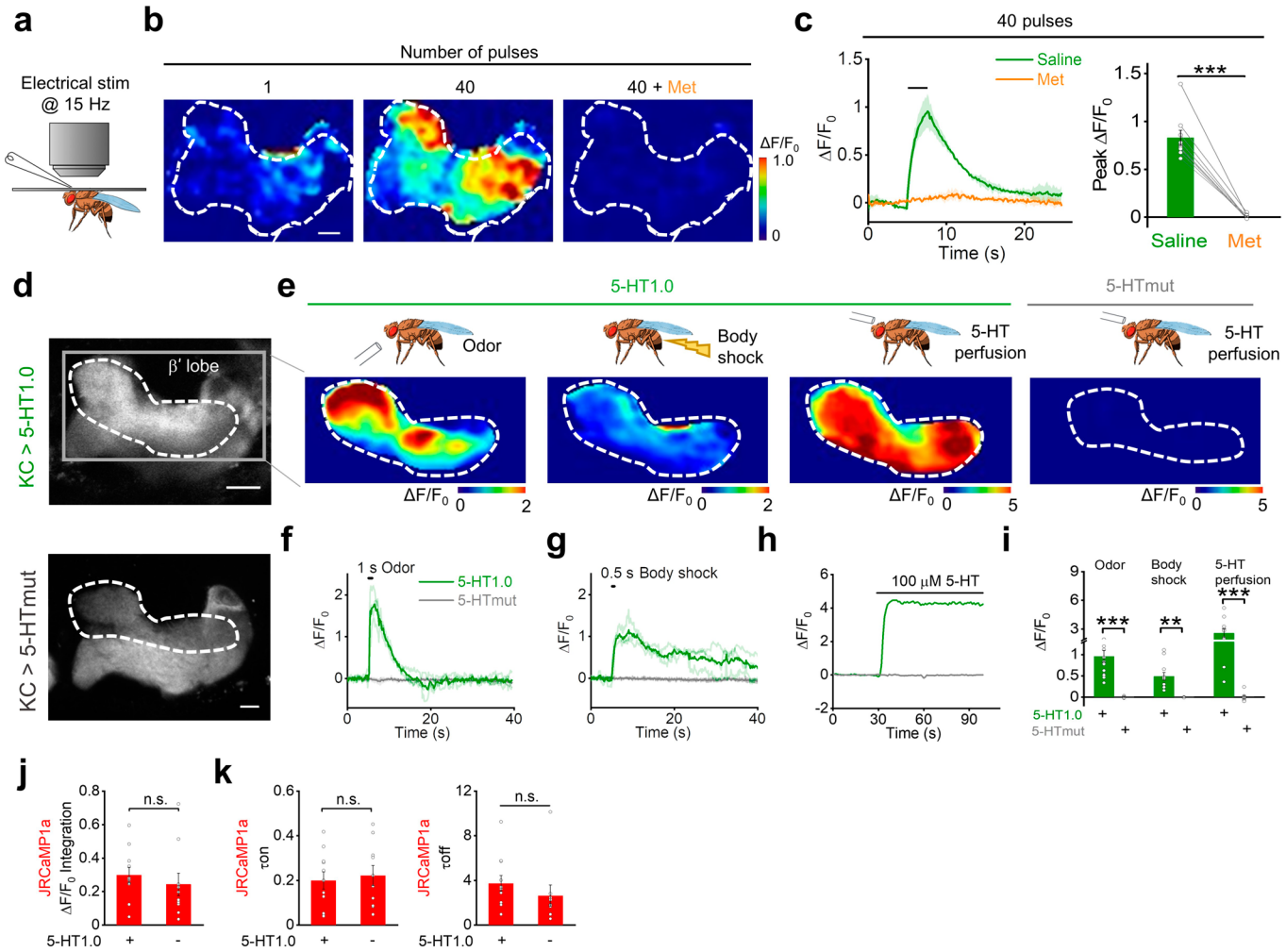


Extended Data Fig. 4 | See next page for caption.

Extended Data Fig. 4 | Further characterization of GRAB$_{5\text{-HT}}$ in cultured HEK293T cells and rat cortical neurons. a, Representative fluorescence and pseudocolor images of HEK293T cells expressing 5-HT1.0 or 5-HTmut before (left) and after (right) application of 10 μM 5-HT. Similar results were observed for more than 10 cells. Scale bar, 20 μm. **b**,**c**, Representative fluorescence traces and group summary of the peak response in HEK293T cells expressing 5-HT1.0 or 5-HTmut; n = 14 and 15 cells from 3 cultures for 5-HT1.0 and 5-HTmut group. Two-tailed Student's t-test was performed. $P = 8.18 \times 10^{-12}$ between 5-HT1.0 and 5-HTmut group. **d**, 5-HT dose-response curves measured in cells expressing 5-HT1.0 or 5-HTmut, the EC_{50} for 5-HT1.0 is shown. n = 3 wells per group with 300–500 cells per well. **e**, Representative normalized fluorescence measured in HEK293T cells expressing 5-HT1.0, EGFP-CAAX, or iGluSnFR during continuous exposure to 488-nm laser (power: 350 μW). **f**, Summary of the decay time constant calculated from the photobleaching curves shown in (**e**). n = 10/3, 14/3, and 12/3 for 5-HT1.0, EGFP-CAAX, and iGluSnFR, respectively. Two-tailed Student's t-test was performed. $P = 2.45 \times 10^{-9}$, 1.90×10^{-9}, 3.05×10^{-8}, and 7.22×10^{-7} between EGFP-CAAX and iGluSnFR without or with Glu, and 5-HT1.0 without or with 5-HT. $P = 4.43 \times 10^{-8}$ and 7.78×10^{-6} between iGluSnFR without or with Glu and 5-HT1.0 without 5-HT. $P = 4.62 \times 10^{-8}$ and 7.05×10^{-6} between iGluSnFR without or with Glu and 5-HT1.0 with 5-HT. **g**, Summary of the brightness measured in HEK293T cells expressing 5-HT1.0 or 5-HT2C-EGFP in the absence or presence of 10 μM 5-HT, normalized to the 5-HT2C-EGFP + 5-HT group; n = 3 wells per group with 300–500 cells per well. **h**,**i**, Intracellular calcium was measured in cells expressing 5-HT1.0 or the 5-HT2C receptor and loaded with the red fluorescent calcium dye Cal590. Representative traces are shown in (**h**), and the peak responses are plotted against 5-HT concentration in (**i**); n = 15/3 for each group. **j**,**k**, Fluorescence response of 5-HT1.0 expressing cells to 5-HT perfusion for two hours. Representative fluorescence images (**j**) and the summary data (**k**) showing the response to 10 μM 5-HT applied at 30 min intervals to cells expressing 5-HT1.0; n = 3 wells per group with 100-300 cells per well. Scale bar, 20 μm. $F_{4,10} = 0.888$, $P = 0.505$ for 0 min, 30 min, 60 min, 90 min and 120 min by one-way ANOVA. **l**, Left, the Gs-coupled cAMP level was detected by pink-Flamindo with or without 5-HT1.0 sensor expression. The exemplar fluorescence response traces of pink-Flamindo without (top) or with 5-HT1.0 sensor (bottom) expression, when treated with 50 μM 5-HT or 50 μM 5-HT + 10 μM Forskolin. Right, quantification data for left. n = 23/3, 23 cells from 3 cultures for each group. Two-tailed Student's t-test was performed. $P = 0.084$ and $P = 0.488$ for 5-HT and 5-HT + FSK group. **m**, Buffering effects of the 5-HT1.0 sensor by luciferase complementation assay. Luminescence signals were measured when treated with different concentrations of 5-HT (left) or 5-HT2C receptor specific agonist CP809101 (right) with or without co-expression of 5-HT1.0 sensor with 5-HT2C receptor. The luminescence signal of cells treated with the control buffer is normalized to 1. Data of 5-HT induced G-protein signaling in 5-HT2C receptor expression group were re-plotted from Fig. 1f. n = 3 wells per group with 100-300 cells per well. Two-tailed Student's t-test was performed. $P = 0.693$, 0.0402, 0.993, 0.340, 0.0618, 0.0691 and 0.127 between 5-HT1.0 and 5-HT1.0 + 5-HT2C with 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, and 10^{-10} M 5-HT. $P = 0.733$, 0.801, 0.346, 0.998, 0.304 and 0.380 between 5-HT1.0 and 5-HT1.0 + 5-HT2C with 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9} M CP809101. **n**, Cultured rat cortical neurons expressing the 5-HTmut sensor were imaged before (left) and after (middle) 5-HT application. These insets in the left and middle fluorescence images show the region with increased contrast. The pseudocolor image on the right shows the change in fluorescence of 5-HTmut in response to 10 μM 5-HT. Similar results were observed for more than 10 neurons. Scale bar, 20 μm. **o**,**p**, Representative trace (**o**) and group summary (**p**) of cultured neurons expressing 5-HT1.0 in response to indicated compounds at 10 μM each; in (**p**), Met was applied where indicated; n = 9/3. Two-tailed Student's t-test was performed. $P = 6.74 \times 10^{-22}$, 1.09×10^{-22}, 1.27×10^{-21}, 3.33×10^{-22}, and 0.0939 between 5-HT^{1st} and DA, NE, His, ACh and 5-HT^{2nd}. $P = 1.97 \times 10^{-11}$ between 5-HT^{2nd} and Met. Data are shown as the mean±s.e.m. in **b**-**d**, **f**, **g**, **i**, **k**-**m**, **p**, with the error bars or shaded regions indicating s.e.m., *$p < 0.05$, ** $p < 0.01$, ***$p < 0.001$, and n.s., not significant.



Extended Data Fig. 5 | Probing endogenous 5-HT release in mouse brain slices. a, Schematic diagram depicting the acute mouse brain slice preparation, with AAV-mediated expression of 5-HT1.0 in the hippocampus. **b**, Representative fluorescence images of the 5-HT1.0 sensor expressed in the mouse hippocampal neurons of brain slices in ACSF (left) and 50 μM 5-HT (right). Similar results were observed from 4 slices. Scale bar, 50 μm. **c**, A magnified view of the rectangular region in (b) showing the 5-HT1.0 sensor response to exogenously applied 50 μM 5-HT; left, fluorescence image; right, corresponding pseudocolor image indicating $\Delta F/F_0$. The arrowheads indicate somata. Scale bar, 15 μm. **d**, Representative traces (left) and quantification (right) of peak $\Delta F/F_0$ of the 5-HT1.0 sensor in response to 50 μM 5-HT from a single soma or neurite (n = 4 slices from 1 mouse). Two-tailed Student's t-test was performed. P = 0.0226 between soma and neurite. **e**, Left, schematic diagram depicting the acute mouse brain slice preparation, with AAV-mediated expression of 5-HT1.0 in the DRN. Middle and right, fluorescence traces (middle) and group data (right) of the change in 5-HT1.0 fluorescence in response to 10 electrical stimuli applied at the indicated frequencies; n = 7 slices from 5 mice. **f**, Summary of the change in 5-HT1.0 fluorescence in response to 6 trains of electrical stimuli (20 pulses at 20 Hz) delivered at 5-min intervals. The responses are normalized to the first train; n = 8 slices from 5 mice. $F_{5,42} = 1.18$, P = 0.335 for 0 min, 5 min, 10 min, 15 min, 20 min, and 25 min by one-way ANOVA. **g**,**h**, Representative fluorescence image, pseudocolor images (**g**), fluorescence traces (**h**, left), and group data (**h**, right) of 5-HT1.0 fluorescence in response to perfusion of 5-HT, 5-HT + Halo, and 5-HT + Met; n = 4 slices from 3 mice for each group. Two-tailed Student's t-test was performed. P = 0.0816 between 5-HT and Halo. P = 0.00297 between 5-HT and Met. **i**, Left, representative FSCV data of 5-HT release in DRN. A specific 5-HT waveform (0.2 V to 1.0 V and ramped down to −0.1 V, and back to 0.2 V at a scan rate of 1000 V/s) was applied to the CFME at a frequency of 10 Hz. Right, current vs time traces are extracted at a horizontal white dashed line shows an immediate increase in 5-HT response after electrical stimulation (20 pulses, 2 ms pulse width, 64 Hz). A cyclic voltammogram (inset) is extracted at the vertical black dashed line shows oxidation and reduction peaks at 0.8 V and 0 V, respectively. **j**, Left, group data of fluorescence response in 5-HT1.0-expressing DRN neurons to electrical stimuli with varied frequencies delivered at 20 pulses. Right, average data of peak 5-HT concentration measured by FSCV at varied stimulating frequencies delivered at 20 pulses; n = 11 neurons from 9 mice. Data are shown as the mean±s.e.m. in **d**-**f**, **h**, **j**, with the error bars or shaded regions indicating s.e.m., *p < 0.05, ** p < 0.01, ***p < 0.001, and n.s., not significant.



Extended Data Fig. 6 | Probing endogenous 5-HT release in *Drosophila in vivo*. a, Schematic drawing showing *in vivo* two-photon imaging of a *Drosophila*, with the stimulating electrode positioned near the mushroom body (MB). **b**,**c**, Representative pseudocolor images (**b**), fluorescence traces, and group summary (**c**) of the change in 5-HT1.0 fluorescence in the MB horizontal lobe in response to 40 electrical stimuli at 15 Hz in control (saline) or 10 μM Met; n = 9 flies for each group. Two-tailed Student's t-test was performed. $P = 2.36 \times 10^{-5}$ between saline and Met. Scale bar, 10 μm. **d**, Fluorescence images measured in the MB of flies expressing 5-HT1.0 or 5-HTmut; the β' lobe is indicated. Scale bar, 10 μm. **e-i**, Representative pseudocolor images (**e**), fluorescence traces (**f-h**), and group summary (**i**) of 5-HT1.0 and 5-HTmut in the MB β' lobe measured in response to a 1-s odor application, a 0.5-s body shock, and application of 100 μM 5-HT; n = 14, 12 and 10 flies for 5-HT1.0 group under odor, body shock and perfusion conditions; n = 9, 5 and 9 flies for 5-HTmut group under odor, body shock and perfusion conditions. Two-tailed Student's t-test was performed. $P = 1.14 \times 10^{-5}$, P = 0.00273, $P = 8.93 \times 10^{-5}$ between 5-HT1.0 and 5-HTmut under odor, body shock and perfusion conditions. **j**,**k**, Quantification data of area under the calcium transient curves (**k**) and the τon, τoff (**j**) in the main Fig. 2r,s; n = 11 and 10 flies for 5-HT1.0$^+$ and 5-HT1.0$^-$ group. Two-tailed Student's t-test was performed. P = 0.497 for calcium signal between two groups. P = 0.710 for τon and P = 0.307 for τoff. Data are shown as the mean ± s.e.m. in **c**, **i**-**k**, with the error bars or shaded regions indicating s.e.m., *p < 0.05, ** p < 0.01, ***p < 0.001, and n.s., not significant.