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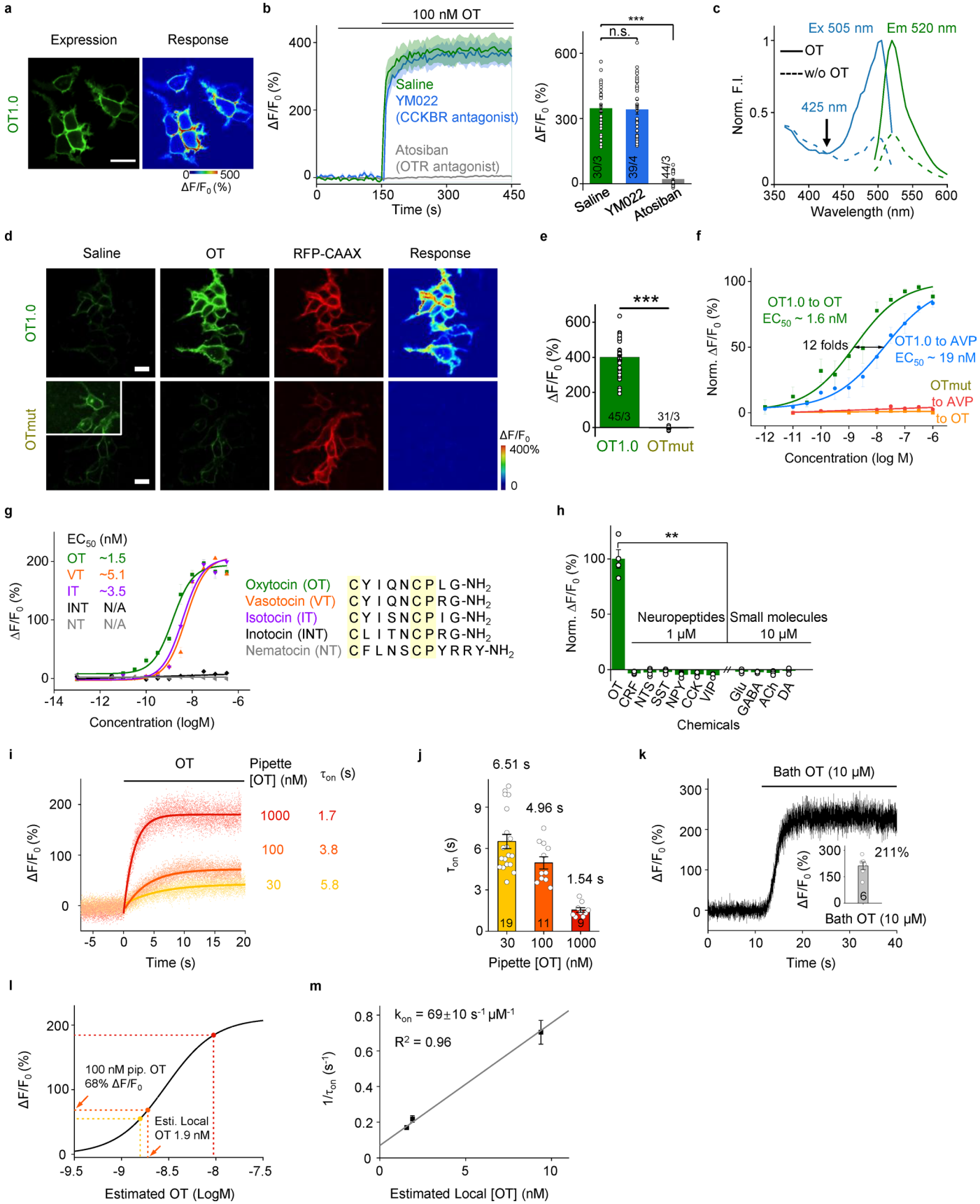

Extended Data Fig. 1 | See next page for caption.

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Extended Data Fig. 1 | Characterization of GRAB$_{OT}$ sensors in HEK293T cells. a. Representative expression and response images of OT1.0 to 100 nM OT in HEK293T cells. Scale bar, 20 μm. **b**. Example traces (left) and summary data (right) of OT1.0-expressing HEK293T cells pre-incubated with saline, 10 μM YM022, or 10 μM Atosiban in response to OT. Saline: n = 30 cells from 3 coverslips [30/3], YM022: n = 39/4, Atosiban: n = 44/3. Two-tailed Student's *t*-tests, p = 0.84 (between Saline and YM022); $p = 9.8 \times 10^{-18}$ (between Saline and Atosiban). **c**. Excitation (Ex) and emission (Em) spectra of the OT1.0 sensor with or without 100 nM OT. **d**. Representative images of OT1.0 and OTmut expressed in HEK293T cells in saline and in the presence of 100 nM OT. Also shown is RFP-CAAX expression, showing localization at the plasma membrane. The images at the right show the change in OT1.0 and OTmut fluorescence in response to OT application. White rectangle with enhanced contrast showing OTmut expressing HEK293T cells in saline. Scale bars, 20 μm. **e**. Summary of the peak change in OT1.0 and OTmut fluorescence measured in HEK293T cells in response to 100 nM OT. OT1.0: n = 45 cells from 3 coverslips; OTmut: n = 31 cells from 4 coverslips. Two-tailed Student's *t*-tests were performed ($p = 5.5 \times 10^{-31}$). **f**. Dose–response curves for OT1.0 and OTmut expressed in HEK293T cells in response to the indicated concentrations of OT and AVP, with the corresponding EC_{50} values shown. The data were normalized to the maximal response measured in OT group. The dosage curves of OT1.0 to OT/AVP were averaged from 9 individual trials, with 3–4 wells per trial. **g**. Dose-response curves for OT1.0 expressed in HEK293T cells in response to the indicated concentrations of OT and its orthologous peptides, with amino acid sequence alignment shown. n = 4 coverslips for each group. **h**. Summary of the peak change in OT1.0 fluorescence measured in HEK293T cells in response to the indicated compounds applied at 1 μM (CRF, NTS, NPY, and VIP) or 10 μM (Glu, GABA, Gly, DA, NE, and 5-HT), normalized to the peak response measured in OT; n = 4 wells per group. CRF, corticotropin-releasing factor; NTS, neurotensin; NPY, neuropeptide Y; VIP, vasoactive intestinal peptide; Glu, glutamate; GABA, γ-aminobutyric acid; Gly, glycine; DA, dopamine; NE, norepinephrine; and 5-HT, 5-hydroxytryptamine (serotonin). One-way ANOVA test was performed for all groups ($F(10, 3.88) = 132.3$, $p = 2.0 \times 10^{-4}$); Dunnett's T3 multiple comparisons tests were performed ($p = 4.6 \times 10^{-3}$, 3.8×10^{-3}, 4.4×10^{-3}, 4.0×10^{-3}, 4.2×10^{-3}, 4.2×10^{-3}, 4.6×10^{-3}, 4.8×10^{-3}, 4.5×10^{-3}, and 4.5×10^{-3} (between OT and CRF, NTS, SST, NPY, CCK, VIP, Glu, GABA, ACh, DA)). **i**. Representative traces of the OT1.0 signal evoked by OT puffing at indicated concentrations. **j**. Summary of the OT1.0 signal time constant at indicated OT concentrations ($n = 19$ cells for 30 nM OT, $n = 11$ cells for 100 nM OT and $n = 9$ cells for 1000 nM OT). **k**. Representative traces and summary $\Delta F/F_0$ of OT1.0 when bath application of OT at saturated concentration using line-scanning mode. $n = 6$ trials. **l**. The calibration curve of OT1.0 dose-dependent fluorescence response in line-scanning mode, which is used to estimate the local OT concentration reaching the cells during local puffing experiments. **m**. The association rate constant of the OT1.0 sensor for OT. Local OT concentrations were estimated from **i** ($n = 19$ cells for 30 nM OT, $n = 11$ cells for 100 nM OT and $n = 9$ cells for 1000 nM OT). $^{**}p < 0.01$, and $^{***}p < 0.001$. Summary data are presented as the mean±s.e.m.

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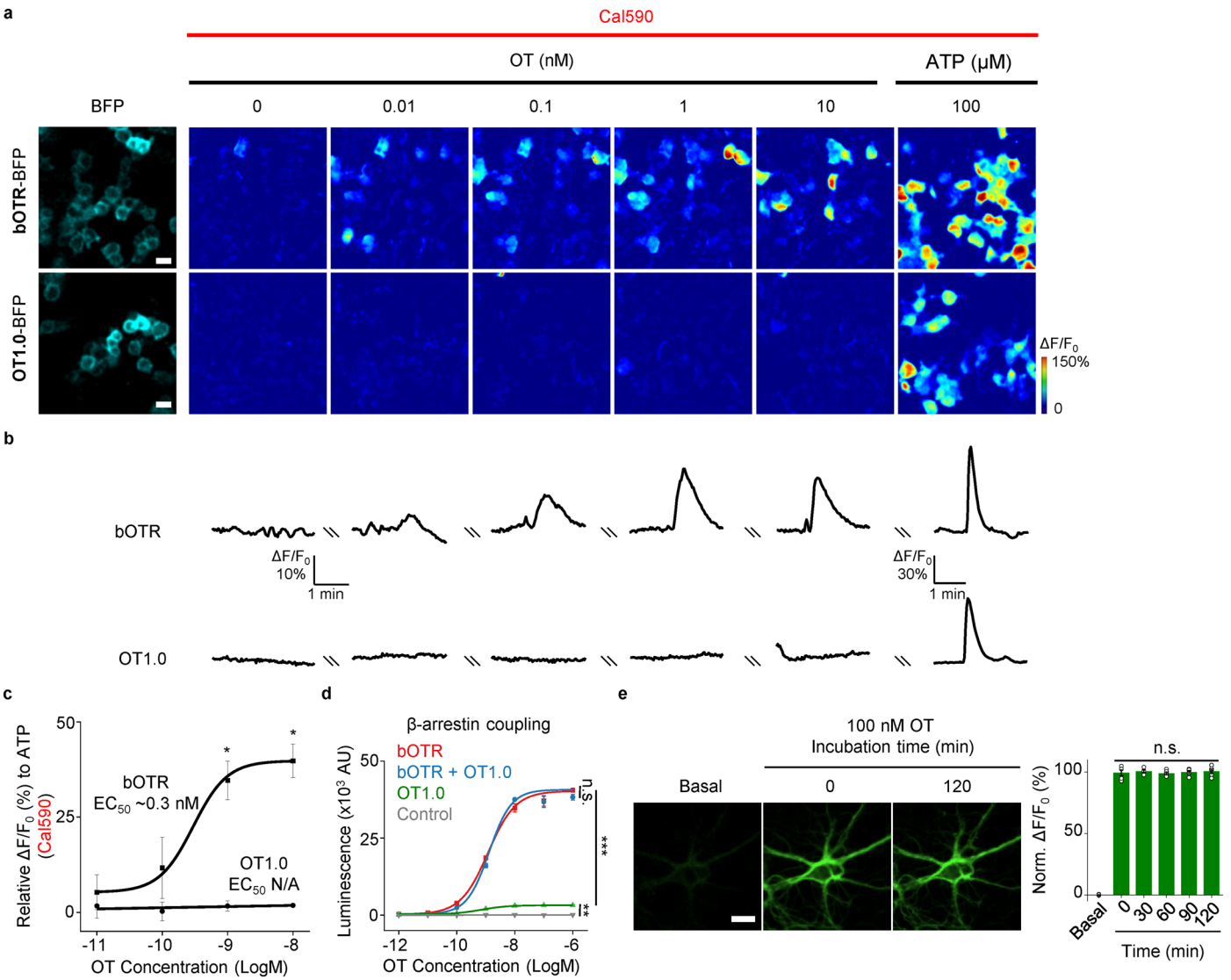

Extended Data Fig. 2 | Negligible downstream signaling coupling of OT1.0 sensor in HEK293T cells. a, **b**. Representative expression images in BFP channel, pseudocolor images (top) and $\Delta F/F_0$ traces (bottom) showing the Ca^{2+} response to the indicated concentrations of OT or ATP in HEK293T cells expressing bOTR-BFP (**a**) or OT1.0-BFP (**b**). **c**. Summary of peak Ca^{2+} $\Delta F/F_0$ for bOTR or OT1.0 expressed HEK293T cells corresponding to (**a** and **b**) at indicated OT concentrations, with the corresponding EC_{50} value shown. The data were normalized to the peak response measured in 100 μM ATP. n = 3 coverslips for each group. Two-tailed Student's *t*-tests were performed between bOTR and OT1.0 (p = 0.56, 0.29, 0.018, and 0.013 for 0.01, 0.1, 1, and 10 nM OT). **d**. β-arrestin coupling was measured using the Tango assay in cells expressing the bovine OTR (bOTR), OT1.0, bOTR and OT1.0, or no receptor (Control). n = 3 wells each. Two-tailed Student's *t*-tests, p = 0.12 (between bOTR and bOTR+OT1.0); $p = 6.8 \times 10^{-7}$ (between bOTR and OT1.0); $p = 2.9 \times 10^{-3}$ (between OT1.0 and Control). **e**. Representative images (left) and summary (right) of the fluorescence change in OT1.0-expressing neurons in response to a 2-hour continuous OT application. n = 5 cultures with >30 cells each. Row matched one-way ANOVA, with the Geisser-Greenhouse correction, F (1.509,6.037) =0.16, p = 0.79. *p < 0.05, **p < 0.01, ***p < 0.001, and n.s., not significant. All scale bars represent 20 μm. Summary data are presented as the mean±s.e.m.

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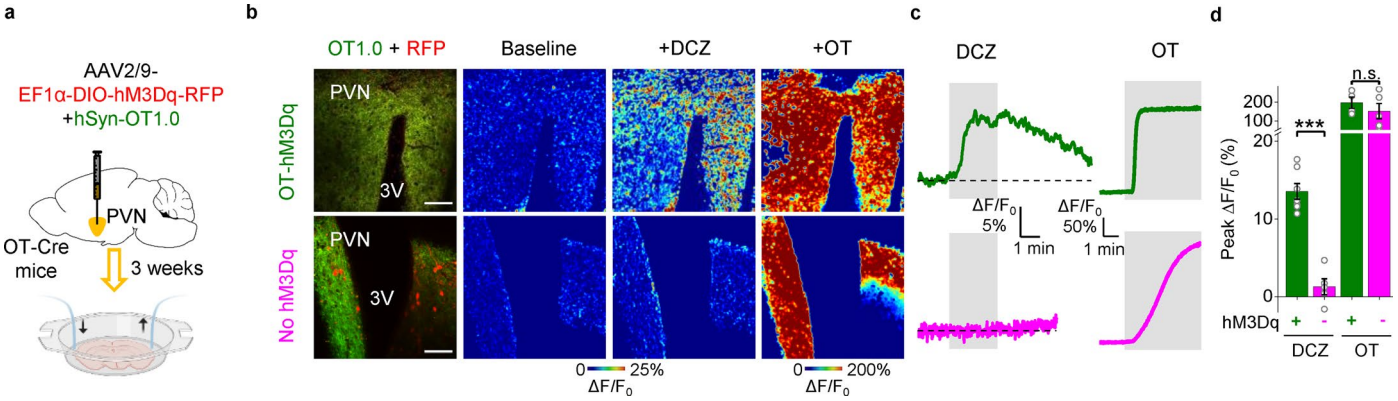

Extended Data Fig. 3 | Chemogenetic activation of oxytocinergic neurons induces OT release. a. Schematic diagram depicting the chemogenetic activation experiments. A mixture of AAVs (EF1α-dio-hM3Dq-mCherry and hSyn-OT1.0) was injected into the PVN of OT-Cre mice. As a control, hSyn-OT1.0 was injected into the PVN of OT-Cre x Ai14 mice (no-hM3Dq). The PVN and third ventricle (3 V) are indicated. **b**. Left: representative 2-photon microscopy merged images of OT1.0 (green channel) and the RFP channel (red, mCherry expression for OT-hM3Dq and tdTomato for no-hM3Dq). Right: responses of the OT1.0 sensor measured in ACSF (baseline), 60 nM DCZ, and 100 nM OT. Scale bars, 100 µm. **c**, **d**. Example OT1.0 traces (**c**) and peak change (**d**) in OT1.0 fluorescence; where indicated, DCZ or OT were applied to the slices. n = 7 slices from 2 mice for OT-hM3Dq and n = 5 slices from 2 mice for no-hM3Dq. Two-tailed Student's *t*-tests were performed ($p = 8.9 \times 10^{-6}$ (left) and 0.41 (right)). ***$p < 0.001$ and n.s., not significant. Summary data are presented as the mean±s.e.m.

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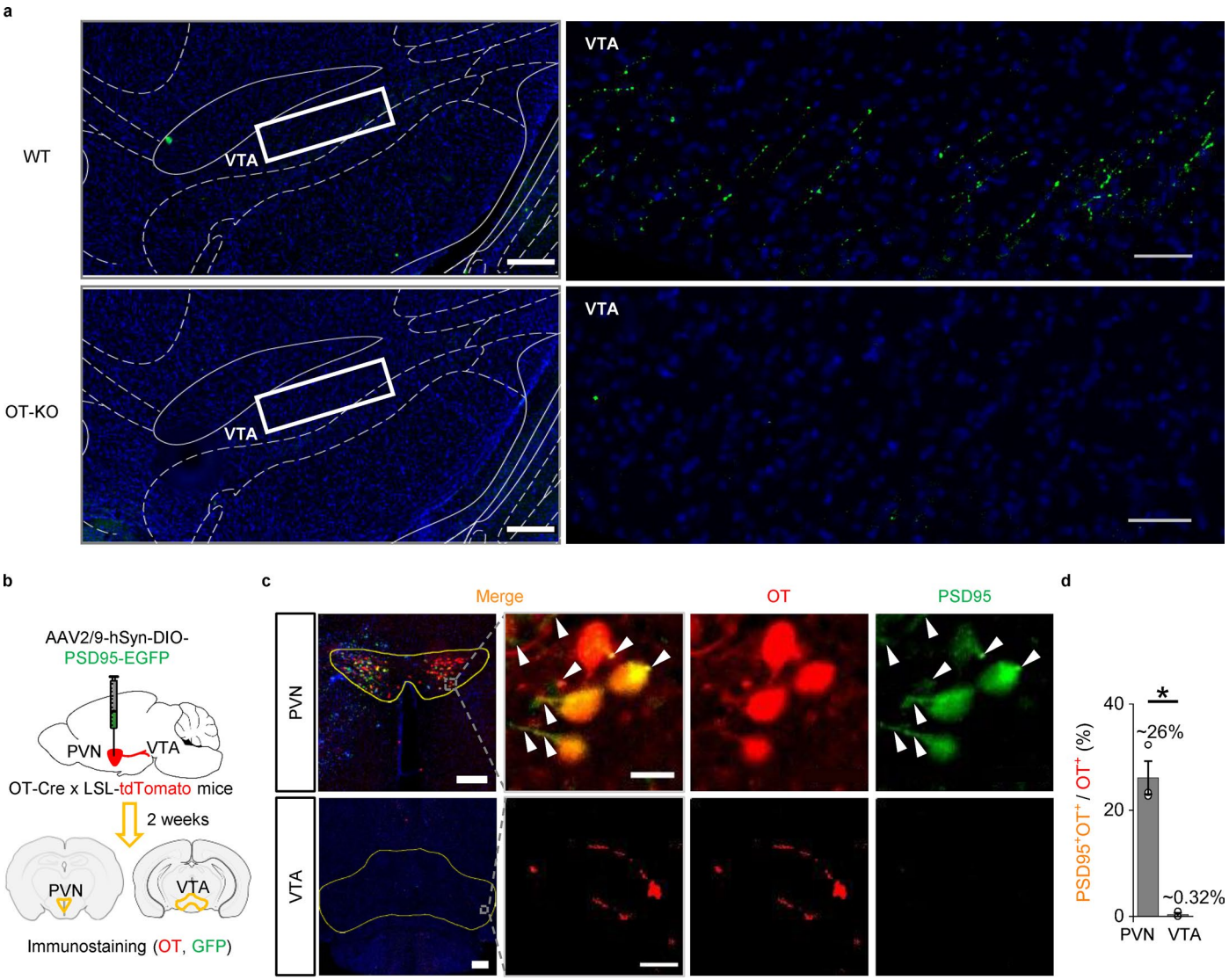

Extended Data Fig. 4 | Immunostaining of OT neurites in the VTA and PVN. **a**. Representative images of OT-positive axons in the VTA of WT and OT-KO mice. Green, OT antibody; blue, DAPI. Scale bars: left, 1 mm; right, 100 μm. **b**-**d**. Schematic drawings depicting the experimental strategy (**b**), representative images (**c**), and quantification data (**d**) showing the colocalization of PSD95-EGFP with OT-positive neurites in the PVN or VTA of coronal slices. Red, OT (indicated by OT antibody in the VTA or tdTomato in the PVN); green, PSD95 (indicated by GFP antibody); blue, DAPI. $n = 3$ slices from 1 mouse. Two-tailed Student's t-tests were performed ($p = 0.014$). Scale bars: left, 200 μm; right, 20 μm. *$p < 0.05$. Summary data are presented as the mean±s.e.m.

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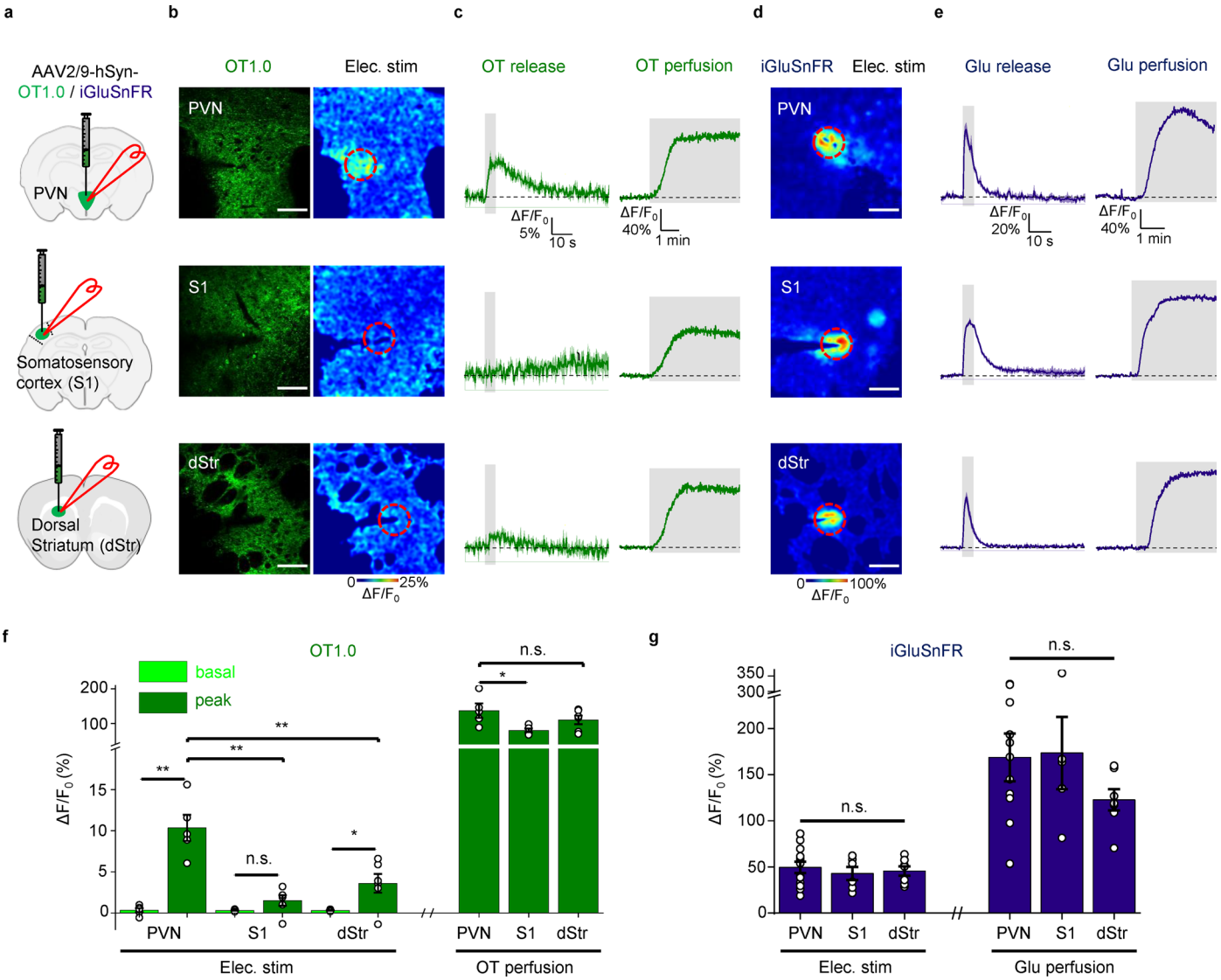

Extended Data Fig. 5 | Electrical stimulation evoked OT and Glutamate release in the PVN, S1 and dStr. a. Schematic illustration depicting the experimental setup for panels (**b**-**g**). **b** and **d**. Representative fluorescence images (left) and $\Delta F/F_0$ pseudocolor images (right) showing the expression and electrical stimulation-induced response of OT1.0 or iGluSnFR in the PVN, S1, and dStr. **c** and **e**. Representative traces of electrical stimulation evoked and 100 nM OT or 100 μM Glu perfusion induced OT1.0 or iGluSnFR signals. **f** and **g**. Summary of the change in OT1.0 or iGluSnFR fluorescence in response to 100 pulses or ligand application ($n = 5$ slices from 4 mice [5/4], 6/3, and 6/3 mice for OT1.0 in the PVN, S1, and dStr, respectively; $n = 12$ slices from 4 mice [12/4], 6/2, and 7/2 mice for iGluSnFR in the PVN, S1, and dStr, respectively.) Two-tailed Student's t-tests were performed (**f**: for electrical stimulation, $p = 2.9 \times 10^{-3}$, 0.11, and 0.034 between basal and peak $\Delta F/F_0$ for PVN, S1, and dStr, respectively; $p = 3.1 \times 10^{-3}$ between PVN and S1; $p = 9.5 \times 10^{-3}$ between PVN and dStr; for OT perfusion, $p = 0.037$ between PVN and S1; $p = 0.27$ between PVN and dStr; **g**: for electrical stimulation, $p = 0.50$ between PVN and S1; $p = 0.63$ between PVN and dStr; $p = 0.77$ between S1 and dStr; for Glu perfusion, $p = 0.92$ between PVN and S1; $p = 0.12$ between PVN and dStr; $p = 0.26$ between S1 and dStr). The data of OT1.0 and iGluSnFR in the PVN are reused from Fig. 5. *p < 0.05, **p < 0.01, and n.s., not significant. All scale bars represent 100 μm. Summary data are presented as the mean±s.e.m.

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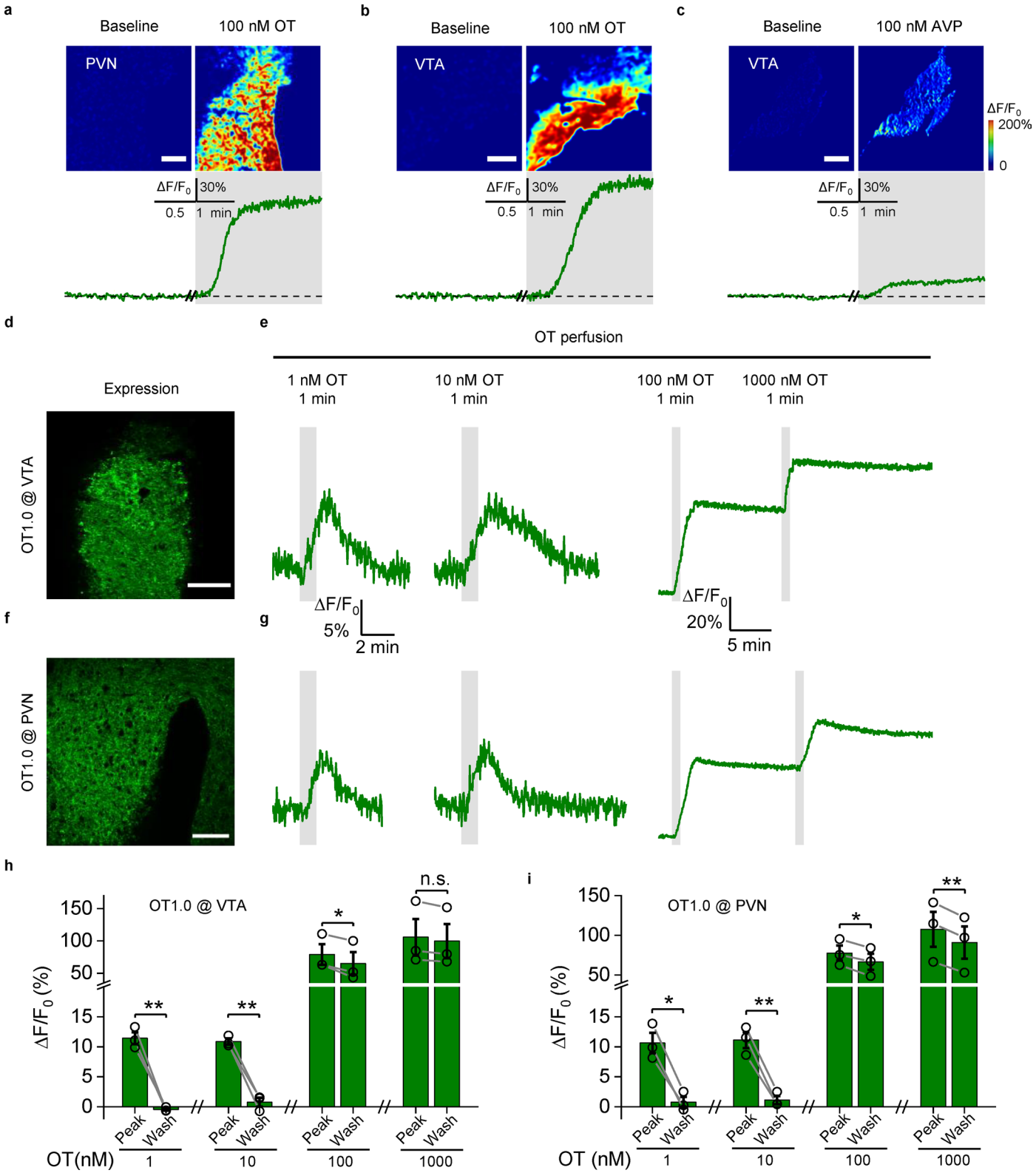

Extended Data Fig. 6 | Application of OT with different concentrations in OT1.0 expressing slices. a-c. Example pseudocolor images (top) and fluorescence traces (bottom) of OT1.0-expressing slices containing the PVN (**a**) or VTA (**b, c**) before and after application of 100 nM OT (**a, b**) or AVP (**c**). **d**, **f**. Representative fluorescence images showing the expression of OT1.0 in the VTA (**d**) and PVN (**f**). **e**, **g**. Example traces of OT1.0 signals in response to 1, 10, 100, and 1000 nM OT application in the VTA (**e**) and PVN (**g**). **h**, **i**. Summary of peak and post-application (wash) $\Delta F/F_0$ in response to 1, 10, 100, and 1000 nM OT in the VTA (**h**) and PVN (**i**). $n = 3$ slices from 3 mice [3/3] and 3/2 mice for OT1.0 in the VTA and PVN, respectively. Two-tailed Student's t-tests were performed (**h**: $p = 0.057$, 0.024, 0.18, and 0.31 (between Peak and Wash) for 1, 10, 100, and 1000 nM OT, respectively; **i**: $p = 0.011$, 8.5×10^{-3}, 0.021, and 9.2×10^{-3} (between Peak and Wash) for 1, 10, 100, and 1000 nM OT, respectively). *p < 0.05, **p < 0.01, and n.s., not significant. All scale bars represent 100 μm. Summary data are presented as the mean±s.e.m.

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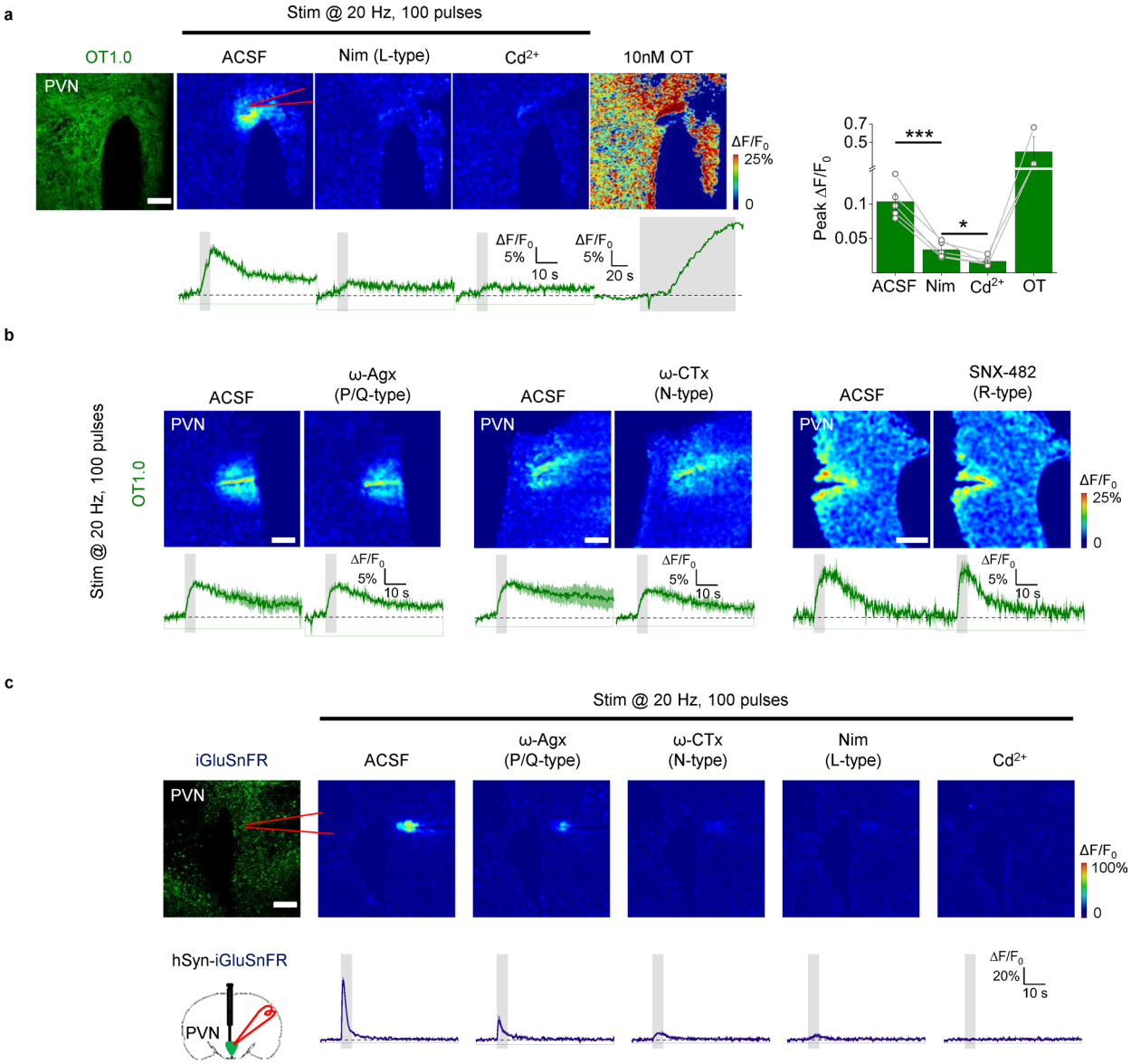

Extended Data Fig. 7 | Dissecting the Ca^{2+} sources underlying somatodendritic OT release. a. Top left: representative image of OT1.0 expressed in the PVN (left). Also shown are example pseudocolor images (top row), corresponding traces (bottom row), and summary of the peak OT1.0 response (right) to 100 electrical stimuli delivered at 20 Hz in ACSF, nimodipine (Nim; 10 μM), Cd^{2+} (200 μM), or 10 nM OT. n = 5 slices from 3 mice for ACSF, Nim, and Cd^{2+}; n = 3 slices from 2 mice for OT. Paired two-tailed Student's *t*-tests were performed ($p = 9.7 \times 10^{-4}$ (left) and 0.031 (right)). **b**. Representative pseudocolor images (top row) and corresponding traces (bottom row) of OT1.0 expressed in the PVN in response to 100 electrical stimuli delivered at 20 Hz in ACSF, ω-Agx-IVA (0.3 μM), ω-CTx (1 μM), or SNX-482 (100 nM) to block P/Q-, N-, and R-type VGCCs, respectively. **c**. Representative fluorescence image of iGluSnFR (top left) and schematic drawing depicting the experimental strategy (bottom left), related to Fig. 3e, f. Example pseudocolor images (top) and traces (bottom) of the change in iGluSnFR fluorescence in response to 100 electrical pulses delivered at 20 Hz in ACSF, ω-Agx-IVA (0.3 μM), ω-CTx (1 μM), nimodipine (Nim; 10 μM), or Cd^{2+} (200 μM) to block P/Q-, N-, L-type or all VGCCs, respectively (slices were sequentially perfused with the indicated blockers). $^{*}p < 0.05$ and $^{**}p < 0.01$. All scale bars represent 100 μm. Summary data are presented as the mean±s.e.m.

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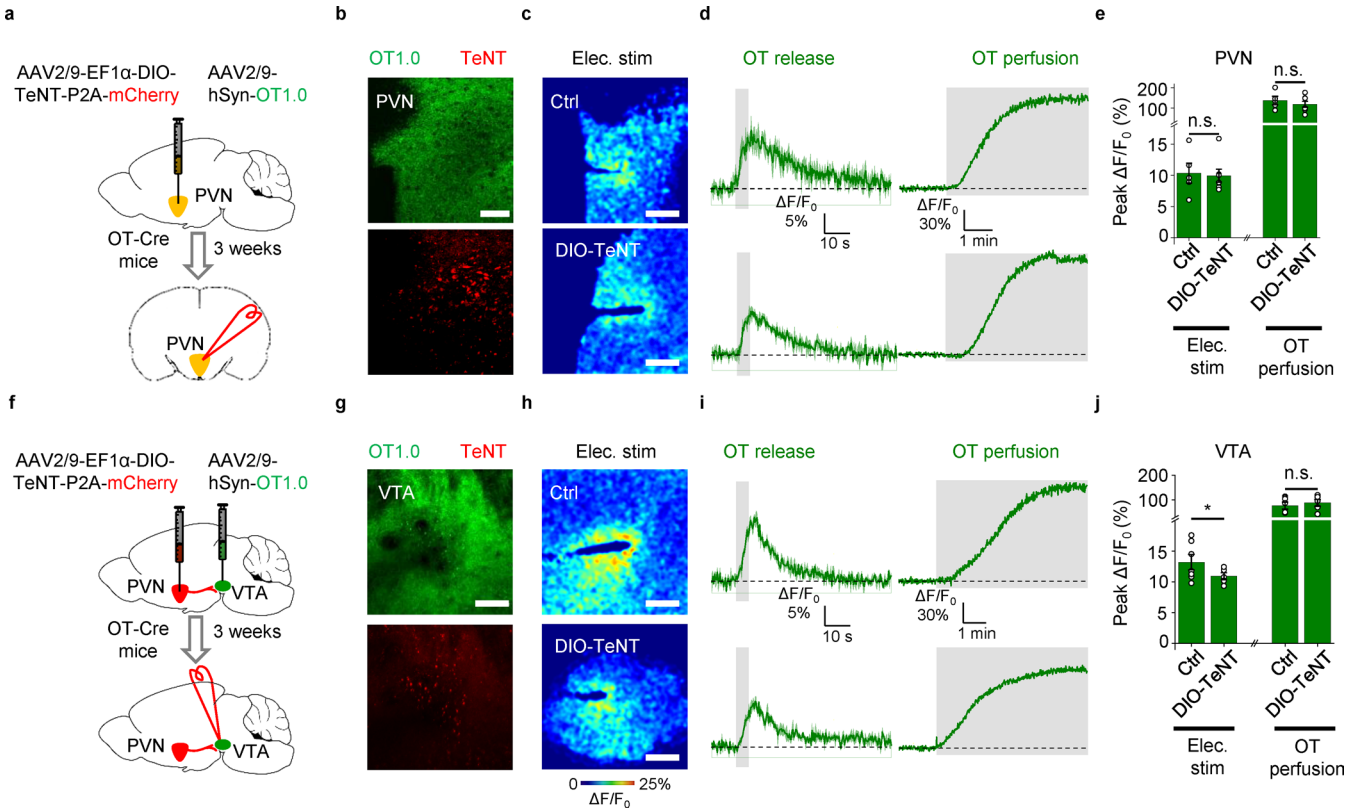

Extended Data Fig. 8 | Somatodendritic OT release is insensitive to cell type-specific expression of TeNT. a-j. Schematic drawings depicting the experimental strategy (**a**, **f**), representative images showing the expression (**b**, **g**) and peak response of OT1.0 sensor (**c**, **h**), average traces (**d**, **i**), and summary (**e**, **j**) of OT1.0 in response to 100 pulses stimulation delivered at 20 Hz or OT perfusion applied under the indicated conditions. $n = 7$ slices from 2 mice [7/2] and 5/2 mice for PVN and VTA, respectively. Two-tailed Student's t-tests were performed (**e**: $p = 0.83$ and 0.47 between Ctrl and DIO-TeNT for electrical stimulation and OT perfusion; **j**: $p = 0.038$ and 0.78 between Ctrl and DIO-TeNT for electrical stimulation and OT perfusion). The data of Ctrl groups in the PVN and VTA are reused from Fig. 5. Scale bars, 100 μm. *$p < 0.05$ and n.s., not significant. Summary data are presented as the mean±s.e.m.

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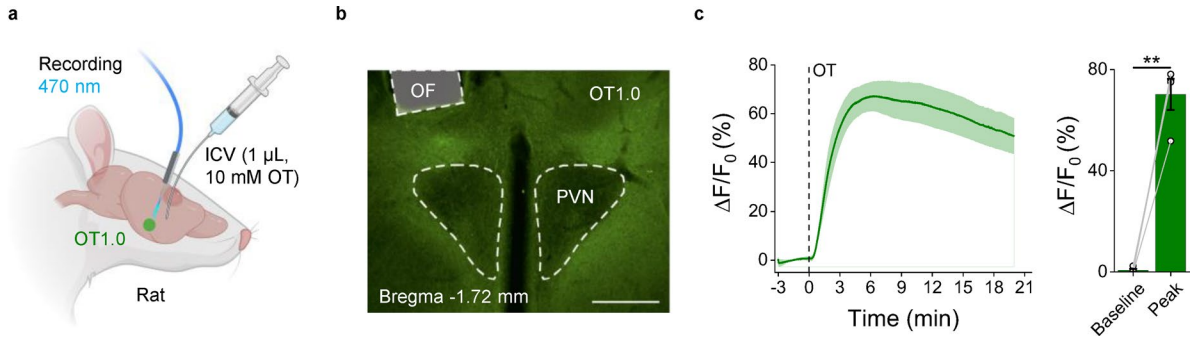

Extended Data Fig. 9 | OT1.0 can detect intraventricularly injected OT in the PVN of rats. a. Schematic diagram depicting the experimental strategy for in vivo recording of OT1.0 in rats. An AAV expressing hSyn-OT1.0 was injected into the PVN of WT Sprague Dawley female rats; optical fibers were placed in the above PVN 2 weeks later, 10 mM OT (1 µL) was injected into the lateral ventricle during recording, and 470- nm light was used to excite the OT1.0 sensor together with isosbestic control signal (405 nm). **b**. Exemplary histological verification of the optic fiber placement and the OT1.0 expression in the periPVN area. OT1.0 was stained with anti-GFP antibody for visualization. OF, optic fiber. Scale bar, 400 µm. **c**. Average trace and quantification of OT1.0 signal. The OT1.0 and isosbestic signals were sampled at 1 Hz. n = 4 rats. Paired two-tailed Student's *t*-tests were performed ($p = 1.3 \times 10^{-3}$). $^{**}p < 0.01$. Summary data are presented as the mean±s.e.m.

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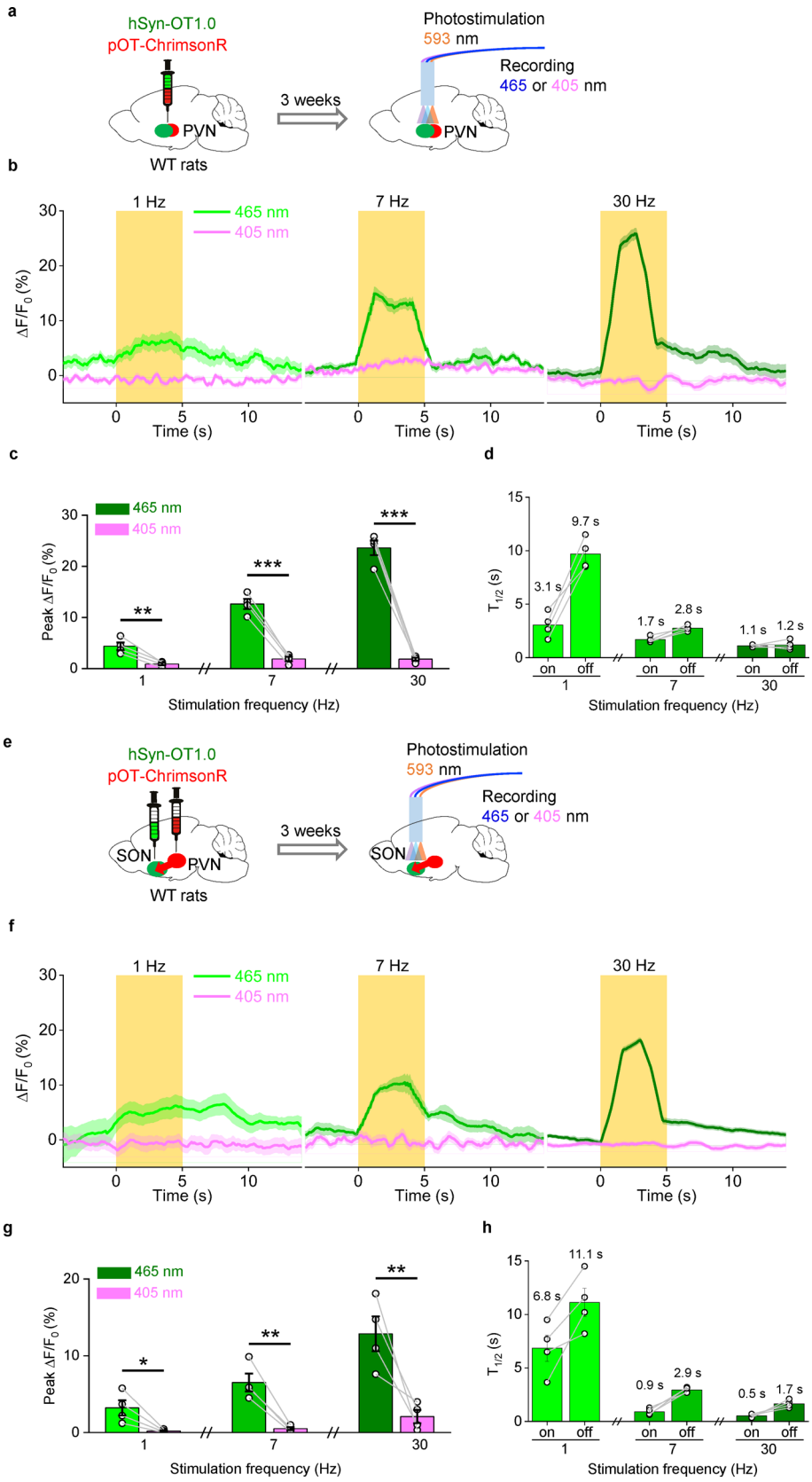

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Extended Data Fig. 10 | Optogenetic activation of neurons induces somatodendritic and axonal OT release in vivo in freely moving rats. **a**. Schematic illustrations depicting the optogenetic activation experiments with both the OT sensor and the optogenetic stimulation by ChrimsonR in the PVN. **b**. Representative traces recorded in the rat PVN of changes in normalized fluorescent emission $\Delta F/F_0$ (525 nm) during excitation at isosbestic control (405 nm, in purple) or sensor wavelength (465 nm, in green) before, during and after stimulation of ChrimsonR with pulses (at 593 nm, in orange) of 10 ms at a frequency of 1, 7 or 30 Hz for a total duration of 5 s). **c**. Summary of the peak changes in OT1.0 fluorescence emission (at 525 nm) during excitation at the sensor wavelength (465 nm, in green) or isosbestic control (405 nm, in purple) in rats PVN (at 1, 7, or 30 Hz photostimulation). n = 4 rats per group. Paired two-tailed Student's *t*-tests were performed between 465 nm and 405 nm ($p = 9.1 \times 10^{-3}$, 6.1×10^{-4}, and 6.6×10^{-4} for 1, 7, and 30 Hz, respectively). **d**. Summary of the rise time ('on') and decay time ('off') constants ($T_{1/2}$) of the OT1.0 response to photostimulation. n = 4 rats per group. **e**. Schematic illustrations depicting the optogenetic activation experiments with OT sensor expressed in the SON and the optogenetic stimulator ChrimsonR expressed in the PVN. **f**. Representative traces recorded in the rat SON of changes in normalized fluorescent emission $\Delta F/F_0$ (525 nm) during excitation at isosbestic control (405 nm, in purple) or sensor wavelength (465 nm, in green) before, during and after stimulation of ChrimsonR (at 593 nm, in orange) with pulses of 10 ms at a frequency of 1, 7, or 30 Hz for a total duration of 5 s). **g**. Summary of the peak change in OT1.0 fluorescence emission (at 525 nm) during excitation at the sensor wavelength (465 nm, in green) or isosbestic control (405 nm, in purple) in rats SON (at 1, 7, or 30 Hz photostimulation). n = 4 rats per group. Paired two-tailed Student's *t*-tests were performed between 465 nm and 405 nm ($p = 0.043$, 8.8×10^{-3}, and 0.034 for 1, 7, and 30 Hz, respectively). **h**. Summary of the rise time ('on') and decay time ('off') constants ($T_{1/2}$) of the OT1.0 response to photostimulation. n = 4 rats per group. $^*p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$. Summary data are presented as the mean±s.e.m.