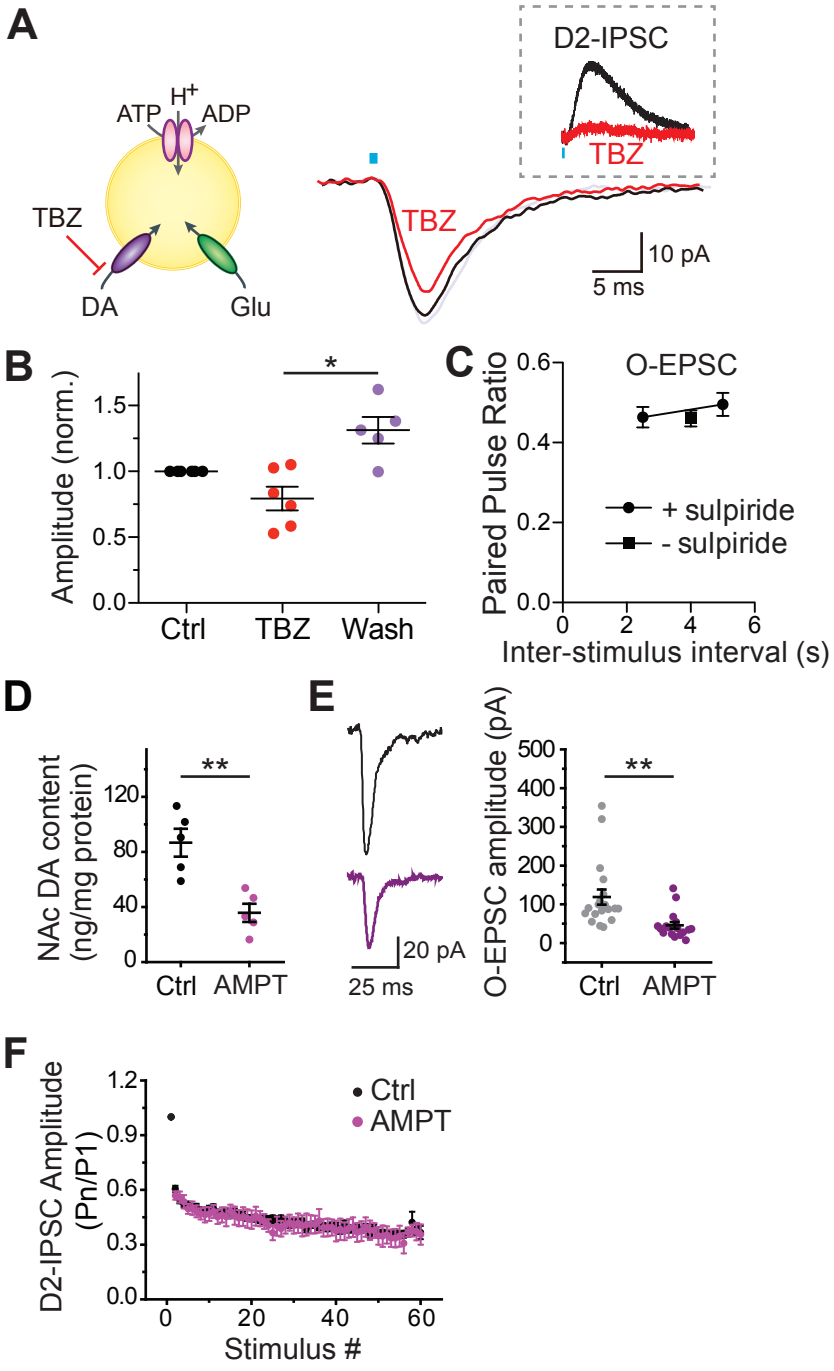


Silm et al., Figure S1

Figure S1. Effects of tetrabenazine (TBZ) and α-methyl-p-tyrosine (AMPT) on glutamate and dopamine release by dopamine neurons. Related to Figure 1.

(A) Diagram (left) shows VMAT2 (purple) and VGLUT2 (green) on a synaptic vesicle containing the vacuolar-type H^+-ATPase, and the inhibition of VMAT2 by TBZ. TBZ slightly reduces the EPSC evoked by stimulation of dopamine neuron inputs with blue light (right). Inset shows essentially complete inhibition of the D2-IPSC by TBZ (5 μM).

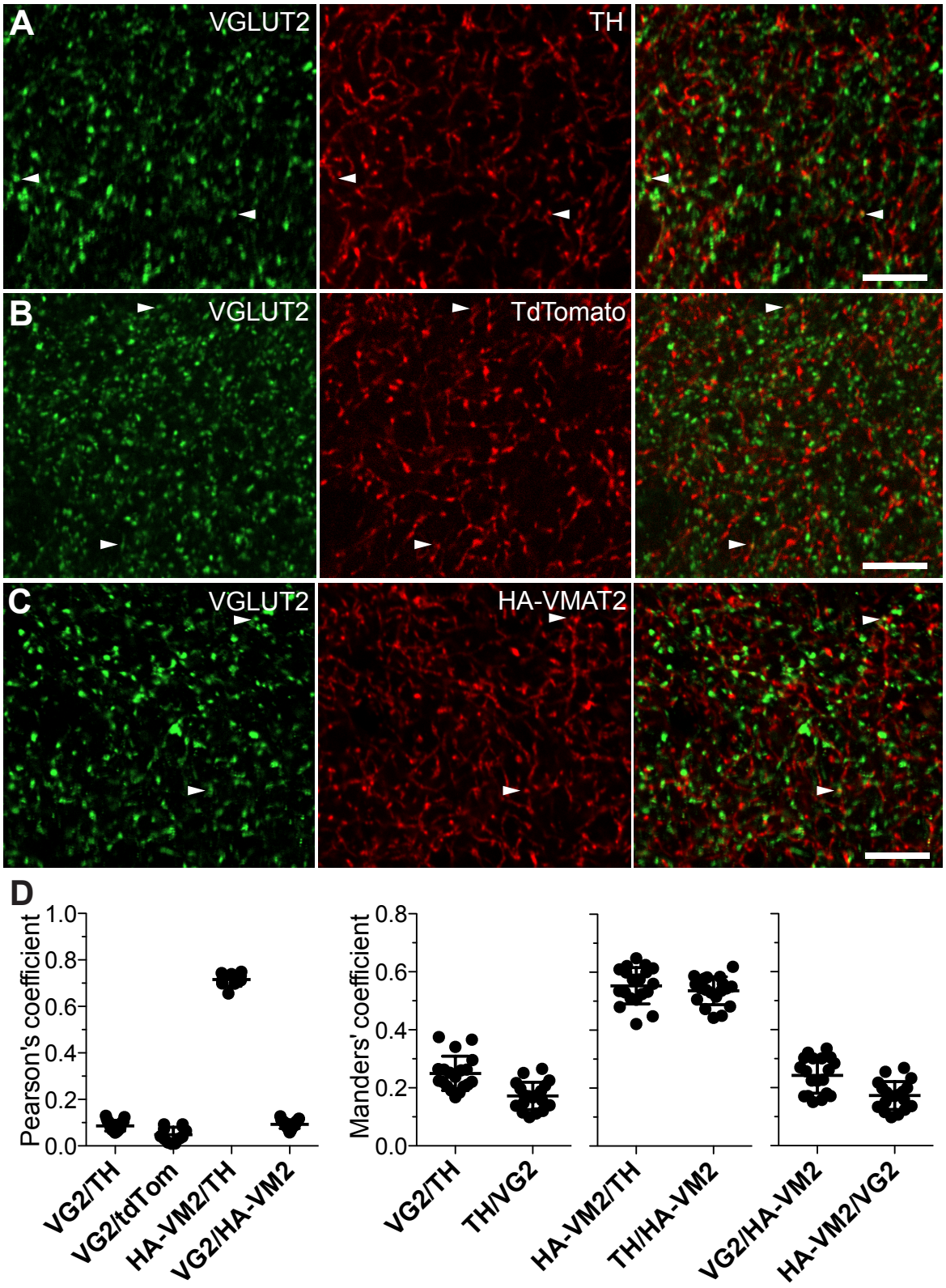
(B) The inhibition of O-EPSC by TBZ becomes apparent only after washout of the drug (for 30 min). *, $p < 0.05$ by Kruskal-Wallis and Dunn's post hoc comparison; n = 5-6 cells

(C) The D2 antagonist sulpiride does not affect the paired pulse ratio (PPR) of the O-EPSC. The PPR at an inter-stimulus interval (ISI) of 4 s in the absence of sulpiride (n = 4 cells) is shown in relation to the PPR at ISIs of 2.5 and 5 s in the presence of sulpiride from Figure 1F.

(D) Intraperitoneal injection of AMPT reduces the dopamine (DA) content of nucleus accumbens (NAc) relative to saline injection (Ctrl). **, $p < 0.01$; n = 5 animals

(E) Intraperitoneal AMPT reduces the amplitude of EPSCs evoked by stimulation of dopamine neuron terminals with light. **, $p < 0.01$; n = 19 (control) and 17 (AMPT) cells

(F) Stimulation at 0.4 Hz shows no difference in the depression of D2-IPSC in mice treated or not treated with AMPT. $p = 0.477$ by one-way ANOVA; n = 19 (control), 8 (AMPT)



Silm et al., Figure S2

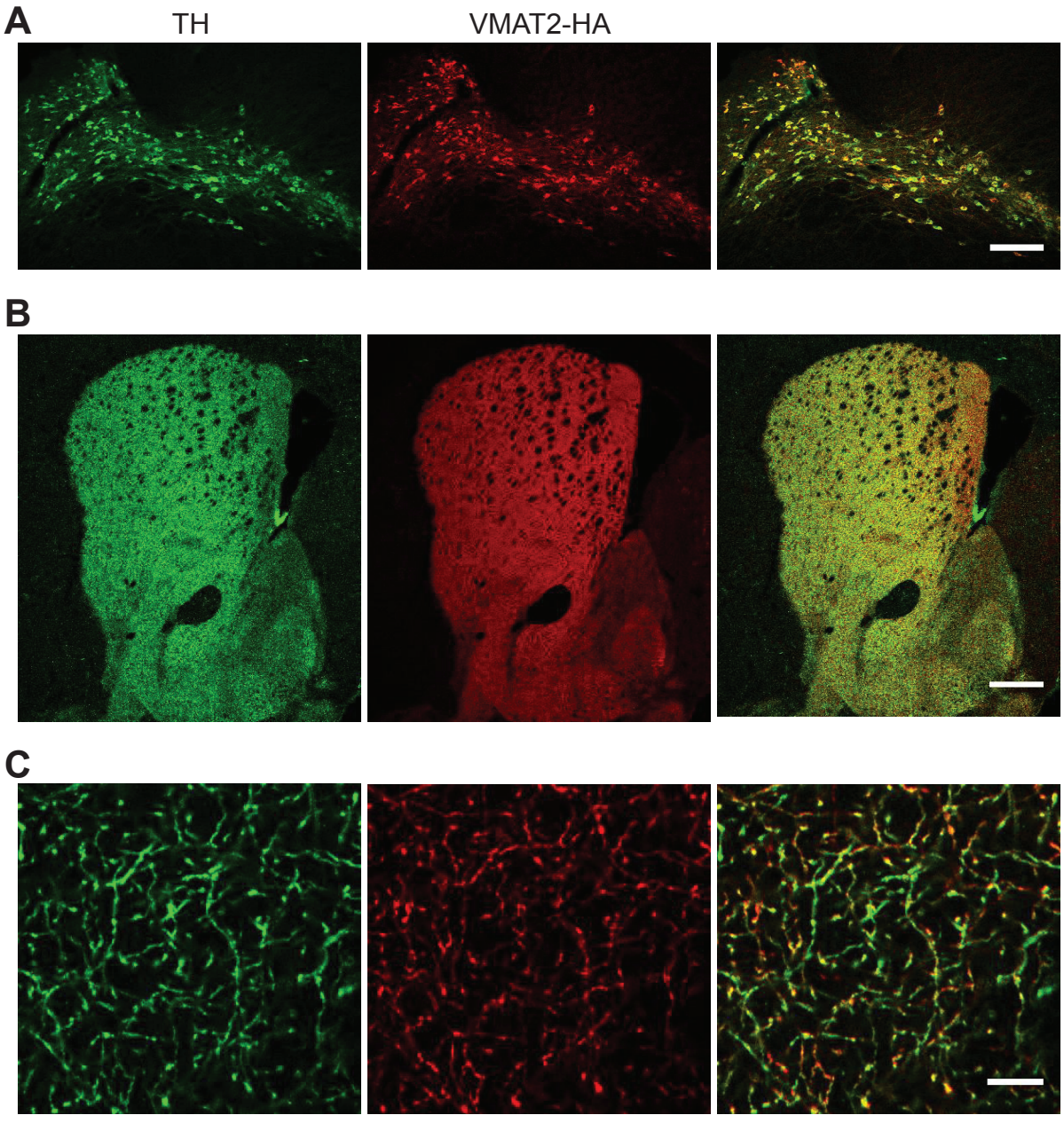
Figure S2. The majority of VGLUT2 in synaptic boutons of the ventral striatum does not occur in dopamine neurons. Related to Figure 3.

(A) Representative image of the mouse ventral striatum double stained for VGLUT2 (green) and tyrosine hydroxylase (TH, red).

(B) Representative image of the ventral striatum from a DATiCre/B6;129S6-Gt(ROSA)26Sor$^{tm9(CAG-tdTomato)Hze}$/J mouse stained for VGLUT2. tdTomato fills the DAT-positive projections.

(C) Representative image of the ventral striatum from a HA-VMAT2 BAC transgenic mouse double stained for VGLUT2 and HA. Arrowheads in A-C indicate colocalization of VGLUT2 with TH (A), tdTomato (B) or HA-VMAT2 (C). Scale bars in A-C indicate 10 μm.

(D) Colocalization of VGLUT2 with the other proteins was quantified using Pearson's (left) and Manders' (right) coefficients.

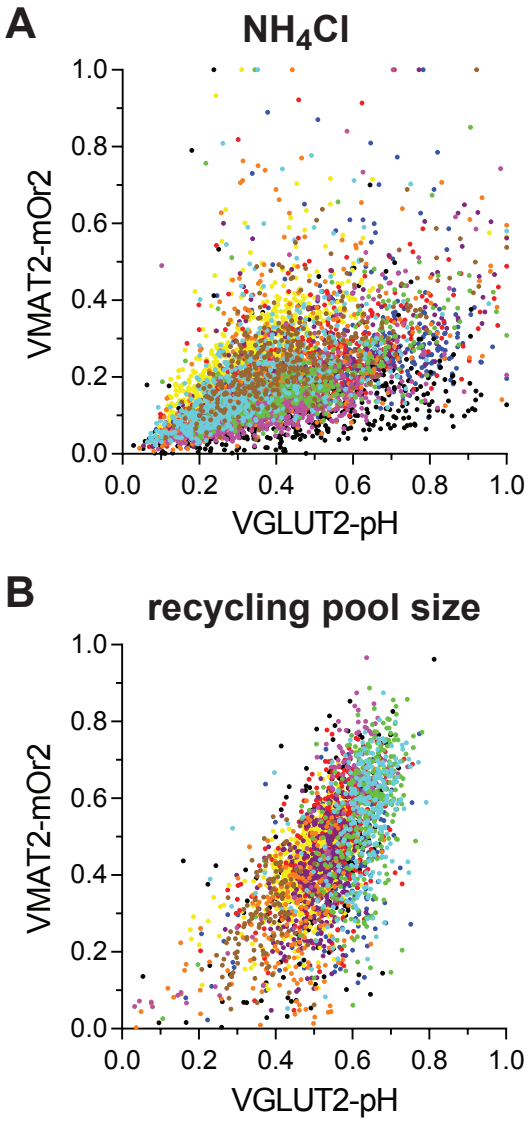


Silm et al., Figure S3

Figure S3. Faithful expression of the HA-VMAT2 BAC transgene by monoamine neurons. Related to Figure 3.

(A,B), Representative images of the midbrain (A) and striatum (B) double stained for tyrosine hydroxylase (TH) and the HA epitope. Scale bars indicate 100 μm (A) and 400 μM (B).

(C) Representative image of the ventral striatum double stained for TH and HA. Scale bar indicates 7 μm.



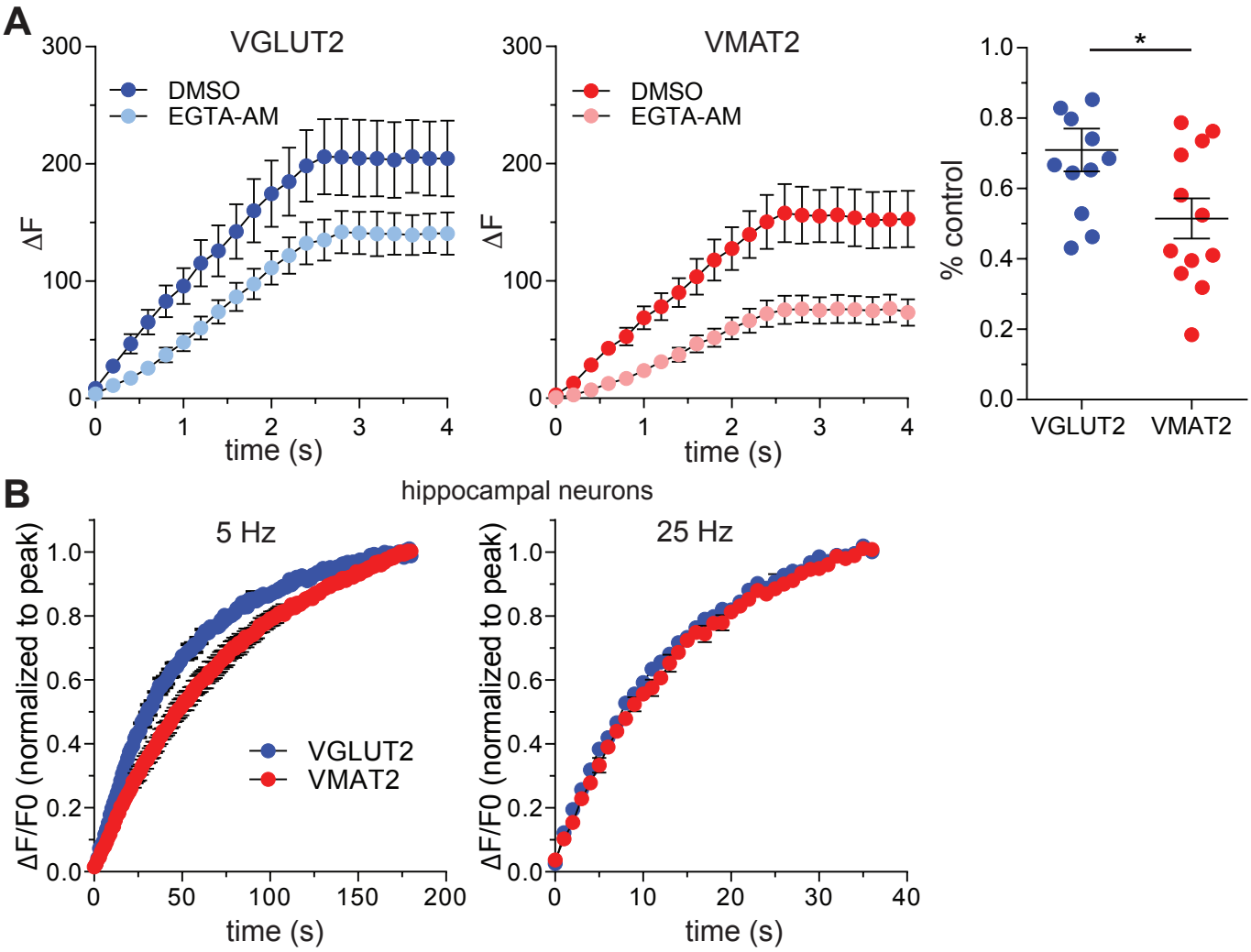
Silm et al., Figure S4

Figure S4. Dual imaging of boutons identified using VGLUT2-pHluorin.

Related to Figure 5.

(A) Hippocampal neurons were transduced with lentiviruses encoding VGLUT2-pHluorin and VMAT2-mOr2 and alkalinization in NH_4Cl used to identify boutons expressing the two fusions. Regions of interest were selected in the GFP channel, and the fluorescence for each protein plotted as a fraction of maximum fluorescence on that coverslip. Each color represents the boutons from a single coverslip. r = 0.555, n = 5797 boutons from 10 coverslips and 3 independent cultures

(B) The cultures were stimulated at 10 Hz for 3 min in bafilomycin, the fluorescence normalized to that in NH_4Cl and regions of interest identified using VGLUT2-pHluorin. The fraction of each reporter responsive to stimulation is plotted for each bouton, and each color represents the boutons from a single coverslip. r = 0.627, n = 3199 boutons from 10 coverslips

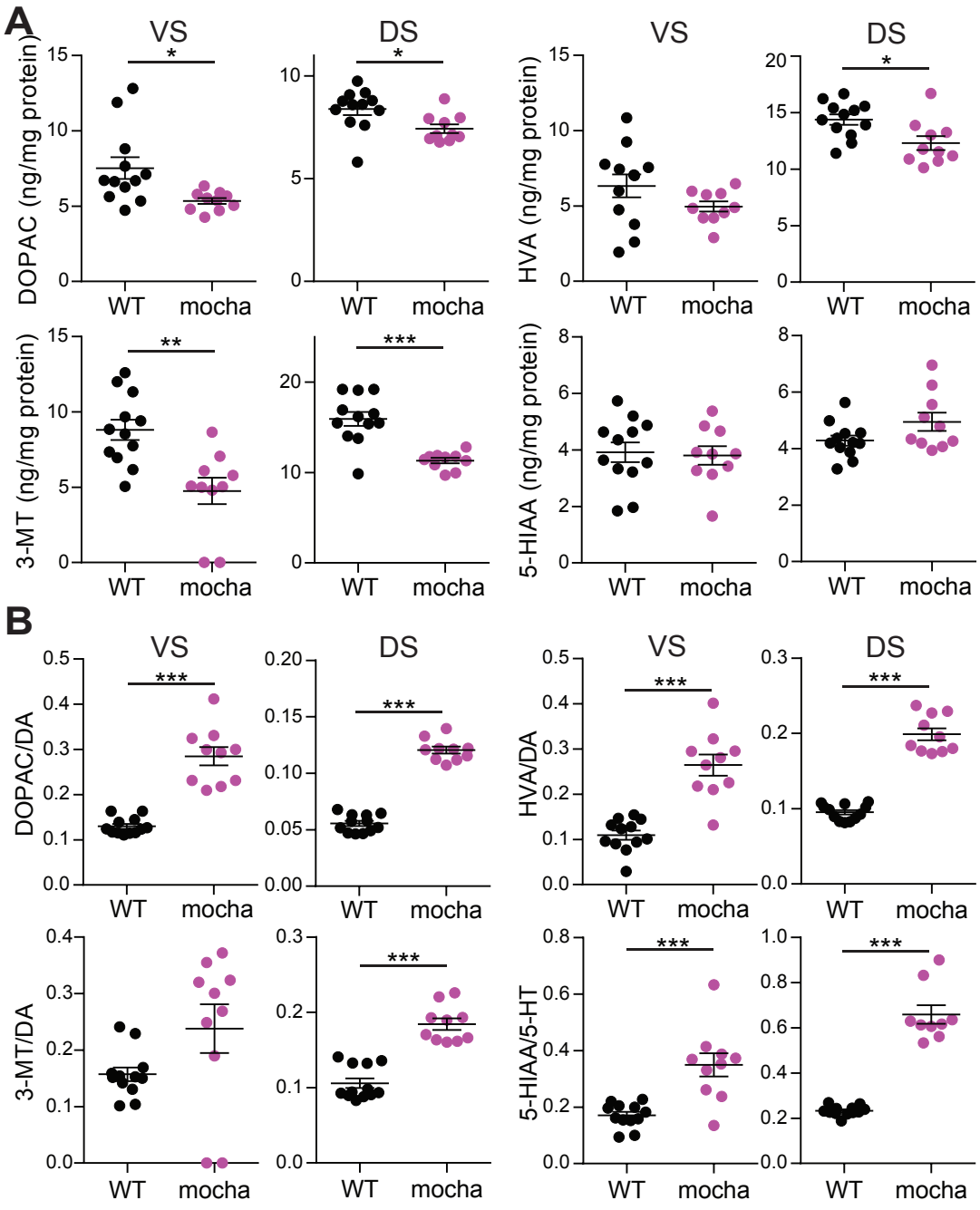


Silm et al., Figure S5

Figure S5. VMAT2 couples more loosely to presynaptic Ca^{++} channels than VGLUT2. Related to Figure 6.

(A) Hippocampal neurons were incubated in EGTA-AM (100 μM) or vehicle and stimulated at 40 Hz for 2.5 s. Left, mean response of VGLUT2-pH in the presence of EGTA-AM (light blue) or vehicle (dark blue) ($p = 0.0545$ by Mann-Whitney test). n = 11 coverslips Middle, mean response of VMAT2-pH in the presence of EGTA-AM (pink) or vehicle (red) (**, $p = 0.0075$ by Mann-Whitney test). n = 12 coverslips Right, EGTA inhibits the response of VMAT2-pH to a greater extent than VGLUT2-pH. The mean fluorescence response for each coverslip in EGTA is expressed relative to the mean control response in vehicle (*, $p = 0.0332$ by Mann-Whitney test). Data indicate mean ± SEM.

(B) The response of VGLUT2-pH is faster than VMAT2-pH in response to 900 AP stimulation at 5 Hz ($p = 0.0430$ by Mann-Whitney test). For 900 AP delivered at 25 Hz, VGLUT2- and VMAT2-pHluorin respond at similar rates ($p = 0.6623$ by Mann-Whitney test). Data indicate mean ± SEM. For 5 Hz stimulation, n = 6 coverslips for VGLUT2-pH and 7 for VMAT2-pHluorin. For 25 Hz stimulation, n = 5 coverslips for VGLUT2-pH and 6 for VMAT2-pH.



Silm et al., Figure S6

Figure S6. Effect of the mocha mutation on metabolites of dopamine and serotonin. Related to Figure 7.

(A) The tissue level of dopamine metabolites DOPAC and 3-MT are reduced in ventral striatum (VS) and dorsal striatum (DS) of *mocha* mice relative to wild type (WT), and HVA is reduced in DS. Loss of AP-3 does not affect the level of serotonin metabolite 5-HIAA. Data indicate mean ± SEM. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ by unpaired t-test

(B) The ratios of DOPAC, HVA and 3-MT to dopamine as well as the ratio of 5-HIAA to serotonin are all increased in both ventral and dorsal striatum of *mocha* mice relative to wild type. Data indicate mean ± SEM. *, $p < 0.05$; ***, $p < 0.001$ by unpaired t-test.

n = 12 for WT and 10 for *mocha*