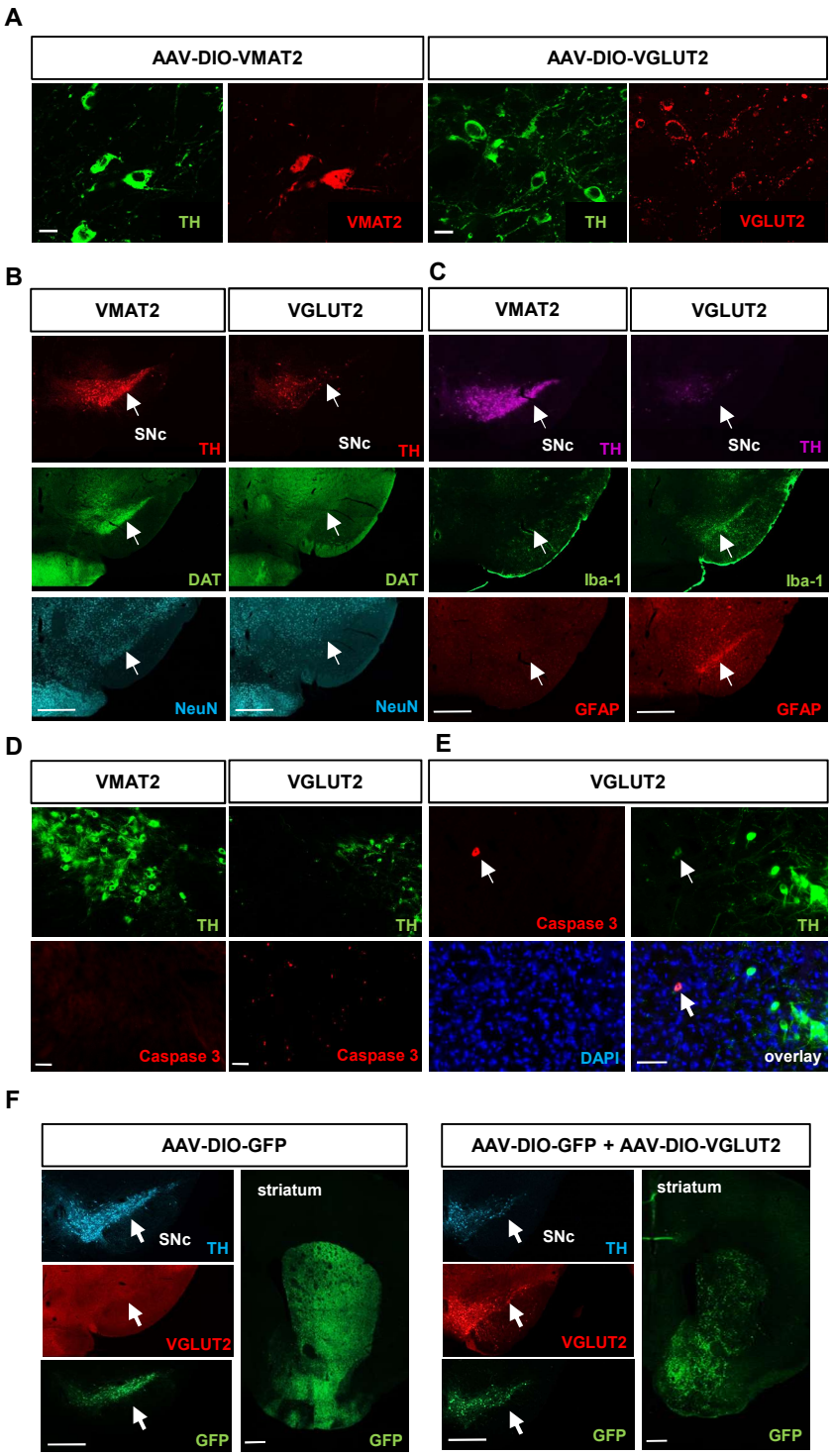
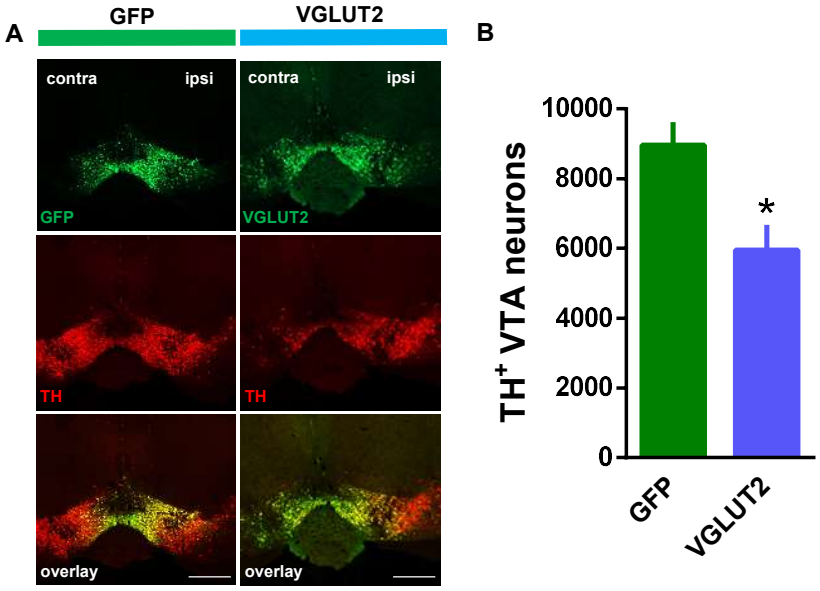



**Supplemental Figure 1**

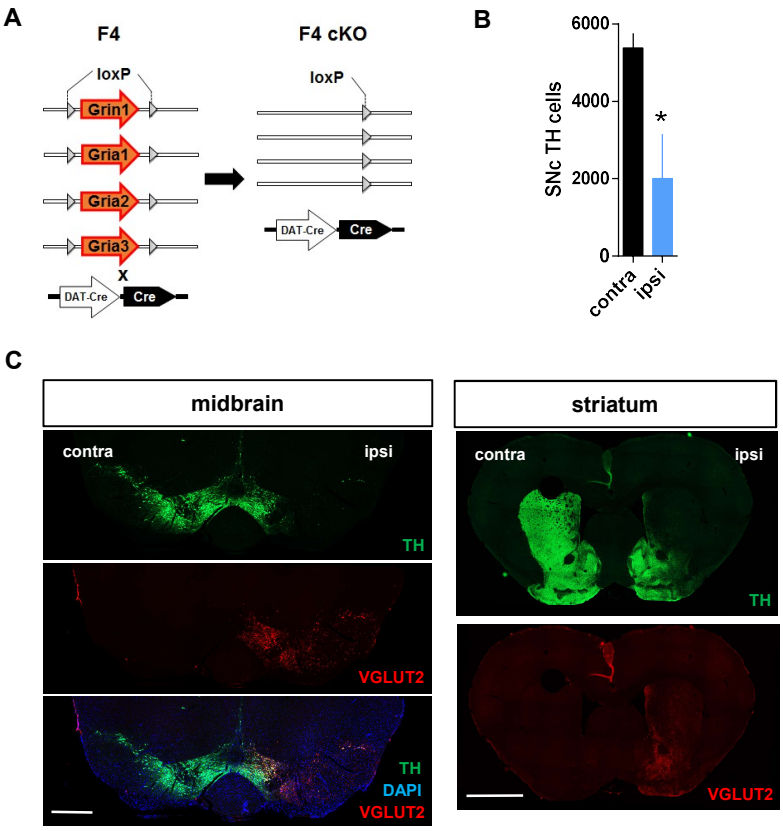
**Supplemental Figure 1: Heterologous expression of VGLUT2 is neurotoxic to SNc dopamine neurons:** (**A**) Images of dopamine neurons after virally induced expression of VMAT2:pHluorin or VGLUT2:HA labeled with antibodies against TH (green) and GFP/pHluorin (red) or HA (red); scale 20 µm. (**B**) Loss of NeuN (blue) and DAT (green) staining in SNc after expression of VGLUT2 (right panel), but not VMAT2 (left panel) correlates with loss of TH (red). (**C**) Upregulation of inflammatory markers GFAP (red) and Iba-1 (green) after VGLUT2 (right panel) but not VMAT2 (right panel) expression in DA neurons; TH (purple). (**D**) Induction of cleaved caspase 3 (red) in VGLUT2 (right panel) but not VMAT2 expressing TH (green) neurons; caspase 3 was detected in midbrains of 6/6 mice after VGLUT2 expression, in 0/3 mice after VMAT2 and 0/3 mice after GFP expression. scale: 50 µm. **(E)** Example image of $TH^+$ (green) cleaved caspase 3 (red) expressing neuron after VGLUT2 expression; scale: 50 µm. **(F)** Co-injection of AAV-DIO-VGLUT2 with AAV-DIO-GFP (right panel) leads to reduced GFP signal in cell bodies and striatal terminals compared to AAV-DIO GFP injection alone (left panel); scales 500 µm.
*Related to Figure 3 and Table 1.*




**Supplemental Figure 2**

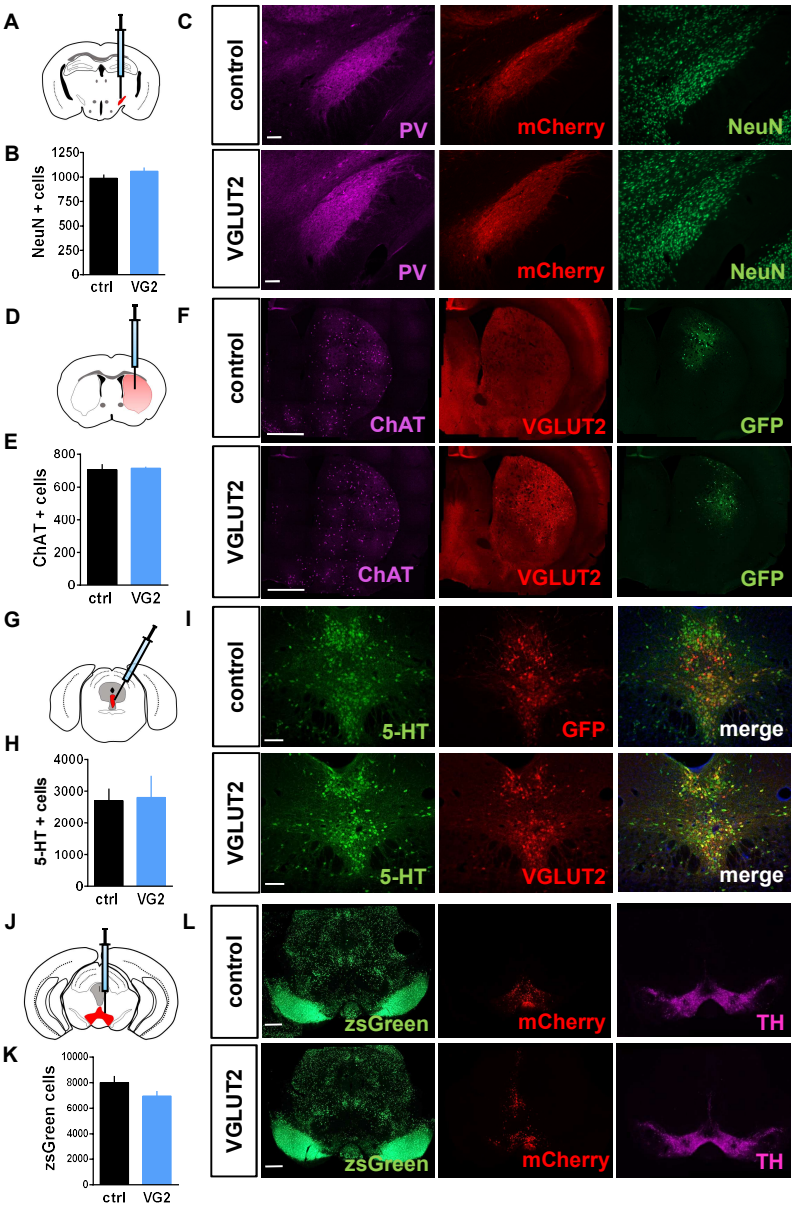
**Supplemental Figure 2: More modest cell loss following heterologous expression of VGLUT2 in adult VTA dopamine neurons:** $DAT^{Cre}$ mice were unilaterally injected with AAV-DIO-VGLUT2 or AAV-DIO-GFP into the VTA and euthanized 21 days after injection. **(A)** Images of coronal sections immunostained against TH (red) and VGLUT2 (green) or GFP (green) show loss of $TH^+$ neurons in VGLUT2- but not GFP-expressing VTA; scale 500 µm. (**B**) TH-positive cell counts in the SNc assessed by unbiased stereology; unpaired t-test, $t=3.1$, $df=4$, $n=3$/group, $^*p<0.05$.

*Related to Figure 3.*




**Supplemental Figure 3**

**Supplemental Figure 3: VGLUT2 driven toxicity does not require NMDA or AMPA glutamate receptors in dopamine neurons:** (**A**) Strategy for the conditional deletion of NMDA- and AMPA-type glutamate receptor subunits in DA neurons. (**B**) Quantification of TH neuron number in the SNc of quadruple cKO mice after injection of AAV-DIO-VGLUT2 compared to the uninjected side. (**C**) Representative sections reveal loss of TH neurons in the SNc (left panel) and striatum (right panel) after injection of AAV-DIO-VGLUT2 into the SNc of quadruple cKO mice. See Table 1 for statistics; *p<0.05.
*Related to Figure 3 and Table 1.*




**Supplemental Figure 4**

**Supplemental Figure 4: Heterologous expression of VGLUT2 in other neuronal populations does not cause their neurodegeneration:** Heterologous expression of VGLUT2 in glutamate neurons of the STN (**A-C**), cholinergic striatal interneurons (**D-F**), DRN 5-HT neurons (**G-I**), or VTA GABA neurons (**J-L**) does not cause their neurodegeneration. (**A**) VGLUT2$^{Cre}$ mice were injected with AAV-DIO-VGLUT2 plus AAV-DIO-mCherry or AAV-DIO-mCherry only (control) into the STN, (**D**) ChAT$^{Cre}$ mice were injected with AAV-DIO-VGLUT2 plus AAV-DIO-GFP or AAV-DIO-GFP only (control) into the dorsal striatum, (**G**) SERT$^{Cre}$ mice were injected with AAV-DIO-VGLUT2 plus AAV-DIO-GFP or AAV-DIO-GFP only (control) into DRN, (**J**) VGAT$^{Cre}$ x Rosa26$^{floxed\text{-}STOP\text{-}zsGreen}$ mice were injected with AAV-DIO-VGLUT2 plus AAV-DIO-mCherry or AAV-DIO-mCherry alone; mice were sacrificed 21 days later for histology and cell counting. (**B)** Quantification of NeuN$^{+}$ cells in the STN of VGLUT2$^{Cre}$ mice, (**E**) ChAT$^{+}$ cells in the dorsal striatum of ChAT$^{Cre}$ mice, (**H**) 5-HT$^{+}$ cells in the DRN of SERT$^{Cre}$ mice, or (**K**) zsGreen$^{+}$ cells in the VTA of VGAT$^{Cre}$ x Rosa26$^{floxed\text{-}STOP\text{-}zsGreen}$ mice. (**C, F, I, L**) Representative images of 30 µm coronal sections through pertinent brain regions were stained with indicated antibodies; scale bar represents 100 µm in **C** and **I**, and 1000 µm in **F** and **L**. See Table 1 for statistics.

*Related to Figure 3 and Table 1.*