

Supplemental Figure Legends

Supplemental Fig. 1 Linearity in the quantification of TH proteins by Western blot.

Serial dilutions of striatal homogenates were prepared from the uninjected side of three mice and subjected to Western blot for TH. The linearity in the relationship between loaded protein level and detected TH signals was evaluated. Black, gray, and open circles represent data sets from individual mice. We employed a range showing linear relationship between actual loaded protein (2.5 to 20 μg , or 0.125 to 1.0 in ratios) and measured TH protein level (0.05 to 1 in ratio). The black line indicates a linear fitting. Dashed line has a slope of one.

Supplemental Fig. 2

A, Summarized quantitative analyses of Western blots for the striatal AADC protein levels. N = 8, 9, 13 and 5 brains for 2, 4, 8 weeks ($Th^{fl/fl}$ mice) and 8 weeks ($Th^{+/+}$ mice) after injection, respectively.

B, The relationship between rotation behavior and dopamine contents. 16 weeks after AAV-Cre injection, the mice were administered with DAT inhibitor, GBR12909 (30 mg/kg, i.p.), and ipsilateral rotation in 60 min was counted. One week later, striatal dopamine contents were measured. The dopamine content and ipsilateral rotation behavior showed significantly negative correlation (Spearman's rank correlation, $p = 0.0350$, $\rho = -0.94$). Gray line shows an exponential curve fitting.

Supplemental Fig.3 The relationship between dopamine and dopamine metabolites.

A, The relationship between HVA and dopamine contents in the $Th^{fl/fl}$ mice 8 weeks after the AAV-Cre injection. The data indicate the ratio of the HVA contents in the AAV-Cre injected side of the striatum normalized to the uninjected side. The open circles indicate individual data. The dotted line has a slope of one, and the solid line indicates a linear fitting. N = 13 mice. Spearman's rank correlation, $p = 0.0008$, $\rho = 0.97$ for HVA vs. dopamine.

B, Summarized ratios of the DOPAC and HVA to dopamine in the $Th^{fl/fl}$ mice 8 weeks after the AAV-Cre injection. (U) and (I) indicate the uninjected and injected sides of the striatum, respectively. Data indicate mean \pm s.e.m..

Supplemental Fig. 4 Western blot analysis of the phospho-TH proteins.

A, pSer40-TH and pSer31-TH levels were examined by Western blot using antibodies against total, pSer40-, and pSer31-TH. Homogenates were prepared 8 weeks after the AAV-Cre injection.

B, C, Summary of scattered plots of pSer40-TH (**B**) and pSer31-TH (**C**) against total TH protein levels. Dotted lines have a slope of one. The protein levels of pSer40-TH and pSer31-TH are correlated to the total TH protein level in the $Th^{fl/fl}$ mice (Spearman's rank correlation, $p = 0.0054$, $\rho = 0.98$ for pSer40-TH vs. total TH; $p = 0.0063$, $\rho = 0.97$ for pSer31-TH vs. total TH). N = 6, 3 for $Th^{fl/fl}$ and $Th^{+/+}$, respectively. Note that there was no trend toward an increase in the ratio of phospho-TH to total TH protein level when total TH protein level was decreased.

Supplemental Fig. 5 Lack of changes in the striatal vMAT2 and DAT protein levels.

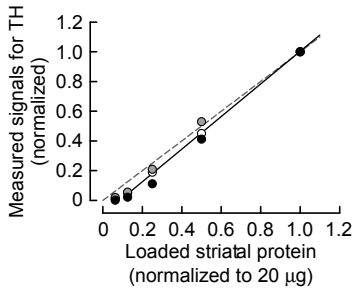
A, Representative Western blot for vMAT2 and DAT. Western blot was performed using crude synaptosomal fractions from the striatum of $Th^{fl/fl}$ mice prepared 8 weeks after AAV-Cre injection. The TH and β -actin protein levels were also examined. (U) and (I) indicate uninjected and injected side of the striatum, respectively. Note that while the TH protein level was reduced in the injected side, vMAT2 and DAT proteins did not show a reduction.

B, Summary of the quantification of Western blot data for vMAT2, DAT, and TH in the $Th^{fl/fl}$ and $Th^{+/+}$ mice. The ratios of protein levels in the injected side to the uninjected side are shown. The open circles indicate the values from individual animals, and the bars indicate the means. n

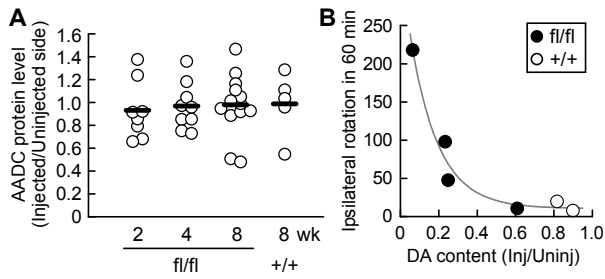
= 7, 3 for the $Th^{fl/fl}$ and $Th^{+/+}$, respectively. $p = 0.31, 0.91,$ and 0.0167 for vMAT2, DAT, and TH, respectively, Mann-Whitney U test.

C, D, Relationship of protein levels between vMAT2 and TH proteins (**C**) or DAT and TH protein (**D**) were examined by scattered plots. The lines indicate linear fitting. There was no significant correlation ($p = 0.42, 0.60$ for vMAT2 vs. TH and DAT vs. TH, respectively, Spearman's rank correlation test).

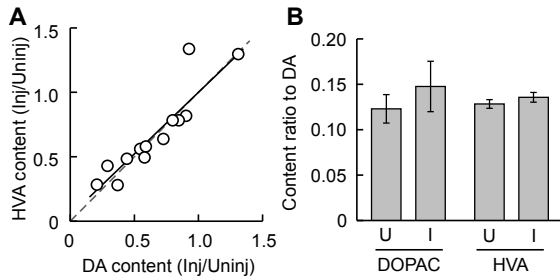
Supplemental Fig. 1



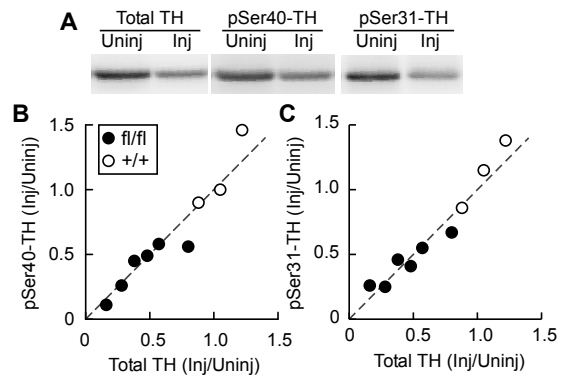
Supplemental Fig. 2



Supplemental Fig. 3



Supplemental Fig. 4



Supplemental Fig. 5

