Online supplemental material

Supplementary Figure 1. qPCR analyses comparing gene expression levels of critical molecular determinants of DA midbrain regionalization, synthesis and release in mouse embryonic fibroblasts (MEF), reprogrammed neurons (iDAN) and FACS-sorted GFP-TH+ E13.5 mouse midbrain DA neurons (DAN). **Data are representative of three independent experiments performed on n = 3 samples per group.**

Supplementary Figure 2. Quantification of tdTomato and GFP expressing iDA neurons transplanted in immunodeficient Rag2/IL2Rgc null mice. Animals received dox respectively for 0, 6, or 12 days or 5 weeks, at which point all animals were sacrificed for analysis. **Data are representative of five independent experiments performed on n = 3 mice.**

Supplementary Figure 3. Quantification of TH-, VMAT2-, AADC- and DAT positive cells among the TH-GFP+ iDA neurons transplanted in 6-OHDA lesioned rats. Data are representative of three independent experiments performed on n = 3 mice.

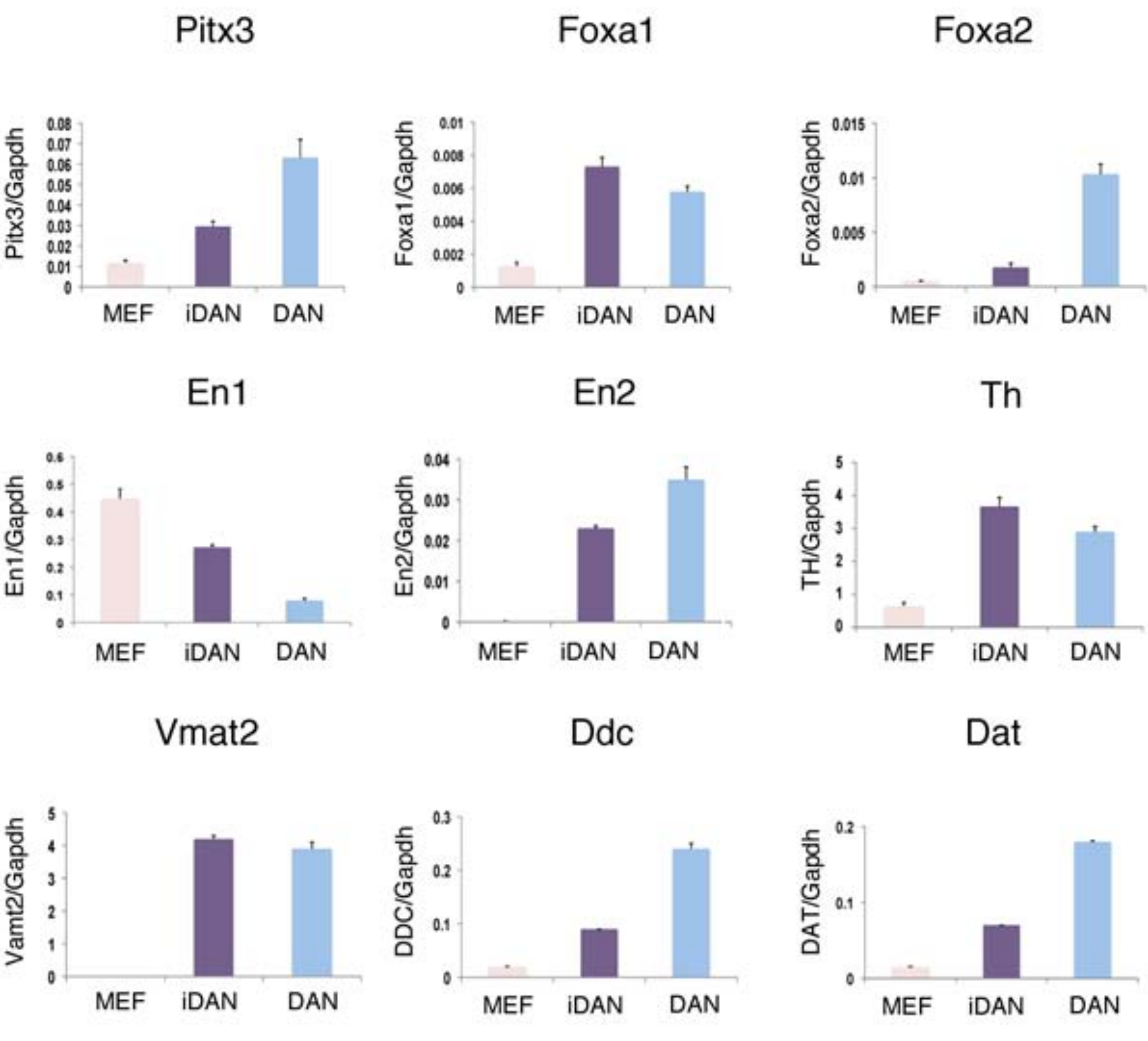
Supplementary Figure 4. Tumorigenicity assays with grafted iDA neurons. (A) Immunohistochemistry of iDA neurons transplanted in Rag2/IL2 Rgc null mice showing Ki67 and GFP staining. (B-D-E) High magnification of grafted cells indicates the absence of Ki67 positive staining. (C) High magnification of the subventricular zone shows endogenous proliferative cells within the same tissue. Scale bars: (A) 600 μm, (B-C) 80 μm, (D-E) 40 μm. **Images are representative of two independent experiments performed on n = 10 mice.**

Supplementary Figure 5. Functional assessment of DREADDs in primary dopaminergic (DA) neurons. (A) Schematic representation of the experimental protocol. The ventral mesencephalon (VM) from TH-GFP mice was dissected and reduced to single cell suspension. Four days after cells were infected with M3Dq and M4Di lentiviral vectors and left in culture for additional 10 days before electrophysiological recordings. Scale bars: (A) 20 μm. (B) Representative traces (upper panels) and quantifications (lower panels) of whole-cell patch clamp recordings in M3Dq-DA neurons showing an increase in action potential firing following CNO (10 μM) administration. (C) Representative traces (upper panels) and quantifications (lower panels) of whole-cell patch clamp recordings in M4Di-DA neurons. CNO administration sustained an evident silencing of action potentials pattern. Data represent means ± SEM; * $p < 0.05$, Two-way ANOVA followed by

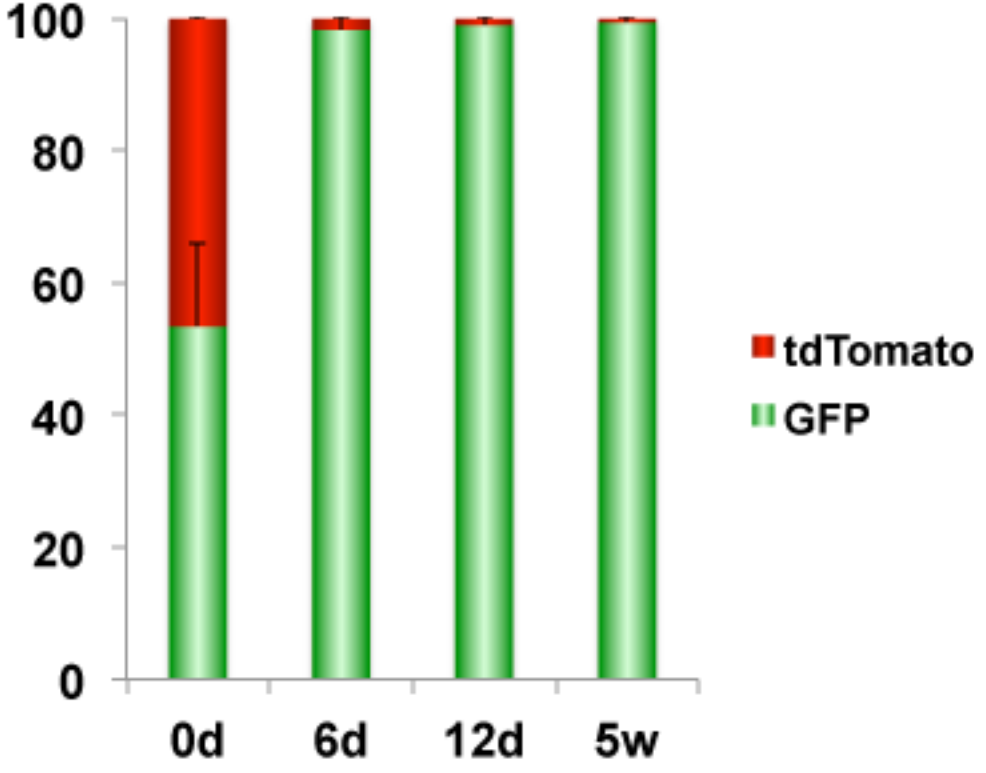
Bonferroni-Dunn post-hoc test. **Data are representative of three independent experiments performed on at least n = 5 cells per group.**

Supplementary table 1. List of primers used for Real Time RT-PCR amplification

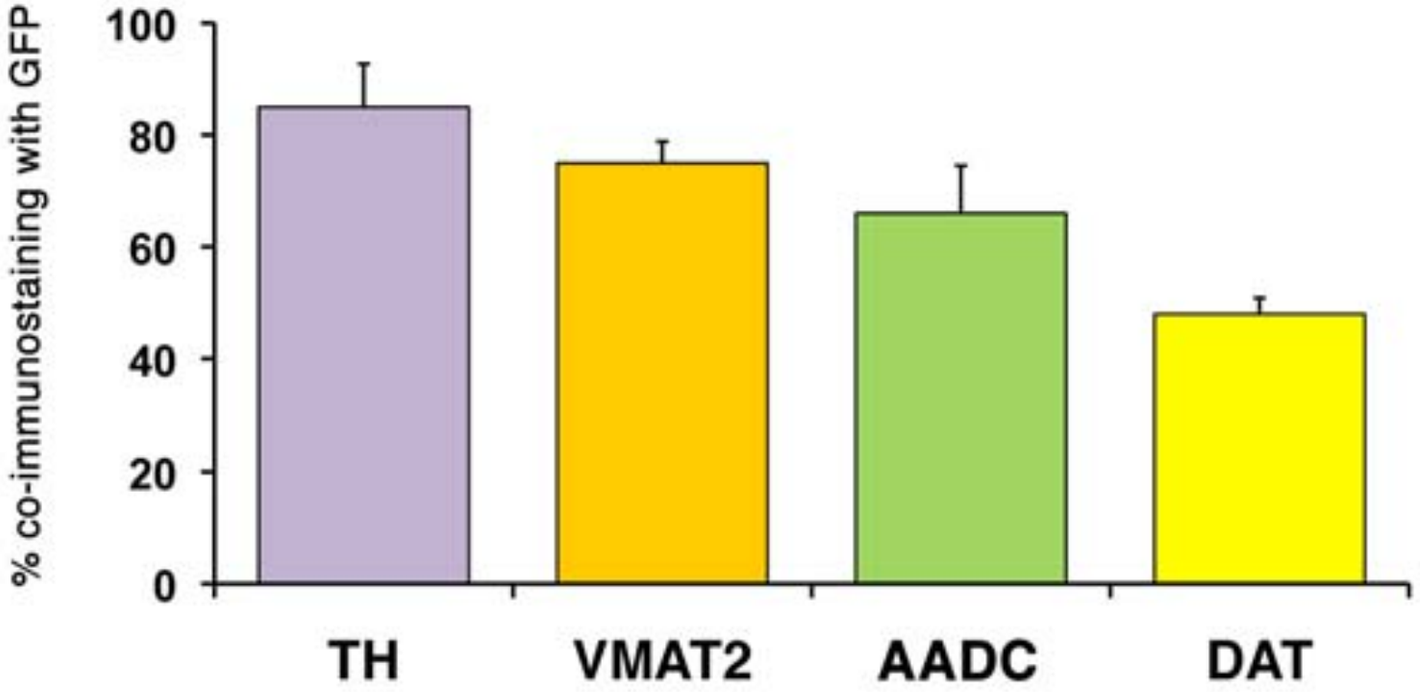
Dell'Anno et al., Figure S1

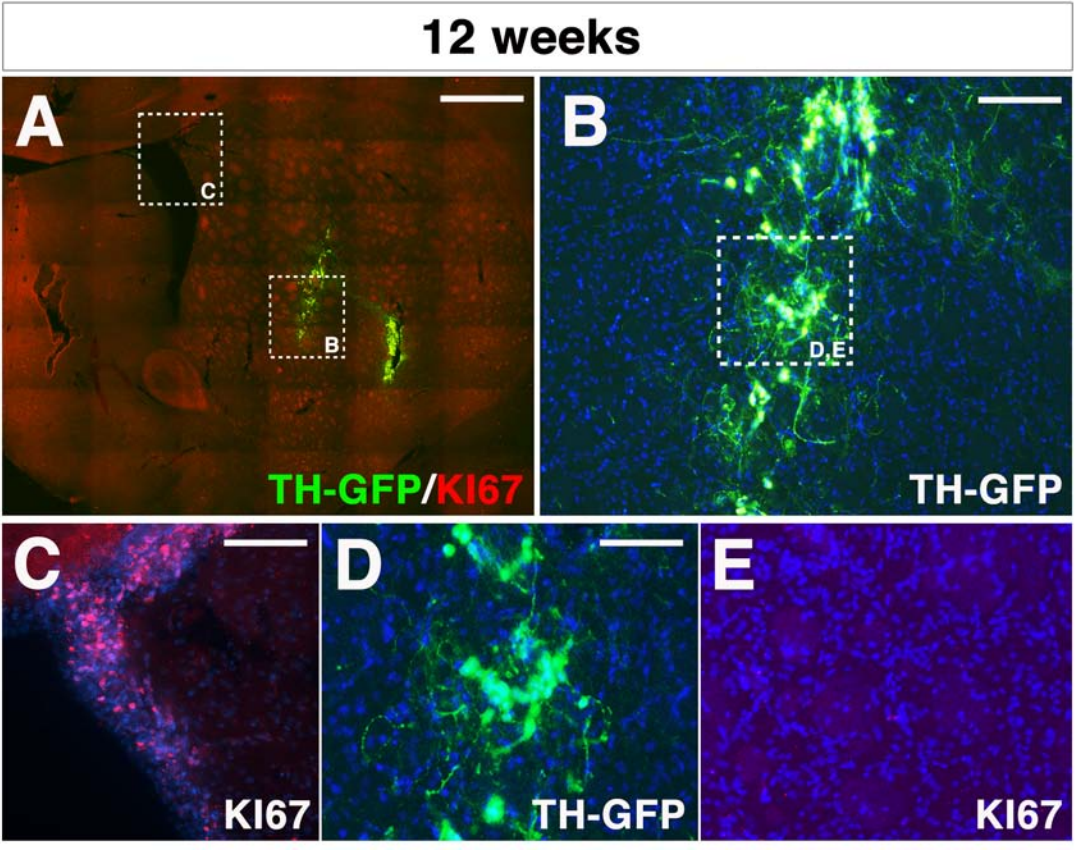


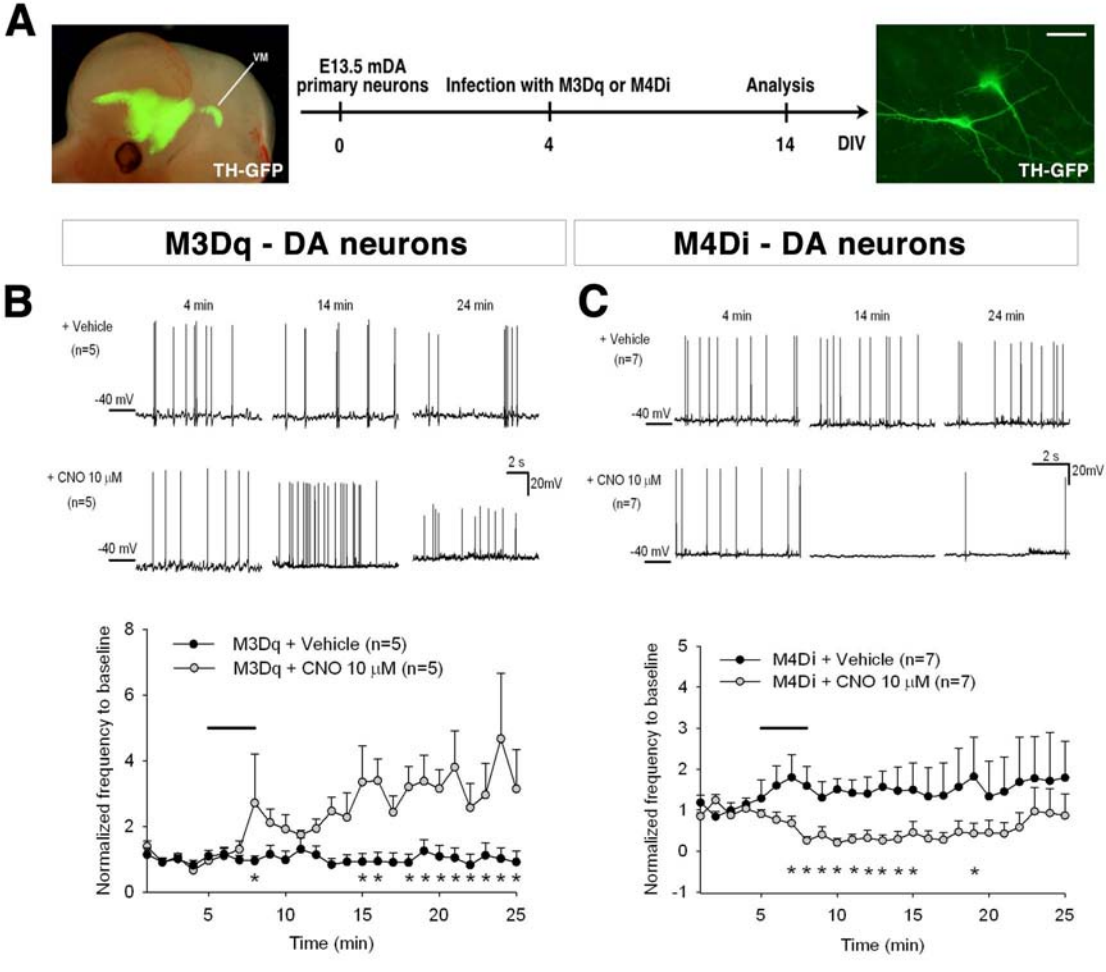
Dell'Anno et al., Figure S2



Dell'Anno et al., Figure S3




12 weeks
A
C
B
TH-GFP/KI67
B
D,E
TH-GFP
C
KI67
D
TH-GFP
E
KI67


A
VM
TH-GFP
E13.5 mDA primary neurons
Infection with M3Dq or M4Di
Analysis
0
4
14
DIV
TH-GFP
M3Dq - DA neurons
M4Di - DA neurons
B
C
4 min
14 min
24 min
+ Vehicle (n=5)
-40 mV
+ CNO 10 μM (n=5)
2 s
20mV
+ Vehicle (n=7)
+ CNO 10 μM (n=7)
M3Dq + Vehicle (n=5)
M3Dq + CNO 10 μM (n=5)
M4Di + Vehicle (n=7)
M4Di + CNO 10 μM (n=7)
Normalized frequency to baseline
Time (min)

Marker	**Forward sequence 5'-3'**	**Reverse sequence 5'-3'**
Dat	TCTGGGTATCGACAGTGCCA	GCAGCTGGAACTCATCGACAA
Ddc	GGGACCACATCCTGCTGTTC	CACACACCCTCCTGGTTGC
En1	CGCCTGGGTCTACTGCACA	TCTTCTTTAGCTTCCTGGTGCG
En2	GACCGGCCTTCTTCAGGTC	GGCCGCTTGTCCTCTTTGT
Foxa1	GAAGGGCATGAGAGCAACGA	ACAGGGACAGAGGAGTAGGCC
Foxa2	ACGAGCCATCCGACTGGAG	GGCGTTCATGTTGCTCACG
Gapdh	CAACTCCCACTCTTCCACCT	CCCTGTTGCTGTAGCCGTAT
Pitx3	GACGCAGGCACTCCACACC	TTCTCCGAGTCACTGTGCTC
Th	CTCACCTATGCACTCACCCGA	GGTCAGCCAACATGGGTACG
Vmat2	TTGCTCATCTGTGGCTGGG	TGGCGTTACCCCTCTCTTCAT