Supporting Information

Garriga-Canut et al. 10.1073/pnas.1206506109

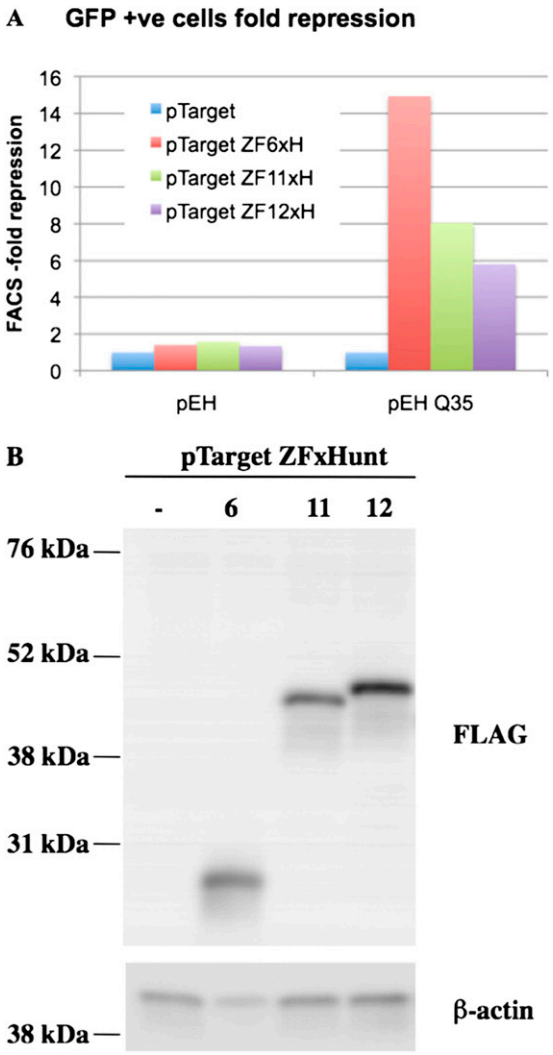

Fig. S1. Comparison of ZF11xHunt and ZF12xHunt in episomal reporter repression. Cells were cotransfected with reporter and zinc finger plasmids. The plasmid pEGFP-HcRed (pEH) reporter plasmid contains EGFP, fused to Q35, under an SV40 promoter. pCMV-ZFxHunt (CMV promoter) expression constructs contain "naked" chains of ZFxHunt (6, 11, or 12 fingers, as indicated). The pTarget vector does not contain zinc finger protein (ZFP) and is used as a control. (*A*) FACS assay measures the fold reduction in EGFP cells in response to different naked zinc fingers. A fivefold repression is equivalent to an 80% reduction. (*B*) FLAG Western blot for ZFP expression shows similar stability and expression levels for all constructs. β-actin staining is used as a loading control.

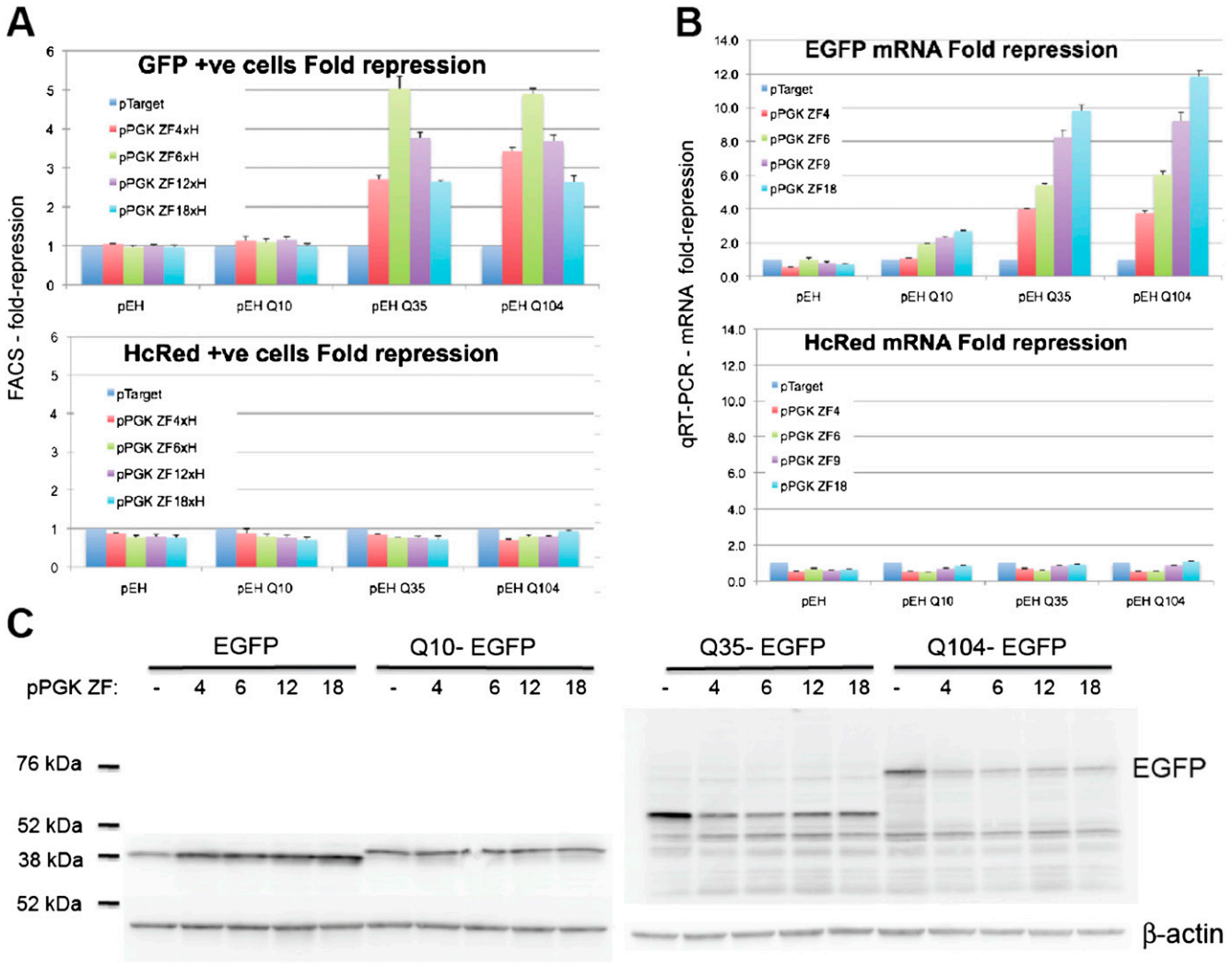

Fig. S2. Episomal reporter repression by phosphoglycerate kinase promoter plasmid (pPGK)-ZFxHunt. Cells were cotransfected with reporter and zinc finger plasmids. The plasmid pEGFP-HcRed (pEH) reporter vector contains EGFP, fused to different-length polyglutamine (polyQ) coding sequences, under an SV40 promoter. A control HcRed gene, under a CMV promoter, measures off-target repression. pPGK-ZF (PGK promoter) expression constructs contain chains of ZFxHunt (0–18 fingers, as indicated). Zinc finger protein (ZFP) is not fused to any effector domain. The pTarget vector does not contain ZFP and is used as a control. (*A*) FACS assay measuring the fold reduction in EGFP or HcRed fluorescent cells in response to different zinc fingers. A fivefold repression is equivalent to an 80% reduction. (*B*) Quantitative RT-PCR (qRT-PCR) assay measures fold repression of EGFP or HcRed mRNA by ZFP. (*C*) EGFP Western blot for ZFP repression of pEH-Qx targets. β-actin staining is used as a loading control.

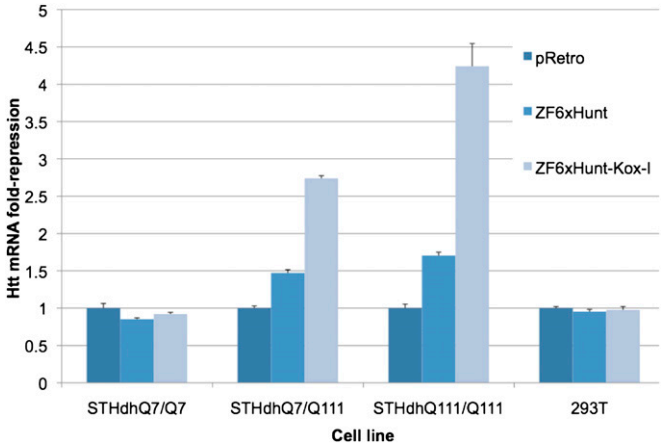

Fig. S3. Repression of chromosomal CAG-repeat genes, 20 d after retroviral zinc finger protein (ZFP) delivery, in homozygous and heterozygous cells. Assays were carried out in wild-type mouse ST*Hdh* cells (Q7/Q7), in homozygous polyQ ST*Hdh* mutants (Q111/Q111), in heterozygous polyglutamine ST*Hdh* mutants (Q7/Q111), and in human HEK293T cells, as indicated. Quantitative RT-PCR was used to compare huntingtin (*HTT*) gene mRNA levels (mRNA fold repression). Mouse primers that do not discriminate between wild-type or mutant *HTT* were used; consequently, repression of *HTT* in heterozygous STHdh cells was approximately half of that was seen in homozygous mutant STHdh cells, suggesting that the levels of repression seen correspond to repression of mutant *HTT*.

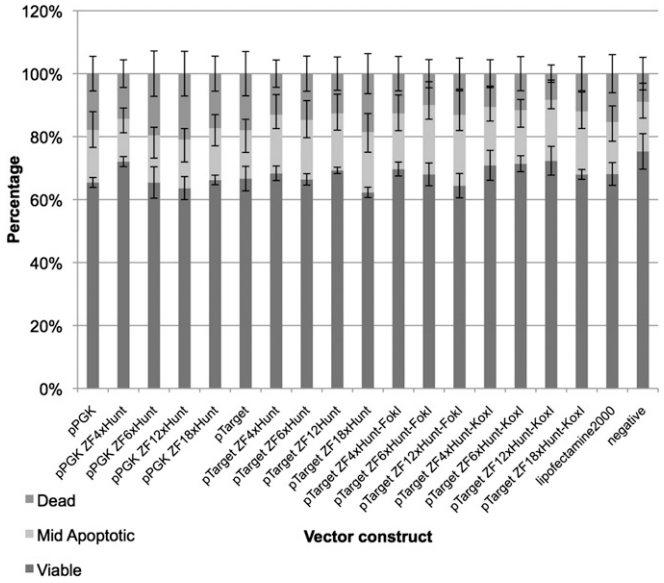

Fig. S4. ZFxHunt toxicity assay. HEK293T cells were transfected with 400 ng of the indicated vector constructs using Lipofectamine 2000 and harvested 48 h after transfection. As a control, Lipofectamine 2000 only or untransfected cells (negative) were used. Cytotoxicity was analyzed using guava cell toxicity [personal cell analysis (PCA)] assay (Millipore Corporation) according to the manufacturer's instructions. Results show the percentage of dead midapoptotic and viable cells. Bars express results of at least three independent experiments. In conclusion, ZFxHunt proteins do not appear to be toxic.

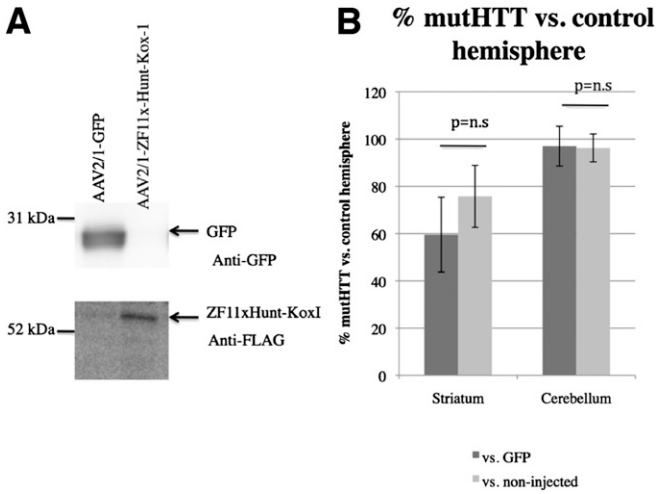

Fig. S5. (*A*) Western blot of dissected striatum shows expression of GFP in one hemisphere and ZF11xHunt–Kox-1 (with FLAG tag) in the other hemisphere, corresponding to injections. (*B*) Quantitative RT-PCR data quantify % of mutant huntingtin (mut*HTT*) gene mRNA vs. control hemisphere in mouse striatal samples injected with ZF11xHunt–Kox-1 in one hemisphere. The other hemisphere was either injected with GFP (n = 3, dark gray) or noninjected (n = 3, light gray). The cerebellum was not injected. There are no statistically significant differences, indicating that the effect observed is due to repression by zinc finger, and not to a toxic effect by GFP [P = 0.18 (striatum) and P = 0.48 (cerebellum), Student's t test]. AAV2/1, adenoassociated virus 2/1; n.s., not significant.

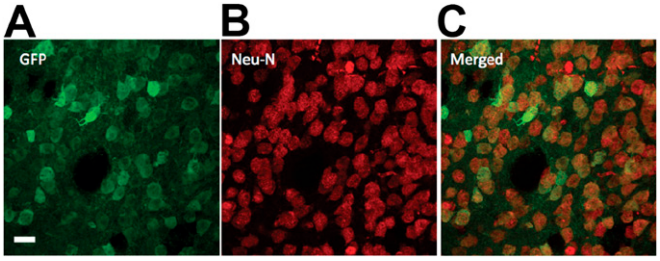

Fig. S6. Assay of the cellular targeting of adenoassociated virus (AAV) 2/1 by immunohistochemistry. (*A*) GFP (green) reveals AAV2/1 transduction in a striatal section. (Scale bar: 10 μm.) (*B*) Anti-NeuN immunofluorescence (red) marks neuronal cell bodies, as previously shown by Burger et al. (1). (*C*) Merged image reveals AAV2/1 transduction of neurons, together with NeuN, showing that transgene expression coincides with a neuronal cell marker. It should be noted that although the AAV1 capsid protein does have neuronal tropism, it efficiently transduces most glial and some ependymal cells.

1. Burger C, et al. (2004) Recombinant AAV viral vectors pseudotyped with viral capsids from serotypes 1, 2, and 5 display differential efficiency and cell tropism after delivery to different regions of the central nervous system. *Mol Ther* 10(2):302–317.

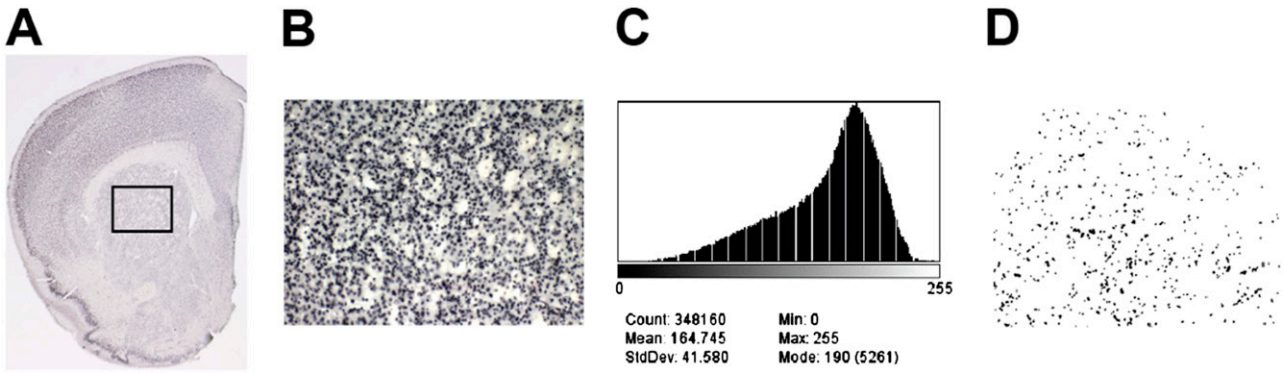

Fig. S7. Quantification of Huntingtin (HTT)-positive aggregates by automatic counting of mutant HTT-positive particles with ImageJ software (National Institutes of Health), using the method of Moncho-Bogani et al. (1). (*A*) Using mutant HTT-immunostained coronal slices, a region of interest sized 650 × 865 μm^2 was selected in the middle of the dorsal striatum (10× objective). (*B*) Background was subtracted, and the image was converted to eight-bit. (*C*) Gray-level histogram was calculated, and a threshold of 70% of the mode was used for binarization (i.e., leaving particles showing a gray level >30% of the mode). (*D*) Image was filtered with an erosion-dilation filter to eliminate noise, and the number of remaining particles was automatically counted. Counts were made in four serial slices, separated by 240 μm, and averaged, providing a single density measure per hemisphere. Counts were then calculated per 0.1 mm^2. Max, maximum; Min, minimum; StdDev, standard deviation.

1. Moncho-Bogani J, Martinez-Garcia F, Novejarque A, Lanuza E (2005) Attraction to sexual pheromones and associated odorants in female mice involves activation of the reward system and basolateral amygdala. *Eur J Neurosci* 21(8):2186–2198.

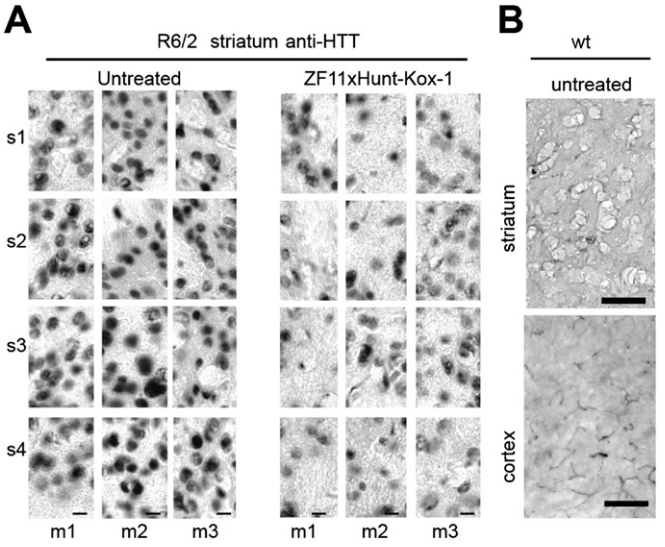

Fig. S8. Anti-Huntingtin (HTT) protein immunostaining of the striatum and cortex of R6/2 and wild type mice. (*A*) Staining R6/2 striata reveals a reduction of the intensity of mutant aggregates with ZF11xHunt–Kox-1 treatment. Data are from three mice (m1–m3) and from four brain sections per mouse (s1–s4). The treated and untreated hemispheres are stained simultaneously within each section. Close-ups are shown to aid visualization (wide-field views are provided in Fig. 6*C*). The automated HTT counting method used wide-field views (Fig. S7). (*B*) Sections from wild-type (wt) mice are shown for comparison and contain no mutant HTT aggregates. (Scale bars: 10 μm for R6/2, 100 μm for wt.)

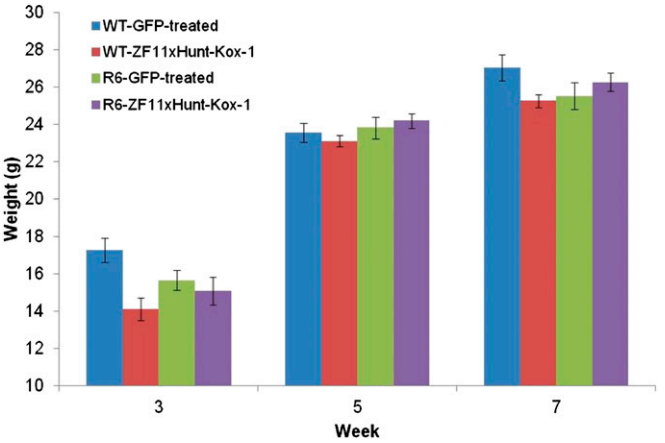

Fig. S9. Weight of R6/2 mice and their wild type (WT) littermate groups across 3 wk during which the rotarod assay was carried out. An ANOVA of repeated measures with group as the between-subject factor and week as the within-subject factor revealed a significant main effect of week ($F_{2,47} = 435$, $P < 0.001$) but not of group ($F_{3,47} = 2.41$, $P = 0.079$). All measures are mean ± SEM. WT-GFP–treated ($n = 14$), WT–ZF11xHunt–Kox-1–treated ($n = 14$); R6/2-GFP–treated ($n = 12$); R6/2–ZF11xHunt–Kox-1–treated ($n = 12$).

Table S1. Student's *t* test analysis of the fold repression data against an expected value of 1

Zinc finger	No. of repeats	Reporter	*P* value
ZF4xHunt	Q0	EGFP	0.894
	Q10		0.363
	Q35		<0.001
	Q104		<0.001
	Q0	HcRed	0.325
	Q10		0.706
	Q35		0.128
	Q104		0.004
ZF6xHunt	Q0	EGFP	0.010
	Q10		0.020
	Q35		<0.001
	Q104		<0.001
	Q0	HcRed	0.158
	Q10		0.468
	Q35		0.540
	Q104		<0.001
ZF11xHunt	Q0	EGFP	0.157
	Q10		0.046
	Q35		<0.001
	Q104		<0.001
	Q0	HcRed	0.221
	Q10		0.990
	Q35		0.848
	Q104		0.109
ZF18xHunt	Q0	EGFP	0.281
	Q10		0.280
	Q35		<0.001
	Q104		<0.001
	Q0	HcRed	0.090
	Q10		0.437
	Q35		0.077
	Q104		0.023

Data for this analysis are taken from the FACS experiment in Fig. 2*B*.

Table S2. Student's *t* test analysis of the fold repression data against an expected value of 1

Zinc finger	No. of repeats	Reporter	*P* value
ZF4xHunt	Q0	EGFP	0.005
	Q10		0.915
	Q35		0.004
	Q104		0.009
	Q0	HcRed	0.282
	Q10		0.207
	Q35		0.444
	Q104		0.644
ZF6xHunt	Q0	EGFP	0.735
	Q10		0.417
	Q35		0.059
	Q104		0.022
	Q0	HcRed	0.575
	Q10		0.038
	Q35		0.386
	Q104		0.994
ZF11xHunt	Q0	EGFP	0.582
	Q10		0.372
	Q35		0.008
	Q104		0.010
	Q0	HcRed	0.046
	Q10		0.318
	Q35		0.282
	Q104		0.495
ZF18xHunt	Q0	EGFP	0.145
	Q10		0.340
	Q35		0.004
	Q104		0.053
	Q0	HcRed	<0.001
	Q10		0.324
	Q35		0.349
	Q104		0.356

Data for this analysis are taken from the quantitative RT-PCR experiment in Fig. 2*D*.

Table S3. CAG-repeat number per gene and corresponding primer sets for quantitative RT-PCR

Gene	CAG repeat length	Forward primer	Reverse primer
EGFP	0–104	CCTGAAGTTCATCTGCACCA	AAGTCGTGCTGCTTCATGTG
HcRed	0	AGATGCTGCGGAAGAAGAAG	GGTACCGTCGACTGCAGAA
hHPRT	N/A	CTTTGCTTTCCTTGGTCAGG	TATCCAACACTTCGTGGGGT
hATN1	16, 22	GTCTCCCTCCGATCTGGATA	CACACTTCCAGGGCTGTAGA
hATXN1	12, 29	CCAGCACCGTAGAGAGGATT	AGCCCTGTCCAAACACAAA
hATXN2	13, 23	GACGCAGCTGAGCAAGTTAG	GAAGGAACGTGGGTTGAACT
hATXN3	7, 14	AGAGCTTCGGAAGAGACGAG	ACTCCCAAGTGCTCCTGAAC
hATXN7	10	AACTGTGTGGCTCACTCTGG	TGGGAAGATGTTACCGTTGA
hCACNA1A	13	GGGAACTACACCCTCCTGAA	CGCTGCTTCTTCTTCCTCTT
hTBP	18, 38	ACGCCGAATATAATCCCAAG	CTTCACTCTTGGCTCCTGTG
hHtt	21, 23	CAGATGTCAGAATGGTGGCT	GCCTTGGAAGATTAGAATCCA
mATN1	3, 10	CACCTGCCTCCACCTCATGGC	ATGCTCCTTGGGGGCCCTGG
mATXN1	2	TGTGGAGAGAATCGAGGAGA	CAGCCCTGTCCAAATACAAA
mATXN2	6, 10	ATCCCAATGCAAAGGAGTTC	CTGCTGATGACCCACCATAG
mATXN3	5, 6	ACCTCGCACTATTCTTGGCT	TGCATCTGTTGGACCTTGAT
mATXN7	5, 7	TGCCCGTGTTCCTCACCGGA	GCGCGGAGACAGTGGTTGCT
mCACNA1A	2, 3	CACTGGCAATAGCAAAGGAA	TTCTTGAGCGAGTTCACCAC
mTBP	3, 13	ACTTCGTGCAAGAAATGCTG	GCTCATAGCTCTTGGCTCCT
mGRK4	N/A	TCCTGGCTTTGAGGAGCCGA	CCACAGCACAGCTCTGCAGCAT
mRgs12	N/A	GGGGGCTCAAGCAGGCATGG	GGGAGCCAGCCTCCGAGTCA
mHtt	4, 7	CAGATGTCAGAATGGTGGCT	GCCTTGGAAGATTAGAATCCA
mHPRT	N/A	GGTTAAGCAGTACAGCCCCA	AGAGGTCCTTTTCACCAGCA
mActb	N/A	GCTTCTTTGCAGCTCCTTCGT	CCAGCGCAGCGATATCG
mAtp5b	N/A	CCACCGACATGGGCACAATGCA	ATGGGCAAAGGTGGTTGCAGGG

Approximate CAG-repeat number for wild-type genes was obtained from GenBank mRNA data. CAG-repeat length: the first number corresponds to pure CAG repeats, and the second number corresponds to broken CAG repeats (containing CAA or CAT). h, human; m, mouse; N/A, not applicable.

Table S4. Striatal volume and cell density are not affected by the genotype of mice or the treatment applied to the hemisphere (repeated-measures ANOVA: genotype, $P > 0.1$; treatment, $P > 0.1$ for both measures)

	Striatal volume, mm^3		Cell density in the striatum, no. of neuronal nuclei/mm^3	
Genotype	Control	ZF11xHunt–Kox-1–treated	Control	ZF11xHunt–Kox-1–treated
Wild type	4.75 ± 0.21	5.08 ± 0.14	1116.23 ± 90.14	991.92 ± 110.71
R6/2	4.75 ± 0.33	5.25 ± 0.39	934.92 ± 197.34	985.47 ± 53.26

Mean ± SEM.

Dataset S1. DNA and protein sequences of zinc finger protein constructs

Dataset S1