

Neuropeptide Y deficiency induces anxiety-like behaviours in zebrafish (*Danio rerio*)

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Supplemental Table 1 Primers used in the study.

Supplemental Fig. 1. Nucleotide and deduced amino acid sequence of the zebrafish NPY.

Nucleotide sequence of zebrafish *npv* was obtained from Genbank (accession No: NM131074).

Exon/intron boundary is represented by vertical lines. Red coloured and underlined characters indicate mature NPY amino acid sequence and gRNA-targeted region, respectively.

Supplemental Fig. 2. Full length gels of electrophoresis for the detection of NPY mutant in

zebrafish. (A-C) Full length gels in HMA analysis of F0 shown in Fig. 1B (A), F1 in Fig. 1D

(B) and F2 in Fig. 1F (C). (D) Full length of gel to detect expression of intact *npv* mRNA in

wild-type fish and mutants shown in Fig. 1G (F2). cDNAs from wild-type fish and mutants

were used for PCR as a template, using primers specific to intact *npv*. *actb* was used as an

internal control.

Supplemental Fig. 3. Sequencing of *npv* mRNA in wild and NPY-KO. The nucleotide

sequence of the gRNA-target region in *npv* was sequenced in wild (upper panel), NPY-KO7

(middle panel) and NPY-KO11 (lower panel).

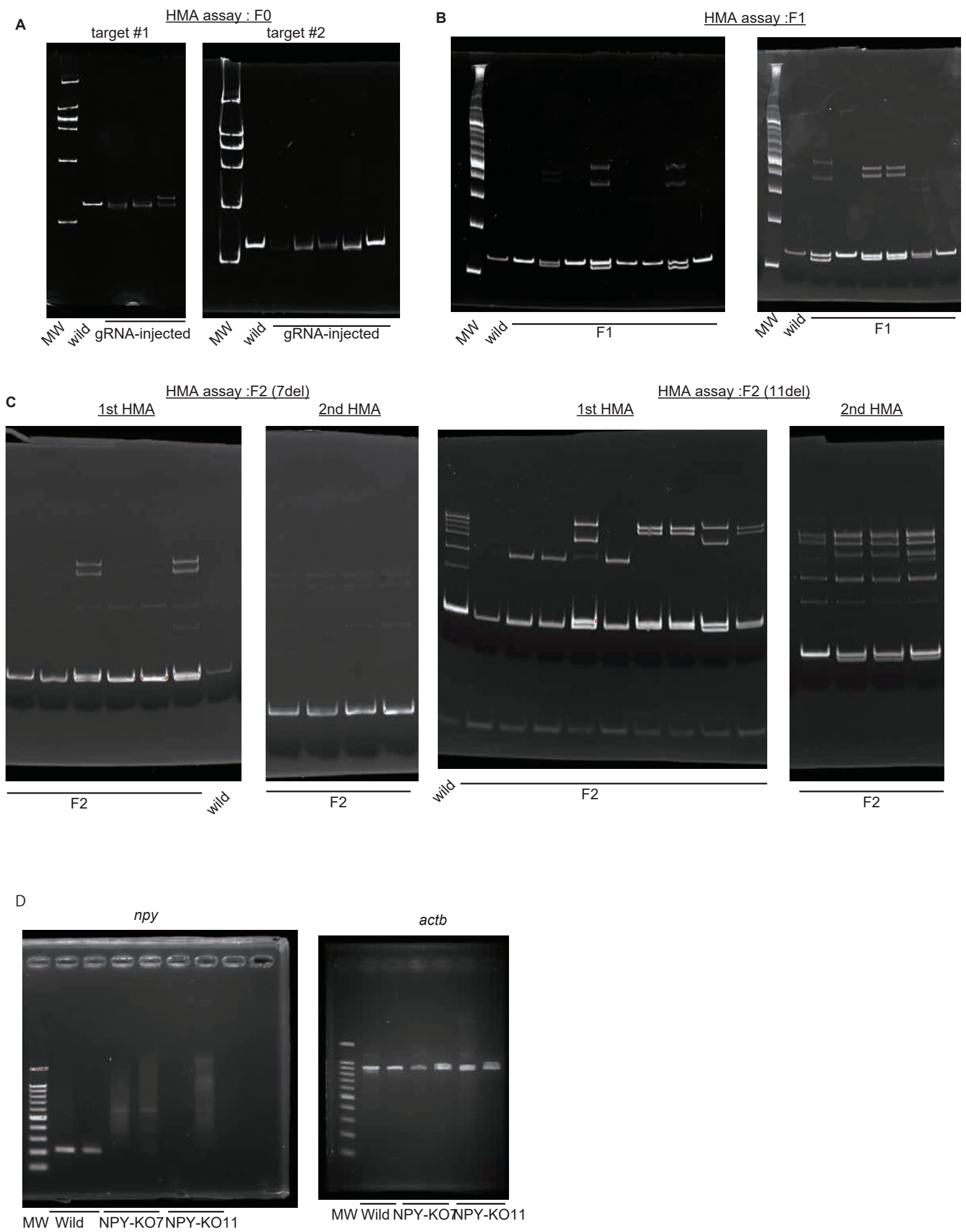
Supplemental Fig. 4. Distribution of NPY-neurons in wild and NPY-KO zebrafish brain.

NPY signals were immuno-stained by NPY-specific antibody and DAB staining. Neuronal cells were detected by Nissl staining. The area around the ventral zone of the periventricular hypothalamus (HV), anterior tuberal nucleus (ATN) and lateral hypothalamic areas (LH) were highlighted with red circle. Black bar indicates 100 μm .

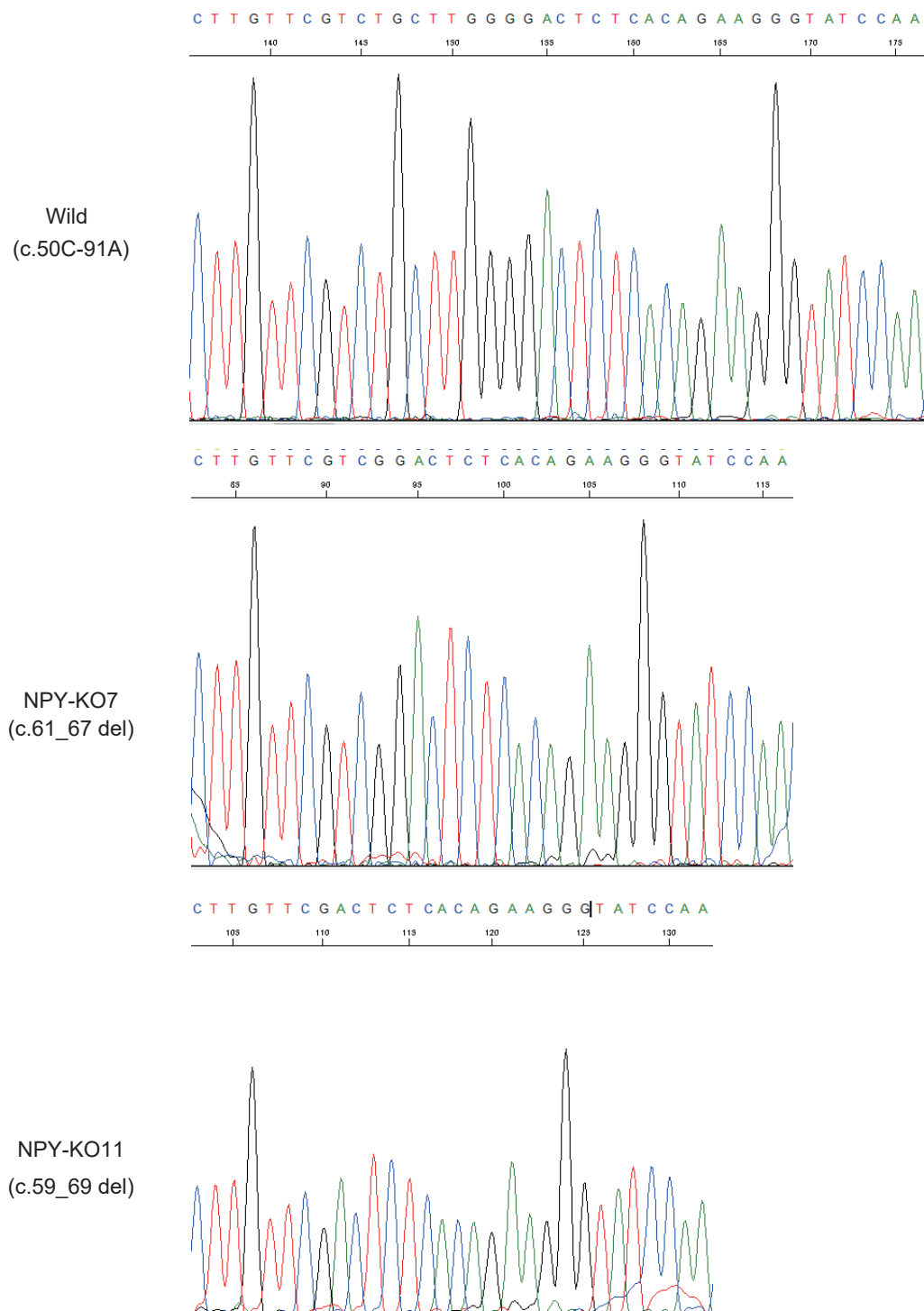
Supplemental table 1 Real-time PCR primers used in this study

Gene	Primers	
<i>orx</i>	5'- TTCATGGCGCTGCTAGCTCA -3'	5'- AATTTAGCGGGCTCCTCCAGC -3'
<i>cck</i>	5'- AGCGTCAACCACCGGATAAA -3'	5'- AGAGGACAGACGGAAACACG -3'
<i>crh</i>	5'- TCGTCCATGATCTTGCGGTT -3'	5'- GAATCTGCACGTGGTTGTCG -3'
<i>pomc</i>	5'- CCCCCTACAAAATGACCCAT -3'	5'- ATCCTTCCTCGGTTGGTCTT -3'
<i>avp</i>	5'- CGCTCTCGTCTGCCTGCTAC -3'	5'- TCTTAAGTCCC GCGCTGCTG -3'
<i>gr</i>	5'- CCCATTGAGGACCAAATCAC -3'	5'- AGTAGAGCATTTGGGCGTTG -3'
<i>mr</i>	5'- TTCGGACTAAAAACGCTGGACG -3'	5'- TCGCCGCAGTCTGAGGTCGT -3'
<i>ist</i>	5'- TCTGGAAAGGCCTGCGGTTA -3'	5'- GCTGTTGGCCGGTTGATTGA -3'
<i>th1</i>	5'- TACATACGGCACGCTTCCTC -3'	5'- GAACCGCACAGAAAACGGTC -3'
<i>th2</i>	5'- AAAGGCTTATGGGGCTGGAC -3'	5'- GCTGCAAGTGTAGGGGTCAT -3'
<i>npv</i>	5'- AAGATGTGGATGAGCTGGGC -3'	5'- TGAATAATACTTGGCGAGCTCCT -3'
<i>actb</i>	5'- AGCACCTGTGCTGCTCACT -3'	5'- CGCCATACAGAGCAGAAGCCA -3'

	10	20	30	40	50	60	
	cccacagagcaagaattccaatcaagatctcaaaaaattccaagacctcattcactgatg						
1						M	20
	70	80	90	100	110	120	
	aatccaaacatgaagatgtggatgagctgggcagcgtgcgcgtttctcttggtcgtctgc						
21	N	P	N	M	K	M	W
	130	140	150	160	170	180	40
	ttggggactctcacagaagggatatccaacaaaacccgacaacccgggagaggacgcacct						
41	L	G	T	L	T	E	G
	190	200	210	220	230	240	60
	gcggaggagctcgccaagtattattcagcactaagacactacatcaacctcataacaagg						
61	A	E	E	L	A	K	Y
	250	260	270	280	290	300	80
	cagaggtatgggaaaaggtcaagcgcgtgacaccttaatttcagaccttctgattggtgaa						
81	Q	R	Y	G	K	R	S
	310	320	330	340	350	360	100
	acagagtcctcgccccagaccagatatgaggatcatttggcatggtgatctcatccactc						
101	T	E	S	R	P	Q	T
	370	380	390	400	410	420	120
	tgtgatgtccatgtgtgccttctgttaacaattttggccaccagatctcataaaactcaaa						
	tgttctgcataaacaacttgctgtgacacaccacagtcatcatcaggatagtgaaaatac						
	tgcagaatgttcaactaacacattacaccataatgtctgtacaaatacgtcaatgtgcgc						
	tctttaggtcatcgaagattcagttcaacaagacagttgtacagagactcattttgtgt						
	atgcgtgttggtgtccattgtaatcccaagtagcaaaggagaacaataatgactgtacagt						
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	cccctga						



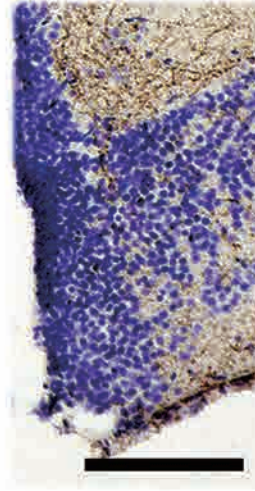
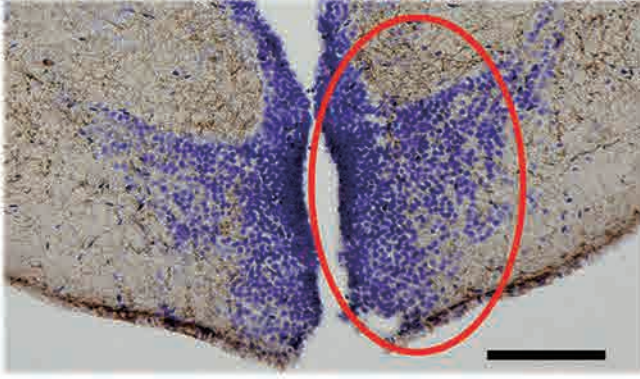
Supplemental Fig. 2.



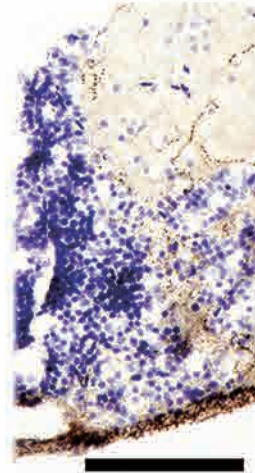
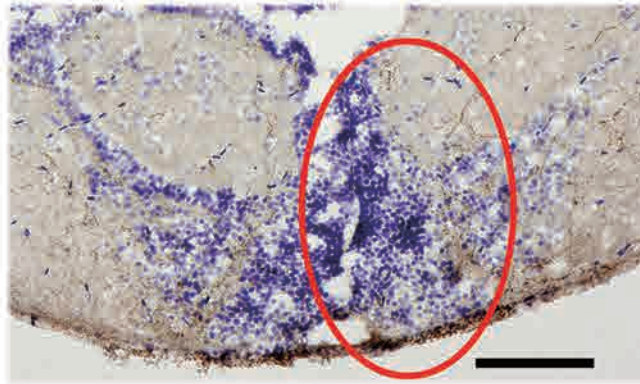
Supplemental Fig. 3.

NPY / Nissl

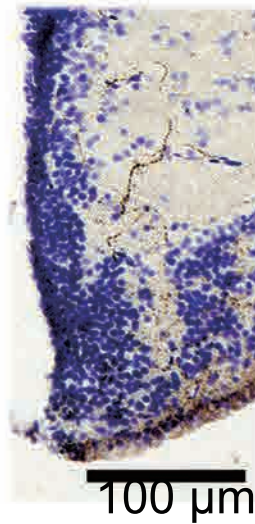
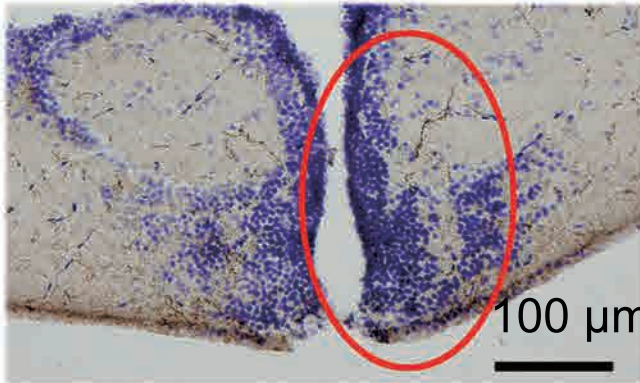
Wild



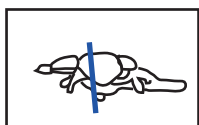
NPY-KO11



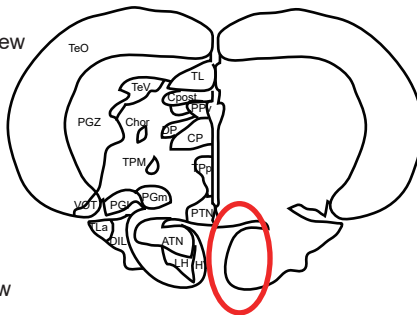
NPY-KO7



Cross Sectional view
at level of blue line in
a sagittals sectional view



Sagittals sectional view



Supplemental Fig.4