

Supplementary Material

Genome editing reveals idiosyncrasy of CNGA2 ion channel- directed antibody immunoreactivity towards oxytocin

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1 Supplementary Figures and Tables

1.1 Supplementary Figures

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A

zFCNGA1	ALTEY PDAKAMLEEKGRQILMKDNL DLELAKQGPDPKDMEKVKIGGVLEDLQTRFAR	599
zFCNGA2a	AVMEY PDAKQVLEERGREILRKQGLLDEGIAQGGLLVMDTEKVERLEGSLDILQARFAR	621
zFCNGA2b	AVIEY PDAQRVLEERGREILRKQGLLDEGIAQGGLLVMDTEKVERLEGSLDILQARFAR	622
zFCNGA3a	ALTEY PDAKAMLEEKGRQILMKDNL DLEAFAAGADPKDLEEKHREGLNLDIMTGKFAK	697
zFCNGA3b	ALSEY PAKKALEEKGRQILMKDNL IDEAAANAGADAKDLEKVNLLGTNLDVMQTKFAR	617
zFCNGA4	TILEY PAKRLLLEEKGRQILTKMGMLEAVEGEGE-VEKTE DKVNRLEGILEMLQTKLAR	530
gFCNGA3	ALTEY LPAKAMLEEKGRQILMKDNL DLELAKQGPDPKVMEEKVIKIGSVLDDLQTRFAR	589
cFCNGA2	AVMEY PDAKQVLEERGREILRKQGLLDEGIAQGGLLVMDTEKVERLEGSLDILQARFAR	621
sbCNGA-cone	ALTEY PDAKAMLEEKGRQILMKDNL DLELAKQGPDPKIEIEKVERMTSMLDVLQTRYAR	615
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zFCNGA1	LLAEQEAAGKLLKRVTKLEKMTSETLM-----E-----	629
zFCNGA2a	LLAQY TATQQLKRLTLECRINHSGSGLSD-----GNE-----	657
zFCNGA2b	LLGEFTATQRLKRLITALERQLCHTIGLVS-----HEM-----	658
zFCNGA3a	LMAEYTSYQKIKQRLITNMEKVKTLRPEDLSE-----VIE-----	733
zFCNGA3b	LMADWTASQRIKRLITNMEKVKTLRPEDLSE-----VVE-----	653
zFCNGA4	LIAELESSARKMMQRVDQLLEQTEGWEGIVAEGAQVEIEERAKIKEREIGDGEGEREK	590
gFCNGA3	LLAEHAAAGKLLKRVTKLEKMTSETLM-----MLD-----	625
cFCNGA2	LLGEY TATQQLKRLTLECRINHSGSGLSD-----GNE-----	657
sbCNGA-cone	LLAQH DATHSKLKHVRVARLEKMLVPPPTPETQ-----MGE-----	651
* : : : : * : * : * :		
zFCNGA1	-----PPPETAD--KTD-----	639
zFCNGA2a	-----STID--GDGTHSEVNIHL-----	673
zFCNGA2b	-----DVESNVNSPRTNSTA-----	673
zFCNGA3a	-----DKKEEK--KTK-----	743
zFCNGA3b	-----DKKDK-----	658
zFCNGA4	EDKEE PLVEGERGEGDGEE SLGEKDKNVGGNEINEEKEEKEI IKTETQQDEKEDEK	648
gFCNGA3	-----PELVIAK--KEEKE-----	637
cFCNGA2	-----STND--GDGTHSEVNIQL-----	673
sbCNGA-cone	-----	651

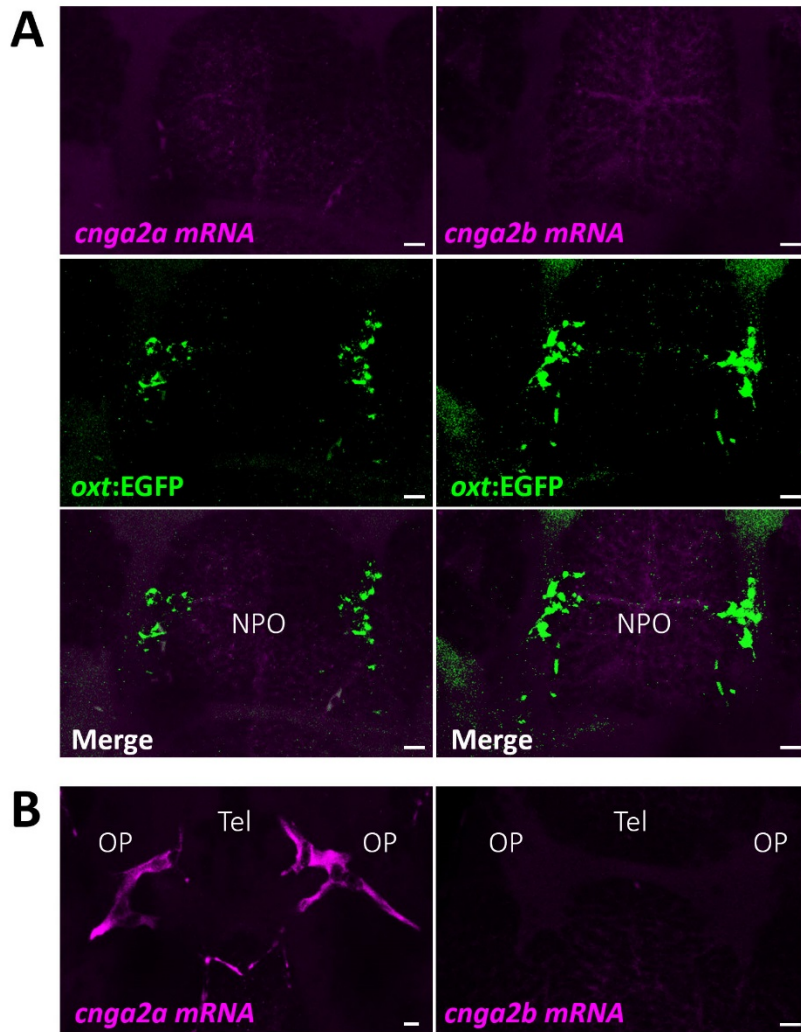
B

zfOXT	-----MSGGLLSAA--ALLCLLSVCSACYISNCPIGKRVSQDWPIR---	40
zFCNGA2a	WINKKTI DEQDVLKNLPNKL-RAEIAINVHLETIKKVRIFQDCEAGLLVELVLKLRPQVF	479
zfAVP	-----MSDSL SVCVLCVLA LSTLSSACYIQNCPRGKRS-QPEPIR---	41
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zfOXT	---QCMPCGPG---DRGRCFGPSICC-----	60
zFCNGA2a	SPGDYICRKGDIGKEMYIIKEGCLAVVADGVTQYAHLAGSCFGEISILNIRSKMGNR	539
zfAVP	---QCMSCGPG---GVGRCFGPSICC-----	61
* * * * *		
zfOXT	-----GEGIGCLVGS PETLRCL EDFLPSPCEMSGKACGYE-GRCAA	101
zFCNGA2a	RTAINRSIGYSDLFCLSKGLMEAVMEY PDAKQVLEEKGR-----QI-----LIK	584
zfAVP	-----GPGLGCVLGSFEAQVCMEEQLSGPCETGGTSCGDRGGRCAA	103
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zfOXT	PGVCCDSEGC SVDQSCVDGDADAAAVNPANSPDLLKLLLSSTHPSR-----	151
zFCNGA2a	EGGLEEVADGIGVEDIEKRVERME D-TIEILQTRFARLLAQYTATQQLKRLTLECR	643
zfAVP	EGICCDSESCAVDPDCPEGS SAGLKS-----ISGETLLRLNLATRRQRPF-----	149
* : * . . : : * : * :		
zfOXT	-----IHQ-----	154
zFCNGA2a	TNHS GSGFLSDGNESTIDGDTNSEVNIHL	673
zfAVP	-----	149

Supplementary Figure 1. Analysis of potential CNGA2a recognition sites for anti-CNGA2a L55/54 mAb.

(A) Alignment of C-terminal CNGA2a 106 amino acid epitope to the C-terminal sequences of the closely related CNGA channel protein sequences from the zebrafish and other species. Amino acid residues marked in red display conserved residues in all sequences. Identical residues between CNGA 2a and CNGA 2b proteins are marked in blue and green-colored letters indicate unique residues for CNGA2a but not CNGA2b. *zf*, zebrafish; *gf*, goldfish; *cf*, catfish; *sb*, striped bass.

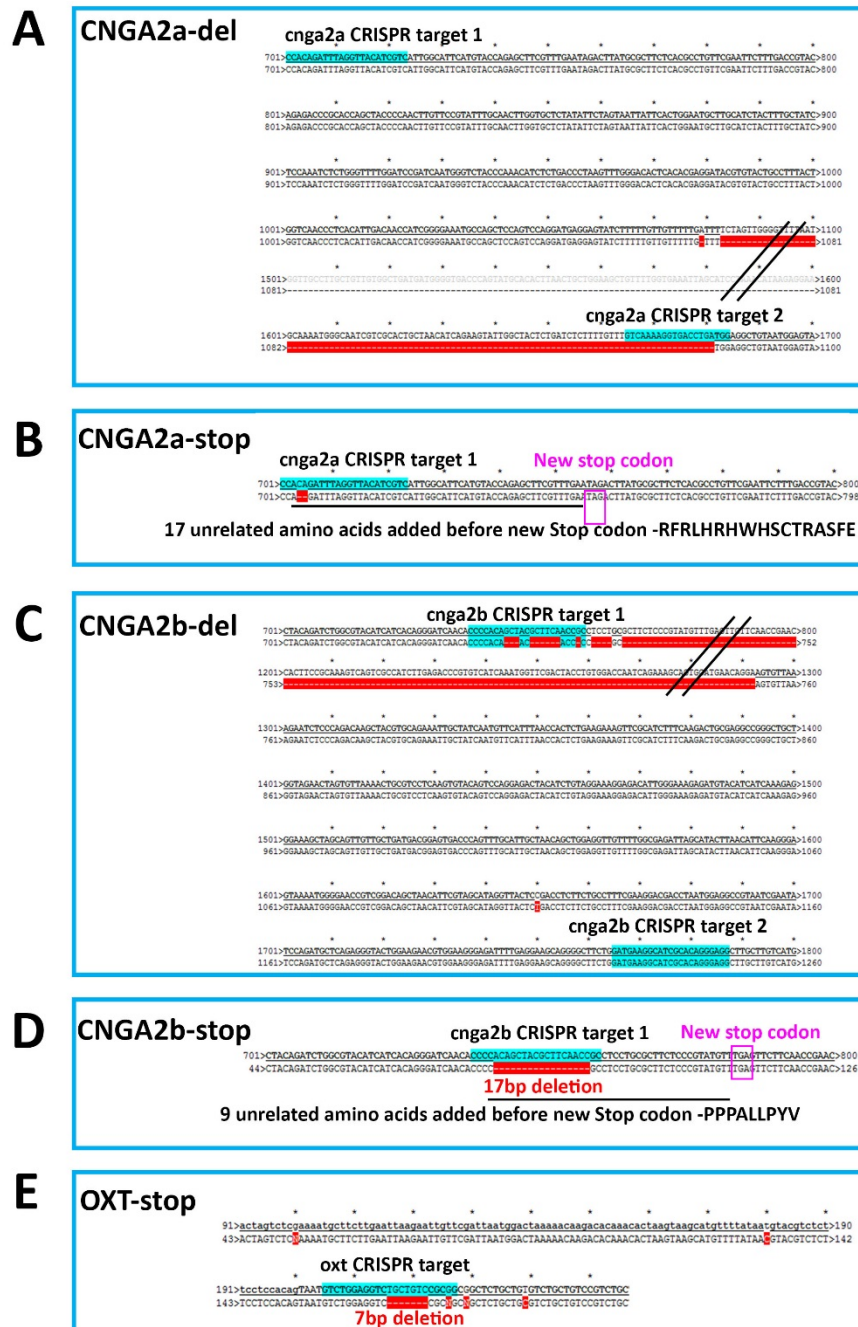
(B) Alignment of CNGA2a C-terminal to oxytocin and arginine-vasopressin precursor protein sequences. Blue-colored letters indicate identical and structurally related amino acid residues that are common between all three protein sequences. Green letters mark common residues between CNGA2a and oxytocin but not arginine-vasopressin precursor sequences.



Supplementary Figure 2. Expression of *cnga2a/b* mRNAs is not detected in zebrafish oxytocin neurons.

(A) Confocal Z-stack images showing fluorescent in situ hybridization (FISH) of transgenic 6-dpf old Tg(*oxt:EGFP*) larvae using probes directed against mRNAs of *cnga2a* and *cnga2b*, followed by anti-EGFP staining. The NPO area with oxytocin cell bodies Tg(*oxt:EGFP*) are shown. No detectable expression of *cnga2a* and *cnga2b* is observed. Scale 10 μ m.

(B) *cnga2a* but not *cnga2b* is expressed in olfactory placode of 6dpf old larvae using probes directed against mRNAs of *cnga2a* and *cnga2b*. Scale 10 μ m.



Supplementary Figure 3. Analysis of *cnga2a* and *cnga2b* CRISPR-induced genomic mutations.

Alignments of wild type and mutant alleles for *cnga2a*-del (A), *cnga2a*-stop (B), *cnga2b*-del (C), *cnga2b*-stop (D) and *oxt* (E) homozygous KO zebrafish. CRISPR-mediated indels are highlighted in red.

1.2 Supplementary Table

Gene	NCBI ID	sgRNA template	Genotyping primers	
			Fwd	Rev
<i>cnga2a</i>	NM_001044746.1	GACGATGTAACCTAAATCTGTGG	AGCTACTTTCATACTCTACAGT	TCGAACAGGCGTGAGAAGCG
		GTCAAAAGGTGACCTGATGGAGG	GCCAGCTCCAGTCCAGGATGA	ATATCTTCCACCCCTATTCCGT
<i>cnga2b</i>	XM_021471593.1	GCGGTTGAAGCGTAGCTGTGGGG	TGGCGTACATCATCACAGGGA	AGTTGGTTCTGGTCTCTGTTCG
		GATGAAGGCATCGCACAGGGAGG	TGGCGTACATCATCACAGGGA	CCAATGCCTGTATGACACAGT
<i>oxl</i>	NM_178291.2	GTCTGGAGGTCTGCTGTCCGCGG	AGACACAAACACTAAGTAAG	AGCAGACGGACAGCAGACAC

Supplementary Table 1. List of primers used in this study