Vocke et al., http://www.jgp.org/cgi/content/full/jgp.201311015/DC1

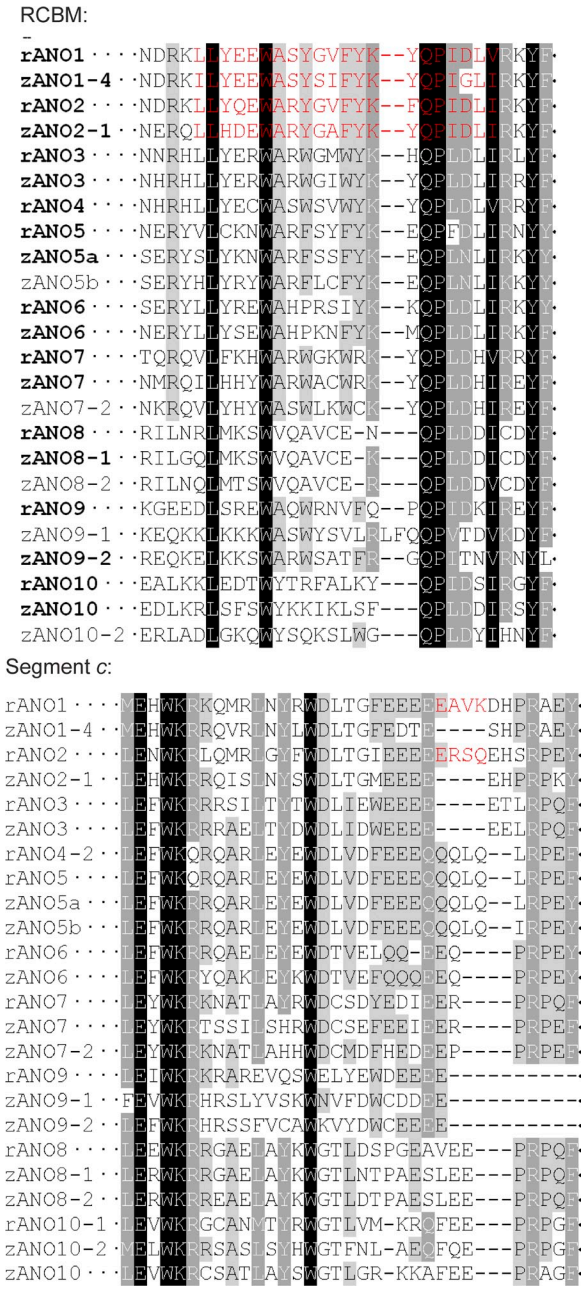

Figure S1. Alignments of rat and zebrafish ANO proteins. The 10 rat homologues ANO 1–10 (prefix r) are compared with those orthologous zebrafish protein sequences (prefix z) that display the highest degree of conservation. Sequences were obtained from the Ensembl database. Indicated percentages of conservation are 100% (black), 80% (dark gray), and 60% (light gray). Red letters indicate RCBM sequences (top alignment) and segment *c* sequences (bottom alignment), each encoded by a separate exon.

TABLE S1

Composition of pipette solutions used for the electrophysiological experiments

Pipette solution	Composition
Measured free Ca^{2+}: 62 μM	130 mM CsCl
	9.98 mM $CaCl_2$
	10 mM HEPES
	10 mM HEDTA
	pH 7.0 using CsOH
Measured free Ca^{2+}: 7.5 μM	133.474 mM CsCl
	8.263 mM $CaCl_2$
	10 mM HEPES
	10 mM HEDTA
	pH 7.0 using CsOH
Measured free Ca^{2+}: 2.6 μM	138.268 mM CsCl
	5.866 mM $CaCl_2$
	10 mM HEPES
	10 mM HEDTA
	pH 7.0 using CsOH
Measured free Ca^{2+}: 2.0 μM	143.582 mM CsCl
	3.209 mM $CaCl_2$
	10 mM HEPES
	10 mM HEDTA
	pH 7.0 using CsOH
Measured free Ca^{2+}: 0.7 μM	147.516 mM CsCl
	1.242 mM $CaCl_2$
	10 mM HEPES
	10 mM HEDTA
	pH 7.0 using CsOH
Ca^{2+}-free pipette solution	150 mM CsCl
	10 mM HEPES
	10 mM HEDTA
	pH 7.0 using CsOH
Bath solution	150 mM CsCl
	10 mM HEPES
	10 mM EGTA
	pH 7.4 using CsOH

Composition of pipette solutions used in whole-cell experiments. Chemicals used were of the highest purity available. Compositions were obtained from Reisert et al. (2003). The indicated free Ca^{2+} concentration of each solution was determined using a calcium electrode (KWIKCAL-2) that was calibrated with the CALBUF-1 series (World Precision Instruments). A single set of pipette solutions was used for the entire project.

TABLE S2

Primers used for cloning, RT-PCR analysis, and mutagenesis

Purpose	Orientation	Primer sequence (5′–3′)
Cloning channels		
Full-length ANO1	Forward	AGAGCTAGCGGCCGCCGATGGGGAGCGCGAGGCCACCATG
	Reverse	TGGATCCGGCTCCAGCGCGCCCCCATGGTA
Full-length ANO2	Forward	AAAGTCGATGCTAGCGACAATGGCAAGAGGAAGCCAG
	Reverse	TTCCCGGGCTCCTACGTTGGTGTGC
Testing for Segment *c*		
SegC+	Forward	AACGTTCCCAGGAACACT
SegC−	Forward	GAAGAAGAACACTCCCG
SegCtest	Reverse	CAATGGAGAACGTCAGGGC
Remove/insert segment *c*		
ANO 1 remove c	Forward	GCTTCGAGGAAGAGGAGGATCATCCCAGAGCAGAG
	Reverse	CTCTGCTCTGGGATGATCCTCCTCTTCCTCGAAGC
ANO2 insert c	Forward	GAAGAGGAAGAAGAACGTTCCCAGGAACACTCCCGGCCTGAATAT
	Reverse	ATATTCAGGCCGGGAGTGTTCCTGGGAACGTTCTTCTTCCTCTTC
Point mutations		
ANO1 L323E	Forward	CAACGACAGGAAACTCGAGTATGAGGAATGGGCAAGCTATGG
	Reverse	CCATAGCTTGCCCATTCCTCATACTCGAGTTTCCTGTCGTTG
ANO1 W327E	Forward	GACAGGAAACTCCTGTATGAGGAAGAGGCAAGCTATGGAGTC
	Reverse	GACTCCATAGCTTGCCTCTTCCTCATACAGGAGTTTCCTGTC
ANO1 L323E/W327E	Forward	CAACGACAGGAAACTCGAGTATGAGGAAGAGGCAAGCTATGGAG
	Reverse	CTCCATAGCTTGCCTCTTCCTCATACTCGAGTTTCCTGTCGTTG
ANO1 V332E/Y336E	Forward	GGGCAAGCTATGGAGAATTCTACAAAGAGCAGCCCATTGACCTGG
	Reverse	CCAGGTCAATGGGCTGCTCTTTGTAGAATTCTCCATAGCTTGCCC
ANO2 L309E/W313E	Forward	GAATGACAGAAAGTTGGAGTATCAGGAAGAGGCACGCTATGGAG
	Reverse	CTCCATAGCGTGCCTCTTCCTGATACTCCAACTTTCTGTCATTC
ANO2 V318E/F322E	Forward	ATGGGCACGCTATGGAGAGTTTTACAAAGAACAACCCATTGACCTCATC
	Reverse	GATGAGGTCAATGGGTTGTTCTTTGTAAAACTCTCCATAGCGTGCCCAT
ANO2 F322E/L327E	Forward	CTATGGAGTGTTTTACAAAGAGCAACCCATTGACGAGATCAGGAAGTATTTTGGAGAG
	Reverse	CTCTCCAAAATACTTCCTGATCTCGTCAATGGGTTGCTCTTTGTAAAACACTCCATAG
Chimaeras		
ANO1-NT Eco105I	Forward	CAGCCCATTGACCTGGTACGTAAATACTTTGGAGAAAAGGTTGGC
	Reverse	GCCAACCTTTTCTCCAAAGTATTTACGTACCAGGTCAATGGGCTG
ANO1-TMR3 EcoRI	Forward	GATTCCCAGCCTATTTCACGAATTCTGTCTCCATCATCTTCATGATC
	Reverse	GATCATGAAGATGATGGAGACAGAATTCGTGAAATAGGCTGGGAATC
ANO2-NT Eco105I	Forward	TTCAACCCATTGACCTCATACGTAAGTATTTTGGAGAGAAAATTGGGC
	Reverse	GCCCAATTTTCTCTCCAAAATACTTACGTATGAGGTCAATGGGTTGAA
ANO2-TMR3 EcoRI	Forward	GTTTCCCAGGATACTTGATGAATTCTGTCTCCATCTTGTTTATG
	Reverse	CATAAACAAGATGGAGACAGAATTCATCAAGTATCCTGGGAAAC

List of the primer sequences used for the PCR protocols in this project. Forward and reverse primers are given for the various tasks. For generating the chimaera, we introduced restriction sites for the indicated endonucleases without altering the amino acid sequences.

REFERENCE

Reisert, J., P.J. Bauer, K.W. Yau, and S. Frings. 2003. The Ca-activated Cl channel and its control in rat olfactory receptor neurons. *J. Gen. Physiol.* 122:349–363. http://dx.doi.org/10.1085/jgp.200308888