# Supporting Information for

$Ca^{2+}$-activated $Cl^-$ currents in the murine vomeronasal organ enhance neuronal spiking but are dispensable for male-male aggression

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**List of Supporting Material**



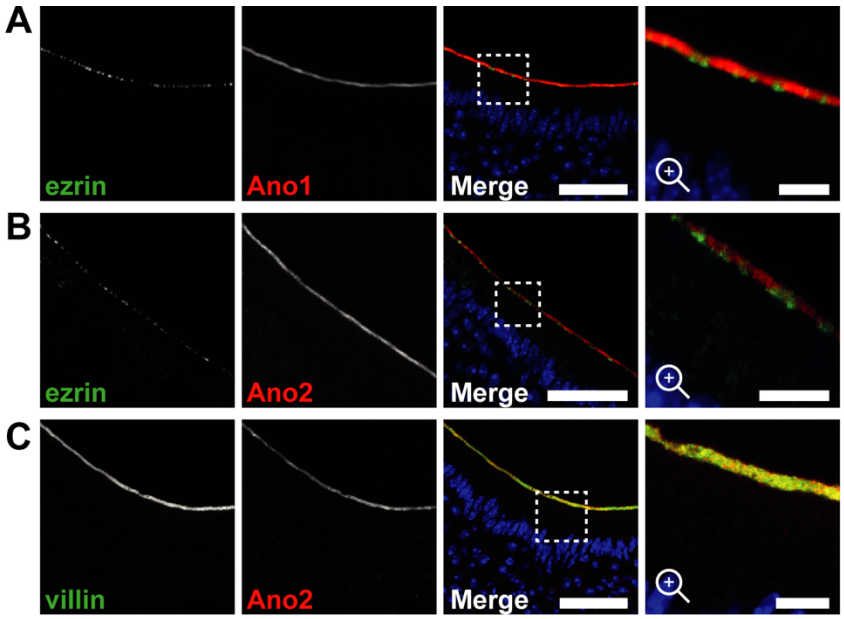


**Figure S 1: Colocalization of Ano1 and Ano2 with markers for microvilli**

*A–B*, coronal VNO sections immunolabeled for ezrin (green) shows staining at the apical border of the sensory epithelium which does not colocalize with Ano1 (red) or Ano2 (red, gpAno2_C1-3). Dashed region is magnified. *C*,

villin (green) colocalizes with Ano2 (red, gpAno2_C1-3). Dashed region is magnified. Bars: 100 µm (magnified images: 50 µm). Nuclei are colored blue in merged images.

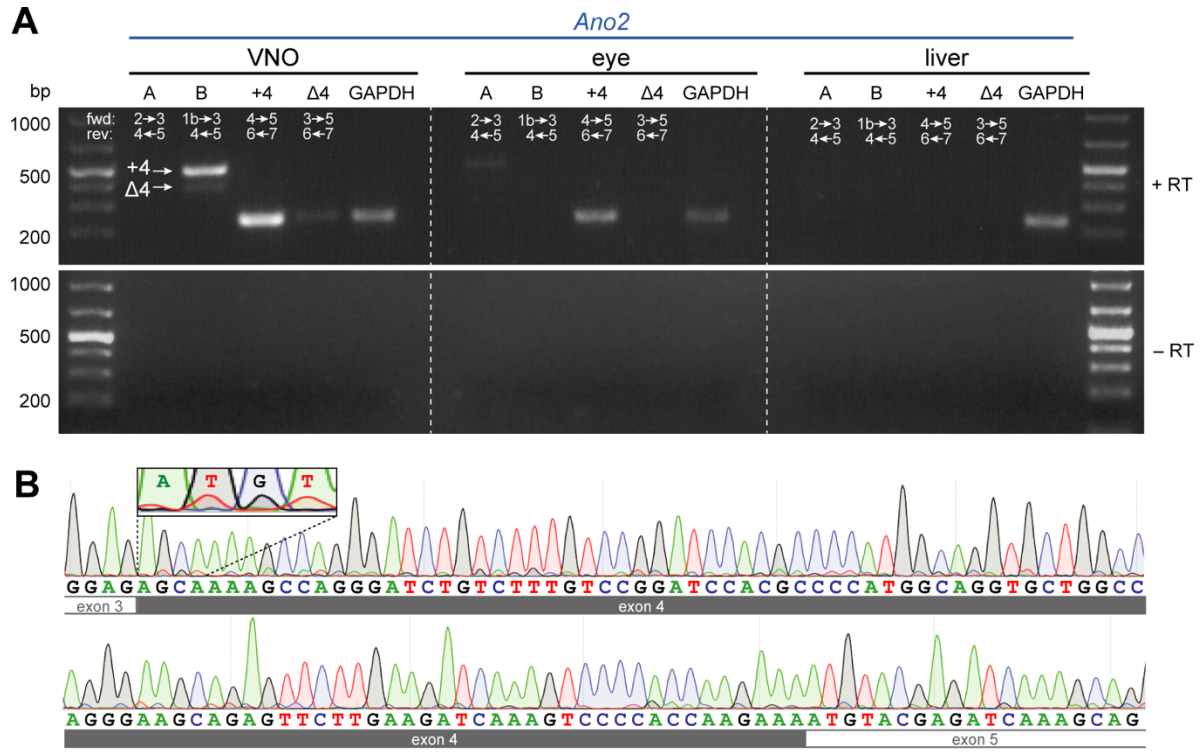


**Figure S 2: Splice isoforms of Ano1 and Ano2 in the VNO**

*A*, RT-PCR on VNO tissue probing different *Ano1* or *Ano2* isoforms in VNO, eye and liver tissue, target exons of forward (fwd) and reverse (rev) primers are indicated, the lower part labelled with "–RT" indicates the control RT-PCR without reverse transcriptase. *B*, *Ano2* sequencing chromatogram of cDNA from the VNO, sequence corresponding to exon 4 is indicated in light grey. An overlapping chromatogram sequence corresponding to exon 5 is magnified in the inset.

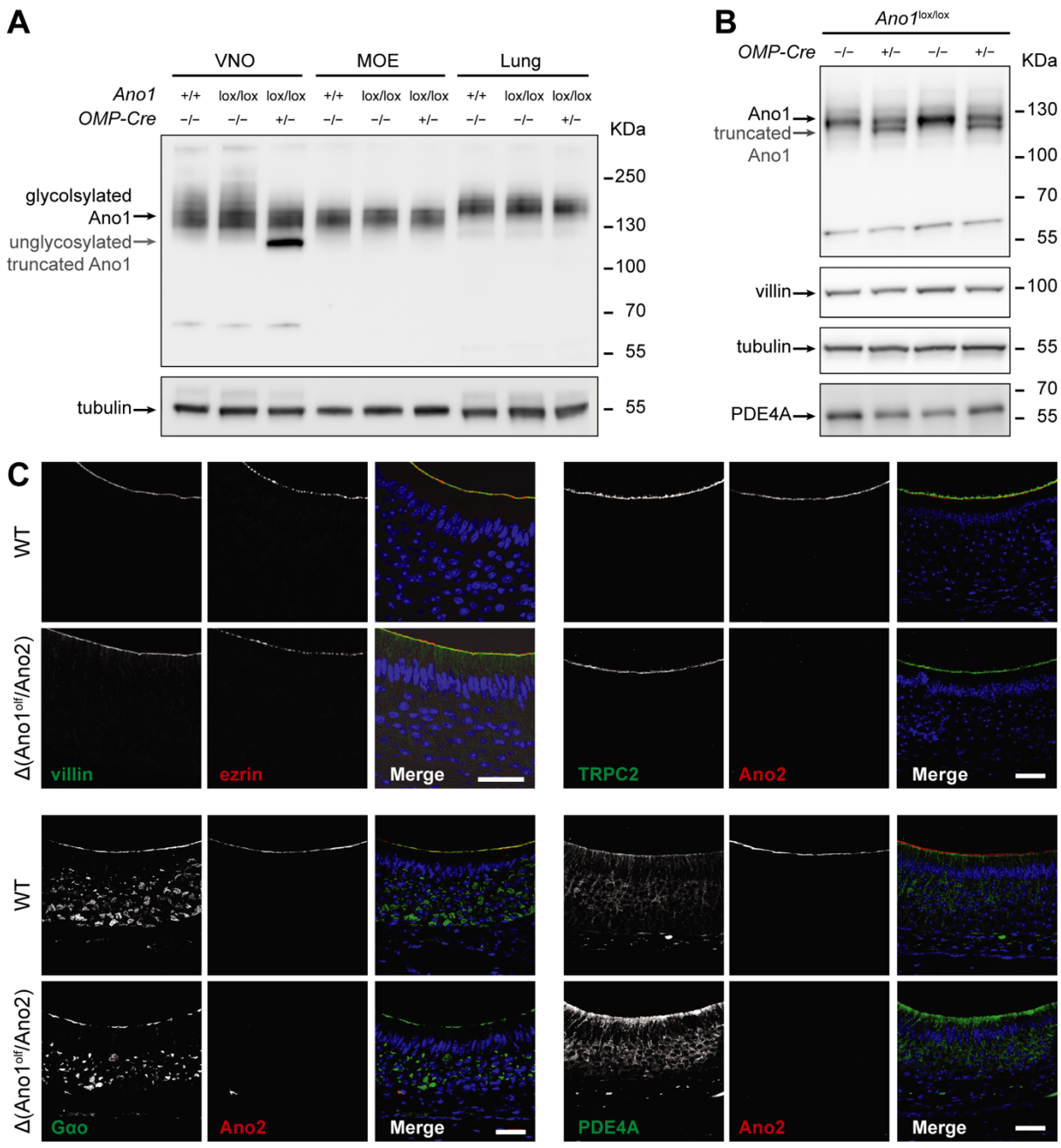


**Figure S 3: Olfactory double knock-out of Ano1 and Ano2**

*A*, Western blot for Ano1 of lysates from different tissues and genotypes, protein load per lane: 40 µg (VNO), 20 µg (MOE), 50 µg (lung), tubulin was used as loading control. *B*, Western blot for Ano1 of N-deglycosylated complete VNO lysates (upper blot) and for villin, tubulin and PDE4A, genotypes are indicated, protein load per lane: 20–40 µg. Arrows indicate the band of the respective proteins. *C*, coronal VNO sections immunolabeled for vomeronasal key proteins villin (green) ezrin (red), Gαo (green), TRPC2 (green) and PDE4A (green) in wild-type and Δ(Ano1$^{olf}$/Ano2) mice. Ano2 (red, gpAno2_C1-3) was costained as reference. Bar: 40 µm. Nuclei are colored blue in merged images.

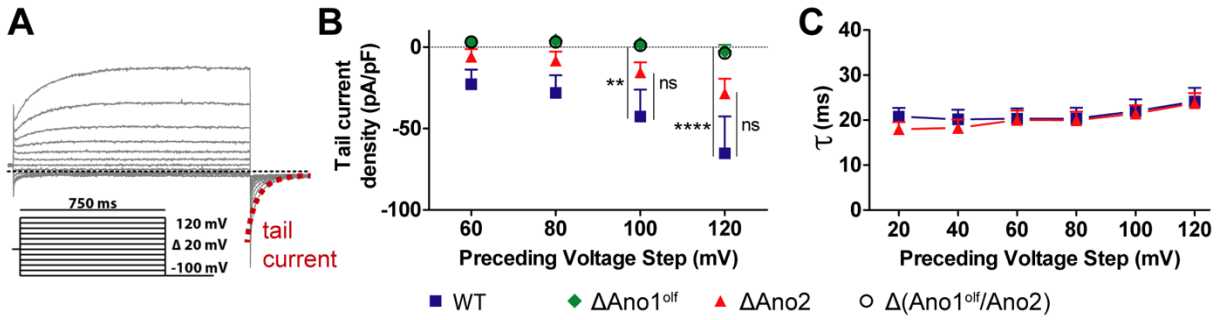


**Figure S 4: $Ca^{2+}$-activated $Cl^-$ tail currents**

*A*, example wild-type voltage clamp current trace demonstrating the tail current curve fit (red dashed line) at the beginning of the −100 mV repolarization step, voltage clamp protocol is shown below. *B*, $Ca^{2+}$-dependent current density extrapolated from exponential fits of $I_{Cl(Ca)}$ tail currents of wild-type, ΔAno1$^{olf}$, ΔAno2 and Δ(Ano1$^{olf}$/Ano2) VSNs. Mean current density after the respective preceding voltage step + SEM, Two-way ANOVA with Bonferroni post-test. ns: $P > 0.05$ (not significant, WT vs. ΔAno2), **: $P \leq 0.01$ (WT vs. ΔAno1$^{olf}$), ****: $P \leq 0.0001$ (WT vs. ΔAno1$^{olf}$). *C*, Deactivation time constants τ from mono-exponential fits of $I_{Cl(Ca)}$ tail currents. Mean of deactivation time constant τ at respective preceding voltage steps + SEM. Measured cells: 19 WT, 13 ΔAno1$^{olf}$, 11 ΔAno2, 8 Δ(Ano1$^{olf}$/Ano2). Free intracellular $Ca^{2+}$ concentration: 1.5 µM.

**Table S1: Primers for RT-PCR**

| Gene | Label | Primer sequence (5'→3') | PCR product | Remarks[#] |
|---|---|---|---|---|
| Ano1 | +14 | GGAGGAGGAGGAAGCTGTCAAGGAT<br>CCGACCAACAAACCGGCCTT | 496 bp | exon 13→14→15 to 18←19,<br>targeting isoform 1 including exon 14 |
| Ano1 | Δ14 | CTTCGAGGAGGAGGAGGATCATCC<br>CCGACCAACAAACCGGCCTT | 490 bp | exon 13→15 to 18←19,<br>targeting isoform 2 excluding exon 14 |
| Ano2 | A | AGTTCCCGAGACCGTTCTGTCATC<br>CTGAACTTCTTTGCGATGCTGCCT | 488 bp | exon 2→3 to 5,<br>targeting isoform A |
| Ano2 | B | CCAGAACACCTGCCAGTCATCAATAA<br>CTGAACTTCTTTGCGATGCTGCCT | 485 bp | exon 1b→3 to 5,<br>targeting isoform B |
| Ano2 | +4 | CCCCACCAAGAAAATGTACGAG<br>GAATCTCGTGTACAATGCGGCT | 233 bp | exon 4→5 to 6←7,<br>targeting isoform including exon 4 |
| Ano2 | Δ4 | GGAGAAGGACTTGGAGATGTACGAG<br>GAATCTCGTGTACAATGCGGCT | 236 bp | exon 3→5 to 6←7,<br>targeting isoform excluding exon 4 |
| Ano2 | +14 | GGAAGAAGAACGTTCCCAGGAA<br>TCTGCCCACAAACCTCCCCT | 523 bp | exon 13→14→15 to 18←19,<br>targeting isoform including exon 14 |
| Ano2 | Δ14 | GGGATCGAAGAGGAAGAAGAACA<br>TCTGCCCACAAACCTCCCCT | 522 bp | exon 13→15 to 18←19,<br>targeting isoform excluding exon 14 |
| GAPDH | GAPDH | ACAGCAACAGGGTGGTGGAC<br>TTTGAGGGTGCAGCGAACTT | ca. 250 bp | targets ubiquitous glyceraldehyde 3-phosphate dehydrogenase as positive control |

[#] exon spanning primers were designed to exclude an amplification of remaining genomic DNA in the sample, flanking primers are indicated with the respective exons they span separated by an arrow (e.g. 2→3)