SUPPLEMENTARY ONLINE MATERIAL

to Billig, Pal, Fidzinski & Jentsch:
Ca^{++}-activated Cl^--currents are dispensable for olfaction

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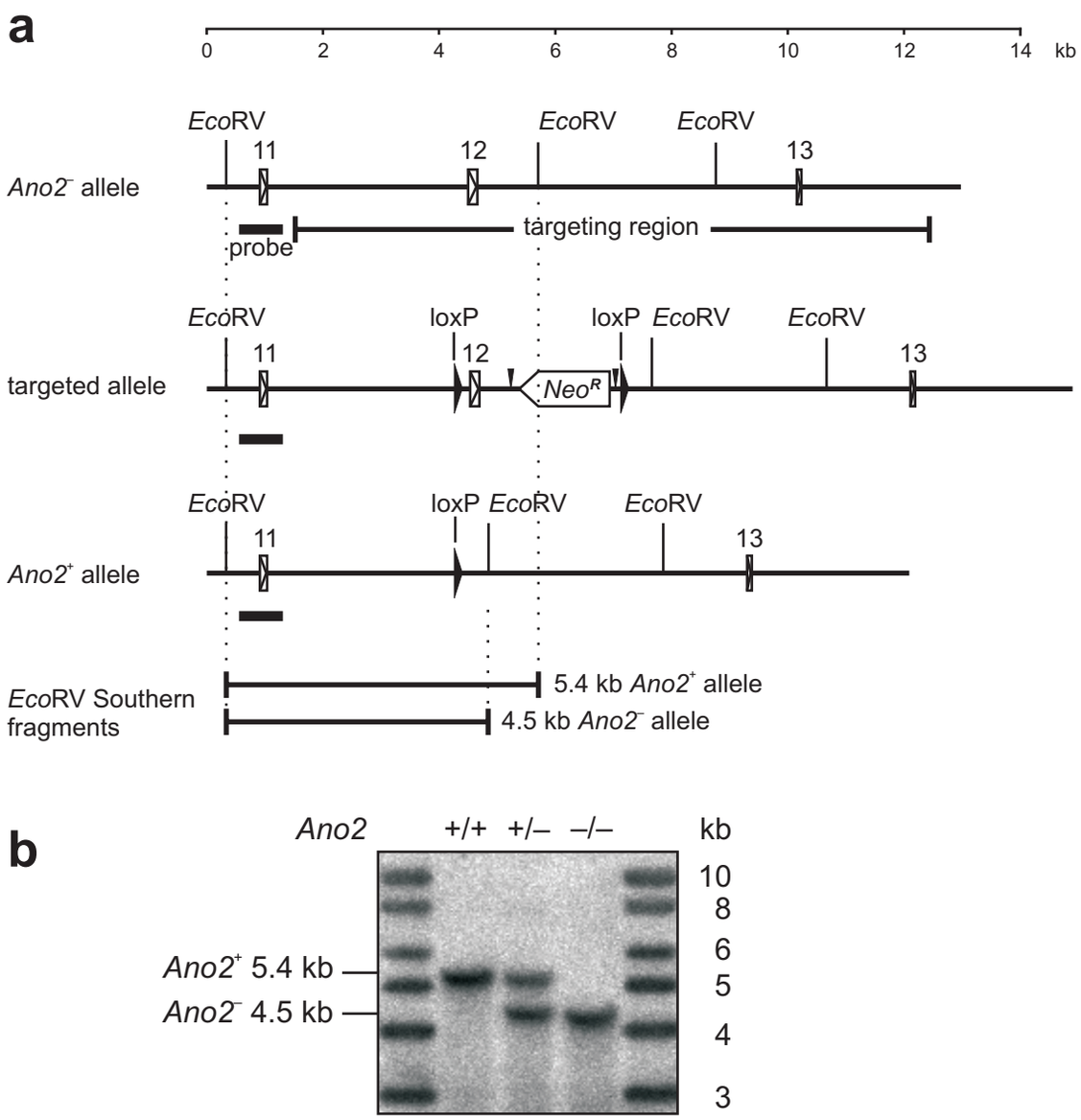
Supplementary Figure 1



Supplementary Figure 1 Generation of *Ano2*$^{-/-}$ mice. (**a**) Targeting strategy for the inactivation of the mouse *Ano2* gene. *Top*, part of the wildtype *Ano2* allele that encompasses exons 11 through 13. Only sequence from the targeting region indicated below was used to construct the targeting vector. Sequence used as probe for Southern analysis of *Eco*RV digested DNA is external to this region. *Below*, in the targeted allele exon 12 is flanked by two loxP sites. Together with the second loxP site, a neomycin selection cassette (*Neo*^{R}) flanked by FRT sites (filled triangles) has been inserted. This allows the removal of the Neo cassette by crossing these mice with FLPe-recombinase expressing 'deleter' mice, resulting in *Ano2*$^{lox/lox}$ mice that may be used for conditional gene disruption. In this work, mice were crossed with Cre-recombinase expressing 'deleter' mice, which by excision of exon 12 results in a null allele (*Ano2*$^{-}$). The *bottom* depicts the expected *Eco*RV fragments for the different alleles when using the external probe for detection. (**b**) Southern blot analysis using *Eco*RV-digested DNA from tail biopsies of *Ano2*$^{+/+}$, *Ano2*$^{+/-}$ and *Ano2*$^{-/-}$ mice confirms successful disruption of *Ano2*.

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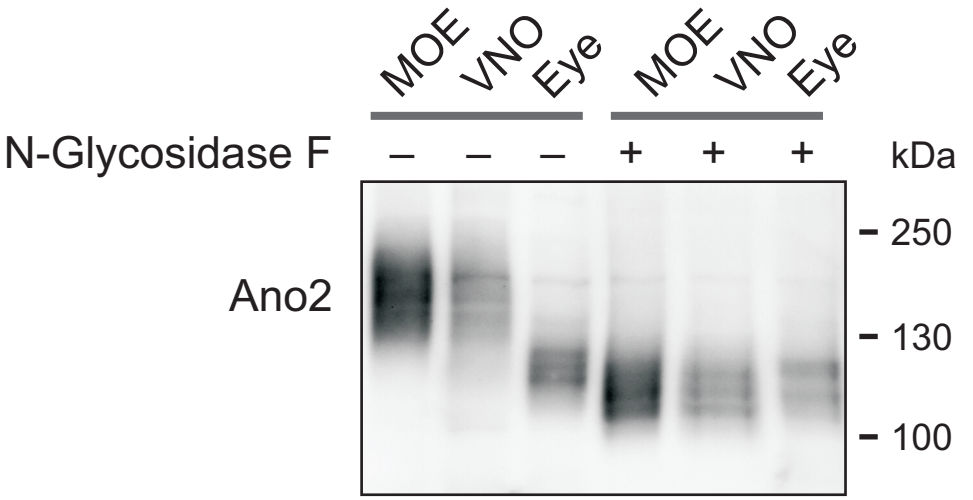
Supplementary Figure 2



Supplementary Figure 2 Glycosylation of Ano2. Ano2 protein runs at higher apparent molecular weights in olfactory tissues than in eye but yields a uniform triplet running at ~110 to ~120 kDa after deglycosylation with N-Glycosidase F. Different bands of this triplet may represent splice variants.

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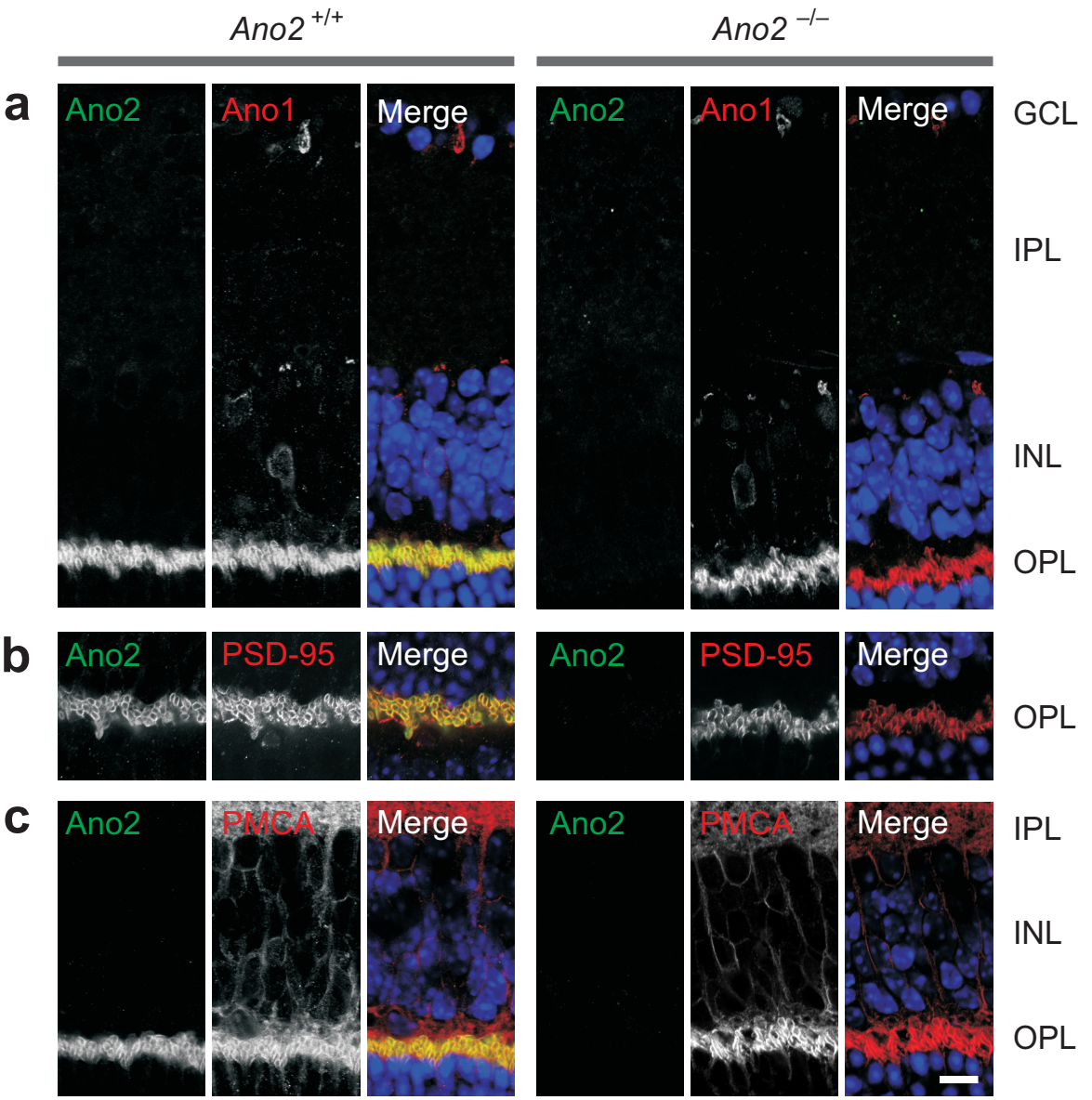
Supplementary Figure 3



Supplementary Figure 3 In the retina Ano2 resides in the outer plexiform layer (OPL) where it co-localizes with Ano1, PSD-95 and PMCA. (**a**) Immunostaining of *Ano2*$^{+/+}$ retina (left) shows localization of Ano2 to synaptic endings of photoreceptors in the OPL and co-localization with Ano1. Lack of signal in *Ano2*$^{-/-}$ tissue (right) confirms specificity of antibody (gpAno2_C1-3). Ano1 expression is not affected by the loss of Ano2. The Ano1 antibody additionally stains cells in the inner nuclear layer (INL) and the ganglion cell layer (GCL). (**b**) Co-localization of Ano2 (rbAno2_N3-3) with PSD-95 in the OPL. PSD-95 is not affected by the loss of Ano2. (**c**) Co-localization of Ano2 (rbAno2_N3-3) with PMCA in the OPL. PMCA is not affected by the loss of Ano2. Nuclei in the merged pictures are marked in blue. Scale bar, 10 m. IPL, inner plexiform layer. Note that the specificity of the Ano1 antibody (ab53212, Abcam) has been demonstrated in mouse colon using *Ano1*$^{-/-}$ tissue as control[46]. However, no such control is currently available for the retina.

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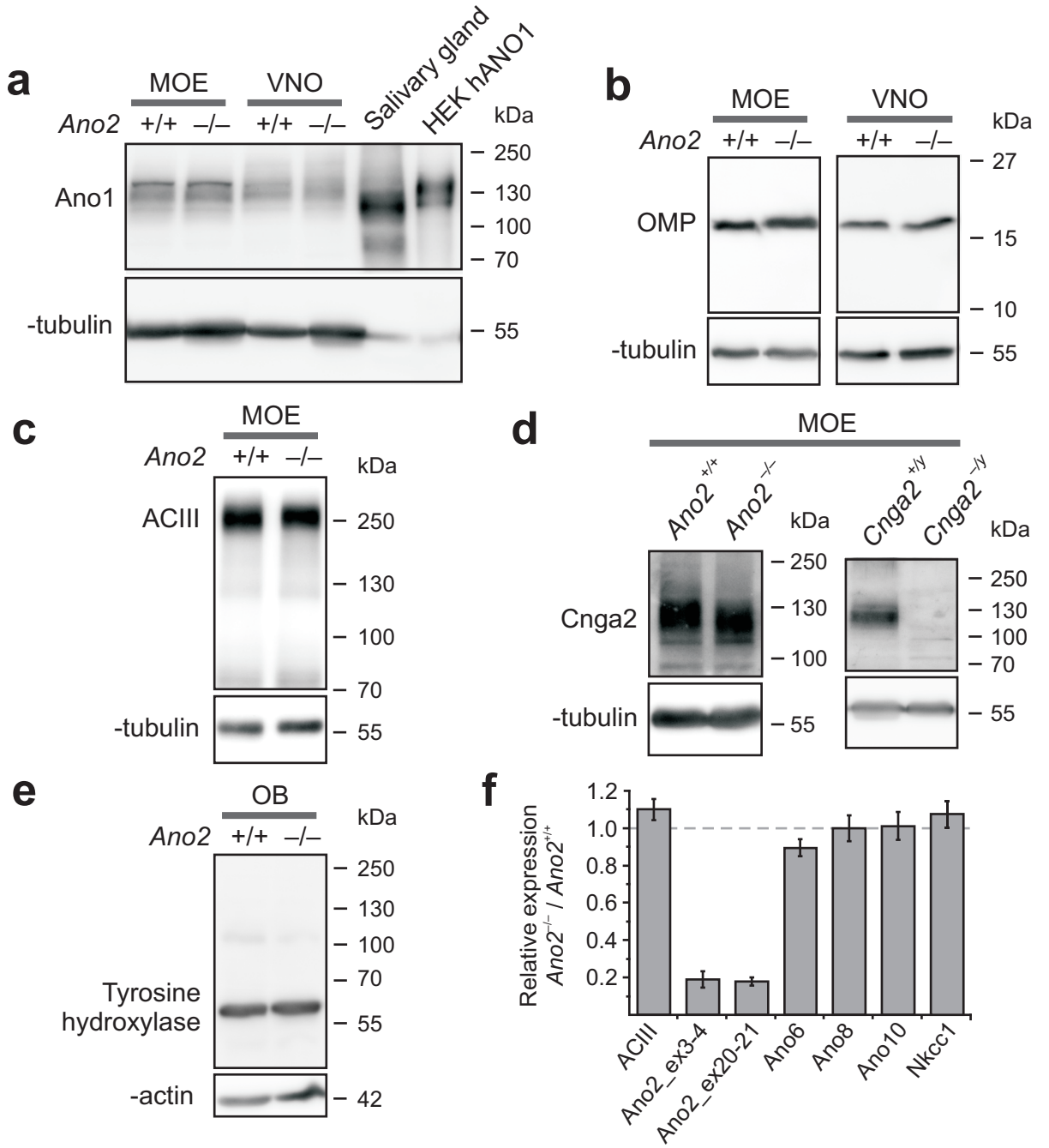
Supplementary Figure 4



Supplementary Figure 4 *Ano2* disruption and expression of other genes. (**a**–**e**) Immunoblots demonstrating unchanged expression of Ano1 and key olfactory signal transduction elements in *Ano2*$^{-/-}$ mice. (**a**) Ano1 immunoblot of extracts from *Ano2*$^{+/+}$ and *Ano2*$^{-/-}$ MOE and VNO, using *Ano2*$^{+/+}$ salivary gland and *Ano1*-transfected (human isoform) HEK cell lysates as positive controls. Ano1 protein levels are unchanged in *Ano2*$^{-/-}$ mice. Amount of protein loaded: 100 g (MOE and VNO), 32 g (salivary gland). (**b**) Levels of OMP, a marker for mature OSNs, are unaffected by disruption of *Ano2*. (**c**) Unchanged levels in *Ano2*$^{-/-}$ MOE of adenylate cyclase III (ACIII), which is specifically expressed in OSNs. (**d**) Unchanged expression levels of the CNG channel subunit Cnga2 in the MOE of *Ano2*$^{-/-}$ mice. Absence of band in extracts from *Cnga2*$^{-/y}$ MOE confirms antibody specificity. (**e**) No effect of *Ano2* disruption on tyrosine hydroxylase expression in the olfactory bulb (OB). -tubulin and -actin served as loading controls. (**f**) Quantitative real-time PCR on MOE of *Ano2*$^{+/+}$ and *Ano2*$^{-/-}$ mice reveals no changes in relative expression levels of *Anoctamin* gene family members *Ano6*, *Ano8* and *Ano10,* and the $Na^+K^+2Cl^-$ cotransporter *Nkcc1. Ano2* is strongly downregulated in the MOE of *Ano2*$^{-/-}$ mice indicating nonsense-mediated RNA decay of transcripts from the targeted allele. Unchanged levels of *ACIII* confirm tissue identity and indicate comparable MOE content in whole turbinate preparations from *Ano2*$^{+/+}$ and *Ano2*$^{-/-}$ mice. Error bars represent s.e.m. Note that MOE and VNO preparations not only contain the olfactory epithelium, but also underlying structures including cartilage and glands.

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