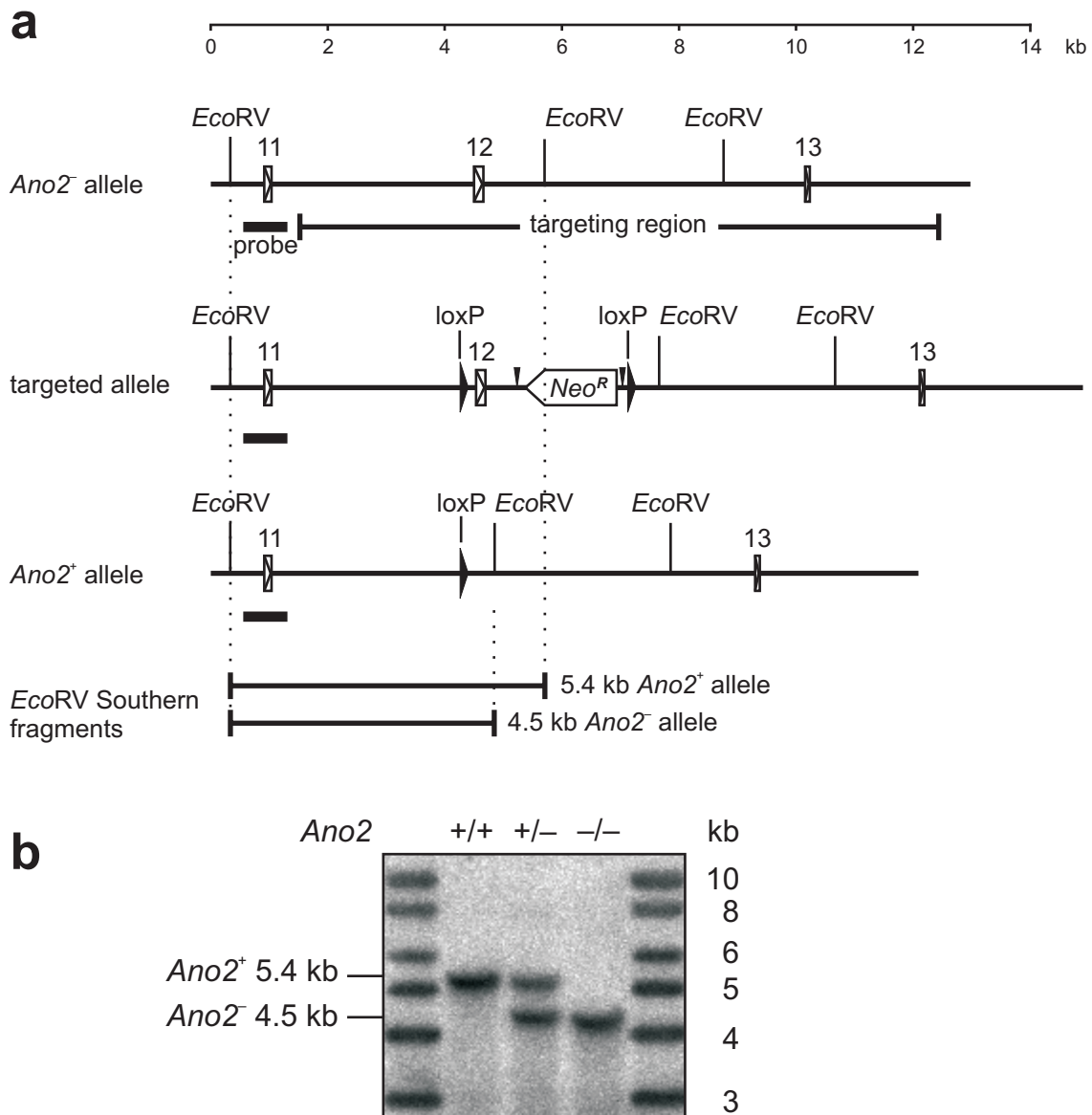


SUPPLEMENTARY ONLINE MATERIAL

to Billig, Pal, Fidzinski & Jentsch:

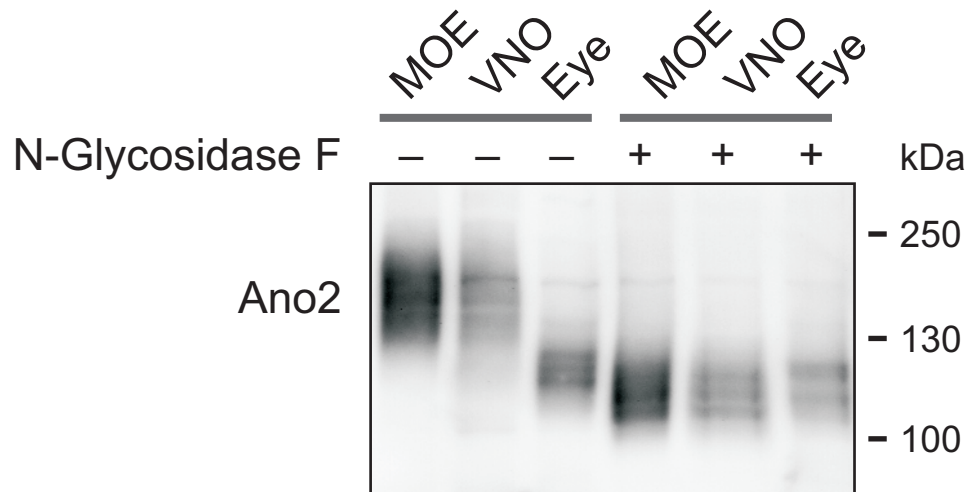
Ca⁺⁺-activated Cl⁻-currents are dispensable for olfaction

Supplementary Figure 1



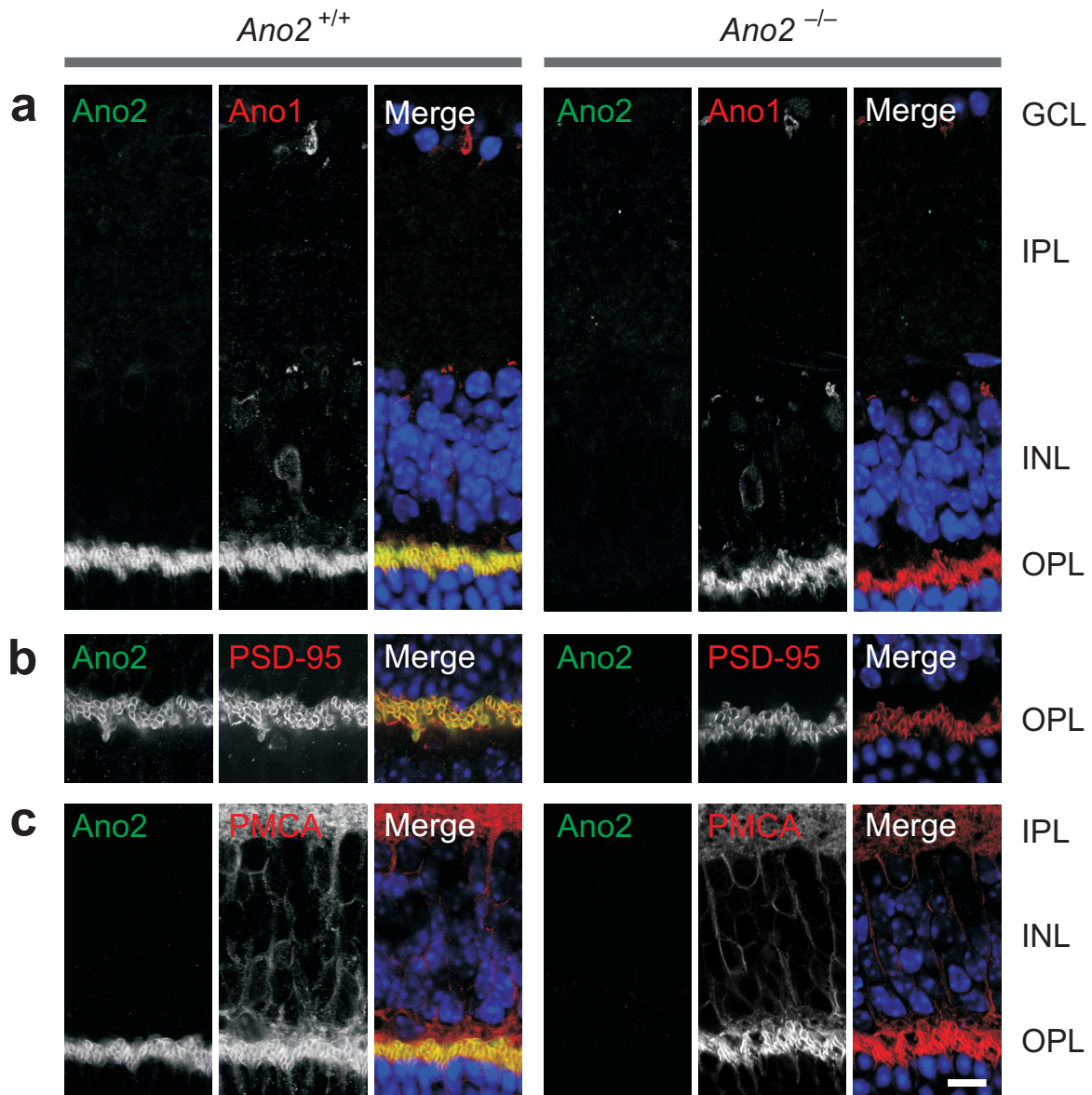
Supplementary Figure 1 Generation of *Ano2*^{-/-} mice. **(a)** Targeting strategy for the inactivation of the mouse *Ano2* gene. *Top*, part of the wildtype *Ano2* allele that encompasses exons 11 through 13. Only sequence from the targeting region indicated below was used to construct the targeting vector. Sequence used as probe for Southern analysis of *EcoRV* 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 *EcoRV* fragments for the different alleles when using the external probe for detection. **(b)** Southern blot analysis using *EcoRV*-digested DNA from tail biopsies of *Ano2*^{+/+}, *Ano2*^{+/-} and *Ano2*^{-/-} mice confirms successful disruption of *Ano2*.

Supplementary Figure 2



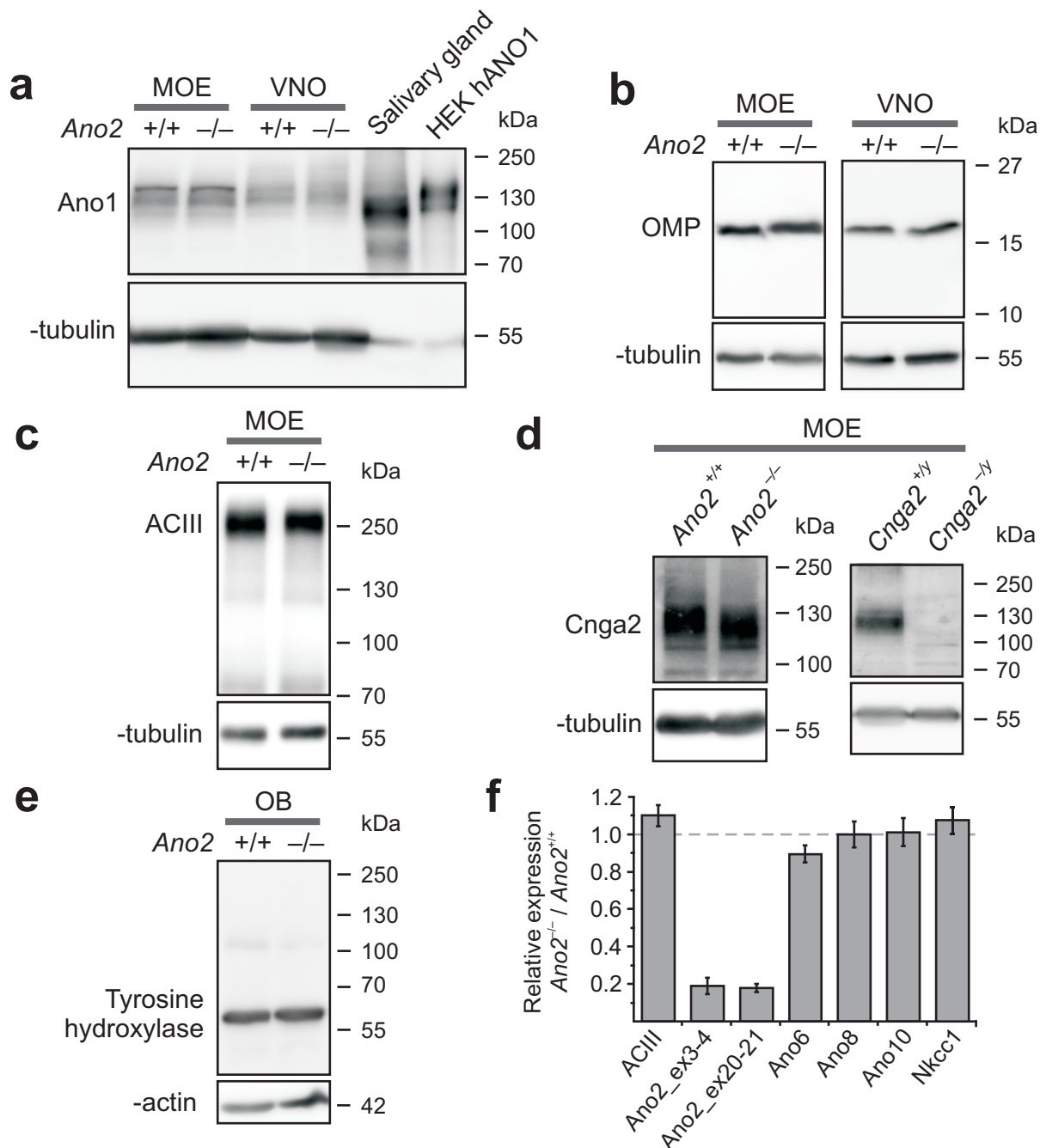
Supplementary Figure 2 Glycosylation of Ano2. Ano2 protein runs at higher apparent molecular weights in olfactory tissues than in eye but yields a uniform triplet running at ~110 to ~120 kDa after deglycosylation with N-Glycosidase F. Different bands of this triplet may represent splice variants.

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 μm. IPL, inner plexiform layer. Note that the specificity of the *Ano1* antibody (ab53212, Abcam) has been demonstrated in mouse colon using *Ano1*^{-/-} tissue as control⁴⁶. However, no such control is currently available for the retina.

Supplementary Figure 4



Supplementary Figure 4 *Ano2* disruption and expression of other genes. (a–e) Immunoblots demonstrating unchanged expression of An1 and key olfactory signal transduction elements in *Ano2*^{-/-} mice. (a) An1 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. An1 protein levels are unchanged in *Ano2*^{-/-} mice. Amount of protein loaded: 100 μ g (MOE and VNO), 32 μ 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*^{-/-} 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.