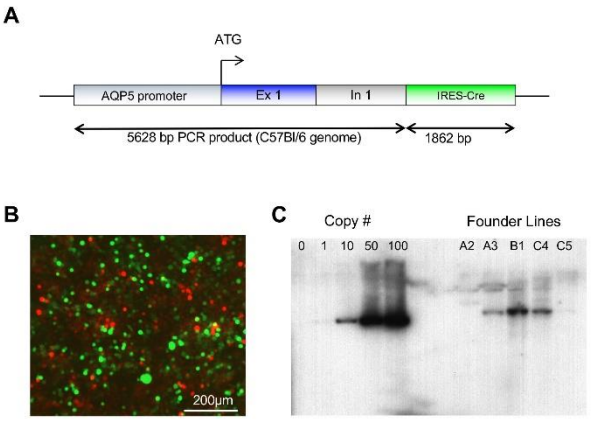
Limaye et al: Supplemental Material

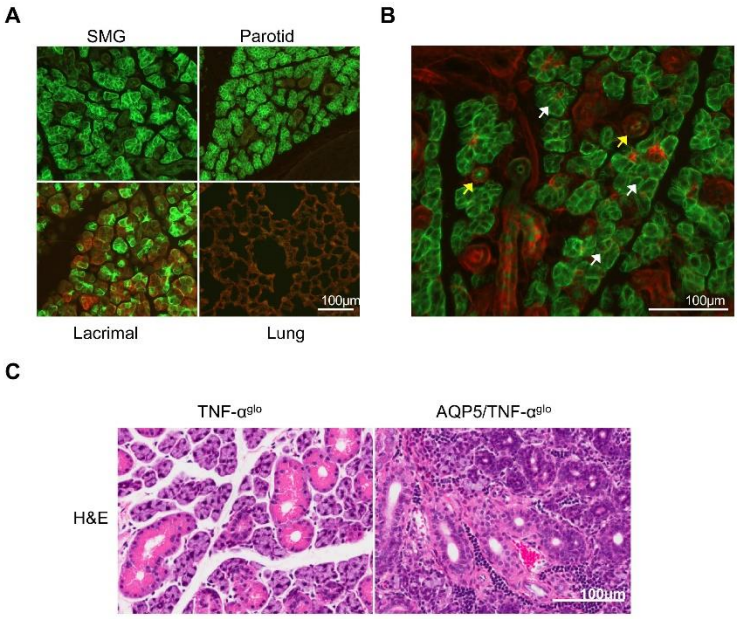
Methods

Generation of the Aquaporin 5-Cre (AQP5-Cre) and AQP5-Cre/TNF-α^{glo} mice

The AQP5 promoter was PCR amplified from an AQP5 genomic BAC clone (Children's Hospital Oakland Research Institute, Oakland, CA) and then subcloned with IRES-BGH-polyA (details listed in Appendix Figure 1a). After first testing recombinase activity *in vitro* using an A5 rat submandibular gland cell line (He et al. 1989) (see Appendix Figure 1b), the transgenic construct was then microinjected into C57BL/6N mouse zygotes. The transgenic founders were initially identified by Southern blot, but subsequently genotyped using PCR with the Cre primers listed in Chen et al. 2014. Founder line C4 was then crossed with mT/mG reporter mice (Jackson Laboratory, stock #007676; Muzumdar et al. 2007) in order to examine the AQP5-Cre expression pattern with Cre-dependent green fluorescence (as detailed in Appendix Figure 2). After determination that Cre recombination was primarily within salivary and lacrimal glands, AQP5-Cre mice were then bred to TNF-α^{glo} mice (AQP5-Cre/ TNF-α^{glo}) (Bradley et al. 2008; see also in main article: Rozas et al. 2016; Hall et al. 2016) for targeted overexpression of TNF-α . All animal studies were approved by the Animal Care and Use Committee of the National Institute of Dental and Craniofacial Research, NIH.



Appendix Figure 1. Development of the AQP5-Cre mice. A) A schematic of the transgene comprised of the AQP5 promoter. A 5.6 kb PCR product was amplified from an AQP5 genomic BAC clone spanning ~4 kb upstream of the ATG start site until the end of the first intron. The AQP5 promoter was then subcloned with IRES-Cre-BGH-polyA (Gift from Dr. Lars von Buchholtz, NIDCR). B) To test for recombinase activity, the transgenic AQP5-Cre vector along with an equal amount of a Cre reporter plasmid (Cre stoplight, Yang et al. 2001 #45150 Addgene Cambridge, MA, USA) was co-transfected into the A5 rat submandibular gland cell line (He et al., 1989) using Lipofectamine 2000 (Thermo Fisher Scientific, MA, USA). Prominent green fluorescence suggested that the AQP5-Cre transgene was expressed in salivary gland cells. C) Southern blot analysis of AQP5- Cre founder lines. Genomic mouse tail DNA was digested with Hind III, while serial dilutions of the control transgene were used to determine copy number. Founder C4 (~5 transgene copies) was used for all further studies.



Appendix Figure 2. AQP5-Cre expression pattern in selected tissues from reporter mice, and inflammation in male AQP5-Cre/ TNF-α^{glo} mouse submandibular glands (H&E staining). A) AQP5-Cre mice were bred with the mT/mG reporter line (Muzumdar et al. 2007) to examine the Cre expression pattern. AQP5-Cre and mT/mG double positive were euthanized at 4 weeks of age and tissues were collected, fixed for one hour in 4% paraformaldehyde (157-8; Electron Microscopy Sciences, Hatfield, PA, USA), then kept in a 30% sucrose solution overnight at 4°C. Tissues were then embedded in OCT (4583; Electron Microscopy Sciences), cryosections were cut at 12 µm, and fluorescence was imaged using an EVOS microscope (Thermo Fisher Scientific). Strong green fluorescence, indicative of Cre-mediated recombination, was detected in the submandibular (SMG), parotid, and lacrimal glands while the red fluorescence, as seen in the lungs, indicates no recombination. B) A higher magnification depicts sites of the Cre recombination within the SMG, which contains both serous and mucous cells. Recombination is predominantly restricted to the acinar cell (white arrows), but some recombination may be seen in the intercalated ducts (yellow arrows). C) Hematoxylin and Eosin (H&E) staining of the SMG from male mice (~5 weeks of age). There were no discernable differences in the degree of inflammation between ♂ (shown) and ♀ mice (see Figure 3 in main article).

Supplemental Material References:

Bradley SV, Hyun TS, Oravecz-Wilson KI, Li L, Waldorff EI, Ermilov AN, Goldstein SA, Zhang CX, Drubn DG, Varela K. 2007. Degenerative phenotypes caused by the combined deficiency of murine HIP1 and HIP1r are rescued by human HIP1. Hum Mol Genet. 16(11): 1279-1292.

Chen PY, Qin L, Zhuang ZW, Tellides G, Lax I, Schlessinger J, Simons M. 2014. The docking protein FRS2α is a critical regulator of VEGF receptors signaling. Proc Natl Acad Sci U S A. 111(15):5514-5519.