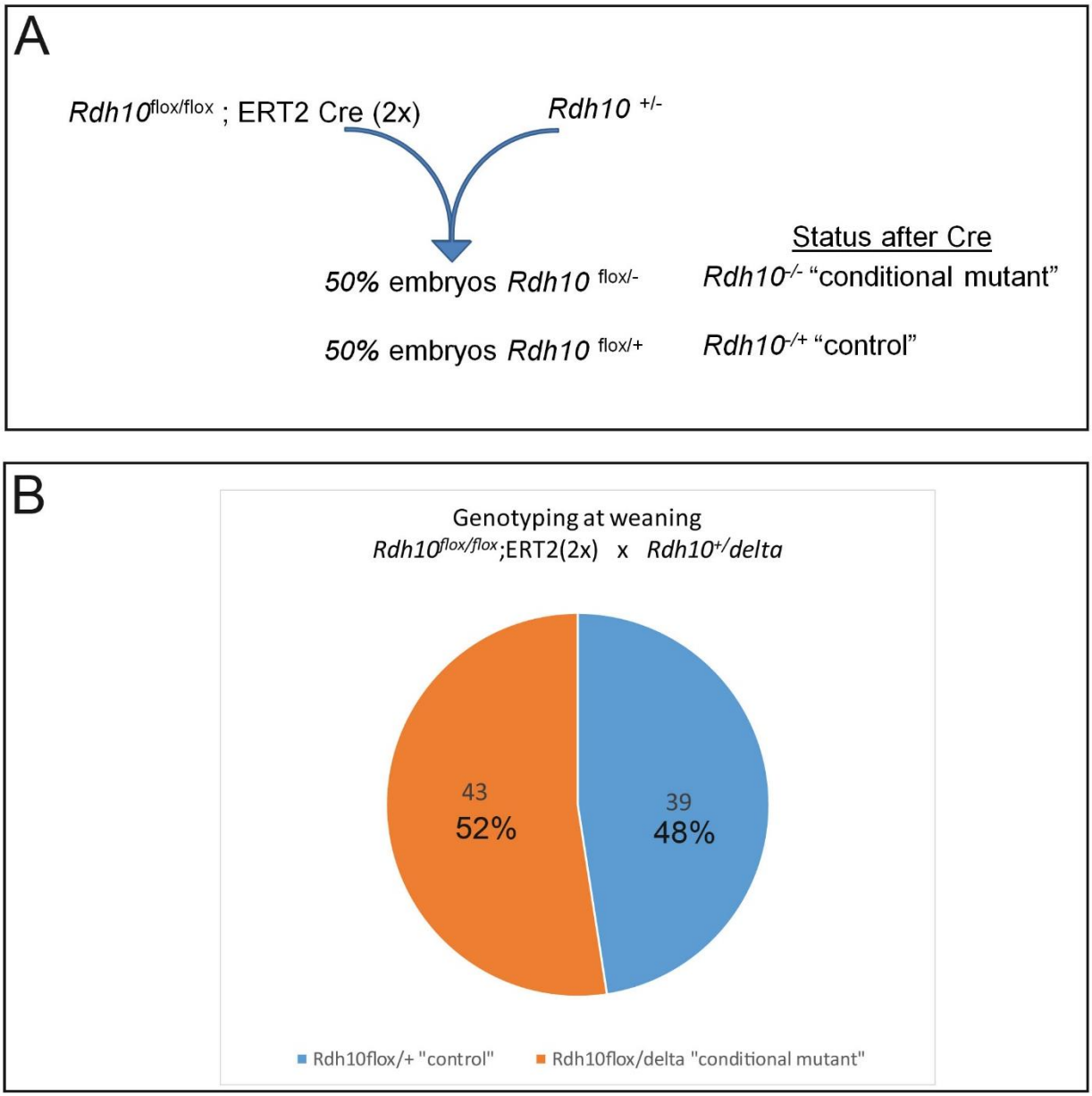
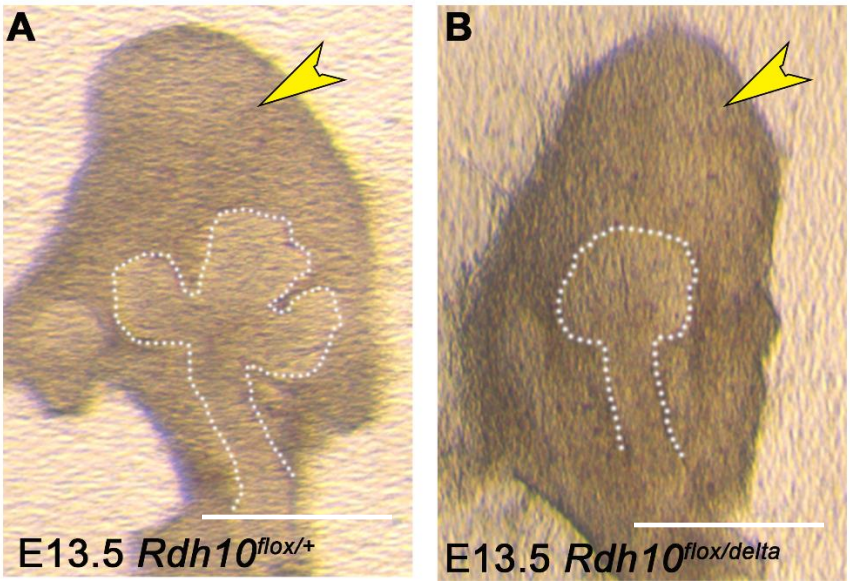
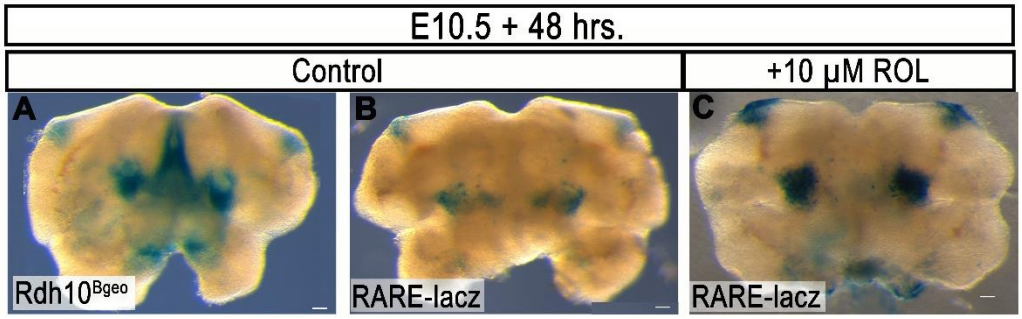


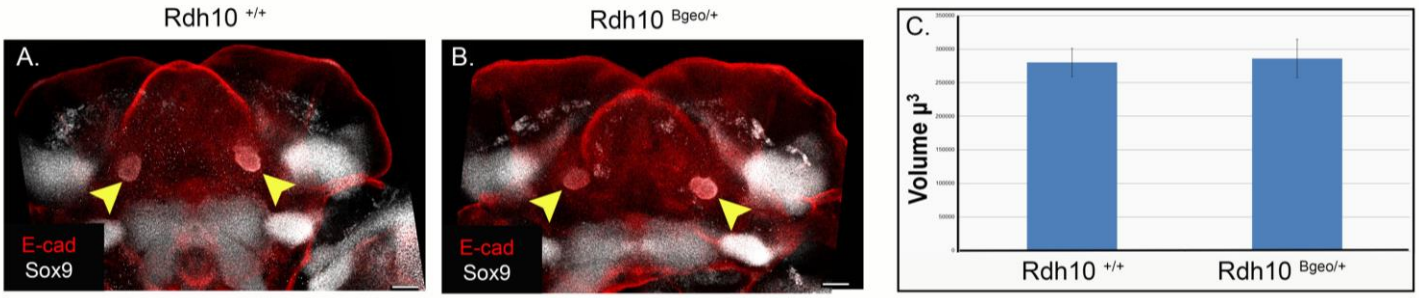
Supplemental Figure 1: Crossing *Rdh10* $^{flox/flox}$; ERT2*2 X *Rdh10* $^{+/delta}$ yields conditional mutant embryos and heterozygous controls in expected 50:50 ratio. (A) Strategy for genetic cross to produce conditional mutant embryos and heterozygous control littermates. When *Rdh10* $^{flox/flox}$; ERT2*2 mice are crossed with *Rdh10* $^{+/delta}$ 50% the resulting pups are *Rdh10* $^{flox/+}$ and 50% are *Rdh10* $^{flox/delta}$. All embryos carry a single copy of ERT2-Cre recombinase. (B) A survey of 10 litters from this cross which were genotyped at weaning. Out of a total of 82 pups, 39 (48%) were *Rdh10*$^{+/flox}$, and 43 (52%) were *Rdh10*$^{delta/flox}$.



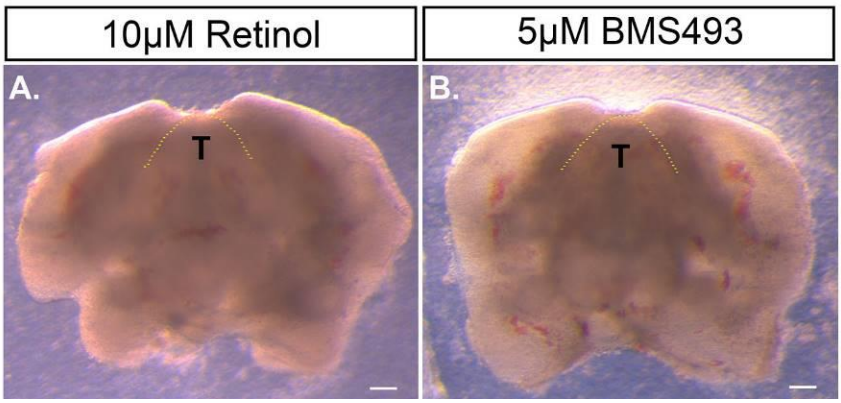
Supplemental Figure 2: SMG mesenchyme condenses in *Rdh10* conditional mutants. Bright field images of representative E13.5 SMG from control (A) (n=16), and *Rdh10* conditional mutant embryos (B) (n=21). Dotted line outlines epithelium. Yellow arrow indicates condensed mesenchyme. scale bar = 100µm.



Supplemental Figure 3: *Rdh10* expression and signaling maintained in *ex vivo* mandible culture. (*A*) Whole mandible from *Rdh10*$^{Bgeo/+}$ mutant embryo. Mandible was dissected out at E10.5, cultured for 48 hours, and stained with X-gal. (*B*) Whole mandible from *RARE-lacZ* mutant embryo. Mandible was dissected out at E10.5, cultured for 48 hours, and stained with X-gal. (*C*) Mandible culture from RARE-lacZ mutant embryo, treated with 10µM retinol, whole mount stained with X-gal. scale bar = 100µm.



Supplemental Figure 4: Heterozygosity for *Rdh10* does not impair SMG initiation. E10.5 mandibles from *Rdh10*$^{+/+}$ and *Rdh10*$^{Bgeo/+}$ were cultured for 48 hours, and then whole mount stained for E-cadherin and SOX9. Images shown are merged max projection of confocal stacks (*A, B*). Volume was calculated for each gland in each condition. Bar graph shows average and standard error bars for each group (n = 6 glands each genotype). scale bar = 100µm.



Supplemental Figure 5: Overall development of cultured mandible explants is normal when treated with retinol or BMS493. Bright field images of cultured mandibles treated with either 10μM retinol or 5μM BMS493 are shown (*A, B*). t, tongue.

Table 1. Mouse strains used in this study

Short name	Official name	Background in this study	Ref.
Rdh10$^{Bgeo/+}$		C57BL/6	(Sandell et al., 2012)
Rdh10$^{flox/flox}$		Mixed (primarily FVB/NJ)	(Sandell et al., 2012)
Rdh10$^{delta/+}$		Mixed (primarily FVB/NJ)	(Sandell et al., 2012)
Cre-ERT2	*Gt(ROSA)26Sor*$^{tm1(cre/ERT2)Tyj}$	Mixed	(Ventura et al., 2007)
RARE-lacZ	Tg(RARE-Hspa1b/lacZ)12Jrt	Mixed	(Rossant et al., 1991)

Table 2. Primers used for qPCR quantification of gene expression

Fgf10	F: 5’ TTTGGTGTCTTCGTTCCCTGT 3’ R: 5’ TAGCTCCGCACATGCCTTC 3’
Lacz	F: 5’ TTATCAGCCGGAAAACCTACC 3’ R: 5’ CTCGCCACTTCAACATCAAC 3’
Gapdh	F: 5’ ACAGTCCATGCCATCACTGCC 3’ R: 5’ GCCTGCTTCACCACCTTCTTG 3’
Actb	F: 5’ GGCTGTATTCCCCTCCATCG 3’ R: 5’ CCAGTTGGTAACAATGCCATGT 3’