Uribe et al., http://www.jcb.org/cgi/content/full/jcb.201503071

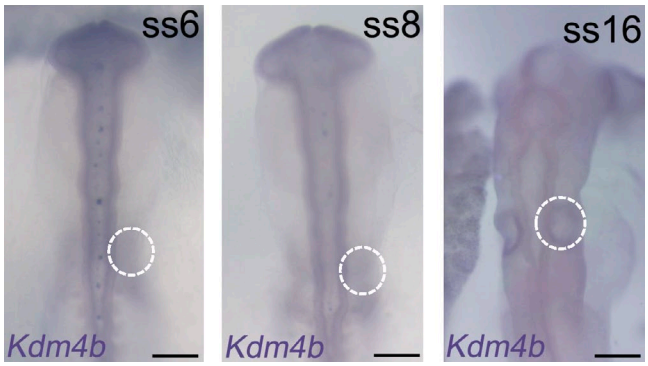

Figure S1. ***Kdm4b* is expressed in the presumptive otic ectoderm.** We assessed the presence of *Kdm4b* transcripts at early stages of otic development by ISH. ss6/st9^{-} (left) and ss8/9^{+} (middle) show presence of *Kdm4b* in the presumptive otic ectoderm (dashed circles). By ss16/st12 (right), *Kdm4b* is evidenced at the border of the invaginating otic vesicle. Bars, 200 µm.

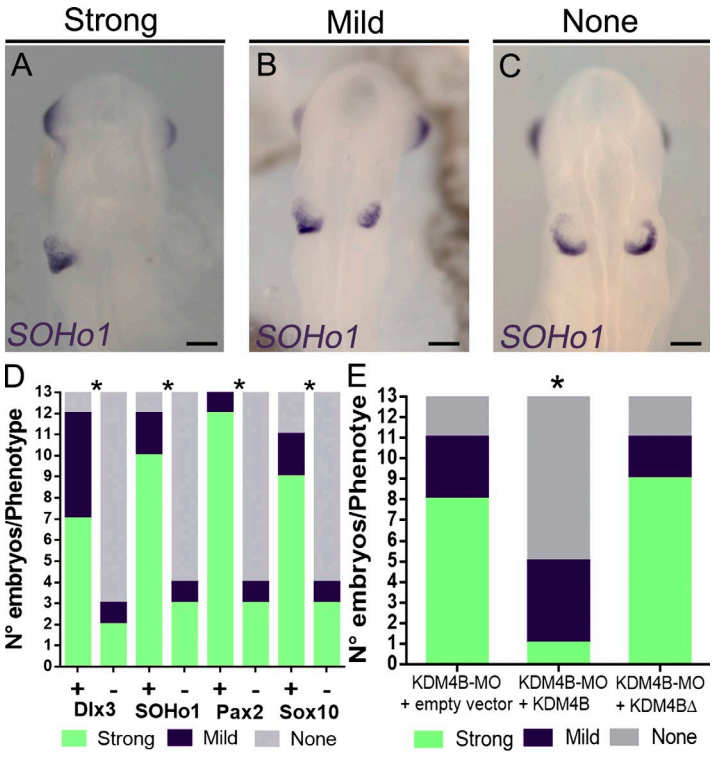

Figure S2. **Strong and mild phenotype caused by KDM4B-MO electroporation.** (A–C) Embryos showing strong (A), mild (B), and no (C) phenotype after KDM4B knockdown when injecting KDM4B-MO on the right side of the embryo. The severity of the phenotype is indicated by *SOHo1* expression, which is lost in the severe phenotypes, and diminishes when the otic domain is reduced. (D) Quantification of the numbers of KDM4B-MO (+)–treated embryos with strong, mild, or nonphenotypes compared with control-MO (–)–treated embryos indicate that the KDM4B-MO treatment induces reduction of the otic domain for all inner ear markers tested. *, $P < 0.0001$ by contingency table followed a χ^2 test. (E) Quantitation of the percentage of KDM4B knockdown (KDM4B-MO plus empty vector), and rescued with the full-length (KDM4B-MO plus KDM4B) or catalytically dead mutant (KDM4B-MO plus KDM4BΔ) embryos with either strong, mild, or nonreduction of the otic domain indicated by the *SOHo1* marker. *, $P < 0.01$; significant differences are compared with the control by contingency table followed a χ^2 test. Bars, 200 µm.

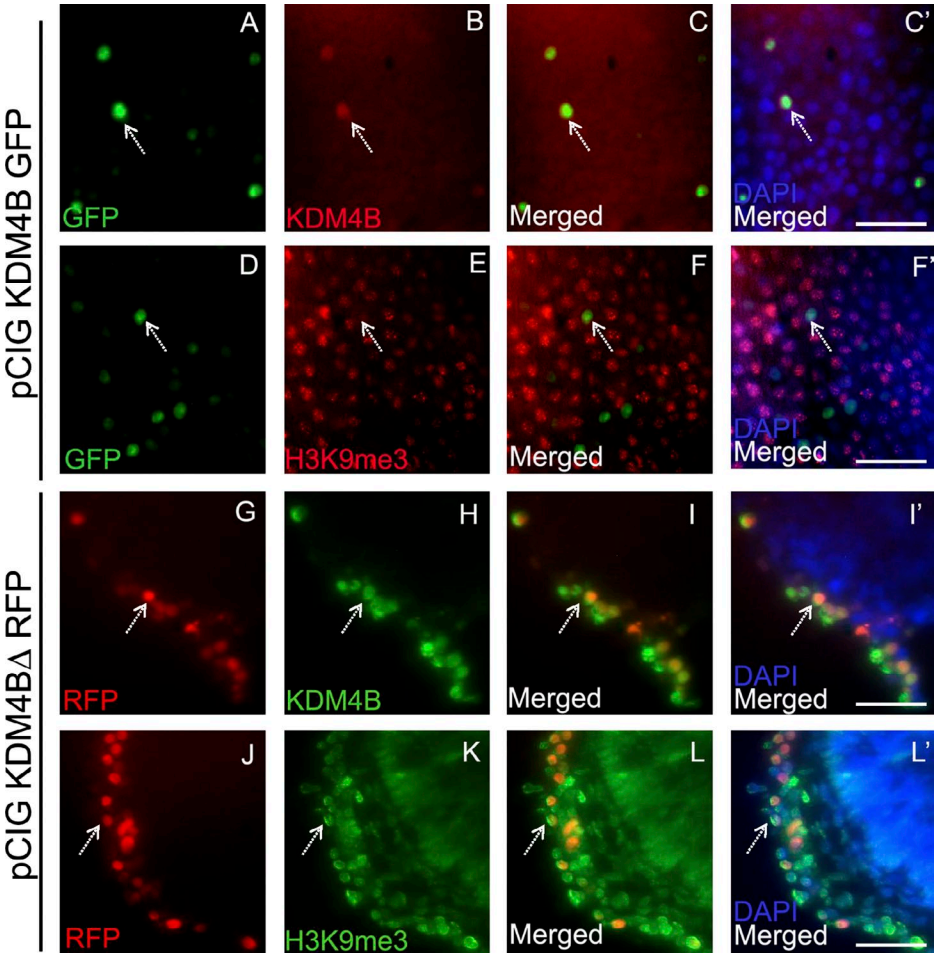

Figure S3. **Characterization of KDM4B and KDM4BΔ overexpressing vectors.** Electroporation of overexpressing vector containing KDM4B (A–C) and KDM4BΔ (G–I) exhibit normal protein expression on the nucleus as indicated by immunohistochemistry using an anti-KDM4B antibody. Overexpression of KDM4B (D–F) is capable of erasing the H3K9me3 mark on electroporated cells (arrows). In contrast, the catalytically dead mutant KDM4BΔ (J–L) fails to alter H3K9me3 abundance on electroporated cells (arrows). Bars, 200 µm.

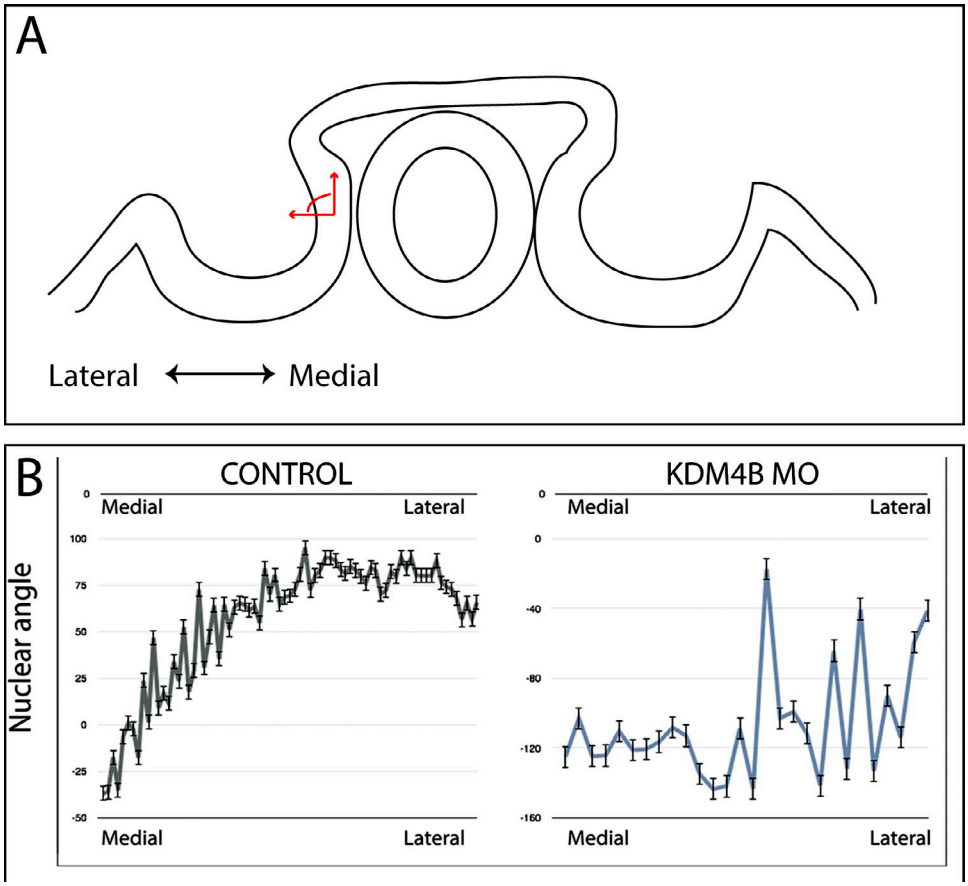

Figure S4. **Depletion of KDM4B affects proper nuclear orientation in the invaginating otic placode.** Nuclei angle measurements were along the mediolateral axis of the otic vesicle at stage 13, as depicted in the schematic in A. Graphical representation of measurements reveal a disrupted nuclear orientation of the otic ectoderm cells after introduction of KDM4B-MO compared with the control side (B). Error bars indicate ±SEM.

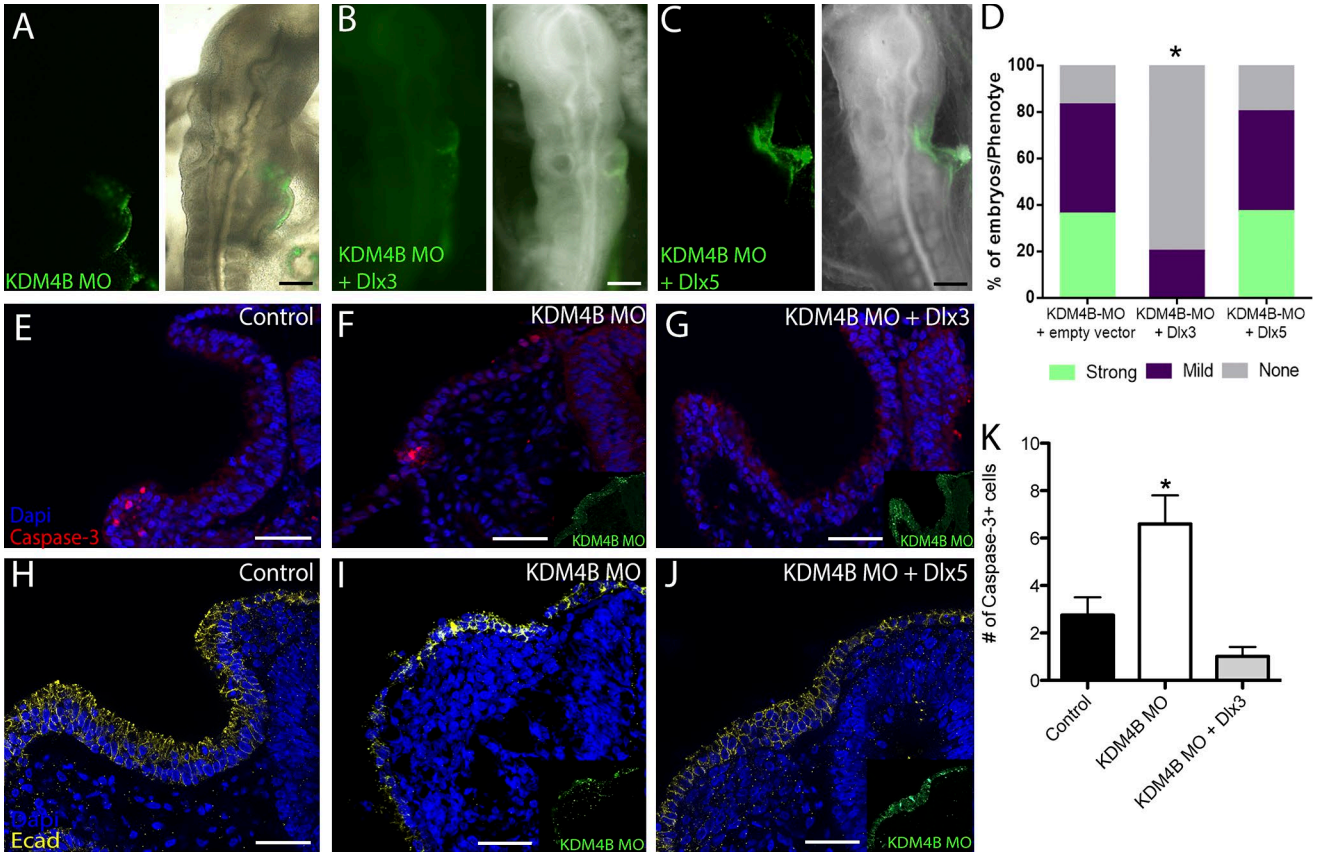

Figure S5. **Coelectroporation of KDM4B-MO with pCIG-Dlx3 was sufficient to rescue cell death and otic vesicle size, whereas coelectroporation of KDM4B-MO with pCIG-Dlx5 was not enough to rescue the phenotype.** (A–C) To assess the rescue of KDM4B-MO–treated embryos with *Dlx3* or the orthologous *Dlx5* gene, we analyzed the severity of the phenotypes upon coelectroporation: KDM4B-MO plus empty vector (A), KDM4B-MO plus *Dlx3* (B), or KDM4B-MO plus *Dlx5* targeted to the otic ectoderm (green; C). (D) Our results show 80% of rescued otic vesicles when electroporating KDM4B-MO plus pCIG-*Dlx3*, but not with *Dlx5*. Data shown are the combined counts from three biological replicates. (E–G and K) Embryos electroporated with either KDM4B-MO plus empty vector or KDM4B-MO plus pCIG-*Dlx3* were analyzed by Caspase3 immunostaining, showing a rescue in cell death. Error bars in K indicate ±SEM. (D and H–J) To provide specificity for the rescue experiments, we coelectroporated KDM4B-MO with pCIG-*Dlx5*. Our data show that *Dlx5* was not sufficient to rescue invagination defects as indicated by E-cad distribution. *, $P < 0.05$ with Student's *t* test. Bars: (A–C) 180 µm; (E–J) 50 µm.

Table S1. **List of primers used for qPCR**

Target gene	Sequence
qPCR primers	
Dlx3 F	5′-GGCTCTTCCTTCACCGACAC-3′
Dlx3 R	5′-GCACCTCGCCGTTCTTGTAG-3′
Pax2 F	5′-GGTGCGACCCTGTGACATTTC-3′
Pax2 R	5′-TCTCCCAAGCGAACATGGTG-3′
Soho1 F	5′-GCCTTCAGCATCGACAGCATC-3′
Soho1 R	5′-TCCGTGGAGAGCGGTGAAAC-3′
Sox10 F	5′-GCAGCATGGAGTCTCCTTGT-3′
Sox10 R	5′-ACTGAGGCCTGGAGATGGAT-3′
GAPDH F	5′-AAAGTCGGAGTCAACGGATTT-3′
GAPDH R	5′-TTGATCACAAGTTTCCCGTTC-3′
ChIP-qPCR primers	
Dlx3 +1kb F	5′-AATCCCAATGAGCCGTCATA-3′
Dlx3 +1kb F	5′-CCCTACGACGATCCCTACAA-3′
Dlx3 −0.5kb F	5′-CAGTCCCAAATTGGTTCAGC-3′
Dlx3 −0.5kb R	5′-GTTCCCAAGGAGCTGAGGA-3′
Pax2 +1.5kb F	5′-AAAGTAGCGACCCCCAAAGT-3′
Pax2 +1.5kb R	5′-CGCCCTTACCTGTTTATGGA-3′
Pax2 −0.5kb F	5′-CCTTCATTTCTTCCCATCTCC-3′
Pax2 −0.5kb R	5′-CCCCAGAAAGACACCGTTAG-3′
Soho1 +1kb F	5′-GACGCTGTGTTTTGCCATT-3′
Soho1 +1kb R	5′-CCTGGCTCTTGGAGAAGATG-3′
Soho1 −0.5kb F	5′-CCAAACCCTCTTCTTCCACA-3′
Soho1 −0.5kb R	5′-GTGCCCTGCTCAGTACCTTC-3′
KDM4BΔ primers	
A KDM4B F	5′-AAAATCGATTCCTGGAAATATGGGGTC-3′
B KDM4Bmut R	5′-CTGTGTAGCCCAAGCAGCCGTTGTCTT-3′
C KDM4Bmut F	5′-GCTGCTTGGGCTACACAGGACATGGATCTC-3′
D KDM4B R	5′-AAACCCGGGTGGTTAAACATGTTGCT-3′