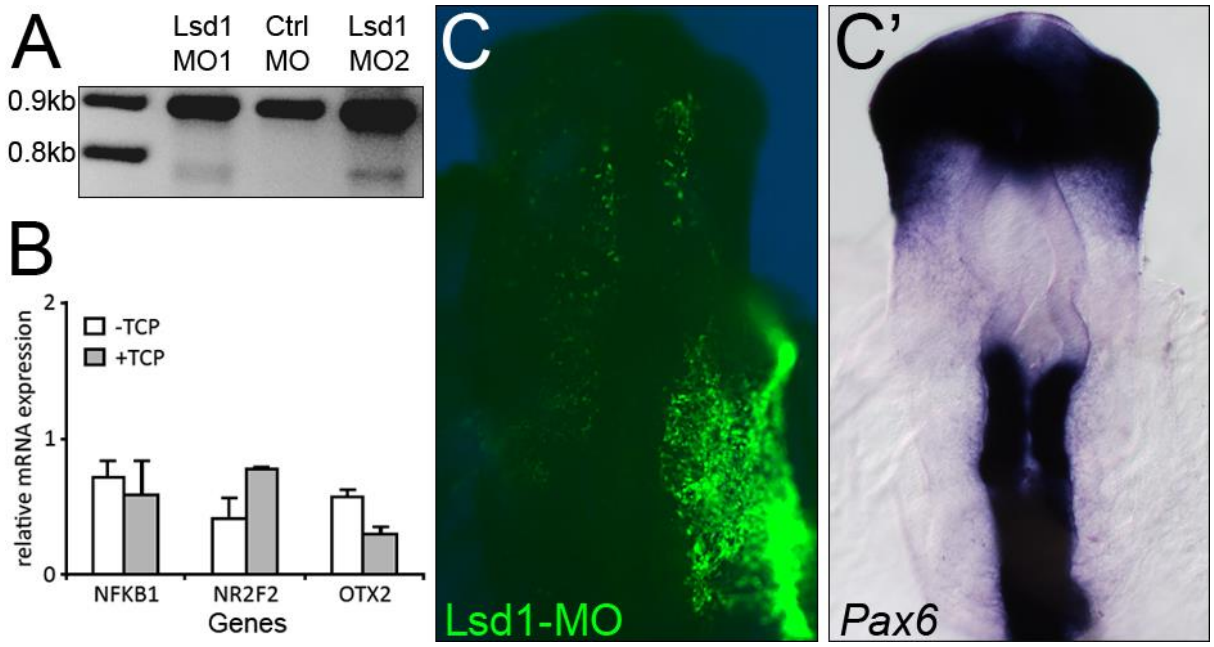

Fig. S1. Lsd1 splice morpholinos and validation of non-otic genes. (A) RT-PCR. Lsd1-MO1 deletes exon 8 and generates an amplicon 95bp smaller than the wild type (top band). Lsd1-MO2 deletes exon 11 and generates an amplicon 91bp smaller than the wild type. (B) Validation of selected upregulated genes by qPCR following pharmacological inhibition of Lsd1 with TCP, normalised to *Sdha*. There is no significant difference between drug treated vs untreated samples. (C) Electroporation of Lsd1-MO (green) and (C') ISH for *Pax6*, an upregulated gene. Note that upregulation of *Pax6* is not observed (n=5).

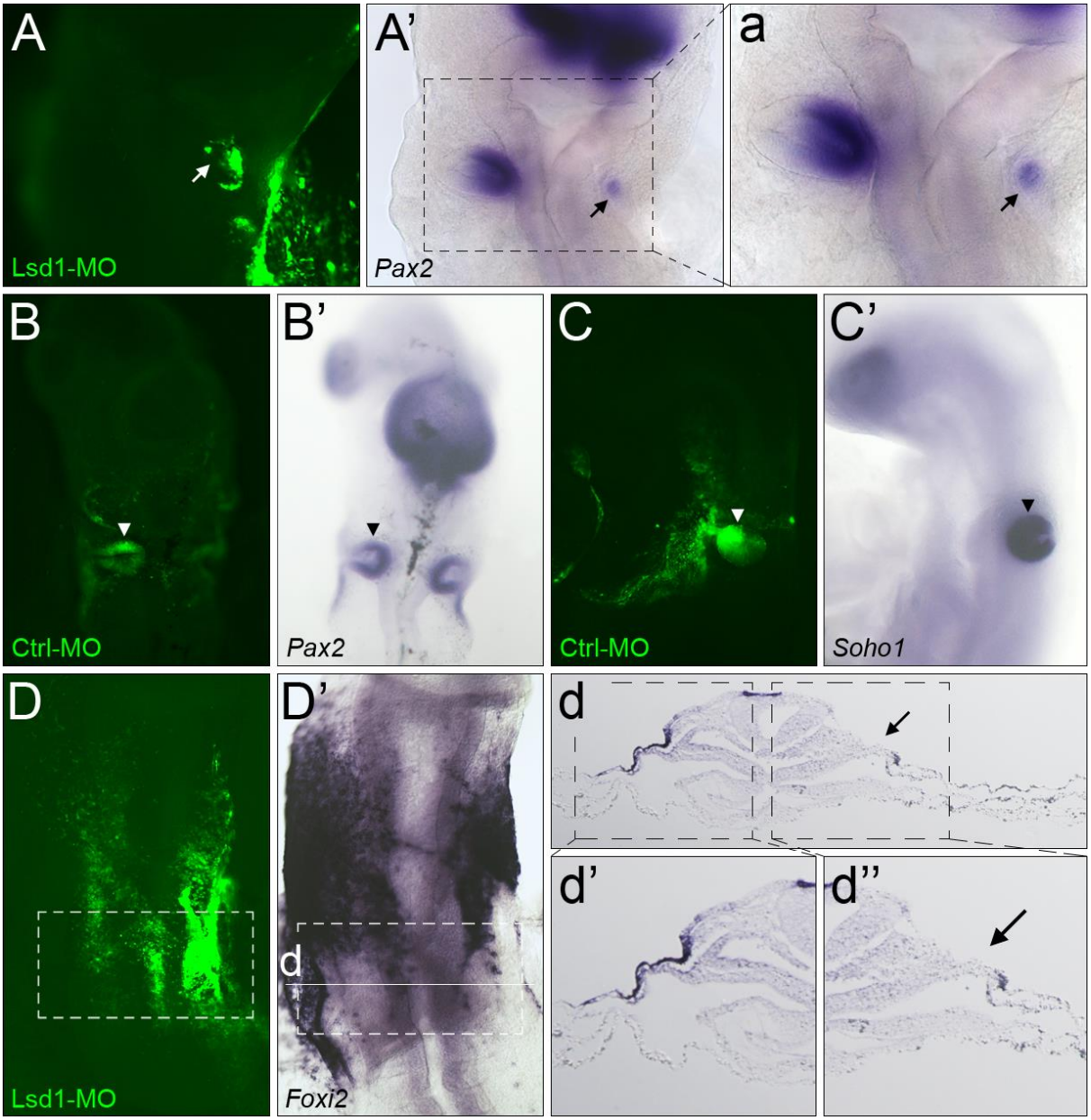

Fig. S2. Lsd1-MO knockdown prevents otic vesicle formation. (A-a) Otic cells electroporated with Lsd1-MO (green) do not express *Pax2* and are unable to form an otic vesicle compared to cells that did not receive the Lsd1-MO (arrow) which continue to express *Pax2*. (B-C') Electroporation of control (Ctrl) –MO does not affect marker gene expression or otic vesicle formation (arrowhead). (D-d") Electroporation of Lsd1-MO in epibranchial regions leads to a reduction in *Foxi2* expression. Arrow indicates electroporated side. (d', d") zoomed images of area inside dashed boxes in panel d.

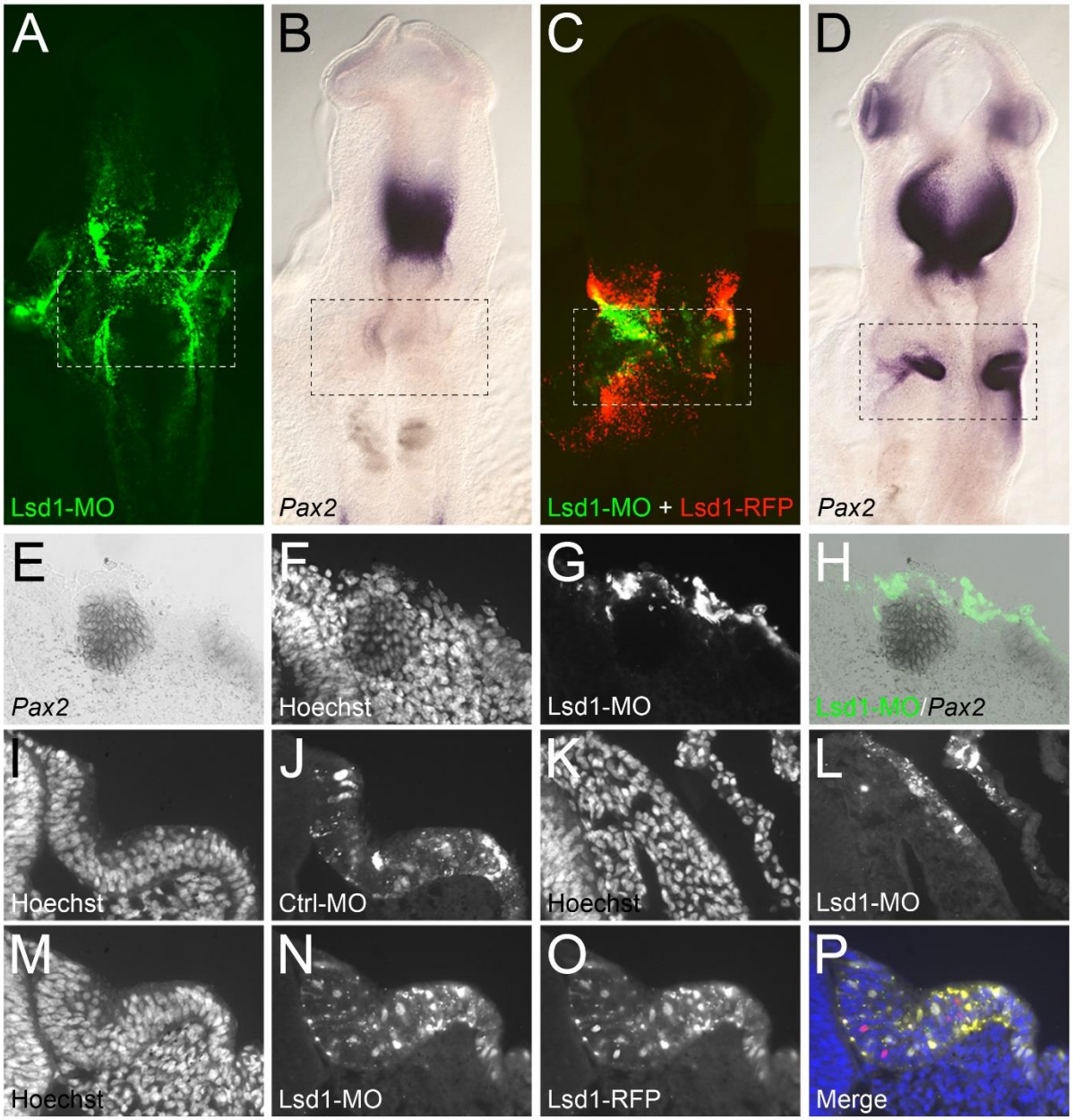

Fig. S3. Lsd1 expression rescues otic placode formation after Lsd1-MO knockdown. (A, B) Lsd1-MO causes loss of placode formation and *Pax2* expression. (C-D) Co-electroporation of Lsd1-MO with full length human Lsd1-RFP rescues *Pax2* expression and subsequent otic cup formation. (E-H) Lsd1-MO+ cells remain in the epithelium and do not contribute to vesicle formation unlike non-targeted, *Pax2+* cells. (I, J) Ctrl-MO do not affect thickening of the otic epithelium or its invagination into an otic cup. (K, L) Lsd1-MO prevents both the thickening and invagination of the epithelium, while co-electroporation of Lsd1-RFP together with Lsd1-MO restores normal morphology (M-P). Hoechst = nuclei.

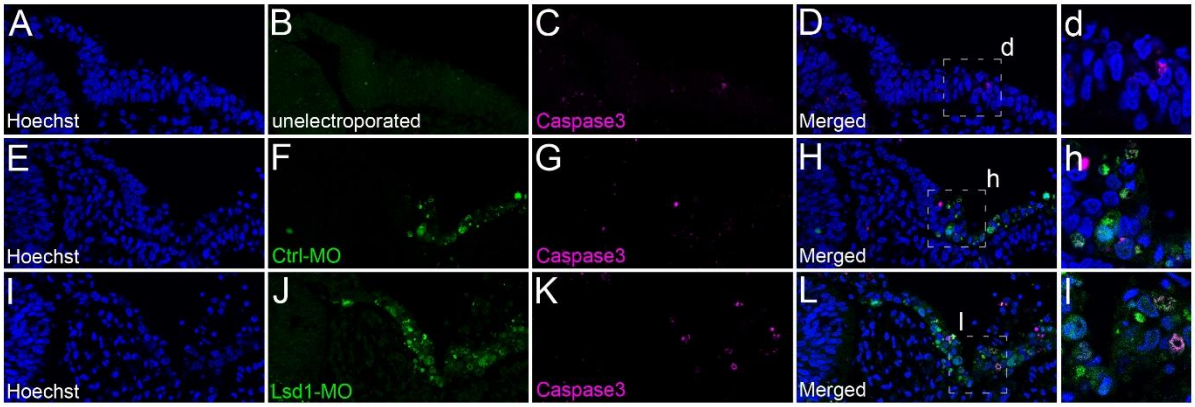

Fig. S4. Lsd1-MO knockdown does not lead to increased apoptosis. (A-d) Unelectroporated placode showing some cleaved caspase 3^{+} cells. (E-h) Ctrl-MO and (I-l) Lsd1-MO show similar levels of apoptotic cells.

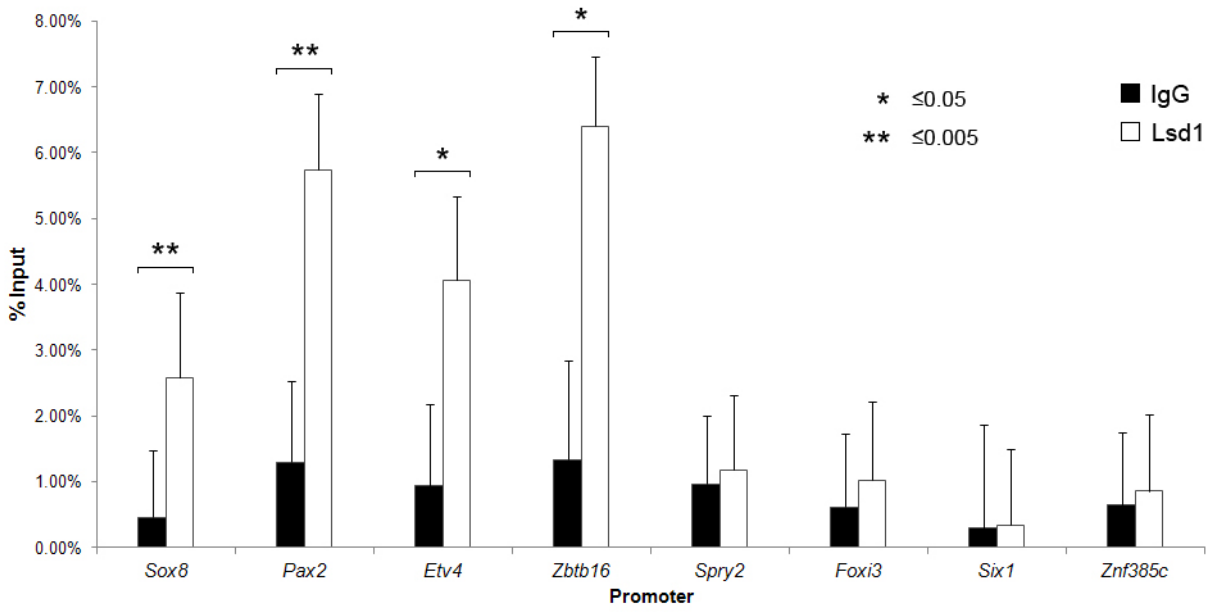

Fig. S5. Lsd1 binding to target otic gene promoters. Lsd1 ChIP-qPCR for OEP and PPR genes shown in Fig4A is represented here as % input. Lsd1 only occupies the promoters of OEP genes.

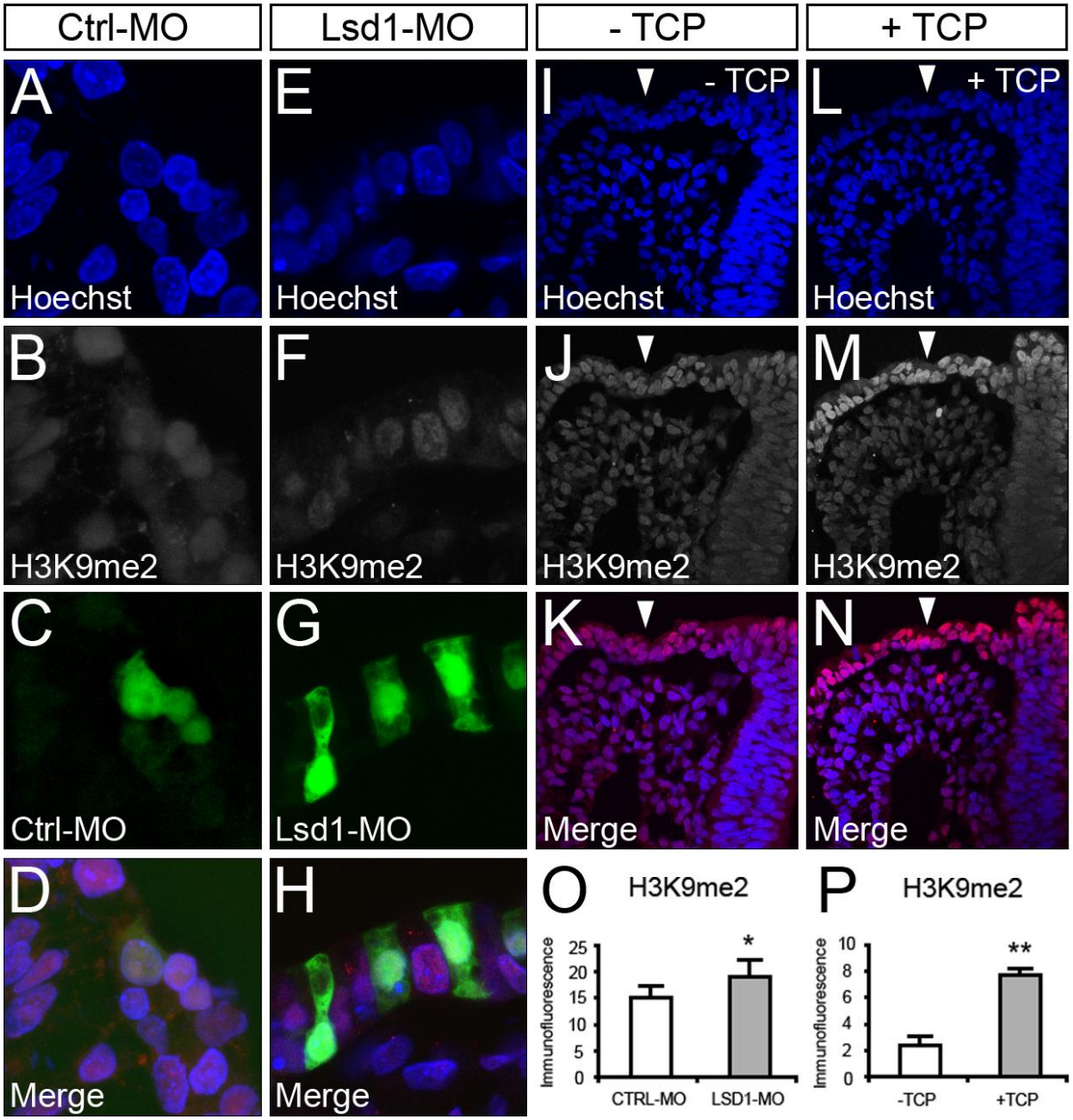

Fig. S6. Lsd1 inhibition increases H3K9me2 in the otic placode. (A-D) Ctrl-MO and (E-H) Lsd1-MO electroporation into the otic region. Nuclei are visualised with Hoechst (blue), H3K9me2 immunofluorescence in grey and MO targeted cells in green. (D, H) Merged images. (I-K) Control (-TCP) and (L-N) TCP treated otic explants showing Hoechst stained nuclei (blue) and H3K9me2 (grey). (K, N) Merged images. Arrowheads point to the epithelium. (O, P) ImageJ was used to measure the intensity of H3K9me2 fluorescence (B, F, J, M) in cells targeted with MO (green) and their neighbours (O) and in TCP treated and untreated epithelium (P). Note that in both morpholino and drug mediated inhibition of Lsd1, there is a significant increase in H3K9me2 levels (p-values: * ≤0.05; ** ≤0.01).

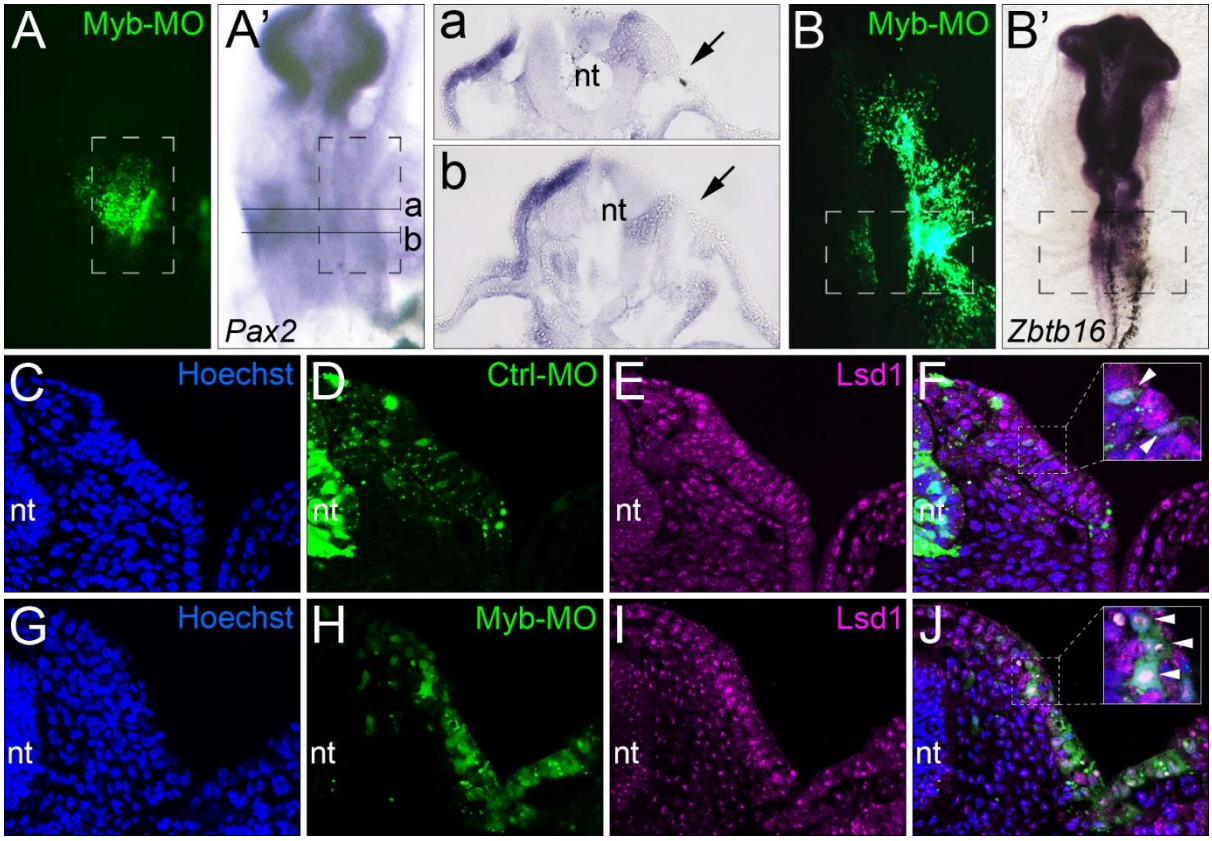

Fig. S7. Myb-MO knockdown leads to loss of otic placode markers but does not affect Lsd1 expression. (A-a) *Pax2* and (B, B') *Zbtb16* are downregulated after Myb-MO knockdown. (C-F) Ctrl-MO and (G-J) Myb-MO electroporation followed by Lsd1 antibody staining. Lsd1 is broadly expressed with slightly stronger expression in the ectoderm and placode. Arrowheads within inset depicting zoom of dashed box area indicate targeted cells. Note that Lsd1 expression persists in the nuclei of Myb-MO cells.

Supplementary Tables

Table S1. NanoString Probeset

Click here to Download Table S/

Table S2. Differential gene expression by NanoString analysis following Lsd1-MO knockdown

Click here to Download Table S0

Table S3. Motif enrichment analysis

Click here to Download Table S3