

TEX15 safeguards neuronal diversity in the mouse olfactory system

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Keywords

Abstract:

Animals detect diverse chemicals using hundreds of chemoreceptor proteins. In many organisms, sensory chemoreceptors obey a one-receptor-type-per-cell rule, which organizes information gathered by sensory neurons¹. A key question remains how receptor expression gets diversified so that hundreds of chemoreceptors get expressed across the population of sensory neurons²⁻⁴. Here we show that diverse odorant receptor (OR) expression in mice depends on *testis expressed gene 15* (*Tex15*). We find that *Tex15* regulates OR expression in neuronal precursor cells. In *Tex15* deficient (*Tex15*^{-/-}) mice, OR transcript levels are increased in precursors, a handful of early-transcribed OR genes get expressed at abnormally high levels, and these ORs come to dominate the olfactory sensory neuron population. This results in a profound reduction in the diversity of OR gene choice as well as disrupted spatial patterning of the olfactory epithelium, without disrupting the one-receptor-type-per-cell rule. *Tex15* encodes a transcriptional repressor⁵, and we show that *Tex15*^{-/-} mice have reduced heterochromatin-associated H3K9me3 on OR genes. Thus, we propose that *Tex15* directs a heterochromatin-based transcriptional repression mechanism that prevents uneven transcriptional onset from skewing OR choice. This mechanism safeguards the cellular diversity that is essential for broadly tuned chemosensation.

Text

Animal sensory systems deploy large families of receptor proteins to detect and distinguish among stimuli present in the environment. Often, these receptors follow a one-receptor-type-per-cell rule, with cells that express the same receptor corresponding to distinct sensory neuron subtypes⁶. This structure is epitomized by the mouse olfactory system, in which each mature olfactory sensory neuron (mOSN) transcribes only one allele of one odorant receptor (OR) gene out of 1,141 protein-coding ORs^{7–10}. Transcription of one OR per cell, termed "OR choice", defines hundreds of distinct mOSN subtypes^{11,12}, each with a characteristic receptor field and downstream synaptic target in the olfactory bulb^{13–15}. As a result, OR choice connects odorant-to-receptor binding to the activation of specific olfactory bulb locations, spatially organizing information from hundreds of receptors for downstream processing¹. The gene regulatory mechanisms governing OR expression have been of keen interest since the genes were first identified¹⁶.

OR gene choice has two key features: singularity, the expression of one OR per cell, and diversity, the expression of the full OR gene repertoire across the OSN population. Singularity is achieved through a positive feedback loop, wherein transcription of one OR allele reinforces its own expression and suppresses transcription of all other OR genes^{17–19}. Diversity is less well understood, but existing evidence suggests an important role for the epigenetic regulation of OR gene chromatin as OSNs differentiate. In differentiating OSN precursors, OR genes become compacted into heterochromatin that is marked by histone H3 lysine 9 trimethylation (H3K9me3)^{20,21}. Lyons et al showed that deleting the H3K9 methyltransferases Ehmt1 (Glp) and Ehmt2 (G9a) reduces OR diversity in a dosage-dependent manner, with the strongest effect in double knockouts². However, the Ehmt1/Ehmt2 double-knockout is embryonic lethal and also disrupts singularity, complicating the interpretation of this phenotype. Nonetheless, the timing of H3K9me3 deposition is suggestive, coinciding with a period of low-level OR co-expression prior to singular choice²¹. Thus, epigenetic regulation of OR transcription in precursors appears critical for diverse OR expression.

To identify regulatory proteins that enable diverse OR gene choice, we examined the expression of heterochromatin associated genes in RNA-seq data from staged OSN precursors¹⁸. We focused on genes that are upregulated at the transition from globose basal cells (GBCs) to immediate neuronal precursors (INPs), which corresponds to the onset of OR transcription and to the onset of H3K9me3 deposition on OR genes²¹. In total, we identified 2,070 genes that are significantly upregulated in INPs relative to GBCs, of which 14 are associated with the gene ontology term heterochromatin formation (Extended Data Fig. 1a-b). Among these, *testis expressed gene 15* (*Tex15*), exhibits the largest increase in expression, with a 35-fold increase in transcript levels at

the INP stage. We next examined *Tex15* expression across differentiation. *Tex15* transcript levels are very low in olfactory stem cells (Horizontal Basal Cells and GBCs), high in INPs and iOSNs, and then low again in mOSNs (Fig. 1a). We confirmed this at the protein level by immunostaining postnatal day 7 (P7) MOE tissue sections from mice bearing an *Ngn1*-GFP reporter, which labels INPs and iOSNs²¹. This revealed *Tex15* immunoreactivity in the nuclei of basal GFP+ cells (INPs), but not in the more apical GFP+ cells (iOSNs) or the OMP+ cells (mOSNs) (Fig. 1b, Extended Data Fig. 1c).

Available evidence suggests that TEX15 is a transcriptional repressor. Most of the protein is intrinsically disordered²², but it has an N-terminal DUF3715 domain that is also found in TASOR1 and TASOR2, which are core components of the HUSH (Human Silencing Hub) complex. In TASOR1/TASOR2, DUF3715 represses transposable element (TE) transcription, and domain-swap experiments show this activity is retained by TEX15's DUF3715 domain⁵. Consistent with this, TEX15 loss in mice increases TE transcription in male germ cells, causing arrested spermatogenesis and infertility^{23,24}. Mutations in *Tex15* have also been linked to male infertility in humans^{25–27}. However, the function of TEX15 outside the male germ line has not been explored.

To investigate the function of TEX15 in the olfactory system, we used a previously characterized *Tex15* mutant allele (*Tex15*^{-/-}) in which most of the coding sequence is replaced by a beta galactosidase-neomycin cassette²⁸. As a first step, we evaluated the overall structure and cellular composition of the main olfactory epithelium (MOE) in *Tex15*^{-/-} mice bearing *Neurog1*-GFP. Tissue sections from *Tex15*^{-/-} mice lack TEX15 immunoreactivity in INPs, leaving only non-specific staining in more basal mesenchymal cells (Fig. 1b). Otherwise, the overall appearance of MOE sections from *Tex15*^{-/-} mice is normal, with distinct layers of *Neurog1*-GFP+ and OMP+ cells.

Loss of mOSN diversity in *Tex15*^{-/-} mice

Next we asked whether the absence of *Tex15* affects OR gene expression by mOSNs. To accomplish this, we crossed the *Tex15*^{-/-} allele together with an *Omp*-GFP reporter allele²⁹, which labels mOSNs, and purified mOSNs by FACS for RNA-seq. Comparison of *Tex15*^{-/-} mOSNs to data from *Tex15* wildtype mOSNs³⁰ revealed widespread changes in OR transcript levels but limited changes in non-OR gene expression (Fig. 1c). The vast majority of protein-coding OR genes are differentially expressed in *Tex15*^{-/-} mOSNs, with 55 (5.06%) OR genes significantly upregulated and 896 (82.35%) significantly downregulated. Many of the downregulated ORs are lost completely; 786 are no longer detected in *Tex15*^{-/-} mOSNs (<0.1 transcripts per million/TPM). OR genes fall into two families, the clustered Class I and the larger, dispersed Class II subfamily; both show a similar effect (Extended Data Fig. 1d). Strikingly, a pair of class II OR genes, *Olf231* and *Olf464*, are upregulated more than 250-fold. These transcript levels

far exceed that of any OR gene in wildtype mOSNs (Extended Data Fig. 1e). Notably, the same two ORs are also among the top expressed ORs in *Ehmt1/Ehmt2* double-knockout mice³¹, suggesting a link between *TEX15* and H3K9 methylation. We also observe downregulation of the trace amine associate receptors (TAARs), another chemoreceptor family expressed by mOSNs, (Extended Data Fig. 1f)³². However, unlike in the male germline, *TE* transcript levels remain largely unchanged, and the few significant changes observed are bidirectional rather than a broad upregulation (Extended Data Fig 1g).

We next examined whether these alterations in OR transcript levels correspond to changed abundance of mOSN subtypes. To address this, we used single-cell RNA-seq (scRNAseq) to analyze the MOEs at multiple ages – postnatal day 3 (P3), postnatal day 14-16 (P14-16), and adult (P56), capturing cells spanning mOSN differentiation (Fig. 1d). We integrated data from 6 pairs of *Tex15*^{-/-} mice and wildtype littermate controls and then isolated and re-clustered the cells in the OSN lineage (*Tex15*^{+/+}= 16,832 cells, *Tex15*^{-/-}=13,543 cells). Expression of cell stage-specific marker genes is similar in both genotypes (Extended Data Fig. 2a), allowing us to define developmental stages using the combined data set, including three substages within the INP population (INP1-3) (Fig. 1e). In our data, *Tex15* transcript levels peak in INP3s, which are greatly reduced in *Tex15*^{-/-} cells, with the remaining transcripts presumably reflecting transcription through the beta galactosidase cassette (Extended Data Fig. 2b). Notably, *Tex15*^{-/-} MOEs show a shift toward differentiation, with significantly fewer immature cells (INP3 and iOSNs) and more mOSNs (Fig. 1e, Extended Data Fig. 2c). Since OR choice regulates OSN maturation^{19,31}, this raises the possibility that cells may choose an OR gene more quickly in *Tex15*^{-/-} mice.

We next examined OR expression in *Tex15*^{-/-} mOSNs. Critically, the vast majority of *Tex15*^{-/-} mOSNs still express only one OR per cell, and OR transcript levels per cell are similar to *Tex15*^{+/+} mOSNs (Fig. 1f, Extended Data Fig. 2d). After excluding mOSNs expressing multiple ORs, we assigned each cell a subtype based on its expressed OR. In *Tex15*^{+/+} mice, we see a broad distribution of mOSN subtypes with the most abundant subtype, *Olfr536* mOSNs, accounting for 1.38% of the total (Fig. 1g). In contrast, *Olfr231* and *Olfr464* together account for nearly half of the mOSNs in *Tex15*^{-/-} mice: 30.7% and 18.0%, respectively. Overall, the *Tex15*^{-/-} results in an mOSN population with fewer subtypes but more cells per subtype, leading to a dramatic reduction in the diversity of the mOSN repertoire (Fig. 1h).

Early-expressed ORs dominate in *Tex15*^{-/-} mice

How does *Tex15* diversify OR choice? Based on its expression timing, we hypothesized that *Tex15* regulates OR transcription in precursors. To test this, we used our

scRNA-seq data to examine OR expression at each stage of differentiation. *Tex15* expression peaks in INP3s (Fig. 2a, Extended Data Fig. 2b), which aligns with the peak of OR co-expression prior to choice, using a threshold of 1 transcript per cell (Fig. 2b). In *Tex15*^{-/-} mOSNs, the rate of OR co-expression is significantly increased, with more OR genes detected per cell (Fig. 2b, Extended Data Fig. 3a). Moreover, we observe that most *Tex15*^{-/-} INP3s express multiple OR genes using a higher threshold of 3 or more transcripts per cell, whereas wildtype INP3s rarely express OR genes at this level (Fig. 2c, Extended Data Fig. 3b). Finally, we observe a significant increase in the total number of OR transcripts detected per cell in *Tex15*^{-/-} INP3s (Fig. 2d, Extended Data Fig. 3c). All of these metrics are similar between *Tex15*^{-/-} and *+/+* at the other cell stages ($p > 0.05$, post hoc t-test). Taken together, these findings demonstrate that the loss of *Tex15* leads to increased OR gene transcription specifically in INP3s.

Next, we asked how the altered OR transcript levels we observe in *Tex15*^{-/-} precursors are related to subsequent OR choice. We split the OR genes into quartiles based upon their transcript levels in *Tex15*^{-/-} mOSNs (Q1-4, $n = 75$ to 76 ORs for each), and defined an additional set containing all ORs that are not detected (ND) (TPM < 0.1 , $n = 786$ ORs) (Extended Data Fig. 3d). In our scRNAseq data, the composition of OR transcripts in *Tex15*^{-/-} INP3s is biased towards the *Tex15*-expressed ORs, and this bias becomes more prominent as differentiation continues to the iOSN and mOSN stages (Fig. 2e). However, OR transcript levels are low in these cells, limiting our ability to quantify individual OR expression. To provide a more sensitive test of this relationship, we examined gene expression in FACS purified Ngn1-GFP⁺ cells from *Tex15*^{-/-} and *Tex15* heterozygous mice. For these experiments, we collected GFP⁺ cells from adult mice 10-days after using methimazole lesioning to induce MOE regeneration. RNAseq revealed pervasive changes in OR gene expression in *Tex15*^{-/-} mice, with ORs accounting for most of the differentially expressed genes (Extended Data Fig. 3e-f). Critically, OR expression changes in Ngn1-GFP⁺ cells are highly correlated with those in OMP-GFP⁺ mOSNs, consistent with altered precursor OR expression skewing the resulting mOSN population (Extended Data Fig. 3g).

Why does altered OR expression in *Tex15*^{-/-} precursors favor specific OR genes? We hypothesized that some OR genes have a transcriptional advantage in INPs that is normally suppressed but revealed in *Tex15*^{-/-}. To test this hypothesis, we examined how OR expression in *Tex15*^{-/-} mOSNs relates to OR expression in *Tex15*^{+/+} INPs¹⁸. Strikingly, the top quartile of OR genes in *Tex15*^{-/-} mOSNs (Q1) are expressed at significantly higher levels in wildtype INPs than the other groups, while ORs not detected in the *Tex15*^{-/-} (ND) are expressed at significantly lower levels (Fig. 2f). Thus, OR choice in *Tex15*^{-/-} mice reflects OR transcription in wildtype INPs.

Next we examined whether choice in *Tex15*^{-/-} mice relates to the timing of OR transcription within the INP stage. We analyzed data from a large scRNA-seq dataset from wildtype OSN differentiation, which includes 154,642 cells from the interval spanning the onset of OR transcription to OR choice³³. Using pseudotime values, we grouped cells from the Class II OR lineage into bins and calculated the mean transcript level of each OR per bin. This analysis revealed a striking relationship between OR transcript levels in *Tex15*^{-/-} mOSNs and OR transcription in precursors (Fig. 2g). ORs that are highly expressed by *Tex15*^{-/-} mOSNs exhibit a wave of strong transcription in early precursors, which subsides prior to choice. Notably, the peak of this wave aligns with the peak of *Tex15* transcript levels. Next, we examined the breadth of OR expression across this population of cells. We calculated the percentage of cells expressing each OR per bin, examining how this varied by group over pseudotime. ORs from Q1 are expressed by a higher percentage of cells, and this expression occurs earlier in pseudotime than ORs from the other groups (Fig. 2h). The prevalence of cells expressing Q1-Q4 ORs decreases in later bins, indicating that transcription is normally repressed after the initial pulse. As a result of this decrease, the expression rates for OR genes from all 5 groups ultimately reach similar levels in iOSNs. Accordingly, we calculated each OR's mean pseudotime value, summarizing its transcription timing before singular choice, and observed the same relationship between *Tex15*^{-/-} transcript levels and early expression (Extended Data Fig. 3h). Therefore, *TEX15* loss favors the choice of early-expressed OR genes that are normally repressed in wildtype precursors.

Assembly of enhancer hubs around the dominant OR genes

Wildtype precursors express *Olf231* very early, even when compared to other Q1 ORs, which may contribute to its dominance in *Tex15*^{-/-} mice (Extended Data Fig. 3i). In contrast, the other dominant OR in *Tex15*^{-/-} MOEs, *Olf464*, resembles other Q1 ORs. However, *Olf464* is distinctive in a different way: it is located between a pair of Greek Islands (GIs) (Extended Data Fig. 3j), which are specialized enhancer elements that control the choice of one Class II OR gene by assembling into an interchromosomal (*trans*) enhancer hub around the chosen OR gene (Fig. 3a)^{30,34}. Indeed, GI proximity is associated with Class II OR expression in *Tex15*^{-/-}, with the top 3 quartiles significantly closer to GIs than ND ORs (Fig. 3b). Thus, we hypothesized that the dominant OR genes, *Olf231* and *Olf464*, preferentially assemble into GI enhancer hubs, stabilizing their choice. To test this, we FACS purified *Tex15*^{-/-} mOSNs and performed in situ Hi-C, comparing the results to existing Hi-C data from *Tex15* wildtype mOSNs³⁰. We first examined whether OR genes and OR enhancers form interchromosomal hubs in *Tex15*^{-/-} mOSNs (Extended Data Fig. 4a-d). The overall pattern of interactions among OR genes and GIs is similar to wildtype, though with a small reduction in mean *trans* contact strength. Otherwise, global features of chromosome folding are preserved in *Tex15*^{-/-} mOSNs. For example, compartment scores are highly correlated between the

two conditions (Extended Data Fig. 4e), and although OR-containing regions show a small increase, most ORs remain in the silent "B" compartment (Extended Data Fig. 4e-f). Notably, compartment scores around upregulated ORs, such as *Olfr464*, are unchanged, consistent with the observation that they remain inactive in most cells (Extended Data Fig. 4g).

Having confirmed that *Tex15*^{-/-} mOSNs form GI enhancer hubs, we next asked whether there is increased association of *Olfr231* and *Olfr464* with these structures. For *Olfr231*, we observe increased long-range Hi-C contacts with Rhodes, a GI enhancer located 81.6 Mb away (Fig. 3c). The increase in Hi-C contacts is strongest over *Olfr231* itself, but also evident on several GIs located near it (Fig. 3d). *Olfr231* also shows significantly increased Hi-C contacts with GIs on different chromosomes, indicating formation of an interchromosomal enhancer hub around it (Fig. 3e-f). For *Olfr464*, the locus already makes strong contacts with GIs in wildtype cells due to the presence of a pair of nearby GIs (Extended Data Fig. 3h). However, interchromosomal contacts to GIs increase specifically over *Olfr464* in *Tex15*^{-/-} mOSNs (Extended Data Fig. 4h-i). Thus, both of the dominant ORs in *Tex15*^{-/-} mice associate with interchromosomal GI hubs.

Even though *Olfr231* and *Olfr464* associate with GI hubs, it remains possible that an orthogonal mechanism drives their dominance in *Tex15*^{-/-} mice. GI hubs are assembled by an adapter protein, LDB1, and deletion of *Ldb1* in mOSNs leads to loss of hubs and reduced OR transcription^{30,35}. Thus we asked whether *Ldb1* is also required for OR gene expression in *Tex15*^{-/-} mOSNs. To accomplish this, we conditionally deleted *Ldb1* using an OMP-cre allele in a *Tex15*^{-/-} background and then profiled gene expression by RNA-seq. We then compared OR expression in double-knockout cells to OMP-cre controls and previously reported OMP-cre *Ldb1* knockouts³⁰. Consistent with enhancer hub dependence, loss of *Ldb1* significantly reduces aggregate OR transcript levels *Tex15*/*Ldb1* double KO mOSNs, as in *Ldb1*KO mOSNs (Fig. 3g, Extended Data 4j). In contrast, the double KO has a much stronger effect on OR transcriptome diversity (Fig. 3h). Strikingly, double KO mOSNs retain the skewed OR expression pattern characteristic of *Tex15*^{-/-} cells but at the reduced overall transcript levels characteristic of *Ldb1*KO cells (Fig. 3i). This indicates that the identity of the chosen OR, determined upstream by the *Tex15*-dependent diversity mechanism, is unaffected by disrupting the hub that sustains its expression. Overall, these results indicate that the *Tex15*^{-/-} biases OR choice without compromising the singularity mechanism, and that these two processes can be genetically dissociated.

TEX15 directs de novo deposition of H3K9me3 on OR genes

Since reduced diversity is seen in H3K9-methyltransferase double knockouts², we hypothesized that TEX15 is involved with directing heterochromatin formation on OR

gene clusters. To test this, we performed native ChIP-seq for H3K9me3 in adult MOE tissue from *Tex15*^{-/-} mice and wildtype controls (Fig. 4a). As expected, we observe reduced H3K9me3 signal on OR genes in *Tex15*^{-/-} tissue (Fig. 4b, Extended Data Fig. 5a-b). Genomewide, H3K9me3 reductions are highly localized to OR gene clusters, standing out against the background of whole chromosomes (Fig. 4c). Differential enrichment analysis confirms that the *Tex15*^{-/-} particularly impacts H3K9me3 on ORs, with OR loci accounting for the vast majority of significantly depleted regions (Fig. 4d). Moreover, as a group, H3K9me3 regions that overlap ORs exhibit a strong reduction in signal relative to the rest of the genome (Fig. 4e).

Since OR genes normally gain H3K9me3 over differentiation, we hypothesized that the reductions we observe in *Tex15*^{-/-} MOE result from impaired H3K9me3 deposition. To test this, we reanalyzed published H3K9me3 data from INPs and mOSNs (Extended Data Fig. 5c-e)²¹, and then compared changes over differentiation to changes in *Tex15*^{-/-} mice. Consistent with our hypothesis, reductions in H3K9me3 in the *Tex15*^{-/-} MOE are almost entirely restricted to regions that gain H3K9me3 over differentiation (Extended Data Fig. 5f). Moreover, regions that gain H3K9me3 over differentiation and overlap ORs show reduced signal in *Tex15*^{-/-}, unlike corresponding non-OR regions (Extended Data Fig. 5g). Taken together, these findings suggest that *Tex15*^{-/-} impairs H3K9me3 deposition over OR genes during differentiation.

***Tex15*^{-/-} disrupts OR spatial patterning**

H3K9me3 has also been linked to the spatial patterning of the MOE. Each mOSN subtype is most abundant at a specific spatial position along the MOE's dorsal-ventral (DV) axis (Fig 5a)^{11,13,33,36,37}. Intriguingly, subtype DV position is linked to the level of H3K9me3 at the corresponding OR locus — dorsal ORs carry higher H3K9me3 than ventral ORs — and mutations that perturb heterochromatin shift the relative expression of dorsal and ventral ORs^{21,33,38}.

Since the *Tex15*^{-/-} reduces H3K9me3 deposition, we asked whether it also impacted the DV patterning of the MOE. First, we used our scRNAseq data to examine the relationship between OR choice and spatially patterned non-OR gene expression. For each mOSN, we assigned an “OR DV score” based on the typical DV position of the expressed OR, as determined in wild-type mice by Brann et al³³. We then compared this to a “cell DV score”, calculated using the transcript levels of non-OR genes that exhibit dorsal or ventral expression, as in Tsukahara et al¹¹. In our wildtype mOSNs, we observe the expected correlation between the cell DV score and OR DV score, but this relationship is disrupted in *Tex15*^{-/-} mOSNs (Fig. 5b). In these cells, we observe a similar distribution of cell DV scores, but very few cells that express ventral ORs (Extended Data Fig. 6a,b). However, spatial patterning is not entirely lost; a correlation

remains between cell DV scores for matched OSN subtypes in *Tex15*^{-/-} and *Tex15*^{+/+} cells ($R=0.54$, $p=1.2e-8$) (Extended Data Fig. 6c). However, dispersion of cell DV scores within each subtype is significantly increased in *Tex15*^{-/-} (Fig. 5c). Taken together, these results indicate the coupling between spatial position and OR gene choice is weakened in *Tex15*^{-/-} mice.

To directly examine how the *Tex15*^{-/-} affects the spatial patterning, we used spatial transcriptomics to simultaneously map DV patterning and OR gene expression in tissue sections from adult MOEs (Fig. 5d). Examining spatially patterned non-OR genes, either individually or via a scaled cell DV score¹¹, we found that the overall DV patterning of the MOE is similar between control and *Tex15*^{-/-} mice (Fig. 5e-f, Extended Data Fig. 6d-e). However, we observe loss of ventral ORs in *Tex15*^{-/-} mice, assessed by calculating an OR-DV score weighted by transcript abundance (Fig. 5g-h, Extended Data Fig. 6f). Consistent with our scRNAseq analysis, we observe that ventral regions of the MOE instead express dorsal ORs. This is not related to spatial variation in *Tex15* expression, as we detect TEX15 protein throughout the MOE (Extended Data Fig. 6g).

If the ventral OR genes are no longer expressed, what ORs take their place? Examination of transcript levels for individual OR genes reveals expression of *Olf231* and *Olf464* throughout the ventral regions of MOE sections from *Tex15*^{-/-} mice (Extended Data Fig. 6h). To examine expression of these ORs at cellular resolution, we generated RNAscope probes for *Olf231* and *Olf464*, as well as *Olf1507*, which is one of the most abundant ventral OR genes in *Tex15*^{+/+} mice (Fig. 5i). As expected from their OR-DV scores, we observe *Olf231* and *Olf464* in dorsal regions of the MOE and *Olf1507* in ventral regions in wildtype tissue sections. In contrast, *Olf1507* is not detected in *Tex15*^{-/-} tissue, while *Olf231* and *Olf464* are abundant throughout the entire ventral MOE. Thus absence of *Tex15* is particularly disruptive to the choice of ventral ORs, which are broadly displaced by *Olf231* and *Olf464*.

Discussion

Our findings establish that *Tex15* safeguards the diversity and spatial patterning of OR choice by repressing an early wave of OR gene transcription that occurs in mOSN precursors. During normal mOSN differentiation, a subset of ORs are transcribed earlier than others (Fig. 5j). *Tex15* is required to repress this early transcription. This allows the broader OR repertoire to get transcribed and places all ORs at a similar transcriptional level (Fig 2g). In *Tex15*^{-/-} mice, this repression fails and the early ORs are transcribed at abnormally high levels in INP3s. We propose that this elevated transcription triggers the positive feedback loop that drives enhancer hub assembly and stable OR choice. Because the dorsal and ventral ORs systematically exhibit different transcriptional timing³³, the *Tex15*^{-/-} also disrupts DV patterning, allowing a few early-expressed dorsal

ORs to dominate. Mechanistically, *Tex15* is known to repress transcription, and we show it promotes the deposition of H3K9me3 heterochromatin at OR loci, two activities that likely reduce the transcription of early-expressed OR genes.

These findings illuminate a fundamental vulnerability in the prevailing model of OR choice. OR transcription initiates and drives stable OR choice through self-reinforcing positive feedback loops^{4,17–19,21,31}. While positive feedback effectively ensures OR singularity, it is inherently unstable and prone to amplifying intrinsic bias. A few ORs with a transcriptional advantage can dominate the choice process, as we observe in *Tex15*^{-/-} mice. This advantage likely comes from multiple sources. For example, some OR genes get a transcriptional head start, perhaps by lying close to a GI or having a promoter particularly active in INP cells. Indeed, we suggest that random mutations occurring over 1,000 OR promoters are likely to evolve highly active promoter sequences by chance, which may explain the early transcription of *Olfr231*. Alternatively, GI proximity may let some OR genes assemble enhancer hubs more efficiently, perhaps accounting for the frequent choice of *Olfr464*'s *Tex15*^{-/-} mice. In any case, our data suggests that *TEX15* acts as a brake or buffer, preventing early transcription from dominating the OR choice. Consistent with this model, our data suggest that OR choice happens faster in *Tex15*^{-/-} mice, resulting in fewer INP3s and iOSNs and more mOSNs. In addition, our data shows that *Tex15*-dependent repression is critical for DV patterning of OR choice. This may be because ventral precursors transiently express dorsal ORs before restricting expression to ventral ORs^{21,33}. The absence of *Tex15* allows these dorsal ORs, particularly *Olfr231* and *Olfr464*, to dominate the ventral MOE. Finally, we note that the precipitous decline in *Tex15* expression between the INP3 and iOSN stages coincides with the switch from a transcriptional competition and spatial patterning regime to a choice regime in which positive feedback drives singular OR expression. It may be that the disappearance of *TEX15* enables the progression to OR choice.

TEX15 has two activities that could reduce OR transcription: transcriptional repression by the N-terminal DUF3715 domain⁵, and facilitating H3K9me3 deposition. But what targets these activities to ORs? *TEX15* is a large protein of 3,049 amino acids, but it lacks predicted DNA binding domains. Indeed, aside from the N-terminal DUF3715 domain, it is dominated by predicted intrinsically disordered regions (IDRs)²². One possible mechanism is suggested by the only other DUF3715-containing proteins, *TASOR* and *TASOR2*, components of the HUSH1 and HUSH2 complexes, respectively. HUSH silences transcription of transposable elements as well as large gene families, such as ZNFs and clustered protocadherins, in somatic cells^{39,40}. If *TEX15* participates in a HUSH-like complex, it could be recruited to ORs by HUSH's MPP8 subunit, which binds H3K9 methylated chromatin, or perhaps the Perphilin subunit, which binds RNA. Notably, this mechanism would be consistent with the reduction in H3K9me3 at OR loci

observed in *Tex15*^{-/-} mice (Fig. 4b-d), since HUSH directs H3K9 methylation by SETDB1. A second possibility is raised by recent reports that IDRs can interact with RNA⁴¹. It may be that the long IDRs of *Tex15* bind and sequester nascent OR transcripts, which would provide a mechanism for both recognition and repression. In any case, given *Tex15*'s tightly restricted expression in the MOE (Extended Data Fig. 5g), in vitro biochemical assays may be necessary to characterize this activity.

We have also recently identified a role for TEX15 in regulating vomeronasal receptor (VR) expression in vomeronasal sensory neurons (VSNs)⁴². In each system where *Tex15* function has been characterized — male germ cell TE silencing, VR expression in VSNs, and now OR expression in OSNs — it regulates large, genomically dispersed gene families. A striking feature of *Tex15* that cuts across all of these contexts is its highly restricted expression pattern. Rather than functioning as a broadly expressed transcriptional repressor, *Tex15* appears to be deployed selectively in specific cell types at specific developmental moments: spermatocytes undergoing meiosis, VSN precursors in the vomeronasal organ undergoing VR choice, and INP3 precursors in the MOE undergoing OR choice. Given its recurring role in regulating large, dispersed gene families, *Tex15* is an attractive candidate regulator in other systems where combinatorial gene expression underlies cellular identity.

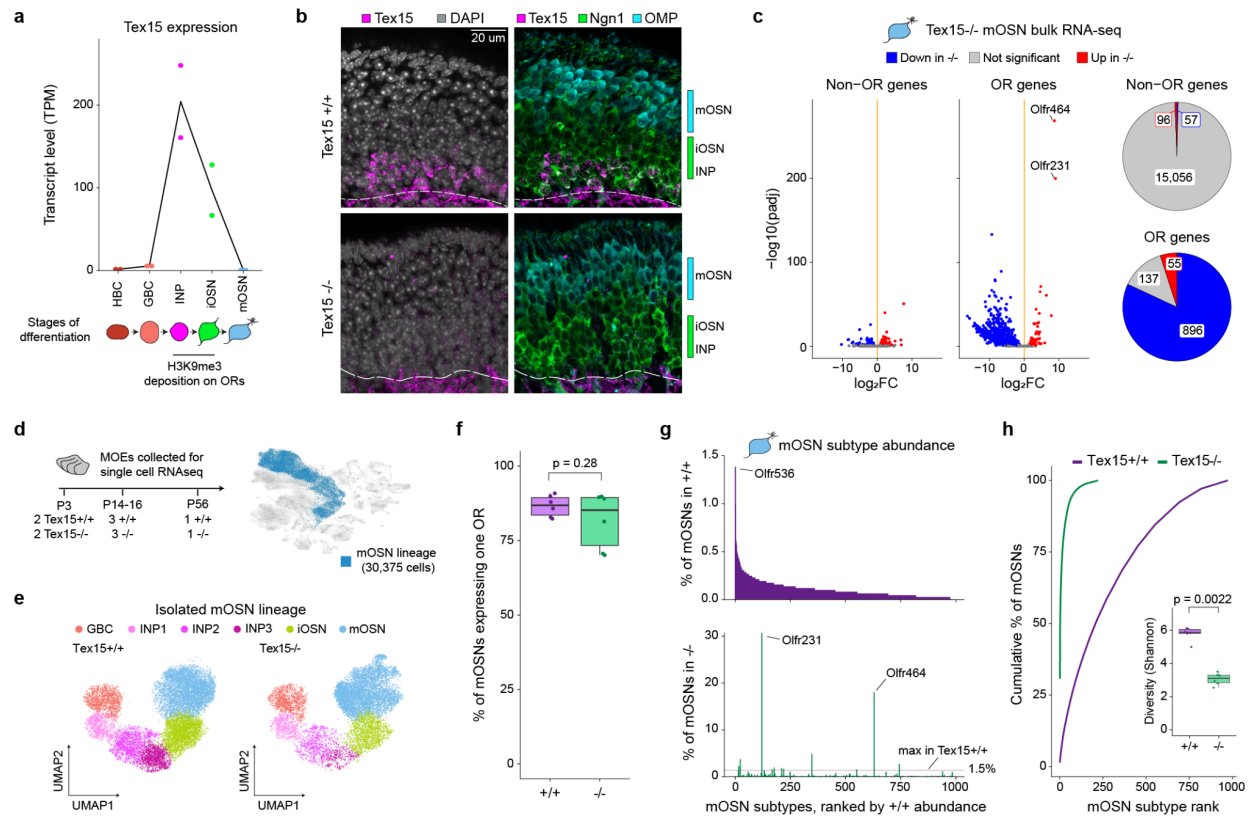
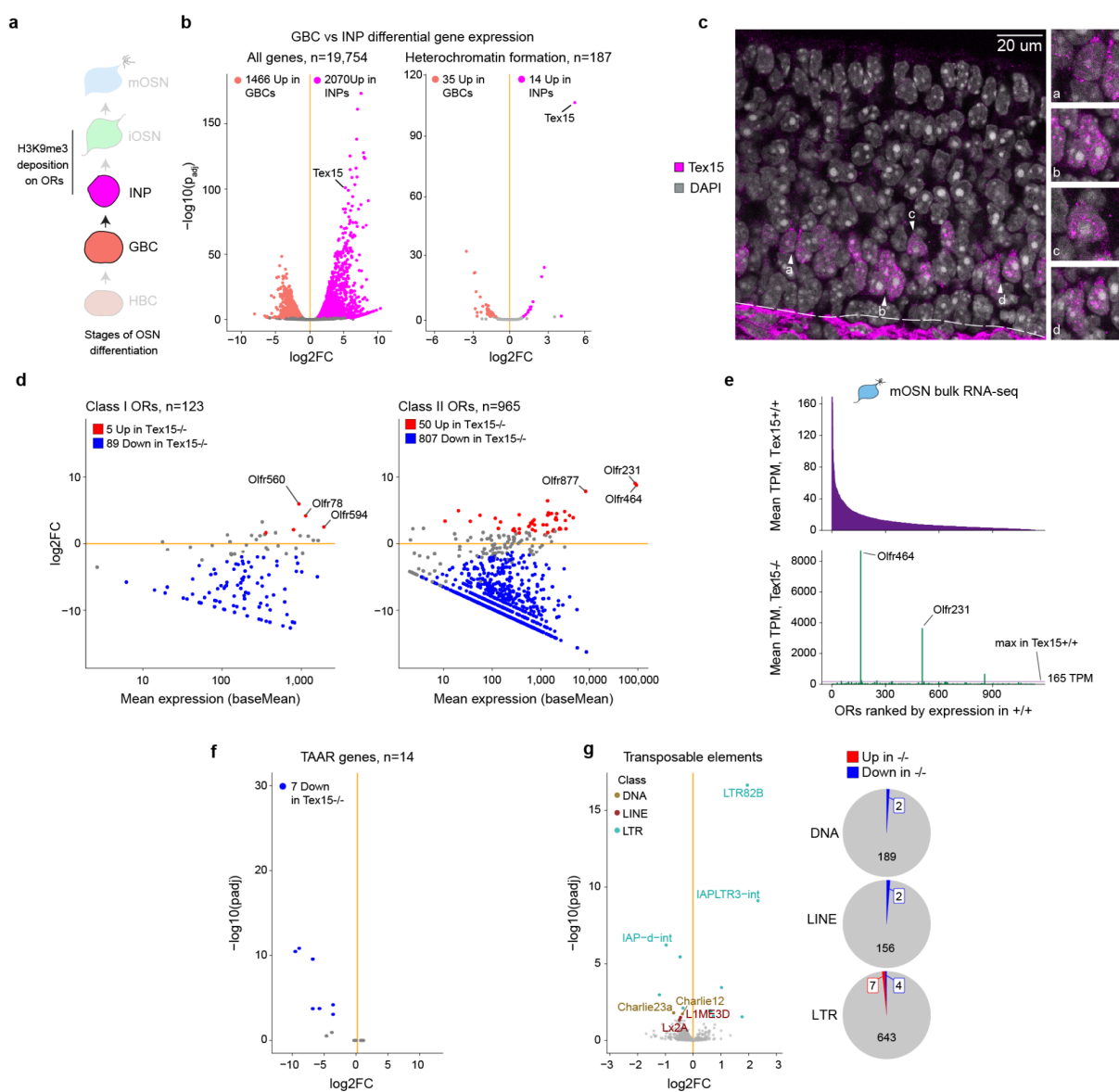
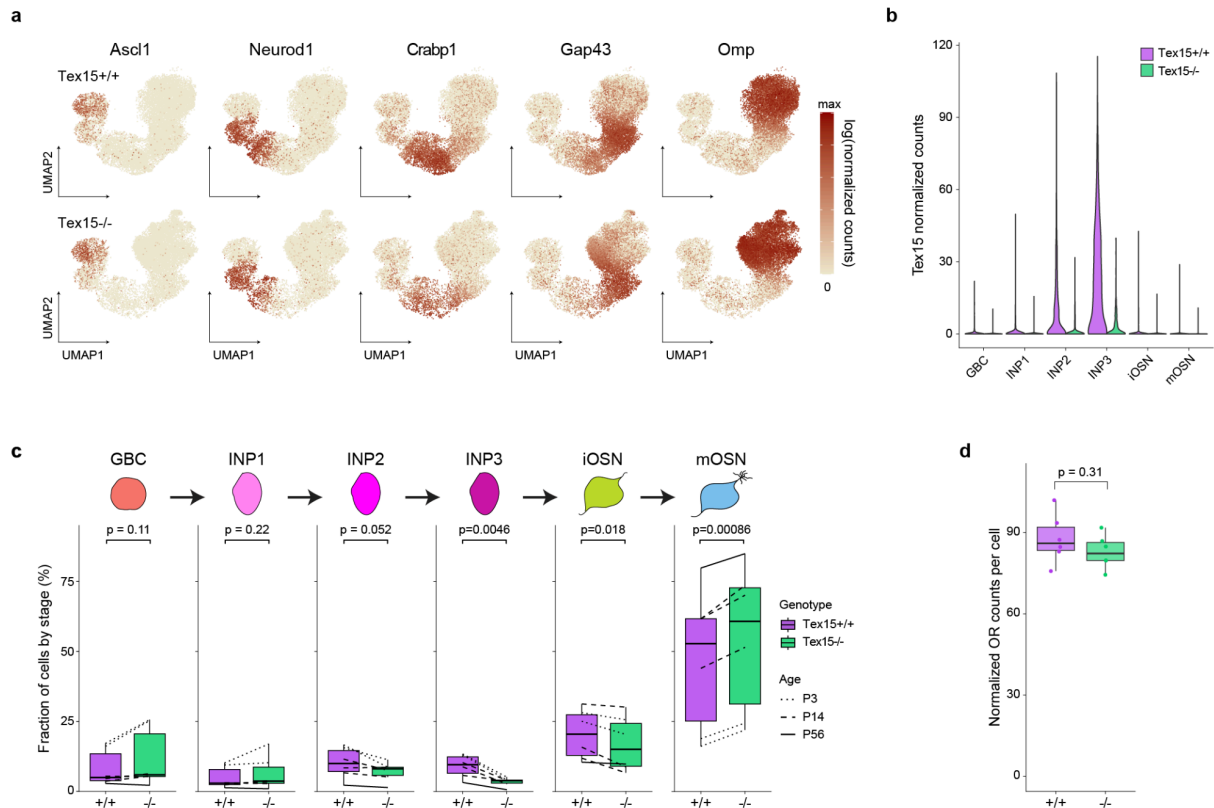


Figure 1. Extreme loss of mOSN diversity in Tex15 $^{-/-}$ mice. **a**, Tex15 transcript levels, in transcripts per million (TPM), in cells purified at different stages of mOSN differentiation. **b**, Immunohistochemistry for Tex15 (magenta), Ngn1-GFP (green - INPs and IOSNs), and OMP (cyan - mOSNs), in MOE tissue sections. Nuclei are labeled with DAPI (gray). **c**, (left) Fold change in transcript levels between Tex15 $^{-/-}$ (n=3) and Tex15 $+/+$ mOSNs (n=5). ORs and non-olfactory receptor genes are plotted separately. (right) Summary of differentially expressed genes from each group (padj < 0.05 for a >50% change in expression, Wald test). **d**, (left) MOE tissue samples analyzed by single-cell RNA-seq. (right) UMAP projection of cells from all 12 libraries, after filtering and integration. Cells in the mOSN lineage are labelled in blue. **e**, Cells in the mOSN lineage after reclustering and assignment to cell stages. The INP population is divided into three stages, INP1-INP3. Tex15 $+/+$ = 16,832 cells, Tex15 $-/-$ = 13,543 cells. **f**, Cells across the neuronal lineage colored by genotype - Tex15 $+/+$ in purple, Tex15 $-/-$ in green. **g**, The percentage of mOSNs expressing a single OR, using a threshold of 3 or more unique transcripts, is not significantly different between Tex15 $+/+$ and Tex15 $-/-$ samples ($p = 0.28$, t-test). **h**, Abundance of individual mOSN subtypes (mOSNs expressing the same OR), ranked by abundance in Tex15 $+/+$ MOEs. The maximum value for wildtype mOSNs (top) is indicated by a dotted line for Tex15 $-/-$ mOSNs (bottom). **i**, Cumulative fraction of mOSNs accounted for by each mOSN subtype, ranked by abundance within each genotype. Inset, the diversity (Shannon diversity index) of the Tex15 $-/-$ mOSN population is significantly reduced compared to the Tex15 $+/+$ ($p = 0.0022$, t-test).



Extended Data 1. Altered receptor gene expression in the olfactory epithelium of Tex15^{-/-} mice. **a**, Schematic illustrating the selection of stage-specific RNA-seq datasets (Pourmorady et al) used to identify factors associated with the onset of H3K9me3 deposition on OR genes. Stages: horizontal basal cells (HBC), globose basal cells (GBC), immature neuronal precursor (INP), immature olfactory sensory neurons (iOSNs), mature olfactory sensory neurons (mOSNs). **b**, (left) Volcano plot showing differential gene expression between GBCs and INPs (n=2 per stage). Tex15 is among the genes with the largest fold change and smallest adjusted p-value. In total, 1,466 genes have significantly higher expression in GBCs and 2,070 in INPs. (right) Genes associated with the GO term "Heterochromatin formation" (n=187). 35 heterochromatin formation genes have significantly higher expression in GBCs and 14 in INPs. Tex15 is the most significantly differentially expressed gene in this set. **c**, Immunohistochemistry for Tex15 (magenta), Nuclei labeled with DAPI (gray), in MOE tissue sections with zoom-ins on cells a-d showing Tex15 protein in the nucleus. **d**, MA plots showing differential expression of Class I and Class II OR genes in Tex15^{+/+} (n=5) and Tex15^{-/-} mOSNs (n=3) by RNA-seq. The top 3 upregulated ORs are labeled. **e**, Mean OR transcripts levels in Tex15^{+/+} and Tex15^{-/-} mOSNs, expressed as transcripts per million (TPM). ORs are ranked by expression in Tex15^{+/+} mOSNs. The maximum transcript level for wildtype mOSNs (top) is indicated by a dotted line for Tex15^{-/-} mOSNs (bottom). **f**, Volcano plot showing differential gene expression of Trace amine associated receptors (TAARs) between Tex15^{-/-} and Tex15^{+/+} mOSNs. **g**, (left) Change in transposable element transcript levels between Tex15^{-/-} and Tex15^{+/+} mOSNs. Significantly differentially expressed elements are color coded by class, with the top 3 from

each class labeled. (right) Summary of differentially expressed TEs by repeat class. For all panels, differentially expressed genes have an adjusted p-value < 0.05 for a >50% change in expression (Wald test).



Extended Data 2. Single cell analysis of Tex15^{-/-} and Tex15^{+/+} MOEs. **a**, UMAP projections in Tex15^{+/+} and Tex15^{-/-} scRNAseq data showing log-normalized transcript levels of marker genes for mOSN differentiation. Ascl1 labels GBCs, Neurod1 labels INP1/2, Crabp1 labels INP2/3, Gap43 labels iOSNs, and OMP labels mOSNs. **b**, Normalized Tex15 counts per cell at each stage of differentiation. **c**, Abundance of cells at each stage of mOSN differentiation in Tex15^{+/+} and Tex15^{-/-} samples. Lines connect paired samples and indicate the age of the mice. The Tex15^{-/-} samples trend towards relatively few cells at the INP2 stage (p = 0.052), and then show a significant decrease at the INP3 (p = 4.57e-3) and iOSN (p = 1.79e-2) stages. However, they have a significant increase in the proportion in mOSNs (p = 8.58e-4) (paired t-test). **d**, Normalized OR counts in Tex15^{+/+} vs Tex15^{-/-} mOSNs (t-test, p = 0.31).

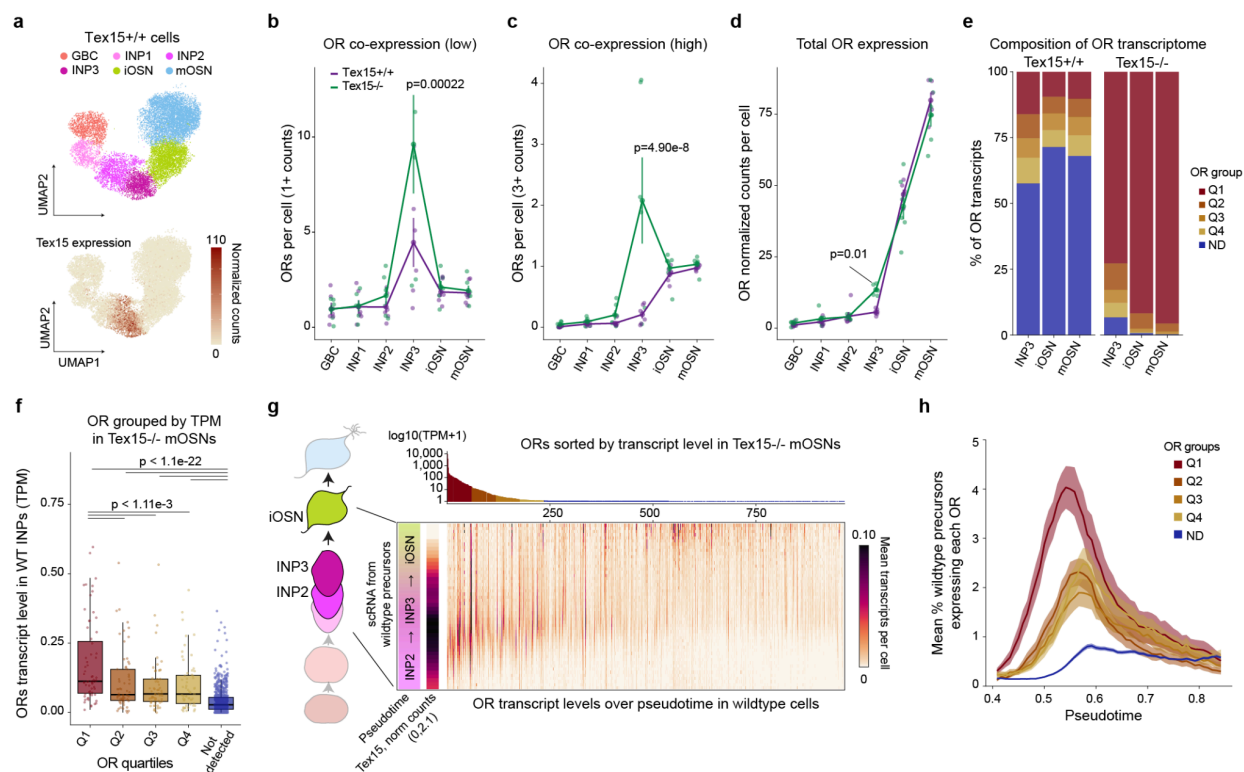
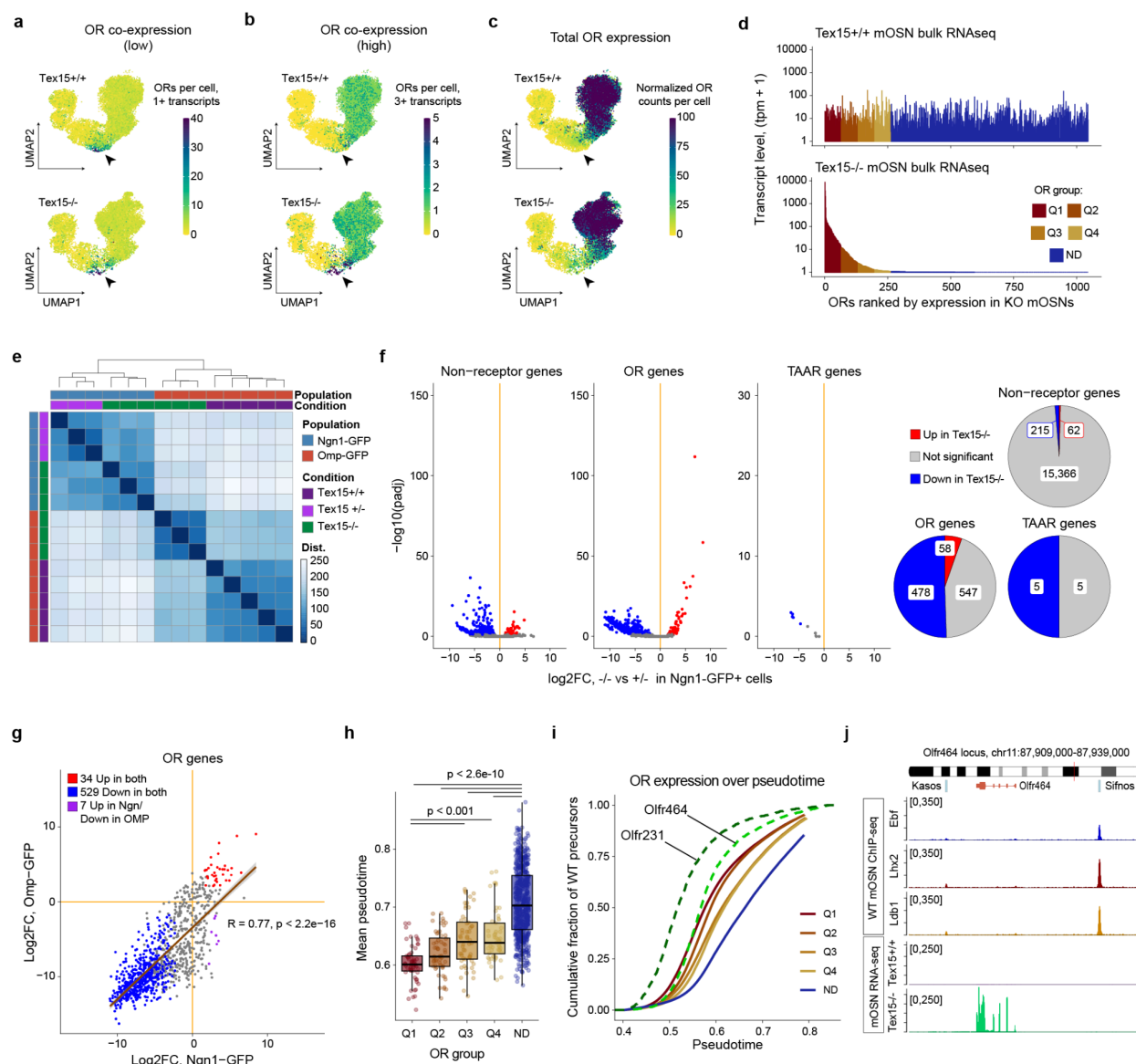


Figure 2: Early-expressed ORs dominate in *Tex15*^{-/-} mice. **a**, *Tex15* transcripts peak in INP2/INP3 cells in *Tex15*^{+/+} mice. **b**, *Tex15*^{-/-} INP3s express more OR genes per cell using a low threshold (1 or more OR counts), which detects increased low-level co-expression ($p=0.00022$, post-hoc t-test, mixed linear model ANOVA). **c**, On average, *Tex15*^{-/-} INP3s express multiple OR genes at a high level (3 or more counts), which is rarely observed in *Tex15*^{+/+} INP3s ($p=4.90e-8$, post-hoc t-test, mixed linear model ANOVA). **d**, Normalized OR counts per cell, which detects atypical high OR expression in *Tex15*^{-/-} INP3s ($p=0.01$, post-hoc t-test, mixed linear model ANOVA). **e**, Relative abundance of OR transcripts, grouped according to the OR expression groups. ORs are split into five groups: ORs expressed in *Tex15*^{-/-} are split into four quartiles ($n=75$ to 76 each, total 302) by expression in *Tex15*^{-/-} and a “not detected” OR group (ND), which are present at less than 0.1 TPM ($n=786$). **f**, In wildtype INP bulk RNA-seq, the Q1 ORs exhibit significantly higher median transcript levels than the Q2 ($p=0.0298$), Q3 ($p=0.00132$), Q4, and ND ORs (adjusted p -values, Dunn test with correction for multiple testing). **g**, (left) scRNA-seq data from $154,642$ wildtype OSN precursors from the INP2 to iOSN stages (Brann et al 2026) was binned based on the pseudotime score for each cell. (right) Mean transcript levels for *Tex15* and each OR over binned pseudotime. ORs are arranged by their level of expression in *Tex15*^{-/-} mOSNs (top). **h**, For each expression group, the mean percentage of cells detected expressing each OR over pseudotime. Line shows mean, shaded area shows SEM. For a-c, points show mean and SEM across *Tex15*^{+/+} and *Tex15*^{-/-} samples ($n=6$ for each). P -values were calculated from post-hoc t-tests on an ANOVA comparing genotypes for each cell population.



Extended Data 3: Early-expressed ORs dominate in *Tex15*^{-/-} mice. **a**, Number of OR genes detected per cell using a low threshold for expression (1 or more transcripts per cell), reveals increased ORs co-expression in the *Tex15*^{-/-} (at arrowhead) **b**, Number of OR genes detected per cell using a higher threshold for expression (3 or more transcripts per cell), reveals *Tex15*^{-/-} INPs co-expressing multiple ORs at unusually high levels. **c**, UMAP projection showing normalized OR counts per cell. **d**, Log-scaled OR transcripts levels (TPM + 1) sorted by level in *Tex15*^{-/-} mOSNs. **e**, Heatmap showing euclidean distance between samples for top-1000 differentially expressed genes in Ngn1-Gfp+ cells (INPs/iOSNs) and OMP-Gfp+ cells (mOSNs) **f**, Change in transcript levels between *Tex15*^{-/-} and *Tex15*^{+/+} Ngn1-Gfp+ cells. ORs and non-olfactory receptor genes are plotted separately. (right) summary of differentially expressed genes from each group (padj < 0.05 for a >50% change in expression, Wald test). **g**, Comparison of log2 fold change in OR gene transcript levels between Ngn1-GFP and OMP-GFP in *Tex15*^{-/-} mice. **h**, The mean pseudotime value, corresponding to timing of OR expression, for each OR expression group. The Q1 ORs are expressed significantly earlier and the ND ORs are expressed significantly later than ORs in most other groups. **i**, The cumulative distribution of OR-expressing cells in wildtype precursors reveals that cells expressing Q1 ORs are abundant earlier in pseudotime than other groups, and the ND ORs appear relatively late. *Olfr231* expressing cells appear very early, even when compared to other Q1 ORs. In contrast, *Olfr464* resembles other Q1 ORs. **j**, *Olfr464* is located between two Greek Islands. ChIP-seq data from Monahan et al 2017 and Monahan et al 2019.

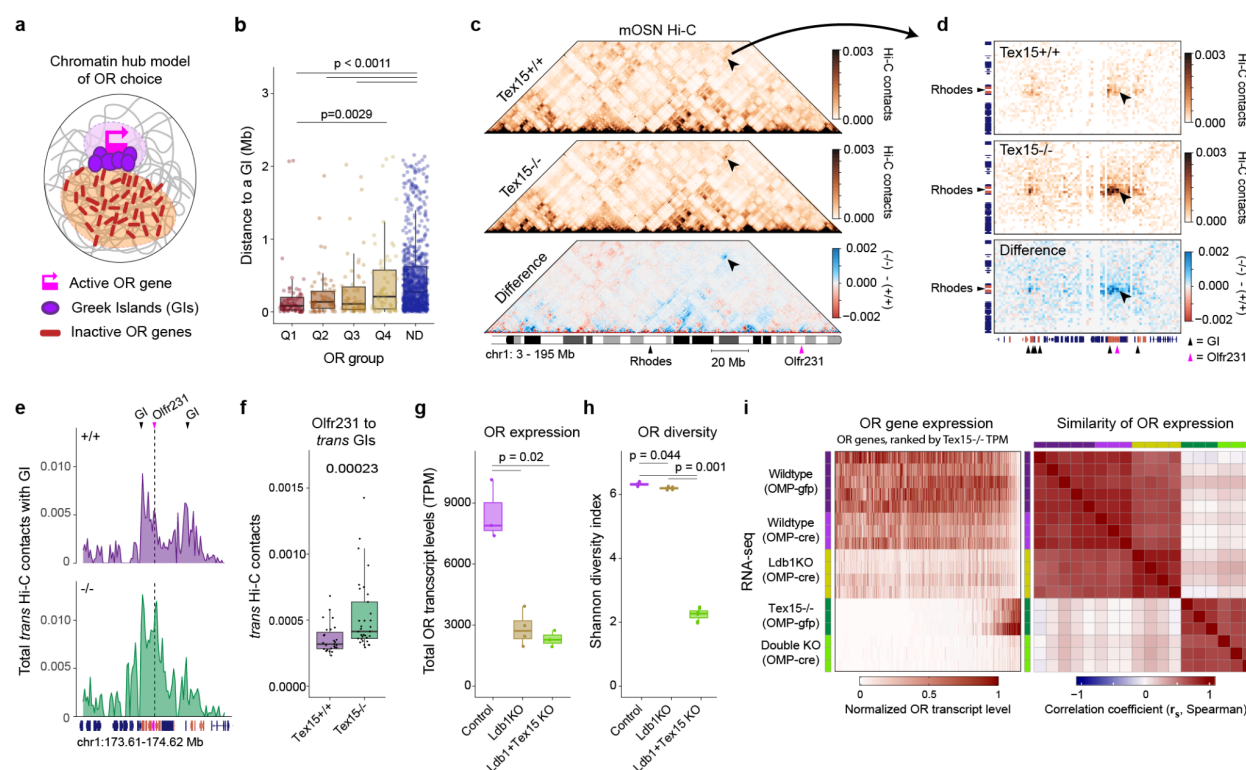
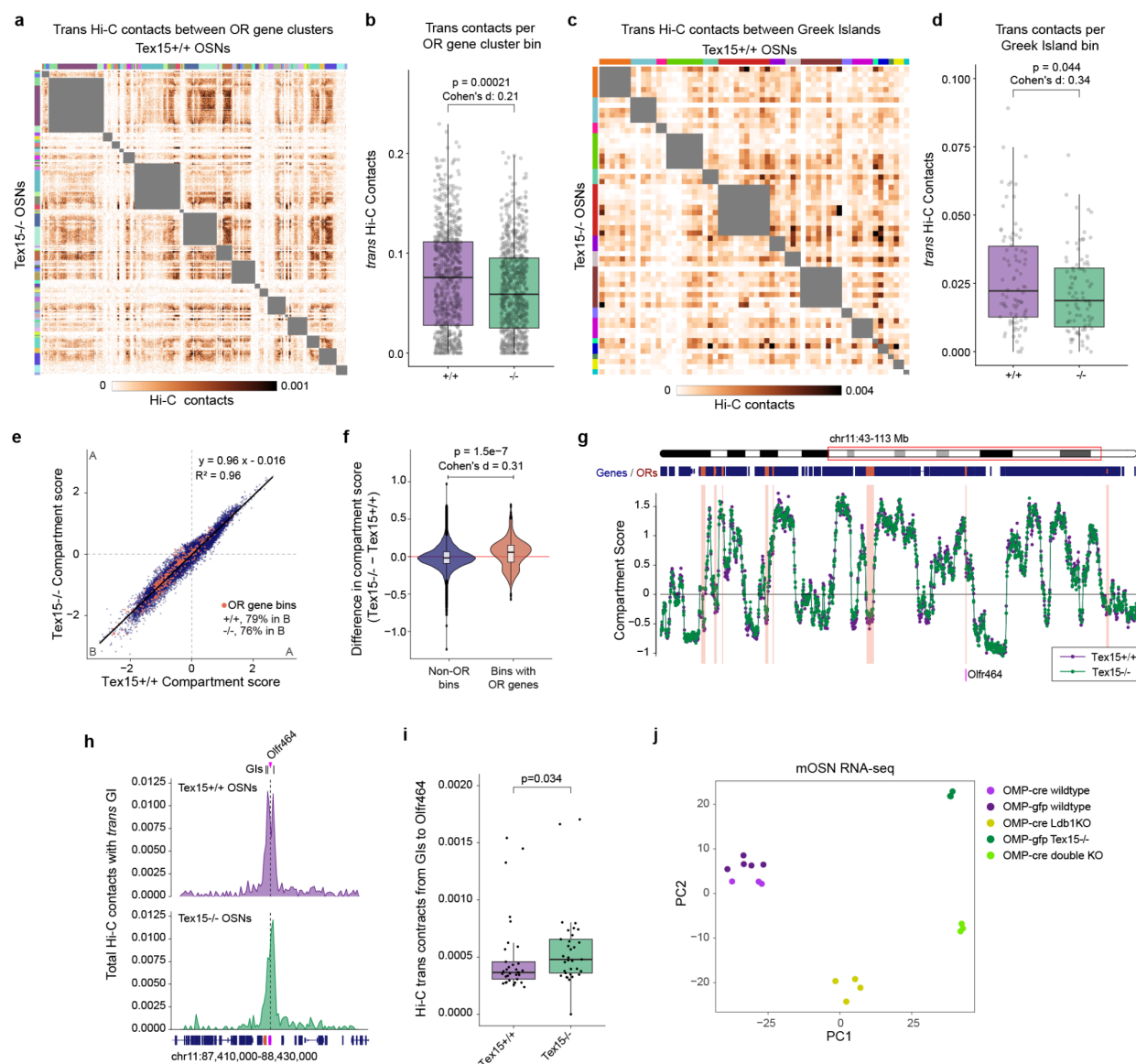


Figure 3: Assembly of enhancer hubs around the dominant OR genes. **a**, a Greek Island (GI) enhancer hub assembles around the active OR gene. **b**, The top 3 quartiles of OR genes in the *Tex15*^{-/-} are significantly closer to GIs than the ND OR genes, and Q1 is also closer than Q4 (Dunn test, corrected for multiple comparisons). **c**, The difference in Hi-C contacts between *Tex15*^{-/-} and *Tex15*^{+/+} mOSNs across a 105 Mb region of chromosome 1. Triangles indicate the location of *Olfr231* and a distant GI, Rhodes. Arrowhead indicates contacts between these loci. 500 Kb resolution **d**, Zoom-in of Hi-C contacts between the Rhodes locus and the *Olfr231* locus, which includes multiple nearby GIs. OR genes are coral, non-OR genes are navy. Arrowheads indicate contacts between Rhodes and *Olfr231* at 25 Kb resolution. **e**, Summed interchromosomal (*trans*) contacts from GIs to the *Olfr231* locus. 10 Kb resolution. **f**, *trans* Hi-C contacts between *Olfr231* and each *trans* GI (Wilcoxon, $p = 0.00023$). **g**, Total OR transcript levels are significantly reduced in *Ldb1*KO OSNs ($n = 4$, from Monahan et al 2019), and *Tex15*/*Ldb1* double-knockout OSNs libraries ($n = 3$) compared to control OMP-cre labeled mOSNs ($n = 3$). (t-test, corrected for multiple comparisons) **h**, Diversity of OR transcripts is significantly reduced in double KO mOSN relative to control ($p = 0.001$, Cohen's $d = 25.0$) than the loss of *Ldb1* alone relative to control ($p = 0.047$, Cohen's $d = 2.79$)(t-test, corrected for multiple comparisons). **i**, (left) OR transcript levels across mOSNs with different genotypes. Each row is a biological replicate. Transcript levels are normalized for each OR gene. ORs are sorted by their transcript levels in *Tex15*^{-/-};OMP-gfp mOSNs. (right) Spearman correlation between OR transcript levels across samples. Panels **b-e**, use balanced Hi-C contacts calculated after merging data from two biological replicates.



Extended Data 4: Genome organization and enhancer hubs in *Tex15*^{-/-} mOSNs. **a**, Heatmap of interchromosomal (*trans*) Hi-C contacts between OR clusters, binned at 25 Kb resolution. *Tex15*^{+/+} data is above the diagonal, *Tex15*^{-/-} below. Interactions between bins located on the same chromosome are masked (gray). Color bars indicate OR clusters. **b**, Quantification of *trans* contacts between OR cluster bins reveal significant but marginal reduction in contacts in *Tex15*^{-/-} OSNs ($p = 0.00024$, Wilcoxon, Cohen's $d = 0.21$). **c**, Heatmap of interchromosomal (*trans*) Hi-C contacts between Greek Islands (GIs), as in **a**, but color bars indicate chromosome. **d**, Quantification of interchromosomal (*trans*) contacts between GIs reveals a small but significant reduction in contacts ($p = 0.043$, Wilcoxon, Cohen's $d = 0.4$). **e**, Compartment scores for the whole genome (blue) and OR cluster loci (coral) are similar between *Tex15*^{+/+} and *Tex15*^{-/-} mOSNs. 50 Kb bins. 79% of OR bins have a negative compartment score value (indicating compartment B) in the *Tex15*^{+/+} and 76% in the *Tex15*^{-/-}. **f**, Bins that overlap OR genes exhibit a small but significant increase in compartment scores in *Tex15*^{-/-} mOSNs ($p = 1.5e-7$, t-test, Cohen's $d = 0.31$). **g**, Signal tracks showing similar compartment scores across chromosome 11, including the region around *Olf464*. **h**, The total Hi-C contacts made across the *Olf464* locus by GIs located on a different chromosome (*trans* interchromosomal contacts) in *Tex15*^{+/+} and *Tex15*^{-/-} mOSNs. **i**, Summed *trans* Hi-C contacts between GIs and the *Olf464* locus in *Tex15*^{+/+} and *Tex15*^{-/-} mOSNs. 10 Kb resolution. **f**, Hi-C contacts between *Olf464* and each *trans* GI (Wilcoxon, $p = 2.5e-05$). **j**, Principal component analysis of bulk-RNA-seq datasets analyzed together with the *Tex15* and *Ldb1* double knockout. Wildtype mOSN labeled with either OMP-Cre and OMP-GFP are similar to each other, otherwise samples from each genotype cluster together as a discrete group. All Hi-C analyses (a-i) show balanced *trans* Hi-C contacts calculated after merging data from two biological replicates.

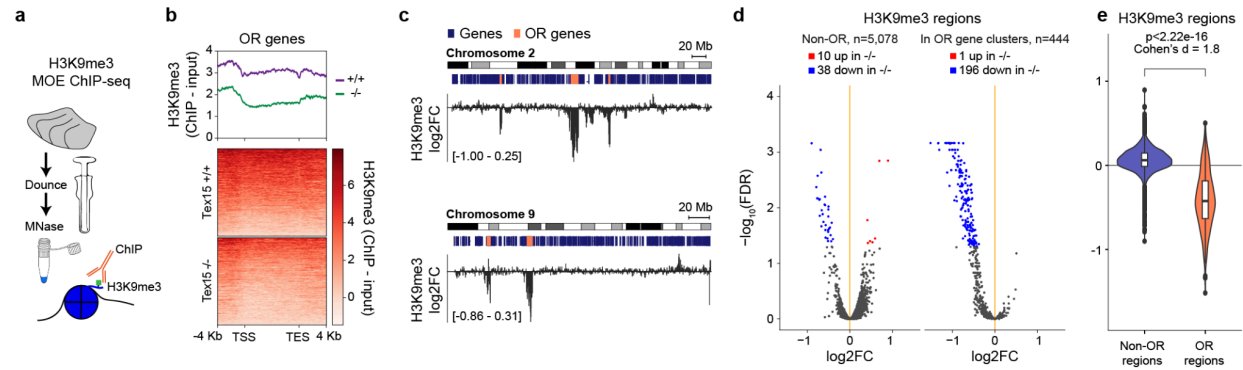
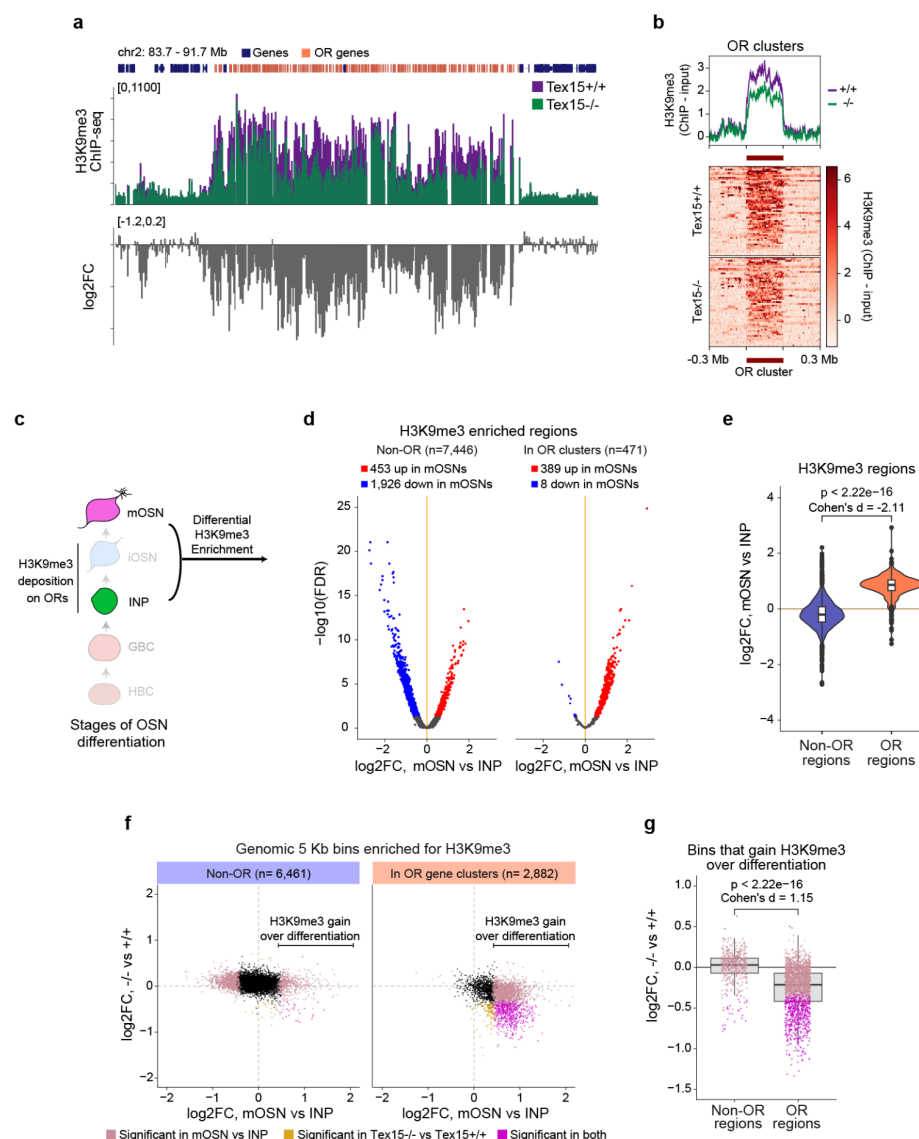


Figure 4: *Tex15* directs de novo deposition of H3K9me3 on OR genes. **a**, H3K9me3 ChIP-seq from MOE of adult *Tex15*^{-/-} mice and wildtype controls. **b**, Heatmap and plot showing H3K9me3 signal over OR genes for a representative experiment. **c**, Fold change in H3K9me3 ChIP-seq signal over the entirety of chromosome 2 and chromosome 9, showing decreased signal over OR genes (coral) compared to all other genes (blue) for a representative experiment. **d**, Differential enrichment analysis of H3K9me3 regions, split based on whether they overlap OR gene clusters. Regions with a significant increase in H3K9me3 signal are in red, decrease in blue (FDR < 0.05, n=4 per condition). **e**, Fold change in ChIP signal in H3K9me3 regions, grouped based on OR cluster overlap. OR regions show a significant reduction in H3K9me3 signal compared to non-OR regions ($p < 2.22 \times 10^{-16}$, t-test, Cohen's $d = 2.04$).



Extended Data 5: H3K9me3 marks on OR gene clusters. **a**, H3K9me3 ChIP-seq signal and log2 fold change over an OR gene cluster on chromosome 2. **b**, Heatmap showing H3K9me3 signal over OR gene clusters and flanking regions. Each row is an OR cluster, which are all scaled to be the same width. The plot above shows the average signal over these regions. **c**, Heatmap showing H3K9me3 signal over OR clusters genes and flanking regions. Each row is an OR gene cluster, which are all scaled to be the same width. The plot above shows the average signal over these regions. Only clusters over 100 Kb in size are included. **c**, Schematic illustrating the selection of cell stages to evaluate the gain of H3K9me3 on OR genes over differentiation. **d**, Differential enrichment analysis for H3K9me3 ChIP from mOSNs and INPs from Bashkirova et al 2023 (n=2 per stage). H3K9me3 enriched regions are split based upon overlap with OR gene clusters. Regions with a significant increase in H3K9me3 signal are in red, decrease in blue (FDR < 0.05). Nearly all regions that overlap an OR cluster gain H3K9me3 over differentiation. **e**, Fold change in ChIP signal for H3K9me3 regions grouped based on OR gene cluster overlap. OR regions show a significant gain in H3K9me3 signal over differentiation compared to non-OR regions ($p < 2.22 \times 10^{-16}$, t-test, Cohen's $d = 2.25$). **f**, Comparison of H3K9me3 fold change over differentiation versus fold change in *Tex15^{-/-}*. All 5 Kb bins enriched for H3K9me3 in either condition are included. Bins are split based upon overlap with OR gene clusters. Bins with significant change in H3K9me3 are colored as indicated (FDR < 0.05). **g**, Fold change in H3K9me3 on 5 Kb bins that exhibit a significant increase in ChIP signal over differentiation. Bins overlapping OR gene clusters show a significant reduction in H3K9me3 signal compared to non-OR regions ($p < 2.22 \times 10^{-16}$, Cohen's $d = 1.15$). Panels **a**, **b** show data from a representative experiment.

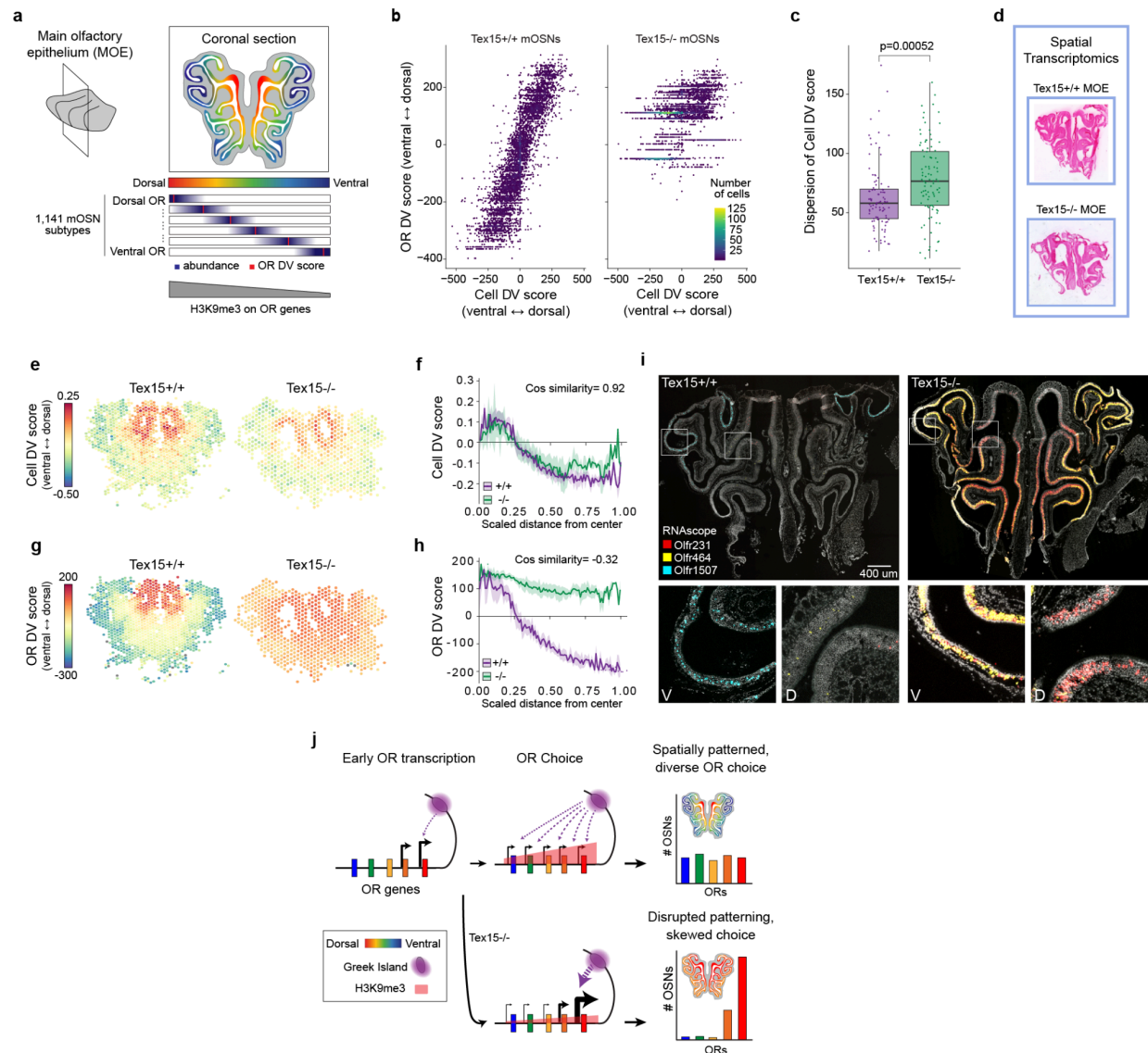
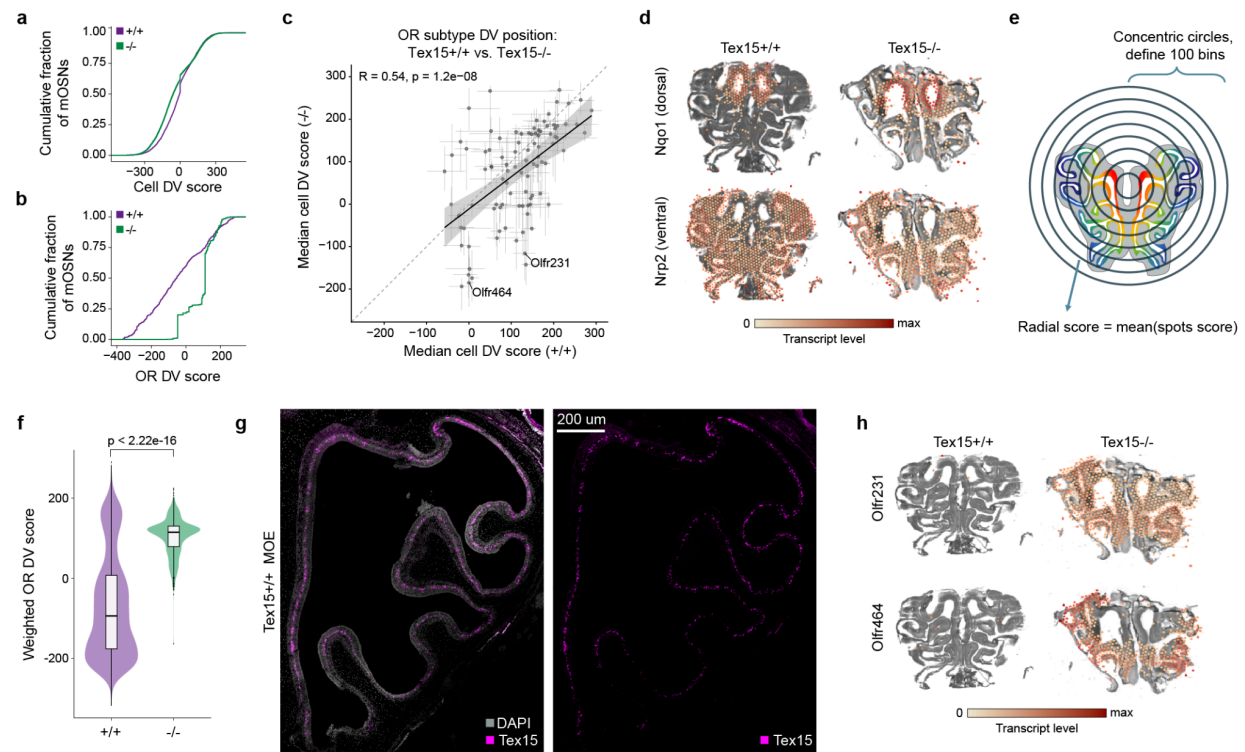


Figure 5: *Tex15*^{-/-} results in disrupted OR spatial patterning. **a**, mOSNs subtypes are most abundant (navy) at specific positions along the dorsal-ventral (DV) axis of the MOE, summarized as an OR-DV score (red). DV score correlates with H3K9me3 levels on OR genes. **b**, The density of mOSNs observed across the range of Cell DV scores (based on non-OR genes) and OR DV scores. **c**, *Tex15*^{-/-} mOSN subtypes exhibit an increased dispersion of Cell DV scores observed within mOSN subtypes (only subtypes with 3+ cells in both conditions, $p=0.00052$, t-test). **d**, 10x Visium spatial transcriptomics analysis of HE-stained coronal sections of *Tex15*^{+/+} ($n=6$) and *Tex15*^{-/-} ($n=3$) MOEs. **e**, Cell DV score across a representative *Tex15*^{+/+} and *Tex15*^{-/-} tissue section. Cell DV score is calculated from transcript levels of dorsally and ventrally patterned genes within each 55 μ m spot. **f**, Cell DV scores versus radial position. Line indicates mean across tissue sections and shaded area indicates SEM. Cos similarity measures how similar the curves are. **g**, OR DV score across a representative *Tex15*^{+/+} and *Tex15*^{-/-} tissue section. OR DV Score is calculated from OR transcript levels within each 55 μ m spot. **h**, OR DV scores versus radial position. Line indicates mean across slides and shaded area indicates SEM. **i**, (top) RNA Scope detection of *Olfr231* (red), *Olfr464* (yellow), and *Olfr1507* (cyan) transcripts in MOE tissue sections. Bar = 400 μ m. (bottom) Larger images of boxed regions from above. **j**, Model for how *Tex15* safeguards mOSN diversity by repressing the transcription of early expressed OR genes. Transcriptional repression and heterochromatin formation equalize OR transcript levels in late precursors, allowing for diverse OR choice. In *Tex15*^{-/-} mice, heterochromatin formation is less efficient and OR transcription in INPs increases rapidly, thereby skewing enhancer hub assembly and choice towards the early expressed ORs.



Extended Data Figure 6: Spatial Pattern of MOE is disrupted in *Tex15*^{-/-}. **a**, Cumulative fraction of *Tex15*^{+/+} and *Tex15*^{-/-} mOSNs across the range of Cell DV scores. **b**, Cumulative fraction of *Tex15*^{+/+} and *Tex15*^{-/-} mOSNs across the range of OR DV scores. **c**, Median cell DV score for mOSNs subtypes from *Tex15*^{+/+} and *Tex15*^{-/-} mice. Only subtypes represented by at least 3 cells in the *Tex15*^{+/+} and *Tex15*^{-/-} are included. Lines represent interquartile range. ($R=0.54$, $p=1.2e-08$, Pearson). **d**, Transcript levels of *Nqo1*, a dorsal marker gene, and *Nrp2*, a ventral marker gene, within each 55 μ m spot detected by spatial transcriptomics of a *Tex15*^{+/+} and *Tex15*^{-/-} MOE representative section from LoupeBrowser. **e**, Schematic illustrating radial analysis strategy used to measure transcript levels along the DV axis across multiple slides. The “radial score” is calculated based upon the average of spot scores at the same radial distance from the center of the tissue, and then expressed as relative distance from center. **f**, The distribution of OR DV scores for spots on slides for *Tex15*^{+/+} ($n=6$) and *Tex15*^{-/-} ($n=3$) MOEs. **g**, Tiledscan showing *Tex15* (magenta) immunoreactivity across half an MOE. Nuclei are labeled with DAPI (gray). Scale bar = 200 μ m. **h**, *Olfr231* and *Olfr464* transcript levels within each 55 μ m spot of a *Tex15*^{+/+} and *Tex15*^{-/-} MOE representative section from Loupe Browser.

Author Contributions

N.Y. and K.M. designed the study and wrote the manuscript. K.M. conducted the bulk-RNA seq experiments and Hi-C experiments. N.Y. and J.K. conducted the single-cell experiments. N.Y. conducted the spatial transcriptomics experiments. N.Y. and A.I. conducted the ChIP-seq experiments. J.D. and N.B.G. conducted the RNAscope experiments. H.P. conducted the immunofluorescence experiments. D.B., S.R.D, K.M., and N.Y, designed and conducted single-cell analyses. C.S., J.Y. and N.Y., designed and conducted spatial transcriptomics analyses. P.J.W. provided the Tex15 null allele as well as technical assistance related to this allele. All other analyses were completed by K.M. and N.Y. with consultation and support from J.D. and D.B.

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Data Availability

All newly generated data used in this study is publicly available in GEO accession number GSE341836. FACS isolated mature OSN Hi-C data and Ldb1 ChIP-seq used in Figure 3 and Extended Figure 3j and Extended Data Figure 4 has been previously published in Monahan et al 2019³⁰ and the data is publicly available in <https://data.4dnucleome.org/> under accession number: 4DNESEPD6KY. WT mOSN ChIP-seq data used in Extended Data Figure 3j is publicly available from Monahan et al 2017⁴³ at GSE93570. For gene expression across cell stages in Fig 1/Extended Data Figure 1, we used previously published data from Pourmorady et al 2024¹⁸, series GSE230380. For comparison of Tex15 single knockout and Tex15 and Ldb1 double knockout bulk-RNA seq data to Ldb1 knockout bulk-RNA seq data in Figure 3, Extended data Figure 4, we used previously published Ldb1 knockout bulk-RNA seq data from Monahan et al 2019³⁰, series GSE112153. For the pseudotime analysis for early transcription of ORs, we

used data from Brann et al 2026³³, publically available under GSE297068. For the heterochromatin deposition across cell stages data in Extended Data Figure 5, we used previously published data from Bashkirova et al 2023²¹, publicly available under GSE158730.

Code availability

All code will be made publicly available in a GitHub repository (https://github.com/MonahanLab/Yusuf_2026), together with formatted reports from R workflow, additional data tables, and annotation files.

Methods

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Kevin Monahan (km1339@dls.rutgers.edu). No statistical methods were used to predetermine sample size. The experiments were not randomized and investigators were not blinded to allocation during experiments and outcome assessment.

Mice

Mice were treated in compliance with the rules and regulations of Rutgers University - New Brunswick - IACUC under protocol number 2020008. All mice were housed in Specific Pathogen Free facilities in ventilated cages with a 12-h light/dark cycle, Mice had ad libitum access to standard rodent chow and water. Mice were group housed by sex. All experiments were performed on dissected whole main olfactory epithelium (MOE) or on freshly isolated, fluorescent activated cell-sorted primary cells collected from whole main olfactory epithelium.

The Tex15 knock-out mouse line was generated and published at Yang et al 2008²⁸. Tex15 mice were crossed with Ngn-gfp²⁰ to label immediate neuronal precursors and immature olfactory sensory neurons (OSNs). Tex15 mice were crossed with Omp-IRES-GFP mice²⁹ to label mature olfactory sensory neurons (mOSNs).

For postnatal day 3, postnatal day 15, and adult single cell RNA-seq experiments, cells were sorted from male and female mice and sorted using the live cell FACs protocol below. For adult bulk RNA-seq, single-cell spatial transcriptomics, Hi-C, ChIP-seq experiments, cells were sorted from male and female mice ranging in age from 6 to 23 weeks. Biological replicate samples were collected and processed separately from different mice and as much as possible, littermate control and null mice were used for the experiments.

Fluorescent Activated Cell Sorting (FAC sorting)

FACs experiments were conducted at Rutgers Cancer Institute of New Jersey Flow Cytometry and Cell Sorting Shared Resource. Mice under P10 were sacrificed directly with cervical dislocation or mice over P10 mice were sacrificed using CO2 followed by cervical dislocation. The main olfactory epithelium (MOE) was dissected and transferred to ice-cold EBSS. The MOE was cut into small pieces with a razor blade, and then dissociated with papain (Worthington Biochemical, LK003163). Diced tissue was added to papain-EBSS, with at most 1-2 adult MOEs/mL or 2-3 young MOEs/mL, and incubated for 30-40 min at 37°C on a rocking platform.

After 30-40 min, tissue was triturated 30 times, the supernatant containing dissociated cells was transferred to a new tube, and the cells were pelleted (300 rcf, 5 min, room temperature). Remaining papain was inhibited by resuspending the cell pellet with Ovomucoid protease inhibitor solution diluted 1:10 in EBSS, and the dissociated cells were pelleted (300 rcf, 5 min, room temperature).

For live cell sorts for native ChIP-seq experiments, dissociated cells were washed once with sort media (PBS with 2% Fetal Bovine Serum), and then resuspended in sort media supplemented with 100 U/mL DNase I, 4 mM MgCl₂, and 500 ng/mL DAPI. These cells were passed through a 40 µm cell strainer, and then FAC sorted. Live cells were selected by gating out DAPI positive cells.

For live cell sorts for single-cell experiments, dissociated cells were washed once with wash media (1xPBS with 10% Fetal Bovine Serum, 5µg/ml Actinomycin, 10µg/ml Anisomycin, 10uM of Triptolide, 25U RNase inhibitor), and then resuspended in sort media (1xPBS with 0.2% Fetal Bovine Serum, 5µg/ml Actinomycin, 10µg/ml Anisomycin, 10uM of Triptolide, 25U RNase inhibitor, 100 U/mL DNase I, 4 mM MgCl₂, and 500 ng/mL DAPI) and collected in collection media¹¹. These cells were passed through a 40 µm cell strainer, and then FAC sorted. Live cells were selected by gating out DAPI positive cells.

For formaldehyde-fixed cell sorts, dissociated cells were resuspended in PBS + 1% methanol-free formaldehyde (Pierce). Cells were fixed at room temperature for 5 min, and then fixation was quenched by adding 1/10th volume of 1.25M glycine. Fixed cells were pelleted (500 rcf, 5 min, room temperature), washed once with sort media, resuspended in sort media, passed through a 40 µm cell strainer, and then FAC sorted.

Native ChIP-seq

The MOE from 9 - 23 weeks old mice of Tex15^{+/+} and Tex15^{-/-} were dissected and native chromatin was prepared as described before in Magklara et al 2011²⁰. Briefly, nuclei were extracted and digested with micrococcal nuclease (MNase, Sigma), and then the digested chromatin was used in chromatin immunoprecipitation assays. The antibodies used were specific to H3 monomethyl lysine-9 (ab8896). All ChIP experiments were performed multiple times using different chromatin preparations.

Bulk RNA-Seq

Live sorted cells were pelleted (15 min, 800 rcf, 4°C), the supernatant was aspirated until 250 µL of media remained, and then the cell pellet was resuspended in 750 µL Trizol LS (ThermoFisher). Total RNA was extracted by adding 200 µL chloroform, vortexing for 15 s, incubating at room temperature for 2 min, then centrifugation at 12,000 rcf for 15 min at 4°C. The aqueous phase was collected and RNA was precipitated with isopropyl alcohol with 10 µg/mL linear acrylamide (ThermoFisher) added as a carrier. The RNA pellet was washed twice with 75% ethanol, dried, then resuspended in nuclease free water. 1 µg of RNA was DNase treated using the TURBO DNA-free Kit (ThermoFisher) according to manufacturer's instructions.

RNA-seq libraries were prepared from DNase-treated RNA using a TruSeq Stranded Total RNA with Ribo-Zero Gold Set B kit (Illumina RS-1222302).

Spatial Transcriptomics

Whole *Tex15*^{-/-} and *Tex15*^{+/+} MOE of 6-7 weeks of age tissue was extracted, flash frozen with isopentane, and prepared for sectioning according to the fresh frozen tissue protocol outlined by 10x Cytassist Spatial Gene Expression for Fresh Frozen Tissue Preparation guide CG000636, Rev A. 10 µm cryosections of tissue were prepared in a clean, RNA-free cryostat on Superfrost slides. Sections were prepared for Hematoxylin & Eosin staining before starting Cytassist workflow to ensure quality and intactness of tissue using 10x Cytassist protocol number CG000614, Rev A. We prepared spatial transcriptome libraries from 6-7 weeks old adult wildtype and *Tex15* knockout MOEs using Visium Cytassist Spatial Gene Expression workflow v4 (CG000495, Rev G). This Cytassist-based method has spatial spots that capture multiple cells within a 55 µm diameter and 45 µm distance between spots. Barcoded cDNA libraries of tissue sections were generated using the Spatial Gene Expression Reagent Kit (10x Genomics, PN-1000521). Data were demultiplexed and processed using SpaceRanger v4.0.1. Reads were aligned to the mm10 2020-A reference mouse transcriptome (10x Genomics) and using 10x mouse probe set 1 and OR transcriptome from Barnes et al 2020⁷.

Single Cell Genomics

MOE was dissected at postnatal day 3, 14 or 56 from *Tex15*^{-/-} and *Tex15*^{+/+} littermate controls. Single cell RNA-seq libraries were generated using either Chromium single cell 3' reagent kits v2 (adult replicates - PN-120267), v3.1 (both postnatal day 3 and 14 replicates PN-1000269, using 10x Chromium single cell 3' protocol CG000315, Rev F) or v4.0 (postnatal day 3, PN-1000686, using 10x Chromium single cell 3' protocol v4.0 CG000731, Rev B).

Hi-C

In situ Hi-C libraries were prepared exactly as described in Monahan et al 2019³⁰.

Sequencing

Sequencing libraries were profiled on a Bioanalyzer 2100 using a high sensitivity DNA kit (Agilent) or Bioanalyzer Tapestation using a D5000 or D1000 DNA kit. Library concentration was determined by KAPA assay (KAPA Biosystems) or with Colibri™ Library Quantification Kit. Libraries were multiplexed and the pooled libraries were sequenced in either Illumina NovaSeq X or NextSeq 500.

Immunofluorescence

MOE was dissected at postnatal day 7 and fixed in 4% PFA for 30 min on ice prior to being embedded in OCT. Coronal cryosections were taken at a thickness of 12 to 14 µm and then air dried for 10 min. Slides were fixed with 4% PFA for 10 min. After fixation, slides were washed with PBST (PBS with 0.1% Triton X-100), blocked in PBST-DS (PBST +4% donkey serum). Slides were stained with primary antibody (*Tex15*, diluted 1:10, *Gfp*, diluted 1:1000, *OMP*, diluted 1:1000) in PBST-DS overnight at 4°C. Slides were then washed, stained with DAPI (2.5

ug/mL) and secondary antibody (Cy3 goat anti-mouse, diluted 1:1000, donkey a-rabbit conjugated to Alexa-488, diluted 1:1000, ThermoFisher) in PBST-DS for 1 hr, washed, and then mounted with Vectashield and imaged. Tex15 antibody was used from Boutros Ghali et al 2025⁴².

Microscopy/Imaging

Confocal Images were collected with a Leica Confocal TCS SP8 built on a DMI8 microscope in the SAS-HGINJ Core Facility. Images were processed in the LAS X software associated with Leica microscope and Fiji software.

Analysis Methods

Bulk RNA-Seq Analysis

Data processing and analysis was performed as previously described Monahan et al 2017⁴³. Adaptor sequences were removed from raw sequencing data with CutAdapt version 5.2. RNA-seq reads were aligned with STAR using a custom transcriptome annotation file based upon ENSEMBL release GRCm38.102, but updated to include expert curated odorant receptor gene annotation Barnes et al 2020 and Dietschi et al 2022^{7,44}. SAMtools was used to select uniquely aligning reads by removing reads with alignment quality below 30 (-q30). RNA-seq data were analysed in R with the DESeq2 package, using a 1.5 fold change threshold for the p-value calculation, with rowmeans threshold set to 2, allowing for 1,088 of 1,141 protein-coding ORs to be detected. Differentially expressed genes were identified on the basis of an adjusted p-value of less than 0.05 for this fold change threshold. DESeq2 was also used to calculate normalized transcript levels as fragments per kilobase per million, which were then used to calculate transcripts per million for each gene. Analysis and plots including all genes for Figure 1a, Extended Figure 1b, Figure 2f uses previously published data from Pourmorady et al 2024¹⁸. All other bulk-RNA-seq analysis uses protein coding genes only for both olfactory receptor and non-olfactory receptor genes. Similar results are obtained if pseudogenes and other noncoding transcripts are included.

Single Cell RNA-Seq Analysis

scRNA-seq fastq files were uniformly processed with a common Nextflow pipeline that ran Cell Ranger (v9.0.1) with a reference based on Ensembl v102 (GRCm10) genome and custom postprocessing to remove double-counted UMIs^{11,33}. Data integration and identification of OSN lineage cells was performed using scvi-tools⁴⁵. In brief, OR genes were excluded and the top 3,000 variable genes were identified using scVI's poisson_gene_selection function. The raw counts for these genes were then used to train a scVI model (n_hidden: 128, n_latent: 30, n_layers: 2, dropout_rate: 0.2, gene_likelihood: "nb"), with a batch key for each replicate, 10x chemistry as a categorical covariate, and total counts as a continuous covariate. A nearest neighbor graph (n_neighbors=25) of the resulting scVI latent embeddings was used for visualization via UMAP and clustered via the Leiden algorithm (resolution=1.6).

Clusters corresponding to OSN lineage cells were manually identified and higher quality non-doublet cells in these clusters with at least 500 genes and less than 20% of UMIs from mitochondrial genes were subclustered, and the above steps starting from variable gene

identification were rerun. Similar custom methods were used for clustering in Brann et al 2026³³. A nearest neighbor graph (n_neighbors=15) of the resulting latent space was constructed, clustered via the Leiden algorithm (resolution=0.6), and visualized via UMAP (min_dist=0.6). The resulting clusters were manually annotated based on known marker genes (e.g. *Ascl1*, *Neurog1*, *Neurod1*, *Gng8*, *Lhx2*, *Hdac2*, *Gap43*, *Stmn2*, *Gnal*, *Adcy3*, *Omp*). Immature and mature OSNs were further iteratively subclustered, gene expression program usages were calculated by applying the GEP loadings identified in Tsukahara et al 2021¹¹, and OR expression in each cell was evaluated based on the expression of functional OR genes at given thresholds (e.g. at least 1 or 3 UMIs).

Hi-C Analysis

Fastq files were aligned to the mouse genome (mm10) using the distiller-nf pipeline version 0.3.4 (<https://github.com/open2c/distiller-nf>). Uniquely mapped reads (mapq > 30) were retained, and duplicate reads were discarded. Cooler was then used to bin contacts into matrices and calculate balanced contact values using default parameters except setting “--mad-max 7” and “--min-nnz 5”, which improves coverage of OR cluster regions. Data pooled from two biological replicates were analysed together, after the results of analyses of individual replicates had been confirmed to be similar. Balanced Hi-C contacts were analyzed and visualized in R using Genova v.1.0⁴⁶.

Native ChIP-Seq Analysis

Sequencing reads were pre-processed by trimming adapters with Cutadapt version 5.2⁴⁷. Trimmed reads were then aligned to the mouse genome (mm10) using Bowtie2 version 1.3.1⁴⁸ with default settings except for maximum insert size set to 1000 (-X 1000), which allows larger fragments to be mapped. Duplicate reads were removed with Samtools version 1.19.2⁴⁹, and then the uniquely mapped, properly paired reads were selected with Samtools (-q 30 -f 3). Native ChIP heatmaps were generated with deeptools⁵⁰ with OR gene bodies re-scaled to 6kb and showing 2kb flanking on each side.

Genome wide analysis of H3K9me3 ChIPseq data was performed in R with csaw⁵¹. Regions enriched for H3K9me3 were identified by performing a binned analysis, in which reads were mapped to 5 Kb and 50 Kb bins spanning the genome. The 50 Kb bins were used to calculate a global background, then enriched 5 Kb bins were identified by filtering for greater than 3-fold enriched relative to the global background. The global background for each library was used to generate a normalization factor to compare read counts between libraries. EdgeR version 4.10.1⁵² was then used to identify bins that were differentially enriched between conditions. The raw p-value for each bin was then corrected by calculating and false discovery rates (FDR). Since H3K9me3 marks tend to cover broad areas, groups of nearby bins were joined into H3K9me3-enriched “regions”, and csaw was used to calculate a region-based p-value and FDR.

To compare the fold change in H3K9me3 signal between cell-stage and *Tex15-/-* experiments, each experiment was first analyzed separately, including the identification of enriched 5 Kb bins and the calculation of fold change in H3K9me3 between conditions. Then the 5 Kb bins that

were significantly enriched in both experiments were selected, and these were used to compare fold changes across experiments.

Visium data processing and spot filtering

10x Genomics Visium spatial transcriptomics data from adult mouse main olfactory epithelium (MOE) sections were analysed in R (v4.3.1) using Seurat 5.5.1⁵³ and Harmony 2.0.5⁵⁴. The final dataset contained six wild-type and three Tex15 knockout MOE sections. Odorant receptor (OR) genes were defined using the OR annotation from Barnes et al 2020⁷. For each spot, the number of detected OR genes and total OR transcript counts were calculated from the Spatial assay counts. Spots were retained if they expressed at least two OR genes and at least three total OR transcripts. Dorsal and ventral non-OR marker genes were taken from Tuskahara et al 2021¹¹. Marker genes were split by annotated direction, yielding 611 dorsal and 739 ventral markers before intersection with genes detected in the Seurat object. To compare dorsal-ventral patterning using radial profiling across MOE sections, spot coordinates were transformed into a normalized radial coordinate. For each section, the centroid of Zone 1 spots was used as the tissue center. Euclidean distance from this centroid was calculated for every spot and min-max normalized within each section to produce a radius from 0 to 1. Spots were assigned to 100 equally spaced radial bins. Mean scaled cell DV score and mean OR-weighted DV score were calculated within each bin for each section. Genotype-level radial profiles were generated by averaging the binned section-level profiles within wild-type and Tex15 knockout groups; shaded intervals show the standard error of the mean across sections.

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