Supplemental Figures:
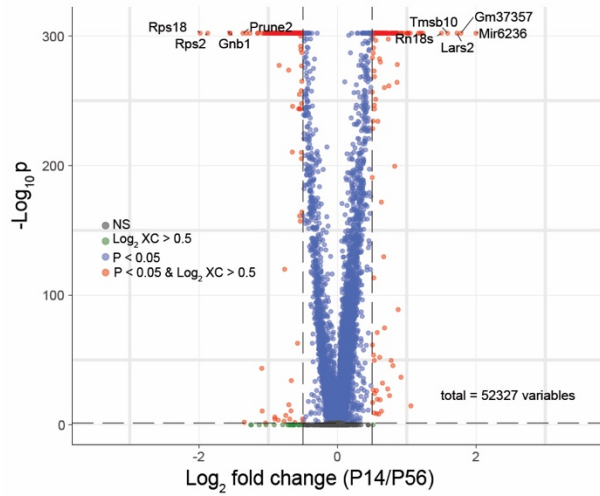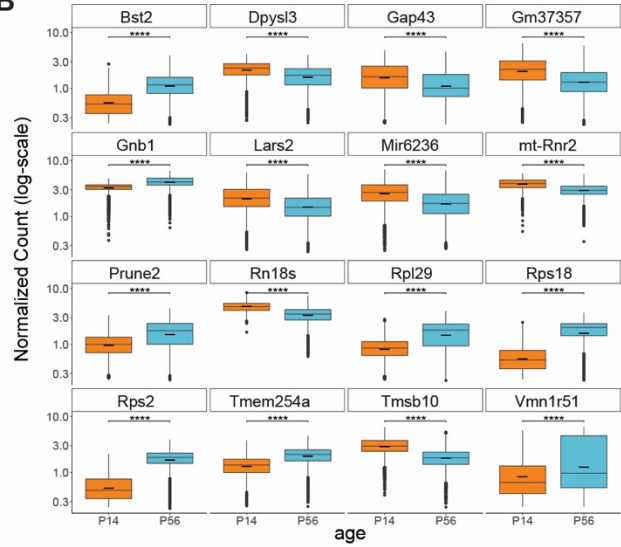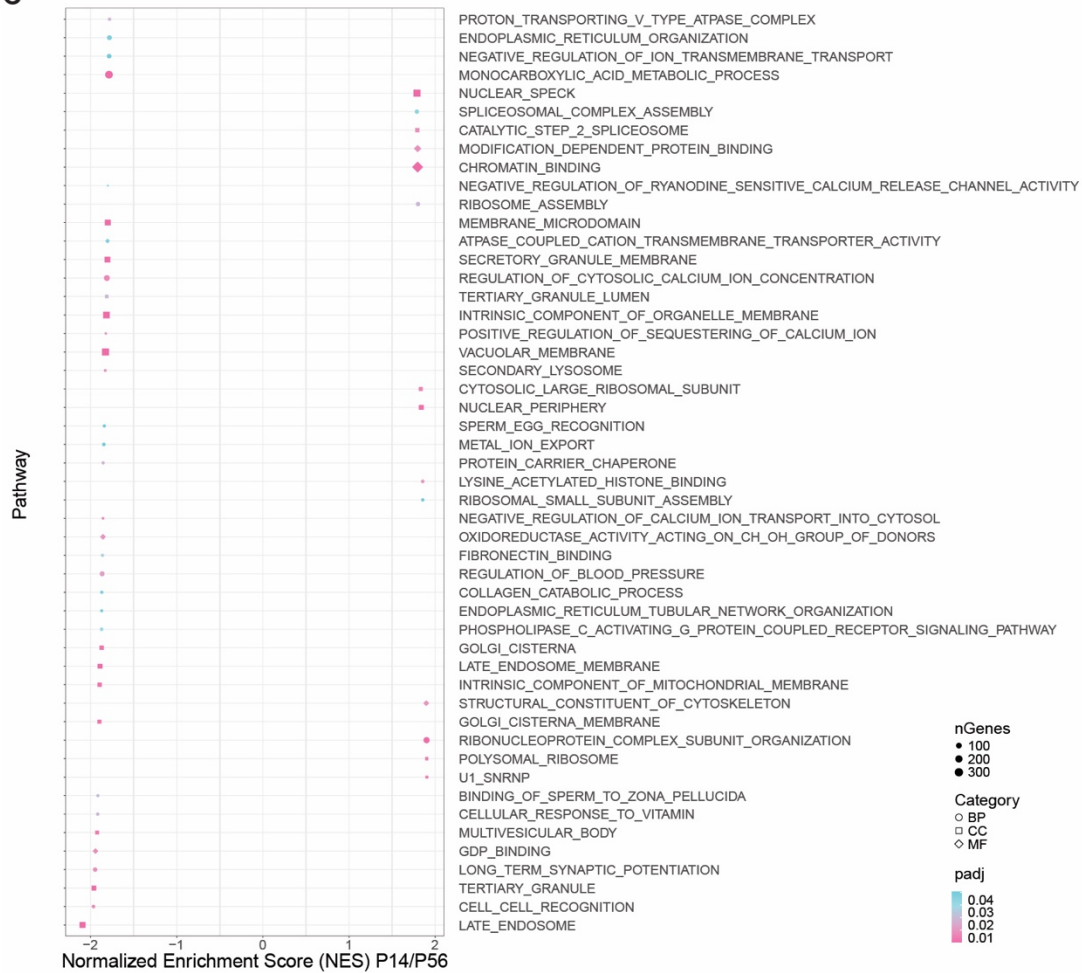
A — Volcano plot: $-\text{Log}_{10}\,p$ versus Log_2 fold change (P14/P56). Labeled genes: Rps18, Rps2, Gnb1, Prune2, Tmsb10, Gm37357, Mir6236, Rn18s, Lars2. Legend: NS, Log_2 XC > 0.5, P < 0.05, P < 0.05 & Log_2 XC > 0.5. total = 52327 variables.

B — Normalized Count (log-scale) boxplots by age (P14, P56) for: Bst2, Dpysl3, Gap43, Gm37357, Gnb1, Lars2, Mir6236, mt-Rnr2, Prune2, Rn18s, Rpl29, Rps18, Rps2, Tmem254a, Tmsb10, Vmn1r51. All marked ****.

C — Pathway dot plot: Normalized Enrichment Score (NES) P14/P56.

PROTON_TRANSPORTING_V_TYPE_ATPASE_COMPLEX
ENDOPLASMIC_RETICULUM_ORGANIZATION
NEGATIVE_REGULATION_OF_ION_TRANSMEMBRANE_TRANSPORT
MONOCARBOXYLIC_ACID_METABOLIC_PROCESS
NUCLEAR_SPECK
SPLICEOSOMAL_COMPLEX_ASSEMBLY
CATALYTIC_STEP_2_SPLICEOSOME
MODIFICATION_DEPENDENT_PROTEIN_BINDING
CHROMATIN_BINDING
NEGATIVE_REGULATION_OF_RYANODINE_SENSITIVE_CALCIUM_RELEASE_CHANNEL_ACTIVITY
RIBOSOME_ASSEMBLY
MEMBRANE_MICRODOMAIN
ATPASE_COUPLED_CATION_TRANSMEMBRANE_TRANSPORTER_ACTIVITY
SECRETORY_GRANULE_MEMBRANE
REGULATION_OF_CYTOSOLIC_CALCIUM_ION_CONCENTRATION
TERTIARY_GRANULE_LUMEN
INTRINSIC_COMPONENT_OF_ORGANELLE_MEMBRANE
POSITIVE_REGULATION_OF_SEQUESTERING_OF_CALCIUM_ION
VACUOLAR_MEMBRANE
SECONDARY_LYSOSOME
CYTOSOLIC_LARGE_RIBOSOMAL_SUBUNIT
NUCLEAR_PERIPHERY
SPERM_EGG_RECOGNITION
METAL_ION_EXPORT
PROTEIN_CARRIER_CHAPERONE
LYSINE_ACETYLATED_HISTONE_BINDING
RIBOSOMAL_SMALL_SUBUNIT_ASSEMBLY
NEGATIVE_REGULATION_OF_CALCIUM_ION_TRANSPORT_INTO_CYTOSOL
OXIDOREDUCTASE_ACTIVITY_ACTING_ON_CH_OH_GROUP_OF_DONORS
FIBRONECTIN_BINDING
REGULATION_OF_BLOOD_PRESSURE
COLLAGEN_CATABOLIC_PROCESS
ENDOPLASMIC_RETICULUM_TUBULAR_NETWORK_ORGANIZATION
PHOSPHOLIPASE_C_ACTIVATING_G_PROTEIN_COUPLED_RECEPTOR_SIGNALING_PATHWAY
GOLGI_CISTERNA
LATE_ENDOSOME_MEMBRANE
INTRINSIC_COMPONENT_OF_MITOCHONDRIAL_MEMBRANE
STRUCTURAL_CONSTITUENT_OF_CYTOSKELETON
GOLGI_CISTERNA_MEMBRANE
RIBONUCLEOPROTEIN_COMPLEX_SUBUNIT_ORGANIZATION
POLYSOMAL_RIBOSOME
U1_SNRNP
BINDING_OF_SPERM_TO_ZONA_PELLUCIDA
CELLULAR_RESPONSE_TO_VITAMIN
MULTIVESICULAR_BODY
GDP_BINDING
LONG_TERM_SYNAPTIC_POTENTIATION
TERTIARY_GRANULE
CELL_CELL_RECOGNITION
LATE_ENDOSOME

nGenes: 100, 200, 300
Category: BP (○), CC (□), MF (◇)
padj: 0.04, 0.03, 0.02, 0.01

Pathway (y-axis); Normalized Enrichment Score (NES) P14/P56 (x-axis)

Fig. S1. Age differences in gene expression.
A Volcano plot of gene expression differences between P14 and P56. **B**. 16 significantly differentially expressed genes with largest positive or negative log$_2$-fold-change values. **C**. 50 significantly enriched GO terms with largest positive or negative log$_2$-fold-change values.
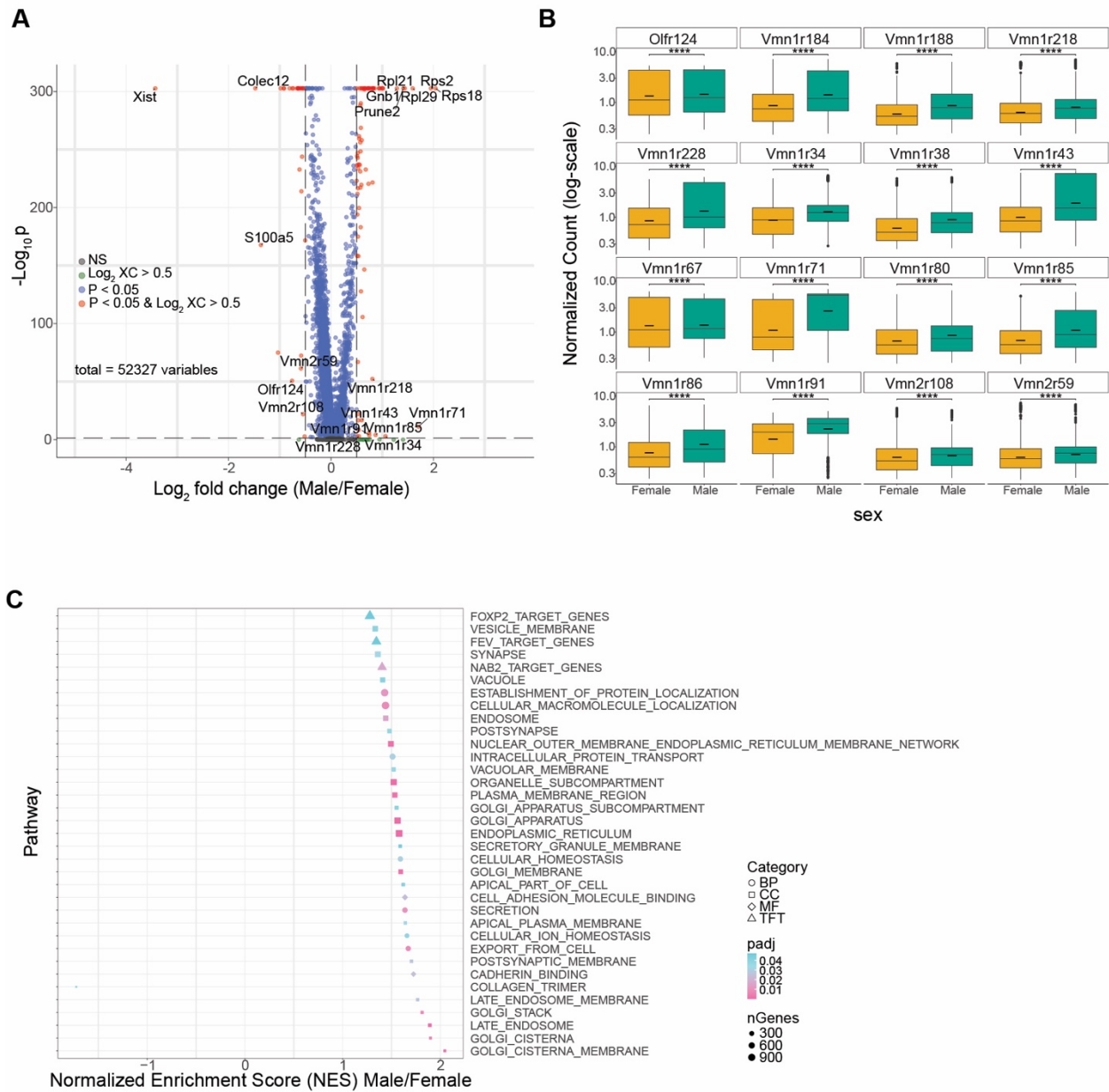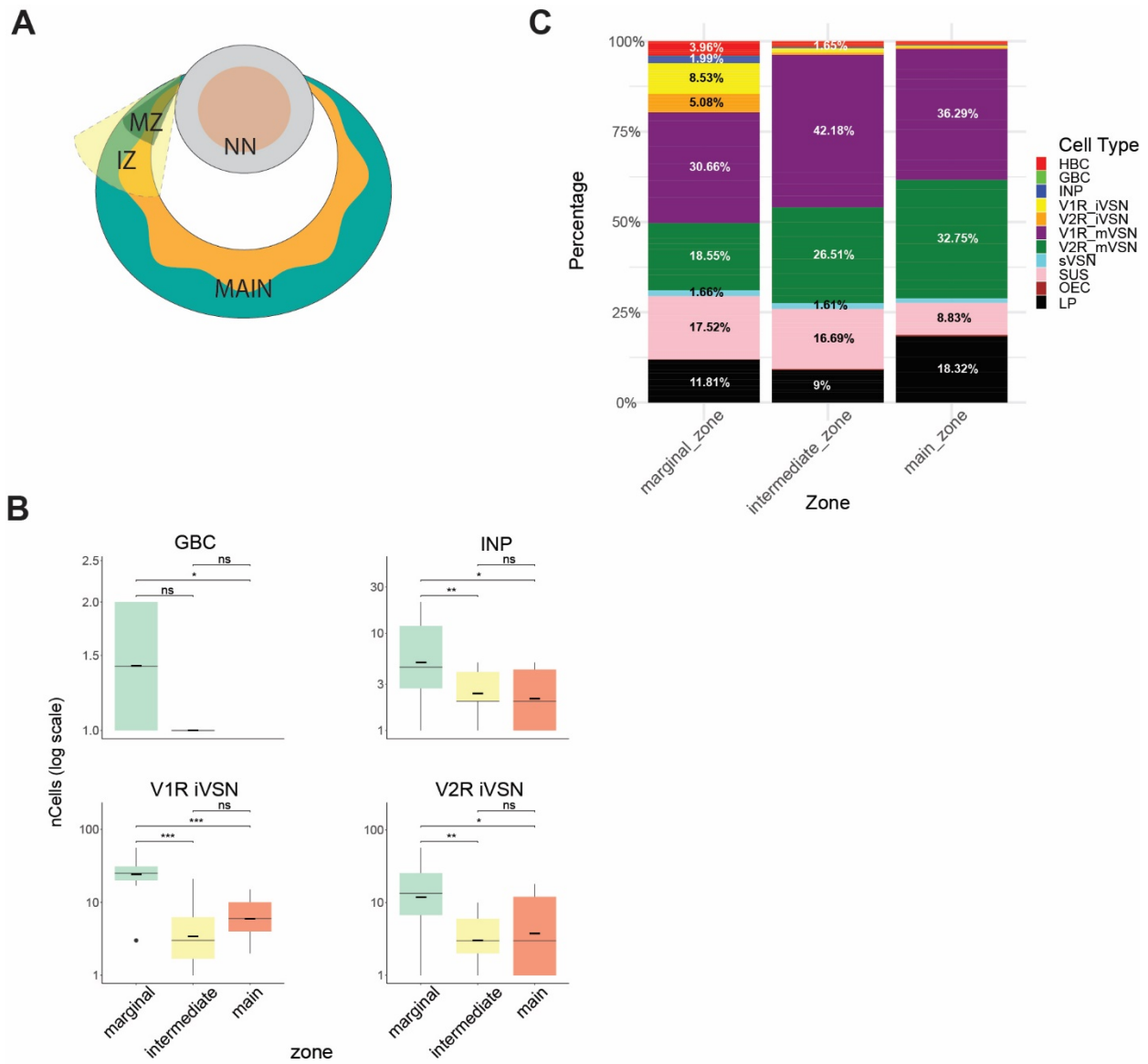
Fig. S2. Sex differences in gene expression.
A Volcano plot of gene expression differences between male and female mice. **B**. 16 significantly differentially expressed chemosensory receptors with largest positive or negative $\log_2$-fold-change values. **C**. 35 significantly enriched GO terms.

Fig. S3. Zonal distribution of cell types in the VNO neuroepithelia.
A. Schematic indicating quantification of cells in the marginal, intermediate, and main zones.
B. Stacked bar-plot of cell-type proportions by VNO zone. **C**. Box plots of GBC, INP, and immature VSN cell counts by zone, across 13 slides.
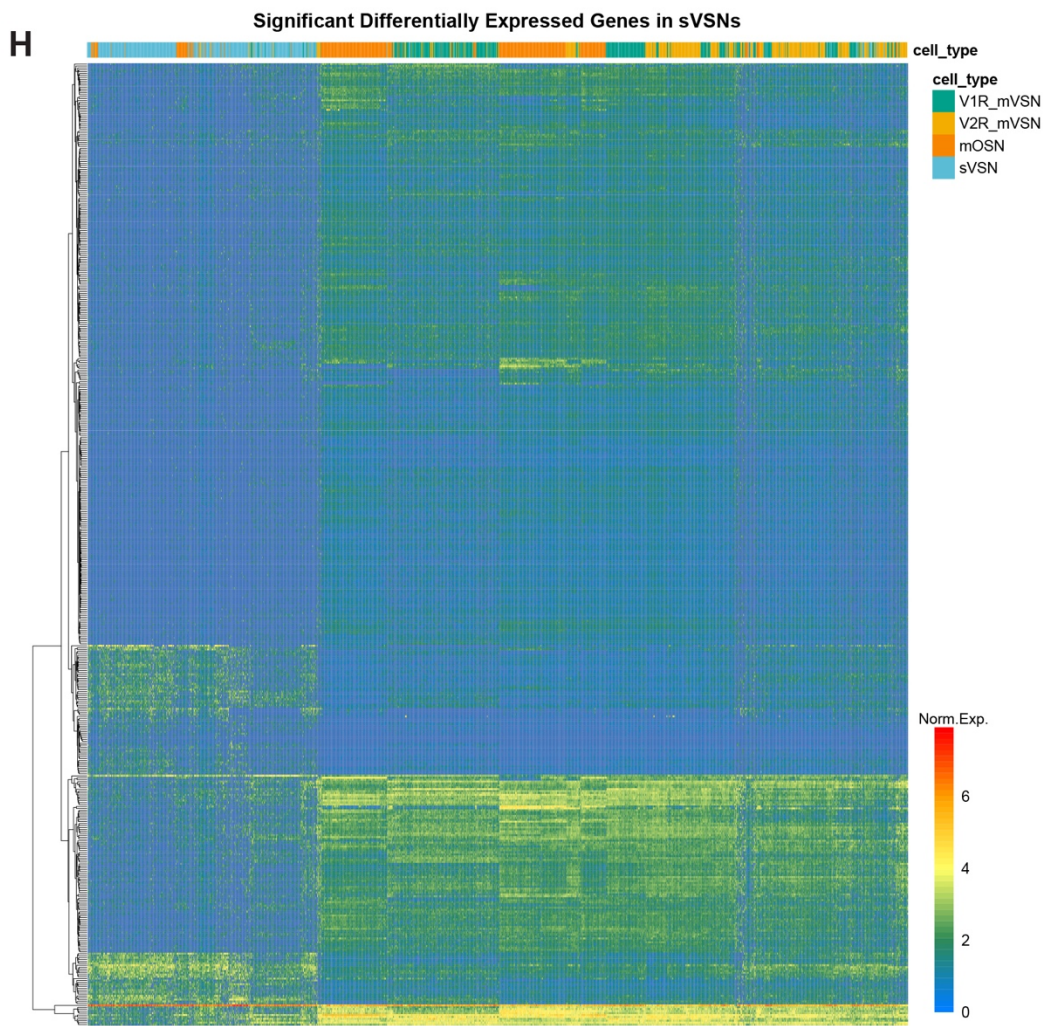
A Gap43

B Stmn2

C Female   Male

D Gnai2 in Mature V2R Neurons

E Total Genes

F Total Counts

G Ribosomal (Percent)

Cell Type: V1R_mVSN   V2R_mVSN   mOSN   sVSN

H Significant Differentially Expressed Genes in sVSNs
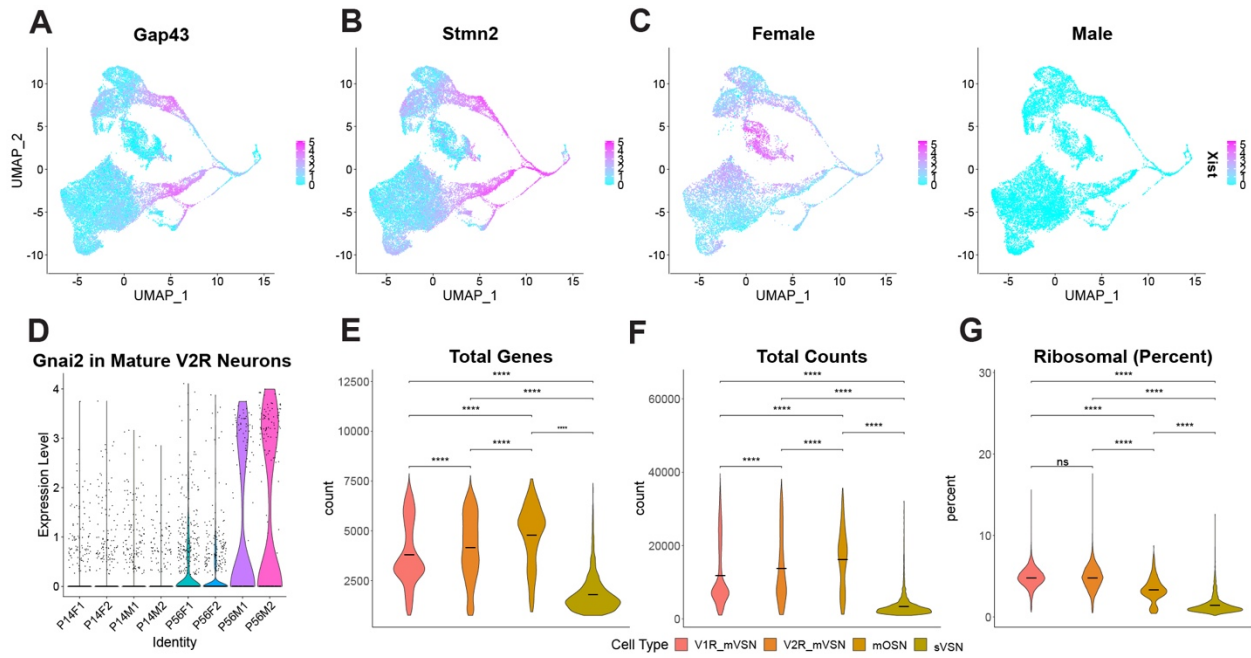
cell_type
V1R_mVSN
V2R_mVSN
mOSN
sVSN

Norm.Exp.

Fig. S4. Neuronal lineage features and differentially expressed genes in sVSNs.
A. and **B**. Normalized Gap43 and Stmn2 expression in the neuronal lineage. **C**. Normalized Xist expression in the neuronal lineage, split by sex. **D**. Violin plot of normalized Gnai2 expression in mature V2R neurons, split by sample. **E-G**. Total number of genes, counts, and percent ribosomal gene expression detected in mature neurons, split by cell type. **H**. Heatmap of 503 significant ($p_{adj} \leq 0.001$, fold-change ≥ 1.5) differentially expressed genes in sVSNs.
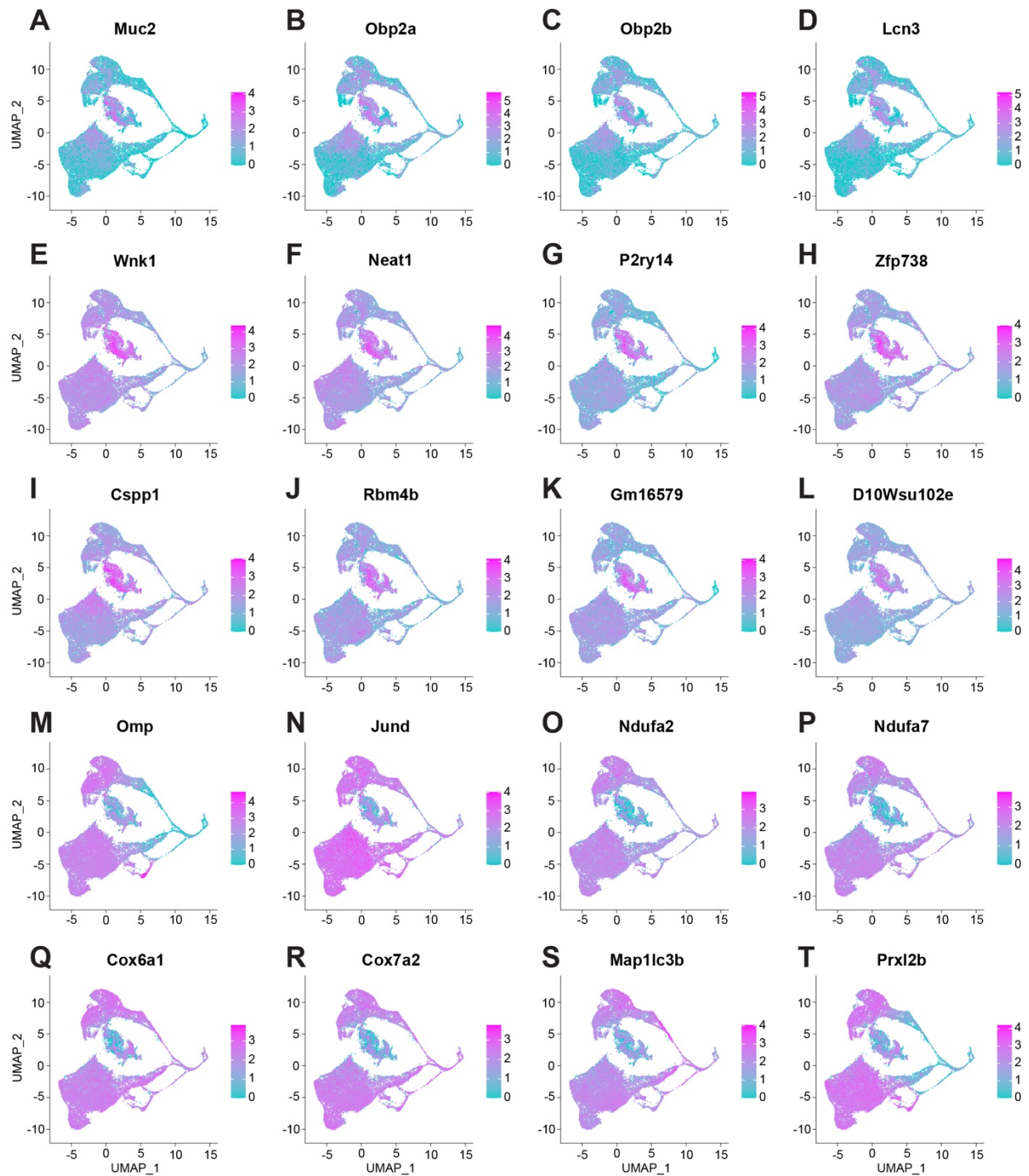
Fig. S5. Significantly regulated genes in sVSNs.

A-D. Feature plots showing odor binding protein gene expression (Muc2, Obp2a, Obp2b, and Lcn3).
E-L. Top upregulated genes in sVSNs. **M-T**. Top downregulated genes in sVSNs.

A

UMAP_2

Cell Type
- V1R_mVSN
- V2R_mVSN
- sVSN
- V1R_iVSN
- V2R_iVSN
- late_INP
- early_INP
- GBC

UMAP_1

B

UMAP_2

PseudoTime
200
150
100
50

UMAP_1

C

- NS
- Log$_2$ FC
- p-value
- p-value and log$_2$ FC

−Log$_{10}$ P

Ndfip1   Stoml3
Atox1 Gnal   S100a5
           Olfr124

Pebp1
Calr4
Zpld2

Gnai2

Log$_2$ fold change

total = 52327 variables

D

mOSN Gene−Set Enrichment Analysis

Pathway

NUCLEAR_OUTER_MEMBRANE_ENDOPLASMIC_RETICULUM_MEMBRANE_NETWORK
ORGANELLE_SUBCOMPARTMENT
ENDOPLASMIC_RETICULUM
MICROTUBULE_CYTOSKELETON
ZBTB7B_TARGET_GENES
SIDE_OF_MEMBRANE
ORGANELLE_INNER_MEMBRANE
MITOCHONDRIAL_PROTEIN_CONTAINING_COMPLEX
NEGATIVE_REGULATION_OF_CATALYTIC_ACTIVITY
ACTIN_CYTOSKELETON
ANCHORING_JUNCTION
CELL_SUBSTRATE_JUNCTION
MICROTUBULE_CYTOSKELETON_ORGANIZATION
MICROTUBULE_BASED_PROCESS
GENERATION_OF_PRECURSOR_METABOLITES_AND_ENERGY
MOTILE_CILIUM
INNER_MITOCHONDRIAL_MEMBRANE_PROTEIN_COMPLEX
9PLUS2_MOTILE_CILIUM
CELLULAR_RESPIRATION
MICROTUBULE_BUNDLE_FORMATION
WOUND_HEALING
PROTON_TRANSPORTING_ATP_SYNTHASE_COMPLEX
CILIARY_BASAL_BODY
BRUSH_BORDER
AEROBIC_RESPIRATION
LARGE_RIBOSOMAL_SUBUNIT
SMALL_RIBOSOMAL_SUBUNIT
OXIDATIVE_PHOSPHORYLATION
STRUCTURAL_MOLECULE_ACTIVITY
MYOSIN_COMPLEX
PROTON_TRANSPORTING_TWO_SECTOR_ATPASE_COMPLEX
CILIUM_ORGANIZATION
ATP_METABOLIC_PROCESS
CILIARY_PLASM
PROTON_TRANSMEMBRANE_TRANSPORTER_ACTIVITY
AXONEME_ASSEMBLY
PROTON_TRANSMEMBRANE_TRANSPORT
CILIUM
POLYSOMAL_RIBOSOME
RIBOSOME
CYTOSOLIC_SMALL_RIBOSOMAL_SUBUNIT
RIBOSOMAL_SUBUNIT
CYTOPLASMIC_TRANSLATION
STRUCTURAL_CONSTITUENT_OF_RIBOSOME
ENDOPLASMIC_RETICULUM_LUMEN
CYTOSOLIC_LARGE_RIBOSOMAL_SUBUNIT
CYTOSOLIC_RIBOSOME

nGenes
- 200
- 400
- 600

Category
- BP
- CC
- MF
- TFT

padj
0.04
0.03
0.02
0.01

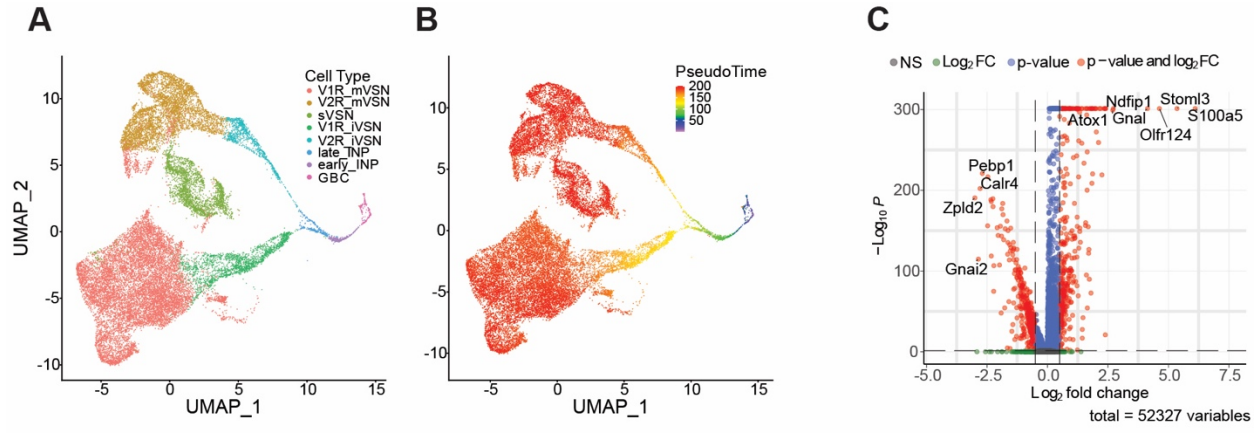Normalized Enrichment Score (NES)

Fig. S6. sVSN Pseudotime, OSN markers and GO Terms.
A. VNO Neuronal lineage cell-types in UMAP space. **B**. V1R, V2R, and sVSN lineage pseudotimes in UMAP space. **C**. Volcano plot of mOSN gene expression versus all other cells from the neuronal lineage. **D.** 45 significant GO terms enriched in OSNs versus all other cells.
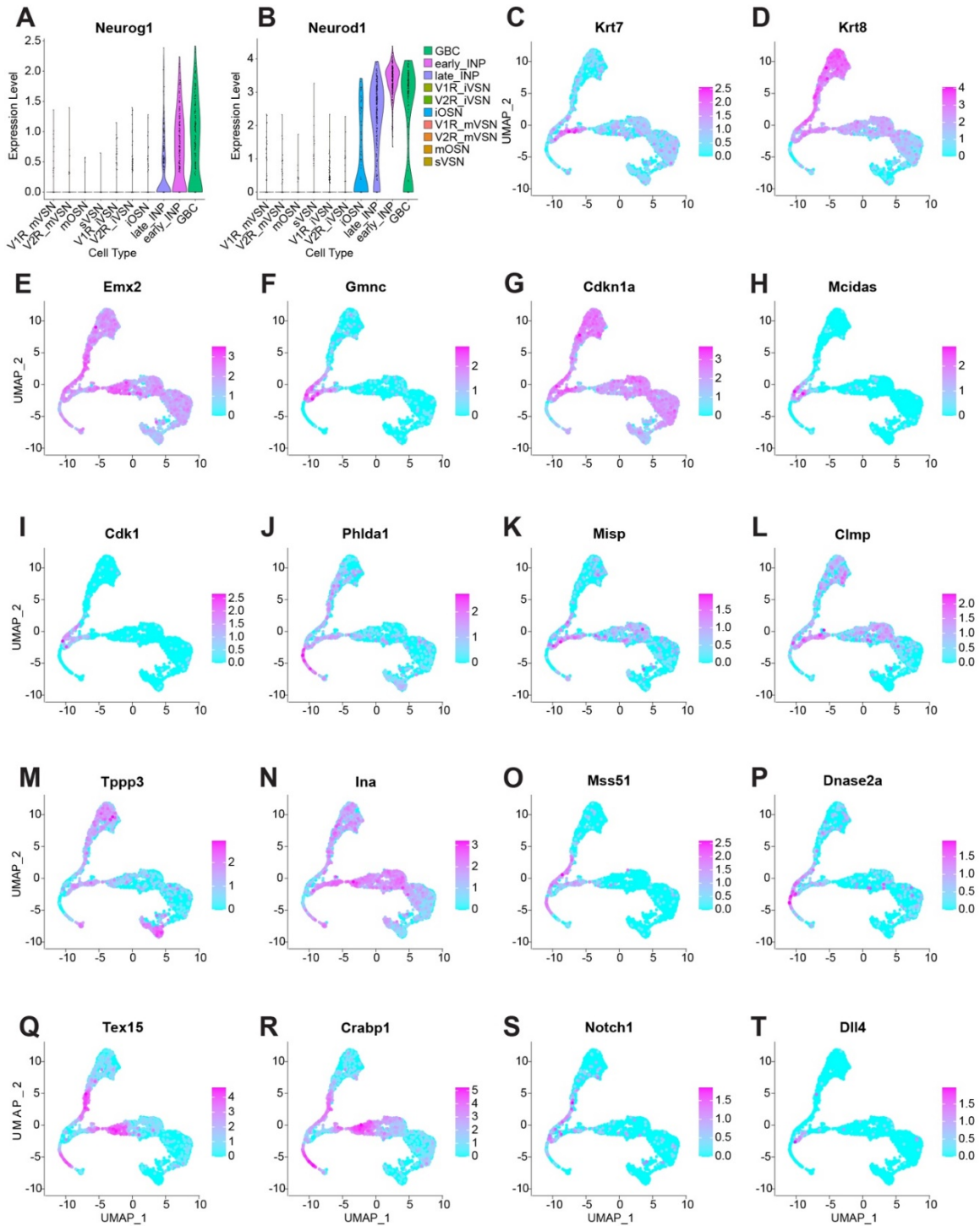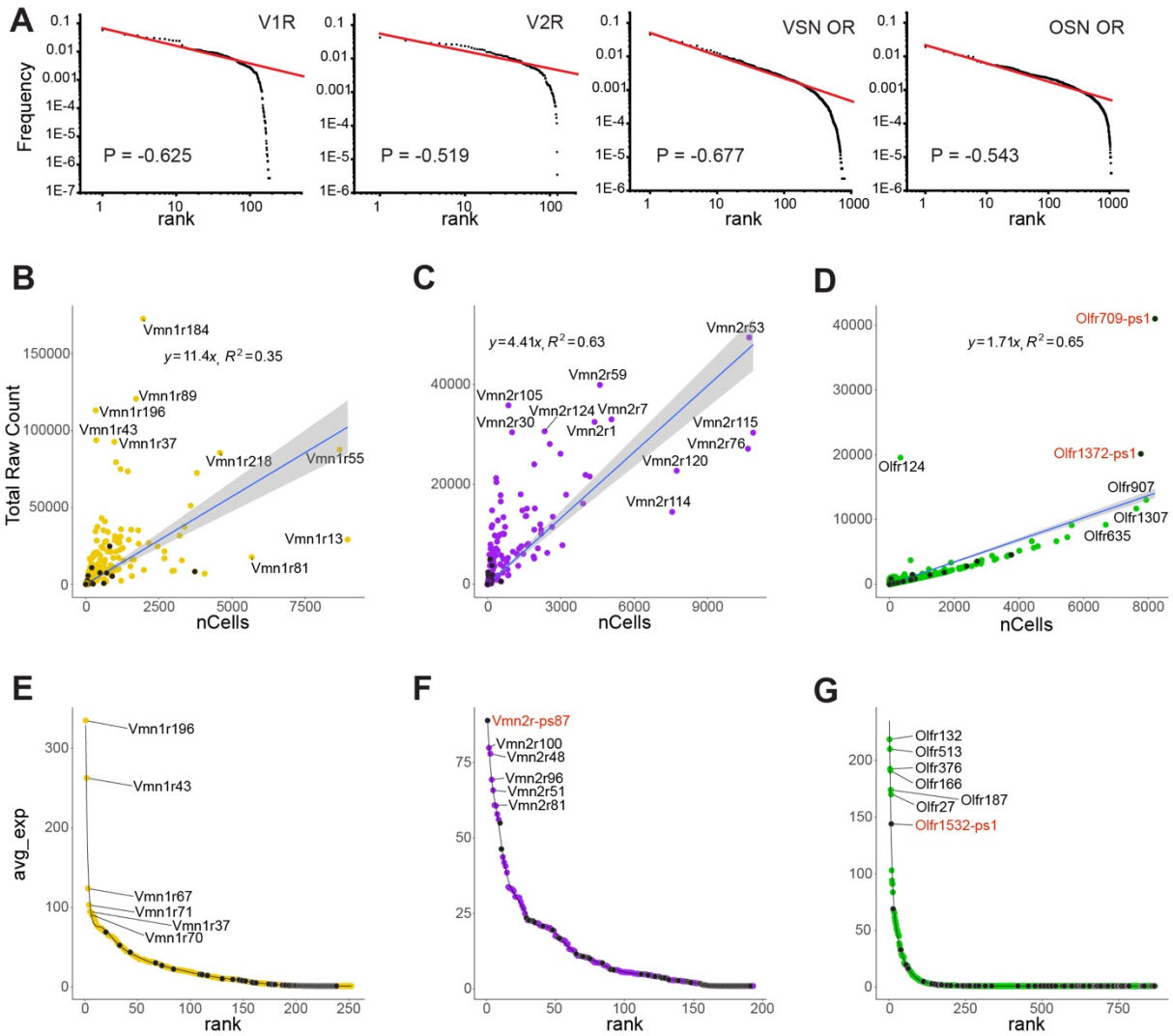
Fig. S7. Genes expressed during INP to immature neuron transition.
A and **B.** Violin plot of normalized expression for Neurog1 (A) and Neurod1 (B), split by cell type.
C-R. Feature plots of normalized gene expression for early differentially expressed genes between V1R, V2R, and OSN lineages. **S** and **T**. Feature plots of normalized expression for Notch1 (S) and Dll4 (T).

Fig. S8. Receptor distribution in the VNO.
A. Log scale rank-frequency distribution plots for V1R, V2R, VSN-OR, and OSN-OR. **B-D**.
Number of cells expressing (nCells) vs. total raw counts for V1R, V2R, and VSN-OR. **E-F**. Rank by average count distributions for V1R, V2R, and VSN-OR.
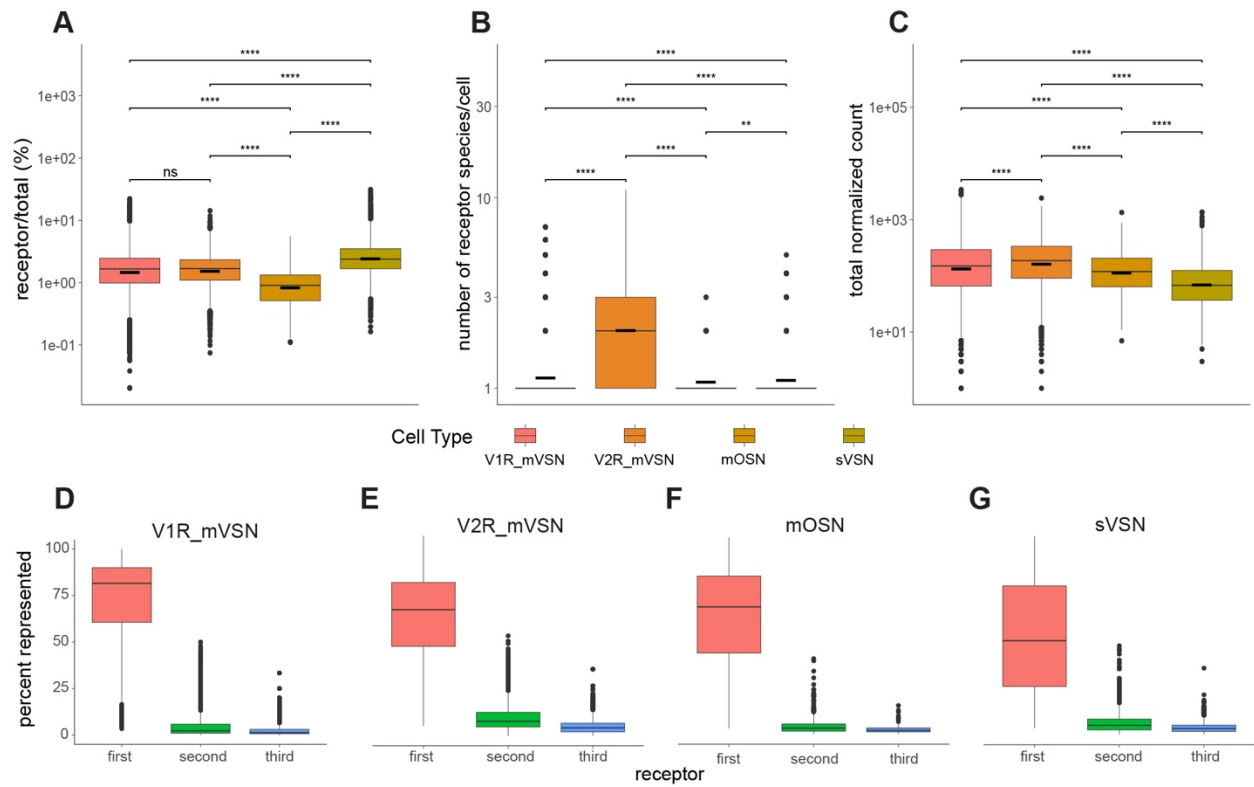
Fig. S9. Co-expression between vomeronasal receptors and transcription factors
A. Distribution density plot showing relationship between similarity of transcription factor (TF) gene expression profiles and receptor sequence similarity. **B-C**. Heatmaps showing the V1R-TF (B) and V2R-TF (C) associations. Heat shows average expression level for TF genes for a given receptor type.

Fig. S10. Receptor expression statistics.
A-C. Percent of all read counts from a receptor gene (A), number of receptor species present per cell (B), and total counts from vomeronasal receptors (C), separated by cell type. **D-G**. Proportions of first, second, and third most expressed receptor gene as percent of total receptor counts, separated by cell type.