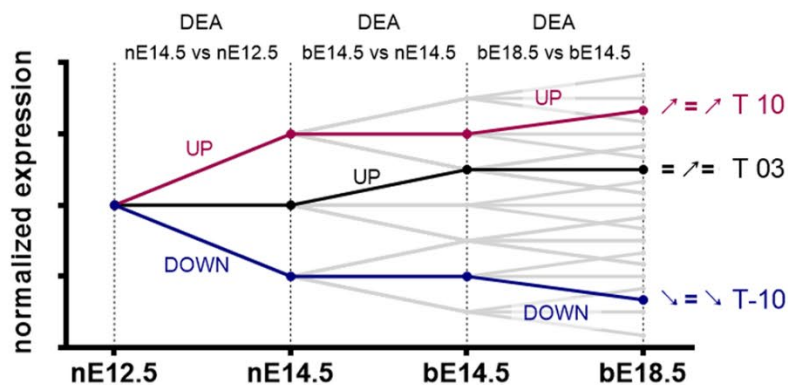


Supplementary Figure 1 | **Principal Component Analysis (PCA) of RNA sequencing data from GnRH⁺ and GnRH⁻ mouse embryonic cells.**

A

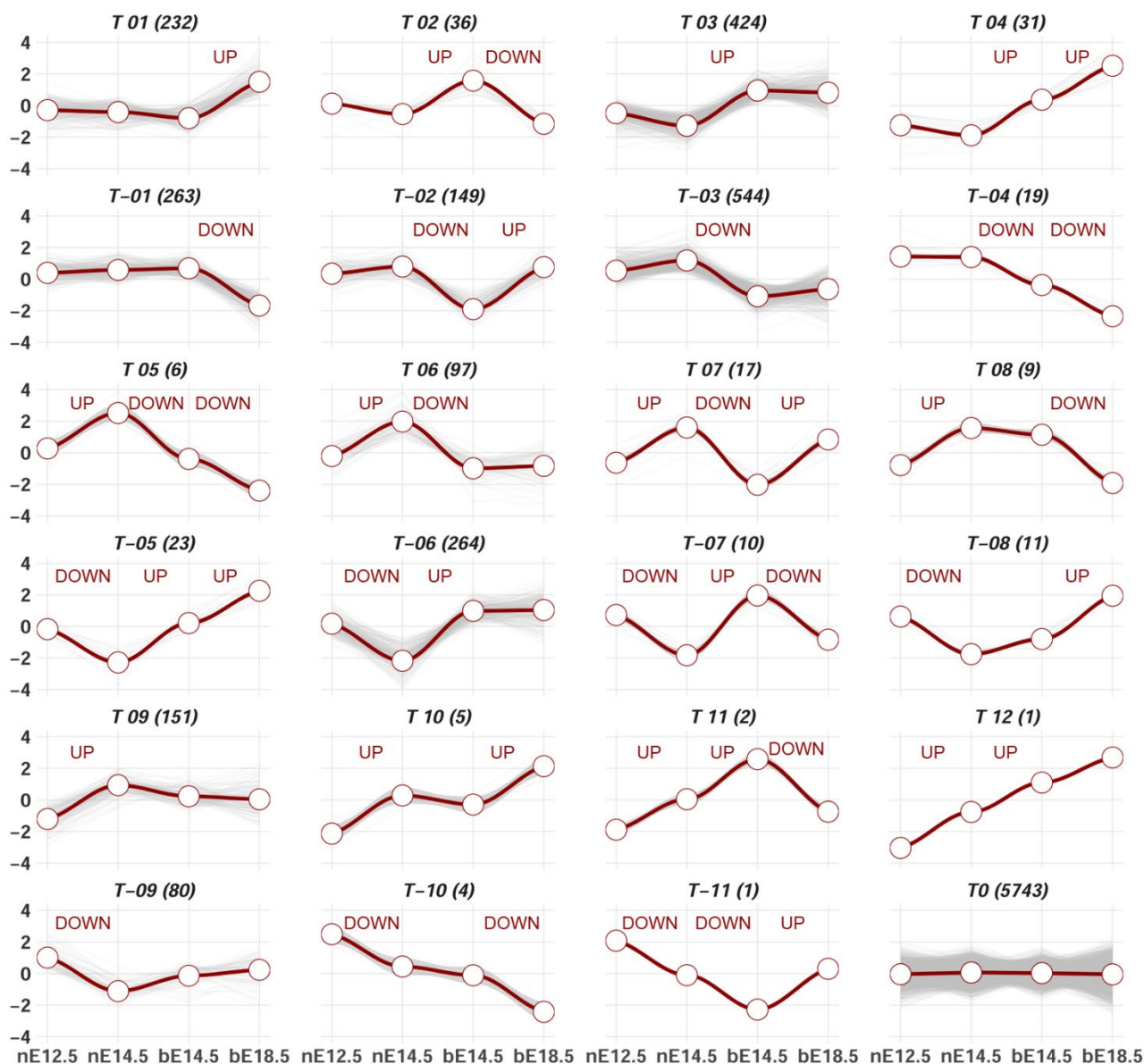
Reconstruction of gene expression trajectories



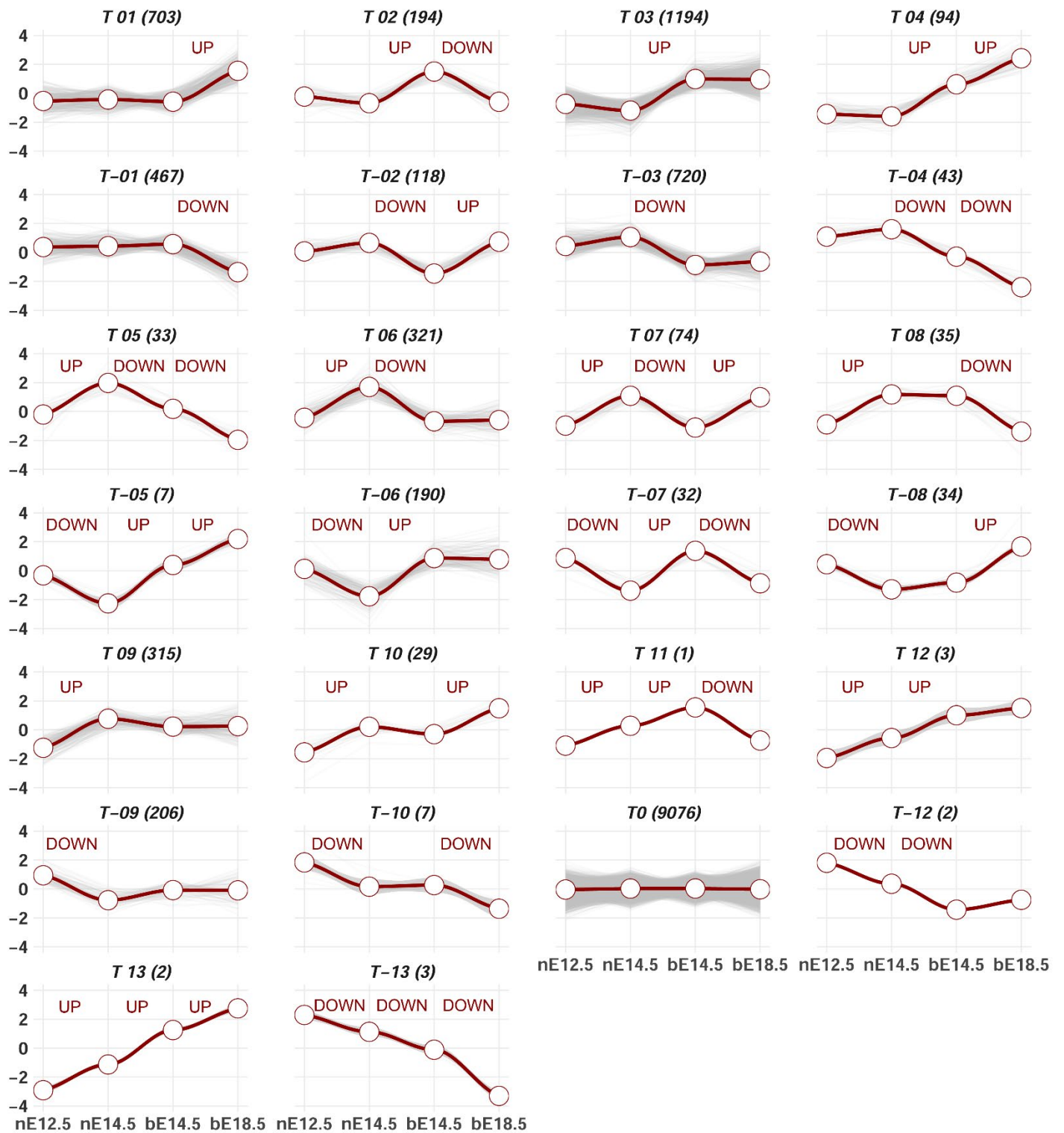
B

T0	===		
T 01	== ↗	T-01	== ↘
T 02	= ↗ ↘	T-02	= ↘ ↗
T 03	= ↗ =	T-03	= ↘ =
T 04	= ↗ ↗	T-04	= ↘ ↘
T 05	↗ ↘ ↘	T-05	↘ ↗ ↗
T 06	↗ ↘ =	T-06	↘ ↗ =
T 07	↗ ↘ ↗	T-07	↘ ↗ ↘
T 08	↗ = ↘	T-08	↘ = ↗
T 09	↗ = =	T-09	↘ = =
T 10	↗ = ↗	T-10	↘ = ↘
T 11	↗ ↗ ↘	T-11	↘ ↘ ↗
T 12	↗ ↗ =	T-12	↘ ↘ =
T 13	↗ ↗ ↗	T-13	↘ ↘ ↘

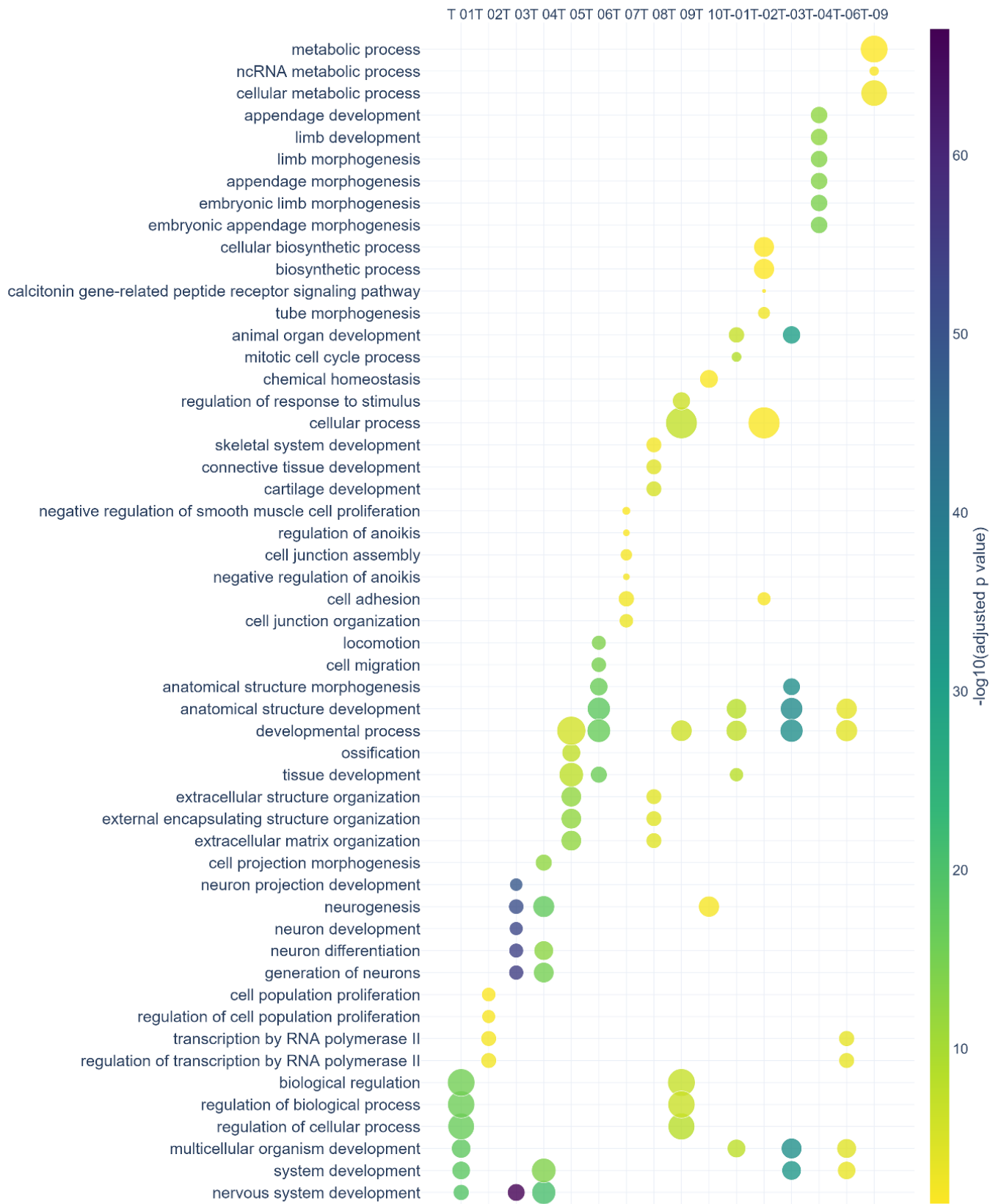
C



Supplementary Figure 2 | **GnRH⁺ cells gene expression dynamics**. (A) Algorithm to assign trajectory names to expression dynamics according to significant expression changes across consecutive developmental stages (UP: Log2FoldChange > 1 and p value < 0.01; DOWN: Log2FoldChange < -1 and p value < 0.01). (B) Pattern description of all possible trajectories in the GnRH transcriptomics dataset. (C) Expression of all detected genes in GnRH⁺ cells organized by trajectory. Grey lines represent individual genes and average gene expression is shown in red.

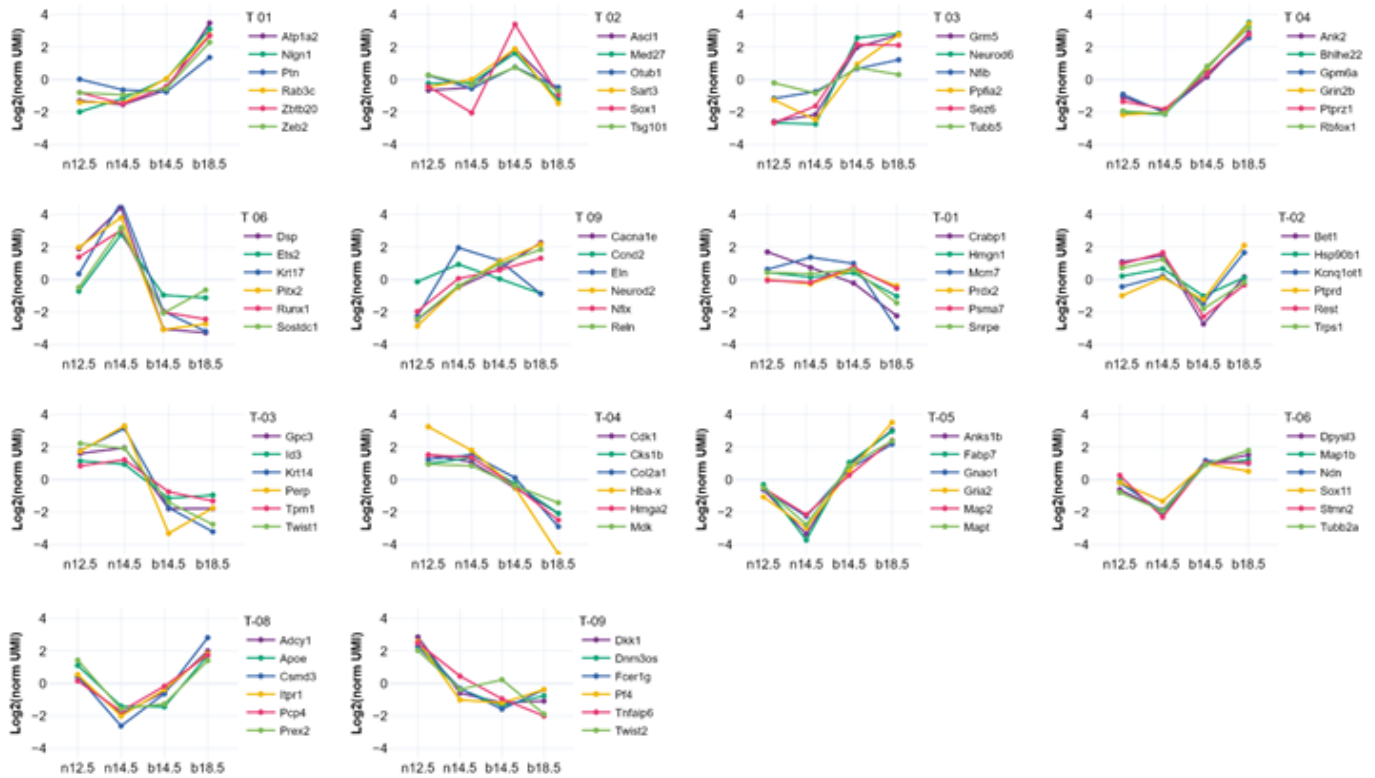


Supplementary Figure 3 | **GnRH⁺ cells gene expression dynamics.** Expression of all detected genes in GnRH⁺ cells organized by trajectory. Average gene expression is shown in red while grey lines represent individual genes.

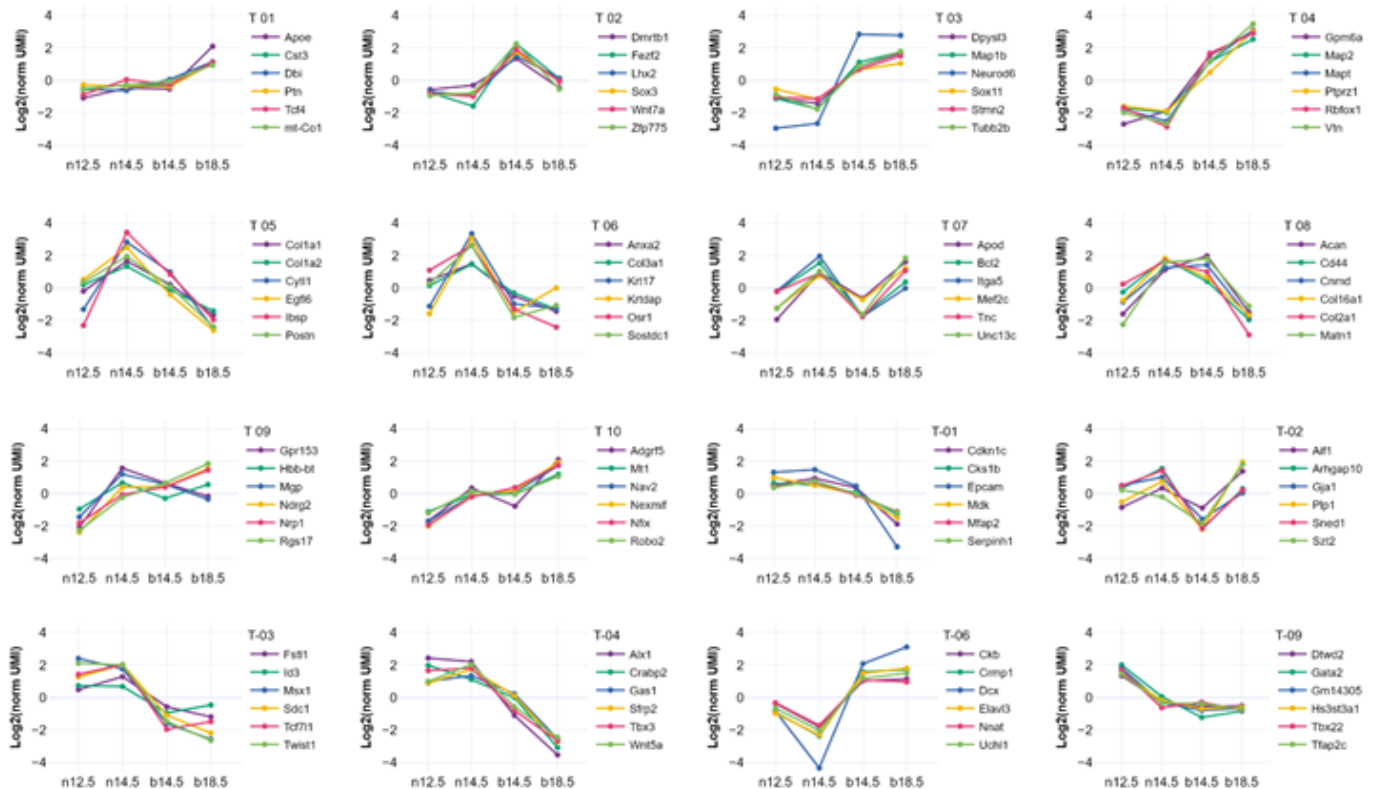


Supplementary Figure 4 | **Functional enrichment analysis in GnRH⁻ cells.** Scatter plot showing the top six significant terms in the main trajectories of GnRH⁻ cells after functional enrichment analysis within biological processes from gene ontology database.

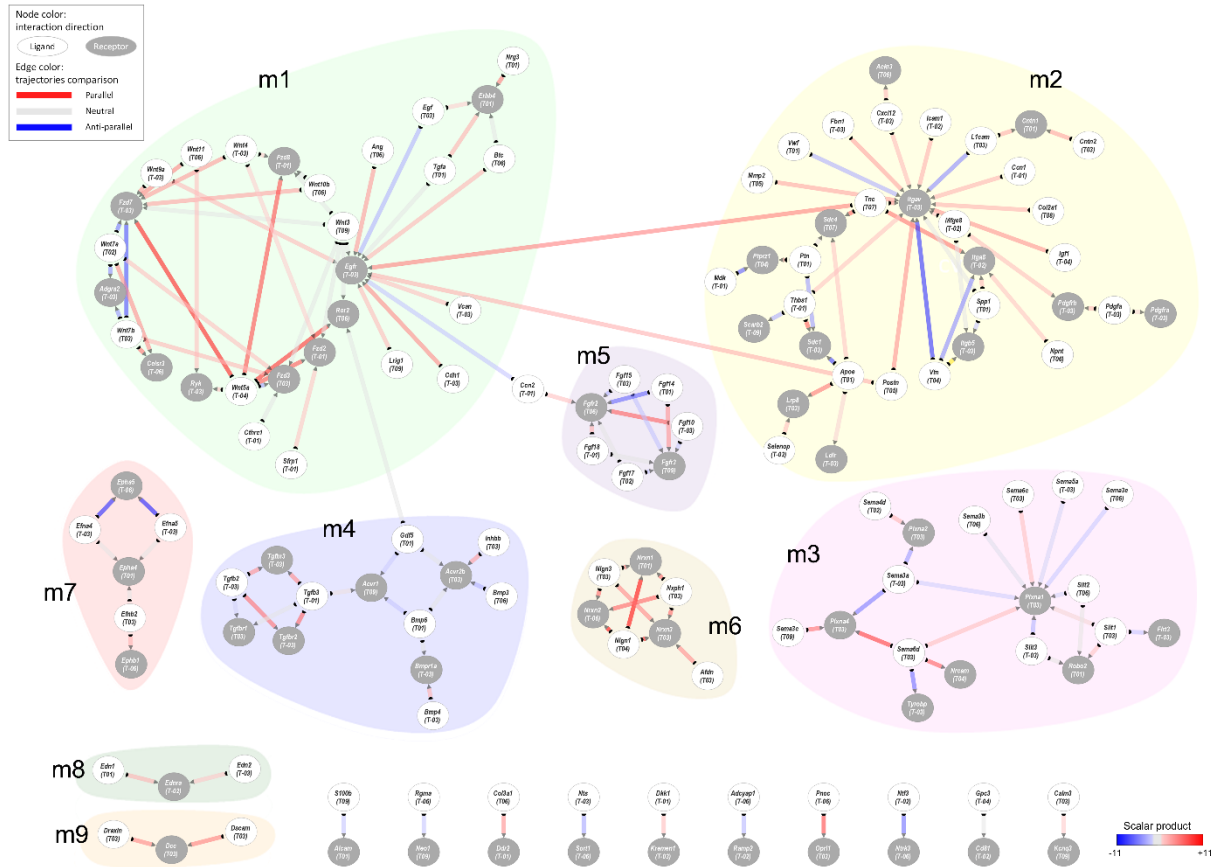
Top candidate genes in GnRH neuron trajectories



Top candidate genes in GnRH negative cell trajectories



Supplementary Figure 5 | **Expression profiles of top genes from trajectories enriched in biological processes.** Normalized expression profiles and trajectory classification of top genes emerging from representative trajectories significant after functional enrichment analysis in both GnRH⁺ and GnRH⁻ cells.



Supplementary Figure 6 | **Full dynamic cell-to-cell communication networks in GnRH neuron development.** PPI network and gene expression profiles of different modules of ligands and their respective receptors (T0 nodes are hidden for visual clarity). Node color discriminates between GnRH neurons (grey) receptors and ligands expressed in the environment (white). The degree of coordinated expression (i.e., scalar product) is color-coded on edges.

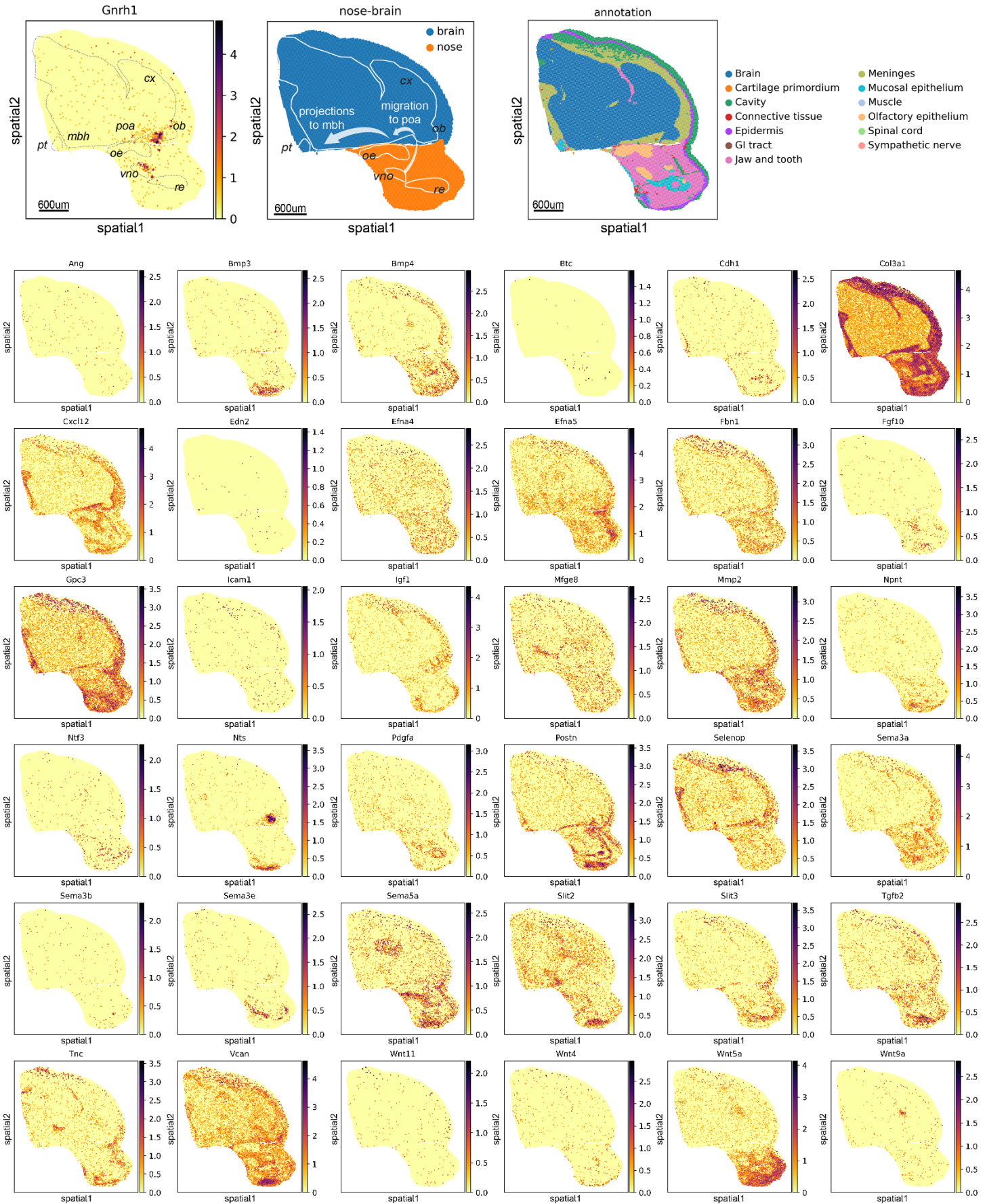


Figure 7 | Spatial transcriptomics validation of CCC-ligand genes with preferential expression in the nasal region during GnRH neuron development.

Embedding plots showing spatial visualization of transcriptomic data reanalyzed from a mouse embryo section at E13.5 (MOSTA). Annotation of anatomical regions relevant for GnRH neuron development followed by spatial visualization transcript encoding CCC-ligands predicted to be preferentially expressed in the nasal region. expression Spot size 25um. *Cx*, cortex; *pt*, pituitary; *mbh*, mediobasal hypothalamus; *poa*, preoptic

area of the hypothalamus; ob, olfactory bulb; oe, olfactory epithelium; vno, vomeronasal organ; re, respiratory epithelium. T, trajectory; SP, spatial pattern.

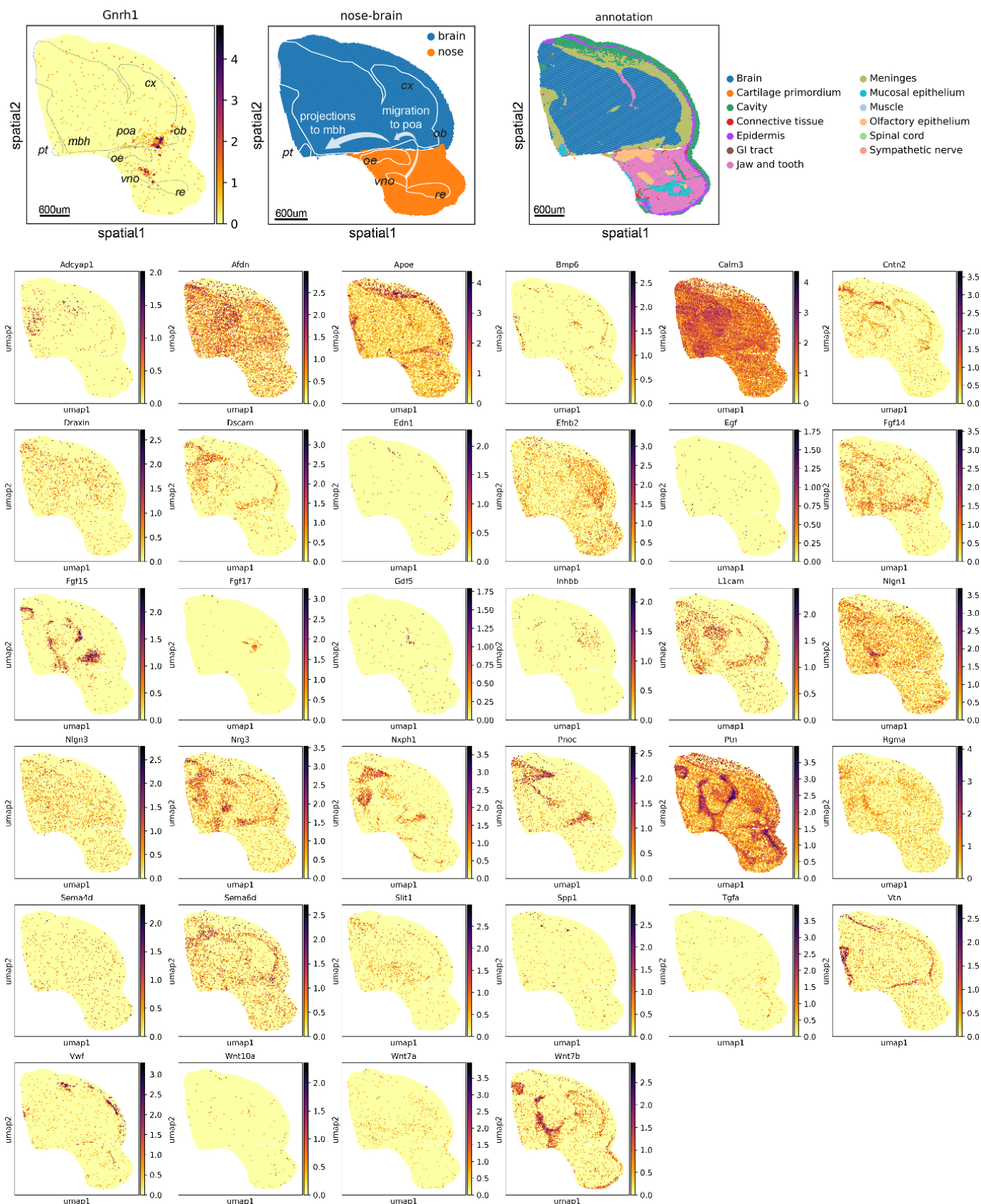


Figure 8 | Spatial transcriptomics validation of CCC-ligand genes with preferential expression in the forebrain region during GnRH neuron development.

Embedding plots showing spatial visualization of transcriptomic data reanalyzed from a mouse embryo section at E13.5 (MOSTA). Annotation of anatomical regions relevant for GnRH neuron development followed by spatial visualization transcript encoding CCC-ligands predicted to be preferentially expressed in the forebrain region. expression Spot size 25um. Cx, cortex; pt, pituitary; mbh, mediobasal hypothalamus; poa, preoptic area of the hypothalamus; ob, olfactory bulb; oe, olfactory epithelium; vno, vomeronasal organ; re, respiratory epithelium. T, trajectory; SP, spatial pattern.



Figure 9 | **Spatial transcriptomics co-localisation analysis of *Gnrh1* transcript with genes encoding CCC-receptors during GnRH neuron development.**

Embedding plots showing spatial visualization of transcriptomic data reanalyzed from a mouse embryo section at E13.5 (MOSTA). Spot size 25um.

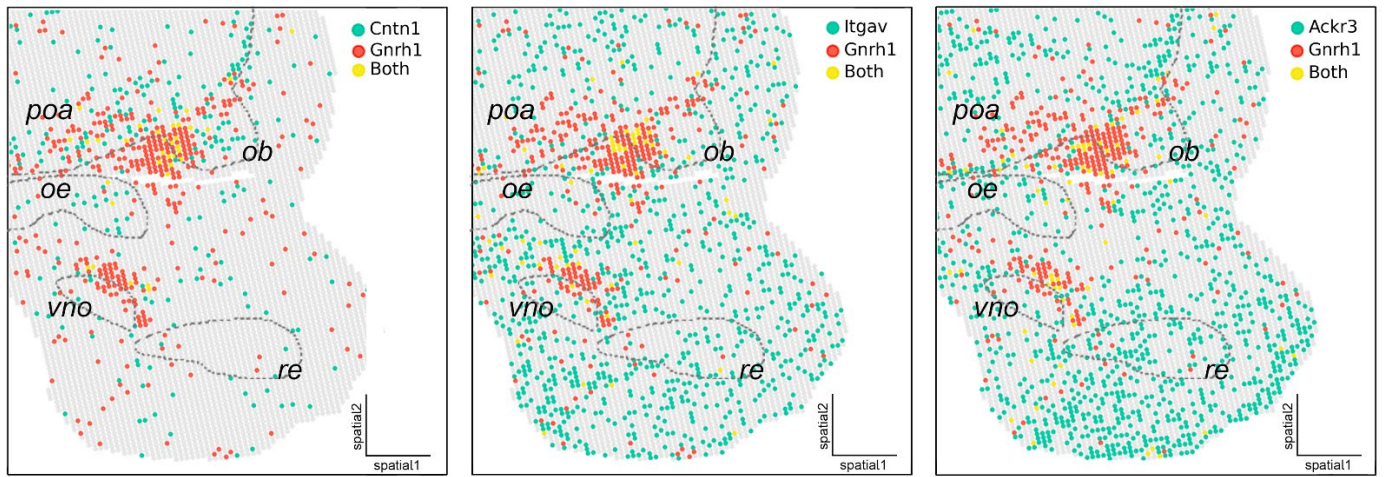


Figure 10 | Heterogeneous co-localisation of *Cntn1*, *Itgav* and *Ackr3* with *Gnrh1* transcript during GnRH neuron development.

Embedding plots illustrating heterogeneous co-localization of *Cntn1*, *Itgav* and *Ackr3* with *Gnrh1* in the context of relevant anatomical regions (gray dotted lines). Spot size 25um. 1090 Cx, cortex; pt, pituitary; mbh, mediobasal hypothalamus; poa, preoptic area of the hypothalamus; 1091 ob, olfactory bulb; oe, olfactory epithelium; vno, vomeronasal organ; re, respiratory epithelium. T, 1092 trajectory; SP, spatial pattern.

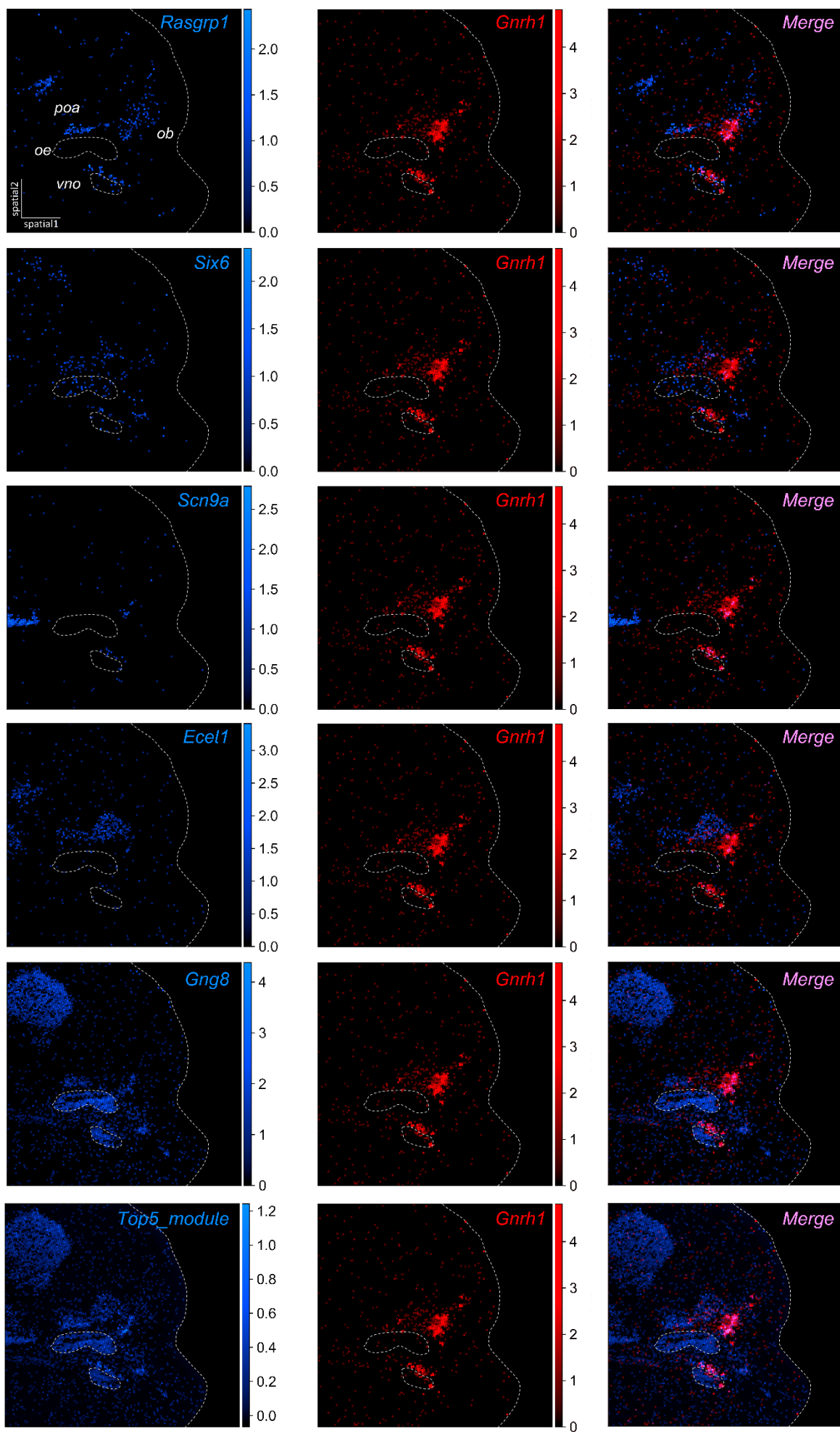


Figure 11 | Spatial transcriptomics analysis of candidate markers for GnRH neurons.

Embedding plots of transcriptomic data reanalyzed from a mouse embryo section at E13.5 (MOSTA) co-localisation of the top 5 GnRH neuron candidate marker genes with *Gnrh1* transcript. Colored scalebars represent relative gene expression intensity. Section border and relevant anatomical regions are outlined (light gray dotted lines). Spot size 25um. *poa*, preoptic area of the hypothalamus; *ob*, olfactory bulb; *oe*, olfactory epithelium; *vno*, vomeronasal organ.

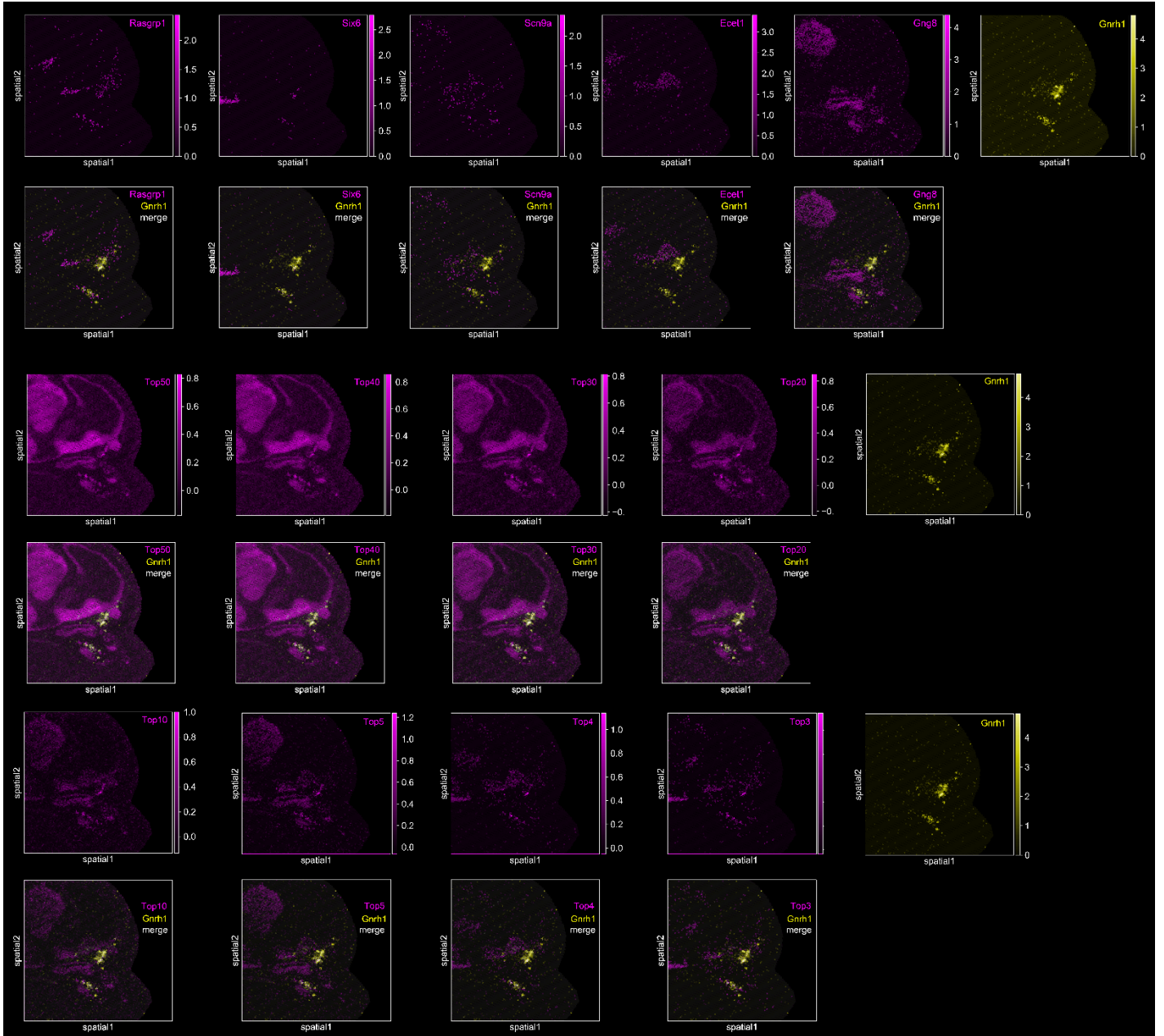


Figure 12 | **Spatial transcriptomics analysis of combined markers for GnRH neurons.**
 Embedding plots showing spatial visualization of transcriptomic data reanalyzed from a mouse embryo section at E13.5 (MOSTA). Spot size 25um.