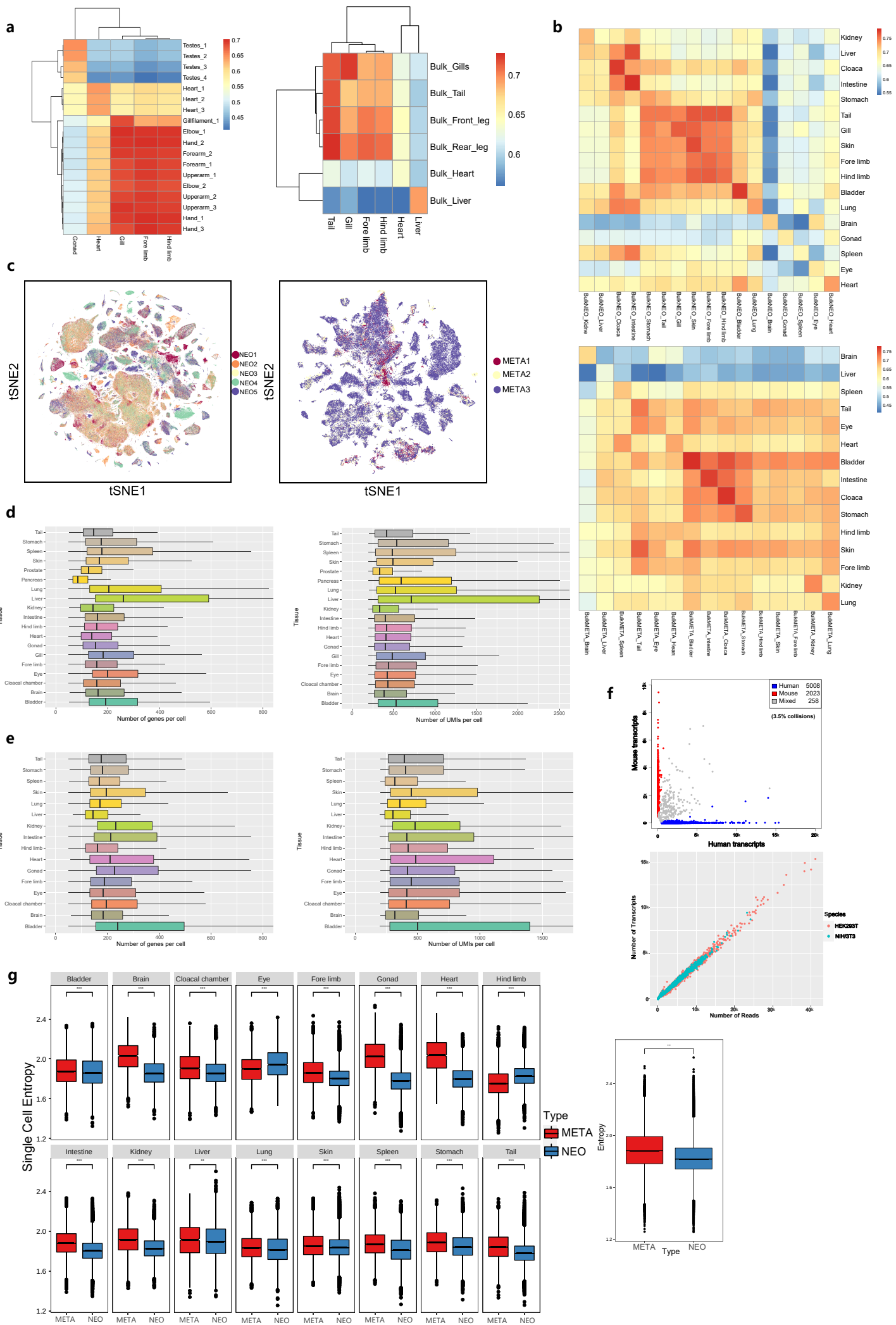


Supplementary Information

Construction of the axolotl cell landscape using combinatorial hybridization sequencing at single cell resolution

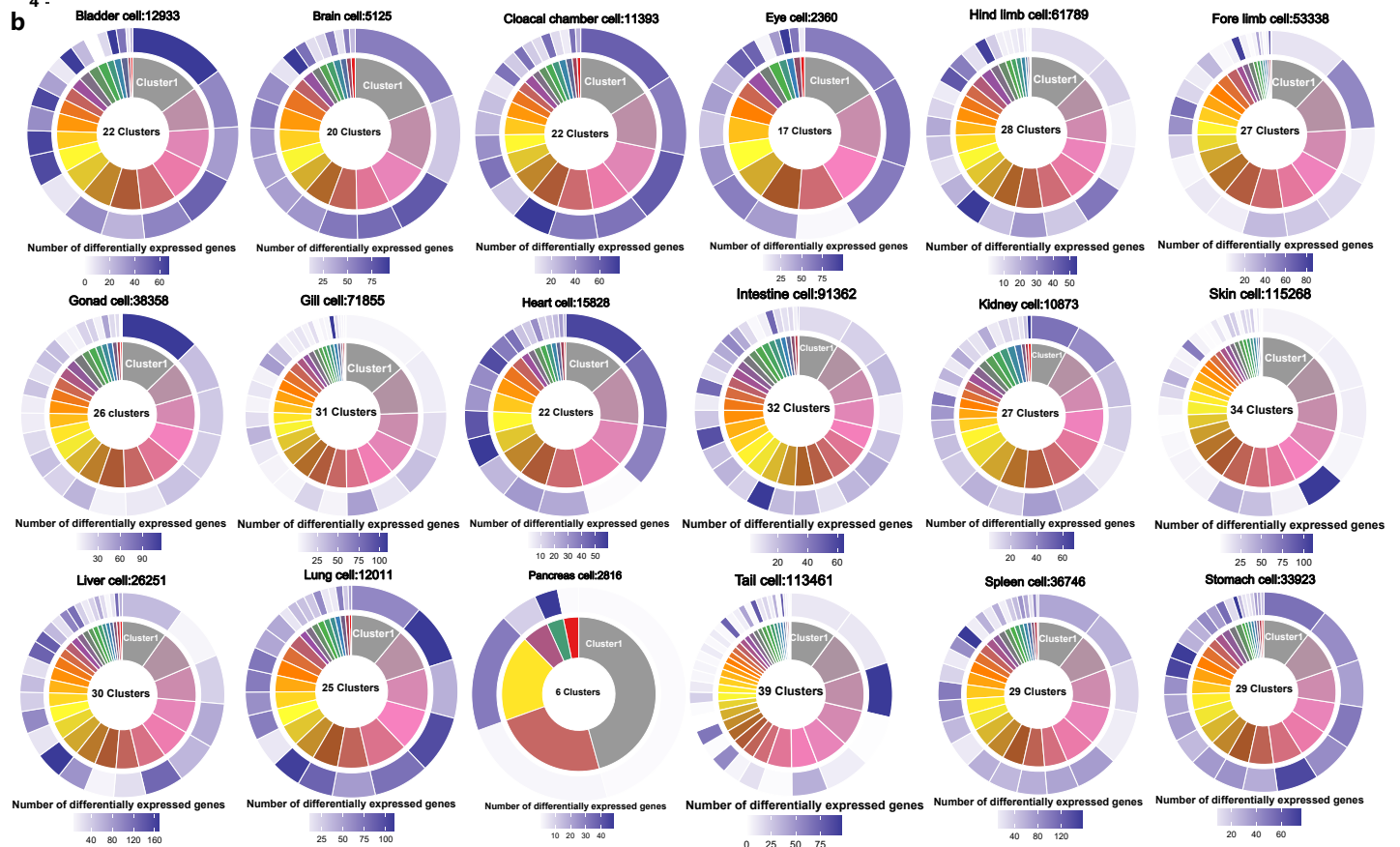
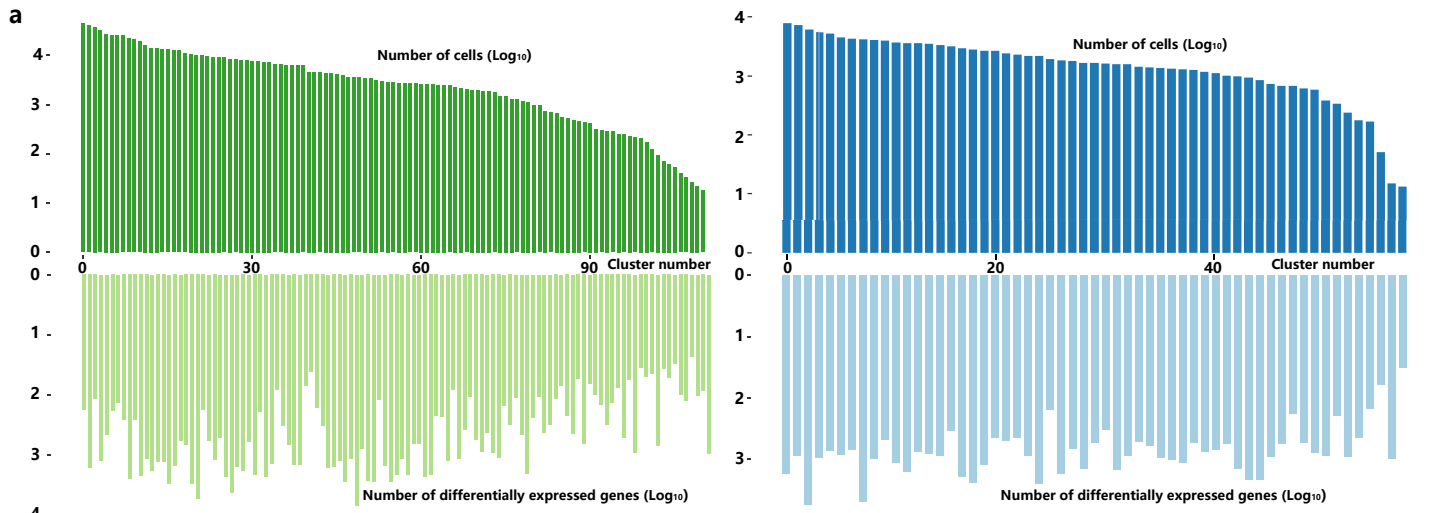
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*Corresponding author. Email: ggj@zju.edu.cn, xhan@zju.edu.cn, jifengfei@gdph.org.cn



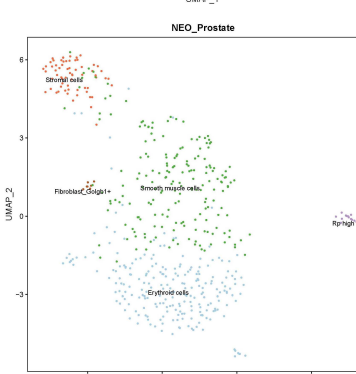
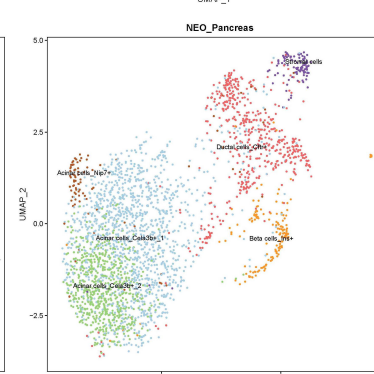
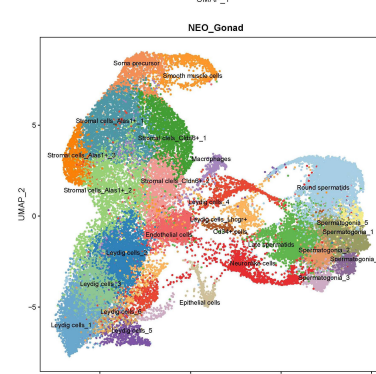
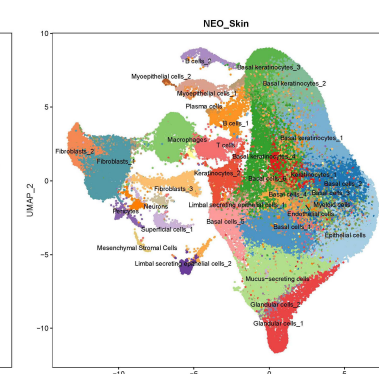
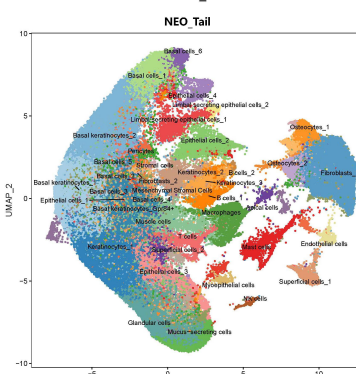
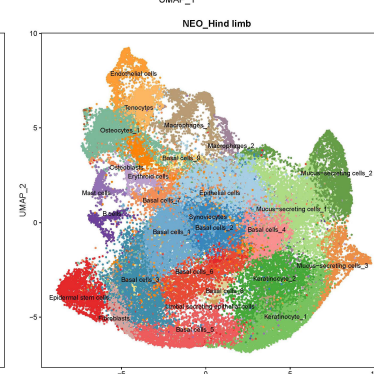
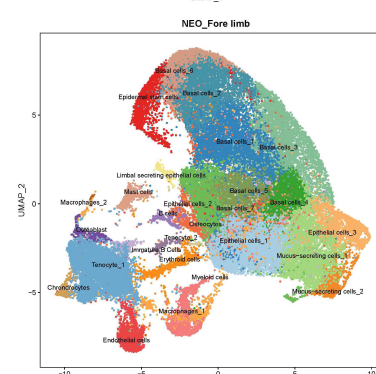
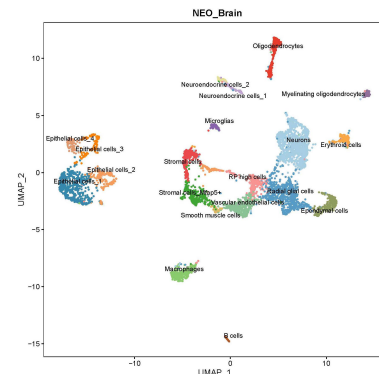
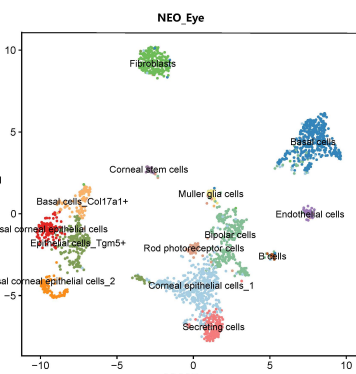
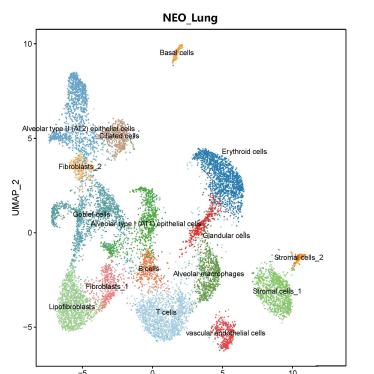
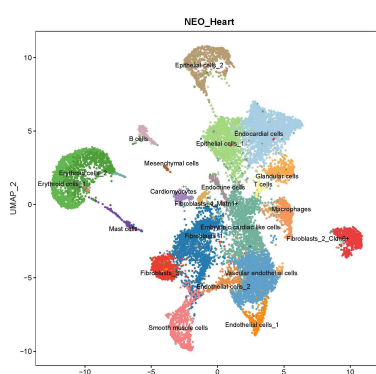
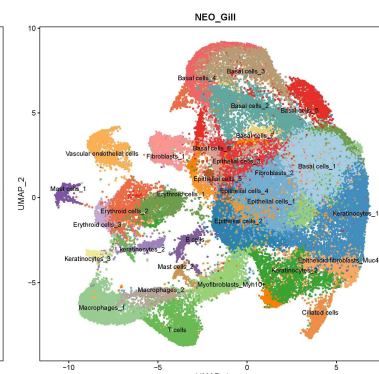
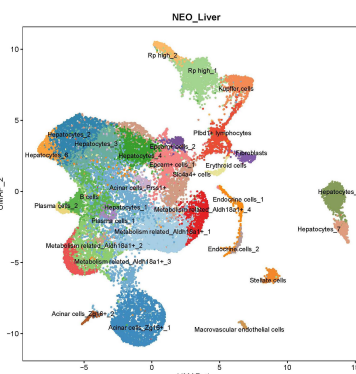
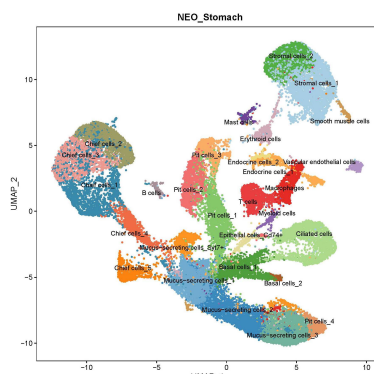
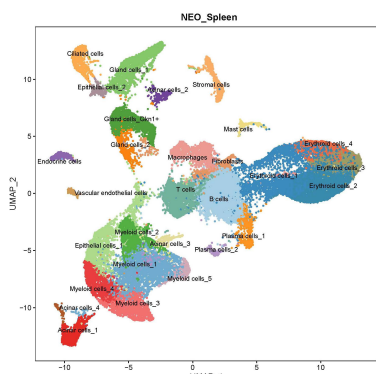
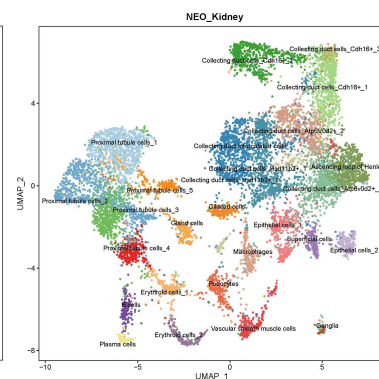
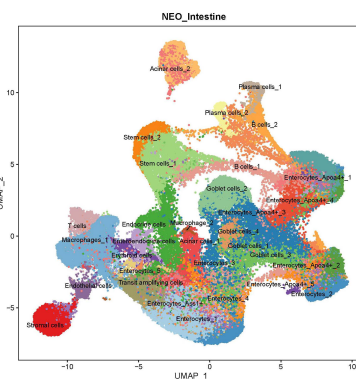
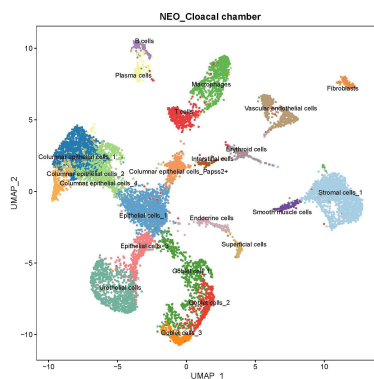
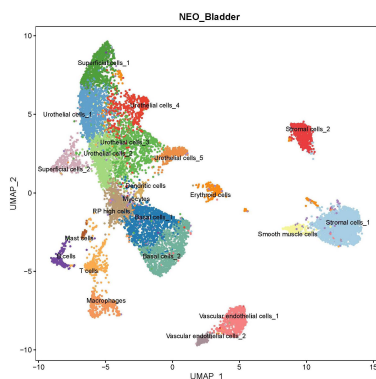
Supplementary Fig 1. Data quality of single cell RNA-seq and bulk RNA-seq datasets.

- (a) Heatmaps showing the neotenic axolotl tissue gene expression correlations between bulk RNA-seq in this work and published RNA-seq work (left¹, right²).
- (b) Heatmap showing the gene expression correlations between tissues bulk RNA-seq (neotenic axolotls: Bulk_NEO, top; metamorphosed axolotls: Bulk_META, bottom) and CH-RNA-seq.
- (c) tSNE plots showing different experiment batch of neotenic axolotls (NEO1-5, left) and metamorphosed axolotls (META1-3, right).
- (d-e) Boxplot showing the number of genes (right) and transcripts (UMIs, left) recovered per cell for each tissue in neotenic and metamorphosed axolotls (n=number of cells in each tissue, see Supplementary Fig. 2 b, c. The boxplots are defined by the 25th and 75th percentiles, with the centre as the median, the minima and maxima extend to the largest value until 1.5 of the interquartile range and the smallest value at most 1.5 of interquartile range, respectively.).
- (f) Scatter plot of showing the number of transcripts (mouse (NIH/3T3) and human (HEK-293T) cells) and unique mapped reads mapping to the human and mouse genome for CH-RNA-seq derived from a mixture of human HEK293T and mouse NIH/3T3 cells (top); Scatter plot of showing the number of transcripts and unique mapped reads in same cells in CH-RNA-seq (bottom)
- (g) Single-cell entropies of combined single cells in each tissue and whole datasets (right) from neotenic and metamorphosed axolotl's data measured by SLICE (n= number of cells, see Supplementary Fig. 2 b, c. The boxplots are defined by the 25th and 75th percentiles, with the centre as the median, the minima and maxima extend to the largest value until 1.5 of the interquartile range and the smallest value at most 1.5 of interquartile range, respectively, ‘**’: p values < 0.01 , ‘***’: p values < 0.001 , p values are as follows: Bladder = $2.9\text{e-}14$, Eye = $1.7\text{e-}10$, in other cases $< 2.22\text{e-}16$, Mann-Whitney-Wilcoxon test was introduced, adjustments p values were made after P value is corrected by Benjamin & Hochberg multiple test).



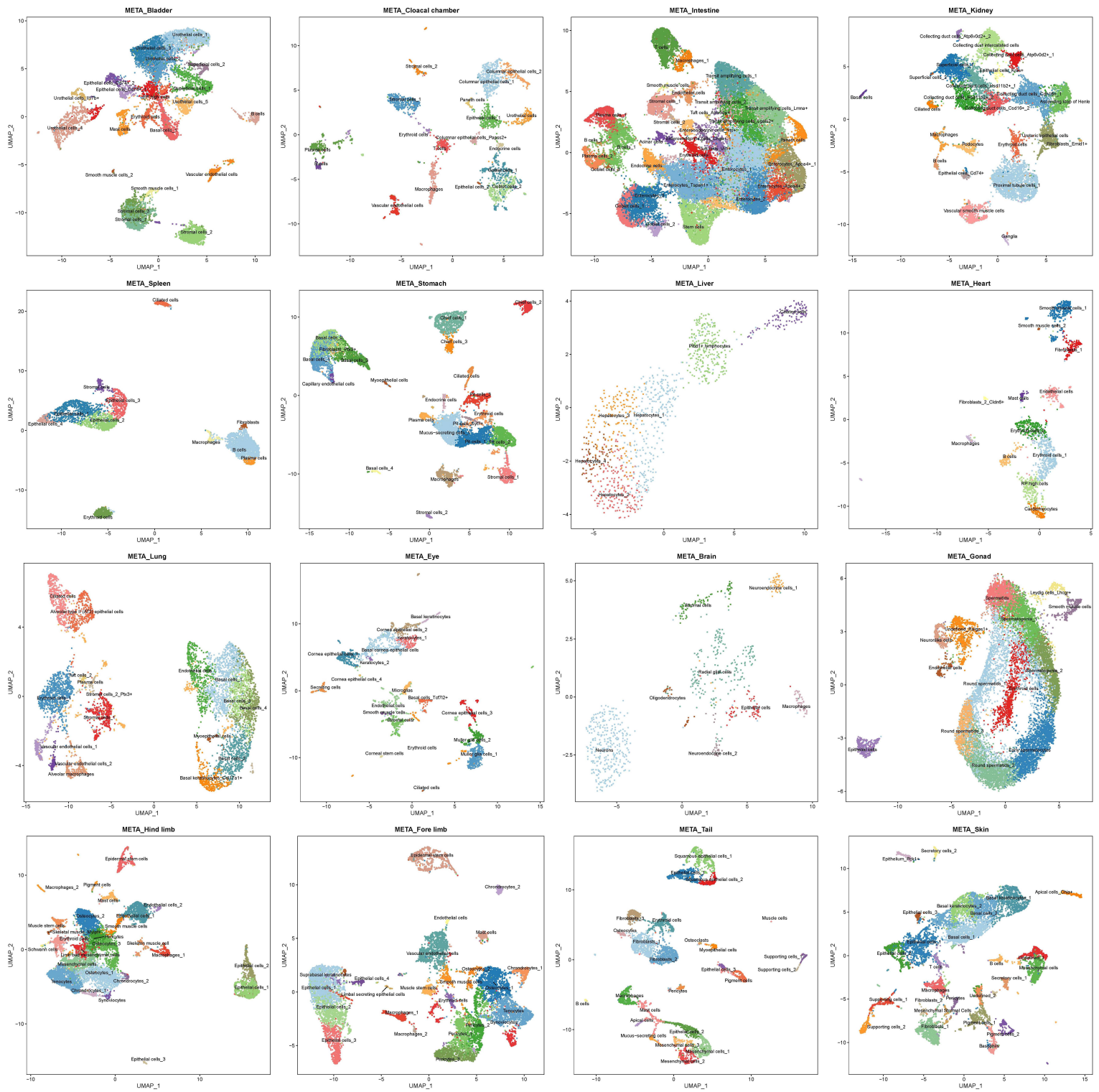
Supplementary Fig 2. Number of differentially expressed genes in CH-RNA-seq datasets of neotenic and metamorphosed axolotls.

- (a) Number of cells and differentially expressed genes in each cluster of merged neotenic axolotl datasets (left) and metamorphosed axolotl datasets (right).
- (b) Color coded number of differentially expressed genes in each cluster from neotenic axolotl tissues (Clusters are clockwise distributed from cluster1).
- (c) Color coded number of differentially expressed genes in each cluster from metamorphosed axolotl tissues (Clusters are clockwise distributed from cluster1).



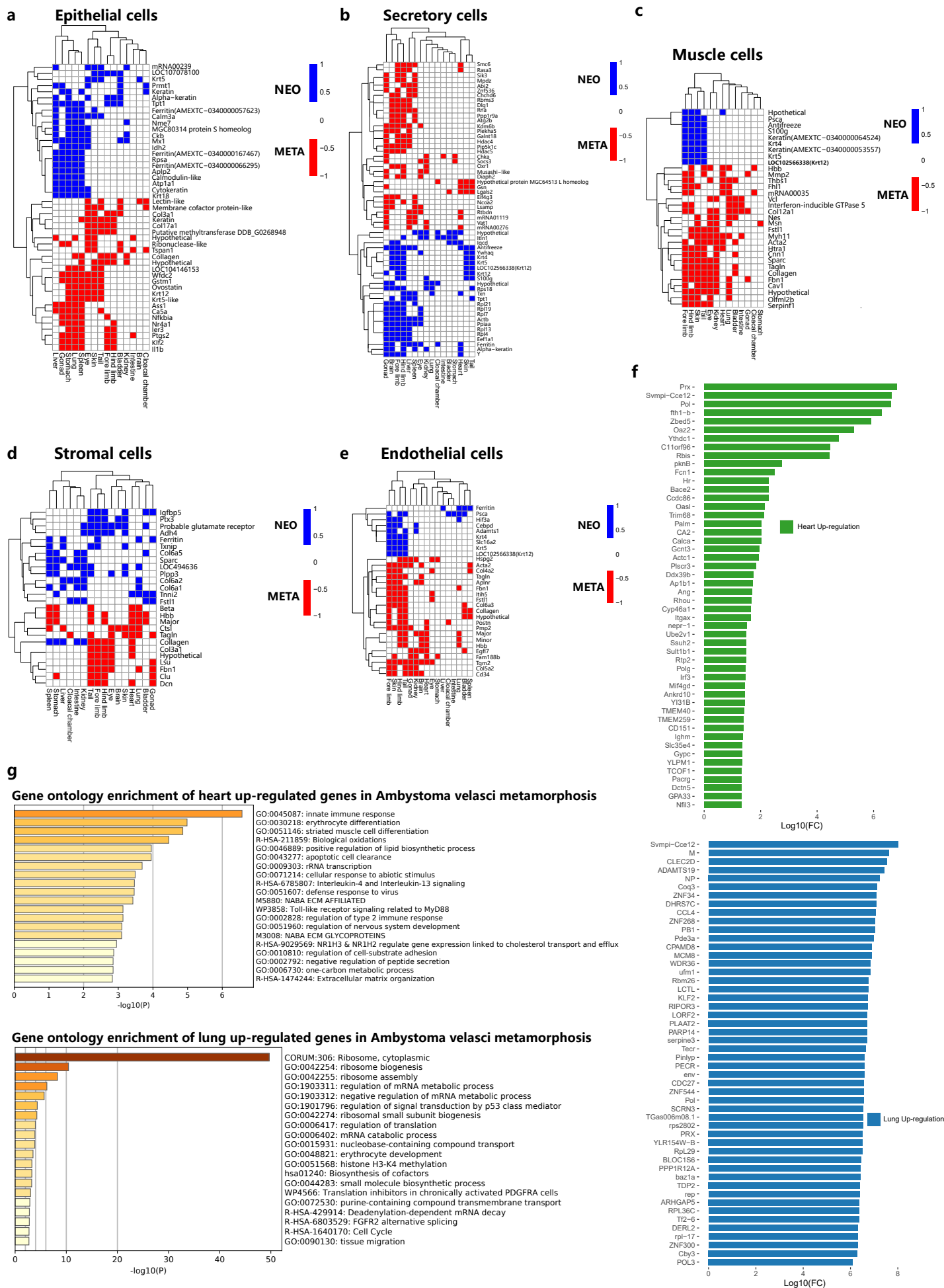
Supplementary Fig 3. Single-cell clustering of neotenic axolotl tissues.

UMAP plots of single cells from 19 tissues in neotenic (NEO) axolotls after low quality cell filtering.



Supplementary Fig 4. Single-cell clustering of metamorphosed axolotl tissues.

UMAP plots single cells from 16 tissues in metamorphosed (META) axolotls after low quality cell filtering.

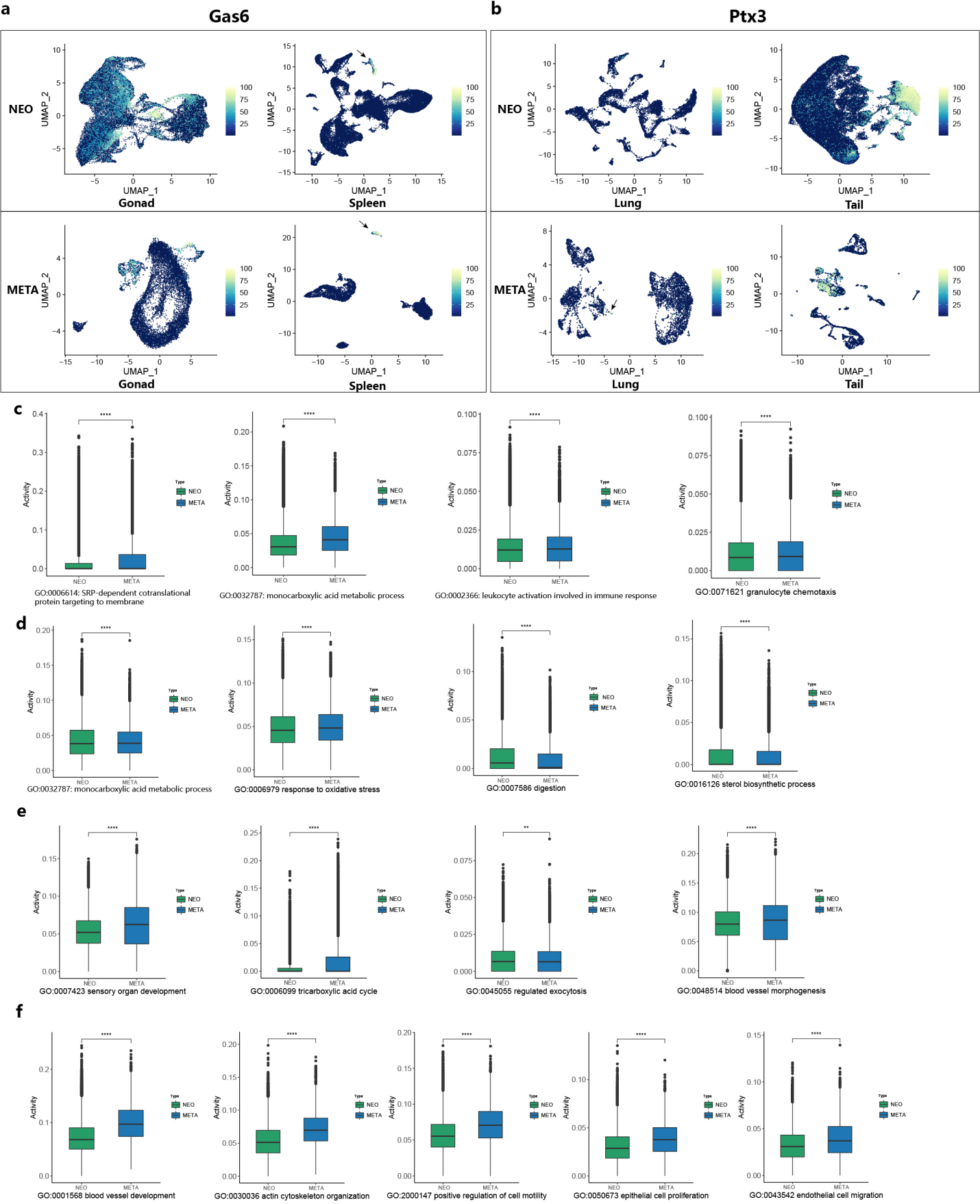


Supplementary Fig 5. Gene expression heterogeneities of non-immune cells between neotenic and metamorphosed axolotls.

(a-e) Clustered differentially expressed genes of neotenic (NEO) and metamorphosed (META) axolotls' epithelial cells (a), secretory cells (b), stromal cells (d), endothelial cells (e), muscle cells (c).

(f) Top upregulated differentially expressed genes in metamorphosed *Ambystoma velasci* lung and heart (stage IV (SIV) or post-metamorphosis at 23 days post-induction³).

(g) Gene ontology enrichment of top upregulated differentially expressed genes in (f) (p values was calculated by the hypergeometric distribution, statistical test is one-sided, adjustments p values were made after p value is corrected by Benjamin & Hochberg multiple test).

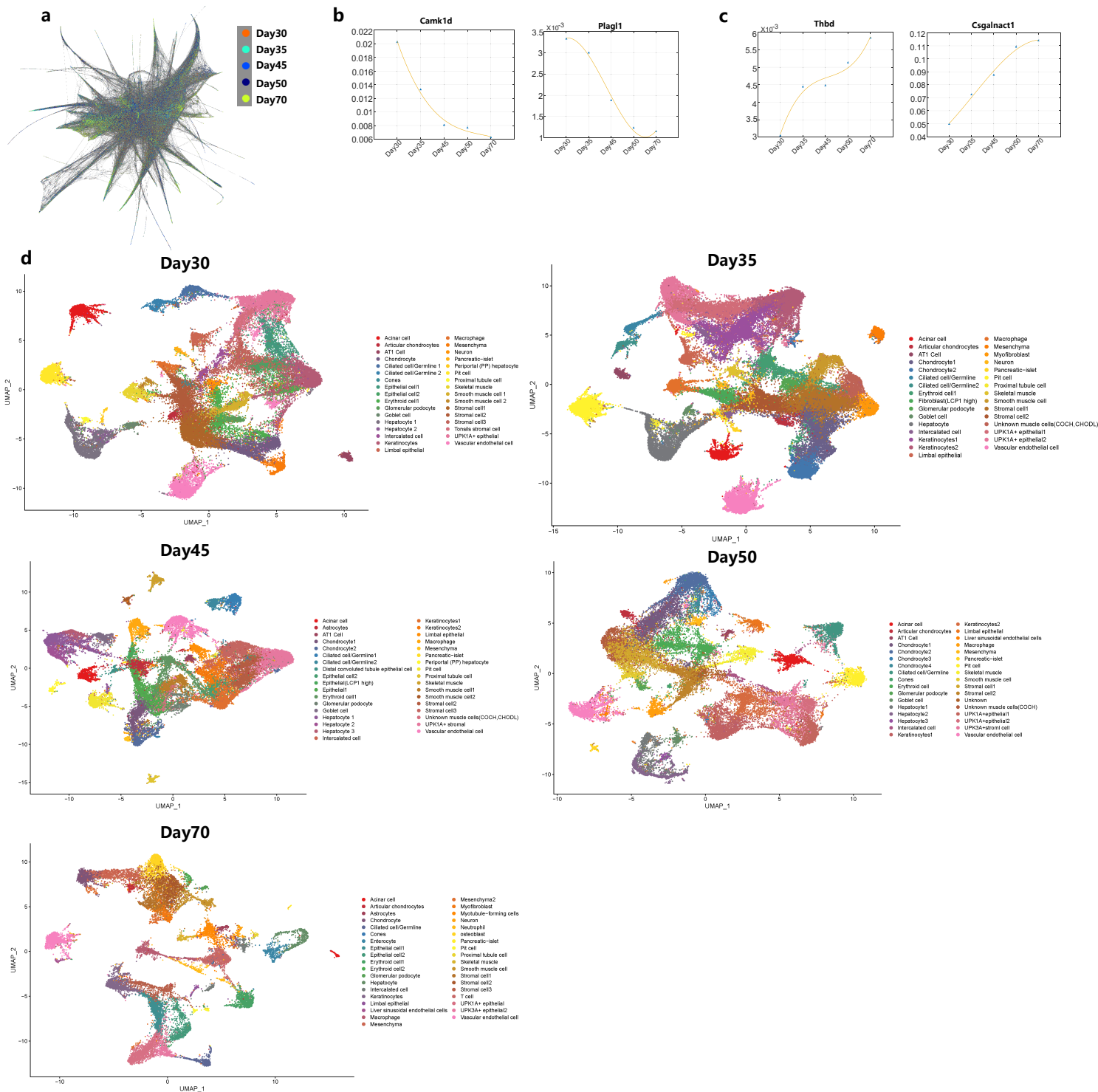


Supplementary Fig 6. Additional gene expression patterns and functions in structural cells of neotenic and metamorphosed axolotls.

(a) Feature plots visualization of *Gas6* in neotenic (NEO) and metamorphosed (META) axolotl's gonad and spleen.

(b) Feature plots visualization of *Ptx3* in neotenic (NEO) and metamorphosed (META) axolotl's lung and tail.

(c-f) Bar plots showing the activities of selected gene ontology (GO) enrichment terms in epithelial cells (c), secretory cells (d), stromal cells (e), endothelial cells (f) from neotenic axolotls (NEO, green) and metamorphosed axolotls (META, blue) (n= number of cells in Fig. 5g, the boxplots are defined by the 25th and 75th percentiles, with the centre as the median, the minima and maxima extend to the largest value until 1.5 of the interquartile range and the smallest value at most 1.5 of interquartile range, respectively. '***': p values < 0.05 , '*****': p values < 0.001 , in all cases, p value $< 2.22 \times 10^{-10}$, t test was introduced, adjustments p values were made after p value is corrected by Benjamin & Hochberg multiple test).



Supplementary Fig 7. Clustering and dynamic gene expression patterns of larval neotenic axolotls at different stage

- (a) SPRING visualization of 217,781 single cells of larval neotenic axolotls' whole organisms, colored by different postfertilization stages.
- (b) Dynamic gene expressions (downregulated) during Day30 to Day70 postfertilization.
- (c) Dynamic gene expressions (upregulated) during Day 30 to Day 70 postfertilization.
- (d) UMAP visualization larval neotenic axolotls whole organisms datasets at five different stages (Day30 to Day70 postfertilization).

Supplementary Reference

1. Bryant DM, *et al.* A Tissue-Mapped Axolotl De Novo Transcriptome Enables Identification of Limb Regeneration Factors. *Cell Rep* **18**, 762-776 (2017).
2. Caballero-Perez J, *et al.* Transcriptional landscapes of Axolotl (*Ambystoma mexicanum*). *Dev Biol* **433**, 227-239 (2018).
3. Janet PM, *et al.* Multi-organ transcriptomic landscape of *Ambystoma velasci* metamorphosis. *Dev Biol* **466**, 22-35 (2020).