

Fig. S1

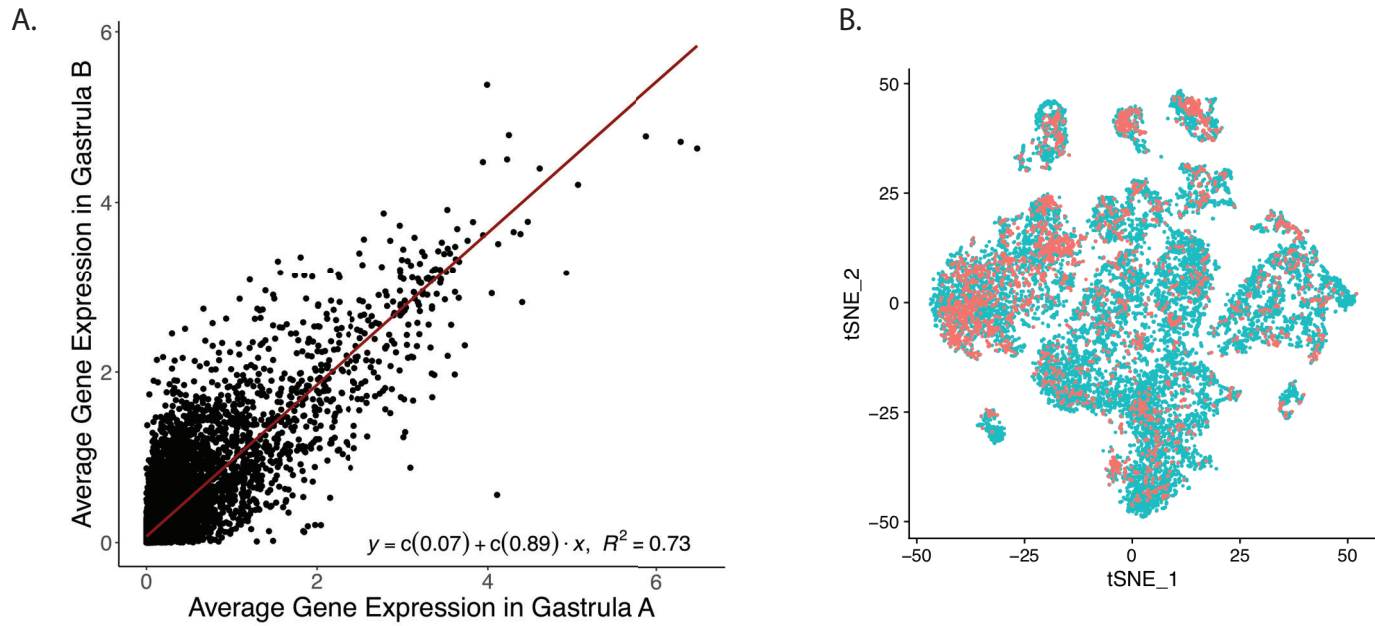


Fig. S1. (A) Comparison of average gene expression between Gastrula A, control from previous experiment (2,435 cells), and Gastrula B, corresponding time point from current analysis (12,763 cells). F-statistic: 5.837e+04 on 1 and 21088 degrees of freedom, p-value: < 2.2e-16. (B) t-SNE plot of early gastrula stage embryos after integration. In red is Gastrula A, in blue is Gastrula B. (dim:20 resolution:0.5)

Fig. S2:

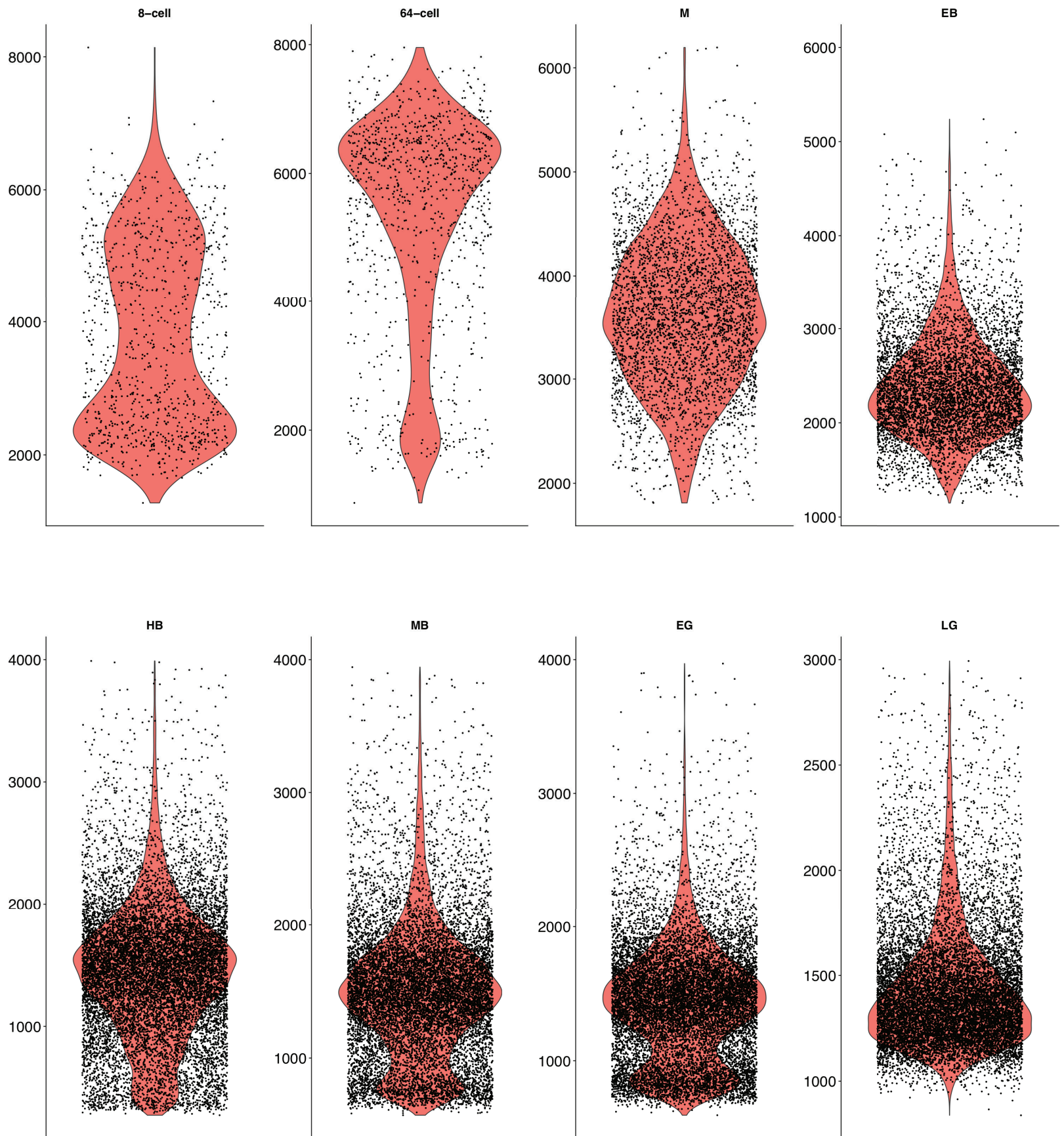


Fig. S2. Total genes detected per cell across developmental time points.

Fig. S3

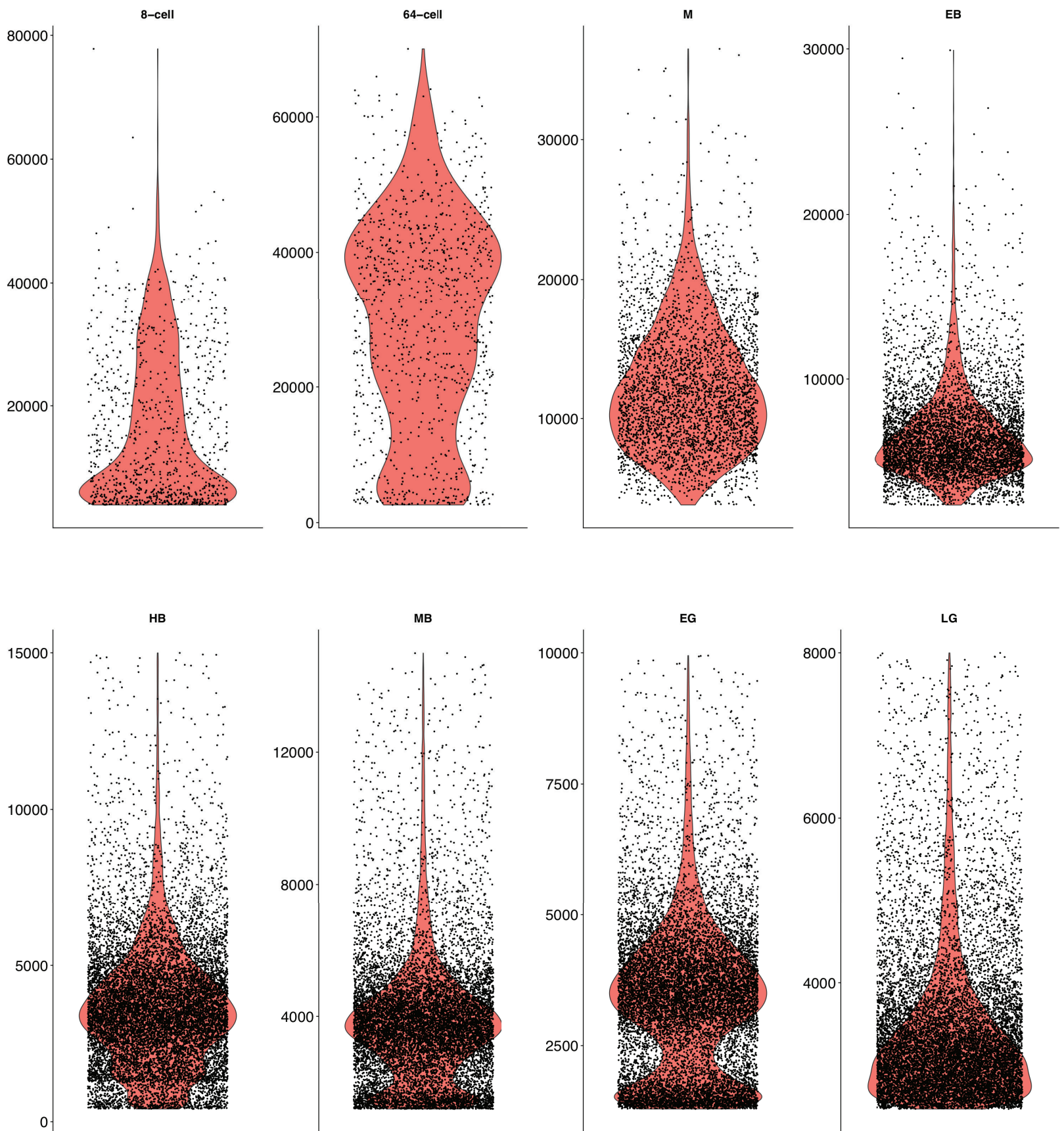


Fig. S3. Total UMIs detected per cell across developmental time points.

Fig. S4: SoxB2

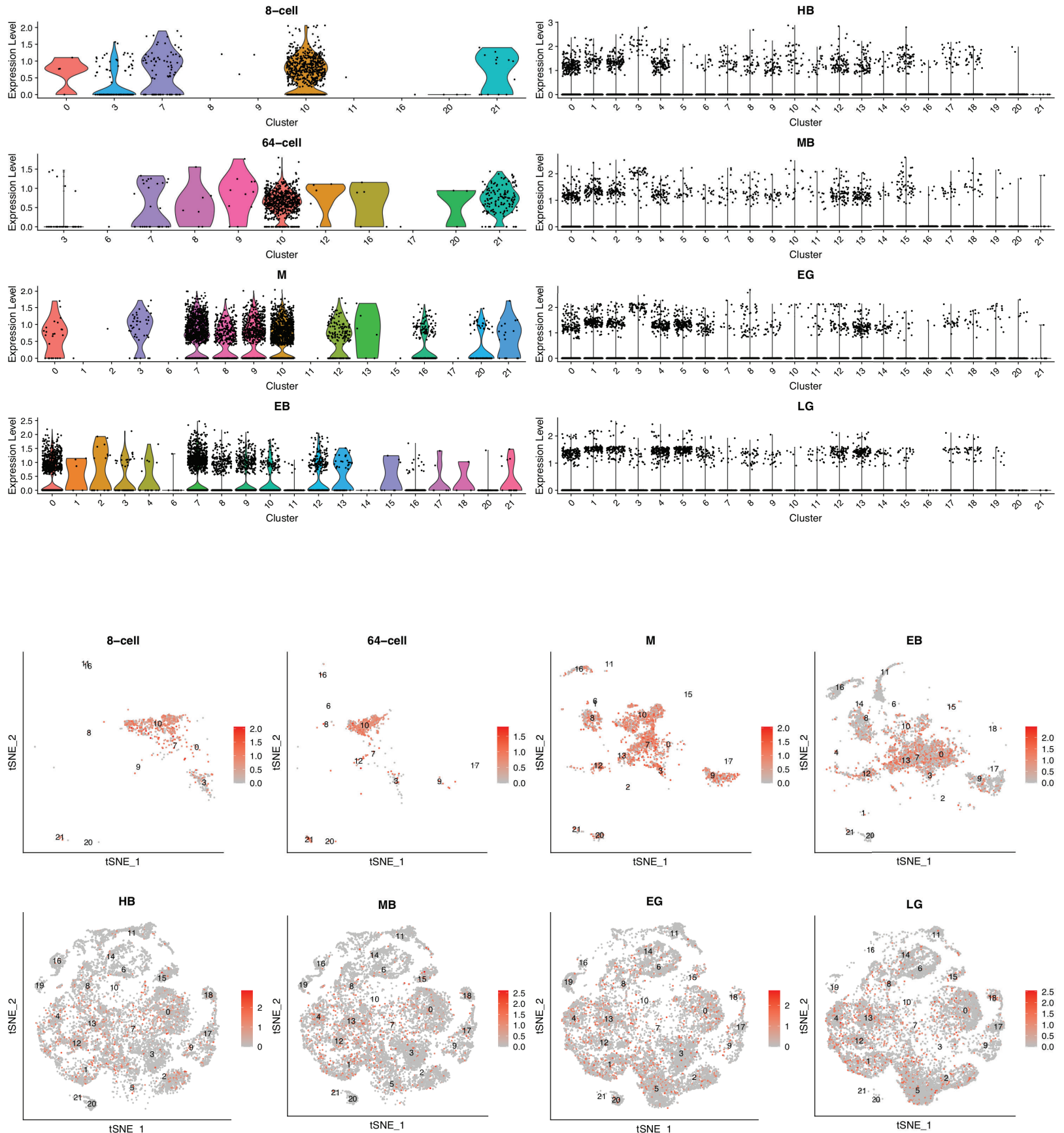


Fig. S4-S7. Violin plots showing expression across different clusters at each developmental stage. Normalized gene expression values at log scale shown per cell cluster identity for each developmental time point. Plots depict probability density at different expression levels. Feature plots showing expression across different clusters at each developmental stage.

Fig. S5: Foxq2

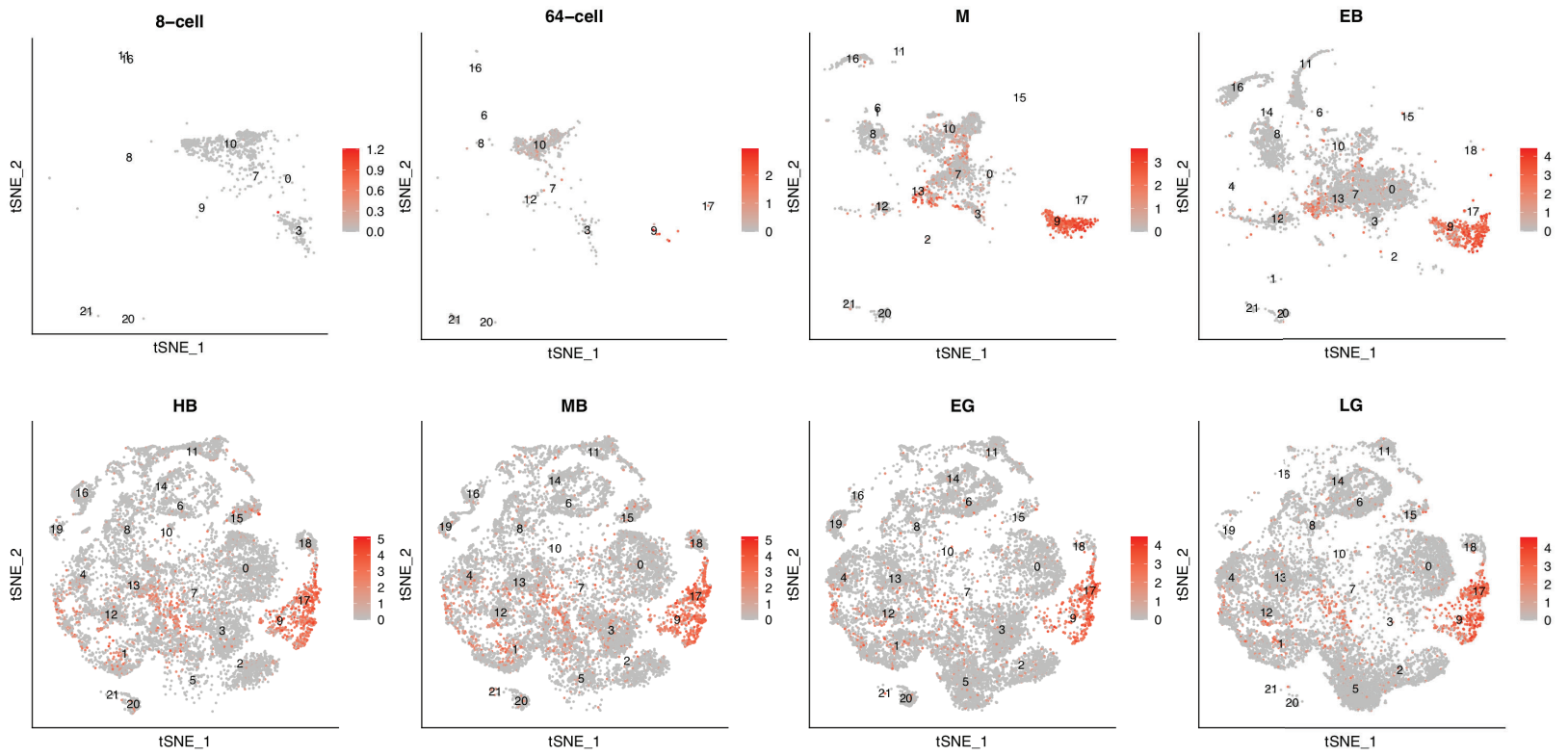
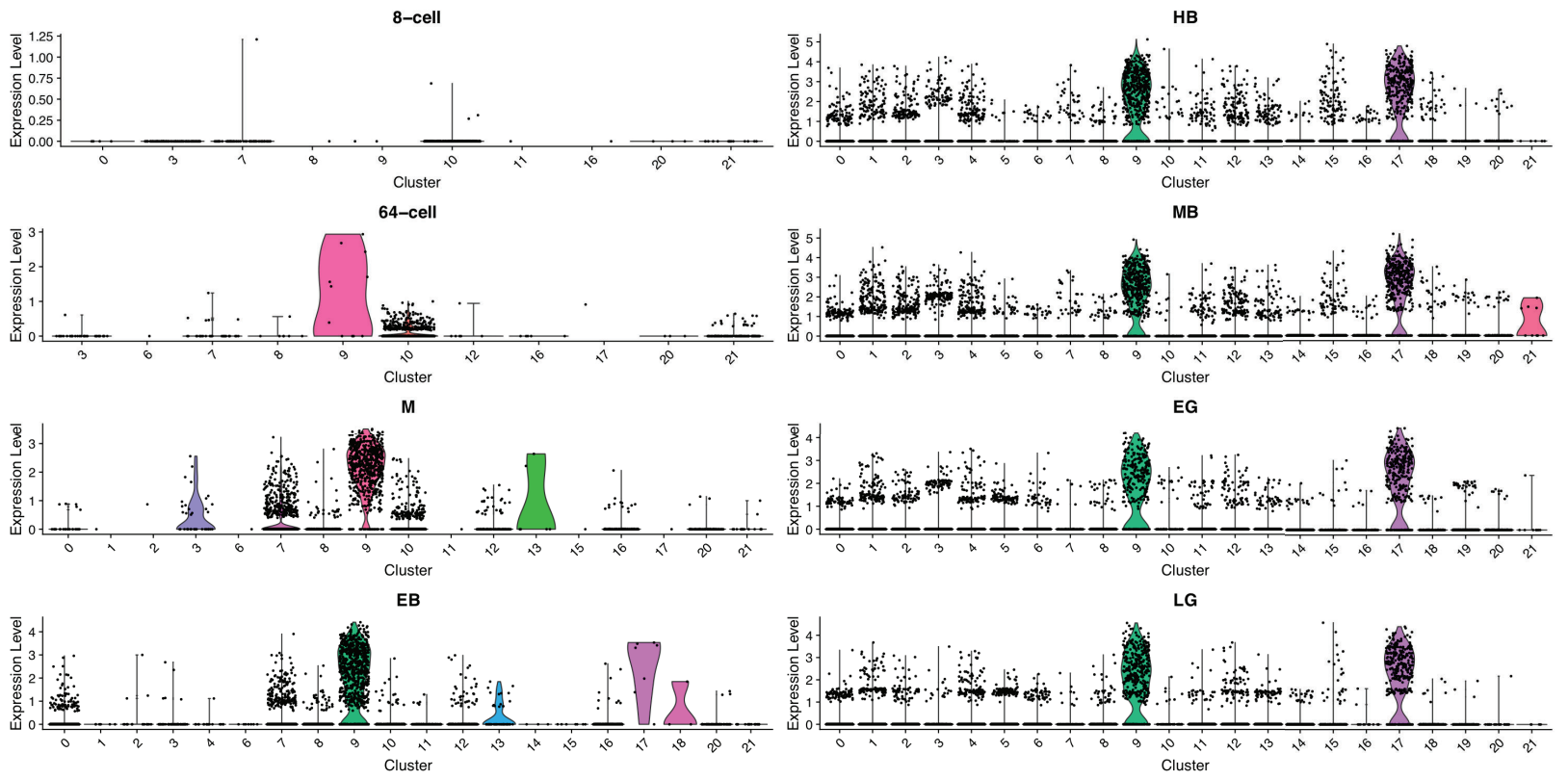


Fig. S6: FoxA

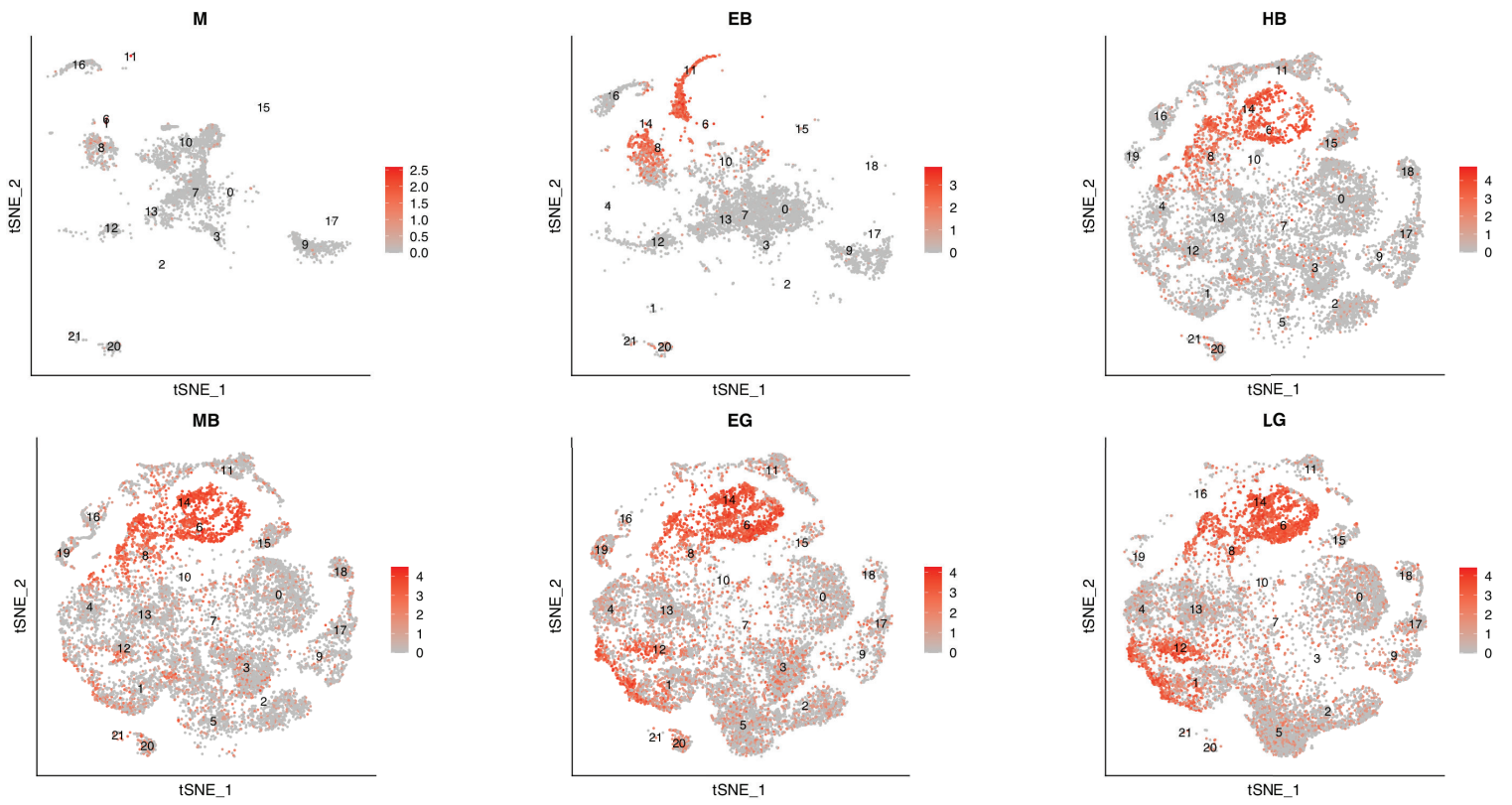
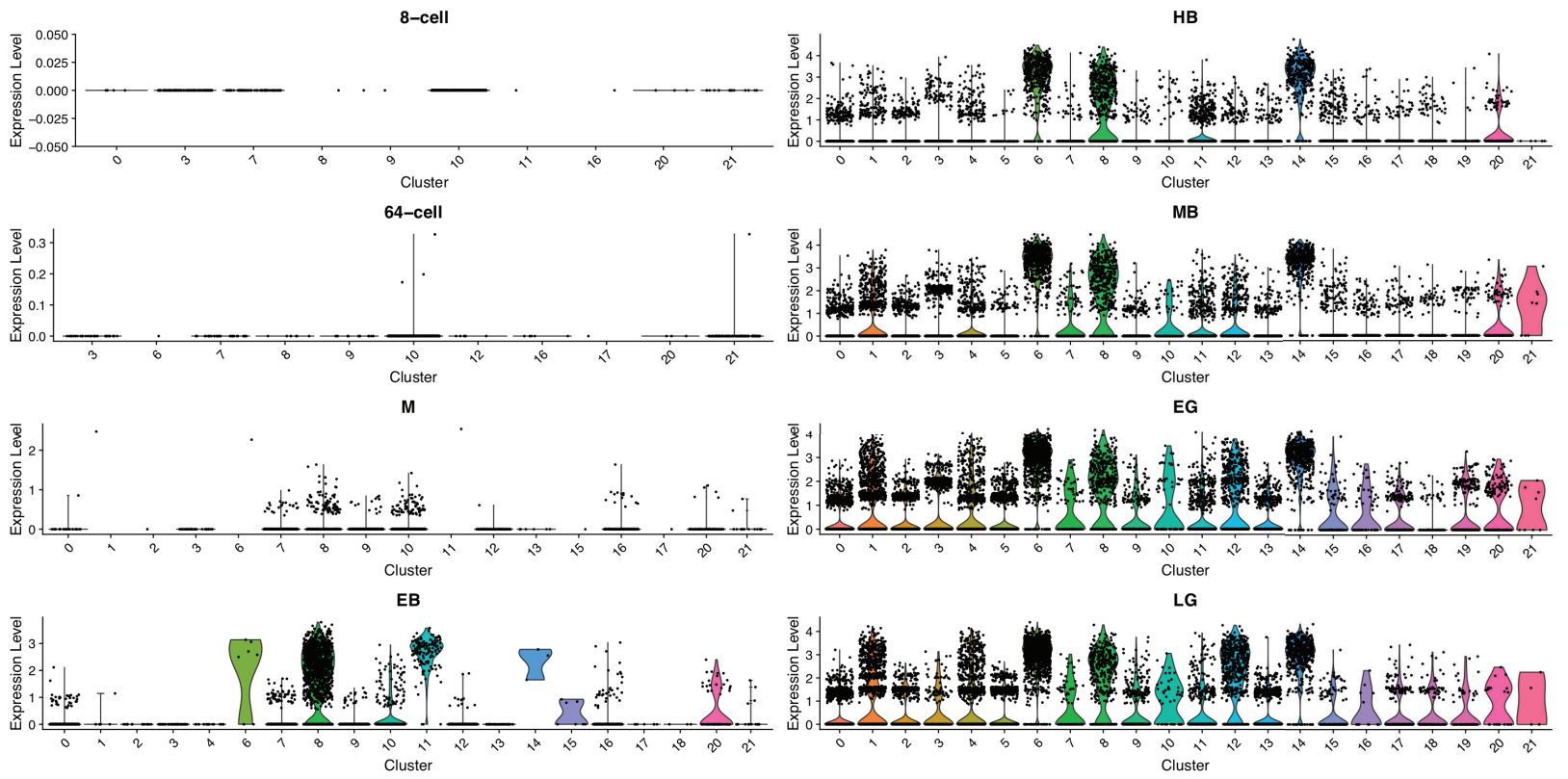


Fig. S7: Endo16

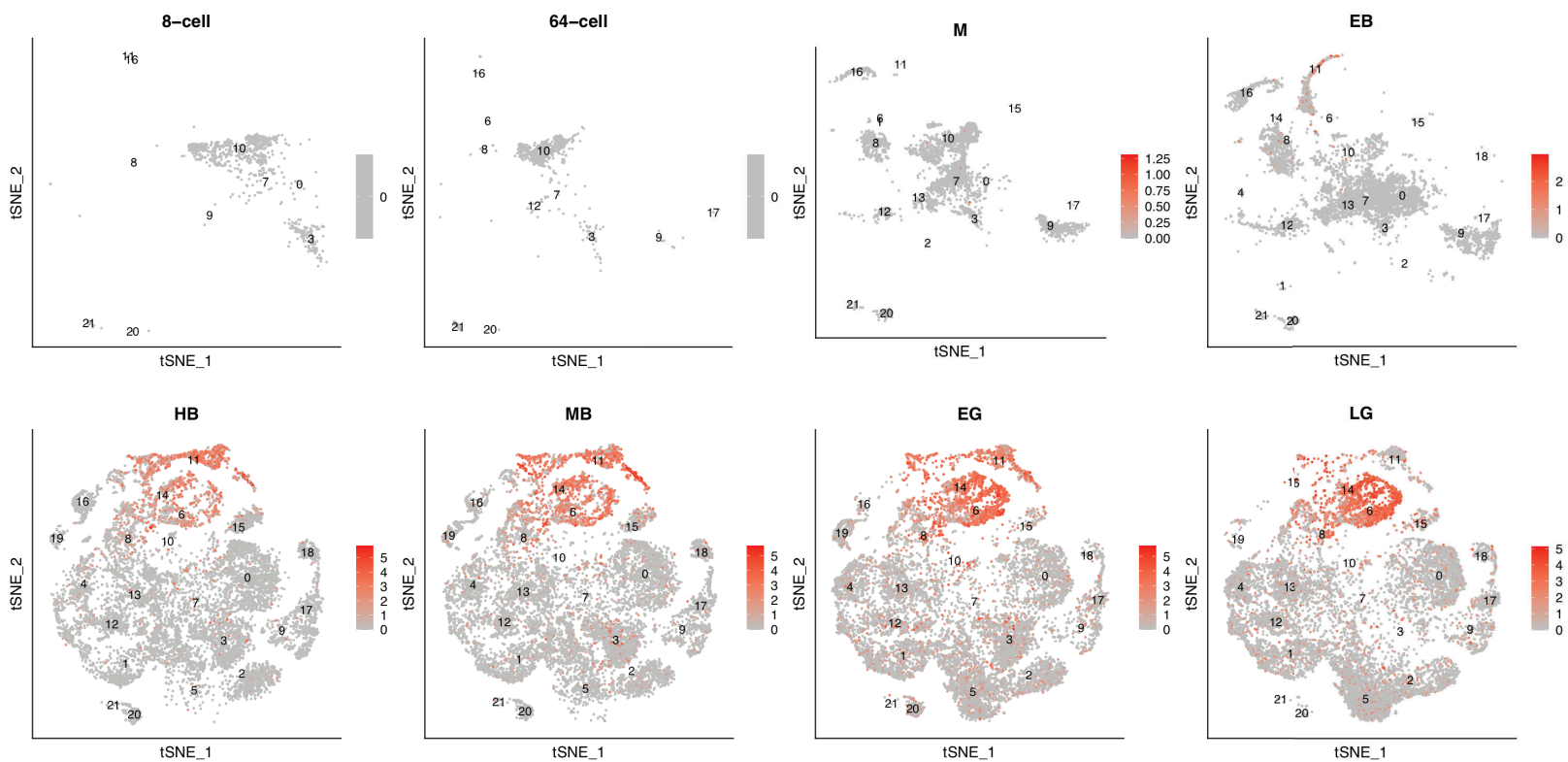
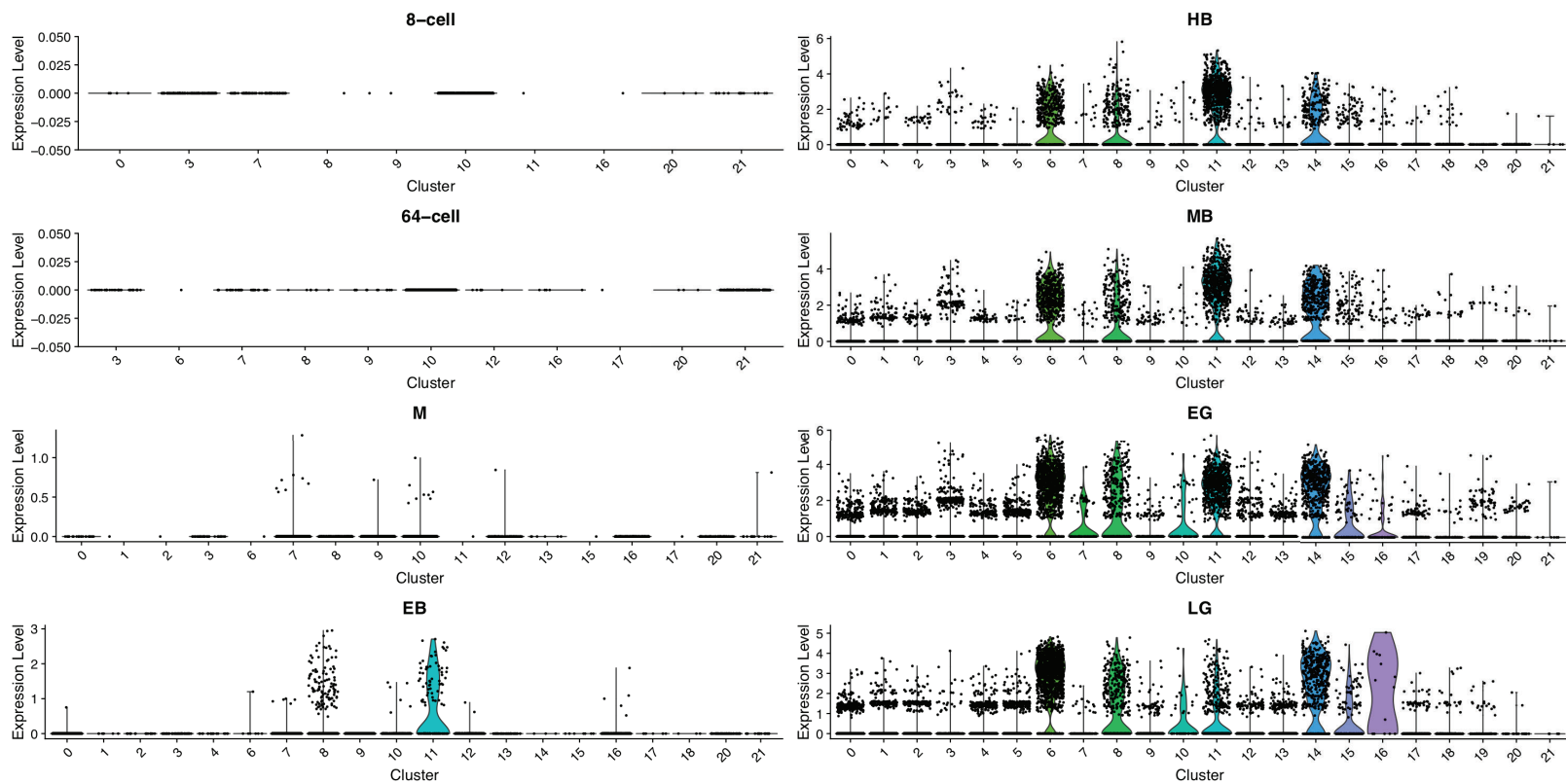


Fig. S8

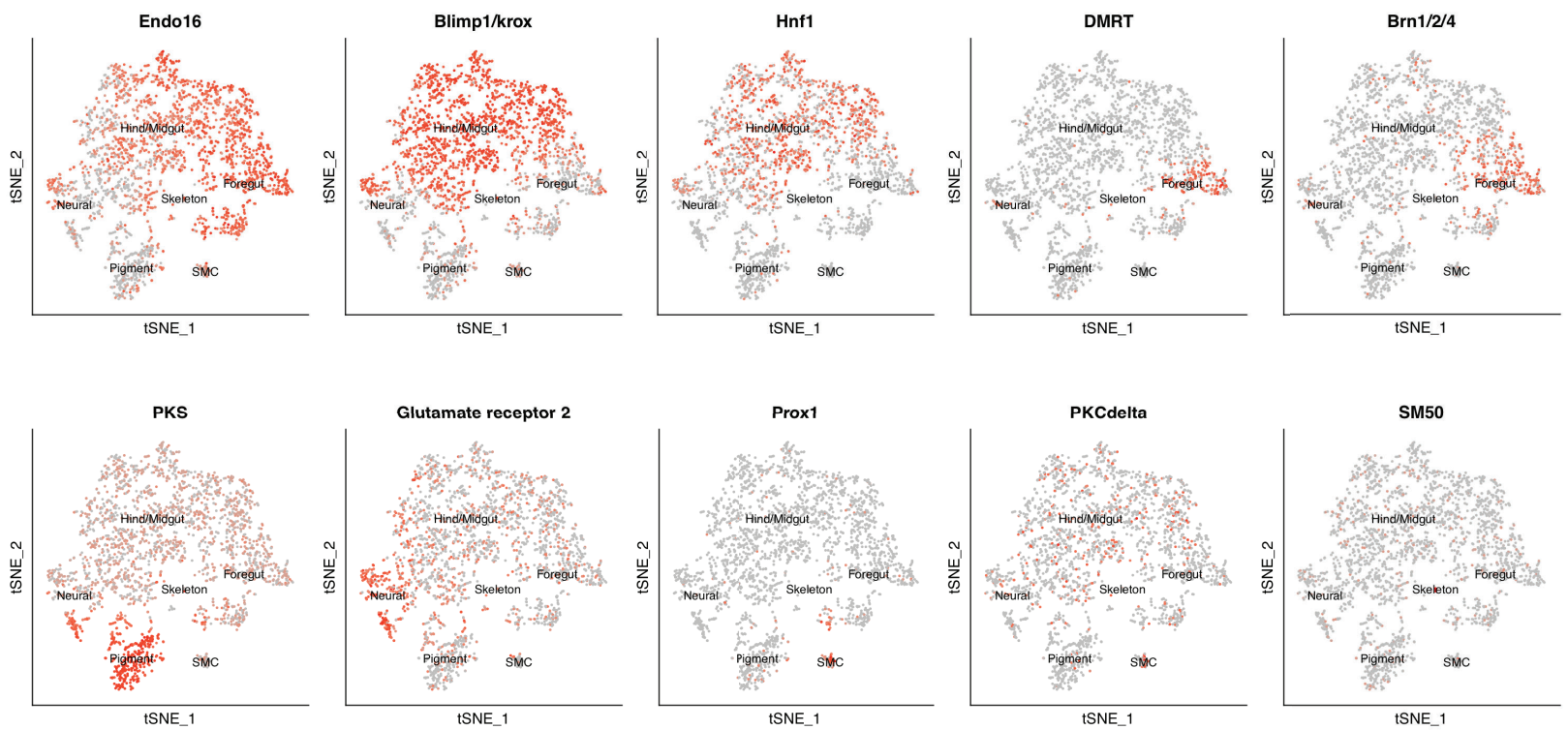
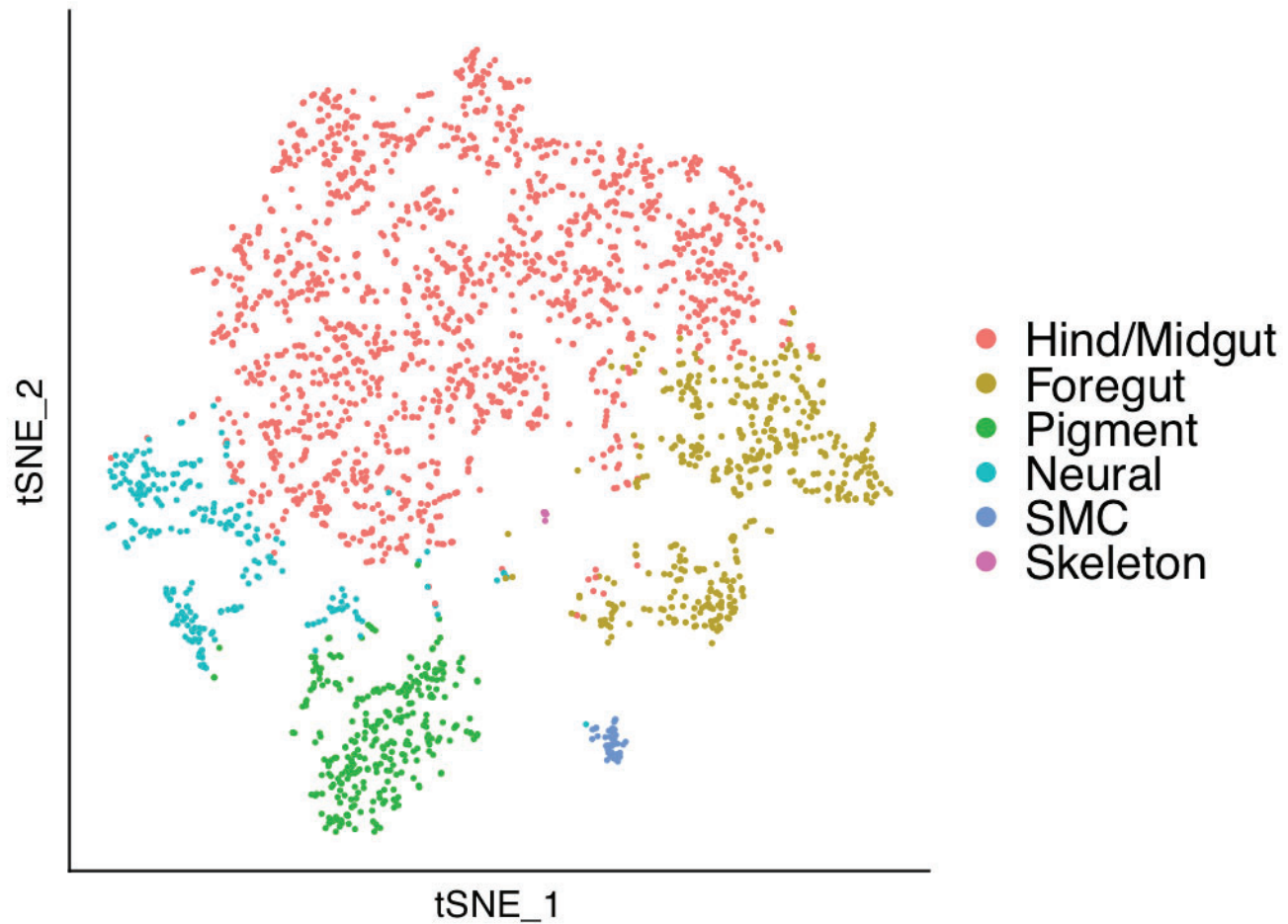


Fig. S8. Subclustering of gut clusters at late gastrula. Endo16-positive clusters 6, 8, 11, and 14 subcluster into seven cell types. Feature plots highlighting subcluster marker genes.

Fig. S9: Alx1

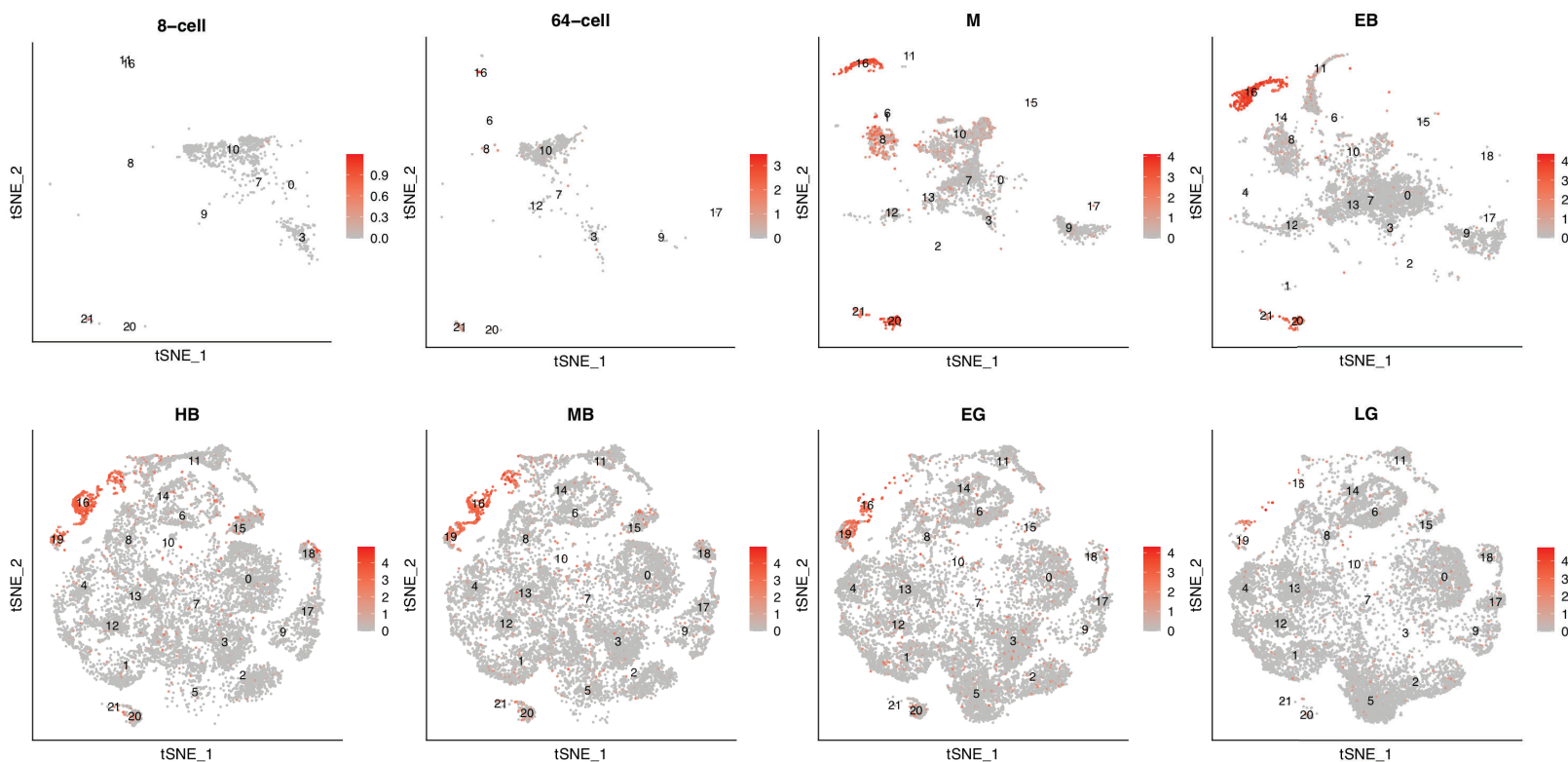
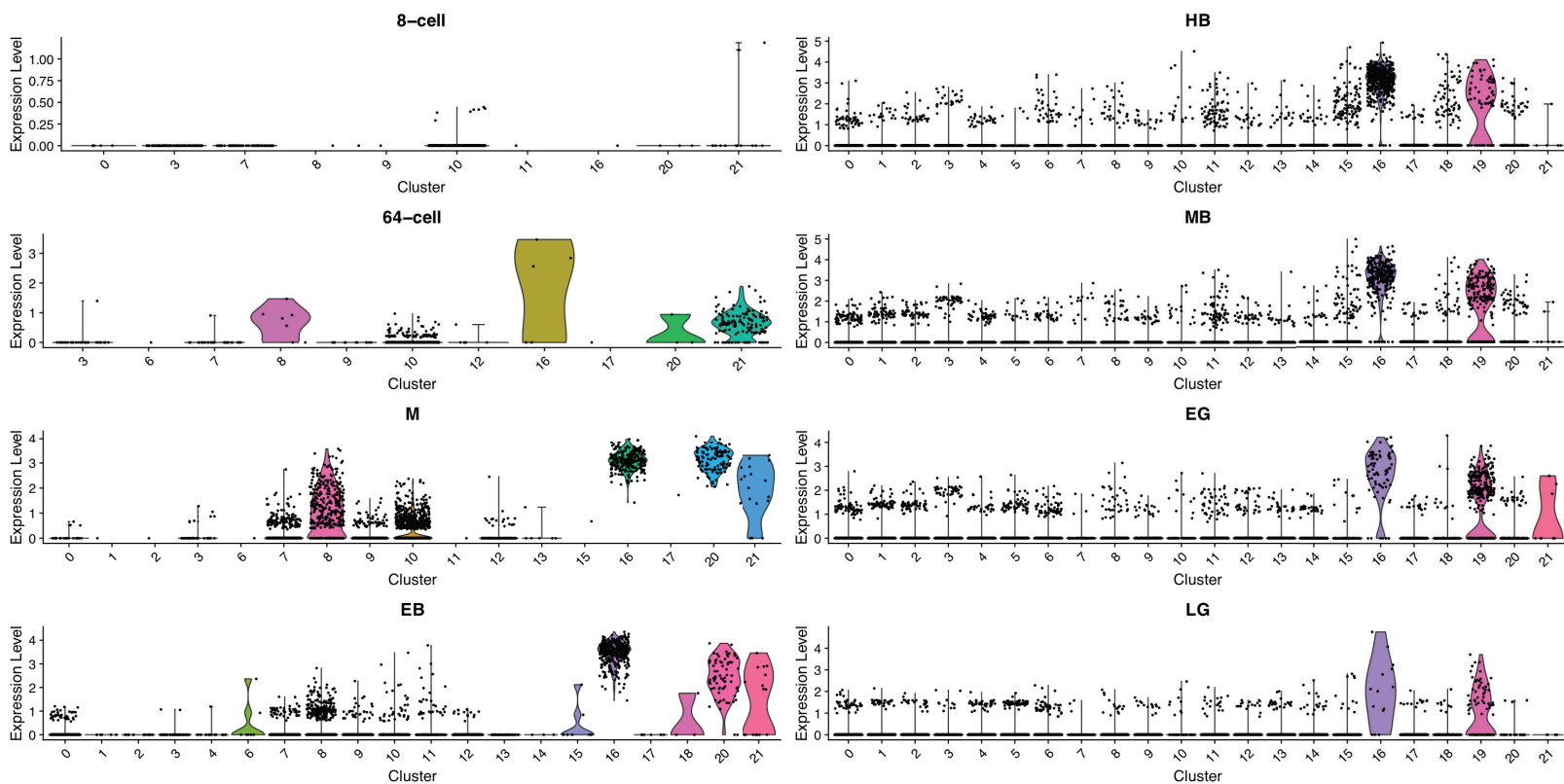


Fig. S9-S15, S17-24. Violin plots showing expression across different clusters at each developmental stage. Normalized gene expression values at log scale shown per cell cluster identity for each developmental time point. Plots depict probability density at different expression levels. Feature plots showing expression across different clusters at each developmental stage.

Fig. S10: SM50

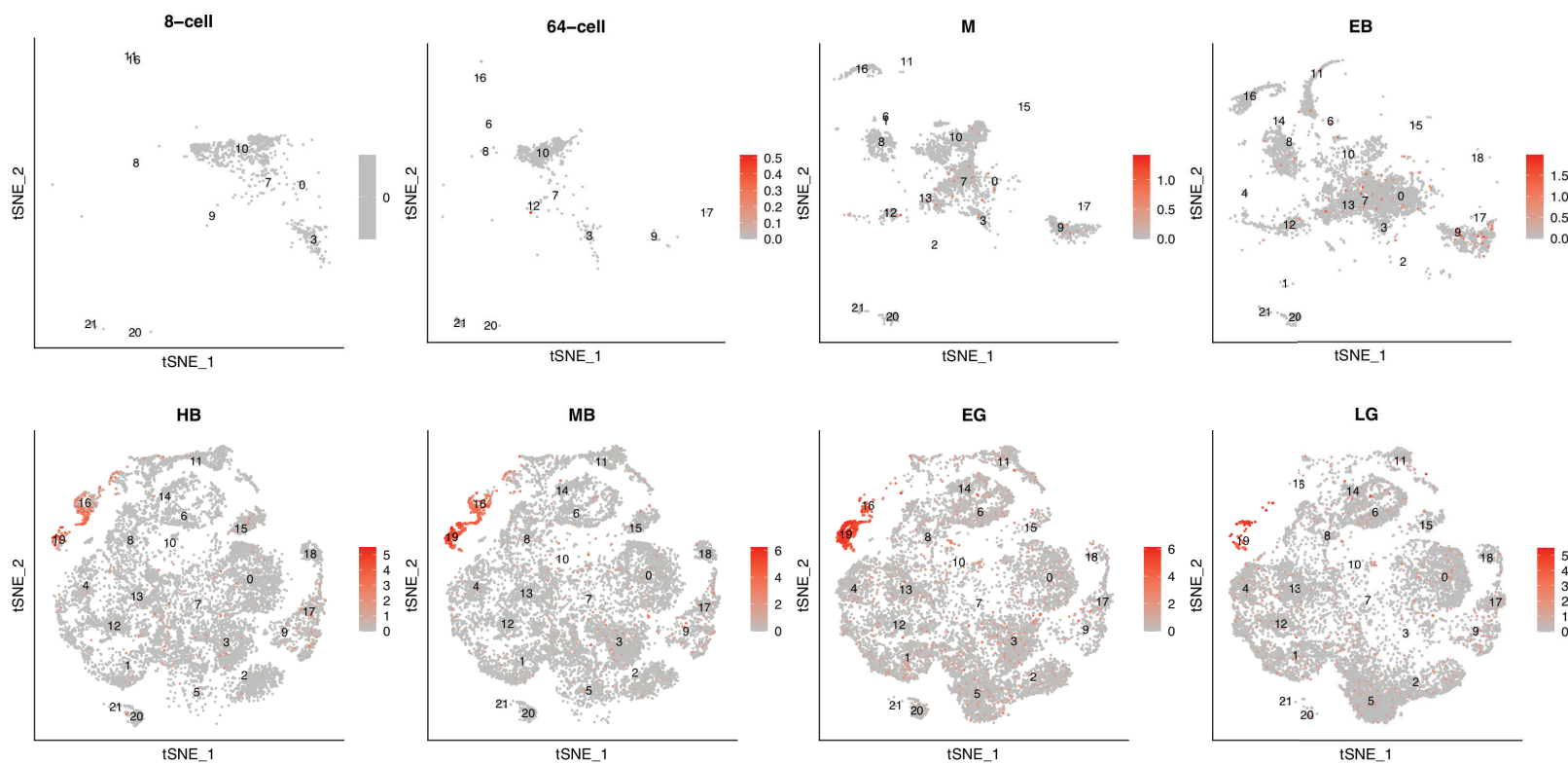
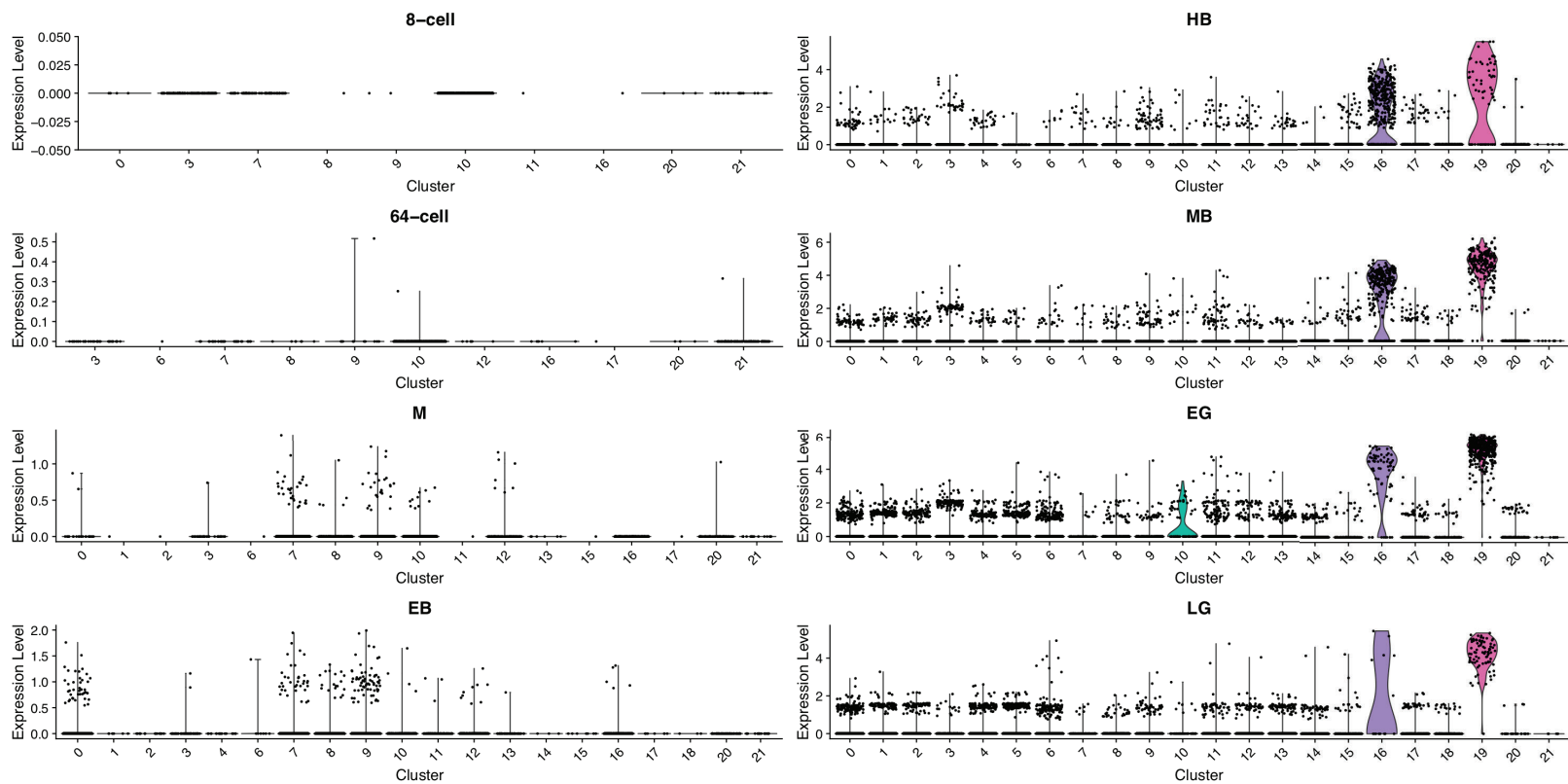


Fig. S11: SM37

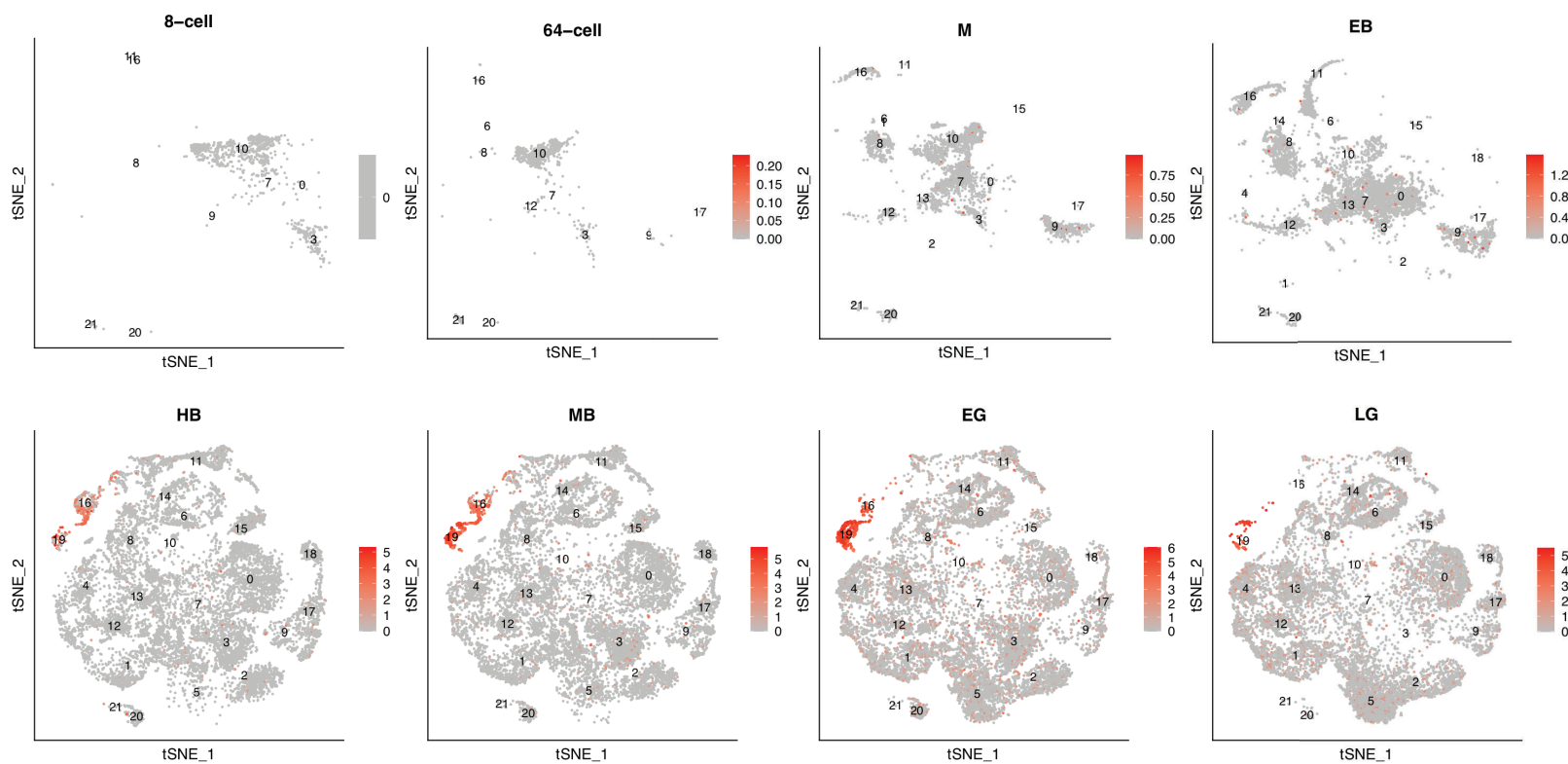
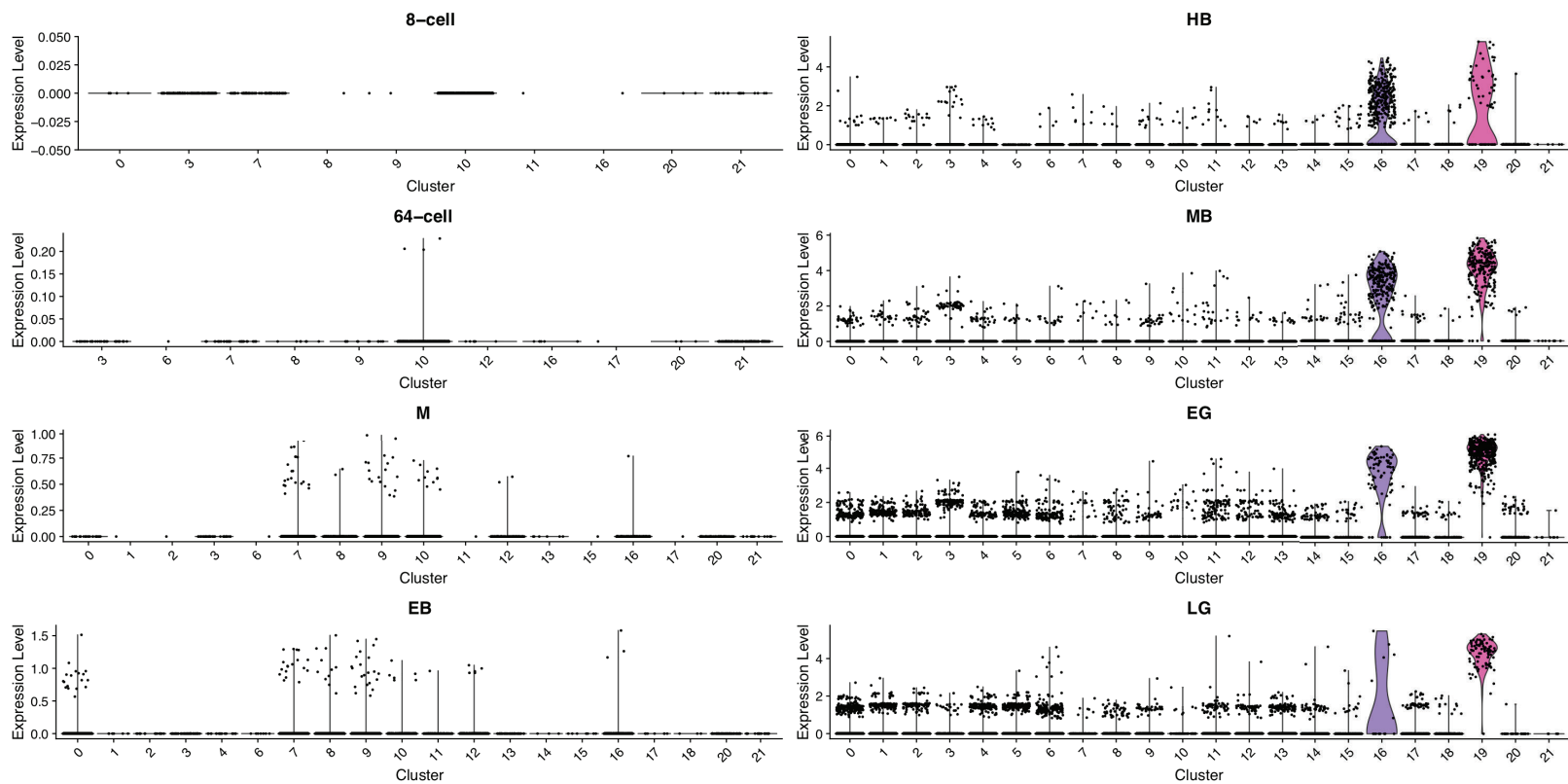


Fig. S12

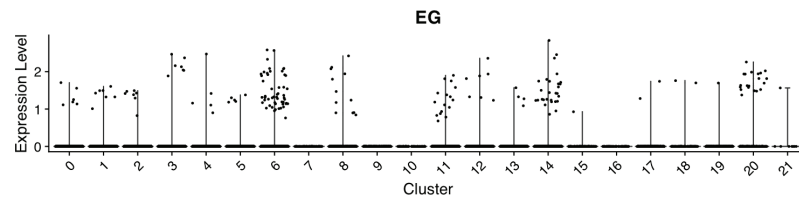


Fig. S12. Violin plot showing enriched expression of FoxY at early gastrula stage in clusters 6, 14, and 20.

Fig. S13: H2A.2.1

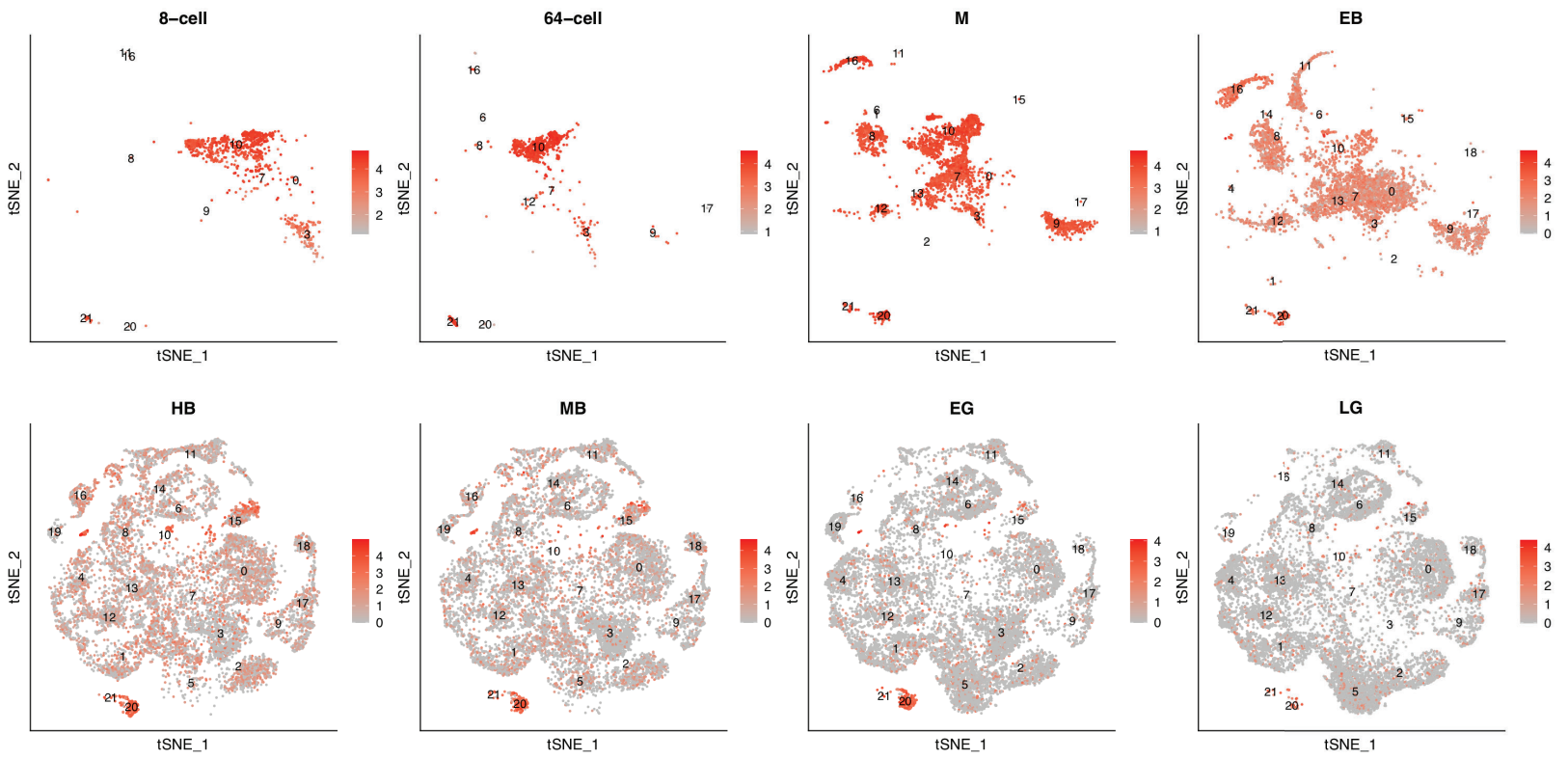
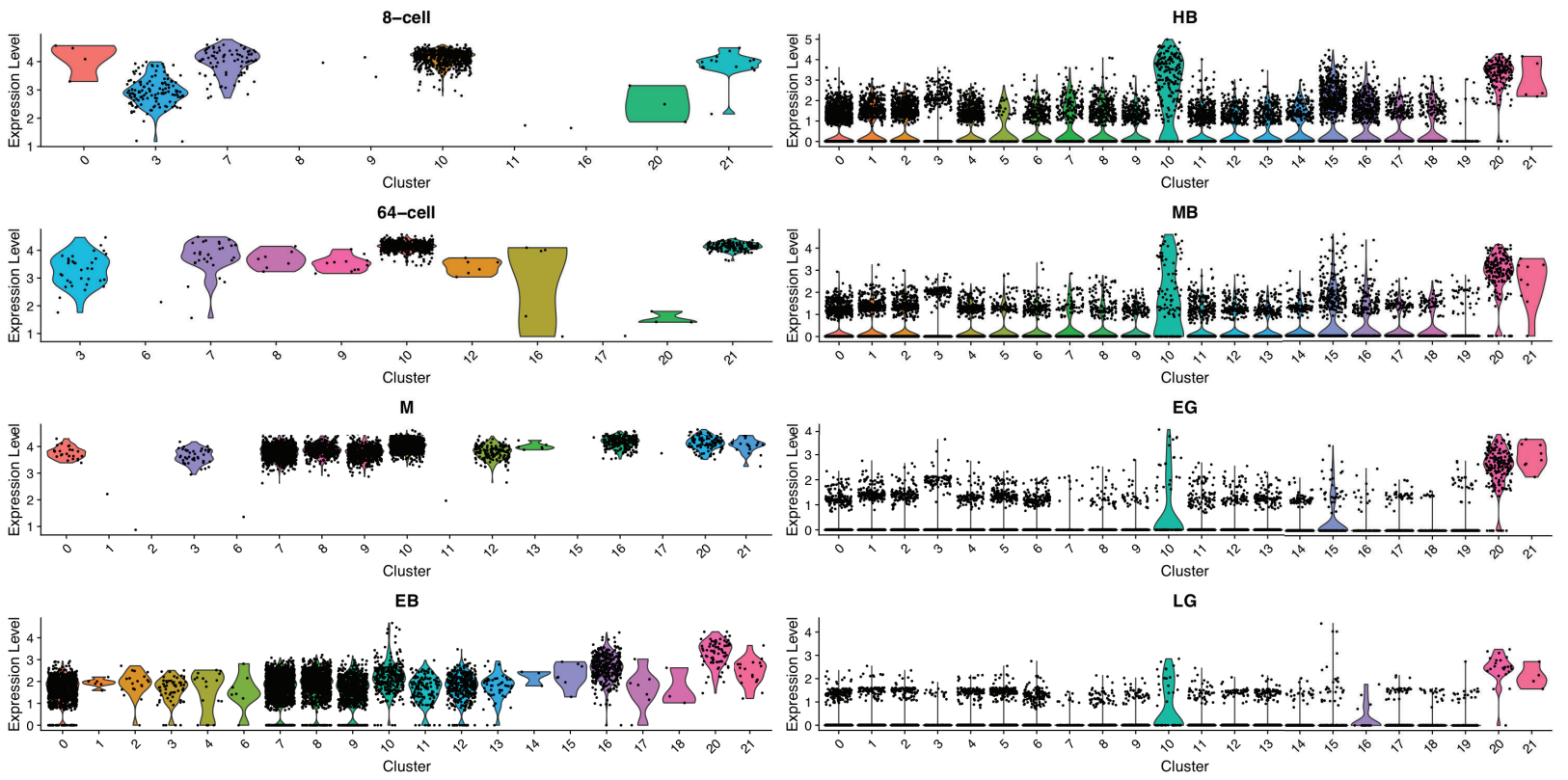


Fig. S14: Vasa

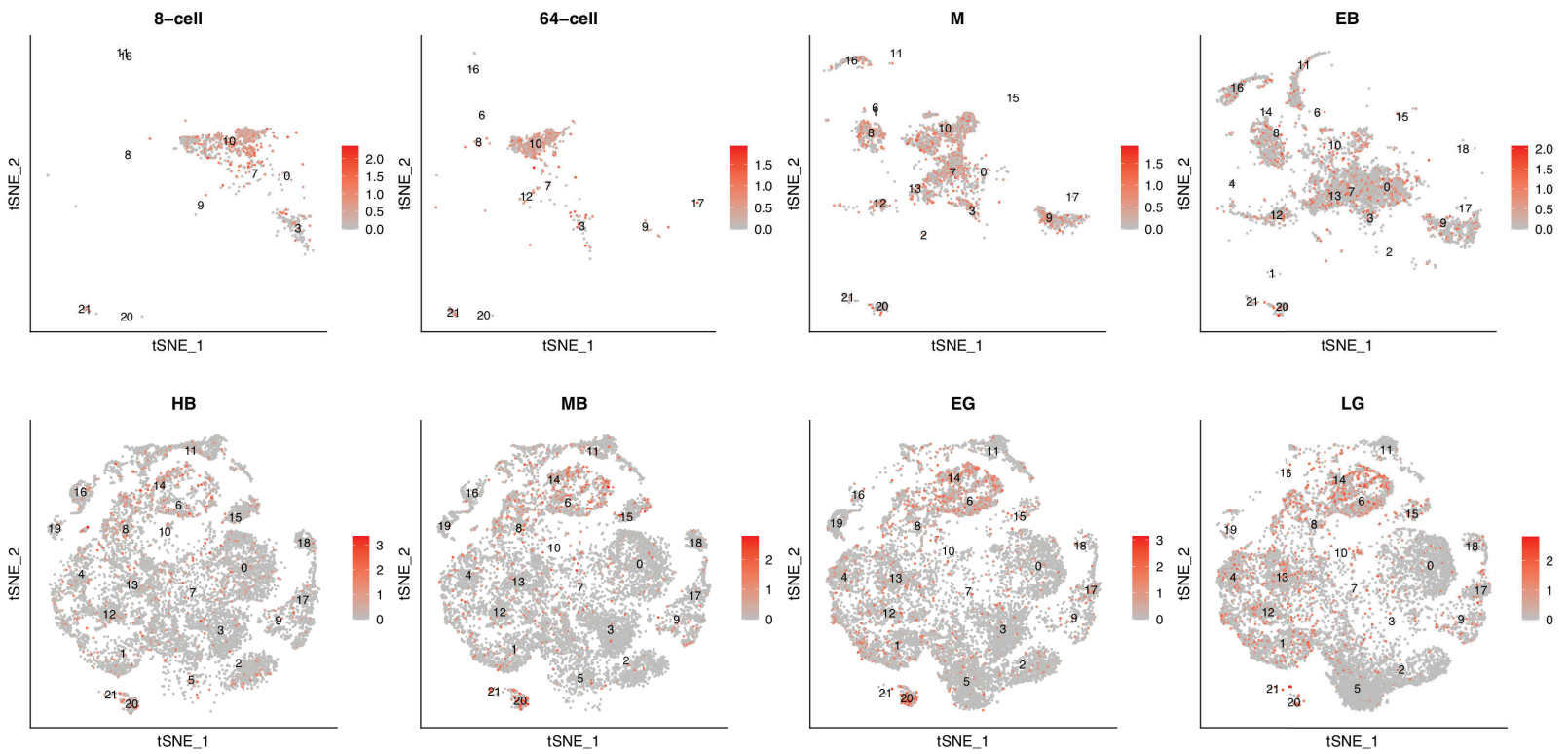
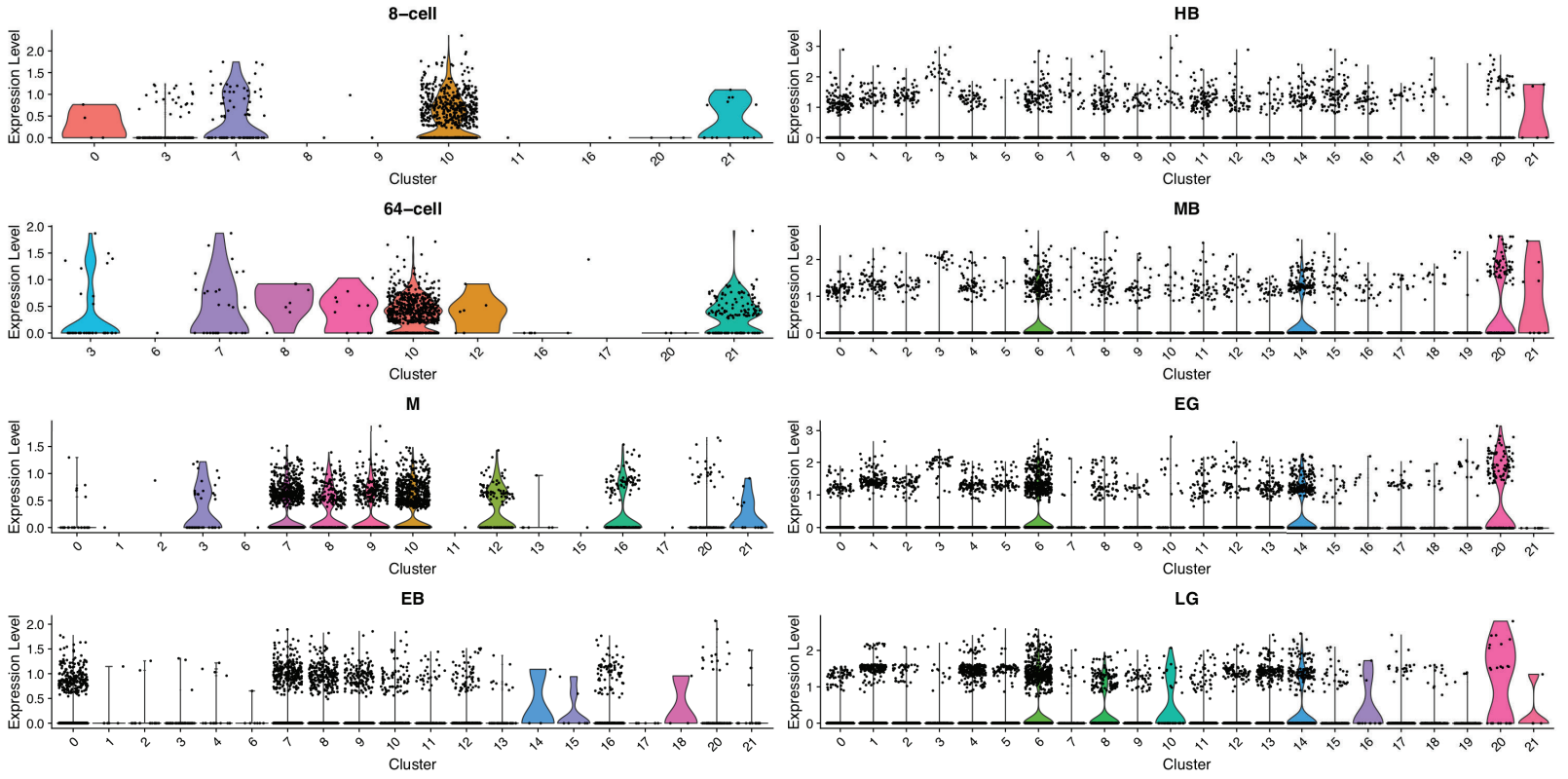


Fig. S15: Seawi

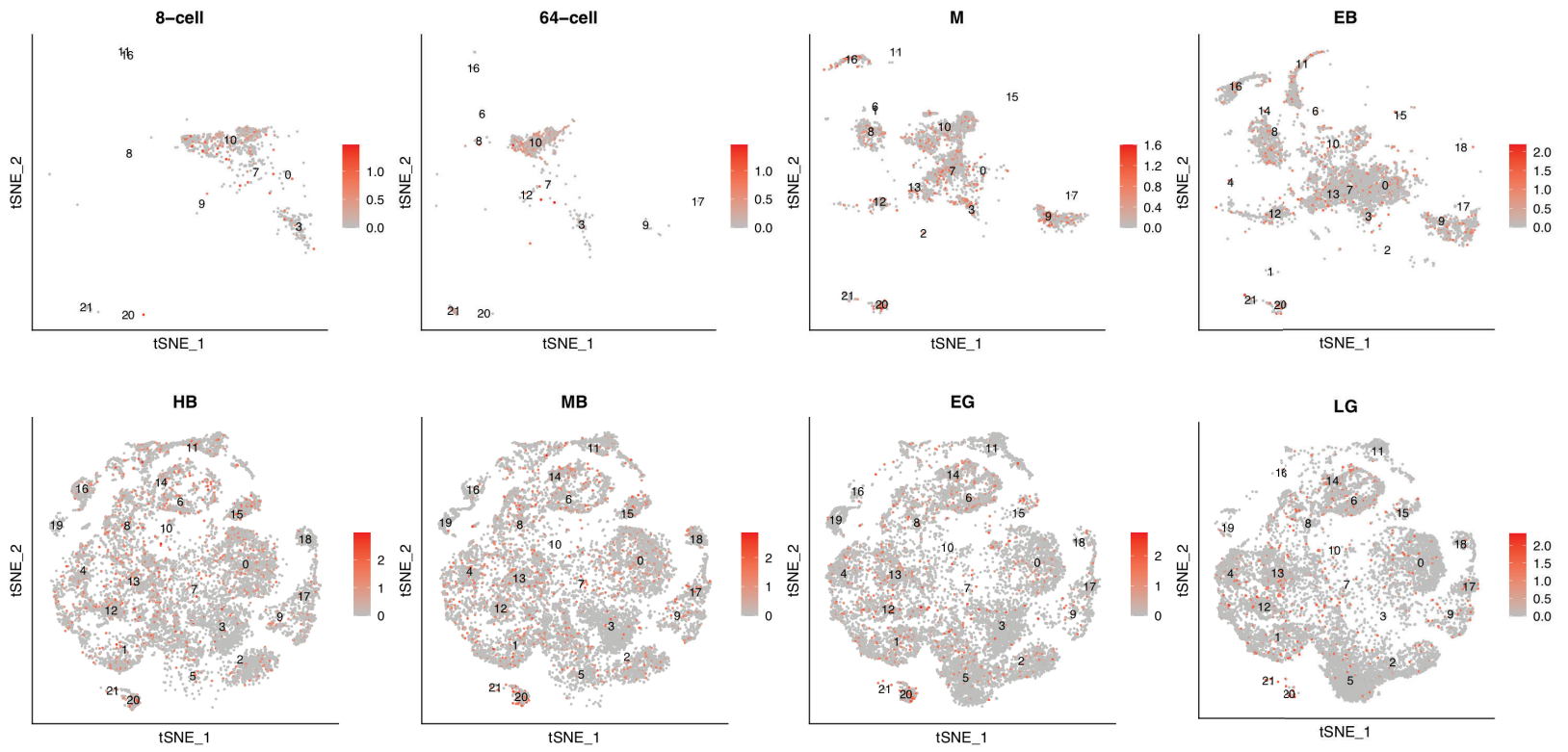
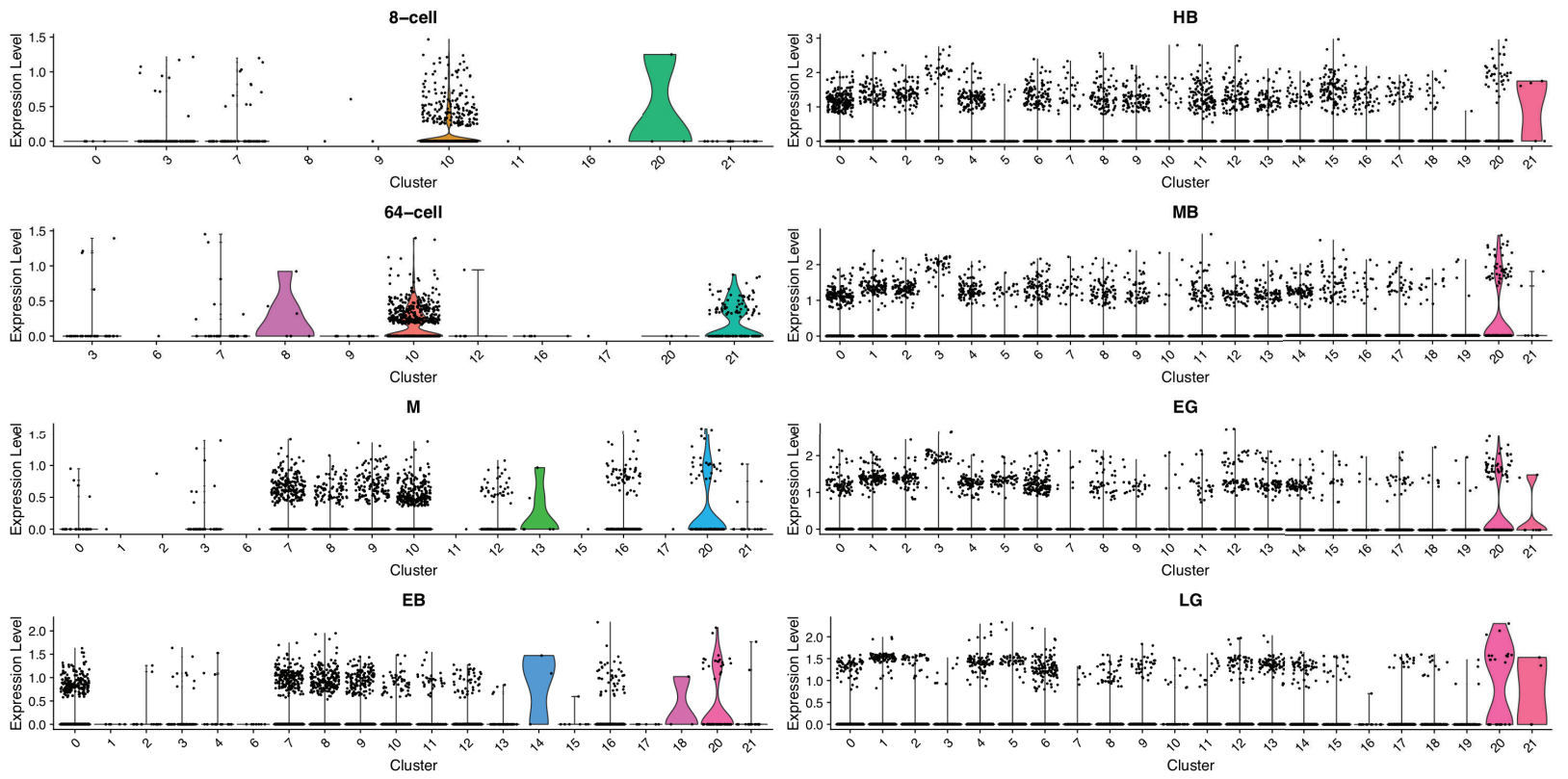


Fig. S16

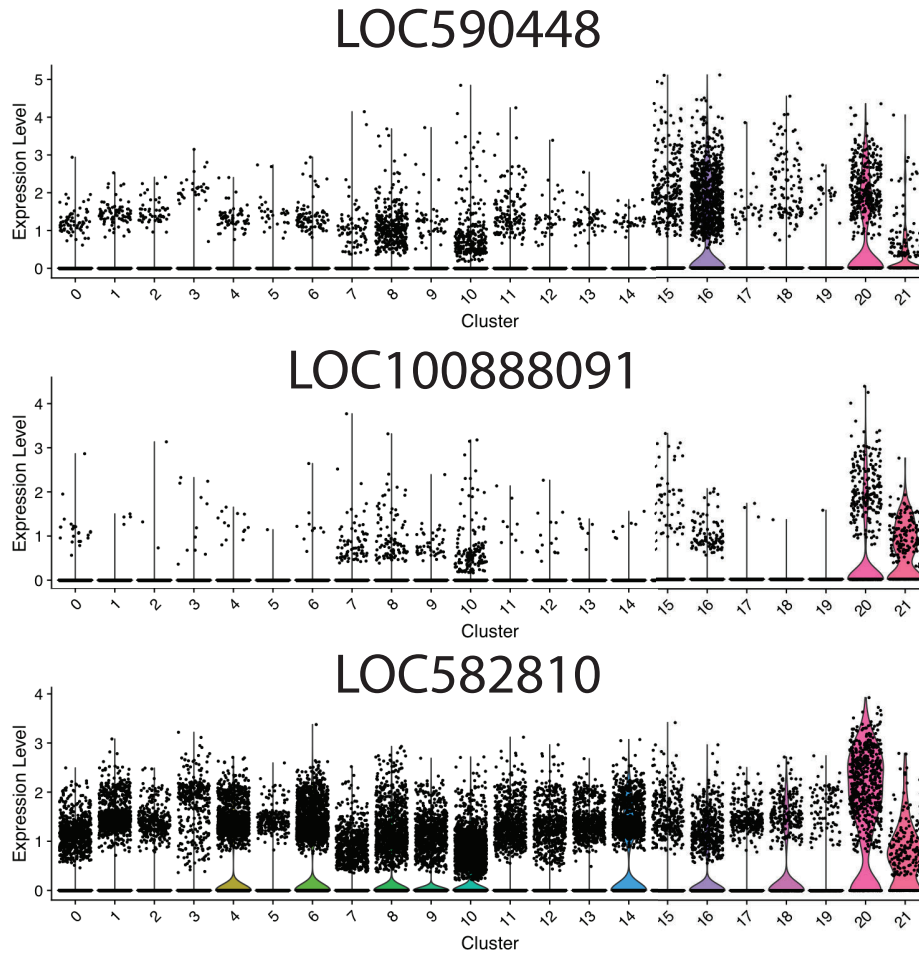


Fig. S16. Violin plots showing enrichment of uncharacterized transcripts in germline cluster 20.

Fig S13: Kruppel

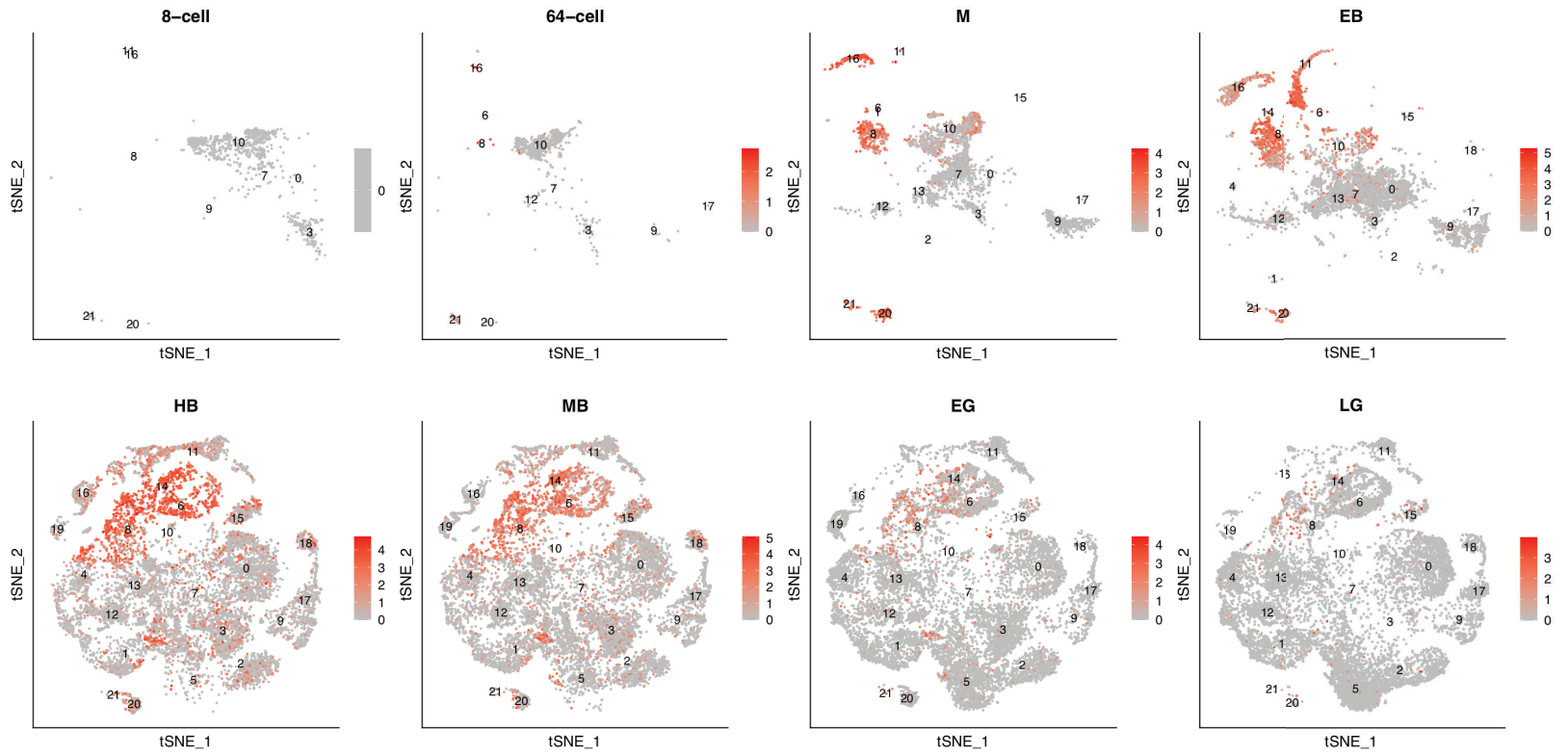
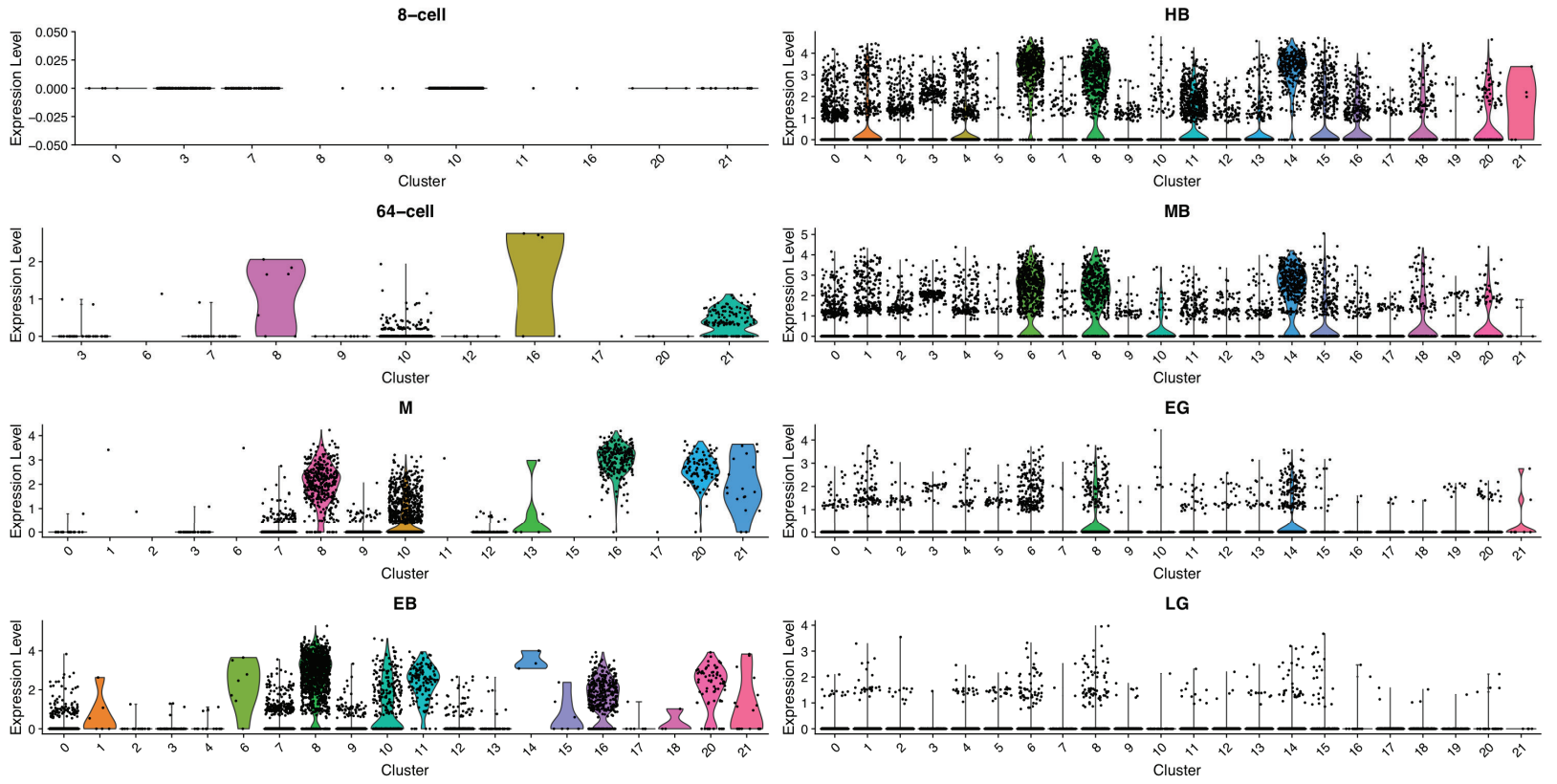


Fig. S18: Odz

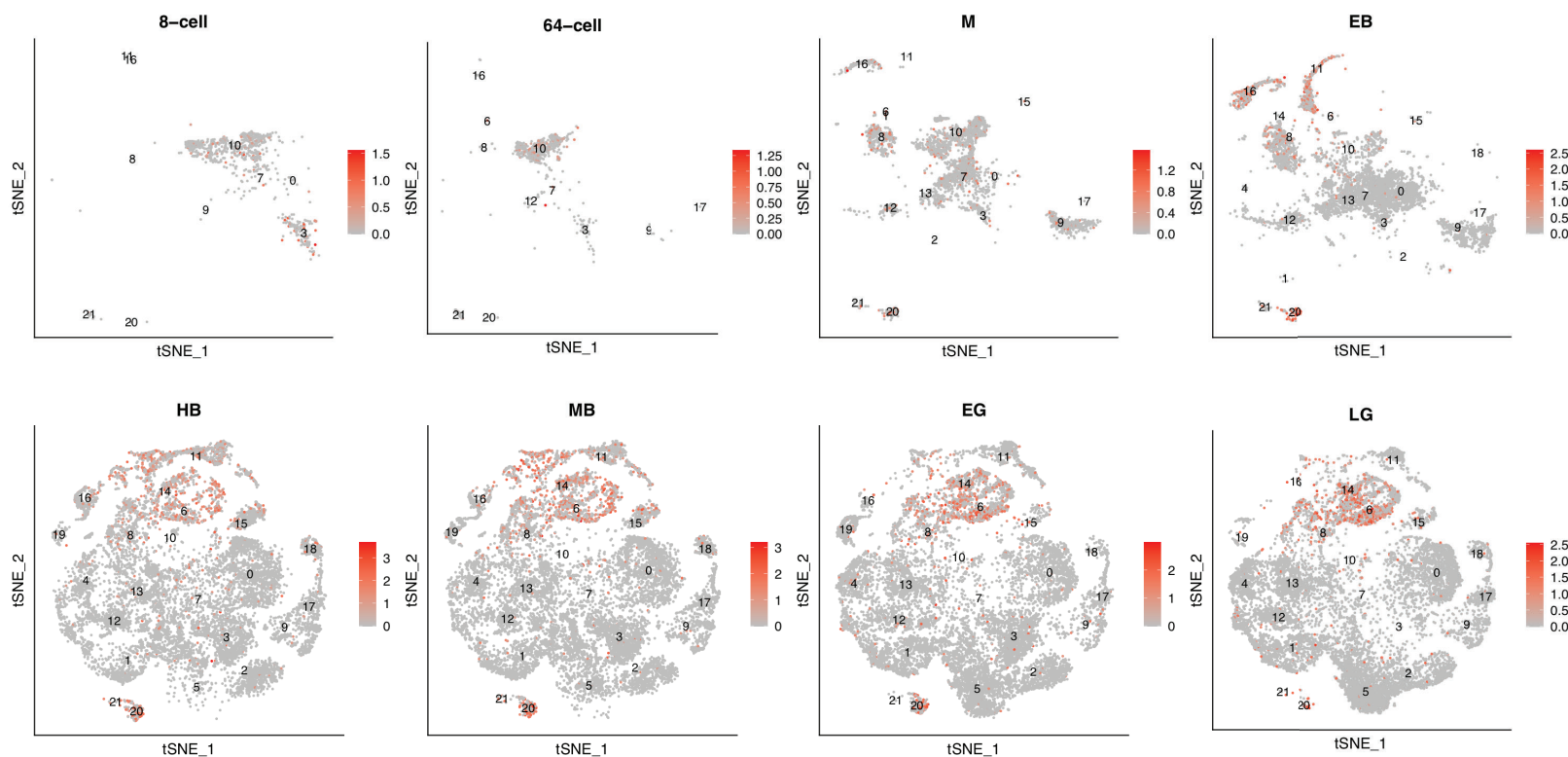
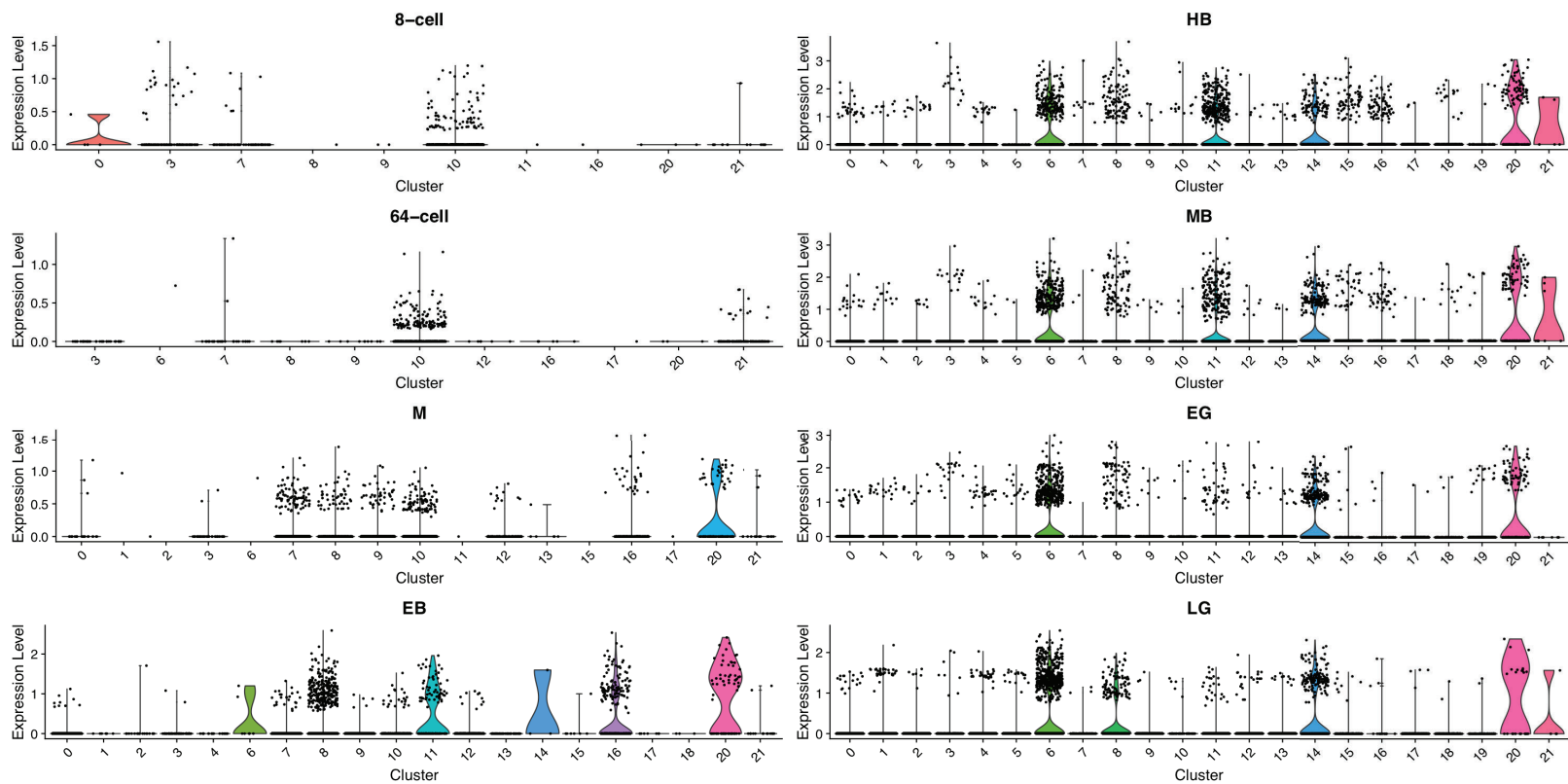


Fig. S19: Stauden

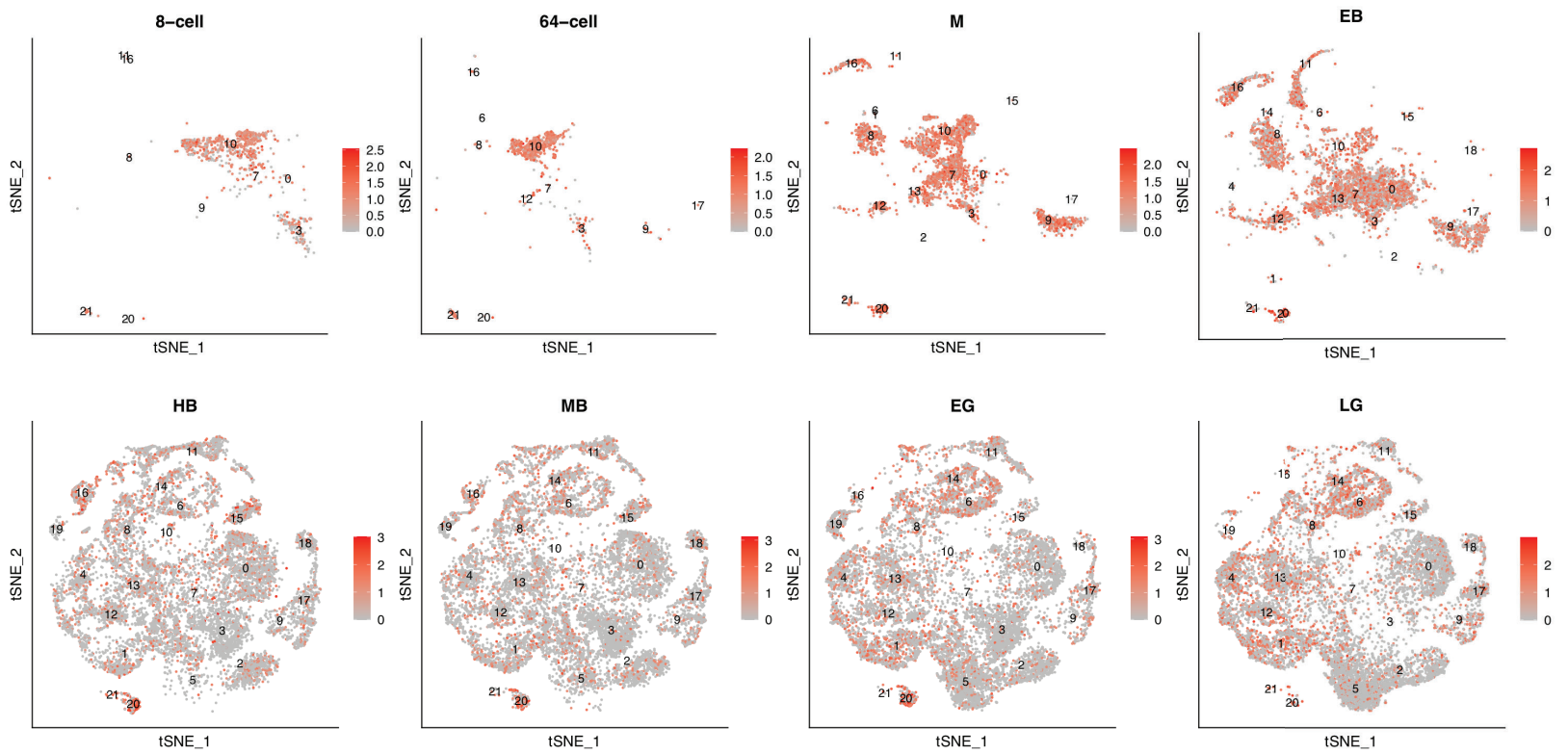
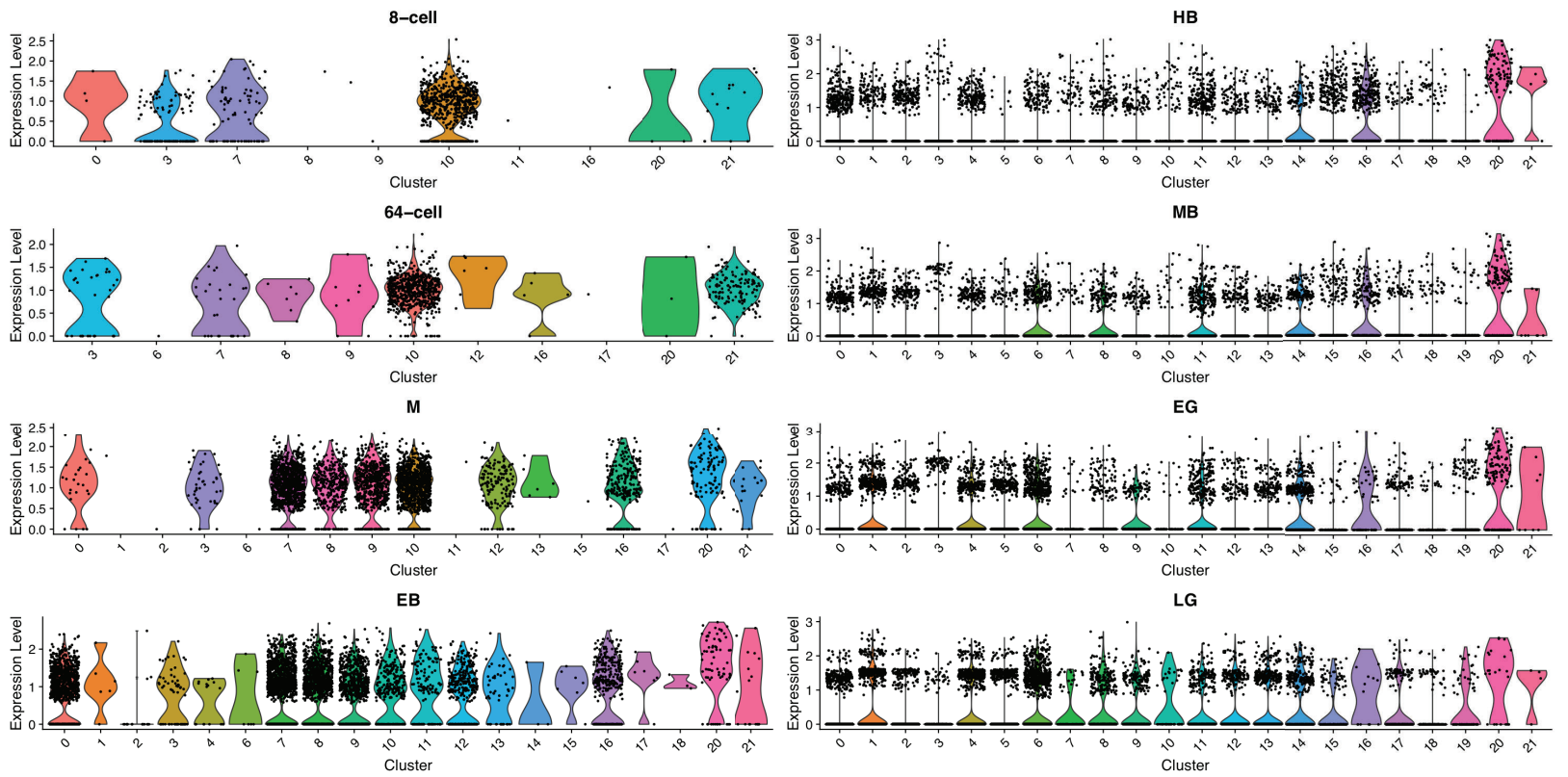


Fig. S20: Maelstrom

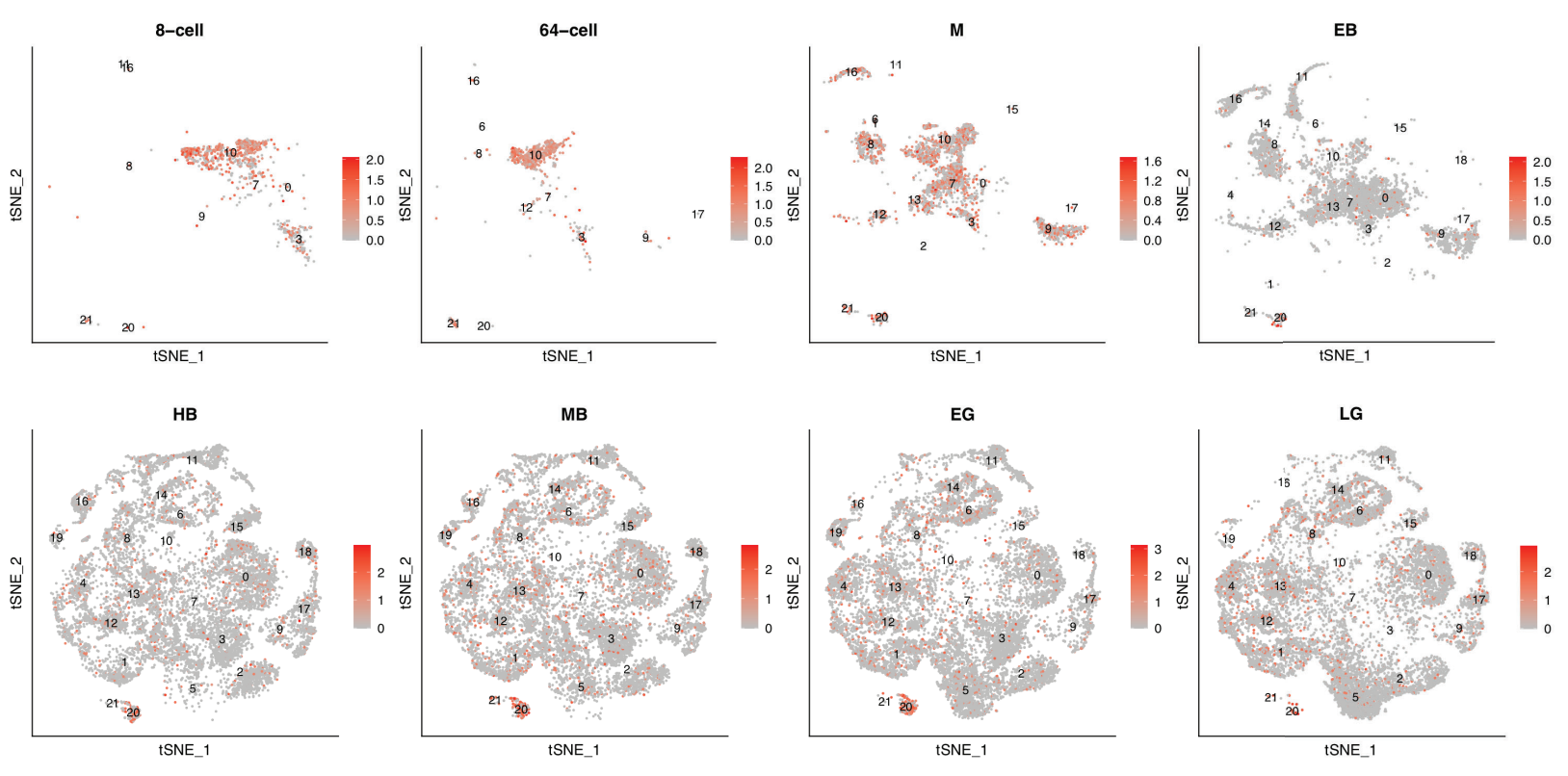
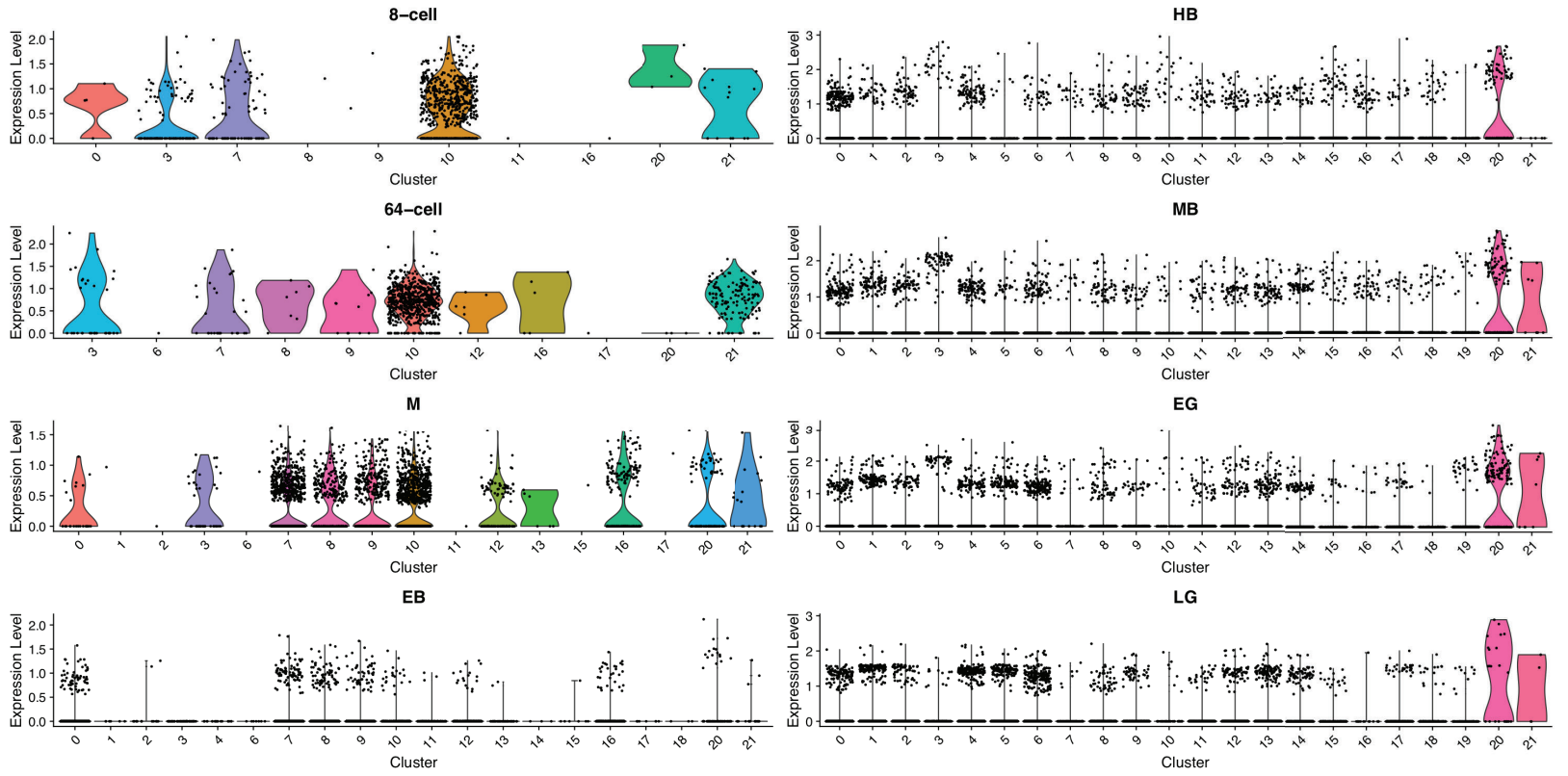


Fig. S21: Gustavus

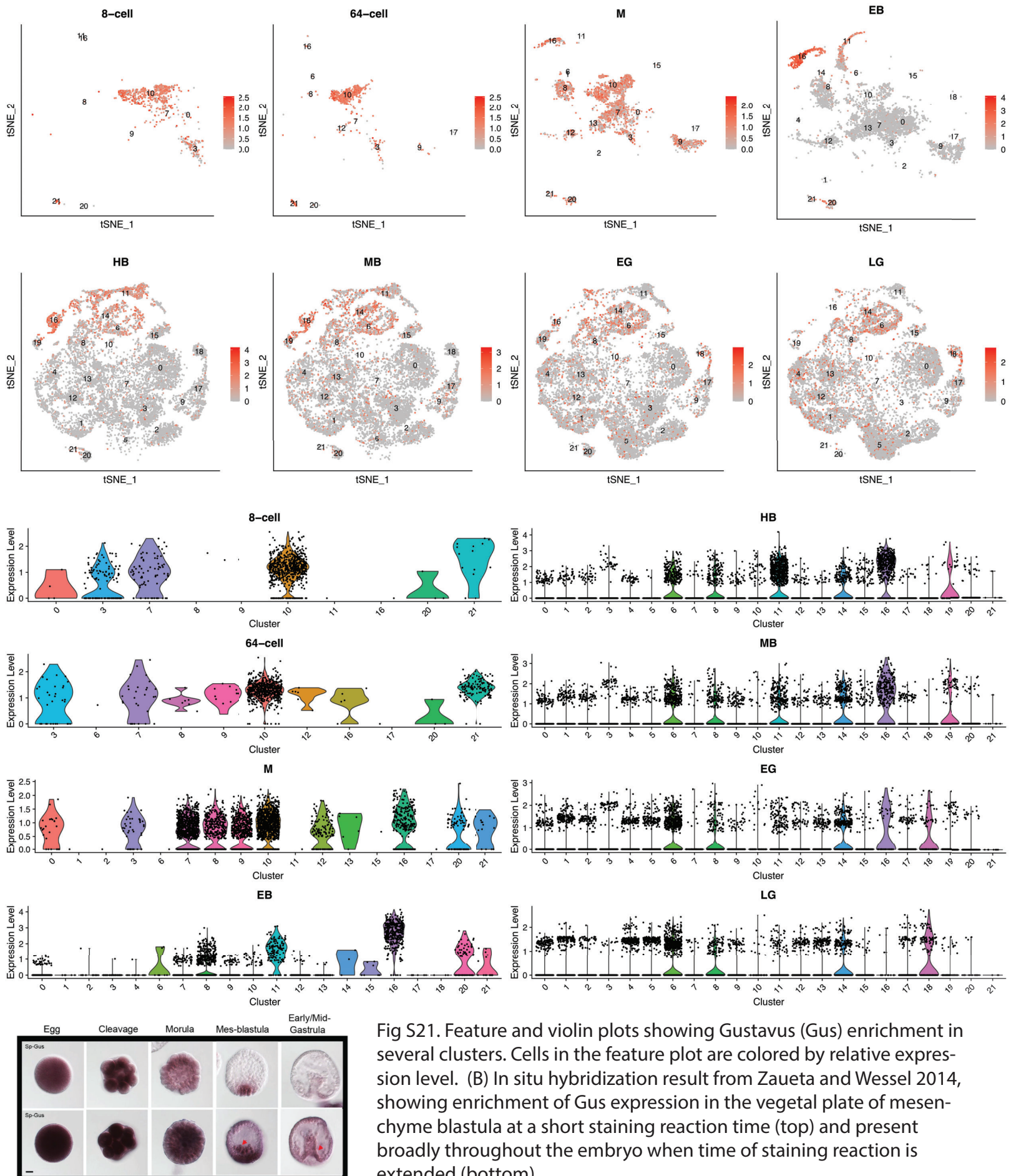


Fig S21. Feature and violin plots showing Gustavus (Gus) enrichment in several clusters. Cells in the feature plot are colored by relative expression level. (B) In situ hybridization result from Zaueta and Wessel 2014, showing enrichment of Gus expression in the vegetal plate of mesenchyme blastula at a short staining reaction time (top) and present broadly throughout the embryo when time of staining reaction is extended (bottom).

Fig. S22: FoxG

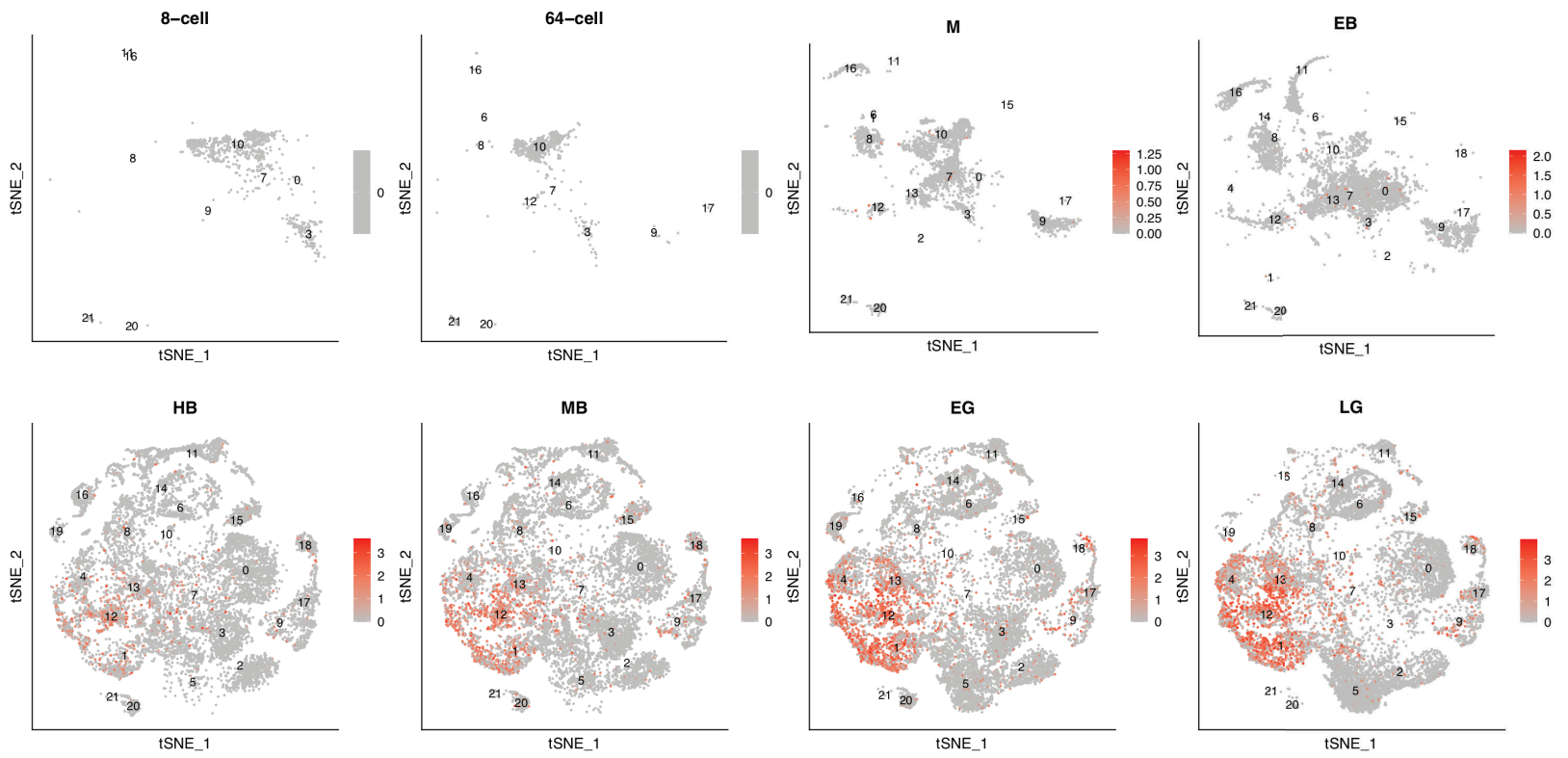
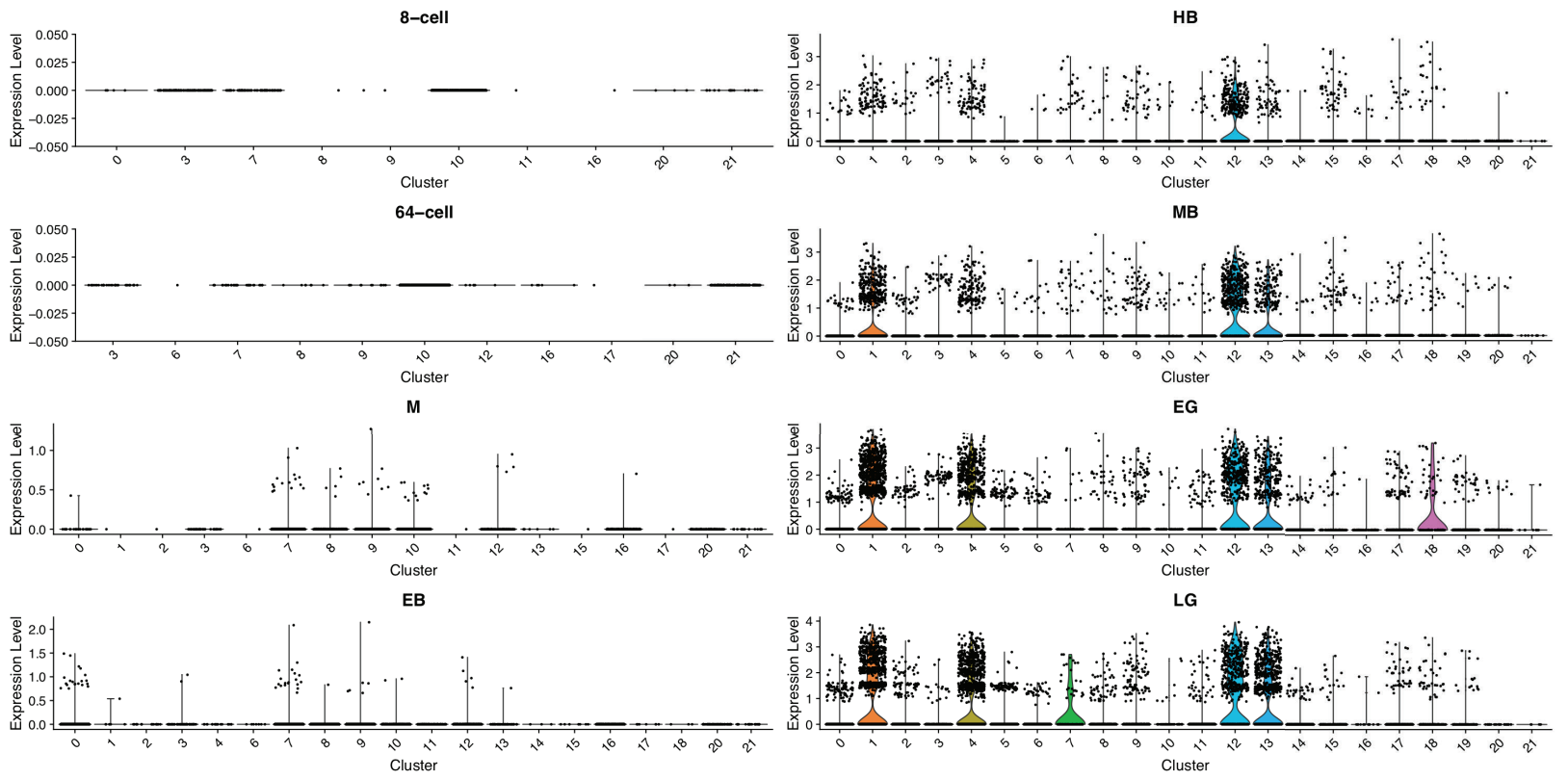


Fig. S23: CHR D

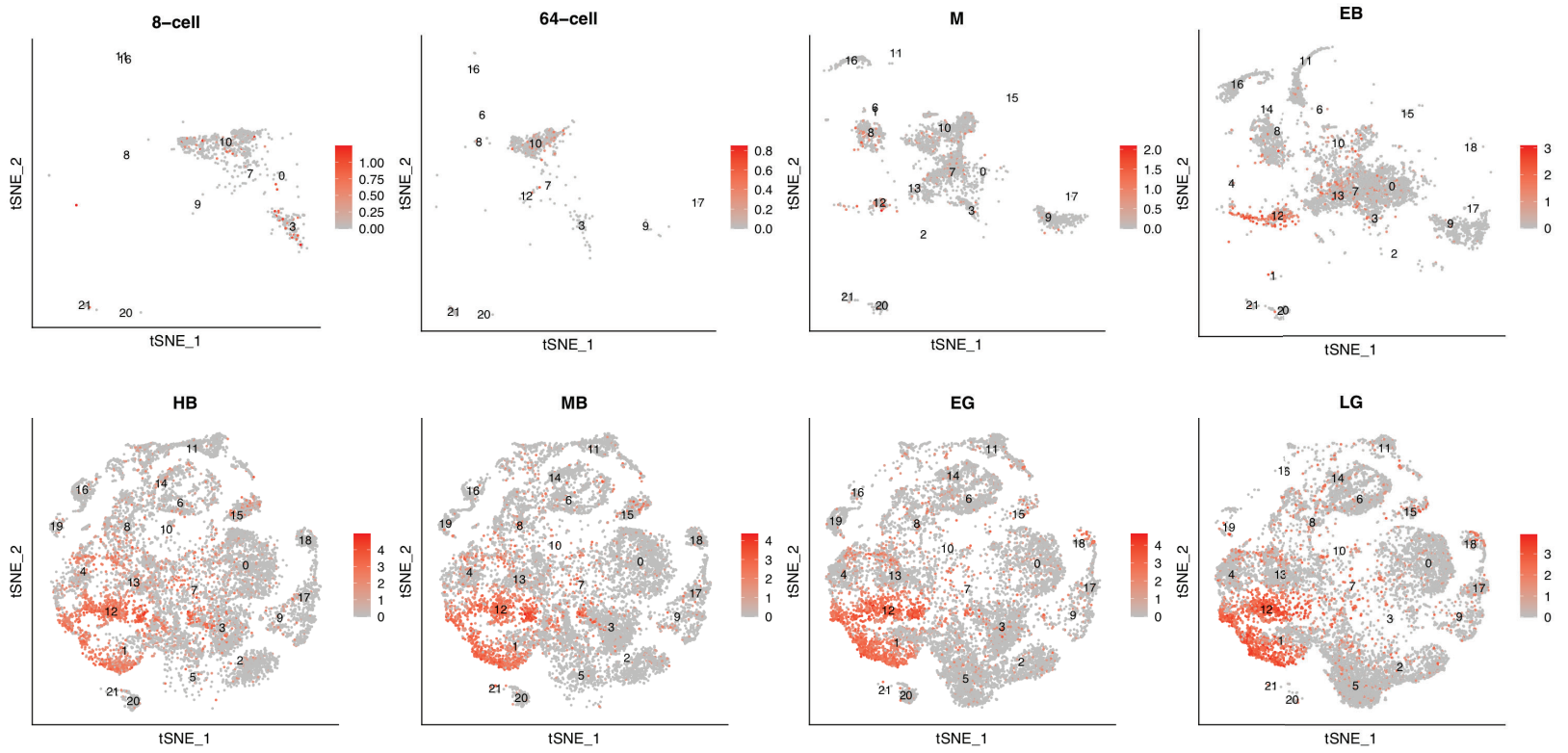
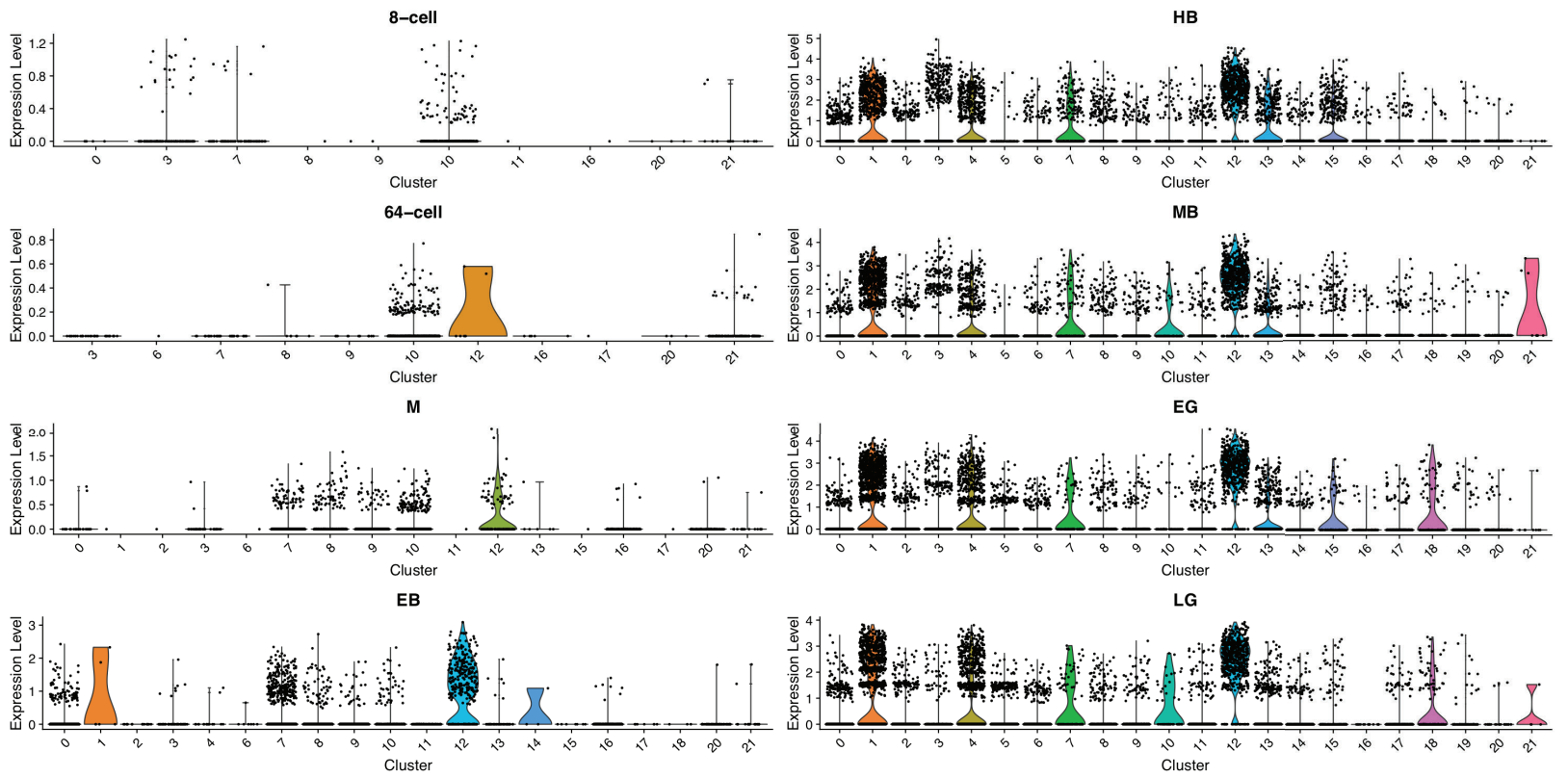


Fig. S24: Lim1

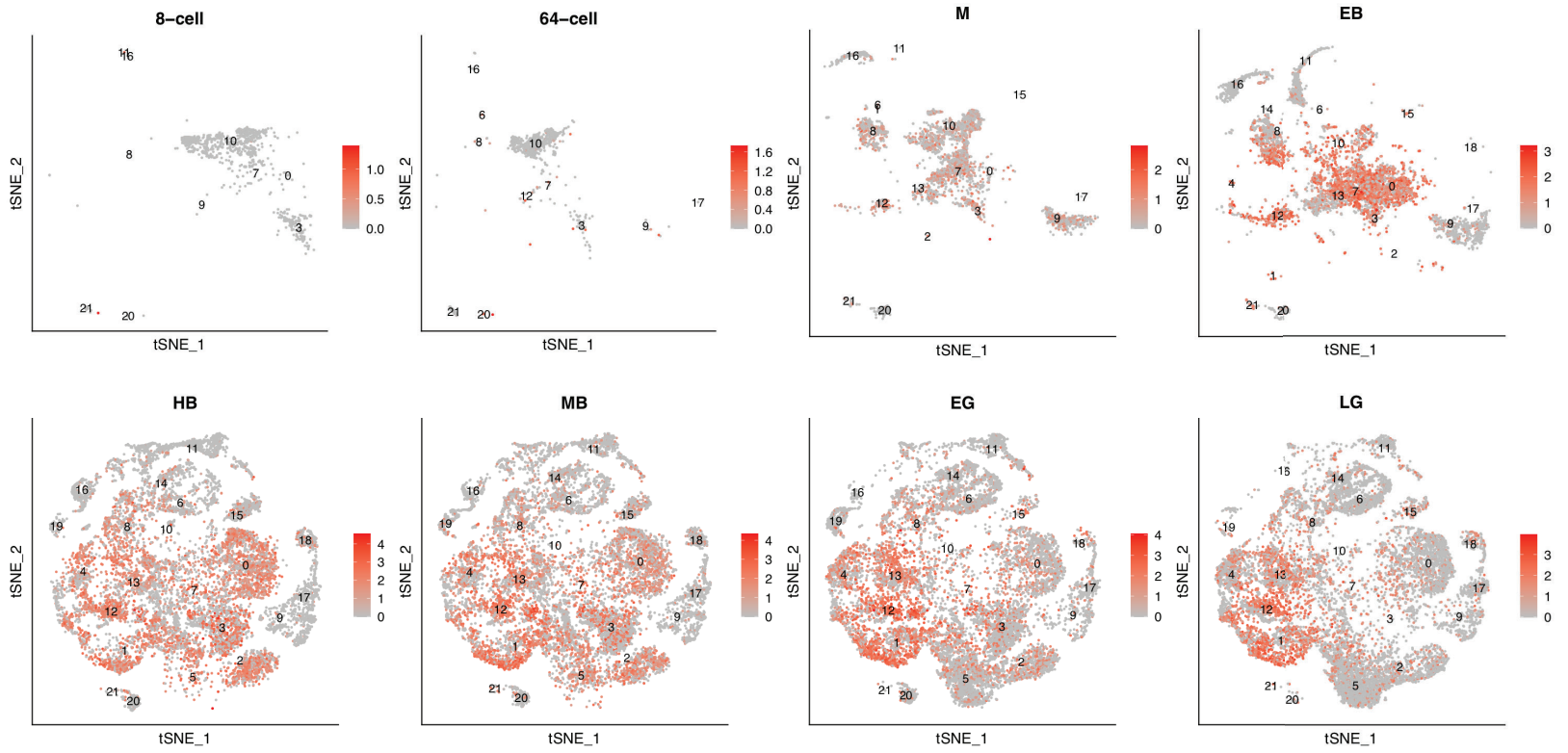
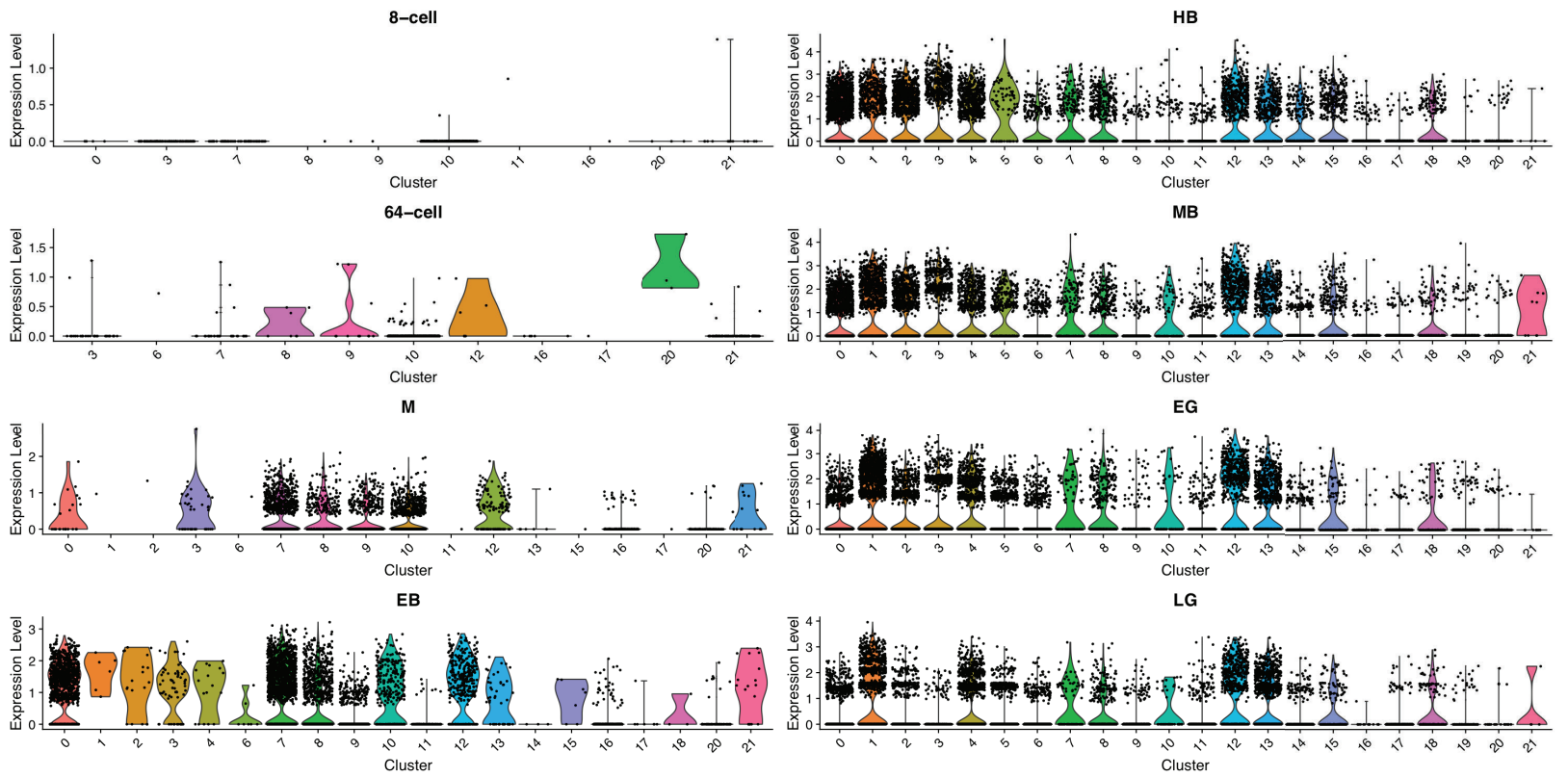


Fig. S25

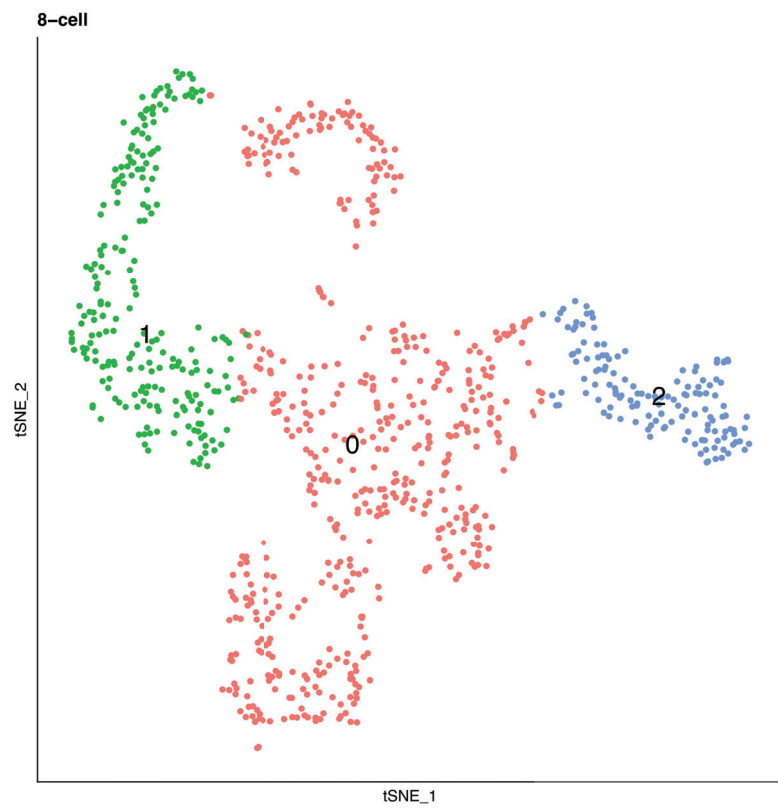


Fig. S25. tSNE plot of 8-cell stage, unintegrated dataset shows the 886 cells cluster into three major cell states. (Clustering parameters used were dims=4, res=0.1.)

Fig. S26

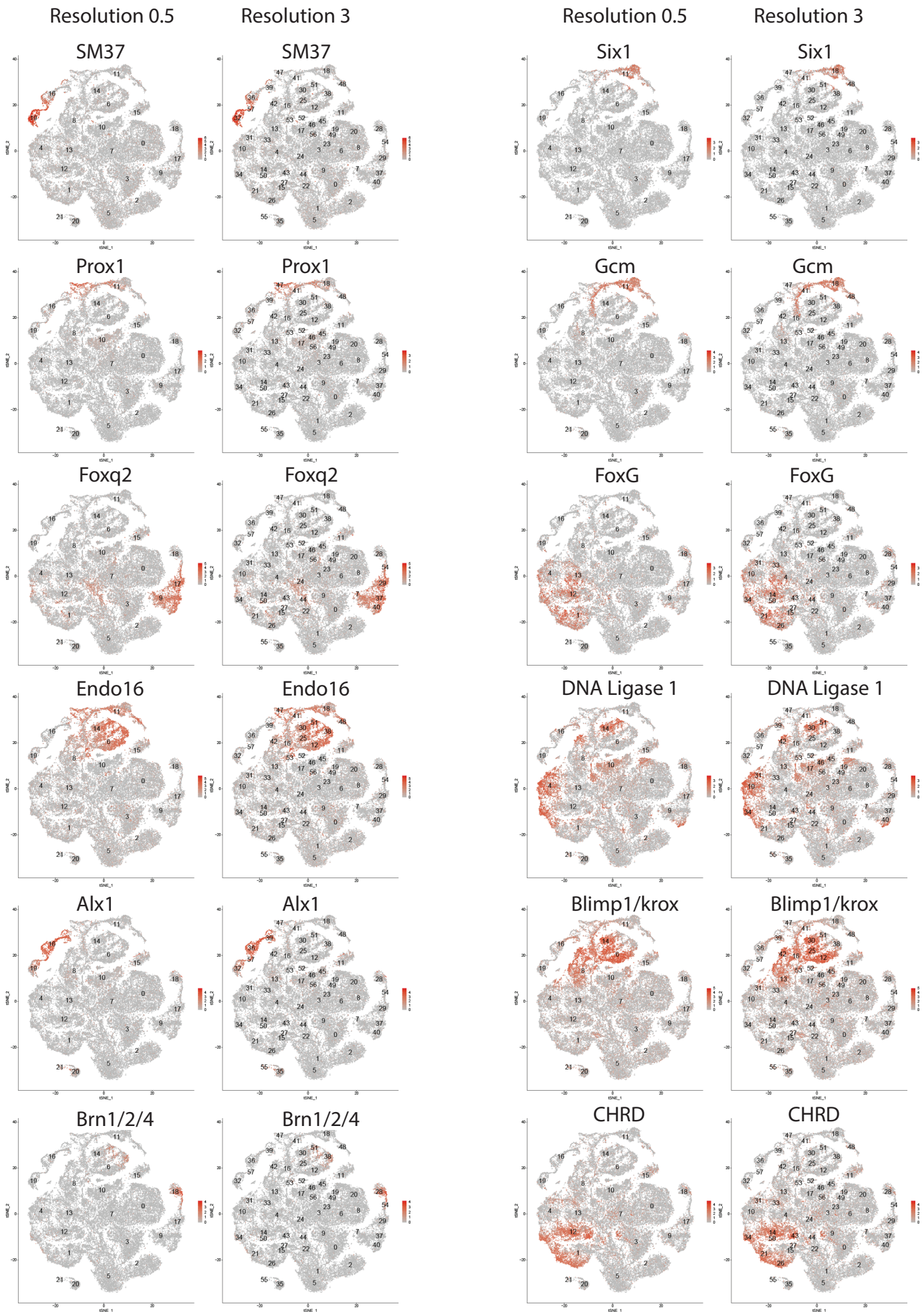


Fig. S26. Feature plots for several marker genes seen at a resolution of 0.5 and 3. Higher resolution differentiates subpopulations of the cell states identified at resolution 0.5. Foregut gene *Brn1/2/4* which is seen in some cells of cluster 14 and 6 (res=0.5) is enriched in clusters 51 and 38 (res=3).

Fig. S27: Cyclin B

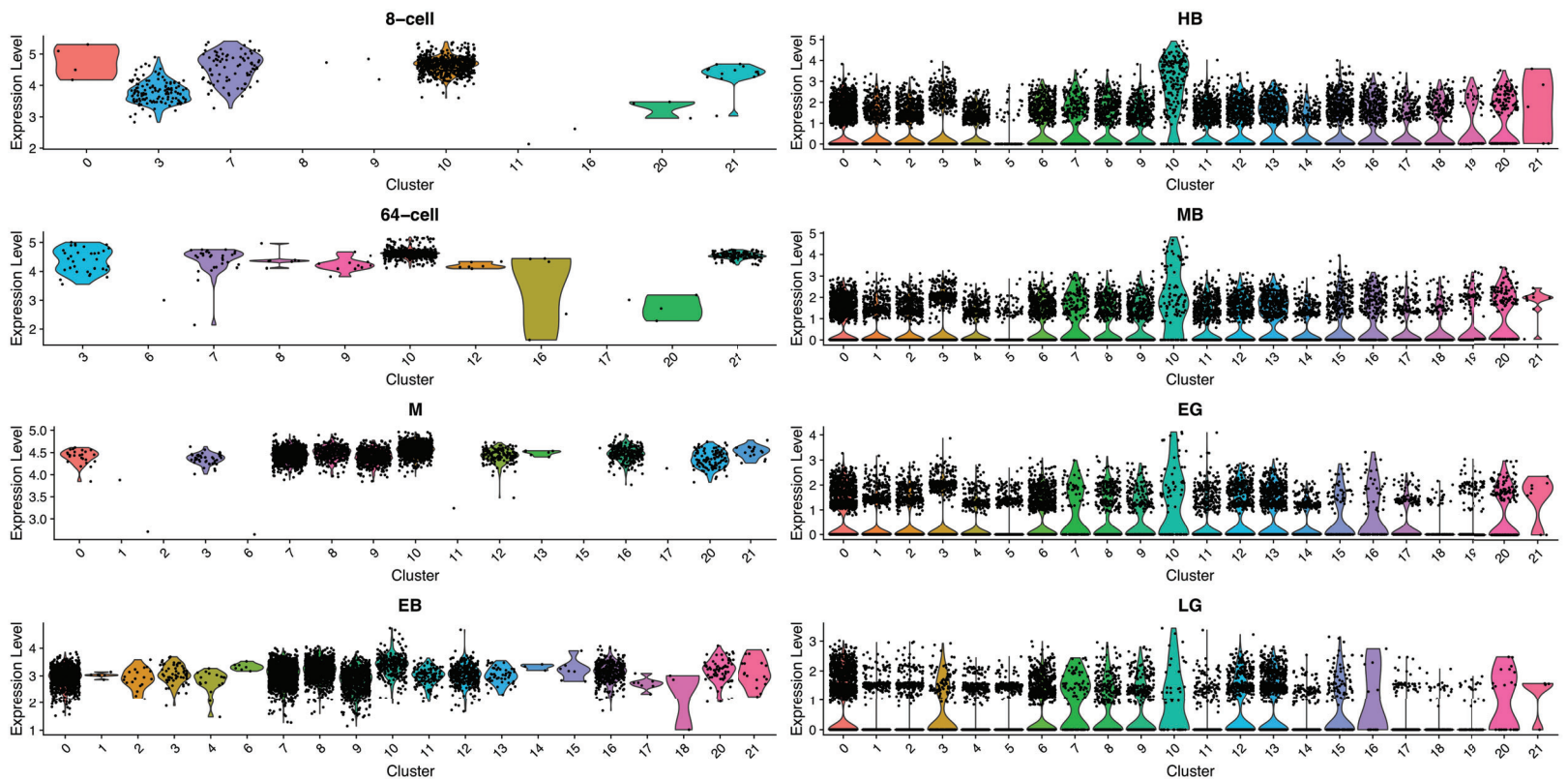


Fig. S27. Violin plots showing Cyclin B expression across different clusters at each developmental stage. Normalized gene expression values at log scale shown per cell cluster identity for each developmental time point. Plots depict probability density at different expression levels.