Fig. S1

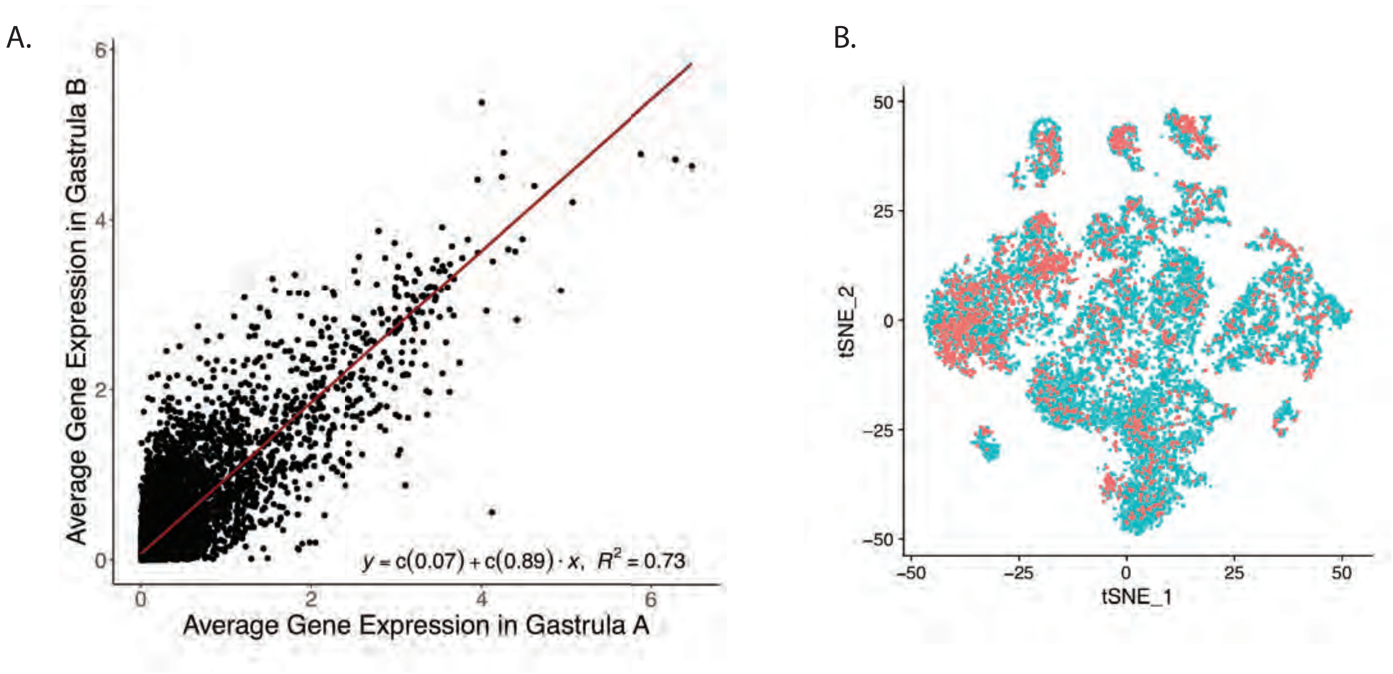


Fig. S1. (A) Comparison of average gene expression between Gastrula A, control from previous experiment (2,435 cells), and Gastrula B, corresponding time point from current analysis (12,763 cells). F-statistic: 5.837e+04 on 1 and 21088 degrees of freedom, p-value: < 2.2e-16. (B) t-SNE plot of early gastrula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

Fig. S2:

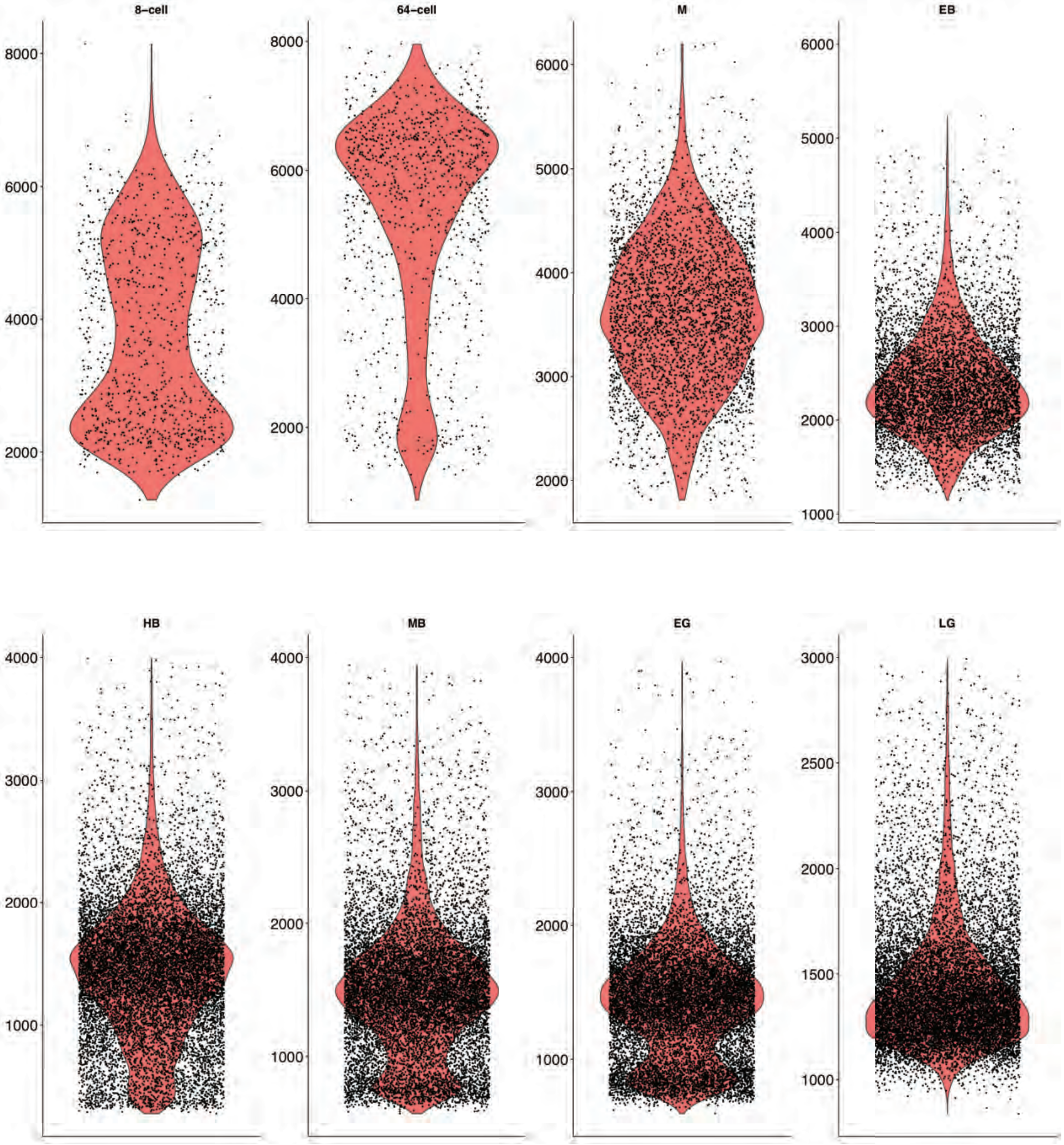


Fig. S2. Total genes detected per cell across developmental time points.

Fig. S3

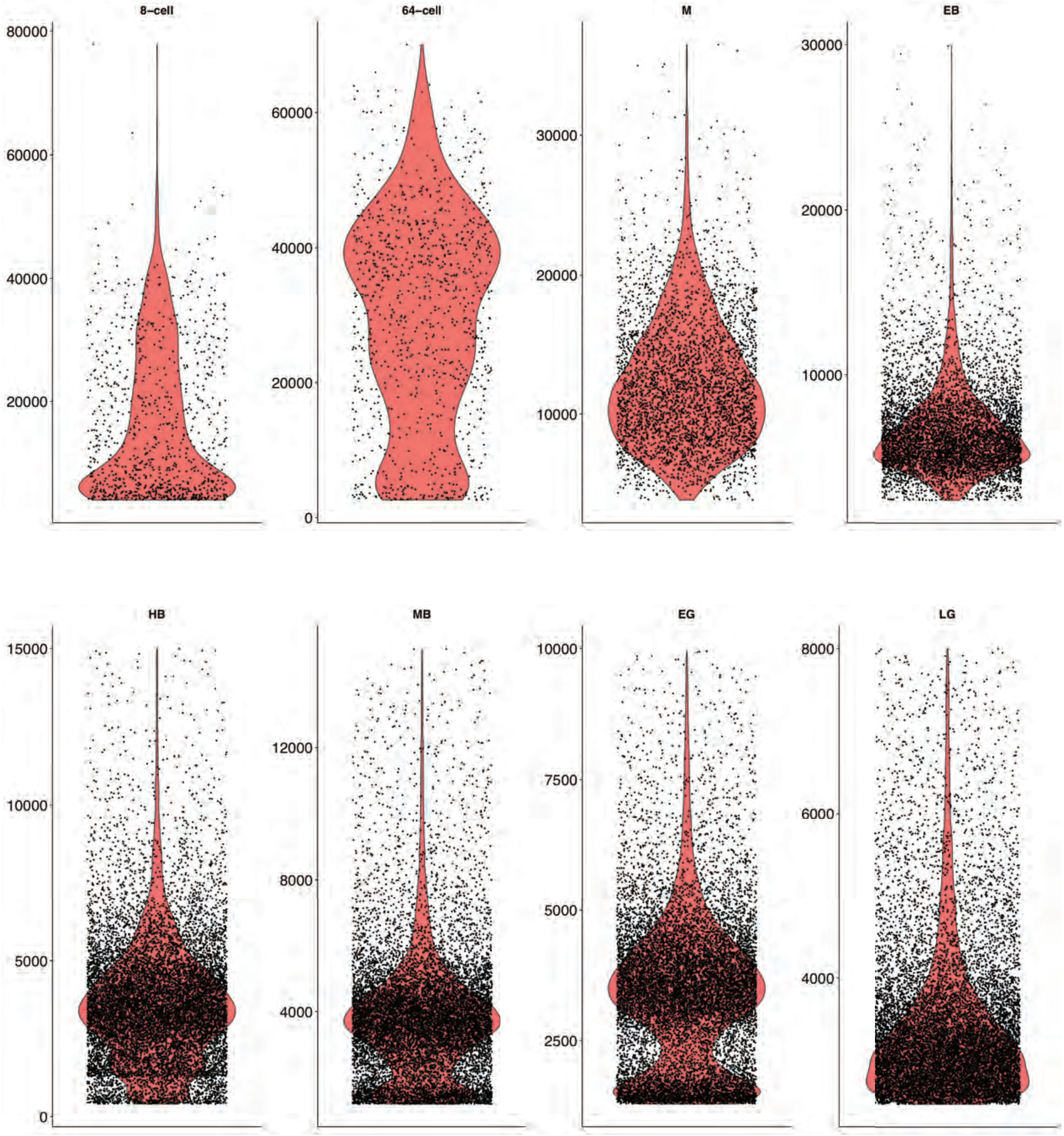


Fig. S3. Total UMIs detected per cell across developmental time points.

Fig. S4: SoxB2

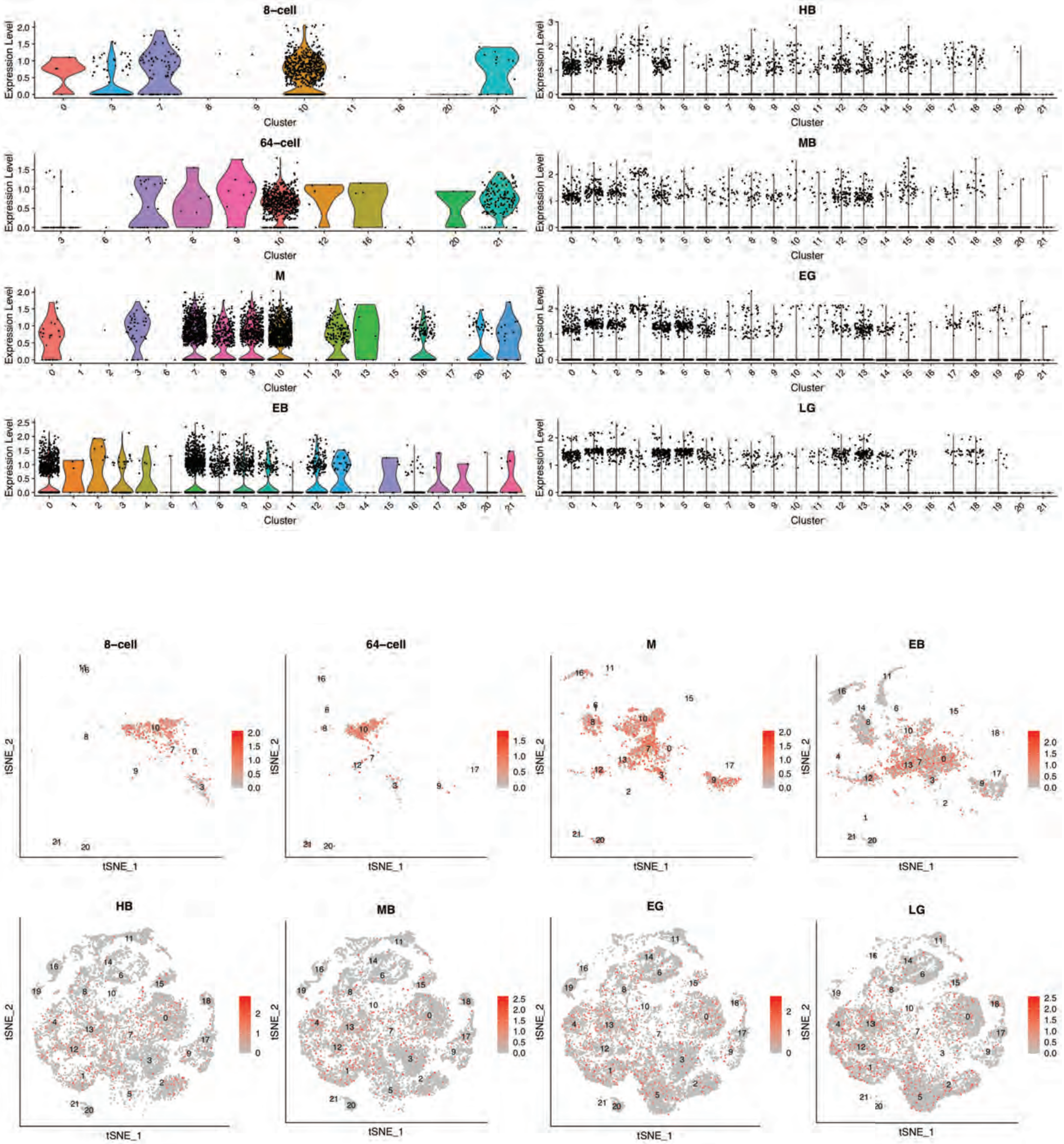
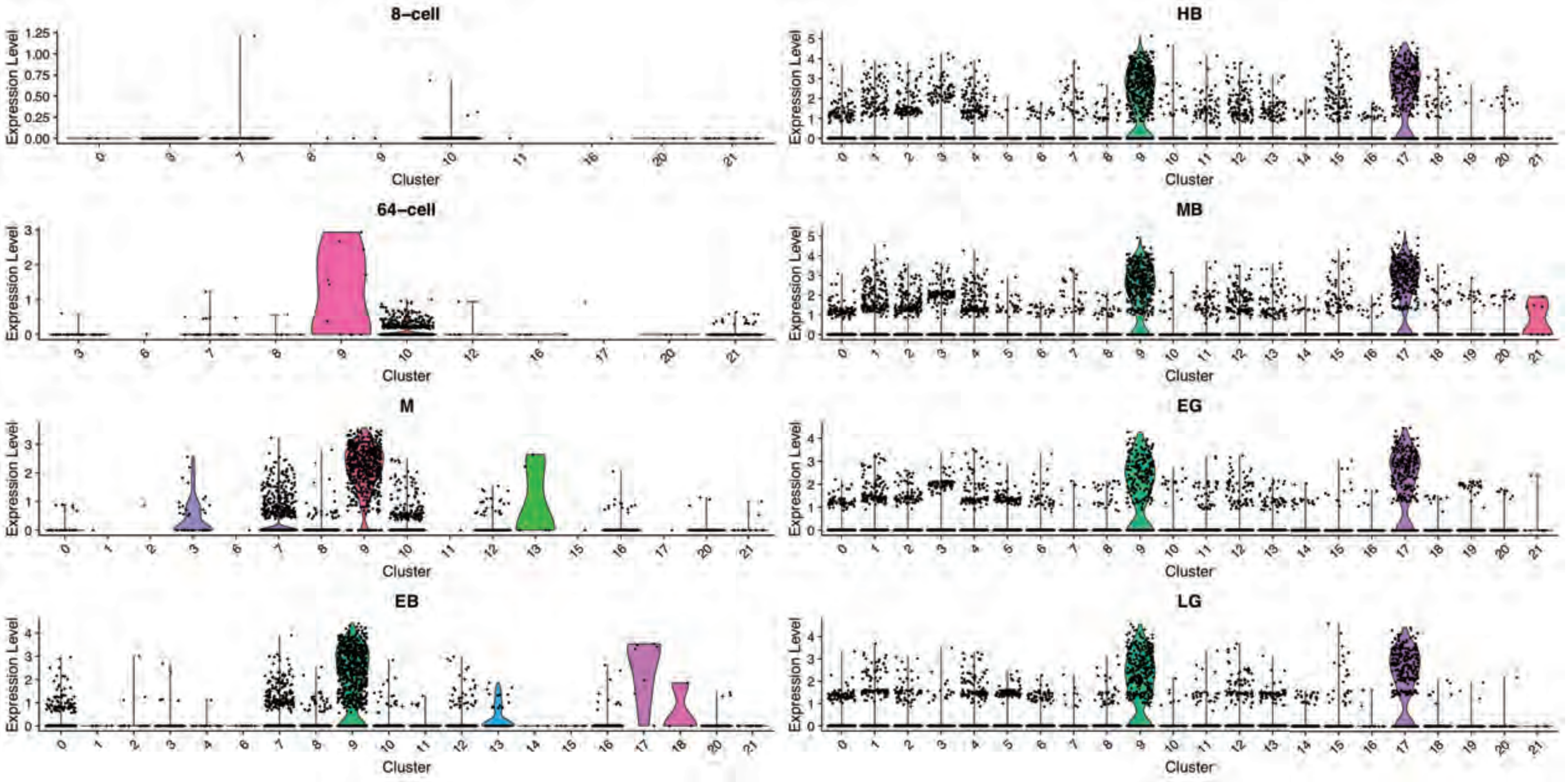


Fig. S4-S7. Violin plots showing expression across different clusters at each developmental stage. Normalized gene expression values at log scale shown per cell cluster identity for each developmental time point. Plots depict probability density at different expression levels. Feature plots showing expression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

Fig. S5: Foxq2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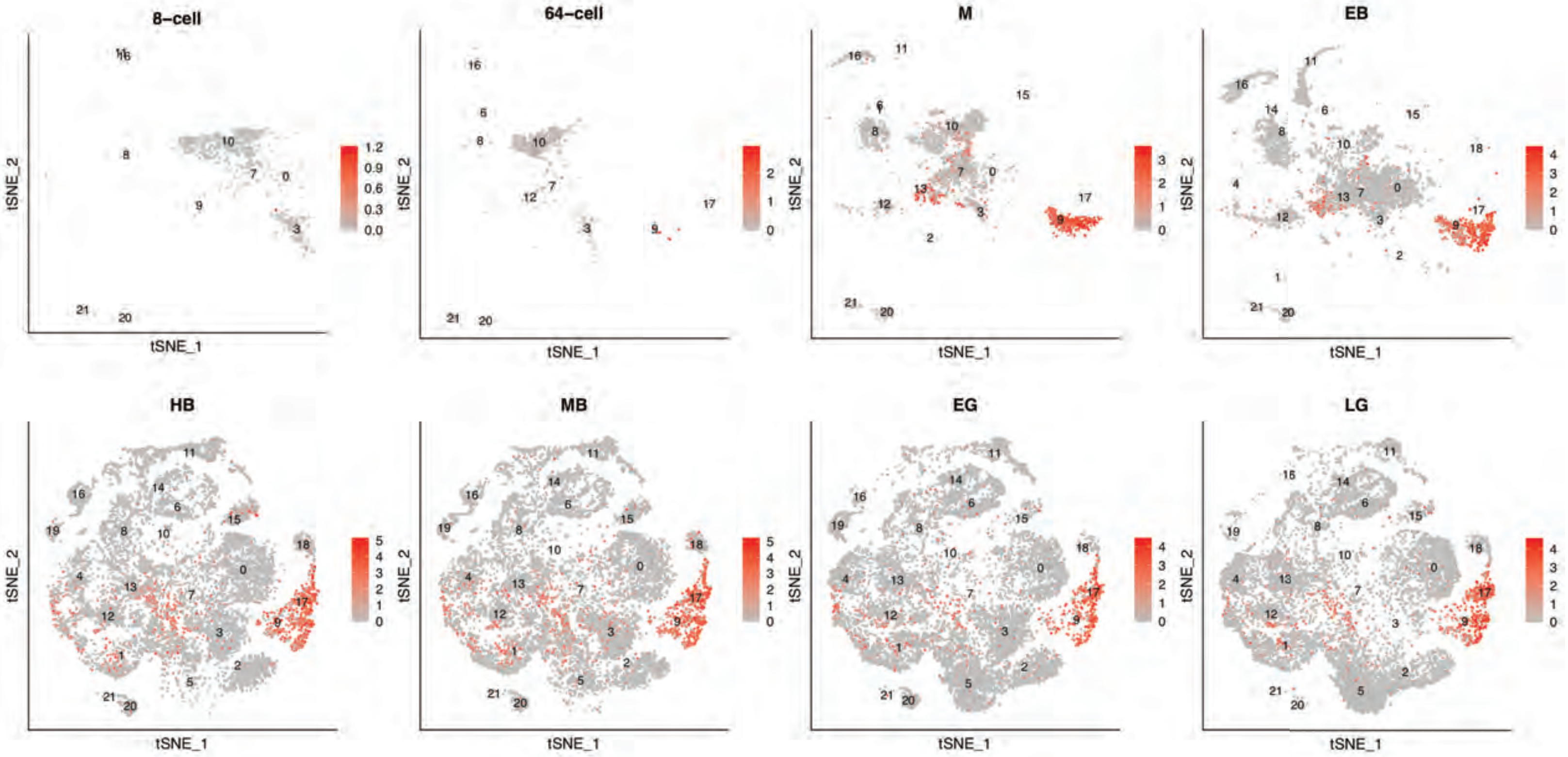
HB
64–cell
MB
M
EG
EB
LG
Expression Level
Cluster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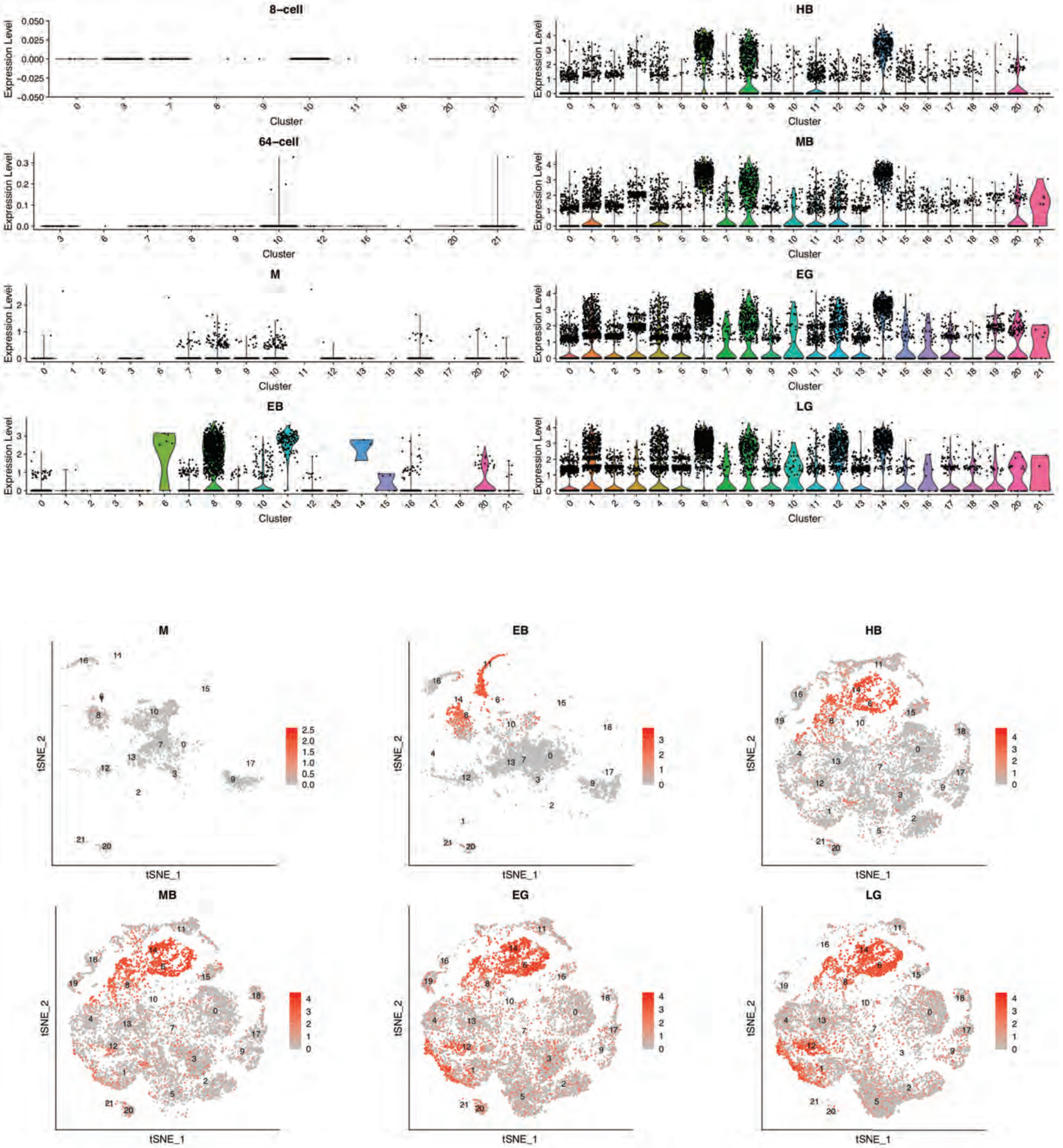
M
EB
HB
MB
EG
LG
tSNE_1
tSNE_2

Fig. S6: FoxA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Expression Level
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Cluster
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EG
EB
LG
M
EB
HB
MB
EG
LG
tSNE_1
tSNE_2
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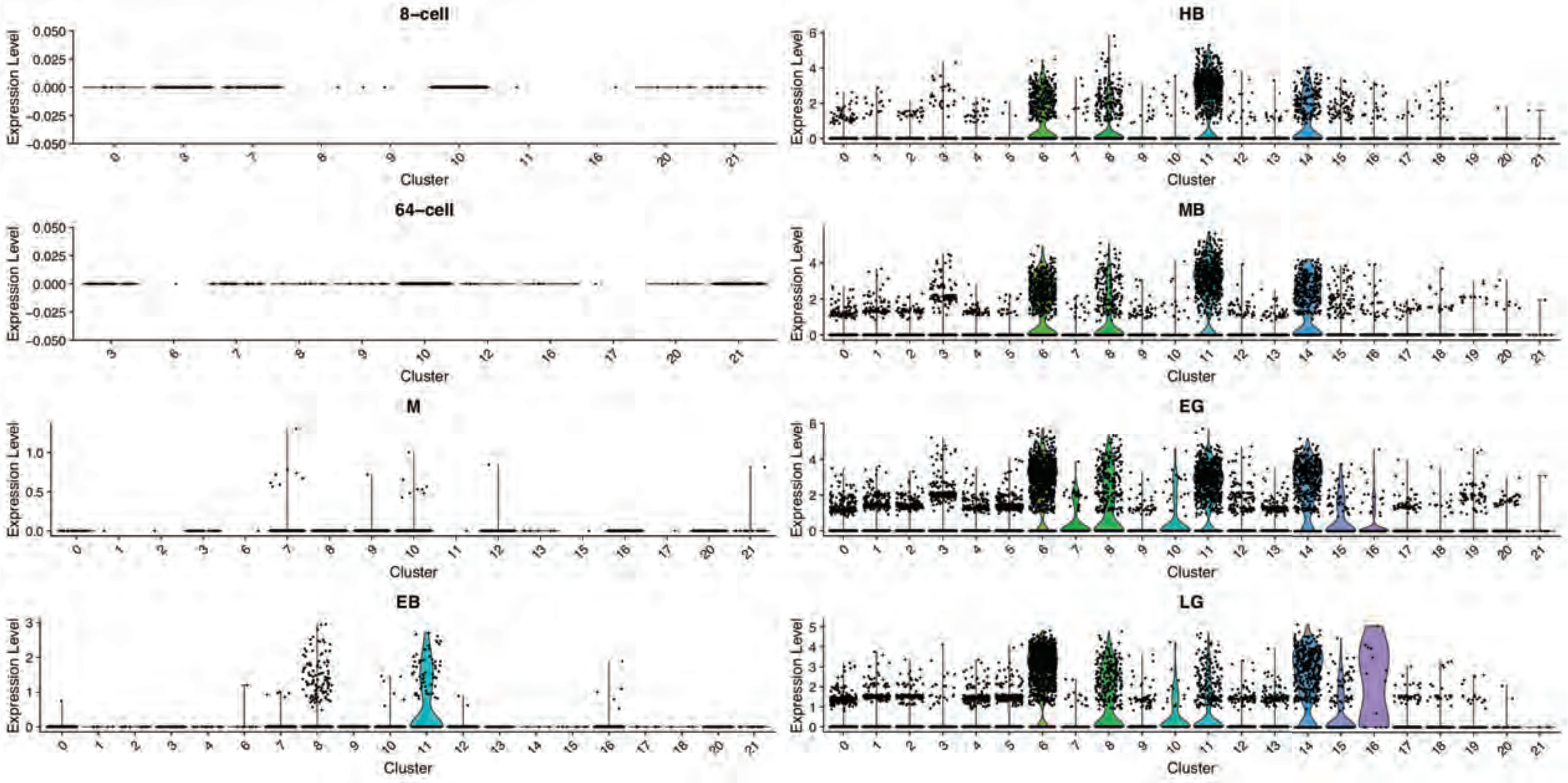
1.5
1.0
0.5
0.0
4
3
2
1
0

Fig. S7: Endo16



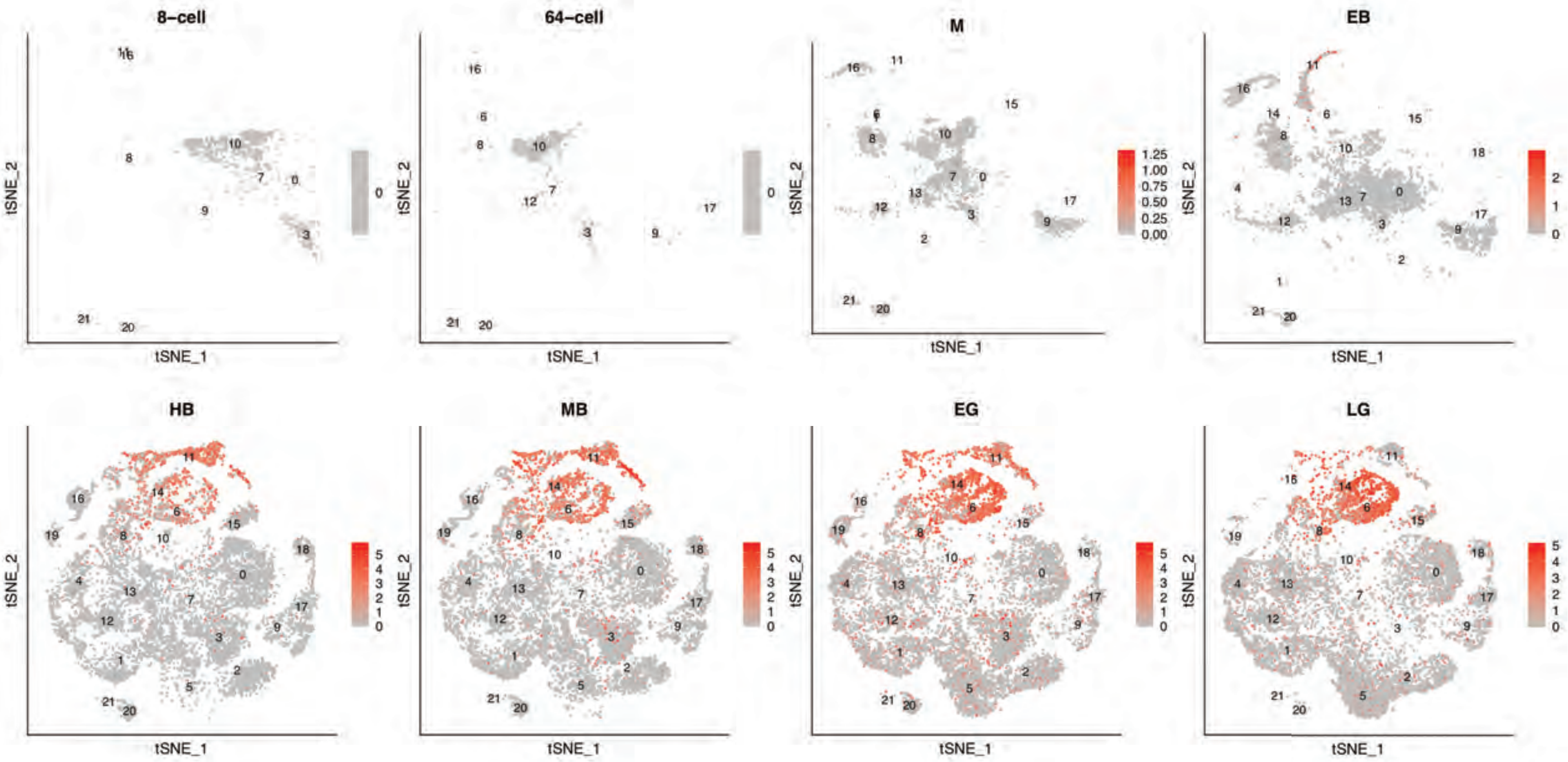


Fig. S8

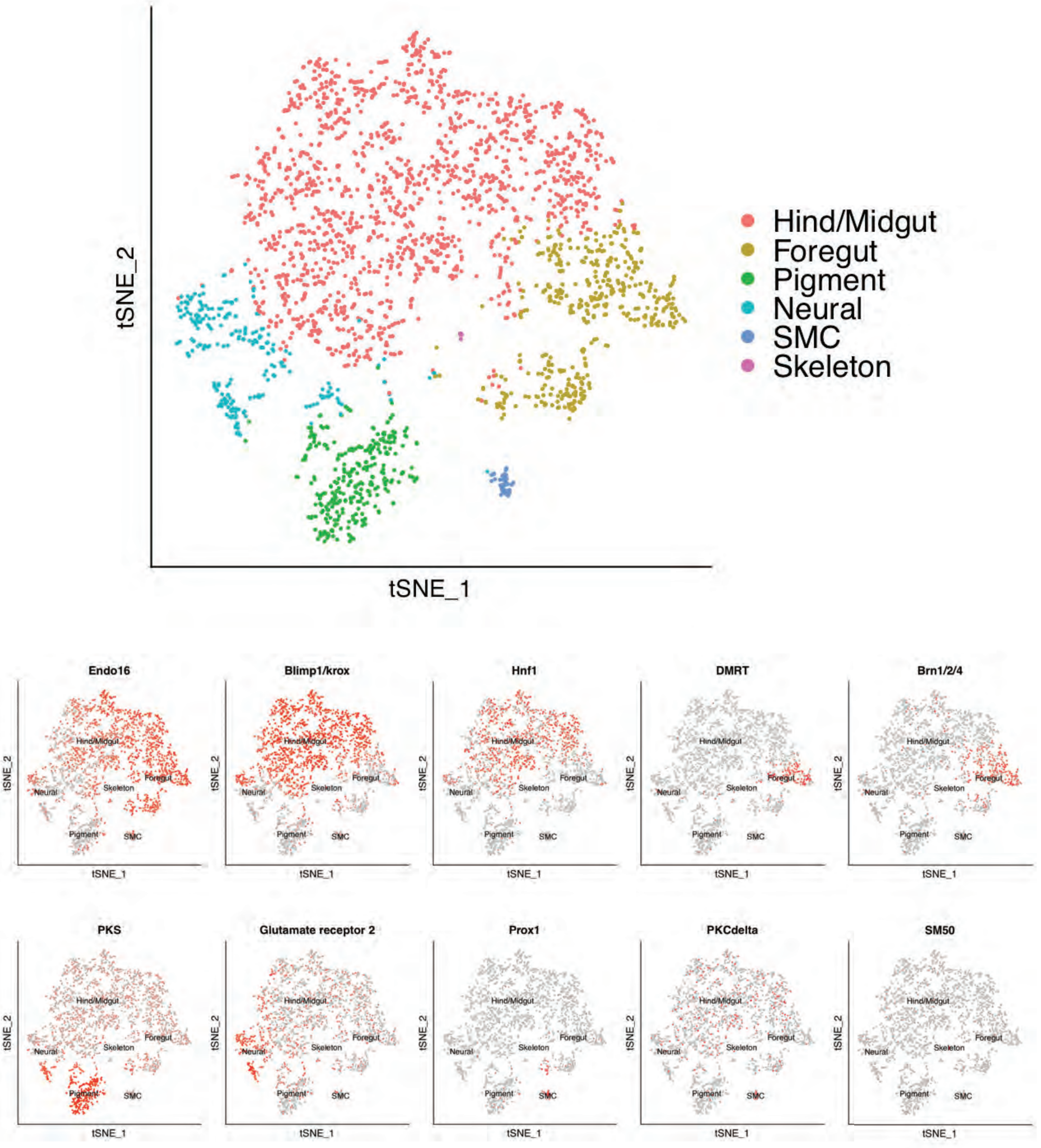


Fig. S8. Subclustering of gut clusters at late gastrula. Endo16-positive clusters 6, 8, 11, and 14 subcluster into seven cell types. Feature plots highlighting subcluster marker genes.

Fig. S9: Alx1

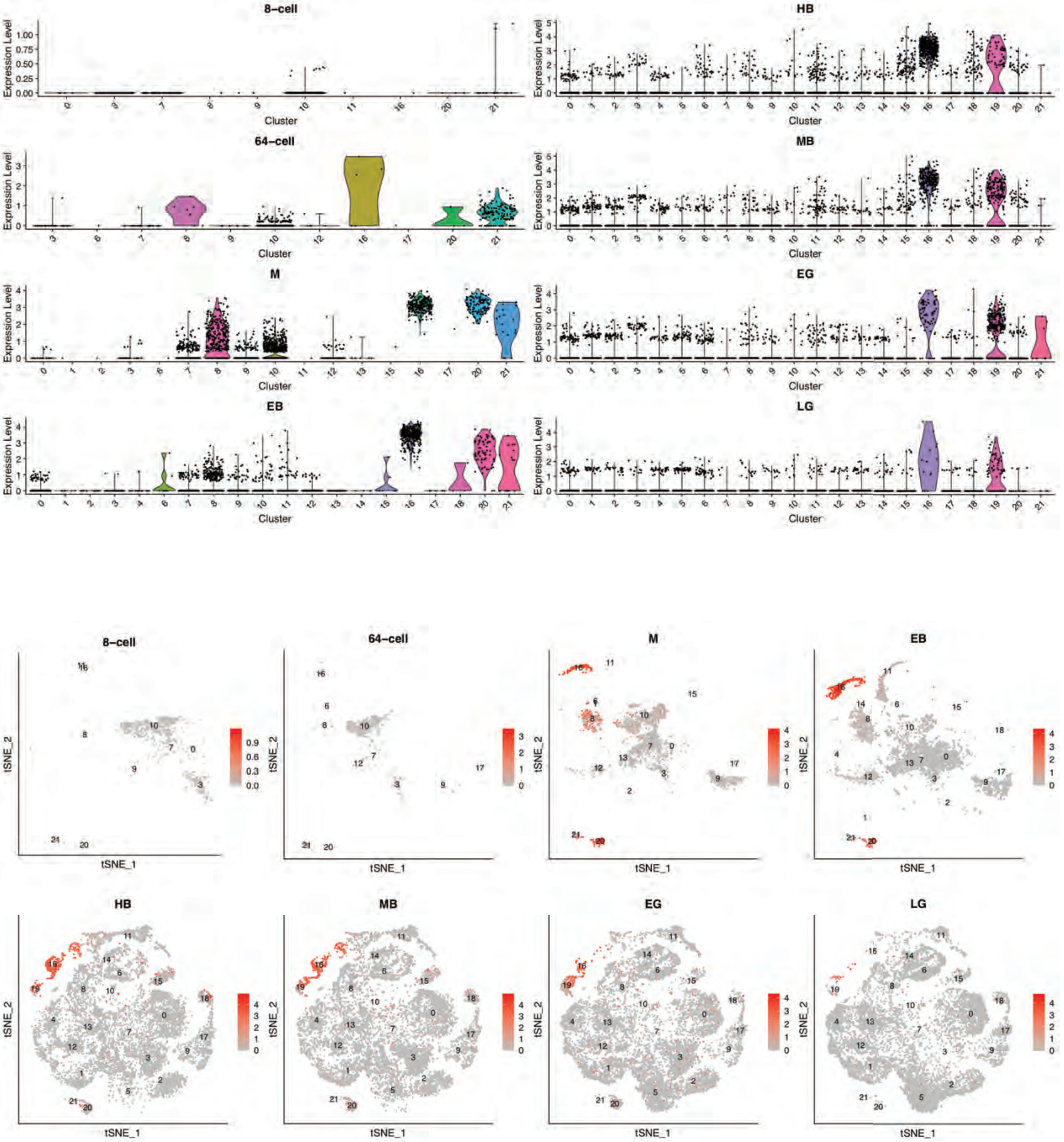
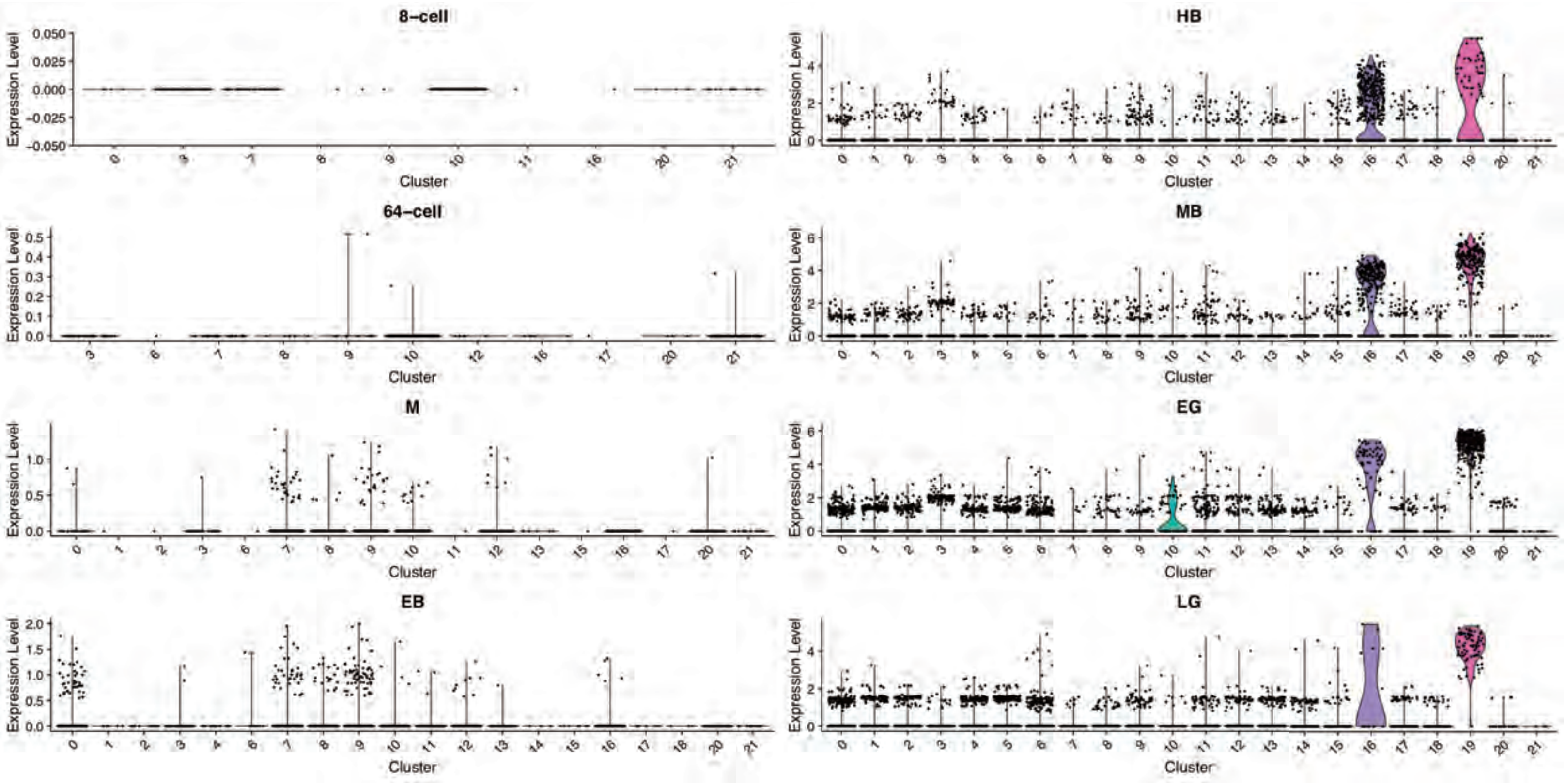


Fig. S9-S15, S17-24. Violin plots showing expression across different clusters at each developmental stage. Normalized gene expression values at log scale shown per cell cluster identity for each developmental time point. Plots depict probability density at different expression levels. Feature plots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

Fig. S10: SM50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HB
64–cell
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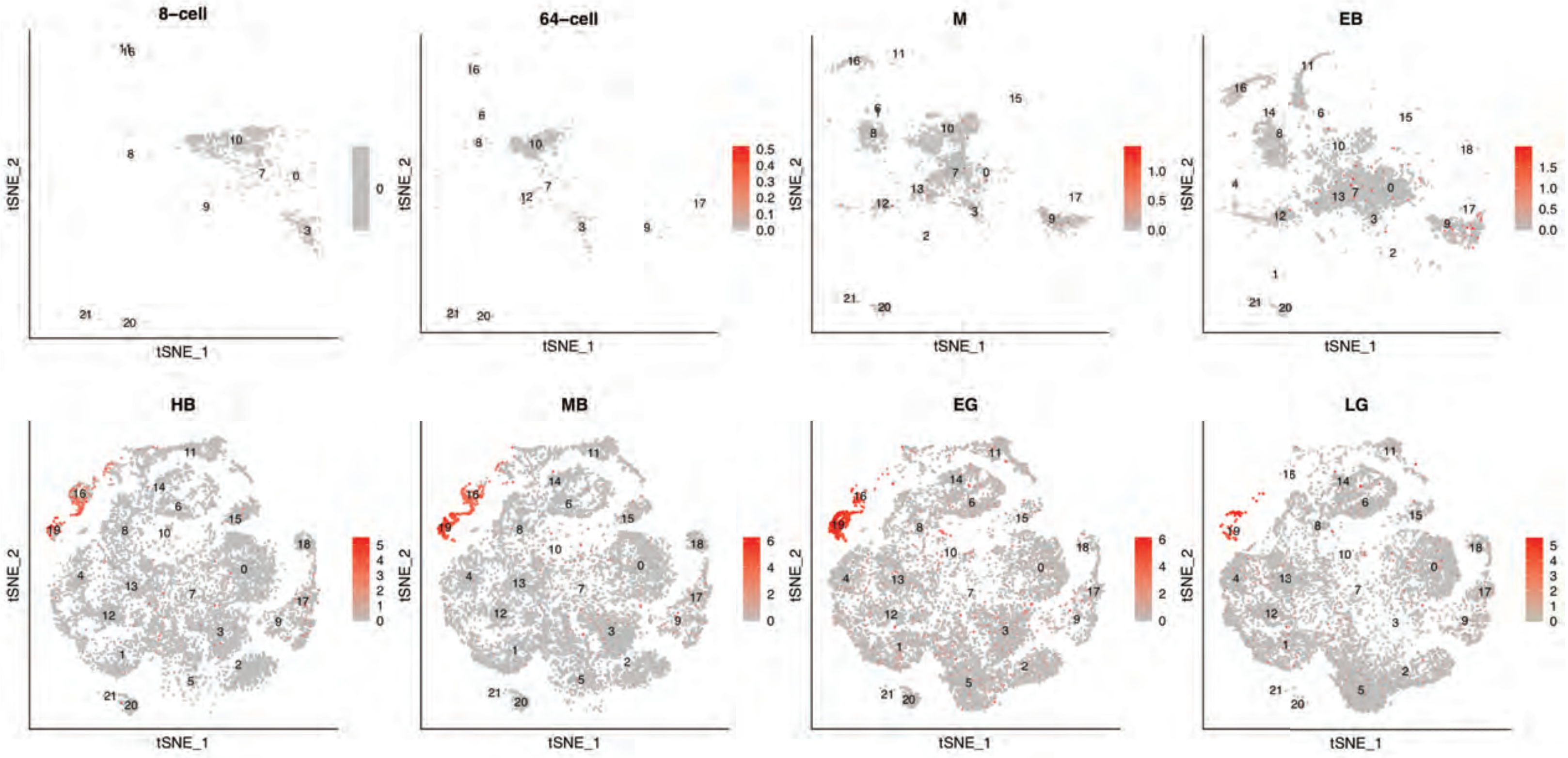
M
EG
EB
LG
Expression Level
Cluster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64–cell
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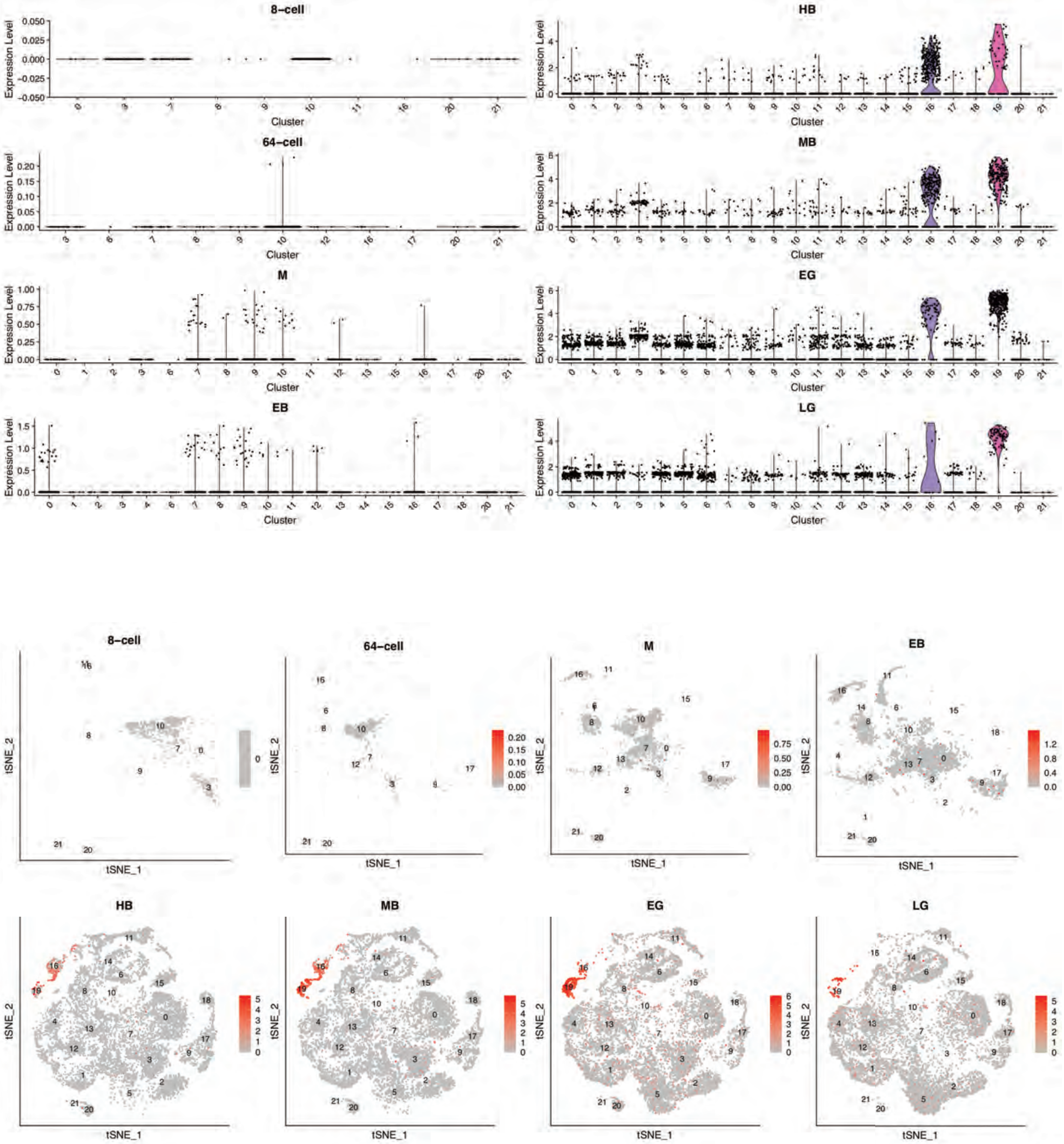
EB
HB
MB
EG
LG
tSNE_1
tSNE_2

Fig. S11: SM37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HB
64–cell
MB
M
EG
EB
LG
Expression Level
Cluster
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EB
HB
MB
EG
LG
tSNE_1
tSNE_2

Fig. S12

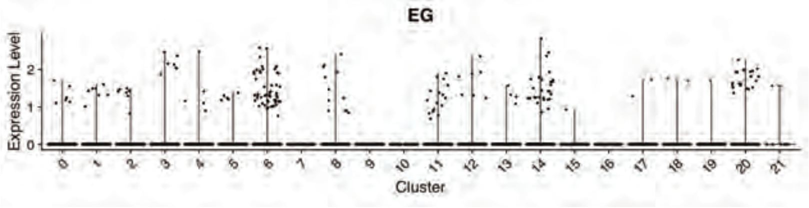
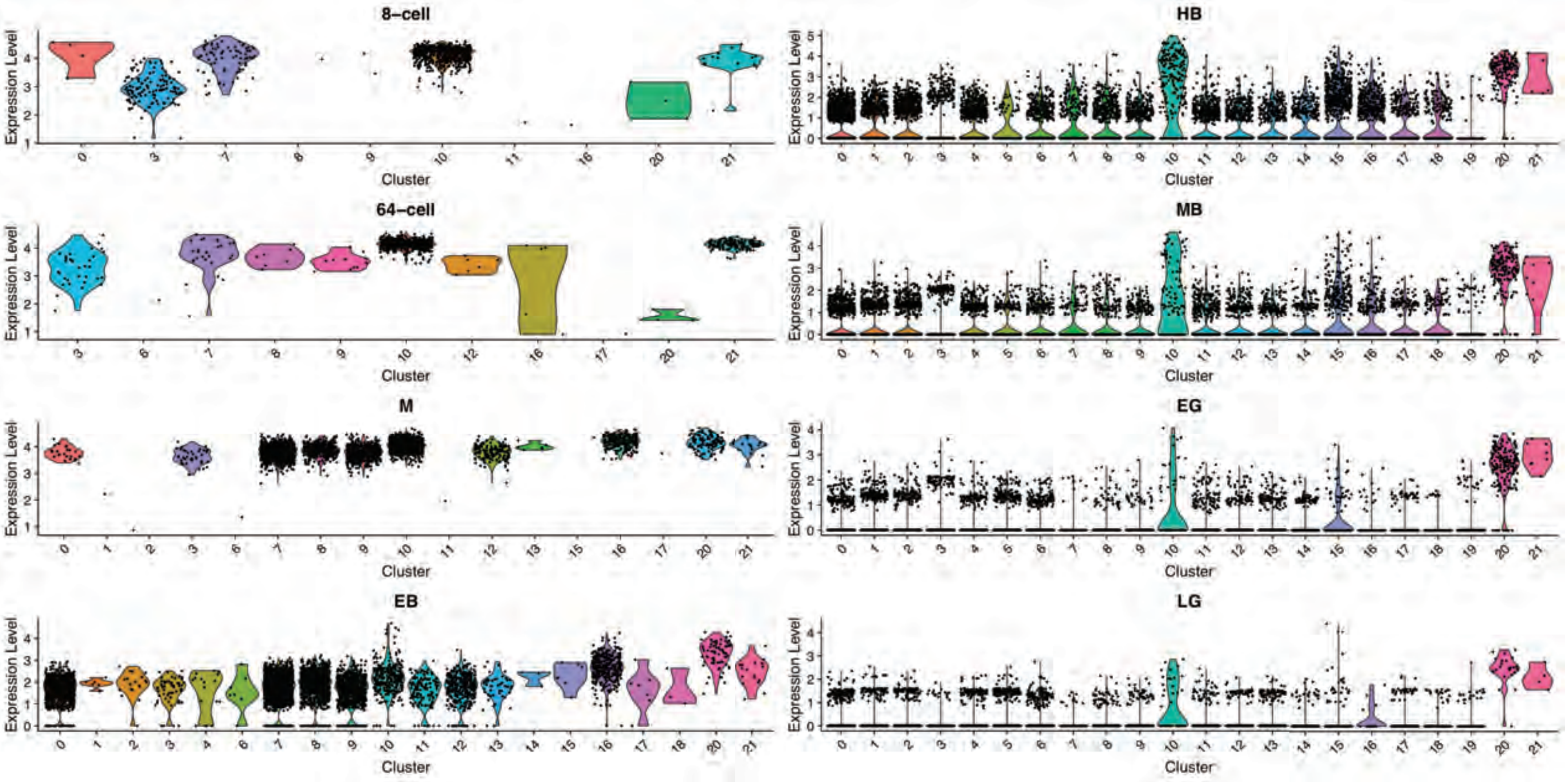


Fig. S12. Violin plot showing enriched expression of FoxY at early gastrula stage in clusters 6, 14, and 20.

Fig. S13: H2A.2.1



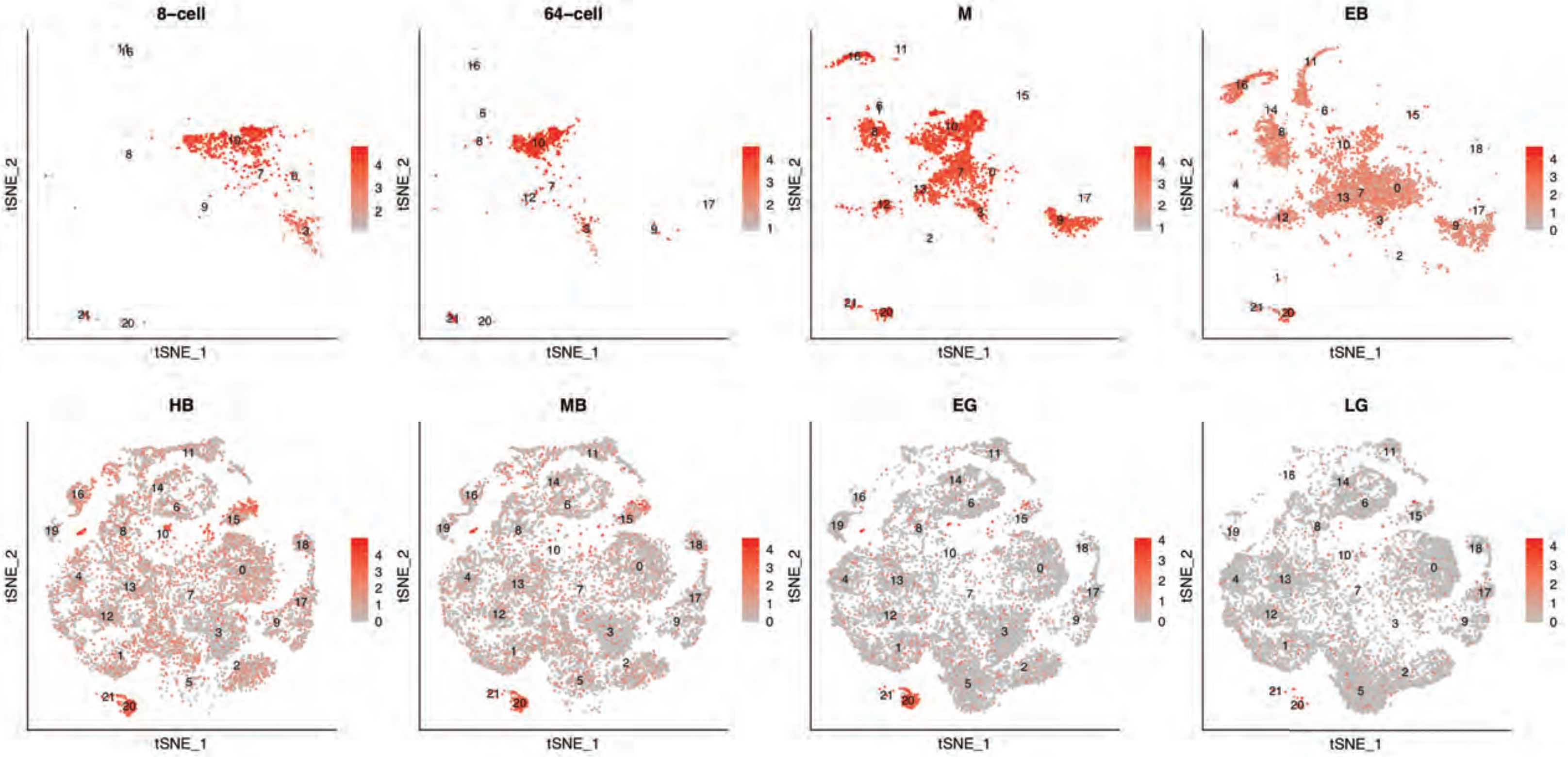
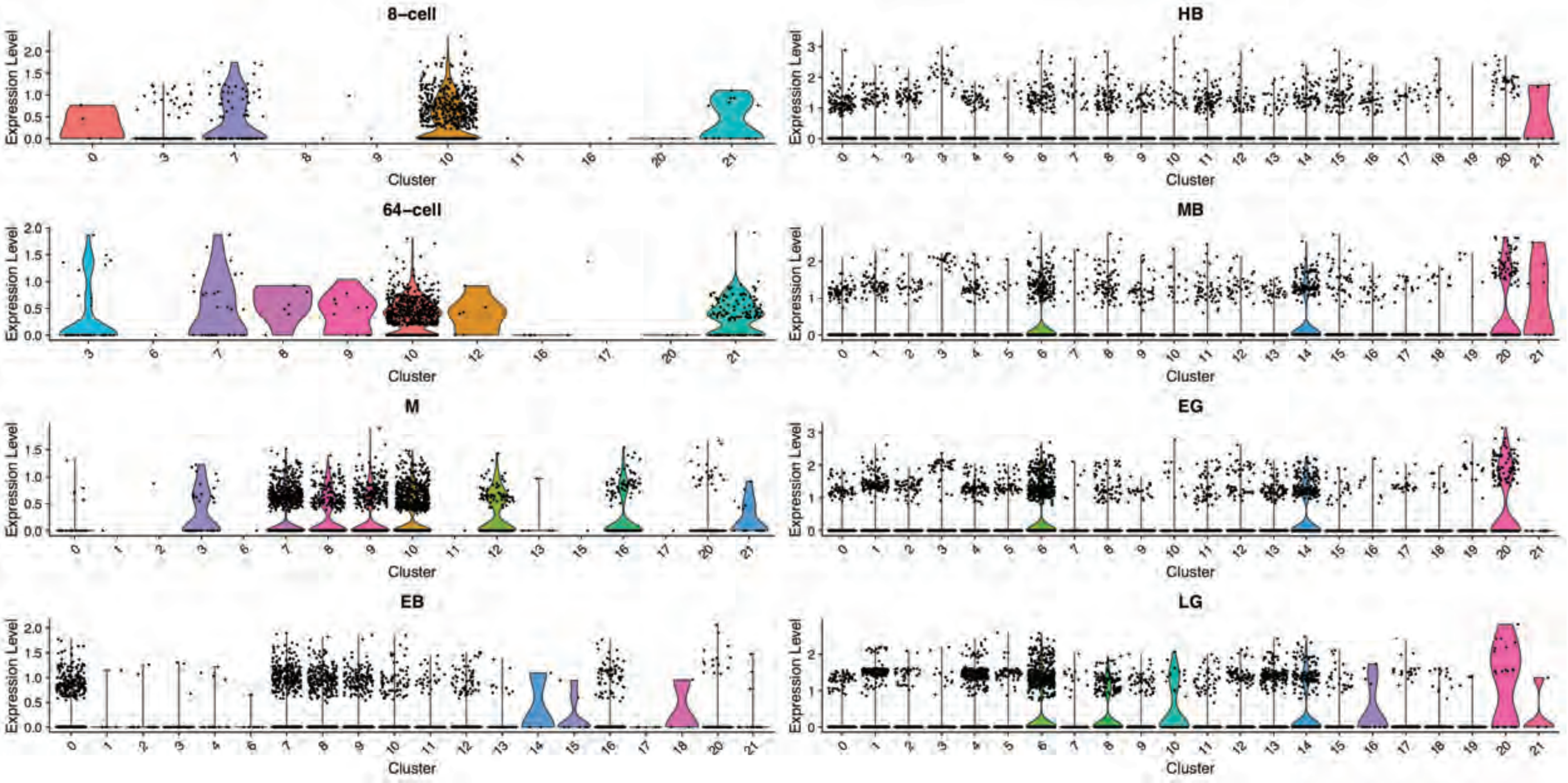


Fig. S14: Vasa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HB
64–cell
MB
M
EG
EB
LG
Expression Level
Cluster

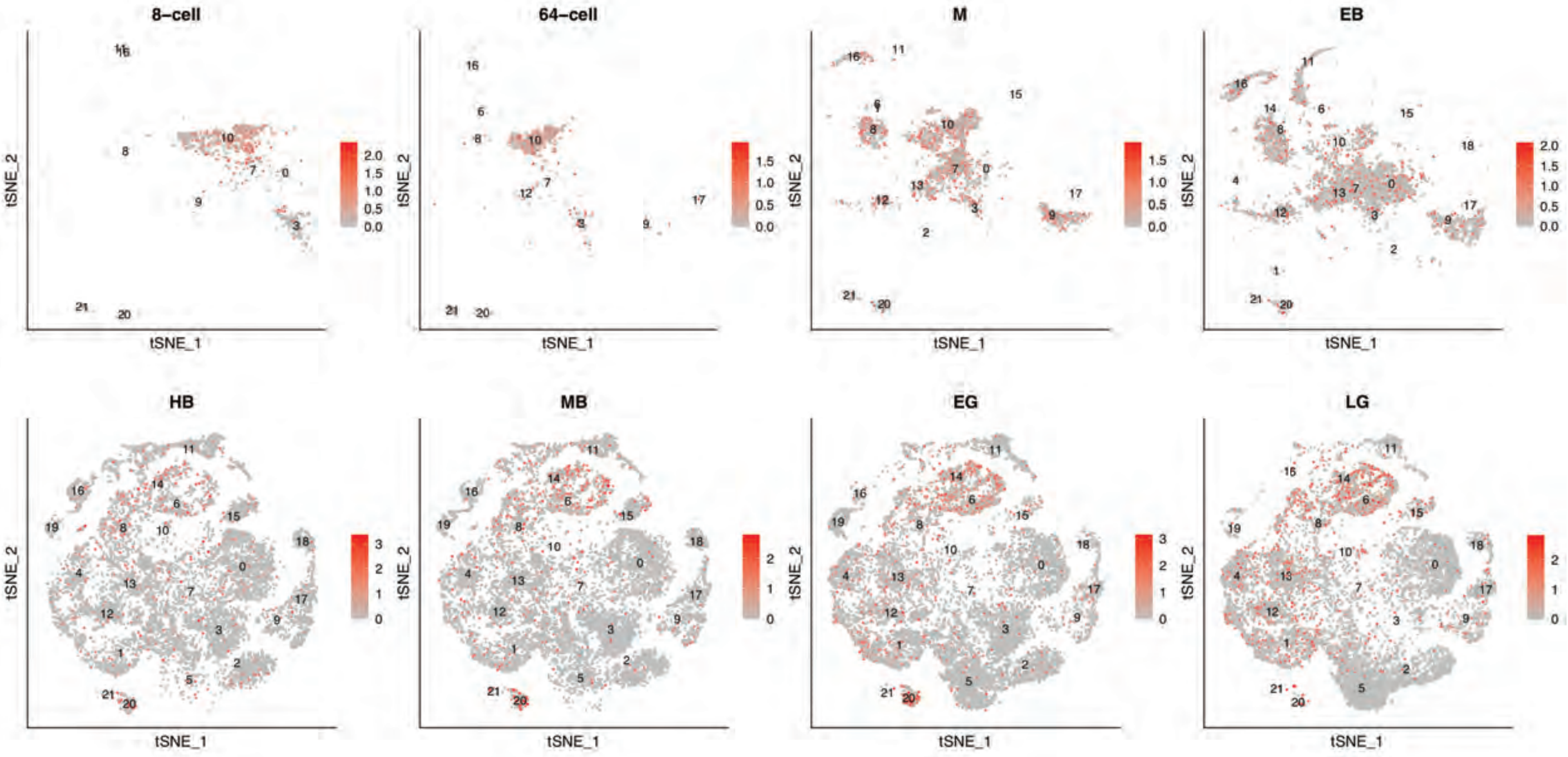
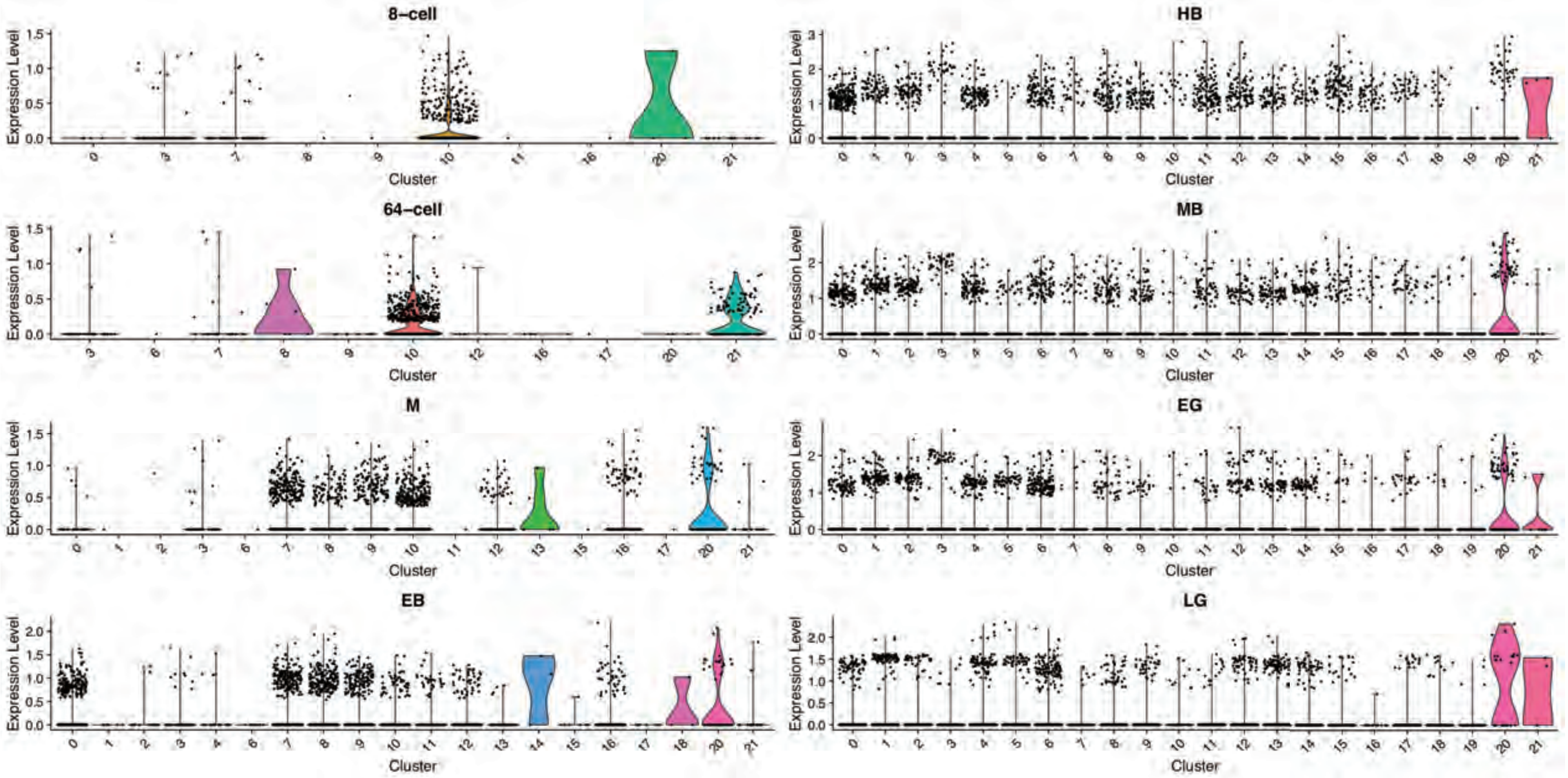

8–cell
64–cell
M
EB
HB
MB
EG
LG
tSNE_1
tSNE_2

Fig. S15: Seawi



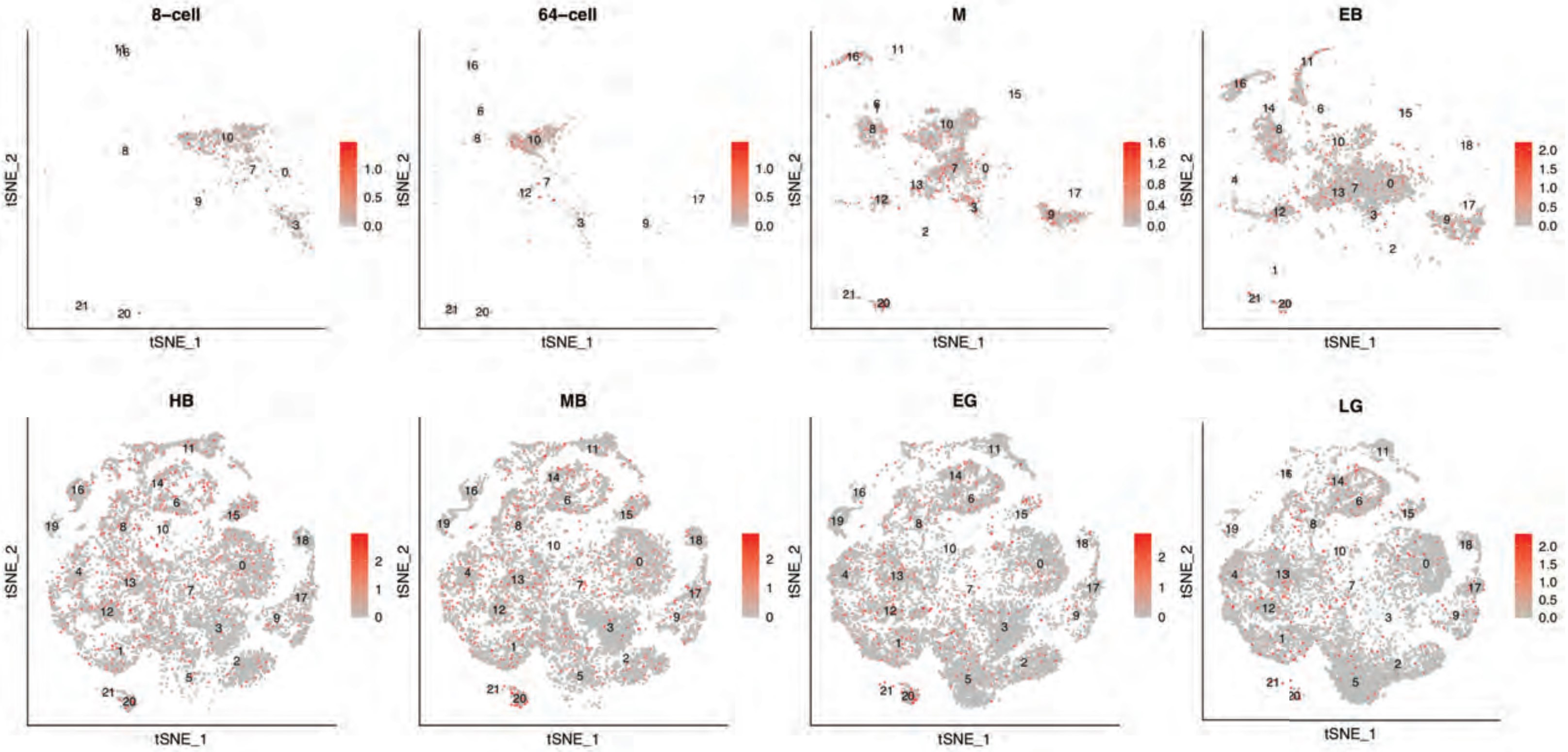
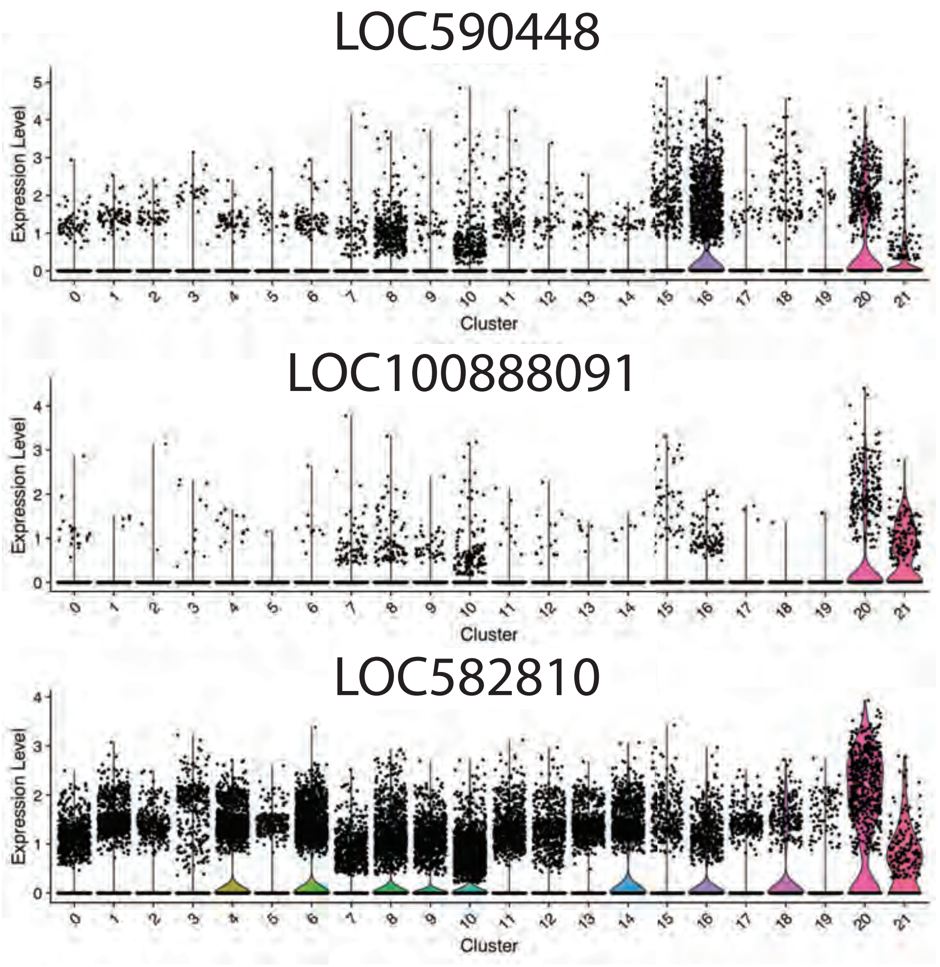


Fig. S16



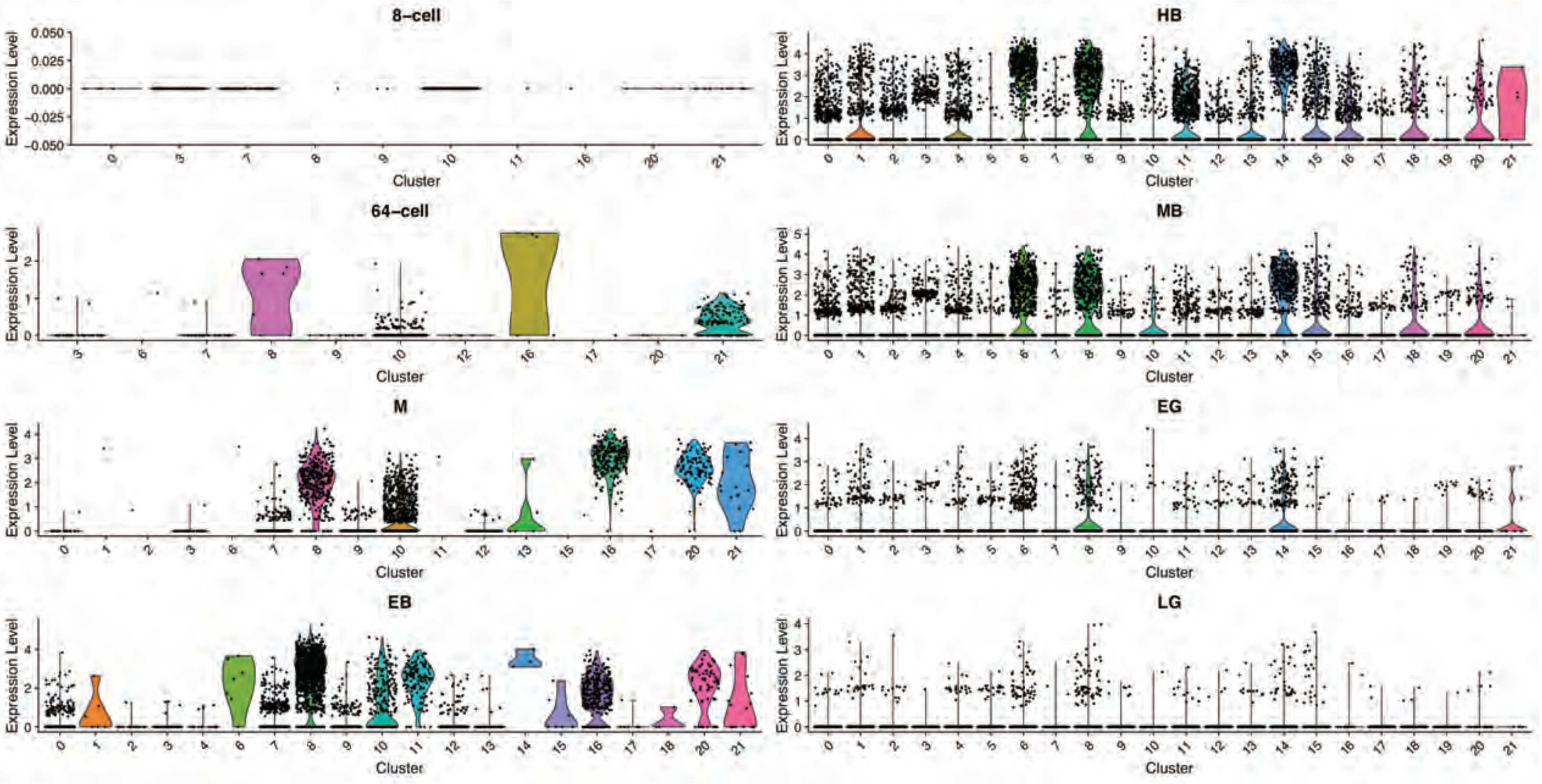
LOC590448

LOC100888091

LOC582810

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

Fig S17: Kruppel



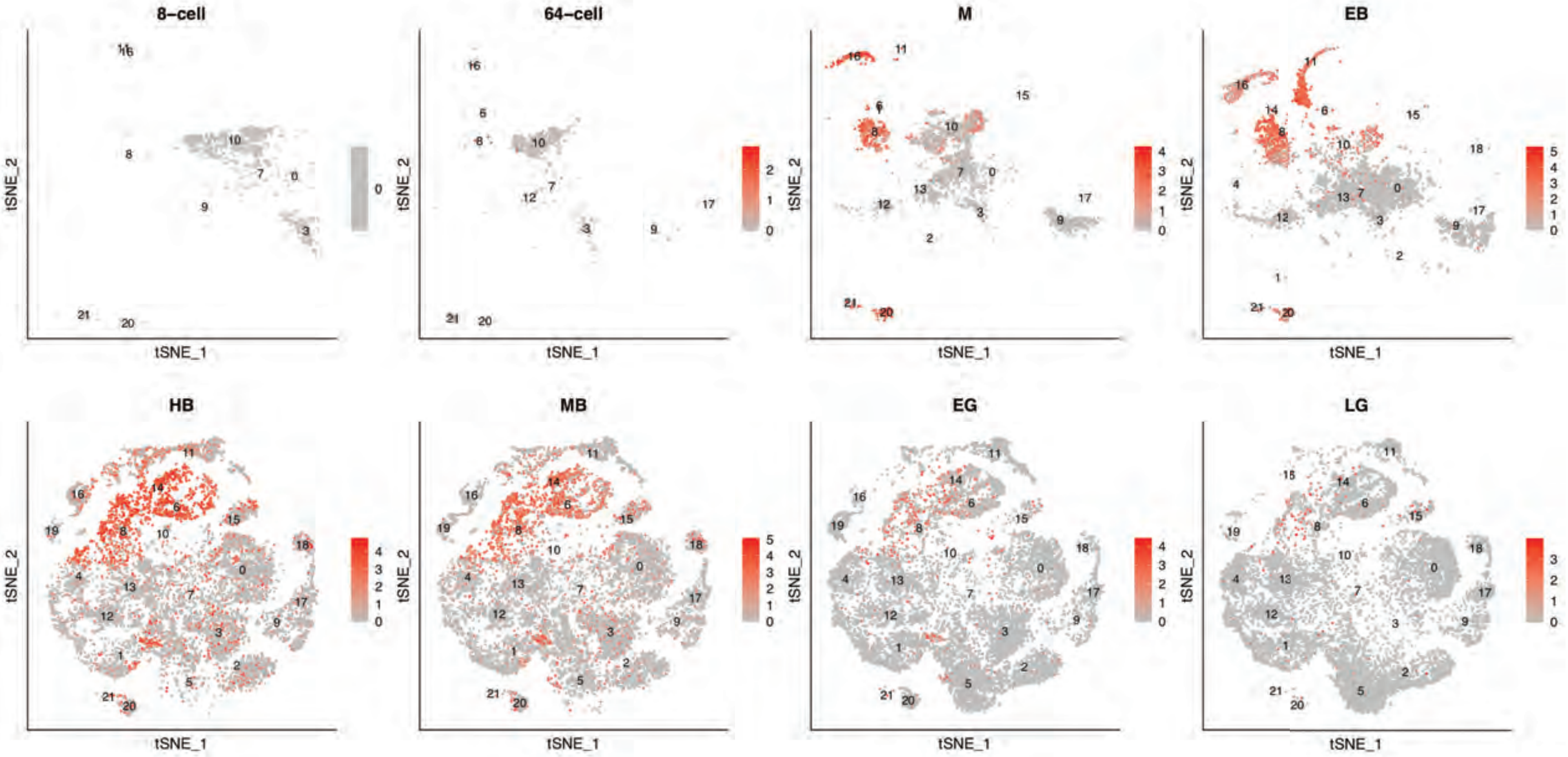
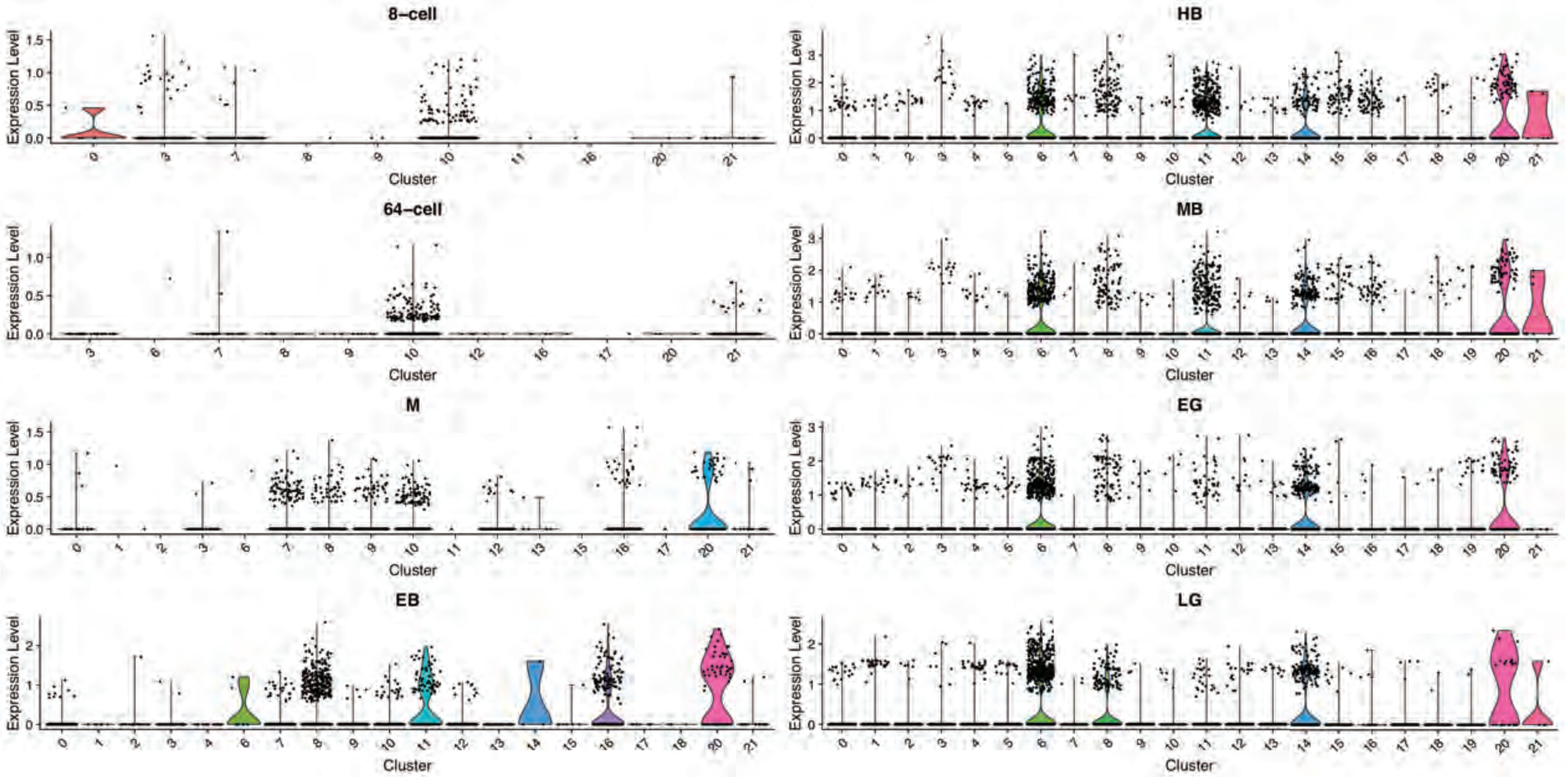


Fig. S18: Odz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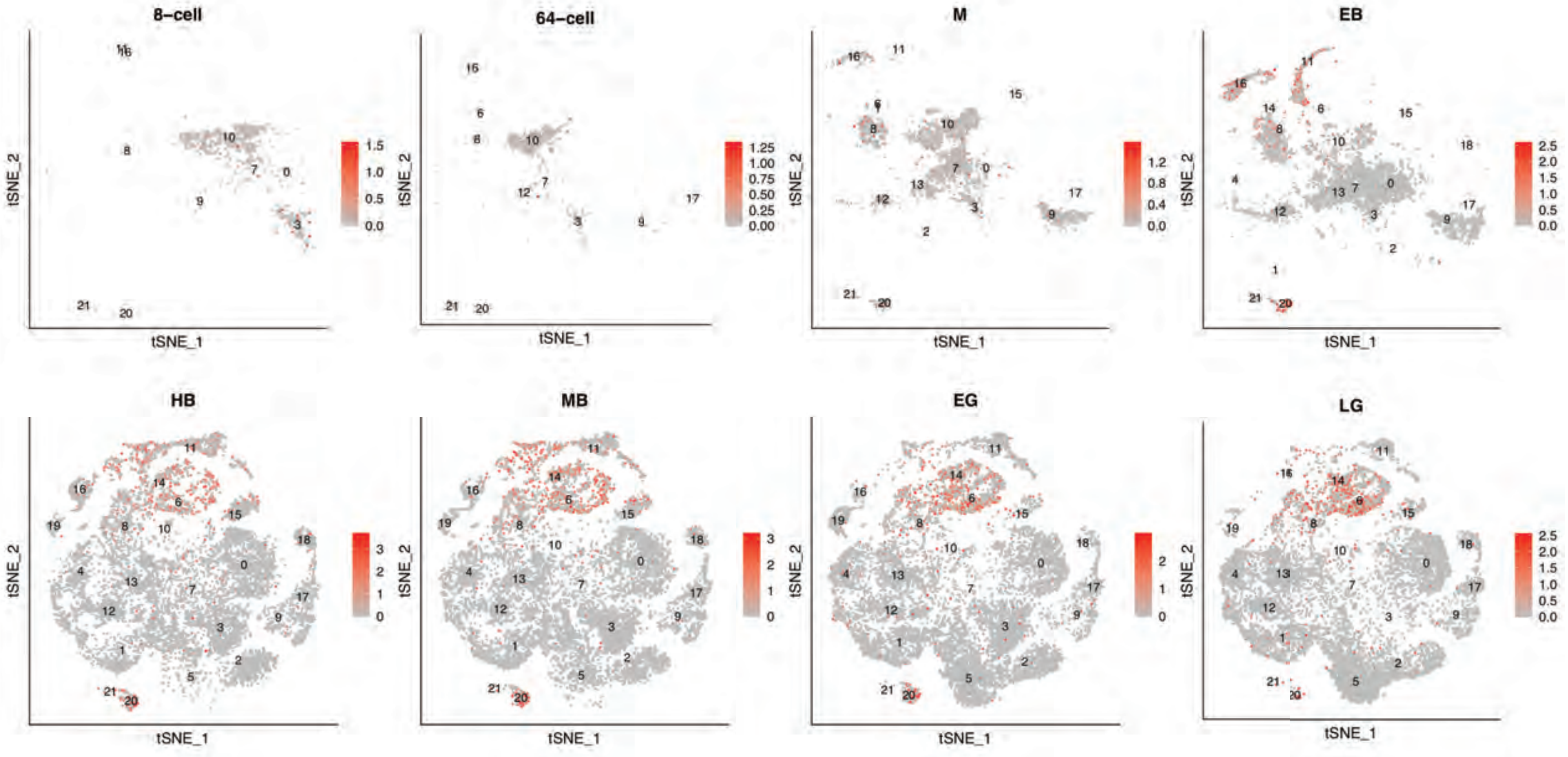
HB
64–cell
MB
M
EG
EB
LG
Expression Level
Cluster


8–cell
64–cell
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EB
HB
MB
EG
LG
tSNE_1
tSNE_2

Fig. S19: Staufen

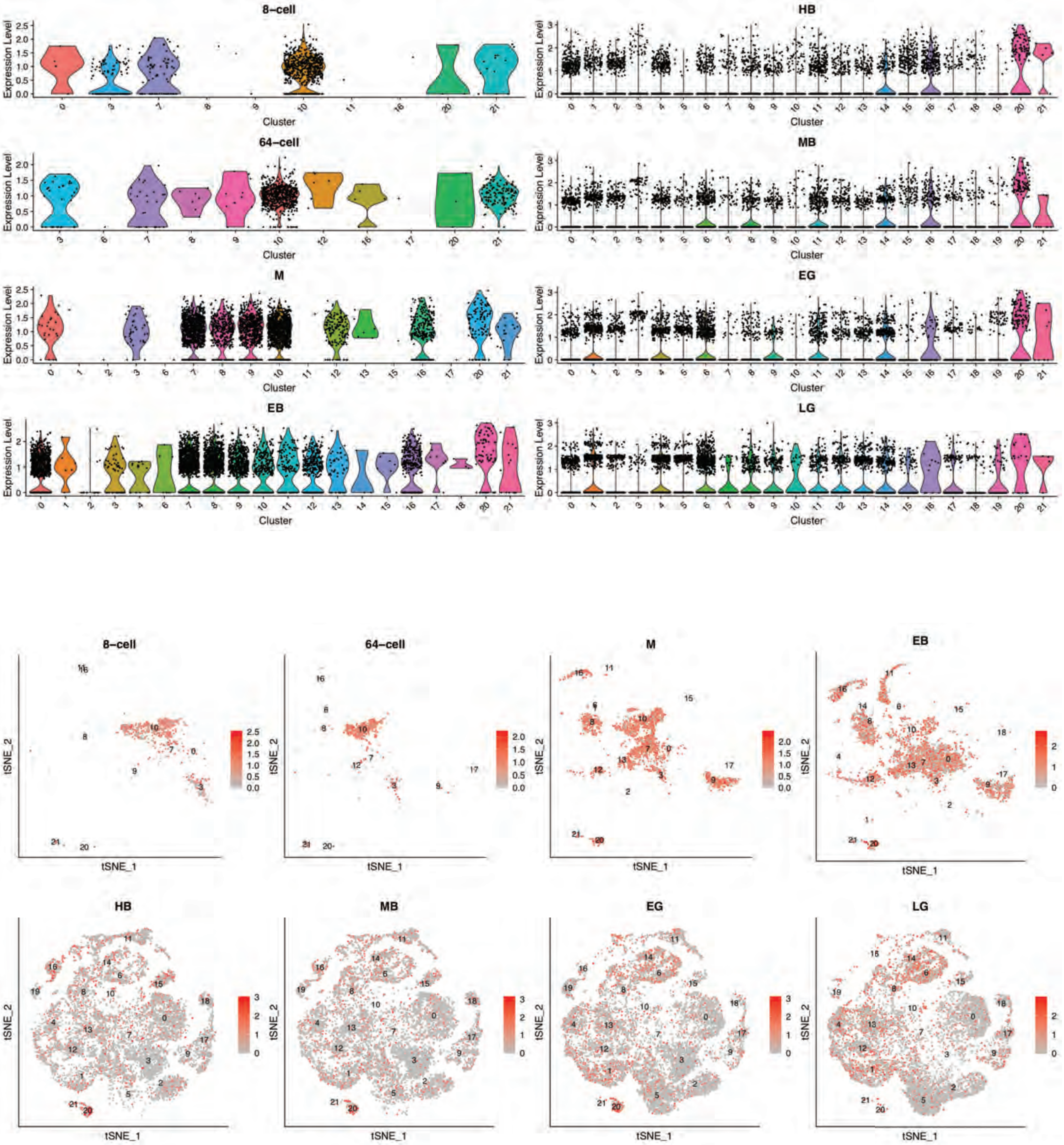


Fig. S20: Maelstrom

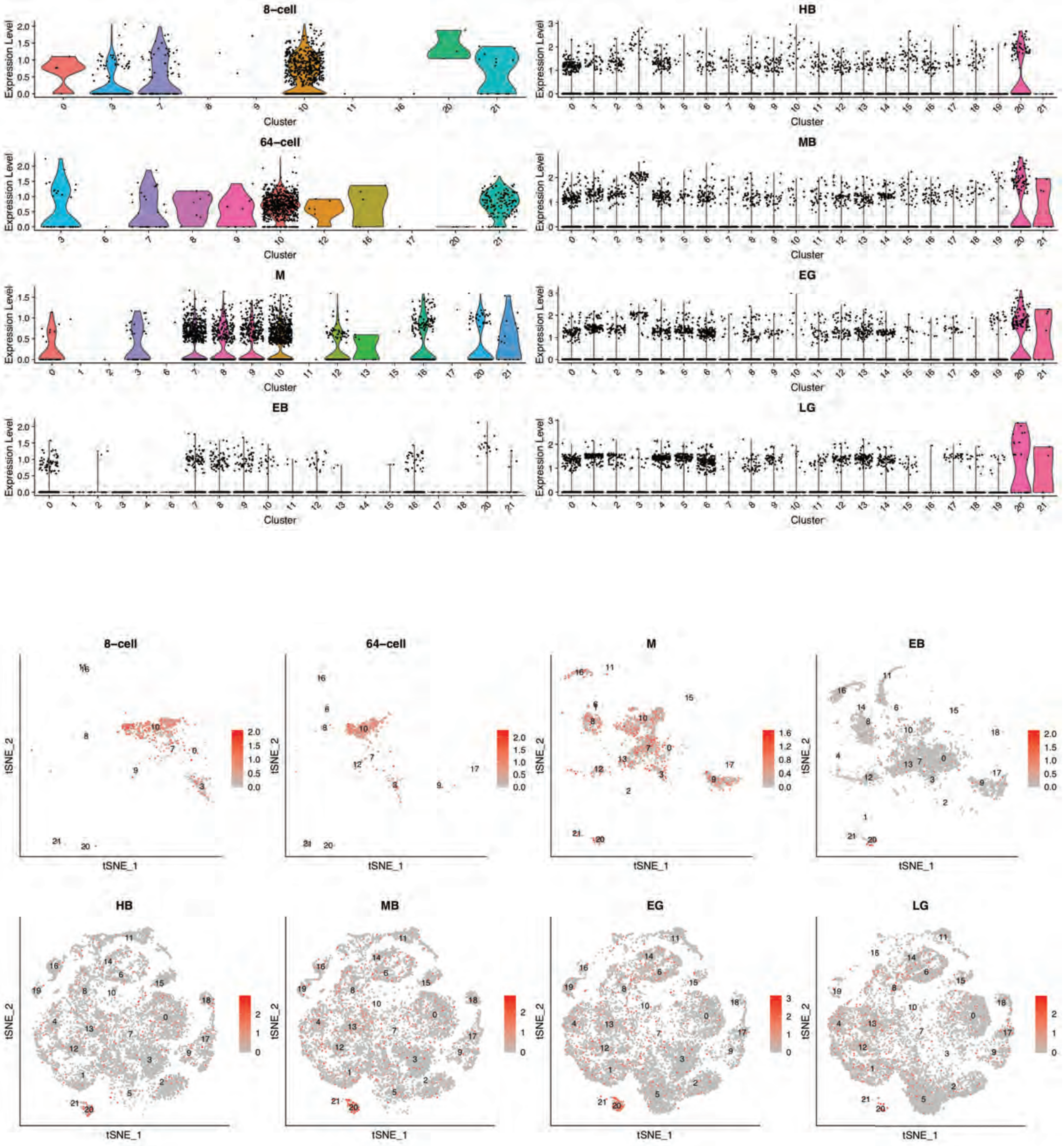
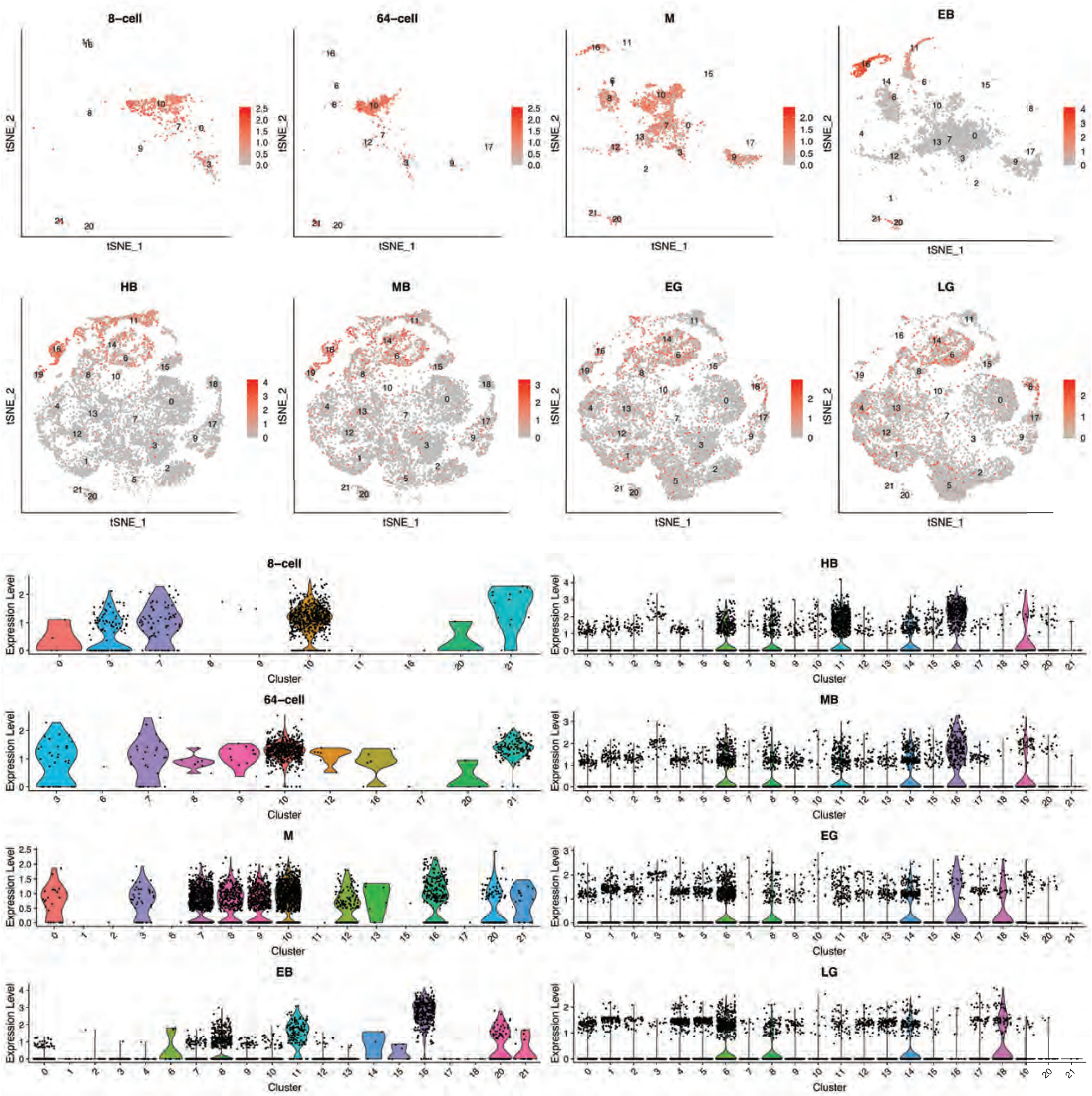


Fig. S21: Gustavus



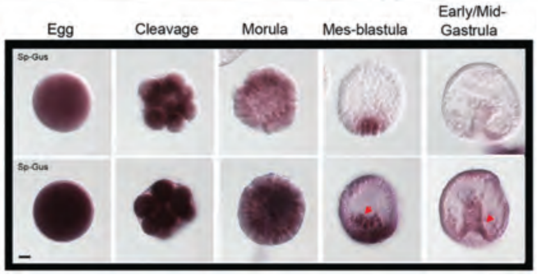
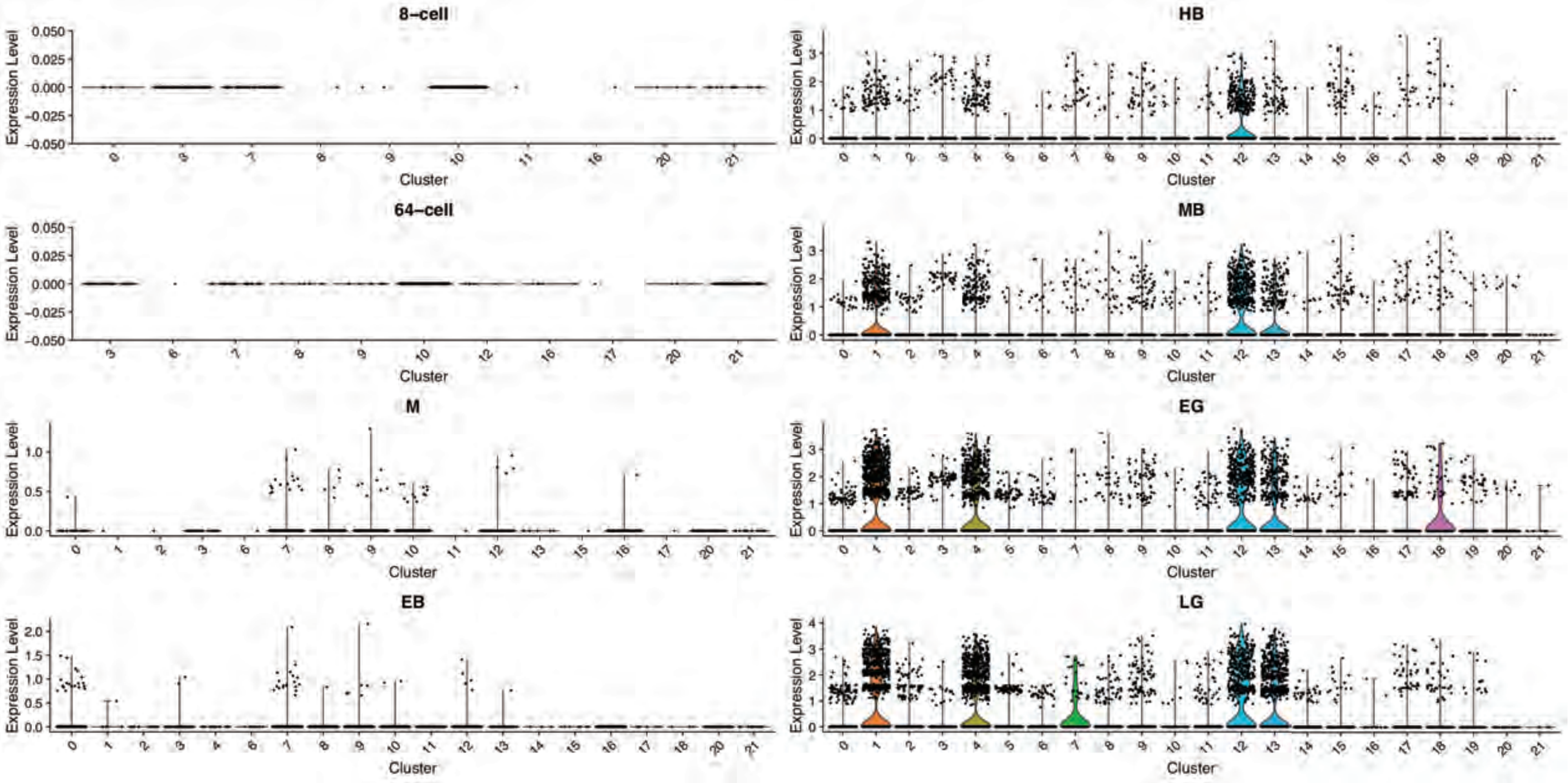


Fig S21. Feature and violin plots showing Gustavus (Gus) enrichment in several clusters. Cells in the feature plot are colored by relative expression level. (B) In situ hybridization result from Zaueta and Wessel 2014, showing enrichment of Gus expression in the vegetal plate of mesenchyme blastula at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

Fig. S22: FoxG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Expression Level
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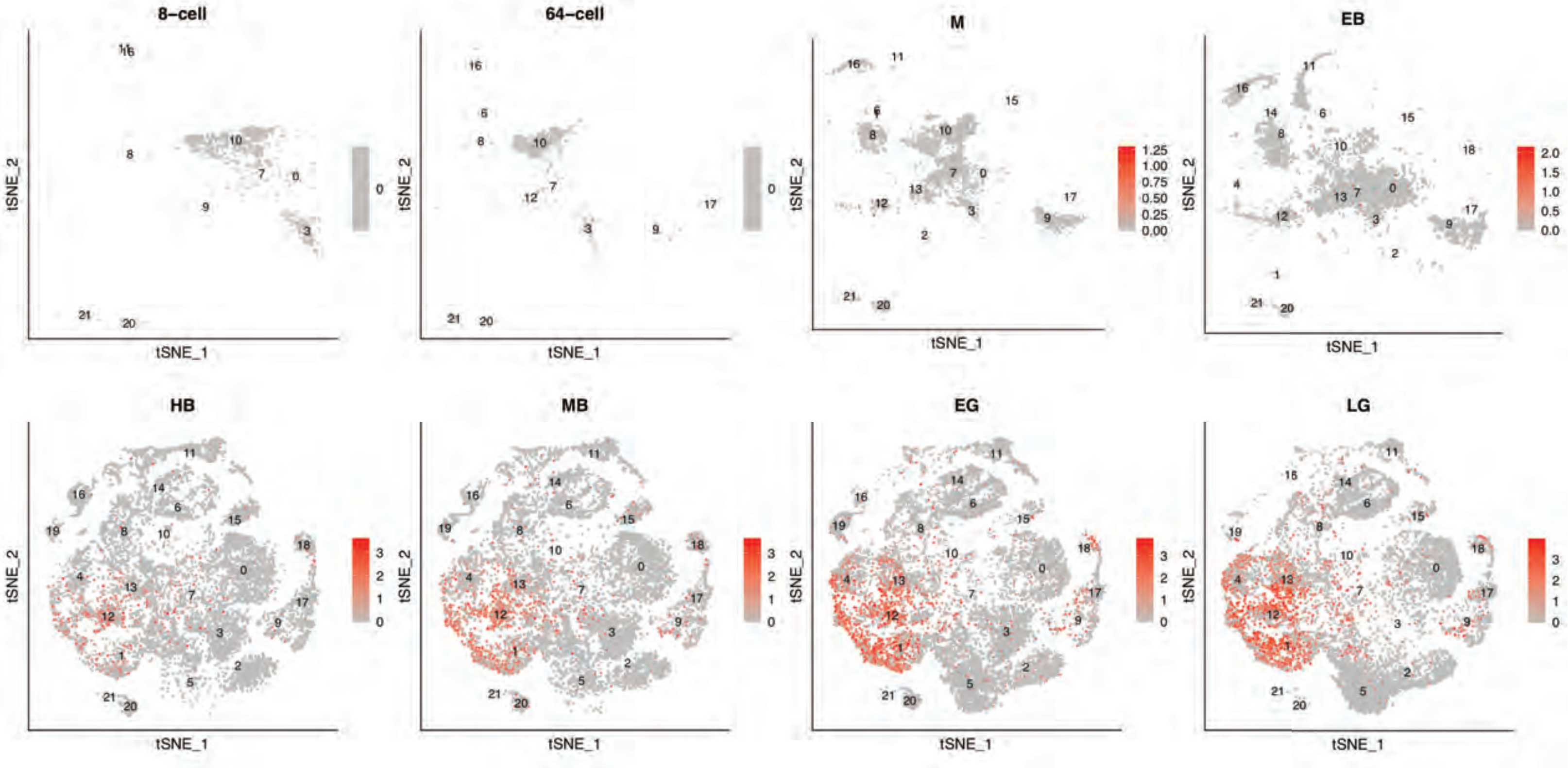
1
2
3
4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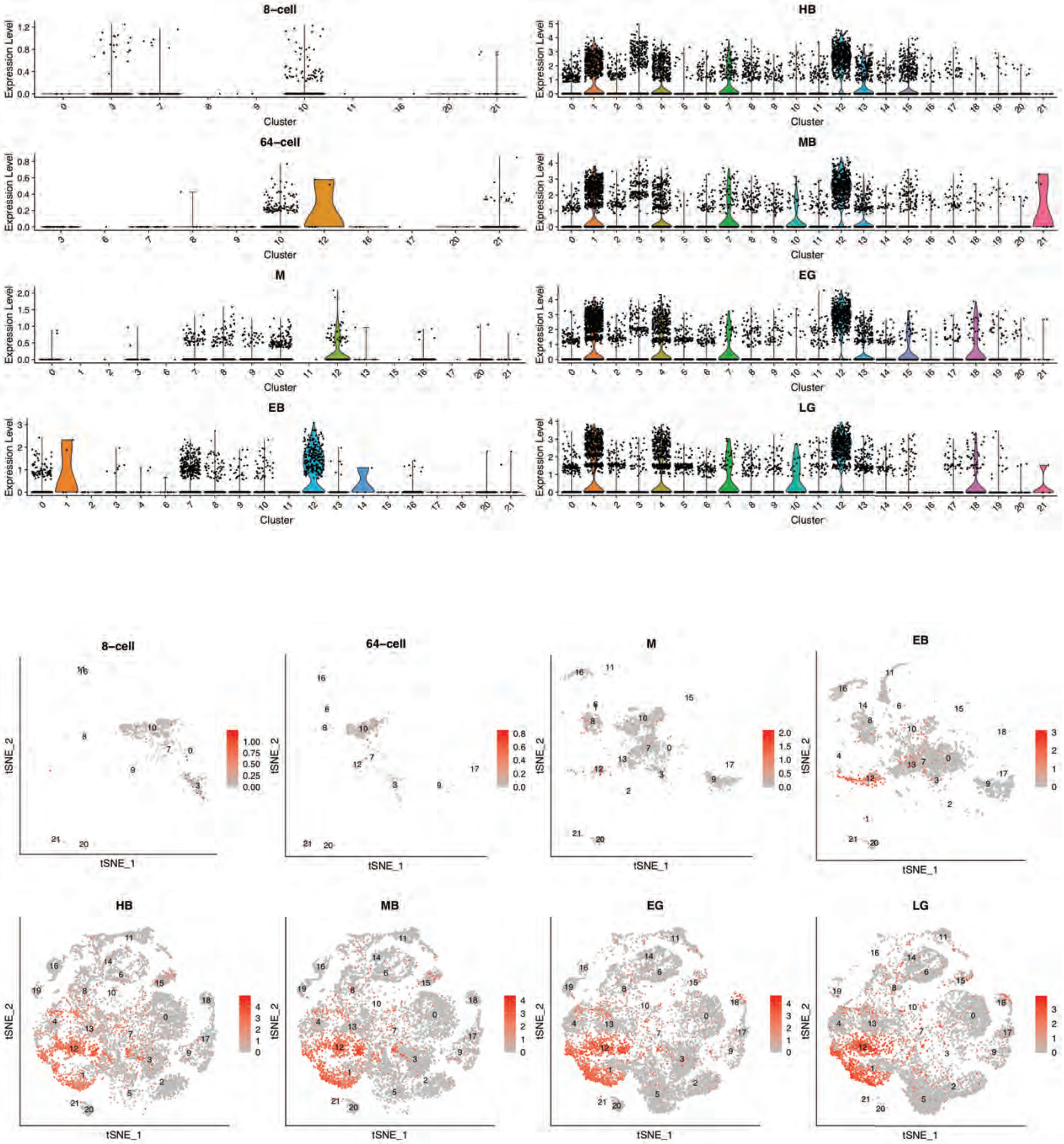
1
2
3

Fig. S23: CHRD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Expression Level
Cluster
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LG
tSNE_1
tSNE_2
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0.0
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0.5
0.0
3
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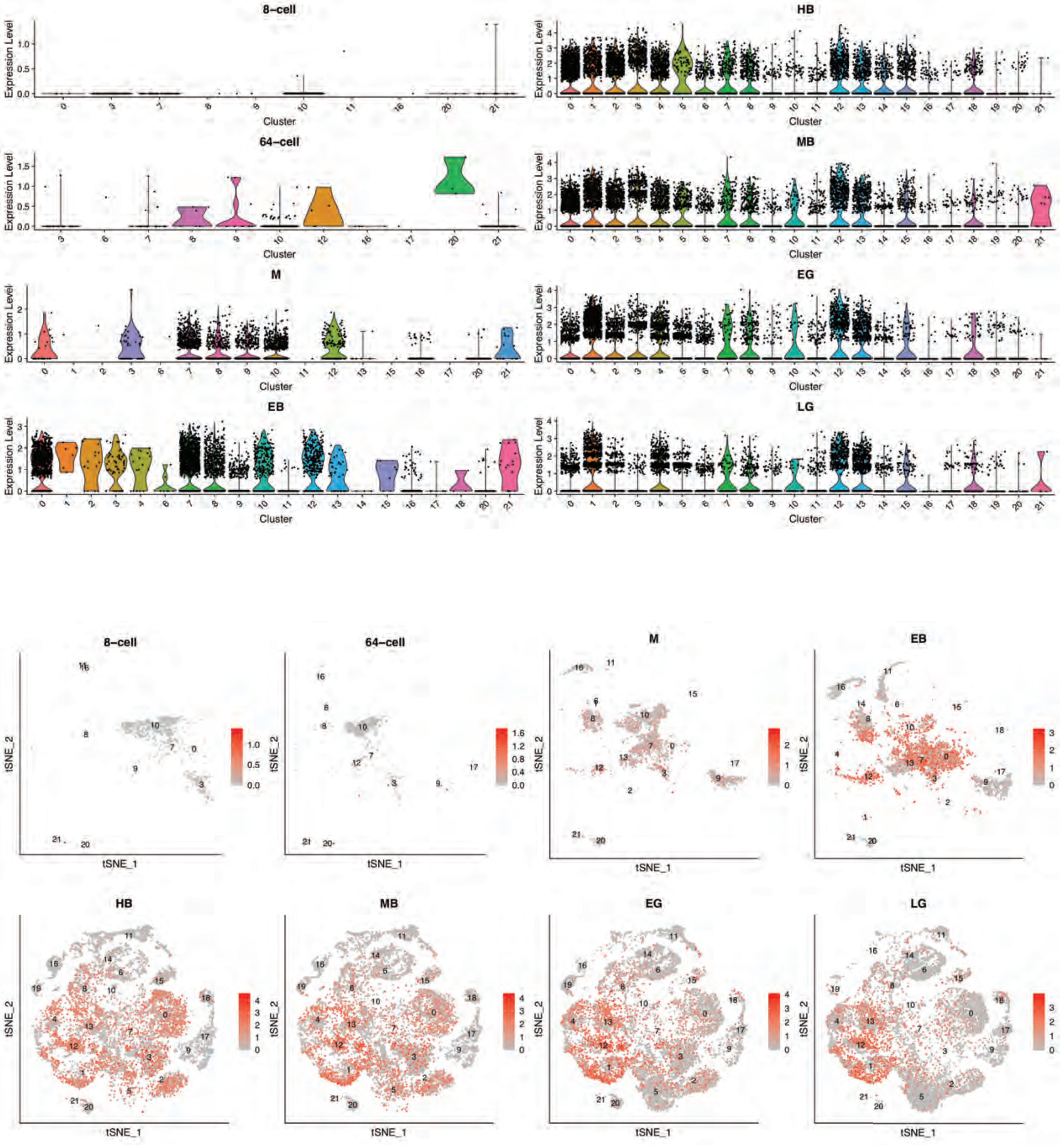
0
4
3
2
1
0

Fig. S24: Lim1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EG
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LG
Expression Level
Cluster
tSNE_1
tSNE_2

Fig. S25

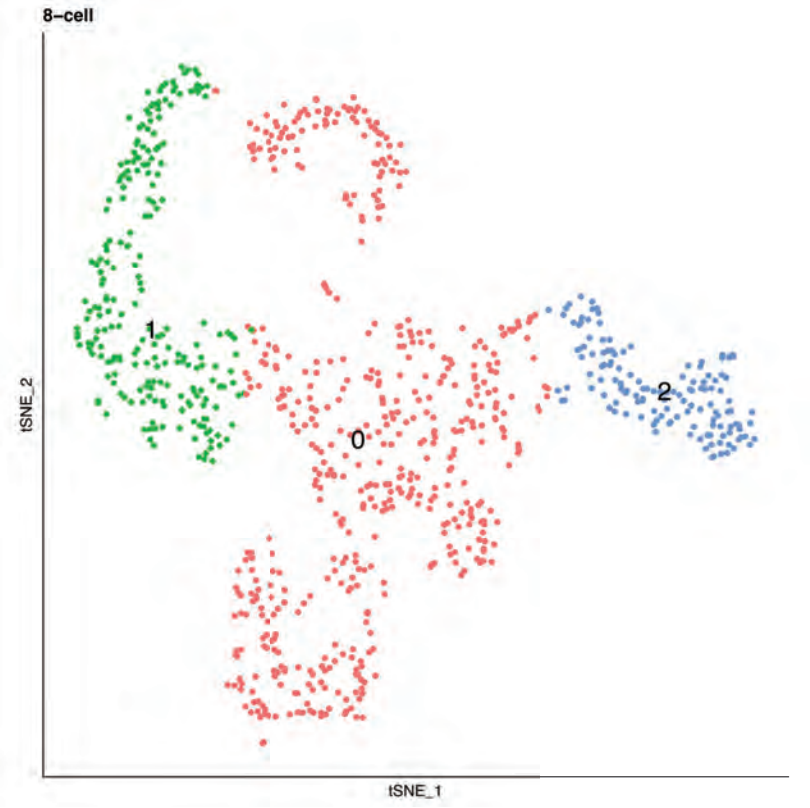

Fig. S25. tSNE plot of 8-cell stage, unintegrated dataset shows the 886 cells cluster into three major cell states. (Clustering parameters used were dims=4, res=0.1.)

Fig. S26

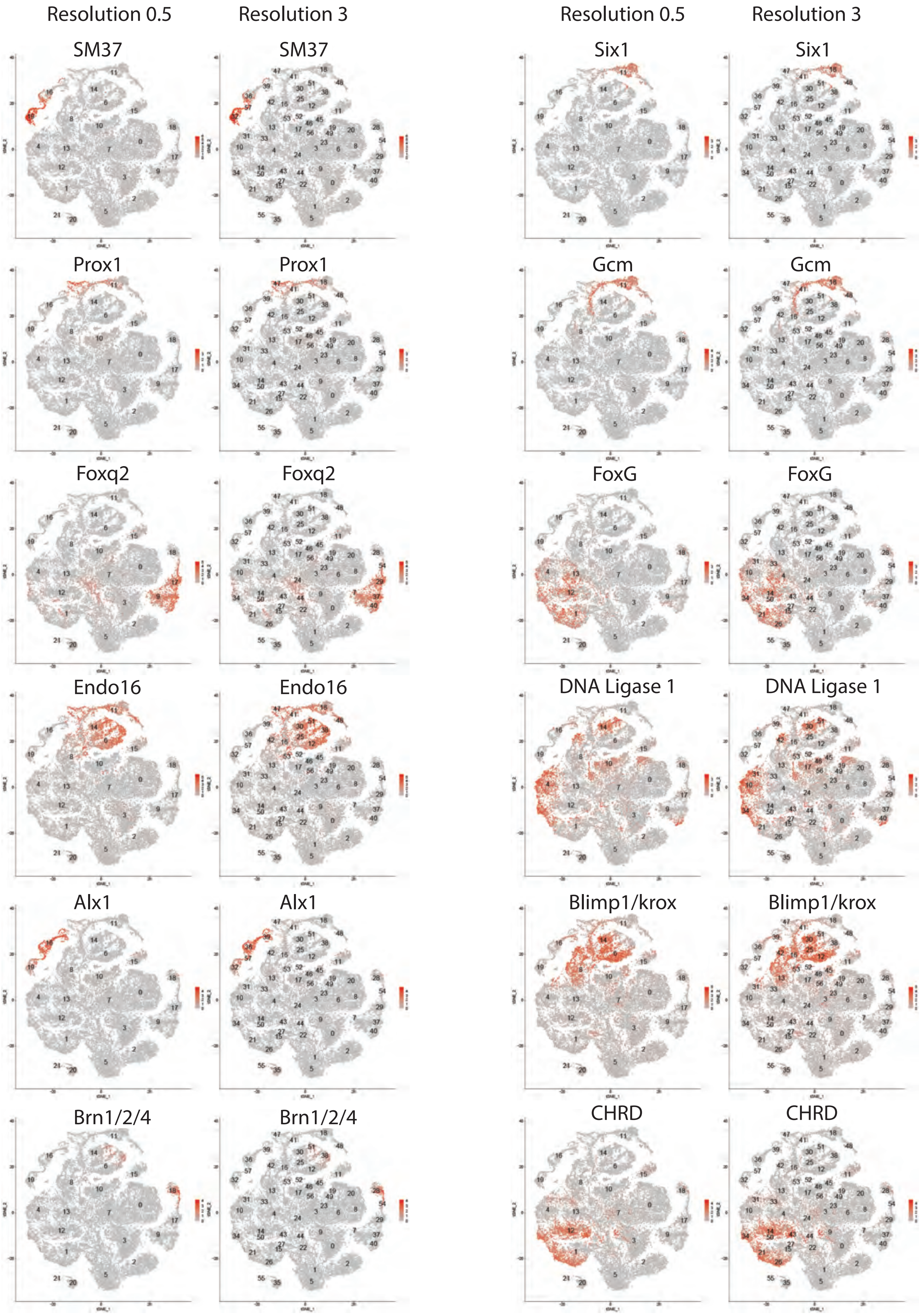

Fig. S26. Feature plots for several marker genes seen at a resolution of 0.5 and 3. Higher resolution differentiates subpopulations of the cell states identified at resolution 0.5. Foregut gene Brn1/2/4 which is seen in some cells of cluster 14 and 6 (res=0.5) is enriched in clusters 51 and 38 (res=3).

Fig. S27: Cyclin B

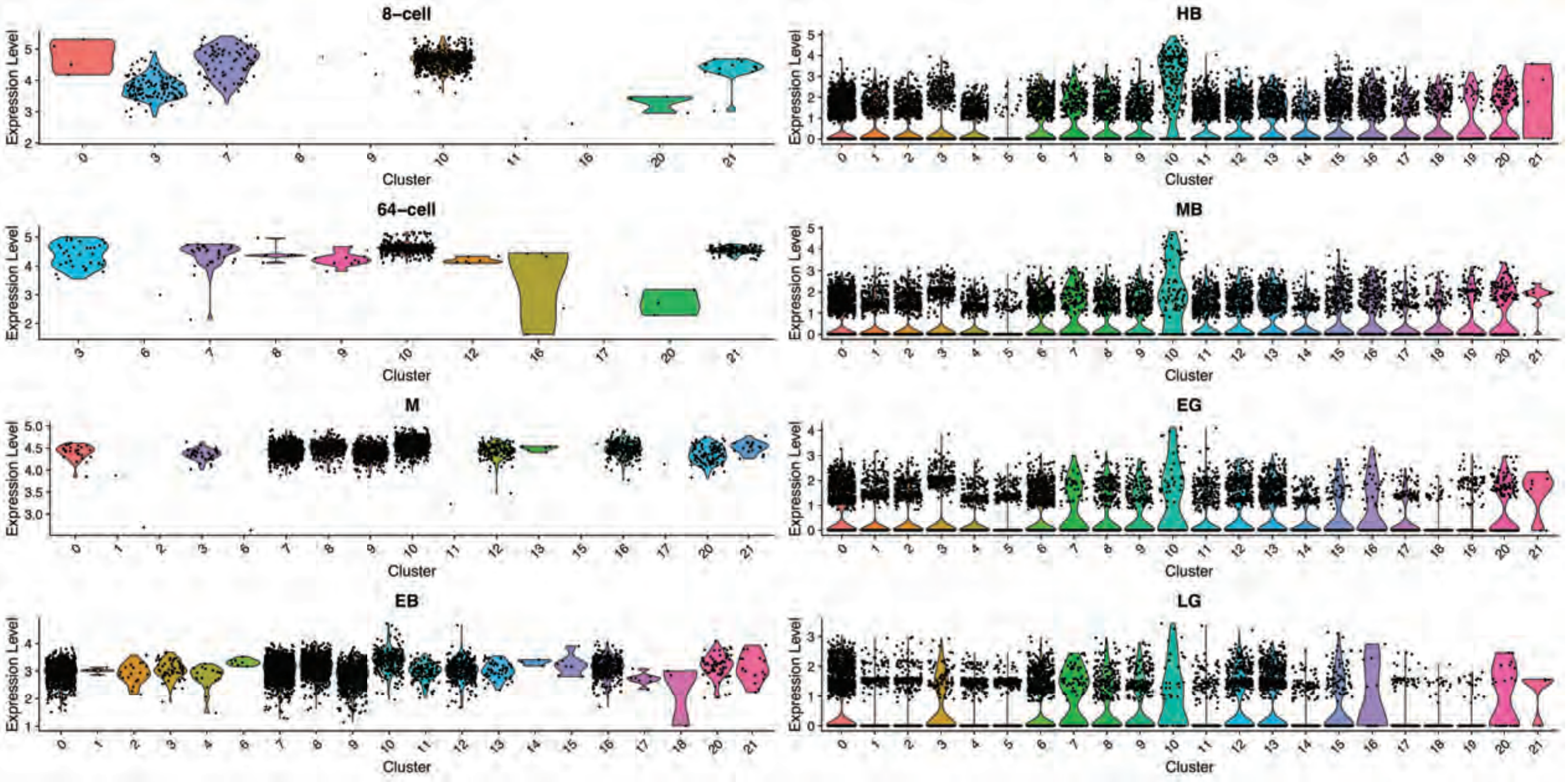

Fig. S27. Violin plots showing Cyclin B expression across different clusters at each developmental stage. Normalized gene expression values at log scale shown per cell cluster identity for each developmental time point. Plots depict probability density at different expression levels.

Table S1. Cell stages

Stage	**Hours post fertilization**	**Number of cells after QC**	**Mean reads per cell**	
			Rep1	Rep2
8-cell	2.75	886	436,520	-
64-cell	6.25	876	418,546	-
Morula	7.25	3,582	113,561	-
Early blastula	11.45	5,992	66,552	-
Hatched blastula	12-15	11,729	57,032	72,845
Mesenchyme blastula	18-20	11,741	66,103	65,176
Early gastrula	24	12,766	59,790	65,293
Late gastrula	26-28	12,827	68,490	57,482

Table S2. Cluster identification

0	**Aboral ectoderm:** ARS, spec1, spec2c, NK2.2, Tbx2/3, Klf7	11	**Pigment cells**: ABCC5a, gcm, Six1, PKS1
1	**Oral ectoderm**: CHRD, FoxG, Hes, hnf6, Lim1	12	**Oral ectoderm**: CHRD, FoxA, FoxG, HMG1, Nk2.1
2	**Ciliated cells**: tektin, cilia and flagella associated proteins	13	**Oral ectoderm**: FoxG, HMG1, Hes, hnf6, Lim1
3	**Neural:** hyalin, ebr1, egf1, glutamate receptor 2, neuronal acetylcholine receptor, synaptotagmin-14, (Hatched Blastula: ebr1, ARS)	14	**Endoderm**: blimp1/krox, endo16, FoxA, GataE, vasa
4	**Oral ectoderm**: CHRD, FoxG, Hes, hnf6, Lim1, otx	15	Major vault protein at late gastrula. (Hatched Blastula: Enriched in proteases, SpAn and Hatching enzyme)
5	**Aboral ectoderm / neural**: glutamate receptor 2, ARS, spec1, spec2c, NK2.2, Klf7	16	**Skeleton:** Alx1, delta, endo16. GataC, SM37, SM50, SM30A, vasa (mesoderm)
6	**Endoderm**: blimp1/krox, endo16, FoxA, GataE, vasa	17	**Neural**: Foxq2, AnkAT-1, NK2.1, Hes (animal ectoderm)
7	**Ciliated cells**: tektin, tubulin, cilia and flagella associated protein	18	**Neural**: Brn1/2/4, SoxC,delta
8	**Endoderm**: blimp1/krox, endo16, eve, FoxA, GATAe, Nk1	19	**Skeleton**: Alx1, erg, GATAc, SM37, SM50, SM30A (mesoderm)
9	**Neural**: foxq2, AnkAT-1, NK2.1 (animal ectoderm)	20	**Germline**: Nanos
10	Enriched in histone H2A.2.1, cyclin A, cyclin B, tektin 3. (Cell population only abundant at early time points going from 73% of population at 8-cell to 0.37% at late gastrula. Morula stage: eve expression enriched)	21	Only four cells at late gastrula. (64-cell stage: PMAR, eve, alx1, ags Morula stage: transient expression of Alx1, transient Blimp, eve)

Table S3. Morula_Cluster16

Click here to download Table S3

Table S4. Morula_Cluster20

Click here to download Table S4

Table S5. Morula_16v20_DifferentialGeneExpression

Click here to download Table S5

Table S6. Nanos_posvneg_DifferentialGeneExpression

Click here to download Table S6

Table S7. Cluster 20 Markers

Click here to download Table S7

Table S8. Differential gene expression in cluster 20 between time points

Click here to download Table S8

Table S9. Cluster Markers for Analysis at Resolution 0.5

Click here to download Table S9

Table S10. Cluster Markers for Analysis at Resolution 3

Click here to download Table S10