SUPPLEMENTAL MATERIALS

Renal single-nuclear transcriptomics identifies novel therapeutic targets in a preclinical model of chronic kidney disease.

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Supplemental Tables

Supplemental Table 1a. Composition of the high cholesterol/high carbohydrate diet (KT324). For a complete list of ingredients, please see Supplementary Table 1b.

Ingredient	**%**
5B4L LabDiet version	79.2223%
Hydrogenated Soy Oil	8.4000%
High Fructose Corn Syrup - 55	5.0000%
Hydrogenated Coconut Oil	4.7000%
Cholesterol	2.0000%
Sodium Cholate	0.6777%
Energy (kcal/g)	%
Protein	16.3
Fat (ether extract)	42.9
Carbohydrates	40.8

Supplemental Table 1b. Full list of ingredients of KT324 diet.

Complete Ingredients List	**Measurements (%, ppm,IU/g,mcg/kg)**
Arginine	0.92%
Histidine	0.47%
Isoleucine	0.95%
Leucine	1.44%
Lysine	1.16%
Methionine	0.36%
Cystine	0.17%
Phenylalanine	0.86%
Tyrosine	0.71%
Threonine	0.67%
Tryptophan	0.22%
Valine	1.09%
Alanine	0.64%
Aspartic Acid	1.54%
Glutamic Acid	3.04%
Glycine	0.56%

Proline	1.09%
Serine	0.81%
Taurine	0.00%
Cholesterol	20,000 ppm
Linoleic Acid	1.45%
Linolenic Acid	0.13%
Arachidonic Acid	0.00%
Omega-3 Fatty Acid	0.13%
Total Saturated Fatty Acids	10.69%
Total Monounsaturated Fatty Acids	4.98%
Polyunsaturated Fatty Acids	1.33%
Neutral Detergent Fiber	11.90%
Acid Detergent Fiber	6.20%
Starch	28.35%
Glucose	2.29%
Fructose	18.64%
Sucrose	1.67%
Lactose	0.01%
Ash	5.90%
Calcium	1.03%
Phosphorus	0.81%
Phosphorus (available)	0.65%
Potassium	0.83%
Magnesium	0.21%
Sulfur	0.13%
Sodium	0.25%
Chloride	0.38%
Fluorine	38.70%
Iron	288 ppm
Zinc	222 ppm
Manganese	40 ppm
Copper	6 ppm
Cobalt	0.30 ppm
Iodine	1.14 ppm
Chromium	2.17 ppm
Selenium	0.36 ppm
Carotene	0.1 ppm
Vitamin A	6 IU/g
Vitamin D-3	1.1 IU/g
Vitamin E	110 IU/kg
Vitamin K (as menadione)	2.9 ppm
Thiamin Hydrochloride	10 ppm
Riboflavin	13.9 ppm
Niacin	64 ppm
Pantothenic Acid	40 ppm
Folic Acid	4.0 ppm
Pyridoxine	6.41 ppm
Biotin	0.4 ppm
Vitamin B-12,	78 mcg/kg

Supplemental Table 2. Composition of the Normal diet (ProBuild Grower and CU Sinclair S-9).

Ingredient-ProBuild Grower	**%**		**Ingredient-CU Sinclair S-9**	**%**
Crude Protein (Min)	18.00%		Crude Protein (Min)	19%
Lysine (Min)	1.15%		Lysine (Min)	0.90%
Crude Fat (Min)	2.00%		Crude Fat (Min)	3.75%
Crude Fiber (Max)	5.00%		Crude Fiber (Max)	3.90%
Calcium (Ca) (Min)	0.32%		Calcium (Ca) (Min)	0.55%
Calcium (Ca) (Max)	0.82%		Calcium (Ca) (Max)	1.05%
Phosphorus (P) (Min)	0.50%		Phosphorus (P) (Min)	0.65%
Salt (NaCl) (Min)	0.23%		Salt(NaCl) (Min)	0.05%
Salt (NaCl) (Max)	0.73%		Salt(NaCl) (Max)	0.55%
Selenium (Se) (Min)	0.30 ppm		Selenium (Se) (Min)	0.30 ppm
Zinc (Zn) (Min)	100.00 ppm		Zinc (Zn) (Min)	125.0 ppm
Phytase (A.Oryzae) (Min)	454 FYT/LB			

Supplemental Table 3: Alignment summary showing estimated and final number of cells in Normal and CKD kidneys (52,213 cells, n=3/group) that were subjected to quality control analysis. Normal controls: D009, D010, D011. CKD: D002, D004, D005.

	D002	**D004**	**D005**	**D009**	**D010**	**D011**
Estimated Number of Cells	**6,771**	**10,035**	**8,912**	**8,707**	**11,068**	**6,367**
Mean Reads per Cell	81,648	46,528	56,862	61,795	41,505	73,941
Median Genes per Cell	2,496	2,318	2,216	1,204	2,078	2,052
Number of Reads	**552,841,532**	**466,911,820**	**506,756,352**	**538,051,075**	**459,378,501**	**470,783,915**
Valid Barcodes	97.60%	97.40%	97.30%	96.60%	96.60%	96.90%
Sequencing Saturation	82.60%	73.60%	75.90%	85.90%	69.10%	80.90%
Q30 Bases in Barcode	93.60%	93.60%	93.60%	93.50%	94.20%	94.20%
Q30 Bases in RNA Read	90.50%	90.60%	91.20%	90.80%	91.10%	92.10%
Q30 Bases in UMI	93.40%	93.30%	93.30%	93.10%	93.00%	93.00%
Reads Mapped to Genome	94.80%	95.10%	95.10%	93.90%	95.20%	95.20%
Reads Mapped Confidently to Genome	91.20%	91.70%	91.70%	88.80%	92.00%	91.90%
Reads Mapped Confidently to Intergenic Regions	12.40%	12.20%	13.40%	12.50%	12.90%	12.60%
Reads Mapped Confidently to Intronic Regions	55.30%	58.90%	60.20%	53.50%	62.90%	60.90%
Reads Mapped Confidently to Exonic Regions	23.40%	20.60%	18.10%	22.90%	16.20%	18.40%
Reads Mapped Confidently to Transcriptome	64.10%	61.40%	54.30%	67.90%	50.90%	61.40%
Reads Mapped Antisense to Gene	14.40%	17.90%	23.70%	8.20%	28.00%	17.70%
Fraction Reads in Cells	74.00%	82.20%	81.10%	52.70%	82.50%	67.70%
Total Genes Detected	29,683	29,745	29,786	29,558	29,656	29,242
Median UMI Counts per Cell	5,715	5,089	4,854	1,916	4,372	4,415
After cell bender	**6,490**	**9,895**	**8,855**	**7,826**	**10,822**	**6,027**

Supplemental Table 4: Alignment summary showing the final number of cells after quality control of Normal and CKD kidneys (39,935 cells, n=3/group). Normal controls: D009, D010, D011. CKD: D002, D004, D005.

Samples	**Total**		**Mito**	**Gene**	**UMI**	**Doublet**		**Failed**	**Passed**
D002	6,490		21	19	139	665		**812**	**5,678**
D004	9,895		103	99	238	1,409		**1,688**	**8,207**
D005	8,855		157	188	328	1,228		**1,583**	**7,272**
D009	7,826		30	347	1,841	952		**2,798**	**5,028**
D010	10,822		102	163	454	1,663		**2,136**	**8,686**
D011	6,072		73	97	409	586		**1,008**	**5,064**

Mito: mitochondria
UMI: unique molecular identifier

Supplemental figure legends

Supplemental Figure 1: Single nuclei RNA-seq (snRNA-seq) workflow of Normal and CKD kidneys (n=3/group).
QC: quality control; UMI: unique molecular identifier.

Supplemental Figure 2: snRNA-seq quality control showing analysis summary showing the percentage (%) of mitochondrial content in cells (density plots) of Normal and CKD kidneys (n=3/group). Normal controls: D009, D010, D011. CKD: D002, D004, D005.

Supplemental Figure 3: snRNA-seq quality control analysis summary showing the mitochondrial content detected in each cell (box plots) of Normal and CKD kidneys (n=3/group). Normal controls: D009, D010, D011. CKD: D002, D004, D005.

Supplemental Figure 4: snRNA-seq quality control showing the number of genes detected in each cell (density plots) of Normal and CKD kidneys (n=3/group). Normal controls: D009, D010, D011. CKD: D002, D004, D005.

Supplemental Figure 5: snRNA-seq quality control showing the number of genes detected in each cell (violin plots) of Normal and CKD kidneys (n=3/group). Normal controls: D009, D010, D011. CKD: D002, D004, D005.

Supplemental Figure 6: snRNA-seq quality control showing analysis summary showing the number of unique molecular identifiers detected in each cell (density plots) of Normal and CKD kidneys (n=3/group). Normal controls: D009, D010, D011. CKD: D002, D004, D005.

Supplemental Figure 7: snRNA-seq quality control showing the number of unique molecular identifiers detected in each cell (violin plots) of Normal and CKD kidneys (n=3/group). Normal controls: D009, D010, D011. CKD: D002, D004, D005.

Supplemental Figure 8: snRNA-seq quality control showing the estimated doublet score (scDBIFinder) detected in each cell (density plots) of Normal and CKD kidneys (n=3/group). Normal controls: D009, D010, D011. CKD: D002, D004, D005.

Supplemental Figure 9: snRNA-seq quality control showing the percentage of mitochondrial content vs. the number of genes detected per cell (scattered plots) of Normal and CKD kidneys (n=3/group). Normal controls: D009, D010, D011. CKD: D002, D004, D005.

Supplemental Figure 10: snRNA-seq quality control showing the correlation (Spearman) of genes detected vs. unique molecular identifiers (scattered plots) of Normal and CKD kidneys (n=3/group). Normal controls: D009, D010, D011. CKD: D002, D004, D005.

Supplemental Figure 11: UMAP visualization of the integrated dataset showing 39,935 nuclei of all kidneys (n=3/group). Normal controls: D009, D010, D011. CKD: D002, D004, D005.

Supplemental Figure 12: UMAP visualization of integrated dataset showing 39,935 nuclei and 30 cell clusters in the kidneys of Normal and CKD pigs (n=3/group).

Supplemental Figure 13: Box plot showing mitochondrial content (%), unique molecular identifier (UMI) per cell, number of genes detected per cell, and estimated doublet content in each cluster (n=3/group).

Supplemental Figure 14: Bar graph visualization of integrated datasets showing the distribution of cells in each cluster (n=3/group). Normal controls: D009, D010, D011. CKD: D002, D004, D005.

Supplemental Figure 15: UMAP visualization of integrated dataset showing 39,935 nuclei and 18 clusters using specific renal cell markers in Normal and CKD pigs (n=3/group).

Supplemental Figure 16: Marker gene expression profile of proximal tubular cells (UMAP) in Normal and CKD pigs (n=3/group).

Supplemental Figure 17: Marker gene expression profile of endothelial cells (UMAP) in Normal and CKD pigs (n=3/group).

Supplemental Figure 18: Marker gene expression profile of connecting tubular cells (UMAP) in Normal and CKD pigs (n=3/group).

Supplemental Figure 19: Marker gene expression profile of loop of Henle cells (UMAP) in Normal and CKD pigs (n=3/group).

Supplemental Figure 20: Marker gene expression profile of α-intercalated cells (UMAP) in Normal and CKD pigs (n=3/group).

Supplemental Figure 21: Marker gene expression profile of principal cells (UMAP) in Normal and CKD pigs (n=3/group).

Supplemental Figure 22: Marker gene expression profile of distal tubular cells (UMAP) in Normal and CKD pigs (n=3/group).

Supplemental Figure 23: Marker gene expression profile of podocytes (UMAP) in Normal and CKD pigs (n=3/group).

Supplemental Figure 24: Marker gene expression profile of renal interstitial cells (UMAP) in Normal and CKD pigs (n=3/group).

Supplemental Figure 25: Marker gene expression profile of β-intercalated cells (UMAP) in Normal and CKD pigs (n=3/group).

Supplemental Figure 26: Marker gene expression profile of immune cells (UMAP) in Normal and CKD pigs (n=3/group).

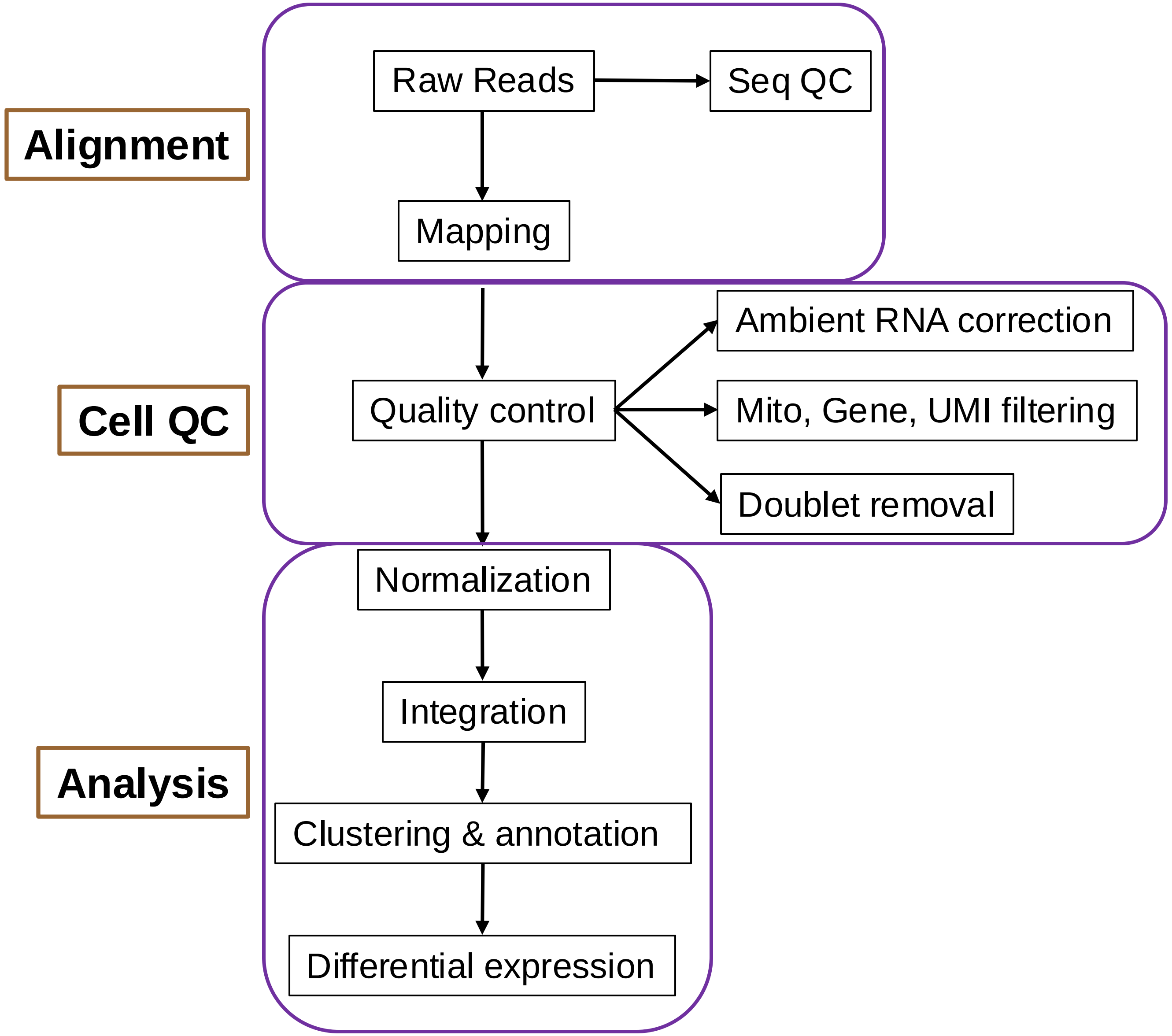
Supplemental Figure 27: Marker gene expression profile of mesangial cells (UMAP) in Normal and CKD pigs (n=3/group).

Supplemental Figure 28: Marker gene expression profile of unknown cells (UMAP, unknown 1) in Normal and CKD pigs (n=3/group).

Supplemental Figure 29: Marker gene expression profile of unknown cells (UMAP, unknown 2) in Normal and CKD pigs (n=3/group).

Supplemental Figure 30: Marker gene expression profile of unknown cells (UMAP, unknown 3) in Normal and CKD pigs (n=3/group).

Supplemental figure 1



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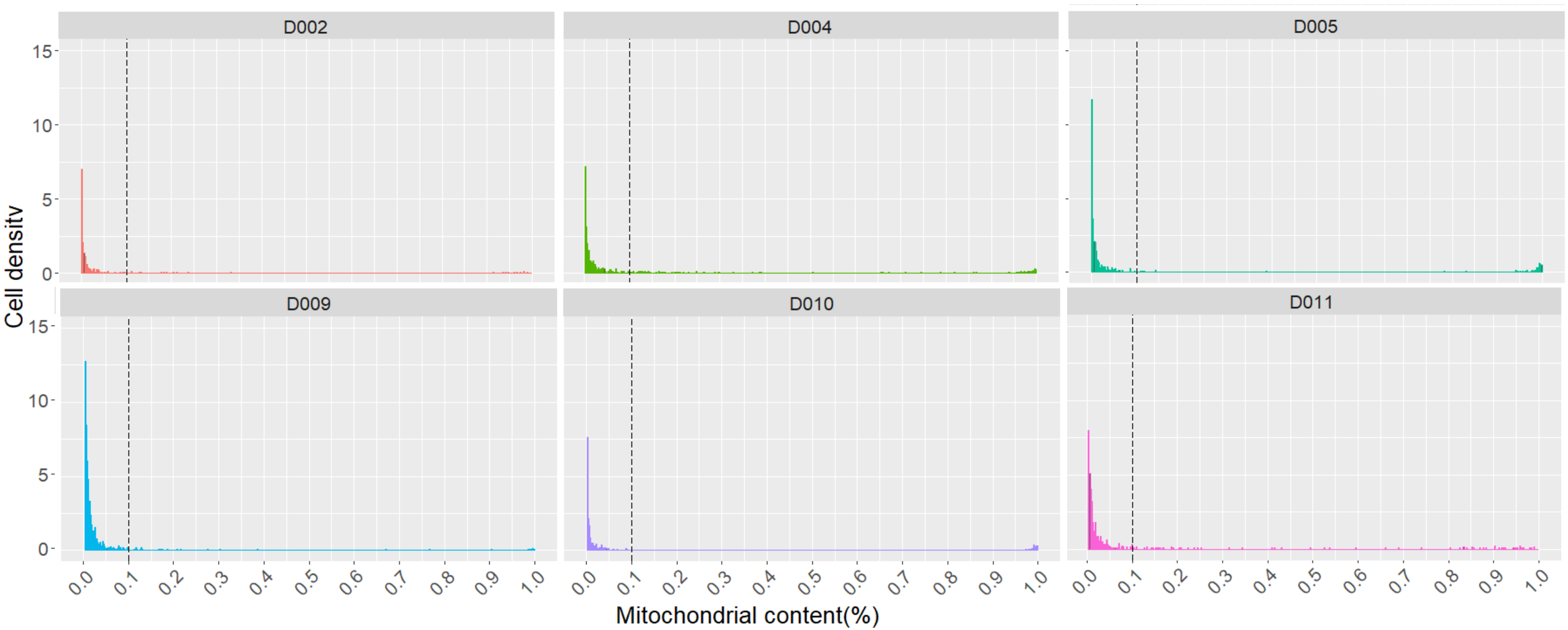

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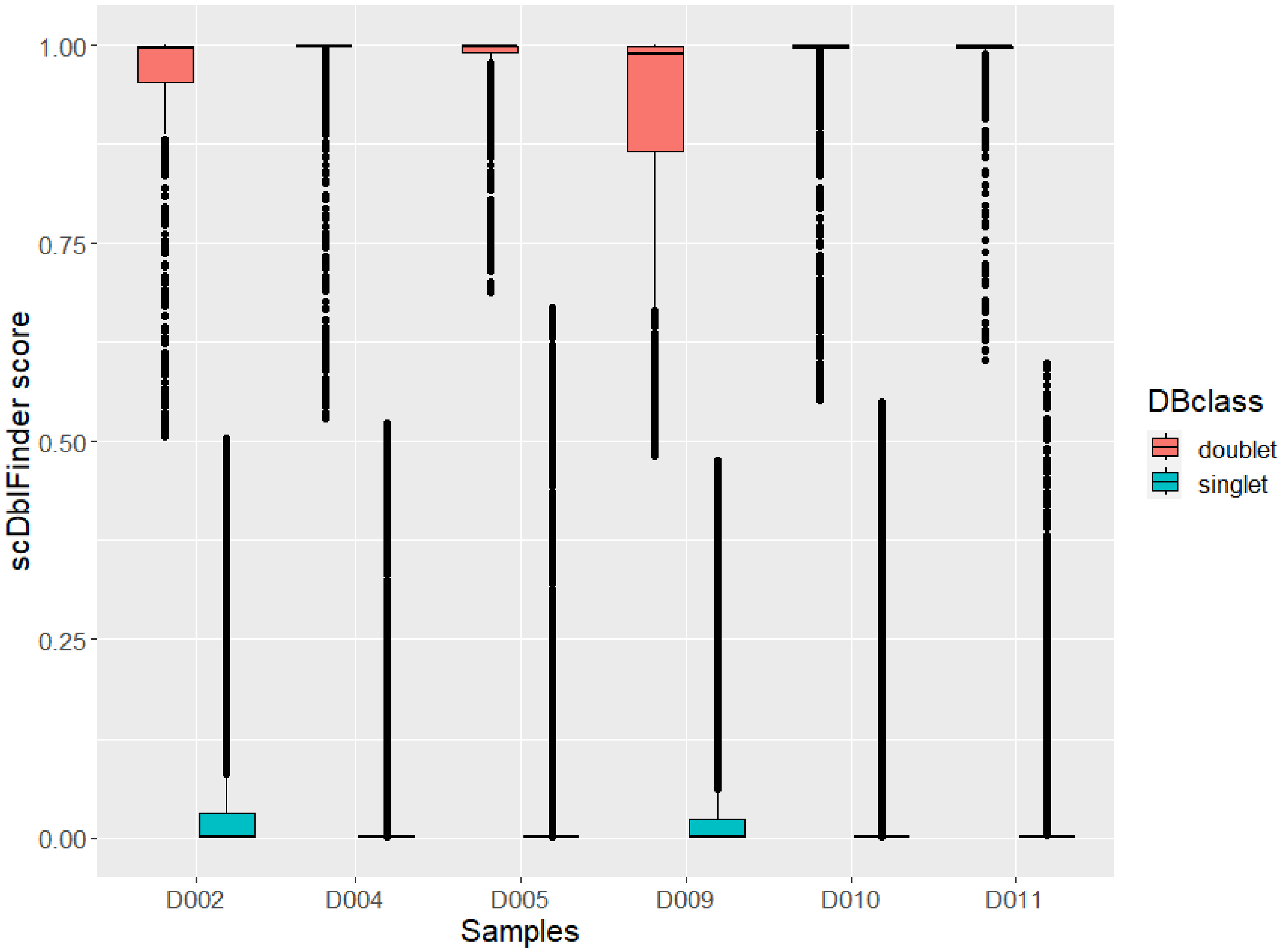

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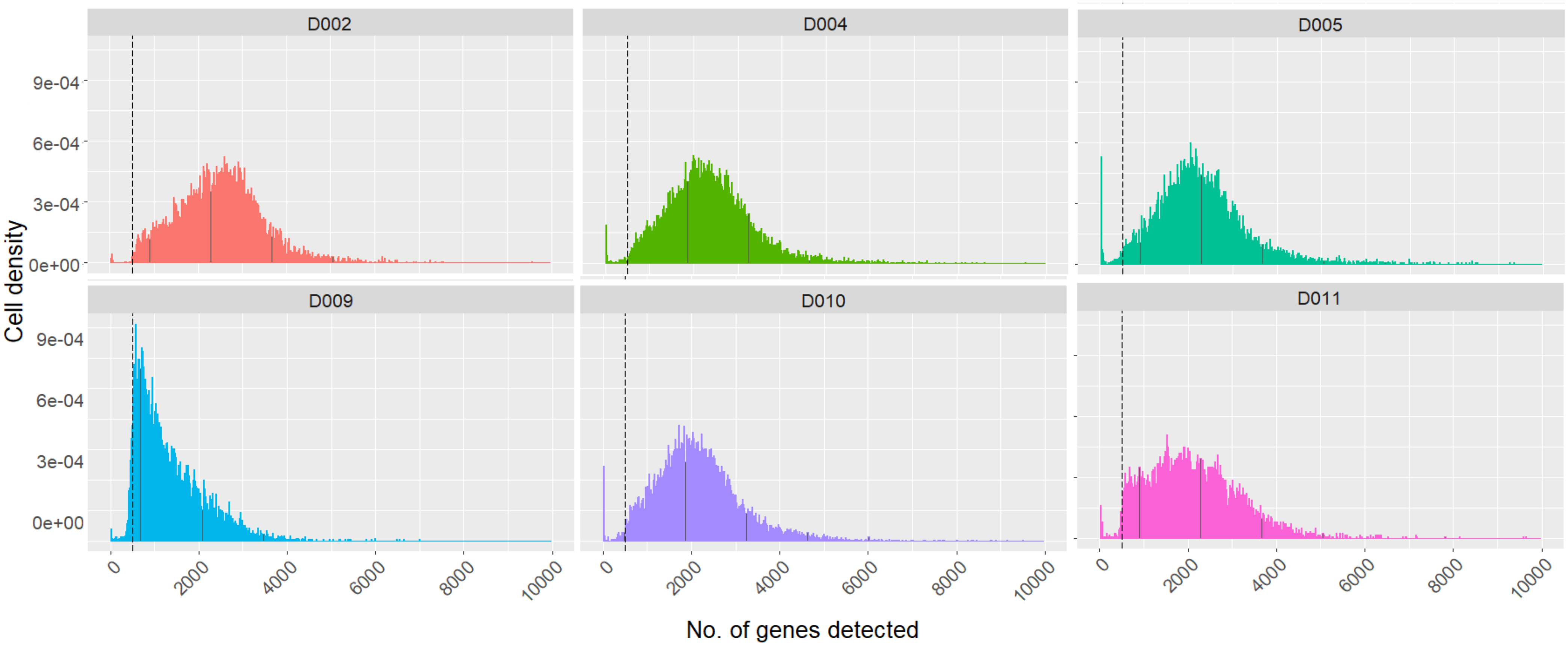

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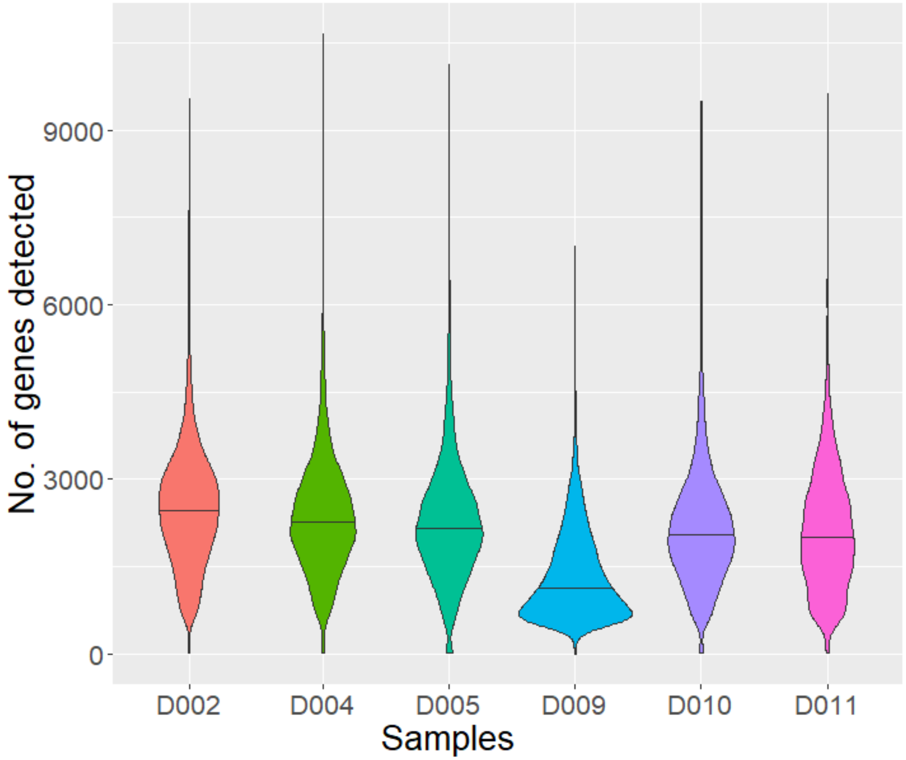

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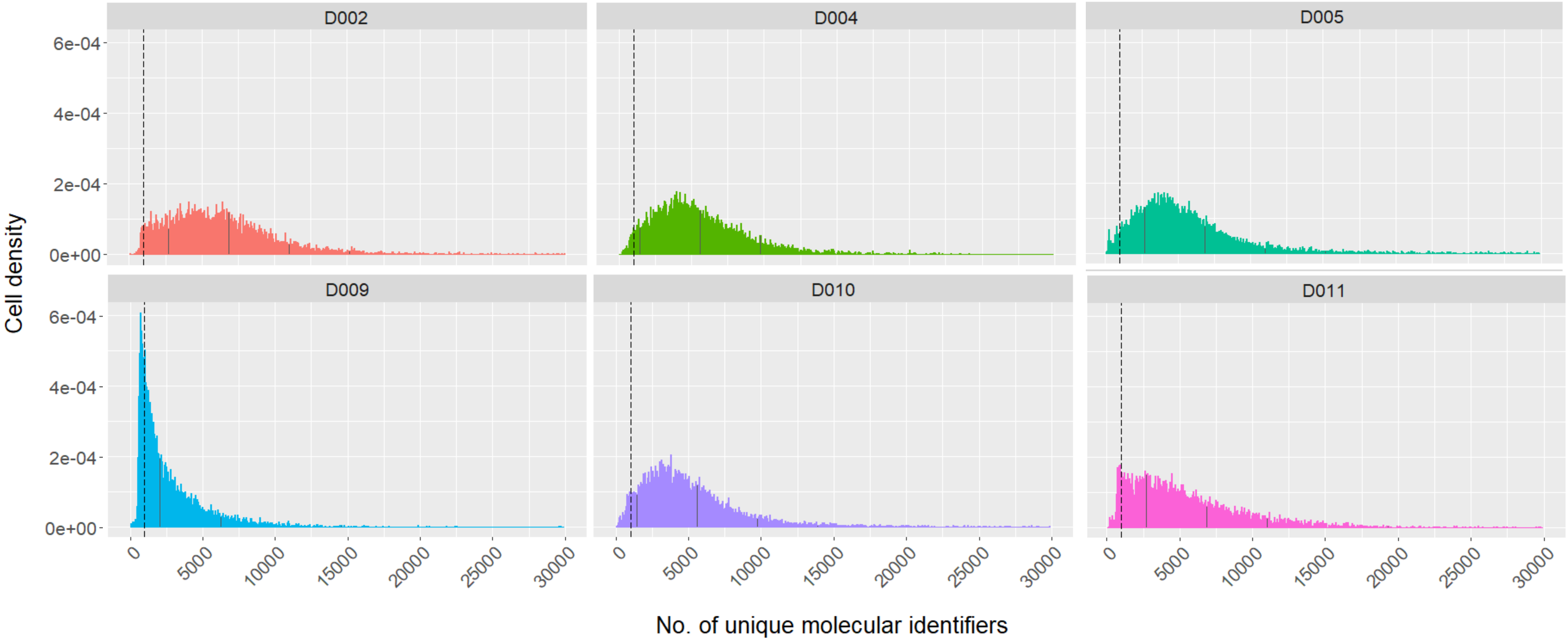

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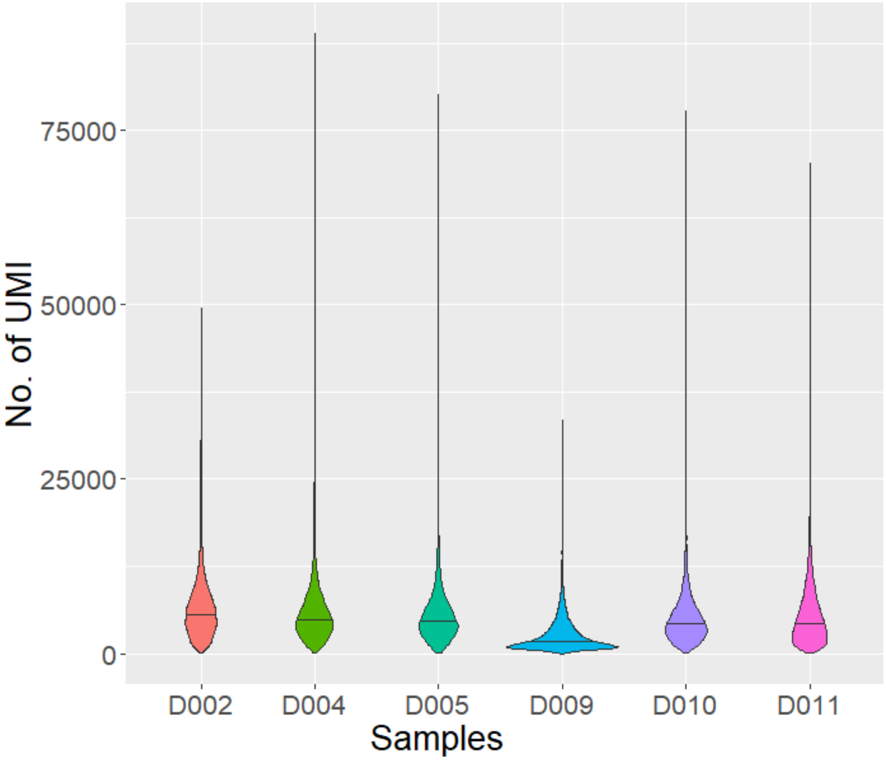

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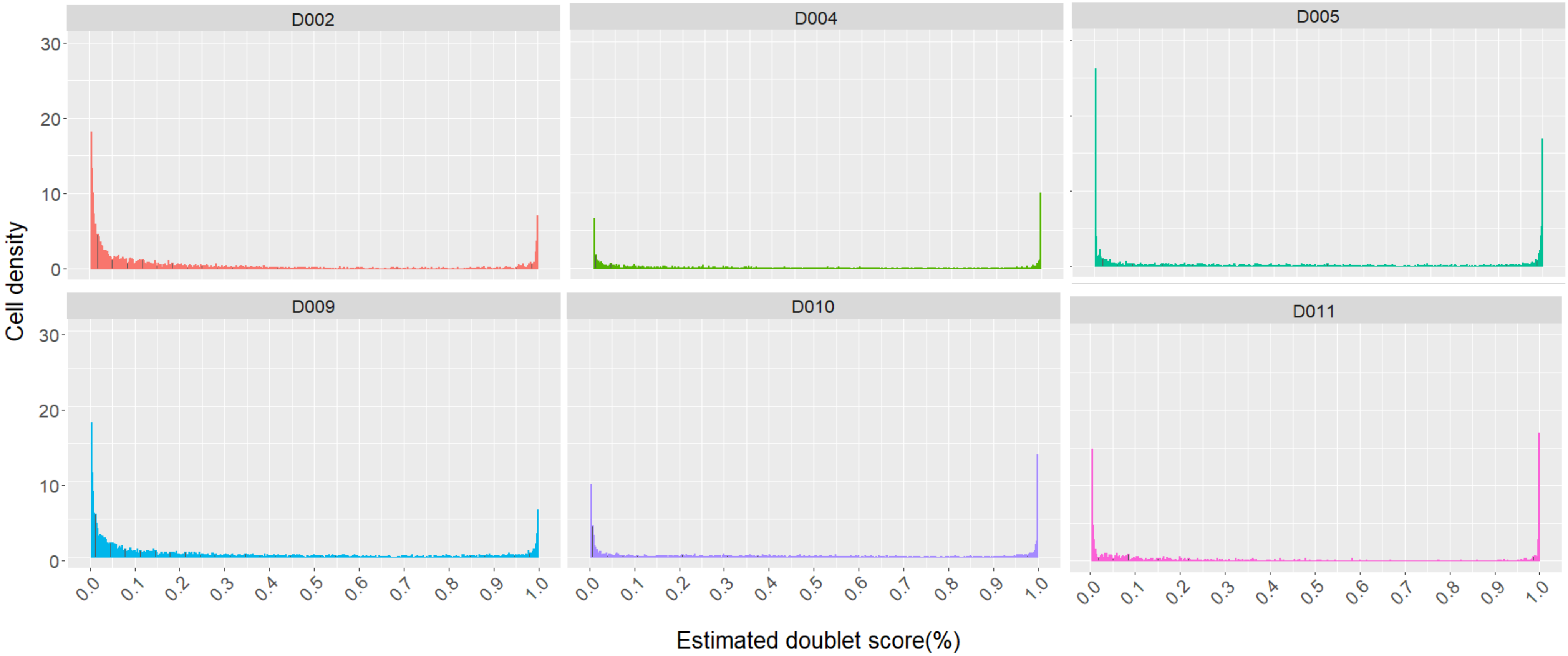

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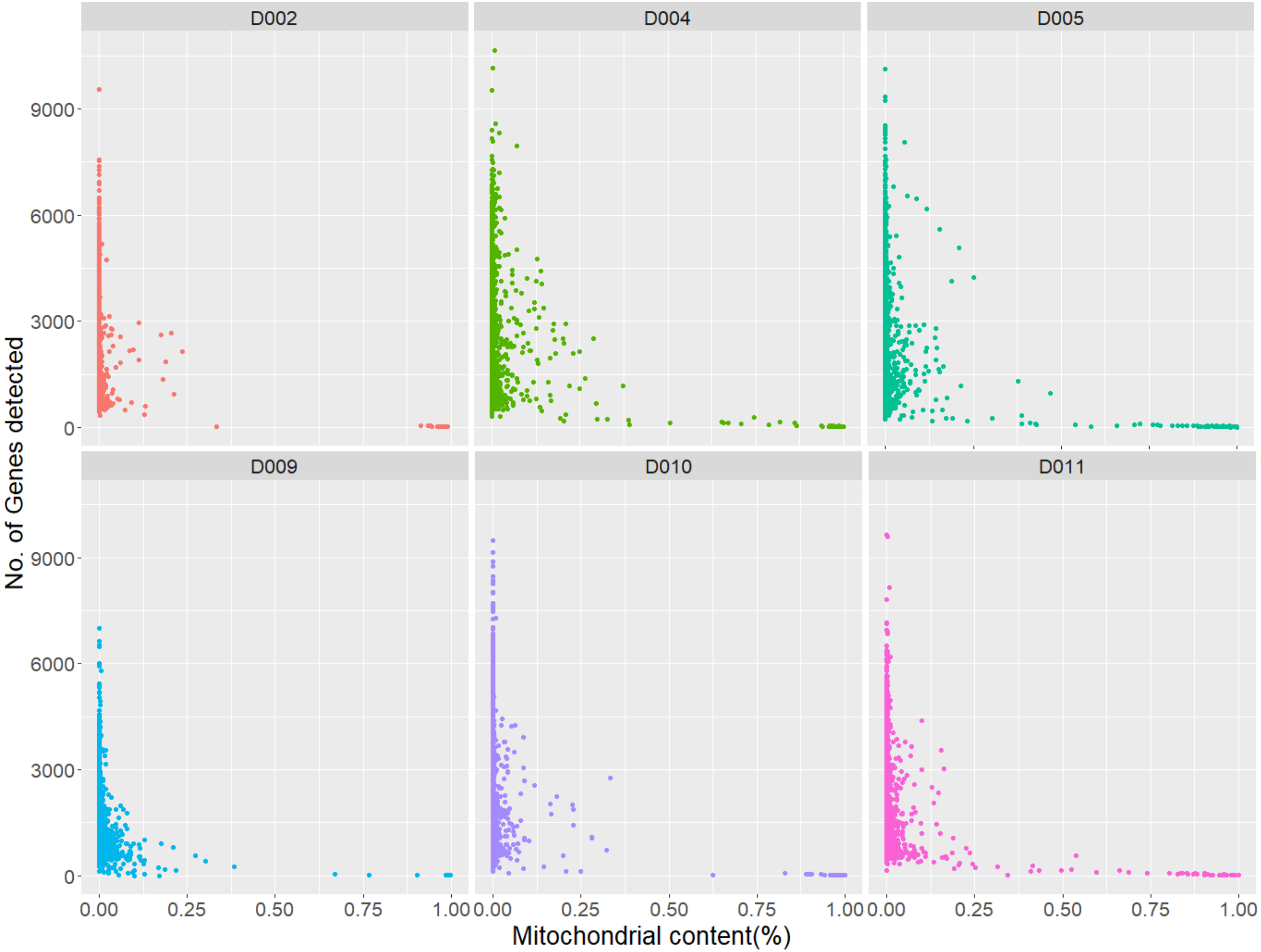

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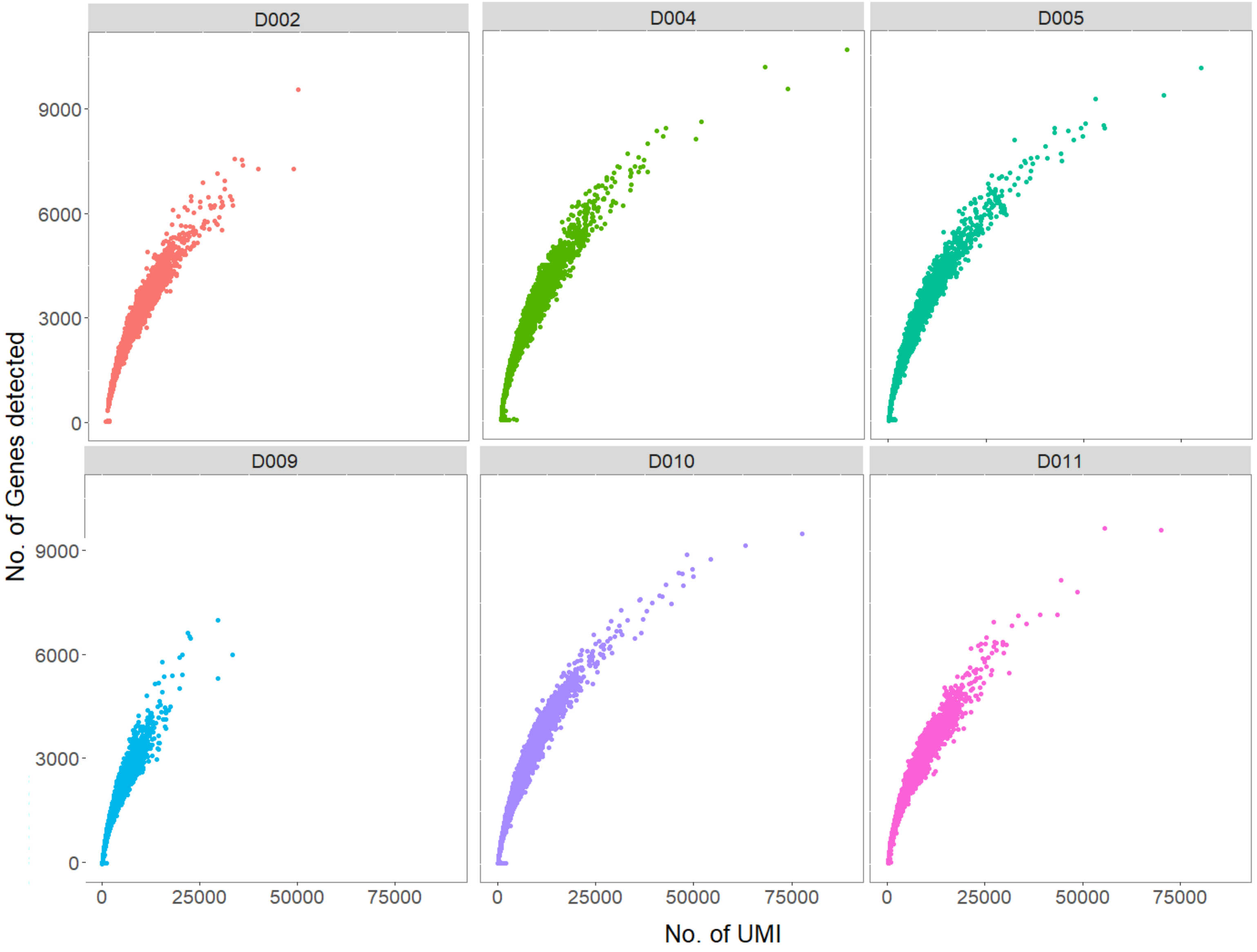

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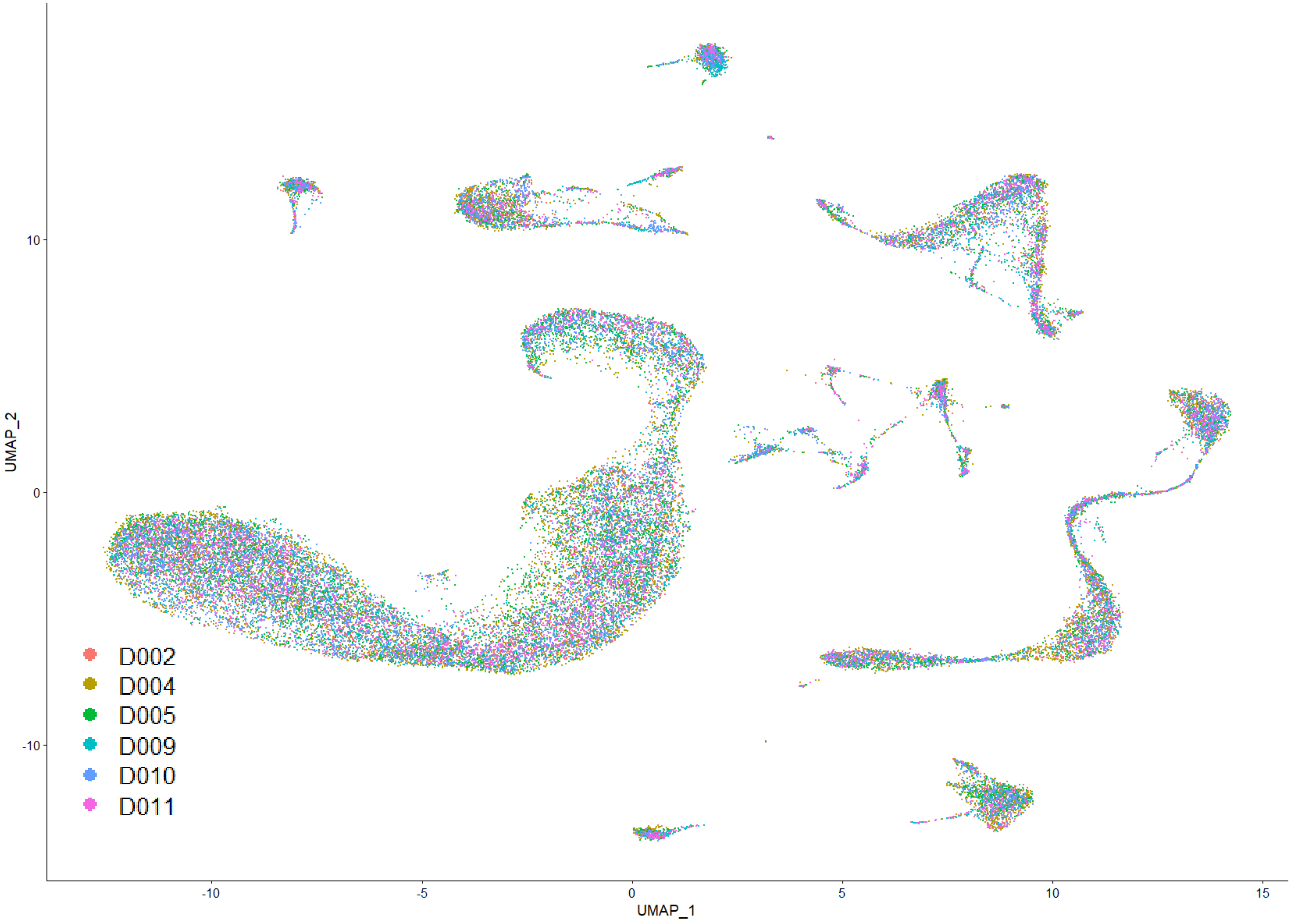

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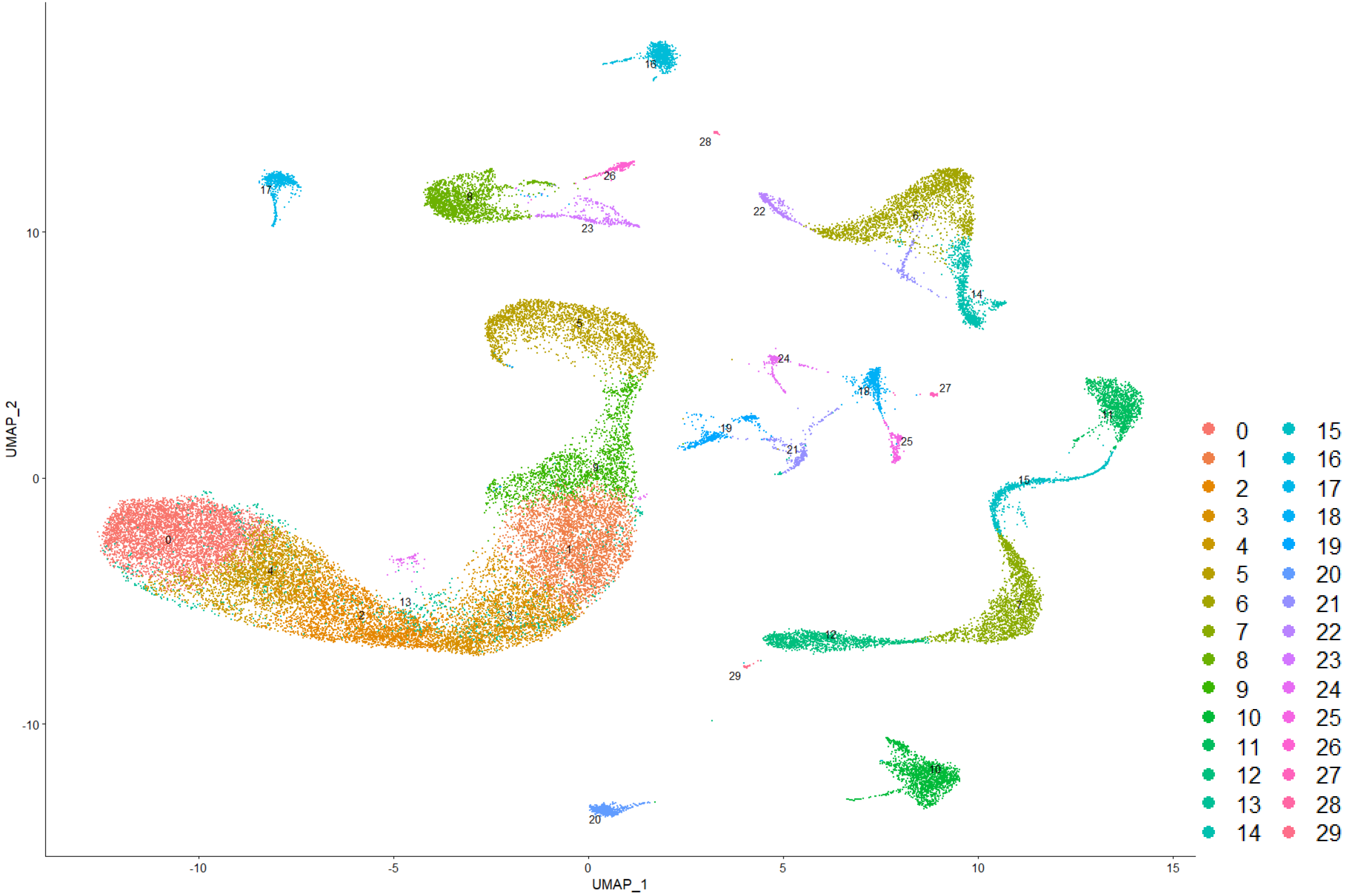

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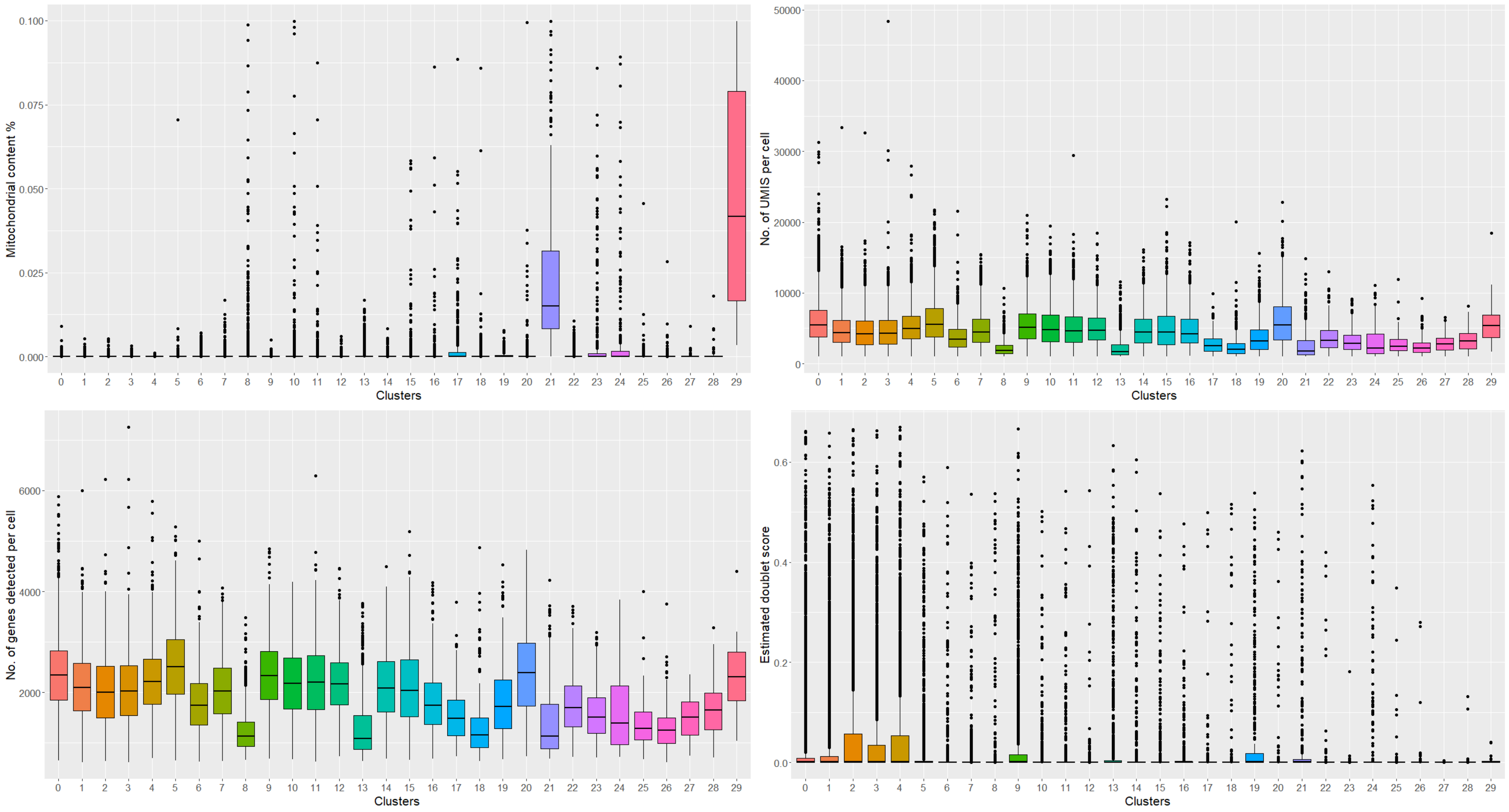

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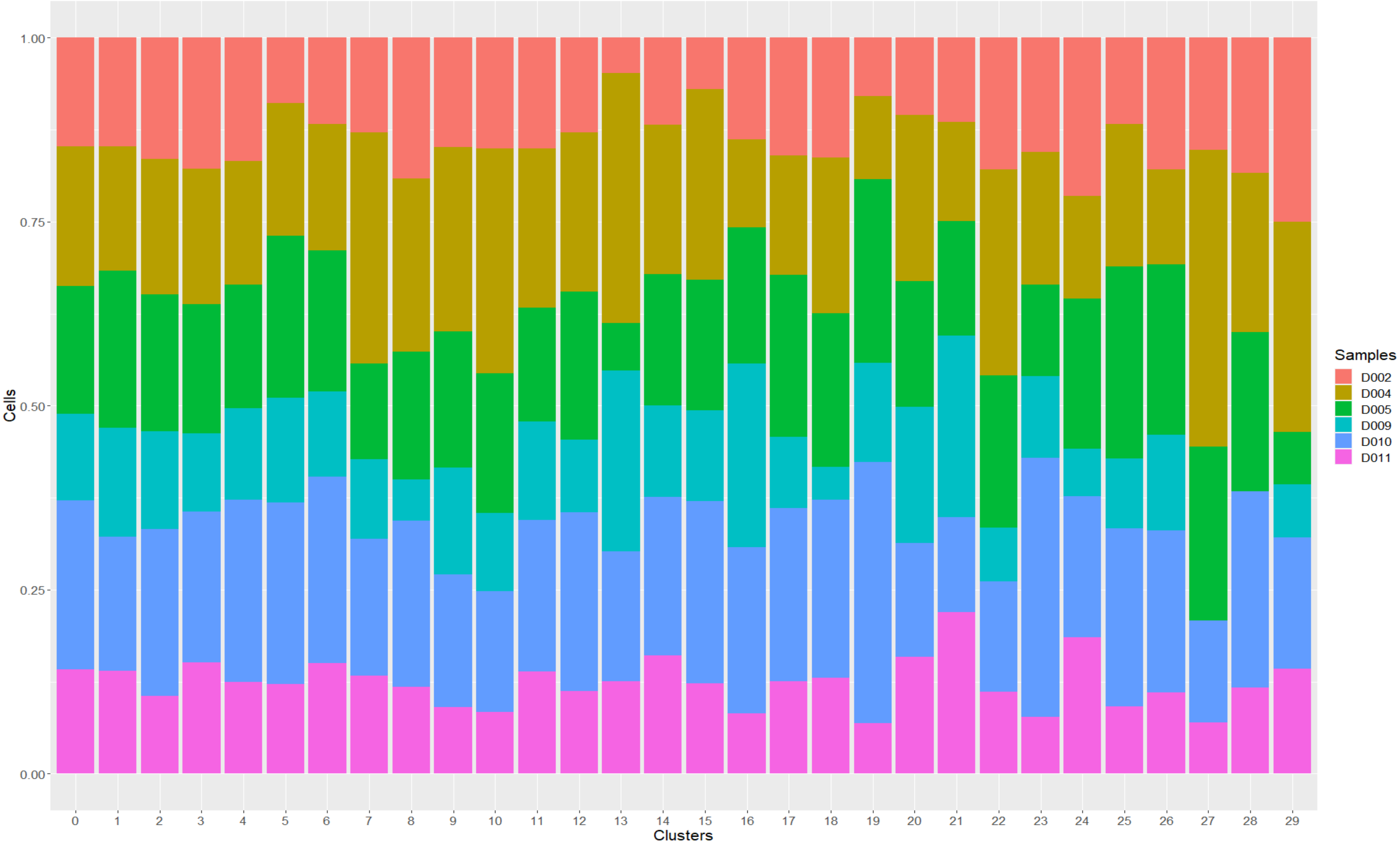

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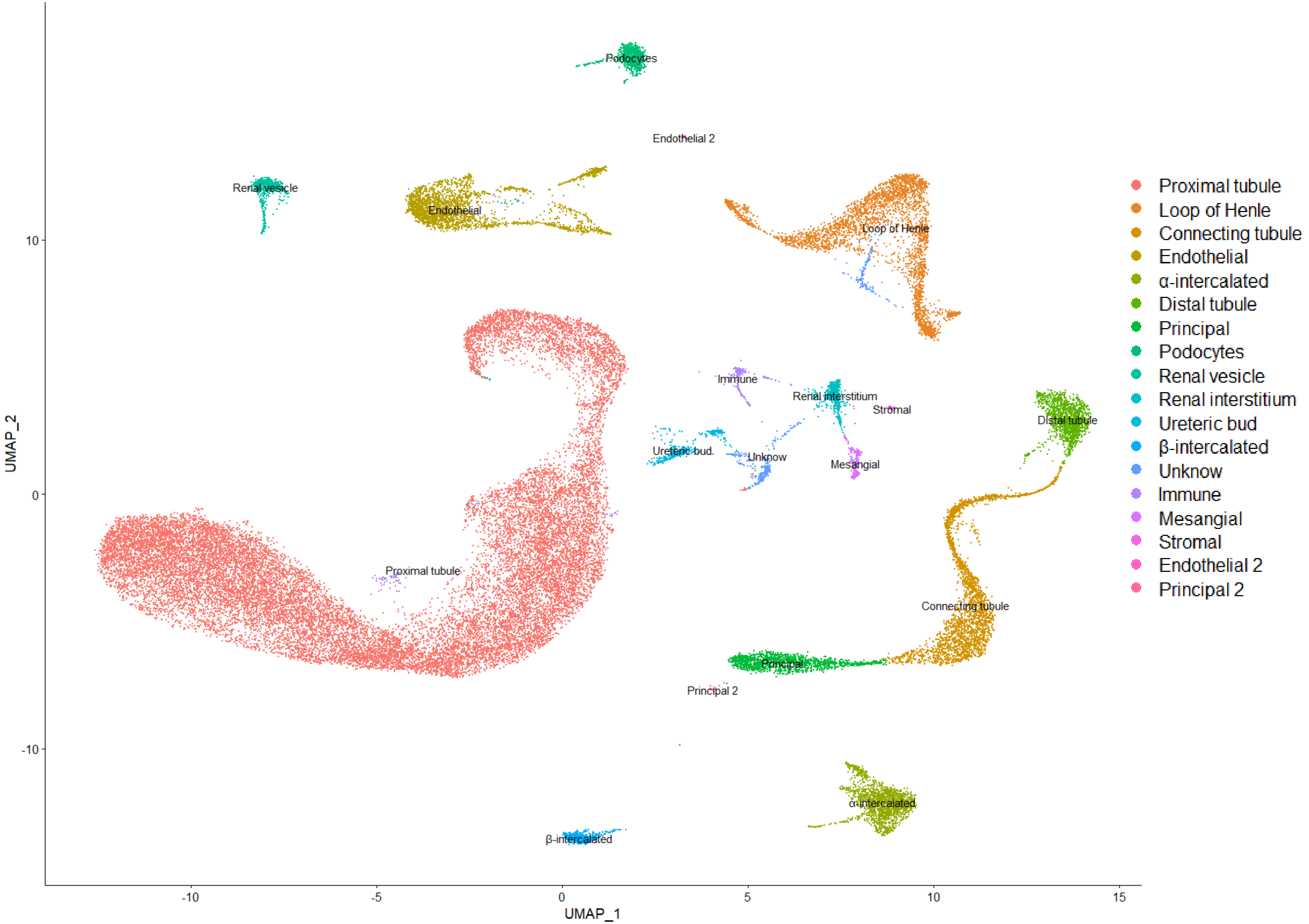

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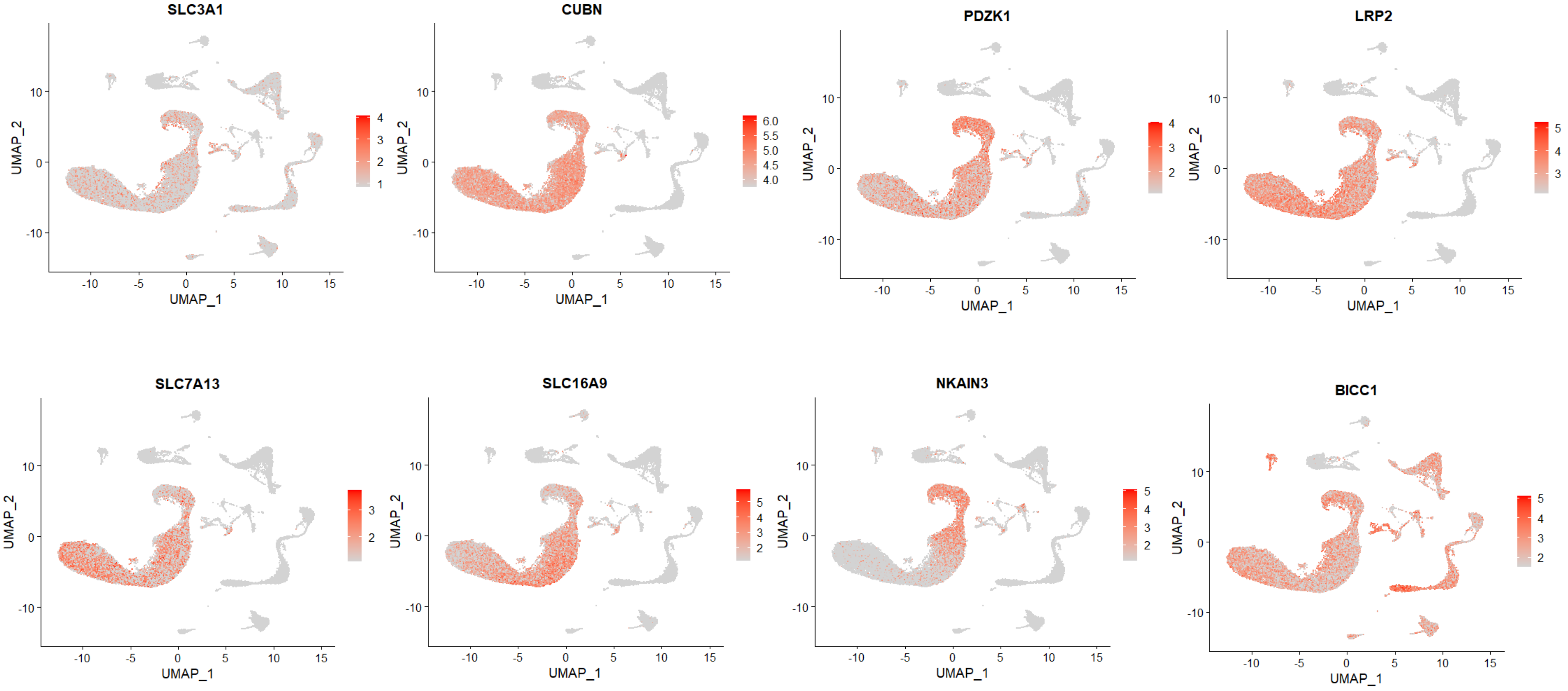

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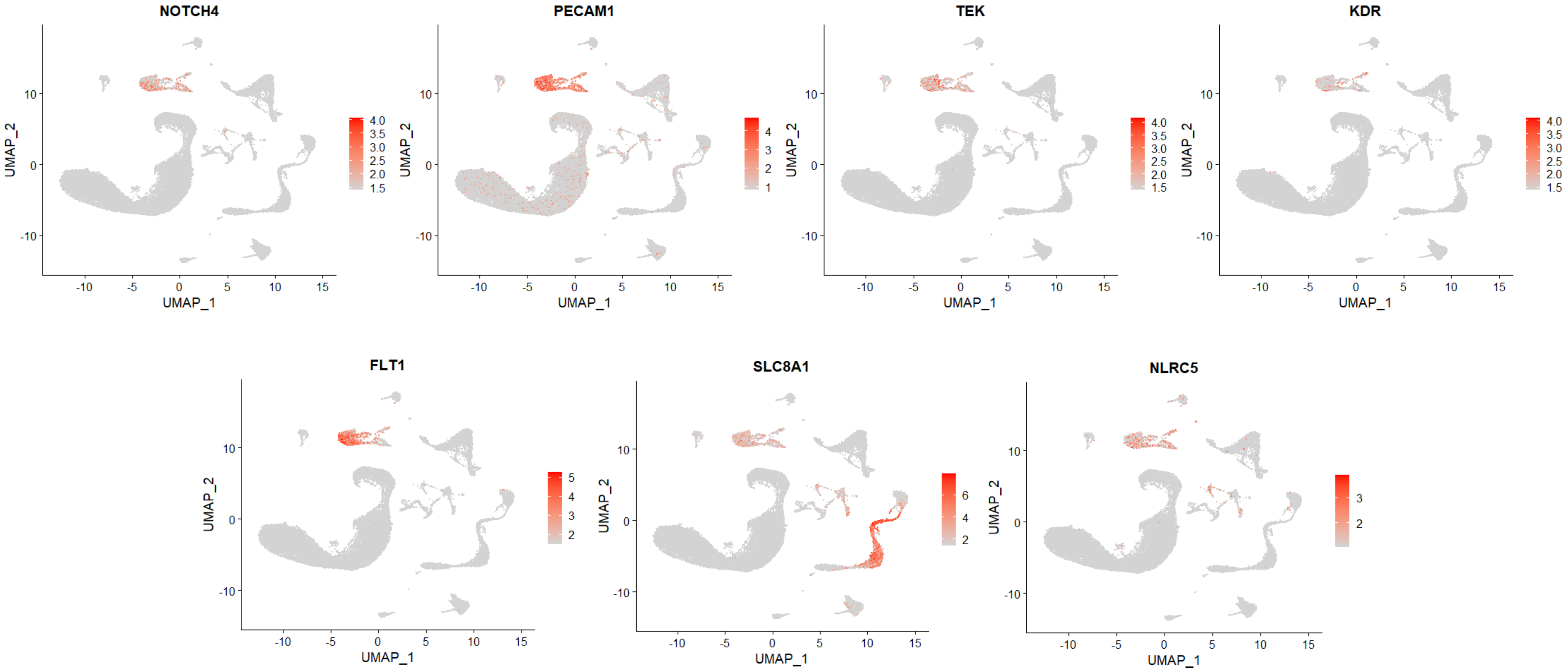

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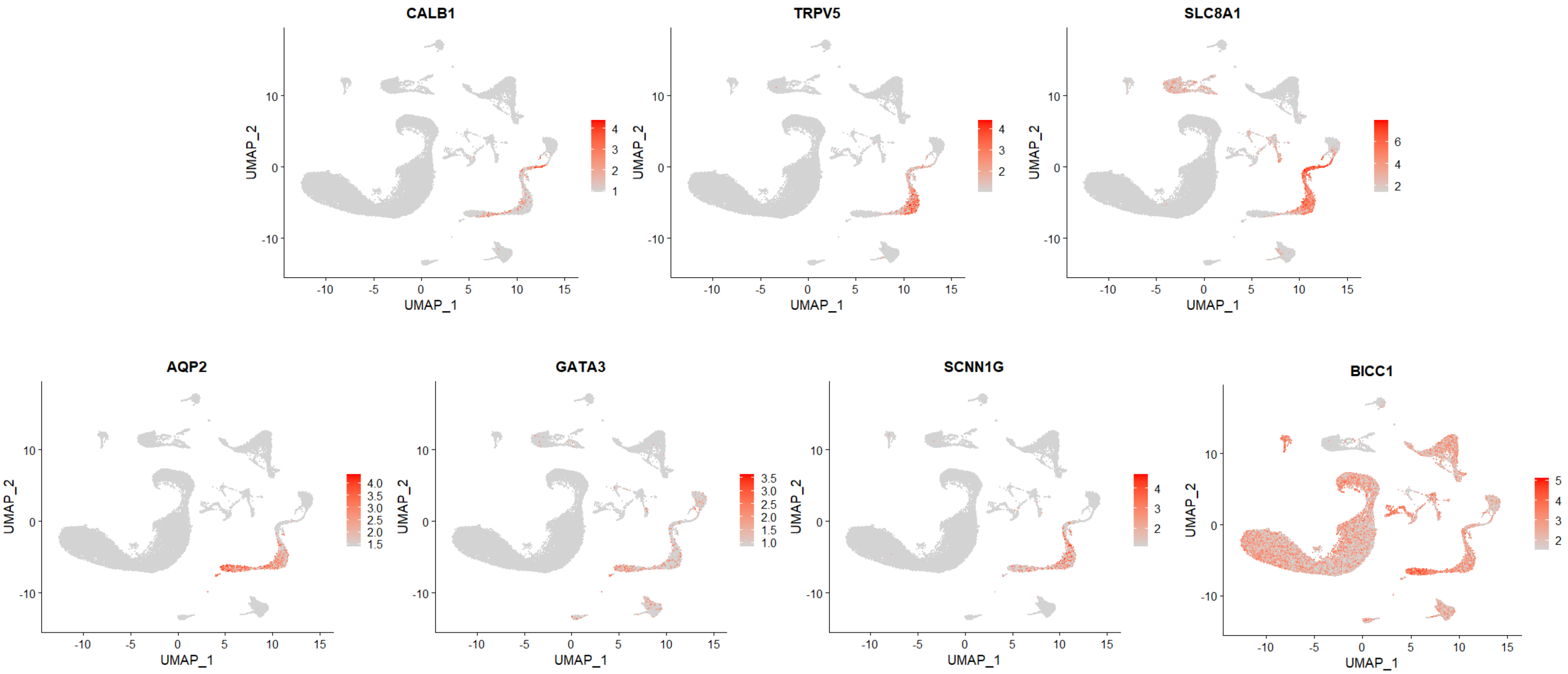

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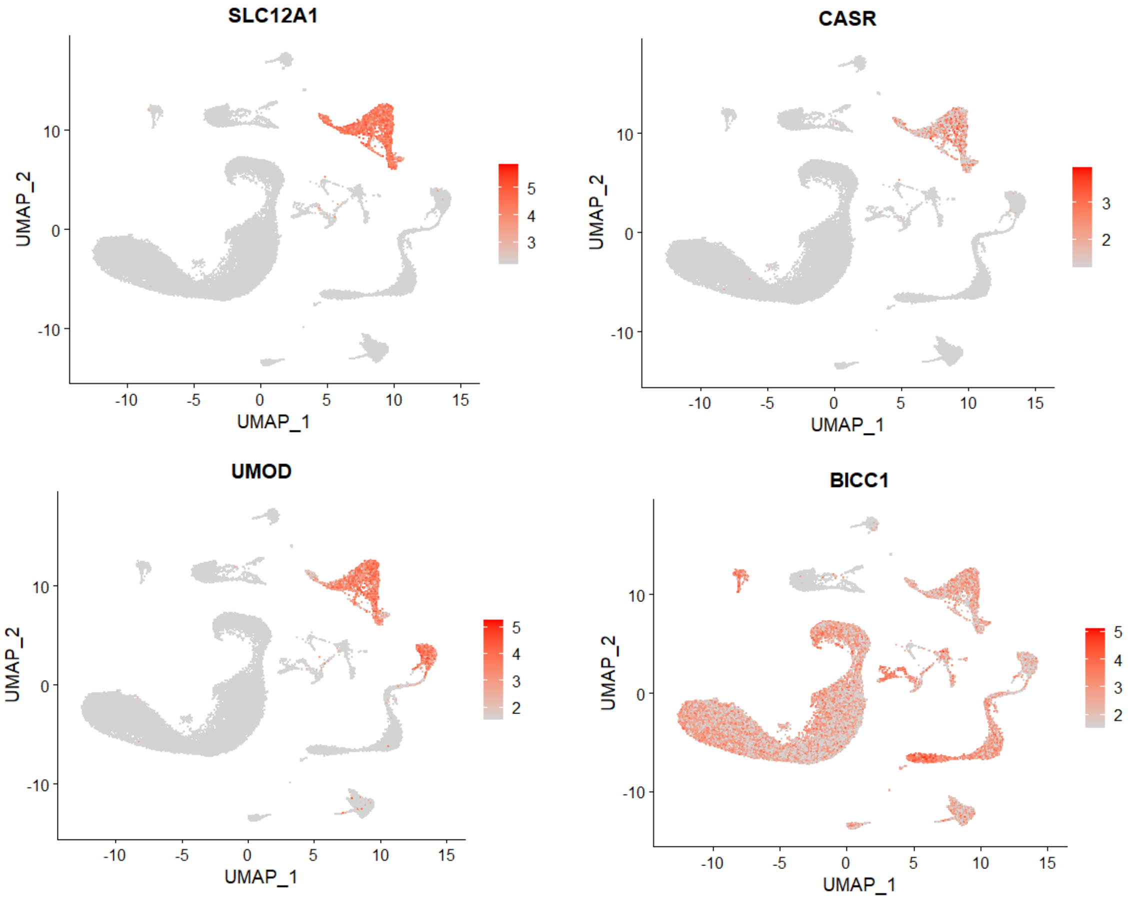

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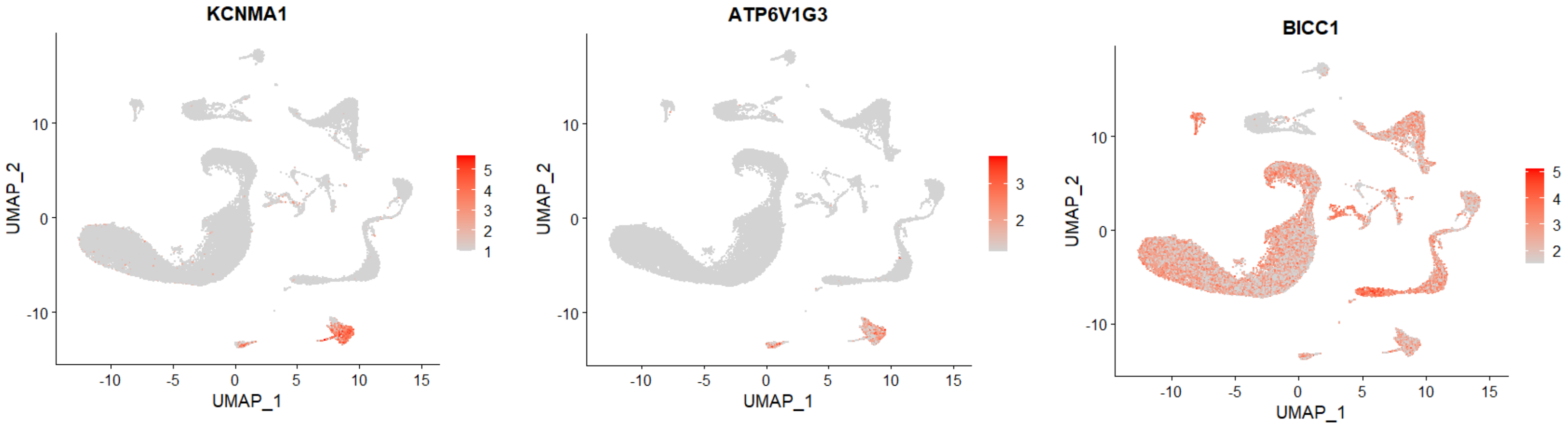

KCNMA1

ATP6V1G3

BICC1

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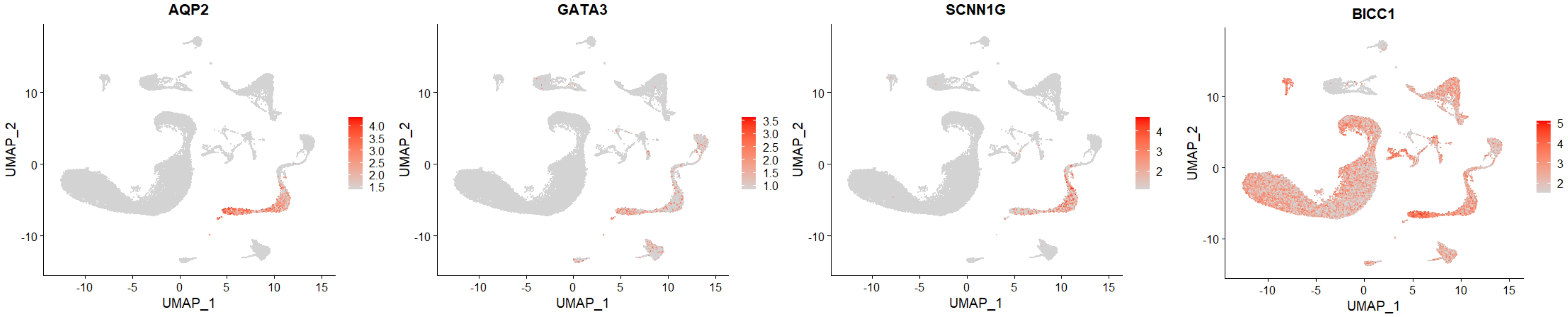

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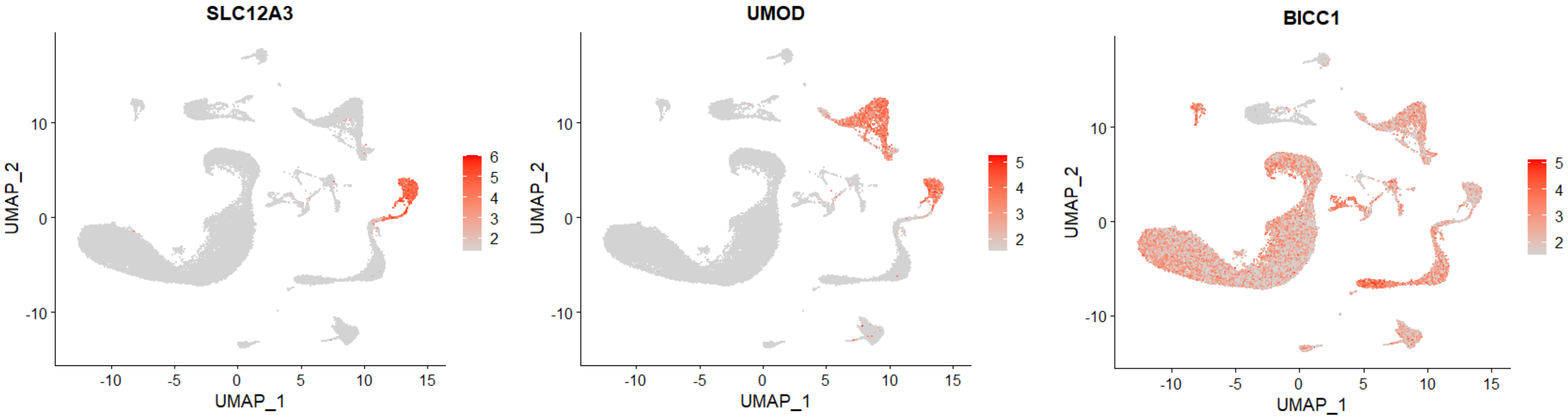

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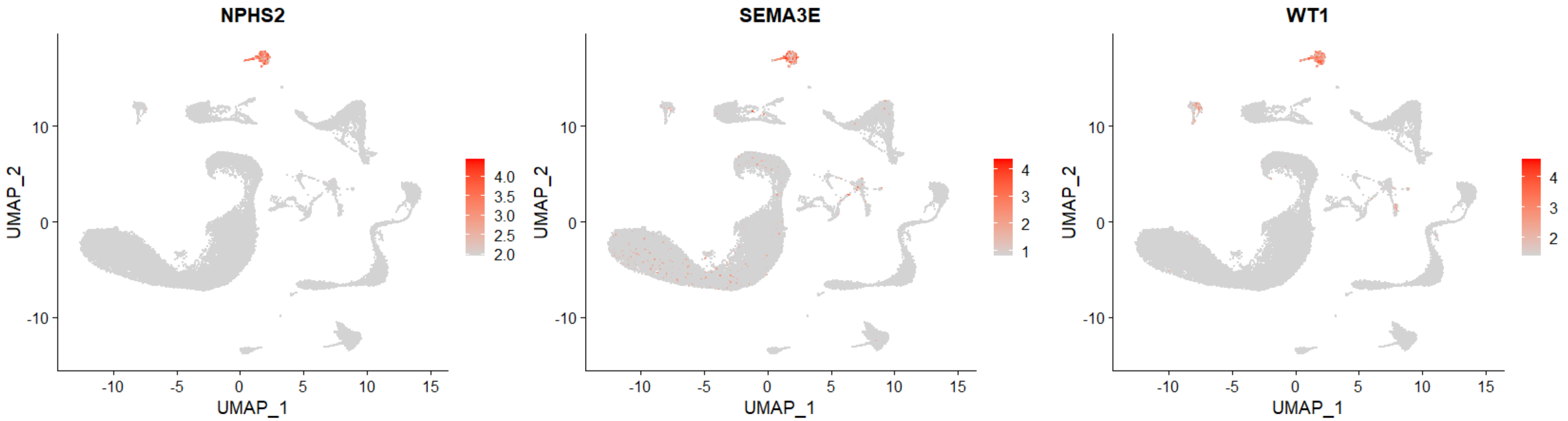

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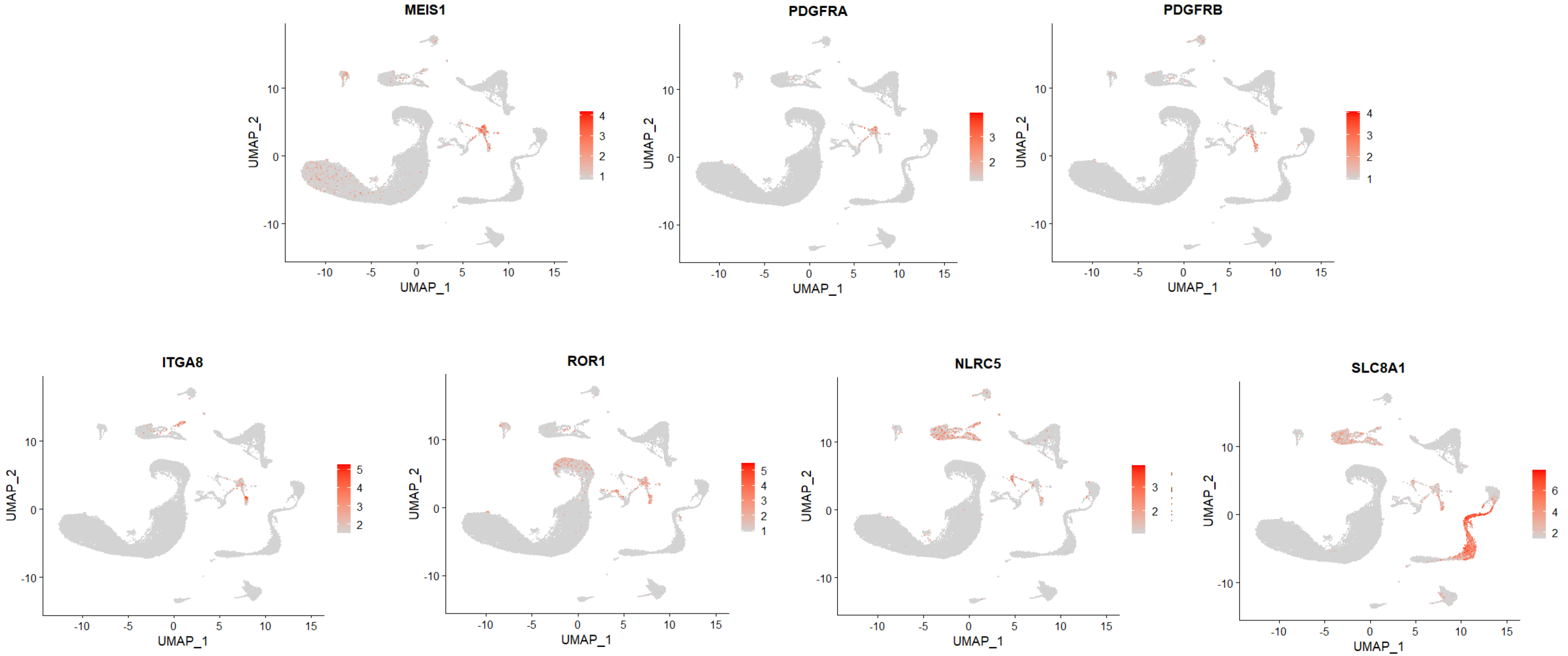

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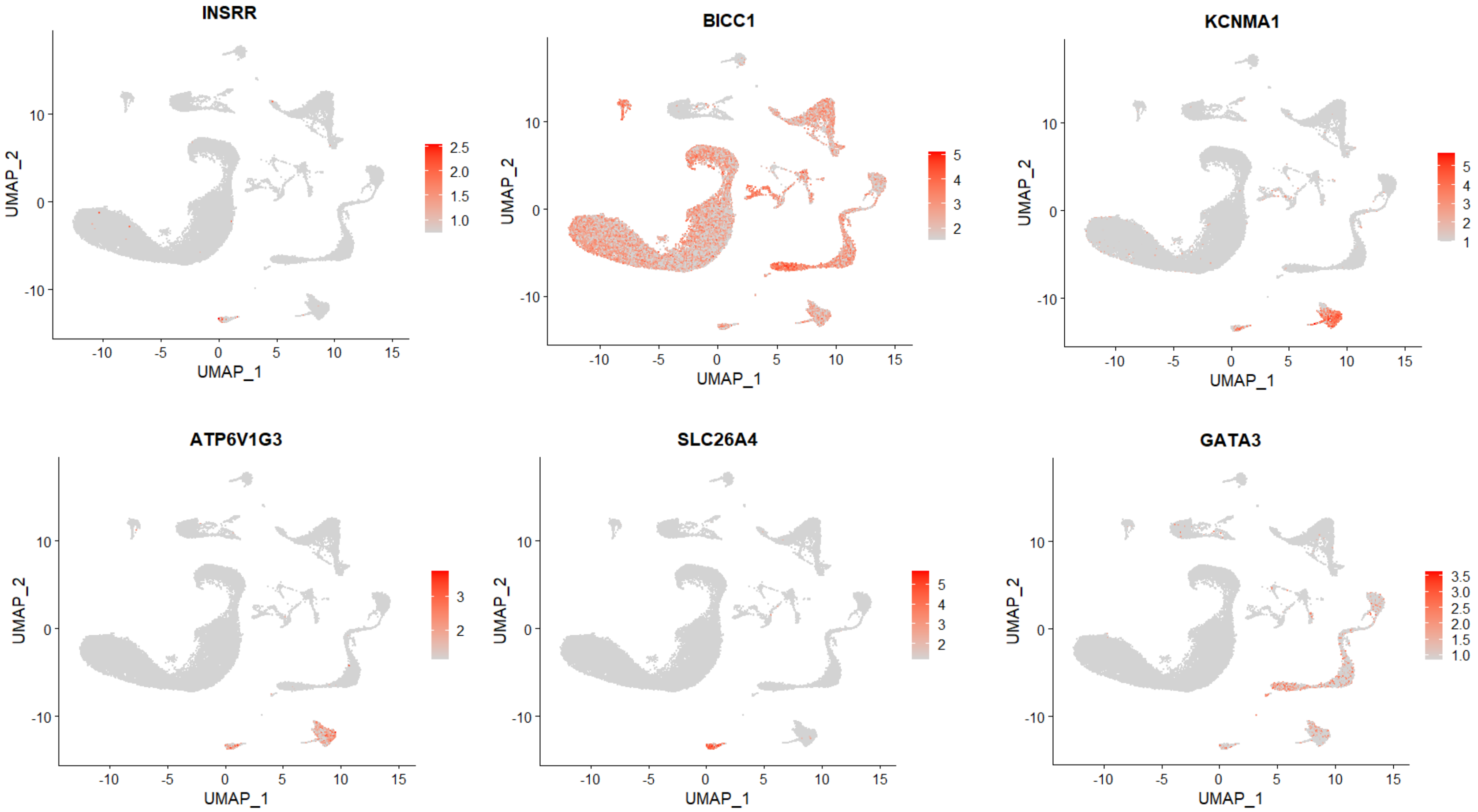

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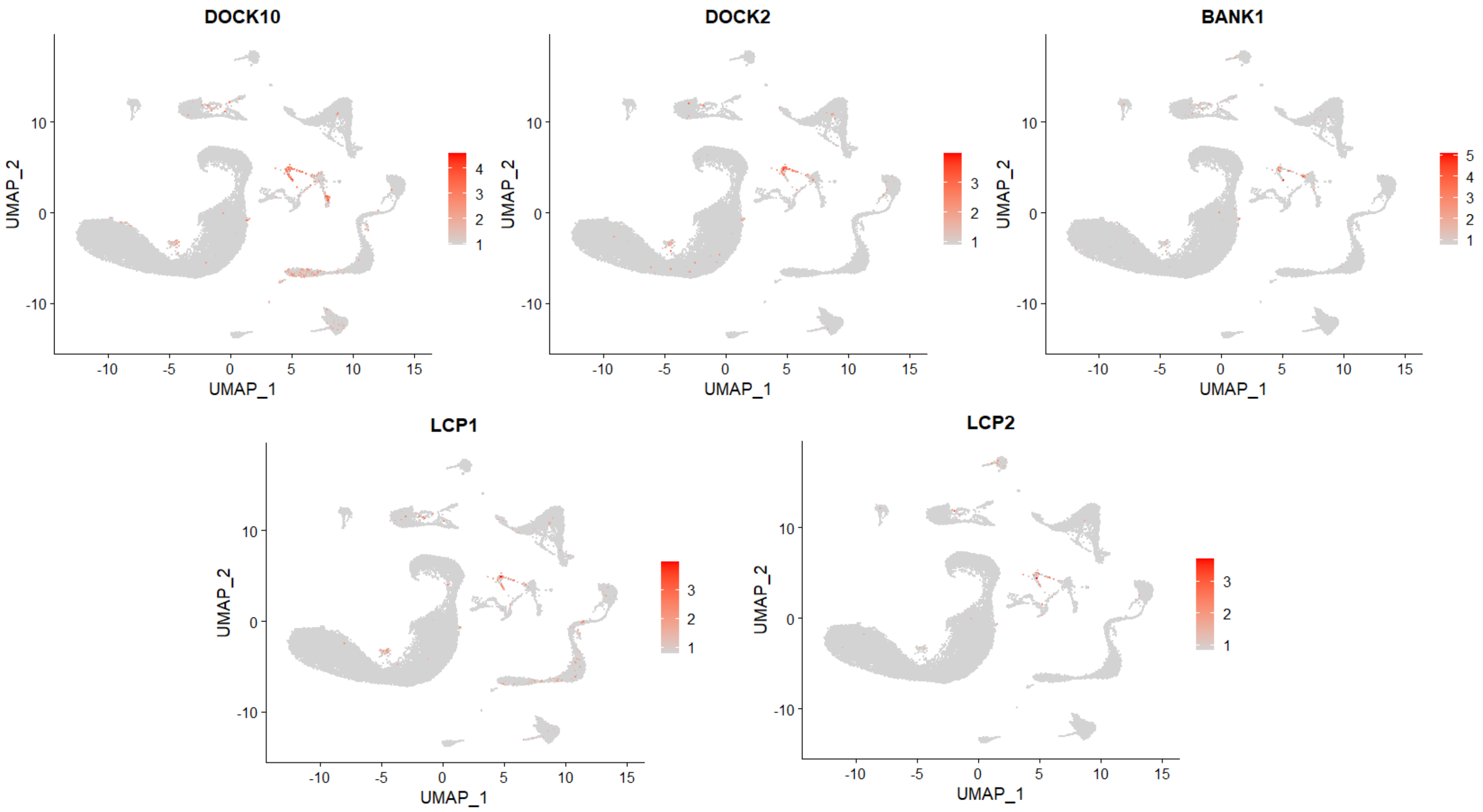

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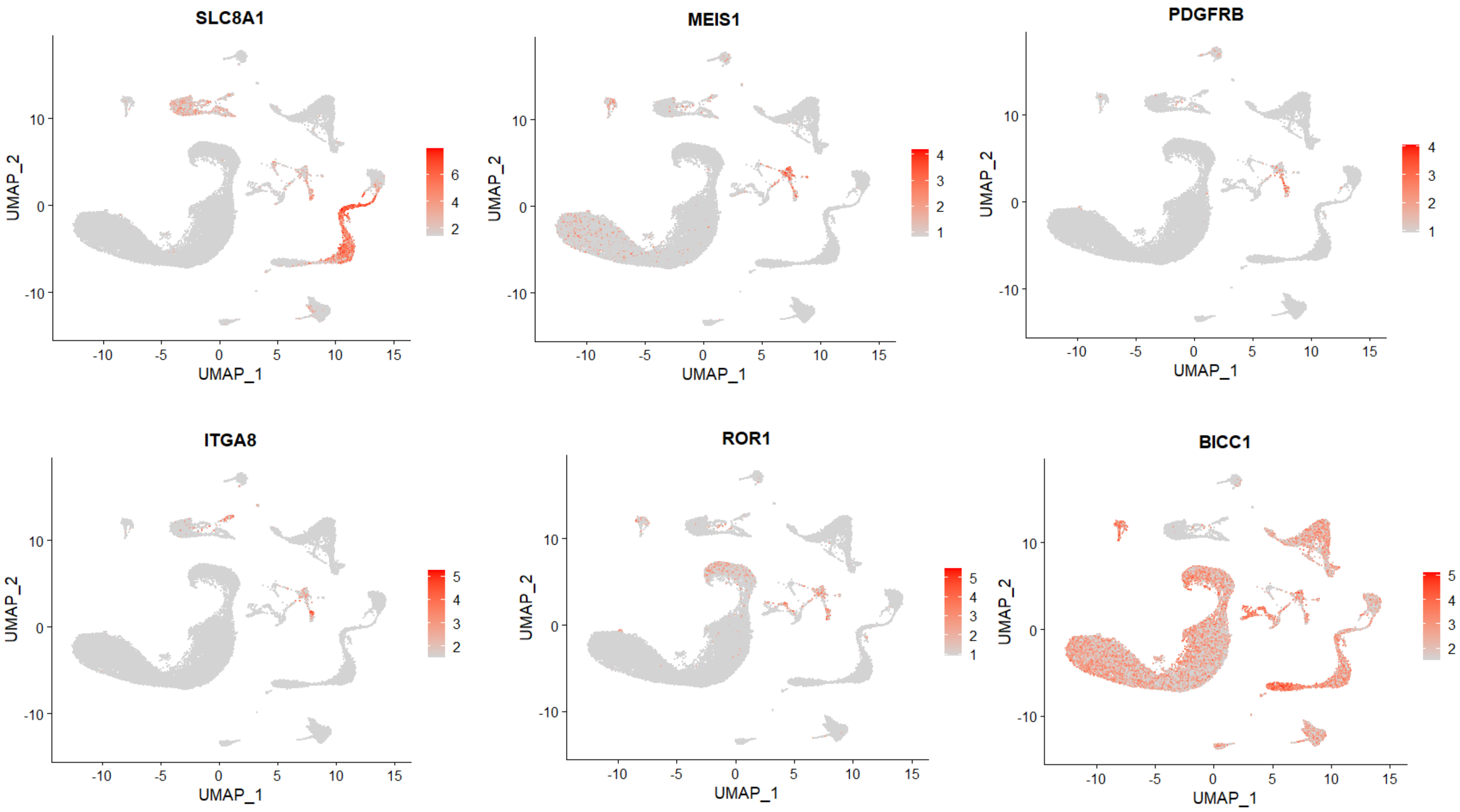

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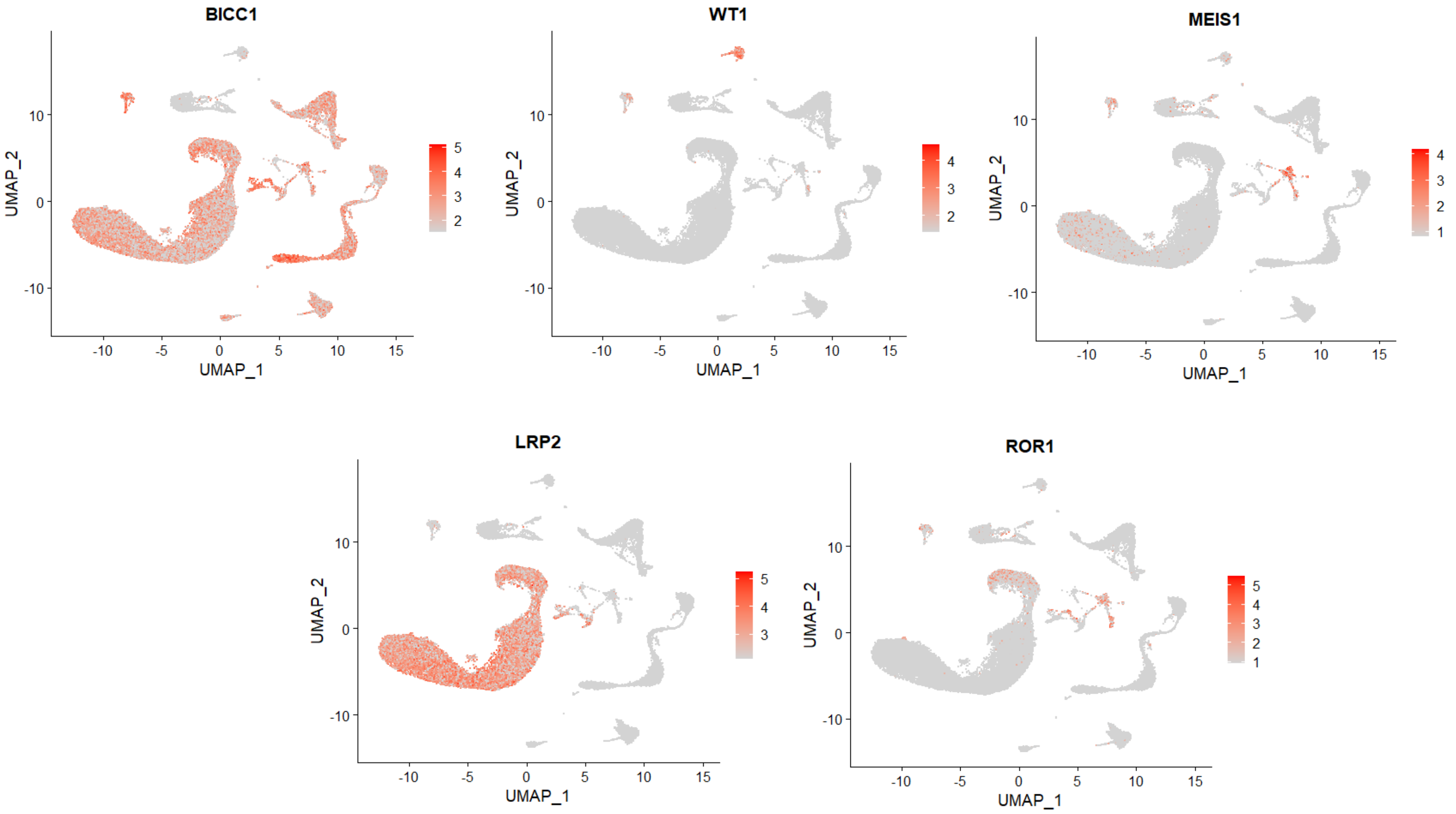

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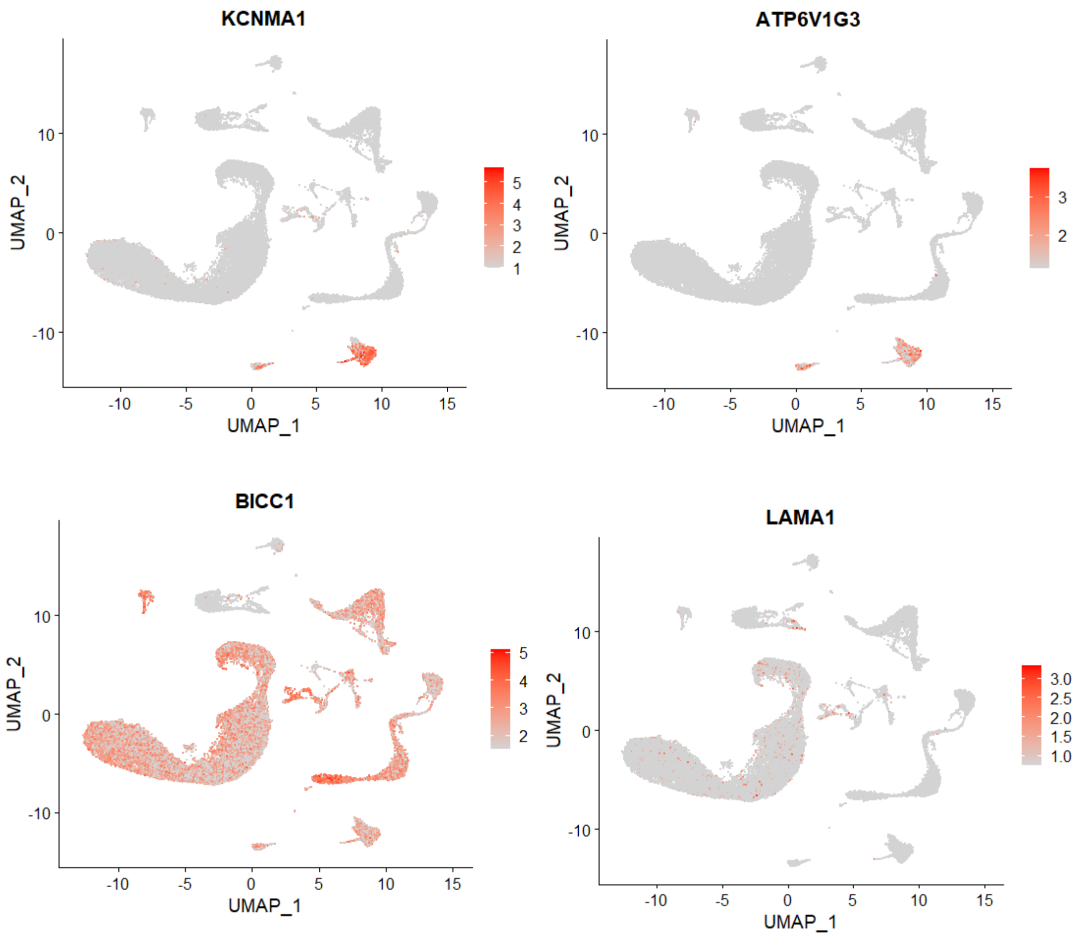

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