

Figure S1. Brightfield images of organoids established from parental tumors.

Figure S2. Histological and β -Catenin expression profiling in medulloblastoma organoids and matched tumors.

A, C. Representative hematoxylin and eosin (H&E) staining of parental tumor tissues and their matched patient-derived organoids (MB1, MB3-MB10). Scale bars: 50 μ m. B, D. Representative immunofluorescence confocal images of β -Catenin expression in the parental tumors and their matched MBOs (MB1, MB3-MB10). Scale bars: 50 μ m. E. Bar graph comparing the overall intensity of β -Catenin in patient-derived organoids versus original tumor tissues across the MB sample series (MB1, MB3-MB10). Values represent mean fluorescence intensity measurements within the entire region. Scatter plot showing the correlation of β -Catenin overall intensity between patient-derived organoids and their corresponding original tumor tissues for each MB sample. The Pearson correlation coefficient (R) and p-value (Pearson correlation coefficient test) are indicated, demonstrating a statistically significant positive correlation. F. Bar graph comparing the positive cell percentage of β -Catenin in patient-derived organoids versus original tumor tissues across the MB sample series (MB1, MB3-MB10). Scatter plot showing the correlation of β -Catenin positive cell percentage between patient-derived organoids and their corresponding original tumor tissues for each MB sample. The Pearson correlation coefficient (R) and p-value (Pearson correlation coefficient test) are indicated, demonstrating a statistically significant positive correlation.

Figure S3. Expression and correlation analysis of KI67, P53, and GAB1 in patient-derived medulloblastoma organoids and matched parental tumors.

A. Representative immunofluorescence confocal images of KI67, P53, and GAB1 expression in parental tumors and their matched MBOs (MB1, MB3-MB10). Scale bars: 50 μ m. B–D. Quantitative comparison of KI67, P53, and GAB1 expression between MBOs and parental tumors. For each marker, upper panels show bar graphs comparing the respective measurements, percentage of KI67-positive cells, percentage of P53 positive cells, and overall GAB1 intensity, in MBOs versus original tumor tissues across the MB series. Lower panels display scatter plots illustrating the correlation of these measurements between MBOs and matched parental tumors for each sample.

Pearson correlation coefficient (R) and p-value (Pearson correlation coefficient test) are indicated.

Figure S4. Expression and correlation analysis of OTX2, SOX2, and GFAP in patient-derived medulloblastoma organoids and matched parental tumors.

A. Representative immunofluorescence confocal images of OTX2, SOX2, and GFAP expression in parental tumors and their matched MBOs (MB1, MB3-MB10). Scale bars: 50 μ m. B–D. Quantitative comparison of OTX2, SOX2, and GFAP expression between MBOs and parental tumors. For each marker, upper panels show bar graphs comparing the percentage of OTX2-positive cells (B), percentage of SOX2-positive cells (C), and overall GFAP intensity (D) in MBOs versus original tumor tissues across the MB series. Lower panels display scatter plots illustrating the correlation of these measurements between MBOs and matched parental tumors for each sample. Pearson correlation coefficients (R) and corresponding p-values (Pearson correlation coefficient test) are indicated for each marker.

Figure S5. Tumor invasion of MBOs into brain organoids in a co-culture model.

A. Brightfield and corresponding fluorescence images showing the progressive infiltration of medulloblastoma organoids (MBOs, red) into brain organoids (blue) over time. The experiment was repeated in MBO4, MBO7, and MBO9 with consistent results. Scale bars: 2000 μ m. B. Faceted line plots depict the invasion ratio (0-1.0) of four distinct MBO lines (MBO2, MBO4, MBO7, MBO9) into brain organoids across co-culture period. The X-axis represents time (in days), and the Y-axis represents the quantitative invasion ratio. C. Brightfield and corresponding fluorescence images illustrate the co-culturing results of red-labeled and blue-labeled cerebral organoids. Scale bars: 2000 μ m.

Figure S6. Expression Profiles of Key Markers and associated Survival Prognosis in MB

A. Expression dot plot representing the key markers identified for all cells. B. Proportions of major cell types, annotated by key marker genes. C. GO analysis of PDS core marker genes, showing the top 20 enriched pathways. D. KEGG analysis of PDS core marker genes, displaying the top 20 ranked pathways. E. Two-panel Kaplan-Meier survival curves stratified by patient age (Old vs. Young; left) and gender (F vs. M; right).

P values were determined by the Mantel-Cox test. F. Kaplan-Meier survival curve for Group 4 medulloblastoma patients stratified by high versus low risk score. P values were determined by the Mantel-Cox test.

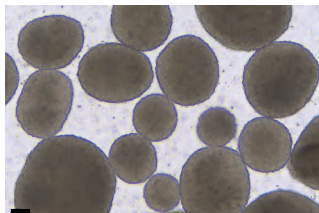
Figure S7. Apoptosis and Cytokine Secretion in MBO-TILs Co-Culture System

A. Representative immunofluorescence (green, apoptotic dye) and matched bright-field images of MBOs co-cultured with autologous TILs or cultured alone. Images derived from independent replicate experiments MBO4, MBO7 and MBO9. Scale bar: 2,000 μm . B. Quantification of apoptotic signal intensity, in the same replicate sets (MBO4, MBO7, MBO9). C-D. Quantification of (C) IFN- γ and (D) TNF- α concentrations in culture supernatants of the same replicate sets (MBO4, MBO7, MBO9).

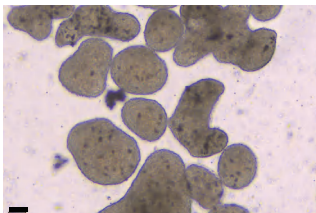
Table S1. Sample information of medulloblastoma organoids.

Table S2. Immunohistochemical Comparison Between 10 MBOs and Corresponding Primary Tumors.

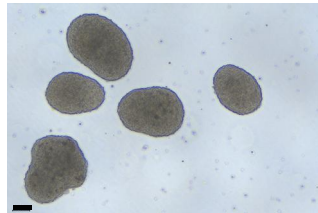
Table S3. scRNA-seq Cluster Annotation and Marker Genes.



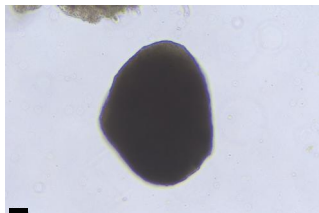
MBO1



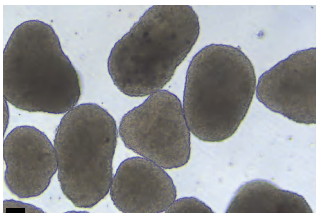
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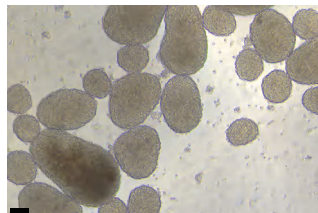
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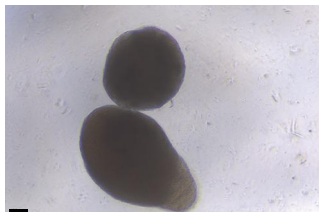
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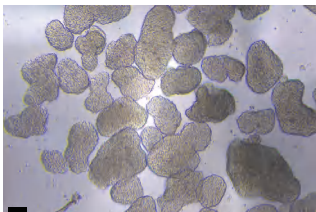
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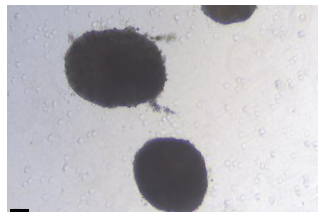
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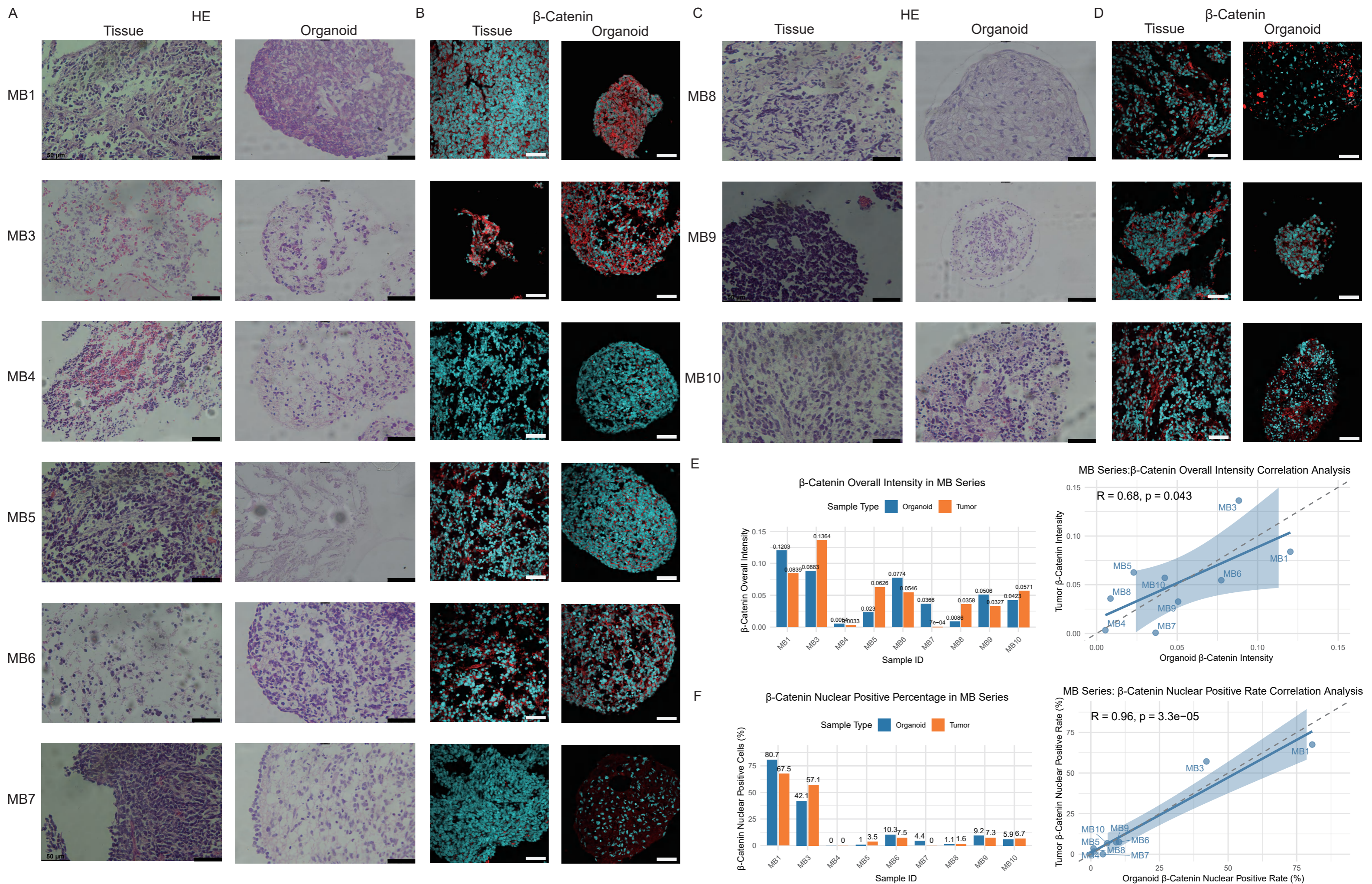
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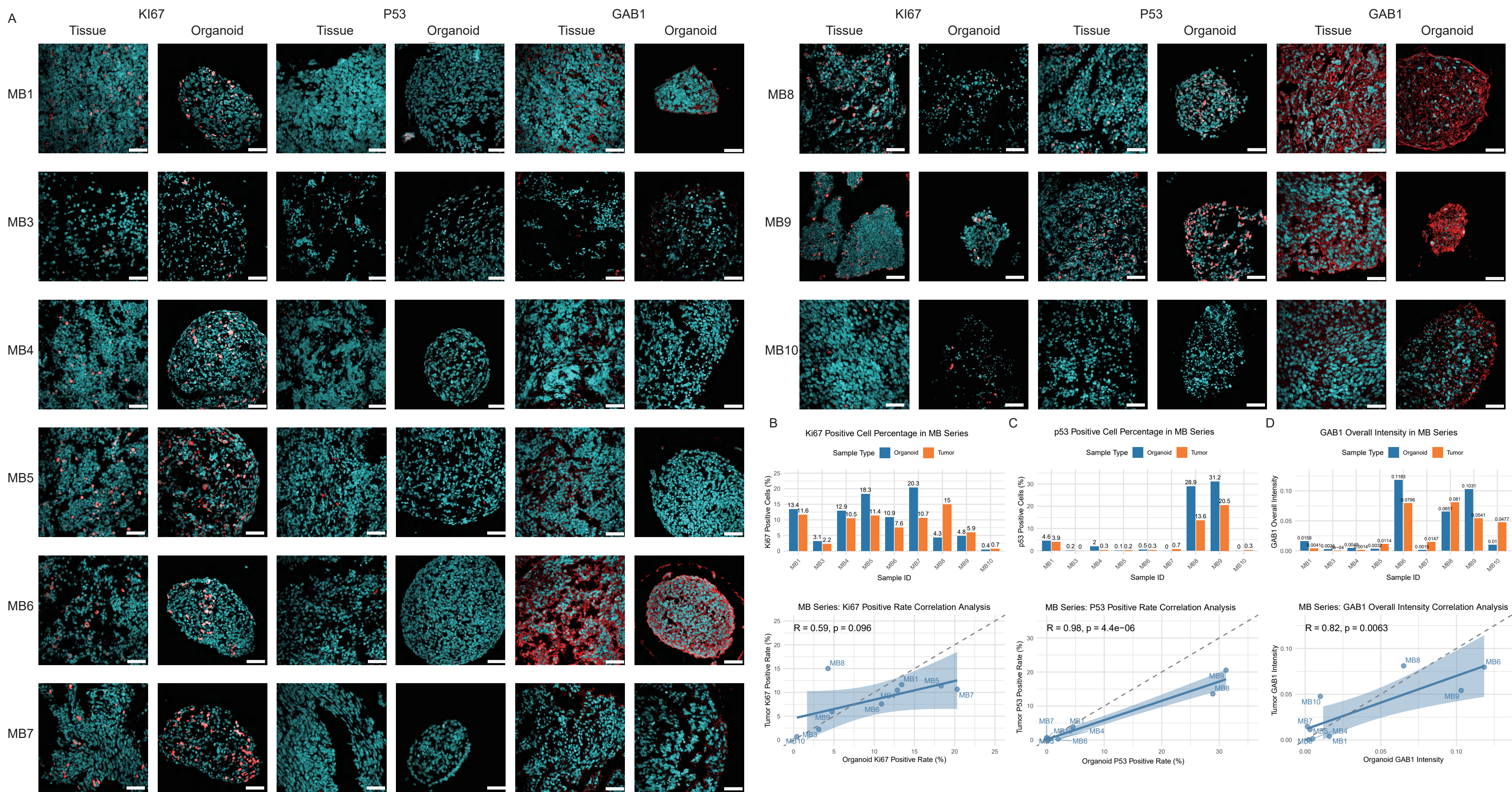


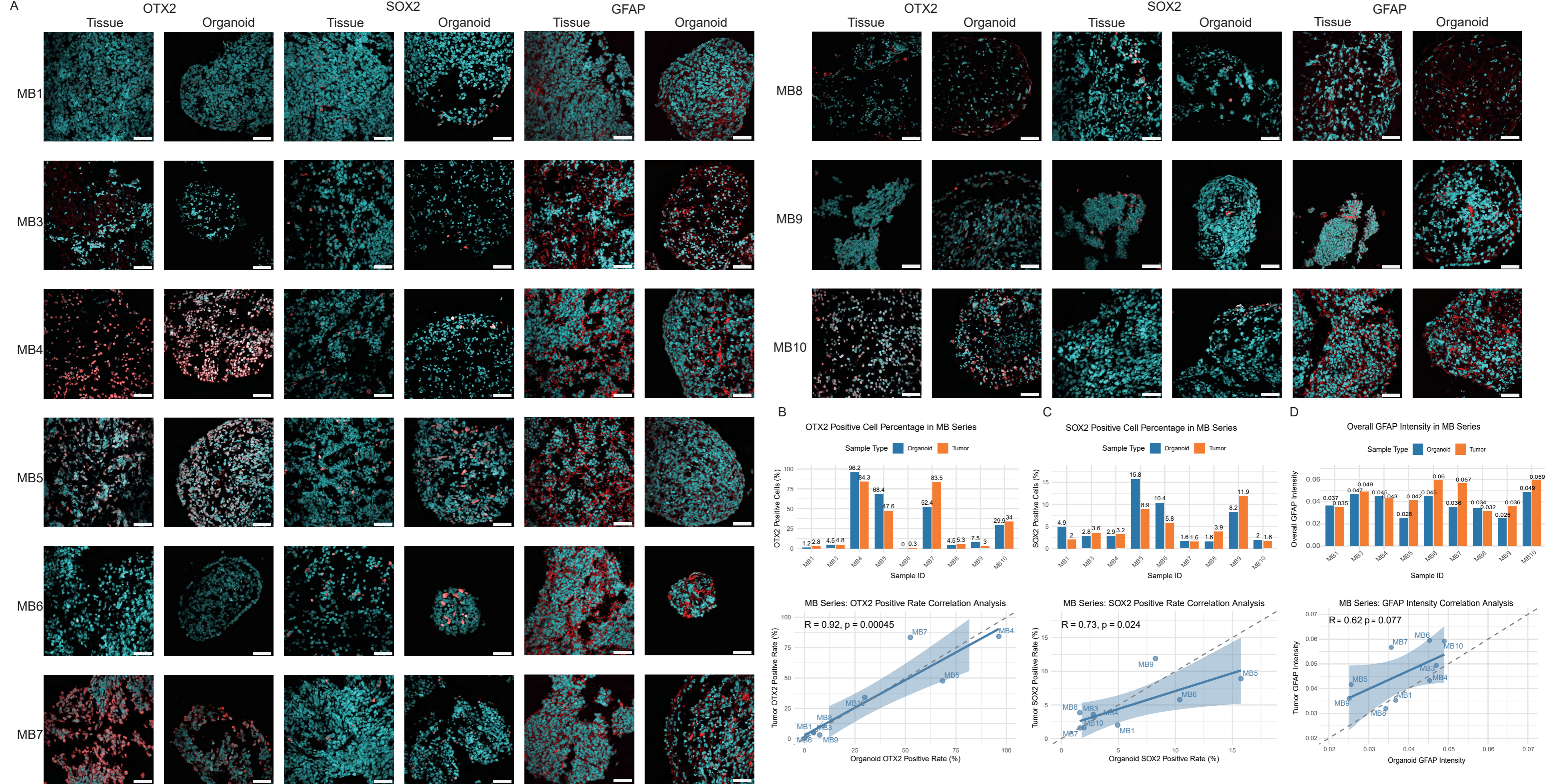
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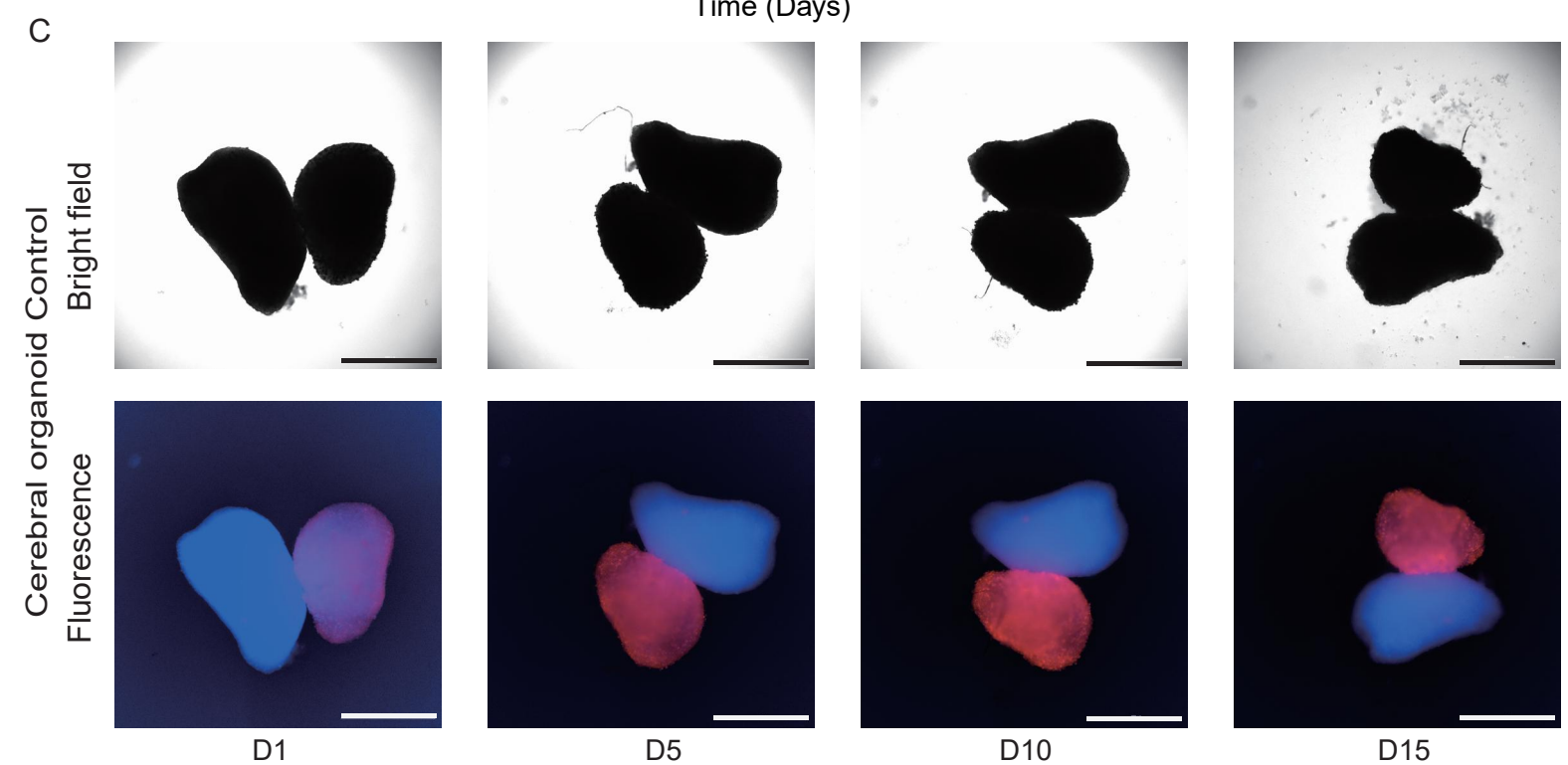
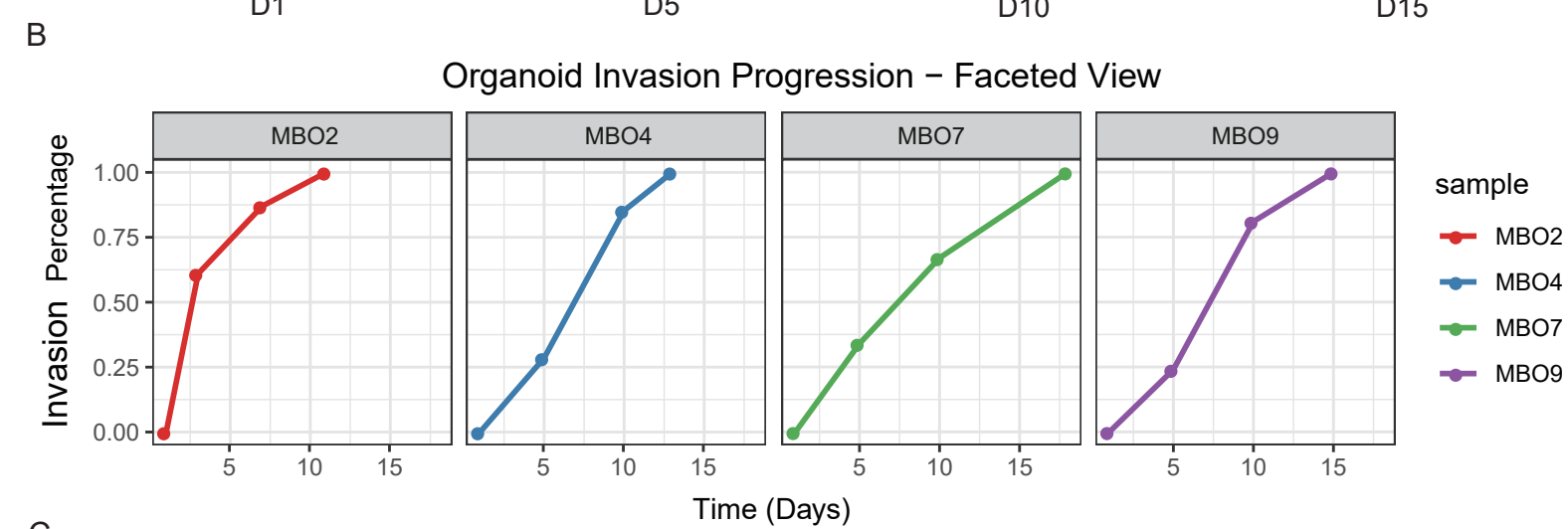
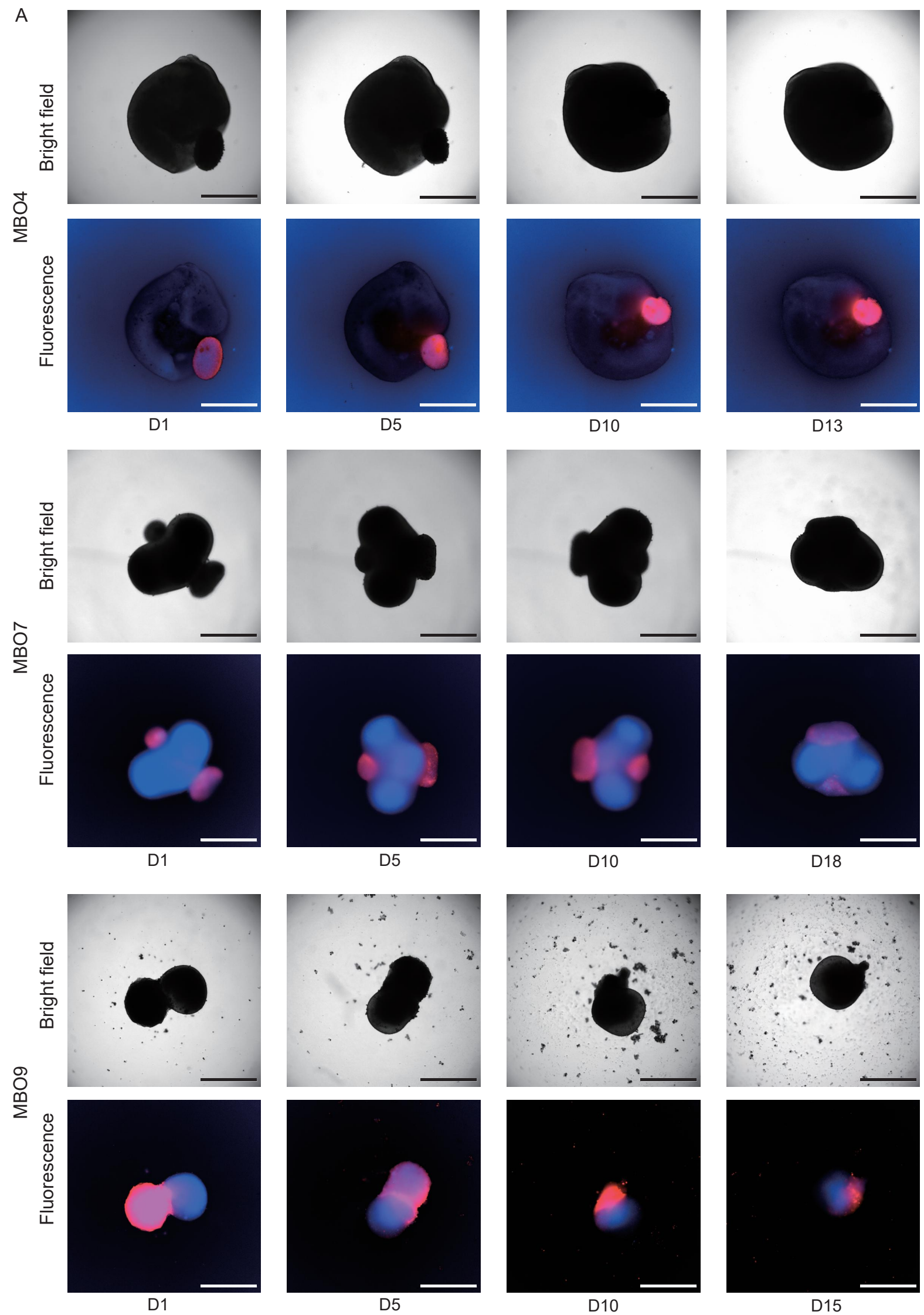


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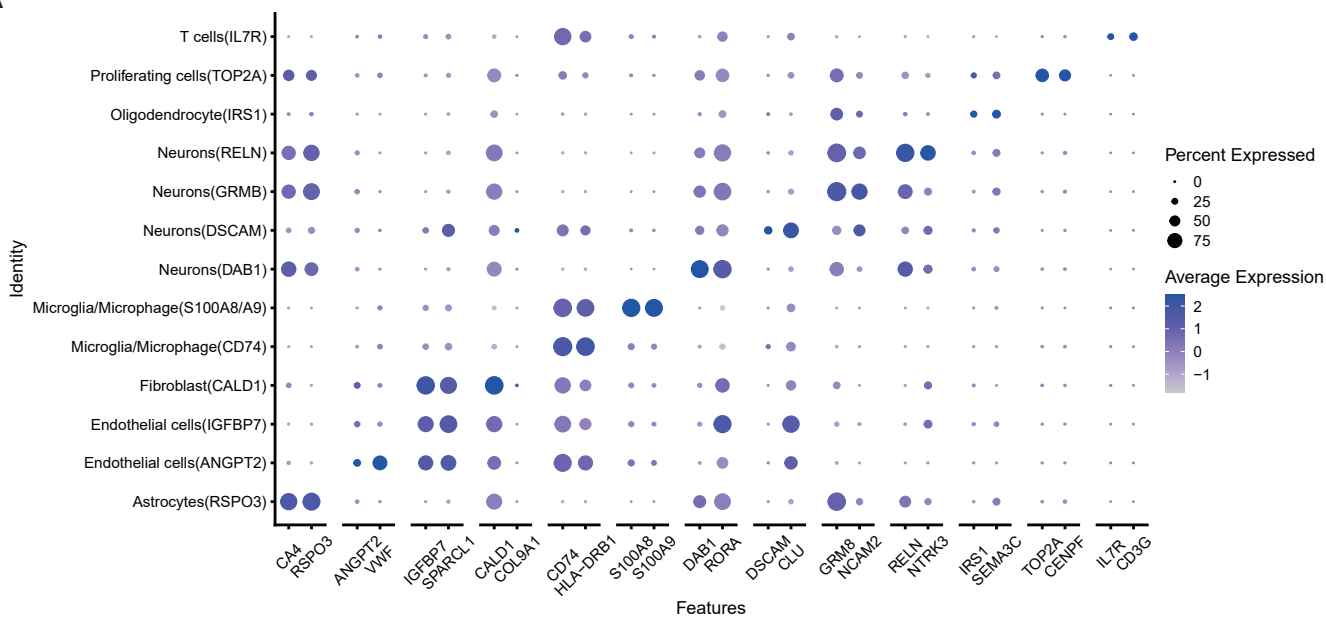




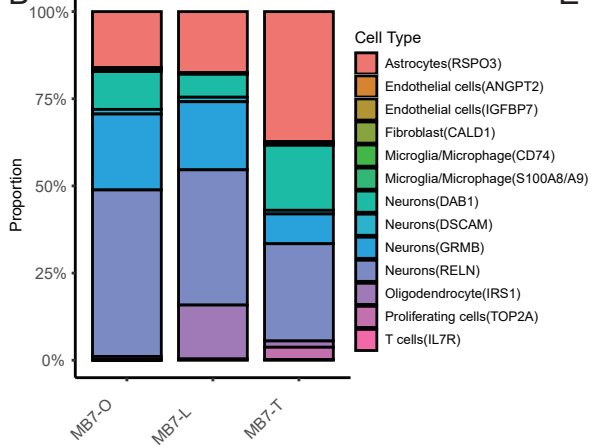




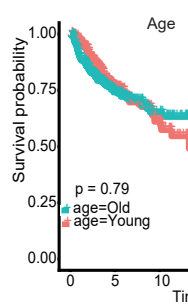
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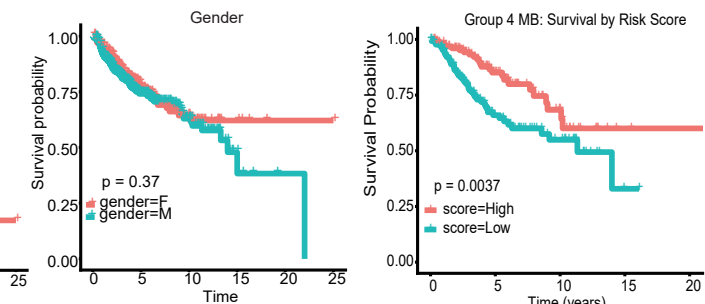
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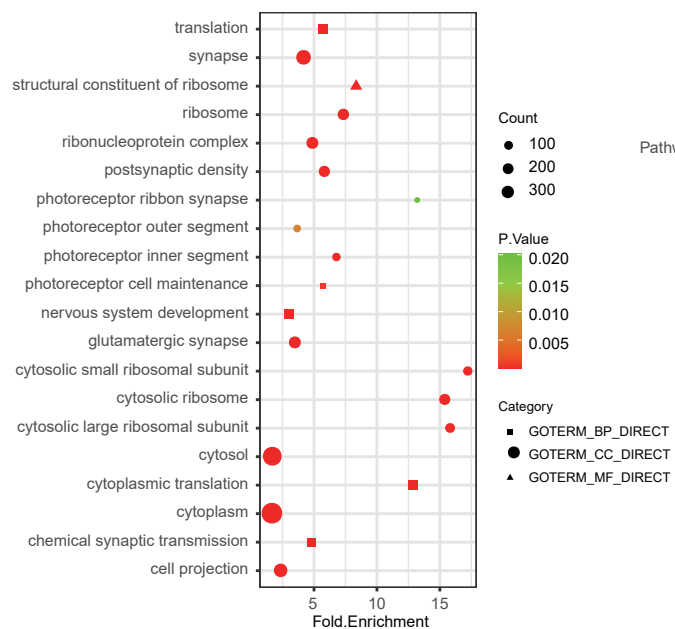
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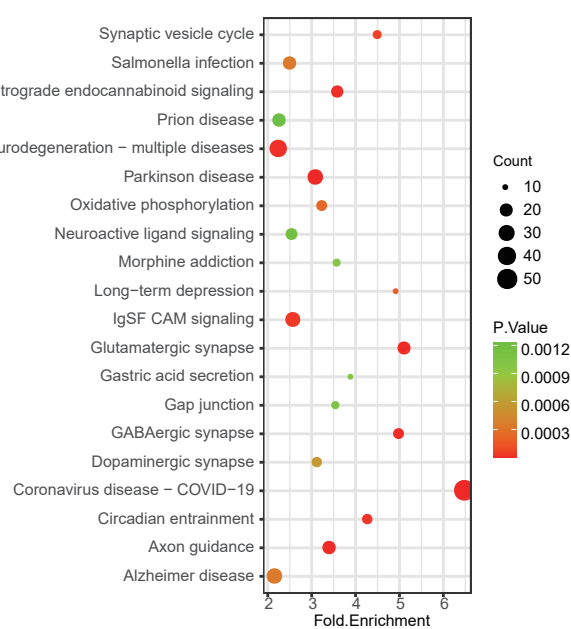
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D



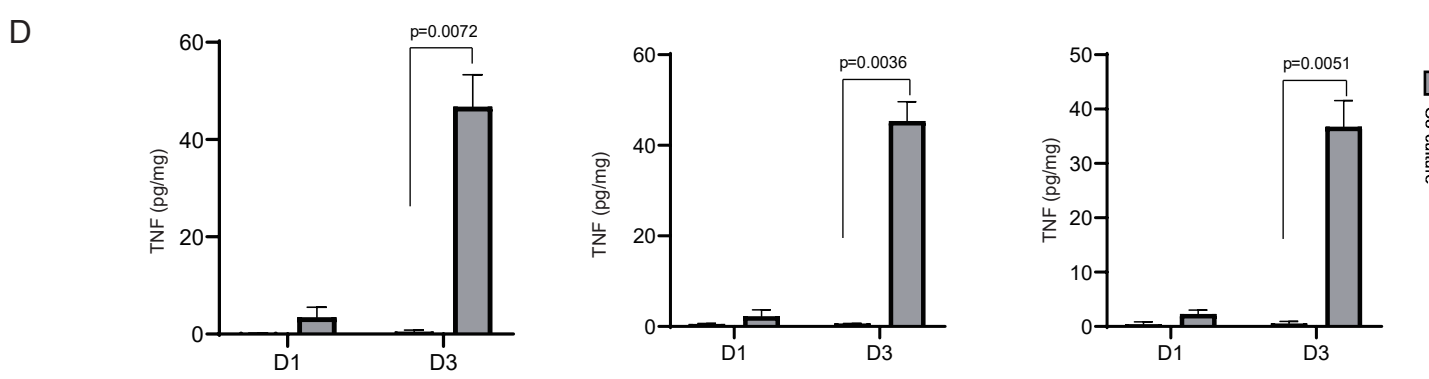
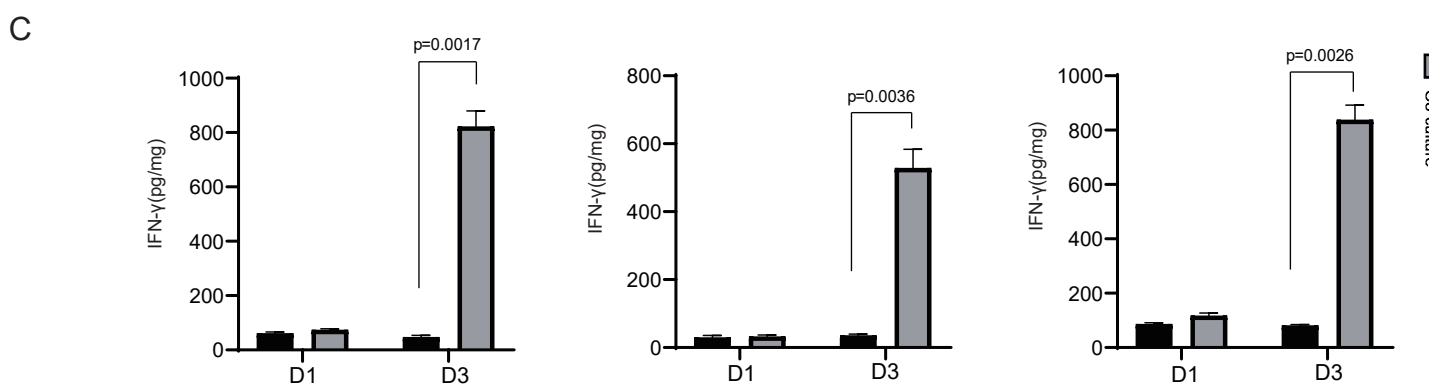
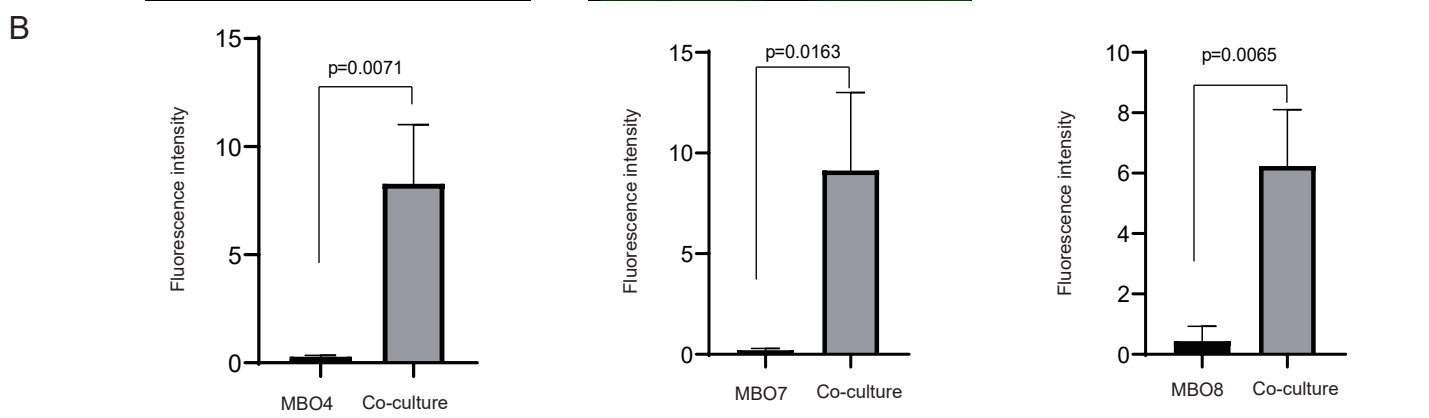
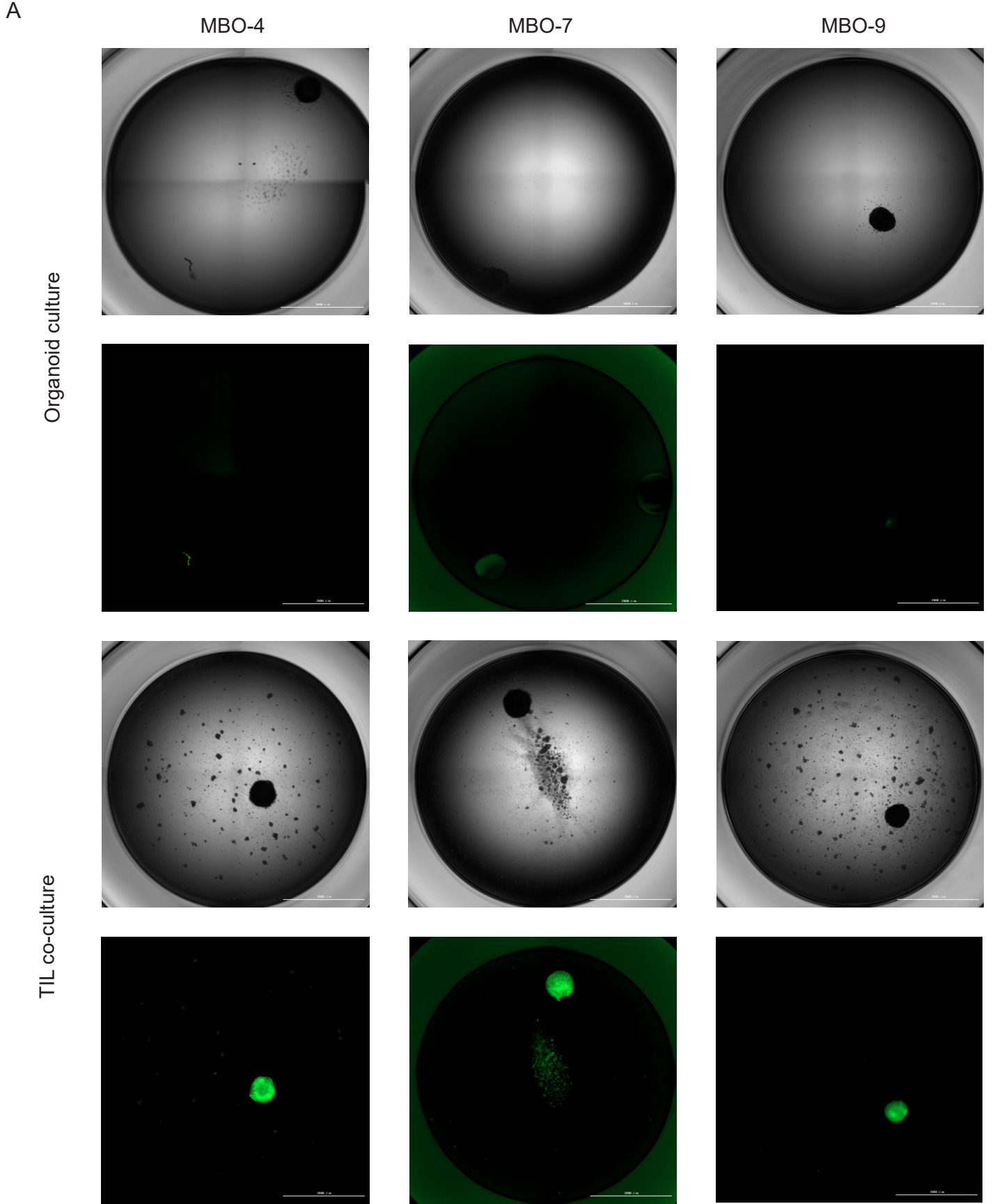


Table S1. Sample information of medulloblastoma

| ID | Date | Gender | Age | Subtype |
|-----------|-------------|---------------|------------|---------------------|
| MB1 | 2023/7/18 | female | 13 | WNT/CTNNB1 mutation |
| MB2 | 2023/8/16 | male | 10 | SHH/TP53 wildtype |
| MB3 | 2023/8/30 | male | 7 | WNT/CTNNB1 mutation |
| MB4 | 2023/12/6 | male | 7 | Group4 |
| MB5 | 2023/12/19 | male | 6 | Group4 |
| MB6 | 2023/1/9 | female | 5 | SHH/TP53 mutation |
| MB7 | 2023/2/5 | female | 10 | Group4 |
| MB8 | 2024/4/3 | female | 9 | SHH/TP53 wildtype |
| MB9 | 2024/4/8 | male | 1 | SHH/TP53 wildtype |
| MB10 | 2024/9/27 | male | 10 | Group4 |

Table S2. Immunohistochemical Comparison Between 10 MBOs and Corresponding Primary Tumors

| Sample | Subtype | Nuclear_Positive_Percentage (%) | | | | | Overall_Intensity | | | |
|--------|-----------|---------------------------------|-------|-------|-------|-------------------|-------------------|--------|--------|--|
| | | KI67 | OTX2 | P53 | SOX2 | β - Catenin | β -Catenin | GAB1 | GFAP | |
| MB1-O | WNT | 13.41 | 1.2 | 4.55 | 4.93 | 80.65 | 0.1203 | 0.0159 | 0.0368 | |
| MB1-T | WNT | 11.63 | 2.84 | 3.87 | 2.02 | 67.53 | 0.0839 | 0.0041 | 0.0353 | |
| MB2-T | SHH/TP53- | 22.05 | 13.73 | 38.47 | 17.55 | 4.93 | 0.0381 | 0.0933 | 0.0307 | |
| MB2-O | SHH/TP53- | 22.5 | 26.47 | 45.05 | 37.37 | 5.49 | 0.0393 | 0.1072 | 0.0225 | |
| MB2-R | SHH/TP53- | 28.61 | 21.99 | 45.91 | 22.78 | 3.28 | 0.0208 | 0.0832 | 0.0376 | |
| MB2-IC | SHH/TP53- | 30.77 | 28.68 | 41.64 | 53.76 | 5.04 | 0.0329 | 0.0691 | 0.026 | |
| MB2-SQ | SHH/TP53- | 35.89 | 16.13 | 38.72 | 69.52 | 8.51 | 0.0435 | 0.0794 | 0.021 | |
| MB3-O | WNT | 1 | 4.49 | 0.16 | 2.8 | 42.11 | 0.0883 | 0.0024 | 0.047 | |
| MB3-T | WNT | 1.9 | 4.85 | 0 | 3.59 | 57.14 | 0.1364 | 3e-04 | 0.0494 | |
| MB4-O | G4 | 12.88 | 96.23 | 1.96 | 7.9 | 0 | 0.0054 | 0.0049 | 0.0453 | |
| MB4-T | G4 | 10.46 | 84.31 | 0.3 | 3.17 | 0 | 0.0033 | 0.0014 | 0.0433 | |
| MB5-O | G4 | 18.32 | 68.38 | 0.1 | 15.75 | 0.97 | 0.023 | 0.0032 | 0.0256 | |
| MB5-T | G4 | 11.37 | 47.65 | 0.2 | 8.9 | 3.55 | 0.0626 | 0.0114 | 0.0417 | |
| MB6-O | SHH/TP53+ | 10.91 | 0 | 0.46 | 10.38 | 10.27 | 0.0774 | 0.1183 | 0.0453 | |
| MB6-T | SHH/TP53+ | 7.57 | 0.34 | 0.28 | 5.4 | 7.54 | 0.0546 | 0.0796 | 0.0595 | |
| MB7-O | G4 | 20.31 | 52.43 | 0 | 1.63 | 4.37 | 0.0366 | 0.0015 | 0.0357 | |
| MB7-T | G4 | 10.66 | 83.51 | 0.72 | 1.58 | 0 | 7e-04 | 0.0147 | 0.0567 | |
| MB8-O | SHH/TP53- | 2.26 | 4.46 | 28.89 | 1.6 | 1.07 | 0.0086 | 0.0651 | 0.0343 | |
| MB8-T | SHH/TP53- | 15.02 | 5.33 | 13.58 | 3.85 | 1.65 | 0.0358 | 0.081 | 0.032 | |
| MB9-O | SHH/TP53- | 4.76 | 7.52 | 31.18 | 8.25 | 9.23 | 0.0506 | 0.1031 | 0.0251 | |
| MB9-T | SHH/TP53- | 5.94 | 3 | 20.53 | 11.9 | 7.35 | 0.0327 | 0.0541 | 0.0361 | |
| MB10-O | G4 | 0.39 | 29.86 | 0 | 1.97 | 5.94 | 0.0423 | 0.01 | 0.049 | |
| MB10-T | G4 | 0.7 | 33.97 | 0.33 | 1.63 | 6.74 | 0.0571 | 0.0477 | 0.0592 | |