Expanded View Figures

Figure EV1. Generating OTCs.

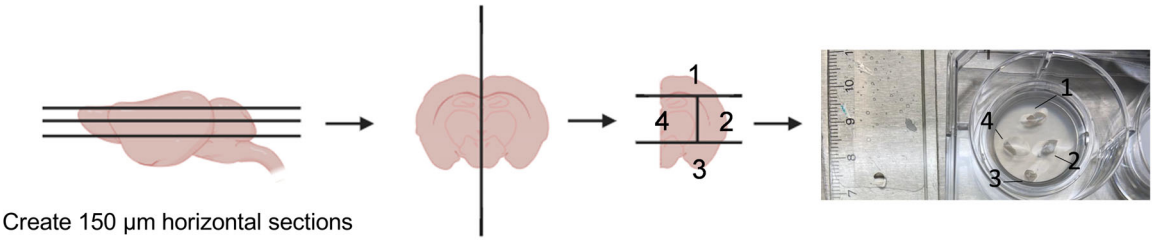
A Schematic setup of generating 150-μm-thick mouse brain slices (OTC) and cutting them along the hemisphere into four intersections and placing them on a Millicell culture insert. Scheme created with BioRender.com.

B 3D rendering of the CAD design of the thermoforming positive molds for the LSFM holders. An array of eight positive molds is shown. The molds have a square 2 × 2 mm cross-section and a height of 5 mm. On the right panel, the real 3D-printed molds array is displayed.

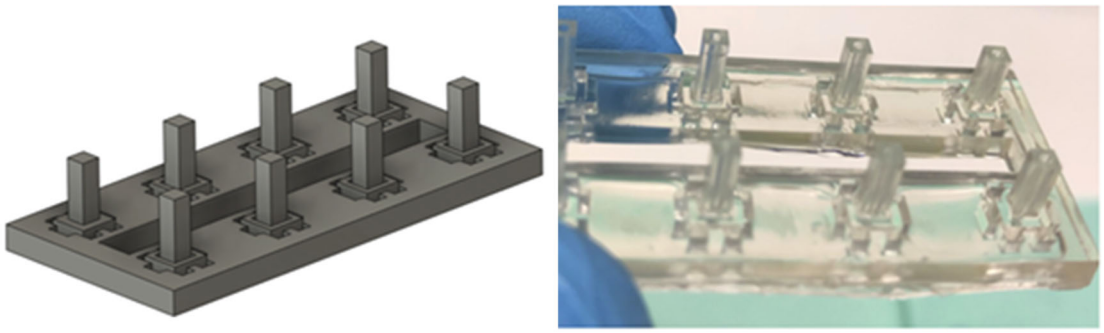
C One thermoformed FEP-foil LSFM holder (3 × 25 mm) glued to a sample holder, followed by an OTC is placed inside (right panel).

D Table depicting the mean and SEM of the tumor cell dispersion of one biological replicate (n = 2 tumors per region) of spheres placed onto different brain regions depicted in (A) using GraphPad Prism 9.

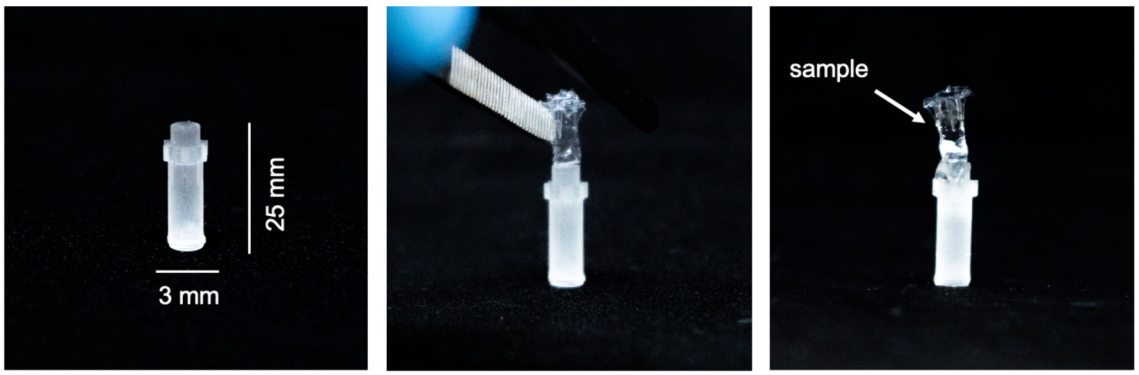
A



B



C



D

Region	Mean [µm]	± SEM [µm]
#1	420.5	0.93
	397.9	14.84
#2	261.0	10.45
	697.9	33.88
#3	305.3	13.73
	438.0	22.68
#4	249.3	22.02
	186.6	15.53

Figure EV1.

Figure EV2. CD9 depletion leads to reduced migration in GS-5 GSCs.

A The expression of CD9 in GS-5 GSCs after lentiviral transduction of a shCD9 plasmid was analyzed via Western blot and quantified in relation to GAPDH expression ($n = 1$).

B False-color images of the sphere migration assay of GS-5 and GS-5 shCD9 GSCs. Time points are displayed for 0 h (false color, cyan) and 48 h (false color, magenta). Scale bars: 100 μm.

C Quantification of the sphere migration assay of GS-5 and GS-5 shCD9 GSCs showed difference in migration upon CD9 depletion. The experiment was performed independently at least three times with eight replicates ($n \geq 24$ biological replicates).

Data information: In (C), data are presented as mean ± SEM. ns, not significant; $^{**}P < 0.01$; $^{****}P < 0.0001$ against GS-5 (unpaired *T*-test with Mann–Whitney test, GraphPad Prism 9).

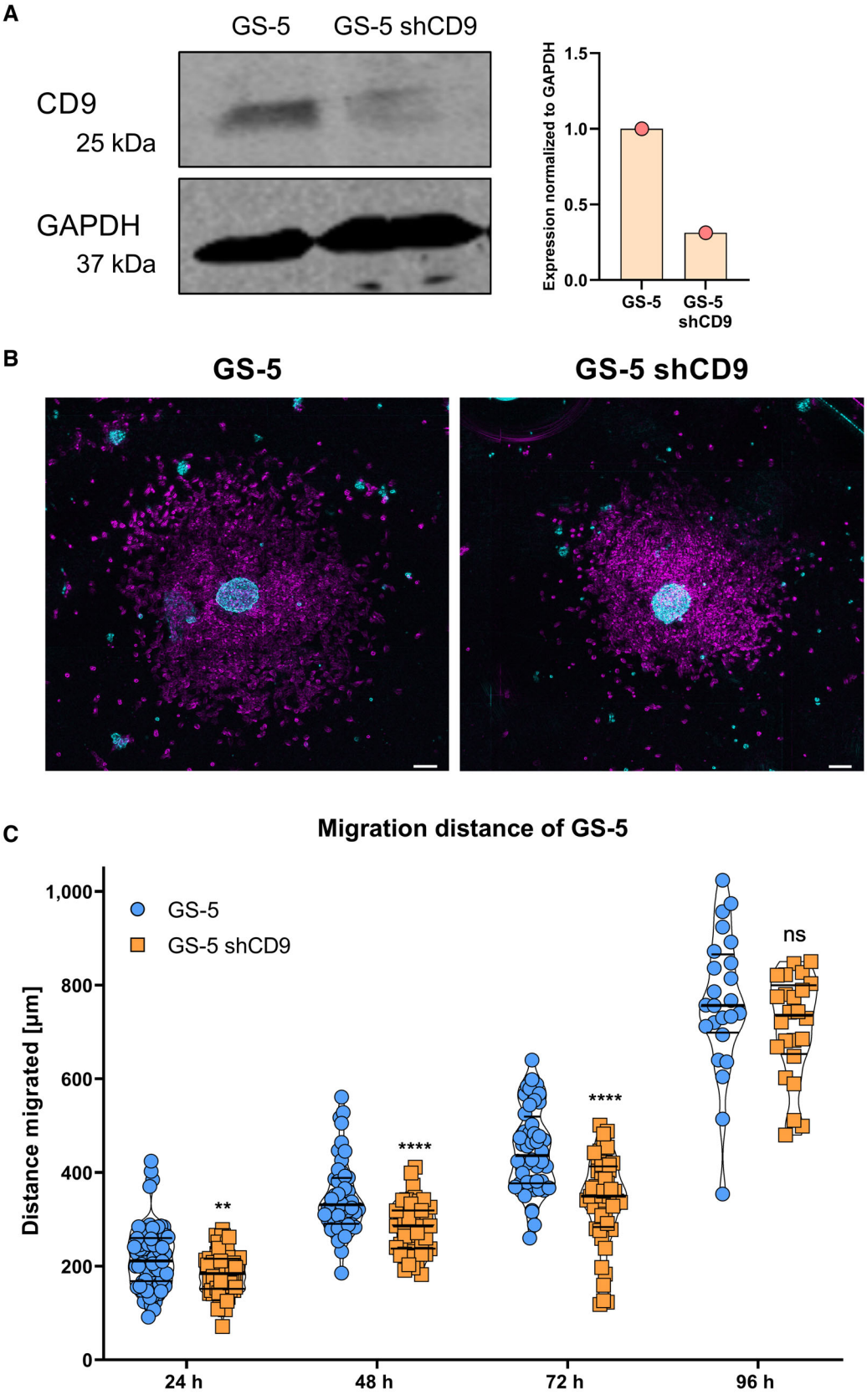


Figure EV2.

Figure EV3. Genetically STAT3 depletion and pharmacological JAK2/STAT3 inhibition affect tumor growth in *ex vivo* organotypic tissue culture in MZ-54 cells.

A Montages of 3D reconstruction of light-sheet images displayed in 90° angles of the same tumor at the latest time point (d5) of MZ-54 wt, MZ-54 STAT3-KO or MZ-54 wt treated with 10 μM WP1066. Arrows display cells which reached their overall maximal migration distance. Scale bars 200 μm.

B Point plot depicting individual fold-changes tumor sizes at the latest time point (d5) generated from epifluorescence microscopy of MZ-54 wt ($n = 20$ tumors), MZ-54 STAT3-KO ($n = 10$ tumors), and MZ-54 wt treated with 10 μM WP1066 ($n = 16$ tumors), being biological replicates for each condition.

C Violin plot depicting single tumor cell dispersion at the latest time point (d5) generated from light-sheet images of MZ- 54. wt $n = 3$ tumors ($n = 103{,}314$ single cells), MZ-54 STAT3-KO $n = 4$ tumors ($n = 2040$ single cells), and MZ-54 treated with 10 μM WP1066 $n = 3$ tumors ($n = 932$ single cells), each point represents one tumor cell.

Data information: In (B, C), data are presented as mean ± SEM. $^{**}P < 0.01$; $^{****}P < 0.0001$ against MZ-54 wt (unpaired *T*-test with Mann–Whitney test, GraphPad Prism 9).

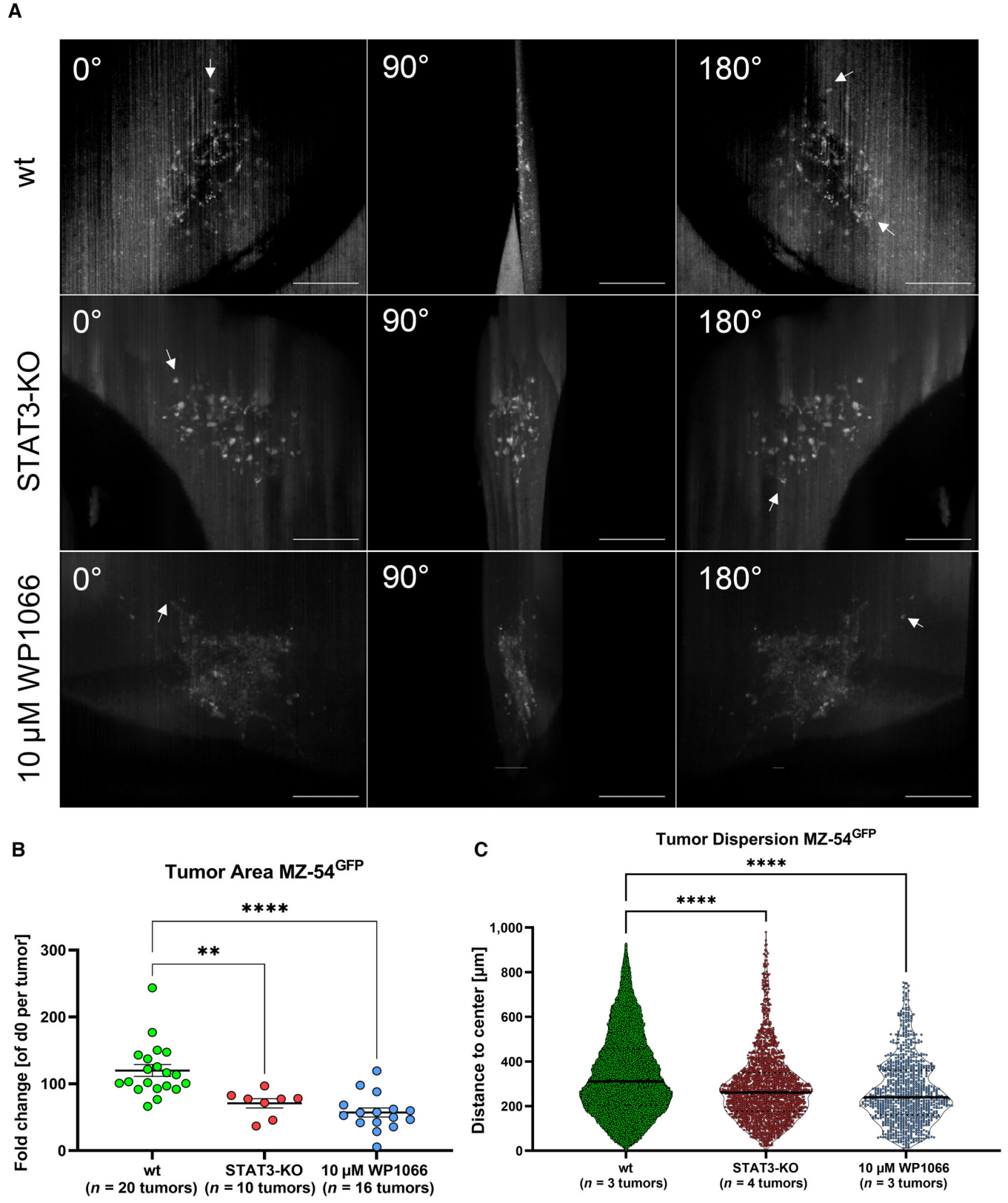


Figure EV3.

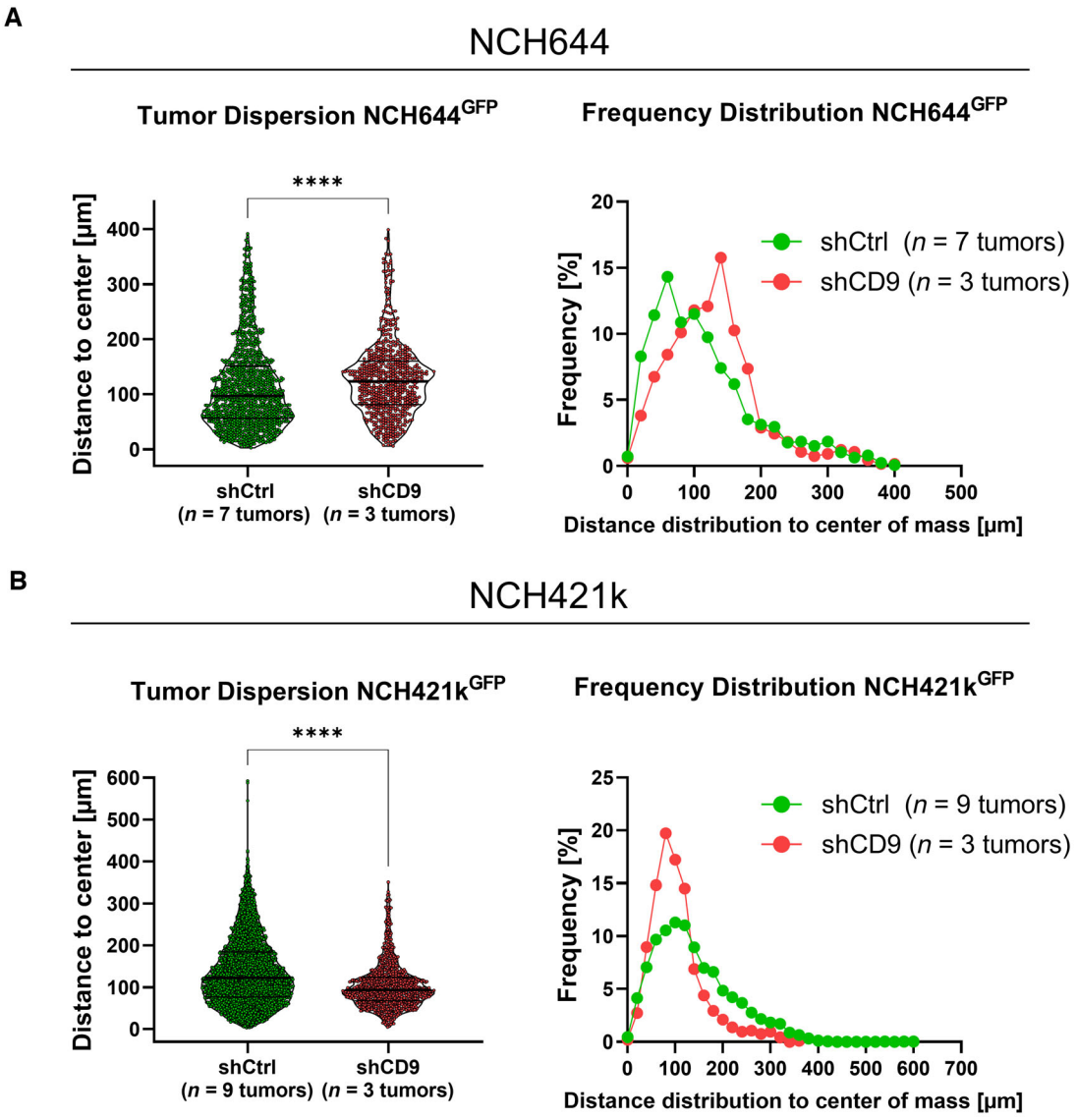


Figure EV4. Quantification of confocal generated pictures observing CD9 depletion performing *ex vivo* organotypic tissue culture using GSC line NCH644 and NCH421k shCtrl and shCD9.

A Violin plot depicting single tumor cell dispersion, each point represents one tumor cell, for NCH644 shCtrl (n = 7 tumors and n = 1,247 single cells) and shCD9 (n = 3 tumors and n = 671 single cells). Frequency distribution showing the relative amount of migration distance in 25 µm bins of NCH644 tumors.

B Violin plot depicting single tumor cell dispersion, each point represents one tumor cell for NCH421k shCtrl (n = 9 tumors and n = 3,345 single cells) and shCD9 (n = 3 tumors and n = 958 single cells). Frequency distribution showing the relative amount of migration distance in 25 µm bins of NCH421k tumors.

Data information: In (A, B), data are presented as mean ± SEM. ****P < 0.0001 against shCtrl cells (unpaired T-test with Mann–Whitney test, GraphPad Prism 9).

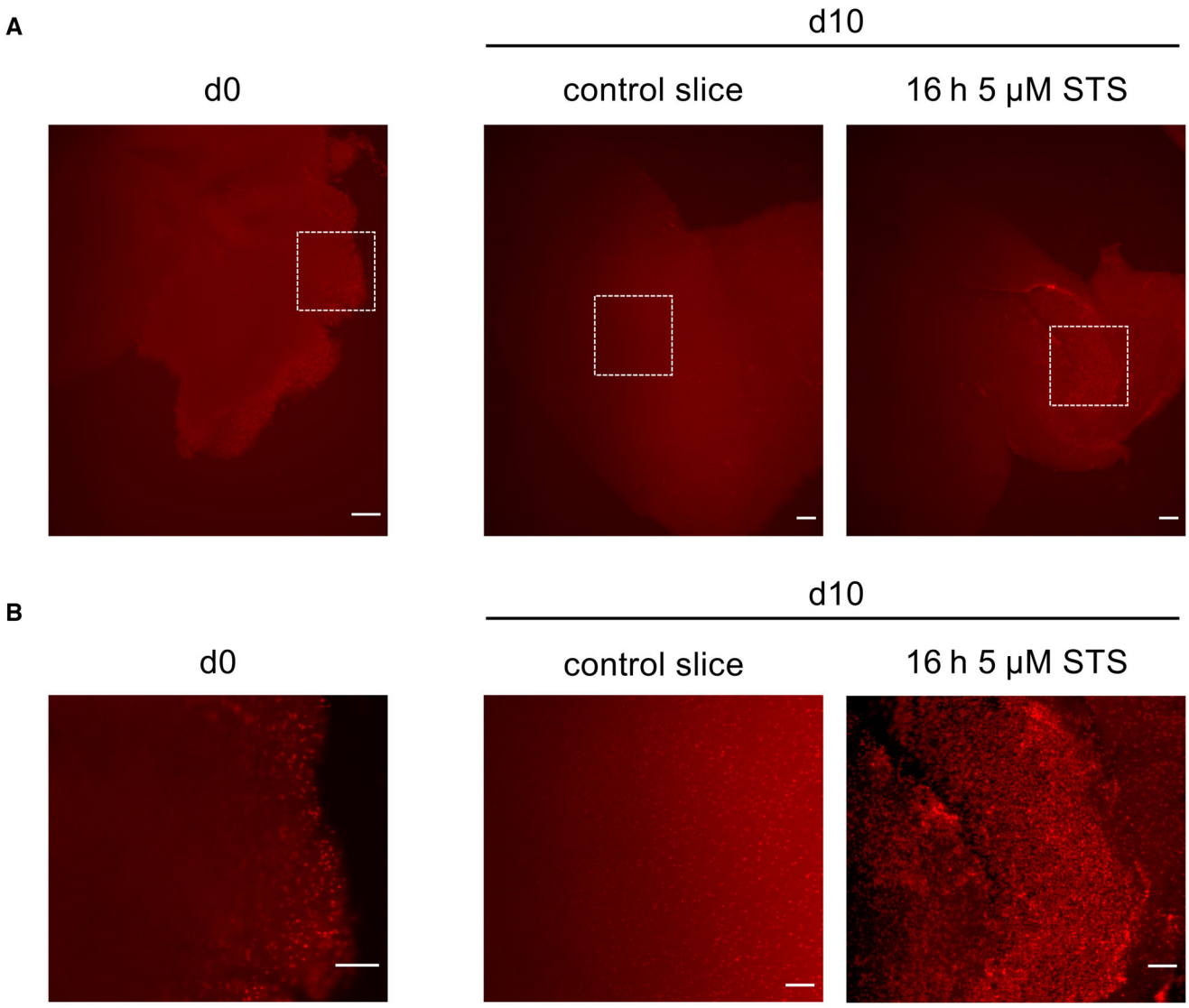


Figure EV5. Integrity of mouse brain slices.

A PI staining of mouse brain slice pictured after d0 and maximum incubation time d10. Slices were incubated on day 9 for 16 h with 5 µM staurosporine (STS) as indicator for cell death. Scale bars 200 µm.

B Close-up of pictures depicted in white boxes in (A) for better visualization. Scale bars 100 µm.