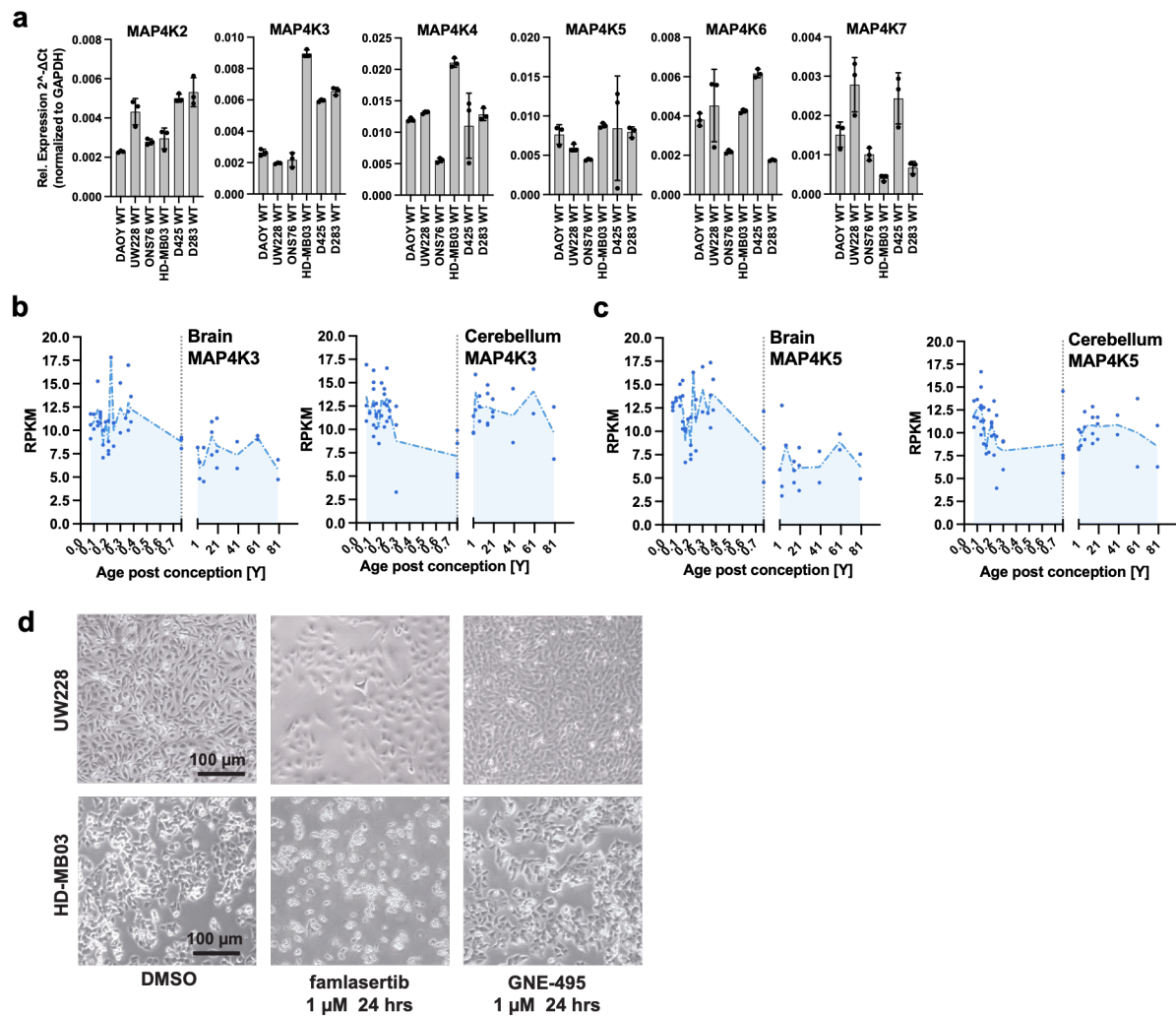


Supplementary Figures:

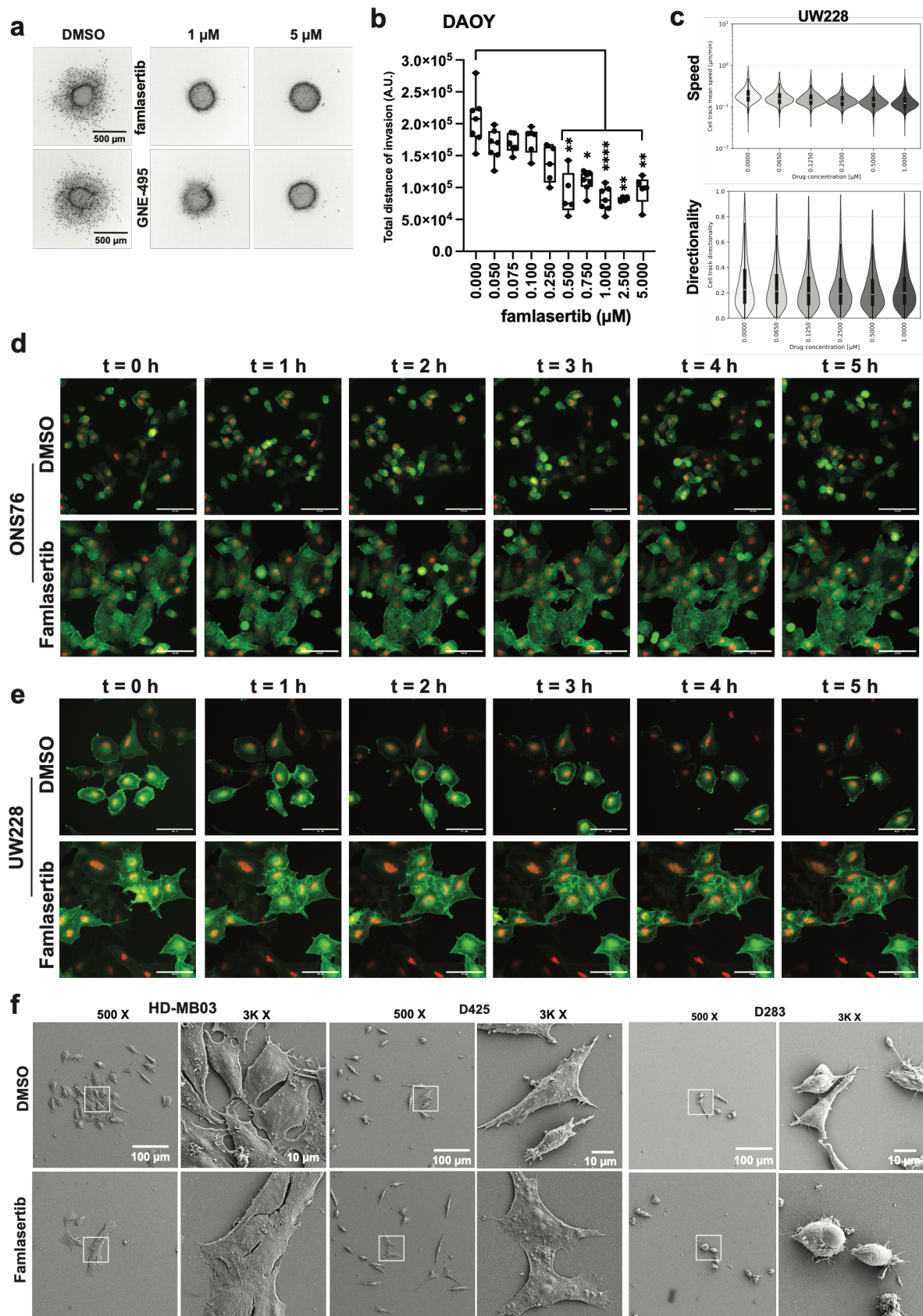
Figure S1



Supplementary Figure S1

a) Comparative analysis of relative MAP4K mRNA expression levels across three SHH (DAOY, UW228 and ONS-76) and three Group 3 (HD-MB03, D425 and D283) MB cell lines, determined by GAPDH-normalized qRT-PCR. **b, c)** MAP4K3 (b) and MAP4K5 (c) expression across the human lifespan in brain (left) and cerebellum (right), assessed by bulk RNA-seq. The x-axis indicates age in years; the vertical dashed line marks birth. The y-axis shows expression values as reads per kilobase per million mapped reads (RPKM). **d)** Representative bright-field images of UW228 and HD-MB03 cells after 24 h exposure to the indicated treatments.

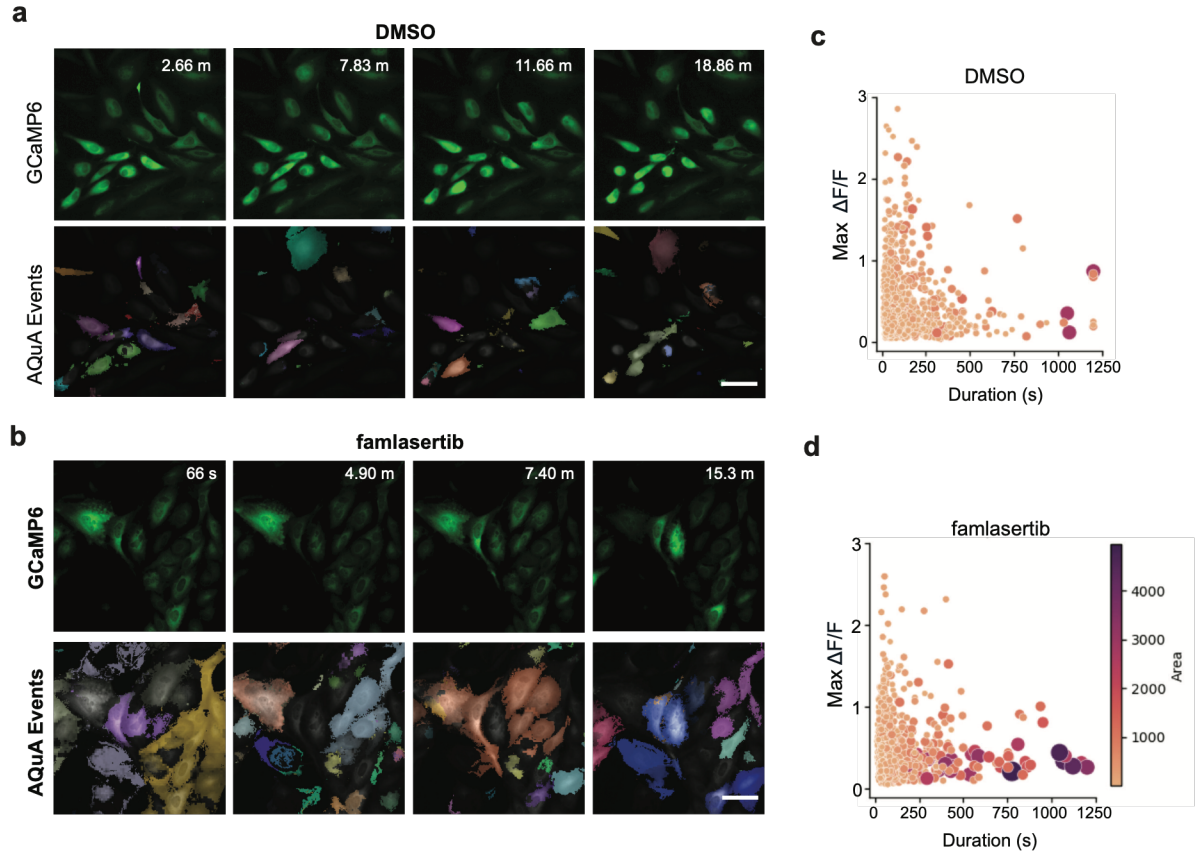
Figure S2



Supplementary Figure S2

a) Representative inverted grayscale images of Hoechst-stained nuclei in collagen I-embedded spheroids at the experimental endpoint shown in (Figure 2a). **b)** Spheroid invasion assay (SIA) comparing the effects of increasing concentrations of famlasertib on bFGF-induced invasiveness in DAOY cells. DMSO served as a solvent control. Box plots show total invasion distance, calculated as the sum of invasion distances measured for each individual cell. Each dot represents one independent spheroid. **c)** Violin plots of mean track speed (f) and directionality (g) of UW228 cells treated with increasing concentrations of famlasertib. Zero concentration refers to DMSO controls. Total number of tracked objects were approximately 10000, distributed across 60 wells, with a minimum of 1250 tracks and 6 wells, and a maximum of 7000 tracks and 18 wells per condition. **d, e)** Still images of ONS-76 (c) and UW228 (d) cells expressing LA-EGFP and mCherryNuc following 24 h exposure to DMSO or 0.5 μ M famlasertib. Scale bar: 100 μ m. **f)** Scanning electron microscopy (SEM) images of HD-MB03, D425 and D283 cells treated with DMSO or 0.5 μ M famlasertib for 24 h. White boxes indicate regions shown at 6 $\times$ higher magnification to the right of each panel.

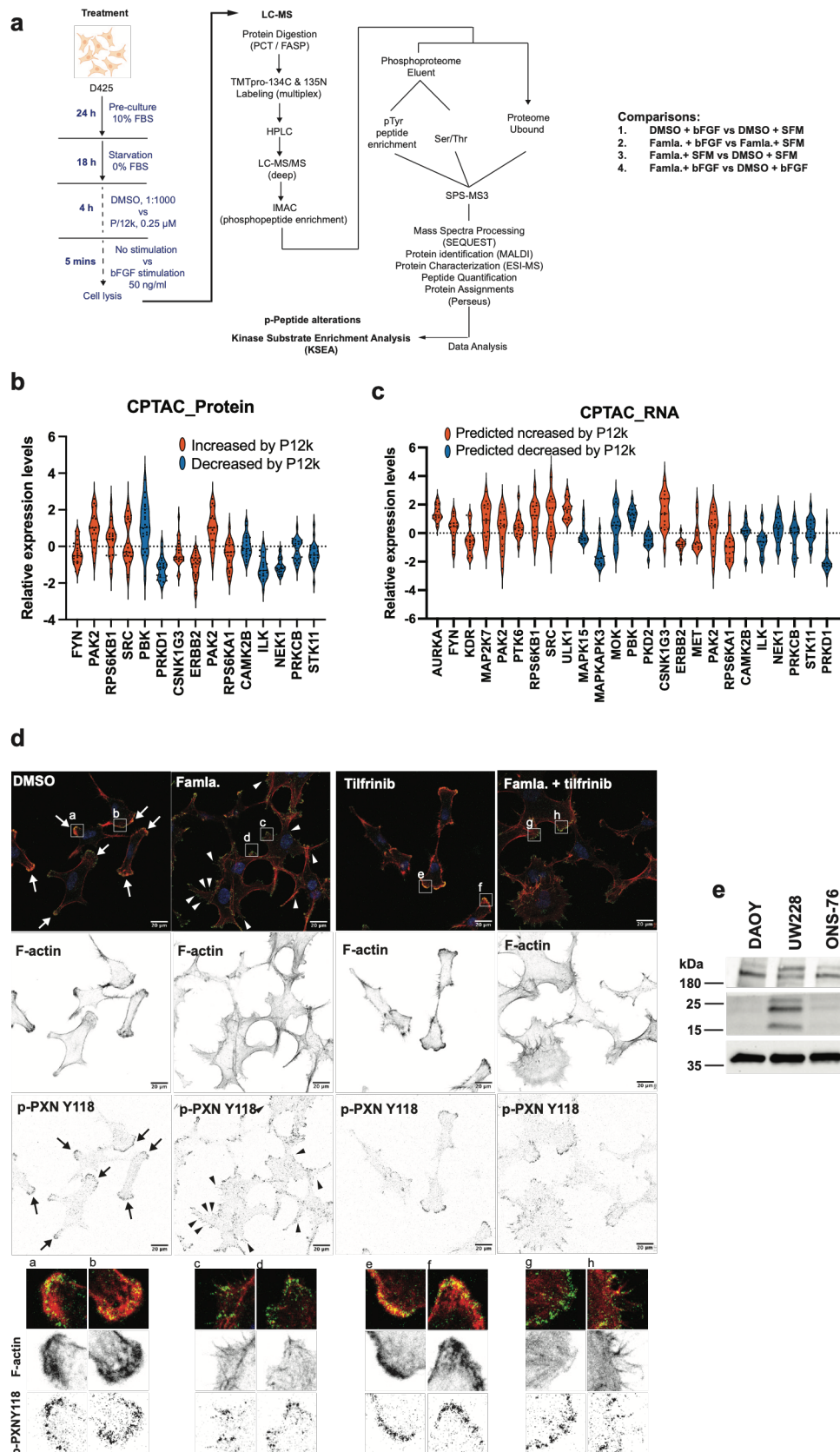
Figure S3



Supplementary Figure S3

a, b) Images illustrating the fluorescence change of the GCaMP6- Ca^{2+} signal (top) and colour-coded, AquA extracted calcium events (bottom) across different time points in DMSO control (**a**) and famlasertib (0.5 μM , (**b**) treated UW228 cells. **c, d**) Ca^{2+} features of individual events in Max $\Delta F/F$, duration and event area in DMSO control (**c**) and famlasertib (**d**) groups. Dots: individual Ca^{2+} events from $n = 4$ of each group, respectively (Total events in DMSO control were 1435 events; in famlasertib were 1082 events).

Figure S4



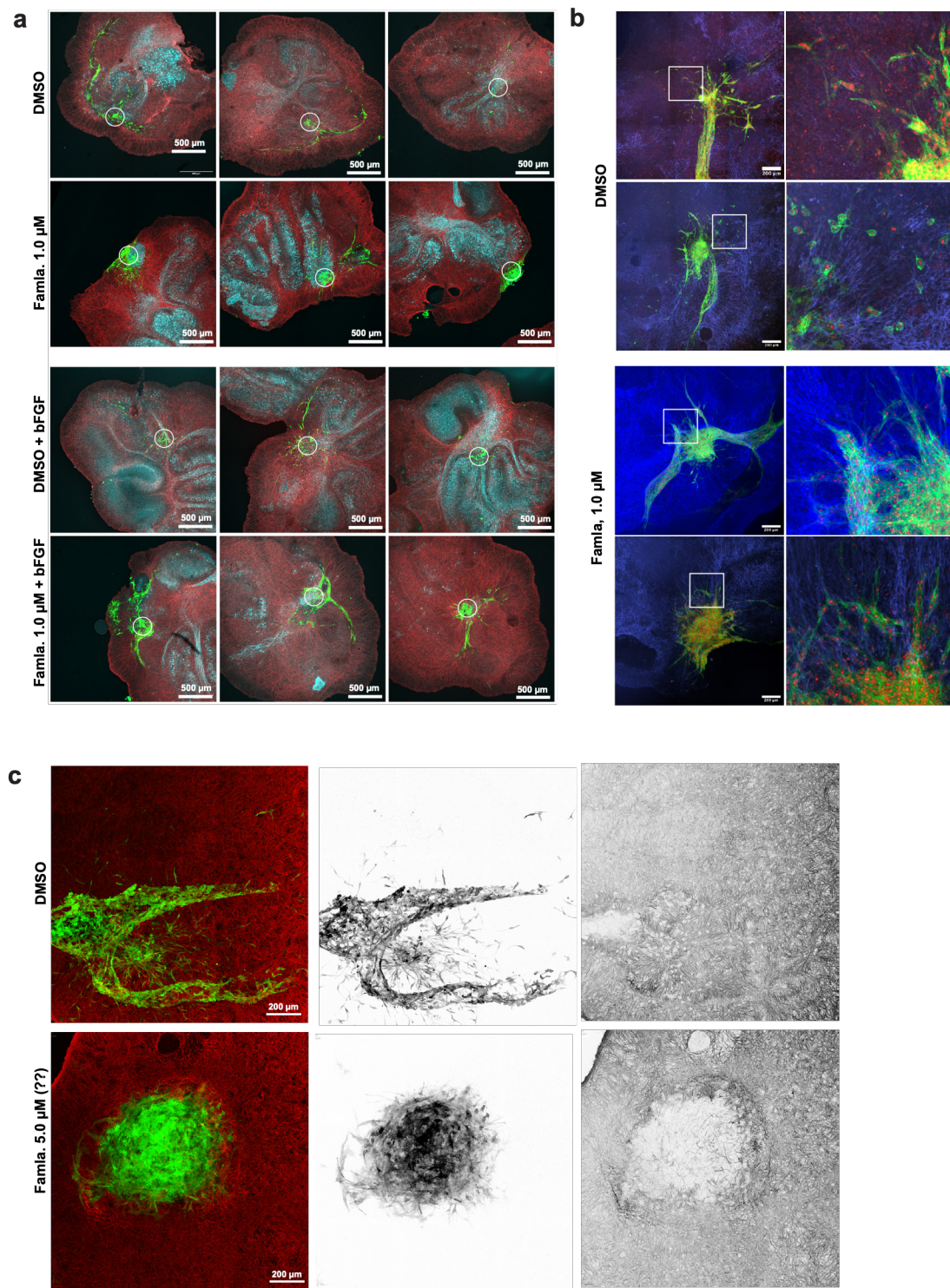
Supplementary Figure S4

a) Schematic overview of the phosphoproteomic workflow and the comparisons performed to identify treatment-dependent alterations. **b)** Correlation analysis between proteins harboring significantly altered phosphosites and corresponding protein expression scores derived from the Clinical Proteomic Tumor Analysis Consortium (CPTAC) dataset. **c)** Correlation analysis between proteins with significantly altered phosphosites and corresponding mRNA expression levels obtained from the CPTAC dataset. **d)** Confocal immunofluorescence analysis (IFA) of p-PXN (Y118) in UW228 cells treated with famlasertib, tilfrinib, or their combination. Inverted grayscale images of F-actin and p-PXN (Y118) are shown to enhance contrast. Regions a–h are displayed below the main panels at 4× magnification and highlight the distribution of p-PXN (Y118) within lamellipodia and cortical regions enriched in F-actin–positive protrusions. **e)** Immunoblot (IB) analysis of TJP1 and bFGF expression across different MB cell models, with GAPDH serving as a loading control.

Supplementary Figure S5

a) Schematic overview of integral membrane proteins of cell junctions (grey boxes), junction-associated proteins, and components of connected signaling pathways (blue boxes). Junction-associated proteins exhibiting increased or decreased phosphorylation upon famlasertib treatment are color coded. Significantly altered phosphosites in junction-associated proteins are shown on the right. **b)** Maximum intensity projections of confocal IFA of ONS-76 cells treated with DMSO or 1 μ M famlasertib for 24 h. **c)** Maximum intensity projections of confocal IFA of MDCK wild-type (WT) and MDCK TJP1 knockout (KO) cells treated with DMSO or 1 μ M famlasertib for 24 h. **d)** Maximum intensity projections of confocal IFA of MDCK wild-type (WT) cells treated with DMSO or 1 μ M famlasertib for 24 h.

Figure S6

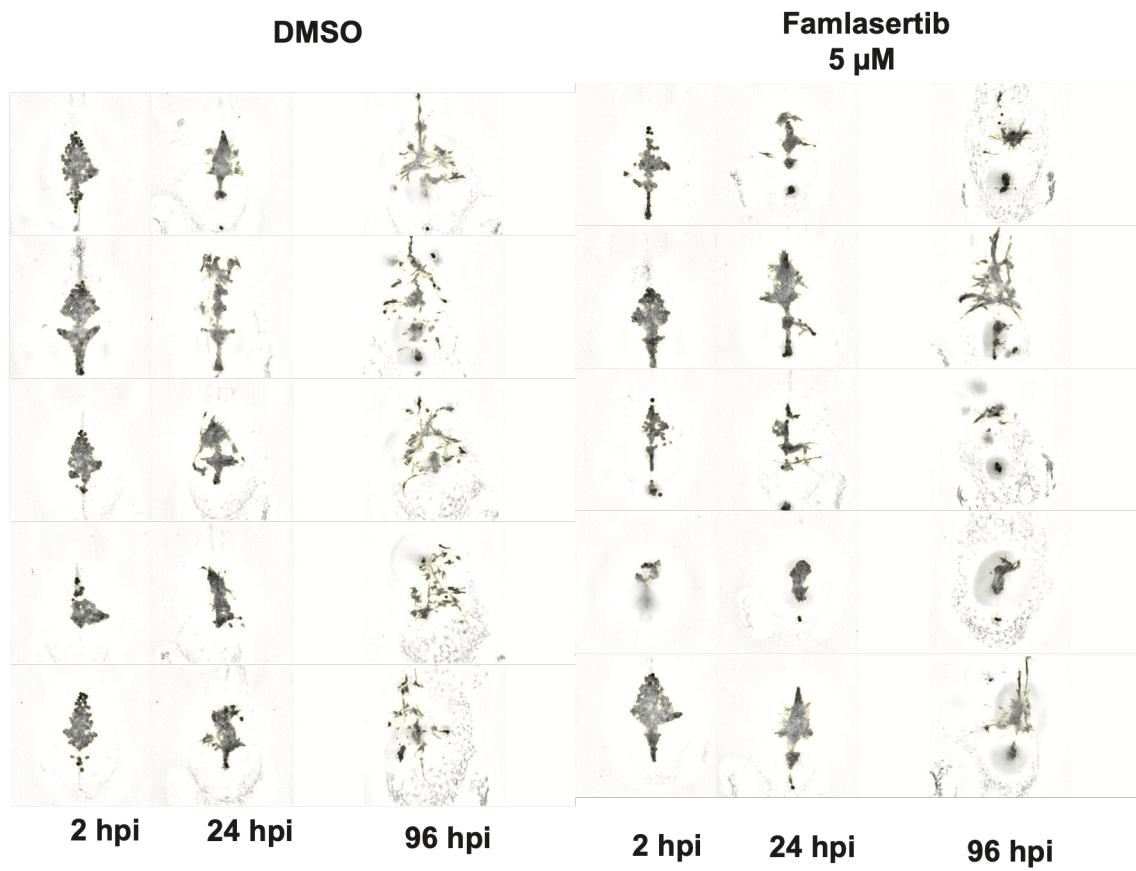


Supplementary Figure S6

a) Maximum intensity projections of confocal IFA of organotypic cerebellar slice cultures implanted with ONS-76 spheroids and treated for 5 days with famlasertib (1 μ M), +/- bFGF (50 ng/ml). Green: LA-EFP, cyan: calbindin (Purkinje cells), red: GFAP (Glial cells). **b)** Maximum intensity projections of confocal IFA of organotypic cerebellar slice cultures implanted with ONS-76 spheroids and treated for 5 days with famlasertib (1 μ M). Green: LA-EFP, Blue: GFAP (Glial cells), red: human nuclei. **c)** Maximum intensity projections of confocal IFA of organotypic cerebellar slice cultures implanted with ONS-76 spheroids and treated for 5 days with

famlasertib (5 μ M). Inverted grayscale images of LA-EGFP (tumor cells) and GFAP (glial cells within the tumor microenvironment) are shown to enhance contrast. Green: LA-EGFP; red: GFAP.

Figure S7



Supplementary Figure S7

Grayscale fluorescence imaging with tumor segmentation highlighting tumor progression. Representative live images of LA-EGFP signal at 2, 24, and 96 hpi in larvae treated with DMSO (n = 5) or famlasertib (n = 5). Tumor segmentation shown with a yellow line.