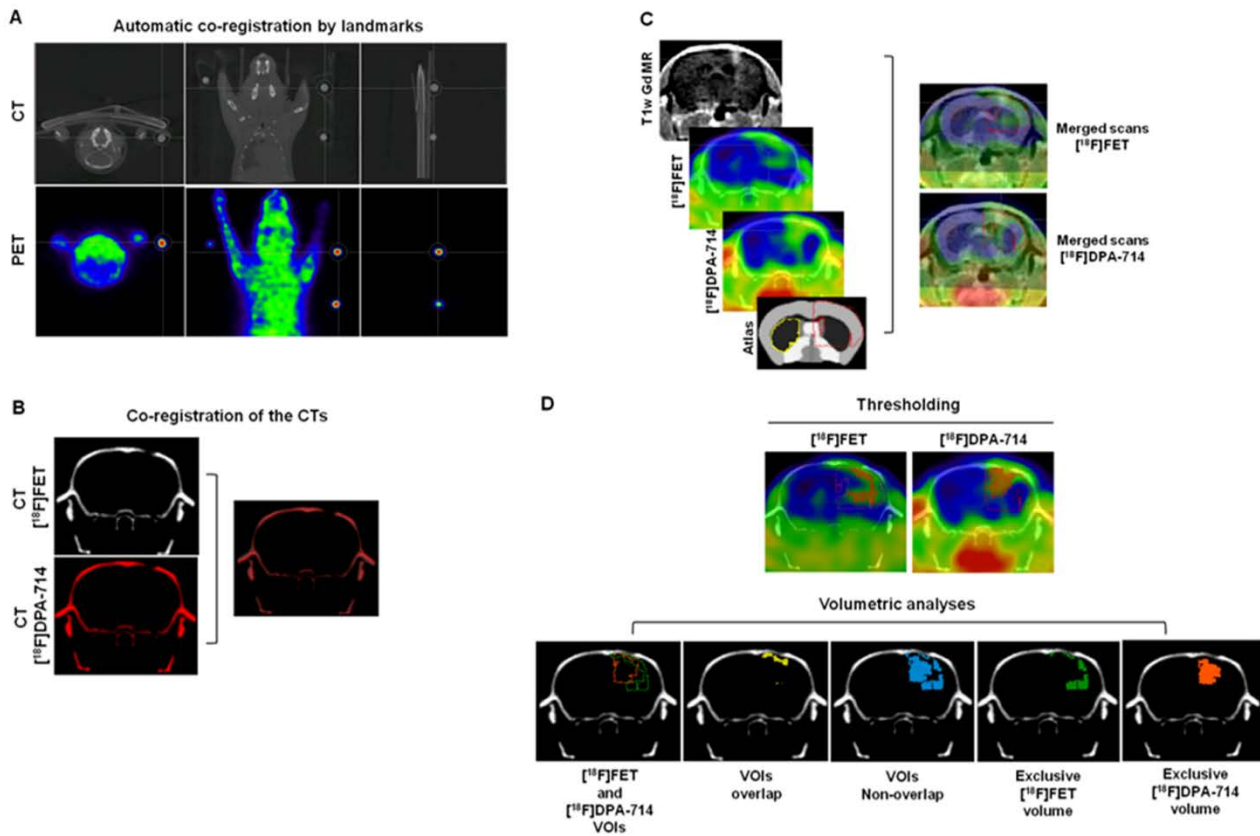
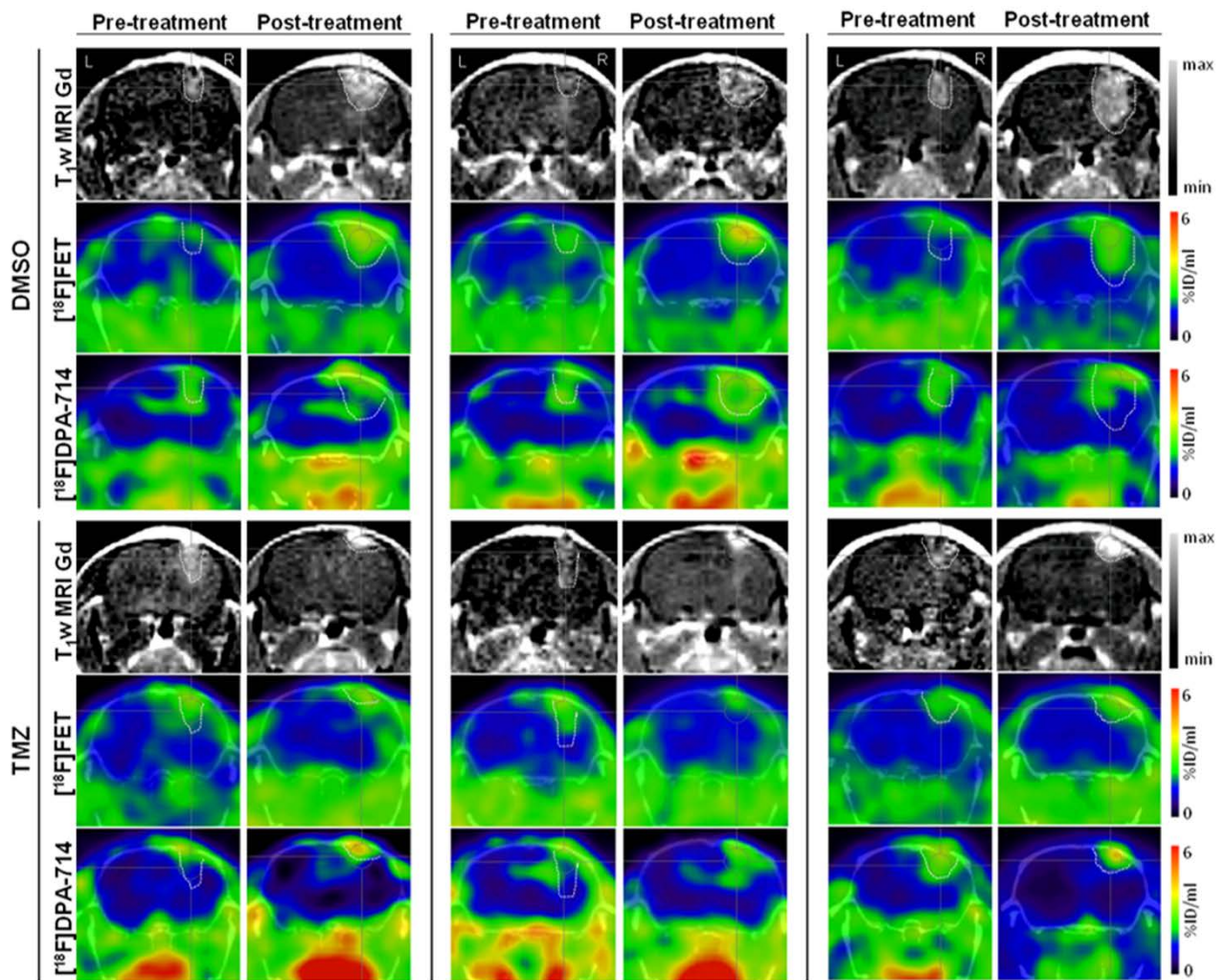


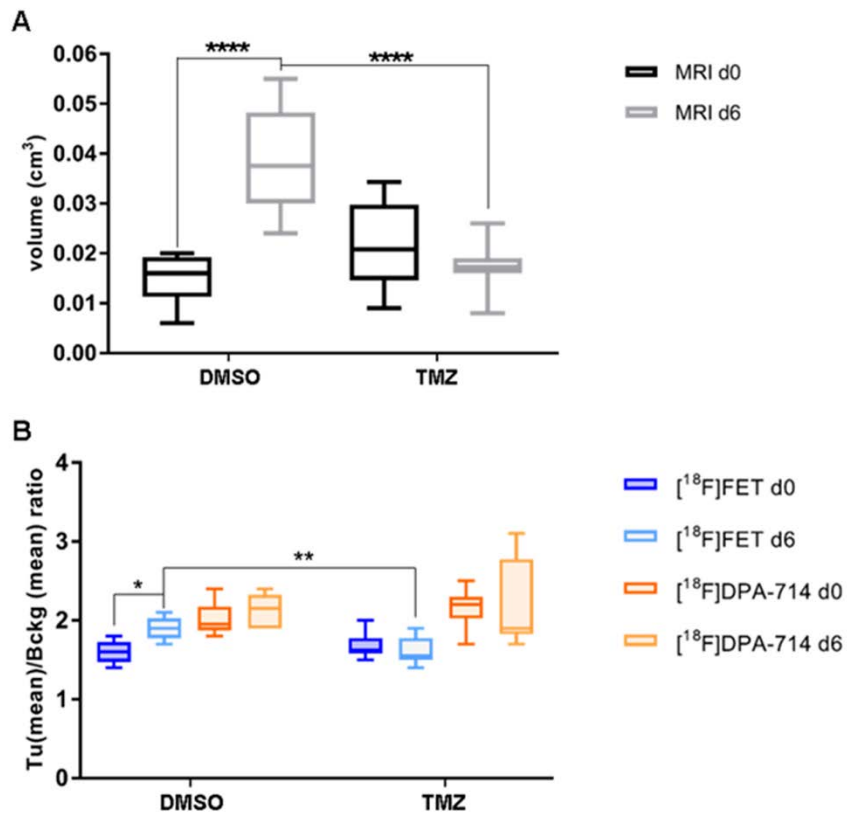
Supplementary Figures



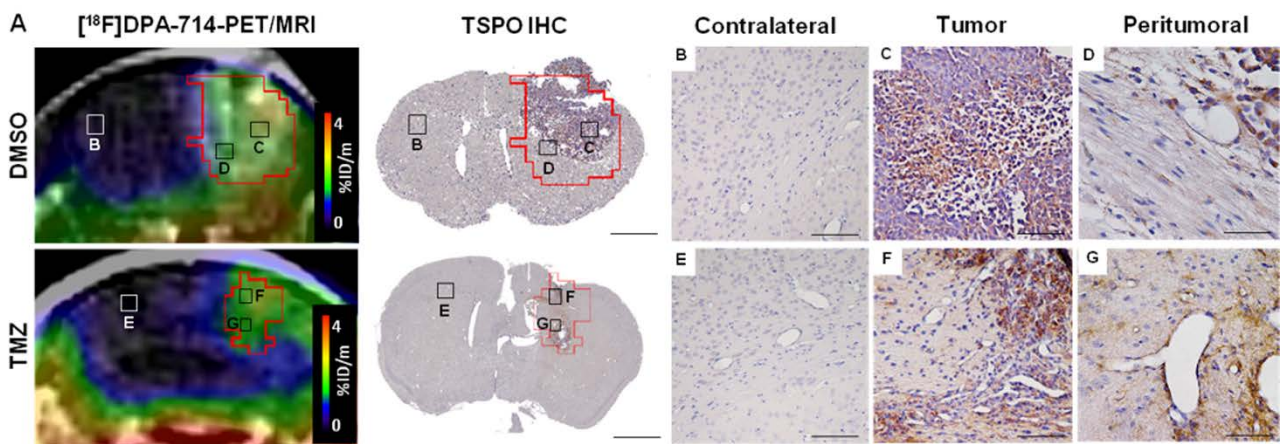
Supplementary Figure S1. Workflow representing the different steps of the data analysis process. Images co-registration and volumetric analysis approach. **(A)** Representative image of the automatic co-registration of CT and PET images using landmarks (circles) as reference. **(B)** Representative image of the co-registration of the two CTs obtained during $[^{18}\text{F}]\text{FET}$ -PET and $[^{18}\text{F}]\text{DPA-714}$ -PET imaging. **(C)** Representative workflow for the co-registration of T1w Gd-MRI with $[^{18}\text{F}]\text{FET}$ -PET, $[^{18}\text{F}]\text{DPA-714}$ -PET and the atlas with the two VOIs. **(D)** Representative images showing the thresholding of the right hemisphere VOI for $[^{18}\text{F}]\text{FET}$ - and $[^{18}\text{F}]\text{DPA-714}$, and the volumetric analyses approach.



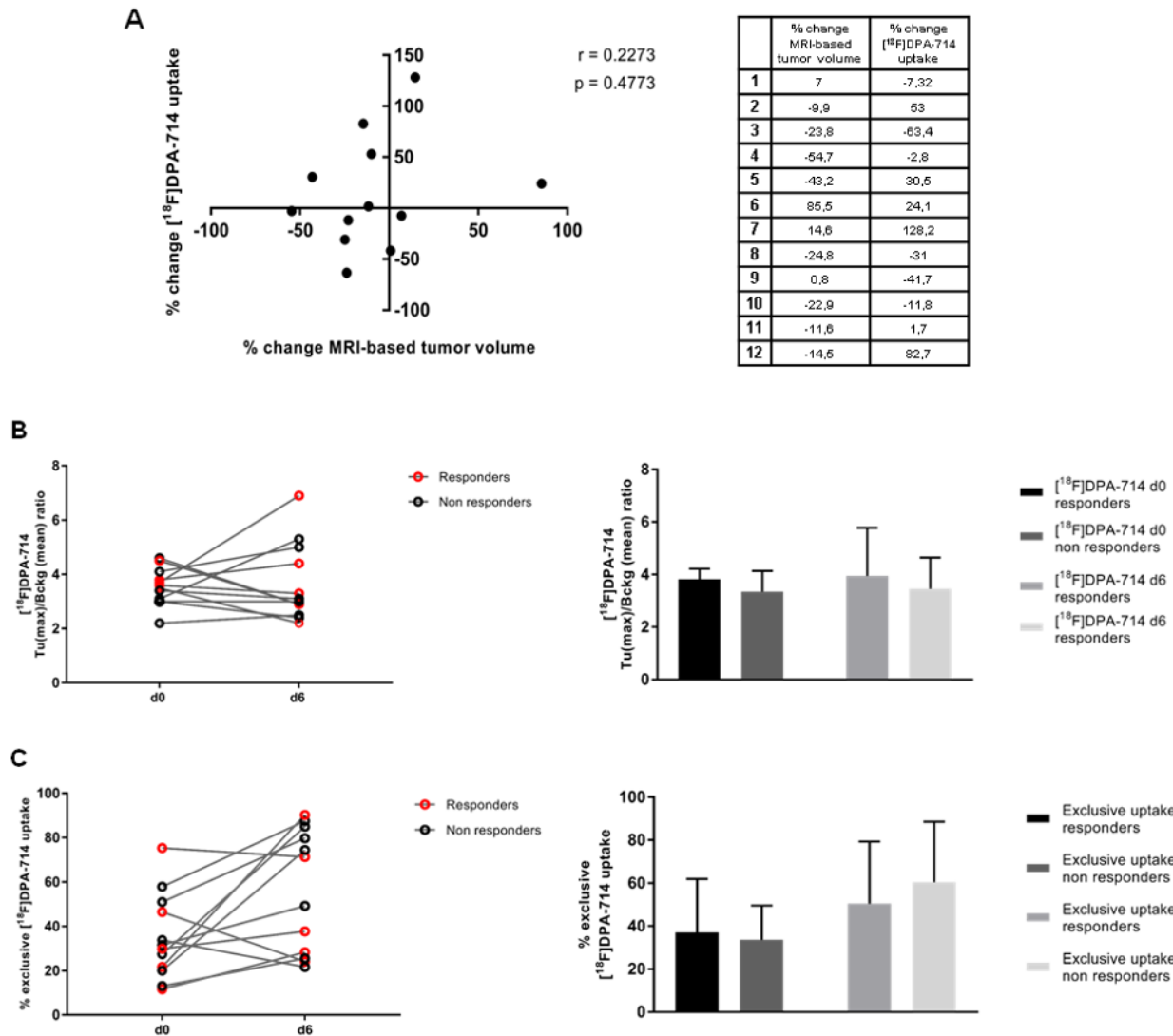
Supplementary Figure S2. Additional multimodal dual-tracer PET/MR images. Representative transaxial T_1w MRI Gd and PET images for $[^{18}F]$ FET and $[^{18}F]$ DPA-714 (top to bottom) fused with CT of control (DMSO) and TMZ-treated animals, pre- and post-treatment (left to right). The dotted line indicates the tumor area depicted by MRI and transferred to PET images. L and R indicate left- and right-hemisphere. DMSO: dimethyl sulfoxide; TMZ: temozolomide.



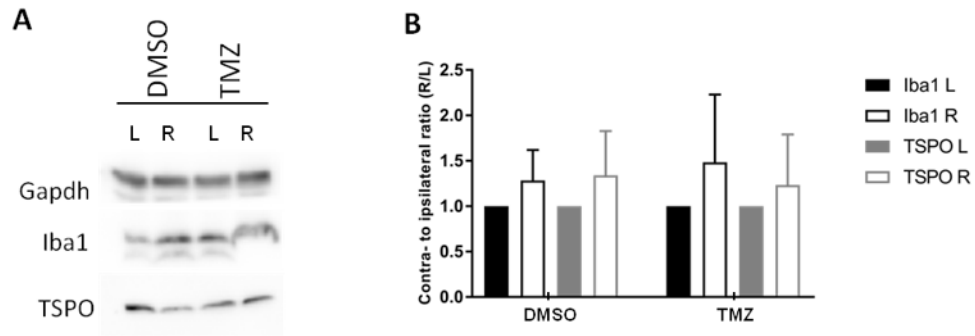
Supplementary Figure S3. Quantification of tumor volume based on T1w CE-MRI and Tu(mean)/Bckg(mean) ratio. Volumetric analyses showed increased tumor volume and [¹⁸F]FET uptake in the DMSO group, and a reduction of tumor volume and [¹⁸F]FET uptake after TMZ treatment, comparing TMZ- and DMSO-treated animals at day 6. **(A)** Quantitative analysis of the tumor volume (cm³/ml) based on T1 MRI with contrast agent (Gadovist) images, pre- and post-treatment. **(B)** Quantitative analysis of [¹⁸F]FET and [¹⁸F]DPA-714 T (mean)/B (mean) uptake ratio after 6 days from the beginning of the therapy with DMSO (vehicle) and 50 mg/kg TMZ. Differences intra- and inter-groups were tested for significance using paired t-Test, Wilcoxon test and Mann-Whitney test with Bonferroni correction.



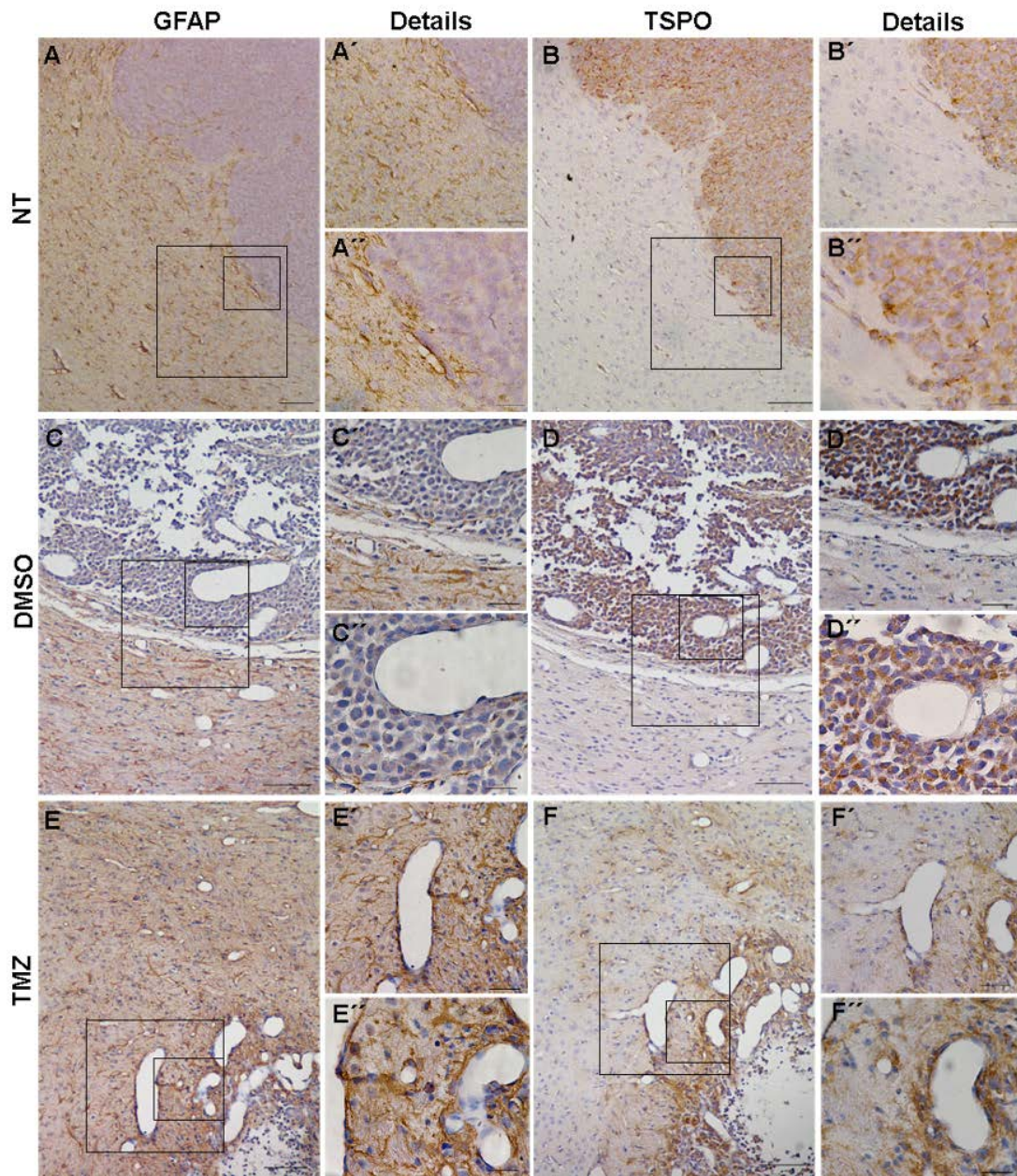
Supplementary Figure S4. Comparison of $[^{18}\text{F}]\text{DPA-714}$ with TSPO histology. Co-registration of PET/MRI images and TSPO IHC showed good spatial agreement. **(A)** Comparison of $[^{18}\text{F}]\text{DPA-714}$ PET/MRI images with immunochemistry for TSPO (scale bar 1000 μm). **(B-G)** Details at higher magnification showing absence of TSPO in the contralateral hemisphere and TSPO reactivity within the tumor tissue (scale bar 100 μm – Contralateral and Tumor - and 50 μm - Peritumoral). To be noticed that the squares representing D (peritumoral area) and E (contralateral side) are placed taking in account the tissue deformation resulting from the alcohol de-hydration process of the histology preparation and for this reason are slightly moved in the TSPO IHC image compared to the PET/MRI image.



Supplementary Figure S5. Case by case analysis of “responders” and “non-responders” TMZ-treated mice showed no correlation between relative change of tumor volume and [¹⁸F]DPA-714 uptake as well as no differences between responders and non-responders regarding [¹⁸F]DPA-714 uptake pre- and post therapy. **(A)** Correlation of the percentage of MRI-based tumor volume change with the percentage change of [¹⁸F]DPA-714 uptake. Single cases data are reported in the table on the right. **(B)** T/B ratios relative to [¹⁸F]DPA-714 uptake pre- and post-treatment with TMZ in responder (red circles) and non responder cases (black circles). Data are also represented in bar graphs as mean \pm SD. **(C)** Percentage of exclusive [¹⁸F]DPA-714 uptake in TMZ-treated animals, responders (red circles) and non responders (black circles). Data are also represented in bar graphs as mean \pm SD.



Supplementary Figure S6. Western blot analysis results. TMZ therapy did not significantly affect TSPO protein levels. **(A)** Representative western-blot for Iba1 and TSPO expression. **(B)** Quantification of the protein levels of Iba1 and TSPO in biological triplicates. All data are shown as ratio ipsi- to contralateral-side (R/L). R = right hemisphere; L = left hemisphere.



Supplementary Figure S7. Histological analysis of GFAP and TSPO in the TME. GFAP IHC revealed the presence of reactive astrocytes surrounding the tumor in controls and DMSO-treated animals, and within the tumor in the TMZ-treated group. TSPO expression resulted from tumor cells in non treated and DMSO-treated groups, while in TMZ-treated animals TSPO is located at the periphery of the remaining tumor mass. Immunohistochemistry for GFAP (A - C - D) and TSPO (B - D - F) performed in histological sections of brains harvested from tumor bearing mice not treated (upper lane), treated with vehicle (DMSO – middle lane) and treated with Temozolomide (TMZ – lower lane), scale bar 1000 μ m (A – F). The bigger squares represent details of GFAP (A', C', E') and TSPO (B', D', F') and are displayed in a 20x magnification, scale bar 50 μ m. The smaller squares represent details of GFAP (A'', C'', E'') and TSPO (B'', D'', F'') in a 40x magnification, scale bar 50 μ m.