Figure S1. MARCKS ED mutants without doxycycline induction. (A) Immunofluorescence imaging of U87 MARCKS effector domain mutants 72 h after PBS vehicle control using the image cytometer Xcyto® 10 (x20 magnification). (B) U87 ATPlite proliferation assay, 5,000 cells per well (n=4; WT P=0.0044; NP P=0.0093). (C) Mean nuclear intensity of U87 mutants calculated on Xcyto10 following 10 Gy RT. (d) Mean nuclear γH2AX intensity of U373 following 8 Gy RT as calculated by Xcyto10 quantitative cell microscopy (n>400). Data are the means ± SEM. Two-way ANOVA with Sidak's multiple comparison test. **P<0.01, ***P<0.001 and ****P<0.0001, compared to CTL; MARCKS, myristoylated alanine-rich C-kinase substrate; ED, effector domain; WT+, V5-tagged MARCKS vector; CTL, control; RT, radiotherapy.

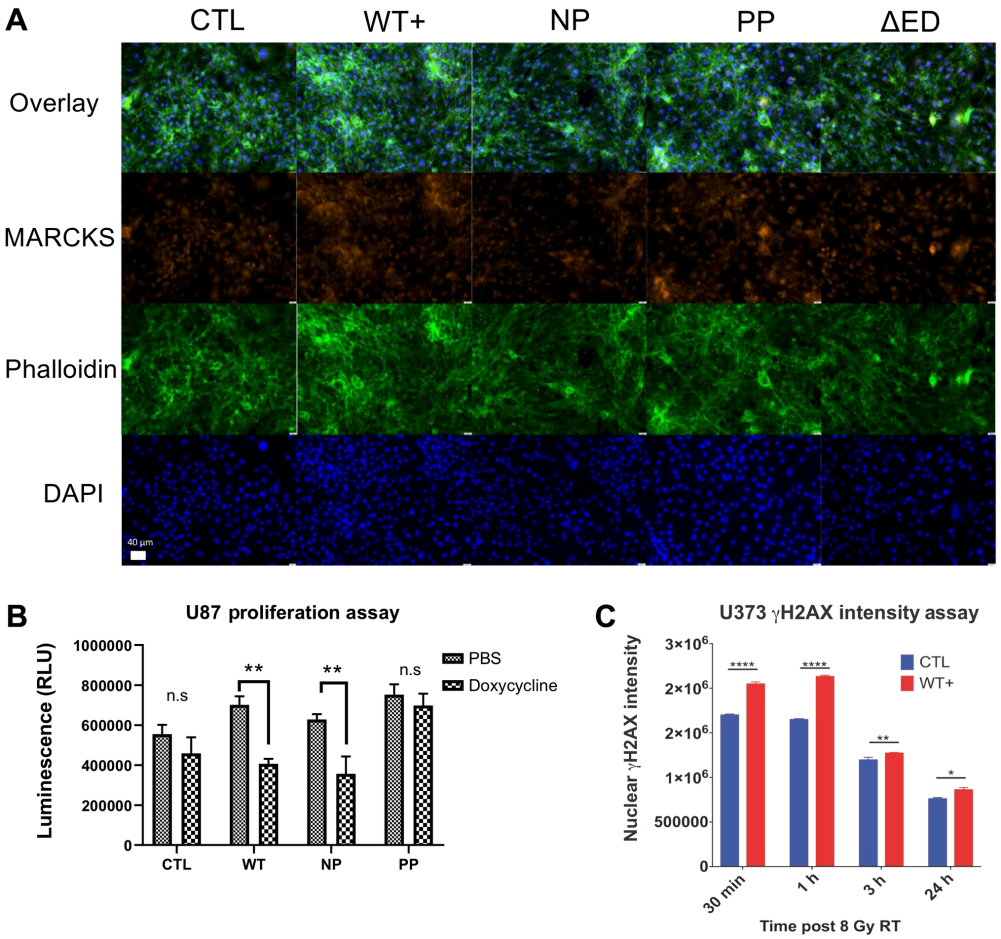

Figure S2. Wild-type MARCKS overexpression in the PTEN-null GBM U373 cell line. (A) Immunofluorescence imaging of U373 MARCKS effector domain mutants 72 h after doxycycline induction using the image cytometer Xcyto10 (x20 magnification). Quantification of MARCKS co-localization with either (B) phalloidin for filamentous actin or (C) DNA for MARCKS nuclear localization association using similarity score. Similarity score is calculated in XcytoView™ and represents the log transformed Pearson's correlation between two separate fluorescent channels within the indicated compartment. Statistical analysis was carried out in Prism using an unpaired t-test. Data are the means ± SEM. ****P<0.0001, compared to control. MARCKS, myristoylated alanine-rich C-kinase substrate; PTEN, phosphatase and tensin homolog; WT+, V5-tagged MARCKS vector; CTL, control.

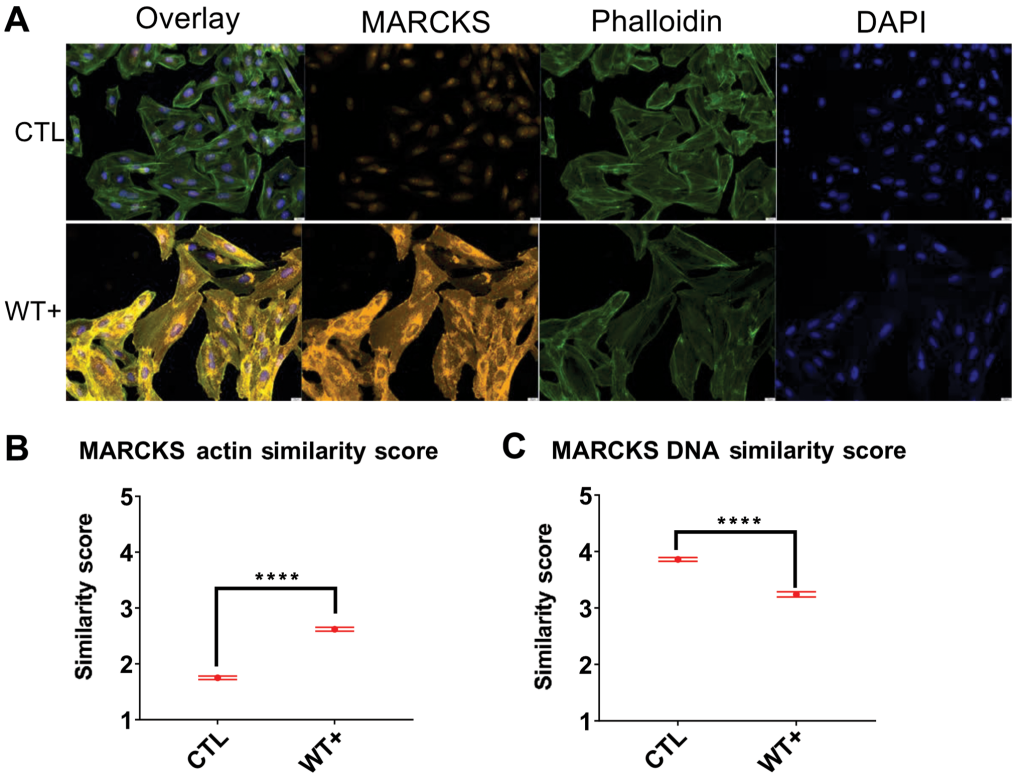

Figure S3. Mitotic counts and Intracranial survival of U87 MARCKS ED mutants. Athymic nude mice were orthotopically injected with 500,000 cells per mouse and monitored for survival by earliest sign of neurologic dysfunction or substantial weight loss (n=5). (A) Mitotic counts determined as mitotic figures per field at x40 magnification by a neuropathologist. Data are the means ± SEM. (B) Survival curves of U87 MARCKS ED mutants. The end of the study period was 100 days. Log-rank (Mantel-Cox) test P=0.0879. (C) Scatter plot of mitotic figures per 40X field by survival with linear regression and goodness of fit R^2 value are displayed. MARCKS, myristoylated alanine-rich C-kinase substrate; ED, effector domain; WT+, V5-tagged MARCKS vector; CTL, control; NP, non-phosphorylatable ED mutant; PP, pseudo-phosphorylated ED mutant.

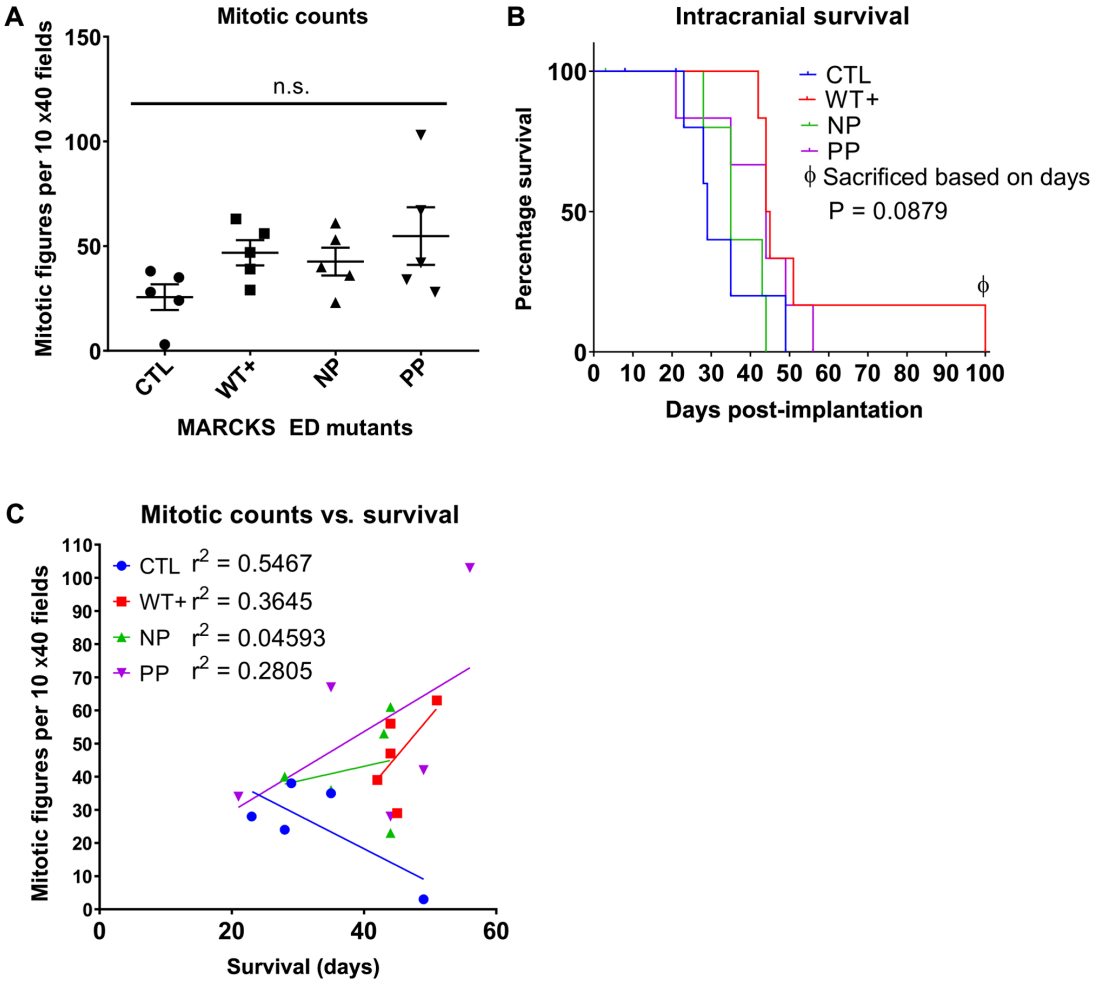

Figure S4. Nuclear and cytoplasmic localization of U87 MARCKS ED mutants. (A) Combined western blots of nuclear and cytoplasmic fractionations of U87 lysate after 72 h doxycycline induction probed for total MARCKS, Lamin A/C (nuclear fraction) and α-tubulin (cytoplasmic fraction). (B-D) Full-length blots of (A). (B) Total MARCKS and clipped product (C) Lamin A/C and (D) α-tubulin. Probing order is as follows: i) Lamin A/c; ii) tubulin; iii) total MARCKS. All probing was carried out on the same membrane with stripping. Cropped boundaries are indicated by the black outline. MARCKS, myristoylated alanine-rich C-kinase substrate; ED, effector domain.

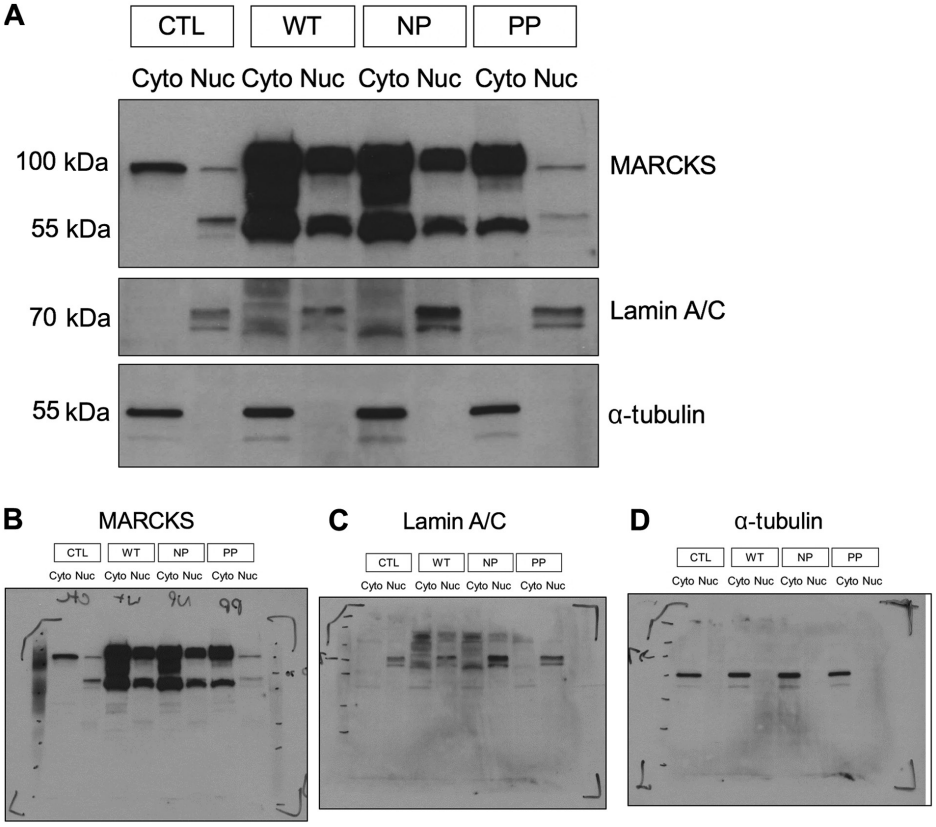

Figure S5. Full-length blots from Fig. 3E. (A) Total MARCKS and clipped product, (B) Phospho-AKT (T308/S473), (C) total AKT, (D) PKCα with stripped p-AKT band visible, and (E) β-actin. Probing order is as follows: i) Total MARCKS; ii) p-AKT (T308/S473); iii) PKCα; iv) total AKT; v) β-actin. All probing was carried out on the same membrane with stripping. Cropped boundaries are indicated by the black outline.