

Supplementary Materials:

TP5, a Peptide Inhibitor of Aberrant and Hyperactive CDK5/p25: A Novel Therapeutic Approach Against Glioblastoma

Emeline Tabouret, Herui Wang, Niranjana Amin, Jinkyu Jung, Romain Appay, Jing Cui, Qi Song, Antonio Cardone, Deric M. Park, Mark R. Gilbert, Harish Pant and Zhengping Zhuang

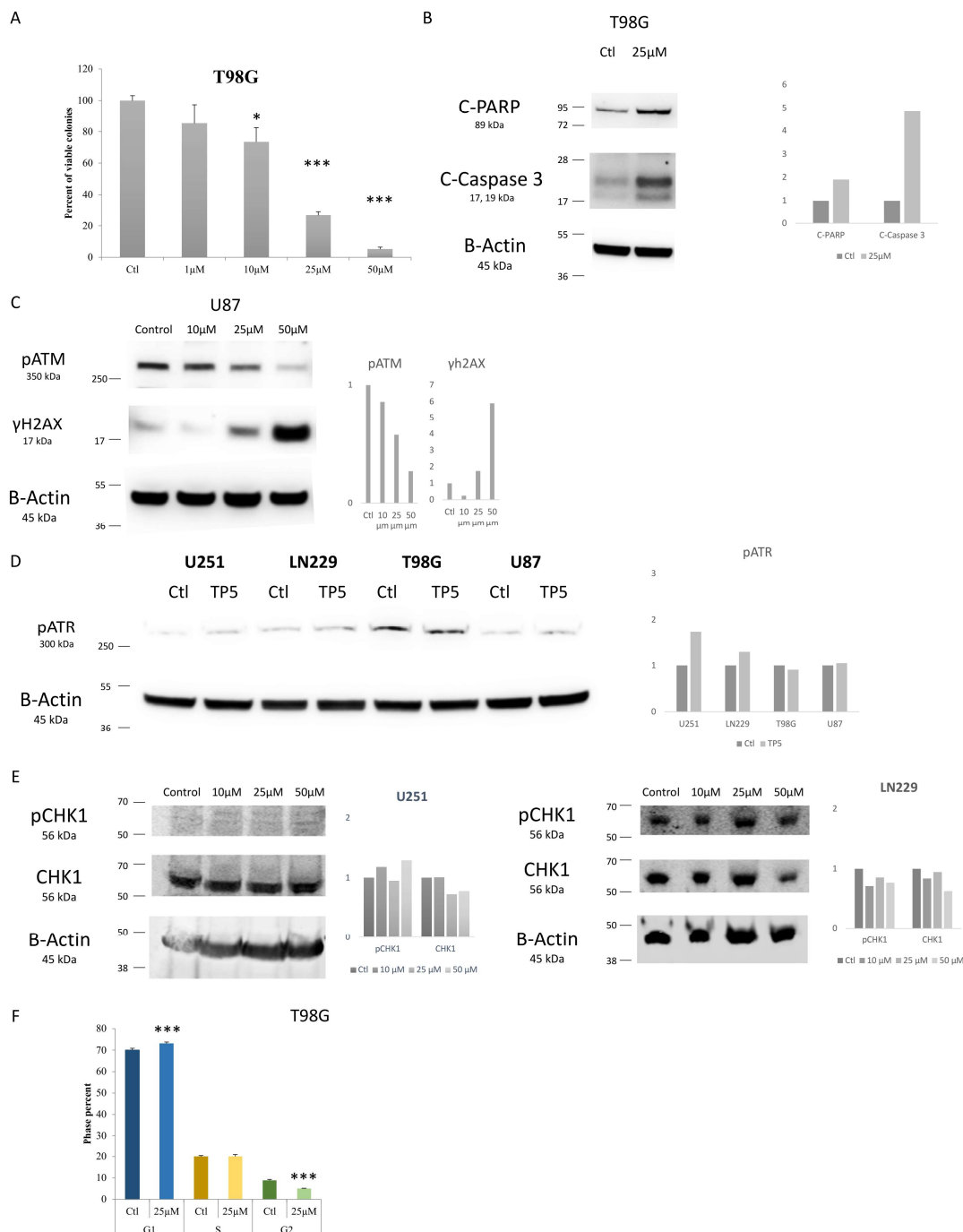


Figure S1. TP5 decreases cell viability by increasing apoptosis and DNA damage. **A.** Clonogenic assay of T98G cells is shown. The bar graphs display quantification of colonies under treatment at indicated concentrations ($N = 3$). (* $p < 0.05$; *** $p < 0.001$). **B.** Protein level of cleaved PARP and cleaved Caspase 3 in T98G cells after 24 h of treatment by TP5 (25 μ M). **C.** Western blot of phosphorylated ATM and γ H2A.X in U87 cells treated by TP5 at indicated concentrations. **D.** Protein level of phosphorylated ATR in U251, LN229, T98G and U87 cells treated by TP5 at 25 μ M. **E.** Protein level of phosphorylated Chk1 and Chk1 in U251 (left) and LN229 (right) cells treated by TP5 at indicated concentrations. **F.** Cell cycle analysis in T98G cells treated by TP5 at 25 μ M for 48 h. The graph bar displays the cell cycle phase quantification ($N = 3$; *** $p < 0.001$).

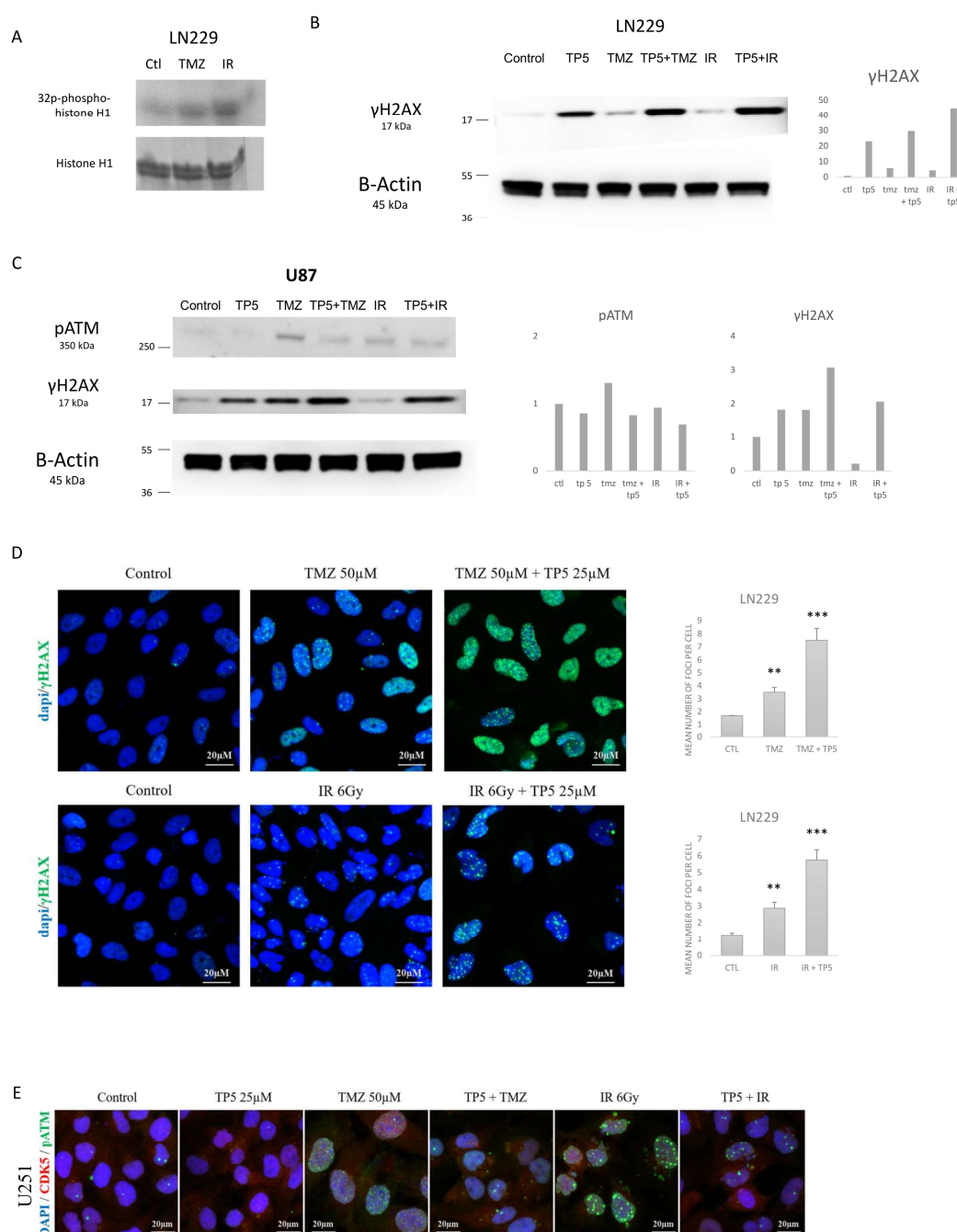


Figure S2. TP5 is synergistic with temozolomide and irradiation by increasing DNA damage. **A.** CDK5 activity is shown by phosphorylated histone 1 in LN229 cells treated as indicated (Ctl: control; TMZ: temozolomide 50 μ M; IR: irradiation 6 Gy). **B.** The protein levels of γ H2A.X is shown by western

blot in LN229 cells treated as indicated (Control; TMZ: temozolomide 50 μ M; TP5: 25 μ M; IR: irradiation 6 Gy). **C.** The protein levels of γ H2A.X and phosphorylated ATM are shown by western blot in U87 cells treated as indicated (Control; TMZ: temozolomide 50 μ M; TP5: 25 μ M; IR: irradiation 6 Gy). **D.** Immunofluorescent staining of γ H2A.X (green) in LN229 cells after treatment as indicated (TMZ: temozolomide; IR: irradiation). **E.** Immunofluorescent staining of phosphorylated ATM (green) and CDK5 (red) in U251 cells after treatment as indicated.

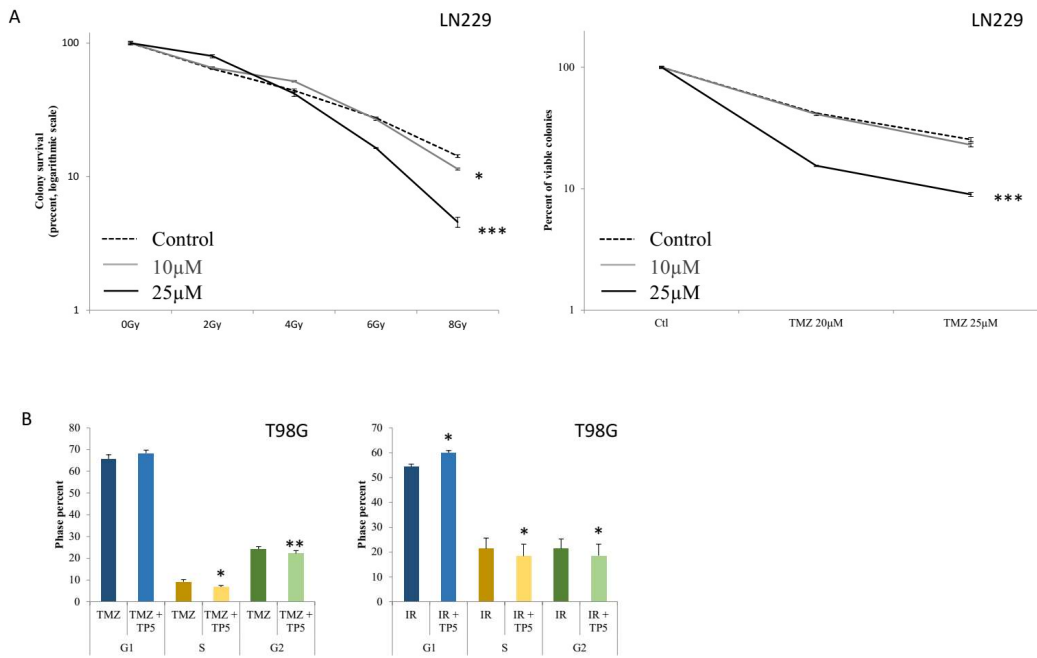


Figure S3. TP5 is synergistic with temozolomide and irradiation. **A.** Synergistic interactions between TP5 and irradiation (left graph) or chemotherapy (TMZ: temozolomide, right panel) are shown by clonogenic growth of LN229 cells treated as indicated (TP5 at 10 μ M and 25 μ M; irradiation from 0 to 8 Gy; TMZ at 20 μ M and 25 μ M) ($N = 3$; * $p < 0.05$; *** $p < 0.001$). **B.** Cell cycle analysis is shown for T98G cells treated by TP5 (25 μ M) and temozolomide (50 μ M) or irradiation (6 Gy) for 48 h. The graph bar displays the cell cycle phase quantification ($N = 3$; * $p < 0.05$; ** $p < 0.01$).

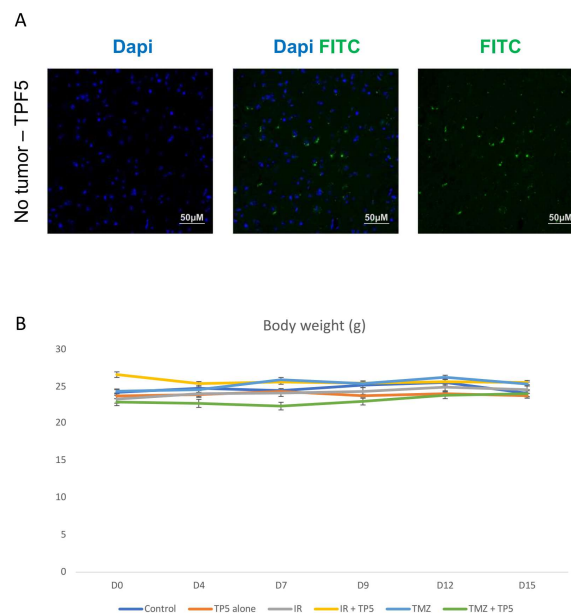


Figure S4. **A.** TPF5 signal (green) in normal brain is shown. **B.** Mouse weight over time is shown.