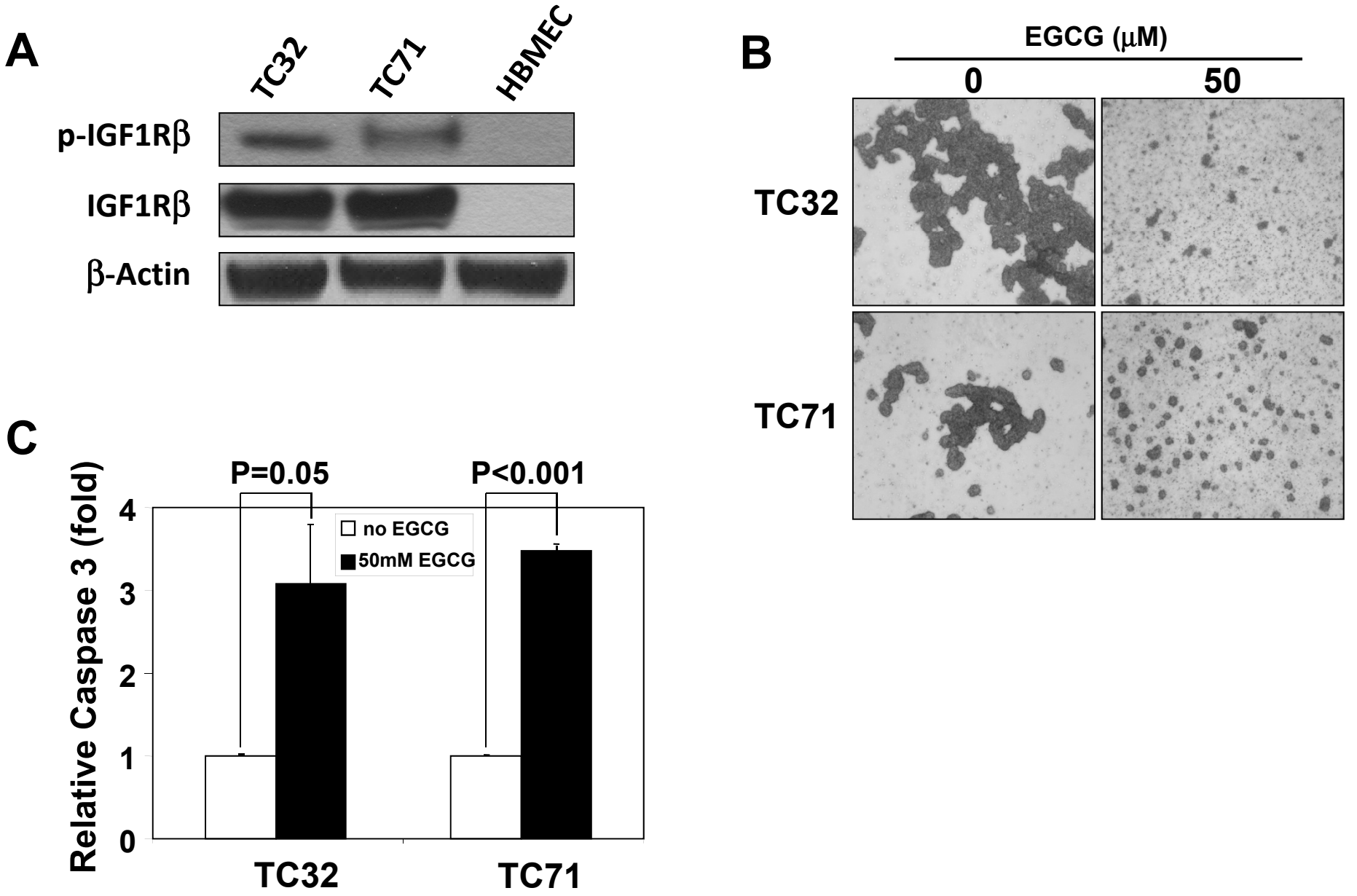

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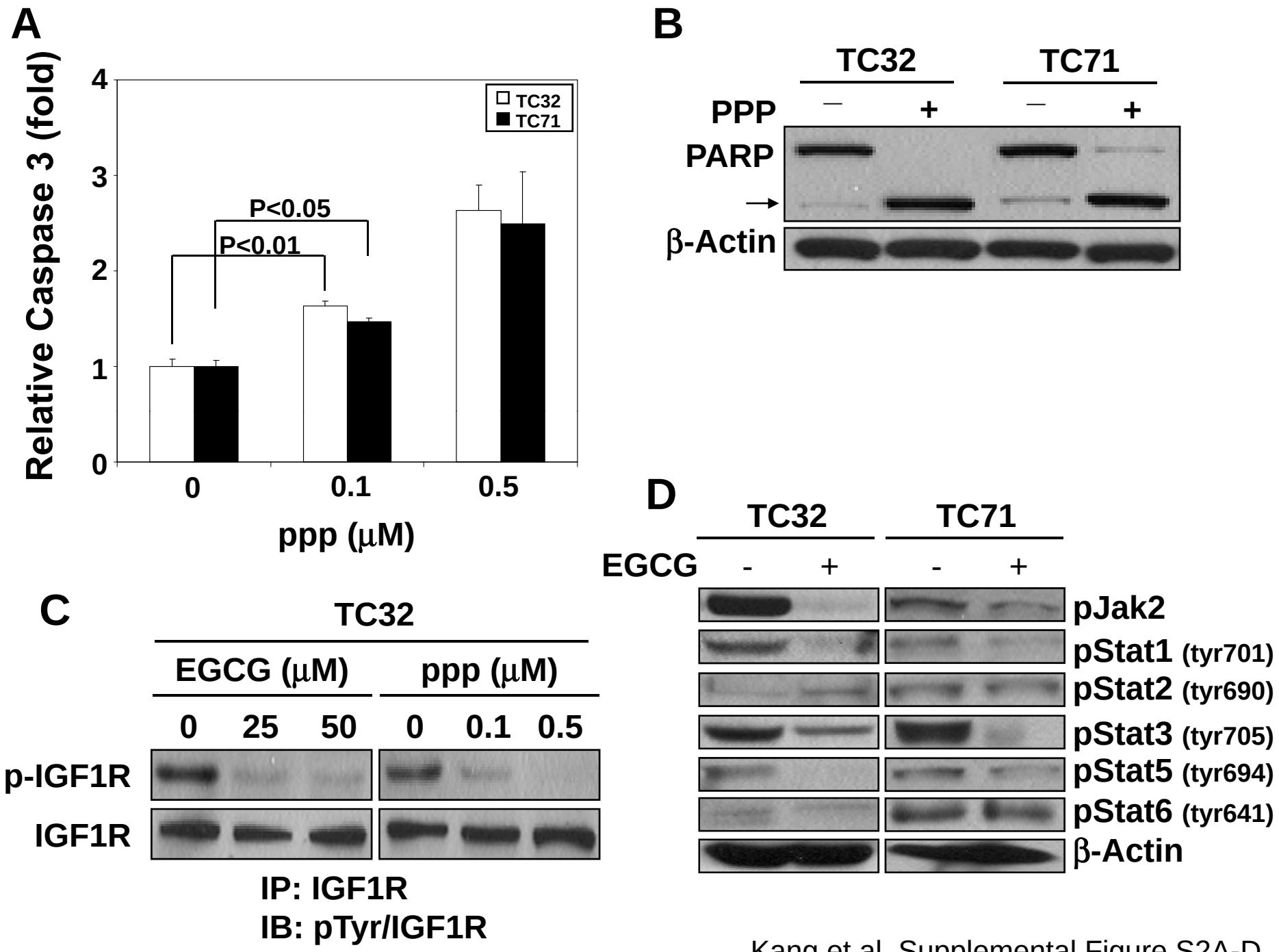

Kang et al. Supplemental Figure S2A-D

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure S1. A, Comparison of IGF1R levels and activation in EFT TC32 and TC71 cells and human brain microvascular endothelial cells (HBMEC). Lysates from cells grown in 10% serum were assayed for expression of phospho-IGF1R (p-IGF1R) and total by standard Western blotting using anti-PARP antibodies. β-actin was used as a loading control. **B,** Effects of EGCG on EFT multicellular spheroid formation. TC32 and TC71 EFT cells were transferred from monolayer cultures to agar-coated plates in the presence or absence of 50 μM EGCG for 12 hr, and then monitored microscopically for spheroid formation. **C,** Effects of EGCG on induction of apoptosis in EFT multicellular spheroids. Spheroid cells treated with or without EGCG (50 μM), were lysed and caspase-3 activity was measured by fluorometry as described for Fig. 2B, and normalized to a value of 1.0 arbitrary unit for untreated spheroid cells. Statistical analysis of data from three separate experiments was done using the Student's *t* test.

Supplemental Figure S2. Effects of EGCG versus PPP on IGF1R activation and induction of apoptosis. **A,** Effects of PPP, a known inhibitor of IGF1R, on apoptosis of EFT cells. TC32 and TC71 cells were grown for 24 hr and then treated with different concentrations of PPP for a further 12 hr. Cells were lysed and caspase-3 activity was measured by fluorometry as described for Fig. 2B after adjusting for protein concentration. Values were normalized to a value of 1.0 arbitrary unit for untreated cells. **B,** Lysates from the same cells were assayed for poly(ADP-ribose) polymerase (PARP) cleavage by immunoblotting using antibodies to PARP. Arrowhead, cleaved PARP. β-actin was used as a loading control. **C,** Comparison of the inhibitory effects of EGCG and PPP on activation of IGF1R in EFT cells. TC32 and TC71 were grown for 24 hr and then treated with the indicated concentrations of PPP and EGCG for a further 12 hr. Cell lysates were subjected to immunoprecipitation (IP) using antibodies to IGF1R, followed by

immunoblotting (IB) with 4G10 anti-phosphotyrosine or anti-IGF1R antibodies as indicated. **D,** Effects of EGCG on activation of Jak2/Stat cascades in EFT cells. TC32 and TC71 cells were grown for 24 h and then treated with EGCG (50 μM) for 12 h. Cell lysates were subjected to immunoblotting with anti-phospho-Jak2 and anti-phospho-Stat antibodies as shown. β-actin was used as a loading control.

Supplemental Figure S3. Effects of EGCG on ETV6-NTRK3 (EN) and myristoylated EN (ENmyr) transformed R- (IGF1R-/-) and R+ (IGF1R re-expressing) mouse embryo fibroblasts (MEFs). **A,** R+ (IGF1R+/EN-), R+EN (IGF1R+/EN+) and R-ENmyr (IGF1R-/ENmyr+) cells (see text for details) were grown for 24 hr and then treated with 50 μM EGCG for 12 hr. Cell lysates were subjected to immunoblotting with anti-IGF1R antibodies as shown to demonstrate lack of IGF1R expression in the R-ENmyr cells. β-actin was used as a loading control. **B,** Effects of EGCG on apoptosis of R+, R+EN and R-ENmyr cells. Cells were grown for 24 hr in 10% serum and then treated with EGCG for 12 hr. Cells were lysed and caspase-3 activity was measured by fluorometry as described for Fig. 2B, after adjusting for protein concentration. Values were normalized to a value of 1.0 arbitrary unit for untreated cells. **C and D,** EGCG blocks soft agar colony formation of IGF1R expressing cells (R+ and R+EN), but not of R-ENmyr cells. Cells were grown in soft agar for 14 days as described in Materials and Methods. After 14 days plates were photographed **(C)** and the colonies were counted **(D)**. Each well is representative of three replicate wells for each condition and statistical analysis was done by Student's *t* test.