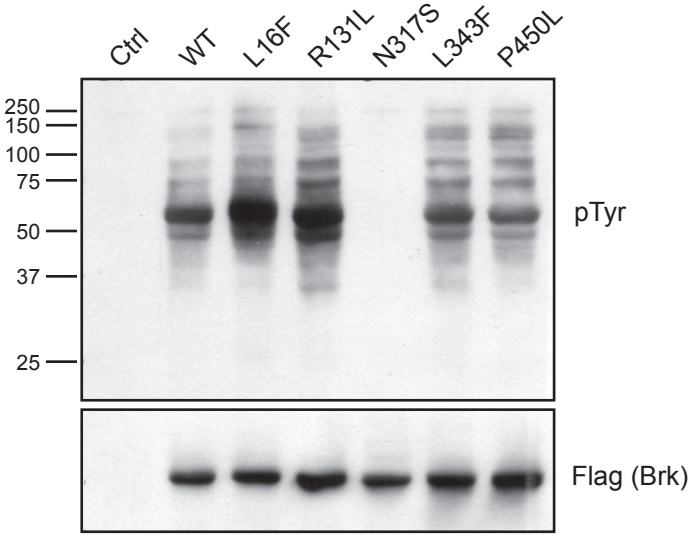
Supplemental figure legends

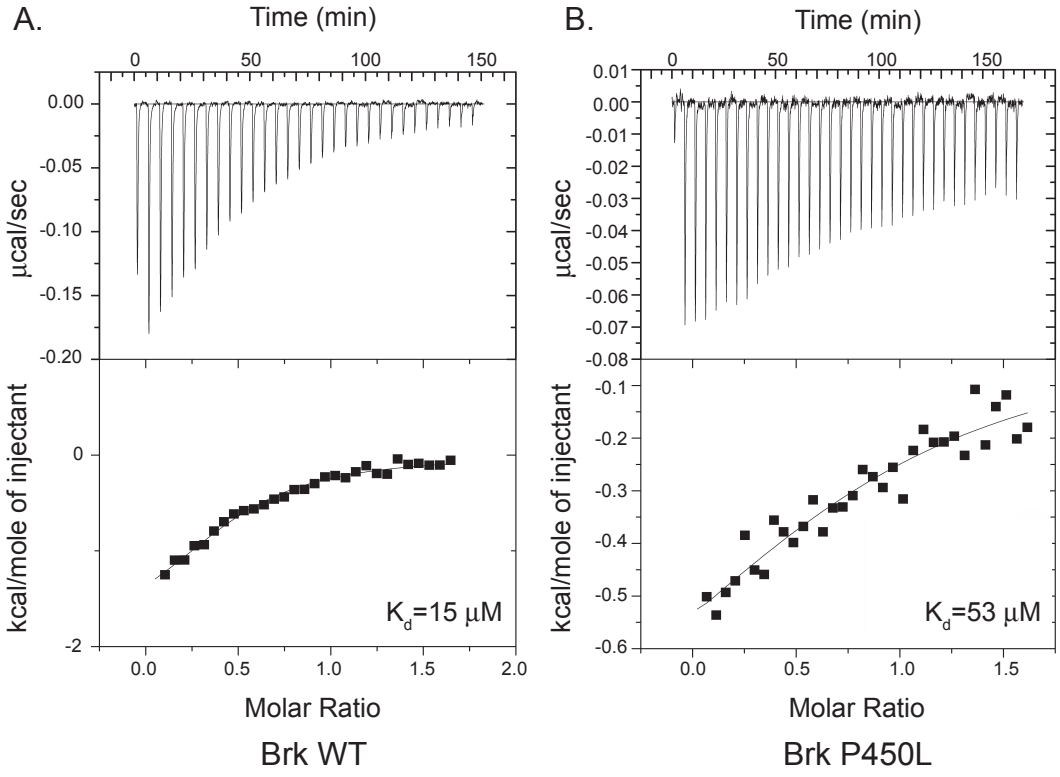
Supplemental Figure 1. Brk mutants affect autophosphorylation. Lysates from 293T cells expressing Flag-tagged wild-type or mutant forms of Brk were probed with anti-phosphotyrosine and Flag antibodies. Molecular weight standards are shown. The gel is representative of five similar experiments.

Supplemental Figure 2. Interaction between the Brk SH2 domain and C-terminal peptides. Representative titrations of 5 μL injections of (A) wild-type peptide (FTS-Y(p)-ENLTG) (1 mM) and (B) P450L peptide (FTS-Y(p)-EN**P**TG) (0.83 mM) into 67.4 μM purified Brk SH2 domain. The dissociation constant calculated using Origin 7.0 was 15 μM and 53 μM, respectively. Note the difference in scale between the two panels; the P450L peptide produced smaller heat changes than the wild-type sequence.

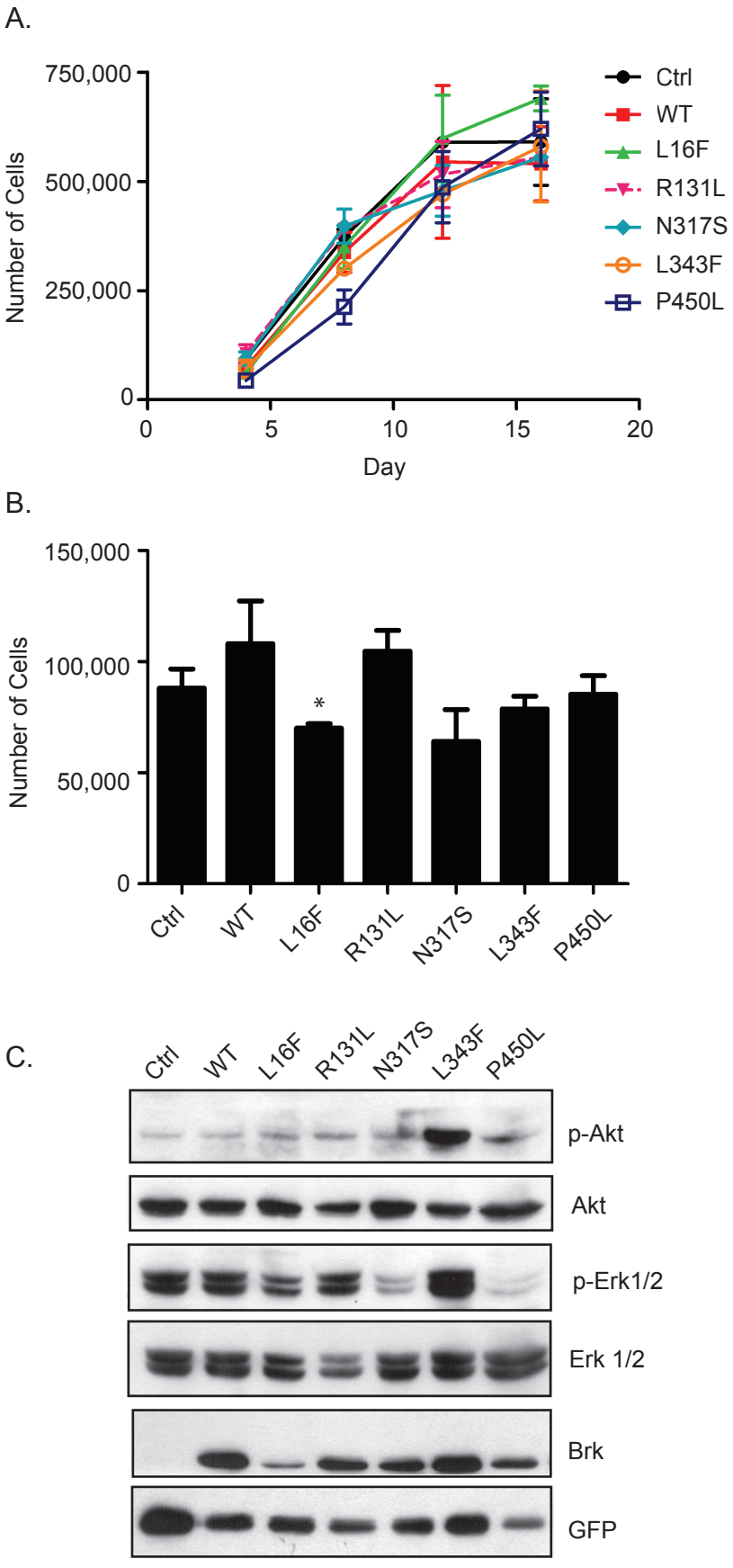
Supplemental Figure 3. Biological effects of Brk mutants in SKBr3 cells. A) SKBr3 cells (20,000) stably expressing wild-type or mutant forms of Brk were plated in 24-well plates. Cells were counted on a hemocytometer on days 4, 8, 12, and 16. B) Stable cells (20,000) were plated in a 24-well low attachment plate. On day 14, the cells were collected and counted using a hemocytometer. The data is an average of two experiments. The error bars show standard deviations. *, $P < 0.05$ compared to Brk WT. C) SkBr3 cells stably expressing wild-type or mutant forms of Brk were probed with anti-phosphoAkt (Ser473), anti-Akt, anti-phospho Erk1/2 (Thr202/Tyr204), anti-Erk 1/2, anti-Brk, and anti-GFP antibodies.



Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3