Supplementary Data for:

“Fyn and Src are Effectors of Oncogenic EGFR Signaling in Glioblastoma Patients”

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SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1. The physical association between EGFR and SFKs is diminished but not abrogated by erlotinib treatment. U87-EGFRvIII cells were treated with erlotinib or vehicle for 24 hours and lysed. Lysates were immunoprecipitated with either pan anti-P-Src Y419 or anti-Fyn antibodies and probed by western blot for EGFR, Src, or Fyn.

Supplementary Figure S2. Specificity of the EGFR immunoprecipitating antibody to EGFR is shown using HIFko transformed mouse astrocytes, which express Fyn and Src but have undetectable levels of EGFR by western blot. HIFko lysates immunoprecipitated with the anti-EGFR antibody showed no Fyn or Src in the resulting pull-downs.

Supplementary Figure S3. Western blot analysis of Fyn on protein lysates prepared from frozen tumor specimens (T) or contralateral normal brain (N) from glioblastoma patients after autopsy. Y420 phosphorylated Fyn was also measured by immunoprecipitating lysates with a Fyn antibody and probing the immunoprecipitates with an antibody recognizing the phosphorylated activating site. 7 representative cases showing higher Fyn expression and activation in tumor lysates are shown.

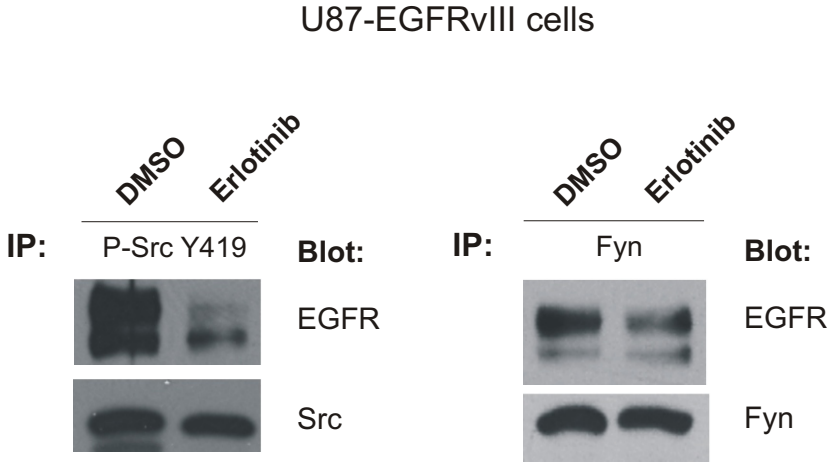
Supplementary Figure S4. The amount of Fyn knockdown achieved for each glioblastoma cell line (x-axis) was highly correlated with its level of invasion reduction (y-axis). Pearson correlation coefficient (r) and *P*-value are indicated.

Supplementary Figure S5. A subset of control and dasatinib-treated mice bearing orthotopic GBM39 tumors were sacrificed at the end of the 17-day treatment period (31 days post-tumor injection) for comparative histological analyses. Control tumors (31 days) immunohistochemically stained for pan phospho-Y419 SFKs exhibited a strong membranous staining pattern whereas P-SFK staining in dasatinib-treated tumors (31 days) was significantly weaker and predominantly cytoplasmic. In dasatinib-treated tumors at the time of recurrence (71 days), the P-SFK staining pattern reverted to that

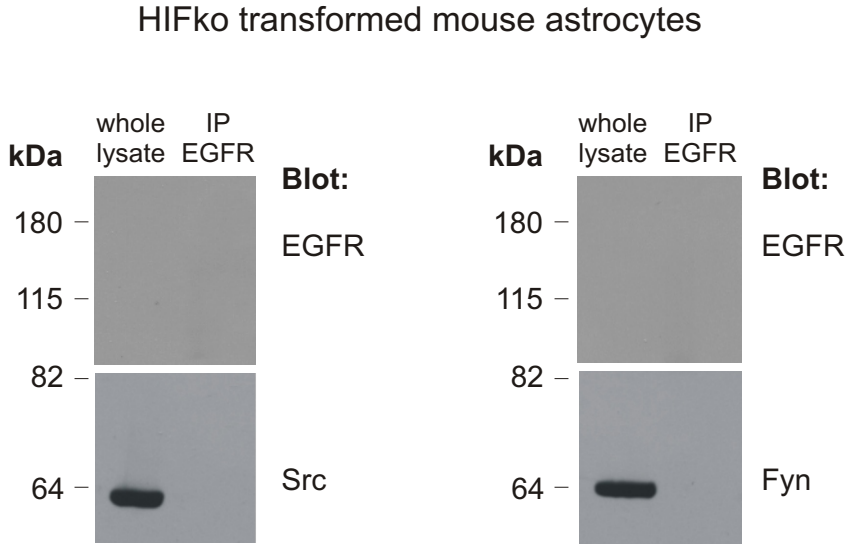
seen in control tumors. Immunohistochemical detection of phospho-Y861 FAK also showed stronger P-FAK staining in control tumors compared to dasatinib-treated tumors (31 days).

Supplementary Figure S6. U87-EGFRvIII-Src cells co-expressing EGFRvIII and activated Src were treated in vitro with mAb 806 (100 μg/ml), dasatinib (100 nM), or a combination of both, then probed for phospho-Src by western blot. The lysosomal marker LAMP-1 is shown as a loading control. Quantification of band intensities after normalization to LAMP-1 is shown below.

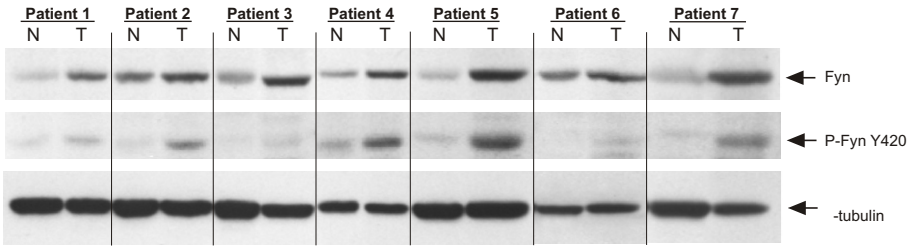
Supplementary Figure 1



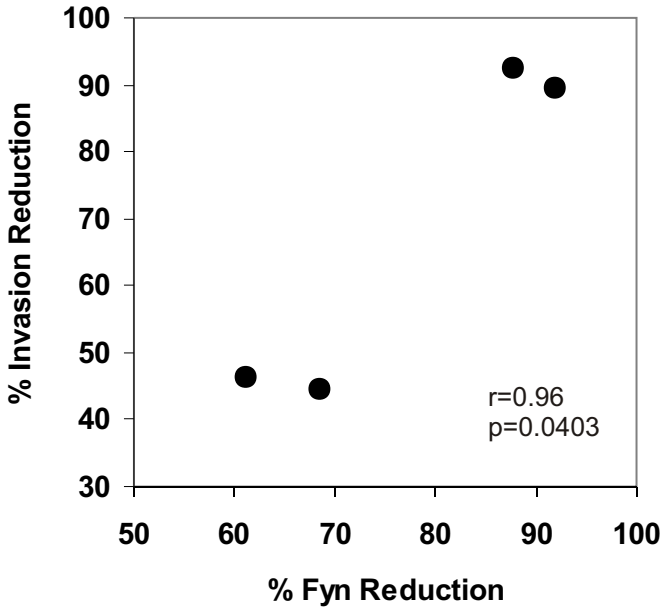
Supplementary Figure 2



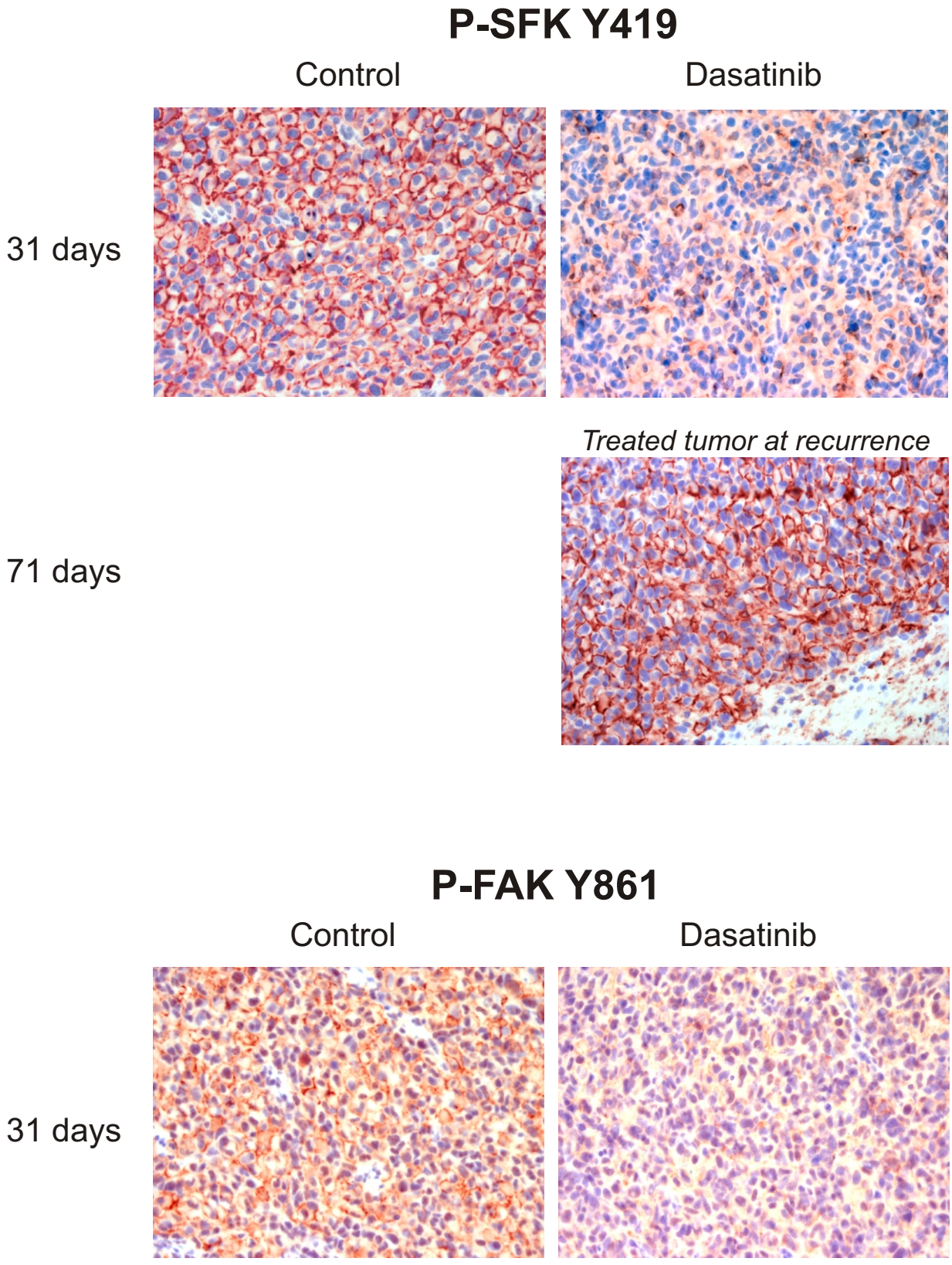
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6