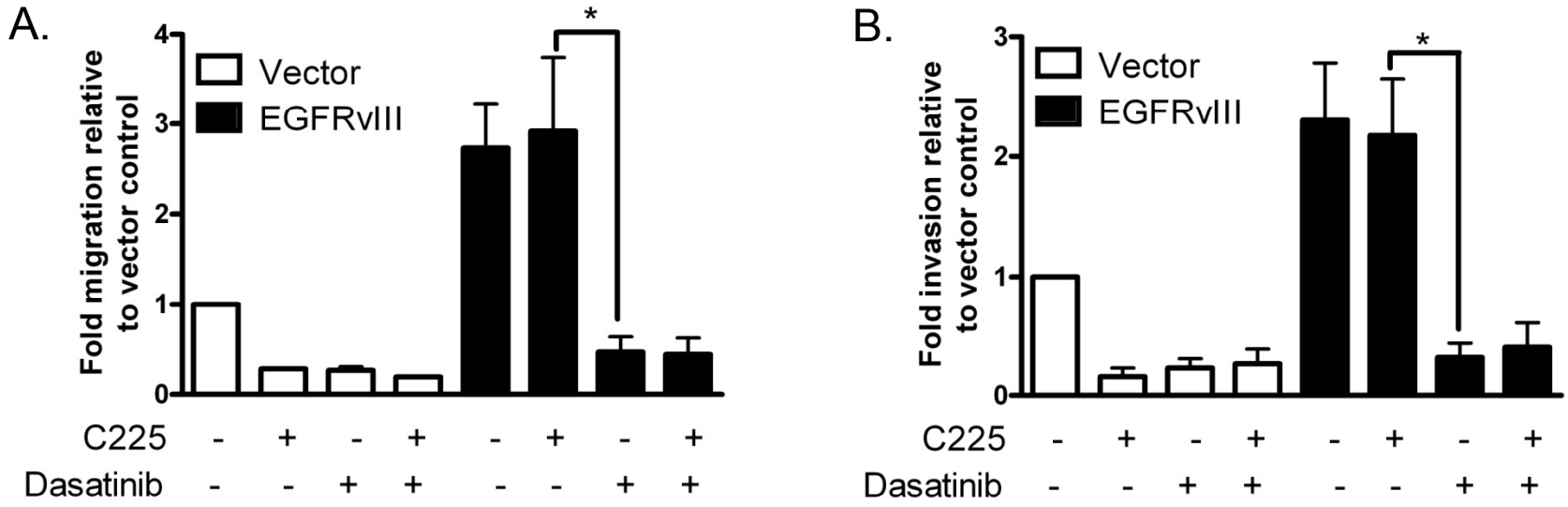
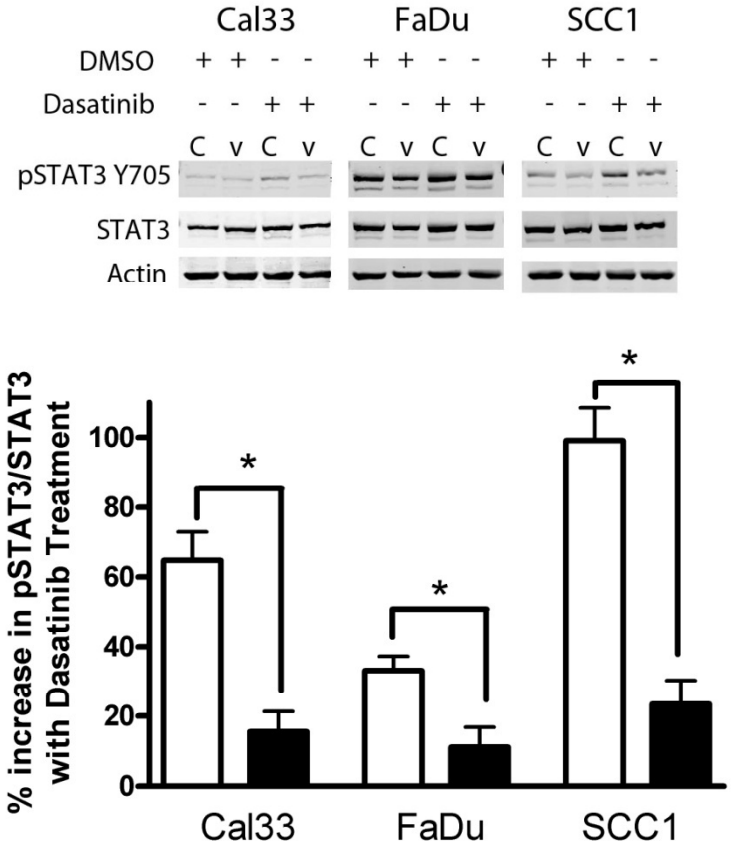


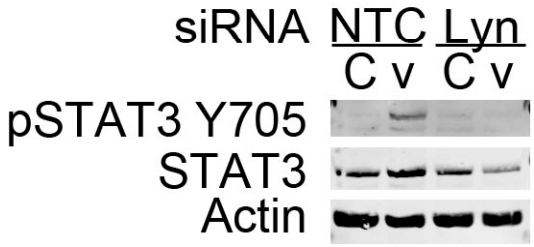
Dasatinib treatment decreases migration and invasion in EGFRvIII expressing HNSCC. The HNSCC cells (FaDu) expressing vector or EGFRvIII were assayed for migration and invasion as indicated in the materials and methods section. (A) Dasatinib reduces EGFRvIII-mediated cell migration. FaDu vector control (open bars) and EGFRvIII (closed bars) expressing cells were assayed for cell motility in the presence of DMSO or 100nM dasatinib (Das). The experiment was repeated 3 times and assessed for significance by the Mann-Whitney test: $p<0.05$. (B) Dasatinib reduces EGFRvIII-mediated cell invasion. FaDu vector control (open bars) and EGFRvIII (closed bars) expressing cells were assayed for cell invasion in the presence of DMSO or 100nM dasatinib (Das). The experiment was repeated 3 times and assessed for significance by the Mann-Whitney test: $p<0.05$.

Supplemental Figure 1

A.

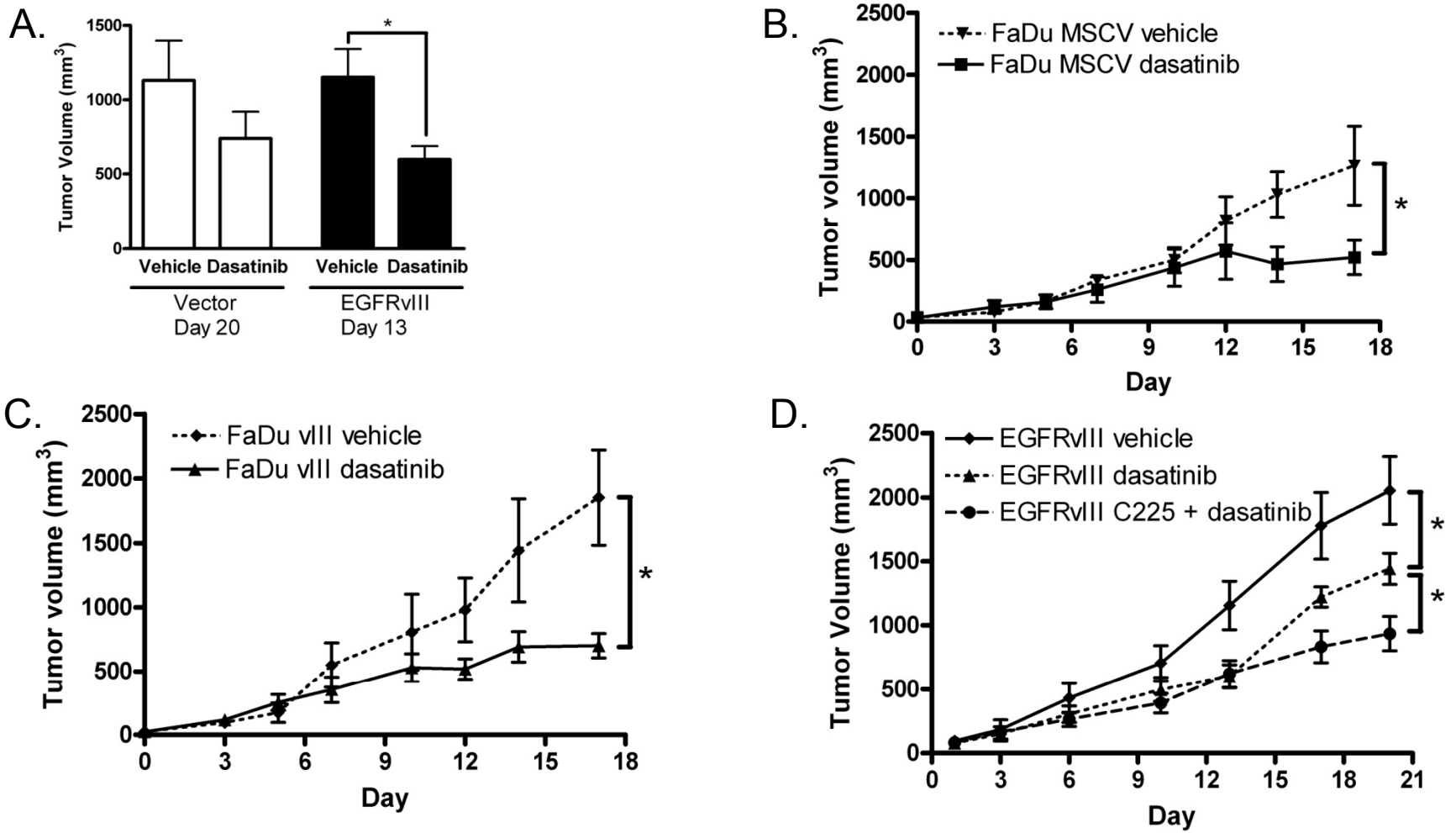


B.



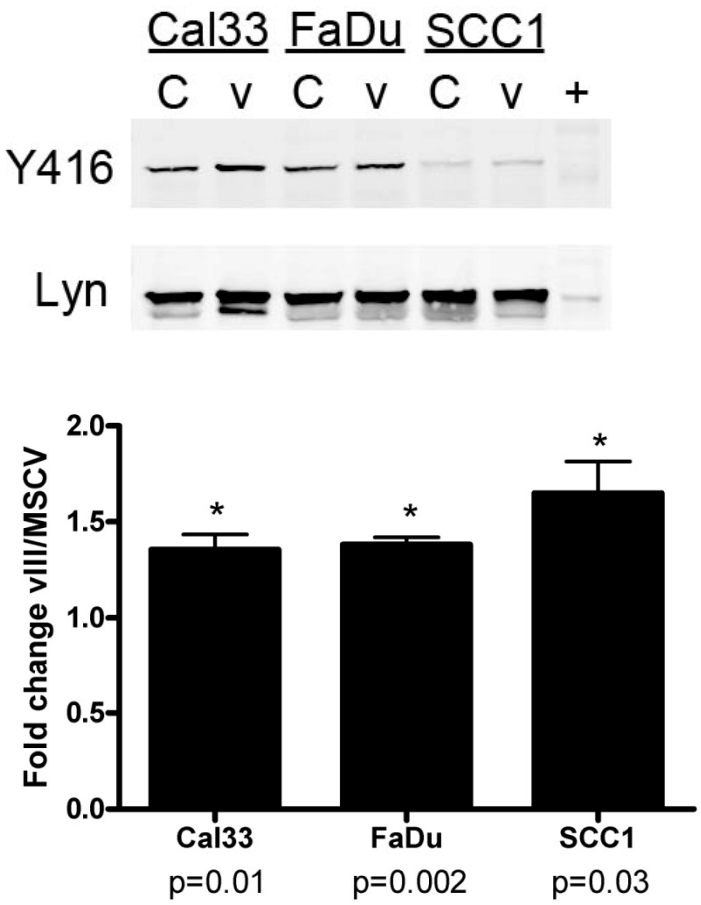
STAT3 expression in dasatinib and Lyn siRNA treated HNSCC cells. (A) EGFRvIII ('V') expressing and vector control ('C') HNSCC cells were cultured for 24 hours in the presence of DMSO or 100nM dasatinib and immunoblotted for STAT3 activation via phosphorylation (antibodies from Cell Signaling Technology: STAT3 and pSTAT3 Y705 XP). Increase in STAT3 phosphorylation with dasatinib treatment is graphed from near infrared measurments. Vector control cells displayed significantly more profound STAT3 activation than EGFRvIII expressing cells in response to dasatinib treatment. Experiment was repeated 3 times with similar results $p<0.05$ (B) UMSCC1 vector and EGFRvIII expressing cells were treated with Lyn siRNA for 48 hours and immunoblotted for expression of total and tyrosine phosphorylated STAT3. STAT3 phosphorylation was decreased in EGFRvIII expressing cells and appeared to be unaffected in vector control cells.

Supplemental Figure 2



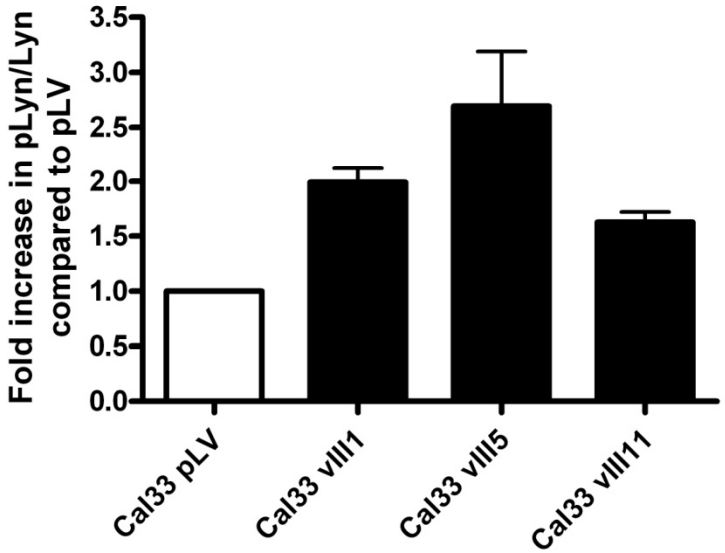
Dasatinib inhibits tumor growth in EGFRvIII expressing xenografts. (A) Tumor volumes from Day 20 of vector control xenografts are plotted with tumor volumes from day 13 of EGFRvIII xenografts to compare the effect of dasatinib treatment on similar tumor volumes at different time points (replot of Figure 4B&C). (B,C) Mice were measured and innoculated as described in materials and methods. $3x10^6$ cells were innoculated per flank of FaDu vector control (MSCV) or FaDu EGFRvIII (vIII). Tumors formed in 5 days. (B) Tumors were assessed for significance by the Mann-Whitney test on day 17: $p = 0.032$, n=5 per group (C) Mann-Whitney test on day 17: $p = 0.008$, n=5 per group. (D) Mice were innoculated, measured and randomized according to the proceedures in materials and methods dividing the mice into 3 groups of 8. Mice were treated with either 80mM citric acid in PBS by oral gavage, dasatinib (50mg/kg daily) by oral gavage, or cetuximab (0.8mg twice/week) by intraperitoneal injection and dasatinib (50mg/kg daily). Day 20 significance was calculated by the Mann-Whitney test: $p = 0.005$ for dasatinib compared to combination treatment.

Supplemental Figure 3



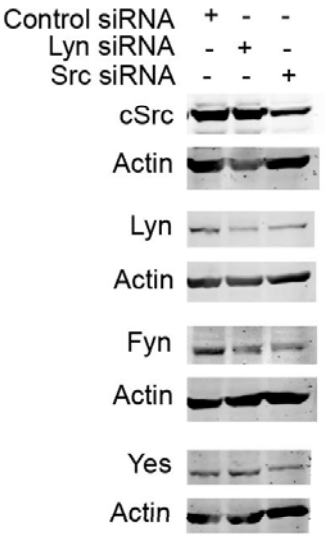
Increased Lyn phosphorylation in EGFRvIII-expressing HNSCC compared to vector-control cells. Cell lines were immunoprecipitated as outlined in materials and methods and 10ug of positive control loaded (K562 cell lysate (Santa Cruz Biotechnology), denoted by "+"). Near infrared quantification was used to determine the fold increase in pLyn for EGFRvIII/vector control cells. Single sample t-test was used to calculate respective p-values. n=4 for each cell line.

Supplemental Figure 4



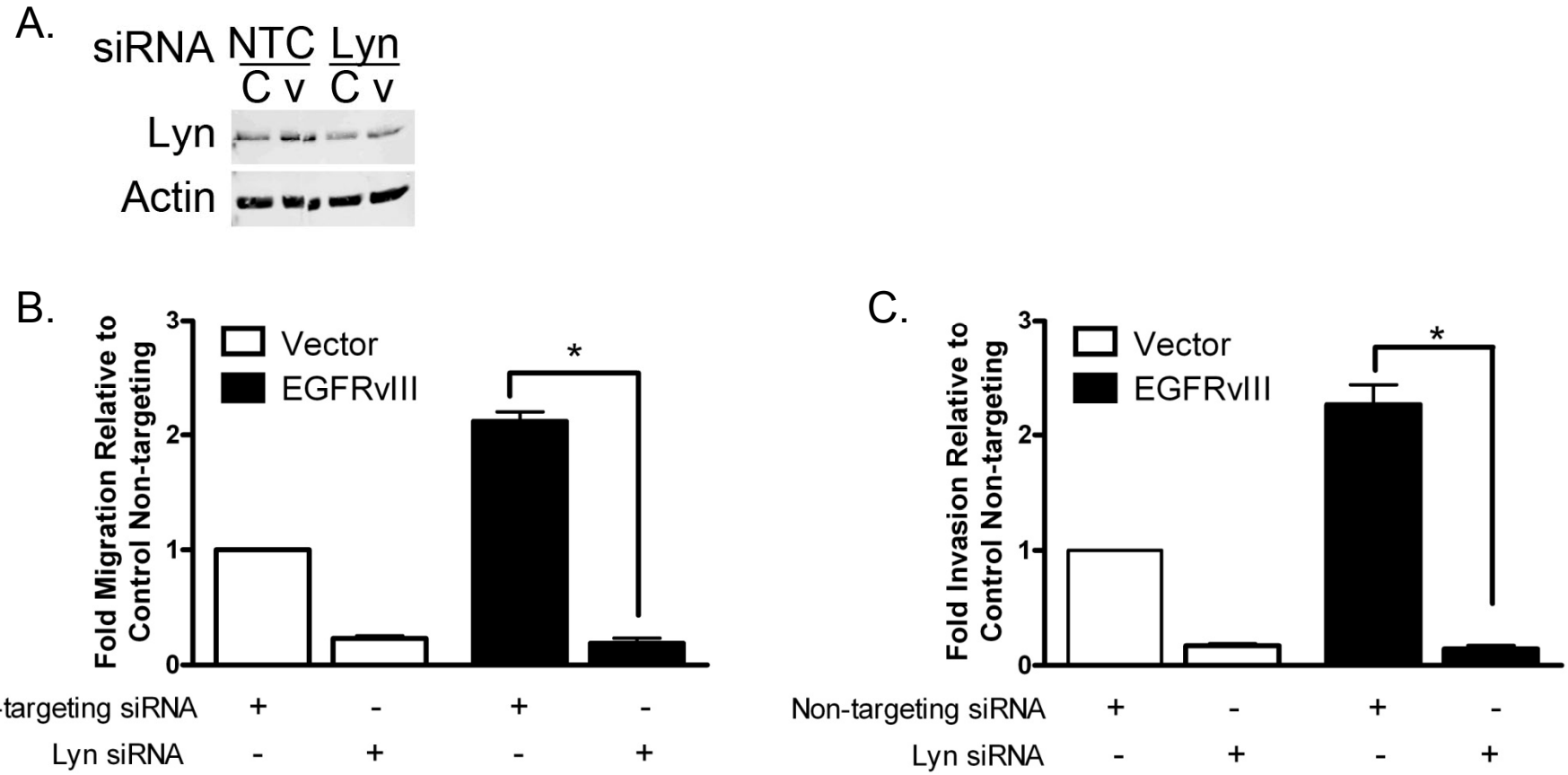
Increase in Lyn phosphorylation in three separate clones of HNSCC cell lines Cal33. Cell lysates from three separate clones of Cal33 cells stably transfected with EGFRvIII were prepared and immunoprecipitated with Lyn antibody as indicated in the materials and methods section. Immunoprecipitates were immunoblotted for phosphorylation using a pan phosphotyrosine antibody (PY99, Santa Cruz Biotechnology). Densitometric quantification was used to calculate the fold change in EGFRvIII expressing clones (closed bars) compared to the vector control clone (pLV, open bar, n=4).

Supplemental Figure 5



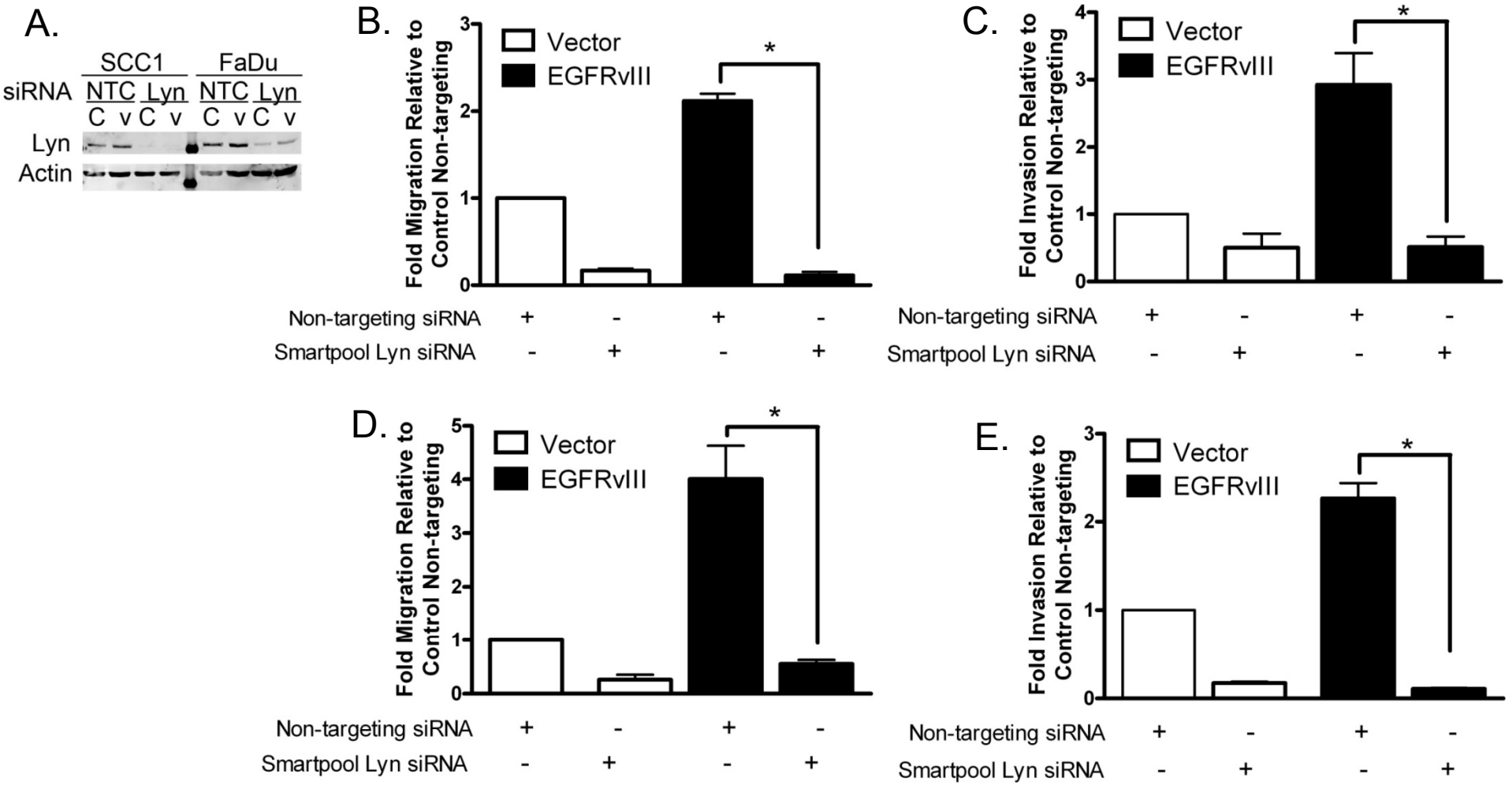
Lyn siRNA specificity. UMSCC1 cells were transfected with control, Src or Lyn siRNA for 48 hours. Cell lysates were probed for cSrc, Lyn, Fyn and Yes (antibodies listed in the materials and methods section) to test siRNA specificity.

Supplemental Figure 6



Knockdown of Lyn inhibits EGFRvIII-mediated HNSCC migration and invasion. (A) FaDu vector and EGFRvIII expressing cells were treated with Lyn siRNA for 48 hours and immunoblotted to confirm Lyn knockdown. (B) FaDu vector control (open bars) and EGFRvIII expressing (closed bars) cells were assayed for cell motility in the presence of non-targeting siRNA or Lyn siRNA. The experiment was repeated 3 times and assessed for significance by the Mann-Whitney test p=0.05. (C) FaDu vector control (open bars) and EGFRvIII expressing (closed bars) cells were assayed for cell invasion in the presence of non-targeting siRNA or Lyn siRNA. The experiment was repeated 3 times and assessed for significance by the Mann-Whitney test p=0.05.

Supplemental Figure 7



Knockdown of Lyn with a second siRNA confirms inhibition of EGFRvIII-mediated HNSCC migration and invasion. (A) UMSCC1/FaDu vector and EGFRvIII expressing cells were treated with Lyn smartpool siRNA (Dharmacon; Lafayette, CO) for 48 hours and immunoblotted to confirm Lyn knockdown. (B) UMSCC1 vector control and EGFRvIII expressing cells were assayed for cell motility in the presence of non-targeting siRNA or Lyn siRNA. The experiment was repeated 3 times and assessed for significance by the Mann-Whitney test p=0.05. (C) UMSCC1 vector control and EGFRvIII expressing cells were assayed for cell invasion in the presence of non-targeting siRNA or Lyn siRNA. n=3; Mann-Whitney p=0.05 (D) FaDu vector control and EGFRvIII expressing cells were assayed for cell motility in the presence of non-targeting siRNA or Lyn siRNA. n=3; Mann-Whitney p=0.05 (E) FaDu vector control and EGFRvIII expressing cells were assayed for cell invasion in the presence of non-targeting siRNA or Lyn siRNA. n=3; Mann-Whitney p=0.05.

Supplemental Figure 8