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Supplementary Materials for

JAK2 inhibition sensitizes resistant EGFR-mutant lung adenocarcinoma to tyrosine kinase inhibitors

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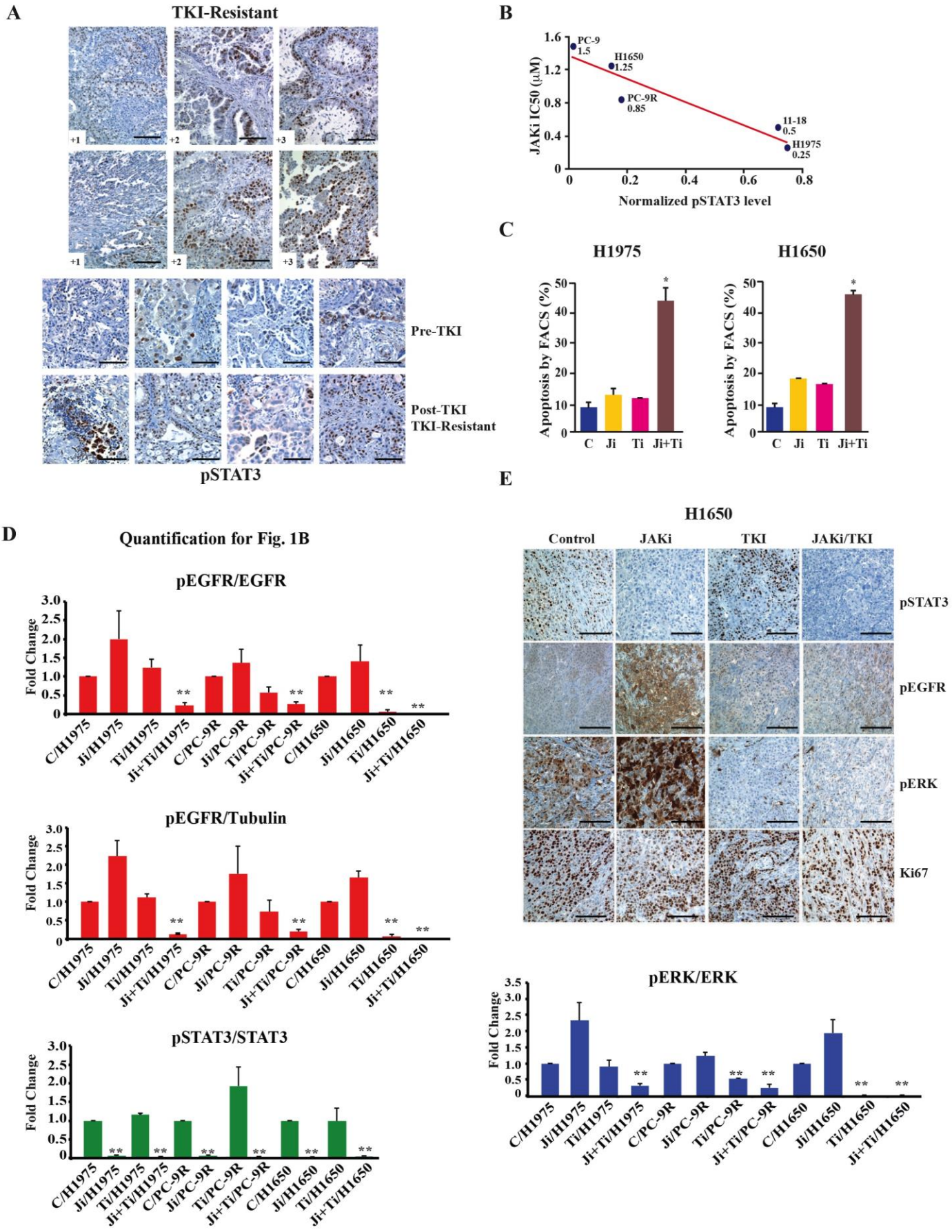
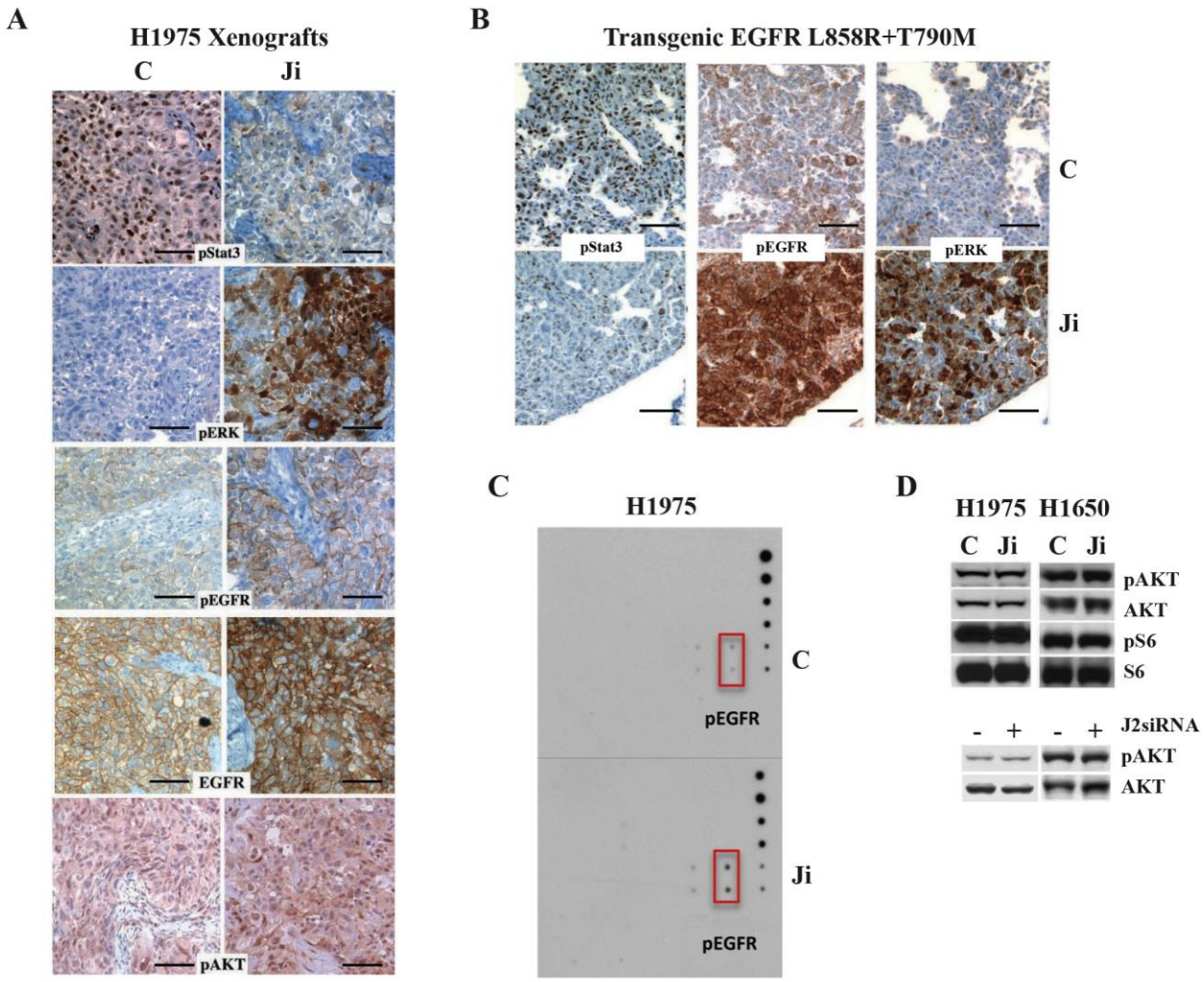

A
TKI-Resistant
+1
+2
+3
Pre-TKI
Post-TKI
TKI-Resistant
pSTAT3
B
JAKi IC50 (μM)
Normalized pSTAT3 level
PC-9
1.5
H1650
1.25
PC-9R
0.85
11-18
0.5
H1975
0.25
C
H1975
H1650
Apoptosis by FACS (%)
C
Ji
Ti
Ji+Ti
D
Quantification for Fig. 1B
pEGFR/EGFR
pEGFR/Tubulin
pSTAT3/STAT3
Fold Change
C/H1975
Ji/H1975
Ti/H1975
Ji+Ti/H1975
C/PC-9R
Ji/PC-9R
Ti/PC-9R
Ji+Ti/PC-9R
C/H1650
Ji/H1650
Ti/H1650
Ji+Ti/H1650
E
H1650
Control
JAKi
TKI
JAKi/TKI
pSTAT3
pEGFR
pERK
Ki67
pERK/ERK

Fig. S1. STAT3 activation and inhibition in TKI-resistant NSCLC. (**A**) Increased abundance of phosphorylated STAT3 (pSTAT3) in TKI-resistant lung cancer specimens. Ten examples of TKI-resistant lung adenocarcinomas were stained for pSTAT3 by IHC. Four samples were matched, where we had tumor prior to erlotinib therapy (Pre-TKI) and upon progression (Post-TKI or TKI-Resistant) Scale bar 100 μm. (**B**) Assessent of growth inhibition and sensitivity to JAK inhibition in relation to pSTAT3 abundance. Correlation of the IC_{50} of AZD1480 (JAKi) in NSCLC cell lines with pSTAT3 abundance. (**C**) EGFR/JAK inhibition increased apoptosis in NSCLC cells. H1975 or H1650 cells were incubated with indicated drugs and combinations for 6 days: Ji: AZD1480-1μM for H1650 and 125nM for H1975; Ti: erlotinib 125nM. Apoptosis was assessed by flow cytometry. Data are mean ± SEM of 3 independent experiments. * $p < 0.05$, Ji+Ti vs. Ji. (**D**) Quantification of blots from Fig. 1B, from three biological replicates. Comparisons made to control. Students 2-tailed t-test ** $p<0.01$. (**E**) Tumor sections from H1650 xenografts treated for 25 days as indicated in Figure 1B were stained for pSTAT3, pEGFR, pERK and Ki67. Scale bar 100 μm.


A
H1975 Xenografts
C
Ji
pStat3
pERK
pEGFR
EGFR
pAKT
B
Transgenic EGFR L858R+T790M
pStat3
pEGFR
pERK
C
Ji
C
H1975
pEGFR
C
pEGFR
Ji
D
H1975 H1650
C Ji C Ji
pAKT
AKT
pS6
S6
- + - + J2siRNA
pAKT
AKT

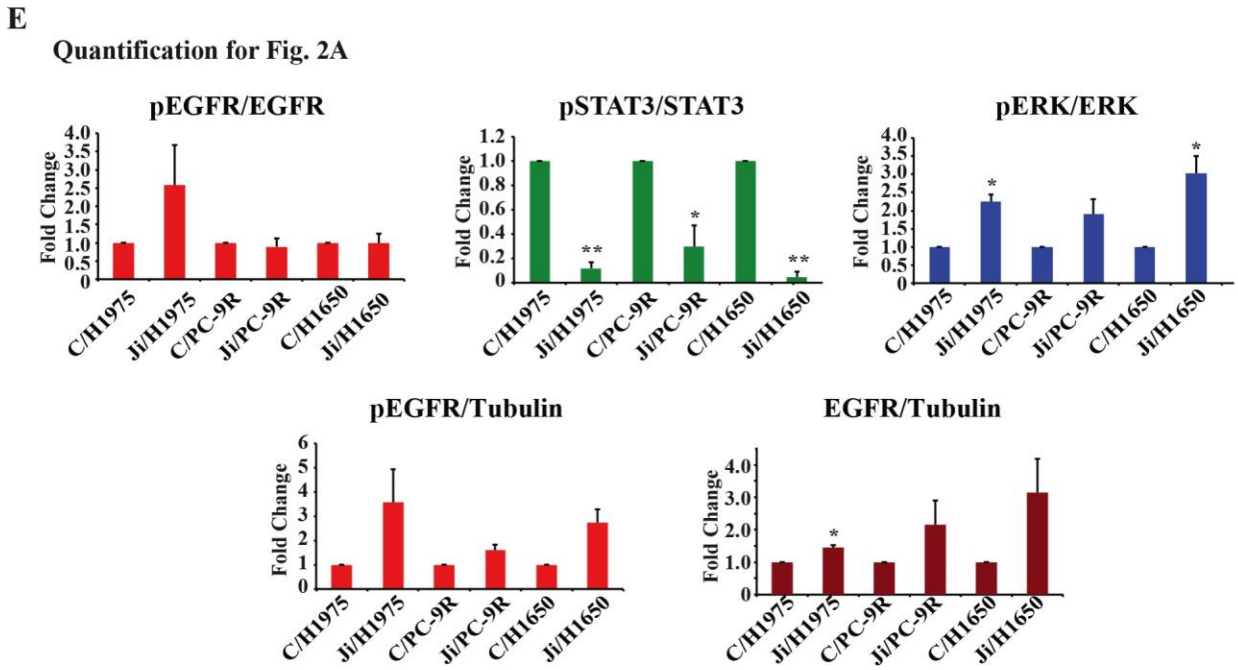

E
Quantification for Fig. 2A
pEGFR/EGFR
pSTAT3/STAT3
pERK/ERK
Fold Change
C/H1975
Ji/H1975
C/PC-9R
Ji/PC-9R
C/H1650
Ji/H1650
pEGFR/Tubulin
EGFR/Tubulin

Fig. S2. JAK2 inhibition increases the abundance of pEGFR and pERK in NSCLC. (**A**) Representative sections from H1975 xenografts treated with AZD1480 (Ji) or vehicle control (C) were stained for phosphorylated STAT3 (pSTAT3), pERK, pEGFR, EGFR and pAKT. Scale bar 100 μm. (**B**) Sections from lung tumors in EGFR L858R+T790M mice treated with AZD1480 (Ji) or vehicle (C) for 6 h were stained for pSTAT3, pEGFR and pERK, Scale bar 100 μm (**C**) RTK array analysis of cell lysates from H1975 treated with Control (C) or Ji. (**D**) Lysates from H1975 and H1650 cells treated with control (C) or AZD1480 (Ji) for 1 hour; or transfected with JAK2 siRNA (JAK2siRNA+) or Scrambled control (JAKsiRNA-) for 36 h, were analyzed for pAKT, AKT, pS6, S6. (**E**) Quantification of blots from Fig. 2A, from three biological replicates. Statistical comparisons made to control. Students 2-tailed t-test * $p < 0.05$; ** $p < 0.01$

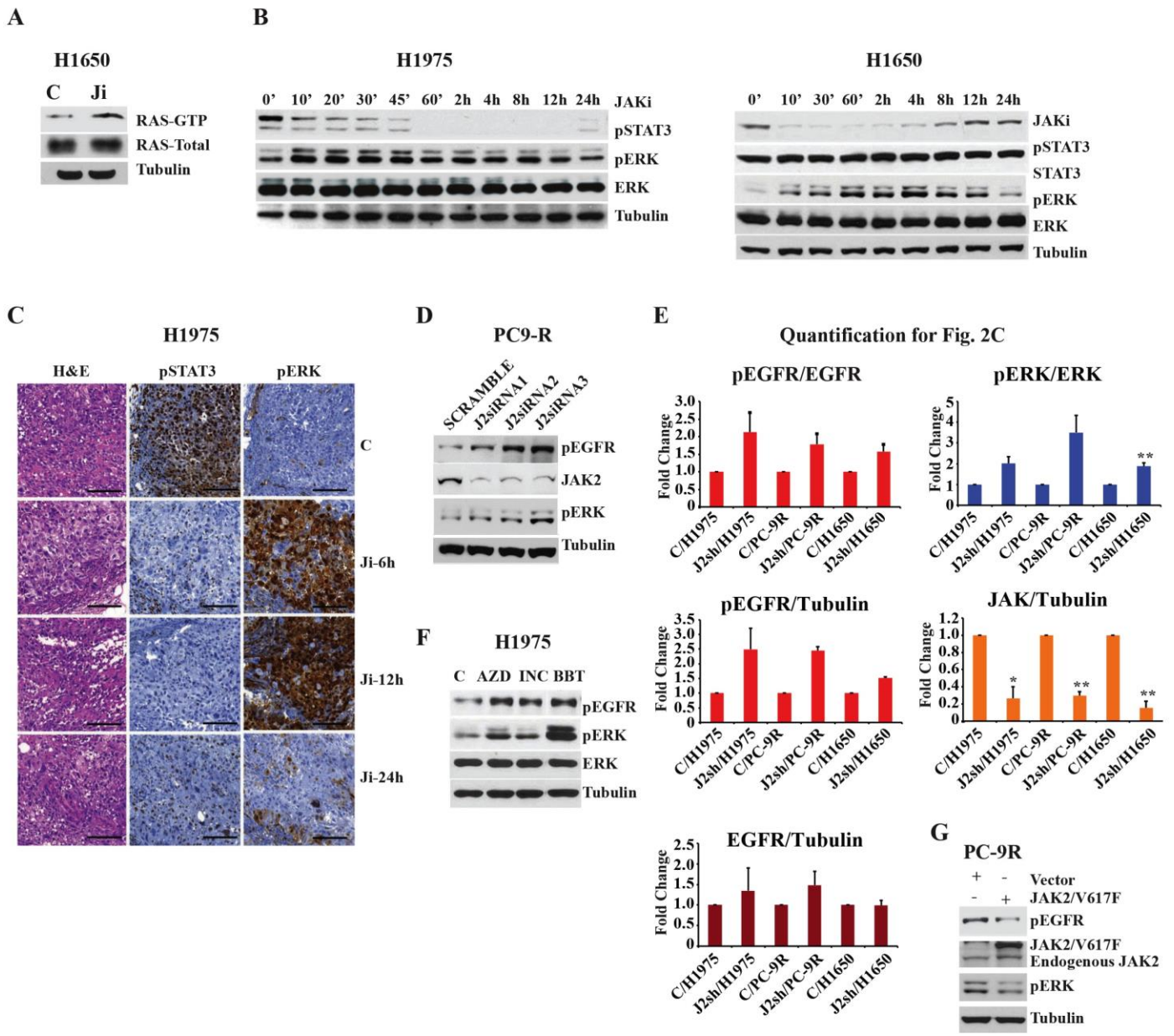


Fig. S3. JAK2 inhibition leads to rapid activation of EGFR/RAS/ERK signaling. (**A**) Western blot analyses of GTP-bound Ras (Ras-GTP), total Ras (Ras-Total) and Tubulin for cell lysates from H1650 cells treated with Ji or control (C) for 1 hour. (**B**) Western blot analyses for pSTAT3, STAT3, pERK, ERK and Tubulin (loading control) of cell lysates from H1975 and H1650 cells treated with JAKi for the indicated times. (**C**) Sections from H1975 xenografts treated with AZD1480 (Ji) or vehicle control (C) for 6, 12, 24 hours were analyzed by H&E staining and immunohistochemistry for pSTAT3 and pERK. Scale bar 100 μm (**D**) Cell lysates from PC-9R cells transfected with 3 different JAK2 siRNAs were analyzed for pEGFR, JAK2, pERK and Tubulin. (**E**) Quantification of blots from Fig. 2C, from three biological replicates. Statistical comparisons made to control. Students 2-tailed t-test * $p < 0.05$; ** $p < 0.01$ (**F**) Lysates from H1975 cells treated with Control (C) and JAK inhibitors: AZD1480 (1 μM, AZD), INCB18424 (0.5 μM, INC), or BBT594 (1 μM, BBT) for 1 hour, were analyzed for pEGFR, pERK, ERK and Tubulin. (**G**) Activated JAK2 decreased EGFR/pEGFR levels in PC-9R cells. Lysates from PC-9R cells transiently transfected with pBABE vector alone (Vector) or an activated form of JAK2 (JAK2/V617F) were analyzed for pEGFR, JAK2, pERK and Tubulin.

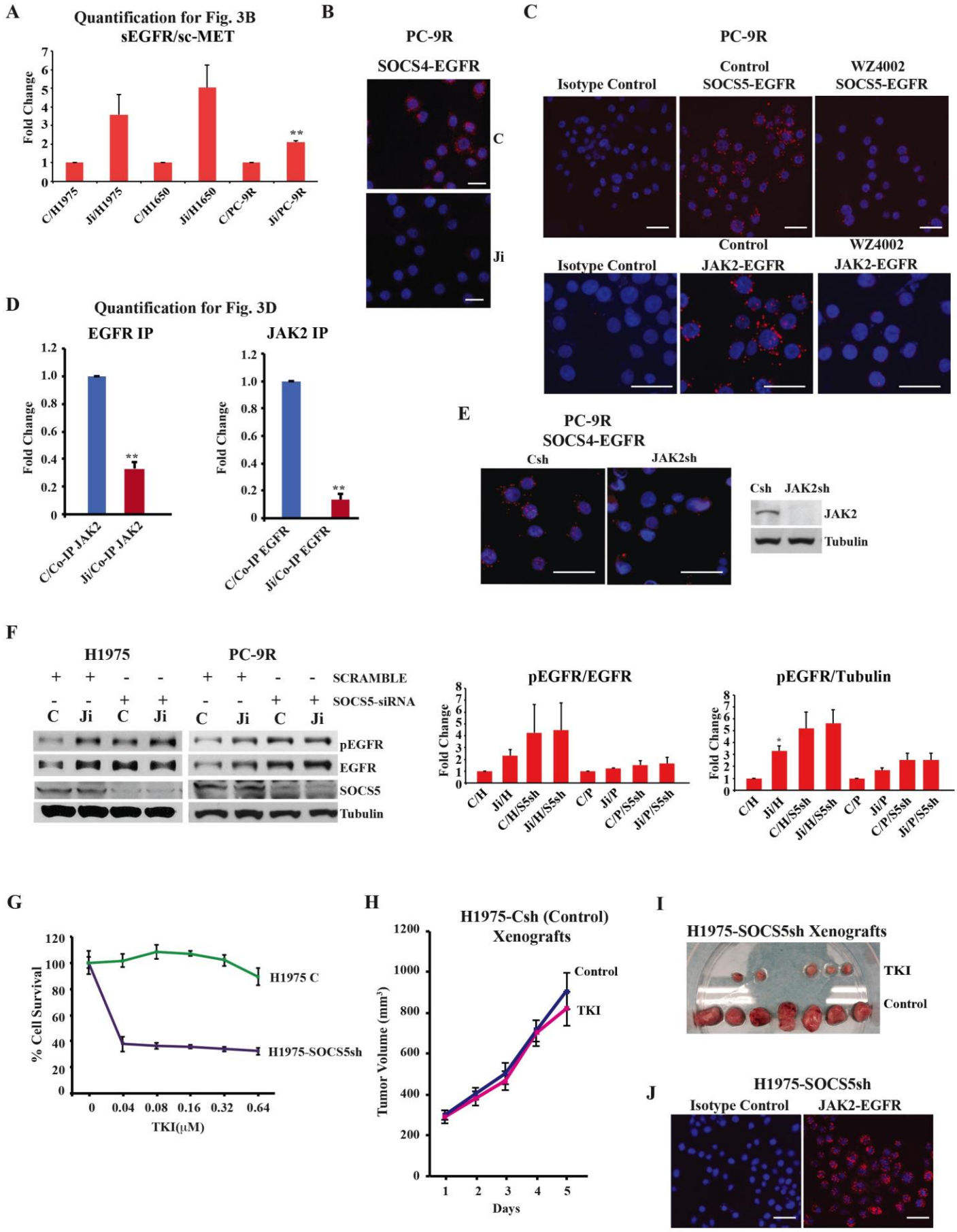

A
Quantification for Fig. 3B
sEGFR/sc-MET
Fold Change
C/H1975
Ji/H1975
C/H1650
Ji/H1650
C/PC-9R
Ji/PC-9R
B
PC-9R
SOCS4-EGFR
C
Ji
C
PC-9R
Isotype Control
Control
SOCS5-EGFR
WZ4002
SOCS5-EGFR
Isotype Control
Control
JAK2-EGFR
WZ4002
JAK2-EGFR
D
Quantification for Fig. 3D
EGFR IP
JAK2 IP
Fold Change
C/Co-IP JAK2
Ji/Co-IP JAK2
C/Co-IP EGFR
Ji/Co-IP EGFR
E
PC-9R
SOCS4-EGFR
Csh
JAK2sh
Csh
JAK2sh
JAK2
Tubulin
F
H1975
PC-9R
SCRAMBLE
SOCS5-siRNA
C
Ji
C
Ji
C
Ji
C
Ji
pEGFR
EGFR
SOCS5
Tubulin
pEGFR/EGFR
pEGFR/Tubulin
Fold Change
C/H
Ji/H
C/H/S5sh
Ji/H/S5sh
C/P
Ji/P
C/P/S5sh
Ji/P/S5sh
G
% Cell Survival
H1975 C
H1975-SOCS5sh
TKI(μM)
H
H1975-Csh (Control)
Xenografts
Tumor Volume (mm³)
Control
TKI
Days
I
H1975-SOCS5sh Xenografts
TKI
Control
J
H1975-SOCS5sh
Isotype Control
JAK2-EGFR

Fig. S4. JAK2 links SOCS5 to EGFR, regulating sensitivity to TKI (**A**) Quantification of blots from Fig. 3B, from three biological replicates. Statistical comparisons made to control. Students 2-tailed t-test ** $p<0.01$. (**B**) PC-9R cells, treated with JAKi (Ji) or Control (C) for 1 hour, were analyzed for SOCS4 and EGFR interactions by Duolink© staining. Scale bar 50 μm (**C**) PC-9R cells treated with Control or a T790M mutant specific TKI (WZ4002) and analyzed for SOCS5-EGFR and JAK2-EGFR interactions by Duolink© staining. Isotype control antibodies were used as a negative control. Scale bar 50 μm (**D**) Quantification of blots from Fig. 3D, from three biological replicates. Statistical comparisons made to control. Students 2-tailed t-test ** $p<0.01$ (**E**) PC-9R cells expressing JAK2shRNA (J2sh) or vector control (Csh) constructs were analyzed for SOCS4 and EGFR interactions by Duolink staining. Scale bar 50 μm. Cell lysates were analyzed for JAK2 and Tubulin. (**F**) Cell lysates from H1975 and PC-9R cells transfected with scrambled control (-) or SOCS5siRNA (+) treated with Ji or control (C) for 1 hour and analyzed for pEGFR, EGFR, SOCS5 and Tubulin. Quantification of blots from three biological replicates is shown. Statistical comparisons made to control. Students 2-tailed t-test * $p<0.05$. (**G**) H1975 Controlsh (C) and H1975-SOCS5sh cells were treated with increasing concentrations of erlotinib (TKI) and cell survival was determined by MTT. (**H**) H1975-Csh xenografts were treated with vehicle (C) and TKI (25mg/kg/day) for 5 days. Tumor volumes were determined daily. Data are mean ± SEM (n=5-7/group). **(I**) Isolated tumors from H1975-SOCS5sh tumor bearing mice treated with vehicle (control) or TKI (from Fig. 3G). (**J**) H1975-SOCS5sh cells were analyzed for JAK2 and EGFR interactions by Duolink staining. Scale bar 50 μm.

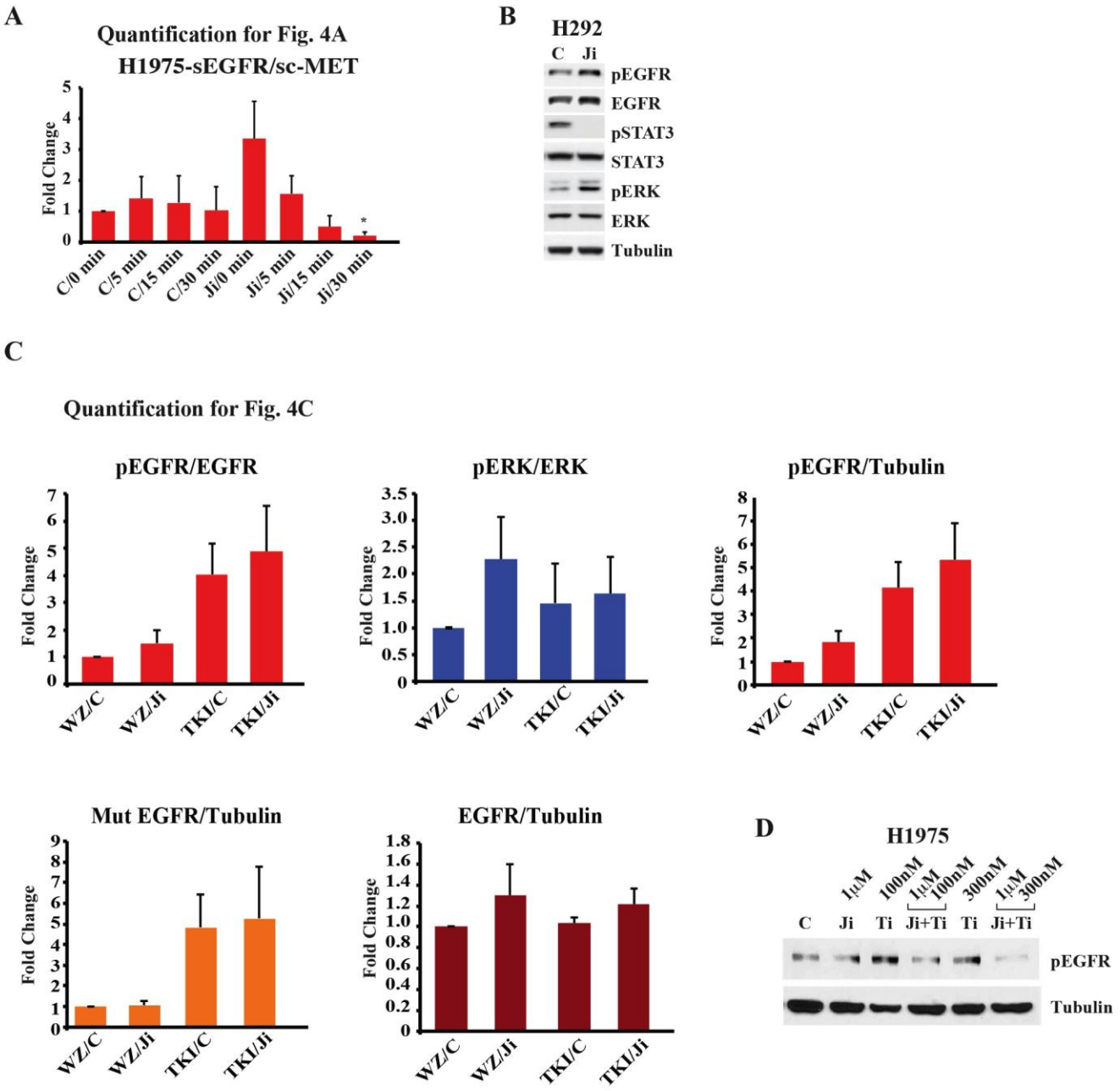


Fig. S5. Quantification of Western blots in Fig. 4, A and C. (**A**) Quantification of blots from Fig. 4A, from three biological replicates. Statistical comparisons made to control. Students 2-tailed t-test * $p<0.05$. (**B**) Western blot for pEGFR, EGFR, pSTAT3, STAT3, pERK, ERK and Tubulin in lysates from H292 cells treated with control (C) or JAK inhibitor (Ji). (**C**) Quantification of blots from Fig. 4C, from three biological replicates. Statistical comparisons made to control. (**D**) Western blot analyses of pEGFR and Tubulin in cell lysates from serum-starved H1975 cells treated with Ji (1μM) for 1 hour, followed by EGF for 30 min, in the presence of erlotinib (Ti) at the indicated concentrations.

A

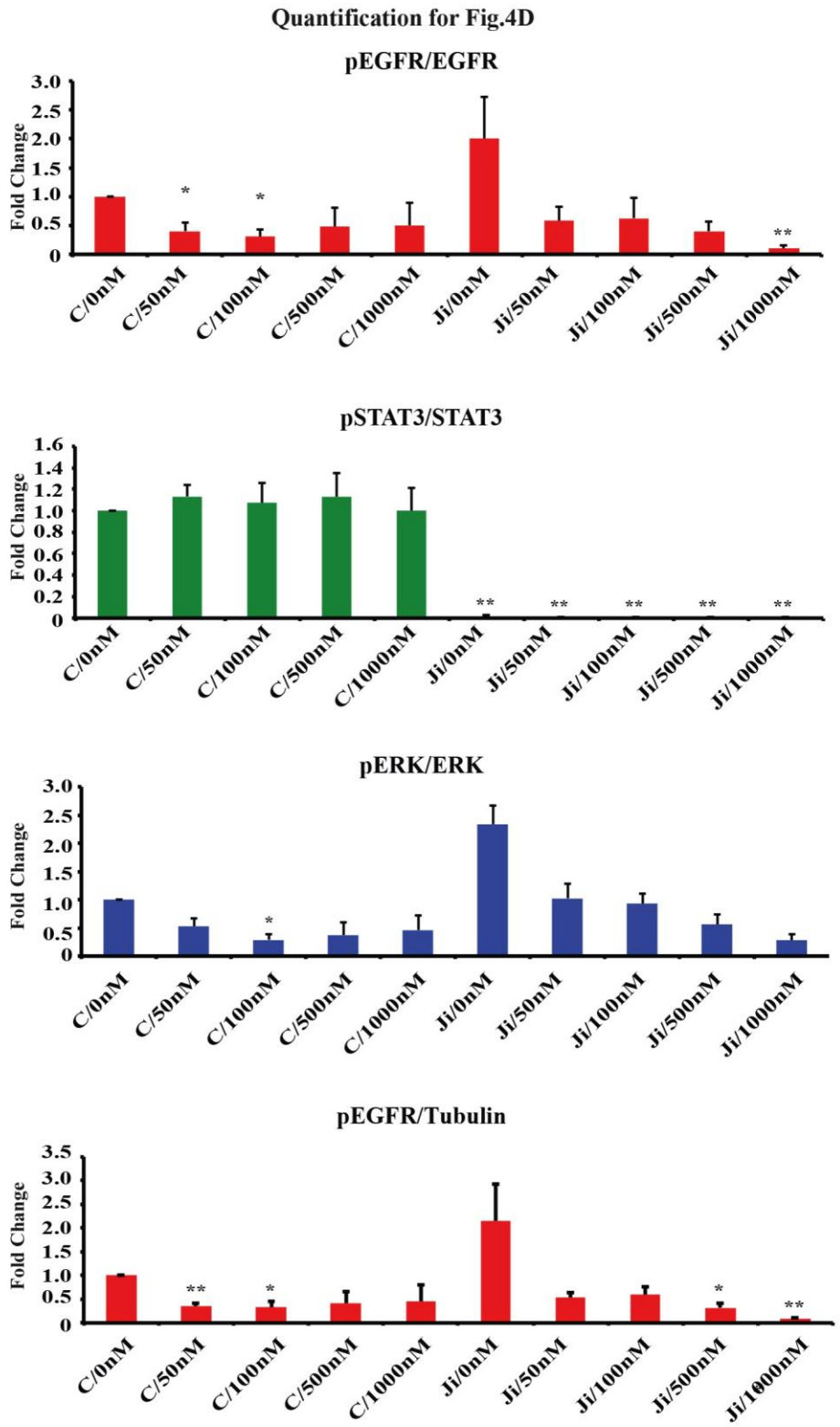


Fig. S6. **Quantification of Western blots in Fig. 4D.** (**A**) Quantification of blots from Fig. 4D, from three biological replicates. Statistical comparisons made to control. Students 2-tailed t-test * $p < 0.05$; ** $p < 0.01$.