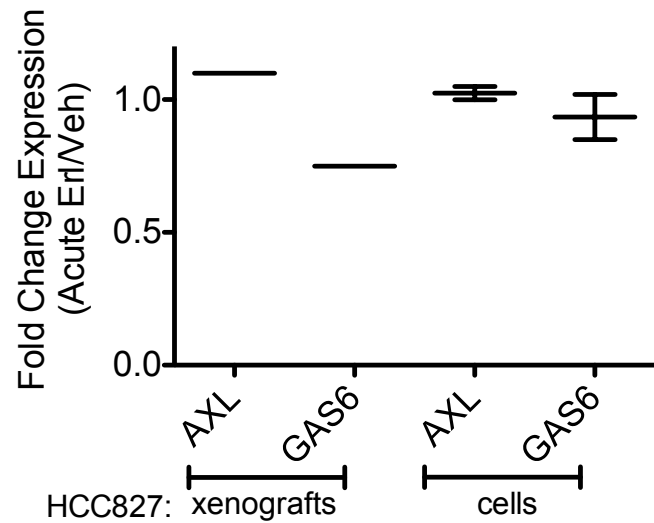


Activation of the *AXL* Kinase Causes Resistance to *EGFR*-Targeted Therapy in Lung Cancer

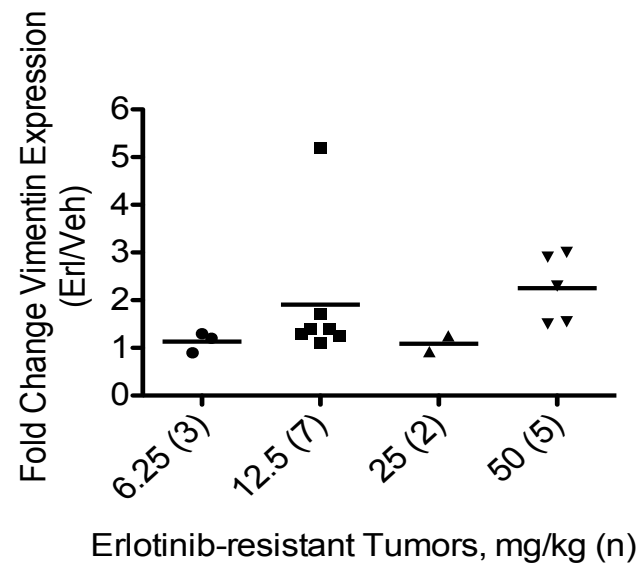
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Supplementary Figure 1.



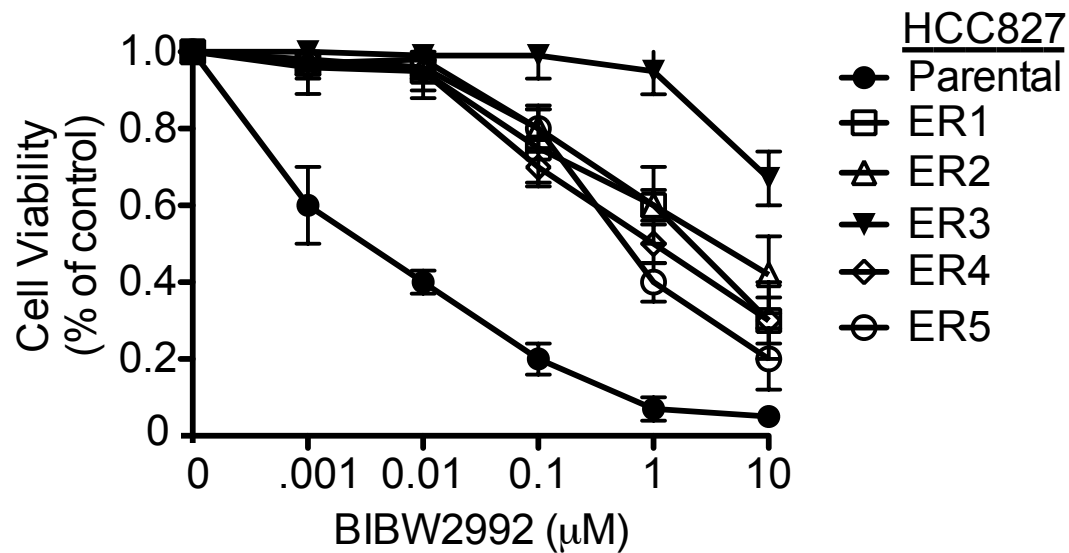
Supplementary Figure 1. Increased AXL and GAS6 levels were not observed in HCC827 tumors or cell lines following acute (48h) treatment with erlotinib. mRNA expression of AXL and GAS6 was measured by Q-RT-PCR and is expressed in erlotinib treated samples relative to vehicle treated samples. n=3 for xenograft and cell line analysis. Data are expressed as mean \pm SEM.

Supplementary Figure 2.



Supplementary Figure 2. mRNA expression of vimentin in HCC827 erlotinib resistant tumor xenografts (treated at the indicated erlotinib doses) relative to vehicle-treated control tumors. The number of tumors analyzed from each treatment cohort is indicated in parentheses. Data are expressed as the mean \pm SEM of the fold change relative to the mean expression of the genes in 2 vehicle treated control xenograft tumors.

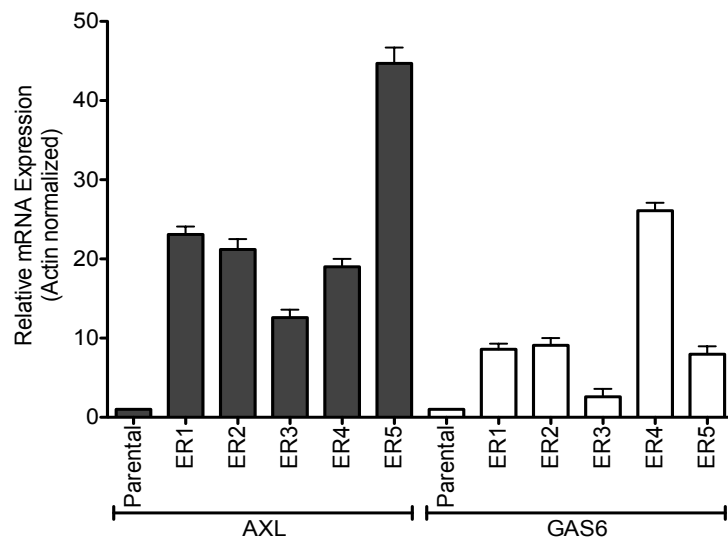
Supplementary Figure 3.



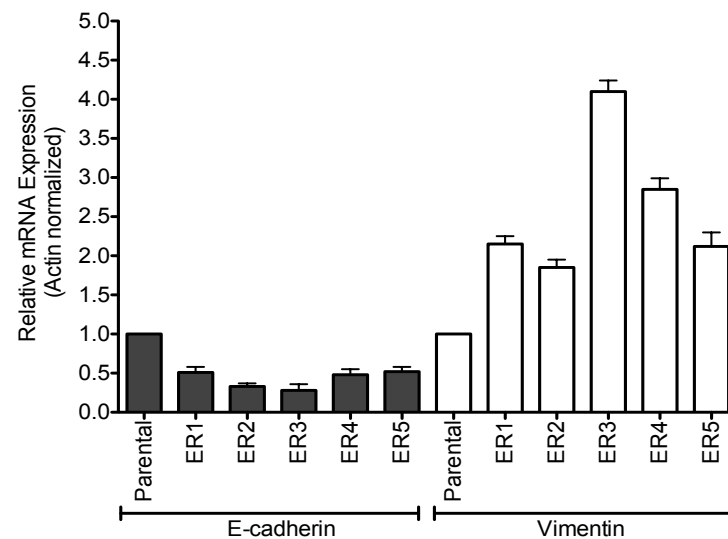
Supplementary Figure 3. HCC827 ER1-ER5 sublines are resistant to treatment with the irreversible EGFR TKI BIBW2992, as measured by CellTiterGLO cell viability assay. Data are from 3 independent experiments and are expressed as percent of vehicle treated cells and mean \pm SEM.

Supplementary Figure 4.

a

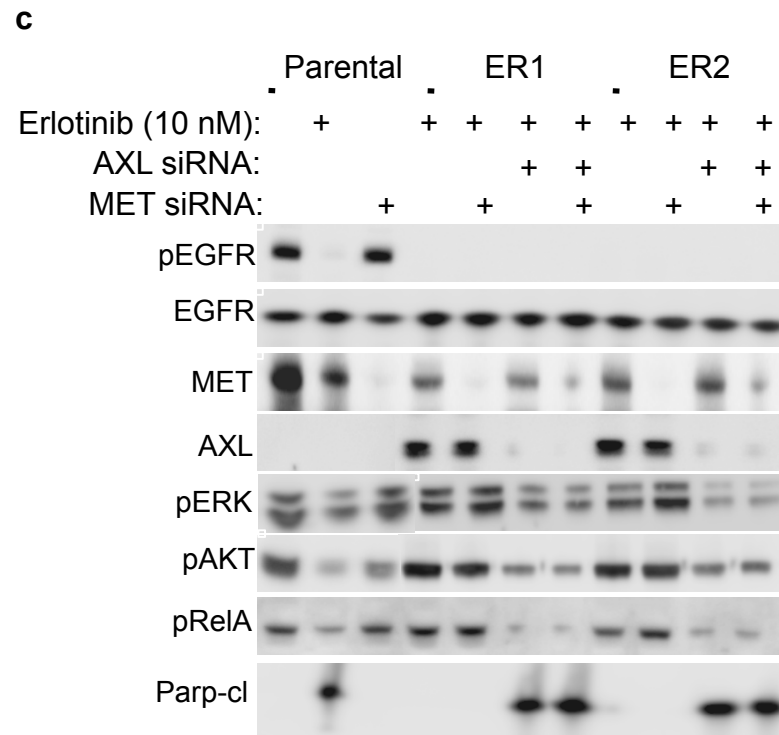
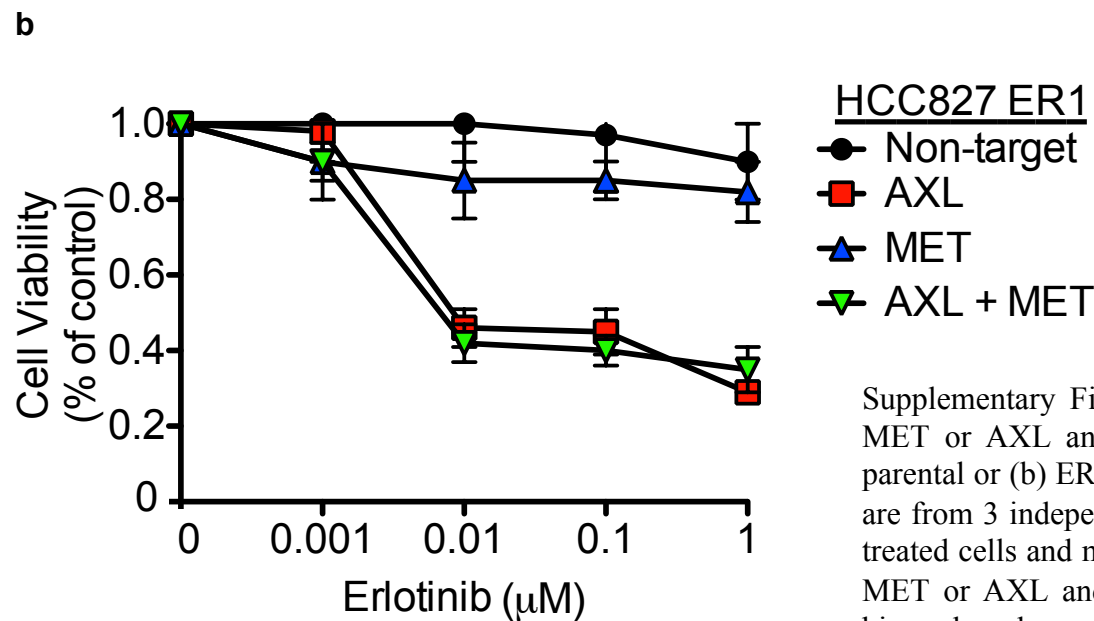
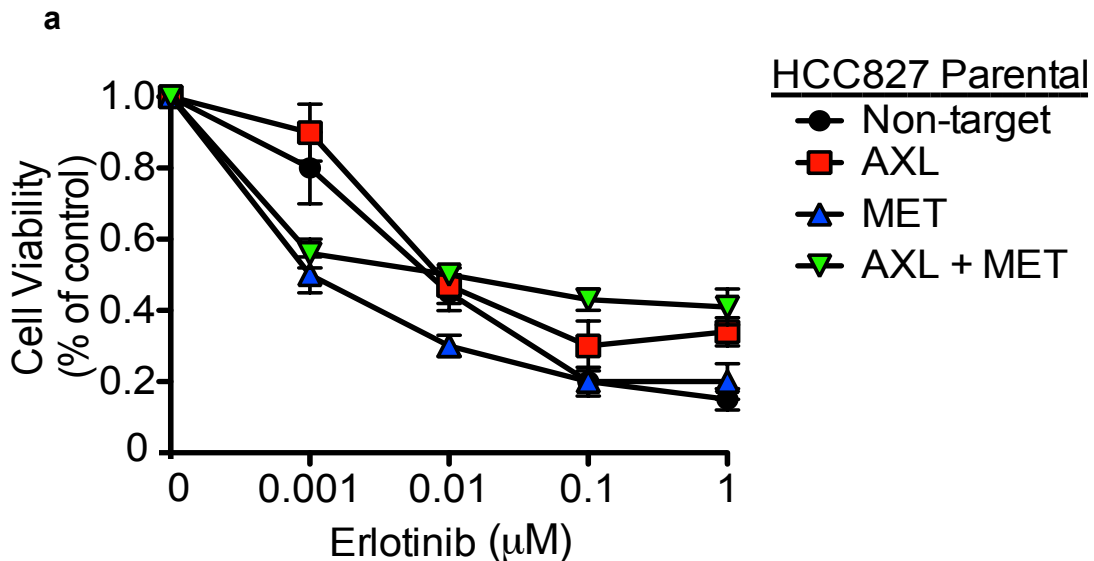


b



Supplementary Figure 4. Expression of the indicated genes by Q RT PCR in the indicated HCC827 cell lines. Data are from 3 independent experiments and are actin normalized and expressed relative to parental cells as the mean \pm SEM.

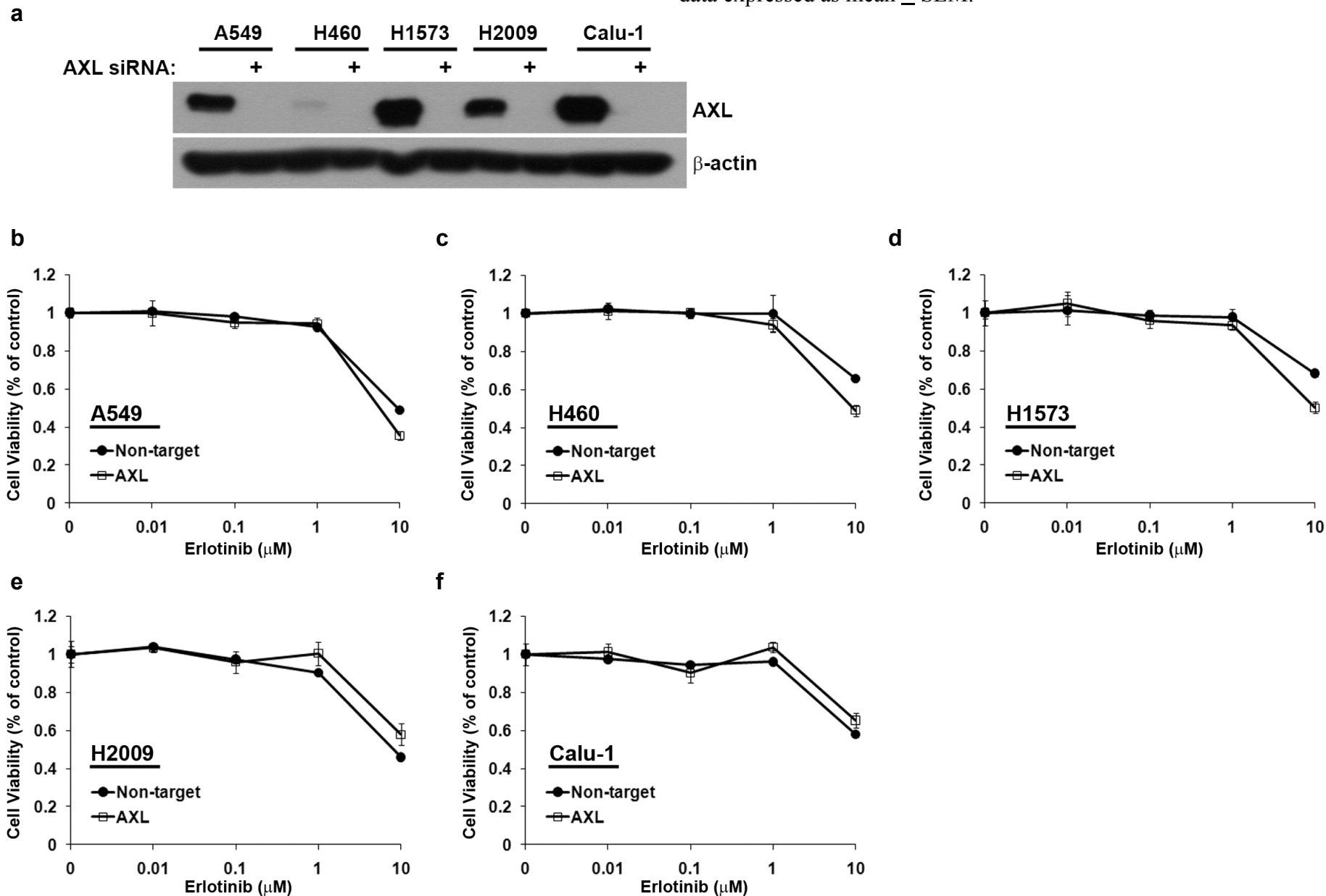
Supplementary Figure 5.



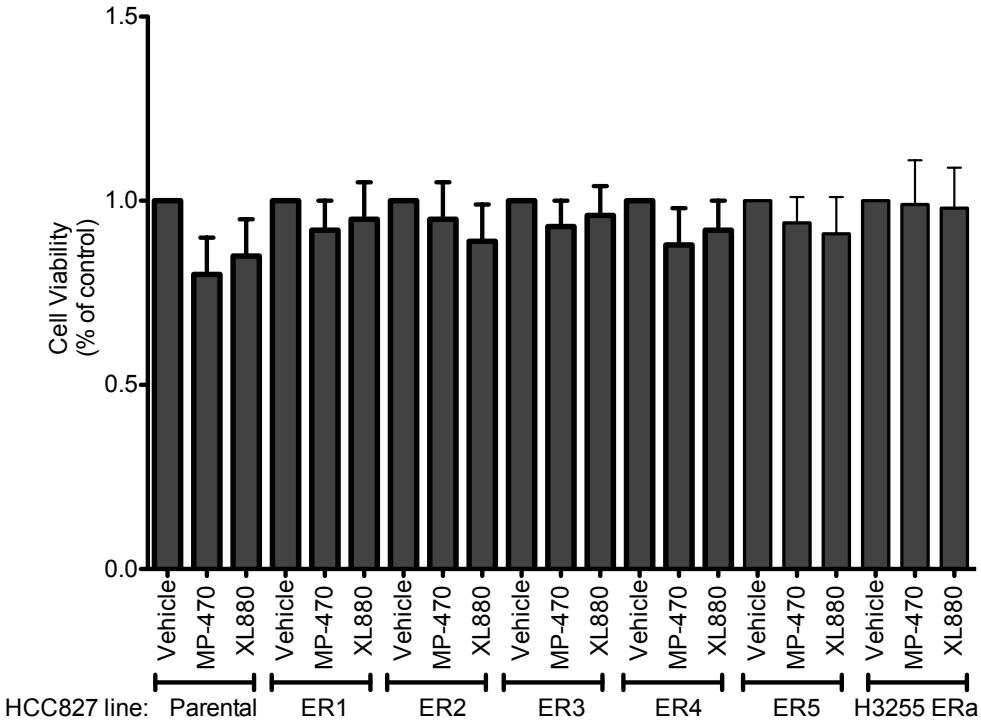
Supplementary Figure 5. a-b, Effect of treatment with non-target, AXL, MET or AXL and MET siRNAs on erlotinib sensitivity in (a) HCC827 parental or (b) ER1 cells, as measured by CellTiterGLO viability assay. Data are from 3 independent experiments and are expressed as percent of vehicle treated cells and mean \pm SEM. c, Effect of treatment with non-target, AXL, MET or AXL and MET siRNAs and erlotinib on the indicated signaling biomarkers by western blot on lysates from treated HCC827 parental, ER1 and ER2 cells. Data represent 3 independent experiments.

Supplementary Figure 6.

Supplementary Figure 6. a-f, Effect of AXL knockdown on the indicated human NSCLC cell lines that express wild type EGFR. n=3, data expressed as mean \pm SEM.

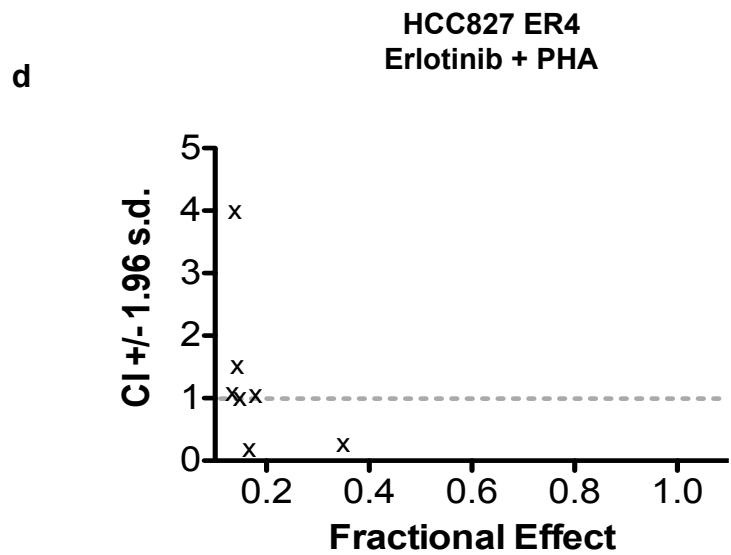
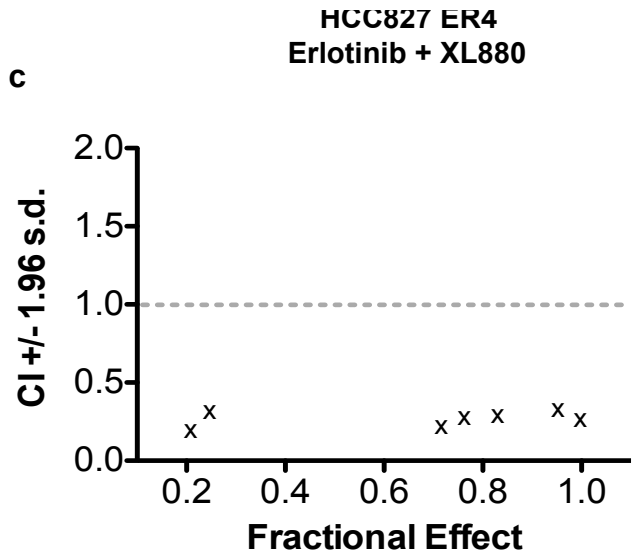
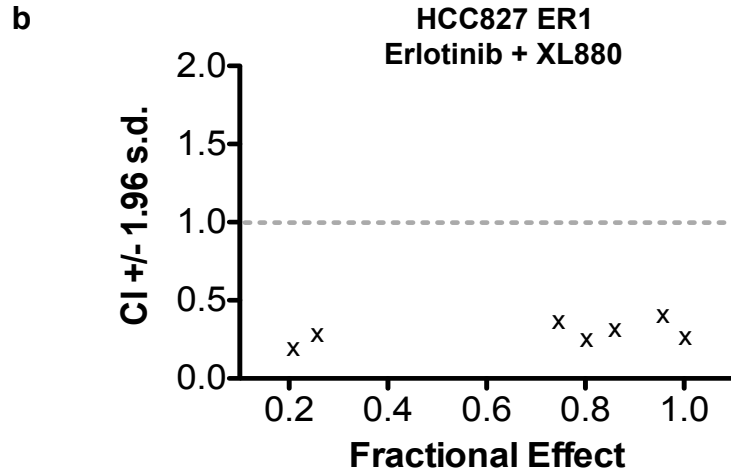
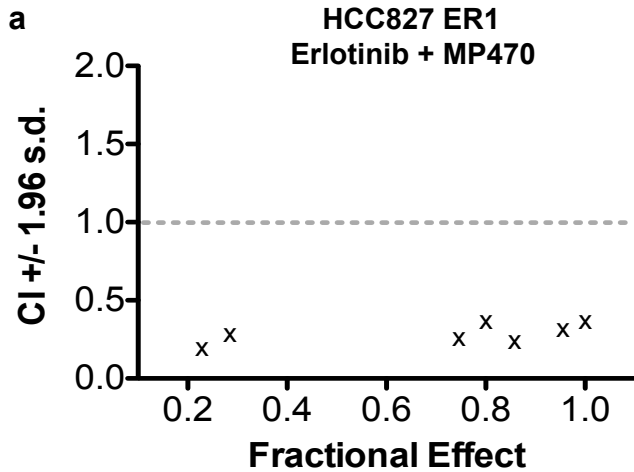


Supplementary Figure 7.



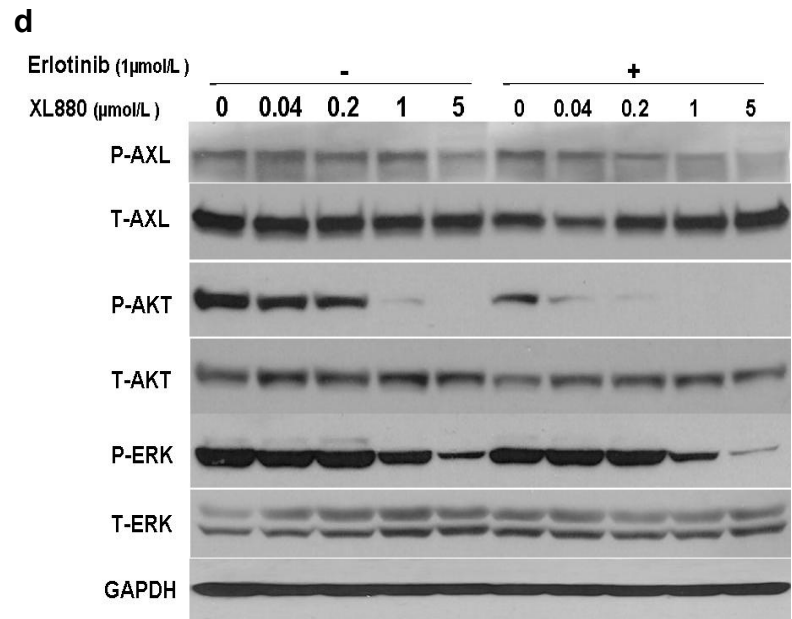
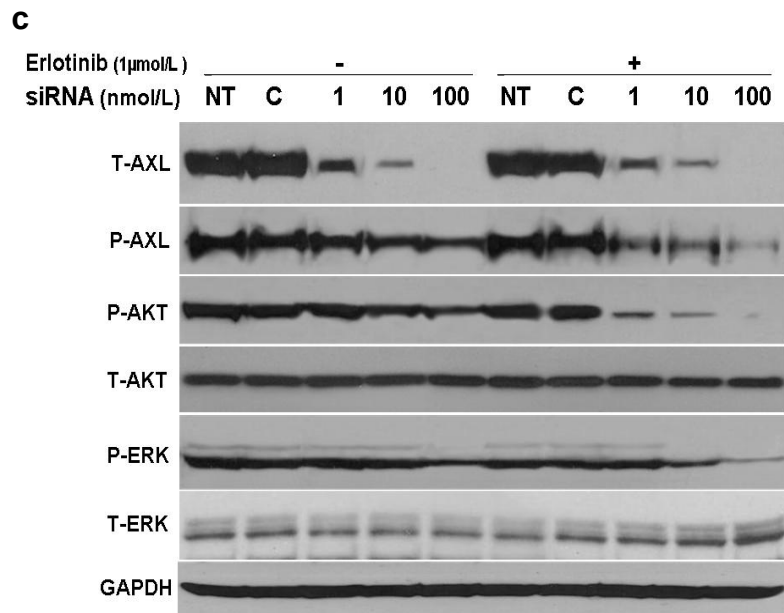
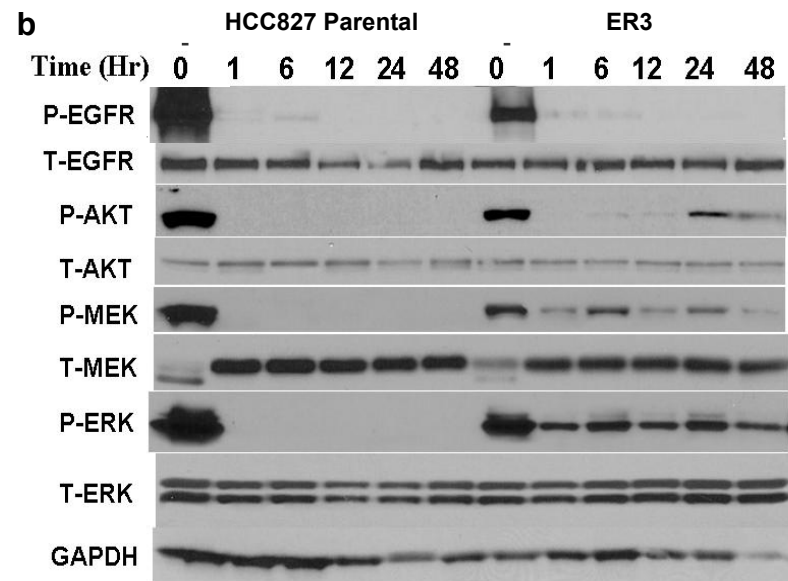
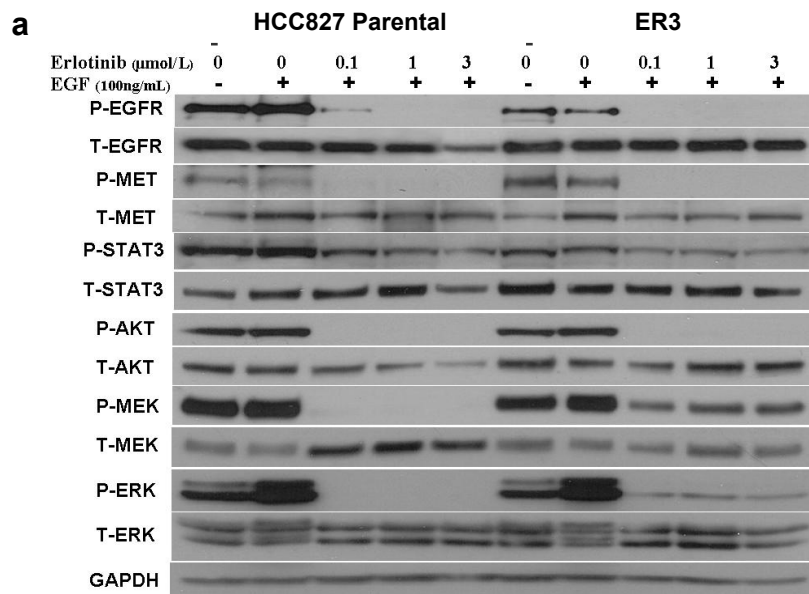
Supplementary Figure 7. Effect of single-agent treatment with either vehicle or MP-470 or XL-880, each at 1 mM, on cell viability in the indicated cell lines as measured by CellTiterGLO assay. n=3, data expressed as mean \pm SEM.

Supplementary Figure 8.



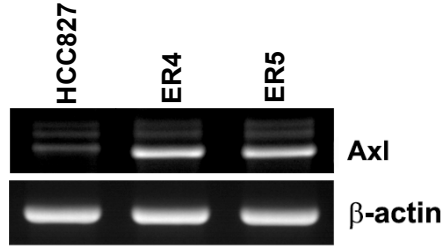
Supplementary Figure 8. a-d, Combination treatment with XL880 and erlotinib, but not PHA and erlotinib, leads to a synergistic decrease in cell viability by combination index analysis. CI values of less than 1, 1, and greater than 1 indicate synergism, additive effect, and antagonism, respectively. The hashed line marks CI = 1. Data are from 3 independent experiments.

Supplementary Figure 9.

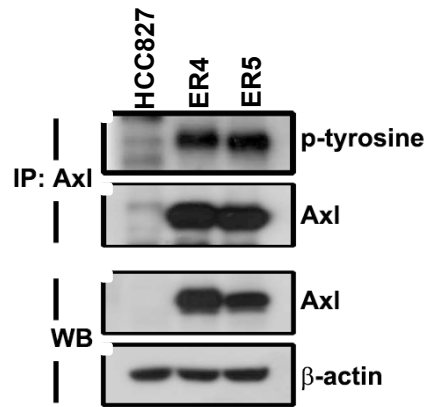


Supplementary Figure 9.

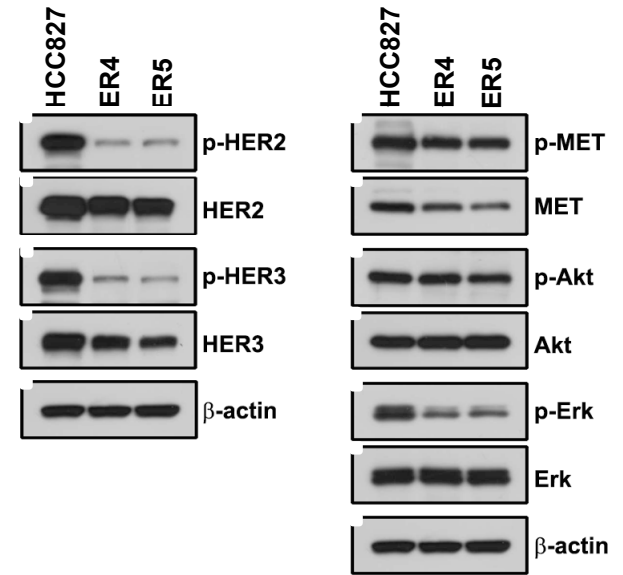
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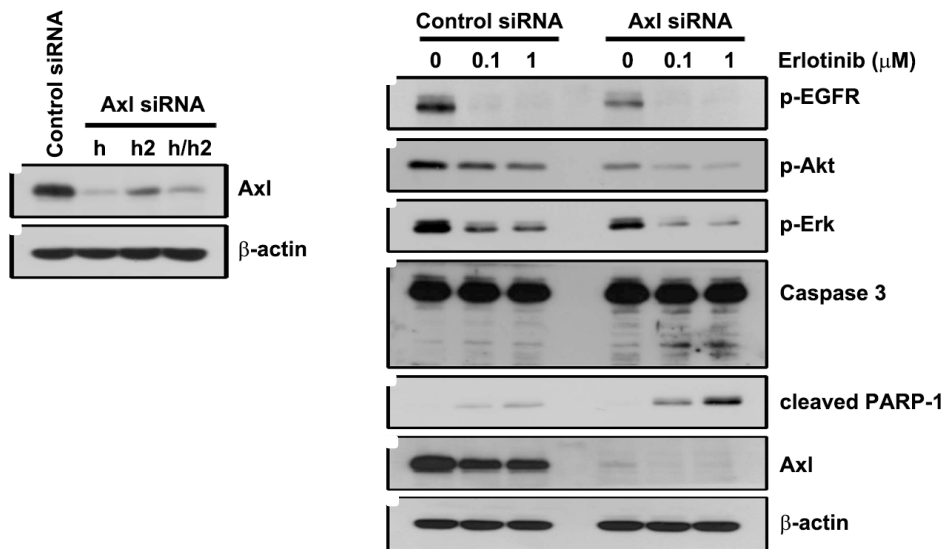
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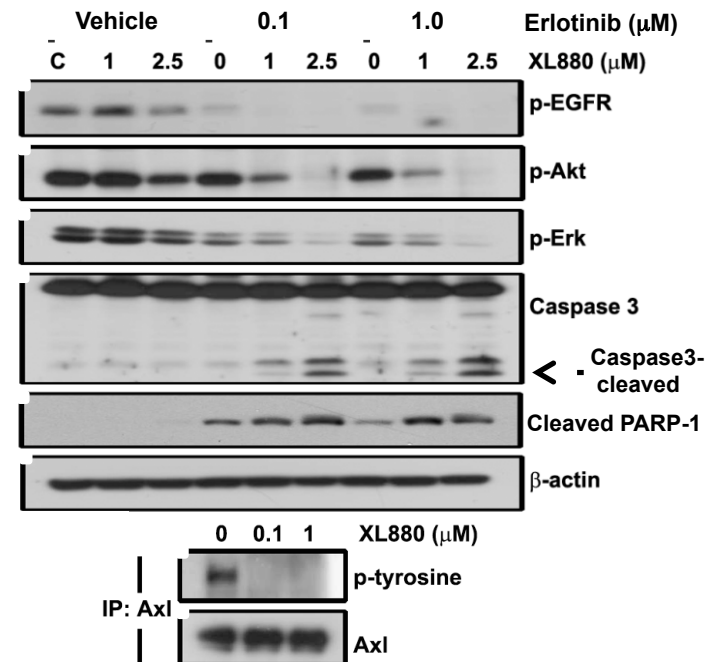
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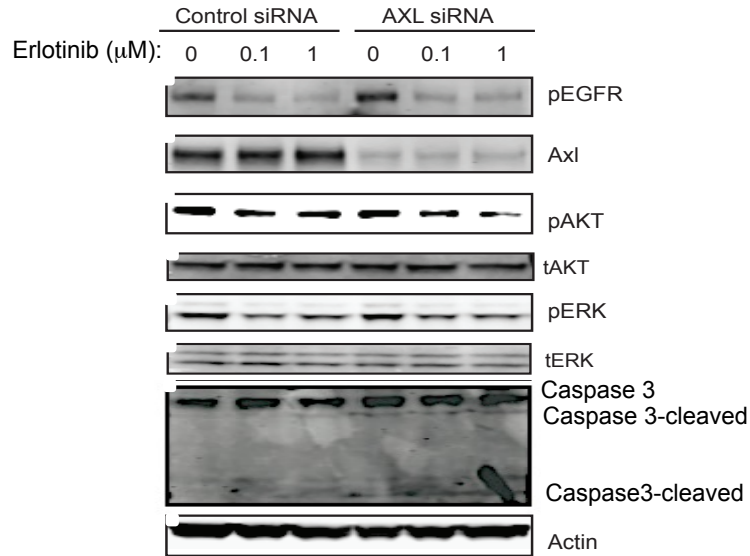


i

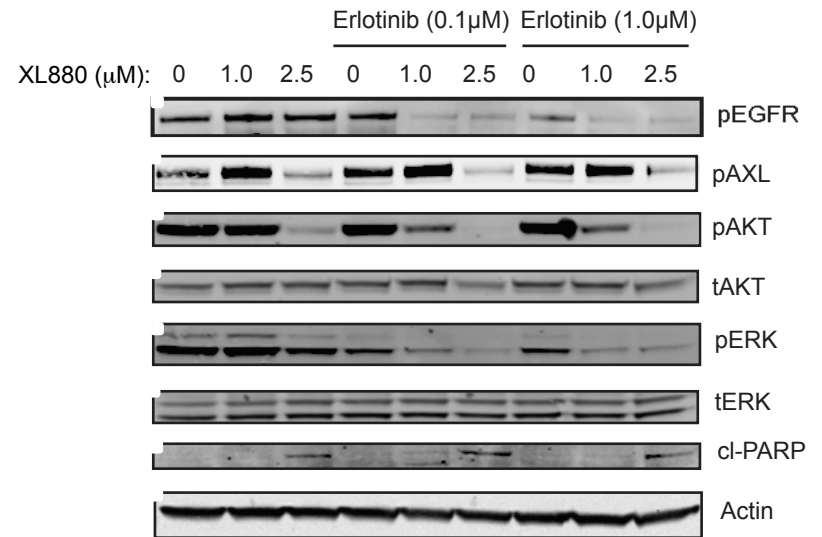


Supplementary Figure 9.

j

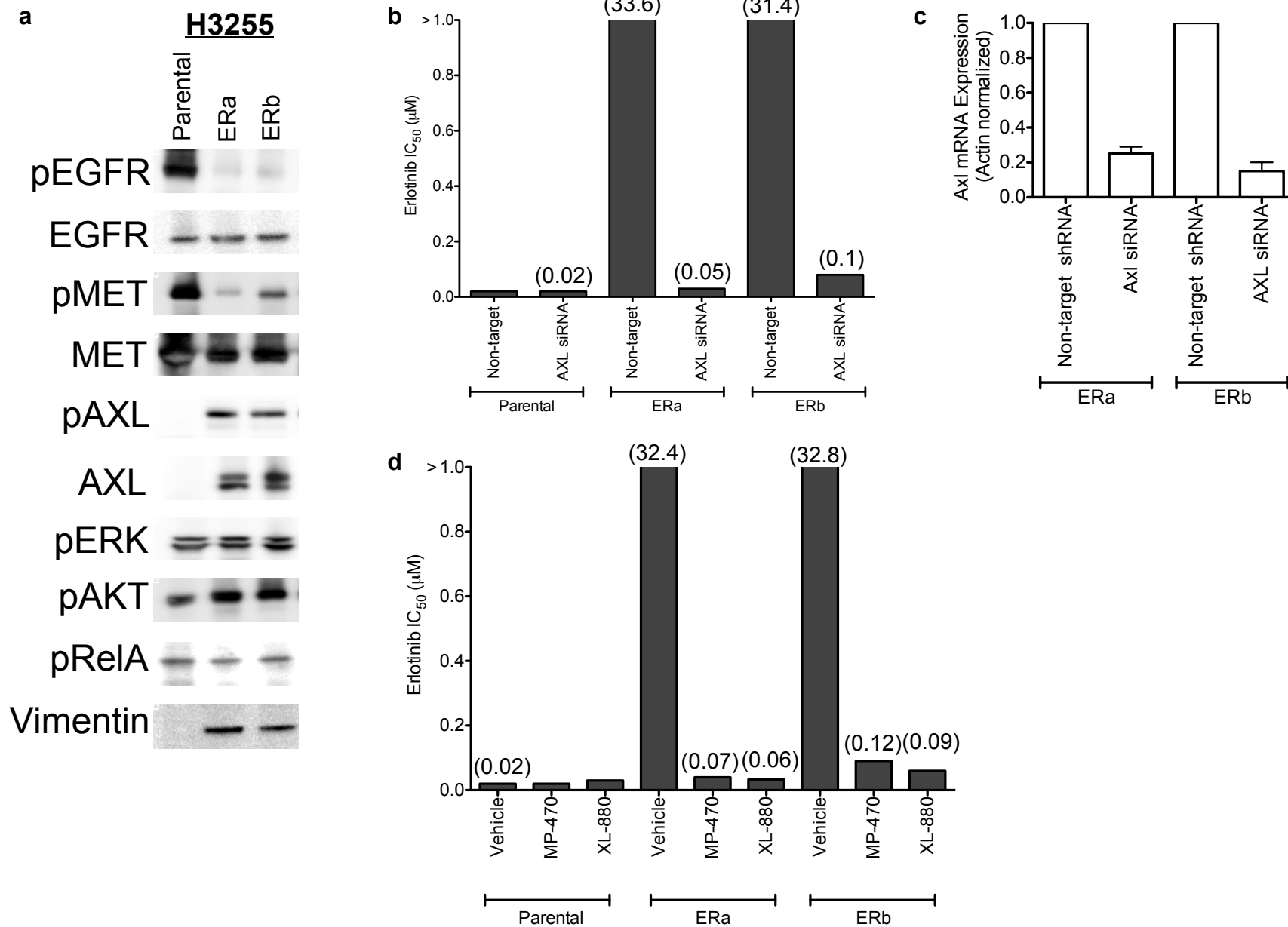


k



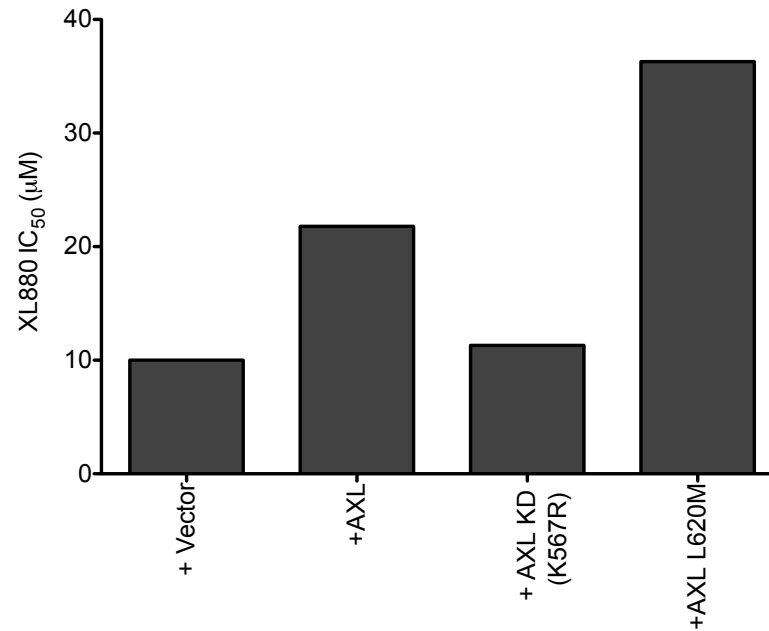
Supplementary Figure 9. a-b, Effects of erlotinib treatment on the indicated biomarkers of pathway activation as measured by western blot on lysates from HCC827 or ER3 cells treated with (a) erlotinib and EGF or (b) erlotinib over a time course. c-d, Effects of AXL inhibition by (c) siRNA or (d) XL880 on the indicated biomarkers of pathway activation as measured by western blots on lysates from HCC827 or ER3 cells. e-f, Expression of AXL by (e) Q RT PCR or (f) western blot on lysates from ER4 and ER5 cells. g, Expression of the indicated proteins by western blot on lysates from ER4 and ER5 cells. h-k, Effects of AXL inhibition by (h,j) siRNA or (i,k) XL880 on the indicated biomarkers of pathway activation as measured by western blots on lysates from HCC827 on (h-i) ER4 or (j-k) ER5 cells treated with vehicle or erlotinib at the indicated doses.

Supplementary Figure 10.



Supplementary Figure 10. AXL overexpression is necessary for erlotinib resistance in H3255 EGFR-mutant NSCLC cells with acquired resistance to erlotinib. a, AXL is overexpressed in the absence of increased pEGFR or pMET. Increased expression of the EMT marker vimentin was also noted in conjunction with AXL overexpression. b, Knockdown of AXL by siRNA restored erlotinib sensitivity in the H3255 ER subclones (erlotinib IC₅₀ shown in mM). c, Validation of siRNA knockdown of AXL in H3255 ER cells as measured by Q-RT-PCR and normalized to H3255 ER cells treated with not-target control. Data are from 3 independent experiments and as mean \pm SEM. d, Erlotinib IC₅₀ in H3255 cells treated with MP-470 or XL-880 at 1 mM concentration. Data represent 3 independent experiments.

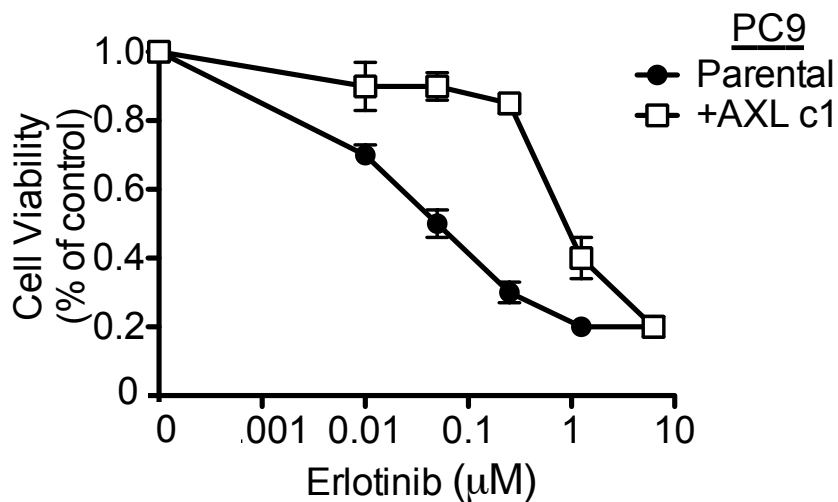
Supplementary Figure 11.



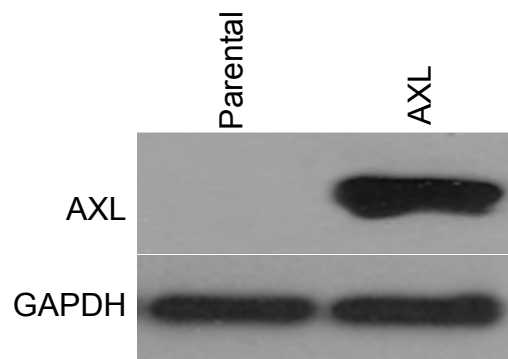
Supplementary Figure 11. Effects of expression of the indicated cDNA constructs encoding AXL or mutants thereof on XL-880 IC₅₀ in HCC827 parental or ER3 cells. Data represent 3 independent experiments.

Supplementary Figure 12.

a

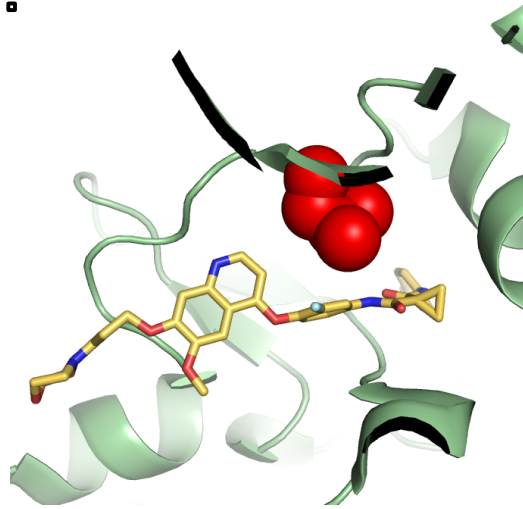


b



Supplementary Figure 12. Effects of ectopic expression of AXL in PC9 cells on (a) erlotinib sensitivity as measured by CellTiterGLO viability assay. $n=3$, data expressed as mean \pm SEM. (b) Western blots for the indicated proteins performed on lysates generated from the parental or AXL overexpressing cells (c1) shown in (a).

Supplementary Figure 13.

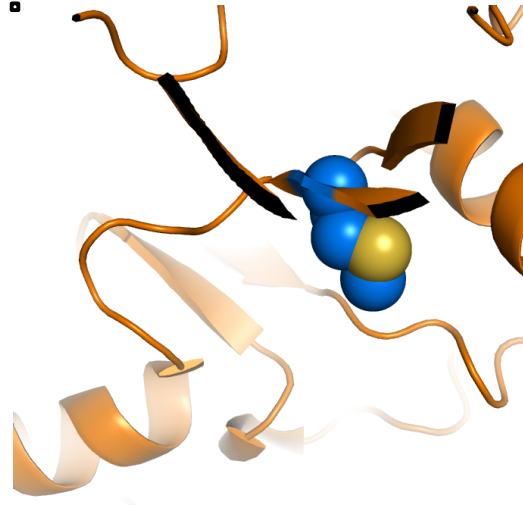


c-Met

L1157 in red

XL880 in yellow

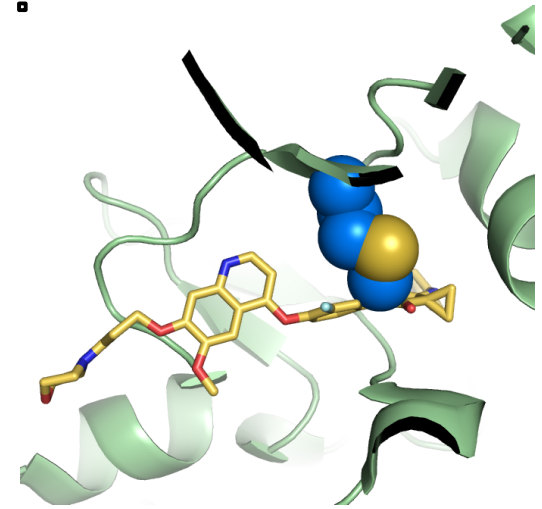
PDB ID: 3L8Q



EGFR T790M

M790 in blue

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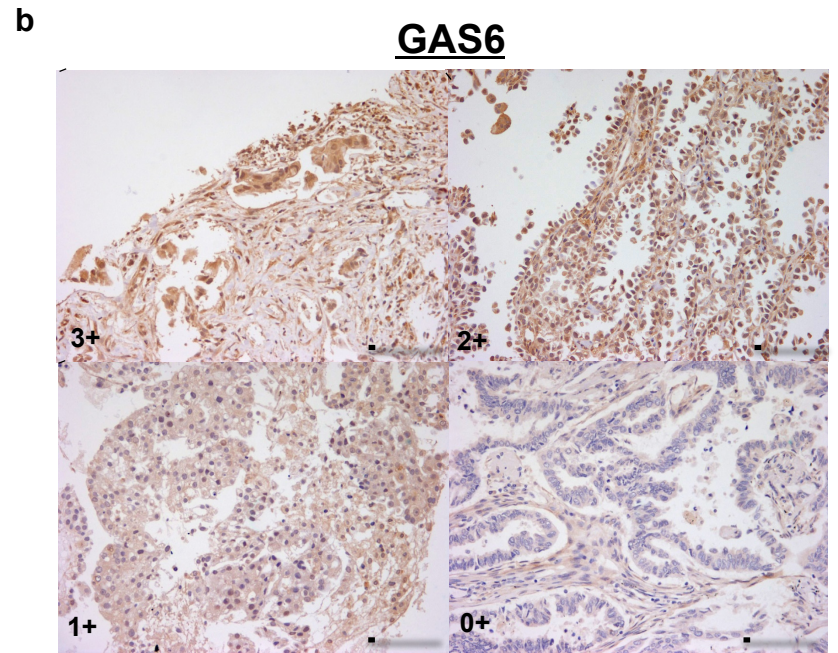
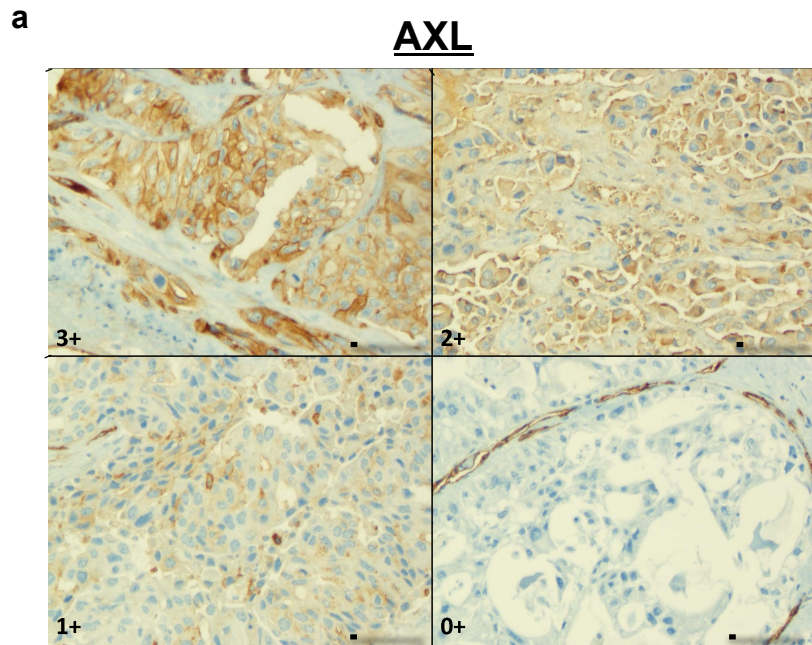


**Axl L620M mutation
expected to clash
with XL880**

Superposition of
M790 (EGFR) onto
c-Met

Supplementary Figure 13. Structural modeling using PDB viewer of the gatekeeper residue in the AXL kinase domain that is predicted to interact with XL880 based on structural analogy to the EGFR T790M gatekeeper residue and to c-MET/XL-880 co-crystal structure.

Supplementary Figure 14.



Supplementary Figure 14. Validation of IHC scoring system for the proteins examined in the paired human EGFR-mutant NSCLC specimens from EGFR TKI treated patients in Tables 2-3 and Figure 5. Scale bar = 100 μ M.

Supplementary Table Legends.

Supplementary Table 1. Genes significantly altered in the group of HCC827 erlotinib-resistant tumor xenografts (n=17) compared to the vehicle-treated control tumors (n=2) (threshold 1 log₂ fold change, P<0.05, unpaired T-test). (hosted as a separate .xls file)

Supplementary Table 2. Probesets significantly altered in HCC827 erlotinib-resistant tumor xenografts (n=17) compared to vehicle-treated control tumors (n=2) (threshold 1 log₂ fold change, P<0.05, unpaired T-test). (hosted as a separate .xls file)

Supplementary Table 3. Genes significantly altered in HCC827 ER1-3 cells compared to vehicle treated HCC827 parental cells (threshold 3 fold absolute change, FDR<0.1). (hosted as a separate .xls file)

Supplementary Table 4. Human AXL and GAS6 Q RT PCR primer sequences.

Supplementary Table 5. Human AXL and EGFR DNA sequencing primer sequences.

Supplementary Table 4. qRT PCR primer sequences.

	Gene Target	Sense	Anti-sense
AXL		5'-AGACATCGCCAGTGGCATG-3'	5'-AGGCGATTCCTGCTTCAGG-3'
GAS6		5'-CATCAACAAGTATGGGTCTCCGT-3'	5'-GTTCTCCTGGCTGCATTCGTTGA-3'

Supplementary Table 5. Sequencing primers for human AXL and EGFR.

AXL	5'-TGAAGAAAGTCCCTTCGTGG-3', 5'-GATCTGTCCATCCCGAAGCC-3', 5'-TGTCAGACGATGGGATGGGC-3', 5'-GCGTCTCCACAGGAAGCCAG-3', 5'-TGGTAGTCAGGTACCGCGTG-3', 5'-TCCAGCTCTGACCTCGTGAC-3', 5'-ATATCCGGGCGTGGAGAACAGC-3', 5'-GAATCCTTAGGGTCTGGCTG-3'.
EGFR	5'-CTGCGTGAGCTTGTTACTCGTGCCTTGG-3', 5'-AGCAGTCACTGGGGGACTTG-3', 5'-GGTGCAGGAGAGGAGAACTGC-3', 5'-GGTTTTCTGACCGGAGGTCC-3', 5'-AGGACCAAGCAACATGGTCAG-3', 5'-TGCATCCGTAGGTGCAGTTTG-3', 5'-GATGGTGGGGGCCCTCCTTT-3', 5'-TCCGGGAACACAAAGACAATA-3', 5'-CTTTCTTTCCGCACCCAGCAGTT-3', 5'-ATCCATCAGGGCACGGTAGAAGTT-3', 5'-AGTGCTGGATGATAGACGCAG-3', 5'-GTCAACAGCACATTGACAGC-3', 5'-AAATTCAGTCTTTGTGGCGC-3'