Article

Supplementary Material: Elevating CDCA3 Levels Enhances Tyrosine Kinase Inhibitor Sensitivity in TKI-Resistant EGFR Mutant Non-Small-Cell Lung Cancer

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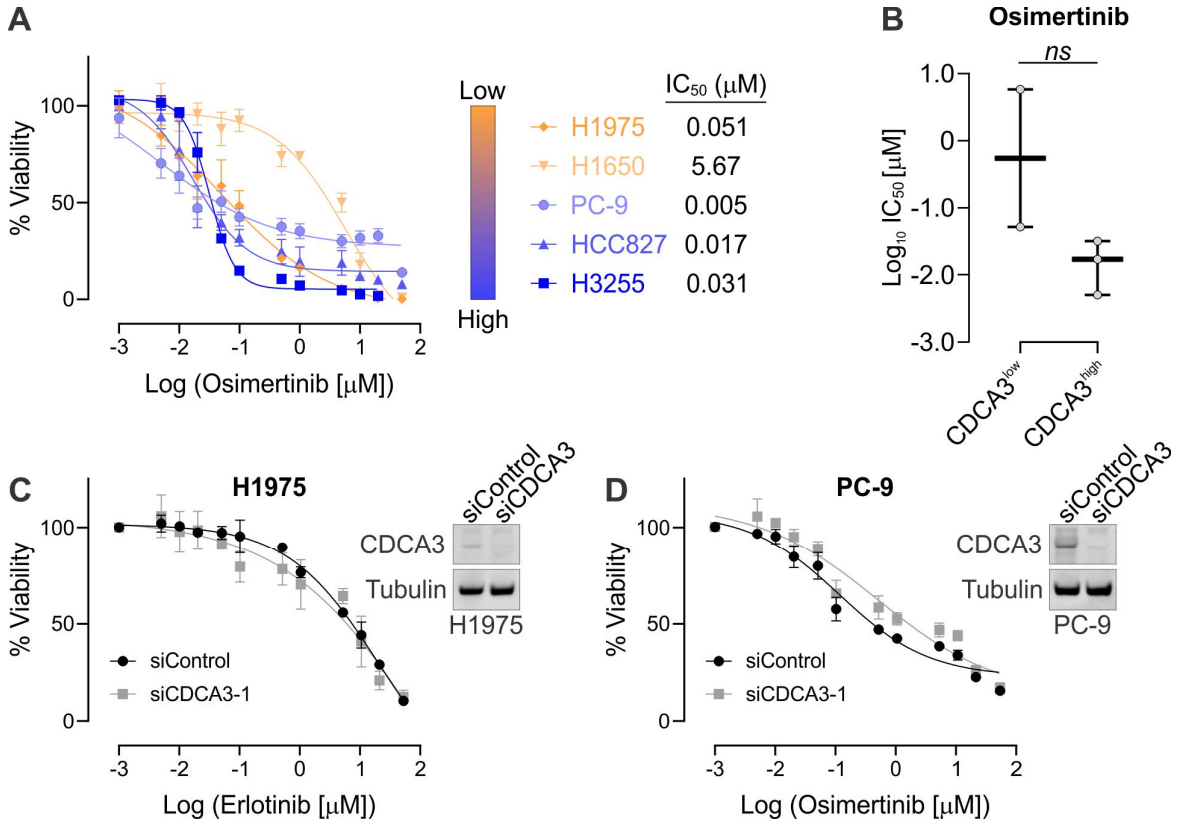

Figure S1. (**A**) *Left panel*, Dose response curves for five EGFR mutant NSCLC cell lines treated with escalating doses of osimertinib. Cells were treated with osimertinib for 72 hours before assessing cell viability. *Right panel*, Cell lines ranked by CDCA3 protein levels where osimertinib potency values (IC_{50}) were calculated using GraphPad Prism and listed for each cell line. n = 4. (**B**) Box and whisker plot showing osimertinib potency (log IC_{50} value) in $CDCA3^{low}$ and $CDCA3^{high}$ EGFR mutant cell lines (unpaired Student's *t* test, ns = not significant). (**C**) *Left panel*, Dose response curves for control (siControl) or CDCA3 depleted (siCDCA3) H1975 cells treated with escalating doses of erlotinib for 72 hours before assessing cell viability. *Right panel*, Endogenous CDCA3 western blot analysis from lysates of control or CDCA3 depleted H1975 cells. Tubulin was used as loading control. (**D**) *Left panel*, Dose response curves for control (siControl) or CDCA3 depleted (siCDCA3) PC-9 cells treated with escalating doses of osimertinib for 72 hours before assessing cell viability. *Right panel*, Endogenous CDCA3 western blot analysis from lysates of control or CDCA3 depleted PC-9 cells. Tubulin was used as loading control.

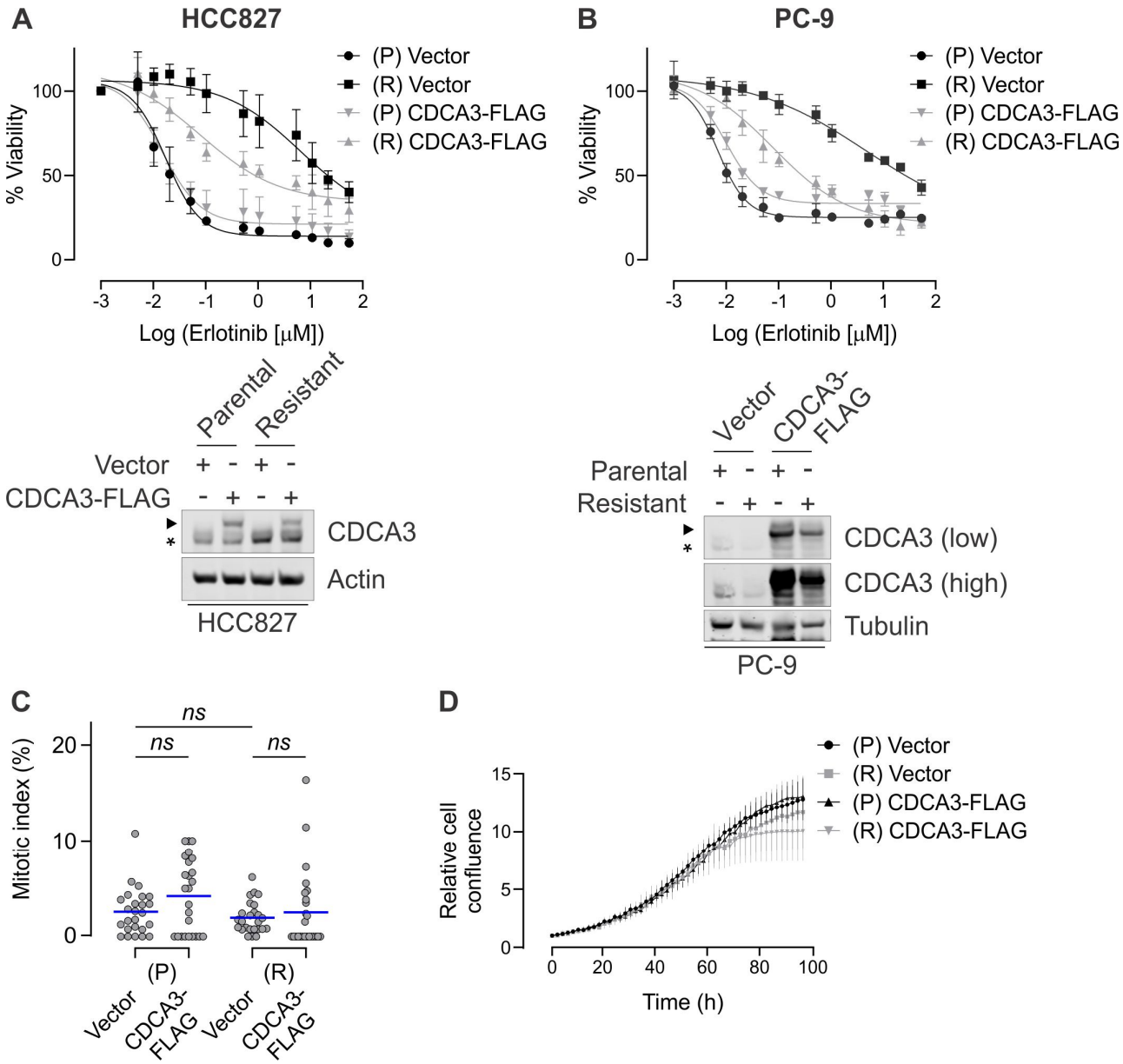

Figure S2. (**A-B**) *Top panels*, Dose response curves for vector transfected or ectopic CDCA3-FLAG overexpression in isogenic parental and resistant (**A**) HCC827 and (**B**) PC-9 cells treated with escalating doses of erlotinib for 72 hours before assessing cell viability. (**A-B**) *Bottom panels*, Western blot analysis of lysates from (**A**) HCC827 and (**B**) PC-9 isogenic parental and TKI resistant cells transfected with empty vector or CDCA3-FLAG. CDCA3 western blot analysis was performed to detect ectopic (*arrowhead*) and endogenous (*asterisk)* CDCA3. Tubulin was used as a loading control. (**C**) Beeswarm plot showing the mitotic index

determined by histone H3 pS10 staining and high throughput immunofluorescence microscopy of vector transfected or CDCA3-FLAG ectopically expressing HCC827 isogenic parental and TKI resistant cells. Data points represent an average percentage of mitotic nuclei per field of view from a minimum of 1100 nuclei (n = 23 fields total). Blue lines indicate median values. ns = not significant. (**D**) Cellular proliferation analysis of vector transfected or CDCA3-FLAG ectopically expressing HCC827 isogenic parental and TKI resistant cells over 96 h using the Incucyte Zoom live cell imaging system.