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Supplementary Materials for

Blocking EGFR palmitoylation suppresses PI3K signaling and mutant KRAS lung tumorigenesis

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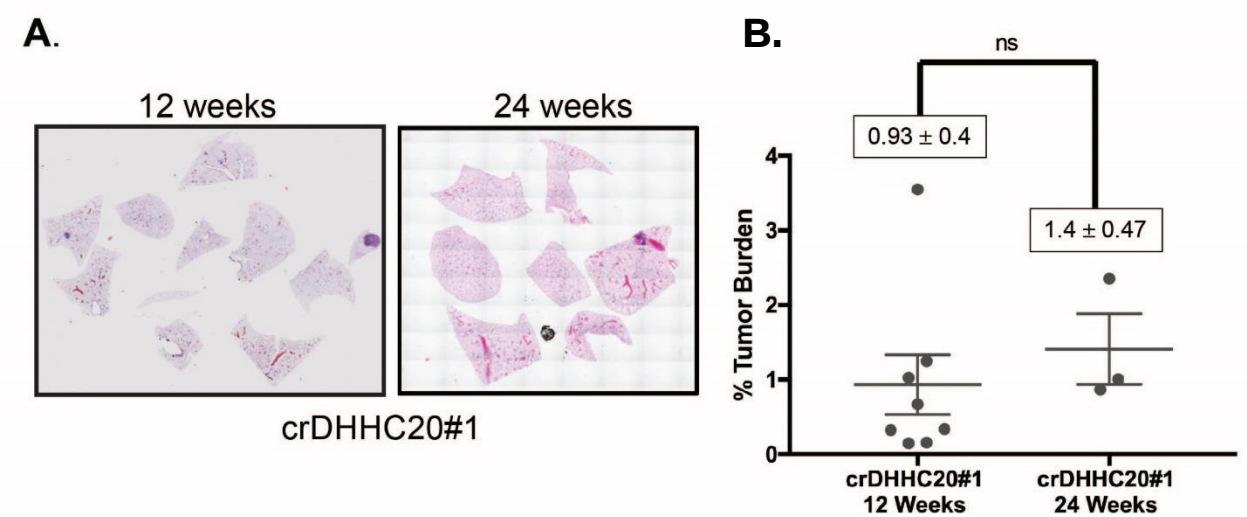

Fig. S1. Tumor burden remains low with extended loss of DHHC20. (**A**) Representative Hematoxylin and Eosin (H&E) 4X stitched images of 3 sgDHHC20#1 mice lungs 24 weeks post-infection. Tumor burden does not increase after 24 weeks. Control mice had to be sacrificed at 13 weeks due to extensive tumor burden. (**B**) Average tumor burden (value indicated by boxed number) of sgDHHC20#1 12 weeks (N = 8 mice), sgDHHC20#1 24 weeks (N = 3 mice) in KRASG12D/+; p53Δ/Δ; Rosa26L-YFP mice 13 weeks and 24 weeks following infection with LentiCRISPRv2Cre respectively, difference is not significant (ns), Student's *t*-test.

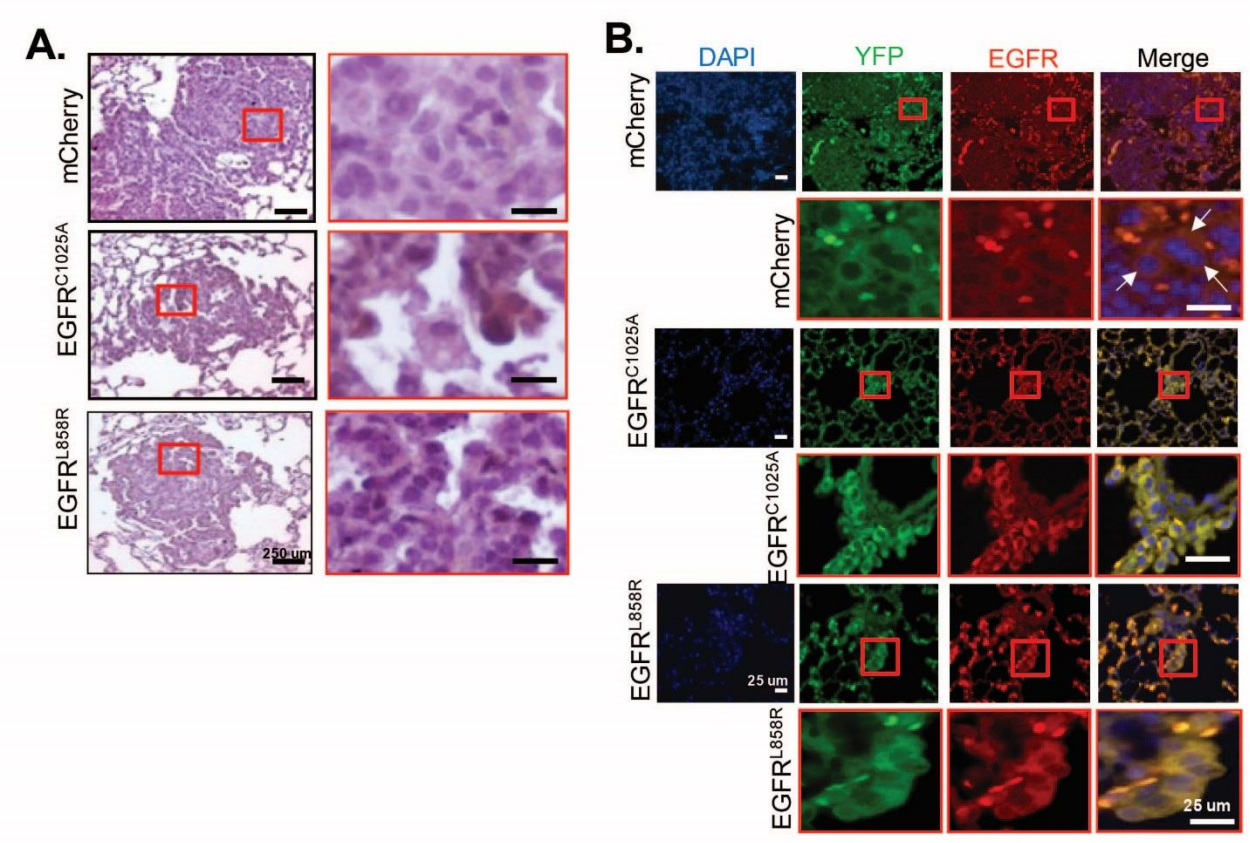

Fig. S2. pCREator (EGFR)-Cre expresses EGFR mutants equivalently in KPY mice. (**A**) IHC images of lungs stained for EGFR from KPY mice 13 weeks following Lenti-pCREator-Cre infection. Representative images Control (mCherry) (top), $EGFR^{C1025A}$ (middle) and $EGFR^{L858R}$ (bottom) at 10X magnification. Red square shows 20X magnification image of indicated region. (**B**) Control, $EGFR^{C1025A}$ and $EGFR^{L858R}$ cohort tissue sections were stained for YFP (green), EGFR (red) and DAPI (blue). Red square shows 20X magnification image of indicated region.

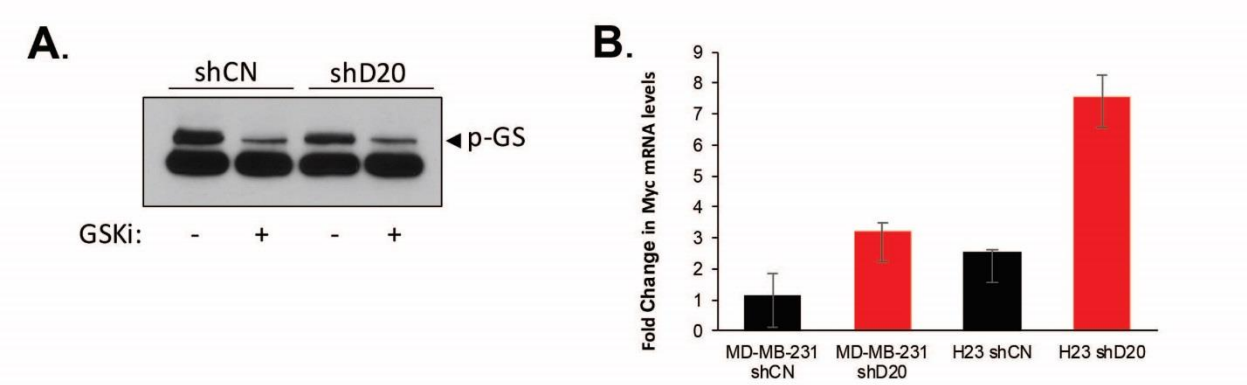

Fig. S3. Myc mRNA levels increase upon inhibition of DHHC20. (**A**) GSK3 inhibitor CHIR-90021 decreases phosphorylation of glycogen synthase in both shCN and shD20 MDA-MB-231 cells. (**B**) Fold change in Myc mRNA levels in MDA-MB-231 and H23 shCon versus shDHHC20 (shD20) cells. Graph represents average calculated fold change ± SD of N = 3 experiments with triplicate well for each condition.

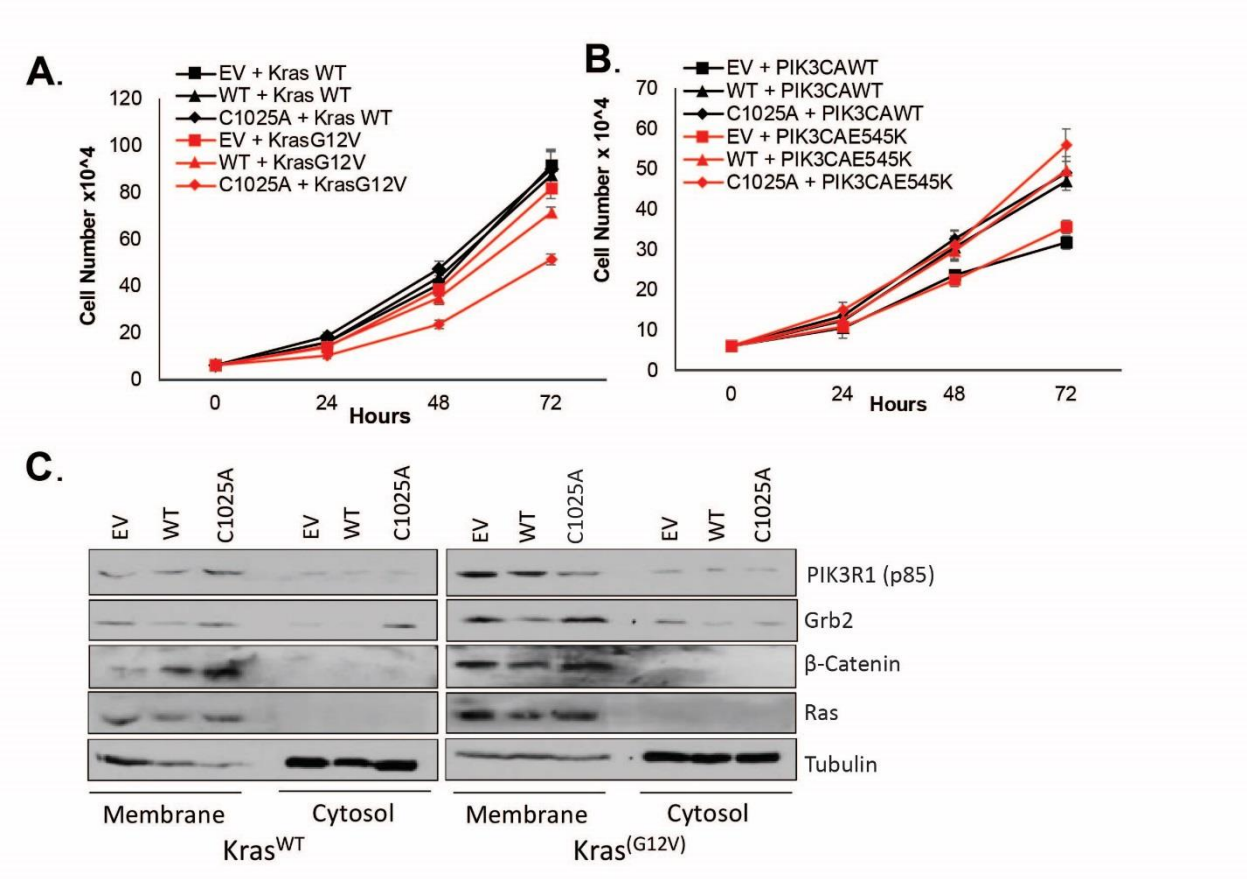

Fig. S4. Presence of mutant KRAS is required to reduce cell growth from loss of EGFR palmitoylation. (**A**) Growth curve of NIH3T3 cells co-expressing $KRAS^{WT}$ (black) or $KRAS^{G12V}$ (red) or with doxycycline inducible, $EGFR^{WT}$ (triangle) or $EGFR^{C1025A}$ (diamond) or empty vector (square). Cell number is expressed as the mean ± SEM of N = 3 experiments with 3 replicates each, *** $P < 0.001$, Student's T-test. (**B**) Growth curve of NIH3T3 cells co-expressing either $PIK3CA^{WT}$ (black) or $PIK3CA^{E545K}$ (red) with doxycycline inducible, $EGFR^{WT}$ (triangl) or $EGFR^{C1025A}$ (diamond) or empty vector (square). Cell number is expressed as the mean ± s.e.m of N = 3 experiments with 3 replicates each. (**C**) Representative immunoblot of the abundance of PIK3R1, Grb2 and RAS, β-catenin, and α-tubulin in the membrane and cytosolic fractions from NIH3T3 cells stably co-expressing wild-type KRAS ($KRAS^{WT}$) or mutant KRAS ($KRAS^{G12V}$) and doxycycline-inducible $EGFR^{WT}$ (WT), $EGFR^{C1025A}$ (C1025A), or empty vector (EV).

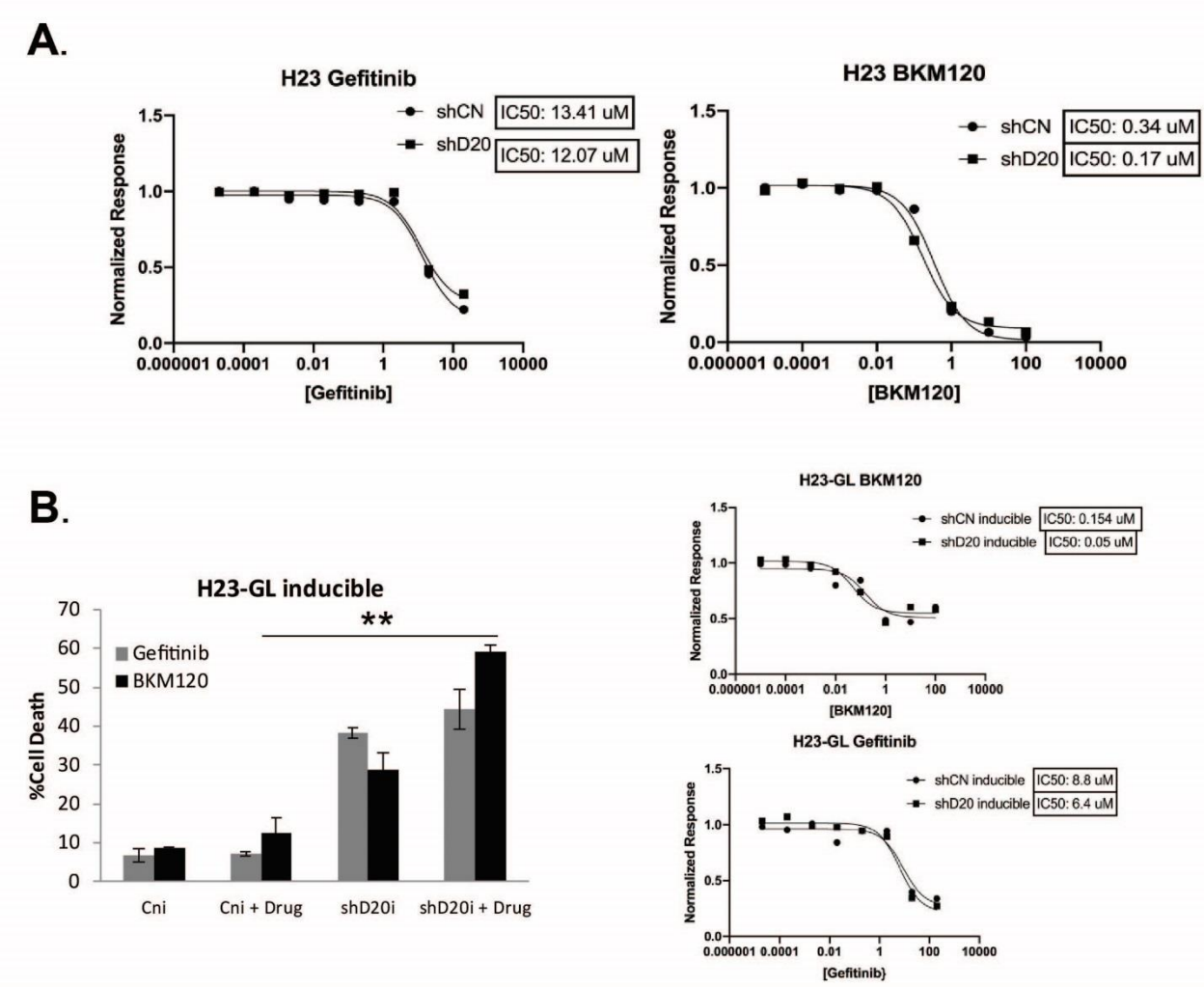

Fig. S5. Inhibition of DHHC20 in H23 cells induces sensitivity to PI3K inhibitor. (**A**) Dose-response curves for H23 shCN and shDHHC20 treated with either Gefitinib or BKM120 at increasing doses for 72 h (N=3) to determine IC50 differences. ***P < 0.001, Two-Way Anova. (**B**) Gefitinib (5 μM) (gray) and BKM120 (500 nm) (black) treatment increases cytotoxicity in inducibly silenced H23-GL (GFP and Luciferase expressing) cells (mean ± s.e.m of N = 3 experiments of triplicate wells per condition). **P < 0.05, Students *t-test.* Dose-response curves H23-GL with inducible shRNA against a control scrambled sequence (shCN) and shD20 cells treated with either Gefitinib or BKM120 at increasing doses for 72 h (N = 3 experiments with triplicate wells per data point) to determine differences in IC50. One representative experiment shown. *** P < 0.001, Two-Way Anova.

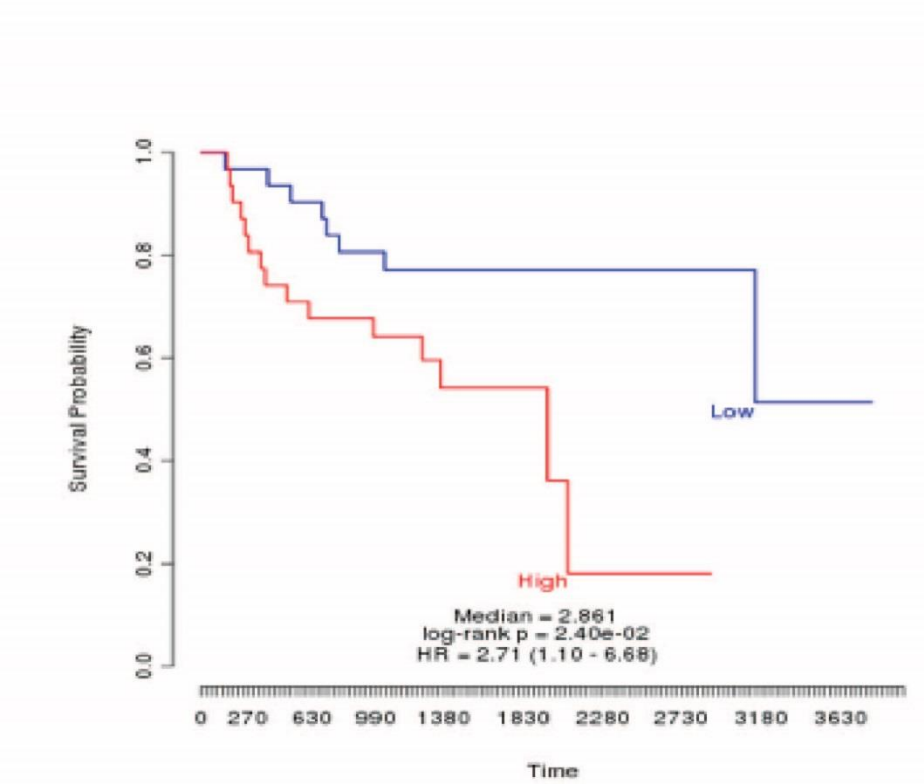

Fig. S6. Low levels of DHHC20 correlate with better survival probability in patients. Representative KM plot for survival probability of low vs. high DHHC20 expression from lung adenocarcinoma. Meta-Z Score = 3.03 from 16 different data sets.