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Supplementary Materials for

A protein network map of head and neck cancer reveals PIK3CA mutant drug sensitivity

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Figs. S1 to S6

Other Supplementary Material for this manuscript includes the following:

MDAR Reproducibility Checklist
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Data S1 to S7

Materials and Methods

Bait cloning

Baits were cloned using the Gateway Cloning System (Life Technologies) into a doxycycline-inducible N-term or C-term 3xFLAG-Tagged vector modified to be Gateway compatible from the pLVX-Puro vector (Clontech) by the Krogan lab. (data S6). Point mutant baits were cloned via site-directed mutagenesis. All expression vectors were sequence validated (Genewiz).

Cell culture, lentivirus production, and stable cell line generation

HEK293T (ATCC, CRL-3216) and CAL-33 were maintained in DMEM (Corning) supplemented with 10% FBS (Gibco) and 1% Penicillin-Streptomycin (Corning). HET-1A was maintained in BEGM™ (Lonza), consisting of Broncho Epithelial Basal medium (BEBM) with the additives of the Bullet kit except GA-1000 (gentamycin-amphotericin B mix). SCC-25 was maintained in DMEM/F12 (Corning) with 10% FBS (Gibco), 1% Penicillin-Streptomycin (Corning) and 400 ng/mL hydrocortisone (Sigma). HET-1A was obtained from American Type Culture Collection and SCC-25 was obtained from Thomas Carey (University of Michigan), CAL-33 were provided by Gerard Milano (University of Nice, Nice, France). All cells were maintained in a humidified 37^{o}C incubator with 5% CO_2. Stably transduced HET-1A, SCC-25, and CAL-33 cell lines were maintained in puromycin (2 μg/mL, 2.5 μg/mL, and 0.7 μg/mL, respectively). Bait expression was induced by 1μg/ml doxycycline for 40 hrs. All cell lines were authenticated by the University of California, Berkeley Cell Culture Facility.

Lentivirus was produced for each bait by packaging 5ug bait vector, 3.33 μg of Gag-Pol-Tat-Rev packaging vector (pJH045 from Judd Hultquist), 1.66 μg of VSV-G packaging vector (pJH046 from Judd Hultquist) with 30 μL of PolyJet (SignaGen). After incubating at room temperature for 25 min, DNA complexes were added dropwise to HEK293T cells (15 cm plate, ~80% confluency). Lentivirus-containing supernatant was collected after 72 hrs and filtered through a 0.45 μm PVDF filter. Lentivirus particles were precipitated with PEG-6000 (8.5% final) and NaCl (0.3 M final) at 4^{o}C for 4-8 hrs. Particles were pelleted via centrifugation at 2,851 *xg* for 20min at 4^{o}C and resuspended in DPBS for a final volume ~800-1000 μL. Stable cell lines were generated by transducing a 10cm plate (~80% confluency) with 200uL of precipitated lentivirus for 24 hrs before selecting with puromycin for a minimum of 2 days.

Affinity Purification

One 10 cm plate of cells (~80% confluency) was washed with ice-cold DPBS and lysed with 300 μL of ice-cold lysis buffer (50 mM Tris pH 7.4, 150 mM NaCl, 1 mM EDTA, 0.5% NP40, 1 mM DTT, 1x protease inhibitor cocktail (Roche, complete mini EDTA free), 125U Benzonase/mL). Lysates were flash-frozen on dry ice for 5-10 min, followed by a 30-45 s thaw in 37^{o}C water bath with agitation, and rotation at 4^{o}C for 15 min. Lysate was clarified by centrifugation at 13000 xg for 15 min at 4^{o}C. A 30 μL lysate aliquot was saved for future BCA assay and western blot.

For FLAG purification, 25 μL of bead slurry (Anti-Flag M2 Magnetic Beads, Sigma) was washed twice with 1 mL of ice-cold wash buffer (50 mM Tris pH 7.4, 150 mM NaCl, 1 mM EDTA) and all of the remaining lysate was incubated with the anti-FLAG beads at 4^{o}C with rotation for 2 hrs. After incubation, flow-through was removed and beads were washed once with

500 µL of wash buffer with 0.05% NP40 and twice with 1 mL of wash buffer (no NP40). Bound proteins were eluted by incubating beads with 15 µL of 100 ug/ml 3xFLAG peptide in 0.05% RapiGest in wash buffer for 15 min at RT with shaking. Supernatants were removed and elution was repeated. Eluates were combined and 10 µL of 8 M urea, 250 mM Tris, 5 mM DTT (final concentration ~1.7 M urea, 50 mM Tris, and 1 mM DTT) was added to give a final total volume of ~45 µL. Samples were incubated at 60ºC for 15 min and allowed to cool to room temperature. IODO was added to a final concentration of 3 mM and incubated at room temperature for 45 min in the dark. DTT was added to a final concentration of 3 mM before adding 1 µg of sequencing-grade trypsin (Promega) and incubating at 37ºC overnight. Samples were acidified to 0.5% TFA (ph<2) with 10% TFA stock and incubated for 30 min before desalting on C18 stage tip (Rainin).

Mass spectrometry data acquisition and analysis

For AP-MS experiments, samples were resuspended in 15 µL of MS loading buffer (4% formic acid, 2% acetonitrile) and 2µL were separated by a reversed-phase gradient over a nanoflow 75µm ID x 25cm long picotip column packed with 1.9µM C18 particles (Dr. Maisch). Peptides were directly injected over the course of a 75 min acquisition into a Q-Exactive Plus mass spectrometer (Thermo), or over the course of a 90 min acquisition into a Orbitrap Elite mass spectrometer. Raw MS data were searched against the uniprot canonical isoforms of the human proteome (downloaded March 21, 2018), and using the default settings in MaxQuant (version 1.6.2.10), with a match-between-runs enabled (*84*). Peptides and proteins were filtered to 1% false discovery rate in MaxQuant, and identified proteins were then subjected to protein-protein interaction scoring. To quantify changes in interactions between WT and mutant baits, we used a label free quantification approach in which statistical analysis was performed using MSstats (*85*) from within the artMS Bioconductor R-package. All raw data files and search results are available from the Pride partner ProteomeXchange repository under the PXD019469 identifier (*86*, *87*). Detailed MS acquisition and MaxQuant search parameters are provided in Table S7.

Targeted proteomic analysis

Targeted proteomic analysis of APMS samples was performed on a Thermo Q-Exactive Plus mass spectrometer using the same HPLC conditions as described for original AP-MS experiments. All peptide and fragment ion selection, as well as quantitative data extraction was performed using Skyline (*88*)**.** Quantitative values were then imported into PRISM 8 software to perform normalization by bait abundance and statistical testing (2-tailed, unpaired t-test).

Protein-protein interaction scoring

Protein spectral counts as determined by MaxQuant search results were used for PPI confidence scoring by both SAINTexpress (version 3.6.1) (*41*) and CompPASS (version 0.0.0.9000) (*22*, *42*). All PPI scoring was performed separately for each cell line. For SAINTexpress, control samples in which bait protein was not induced by addition of doxycycline were used. For CompPASS, a stats table representing all WT baits was used. After scoring, the CompPASS WD and Z-score were normalized within a given bait for each cell line. The total list of candidate PPIs was filtered to those that met the following criteria: SAINTexpress BFDR =< 0.05, WD percentile by bait >= 0.95, and Z-score percentile by bait >= 0.95. PPIs passing all 3 of these criteria were considered to be high-confidence PPIs. To enable visualization and analysis of PPIs by confidence score among these 3 criteria, we also calculated a PPI score: [(WD percentile

by bait + Z-score percentile by bait)/2) + (1-BFDR)] / 2. This score places both the PPI confidence from SAINTexpress and CompPASS on a zero to 1 scale, with 1 being the highest confidence, and then takes the weighted average of these confidence scores.

Permutation test

A permutation test was performed in which genes were drawn from the list of all genes detected in the global protein abundance analysis of the parental cell lines. The null distribution of the average number of samples with variation was learned from 10,000 random gene lists of equal size to the set of interacting partners. This permutation test was performed individually for mutations (excluding silent mutations), CNVs, and mRNA expression. The information for observed variation of each gene is collected from the TCGA head and neck cancer cohort (firehose legacy; downloaded from cbioportal.org/datasets).

Differential interaction scoring

To compare PPIs across cell lines, we developed a method for calculating a differential interaction score (DIS) and a corresponding false discovery rate (FDR) using AP-MS data across multiple cell lines. This approach uses the SAINTexpress score *(38)*, which is the probability of a PPI being bonafide in a single cell line. Here we let S_c(b, p) be the SAINTexpress score of a specific PPI denoted as (b, p) in a cell line *c*. Given that PPIs are independent events across different cell lines, we computed the differential interaction score for each PPI (b, p) as the product of the probability of a PPI being present in both cancer cell lines but absent in the HET-1A normal cell line as follow for each PPI:

DIScancer(b,p)=SCAL-33(b,p)SSCC-25(b,p)[1-SHET-1A(b,p)]

This differential interaction score highlights PPIs that are strongly conserved across two cancer cell lines, but not shared by the normal cell line. Additionally, we can highlight PPIs that are present in the control HET-1A cell line, but depleted in both cancer cell lines as follows:

DISnormal(b,p)=[1-SCAL-33(b,p)][1-SSCC-25(b,p)]SHET-1A(b,p)

We further merged these two DIS scores to define a single score for each PPI, where if $DIS_{cancer} > DIS_{normal}$, the DIS is assigned a positive (+) sign, while if $DIS_{cancer} < DIS_{normal}$, the unified DIS is assigned a negative (-) sign. In this way, the DIS for each PPI is represented by a continuum, in which negative DIS scores represent PPIs depleted in HNSCC, while positive DIS scores represent PPIs enriched in HNSCC. Additionally, for all differential interaction scores that we calculated, we also computed the Bayesian false discovery rate (BFDR) *(38)* estimates at all possible thresholds (*p**) as follows:

FDR(p*)=i,i(1-DIS(pi,pj))I{DIS(pi,pj)>p*}i,jI{DIS(pi,pj)>p*}, where I{A} is 1 when A is True and 0 otherwise.

Note, while these scores were used for comparison across 3 cell lines, it can also be used more simply to compare between any two cell lines. Such a comparison is calculated as follows where $DIS_{LineA/LineB}$ results in PPIs specific to cell line A have a positive DIS value, while PPIs specific to cell line B results in a negative DIS value:

DISCAL-33/HET-1A(p1,p2)=SCAL-33(p1,p2)(1-SHET-1A(p1, p2)) or
DISSCC-25/HET-1A(p1,p2)=SSC-25(p1,p2)(1-SHET-1A(p1, p2)) or
DISSCC-25/CAL-33(p1,p2)=SSCC-25(p1,p2)(1-SCAL-33(p1, p2)).

NanoBiT Gαi1 dissociation assay

The NanoBiT G-protein dissociation assay, based on a split-luciferase system, was performed as previously described with some modifications *(87)*. All DNA constructs were provided by Dr. Asuka Inoue (Tohoku University, Japan). NanoBiT plasmids (pCAGGS) include Gαi1-LgBiT, Gβ1-native, and SmBiT-Gγ2 (CAAX C68S mutant). Gαi-DREADD (pcDNA3.1) was used as a synthetic Gαi-coupled GPCR. Briefly, CAL-33 and HET-1A cells were seeded on poly-D-lysine coated (Sigma, Cat# P7280), opaque, white 96-well plates (Falcon Cat# 353296). The following day cells were transfected with NanoBiT and receptor plasmids using Lipofectamine 3000 (ThermoFisher Scientific, Cat# L3000008) according to manufacturer recommendations for a 12-well scale (10µL transfection mix to each well). The NanoBiT plasmids were mixed at a ratio of 100ng Gαi1-LgBiT, 500ng Gβ1, 500ng SmBiT-Gγ2, and 200ng of receptor if needed. For gene knockdown experiments, 10pmol of pooled siControl (Dharmacon, Cat# D-001810-10-20), siFGFR3 (Mission siRNA, Cat# SIHK0780, SIHK0781, SIHK0782), or siDaple (Dharmacon, Cat# L-033364-01-0005) was included in the plasmid mix. Media was changed the following day. Two days after transfection, media was aspirated from each well and washed once with HBSS. Cells were incubated in HBSS with a final concentration of 5µM native coelenterazine (Biotium, Cat# 10110-1) for 30 minutes at room temperature protected from light. Basal luminescence was read and ligand prepared for final concentrations of 10ng/mL human bFGF (Roche Cat# 11123149001) and 10µM clozapine-N-oxide (Cayman Chemical, Cat# NC1044836). After ligand addition, luminescence was read in kinetic loops (each well ~every 30 seconds) for 60 minutes total (Tecan Spark). Raw luminescent values were normalized to the corresponding basal value for each well and subsequently to the mean vehicle ratio (raw/basal) at time 0. Significance was calculated using a one-way ANOVA at the 60 minute time point.

Scratch migration assay

CAL-33 cells were seeded on 12-well plates coated with 10µg/mL fibronectin in PBS (Sigma Aldrich, Cat# F2006-1MG). Once cells reached confluence, a vertical scratch was made with a pipette tip and washed well with PBS before adding serum-free media. Cells were stimulated with vehicle, 10ng/mL bFGF, or 1% serum for 24 hours. Images were taken at the 0 and 24 hour time points (2X magnification) and the scratch area was quantified using ImageJ. Percent scratch closure was calculated for each well and significance assessed using a one-way ANOVA.

Phosphorylated PAK, ERK, and siRNA knockdown confirmation immunoblots

CAL-33 and HET-1A cells were seeded on poly-D-lysine-coated 6-well plates. Cells were transfected with siRNA using Lipofectamine RNAiMAX (Thermo Fisher Scientific, Cat# 100014472) according to manufacturer recommendations. After overnight serum starvation, cells were stimulated with vehicle, 10ng/mL bFGF, or 10µM CNO. Cells were washed once with PBS and lysed in RIPA buffer (50mM Tris-HCl pH 6.8, 150mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) with protease and phosphatase inhibitors (Bimake, Cat# B14001, B15001-A/B). Lysates were briefly sonicated and cleared by centrifugation before boiling in Laemmli sample buffer (Bio-Rad Cat# 1610747). After separation on 10% acrylamide gels and transfer to PVDF membranes, membranes were blocked with 2% BSA in TBST before incubating

with antibodies. Primary antibodies against phospho-PAK1(S199/204)/PAK2(S192/197) (1:1000, Cell Signaling Technology, Cat# 2605), PAK1 (1:2000, Cell Signaling Technology, Cat# 2602), PAK2 (1:2000, Cell Signaling Technology, Cat# 2608), pERK (1:2000, Cell Signaling Technology, Cat# 9106), ERK (1:2000, Cell Signaling Technology, Cat# 9102), FGFR3 (1:2000, OriGene, Cat# TA801078), Daple (1:1000, Millipore EMD, Cat # ABS515), and GAPDH (1:10000, Cell Signaling Technology, Cat# 2118) were used. After washing with TBST, membranes were incubated in secondary goat anti-rabbit HRP (1:20000, Southern Biotech, Cat# 4010-05) and goat anti-mouse HRP (1:20000, Southern Biotech, Cat# 1010-05) antibodies for chemiluminescent development.

CDX3379 treatment in vivo and in vitro experiments

All the animal studies using HNSCC tumor xenografts were approved by the University of California, San Diego Institutional Animal Care and Use Committee (IACUC), with protocol ASP # S15195. All mice were obtained from Charles River Laboratories (Worcester, MA). To establish tumor xenografts, HNSCC cells were transplanted into both flanks (2 million per tumor) of female athymic mice (nu/nu, 4–6 weeks of age and weighing 16–18 g). Mice were fed with doxycycline food (6g/kg) from Newco Distributors (Rancho Cucamonga, CA, USA) to induce PIK3CA expression. When average tumor volume reached 100 mm^3, the mice were randomized into groups and treated by intraperitoneal (IP) injection with vehicle (PBS) or CDX3379 (10mg/kg, twice a week) for approximately 15 days. The mice were sacrificed at the indicated time points (or when mice succumbed to disease, as determined by the ASP guidelines).

Phosphorylated HER3 immunoblots

Wild-type (WT) or mutant PIK3CA with FLAG-tag were expressed by lentiviral transduction in SCC-25 cells. Collected cells were washed with ice-cold PBS twice and then lysed with RIPA lysis buffer (150 mM Tris, pH 7.4, 100 mM NaF, 120 mM NaCl, 100 mM sodium orthovanadate, with 1 tablet protease inhibitor cocktail (Roche 31075800) and 1 tablet phosphatase inhibitor cocktail (Roche 04906837001) added. Lysates (30 μg) were resolved by SDS-PAGE, transferred to PVDF membranes (Bio-Rad #1620177), and incubated with primary antibodies (1:1000) at 4^{o}C overnight. Membranes were then washed and incubated with Goat Anti-Rabbit lgG(H+L)-HRP Conjugated secondary antibodies (1:5000) (Bio-Rad #170-6515) for 1 hr at room temperature, followed by washing four times with TBST. Antibodies against P-HER3-Y1197 (#4561) and HER3 (#12708) were from Cell Signaling Technology, and anti-B-tubulin (ab6276) was from Abcam. Blots were quantified with ImageJ software, and the intensity of P-HER3-Y1197 signal was normalized to FLAG-PIK3CA intensity.

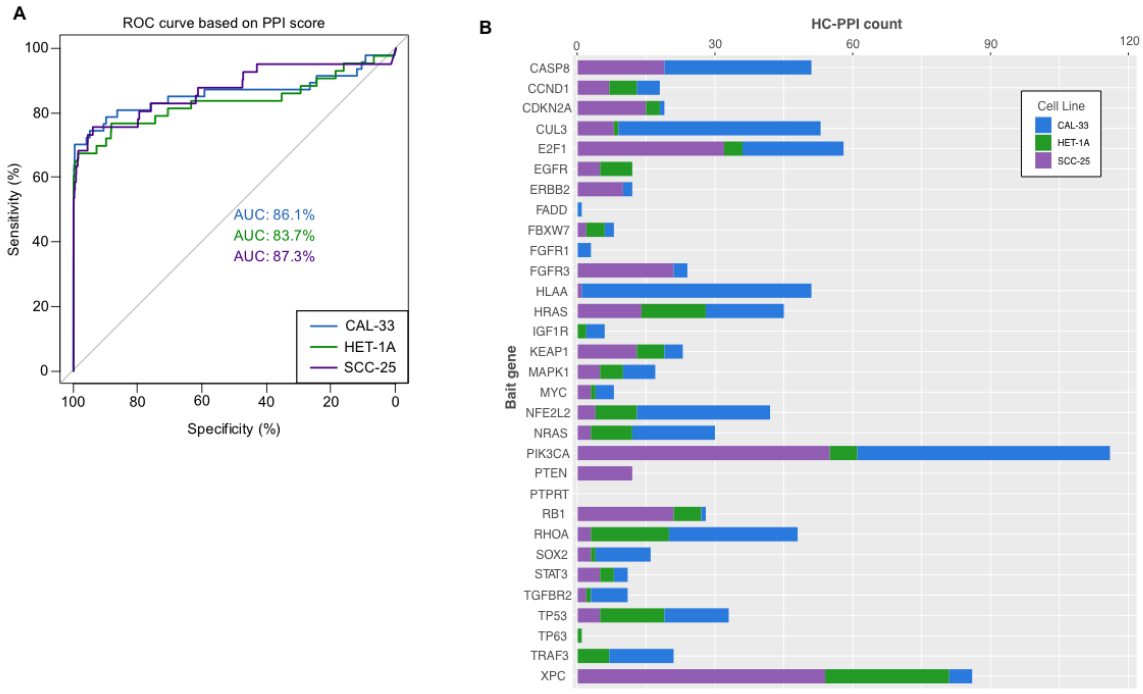
IAS background network

The integrated associated stringency (IAS) network was derived from integration of five major types of protein pairwise relationships recorded in public databases: (1) physical protein-protein interaction; (2) mRNA co-expression; (3) protein co-expression; (4) co-dependence (correlation of cell line growth upon gene knockouts); (5) sequence-based relationships. A broad survey created a compendium of 127 network features used as inputs to a random forest regression model, trained to best recover the proximity of protein pairs in the Gene Ontology (GO). The final IAS score, ranging from 0 to 1, quantifies all pairwise associations among 19035 human proteins. In this study, we displayed stringent protein interactions with IAS > 0.3 when the IAS network was used in figures. More details are described in the companion paper *(50)*.

Data analysis

Instant Clue software was used for the generation and statistical analysis of some figures (*88*). Heatmaps were generated with Morpheus (https://software.broadinstitute.org/morpheus).

Fig. S1.



C

	prognostic	non−prognostic	p−value
Preys (CAL−33)	24	321	0.0231
Preys (SCC−25)	28	267	0.0001
Preys (HET−1A)	14	130	0.0066
Total genes	790	18880	NA

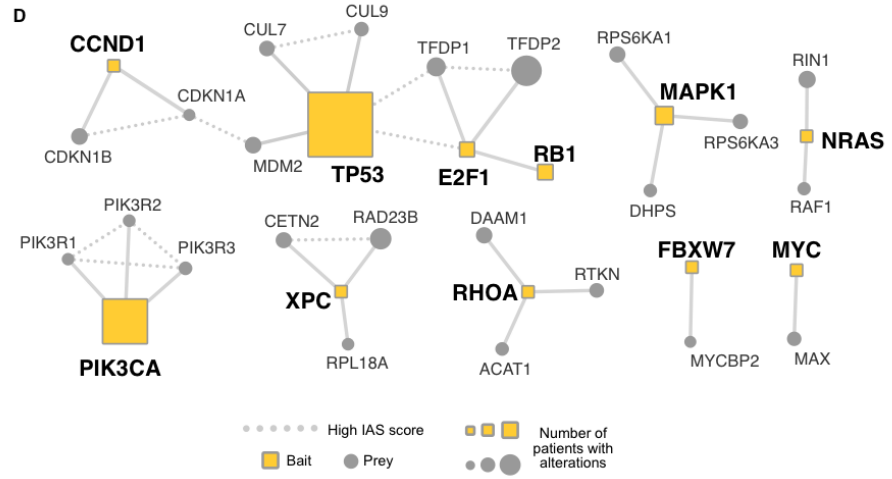


Figure S1. Overview of HC-PPI detection. (**A**) Receiver operating characteristic (ROC) curve illustrating high recovery of gold standards (sensitivity). (**B**) Number of HC-PPIs per cell line for each bait. (**C**) Each prey protein was queried in the Human Pathology Atlas,(*83*) and the significance of association between its mRNA expression and survival in TCGA head and neck cancer patients was scored using the log-rank p-value of the Kaplan-Meier analysis. Proteins with survival p-values lower than 0.001 were defined as prognostic. The enrichment of prognostic proteins in preys was evaluated by Fisher's exact test (3rd column

in the table). (**D**) HC-PPIs that were detected across all cell lines (n = 24). PPIs between preys from public databases having a high IAS score (see Material and methods) are also plotted as dotted lines (*55*).

Fig. S2.

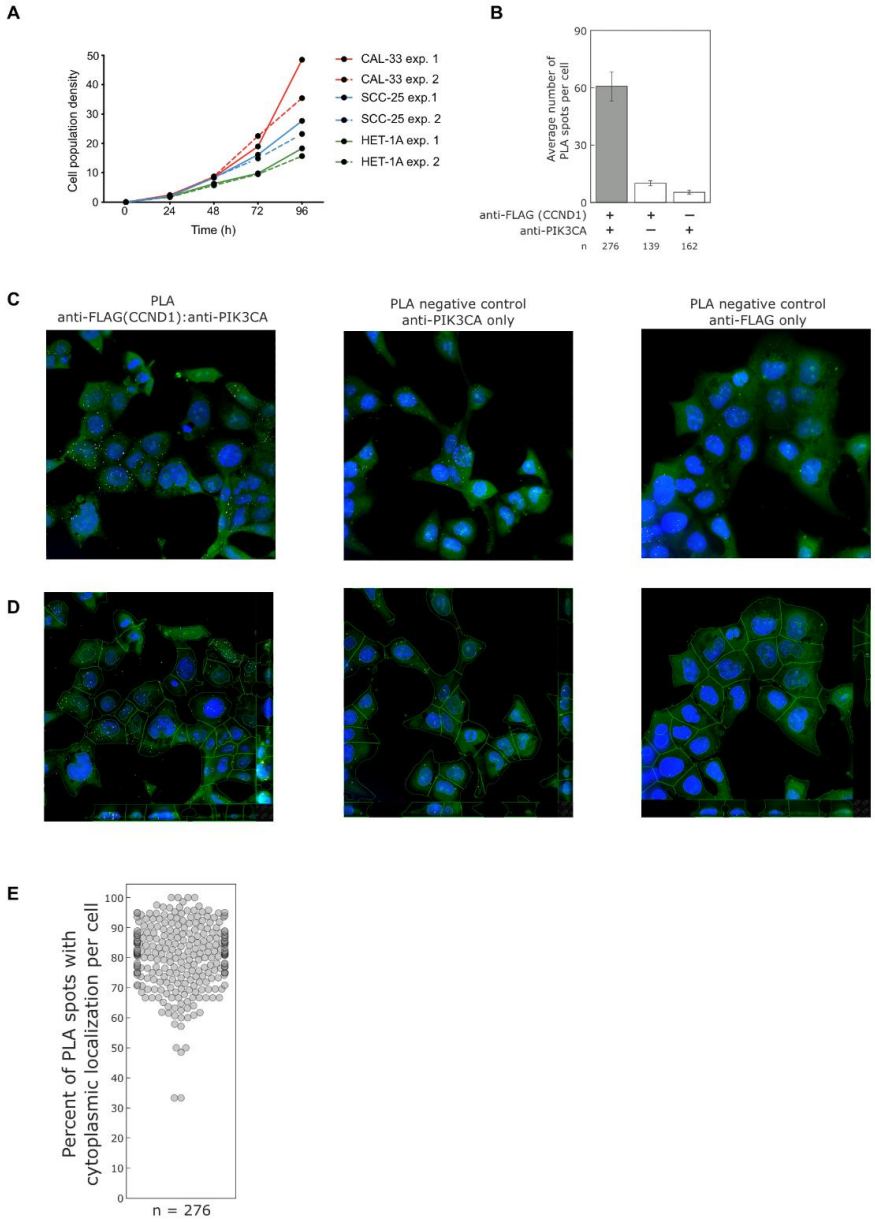


Figure S2. CCND1 PPI validation. (**A**) Quantification of cell population density over time. Each cell line was analyzed in duplicate. Average doubling times were 17.6h, 19.9h, and 23.1h for CAL-33, SCC-25, and HET-1A cells, respectively. (**B**) The average number of PLA spots per cell (error bars represent 95% confidence interval). (**C**) Confocal microscopy images with DAPI nuclear staining (blue), and PLA signal (white) for the CCND1:PIK3CA interaction, and CellMask labeling of the entire cell (green). (**D**) Representative 3D segmented images with a single slice and orthogonal views with segmented binary overlays (left column) and corresponding max intensity projection showing total PLA interactions in each image. PLA signal (whilte) for the CCND1:PIK3CA interaction, and DAPI nuclear staining (blue) is shown for Het1A CCND1-FLAG cells (E). (**E**) Quantification of the percent of PLA spots with cytoplasmic localization in each cell.

Fig. S3.

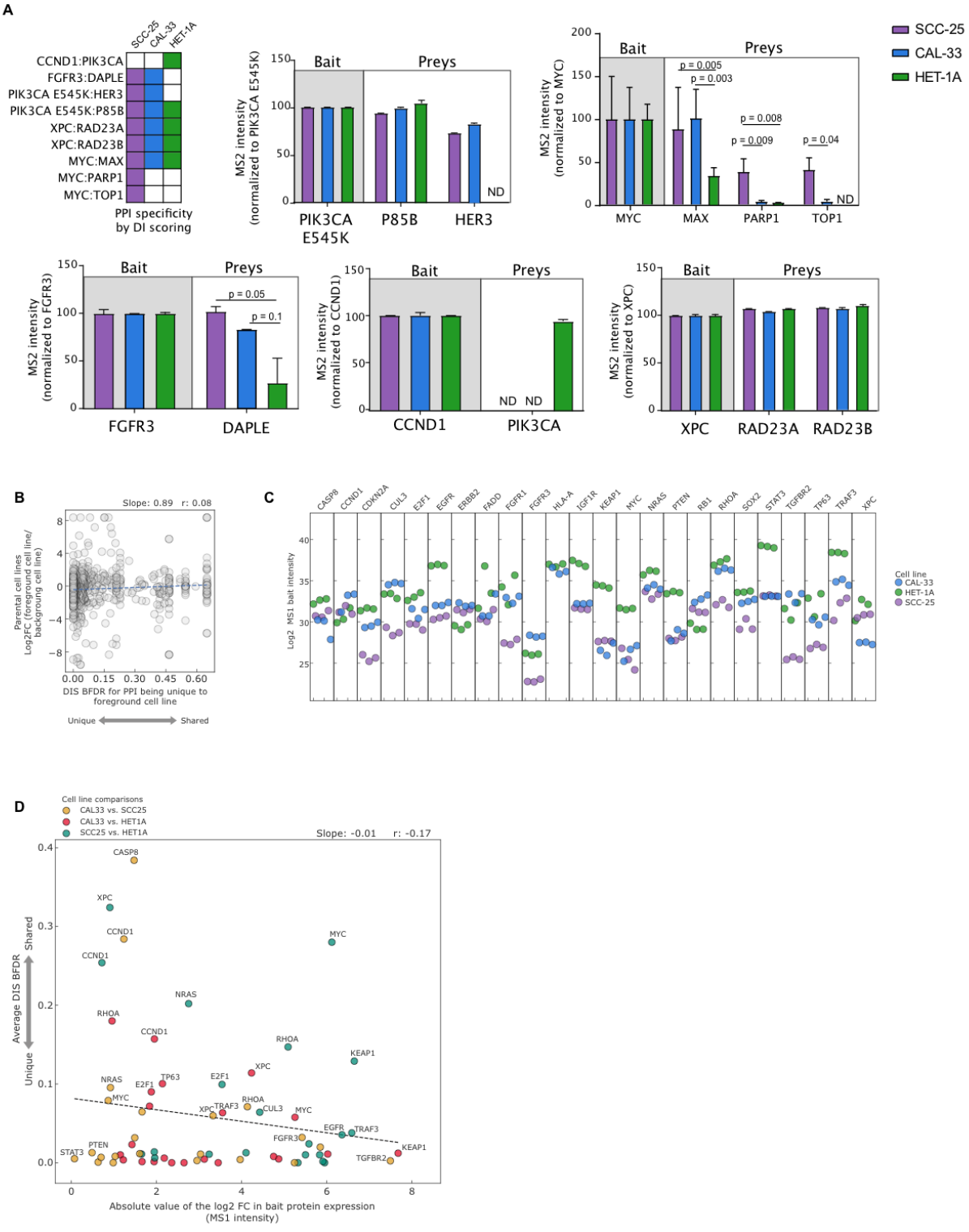


Fig. S3. Properties of differentially interacting proteins. (A) Overview of PPI enrichment as determined by DIS values for a selection of PPIs and targeted proteomic analysis for these PPIs measuring bait and prey protein abundances. All prey quantification is normalized to the bait level

expression in the respective cell line (p-values are the result of a 2-tailed unpaired t-test). (B) Evaluation of the relationship between prey protein abundance and PPI enrichment. Specifically, the DIS BFDR for each PPI is compared to the abundance of the prey protein in the parental cell lines. (C) The expression level of baits across each cell line as measured by the MS1 intensity in APMS experiments. Note, for clarity, only baits for which only WT protein sequences were analyzed are displayed. (D) Evaluation of the relationship between PPI enrichment and bait expression level differences between cell lines. For pairwise comparisons between cell lines the average of all DIS BFDR for all PPIs for a given bait is compared to the absolute value of the log2 fold-change in the bait protein expression (from panel C) between those two cell lines.

Fig. S4.

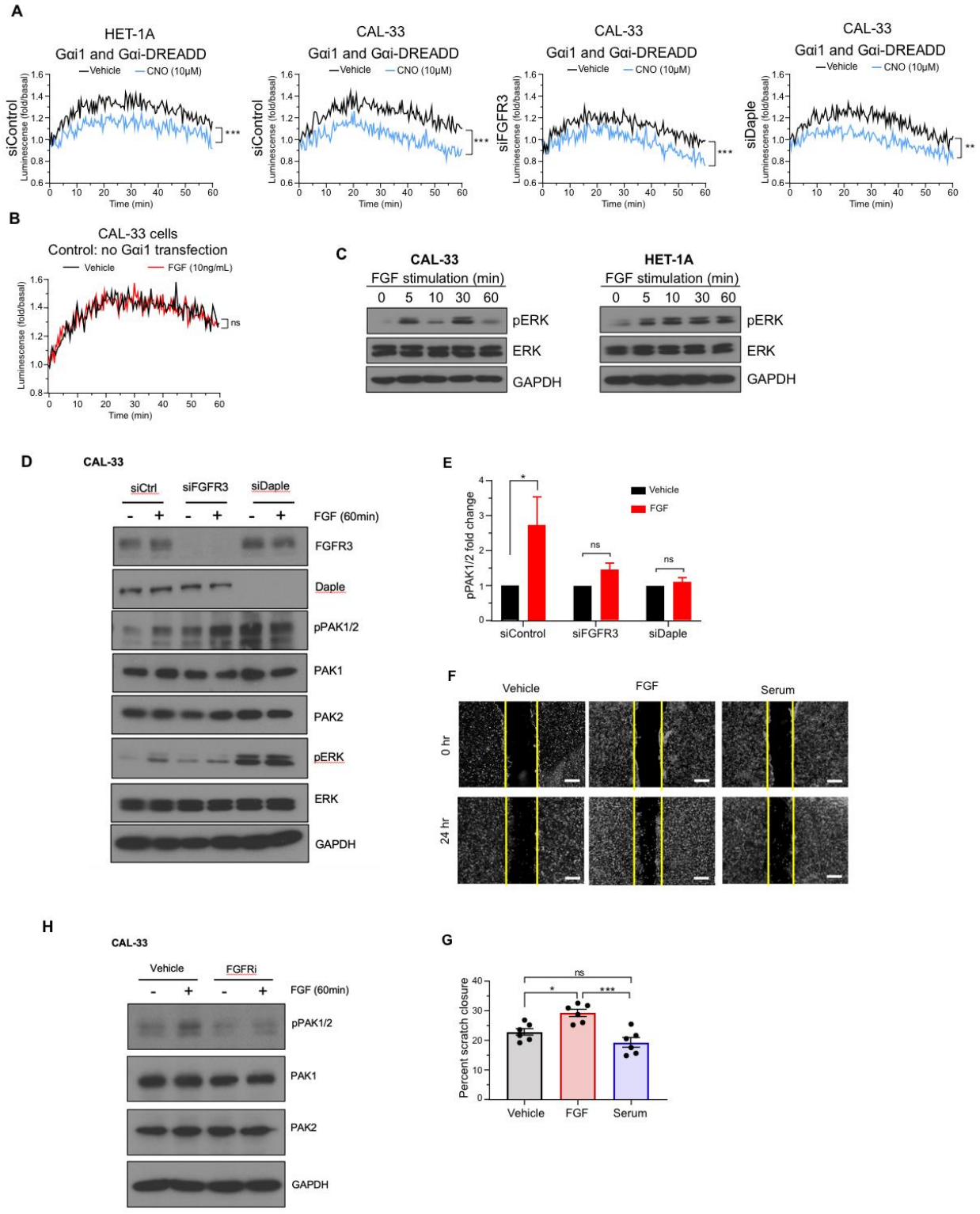

A
HET-1A
Gαi1 and Gαi-DREADD
CAL-33
Gαi1 and Gαi-DREADD
CAL-33
Gαi1 and Gαi-DREADD
CAL-33
Gαi1 and Gαi-DREADD
Vehicle
CNO (10μM)
siControl
siFGFR3
siDaple
Luminescense (fold/basal)
Time (min)
B
CAL-33 cells
Control: no Gαi1 transfection
Vehicle
FGF (10ng/mL)
ns
C
CAL-33
HET-1A
FGF stimulation (min)
0 5 10 30 60
pERK
ERK
GAPDH
D
CAL-33
siCtrl
siFGFR3
siDaple
FGF (60min)
FGFR3
Daple
pPAK1/2
PAK1
PAK2
pERK
ERK
GAPDH
E
pPAK1/2 fold change
Vehicle
FGF
ns
siControl
siFGFR3
siDaple
F
Vehicle
FGF
Serum
0 hr
24 hr
H
CAL-33
Vehicle
FGFRi
FGF (60min)
pPAK1/2
PAK1
PAK2
GAPDH
G
Percent scratch closure
ns
Vehicle
FGF
Serum

Fig. S4. HNSCC-enriched FGFR3:Daple interaction mediates activation of Gαi. (A) Luminescence was measured in HET-1A cells transfected with Gαi NanoBiT and Gαi-DREADD as well as CAL-33 cells transfected with Gαi NanoBiT, Gαi-DREADD, and siRNA (control, FGFR3, or Daple). Cells were stimulated with CNO (10μM) and luminescence was measured over 60 minutes with a decrease in luminescent signal demonstrating Gαi activation (***$P < 0.001$ and **$P < 0.01$ when compared with the vehicle-treated group). (B) Luminescence was measured over 60 minutes in mock transfected CAL-33 cells stimulated with FGF (10ng/mL). (C) ERK phosphorylation over a time course was measured by immunoblotting in CAL-33 and HET-1A cells stimulated with FGF (10ng/mL). (D-E) PAK1/2 autophosphorylation measured by immunoblot analysis in CAL-33 cells transfected with siRNA (control, FGFR3, or Daple) and stimulated with FGF (10ng/mL) (*$P < 0.05$ when compared with the vehicle-treated group). (F-G) A vertical scratch was introduced to fibronectin-plated CAL-33 cells and cells were stimulated with FGF (10ng/mL) or serum (1%) in the presence of mitomycin C. (F) Images were taken at 0 and 24 hours after FGF stimulation (scale bar = 250μm), and replicate scratch closures were quantified (G, (*$P < 0.05$, ***$P < 0.001$ when compared with the vehicle-treated group)). (H) PAK1/2 autophosphorylation measured by immunoblot analysis in CAL-33 cells stimulated with FGF (10ng/mL) and/or treated with the 0.5μM of the pan FGFR inhibitor Infigratinib.

Fig. S5.

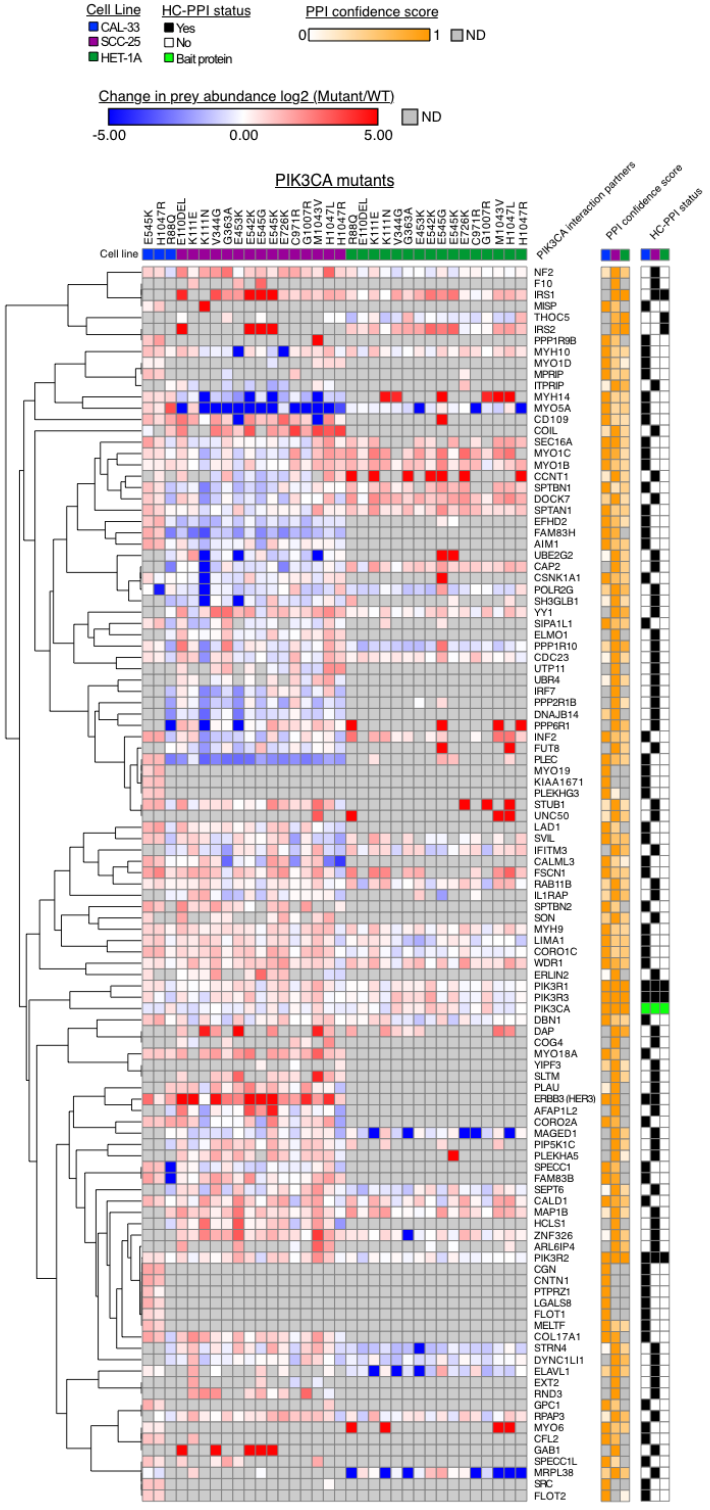
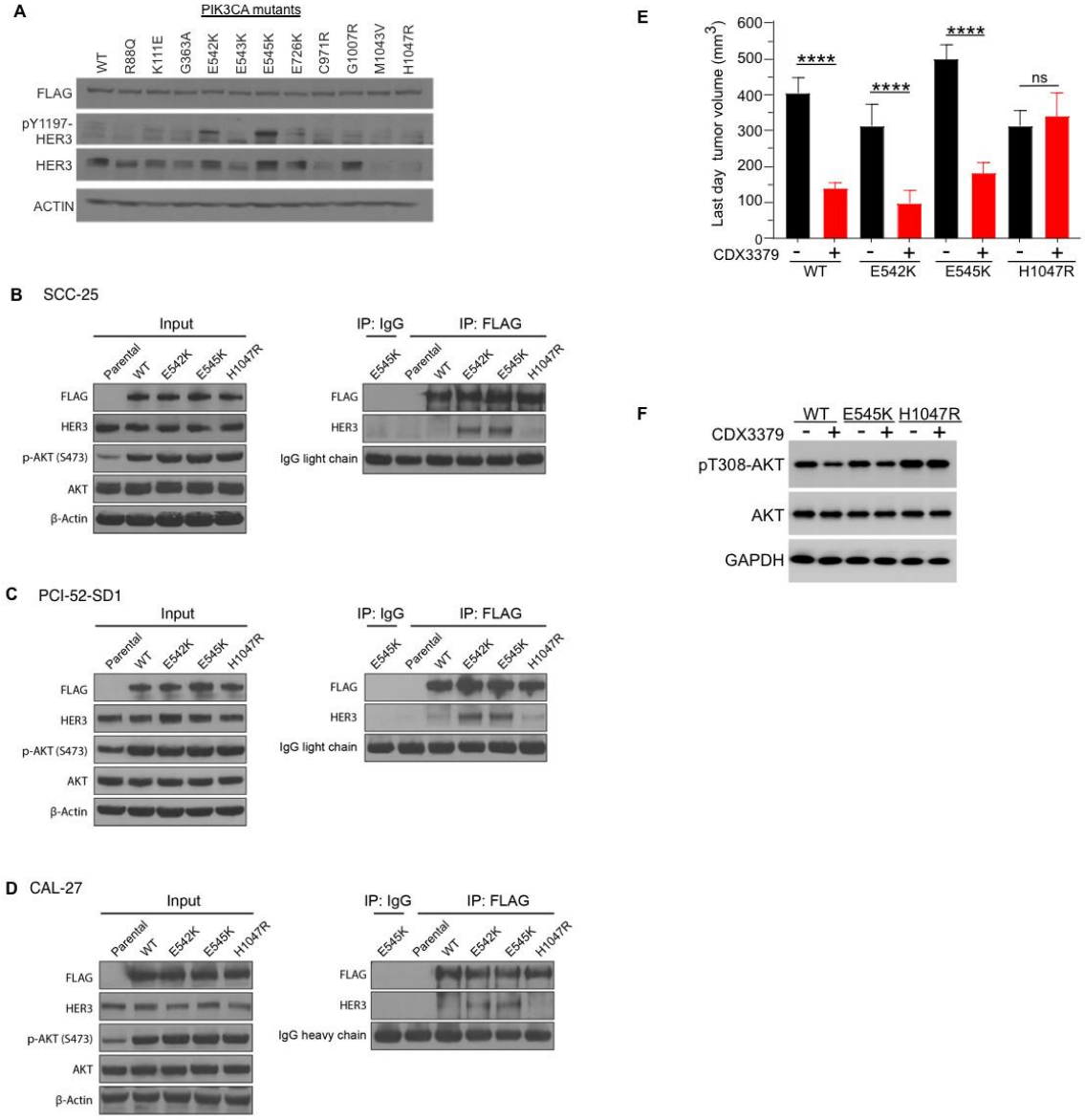


Figure S5. PIK3CA mutant interactome. Quantification of PIK3CA PPIs across all cell lines and mutants. For each interaction partner the PPI confidence score is reported, which is an average of the confidence scores reported from SAINTexpress and CompPASS score (see Fig. 1G and Materials and methods for details). Additionally, the designation of that PPI as a HC-PPI in each cell line is designated.

Fig. S6.



Supplementary Figure 6. Mutation dependent HER3 interactions and cellular response to CDX3379 treatment. (**A**) Immunoblot of total and phosphorylated HER3 (Y1197), total HER3, Actin (loading control), and FLAG peptide in SCC-25 cells expressing a panel of FLAG-tagged PIK3CA mutants. (**B-D**) Interaction between PI3Kα-FLAG and endogenous HER3 in HNSCC cells by co-immunoprecipitation. (A) SCC25, (B) Cal27 and (C) PCI-52-SD1 cells were engineered to stably express doxycycline-inducible, FLAG-tagged canonical PIK3CA mutation (E542K, E545K or H1047R), and wild-type PIK3CA (WT). Cells cultured in the presence of doxycycline were harvested at 48 hr. Protein lysate was subjected to co-immunoprecipitation assay using mouse anti-FLAG magnetic beads, and protein A IgG was used for negative control. The precipitated proteins were analyzed by immunoblotting with the indicated antibodies. The input represented 2% of the total protein lysate used for the immunoprecipitations, and β-Actin was used for loading control. (**E**) Last day tumor volume (****P < 0.0001 when compared with the control-treated group). (**F**) Representative immunoblot of phosphorylated Akt (T308), total Akt, and GAPDH (loading control), in CAL-27 cells expressing WT, E545K, or H1047R PIK3CA. Cells were treated *in vitro*

with either DMSO or the CDX3379 (1μg/ml, 1hr).

Data S1. List of baits analyzed per cell line
Data S2. Complete list of scored PPIs.
Data S3. Filtered list of only high-confidence PPIs, and cross comparison of those interactions across cell lines.
Data S4. DIS analysis of PPIs.
Data S5. Mutant regulation of PPIs.
Data S6. Bait cloning information
Data S7. MS acquisition parameters.