**Supplemental Methods**

*Whole Exome Sequencing Analysis*

Nextera Rapid Capture whole exome sequencing data for SUM229 EpCAM/CD49f positive or negative sorted populations was aligned to GRCh37/hg19 using BWA-MEM v 0.7.9a using -Y -M -R options and duplicates were subsequently marked using Biobambam v 0.0.172. ABRA (1) realignment was employed for indel detection and to improve variant allele frequency estimation. The resulting ABRA .bam files were piped to FreeBayes (2) v 1.1.0-54 for generation of a germline variant call file whereby SUM-229 positive and negative samples were unified by nucleotide variant position. VCFtools (3) v 0.1.15 was employed to limit variant call file entries to Nextera Rapid Capture targeted regions. Variants were annotated using SNPEff (4) v 4.3t and only variants supported by a Quality score of > 30 were considered for tabulation.

*Fluorescence-Activated Cell Sorting*

Approximately 50 million SUM-229PE cells were stained and sorted by fluorescence-activated cell sorting (FACS). Media was aspirated from the plates and cells were washed with PBS. Cells were trypsinized for approximately 5 minutes with 0.05% Trypsin-EDTA solution (Gibco cat. # 25300054), then resuspended in HF Media (Hank’s Balanced Salt Solution (Gibco cat. # 14025092) with 2% FBS and 5 mM EDTA). Cells were resuspended in 1 mL of HF media at a concentration of 1 x 106 cells/mL and stained with 50 L of EpCAM (EpCAM-FITC, Stem Cell Technologies cat. # 60136FI) and 10 L of CD49f (CD49f-PE-Cy5, BD Biosciences cat. #551129) antibodies for 30 minutes in the dark at 4C. Cells were pelleted at 200 x g, and media was aspirated. Cells were washed twice with 10 mL of HF media, then filtered with a 30 m sterile cell strainer (Partec cat. # 04-004-2326). EpCAM-positive, CD49f-positive cells and EpCAM-negative, CD49f-negative cells were sorted at single-cell purity using the FACSDiva software on the BD FACS Aria II.

*Cell Lysis and Immunoblotting*

KRAS expression was detected using a mouse polyclonal anti-human KRAS antibody (Santa Cruz cat. # sc-30). CXCR7 expression was detected using a rabbit polyclonal anti-human CXCR7 antibody (Proteintech cat. # 20423-1-AP). ERK2 expression was detected using a rabbit polycolonal ERK2 antibody (Santa Cruz cat. # sc-154). Rabbit primary antibodies were detected using an HRP-conjugated donkey anti-rabbit secondary antibody (Jackson Laboratories cat. # 711-035-152). Mouse primary antibodies were detected using an HRP-conjugated goat anti-mouse secondary antibody (Invitrogen cat. # 31432). Media was aspirated and plate was washed 1X with ice-cold PBS, then plate was aspirated again. 250 L of lysis buffer (20 mM Tris-HCl, pH 8.0, 1% NP-40, 10% glycerol, 137 mM NaCl, 2 mM EDTA, 2.5 mM NaVO4, 1 EDTA-free protease inhibitor cocktail tablet per 25 mL (Roche cat. # 11873580001), 1% phosphatase inhibitor cocktail 2 (Sigma cat. # P5726), and 1% phosphatase inhibitor 3 (Sigma cat. # P0044)) was added to plate and cells were scraped from the plate using a cell lifter, then lysed by vigorously pipetting cells up and down. Cell debris was pelleted at 16,000 x g, and supernatant was transferred to a new tube. Protein concentration was calculated by Bradford Assay using a bovine serum albumin standard solution. 50 g of lysate was added to each lane of 8% SDS-PAGE gel, and run at 120V for approximately 2 hrs. Gel was transferred overnight to nitrocellulose membrane in transfer buffer (25 mM Tris-HCl, 192 mM glycine, pH 8.3 with 20% methanol) at constant current of 180 mA. Membranes were blocked for 1 hr with TBST (50 mM Tris-HCl, 150 mM NaCl, pH 7.5, 0.1% Tween-20) + 5% powdered milk, washed three times with TBST, then incubated overnight in primary antibody diluted 1:1000 in TBST + 5% milk. Membranes were washed three times with TBST, then incubated for 1 hr in secondary antibody diluted 1:5000 in TBST + 5% milk. Membranes were washed three times, then developed using SuperSignal Pico Plus Chemiluminescent Substrate (Thermo Fisher cat. # 34577). Membranes were imaged using the BioRad Chemidoc Touch Imaging System.

*RNA Sequencing*

Three biological replicates were used for each treatment condition. 10 cm plates were aspirated and washed with PBS, then 600 L of RPE Buffer Plus with 2-mercaptoethanol was added to the plate. Cells were scraped using a cell lifter and then lysed by mechanical disruption using a 20-gauge needle. Total RNA was isolated using the RNeasy Plus Kit (Qiagen cat. # 74134). RNA Sequencing library was prepared with 4 g of total RNA using KAPA stranded mRNA Sequencing kit (Roche cat. # 07962193001) and Illumina TruSeq sample indexes following the manufacturer’s protocol with the following exception: half of the adapter-ligated purified cDNA library was amplified with 10 cycles of PCR. The remaining library was stored at -20C. Samples were pooled and sequenced as a 12-plex library pool using the 75-cycle NextSeq 500/550 High Output v2 sequencing kit (Illumina cat. # FC-404-2005) on the Illumina NextSeq500 to yield approximately 4 x 107 reads per sample. Sequenced reads were filtered, aligned, and sorted as previously described (5). Expressed genes were filtered from the total transcriptome using mean expression of 25 reads. Differentially expressed genes were identified using DESeq2 package in R (6), filtering by mean expression of 25 reads, log2 fold-change > 1 or < -1, and adjusted p-value of less than 0.05. GO analysis of the molecular function of differential genes was performed using the Enrichr database (7).

*Identification of Differential regions of FAIRE enrichment*

Regions of FAIRE signal enrichment (ROE) were identified for both POS and NEG (MACS2 v2.1.2) with default parameters. The Score per million (SPM) was calculated for each region of enrichment by dividing the MACS2 derived -log10(p-value) by the total -log10(p-value) then by 1,000,000 for each sample. A union set of all FAIRE ROE was created where any overlapping ROE was resolved by keeping the ROE with the highest SPM value. The union set was then filtered to exclude any FAIRE ROE that fell near TSS (-300 bp : TSS : +500 bp). Raw read counts for each of the FAIRE ROE was tabulated using the featureCounts function in the Rsubread R package (v. 1.34.7) (8). Differential FAIRE regions between POS\_DMSO and NEG\_DMSO experimental conditions were identified using DESeq2 (v. 1.24.0) at a padj < 0.05 with the following parameter: betaPrior=T (6). The heatmap of differential FAIRE ROE was rendered using average normalized signal for each ROE unique to POS\_DMSO and NEG\_DMSO experimental conditions calculated with the python library pyBigWig v0.3.17, and plotted using the heatmap method in the python package Seaborn (v. 0.9.0) with parameter robust=True, which calculates the colormap range with robust quantiles.

*Identification of Differential Regions of H3K27ac Enrichment*

Regions of H3K27ac enrichment (ROE) were defined with method callpeak in the MACS2 package (v2.1.2) with the options –broadpeak -c [match input control], and –max-gap 1000. MACS broadpeaks were than ranked by size, and the top 3% of H3K27ac regions of enrichment were filtered out. The top 3% threshold is an approximation of the cutoff used for super enhancer identification, and since our focus was not on super enhancers, we removed these large regions from our downstream analyses. The summit of each H3K27ac ROE was identified using the refinepeak method in MACS2 package using default parameters. A union set of H3K27ac regions of enrichment identified across all conditions and replicates was created, where a +/- 500 bp window was put around each summit, and any overlapping regions was resolved by taking the ROE with the higher refinepeak score. Any H3K27ac ROE that overlapped with promotor region of a gene defined as +300 upstream of TSS and -500 bp downstream was excluded from downstream analyses. Unnormalized read count at each H3K27ac ROE was tabulated with the featureCounts function in the Rsubread R package (v. 1.34.7) (8). Differential regions of H3K27ac enrichment were then defined using DESeq2 (v. 1.24.0) at a padj < 0.05, with the parameter betaPrior=T (6).

*Differential JUNB Binding Analysis*

JUNB peaks were defined using MACS2 (v2.1.2) with default parameters. Score per million (SPM) values were calculated for each peak by dividing the MACS2 derived -log10(p-value) by the total -log10(p-value) then by 1,000,000 for each sample. A union set of all JUNB peaks was created where any overlapping peaks (summit +/- 100 bp) were resolved by keeping the peak with the highest SPM value. Raw read counts for each of the JUNB peaks was tabulated using the featureCounts function in the Rsubread R package (v. 1.34.7) (8). Differential JUNB peaks between POS\_DMSO and NEG\_DMSO experimental conditions were identified using DESeq2 (v. 1.24.0) at a padj < 0.05 (6).

*Motif Analysis*

Enriched DNA sequence motifs at FAIRE ROE unique to the POS subpopulation were identified using the findMotifsGenome.pl method in the HOMER package (v. 4.10.3) (9). Input FAIRE ROE were defined as summit of each FAIRE ROE as defined by MACS2 (v. 2.1.2) +/- 100 bp (10). Background for this analysis was defined as the FAIRE ROE that were common to both the POS and NEG subpopulations. Motifs at differential regions of H3K27ac enrichment (in acute and chronic treatment conditions) were also identified using the findMotifsGenome.pl method in the HOMER (v. 4.10.3) (9). Motif analysis was performed on gained or lost regions of H3K27ac unique to POS/NEG cells relative to regions of H3K27ac unchanged after trametinib treatment unique to POS/NEG cells.

*Chromatin Immunoprecipitation Sequencing*

ChIP-Seq for H3K27ac was performed using the ChIP-grade antibody for histone H3K27ac (Active Motif cat. # 39133). ChIP-Seq for JUNB was performed using the ChIP-grade antibody for JUNB (Cell Signaling cat. #3753S). Cells were fixed with 1% formaldehyde for 10 minutes at room temperature, then quenched for five minutes using 1 mM Glycine. Media was discarded and cells were scraped on ice using a cell lifter. Cells were centrifuged 3K RPM at 4C, then supernatant was aspirated and the pellet was flash frozen in liquid nitrogen. Antibody was conjugated to Protein A Dynabeads (Life Technologies cat. # 1002D) for 4 hours at 4C in PBS + 0.5% BSA. Crosslinked pellets were resuspended in lysis buffer 1 to permeabilize plasma membrane, and centrifuged for 5 minutes at 4C at 200 x g. Nuclei were resuspended in lysis buffer, and centrifuged for 5 minutes at 4C at 200 x g. Chromatin was resuspended in sonication buffer, sonication beads added and chromatin was sonicated at 4C for 15 cycles; 30 seconds on, 30 seconds off. Beads were then washed three times with a low salt sonication buffer, then washed once with a high salt sonication buffer, then washed once with a LiCl wash buffer, then washed once with TE buffer. Fragmented chromatin was eluted from the beads with elution buffer at 65C for 15 minutes, vortexing every two minutes. Eluted chromatin and Dynabeads were placed on the magnet, and eluted chromatin was transferred to a fresh tube. Crosslinking was reversed by incubating the eluted chromatin at 65C overnight. The following day, 0.2 g/mL RNase A (Sigma cat. # R4642) was added to the samples and incubated for 1 hr at 37C. Protein in the sample was digested using 0.2 g/mL Proteinase K (Ambion cat. # 25530-015) at 55C. Fragmented DNA was purified using the Qiagen MinElute Kit (Qiagen cat. # 28004). 50 ng of DNA was used for library preparation using the KAPA stranded HyperPrep Kit (Roche cat. # 07962347001) and Illumina TruSeq Indexed Adapters according to the manufacturer’s instructions. Dual size selection was performed after 18 cycles of PCR amplification of the cDNA library according to manufacturer recommendations. A multiplexed pool of 12 libraries was sequenced using the 75-cycle NextSeq 500/550 High Output v2 sequencing kit (Illumina cat. # FC-404-2005) on the Illumina NextSeq500 to yield approximately 3.5 x 107 reads per sample. Cutadapt (v.1.12) was used to remove adaptor sequences from reads. Reads were then filtered with FASTX-Toolkit (v0.0.12) including options -Q 33, -p 90, and q 20. Reads were then aligned to the hg19 genome using STAR (v2.5.2b) with the following parameters: --outFilterMismatchNmax 2, --chimSegmentMin 15, --chimJunctionOverhangMin 15, --outSAMtype BAM Unsorted, --outFilterType BySJout, --outFilterScoreMin 1, --outFilterMultimapNmax 1. Track-level H3K27ac signal was RPM normalized and visualized using UCSC genome browser.

*Comparison of Differential Regions of H3K27ac Signal Identified After Chronic and Acute Trametinib Treatment*

Significance of observed overlap between differential regions of H3K27ac signal following either chronic or acute trametinib treatment was determined using a permutation test (Fig 3F). Differential regions of H3K27ac signal identified after chronic treatment were held constant for each permutation and then compared to randomly assigned size and chromosome matched regions identified after acute treatment using bedtools shuffle and intersect (v. 2.29.0). Each comparison was run 1000 times and the p-value was calculated as the number of times the overlap was greater than observed overlap divided by total number of iterations.

*Cell Cycle Analysis*

Approximately 1 million cells were plated in a 10 cm tissue culture plate, and treated for the time indicated. Treated cells were washed with PBS, trypsinized for 5 minutes, then quenched with media. Cells were pelleted at 200 x g, and media was aspirated. Cells were resuspended in 300 L 50% FBS in PBS. Ice-cold 70% ethanol was added dropwise, and cells were fixed overnight at 4C. Fixed cells were pelleted at 200 x g, then washed twice with PBS, and resuspended the fixed cells in 500 L of a propidium iodide solution (BD Biosciences cat. # 556463). Propidium iodide staining was measured by quantifying peak area of each cell using flow cytometry running Summit flow cytometry software (version 4.3) on the Cyan ADP Flow Cytometer (Beckman Coulter). Data was normalized by adjusting the PE-TexRd channel voltage so that the mean of the G1 peak measured 100 counts. Cell cycle analysis was performed using FlowJo (version 10.0.8). Cells were gated to remove dead and doublet cells, then frequency of each cell cycle phase was determined using the Watson method in the cell cycle analysis package of FlowJo.

*Beta-Galactosidase Staining*

Cells were stained using Senescence -Galactosidase Staining Kit (Cell Signaling Technology cat. # 9860) according to the manufacturer’s instructions. Cells were cultured in a 35 mm cell culture dish. Media was aspirated and cells were washed with PBS. Cells were fixed for 10-15 minutes at room temperature using 1 mL of the 1X Fixative Solution prepared as recommended. Plate was rinsed twice with PBS, then 1 mL of the -Galactosidase Staining Solution was added to the plate, which was sealed with parafilm and incubated overnight at 37C in a dry incubator. Plates were stored in 70% glycerol at 4C.

*Xenograft Growth Assay*

All mice were treated in accordance with protocols approved by the Institutional Care and Use Committee for animal research at The University of North Carolina. Female NOD *scid* gamma mice were given a mammary fat pad injection of 2 x 106 POS, POS R1, NEG, or NEG R cells suspended in 50% Matrigel. Tumor volume was calculated by caliper measurements ((width)2 x (length)) / 2 every three days until tumors reached 100 mm3, at which point mice were given control chow or 0.3 mg/kg/day trametinib chow. Mice injected with trametinib resistant cell lines POS R1 and NEG R were given 0.3 mg/kg/day trametinib chow three days before xenograft injection and remained on trametinib chow through the duration of the study. Number of mice for each arm of the xenograft study: POS on control chow (n=6), POS on trametinib chow (n=6), NEG on control chow (n=12), POS R1 on trametinib chow (n=6), NEG R on trametinib chow (n=6). Tumor volume of NEG and NEG R tumors did not reach the 100 mm3 minimum volume, and after 90 days the NEG and NEG R arms of the study were terminated.

*RNA Isolation From Fixed Isolated and Parental Cells*

Approximately 10 million SUM-229PE cells were treated for 24 hours with 30 nM trametinib or DMSO control. Additionally, 5 million POS cells and 5 million were treated with 30 nM trametinib or DMSO control. Cells were fixed using the MARIS method as previously described (11). Cells were trypsinized for 5 minutes with 0.5% Trypsin-EDTA (Gibco cat. # 25300-0054) at 37°C, then trypsin was quenched with culture media. Cells were pelleted at 200 x g, then washed 2X with ice-cold molecular biology grade PBS (MBG-PBS). Cells were fixed and permeabilized with permeabilization buffer (4% PFA (Electron Microscopy Sciences cat. # 30525-89-4), 0.1% saponin (Sigma-Aldrich cat. # 47036), with 1:100 RNasin Plus RNase Inhibitor (Promega cat. # N2615) in MGB-PBS) for 30 min. at 4°C. Cells were pelleted at 3000 x g for 3 min. and washed 2X with wash buffer (0.2% BSA , 0.1% saponin and 1:100 RNasin Plus RNase Inhibitor in MBG-PBS). SUM-229PE cells were resuspended to a concentration of 1 X 106 cells/mL in staining buffer (1% BSA, 0.1% saponin, and 1:25 RNasin Plus RNase Inhibitor in MBG-PBS) and stained with50 mL of EpCAM (EpCAM-FITC, Stem Cell Technologies cat. # 60136FI) and 10 mL of CD49f (CD49f-PE-Cy5, BD Biosciences cat. #551129) antibodies for 30 minutes in the dark at 4°. Cells were washed twice with wash buffer, then resuspended in sorting buffer (0.5% BSA, 1:25 RNasin Plus RNase Inhibitor). Isolated POS and NEG cells were resuspended to a concentration of 1 X 106 cells/mL in sorting buffer and kept on ice while SUM-229PE cells were stained and sorted. Following SUM-229PE sorting, the sorted populations, as well as isolated POS and NEG cells, were pelleted at 3000 x g for 5 min at 4°C. Total RNA was isolated using the RecoverAll Total Nucleic Acid Isolation Kit (Ambion cat. # AM1975), beginning at the protease digestion step of the manufacturer’s protocol with the following modification: cells were digested for 3 hrs. at 50°C. RNA-Seq libraries were then prepared as described herein.

*Single Cell RNA Sequencing*

Single cell RNA Sequencing of approximately 5000 POS cells and 5000 POS R1 cells was performed according to the manufacturer specification (10X Genomics cat. # 1000092, 1000074, 120262). Media was aspirated and cells were washed with PBS, then trypsinized with 0.05% Trypsin for 5 minutes. Media was added to stop digestion and cells were centrifuged at 200 x g for 5 minutes. Cells were resuspended in 1 mL of media, then filtered with a 30 m filter and counted. Cells were washed twice with 0.04% BSA, then strained using a FlowMi tip strainer (Sigma cat. # BAH136800040) to minimize sample loss. Single cell libraries were prepared according to the manufacturer’s instructions. Library concentration was calculated by Agilent Tapestation, and library was amplified using 8 cycles of PCR according to manufacturer’s recommendation. Library was sequenced using the 150-cycle NextSeq 500/550 High Output v2.5 sequencing kit (Illumina cat. # 20024907) on an Illumina NextSeq 500 Sequencer. The cellranger mkfastq pipeline of 10x Genomics Cell Ranger v. 2.1.0 software was utilized to demultiplex 3’ Chromium v2 sequence reads. The resulting FASTQ files were piped to cellranger count for alignment, filtering, barcode counting, and UMI counting to generate the gene-barcode matrix for single-cell *KRAS* expression estimates of each SUM-229 subpopulation.

*Scanning and analysis of IF images*

Slides containing RNAscope fluorescently labeled cells were scanned either in the Aperio ScanScope FL or the Aperio Versa Digital Pathology Scanner using a 20X objective (Leica Biosystems). Images were archived in TPL’s eSlide Manger database (Leica Biosystems). Images were manually annotated for regions of interest using Tissue Studio software (Definiens Inc.Tissue Studio version 2.7 with Tissue Studio Library version 4.4.2), specifically the Nuclei and Simulated Cells algorithm in the IF Portal, was then used to detect and enumerate cells that contained the cy5-labeled KRAS RNA. Briefly, nuclei were digitally detected by the presence of DAPI stain (nuclear counterstain). From these nuclei, a cell simulation was performed, and cells margins were grown out from nuclear boundaries. Positivity thresholds for KRAS RNA probe were determined by measuring the staining intensities both inside and outside simulated cells. Once thresholds were set, the algorithm evaluated each cell individually for the presence of KRAS RNA positive signal. Cells were separated by the algorithm into negative, low positive, medium positive, or high positive categories based on the signal intensity. H-Score values were determined using the following formula: [1 × (% cells 1+)] + [2 × (% cells 2+)] + [3 × (% cells 3+)].

*Crystal Violet Colony Formation Assay*

10,000 cells per well were plated in 6-well plates with three replicates for each condition at each timepoint. Media was changed every three days throughout the treatment period. Following treatment, cells were washed with PBS, fixed with ice-cold methanol for 10 minutes at -20C, then stained for 20 minutes with a 20% methanol solution containing 0.5% crystal violet dye. Stain was aspirated, and wells were rinsed and dried before imaging. Stain was solubilized in 1 mL of 30% acetic acid solution, and absorbance of dye from each well was measured at 600 nm.

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