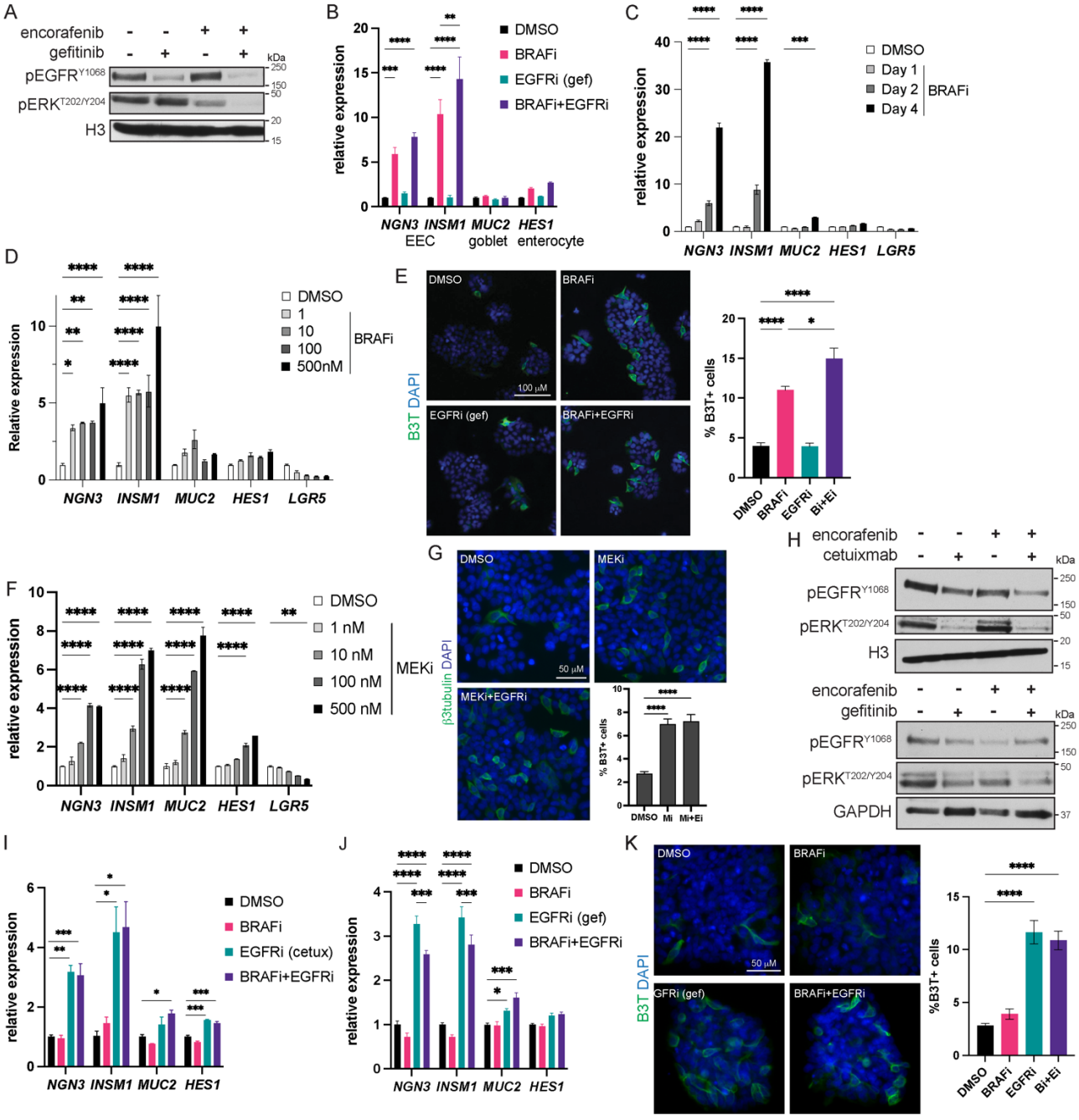
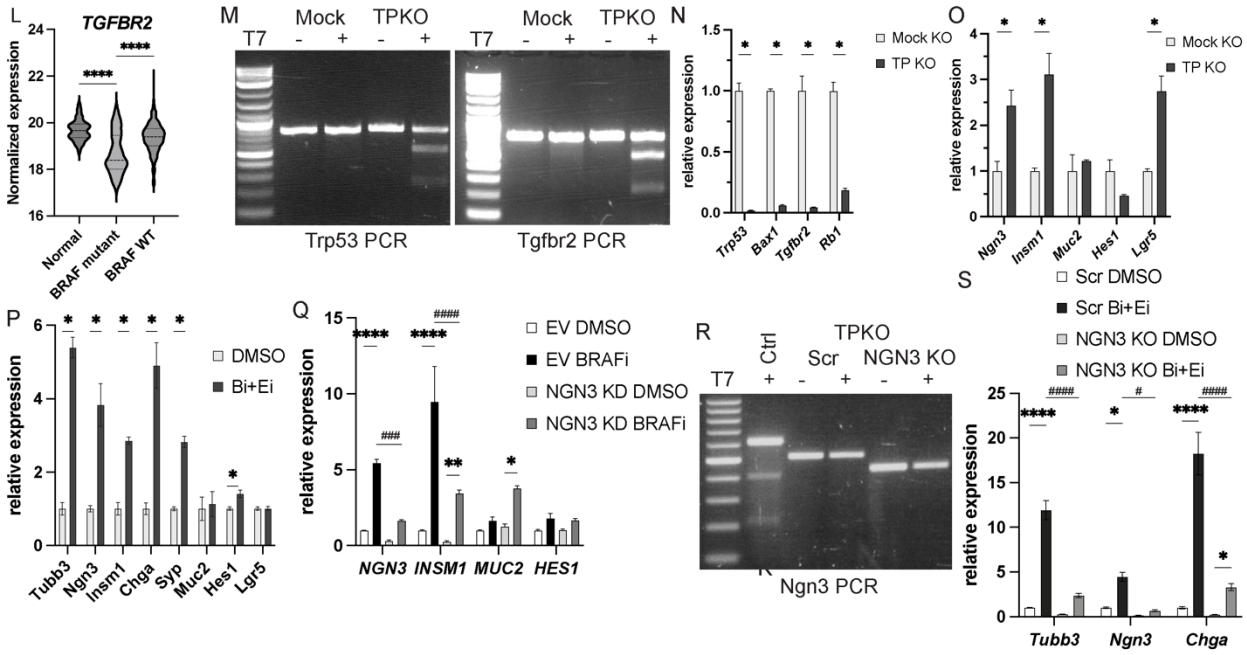
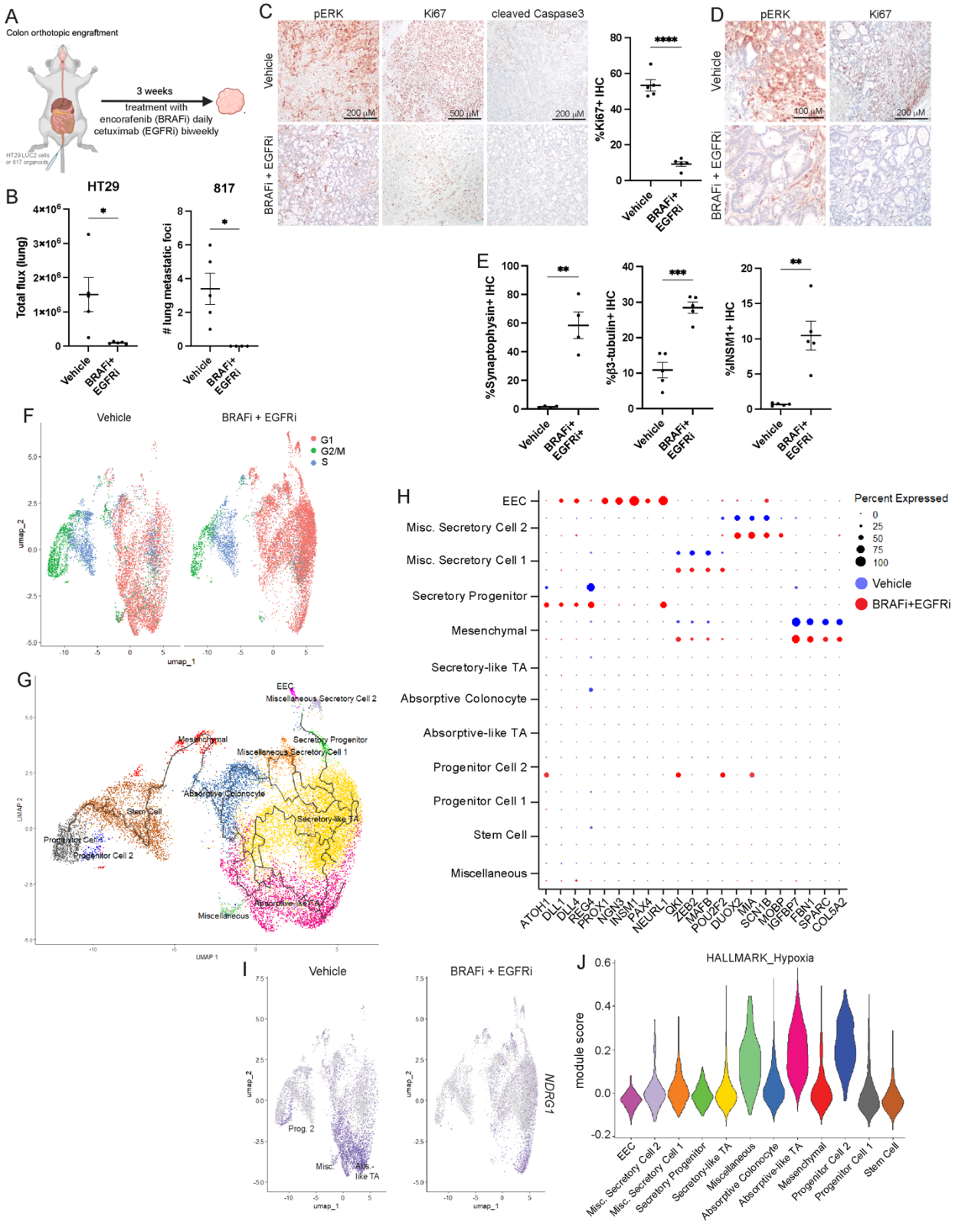

A
encorafenib
gefitinib
kDa
pEGFR^{Y1068}
pERK$^{T202/Y204}$
H3
B
relative expression
DMSO
BRAFi
EGFRi (gef)
BRAFi+EGFRi
NGN3 INSM1 MUC2 HES1
EEC goblet enterocyte
C
relative expression
DMSO
Day 1
Day 2
Day 4
BRAFi
NGN3 INSM1 MUC2 HES1 LGR5
D
Relative expression
DMSO
1
10
100
500nM
BRAFi
NGN3 INSM1 MUC2 HES1 LGR5
E
DMSO
BRAFi
EGFRi (gef)
BRAFi+EGFRi
100 μM
B3T DAPI
% B3T+ cells
DMSO BRAFi EGFRi Bi+Ei
F
relative expression
DMSO
1 nM
10 nM
100 nM
500 nM
MEKi
NGN3 INSM1 MUC2 HES1 LGR5
G
DMSO
MEKi
MEKi+EGFRi
50 μM
β3tubulin DAPI
% B3T+ cells
DMSO Mi Mi+Ei
H
encorafenib
cetuximab
kDa
pEGFR^{Y1068}
pERK$^{T202/Y204}$
H3
encorafenib
gefitinib
kDa
pEGFR^{Y1068}
pERK$^{T202/Y204}$
GAPDH
I
relative expression
DMSO
BRAFi
EGFRi (cetux)
BRAFi+EGFRi
NGN3 INSM1 MUC2 HES1
J
relative expression
DMSO
BRAFi
EGFRi (gef)
BRAFi+EGFRi
NGN3 INSM1 MUC2 HES1
K
DMSO
BRAFi
GFRi (gef)
BRAFi+EGFRi
50 μM
B3T DAPI
%B3T+ cells
DMSO BRAFi EGFRi Bi+Ei



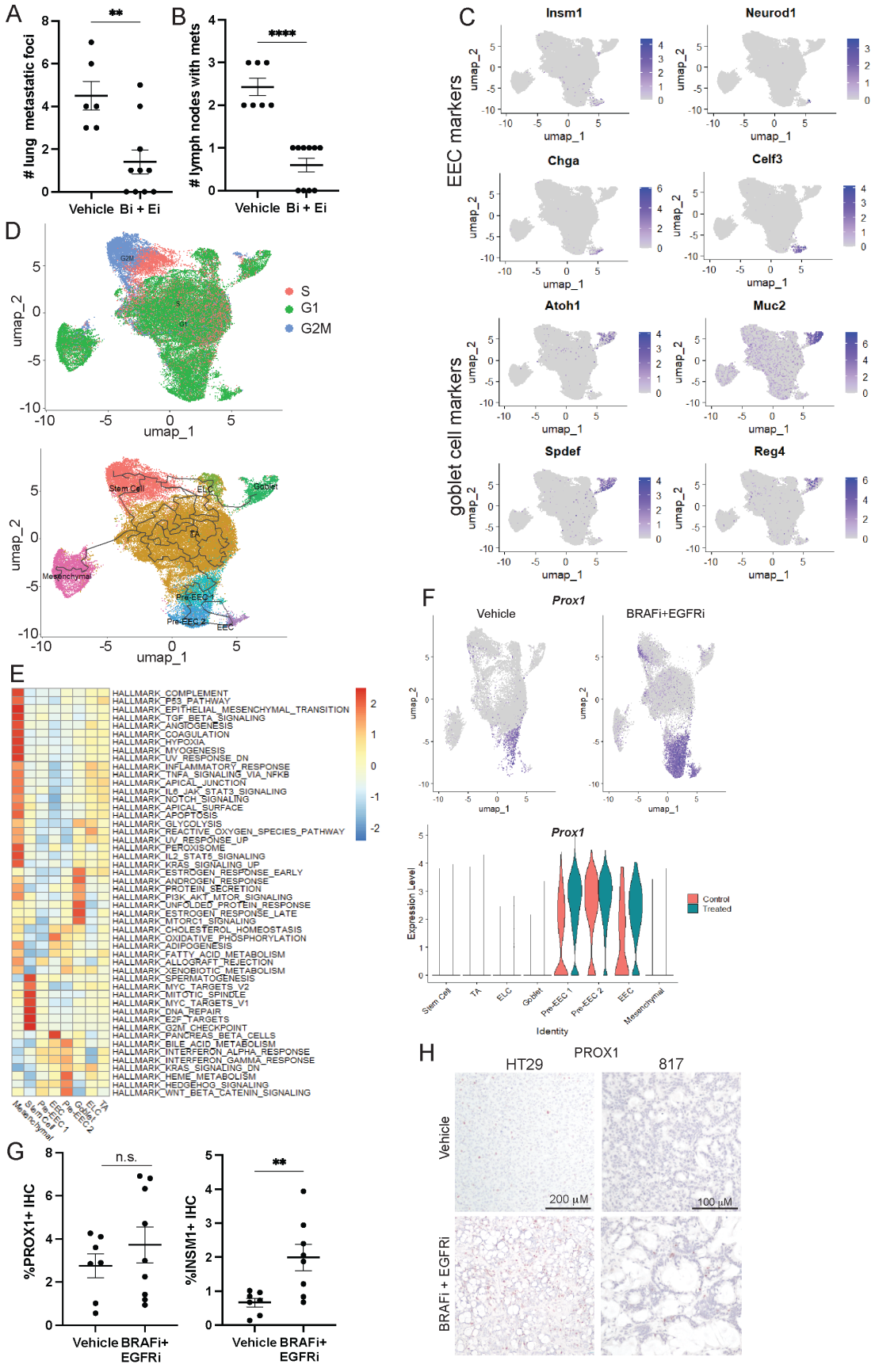
Supplementary Figure S1. MAPK pathway inhibition enriches for EECs in *BRAF* mutant CRC. A) Western blots of HT29 cells treated with DMSO or 2.5 nM encorafenib (BRAFi) alone or in combination with 500 nM gefitinib (EGFRi, gef) for 48H. (B) Relative gene expression of indicated genes in HT29 cells treated as in A. Gene expression was normalized to the housekeeping gene *RHOA* and then to the DMSO treated cells. Graph represents mean +/- SEM. N=3. (C) Relative gene expression of indicated genes in HT29 cells treated with DMSO or 2.5 nM encorafenib (BRAFi) for the indicated number of days. Data presented as in B. (D) Relative gene expression of the indicated genes in HT29 cells treated with the indicated concentration of encorafenib (BRAFi) for 48H. Data presented as in B. (E) Immunofluorescence for β3-tubulin (B3T) in HT29 cells treated as in A for 72H. Graph is the %β3-tubulin+ cells of the total number of cells per field. N=3. (F) Relative gene expression of indicated genes in HT29 cells treated with the indicated concentrations of binimetinib (MEKi) for 48H. Data presented as in B. (G) Immunofluorescence for β3-tubulin (B3T) in HT29 cells treated with 50 nM binimetinib (MEKi, Mi) with or without 500 nM gefitinib (EGFRi, Ei) for 72H. Data presented as in E. (H) Western blots of NCI-H508 cells treated with DMSO or 2.5 nM encorafenib alone or in combination with in combination with 10 μg/ml cetuximab (top) or 250 nM gefitinib (bottom) for 48H. (I) Relative gene expression of indicated genes in NCI-H508 cells treated with DMSO or 2.5 nM encorafenib (BRAFi) alone or in combination with 10 μg/ml cetuximab (EGFRi, cetux) for 48H. Data presented as in B. (J) Relative gene expression of indicated genes in NCI-H508 cells treated with DMSO or 2.5 nM encorafenib (BRAFi) alone or in combination with 250 nM gefitinib (EGFRi, gef) for 48H. Data presented as in B. (K) Immunofluorescence for β3-tubulin (B3T) in NCI-H508 cells treated as in I for 72H. Data presented as in E. (L) Normalized expression of *TGFBR2* in the indicated samples from the TCGA colon adenocarcinoma data. (M) T7 endonuclease 1 mismatch detection assay using primers that span the targeted regions for CRISPR knockout (KO) for Trp53 and Tgfbr2 in mock and TP (Trp53 + Tgfbr2) KO mouse colon CRC organoids. Gels are cropped. (N) and (O) Relative gene expression of the indicated genes in mock and TP KO mouse organoids. Data presented as in B. (P) Relative gene expression of the indicated genes in TP KO organoids treated with DMSO or 2.5 nM encorafenib (Bi) and 500 nM gefitinib (Ei) for 72H. Data presented as in B. (Q) Relative gene expression of the indicated genes in empty vector (EV) and NGN3 knockdown (KD) HT29 cells treated as in C for 48H and presented as in B. (R) T7 endonuclease 1 mismatch detection assay using primers that span

the targeted regions for CRISPR knockout (KO) for Ngn3 in mock and Ngn3 KO mouse colon TP KO CRC organoids. Ctrl is a control template used as positive control for T7 activity. Gels are cropped. (S) Relative gene expression of the indicated genes in scramble KO (Scr) and NGN3 KO TP KO organoids treated as in P and presented as in B. Significance was determined by ordinary one-way ANOVA with Tukey pairwise multiple comparison testing (B,C,D,E,F,G,I,J,K,L,Q,S) and Student's t-test (N,O,P). *P≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, **** P ≤ 0.0001 relative to DMSO or Mock control. $^{\#}$P≤ 0.05, $^{\#\#\#}$P≤ 0.001, $^{\#\#\#\#}$P≤ 0.0001 relative to EV Bi or Scr Bi+Ei.

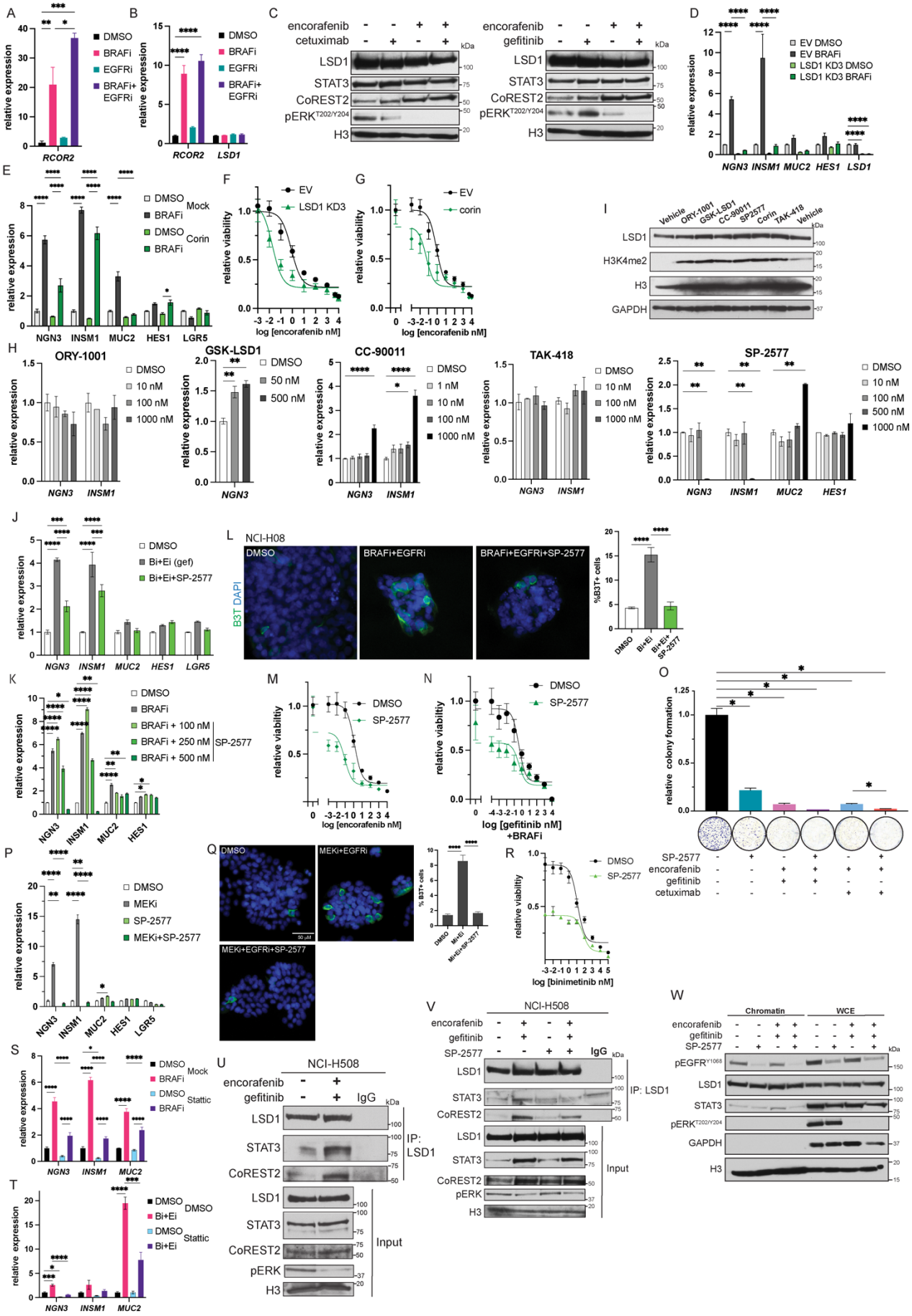


Supplementary Figure S2. Effect of BRAFi plus EGFRi on tumor cell type composition in *BRAF*^{V600E} CRC. (A) Diagram of HT29 and 817 colon orthotopic model and treatment. (B) Total

ex vivo flux of lungs or number of metastatic foci in lungs from mice with colon orthoptic HT29 or 817 tumors, respectively. Each dot represents the signal from lung from one mouse. Lines represent mean +/- SEM. (C&D) Representative IHC for indicated proteins in (C) HT29 or (D) 817 tumor sections from mice treated as indicated. (E) Scoring of HT29 tumor IHC staining of indicated proteins. Each point represents the scoring for one tumor. Lines represent mean -/+ SEM. (F&G) UMAP dot plots detailing predicted (F) cell cycle phase and (G) trajectory analysis of 817 scRNAseq samples. (H) Dot plot showing marker gene expression in vehicle and BRAFi+EGFRi treated samples across all annotated cell types. The size of the dot is proportional to the percentage of cells that express a given gene, and the color scale indicates the average scaled gene expression within the specific cell population. Blue and red dots are expression levels in cells from vehicle and BRAFi+EGFRi tumors, respectively. (I) UMAP dot plots of normalized expression values of *NDRG1* separated by treatment. (J) Module scores for the HALLMARK_hypoxia gene set for each cluster in the 817 scRNAseq data.


A
lung metastatic foci
Vehicle
Bi + Ei
**
B
lymph nodes with mets
****
C
EEC markers
goblet cell markers
Insm1
Neurod1
Chga
Celf3
Atoh1
Muc2
Spdef
Reg4
umap_1
umap_2
D
G2M
S
G1
E
HALLMARK_COMPLEMENT
HALLMARK_P53_PATHWAY
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
HALLMARK_TGF_BETA_SIGNALING
HALLMARK_ANGIOGENESIS
HALLMARK_COAGULATION
HALLMARK_HYPOXIA
HALLMARK_MYOGENESIS
HALLMARK_UV_RESPONSE_DN
HALLMARK_INFLAMMATORY_RESPONSE
HALLMARK_TNFA_SIGNALING_VIA_NFKB
HALLMARK_APICAL_JUNCTION
HALLMARK_IL6_JAK_STAT3_SIGNALING
HALLMARK_NOTCH_SIGNALING
HALLMARK_APICAL_SURFACE
HALLMARK_APOPTOSIS
HALLMARK_GLYCOLYSIS
HALLMARK_REACTIVE_OXYGEN_SPECIES_PATHWAY
HALLMARK_UV_RESPONSE_UP
HALLMARK_PEROXISOME
HALLMARK_IL2_STAT5_SIGNALING
HALLMARK_KRAS_SIGNALING_UP
HALLMARK_ESTROGEN_RESPONSE_EARLY
HALLMARK_ANDROGEN_RESPONSE
HALLMARK_PROTEIN_SECRETION
HALLMARK_PI3K_AKT_MTOR_SIGNALING
HALLMARK_UNFOLDED_PROTEIN_RESPONSE
HALLMARK_ESTROGEN_RESPONSE_LATE
HALLMARK_MTORC1_SIGNALING
HALLMARK_CHOLESTEROL_HOMEOSTASIS
HALLMARK_OXIDATIVE_PHOSPHORYLATION
HALLMARK_ADIPOGENESIS
HALLMARK_FATTY_ACID_METABOLISM
HALLMARK_ALLOGRAFT_REJECTION
HALLMARK_XENOBIOTIC_METABOLISM
HALLMARK_SPERMATOGENESIS
HALLMARK_MYC_TARGETS_V2
HALLMARK_MITOTIC_SPINDLE
HALLMARK_MYC_TARGETS_V1
HALLMARK_DNA_REPAIR
HALLMARK_E2F_TARGETS
HALLMARK_G2M_CHECKPOINT
HALLMARK_PANCREAS_BETA_CELLS
HALLMARK_BILE_ACID_METABOLISM
HALLMARK_INTERFERON_ALPHA_RESPONSE
HALLMARK_INTERFERON_GAMMA_RESPONSE
HALLMARK_KRAS_SIGNALING_DN
HALLMARK_HEME_METABOLISM
HALLMARK_HEDGEHOG_SIGNALING
HALLMARK_WNT_BETA_CATENIN_SIGNALING
Mesenchymal
Stem Cell
Pre-EEC 1
EEC
Pre-EEC 2
Goblet
ELC
TA
F
Prox1
Vehicle
BRAFi+EGFRi
Expression Level
Identity
Control
Treated
G
%PROX1+ IHC
%INSM1+ IHC
n.s.
**
Vehicle
BRAFi+ EGFRi
H
PROX1
HT29
817
Vehicle
BRAFi + EGFRi
200 μM
100 μM

Supplementary Figure S3. Effect of BRAFi plus EGFRi on tumor cell type composition in a syngeneic model of *BRAF*^{V600E} CRC. (A) Number of metastatic foci in lungs from mice with colon orthotopic TP KO tumors treated with encorafenib (Bi) or gefitinib (Ei) as determined by IVIS imaging. Each dot represents the lung from one mouse. Lines represent mean +/- SEM. (B) Number of lymph nodes with metastases per mouse as determined by IVIS imaging. Each dot represents the number of positive lymph nodes in one mouse. Lines represent mean +/- SEM. (C) UMAP dot plots of normalized expression values of marker genes representative of EEC and goblet cell populations in TP KO scRNAseq samples. (D) UMAP dot plots detailing predicted cell cycle phase and trajectory analysis of TP KO scRNAseq samples. (E) Heatmap of enrichment scores for Hallmark gene sets by cell cluster in the TP KO scRNAseq data. (F) UMAP dot plot and violin plot of normalized *Prox1* expression in TP KO scRNAseq data separated by treatment type. (G) %PROX1 positive IHC staining of TP KO tumors as quantified by ImageJ. Each dot represents an individual tumor. Lines represent mean -/+ SEM. (H) Representative PROX1 IHC in HT29 and 817 colon orthotopic tumors. Significance was determined by Student's t-test. *P≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, **** P ≤ 0.0001, n.s. – not significant.


A
relative expression
DMSO
BRAFi
EGFRi
BRAFi+ EGFRi
RCOR2
B
relative expression
DMSO
BRAFi
EGFRi
BRAFi+ EGFRi
RCOR2
LSD1
C
encorafenib
cetuximab
LSD1
STAT3
CoREST2
pERKT202/Y204
H3
kDa
encorafenib
gefitinib
LSD1
STAT3
CoREST2
pERKT202/Y204
H3
kDa
D
relative expression
EV DMSO
EV BRAFi
LSD1 KD3 DMSO
LSD1 KD3 BRAFi
NGN3
INSM1
MUC2
HES1
LSD1
E
relative expression
DMSO
BRAFi
Mock
DMSO
BRAFi
Corin
NGN3
INSM1
MUC2
HES1
LGR5
F
relative viability
EV
LSD1 KD3
log [encorafenib nM]
G
relative viability
EV
corin
log [encorafenib nM]
I
Vehicle
ORY-1001
GSK-LSD1
CC-90011
SP2577
Corin
TAK-418
Vehicle
kDa
LSD1
H3K4me2
H3
GAPDH
H
ORY-1001
relative expression
DMSO
10 nM
100 nM
1000 nM
NGN3
INSM1
GSK-LSD1
relative expression
DMSO
50 nM
500 nM
NGN3
CC-90011
relative expression
DMSO
1 nM
10 nM
100 nM
1000 nM
NGN3
INSM1
TAK-418
relative expression
DMSO
10 nM
100 nM
1000 nM
NGN3
INSM1
SP-2577
relative expression
DMSO
10 nM
100 nM
500 nM
1000 nM
NGN3
INSM1
MUC2
HES1
J
relative expression
DMSO
Bi+Ei (gef)
Bi+Ei+SP-2577
NGN3
INSM1
MUC2
HES1
LGR5
L
NCI-H08
DMSO
BRAFi+EGFRi
BRAFi+EGFRi+SP-2577
B3T DAPI
%B3T+ cells
DMSO
Bi+Ei
Bi+Ei+ SP-2577
K
relative expression
DMSO
BRAFi
BRAFi + 100 nM
BRAFi + 250 nM
BRAFi + 500 nM
SP-2577
NGN3
INSM1
MUC2
HES1
M
relative viability
DMSO
SP-2577
log [encorafenib nM]
N
relative viability
DMSO
SP-2577
log [gefitinib nM] +BRAFi
O
relative colony formation
SP-2577
encorafenib
gefitinib
cetuximab
P
relative expression
DMSO
MEKi
SP-2577
MEKi+SP-2577
NGN3
INSM1
MUC2
HES1
LGR5
Q
DMSO
MEKi+EGFRi
MEKi+EGFRi+SP-2577
% B3T+ cells
DMSO
Bi+Ei
Bi+Ei+SP-2577
R
relative viability
DMSO
SP-2577
log [binimetinib nM]
S
relative expression
DMSO
BRAFi
Mock
DMSO
Stattic
BRAFi
NGN3
INSM1
MUC2
T
relative expression
DMSO
Bi+Ei
DMSO
Stattic
Bi+Ei
NGN3
INSM1
MUC2
U
NCI-H508
encorafenib
gefitinib
IgG
kDa
LSD1
STAT3
CoREST2
IP: LSD1
LSD1
STAT3
CoREST2
pERK
H3
Input
V
NCI-H508
encorafenib
gefitinib
SP-2577
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kDa
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STAT3
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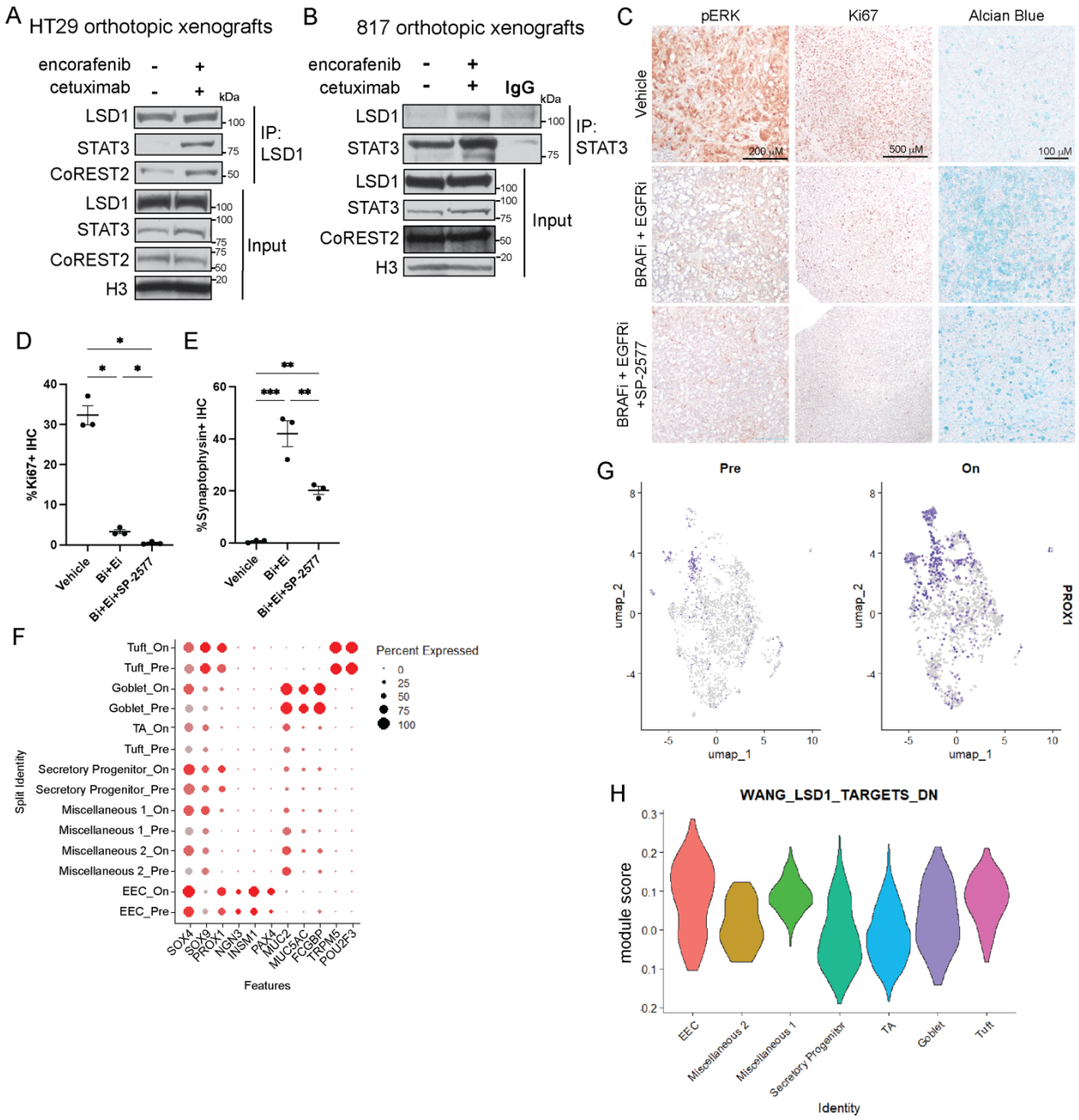
LSD1
STAT3
CoREST2
pERK
H3
Input
W
Chromatin
WCE
encorafenib
gefitinib
SP-2577
kDa
pEGFRY1068
LSD1
STAT3
pERKT202/Y204
GAPDH
H3

Supplementary Figure S4. LSD1 depletion blocks MAPK pathway inhibition-induced increase in EECs. (A) Relative gene expression of indicated genes in HT29 cells treated with DMSO or 2.5 nM encorafenib (BRAFi) and 20 μg/ml cetuximab (EGFRi) for 48H. Gene expression was normalized to the housekeeping gene *RHOA* and then to the DMSO treated cells. Graph represents mean +/- SEM. N=3. (B) Relative gene expression of indicated genes in HT29 cells treated with DMSO or 2.5 nM encorafenib (BRAFi) and/or 500 nM gefitinib (EGFRi) for 48H. Data presented as in A. (C) Western blot of HT29 cells treated as indicated and as in A and B. (D) Relative gene expression in empty vector (EV) and LSD1 knockdown (KD) HT29 cells treated with DMSO or 2.5 nM encorafenib (BRAFi) for 48H. Data presented as in A. LSD1 KD3 is performed using distinct shRNA from those used in primary Figure 1A and 1B. (E) Relative gene expression of indicated genes in HT29 cells treated with DMSO or 2.5 nM encorafenib with or without 50 nM corin for 48H. Data is normalized and presented as in A. (F) Encorafenib dose response curve of EV and LSD1 HT29 cells treated for 72H. Viability was normalized to each group's respective non-encorafenib treated cells. (G) Encorafenib dose response curve of HT29 cells treated with 50 nM corin for 72H. Normalized as in F. (H) Relative gene expression in HT29 cells treated with the indicated concentrations of the indicated LSD1 inhibitors. Graph represents mean +/- SEM. N=3. (I) Western blot of cell lysates prepared from HT29 cells treated with 10 nM of the indicated LSD1 inhibitors. (J) Relative gene expression of indicated genes in NCI-H508 cells treated with DMSO or 2.5 nM encorafenib plus 250 nM gefitinib (EGFRi, Ei, gef) for with or without 1 μM SP-2577 for 48H. Data is normalized and presented as in A. (K) Relative gene expression of indicated genes in HT29 cells treated with DMSO or 2.5 nM encorafenib with or without the indicated concentration of SP-2577 for 48H. Data is normalized and presented as in A. (L) Immunofluorescence for β3-tubulin in NCI-H508 cells treated as in J for 72H. Graph is the %β3-tubulin+ cells of the total number of cells per field. N=3. (M) Encorafenib dose response curve of HT29 cells treated with 500 nM SP-2577 for 72H. Normalized as in F. (N) Gefitinib dose response curve of NCI-H508 cells treated with 2.5 nM encorafenib (BRAFi) and DMSO or 500 nM SP-2577 for 72H. Normalized as in F. (O) Colony formation assay of HT29 cells treated with DMSO or 2.5 nM encorafenib and 500 nM gefitinib or 20 μg/ml cetuximab with or without 1 μM SP-2577 for 6 days. Data is normalized to DMSO treated cells. Graph represents mean +/- SEM. N=3. (P) Relative gene expression of indicated genes in HT29 cells treated with DMSO or 20 nM binimetinib (MEKi) with or without 500 nM SP-2577 for 48H. Data is normalized and presented as in A. (Q) Immunofluorescence for β3-tubulin in HT29 cells treated with DMSO or 20 nM binimetinib (MEKi; Mi) and 500 nM gefitinib (EGFRi, Ei) with or without 500 nM SP-2577for 72H. Data presented as in L. (R) Binimetinib dose response curve of HT29 cells treated with 500 nM SP-2577 for 72H. Viability was normalized to cells treated with DMSO only. (S) Relative gene expression of indicated genes in HT29 cells treated with DMSO or 2.5 nM encorafenib (BRAFi) with or without 2 μM Stattic (STAT3i) for 48H. Data is normalized and presented as in A. (T) Relative gene expression of indicated genes in NCI-H508 cells treated with DMSO or 2.5 nM encorafenib (Bi) and 250 nM gefitinib (Ei) with or without 5 μM Stattic (STAT3i) for 48H. Data is normalized and presented as in A. (U) LSD1 coIP in nuclear lysates prepared from NCI-H508 cells treated with DMSO or 2.5 nM encorafenib plus 250 nM gefitinib for 4H. (V) LSD1 coIP in nuclear lysates prepared from NCI-H508 cells pre-treated with DMSO or 1 μM SP-2577 for 24H prior to treatment with DMSO or 1 μM SP-2577 with or without 2.5nM encorafenib plus 250 nM geftinib for 4H. (W) Western blot of chromatin and whole cell extract (WCE) fractions prepared from HT29 cells pre-treated with DMSO or 1 μM SP-2577 for 24H prior to treatment of DMSO or 1 μM SP-2577 with or without 2.5 nM encorafenib plus 500 nM gefitinib for 4H. Significance was determined by ordinary one-way ANOVA with Tukey pairwise multiple comparison testing for all panels except O, which was analyzed by Brown-Forsythe and Welch ANOVA test with Dunnett's T3 multiple comparisons test. *P≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, **** P ≤ 0.0001.



Supplementary Figure S5. Epithelial cancer cell types present in patient samples of *BRAF*^{V600E} CRC. (A) LSD1 coIP in nuclear lysates prepared from HT29 colon orthotopic tumors from vehicle or encorafenib + cetuximab treated NSG mice. The same tumor lysates were also used in the experiment in 5G, so the input blots are the same. (B) STAT3 coIP in nuclear lysates prepared from 817 colon orthotopic tumors from vehicle or encorafenib + cetuximab treated NSG mice. All images of western blots are cropped. (C) Representative IHC for indicated proteins in HT29 orthotopic tumor sections from mice treated as indicated. (D and E) Scoring of IHC staining of indicated proteins. Each point represents the scoring for one tumor. Lines represent mean -/+ SEM. (F) Dot plot showing marker gene expression in pretreatment and on treatment samples across all annotated cell types. The size of the dot is proportional to the percentage of cells that express a given gene, and the color scale indicates the average scaled gene expression within the specific cell population. (G) UMAP dot plot of normalized *PROX1* expression in samples from patient with *BRAF*^{V600E} CRC scRNAseq data separated by

treatment type. (H) Module scores for the Wang LSD1 Targets Down gene set for each cluster in the scRNAseq data from patients with *BRAF*^{V600E} CRC.