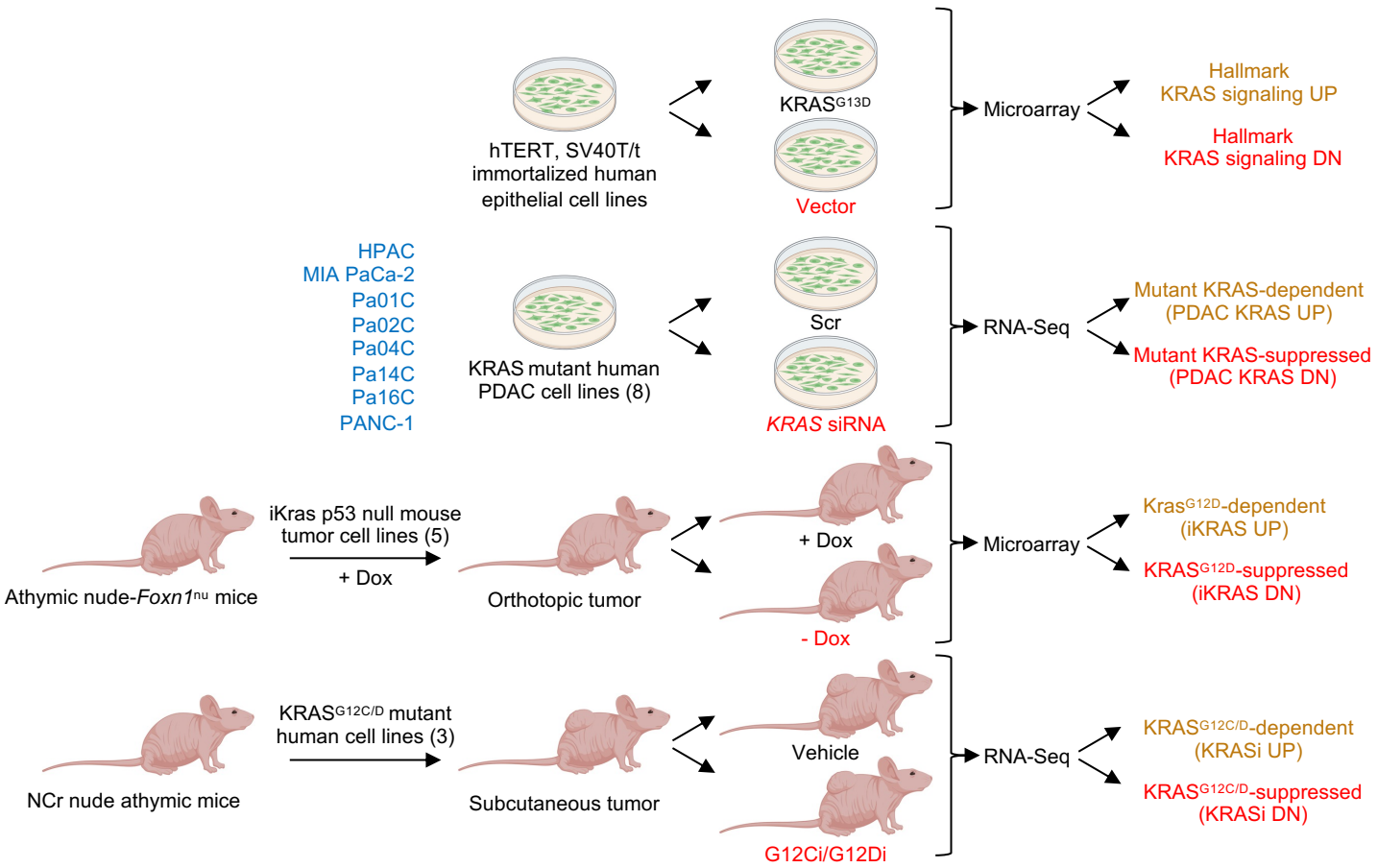
figure S1

A



Mutant KRAS-dependent transcriptome analyses					
Study	**PDAC model**	**KRAS inhibition**	**Method**	**Strengths**	**Limitations**
Liberzon et al. (Cell Syst, 2015)	Compiled from KRAS^{G13D} overexpressing epithelial cells and correlated gene sets	KRAS^{G13D} overexpression	Microarray	• Emergent programming • Mostly isogenic comparisons	• Limited interpretation of founding gene sets • Limited precision
This study	KRAS-mutant PDAC cell lines	*KRAS* targeted siRNA (24 h) and loss of KRAS gene and protein expression	RNA-Seq	• Multiple (8) human PDAC cell lines reflect genetic heterogeneity of human PDAC	• In vitro cell culture environment • Potential off-target siRNA activities
Ying et al. (Cell, 2012)	Doxycycline-inducible Kras^{G12D}; Tp53$^{-/-}$ mouse cell line-induced orthotopic tumors	Doxycycline withdrawal (24 h) and loss of Kras^{G12D} protein expression	Microarray	• In vivo 3D tumor • Pancreas stroma • Regulation of mutant Kras expression	• Mouse • Genetic simplicity of PDAC tumors • RNA quality
Hallin et al. (Cancer Discov, 2020) Hallin et al. (Nat Med, 2022)	KRAS^{G12C} and KRAS^{G12D}-mutant human PDAC cell line-induced mouse xenograft tumors	KRAS$^{G12C/D}$ inhibitor treatment (24 h) and inhibition of KRAS effector binding	RNA-Seq	• In vivo 3D tumor; genetic heterogeneity of human PDAC • Pharmacologic inhibition of mutant KRAS function	• Subcutaneous nonphysiologic location • Mouse stroma-containing tumors • RNA quality

B

PDAC cell line characteristics						
Cell line	**Driver gene mutations**				**Sex**	**Site**
	KRAS	***TP53***	***CDKN2A***	***SMAD4***		
AsPC-1	G12D[a]	C135Afs*35	L78Hfs*41	R100T	Female	Metastatic
HPAC	G12D/WT	WT	E120Ter	D52N_fs*?	Female	Primary
HPAF-II	G12D[a]	P151S	[R29G; A34V];L32_del_fs	WT	Male	Metastatic
MIA PaCa-2	G12C[a]	R248W	Deletion	WT	Male	Primary
Pa01C	G12D/WT	T155P	P70del_fs*74	Deletion	Male	Metastatic
Pa02C	Q61H[a]	L257P	Deletion	Deletion	Male	Metastatic
Pa04C	G12V[a]	Deletion	Deletion	WT	Male	Metastatic
Pa14C/Panc 08.13	G12D/WT	L344P	[R87_F90_del_fs*54];[R87W; F90C]	WT	Male	Primary
Pa16C/Panc 10.05	G12D/WT	I255N	CDKN2A-201 expression loss	WT	Male	Primary
PANC-1	G12D/WT	R273H	Deletion	WT	Male	Primary
SW1990	G12D[a]	Q533Ter	NA	NA	Male	Metastatic

[a]Homozygous; NA, not available

figure S1

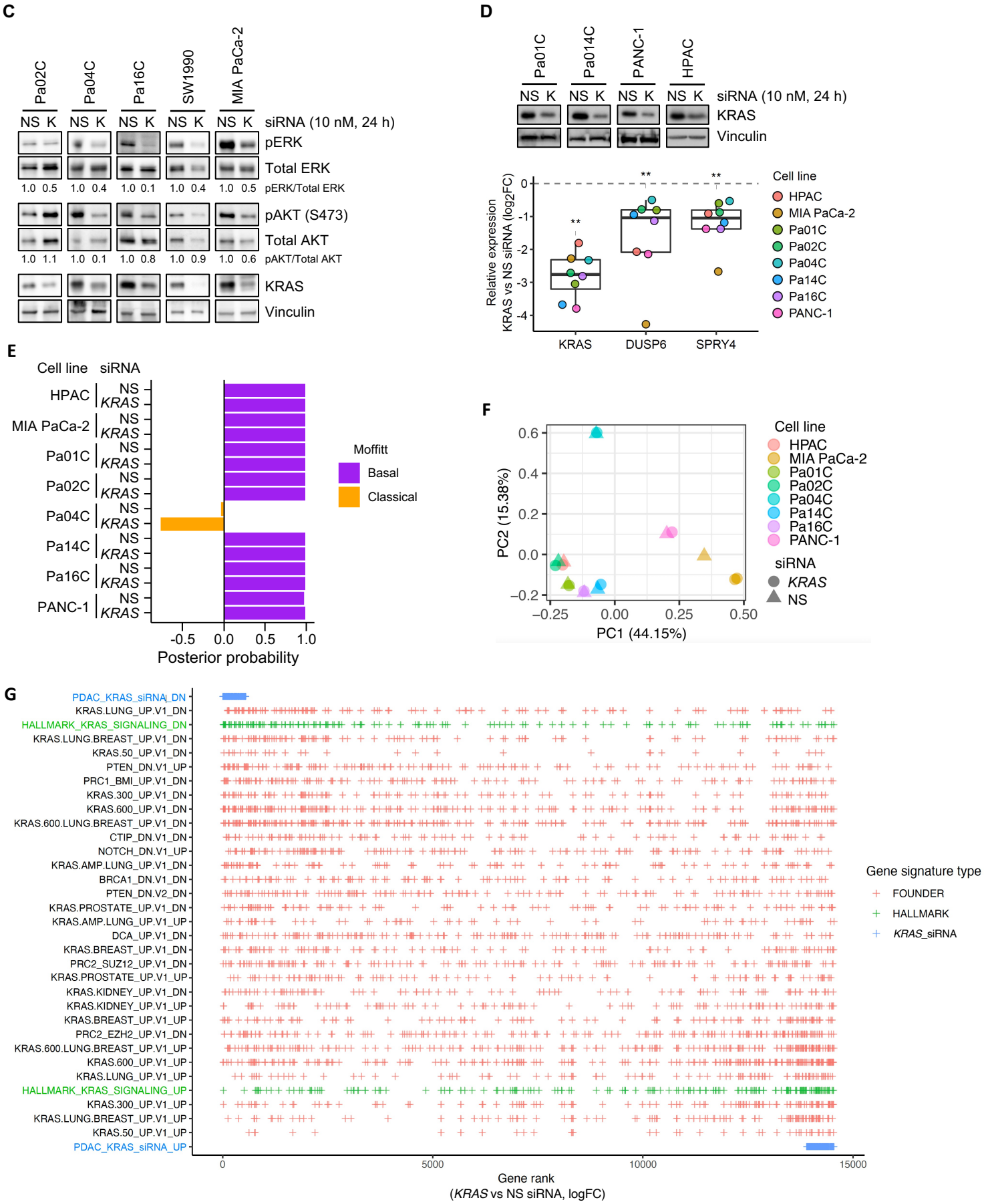
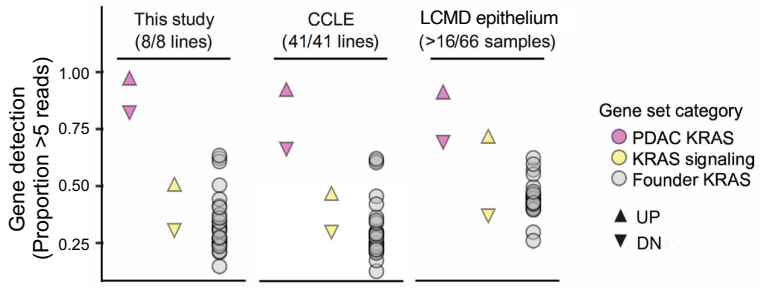

C
Pa02C
Pa04C
Pa16C
SW1990
MIA PaCa-2
NS K NS K NS K NS K NS K
siRNA (10 nM, 24 h)
pERK
Total ERK
1.0 0.5 1.0 0.4 1.0 0.1 1.0 0.4 1.0 0.5 pERK/Total ERK
pAKT (S473)
Total AKT
1.0 1.1 1.0 0.1 1.0 0.8 1.0 0.9 1.0 0.6 pAKT/Total AKT
KRAS
Vinculin
D
Pa01C
Pa014C
PANC-1
HPAC
NS K NS K NS K NS K
siRNA (10 nM, 24 h)
KRAS
Vinculin
Relative expression
KRAS vs NS siRNA (log2FC)
0
-1
-2
-3
-4
**
**
**
KRAS
DUSP6
SPRY4
Cell line
HPAC
MIA PaCa-2
Pa01C
Pa02C
Pa04C
Pa14C
Pa16C
PANC-1
E
Cell line siRNA
HPAC NS KRAS
MIA PaCa-2 NS KRAS
Pa01C NS KRAS
Pa02C NS KRAS
Pa04C NS KRAS
Pa14C NS KRAS
Pa16C NS KRAS
PANC-1 NS KRAS
-0.5 0.0 0.5 1.0
Posterior probability
Moffitt
Basal
Classical
F
PC2 (15.38%)
0.6
0.4
0.2
0.0
−0.2
−0.25 0.00 0.25 0.50
PC1 (44.15%)
Cell line
HPAC
MIA PaCa-2
Pa01C
Pa02C
Pa04C
Pa14C
Pa16C
PANC-1
siRNA
KRAS
NS
G
PDAC_KRAS_siRNA_DN
KRAS.LUNG_UP.V1_DN
HALLMARK_KRAS_SIGNALING_DN
KRAS.LUNG.BREAST_UP.V1_DN
KRAS.50_UP.V1_DN
PTEN_DN.V1_UP
PRC1_BMI_UP.V1_DN
KRAS.300_UP.V1_DN
KRAS.600_UP.V1_DN
KRAS.600.LUNG.BREAST_UP.V1_DN
CTIP_DN.V1_DN
NOTCH_DN.V1_UP
KRAS.AMP.LUNG_UP.V1_DN
BRCA1_DN.V1_DN
PTEN_DN.V2_DN
KRAS.PROSTATE_UP.V1_DN
KRAS.AMP.LUNG_UP.V1_UP
DCA_UP.V1_DN
KRAS.BREAST_UP.V1_DN
PRC2_SUZ12_UP.V1_DN
KRAS.PROSTATE_UP.V1_UP
KRAS.KIDNEY_UP.V1_DN
KRAS.KIDNEY_UP.V1_UP
KRAS.BREAST_UP.V1_UP
PRC2_EZH2_UP.V1_DN
KRAS.600.LUNG.BREAST_UP.V1_UP
KRAS.600_UP.V1_UP
KRAS.LUNG_UP.V1_UP
HALLMARK_KRAS_SIGNALING_UP
KRAS.300_UP.V1_UP
KRAS.LUNG.BREAST_UP.V1_UP
KRAS.50_UP.V1_UP
PDAC_KRAS_siRNA_UP
0
5000
10000
15000
Gene rank
(KRAS vs NS siRNA, logFC)
Gene signature type
FOUNDER
HALLMARK
KRAS_siRNA

figure S1

H


This study
(8/8 lines)
CCLE
(41/41 lines)
LCMD epithelium
(>16/66 samples)
Gene detection
(Proportion >5 reads)
1.00
0.75
0.50
0.25
Gene set category
PDAC KRAS
KRAS signaling
Founder KRAS
UP
DN

I

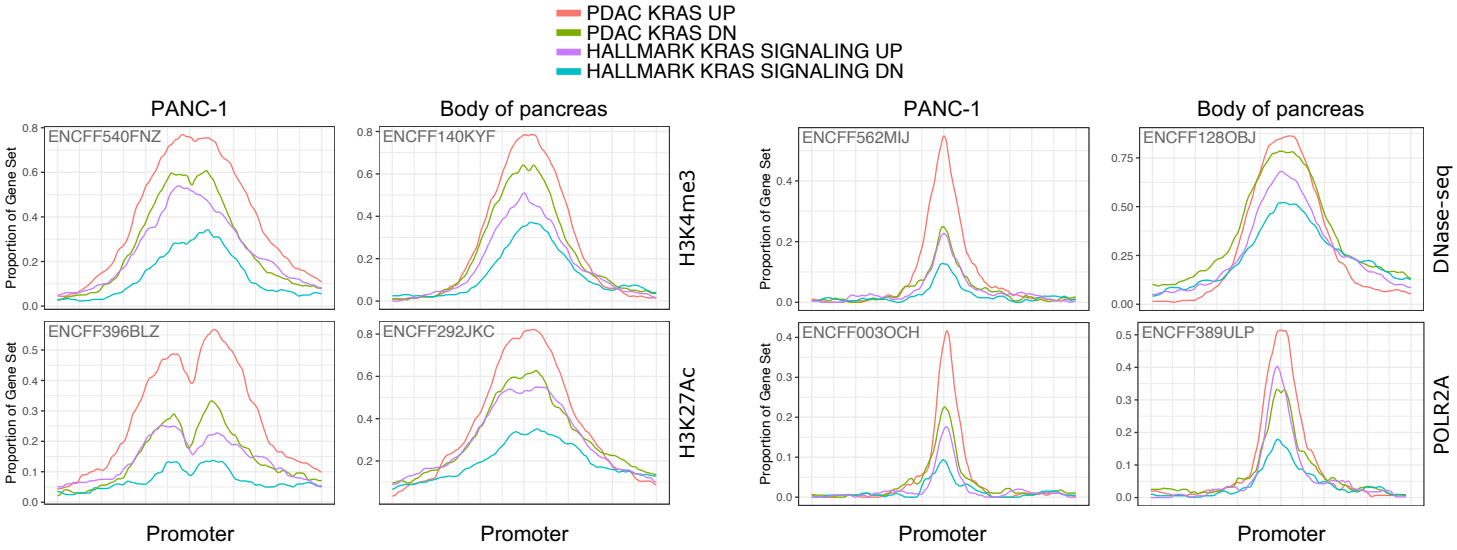

PDAC KRAS UP
PDAC KRAS DN
HALLMARK KRAS SIGNALING UP
HALLMARK KRAS SIGNALING DN
PANC-1
Body of pancreas
PANC-1
Body of pancreas
ENCFF540FNZ
ENCFF140KYF
ENCFF562MIJ
ENCFF128OBJ
ENCFF396BLZ
ENCFF292JKC
ENCFF003OCH
ENCFF389ULP
H3K4me3
H3K27Ac
DNase-seq
POLR2A
Proportion of Gene Set
Promoter

figure S1

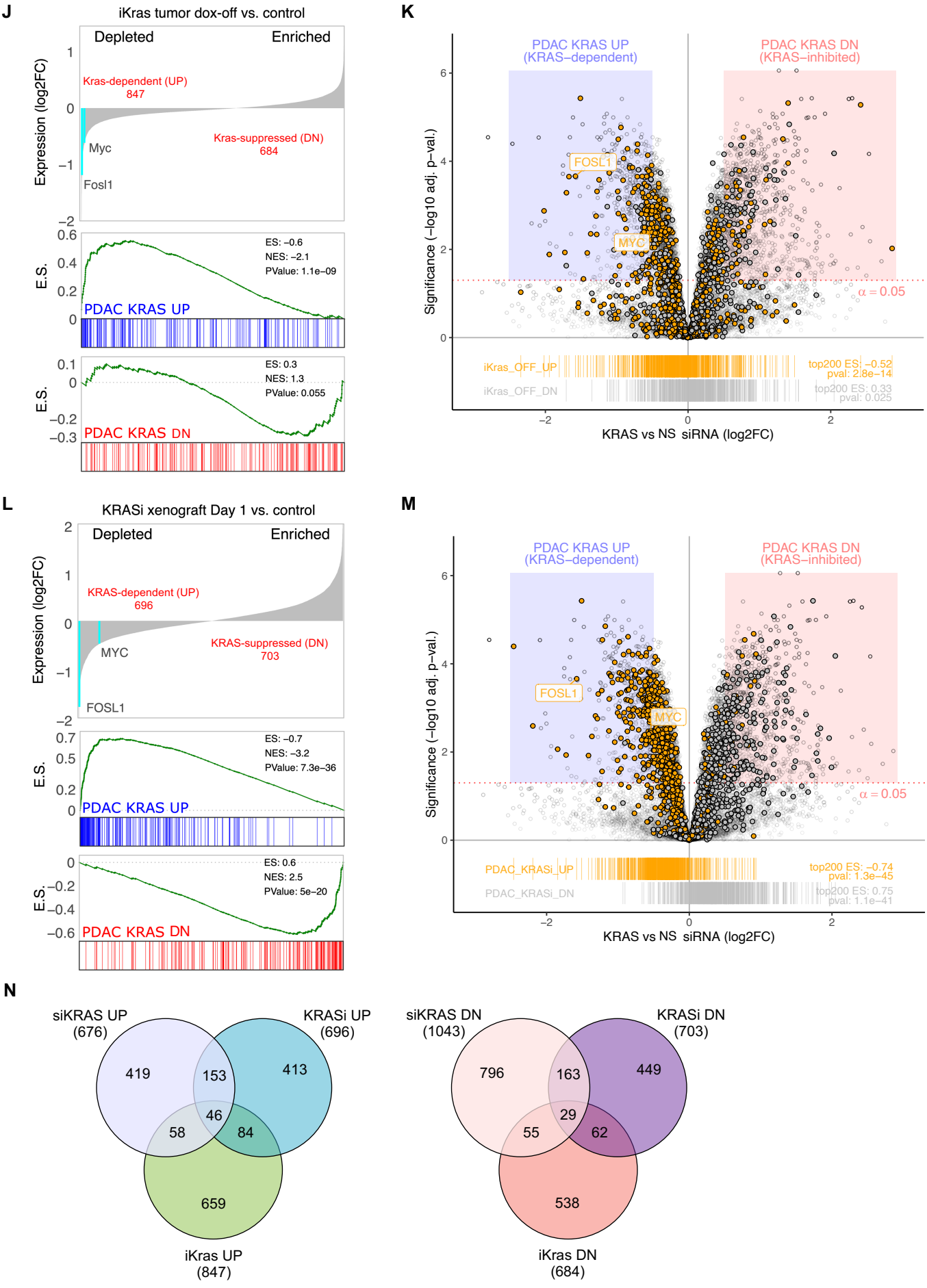
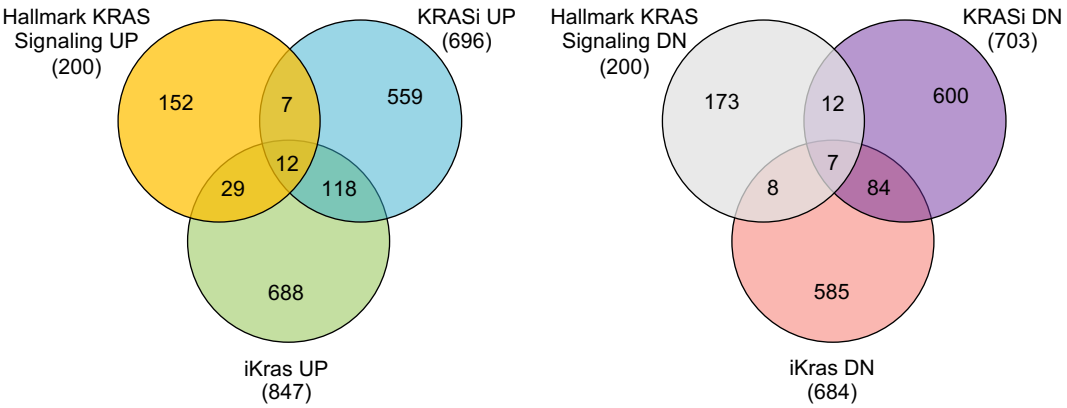
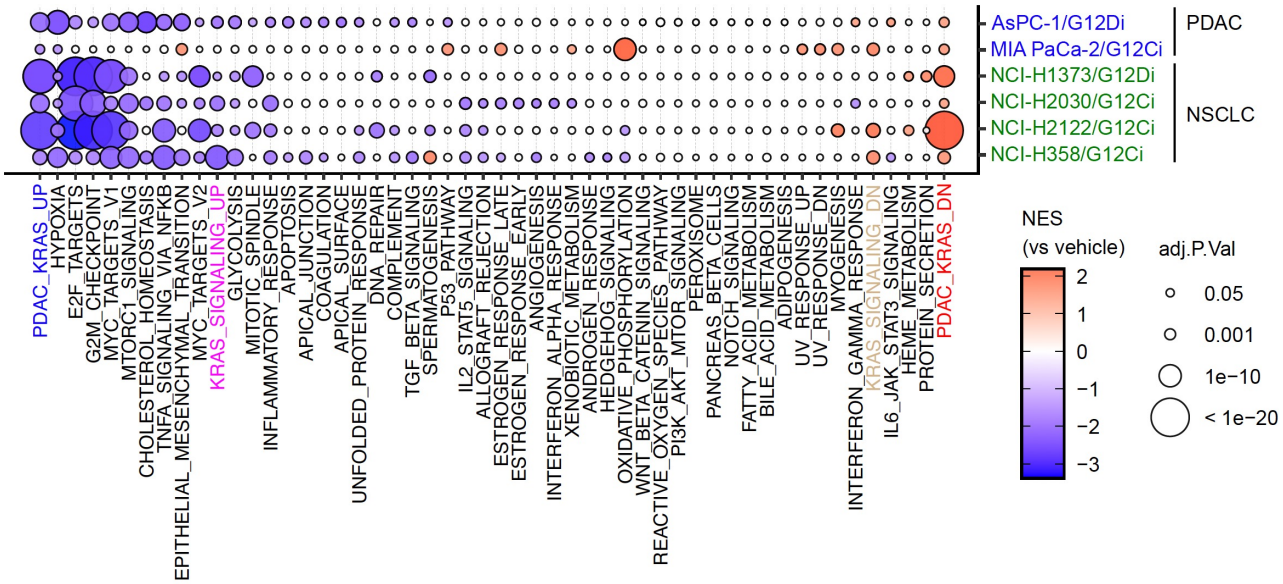

J
iKras tumor dox-off vs. control
Depleted
Enriched
Expression (log2FC)
Kras-dependent (UP)
847
Kras-suppressed (DN)
684
Myc
Fosl1
E.S.
ES: −0.6
NES: −2.1
PValue: 1.1e−09
PDAC KRAS UP
ES: 0.3
NES: 1.3
PValue: 0.055
PDAC KRAS DN
K
PDAC KRAS UP
(KRAS−dependent)
PDAC KRAS DN
(KRAS−inhibited)
FOSL1
MYC
Significance (−log10 adj. p−val.)
α = 0.05
iKras_OFF_UP
top200 ES: −0.52
pval: 2.8e−14
iKras_OFF_DN
top200 ES: 0.33
pval: 0.025
KRAS vs NS siRNA (log2FC)
L
KRASi xenograft Day 1 vs. control
KRAS-dependent (UP)
696
KRAS-suppressed (DN)
703
MYC
FOSL1
ES: −0.7
NES: −3.2
PValue: 7.3e−36
ES: 0.6
NES: 2.5
PValue: 5e−20
M
PDAC_KRASi_UP
top200 ES: −0.74
pval: 1.3e−45
PDAC_KRASi_DN
top200 ES: 0.75
pval: 1.1e−41
N
siKRAS UP
(676)
KRASi UP
(696)
419
153
413
46
58
84
659
iKras UP
(847)
siKRAS DN
(1043)
KRASi DN
(703)
796
163
449
29
55
62
538
iKras DN
(684)

figure S1

O


Hallmark KRAS Signaling UP (200)
KRASi UP (696)
iKras UP (847)
152
7
559
12
29
118
688
Hallmark KRAS Signaling DN (200)
KRASi DN (703)
iKras DN (684)
173
12
600
7
8
84
585

P


AsPC-1/G12Di
MIA PaCa-2/G12Ci
NCI-H1373/G12Di
NCI-H2030/G12Ci
NCI-H2122/G12Ci
NCI-H358/G12Ci
PDAC
NSCLC
PDAC_KRAS_UP
HYPOXIA
E2F_TARGETS
G2M_CHECKPOINT
MYC_TARGETS_V1
MTORC1_SIGNALING
CHOLESTEROL_HOMEOSTASIS
TNFA_SIGNALING_VIA_NFKB
EPITHELIAL_MESENCHYMAL_TRANSITION
MYC_TARGETS_V2
KRAS_SIGNALING_UP
GLYCOLYSIS
MITOTIC_SPINDLE
INFLAMMATORY_RESPONSE
APOPTOSIS
APICAL_JUNCTION
COAGULATION
APICAL_SURFACE
UNFOLDED_PROTEIN_RESPONSE
DNA_REPAIR
COMPLEMENT
TGF_BETA_SIGNALING
SPERMATOGENESIS
P53_PATHWAY
IL2_STAT5_SIGNALING
ALLOGRAFT_REJECTION
ESTROGEN_RESPONSE_LATE
ESTROGEN_RESPONSE_EARLY
ANGIOGENESIS
INTERFERON_ALPHA_RESPONSE
XENOBIOTIC_METABOLISM
ANDROGEN_RESPONSE
HEDGEHOG_SIGNALING
OXIDATIVE_PHOSPHORYLATION
WNT_BETA_CATENIN_SIGNALING
REACTIVE_OXYGEN_SPECIES_PATHWAY
PI3K_AKT_MTOR_SIGNALING
PEROXISOME
PANCREAS_BETA_CELLS
NOTCH_SIGNALING
FATTY_ACID_METABOLISM
BILE_ACID_METABOLISM
ADIPOGENESIS
UV_RESPONSE_UP
UV_RESPONSE_DN
MYOGENESIS
INTERFERON_GAMMA_RESPONSE
KRAS_SIGNALING_DN
IL6_JAK_STAT3_SIGNALING
HEME_METABOLISM
PROTEIN_SECRETION
PDAC_KRAS_DN
NES
(vs vehicle)
2
1
0
−1
−2
−3
adj.P.Val
0.05
0.001
1e−10
< 1e−20