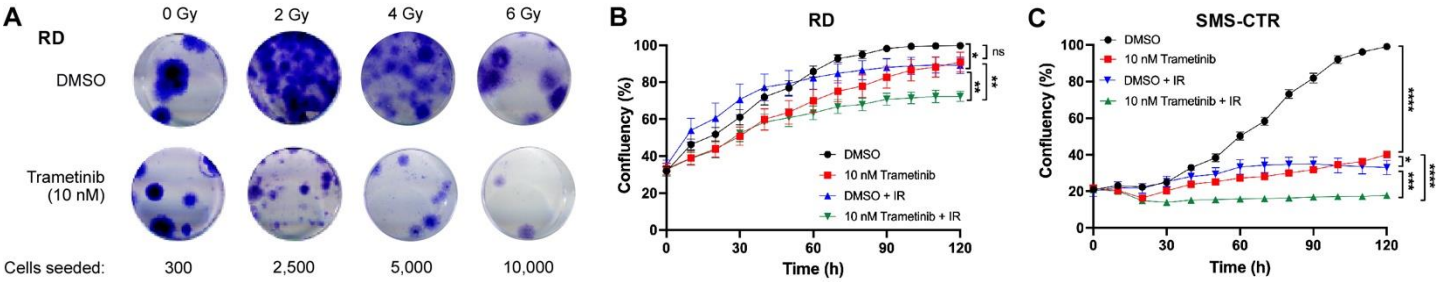
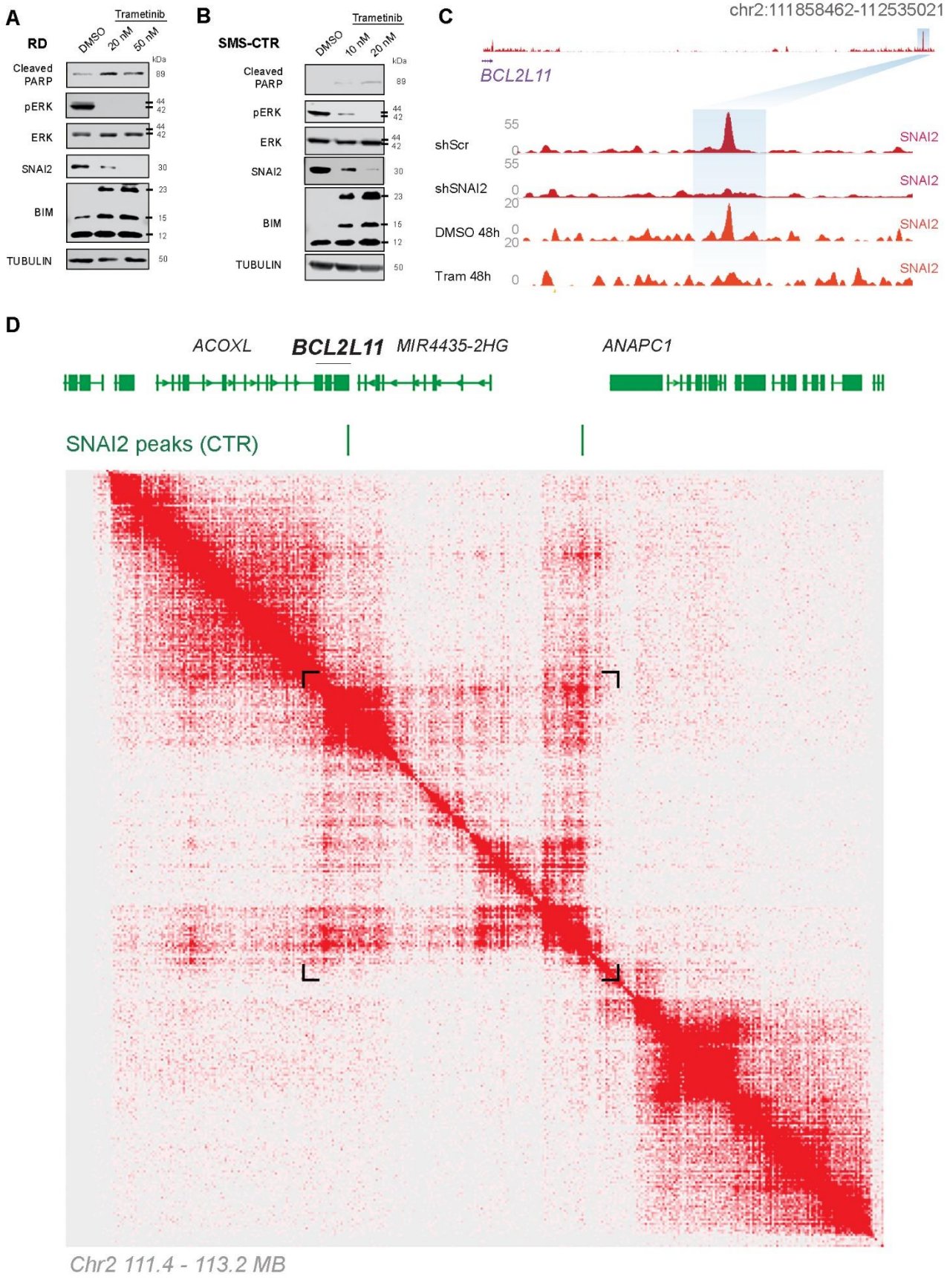


Supplemental Figure 1.

A. Representative colony formation wells from RD cells treated with DMSO or 10 nM trametinib then exposed to the indicated IR dose.

B, C. Average percent confluency of RD (B) and SMS-CTR (C) cells after DMSO and trametinib treatment, with and without IR exposure. Error bars represent ± 1 SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ by one-way ANOVA with Dunnett's multiple comparisons test.

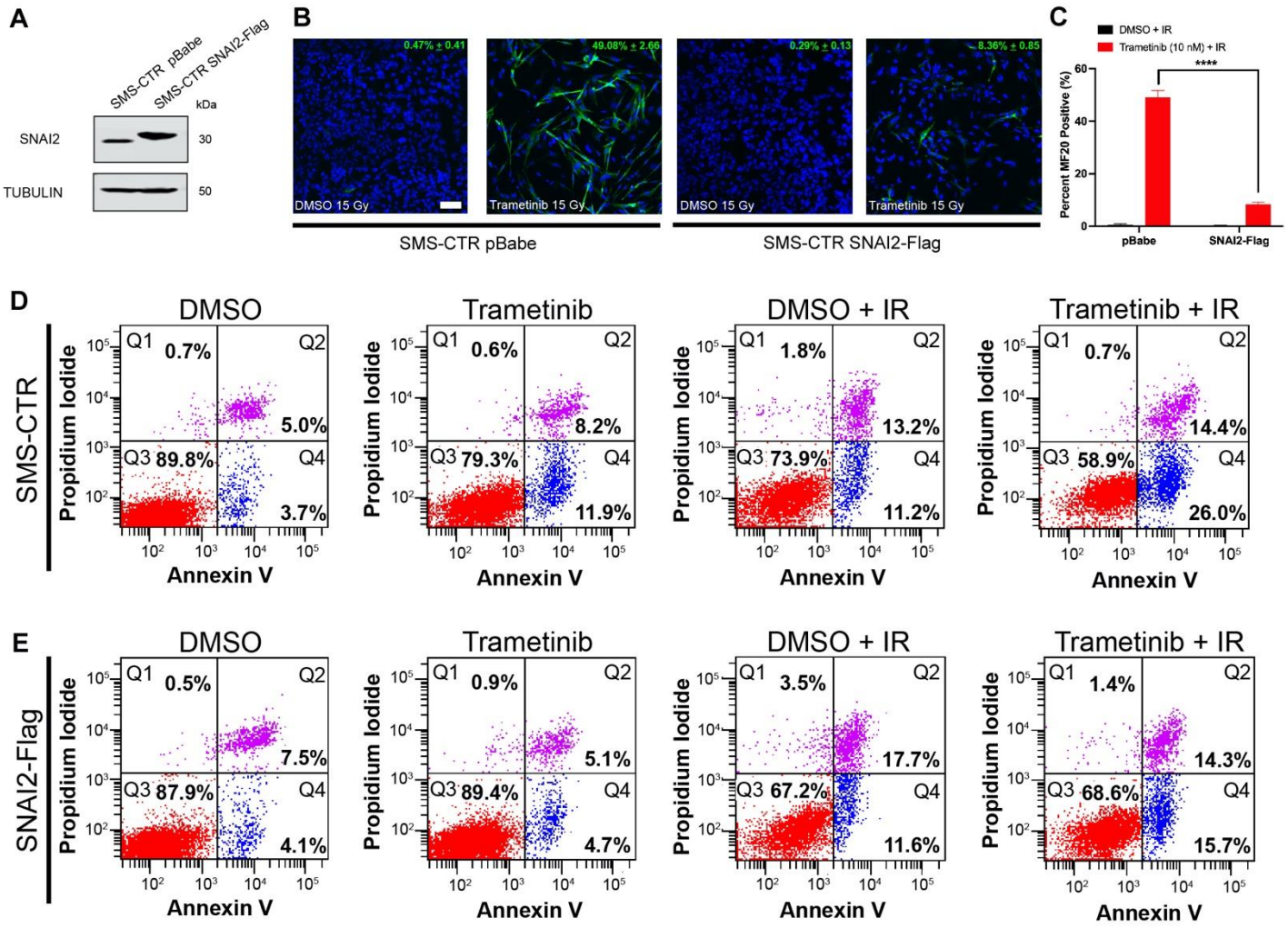

A
RD
Trametinib
DMSO
20 nM
50 nM
kDa
Cleaved PARP
89
pERK
44
42
ERK
44
42
SNAI2
30
BIM
23
15
12
TUBULIN
50
B
SMS-CTR
Trametinib
DMSO
10 nM
20 nM
kDa
Cleaved PARP
89
pERK
44
42
ERK
44
42
SNAI2
30
BIM
23
15
12
TUBULIN
50
C
chr2:111858462-112535021
BCL2L11
shScr
55
0
SNAI2
shSNAI2
55
0
SNAI2
DMSO 48h
20
0
SNAI2
Tram 48h
0
SNAI2
D
ACOXL
BCL2L11
MIR4435-2HG
ANAPC1
SNAI2 peaks (CTR)
Chr2 111.4 - 113.2 MB

Supplemental Figure 2.

A, B. Cleaved PARP, pERK, total ERK, SNAI2, and BIM protein expression immunoblots in DMSO- and trametinib-treated RD (A) and SMS-CTR (B) cells treated with DMSO and increasing doses of trametinib for 24h.

C. ChIP-seq SNAI2 binding peaks of the downstream enhancer region of *BCL2L11* in SMS-CTR cells treated with DMSO or trametinib (10 nM) for 48h, compared to SNAI2 binding peaks seen in RD shScr or shSNAI2 cells.

D. Topologically associated domain (TAD) for *BCL2L11/BIM* in IMR90 cells. Annotated in the image are the two SNAI2 bound distil *BCL2L11* enhancers.



Supplemental Figure 3.

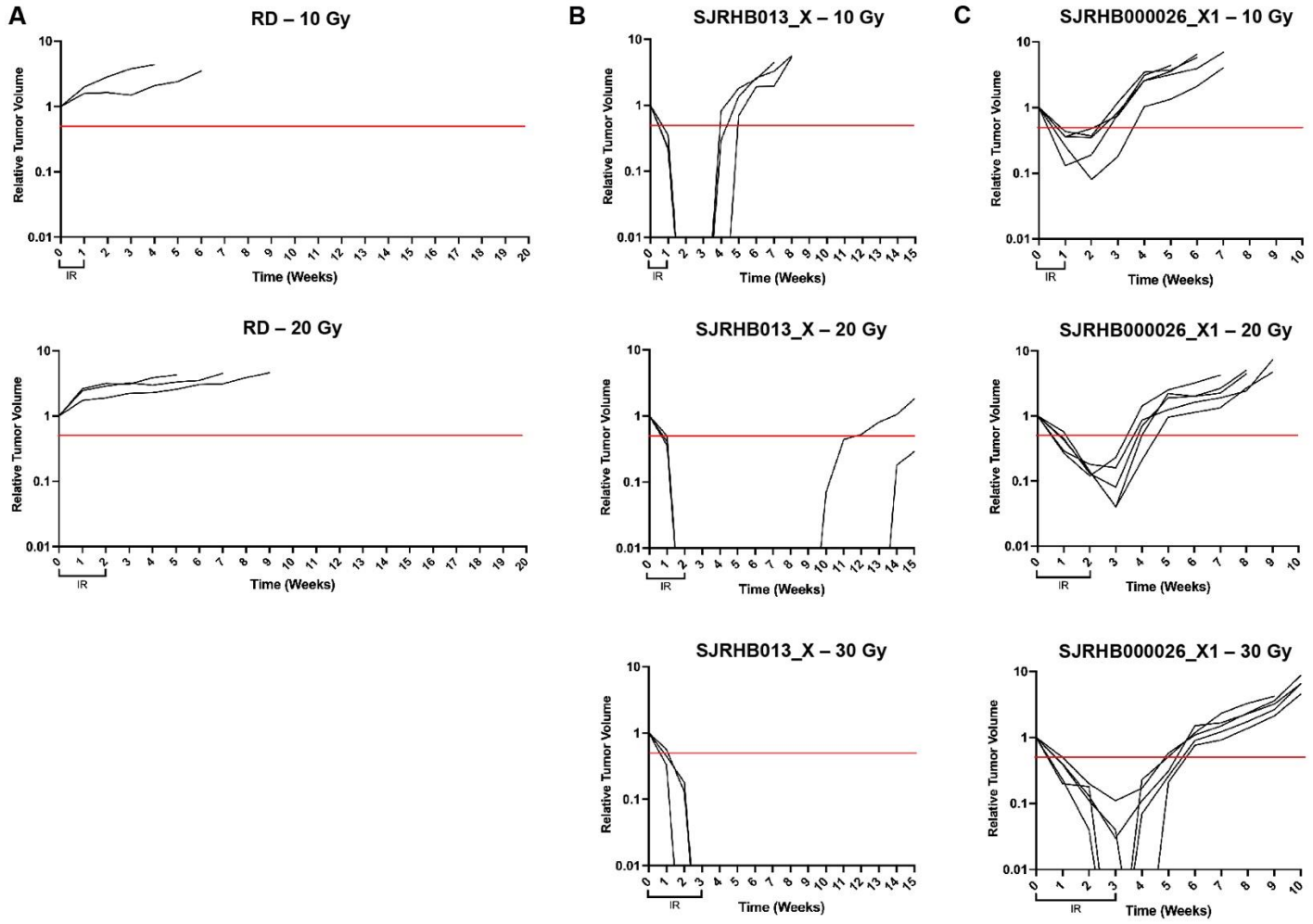
A. SNAI2 protein expression in SMS-CTR pBabe and SNAI2-Flag overexpressing cell lines.

B. Representative confocal microscopy images of SMS-CTR pBabe and SNAI2-Flag cells treated with DMSO or trametinib after IR exposure, immunostained with myogenic differentiated myosin MF20 antibody. Scale bar = 100 μm.

C. Quantification of average MF20-positive cells in DMSO- and trametinib-treated SMS-CTR pBabe and SNAI2-Flag cells after IR exposure. Error bars represent ±1 SD. ns = not significant, ****$p<0.0001$ by a two-way ANOVA with a posthoc Sidak's multiple comparison test.

D. Flow cytometry plots showing propidium iodide vs. annexin V staining of DMSO- and trametinib-treated SMS-CTR pBabe cells after non-IR and IR conditions.

E. Flow cytometry plots showing propidium iodide vs. annexin V staining of DMSO- and trametinib-treated SMS-CTR SNAI2-Flag cells after non-IR and IR conditions.



Supplemental Figure 4.

A–C. Relative tumor volume (RTV) of RD (A), SJRHB013_X (B), and SJRHB000026_X1 (C) tumors receiving increasing doses of IR (10–30 Gy) for IR sensitivity studies. Red line depicts 0.5 RTV.

Supplemental Table 1.

FN-RMS Cell Line	**RAS Mutation**	**p53 Status**
RD	$NRAS^{Q61H}$ (43)	R248W mutation (42)
SMS-CTR	$HRAS^{Q61K}$ (43)	△Nt 1236-1239 (42, 43)
Rh36	$HRAS^{Q61K}$ (43)	Wild type (43)
FN-RMS PDX		
SJRHB013_X	$NRAS^{Q61K}$ (40)	Wild type (40)
SJRHB000026_X1	$HRAS^{G13R}$ (40)	Wild type (40)

Supplemental Table 2.

REAGENT/RESOURCE	SOURCE	IDENTIFIER
phospho ERK	CST	Cat# 4370, RRID:AB_2315112
ERK	CST	Cat# 9102, RRID:AB_330744
Slug (C19G7)	CST	Cat# 9585, RRID:AB_2239535
SNAI1 (L70G2)	CST	Cat# 3895s, RRID:AB_2191759
BIM (C34C5)	CST	Cat# 2933, RRID:AB_1030947
Cleaved PARP (Asp214) (D64E10)	CST	Cat# 5625, RRID:AB_10699459
GAPDH	CST	Cat# 2118, RRID:AB_561053
β-Tubulin	Abcam	Cat# Ab6046, RRID:AB_2210370
HRP anti-rabbit	CST	Cat# 7074, RRID:AB_2099233
HRP anti-mouse	GE Healthcare	Cat# NA93IV, RRID:AB_772210
Myosin Heavy Chain (MF20)	DSHB	Cat# MF 20, RRID:AB_2147781
Alexa Flour 488 goat anti-mouse	Invitrogen	Cat# A11029, RRID:AB_138404