
Supplementary information

RBFox2 modulates a metastatic signature of alternative splicing in pancreatic cancer

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RBFOX2 modulates a metastatic signature of alternative splicing in pancreatic cancer

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Supplementary figure 1. Raw data of immunoblot scans from Figure.1

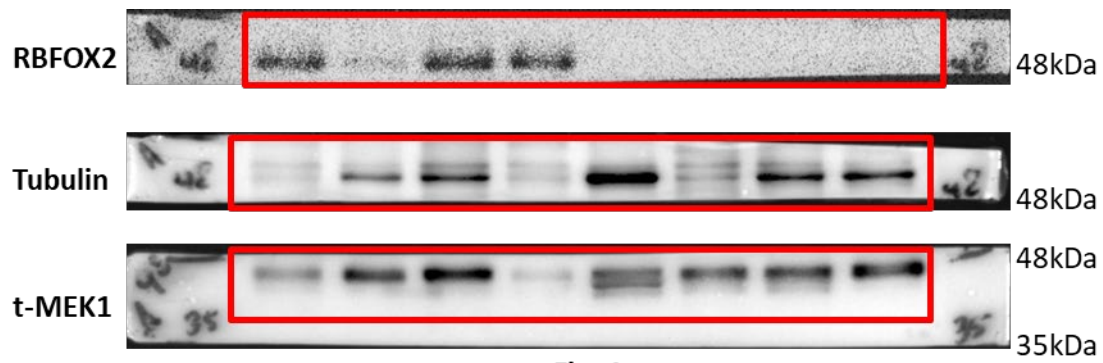
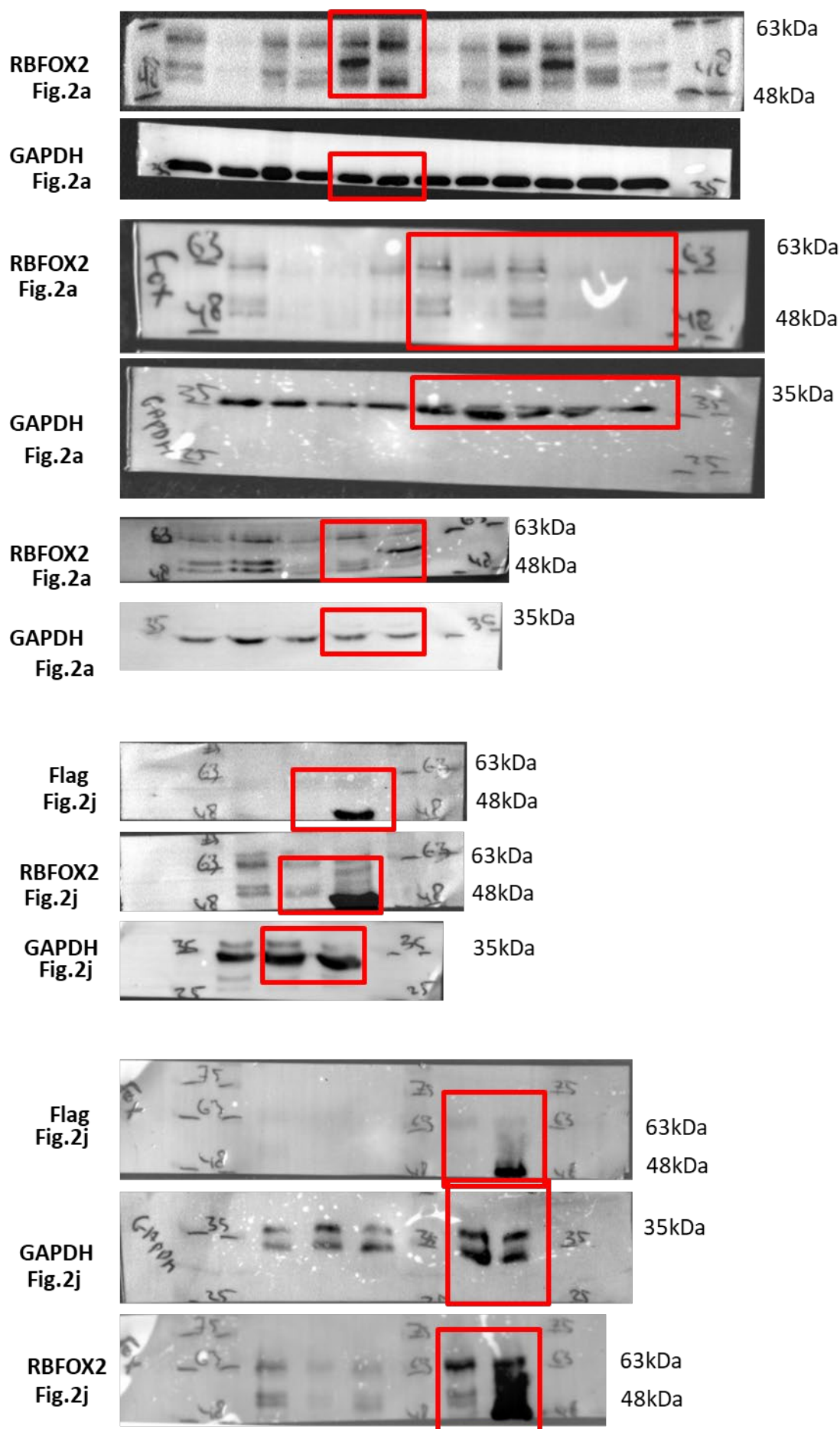


Fig. 1e

Supplementary figure 2. Raw data of immunoblot scans from Figure.2



Supplementary figure 3. Raw data of PCR gels scans from Figure.4

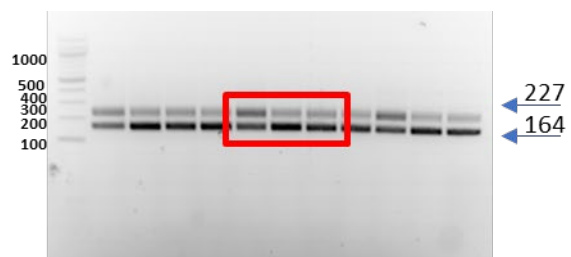


Fig. 4b

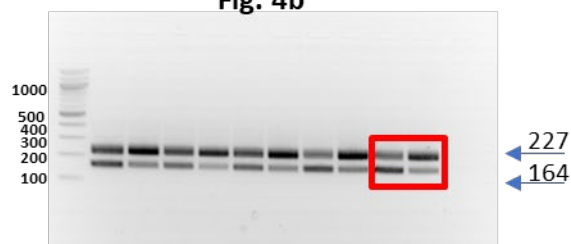


Fig. 4b



Fig. 4i

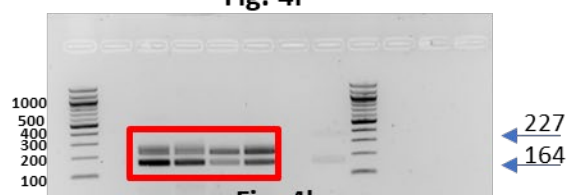


Fig. 4l



Fig. 4e

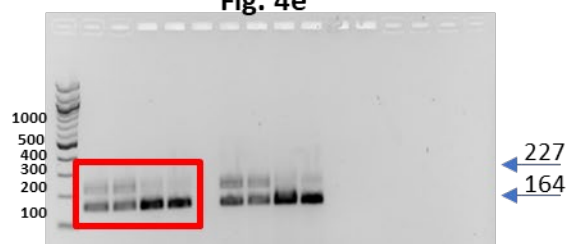
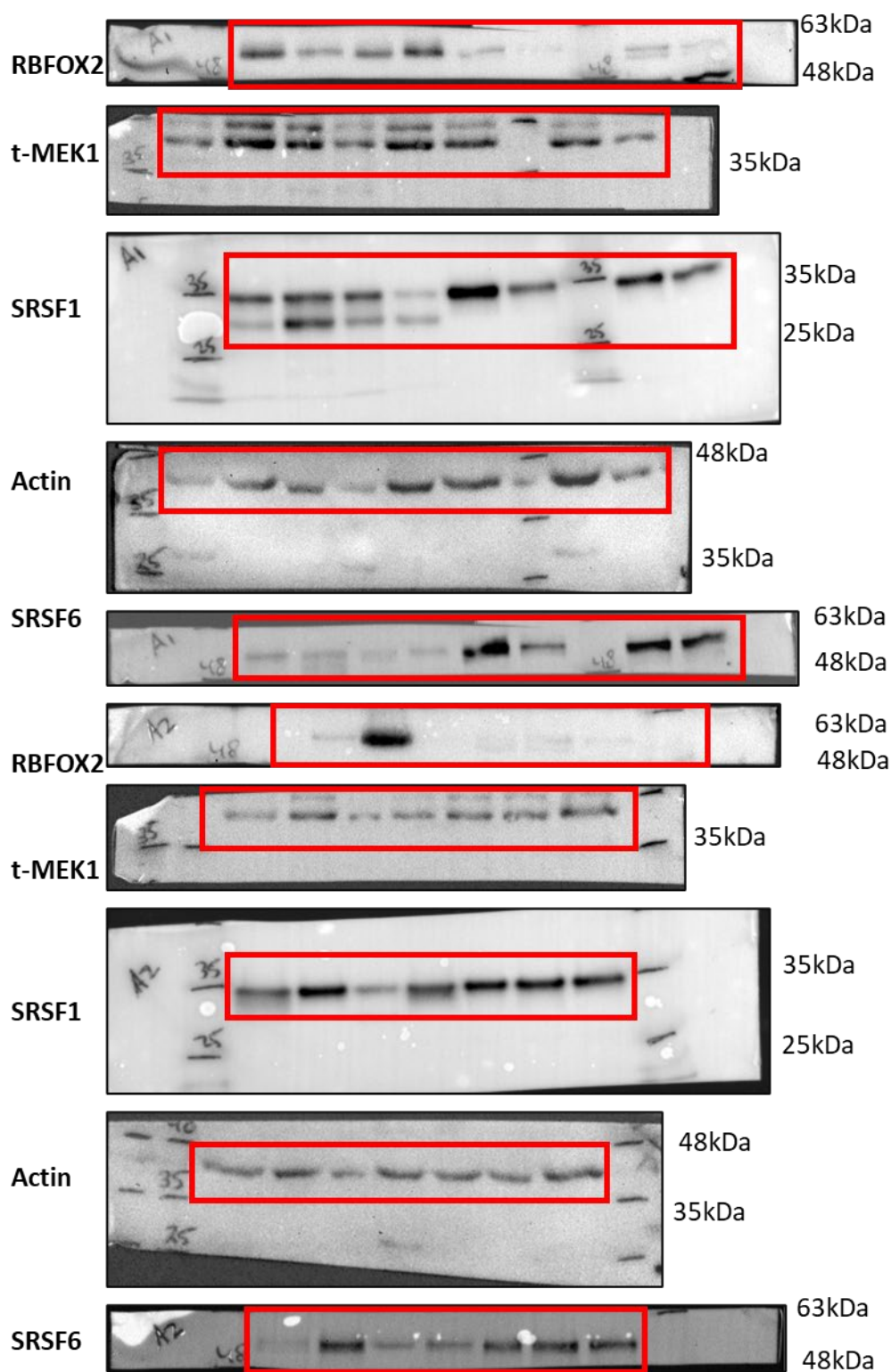


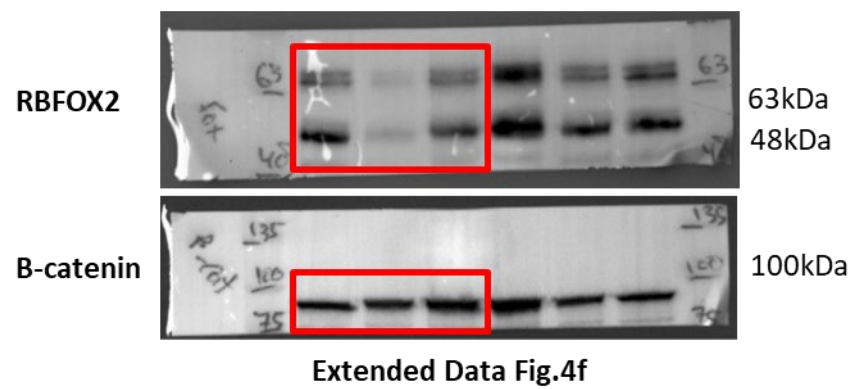
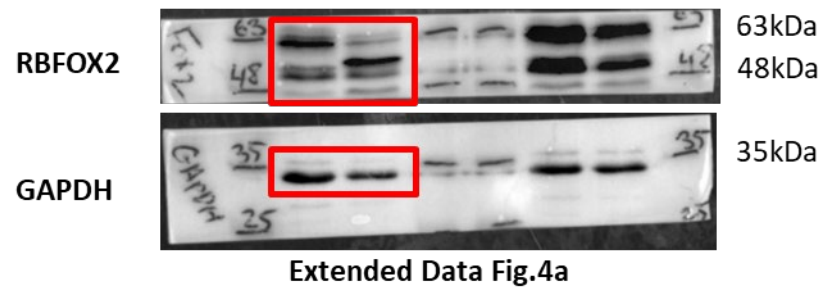
Fig. 4h

Supplementary figure 4. Raw data of immunoblot scans from extended data figure.3

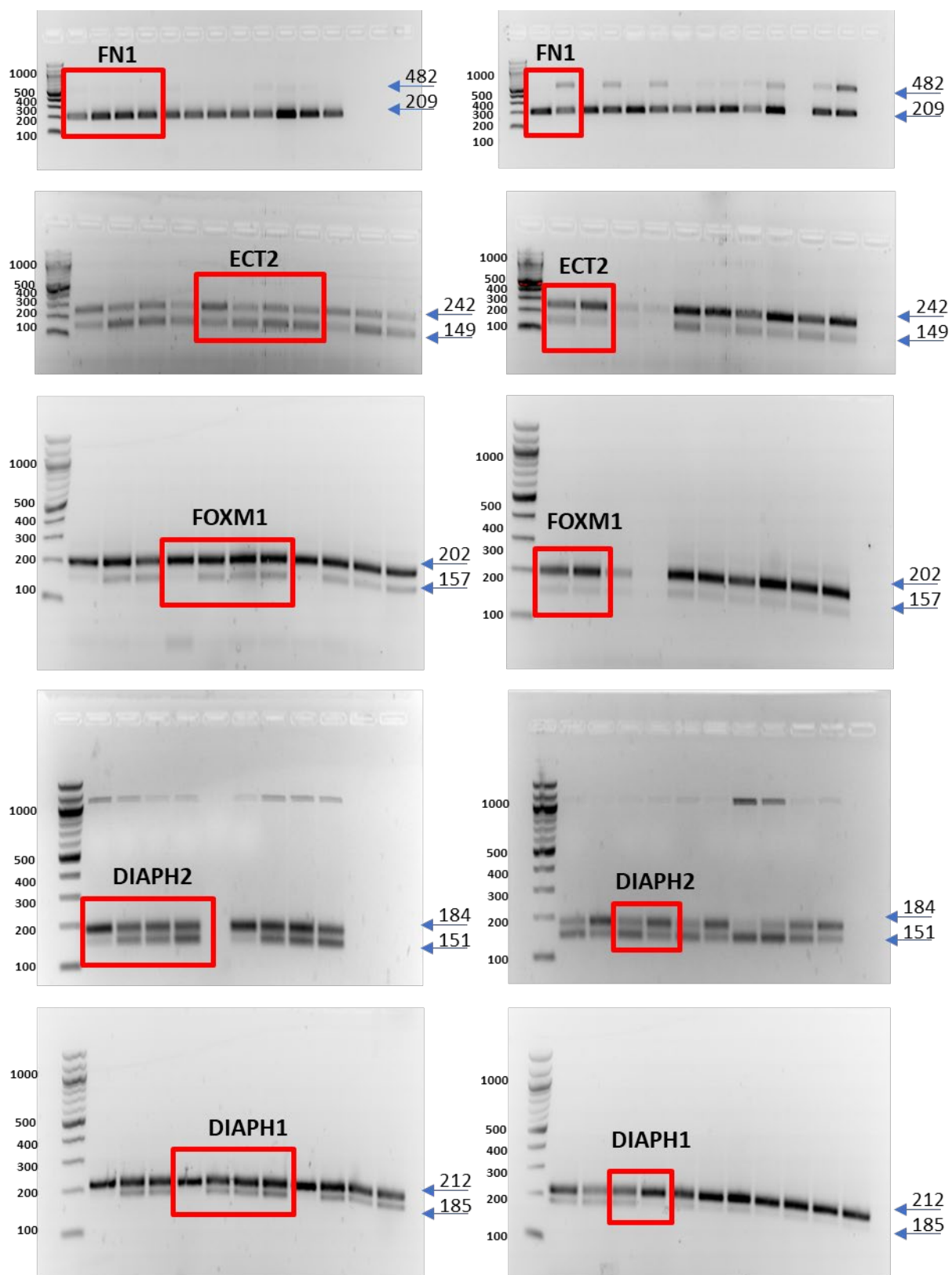


Extended Data Figure.3a

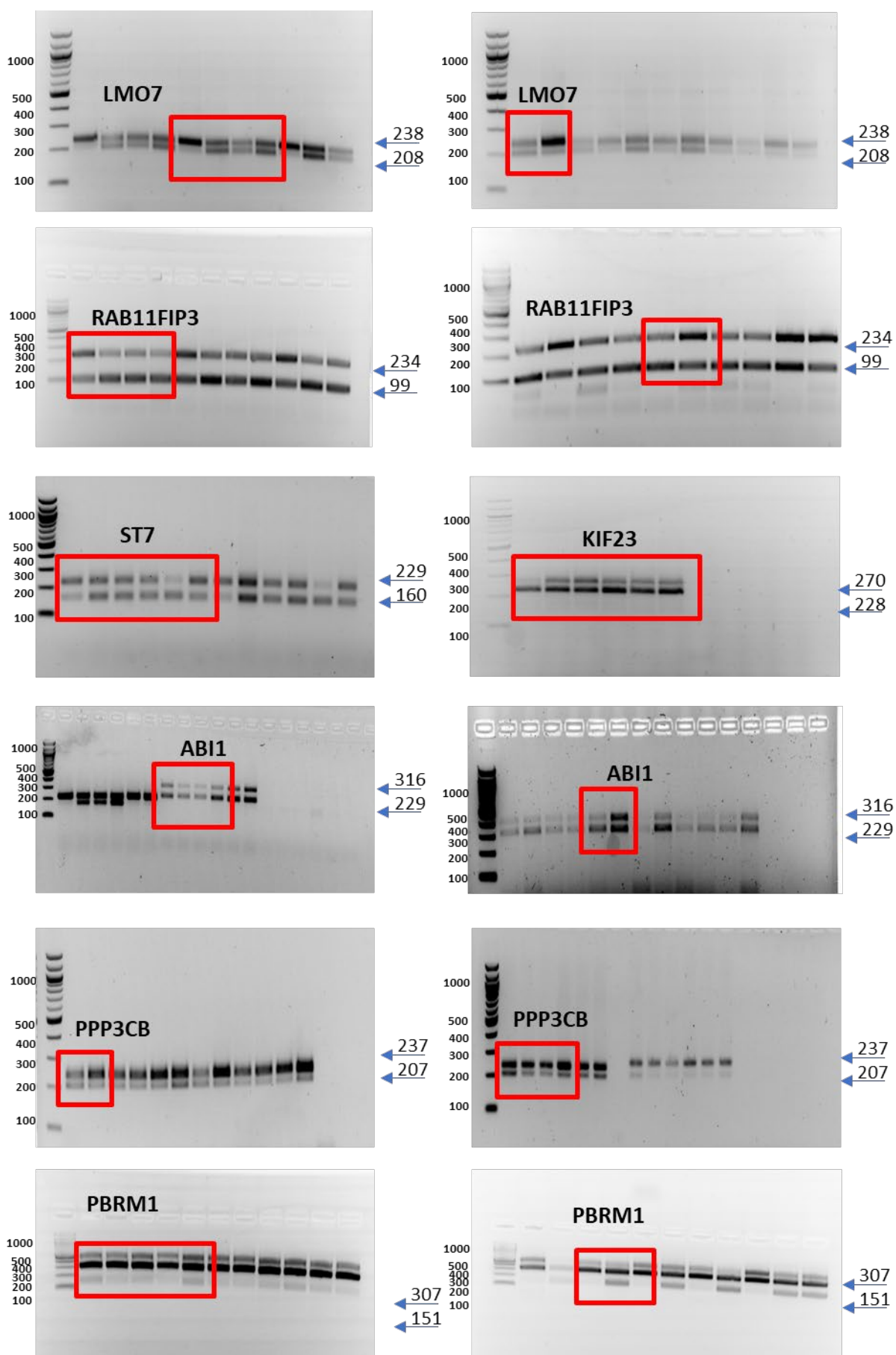
Supplementary figure 5. Raw data of immunoblot scans from extended data fig.4



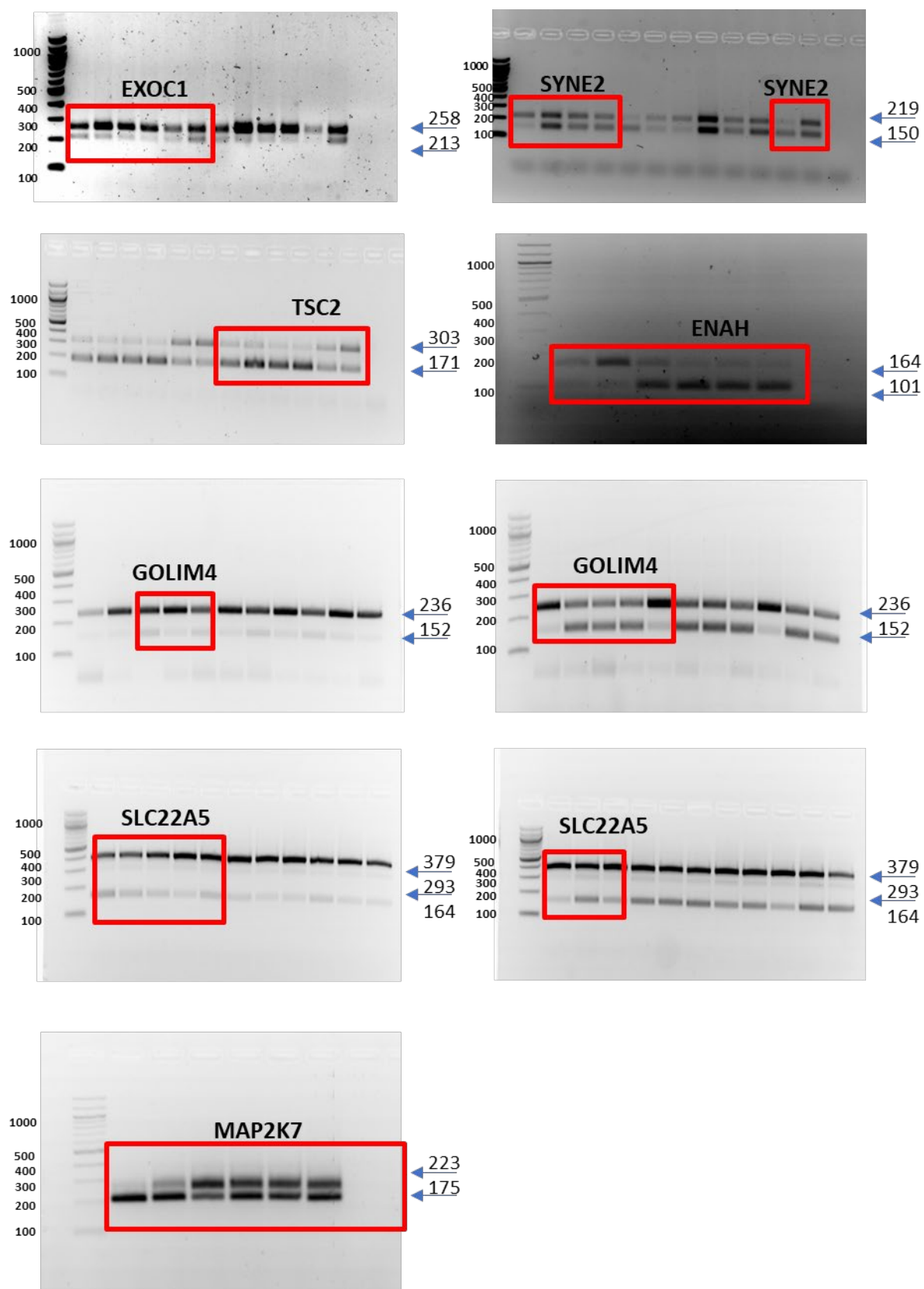
Supplementary figure 6. Raw data of PCR gels scans from extended data figure.6



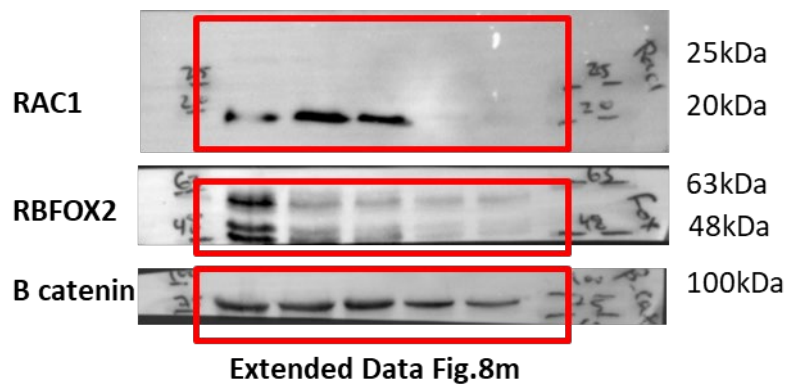
Supplementary figure 6. Raw data of PCR gels scans from extended data figure.6



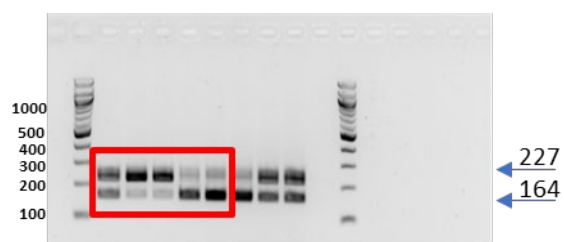
Supplementary figure 6. Raw data of PCR gels scans from extended data figure.6



Supplementary figure 7. Raw data of immunoblot scans from extended data figure.8

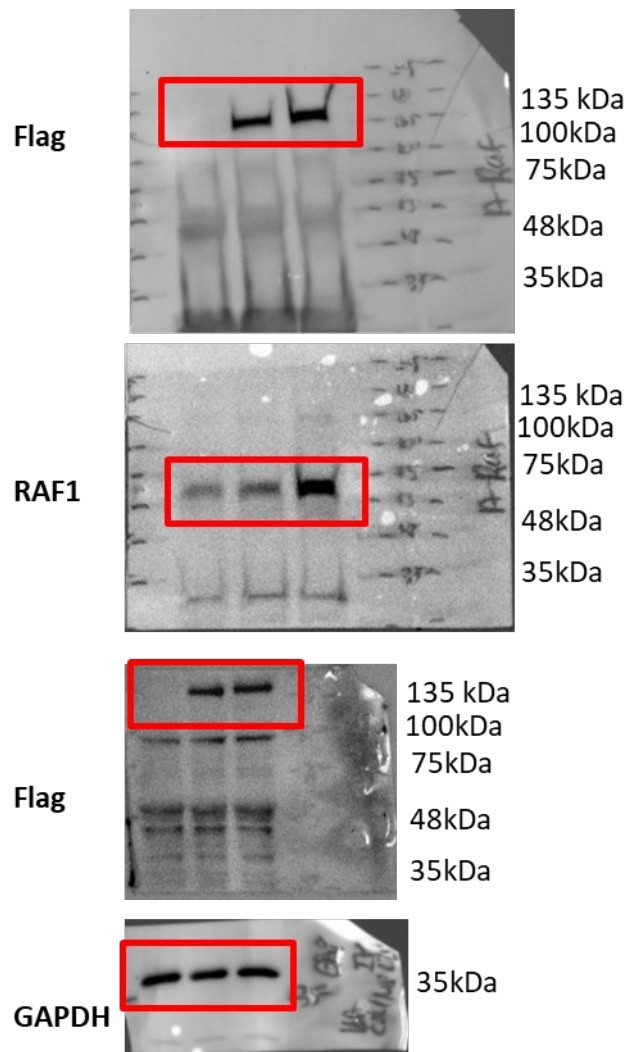


Supplementary figure 8. Raw data of PCR gels scans from extended data figure.9



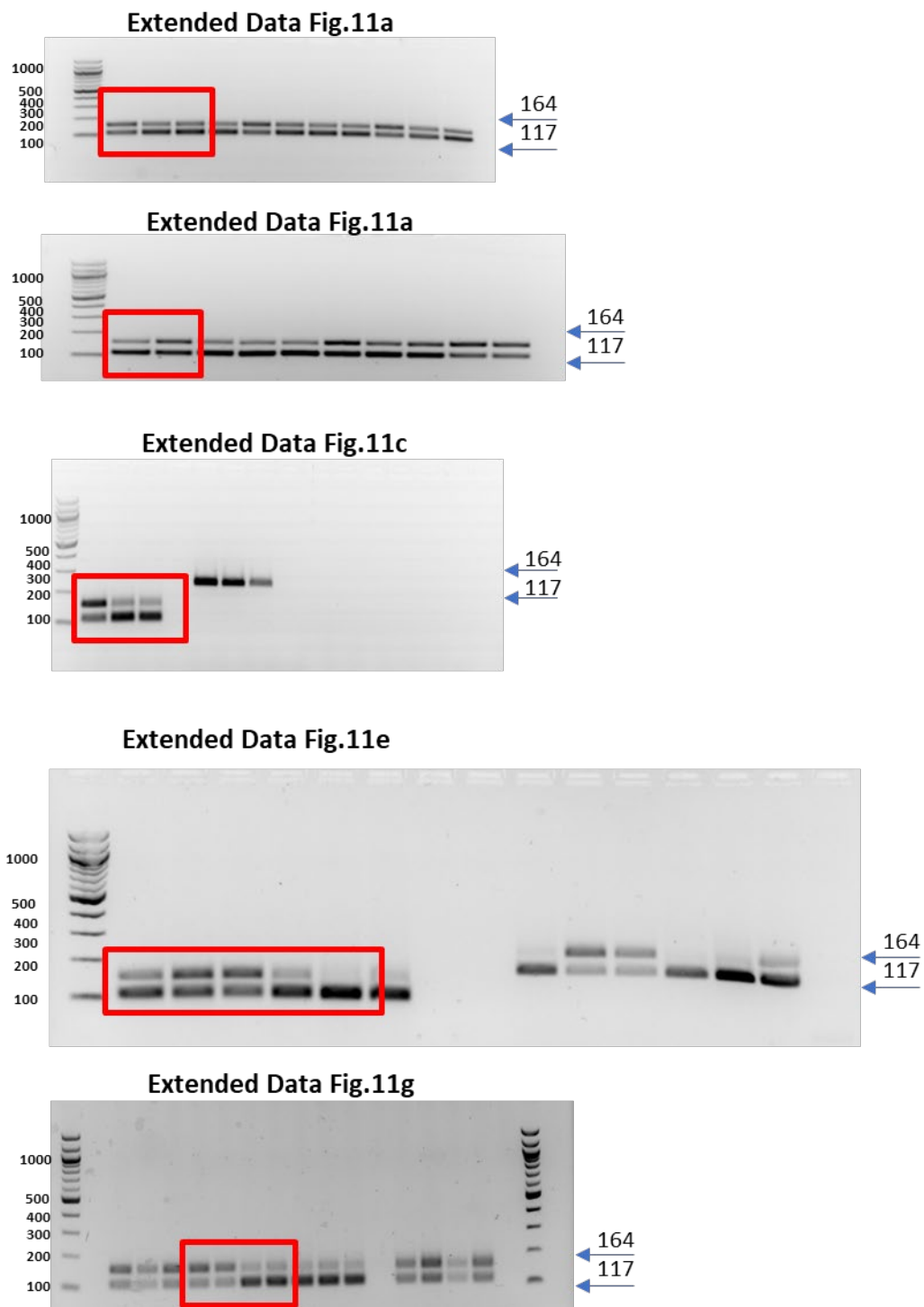
Extended Data Fig.9e

Supplementary figure 9. Raw data of immunoblot scans from extended data figure.10

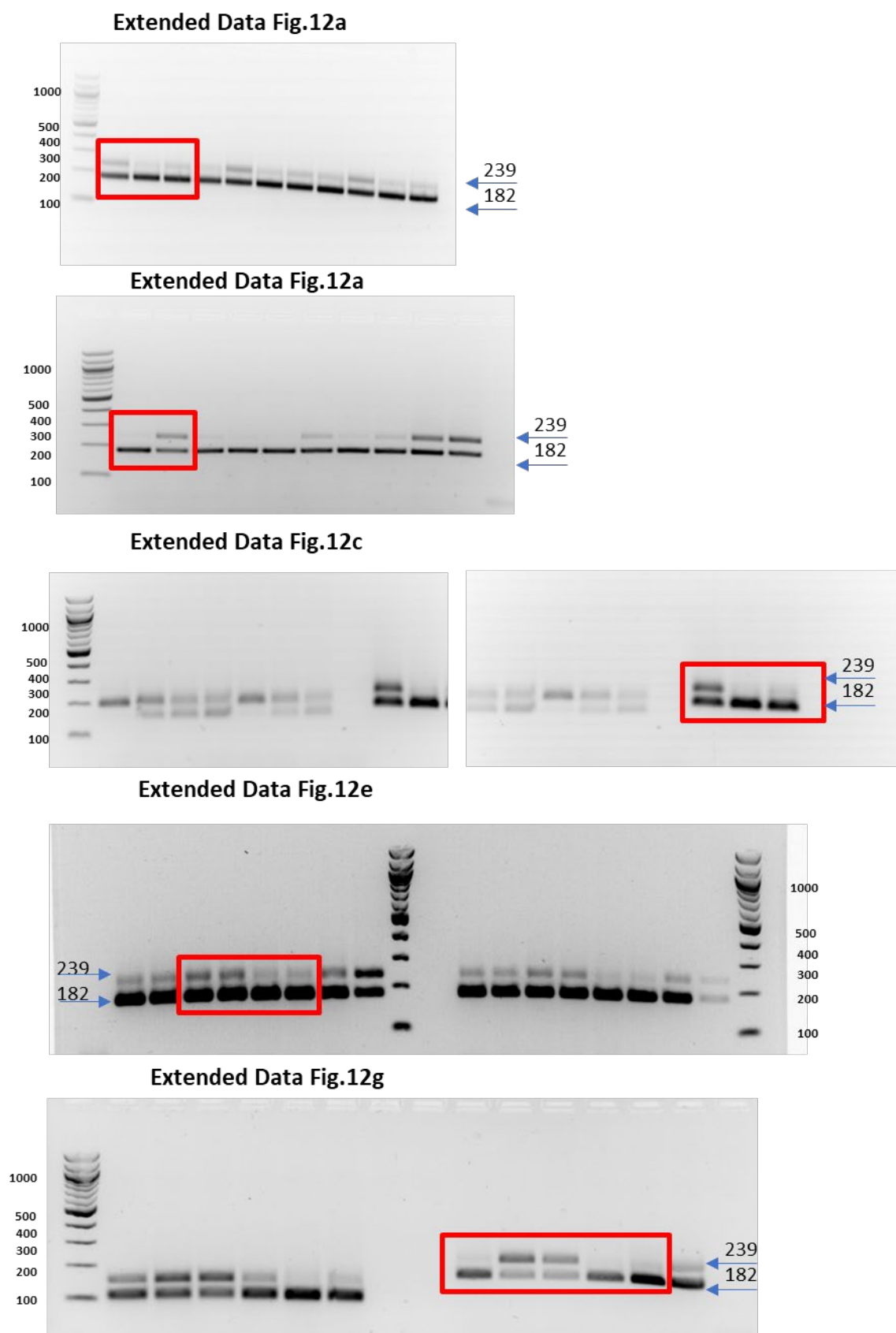


Extended Data Fig.10h

Supplementary figure 10. Raw data of PCR gels scans from extended data figure.11

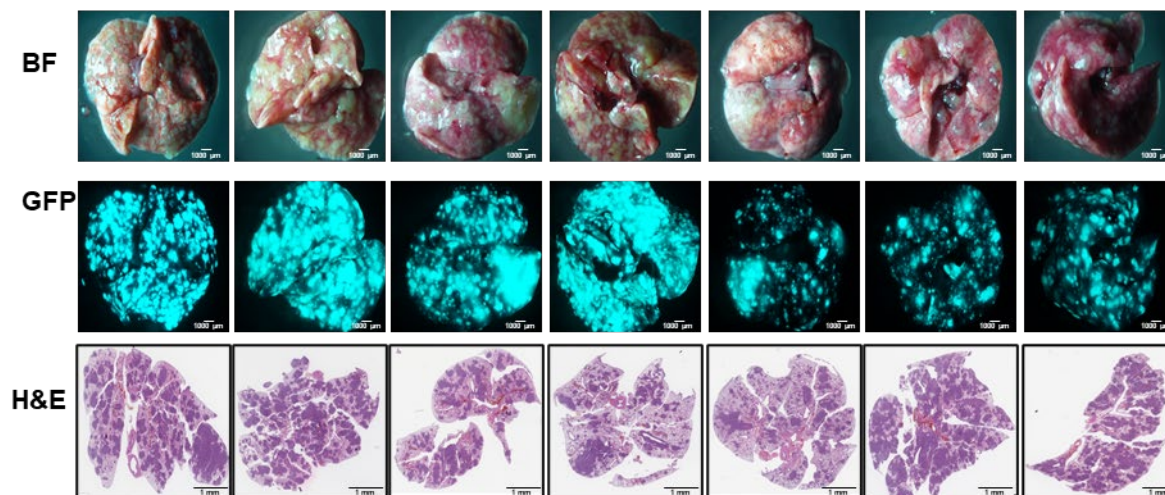


Supplementary figure 11. Raw data of PCR gels scans from extended data figure.12

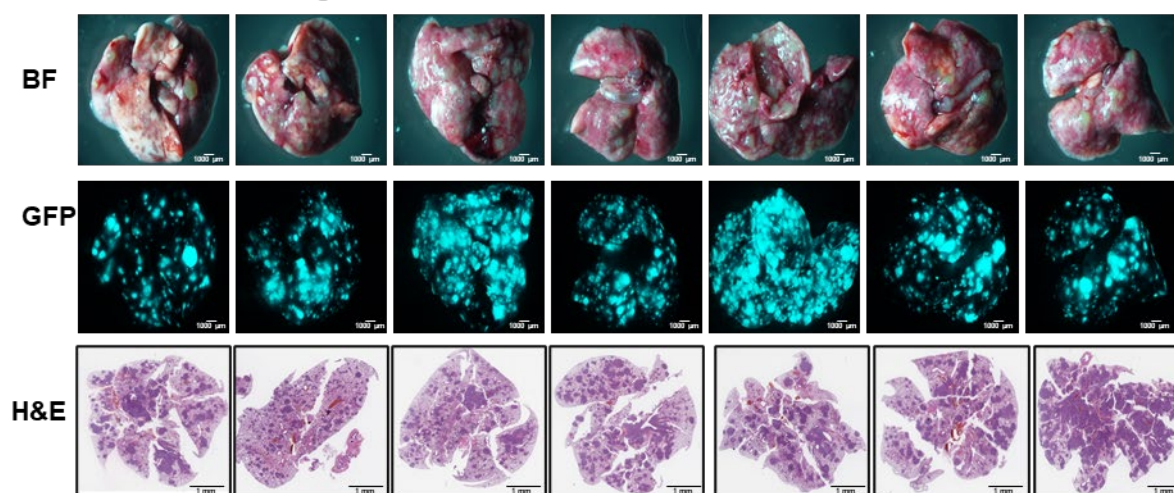


Supplementary figure 12. Lung histology samples related to figure 3g.
(n=7 mice/group)

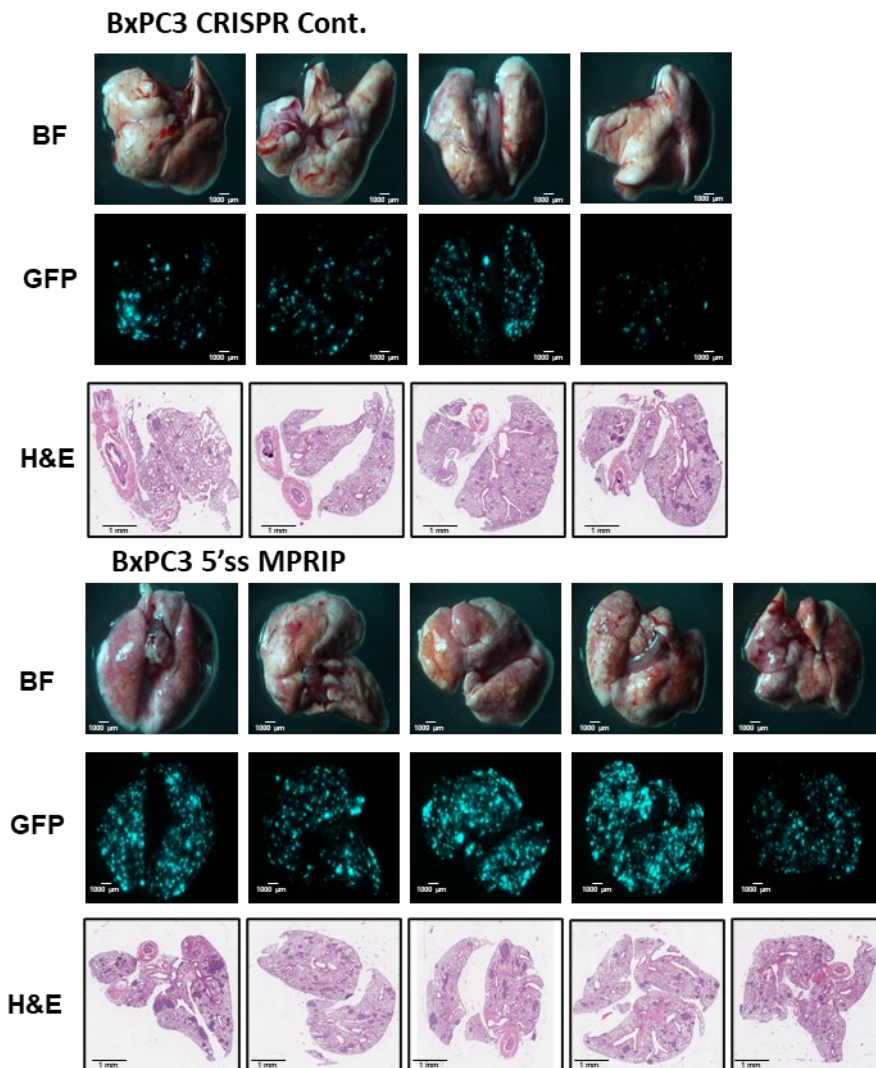
BxPC3 RBFOX2 sgRNA-1 Vehicle



BxPC3 RBFOX2 sgRNA-1 MBQ-167

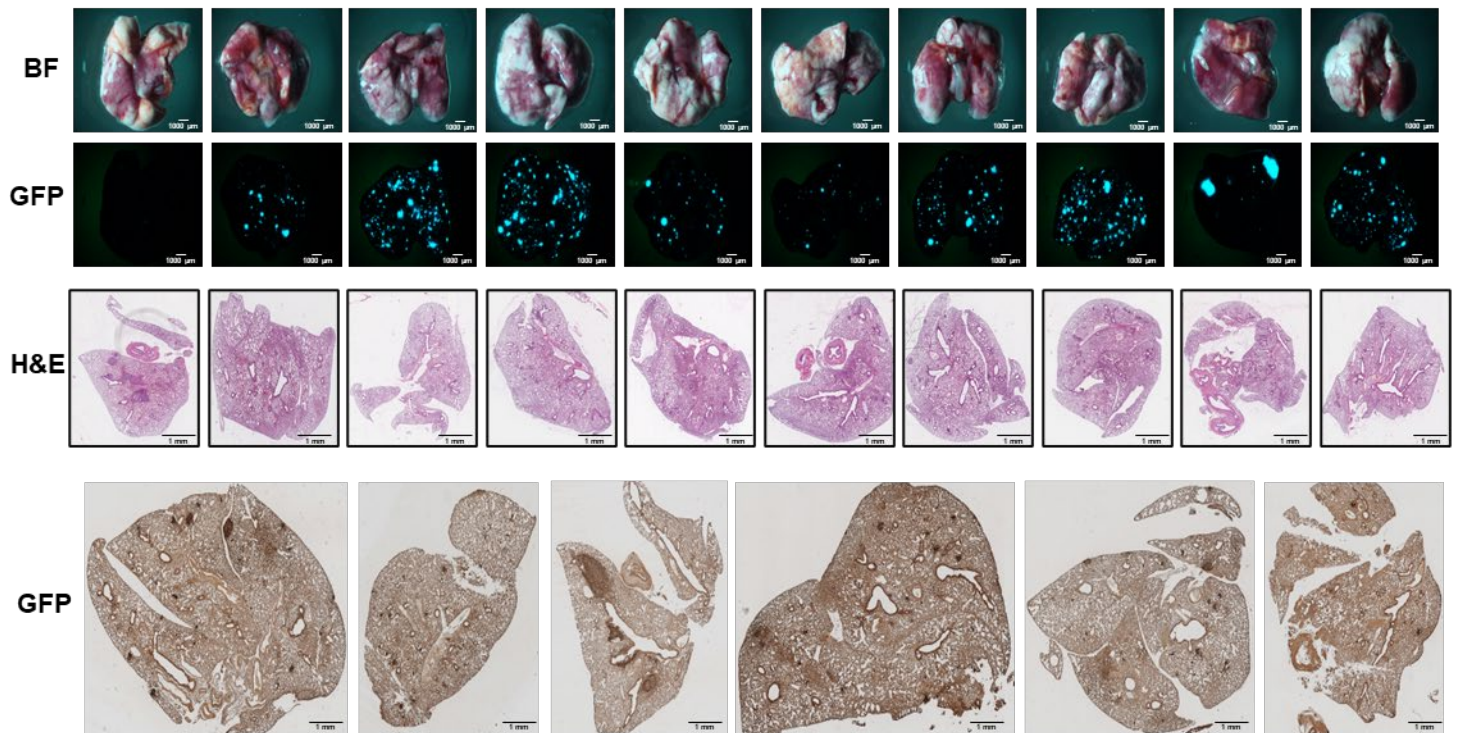


Supplementary figure 13. Lung histology samples related to figure 4h.
(n=4 mice for CRISPR Cont. and, n=5 mice for 5'ss MPRIP sgRNA).

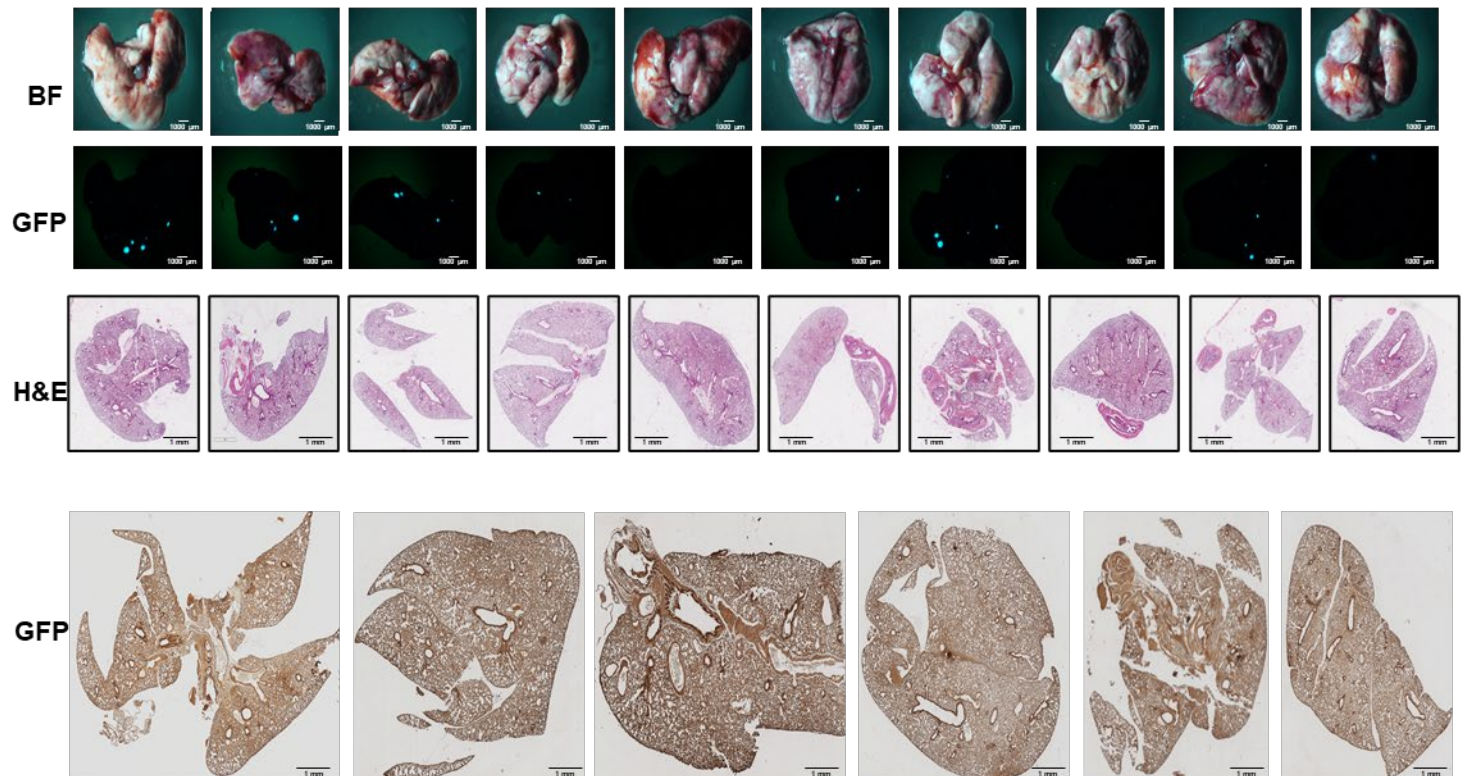


Supplementary figure 14. Lung histology samples related to figure 41
(n=10 mice/ group)

X50 CRISPR Cont.

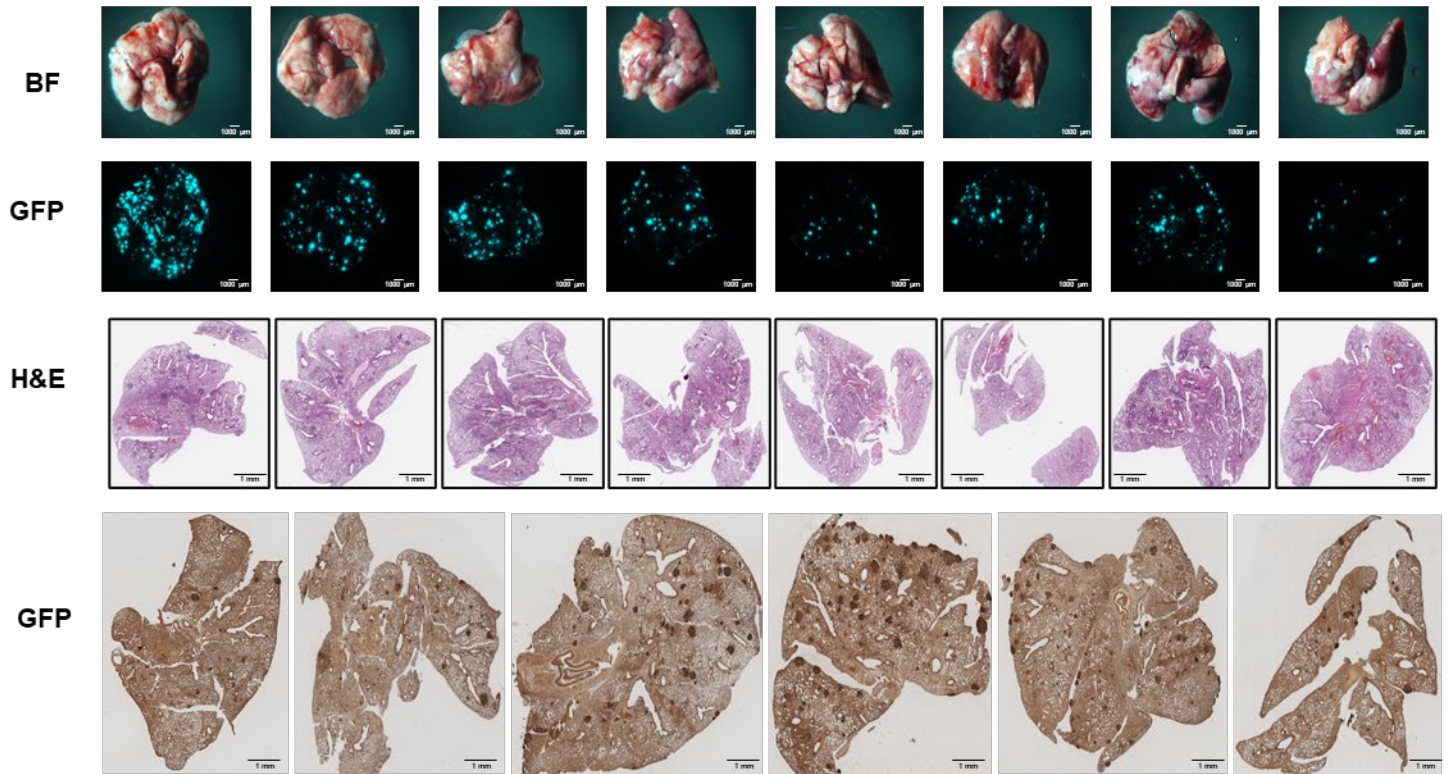


X50 DS-24 MPRIP

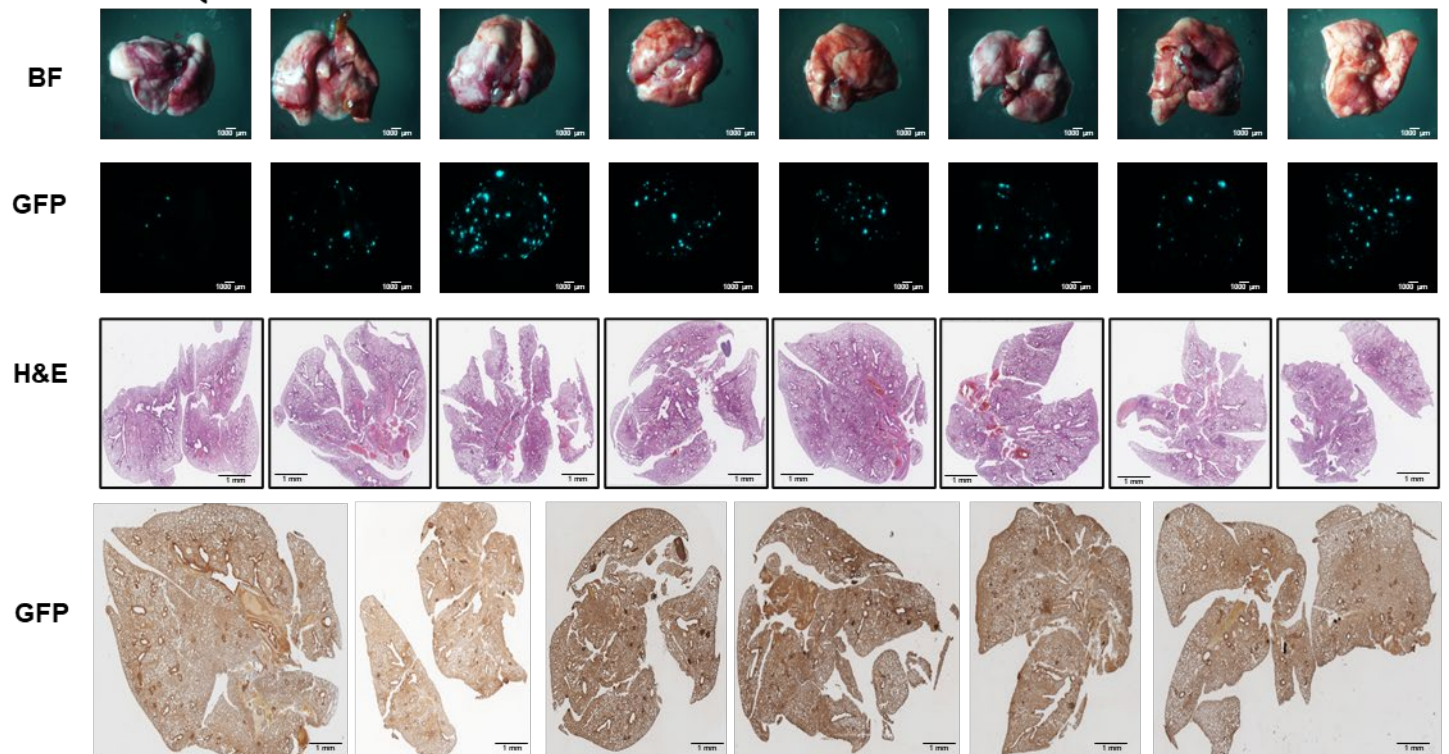


Supplementary figure 15. Lung histology samples related to extended data figure 8a.
(n=8 mice/group)

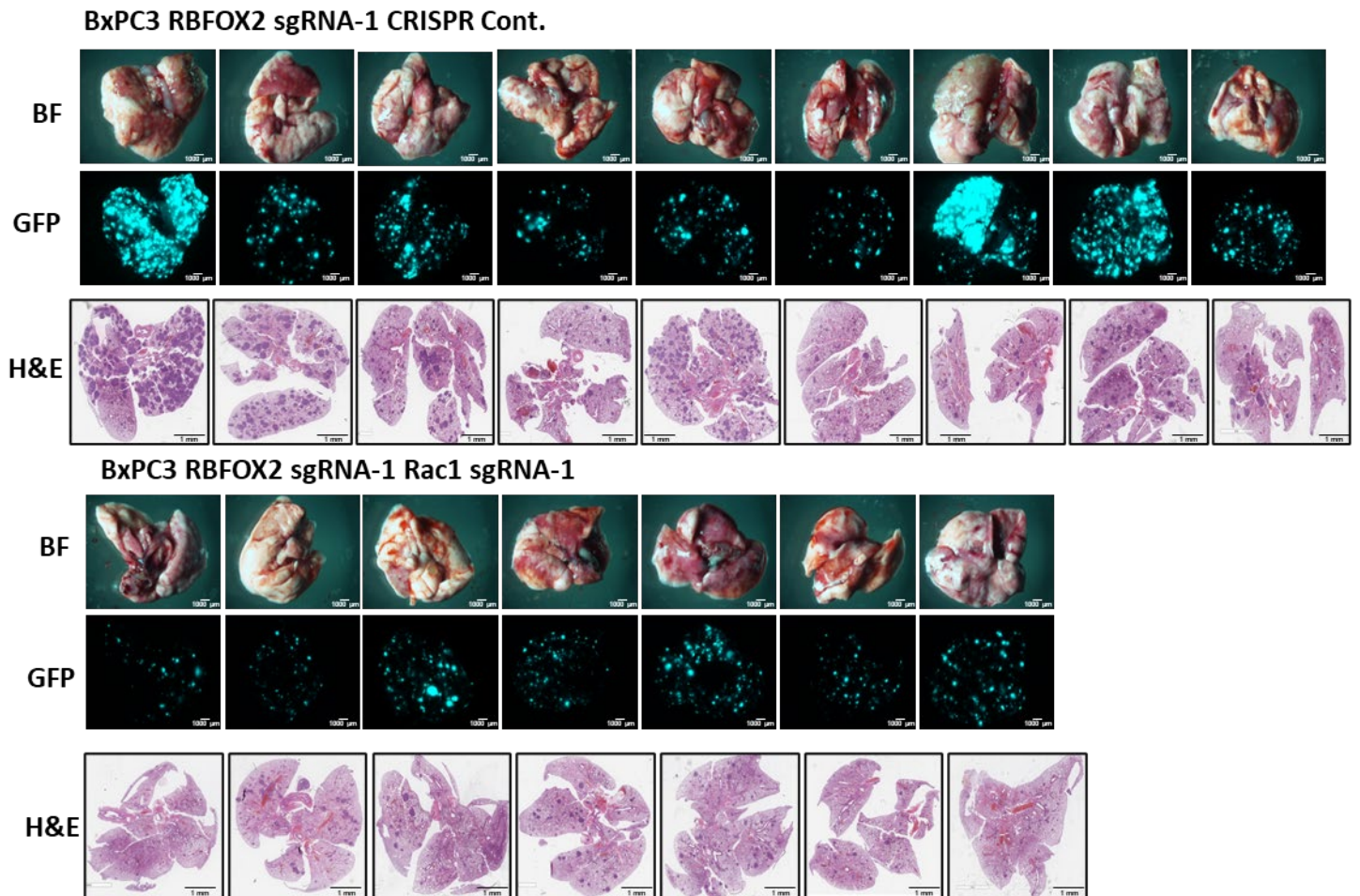
X50 Vehicle



X50 MBQ-167

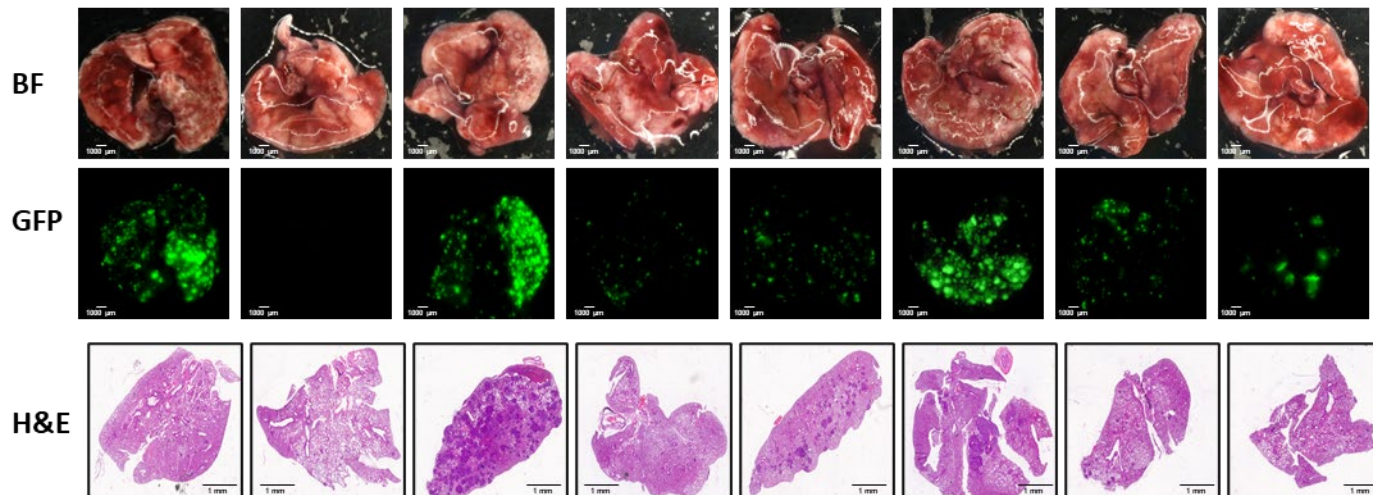


Supplementary figure 16. Lung histology samples related to extended data figure 8p
(n=9 for CRISPR Cont, n=7 sgRNA-1 Rac1)

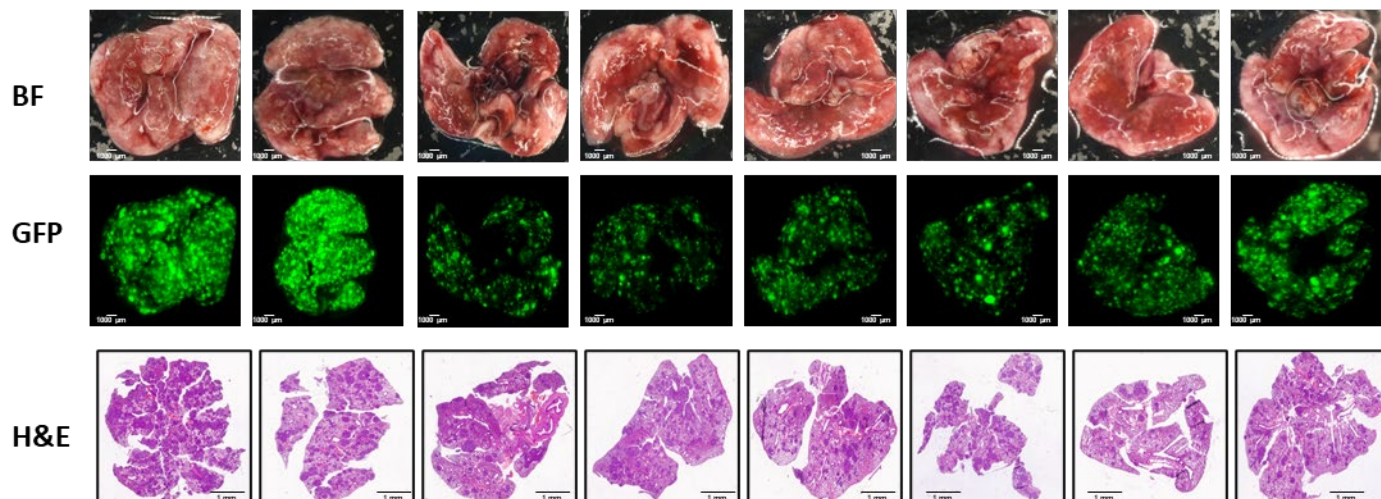


Supplementary figure 17. Lung histology samples related to extended data figure 11g.
(n=8 mice/group for CRISPR Cont. and 3' ss MYL6 sgRNAs)

BxPC3 CRISPR Cont.

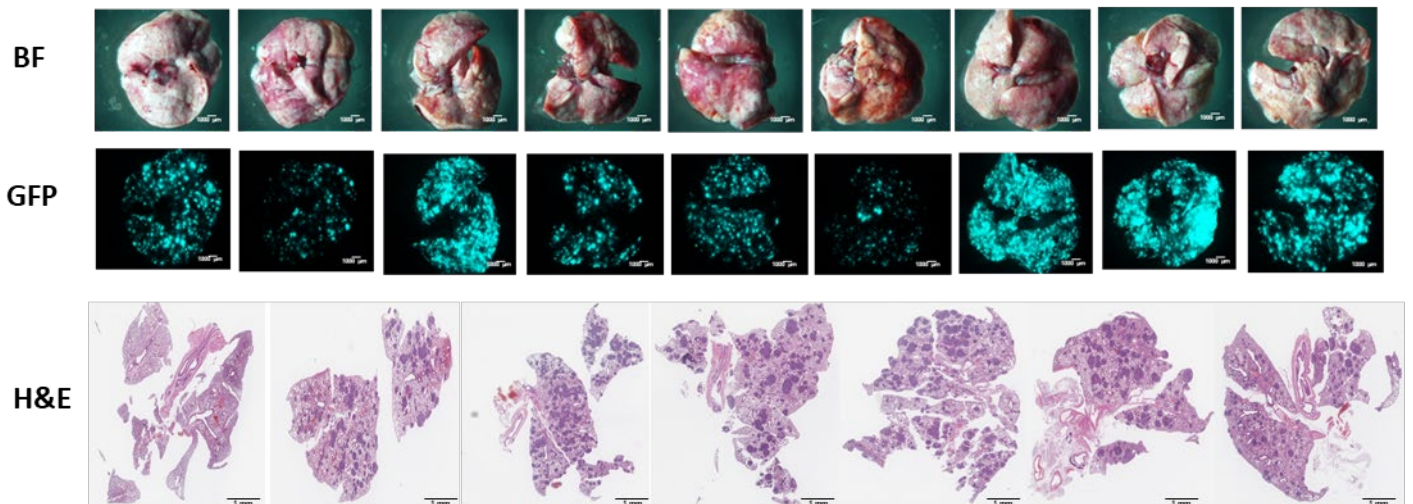


BxPC3 3'ss MYL6

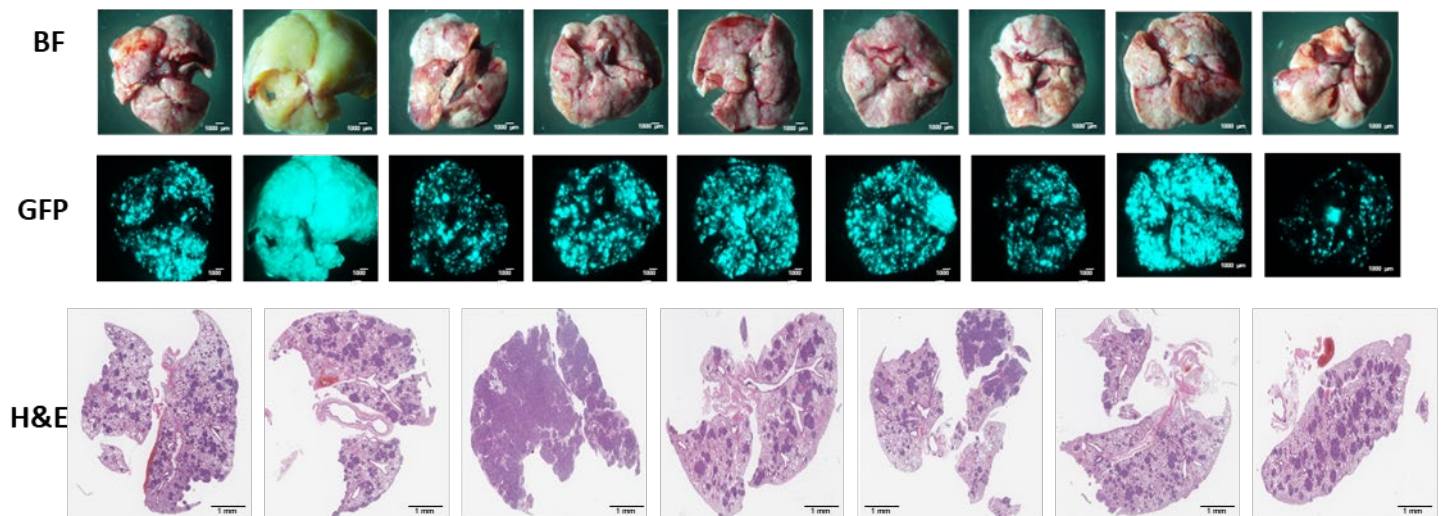


Supplementary figure 18. Lung histology samples related to extended data figure 12g.
(n=9 mice/group for CRISPR Cont. and 3' ss CLSTN1 sgRNAs)

BxPC3 CRISPR Cont.



BxPC3 3'ss CLSTN1



Supplementary Table 1. Genetic alterations and clinical data of PDA patients.

Related to figs.1a-b.

Supplementary Table 2. Differentially spliced events in PDA patient samples, primary tumors versus metastatic tumors.

Related to fig.1b .

Supplementary Table 3. Sequence motif enrichment analysis.

Related to fig.1d and extended data figs.2a and 5f.

Supplementary Table 4. Reactome analysis.

Related to figs.1c,3c and extended data figs.2b and 5d-e.

Supplementary Table 5. Differentially gene expression changes in PDA patient samples, primary tumors versus metastatic tumor.

Related to extended data fig.1h.

Supplementary Table 6. Differentially spliced events in RBFOX2 manipulated cell lines. manipulated cell lines: (X50 Pwzl(-) vs. X50 OE RBFOX2) vs. (BxPC3 CRISPR Cont. vs. BxPC3 sgRNA-1/2 RBFOX2). Related to figs. 3a-b and extended data figs.5a-c.

Supplementary Table 7. Comparisons of RBFOX2 target genes to known RBFOX2 target genes.

Related to extended data figs.1b and 5d-e.

Supplementary Table 8. Serine-threonine kinome analysis for MPRIP isoforms.

Related to extended data fig.9b.

Supplementary Table 9. Mass spectrometry analysis for MPRIP isoforms.

Related to extended data figs.9c-g.

Supplementary Table 10. sgRNAs sequences

RBFOX2 sgRNA-1	GTGGGAATTCCATTCTGCGG
RBFOX2 sgRNA-2	GAAGGTGGAGCACAGACAGA
sgRNA control	GGTGTGCGTATGAAGCAGTG
Target sequence for RBFOX2 EIJ sgRNA	TTTGCTTAGCAGACAGAAGG
MPRIP exon 23 3'ss sgRNA	GCTCAATTACGGACTGTCAG
MPRIP exon 23 5'ss sgRNA	CGCTTCCCGGCCCATGCAGG
MYL6 exon 6 3'ss sgRNA	ATATGCCTCACAAACGCTGC
CLSTN1 exon 10 5'ss sgRNA	CTGTGGCCTCTGCAGGTTGC
CLSTN1exon 10 3'ss sgRNA	TTCAACTCCTGAAAACCTCTG
DS-24 MPRIP sgRNA	CTGTGGGCATGGAATGCGTG
Rac1 sgRNA-1	ACACTTGATGGCCTGCATCA
Rac1 sgRNA-2	ATTTAAGATACTTACACAGT

Supplementary Table 11. PCR primers sequences

RT-PCR primers for splicing validation	
MPRIP	AAAGCAACCCTGACTTCTTGA CTCAACTTGGATGGGACACA
MYL6	GCATGAGGACAGCAATGGTT CAAAATTCACACAGGGAAAGG
CLSTN1	AATGGCACCCTACGTCCTC TCGGAAAACTGGGTCATGT
FOXMI	GACATGTTTGTCCGGGAGAC GGAGTTCGGTTTTGATGGTC
LMO7	ACTGCGTTACCCTTCAATCG GCTGACGCAAGTTTTGAACA
RAB11FIP3	GCTGTCCCCAGAGACCCTAT GCCACCTTCTTGCTGGAGAG
GOLIM4	GACAACTAAGGAAAGCACACCA CACCTGGGTCACATTTTGCT
ECT2	GAGATGCCTCAGATTGAAACAA TCAAATTCCGGAGAATCCAA
MAP2K7	AGGATCGACCTCAACCTGGA GGGGTGTGAACAGGGTTG
ENAH	CAAATACAATGAATGGCAGCA GTCAAGTCCTTCCGTCTGGA
DIAPH1	GACCCGGGACAAGAAGAAG ATGACTGTGCTGTGGGATCA
KIF23	TGCAAAGCAGAAGAGGTTGA GAAACTTCAAAAGCCTCCTCA
PBRM1	GTGATTAAGGCCCAACACCC CTGGTGCTGGAGTCCCTAC
SLC22A5	GCAGTCCCACAACATTCTGG CTCGGCTGTGTACACGTAGA
FN1	TTGATAACCTGAGTCCCGGC TGAGTAACGCACCAGGAAGT
ABI1	TTCCCAGTATGGCACAATGA GGGAGGTGGAGAGTCATCAA
ST7	ATGCAGAAAGCCTGGAGAGA CCTTCAGGGCCTGCTTAAAT
SYNE2	CCTCTCACGAAGAGGACGAG CAGGTGGAACATTCCTGTCA
TSC2	CTCAACGAGAGACCCAAGAG AGCCGTGAAGTTGGAGAAGA
PPP3CB	ACAGGGATGTTGCCTAGTGG GTGGTTCTCAGTGGCATGTG
EXOC1	TGAAGTTGCAAAGATCAAGATGA TCAAATTTGGTCCTGTCAGC
DIAPH2	GCCAATGAAGAGGAAACGAA ATAATGGCTGAGCTGCAGGA

Quantitative RT-PCR primers	
RBFOX2	TGGAAATTAAGCCCAGTAGTTG
	TGATACCCCTCTTCCTGA
18S rRNA	GTAACCCGTTGAACCCCAT
	CCATCCAATCGGTAGTAGCG

Supplementary Table 12. Primary and secondary antibodies

RBFOX2	Sigma	#006240	Prestige Antibodies® Immunoblotting	Immunoblotting: 0.4 µg/mL
SRSF1		(Cáceres et al. 1997)	mAb AK96 culture supernatant	Immunoblotting: 1:1000
SRSF6		(Fu and Maniatis 1990)	mAb 8-1-28 culture supernatant	Immunoblotting: 1:1000
Tubulin	Abcam	#ab6160	[YL1/2]	Immunoblotting: 1:10000
Flag	Sigma	# F3165	clone M2	Immunoblotting: 10 µg/mL
GAPDH	Sigma	# G9545	polyclonal	Immunoblotting: 0.2 µg/mL
β-catenin	Abcam	#ab6302	polyclonal	Immunoblotting: 1:4000
β-Actin	Santa Cruz	#sc-1616	I-19	Immunoblotting: 1:1000
total-MEK 1/2	Cell Signaling	#8727	D1A5	Immunoblotting: 1:1000
Paxillin	BD Biosciences	BD61240	5, Clone 349/Paxillin (RUO)	Immunofluorescence: 1:1000
Rac1	Cytoskeleton, Inc.	ARC03		Immunoblotting: 1:500
A-Raf	Santa Cruz	sc-408	C-20	Immunoblotting: 1:500
GFP	abcam	#ab6673		Immunohistochemistry 1:1000
Anti-FLAG	Sigma	#A2220	M2 Affinity gel	Immunoprecipitation 30 µg/µL
ImmPRESS HRP anti-Goat IgG polymer)	Vector Laboratories	#MP-7401		
Peroxidase-conjugated AffiniPure Goat Anti-Mouse IgG (H+L)	Jackson ImmunoResearch Inc.	# 115-035-003		Immunoblotting: 1:10000

Peroxidase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L)	Jackson ImmunoResearch Inc.	,# 111-035-003		Immunoblotting: 1:10000
Peroxidase-conjugated AffiniPure donkey Anti- goat IgG (H+L),	Jackson ImmunoResearch Inc.	# 705-035-003		Immunoblotting: 1:10000
Alexa Fluor® 488 AffiniPure Goat Anti-Mouse IgG (H+L)	Jackson ImmunoResearch Inc.	115-545-003		Immunofluorescence: 1:800
Donkey Anti-Rat IgG H&L (HRP) preadsorbed	Abcam	ab102265		Immunoblotting: 1:10000