Supplementary Figure S1

A

Panc02

KRAS - codon 32 TAT

T G G A T G A G T A C G A C C C T A C G A

KRAS - codon 12 GGT

T T G G A G C T G G T G G C G T A G G C A

TP53 - codon 175 CGC

G T C G T G A G A C G C T G C C C C C A C C

B

BxPC3

KRAS - codon 12 GGT

T T G G A G C T G G T G G C G T A G G C A

TP53 - codon 175 CGC

T T G T G A G G C G C T G C C C C C A C C
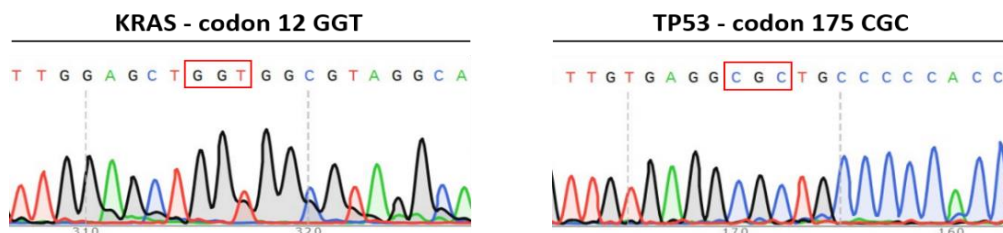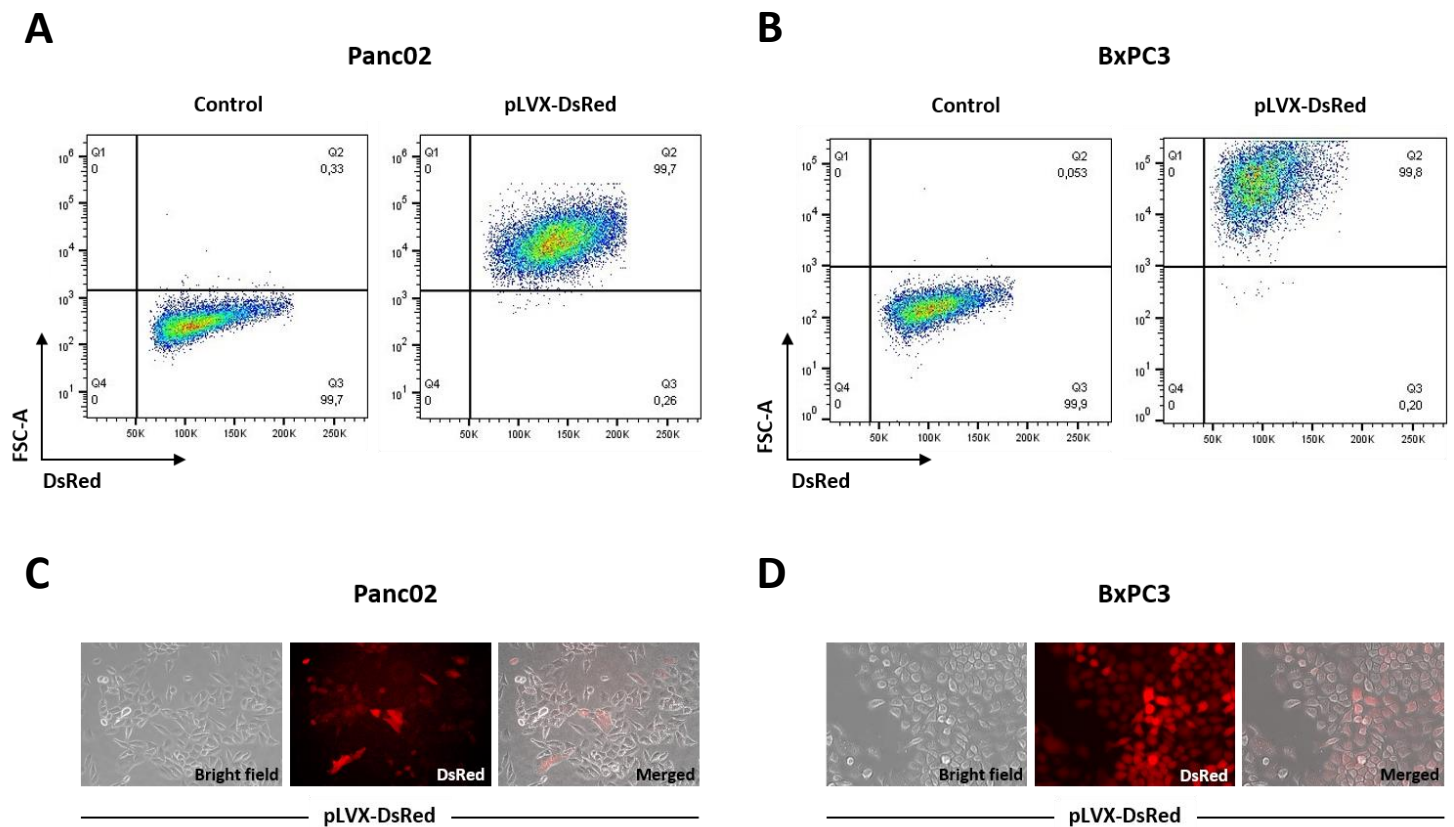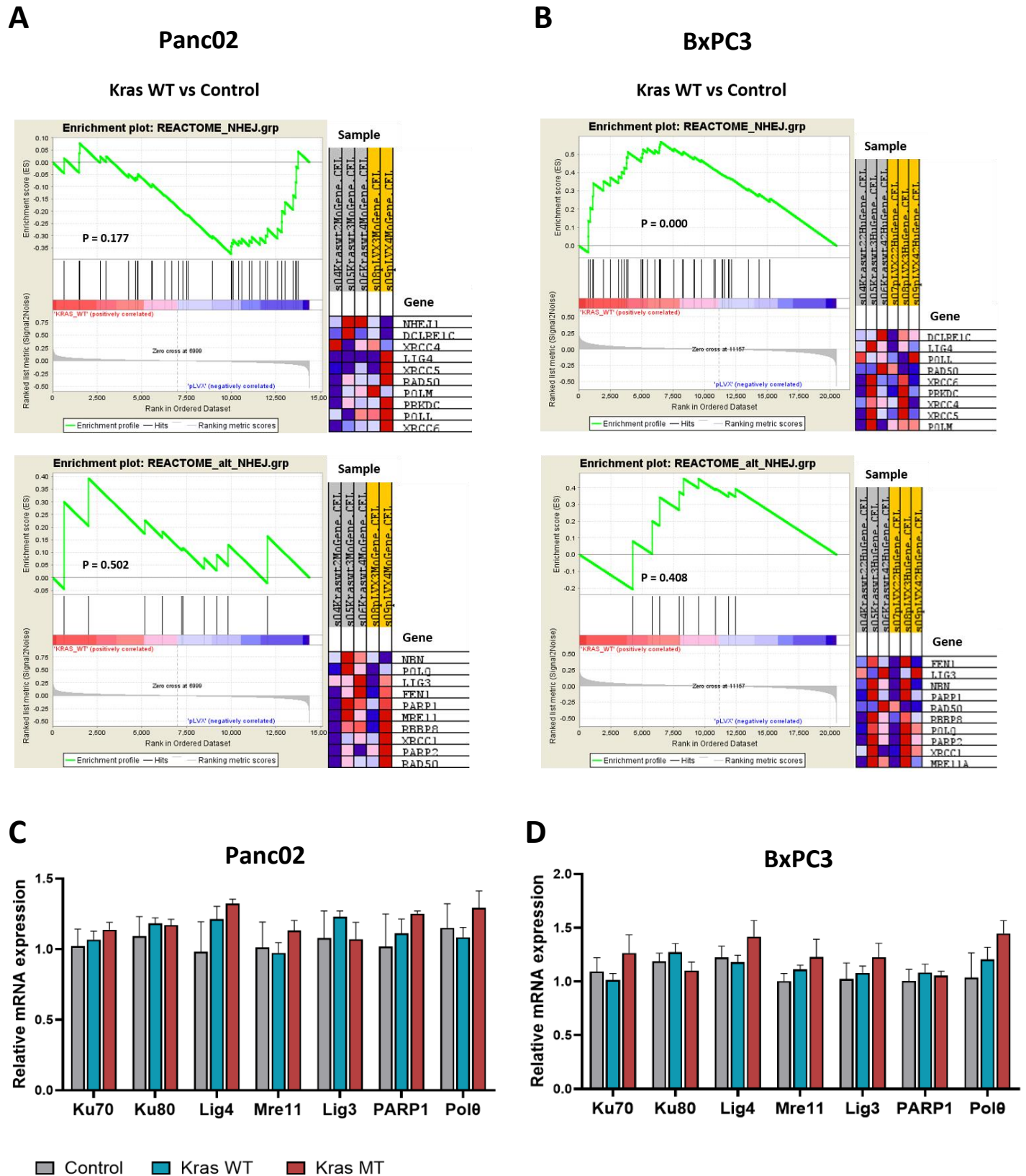
Supplementary Figure S1. Detection of mutations in *KRAS* exon 2, *TP53* exon 5 in pancreatic cancer cell lines. **(A)** Representative sequence analysis for each case in Panc02 mouse pancreatic cancer cell line is presented. The rectangle contains the codons in which hotspot mutations were expected. The arrow indicates the synonymous SNP in codon 32 of *KRAS* gene. **(B)** Representative sequence analysis for each case in BxPC3 human pancreatic cancer cell line is presented. No somatic mutations in KRAS and TP53 genes were found in this pancreatic cancer cell line.

Supplementary Figure S2

A Panc02

B BxPC3
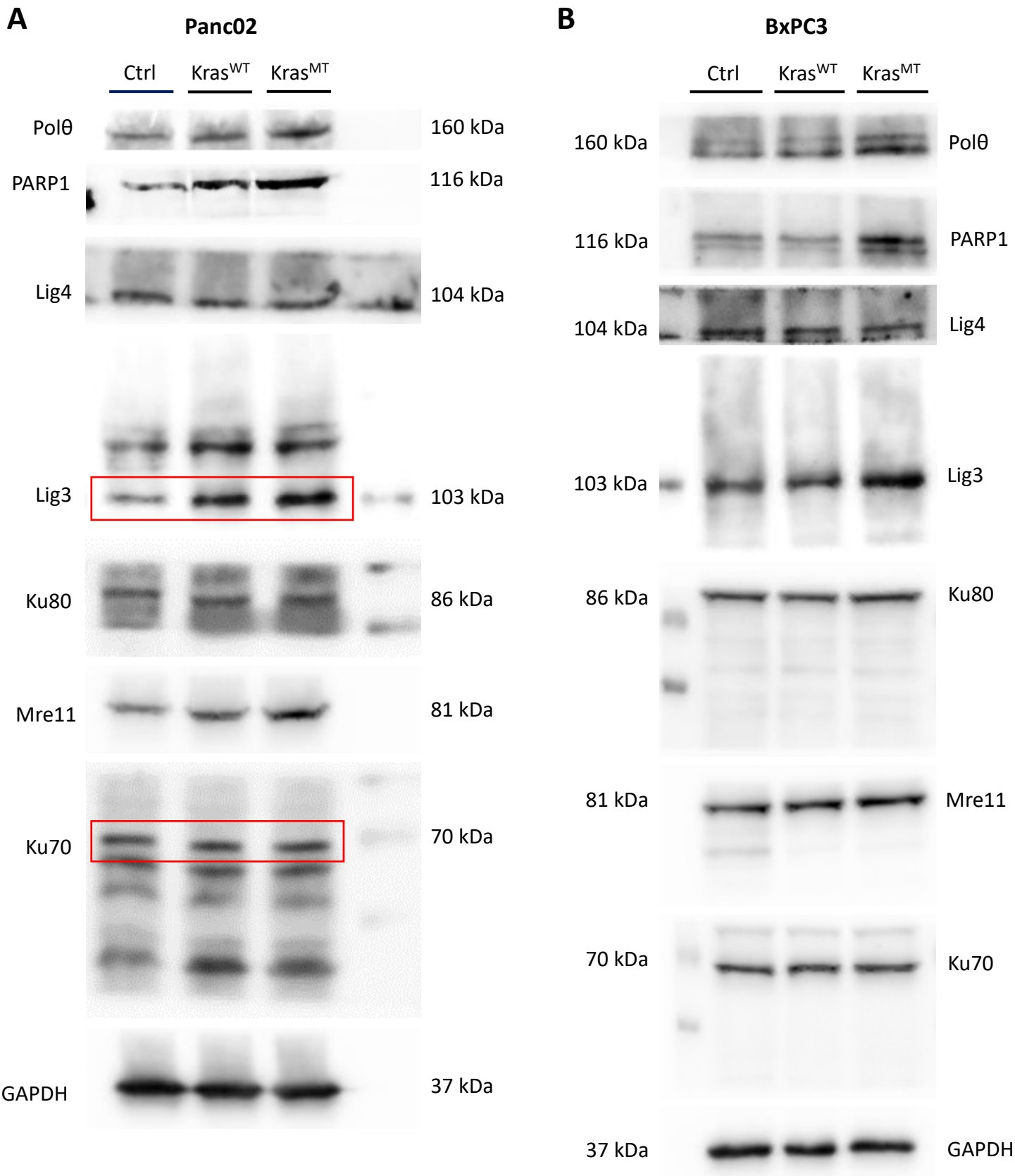

C Panc02

D BxPC3

Supplementary Figure S2. Lentiviral transduction of pLVX-DsRed, KRAS wild-type and mutagenic KRAS plasmids in pancreatic cancer cell lines. (A-B) The transduction efficiency of pLVX-DsRed plasmid in **(A)** Panc02 and **(B)** BxPC3 cell line was demonstrated by flow cytometry and microscopic analysis. **(C-D)** Representative images of **(C)** Panc02 and **(D)** BxPC3 cells expressing pLVX-DsRed plasmid.

Supplementary Figure S3

A

Panc02

Kras WT vs Control

B

BxPC3

Kras WT vs Control

C

D

□ Control ■ Kras WT ■ Kras MT

Supplementary Figure S3. Kras does not regulate the expression level of alt-EJ components at the transcriptional level. **(A-B)** Gene Set Enrichment Analysis plots for the Reactome NHEJ and alt-EJ in Panc02 and BxPC3 cells are shown. The heatmap on the right side of each panel visualizes the genes contributing the most to the enriched pathway. The green curve corresponds to the ES (enrichment score) curve, the running sum of the weighted enrichment score in GSEA. P value are reported within each graph (Panc02 control, n=2; Panc02 KrasWT, n=3; BxPC3 control, n=3; KrasWT, n=3). **(C-D)** mRNA expression of alt-EJ and c-NHEJ components in Panc02 and BxPC3 cells upon exogenous expression of KrasWT and mutagenic Kras[G12D]. Relative mRNA levels were normalized to 5S RNA of alt-EJ and c-NHEJ factors from three independent experiments (mean ± SD; *$P < 0.05$ is considered as significant; Student's t-test).

Supplementary Figure S4

A — Panc02

B — BxPC3

C

Panc02	Control	Kras WT	Kras MT
Polθ/GAPDH	1	2,129	4,109
PARP1/GAPDH	1	3,361	4,999
Lig4/GAPDH	1	0,865	0,931
Lig3/GAPDH	1	3,745	4,625
Ku80/GAPDH	1	0,947	0,976
Mre11/GAPDH	1	1,613	2,375
Ku70/GAPDH	1	0,987	1,093

BxPC3	Control	Kras WT	Kras MT
Polθ/GAPDH	1	1,603	2,662
PARP1/GAPDH	1	1,341	2,862
Lig4/GAPDH	1	1,149	1,067
Lig3/GAPDH	1	1,271	1,946
Ku80/GAPDH	1	1,038	1,282
Mre11/GAPDH	1	1,342	1,512
Ku70/GAPDH	1	1,101	1,174

Supplementary Figure S4. Whole western blot and densitometry readings/intensity ratio of alt-EJ and c-NHEJ components. (A-B) Western blot analysis of total lysate from transduced **(A)** Panc02 and **(B)** BxPC3 cells. The level of Polθ, PARP1, Lig4, Lig3, Ku80, Mre11 and Ku70 were analyzed. GAPDH was used as a loading control. Red rectangles are the selected for figure 1. **(C)** Densitometry readings/intensity ratio of each band of total blot from Panc02 and BxPC3 cells.

Supplementary Table S1

ID	Strain	Gender	Age (days)	PDA	Metastasis	Other
873	KC	F	289	N	N	
931	KC	F	164	N	N	
938	KC	M	96	N	N	
1034	KC	M	161	N	N	
1161	KC	M	132	Y	N	
872	KC	M	440	N	N	
878	KC	M	431	N	N	
916	KC	M	400	N	N	
1003	KC	M	326	Y	N	
251	KC	M	258	N	N	
208	KC	F	286	Y	Y' liver	Pancreatobiliary type IPMN
1151	KC	F	209	Y	N	Sarcomatoid dedifferentiation, Fatty Infiltration (FI)
957	KC	M	412	N	N	
967	KC	M	407	N	N	
994	KC	M	413	N	N	
1033	KC	F	440	Y	N	Sarcomatoid
1099	KC	M	440	Y	N	Serous microcystic adenoma
1116	KC	M	420	N	N	
1117	KC	M	420	N	N	
1126	KC	F	418	N	N	Serous microcystic adenoma
1050	qKC	F	274	Y	Y' liver, lung	Sarcomatoid, Fatty Infiltration (FI)
990	qKC	M	397	Y	N	
959	qKC	F	440	N	N	Serous microcystic adenoma
960	qKC	F	440	N	N	
1039	qKC	F	387	Y	Y' liver	Sarcomatoid, Fatty Inflltration (FI)
981	qKC	F	440	Y	N	
988	qKC	M	440	N	N	
1041	qKC	M	404	Y	N	
1080	qKC	F	360	Y	N	
1013	qKC	F	440	N	N	
1090	qKC	M	375	Y	Y' liver	
1046	qKC	F	446	N	N	
1086	qKC	F	440	Y	N	
1089	qKC	M	424	Y	Y' liver, Y' lung	Sarcomatoid, Fatty Infiltration (FI)

Supplementary Table S1. Clinical spectrum of disease in KC and qKC mice. Histopathological analysis of animals used in the survival curve (N = no, Y = yes).