

APPENDIX

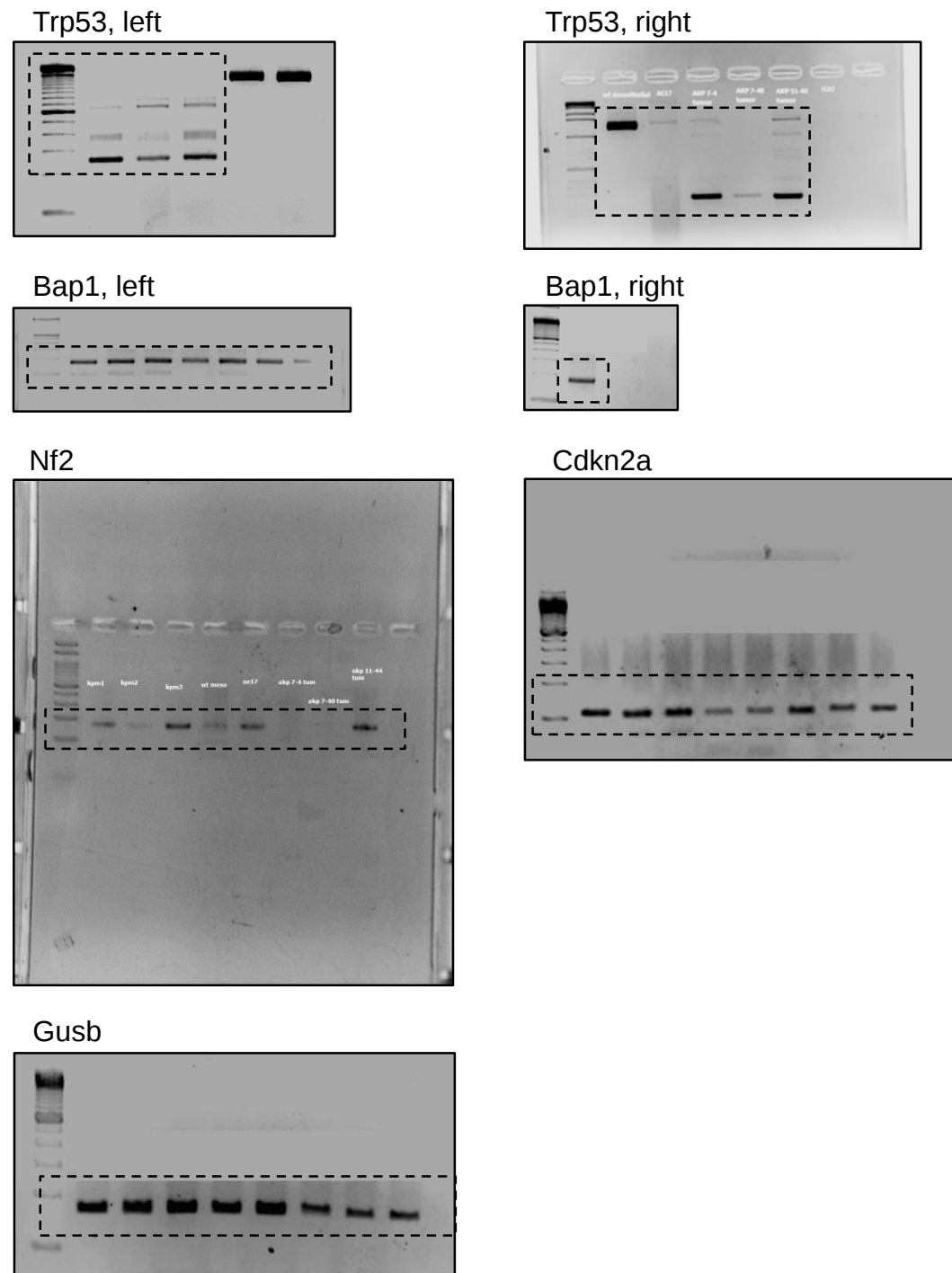
KRAS signaling in malignant pleural mesothelioma

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Table of Contents

Appendix Element	Page
Appendix Title Page.....	S1
Appendix Figure.....	S2
Appendix Tables.....	S3

Appendix Figure



Appendix Fig S1. Uncropped PCR gels from Fig 7E.

Cropboxes indicate the gel areas shown in the main Figure.

Appendix Tables

Appendix Table S1. Clinical attributes of 45 patients with pleural effusions from Munich, Germany, studied by digital droplet (dd)PCR.

Attribute	MPM	LUAD	Other cancers	Benign
Patient number (<i>n</i>)	12	16	12	5
Age (years) ^a	71(68-77)	72(56-75)	63(50-75)	62(21-86)
Sex (female/male; <i>n</i>)	3/9	7/9	7/5	3/2
Smoking status (never/ former/ current; <i>n</i>)	8/4/0	10/4/2	9/0/3	3/0/2
Smoking exposure (pack-years) ^a	0(0-10)	0(0-20)	0(0-40)	0(0-100)
Overall survival (years) ^a	3.1(1.0-3.6)	2.6(0.3-3.2)	2.6(0.2-3.1)	3.1(3.0-3.3)
Death events/ Censored observations (<i>n</i>)	4/8	6/10	4/8	0/5
<i>KRAS</i> ^{G12/13} droplets (%) ^a	0(0-6)	0(0-1)	0(0-0)	0(0-0)
<i>KRAS</i> ^{Q61} droplets (%) ^a	0(0-0)	0(0-0)	0(0-8)	0(0-0)
<i>TP53/TERT</i> droplets pleural fluid (%) ^a	108(74-133)	98(75-130)	93(60-103)	104(55-177)
<i>TP53/TERT</i> droplets pleural cells (%) ^a	89(64-149)	82(54-105)	88(69-103)	70(59-73)
Erythrocytes (10 ⁶ /mm ³) ^a	4.2(1.4-27.8)	1.9(1.2-32.3)	8.5(1.2-32.0)	1.9(0.8-9.0)
Nucleated cells (10 ³ /mm ³) ^a	1.4(0.9-4.6)	1.5(0.7-4.5)	1.5(1.0-3.4)	3.9(1.9-7.7)
Large mononuclear cells (%) ^a	44(18-71)	43(32-56)	49(41-73)	25(2-88)
Neutrophils (%) ^a	2(0-7)	9(3-11)	7(2-29)	2(2-98)
Small mononuclear cells (%) ^a	47(6-64)	44(23-54)	31(10-53)	18(0-73)
Eosinophils (%) ^a	0(0-16)	1(0-5)	1(0-1)	0(0-45)

^a Data are presented as median(95%confidence interval).

Appendix Table S2. Clinical attributes of 33 donors of malignant pleural effusions due to malignant pleural mesothelioma from Nantes, France for establishment of cell lines studied by CytoScan arrays.

Attribute	
Patient number (<i>n</i>)	33
Age (years) ^a	72(65-75)
Sex (female/male/unknown; <i>n</i>)	6/26/1
Asbestos exposure (yes/ no/ unknown; <i>n</i>)	22/8/3
MPM subtype (epithelioid/ biphasic/ sarcomatoid/ unspecified) ^a	27/2/2/2
Overall survival (years) ^a	0.8(0.5-1.0)
Death events/ Censored observations (<i>n</i>)	4/29

^a Data are presented as median(95%confidence interval).

Appendix Table S3. Clinical attributes of 10 donors of malignant pleural effusions due to lung adenocarcinoma (LUAD) and malignant pleural mesothelioma (MPM) from Nantes, France and of 17 donors of MPM tissues from Istanbul, Turkey, which were study by digital droplet PCR.

Attribute	Nantes LUAD	Nantes MPM	Istanbul MPM
Patient number (<i>n</i>)	4	6	17
Age (years) ^a	64(49-70)	66(56-85)	67(54-70)
Sex (female/male/unknown; <i>n</i>)	1/3	0/6	5/12
Metastasis (yes/no; <i>n</i>)	1/3/0	0/6	3/14
MPM subtype (epithelioid/ biphasic/ sarcomatoid/ unspecified) ^a	-	4/1/1/0	11/4/1/1

^a Data are presented as median(95%confidence interval).

Appendix Table S4. Top differentially expressed genes in *KRAS*^{G12D};*Trp53*^{f/f} malignant pleural mesothelioma cell lines (KPM) versus pleural mesothelial cells (PMC).

Gene symbol	Gene ID	<i>P</i> ^a	FDR <i>q</i> ^b	Δ GE ^c
<i>Fkbp10</i>	ENSMUSG00000001555	2,69E-06	3.66E-04	+11,478
<i>Cxcl5</i>	ENSMUSG00000029371	1,17E-06	1.27E-04	+11,160
<i>Tm4sf1</i>	ENSMUSG00000027800	7,75E-06	6.29E-04	+11,090
<i>Npdc1</i>	ENSMUSG00000015094	4,54E-06	4.12E-04	+10,777
<i>Etv4</i>	ENSMUSG00000017724	6,55E-06	5.49E-04	+10,605
<i>Cdc42ep1</i>	ENSMUSG00000049521	8,70E-06	6.92E-04	+10,439
<i>Gjb3</i>	ENSMUSG00000042367	2,32E-05	1.58E-03	+10,230
<i>Flnc</i>	ENSMUSG00000068699	1,44E-05	1.06E-03	+10,153
<i>Foxa2</i>	ENSMUSG00000037025	7,90E-05	4.17E-03	+10,114
<i>Cck</i>	ENSMUSG00000032532	2,37E-05	1.60E-03	+9,950
<i>C1qc</i>	ENSMUSG00000036896	6,77E-18	2,87E-14	-12,792
<i>C1qa</i>	ENSMUSG00000036887	1,57E-18	1,33E-14	-13,319
<i>Tyrobp</i>	ENSMUSG00000030579	2,12E-17	6,00E-14	-13,392
<i>Cd79b</i>	ENSMUSG00000040592	2,66E-17	6,45E-14	-13,522
<i>Lyz1</i>	ENSMUSG00000069515	2,81E-18	1,59E-14	-14,652
<i>Hbb-bs</i>	ENSMUSG00000052305	5,05E-05	2.89E-03	-15,190
<i>C1qb</i>	ENSMUSG00000036905	1,23E-18	1,33E-14	-15,336
<i>Hba-a2</i>	ENSMUSG00000069917	3,39E-06	3.20E-04	-15,518
<i>Hba-a1</i>	ENSMUSG00000069919	3,58E-07	4,82E-05	-18,064
<i>Hbb-bt</i>	ENSMUSG00000073940	1,55E-07	2,33E-05	-18,539

^a *P*, probability value by two-way ANOVA with Bonferroni post-test.

^b FDR *q*, false discovery rate probability value.

^c Δ GE, differential gene expression between KPM and PMC; a positive Δ GE indicates overexpression by KPM, while a negative Δ GE by PMC cells.

Appendix Table S5. PCR primers used for these studies

Method ^a	Primer	Sequence	Amplicon (bp)
Seq	KrasF1	CCATTTTCGGACCCGGAG	904
	KrasR1	CTTTAGTCTCTTCCACAGGCA	
	KRASf2	TCCCAGGTGCGGGAGAGAG	846
	KRASr2	GCTAACAGTCTGCATGGAGCAGG	
	Bap1F	TATGCAATTGGCAATGCCCC	928
	Bap1R	CTGACAGCTGCCCATCTGAA	
RT-PCR	Trp53F	GTAGCTTCAGTTCATTGGGA	1450
	Trp53R	GAAGTCATAAGACAGCAAGGA	
	TP53F	CGCAGTCAGATCCTAGCGTC	551
	TP53R	ACCATCGCTATCTGAGCAGC	
RT-PCR & qPCR	MycoplasmaF	GGGAGCAAACAGGATTAGATACCCT	270
	MycoplasmaR	TGCACCATCTGTCACTCTGTTAACCTC	
	GusbF	TTACTTTAAGACGCTGATCACC	165
	GusbR	ACCTCCAAATGCCCATAGTC	
	Nf2F	CTGAAAATGCTGAGGAGGAGC	155
	Nf2R	ATAGTCGCCATACTTGGCCTG	
	Bap1F	TGCCATCAGATACAAGCGGA	173
	Bap1R	CAGCCCCACTGCTAGTCTTG	
	Cdkn2aF	CGAACTCTTTCGGTCGTACCC	102
	Cdkn2aR	GGTCCTCGCAGTTCGAATCTG	
	GUSBF	CTACTTGAAGATGGTGATCGCTC	118
	GUSBR	ACAGATCACATCCACATACGG	
	TP53F	AGTCTACCTCCCGCCATAAA	173
	TP53R	GCTTCTGACGCACACCTATT	

^a Method: Seq, sequencing; RT-PCR, reverse transcriptase polymerase chain reaction; qPCR, quantitative real-time polymerase chain reaction.

Appendix Table S6. Antibodies used for these studies

Method ^a	Target ^b	Provider ^c	Catalog #	Dilution	Conjugate ^d
IHC	PCNA	Abcam	ab2426	1:2000	-
	CAL	Abcam	ab203055	1:400	-
	PDPN	Merck	ABT34	1:1000	-
	OPN	Abcam	ab8448	1:100	-
	KRT5/6	Merck	MAB3412	1:200	-
	SFTPC	Santa Cruz	FL-197	1:100	-
	BAP1	Santa Cruz	Sc-28383	1:100	-
	WT1	Bioworld	BS6239	1:100	-
	VIM	Elabscience	E-AB-36172	1:1000	-
	MSLN	Biomol	V3821-20UG	1:100	-
FC	CD11b	eBioscience	12-0112	0.1µg/10 ⁶ cells	PE
	Gr1	eBioscience	25-5931-82	0.1µg/10 ⁶ cells	PE-Cy7

^a Method: IHC, immunohistochemistry; FC, flow cytometry.

^b Target: PCNA, proliferating cell nuclear antigen; CAL, calretinin; PDPN, podoplanin; OPN, osteopontin; KRT, cytokeratin; SFTPC, surfactant protein C; BAP1, *BRCA1* associated protein-1; CD11b, cluster of differentiation molecule 11B; Gr1, Gr1 antigen.

^c Provider: Abcam, Cambridge, UK; eBioscience, San Diego, CA; Santa Cruz Biotechnology, Santa Cruz, CA; Merck Millipore, Darmstadt, Germany; Bioworld, Philadelphia, PA; Elabscience, Houston, TX; Biomol, Hamburg, Germany.

^d Conjugate: PE, phycoerythrin; PE-Cy7, phycoerythrin-cyanin 7.

Appendix Table S7. Number of experimental mice (*n*) used for these studies

Strain designation	Jackson Laboratory ID #	Short strain designation	<i>n</i>
<i>C57BL/6J</i>	000664	<i>C57BL/6</i>	177
B6.129(Cg)-Gt(ROSA)26Sor ^{tm4(ACTB-tdTomato,-EGFP)Luo/J}	007676	<i>mT/mG</i>	40
B6.129S4- <i>Kras</i> ^{tm4Tyj/J}	008179	<i>KRAS</i> ^{G12D}	40
B6.129P2- <i>Trp53</i> ^{tm1Brn/J}	008462	<i>Trp53f/f</i>	33
FVB-Tg(CAG-luc,-GFP)L2G85Chco/J	008450	CAG.Luc.eGFP	5
Intercrossed mice		<i>Trp53f/Wt</i>	11
		<i>KRAS</i> ^{G12D} ; <i>Trp53f/Wt</i>	11
		<i>KRAS</i> ^{G12D} ; <i>Trp53f/f</i>	115
Total			432