

Appendix

ADAM17 selectively activates the IL-6 trans-signaling/ERK MAPK axis in KRAS-addicted lung cancer

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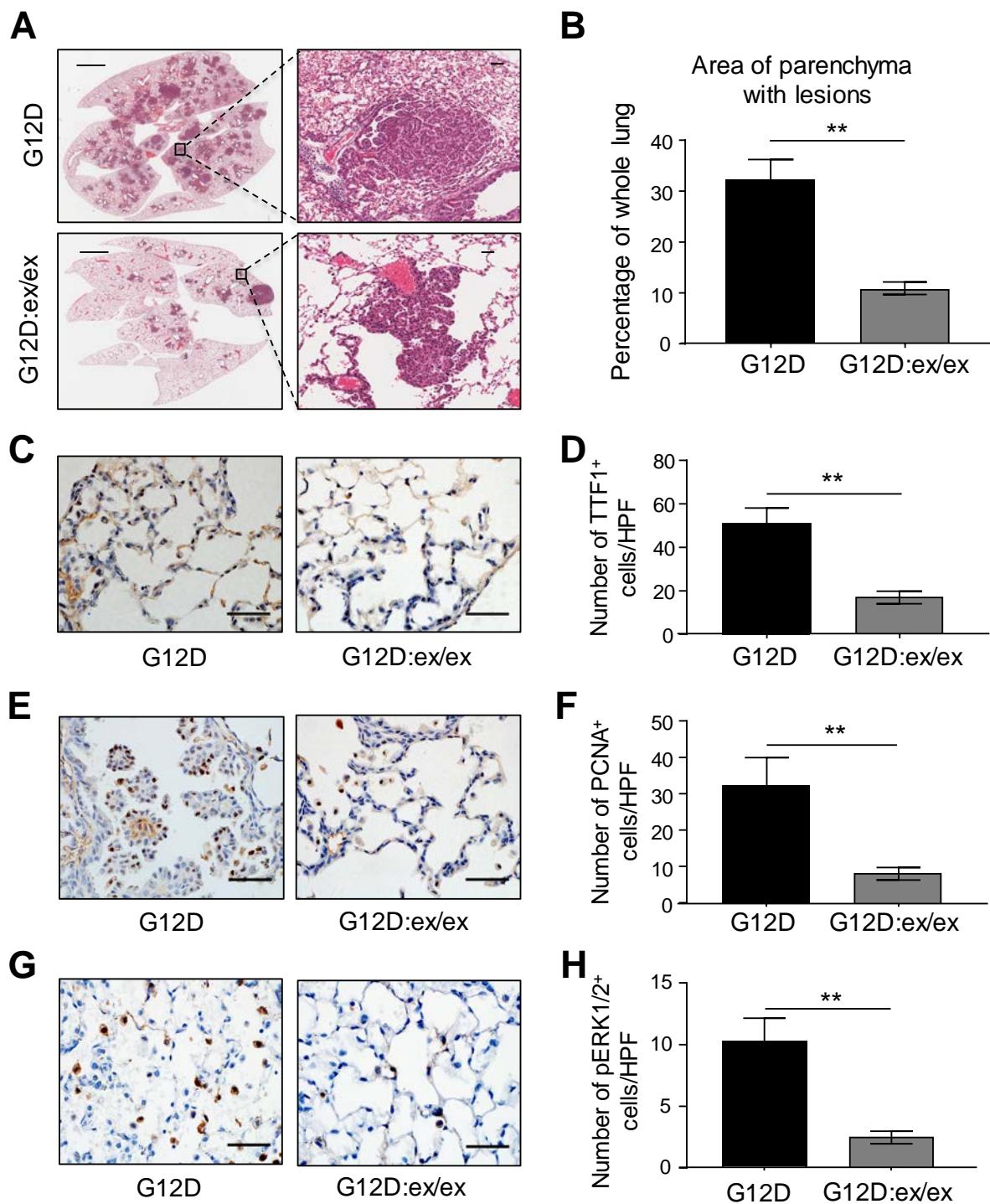
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Appendix Figure S1. Genetic reduction of ADAM17 expression suppresses the 12-week-old LAC phenotype of *Kras*^{G12D} mice.

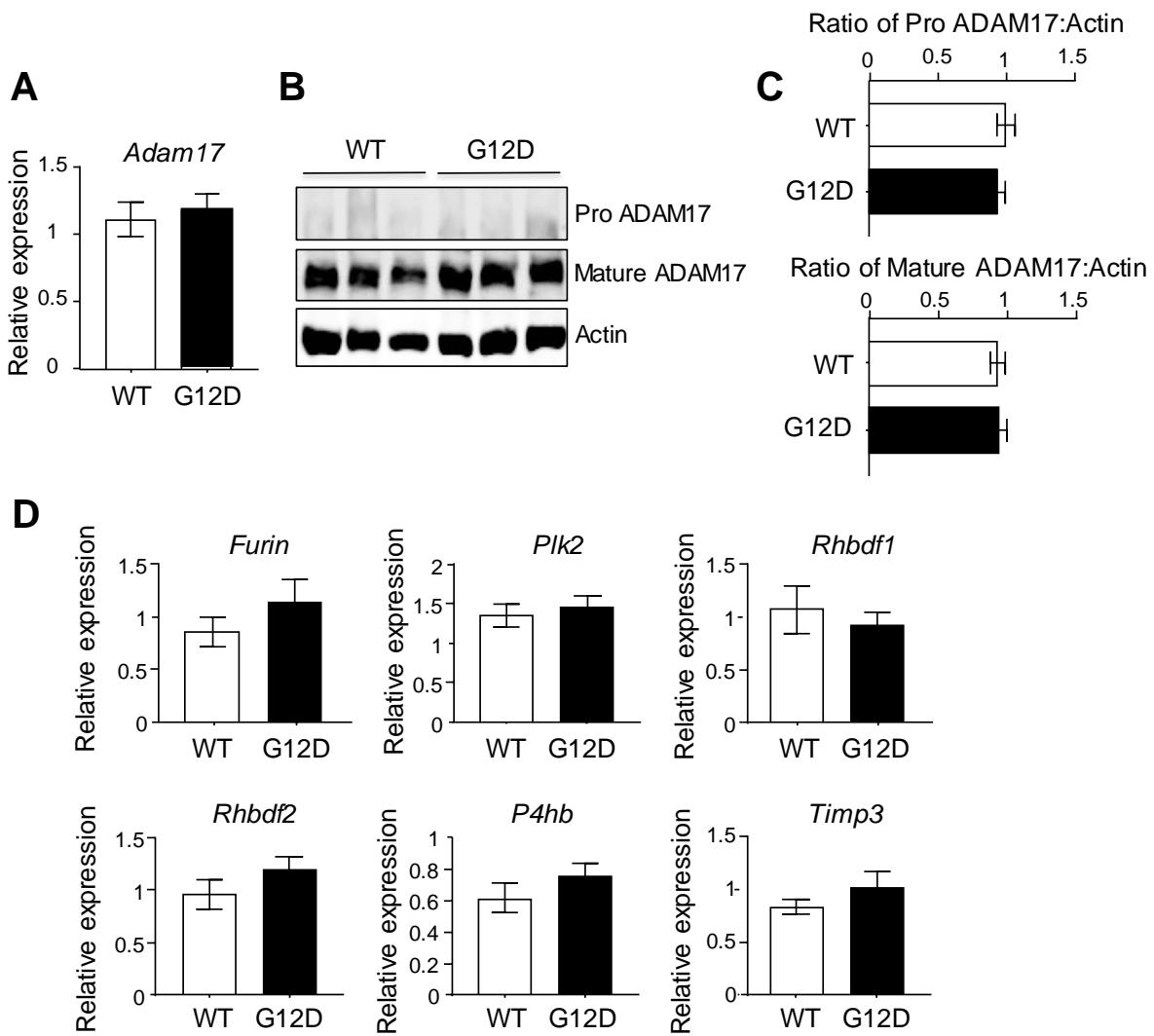
A Representative low power (left) and high power (right) photomicrographs of H&E-stained lung cross-sections from *Kras*^{G12D} and *Kras*^{G12D}:*Adam17*^{ex/ex} mice at 12 weeks following Ad-Cre inhalation. Scale bars, 3mm (left) and 300μm (right).

B Quantification of lung parenchyma area occupied by tumor lesions in the indicated genotypes from (A). Data are presented as the mean ± SEM (n = 6). **P<0.01, Student's *t*-test.

C, E, G Representative high power photomicrographs of lung cross-sections from *Kras*^{G12D} and *Kras*^{G12D}:*Adam17*^{ex/ex} mice at 12 weeks following Ad-Cre inhalation that were stained with antibodies against TTF-1 (C), PCNA (E) and pERK1/2 MAPK (G). Scale bars, 100μm.

D, F, H Quantification of positive cells per high power field (HPF) stained for TTF-1 (D), PCNA (F) and pERK1/2 MAPK (H) in lungs of the indicated genotypes from (C), (E) and (G), respectively, at 12 weeks post Ad-Cre inhalation. Data are presented as the mean ± SEM (n = 5). **P<0.01, Student's *t*-test.

Exact *P* values are specified in Appendix Table S4.



Appendix Figure S2. Unaltered expression status of ADAM17 and its regulators in the lungs of *Kras*^{G12D} mice.

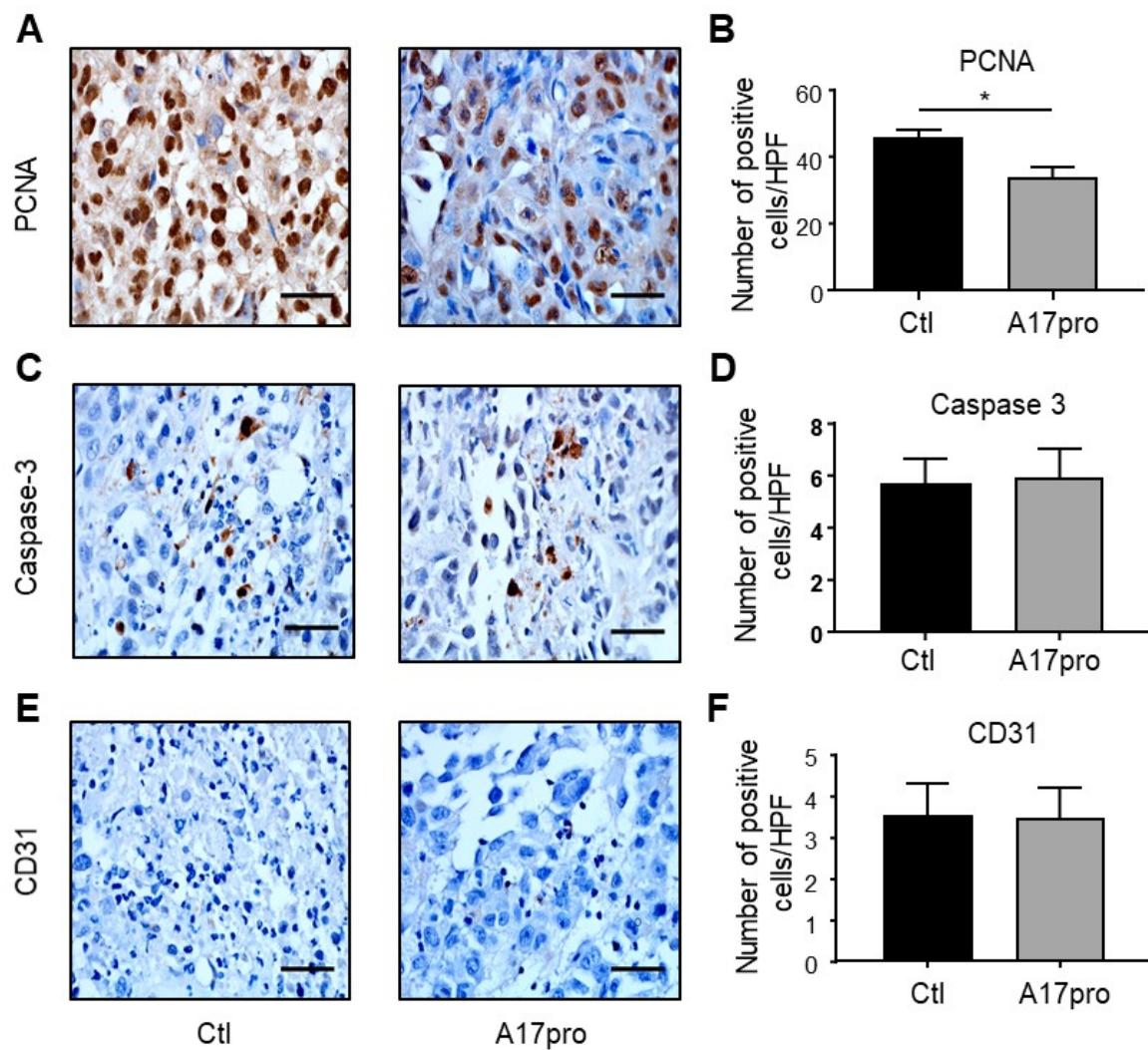
A qPCR gene expression analysis of *Adam17* on lung cDNA from *Kras*^{WT} and *Kras*^{G12D} mice at 6 weeks post inhalations. Expression data are normalized against 18S rRNA. Data are presented as the mean \pm SEM (n = 6 per genotype).

B Immunoblots of representative *Kras*^{WT} and *Kras*^{G12D} lung lysates with antibodies against ADAM17 (detecting pro and mature forms) and Actin. Note that for the Actin blot, lanes 4-6 are reproduced in lanes 1-3 of the Actin blot in Fig 4L.

C Densitometric semi-quantitative analysis of pro and mature ADAM17 protein levels in lung lysates from the mice indicated in (B). Relative expression was determined against Actin. Data are presented as the mean ± SEM (n = 6 per genotype).

D qPCR expression analysis of the indicated genes in lungs of *Kras*^{WT} and *Kras*^{G12D} mice at 6 weeks post inhalations. Expression data are normalized against *18S rRNA*. Data are presented as the mean ± SEM (n = 6 per genotype).

Exact *P* values are specified in Appendix Table S4.



Appendix Figure S3. Reduced proliferation, but not apoptosis or angiogenesis, is associated with suppressed tumorigenesis in an A17 pro-treated KRAS mutant LAC PDX.

A, C, E Representative high power photomicrographs of lung cross-sections from a *KRAS* mutant PDX treated with vehicle control (Ctl) or A17pro (1mg/kg) every second day that were stained with antibodies against PCNA (A), cleaved Caspase-3 (C) and CD31 (E). Scale bars, 100 μ m.

B, D, F Quantification of positive cells per high power field (HPF) stained for PCNA (B), cleaved Caspase-3 (D) and CD31 (F) in the above treated *KRAS* mutant PDX. Data are presented as the mean \pm SEM ($n = 5$ per genotype). * $P < 0.05$, Student's *t*-test.

Exact *P* values are specified in Appendix Table S4.

Appendix Table S1. Clinicopathological features of patients used for immunohistochemical staining of pADAM17 and pERK1/2.

	LAC	Non-LAC
Mean age		
Years (range)	69.8 (58-87)	62.0 (47-71)
Sex [number (%)]		
Male	10 (55.6)	6 (75)
Female	8 (44.4)	2 (25)
Smoking history [number (%)]		
Never	4 (22.2)	2 (25)
Former	10 (55.6)	6 (75)
Current	2 (11.1)	0 (0)
Unknown	2 (11.1)	0 (0)
Tumor stage¹ [number (%)]		
I	12 (66.7)	NA
II	4 (22.2)	NA
Unknown	2 (11.1)	NA
KRAS status [number (%)]		
Wild-type	13 (72.2)	NA
Mutant	5 (27.8)	NA

¹American Joint Committee on Cancer staging for non-small cell lung cancer based on tumor (T)/node (N)/metastasis (M) classification; Stage I (IA/IB), Stage II (IIA/IIB), Stage III (IIIA/IIIB), Stage IV. NA, not applicable.

Appendix Table S2. Antibody dilutions used for immunoblotting, immunofluorescence and immunohistochemistry.

Immunohistochemistry	
Antibody	Dilution
pThr ⁷³⁵ -ADAM17	1:100
pThr ²⁰² /pTyr ²⁰⁴ -ERK1/2	1:100
pThr ¹⁸⁰ /pTyr ¹⁸² -p38 MAPK	1:100
B220	1:100
CD3	1:100
CD45	1:100
Ly6G	1:100
CD31	1:100
TTF-1	1:100
PCNA	1:5000
Cleaved Caspase-3	1:100
F4/80	1:150
Cleaved Notch1	1:100
Immunofluorescence	
Antibody	Dilution
ADAM17	1:100
CD31	1:100
pThr ⁷³⁵ -ADAM17	1:100
CD45	1:100
CC10	1:100
SP-C	1:100
IL-6R	1:100
TGF α	1:100

Nrg1	1:100
EGF	1:100
Podoplanin	1:100
Alexa Fluor secondary antibodies	1:200

Immunoblotting

Antibody	Dilution
ADAM17	1:500
pThr ⁷³⁵ -ADAM17	1:500
Myc	1:1000
pTyr ⁷⁰⁵ -STAT3	1:1000
pSer ⁷²⁷ -STAT3	1:1000
STAT3	1:1000
pSer ⁴⁷³ -AKT	1:1000
AKT	1:1000
pThr ²⁰² /pTyr ²⁰⁴ -ERK1/2	1:1000
ERK1/2	1:1000
pThr ¹⁸⁰ /pTyr ¹⁸² -p38 MAPK	1:1000
p38 MAPK	1:1000
pTyr ¹⁰⁶⁸ -EGFR	1:1000
EGFR	1:1000
pSer ⁸² -HSP27	1:1000
Cleaved Notch1	1:500
IL-6R	1:500
Nrg1	1:1000
pTyr ¹²⁸⁹ -ErbB3	1:1000
ErbB3	1:1000
TGF α	1:500

Actin

1:500

Appendix Table S3. Primer sequences used for qPCR.

Gene	Forward	Reverse
<i>Ccnd1</i>	AGTGCCTGCAGAAGGAGATT	AGGAAGCGGTCCAGGTAGTT
<i>Ccnb1</i>	TAGGGTGTCTTCGAATCGGGG	ACGCTTGGCGCCTGCCATA
<i>Cdc42</i>	GAAAATGTGAAAGAAAAGTGG	TCTGGAGTAATAGGCTTCTGT
<i>Myc</i>	CGGACACACAACGTCTGGAA	AGGATGTAGGCAGGTGGCTTT
<i>Il1b</i>	CAACCAACAAGTGATATTCTCCATG	GATCCACACTCTCCAGCTGCA
<i>Il7a</i>	ACCGCAATGAAGACCCTGAT	TCCCTCCGCATTGACACA
<i>Il6</i>	ATGGATGCTACCAAACCTGGAT	TGAAGGACTCTGGCTTGTCT
<i>Cxcl2</i>	AACATCCAGAGCTTGAGTGTGA	TTCAGGGTCAAGGCAAACCTT
<i>Vegf</i>	GCACACAGGACGGCTTGAAGA	CCCACGACAGAAGAGCAGA
<i>Mmp2</i>	GCTCTATGGGCCCTCCCCG	CGTGGTGTCACTGTCCGCCA
<i>Il6r</i>	AAGCAGCAGGCAATGTTACC	CATAAATAGTCCCCAGTGTGCG
<i>Hes1</i>	ACCTTCCAGTGGCTCCTC	TTTAGTGTCCGTAGAACAGAGAG
<i>Hey1</i>	GCCGAAGTTGCCCGTTATCTG	TGTGTGGGTGATGTCCGAAGG
<i>Furin</i>	GGCTCTAAGAAAGGGGGTTG	TGCAAAAGGCACATCAGAAG
<i>Plk2</i>	CGGTGCTGAAATACTTTCTCAT	TCGGGAGATCACCACCAT
<i>Rhbd1</i>	AGCTATGTCAAGCGGCAGAT	TTCCGAAGTACCGAGTCCAC
<i>Rhbd2</i>	TGCCTGACGATGTCTTGAG	TAGCTCGGTTGAGCCAGTT
<i>P4hb</i>	TGACCTGGCTCAGCAGTATG	AAACCGATGACCGTCACTTC
<i>Timp3</i>	TCCTAGACCCAGTTCCATATACACTTC	TTGGACTTCTGCCAACCTCCTT

Appendix Table S4. *P* values for differences between experimental groups in figures.

Figure 1B				
Student's <i>t</i>-test		Significant?	Summary	<i>P</i> value
G12D	G12D:ex/+	Yes	*	0.0347
G12D	G12D:ex/ex	Yes	***	0.0001
G12D:ex/+	G12D:ex/ex	Yes	***	0.0001
Figure 1C				
Student's <i>t</i>-test		Significant?	Summary	<i>P</i> value
G12D	G12D:ex/+	No	ns	0.1236
G12D	G12D:ex/ex	Yes	***	0.0001
G12D:ex/+	G12D:ex/ex	Yes	*	0.0260
Figure 1E				
Student's <i>t</i>-test		Significant?	Summary	<i>P</i> value
WT	G12D	Yes	**	0.0022
WT	G12D:ex/+	Yes	**	0.0022
WT	G12D:ex/ex	No	ns	0.3939
G12D	G12D:ex/+	Yes	**	0.0043
G12D	G12D:ex/ex	Yes	**	0.0022
G12D:ex/+	G12D:ex/ex	Yes	*	0.0152
Figure 2B				
Student's <i>t</i>-test		Significant?	Summary	<i>P</i> value
WT	G12D	Yes	**	0.0022
WT	G12D:ex/ex	No	ns	0.2403
G12D	G12D:ex/ex	Yes	**	0.0022
Figure 2C				
Student's <i>t</i>-test		Significant?	Summary	<i>P</i> value
<i>Ccnd1</i>				
WT	G12D	Yes	*	0.0181
WT	G12D:ex/ex	No	ns	0.3374
G12D	G12D:ex/ex	Yes	*	0.0428
<i>Ccnb1</i>				
WT	G12D	Yes	*	0.0110
WT	G12D:ex/ex	No	ns	0.0635
G12D	G12D:ex/ex	Yes	**	0.0034
<i>Cdc42</i>				
WT	G12D	Yes	*	0.0124
WT	G12D:ex/ex	No	ns	0.4533
G12D	G12D:ex/ex	Yes	*	0.0462
<i>Myc</i>				
WT	G12D	Yes	*	0.0181
WT	G12D:ex/ex	No	ns	0.4026
G12D	G12D:ex/ex	Yes	*	0.0290
Figure 2E				
Student's <i>t</i>-test		Significant?	Summary	<i>P</i> value
G12D	G12D:ex/ex	Yes	**	0.0079
Figure 2G				
Student's <i>t</i>-test		Significant?	Summary	<i>P</i> value
WT	G12D	Yes	**	0.0022
WT	G12D:ex/ex	Yes	**	0.0022
G12D	G12D:ex/ex	Yes	**	0.0022
Figure 3H				
Student's <i>t</i>-test		Significant?	Summary	<i>P</i> value
Kras ^{Kras}	Kras ^{Kras:ex/ex}	No	ns	0.8502

Figure 4B				
Student's t-test		Significant?	Summary	P value
WT	G12D	Yes	**	0.0079
Figure 4D				
Student's t-test		Significant?	Summary	P value
WT	G12D	Yes	*	0.0317
Figure 4F				
Student's t-test		Significant?	Summary	P value
WT	G12D	Yes	**	0.0079
WT	G12D	No	ns	0.8413
Figure 4H				
Student's t-test		Significant?	Summary	P value
Ctl	SB	Yes	**	0.0079
Ctl	U0	No	ns	>0.9999
SB	U0	Yes	*	0.0317
Figure 4J				
Student's t-test		Significant?	Summary	P value
Ctl	SB	Yes	**	0.0079
Ctl	SB	Yes	*	0.0317
Figure 4K				
Student's t-test		Significant?	Summary	P value
Ctl	SB	Yes	**	0.0012
Ctl	SB	Yes	**	0.0095
Figure 4N				
Student's t-test		Significant?	Summary	P value
G12D	G12D:ex/ex	Yes	**	0.0079
Figure 5A				
Student's t-test		Significant?	Summary	P value
		sIL-6R		
WT	G12D	Yes	*	0.0334
WT	G12D:ex/ex	No	ns	0.3577
G12D	G12D:ex/ex	Yes	**	0.0016
		TNFα		
WT	G12D	No	ns	0.8481
WT	G12D:ex/ex	No	ns	0.4436
G12D	G12D:ex/ex	No	ns	0.4931
Figure 5E				
Student's t-test		Significant?	Summary	P value
Ctl	1F7	Yes	*	0.0357
Ctl	25F10	No	ns	0.1429
1F7	25F10	No	ns	0.1000
Figure 6B				
Student's t-test		Significant?	Summary	P value
Norm	LAC	Yes	*	0.0228
Figure 6C				
Student's t-test		Significant?	Summary	P value
WT	MUT	Yes	*	0.0228
WT	MUT	Yes	**	0.0056
Figure 6H				
Student's t-test		Significant?	Summary	P value
HBEC	A549	Yes	**	0.0012
HBEC	H23	Yes	**	0.0043
HBEC	H2228	Yes	**	0.0012
A549	H23	No	ns	0.1490

A549	H2228	Yes	**	0.0012
H23	H2228	Yes	**	0.0025

Figure 6I

Student's <i>t</i>-test		Significant?	Summary	P value
Ctl	KO	Yes	**	0.0043

Figure 6J

Student's <i>t</i>-test		Significant?	Summary	P value
Ctl	KO	Yes	*	0.0286

Figure 6K

Student's <i>t</i>-test		Significant?	Summary	P value
Ctl	KO	Yes	***	0.0006

Figure 6L

Multiple Student's <i>t</i>-test		Significant?	Summary	P value
(0)	Ctl	KO	No	ns
(3)	Ctl	KO	No	ns
(6)	Ctl	KO	Yes	*
(9)	Ctl	KO	Yes	*
(12)	Ctl	KO	Yes	*
(15)	Ctl	KO	Yes	*
(18)	Ctl	KO	Yes	*
(21)	Ctl	KO	Yes	*

Figure 6M

Student's <i>t</i>-test		Significant?	Summary	P value
Ctl	KO	Yes	**	0.0079

Figure 7B

Student's <i>t</i>-test		Significant?	Summary	P value
Ctl	A17pro	Yes	**	0.0057

Figure 7D

Student's <i>t</i>-test		Significant?	Summary	P value
Ctl	A17pro	Yes	**	0.0072

Figure 7F

Student's <i>t</i>-test		Significant?	Summary	P value
Ctl	A17pro	Yes	**	0.0022

Figure 7H

Student's <i>t</i>-test		Significant?	Summary	P value
Ctl	A17pro	Yes	*	0.0481

Figure 7I

Student's <i>t</i>-test		Significant?	Summary	P value
Ctl	A17pro	Yes	*	0.0411

Figure 7J

Multiple Student's <i>t</i>-test		Significant?	Summary	P value
(0)	Ctl	A17pro	No	ns
(2)	Ctl	A17pro	No	ns
(4)	Ctl	A17pro	No	ns
(6)	Ctl	A17pro	Yes	*
(8)	Ctl	A17pro	Yes	*
(10)	Ctl	A17pro	Yes	*
(12)	Ctl	A17pro	Yes	*
(14)	Ctl	A17pro	Yes	*

Figure 7K

Student's <i>t</i>-test		Significant?	Summary	P value
Ctl	A17pro	Yes	*	0.0411

Figure 7M

Student's <i>t</i>-test	Significant?	Summary	P value

Ctl	A17pro	Yes	*	0.0240
Figure EV1A				
Student's t-test G12D	G12D:ex/ex	Significant? Yes	Summary **	P value 0.0022
Figure EV1C				
Student's t-test G12D	G12D:ex/ex	Significant? Yes	Summary *	P value 0.0152
Figure EV1D				
Student's t-test G12D	G12D:ex/ex	Significant? Yes	Summary **	P value 0.0043
Figure EV1E				
Student's t-test G12D	G12D:ex/ex	Significant? Yes	Summary *	P value 0.0433
Figure EV1F				
Student's t-test G12D	G12D:ex/ex	Significant? Yes	Summary *	P value 0.0455
Figure EV2B				
Student's t-test WT	G12D	Significant? Yes	Summary **	P value 0.0022
WT	G12D:ex/ex	Significant? Yes	Summary **	P value 0.0043
G12D	G12D:ex/ex	Significant? Yes	Summary *	P value 0.0411
Figure EV2D				
Student's t-test WT	G12D	Significant? Yes	Summary **	P value 0.0022
WT	G12D:ex/ex	Significant? Yes	Summary *	P value 0.0411
G12D	G12D:ex/ex	Significant? No	Summary ns	P value 0.0931
Figure EV2F				
Student's t-test WT	G12D	Significant? Yes	Summary *	P value 0.0260
WT	G12D:ex/ex	Significant? No	Summary ns	P value 0.5887
G12D	G12D:ex/ex	Significant? Yes	Summary *	P value 0.0152
Figure EV2H				
Student's t-test WT	G12D	Significant? No	Summary ns	P value 0.1667
WT	G12D:ex/ex	Significant? No	Summary ns	P value 0.9069
G12D	G12D:ex/ex	Significant? No	Summary ns	P value 0.2900
Figure EV2I				
<i>IIIb</i>				
Student's t-test G12D	G12D:ex/ex	Significant? Yes	Summary **	P value 0.0032
<i>III7a</i>				
Student's t-test G12D	G12D:ex/ex	Significant? Yes	Summary *	P value 0.0353
<i>Il6</i>				
Student's t-test G12D	G12D:ex/ex	Significant? Yes	Summary *	P value 0.0489
Figure EV2K				
Student's t-test G12D	G12D:ex/ex	Significant? No	Summary ns	P value 0.3651
Figure EV2M				
Student's t-test G12D	G12D:ex/ex	Significant? No	Summary ns	P value 0.1688
Figure EV2N				
<i>Cxcl2</i>				

	Student's <i>t</i>-test	Significant?	Summary	P value
G12D	G12D:ex/ex	No	ns	0.1775
	<i>Vegf</i>			
	Student's <i>t</i>-test	Significant?	Summary	P value
G12D	G12D:ex/ex	No	ns	0.5887
	<i>Mmp2</i>			
	Student's <i>t</i>-test	Significant?	Summary	P value
G12D	G12D:ex/ex	No	ns	0.6623
Figure EV3C				
	Student's <i>t</i>-test	Significant?	Summary	P value
G12D	G12D:ex/ex	No	ns	0.0649
Figure EV3D				
	<i>Hes1</i>			
	Student's <i>t</i>-test	Significant?	Summary	P value
G12D	G12D:ex/ex	No	ns	0.1797
	<i>Hey1</i>			
	Student's <i>t</i>-test	Significant?	Summary	P value
G12D	G12D:ex/ex	No	ns	0.3095
Figure EV5A				
	Student's <i>t</i>-test	Significant?	Summary	P value
Ctl	KO	Yes	**	0.0012
Figure EV5B				
	Student's <i>t</i>-test	Significant?	Summary	P value
Ctl	KO	Yes	**	0.0043
Figure EV5C				
	Student's <i>t</i>-test	Significant?	Summary	P value
Ctl	KO	Yes	**	0.0022
Figure EV5D				
	Student's <i>t</i>-test	Significant?	Summary	P value
Ctl	A17pro	Yes	**	0.0022
Ctl	A17pro	Yes	***	0.0003
Figure EV5E				
	Student's <i>t</i>-test	Significant?	Summary	P value
Ctl	A17pro	Yes	*	0.0254
Ctl	A17pro	Yes	***	0.0007
Figure EV5F				
	Student's <i>t</i>-test	Significant?	Summary	P value
Ctl	A17pro	Yes	*	0.0424
Ctl	A17pro	Yes	**	0.0022
Figure EV5H				
	Student's <i>t</i>-test	Significant?	Summary	P value
Ctl	KO	Yes	*	0.0286
Ctl	GW	Yes	*	0.0286
Ctl	A17pro	Yes	*	0.0286
KO	GW	No	ns	0.2739
KO	A17pro	No	ns	0.7490
GW	A17pro	No	ns	0.1402
Figure EV5I				
	Student's <i>t</i>-test	Significant?	Summary	P value
Ctl	GW	Yes	*	0.0496
Ctl	GW	Yes	*	0.0295
Figure EV5J				
	Student's <i>t</i>-test	Significant?	Summary	P value
Ctl	GW	Yes	*	0.0466

Ctl	GW	Yes	*	0.0357
Figure EV5K				
	Student's t-test	Significant?	Summary	P value
Ctl	GW	Yes	**	0.0079
Ctl	GW	Yes	**	0.0079
Figure S1B				
	Student's t-test	Significant?	Summary	P value
G12D	G12D:ex/ex	Yes	**	0.0022
Figure S1D				
	Student's t-test	Significant?	Summary	P value
G12D	G12D:ex/ex	Yes	**	0.0022
Figure S1F				
	Student's t-test	Significant?	Summary	P value
G12D	G12D:ex/ex	Yes	**	0.0079
Figure S1H				
	Student's t-test	Significant?	Summary	P value
G12D	G12D:ex/ex	Yes	**	0.0079
Figure S2A				
	Student's t-test	Significant?	Summary	P value
WT	G12D	No	ns	0.7221
Figure S2C				
	Student's t-test	Significant?	Summary	P value
WT	G12D	No	ns	0.2046
WT	G12D	No	ns	0.9372
Figure S2D				
<i>Furin</i>				
	Student's t-test	Significant?	Summary	P value
WT	G12D	No	ns	0.5476
<i>Plk2</i>				
	Student's t-test	Significant?	Summary	P value
WT	G12D	No	ns	0.8413
<i>Rhbd1</i>				
	Student's t-test	Significant?	Summary	P value
WT	G12D	No	ns	>0.9999
<i>Rhbd2</i>				
	Student's t-test	Significant?	Summary	P value
WT	G12D	No	ns	0.4206
<i>P4hb</i>				
	Student's t-test	Significant?	Summary	P value
WT	G12D	No	ns	0.2783
<i>Timp3</i>				
	Student's t-test	Significant?	Summary	P value
WT	G12D	No	ns	0.3326
Figure S3B				
	Student's t-test	Significant?	Summary	P value
Ctl	A17pro	Yes	*	0.0133
Figure S3D				
	Student's t-test	Significant?	Summary	P value
Ctl	A17pro	No	ns	0.8468
Figure S3F				
	Student's t-test	Significant?	Summary	P value
Ctl	A17pro	No	ns	0.9496