

Supplementary Information

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TP53, CDKN2A/P16, and NFE2L2/NRF2 regulate the incidence of pure- and combined-small cell lung cancer in mice

Samera H. Hamad, Stephanie A. Montgomery, Jeremy M. Simon; Brittany M. Bowman, Kyle B. Spainhower, Ryan M. Murphy, Erik S. Knudsen, Suzanne E. Fenton, Scott H. Randell, Jeremiah R. Holt, D. Neil Hayes, Agnieszka K. Witkiewicz, Trudy G. Oliver, M. Ben Major* and Bernard E. Weissman*

***To whom the correspondence should be addressed**

M. Ben Major, PhD: Washington University in St. Louis, 660 Euclid Ave. Avenue Box 8228, St. Louis, MO 63110. Tel: 314-273-3675. Fax 314-362-7463 Email: bmajor@wustl.edu

Bernard E. Weissman, PhD: Lineberger Cancer Center, Room 31-322, University of North Carolina, Chapel Hill, NC, 27599-7295. Tel: 919-966-7533. Fax: 919-966-9673. Email: weissman@med.unc.edu

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Supplementary Table 1a. Tumors observed in experiment #1						
Genotype	ID	Sex	Lung	Brain	Liver	Other Tumors
<i>Trp53^{fl/fl};p16^{fl/fl};Nrf2^{+/+}</i> (n=19)	117	Female	ND	ND	ND	
	173	Female	ND	ND	ND	
	174	Female	ND	ND	ND	
	175	Female	ND	ND	ND	
	200	Female	ND	ND	ND	Salivary tumor
	201	Female	ND	ND	ND	
	202	Female	ND	ND	ND	
	224	Female	ND	ND	ND	
	170	Male	C-SCLC	ND	Lymphoproliferative	Lymphoma in Spleen
	111	Male	Poorly differentiated tumor	NT	NT	
	192	Male	Mets from PTT	ND	Mets from PTT	Parathyroid tumor (PTT); salivary tumor
	171	Male	ND	ND	ND	SCC from Ear
	199	Male	ND	ND	ND	Salivary tumor
	193	Male	ND	ND	ND	
	194	Male	ND	ND	ND	
	195	Male	ND	ND	ND	
	198	Male	ND	ONB	ND	
	222	Male	ND	ND	ND	
982	Male	ND	ND	ND		
<i>Trp53^{fl/fl};p16^{fl/fl};Nrf2^{E79Q/+}</i> (n=18)	294	Female	C-SCLC	ONB	Mets from lung	Mets in Lymph node
	218	Female	ND	ND	ND	Spindle cell sarcoma
	227	Female	ND	ONB	ND	
	226	Female	ND	ND	Lymphoma	Lymphoma in lymph node
	260	Female	ND	ND	ND	SCC from Ear
	261	Female	ND	ND	ND	
	180	Female	ND	ND	ND	
	181	Female	ND	ND	ND	
	217	Female	ND	ND	ND	
	296	Female	ND	ND	ND	
	987	Female	ND	ND	NT	
	291	Male	P-SCLC	ND	ND	
	114	Male	P-SCLC	ND	ND	
	179	Male	P-SCLC	ND	ND	
	223	Male	ND	ND	Hepatocellular adenoma	
	253	Male	ND	ND	ND	
	254	Male	ND	ONB	ND	
	302	Male	ND	ND	ND	

Tumors and benign lesions developed in *Trp53^{fl/fl};p16^{fl/fl};Nrf2^{+/+}* and *Trp53^{fl/fl};p16^{fl/fl};Nrf2^{E79Q/+}* mice from Experiment #1: ND: None detected ; C-SCLC: Combined Small Cell Lung Cancer; NT: No tissue; PTT: Parathyroid tumor; SCC: Squamous Cell Carcinoma; ONB: Olfactory Neuroblastoma; P-SCLC: Pure Small Cell Lung Cancer. The darker shaded rows indicate invasive lung tumors, while the lighter shade indicates other types of lung tumors.

Supplementary Table 1b. Tumors observed in experiment #2

Genotype	ID	Sex	Lung	Brain	Liver	Other Tumors
<i>Trp53^{fl/fl};p16^{fl/fl};Nrf2^{+/+}</i> (n=19)	444	Female	C-SCLC	ND	ND	
	445	Female	C-SCLC	ND	Mets from lung	
	451	Female	C-SCLC	ONB	ND	
	456	Female	C-SCLC	ONB	ND	
	446	Female	P-SCLC	ND	ND	SCC from Skin; Mets in lymph node
	448	Female	P-SCLC	ONB	ND	
	452	Female	Poorly differentiated tumor	NT	ND	Metastasis to diaphragm
	436	Female	ND	ONB	ND	
	455	Female	ND	ND	ND	
	457	Female	ND	ND	ND	
	432	Male	C-SCLC	ONB	ND	
	458	Male	C-SCLC	ND	ND	
	427	Male	P-SCLC	ND	ND	
	437	Male	P-SCLC	ONB	ND	
	440	Male	P-SCLC	ONB	ND	
	441	Male	ND	ONB	ND	
	431	Male	ND	ONB	ND	
	450	Male	ND	ND	ND	
	463	Male	ND	ONB	ND	
<i>Trp53^{fl/fl};p16^{fl/fl};Nrf2^{E79Q/+}</i> (n=19)	466	Female	C-SCLC	ONB	Mets from lung	ONB with local invasion
	467	Female	C-SCLC	ONB	Mets from lung	
	610	Female	C-SCLC	ND	ND	
	483	Female	P-SCLC	ONB	ND	
	454	Female	P-SCLC	ND	ND	
	609	Female	P-SCLC	ONB	ND	Salivary gland tumor
	449	Female	Tumor-Unknown Origin	ONB	ND	
	453	Female	ND	ONB	ND	
	447	Female	ND	ONB	ND	
	481	Male	P-SCLC	ND	ND	
	459	Male	C-SCLC	ND	ND	
	462	Male	C-SCLC	ND	ND	
	428	Male	C-SCLC	ND	Mets from lung	
	641	Male	C-SCLC+ P-SCLC	ONB	ND	Salivary gland tumor; lung lymph node metastasis of lung adenocarcinoma
	439	Male	P-SCLC	ND	ND	
	460	Male	P-SCLC	ND	ND	
	429	Male	P-SCLC	ND	ND	
	618	Male	P-SCLC	ND	ND	
	430	Male	Poorly differentiated neoplasia	ND	ND	Thymic Lymphoma

Tumors and benign lesions developed in *Trp53^{fl/fl};p16^{fl/fl};Nrf2^{+/+}* and *Trp53^{fl/fl};p16^{fl/fl};Nrf2^{E79Q/+}* mice from Experiment #2: C-SCLC: Combined Small Cell Lung Cancer; ND: None detected; ONB: Olfactory Neuroblastoma; P-SCLC: Pure Cell Lung Cancer; SCC: Squamous Cell Carcinoma; NT: No Tissue. The darker shaded rows indicate invasive lung tumors, while the lighter shade indicates other types of lung tumors.

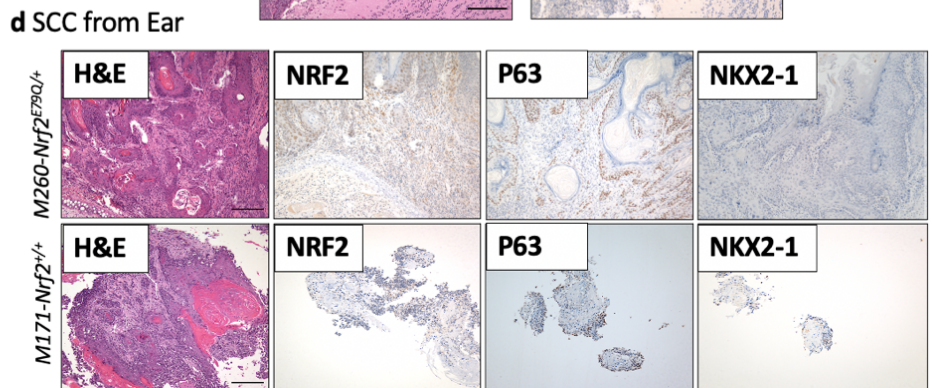
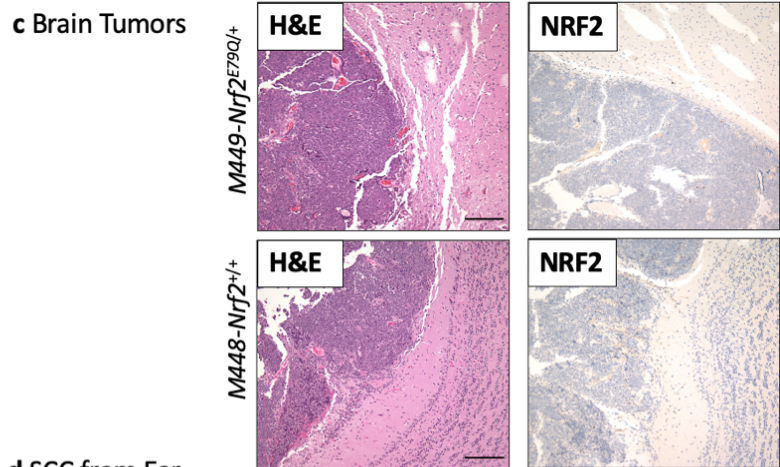
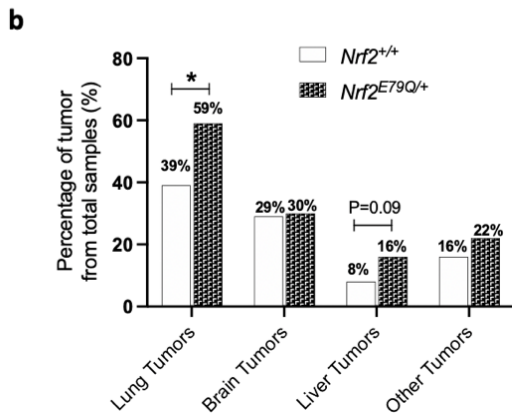
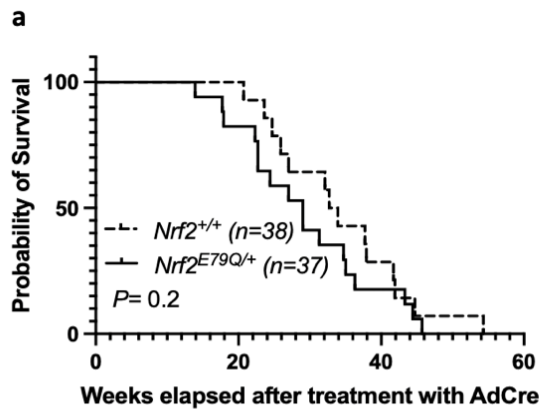
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Supplementary Table 2. Total numbers of pure small cell lung cancer (P-SCLC) found in mice used for serial sectioning of lung tissue			
Experimental group (number of mice used to sectioning their lung tissue)	Number of mice with P-SCLC	Total number of P-SCLC	Average of survival after treatment with Ad-CMV-Cre, Average \pm SD (Range) weeks
<i>Trp53^{fl/fl};p16^{fl/fl};Nrf2^{+/+}</i> (n=9)	4	27	33 \pm 15 (15 – 54)
<i>Trp53^{fl/fl};p16^{fl/fl};Nrf2^{E79Q/+}</i> (n=9)	8	127	24 \pm 5 (16 – 33)

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Supplementary Fig. 1 **a** Survival curve of $Trp53^{fl/fl};p16^{fl/fl};Nrf2^{+/+}$ versus $Trp53^{fl/fl};p16^{fl/fl};Nrf2^{E79Q/+}$ mice (all mice) after treatment with Ad-CMV-Cre; **b** Percentages of tumors developed by $Trp53^{fl/fl};p16^{fl/fl};Nrf2^{+/+}$ and $Trp53^{fl/fl};p16^{fl/fl};Nrf2^{E79Q/+}$ mice, * $P<0.05$; **c** H&E and NRF2 labeling of olfactory bulb neuroblastoma, scalebar=200 μ m; **d** H&E and representative IHC for NRF2, P63 and NKX2-1 of Squamous Cell Carcinoma (SCC) from ears in $Trp53^{fl/fl};p16^{fl/fl};Nrf2^{+/+}$ and $Trp53^{fl/fl};p16^{fl/fl};Nrf2^{E79Q/+}$ mice, scalebar=200 μ m. Images were taken using a BX61-Neville microscope.

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Positively Labelled Cells		Labelling Intensity	
Percent	Score	Intensity	Score
≤ 5%	0	Absent	0
6%-25%	1	Weak	1
26%-50%	2	Moderate	2
51%-75%	3	Strong	3
76%-100%	4		

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Supplementary Table 4a. IHC score* for markers in combined small cell lung cancer (C-SCLC) of *Trp53^{fl/fl};p16^{fl/fl};Nrf2^{E79Q/+}* and *Trp53^{fl/fl};p16^{fl/fl};Nrf2^{+/+}* mice; NT: No Tissue

Experimental Group	ID	NRF2		NKX2-1		P63		SPC		INSM1		Phospho-RB1	
		Positively Labelled Cells	Labelling Intensity	Positively Labelled Cells	Labelling Intensity	Positively Labelled Cells	Labelling Intensity	Positively Labelled Cells	Labelling Intensity	Positively Labelled Cells	Labelling Intensity	Positively Labelled Cells	Labelling Intensity
<i>Nrf2^{+/+}</i>	170	0	1	3	3	0	0	NT	NT	NT	NT	NT	NT
	432	0	0	3	3	0	0	NT	NT	NT	NT	NT	NT
	444	0	0	3	3	0	0	0	0	4	2	1	1
	445	0	0	3	3	0	0	0	0	4	2	1	3
	456	0	0	3	3	0	0	NT	NT	NT	NT	NT	NT
	458	0	0	3	3	0	0	0	0	4	3	1	2
<i>Nrf2^{E79Q/+}</i>	294	0	1	4	3	0	0	0	0	3	2	NT	NT
	428	0	0	3	3	0	0	0	0	3	2	2	3
	459	0	0	3	3	0	0	0	0	4	1	NT	NT
	462	0	0	4	2	0	0	NT	NT	NT	NT	NT	NT
	466	0	0	4	2	0	0	0	0	4	2	2	1
	467	0	0	4	2	0	0	NT	NT	NT	NT	NT	NT
	610	0	0	4	3	0	0	0	0	4	3	1	3
	641	0	0	4	3	0	0	0	0	4	3	NT	NT

*Scoring as shown in Supplementary Table 3

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Supplementary Table 4b. IF score* for markers of combined small cell lung cancer (C-SCLC) of *Trp53^{fl/fl};p16^{fl/fl};Nrf2^{E79Q/+}* and *Trp53^{fl/fl};p16^{fl/fl};Nrf2^{+/+}* mice.

Experimental Group	ID	ASCL1		SYP		CHG-A		Phospho-HH3	
		Positively Labelled Cells	Labelling Intensity	Positively Labelled Cells	Labelling Intensity	Positively Labelled Cells	Labelling Intensity	Positively Labelled Cells	Labelling Intensity
<i>Nrf2^{+/+}</i>	444	4	2	3	2	2	1	3	3
	445	3	2	2	1	1	1	3	3
	458	4	3	4	2	1	1	4	3
<i>Nrf2^{E79Q/+}</i>	428	4	3	3	3	3	2	4	3
	466	4	3	3	2	2	1	4	3
	610	4	3	4	3	3	2	4	3

*Scoring as shown in Supplementary Table 3

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Supplementary Table 5a. IHC score* of markers of pure small cell lung cancer (P-SCLC) of *Trp53^{fl/fl};p16^{fl/fl};Nrf2^{E79Q/+}* and *Trp53^{fl/fl};p16^{fl/fl};Nrf2^{+/+}* mice; NT: No Tissue

Experimental Group	ID	NRF2		P63		NKX2-1		CGRP		Phospho-RB1		
		Positively Labelled Cells	Labelling Intensity	Positively Labelled Cells	Positively Labelled Cells	Positively Labelled Cells	Labelling Intensity	Positively Labelled Cells	Labelling Intensity	Positively Labelled Cells	Labelling Intensity	
<i>Nrf2^{+/+}</i>	427	0	0	0	3	3	3	3	3	3	1	2
<i>Nrf2^{E79Q/+}</i>	114	3	2	NT	NT	NT	NT	NT	NT	NT	NT	NT
	429	2	2	2	4	4	3	3	3	NT	NT	NT
	439	3	2	0	3	3	3	4	3	NT	NT	NT
	460	0	1	0	4	4	3	4	3	NT	NT	NT
	483	4	1	0	4	4	3	3	3	4	2	2
	618	0	1	0	3	3	3	4	3	4	2	2
	641	2	2	1	NT	NT	3	NT	NT	NT	NT	NT

*Scoring as shown in Supplementary Table 3

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Supplementary Table 5b. IF score* of markers of pure small cell lung cancer (P-SCLC) of *Trp53^{fl/fl};p16^{fl/fl};Nrf2^{E79Q/+}* and *Trp53^{fl/fl};p16^{fl/fl};Nrf2^{+/+}* mice.

Experimental Group	ID	ASCL1		SYP		Phospho-HH3	
		Positively Labelled Cells	Labelling Intensity	Positively Labelled Cells	Labelling Intensity	Positively Labelled Cells	Labelling Intensity
<i>Nrf2^{+/+}</i>	427	4	3	3	2	1	3
<i>Nrf2^{E79Q/+}</i>	483	4	3	2	1	1	2
	618	4	3	2	1	1	3

*Scoring as shown in Supplementary Table 3

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