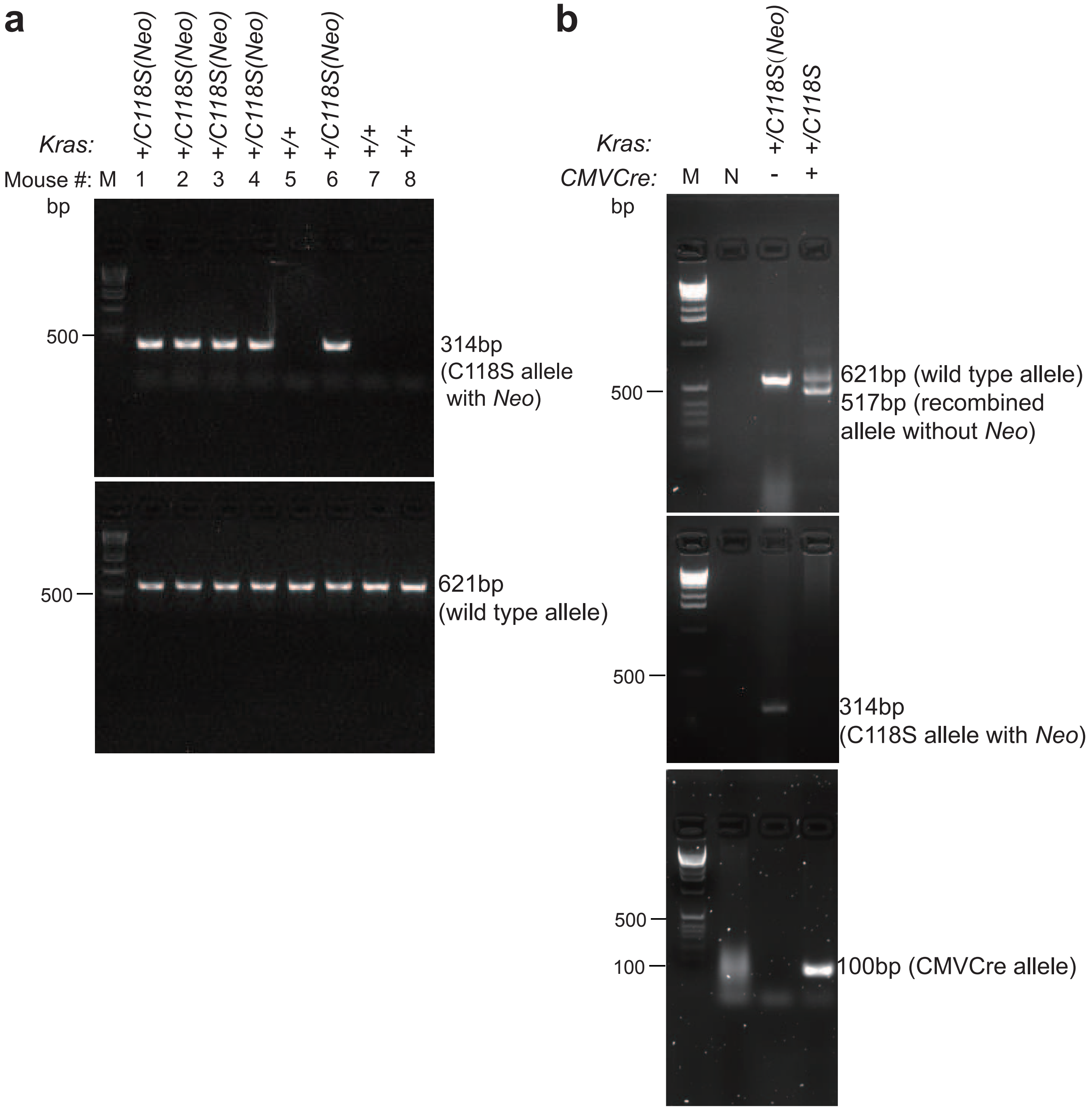
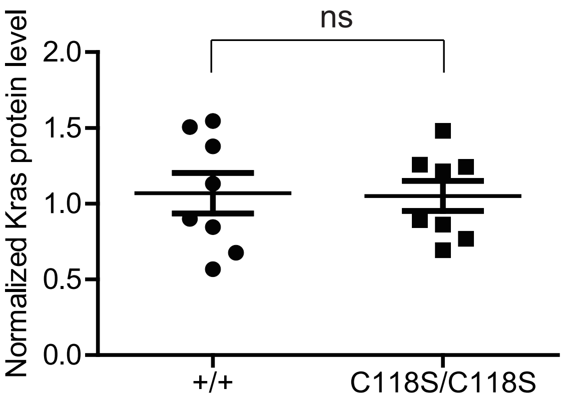
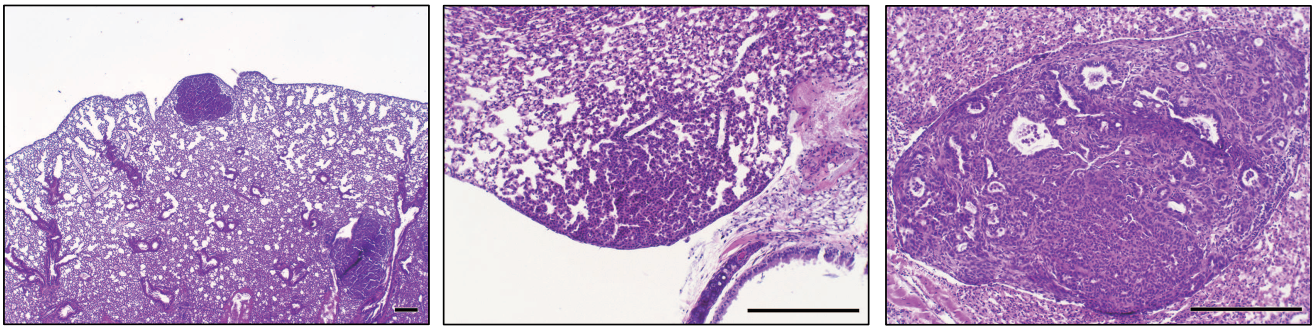


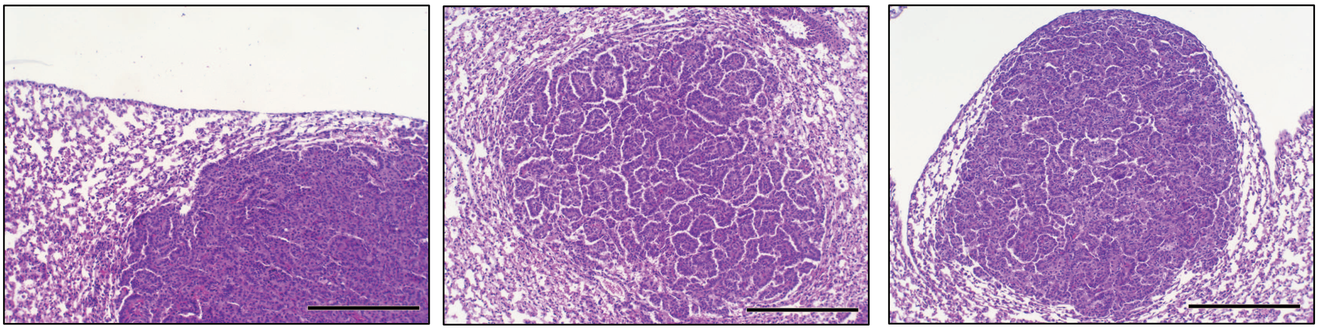
Supplementary Figure 1 | Identification of successful excision of the *Neo* cassette in the *Kras*^{C118S} allele. (**a**) Full-length gels of PCR amplification of genomic DNA from eight mice (numbered from 1 to 8) from the breeding of a *Kras*$^{+/C118S(Neo)}$ mouse and a *Kras*$^{+/+}$ (BL6) mouse using the primer pair P3+P4 to amplify a 314 bp product specific to the *Kras*$^{C118S(Neo)}$ allele (upper panel) or using the primer pair P3+P5 to amplify a 621 bp product specific to the wild type (+) *Kras* allele (lower panel), identifying the genotype as *Kras*$^{+/C118S(Neo)}$ or *Kras*$^{+/+}$ (labeled on top). (**b**) Full-length gels of PCR amplification of genomic DNA from a *Kras*$^{+/C118S(Neo)}$ mouse without transgenic *CMVCre* and a *Kras*$^{+/C118S}$ mouse with transgenic *CMVCre* using the primer pair P3+P5 to amplify a 621 bp product specific to the wild type (+) *Kras* allele and/or a 517 bp product specific to the *Kras*^{C118S} allele after successful excision of the *Neo* cassette (upper panel), using the primer pair P3+P4 to amplify a 314 bp product specific to the *Kras*$^{C118S(Neo)}$ allele with the *Neo* cassette (middle panel), or using the *CMVCre* genotyping primer pair P16+P17 to amplify a 100 bp product specific to *CMVCre* (lower panel). M: 1kb marker; N: negative (no DNA) control. All the primers are listed in Supplementary Table 4.



Supplementary Figure 2 | Similar Kras protein levels in lung tissue isolated from *Kras*$^{+/+}$ and *Kras*$^{C118S/C118S}$ mice. Quantification of Kras protein levels (relative to tubulin and actin) from immunoblot of lysates derived from lung tissue of eight *Kras*$^{+/+}$ (+/+) and eight *Kras*$^{C118S/C118S}$ (C118S/C118S) mice. Bars: Mean ± SEM. ns: non-significant (P>0.05), as determined by two-tailed unpaired Student's t test.

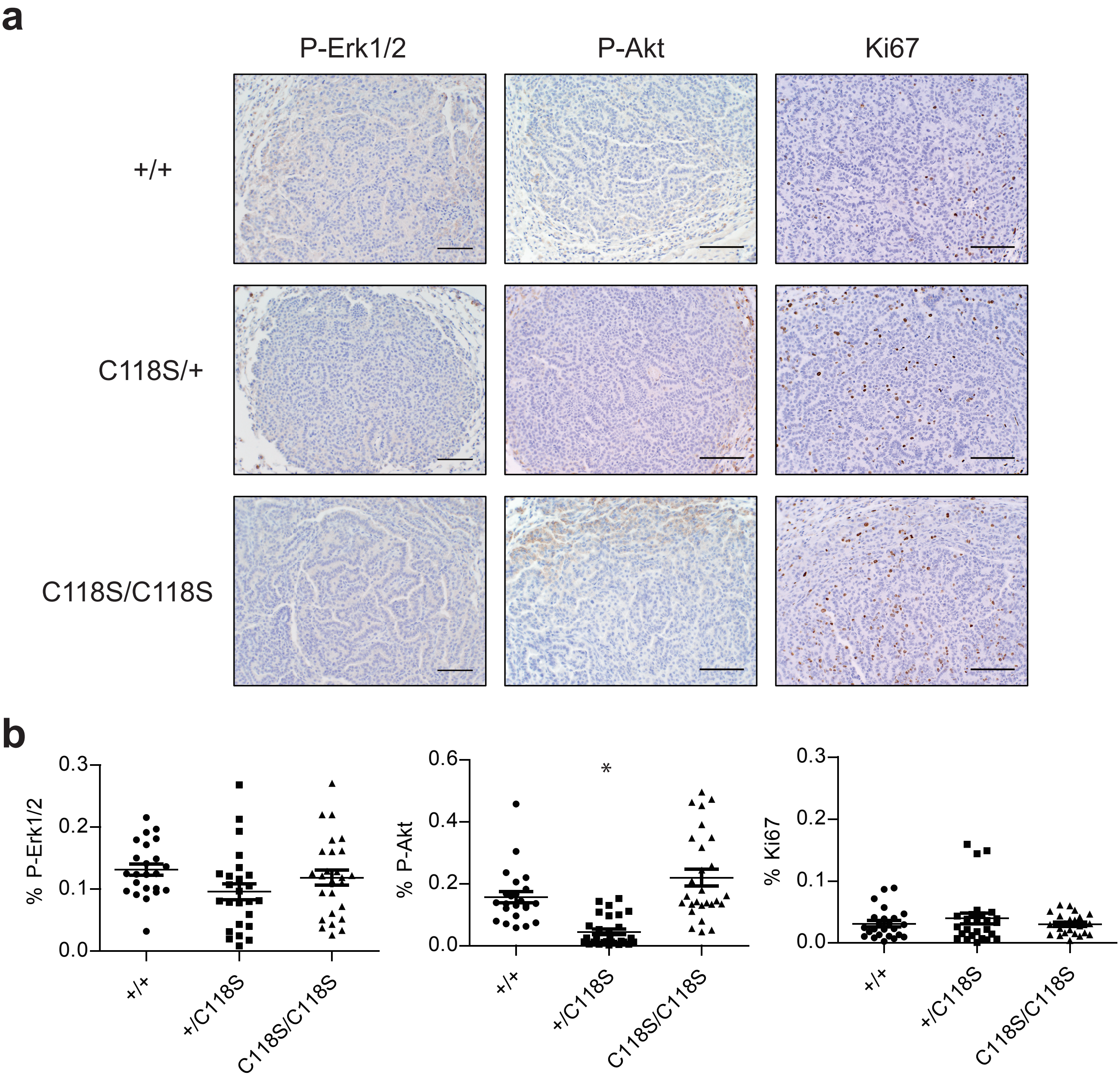


adenoma (2X) AAH (10X) adenocarcinoma (10X)

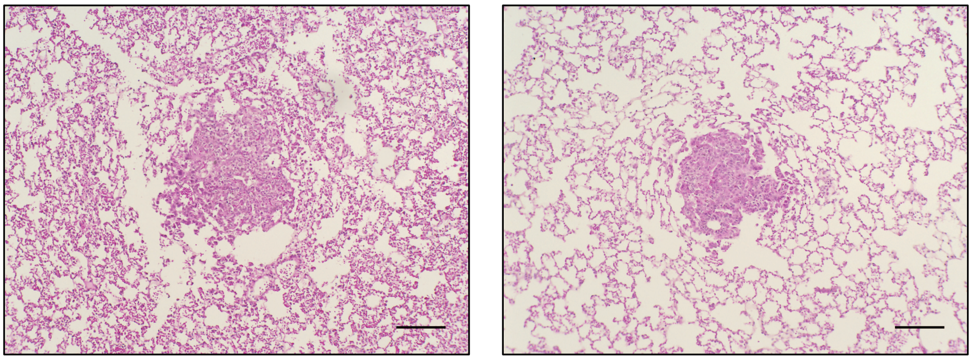


solid adenoma (10X) papillary adenoma (10X) solid/papillary adenoma (10X)

Supplementary Figure 3 | Representative images of different types of tumors detected in H&E-stained lung sections from urethane-treated mice. Scale bar: 300 µm. 2X or 10 X: magnification.

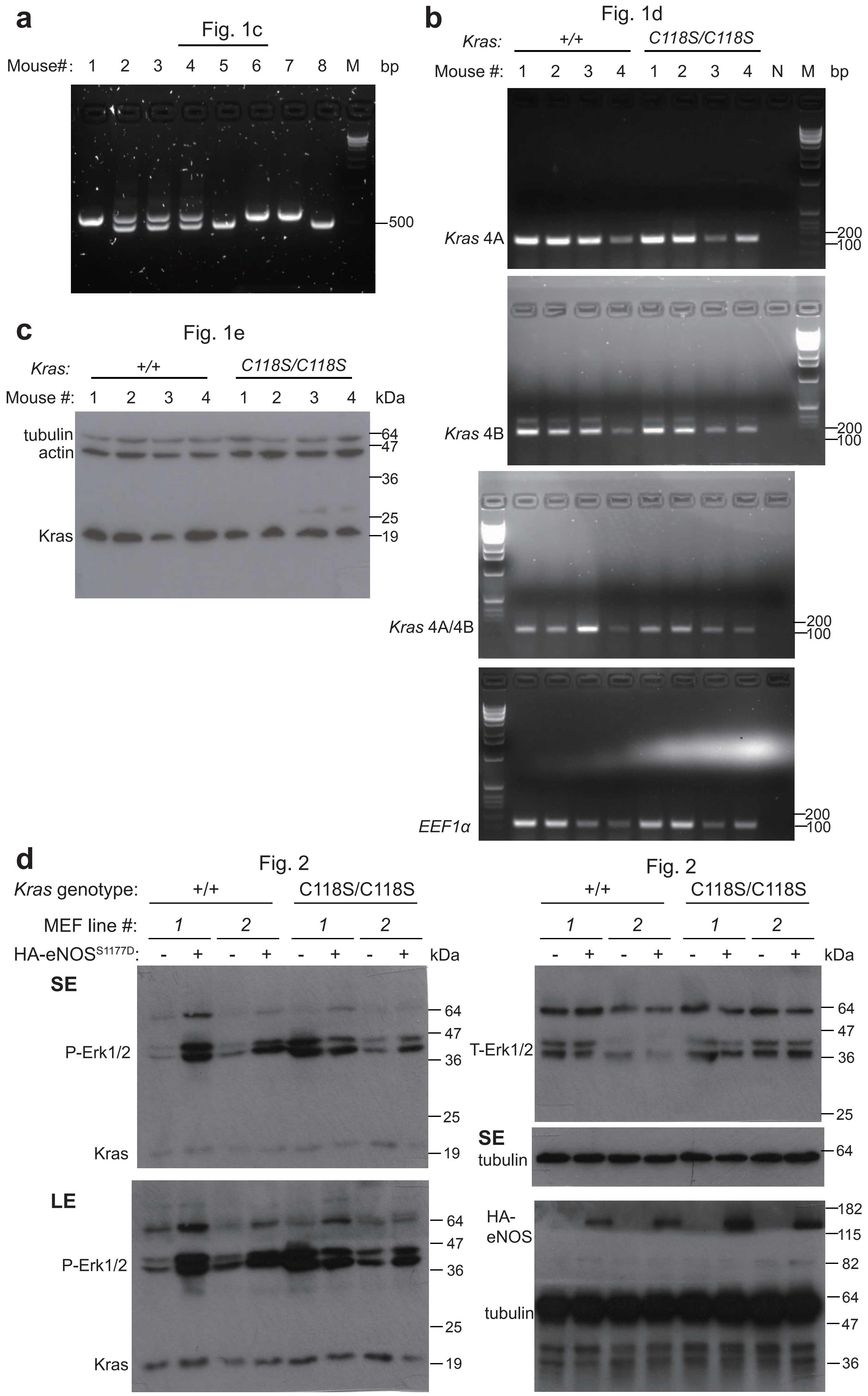


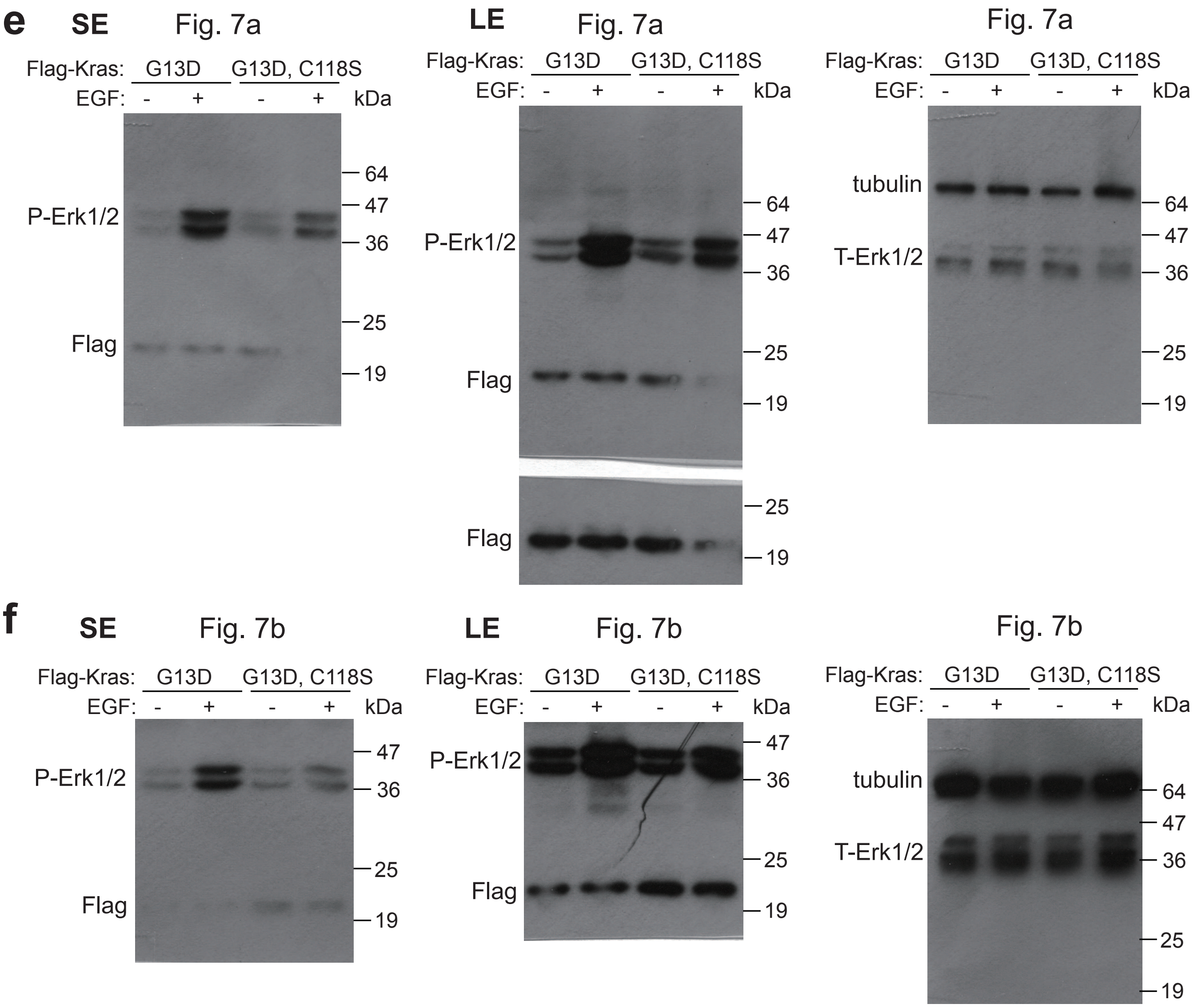
Supplementary Figure 4 | P-Erk1/2, P-Akt, and Ki67 immunohistochemical analysis of lung tumors. (**a**) Representative photograph (scale bar: 100 μm) and (**b**) quantification of the percent positive staining for P-Erk1/2, P-Akt, or Ki67 per tumor area (bars: mean ± SEM) from four to six high-power (20X) random fields of lung sections from five mice from each of the *Kras*$^{+/+}$ (+/+), *Kras*$^{+/C118S}$ (+/C118S), and *Kras*$^{C118S/C118S}$ (C118S/C118S) cohorts. No significant difference was noted except in one case (*: P<0.001 between C118S/+ versus +/+ or C118S/C118S).



Kras$^{LSL\text{-}G12D/+}$ *Kras*$^{LSL\text{-}G12D/C118S}$

Supplementary Figure 5 | A representative image of an H&E stained lung section from an AdCre-treated *Kras*$^{LSL\text{-}G12D/+}$ or *Kras*$^{LSL\text{-}G12D/C118S}$ mouse at 4X magnification. Scale bar: 300 µm.


a
Fig. 1c
Mouse#: 1 2 3 4 5 6 7 8 M bp
500
b
Fig. 1d
Kras: +/+ C118S/C118S
Mouse #: 1 2 3 4 1 2 3 4 N M bp
Kras 4A
200
100
Kras 4B
200
100
Kras 4A/4B
200
100
EEF1α
200
100
c
Fig. 1e
Kras: +/+ C118S/C118S
Mouse #: 1 2 3 4 1 2 3 4 kDa
tubulin
actin
Kras
64
47
36
25
19
d
Fig. 2
Kras genotype: +/+ C118S/C118S
MEF line #: 1 2 1 2
HA-eNOS^S1177D: - + - + - + - + kDa
SE
P-Erk1/2
Kras
64
47
36
25
19
LE
P-Erk1/2
Kras
64
47
36
25
19
Fig. 2
+/+ C118S/C118S
1 2 1 2
- + - + - + - + kDa
T-Erk1/2
64
47
36
25
SE
tubulin
64
HA-eNOS
tubulin
182
115
82
64
47
36



Supplementary Figure 6 | Full-length gels and blots for figures. Full-length gels and blots for (**a**) Fig. 1c, (**b**) Fig. 1d, (**c**) Fig. 1e, (**d**) Fig. 2, (**e**) Fig. 7a, (**f**) Fig. 7b. M: Marker; N: negative control; SE: short exposure; LE: long exposure.

Supplementary Table 1. Histological characterization of lung tumors from urethane-treated mice.

***Kras* genotype**	**Number of lung sections**	**Tumor incidence**	**Mean ± SEM tumors per section**	**Total number of tumors**	**Tumor incidence by classification**		
					AAH	AD	AC
+/+	27	85.2%	2.96 ± 0.44	80	11.3%	85.0%	3.8%
+/C118S	25	80.0%	2.28 ± 0.326	57	14.0%	84.2%	1.8%
C118S/C118S	25	80.0%	2.00 ± 0.44	50	16.0%	82.0%	2.0%

Supplementary Table 2. Subtypes of lung adenomas from urethane-treated mice.

***Kras* genotype**	**Total number of adenomas**	**Adenoma incidence by classification**		
		Solid	Papillary	Solid/ Papillary
+/+	68	39.7%	16.2%	44.1%
+/C118S	48	37.5%	4.2%	58.3%
C118S/C118S	41	43.9%	22.0%	34.2%

Supplementary Table 3. ***Kras* sequencing analysis from lung tumors of urethane-treated *Kras*$^{+/C118S}$ mice.**

Q61 mutation	**Total**	**Q61L**	**Q61R**
Present on native *Kras* allele	20	8	12
Present on C118S *Kras* allele	12	0	12
Present on both *Kras* alleles	3	1	2
Absent from both *Kras* alleles	21		
Only one *Kras* alleles recovered	9		
Total tumors analyzed	65	9	26

Supplementary Table 4. PCR primers.

Primer	**Sequence**
P1 (*Kras* RT-PCR F)	atgactgaatataaacttgtgg
P2 (*Kras* RT-PCR R)	ttacataattacacactttgtc
P3 (*Kras*^{C118S} genotyping F)	agaacaaattaaaagagtaaaggac
P4 (*Kras*^{C118S} genotyping R-targeting vector)	ccaagctagcttggctggacgtaaa
P5 (*Kras*^{C118S} genotyping R-*Kras* genomic)	atgtaaaatgtactctagacggaac
P6 (343F *Kras* RT-PCR)	tatagggcgaattggagctcatgactgagtataaacttgtg
P7 (343R *Kras* RT-PCR)	cggtatcgataagctttcacataactgtacaccttgtc
P8 (*Kras* 4A RT-PCR F)	gggctttctttgtgtatttgc
P9 (*Kras* 4A RT-PCR R)	caatgtataaaaagcatcctcca
P10 (*Kras* 4B RT-PCR F)	tgcaatgagggaccagtaca
P11 (*Kras* 4B RT-PCR R)	tagaaggcatcgtcaacacc
P12 (*Kras* 4A/4B RT-PCR F)	acttgtggtggttggagctg
P13 (*Kras* 4A/4B RT-PCR R)	ccctccccagttctcatgta
P14 (*EEF1α* RT-PCR F)	ggattgccacacggctcacatt
P15 (*EEF1α* RT-PCR R)	ggtggatagtctgagaagctctc
P16 (CMV-Cre genotyping F)	gcggtctggcagtaaaaactatc
P17 (CMV-Cre genotyping R)	gtgaaacagcattgctgtcactt