Supplementary Data

Supplemental Figure 1: *Arf* loss accelerates disease. [A] *Arf* $^{-/-}$ mice exhibited significantly reduced overall survival after urethane injection (logrank test for trend, $P < 0.0001$). **[B]** *Arf* $^{-/-}$ animals developed larger lung tumors than their *Arf* $^{+/-}$ and *Arf* $^{+/+}$ littermates (**** $P < 0.0001$). Analysis was performed on the same dataset as Fig. 1D, but tumors were plotted individually and statistical comparisons between genotypes were performed using the Mann Whitney test.

Supplemental Figure 2: Expression of the cell proliferation marker Ki67. Although not reaching statistical significance, adenocarcinomas from *Arf* $^{-/-}$ animals displayed a clear trend toward increased Ki67 staining compared to *Arf* $^{+/+}$ ($P = 0.0831$; $n = 40$ *Arf* $^{+/+}$ and 96 *Arf* $^{-/-}$ AC fields counted).

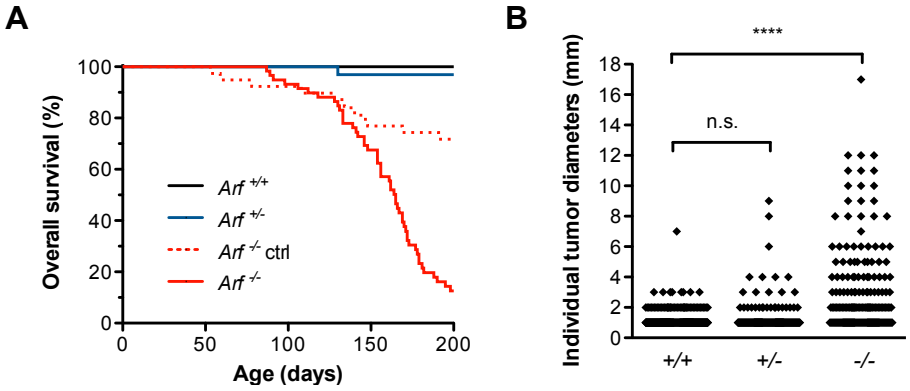
Supplemental Figure 3: Absence of apoptosis in tumors of all genotypes. Cleaved caspase 3 staining of adenomas and adenocarcinomas from *Arf* $^{+/+}$ and *Arf* $^{-/-}$ mice. All fields examined were largely or entirely negative for immunoreactivity. A lung adenocarcinoma from an irradiated (4Gy/4hr) *Arf* $^{-/-}$ mouse serves as positive control. Arrowheads identify CC3-positive cells. Scale bars 100 μm.

Supplemental Figure 4: Activation of the RAS pathway in urethane-induced tumors. [A] The codon 61 glutamine (Q) residue of *Kras* in both *Arf* $^{+/+}$ and *Arf* $^{-/-}$ lung tumors was mutated to leucine (L) or arginine (R). The glycine (G) 12 and 13 amino acids were unaffected ($n = 4$ each genotype). **[B]** Relative intensities of phosphorylated versus total ERK1/2 in nuclear and cytoplasmic protein fractions from adenocarcinomas of both genotypes, as shown in Figure 6A.

Nuclear phosphorylated ERK1/2 levels appear to be increased in *Arf* $^{-/-}$ adenocarcinomas compared to *Arf* $^{+/+}$, although the difference does not reach statistical significance (P = 0.1892 for nuclear fraction, P = 0.3089 for cytoplasmic fraction). **[C]** IHC reveals predominantly nuclear localization of phospho-ERK1/2 protein in tumors from *Arf* $^{-/-}$ animals. In contrast, tumors isolated from wild-type animals exhibited cytoplasmic phospho-ERK1/2 expression. Scale bars 100 μm.

Supplemental Figure 5: ARF-p53 signaling pathway in lung tumors. **[A]** ARF protein was undetectable by IHC in normal lung bronchioles, alveolar spaces, and stroma. Scale bars 100 μm. **[B]** ARF and p21 co-localized in lung tumors from *Arf* $^{+/+}$ mice (top), but staining for phospho-histone H2A.X was largely negative. In *Arf* $^{-/-}$ tumors (bottom), p21 staining correlated with H2AX^{+} tumor regions. Insets show magnified, matched regions of staining. Scale bars 100 μm. **[C]** Quantitative RT-PCR identified decreased *Mdm2* expression in *Arf*-deficient lung adenocarcinomas compared to wild-type (n = 6 samples each genotype; * P = 0.0241). **[D]** Quantitative RT-PCR found no significant difference in *Bax* expression in *Arf*-deficient lung adenocarcinomas compared to wild-type (n = 5 samples each genotype; P = 0.0914). **[E]** All tumors examined were negative for phosphorylated p53 (Ser15) expression by Western blot. Irradiated spleen was used as positive control, and β-actin was used as loading control. The arrow indicates β-actin band.

Busch_SuppFig1



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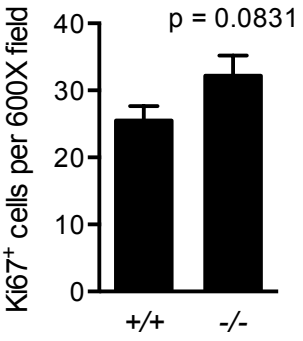

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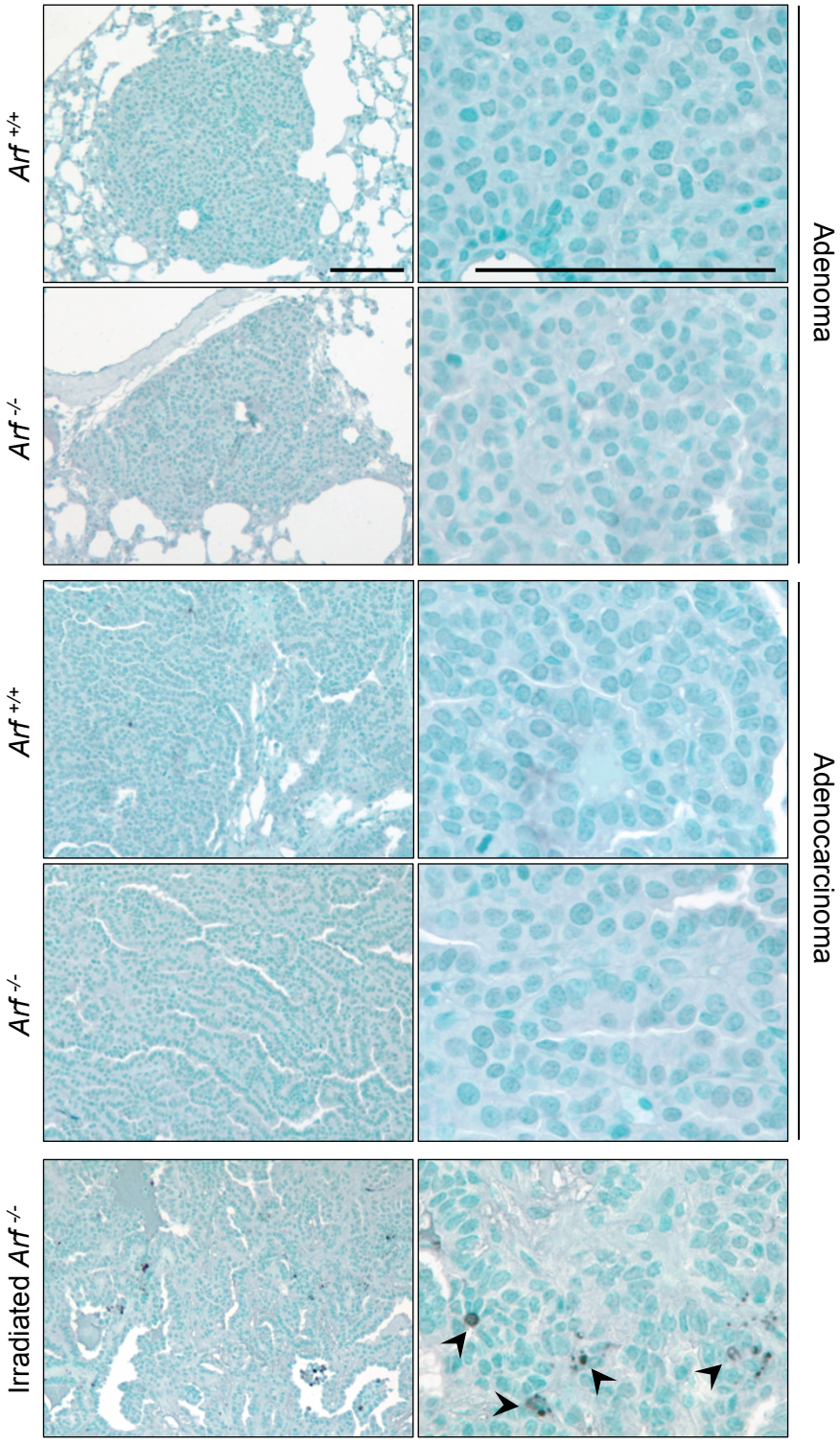

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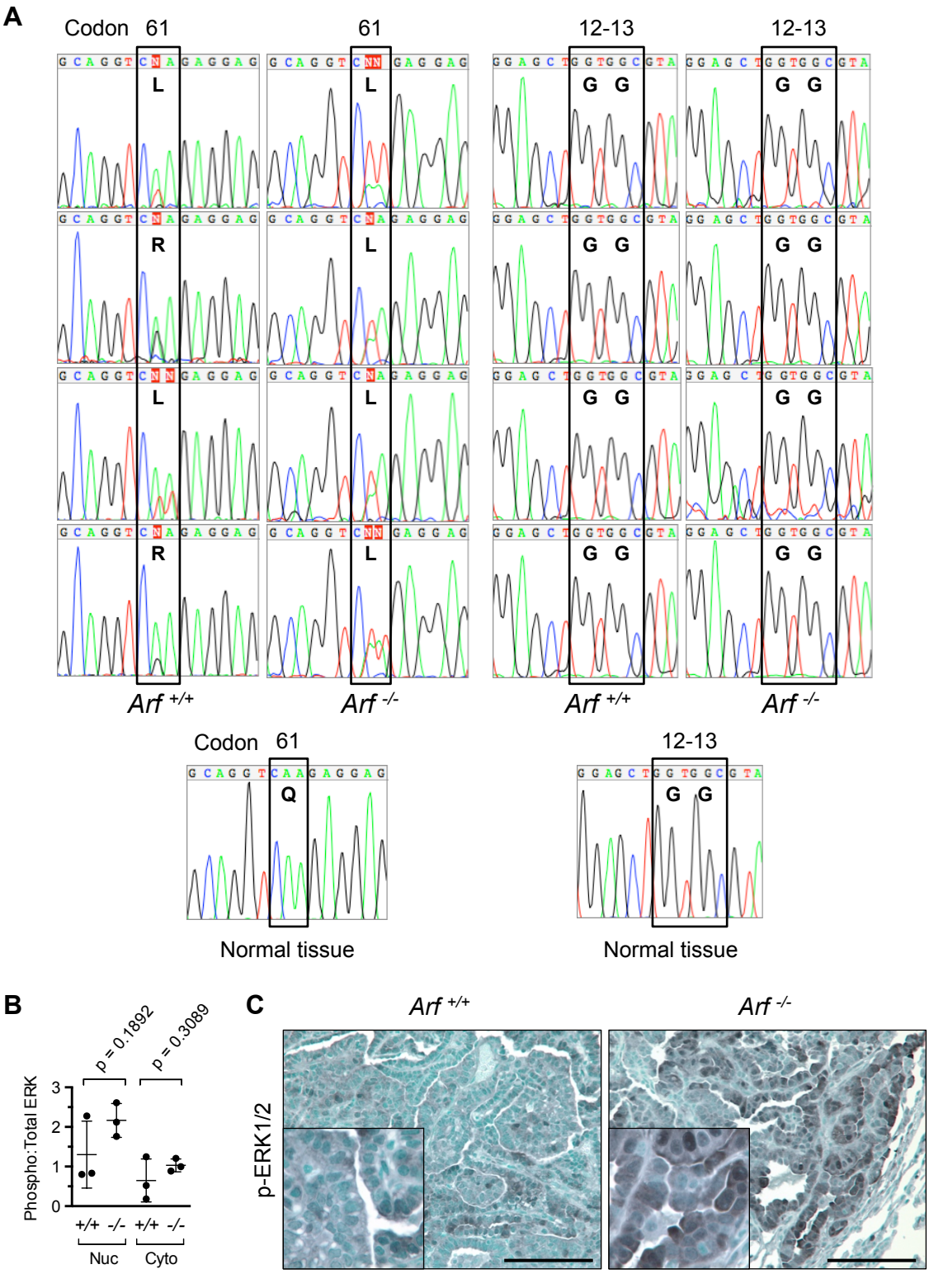

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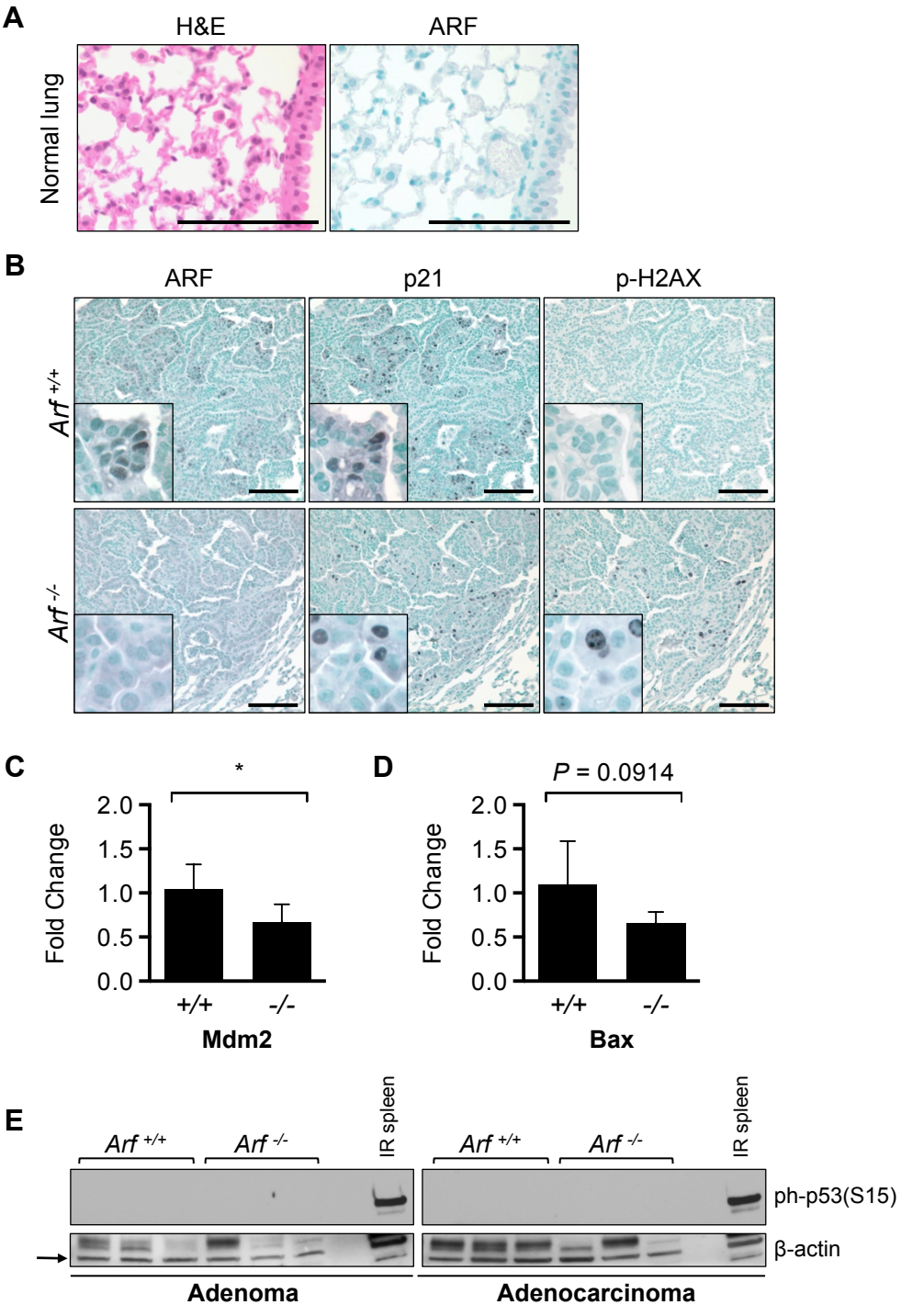

Supplemental Table 1 - Mutations in *Trp53* in Urethane-induced Lung Adenocarcinomas[1]

ID	Genotype	Tissue	*Trp53* mutation	Amino Acid Change	Type of Mutation	Region	Functional Domain
1	*Arf*$^{+/+}$	AC	CTG -> CCG (558bp)	L134P	Missense	Exon 5	DNA Binding
2	*Arf*$^{+/+}$	AC	NM[2]				
3	*Arf*$^{+/+}$	AC	TGC -> TGT (979bp)	C274C	Synonymous	Exon 8	--
4	*Arf*$^{+/+}$	AC	NM				
5	*Arf*$^{+/+}$	AC	NM				
6	*Arf*$^{+/+}$	AC	NM				
7	*Arf*$^{+/+}$	AC	CAG -> CGG (723bp)	Q189R	Missense	Exon 6	DNA Binding
8	*Arf*$^{+/+}$	AC	ACC -> GCC (1133bp)	N326A	Missense	Exon 9	DNA Binding
9	*Arf*$^{-/-}$	AC	NM				
10	*Arf*$^{-/-}$	AC	CCT -> CCC (358bp)	P67P	Synonymous	Exon 4	--
11	*Arf*$^{-/-}$	AC	A (453bp)	Stop codon at 129	Frameshift	Exon 4	Transactivation
12	*Arf*$^{-/-}$	AC	AGC ->AAC (612bp)	S152N	Missense	Exon 5	DNA Binding
13	*Arf*$^{-/-}$	AC	NM				
14	*Arf*$^{-/-}$	AC	NM				
15	*Arf*$^{-/-}$	AC	CAG ->> CGG (180bp)	Q8R	Missense	Exon 2	Transactivation
16	*Arf*$^{-/-}$	AC	NM				
NL	*Arf*$^{+/+}$	Lung	NM				
NL	*Arf*$^{-/-}$	Lung	NM				

[1] Sequences aligned to *Trp53* transcript VEGA OTTMUST00000013379 (1772bp) - CCDS36193.1 1173nt (158-1330bp); [Exon2-11]; 390 amino acids.

[2] NM, no mutation