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Supplementary Information for

AGO2 promotes tumor progression in KRAS-driven mouse models of non-small cell lung cancer

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Figures S1 to S6

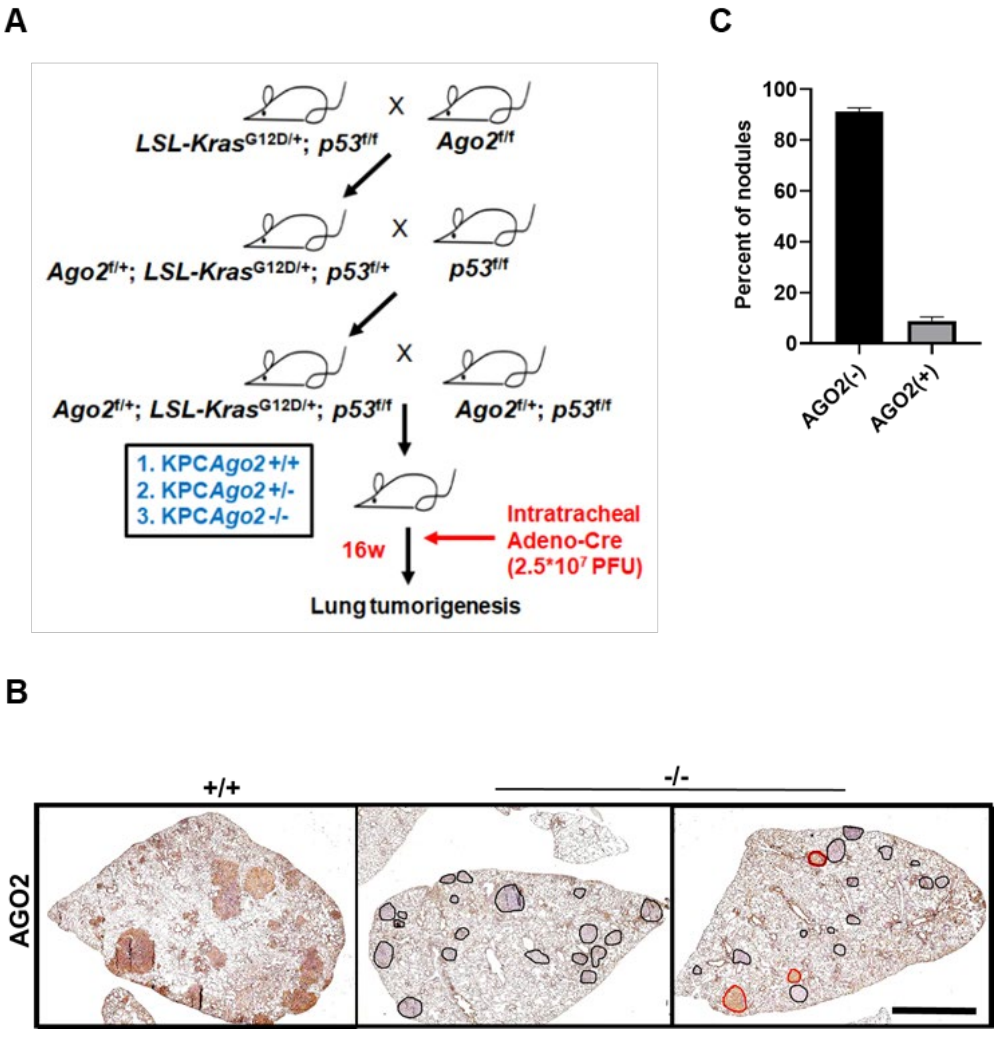


Fig. S1. (A) Breeding scheme employed in generation of *KPC-Ago2-/-* mice. **(B)** Immunohistochemistry for AGO2 in lung nodules of *KPC-Ago2+/+* (+/+) and *KPC-Ago2-/-* (-/-) mice. In -/- images, black circles indicate AGO2(-) nodules, red circles indicate AGO2(+) nodules. Scale 2.5 mm. **(C)** Percentage of AGO2(-) and AGO2(+) nodules in lung cross sections of *KPC-Ago2-/-* mice.

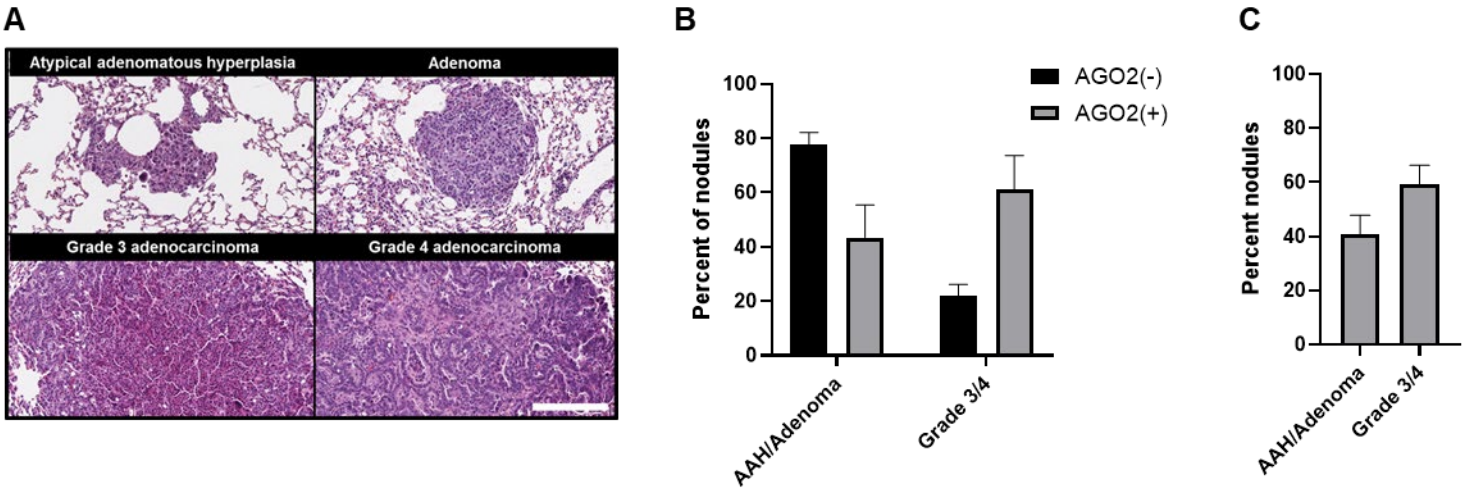


Fig. S2. (A) Pathological grading of lung lesions in *KPC-Ago2+/+* mice. H/E staining. Scale 200 µm. **(B)** Percentage of AGO2(-) and AGO2(+) nodules from lung cross sections of *KPC-Ago2-/-* mice pathologically characterized as (1) atypical adenomatous hyperplasia (AAH)/ adenoma hyperplasia pathology versus (2) grade 3/ grade 4 adenocarcinoma. **(C)** Percentage of all nodules from *KPC-Ago2+/+* mice pathologically characterized as 1) atypical adenomatous hyperplasia (AAH)/ adenoma hyperplasia pathology, and (2) grade 3/ grade 4 adenocarcinoma.

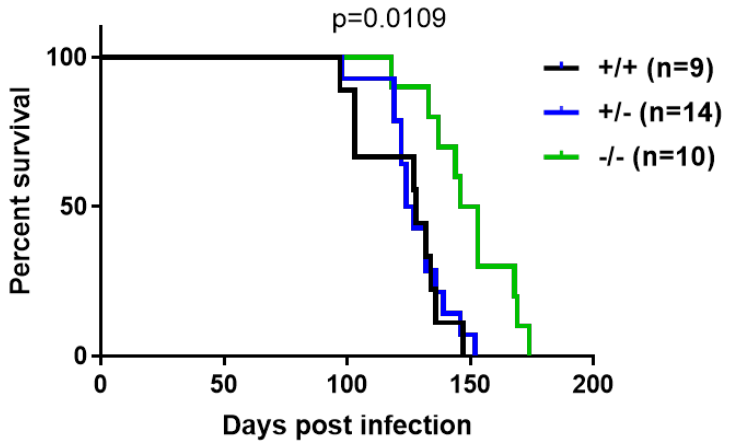
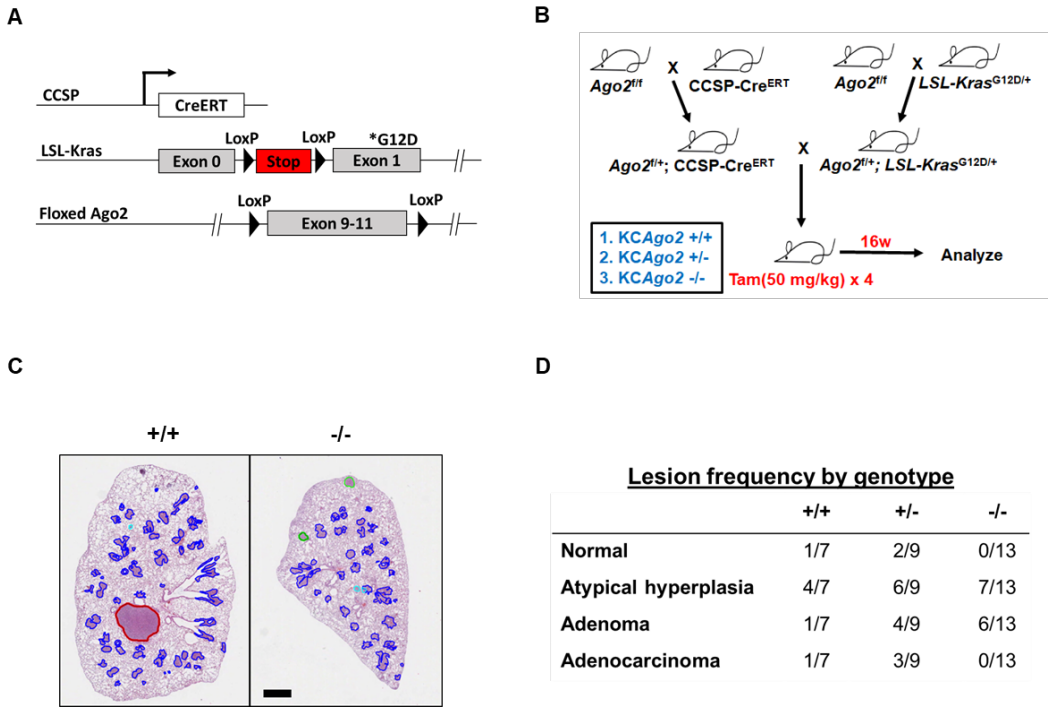


Fig. S3. Kaplan-Meier plot demonstrating improved survival in *KPC-Ago2-/-* (-/-) mice (median 149.5 days) versus *KPC-Ago2+/+* (+/+) (median 128.0 days) and *KPC-Ago2+/-* (+/-) mice (median 125.5 days).



Lesion frequency by genotype

	+/+	+/-	-/-
Normal	1/7	2/9	0/13
Atypical hyperplasia	4/7	6/9	7/13
Adenoma	1/7	4/9	6/13
Adenocarcinoma	1/7	3/9	0/13

Fig S4. CCSP Cre-driven KC lung adenocarcinoma model. (A) Diagram showing activation of *KrasG12D* and ablation of *Ago2* via the *CCSP* Cre (expressed in Clara cells). **(B)** Breeding scheme for generation of *KC* mice with wild-type (+/+), heterozygous knockout (+/-) or homozygous knockout (-/-) of *Ago2*. **(C)** H/E stained lung cross sections from (+/+) and (-/-) mice with lesion types indicated as follows: Adenocarcinoma (red), adenoma (green), atypical hyperplasia (blue). Scale 1 cm) **(D)** Fraction of total mice in which any of the indicated lesions were observable in lung cross sections (3 non-consecutive sections evaluated in each mouse).

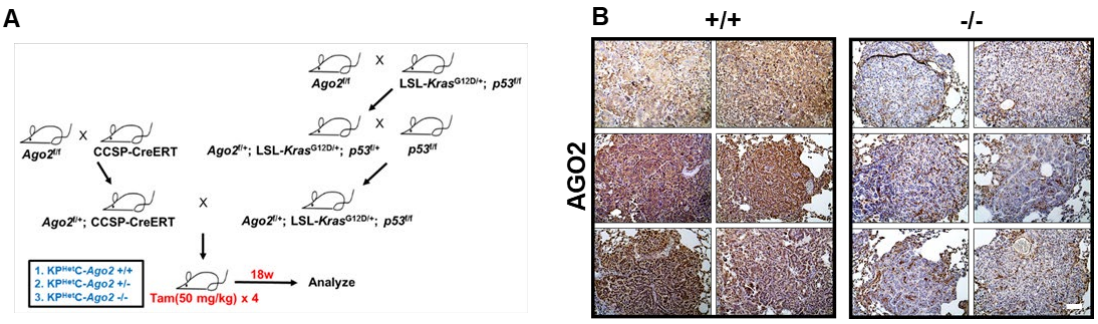


Fig S5. (A) *KP^{Het}C* breeding scheme **(B)** AGO2 staining in lung nodules of *KP^{Het}C-Ago2+/+* (+/+) and *KP^{Het}C-Ago2-/-* (-/-) mice. Scale 100μm.

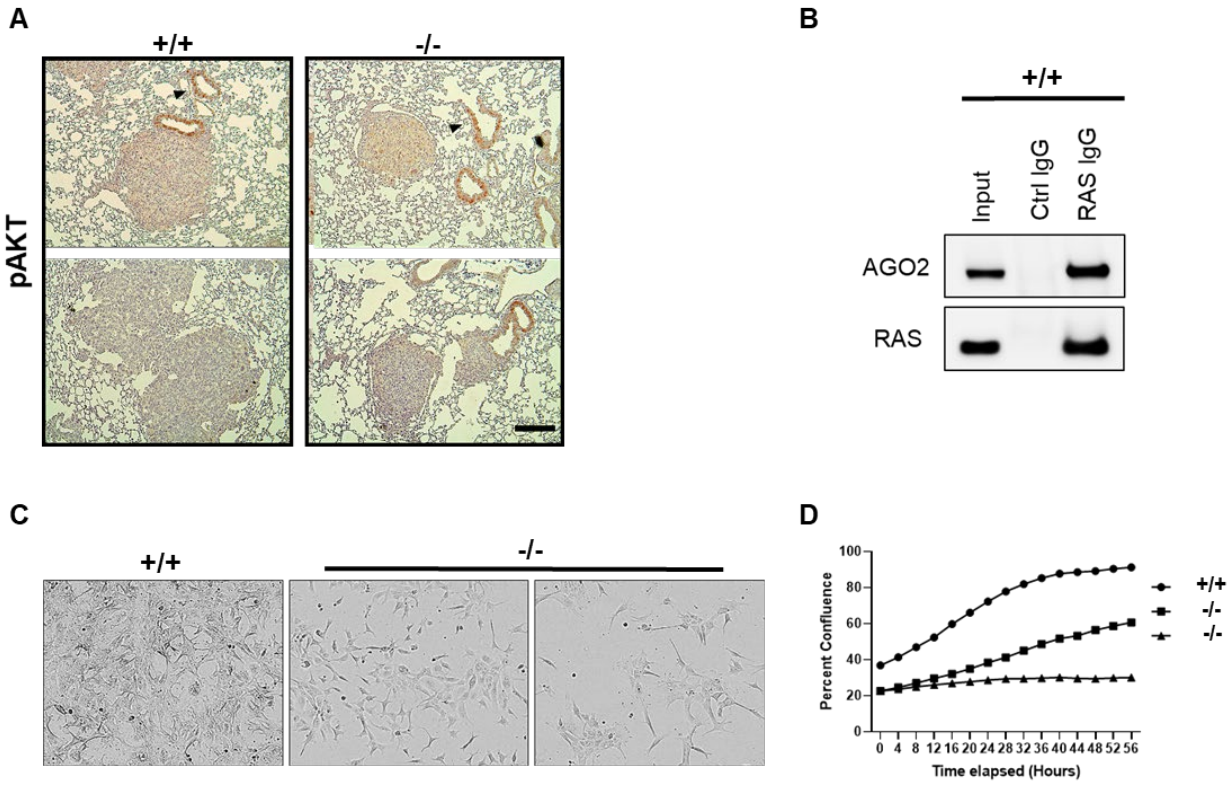


Fig S6. (A) pAKT immunohistochemistry of lung cross sections from *KPC-Ago2+/+ (+/+)* versus *KPC-Ago2-/-* (-/-) mice. Arrowheads demonstrate ducts with prominent staining. Scan staining is apparent in some nodules. Scale 100μm. **(B)** AGO2 immunoprecipitation with RAS IgG performed on *KPC-Ago2+/+* organoid-derived cell lysate. Ctrl indicates control IgG. **(C)** 2D-cultured cells derived from *KPC-Ago2+/+* (+/+) and *KPC-Ago2-/-* (-/-) organoids. (20x magnification) **(D)** Proliferation (Incucyte imaging system) of cells indicated in (C).