Supplementary Information

Adaptive immunity is required for durable responses to alectinib in murine models of EML4-ALK lung cancer

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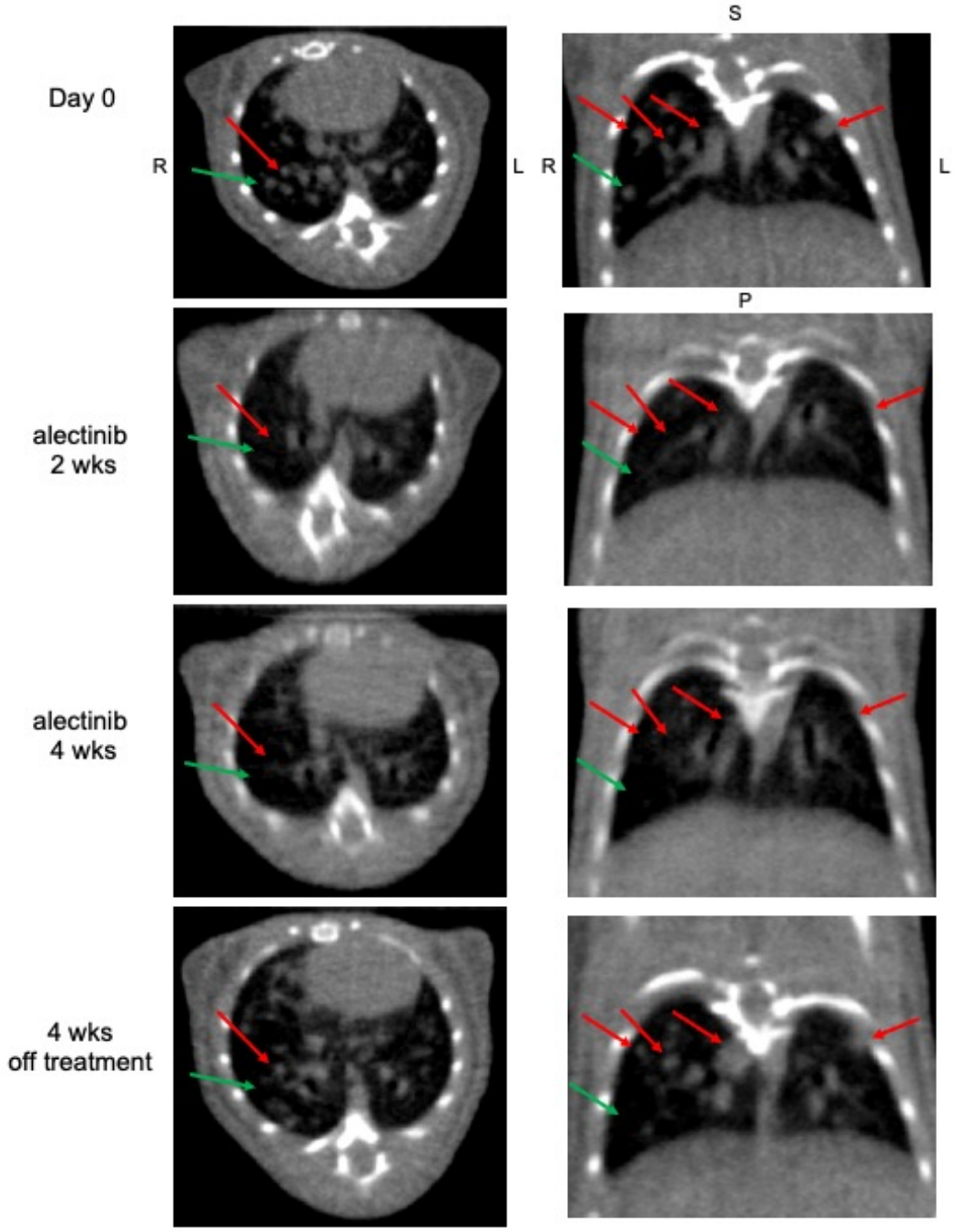
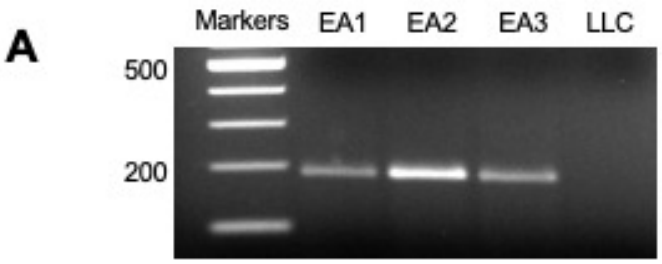


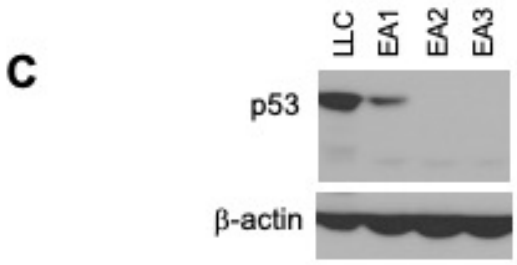
Supplementary Figure S1. Alectinib sensitivity of primary murine EML4-ALK tumors. Recombinant adenoviruses encoding Cas9 and gRNAs targeting *Eml4* and *Alk* were instilled intratracheally into C57BL/6 mice. The mice were routinely monitored by μCT for emergence of lung tumors and after 8 weeks (Day 0 in figure), mice were submitted to daily oral gavage with alectinib (20 mg/kg) and μCT

continued on a weekly basis. Following 4 weeks of alectinib treatment, therapy was terminated and μCT imaging continued. Serial images of an alectinib-treated mouse (representative of 4 others) are shown. R = right, L = left, S = superior, P = posterior. Red arrows identify lesions that shrank upon alectinib treatment and subsequently re-grew following therapy termination. Green arrows identify lesions that shrank with treatment and did not regrow upon termination of alectinib therapy.

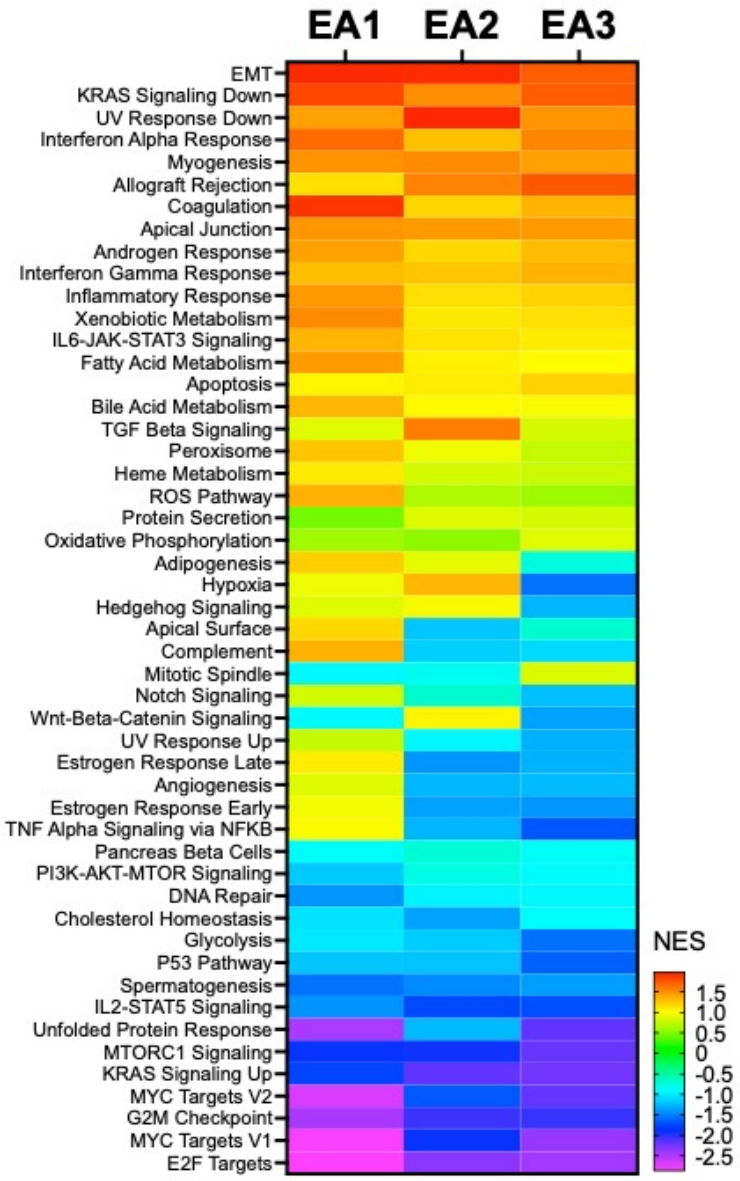


B

	RNA expression, CPM			
Gene	EA1	EA2	EA3	LLC
ALK	646	1204	700	0
MAPK1	1756	2602	1577	1664

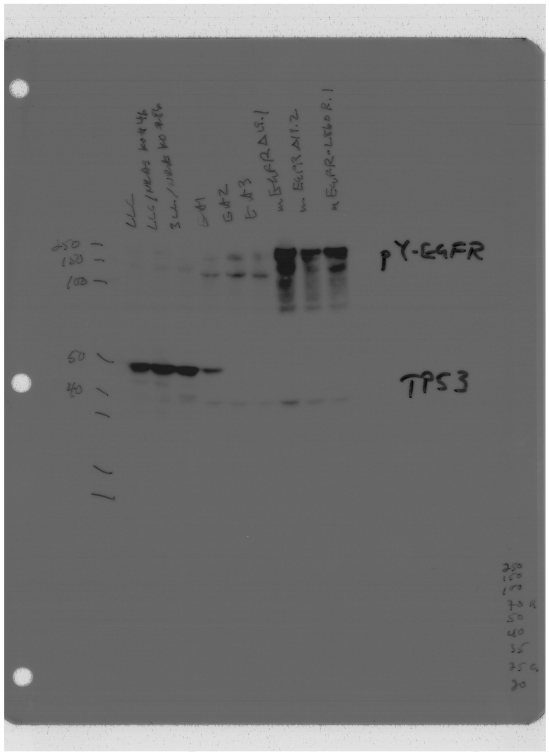


Supplementary Figure S2. RNA expression of EML4-ALK in murine cell lines. A, RNA was purified from EA1, EA2 and EA3 cells and submitted to reverse transcription PCR to amplify the coding sequences expressed from the rearranged EML4-ALK gene fusion. **B**, RNAseq data from the cell lines was queried for ALK mRNA expression with MAPK1 as a control house-keeping gene. The LLC cell line is a KRAS mutant line that does not express ALK. **C**, Cell extracts were prepared from the indicated murine cell lines and submitted to immunoblot analysis for TP53 and β-actin as a loading control. LLC cells bear a missense mutation in TP53, EA1 was developed in TP53 wild-type mice and EA2 and EA3 were generated in TP53 null mice.

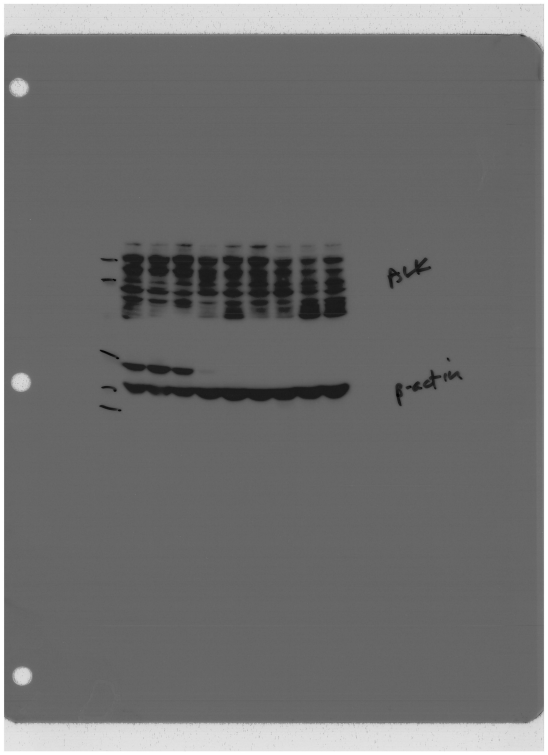


Supplementary Figure S3. Gene-set enrichment analysis of RNAseq data from murine EML4-ALK cell lines treated *in vitro* with alectinib. RNA was purified from EA1, EA2 and EA3 cells treated *in vitro* with DMSO or alectinib (100 nM) for 1-5 days and submitted for RNAseq. For this analysis, the different time points were considered as replicates (n=4) and DMSO vs. alectinib-treated samples analyzed with GSEA using the Hallmark Pathways. The heatmap presents the normalized enrichment scores (NES) in the alectinib-treated samples (see color bar for relative scores).

A.



B.



Supplementary Figure S4. Un-cropped western blot images accompanying supplementary figure S2. The untouched, full western blot images that accompany supplementary figure S2, which have not been altered or cut. **A**, TP53-probed exposure. **B**, β-actin exposure. The original exposed images contain extra lanes with cell lines that are not relevant to this study. Additionally, these membranes were probed for proteins that are not relevant to this study.