**Supplementary Figure S1. ALK Tg mice develop lung multifocal adenocarcinomas similar to human ALK-rearranged NSCLC.**

(A) Schematic representation of DNA constructs used to generate ALK Tg mice expressing human TFG-ALK or EML4-ALK protein in lung epithelium under the human lung specific SP-C promoter. SP-C, surfactant protein C. (B) RT-PCR performed on RNA extracted from different tissues of TFG-ALK or EML4-ALK mice. (C) Quantitative RT-PCR (qRT) performed to detect the levels of ALK mRNA. hNSCLC cell lines are ALK-rearranged cell lines, H3122 and H2228. Horizontal bars represent means. (D) Western Blot to check ALK protein expression in both EML4-ALK and TFG-ALK mice compared to human ALK-rearranged NSCLC cell lines, H3122 and H2228. Membranes were blotted with the indicated antibodies. The lines between the blots indicate cut lanes on the same gel. (E) Representative images of histological section of the lungs of EML4-ALK (left panels) or TFG-ALK (right panels) mice stained for hematoxylin and eosin (H&E). Scale bars, 1mm (top) and 50µm (bottom). (F) ALK IHC in normal lung and in tumor bearing lung in ALK mice. IHC shows ALK protein expression only in the tumor nodule. Scale bar, 100µm. (G) Ki-67 staining in ALK mice. Scale bar, 100µm. (H and I) Development of lung tumors in EML4-ALK (H) or TFG-ALK (I) mice at the indicated time-points. Representative T2w coronal MRI sections of lungs are shown. (L) Overall survival of EML4-ALK and TFG-ALK Tg mice plotted in Kaplan-Meier curves.

**Supplementary Figure S2. Therapeutic ALK vaccine impairs tumor growth of ALK-rearranged lung tumors.**

 (A) Representative T2w coronal MRI sections of lungs of TFG-ALK mice. Control vaccinated mice (Ctrl) or ALK vaccinated (Vax) at 4 weeks of age (Pre-vax) and at 12 and 20 weeks of age (Post-vax). (B and C) Total tumor volume in the lungs of EML4-ALK (B) and TFG-ALK (C) mice was monitored by MRI at the indicated time points. (D) Tumor growth fold change evaluated 8 weeks after ALK vaccination in EML4-ALK mice vaccinated at younger or older age (4 *vs* 12 weeks). Data are represented as mean (±SEM). Two-tailed Student’s *t* tests were used to calculate the pvalues shown. \*\*, *P*<0.005; \*\*\*, *P*<0.0005; \*\*\*\*, *P*<0.0001.

**Supplementary Figure S3. Immunophenotype of EML4-ALK transgenic mice**

(A-D) Lung immune infiltrates of WT mice (n= 5) or 16-week-old EML4-ALK mice (n= 9) were stained with antibodies to CD45, CD3, CD4, CD8 (A), CD45, CD19 (B), CD45, CD3, NKp46, CD49 (C), CD45, GR1, CD11b (D) and analyzed by FACS. Data are represented as mean (±SEM). (E) Lung immune infiltrates from WT mice (n= 5 mice) or EML4-ALK mice at 12 weeks of age (n= 5 mice) and at 16 weeks of age (n= 9 mice) were stained with antibodies to CD3, PD-1, LAG-3 and TIM-3 and analyzed by FACS. Data are represented as mean (±SEM). (G) Lung sections from 12-week-old or 16-week-old EML4-ALK mice were stained with anti-CD3 and Foxp3 antibodies. Percentages of CD3+ and Foxp3+ tumor infiltrating lymphocytes (TIL) were calculated relative to tumor cells. Histograms represent the mean (±SEM) percentage in at least 3 tumors from 3 independent mice for each group. Two-tailed Student’s t tests were used to calculate the p values shown. \*, *P*<0.05; \*\*, *P*<0.005; \*\*\*, *P*<0.0005.

**Supplementary Figure S4. Human ALK-rearranged NSCLC show a pattern of low expression of T cell markers.**

(A-C) Heatmaps of genes enriched for T cell markers of ALK-rearranged NSCLC *vs* EGFR mutated NSCLC (L858R or EGFR-Del19) (A), or *vs* K-RAS mutated NSCLC; FDR q-Value: 0.001 (B), or *vs* K-RAS/EGFR/ALK negative NSCLC; FDR q-Value: 0.00046 (C). (D) Genes in the GSEA for T cell marker were ranked by signal-to-noise metric to measure the difference of gene expression in two compared groups. The table reports the *p* value (significance *P*<0.05) for each gene. Genes with significantly lower expression levels between EML4-ALK rearranged and other NSLCL subtypes are highlighted in orange boxes.

**Supplementary Figure S5. PD-L1 expression is dependent on ALK kinase activity in ALK-rearranged NSCLC cells.**

(A) Levels of PD-L1 mRNA in lung tumors of mice with different genotypes (EML4-ALK; EGFRL858R; K-RasG12V) at 10 weeks of age. qRT-PCR analysis performed on cDNA of tumors from 5 mice for each genotype. Horizontal bars represent means. (B) FACS analysis of PD-L1 expression on CD45+ cells and CD45-/EpCAM+ cells in EML4-ALK transgenic mice. PD-L1 and isotype control staining is shown in clear black and in gray filled lines, respectively. (C) Western blot of human ALK-rearranged NSCLC cells, H2228 and DFCI032, treated with different concentrations of crizotinib at the indicated time points. Membranes were blotted with the indicated antibodies. (D) PD-L1 protein expression was evaluated by flow cytometry in H2228 and DFCI032 treated with crizotinib for 48 hours. (E) qRT-PCR for PD-L1 mRNA expression in H2228 and DFCI032 cell lines treated with crizotinib for 96h. (F-G) H3122 cells were infected with a lentivirus vector containing a doxycycline-inducible (TTA) shRNA against ALK (A5) or a control scrumble shRNA (A5M). Cells were treated with doxycycline for 72h to knock-down ALK expression (F) and analysed by qRT-PCR for PD-L1 mRNA expression (G). The NPM-ALK translocated ALCL cell line, SU-DHL1, was used as a control. Data are represented as mean (±SEM). Two-tailed Student’s *t* test was used to calculate the p values shown. \*, *P*<0.05; \*\*, *P*<0.005; \*\*\*, *P*<0.0005.

**Supplementary Figure S6. Schematic protocols of the *in vivo* treatment with anti-PD-1 blocking antibody.**

(A) Schematic representation of ALK vaccination protocol combined with blockade of PD-1/PD-L1 immune checkpoint by anti-PD-1 antibody in Balb/c mice injected i.v. with EML4-ALK ASB-XIV or EML4-ALK/PD-L1 ASB-XIV cells. (B) Schematic representation of the treatment with anti-PD-1 blockade antibody in EML4-ALK transgenic mice. At 12 weeks of age ALK mice were screened by MRI and stratified according to their tumor burden. Mice were treated 5 times i.p. with the isotyope control or with anti-PD-1 antibody (200 g). Mice were screened by MRI to measure tumor volume at the end of treatment (14 weeks of age) and at 4 weeks after treatment suspension (18 weeks of age).

**Supplementary Figure S7. ALK vaccine can be combined with ALK inhibitor TAE684.**

(A)Schematic representation of the ALK vaccine combined with TAE684 treatment (25mg/kg for 2 weeks) in EML4-ALK mice. MRI, Magnetic Resonance Imaging. (B-C) The number of neoplastic nodules (B) and the tumor volume (C) were monitored by MRI analysis at the indicated time points. Data are represented as mean (±SEM). Two-tailed Student’s *t* tests were used to calculate the p values shown. \*, *P*<0.05; \*\*, *P*<0.005.

**Supplementary Figure S8. Immunophenotype of intratumoral T cells in ALK vaccinated mice.**

(A) ALK vaccine induces an increase of PD-1 expression on CD8+ T lymphocytes in EML4-ALK transgenic mice. Expression levels of PD-1 in CD3+ cells (top panels) and in CD3+ CD8+ cells (bottom panels) in control vaccinated mice (Ctrl) and in ALK-vaccinated (Vax) mice. (B) Histograms show the mean percentage of CD3+ cells that express PD-1 (PD-1+) and high levels of PD-1 (PD-1+hi) (top) and the mean percentage of CD8+ that express PD-1 (PD-1+) and high levels of PD-1 (PD-1+hi) in the CD3+ population (bottom) in control vaccinated mice (Ctrl) and in ALK-vaccinated (Vax) mice. Data are represented as mean (±SEM). (C) Depletion of Treg cells restores the specific CTL activity against ALK in mice ALK vaccinated at 12 weeks of age. Cytotoxicity displayed by 12-week-old EML4-ALK mice vaccinated with ALK-DNA with or w/o Treg depletion by anti-CD-25 antibody. Horizontal bars represent means. Two-tailed Student’s *t* tests were used to calculate the p values shown. \*, *P*<0.05; \*\*, *P*<0.005; \*\*\*, *P*<0.0005; \*\*\*\*, *P*<0.0001.