**Supplementary Materials**

**Supplementary Table S1.** Detailed information about the antibodies used.

**Supplementary Table S2.** Proportions of listed immune cell subsets in the tumor microenvironment of NSCLC GEMs were evaluated by flow cytometry utilizing indicated lineage markers and proteins. TAMs; Tumor-associated macrophages, TANs; Tumor-associated neutrophils.

**Supplementary Table S3.** Tumor cells from treated KP mice were subjected to RNA-sequencing. Transcripts (calculated as Log2FC over vehicle) were ranked with a Padj<0.05 cut-off and only up to top 50 most up- (A) or down-regulated (B) genes are shown.

**Supplementary Figure S1. Kinetics of tumor growth in KP mice treated with anti-PD-1.**

KP mice with starting tumor burdens of approximately 100 mm3 were intraperitoneally injected 3x/week for 5-6 weeks with α-PD-1 and tumor growth was monitored weekly by MRI. Shown is the kinetics of tumor growth over 5-8 weeks for individual mice enrolled in indicated treatment group.

**Supplementary Figure S2. Immune cell proportions in the tumors of treated KP mice.**

Cell suspensions from tumor nodules of KP mice treated for two weeks with vehicle or JQ1 and/or α-PD-1 were analyzed by multi-parameter flow cytometry. Shown are absolute cell counts of indicated leukocyte subsets within the tumors of mice under each treatment. Data are mean ± SEM for 4-5 mice per group. TAM; tumor-associated macrophages, TAN; tumor-associated neutrophils.

**Supplementary Figure S3. PD-1 is prominently expressed on tumor-infiltrating T cells in GEMM of NSCLC.**

Multi-parameter FACS analysis was conducted on single cell suspensions obtained from lung tumor or spleen of KP mice. Shown are representative dot plots for the expression of PD-1 (left) and CTLA-4 (right) on CD3+Foxp3- T cells in the tumor (red) versus the spleen (blue).

**Supplementary Figure S4. Decreased expression of PD-1 and CTLA-4 on tumor-infiltrating CD4+ T cells under JQ1 treatment.**

Immune profiling was performed on tumors of treated KP mice as described in figure 1. (A, B) Representative histograms (left) and summary (right) of expression levels for (A) PD-1 and (B) CTLA-4 on tumor-infiltrating CD4+ T cells. (C) Percent of CD4+ T cells that secreted IFN-γ following ex-vivo stimulation as described in Figure 2C. (D) Correlation graph depicting association between CD8+ T cells that are PD-1+ in the tumors of KP mice and % that are IFN-γ+ as evaluated in the functional studies.

**Supplementary Figure S5. Increased expression of MHC class I on tumor-infiltrating tumor-associated macrophages (TAMs).**

(A-C) Representative histograms (left) and summary (right) of expression levels for (A) H-2 (MHC I), (B) I-A/I-E (MHC II), and (C) PD-L1 on tumor-infiltrating CD45+CD11bdimCD11c+ tumor-associated macrophages (TAMs). Data are mean ± SEM for 4-5 mice per group. MFI; mean fluorescent intensity.

**Supplementary Figure S6. Combination of JQ1 and PD-1 blockade evokes unique genomic changes in tumors of treated KP mice.**

KP tumor nodules were collected from mice treated for two weeks with vehicle, JQ1 and/or α-PD-1 after which RNA-sequencing was performed. Shown is the hierarchical clustering of samples based on relative expression levels of the top 100 transcripts most sensitive to each treatment expressed as a heat map.

**Supplementary Figure S7. Overall survival of tumor-bearing nude mice treated with JQ1 or anti-PD-1.**

Athymic nude mice received 2x106 KP tumor cells via intra-tracheal instillation. Upon tumor establishment as confirmed by MRI, mice were intraperitoneally injected daily with JQ1 or 3x/week with anti-PD-1 and tumor growth was monitored by MRI. Shown is the overall survival of mice under each indicated treatment with corresponding median survival time in days. Data are from 4 mice/treatment group.

**Supplementary Figure S8. JQ1 and/or anti-PD-1 treatment facilitated enhanced effector function of tumor-infiltrating CD8+ T cells.**

1x105 sorted CD3+Foxp3- T cells were added to 1x103 tumor cells in 96-well plate and incubated at 370C for 24 hours in the presence of 5IU/ml of IL-2 after which cells were washed and subjected to FACS analysis. (A, B) Representative histograms (left) and summary (right) of expression levels for (A) CD107a and (B) Perforin in the tumor-associated T cells as evaluated by intracellular staining. For tumor cell death, a viability dye was used to discriminate between live and dead cells. Cultures with no added T cells served as a control. (C) Proportion of dead tumor cells under each co-culture condition. Data are mean ± SEM for 3 mice per group.

**Supplementary Figure S9. Combination of JQ1 and α-PD-1 did not have substantial deleterious effects on tumor-associated T cells.**

 (A, B) Mouse-derived organotypic tumor (MDOT) cultures were established from gently dissociated KP tumors after which generated tumor spheroids were cultured in the presence of complete media containing JQ1 (250 nM) and α-PD-1 (10 ug/ml). DMSO served as control. After 3 days in culture, immunofluorescent staining was performed using antibodies against CD3 (T cells) and EpCAM (tumor cells). Images were captured using a Nikon Eclipse 80i fluorescence microscope equipped with CoolSNAP CCD camera (Roper Scientific). NIS elements Imaging software was used to create merged images. (A) Representative image of a tumor spheroid under each condition. Magnification; x20. (B) Proportion of CD3+ T cells of total cells as enumerated by ImageJ software.

**Supplementary Figure S10. Tumor-infiltrating KLRG1+ CD4+Foxp3+ Tregs exhibit a highly activated profile but resemble natural peripheral Tregs.**

(A) Representative histograms of expression levels of indicated markers of T cell activation and the anti-apoptotic protein Bcl-2 on indicated subsets of tumor-infiltrating Tregs or Tregs in the spleen of tumor-bearing, untreated KP mice. (B) Representative histograms of expression levels for Helios and Neuropilin-1 on indicated Treg or conventional CD4+ T cell populations. Data are representative of 5 mice.

**Supplementary Figure S11. Tumor-infiltrating KLRG1+ CD4+Foxp3+ Tregs are not derived from CD4+CD25- cells.**

(A) Schematics of orthotopic tumor implantation and adoptive transfer studies. 2x106 KP tumor cells were injected trans-thoracically into athymic nuce mice. Upon tumor establishment as confirmed by MRI, unfractionated CD4+ T cells or cells depleted of the CD25+ fraction isolated from the spleen of KP mice were transferred i.v. into these tumor-bearing mice after which the tumors were subjected to phenotypic assessments by FACS analysis. (B) Representative CD4/CD25 profile of input bulk (top) or CD25-depleted (bottom) CD4+ T cells and the corresponding expression of Foxp3 and KLRG1 from indicated gates. Percent of positive population are shown in red for each histogram. (C) Representative CD25/Foxp3 profile of gated CD45+CD3+CD4+ T cells present in the tumors of bulk CD4+ T cell recipient mice (top) or mice that received CD4+CD25-depleted cells (bottom) and the corresponding expression of KLRG1 for depicted gates. Percent of positive population are shown in red for each histogram. Data are representative of 4 mice/group.

**Supplementary Figure S12. Tumor-associated KLRG1+ Tregs express higher CTLA-4 and demonstrate enhanced suppressive function.**

Phenotypic assessments were conducted by FACS on tumor cell suspensions from untreated KP mice to evaluate the phenotype of tumor-infiltrating CD4+Foxp3+ Tregs. (A) Representative histograms of CTLA-4 expression on the KLRG1-positive and negative subsets. CD4+CD25hi Tregs within the tumors of untreated KP mice were sorted and co-cultured with CFSE-labeled CD4+CD25- responder T cells isolated from the spleen of the same mouse. Cells were stimulated with ɑ-CD3 in the presence of T-depleted splenocytes as APCs for 3 days. (B) Summary of percent of responder cells proliferating in the presence of KLRG1+ (red) or KLRG1- (blue) Tregs at indicated Treg: T cell ratios based on CFSE dilution. Data in (B) are mean ±SEM of 3 independent experiments. \* indicates p-value ˂ 0.05.

**Supplementary Figure S13. Decreased expression of Treg signature proteins and Bcl-2 on tumor-infiltrating KLRG1+ Treg subset under JQ1 treatment.**

Immune profiling was performed on tumors of treated KP mice as indicated. Summary of expression levels for Foxp3, CTLA-4, GITR, and Bcl-2 on gated KLRG1+ versus KLRG1- Treg subsets within CD45+CD3+CD4+Foxp3+ cells as evaluated by FACS. MFI; mean fluorescent intensity. Data are mean ± SEM for 4-5 mice per group.