Supplemental material

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Supplementary Figure 1. (A) OS curves for LYN in NSCLC patients from the TCGA database. Expression was stratified by the median and the Log-rank test was used for the statistical analysis. **(B-C)** Quantification of FOXP3+CD4+ (Treg) cells in LUSC specimens from University Clinic of Navarra. The percentage of Tregs in YES1 high tumors (upper quartile) was compared to the rest of samples (B) or to the lower quartile (C). **(D-E)** Percentage of CD4+ (D) and CD8+ (E) cells in YES1 high or YES1 low NSCLC specimens from the University Clinic of Navarra. **(F)** Relative abundance of Treg cells in LUSC specimens from the TCGA database analyzed with CIBERSORT. **(G)** Individual follow-up of tumor growth of 393P cells injected in athymic nude mice. **(H)** Individual follow-up of the tumor volume in the 393P model shown in Figure 3A. **(I)** Individual follow-up of the tumor volume in the UN680 model *in vivo* shown in Figure 3F.

Supplementary Figure 2. (A) Western blotting of phospho SFKs (pSFKs), phospho STAT3 (Y705) (pSTAT3), STAT3, phospho-ERK1/2 (pERK 1/2), ERK1/2, phospho-AKT (ser473) (pAKT), AKT and β-actin performed with protein extracts coming from a subcutaneous *in vivo* experiment (short-term treatment) with 393P cells. **(B)** Densitometric quantification of the protein levels corresponding to pSFKs after normalization with β actin expression. **(C)** Western blotting for YES1 in the 393P cell line infected with a shRNA control (sh-scramble) or a shRNA targeting YES1 (sh-YES1). **(D)** *In vivo* growth of 393P tumors after injection of transduced cells (either sh-scramble or sh-YES1) into Sv/129 mice, with or without anti-PD-1 treatment (100 µg, days 6, 9 and 12).

Supplementary Figure 3. (A) Evolution of subcutaneous growth of 393P tumors undergoing single or combined treatments with dasatinib (30 mg/kg, daily) and/or anti-PD-1 (100 µg, days 4, 7 and 10). At day 14 of the experiment, animals (n=8 per group)

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were sacrificed and tumors harvested for flow cytometry analysis of the tumor-immune infiltrate. **(B-F)** Flow cytometry showing percentage of tumor-infiltrating macrophages (F4/80+), B cells (CD19+), NK cells (NK1.1+), M-MDSCs (Ly6C^{high} Ly6G$^{low/-}$) and PMN-MDSC (Ly6G^{high} Ly6C ^{low}) cells. All data were referred to the percentage of CD45+ cells. **(G-I)** PD-L1 median fluorescence intensity (MFI) in M-MDSC, PMN-MDSC and macrophages. **(J-K)** PD-L1 MFI in 393P (left) and UN680 (right) cells after addition of dasatinib (5µM or 10µM for 72h) and IFNɣ (500 U/mL) *in vitro*.

Supplementary Figure 4. (A-B) Flow cytometry gating strategy for tumor-infiltrating B cells, NK cells, CD8 T cells, CD4 T cells, Tregs and MDSCs. After excluding doublets, leukocytes were selected using leukocyte common antigen CD45. Within CD45-positive cells, B cells, NK cells, CD8 T cells, CD4 T cells and CD11b+ cells were delimited as the CD19+, NK1.1+, CD8+ CD4+ cells and CD11b+ cells, respectively. Tregs were gated from CD4 T cells as the double positive population for CD25 and FOXP3 markers. M-MDSCs and PMN-MDSCs were gated from CD11b+ cells as Ly6C^{high} Ly6G$^{low/-}$ and Ly6G^{high} Ly6C^{low}, respectively.

Supplementary Figure 5. (A-D) Multiplex immunofluorescence (mIF) analysis of CD4+, macrophages (F4/80+), CD8+ and CD31+ cells in 393P tumors (n=8). **(E)** Representative mIF images to show CD8+, CD4+, F4/80+ and CD31+ cells (upper panel) or all markers (lower panel) in one of the tumors. **(F)** Tumor growth of 393P cells subcutaneously injected in Sv/129 mice for studying circulating CD4+, CD8+ and Tregs by flow cytometry. Data are expressed as mean ± SEM and were analyzed with a one-way ANOVA test followed by a post-hoc Bonferroni test. **p<0.01; ***p<0.001.

Supplementary Figure 6. (A) Flow cytometry analysis of the CD8+ T cell population after treatment with anti-CD8α depleting antibody (100 µg, days 2, 6, 10 and 14) in

393P tumors. **(B-F)** Densitometric quantification of the protein levels corresponding to

pLCK (B), pSTAT5 (C), pSMAD3 (D), STAT5 (E) and SMAD3 (F).