

Expanded View Figures

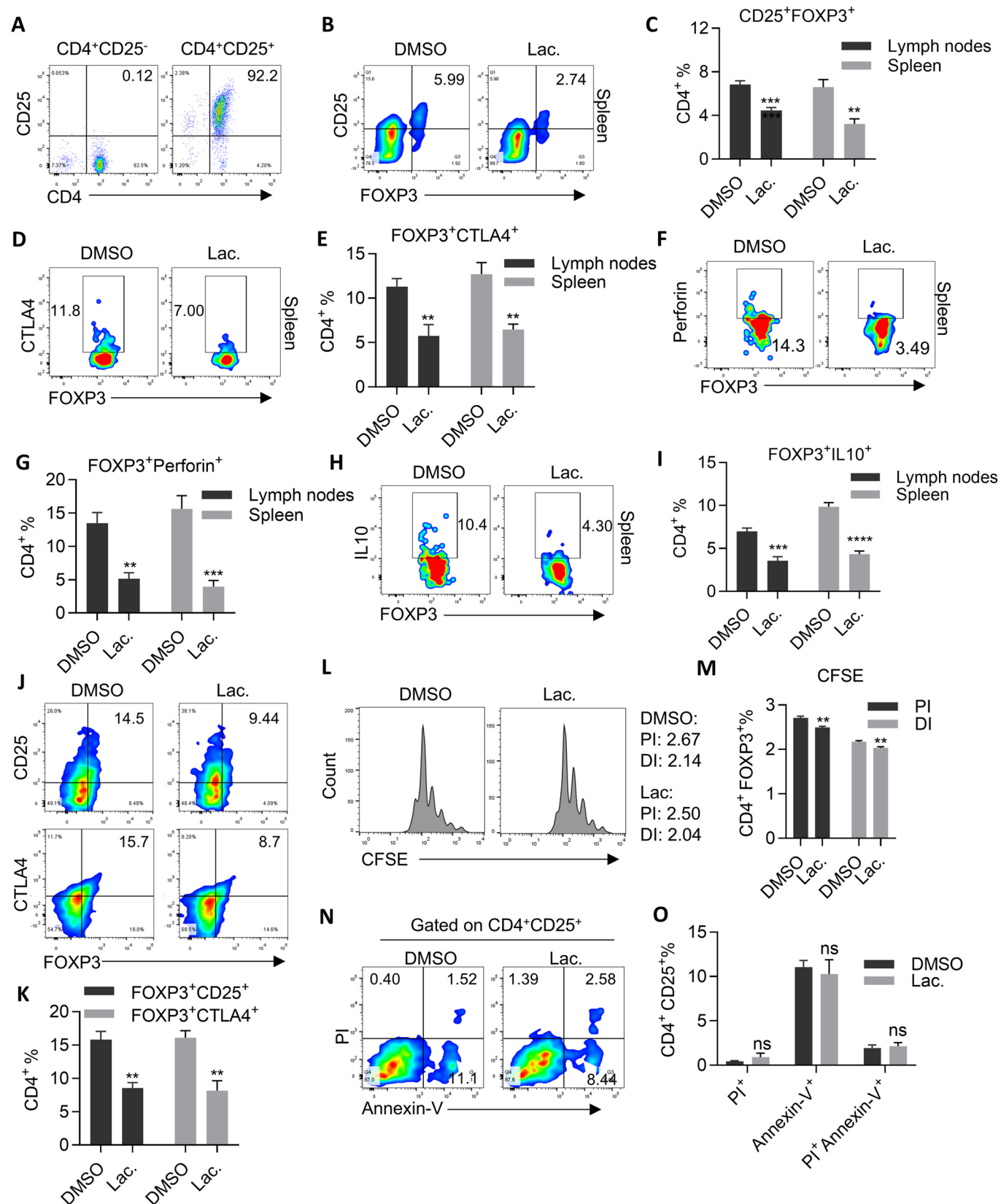


Figure EV1. Impact of Lac on Tregs.

(A) Tregs-purifying efficiency from mouse lymph nodes. Conventional CD4⁺ T (CD4⁺CD25⁻) or Treg (CD4⁺CD25⁺) cells were purified from lymph nodes by negative selection with MACS before flow cytometry assay. (B-I) Flow cytometry analysis of the impact of Lac on marker protein expression by Tregs. The gating of FOXP3⁺CD25⁺ (B), FOXP3⁺CTLA4⁺ (D), FOXP3⁺Perforin⁺ (F) and IL10⁺ (H) populations were determined against their FMO; Statistics analysis of Fig. 1D and EV1B (EV1C), Fig. 1E and EV1D (EV1E), Fig. 1F and EV1F (EV1G), Fig. 1G and EV1H (EV1I). Data are representative of three independent experiments and were analyzed by Student's *t* test. Error bars denote mean $\pm$ SD. *P* value: FOXP3⁺CD25⁺ (Lymph nodes), ****P* = 0.0006; FOXP3⁺CD25⁺ (Spleen), ***P* = 0.0024; FOXP3⁺CTLA4⁺ (Lymph nodes), ***P* = 0.0035; FOXP3⁺CTLA4⁺ (Spleen), ***P* = 0.0017; FOXP3⁺Perforin⁺ (Lymph nodes), ***P* = 0.0014; FOXP3⁺Perforin⁺ (Spleen), ****P* = 0.0008; FOXP3⁺IL10⁺ (Lymph nodes), ****P* = 0.0006; FOXP3⁺IL10⁺ (Spleen), *****P* < 0.0001. (J) Lac suppresses Tregs differentiation in vitro. Representative flow plots were gated on CD4⁺ population for analysis. The FOXP3⁺CD25⁺ (upper panel) or FOXP3⁺CTLA4⁺ (lower panel) populations were determined against their FMO, respectively. (K) Statistics analysis of Fig. EV1J. Data are representative of three independent experiments and were analyzed by Student's *t* test. Error bars denote mean $\pm$ SD. *P* value: FOXP3⁺CD25⁺, ***P* = 0.0010; FOXP3⁺CTLA4⁺, ***P* = 0.0016. (L) Lac suppresses the proliferation of Tregs in vitro. Proliferation of Tregs were determined by dilution of CFSE through flow cytometry analysis. (M) Statistics of the percentages of proliferating Tregs of Fig. EV1L. Data are representative of three independent experiments and were analyzed by Student's *t* test. Error bars denote mean $\pm$ SD. *P* value: PI, ***P* = 0.0014; DI, ***P* = 0.0041. (N) Impact of Lac on apoptosis of Tregs. Cells were stained with PI and Annexin V-FITC for flow cytometry analysis. (O) Statistics of apoptosis population of Fig. EV1N. Data are representative of three independent experiments and were analyzed by Student's *t* test. Error bars denote mean $\pm$ SD. *P* value: PI⁺, *P* = 0.1653; Annexin V⁺, *P* = 0.4850; Annexin V⁺PI⁺, *P* = 0.4890. Source data are available online for this figure.

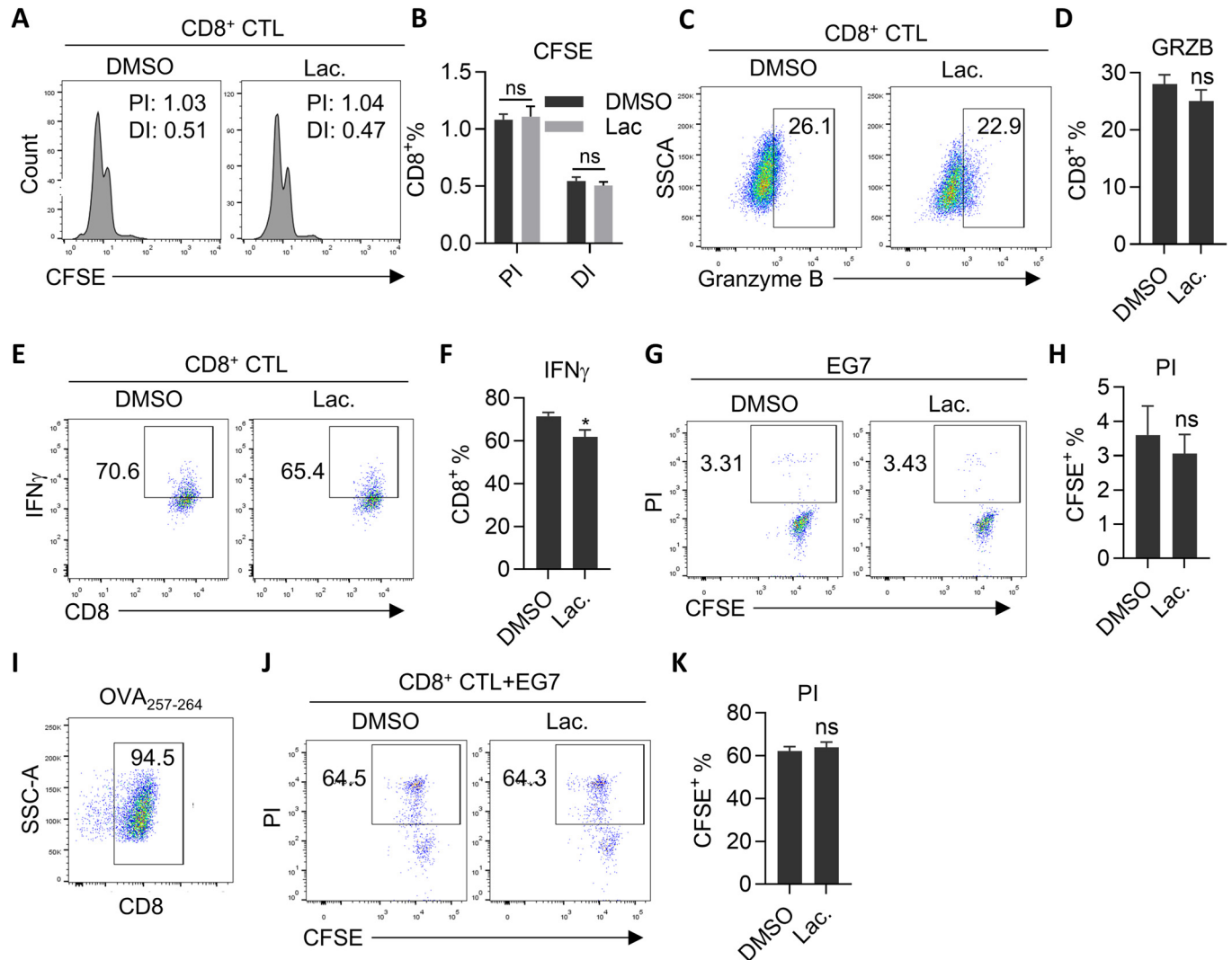


Figure EV2. Impact of Lac on the phenotype of CD8⁺ T cells.

(A) Lac exhibits limited impact on the proliferation of CD8⁺ T cells. Proliferation of OT-I cells were determined by dilution of CFSE through flow cytometry analysis on CD8a positive population. (B) Statistics of percentages of proliferating OT-I CTLs of Fig. EV2A. Data are representative of three independent experiments and were analyzed by Student's *t* test. Error bars denote mean $\pm$ SD. *P* value: PI, *P* = 0.6702; DI, *P* = 0.2193. (C–F) The mature CTLs and EG7 cells (CTLs:EG7 = 5:2) were co-cultured in the presence of DMSO or Lac (2 μ M) for 6 h, followed by incubation with monensin for another 6 h. The cells were then stained with anti-CD8a and GRZB (EV2C) or anti-CD8a and IFN γ (EV2E) for flow cytometry analysis. Statistics of GRZB (EV2D) and IFN γ (EV2F) expression. Data are representative of three independent experiments and were analyzed by Student's *t* test. Error bars denote mean $\pm$ SD. *P* value: GRZB, *P* = 0.1202; IFN γ , **P* = 0.0114. (G) Lac exhibits no toxicity to EG7 cells. Toxicity of EG7 cells was determined by analyzing PI/CFSE double-positive population. (H) Statistics of the percentages of CFSE⁺PI⁺ EG7 cells of Fig. EV2G. Data are representative of three independent experiments and were analyzed by Student's *t* test. Error bars denote mean $\pm$ SD. *P* value: *P* = 0.4103. (I) Purity of OVA₂₅₇₋₂₆₄ peptide-induced splenic OT-I CTLs. (J) Impact of Lac treatment on cytotoxicity of CTLs. (K) Statistics of the percentages of PI/CFSE double-positive EG7 cells of Fig. EV2J. Data are representative of three independent experiments and were analyzed by Student's *t* test. Error bars denote mean $\pm$ SD. *P* value: *P* = 0.3614. Source data are available online for this figure.

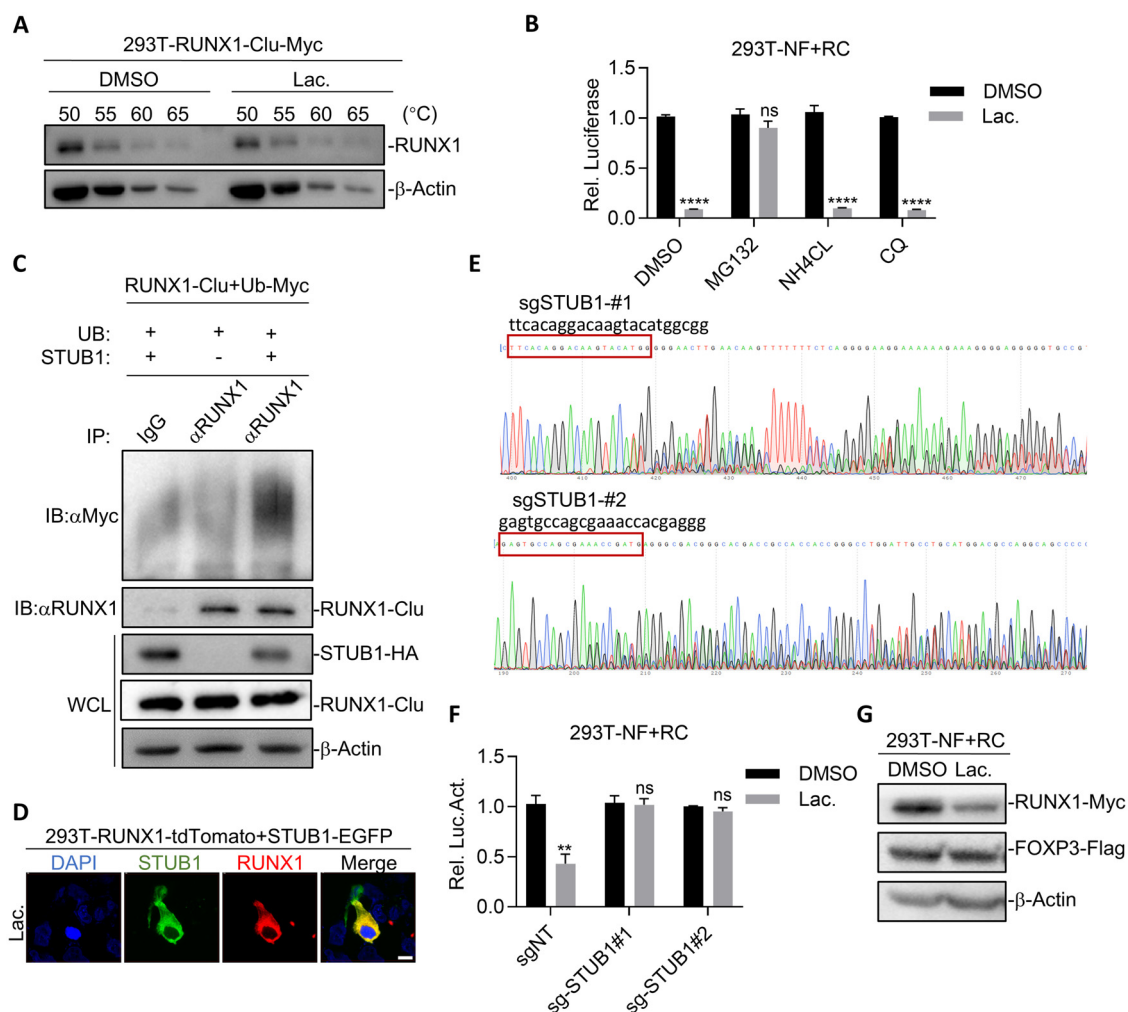


Figure EV3. STUB1 is required for Lac-induced RUNX1 degradation through proteasome pathway.

(A) Lac doesn't change the thermal stability of ectopically expressed RUNX1. (B) MG132, but not NH4Cl or CQ, stabilizes luciferase activity in response to Lac treatment in 293T-NF + RC cells. Data are representative of three independent experiments and were analyzed by Student's *t* test. Error bars denote mean $\pm$ SD. *P* value: DMSO, *****P* < 0.0001; MG132, *P* = 0.0537; NH4Cl, *****P* < 0.0001; CQ, *****P* < 0.0001. (C) STUB1-mediated induction of ubiquitination of RUNX1. (D) Lac promotes STUB1-mediated redistribution of RUNX1. Scale bar: 10 μ m. (E) Sequence chromatograms of 293T-sgSTUB1 cells. (F) STUB1 knockout stabilizes the luciferase activity of 293T-NF + RC in response to Lac treatment in bimolecular fluorescence complementation assay. Data are representative of three independent experiments and were analyzed by Student's *t* test. Error bars denote mean $\pm$ SD. *P* value: sgNT, ***P* = 0.0013; sgSTUB1#1, *P* = 0.7501; sgSTUB1#2, *P* = 0.0811. (G) Lac promotes the degradation of RUNX1 but not FOXP3 in 293T-NF + RC cells. Source data are available online for this figure.

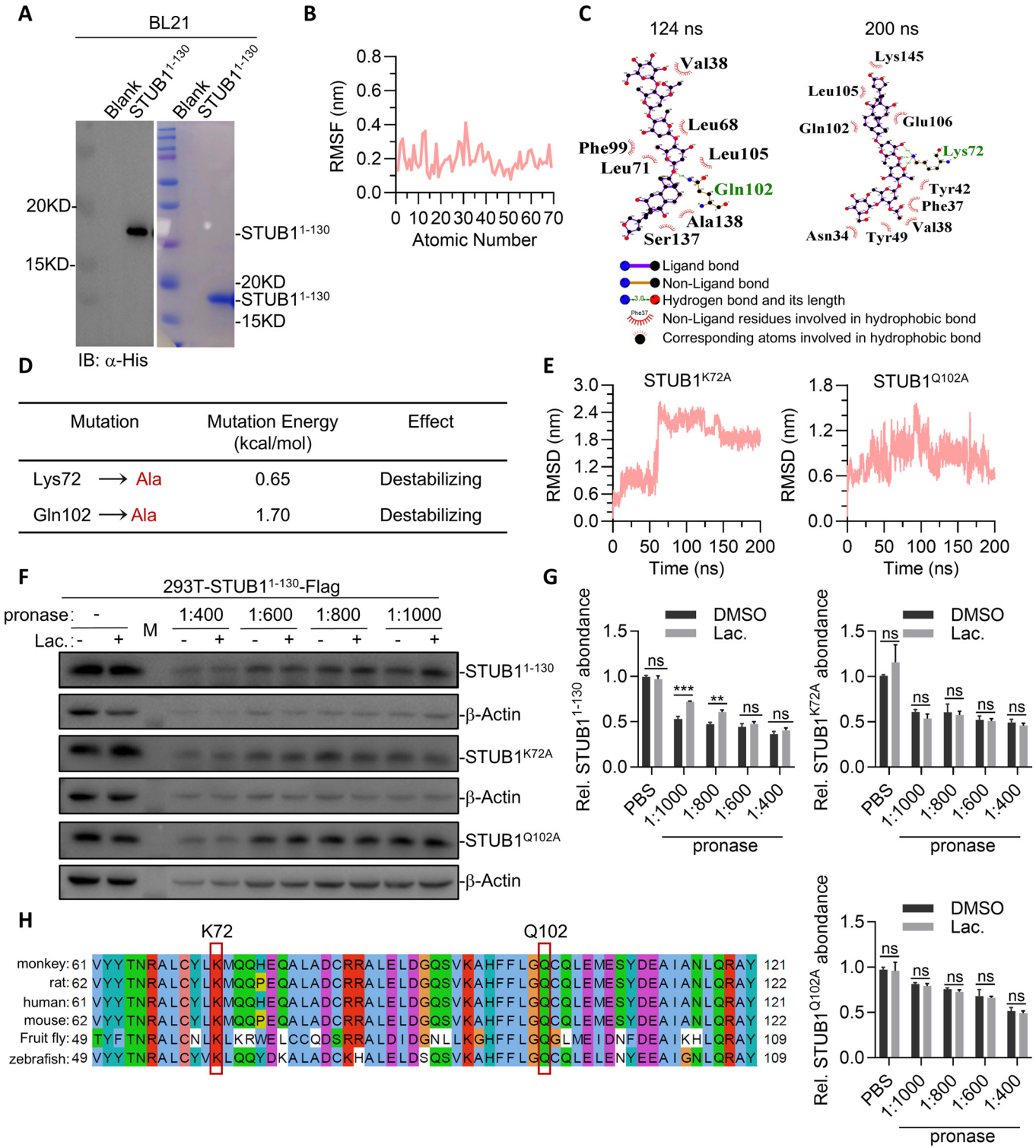
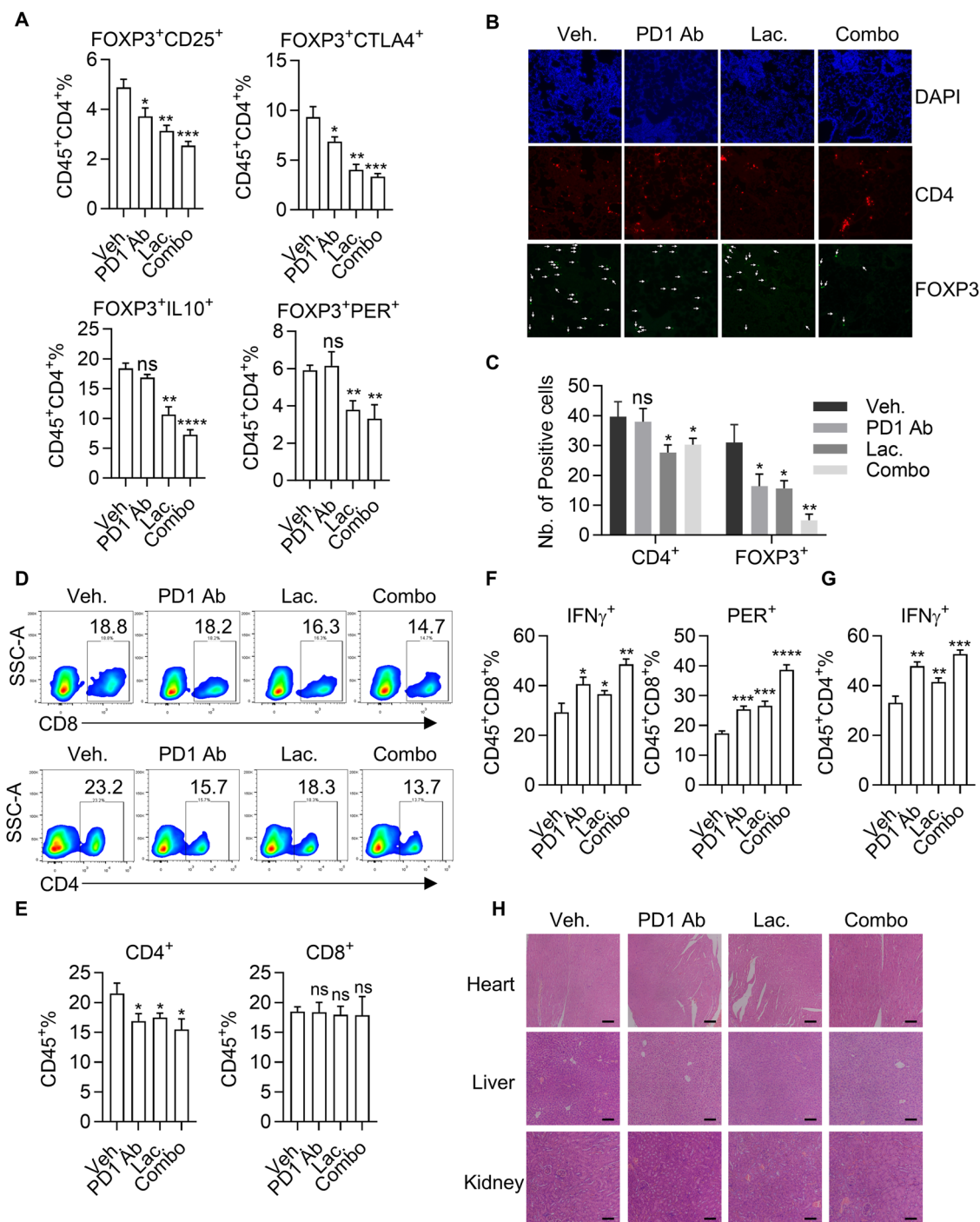


Figure EV4. Lac directly binds STUB1 at its TPR domain to enhance its affinity to RUNX1.

(A) IB and coomassie brilliant blue staining of purified STUB1¹⁻¹³⁰. IB (left panel) and SDS-PAGE (right panel) analysis of purified STUB1¹⁻¹³⁰ protein. (B) The root-mean-square deviations (RMSF) plot for docked Lac during MD simulations. (C) Ligplot of 2-D interaction of Lac-STUB1 at 124 ns and 200 ns in the MD simulations (The red arcs for hydrophobic interactions; green lines for hydrogen bonds). (D) Free energies predicted for bindings of Lac-STUB1^{K72A} (Lys72 to Ala) and Lac-STUB1^{Q102A} (Gln102 to Ala) with Lac. (E) Time-dependent RMSD plot of docked Lac in single-point mutants of STUB1 (K72A and Q102A) during molecular dynamics simulation. (F) Lac fails to protect ectopically expressed mutant STUB1 from pronase digestion through DARTS assay. (G) Statistics of relative abundance of mutant STUB1 of (F). Data are representative of three independent experiments and were analyzed by Student's *t* test. Error bars denote mean $\pm$ SD. *P* value: PBS, *P* = 0.3196, 1: 1000, ****P* = 0.0005, 1: 800, ***P* = 0.0021, 1: 600, *P* = 0.2729, 1: 400, *P* = 0.1308 for STUB1¹⁻¹³⁰; PBS, *P* = 0.2618, 1: 1000, *P* = 0.0892, 1: 800, *P* = 0.6736, 1: 600, *P* = 0.5848, 1: 400, *P* = 0.2594 for STUB1¹²⁷⁻²²⁶; PBS, *P* = 0.8264, 1: 1000, *P* = 0.3046, 1: 800, *P* = 0.1404, 1: 600, *P* = 0.7153, 1: 400, *P* = 0.4499 for STUB1²²³⁻³⁰³. (H) Sequence homology of STUB1 TPR domain. Alignment of STUB1 sequences from human (*H. sapiens*, 005852.2), zebrafish (*D. rerio*, 021325787.1), mouse (*M. musculus*, 062693.1), rat (*R. norvegicus*, 001020796.2), monkey (*M. mulatta*, 001244487.1), Fruit fly (*D. melanogaster*, 477441.1). Source data are available online for this figure.



◀ **Figure EV5. Lac synergizes with PD-1 inhibitor to treat mutant KRAS-driven lung cancer.**

(A) Statistics of the proportion of FOXP3⁺CD25⁺, FOXP3⁺CTLA4⁺, FOXP3⁺IL10⁺ and FOXP3⁺PER⁺ Tregs in tumor of Fig. 7G, H. Data are representative of three independent experiments and were analyzed by Student's *t* test. Error bars denote mean $\pm$ SD. *P* value: Veh. vs PD-1 Ab, **P* = 0.0127, Veh. vs Lac, ***P* = 0.0015, Veh. vs Combo, ****P* = 0.0004 for FOXP3⁺CD25⁺; Veh. vs PD-1 Ab, **P* = 0.0218, Veh. vs Lac, ***P* = 0.0016, Veh. vs Combo, ****P* = 0.0007 for FOXP3⁺CTLA4⁺; Veh. vs PD-1 Ab, *P* = 0.0624, Veh. vs Lac, ***P* = 0.0010, Veh. vs Combo, *****P* < 0.0001 for FOXP3⁺IL10⁺; Veh. vs PD-1 Ab, *P* = 0.6317, Veh. vs Lac, ***P* = 0.0027, Veh. vs Combo, ***P* = 0.0049 for FOXP3⁺PER⁺. (B) Immunofluorescence analysis of Tregs infiltration. Scale bar: 100 μ m. (C) Statistics of the Tregs infiltration (CD4⁺; FOXP3⁺) in tumor of Fig. EV5B. Data are representative of three independent experiments and were analyzed by Student's *t* test. Error bars denote mean $\pm$ SD. *P* value: Veh. vs PD-1 Ab, *P* = 0.6870, Veh. vs Lac, **P* = 0.0210, Veh. vs Combo, **P* = 0.0412 for CD4⁺; Veh. vs PD-1 Ab, **P* = 0.0246, Veh. vs Lac, **P* = 0.0151, Veh. vs Combo, ***P* = 0.0021 for FOXP3⁺. (D) Lac or PD-1 antibody treatment did not significantly alter the infiltration of CD4⁺ and CD8⁺ T cells into tumors. Representative flow plots were gated on CD45⁺ population for analysis. (E) Statistics of the proportion of CD8⁺ (right panel) and CD4⁺ (left panel) in tumor of Fig. EV5D. Data are representative of three independent experiments and were analyzed by Student's *t* test. Error bars denote mean $\pm$ SD. *P* value: Veh. vs PD-1 Ab, **P* = 0.0191, Veh. vs Lac, **P* = 0.0195, Veh. vs Combo, **P* = 0.0129 for CD4⁺; Veh. vs PD-1 Ab, *P* = 0.9277, Veh. vs Lac, *P* = 0.5793, Veh. vs Combo, *P* = 0.7526 for CD8⁺. (F) Statistics of the proportion of CD8⁺IFN γ ⁺ (left panel) and CD8⁺PER⁺ (right panel) in tumor of Fig. 7I. Data are representative of three independent experiments and were analyzed by Student's *t* test. Error bars denote mean $\pm$ SD. *P* value: Veh. vs PD-1 Ab, **P* = 0.0124, Veh. vs Lac, **P* = 0.0318, Veh. vs Combo, ***P* = 0.0013 for IFN γ ⁺; Veh. vs PD-1 Ab, ****P* = 0.0005, Veh. vs Lac, ****P* = 0.0007, Veh. vs Combo, *****P* < 0.0001 for PER⁺. (G): Statistics of the proportion of CD4⁺IFN γ ⁺ in tumor of Fig. 7J. Data are representative of three independent experiments and were analyzed by Student's *t* test. Error bars denote mean $\pm$ SD. *P* value: Veh. vs PD-1 Ab, ***P* = 0.0013, Veh. vs Lac, ***P* = 0.0090, Veh. vs Combo, ****P* = 0.0004 for IFN γ ⁺. (H) Representative images of H&E staining of the indicated organs of treated mice. Scale bar: 100 μ m. Source data are available online for this figure.