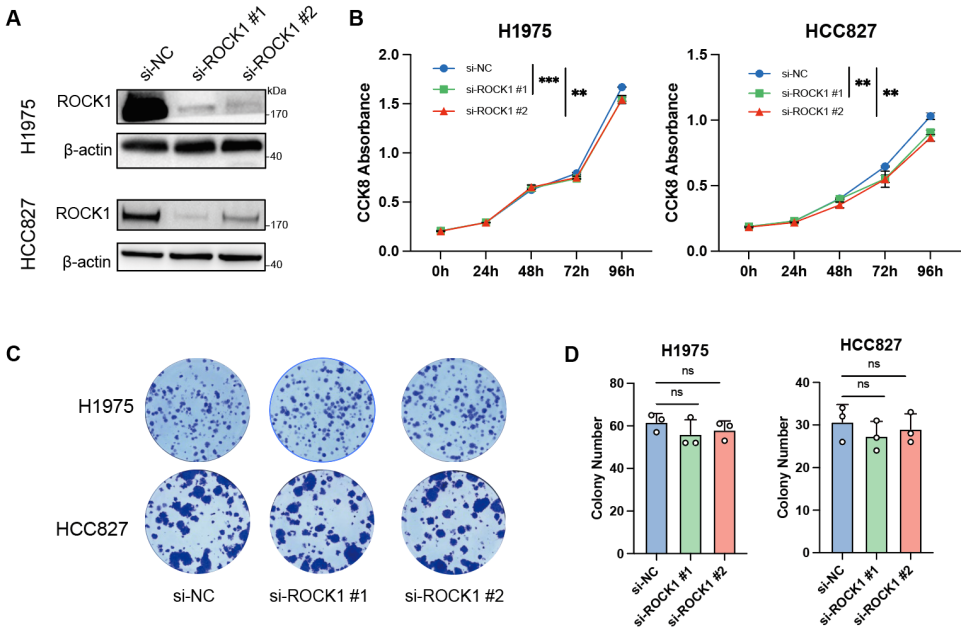
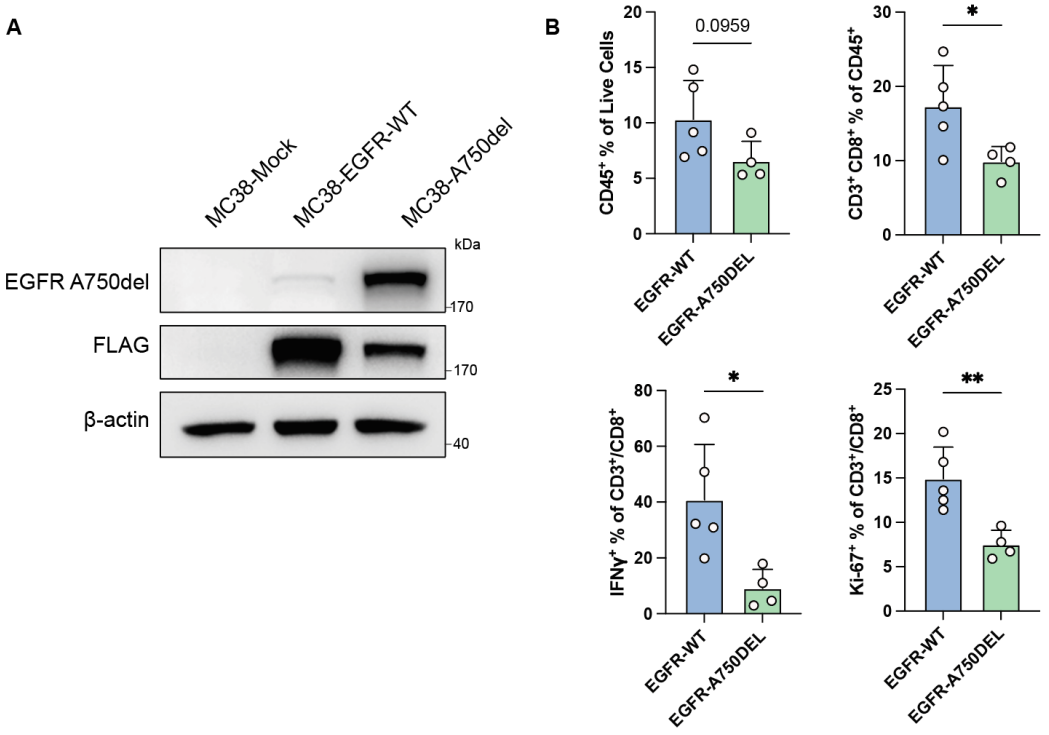
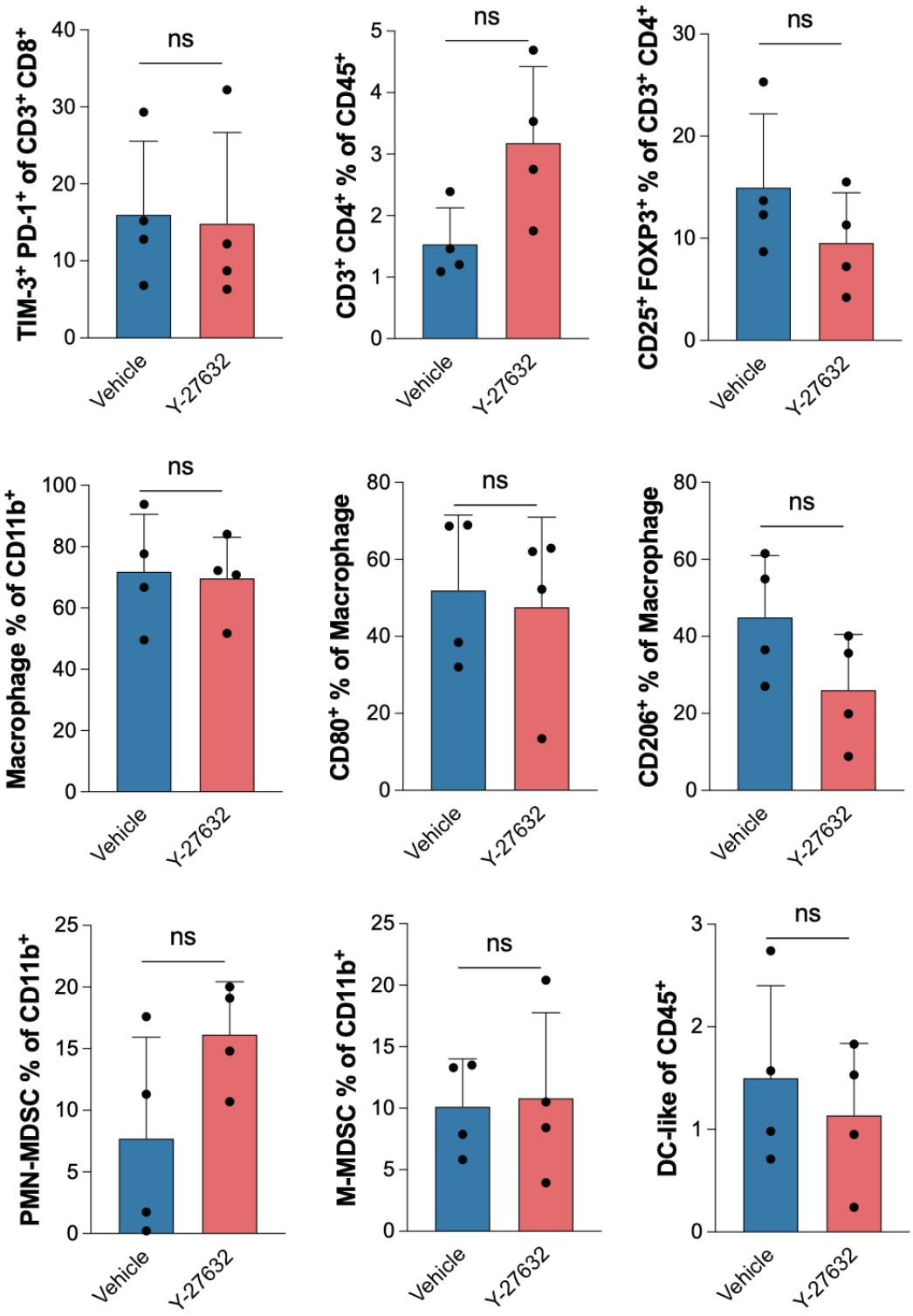


Supplementary Figure 1. ROCK1 knockdown shows little effect on cell proliferation. (**A**) Immunoblotting of ROCK1 in H1975 (upper) and HCC827 (lower) cells treated with ROCK1 knockdown siRNA. Nonsilencing siRNA was used as negative control. (**B**) Proliferation of H1975 (left) and HCC827 (right) cells treated with ROCK1 knockdown siRNA measured by CCK8 absorbance. Nonsilencing siRNA was used as negative control. (**C**) Representative image of colony formation assay showing H1975 and HCC827 cells treated with ROCK1 knockdown siRNA. Nonsilencing siRNA was used as negative control. (**D**) Comparison of colony number of H1975 and HCC827 cells treated with ROCK1 knockdown siRNA. Nonsilencing siRNA was used as negative control. ns, not significant; **, $P<0.01$; ***, $P<0.001$; two tailed Student *t* test.

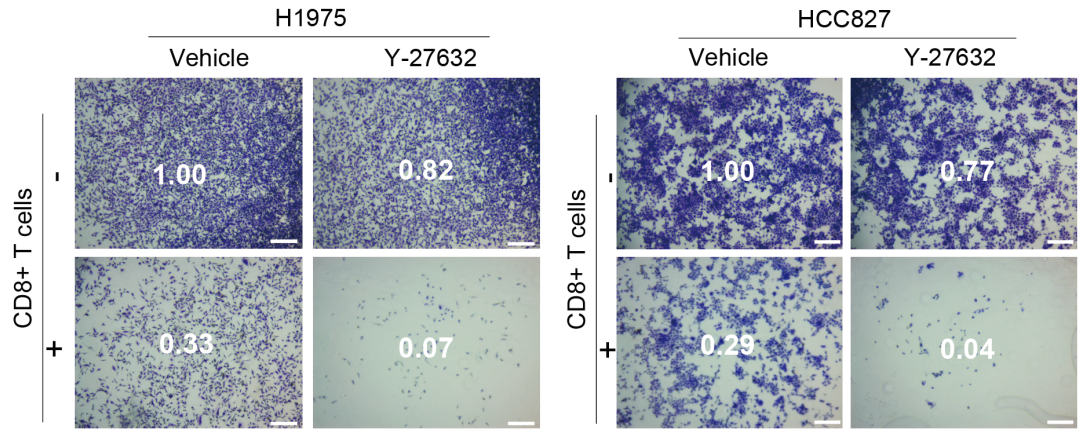


Supplementary Figure 2. Immunosuppressive microenvironment in EGFR-mutant tumor model. (**A**) Immunoblotting of EGFR A750del and FLAG in MC38 cells transfected with wildtype or A750del mutant EGFR. Empty vector (Mock) was used as negative control. (**B**) Flow cytometry analysis showing the proportion of CD45+ cells, CD3+CD8+ T cells, and the expression of IFNγ+ and Ki-67+ in CD3+CD8+ T cells in EGFR-WT and EGFR-A750del mutant tumors (n=5, means±SD). *, $P<0.05$; **, $P<0.01$; two-tailed Student *t* test.

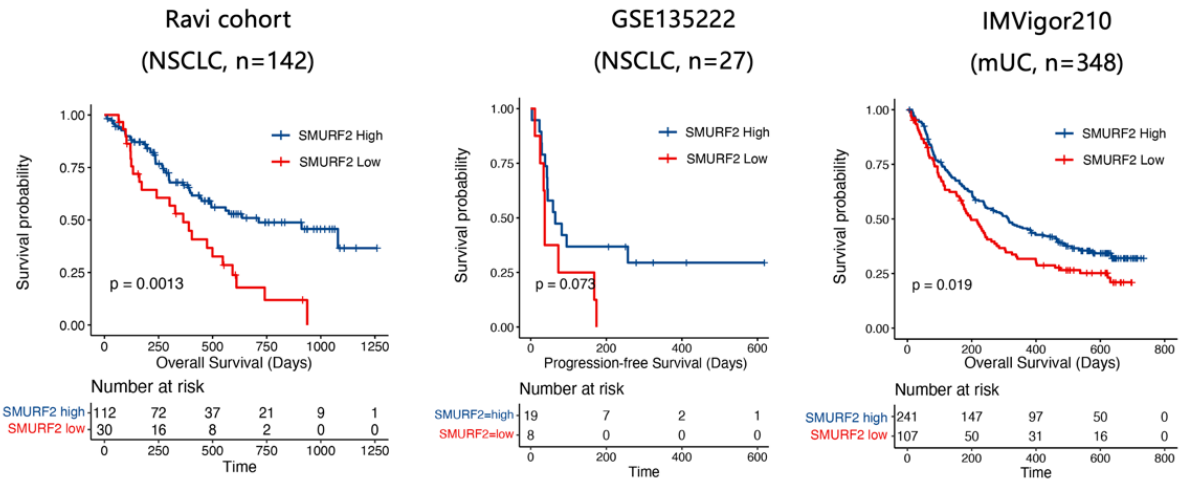


Supplementary Figure 3. Comprehensive analysis of tumor immune microenvironment following ROCK1 inhibition in MC38-EM tumors. Flow cytometry analysis showing the proportion of TIM-3+PD-1+ in CD3+CD8+ T cells, CD3+CD4+ T cells, CD25+FOXP3+ regulatory T cells (Tregs), macrophages (F4/80+

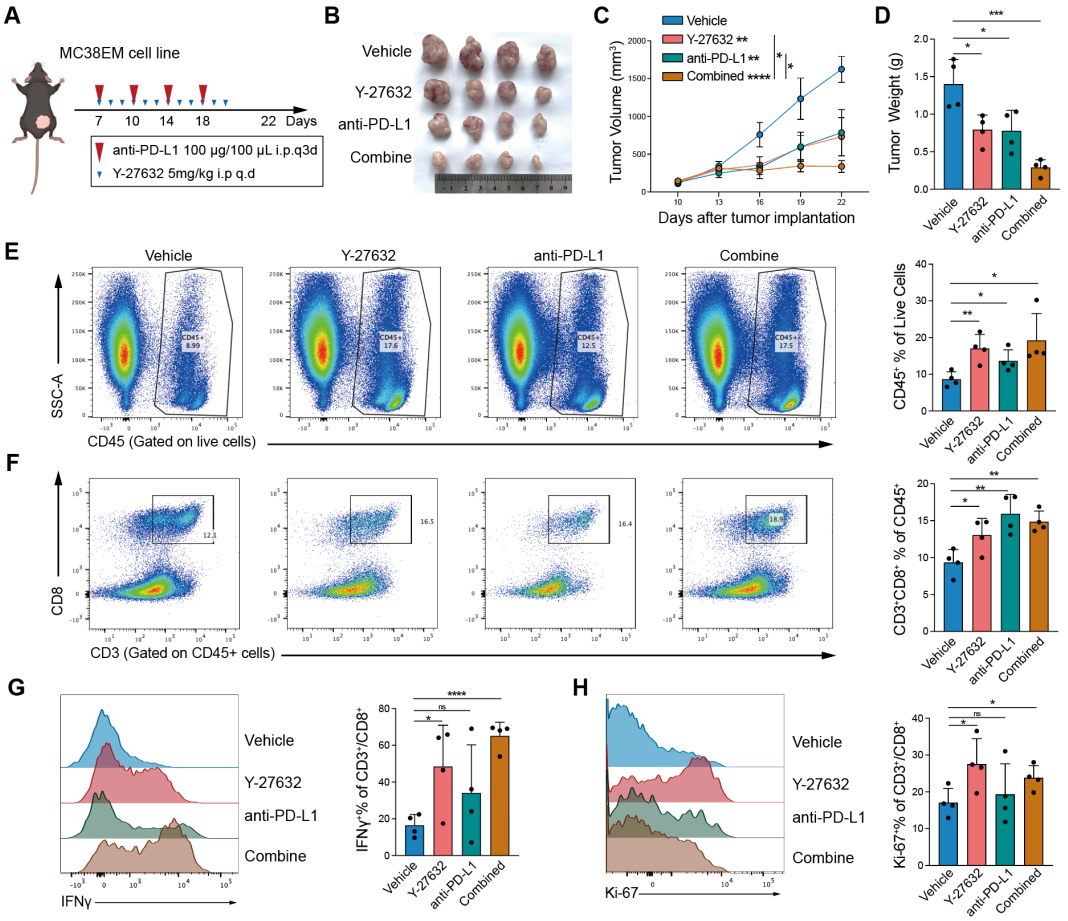
LY6G-low of CD11b+), M1-like macrophages (CD80+), M2-like macrophages (CD206+), PMN-MDSCs, M-MDSCs, and dendritic cells (DC-like) in MC38-EM tumors treated with vehicle or Y-27632 (n=4, means±SD). ns, not significant; two-tailed Student *t* test.



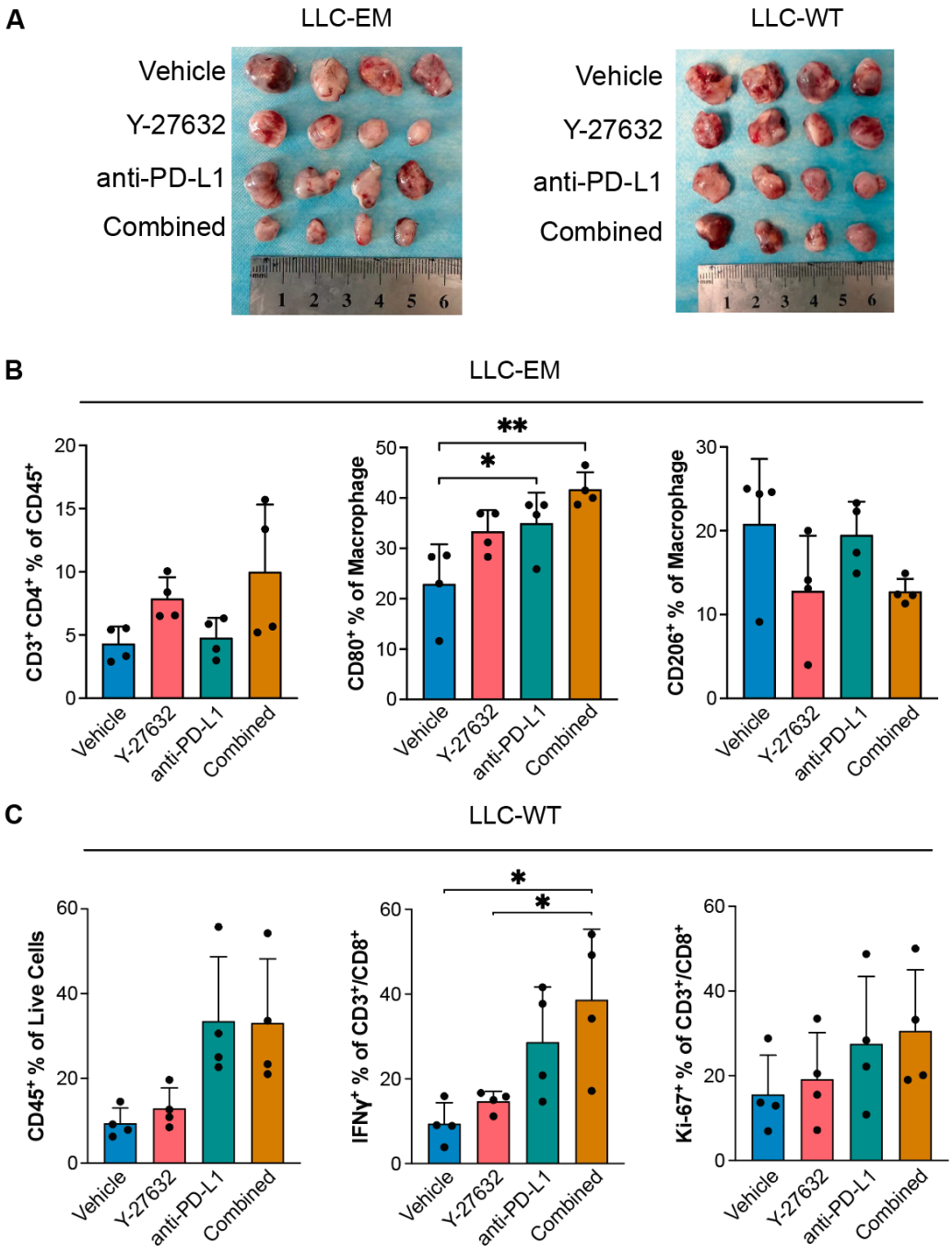
Supplementary Figure 4. Inhibition of ROCK1 using Y-27632 promotes CD8+ T cell-mediated tumor cell killing. H1975 (left) and HCC827 (right) cells were pre-treated with vehicle or Y-27632 for 48 hours, then cocultured with or without CD8+ T cells. The normalized absorbance relative to the vehicle control without CD8+ T cells is shown in each panel. Scale bars, 100 μm.



Supplementary Figure 5. SMURF2 expression correlates with better prognosis in patients receiving immunotherapy. Kaplan-Meier survival analysis of patients stratified by SMURF2 expression in the Ravi cohort (NSCLC, n=142; overall survival), GSE135222 cohort (NSCLC, n=27; progression-free survival), and IMVigor210 cohort (mUC, n=348; overall survival). *P* values were determined by log-rank test.

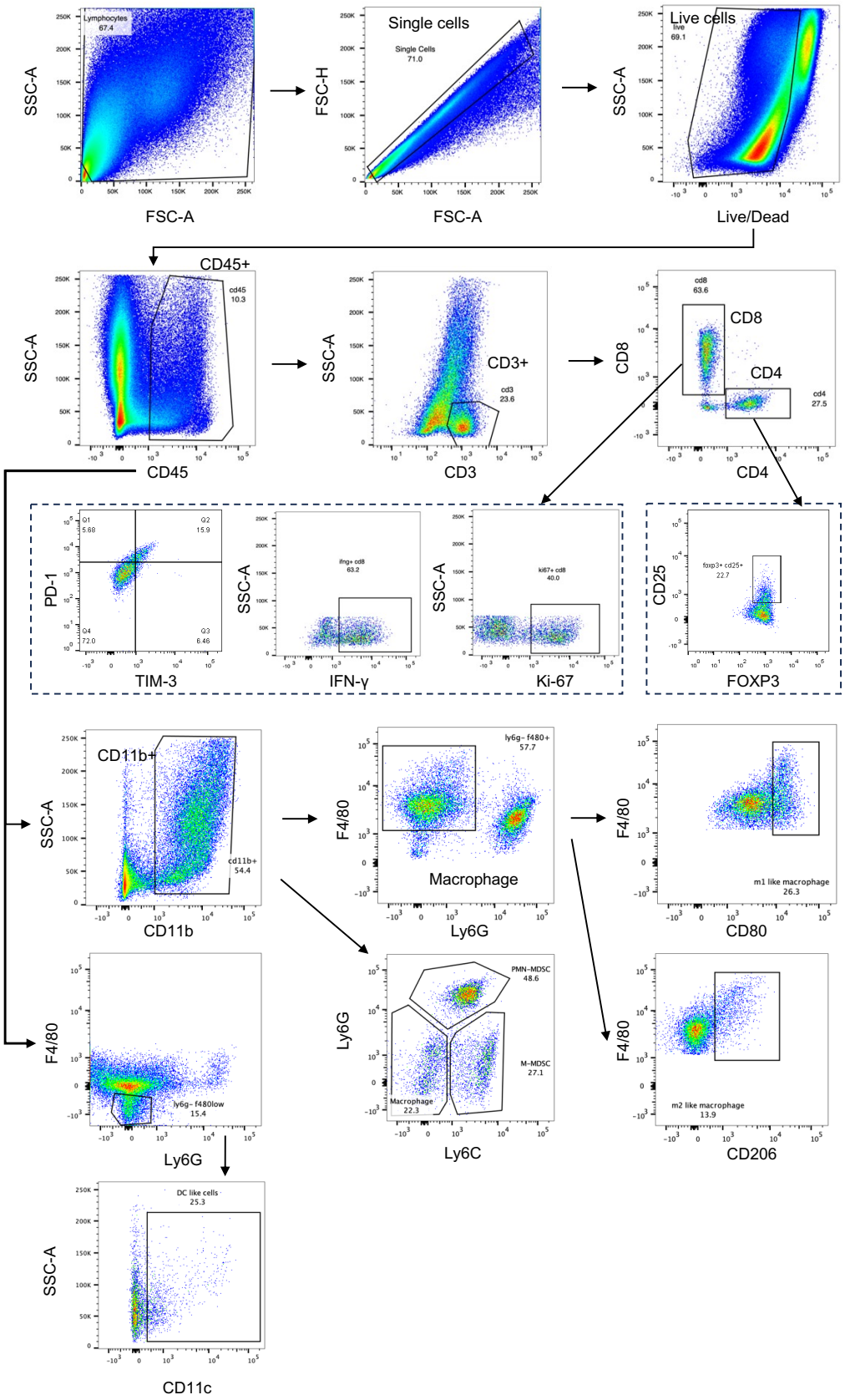


Supplementary Figure 6. ROCK1 inhibition enhances anti-tumor efficacy of anti-PD-L1 therapy in MC38-EM tumors. (**A**) Experimental design to evaluate the in vivo effect of vehicle, Y-27632, anti-PD-L1, or combined therapy (Y-27632 + anti-PD-L1) in C57BL/6 mice bearing MC38-EM tumors. (**B**) Macroscopic image of excised MC38-EM tumors. (**C** and **D**) Tumor growth curves (C) and tumor weight (D) of MC38-EM tumors treated with vehicle, Y-27632, anti-PD-L1, or combined therapy (n=4, means±SD). (**E** and **F**) Representative flow cytometry plots and analysis showing the proportion of CD45+ cells (E) and CD3+CD8+ T cells (F) in tumors (n=4, means±SD). (**G** and **H**) Representative flow cytometric histograms and analysis showing the expression of IFNγ+ (G) and Ki-67+ (H) in CD3+CD8+ T cells in tumors (n=4, means±SD). ns, not significant; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; ****, $P<0.0001$; two-tailed Student *t* test.



Supplementary Figure 7. Macroscopic tumor images and additional immune profiling of LLC-EM and LLC-WT tumors. (**A**) Macroscopic images of excised LLC-EM (left) and LLC-WT (right) tumors treated with vehicle, Y-27632, anti-PD-L1, or combined therapy. (**B**) Flow cytometry analysis showing the proportion of CD3+CD4+ T cells (left), M1-like macrophages (CD80+, middle), and M2-like macrophages (CD206+, right) in LLC-EM tumors treated with vehicle, Y-27632, anti-

PD-L1, or combined therapy (n=4, means±SD). (**C**) Flow cytometry analysis showing the proportion of CD45+ cells (left), IFNγ+ in CD3+CD8+ T cells (middle), and Ki-67+ in CD3+CD8+ T cells (right) in LLC-WT tumors treated with vehicle, Y-27632, anti-PD-L1, or combined therapy (n=4, means±SD). ns, not significant; *, $P<0.05$; **, $P<0.01$; one-way ANOVA with Tukey's post-hoc test for multi-group comparisons.



Supplementary Figure 8. Flow cytometry gating strategy. Representative gating strategy for the identification of immune cell populations in tumor tissues.