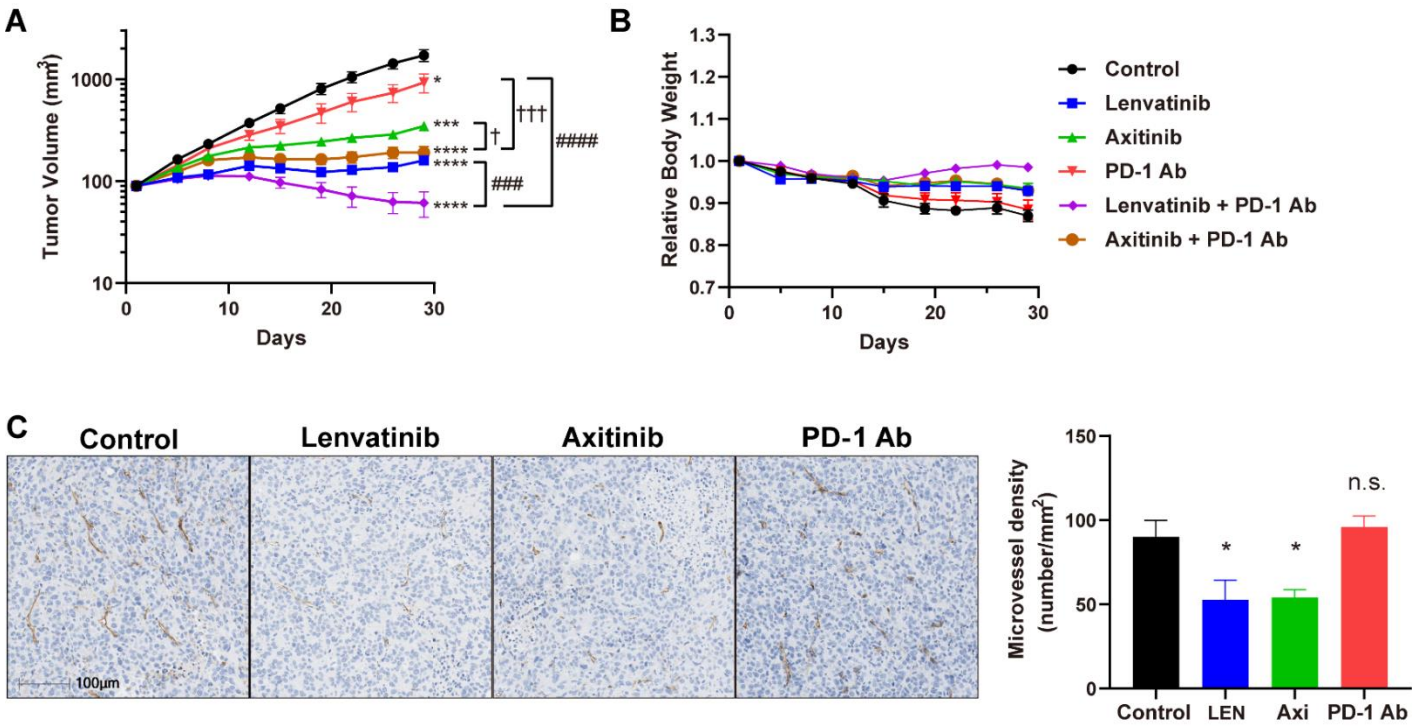
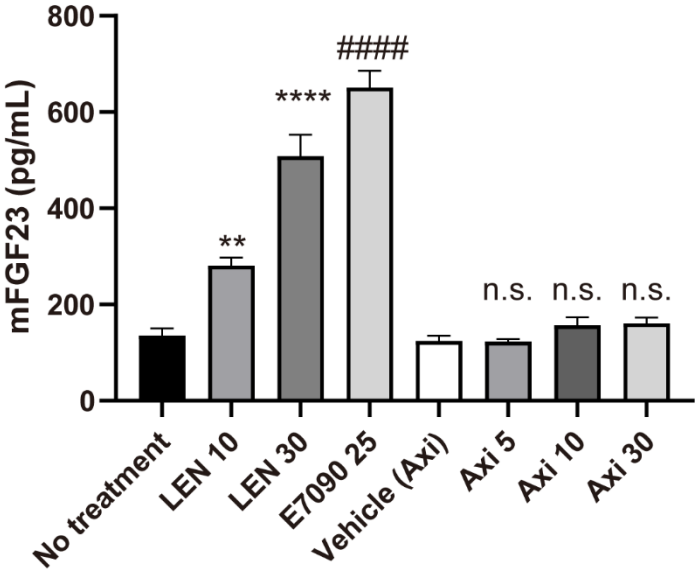
Supplementary Figure S1



Supplementary Figure S1. Tumor growths, relative body weights, and microvessel density in the RAG tumor model. Mice bearing RAG tumors were allocated into each treatment group on day 1 (when tumor volumes were approximately 90 mm^3) and then treated with lenvatinib at 10 mg/kg once daily, axitinib at 10 mg/kg twice daily, or anti-PD-1 mAb at 200 μg/mouse twice weekly for 4 weeks. Data from 2 independent experiments were combined (n = 20). **A**. Tumor growth curves (days 1–29). Data are shown as mean ± SEM. **P* < 0.05, ****P* < 0.001, *****P* < 0.0001 compared with control group; ###*P* < 0.001, ####*P* < 0.0001 compared with lenvatinib plus anti-PD-1 mAb combination group; †*P* < 0.05, †††*P* < 0.001 compared with axitinib plus anti- mAb combination group (Dunnett's multiple comparison test after logarithmic transformation). **B.** Relative body weight during treatment period (days 1–29). Data are presented as means ± SEM. **C**. Antiangiogenic activities of lenvatinib, axitinib, and anti-PD-1 mAb in the RAG model. Mice bearing RAG tumors were allocated into treatment groups on day 1, when tumor volumes were approximately 110 mm^3, and then treated with lenvatinib at 10 mg/kg once daily, axitinib at 10 mg/kg twice daily, or anti-PD-1 mAb at 200 μg/animal twice weekly for 1 week. Tumor sections were stained with anti-CD31 antibody. Left panel, representative images from each group. Bars, 100 μm. Right

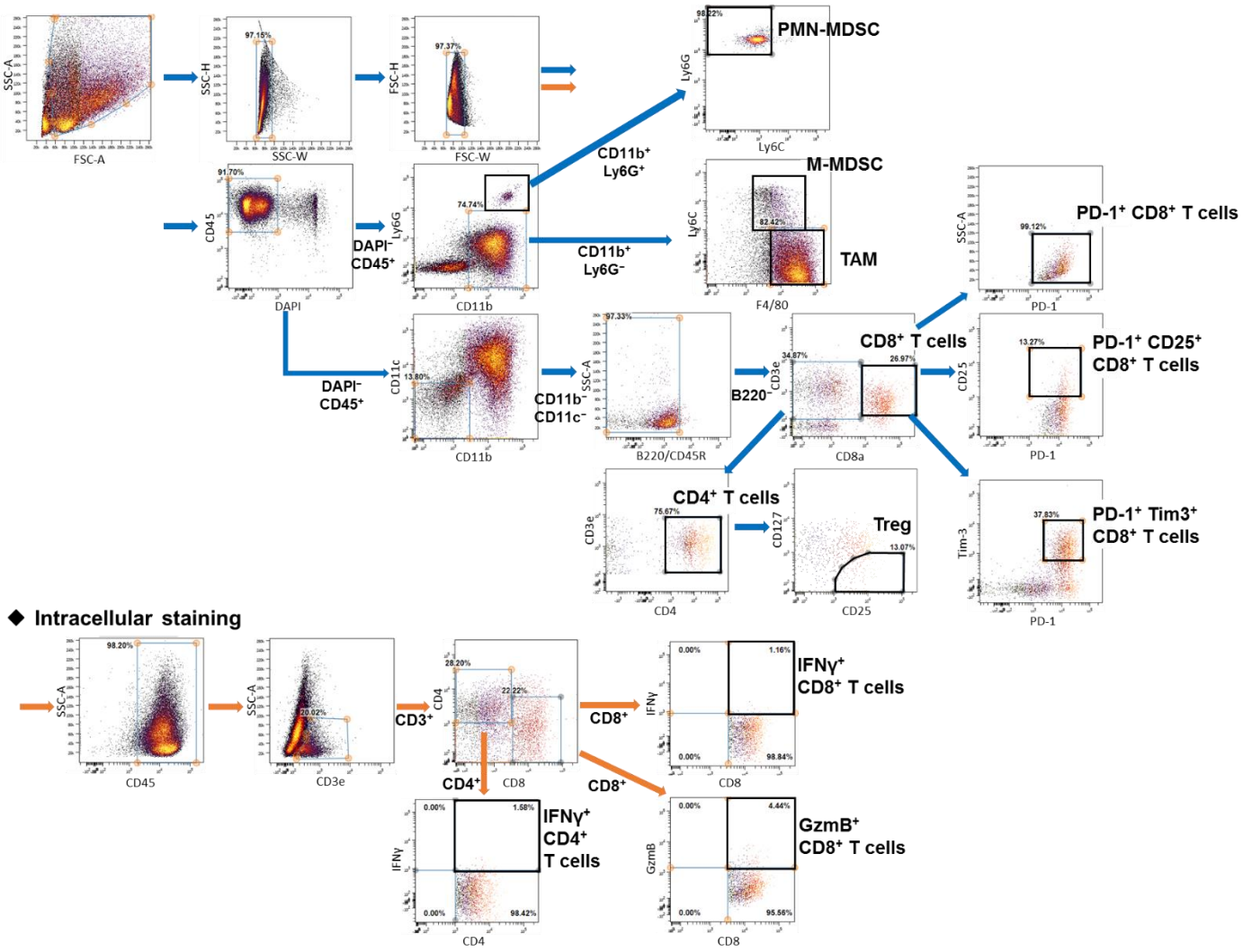
panel, microvessel density (number of microvessels per square millimeter) in each treatment group. Data are shown as mean + SEM (n = 5). *$P < 0.05$ unpaired *t*-test vs control group. PD-1 Ab, anti-PD-1 antibody; LEN, lenvatinib; Axi, axitinib.

Supplementary Figure S2



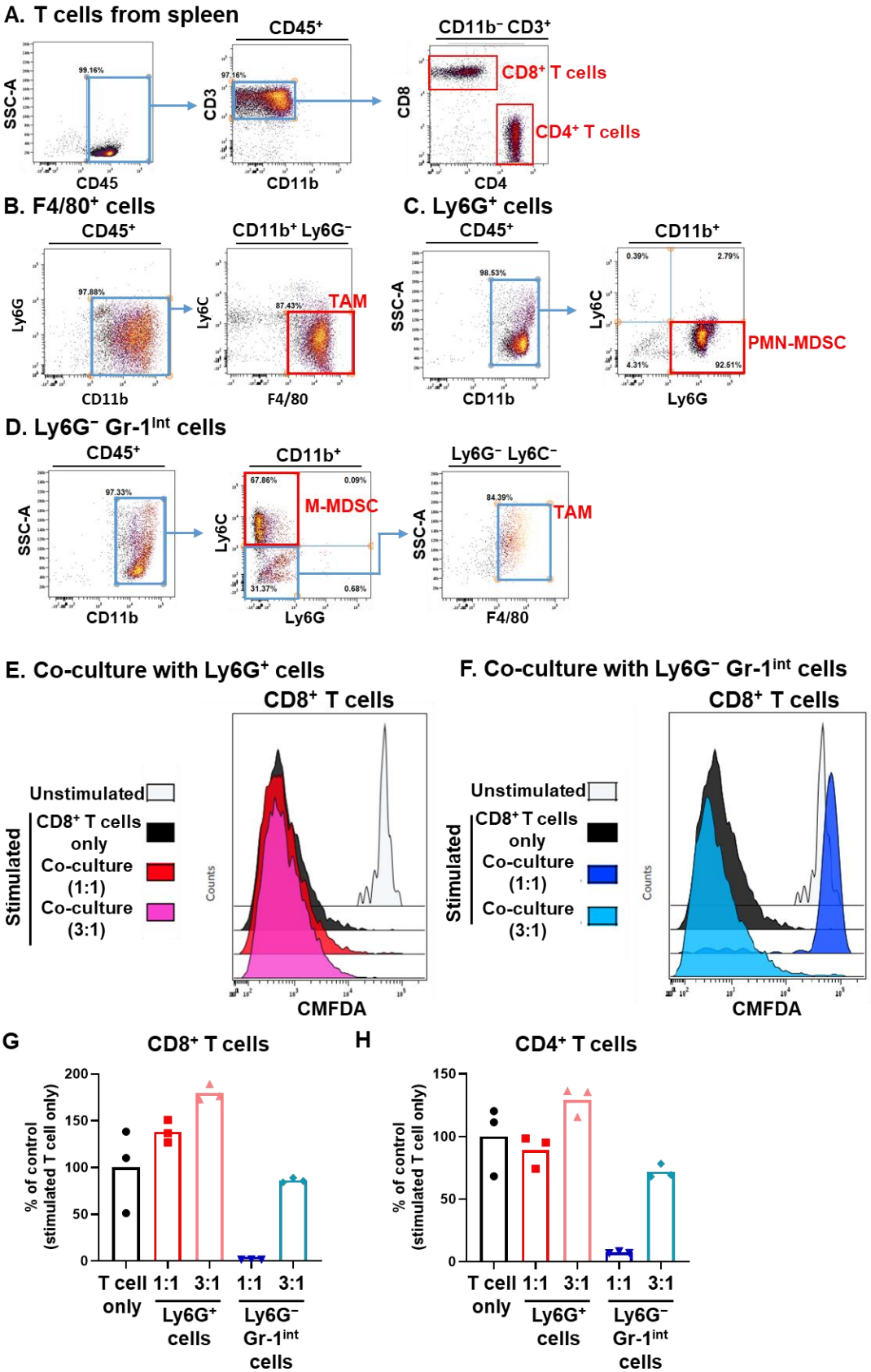
Supplementary Figure S2. Plasma levels of mouse FGF23 after treatment with lenvatinib or axitinib. Balb/c mice received a single dose of lenvatinib at 10 or 30 mg/kg, a single dose of E7090 at 25 mg/kg, twice-daily doses of the vehicle for axitinib or axitinib at 5, 10, or 30 mg/kg. At 24 hours after the first administration, mice were anesthetized, blood was withdrawn, and plasma was prepared. The mouse plasma levels of FGF-23 shown represent data pooled from two independent experiments (n = 16; lenvatinib at 10 mg/kg, n = 15). Data are shown as mean ± SEM. $^{**}P < 0.01$, $^{****}P < 0.0001$ Dunnett's multiple comparison test vs no-treatment group (black bar), $^{####}P < 0.0001$ unpaired *t*-test vs no-treatment group (black bar). n.s., not significant, Dunnett's multiple comparison test vs vehicle (Axi) group (white bar). LEN, lenvatinib; Axi, axitinib.

Supplementary Figure S3



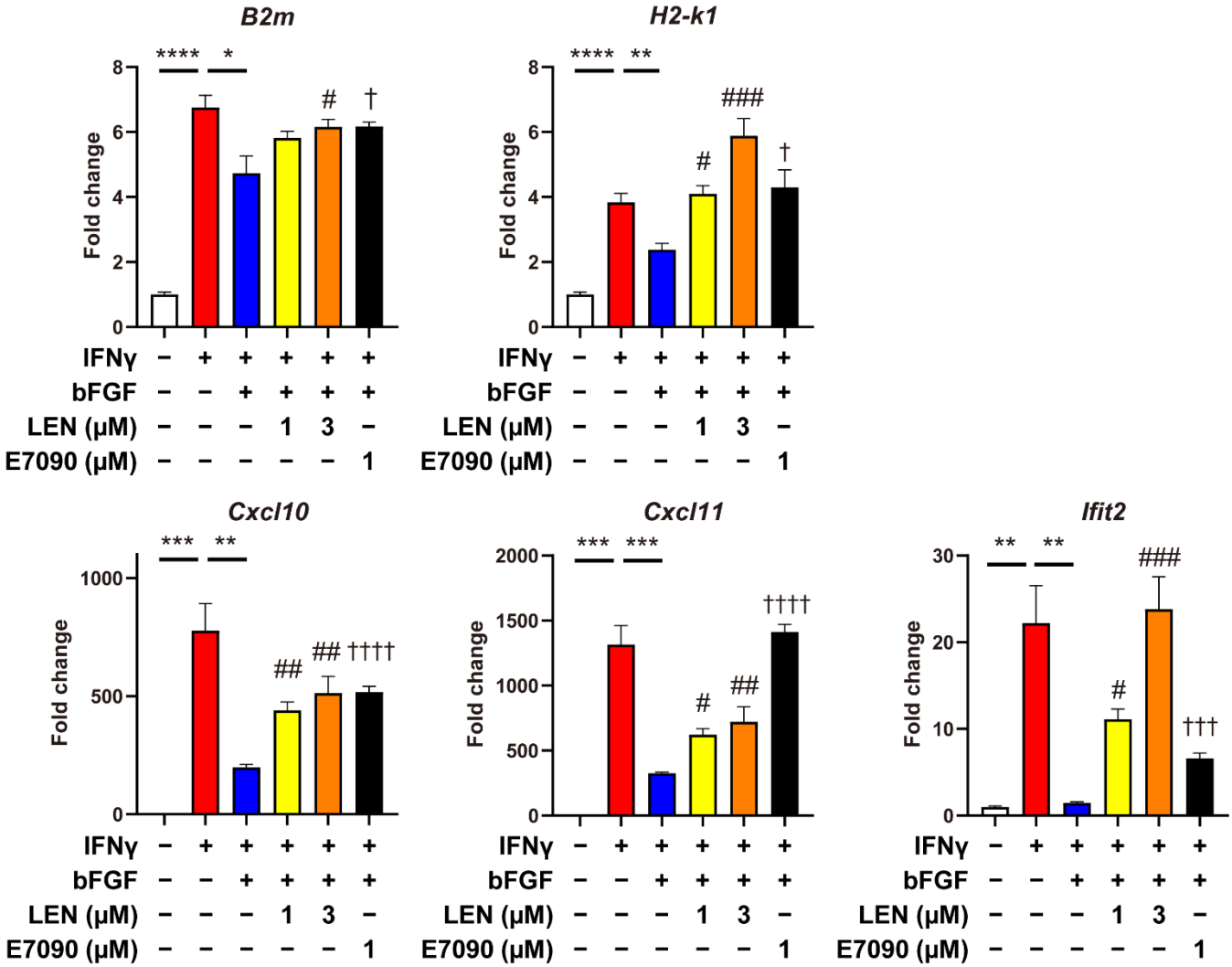
Supplementary Figure S3. Gating strategy for the flow cytometry analysis of RAG tumors. Immune cell populations of TAMs, PMN-MDSCs, M-MDSCs, $CD8^+$ T cells, PD-1^+ $CD8^+$ T cells, IFNγ^+ $CD8^+$ T cells, GzmB$^+$ $CD8^+$ T cells, PD-1^+ Tim3^+ $CD8^+$ T cells, PD-1^+ $CD25^+$ $CD8^+$ T cells, $CD4^+$ T cells, IFNγ^+ $CD4^+$ T cells, and Treg were gated as indicated. TAM, tumor-associated macrophage; PMN-MDSCs, polymorphonuclear myeloid-derived suppressor cells; M-MDSCs, monocytic MDSCs; Treg, regulatory T cells.

Supplementary Figure S4



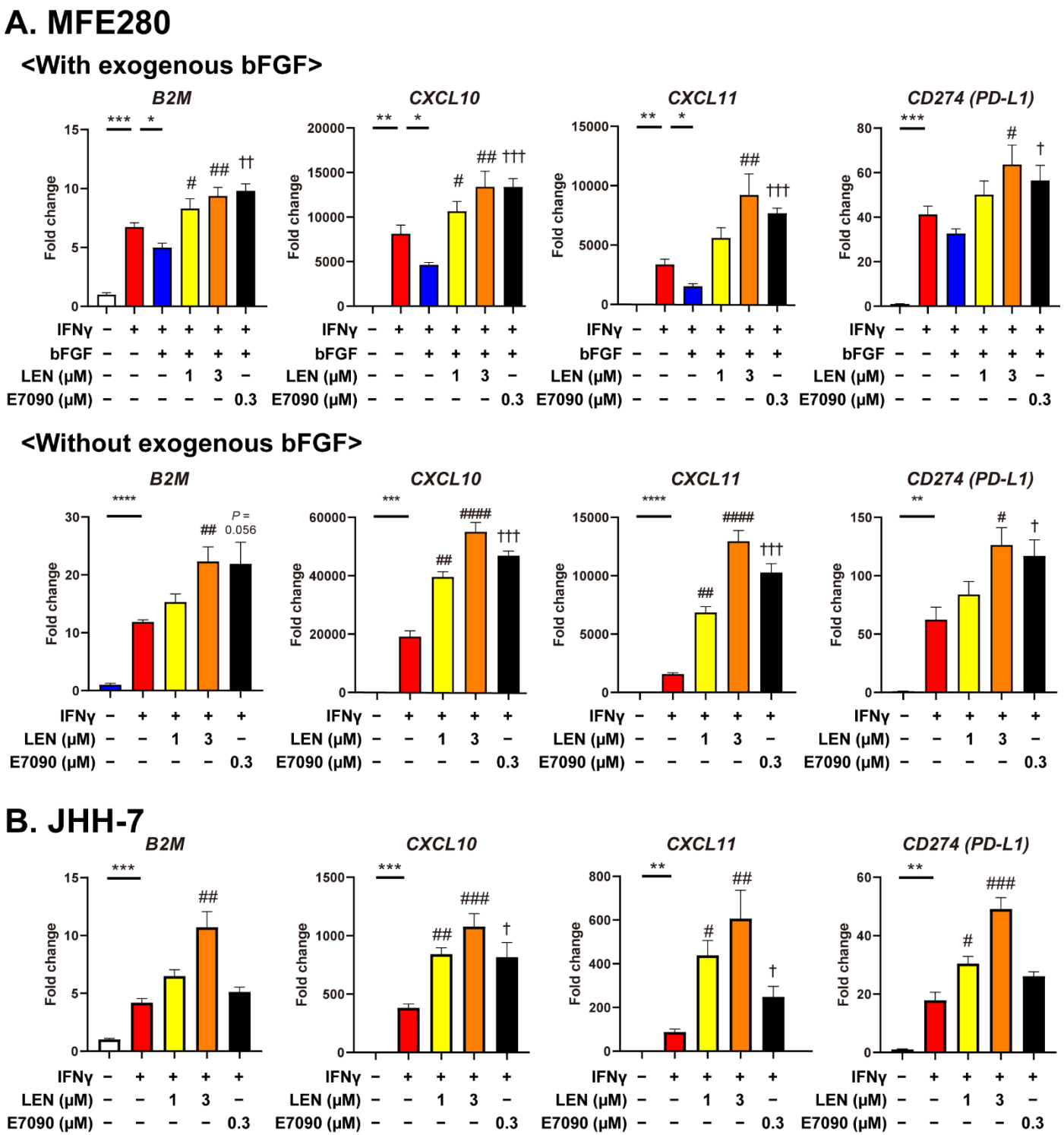
Supplementary Figure S4. Flow cytometry of isolated T cells, Ly6G$^+$ cells, Ly6G$^-$ Gr-1^{int} (Gr-1^{intermediate}) cells, and F4/80$^+$ cells and ex vivo co-culture assay of T cells with Ly6G$^+$ cells and Ly6G$^-$ Gr-1^{int} cells isolated from RAG tumors. Naïve T cells were isolated from the spleens of Balb/c mice with RAG tumors. F4/80$^+$ cells, Ly6G$^+$ cells, and Ly6G$^-$ Gr-1^{int} cells were isolated from RAG tumors. **A–D.** The isolated cells were analyzed by flow cytometry. **A.** The populations of CD8$^+$ T cells (CD45$^+$ CD3$^+$ CD11b$^-$ CD8$^+$) and CD4$^+$ T cells (CD45$^+$ CD3$^+$ CD11b$^-$ CD4$^+$) in naïve T cells isolated from spleen. **B.** The population of TAMs (CD45$^+$ CD11b$^+$ Ly6G$^-$ Ly6C$^-$ F4/80$^+$) in F4/80$^+$ cells isolated from tumors. **C.** The population of PMN-MDSCs (CD45$^+$ CD11b$^+$ Ly6G$^+$ Ly6C$^-$) in Ly6G$^+$ cells. **D.** The populations of M-MDSCs (middle; CD45$^+$ CD11b$^+$ Ly6G$^-$ Ly6C$^+$) and small populations of contaminated TAMs (right; CD45$^+$ CD11b$^+$ Ly6G$^-$ Ly6C$^-$ F4/80$^+$) in isolated Ly6G$^-$ Gr-1^{int} cells. **E-H.** Ex vivo co-culture assay of T cells with Ly6G$^+$ cells and Ly6G$^-$ Gr-1^{int} cells. Naïve T cells were labeled with CMFDA dye (CellTracker), stimulated with anti-CD3/CD28 beads, and co-cultured with Ly6G$^+$ cells and Ly6G$^-$ Gr-1^{int} cells for 4 days at the indicated ratios (T cells: Ly6G$^+$ cells or Ly6G$^-$ Gr-1^{int} cells). The proliferation of T cells (CD4$^+$ T cells, CD45$^+$ CD3$^+$ CD4$^+$; CD8$^+$ T cells, CD45$^+$ CD3$^+$ CD8$^+$) was analyzed via flow cytometry. **E** and **F.** Histograms of CMFDA-labeled CD8$^+$ T cells co-cultured with (**E**) Ly6G$^+$ cells or (**F**) Ly6G$^-$ Gr-1^{int} cells. **G** and **H.** Proliferation of (**F**) CD8$^+$ T cells and (**H**) CD4$^+$ T cells co-cultured with Ly6G$^+$ cells or Ly6G$^-$ Gr-1^{int} cells compared with T cell single culture (T cells only). Data are representative of two independent experiments performed in triplicate.

Supplementary Figure S5



Supplementary Figure S5. Inhibitory effects of FGFR signaling on expression levels of IFNγ-induced genes in RAG cells. RAG cells were treated first with lenvatinib at 1 or 3 μM or with E7090 at 1 μM for 1 hour and then with bFGF (10 ng/mL) for 23 hours. Afterward, cells were stimulated with IFNγ (5 ng/mL) for 24 hours. Expression levels of IFNγ-target genes were determined through qRT-PCR analysis. Data were normalized according to the expression level of the *Gapdh* gene. Data are presented as means + SEM of 4 independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ unpaired *t*-test between groups; #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$, Dunnett's multiple comparison test vs bFGF+IFNγ-treated group (blue bar); †$P < 0.05$, †††$P < 0.001$, ††††$P < 0.0001$, unpaired *t*-test vs bFGF+IFNγ-treated group (blue bar). bFGF, basic fibroblast growth factor; LEN, lenvatinib.

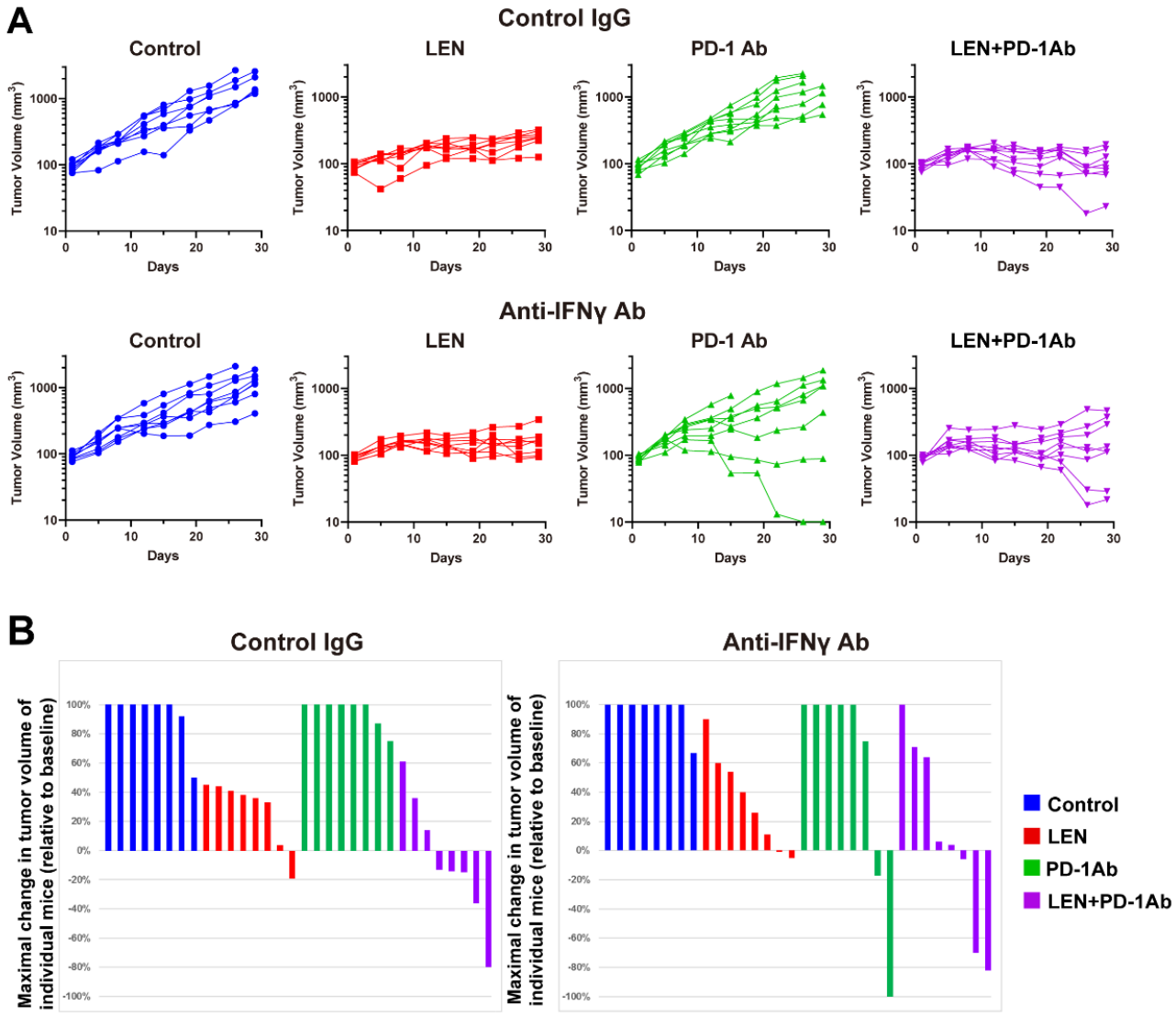
Supplementary Figure S6



Supplementary Figure S6. Inhibitory effects of FGFR signaling on expression levels of IFNγ-induced genes in human cancer cells. **A**. (upper panel; with exogenous bFGF) MFE280 cells were treated first with lenvatinib at 1 or 3 μM or with E7090 at 0.3 μM

for 1 hour and then treated with bFGF (10 ng/mL) for 23 hours. Afterward, cells were stimulated with IFNγ (5 ng/mL) for 24 hours. (lower panel; without exogenous bFGF) MFE280 cells were treated with lenvatinib at 1 or 3 μM or with E7090 at 0.3 μM for 24 hours and then stimulated with IFNγ (5 ng/mL) for 24 hours. **B.** JHH-7 cells were treated first with lenvatinib at 1 or 3 μM or with E7090 at 0.3 μM for 24 hours and then stimulated with IFNγ (5 ng/mL) for 24 hours. Expression levels of IFNγ-target genes were determined by using qRT-PCR analysis. Data were normalized according to the expression level of the *GAPDH* gene. Data are presented as means + SEM of 3 independent experiments. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001, unpaired *t*-test between groups; #*P* < 0.05, ##*P* < 0.01, ###*P* < 0.001, ####*P* < 0.0001, Dunnett's multiple comparison test (**A**, upper panel) vs bFGF+IFNγ-treated group (blue bar) or (**A**, lower panel and **B**) vs IFNγ-treated group (red bar). †*P* < 0.05, ††*P* < 0.01, †††P < 0.001, unpaired *t*-test (**A**, upper panel) vs bFGF+IFNγ-treated group (blue bar) or (**A**, lower panel and **B**) vs IFNγ-treated group (red bar). bFGF, basic fibroblast growth factor; LEN, lenvatinib.

Supplementary Figure S7



Supplementary Figure S7. Effects of anti-IFNγ antibody on antitumor activity with lenvatinib, anti-PD-1 Ab, and its combination treatments. Individual tumor volumes and waterfall plots with anti-IFNγ blocking antibody in the RAG model. Mice bearing RAG tumors were injected intraperitoneally with anti-IFNγ mAb or control IgG1 at 300 μg/animal 2 days before allocation to treatment groups (ie, day –1) and twice weekly thereafter. On day 1, when tumor volumes were approximately 90 mm^3, mice were allocated randomly into treatment groups. Lenvatinib was orally administered at 10 mg/kg once daily, and anti-PD-1 mAb was injected intraperitoneally at 200 μg/mouse

twice weekly for 4 weeks. **A**. Tumor volumes of individual mice. (upper panel) Control IgG-treatment group, (lower panel) anti-IFNγ mAb treatment group. **B**. Waterfall plots showing the greatest percent change from baseline in the RAG model after day 8 from the initiation of each treatment. LEN, lenvatinib; PD-1 Ab, anti-PD-1 antibody.