Salmonella typhimurium suppresses tumor growth via the pro-inflammatory cytokine interleukin-1β

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SUPPLEMENTARY DATA

Supplement 1.

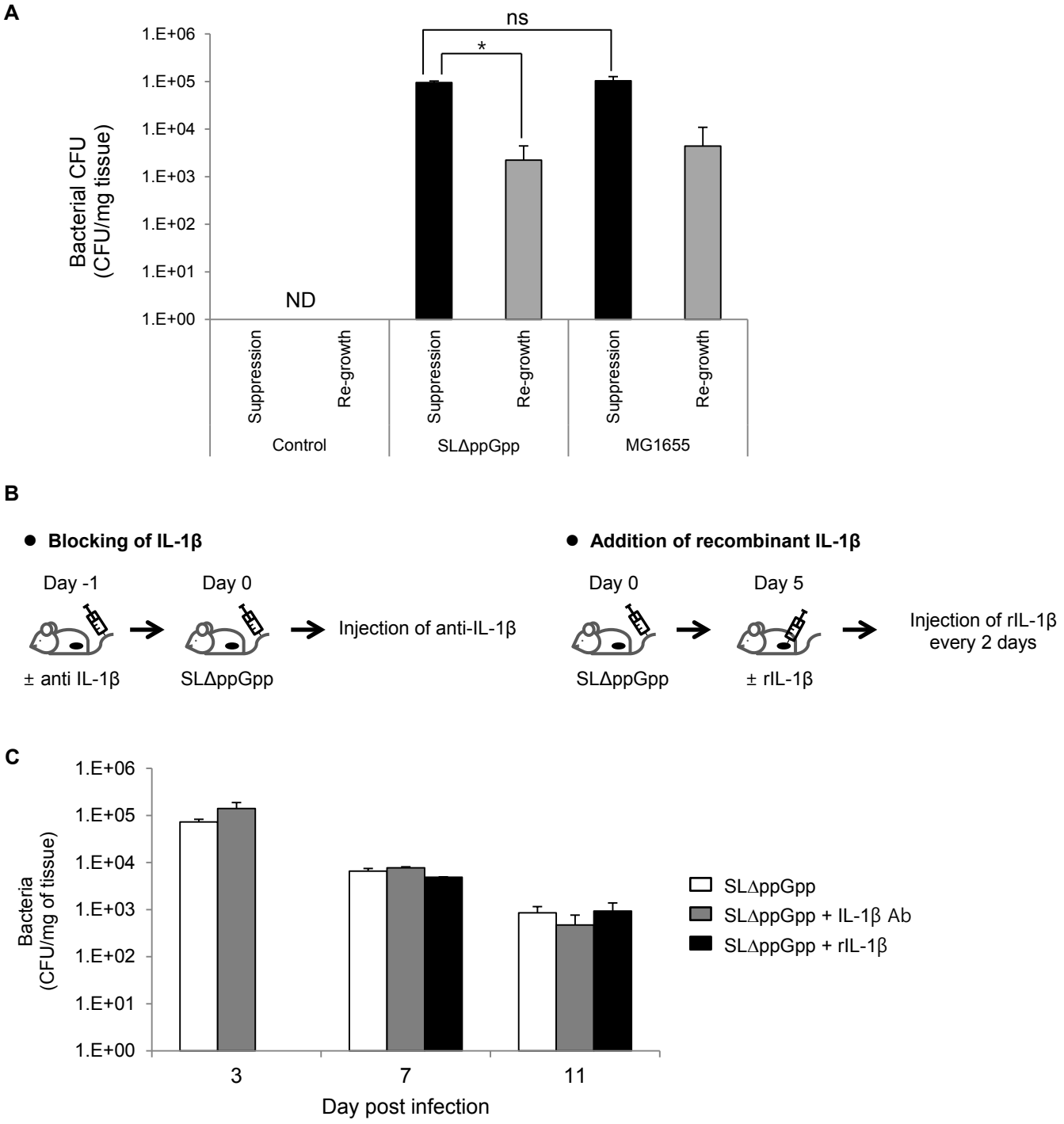

Figure S1. Number of bacteria in tumor tissue.
(A) *E. coli* (MG1655; 5×10^7 CFU) or *S. typhimurium* (SLΔppGpp; 4.5×10^7 CFU) were injected into tumor-bearing mice, and the number of bacteria in the tumor tissues was counted during the suppression stage (3 dpi) and recurrence stage (when tumor sizes reach around 1200mm^3). (B) Protocols for co-treatment with an IL-1β blocking antibody (left) or recombinant IL-1β (right), plus PBS or SLΔppGpp. (C) CT26 cells were transplanted into mice. Mice then received an intravenous (i.v.) SLΔppGpp (white bars). IL-1β depletion: mice received an i.v. injection of anti-IL-1β-specific antibody (IL-1β Ab) 1 day before SLΔppGpp treatment (Day -1). The antibody was then injected twice a week for 2 weeks (grey bars). Treatment with recombinant IL-1β (rIL-1β): mice received an intratumoral (i.t.) injection of rIL-1β on 5 dpi (black bars), followed by another i.t injection every other day up until 11 dpi. The number of bacteria in the tumor tissues was counted at 3, 7 and 11 dpi. Data represent the mean ± SD of three independent experiments. *P < 0.001.

Supplement 2.

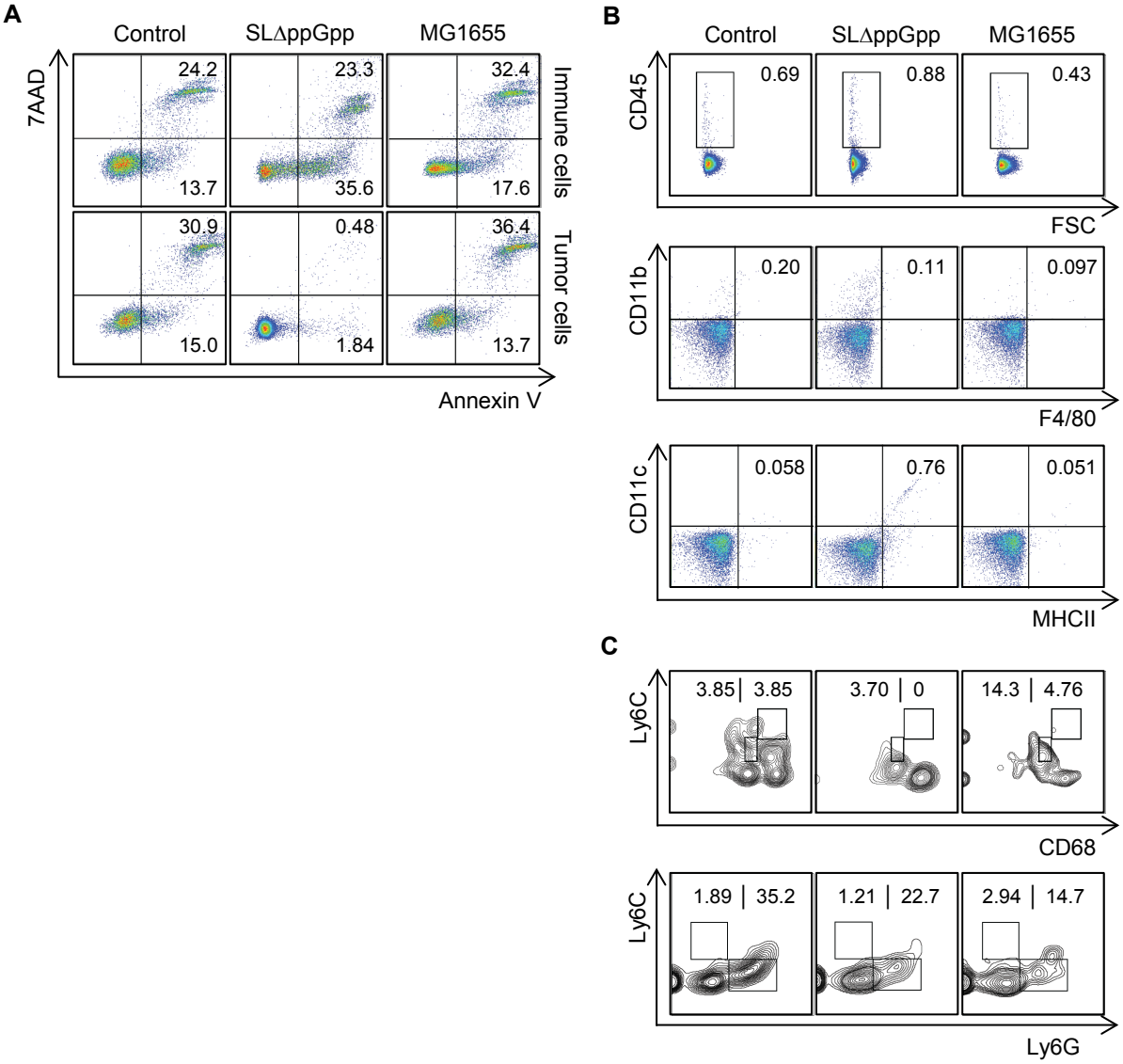

Figure S2. Immune cell population in tumor at the re-growing stage.
(A) Apoptosis was examined in tumor-resident cells at re-growing stage using Annexin V and 7AAD. Cells isolated from tumor (tumor volume > 1200 mm^3 or at 15 dpi) were separated into tumor cells and infiltrating immune cells using magnetic bead method. The upper and lower panels show infiltrating immune cells and tumor cells, respectively. (B) The immune cell population at the re-growing stage was examined using multi-color flow cytometry. The indicated percentages represented hematopoietic cells (CD45+), neutrophils (CD11b+Gr1+), macrophages (CD11b+F4/80+), and dendritic cells (CD11c+MHCII+), respectively. (C) The population of M1/M2 macrophages was examined by measuring CD68 and Ly6C expression in CD11b+F4/80+ cells (M1: $CD68^{int}$ $Ly6C^{int}$, M2: $CD68^{hi}$ $Ly6C^{hi}$). MDSCs within CD11b+ cell population were categorized as M-MDSC (monocytic MDSC, CD11b+$Ly6C^{hi}$Ly6G-) or PMN-MDSC (polymorphonuclear MDSC, CD11b+ $Ly6C^{low}$ Ly6G+). Data are representative of two indi vidual experiments, each with similar results.

Supplement 3.

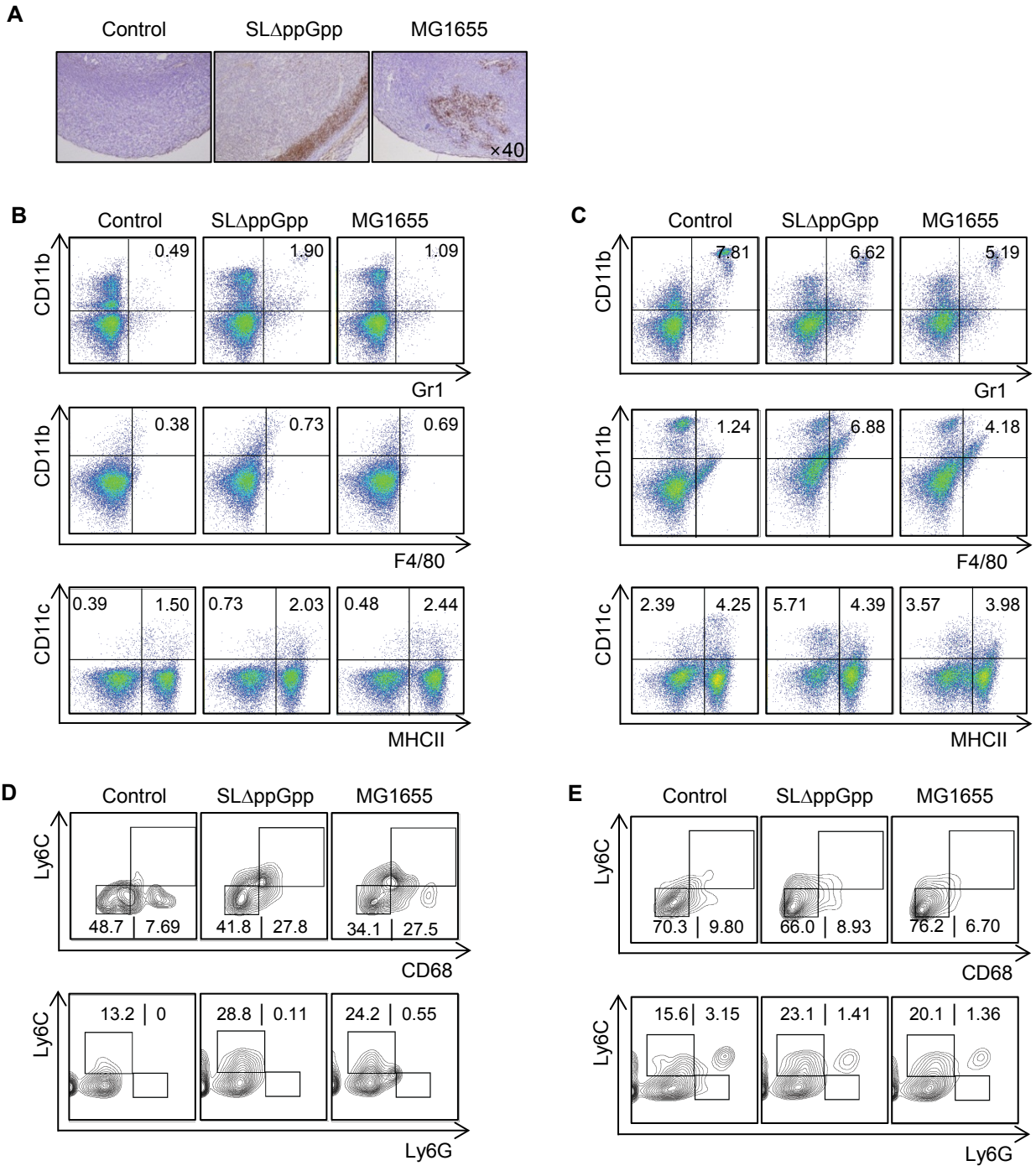

Figure S3. Colonization by SLΔppGpp leads to increased tumor infiltration by immune cells.

(A) Neutrophils infiltrating into the tumor were examined by immunohistochemistry as described in the legend to Figure 2A. Brown signals indicate Ly-6G/Ly-6C+ cells. Sections were counterstained with hematoxylin. In addition to the experiments depicted i n Figure 2B, the proportion of the immune cells in tumor-draining lymph nodes (B) and s pleens (C) were analyzed by flow cytometry at 2dpi. The indicated percentages represen t neutrophils (CD11b+Gr1+), macrophages (CD11b+F4/80+), and dendritic cells (CD11c +MHCII+), respectively. The population of M1/M2 macrophages was examined by measuring CD68 and Ly6C expression in CD11b+F4/80+ cells (M1: $CD68^{int}$ $Ly6C^{int}$, M2: $CD68^{hi}$ $Ly6C^{hi}$). MDSCs within CD11b+ cell population were categorized as M-MDSC (monocytic MDSC, CD11b+ $Ly6C^{hi}$ Ly6G-) and PMN-MDSC (polymorphonuclear MDSC, CD11b+ $Ly6C^{low}$ Ly6G+). M1/M2 and MDSC population in cells isolated from tumor-draining lymph nodes (D) and spleen (E) were analyzed. Data are representative of two individual experiments,each with similar results.

Supplement 4.

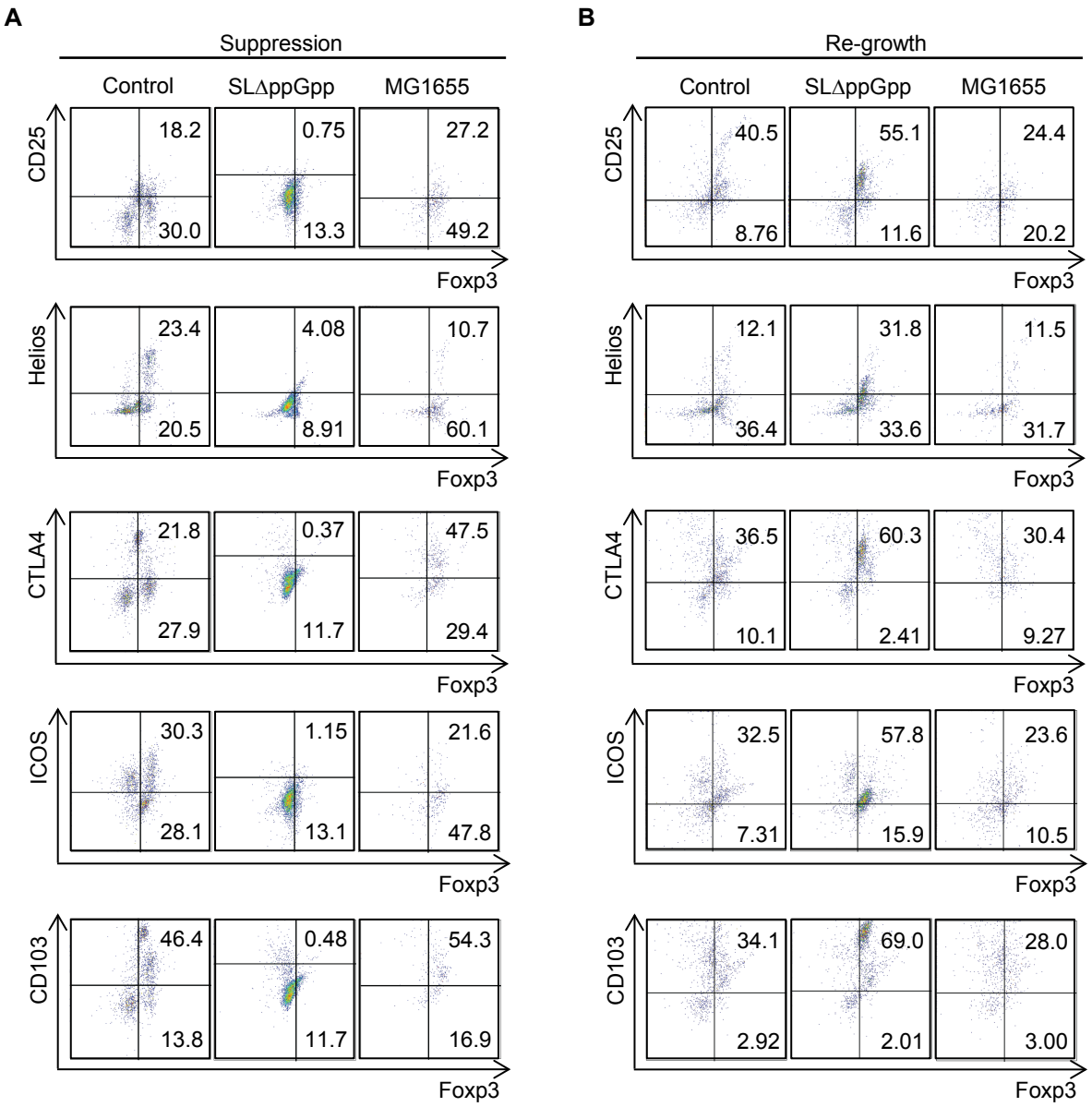

Figure S4. SLΔppGpp reduced the numbers and altered characteristics of Treg. Cells were stained with CD4 and Foxp3 to compare the proportion of Tregs among c ontrol, MG1655-, or SLΔppGpp-treated tumors. CD4+-gated cells are shown in each plot. The CD4+Foxp3+ population was examined by co-staining with Treg-related m arkers, including CD25, Helios, CTLA4, ICOS, and CD103. The numbers are indicative of each population in tumor tissue at the suppression (2 dpi) (A) and re-growing (at 15 dpi or when tumor volume exceeded 1200 mm^3) (B) stages. Data are representati ve of two individual experiments, each with similar results.

Supplement 5.

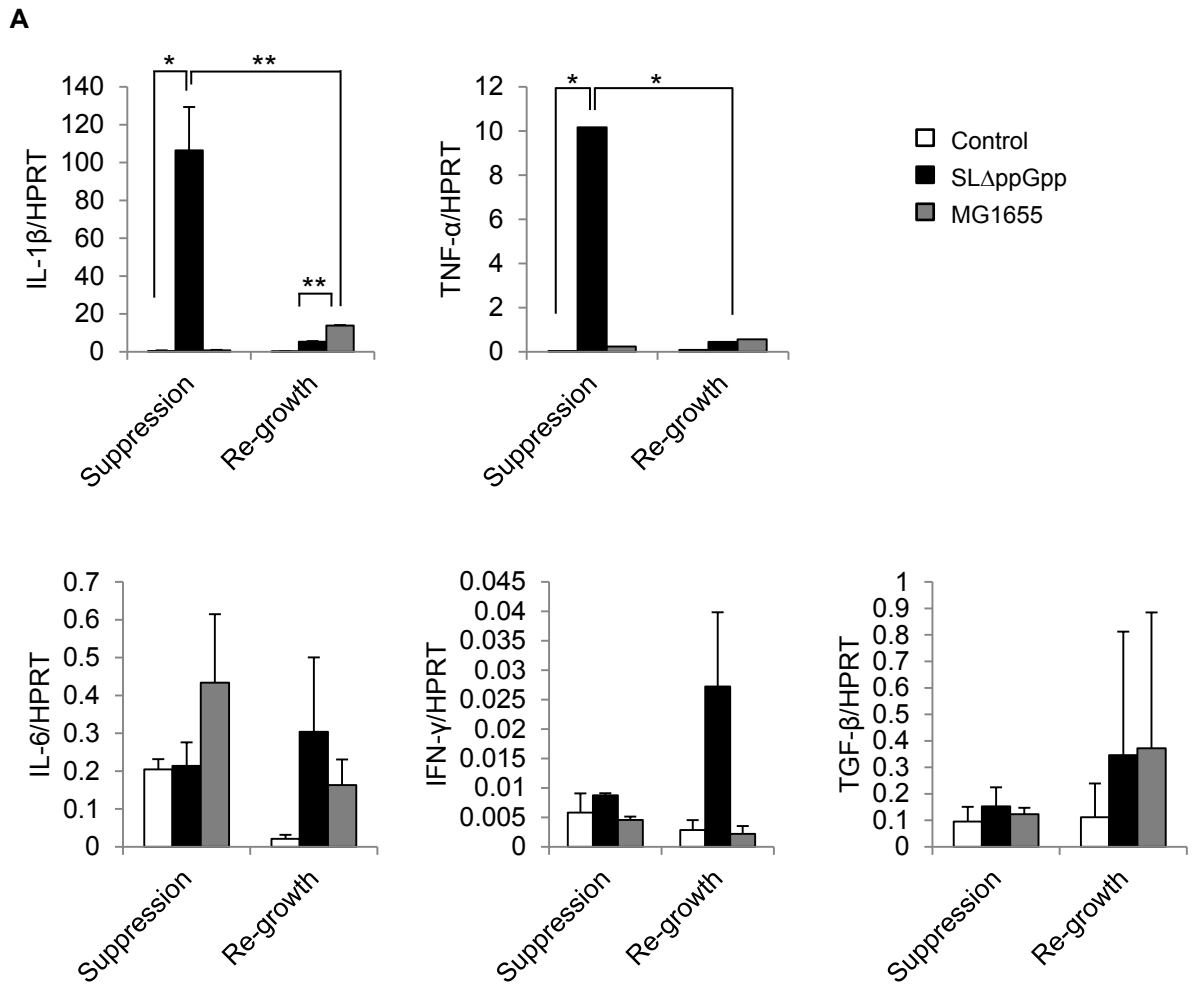

Figure S5. Cytokine profile in SLΔppGpp- or MG1655-colonized tumors during the suppression and re-growing stages.

(A) Cytokine expression patterns at the suppression (2dpi) and re-growing (tumor volume > 1200 mm^3 or at 15 dpi) stages were examined by RT-PCR after intravenous injection of PBS (control) or bacteria into tumor-bearing mice. Data are expressed as the mean ± SD of three separate experiments. (B) Cells isolated from the tumors at both stages were lysed, and the levels of caspase-1 and IL-1β were examined by western blotting. Results are representative of at least three individual experiments. *P < 0.05, **P < 0.005, and *** P < 0.001.

Supplementary 6

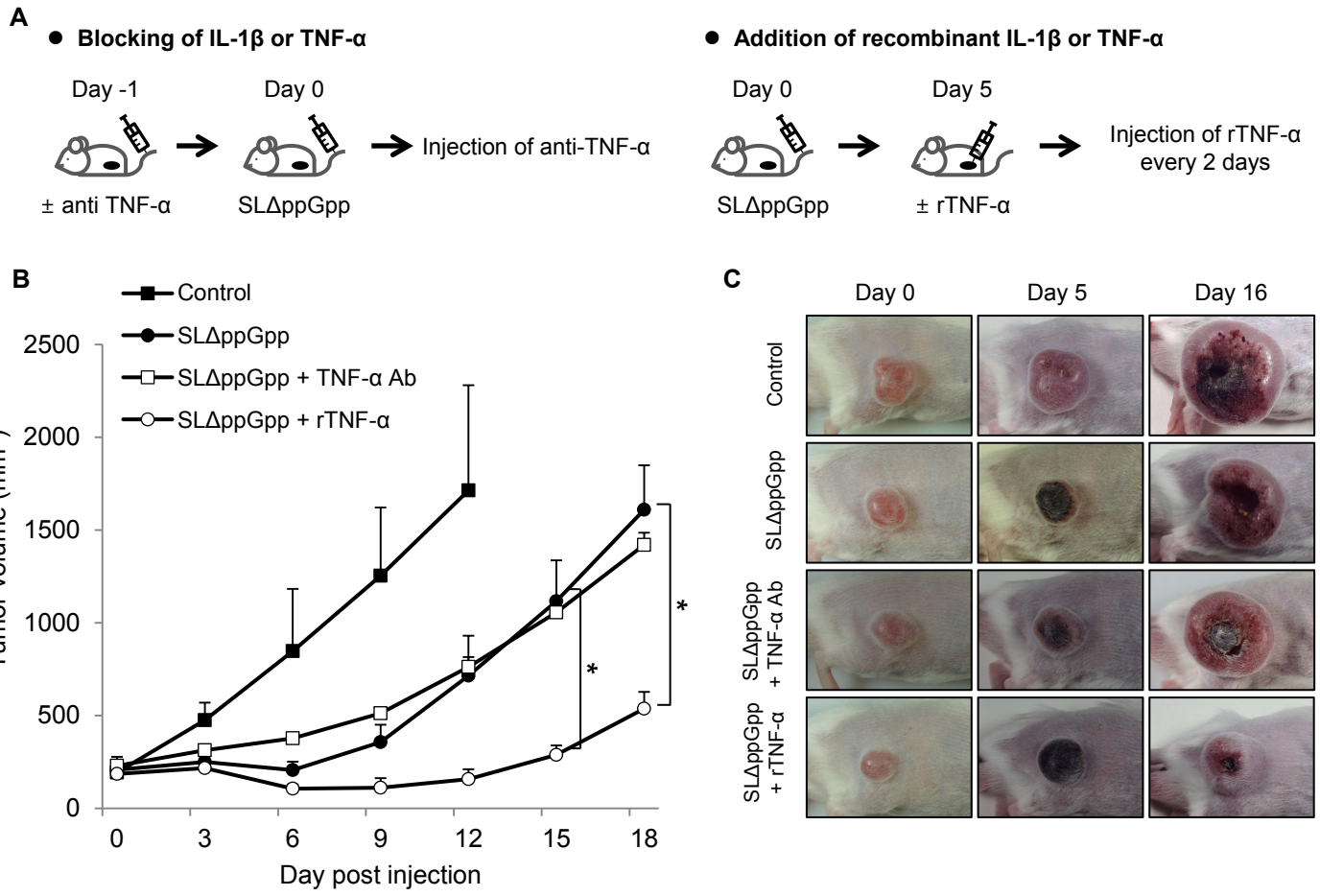

Figure S6. Role of TNF-α in SLΔppGpp-mediated cancer therapy.
(A) Protocols for co-treatment with a TNF-α blocking antibody (left) or TNF-α (right) plus PBS or SLΔppGpp. (B) CT26 cells were transplanted into mice. Mice then receive d an intravenous (i.v.) injection of PBS (black squares) or SLΔppGpp (black circles). To deplete TNF-α, mice were treated with an anti-TNF-α antibody (TNF-α Ab) 1 day bef ore SLΔppGpp treatment (Day -1). The antibody was then injected twice a week for 2 weeks (open squares). For treatment with recombinant TNF-α (rTNF-α), mice received rTNF-α via an intra-tumoral (i.t.) injection on Day 5 post-injection (pi) of SLΔppGpp (open circles), followed by another i.t. injection every other day up until 11 dpi. (E) Ph otographs of representative animals in each group were taken before (0 dpi) and aft er treatment (5 and 16 dpi). Data represent the mean ± SD. Results from at least thre e individual experiments are shown. *P < 0.05.