Powerful anti-colon cancer effect of modified nanoparticle-mediated IL-15 immunogene therapy through activation of the host immune system

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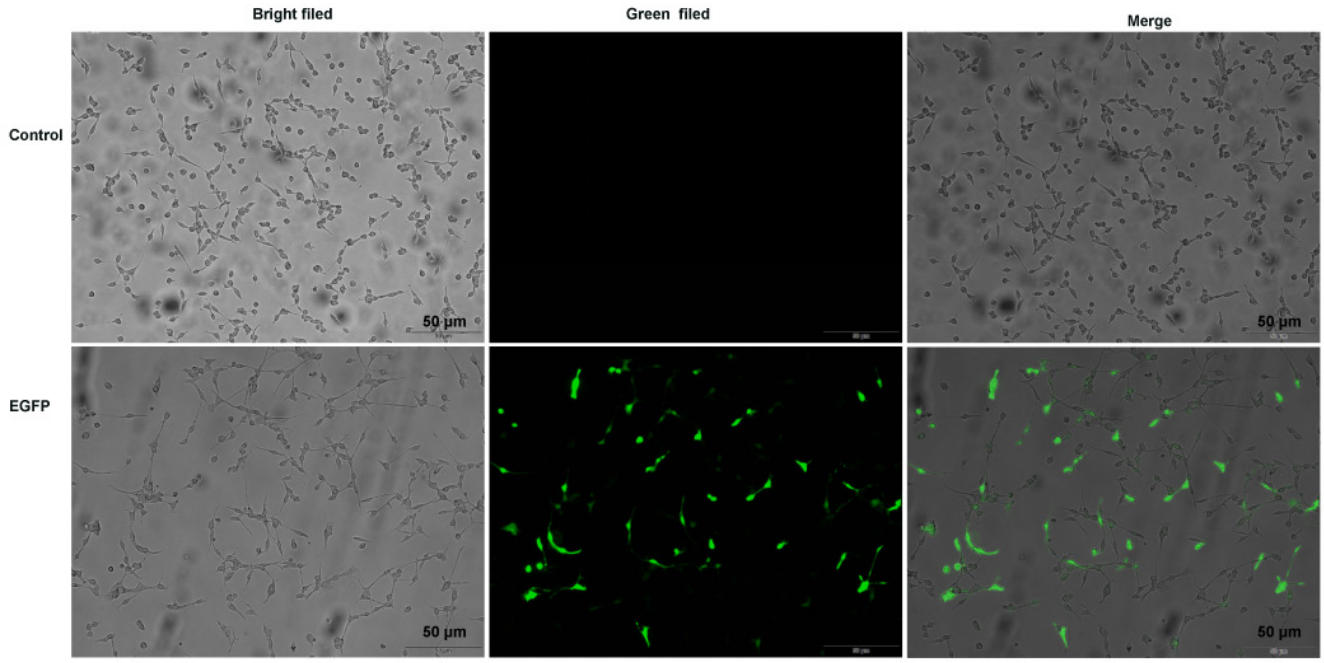
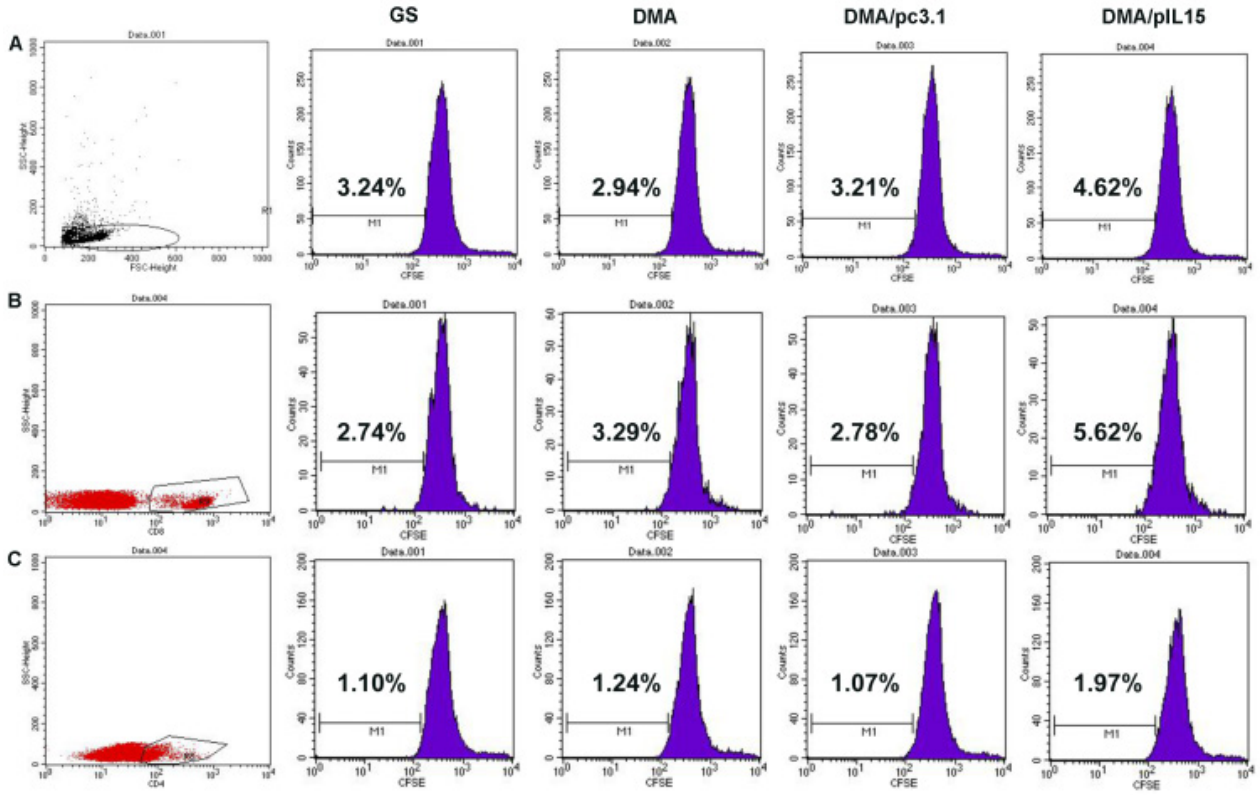
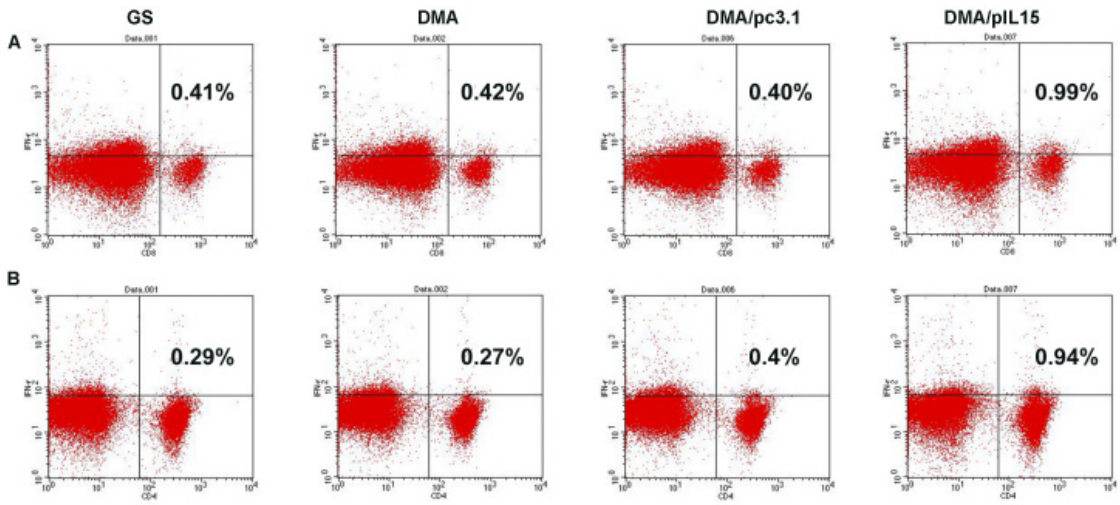


Supplementary figure 1. Measurement of transfection efficiency *in vitro* and *in vivo*. DMA containing pEGFP (4 ug) was used to transfect CT26 cells at a weight ratio of 1:10 pEGFP to DMA. Transfection efficiency was measured by fluorescence microscopy after 48 h



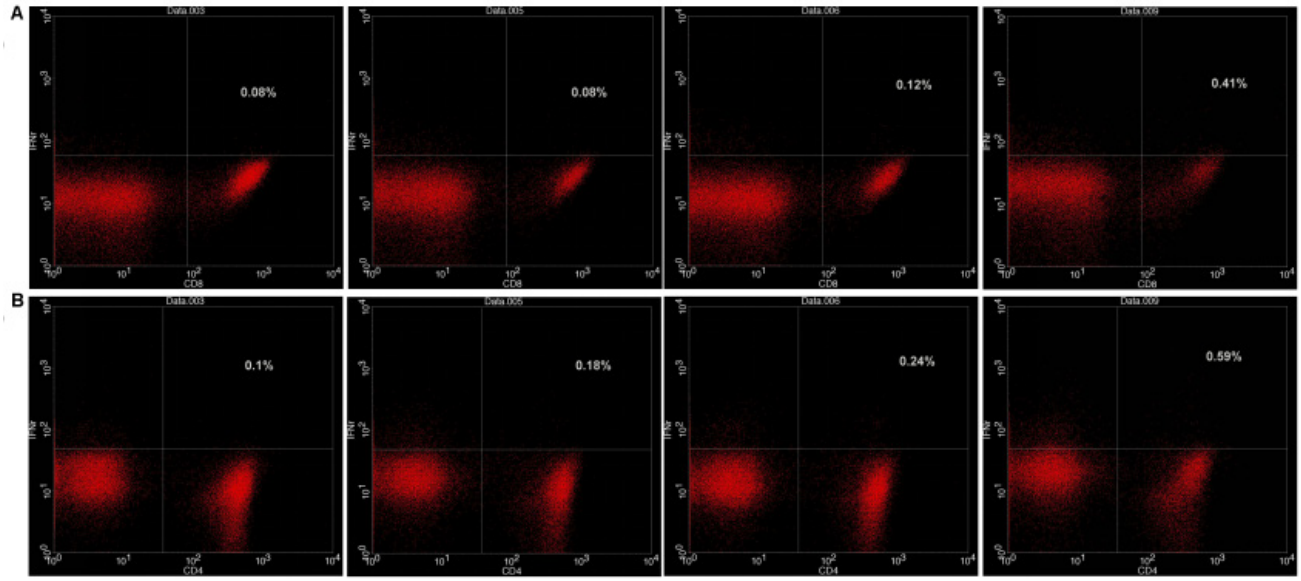
Supplementary Figure 2. Lymphocytes stimulated by the supernatant of transgenic tumor cells.

When CT26 cells were transfected with DMA, DMA/pc3.1, or DMA/pIL15, or treated with GS as a negative control, for 72 h, the supernatant from different treatments were used to culture lymphocytes over the course of 24 h. **(A)** Proliferation of lymphocytes was measured by flow cytometry with CFSE staining. **(B)** Proliferation of CD8+ lymphocytes and **(C)** proliferation of CD4+ lymphocytes were measured by flow cytometry.

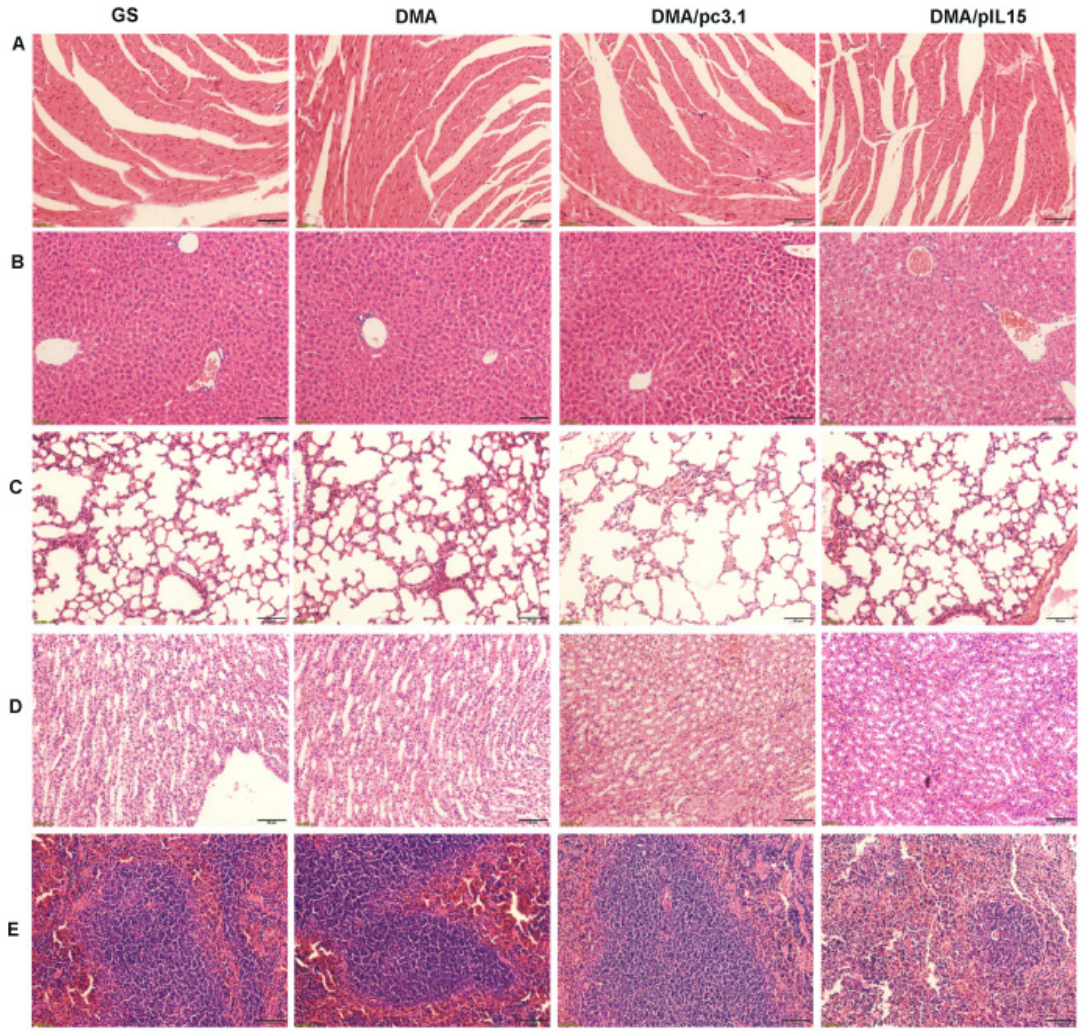


Supplementary Figure 3. Lymphocytes stimulated by the supernatant of transgenic tumor cells

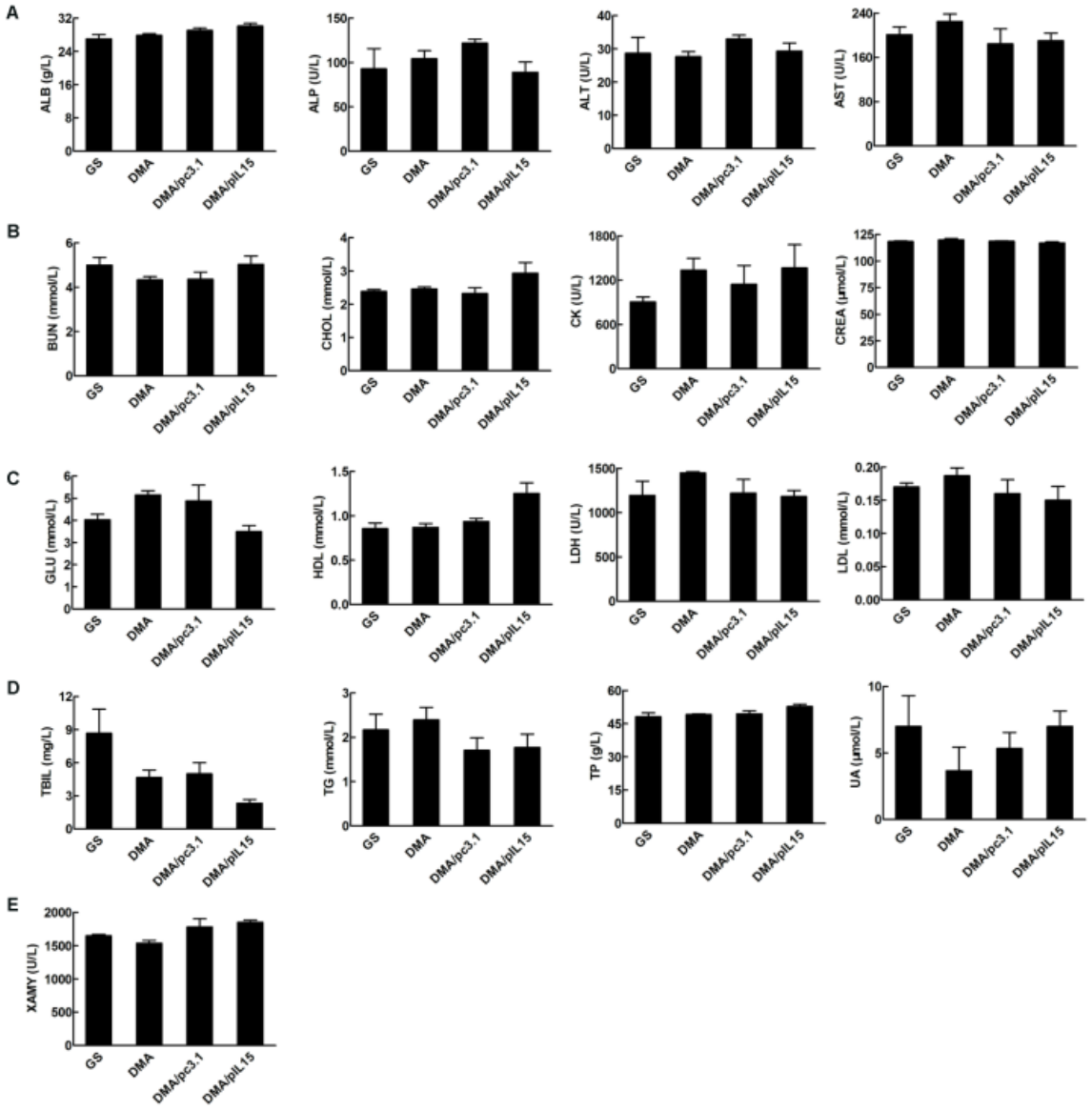
When CT26 cells were transfected with DMA, DMA/pc3.1, or DMA/pIL15, or treated with GS as a negative control, for 72 h, the supernatant from different treatments were used to culture lymphocytes over the course of 24 h. Increases in **(A)** CD8+ IFN-γ+ and **(B)** CD4+ IFN-γ+ were also observed by flow cytometry.



Supplementary Figure 4. Proliferation of lymphocytes and increase in the numbers of cytotoxic T cells in the peritoneal model. Splenic cells from the peritoneal model (GS, DMA, DMA/pc3.1, and DMA/pIL15) were collected after the final treatment.The label of the x-axis shows the ratio of effector cells to ^{51}Cr-labeled target cells. Increases in the number of **(A)** CD8+IFN-γ+ and **(B)** CD4+IFN-γ+ cytotoxic T cells were observed in the DMA/pIL15 group relative to the other groups.

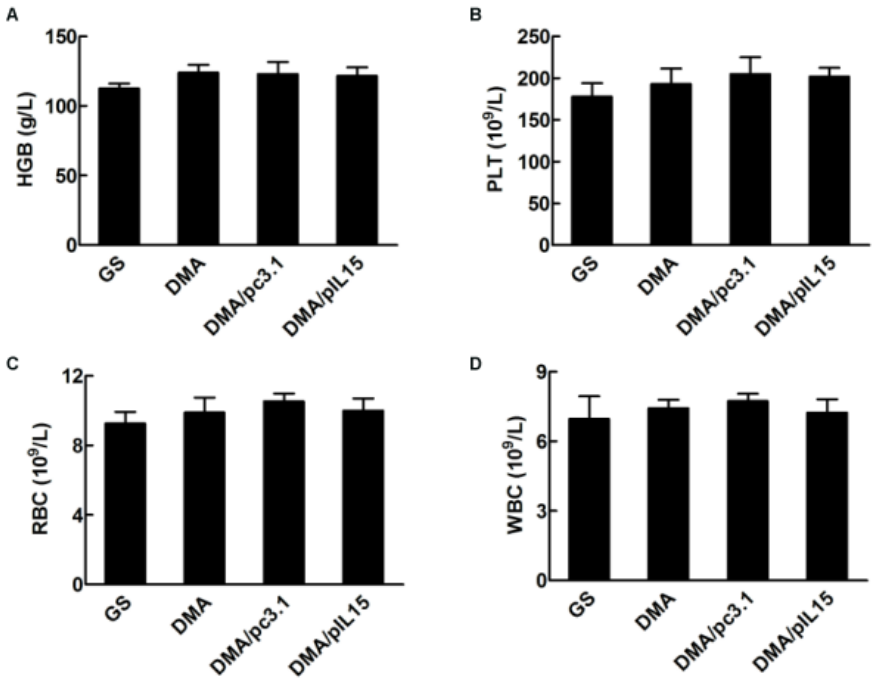


Supplementary Figure 5. Toxicity assessment *in vivo* with pathological sections. Histological examinations of H&E-stained sections of vital organs from mice in the different treatment groups: **(A)** heart, **(B)** liver, **(C)** lung, **(D)** kidney, and **(E)** spleen. No significant pathological changes were detected.



Supplementary Figure 6. Safety and toxicity evaluations by serological biochemical analysis.

(A) Levels of albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were assessed in serum from mice in the different treatment groups. **(B)** Blood urea nitrogen (BUN), cholesterol (CHOL), creatine kinase (CK), and creatinine (CREA) were assessed. **(C)** Levels of glucose (GLU), high density lipoprotein-cholesterol (HDL), lactate dehydrogenase (LD), and low density lipoprotein-cholesterol (LDL) were assessed. **(D)** Levels of total bilirubin (TBIL), triglycerides (TG), total protein (TP), and uric acid (UA) were assessed. **(E)** Levels of serum amylase (XAMY) were assessed. All tests were within normal range. No significant differences were observed across the four groups (GS, DMA, DMA/pc3.1, and DMA/pIL15).



Supplementary Figure 7. Safety and toxicity evaluations by routine blood tests. Levels of **(A)** hemoglobin (HGB), **(B)** platelets (PLT), **(C)** red blood cells (RBCs), and **(D)** white blood cells (WBC) were determined. Results from all four groups were within normal ranges.