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Supplementary Materials for

Blockade of TNFR2 signaling enhances the immunotherapeutic effect of CpG ODN in a mouse model of colon cancer

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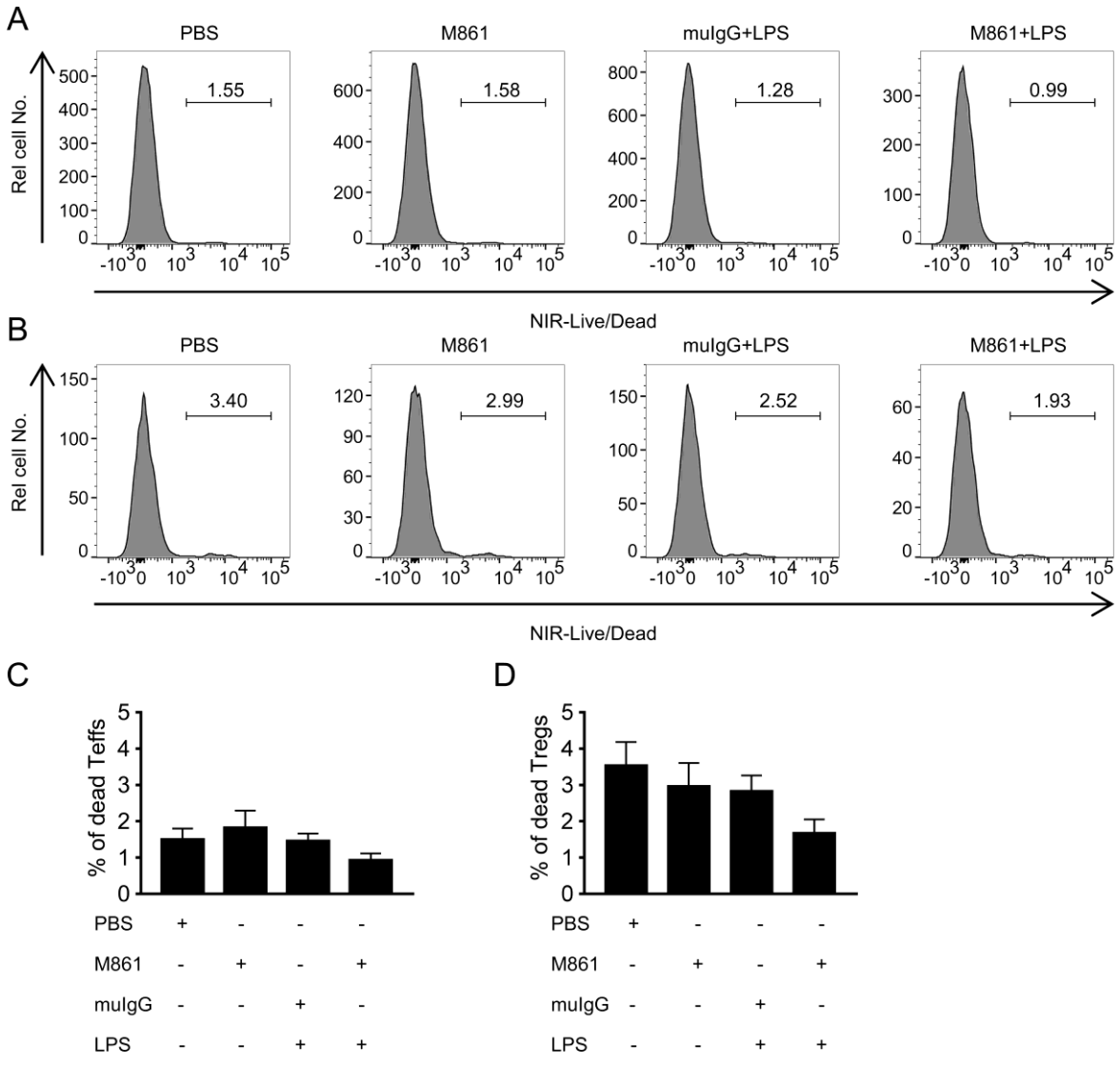

Fig S1. M861 does not induce the death of T_{reg} cells in LPS-treated mice. (A-D) Wild-type (WT) B6 mice were injected intraperitoneally with PBS, LPS (200 μg), M861(100 μg), mouse immunoglobulin G (muIgG), or a combination thereof, as indicated. After 72 hours, mice were sacrificed and splenic cells were stained with LIVE/DEAD® Fixable Near-IR Dead Cell Stain Kit. The proportion of dead cells, as indicated by the Near-IR positive staining, was analyzed by FACS, gating for $CD4^+Foxp3^-$ T_{eff} cells (A and C) or $CD4^+Foxp3^+$ T_{reg} cells (B and D). FACS plots are representative and data are means ± SEM of three independent experiments. Number within each FACS histogram shows the percentage of gated cells.

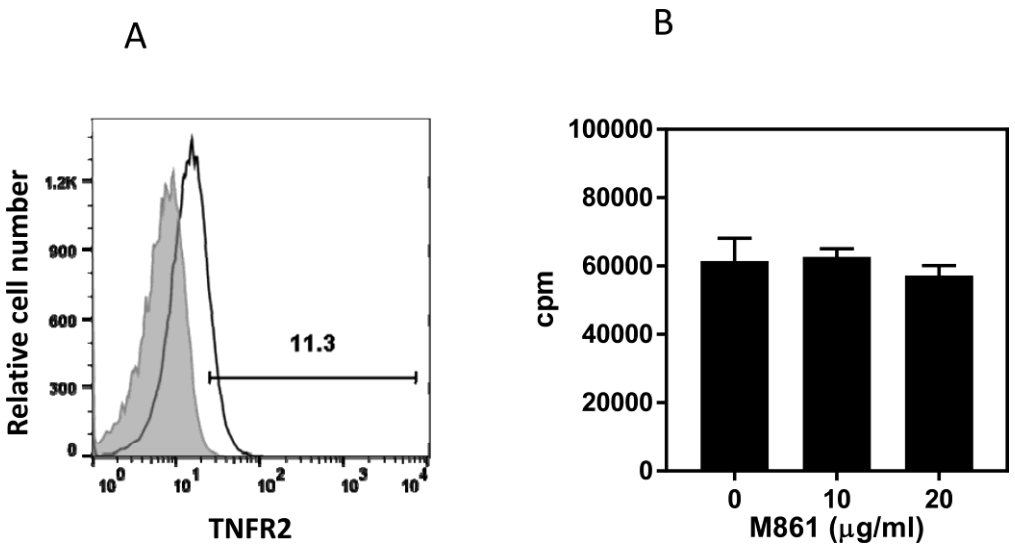

Fig S2. M861 does not inhibit the proliferation of TNFR2-expressing CT26 tumor cells. **(A)** Surface abundance of TNFR2 on CT26 tumor cells as analyzed by FACS. Gray histogram: isotype control; solid line histogram: PE-TNFR2 staining. Number in the FACS plot represents the proportion of $TNFR2^+$ cells. Data are representative of three independent experiments. **(B)** CT26 cells were seeded into 96 well-plate at 5×10^4 cells/well. The cells were cultured with media alone, or with 10 or 20 μg/ml of M861. After 72 hours, the proliferation of cells was measured by ^{3}H-thymidine incorporation assay. Data are means ± SEM of three independent experiments.