Supplementary information

Ionizable lipid nanoparticle-mediated TRAIL mRNA delivery in the tumor microenvironment to inhibit colon cancer progression

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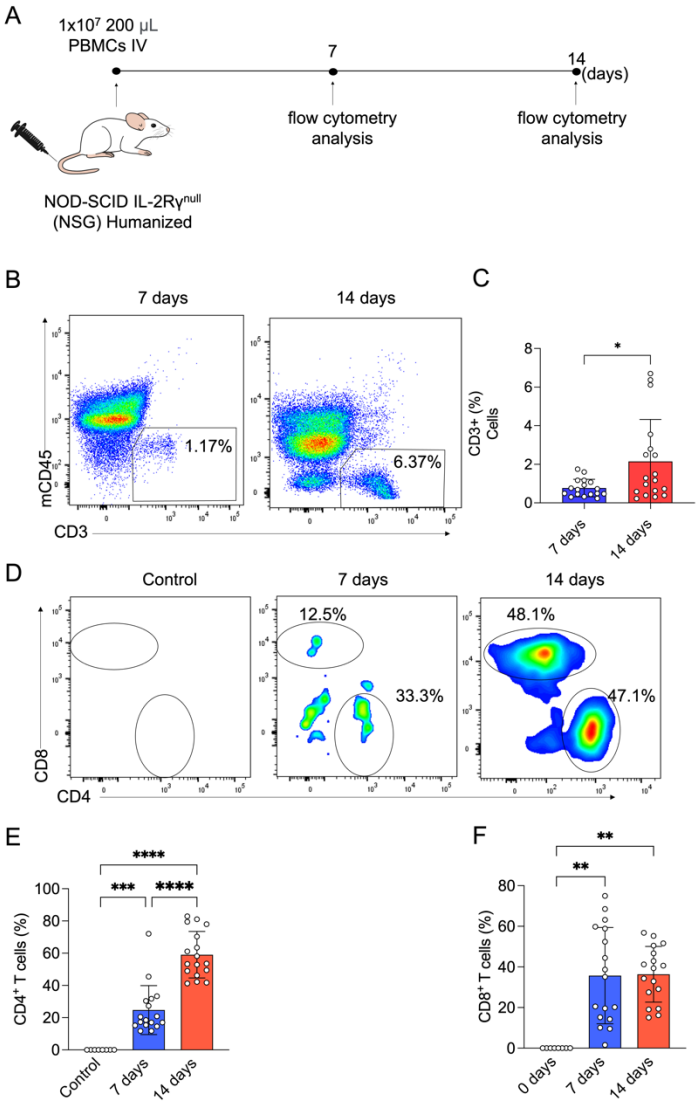

Fig. S1: Establishment of humanized mice from human peripheral blood mononuclear cells (PBMCs)**. (A)** Schematic representation of the humanization of NSG mice by transplantation of PBMCs. NSG mice were humanized by intravenously injecting 10^7 PBMCs in a volume of 200 µL. Humanization rate analysis was conducted at 7 days and 14 days post-transplantation to evaluate the level of human cell engraftment. **(B)** Gating strategy for human CD3+ cells and lymphocytes (n = 17-18 samples/group). **(C)** Frequency quantification of human CD3+/mCD45- cells in the peripheral blood of humanized NSG mice 7 days and 14 days post-humanization. **(D)** Gating strategy for human lymphocytes. Frequency quantification of human **(E)** CD4+ T and **(F)** CD8+ T cells in the peripheral blood of NSG mice 7 days and 14 days post-humanization (n = 8-17 samples/group). Data are presented as mean ± SD. Unpaired t-test (*p < 0.05; ** p < 0.01; *** p < 0.001 and **** p < 0.0001).

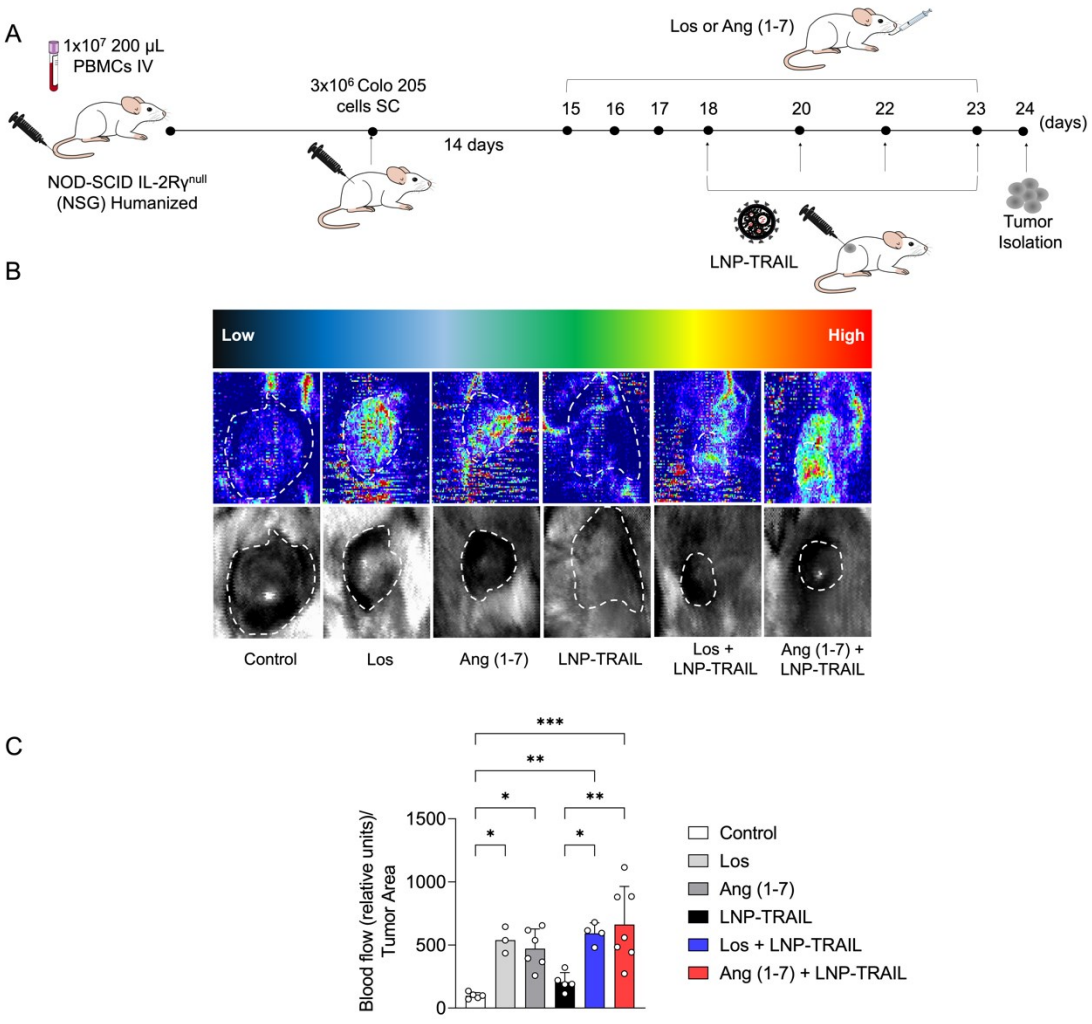

Fig. S2: TME normalization increases blood flow in humanized NSG mice. (A) Schematic representation of NSG mice humanization and subcutaneous Colo 205 tumor cells transplantation following treatments with Los, Ang (1-7), and LNP-TRAIL. **(B)** Representative blood flow images in tumor tissue 24 days post-treatment. Warmer colors represent higher perfusion levels, while the dashed area denotes the tumor region. **(C)** Quantification of blood flow in tumor tissues of humanized mice 24 days post-treatment (n = 3-7/group). Data are presented as mean ± SD. One-way ANOVA followed by Tukey's multiple comparison test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

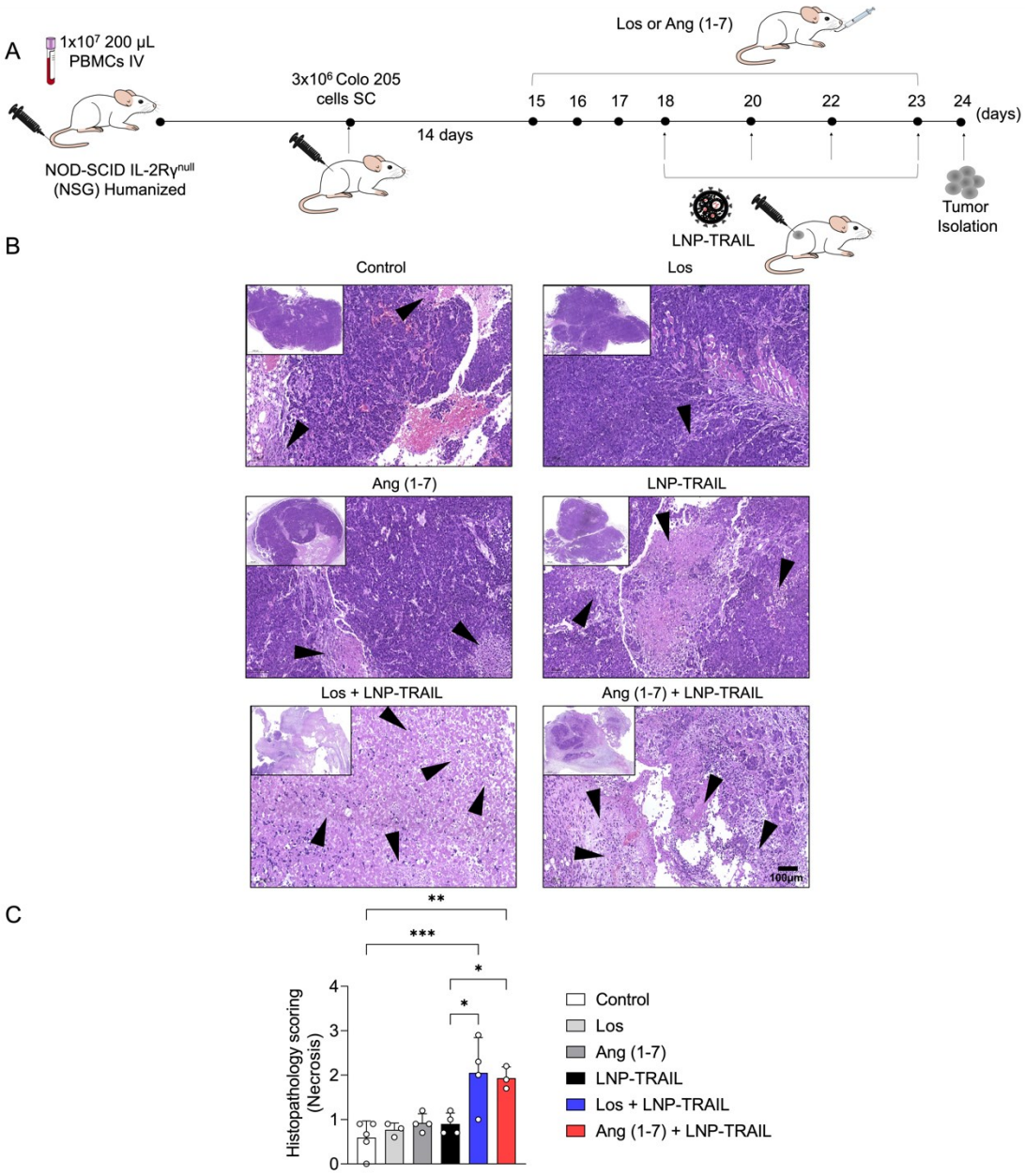

Fig. S3. LNP-TRAIL combined with TME normalization increases the area of necrosis tumoral. (A) Schematic representation of NSG mice humanization and subcutaneous Colo 205 tumor cells transplantation following treatments with Los, Ang (1-7), and LNP-TRAIL. **(B)** Tumor sections were stained with hematoxylin and eosin (H&E). Histopathological analysis at 40× magnification. Black arrows indicate the area of tumor necrosis. **(C)** Histopathology scoring (necrosis) in humanized mice treated with LNP-TRAIL combined with TME normalization (n = 3-5 samples/group). Data are presented as mean ± SD. One-way ANOVA followed by Tukey's multiple comparison test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

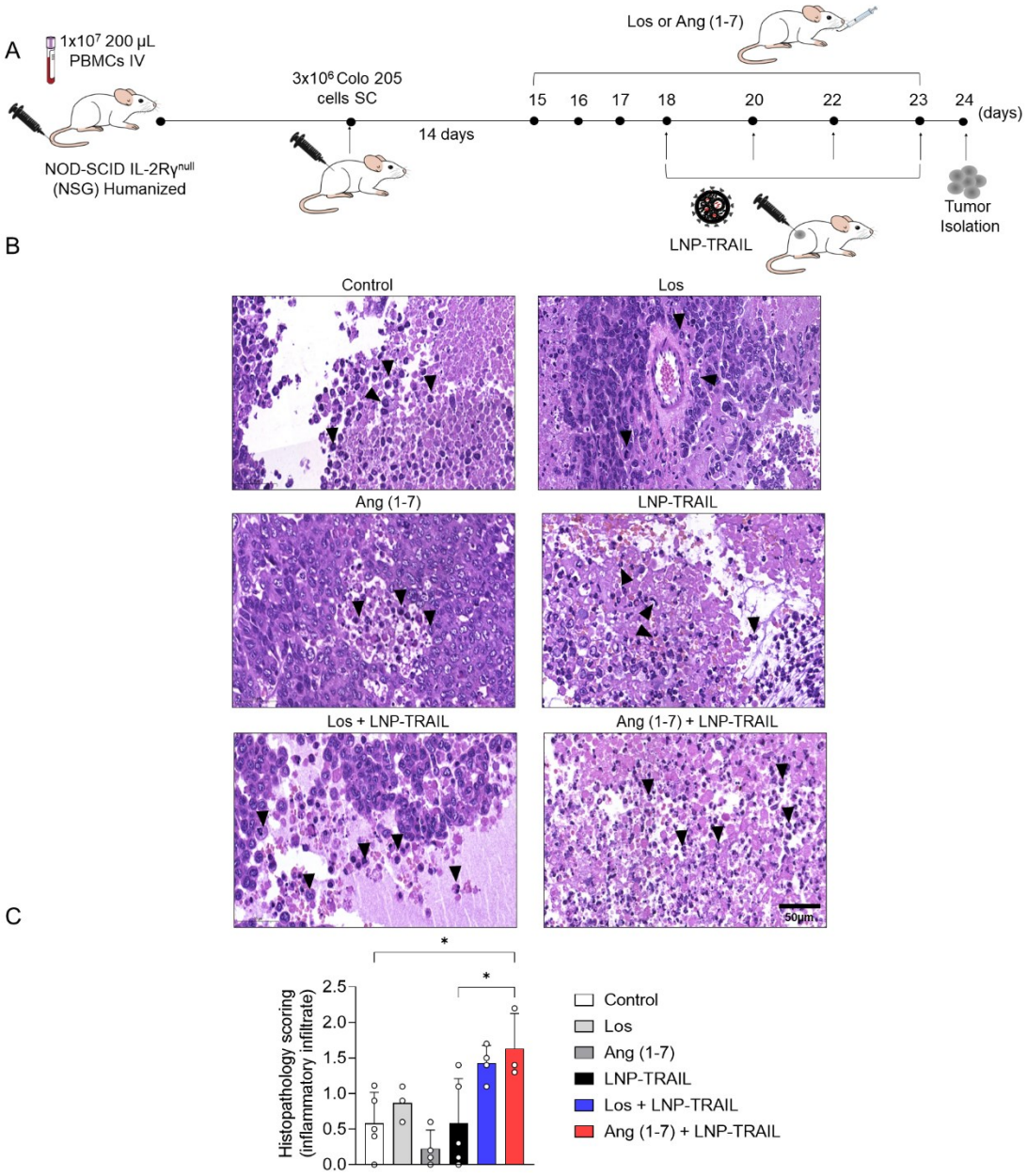

Fig. S4: TME normalization increases the inflammatory infiltrate. (A) Schematic representation of NSG mice humanization and subcutaneous Colo 205 tumor cells transplantation following treatments with Los, Ang (1-7), and LNP-TRAIL. **(B)** Tumor tissues stained with hematoxylin and eosin (H&E). Histopathological analysis at 40× magnification, with black arrows indicating the immune cell infiltrate. **(C)** Histopathology scoring (inflammatory infiltrate) in humanized mice treated with LNP-TRAIL combined with Los or Ang (1-7) (n = 3-5 samples/group). Data are presented as mean ± SD. One-way ANOVA followed by Tukey's multiple comparison test (* $p < 0.05$).

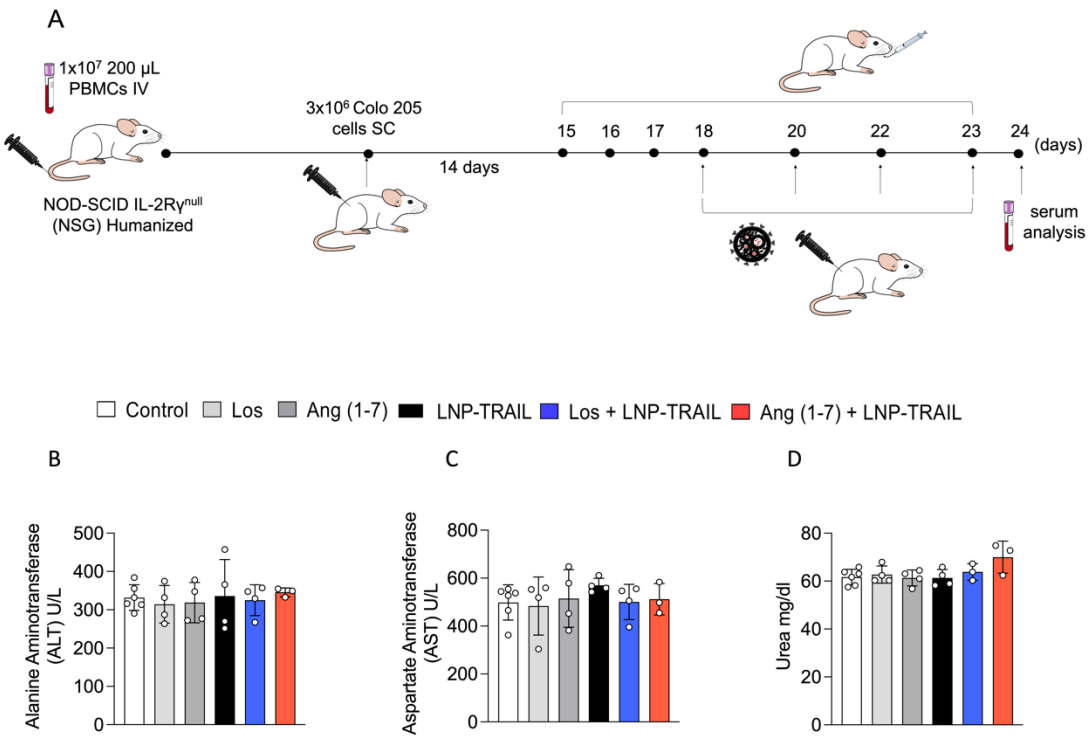

Fig. S5: The administration of LNP-TRAIL does not impact liver and kidney function. **(A)** Schematic representation of NSG mice humanization and subcutaneous Colo 205 tumor cells transplantation following treatments with Los, Ang (1-7), and LNP-TRAIL. (**B**) Alanine Transaminase (ALT) (**C**) and Aspartate Transaminase (AST) levels measured in (U/mL), along with (**D**) Urea levels measured in (mg/dL) (n = 4-6 samples/group). Data are presented as mean ± SD. Statistical analysis was conducted using one-way ANOVA followed by Tukey's multiple comparison test.

Table S1. Antibodies list for flow cytometry

Antigen	Fluorochrome	Clone	Concentration	Company
CD3	Super Bright 645	OKT3	1/100	ThermoFisher
CD8a	eFluor 450	RPA-TB	1/100	ThermoFisher
CD4	Super Bright 600	RPA-T4	1/1000	ThermoFisher
LIVE/DEAD	Acqua		1/1000	ThermoFisher
CD253	PE	RIK-2	1/100	ThermoFisher
Hu-CD45RO	PE-eFluor 610	UCHL1	1/100	ThermoFisher
mCD45	Pacific Orange	30-F11	1/200	ThermoFisher
Ki67	AlexaFluor 700	SolA15	1/100	ThermoFisher
PD-1	PE-Cyanine7	EBioJ105 (J105)	1/100	ThermoFisher
CTLA-4	APC-eFluor 780	14D3	1/100	ThermoFisher