

Supporting Information

Tumor-targeted Gene Therapy with Lipid Nanoparticles Inhibits Tumor - Associated Adipocytes and Remodels the Immunosuppressive Tumor Microenvironment in Triple-Negative Breast Cancer

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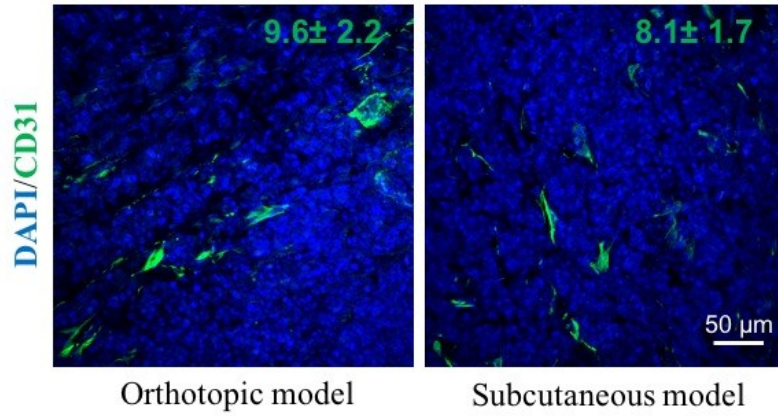
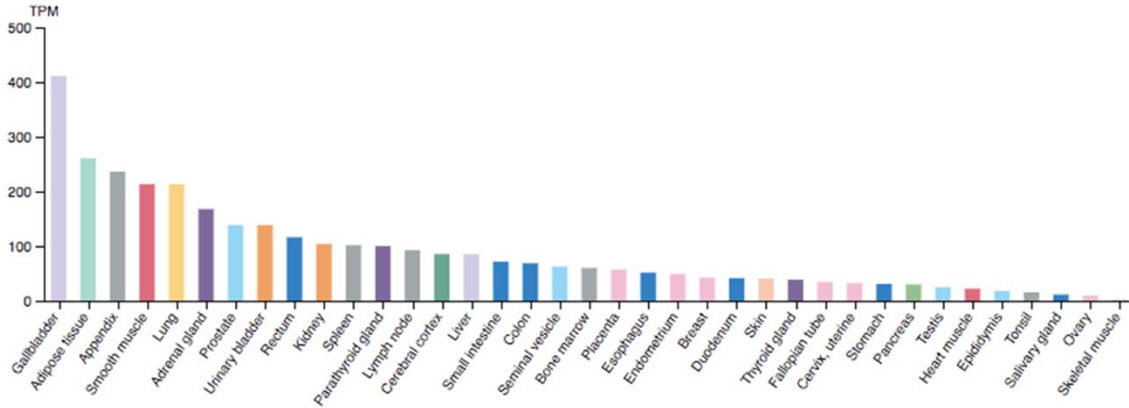
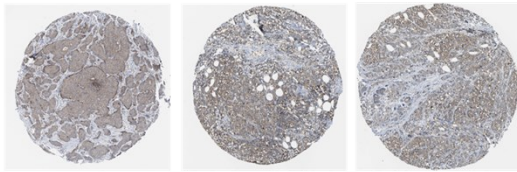


Figure S1. Immunofluorescence staining of tumor samples from orthotopic model and subcutaneous model using anti-CD31 antibody (green). Cell nuclei were stained as blue using DAPI. Five random fields were chosen for statistical analysis in each treatment groups. Images were analyzed by Image J software and quantified with GraphPad 6.0. Scale bar represents 50 μm.

A



B



C

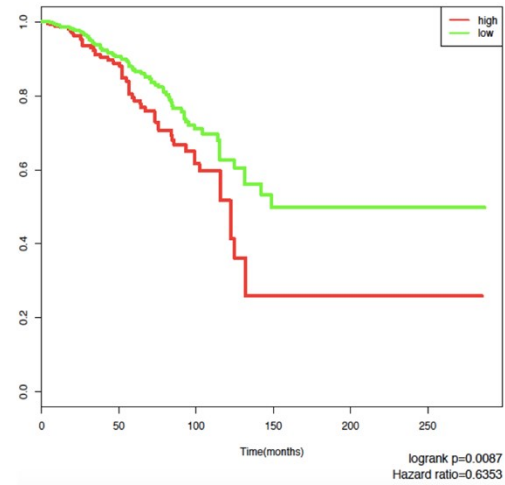


Figure S2. A. The CCL2 mRNA levels among different tissues/organs. B. Representative clinical tumor tissue from TNBC patients, cited from The Human Protein Atlas. High expression of CCL2 was found in human TNBC samples. C. High CCL2 expression correlates with shorter survival and poor prognosis in human TNBC. Data obtained from TCGA database. $p = 0.0087$.

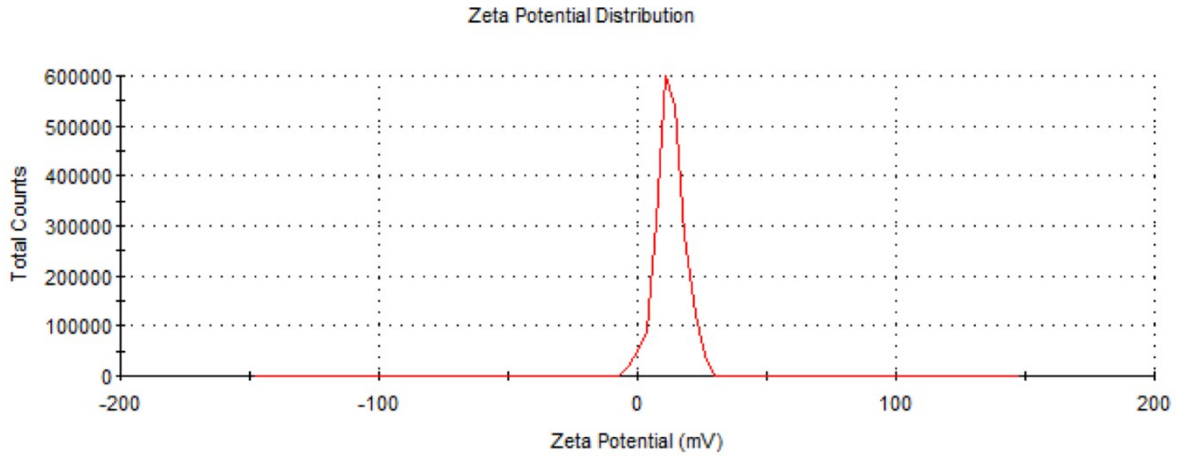


Figure S3. The zeta potential of final LPD NPs measured by DLS.

Sample Name	RBC (M/uL)	HGB (g/dL)	PLT (K/uL)	WBC (K/uL)	NEUT (K/uL)	LYMPH (K/uL)	MONO (%)
PBS	7.5±0.4	11.7±1.6	16.1±1.8	8.7±1.1	6.5±1.2	2.1±1.8	7.9±2.5
pPD-L1 trap	7.8±1.1	12.1±1.5	14.3±0.9	8.5±1.3	6.6±2.4	3.2±0.6	6.1±1.1
pGFP	8.2±0.5	11.8±1.7	14.9±2.4	8.2±0.9	5.9±1.4	2.9±1.1	8.4±1.3
pCCL2 trap	7.4±1.5	12.4±0.4	13.6±1.3	8.9±1.1	6.6±1.2	3.4±0.7	7.9±1.2
Combo trap	7.7±1.2	10.9±0.8	11.7±2.4	7.7±1.4	5.6±1.9	2.4±0.9	6.9±0.7
Normal Range	6.5-10.1	10.1-16.1	7.8-15.4	2.6-10.1	2.5-7.5	1-4.8	2.0-10.0

Table S1. Whole cell counts of 4T1 bearing mice in different treatment groups.

Antibodies	Company	Application
Alexa Fluor®647 anti-mouse CD3 Antibody	Biolegend	IF, Flow
Alexa Fluor®594 anti-mouse CD4 Antibody	Biolegend	Flow
eFluro 450 anti-mouse CD8a antibody	Biolegend	Flow
PE/Cyanine 7 anti-mouse CD11c Antibody	Biolegend	Flow
APC anti-mouse CD62L antibody	Biolegend	Flow
Alexa Fluor® 488 anti-mouse CD11b Antibody	Biolegend	Flow
Alexa Fluor® 647 anti-mouse Ly-6G/Ly-6C (Gr-1) Antibody	Biolegend	Flow
Alexa Fluor® 488 anti-mouse NK1.1 Antibody	Biolegend	Flow
PE anti-mouse F4/80 Antibody	Biolegend	Flow
PerCP/Cyanine 5.5 anti-mouse CD206 Antibody	Biolegend	Flow
APC/Cyanine 7 anti-mouse CD86 Antibody	Biolegend	Flow
FIFC anti-mouse CD45 Antibody	Biolegend	Flow
APC anti-mouse CD31 Antibody	Biolegend	IF
Alexa Fluor® 488 anti- α -SMA Antibody	Invitrogen	IF

IF: immunofluorescence; Flow: flow cytometry

Table S2. Antibodies used in this study.

Primers	Applied Biosystems/Ref	Assay method
Mouse IFN- γ	Mm01168134_m1	TaqMan [®]
Mouse TNF- α	Mm00443260_g1	TaqMan [®]
Mouse TGF- β	Mm01178820_m1	TaqMan [®]
Mouse IL-10	Mm01288386_m1	TaqMan [®]
Mouse CXCL9	Mm00434946_m1	TaqMan [®]
Mouse CXCL10	Mm04214185_m1	TaqMan [®]
Mouse CCL2	Mm00441242_m1	TaqMan [®]
Mouse IL-1 β	Mm00434228_m1	TaqMan [®]
Mouse GAPDH	Mm99999915_g1	TaqMan [®]
Mouse MMP2	Mm00439498_m1	TaqMan [®]
Mouse MMP9	Mm00442991_m1	TaqMan [®]
Mouse MMP13	Mm00439491_m1	TaqMan [®]
Mouse PDGF-C	Mm00439560_m1	TaqMan [®]
Mouse IL-12	Mm00434169_m1	TaqMan [®]
Mouse CXCL12	Mm00445553_m1	TaqMan [®]
Mouse CXCL13	Mm04214185_s1	TaqMan [®]

Table S3. Primers for RT-PCR used in this study.