Supplementary Material for

Nano-delivery of fraxinellone remodels tumor microenvironment and facilitates therapeutic vaccination in desmoplastic melanoma

Lin Hou[1, 2, 3, 4, #], Qi Liu[1, #], Limei Shen[1], Yun Liu[1], Xueqiong Zhang[1], Fengqian Chen[5], and Leaf Huang[1, *]

[1]Division of Pharmacoengineering and Molecular Pharmaceutics and Center for Nanotechnology in Drug Delivery, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA; [2]School of Pharmaceutical Sciences, Zhengzhou University, 100 Kexue Avenue, Zhengzhou 450001, China; [3]Collaborative Innovation Center of New Drug Research and Safety Evaluation, Henan Province, China; [4]Key Laboratory of Targeting and Diagnosis for Critical Diseases, Henan Province, China; [5]Department of Environmental Toxicology, The Institute of Environmental and Human Health (TIEHH) and the Center for Biotechnology & Genomics, Texas Tech University, Lubbock, TX 79416, USA.

[#] These authors contributed equally

[*] Corresponding author: leafh@email.unc.edu

Supplementary Material

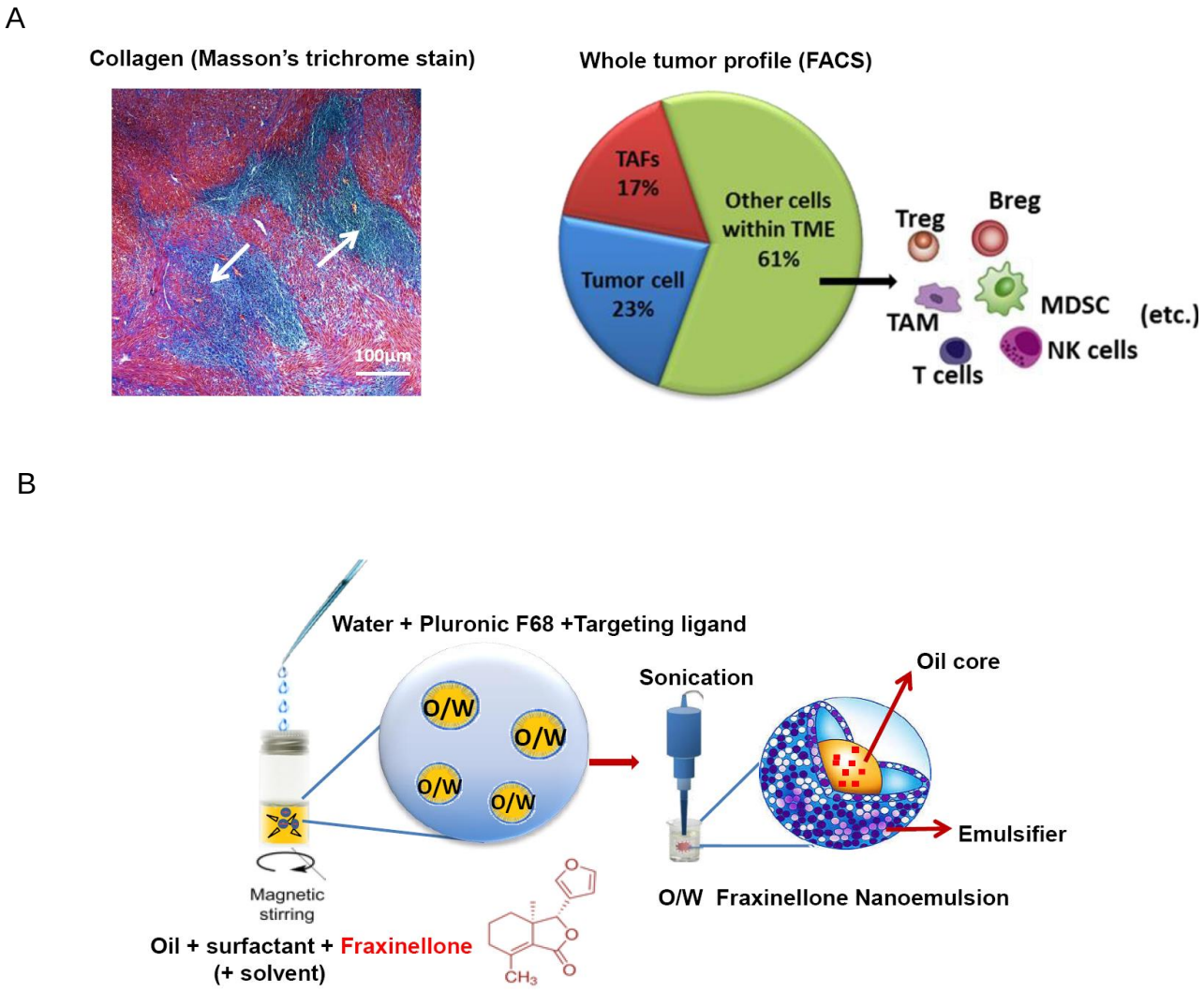

Figure S1. Design of Frax NE combined with vaccine to remodel fibrotic TME and facilitate immunotherapy. (A) Stroma-rich TME comprised of different kinds of cell population. **(B)** Scheme depicting the preparation process of Frax NE.

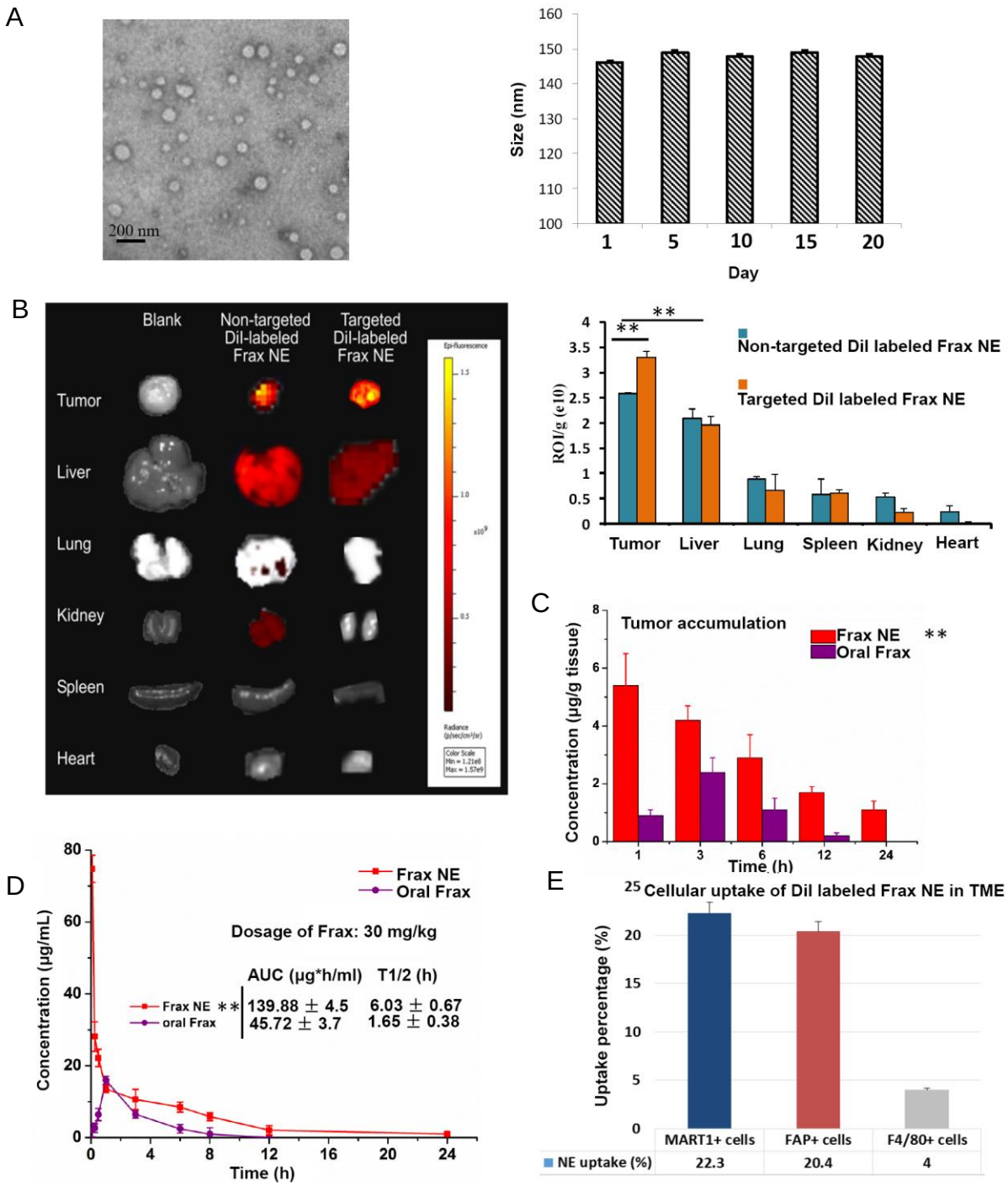

Figure S2. Preparation and characterization of Frax NE *in vitro* and *in vivo*. (A) TEM image and *in vitro* stability of Frax NE. **(B)** IVIS image and quantitative analysis of DiI-labeled Frax NE with or without targeting ligand in BPD6 tumor bearing mice. **(C)** Quantitative analysis for tumor accumulation of Frax *in vivo* using LC/MS. **(D)** PK analysis of Frax NE *in vivo* using LC/MS. **(E)** Cellular uptake of DiI-labeled Frax NE in TME, measured by flow cytometry. (n = 3, * P < 0.05, ** P < 0.01, *** P < 0.001)

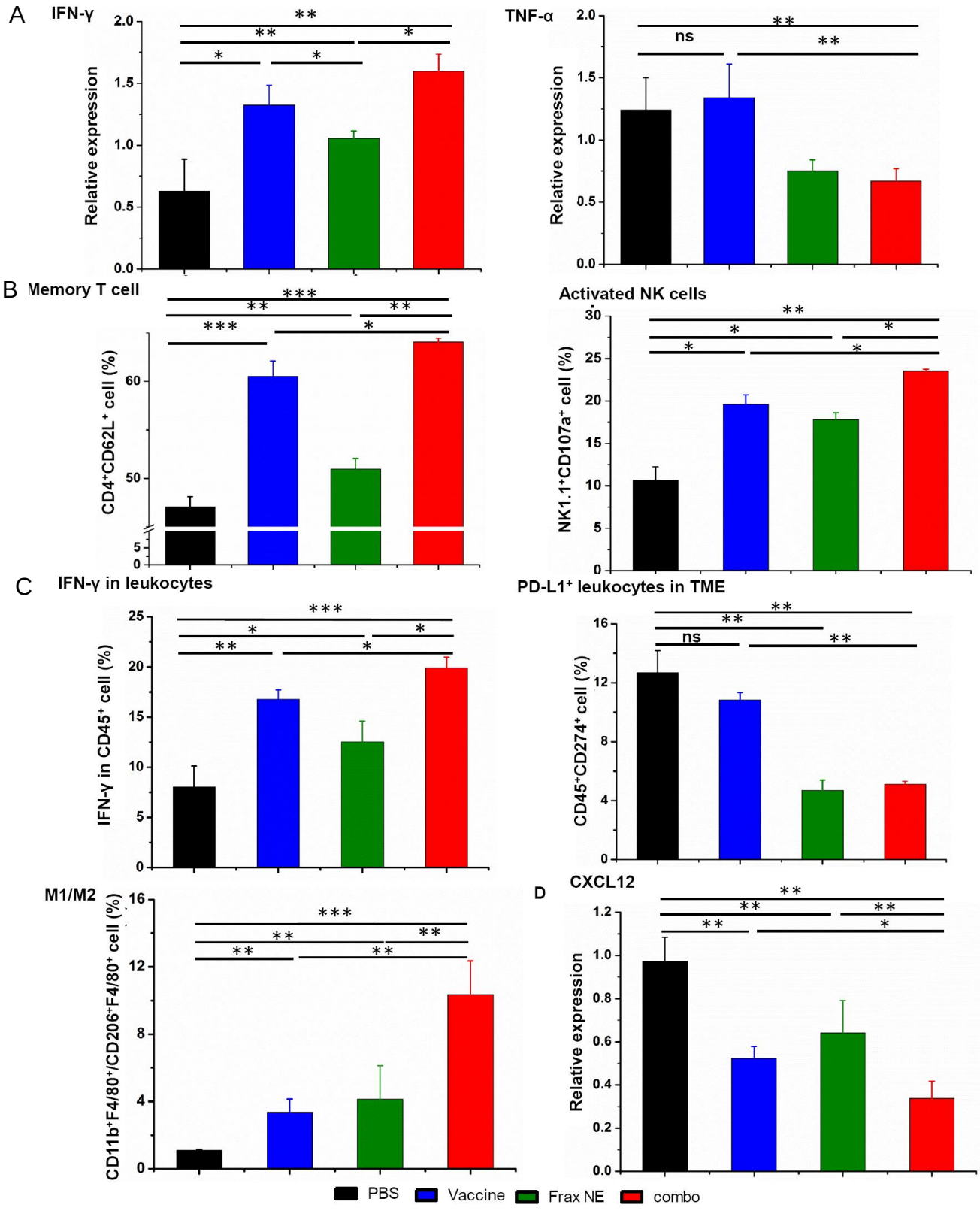

Figure S3. (A) Changes in mRNA expression levels of IFN-γ and TNF-α detected by quantitative RT-PCR. **(B-C)** Changes of immune cells (memory T cells and active NK cells) in lymph nodes or in whole tumor, measured by flow cytometry. **(D)** Changes of cytokines in TME using flow cytometric analysis and quantitative RT-PCR. (n = 6, * P < 0.05, ** P < 0.01, *** P < 0.001)

Table S1. Antibody Summary

Antibody	Company	Catalog number
α-SMA	Abcam	ab184675
CUGBP-1	Santa Cruz Biotechnology	sc-20003 AF647
CD3	Biolegend	100322
CD8	Biolegend	100758
CD4	Biolegend	100446
CD62L	Biolegend	104420
NK1.1	Biolegend	108718
CD11b	Biolegend	101217
Gr1	Biolegend	108418
CD1d	Biolegend	123508
CD19	Biolegend	115552

Table S2. Primer Summary

Primer	Company	Catalog number
TGF-β	ThermoFisher Scientific	Mm01178820_m1
CUGBP1	ThermoFisher Scientific	Mm04279608_m1
CCL2	ThermoFisher Scientific	Mm00441242_m1
IL6	ThermoFisher Scientific	Mm00446190_m1
CXCL13	ThermoFisher Scientific	Mm04214185_m1
IGF-1	ThermoFisher Scientific	Mm00439560_m1
FGF-2	ThermoFisher Scientific	Mm01285715_m1
CXCL12	ThermoFisher Scientific	Mm00445553_m1