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Supplementary Materials for

P-selectin is a nanotherapeutic delivery target in the tumor microenvironment

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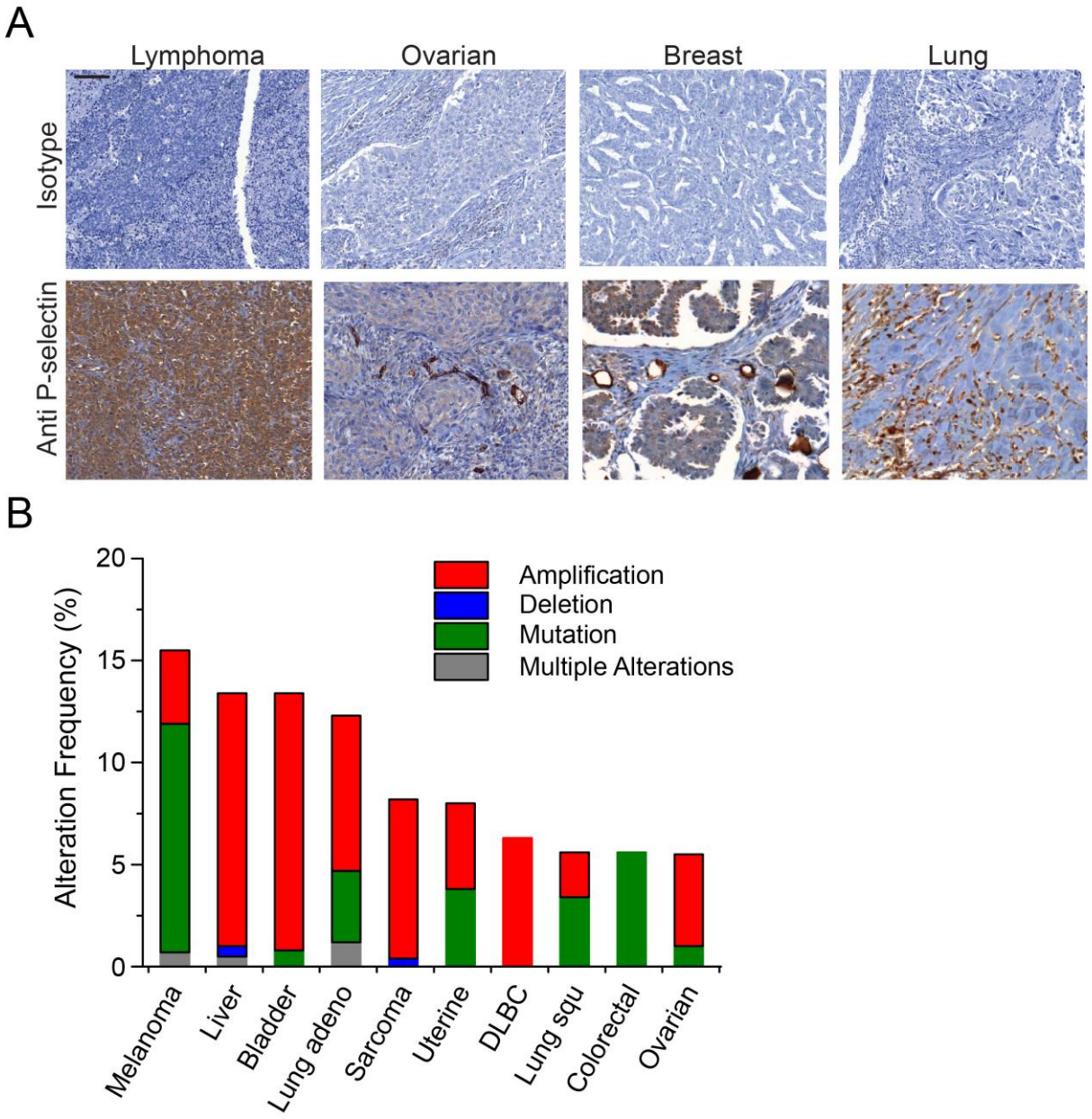

Figure S1. **Isotype controls and major genomic alterations. (A)** Isotype control immunohistochemical staining of adjacent sections from tumor microarrays in Figure 1. Scale bar = 50 µm. **(B)** Major genomic alterations of *SELP* found in the TCGA database. Abbreviations: DLBC = diffuse large B-cell lymphoma; lung squ = lung squamous cell carcinoma, lung adeno = lung adenocarcinoma.

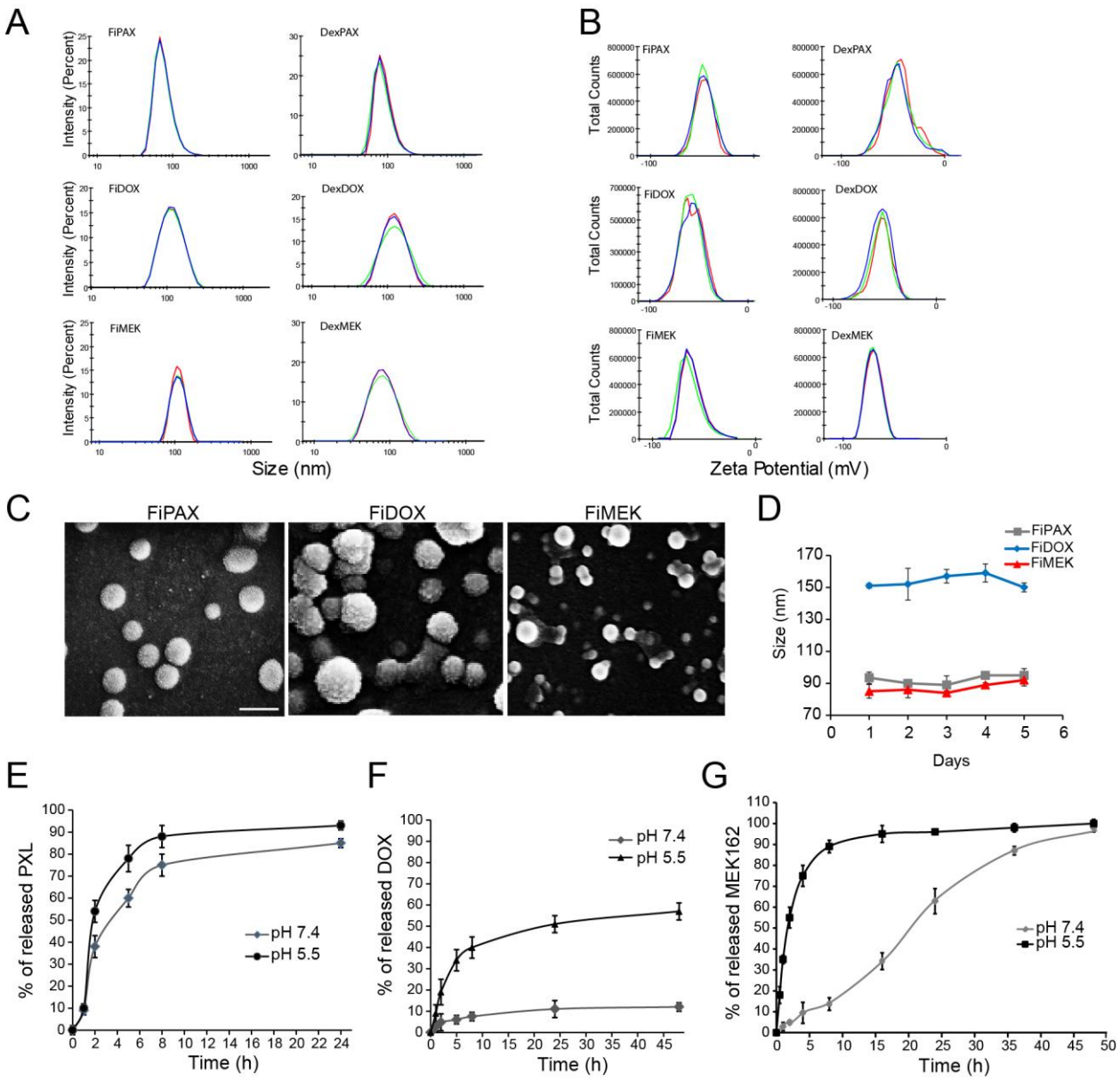

Figure S2. **Nanoparticle characterization. (A)** Size measurements using dynamic light scattering (DLS) in PBS. **(B)** Zeta potential (surface charge) of nanoparticles in deionized water. **(C)** Scanning electron microscopy (SEM) images. Scale bar = 100 nm. **(D)** Stability of nanoparticles in growth medium containing 10% FBS, evaluated by measuring particle size. **(E)** Drug release profile of paclitaxel, **(F)** doxorubicin, and **(G)** MEK162 from their respective fucoidan nanoparticles. Error bars are SD of mean.

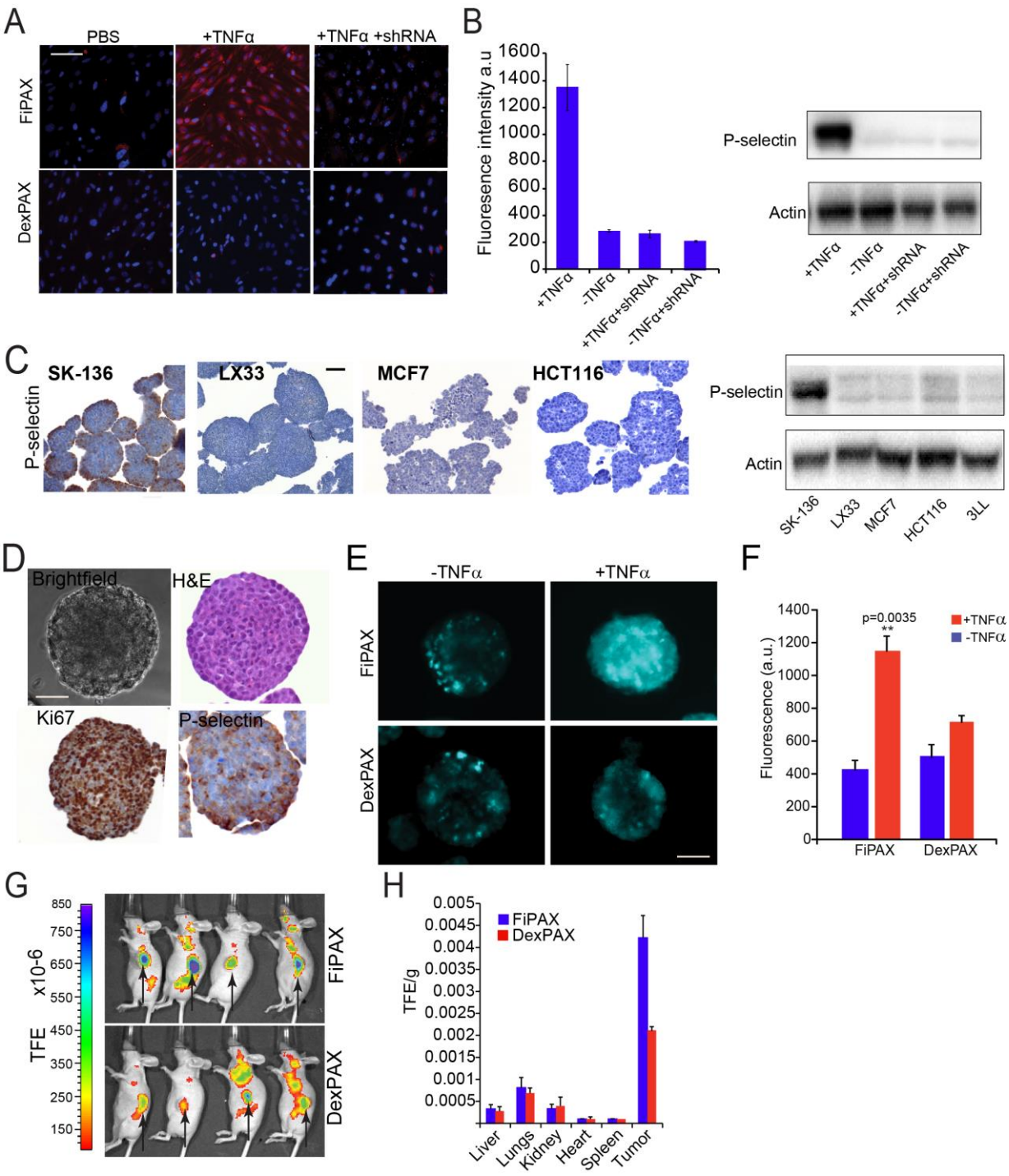

Figure S3. **In vitro and in vivo nanoparticle targeting studies. (A)** Binding of targeted (FiPAX) and control (DexPAX) nanoparticles to activated endothelial cells (bEnd.3) assessed by fluorescence microscopy. shRNA against P-selectin was added to knock down P-selectin expression before the experiment. (Red) Near-infrared fluorophore emission from the nanoparticles. (Blue) Hoescht nuclear stain. Scale bar = 10 μm. **(B)** Left: Quantification of

nanoparticle fluorescence in the treated cells. Right: Western blot of P-selectin expression in bEnd.3 cells upon treatment with TNFα and shRNA against P-selectin. **(C)** Left: Immunohistochemical staining of P-selectin expression in several cell lines. Scale bar = 50 μm. Right: P-selectin expression in cell lines measured by Western blot. **(D)** Brightfield and immunohistochemical staining of P-selectin, H&E, and Ki67 expression in SK-136 tumor spheroids. **(E)** Fluorescence microscopy of near-infrared dye emission from nanoparticles in SK-136 tumor spheroids at the bottom of the Transwell assay plates. Scale bar = 20 μm. **(F)** Quantification of nanoparticle emission in tumor spheroids. **(G)** In vivo fluorescence images of targeted and control nanoparticles in the P-selectin-expressing JHU-LX33 PDX model. Arrows indicate location of the tumors. **(H)** Nanoparticle biodistribution in organs and tumor, calculated from ex vivo fluorescence images as total fluorescence efficiency divided by organ weight. Bar graphs are shown as mean ±SD.

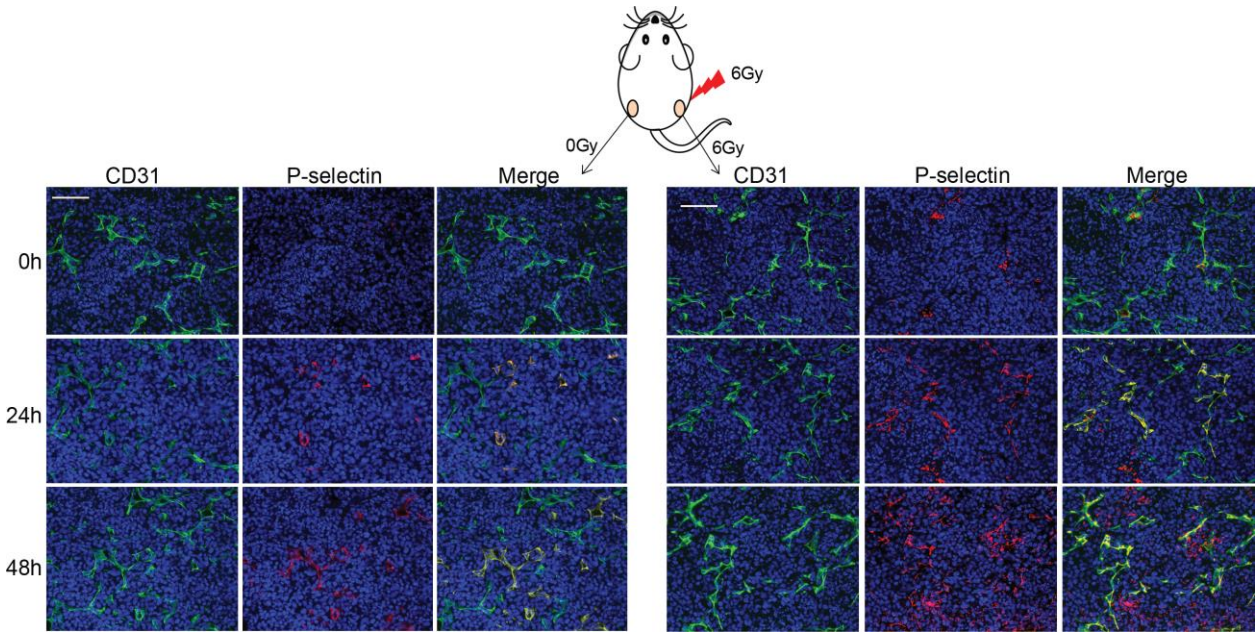

Figure S4. P-selectin and CD31 expression in irradiated and contralateral unirradiated tumors. Immunofluorescence microscopy of tumor slices resected 0, 24, and 48 h after treatment and stained for CD31 (green), P-selectin (green), and DAPI (blue). Scale bar = 150 µm.

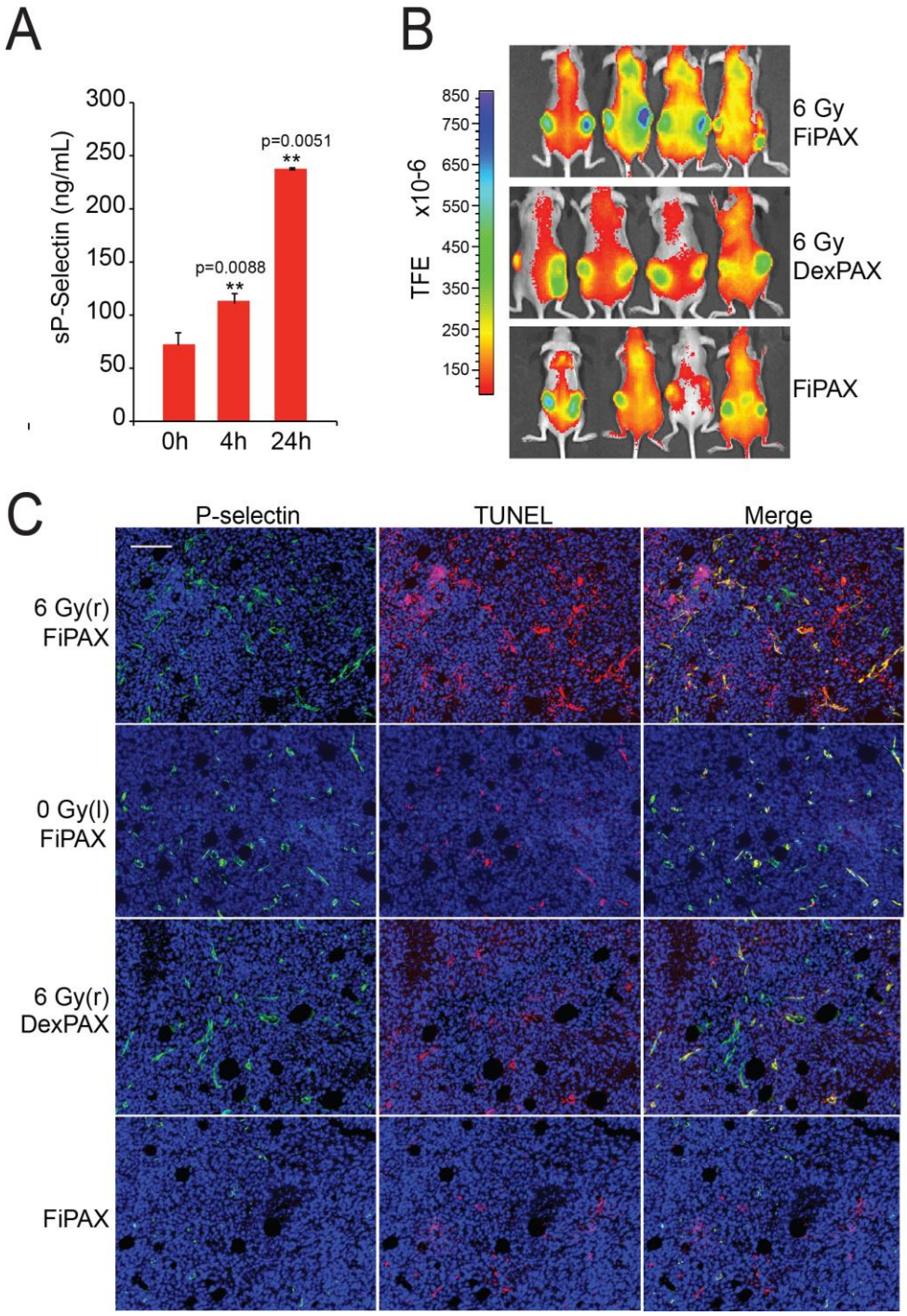

Figure S5. Effect of ionizing radiation on P-selectin and nanoparticle localization. (A) Quantification of soluble P-selectin in the blood upon radiation treatment, as measured by ELISA assay. Data shown as mean ±SD. **(B)** Whole-body imaging of nanoparticles in the 3LL bilateral tumor model. The 6 Gy FiPAX group denotes mice irradiated on the right flank tumors before injection of FiPAX nanoparticles. The 6 Gy DexPax group was irradiated similarly before injection of DexPAX nanoparticles. The FiPAX group was injected with FiPAX nanoparticles

but was not irradiated. **(C)** Immunofluorescence microscopy of tumor tissue slices resected 24 h after treatments, stained for P-selectin (green), TUNEL (red), and DAPI (blue). The tissues denoted 6Gy(r) and 0Gy(l) refer to the irradiated right tumor and non-irradiated left tumor in the irradiated mouse, respectively. The tissue sample without either label received FiPAX nanoparticles only and did not receive radiation. Scale bar = 150 μm.

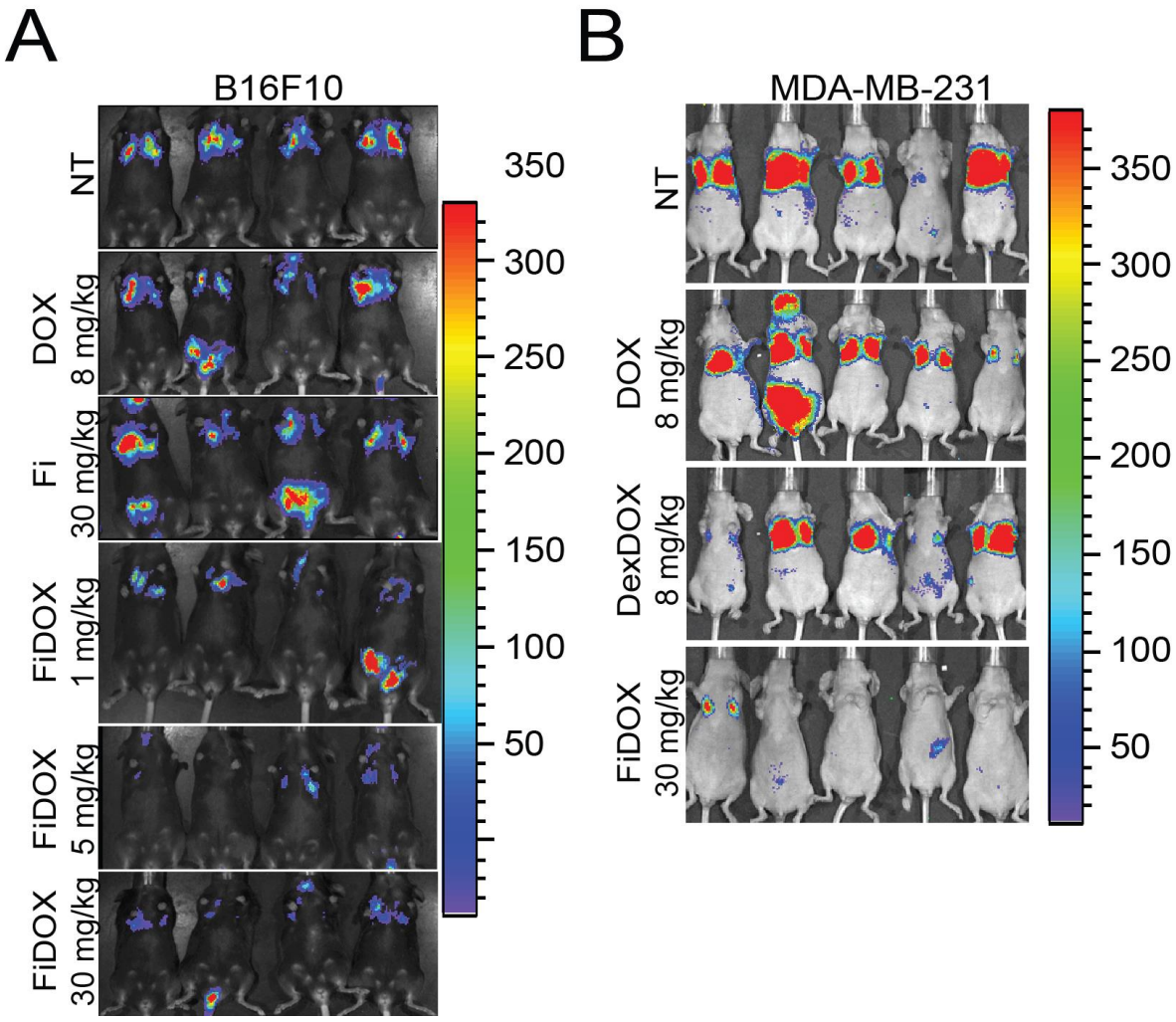

Figure S6. **In vivo bioluminescence of metastatic models 7 days after treatments. (A)** B16F10 melanoma lung metastasis model. **(B)** MDA-MB-231 breast cancer lung metastasis model.

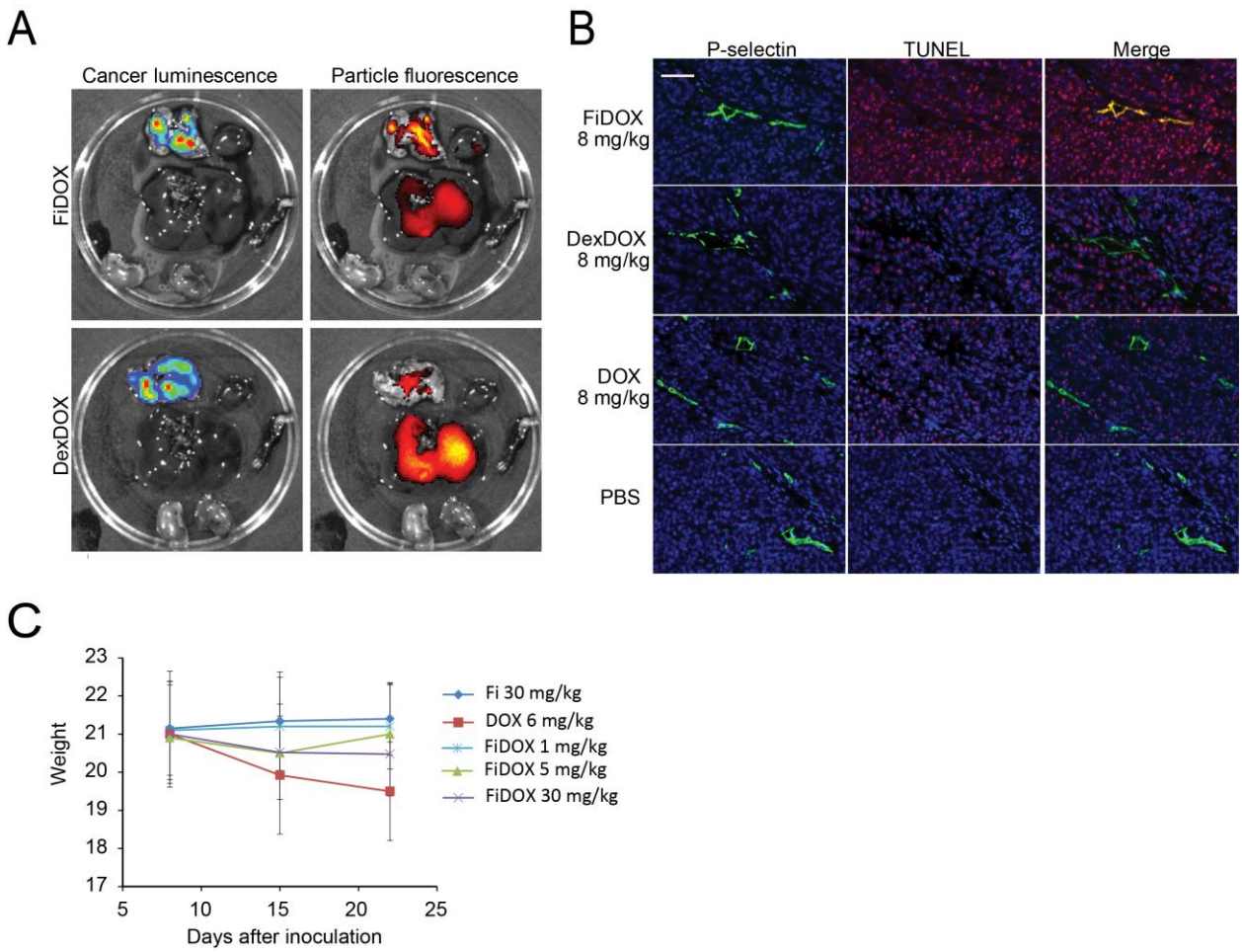

Figure S7. B16F10 melanoma model assessments. (A) Bioluminescence and fluorescence images of organs ex vivo, 24 h after administration of nanoparticles. (Left) Bioluminescence. (Right) Fluorescence captured with 745 nm excitation filter and 820 nm emission filter, to observe nanoparticle emission. **(B)** Immunofluorescence microscopy of tumor slices resected 24 h after treatment and stained for P-selectin (green), TUNEL (red), and DAPI (blue). **(C)** Weight of mice measured after the indicated treatments, which were administered 7 days after inoculation with B16F10 cells. Data are presented as the mean of 5 mice per condition. FiDOX concentrations are listed in terms of the amount of doxorubicin encapsulated in the nanoparticle. Data shown as mean ±SD.

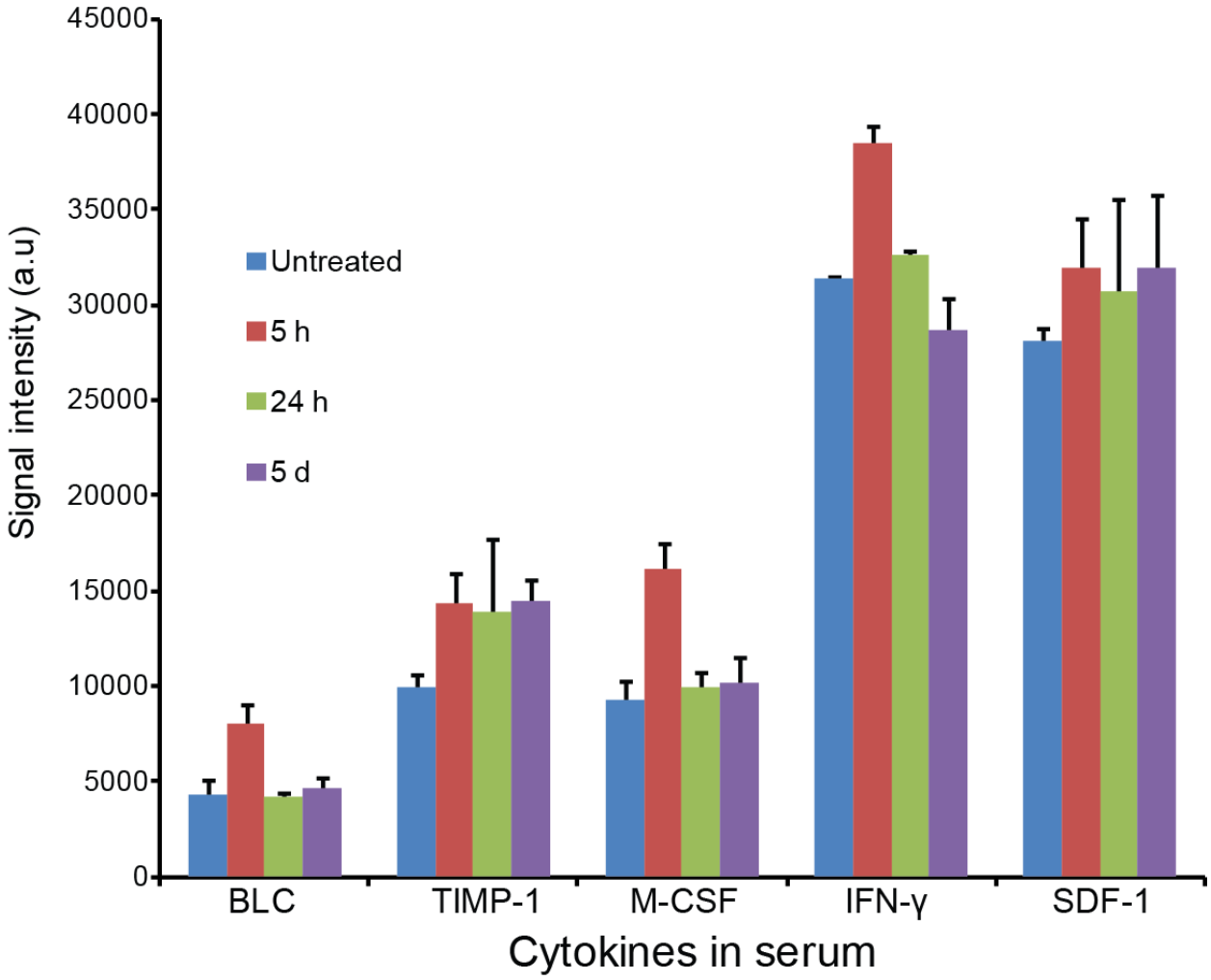

Figure S8. Cytokine profile in the serum of healthy mice injected with FiPAX nanoparticles. Cytokines were measured by ELISA assay. BLC = B lymphocyte chemoattractant, M-CSF = macrophage colony stimulating factor, TIMP-1 = tissue inhibitor of metalloproteinases, IFN-γ = interferon type II, SDF-1 = stromal cell-derived factor 1, also known as C-X-C motif chemokine 12 (CXCL12). Data shown as mean ±SD.

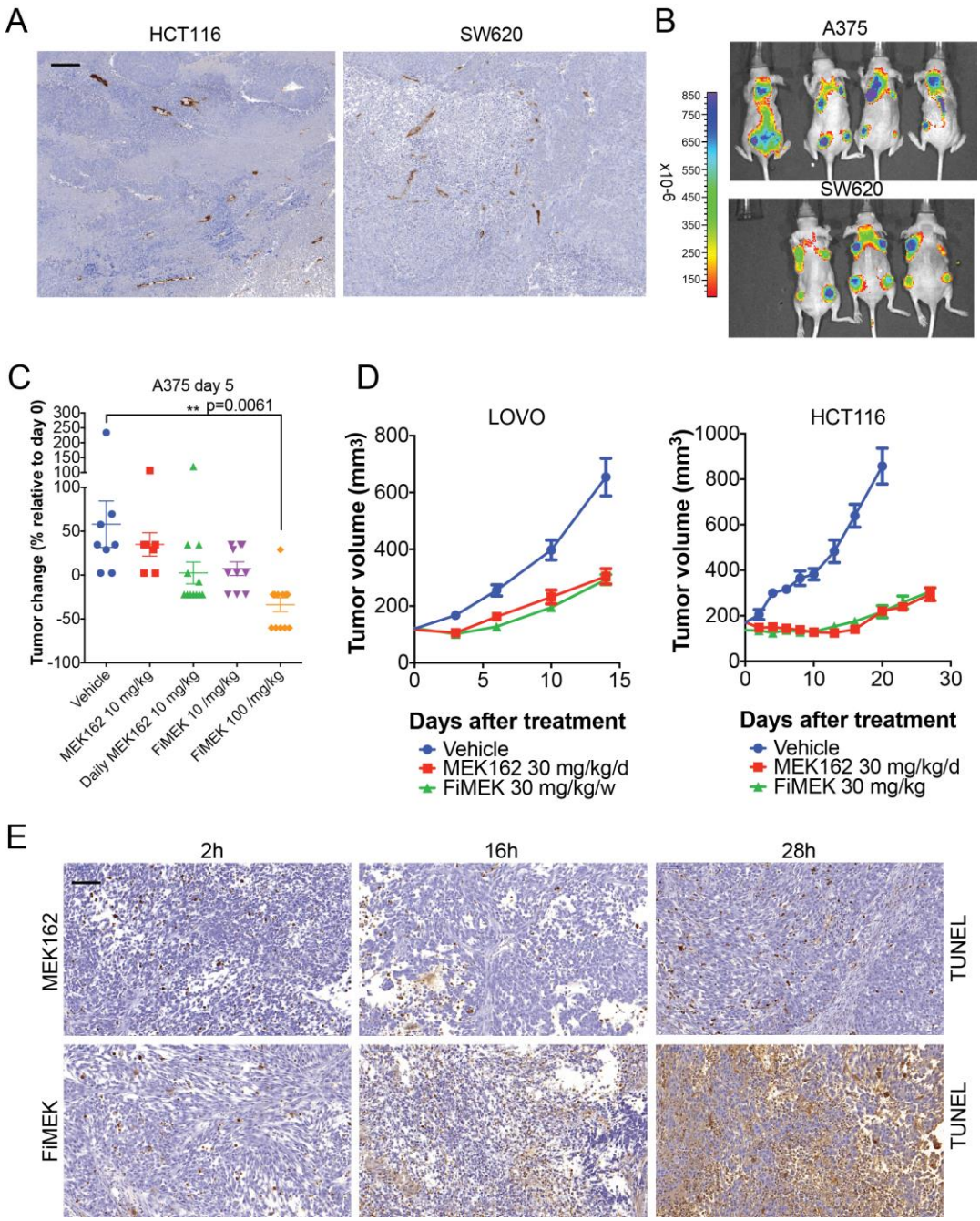

Figure S9. **P-selectin–targeted nanoparticle delivery of a MEK/ERK inhibitor. (A)** Immunohistochemical staining of P-selectin expression in HCT116 and SW620 xenografts. Scale bar = 75 µm. **(B)** Whole body imaging of FiMEK nanoparticles in A375 and SW620 xenografts 24 h after administration. **(C)** Change in tumor size, measured on day 5 after tumor inoculation. **(D)** Tumor growth inhibition in two murine xenograft models of colorectal cancer. Data shown as mean ±SD. **(E)** Evaluation of apoptosis after a single-dose administration of MEK162 or FiMEK nanoparticles in an HCT116 xenograft model. Scale bar = 50 µm**.** FiMEK

concentrations are listed in terms of the amount of MEK162 encapsulated in the nanoparticle. Error bars are SD of mean.

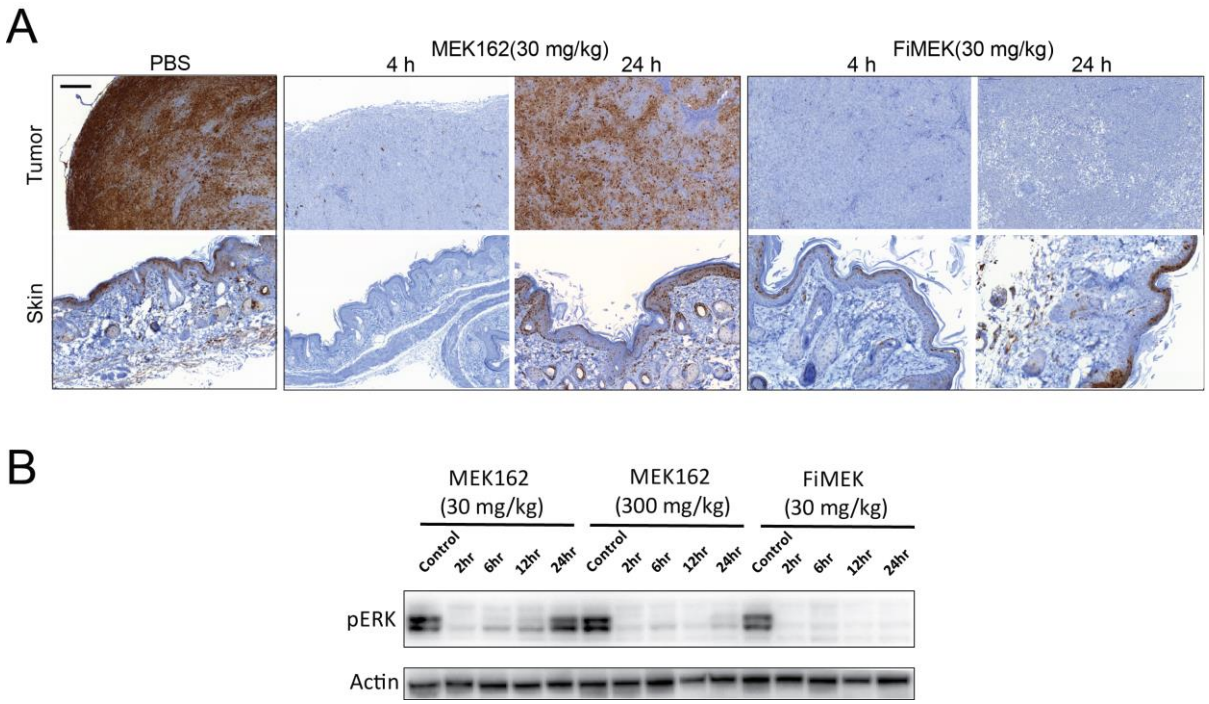

Figure S10. Kinetics of pERK inhibition in an HCT116 xenograft model. (A) Immunohistochemistry of pERK in skin and tumors of mice treated with MEK162 (30 mg/kg) or FiMEK nanoparticles (30 mg/kg) at 4 h and 24 h after administration. Scale bar = 50 μm. **(B)** Western blot of pERK expression in the tumor after treatments with free MEK162 (30 mg/kg or 300 mg/kg) or FiMEK (30 mg/kg).

Drug	Fi	Dex
Paclitaxel	105±4.2	102±3
MEK162	85±3.6	86±7.4
Doxorubicin	150±8.1	146±4.4

Table S1. Nanoparticle sizes in nanometers, as measured by DLS.

Parameters (units)	**Control (NT)**	**FiDOX**	**FiPAX**
WBCs (K/μL)	6.90±0.91	4.71±0.28	5.21±0.70
NE (K/μL)	3.71±3.23	1.46±0.12	1.61±0.48
LY (K/μL)	3.03±2.41	3.20±0.29	3.51±0.26
MO (K/μL)	0.15±0.05	0.06±0.03	0.07±0.02
EO (K/μL)	0.03±0.02	0.01±0.01	0.01±0.01
BA (K/μL)	0.00±0.00	0.00±0.00	0.00±0.00
RBC (M/μL)	10.30±0.69	10.55±0.60	10.55±0.54
Hb (g/dL)	13.30±0.99	13.87±0.50	13.70±0.52
HCT (%)	45.05±2.05	45.73±1.96	45.30±2.78
MCV (fL)	43.75±0.92	43.40±1.14	42.90±0.95
MCH (pg)	12.90±0.14	13.20±0.61	13.00±0.40
MCHC (g/dL)	29.50±0.85	30.40±0.69	30.30±0.89
PLT (K/μL)	1082.00±203.65	753.33±50.08	890.33±125.92

Table S2. Complete blood count conducted 24 hours after intravenous administration of nanoparticles in healthy mice.