Supporting Information

Hypoxia-tropic Protein Nanocages for Modulation of Tumor- and Chemotherapy-associated Hypoxia

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Table S1. Pharmacokinetic parameters of non-PEGylated FTn and PEG-FTn$^{75\%}$ in mice after systemic administration via tail vein.

	Non-PEGylated FTn	PEG-FTn$^{75\%}$
C_{max} (μg/mL)	697.3 ± 9.1	1192 ± 20
$t_{1/2}$ (min)	25.20 ± 0.22	73.83 ± 0.29
AUC_{last} (μg·min/mL)	17967 ± 449	102842 ± 4614
AUC_{INF} (μg·min/mL)	21824 ± 654	126874 ± 5596
MRT_{last} (min)	19.60 ± 0.18	57.65 ± 0.12
MRT_{INF} (min)	33.15 ± 0.53	101.0 ± 0.2

C_{max}, maximum concentration; $t_{1/2}$, time of plasma half-life; AUC, area under the curve; MRT, mean residence time. Data are presented as mean ± s.e.m.

Table S2. Comparison chart of pH and urea methods in preparing hybrid PEG-FTn.

	Preparation time	AF loaded number	Protein recovery yield
pH method	< 8 h	18 ± 6	< 30%
Urea method	> 24 h	62 ± 4	> 80%

Table S3. Pharmacokinetic parameters of free AF and PEG-FTn$^{75\%}$/AF in mice after systemic administration via tail vein.

	Free AF	PEG-FTn$^{75\%}$/AF
C_{max} (μg/mL)	0.35 ± 0.06	3.71 ± 0.58
AUC_{last} (μg·h/mL)	0.18 ± 0.01	13.02 ± 0.93
MRT_{last} (h)	1.39	5.91

C_{max}, maximum concentration; AUC, area under the curve; MRT, mean residence time. Data are presented as mean ± s.e.m.

Table S4. Serum biochemistry analysis

Biochemistry parameters	Saline	PEG-FTn$^{75\%}$ (320 mg/kg)	AF (10 mg/kg)	PEG-FTn$^{75\%}$/AF (10 mg/kg)
AST (U/L)	69.3 ± 2.9	72.5 ± 6.5	60.3 ± 3.5	72.5 ± 4
ALT (U/L)	26 ± 2.3	19 ± 2	22.3 ± 2.1	24.5 ± 2
BUN (mg/dL)	20.3 ± 1.9	24.8 ± 1.8	21.3 ± 1.2	21.2 ± 1.8
ALP (U/L)	105.7 ± 3.1	91.8 ± 2.6	77.3 ± 3.7*	102.3 ± 3.3
CA (mg/dL)	8.7 ± 0.3	8.9 ± 0.4	8.8 ± 0.5	9.1 ± 0.4
GLU (mg/dL)	186 ± 5.5	215.8 ± 5.7	194.8 ± 5.6	187.3 ± 4.4
LDH (U/L)	192 ± 2.2	191.3 ± 8.5	193.3 ± 6.4	218.5 ± 6.5
GGT (U/L)	3 ± 0	3± 1.1	2.5 ± 0.7	3.3 ± 0.7
TPROT (g/dL)	4.6 ± 0.4	4.8 ± 0.4	4.7± 0.6	5.3± 0.7
ALB (g/dL)	2.8 ± 0.3	2.9 ± 0.2	2.6 ± 0.5	2.8 ± 0.4
TBILI (mg/dL)	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2
CREAT (mg/dL)	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0	0.3 ± 0
CK (U/L)	592.7 ± 10.7	528.3 ± 9.3	590.5 ± 14.8	629.5 ± 19.4
PHOS (mg/dL)	5.8 ± 0.3	6.7 ± 0.9	6.5 ± 0.9	8.3 ± 1.3

*indicates statistically significant differences compared to saline-treated group

Biochemistry parameters:
AST, aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood urea nitrogen; ALP, alkaline phasphatase; CA, calcium; GLU, glucose; LDH, lactate dehydrogenase; GGT, gamma glutanyl transferase; TPROT, total protein concentration; ALB, albumin; TBILI, total bilirubin; CREAT, creatinine; CK, creatine kinase; PHOS, phosphate.

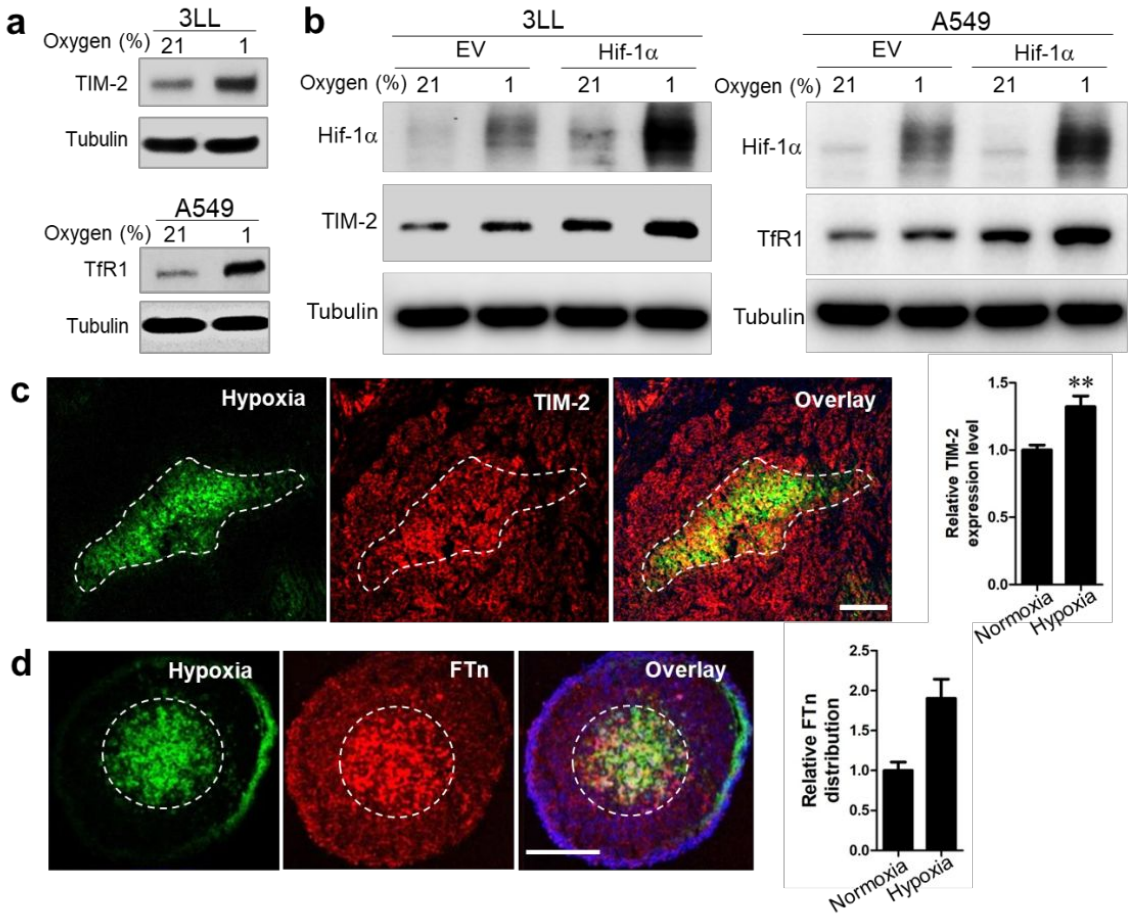

Figure S1. Elevation of TfR1 and TIM-2 expression in A549 human non-small cell lung carcinoma and 3LL mouse Lewis lung carcinoma, respectively, under hypoxia and hypoxia-tropism of FTn. (a) Western blot of TfR1 and TIM-2 in A549 and 3LL cells, respectively, that are incubated at 21% (normoxia) or 1% (hypoxia) O_2. (b) Western blot of HIF-1α and FTn receptor (i.e. TfR1 and TIM-2) expression in 3LL and A549 cells treated with either empty (EV) or HIF-1α-expressing plasmid vectors at 21% and 1% O_2, respectively. (c) Co-localization of hypoxic areas (green) and TIM-2 expression (red) within 3LL-based flank tumor. (Left) Representative confocal images and (Right) image-based quantification of TIM-2 expression in normoxic and hypoxic tumor areas. Scale bar = 100 μm. (d) Cross section images of 3LL multicellular spheroid showing the distribution of FTn (red) in hypoxic area (green). Blue color represents cell nuclei. (Left) Representative confocal images and (Right) image-based quantification of FTn in normoxic and hypoxic tumor areas. Scale bar = 200 μm. **P* < 0.05, ***P* < 0.01.

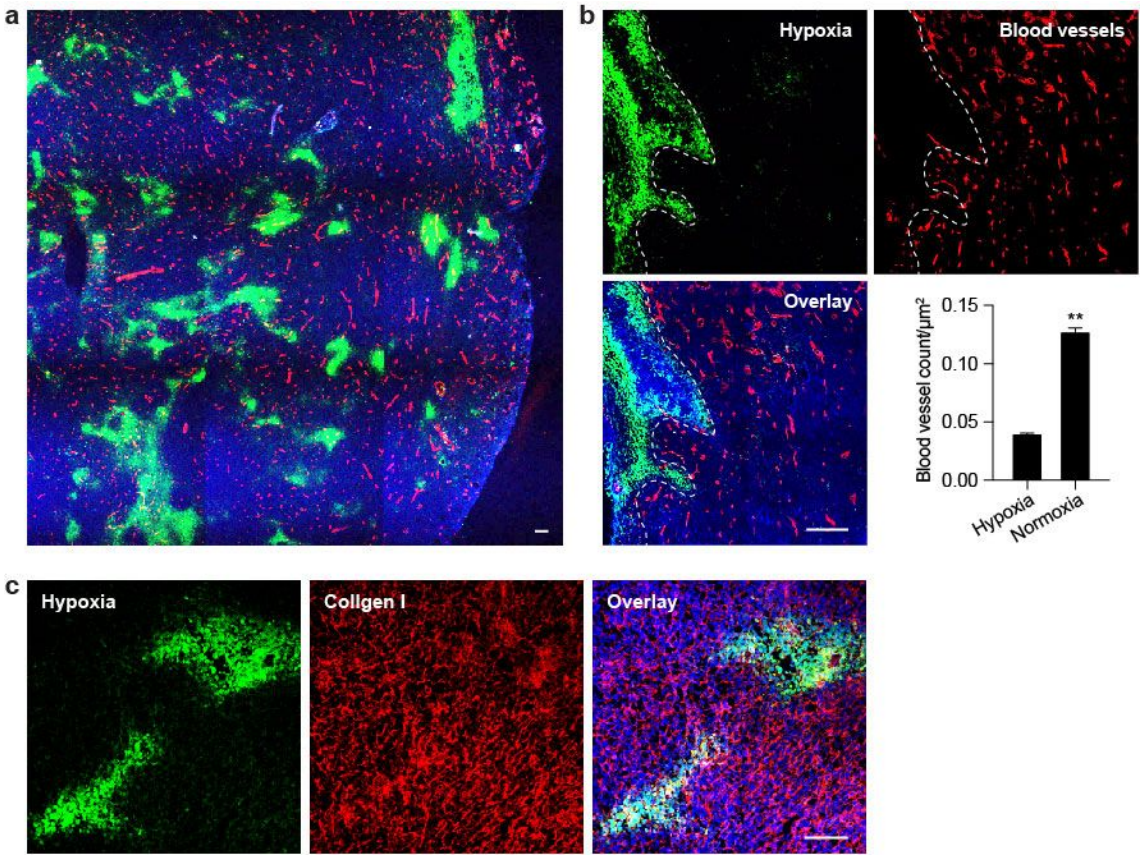

Figure S2. (a) Co-localization of hypoxic areas within a 3LL-based flank tumor (green) and blood vessels (red). Blue color represents cell nuclei. (b) Representative confocal images in an enlarged area (Left) and image-based quantification of blood vessel distribution within hypoxic and normoxic tumor areas (Right). (c) Representative confocal images of distribution of hypoxia and collagen I in subcutaneously established 3LL tumor. Scale bar = 100 μm. $^{**}P < 0.01$.

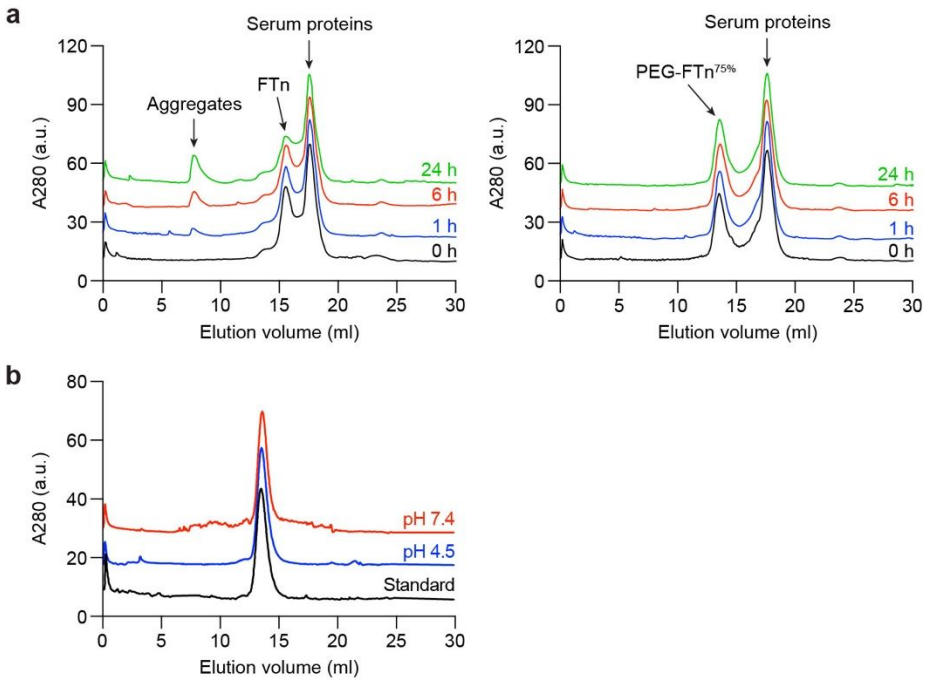

Figure S3. Colloidal stability of PEG-FTn$^{75\%}$ in physiological conditions assessed by size exclusion chromatography. (a) Stability of (Left) FTn and (Right) PEG-FTn$^{75\%}$ in 25% human serum over incubation times (0, 1, 6 and 24 h). (b) Stability of PEG-FTn$^{75\%}$ after a 24 h incubation in pH 4.5 and pH 7.4 buffers.

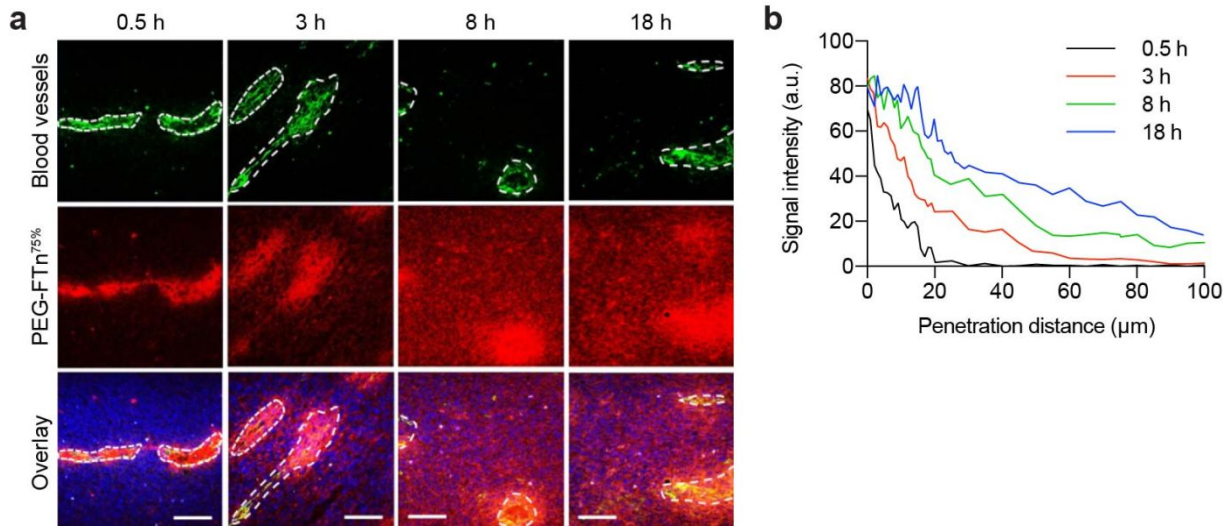

Figure S4. Penetration of systemically administered PEG-FTn$^{75\%}$ into a 3LL-based flank tumor over time following extravasation. (a) Representative confocal images showing penetration of PEG-FTn$^{75\%}$ (red) into the flank tumor (blue: DAPI) from blood vessels (green) at different time points after the administration. Scale bar = 100 μm. (b) Image-based quantification of PEG-FTn$^{75\%}$ penetration into the flank tumor from blood vessels over time (n = 4).

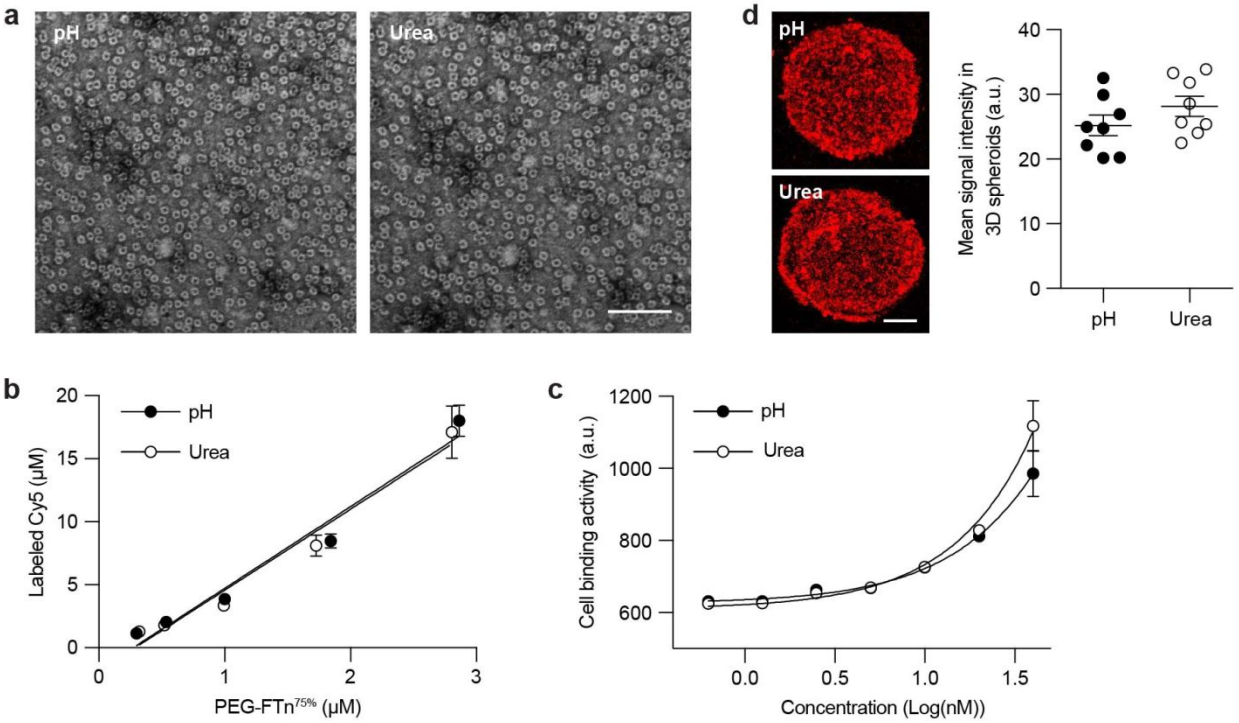

Figure S5. Comparison of PEG-FTn$^{75\%}$ prepared using two different approaches, including pH adjustment and urea gradient methods. (a) Representative TEM images of PEG-FTn$^{75\%}$ prepared using (Left) pH adjustment and (Right) urea gradient methods. Scale bar = 50 nm. (b) Quantification of surface-associated Cy5-labeld ferritin subunits on PEG-FTn$^{75\%}$ prepared by different methods. (c) Concentration-dependent 3LL cell bindings of PEG-FTn$^{75\%}$ prepared by different methods. (d) Penetration of PEG-FTn$^{75\%}$ prepared by different methods through 3LL-based multicellular spheroids. (Left) representative confocal images and (Right) image-based quantification of PEG-FTn$^{75\%}$ penetration through the spheroids. Scale bar = 100 μm.

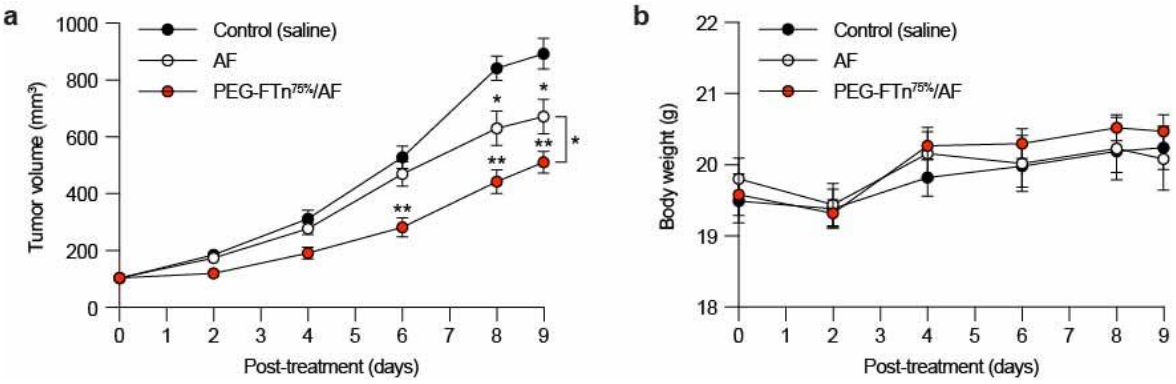

Figure S6. Systemic treatment of 3LL-based flank tumors with PEG-FTn$^{75\%}$/AF. (a) Tumor growth and (b) change in the body weight over time following the treatment with saline, AF or PEG-FTn$^{75\%}$/AF (n = 10). *P < 0.05, **P < 0.01.

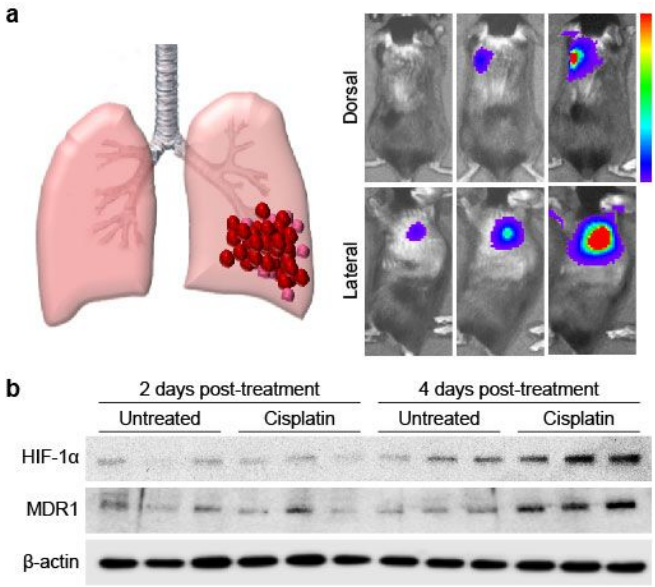

Figure S7. Characterization and response to systemic cisplatin treatment of orthotopic lung tumors. (a) Establishing 3LL-based orthotopic lung tumors. (Left) Schematic illustration and (Right) representative bioluminescence images of the orthotopic tumors established in the left lung. (b) Western blots of HIF-1α and MDR1 expression in the orthotopic lung tumor 2 and 4 days after the systemic treatment with normal saline (control) or 2 mg/kg cisplatin.

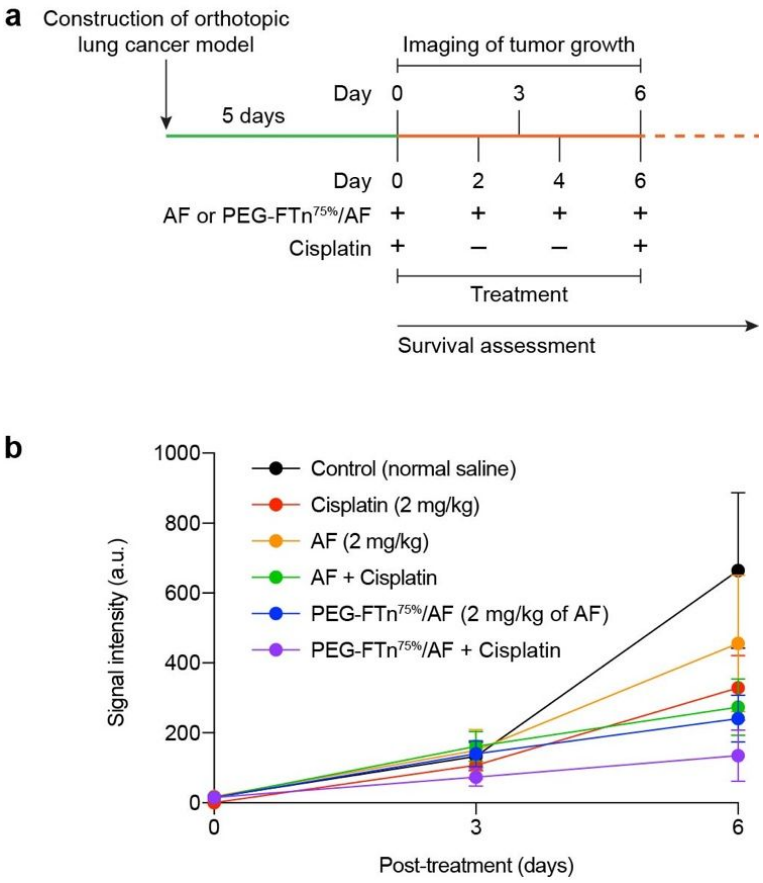

Figure S8. Combined treatment of 3LL-based orthotopic lung tumors with PEG-FTn$^{75\%}$/AF and cisplatin. (a) Treatment schedule for the combined treatment of cisplatin with AF or PEG-FTn$^{75\%}$/AF. (b) Bioluminescence-based assessment of tumor growth 3 and 6 days after the initiation of treatments.

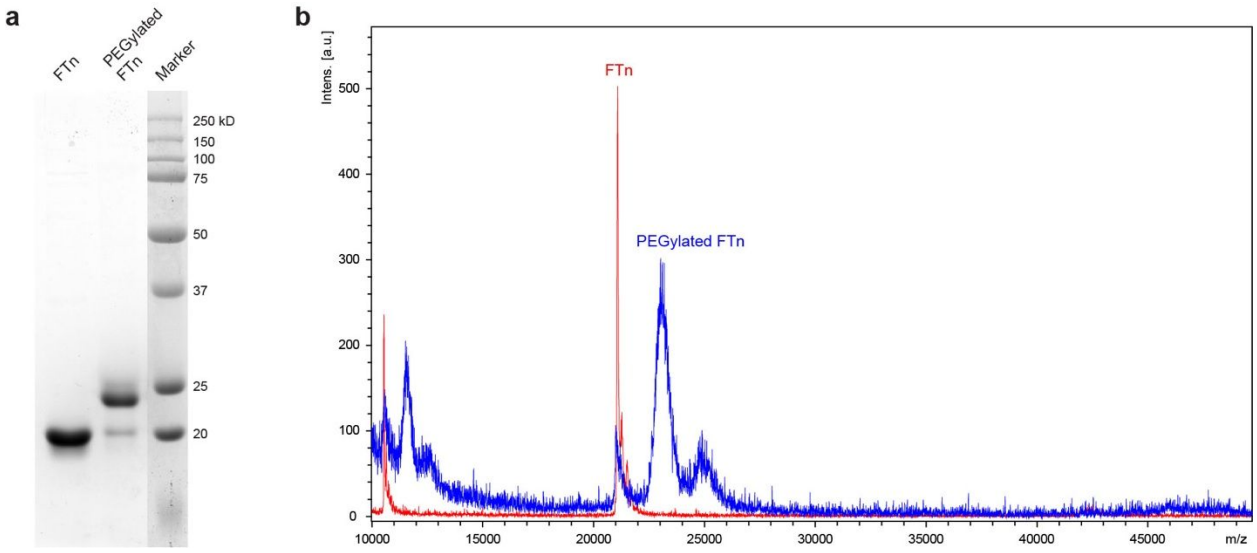

Figure S9. Characterization of PEGylated FTn. (a) Denaturing SDS-PAGE of non-PEGylated and PEGylated ferritin proteins. The protein band was stained using coomassie blue stain. (b) MALDI-TOF MS analysis of non-PEGylated (red) and PEGylated (blue) ferritin proteins.