Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Metal Ion Assisted Interface Re-engineering of Ferritin Protein Nanocage for Enhanced Biofunctions and Cancer Therapy

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Primers used in this study

Mutant A primers:

Primer	Sequence (5'-3')
MuA1-F	accagcgaggtggacgaatcttcctgcaggatataaagaaacctgaccgtgatgactggg
MuA1-R	tagtgactgattcacactcttttccaagtgcagtgcacactccattgcattcagcccgctctcccagtcatcacg
MuA2-F	atctcatgaagagCATgaacatgctCATaaactgatgaagctgcagaaccagcgaggtgg
MuA2-R	tcttgtcagtagccagtttgtgaagttccagtagtgactgattcac
MuA3-F	cgggatgatgtggccctgaagaactttgccaaatactttCATcatcaatctcatgaagag
MuA3-R	tcattcaggtaatgcgtctcaatgaagtcacataagtggggatcattcttgtcagtagcc
MuA4-F	agttgtatgcctcctacgtctatctgtccatgtcttgttattttgaccgggatgatgtgg
MuA4-R	gtaagttggtcacgtggtcacccagttctttaatggatttcacctgctcattcaggtaats
MuA5-F	catgccatgggcaccaccgcgtctccctcgcaagtgcgccagaactaccaccaggactcggaggctgccatcaaccgcca gatcaacctggagttgtatgcctc
MuA5-R	cggctcgagttagctctcatcaccgtgtcccagggtgtgcttgtcaaagagatattctgccatgccagattcaggggctcccat cttgcgtaagttggtcacg

Mutant B primers:

Primer	Sequence (5'-3')
MuB1-F	accagcgaggtggacgaatcttcctgcaggatataaagaaacctgaccgtgatgactggg
MuB1-R	tagtgactgattcacactcttttccaagtgcagtgcacactccattgcattcagcccgctctcccagtcatcacg
MuB2-F	atctcatgaagagagggaacatgctCATaaactgatgCATctgcagaaccagcgaggtgg
MuB2-R	tcttgtcagtagccagtttgtgaagttccagtagtgactgattcac
MuB3-F	cgggatgatgtggccctgaagaactttCATaaatactttCATcatcaatctcatgaagag
MuB3-R	tcattcaggtaatgcgtctcaatgaagtcacataagtggggatcattcttgtcagtagcc
MuB4-F	agttgtatgcctcctacgtctatctgtccatgtcttgttattttgaccgggatgatgtgg
MuB4-R	gtaagttggtcacgtggtcacccagttctttaatggatttcacctgctcattcaggtaat
MuB5-F	Catgccatgggcaccaccgcgtctccctcgcaagtgcgccagaactaccaccaggactcggaggctgccatcaaccgc cagatcaacctggagttgtatgcctc
MuB5-R	Cggctcgagttagctctcatcaccgtgtcccagggtgtgcttgtcaaagagatattctgccatgccagattcaggggctccc atcttgcgtaagttggtcacg

Mutant C primers:

Primer	Sequence (5'-3')
MuC1-F	accagcgaggtggacgaatcttcctgcaggatataaagaaacctgaccgtgatgactggg
MuC1-R	tagtgactgattcacactcttttccaagtgcagtgcacactccattgcattcagcccgctctcccagtcatcacg
MuC2-F	atctcatgaagagagggaacatgctgagaaactgatgaagctgcagaaccagcgaggtgg

MuC2-R	tcttgtcagtagccagtttgtgaagttccagtagtgactgattcac
MuC3-F	cgggatgatgtggccctgaagaactttgccaaatactttctccatcaatctcatgaagag
MuC3-R	tcATGcaggtaatgcgtctcaatgaagtcacataagtggggatcattcttgtcagtagcc
MuC4-F	agttgtatgcctcctacgtctatctgtccatgtcttgttattttgaccgggatgatgtgg
MuC4-R	gtaagttggtcacgtgATGacccagttcATGaatggaATGcacctgctcATGcaggtaat
MuC5-F	catgccatgggcaccaccgcgtctccctcgcaagtgcgccagaactaccaccaggactcggaggctgccatcaaccgc cagatcaacctggagttgtatgcctc
MuC5-R	cggctcgagttagctctcatcaccgtgtcccagggtgtgcttgtcaaagagatattctgccatgccagattcaggggctccc atcttgcgtaagttggtcacg

PCR conditions

Steps	**1**	**2**	**3**	**4**	**5**
Primers	MuA(B or C)1-F, MuA(B or C)1-R	MuA(B or C)2-F, MuA(B or C)2-R	MuA(B or C)3-F, MuA(B or C)3-R	MuA(B or C)4-F, MuA(B or C)4-R	MuA(B or C)5-F, MuA(B or C)5-R

For specific PCR procedure, five rounds PCR are as follows:

Cycle number	**Denaturation**	**Annealing**	**Extension**	**Final extension**
1	2 min at 95 ºC			
2-19	30 s at 95 ºC	30 s at 58 ºC	1 min at 72 ºC	
20				10 min at 72 ºC

Table S1. Computational calculation of interface interacting atoms and areas between two nearby ferritin subunits in ferritin variants.

	Structure 1				Structure 2				Interface area, Å^2
	Range	N_{at}	N_{res}	Surface Å^2	Range	N_{at}	N_{res}	Surface Å^2	
MutA	A	154	37	9617	B	159	36	9617	1478.6
MutB	A	163	35	9360	B	160	39	9338	1509.9
MutC	A	142	36	9384	B	137	36	9384	1285.3
Fn	A	128	32	9373	B	150	36	9367	1297.7

MutA: Mutant A ferritin; MutB: Mutant B ferritin; MutC: Mutant C ferritin; Fn: Ferritin

$^{i}\mathbf{N}_{\mathbf{at}}$ indicates the number of interfacing atoms in the corresponding structure.

$^{i}\mathbf{N}_{\mathbf{res}}$ indicates the number of interfacing residues in the corresponding structure.

Surface Å^2 is the total solvent accessible surface area in square Ångstroms.

Interface area in Å^2, calculated as difference in total accessible surface areas of isolated and interfacing structures divided by two.

Table 2. Titration of $^{64}Cu^{2+}$ incorporation ratio in ferritin variants

	Cu^{2+} concentration (μM)	1	10	50	100	1000
Mutant A	Labeling yield (%)	100	91.15±0.55	23.95±12.89	20.16±3.76	2.66±2.33
	Number of binding sites per ferritin	N/A	8.09±2.33	10.05±8.5	18.44±7.43	18.14±13.04
	Cu^{2+} concentration (μM)	1	10	50	100	1000
Mutant B	Labeling yield (%)	100	92.74±1.96	32.11±2.2	14.49±3.76	1.89±0.32
	Number of binding sites per ferritin	N/A	9.83±0.2	17.02±1.17	15.36±3.99	20±3.44
	Cu^{2+} concentration (μM)	1	10	50	100	1000
Mutant C	Labeling yield (%)	31.79±10.33	5.8±0.82	2.37±0.85	0	0
	Number of binding sites per ferritin	0.17±0.05	0.31±0.04	0.63±0.23	N/A	N/A
	Cu^{2+} concentration (μM)	1	10	50	100	1000
Native Fn	Labeling yield (%)	26.67±10.53	5.33±0.94	2.17±0.26	0	0
	Number of binding sites per ferritin	0.21±0.03	0.46±0.13	0.94±0.24	N/A	N/A

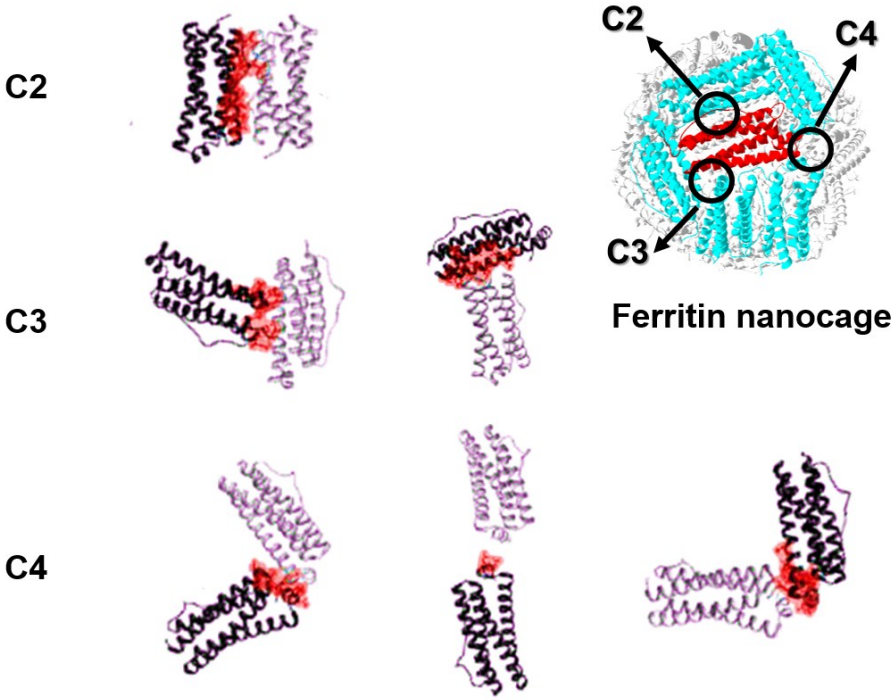

Figure S1. Six inter-subunit interfaces of ferritin heavy chain as shown in red color. C2, C3, C4 interfaces are highlighted with black circles.

1st Round	2nd Round	3rd Round	4th Round	5th Round
107bp	200bp	293bp	387bp	567bp

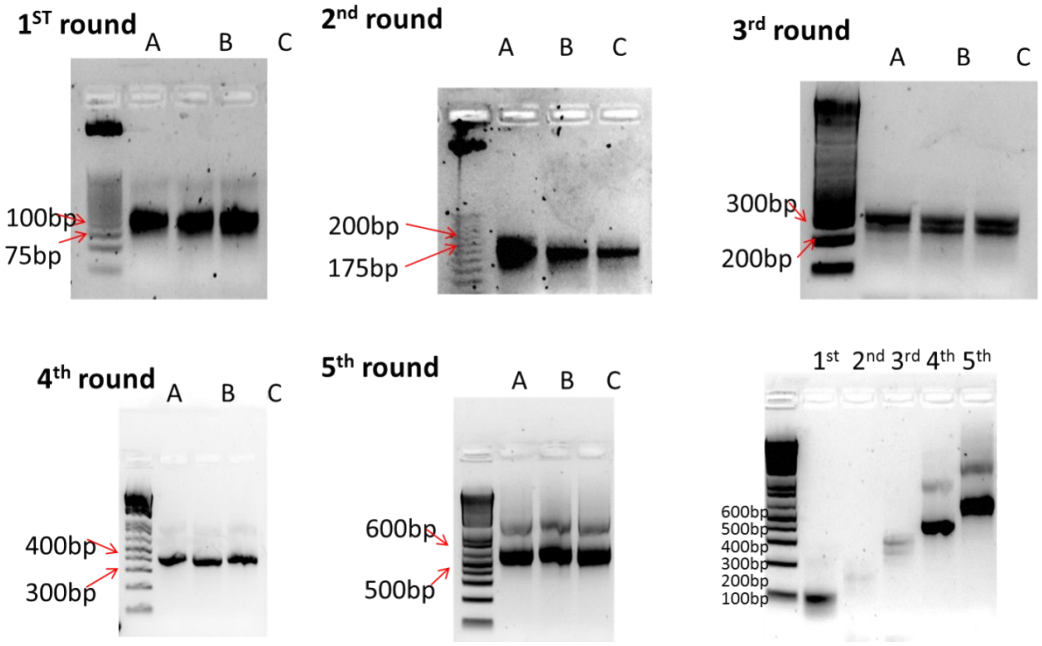

Figure S2. Step-wise site-directed mutagenesis of native ferritin. 1% agarose gels were used in the entire experiment.

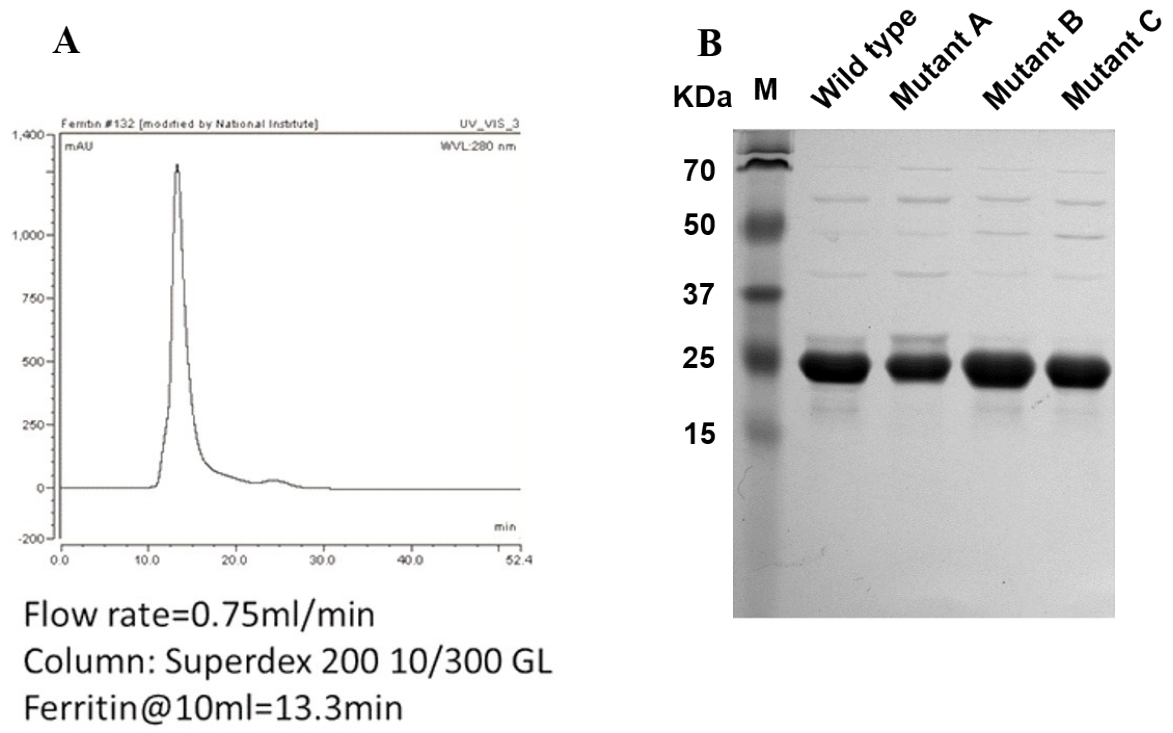

Figure S3. (A) GPC purification of expressed ferritin variants. (B) SDS-PAGE analysis of purified ferritin variants. The dark band below 25KDa is the monomer, and other bands above 25KDa are multimers.

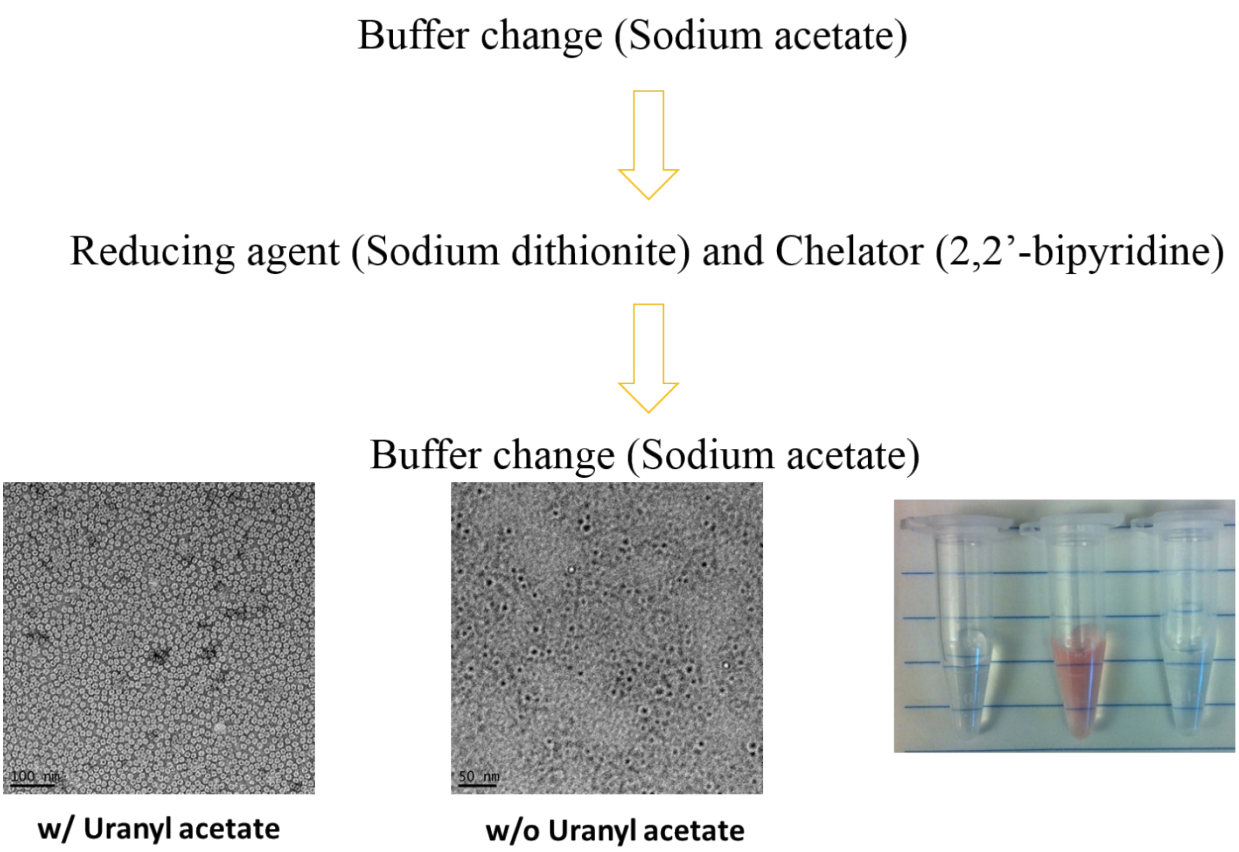

Figure S4. Procedure and images of metal ion removal from purified ferritins for subsequent $^{64}Cu^{2+}$ labeling.

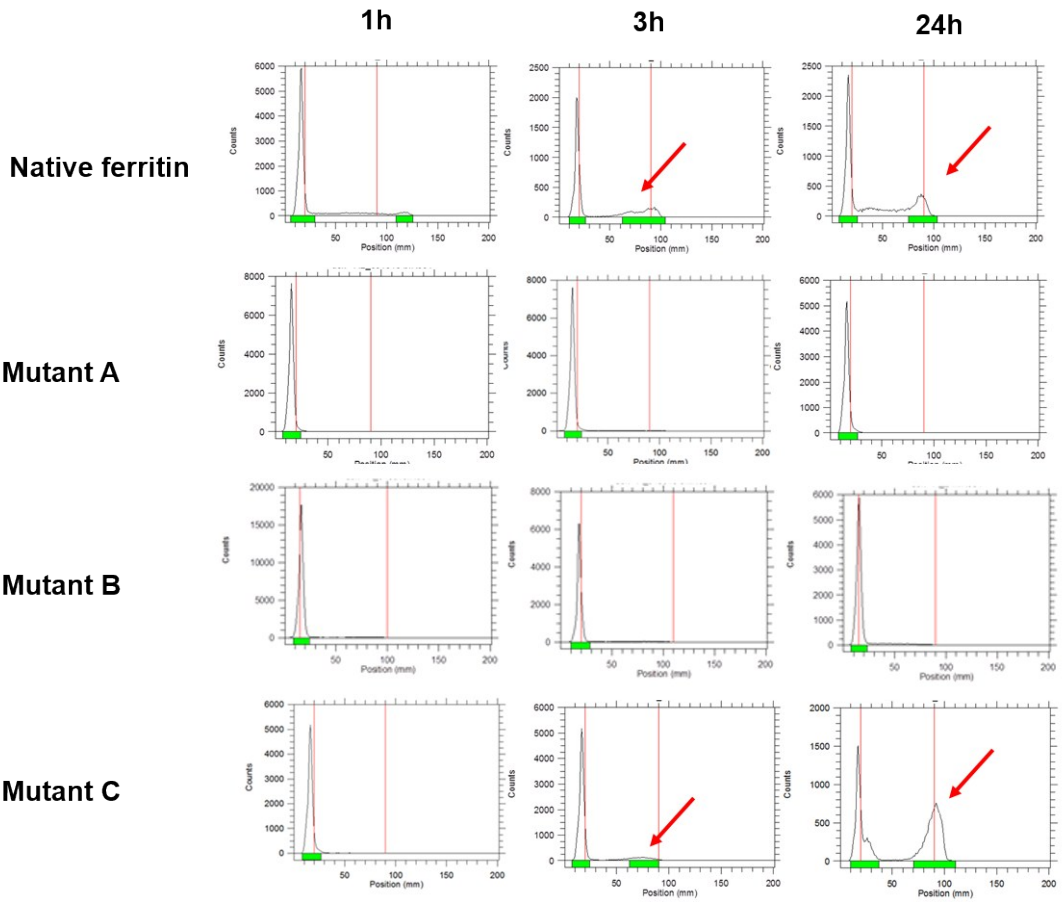

Figure S5. TLC analysis of the stability of $^{64}Cu^{2+}$ coordination in ferritin variants in mouse serum.

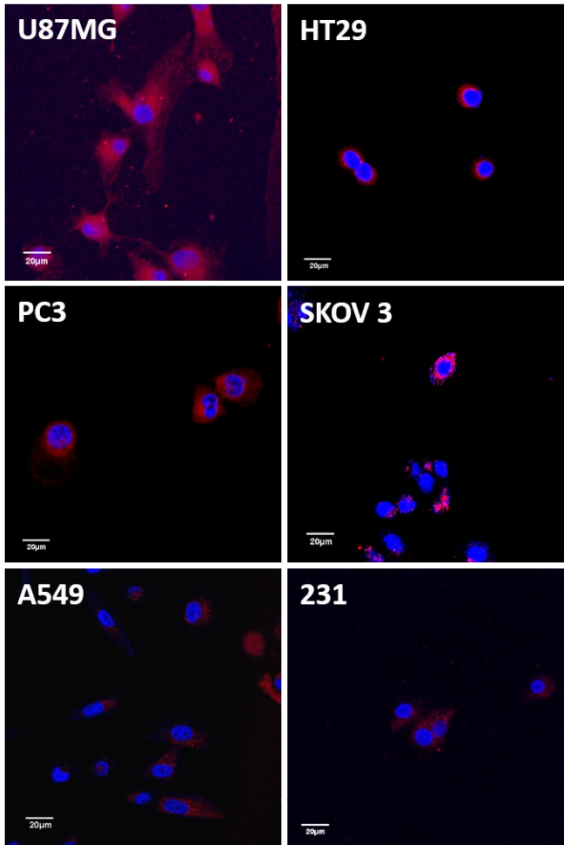

Figure S6. Uptake of Cy5.5 labeled ferritin nanoprobes (mutant B) by different cancer cells. Cell nucleus are counterstained with DAPI. Scale bar: 20μm.

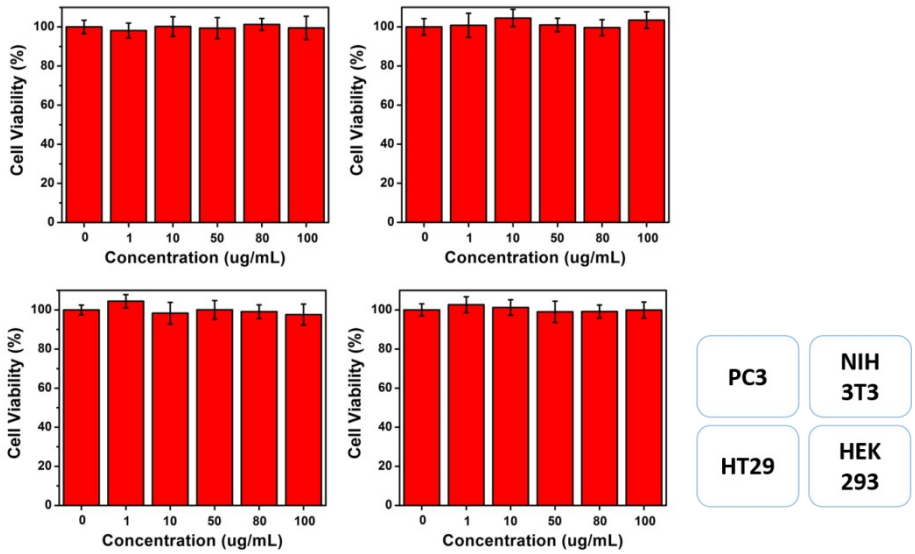

Figure S7. MTT assay of mutant B ferritin nanoprobe in different cancer cells (n=6).

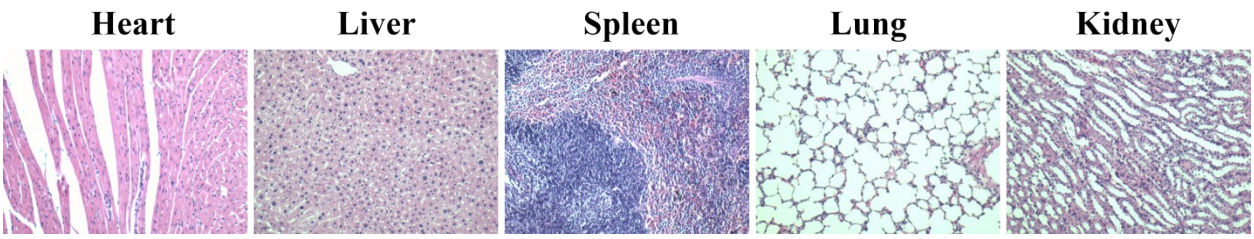

Figure S8. H&E staining of major organs after injection of the free ferritin nanoprobe (mutant B) in PET imaging.

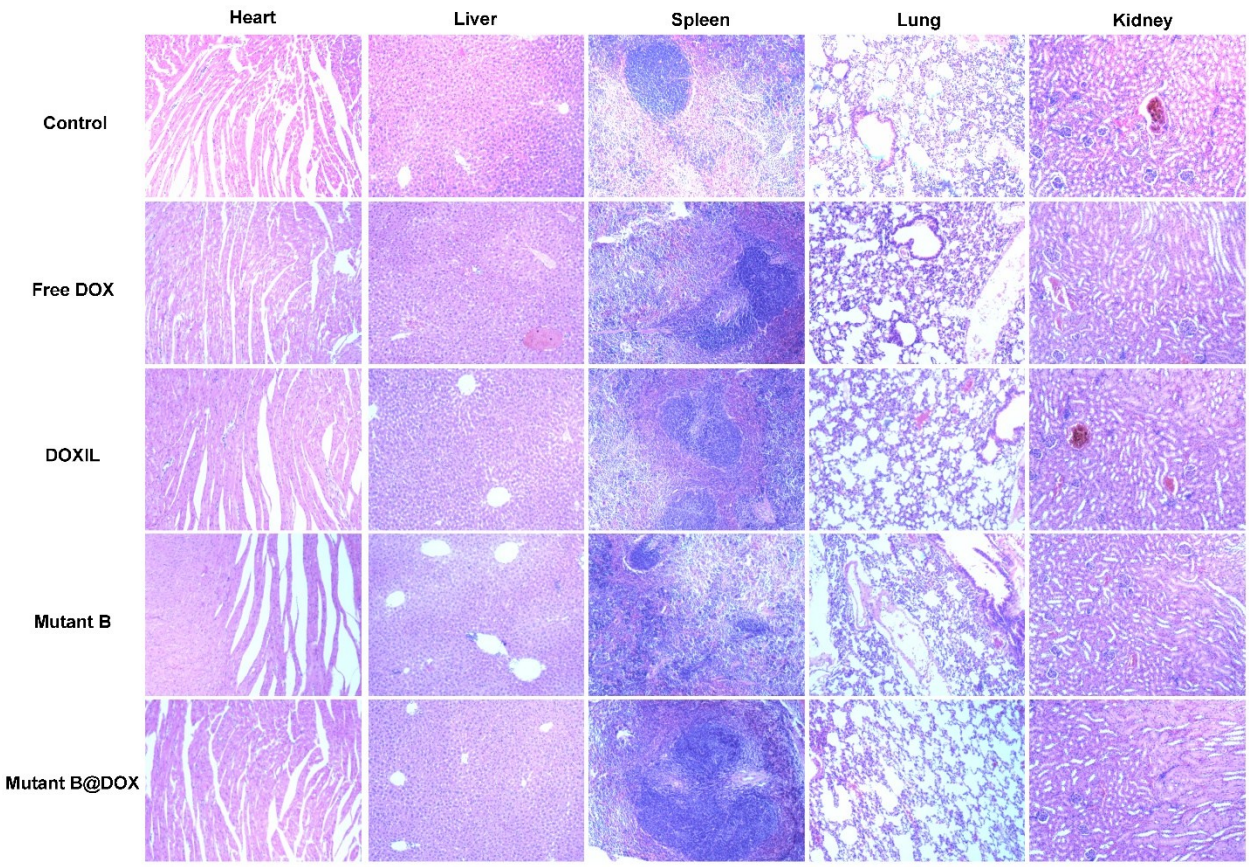

Figure S9. H&E stained imaging of main organs from various treatment groups.