Supporting Information

E. coli Nissle 1917-Derived Minicells for Targeted Delivery of Chemotherapeutic Drug to Hypoxic Regions for Cancer Therapy

Content:

I. RED/ET recombination method

The EcN with the plasmid of pSC101-BAD-gbaA-tet was grown in LB medium at 37 ℃ for overnight. Next, the bacteria was transferred into the fresh medium by 2% and cultured at 30 ℃ for another 2 h. Further, 20 μL of 10% L-arabinose was added into the medium and shaken at 37 ℃ for 40 min. Then, the EcN was gathered and washed for two times using chilled millipore water. Further, the gathered pellet was resuspended in 100 μL millipore water and mixed with 3 ~ 5 μg PCR products (chloromycetin gene (*Cm*) with homologous sequences to the ends of *minCD* gene), which was transferred into 1 mm chilled electroporation cuvettes. Electroporation was conducted at 1350 V using Electroporator 2510 (Eppendorf, Germany). The electroporation products in the cuvette was carefully resuspended in 1 mL of LB medium without antibiotics and incubated at 37 ℃ for 1 h. Finally, the bacteria was collected by centrifugation and streaked on one LB agar plate containing 25 μg/mL chloromycetin for screening the positive strains.

II. Gene sequence of Lpp'-OmpA-SSG-pHLIP-Histag

1. The DNA fragment of Lpp'-OmpA-SSG-pHLIP-Histag contains Lpp' (lowcase letter), OmpA (black color), pHLIP (red color), six His-tag (green color) and nucleotides of flexible amino acids (blue color).

atgaaagctactaaactggtactgggcgcggtaatcctgggttctactctgctggcaggttgctccagcaacgctaaa
atcgatcagggtatcCCGTATGTTGGCTTTGAAATGGGTTACGACTGGTTAGGTCGT
ATGCCGTACAAAGGCGACAACATCAACGGCGCATACAAAGCTCAGGGCGT
TCAGCTGACCGCTAAACTGGGTTACCCAATCACTGACGATCTGGACATCTA

CACTCGTCTGGGTGGTATGGTATGGCGTGCAGACACCAAGGCTAACGTACC

TGGTGGCGCATCCTTTAAAGACCACGACACCGGCGTTTCTCCGGTCTTCGC

TGGCGGTGTTGAGTATGCGATCACTCCTGAAATCGCTACCCGTCTGGAATAC

CAGTGGACCAACAACATCGGTGACGCACACACCATCGGCACTCGTCCGGA

CAACggtatcccgtccagcggtGCTGCTGAACAGAACCCGATCTACTGGGCTCGTTA

CGCTGACTGGCTGTTTACCACCCCGCTGCTGCTGCTGGATCTGGCTCTGCT

GGTCGACGCGGACGAAGGCACTGGCggttccagccatcatcatcatcatcacTAA

2. Amino acid sequence of pHLIP

   AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTG

III. Primers

Table S1 Primers

Name of primer	Nucleotide sequence
KTminCD1	AGGATGTCAAACACGCCAATCGAGCTTAAAGGCAGTAGCTTCACT TTATAAGCAGCATCACCCGACGCACTT
KTminCD2	TGGCTGTGTTTTTCTTCCGCGAGAGAAAGAAATCGAGTAATGCCA TAACCTGACCTCCTTTACGCCCCGCCCTGCCAC
IDCD1	AAA TCC TGT CCC TGT TG
IDCD2	ACT GCG GTA AAT GCG GT
Luc1	ATA AGA ATG CGG CAT GAG CGC GAA GTT ACA TTA ATA A
Luc2	CCG CTC GAG TCT AGA TCA ACT ATT AAA TGC TTG GTT TAA GC
Tet-R	CAT GCC ATG GTG AGG GCG GTC ATT CTA CG
Tet-F	CTT CTT AAA GTT AAA CAA AAT TAT TTC TAG AGC AGT CAC CCT CAC TTT TCG
pHLIP-R	ATT TTG TTT AAC TTT AAG AAG GAG ATA TAC CAT GAA AGC CAC CAA ACT GGT
pHLIP-F	CCC AAG CTT TTA ATG ATG GTG ATG AT

IV. Supporting information

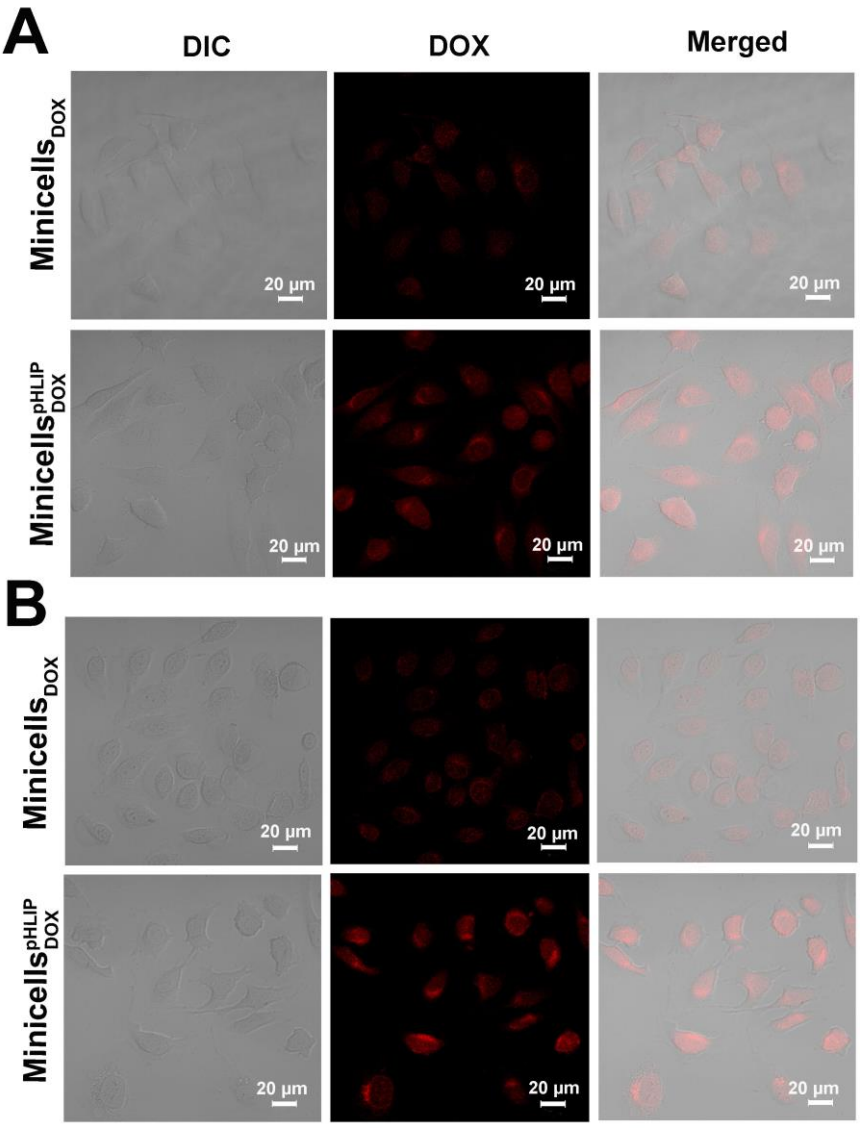

Figure S1. Confocal imaging of the internalization of minicells$_{DOX}$ and minicells$^{pHLIP}_{DOX}$ by MCF-7 tumor cells in pH 7.4 cell culture medium (A) and pH 6.5 culture medium (B).

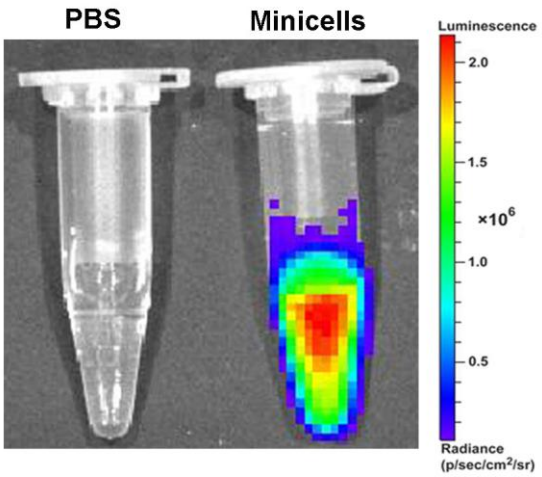

Figure S2. Bioluminescence density of minicells (1×10^9 cells/mL).

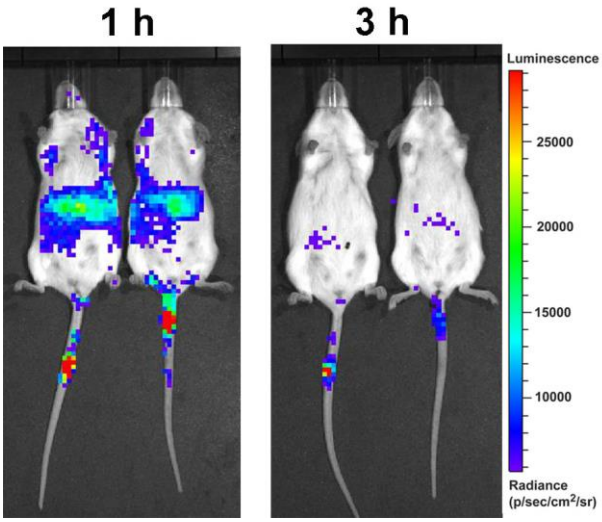

Figure S3. *In vivo* distribution of ECN in healthy BALB/c mice. The bioluminescence signals in the mice were imaged at 1 h and 3 h after i.v. administration of 2×10^6 EcN.

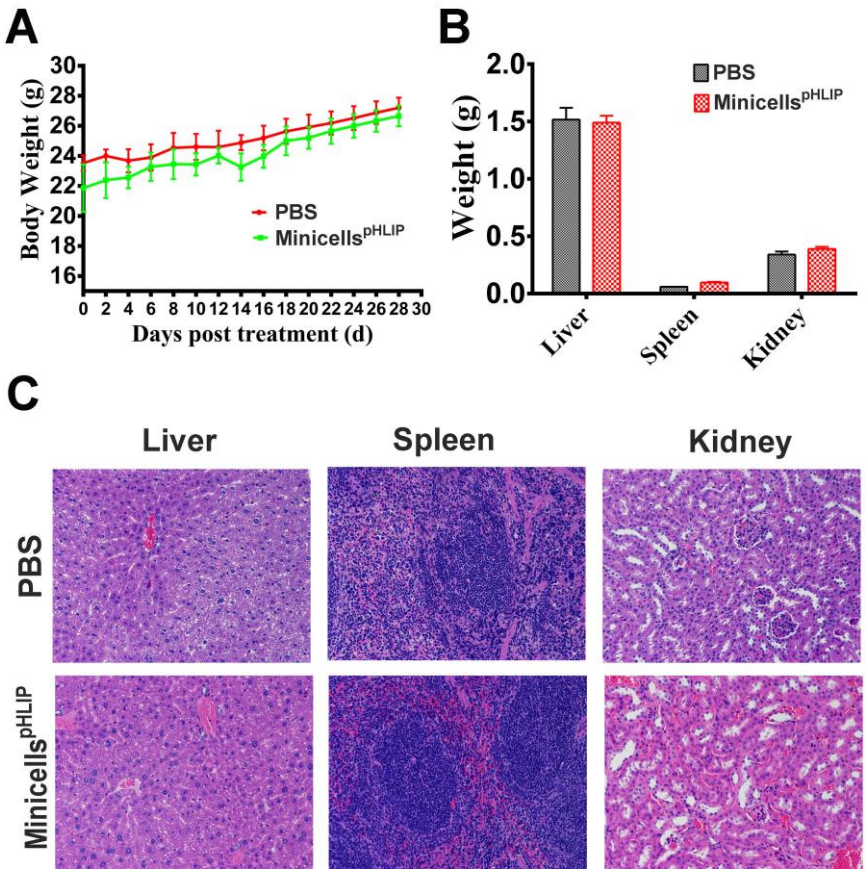

Figure S4. *In vivo* toxicity assay. Healthy BALB/c mice (n = 10) were weekly i.v. treated by PBS or minicells^{pHLIP} for one month. The weights of the mice were recorded every two days (A). At sacrifice, the livers, spleens and kidneys were excised and weighted (B). (C) H&E staining were used to analyze the pathological characteristics of liver, spleen and kidney (200 X).