

# Bioengineered Human Serum Albumin Fusion Protein as Target/Enzyme/pH Three-Stage Propulsive Drug Vehicle for Tumor Therapy

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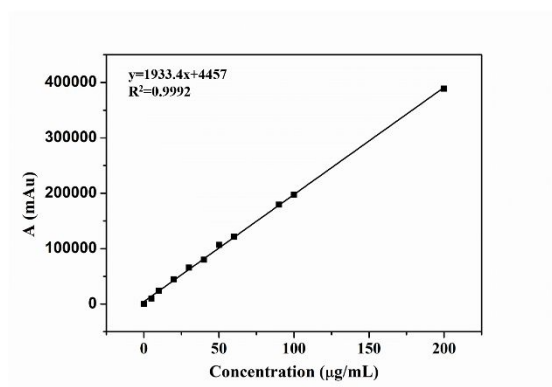
Component	Volume
Primers Star Max (2X)	25 $\mu$ L
Upstream primer	2 $\mu$ L
Downstream primer	2 $\mu$ L
Ultra-pure water	20.5 $\mu$ L
HSA Template	2.5 $\mu$ L

**Table S2.** *The condition of double enzyme digestion*

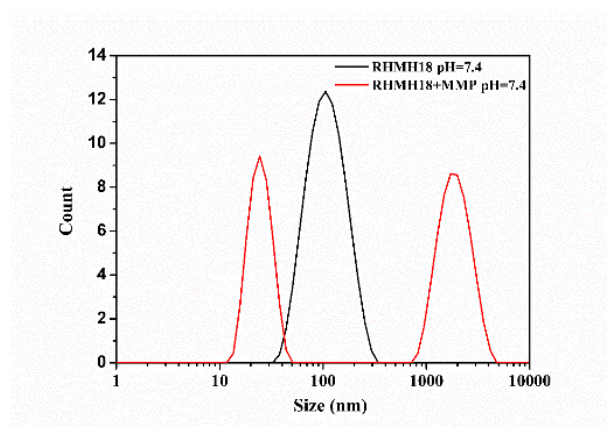
Component	Volume
DNA	3 $\mu$ L
FastDigest Buffer	2 $\mu$ L
Xho I	1 $\mu$ L
Not I	1 $\mu$ L
Ultra-pure water	13 $\mu$ L

**Table S3.** *The condition of plasmid linkage*

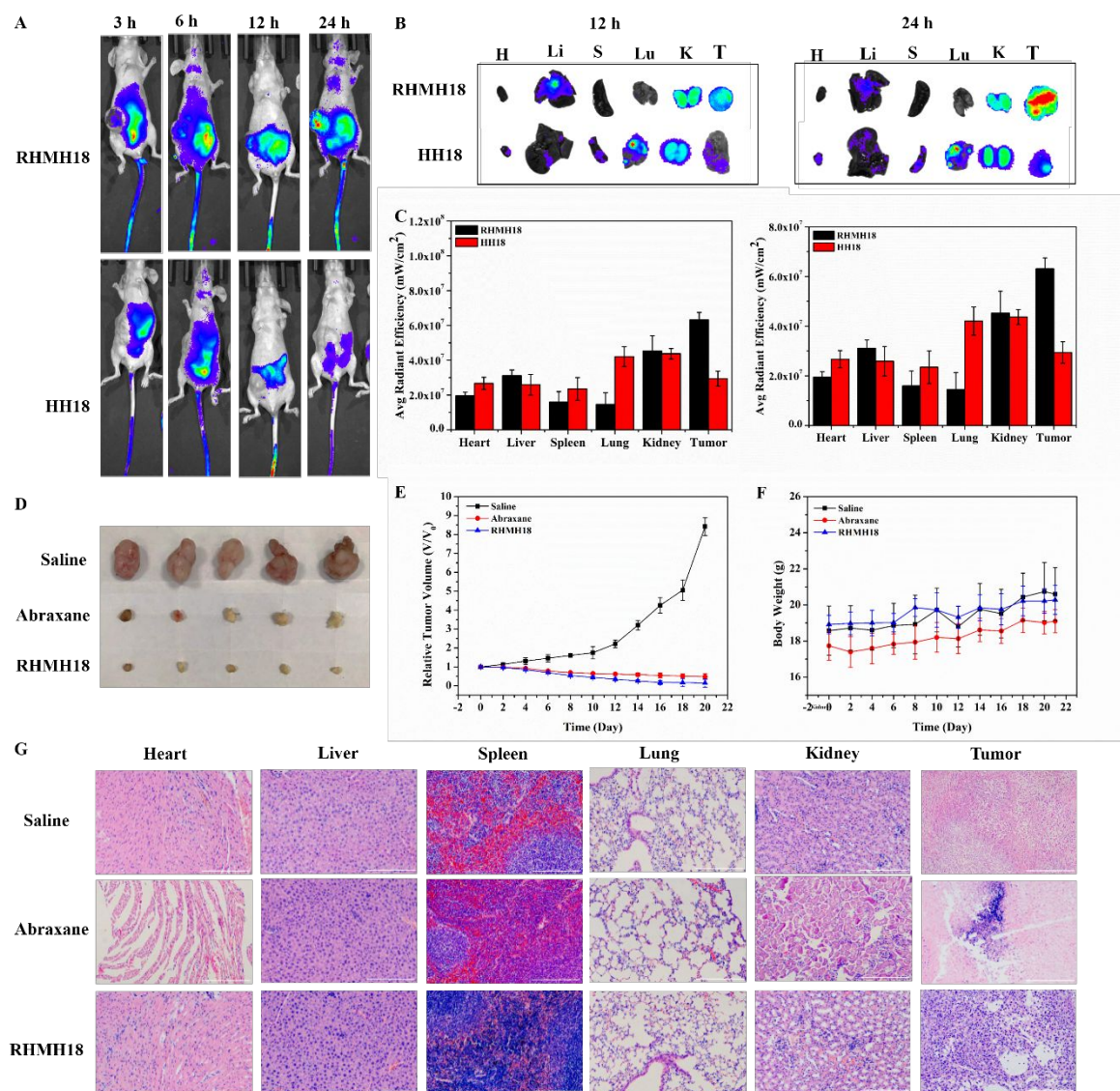
Component	Volume
T4 ligase	1 $\mu$ L
FastDigest Buffer	1 $\mu$ L
pPICZ $\alpha$ A (after digestion)	1 $\mu$ L
DNA (after digestion)	1 $\mu$ L
Ultra-pure water	16 $\mu$ L



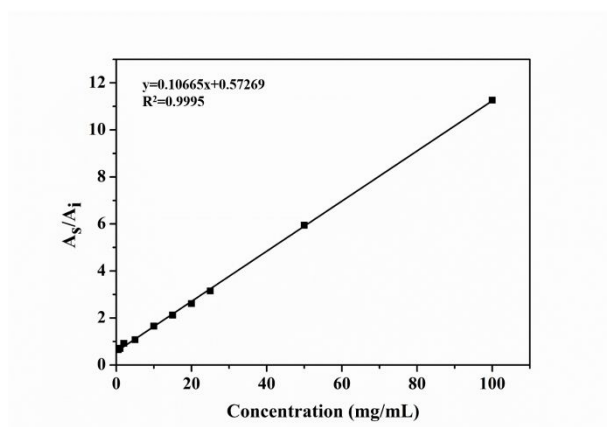
**Figure S1** The concentration-absorption curve of PTX in PBS: methanol=45:55.



**Figure S2** Size difference of RHHM18 NPs before and after adding MMP-2 for 24 h



**Figure S3** (A) NIR fluorescence images of A549 tumor bearing mice after injection of fusion HSA nanomedicine at different time intervals. (B) *Ex vivo* NIR fluorescence images of major organs and tumors after different treatments at 12 h and 24 h. (C) Quantitative analysis of NIR fluorescence intensities of major organs and tumors after different treatments at 12 h and 24 h. (D) Optical images of tumor status stripped from mouse. (E) Relative volumes of tumors. (F) Body weight changes. (G) H&E images of the excised tumor and normal organs.



**Figure S4** Standard curve of paclitaxel concentration and peak area in plasma samples.