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Supporting Information

In vivo safety evaluation and tracing of arginylglycylaspartic acid-engineered phage nanofiber in murine model

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Table S1. Primers used in this study

Name	Sequence (5' —> 3')	Product Length
Mouse CD86	FW GCCCATTTACAAAGGCTCAA RE TGTTCCTGTCAAAGCTCGTG	156
Mouse TNFa	FW GAACTGGCAGAAGAGGCACT RE GGTCTGGGCCATAGAACTGA	201
Mouse IL-10	FW GCCTTATCGGAAATGATCCA RE TTTTCACAGGGGAGAAATCG	158
Mouse β-Actin	FW GTCCCTCACCCTCCCAAAA RE GCTGCCTCAACACCTCAACC	266

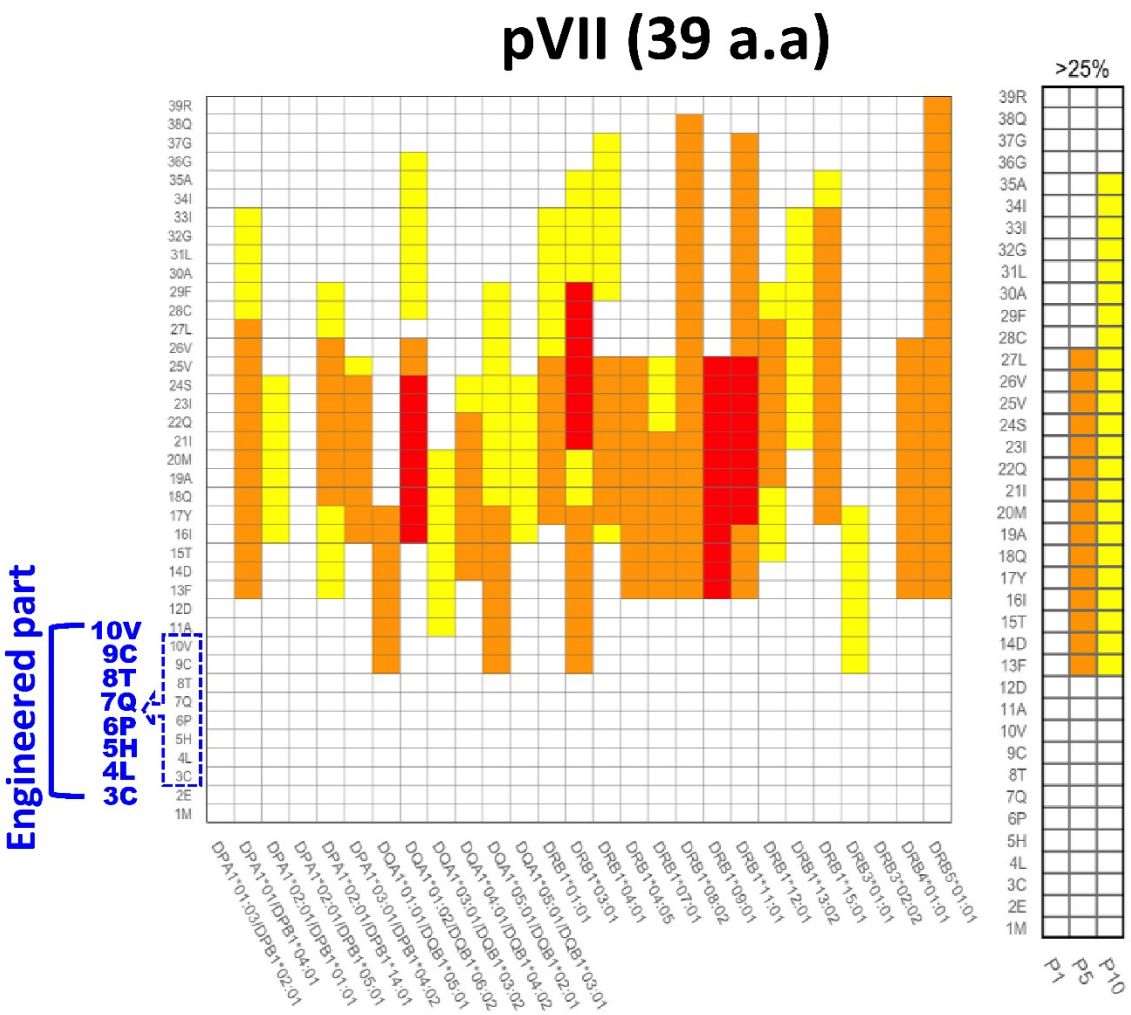


Figure S1. Enlarged image of in silico immunogenicity studies on the pVII coat protein of the engineered YSY184 phage.

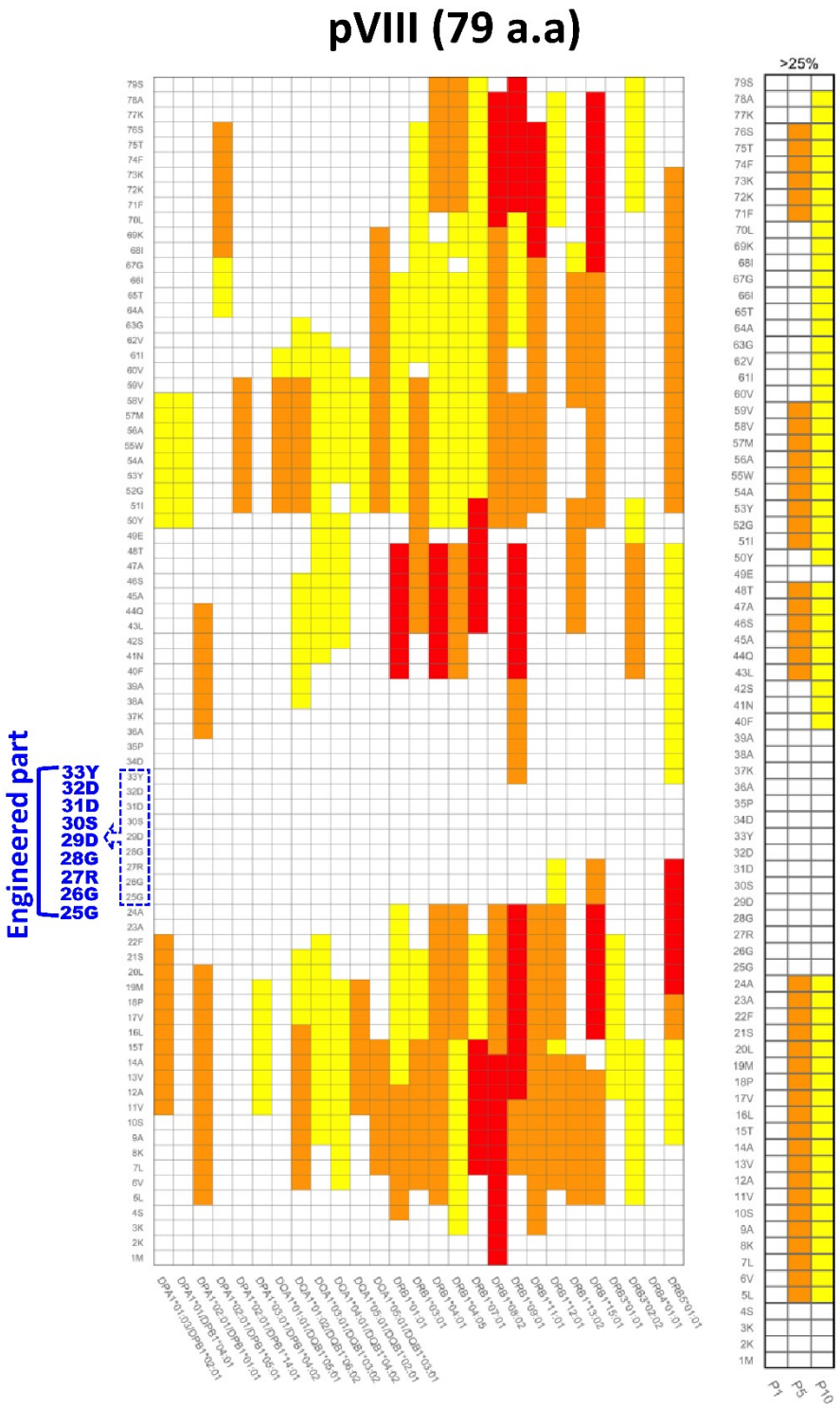
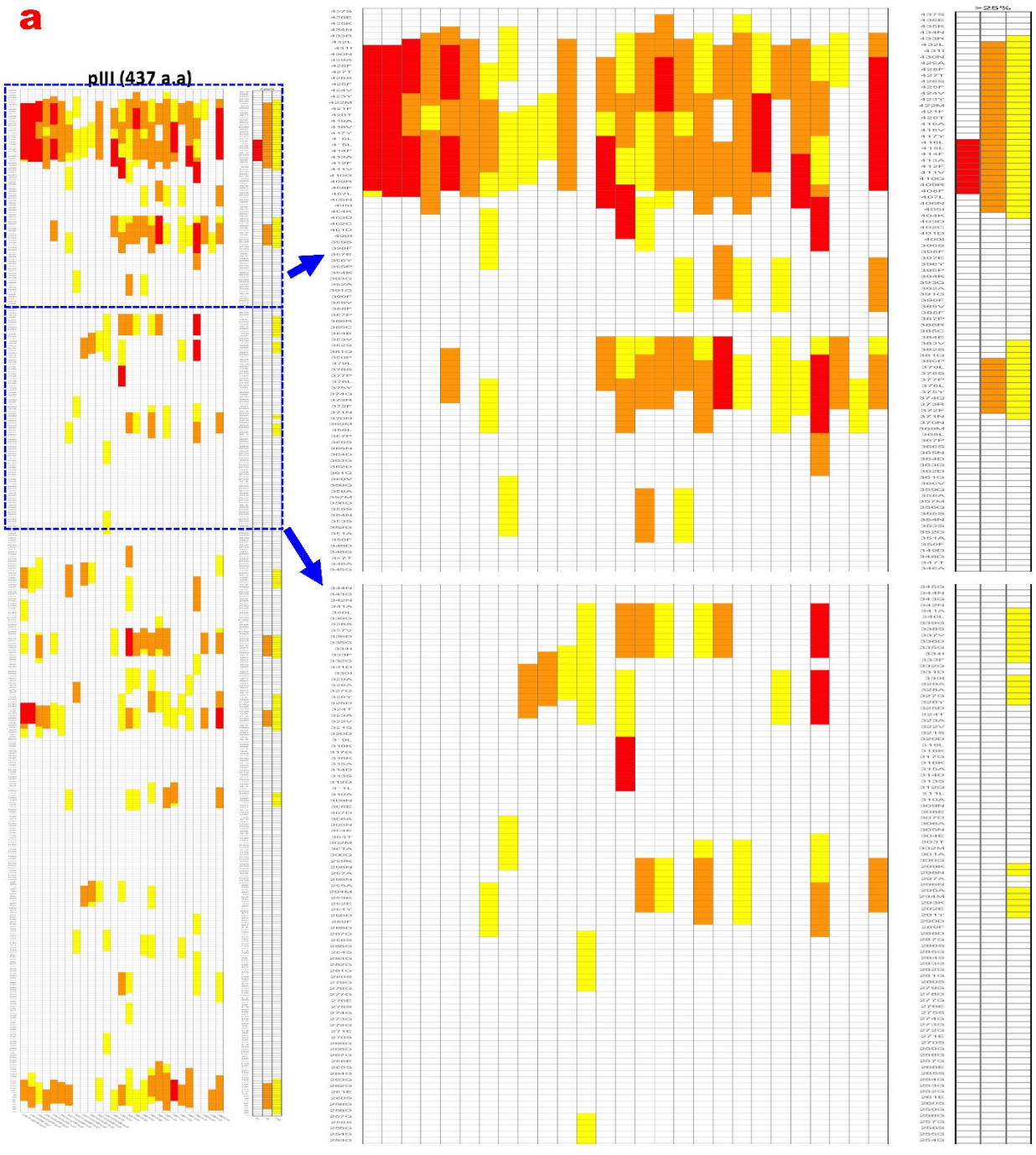
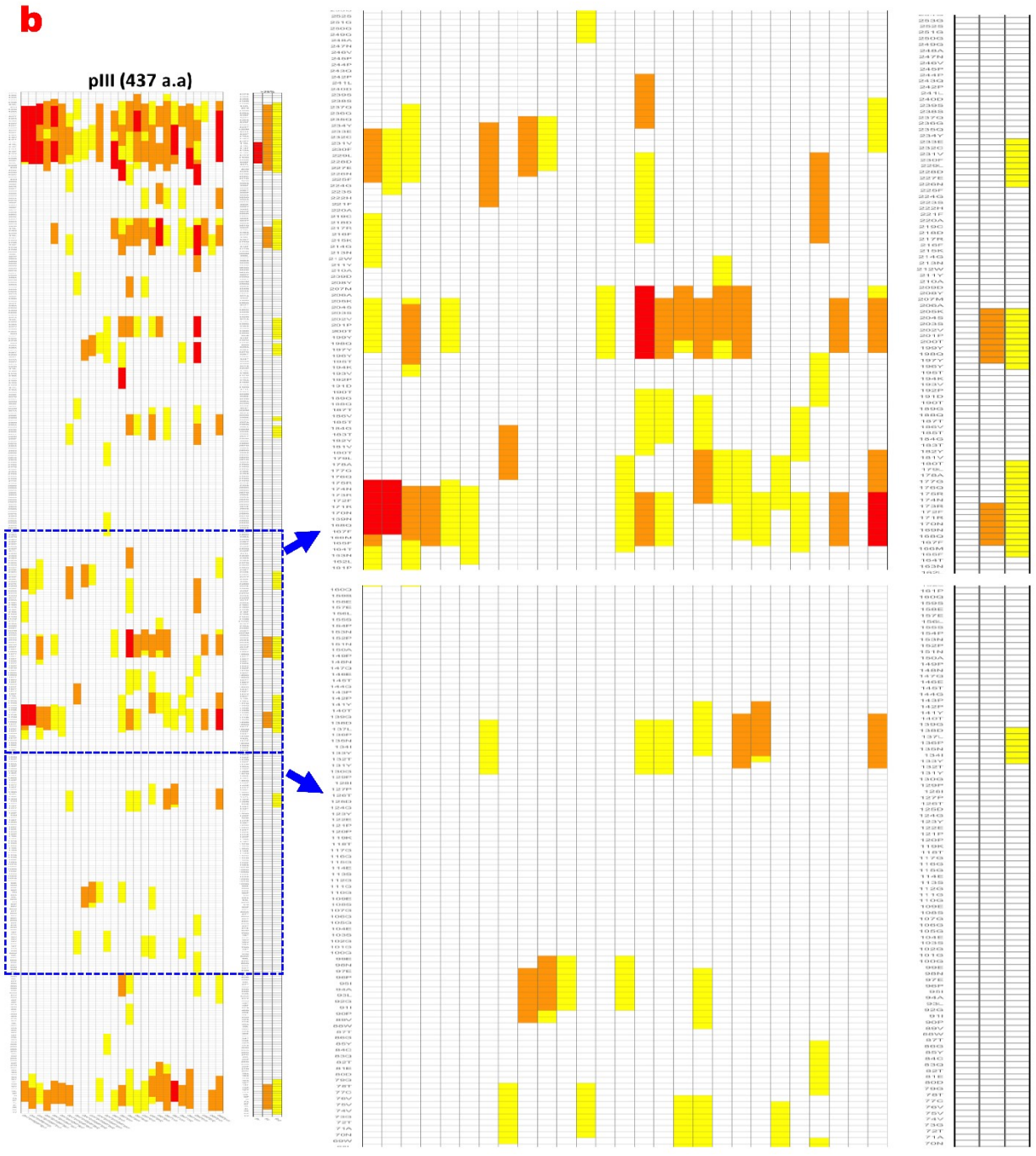


Figure S2. Enlarged image of *in silico* immunogenicity studies on the pVIII coat protein of the engineered YSY184 phage.


a
pIII (437 a.a)
>26%


b
pIII (437 a.a)

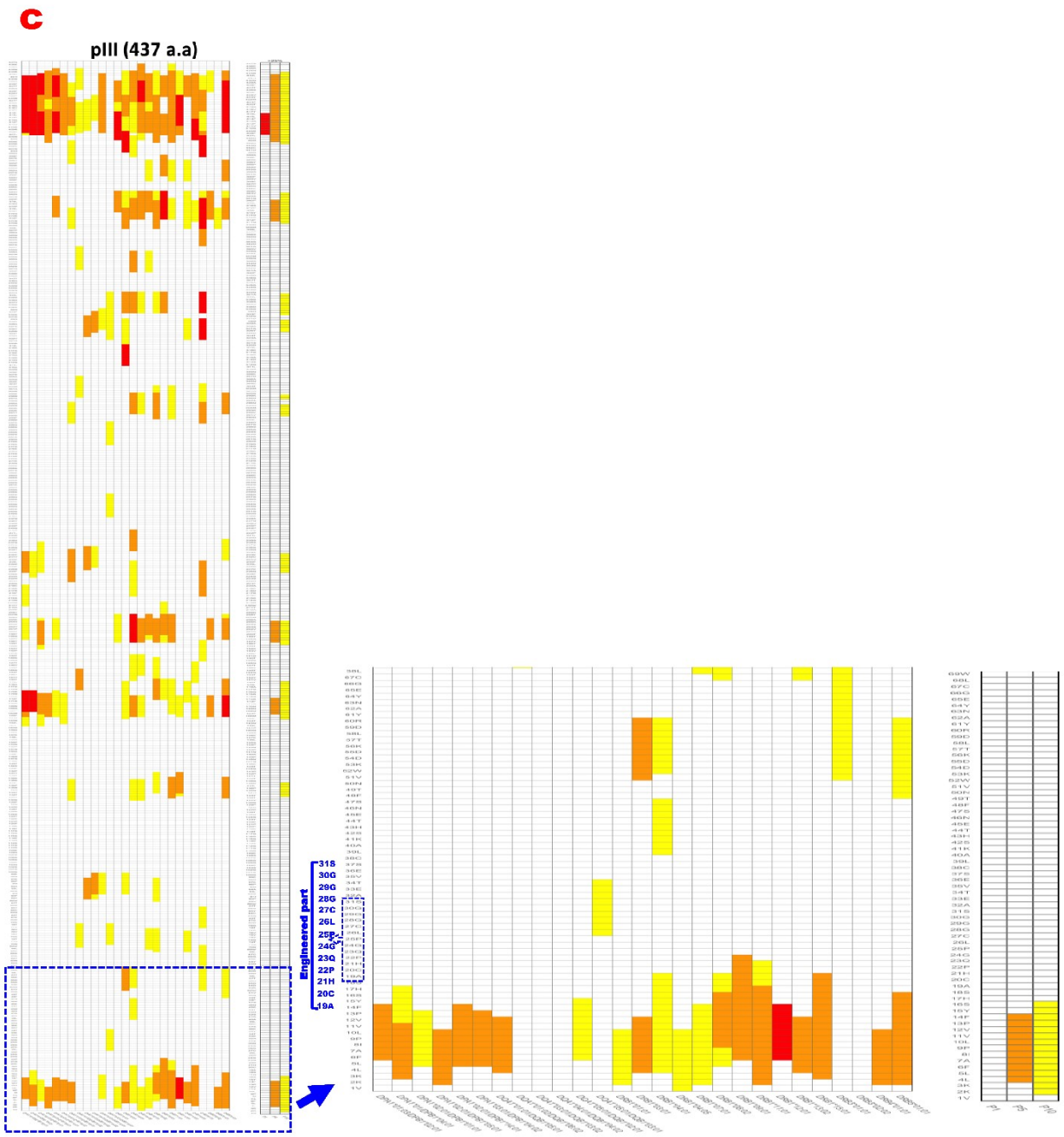


Figure S3. Enlarged image of in silico immunogenicity studies on the pIII coat protein of the engineered YSY184 phage (a) from 437S to 253G, (b) 252S to 69 W, and (c) 68 L to 1V.

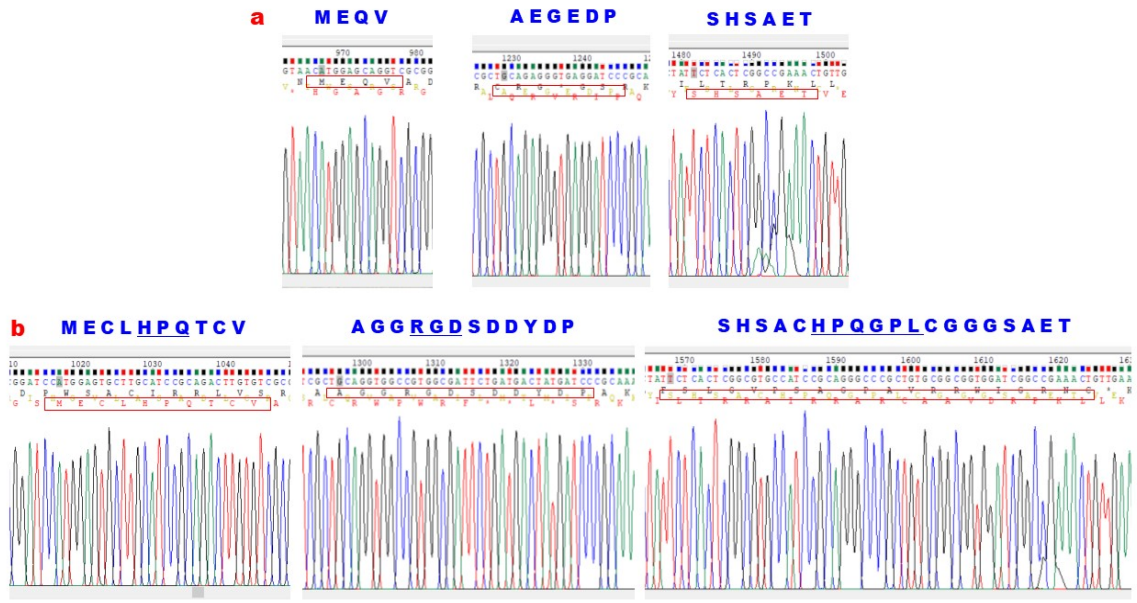


Figure S4. Gene sequencing results showing that the functional pVII, pVIII, and pIII coat protein sequences for both the WT (a) and engineered YSY184 (b) phages perfectly matched the native gene sequences.

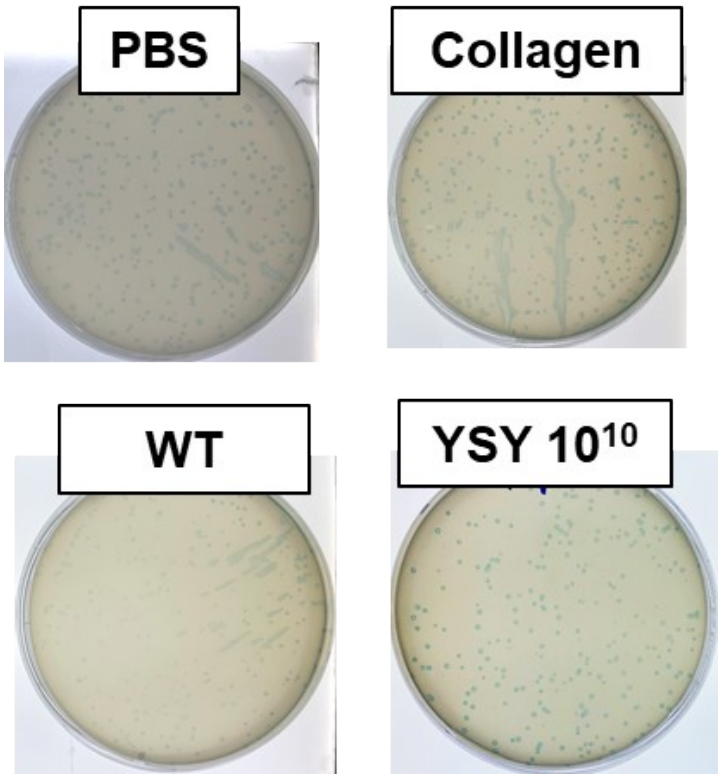


Figure S5. Representative plaque titration images corresponding to Figure 4.

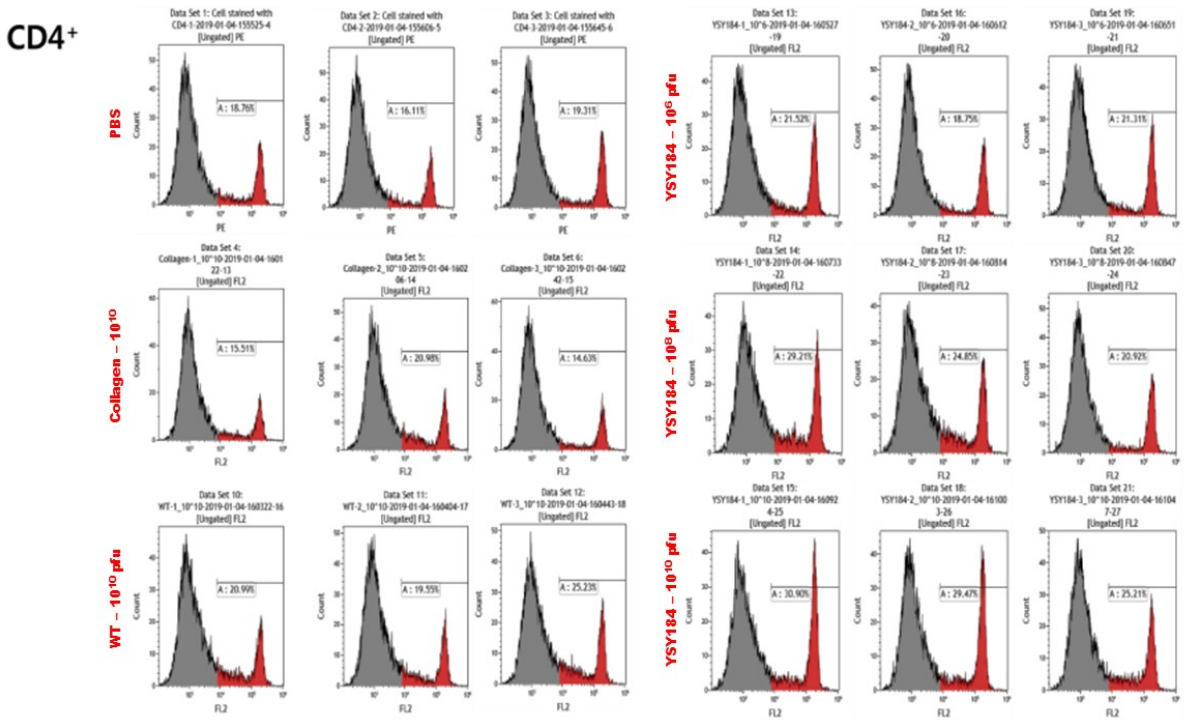


Figure S6. Flow cytometry results of the $CD4^+$ T cell population in the spleen for the phosphate-buffered saline (PBS)-, collagen (10^{10} molecules)-, wild-type (WT) (10^{10} pfu)-, YSY184 (10^6 pfu)-, YSY184 (10^8 pfu)-, and YSY184 (10^{10} pfu)-treated groups.

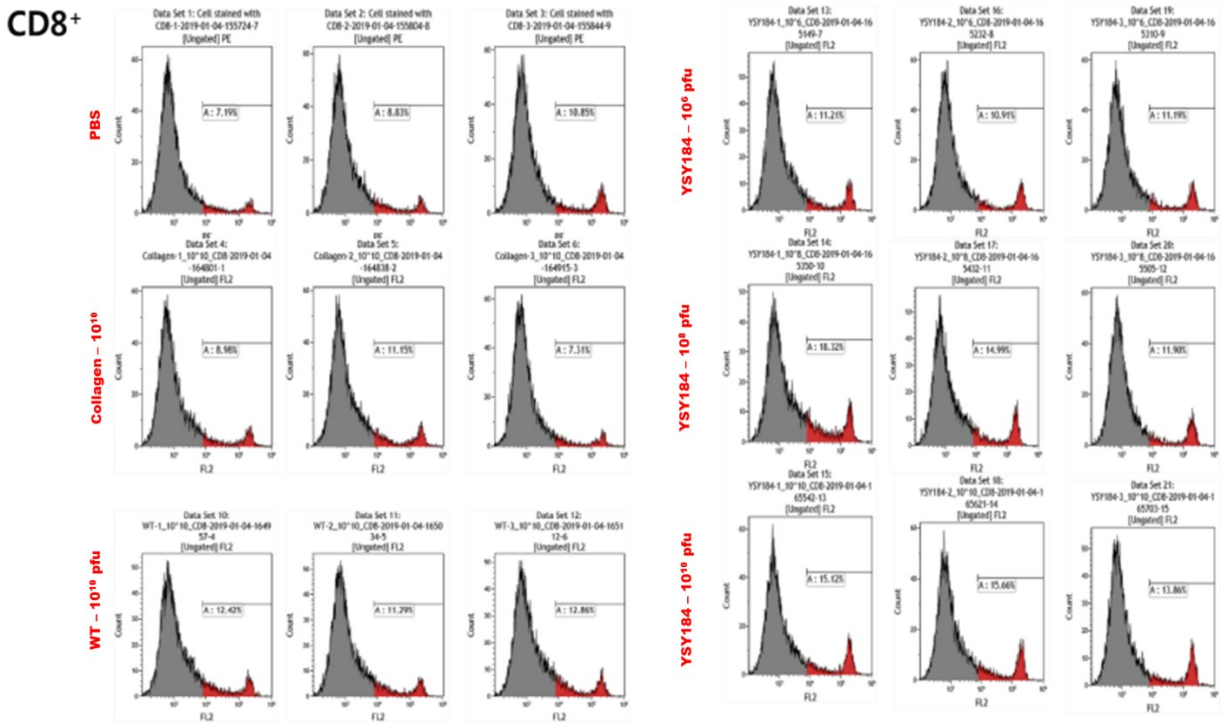


Figure S7. Flow cytometry results of the $CD8^+$ T cell population in the spleen for the phosphate-buffered saline (PBS)-, collagen (10^{10} molecules)-, wild-type (WT) (10^{10} pfu)-, YSY184 (10^6 pfu)-, YSY184 (10^8 pfu)-, and YSY184 (10^{10} pfu)-treated groups.

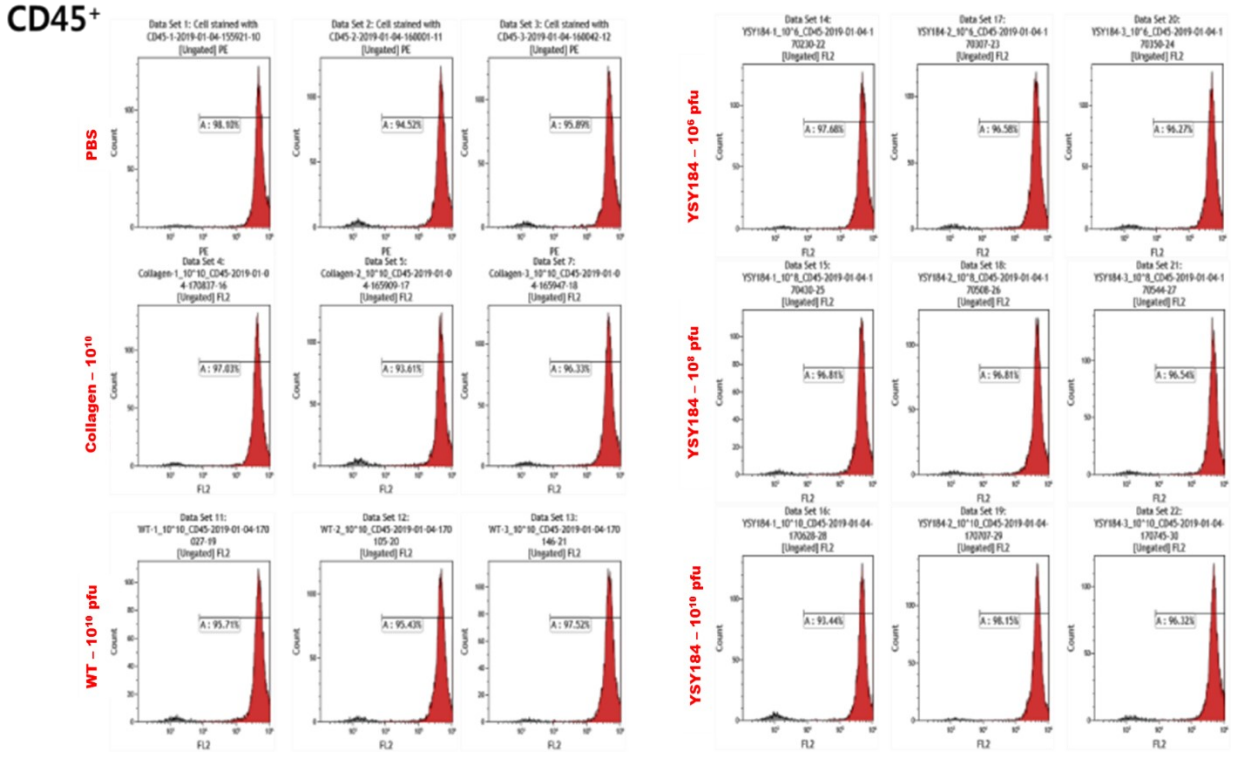


Figure S8. Flow cytometry results of the CD45^{+} T cell population in the spleen for the phosphate-buffered saline (PBS)-, collagen (10^{10} molecules)-, wild-type (WT) (10^{10} pfu)-, YSY184 (10^{6} pfu)-, YSY184 (10^{8} pfu)-, and YSY184 (10^{10} pfu)-treated groups.

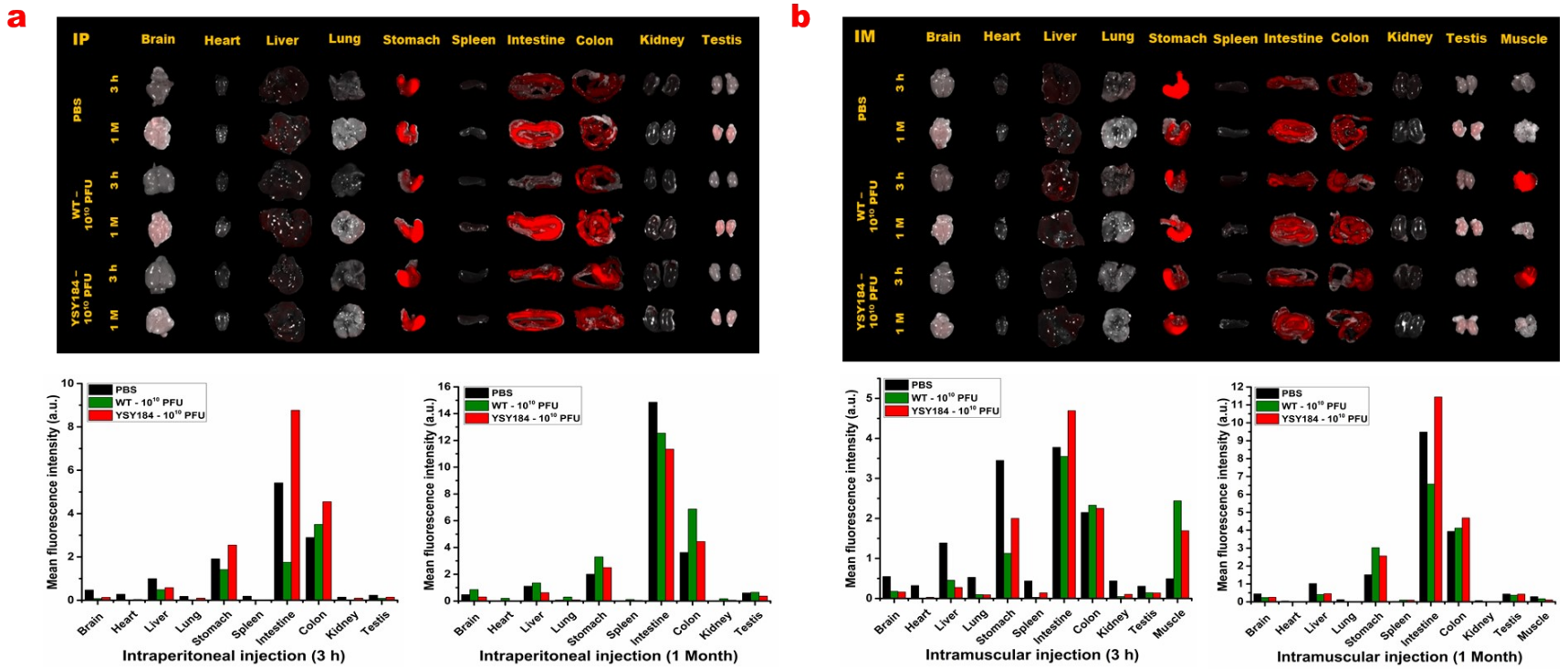


Figure S9. Biodistribution of the phages after intraperitoneal and intramuscular administration. (a) Organs procured 3 h and 1 month after intraperitoneal phage administration; (b) Organs harvested 3 h and 1 month after intramuscular phage administration. All fluorescence images were visualized using the FOBI green channel of a fluorescent *in vivo* imaging system.

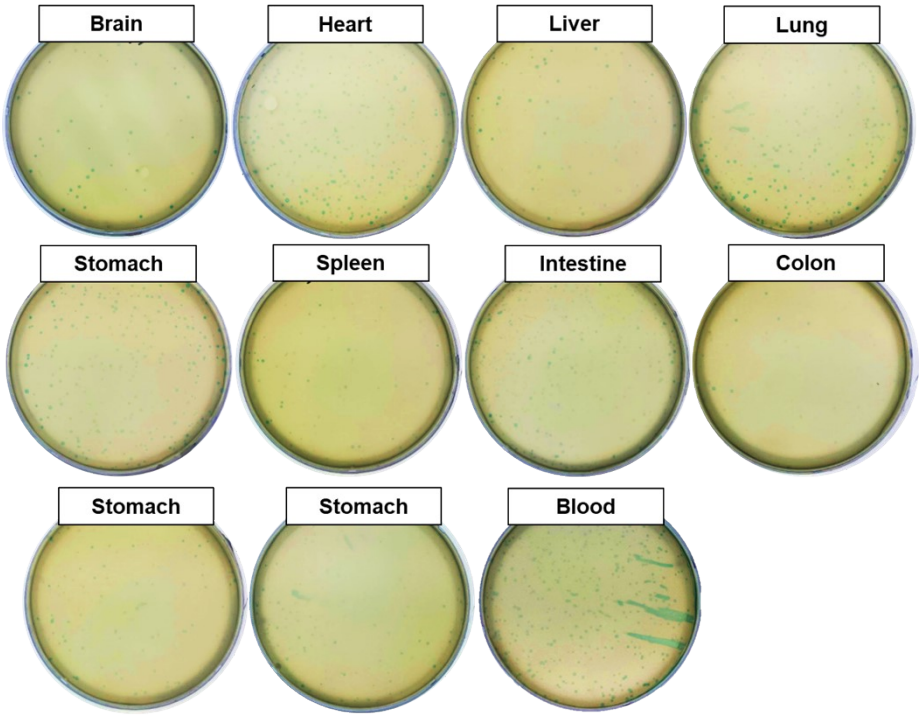


Figure S10. Representative plaque titration images corresponding to Figure 9.

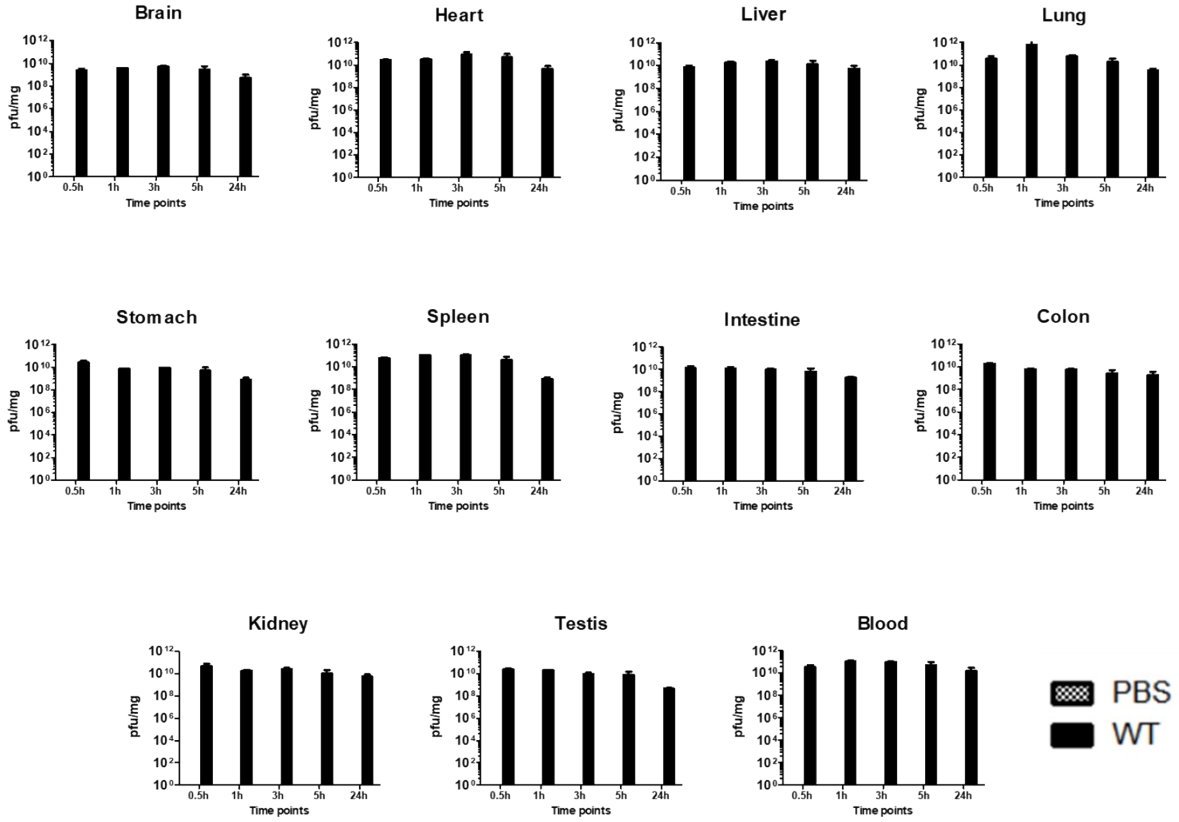


Figure S11. The biodistribution patterns of phages after intraperitoneal administration were analyzed using plaque titration in organs procured at different time intervals for the phosphate-buffered saline (PBS)- and wild-type (WT)-treated groups. Mice (n = 3 per group) were administered the selected phage once via intraperitoneal injection. Blood and tissue samples were collected from the mice in each group at 0.5, 1, 3, 5, and 24 h after phage injection. Phage titers were determined using a fresh layer of an appropriate bacterial host strain on top agar. The results are presented as means ± SEM

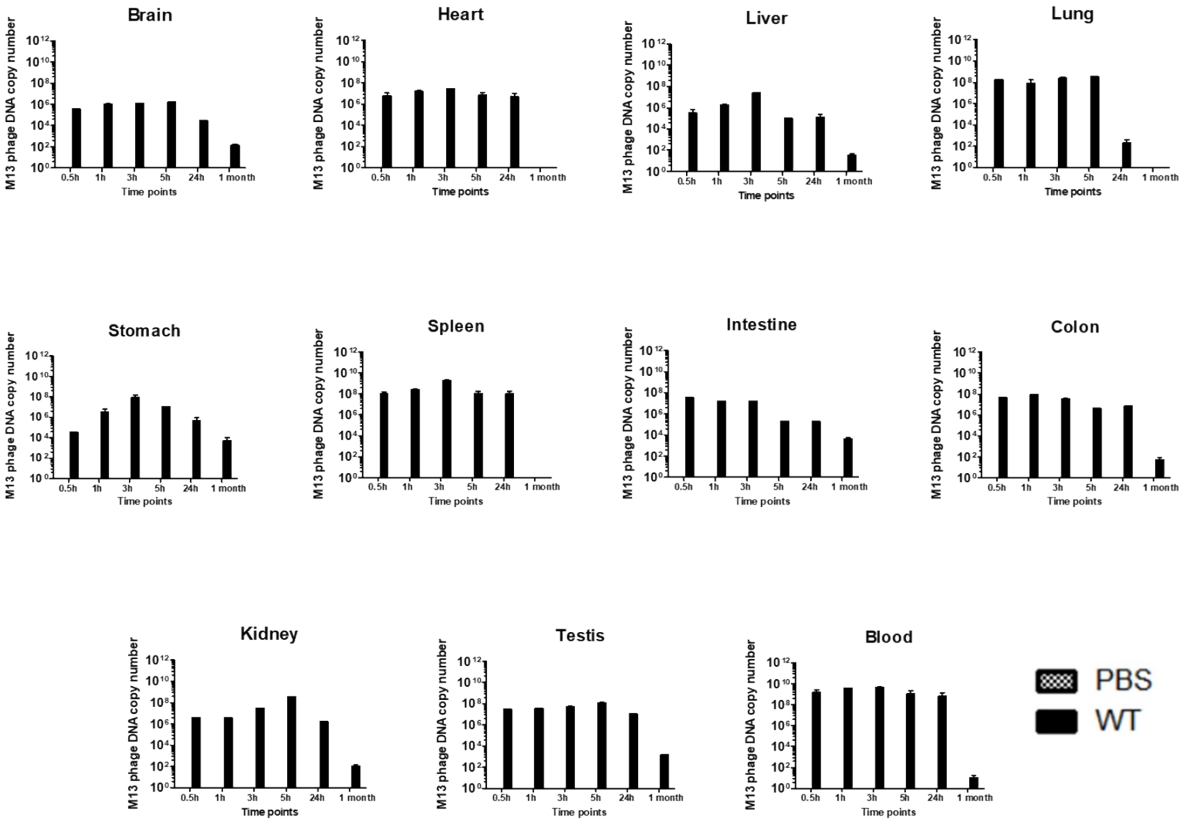


Figure S12. Quantification of phage DNA copy numbers distributed in the procured organs of mice after intraperitoneal administration of phosphate-buffered saline (PBS)- and wild-type (WT)-treated groups at different time intervals. Mice (n = 3 per group) were administered the selected phages via intraperitoneal injection. Blood and tissue samples were collected from the mice in each group at 0.5, 1, 3, 5, and 24 h after phage injection. Viral DNA was extracted from each sample and quantitative PCR was performed. The results are presented as means ± SEM

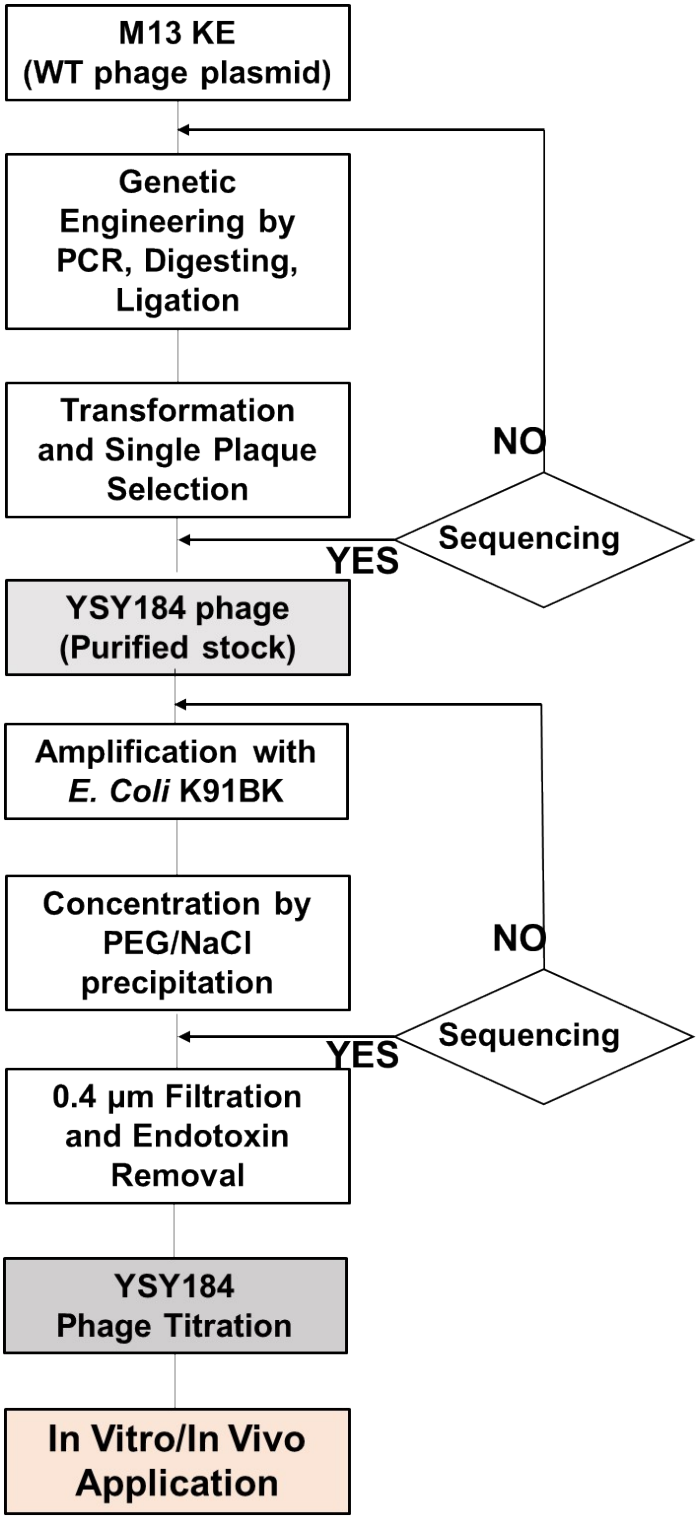


Figure S13. A flow chart showing YSY184 engineering, amplification, and purification.

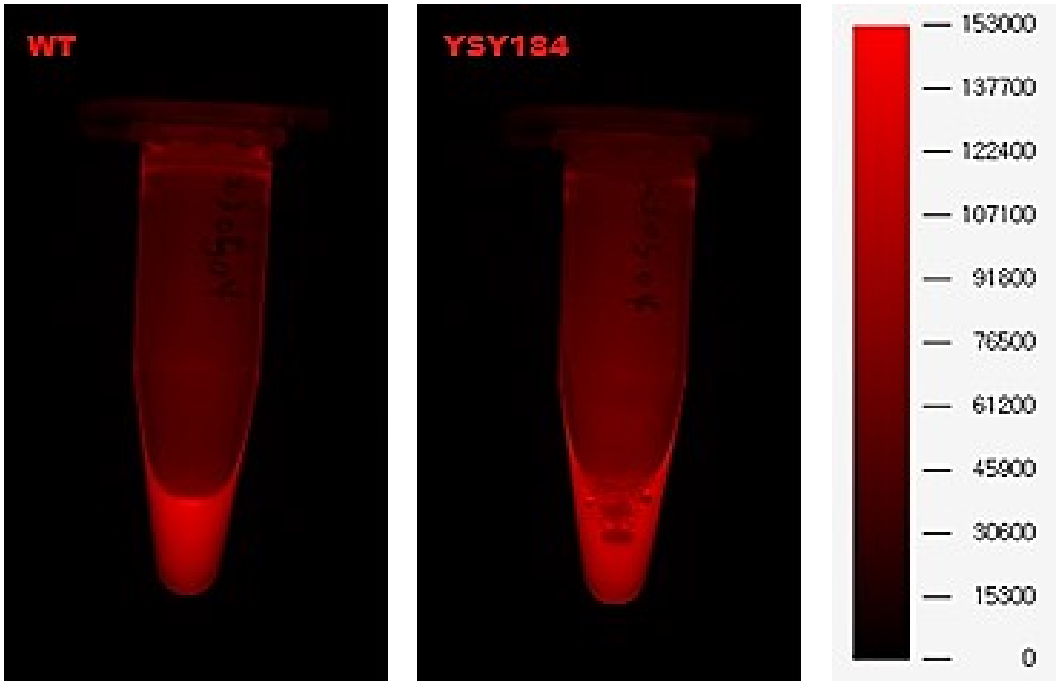


Figure S14. Fluorescence images of Texas Red-labeled wild-type (WT) and YSY184 phages, which were recorded using a fluorescence *in vivo* imaging system (FOBI, Neo Science, Suwon, South Korea).