

Supporting Information for

Original article

## Hyperthermia based individual *in situ* recombinant vaccine enhances lymph nodes drainage for *de novo* antitumor immunity

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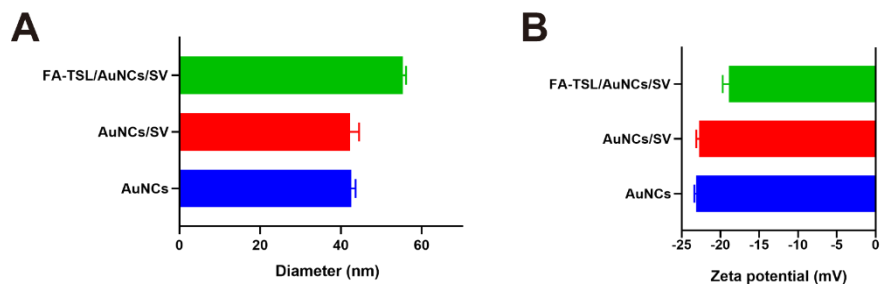
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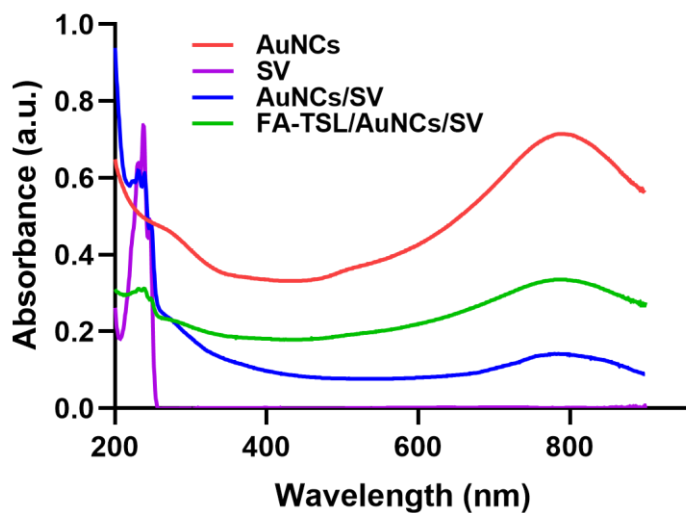
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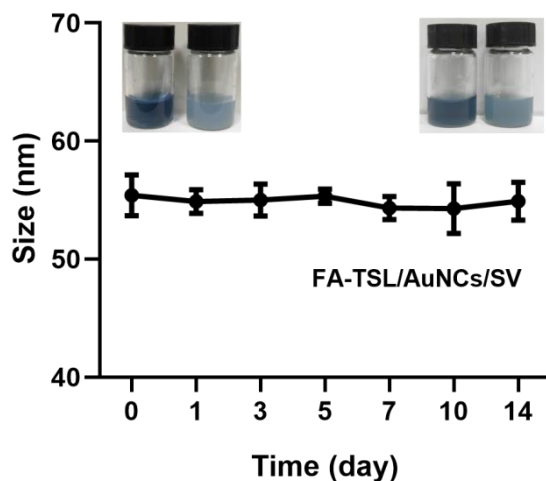
**Running title:** Individual *in situ* recombinant vaccine for enhancing immunotherapy



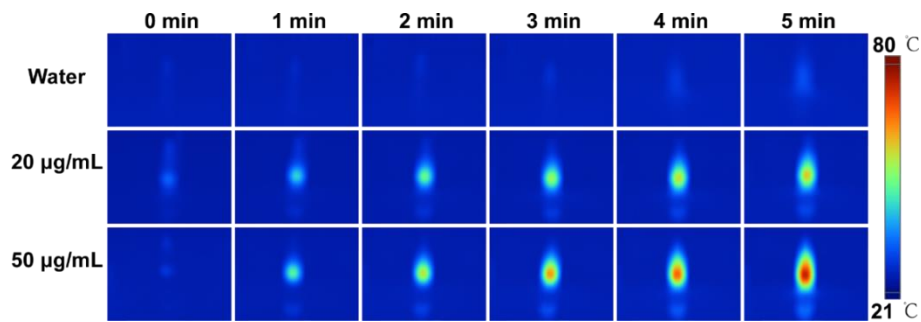
**Figure S1** (A) Particle size and (B) Zeta potential of AuNCs, AuNCs/SV, FA-TSL/AuNCs/SV.



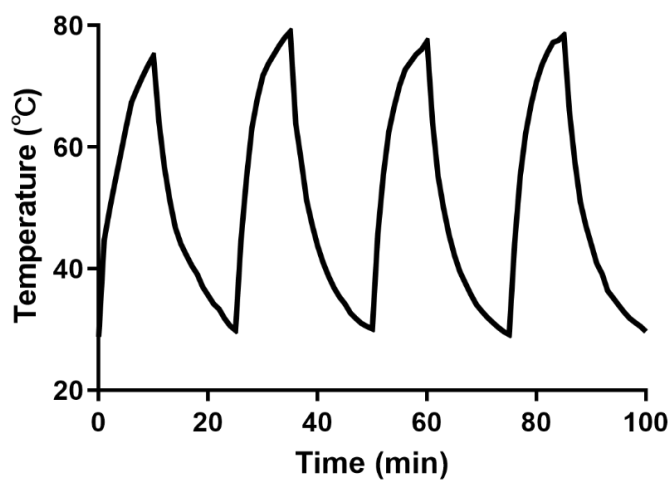
**Figure S2** UV-Vis spectrum.



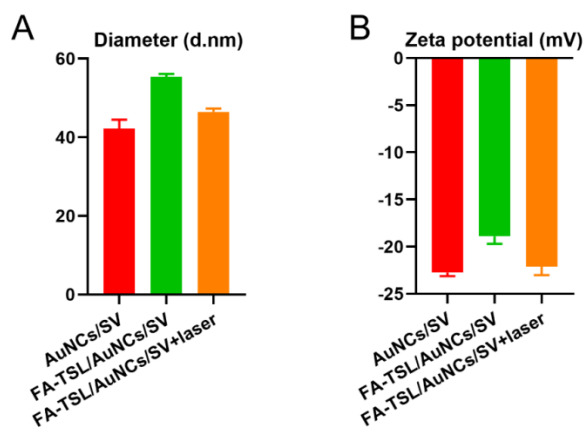
**Figure S3** The stability of FA-TSL/AuNCs/SV.



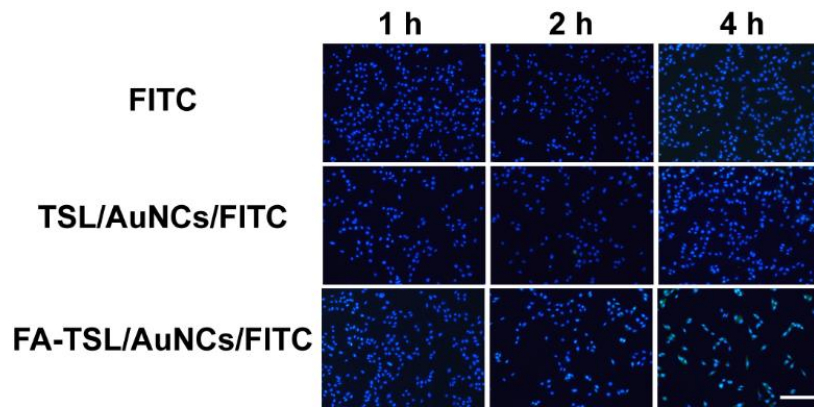
**Figure S4** Thermal images of water, Au (20 and 50  $\mu\text{g/mL}$ ) exposed to 808 nm laser irradiation ( $1.5 \text{ W/cm}^2$ ).



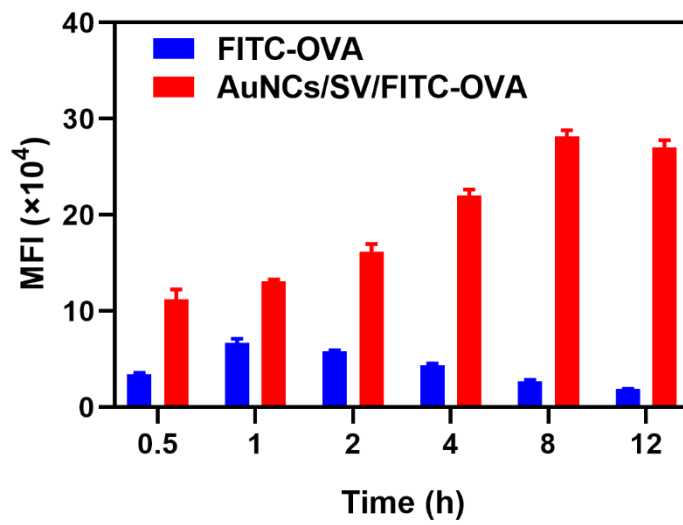
**Figure S5** Photostability of FA-TSL/AuNCs/SV during 808 nm laser irradiation ( $1.5 \text{ W/cm}^2$ ).



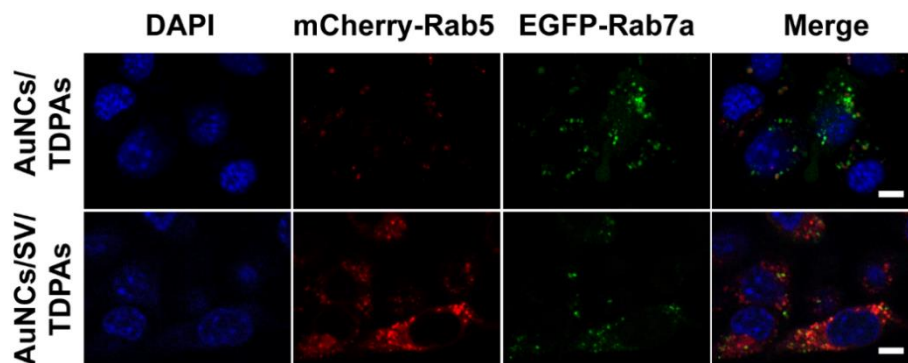
**Figure S6** Size (A) and zeta potential (B) of AuNCs/SV, FA-TSL/AuNCs/SV and FA-TSL/AuNCs/SV+laser ( $n = 3$ ).



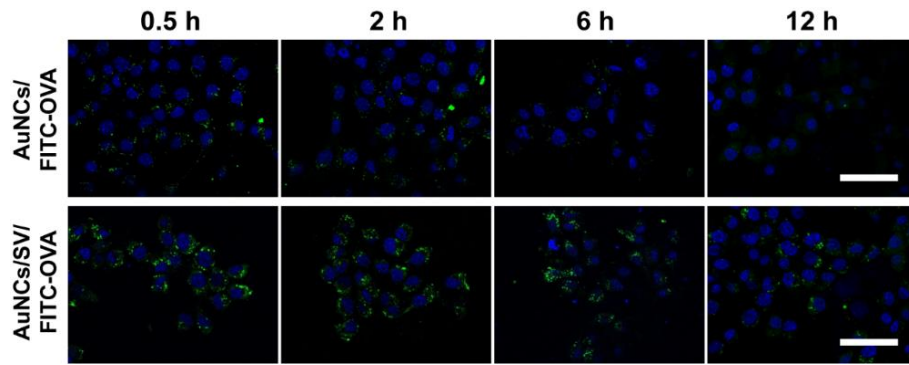
**Figure S7** Cellular uptake (scale bar = 100  $\mu\text{m}$ ).



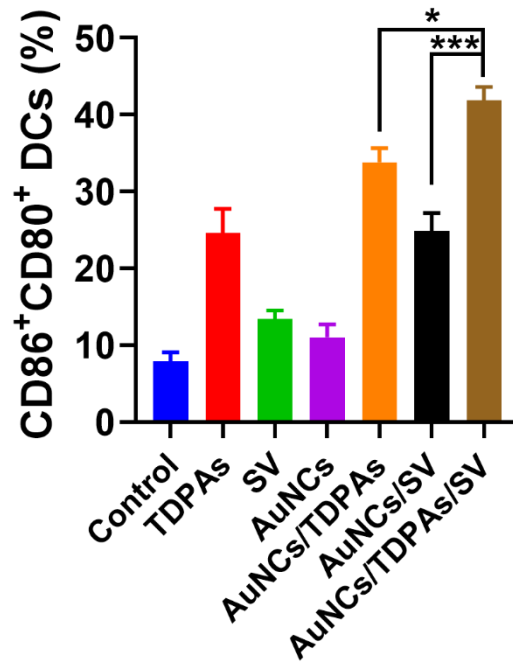
**Figure S8** The flow cytometry analysis of uptake efficiency on BMDCs ( $n = 3$ ).



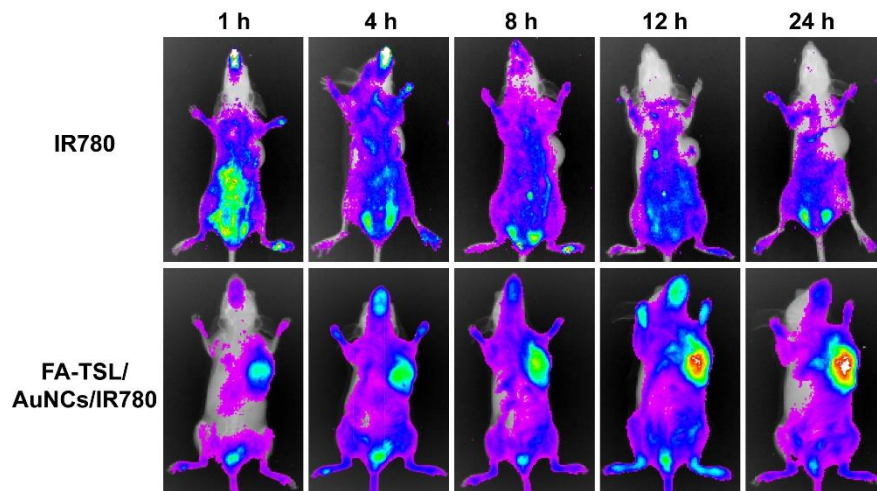
**Figure S9** CLSM image of tumor cells expressing mCherry-Rab5 and EGFP-Rab7a in BMDCs treated with AuNCs/TDPAs and AuNCs/SV/TDPAs. Scale bar = 10  $\mu\text{m}$ .



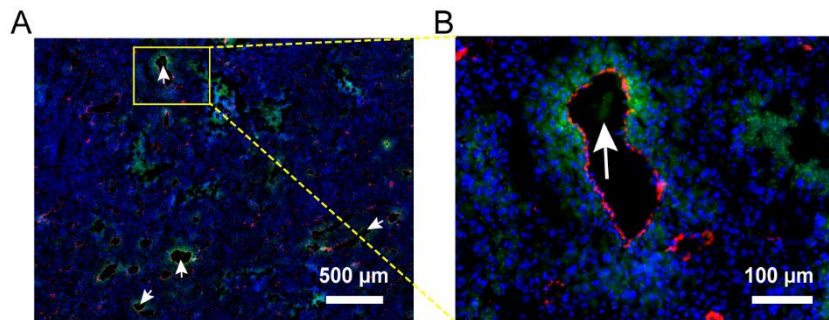
**Figure S10** AuNCs/SV/FITC–OVA shows prolonged antigen retention in BMDC compared with AuNCs/ FITC–OVA. Scale bar = 50  $\mu$ m.



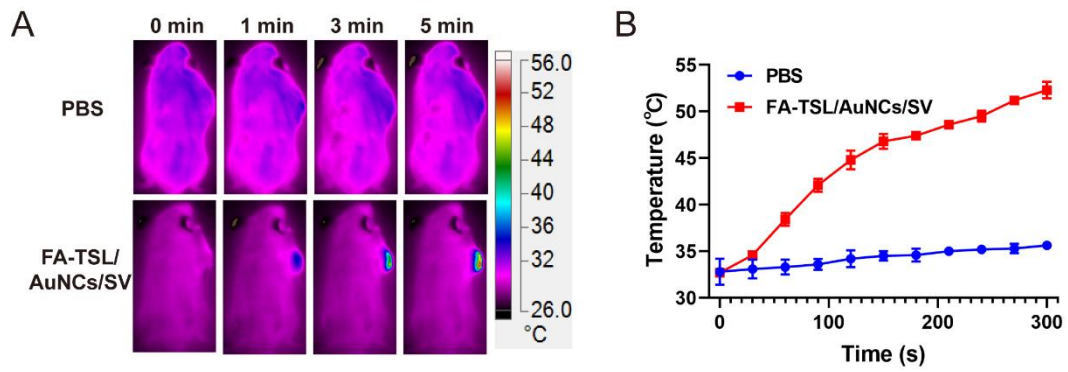
**Figure S11** Quantitative analysis of CD86<sup>+</sup>CD80<sup>+</sup> DCs ( $n = 3$ ).



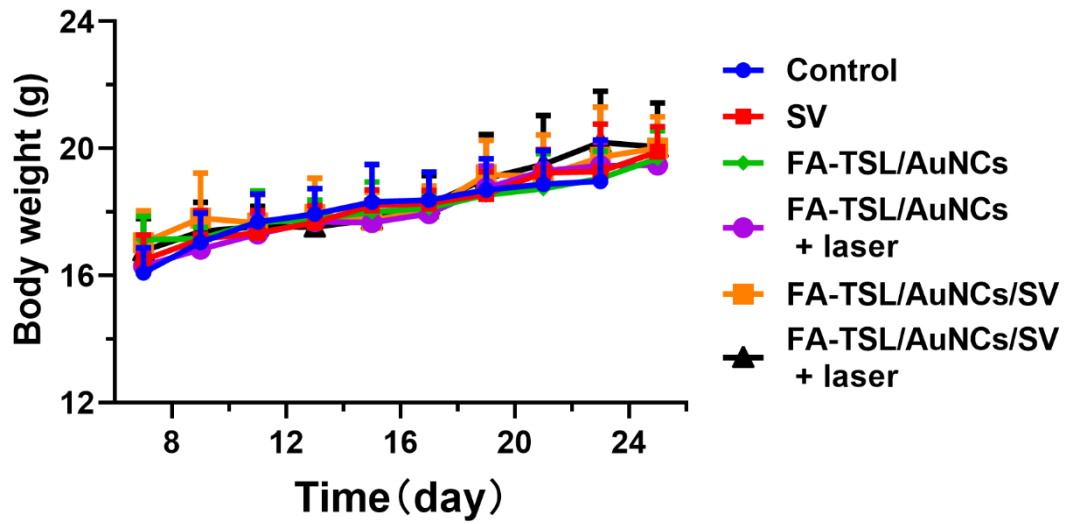
**Figure S12** Biodistribution of IR780 and FA-TSL/AuNCs/IR780.



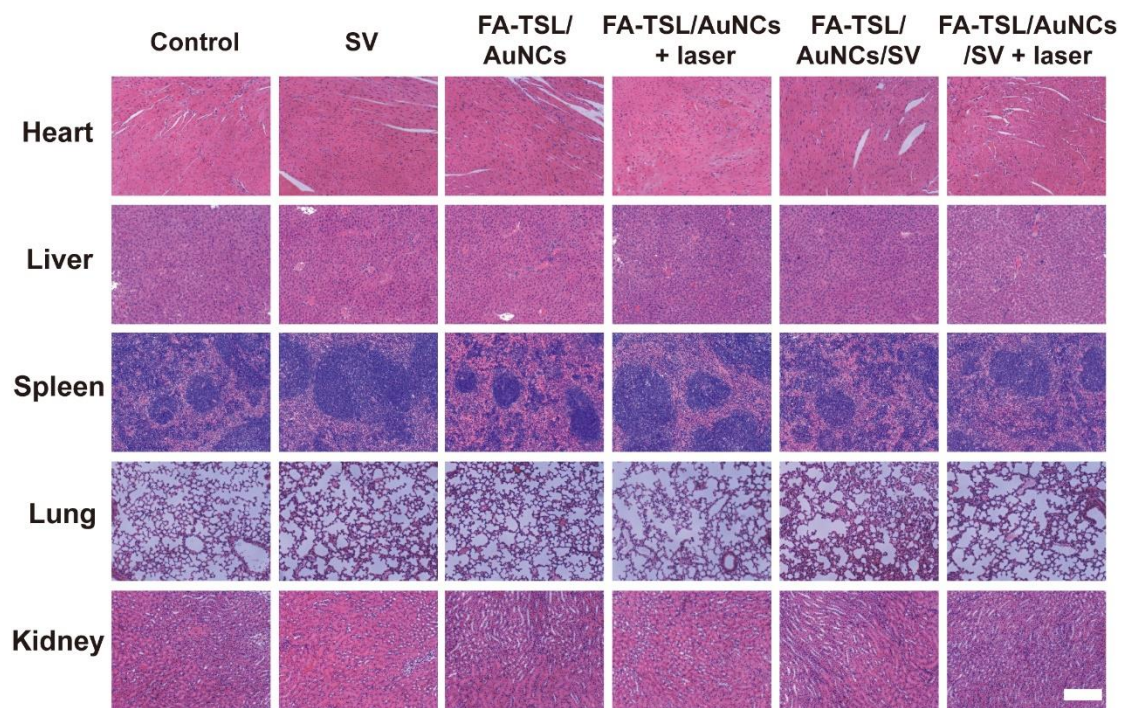
**Figure S13** Representative images of recombinant vaccine drainage to LNs *via* lymphatic vessels (red, podoplanin; green, AuNCs/FITC; blue, DAPI). Scale bar = 100 μm.



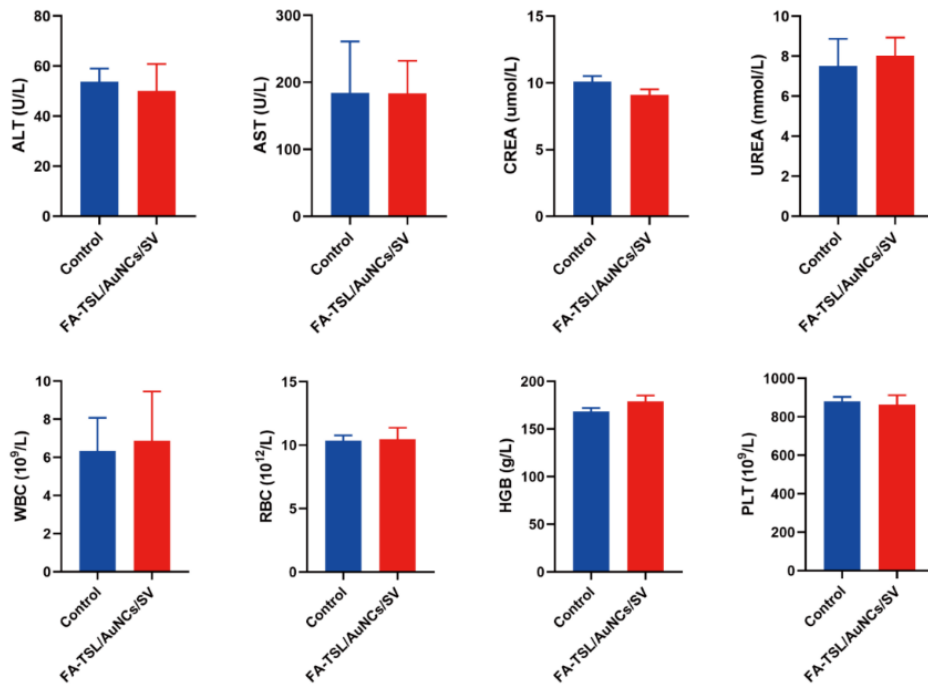
**Figure S14** (A) *In vivo* IR thermal images and (B) corresponding temperature changes.



**Figure S15** Body weight ( $n = 6$ ).

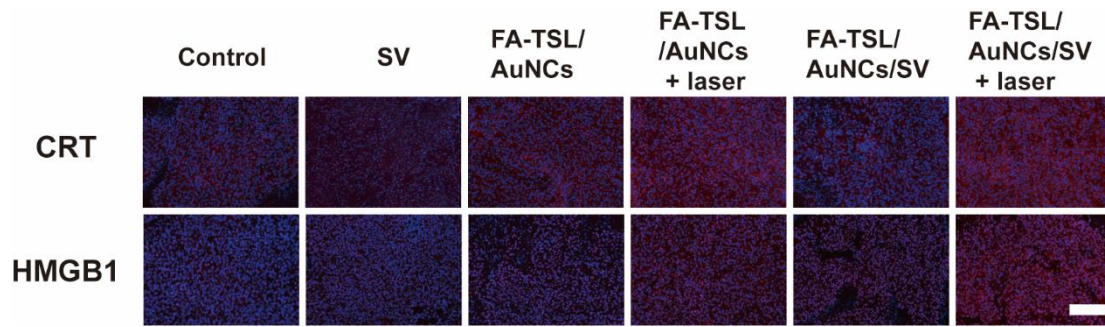


**Figure S16** H&E staining of main organs (scale bar = 200  $\mu\text{m}$ ).

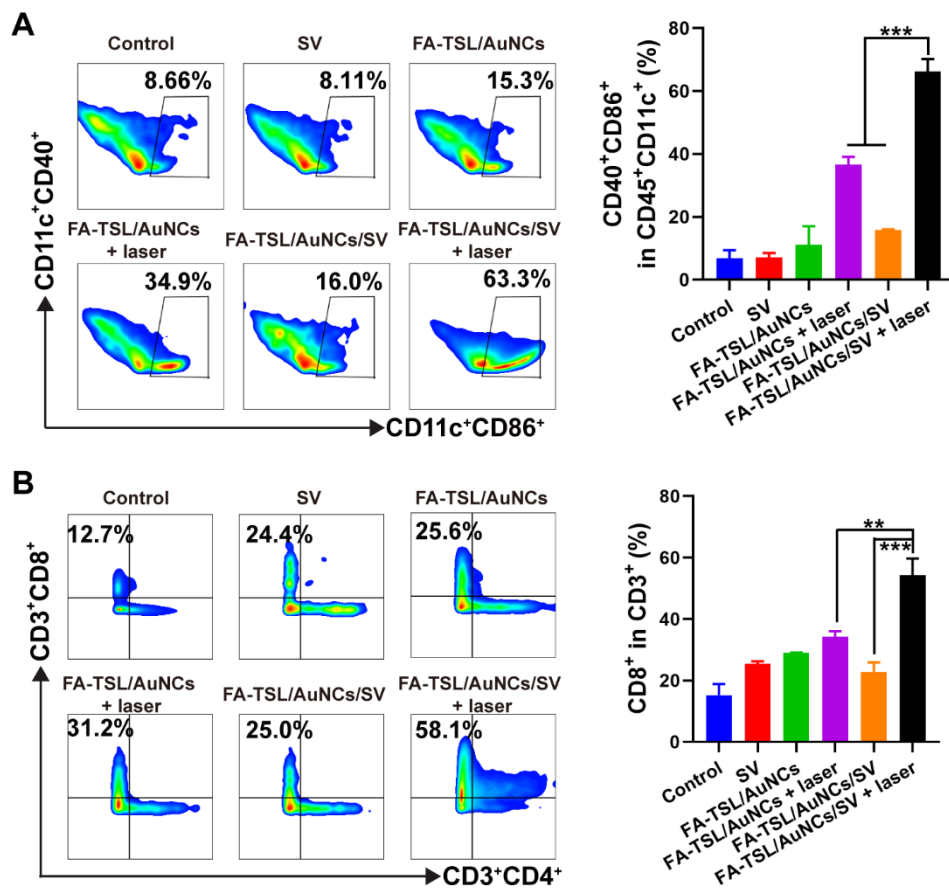


**Figure S17** Serum biochemical analysis and blood routine data ( $n = 3$ ).

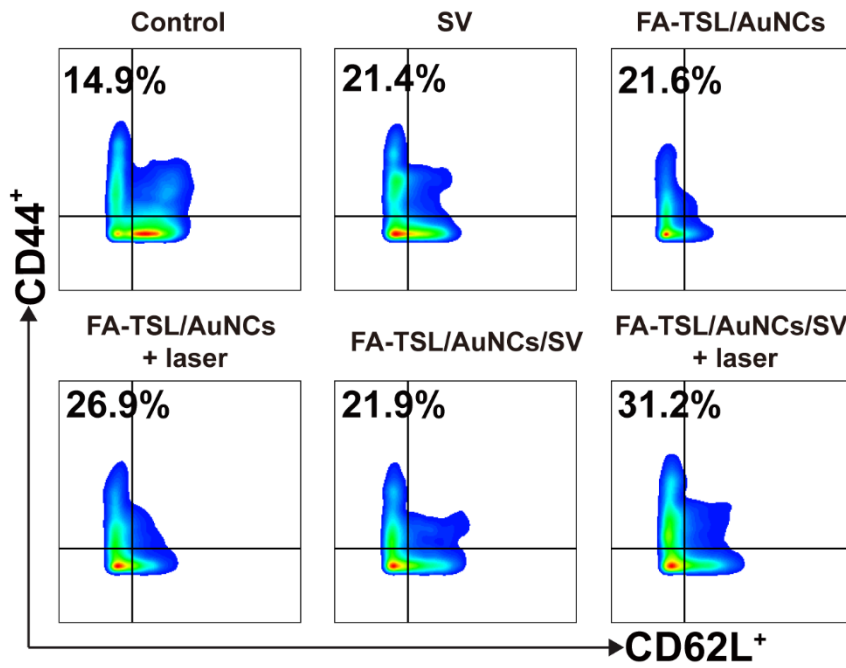




**Figure S18** The expression of CRT and HMGB1 in tumor tissues (scale bar = 200  $\mu$ m).



**Figure S19** (A) DCs maturation and (B) CTLs in tumor tissue ( $n = 3$ ).



**Figure S20** T<sub>EM</sub> (gated on CD3<sup>+</sup> and CD8<sup>+</sup>) in spleens ( $n = 3$ ).