Supporting information

Redirecting Natural Killer Cells to Potentiate Adoptive Immunotherapy in Solid Tumor through Stabilized Y-type Bi-specific Aptamer

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Table S1. The detailed information of aptamer sequences

Name	Sequence from 5' to 3'
FAM-labeled TLS11a aptamer	FAM-CGACGGTAGGATCAATCATGGCCAGCATCCCCATGTGAACAATCGCATTGTGATTGTTACGGTTTCCGCCTCATGGACGTGCTG
FAM-labeled Random - TLS11a aptamer	FAM-CGACGGTAGGATCAATCATGGCCATCGTACTCGTATTAGCGAGCCCGTCGGGCTGGATCCAGCTCCGACATTAGGAATGCGATG
Cy5-labeled CD16 aptamer	Cy5-GGCCATGTGTATGTGGGCCACTGCGGGGGTCTATACGTGAGGAAGAAGTGGGCAGGTCCCCACATACTTTGTTGATCCTACCGTCGTT
Cy5-labeled CD16 random aptamer	Cy5-GGCCATGTGTATGTGGGCCGCAGTGCGTGGCAACAGGAGCGCATGCATTAGACGGCTACCCACATACTTTGTTGATCCTACCGTCGTT

The letters in red represent mutations, and the letters with underline represent the functional sequences of CD16 or TLS11a Ap. The letters unlabeled was the Y-shaped backbone.

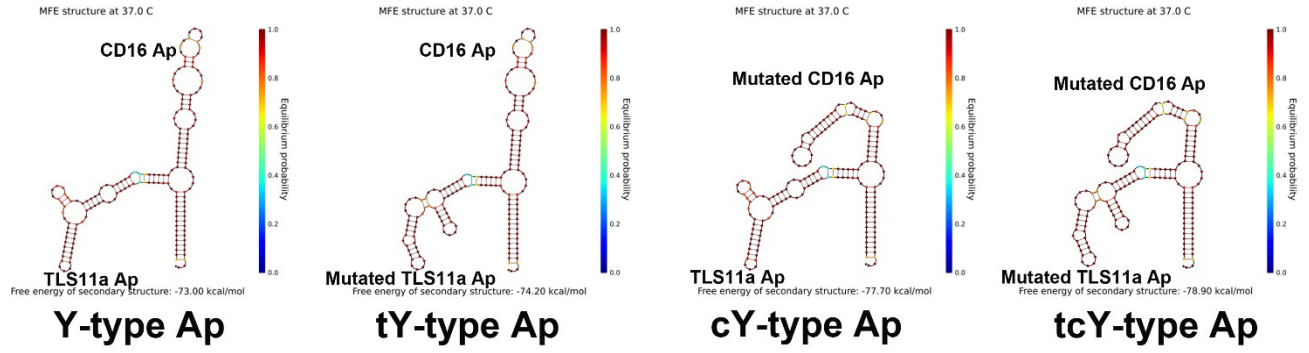

Figure S1. The structures of Y-shape Ap and mutated Y-shape Ap (tY-type Ap, cY-type Ap and tcY-type Ap) were analyzed by NUPACK.

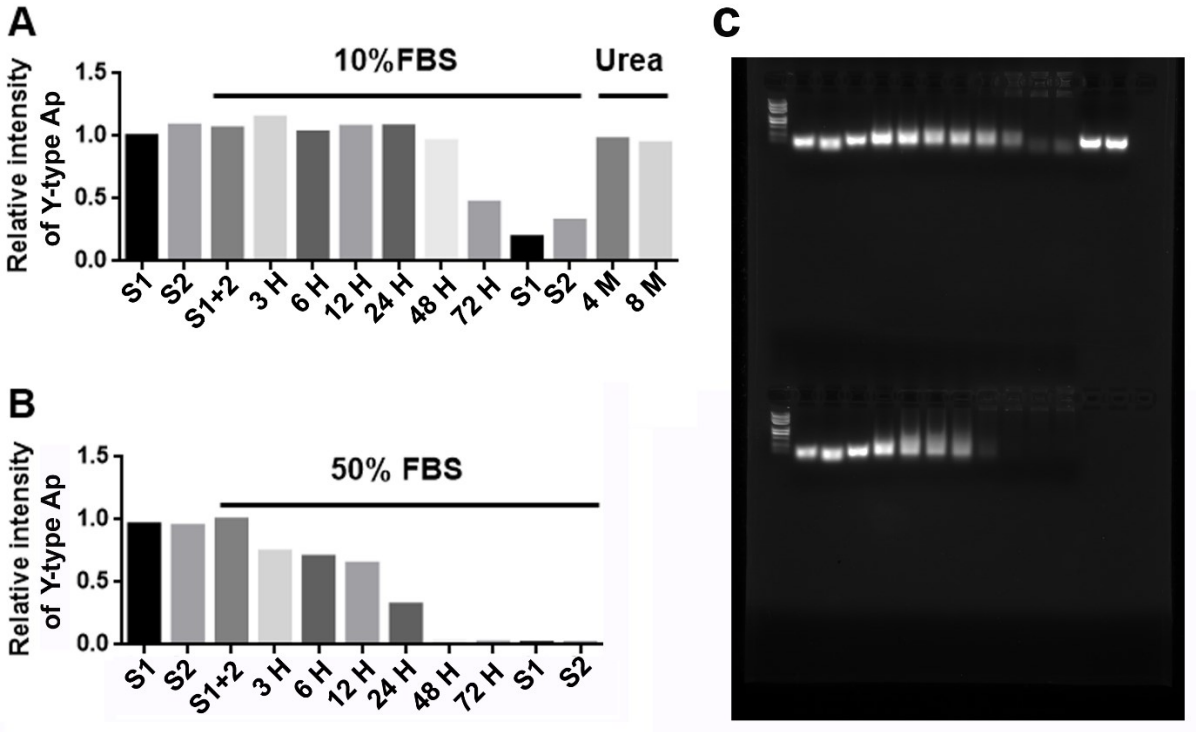

Figure S2. (A). Quantification of bio-stability of single-strand TLS11a Ap (S1), single-strand CD16 Ap (S2) and Y-type Ap in serum (10%, lane 4 to 9) and in urea (4 M and 8 M, lane 12 to 13) with different co-incubation time. (B). Quantification of bio-stability of single-strand TLS11a Ap (S1),

single-strand CD16 Ap (S2) and Y-type Ap in serum (50%, lane 4 to 9) with different co-incubation time. (C). The original image of gel electrophoresis experiment (Figure 1B).

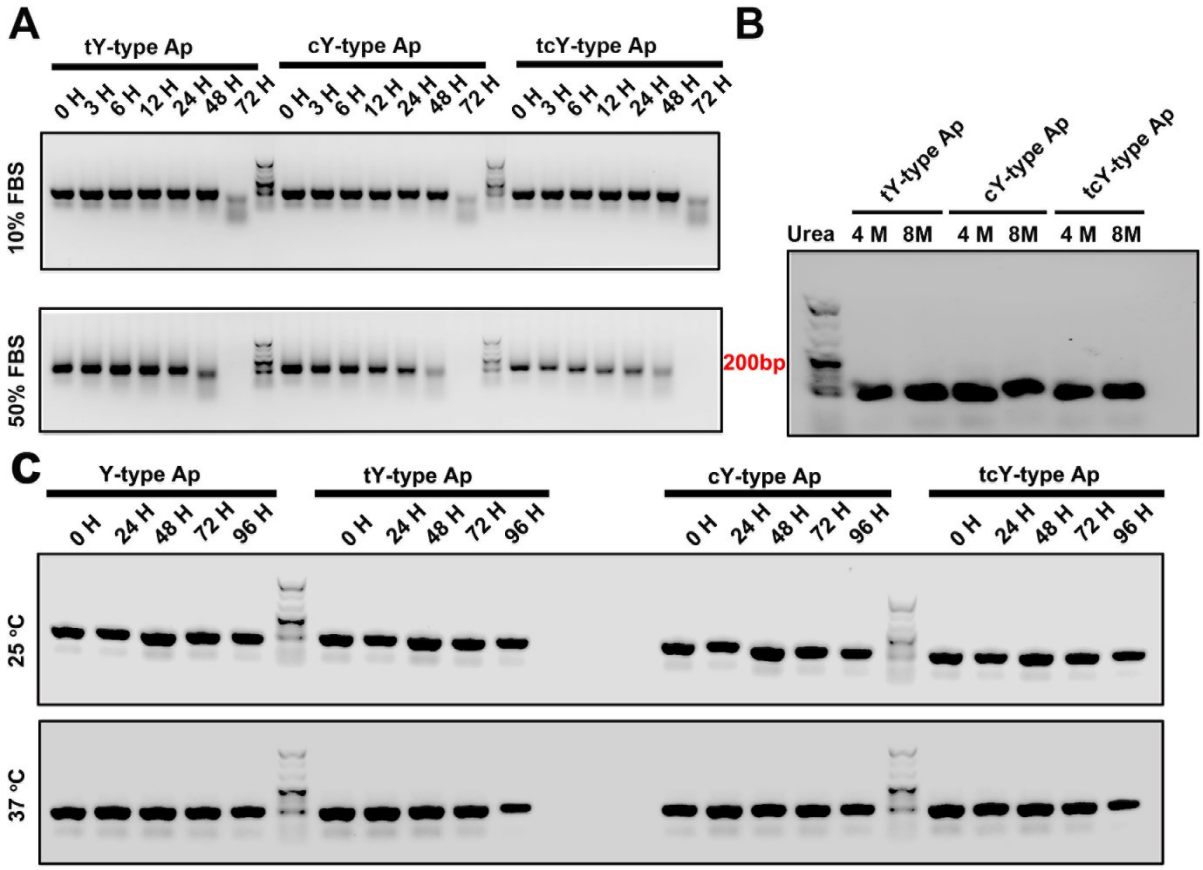

Figure S3. (A). Agarose Gel Electrophoresis of mutated Y-type Ap in 10% and 50% FBS for different co-incubation time. (B). The mutated Y-type Ap was co-incubated in urea (4 M or 8 M) for 2 h. (C)**.** Y-type Ap and mutated Y-type Ap stored in TM buffer at 25℃ and 37℃ for different time, respectively.

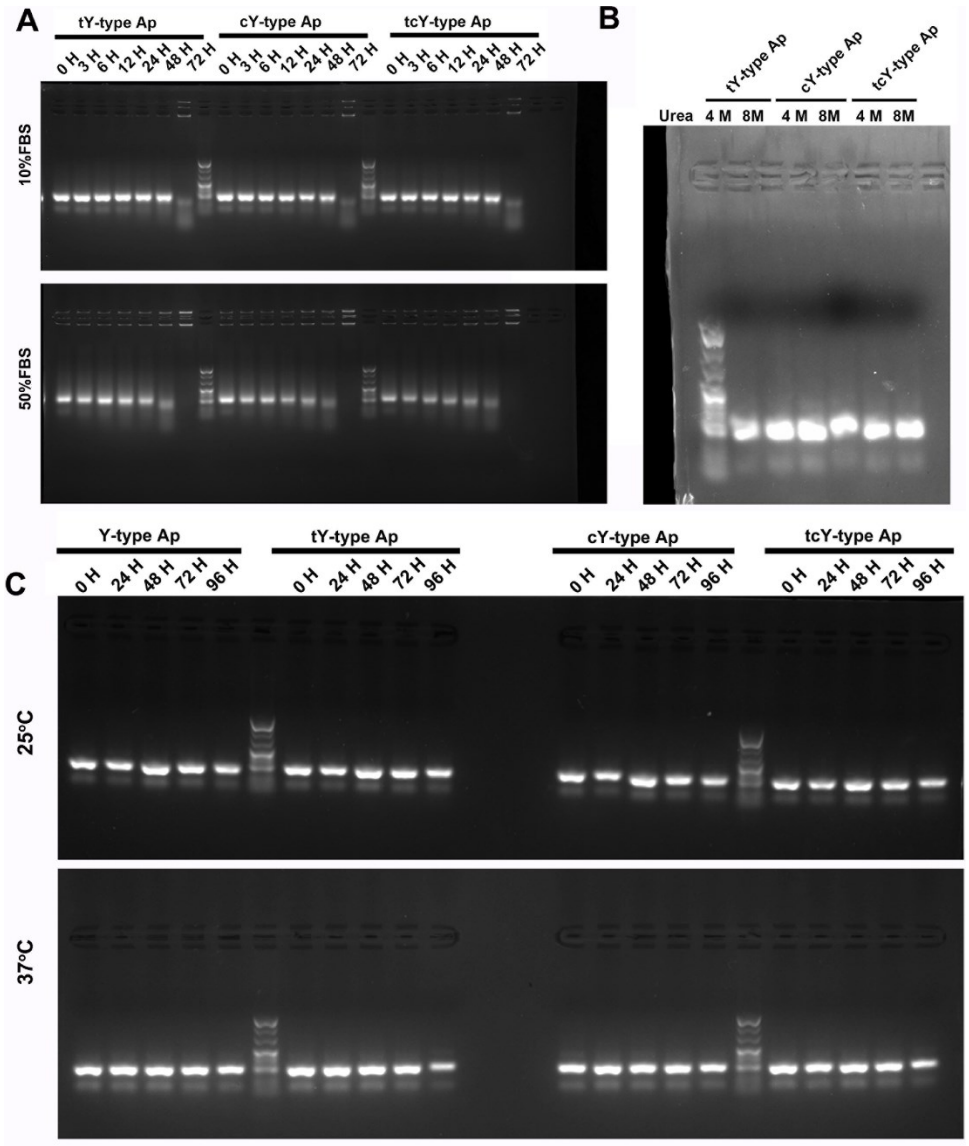

Figure S4. The original images of gel electrophoresis experiment in Figure S3.

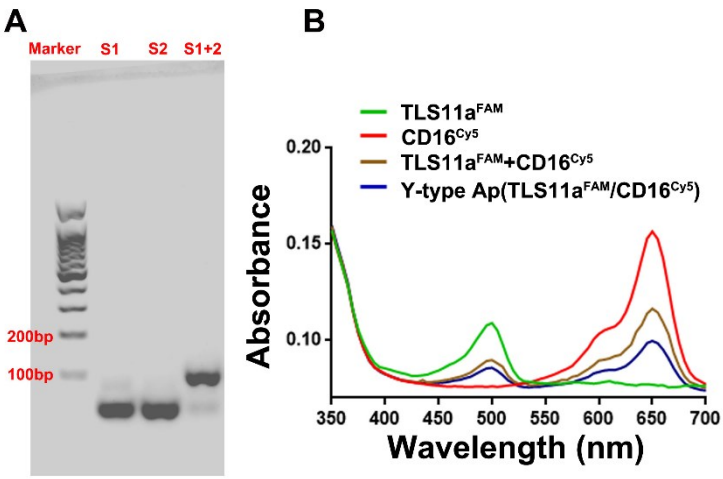

Figure S5. (A). Agarose gel electrophoresis of S1 (TLS11A Ap), S2 (CD16 Ap) and S1+2 (Y-type Ap). (B). The UV-vis-NIR absorbance of TLS11a^{FAM} Ap, CD16^{Cy5} Ap, the mixture of TLS11a^{FAM} / CD16^{cy5} and self-assembled Y-type Ap in TM buffer solution.

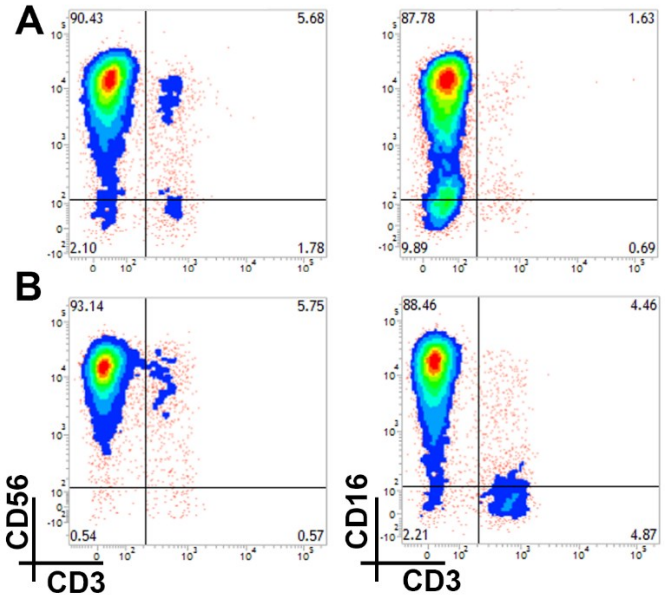

Figure S6. (A). The activation of NK cells was analyzed by staining $CD3^-$ / $CD56^+$ and $CD3^-$ / $CD16^+$. (B). The phenotype of NK cells treated by Y-type Ap (0.2 μM) for 48 h.

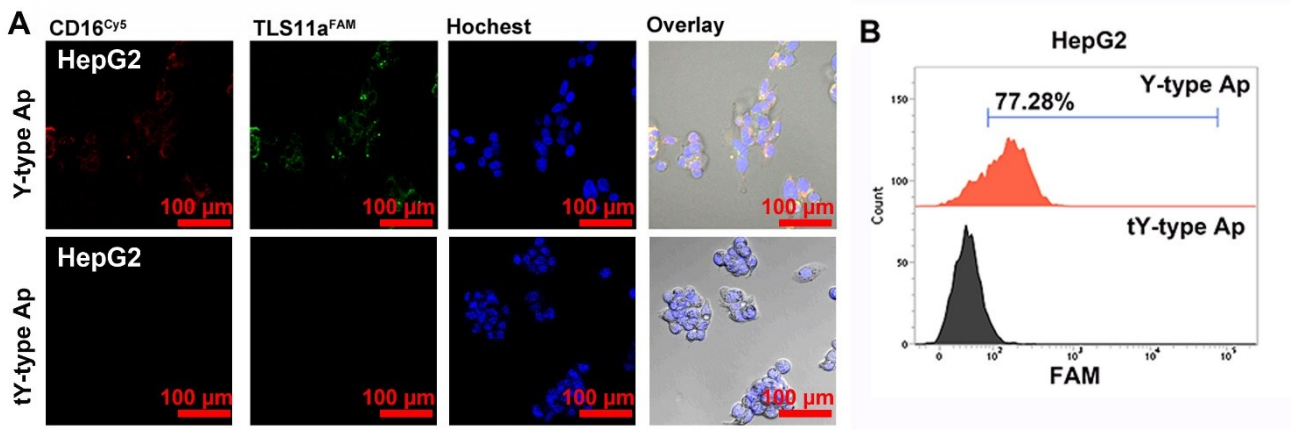

Figure S7. The specific binding ability of Y-type Ap was analyzed by CLSM (A) and FACS (B) after co-incubation of Y-type Ap or mutated Y-type Ap with HepG2 cells for 30 min.

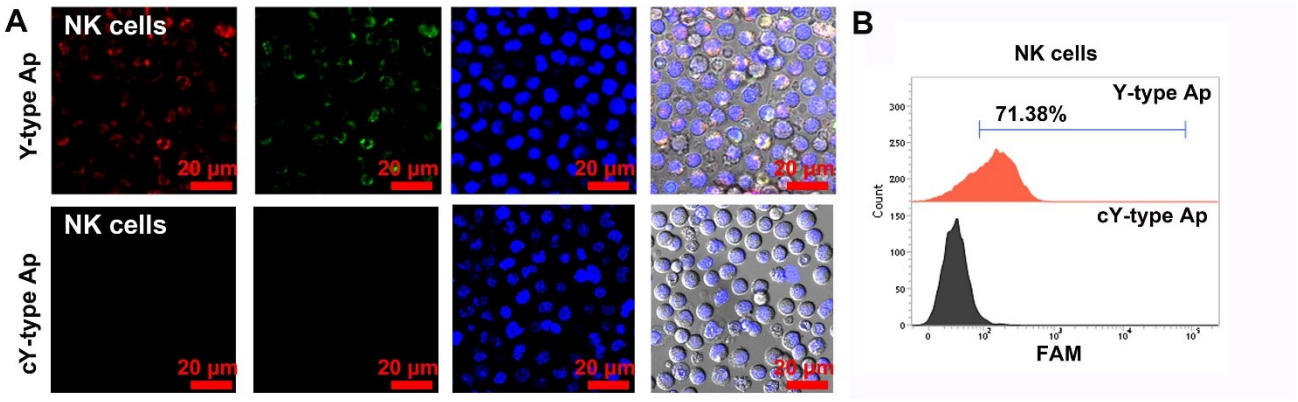

Figure S8. The specific binding ability of Y-type Ap was analyzed by CLSM (A) and FACS (B) after co-incubation of Y-type Ap or mutated Y-type Ap with NK cells for 30 min.

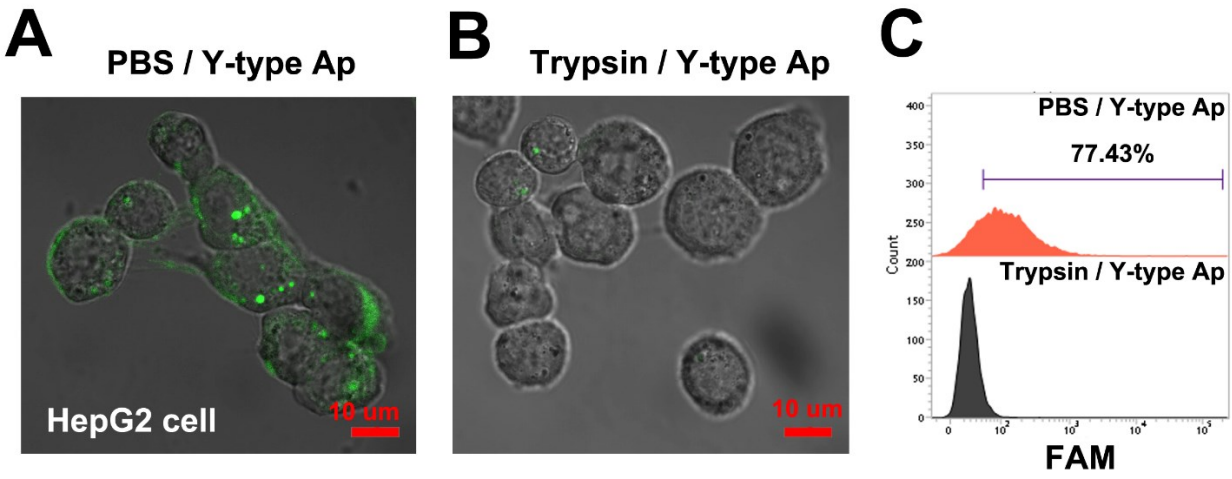

Figure S9. CLSM image of HepG2 cells treated with PBS (A) or trypsin (B) and then co-incubated with Y-type Ap. (**C**)**.** Quantitative analysis of FAM from Y-type Ap by flow cytometry.

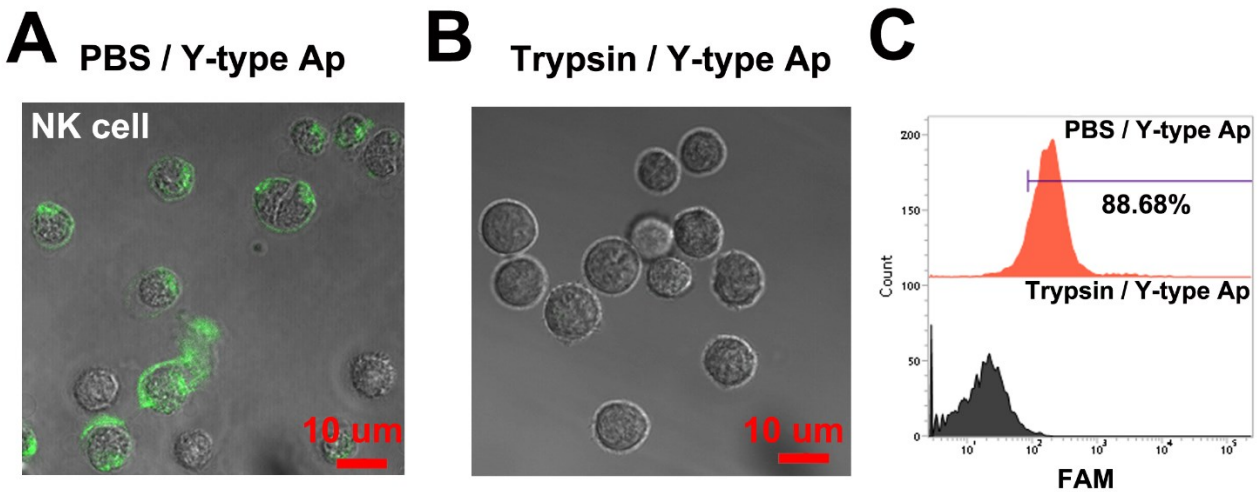

Figure S10. CLSM image of NK cells treated with PBS (A) or trypsin (B) and then co-incubated with Y-type Ap. (C). Quantitative analysis of FAM from Y-type Ap by flow cytometry.

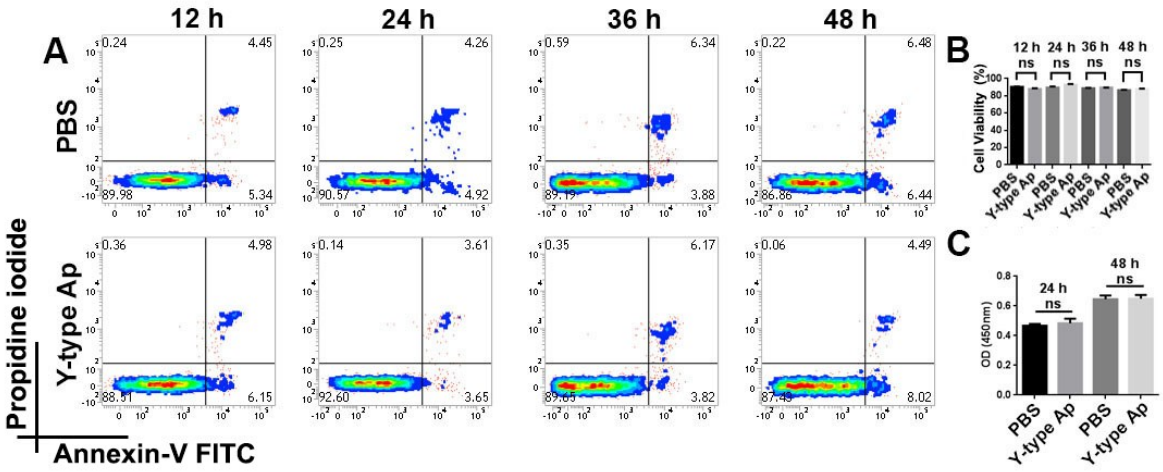

Figure S11. (A). Apoptosis of NK cells incubated with or without Y-type Ap (0.2 μM) for 12, 24, 36, 48 h, respectively. (B). Quantification of apoptosis and necrosis (%) of NK cells after treatment with or without adding Y-type Ap (0.2 μM) for 12, 24, 36, 48 h, respectively. The data are presented as the mean ± SD, n=3. **C.** The relative growth rates of NK cells incubated with or without Y-type Ap (0.2 μM) for 24 h and 48 h, respectively, and analyzed by cell counting kit-8 (CCK8) assays. The statistical analysis was performed with the ANOVA analysis (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). The data are presented as the mean ± SD, n=5.

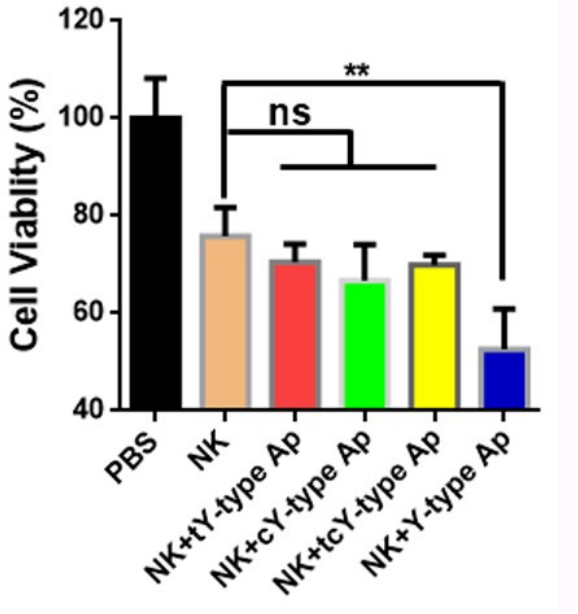

Figure S12. The cytotoxicity of NK cells combined with Y-type Ap or mutant Y-type Ap evaluated by LDH assay. The statistical analysis was performed with the ANOVA analysis (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). The data are presented as the mean ± SD, n=5.

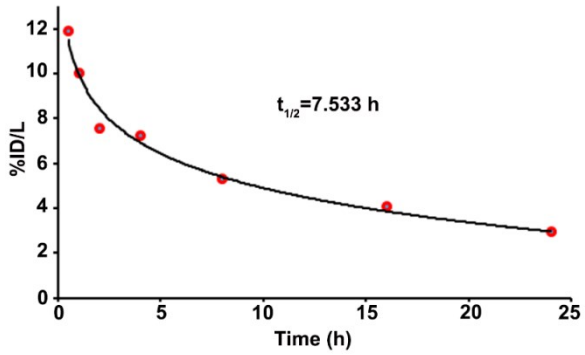

Figure S13. The half-life of the Y-type Ap *in vivo*. the half-life of Y-type Ap in mice mode through analysis the content of Y-type Ap by the standard curve of Cy5 labeled Y-type Ap from 0.0625 to 1 μM ($Y = 408.92x + 23.652$, $R^2 = 0.995$) in the mice serum.

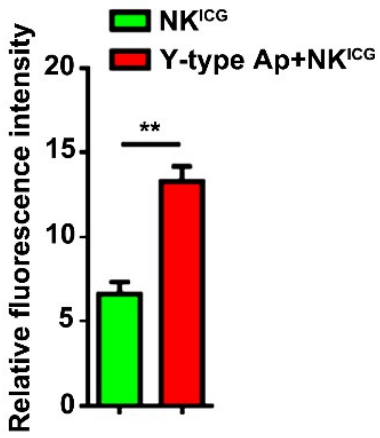

Figure S14. Quantification of fluorescence intensity of ICG labeled NK cells in tumor tissue from Figure 5D through Image J software.

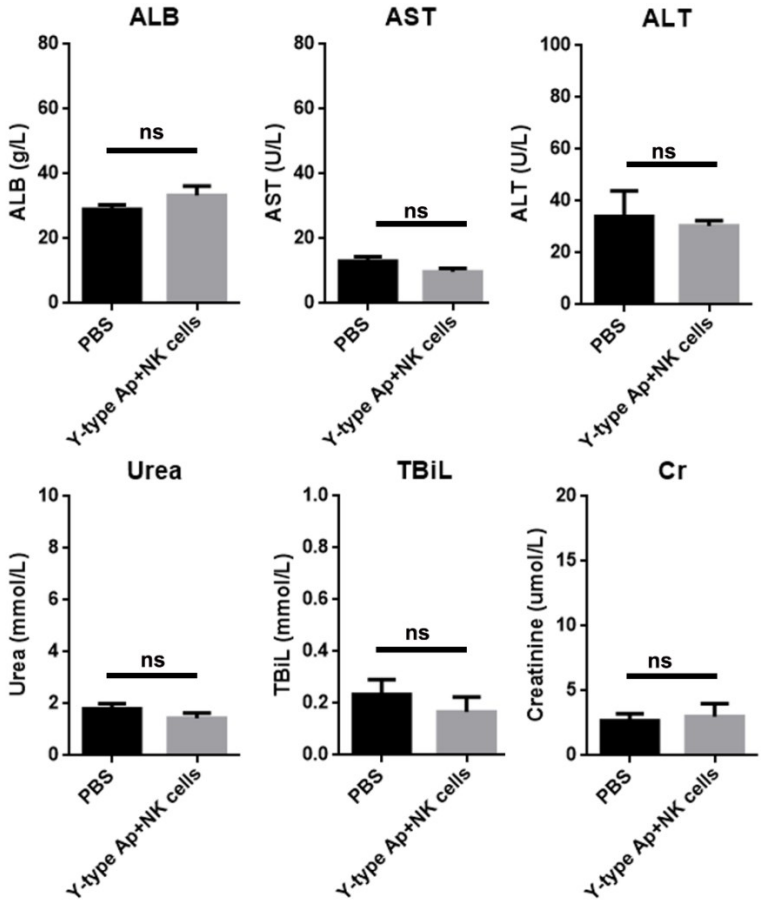

Figure S15. The liver and kidney functionality of the animal treated with PBS and NK cells combined with Y-type Ap after 3 days.