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Supplemental information

**An epitope encoded by uORF
of *RNF10* elicits a therapeutic
anti-tumor immune response**

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Table S1. Primers used in the qRT-PCR for indicated genes.

Gene name	Sequence
<i>mmu-RNF10</i>	5'-CCGGCGAGTCTAAACCCAAG-3'(forward) 5'-CGACGGGACTGGTTGCTAAAA-3'(reverse)
<i>mmu-ACTB</i>	5'-GGCTGTATTCCCCTCCATCG-3'(forward) 5'-CCAGTTGGTAACAATGCCATGT-3'(reverse)
<i>hsa-RNF10</i>	5'-AAGGAACTCTTTTTACAGGCCAA-3'(forward) 5'-AGATGGCACTTCATGGCTACA-3'(reverse)
<i>hsa-ACTB</i>	5'-GATGCGTTGTTACAGGAAGTCC-3'(forward) 5'-GGCACGAAGGCTCATCATTCA-3'(reverse)

Table S2. Information of patients whose peripheral blood were used for RNF10 uPeptide immunogenic assays.

Patients ID	Age	Histology grade	sex	Tumor size (mm)
SYM201	38	II	man	43*36
SYM202	51	III	female	41*27
SYM203	46	III	man	37*22

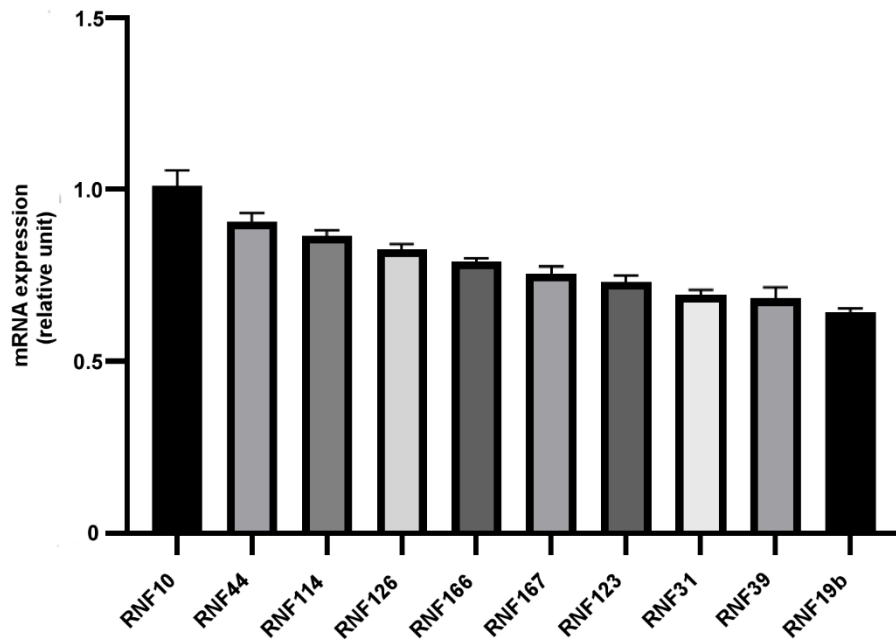


Figure S1. *RNFs* mRNA levels in CT26 cells were detected by qRT-PCR.

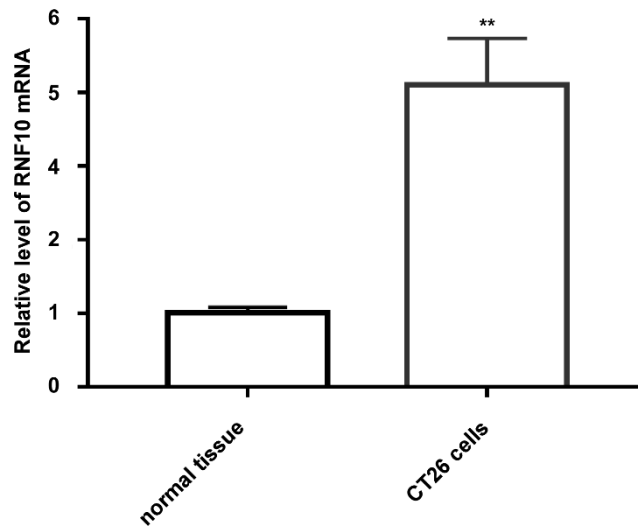


Figure S2. *RNF10* mRNA levels in CT26 cells (n=3) and normal colon tissues (n=3) of BALB/c were detected by q-PCR. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, CT26 cells compared with normal tissue.

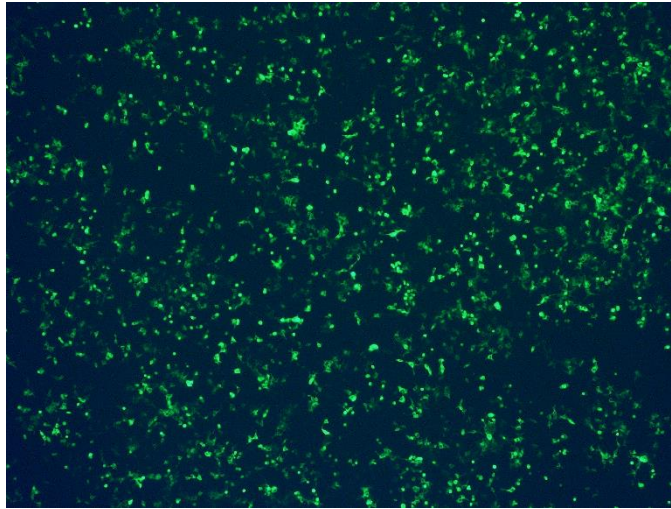


Figure S3. Representative pictures of EGFP-tagged uORF vector was detected 24 h after transfection of HEK293T cells.

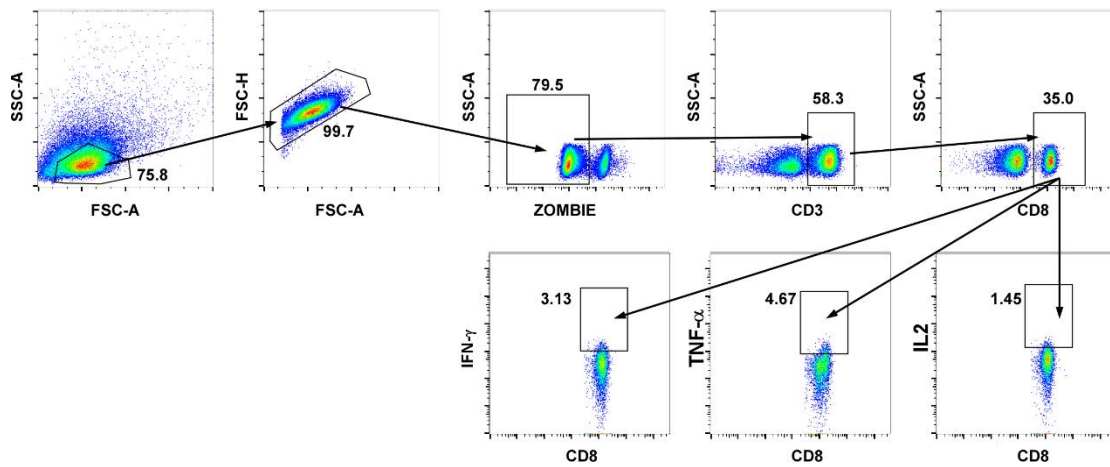


Figure S4. The gating strategy for CD8+ T cells used to determine these gates of IFN- γ +, TNF- α or IL2+ CD8+ T cells.

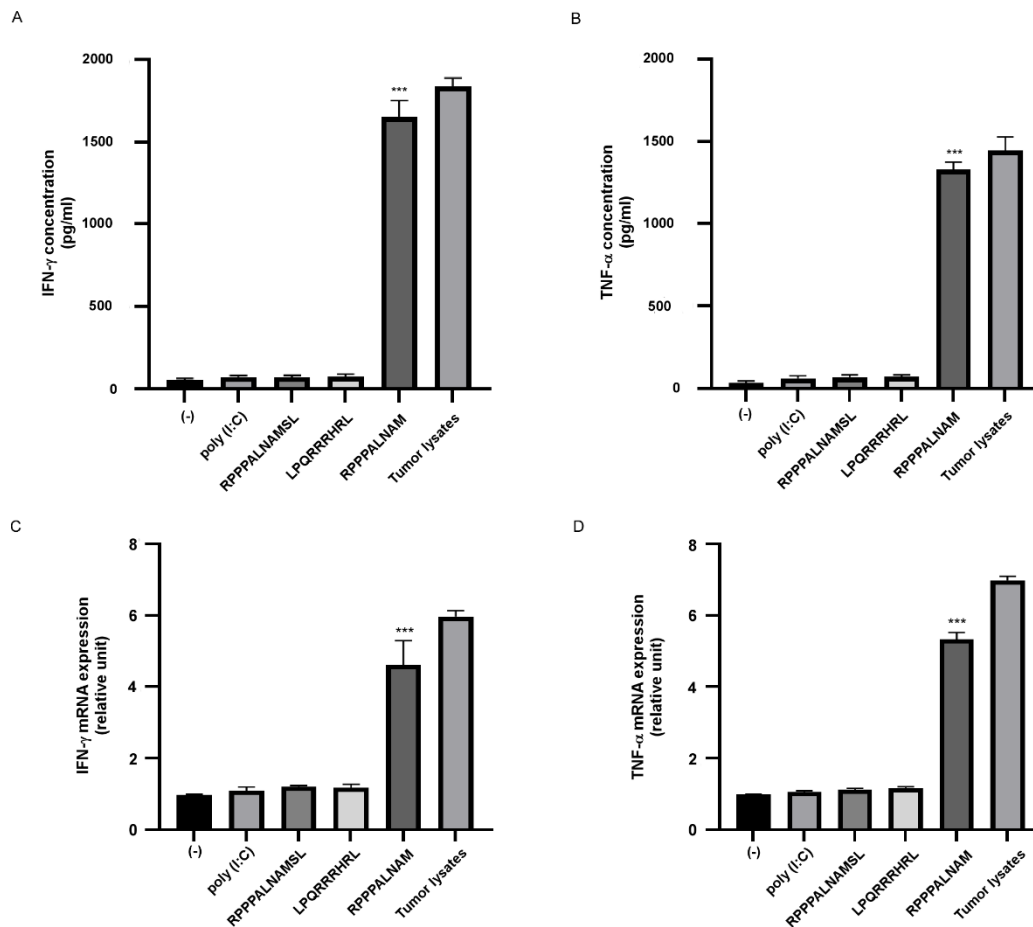


Figure S5. A, B. Splenocytes of vaccinated mice were restimulated with the RNF10 uPeptide antigens used for vaccination. The production of IFN- γ and TNF- α of splenocytes to the RNF10 uPeptide was assessed by ELISA.

C, D. Splenocytes of mice injected with CT26 cells and vaccinated with peptides and the polyinosinic:polycytidylic acid (poly[I:C]) adjuvant (CT26, n = 3) were tested for the production of cytokine by qRT-PCR.

*P < 0.05, **P < 0.01, ***P < 0.001 compared with untreated T cells (-). P-values were determined by two-tailed one-way ANOVA with Dunnett's multiple-comparisons test.

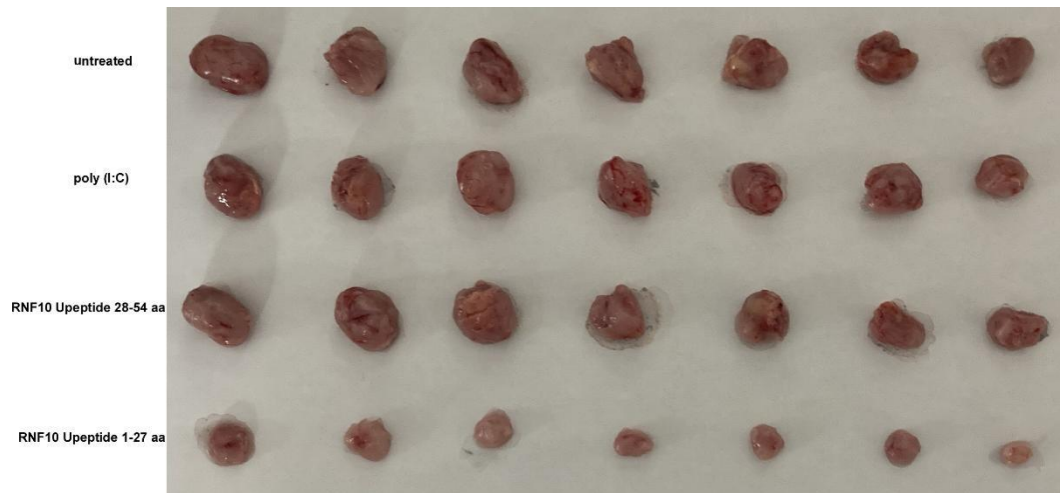


Figure S6. Images of subcutaneous tumors in each group.

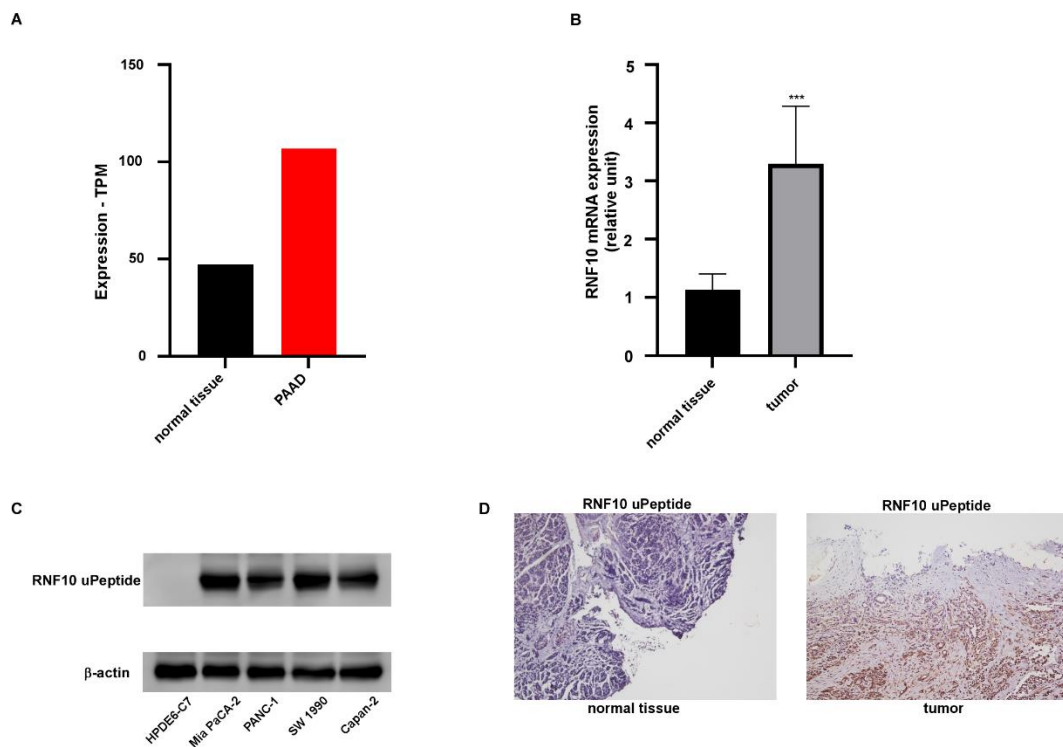


Figure S7. Human RNF10 uPeptide overexpressed in PAAD. A. The expression of *RNF10* mRNA in GEPIA 2. B. The expression of *RNF10* mRNA in PAAD tissue and normal tissue tested by qRT-PCR. C. The expression of RNF10 uPeptide was detected in benign HPDE6-C7 cells and malignant MIA PaCa-2 cells, PANC-1 cells, SW1990 cells and Capan-2 cells by immunoblotting. D. The expression of RNF10 uPeptide was assessed in PAAD tissue and normal tissue by IHC.

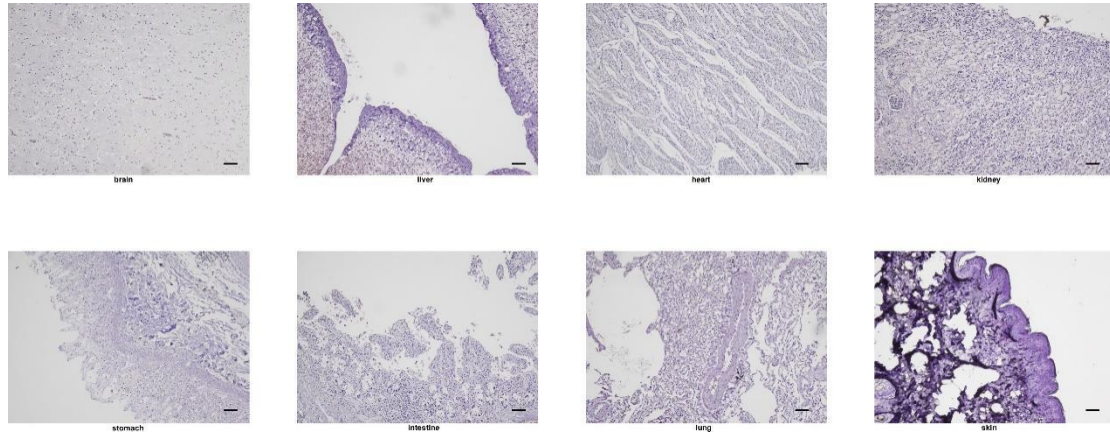


Figure S8. The expression of RNF10 uPeptide was assessed in some vital organs by IHC.

allele	peptide	rank
HLA-A*02:01	RLFGQQQRA	0.2
HLA-A*02:01	RLFGQQQRATA	0.71
HLA-A*02:01	RLGGASRRV	1.1
HLA-A*02:01	LRLFGQQQRA	1.8
HLA-A*02:01	LLRLFGQQQRA	2

Figure S9. The binding predictions for RNF10 uPeptides to HLA-A2 using the Immune Epitope Database (version 2.5) analysis resource NetMHCpan (ver. 4.1) algorithm.

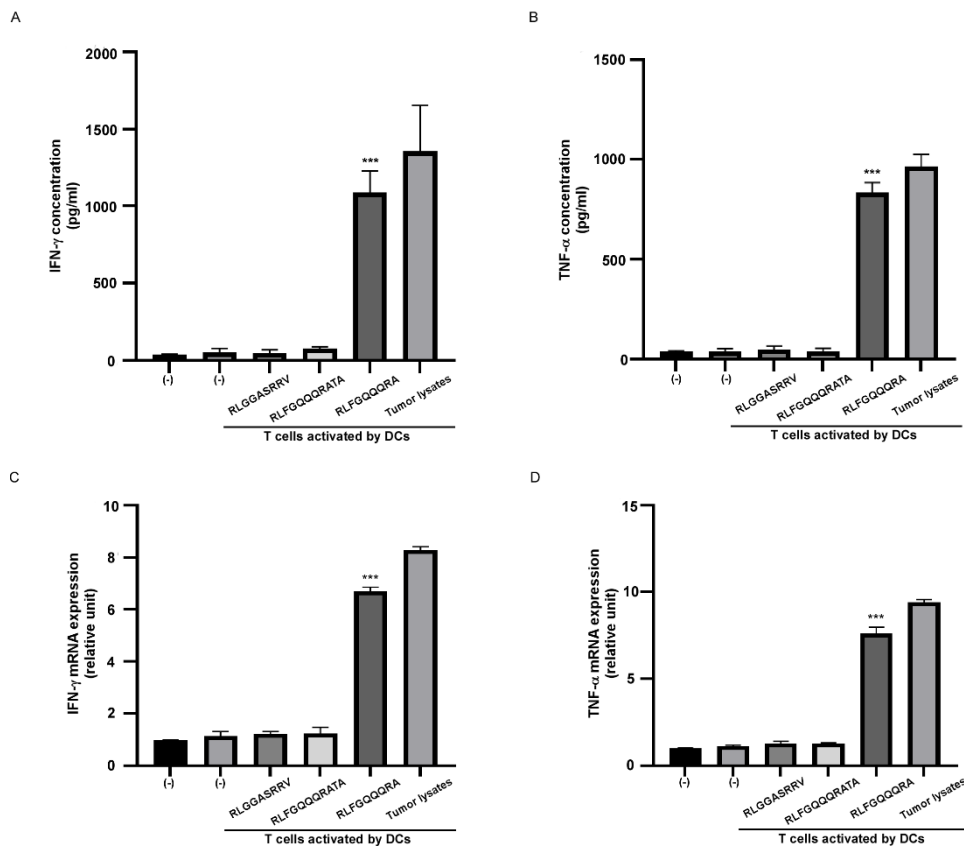


Figure S10. RNF10 uPeptide elicits human T cell immune response.

A, B. T cells were primed by autologous DCs loaded with uPeptide antigens. The production of IFN- γ and TNF- α of T cells to the RNF10 uPeptide was assessed by ELISA. C, D. The primed T cells were re-stimulated with uPeptide antigens overnight and evaluated by qRT-PCR.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with untreated T cells (-). P-values were determined by two-tailed one-way ANOVA with Dunnett's multiple-comparisons test.

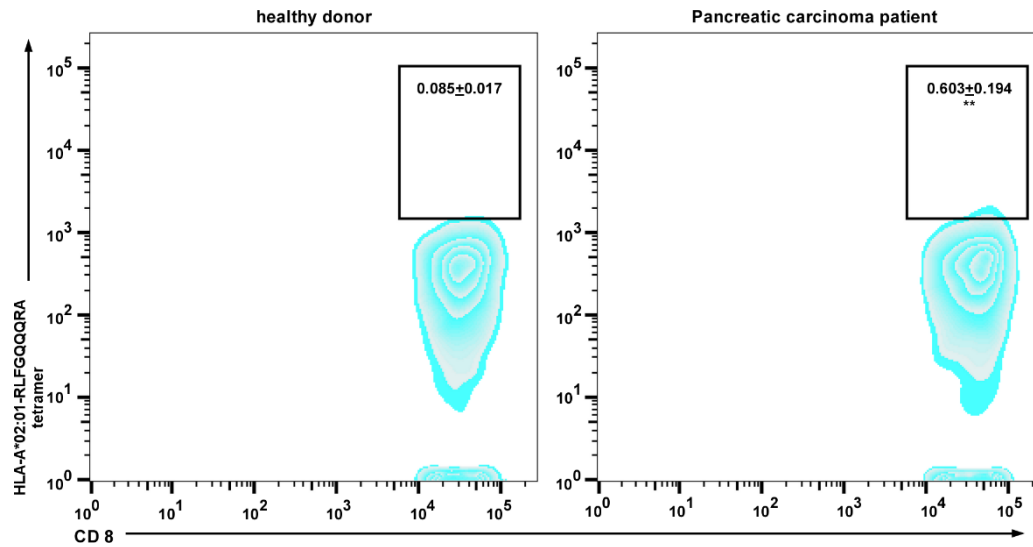


Figure S11. Representative flow cytometry plots and quantitation of RLFQQQRA-tetramer+ CTLs in the peripheral blood of healthy donor and pancreatic carcinoma patients with uPeptide of RNF10 expression (mean \pm s.d.; healthy donor: n = 3, patient: n = 3). *P < 0.05, **P < 0.01, ***P < 0.001, by t-test.

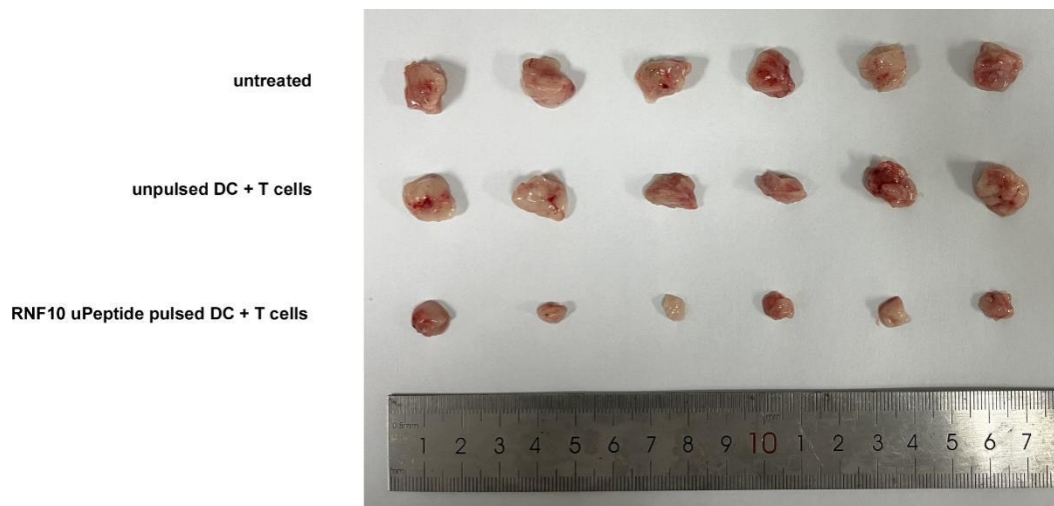


Figure S12. Images of PDX tumors in each group.