

## Experimental Supporting Information

### Materials and methods.

**Chemicals and materials:** Fmoc-amino acids and HBTU were obtained from GL Biochem (Shanghai, China); Phosphorylated antigen peptides, biotin-conjugated phosphorylated antigen peptides and phosphorylated antigen peptide-conjugated keyhole limpet hemocyanin (KLH) proteins were ordered from GL Biochem (Shanghai, China); Lambda Protein Phosphatase (Lambda PP) was bought from NEB; Alkaline phosphatase (ALP) was bought from Takara Bio; All the other chemical solvents and reagents were purchased on experimental reagents and technical safety management platform of Nankai University.

**General methods:**  $^1\text{H}$  NMR spectra were obtained on Varian Mercury Vx-300 (America). HR-MS were acquired at Agilent 6520 Q-TOF LC/MS (America). HPLC was conducted at LUMTECH HPLC (Germany) system using a C18 RP column with MeOH (0.05% of TFA) and water (0.05% of TFA) as the eluents. LC-MS was conducted at the Shimadzu LCMS-2020 system (Japan). TEM characterization was performed at the HITACHI HT7700 Exalens system (Japan). Rheology was performed on an TA instrument ARES 1500ex system (America). Circular dichroism was performed at the BioLogic MOS-450 system (France). Fluorescence spectrum was measured using BioTek Synergy 4 system (America).

**Preparation of peptides:** Nap-G<sup>D</sup>F<sup>D</sup>Fp<sup>D</sup>Y-COOH and Nap-G<sup>D</sup>F<sup>D</sup>F<sup>D</sup>Y-COOH were synthesized by standard solid phase synthesis (SPPS) using 2-chlorotrityl chloride resin and N-Fmoc protected amino acids whose side chain were properly protected. The crude products were further purified with reverse phase high performance liquid chromatography(HPLC).

**Transmission electron microscopy (TEM):** 10  $\mu$ L hydrogel was dripped on a carbon coated copper mesh. 15 seconds later, removed the hydrogel carefully with a filter paper. Washed the copper mesh one to three times with double distilled water. Subsequently, dripped 10 $\mu$ L uranyl acetate on the copper mesh for 1 minutes. Finally, dried the copper mesh in a desiccator overnight for TEM measurement.

**Preparation of pY-Gel:** 5mg Nap-G<sup>DFDFD</sup>pY-COOH was dissolved in 1mL PBS buffer in a glass bottle and the pH value was adjusted to 7.4 using 1M NaCO<sub>3</sub>. We then added 2 equiv Ca<sup>2+</sup> (12.8  $\mu$  L of 1M CaCl<sub>2</sub>) to the solution and drastically shook the bottle. The pY-Gel formed immediately.

**Preparation of Y-Gel:** Weighed 5mg of Nap-G<sup>DFDFD</sup>Y-COOH into a glass bottle. 1mL PBS buffer and 6  $\mu$  L of 1M NaCO<sub>3</sub> were added to the bottle. Heated the bottle on the flame of an alcohol burner with constantly shook until the powder was completely dissolved. As the temperature of the solution decreased, Y-Gel would gradually formed. The finally pH value of Y-Gel was 7.4.

**Preparation of vaccine:** Weighed 10 mg of Nap-G<sup>DFDFD</sup>pY-COOH and dissolved it in 1 mL PBS buffer to prepare a 10 mg/mL stock solution, the pH value was adjusted to 7.4 using 1M NaCO<sub>3</sub>. To prepare 1 mL hydrogel vaccine, 500  $\mu$ L stock solution was mixed with KLH protein-coupled phosphorylated antigen in PBS solution, and the final volume was 1 mL. 12.8  $\mu$  L of 1M CaCl<sub>2</sub> was then added with vortex immediately to form the co-assembled hydrogel.

**Conversion rate of phosphorylated peptides *in vitro*:** We prepare 1mL of pY-Gel containing 1mg/mL phosphorylated peptides (TpSN, KpTL and CpTW), then separate the hydrogels into centrifuge tubes with 50 $\mu$ L per tube. We add 50 $\mu$ L of lambda PP (400U/mL) to hydrogels and put the tubes in a 37 °C incubator. At the predetermined

time point, we take out one tube and dissolve the sample with 300 $\mu$ L of methyl alcohol, which is then used for HPLC analyst after filtration with a 0.45  $\mu$ m filter.

**Preparation of RBITC-tagged phosphorylated peptide-conjugated KLH protein:**

First, 3 mg RBITC was dissolved in 20 $\mu$ L anhydrous dimethyl sulfoxide. 3mg phosphorylated peptide-conjugated KLH protein (KLH-TpSN, KLH-KpTL or KLH-CpTW) was dissolved in 5mL pH 9.0 K<sub>2</sub>CO<sub>3</sub> (2mM) solution. Then 5 $\mu$ L fresh RBITC solution was added into the protein solutions. The reactions were shaken at 4 $^{\circ}$ C overnight. The reaction mixture was then suffered from dialysis to remove dimethyl sulfoxide and the unreacted RBITC. The obtained RBITC-tagged phosphorylated peptide-conjugated KLH proteins were named RBITC-KLH-TpSN, RBITC-KLH-KpTL and RBITC-KLH-CpTW, respectively.

**Animal experiments:** All animal studies were performed in compliance with the guidelines set by the Tianjin Committee of Use and Care of Laboratory Animals, and the overall project protocols were approved by the Animal Ethics Committee of Nankai University. 6 weeks old C57BL/6 mice were bought from Beijing Vital River Laboratory Animal Technology Co., Ltd and fed in SPF animal center of college of life sciences, Nankai University. The mice were vaccinated in subcutaneous injections at the predetermined time points. The peripheral blood of mice was gathered from superior ophthalmic vein using blood-taking needles at the predetermined time points.

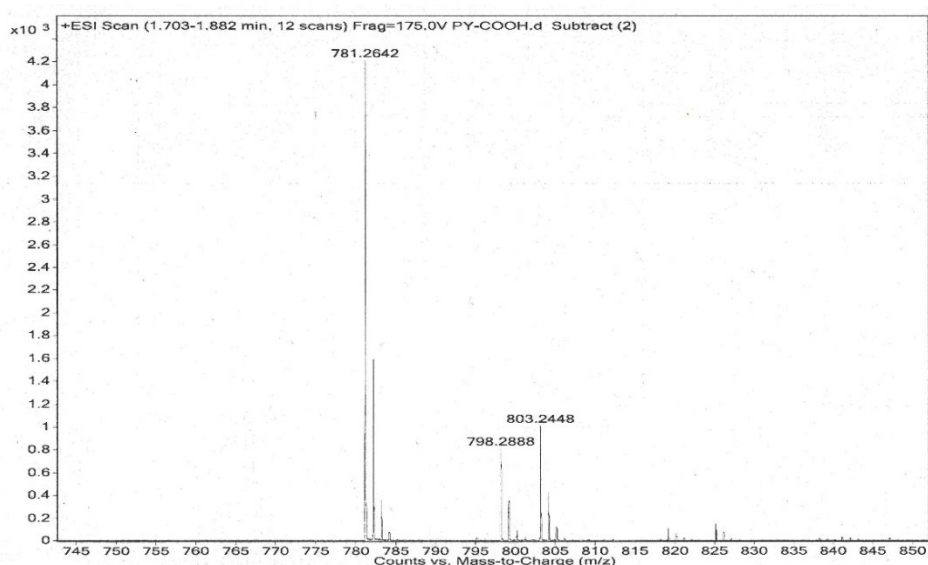
**Separation of serum:** Collected peripheral blood was incubated at 37  $^{\circ}$ C for 30 minutes and then centrifuged at 4000rpm for 10 minutes. Sera were carefully collected to new centrifuge tubes using pipettor and stored at -80 $^{\circ}$ C.

**Determination of Antibody titer:**Antibody titer was examined using BAS-ELISA. ELISA plates were incubated with 100 $\mu$ L/well streptavidin (20ug/mL in PBS buffer) at 4 $^{\circ}$ C overnight. Removed the solution and washed the plates with PBST three times.

Subsequently, incubated the plates with 100 $\mu$ L/well biotin-antigenic peptide (20 $\mu$ M in PBS buffer) at room temperature for 30 minutes. Next, washed the plates with PBST three times and the plates were blocked with 200 $\mu$ L/well BSA (1% in PBS buffer) at room temperature for 1 hour. Abandoned the solution and washed the plates with PBST three times again. Serum samples diluted in appropriate proportion with BSA were added to the first row of wells (100 $\mu$ L/well) and performed a further dilution using double dilution method. The samples were incubated at room temperature for 2 hours. Hereafter, abandoned the solution and washed the plates seven times with PBST. And incubated the plates with 100 $\mu$ L/well HRP-conjugated IgG antibody (1:5000 diluted in 1% BSA) at room temperature for 1 hour. After that, washed the plated seven times with PBST. Incubated the plates with 100 $\mu$ L/well TMB substrate solution for 20min in dark and end the reaction with 100 $\mu$ L/well stop solution (2M H<sub>2</sub>SO<sub>4</sub>). In the end, measured the optical density at 450 nm with a microplate reader.

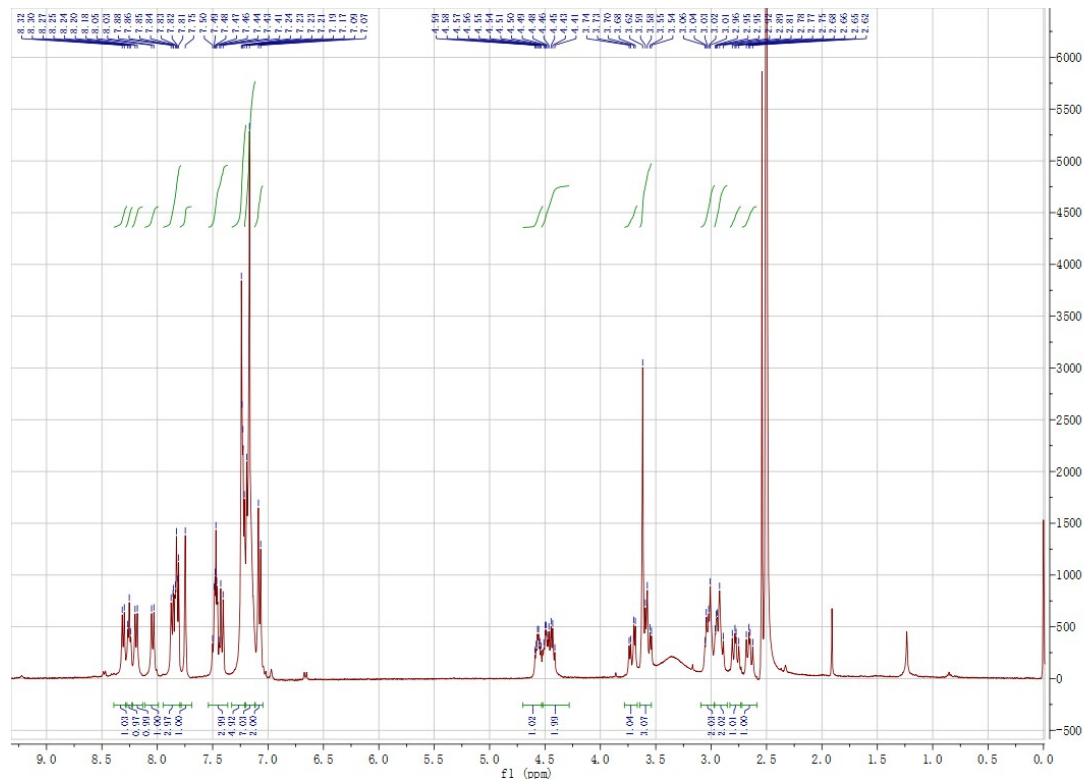
**Table S-1.** Phosphorylated peptides as antigens used in the study

Abbreviations	Sources	Sequences
TpSN	MRCL3 [Ovis aries]; Ser20(12-27)	KKRPQRAT(pS)NVFAMFD
CpTW	FKHR Thr-24; (19–31)	RPRSC(pT)WPLPRPE
KpTL	Human Plk1; Thr210(205-214)	GERKK(pT)LCGT

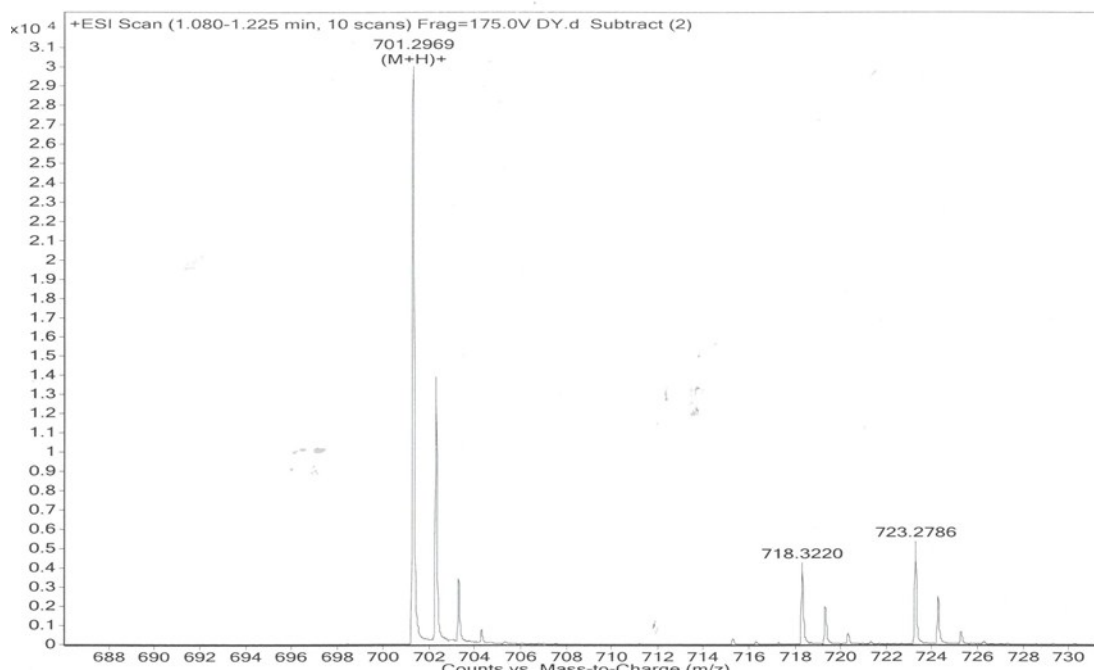


**Figure S-1.** HR-MS spectrum of Nap-G<sup>D</sup>F<sup>D</sup>F<sup>D</sup>pY.

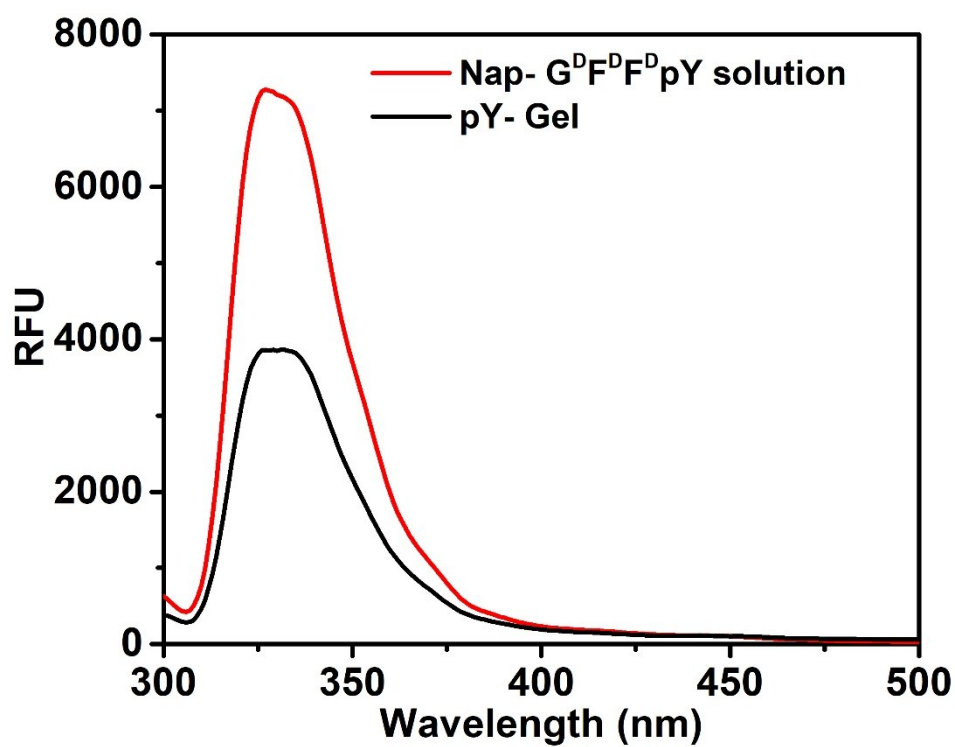
$^1\text{H}$  NMR (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$  8.31 (d,  $J = 7.6$  Hz, 1H), 8.25 (t,  $J = 5.5$  Hz, 1H), 8.19 (d,  $J = 8.2$  Hz, 1H), 8.04 (d,  $J = 8.3$  Hz, 1H), 7.95 – 7.78 (m, 3H), 7.75 (s, 1H), 7.54 – 7.36 (m, 3H), 7.23 (dd,  $J = 6.9$ , 3.8 Hz, 5H), 7.18 (d,  $J = 10.0$  Hz, 7H), 7.08 (d,  $J = 8.2$  Hz, 2H), 4.56 (td,  $J = 9.2$ , 4.5 Hz, 1H), 4.47 (ddd,  $J = 21.4$ , 11.0, 6.5 Hz, 2H), 3.71 (dd,  $J = 16.8$ , 5.6 Hz, 1H), 3.60 (d,  $J = 16.7$  Hz, 3H), 3.09 – 2.97 (m, 2H), 2.97 – 2.85 (m, 2H), 2.78 (dd,  $J = 13.9$ , 9.8 Hz, 1H), 2.65 (dd,  $J = 13.6$ , 9.6 Hz, 1H).



**Figure S-2.**  $^1\text{H}$  NMR spectrum of Nap-G $^{\text{D}}\text{F}^{\text{D}}\text{F}^{\text{D}}\text{D}^{\text{D}}$ pY.



**Figure S-3.** HR-MS spectrum of Nap-G $^{\text{D}}\text{F}^{\text{D}}\text{F}^{\text{D}}\text{D}^{\text{D}}$ Y.



*Figure S-4.* Fluorescence spectra of PBS solutions of Nap-G<sup>D</sup>F<sup>D</sup>F<sup>D</sup>pY and pY-Gel, the sample was diluted to 1mM using PBS, excitation wavelength at 272nm.