Supplementary Information

Remodeling Fibrotic Tumor Microenvironment of Desmoplastic Melanoma to Facilitate Vaccine Immunotherapy

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Preparation Lipid calcium-phosphate nanoparticle (LCP NP)

A well-dispersed oil phase was formed with 20 mL mixture solution containing cyclohexane/Igepal CO-520 (71:29, v/v) and 600 μL of 2.5 M $CaCl_2$ containing modified $BRAF^{V600E}$ peptide, immune-stimulator adjuvant CpG ODN. Meanwhile, 600 μL Na_2HPO_4 (12.5 mM, pH = 9.0) and 400 μL DOPA (20 mM) was added in oil phase, stirring two phases for 30 min. Then cores deposited when 40 mL ethanol was added and collected using centrifugation (10,000 g × 15 min). One hundred μL of 20 mM DOTAP, 20 μL of 20 mM DSPE-PEG-2000, 20 μL DSPE-PEG-mannose (20 mM) and 100 μL Cholesterol in chloroform were mixed with 1 mL CaP cores. After removing of chloroform using reduced pressure, the final LCP NP was dispersed in 100 μL glucose (5 %).

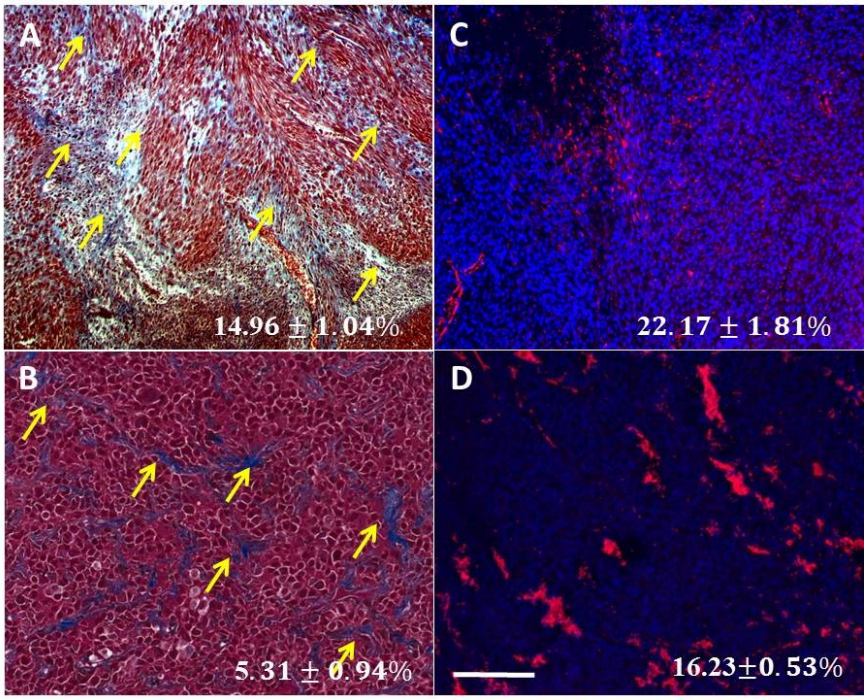

Fig. S1 The difference of collagen fiber and TAFs between murine DM model (BPD6 tumor model) and murine non-DM model (B16F10 tumor model). Masson's trichrome staining illustrating collagen morphology within BPD6 tumor model (A) and B16F10 tumor model (B), and also α-SMA staining illustrating TAFs within BPD6 tumor model (C) and B16F10 tumor model (D). For (A) and (B), the blue color represents collagen fibers (pointed out by yellow arrows). For (C) and (D), the red color represents α-SMA (as TAF marker). The scale bar represents 200 μm.

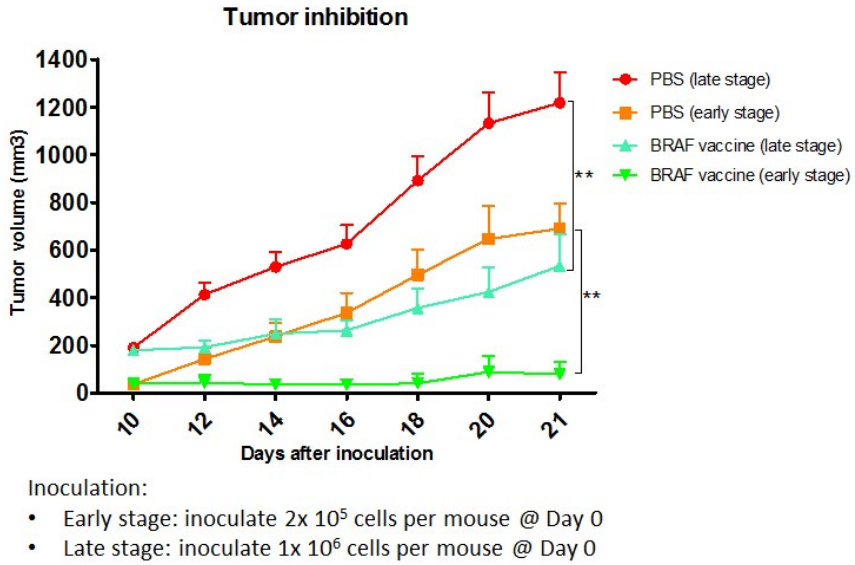

Fig. S2 The BRAF peptide vaccine showed inhibition of tumor growth at different stages of the murine DM model. C57BL/6 mice were inoculated with 2×10^5 BPD6 cells per mouse for "early stage" tumor growth inhibition study, or 1×10^6 BPD6 cells per mouse for "late stage" tumor growth inhibition study. Tumor cells were inoculated on mice subcutaneously on day 0, and vaccination was given on day 10 (n=5). **$p < 0.01$. At early stage, vaccine alone showed long-lasting tumor inhibition, while at advanced late stage tumor, vaccination could only achieve partial efficacy.

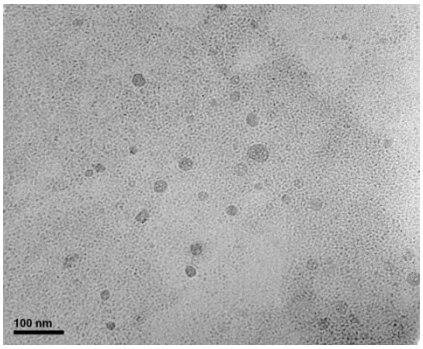

Fig. S3 Characterization of LCP-BRAF peptide vaccine.

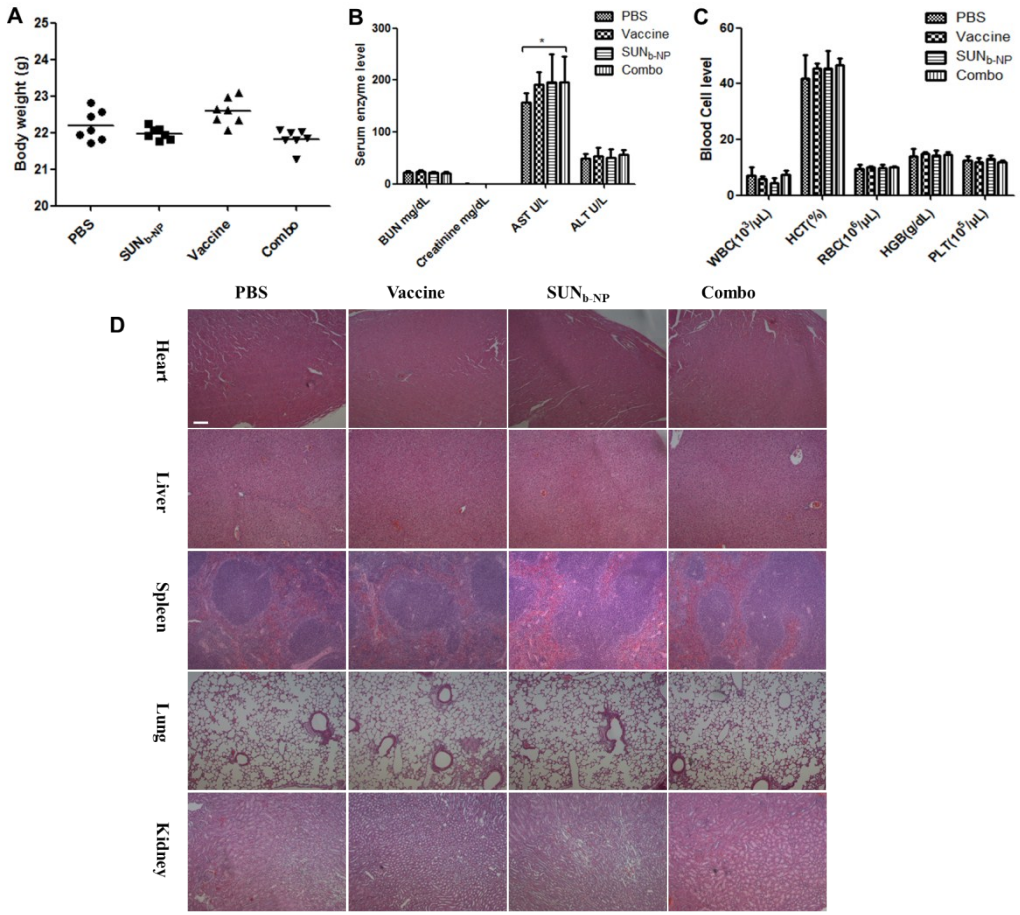

Fig. S4 Safety evaluations. Throughout the tumor inhibition study, mice body weights were monitored every two days. Blood samples, major organs and tumor tissue were harvested at the endpoint of study, when mice were humanely sacrificed (on day 24 after tumor cell inoculation). (**A**) Body weight change at endpoint day of study. (**B**) Liver, kidney function assays and (**C**) whole blood cell analysis after treatment. (**D**) H&E stained heart, liver, spleen, lung, and kidney sections from tumor-bearing mice after treatment. The white scale bar represents 100 μm. Results were expressed as the mean ± S.D. (n = 4-5). *p < 0.05,

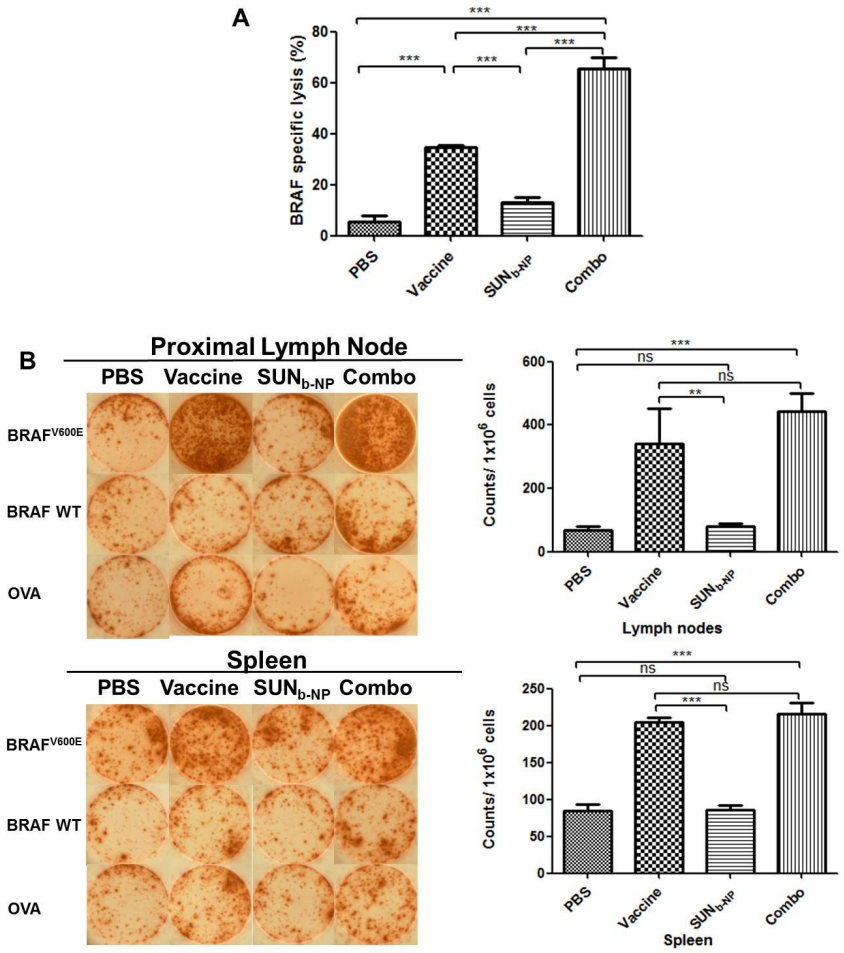

Fig. S5 Antigen-specific immune response. (A) CTL response after vaccination *in vivo*, n=5. (B) IFN-γ production after vaccination was detected using ELISPOT assay. **p < 0.01, ***p < 0.001 and ns, not significant. n = 3.

Tab. S1 Antibodies used in the study

Antibodies	Company	Catalog	Application
Anti-αSMA	Abcam	Ab5694	IF
Anti-CD31	Abcam	Ab28364	IF
Anti-CD8 (PE-conjugated)	BD	553032	flow cyt
Anti-CD8 (FITC-conjugated)	BD	553031	IF
Anti-CD4 (FITC-conjugated)	BD	561828	IF, flow cyt
Anti-FOXP3 (PE-conjugated)	BD	560408	IF, flow cyt
Anti-CD11b (FITC-conjugated)	BD	553310	IF, flow cyt
Anti-Gr1 (Ly-6G and Ly-6C) (PE-conjugated)	BD PharmingenTM	553128	IF, flow cyt
APC Rat IgG2b,	BD PharmingenTM	553991	flow cyt
Anti-Rabbit IgG (Alex Fluor® 647 Conjugate)	Cell Signaling	4414	IF, flow cyt
Goat Anti-Rabbit IgG-HRP	Santa Cruz	Sc-2030	WB
Phospho-Stat3 (Ser727)	Cell Signaling	9134	WB
Stat3 (79D7)	Cell Signaling	4904	WB
Phospho-Akt (Ser473)	Cell Signaling	4060	WB
Akt (C67E7)	Cell Signaling	4691	WB
PD-L1	Invitrogen	PA5-20343	WB
GAPDH	Santa Cruz	I3015	WB

Tab. S2 Characterization of $SUN_{b\text{-}NP}$ (n=3)

	Blank NP	$SUN_{b\text{-}NP}$
Size (nm)	116.4±3.3	85.7±2.3
Zeta (mV)	-17.0±0.3	7.0±0.2

Tab. S3 *In vivo* pharmacokinetic parameters of ^{3}H-labeled $SUN_{b\text{-}NP}$ and SUN solution in DM tumor-bearing C57BL/6 mice hosts (n = 5)

	^{3}H SUN solution	**^{3}H $SUN_{b\text{-}NP}$**
K (h^{-1})	0.10±0.03	0.01±0.01
$AUC_{0\text{-}t\ (mg.h/mL)}$	632.2±326.5	1381.9±442.6
$AUC_{0\text{-}\infty\ ((mg.h/mL)}$	688.1±367.3	1698.3±406.2
$T_{1/2}$ (h)	7.5±0.8	5.3±0.6