Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information

In vivo targeting of tumor-antigen encoded DNA vaccine to dendritic cells in combination with tumor-selective chemotherapy eradicates established mouse melanoma

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Table S1. Sizes and Zeta potentials (ξ) of Liposomes of CGKRK-Lipopeptide

Drug/siRNA entrapped in liposome containing CGKRK-lipopeptide 1	**Hydrodynamic diameter(nm)**	**Zeta Potentials(mV)**
WP1066	181 ± 3	5.9 ± 1.2
STAT3siRNA	196 ± 2	4.6 ± 2.1
WP1066 & STAT3siRNA	206 ± 4	5.1 ± 1.9

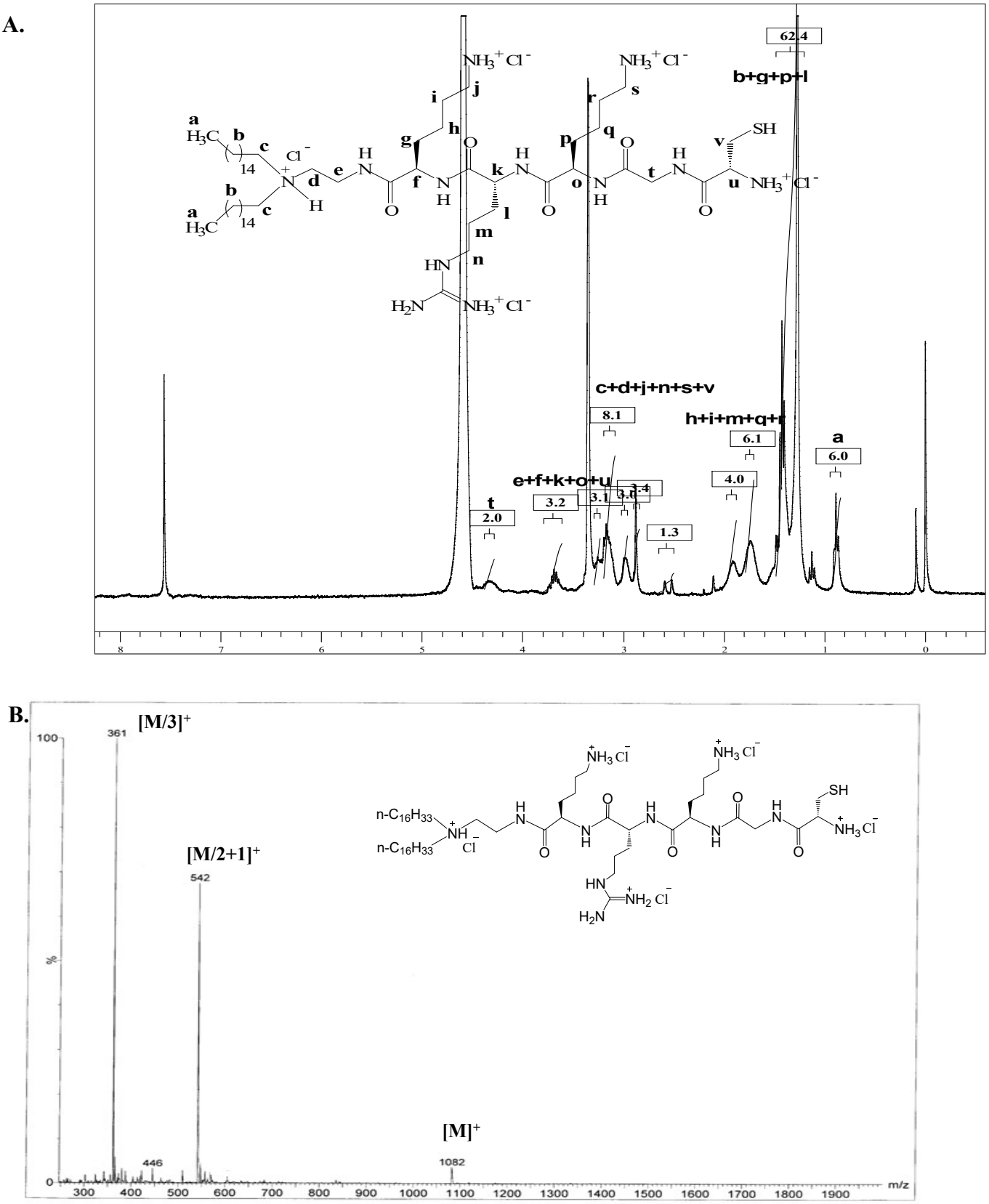


Figure S1. ^{1}H NMR (**A**) and ESI-MS (**B**) of CGKRK-lipopeptide **1** (**CLP, Figure 1**).

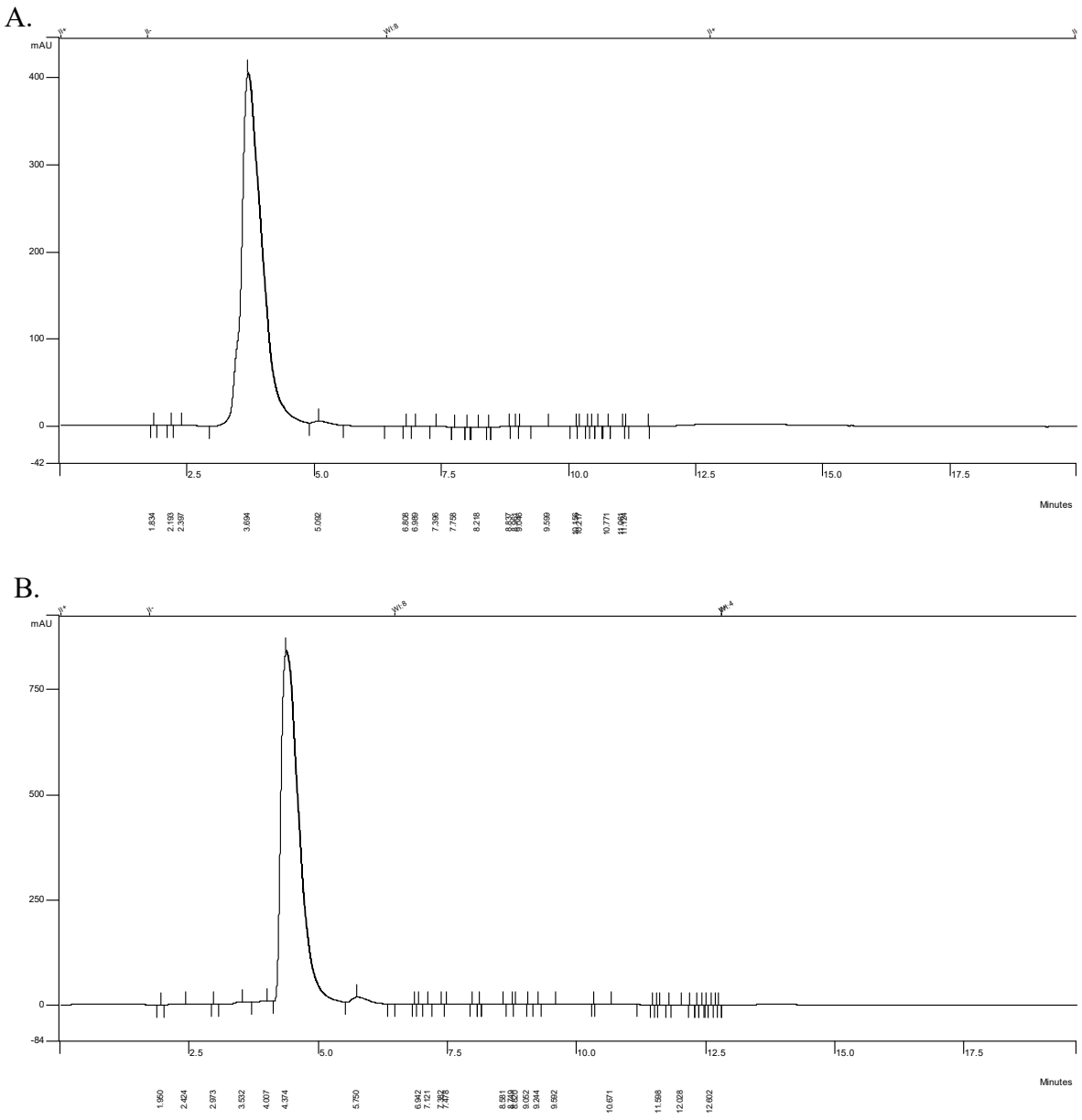


Figure S2. HPLC Chromatogram of CGKRK-lipopeptide **1**, Mobile Phase: Methanol (**A**); Methanol:Water, 95:5, v/v, (**B**). **HPLC Conditions:** System: Varian 1100 series, Column: Lichrospher® 100, RP-18e (5 µm), Flow Rate: 1.0 mL/min (0-20 min), Typical Column Pressure: 60-65 Bars, Detection: UV at 210 nm.

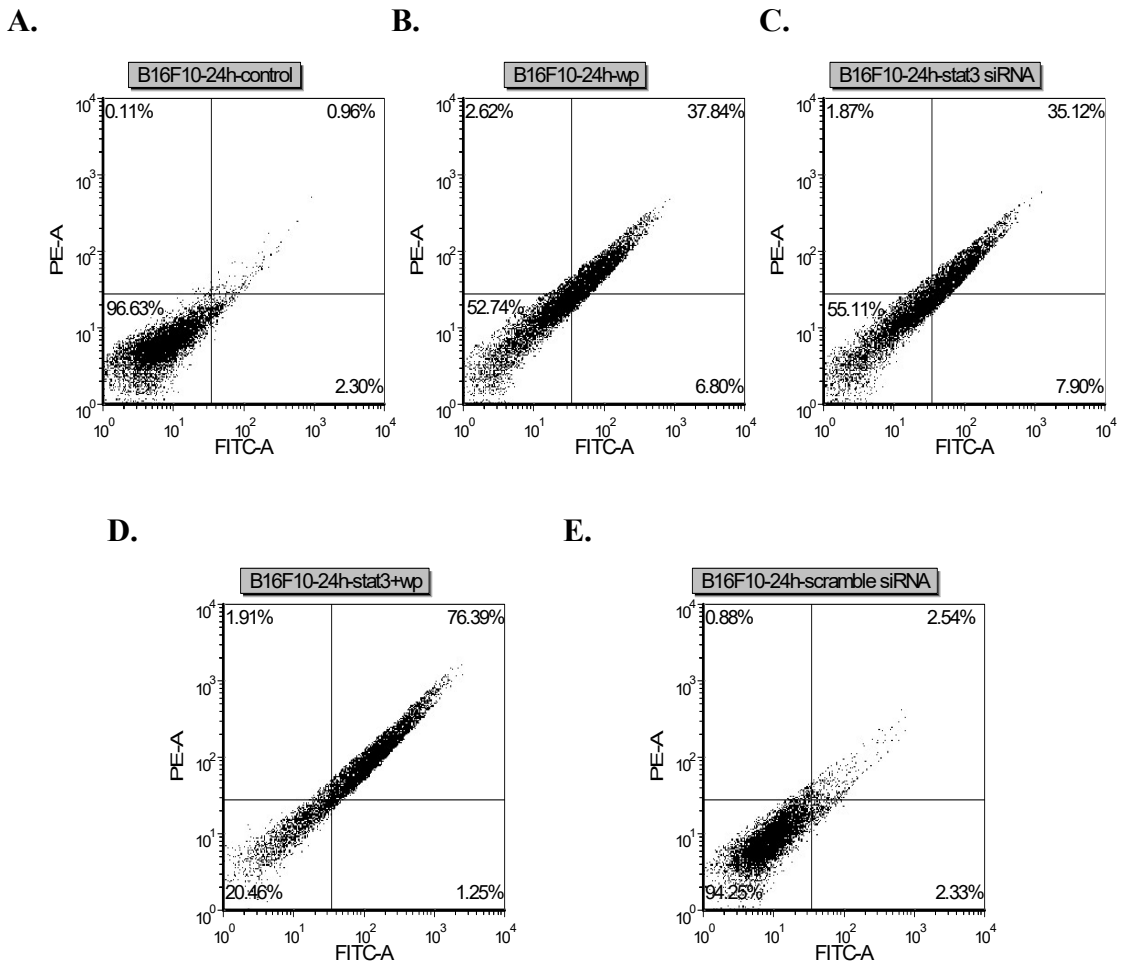


Figure S3. Synergistic effects of liposomally co-loaded STAT3siRNA & WP1066 in inducing apoptosis in B16F10 cells. B16F10 cells were treated with tumor-selective liposomes of **CLP** containing: only WP1066 (**B**); only STAT3siRNA (**C**); both STAT3siRNA & WP1066 (**D**); and scrambled siRNA (**E**). Both untreated (**A**) and treated cells were stained with FITC-Annexin V and propidium iodide (PI) for flow cytometric analysis. The horizontal and vertical axes represent cells labeled with FITC-Annexin V and PI, respectively in the dot plot. Dots in the upper right quadrant represent late apoptotic cells (positive for both Annexin V and PI). Data shown here are representative of two separate experiments.

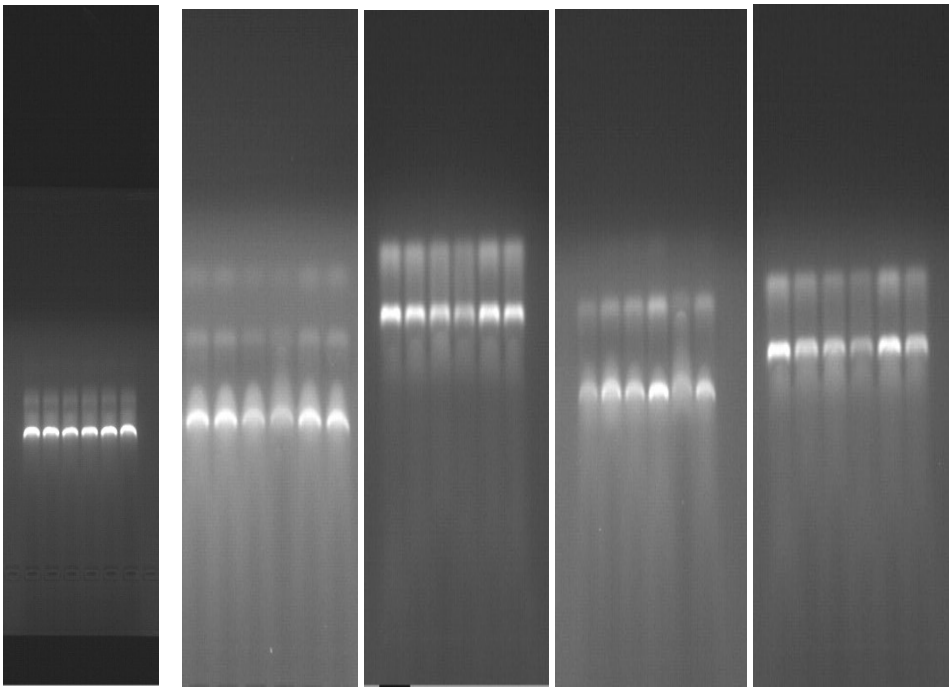


Figure S4. Original gel images used for drawing **Figure 3B** in the text

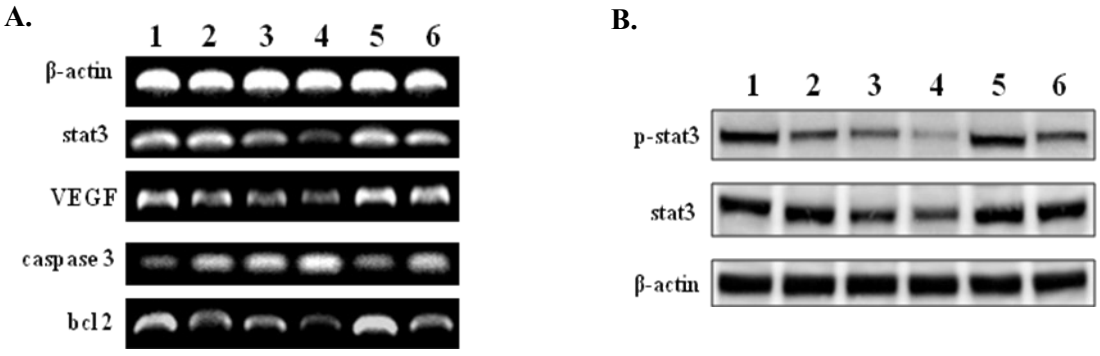


Figure S5. Liposomal formulations of combined therapeutics (STAT3siRNA & WP1066) show synergistic effects in inhibiting STAT3 activation in B16F10 cell. mRNA levels (**A**) and protein expressions (**B**) of indicated genes involved in STAT3 signaling pathway were measured by RT-PCR and Western blotting respectively. Lane 1, untreated cells; lane 2, cells treated with targeted liposomal WP1066; lane 3, cells treated with targeted liposome containing STAT3siRNA; lane 4, cells treated with targeted liposome containing both WP1066 & STAT3siRNA; lane 5, cells treated with targeted liposome containing scrambled siRNA and lane 6, cells treated with targeted liposome containing both WP1066 & scrambled siRNA. Data shown here are representative of two separate experiments.

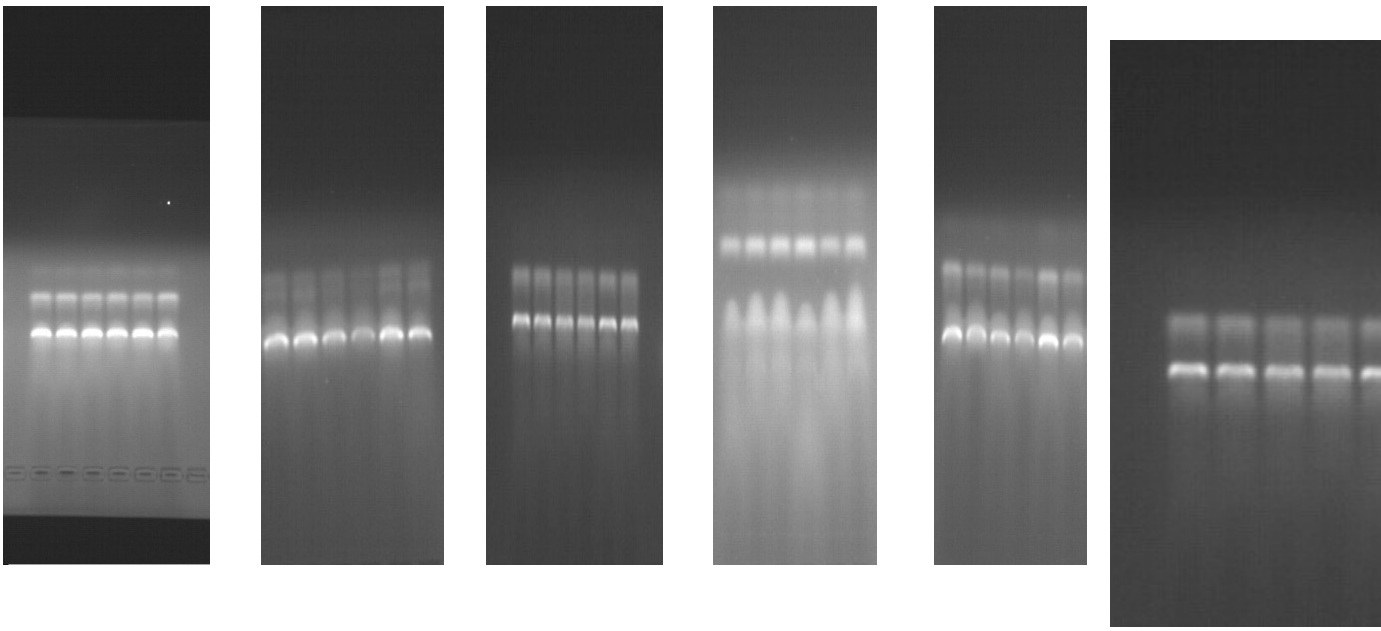


Figure S6. Original gel images used for drawing **Figure S5A**

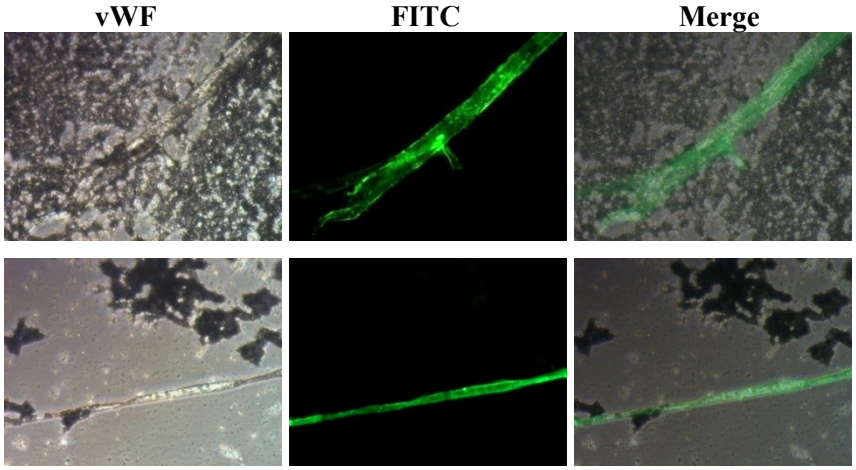


Figure S7. Liposomes of **CLP** can deliver liposomally encapsulated siRNA to tumor vasculatures. On day 18 after tumor inoculation, mice were intravenously injected with FITC labeled siRNA encapsulated in liposomes of **CLP**. Tumors were excised 24 h post injection and tumor cryosections were immunostained with anti-vWF antibody (markers of tumor endothelial cells). The stained slides were put under an inverted fluorescence microscope and the snaps were taken in bright field for blood vessel and in green field for FITC. All the images were taken at 10X magnification.

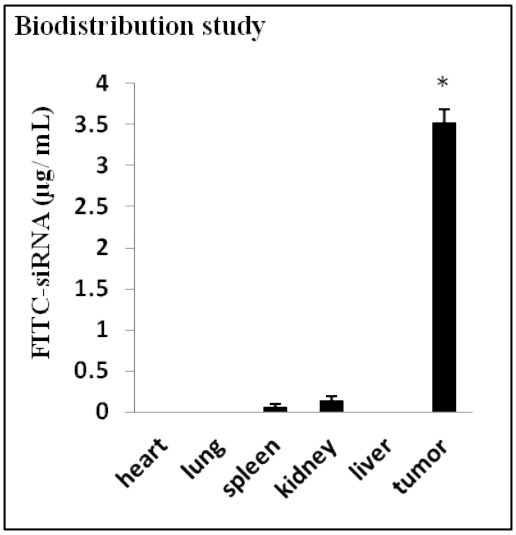
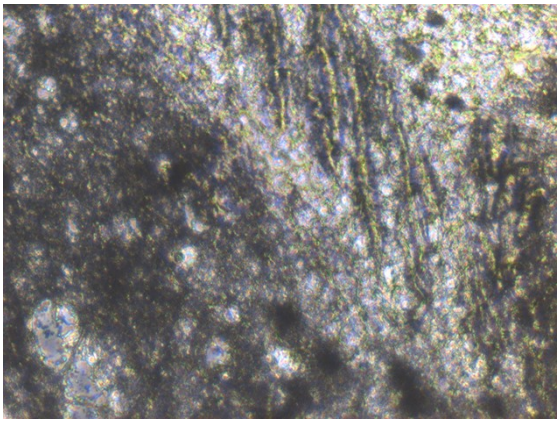
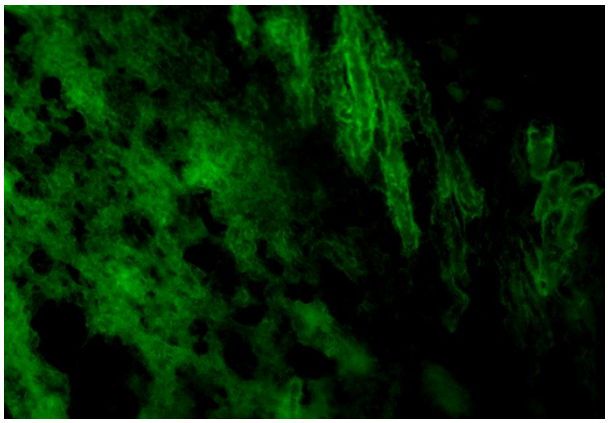


Figure S8. Liposomes of **CLP** selectively target liposomally encapsulated FITC-labeled siRNA to tumor sites under systemic settings. After 18 d of B16F10 tumor inoculation, when tumor volume reached ~1500 mm^3, mice were injected with a single dose of FITC labeled siRNA (6 µg/mice) encapsulated in liposomes of **CLP**. After 24 h, lung, liver, tumor, kidney, spleen, heart tissues were obtained. Tissue siRNA concentrations were quantified by fluorescence measurements. To correct for tissue autofluorescence, untreated control tissues were similarly extracted. For each sample, the background fluorescence was subtracted, and the remaining counts were converted to concentration of siRNA using standard graph of fluorescence vs FITC labeled siRNA concentration (*$P < 0.005$). The observed fluorescence intensities and autofluorescence values for each organ which were used in drawing **Figure S8** are provided below in the Excel Sheet **Table S2**, Supporting Information.

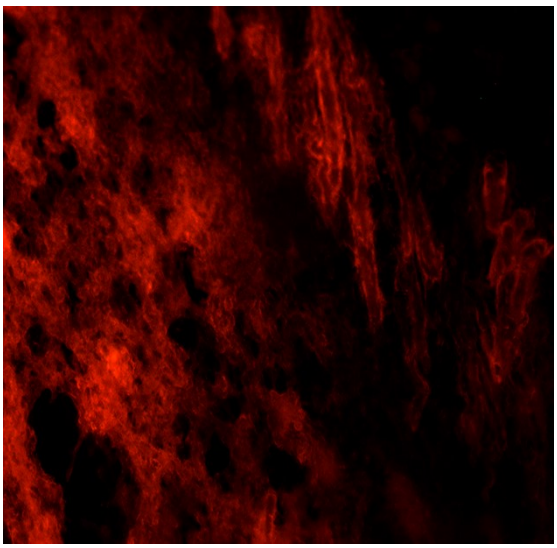
Figure S9. Original colored image of **Figure 4B** in the text.



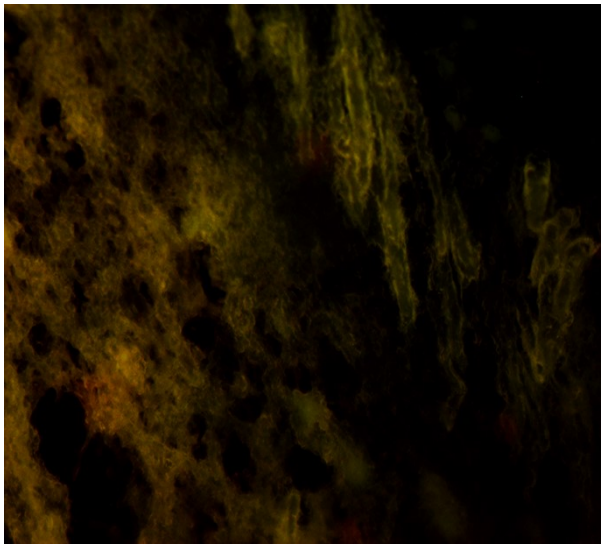
Bight field of tumor cryosection



TUNEL positive (green) cells



VE-Cadherin positive (red) cells



Merged image of TUNEL and VE-Cadherin positive cells

Figure S10. The original snap shots of **Figure 4C** in the text.

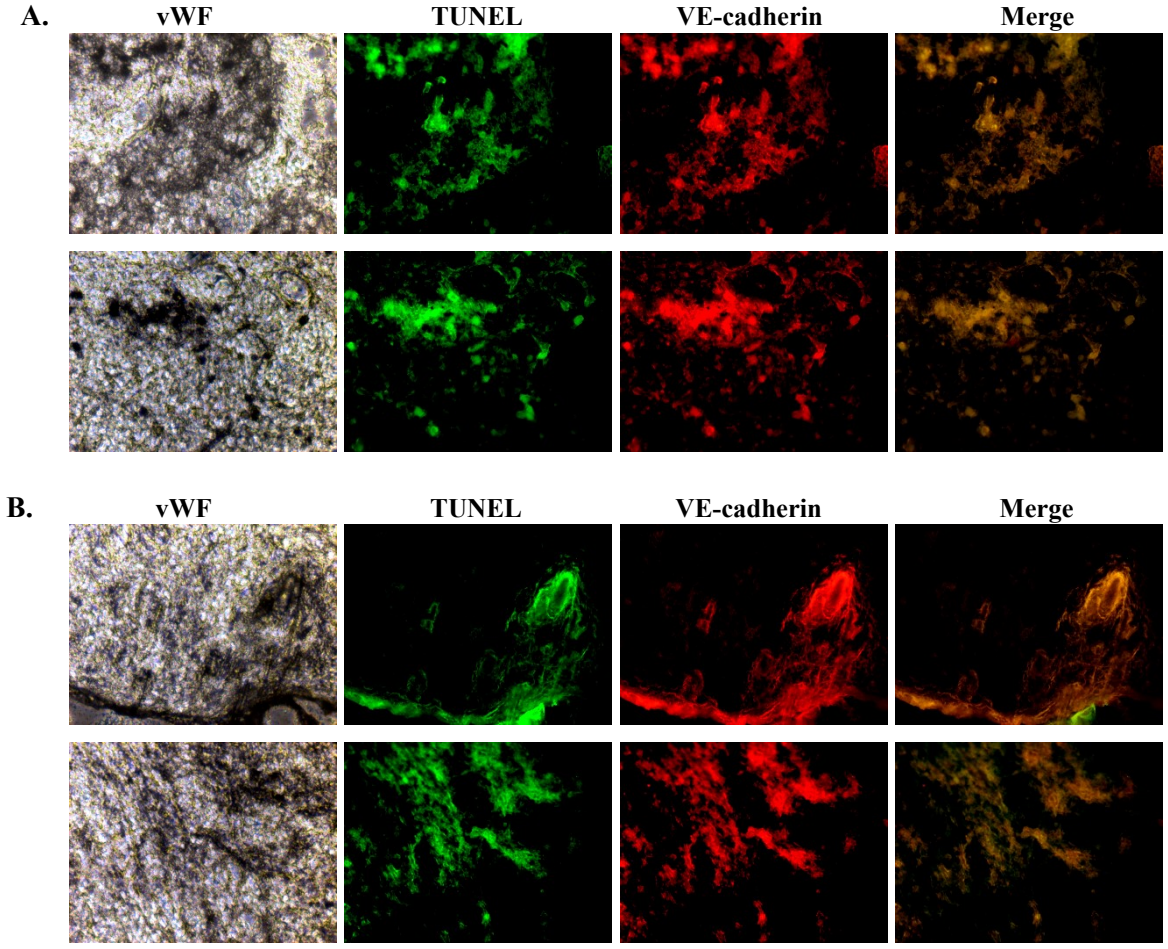


Figure S11. Tumor growth inhibition is mediated through apoptosis of tumor endothelial cells. **A.** Mice *i.v.* administered with tumor vasculature targeting liposomes of CGKRK-lipopeptide containing WP1066, **B.** Mice *i.v.* administered with tumor vasculature targeting liposomes of CGKRK-lipopeptide containing STAT3siRNA. On day 24 post tumor inoculation, the fixed tumor cryosections from each group were immunostained with anti-vWF & anti-VE-cadherin antibodies (markers of tumor endothelial cells) and TUNEL assay kit (markers for apoptotic cells, green). All the images were taken at 10X magnification.

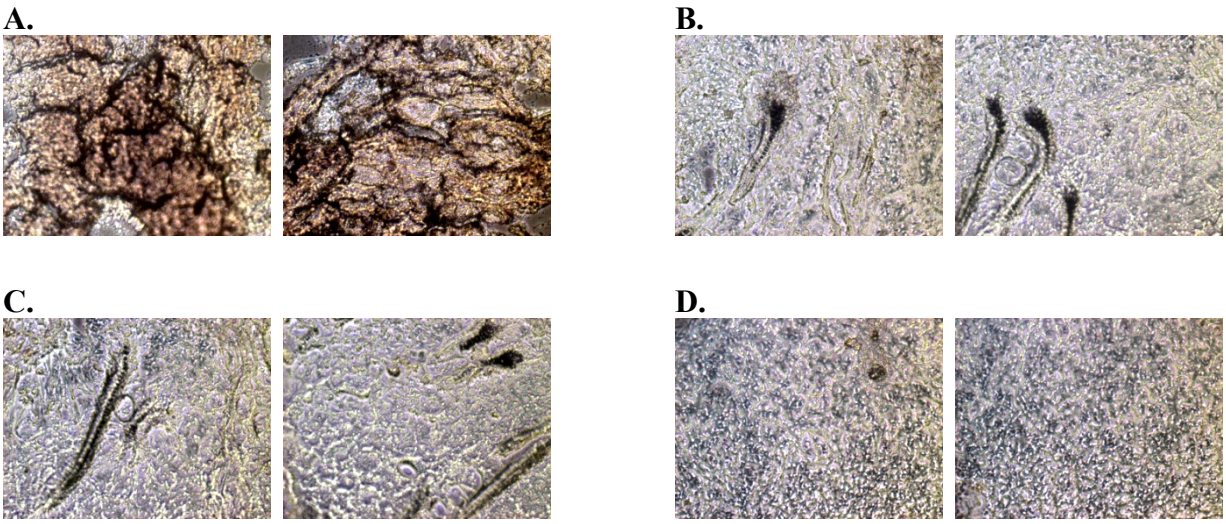


Figure S12. Microvessel densities around tumors get significantly reduced by *i.v.* administration of tumor vasculature targeting liposomal formulations of WP1066 & STAT3siRNA. B16F10 melanoma cell inoculated mice were randomly sorted into four groups (n = 5 for each group). **A.** mice *i.v.* injected with only vehicle (5% glucose); Mice *i.v.* administered with tumor-selective liposomes of CGKRK-lipopeptide containing: only WP1066 (**B**); only STAT3siRNA (**C**); both WP1066 and STAT3siRNA (**D**). On day 24 post tumor inoculation, the fixed tumor cryosections from each group were immunostained with polyclonal anti-CD31 antibody (a widely used marker for tumor microvasculature densities) as described in the text. The immunostained cryosections were then observed in bright fields at 20X magnification.

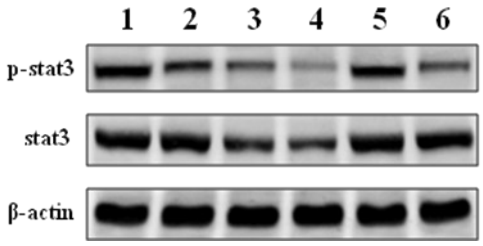


Figure S13. WP1066 & STAT3 siRNA co-encapsulated in liposomes of **CLP** show synergistic effects in inhibiting STAT3 phosphorylation *in vivo*. Both the treated and untreated melanoma tumor bearing mice were sacrificed, tumors excised and B16F10 cells were isolated from tumor. Isolated cells were lysed and processed for immunoblotting with antibodies against STAT3, *p*-STAT3 and β-actin (control). lane 1, untreated cells; lane 2, cells treated with targeted liposomal WP1066; lane 3, cells treated with targeted liposome containing STAT3siRNA; lane 4, cells treated with targeted liposome containing both WP1066 & STAT3siRNA; lane 5, cells treated with targeted liposome containing scrambled siRNA and lane 6, cells treated with targeted liposome containing both WP1066 & scrambled siRNA. Data shown here are representative of two separate experiments.

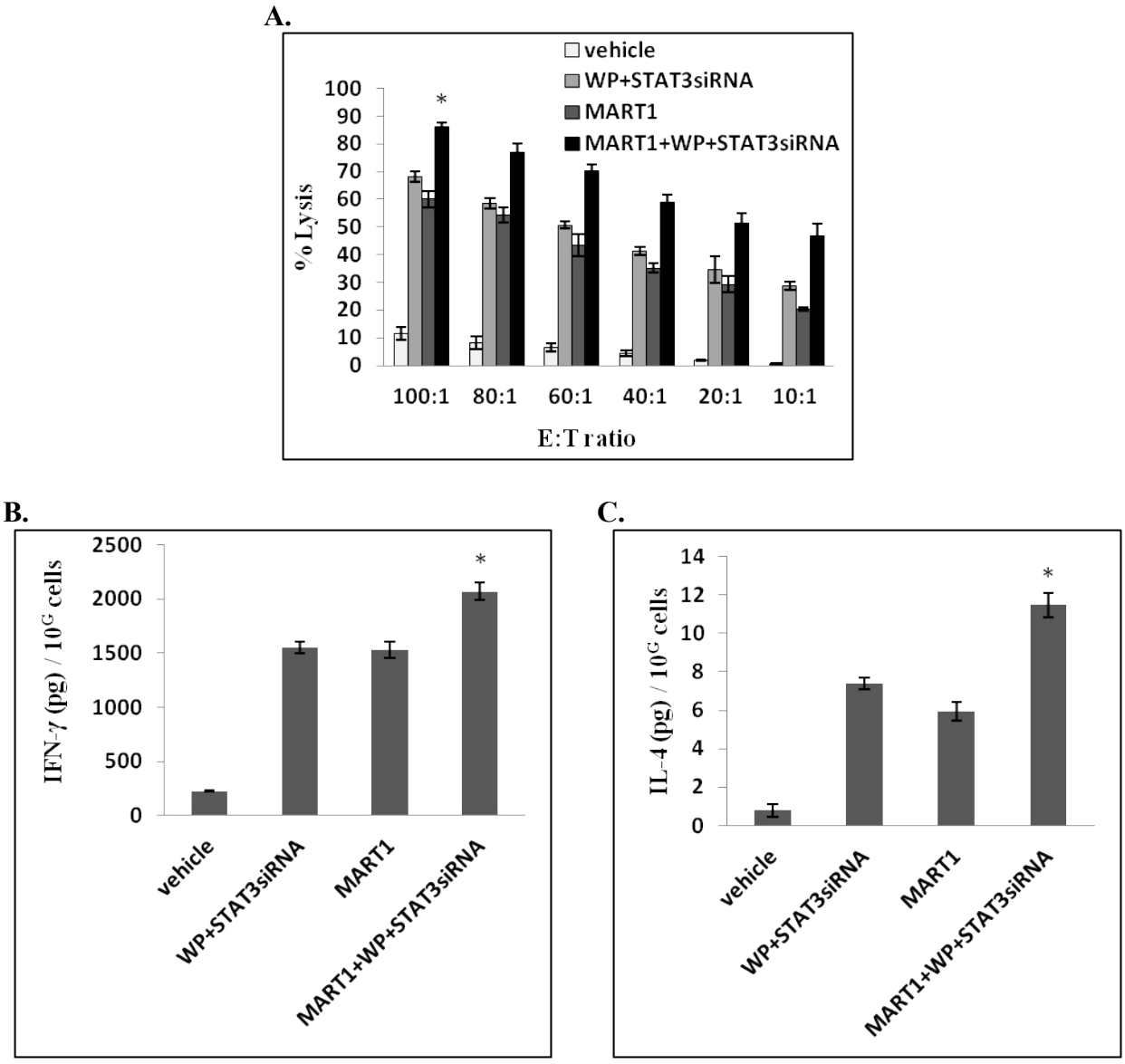
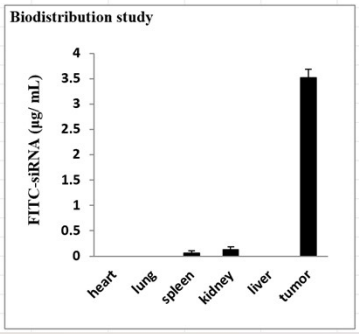


Figure S14. Long-lasting protection against melanoma is induced majorly by melanoma specific cellular immune response. **A.** Tumor bearing C57BL/6 mice were immunized (s.c) with lipoplexes of p-CMV-MART1 complexed with liposomes of **CL** on day 15, 17 and 19 after tumor inoculation. One week after the last immunization, splenocytes were collected and percentages of target melanoma cell lysis (CTL responses) were measured (*P < 0.005 versus vehicle). **B** & **C.** Degrees of IFN-γ & IL-4 secretion. One week post last immunization, splenocytes were collected and used after 3 days stimulation with target B16F10 cells. Amounts of IFN-γ & IL-4 released in the co-culture were determined by ELISA Reader. Data shown here are representative of two separate experiments (*P < 0.005 versus vehicle).

Table S2. The measured fluorescence and autofluorescence intensities for each organ used for drawing the biodistribution profile Figure S8.

24hrs	FITC	6ug	100 µl from the stock (FITC-siRNA 1 µg/mL) gives value ~1000												
			heart			lung			spleen						
cont		5086	5004		6095	6047		3635	3769						
16cg+FITC		4563	4698		5557	5347		3669	3859						
			kidney			tumor			liver						
cont		17287	17759		3069	2945		18526	17589						
16cg+FITC		17379	17930		6459	6585		17656	18251						
	heart	0		tumor	3390	3.39	3.515	0.176777		spleen	34	0.034	0.062	0.039598	
					3640	3.64					90	0.09			
	lung	0													
				kidney	92	0.092	0.1315	0.055861							
	liver	0			171	0.171									
			FITC-siRNA (µg/ mL)												
		heart	0	0											
		lung	0	0											
		spleen	0.062	0.039598											
		kidney	0.1315	0.055861											
		liver	0	0											
		tumor	3.515	0.176777											



16cg+FITC = Fluorescence intensities observed in each organ isolated from mice *i.v.* treated with liposomes of CGKRK-lipopeptide (**CLP**) containing encapsulated FITC-labeled siRNA;

Cont: Autofluorescence intensities for each organ of untreated control mice (which were subtracted from the corresponding measured values in the organ of treated mice in constructing **Figure S8**).

The replicate values shown in the spread sheet for each organ are the fluorescence intensities values measured in each mouse (n = 2) the averages of which were used for drawing the biodistribution profile **Figure S8**.