Supporting information for

Nanoparticle-Mediated Trapping of Wnt Family Member 5A in Tumor Microenvironments Enhances Immunotherapy for B-Raf Proto-Oncogene-Mutant Melanoma

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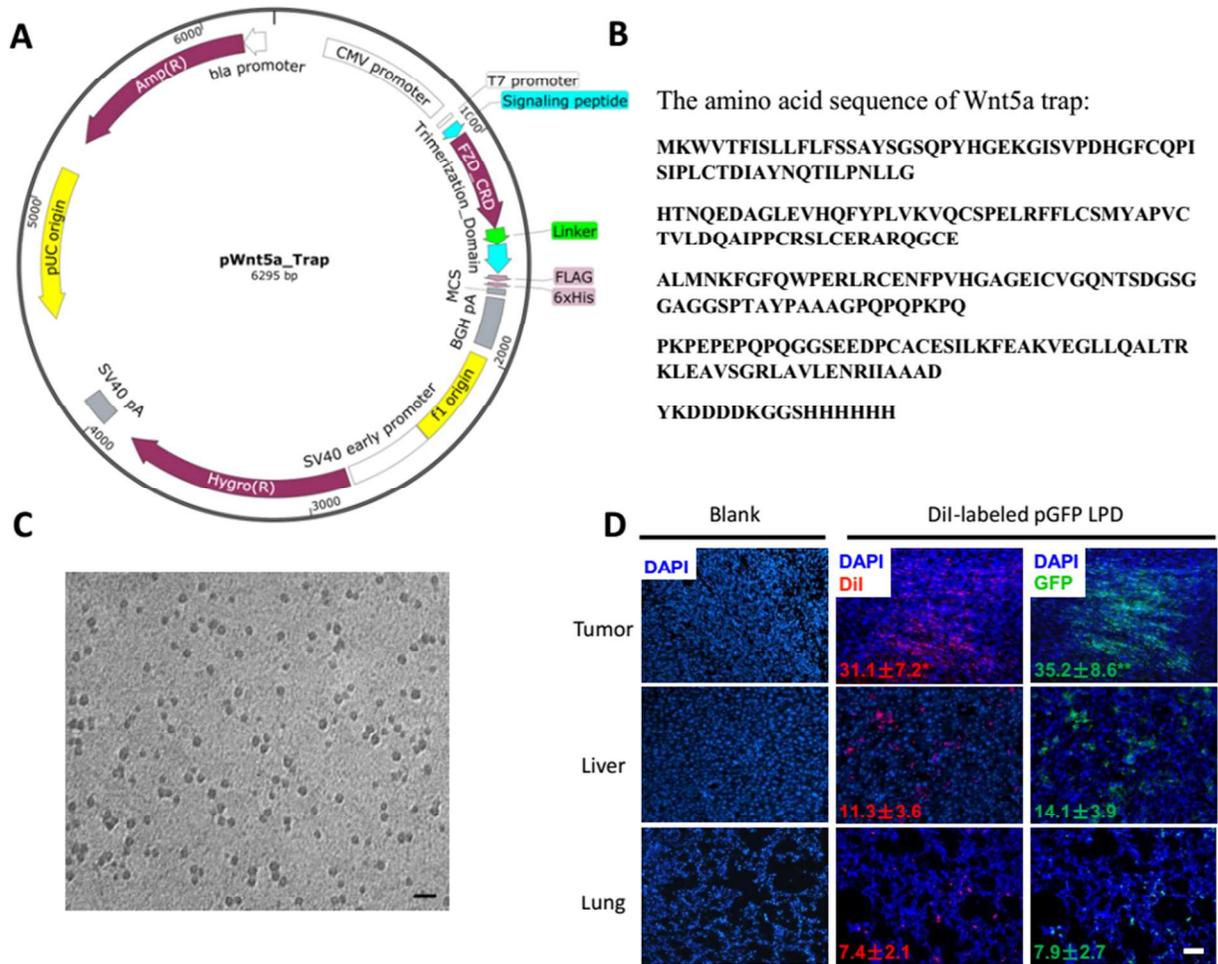[#]Contributed equally
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Supporting Information
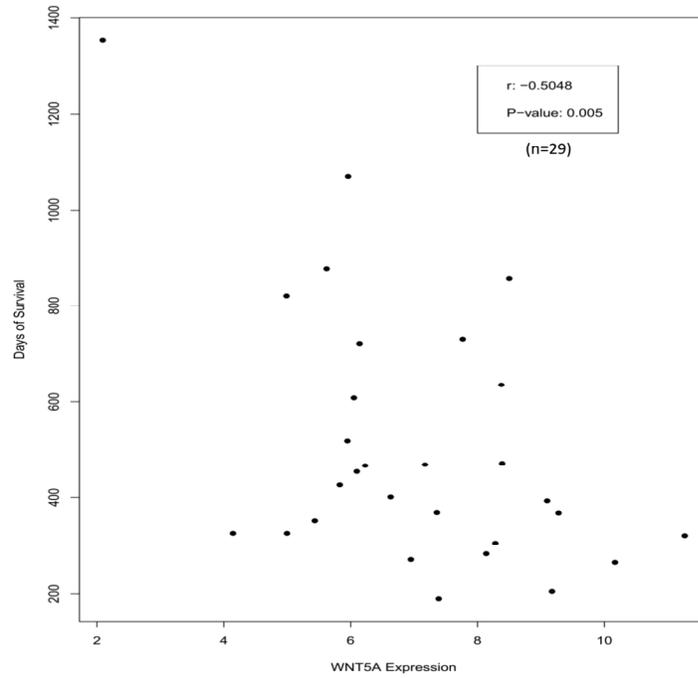
Figure S1. Wnt5a is a key molecule controlling the immunosuppressive desmoplastic TME.
Excessive Wnt5a correlates with poorer patient overall survival. n = 29. *: $p < 0.05$, **: $p < 0.01$.

A

pWnt5a_Trap
6295 bp

B

The amino acid sequence of Wnt5a trap:

MKWVTFISLLFLFSSAYSGSQPYHGEKGISVPDHGFCQPI
SIPLCTDIAYNQTILPNLLG

HTNQEDAGLEVHQFYPLVKVQCSPELRFFLCSMYAPVC
TVLDQAIPPCRSLCERARQGCE

ALMNKFGFQWPERLRCENFPVHGAGEICVGQNTSDGSG
GAGGSPTAYPAAAGPQPQPKPQ

PKPEPEPQPQGGSEEDPCACESILKFEAKVEGLLQALTR
KLEAVSGRLAVLENRIIAAAD

YKDDDDKGGSHHHHHH

C

D

Figure S2. Local distribution and expression of Wnt5a trapping. (A) The plasmid map for the Wnt5a trap. (B) The amino acid sequence of the monomeric version of Wnt5a trap. (C) TEM image of LPD NP (vector for encapsulating plasmid). Scale bar indicates 300 nm. (D) Fluorescence images of DiI distribution in tumor, liver and lung. Numbers indicate % cells taken up DiI (red) or expressed GFP (green). n = 3. Scale bar indicates 300 μm. *: $p < 0.05$, **: $p < 0.01$.

Size=104nm, PDI=0.195


Zeta potential=41.6mV
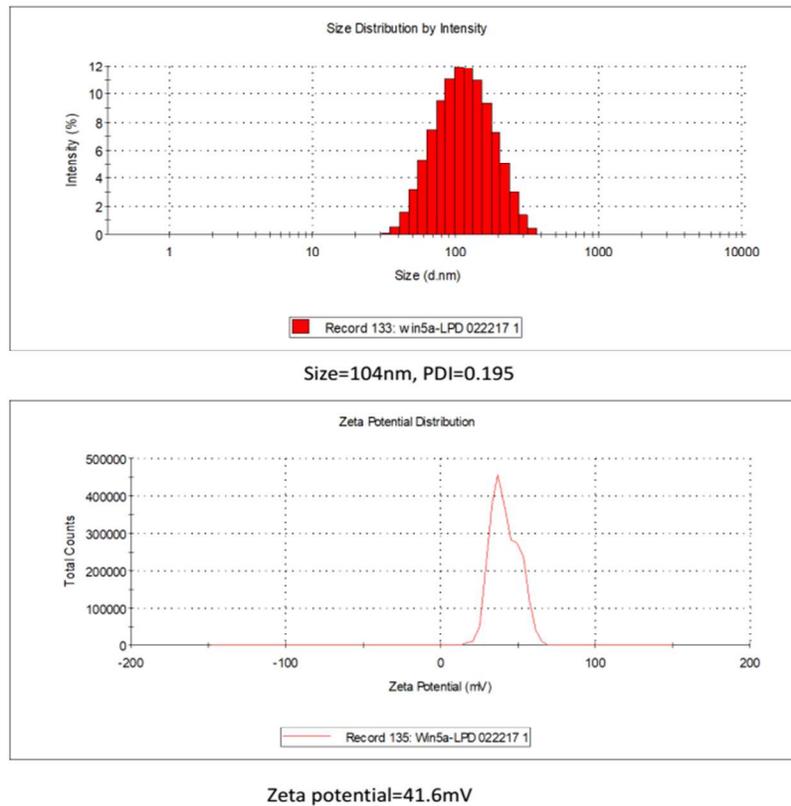
Figure S3. Size and zeta potential of the Wnt5a trap. DLS depicting size (104 nm), PDI (0.195) and Zeta potential (41.6 mV) of trap cDNA encapsulated LPD NPs.
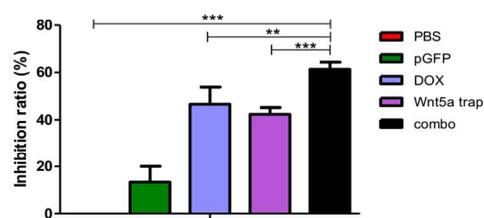

Figure S4. Combination therapy significantly inhibited tumor progression. Figure depicts tumor weight inhibition ratio on day 27 (tumor inhibition study endpoint). n = 8. **: $p < 0.01$, ***: $p < 0.001$.
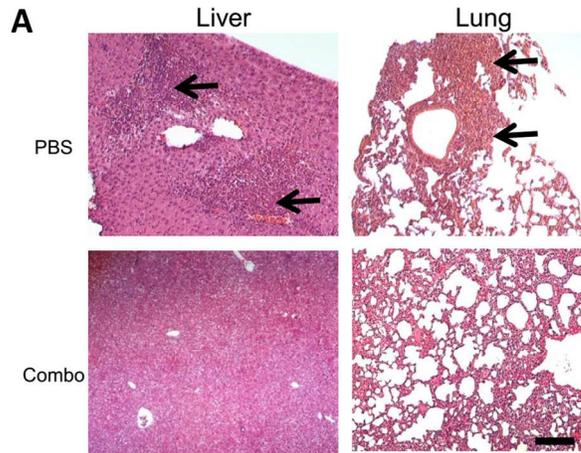
Figure S5. Combination therapy demonstrated long-lasting overall immune response. **(A)** H&E staining shows the histology of tumor metastasis in the major organs. Black arrows indicate metastasis loci. Scale bar indicates 300 μm.
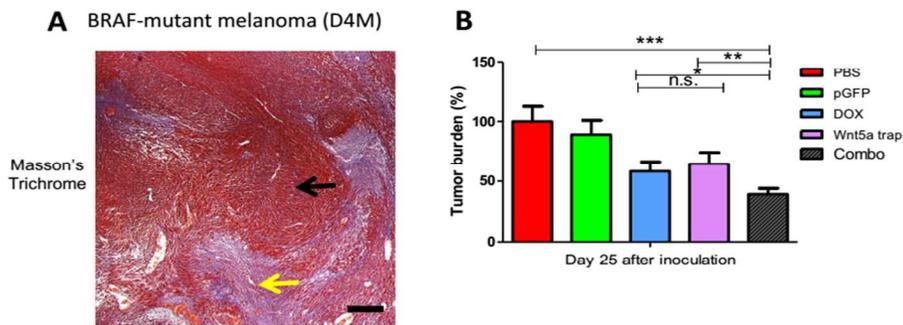
Figure S6. Second model of BRAF-mutant melanoma. (A) D4M cell line inoculated mice were used as the second BRAF-mutant model, where different treatments were applied accordingly. Masson's trichrome staining depicting collagen morphology in untreated tumor. Black arrow indicates tumor nest, yellow arrow indicates surrounding stromal region. Scale bar indicates 300 μm. **(B)** Effectiveness of therapies were compared by monitoring tumor growth every 2-3 days, on day 25 after inoculation, tumor burden between groups were compared and quantified. n = 5, n.s.: $p > 0.05$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.
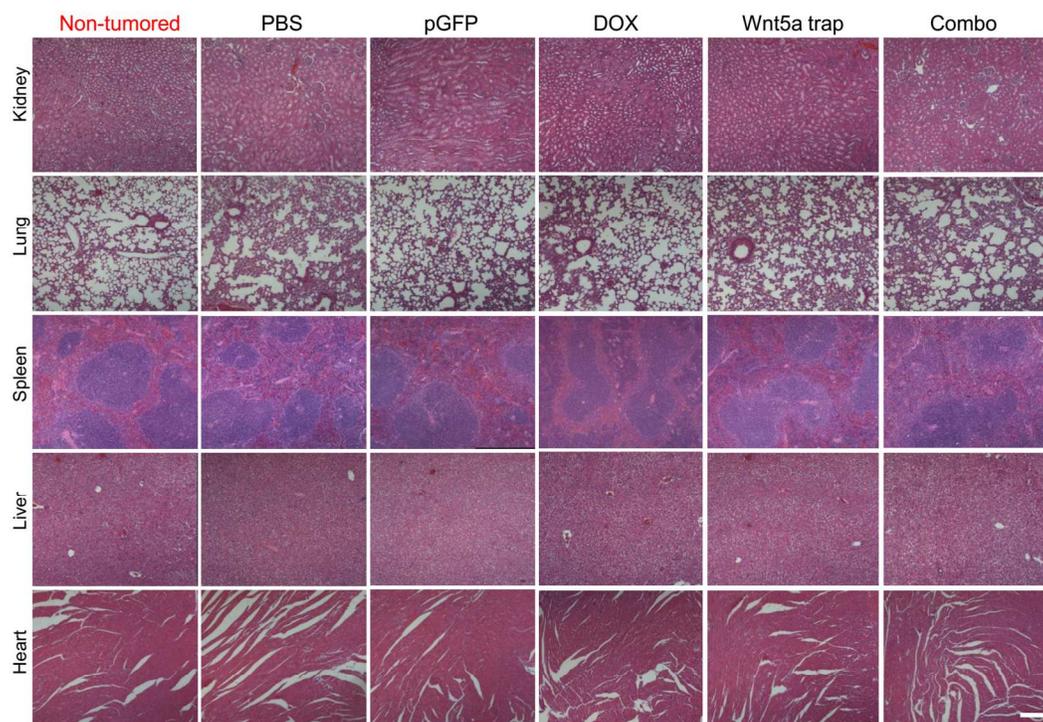
Figure S7. H&E morphology under different therapies. At the endpoint of tumor inhibition study, mice were humanely euthanized, major organs were collected, sectioned, and stained for H&E analysis. Non-tumored mice were also examined as control. Scale bar indicates 300 μm.

Table S1. Antibody list

Antibodies	Company	Catalog	Application
Anti-CD8 (PE-conjugated)	BD	553032	flow cyt, IF
Anti-CD4 (FITC-conjugated)	BD	561828	flow cyt, IF
Anti-CD11b (FITC-conjugated)	BD	553310	flow cyt
Anti-Gr1 (Ly-6G and Ly-6C) (PE-conjugated)	BD Pharmingen^{TM}	553128	flow cyt
Anti-CD45 (FITC-conjugated)	BD Pharmingen^{TM}	553080	flow cyt
Anti-CD62L (FITC-conjugated)	BD Pharmingen^{TM}	553150	flow cyt, IF
Anti-CD44 (FITC-conjugated)	BD Pharmingen^{TM}	553133	IF
Anti-CD103 (PE-conjugated)	Biolegend	121406	flow cyt
Hoechst 33342	ThermoFisher	H1399	IF
ProLong™ Diamond Antifade Mountant with DAPI	ThermoFisher	P36971	IF
Anti-CD206 (FITC-conjugated)	Biolegend	141704	flow cyt
Anti-F4/80 (FITC-conjugated)	BD Pharmingen^{TM}	565410	flow cyt
Anti-CD274 (PE-conjugated)	BD Pharmingen^{TM}	558091	flow cyt
Anti-CD11c (FITC-conjugated)	BD Pharmingen^{TM}	557400	flow cyt
Anti-CD69 (FITC-conjugated)	BD Pharmingen^{TM}	553236	flow cyt
Anti-MHCII (PE-conjugated)	BD Pharmingen^{TM}	553570	flow cyt
Anti-Wnt5a	Abcam	Ab72583	WB, IF
Anti-CRT	Abcam	Ab2907	IF
Anti-HMGB1	Abcam	Ab18256	IF
GAPDH	Santa Cruz	I3015	WB

Table S2. Primer list for real-time PCR

Antibodies	Applied Biosystems/Ref
Mouse IFN-γ	Mm01168134_m1
Mouse IL12α	Mm00434169_m1
Mouse TNF-α	Mm00443260_g1
Mouse TGF-β	Mm01178820_m1
Mouse CCL2	Mm00441242_m1
Mouse GAPDH	Mm99999915_g1