Supplementary Figures

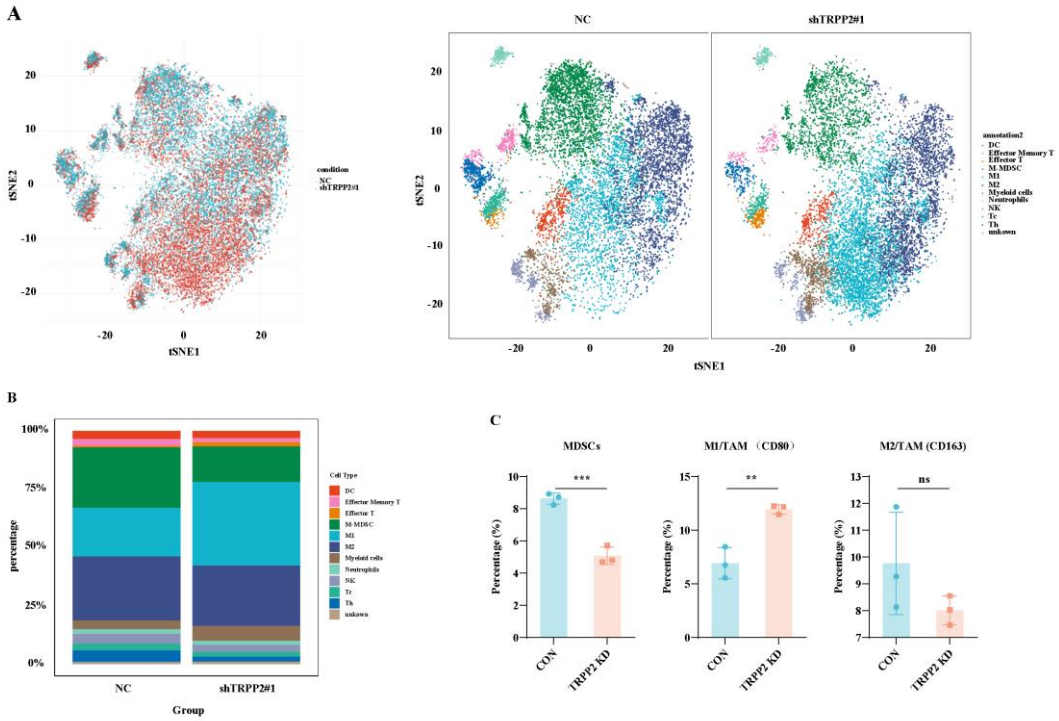


Fig.S1 FC analysis of the distribution of various immune cell subsets in HNC tumors following TRPP2 knockdown. (A-B) FC analysis outlining the distribution and percentage changes of major immune cell populations in HNC tumor tissues following TRPP2 knockdown. **(C)** Tumor-infiltrating MDSCs (Gr-1$^+$MHCII$^+$), M1-type TAMs (CD11b$^+$F4/80$^+$CD80$^+$), and M2-type TAMs (CD11b$^+$F4/80$^+$CD163$^+$) were quantified in control and TRPP2-knockdown groups by FC. ***P*<0.01 and ****P*<0.001. ns, not significant.

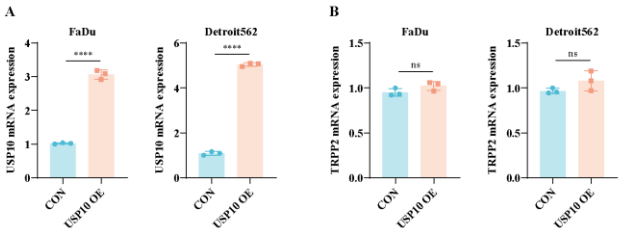


Fig.S2 USP10 overexpression does not affect TRPP2 mRNA expression. RT-qPCR analysis of USP10 (A) and TRPP2 (B) mRNA levels in HNC cells overexpressing USP10 or vector control. *****P*<0.0001. ns, not significant.

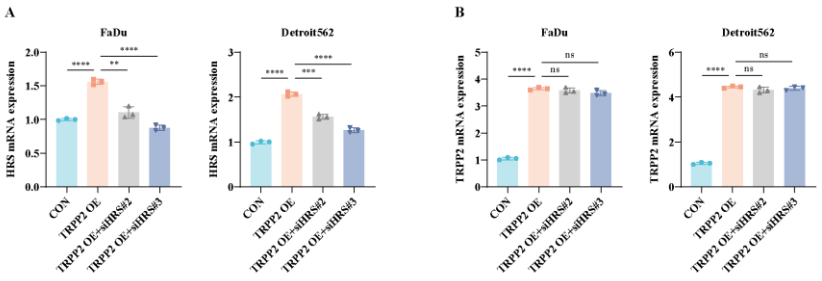


Fig.S3 HRS knockdown does not affect TRPP2 mRNA expression. RT-qPCR analysis of HRS (A) and TRPP2 (B) mRNA levels in TRPP2-overexpressing HNC cells transfected with siHRS or siCON. ***P*<0.01, ****P*<0.001 and *****P*<0.0001. ns, not significant.

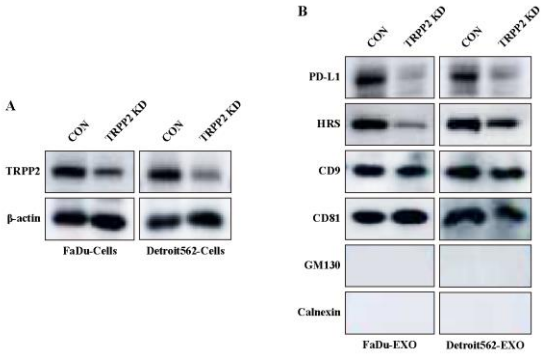


Fig.S4 TRPP2 knockdown reduces PD-L1 and HRS levels in exosomes. (A) Western blotting analysis of TRPP2 expression in cell lysates from control and TRPP2-knockdown HNC cells. **(B)** Western blotting analysis of exosomal PD-L1, HRS, CD9, CD81, GM130, and Calnexin in exosomes isolated from control and TRPP2-knockdown cells.

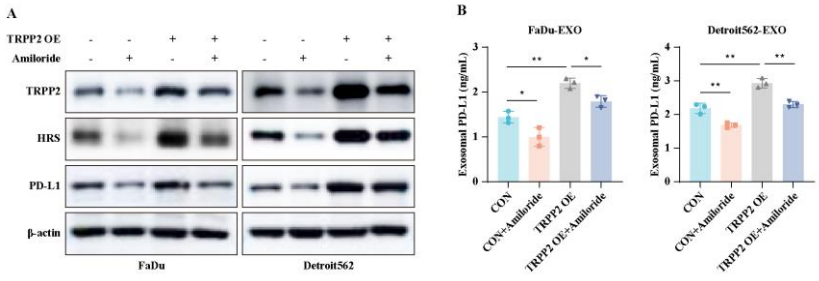


Fig.S5 TRPP2 channel inhibition reduces PD-L1 and HRS expression and exosomal PD-L1 secretion. (A) Western blotting analysis of TRPP2, HRS, and PD-L1 protein levels in HNC cells treated with amiloride (100 μM) for 48 h. **(B)** ELISA quantification of exosomal PD-L1 levels from the same treatment groups.

*P<0.05 and **P<0.01.

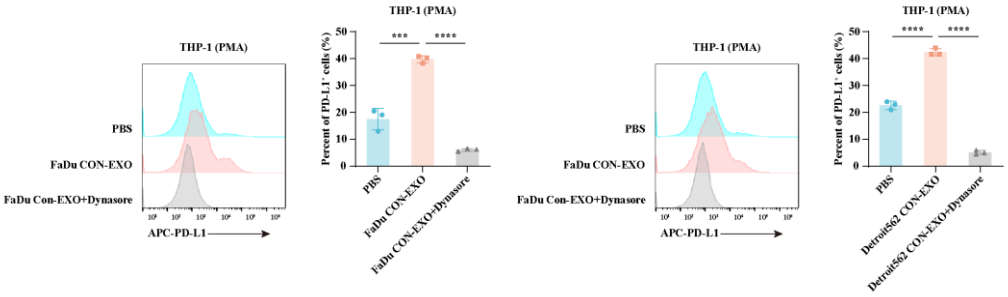


Fig.S6 Phagocytosis is required for exosomal PD-L1 transfer to recipient macrophages. FC analysis of surface PD-L1 expression on macrophages after co-incubation with exosomes in the presence of Dynasore (80 μM). ***P<0.001 and ****P<0.0001.

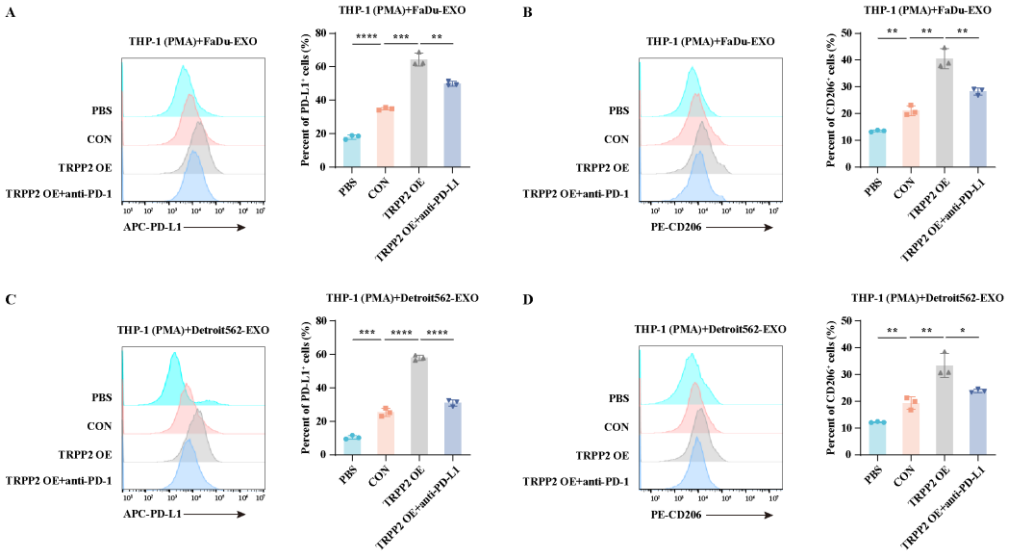


Fig.S7 Neutralization of exosomal PD-L1 attenuates macrophage M2 polarization. (A) FC analysis of PD-L1$^+$ macrophage populations after treatment with TRPP2-overexpressing cell-derived exosomes in the presence of anti-PD-L1 neutralizing antibody (10 μg/mL). **(B)** FC analysis of CD206$^+$ macrophage populations under the same conditions. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001.

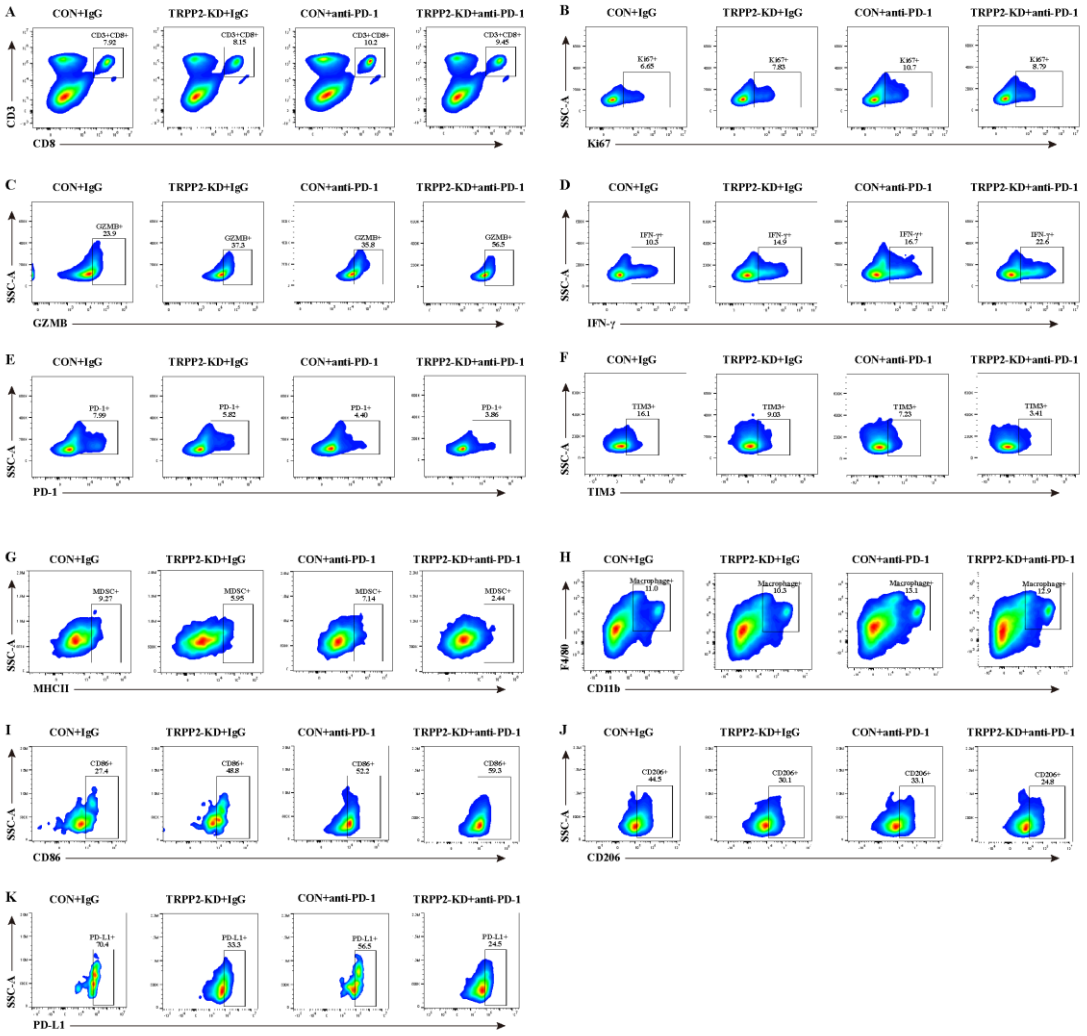


Fig.S8 Combined treatment of TRPP2 knockdown and anti-PD-1 mAb therapy reduces PD-L1-positive TAMs infiltration and inhibits CD8$^+$T cell function in HNC. (A-F) Representative FC data depict the infiltration levels of CD8$^+$ T cells (CD3$^+$CD8$^+$) in mouse tumor tissue after combined treatment, including their proliferation (CD8$^+$Ki67$^+$), effector cytokine expression (CD8$^+$GZMB$^+$, CD8$^+$IFN-γ$^+$), and inhibitory receptor expression (CD8$^+$PD-1$^+$, CD8$^+$TIM-3$^+$). (G-K) FC profiles show the infiltration levels of MDSCs (Gr1$^+$MHCII$^+$), TAMs (GR-1$^-$CD11b$^+$F4/80$^+$), M1-type TAMs (CD11b$^+$F4/80$^+$CD86$^+$), M2-type TAMs (CD11b$^+$F4/80$^+$CD206$^+$), and PD-L1$^+$TAMs (CD11b$^+$F4/80$^+$CD206$^+$PD-L1$^+$) in mouse tumor tissue following the combined treatment of TRPP2 knockdown and anti-PD-1 mAb therapy.

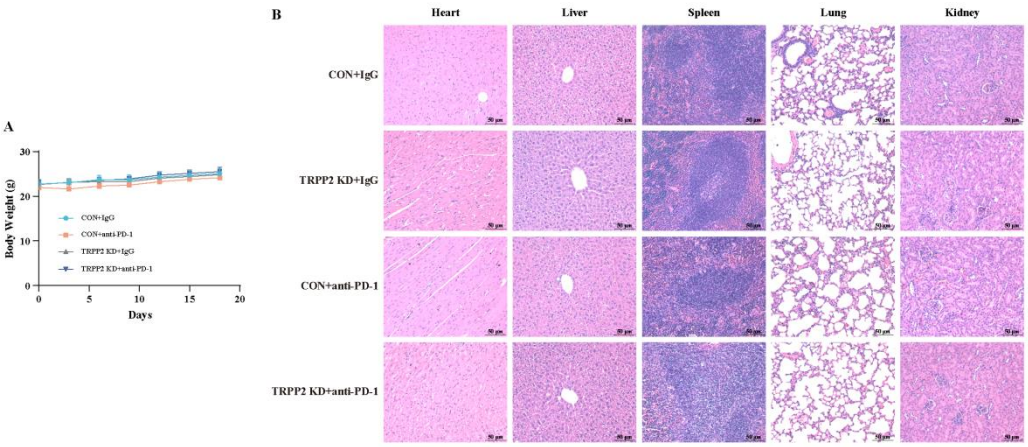


Fig.S9 Biosafety evaluation of combination therapy *in vivo*. (A) Body weight curves of mice in each treatment group measured every 3 days throughout the treatment course. **(B)** Biosafety evaluation via H&E staining of major organs (heart, liver, spleen, lung, kidney) showing no significant morphological toxicity across treatment groups.

Supplementary Tables

Table S1. The sequences of siRNAs.

Sequence Name	Sequence（5' to 3'）
siCON	UUCUCCGAACGUGUCACGUTT
HRS (siRNA#1)	ACAGGAAAGCGGAGGGAAATT
HRS (siRNA#2)	AGAGACAAGUGGAGGUAAATT
HRS (siRNA#3)	AGUCAGAGGCGGAGGAGAATT

Table S2. Antibody used in this work.

Antibody name	Catalog number	Manufacturer
TRPP2	ab51250	Abcam
TRPP2	sc-374344	Santa cruz
PD-L1	13684	CST
PD-L1	Ab205921	Abcam
PD-L1	66248	Proteintech
PD-L1	MABC1115	Sigma

APC-PD-L1	329708	Biolegend
PE-CD206	321105	Biolegend
FITC-CD9	987206	Biolegend
FITC-CD63	353007	Biolegend
IgG	30000	Proteintech
β-actin	CW0096	CWBIO
CD9	AF5139	Affinity
CD81	DF2306	Affinity
HRS	15087	CST
Ki67	27309	Proteintech
Ubiquitin	80992	Proteintech
HA-Tag	M20003	Abmart
Flag-Tag	M20008	Abmart
USP10	19374	Proteintech
LC3	14600	Proteintech
CD206	86163	CST
Arg-1	11285	Service
iNOS	2226	Proteintech
EEA1	sc-53939	Santa cruz
TFRC	sc-393719	Santa cruz
RAB7	sc-376362	Santa cruz
RAB11	2413	CST
LAMP1	sc-20011	Santa cruz
Goat anti-mouse	2305	ZSGB-BIO
Goat anti-rabbit	2301	ZSGB-BIO
BD Horizon™ Fixable Viability Stain 700	564997	BD Bioscience
BD Pharmingen™ APC-Cy™7 Rat Anti-Mouse CD45	561037	BD Bioscience
BD Horizon™ RB705 Hamster Anti-Mouse CD3e	570648	BD Bioscience
BD Horizon™ BB515 Rat Anti-Mouse CD8a	564422	BD Bioscience

BD Pharmingen™ PE Rat Anti-Mouse IFN-γ	562020	BD Bioscience
PE/Cyanine7 anti-human/mouse Granzyme B	396409	Biolegend
BD Horizon™ BV421 Hamster Anti-Mouse PD-1	562584	BD Bioscience
BD Horizon™ BV650 Mouse Anti-Ki-67	563757	BD Bioscience
BD Pharmingen™ APC Rat Anti-Mouse TIM3	568797	BD Bioscience
BD OptiBuild™ RY586 Rat Anti-Mouse F4/80	753430	BD Bioscience
BD Pharmingen™ FITC Rat Anti-CD11b	561688	BD Bioscience
BD OptiBuild™ RB705 Rat Anti-Mouse PD-L1	758168	BD Bioscience
BD Pharmingen™ APC Rat Anti-Mouse CD206	568809	BD Bioscience
BD Pharmingen™ PE-Cy™7 Rat Anti-Mouse CD86	560582	BD Bioscience
BD OptiBuild™ RY610 Rat Anti-Mouse GR-1	759333	BD Bioscience
MO MHC Class II BV650	406-5321-80	eBioscience

Table S3. qRT-PCR primer sequences.

Primer name	**Primer sequences（5' to 3'）**
PD-L1-F	TGGCATTTGCTGAACGCATTT
PD-L1-R	TGCAGCCAGGTCTAATTGTTTT
HRS-F	CTCCTGTTGGAGACAGATTGGG
HRS-R	GTGTGGGTTCTTGTCGTTGAC
STAM-F	CGTGTCCTTGGCTATCACTGA
STAM-R	GGCTGGTGTAGATATTGCAGAAG
USP10-F	ATTGAGTTTGGTGTCGATGAAGT
USP10-R	GGAGCCATAGCTTGCTTCTTTAG
PKD2-F	CTCTGGGGAACAAGACTCATGG
PKD2-R	TCATCATGCCGTAGGTCAAGA