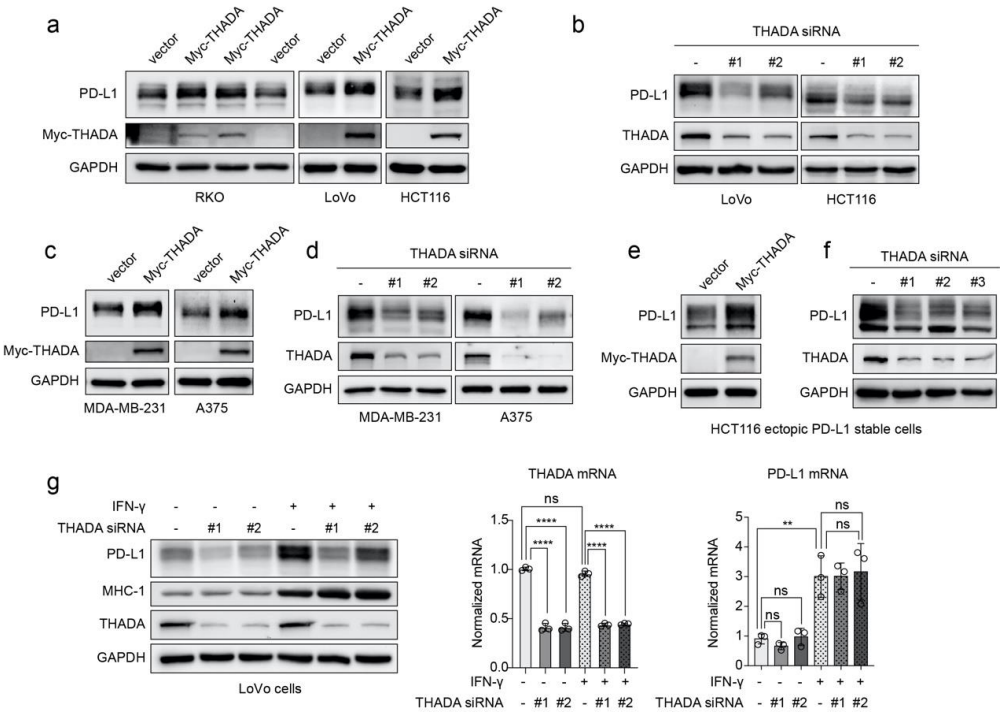
Supplementary Information

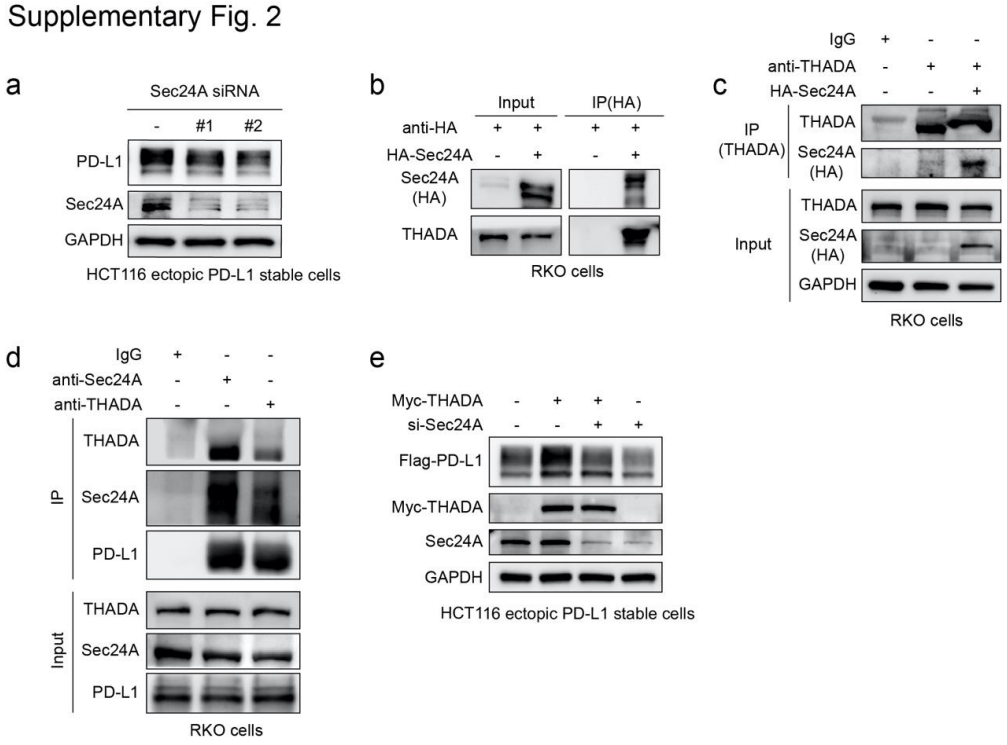
Supplementary Figures

Supplementary Fig.1



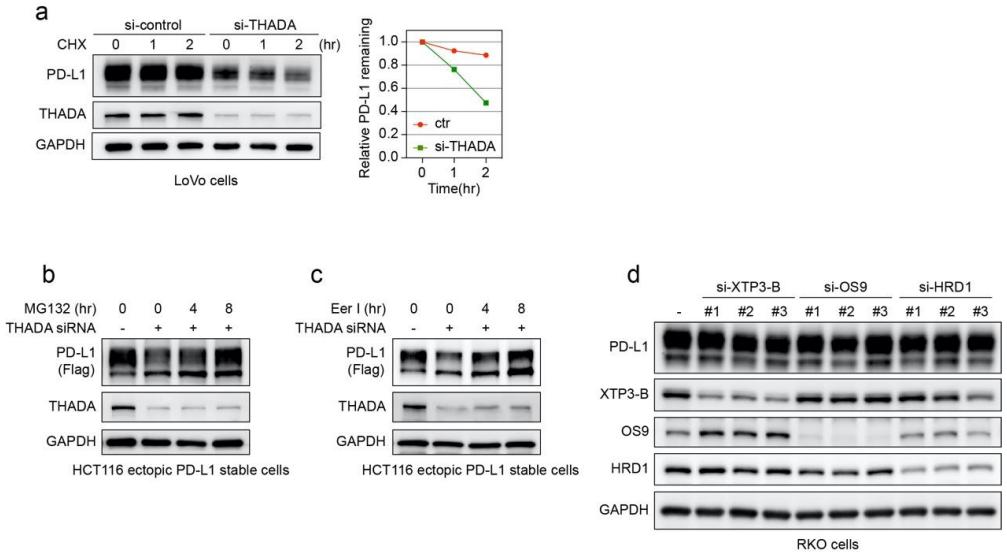
Supplementary Fig.1 THADA positively regulated PD-L1. a-**d**, Western blot showing the effect of THADA overexpression (**a**,**c**) and depletion (**b**,**d**), respectively, on PD-L1 expression in the indicated cells. The experiments were repeated three times independently with similar results. **e**,**f**, Western blot showing the effect of THADA overexpression (**e**) and depletion (**f**) on exogenous PD-L1 expression in HCT116 ectopic PD-L1 stable cells. The experiments were repeated three times independently with similar results. **g**, LoVo cells transfected with two distinct THADA siRNAs and co-incubated with IFN-γ (100ng/mL, 24h), subjected to western blot (left) and RT-PCR (right). Values are means ± s.d. from n = 3 independent experiments. Statistical differences were evaluated by ANOVA post-hoc test (Tukey). **, P<0.002; ****, P<0.0001; ns, no significance.

Supplementary Fig. 2

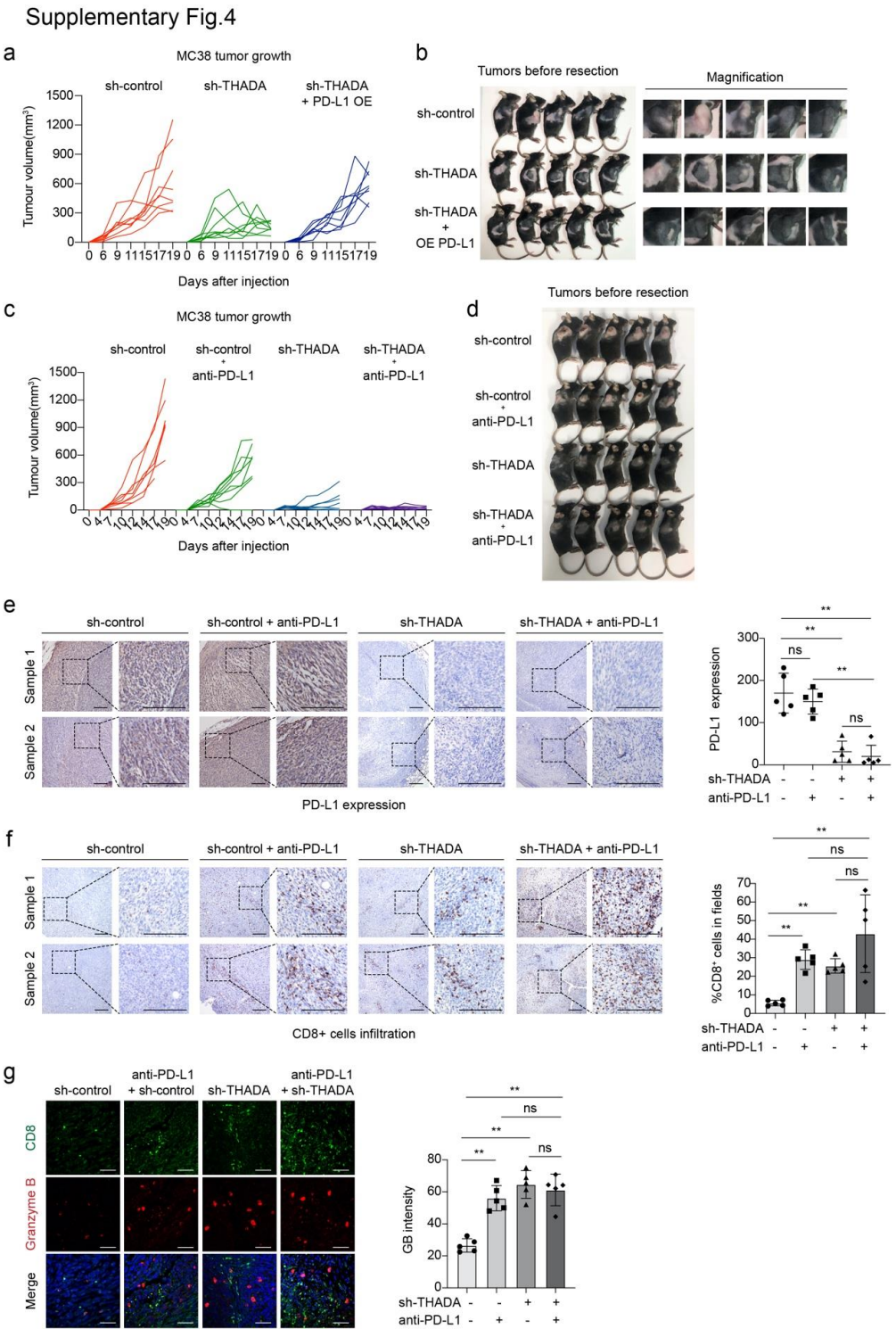


Supplementary Fig. 2 THADA required for Sec24A-dependent vesicle trafficking of PD-L1. a, Western blot showing the effect of Sec24A depletion on exogenous PD-L1 expression in HCT116 ectopic PD-L1 stable cells. **b**,**c**, Co-IP assays showing the interaction between THADA and Sec24A in RKO cells. **d**, Co-IP assay showing the interactions among THADA, Sec24A and PD-L1 in RKO cells. **e**, Overexpression of Myc-tagged THADA and depletion of Sec24A as indicated in denoted cells and subjected to western blot with indicated antibodies.

Supplementary Fig.3



Supplementary Fig.3 THADA depletion induced ER-associated degradation of PD-L1. a, Left, LoVo cells transfected with THADA siRNAs and co-incubated with cycloheximide (CHX) (50μg/mL) for denoted time points, and subjected to western blot with indicated antibodies. Right, quantification of gray value of remained PD-L1. The experiment was repeated three times independently with similar results. **b**,**c**, Western blot showing the effect of THADA depletion on PD-L1 expression in the absence or presence of proteasomal inhibitor MG132 (10μM) and ERAD inhibitor Eer I (10μM). **d**, Western blot evaluating PD-L1 expression transfected with XTP3-B, HRD1 and OS9 siRNAs as denoted. The experiment was repeated three times independently with similar results.



Supplementary Fig.4 Targeting THADA improved PD-L1-dependent tumor immune response in vivo. a, Tumor growth rate of each individual inoculated with indicated MC38 stable clones (n=8 per group). **b**, Representative individuals bearing

tumors before resection and the magnified images of tumors on mice. **c**, Tumor growth rate of each individual that received indicated treatment after inoculation of indicated MC38 stable clones (n=8 per group). **d**, Representative individuals bearing tumors before resection. **e**, Left, immunohistochemistry assay showing mouse PD-L1 expression in indicated tumor tissues. Dashed boxes denote the representative fields to be magnified. Scale bars, 200μm. Right, statistical result for PD-L1 expression assessed using H score. Values are means ± s.d. from five independent samples from each group. Statistical differences were evaluated by ANOVA post-hoc test. **, $P < 0.05$. ns, no significance. **f**, Left, immunohistochemistry assay showing CD8+ cell infiltration in indicated tumor tissues. Dashed boxes denote the representative fields to be magnified. Scale bars, 200μm. Right, quantification of CD8+ cell infiltration in indicated tumor tissues. Values are means ± s.d. from five independent samples from each group. The P value was determined by ANOVA post-hoc test. **, $P < 0.05$. ns, no significance. **g**, Immunofluorescence assays showing CD8+ T cell infiltration and granzyme B release in indicated tumor tissues. Scale bars, 50μm. Values are means ± s.d. from five independent samples of each group. Statistical differences were evaluated by ANOVA post-hoc test. **, $P < 0.05$. ns, no significance.

Supplementary Table

Supplementary Table 1. Sequences of siRNAs used in this paper.

Name	Sense	Antisense
si-THADA#1	GCGAAUAGCUAGAGCUCAUTT	AUGAGCUCUAGCUAUUCGCTT
si-THADA#2	GCAGUGAUCCUUCAUCUAATT	UUAGAUGAAGGAUCACUGCTT
si-THADA#3	GCACAGAAAUUGUUUCCAUTT	AUGGAAACAAUUUCUGUGCTT
si-Sec24A#1	GCCAGAGUUUGUUAGACAATT	UUGUCUAACAAACUCUGGCTT
si-Sec24A#2	GCUGAUGUUCAAGCAAUUUTT	AAAUUGCUUGAACAUCAGCTT
si-OS9#1	GCGGAUUUGAUUCGAUUCATT	UGAAUCGAAUCAAAUCCGCTT

si-OS9#2	CGACCAAGGAUGACAGUAATT	UUACUGUCAUCCUUGGUCGTT
si-OS9#3	GGUCCAAGUGCGACCUUAATT	UUAAGGUCGCACUUGGACCTT
si-XTP3-B#1	GCAGUUGUUCCUACAGAAUTT	AUUCUGUAGGAACAACUGCTT
si-XTP3-B#2	GGAAUAUGUUGGCCAAGAATT	UUCUUGGCCAACAUAUUCCTT
si-XTP3-B#3	GGACAACCCACAUAUCCAATT	UUGGAUAUGUGGGUUGUCCTT
si-HRD1#1	GCUCUUUCACUGCCGCAUUTT	AAUGCGGCAGUGAAAGAGCTT
si-HRD1#2	GCAUGGCAGUCCUGUACAUTT	AUGUACAGGACUGCCAUGCTT
si-HRD1#3	UCAUCUGCCGAGAAGAGAUTT	AUCUCUUCUCGGCAGAUGATT
Negative control	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT

Supplementary Table 2. Sequences of lentiviral shRNA used in this paper.

Name	Sequence
shTHADA#1	GCAGCCTTCTGTCACTTTACA
shTHADA#2	GCTGGAAAGGAACACATTAGT

Supplementary Table 3. DNA primer sequences for quantitative real-time PCR.

Name	Coding	Anticoding
PD-L1 (human)	TGGCATTTGCTGAACGCATTT	TGCAGCCAGGTCTAATTGTTTT
PD-L1 (mouse)	GACGCAGGCGTTTACTGCT	GCGGTATGGGGCATTGACTTT
GAPDH	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG

ACTB (mouse)	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
THADA (human)	TCACGGATGGAGTGTCACAAA	TGAATAGTGGGATCACACATGC
THADA (mouse)	CCCTGGCATGTTCCTCTTACT	GCGACCACACCTGATAATGAA
Sec24A (human)	CTACACCAATGCCTTCTA	GCTGTGGATGGATAGTTA