Table. S1. Antibodies used in this study

Antigen	Cat #	Manufacturer	Dilution
PD-L1	17952-1-AP	Proteintech	1:100(IP), 1:100 (IHC)
PD-L1	66248-1-Ig	Proteintech	1:1000 (WB)
ENO1	11204-1-AP	Proteintech	1:1000 (WB), 1:50 (IP), 1:200 (IHC)
β-actin	12262s	Cell Signaling Technology	1:10000 (WB)
STUB1	55430-1-AP	Proteintech	1:1000 (WB)
T7	A190-117A	BETHYL	1:2000 (WB), 1:100 (IP)
V5	66007-1-Ig	Proteintech	1:200 (IP)
V5	14440-1-AP	Proteintech	1:2000 (WB)
HA	sc-7392	Santa Cruz	1:100 (IP)
HA	sc-805	Santa Cruz	1:2000 (WB)
Ub	ab7780	Abcam	1:1000 (WB)

WB: Western blot
IP: Immunoprecipitation
IHC: Immunohistochemistry

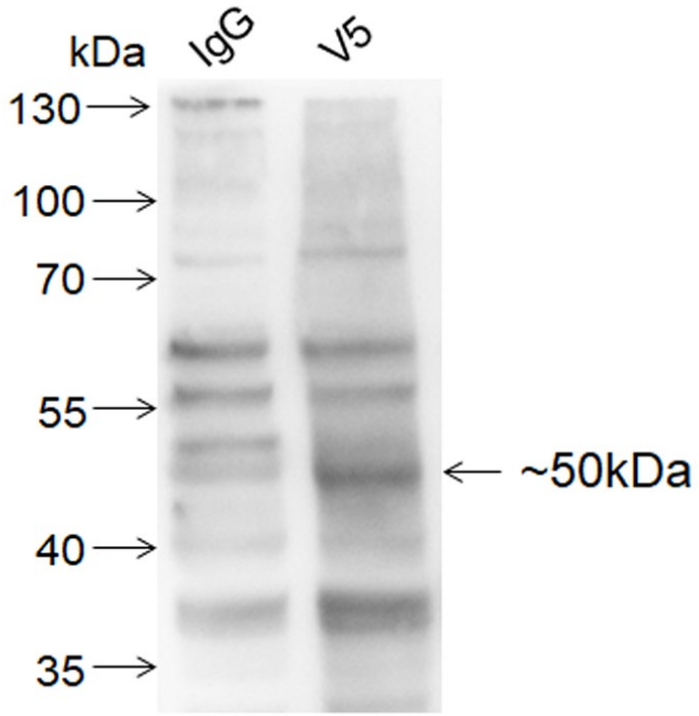
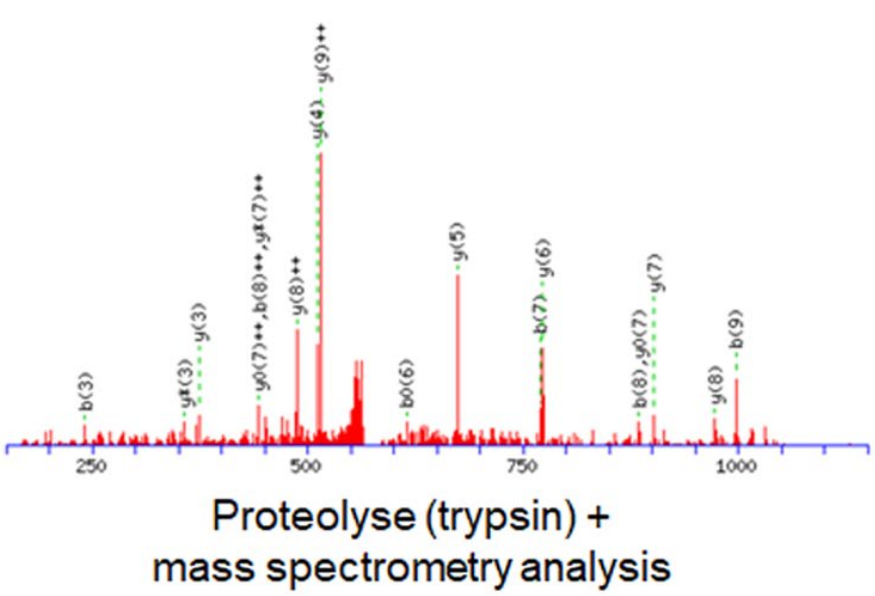


Fig. S1 293T cells were transiently transfected with V5-PD-L1 plasmid. Immunoprecipitation was performed as described in 'Materials and Methods'. Coomassie blue staining showed the appearance of a ~50 kDa band in V5-bound immunoprecipitates.



OS=Homo sapiens GN=ENO1
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GKGVSKAVEHINKTIAPALVSKKLNVTEQ
EKIDKLMIEMDGTENKSKFGANAILGVSL
AVCKAGAVEKGVPLYRHIADLAGNSEVIL
PVPAFNVINGGSHAGNKLAMQEFMILPV
GAANFREAMRIGAEVYHNLKNVIKEKYG
KDATNVGDEGGFAPNILENKEGLELLKT
AIGKAGYTDKVVIGMDVAASEFFRSGKY
DLDFKSPDDPSRYISPDQLADLYKSFIKD
YPVVSIEDPFDQDDWGAWQKFTASAGI
QVVGDDLTVTNPKRIAKAVNEKSCNCLL
LKVNQIGSVTESLQACKLAQANGWGVM
VSHRSGETEDTFIADLVVGLCTGQIKTGA
PCRSERLAKYNQLLRIEEELGSKAKFAG
RNFRNPLAK

Fig. S2 Mass spectrometry analysis identified ENO1 as a principal interaction partner of PD-L1. PD-L1 was immunoprecipitated from lysates of V5-PD-L1-transfected 293T cells, and un-transfected cells as a control. Immunoprecipitates were analyzed by mass spectrometry. ENO1 was identified by a unique peptide and absent from the control immunoprecipitation.

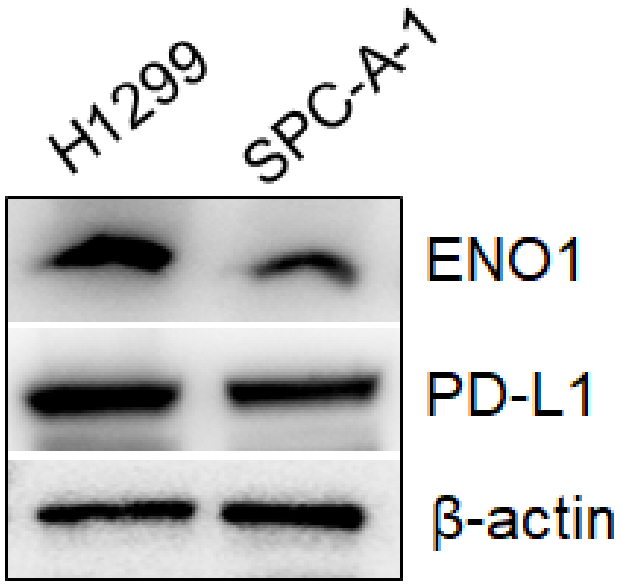


Fig. S3 Western blot revealed that ENO1 and PD-L1 have obvious expression in natural H1299 and SPC-A-1 cells.

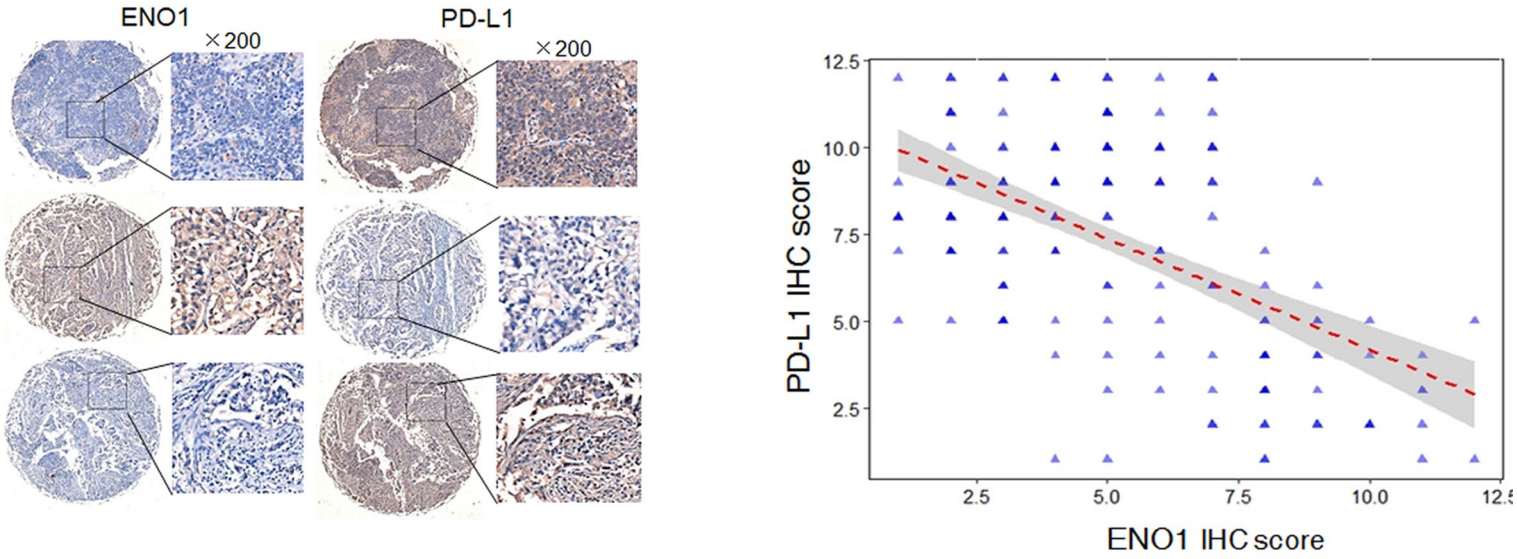


Fig. S4 The half-violin chart was used to display clearly data distribution and frequency of patients.

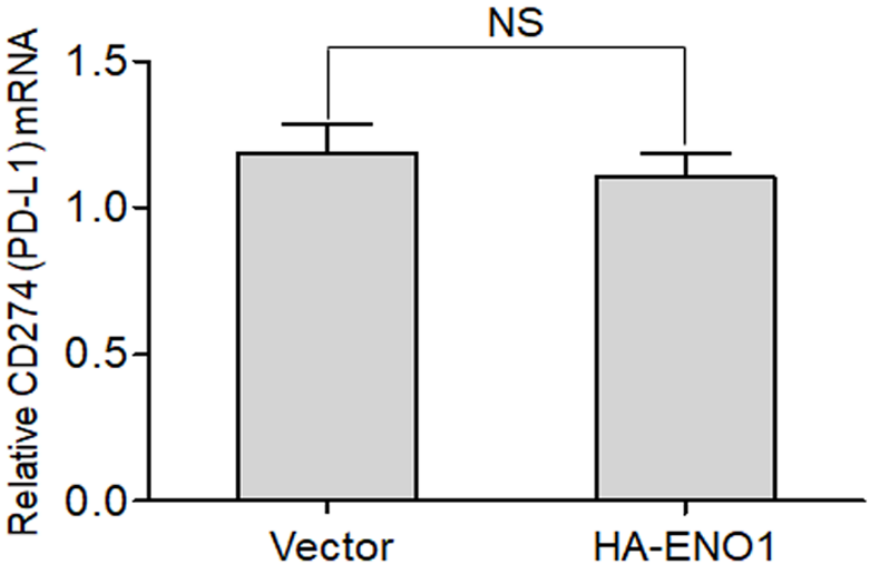
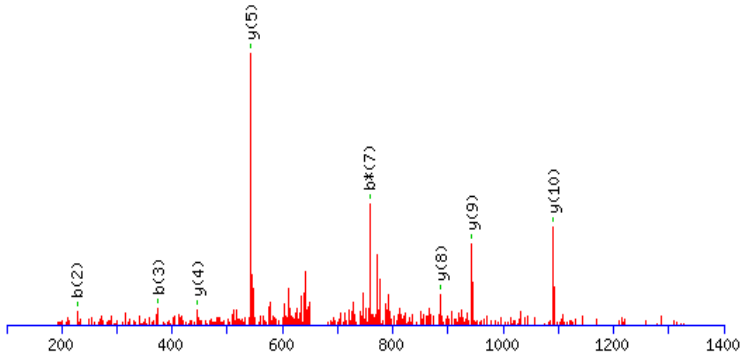


Fig. S5 H1299 cells were transiently transfected with HA-ENO1 or empty vector. PD-L1 mRNA was determined by real-time PCR and normalized against GAPDH. Error bars represent ±S.D. of triplicate experiments. The two-tailed Student's *t*-test was used. NS denotes no significance.

A



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MKGKEEKEGGARLGAGGGSPEKSPSAQEL
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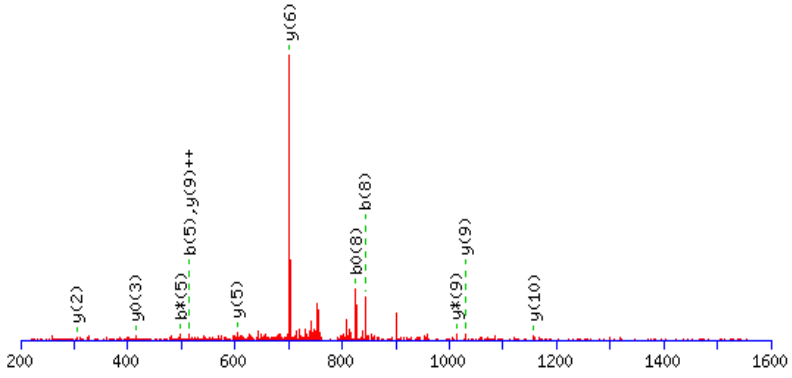
AVYYTNRALCYLKMQQHEQALADCRRALEL
DGQSVKAHFFLGQCQLEMESYDEAIANLQR
AYSLAKEQRLNFGDDIPSALRIAKKKRWNSI
EERRIHQESELHSYLSRLIAAERERELEECQ
RNHEGDEDDSHVRAQQACIEAKHDKYMAD
MDELFSQVDEKRKKRDIPDYLCGKISFELMR
EPCITPSGITYDRKDIEEHLQRVGHFDPVTR
SPLTQEQLIPNLAMKEVIDAFISENGWVEDY

B



OS=Homo sapiens GN=TRIM21
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CISQVGKGGGSVCPVCRQRFLLKNLRPNRQLANMVNNL
KEISQEAREGTQGERCAVHGERLHLFCEKDGKALCWVC
AQSRKHRDHAMVPLEEAAQEYQEKLQVALGELRRKQEL
AEKLEVEIAIKRADWKKTVETQKSRIHAEFVQQKNFLVEE
EQRQLQELEKDEREQLRILGEKEAKLAQQSQALQELISE
LDRRCHSSALELLQEVIIVLERSESWNLKDLDITSPELRS
VCHVPGLKKMLRTCAVHITLDPDTANPWLILSEDRRQVR
LGDTQQSIPGNEERFDSYPMVLGAQHFHSGKHYWEVD
VTGKEAWDLGVCRDSVRRKGHFLLSSKSGFWTIWLWN
KQKYEAGTYPQTPLHLQVPPCQVGIFLDYEAGMVSFYNI
TDHGSLIYSFSECAFTGPLRPFFSPGFNDGGKNTAPLTL
CPLNIGSQGSTDY

Fig. S6 A, B Mass spectrometry analysis identified STUB1 and TRIM21 as potential interaction partners of PD-L1. PD-L1 was immunoprecipitated from lysates of V5-PD-L1-transfected 293T cells, and un-transfected cells as a control. Immunoprecipitates were analyzed by mass spectrometry. STUB1 and TRIM21 were identified by a unique peptide.

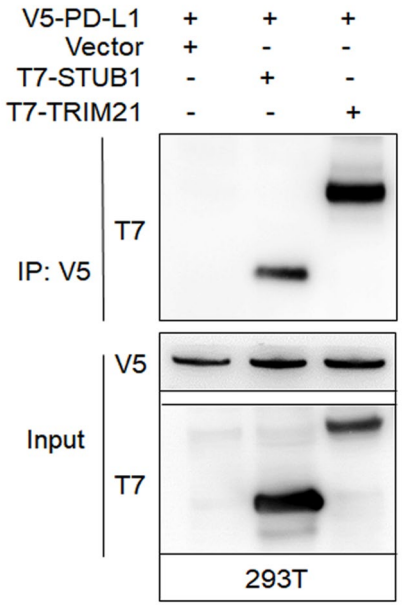

Fig. S7 293T cells were transiently transfected with V5-PD-L1 and T7-STUB1 or T7-TRIM21 plasmids. The lysates were immunoprecipitated with anti-V5 antibody, followed by western blot analysis with anti-T7 antibody. 10% whole cell lysates (input) were probed for the expression of exogenous proteins.

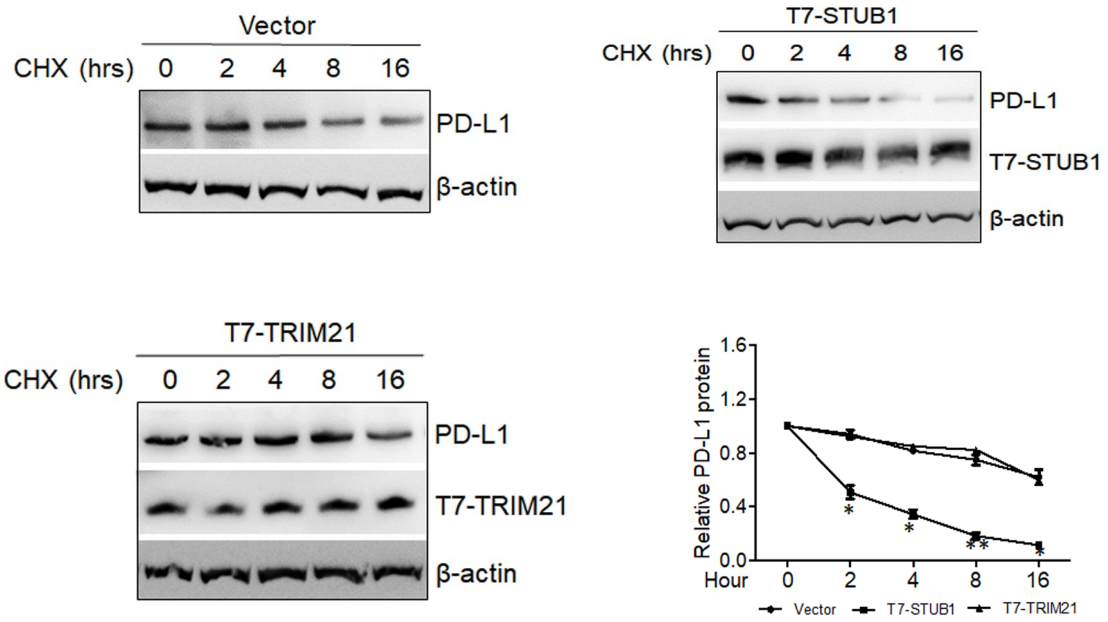

Fig. S8 H1299 cells were transfected with T7-STUB1 or T7-TRIM21, stability of PD-L1 protein was analyzed by western blot. PD-L1 expression levels were quantified by densitometric analysis (Totallab 2.01), statistically analyzed from three independent experiments and presented on the lower right panel. *P< 0.05; **P< 0.01.

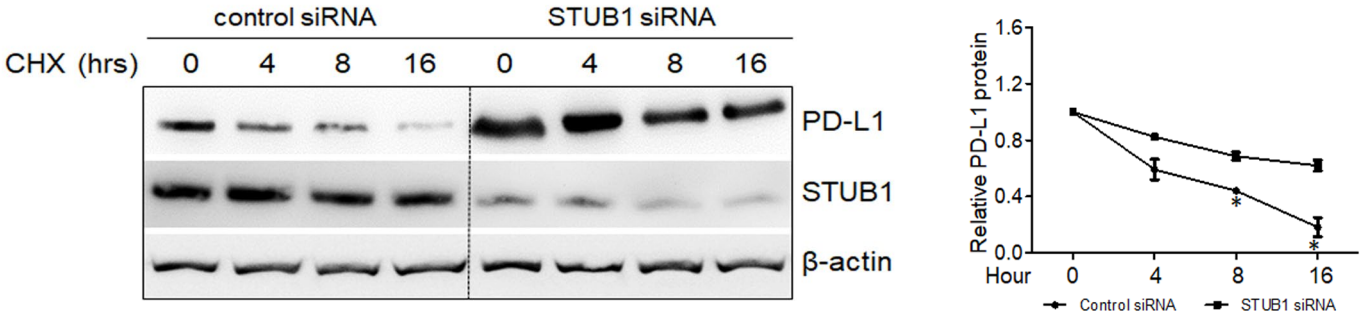

Fig. S9 Western blot analysis of PD-L1 stability in STUB1-knockdown H1299 cells. PD-L1 expression levels were quantified by densitometric analysis (Totallab 2.01). Statistical analysis from three independent experiments was presented on the right panel. *P< 0.05.

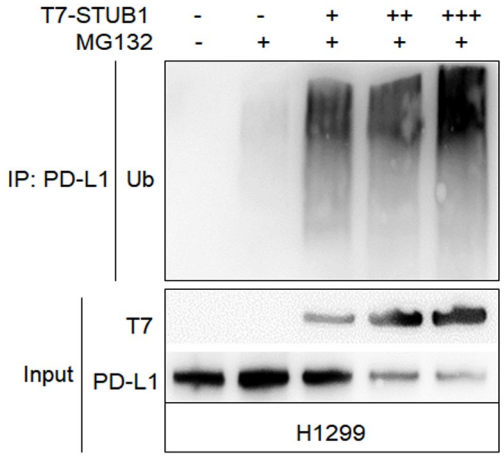

Fig. S10 H1299 cells were transiently transfected with the different dose of T7-STUB1 plasmids. PD-L1 ubiquitination was analyzed.

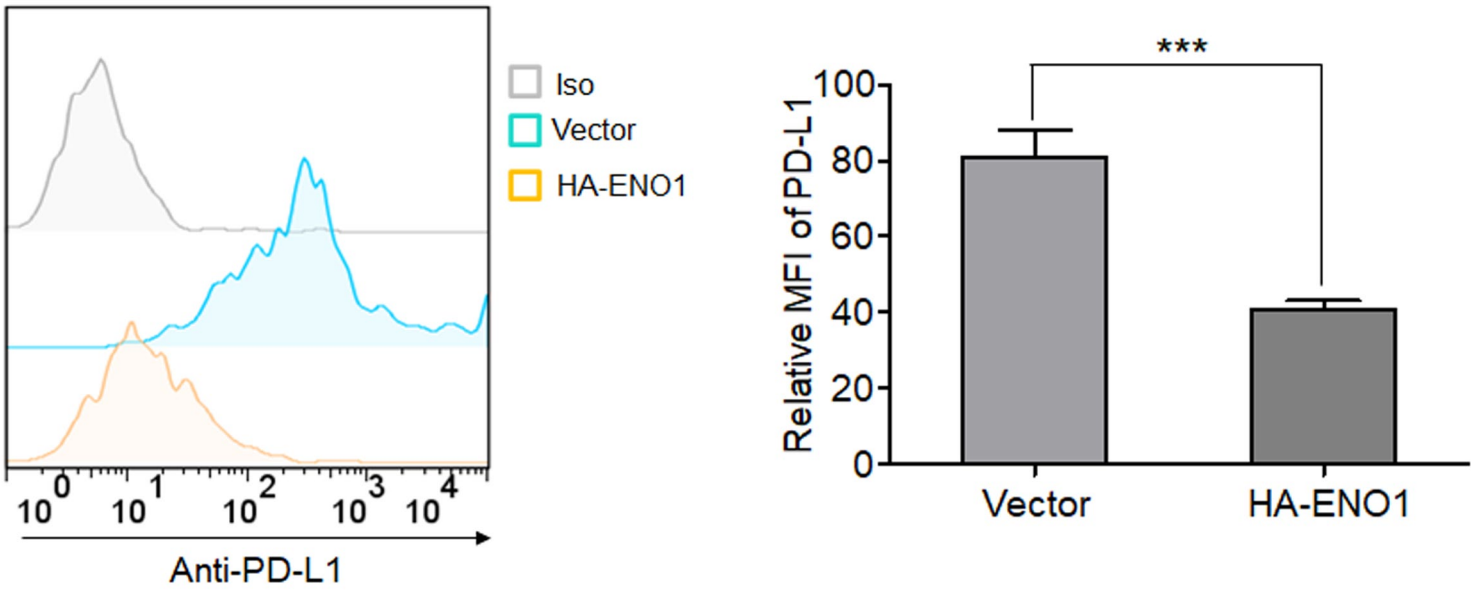

Fig. S11 LLC cells stably transfected with empty or HA-mENO1 vector were treated with IFN-γ at 50ng/ml for 12h, then subjected to flow cytometric analysis of cell surface mPD-L1 using APC-mPD-L1 antibody. mENO1/PD-L1 indicated mouse ENO1/PD-L1.

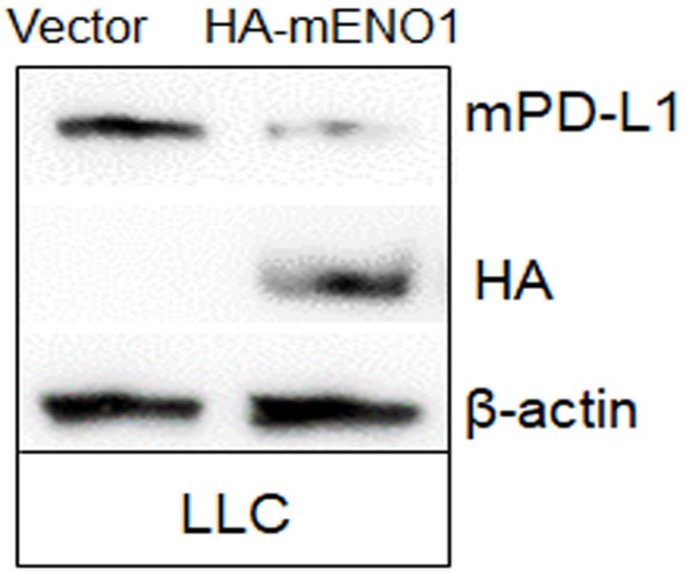

Fig. S12 Western blot revealed the expression of mouse PD-L1 in control and HA-mENO1-overexpressing LLC stable cells. β-actin was served as a loading control. mENO1 indicated mouse ENO1.

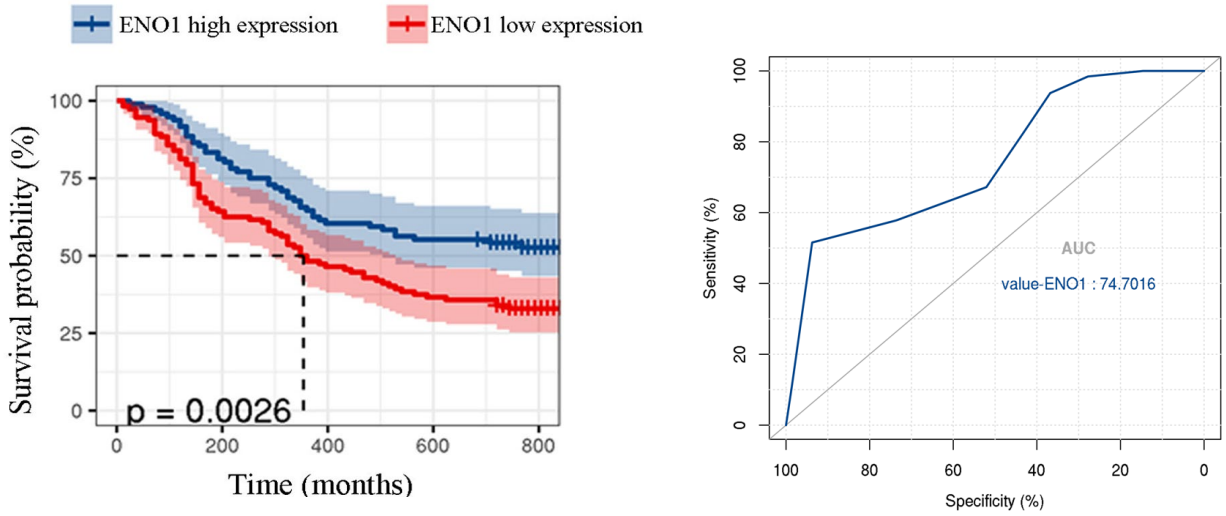

Fig. S13 Kaplan-Merier survival curves showed low expression level of ENO1 was significantly correlated with poor survival of lung cancer (left panel). ROC analysis of the sensitivity and specificity for the prognosis of overall survival by ENO1 expression model (right panel). P-values were calculated by log-rank test. mENO1 indicated mouse ENO1.