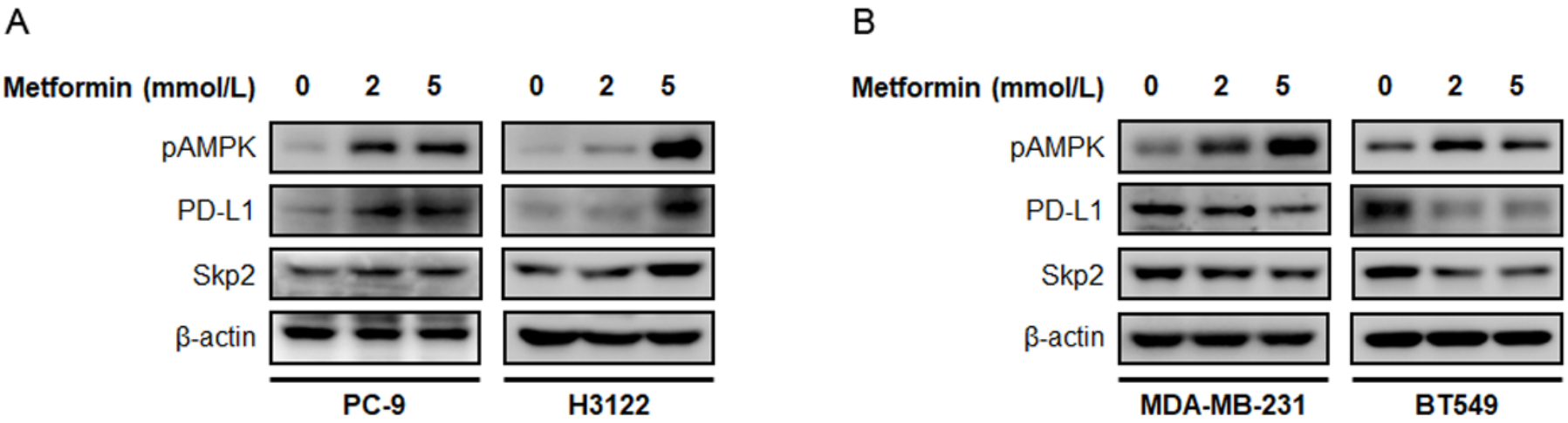
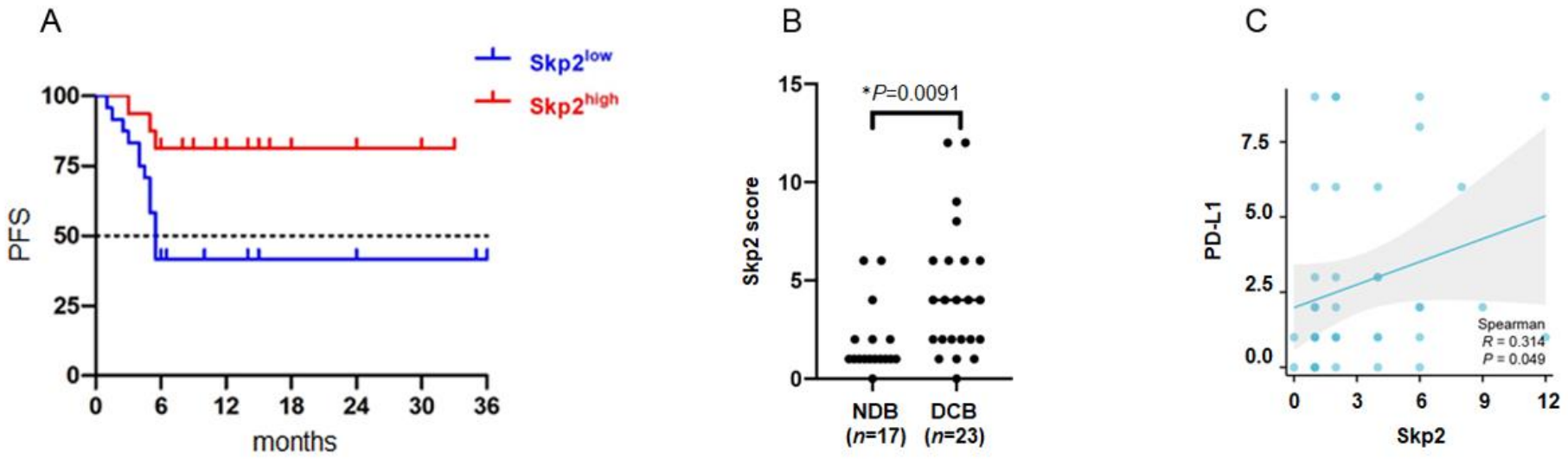
Figure S1



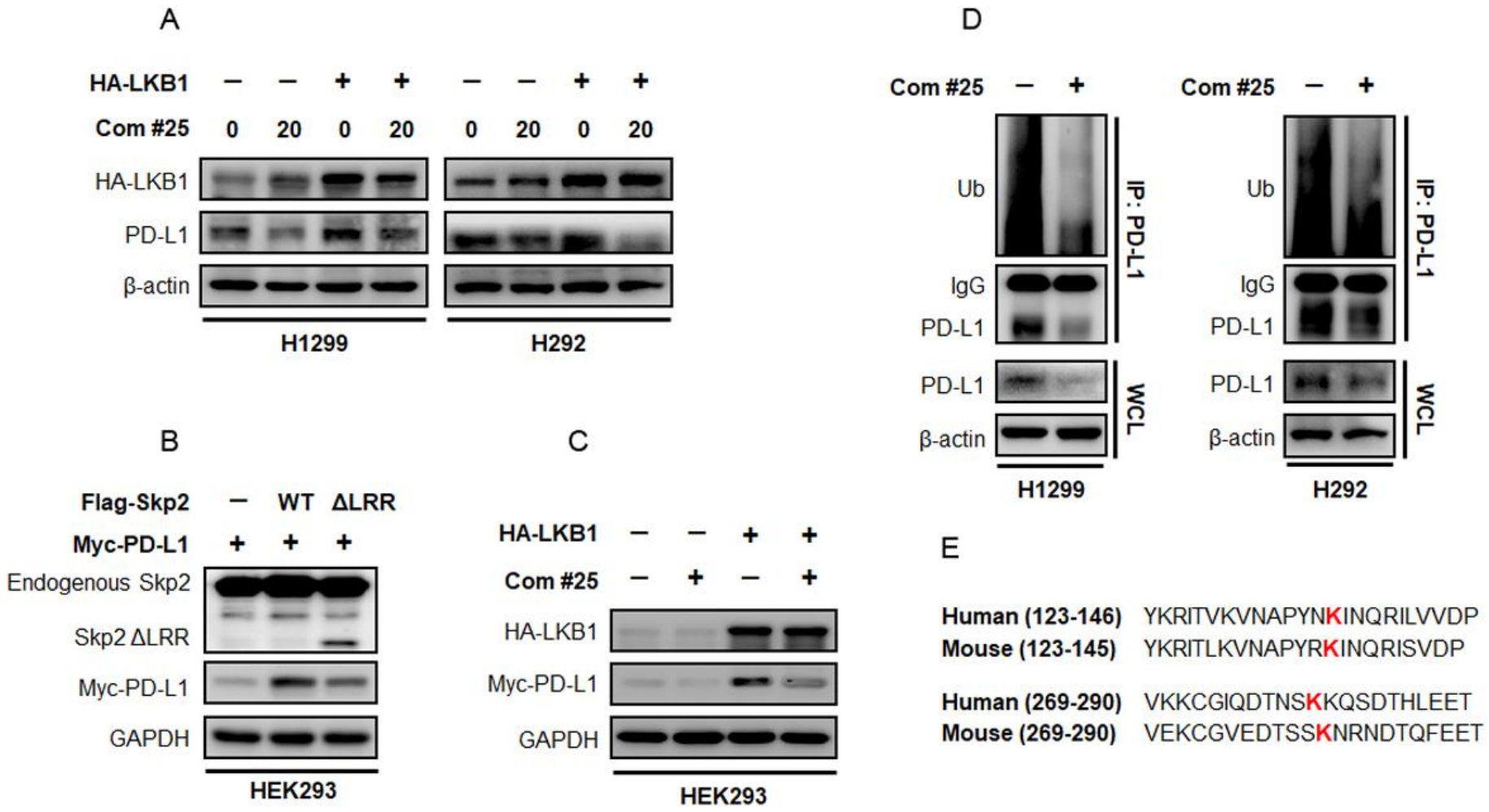
Supplementary Figure S1. Effect of metformin treatment on PD-L1 in NSCLC and breast cancer. (A) PC-9 and H3122 NSCLC cells were treated with increasing concentrations of metformin for 48 h. Expression of pAMPK, PD-L1 and Skp2 was determined by immunoblotting. (B) MDA-MB-231 and BT549 breast cancer cells were treated with metformin and analyzed for pAMPK, PD-L1 and Skp2 expression.

Figure S2



Supplementary Figure S2. Effect of Skp2 on the therapeutic outcomes of anti-PD-1/PD-L1 immunotherapy in patients with NSCLC. (A) Progression-free survival analysis of NSCLC patients receiving anti-PD-1/PD-L1 immunotherapy with different Skp2 status. (B) The distribution of Skp2 IHC-H score in NSCLC patients with favorable response (DCB) and unfavorable response (NDB) to anti-PD-1/PD-L1 immunotherapy. (C) The linear regression analysis of IHC H-score to determine the correlation between Skp2 and PD-L1.

Figure S3


A
HA-LKB1 – – + + – – + +
Com #25 0 20 0 20 0 20 0 20
HA-LKB1
PD-L1
β-actin
H1299
H292
B
Flag-Skp2 – WT ΔLRR
Myc-PD-L1 + + +
Endogenous Skp2
Skp2 ΔLRR
Myc-PD-L1
GAPDH
HEK293
C
HA-LKB1 – – + +
Com #25 – + – +
HA-LKB1
Myc-PD-L1
GAPDH
HEK293
D
Com #25 – +
Com #25 – +
Ub
IgG
PD-L1
IP: PD-L1
PD-L1
β-actin
WCL
H1299
H292
E
Human (123-146) YKRITVKVNAPYNKINQRILVVDP
Mouse (123-145) YKRITLKVNAPYRKINQRISVDP
Human (269-290) VKKCGIQDTNSKKQSDTHLEET
Mouse (269-290) VEKCGVEDTSSKNRNDTQFEET

Supplementary Figure S3. Skp2 promoted PD-L1 protein ubiquitination. (A) HA-tagged LKB1 was overexpressed in H1299 and H292 cells and the resultant cells were treated with 20 μmol/L compound #25 for 48 h. Expression of LKB1 and PD-L1 was evaluated by Western blot analysis. (B) The Flag-tagged Skp2 WT or ΔLRR mutant along with Myc-tagged PD-L1 were expressed in HEK293 cells. The effect of Skp2 on exogenous PD-L1 protein expression was assessed by immunoblotting. (C) Myc-tagged PD-L1 was stably overexpressed in HEK293 cells. The cells were transfected with HA-LKB1 and treated with or without compound #25 (20 μmol/L) for 48 h. The effect of compound #25 on LKB1-induced PD-L1 expression was determined by Western blot analysis. (D) Effect of inhibiting Skp2 E3 ligase activity by compound #25 on PD-L1 protein ubiquitination in H1299 and H292 cells. (E) Alignment analysis of conserved lysine residues on human PD-L1 and mouse PD-L1 protein. Potential ubiquitination sites on PD-L1 were highlighted in red.