A

Human CD274 3'-UTR

$_{526}$ AUAUUGUAGUAGAUGUUACAAUUUUGUCGCCAAACUAAACUUGCUGCUUAAUGAUUUGCUCACAUCUAGUAAAAC $_{600}$

$_{601}$ AUGGAGUAUUUGU $_{613}$

miR-503
miR-497
miR-424
miR-195 ← miRNA family
miR-16
miR-15b
miR-15a

B

Human CD80 3'-UTR

$_{401}$ CAUAUAUAUGUCAGGCAAAGUGCUGCUGGAAGUAGAAUUUGUCCAAUAACAGGUCAACUUCAGAGACUAUCUGA $_{474}$

miR-497
miR-424
miR-195 ← miRNA family
miR-16
miR-15b
miR-15a

$_{486}$ AGAGUAGAAGAUUUUAUGCUGCUUUUACAAAAGCCCAAUGUAAUGCAUAGGAAGUAUGGCAUGAACAUCUUUAGG $_{562}$

miR-497
miR-424
miR-195 ← miRNA family
miR-16
miR-15b
miR-15a

$_{601}$ CAUUUUUUUCAUUGUGUUCUCUAUUGCUGCUCUCUCACUCCCCCAUGAGGUACAGCAGAAAGGAGAACUAUCC $_{672}$

miR-497
miR-424
miR-195 ← miRNA family
miR-16
miR-15b
miR-15a

$_{1201}$ UUAAAGCCUUUAGAGCCAGCCCAUGGCUUUAGCUACCUCACUAUGCUGCUUCACAAACCUUGCUCCUGUG $_{1270}$

miR-497
miR-424
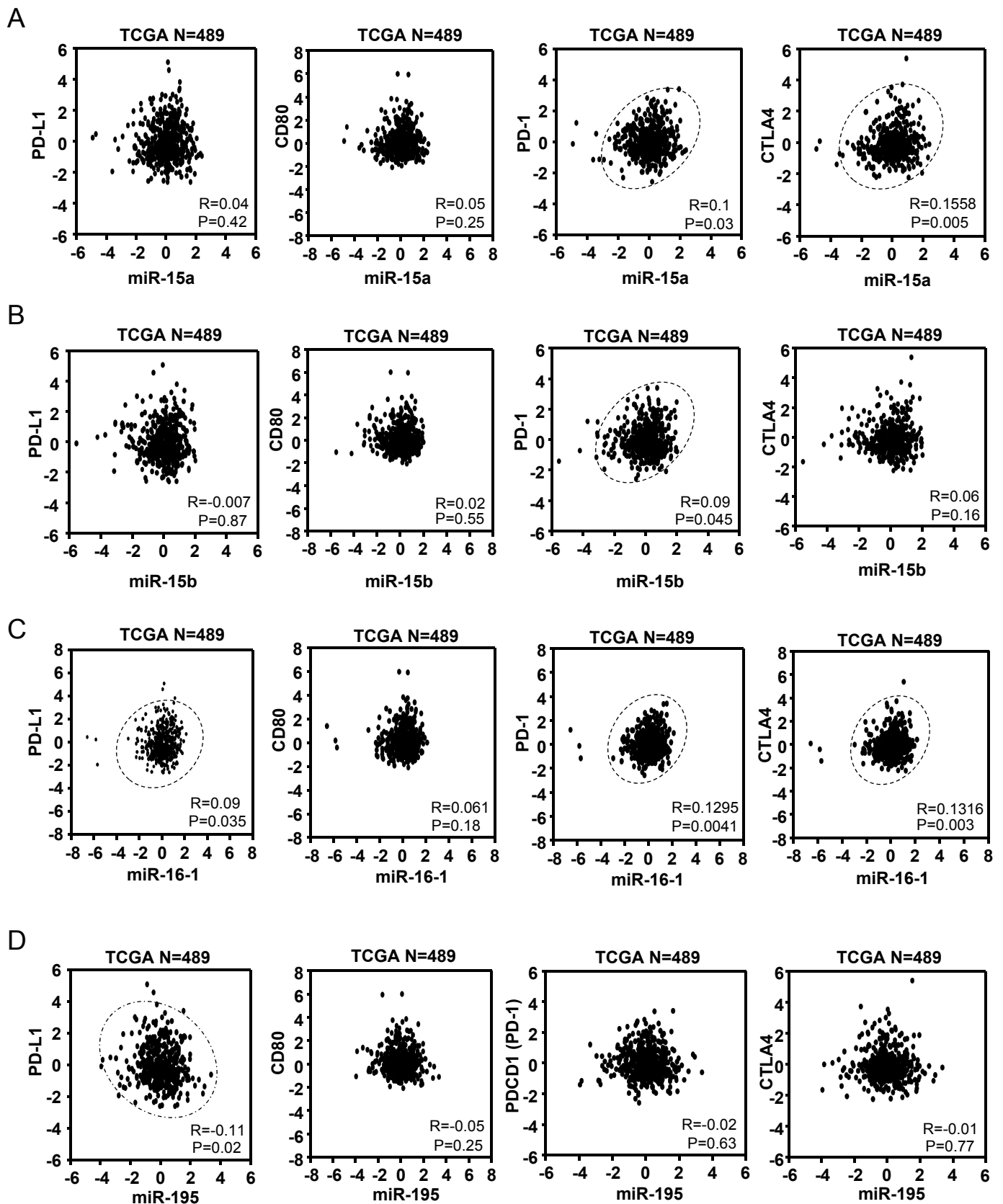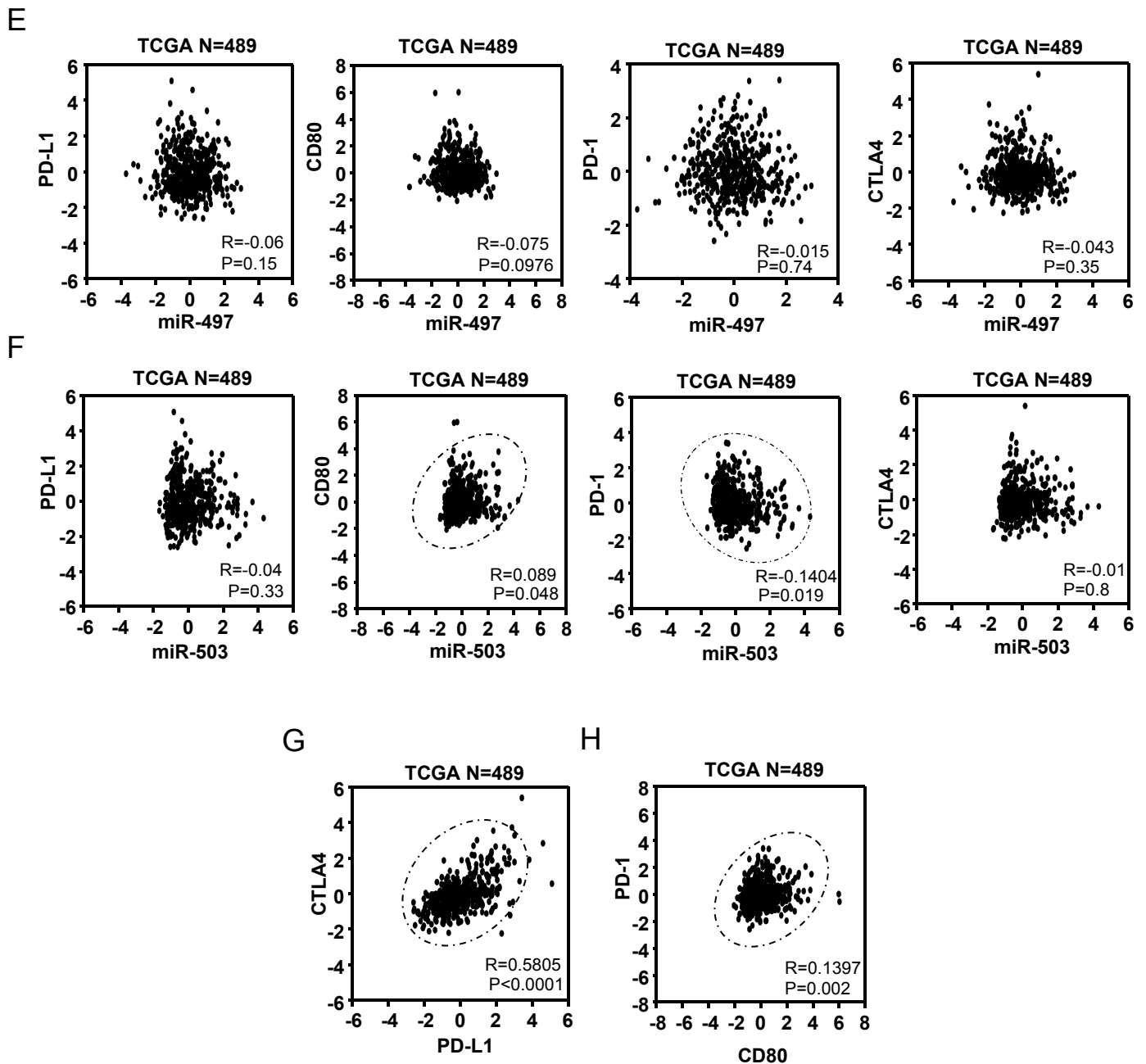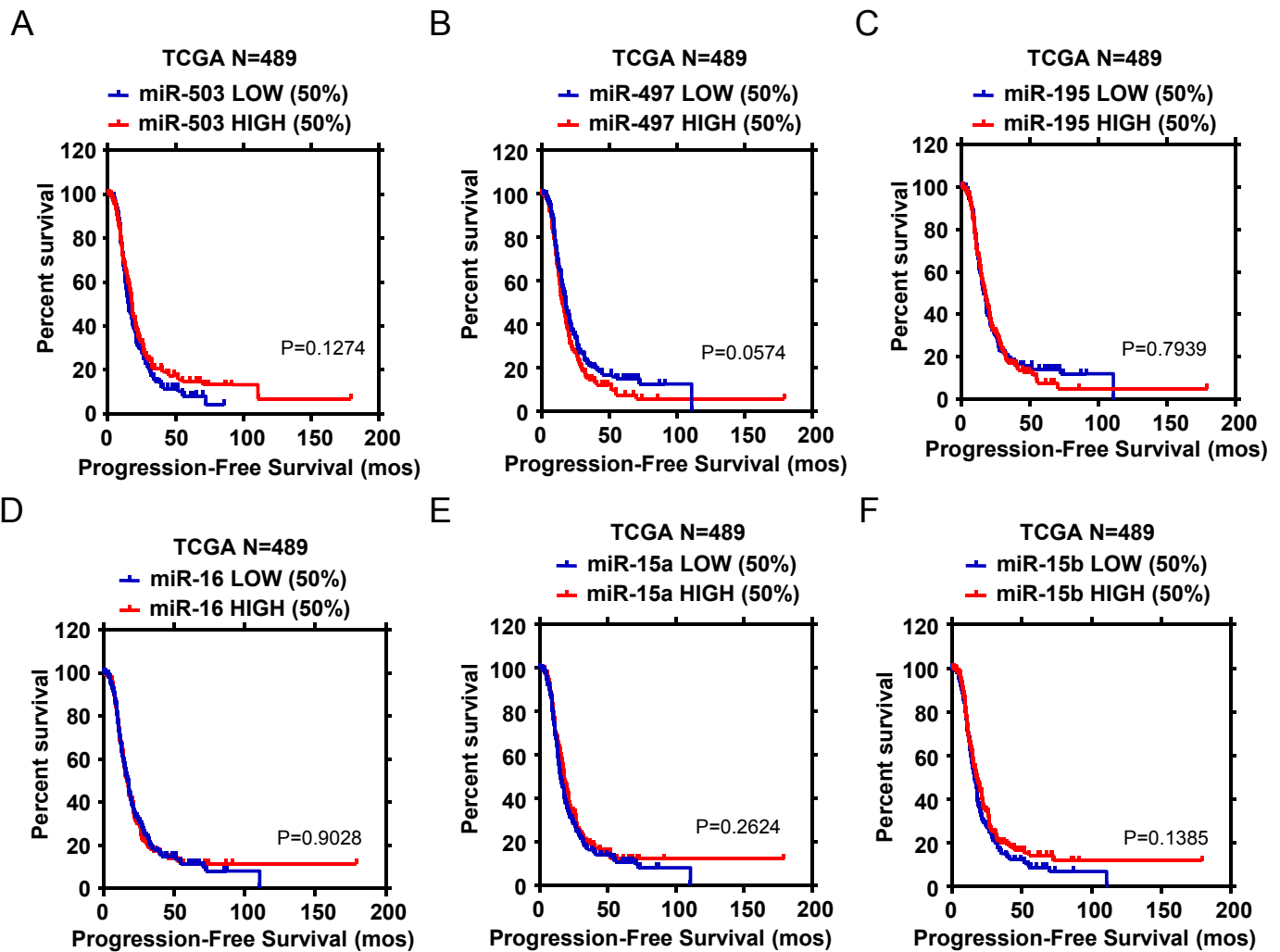miR-195 ← miRNA family
miR-16
miR-15b
miR-15a

Supplementary Figure 1. PD-L1 and CD80 are identified as potential targets of the miR-15a/15b/16/195/424/497/503 family using the public database microRNA.org. (A) PD-L1 was potential target of miR-15a/15b/16/195/424/497/503 family (B) CD80 was potential target of miR-15a/15b/16/195/424/497/503 family.
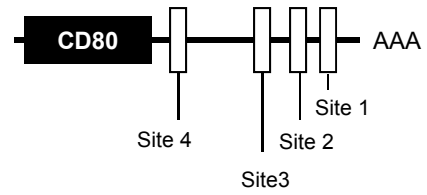
A

TCGA N=489	TCGA N=489	TCGA N=489	TCGA N=489
PD-L1 vs miR-15a R=0.04 P=0.42	CD80 vs miR-15a R=0.05 P=0.25	PD-1 vs miR-15a R=0.1 P=0.03	CTLA4 vs miR-15a R=0.1558 P=0.005

B

TCGA N=489	TCGA N=489	TCGA N=489	TCGA N=489
PD-L1 vs miR-15b R=-0.007 P=0.87	CD80 vs miR-15b R=0.02 P=0.55	PD-1 vs miR-15b R=0.09 P=0.045	CTLA4 vs miR-15b R=0.06 P=0.16

C

TCGA N=489	TCGA N=489	TCGA N=489	TCGA N=489
PD-L1 vs miR-16-1 R=0.09 P=0.035	CD80 vs miR-16-1 R=0.061 P=0.18	PD-1 vs miR-16-1 R=0.1295 P=0.0041	CTLA4 vs miR-16-1 R=0.1316 P=0.003

D

TCGA N=489	TCGA N=489	TCGA N=489	TCGA N=489
PD-L1 vs miR-195 R=-0.11 P=0.02	CD80 vs miR-195 R=-0.05 P=0.25	PDCD1 (PD-1) vs miR-195 R=-0.02 P=0.63	CTLA4 vs miR-195 R=-0.01 P=0.77

Supplementary Figure 2. Relevance of PD-L1/PD-1/CD80/CTLA-4 and the miR-15a/15b/16/195//497/503 family in a TCGA dataset composed of 489 ovarian cancer patient samples. (A-F) The relevance of PD-L1/PD-1/CD80/CTLA-4 and miR-15a/15b/16/195/497/503 family in a 2011 TCGA dataset. (G) PD-L1 expression levels were positively correlated with CTLA-4 expression levels in human ovarian cancer (r = 0.5805, p < 0.0001). (H) CD80 expression levels were positively correlated with PD-1 expression levels in human ovarian cancer (r = 0.1397, p = 0.002).

Supplementary Figure 3. Disease progression-free survival (PFS) was evaluated for miR-15a/15b/16/195/424/497/503 family using Kaplan-Meier analysis in the TCGA dataset. (A-F) Kaplan-Meier analysis was conducted to evaluate the correlation between the disease PFS of the patients and the expression of miR-15a/15b/16/195/497/503 family members in tumour samples. The miR-15a/15b/16/195/497/503 expression was used to assign the samples to high (upper 50th percentile) or low (lower 50th percentile) groups.

A

Site 1 (pos. 414-430)

ugucAGGCA––AAGUGCUGCUg
   | || || |||||||
aaguUUUGUACUUAACGACGAc

hsa-miR-424(322)

Site 4 (pos. 1235-1250)

cuACCUCACUAUGCUGCUu
  || | | | |||||||
uuUGUACUUA–ACGACGAc

hsa-miR-424(322)

Supplementary Figure 4. miR-424(322) targeting sites in 3'-UTRs of CD80 are shown.
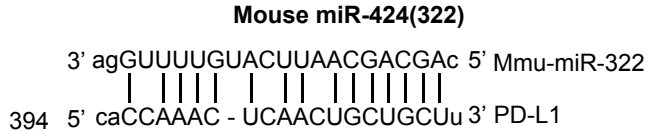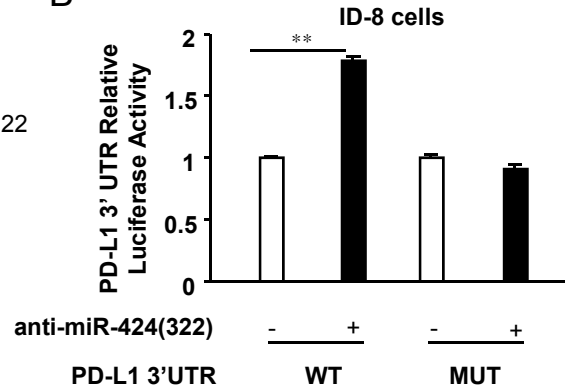(A) The miR-424(322) targeting sites in 3'-UTRs of human CD80 are shown.

A

B

Q1: Early apoptosis；Q3: Background;
Q2: Necrosis and late apoptosis; Q4: live

C

Supplementary Figure 5. miR-424(322) influences TNF-α induced PD-L1-associated CD8+ T cells apoptosis in a Skov3 (CP)/T cells co-culture model. Skov3 (CP) cells with stable overexpression of miR-424(322) or miR-Src were first exposed to TNF-a for 24 h in the presence or absence of anti-PD-L1. (A) Culture media were assayed for IL-10 using a cytokine ELISA assay. ** p ≤ 0.01. The results represent the mean ± SEM from three independent experiments. (B-C) T cells were subsequently co-cultured with mitomycin C-treated Skov3 (CP) cells for 24 h. T cells were sorted by FACS, stained with PE anti-PD-L1, Alexa Fluor 488 anti-annexin V and APC anti-CD8 and analysed by flow cytometry for T-cell apoptosis in the PD-L1+/CD8+ population. ** p ≤ 0.01. The results represent the mean ± SEM from three independent experiments, and the densitometric level of the apoptosis ratio is shown.
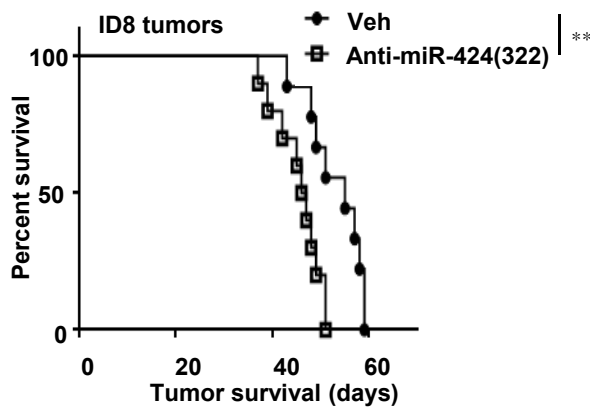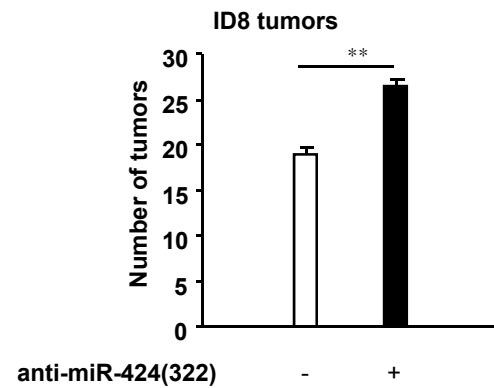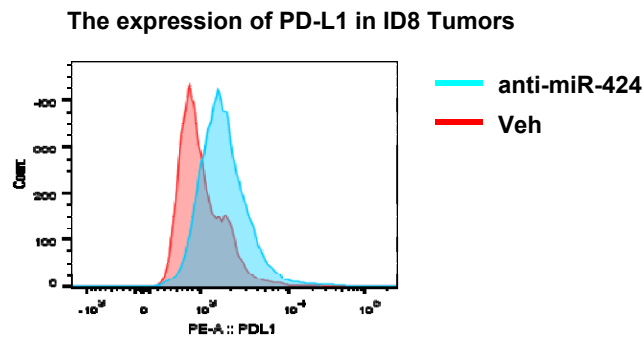
A

Mouse miR-424(322)

3' agGUUUUGUACUUAACGACGAc 5' Mmu-miR-322

394 5' caCCAAAC - UCAACUGCUGCUu 3' PD-L1

B

ID-8 cells

**

PD-L1 3' UTR Relative Luciferase Activity

anti-miR-424(322)   -   +   -   +

PD-L1 3'UTR   WT   MUT

C

ID8 tumors

● Veh
☐ Anti-miR-424(322)   **

Percent survival

Tumor survival (days)

D

ID8 tumors

**

Number of tumors

anti-miR-424(322)   -   +

E

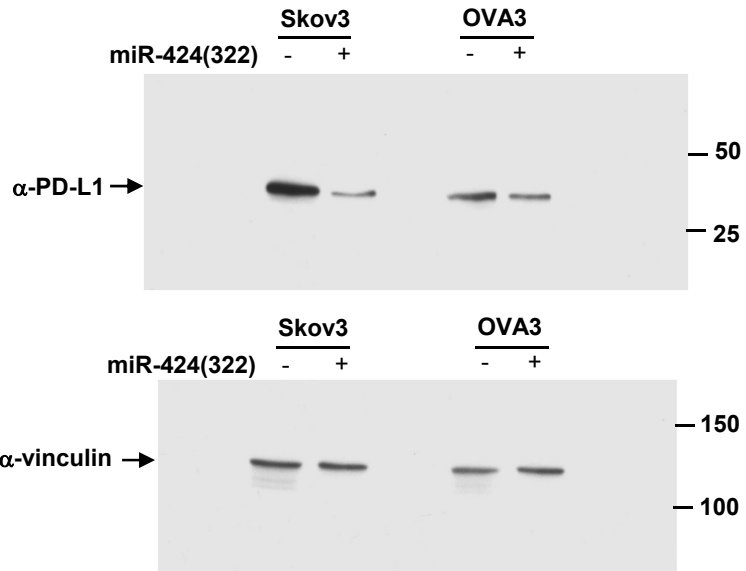The expression of PD-L1 in ID8 Tumors
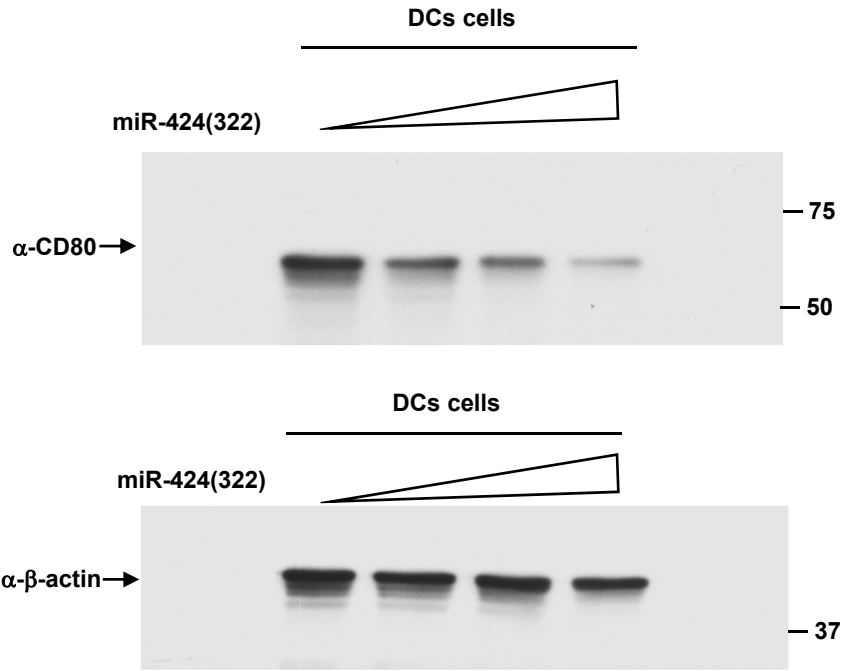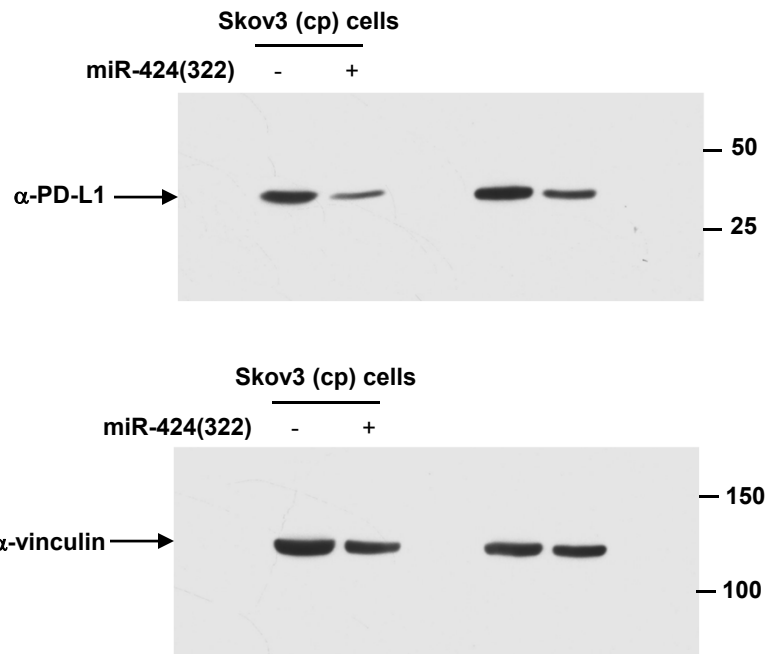
anti-miR-424
Veh

Count

PE-A :: PDL1

Supplementary Figure 6. miR-424(322) inhibits ID8 tumour growth. (A) The miR-424(322) targeting sites in the 3'-UTR of mouse PD-L1 are shown. (B) The luciferase vectors that contained the mouse wild-type (WT) and mutant (MUT) PD-L1 3'-UTR regions were co-transfected into ID8 cells with anti-miR-424(322) or vector control. The relative luciferase/*Renilla* activities were analysed in the cells 48 h after the transfection. The results represent the mean $\pm$ SEM from three independent experiments. ** p ≤ 0.01. (C-E) ID8 cells (5×106) were injected into the syngeneic C57BL/7 mice, followed by miR-424(322) inhibitor (anti-miR-424(322)) or vehicle (Veh) treatment. (C) Kaplan-Meier survival analysis of tumour-bearing mice in different treatment groups. ** p ≤ 0.01. (D) The number of tumours was determined in different treatment groups. ** p ≤ 0.01. Bar graphs are shown as the mean $\pm$ SEM (n = 12 mice/group). (E) FACS analysis of cell-surface PD-L1 expression in ID8 tumours. PD-L1 expression was increased in anti-miR-424(322) treated ID8 tumours.
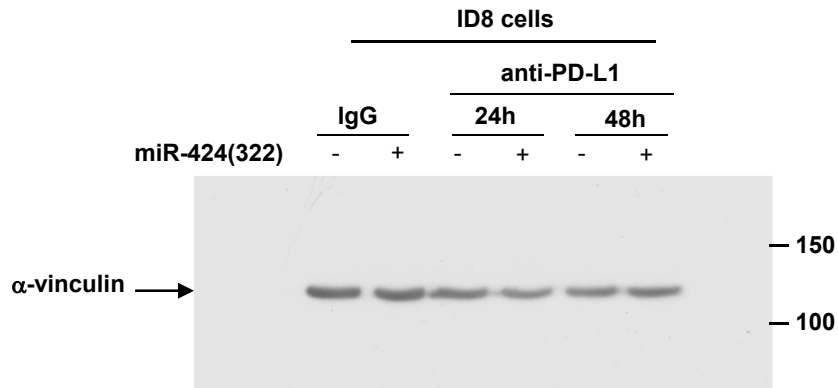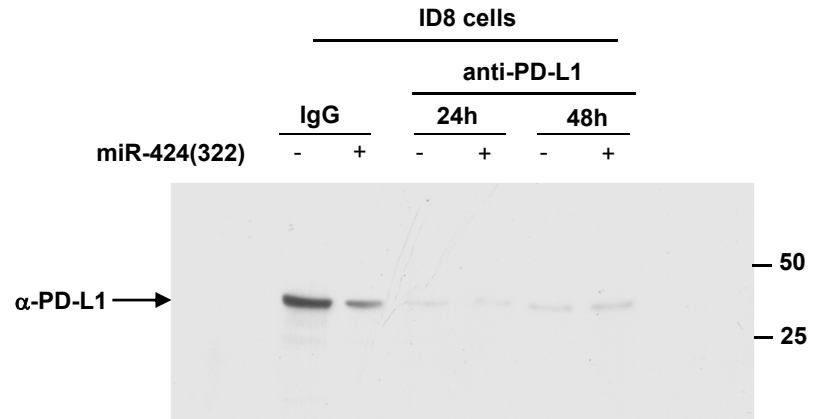
Supplementary Figure 7. Original blots for western blots in Figure 2E.
miR-424(322) overexpression in Skov3 and OVA3 human cancer cells
decreased the protein levels of PD-L1.

DCs cells

miR-424(322)

α-CD80 →

— 75

— 50

DCs cells

miR-424(322)

α-β-actin →

— 37

Supplementary Figure 8. Original blots for western blots in Figure 2F.
miR-424(322) overexpression in DC cells decreased the protein levels of CD80.

Supplementary Figure 9. Original blots for western blots in Figure 3E. miR-424(322) overexpression in Skov3 (cp) cells decreased the protein levels of PD-L1.

Supplementary Figure 10. Original blots for western blots in Figure 5B. miR-424(322) overexpression in ID8 cells decreased the protein levels of PD-L1.

Supplementary Table 1. Clinical characteristics of ovarian cancer patients.

Patient Characteristics (N = 42)

Characteristic	PFS > 6 (N = 21)	PFS < 6 (N = 21)
Age		
< 50	5	4
> 50	16	17
Stage		
I-II	6	3
III-IV	15	18
Grade		
0	0	0
1	1	0
2	5	5
3	15	16
Histologic subtypes		
High-grade Serous	13	17
Low-grade Serous	2	2
Mucinous	3	1
Endometrioid	3	1
Debulking status		
Optimal (≤1cm)	8	7
Suboptimal (>1cm)	13	14
Chemotherapy response		
Sensitive	21	0

Characteristic	PFS > 6 (N = 21)	PFS < 6 (N = 21)

Resistant	0	21