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Supplemental Material to:

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Tumor suppressor gene PDCD4 negatively regulates autophagy by inhibiting the expression of autophagy-related gene ATG5

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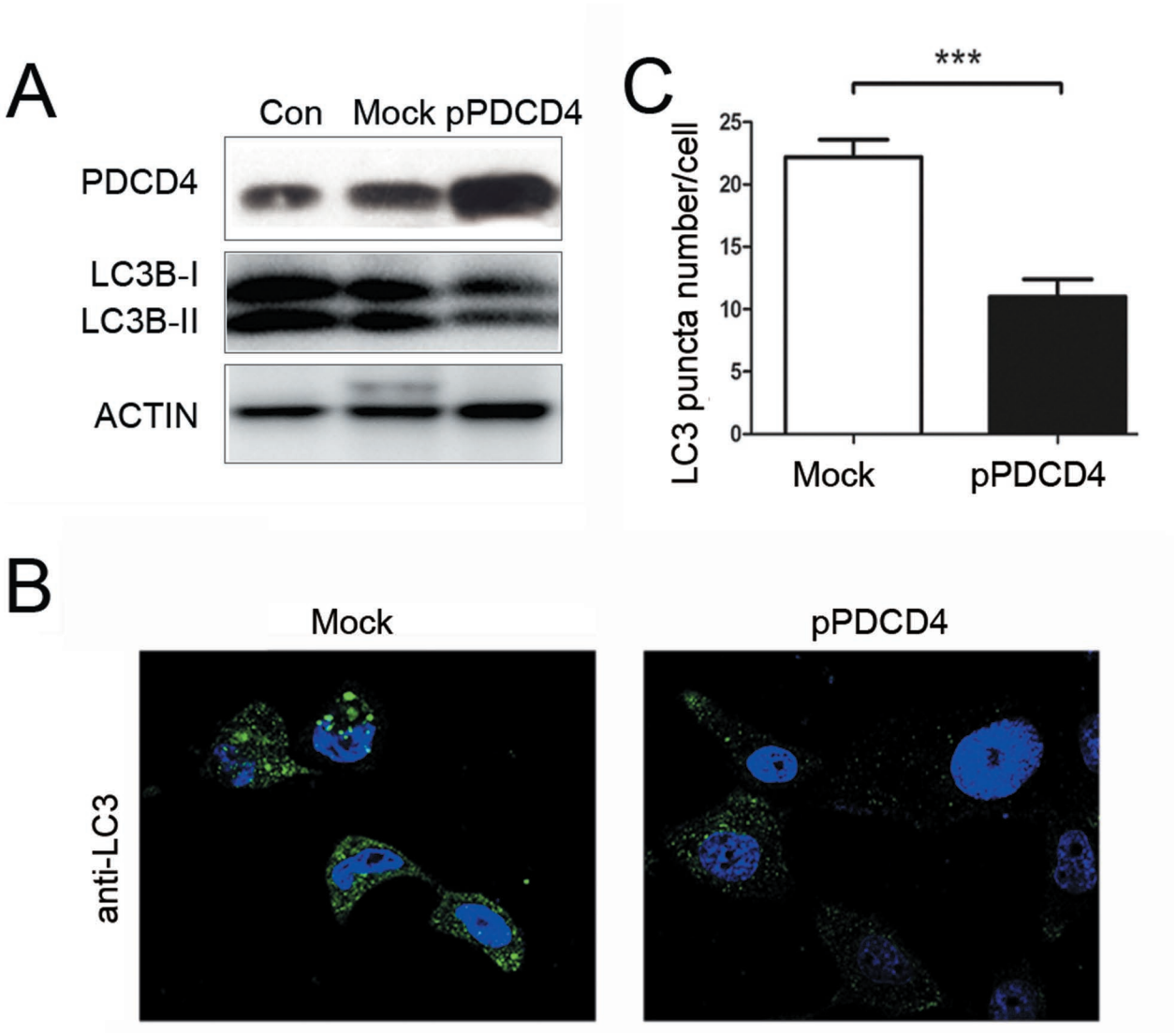
A
Con
Mock
pPDCD4
PDCD4
LC3B-I
LC3B-II
ACTIN
C
***
LC3 puncta number/cell
25
20
15
10
5
0
Mock
pPDCD4
B
Mock
pPDCD4
anti-LC3

Figure S1

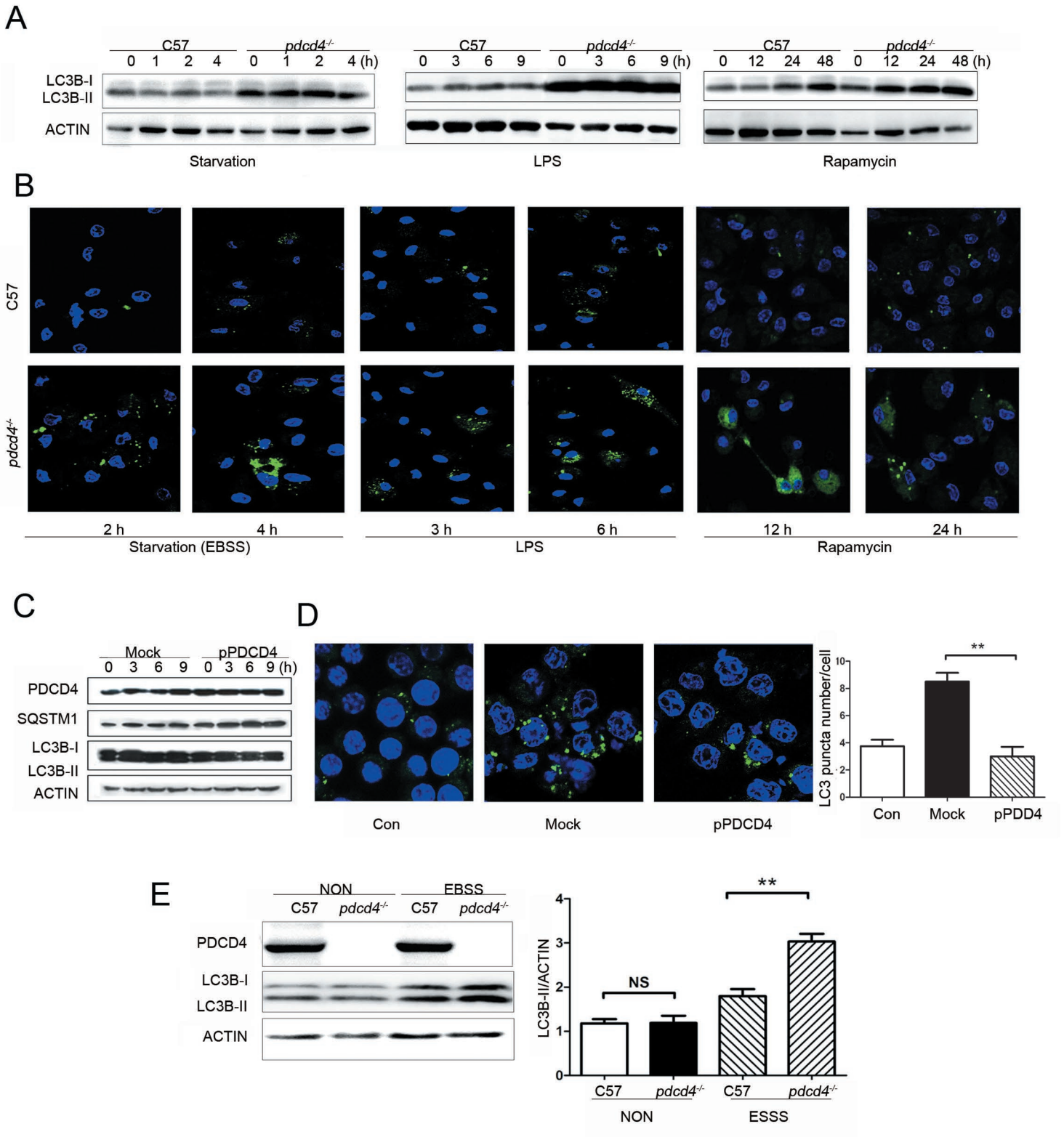
A
C57
pdcd4-/-
0 1 2 4 0 1 2 4 (h)
LC3B-I
LC3B-II
ACTIN
Starvation
0 3 6 9 0 3 6 9 (h)
LPS
0 12 24 48 0 12 24 48 (h)
Rapamycin
B
C57
pdcd4-/-
2 h
4 h
Starvation (EBSS)
3 h
6 h
LPS
12 h
24 h
Rapamycin
C
Mock
pPDCD4
0 3 6 9 0 3 6 9 (h)
PDCD4
SQSTM1
LC3B-I
LC3B-II
ACTIN
D
Con
Mock
pPDCD4
LC3 puncta number/cell
**
Con
Mock
pPDD4
E
NON
EBSS
C57
pdcd4-/-
C57
pdcd4-/-
PDCD4
LC3B-I
LC3B-II
ACTIN
LC3B-II/ACTIN
NS
**
C57
pdcd4-/-
C57
pdcd4-/-
NON
ESSS

Figure S2

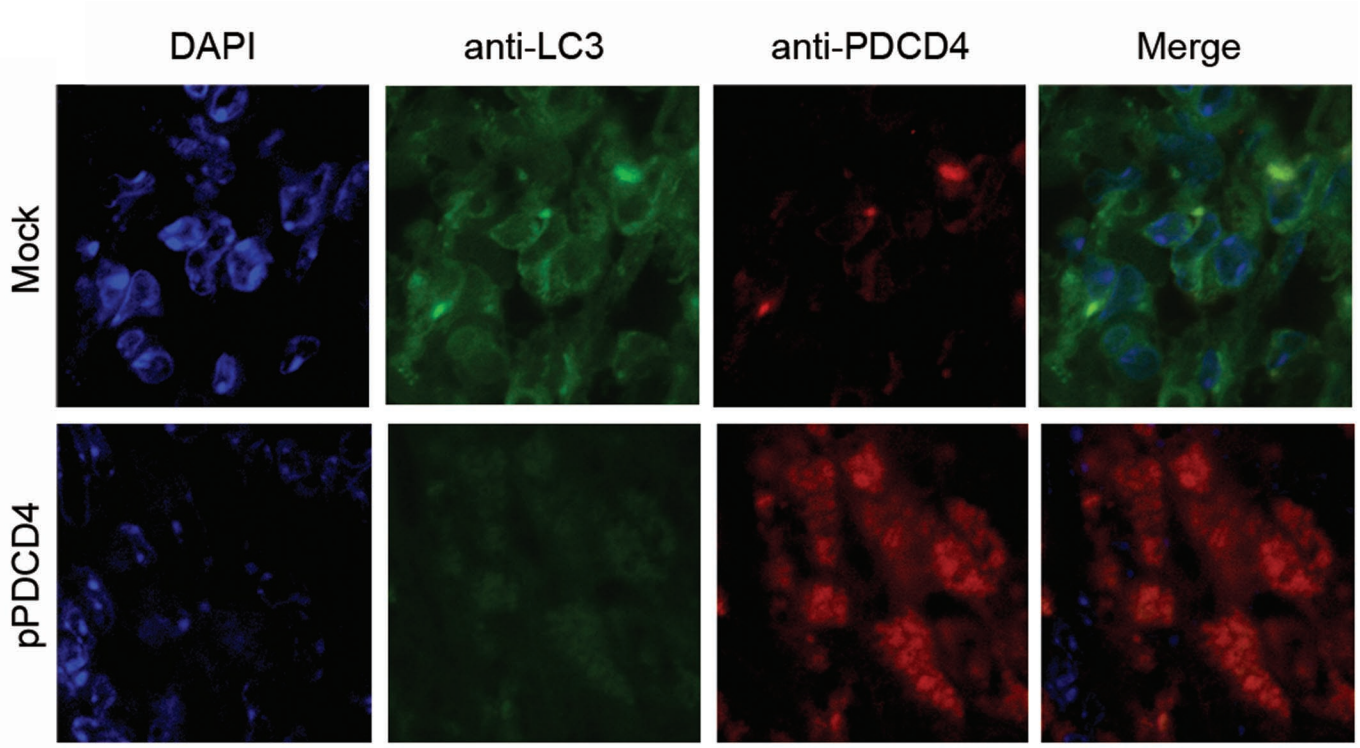

Figure S3

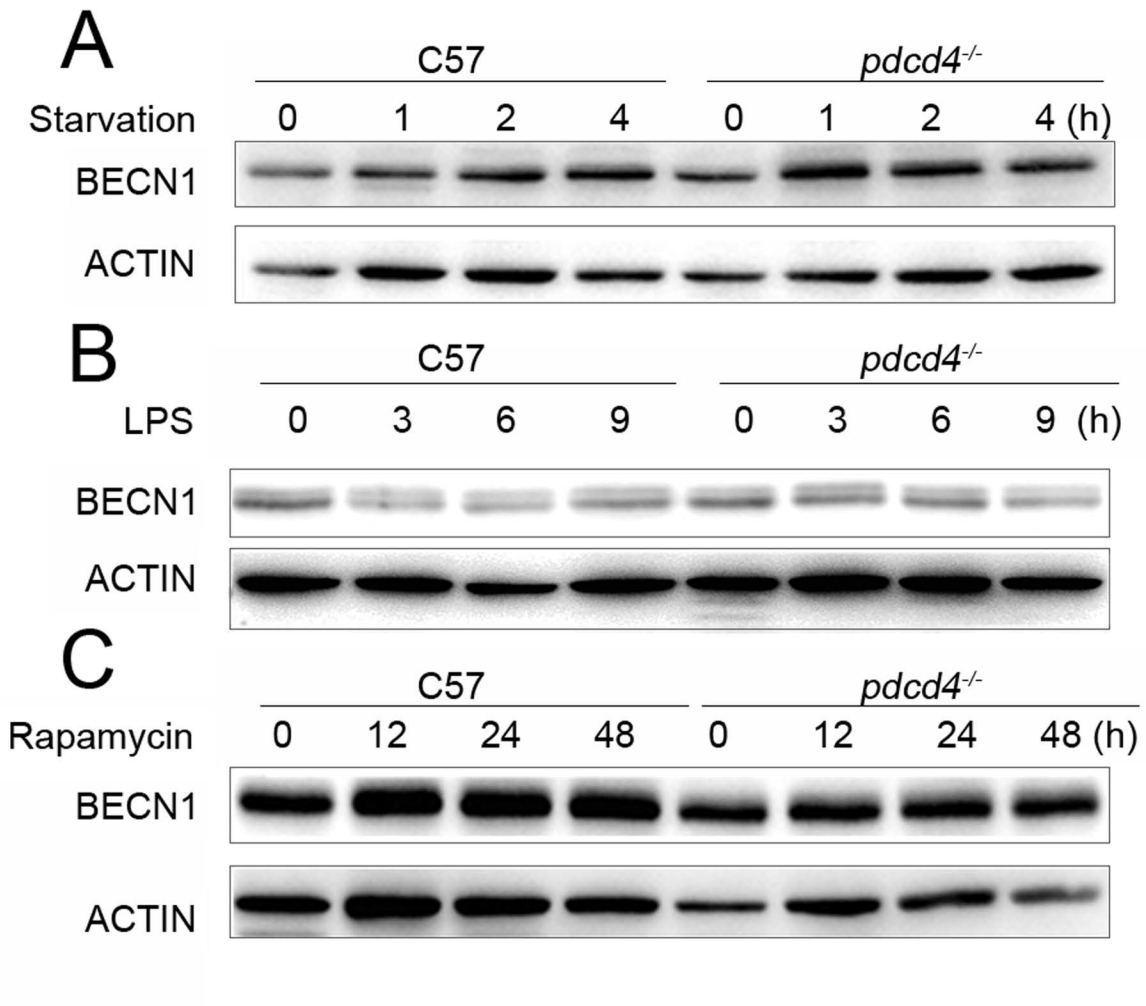
A
C57
pdcd4-/-
Starvation
0 1 2 4 0 1 2 4 (h)
BECN1
ACTIN
B
C57
pdcd4-/-
LPS
0 3 6 9 0 3 6 9 (h)
BECN1
ACTIN
C
C57
pdcd4-/-
Rapamycin
0 12 24 48 0 12 24 48 (h)
BECN1
ACTIN

Figure S4

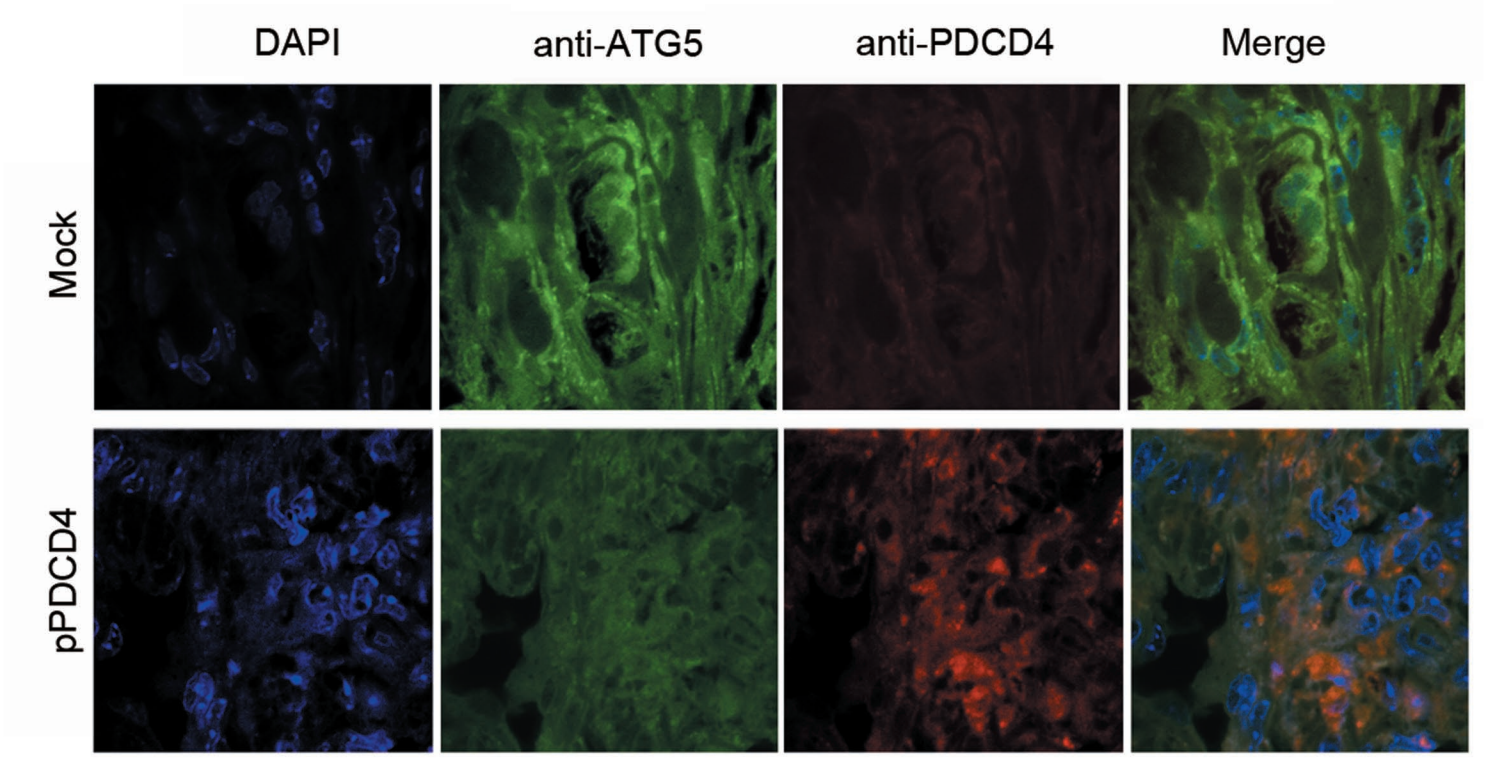

Figure S5

Figure S1. PDCD4 suppresses autophagy in Caov3 cells. Caov3 cells were transfected using *pDsRed2-N1* (Mock) and *pDsRed2-N1-PDCD4* (pPDCD4) plasmids. Twenty-four hours later after starvation for 2 h, (**A**) cells were subjected to western blot analysis to demonstrate PDCD4, LC3B-I/LC3B-II expression in Caov3 cells. (**B and C**) LC3 puncta were detected by immunofluorescence under confocal laser microscopy and quantified as described above. The results shown are means ± SD; **p < 0.01

Figure S2. PDCD4 suppresses autophagy in immune cells. (**A and B**) Primary peritoneal macrophages from C57BL/6J (C57) or *Pdcd4* knockout (*pdcd4*$^{-/-}$) mice were starved, or treated with LPS, or rapamycin respectively. (**A**) LC3B-I/LC3B-II expression level was detected by western blot analysis. (**B**) The cells were inspected under confocal laser microscopy to detect LC3 puncta by immunofluorescence. (**C and D**) RAW264.7 cells were transfected using *pDsRed2-N1* (Mock) and *pDsRed2-N1-PDCD4* (pPDCD4) plasmids, 24 h later, treated with LPS. (**C**) Western blot analysis demonstrated LC3B-I/LC3B-II expression. (**D**) The cells were inspected under confocal laser microscopy to detect LC3 puncta by immunofluorescence. LC3 puncta per cell were quantified as described above. The results shown are means ± SD; **p < 0.01. (**E**) Spleen cells from C57 and *pdcd4*$^{-/-}$ mice were isolated and cultured for 24 h to discharge adhesive macrophages. The suspension lymphocytes were collected and treated with or without EBSS for another 24 h, and then subjected to western bolt analysis. PDCD4, LC3B-I/LC3B-II expression were detected. Data were quantified by Quantity One. The results shown are means ± SD; **p < 0.01.

Figure S3. Colocalization between PDCD4 and LC3 in murine xenograft tumors. Xenograft tumors paraffin sections treated by injection with mock vector (Mock), PDCD4 plasmid (pPDCD4) were stained using an anti-PDCD4 antibody followed by a Rhodamine-conjugated secondary antibody (Red) and LC3 puncta using an anti-LC3 antibody followed by a FITC-conjugated secondary antibody (green).

Figure S4. PDCD4 suppresses autophagy in a BECN1-independent manner in immune cells. Primary peritoneal macrophages from C57BL/6J (C57) or *Pdcd4* knockout (*pdcd4*$^{-/-}$) mice were subjected to starvation (**A**), LPS (**B**), rapamycin (**C**) respectively, and subjected to western blot analysis to detect BECN1 protein level.

Figure S5. PDCD4 suppresses ATG5 expression in in murine xenograft tumors. Xenograft tumors paraffin sections of nude mice treated by injection with mock vector (Mock), PDCD4 plasmid (pPDCD4) respectively were inspected to IF to detect PDCD4 using an anti-PDCD4 antibody followed by a Rhodamine-cojugated secondary antibody and ATG5 using an anti-ATG5 antibody followed by a FITC-conjugated secondary antibody.