Figure S4

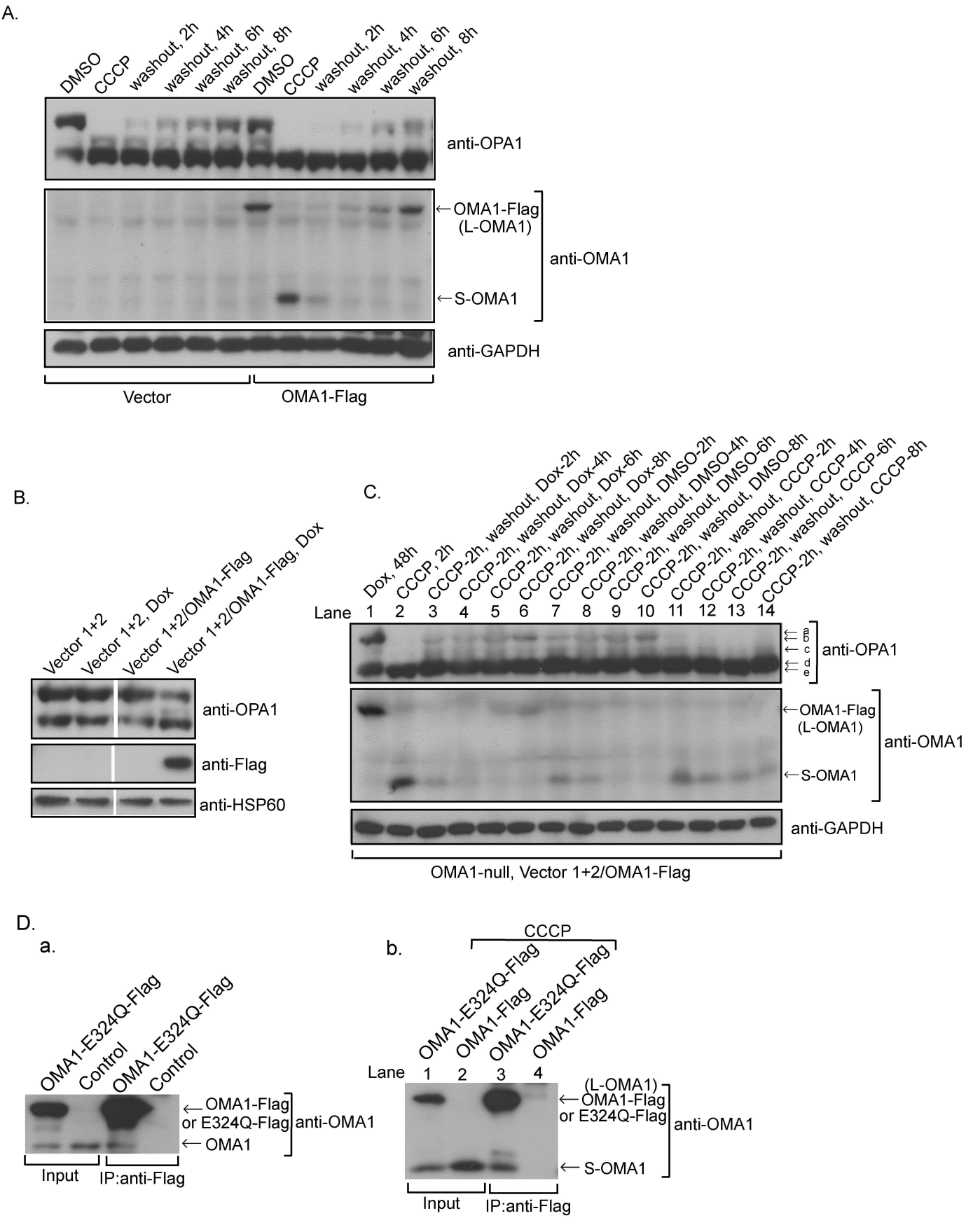
A.
DMSO
CCCP
washout, 2h
washout, 4h
washout, 6h
washout, 8h
DMSO
CCCP
washout, 2h
washout, 4h
washout, 6h
washout, 8h
anti-OPA1
←OMA1-Flag
(L-OMA1)
←S-OMA1
anti-OMA1
anti-GAPDH
Vector
OMA1-Flag
B.
Vector 1+2
Vector 1+2, Dox
Vector 1+2/OMA1-Flag
Vector 1+2/OMA1-Flag, Dox
anti-OPA1
anti-Flag
anti-HSP60
C.
Dox, 48h
CCCP, 2h
CCCP-2h, washout, Dox-2h
CCCP-2h, washout, Dox-4h
CCCP-2h, washout, Dox-6h
CCCP-2h, washout, Dox-8h
CCCP-2h, washout, DMSO-2h
CCCP-2h, washout, DMSO-4h
CCCP-2h, washout, DMSO-6h
CCCP-2h, washout, DMSO-8h
CCCP-2h, washout, CCCP-2h
CCCP-2h, washout, CCCP-4h
CCCP-2h, washout, CCCP-6h
CCCP-2h, washout, CCCP-8h
Lane 1 2 3 4 5 6 7 8 9 10 11 12 13 14
a
b
c
d
e
anti-OPA1
←OMA1-Flag
(L-OMA1)
←S-OMA1
anti-OMA1
anti-GAPDH
OMA1-null, Vector 1+2/OMA1-Flag
D.
a.
OMA1-E324Q-Flag
Control
OMA1-E324Q-Flag
Control
←OMA1-Flag
or E324Q-Flag
←OMA1
anti-OMA1
Input
IP:anti-Flag
b.
CCCP
OMA1-E324Q-Flag
OMA1-Flag
OMA1-E324Q-Flag
OMA1-Flag
Lane 1 2 3 4
(L-OMA1)
←OMA1-Flag
or E324Q-Flag
←S-OMA1
anti-OMA1
Input
IP:anti-Flag

Figure S4. S-OMA1 is associated with OPA1 processing.

A. WT or WT cells stably expressing OMA1-Flag were treated with CCCP (20μM, 2h), and then washout and continue to grow for 2, 4, 6 or 8h, the recovery of L-OPA1 were check by Western blot using anti-OPA1 antibody, cell lysates were also analyzed by Western blot using anti-OMA1 and anti-GAPDH antibodies.

B. OMA1$^{-/-}$ MEFs were infected with pRetroX-Tet-On Advanced (vector 1) plus pRetro-Tight-Pur (Vector 2) retrovirus or pRetroX-Tet-On Advanced plus pRetro-Tight-Pur/OMA1-Flag (Vector 2/OMA1-Flag) retrovirus, two Double Stable RetroX-Tet-On Advanced cell lines were generated, two cell lines were incubated with or without doxycycline (Dox; a tetracycline derivative, 500ng/ml) for 48h, and then cell lysates were check by Western blot with anti-OPA1, anti-Flag and anti-HSP60 antibodies.

C. The Double Stable RetroX-Tet-On Advanced cell line (established in OMA1$^{-/-}$ MEFs) containing pRetro-Tight-Pur/OMA1-Flag were incubated with Dox for 48h to induce the expression of OMA1-Flag, then washout and treated with CCCP (20μM, 2h) to process all L-OPA1, after CCCP washout, cells were treated with Dox, DMSO or CCCP (20μM) for 2, 4, 6 or 8h, the recovery of L-OPA1 or OMA1 were assessed by Western blot using anti-OPA1 or anti-OMA1 antibody, cell lysates were also analyzed by Western blot with anti-GAPDH antibody.

D. (a) OMA1-E324Q-Flag were stably expressed in HeLa cells and the cell lysates were immunoprecipitated with anti-Flag M2 affinity gel, Western blot analysis were then performed with anti-OMA1 antibody; (b) HeLa cells expressing OMA1-Flag or OMA1-E324Q-Flag were treated with CCCP, the lysates were then immunoprecipitated with Flag-M2 beads (anti-Flag resin), the IP products were analyzed by Western blot using anti-OMA1 antibody.