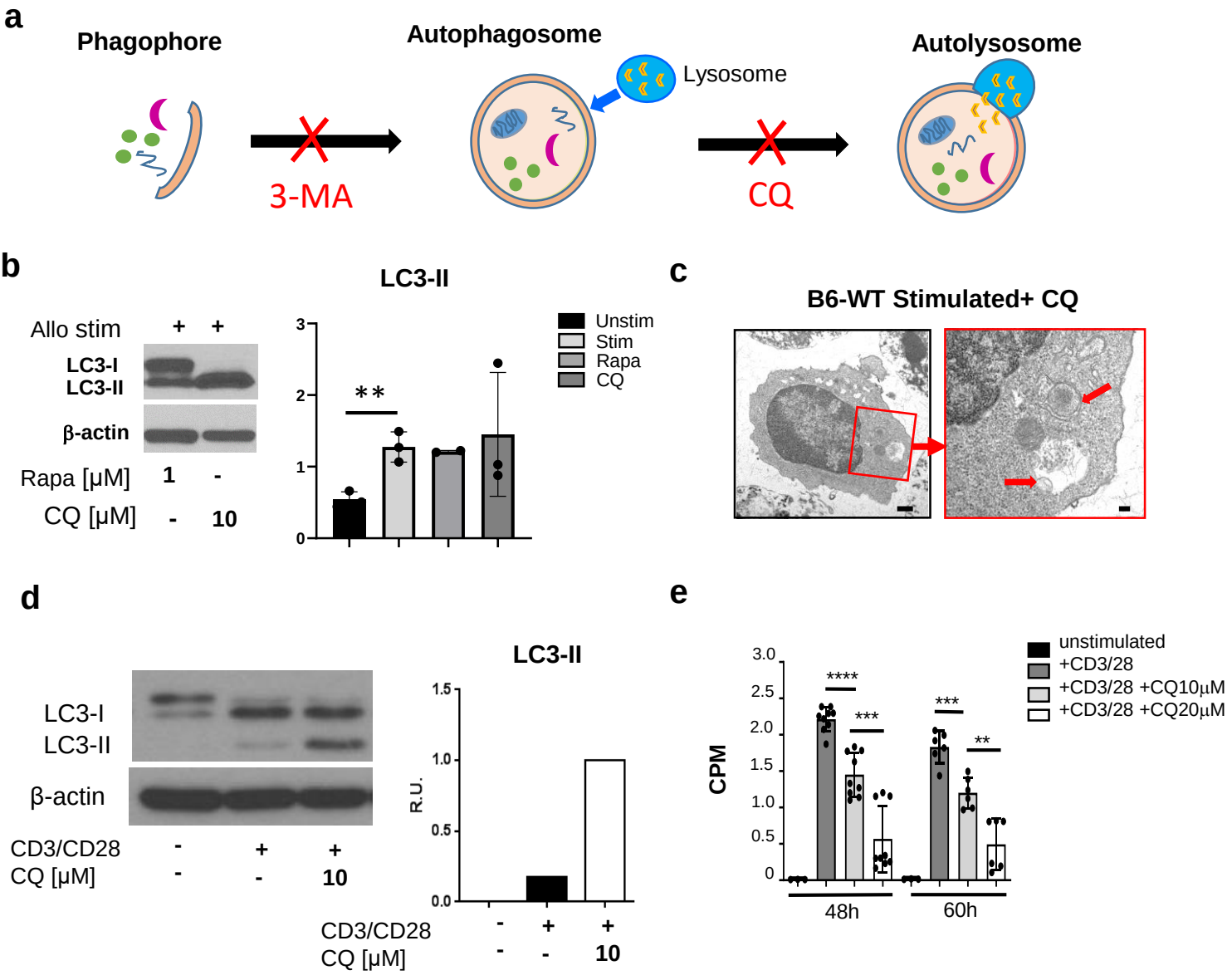
a
Phagophore
Autophagosome
Lysosome
Autolysosome
3-MA
CQ
b
LC3-II
Allo stim + +
LC3-I
LC3-II
β-actin
Rapa [μM] 1 -
CQ [μM] - 10
**
Unstim
Stim
Rapa
CQ
c
B6-WT Stimulated+ CQ
d
LC3-II
LC3-I
LC3-II
β-actin
CD3/CD28 - + +
CQ [μM] - - 10
R.U.
CD3/CD28 - + +
CQ [μM] - - 10
e
CPM
****
***
***
**
48h
60h
unstimulated
+CD3/28
+CD3/28 +CQ10μM
+CD3/28 +CQ20μM
f
Annexin-V+ T cells (%)
****
****
***
+CD3/28
+CD3/28 +CQ10mM
+CD3/28 +CQ20mM

SFig. 1 Autophagy is induced with allogeneic and non-specific TCR stimulation. Whole-cell lysates were prepared from purified splenic T_{con} cells and stimulated for 24hours with anti-CD3/CD28 antibodies in the absence or presence of chloroquine (CQ) 10µM. (a) Schematic of autophagy inhibitors, CQ and 3-Methyladenine (3-MA), illustrating site of inhibition (b) Allogenic activated T cells (right) alone or treated with rapamycin (1µM) or chloroquine (CQ; 10µM) were prepared for western blot analysis for the presence of LC3-I and LC3-II bands. Purified T_{con} cells were used as controls. Graph of the relative intensities (left) of LC3 II bands in relative units (R.U.) after their quantification and normalization to β-actin. Left panel: Western blot for the presence of LC3-II bands. Right panel: graph of the relative intensities of LC3 II bands after their quantification and normalization to β-actin in 3 separate experiments. After 4 days of allogenic stimulation, T_{con} and activated T cells were processed for electron microscopy (c) Activated T cells were compared to allogeneic activated T cells treated with 10 uM CQ. The red arrows indicate the location of identifiable autophagosomes in the cell cytoplasm. Unstimulated C57BL/6 T cells were used as controls (scale bars, 500 nm whole cells; 100 nm red box). (d) Purified splenic T_{con} cells were stimulated for 24hours with anti-CD3/CD28 antibodies in the presence or absence or of chloroquine (CQ) 10 µM and rapamycin (Rapa) 1 mM. Activated T cells (right) were prepared for western blot analysis for the presence of LC3-I and LC3-II bands. Purified T_{con} cells were used as controls. Graph of the relative intensities (left) of LC3 II bands in relative units (R.U.) after their quantification and normalization to β-actin. (e) CD90.2-positive T cells were sorted from the spleens of C57B/6 mice and stimulated with plate-bound anti-CD3 and soluble anti-CD28 antibodies, then analyzed for T cell proliferation based on ^{3}H-thymidine incorporation at 48 and 60 hours. The data in counts per minute (CPM) are the mean ± SEM of triplicates and are representative of 2 from 3 independent experiments. (f) Cells were analyzed at 48 hours after CD3/28 stimulation by flow cytometry. The graph shows the mean percentage of Annexin-V-positive T cells ± SEM of triplicates representative of 2 independent experiments. Data *P< 0.05; **P< 0.01; ***P< 0.001; ****P< 0.0001.