Figure S1. CQ inhibited the autophagic lysosomal degradation of CTLA-4 in T cells. (A) Sorted T cells were activated with anti-CD3/28 for 12 h and then treated with CQ or PBS for 6 h before collected to detect the expression of CTLA-4 and P62 with immunoblot analysis. (B) Endogenous co-IP of CTLA-4 and P62 in murine T cell lysate. Naïve CD4⁺ T cells were obtained by FACS and activated with anti-CD3/CD28 antibodies for 24 h before lysis. Antibodies against CTLA-4 were used to pull down target proteins and their interaction partners, which were resolved under denaturing conditions and visualized by immunoblotting. Data are representative of three independent experiments.

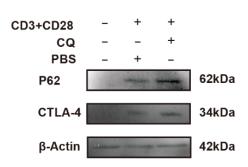
Figure S2. Impact of Chloroquine dose escalation on survival and weight in mice.

8-week-old C57 male mice were randomly divided into 5 groups (8 mice in each group) and i.p. with different doses of CQ (400ug/d, 800ug/d, 1200ug/d, 1600ug/d) or PBS for 14 days. (A) Survival rates were calculated. (B) Weight of mice was recorded, and the ratio of weight after treatment and original weight was calculated. Data are the mean \pm SD and representative of three independent experiments. **p*<0.05; unpaired Student's test.

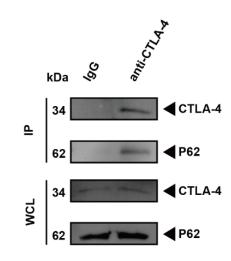
Figure S3. CQ does not influence Ctla-4 mRNA expression of human CD4⁺ and CD8⁺ T cells. (A) Histograms show the relative expression of CTLA-4 among CD4⁺ cells in three samples after different treatments by quantitative reverse transcriptase-PCR (qRT-PCR). (B) Histograms show the relative expression of CTLA-4 among CD8⁺ cells in three samples after different treatments by quantitative reverse transcriptase-PCR (qRT-PCR). Data are the mean \pm SD and representative of three independent experiments. *p<0.05; unpaired Student's test.

Figure S4. Effect of combined Chloroquine and Rapamycin treatment on allograft

survival. (A) Sorted T cells were activated with anti-CD3/28 for 12 h, and then treated with CQ, rapamycin, CQ plus rapamycin or vehicle for 6 h before analyzed by FCM. Histogram shows CTLA-4 MFI of T cells after different treatments. (B) Percentage of heart graft survival with time after transplantation (n=6). Data are the mean \pm SD and representative of three independent experiments. *p<0.05; unpaired Student's test or Mann-Whitney test.



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