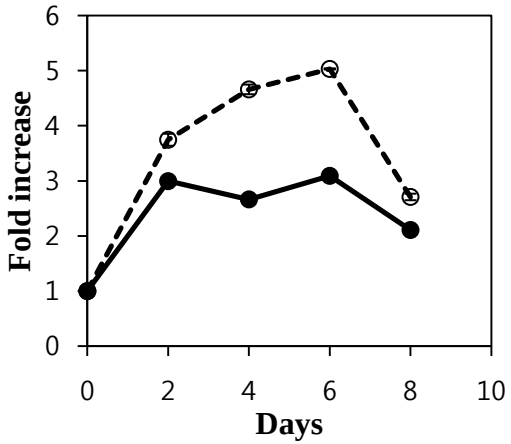
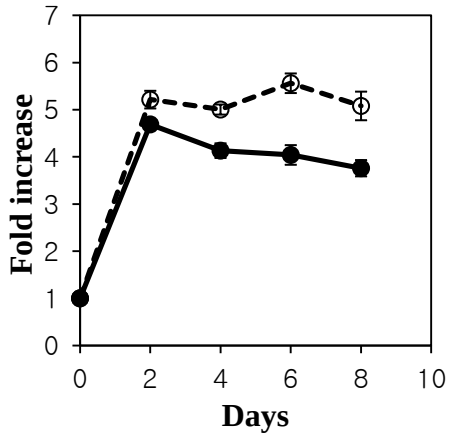
Supplemental figure 1

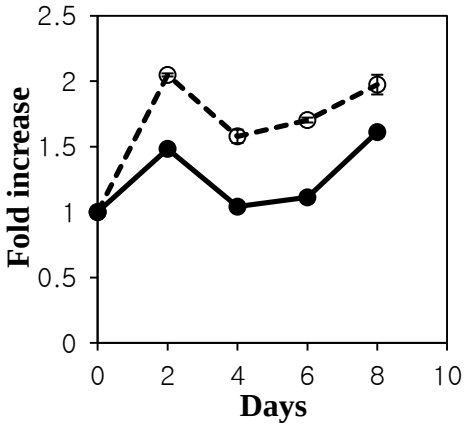
(A) Mitochondria content



(B) Mitochondrial $O_2^{\bullet}$

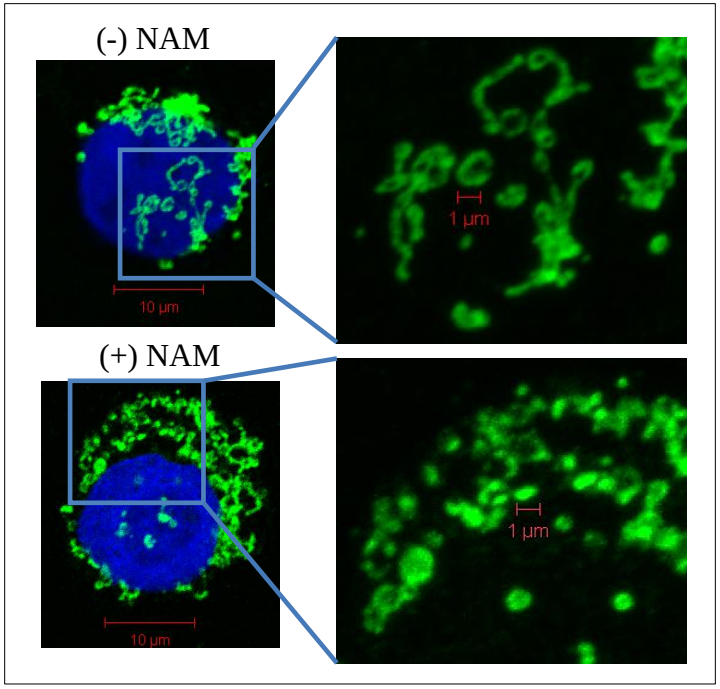


(C) Mitochondrial $^{\bullet}HO$



Effect of NAM treatment on mitochondria and ROS contents in another sample of activated T cells. $CD8^+$ T cells from a donor different from that used in Fig. 1 were treated identically, and the change in the contents of mitochondria (A), mitochondrial superoxide (B) or hydroxyl radical (C) are presented. Basically, the patterns of the activation-induced increase of mitochondria and ROS are similar to those in Fig.1. And, the attenuation of these changes was similarly achieved by NAM treatment.

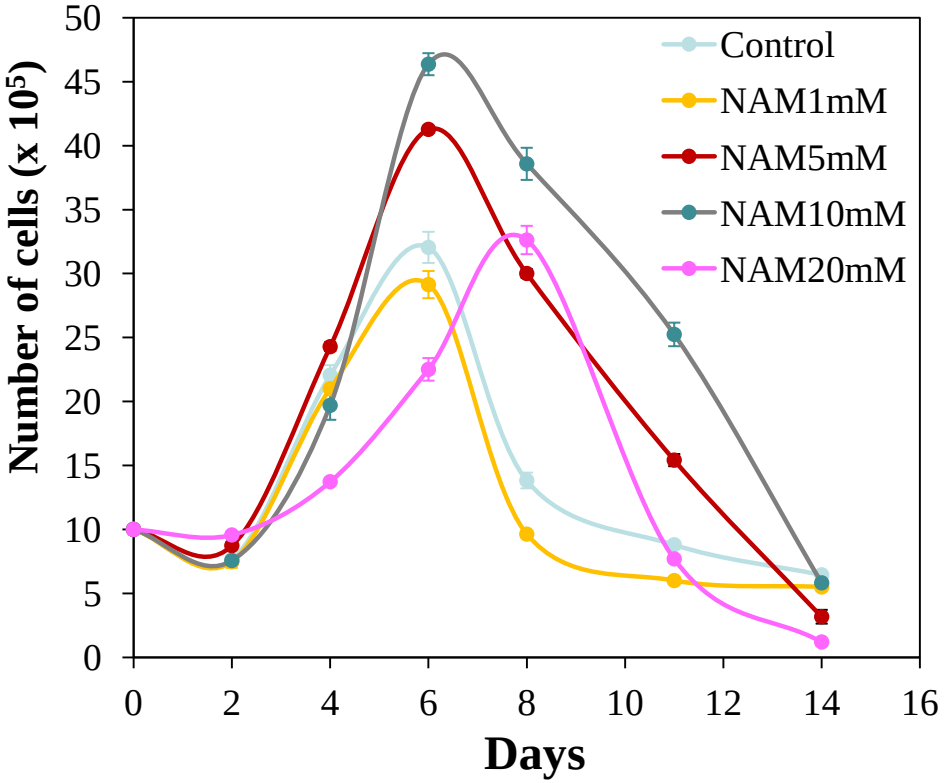
Supplemental figure 2



T cells were mounted on the cover slips coated with 1% gelatin in a 24-well plate by centrifugation. After fixation in 3% formaldehyde, and permeabilization by 0.1% Triton x-100, the cover slips were blocked in 10% FBS, incubated with antibody for LC3 or TOM20 (Santa Cruz), and further probed with Alexa 488-labeled antibody (Santa Cruz). After counter-staining with DAPI, the slides were viewed under confocal microscope.

In fibroblasts, the NAM-mediated autophagy activation was shown to accompany the fragmental change in mitochondria structure [17, 18], which likely facilitates autophagy of lengthy mitochondria [23]. A similar change was observed in T cells activated in the presence of NAM. While mitochondria appeared filamentous in the cells activated in the absence of NAM ((-)NAM), those activated in the presence of NAM appeared as dots and small circles. This supports the possibility of the autophagic removal of mitochondria upon NAM treatment during T cell activation. (In these confocal micrographs, pieces of mitochondria were observed on nucleus. This, seen in other cells in the slides as well, might be an artifact originating from the centrifugation-assisted mounting of T cell.)

Supplemental figure 3



Effect of different amount of NAM on the level of T cell population expansion. A million naive $CD8^+$ T cells were isolated from a healthy donors and treated with Dynalbeads-anti-CD3/anti-CD28 in a medium supplemented without or with 1, 5, 10, or 20 mM NAM. On every second or third day, cells were spun down and fed with fresh media containing NAM while viable cells were counted. It appears that either 5 or 10 mM NAM has positive effect on the level of T cell expansion. NAM at 1 mM dose has no effect, and NAM at 20 mM dose caused a delay in activation-induced cell expansion without a positive effect on the level of expansion.