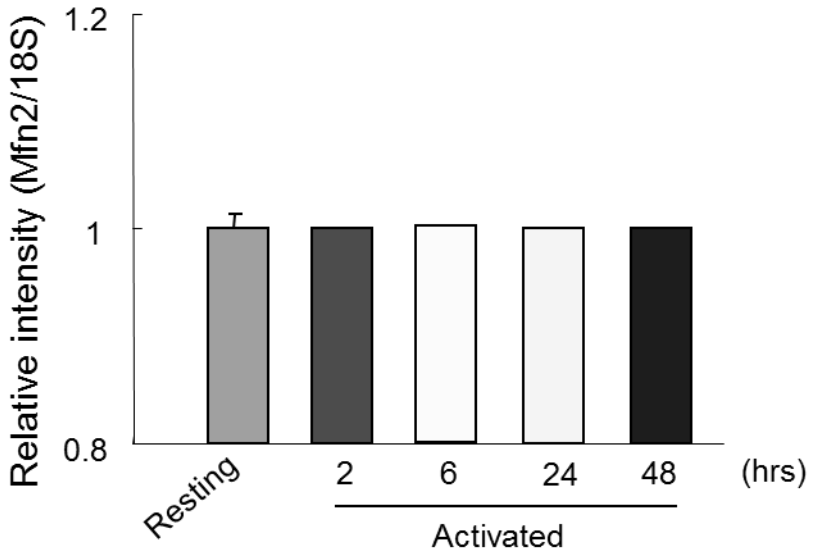
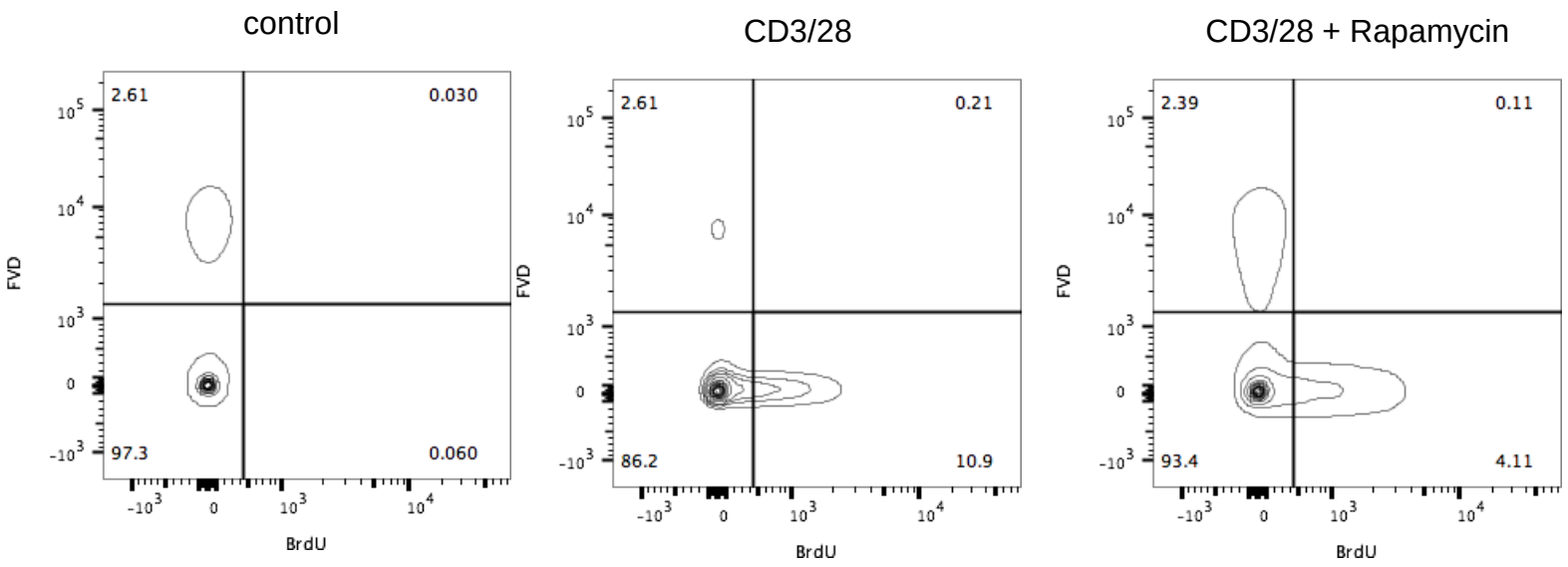
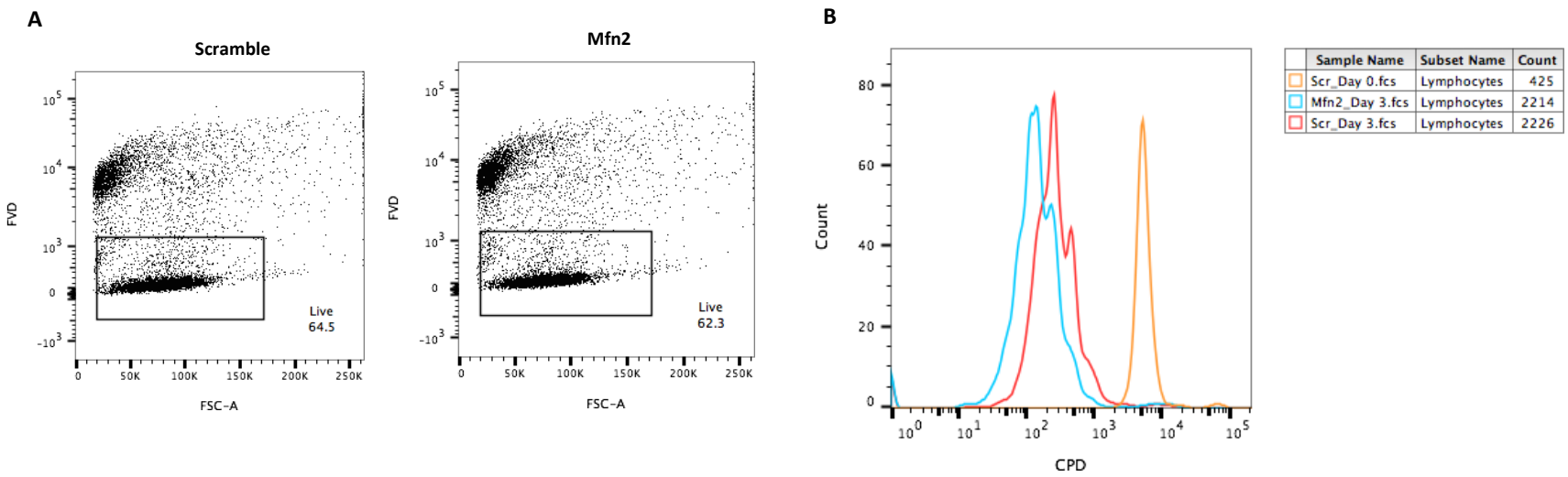


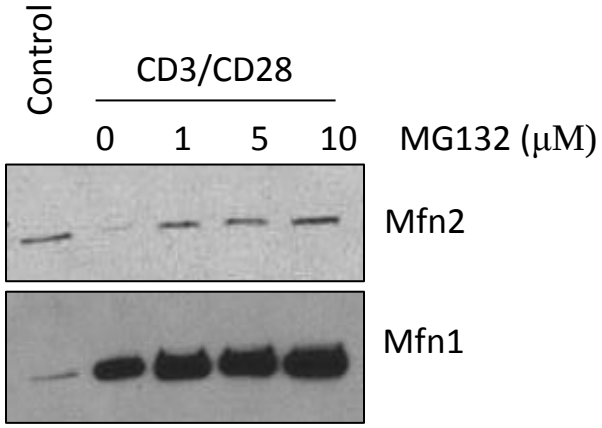
SUPPLEMENTAL FIGURE 1. RNA levels of Mfn2 in resting human T cells and T cells activated with α-CD3 and α-CD28 antibodies for different time periods (2, 6, 24, and 48 hrs) were assayed by real time PCR (n=3, performed in triplicate).



SUPPLEMENTAL FIGURE 2. Rapamycin treatment inhibited BrdU incorporation. Primary human T cells were activated with or without αCD3 and αCD28 antibodies in the presence or absence of rapamycin for 48 hrs. BrdU was added for last 24 hrs. The cells were then processed and stained according to the BrdU Staining Kit and Fixable Viability Dye eFluor 780 provided by the manufacturer (eBioscience). The stained cells were measured with Canto II flow cytometer (BD Bioscience), and the data were analyzed with the FlowJo software (Tree Star). Gating for BrdU positive cells was done using a sample of activated cells without BrdU treatment. One representative experiment out of two experiments is shown here.



SUPPLEMENTAL FIGURE 3. Primary human T cells were transfected with either scrambled siRNA (Scrambl) or Mfn2-specific siRNA (Mfn2) by electroporation. After 24 hours of culturing, cells were labeled with the Cell Proliferation Dye (CPD) eFluor 670 (eBioscience) as described by the protocol provided by the manufacturer with the following exception. Cells were stained with 2.5 µM dye (final) for 5 minutes at room temperature. The stained cells were activated with plate bound antibodies against CD3 and CD28 for 48 hours. The cells were expanded for another 24 hours in the presence of IL-2. (A) The cells were then stained with the Fixable Viability Dye (FVD) eFluor 780 according to the protocol provided by the manufacturer (eBioscience). (B) Live cells were measured for CPD staining with Canto II flow cytometer (BD Bioscience) and analyzed with the FlowJo software (Tree Star). Viable cells were gated initially based on the exclusion of the FVD dye. One representative experiment out of two experiments is shown here.



SUPPLEMENTAL FIGURE 4. Activation-induced downregulation of Mfn2 was mediated by proteasome. Primary human T cells were activated with or without α-CD3 and α-CD28 antibodies for 48 hrs. MG132 was added to the cultures for last 8 hrs. Whole cell lysates were prepared, and an equal amount of lysates (25 μg) were analyzed by western blot analysis. One representative experiment out of two experiments is shown.