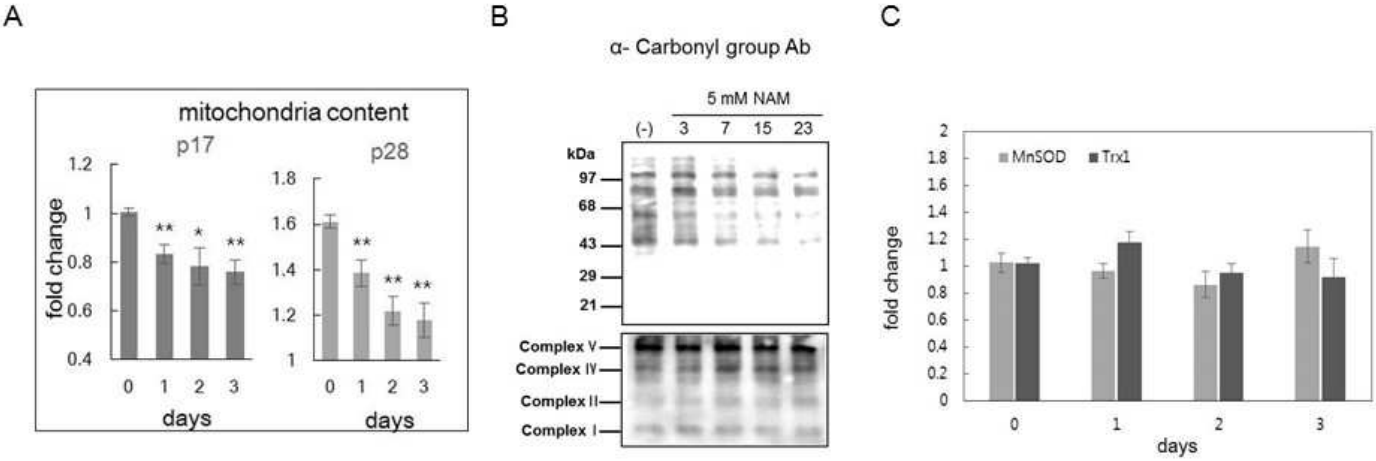
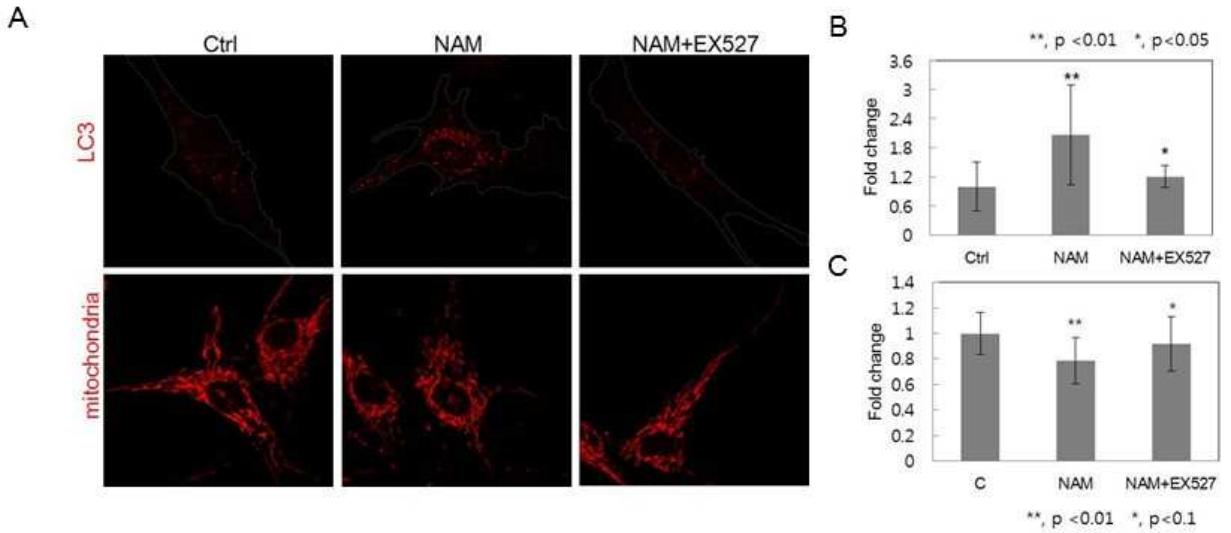
Supplemental Fig. 1



S1. Effect of NAM in the content and protein oxidation of mitochondria and levels of MnSOD and Trx1 expression. (A) Human fibroblasts at passage 17 or passage 28 were cultivated in the medium containing 5 mM NAM for 1, 2, or 3 days and stained with NAO and applied to flow cytometric quantitation of mitochondria content. In 3 days, mitochondria content decreased by 21.6 % and 25.4 % in p17 and p28 cells, respectively. (B) Decline in the levels of protein oxidation in the mitochondria upon NAM treatment. Fibroblasts were incubated for 3, 7, 15, and 23 d in the presence of 5 mM NAM, collected and fractionated for mitochondria. The same amount of mitochondrial proteins was loaded on SDS-PAGE, and the blotted proteins were treated with 2,4 dinitrophenylhydrazine and incubated with an antibody (ab6306, Abcam) against dinitrophenyl moiety (top). The filter was stripped and reprobed with OxPhos antibody (bottom). (C) NAM-treated fibroblasts were collected at days 1, 2, or 3, and mRNAs were isolated and applied to quantitative RT-PCR for the RNAs of MnSOD and Thioredoxin 1 (Trx1). Primers of the following sequences were used: MnSOD, 5'-TTCTGGACAAACCTCAGCCC-3' and 5'-CGTTTGATGGCTTCCAGCA-3'; Trx, 5'-CAGGGGAAT GAAAGAAAGG-3' and 5'-CAAGGTGAAGCAGATCG-3'; β-actin, 5'-TTTGAATGATGAGCCTTTGTG-3' and 5'-TCAGTGT ACAGGTAAGCCCT-3'. There was no significant change in the expression levels of MnSOD and Trx1 upon NAM treatment.

Supplemental Fig. 2



S2. Dependence of SIRT1 activity on NAM-induced mitophagy. Role of SIRT1 in NAM-induced mitophagy was confirmed by checking the effects of SIRT1 inhibition on autophagy and mitochondria fragmentation induced by NAM treatment. Fibroblasts were treated with NAM for 1 day alone or with EX527, a potent inhibitor of SIRT1. Cells were subjected to confocal imaging for LC3 or mitochondria to check either autophagosome formation (panels LC3) or mitochondria fragmentation (panels mitochondria) (A). The number of red colored LC3 puncta and the length of mitochondria filaments were measured using ImageJ. The numbers in 20 cells were averaged, and the means were plotted in (B) and (C), respectively. $**p < 0.01$; $*p < 0.05$ (compared with the untreated control, one-way ANOVA).