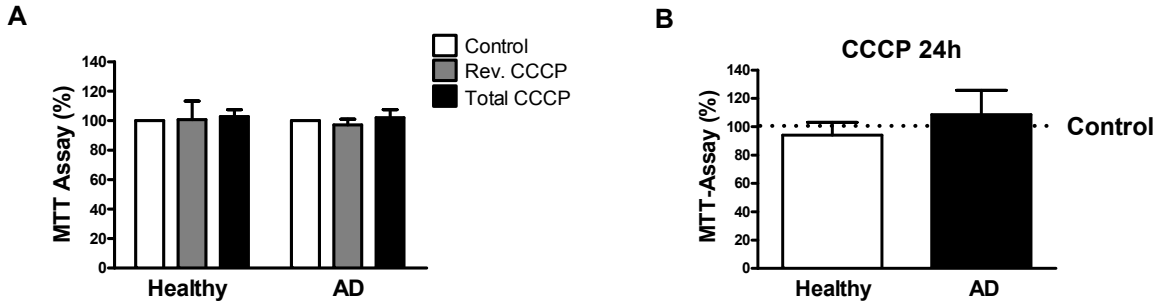


Figure S1. Viability of fibroblasts was not compromised during experimental conditions. (**A**) MTT assay of healthy and AD fibroblasts in a reversible treatment with CCCP (20 μM) for 6 h and then allowed to recover for 1 h (reversible CCCP) or treated for 7 h with CCCP (total CCCP). (**B**) MTT assay after the treatment with CCCP for 24 h. Analysis of four healthy/AD age-matched samples using the following couples: AG11362/AG05809, AG07310/AG06869, AG05813/AG06263 and AG05813/AG06263. All graphs show means and standard deviations of the indicated healthy/AD age-matched couple samples.

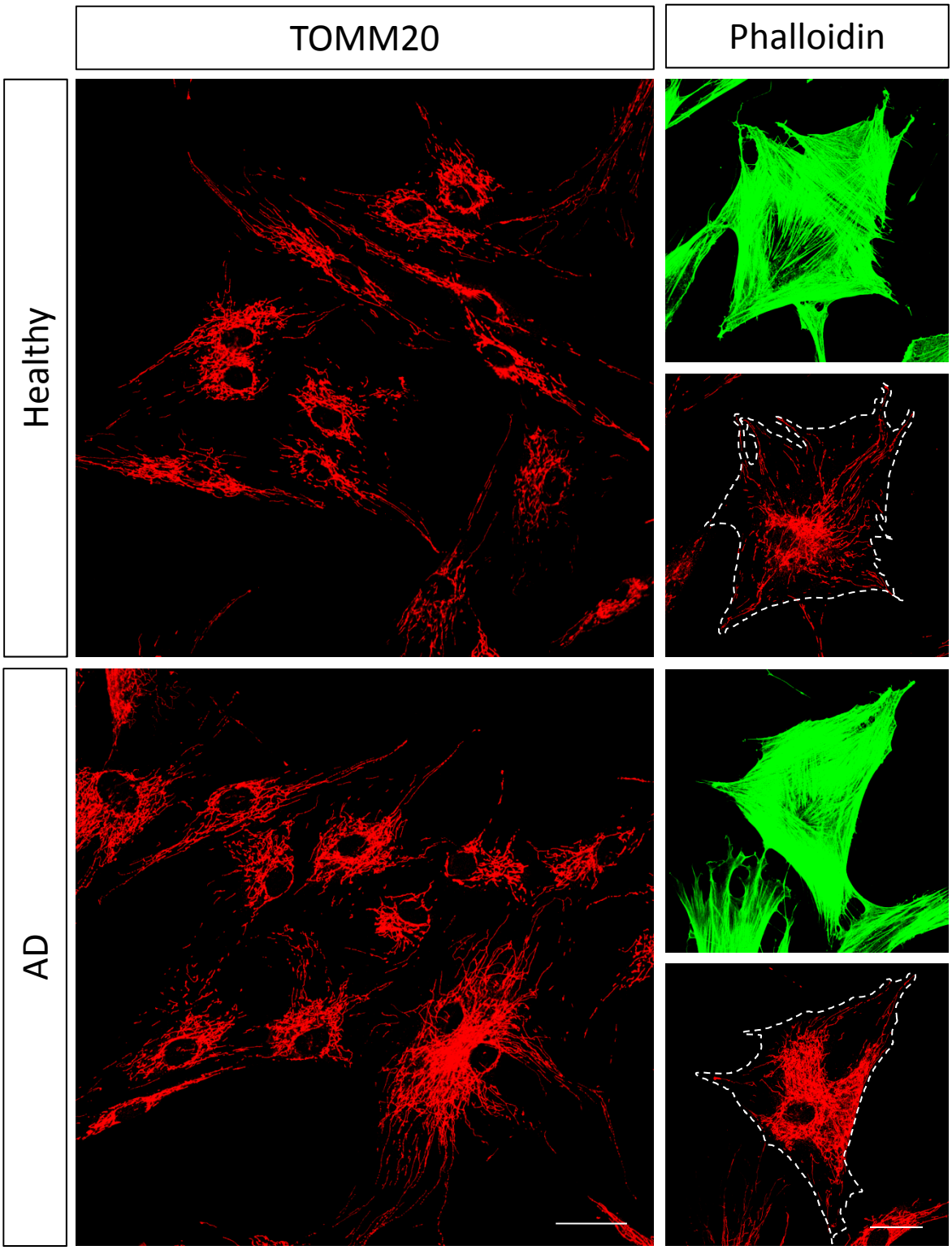


Figure S2. SAD fibroblasts exhibited increased mitochondrial surface. Representative confocal images of healthy and SAD fibroblasts showing TOMM20 in red in basal conditions. Phalloidin staining were used to delineate the boundary of the cell. Analysis of three healthy/AD age-matched samples using the following couples: AG11362/AG05809, AG07803/AG06262 and AG05813/AG06263. Results were quantified in Fig. 1E. Scale bar: 20 and 40 µm respectively.

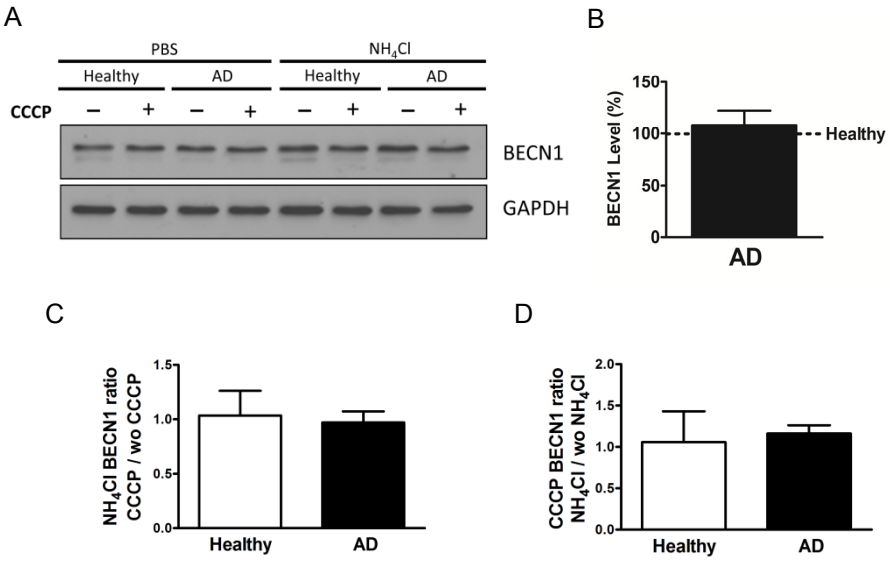


Figure S3. BECN1 expression during autophagy process.
(**A**) Representative Western blot of BECN1 expression in the cells treated with CCCP (20 μM) for 24 h, when indicated, followed by an additional treatment of PBS or NH_4Cl (15 mM) for 6 h in the presence or absence of CCCP. (**B**) Quantification of the levels of BECN1 under basal conditions. (**C**) Quantification of BECN1 synthesis ratio represented as the values of the cells treated with CCCP and NH_4Cl with respect to the condition without CCCP but maintaining NH_4Cl treatment. (**D**) Quantification of BECN1 degradation ratio obtained by the relation between the values of the cells treated with CCCP and NH_4Cl and the ones without NH_4Cl but maintaining CCCP treatment. All graphs show means and standard deviations of the following healthy/AD couples AG11362/AG05809, AG07310/AG06869, AG07803/AG06262 and AG05813/AG06263.

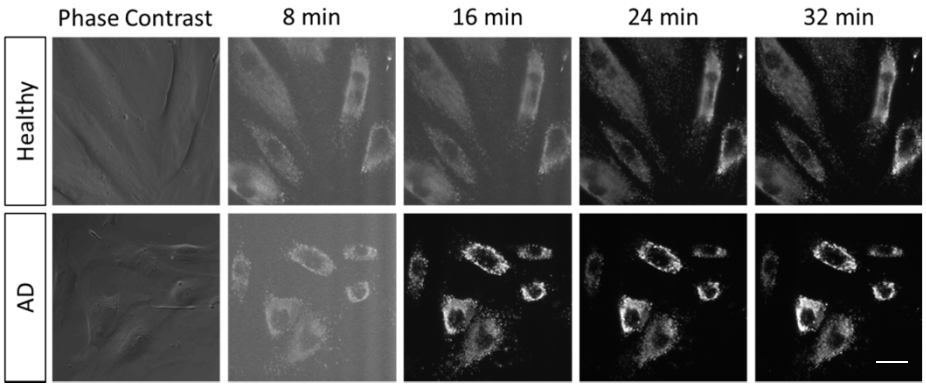


Figure S4. CTSB activity along time. Representative field of fibroblasts showing phase contrast and fluorescence images exhibiting the time course of intracellular CTSB activity as red fluorescence by using the fluorogenic substrate Magic Red MR-(RR)2. The couples used for this experiment were: AG11362/AG05809, AG05813/AG06263 and AG07310/AG06869. Results were quantified in Fig. 3E. Scale bar: 40 μm.

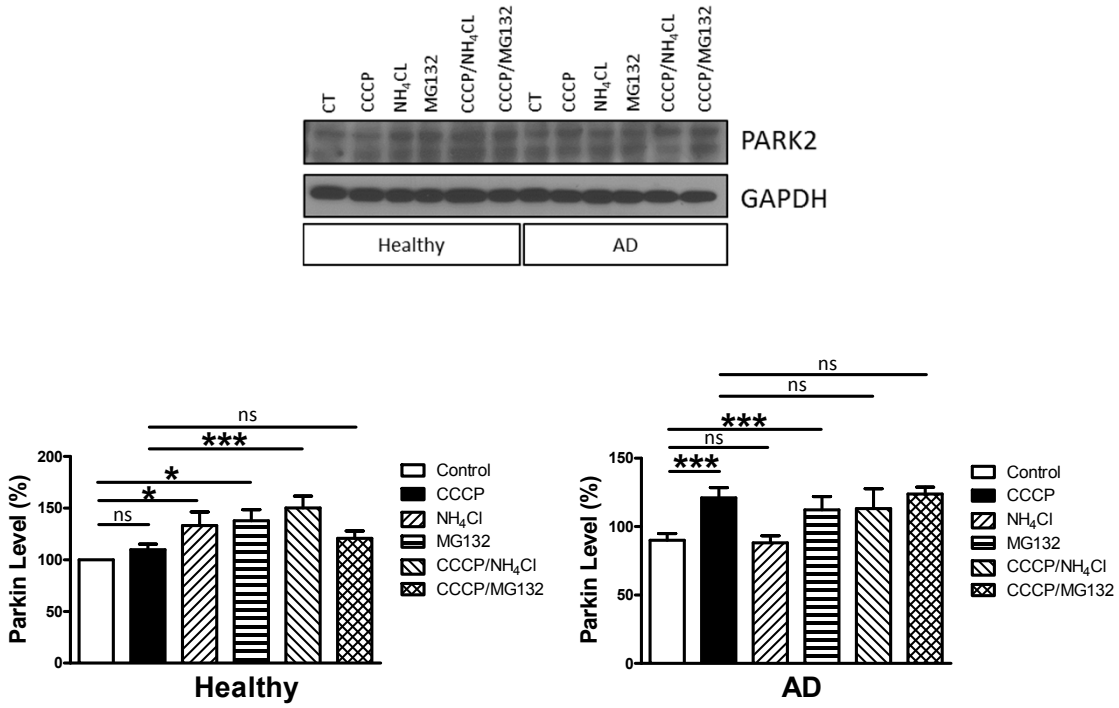


Figure S5. Degradation flux of PARK2 by autophagy is impaired in SAD fibroblasts. Representative Western blot and quantification of PARK2 levels in cells treated or 3 h with 20 μM CCCP followed by 15 mM NH_4Cl and 10 μM MG132 for 6 h using the following cells: AG11362/AG05809, AG07310/AG06869 and AG05813/AG06263. ns: not significant, *p<0.05, ***p<0.001.

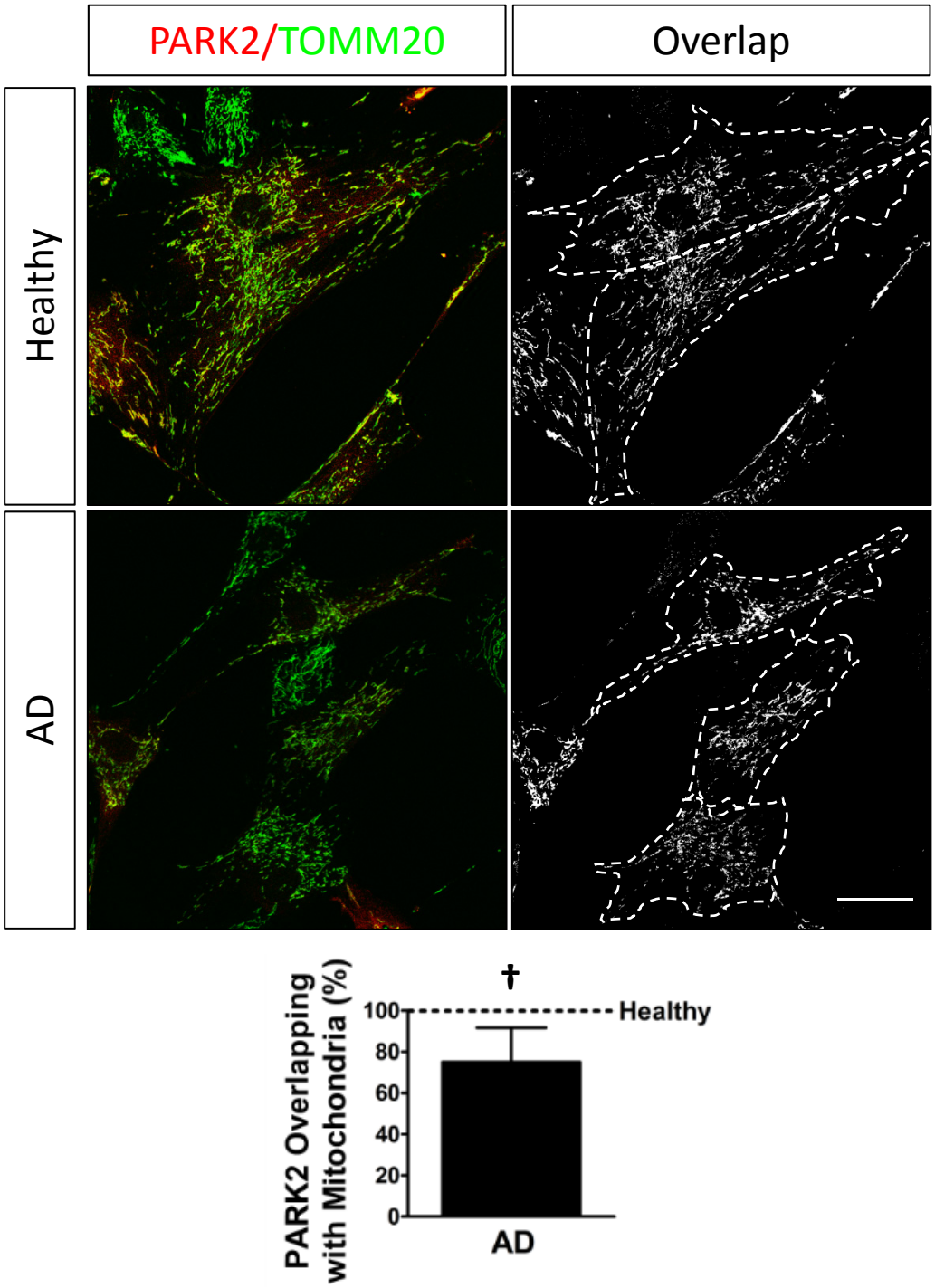


Figure S6. Study of the colocalization of PARK2 and TOMM20 during mitophagy. Representative confocal microscopy immunofluorescence images showing PARK2 in red and TOMM20 in green in cells treated with 20 µM CCCP for 1 h. On the right, binary images representing the colocalization of both labels and dotted line delimits cytoplasm of each cell according to phalloidin label (not shown). Quantification of the colocalization between PARK2 and TOMM20 expressed as area occupied by the overlapping elements per cell. Graph shows means and standard deviation of the results obtained using the following healthy/AD couples AG11362/AG05809, AG07310/AG06869 and AG05813/AG06263. †$p < 0.08$. Scale bars: 40 µm.

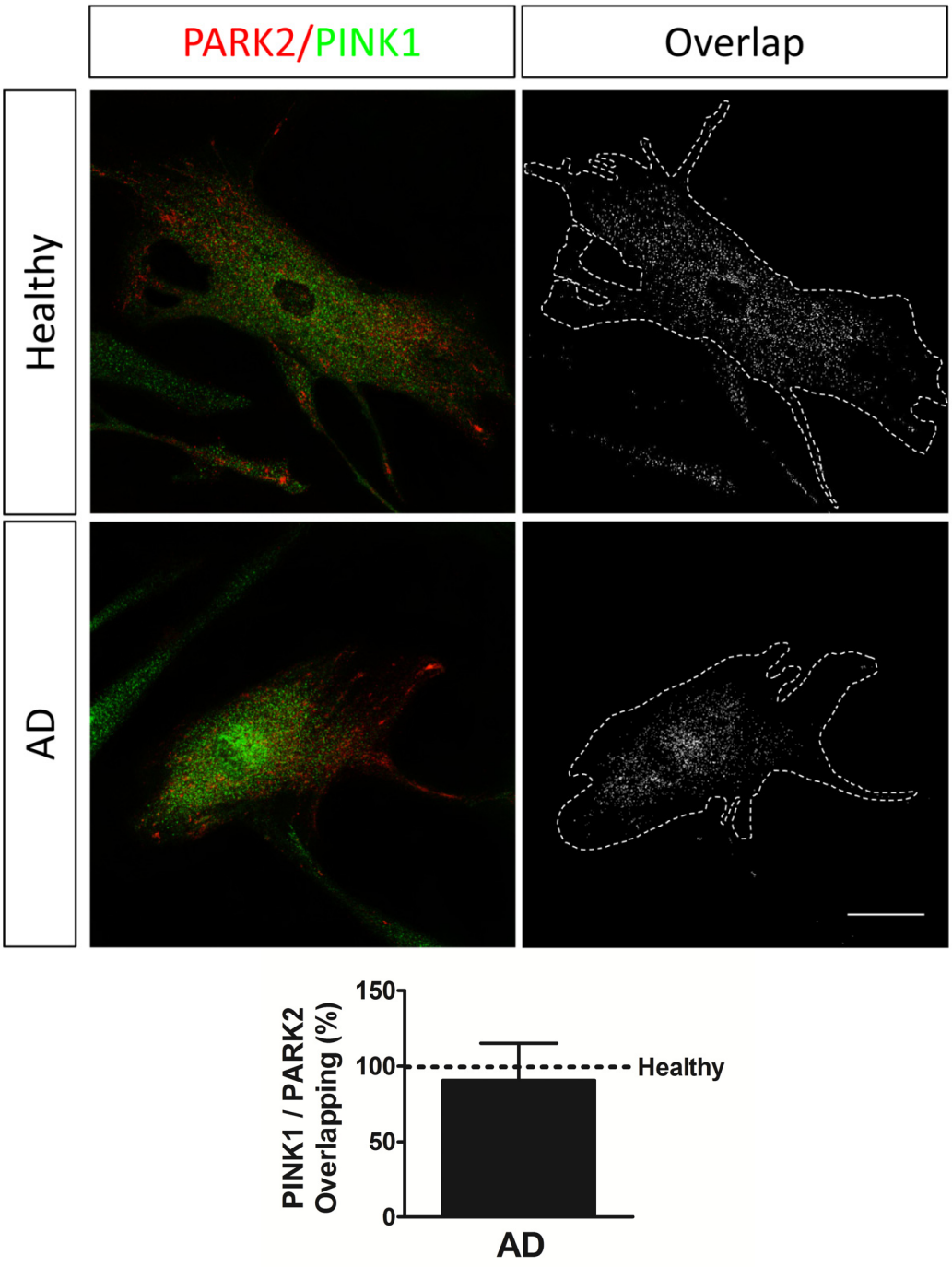


Figure S7. Study of the colocalization of PARK2 and PINK1 during mitophagy. Representative confocal microscopy immunofluorescence images showing PARK2 in red and PINK1 in green in cells treated with 20 µM CCCP for 1 h. On the right, binary images representing the colocalization of both labels and dotted line delimits cytoplasm of each cell determined by phalloidin label (not shown). Quantification of the colocalization between PARK2 and PINK1 expressed as area occupied by the overlapping elements per cell. Graph shows means and standard deviation of the results obtained using the following healthy/AD couples AG11362/AG05809, AG07310/AG06869 and AG05813/AG06263. Scale bars: 40 µm.

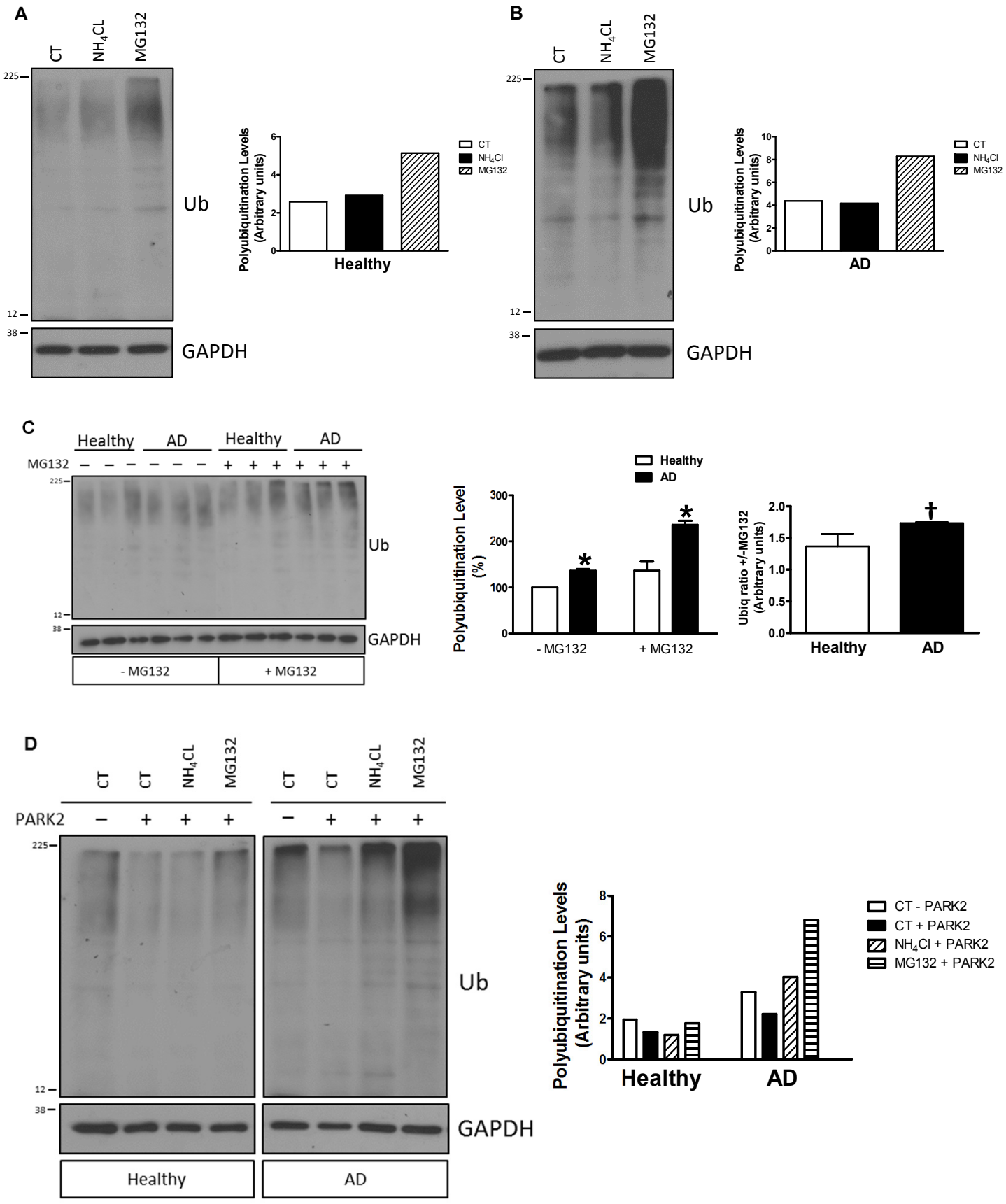


Figure S8. Study of polyubiquitinated protein degradation. (**A**) Representative Western blot and quantification of ubiquitination levels after the treatment with 15 mM NH_4Cl and 10 µM MG132 for 6 h in healthy cells. (**B**) Similar analysis as in (**A**) in SAD fibroblasts. (**C**) Representative Western blot and quantification of healthy and AD ubiquitination levels after the treatment 10 µM MG132 for 6 h. The following couples were used for AG11362/AG05809, AG05813/AG06263 and AG07310/AG06869. (**D**) Representative Western blot and quantification of ubiquitination levels when the cells were infected or not with a lentivector encoding WT *PARK2* and treated with 15 mM NH_4Cl and 10 µM MG132 for 6h. The couple used for (**A, B** and **D**) was: AG11362/AG05809.

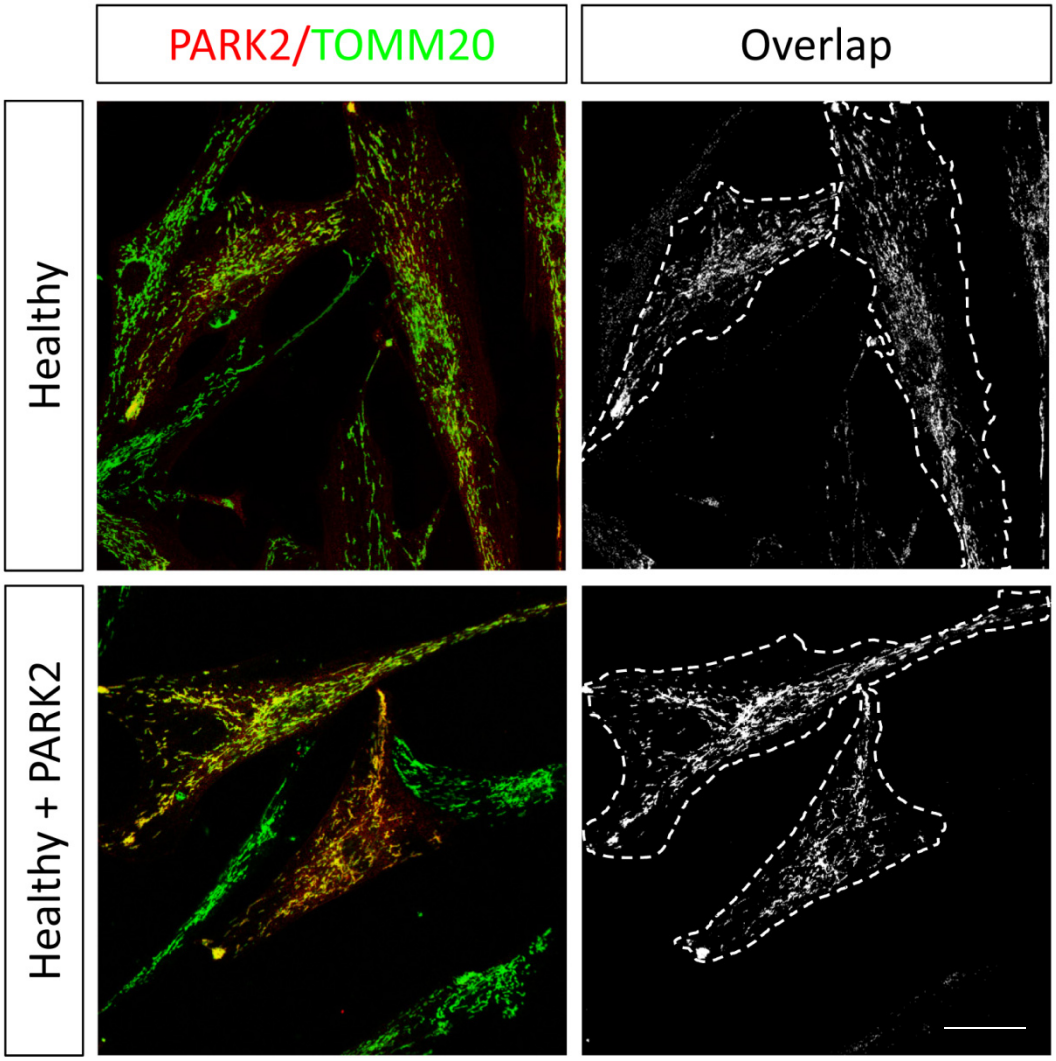


Figure S9. Translocation of PARK2 to mitochondria in healthy cells. Confocal microscopy images showing PARK2 in red and TOMM20 as a mitochondria constitutive marker in green of healthy fibroblasts infected with a lentivector encoding PARK2 treated with CCCP (20 µM) for 1 h. On the right, binary images representing the colocalization of both labels and dotted line delimits cytoplasm of each cell determined by phalloidin label (not shown). The couples used for this experiment were: AG11362/AG05809, AG07310/AG06869 and AG05813/AG06263. Results were quantified in Fig. 6H. Scale bar: 40 µm.

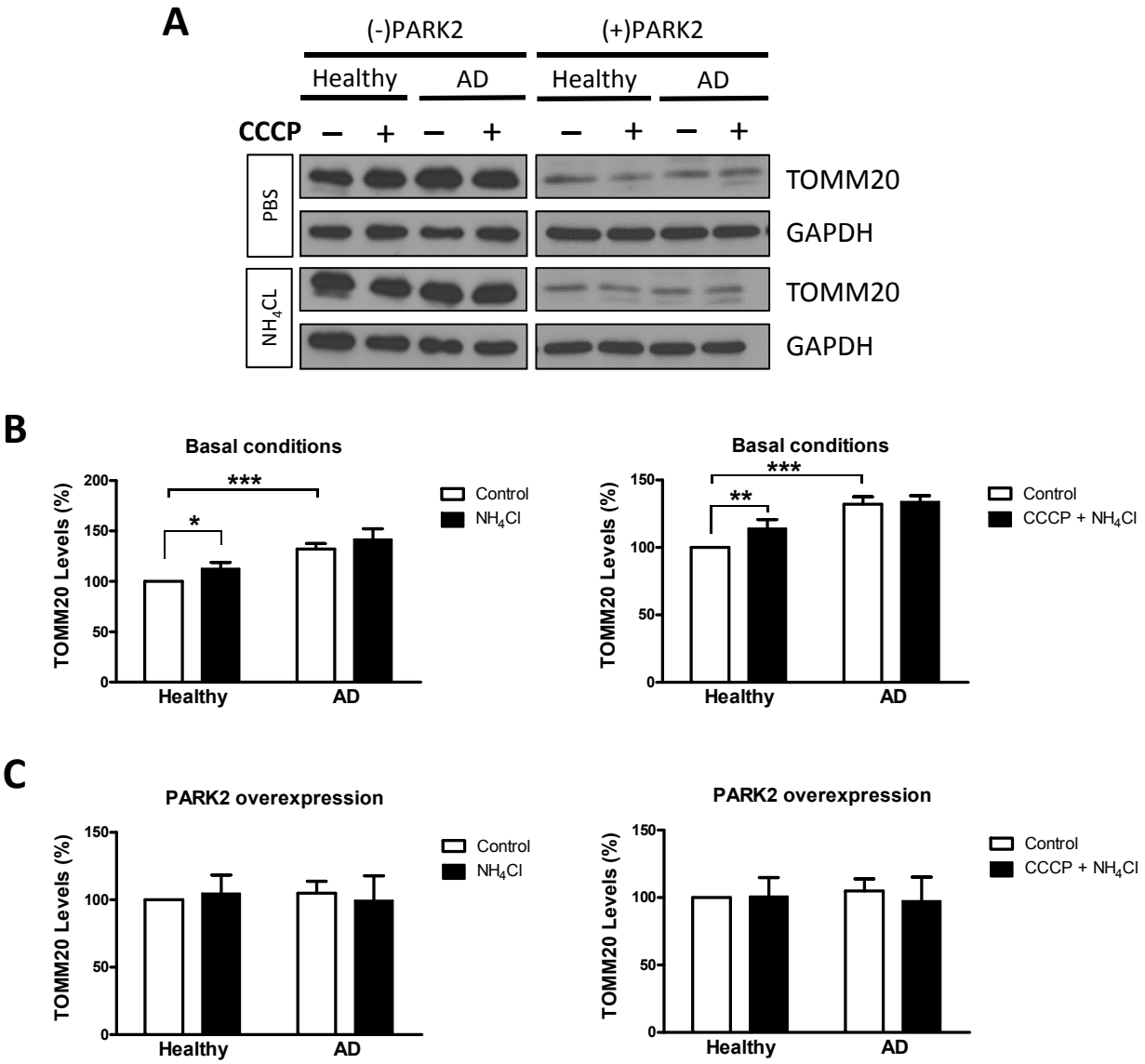
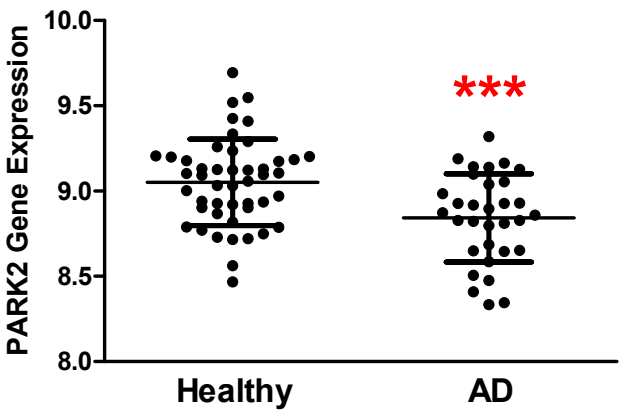
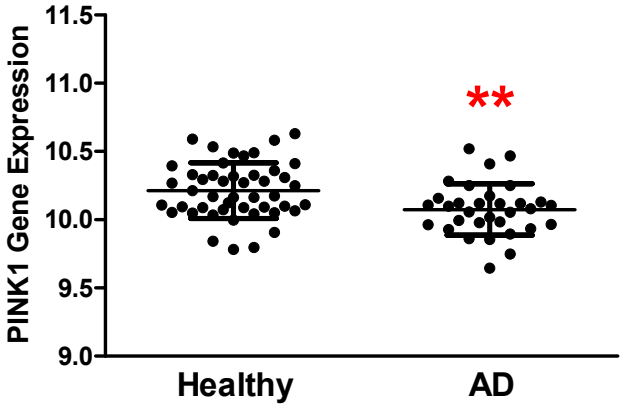


Figure S10. TOMM20 recycling by autophagy is impaired in SAD fibroblast. (**A**) Representative Western blot of TOMM20 expression in fibroblasts infected with a lentivector encoding WT PARK2. Then, cells were treated with CCCP (20 μM) followed by an additional treatment of PBS or NH_4Cl (15 mM). (**B**) Quantification of Western Blot showing the levels of TOMM20 under basal condition after treatment with NH_4Cl or CCCP plus NH_4Cl. (**C**) Quantification of Western Blot showing the levels of TOMM20 when PARK2 is overexpressed after treatment with NH_4Cl or CCCP plus NH_4Cl. The following couples were used: AG11362/AG05809, AG05813/AG06263 and AG07310/AG06869. **p*<0.05, ***p*<0.01, ****p*<0.001.





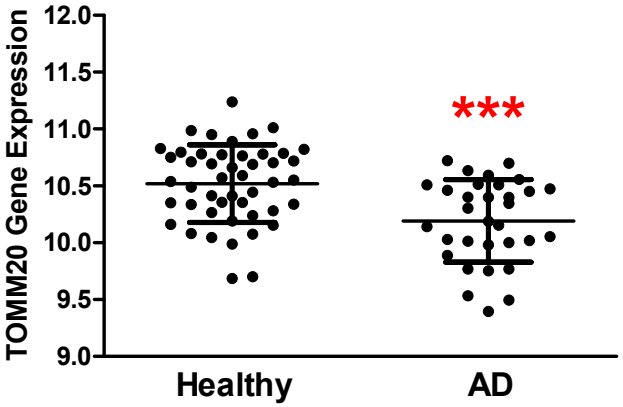


Figure S11. Decreased gene expression of mitophagy-related proteins in AD patient brains. Graphs show expression profiling by array (Affymetrix Human Gene 1.0 ST Array) of PARK2, PINK1 and TOMM20 of human brain samples classified into healthy (n= 47) vs AD (n=32) retrieved from the Hisayama study. Differences in PARK2, PINK1 and TOMM20 expression between healthy and AD were calculated using Student's T test. $^{**}p<0.01$, $^{***}p<0.001$