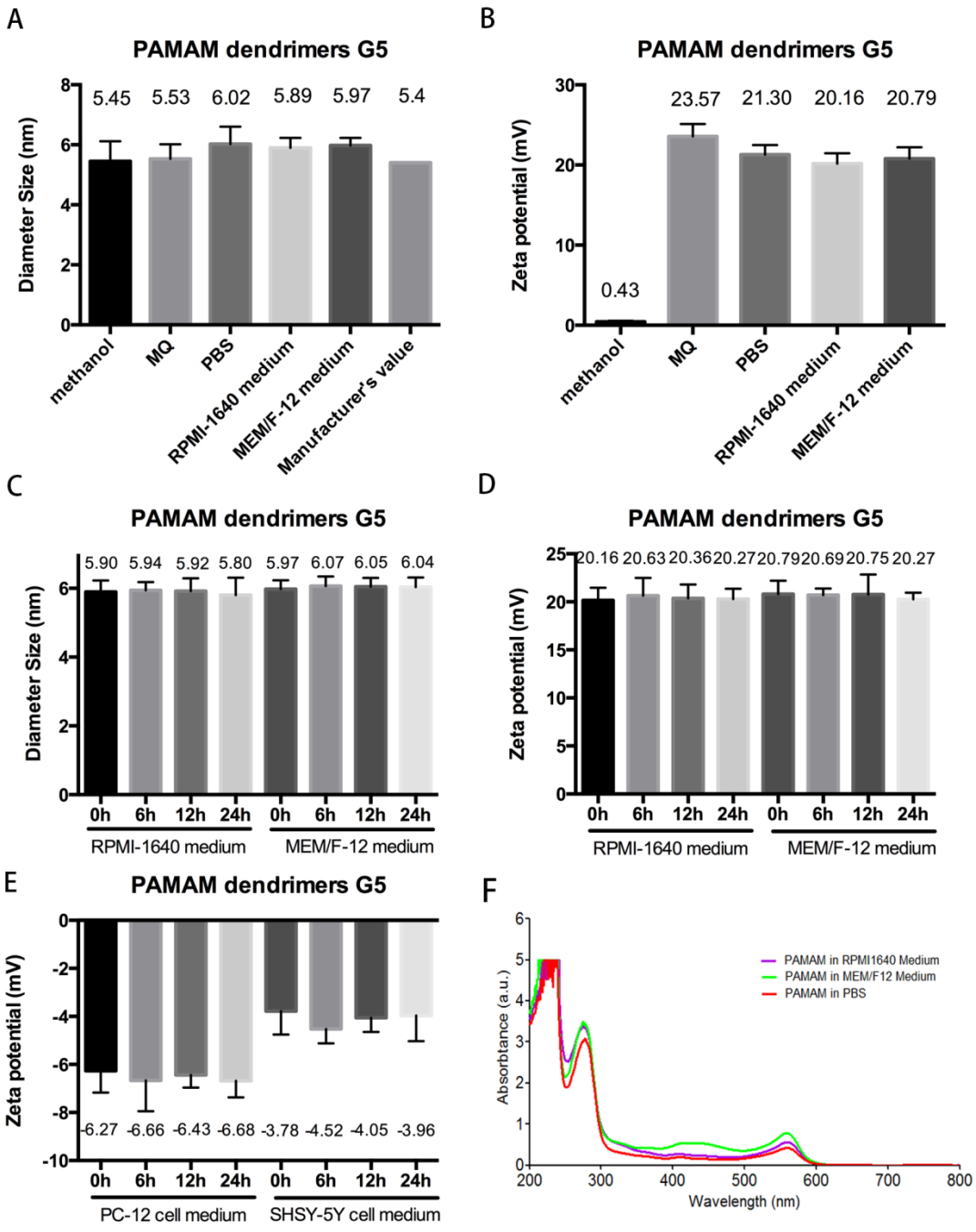
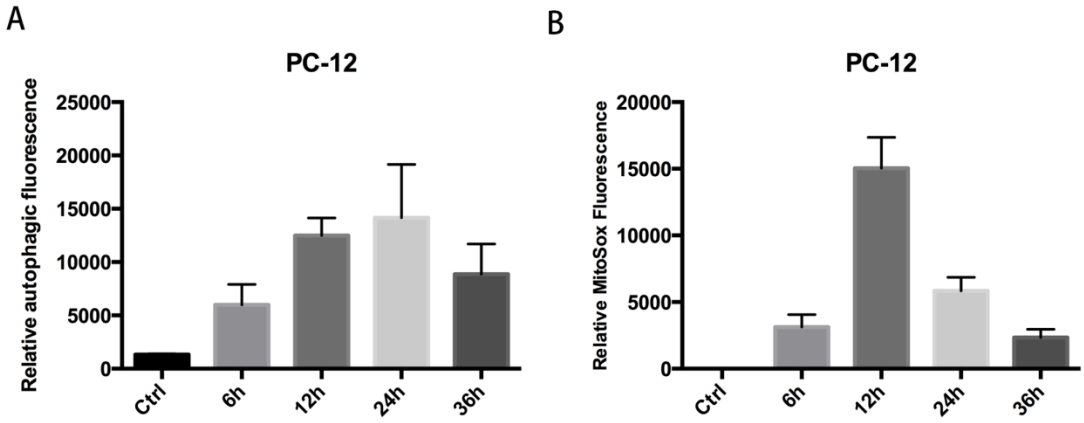
Supplementary Material



Supplemental Figure 1 Characterization of PAMAM dendrimers G5

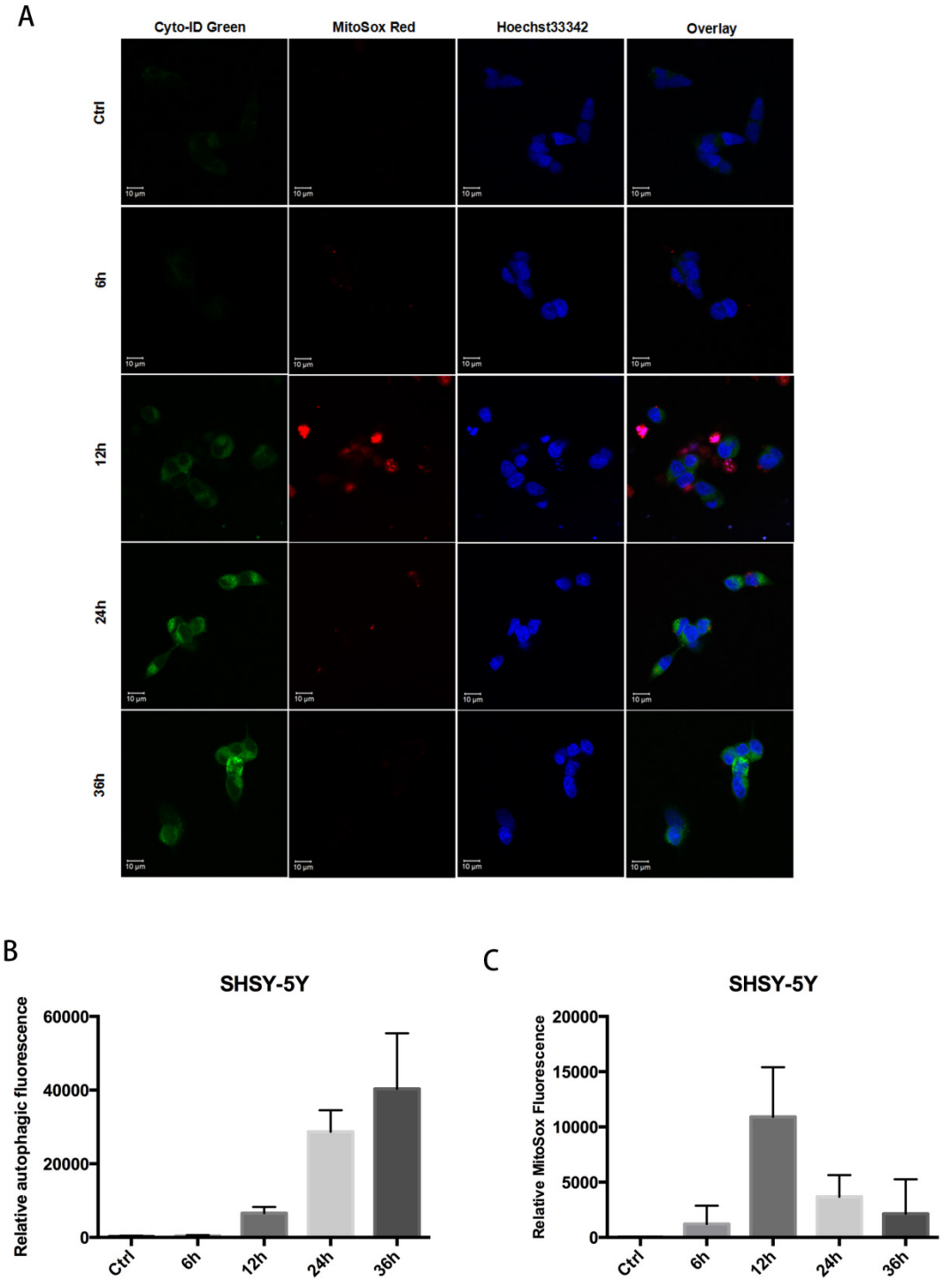
(A) The size distributions and (B) the zeta potentials of the dendrimers were measured by using photon correlation spectroscopy. The measurements were performed on 20 μM of PAMAM dendrimers solutions at 23 ℃in methanol; Milli Q distilled water; PBS; RPMI-1640 medium; or MEM/F-12 medium. Each sample

was measured three times. (C) The size distributions of the dendrimers were performed on 20 μM of PAMAM dendrimers solutions at 23 ℃in RPMI-1640 medium and MEM/F-12 medium at different times. (D) The zeta potentials of the dendrimers were measured on 20 μM of PAMAM dendrimers solutions at 23 ℃in RPMI-1640 medium or MEM/F-12 medium at different times. (E) The zeta potentials of the dendrimers were measured on 20 μM of PAMAM dendrimers solutions at 23 ℃in PC-12 cell medium or SHSY-5Y cell medium at different times. (F) UV/visible absorption spectroscopic analysis of PAMAM dendrimers solutions in PBS; RPMI-1640 medium; or MEM/F-12 medium was performed using a Perkin Elmer Lambda 900 UV/visible/NIR absorption spectrometer. Equal molarities of 15 μM of PAMAM dendrimers G5 were used for spectroscopic analysis.



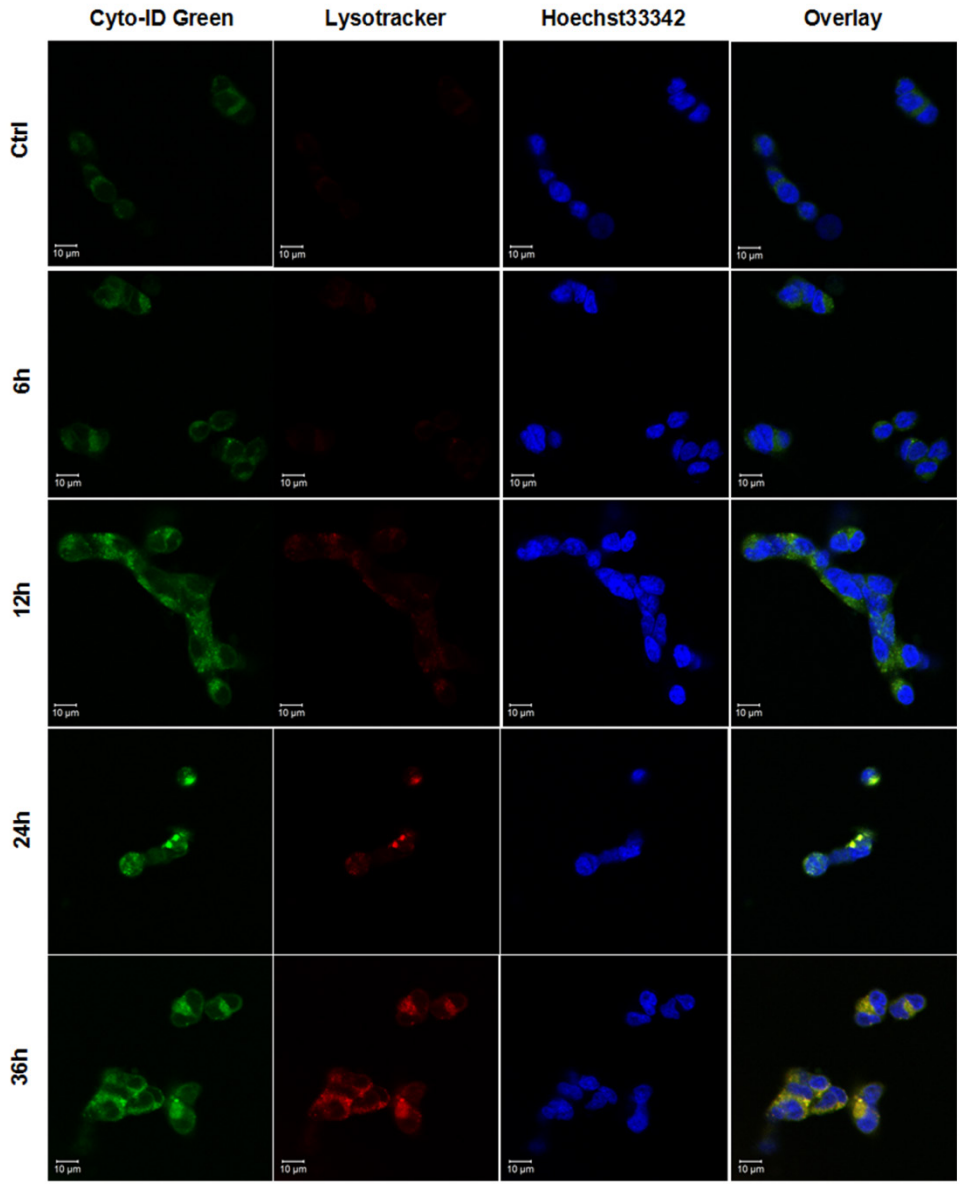
Supplemental Figure 2 Relative quantitative analysis of relative autophagic fluorescence and oxidative fluorescence intensity in PAMAM dendrimers-treated PC-12 cells

(A) The relative autophagic fluorescence intensity was quantified by confocal microscopy software. All data were presented as the means ± SD of more than three samples. (B) The relative oxidative fluorescence intensity was quantified by confocal microscopy software. All data were presented as the means ± SD of more than three samples.



Supplemental Figure 3 Autophagy and oxidative stress were triggered by PAMAM dendrimers in SHSY-5Y cells

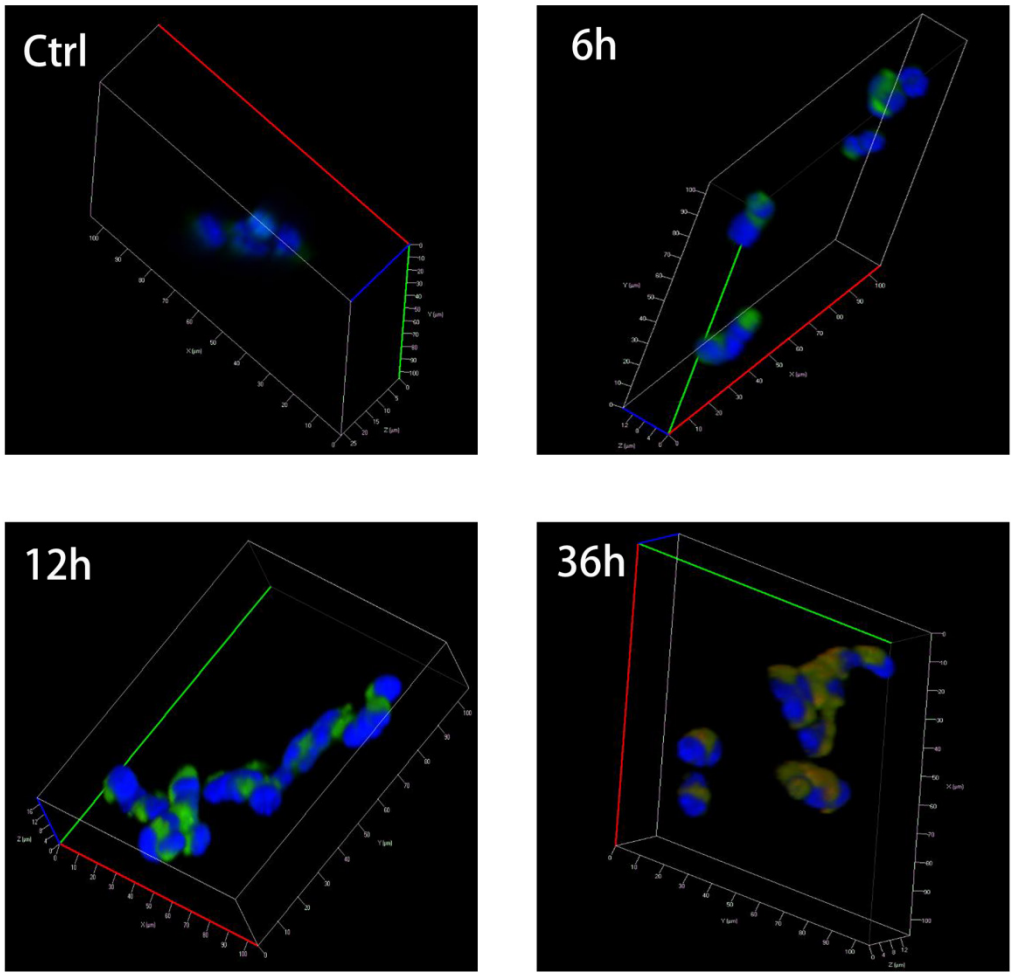
(A) Cells were plated in cell culture dishes with glass bottom. After treated with 10 μg/mL of PAMAM dendrimers G5 for 6h, 12h, 24h, and 36h, cell samples were stained with Cyto-ID Green autophagy dye and MitoSox Red dye and analyzed by confocal microscopy. Untreated cells were used as negative control. (B) and (C) The relative autophagic fluorescence and oxidative fluorescence intensity were quantified by confocal microscopy software. All data were presented as the means ± SD of more than three samples.



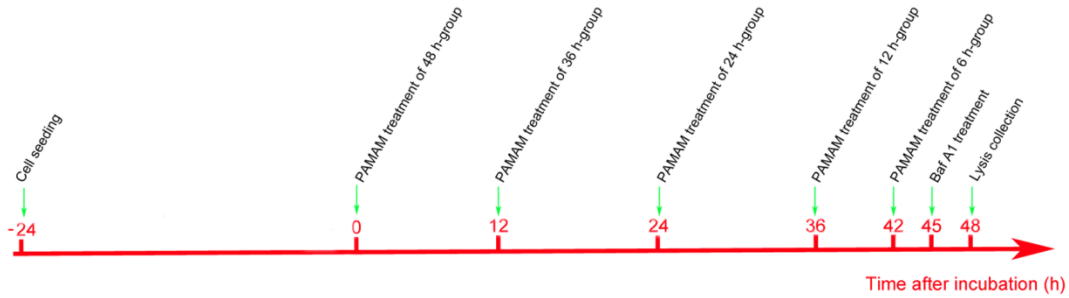
Supplemental Figure 4 Autophagic flux was induced by PAMAM dendrimers in SHSY-5Y cells.

Cells were plated in cell culture dishes with glass bottom. After treated with 10 μg/mL of PAMAM dendrimers

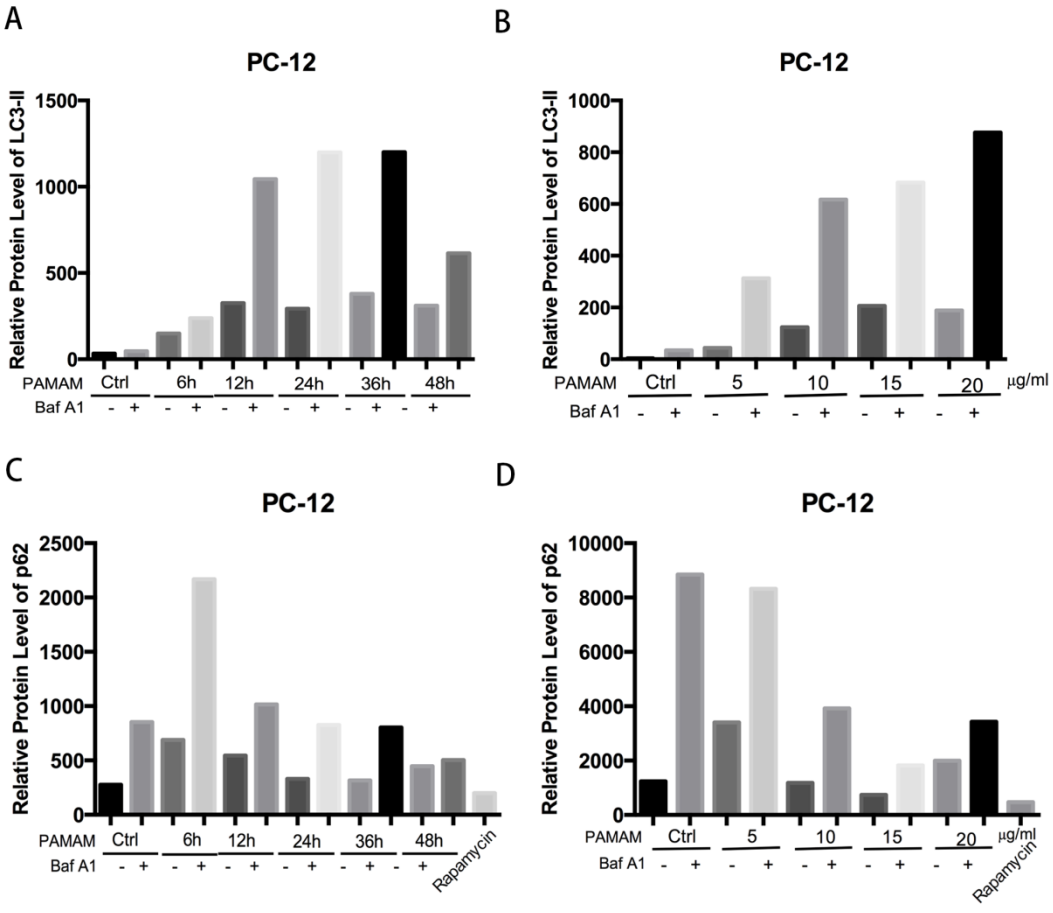
G5 for 6h, 12h, 24h, and 36h, cell samples were stained with Cyto-ID Green dye and Lysotracker Red dye and analyzed by confocal microscopy. Untreated cells were used as negative control.



Supplemental Figure 5 Zoomed-in images with a z-stack showed co-localization of autophagosome-related fluorescence and lysosomes-related fluorescence in PAMAM dendrimers-induced autophagic flux.



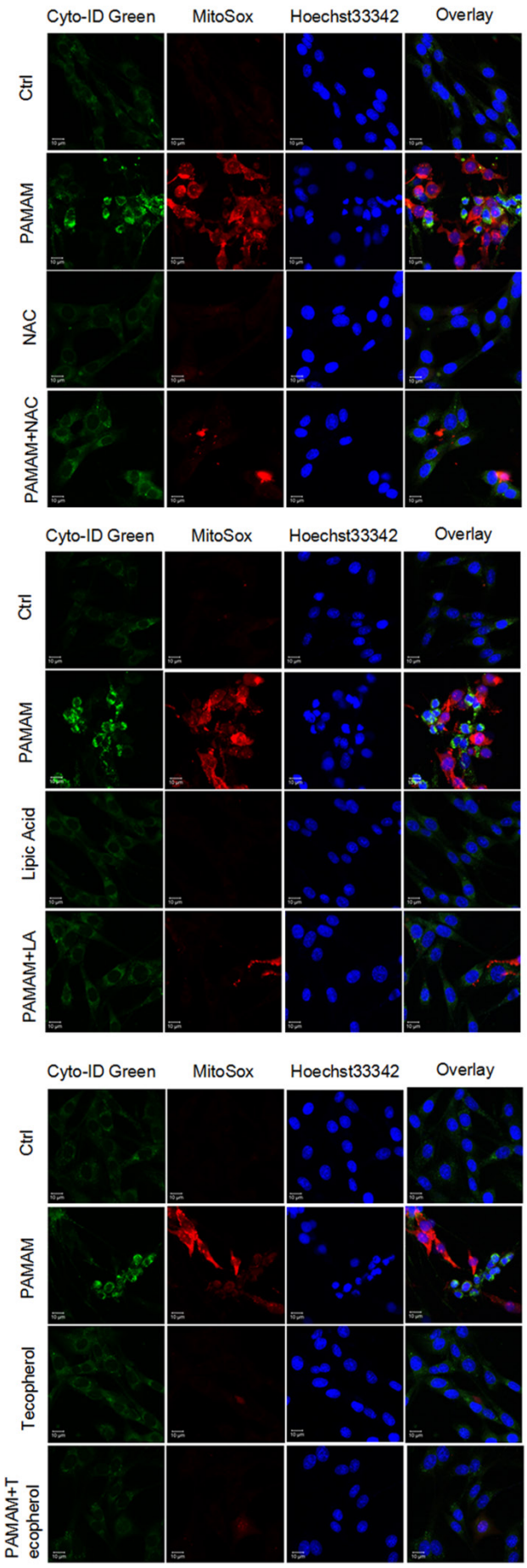
Supplemental Figure 6 Scheme of Baf A1 treatment in PAMAM dendrimers-treated PC-12 cells



Supplemental Figure 7 Relative protein level of LC3-II and p62 in PAMAM dendrimers-induced

autophagic flux

(A) After exposure to 10μg/mL of PAMAM dendrimers G5 for indicated times, PC-12 cells were treated with or without 20 nM of Baf A1 for another 3 h. Changes in the expression of LC3 was examined by Western blot. The relative protein level of LC3-II was quantified by ImageJ software. (B) After exposure to a series of concentrations of PAMAM dendrimers for 24 h, PC-12 cells were treated with or without 20 nM of Baf A1 for another 3 h. Changes in expression of LC3 was examined by Western blot. The relative protein level of LC3-II was quantified by ImageJ software. (C) After exposure to 10μg/mL of PAMAM dendrimers G5 for indicated times, PC-12 cells were treated with or without 20 nM of Baf A1 for another 3 h. Changes in the expression of p62 was examined by Western blot. Cells treated by 10 nM of rapamycin for 24 h were as positive control. The relative protein level of p62 was quantified by ImageJ software. (D) After exposure to a series of concentrations of PAMAM dendrimers for 24 h, PC-12 cells were treated with or without 20 nM of Baf A1 for another 3 h. Changes in expression of p62 were examined by Western blot. Cells treated by 10 nM of rapamycin for 24 h were as positive control. The relative protein level of p62 was quantified by ImageJ software.


Cyto-ID Green
MitoSox
Hoechst33342
Overlay
Ctrl
PAMAM
NAC
PAMAM+NAC
Cyto-ID Green
MitoSox
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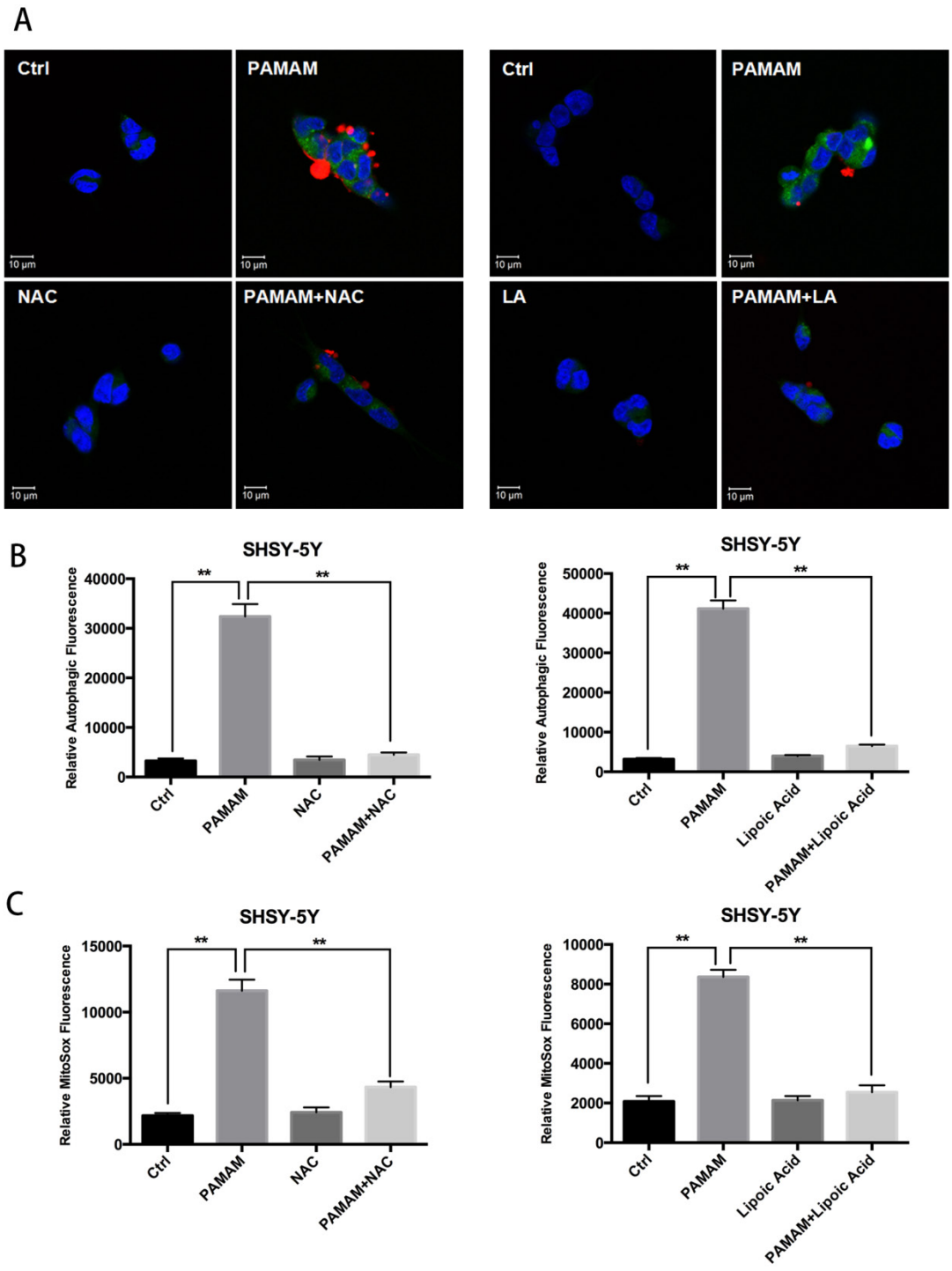
Overlay
Ctrl
PAMAM
Lipic Acid
PAMAM+LA
Cyto-ID Green
MitoSox
Hoechst33342
Overlay
Ctrl
PAMAM
Tecopherol
PAMAM+Tecopherol

Supplemental Figure 8 The larger versions of confocal images on Suppression of reactive oxygen species impaired PAMAM dendrimers-induced autophagic effects

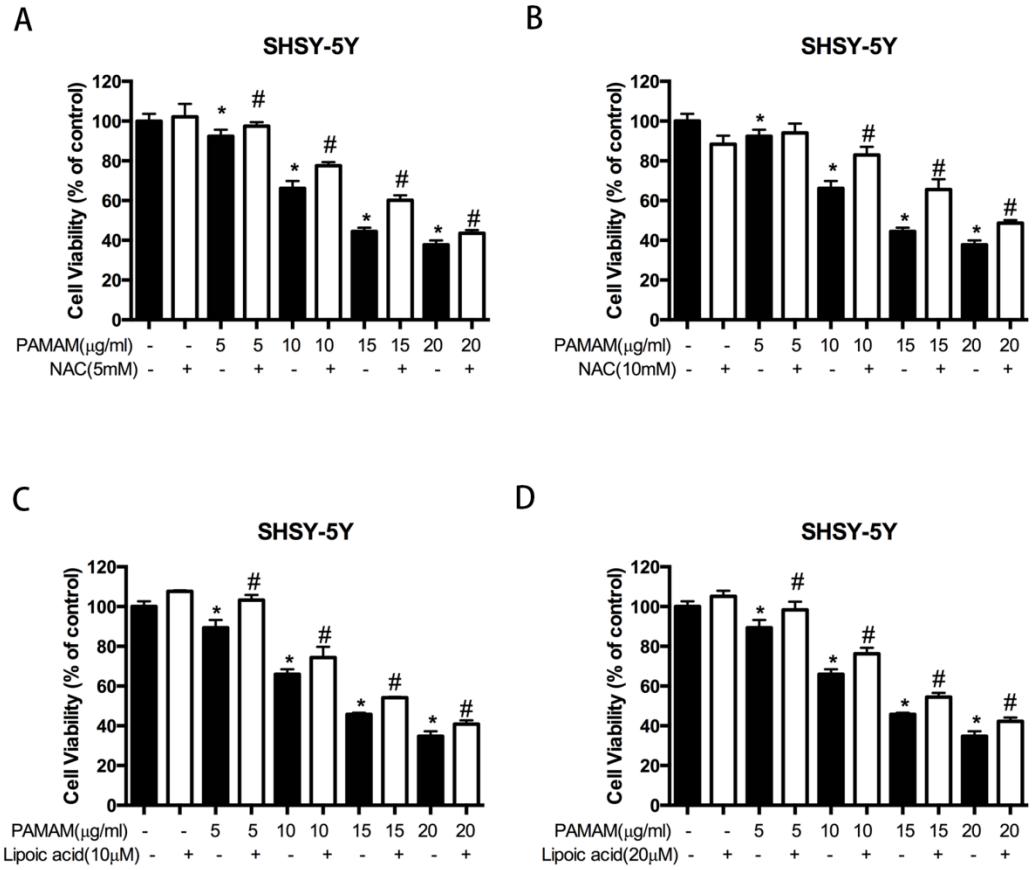
PC-12 cells were incubated with or without PAMAM dendrimers in the presence or absence of antioxidants for 24h. Cell samples were stained with Cyto-ID Green autophagy dye and MitoSox Red dye and analyzed by confocal microscopy.



Supplemental Figure 9 Suppression of reactive oxygen species impaired PAMAM dendrimers-induced autophagic effects in SHSY-5Y cells.

(A) SHSY-5Y cells were incubated with or without PAMAM dendrimers in the presence or absence of antioxidants for 24h. Cell samples were stained with Cyto-ID Green dye and MitoSox Red dye and analyzed

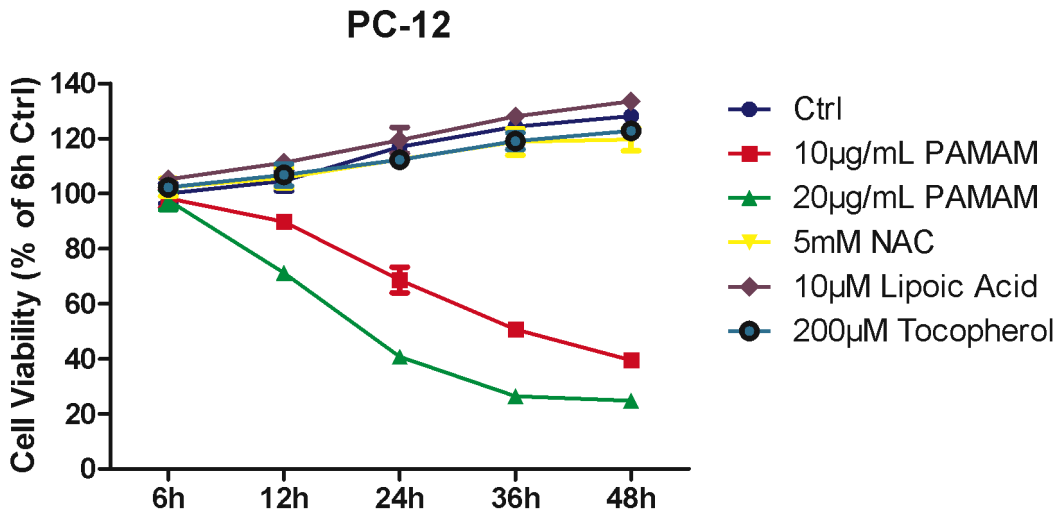
by confocal microscopy. (B) and (C)The relative oxidative fluorescence intensity was quantified by confocal microscopy software. All data were presented as the means ± SD of more than three samples. *p<0.05 versus Ctrl, or versus PAMAM dendrimers G5-treated group.



Supplemental Figure 10 Blocking reactive oxygen species reduced PAMAM dendrimers-induced SHSY-5Y neuronal cell death.

(A) and (B) SHSY-5Y cells were incubated with or without PAMAM dendrimers in the presence or absence of NAC for 24h. Cell growth inhibition was analyzed by CCK-8 assay. All data were presented as the means ± SD of four samples. *p<0.05 versus Ctrl, and #p<0.05 versus PAMAM dendrimers G5-treated group. (C) and (D) SHSY-5Y cells were incubated with or without PAMAM dendrimers in the presence or absence of lipic acid for 24h. Cell growth inhibition was analyzed by CCK-8 assay. All data were presented as the means ± SD

of four samples. *p<0.05 versus Ctrl, and #p<0.05 versus PAMAM dendrimers G5-treated group.



Supplemental Figure 11 Cell viability curves for PAMAM dendrimers and antioxidant treatments on PC-12 cells

PC-12 cells were incubated with or without PAMAM dendrimers (10 μg/mL and 20 μg/mL), antioxidants NAC (5mM), lipic acid (10μM), or tocopherol (200μM) for different times. Cell growth inhibition was analyzed by CCK-8 assay. All data were presented as the means ± SD of four samples.