**Supplementary Figure Legends**

**Figure S1. Expression of autophagic proteins in ANSCs *in vitro.*** ANSCs were isolated and expanded in culture. After seeding on matrigel-coated coverslip, they were fixed and stained for autophagic markers. Expression of Ambra1 (**A**) and Beclin 1 (**B**) in Nestin-positive ANSCs *in vitro*. (**C**) Expression of LC3 in Sox2-positive ANSCs *in vitro* (Scale bars, 10 μm). Cell nuclei are detected by Dapi (blue).

**Figure S2. Autophagy impacts the proliferating potential of ANSCs *in vitro.***

(**A**) Inhibition of autophagy in ANSCs by means of 3-methyl-adenine (3-MA) *in vitro*. ANSCs were pre-treated with 3MA for 24h and then medium was replaced with normal medium for other 4 days. Representative images of untreated (Ctrl, control) and 3MA-treated (3MA) neurospheres (Scale bar, 200 μm). As indicated by the graph (right), the diameter (mean ± SEM) of secondary neurospheres is significantly reduced by autophagy inhibition (*n*=3). (**B**) Dissociated neurospheres from different *Beclin 1* genotypes were plated on matrigel-coated coverslips for 20 min and then fixed for immunostaining. Representative confocal microscope images of proliferating cells (Ki67-positive) in *Beclin 1*$^{+/-}$and wild type ANSCs *in vitro* are shown. The number of Ki67 positive cells, indicated by the arrowheads (over total Dapi-positive cells, in blue) is significantly reduced in dissociated ANSCs from *Beclin 1*$^{+/-}$ in comparison with the wild type (see graph at right). Values represent mean ± SEM, (*n*=6), **P*<0.05, ***P*<0.01.

**Figure S3. Autophagy impairment enhances Etoposide-induced cell death in ANSCs, *in vitro*.** (**A**) ANSCs under proliferating conditions were divided into three groups: untreated (Ctrl, control), treated with Etoposide (Eto) and with Eto in combination with 3MA. Cell lysates were obtained and subjected to immunoblotting. The apoptotic markers CPARP and CCasp3 are strongly increased in Eto-treated samples in which autophagy has been inhibited (graphs show corresponding densitometric analysis of the bands, (*n*=3). (**B**) Representative confocal microscope images of secondary neurospheres derived from *Beclin 1*$^{+/-}$ and wild-type mice upon Etoposide-induced cell death. Apoptotic cells were detected as those stained by CCasp3. The graph (right) shows a significant increase in the number of dying cells in *Beclin 1*$^{+/-}$ neurospheres when compared with wild type ones. Values represent mean ± SEM, (*n*=6),*P<0.05; ***P<0.001.

**Figure S4. Autophagy is enrolled during differentiation of ANSCs.** Isolated and expanded ANSCs were allowed to differentiate by a differentiation medium. At the indicated time points, cells were fixed for immunostaining or collected for protein extraction and immunoblotting. (**A**) Representative confocal microscope images of cells at 12h and 72h after induction of differentiation. As long as the cells were kept in differentiation medium, as expected, they exhibited a marked increase of TuJ1 (red). Dapi staining (blue) tags cell nuclei (Scale bar, 20 μm). (**B**) Immunoblot of TuJ1 expression in cells cultured in differentiation medium for 3 h, 24h and 72h. In line with results shown in (A), a significant linear increase of TuJ1 is observed. GAPDH, loading control. The TuJ1/GAPDH *ratio* (graph at right, reporting densitometric analysis) is shown as mean ± SEM. (**C**) GFP-LC3 mice derived ANSCs were allowed to differentiate by differentiation medium in the presence of CHQ during the last hour of culture. Representative confocal microscope images of cells at 12h and 72h after induction of differentiation are shown. High magnification of the GFP-LC3 staining regions corresponding to the insert are shown at the right of each panel. TuJ1-positive cells (red) and GFP-LC3 dots (green) co-localization suggest that immature neurons in active differentiation (TuJ1-positive), exhibit high numbers of GFP-LC3 dots/cells (Scale bar, 5 μm). Values represent mean ± SEM ($n$=3);*$P$<0.05.

**Figure S5. Differentiating *Ambra1*$^{+/gt}$ and *Beclin 1*$^{+/-}$ ANSCs show autophagy impairment.** Isolated and expanded wild type and *Ambra1*$^{+/gt}$ or *Beclin 1*$^{+/-}$ ANSCs were cultured under differentiating conditions. Proteins were extracted and analyzed by immunoblot for LC3 at 24h after differentiation in the absence or presence of CHQ during the last hour of culture. (**A-B**) Representative immunoblotting of *Ambra1*$^{+/gt}$ (**A**) and *Beclin 1*$^{+/-}$ (**B**) cell-derived protein extracts compared with their corresponding wild type controls. GAPDH, loading control. The *ratio* of LC3II/GAPDH (mean ± SEM) in CHQ-treated *versus* untreated cells is reported in the corresponding right graphs ($n$=4).