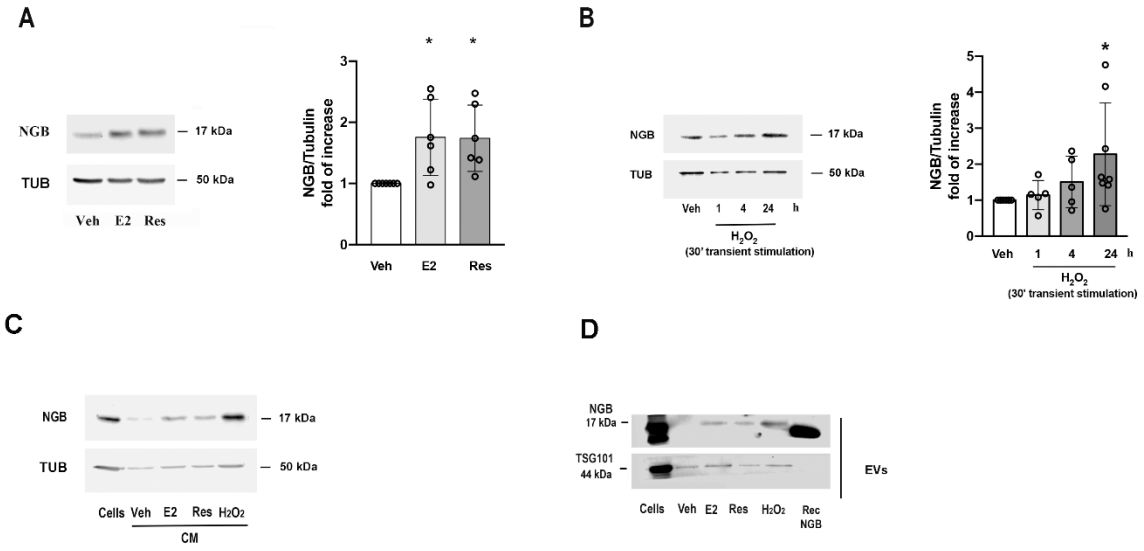
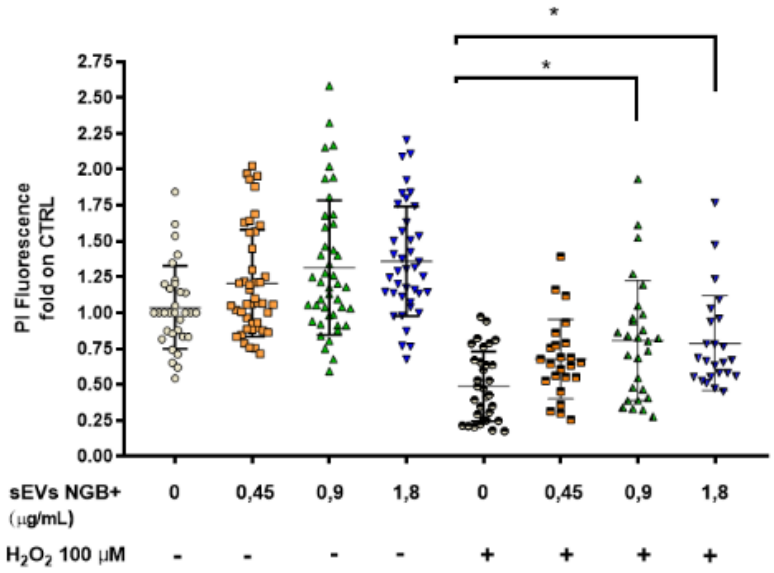
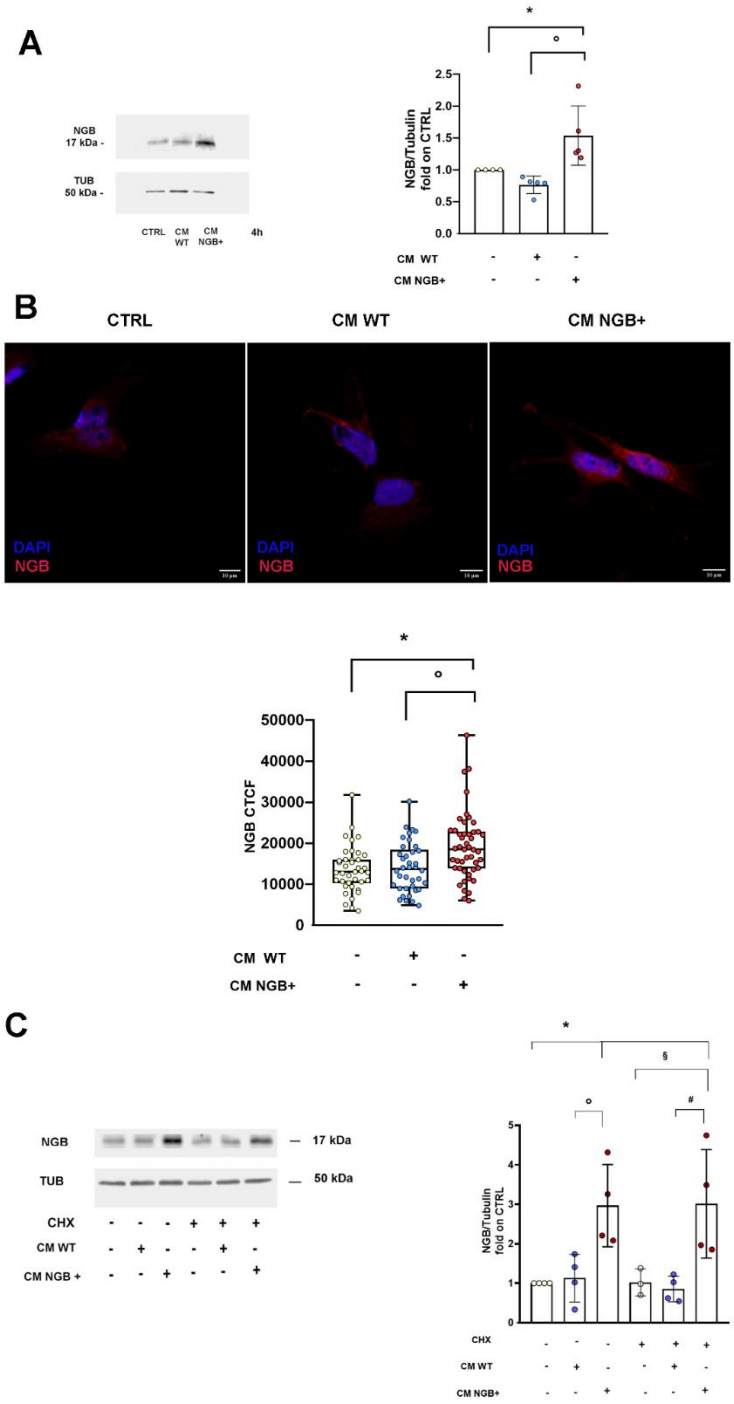
Supplemental data



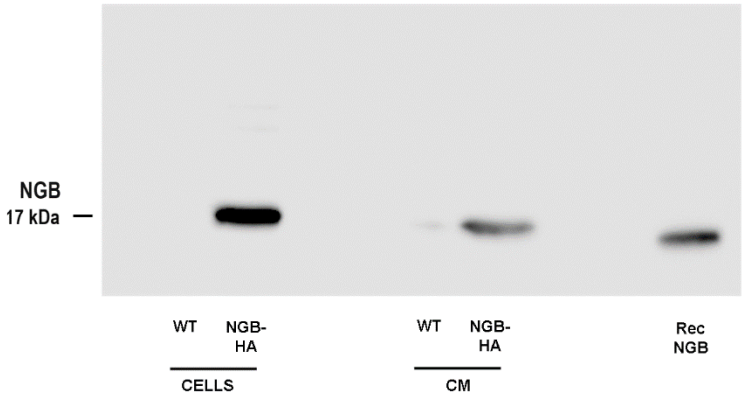
Supplemental Figure 1 Effects of globin inducers on intracellular and extracellular NGB levels. SH-SY5Y were transiently treated with the selected compounds for 30 minutes, followed by cell maintenance in a stimulation-free medium for the indicated time. Briefly, SH-SY5Y cells (cell lysate) were treated with either E2 (1 nM), Res (100 nM) for 30 min and collected after 24h of cell culturing. The transient stimulation with both E2 and Res increased the intracellular NGB levels after 24h of cell culture (A). On the other side, the protein levels of NGB were explored at different time points after the transient (30 min) stimulation with H_2O_2 (50 µM) for evaluating the effect at both short and long term. Obtained results indicate that, NGB level significantly increases only at 24h, without any significant increase after 1h and 4h (B). Representative blots and densitometric analysis were obtained from at least four independent experiments. The levels of tubulin were evaluated on the same filter as cell lysate loading control. Data shown are means ± SD. Statistical significance was determined with ANOVA followed by Tukey-Kramer post-test vs Veh condition (*). Western blot analysis was performed on NGB expression levels in conditioned media (CM) (C) or extracellular vesicle (EVs) fraction (D) generated from SH-SY5Y cells treated with E2, Res or H2O2 for 30 min and kept in cell culture for 24h. All the examined NGB inducers appear to promote the NGB accumulation in both CM and extracellular vesicle fraction. Western blot images are representative of at least two different experiments with similar results. The levels of tubulin was evaluated on the same filter as marker of passively released protein in CM (C). The common exosomes marker TSG101 was used as loading control for EVs (D). Where indicated, recombinant NGB protein (2.5 ng) was used as a control.



Supplemental Figure 2 Effects of NGB-enriched sEVs on H_2O_2-induced cytotoxicity NGB-enriched sEVs were used at different concentrations (0.45; 0.9; 1.8 µg/mL) as pre-treatment (4h) before the stimulation with H_2O_2 (100 µM; 24h). Cell quantification was performed by analyzing cell DNA content obtained by propidium iodide assay on fixed and permeabilized cells. Statistical significance was determined with ANOVA followed by Tukey-Kramer post-test vs H_2O_2 condition (*).



Supplemental figure 3 Levels of intracellular NGB protein in SH-SY5Y treated with WT and NGB+ conditioned medium Western blot analysis of NGB levels in SH-SY5Y cells cultured for 4h with CM WT or CM NGB+ (A). Representative blot (left) and relative densitometric analysis (right) obtained from at least three independent experiments are reported. The levels of the tubulin protein were used as internal loading control. Representative immunofluorescence image (B) of SH-SY5Y cells treated for 4h with complete conditioned medium obtained from WT (CM WT) or NGB overexpressing cells (CM NGB+) stained with 4',6-Diamidino-2-Phenylindole (DAPI, blue) for nuclei and anti-NGB antibody (red). Bar graphs report the value of Corrected Total Cell Fluorescence (CTCF) for NGB signal obtained by the analysis of ~ 30 cells from three independent experiments. Representative blot (left) and relative densitometric analysis (right) (C) obtained from four independent experiments reporting NGB levels in SH-SY5Y cells cultured for 24h with CM WT or CM NGB+ in presence or absence of the protein synthesis inhibitor cycloheximide (CHX; 3.5 μM; 6h pre-stimulation). In all cases, statistical significance was determined with ANOVA followed by Tukey-Kramer post-test vs CTRL condition (*) or CM WT (°) or CHX (§) or CHX and CM WT (#) stimulation.



Supplemental figure 4 Total western blot image of NGB staining in cell lysate and conditioned media from SH-SY5Y WT and NGB-overexpressing cells. Conditioned media (600 µL) collected from cells seeded (5 $X10^5$/well) in 6-well plates, were concentrated with AMICON Ultra-0.5 with 3 kDa cut-off to ~ 40 µL. Solubilized protein from cell lysate (30 µg) and conditioned media were resolved by 13.5% SDS-PAGE. Recombinant NGB protein (2.5 ng) was used as a control.