**SUPPLEMENTARY FIGURE LEGENDS.**

**Supp. Figure 1. DT-EGF causes caspase dependent death in epithelial tumor cells and caspase independent death in glioblastoma cells.** Panel A, caspase3/7 activity in epithelial (HN12 and HeLa) or glioblastoma (U373MG and U87MG) cells treated with DT-EGF. Both epithelial cell lines activated caspase 3/7 activity while neither glioblastoma cell line displayed caspase activation. Panel B, Caspase activation in HeLa and U87MG cells after treatment with DT-EGF or Lexatumumab. Both cell lines activated caspases in response to lexatumumab however even at 48 hours there was no caspase activity in the U87MG cells. Panel C, inhibition of caspases with the caspase inhibitor zVAD-fmk inhibited HeLa cell death as determined by MTS assay 24 hours after treatment with DT-EGF. There was no effect on DT-EGF-induced death in U87MG glioblastoma cells. These data indicate that DT-EGF kills the glioblastoma cells via a caspase-independent mechanism and the epithelial cells via a caspase-dependent mechanism.

**Supp. Figure 2. siRNA knockdown of autophagy regulators.** U87MG cells were transfected with siRNAs targeting Atg5, Atg7, Atg12, Beclin 1 or the EGFR. 3 days after transfection protein samples were analyzed by western blotting indicating that all the siRNAs significantly reduced expression of their targets.

**Supp. Figure 3. DT-EGF causes autophagosome maturation.** U87MG cells expressing a tandem GFP-mCherry-tagged LC3 construct were treated as indicated with DT-EGF or trehalose. At early time points, many of the red fluorescent puncta are also labeled with green fluorescence indicating autophagosomes. At later time points when the autophagosomes fuse with lysosomes, green fluorescence is lost but red fluorescence is retained. Both DT-EGF and trehalose promote autophagosome formation and maturation.

**Supp. Figure 4. DT-EGF does not cause LC3 aggregation in epithelial tumor cells.** HN12 and HeLa cells stably expressing GFP-LC3 were treated with DT-EGF or trehalose and aggregation of GFP-LC3 was determined by fluorescence microscopy. Both cell lines displayed marked LC3 aggregation in response to trehalose treatment but not when treated with DT-EGF. These data indicate that DT-EGF does not cause autophagy in the epithelial tumor cells.

**Supp. Figure 5.** DT-EGF induces autophagy and cell death in SNB19 and 8MGBA cells. Panel A. Cell viability assay with increasing doses of DT-EGF on SNB19 and 8MGBA glioblastoma cell lines, both cell lines are killed by DT-EGF.

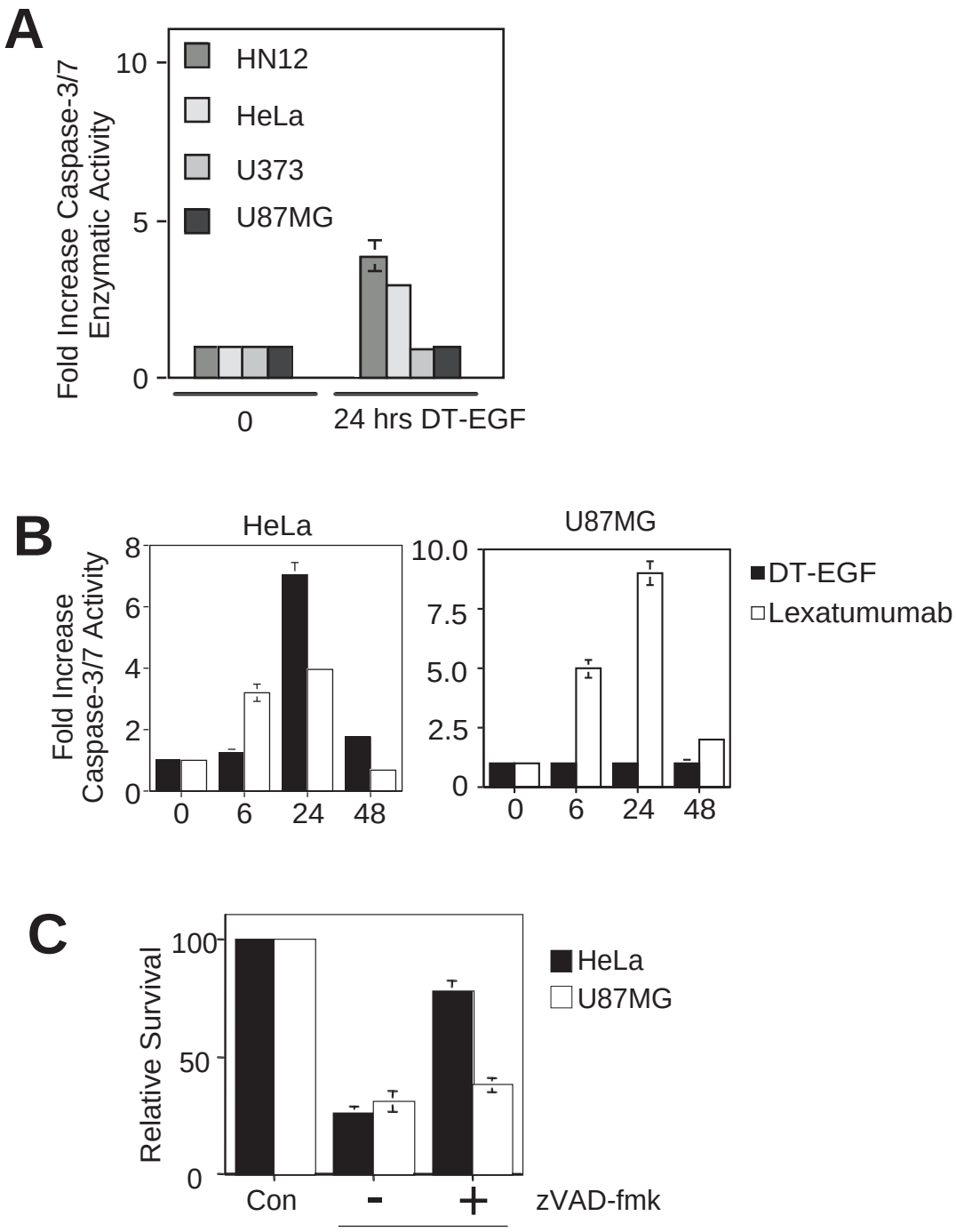
Panel B. Processing of the autophagy cargo protein betaine homocysteine methyltransferase (BHMT). The arrow indicates the processed form of the protein that is produced in autophagolysosomes. SNB19 and 8MGBA cells display DT-EGF-induced BHMT processing. GFP-Myc indicates the loading control expressed from the same construct (26).

**Supp. Figure 6. Trehalose treatment to increase autophagy inhibits DT-EGF-induced HN12 cell death.** Panel A., HN12 cells were pre-treated for 24 hours with trehalose to induce autophagy then treated with increasing doses of DT-EGF for 24 hours. After washing to remove the drug, surviving cells were grown for two weeks then colonies assessed after crystal violet staining. Trehalose treatment protects against DT-EGF-induced death at multiple doses. Panel B, HN12 cells were pre-treated with trehalose then treated with DT-EGF and followed by timelapse microscopy. Trehalose treatment delayed morphological signs of cell death.

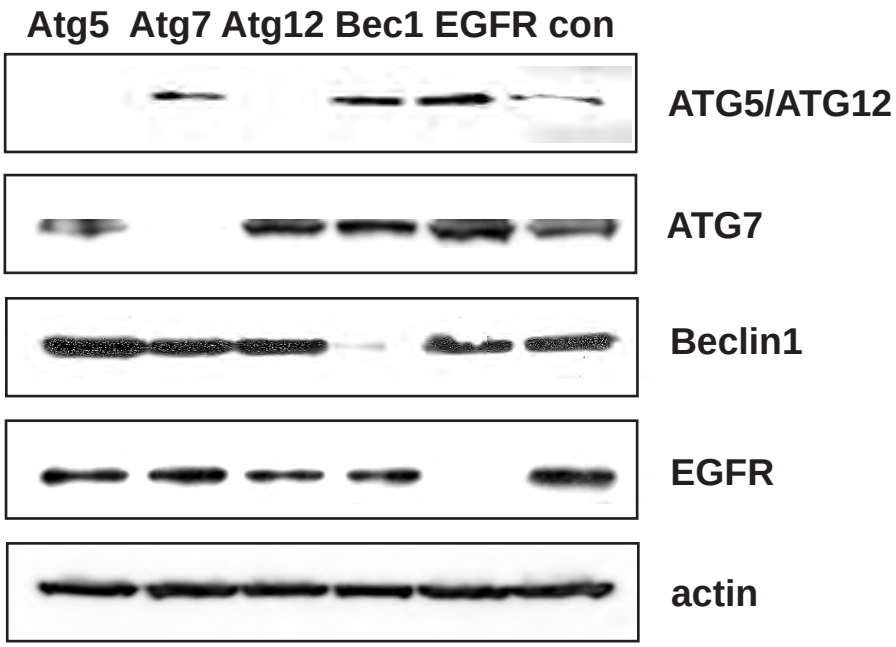
**Supp. Figure 7. Co-localization of HMGB1 and LC3.** U87 cells expressing GFP-HMGB1 and mCherry-LC3 were treated with DT-EGF. Before treatment, all HMGB1 is located in the nucleus; 48 hours after DT-EGF treatment HMGB1 is located in the cytoplasm and in puncta. Some but not all of the puncta (arrowed) co-localize with larger LC3-containing puncta.

**Supp. Figure 8. HMGB1 release.** U87MG cells treated with DT-EGF with and without Atg5 knockdown were followed by time-lapse microscopy for release of GFP-HMGB1. Atg5 knockdown prevents HMGB1 release. HeLa cells treated with DT-EGF retain GFP-HMGB1.

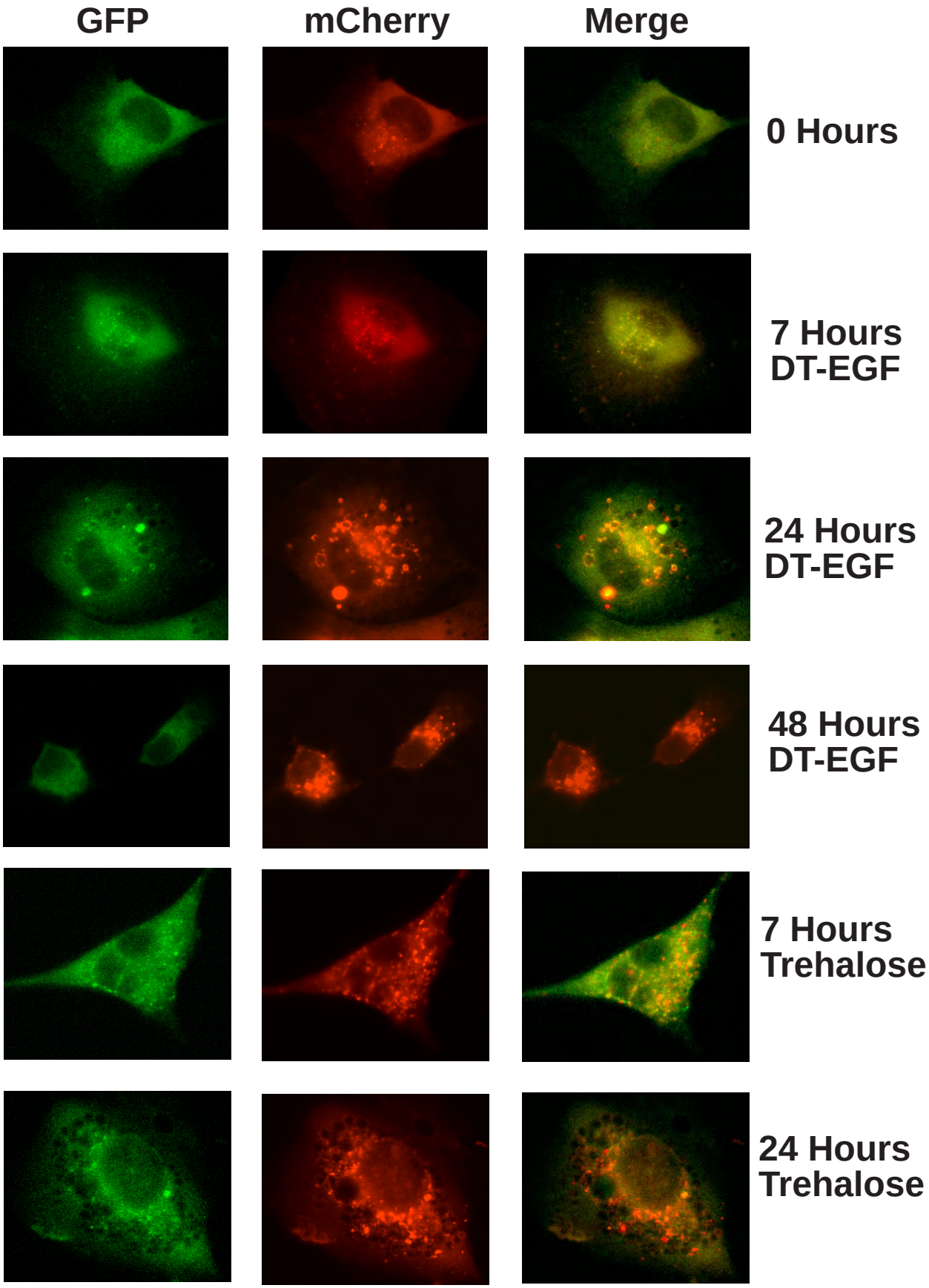
# Supplementary Figure 1



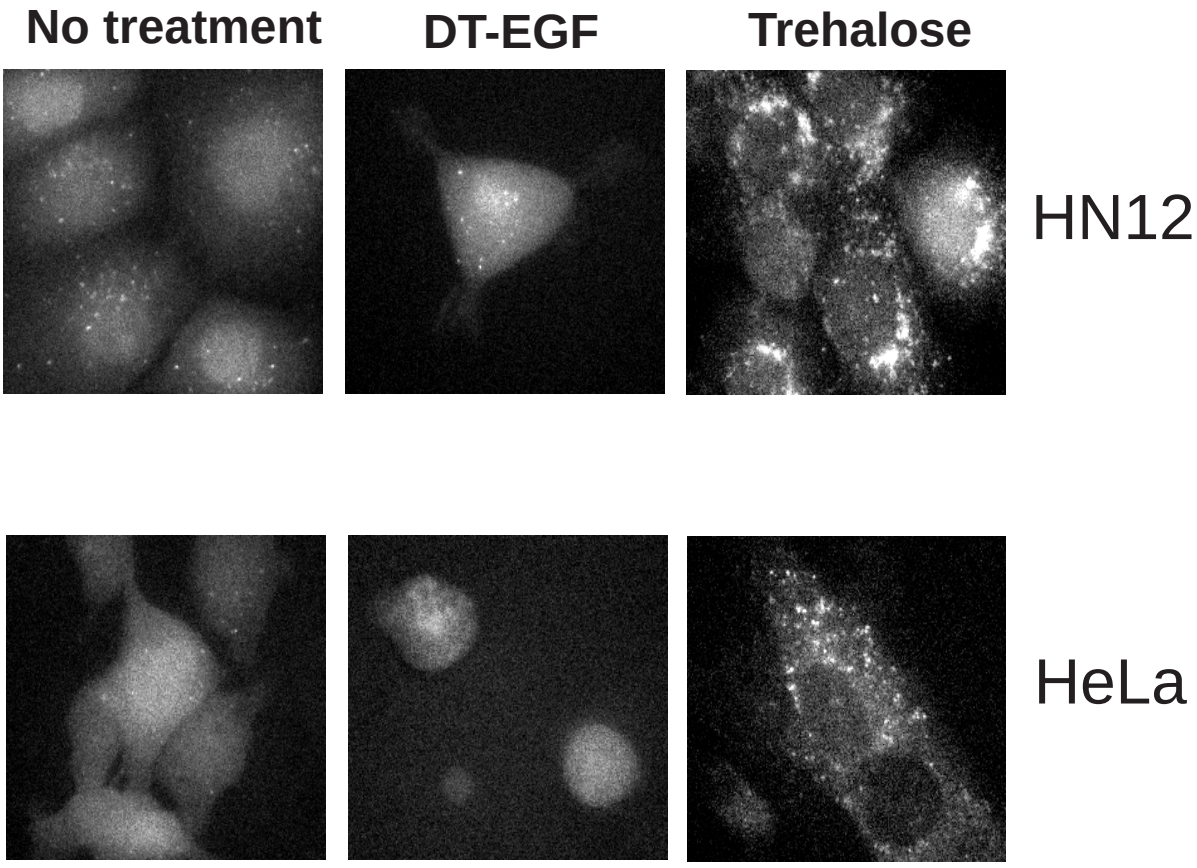
# Supplementary Figure 2



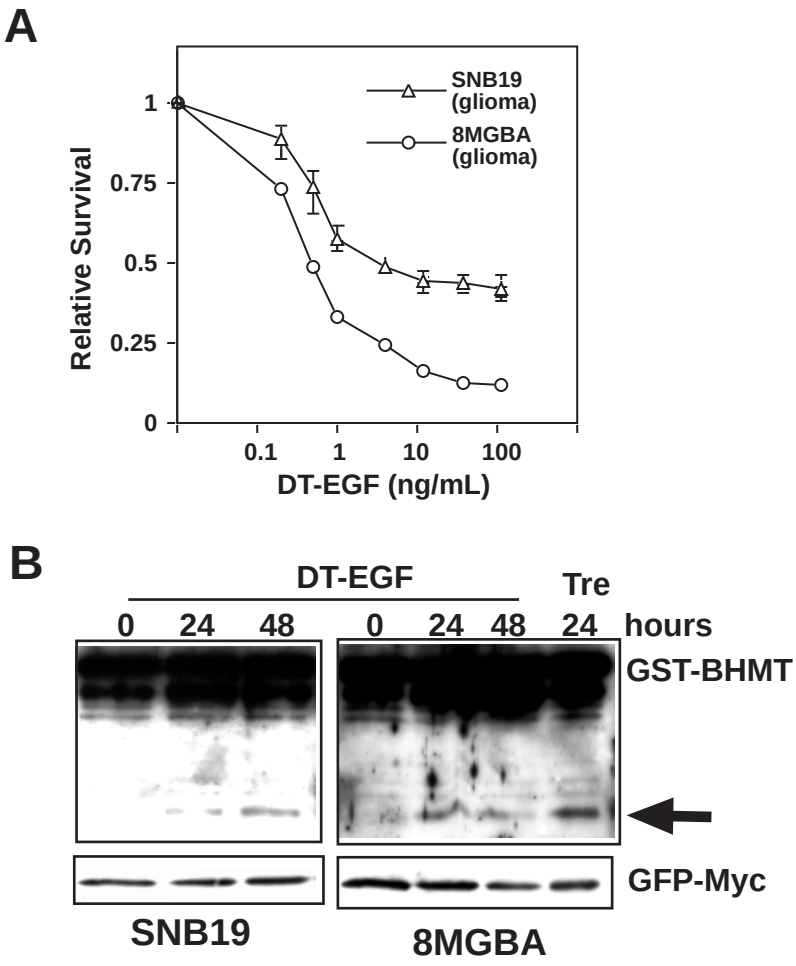
# Supplementary Figure 3



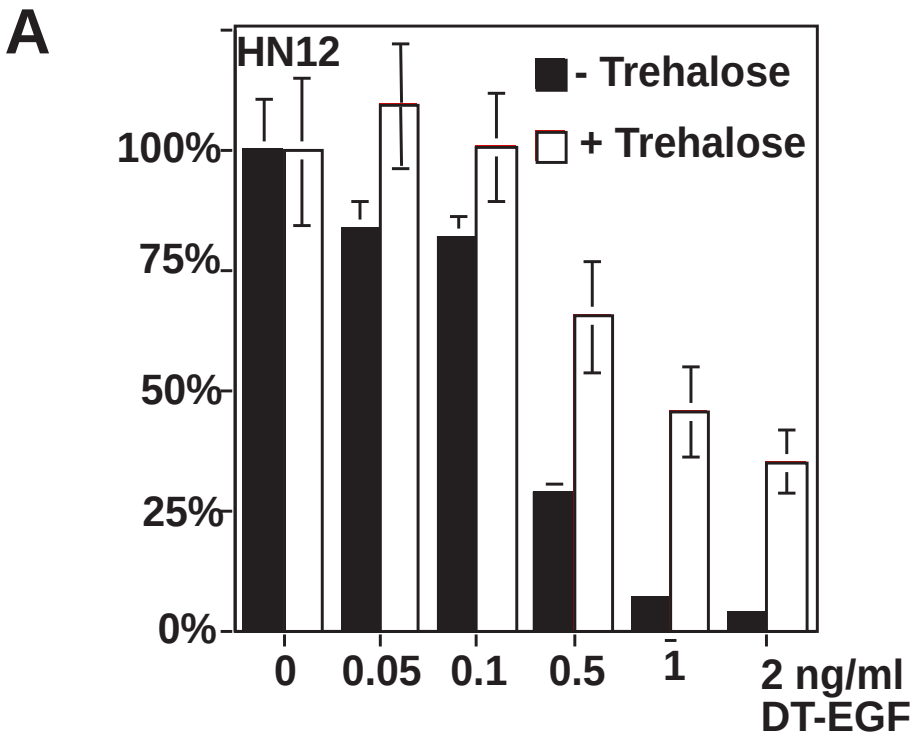
# Supplementary Figure 4

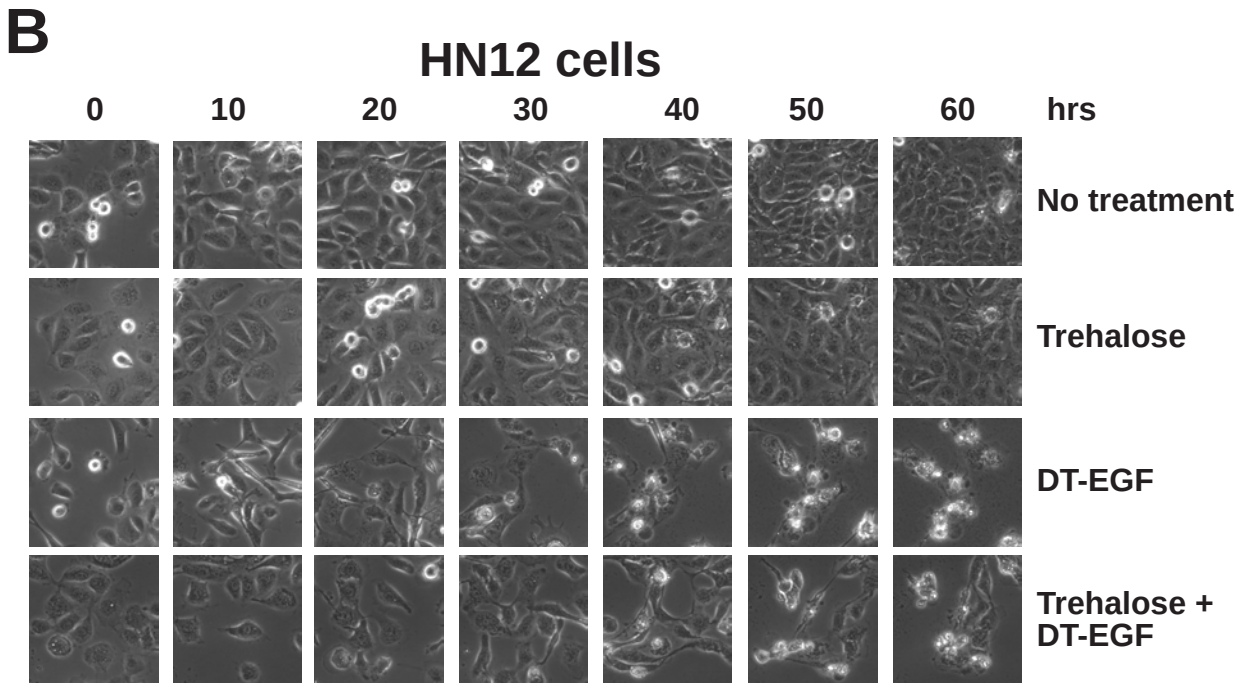


# Supplementary Figure 5

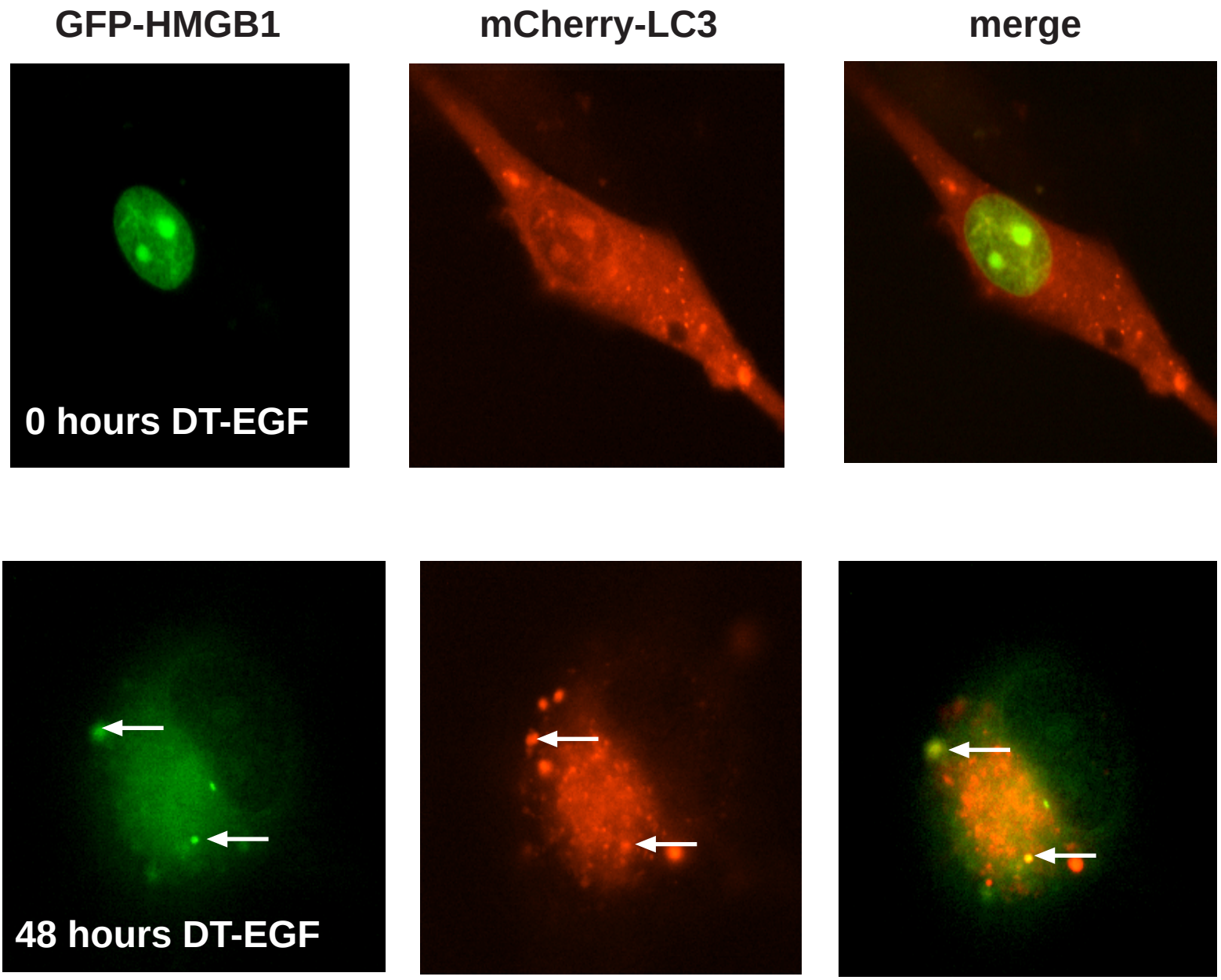


# Supplementary Figure 6



# Supplementary Figure 7



# Supplementary Figure 8