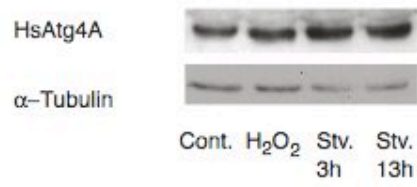
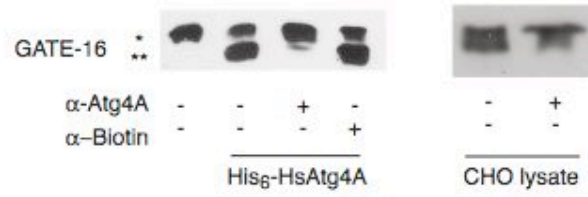


A



B



C



Supplementary Figure 4. (A) HsAtg4A levels are not affected by starvation or oxidation. HEK 293 cells were grown in a control medium (α -MEM, 10% FCS) in the presence or absence of 1 mM H_2O_2 for 1 h, or starved for 3 h or 13 h in EBSS. Lysates (40 μ g) obtained in Ripa buffer were run on 10% SDS-PAGE and analyzed by Western blot, using anti-HsAtg4 polyclonal antibodies. **(B) anti-HsAtg4A antibodies inhibit the cleavage of GATE-16 by cell lysates.** Left panel: affinity purified anti-HsAtg4A antibody or anti-Biotin IgG (1 μ g) were preincubated with recombinant His₆-HsAtg4A (0.1 μ g) in 50 KT buffer for 10 min at 25°C before addition of His₆-GATE-16-HA (0.3 μ g) and 1 mM DTT to the reaction. As control, His₆-GATE-16-HA was incubated in 50 KT containing 1 mM DTT in the absence or presence of His₆-HsAtg4A, without antibodies. Incubation then proceeded at 30°C for 45 min and the reaction was stopped by addition of sample buffer. Samples were resolved on 15% SDS-PAGE and subsequently analyzed by Western blot, using anti-His monoclonal antibodies. Right panel: lysates obtained in Ripa buffer from non-treated CHO cells (5 μ g) were preincubated in the presence or absence of affinity purified anti-HsAtg4 antibody (5 μ g) for 10 min at 25°C before addition of His₆-GATE-16-HA (0.3 μ g) and 1 mM DTT to the reaction. Incubation then proceeded at 30°C for 45 min and the samples were analyzed as in the left panel. The experiment was repeated 3 times; representative blots are shown. **(C) Oxidation inhibits the delipidation activity of endogenous Atg4.** CHO cells stably transfected with GFP-LC3 were incubated in the presence or absence of 1 mM H_2O_2 for 30 min, after which the cells were harvested, homogenized and fractionated with a Dounce homogenizer as explained in Supplementary Materials and Methods. Fractions collected from $\sim 5 \times 10^6$ cells were run on 10% SDS-PAGE and analyzed by Western blot with anti-GFP antibodies. The experiment was repeated 4 times: a representative blot is shown. (*) indicates non-lipidated GFP-LC3 and (**) indicates lipidated GFP-LC3.