For GST-LC3B, as previously described [80], we inserted human LC3B cDNA in a pGEX-4T1 vector. The last five amino acids of LC3B were deleted, to mimic the cleavage by ATG4. After the transformation of the pGEX-4T1 vector encoding GST-LC3B in *E. coli* Rosetta (DE3) pLysS cells, cells were grown in LB medium at 37°C until an OD_{600} of 0.8-1, protein expression was induced with 1 mM IPTG for 4 h at 37°C. Cells were collected by centrifugation and resuspended in lysis buffer (50 mM HEPES pH 7.5, 300 mM NaCl, 2 mM $MgCl_2$, 2 mM β-mercaptoethanol, cOmplete EDTA-free protease inhibitors (Roche), and DNase (Sigma)). Cell lysates were sonicated twice for 30 s. Lysates were cleared by centrifugation at 140,000 x*g* for 30 min at 4°C in a Beckman Ti45 rotor. The supernatant was collected and incubated with pre-equilibrated Glutathione Sepharose 4B beads (GE Healthcare) for 2 h at 4°C with gentle shaking to bind GST-LC3B. Samples were centrifuged to pellet the beads and remove the unbound lysate. Beads were then washed twice with wash buffer (50 mM HEPES pH 7.4, 300 mM NaCl, 1 mM DTT), once with high salt wash buffer (50 mM HEPES pH 7.4, 700 mM NaCl, 1 mM DTT), and two more times with wash buffer (50 mM HEPES pH 7.4, 300 mM NaCl, 1 mM DTT). Proteins were eluted overnight with 20 mM reduced L-glutathione in 50 mM HEPES pH 7.4, 300 mM NaCl, 1 mM DTT buffer. The supernatant was collected, filtered through a 0.45 µm syringe filter, and concentrated using a 10 kDa cut-off Amicon filter (Merck Millipore), and loaded onto a pre-equilibrated Superdex 75 16/600 column (Cytiva). Proteins were eluted with SEC buffer (25 mM HEPES pH 7.4, 150 mM NaCl, 1 mM DTT). Fractions were analyzed by SDS-PAGE and Coomassie staining. Fractions containing purified GST-LC3B were pooled. After concentrating the purified protein, the protein was aliquoted and snap-frozen in liquid nitrogen. Proteins were stored at -80°C.