Summary

Macroautophagy (hereafter autophagy) is an intracellular bulk degradation system that is highly conserved in all eukaryotic cells. It is governed by a large number of core and accessory autophagy protein (ATGs) and is involved in many physiological and pathological processes. Autophagy also constitutes an important mechanism in cell autonomous immunity and we previously found that infection of *Dictyostelium discoideum* with medically relevant pathogens caused differential regulation of the core autophagy genes ATG8a, ATG8b, ATG9, ATG12 and ATG16. Here, we describe the phenotypes of the previously reported ATG9$^-$, of ATG16$^-$ and ATG9$^-$/16$^-$ cells and of cells that express ATG16-GFP in the knock-out mutant background. ATG16 deficiency caused an increase in the expression of several core autophagy genes, among them *atg9* and the two *atg8* paralogues. This single and double ATG9 and ATG16 knock-out mutants had complex phenotypes and displayed severe and comparable defects in pinocytosis and phagocytosis. Uptake of *L. pneumophila* was reduced. In addition, ATG9$^-$ and ATG16$^-$ cells had dramatic defects in autophagy, development and proteasomal activity which were much severe in the ATG9$^-$/16$^-$ double mutant. Mutant cells showed an increase in poly-ubiquitinated proteins and contained large ubiquitin-positive protein aggregates which partially colocalised with ATG16-GFP in ATG9$^-$/16$^-$ cells. We also identified PSMD1 and PSMD2, which are both components of the 19S proteasome, as novel interacting proteins of ATG16. The interaction of PSMD1 and ATG16 is direct. Further analysis showed that overexpressed PSMD1 and PSMD2 localized to punctae structures whose formation was dependent on ATG16. The more severe autophagic, developmental and proteasomal phenotypes of ATG9$^-$/16$^-$ cells imply that ATG9 and ATG16 likely function in parallel in autophagy and have in addition autophagy-independent functions in further cellular processes. The interaction between ATG16 and proteasomal subunits suggests a direct link between autophagy and the ubiquitin proteasome system (UPS).