



**Figure S4 A model of endocytic maturation in mammalian cells.**

Upon entry into the cell, some cargoes first traffic through a pre-Rab5 compartment. Subsequently, Rab5 associates with these vesicles. Often, these are referred to as 'early endosomes'. Next, the kinase Vps34 is recruited to these endosomes by Rab5, thus generating phosphatidylinositol 3-phosphate (PI3P) on the membrane of these vesicles. This phosphoinositide modification is recognized by the 2xFYVE reporter. These vesicles also have a tendency to fuse and become larger. Next, proteins of the ESCRT complex are recruited to early endosomes by binding PI3P present on the membrane of these vesicles. The ESCRT complex proteins are responsible for formation of intraluminal vesicles. Subsequently, the protein Mon1/Sand is recruited to these endosomes. The activity of Mon1/Sand results in displacement of Rab5 and in concomitant recruitment of Rab7. These Rab7-positive vesicles are often referred to as 'late endosomes'. Finally, Rab7 promotes the fusion of late endosomes with lysosomes.