© 2018. Published by The Company of Biologists Ltd | Development (2018) 145, dev146506. doi:10.1242/dev.146506

REVIEW

Autophagy in stem cells: repair, remodelling and metabolic reprogramming

Patricia Boya[1,*], Patrice Codogno[2] and Natalia Rodriguez-Muela[1,*]

ABSTRACT

Autophagy is a catabolic pathway by which cellular components are delivered to the lysosome for degradation and recycling. Autophagy serves as a crucial intracellular quality control and repair mechanism but is also involved in cell remodelling during development and cell differentiation. In addition, mitophagy, the process by which damaged mitochondria undergo autophagy, has emerged as key regulator of cell metabolism. In recent years, a number of studies have revealed roles for autophagy and mitophagy in the regulation of stem cells, which represent the origin for all tissues during embryonic and postnatal development, and contribute to tissue homeostasis and repair throughout adult life. Here, we review these studies, focussing on the latest evidence that supports the quality control, remodelling and metabolic functions of autophagy during the activation, self-renewal and differentiation of embryonic, adult and cancer stem cells.

KEY WORDS: Autophagy, Cancer stem cells, Mitophagy, Muscle stem cells, Reprogramming, Stem cells

Introduction

Autophagy is an intracellular catabolic mechanism by which cell components, including proteins, lipids and whole organelles, are degraded and recycled inside lysosomes (Galluzzi et al., 2017a). The degradation products are then transported back to the cytoplasm and are used to sustain cell homeostasis. Autophagy is a fundamental metabolic response to nutrient and oxygen deprivation, as well as an essential cytoplasmic quality control process (Boya et al., 2013; Galluzzi et al., 2017a). In recent years, studies have shown that autophagy plays a crucial role in various cell types, including neurons, muscle and cancer cells. However, it has also emerged that autophagy is key for the functioning and maintenance of various populations of stem cells (SCs), acting to promote their quiescence, maintain their stemness and self-renewal, and mediate their differentiation.

SCs give rise to the body's key cell lineages during embryogenesis, and also participate in tissue homeostasis and repair postnatally and throughout adult life. They divide to self-renew and to produce daughter cells that can undergo differentiation (García-Prat et al., 2017). These differentiation processes require cell remodelling, which is achieved by the autophagic elimination of structures and cell components that are no longer needed (Mizushima and Levine, 2010). Because most SCs are long-lived and need to remain functional for the life span of the organism, robust quality control mechanisms are essential for their subsistence. Autophagy, the main cellular quality control pathway, is thus thought to play a crucial role in sustaining SC homeostasis. In recent years, a number of studies have also revealed that autophagy contributes to the control of metabolism (Esteban-Martínez et al., 2017a; Kaur and Debnath, 2015) and can thus regulate the metabolism, identity and function of SCs. Indeed, the view that SCs are metabolically distinct from their differentiated counterparts, and that these metabolic features are essential for maintaining SC identity, is well-supported in the literature (Shyh-Chang et al., 2013). The fact that most SCs appear to rely more on glycolysis to generate ATP than on oxidative phosphorylation (Shyh-Chang and Ng, 2017) has been linked to the hypoxic milieu of SC niches. However, increased glycolysis is a crucial step in the conversion of differentiated cells into induced pluripotent stem cells (iPSCs; see Glossary, Box 1), suggesting that glycolytic metabolism is a requirement to maintain stemness rather than being an adaptation to the SC environment (Folmes et al., 2011; Shyh-Chang and Ng, 2017). Overall, this growing body of evidence suggests that autophagy helps to preserve SC function by simultaneously regulating cell remodelling and metabolism and serving as an important quality control mechanism.

In this Review, we discuss our current understanding of the mechanisms by which autophagy preserves SC function. We also highlight the key consequences of autophagy dysregulation in SCs on tissue development and maintenance, and discuss how SCs respond to stressful conditions. The functions of autophagy in somatic cells and disease models have been reviewed elsewhere (Schneider and Cuervo, 2014) and are therefore not discussed here.

Autophagy: induction, molecular machinery and general functions

The activation of autophagy during periods of starvation is an evolutionarily conserved response in eukaryotes (Kaur and Debnath, 2015). Under these conditions, the cell uses protein and lipid degradation to adapt its metabolism and fulfil its energy needs. Accordingly, the pharmacological or genetic downregulation of autophagy results in rapid cell death in starvation conditions (Boya et al., 2005). Other stressors, such as hypoxia, oxidative stress and infection, can also induce autophagy (Fig. 1).

The process of autophagy involves members of the autophagy-related (ATG) family of proteins. Autophagy induction is controlled by mTOR and AMP-activated protein kinase (AMPK) signalling pathways, which regulate the assembly and activation of an ATG1/ULK1 complex that in turn triggers formation of the phosphatidylinositol 3-kinase (PI3K) complex (Fig. 1). This complex regulates the incorporation of phosphatidylinositol 3-phosphate into the phagophore membrane from which autophagosomes (see Glossary, Box 1) are generated (Hurley and Young, 2017). Next, two conjugation reactions catalysed by ATG7 are necessary for autophagosome formation: one relies on ATG7

[1]Department of Cellular and Molecular Biology, Centro de Investigaciones Biológicas, CSIC, Madrid, Spain. [2]Institut Necker-Enfants Malades (INEM), INSERM U1151-CNRS UMR 8253, Université Paris-Descartes, Sorbonne Paris Cité, Paris, France.

*Authors for correspondence (patricia.boya@csic.es; nrodriguez@cib.csic.es)

P.B., 0000-0003-3045-951X; P.C., 0000-0002-5492-3180; N.R., 0000-0003-1065-1870

Development (2018) 145, dev146506. doi:10.1242/dev.146506

Box 1. Glossary

Autophagic flux. The rate at which lysosomes degrade autophagy substrates. It is a measurement used as an indicator of the efficiency of autophagy activity that can be assessed by comparing the number of autophagosomes in the presence and absence of lysosomal inhibitors.

Autophagosomes. Transient, double-membrane vesicles that engulf cytoplasmic components, including entire organelles, and deliver them to lysosomes for degradation.

Blastocyst. The mammalian pre-implantation/early-stage embryo.

FOXO transcription factors. Evolutionarily conserved regulators of the expression of genes involved in cellular metabolism and resistance to oxidative stress.

Induced pluripotent stem cells (iPSCs). Somatic cells, typically skin or blood cells, that have been reprogrammed back into an embryonic-like pluripotent state through overexpression of a cocktail of transcription factors.

Mitophagy. The specific removal of mitochondria by the process of autophagy.

NuRD complex. A macromolecular chromatin remodelling complex that regulates gene transcription, genome integrity and cell cycle progression, and is essential for embryo development and ESC self-renewal/differentiation among other functions.

Primary cilium. A non-motile microtubule-based organelle that acts as a cellular antenna sensing environmental cues linked to the cell cycle.

Ubiquitin proteasome system (UPS). The catabolic system that predominantly degrades short half-life, properly folded and misfolded, cytoplasmic and nuclear proteins that have been ubiquitylated, producing as a result short peptides.

Zygote-to-embryo transition. The stage of development following fertilization in which the molecular programmes of the fertilized oocyte are degraded. Genetic and epigenetic reprogramming changes occur resulting in activation of the embryonic molecular programmes (days 0-3).

and ATG10, which induce the conjugation of ATG5 to ATG12 in the context of a multiprotein complex containing autophagy-related 16-like 1 (ATG16L1); the other results in the conjugation of phosphatidylethanolamine to LC3 (MAP1LC3) to form the autophagosome-bound form of LC3 called LC3-II. The continuous assembly of these complexes and the delivery of lipids via ATG9, the only multi-membrane-spanning ATG protein, allow the autophagosomal membrane to elongate and close to form the mature autophagosome (Hurley and Young, 2017). The later stages of autophagy are controlled by molecules that regulate autophagosome fusion with lysosomes, such as LAMPs and RAB7, and by lysosomal acidic hydrolases that regulate the degradation of the autophagy cargo (Fig. 1). The final degradation products then translocate to the cytoplasm to be recycled for use in new anabolic reactions to sustain cell homeostasis. Because autophagy is a highly dynamic process, the blockade of one of its stages, or impaired lysosomal function or biogenesis, leads to the accumulation of autophagosomes, ultimately disrupting or diminishing autophagic flux (see Glossary, Box 1) (Boya et al., 2013).

Development and differentiation are often accompanied by large-scale cellular and tissue remodelling, which is mediated by autophagy (Mizushima and Komatsu, 2011). At the cellular level, autophagy is essential for the differentiation of many cell types, including adipocytes, erythrocytes, lymphocytes and neurons (Mizushima and Levine, 2010). In addition to its role in intracellular quality control and metabolic regulation during cell differentiation, autophagy provides a rapid and efficient means of altering the composition of the cytosol, and is involved in controlling cell size through the degradation of receptors, organelles and transcription factors, all of which are processes that are crucial for cell differentiation (Mizushima and Levine, 2010). Given that autophagy is essential for the elimination of unnecessary or harmful components from cells, and is a key regulator of cell metabolism, its dysregulation has significant pathological consequences. Indeed, autophagy is implicated in a plethora of pathologies, including neurodegenerative, metabolic and immune diseases (Boya et al., 2016; Deretic et al., 2015; Galluzzi et al., 2015; Menzies et al., 2017; Stienstra et al., 2014). Defects in proteostasis and autophagy have also been described to occur in ageing, and autophagy is proposed to underlie the beneficial effects of caloric restriction (Kaushik and Cuervo, 2015; Madeo et al., 2015).

Autophagy can also be a highly selective process, allowing specific cytoplasmic components to be delivered to lysosomes via

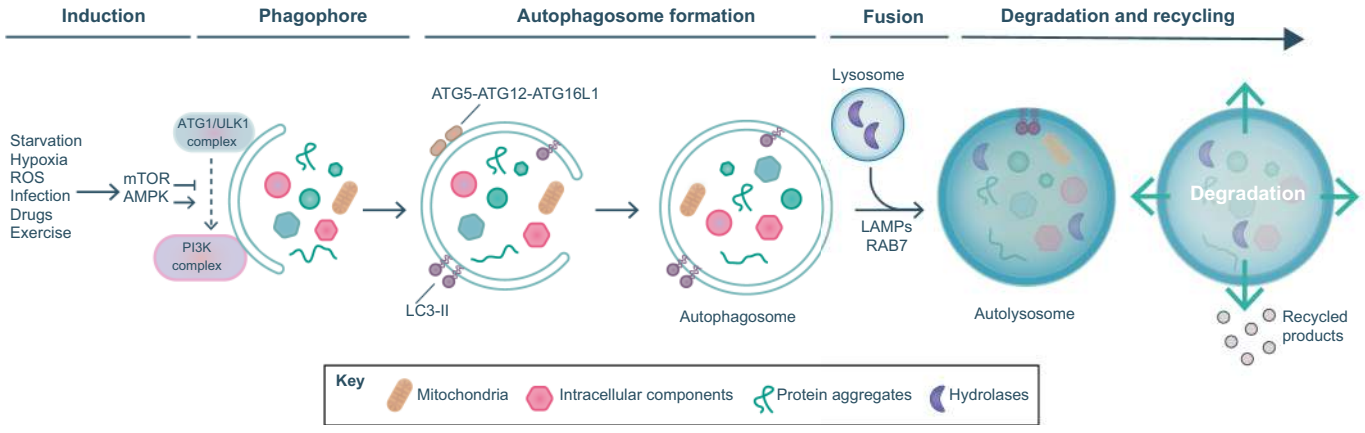

Fig. 1. The molecular machinery implicated in autophagy. The induction of autophagy is controlled by the mTOR and AMPK signalling pathways and relies on the assembly and activation of two macromolecular complexes: ATG1/ULK1 (composed of ULK1, FIP200, ATG13 and ATG101) and the Class III PI3K complex (composed of BECN1, ATG14, AMBRA1, VPS34, VPS15 and UVRAG). Next, two conjugation reactions are necessary for autophagosome formation. In the first, mediated by ATG7 and ATG10, ATG5 and ATG12 are conjugated and bind to ATG16L. In the second, catalysed by ATG7 and ATG3 together with the ATG12-ATG5:ATG16L complex, LC3 conjugates to the lipid phosphatidylethanolamine (PE) to generate LC3-II, which facilitates its anchoring at the autophagosomal membrane. Once formed, the autophagosome then fuses with a lysosome, a process that involves several lysosomal proteins including LAMPs and RAB7. After degradation by the action of lysosomal hydrolases, the final products, which include amino acids, lipids and nucleotides, translocate to the cytoplasm to be used in new anabolic reactions to sustain cell homeostasis.

Development (2018) 145, dev146506. doi:10.1242/dev.146506

specific cargo-recognition proteins called autophagy receptors (Khaminets et al., 2016). The selective autophagy of mitochondria, a process termed mitophagy (see Glossary, Box 1), can also occur and allows damaged or unwanted mitochondria to be engulfed into autophagosomes for lysosomal delivery and degradation (Ashrafi and Schwarz, 2013). Under conditions of mitochondrial stress, for instance, the kinase PINK1 activates the ubiquitin ligase PRKN, which then ubiquitylates mitochondrial proteins that recruit autophagy receptors that bridge between mitochondria and autophagosomes (Lazarou et al., 2015). Mitochondria are also eliminated in developmental contexts, for example during cell differentiation, in a process named programmed mitophagy. Programmed mitophagy has been observed during erythrocyte and lens maturation and during neuronal differentiation (Ashrafi and Schwarz, 2013; Esteban-Martínez and Boya, 2017; Ney, 2015). In addition, mitophagy facilitates cell remodelling while also acting as a quality control mechanism by eliminating potential sources of oxidative stress (Takamura et al., 2011). Finally, recent evidence has demonstrated that mitophagy regulates a metabolic shift towards glycolysis in several contexts, such as during neuronal differentiation and macrophage activation (Esteban-Martínez et al., 2017b), indicating a link between mitophagy and metabolic reprogramming (Esteban-Martínez and Boya, 2017). How mitophagy controls SC homeostasis is further described in Box 2.

Autophagy and stem cells

Given their unique properties of self-renewal, multipotency, differentiation and quiescence in adult tissues, SCs must strictly control their rates of protein and organelle turnover and of ATP production (Guan et al., 2013; Vessoni et al., 2012). Metabolic regulation is now widely believed to function as a general mechanism for controlling SC quiescence (García-Prat et al., 2017). However, a growing body of evidence indicates that autophagy is also required for SC quality control and for maintaining the cellular homeostasis of SCs (García-Prat et al., 2017; Guan et al., 2013).

Depending on their source, SCs can be classified as embryonic stem cells (ESCs) or adult SCs. ESCs are derived from the inner cell mass (ICM) of blastocysts (see Glossary, Box 1), around 3.5-5.5 days after fertilization in the case of mouse embryos and 4-9 days after fertilization for human embryos. They are grown *in vitro* and are pluripotent, meaning they can produce all the cells of the embryo proper (but not the placental lineages). By contrast, adult SCs are found in tissues and organs after they have completed their development. These multipotent cells have a restricted potency compared with ESCs, and only give rise to a subset of cell types to replace and repair specific tissues. Some of the best-studied types of adult SCs include haematopoietic stem cells (HSCs), neural stem cells (NSCs) and muscle stem cells (better known as satellite cells) (Rumman et al., 2015). Adult SCs are also found in tissues that exhibit high turnover, such as the intestine and skin. Such SCs are responsible not only for tissue repair after damage but also for maintaining normal tissue turnover. Conversely, HSCs are maintained in a quiescent or very low-cycling state for months, a state that they abandon to repopulate the blood in response to haematopoietic stress, and muscle satellite cells are maintained in quiescence for most of their life and only divide in response to tissue damage (Rumman et al., 2015). Maintaining a balance between stemness and differentiation is therefore of crucial importance for SCs. Excessive cell differentiation depletes the SC population (leading to SC exhaustion) and promotes ageing or decay. On the contrary, excessive SC proliferation can give rise to cancer. Thus, quality control mechanisms are essential to preserve adult SC homeostasis and the capacity of SCs to respond rapidly to environmental stressors, damage and differentiation signals to sustain tissue regeneration. Below, we review recent studies that demonstrate the essential role of autophagy in maintaining embryonic and adult SC homeostasis. We describe how autophagy functions as an intracellular quality control and repair mechanism in SCs, and how autophagy remodels cellular morphology by eliminating, for example, organelles and stemness factors that control cellular reprogramming. We also review why and how autophagy controls metabolism to sustain energy homeostasis in SCs.

Box 2. Mitophagy in stem cells

A growing body of evidence indicates that mitophagy constitutes a prominent pathway controlling SC homeostasis. The role of mitophagy in the regulation of SC fate is associated with its quality control function as well as its ability to regulate cellular metabolism. For example, mitophagy prevents senescence by removing damaged mitochondria, the main source of ROS, and thereby limits ROS-induced genome damage, which is essential to maintain stemness (Garcia-Prat et al., 2016; Ho et al., 2017; Ma et al., 2015; Paik et al., 2009; Pan et al., 2013; Renault et al., 2009; Sena and Chandel, 2012; Tan and Wong, 2017). The lower mitochondrial number in HSCs and ESCs is associated with reduced reliance on aerobic metabolism (Kondoh et al., 2007; Shyh-Chang et al., 2013; Shyh-Chang and Ng, 2017), which results in the generation of fewer ROS. Mitophagy also maintains low ROS levels during the reprogramming of somatic cells into iPSCs through autophagy-related proteins including PINK1 and ATG3 (Liu et al., 2016; Vazquez-Martin et al., 2016; Xiang et al., 2017). Indeed, loss of PINK1-dependent mitophagy is sufficient to dramatically decrease the speed and efficiency of iPSC reprogramming from mouse embryonic fibroblasts (Vazquez-Martin et al., 2016). In line with this, iPSCs from *Pink1* knockout mice show decreased glycolytic metabolism and a strong tendency to differentiate. Mitophagy also removes paternal mitochondria from fertilized oocytes, a process initially described in nematodes and flies and recently in mouse embryos (Al Rawi et al., 2011; Rojansky et al., 2016; Sato and Sato, 2011). Lastly, recent evidence has demonstrated a pivotal role for mitophagy in regulating a metabolic shift towards glycolysis during mouse developmental neurogenesis (Esteban-Martínez et al., 2017a; Esteban-Martinez et al., 2017b). Further studies are needed to unravel the molecular mechanisms underlying mitophagy-dependent metabolic reprogramming and to determine whether targeting mitophagy could constitute a useful strategy to promote the quiescence and/or differentiation of SCs.

Autophagy in early development and embryonic stem cells

After fertilization, the mammalian zygote is reprogrammed to form pluripotent cells located in the ICM of blastocysts. This reprogramming, which requires the epigenetic modification of maternal and paternal genomes, the expression of pluripotency genes, and the removal of inherited maternal proteins, involves both the ubiquitin proteasome system (UPS; see Glossary, Box 1) and autophagy (DeRenzo and Seydoux, 2004). For example, the participation of autophagy in cell reprogramming during the zygote-to-embryo transition (see Glossary, Box 1) has been documented (Hanna et al., 2010; Jopling et al., 2011). Autophagy is also essential in the very early stages of mouse embryogenesis, and is required for the embryo to reach the 4- to 8-cell stage (Tsukamoto et al., 2008; Wang et al., 2013b). The relevance of autophagy in this process was first evidenced in fertilized mouse oocytes lacking *Atg5*, which do not proceed beyond this stage if

Development (2018) 145, dev146506. doi:10.1242/dev.146506

fertilized with *Atg5*-null sperm, and therefore fail to form the blastocyst and the ICM (Tsukamoto et al., 2008). More recently, it has been proposed, based on the well-documented induction of autophagy during the 4- to 8-cell stage, that markers of autophagy activity could be used in the future to determine embryonic viability in the field of assisted reproduction (Tsukamoto et al., 2014).

The induction of autophagy during embryonic reprogramming and early embryonic development seems to be controlled by different molecular mechanisms. The initial pulse of autophagy induced by fertilization is independent of mTORC1 activity; it is possible that calcium oscillations triggered by fertilization initiate autophagic responses instead (Tsukamoto et al., 2008; Yamamoto et al., 2014). However, once the 4- to 8-cell stage of embryogenesis is reached, the downregulation of mTOR expression, which is mediated by Sox2 and the nucleosome remodelling and deacetylase complex (NuRD, see Glossary, Box 1), becomes indispensable for autophagy induction, similar to the situation seen during the reprogramming of somatic cells into iPSCs (Wang et al., 2013b) (as discussed later). Interestingly, during this period, overactivation of autophagy with the mTOR inhibitor rapamycin accelerates embryonic reprogramming and the formation of the blastocyst (Wang et al., 2013b). Autophagy is also essential for the removal of maternal material that otherwise blocks the reprogramming process (DeRenzo and Seydoux, 2004), and provides recycled amino acids, nucleotides and sterols that are crucial for maintaining cellular energy homeostasis prior to pre-implantation, after which cells have access to transplacental nutrients (Tsukamoto et al., 2008).

Interestingly, autophagy appears to be dispensable for the later stages of embryogenesis. Indeed, mice null for *Atg3*, *Atg5*, *Atg7*, *Atg9* or *Atg16L1* are not embryonically lethal, although they are born with reduced body weight and generally die 1 to 2 days after birth, possibly owing to suckling defects caused by deficient neurological development (Mizushima and Levine, 2010). It is thought that the neonatal survival of these mutant embryos is due to the presence of maternally inherited ATG proteins in the oocyte cytoplasm (Mizushima and Levine, 2010; Tsukamoto et al., 2008). However, this is not the case for mice that are null for the genes encoding the phagophore-forming BECN1, AMBRA1 or FIP200 (RB1CC1) proteins, which are embryonically lethal. The origin of such phenotypic differences among ATG gene knockout mouse models is unclear. It is possible that the embryonic death affecting BECN1, AMBRA1 and FIP200-deficient mice is due to other (i.e. autophagy-independent) functions of these proteins, as has been recently demonstrated for FIP200 (Chen et al., 2016), for example. Different degrees of functional redundancy or compensatory mechanisms for the different ATG proteins have also been postulated (Mizushima and Levine, 2010). Moreover, it has also been suggested that the UPS can compensate for the absence of autophagic activity in these autophagy-deficient ESCs (Lee et al., 2017; Vilchez et al., 2012). Indeed, crosstalk between the UPS and autophagy has been observed in human ESCs, which show high levels of proteasome activity that progressively decline during differentiation, coinciding with an increase in autophagy, which possibly then degrades damaged or unnecessary proteins and organelles (Vilchez et al., 2012). FOXO transcription factors (see Glossary, Box 1) have also been shown to regulate autophagy and the UPS (Sandri, 2012; Webb and Brunet, 2014). For instance, FOXO1 has been reported to be essential for maintaining human and mouse ESC pluripotency, probably by controlling OCT4 (POU5F1) and SOX2 expression (Zhang et al., 2011), and, more recently, it has been shown that FOXO1 regulates the expression of autophagy genes and maintains a high level of autophagic flux in mouse ESCs (Liu et al., 2017).

Autophagy has also been linked to the phagocytosis of apoptotic cells by either neighbour cells or professional phagocytes, a process that is required for proper metazoan development and adult tissue homeostasis. Qu and co-workers were the first to show that mouse ESCs lacking *Atg5* or *Becn1* fail to cavitate during embryoid body (EB) generation (Qu et al., 2007); these EBs display defective ATP production, which results in deficient expression of the engulfment signals required for the phagocytosis of dead cells. This phenotype could be reversed by the addition of methylpyruvate, a cell-permeant intermediate of glucose metabolism, which is incorporated into the mitochondrial tricarboxylic acid cycle. Similar findings were also shown in *Caenorhabditis elegans* and mouse embryonic development, in which autophagy is required for the proper clearance of apoptotic cells (Cheng et al., 2013; Huang et al., 2013; Li et al., 2012; Mellén et al., 2008, 2009).

Finally, it has been shown that autophagy promotes morphological changes associated with SC differentiation (Vessoni et al., 2012). For example, autophagy contributes to the degradation of the midbody ring (MB) during ESC differentiation, a process that can be triggered by either starvation or treatment with rapamycin (Kuo et al., 2011). The MB is a circular structure that forms an intercellular bridge after cytokinesis, and is required for the separation of daughter cells (Schink and Stenmark, 2011). Studies have provided some indication of how autophagy removes MBs, via a process known as midbophagy, which involves a complex consisting of p62 (SQSTM1), ALFY (WDFY3) and TRAF6 (Isakson et al., 2013), interactions between NBR1 and CEP55 (Kuo et al., 2011), and the participation of TRIM17 (Mandell et al., 2016). A better understanding of how these structures are cleared by autophagy and how they can avoid autophagosomal degradation could greatly advance our understanding of how SC pluripotency and differentiation are controlled. Studies have also demonstrated that the primary cilium (see Glossary, Box 1) emerges from ESCs after induced lineage specification and activates autophagy. This results in the inactivation of nuclear factor erythroid-related factor 2 (Nrf2; Nfe2l2), likely by autophagy-mediated degradation of its positive regulator p62, promoting the transcriptional activation of the pluripotency factors OCT4 and NANOG and directing ESCs towards a neuroectodermal fate (Jang et al., 2016). This induction of autophagy is not observed during mesoderm differentiation, indicating that autophagy is required for the degradation of organelles and proteins only in specific differentiated cells, rather than for the removal of damaged proteins. In conclusion, autophagy is required in ESCs to fulfil the energy requirements for cell remodelling and metabolic and proteostatic changes, and for the engulfment and clearance of apoptotic cells.

Autophagy in neural stem cells

A number of studies have investigated the role of autophagy in embryonic and adult NSCs. Our group has demonstrated that the expression of *Atg7*, *Becn1*, *LC3* and *Ambra1* is markedly increased in cultured embryonic mouse olfactory bulb (OB)-derived NSCs during the initial period of neuronal differentiation (Vazquez et al., 2012). Furthermore, it was shown that *Ambra1* and *Atg5* deficiency decreases neurogenesis, a phenotype reversed by methylpyruvate supplementation, suggesting that progenitor cells activate autophagy to meet their high energy demands (Vazquez et al., 2012). Defective neurogenesis in the mouse cerebral cortex during early brain development has also been reported following *Atg5* knockdown (Lv et al., 2014). A recent study using *Atg16L1* hypomorph mice and primary neurons showed that NOTCH, a plasma membrane receptor and master regulator of neuronal

Development (2018) 145, dev146506. doi:10.1242/dev.146506

development, is taken up into ATG16L1-positive autophagosome-precursor vesicles and modulates neurogenesis (Wu et al., 2016). Inhibition of mTOR via the autophagy-related protein Eva1a (also known as Tmem166) has also been linked to mouse NSC self-renewal and differentiation (Li et al., 2016).

In the adult mammalian brain, the best studied NSCs are those located in the subventricular zone (SVZ) of the lateral ventricles and in the subgranular zone (SGZ) of the hippocampal dentate gyrus (Doetsch et al., 1999; Palmer et al., 1997). These niches, like other SC niches in the body, are hypoxic, a condition required for stemness. NSCs, unlike terminally differentiated neurons, can expand through self-renewal and differentiate into several neural lineages. Oxidative stress, which is marked by elevated levels of reactive oxygen species (ROS), is one of the best-known factors inhibiting SC proliferation. Autophagy appears to maintain a low level of ROS in order to sustain the slow cycling of NSCs. Interestingly, the absence of FOXO1, FOXO3 and FOXO4 (i.e. in triple-null mice) leads to increased ROS production in the NSC pool, and this is accompanied by an initial increase in NSC proliferation followed by an abrupt reduction of the NSC pool and reduced neurogenesis (Paik et al., 2009; Palmer et al., 1997; Renault et al., 2009). It has been suggested that FOXO3 regulates the NSC pool by promoting quiescence, preventing premature differentiation and controlling oxygen metabolism (Renault et al., 2009). Once in the nucleus, FOXO3a might promote the expression of mitophagy genes to facilitate mitochondrial clearance, and it is therefore likely that FOXO activation decreases ROS levels via the induction of mitophagy as a protective mechanism to counterbalance mitochondrial stress (Tan and Wong, 2017). However, there is some disagreement regarding whether adult NSCs have lower or higher endogenous ROS levels than their differentiated progeny and the extent to which this could influence their self-renewal properties. A study of NSCs in the mouse SVZ reported high levels of ROS, on which the self-renewal and neurogenesis capabilities of these cells depend (Le Belle et al., 2011). The authors proposed that NSCs might maintain high ROS levels during highly proliferative stages of development, and lower levels during quiescence, suggesting a mechanism for antioxidant regulation.

Several other studies have also described the autophagy-mediated control of NSC proliferation and differentiation. For example, the downregulation of *Ambra1* and *Becn1* results in the decreased proliferation and increased apoptosis of mouse NSCs (Yazdankhah et al., 2014), and downregulation of miR-34a gives rise to increased expression of synaptic proteins and Atg9a, which appear to also regulate mouse NSC differentiation *in vitro* (Morgado et al., 2015). Together, these observations suggest that autophagy participates in the regulation of ROS levels through mitophagy, thereby controlling adult NSC proliferation, although the involvement of other molecular players or signalling pathways cannot be ruled out. Further studies will be required to identify whether crosstalk exists between autophagy and other catabolic systems in controlling NSC stemness, as well as the mechanisms that regulate the relative quiescence of NSCs during adult life.

The regulation of adult neurogenesis by autophagy has received surprisingly little research attention, with only a handful of studies supporting a role for autophagy in the maintenance and differentiation of adult SCs into different neuronal lineages. Wang and colleagues reported that ablation of *FIP200*, but not of *Atg5*, *Atg16L1* or *Atg7*, results in increased ROS that leads to a progressive loss of NSCs caused by p53 (Trp53)-dependent apoptotic and cell cycle arrest (Wang et al., 2016a, 2013a). The authors showed that *FIP200*-null NSCs, but not NSCs deficient for other autophagy genes, display p62 aggregates and increased SOD1 retained in the cytoplasm, leading to increased levels of superoxide (Wang et al., 2016a). Loss of FIP200 causes defects in neuronal differentiation and both failures can be rescued by treatment with the antioxidant N-acetylcysteine. Interestingly, *FIP200* deletion causes NSC depletion in the postnatal mouse brain but does not affect embryonic NSCs (Wang et al., 2013a). The same group recently showed that autophagy regulates NSCs through cell-extrinsic mechanisms. They demonstrated that FIP200 regulates the differentiation of NSCs in the mouse postnatal SVZ by restricting microglia infiltration and activation (Wang et al., 2017). However, other studies, using different genetic approaches to modulate gene expression, found that *Atg5* deletion impairs adult neurogenesis in the SGZ of the mouse hippocampus (Xi et al., 2016) and that *Atg5* downregulation leads to increased proliferation and decreased differentiation of mouse embryonic cortical neural precursor cells (Lv et al., 2014). It is therefore possible that autophagy is required for the maintenance and differentiation of NSCs at different stages of postnatal life.

Some authors have proposed very different roles for autophagy in the maintenance of adult rat hippocampal NSCs. Chung and co-workers found that rat hippocampal NSCs undergo autophagic cell death in response to insulin withdrawal despite the presence of intact apoptotic machinery, and that this effect is suppressed by the knockdown of *Atg7* (Chung et al., 2015). Conversely, Yu and colleagues reported that the tight regulation of calpains – a family of calcium-dependent cytosolic proteases – by the proteasome and by Ca^{2+} levels switches the mode of hippocampal NSC cell death from being autophagic, when calpain levels are low, to apoptotic, when calpains levels are high (Yu et al., 2008). These apparently contradictory findings demonstrate that there is still much to be discovered in order to fully understand the role of autophagy in adult neurogenesis. In recent years, other populations of NSCs have been detected in other regions of the mouse adult nervous system, such as Müller glia in the retina (Jorstad et al., 2017) and nestin-expressing progenitors in the cerebellum (Wojcinski et al., 2017), and it has been shown that these cells are able to repopulate their respective tissues upon induced damage. Mouse adult spinal cord glial cells can also be reprogrammed *in vivo* to generate neurons upon injury (Wang et al., 2016b). These studies constitute a compelling incentive to elucidate the molecular mechanisms through which autophagy mediates neuronal differentiation, as that knowledge could be applied to stimulate these resident SCs for adult brain repair.

Autophagy in haematopoietic stem cells

HSCs sustain the production of all blood cells (Fig. 2). The first HSCs appear in mid-gestation in mouse embryos and progressively colonize the foetal liver, the main haematopoietic organ during these embryonic stages. Just before birth, the bone marrow replaces the liver as the main reservoir of HSCs. In adulthood, haematopoiesis is maintained by multipotent bone marrow-resident HSCs (Hirschi, 2012). These quiescent HSCs self-renew but can enter the cell cycle and differentiate into multipotent progenitors during physiological haematopoiesis (Crisan and Dzierzak, 2016) to balance the massive destruction of blood cells that occurs daily, and also as a consequence of haematological stress (e.g. bone marrow transplantation, cytotoxic drugs, radiation). Dysregulation of the fine balance between quiescence, self-renewal and differentiation can result in the development of blood disorders, such as anaemia and leukaemia (Ansó et al., 2017). Understanding the mechanisms that protect HSCs from damage is therefore essential to treat haematopoietic malignancies.

Development (2018) 145, dev146506. doi:10.1242/dev.146506

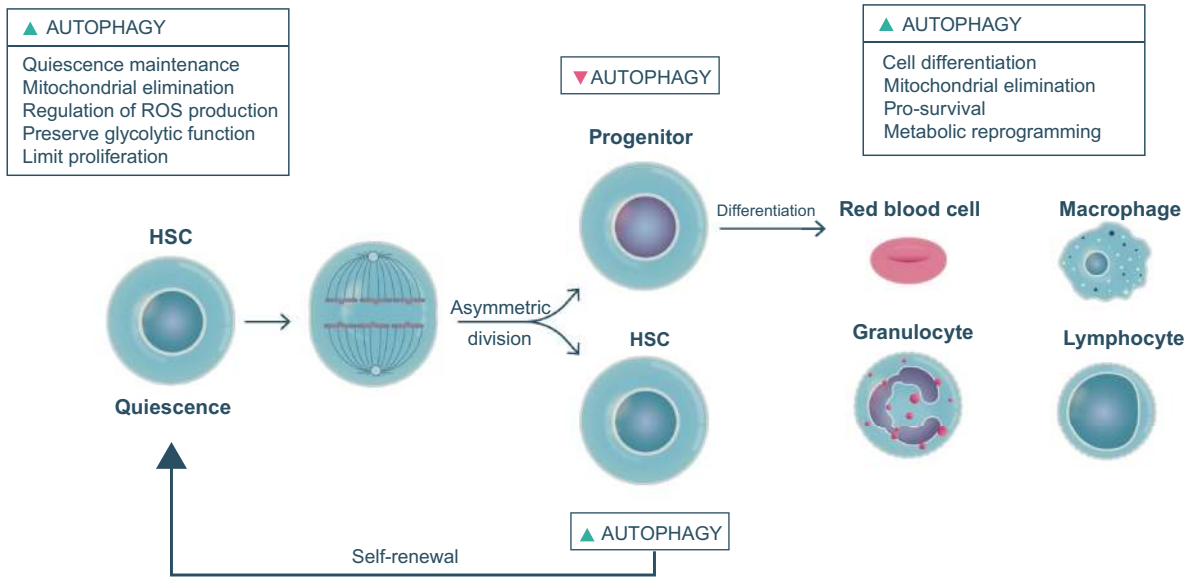

Fig. 2. The role of autophagy in preserving haematopoietic stem cell homeostasis. Autophagy contributes in several ways to the preservation of HSC quiescence. It regulates glycolysis and mitochondrial elimination, and limits proliferation and ROS production. In response to HSC activation, an asymmetric division produces another HSC (self-renewal) and a progenitor cell that displays reduced autophagy compared with HSCs. These progenitor cells differentiate into several lineages, and autophagy is also essential for this final differentiation step, acting as a cell remodelling mechanism, protecting cells from cell death, and influencing cellular metabolism.

Single-cell RNA sequencing has recently demonstrated high levels of transcription activity of autophagy-related genes during foetal HSC formation in mouse embryos (Hu et al., 2017; Zhou et al., 2016). Unlike adult SCs, which are mainly quiescent, foetal HSCs undergo rapid cycling. The cytofluorimetric analysis of cells isolated from transgenic GFP-LC3 mice, which express the autophagosomal marker LC3 tagged to GFP (Mizushima et al., 2004), has revealed increased autophagic flux activity in HSCs, compared with their differentiated progeny (Watson et al., 2015). Autophagy is also increased in HSCs from GFP-LC3 mice after cytokine withdrawal and is induced in a FOXO3a-dependent manner in response to metabolic stress (Warr et al., 2013). These data indicate that HSCs exhibit a high degree of basal and induced autophagy, supporting the view that autophagy is crucial for preserving HSC function.

Autophagy also plays an important role in maintaining foetal and adult HSCs. The conditional deletion of *FIP200* in mouse HSCs (i.e. in FIP200$^{flox/flox}$; Tie2-Cre mice) results in a massive reduction in the number of liver embryonic HSCs, resulting in foetal/perinatal lethality (Liu et al., 2010). Competitive reconstitution experiments in lethally irradiated recipients show that *FIP200*-null foetal liver HSCs also fail to provide long-term multilineage reconstitution. At the cellular level, these cells display increased mitochondrial mass and elevated ROS, and a slight increase in bromodeoxyuridine (BrdU) incorporation, suggesting that the observed exhaustion of HSCs might be due to increased proliferation following autophagy downregulation (Liu et al., 2010). Autophagy is also essential for adult HSC function. Indeed, foetal and adult HSCs isolated from Atg7$^{flox/flox}$; Vav-Cre mice are unable to form secondary colonies in colony-forming assays, and in transplantation experiments *Atg7*-deficient HSCs are unable to rescue lethally irradiated hosts (Mortensen et al., 2011). Interestingly, foetal liver *Atg7*-deficient HSCs can rescue lethally irradiated recipients, suggesting that Atg7 is less crucial for foetal than for adult HSC function. Mice with *Atg7*-deficient HSCs also have increased numbers of mitochondria as well as elevated oxidative stress, DNA damage and cell proliferation, which might promote the onset of blood malignancies observed in these mice (Mortensen et al., 2011). A similar phenotype has also been documented in Atg5$^{flox/flox}$; Vav-Cre mice (Watson et al., 2015), and heterozygous loss of *Atg5* in a model of acute myeloid leukaemia leads to increased HSC proliferation *in vitro* and to the development of more aggressive leukaemias *in vivo* (Watson et al., 2015). Deleting *Atg12* in 4-week-old mice using the pan-haematopoietic promoter Mx, which responds to the synthetic analogue of double-stranded RNA polyIpolyC, results in defective HSC self-renewal and myeloid-biased differentiation (Ho et al., 2017). Interestingly, this myeloid lineage expansion is also observed in other autophagy-deficient HSCs (Liu et al., 2010). In conclusion, these data support the view that autophagy preserves HSC stemness by reducing oxidative damage and limiting HSC proliferation.

The importance of mitophagy in the haematopoietic system has also been recognized. Mice deficient for the mitophagy receptor BNIP3L (also known as NIX) develop anaemia as a result of the defective elimination of mitochondria in red blood cells (Diwan et al., 2007; Sandoval et al., 2008). Recent findings also indicate that mitophagy is important not only for the elimination of mitochondria from mature erythrocytes but also to preserve stemness in HSCs (Ito et al., 2016). Ito and co-workers demonstrated that in a newly described HSC population [Tie2 (Tek)-positive cells; hereafter Tie2^{+}] the upregulation of two essential mitophagy regulators, PINK1 and PRKN, results in high levels of mitophagy that maintain HSC division potential. Silencing *Pink1* and *Prkn* results in a failure to reconstitute the haematopoietic system in irradiated recipient mice (Ito et al., 2016). Interestingly, in single-cell gene expression assays this Tie2^{+} population displays increased expression of fatty acid oxidation and peroxisome proliferator-activated receptor-delta (PPAR) genes. PPAR agonist treatment results in increased mitophagy through enhanced transcriptional expression of *Pink1* via FOXO3 signalling, in agreement with earlier data showing that FOXO3-dependent autophagy preserves HSC function (Warr et al.,

Development (2018) 145, dev146506. doi:10.1242/dev.146506

2013). These findings contrast with those of another study in which HSCs from *Prkn*-deficient animals were found to reconstitute the blood in transplantation experiments (Ho et al., 2017). Further studies are therefore required to understand better the putative role of mitophagy in HSC function (Joshi and Kundu, 2013).

The ability of HSCs to differentiate depends on their ability to activate mitochondrial oxidative phosphorylation (Kohli and Passegué, 2014). Accordingly, deletion of the mitochondrial phosphatase PTPMT1, which regulates the transition from anaerobic glycolysis to oxidative phosphorylation, results in accumulation of HSCs that are unable to differentiate (Yu et al., 2013). PTPMT1-Vav-Cre mice display important alterations in the HSC pool, as well as cell cycle delay and differentiation defects due to an inability to upregulate mitochondrial fatty acid oxidative metabolism. Moreover, deletion of the same gene in myeloid or lymphoid lineage progenitors does not impair normal development, indicating that PTPMT1 plays a pivotal role in HSCs but is not essential in late lineage progenitors (Yu et al., 2013). Other data support the existence of a close relationship between mitochondrial metabolism and HSC function. A recent report demonstrated that loss of the mitochondrial complex III subunit, Rieske iron sulfur protein (RISP), in mouse foetal HSCs has no effect on cell proliferation but does alter differentiation, leading to severe anaemia and embryonic death. Furthermore, deletion of the same gene in adult HSCs leads to the loss of HSC quiescence and to increased BrdU incorporation, indicative of cell cycle entry, and results in severe deficiencies in red and white cells as well as platelets (Ansó et al., 2017).

As mentioned earlier, autophagy is crucial for the later stages of the differentiation of many blood cell types, as evidenced by the dramatic alterations in cell function and differentiation observed following cell-specific autophagy blockade. Interestingly, GATA1, a master regulator of haematopoiesis, regulates several autophagy genes (Kang et al., 2012). Phenotypically, dysregulated autophagy is characterized by alterations in cellular quality control and by marked metabolic changes, which affect, for example, the glycolytic shift that occurs during proinflammatory macrophage activation (Esteban-Martínez and Boya, 2017; Riffelmacher et al., 2017). Autophagy activity is therefore not only necessary to sustain the self-renewal of HSCs but also to control the terminal differentiation of different blood cell types to maintain haemostasis (Fig. 2).

Autophagy and muscle stem cells

Skeletal muscle is composed of muscle fibres (myofibres), which consist of multinucleated syncytial cells. These postmitotic cells are unable to sustain muscle growth and repair, which instead relies on a unique population of muscle stem cells, also known as satellite cells. Named for their location beneath the basal lamina of muscle fibres, these are somite-derived myoblasts that have not fused to other myoblasts and remain as stem cells throughout adult life (Wang and Rudnicki, 2011).

During muscle development, embryonic myoblasts differentiate to generate primary myofibres that will serve as template fibres for subsequent myogenesis before birth. Later, rapid and extensive proliferation of postnatal myoblasts is responsible for further muscle growth and maturation during neonatal myogenesis (Wang and Rudnicki, 2011). By the third week of life in mice, the muscle is mature, and the number of satellite cells reaches a steady state as they enter quiescence (Fig. 3). Quiescent satellite cells are then activated in response to muscle damage and enter the cell cycle to give rise to committed proliferating muscle precursors, which differentiate and fuse to repair the damaged muscle. As in the case of HSCs, transplantation experiments are often used to evaluate the capacity of self-renewal to replenish the SC pool and to generate committed descendants that will proliferate and differentiate to orchestrate tissue repair.

Studies have only recently begun to investigate the role of autophagy in satellite cells. In *Atg7*-deficient mouse satellite cells (generated by crossing Pax7-Cre with $Atg7^{flox/flox}$ mice), satellite cell numbers are severely reduced (García-Prat et al., 2016). At the cellular level, this autophagy deficiency is very similar to the phenotype observed in aged satellite cells, which are characterized by increased oxidative stress, DNA damage, accumulation of p62 and ubiquitin aggregates, damaged mitochondria and presence of senescence markers, including p16INK4a (CDKN2A), p21CIP1 (CDKN1A) and P15INK4b (CDKN2B) (Fig. 3). Interestingly, satellite cells isolated from aged mice show a reduction in

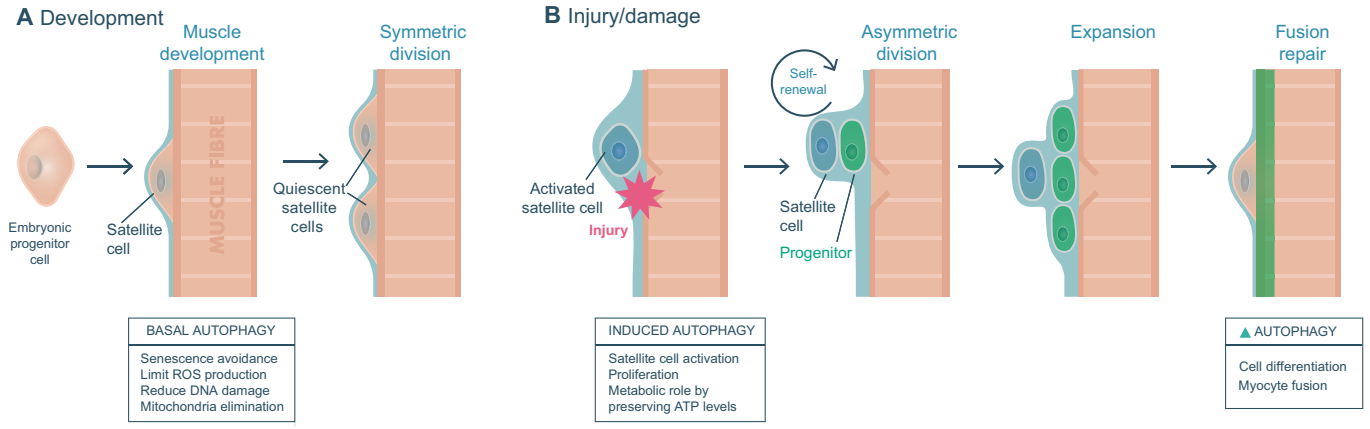

Fig. 3. Autophagy is essential for preventing senescence and aging in muscle satellite cells. Schematic of mammalian muscle development and repair. (A) During muscle development, embryonic muscle progenitor cells migrate to the myofibres and, by 3 weeks of age in mice, give rise to a pool of mature adult satellite cells by symmetric division. Basal autophagy helps to preserve quiescence in these satellite cells by preventing senescence and by limiting ROS production and DNA damage, probably via increased mitophagy. (B) In response to muscle injury or damage, satellite cells are activated and divide to self-renew and produce a progenitor cell that proliferates and expands. They depend on autophagy to provide metabolic substrates to fuel their activation and subsequent proliferation. During the later phases of muscle regeneration, increased autophagy is also required for the final stages of myocyte differentiation during fibre fusion.

Development (2018) 145, dev146506. doi:10.1242/dev.146506

autophagic flux, which is completely restored *in vivo* in response to mTOR inhibition with rapamycin or administration of spermidine, a natural polyamine that has been shown to extend the lifespan of mice in an autophagy-dependent manner (Eisenberg et al., 2016). These data demonstrate the potential role of autophagy in preserving muscle homeostasis and preventing age-dependent senescence (García-Prat et al., 2016). Interestingly, a recent study found that adult mouse satellite cells express genes involved in autophagy in a circadian manner, and aged satellite cells exhibit a markedly reduced capacity to cyclically recycle damaged components that are generated on a daily basis (Solanas et al., 2017).

In addition to the role of basal autophagy in maintaining SC stemness, induced autophagy is also essential for supporting the bioenergetic demands generated during satellite cell activation (Tang and Rando, 2014). When autophagy is acutely downregulated by pharmacological or genetic approaches in isolated *ex vivo* mouse myofibres or in sorted satellite cells, reduced BrdU incorporation during spontaneous activation in culture is observed, suggesting a delay in satellite cell activation (Fig. 3). Moreover, autophagy downregulation in mouse satellite cells reduces ATP levels, which can be restored by supplementing the cultures with sodium pyruvate, which also partially restores levels of cell proliferation (Tang and Rando, 2014).

Autophagy is also essential in the later stages of muscle cell differentiation, with autophagy blockade resulting in altered myocyte fusion and myotube formation during muscle differentiation (Fortini et al., 2016; Sin et al., 2016). Interestingly, autophagy is activated during the early, compensatory regenerative stages in the *mdx* mouse model of Duchenne muscular dystrophy, and impaired autophagy activation in late stages of disease progression correlates with fibrotic tissue deposition and with diminished regeneration in dystrophic muscles (Fiacco et al., 2016). These findings indicate that autophagy is essential for preserving muscle homeostasis, serving as a quality control mechanism by preventing satellite cell senescence and meeting the bioenergetic demands of satellite cells during activation (Fig. 3).

Autophagy in somatic reprogramming and iPSC generation

The advent of cell reprogramming methodologies has enabled the generation of iPSCs. These are pluripotent SCs generated from differentiated somatic cells that are reset to a pluripotent state (Yamanaka and Blau, 2010) by the pluripotency transcription factors, which include Oct4, Sox2, Klf4 and Myc (together called OSKM) (Takahashi and Yamanaka, 2006; Yamanaka, 2012). In recent years, a number of studies have revealed that autophagy is implicated in this reprogramming process, particularly during the early stages (Buckley et al., 2012; Hansson et al., 2012; Tsukamoto et al., 2008; Wang et al., 2013b) (Fig. 4). For example, the deletion of *Atg5*, *Atg3* or *Atg7* in mouse embryonic fibroblasts impairs reprogramming efficiency (Tsukamoto et al., 2008; Wang et al., 2013b). Furthermore, autophagy degrades nuclear pluripotency-associated proteins that are normally only transiently expressed (Cho et al., 2014). Transducing mouse fibroblasts with OSKM triggers a transient pulse of increased autophagy from day 1 that peaks the following day and subsequently declines to basal levels by day 3, correlating with mTOR downregulation at both the mRNA and protein levels (Menendez et al., 2011; Wang et al., 2013b). This transient mTOR inhibition is essential, as demonstrated by experiments in which cells treated with rapamycin during the first 3 days after OSKM transduction show increased efficiency of cell reprogramming; by contrast, rapamycin treatment at later stages, or very high concentrations of rapamycin, abolish iPSC generation, suggesting that autophagy needs to be downregulated soon after it peaks (Chen et al., 2011; He et al., 2012). Accordingly, iPSC reprogramming is also inhibited by increasing mTOR activity by knocking down the mTOR negative regulator Tsc2 or by using $Tsc2^{-/-}$ mouse embryonic fibroblasts in which mTOR activity is hyperactivated (He et al., 2012). At the molecular level, it has been shown that Sox2, which is one of the OSKM factors, controls mTOR expression via the NuRD complex (Hu and Wade, 2012; Wang et al., 2013b). During the early stages of cell reprogramming, the NuRD complex is recruited by Sox2 to a repressive region of the mTOR promoter to mediate its transcriptional repression, and it dissociates from it 2 days after the induction of reprogramming. However, little is known about the feedback mechanisms that shut down the activity of the NuRD complex to enable the transcription and translation of mTOR to resume in the later stages of reprogramming (Rais et al., 2013). Such mechanisms might be essential for establishing the pluripotency signalling network required for cell reprogramming.

A role for mitochondria and mitophagy has also been implicated in iPSCs. As in ESCs, the mass, maturation status and number of mitochondria are significantly reduced in iPSCs compared with somatic cells (Armstrong et al., 2010; Facucho-Oliveira and St John, 2009; Prigione et al., 2010; Sena and Chandel, 2012; St John et al., 2006), suggesting that pluripotent SCs rely more heavily on glycolysis for energy production (Fig. 4). Although it is commonly accepted that mitophagy plays an important role in creating the conditions necessary to establish pluripotency (Vessoni et al., 2012; Wang et al., 2013b) and mediates mitochondrial rejuvenation to prevent iPSC differentiation, the form of autophagy involved and the molecular mechanisms that govern these processes remain a

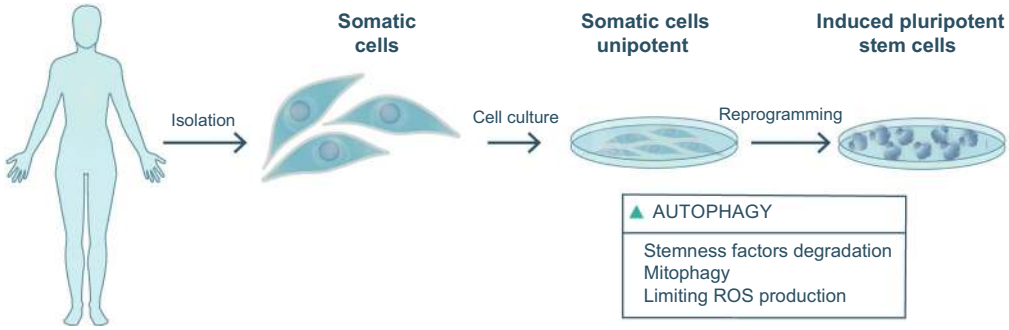

Fig. 4. The role of autophagy during reprogramming. iPSCs can be generated from healthy donors or diseased patients by reprogramming somatic cells, such as skin fibroblasts, via the transient expression of several transcription factors. Reprogramming is dependent upon autophagy, which promotes the degradation of stemness factors and mediates mitochondrial degradation by mitophagy, thus limiting ROS production and modulating metabolism.

Development (2018) 145, dev146506. doi:10.1242/dev.146506

matter of debate (Vessoni et al., 2012; Wang et al., 2013b). A recent study reported that mitophagy is essential for iPSC reprogramming and is regulated by Atg5-independent, AMPK-dependent autophagy (Ma et al., 2015). However, how mitophagy is controlled and how it functions in pluripotency reprogramming remains to be elucidated.

Autophagy in cancer stem cells

The role of autophagy in cancer stem cells (CSCs) has been studied in detail (Auberger and Puissant, 2017; Hamaï et al., 2014). Below, we briefly summarize how the autophagic-lysosomal pathway contributes to the unique characteristics of CSCs. We also discuss the potential value of targeting autophagy as a means of eradicating CSCs.

Recent *in vivo* lineage-tracing approaches support the involvement of CSCs in many cancers (Beck and Blanpain, 2013; Pattabiraman and Weinberg, 2014; Singh et al., 2015). Similar to other SCs, CSCs can self-renew (Beck and Blanpain, 2013) but they also have a potential for malignancy (Beck and Blanpain, 2013; Pattabiraman and Weinberg, 2014; Tam and Weinberg, 2013). Of note, they are highly resistant to cancer therapy and recent evidence suggests they can initiate tumour metastasis (de Sousa e Melo et al., 2017; Lawson et al., 2015; Massagué and Obenauf, 2016; Pascual et al., 2017). Moreover, it has been shown that CSCs harbour the ability to convert into non-cancer SCs and vice versa, a phenomenon known as CSC plasticity (Beck and Blanpain, 2013; Pattabiraman and Weinberg, 2014; Singh et al., 2015). This phenomenon of CSC plasticity increases the complexity of the relationship between autophagy and CSCs. Thus, although active autophagy is a recognized hallmark of tumours (Galluzzi et al., 2015; White, 2015), it can serve as a tumour-suppressing mechanism or can promote tumour formation, depending on the type of cancer and the stage of development (Galluzzi et al., 2015). Autophagy is also implicated in the crosstalk between cancer cells and the microenvironment, host tissues and the immune system (Galluzzi et al., 2017b, 2015; Zhong et al., 2016). Compelling evidence indicates that autophagy is a major cellular pathway involved in the origin, maintenance and differentiation of CSCs (Auberger and Puissant, 2017; Guan et al., 2013; Hamaï et al., 2014; Pan et al., 2013); CSCs reside in hypoxic, nutrient-poor and acidic environments, conditions known to induce autophagy, and many studies have shown that CSCs are highly responsive to these stimuli (Fig. 5) and, hence, that the basal rate of autophagy is frequently higher in CSCs than in non-cancer SCs.

Given the multiple functions of autophagy in CSCs (Fig. 5), a number of studies have investigated if and how autophagy can

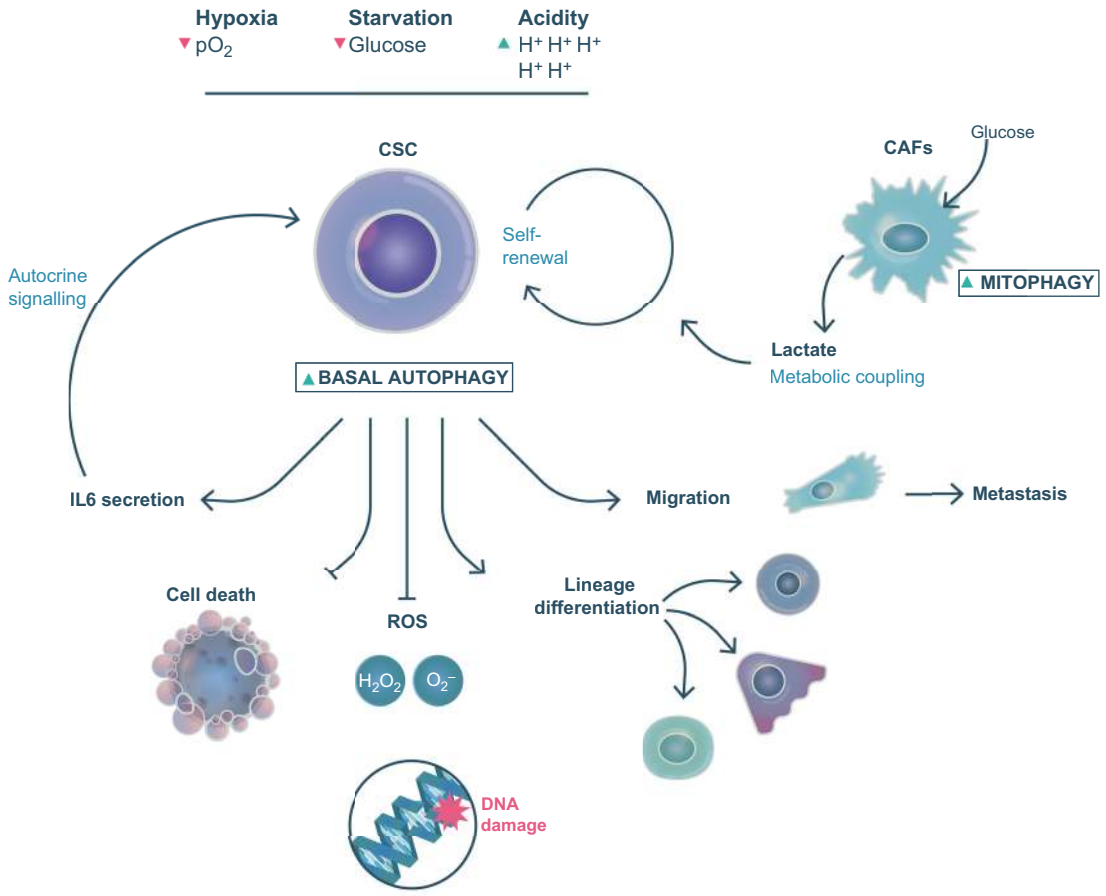

Fig. 5. The roles of autophagy in cancer stem cells. Distinctive features of the CSC niche, including hypoxia, reduced nutrient availability and acidity (H^+), promote high levels of basal autophagy in CSCs. Mitophagy also occurs in cancer-associated fibroblasts (CAFs), resulting in glucose fermentation to lactate, which is used to fuel the growth of CSCs. Autophagy is also important for the self-renewal of CSCs via IL6-mediated autocrine signalling. High levels of autophagic flux protect CSCs from cell death and from DNA damage induced by high ROS concentrations. Autophagy also regulates lineage differentiation and cell migration, thereby influencing metastasis in other tissues.

Development (2018) 145, dev146506. doi:10.1242/dev.146506

influence tumorigenesis. It has been shown that autophagy supports the survival of human breast malignant precursor cells but that treatment with chloroquine, a lysosomotropic inhibitor of autophagy, blocks the generation of breast ductal carcinoma *in situ* spheroids *in vitro* and abrogates xenograft tumour formation (Espina et al., 2010). The silencing of *BECN1* and *ATG7* impairs the *in vitro* self-renewal of ALDH1-positive breast cancer cell lines or CSCs isolated from human breast cancer specimens and inhibits their growth in xenografts in mice (Gong et al., 2013; Yue et al., 2013). Similarly, the silencing of *ATG7*, *ATG12* or *ATG8/LC3* impairs the *in vitro* growth of $CD44^{+}CD24^{-/low}$ breast cancer stem cells (Cufi et al., 2011; Maycotte et al., 2015). Interestingly, the inhibition of autophagy in $CD44^{+}CD24^{-}$ breast CSCs decreases the secretion of IL6 (Maycotte et al., 2015), a cytokine important for CSC maintenance (Iliopoulos et al., 2011).

The role of autophagy in the survival of CSCs and the maintenance of stemness has also been described in other tumour types (Auberger and Puissant, 2017; Hamaï et al., 2014; Lin et al., 2015; Marcucci et al., 2017; Ojha et al., 2015). However, the role of autophagy in CSCs is probably more complex, as demonstrated by the fact that autophagy inhibition decreases the viability of chronic myeloid leukaemia $CD34^{+}$ progenitor cells, whereas its inhibition in HSCs favours the expansion of acute myeloid leukaemia progenitor cells (Auberger and Puissant, 2017). In summary, these data underscore the importance of autophagy in CSC function but highlight the need for further studies to investigate the potential of cancer therapies that target autophagy in CSCs.

Conclusions

In this Review, we have discussed studies that have provided important insights into the pivotal roles that autophagy plays in embryonic and adult SCs, including in the maintenance of stemness, the promotion of cellular reprogramming and the differentiation of SCs (summarized in Fig. 6). Together, these findings indicate that: (1) autophagy is used for cell remodelling to degrade organelles and stemness factors during SC reprogramming, activation or differentiation; (2) autophagy-mediated cell repair and quality control mechanisms are essential to preserve homeostasis in most if not all SCs, and this is usually associated with eliminating damaged mitochondria, the most usual source of cellular ROS; and (3) autophagy and mitophagy are essential to preserve the energy homeostasis and metabolic reprogramming that allow different SC types to maintain quiescence, self-renewal, activation and differentiation. Accordingly, autophagy deficiency results in significant alterations in SC function including SC exhaustion, senescence, aging and cell death (Fig. 6).

Although pharmacological approaches to modulate SC fate have shown some promise (Angelos et al., 2017; Bouchez et al., 2011; Fares et al., 2014; Rentas et al., 2016), few studies have successfully modulated SC function by targeting autophagy. SMER28, a small molecule capable of inducing autophagy (Sarkar et al., 2007), has been used to reverse erythropoiesis abnormalities in patients with Diamond–Blackfan anaemia (DBA), a congenital disorder characterized by severely diminished red blood cell production due to defective erythroid progenitor differentiation (Doulatov et al., 2017). Classical autophagy-modulating approaches, such as

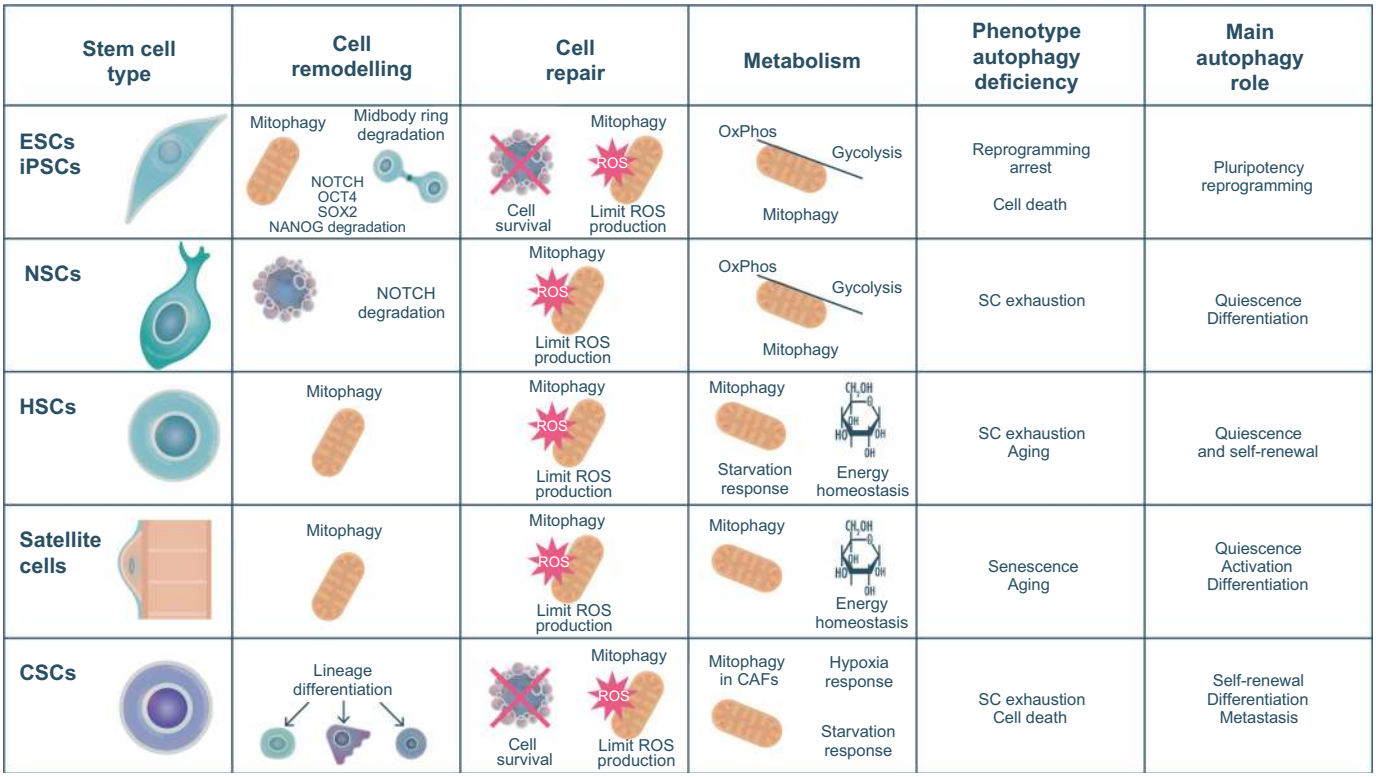

Fig. 6. A summary of the main roles of autophagy in stem cells. Autophagy has three main roles that preserve proper SC function. Cell remodelling is associated with organelle and stemness factor degradation in pluripotent cells and during reprogramming, and with apoptotic cell degradation and differentiation for many SC types. Cell repair functions to limit ROS production by eliminating damaged mitochondria, thereby promoting cell survival. Lastly, autophagy influences cellular metabolism: for example, mitophagy has been shown to promote the glycolytic shift in reprogramming as well as to control energy homeostasis after nutrient and oxygen deprivation. In line with this, autophagy deficiency results in alterations in pluripotency, reprogramming, cell death, exhaustion, senescence and aging. CAFs, cancer-associated fibroblasts; OxPhos, oxidative phosphorylation.

rapamycin or spermidine treatment, caloric restriction, and low protein diets, have also been shown to preserve HSC and muscle satellite cell function (Cerletti et al., 2012; Fiacco et al., 2016; García-Prat et al., 2016; Kohli and Passegué, 2014). These studies suggest that SC function could indeed be modulated by targeting autophagy, a finding that has relevant therapeutic implications.

A better understanding of the molecular mechanisms through which autophagy regulates the function of SCs and their differentiation into specific cell types could hold significant promise for the development of new therapies for haematological, muscular and neurological diseases, as well as for some cancers. Other questions on the role of autophagy in SC maintenance and differentiation remain unanswered. For example, it is still unclear whether autophagy failure during ESC expansion or differentiation has deleterious consequences in the adult organism. How autophagy is involved in maintaining adult stem cell quiescence, exit and re-entry into this cellular stage is an emerging field that has only recently started to be explored in HSCs and muscle satellite cells and remains dark for other SC types, such as NSCs. Furthermore, we also emphasize the need to finely decipher the distinct autophagy requirements for healthy adult SCs and ESCs versus CSCs in order to find autophagy-based targets for potential anti-cancer therapies and to prevent malignant reprogramming into CSCs. Our knowledge on the understanding of autophagy function in SC biology has expanded dramatically in the last decade. However, although it is clear that autophagy is a key determinant of SC self-renewal, stemness and differentiation, many aspects of this relationship remain unclear. Answering these questions will increase our insight into SC biology and human development, improve the efficiency of iPSC reprogramming and differentiation protocols, and facilitate the design of strategies to delay the onset of degenerative and age-associated diseases.

Acknowledgements
We thank O. Howard for English language editing. The authors apologize for work not being mentioned owing to space limitations.

Competing interests
The authors declare no competing or financial interests.

Funding
P.B. is supported by Spain's Ministerio de Economía y Competitividad [BFU2015-65623 (FEDER funding) and BFU2015-71869-REDT], European Cooperation in Science and Technology (COST) Action Transautophagy (CA15138) and the European Union Horizon 2020 Marie Skłodowska-Curie Innovative Training Networks (ITN-ETN) under grant agreement 765912. N.R.-M. is supported by a grant from the Muscular Dystrophy Association (MDA376743) and a Juan de la Cierva Incorporación fellowship from Spain's Ministerio de Economía y Competitividad. P.C. is supported by funding from the Institut National de la Santé et de la Recherche Médicale (INSERM), the Centre National de la Recherche Scientifique (CNRS), and Université Paris Descartes-Sorbonne Paris Cité and grants from the Institut National Du Cancer (INCa) and the Agence Nationale de la Recherche (ANR).

References
Al Rawi, S., Louvet-Vallee, S., Djeddi, A., Sachse, M., Culetto, E., Hajjar, C., Boyd, L., Legouis, R. and Galy, V. (2011). Postfertilization autophagy of sperm organelles prevents paternal mitochondrial DNA transmission. *Science* **334**, 1144-1147.

Angelos, M. G., Ruh, P. N., Webber, B. R., Blum, R. H., Ryan, C. D., Bendzick, L., Shim, S., Yingst, A. M., Tufa, D. M., Verneris, M. R. et al. (2017). Aryl hydrocarbon receptor inhibition promotes hematolymphoid development from human pluripotent stem cells. *Blood* **129**, 3428-3439.

Ansó, E., Weinberg, S. E., Diebold, L. P., Thompson, B. J., Malinge, S., Schumacker, P. T., Liu, X., Zhang, Y., Shao, Z., Steadman, M. et al. (2017). The mitochondrial respiratory chain is essential for haematopoietic stem cell function. *Nat. Cell Biol.* **19**, 614-625.

Armstrong, L., Tilgner, K., Saretzki, G., Atkinson, S. P., Stojkovic, M., Moreno, R., Przyborski, S. and Lako, M. (2010). Human induced pluripotent stem cell lines show stress defense mechanisms and mitochondrial regulation similar to those of human embryonic stem cells. *Stem Cells* **28**, 661-673.

Ashrafi, G. and Schwarz, T. L. (2013). The pathways of mitophagy for quality control and clearance of mitochondria. *Cell Death Differ.* **20**, 31-42.

Auberger, P. and Puissant, A. (2017). Autophagy, a key mechanism of oncogenesis and resistance in leukemia. *Blood* **129**, 547-552.

Beck, B. and Blanpain, C. (2013). Unravelling cancer stem cell potential. *Nat. Rev. Cancer* **13**, 727-738.

Bouchez, L. C., Boitano, A. E., de Lichtervelde, L., Romeo, R., Cooke, M. P. and Schultz, P. G. (2011). Small-molecule regulators of human stem cell self-renewal. *Chembiochem* **12**, 854-857.

Boya, P., Gonzalez-Polo, R.-A., Casares, N., Perfettini, J., Dessen, P., Larochette, N., Metivier, D., Meley, D., Souquere, S., Yoshimori, T. et al. (2005). Inhibition of macroautophagy triggers apoptosis. *Mol. Cell. Biol.* **25**, 1025-1040.

Boya, P., Reggiori, F. and Codogno, P. (2013). Emerging regulation and functions of autophagy. *Nat. Cell Biol.* **15**, 713-720.

Boya, P., Esteban-Martínez, L., Serrano-Puebla, A., Gómez-Sintes, R. and Villarejo-Zori, B. (2016). Autophagy in the eye: development, degeneration, and aging. *Prog. Retin. Eye Res.* **55**, 206-245.

Buckley, S. M., Aranda-Orgilles, B., Strikoudis, A., Apostolou, E., Loizou, E., Moran-Crusio, K., Farnsworth, C. L., Koller, A. A., Dasgupta, R., Silva, J. C. et al. (2012). Regulation of pluripotency and cellular reprogramming by the ubiquitin-proteasome system. *Cell Stem Cell* **11**, 783-798.

Cerletti, M., Jang, Y. C., Finley, L. W., Haigis, M. C. and Wagers, A. J. (2012). Short-term calorie restriction enhances skeletal muscle stem cell function. *Cell Stem Cell* **10**, 515-519.

Chen, T., Shen, L., Yu, J., Wan, H., Guo, A., Chen, J., Long, Y., Zhao, J. and Pei, G. (2011). Rapamycin and other longevity-promoting compounds enhance the generation of mouse induced pluripotent stem cells. *Aging Cell* **10**, 908-911.

Chen, S., Wang, C., Yeo, S., Liang, C.-C., Okamoto, T., Sun, S., Wen, J. and Guan, J.-L. (2016). Distinct roles of autophagy-dependent and -independent functions of FIP200 revealed by generation and analysis of a mutant knock-in mouse model. *Genes Dev.* **30**, 856-869.

Cheng, S., Wu, Y., Lu, Q., Yan, J., Zhang, H. and Wang, X. (2013). Autophagy genes coordinate with the class II PI/PtdIns 3-kinase PIKI-1 to regulate apoptotic cell clearance in C. elegans. *Autophagy* **9**, 2022-2032.

Cho, Y.-H., Han, K.-M., Kim, D., Lee, J., Lee, S.-H., Choi, K.-W., Kim, J. and Han, Y.-M. (2014). Autophagy regulates homeostasis of pluripotency-associated proteins in hESCs. *Stem Cells* **32**, 424-435.

Chung, K. M., Park, H., Jung, S., Ha, S., Yoo, S.-J., Woo, H., Lee, H. J., Kim, S. W., Kim, E.-K., Moon, C. et al. (2015). Calpain determines the propensity of adult hippocampal neural stem cells to autophagic cell death following insulin withdrawal. *Stem Cells* **33**, 3052-3064.

Crisan, M. and Dzierzak, E. (2016). The many faces of hematopoietic stem cell heterogeneity. *Development* **143**, 4571-4581.

Cufi, S., Vazquez-Martin, A., Oliveras-Ferraros, C., Martin-Castillo, B., Vellon, L. and Menendez, J. A. (2011). Autophagy positively regulates the CD44(+) CD24(−/low) breast cancer stem-like phenotype. *Cell Cycle* **10**, 3871-3885.

DeRenzo, C. and Seydoux, G. (2004). A clean start: degradation of maternal proteins at the oocyte-to-embryo transition. *Trends Cell Biol.* **14**, 420-426.

Deretic, V., Kimura, T., Timmins, G., Moseley, P., Chauhan, S. and Mandell, M. (2015). Immunologic manifestations of autophagy. *J. Clin. Invest.* **125**, 75-84.

de Sousa e Melo, F., Kurtova, A. V., Harnoss, J. M., Kljavin, N., Hoeck, J. D., Hung, J., Anderson, J. E., Storm, E. E., Modrusan, Z., Koeppen, H. et al. (2017). A distinct role for Lgr5+ stem cells in primary and metastatic colon cancer. *Nature* **543**, 676-680.

Diwan, A., Koesters, A. G., Odley, A. M., Pushkaran, S., Baines, C. P., Spike, B. T., Daria, D., Jegga, A. G., Geiger, H., Aronow, B. J. et al. (2007). Unrestrained erythroblast development in Nix−/− mice reveals a mechanism for apoptotic modulation of erythropoiesis. *Proc. Natl. Acad. Sci. USA* **104**, 6794-6799.

Doetsch, F., Caillé, I., Lim, D. A., García-Verdugo, J. M. and Alvarez-Buylla, A. (1999). Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell* **97**, 703-716.

Doulatov, S., Vo, L. T., Macari, E. R., Wahlster, L., Kinney, M. A., Taylor, A. M., Barragan, J., Gupta, M., McGrath, K., Lee, H. Y. et al. (2017). Drug discovery for Diamond-Blackfan anemia using reprogrammed hematopoietic progenitors. *Sci. Transl. Med.* **9**, eaah5645.

Eisenberg, T., Abdellatif, M., Schroeder, S., Primessnig, U., Stekovic, S., Pendl, T., Harger, A., Schipke, J., Zimmermann, A., Schmidt, A. et al. (2016). Cardioprotection and lifespan extension by the natural polyamine spermidine. *Nat. Med.* **22**, 1428-1438.

Espina, V., Mariani, B. D., Gallagher, R. I., Tran, K., Banks, S., Wiedemann, J., Huryk, H., Mueller, C., Adamo, L., Deng, J. et al. (2010). Malignant precursor cells pre-exist in human breast DCIS and require autophagy for survival. *PLoS ONE* **5**, e10240.

Esteban-Martínez, L. and Boya, P. (2017). BNIP3L/NIX-dependent mitophagy regulates cell differentiation via metabolic reprogramming. *Autophagy* 1-3.

Esteban-Martínez, L., Sierra-Filardi, E. and Boya, P. (2017a). Mitophagy, metabolism, and cell fate. *Mol. Cell. Oncol.* **4**, e1353854.
Esteban-Martínez, L., Sierra-Filardi, E., McGreal, R. S., Salazar-Roa, M., Marino, G., Seco, E., Durand, S., Enot, D., Grana, O., Malumbres, M. et al. (2017b). Programmed mitophagy is essential for the glycolytic switch during cell differentiation. *EMBO J.* **36**, 1688-1706.
Facucho-Oliveira, J. M. and St John, J. C. (2009). The relationship between pluripotency and mitochondrial DNA proliferation during early embryo development and embryonic stem cell differentiation. *Stem Cell Rev.* **5**, 140-158.
Fares, I., Chagraoui, J., Gareau, Y., Gingras, S., Ruel, R., Mayotte, N., Csaszar, E., Knapp, D. J., Miller, P., Ngom, M. et al. (2014). Cord blood expansion. Pyrimidoindole derivatives are agonists of human hematopoietic stem cell self-renewal. *Science* **345**, 1509-1512.
Fiacco, E., Castagnetti, F., Bianconi, V., Madaro, L., De Bardi, M., Nazio, F., D'Amico, A., Bertini, E., Cecconi, F., Puri, P. L. et al. (2016). Autophagy regulates satellite cell ability to regenerate normal and dystrophic muscles. *Cell Death Differ.* **23**, 1839-1849.
Folmes, C. D. L., Nelson, T. J., Martinez-Fernandez, A., Arrell, D. K., Lindor, J. Z., Dzeja, P. P., Ikeda, Y., Perez-Terzic, C. and Terzic, A. (2011). Somatic oxidative bioenergetics transitions into pluripotency-dependent glycolysis to facilitate nuclear reprogramming. *Cell Metab.* **14**, 264-271.
Fortini, P., Ferretti, C., Iorio, E., Cagnin, M., Garribba, L., Pietraforte, D., Falchi, M., Pascucci, B., Baccarini, S., Morani, F. et al. (2016). The fine tuning of metabolism, autophagy and differentiation during in vitro myogenesis. *Cell Death Dis.* **7**, e2168.
Galluzzi, L., Pietrocola, F., Bravo-San Pedro, J. M., Amaravadi, R. K., Baehrecke, E. H., Cecconi, F., Codogno, P., Debnath, J., Gewirtz, D. A., Karantza, V. et al. (2015). Autophagy in malignant transformation and cancer progression. *EMBO J.* **34**, 856-880.
Galluzzi, L., Baehrecke, E. H., Ballabio, A., Boya, P., Bravo-San Pedro, J. M., Cecconi, F., Choi, A. M., Chu, C. T., Codogno, P., Colombo, M. I. et al. (2017a). Molecular definitions of autophagy and related processes. *EMBO J.* **36**, 1811-1836.
Galluzzi, L., Bravo-San Pedro, J. M., Demaria, S., Formenti, S. C. and Kroemer, G. (2017b). Activating autophagy to potentiate immunogenic chemotherapy and radiation therapy. *Nat. Rev. Clin. Oncol.* **14**, 247-258.
García-Prat, L., Martínez-Vicente, M., Perdiguero, E., Ortet, L., Rodríguez-Ubreva, J., Rebollo, E., Ruiz-Bonilla, V., Gutarra, S., Ballestar, E., Serrano, A. L. et al. (2016). Autophagy maintains stemness by preventing senescence. *Nature* **529**, 37-42.
Garcia-Prat, L., Sousa-Victor, P. and Munoz-Canoves, P. (2017). Proteostatic and metabolic control of stemness. *Cell Stem Cell* **20**, 593-608.
Gong, C., Bauvy, C., Tonelli, G., Yue, W., Delomenie, C., Nicolas, V., Zhu, Y., Domergue, V., Marin-Esteban, V., Tharinger, H. et al. (2013). Beclin 1 and autophagy are required for the tumorigenicity of breast cancer stem-like/progenitor cells. *Oncogene* **32**, 2261-2272.
Guan, J.-L., Simon, A. K., Prescott, M., Menendez, J. A., Liu, F., Wang, F., Wang, C., Wolvetang, E., Vazquez-Martin, A. and Zhang, J. (2013). Autophagy in stem cells. *Autophagy* **9**, 830-849.
Hamaï, A., Codogno, P. and Mehrpour, M. (2014). Cancer stem cells and autophagy. *J. Cancer Stem Cell Res.* **2**, e1005.
Hanna, J. H., Saha, K. and Jaenisch, R. (2010). Pluripotency and cellular reprogramming: facts, hypotheses, unresolved issues. *Cell* **143**, 508-525.
Hansson, J., Rafiee, M. R., Reiland, S., Polo, J. M., Gehring, J., Okawa, S., Huber, W., Hochedlinger, K. and Krijgsveld, J. (2012). Highly coordinated proteome dynamics during reprogramming of somatic cells to pluripotency. *Cell Rep.* **2**, 1579-1592.
He, J., Kang, L., Wu, T., Zhang, J., Wang, H., Gao, H., Zhang, Y., Huang, B., Liu, W., Kou, Z. et al. (2012). An elaborate regulation of Mammalian target of rapamycin activity is required for somatic cell reprogramming induced by defined transcription factors. *Stem Cells Dev.* **21**, 2630-2641.
Hirschi, K. K. (2012). Hemogenic endothelium during development and beyond. *Blood* **119**, 4823-4827.
Ho, T. T., Warr, M. R., Adelman, E. R., Lansinger, O. M., Flach, J., Verovskaya, E. V., Figueroa, M. E. and Passegué, E. (2017). Autophagy maintains the metabolism and function of young and old stem cells. *Nature* **543**, 205-210.
Hu, G. and Wade, P. A. (2012). NuRD and pluripotency: a complex balancing act. *Cell Stem Cell* **10**, 497-503.
Hu, Y., Huang, Y., Yi, Y., Wang, H., Liu, B., Yu, J. and Wang, D. (2017). Single-cell RNA sequencing highlights transcription activity of autophagy-related genes during hematopoietic stem cell formation in mouse embryos. *Autophagy* **13**, 770-771.
Huang, S., Jia, K., Wang, Y., Zhou, Z. and Levine, B. (2013). Autophagy genes function in apoptotic cell corpse clearance during C. elegans embryonic development. *Autophagy* **9**, 138-149.
Hurley, J. H. and Young, L. N. (2017). Mechanisms of autophagy initiation. *Annu. Rev. Biochem.* **86**, 225-244.
Iliopoulos, D., Hirsch, H. A., Wang, G. and Struhl, K. (2011). Inducible formation of breast cancer stem cells and their dynamic equilibrium with non-stem cancer cells via IL6 secretion. *Proc. Natl. Acad. Sci. USA* **108**, 1397-1402.
Isakson, P., Lystad, A. H., Breen, K., Koster, G., Stenmark, H. and Simonsen, A. (2013). TRAF6 mediates ubiquitination of KIF23/MKLP1 and is required for midbody ring degradation by selective autophagy. *Autophagy* **9**, 1955-1964.
Ito, K., Turcotte, R., Cui, J., Zimmerman, S. E., Pinho, S., Mizoguchi, T., Arai, F., Runnels, J. M., Alt, C., Teruya-Feldstein, J. et al. (2016). Self-renewal of a purified Tie2+ hematopoietic stem cell population relies on mitochondrial clearance. *Science* **354**, 1156-1160.
Jang, J., Wang, Y., Lalli, M. A., Guzman, E., Godshalk, S. E., Zhou, H. and Kosik, K. S. (2016). Primary cilium-autophagy-Nrf2 (PAN) axis activation commits human embryonic stem cells to a neuroectoderm fate. *Cell* **165**, 410-420.
Jopling, C., Boue, S. and Izpisua Belmonte, J. C. I. (2011). Dedifferentiation, transdifferentiation and reprogramming: three routes to regeneration. *Nat. Rev. Mol. Cell Biol.* **12**, 79-89.
Jorstad, N. L., Wilken, M. S., Grimes, W. N., Wohl, S. G., VandenBosch, L. S., Yoshimatsu, T., Wong, R. O., Rieke, F. and Reh, T. A. (2017). Stimulation of functional neuronal regeneration from Muller glia in adult mice. *Nature* **548**, 103-107.
Joshi, A. and Kundu, M. (2013). Mitophagy in hematopoietic stem cells: the case for exploration. *Autophagy* **9**, 1737-1749.
Kang, Y.-A., Sanalkumar, R., O'Geen, H., Linnemann, A. K., Chang, C.-J., Bouhassira, E. E., Farnham, P. J., Keles, S. and Bresnick, E. H. (2012). Autophagy driven by a master regulator of hematopoiesis. *Mol. Cell. Biol.* **32**, 226-239.
Kaur, J. and Debnath, J. (2015). Autophagy at the crossroads of catabolism and anabolism. *Nat. Rev. Mol. Cell Biol.* **16**, 461-472.
Kaushik, S. and Cuervo, A. M. (2015). Proteostasis and aging. *Nat. Med.* **21**, 1406-1415.
Khaminets, A., Behl, C. and Dikic, I. (2016). Ubiquitin-dependent and independent signals in selective autophagy. *Trends Cell Biol.* **26**, 6-16.
Kohli, L. and Passegué, E. (2014). Surviving change: the metabolic journey of hematopoietic stem cells. *Trends Cell Biol.* **24**, 479-487.
Kondoh, H., Lleonart, M. E., Nakashima, Y., Yokode, M., Tanaka, M., Bernard, D., Gil, J. and Beach, D. (2007). A high glycolytic flux supports the proliferative potential of murine embryonic stem cells. *Antioxid. Redox Signal.* **9**, 293-299.
Kuo, T.-C., Chen, C.-T., Baron, D., Onder, T. T., Loewer, S., Almeida, S., Weismann, C. M., Xu, P., Houghton, J.-M., Gao, F.-B. et al. (2011). Midbody accumulation through evasion of autophagy contributes to cellular reprogramming and tumorigenicity. *Nat. Cell Biol.* **13**, 1214-1223.
Lawson, D. A., Bhakta, N. R., Kessenbrock, K., Prummel, K. D., Yu, Y., Takai, K., Zhou, A., Eyob, H., Balakrishnan, S., Wang, C.-Y. et al. (2015). Single-cell analysis reveals a stem-cell program in human metastatic breast cancer cells. *Nature* **526**, 131-135.
Lazarou, M., Sliter, D. A., Kane, L. A., Sarraf, S. A., Wang, C., Burman, J. L., Sideris, D. P., Fogel, A. I. and Youle, R. J. (2015). The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy. *Nature* **524**, 309-314.
Le Belle, J. E., Orozco, N. M., Paucar, A. A., Saxe, J. P., Mottahedeh, J., Pyle, A. D., Wu, H. and Kornblum, H. I. (2011). Proliferative neural stem cells have high endogenous ROS levels that regulate self-renewal and neurogenesis in a PI3K/Akt-dependant manner. *Cell Stem Cell* **8**, 59-71.
Lee, H. J., Gutierrez-Garcia, R. and Vilchez, D. (2017). Embryonic stem cells: a novel paradigm to study proteostasis? *FEBS J.* **284**, 391-398.
Li, W., Zou, W., Yang, Y., Chai, Y., Chen, B., Cheng, S., Tian, D., Wang, X., Vale, R. D. and Ou, G. (2012). Autophagy genes function sequentially to promote apoptotic cell corpse degradation in the engulfing cell. *J. Cell Biol.* **197**, 27-35.
Li, M., Lu, G., Hu, J., Shen, X., Ju, J., Gao, Y., Qu, L., Xia, Y., Chen, Y. and Bai, Y. (2016). EVA1A/TMEM166 regulates embryonic neurogenesis by autophagy. *Stem Cell Reports* **6**, 396-410.
Lin, Y.-H., Huang, Y.-C., Chen, L.-H. and Chu, P.-M. (2015). Autophagy in cancer stem/progenitor cells. *Cancer Chemother. Pharmacol.* **75**, 879-886.
Liu, F., Lee, J. Y., Wei, H., Tanabe, O., Engel, J. D., Morrison, S. J. and Guan, J.-L. (2010). FIP200 is required for the cell-autonomous maintenance of fetal hematopoietic stem cells. *Blood* **116**, 4806-4814.
Liu, K., Zhao, Q., Liu, P., Cao, J., Gong, J., Wang, C., Wang, W., Li, X., Sun, H., Zhang, C. et al. (2016). ATG3-dependent autophagy mediates mitochondrial homeostasis in pluripotency acquirement and maintenance. *Autophagy* **12**, 2000-2008.
Liu, P., Liu, K., Gu, H., Wang, W., Gong, J., Zhu, Y., Zhao, Q., Cao, J., Han, C., Gao, F. et al. (2017). High autophagic flux guards ESC identity through coordinating autophagy machinery gene program by FOXO1. *Cell Death Differ.* **24**, 1672-1680.
Lv, X., Jiang, H., Li, B., Liang, Q., Wang, S., Zhao, Q. and Jiao, J. (2014). The crucial role of Atg5 in cortical neurogenesis during early brain development. *Sci. Rep.* **4**, 6010.
Ma, T., Li, J., Xu, Y., Yu, C., Xu, T., Wang, H., Liu, K., Cao, N., Nie, B.-M., Zhu, S.-Y. et al. (2015). Atg5-independent autophagy regulates mitochondrial clearance and is essential for iPSC reprogramming. *Nat. Cell Biol.* **17**, 1379-1387.
Madeo, F., Zimmermann, A., Maiuri, M. C. and Kroemer, G. (2015). Essential role for autophagy in life span extension. *J. Clin. Invest.* **125**, 85-93.
Mandell, M. A., Jain, A., Kumar, S., Castleman, M. J., Anwar, T., Eskelinen, E.-L., Johansen, T., Prekeris, R. and Deretic, V. (2016). TRIM17 contributes to

autophagy of midbodies while actively sparing other targets from degradation. *J. Cell Sci.* **129**, 3562-3573.
Marcucci, F., Ghezzi, P. and Rumio, C. (2017). The role of autophagy in the cross-talk between epithelial-mesenchymal transitioned tumor cells and cancer stem-like cells. *Mol. Cancer* **16**, 3.
Massagué, J. and Obenauf, A. C. (2016). Metastatic colonization by circulating tumour cells. *Nature* **529**, 298-306.
Maycotte, P., Jones, K. L., Goodall, M. L., Thorburn, J. and Thorburn, A. (2015). Autophagy supports breast cancer stem cell maintenance by regulating IL6 secretion. *Mol. Cancer Res.* **13**, 651-658.
Mellén, M. A., de la Rosa, E. J. and Boya, P. (2008). The autophagic machinery is necessary for removal of cell corpses from the developing retinal neuroepithelium. *Cell Death Differ.* **15**, 1279-1290.
Mellén, M. A., de la Rosa, E. J. and Boya, P. (2009). Autophagy is not universally required for phosphatidyl-serine exposure and apoptotic cell engulfment during neural development. *Autophagy* **5**, 964-972.
Menendez, J. A., Vellon, L., Oliveras-Ferraros, C., Cufí, S. and Vazquez-Martin, A. (2011). mTOR-regulated senescence and autophagy during reprogramming of somatic cells to pluripotency: a roadmap from energy metabolism to stem cell renewal and aging. *Cell Cycle* **10**, 3658-3677.
Menzies, F. M., Fleming, A., Caricasole, A., Bento, C. F., Andrews, S. P., Ashkenazi, A., Füllgrabe, J., Jackson, A., Jimenez Sanchez, M., Karabiyik, C. et al. (2017). Autophagy and neurodegeneration: pathogenic mechanisms and therapeutic opportunities. *Neuron* **93**, 1015-1034.
Mizushima, N. and Komatsu, M. (2011). Autophagy: renovation of cells and tissues. *Cell* **147**, 728-741.
Mizushima, N. and Levine, B. (2010). Autophagy in mammalian development and differentiation. *Nat. Cell Biol.* **12**, 823-830.
Mizushima, N., Yamamoto, A., Matsui, M., Yoshimori, T. and Ohsumi, Y. (2004). In vivo analysis of autophagy in response to nutrient starvation using transgenic mice expressing a fluorescent autophagosome marker. *Mol. Biol. Cell* **15**, 1101-1111.
Morgado, A. L., Xavier, J. M., Dionisio, P. A., Ribeiro, M. F. C., Dias, R. B., Sebastião, A. M., Solá, S. and Rodrigues, C. M. (2015). MicroRNA-34a modulates neural stem cell differentiation by regulating expression of synaptic and autophagic proteins. *Mol. Neurobiol.* **51**, 1168-1183.
Mortensen, M., Soilleux, E. J., Djordjevic, G., Tripp, R., Lutteropp, M., Sadighi-Akha, E., Stranks, A. J., Glanville, J., Knight, S., Jacobsen, S.-E. et al. (2011). The autophagy protein Atg7 is essential for hematopoietic stem cell maintenance. *J. Exp. Med.* **208**, 455-467.
Ney, P. A. (2015). Mitochondrial autophagy: origins, significance, and role of BNIP3 and NIX. *Biochim. Biophys. Acta* **1853**, 2775-2783.
Ojha, R., Bhattacharyya, S. and Singh, S. K. (2015). Autophagy in cancer stem cells: a potential link between chemoresistance, recurrence, and metastasis. *BioResearch Open Access* **4**, 97-108.
Paik, J.-H., Ding, Z., Narurkar, R., Ramkissoon, S., Muller, F., Kamoun, W. S., Chae, S.-S., Zheng, H., Ying, H., Mahoney, J. et al. (2009). FoxOs cooperatively regulate diverse pathways governing neural stem cell homeostasis. *Cell Stem Cell* **5**, 540-553.
Palmer, T. D., Takahashi, J. and Gage, F. H. (1997). The adult rat hippocampus contains primordial neural stem cells. *Mol. Cell. Neurosci.* **8**, 389-404.
Pan, H., Cai, N., Li, M., Liu, G.-H. and Izpisua Belmonte, J. C. (2013). Autophagic control of cell 'stemness'. *EMBO Mol. Med.* **5**, 327-331.
Pascual, G., Avgustinova, A., Mejetta, S., Martín, M., Castellanos, A., Attolini, C. S.-O., Berenguer, A., Prats, N., Toll, A., Hueto, J. A. et al. (2017). Targeting metastasis-initiating cells through the fatty acid receptor CD36. *Nature* **541**, 41-45.
Pattabiraman, D. R. and Weinberg, R. A. (2014). Tackling the cancer stem cells - what challenges do they pose? *Nat. Rev. Drug Discov.* **13**, 497-512.
Prigione, A., Fauler, B., Lurz, R., Lehrach, H. and Adjaye, J. (2010). The senescence-related mitochondrial/oxidative stress pathway is repressed in human induced pluripotent stem cells. *Stem Cells* **28**, 721-733.
Qu, X., Zou, Z., Sun, Q., Luby-Phelps, K., Cheng, P., Hogan, R. N., Gilpin, C. and Levine, B. (2007). Autophagy gene-dependent clearance of apoptotic cells during embryonic development. *Cell* **128**, 931-946.
Rais, Y., Zviran, A., Geula, S., Gafni, O., Chomsky, E., Viukov, S., Mansour, A. A., Caspi, I., Krupalnik, V., Zerbib, M. et al. (2013). Deterministic direct reprogramming of somatic cells to pluripotency. *Nature* **502**, 65-70.
Renault, V. M., Rafalski, V. A., Morgan, A. A., Salih, D. A. M., Brett, J. O., Webb, A. E., Villeda, S. A., Thekkat, P. U., Guillerey, C., Denko, N. C. et al. (2009). FoxO3 regulates neural stem cell homeostasis. *Cell Stem Cell* **5**, 527-539.
Rentas, S., Holzapfel, N., Belew, M. S., Pratt, G., Voisin, V., Wilhelm, B. T., Bader, G. D., Yeo, G. W. and Hope, K. J. (2016). Musashi-2 attenuates AHR signalling to expand human haematopoietic stem cells. *Nature* **532**, 508-511.
Riffelmacher, T., Richter, F. C. and Simon, A. K. (2017). Autophagy dictates metabolism and differentiation of inflammatory immune cells. *Autophagy* 1-8.
Rojansky, R., Cha, M. Y. and Chan, D. C. (2016). Elimination of paternal mitochondria in mouse embryos occurs through autophagic degradation dependent on PARKIN and MUL1. *Elife* **5**, e17896.
Rumman, M., Dhawan, J. and Kassem, M. (2015). Concise review: quiescence in adult stem cells: biological significance and relevance to tissue regeneration. *Stem Cells* **33**, 2903-2912.
Sandoval, H., Thiagarajan, P., Dasgupta, S. K., Schumacher, A., Prchal, J. T., Chen, M. and Wang, J. (2008). Essential role for Nix in autophagic maturation of erythroid cells. *Nature* **454**, 232-235.
Sandri, M. (2012). FOXOphagy path to inducing stress resistance and cell survival. *Nat. Cell Biol.* **14**, 786-788.
Sarkar, S., Perlstein, E. O., Imarisio, S., Pineau, S., Cordenier, A., Maglathlin, R. L., Webster, J. A., Lewis, T. A., O'Kane, C. J., Schreiber, S. L. et al. (2007). Small molecules enhance autophagy and reduce toxicity in Huntington's disease models. *Nat. Chem. Biol.* **3**, 331-338.
Sato, M. and Sato, K. (2011). Degradation of paternal mitochondria by fertilization-triggered autophagy in C. elegans embryos. *Science* **334**, 1141-1144.
Schink, K. O. and Stenmark, H. (2011). Cell differentiation: midbody remnants-junk or fate factors? *Curr. Biol.* **21**, R958-R960.
Schneider, J. L. and Cuervo, A. M. (2014). Autophagy and human disease: emerging themes. *Curr. Opin. Genet. Dev.* **26C**, 16-23.
Sena, L. A. and Chandel, N. S. (2012). Physiological roles of mitochondrial reactive oxygen species. *Mol. Cell* **48**, 158-167.
Shyh-Chang, N. and Ng, H.-H. (2017). The metabolic programming of stem cells. *Genes Dev.* **31**, 336-346.
Shyh-Chang, N., Daley, G. Q. and Cantley, L. C. (2013). Stem cell metabolism in tissue development and aging. *Development* **140**, 2535-2547.
Sin, J., Andres, A. M., Taylor, D. J. R., Weston, T., Hiraumi, Y., Stotland, A., Kim, B. J., Huang, C., Doran, K. S. and Gottlieb, R. A. (2016). Mitophagy is required for mitochondrial biogenesis and myogenic differentiation of C2C12 myoblasts. *Autophagy* **12**, 369-380.
Singh, A. K., Arya, R. K., Maheshwari, S., Singh, A., Meena, S., Pandey, P., Dormond, O. and Datta, D. (2015). Tumor heterogeneity and cancer stem cell paradigm: updates in concept, controversies and clinical relevance. *Int. J. Cancer* **136**, 1991-2000.
Solanas, G., Peixoto, F. O., Perdiguero, E., Jardi, M., Ruiz-Bonilla, V., Datta, D., Symeonidi, A., Castellanos, A., Welz, P. S., Caballero, J. M. et al. (2017). Aged stem cells reprogram their daily rhythmic functions to adapt to stress. *Cell* **170**, 678-692 e620.
Stienstra, R., Haim, Y., Riahi, Y., Netea, M., Rudich, A. and Leibowitz, G. (2014). Autophagy in adipose tissue and the beta cell: implications for obesity and diabetes. *Diabetologia* **57**, 1505-1516.
St John, J. C., Amaral, A., Bowles, E., Oliveira, J. F., Lloyd, R., Freitas, M., Gray, H. L., Navara, C. S., Oliveira, G., Schatten, G. P. et al. (2006). The analysis of mitochondria and mitochondrial DNA in human embryonic stem cells. *Methods Mol. Biol.* **331**, 347-374.
Takahashi, K. and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**, 663-676.
Takamura, A., Komatsu, M., Hara, T., Sakamoto, A., Kishi, C., Waguri, S., Eishi, Y., Hino, O., Tanaka, K. and Mizushima, N. (2011). Autophagy-deficient mice develop multiple liver tumors. *Genes Dev.* **25**, 795-800.
Tam, W. L. and Weinberg, R. A. (2013). The epigenetics of epithelial-mesenchymal plasticity in cancer. *Nat. Med.* **19**, 1438-1449.
Tan, S. and Wong, E. (2017). Mitophagy transcriptome: mechanistic insights into polyphenol-mediated mitophagy. *Oxid. Med. Cell. Longev.* **2017**, 9028435.
Tang, A. H. and Rando, T. A. (2014). Induction of autophagy supports the bioenergetic demands of quiescent muscle stem cell activation. *EMBO J.* **33**, 2782-2797.
Tsukamoto, S., Kuma, A., Murakami, M., Kishi, C., Yamamoto, A. and Mizushima, N. (2008). Autophagy is essential for preimplantation development of mouse embryos. *Science* **321**, 117-120.
Tsukamoto, S., Hara, T., Yamamoto, A., Kito, S., Minami, N., Kubota, T., Sato, K. and Kokubo, T. (2014). Fluorescence-based visualization of autophagic activity predicts mouse embryo viability. *Sci. Rep.* **4**, 4533.
Vazquez, P., Arroba, A. I., Cecconi, F., de la Rosa, E. J., Boya, P. and De Pablo, F. (2012). Atg5 and Ambra1 differentially modulate neurogenesis in neural stem cells. *Autophagy* **8**, 187-199.
Vazquez-Martin, A., Van den Haute, C., Cufi, S., Corominas-Faja, B., Cuyàs, E., Lopez-Bonet, E., Rodriguez-Gallego, E., Fernández-Arroyo, S., Joven, J., Baekelandt, V. et al. (2016). Mitophagy-driven mitochondrial rejuvenation regulates stem cell fate. *Aging (Albany NY)* **8**, 1330-1352.
Vessoni, A. T., Muotri, A. R. and Okamoto, O. K. (2012). Autophagy in stem cell maintenance and differentiation. *Stem Cells Dev.* **21**, 513-520.
Vilchez, D., Boyer, L., Morantte, I., Lutz, M., Merkwirth, C., Joyce, D., Spencer, B., Page, L., Masliah, E., Berggren, W. T. et al. (2012). Increased proteasome activity in human embryonic stem cells is regulated by PSMD11. *Nature* **489**, 304-308.
Wang, Y. X. and Rudnicki, M. A. (2011). Satellite cells, the engines of muscle repair. *Nat. Rev. Mol. Cell Biol.* **13**, 127-133.
Wang, C., Liang, C.-C., Bian, Z. C., Zhu, Y. and Guan, J.-L. (2013a). FIP200 is required for maintenance and differentiation of postnatal neural stem cells. *Nat. Neurosci.* **16**, 532-542.

Wang, S., Xia, P., Ye, B., Huang, G., Liu, J. and Fan, Z. (2013b). Transient activation of autophagy via Sox2-mediated suppression of mTOR is an important early step in reprogramming to pluripotency. *Cell Stem Cell* **13**, 617-625.
Wang, C., Chen, S., Yeo, S., Karsli-Uzunbas, G., White, E., Mizushima, N., Virgin, H. W. and Guan, J.-L. (2016a). Elevated p62/SQSTM1 determines the fate of autophagy-deficient neural stem cells by increasing superoxide. *J. Cell Biol.* **212**, 545-560.
Wang, L.-L., Su, Z., Tai, W., Zou, Y., Xu, X.-M. and Zhang, C.-L. (2016b). The p53 pathway controls SOX2-mediated reprogramming in the adult mouse spinal cord. *Cell Rep.* **17**, 891-903.
Wang, C., Yeo, S., Haas, M. A. and Guan, J.-L. (2017). Autophagy gene FIP200 in neural progenitors non-cell autonomously controls differentiation by regulating microglia. *J. Cell Biol.* **216**, 2581-2596.
Warr, M. R., Binnewies, M., Flach, J., Reynaud, D., Garg, T., Malhotra, R., Debnath, J. and Passegué, E. (2013). FOXO3A directs a protective autophagy program in haematopoietic stem cells. *Nature* **494**, 323-327.
Watson, A. S., Riffelmacher, T., Stranks, A., Williams, O., De Boer, J., Cain, K., MacFarlane, M., McGouran, J., Kessler, B., Khandwala, S. et al. (2015). Autophagy limits proliferation and glycolytic metabolism in acute myeloid leukemia. *Cell Death Discov.* **1**, 15008.
Webb, A. E. and Brunet, A. (2014). FOXO transcription factors: key regulators of cellular quality control. *Trends Biochem. Sci.* **39**, 159-169.
White, E. (2015). The role for autophagy in cancer. *J. Clin. Invest.* **125**, 42-46.
Wojcinski, A., Lawton, A. K., Bayin, N. S., Lao, Z., Stephen, D. N. and Joyner, A. L. (2017). Cerebellar granule cell replenishment postinjury by adaptive reprogramming of Nestin+ progenitors. *Nat. Neurosci.* **20**, 1361-1370.
Wu, X., Fleming, A., Ricketts, T., Pavel, M., Virgin, H., Menzies, F. M. and Rubinsztein, D. C. (2016). Autophagy regulates Notch degradation and modulates stem cell development and neurogenesis. *Nat. Commun.* **7**, 10533.
Xi, Y., Dhaliwal, J. S., Ceizar, M., Vaculik, M., Kumar, K. L. and Lagace, D. C. (2016). Knockout of Atg5 delays the maturation and reduces the survival of adult-generated neurons in the hippocampus. *Cell Death Dis.* **7**, e2127.
Xiang, G., Yang, L., Long, Q., Chen, K., Tang, H., Wu, Y., Liu, Z., Zhou, Y., Qi, J., Zheng, L. et al. (2017). BNIP3L-dependent mitophagy accounts for mitochondrial clearance during three factors induced somatic cell reprogramming. *Autophagy* **13**, 1543-1555.
Yamamoto, A., Mizushima, N. and Tsukamoto, S. (2014). Fertilization-induced autophagy in mouse embryos is independent of mTORC1. *Biol. Reprod.* **91**, 7.
Yamanaka, S. (2012). Induced pluripotent stem cells: past, present, and future. *Cell Stem Cell* **10**, 678-684.
Yamanaka, S. and Blau, H. M. (2010). Nuclear reprogramming to a pluripotent state by three approaches. *Nature* **465**, 704-712.
Yazdankhah, M., Farioli-Vecchioli, S., Tonchev, A. B., Stoykova, A. and Cecconi, F. (2014). The autophagy regulators Ambra1 and Beclin 1 are required for adult neurogenesis in the brain subventricular zone. *Cell Death Dis.* **5**, e1403.
Yu, S.-W., Baek, S.-H., Brennan, R. T., Bradley, C. J., Park, S. K., Lee, Y. S., Jun, E. J., Lookingland, K. J., Kim, E.-K., Lee, H. et al. (2008). Autophagic death of adult hippocampal neural stem cells following insulin withdrawal. *Stem Cells* **26**, 2602-2610.
Yu, W.-M., Liu, X., Shen, J., Jovanovic, O., Pohl, E. E., Gerson, S. L., Finkel, T., Broxmeyer, H. E. and Qu, C.-K. (2013). Metabolic regulation by the mitochondrial phosphatase PTPMT1 is required for hematopoietic stem cell differentiation. *Cell Stem Cell* **12**, 62-74.
Yue, W., Hamaï, A., Tonelli, G., Bauvy, C., Nicolas, V., Tharinger, H., Codogno, P. and Mehrpour, M. (2013). Inhibition of the autophagic flux by salinomycin in breast cancer stem-like/progenitor cells interferes with their maintenance. *Autophagy* **9**, 714-729.
Zhang, X., Yalcin, S., Lee, D.-F., Yeh, T.-Y. J., Lee, S.-M., Su, J., Mungamuri, S. K., Rimmelé, P., Kennedy, M., Sellers, R. et al. (2011). FOXO1 is an essential regulator of pluripotency in human embryonic stem cells. *Nat. Cell Biol.* **13**, 1092-1099.
Zhong, Z., Sanchez-Lopez, E. and Karin, M. (2016). Autophagy, inflammation, and immunity: a troika governing cancer and its treatment. *Cell* **166**, 288-298.
Zhou, F., Li, X., Wang, W., Zhu, P., Zhou, J., He, W., Ding, M., Xiong, F., Zheng, X., Li, Z. et al. (2016). Tracing haematopoietic stem cell formation at single-cell resolution. *Nature* **533**, 487-492.