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GCN2 can promote autophagy initiating events independently of mTORC1 inhibition

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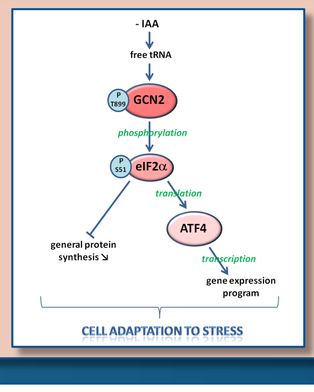
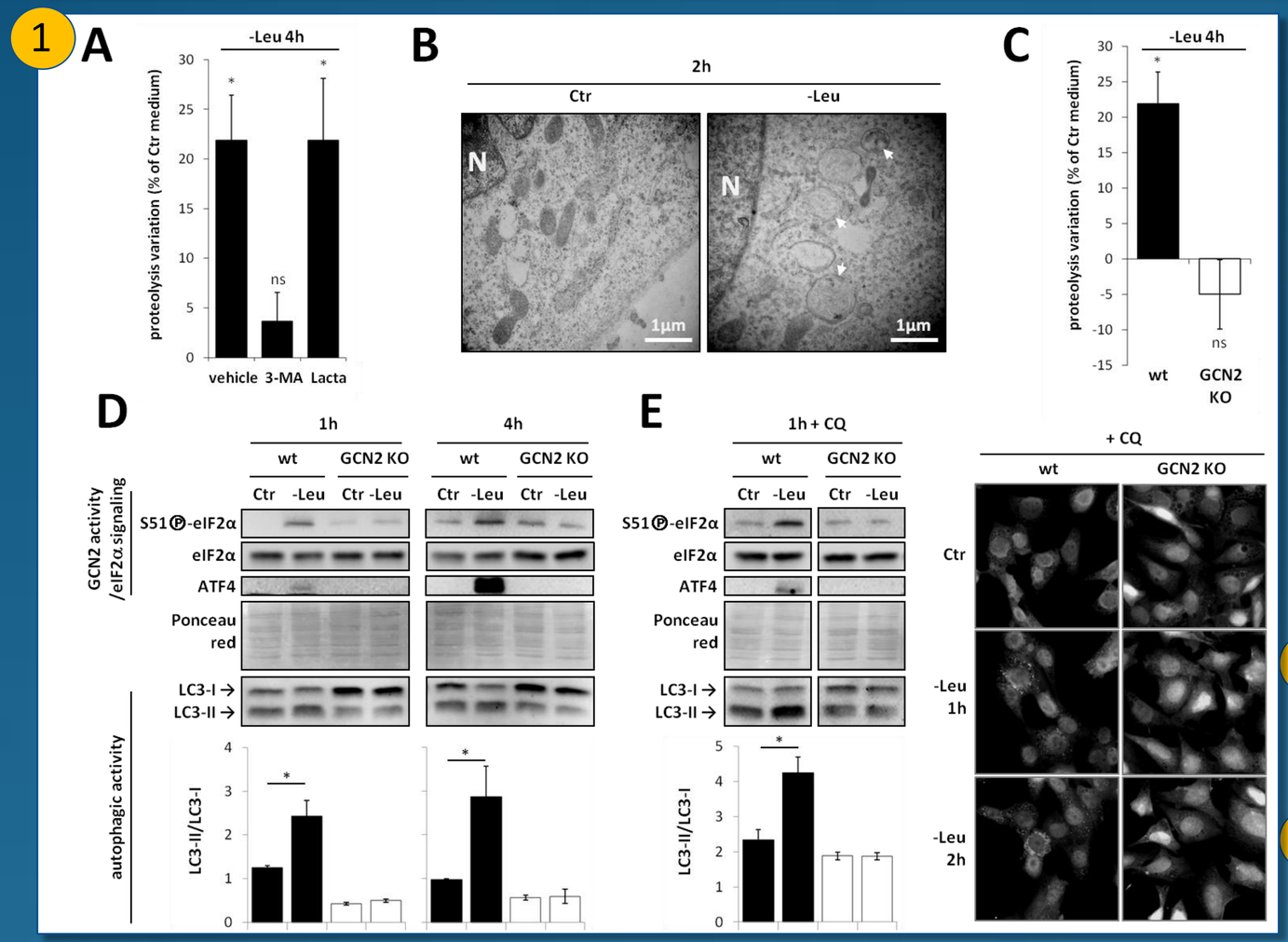
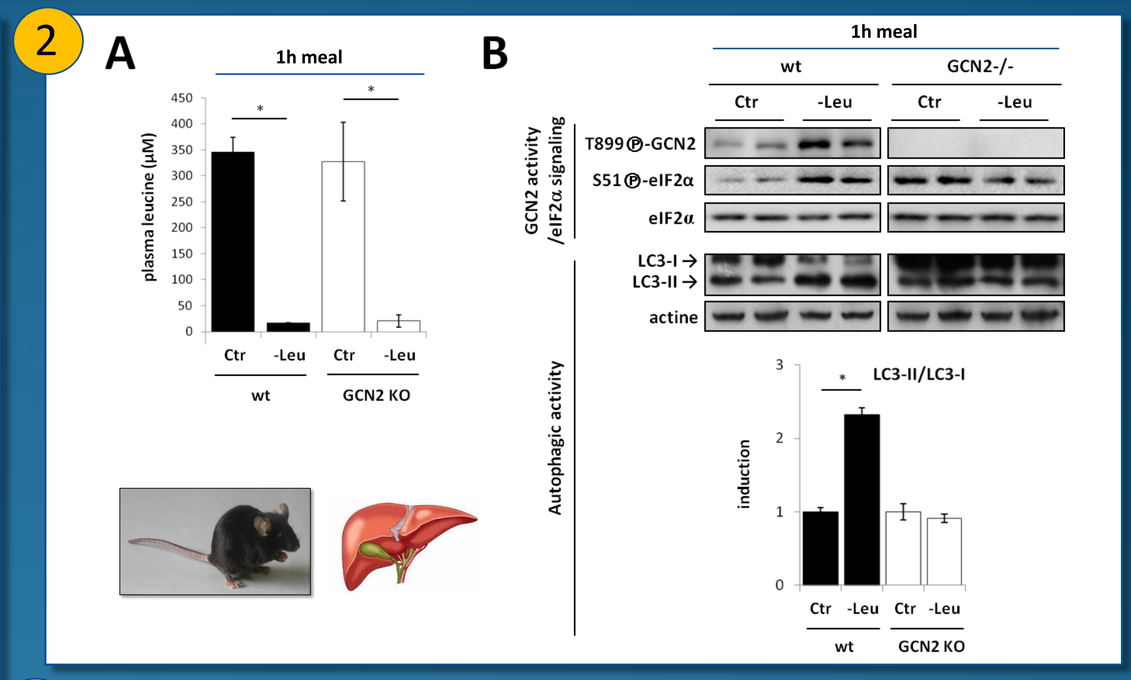
INTRODUCTION:

The imbalances in dietary amino acid (AA) supply, including deficits in one or more indispensable amino acids (IAA), are stressful conditions for the organism that needs to modulate a number of physiological functions in order to adapt to this situation. In particular, the release of free AA by degradation of functional proteins can rapidly become necessary, notably by macro-autophagy (hereafter "autophagy"). This process can be up-regulated within minutes inside cells in response to a number of stresses, by post-translational modifications of autophagy-related proteins already present in the cytosol. Until now, the activation of autophagy resulting from amino acid deficiencies has been considered exclusively as a consequence of mTORC1 inhibition. The protein kinase GCN2 is activated upon IAA scarcity in order to promote cell adaptation to a nutritional stress condition. Under IAA limitations, GCN2 is activated within minutes by uncharged transfer RNAs. By phosphorylating eIF2α on serine 51, GCN2 diminishes the overall protein synthesis rate, while simultaneously triggering a gene expression program via the translational upregulation of the transcription factor ATF4 (Figure). Our recent work has shown that the GCN2/p-eIF2α/ATF4 signaling pathway plays an essential role in inducing the transcription of a number of autophagy-related genes for the maintenance of high levels of autophagy during IAA deficiencies (B'chir et al., 2013). **In the present study we sought to determine whether GCN2 could play a role in regulating the early stages of autophagy activation.**

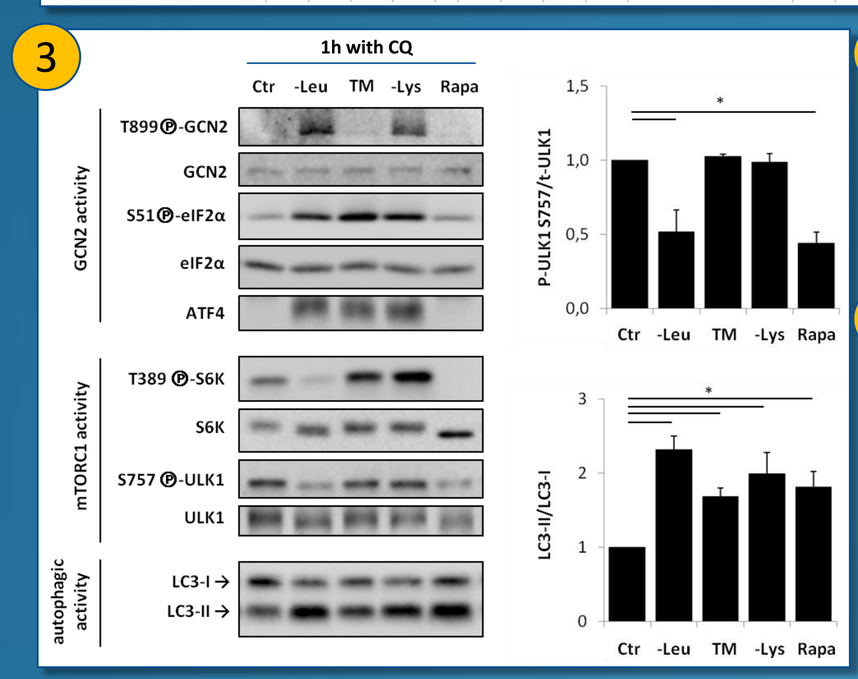






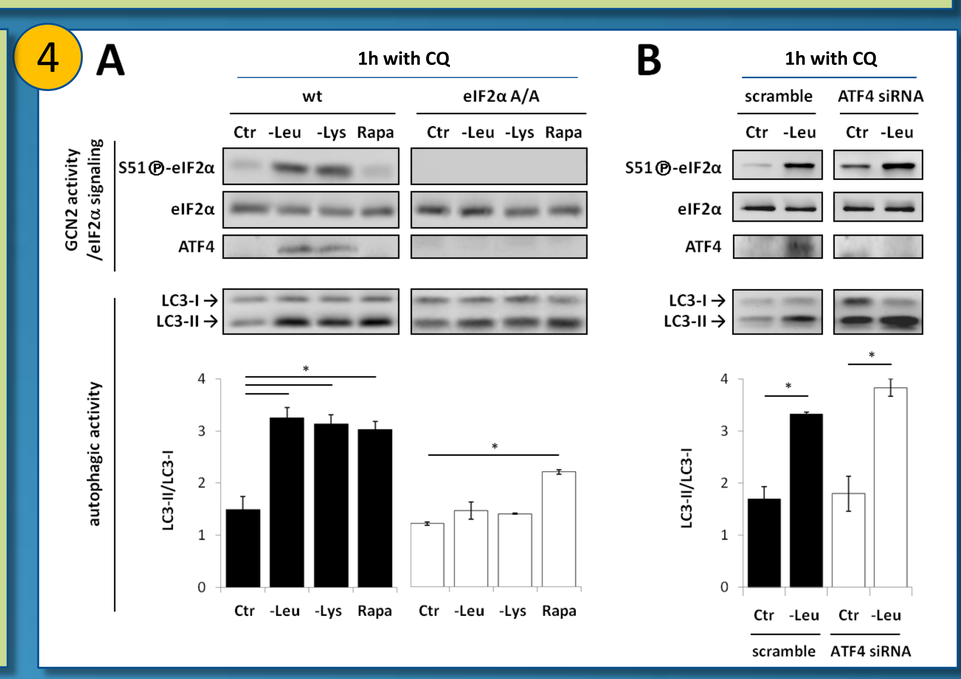
1 GCN2 is required for the short-term increase in autophag flux resulting from leucine starvation in MEFs.
A. Autophagy-dependent up-regulation of proteolysis upon leucine starvation (3-MA, 3-methyladenine; Lacta, Lactacystin). **B.** Increase in autophagic vacuolaes following leucine starvation. **C.** The increase in proteolysis rate following leucine starvation (-Leu, 4h) requires GCN2. **D, E.** GCN2 is required for up-regulating autophagy in response to leucine starvation as analyzed by LC3 immunoblotting and LC3 immunofluorescence (CQ, chloroquine 20 µM). *$p < 0.05$.

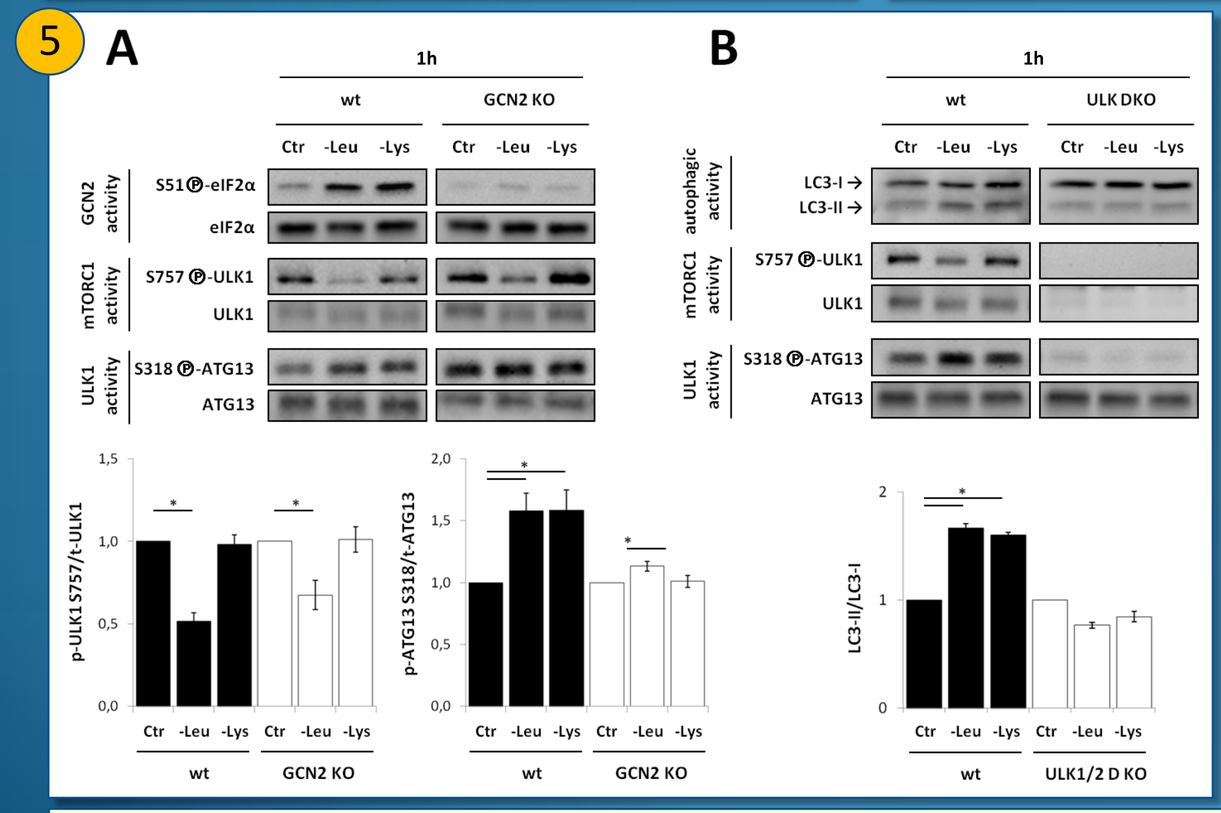
2 GCN2 is required for early activation of autophagy in the liver of mice following the consumption of a 1h leucine-devoid meal.
A. Plasma leucine following a 1h control (Ctr)- or leucine-devoid (-Leu) meal. **B.** The up-regulation of liver autophagy resulting from a 1h-leucine devoid meal requires GCN2 activation. *$p < 0.05$.



3 The short-term activation of autophagy during one IAA deficiency is associated with GCN2 activation, but can occur independently of mTORC1 inhibition in MEFs.
Wild-type MEFs were cultured for 1h either in the control medium (Ctr) or in a medium lacking leucine (-Leu) or lysine (-Lys) or supplemented with tunicamycin (TM) or rapamycin (Rapa), in the presence of CQ (20 µM). Lysine starvation and TM treatment resulted in early activation of autophagy irrespectively to p-S757-ULK1 dephosphorylation.*$p < 0.05$.

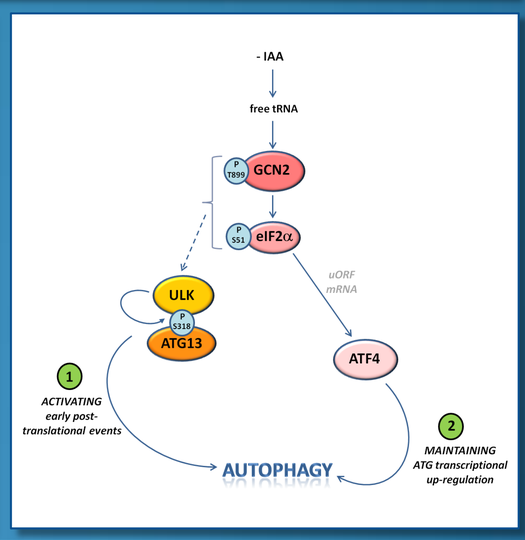
4 eIF2α phosphorylation is necessary whereas ATF4 expression is dispensable for the early events of autophagy activation resulting from leucine starvation in MEFs.
MEFs were cultured for 1h either in the control medium (Ctr) or in a medium lacking leucine (-Leu) or lysine (-Lys) or supplemented with rapamycin (Rapa), in the presence of CQ (20 µM). **A.** The early up-regulation of autophagic flux resulting from leucine- or lysine- starvation is impaired in a non-phosphorylatable eIF2a-S51A MEF mutant. **B.** MEFs were beforehand transfected with an ATF4 siRNA. ATF4 upregulation was not required for activating the early steps of autophagy initiation triggered by leucine starvation. *$p < 0.05$.





5 ULK kinase is activated in a GCN2-dependent manner during one IAA starvation and is required for early activation of autophagy.

Inside the pre-initiation complex, once activated the ULK kinase phosphorylates ATG13 on Ser318. Thus, phosphorylation of this residue can be used as a read-out of ULK activity (Petherick 2015; Joo 2011). MEFs were cultured for 1h either in the control medium (Ctr) or in a medium lacking leucine (-Leu) or lysine (-Lys). **A.** Leucine or lysine starvation led to the up-regulation of S318-ATG13 phosphorylation. This event required GCN2 but could be dissociated from p-S757-ULK1 dephosphorylation that reflects mTORC1 inhibition. **B.** ULK1/2 double knock-out MEFs were used in order to test whether ULK kinase was required in these effects. The absence of ULK completely abolished ATG13-S318 phosphorylation and LC3 processing triggered by a 1h leucine- or lysine-starvation. Altogether these results provide evidence that the activation of ULK is required for the GCN2-dependent activation of autophagy. *$p < 0.05$.



CONCLUSION

This study provides evidences that the early up-regulation of autophagy during IAA deficiencies can be promoted by GCN2 activation and eIF2α phosphorylation independently of ATF4 transcription factor. Furthermore, our data show that this GCN2-dependent mechanism can occur without concomitent inhibition of mTORC1 and involves the ULK/ATG13 pre-initiation complex. Further studies are needed to understand how GCN2 activation/eIF2α phosphorylation and ULK are connected.

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