**Supplementary Material**

1. **Additional Methods**
2. **Table S1.** List of Taq-man primers for quantitative real time PCR
3. **Table S2.** List of antibodies for western blot analysis
4. **Figure S1.** Effect of E2F1 overexpression on LC3-associated autophagosome formation

**Additional Methods:**

**Fluorescence microscopy**

For indirect fluorescence, HEK293 cells were grown on glass cover slips and transfected with 200 ng/well of wild-type E2F1 (E2F1) or mutant E2F1 (E2F1E132, plasmid that encodes for E2F1 protein with mutated DNA binding site as a result of a point mutation at amino acid number 132 of A to G, as previously described)75 expression plasmids and treated with TNF (10 ng/ml) for 24 h. Next, cells were washed twice with phosphate-buffered saline (PBS) containing 0.5% BSA (wash buffer), followed by a 40 min incubation at room temperature with 4% paraformaldehyde (Sigma-Aldrich St. Louis, MO) supplemented with 0.1% Triton X-100 (Sigma-Aldrich St. Louis, MO). After three washes with wash buffer, cells were immunostained with anti-LC3B (Cell Signaling, Beverly, MA) for 1 h. Cells were washed again 3 times with wash buffer and incubated with Alexa Fluor for additional 1 hour. After 3 washes, cover slips were placed on slides and the cell samples were used for image analysis and acquisition with Nikon Eclipse TS100 (Nikon Imaging Inc., Tokyo, Japan). For direct fluorescence, HEK293 cells were grown on glass cover slips and transfected with 200 ng/well of wild-type E2F1 (E2F1) or mutant E2F1 (E2F1E132)expression plasmids and with 200 ng/ml of EGFP-LC3 plasmid. 4 h after transfection, cells were treated with TNF 10 ng/ml in the presence or absence of BafA1 (0.1 μM) for 24 h. Next, cells were washed twice with phosphate-buffered saline (PBS) containing 0.5% BSA (wash buffer), followed by a 40 min incubation at room temperature with 4% paraformaldehyde. After three washes with wash buffer, cover slips were placed on slides and the cell samples were used for image analysis with Nikon Eclipse TS100 (Nikon Imaging Inc., Tokyo, Japan).

**Table S1.** List of Taq-man primers for quantitative real time PCR.

|  |  |
| --- | --- |
| **Reference gene** | |
| *Hprt1* | Mm00446968\_m1 |
| *RNA18S1* | Hs0392899\_g1 |
| **Detected genes** | |
| *E2F1*  *E2f1* | Hs00153451\_m1  Mm00432936\_m1 |
| *MAP1LC3B*  *Map1lc3b* | Hs00797944\_s1  Mm00782868\_s1 |
| *ATG5*  *Atg5* | Hs00169468\_m1  Mm00504340\_m1 |
| *ATG7*  *Atg7* | Hs00197348\_m1  Mm00512209\_m1 |

**Table S2.** List of antibodies for western blot analysis.

|  |  |  |
| --- | --- | --- |
| **Antibody name** | **Catalog number** | **Manufacturer** |
| ATG12 | 2010 | Cell Signaling Technology |
| p-AKT (Ser473) | 9271 |
| t-AKT | 9272 |
| p-GSK3B | 9331 |
| t-GSK3B | 9315 |
| ATG12 | 4180 |
| PCNA | 13110 |
| CDK4 | 12790 |
| E2F1 | sc-193 | Santa Cruz Biotechnology |
| MAP1LC3B | L7543 | Sigma-Aldrich |
| SQSTM1/p62 | P0067 |
| MKI67 | AV41054 |
| ATG5 | A0856 |
| ACTB | A5441 |

**Figure S1.**

