Supplementary Data.

Supplementary Methods.

Cell lines and transfection. Human HeLa cells and C2C12 skeletal muscle cells were maintained in Dulbecco's Modified Eagle Medium (DMEM, Gibco) supplemented with 10% Foetal Bovine Serum (FBS, Gibco), 1% Penicillin/streptomycin and 2% Hepes. Murine 1C9 myoblasts obtained from an immortomouse were allowed to proliferate in DMEM, 10% FBS, 1% Penicillin/streptomycin and 2% Glutamine with 1/100 γ-interferon at 33ºC in 5% CO_2. Cells were treated for various times with 20 μg/ml cytochalasin-D (Sigma), 10 μM nocodazole (Sigma), 1-5 μg/ml actinomycin D (Sigma), 50 μM staurosporine (MBL), 2 μM rapamycin (Calbiochem), 10 mM 3-methyladenine (3MA) or 50 μM chloroquine (Sigma). Amino acid starvation was induced by incubating the cells in Hank's Balanced Salt Solution (HBSS, Gibco) with 10% dialyzed FBS, 2% Hepes and 1% Penicillin/streptomycin. C2C12 myoblasts were infected with lentiviruses encoding scrambled RNA or DOR siRNAs (siRNA1 sequence is GTGGATGGCTGGCTCATCA, and siRNA2 is AGTGTTGCAACGGCAGAAT) as previously described (Baumgartner *et al.*, 2007)

For transient transfection assays, cells were typically plated onto a 100-mm cell plate and transfected with 6 μg DNA using calcium and phosphate solutions. After 18 h of incubation at 37ºC in 5% CO_2, the medium was removed and fresh DMEM was added to the cells. Transfection efficiency was analyzed by flow cytometric analysis of GFP expression. Full-length DOR cDNA was PCR-amplified and cloned as previously described (Baumgartner *et al.*, 2007). The vector containing the GFP-LC3 construct was kindly supplied by Dr. Sergio Lavandero (Universidad de Chile, Chile), and the vector containing GFP (Green Fluorescent Protein) was provided by Dr Joan Bertran (Universitat de Barcelona, Spain). The GFP-GATE16 construct was kindly provided by Dr Zvulun Elazar's group (Weizmann Institute of Science, Israel). The LAMP1-GFP plasmid was provided by Dr Lippinct-Schwatz (NIH, Bethesda). His-LC3 was constructed by In-Fusion™ Dry-down PCR (Clontech, USA). DORmut plasmid was generated by mutagenesis (L36A, I37A, I38A and L40A) (QuickChange Site-Directed Mutagenesis Kit, Stratagene).

Immunofluorescence assays. After specific treatments, cells were fixed in 4% paraformaldehyde and washed twice with PBS. Immunocytochemistry assays were performed using anti-DOR (1/150) generated in our laboratory (Baumgartner *et al.*, 2007), anti-LC3 (clone 5F10, Nanotools), anti-SC-35 (BD Pharmingen) (marker of splicing speckles), or anti-GFP antibodies (BD Pharmingen) (to detect GFP-LC3 or GFP-GATE-16). Hoechst (1/2000) (Molecular Probes, Oregon USA) was used to label DNA. Cells were mounted in Mowiol (Sigma). Fat bodies from feeding and wandering third instar larvae were dissected in PBS, and then incubated for 10 s in 1µM LysoTracker Red DND 99 (Molecular Probes), 1µM Hoechst 33342 in PBS. Fat bodies were transferred to PBS on glass slides, covered, and immediately photographed live in a Zeiss Axioplan fluorescent microscope. Proliferative and amino acid-starved HeLa cells were incubated 1 h with 50 nM Lysotracker Blue DND-22 (Invitrogen) and then fixed with 4% paraformaldehyde and washed twice with PBS.

Immunofluorescence microscopy of cells was performed with a Leica TCS SP5 (Leica Lasertechnik GmbH, Manheim, Germany) confocal scanning microscope adapted to an inverted LEICA DMI 6000CS microscope to the sequential acquisition of multiple cellular staining. Samples were scanned using a 63x Leitz objective (oil) and a zoom ranging from 1 to 3.5 to analyze intracellular regions using the software LAS AF from LEICA. The fluorochromes used (DAPI, GFP and Alexa-Fluor 561) were excited with the 405 (UV), 488 and 561 laser lines respectively. To avoid bleed-through effects (i.e crosstalk of different fluorescence emission) in double or triple staining experiments, each dye was scanned independently. Moreover, we demonstrated that the green channel does not emit in the red channel by doing separate acquisitions, and exciting in one wavelength (green 488nm) and acquiring images in the higher one (red 561nm). The projection format was 512x512 or 1024x1024. Images were acquired from 10 to 20 optical sections depending on the cell type analyzed. Figures were assembled from the TIFF and LIF files using open software ImageJ (author: Wayne Rasband, National Institute of Mental Health, Bethesda, Maryland, USA)**.** The percentage of cells with GFP-LC3-positive or endogenous LC3 positive punctate structures was obtained by counting 100 positive cells in each working condition of 4 independent experiments and results were expressed as the mean ± standard error.

Image analysis was evaluated using PDM which is the Product of the Differences from the Means: PDM = (red intensity- mean red intensity)×(green intensity – mean green intensity) (Li et al., 2004), it is calculated and normalized for each pixel of an image and

expresses the degree of colocalization of two signals with a value varying from -1 to +1. -1 represents maximal exclusion, negative values express asynchronous localization, positive values express synchronous localization (or colocalization) and +1 expresses maximal colocalization. PDM images and Pearson's coefficients (Manders et al., 1993) were calculated using Image J "ICA plugin" (Tony Collins, McMaster University, Ontario) after having defined and removed the background noise from the original pictures (N=10). On the images (Figure 2), non colocalizing pixels (negative values of PDM) are represented with a blue intensity gradient while colocalizing values are shown with a gradient of yellow. For clarity, pixels that have values below average in both channels are excluded (they give otherwise a positive PDM). Images in figures are projections of PDM images calculated in each plane. For each experimental condition, Pearson's coefficients (Manders et al., 1993) are reported in supplementary data. To achieve a qualitative 3D vision of protein co-localization, we used the IMARIS 6.1.0 software (Bitplane, Zurich, Switzerland). In many fields of research, correlation analysis with the Pearson coefficient gives values ranging from 1 to -1. When evaluated from fluorescent images, a value close to 1 indicates reliable co-localization.

Yeast two-hybrid cloning. A human adult skeletal muscle cDNA library (Invitrogen) was screened by the yeast two-hybrid assay to gain insight into potential physical and/or functional partners of DOR. The DNA binding domain (BD) of GAL4 (aa 1-147) is enclosed in the pGBKT7 vector, which encodes the TRP1 gene for detection in yeast. The activation domain (AD) of GAL4 (aa 768-881) is enclosed in the pADT7 vector, which encodes the LEU2 gene for detection in yeast. As a bait for screening with yeast *GAL4* two-hybrid system Matchmaker 3 (Clontech ®), human DOR protein sequence was subcloned in the pGBKT7 vector in-frame with the DNA BD of *GAL4*. Furthermore, selected regions of the protein were also subcloned to obtain a more detailed map of the interactions between DOR and muscle proteins. The regions selected were the N-terminal half (positions 1-120) and C-terminal half of the protein (positions 120-220). DOR-pGBKT7 was co-transformed with the entire library enclosed in pACT2 in yeast strain PJ69-4A (James *et al.*, 1996), and then plated onto selective 150-mm plates (TRP negative, 25mM 3-AT (3-amino-1, 2, 4-triazole)). The clones were then left to grow for 10 days and more than 500 clones were obtained. Of these, 250 were randomly selected on the basis of their capacity to grow under selective pressure. DNA containing BD and AD regions from 230 clones was isolated using a yeast

miniprep assay and only 220 clones were successfully incorporated into KC8 bacterial cells. Plasmids from library clones were then isolated from the yeast and recovered using a standard bacterial minipreps. The DNA obtained was subjected to specific restriction analysis in order to excise the prey inserts from the carrying vector. Once classified by length (small clones were discarded), the remaining clones were subsequently identified by sequencing. GenBank and European Molecular Biology Laboratory databases were screened for similar sequences by using the GCG program FASTA.

Pull-down, immunoprecipitation and Western blot assays. HeLa cells were transfected as previously described with His-DOR and GFP-GATE16 or GFP-LC3 alone or in combination. As a negative control group, cells were transfected with DOR without the His-tag and processed in the same way as the other groups. Cells were then incubated in DMEM (basal state) or in HBSS (amino acid starvation) for 1 h at 37ºC and 5% CO_2. After washing two times with PBS, extranuclear lysates were obtained by incubating with lysis buffer for 1 h at 4ºC. In parallel, Ni-NTA agarose beads (Qiagen) (Ros-Baro *et al.*, 2001) were equilibrated with lysis buffer for 1 h at 4ºC. After centrifugation at 10,000 x *g* for 10 min at 4ºC, the supernatant of each fraction was mixed with Ni-NTA beads and these were incubated overnight at 4ºC. Fractions were then centrifuged at 10,000 x *g* for 10 min at 4ºC and then washed twice with the washing buffer. To elute the immunoprecipitate complex, Ni-NTA beads were washed with 45 µl of elution buffer and then centrifuged at 16,000 x *g* for 10 min at 4ºC. Fractions were prepared with Laemmli sample buffer x 4 with DTT, boiled at 95ºC for 5 min and then loaded on 10% SDS-PAGE.

Immunoprecipitation assays were performed in C2C12 myotubes under basal or starvation (HBSS for 20 min) conditions. Endogenous LC3 was immunoprecipitated with anti-LC3 monoclonal antibody (MBL) together with Protein-G Sepharose® (Sigma). Samples were incubated overnight with lysis buffer at 4ºC. After centrifugation at 2,000 x g for 3 min at 4ºC, fractions were washed 3 times with lysis buffer. To elute the immunoprecipitates, pellets were loaded with 25 µl of Laemmli sample buffer x 4 with DTT, boiled at 95ºC for 5 min, centrifuged for 1 min at 13,000 x g and then loaded on 15% SDS-PAGE.

Western blotting was performed using a monoclonal antibody against GFP (BD Pharmigen), and polyclonal antibodies directed against LC3 (MBL) or DOR proteins.

Middle-long half-life protein degradation assay. Degradation of long-lived proteins was measured following a standard method (Ogier-Denis *et al.*, 1996). After 48 h of transfection, cells were labelled for 24 h with 1 μCi/ml L-(^{14}C) valine (Amersham CFB75). HeLa cells were rinsed with PBS, and incubated for 4 h in either complete growth medium or in HBSS containing 10 mM Hepes and supplemented with cold valine (10 mM) in both cases. Scramble and siRNA1 C2C12 cells were also rinsed with PBS, and incubated for 4 h in either complete growth medium or in DMEM containing 10 mM 3MA and supplemented with cold valine (10 mM) as mentioned for HeLa cells. At 1 h, 2.5 h and 4 h of incubation, 500 μl of the medium were withdrawn. At the end of the incubation time, cells were scrapped with 500 μl ice-cold PBS and transferred to an Eppendorf tube. Each fraction was precipitated in 10% trichloroacetic acid (TCA) for 30 min at 4ºC and then centrifuged at 1000 x *g* for 20 min at 4ºC. Pellets were resuspended with 200 μl NaOH 0.2 N 0.4% deoxycholate. Each fraction was counted in 4 ml of scintillation liquid (Ecolite™). The percentage of protein degradation was estimated as previously described (Vargas *et al.*, 1989) from the ratio of the TCA-soluble radioactivity released to the medium at the different chase times and the TCA-precipitable radioactivity present in the cells at the start of the chase (Fuertes *et al.*, 2003). Radioactivity released in the non-soluble TCA fraction of the medium was negligible.

Transmission electron microscopy. Untreated and amino acid-starved DOR-transfected HeLa cells were cultured for two days. . Scramble and siRNA1 C2C12 cells were cultured as myoblasts. Cells were then washed two times with phosphate buffer 0.1 M at room temperature. For fixation, cells were incubated in 2.5% glutaraldehyde solution at room temperature for 70 min. Following post-fixation in 1% osmium tetroxide and 0.8% K3Fe(CN)6 in 0.1 M phosphate buffer at 4ºC for 135 min, they were washed with highly pure water and samples were kept overnight in 0.1 M phosphate buffer. Fed or starved larvae were dissected in PBS and their anterior halves were inverted. The inverted carcasses were fixed overnight at 4ºC in 2% paraformaldehyde, 4% glutaraldehyde, 2% sucrose in 100 mM phosphate buffer and post-fixed in 2% paraformaldehyde in 100 mM phosphate overnight at 4ºC. Afterwards samples were dehydrated at 4ºC under shaking in graded solutions of acetone (50%, 70% and 90%) in

highly pure water. Samples were then gradually infiltrated with Eponate 12 Resin (TED PELLA 18010) and polymerization of the resin was processed for 48 h at 60ºC. Thin sections (50-nm) were cut using a Leica EM UC6 (Leica, Vienna) and mounted on bare 200-mesh copper grids. Sections were stained with uranyl acetate 2% for 30 min, then washed with highly pure water and finally incubated for 5 min with lead citrate and air dried. Sample sections were viewed on an FEI CM-12 transmission electron microscope.

Drosophila **strains and transgenic lines.** w1118; P{GD5124}v41186; P{GD5124}v41183/TM6; v105330 and tubulin-Gal4 were obtained from the Bloomington stock center (http://fly.bio.indiana.edu) and from the Vienna Drosophila RNAi Center (VDRC). Eggs were collected on standard apple juice agar plates at 25ºC. Upon hatching, 40 first instar larvae were transferred to a vial containing fresh fly food supplemented with yeast paste. Feeding and wandering third instar larvae were used for RNA extraction, lysotracker staining and EM experiments.

RNA extraction and QRT-PCR. RNA from five wild-type and RNAi third instar larvae was extracted using TRIzol reagent (Invitrogen). RNA was reverse-transcribed with the SuperScript RTIII kit (Invitrogen). PCRs were performed using the StepOnePlus real-time PCR machine (Applied Biosystems) and the ABI SYBR green reagents. All measurements were normalized to rp49.

Legends to supplementary figures.

Supplementary Figure 1. DOR shows a short half-life and its expression is enhanced by proteasomal inhibitors.

Panel A. DOR-transfected HeLa cells were incubated for a range of times with 100 μg/ml of cycloheximide. Thereafter, cell extracts were obtained and Western blot assays were performed by using anti-DOR and anti-β-actin antibodies. Values of DOR protein abundance are expressed as percentage of basal value (time 0).

Panels B and C. DOR-transfected HeLa cells or 1C9 muscle cells were incubated for 1 h in the presence of 20 mg/ml MG-132 (a 26S proteasome inhibitor). Thereafter, lysates were obtained and Western blot assays were performed by using anti-DOR and anti-β-actin antibodies. DOR protein abundance is expressed as a percentage of control values, *, indicates a significant difference, $P<0.05$.

Supplementary Figure 2. DOR moves out of the nucleus in response to cellular stress.

The cellular distribution of DOR is modulated by inhibitors of the cytoskeletal network, by inhibitors of transcription or by changes in the phosphorylation state of the cell. Transiently transfected HeLa cells were incubated for 1 h with disruptors of actin (Cytochalasin D) or microtubule (Nocodazole) networks or with transcription inhibitors (Actinomycin D). Transfected HeLa cells and 1C9 myoblasts were incubated with an inhibitor of protein kinases (50 μM staurosporine for 1 h). Control (untreated) or inhibitor-treated cells were fixed with 4% paraformaldehyde and the intracellular localization of DOR was determined by indirect immunofluorescence (red). Nuclei were labelled with Hoechst and detected in blue. Specificity control of the effect on DOR re-localization after drug treatment was assessed by staining of the nuclear protein, SC-35 (marker of splicing speckles) (stained in green). Scale bars, 10μm.

Supplementary Figure 3. Effects of amino acid starvation, chloroquine and 3MA on the cellular localization of DOR protein.

Panel A. Image analysis on the DOR intracellular distribution in HeLa cells subjected to activation of autophagy. HeLa cells were transiently transfected with DOR and incubated for 1 h with DMEM (Basal), Hank's Balanced Salt Solution (HBSS)

(Starvation), or with 50 μM chloroquine, or 10 mM 3MA. DOR intracellular localization was analyzed by indirect immunofluorescence. See details in legend to Figure 1.

Panel B: Image analysis on the cellular distribution of endogenous DOR in C2C12 muscle cells. C2C12 myoblasts were incubated for 20 min at 37ºC with DMEM (Basal) or HBSS (Starvation). DOR intracellular localization was analyzed by indirect immunofluorescence.

Panels C. Image analysis on the DOR intracellular distribution in HeLa cells subjected to activation of autophagy and its reversal. HeLa cells were transiently transfected with DOR and incubated for 1 h with DMEM (Basal) or HBSS (Starvation). Cells were then incubated with normal DMEM medium for 1 additional hour (starvation + GM). DOR intracellular localization was analyzed by indirect immunofluorescence.

Cellular localization of DOR was analyzed in 100 randomly chosen transfected HeLa cells or C2C12 cells in each experiment. Cells were categorized as having DOR exclusively located in the cytosol or also in nucleus. Data are mean± SEM of 4 independent experiments.

Supplementary Figure 4. Effects of amino acid starvation, chloroquine and rapamycin on the cellular localization of DOR and LC3 proteins.

Analysis on the intracellular distribution of DOR or GFP-LC3 in HeLa cells subjected to activation of autophagy. HeLa cells were transiently transfected with DOR and GFP-LC3 and incubated for 1 h with DMEM (Basal), HBSS (Starvation), 50 μM chloroquine, or 2 μM rapamycin. Intracellular localization of DOR and GFP-LC3 was analyzed by immunofluorescence. Cellular localization of DOR or GFP-LC3 was analyzed in 100 randomly chosen transfected HeLa cells in each experiment. Cells were categorized as having DOR or GFP-LC3 exclusively located in the cytosol or also in nucleus. Data are mean± SEM of 4 independent experiments. Image analysis indicated a substantial co-localization of DOR and GFP-LC3 proteins in starvation (Pearson coefficient of co-localization was 0.82±0.10 (mean±standard deviation), in starvation plus chloroquine (Pearson coefficient was 0.85±0.17), or in the presence of rapamycin (Pearson coefficient was 0.81±0.08).

Supplementary Figure 5. DOR interacts with LC3 and with GATE-16.

Panel A. Pull-down assays: His-DOR interacts with GFP-LC3 under basal or amino acid-starved conditions. HeLa cells were transiently transfected with the His-DOR plasmid alone or in combination with the GFP-LC3 construct and incubated under basal conditions or subjected to amino acid starvation for 1 h. Cells were also transfected with the DOR plasmid. Lysates were subjected to pull-down with Ni-NTA beads. Western blot assays were done by using specific antibodies directed against DOR and GFP (GFP-LC3).

Panel B. Pull-down assays: His-LC3 interacts with DOR under basal or amino acid-starved conditions. HeLa cells were transiently transfected with the His-LC3 plasmid alone or in combination with the DOR construct and incubated under basal conditions or subjected to amino acid starvation for 1 h. Cells were also transfected with myc-LC3. Lysates were subjected to pull-down with Ni-NTA beads. Western blot assays were done by using specific antibodies directed against DOR and LC3 (His-LC3).

Panel C. Immunoprecipitation assay: endogenous DOR interacts with endogenous LC3. C2C12 cells were incubated under basal conditions or subjected to amino acid starvation for 20 minutes. Lysates were subjected to immunoprecipitation with monoclonal antibody against LC3 and Protein-G Sepharose or with an irrelevant antibody (lane C1). The anti-LC3 antibody and the Protein-G Sepharose were also processed in the absence of cell lysate (lane C2). Western blot assays were done by using specific antibodies directed against DOR and LC3.

Panel D. Yeast two-hybrid analysis. Full length DOR (DOR), the N-terminal region (1-120), and the C-terminal region (120-220) were cloned in pGBKT7 and then co-transformed with GATE-16 cloned in pACT2. Clones were grown in plates and selected as described in Material and Methods. +++: indicates maximal growth, + indicates mild growth, and – indicates no growth.

Panel E. Pull-down assays: His-DOR interacts with GFP-GATE-16. HeLa cells were transiently transfected with the His-DOR plasmid alone or in combination with the GFP-GATE-16 and incubated under basal conditions or subjected to amino acid starvation for 1 h. Lysates were subjected to pull-down with Ni-NTA beads. Western blot assays were done by using specific antibodies directed against DOR and GFP (GFP-GATE-16). A negative control consisting in lysates from HeLa cells transfected with DOR without the His-tag was included.

Panel F. Starvation re-localizes DOR and GATE-16 from the nucleus to the cytoplasm. Confocal images of HeLa cells transiently transfected with DOR and GFP-GATE-16. DOR is shown in red and GFP-GATE16 in green. Basal cells were incubated with DMEM. Autophagy was activated by incubating the cells for 1 h in HBSS (Starvation). Cells were also incubated with HBSS containing 50 μM chloroquine. Scale bars, 10 μm. Panel G. Co-localization between DOR and GFP-GATE16 and between DOR and GFP-LC3 during starvation was qualitatively determined by using the program IMARIS (Pearson coefficient was 0.819). The co-localization between the two signals is shown in yellow.

Supplementary Figure 6. Effects of a mutation in the NES motif of DOR protein.
Panel A. Cellular distribution of a mutant form of DOR (L36A/I37A/I38A/L40A) and GFP-LC3 in transiently transfected HeLa cells incubated with DMEM (control), with Hank's Balanced Salt Solution (HBSS) (Starvation) (1 h), or with 2 μM rapamycin (3 h). Cells were fixed with 4% paraformaldehyde and the intracellular localization of DOR was determined by indirect immunofluorescence (red). Nuclei were labelled with Hoechst and detected in blue. Scale bars, 10μm.
Panel B. Mutant (L36A/I37A/I38A/L40A) DOR over-expression does not enhance autophagosome formation. GFP-LC3-positive vacuoles were counted in 100 transfected HeLa cells (either transfected with GFP-LC3 and an irrelevant protein (TRα1) or with GFP-LC3 and mutant DOR) in 3 independent experiments. *, indicates significant effects of starvation, $P<0.001$; τ indicates significant effects caused by overexpression of DOR mutant, $P<0.001$.

REFERENCES

1. Baumgartner,B.G., Orpinell,M., Duran,J., Ribas,V., Burghardt,H.E., Bach,D., Villar,A.V., Paz,J.C., Gonzalez,M., Camps,M., Oriola,J., Rivera,F., Palacin,M., and Zorzano,A. (2007). Identification of a novel modulator of thyroid hormone receptor-mediated action. *PLoS. ONE.,* **2,** e1183.

2. Fuertes,G., Martin De Llano,J.J., Villarroya,A., Rivett,A.J., and Knecht,E. (2003). Changes in the proteolytic activities of proteasomes and lysosomes in human

fibroblasts produced by serum withdrawal, amino-acid deprivation and confluent conditions. *Biochem. J.,* **375,** 75-86.

3. James,P., Halladay,J., and Craig,E.A. (1996). Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. *Genetics,* **144,** 1425-1436.

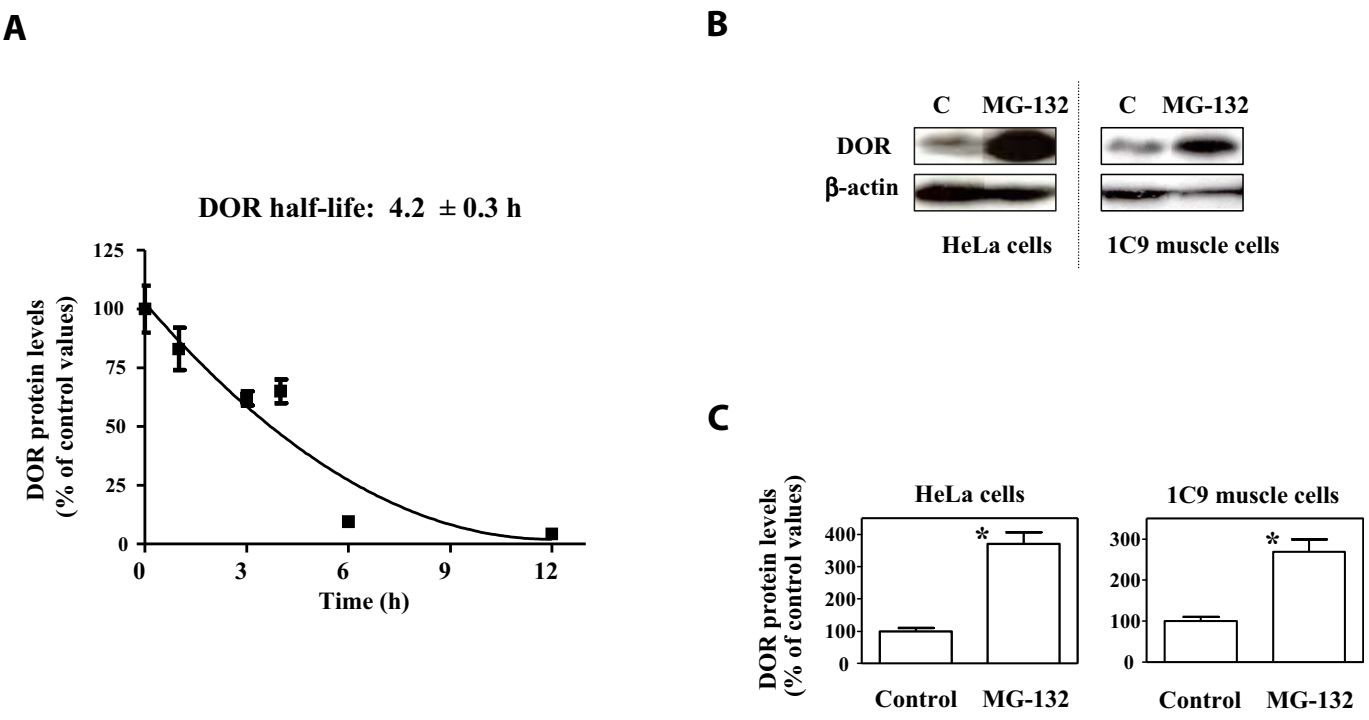
4. Li,Q., Lau,A., Morris,T.J., Guo,L., Fordyce,C.B., and Stanley,E.F. (2004). A syntaxin 1, Galpha(o), and N-type calcium channel complex at a presynaptic nerve terminal: analysis by quantitative immunocolocalization. *J. Neurosci.,* **24,** 4070-4081.

5. Manders E.E.M., Verbeek F.J., and Aten J.A., (1993). Measurement of co-localisation of objects in dual-colour confocal images, *J. Microscopy*, **169**, 375-382.

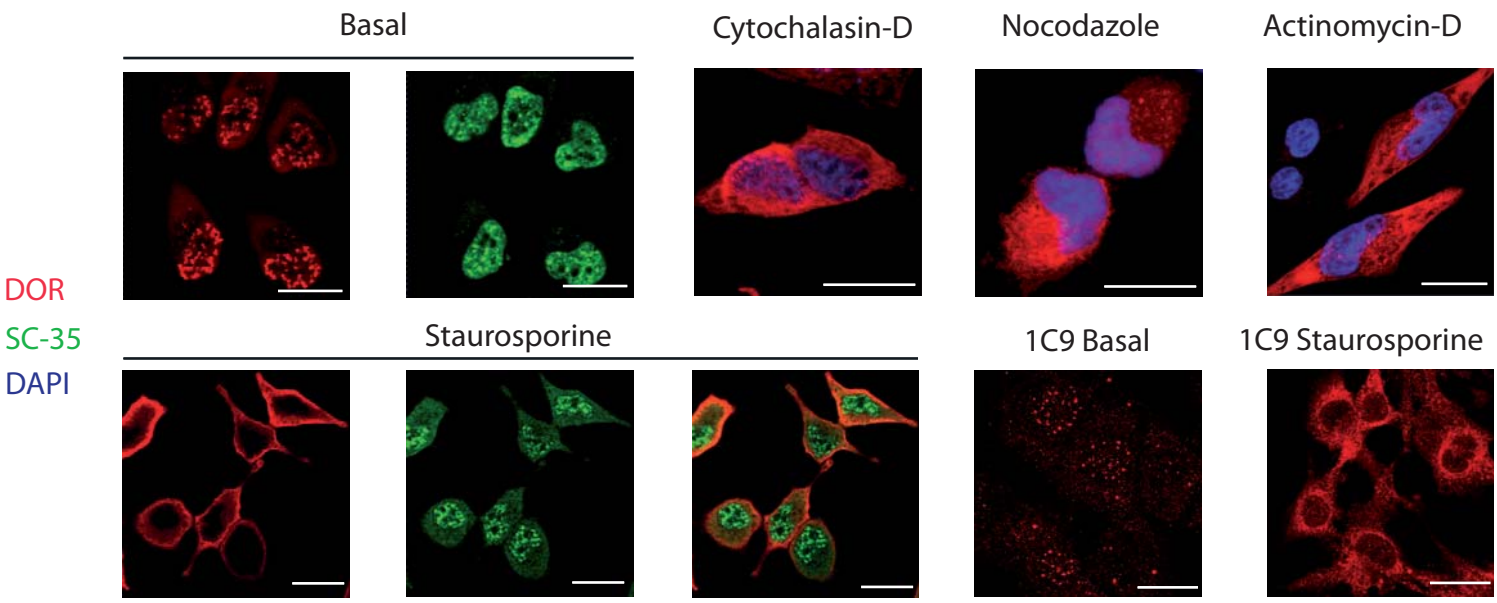
6. Ogier-Denis,E., Houri,J.J., Bauvy,C., and Codogno,P. (1996). Guanine nucleotide exchange on heterotrimeric Gi3 protein controls autophagic sequestration in HT-29 cells. *J. Biol. Chem.,* **271,** 28593-28600.

7. Ros-Baro,A., Lopez-Iglesias,C., Peiro,S., Bellido,D., Palacin,M., Zorzano,A., and Camps,M. (2001). Lipid rafts are required for GLUT4 internalization in adipose cells. *Proc. Natl. Acad. Sci. U. S. A,* **98,** 12050-12055.

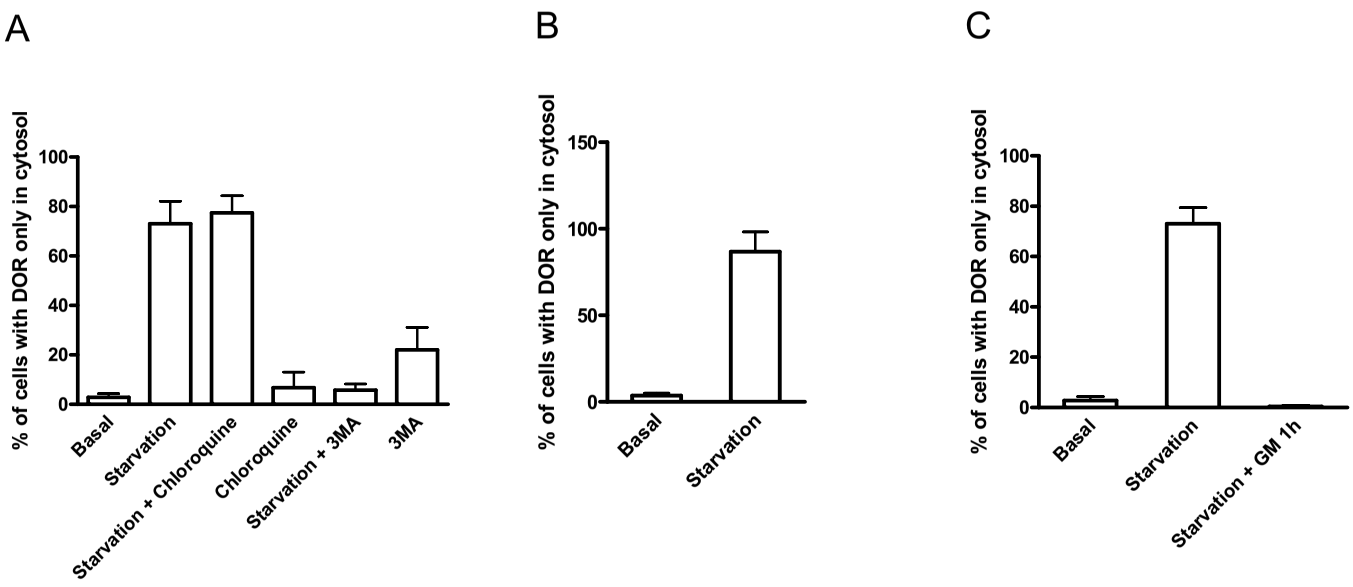
8. Vargas,J.L., Aniento,F., Cervera,J., and Knecht,E. (1989). Vanadate inhibits degradation of short-lived, but not of long-lived, proteins in L-132 human cells. *Biochem. J.,* **258,** 33-40.



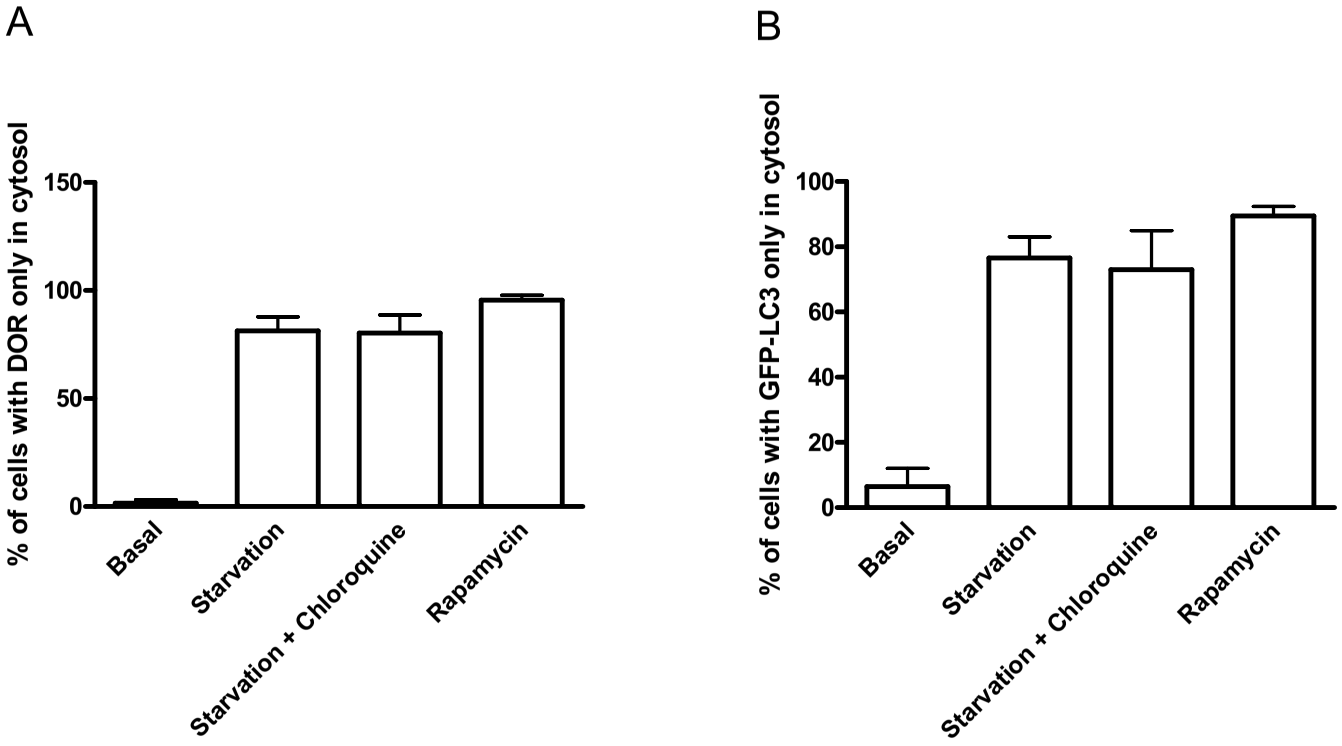
Supplementary Figure 1



Supplementary Figure 2



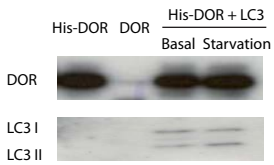
Supplementary Figure 3



Supplementary Figure 4

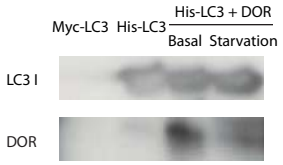
A

Pull-down: His-DOR



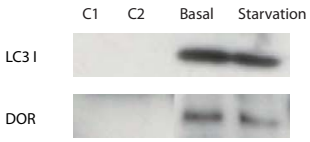
B

Pull-down: His-LC3



C

IP: anti-LC3

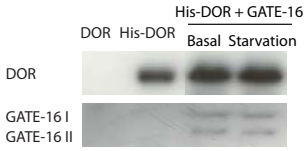


D

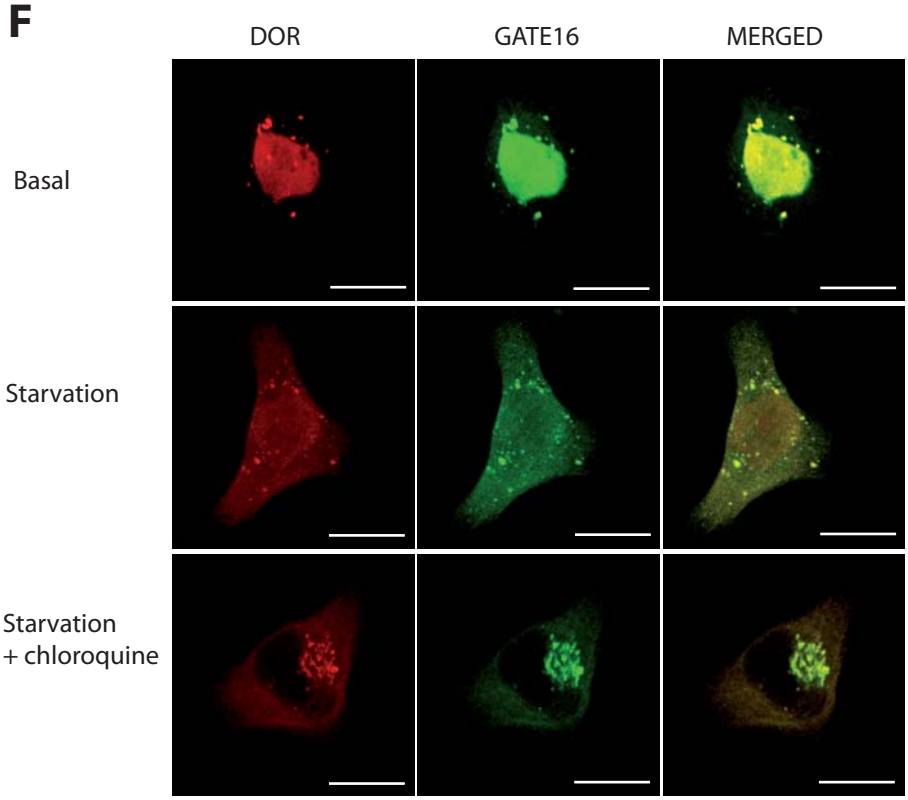
		3AT Concentration			
pGBKT7	pACT2	0	15	20	40
Empty vector	Empty vector	+	-	-	-
DOR		+	-	-	-
DOR (1-120)		+	-	-	-
DOR (120-220)		+	-	-	-
Empty vector	GATE-16	+	-	-	-
DOR		+++	+++	+++	+++
DOR (1-120)		+++	+++	+++	+++
DOR (120-220)		+	+	+	-

E

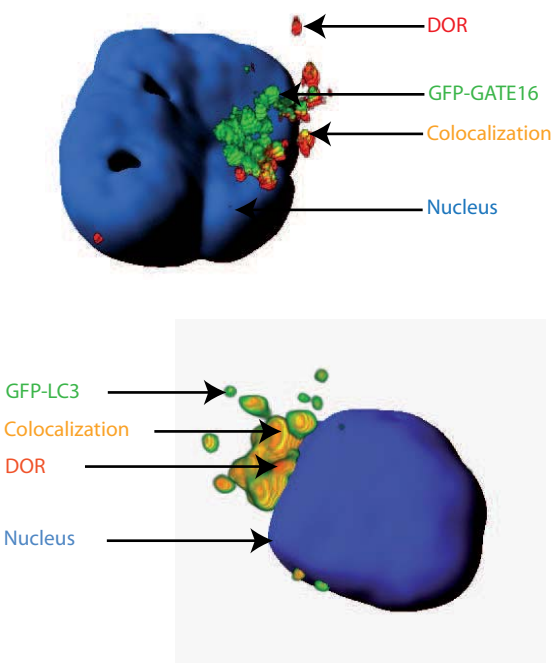
Pull-down: His-DOR



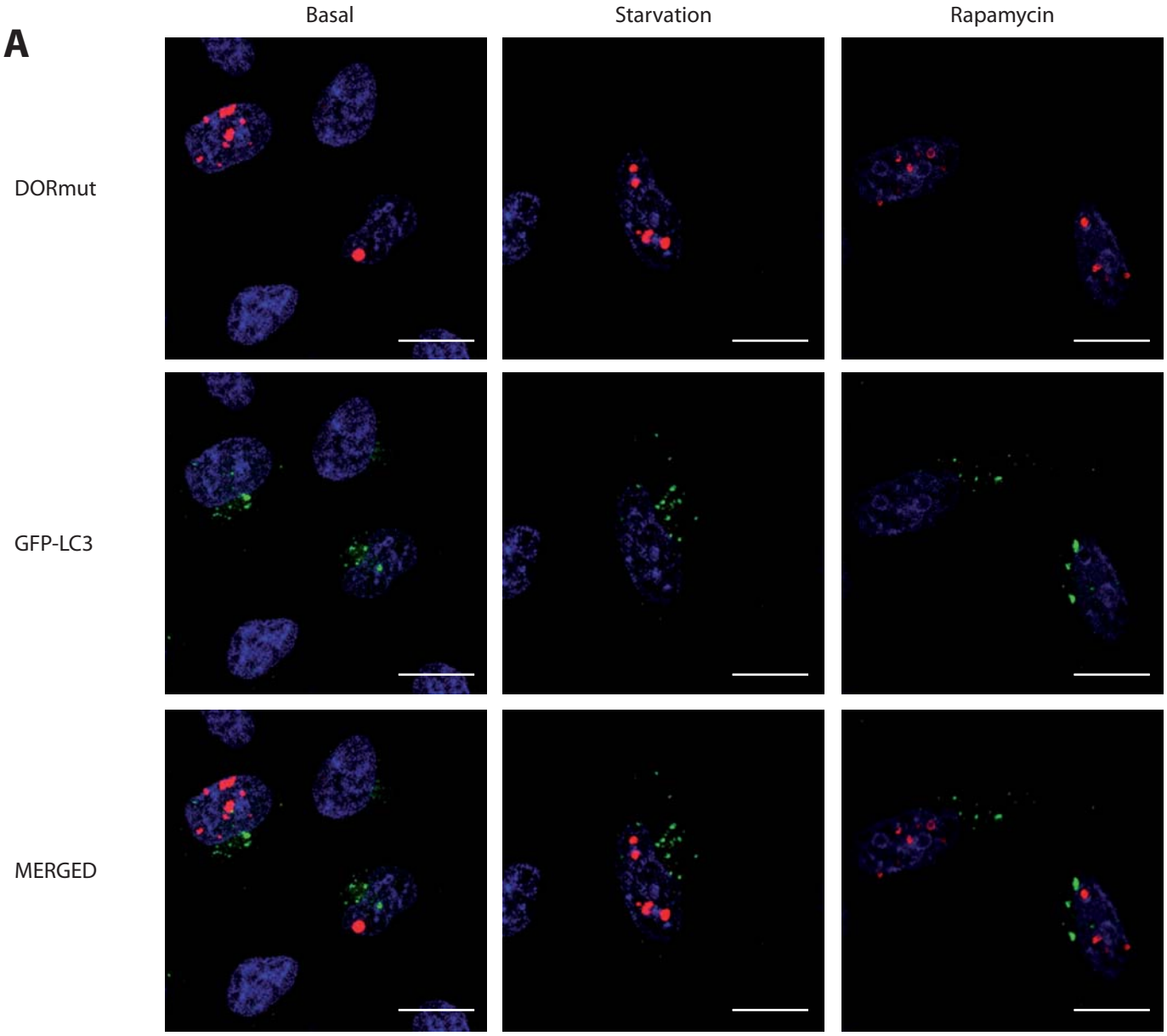
F



G IMARIS



Supplementary Figure 5



Supplementary Figure 6