



HAL
open science

mRNA and protein dataset of autophagy markers (LC3 and p62) in several cell lines

Rubén Gómez-Sánchez, Sokhna M.S. Yakhine-Diop, Mario Rodríguez-Arribas, José M. Bravo-San Pedro, Guadalupe Martínez-Chacón, Elisabet Uribe-Carretero, Diana C.J. Pinheiro de Castro, Elisa Pizarro-Estrella, José M. Fuentes, Rosa A. González-Polo

► To cite this version:

Rubén Gómez-Sánchez, Sokhna M.S. Yakhine-Diop, Mario Rodríguez-Arribas, José M. Bravo-San Pedro, Guadalupe Martínez-Chacón, et al.. mRNA and protein dataset of autophagy markers (LC3 and p62) in several cell lines. *Data in Brief*, 2016, 10.1016/j.dib.2016.02.085 . hal-01289419v1

HAL Id: hal-01289419

<https://hal.sorbonne-universite.fr/hal-01289419v1>

Submitted on 16 Mar 2016 (v1), last revised 31 Mar 2016 (v2)

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License



ELSEVIER

Contents lists available at ScienceDirect

Data in Brief

journal homepage: www.elsevier.com/locate/dib

Data article

mRNA and protein dataset of autophagy markers (LC3 and p62) in several cell lines

Rubén Gómez-Sánchez^{a,b,1}, Sokhna M.S. Yakhine-Diop^{a,c,1},
 Mario Rodríguez-Arribas^{a,c,1}, José M. Bravo-San Pedro^{a,d,e,f,g,h},
 Guadalupe Martínez-Chacón^{a,c}, Elisabet Uribe-Carretero^{a,c},
 Diana C.J. Pinheiro de Castro^{a,c}, Elisa Pizarro-Estrella^{a,c},
 José M. Fuentes^{a,c,1}, Rosa A. González-Polo^{a,c,1}

^a Centro de Investigación Biomédica en Red en Enfermedades Neurodegenerativas (CIBERNED), Spain

^b Department of Cell Biology, University of Groningen, University Medical Center Groningen, A. Deusinglaan 1, 9713 AV Groningen, The Netherlands

^c Departamento de Bioquímica, Biología Molecular y Genética. Facultad de Enfermería y Terapia Ocupacional. Avda de la Universidad S/N C.P. 10003 Cáceres, Spain

^d Equipe 11 labellisée Ligue contre le Cancer, Centre de Recherche des Cordeliers, 75006 Paris, France

^e INSERM U1138, 75006 Paris, France

^f Université Paris Descartes/Paris V, Sorbonne Paris Cité, 75006 Paris, France

^g Université Pierre et Marie Curie/Paris VI, 75006 Paris, France

^h Gustave Roussy Comprehensive Cancer Institute, 94805 Villejuif, France

ARTICLE INFO

Article history:

Received 21 December 2015

Received in revised form

23 February 2016

Accepted 29 February 2016

Keywords:

Autophagy
 LC3
 p62
 Western-blot

ABSTRACT

We characterized the dynamics of autophagy in vitro using four different cell systems and analyzing markers widely used in this field, i.e. LC3 (microtubule-associated protein 1 light chain 3; protein recruited from the cytosol (LC3-I) to the autophagosomal membrane where it is lipidated (LC3-II)) and p62/SQSTM1 (adaptor protein that serves as a link between LC3 and ubiquitinated substrates), “Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition)” (Klionsky et al., 2016) [1]. Data provided include analyses of protein levels of LC3 and p62 by Western-blotting and endogenous immunofluorescence experiments, but also p62 mRNA

DOI of original article: <http://dx.doi.org/10.1016/j.ydbio.2016.02.033>

Abbreviations: Baf. A1, Bafilomycin A1F; EBSS, Earle's Balanced Salt Solution; FBS, Fetal bovine serum; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; HFs, Human fibroblasts; LC3, Microtubule-associated protein 1 light chain 3; MEFs, Mouse embryonic fibroblasts; PBS, Phosphate-buffered saline; qPCR, Quantitative PCR; SB, Sample buffer; SDS, Sodium dodecyl sulfate; TBST, Tris-buffered saline with Tween 20

E-mail addresses: jfuentes@unex.es (J.M. Fuentes), rosapolo@unex.es (R.A. González-Polo).

¹ These authors contributed equally to this work.

<http://dx.doi.org/10.1016/j.dib.2016.02.085>

2352-3409/© 2016 Published by Elsevier Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

levels obtained by quantitative PCR (qPCR). To monitor the turnover of these autophagy markers and, thus, measure the flux of this pathway, cells were under starvation conditions and/or treated with bafilomycin A1 (Baf. A1) to block fusion of autophagosomes with lysosomes.

© 2016 Published by Elsevier Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Specifications Table

Subject area	<i>Biochemistry and Molecular Biology</i>
More specific subject area	<i>Autophagy</i>
Type of data	<i>Figures</i>
How data was acquired	<i>Western-blotting (SDS-gel electrophoresis and semi-dry transfer; Bio-Rad equipment and ImageJ software), immunofluorescence (inverted microscope (OLYMPUS IX-51) and ImageJ software), quantitative PCR (qPCR) (Applied Biosystems 7500 PCR real system), data statistical analysis (SPSS software).</i>
Data format	<i>Analyzed</i>
Experimental factors	<i>Four cell lines (Mouse embryonic fibroblasts (MEFs), Human fibroblasts (HFs), SH-SY5Y human neuroblastoma cells, and N27 rat dopaminergic cells). Starvation-induced autophagy by incubation with Earle's Balanced Salt Solution (EBSS) medium, bafilomycin A1 (Baf. A1) treatment (blocking autophagosome-lysosome fusion), and dual treatment (Baf. A1 + EBSS).</i>
Experimental features	<i>Western-blotting analysis of LC3 and p62 proteins using Sample buffer (SB) 1X lysis buffer, detection of endogenous LC3 and p62 immunofluorescence and p62 mRNA levels by qPCR.</i>
Data source location	<i>Not applicable</i>
Data accessibility	<i>Data is within this article</i>

Value of the data

- This data provides characterization of basal macroautophagy flux and response to classical inducers (EBSS) and inhibitors (Baf. A1) of this mechanism in MEFs, HFs, SH-SY5Y and N27 cell lines.
- The data would be valuable for further studies of autophagy dynamics in these four cell lines.
- This data could give a basis for further experiments on revealing the underlying mechanism of autophagy in these cell lines.
- The data support the development of transcription analysis of autophagy markers.

1. Data

In order to understand autophagy dynamics and develop a robust methodological assay, we used four cell models: MEFs, HFs, SH-SY5Y human neuroblastoma cells, and N27 rat mesencephalic dopaminergic cells. LC3 and p62 protein levels were analyzed by Western-blotting assay, using SB 1X as a lysis buffer (Fig. 1). Furthermore, we measured the endogenous immunostaining of LC3 and p62 proteins in all cell models used in this report (Fig. 2). To complete this data analysis, p62 mRNA expression was performed in all cell lines (Fig. 3).

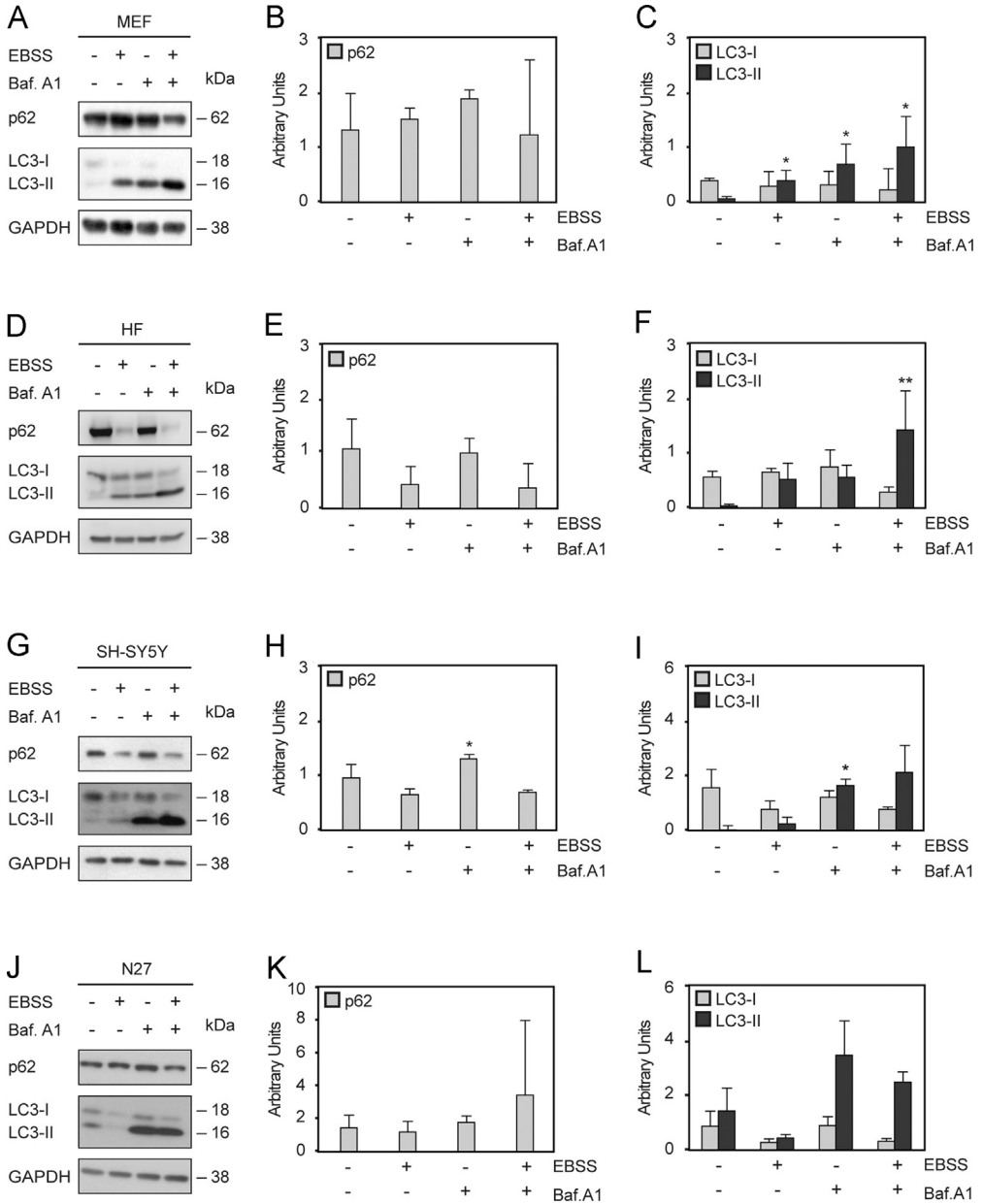


Fig. 1. LC3 and p62 modulation after EBSS ± Baf. A1 treatment in four cell models. Cells were treated with EBSS, Baf. A1 and dual treatment for 4 h, as described in Section 2. Representative blots of 3 independent experiments of MEFs (A), HF (D) SH-SY5Y (G) and N27 rat dopaminergic cells (J) are shown. GAPDH was used as a loading control. p62 densitometry expressed in arbitrary units is shown in panels B (MEFs), E (HF), H (SH-SY5Y) and K (N27). LC3 (isoforms I and II) densitometry expressed in arbitrary units is shown in panels C (MEFs), F (HF), I (SH-SY5Y) and L (N27). Data represent the mean ± SEM; $n=3$ (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, related to the corresponding non-treated condition).

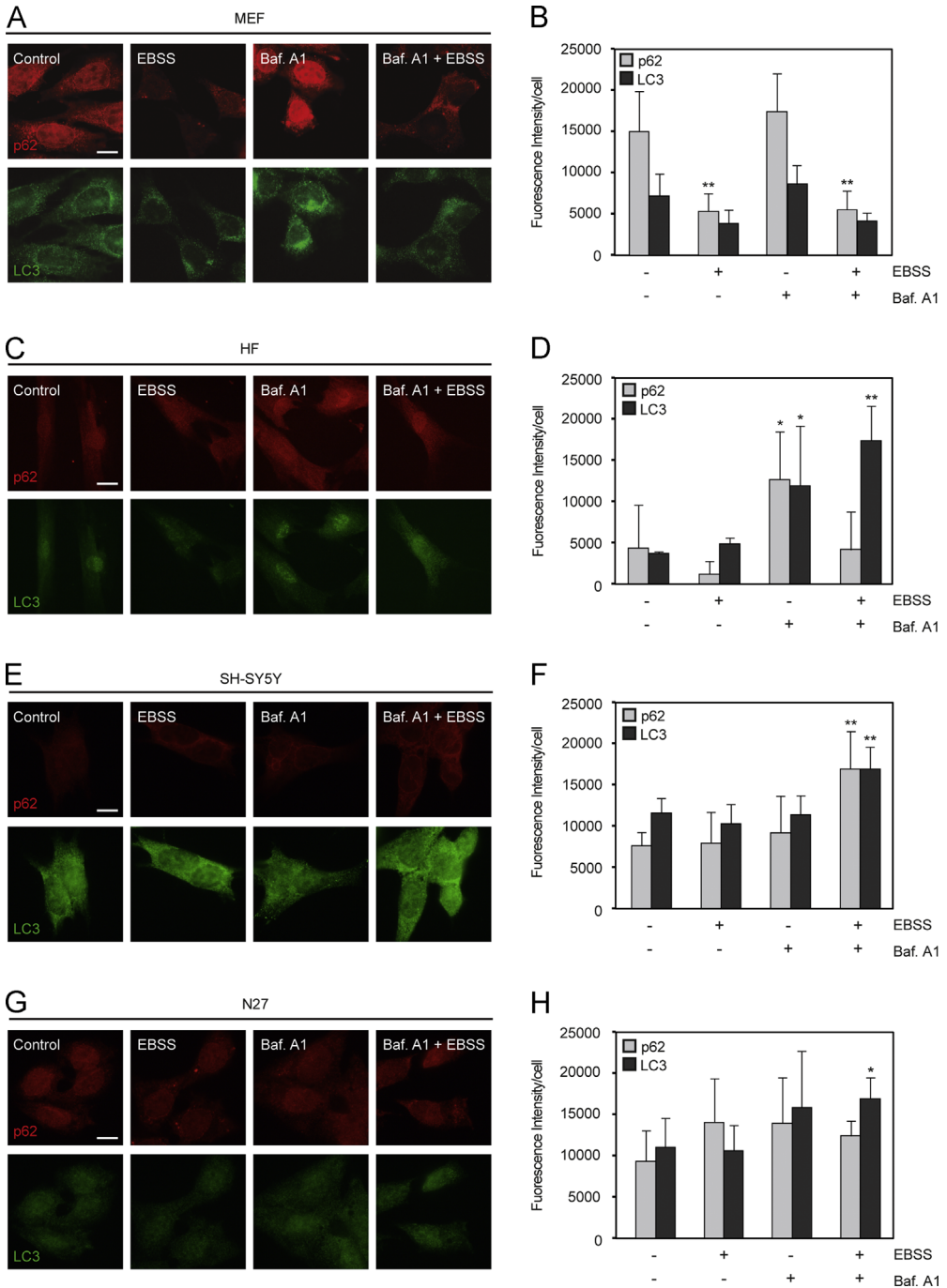


Fig. 2. Endogenous immunofluorescence of LC3 and p62 after EBSS \pm Baf. A1 treatment in four cell models. Cells were treated with EBSS, Baf. A1 and dual treatment for 4 h, as described in Section 2. Representative images from MEFs (A), HFs (C), SH-SY5Y (E) and N27 cells (G) are shown. Scale bars equate to 10 μ m. Histograms show quantification of fluorescence intensity per cell from MEFs (B), HFs (D), SH-SY5Y (F) and N27 cells (H). Data represent the mean \pm SEM; $n=3$ ($*p \leq 0.05$, $**p \leq 0.01$, related to the corresponding non-treated condition).

217 2. Experimental design, materials and methods

218

219 2.1. Cell culture and treatments

220

221 To perform this data analysis, we used four cell lines: MEFs, HF, SH-SY5Y and N27 rat mesen-
 222 cephalic dopaminergic cells. The culture media for MEFs, HF and SH-SY5Y were Dulbecco's Modified
 223 Eagle Medium-High Glucose (Sigma-Aldrich, D6546) supplemented with 10% fetal bovine serum
 224 (FBS) (Sigma-Aldrich, F7524), 1% L-glutamine (Sigma-Aldrich, G7513) and penicillin-streptomycin
 225 (Hyclone, SV30010). For N27 cell line, the culture medium was made of RPMI 1640 medium (1X)
 226 (Hyclone, SH30096.01) supplemented with 10% FBS, L-glutamine and penicillin-streptomycin.

227 Cells were seeded at densities of 3×10^5 (MEFs), 1×10^6 (HFs), 2×10^6 (SH-SY5Y) and 4×10^5 (N27)
 228 in 75-cm² tissue culture flasks and incubated at 37 °C under saturating humidity in 5% CO₂/95% air.
 229 Confluent cells (~80%) were trypsinized and plated into a 6 or 24-well plates at densities of 3×10^4
 230 cells/mL (MEFs and HFs), 1×10^5 cells/mL (SH-SY5Y) and 3.5×10^4 cells/mL (N27).

231 After 24 h (h), the culture medium was replaced with different treatments (Control, EBSS, Baf. A1
 232 and Baf. A1 + EBSS). To induce autophagy by starvation conditions, the culture medium was replaced
 233 with EBSS (Sigma-Aldrich, E2888). To block fusion between autophagosomes and lysosomes, cells
 234 were incubated with 100 nM Baf. A1 (LC Laboratories, B-1080). For combined treatment, cells were
 235 preincubated with 100 nM Baf. A1 for 1 h and then they were washed with phosphate-buffered saline
 236 (PBS) 1X and treated with 100 nM Baf. A1 and EBSS. All treatments lasted 4 h.

237

238

239

240

241

242

243

244

245

246

247

248

249

250

251

252

253

254

255

256

257

258

259

260

261

262

263

264

265

266

267

268

269

270

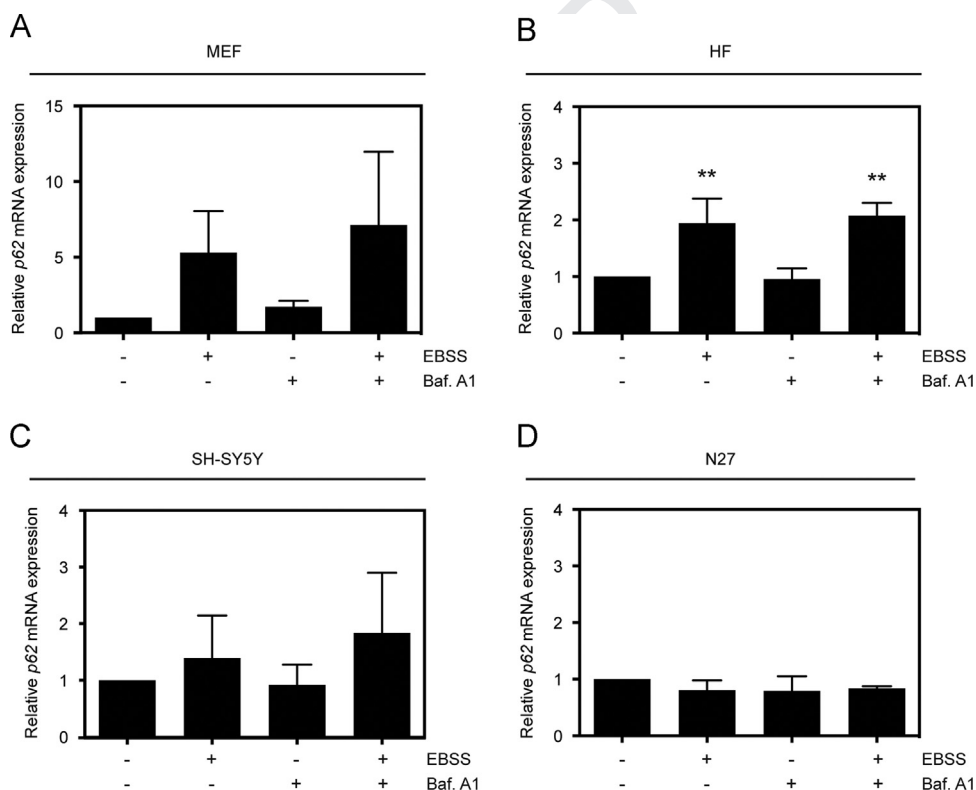


Fig. 3. p62 mRNA analysis after EBSS ± Baf. A1 treatment in four cell models. Cells were treated with EBSS, Baf. A1 and dual treatment for 4 h, as described below. Quantification of p62 mRNA expression levels from all lines studied is shown: MEFs (A), HFs (B), SH-SY5Y (C) and N27 cells (D). Relative expression was determined using GAPDH as a housekeeping gene. Data represent the mean ± SEM; n=3 (**p ≤ 0.01, related to the corresponding non-treated condition).

2.2. Western-blotting

Following treatments, cells were processed as described previously [2]. Basically, cells were washed with PBS 1X and lysed in sample buffer (SB) 1X (2% (v/v) sodium dodecyl sulfate (SDS), 10% (v/v) glycerol, and 50 mM Tris-HCl, pH 6.8, in distilled water) by pipetting until homogenization. Protein concentration was measured based on the bicinchoninic acid assay, using bovine serum albumin as a standard. Samples were heated at 95 °C for 10 min (min) before their quantification.

Equal amounts of protein (25–40 µg/condition) were resolved by 12% SDS-gel electrophoresis and transferred to polyvinylidene fluoride membranes, according to a partially modified conventional protocol [3]. Immunodetection included transferring (15 V during 15 min, per each membrane) and blocking of the membrane with WB blocking solution (10% w/v fat free milk in Tris-buffered saline with Tween 20 (TBST)) for 1 h at room temperature. After washing the membranes 2 times with TBST 1X, blots were incubated with the corresponding primary antibody: p62/SQSTM1 (1:5000) (BD Transduction Laboratories, 610498), LC3-B (1:5000) (Sigma-Aldrich, L7543) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (1:5000) (Millipore, MAB374) at 4 °C overnight, unless GAPDH (1 h at room temperature). The membranes were washed 2 times with TBST 1X and subsequently incubated with their respective HRP-conjugated secondary antibodies (1:10000) (Bio-Rad, 170–6515 and 170-5047 for rabbit and mouse antibodies, respectively) for 1 h at room temperature. Detection of bound antibodies was visualized by chemiluminescence using ECL substrate (Thermo Scientific, 32106). Quantification data analysis was performed using ImageJ software (NIH), establishing GAPDH protein levels as a loading control.

2.3. Immunofluorescence

For the detection of endogenous p62 and LC3B, cells were seeded on coverslips, fixed with paraformaldehyde (0.4% in PBS 1X) and permeabilized with Triton X-100 solution (0.1% in PBS 1X) for 10 min. To block non-specific binding, cells were incubated with 10% FBS in PBS 1X for 20 min, followed by incubation with primary antibodies anti-p62 (1:500) and anti-LC3B (1:500) for 1 h at room temperature. After that, cells were incubated with Alexa Fluor 488 anti-rabbit (1:1000) (Molecular Probes, A-11034) and 568 anti-mouse (1:1000) (Molecular Probes, A-11004) secondary antibodies for LC3 and p62, respectively. Finally, coverslips were mounted on microscope slides, by using fluoromount-G (SouthernBiotech, 0100–01) medium. Images were taken by using an inverted fluorescence microscope (Olympus, IX-51) equipped with a camera (Olympus, DP70). The quantitative measurement of the fluorescence signal was performed using ImageJ software analyzing at least 200 cells per condition. Immunofluorescence procedure was developed as previously described [2].

2.4. Quantitative PCR

Total RNA was extracted by RNeasy Mini Kit (Qiagen, 74104); 500 ng of total RNA were reverse-transcribed into complementary DNA by using a QuantiTect Reverse Transcription Kit (Qiagen, 205311), both according to the manufacturer's protocol. p62 mRNA expression was measured by qPCR with KAPA SYBR Fast reagents (KK4601), by using the primers described above. GAPDH gene expression was used as an endogenous control, and the expression level was calculated by using the ($2^{-\Delta\Delta C_t}$) ratio [4].

2.5. Statistical analyses

Each experiment was repeated at least three times. The data were analyzed by two-tailed unpaired Student's t-test and ANOVA test where applicable, and all comparisons with a p value less than 0.05 ($p < 0.05$) were considered statistically significant ($***p < 0.001$, $**p < 0.01$, $*p < 0.05$). Non-significant results are not indicated in the figures. The data are expressed as the mean \pm the standard error of the mean (SEM).

Acknowledgments

We thank George Auburger (Experimental Neurology, Goethe University Medical School, Frankfurt am Main, Germany) for the MEFs, Adolfo López de Munaín (Neurology service, Instituto BioDonostia, Hospital Donostia, San Sebastian, Spain) for the HF, and Anumantha G. Kanthasamy (Iowa State University, Ames, IA) for the N27 cells. M. R.-A. was supported by a FPU predoctoral fellowship (FPU13/01237) from Ministerio de Educación, Cultura y Deporte, Spain. R. G.-S. was supported by a Marie Skłodowska-Curie Individual Fellowship (IF-EF) from the European Commission. J. M. F. received research support from the Ministerio de Economía y Competitividad, Spain, CIBERNED (CB06/05/004 and PI12/02280). R. A. G.-P. was supported by a "Contrato destinado a la retención y atracción del talento investigador, TA130009" from Junta de Extremadura, Spain, and she received research support from Ministerio de Economía y Competitividad, Spain (PI14/00170). This work is supported also by "Fondo Europeo de Desarrollo Regional" (FEDER), from European Union. The authors also thank FUNDESALUD for helpful assistance.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.dib.2016.02.085>.

References

- [1] D.J. Klionsky, K. Abdelmohsen, A. Abe, et al., Guidelines for the use and interpretation of assays for monitoring autophagy (3rd ed.), *Autophagy* 12 (1) (2016) 1–222.
- [2] R. Gomez-Sanchez, E. Pizarro-Estrella, S.M. Yakhine-Diop, et al., Routine Western blot to check autophagic flux: cautions and recommendations, *Anal. Biochem.* 477 (2015) 13–20.
- [3] J.M. Fuentes, A.M. Lompre, J.V. Moller, et al., Clean Western blots of membrane proteins after yeast heterologous expression following a shortened version of the method of Perini et al, *Anal. Biochem.* 285 (2) (2000) 276–278.
- [4] M.W. Pfaffl, A new mathematical model for relative quantification in real-time RT-PCR, *Nucleic Acids Res.* 29 (9) (2001) e45.