Landes Bioscience www.landesbioscience.com

Supplemental Material to:

Melissa Nassif, Vicente Valenzuela, Diego Rojas-Rivera, René Vidal, Soledad Matus, Karen Castillo, Yerko Fuentealba, Guido Kroemer, Beth Levine, and Claudio Hetz

Pathogenic role of BECN1/Beclin 1 in the development of amyotrophic lateral sclerosis

Autophagy 2014; 10(7)
http://dx.doi.org/10.4161/auto.28784

www.landesbioscience.com/journals/autophagy/article/28784

TableS1. Visual observation scores. One of the disease parameters evaluated to determinate the onset and duration of the disease is the visual symptoms.

Code	Visual observation	Scores
C1	slight limb-clasping	1
C2	moderated limb-clasping	3
C3	severe limb-clasping	5
T1	slight tremor felt in one of the hind-limbs	1
T2	moderated tremor felt in one of the hind-limbs	3
T3	severe tremor felt in one of the hind-limbs	5
S1	moderated dirty appearance	1
S2	severe dirty appearance	3
A1	moderated backbone arching	2
A2	obvious backbone arching	5
P1	slight paralysis	2
P2	moderated paralysis	3
P3	severe paralysis	5

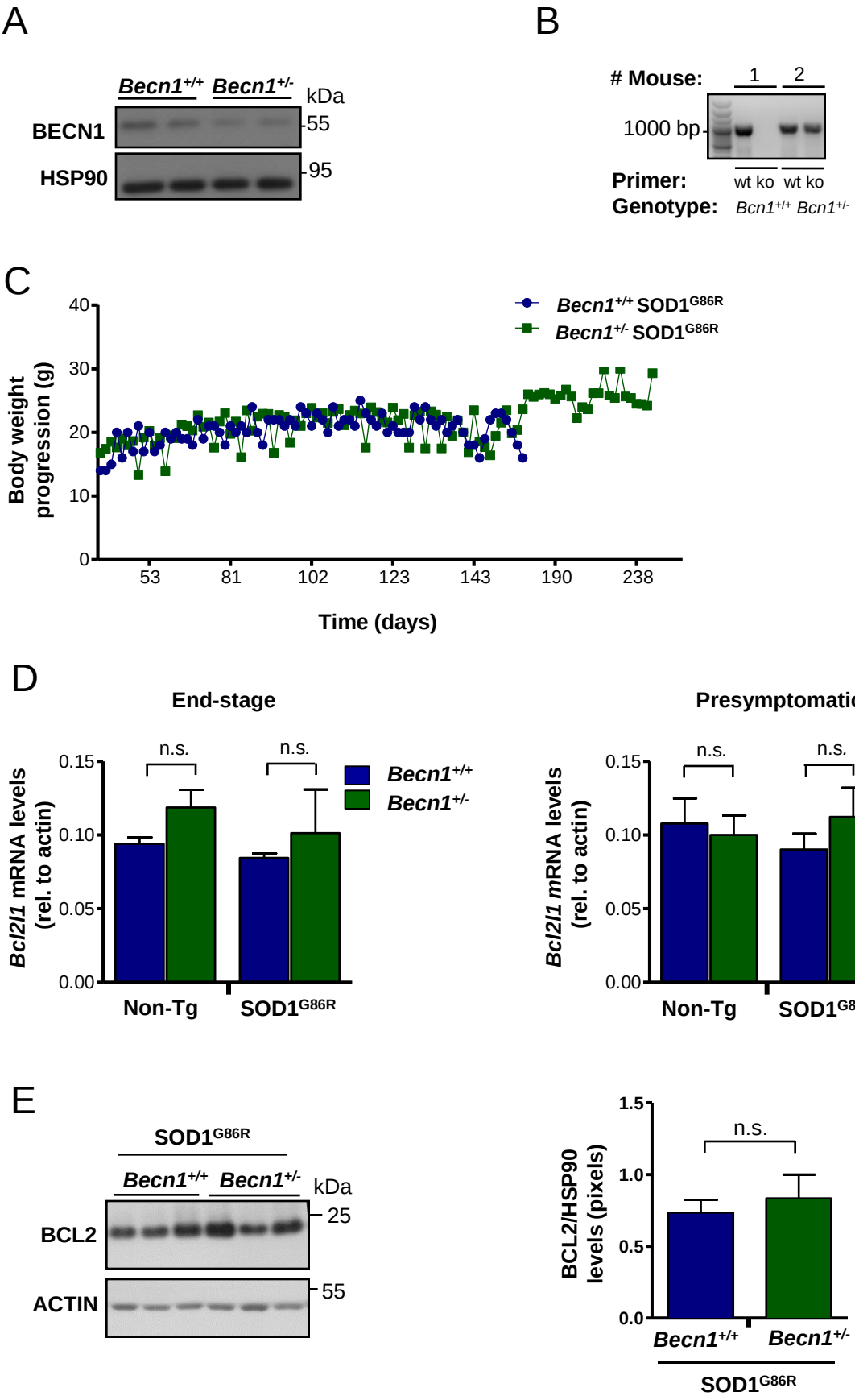
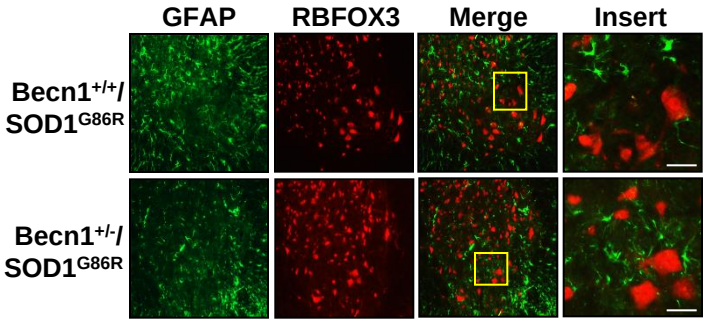

A
Becn1+/+ Becn1+/-
kDa
BECN1
55
HSP90
95
B
Mouse:
1
2
1000 bp
Primer:
wt ko wt ko
Genotype:
Bcn1+/+ Bcn1+/-
C
Becn1+/+ SOD1G86R
Becn1+/- SOD1G86R
Body weight progression (g)
40
30
20
10
0
53
81
102
123
143
190
238
Time (days)
D
End-stage
Presymptomatic
Bcl2l1 mRNA levels (rel. to actin)
0.15
0.10
0.05
0.00
n.s.
n.s.
Becn1+/+
Becn1+/-
Non-Tg
SOD1G86R
E
SOD1G86R
Becn1+/+ Becn1+/-
kDa
BCL2
25
ACTIN
55
BCL2/HSP90 levels (pixels)
1.5
1.0
0.5
0.0
n.s.
Becn1+/+
Becn1+/-
SOD1G86R

Figure S1


GFAP
RBFOX3
Merge
Insert
Becn1+/+/
SOD1G86R
Becn1+/-/
SOD1G86R

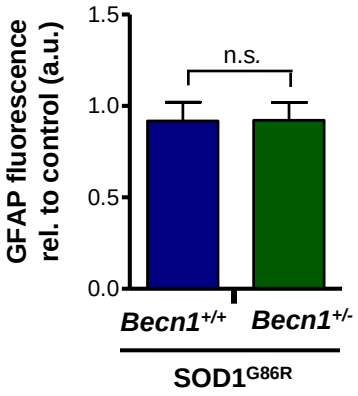

GFAP fluorescence
rel. to control (a.u.)
1.5
1.0
0.5
0.0
n.s.
Becn1+/+
Becn1+/-
SOD1G86R

Figure S2

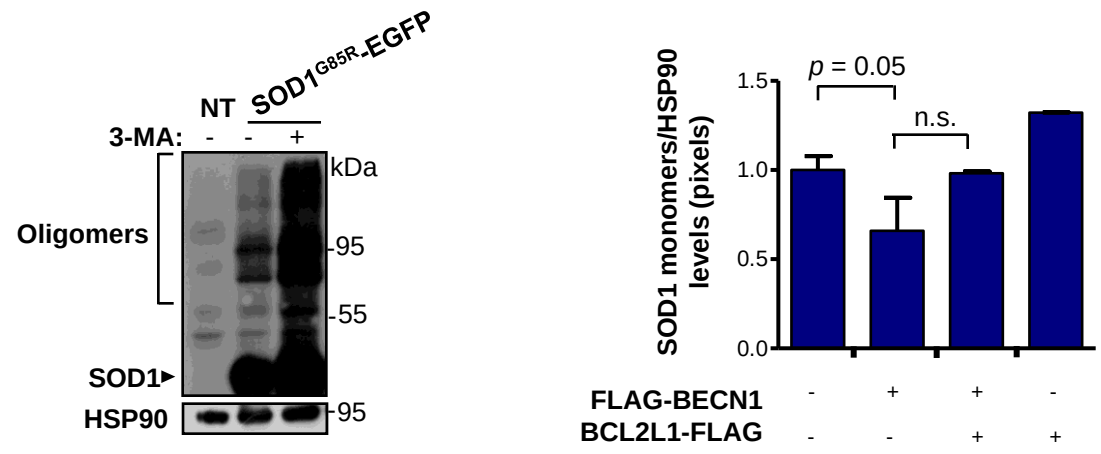
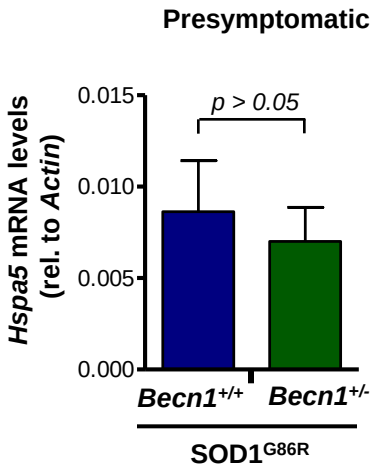
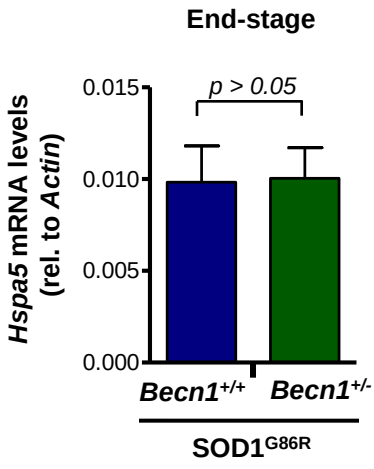
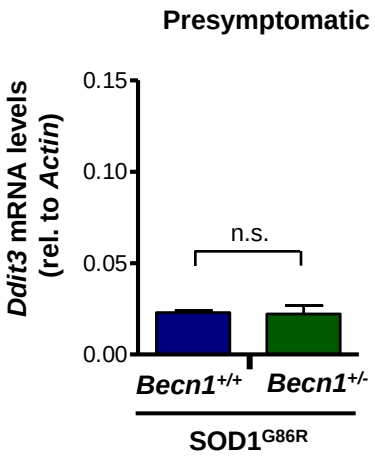

A
NT
SOD1G85R-EGFP
3-MA: - - +
kDa
Oligomers
95
55
SOD1
HSP90
95
B
p = 0.05
n.s.
SOD1 monomers/HSP90 levels (pixels)
1.5
1.0
0.5
0.0
FLAG-BECN1 - + + -
BCL2L1-FLAG - - + +
C
SOD1G85R-EGFP: - + + +
FLAG-BECN1: - - + +
3-MA: - - - +
kDa
Oligomers
95
55
SOD1
HSP90
95

Figure S3


Presymptomatic
Hspa5 mRNA levels (rel. to Actin)
0.015
0.010
0.005
0.000
p > 0.05
Becn1+/+
Becn1+/-
SOD1G86R


End-stage
Hspa5 mRNA levels (rel. to Actin)
0.015
0.010
0.005
0.000
p > 0.05
Becn1+/+
Becn1+/-
SOD1G86R


Presymptomatic
Ddit3 mRNA levels (rel. to Actin)
0.15
0.10
0.05
0.00
n.s.
Becn1+/+
Becn1+/-
SOD1G86R

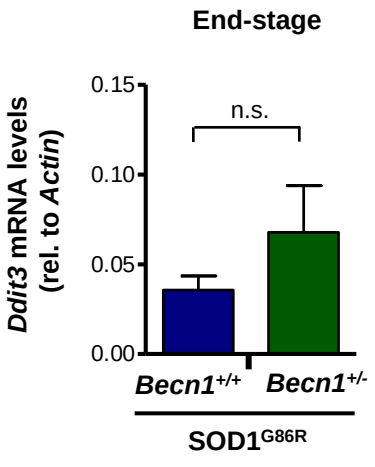

End-stage
Ddit3 mRNA levels (rel. to Actin)
0.15
0.10
0.05
0.00
n.s.
Becn1+/+
Becn1+/-
SOD1G86R

Figure S4

Figure S1. *Becn1* heterozygoous mice. (**A**) To confirm the functional reduction of BECN1 1 in *Becn1*$^{+/-}$ animals, the levels of BECN1 were monitored in spinal cord extracts from *Becn1*$^{+/+}$ and *Becn1*$^{+/-}$ mice by western blot. HSP90 was used as a loading control. (**B**) Example of a DNA-tail genotyping by PCR followed by an electrophoresis on an agarose gel. In the figure we show the representative genotying of 2 animals, a *Becn1*$^{+/+}$ and *Becn1*$^{+/-}$ mouse. Two separate PCR reactions were performed for each DNA-tail, with 2 pairs of primers to amplify the wild-type allele (monitored as a loading control) or the "knockout" (ko) allele. (**C**) Variation of the body weight over time in experimental *Becn1*$^{+/+}$ SOD1^{G86R} and *Becn1*$^{+/-}$ SOD1^{G86R}. Mice started to be monitored from 30 d old until the day of their sacrifice. (**D**) Levels of *Bcl2l1* were determined by real-time PCR in *Becn1*$^{+/+}$ SOD1^{G86R} and *Becn1*$^{+/-}$ SOD1^{G86R} and non-transgenic spinal cord tissue at the presymptomatic and end-of-disease stages. Mean and standard error are presented for the analysis of 4 animals per group. The *P* value was calculated with the Student *t* test. n.s., nonsignificant differences. (**E**) BCL2 levels were evaluated in spinal cord tissue from *Becn1*$^{+/+}$ SOD1^{G86R} and *Becn1*$^{+/-}$ SOD1^{G86R} mice in the same samples/gel shown in Figure 4F. Actin was used a loading control (same image from Figure 4F). Right panel: Quantification of BCL2 levels. Mean and standard error are presented.

Figure S2. GFAP and RBFOX3 in presymptomatic SOD1^{G86R} mice. (**A**) Immunofluorescence analysis of the expression of GFAP (astrocytes, green) and RBFOX3 (neurons, red) after indirect immunofluorescent staining of spinal cord tissue derived from *Becn1*$^{+/+}$ SOD1^{G86R} and *Becn1*$^{+/-}$ SOD1^{G86R} at the presymptomatic stage of disease. A merged image is also presented together with a zoom of the selected area (yellow square). A representative image is presented of the analysis of 3 independent animals for group. Scale bars: 10 μm. Right panel: Quantification of the GFAP signal intensity is presented for 3 animals from each genotype.

Mean and standard deviation are presented. *P* value was calculated with the Student *t* test. n.s., nonsignificant differences.

Figure S3. Autophagy inhibition increases SOD1^{G85R}-EGFP aggregation. (**A**) NSC34 cells expressing SOD1^{G85R}-EGFP were treated with 10 mM 3-MA for 16 h or left untreated (control), and then the generation of mutant SOD1 aggregation was analyzed by western blot. Results are representative from at least 3 independent experiments. (**B**) NSC34 cells were cotransfected with SOD1^{G85R}-EGFP, FLAG-BECN1 in the presence or absence of FLAG-BCL2L1 expression vectors. After 72 h, SOD1 protein oligomers were measured in cell extracts prepared in 1% Triton X-100?? by western blot. HSP90 levels were monitored as loading control. Then quantification of relative SOD1 levels of the monomeric species from 3 independent experiments was performed, normalized to the values obtained in control cells only expressing mutant SOD1. Mean and standard error are presented. Western blots are shown in Figure 6C. (**C**) NSC34 cells expressing SOD1^{G85R}-EGFP together with FLAG-BECN1 were treated with 10 mM 3-MA for 16 h or left untreated (control), and then the generation of mutant SOD1 aggregation was analyzed by western blot. HSP90 levels were monitored as loading control.

Figure S4. Expressions of ER stress markers in SOD1^{G86R} mice heterozygous for *Becn1*. Expression levels of the mRNA of *Hspa5* and *Ddit3* were determined by real-time PCR in *Becn1*$^{+/+}$ SOD1^{G86R} and *Becn1*$^{+/-}$ SOD1^{G86R} spinal cord in presymptomatic and end-stage of the disease. Mean and standard error are presented for the analysis of 4 animals per group. *P* value was calculated with the Student *t* test. n.s., nonsignificant differences.