LEGENDS TO SUPPLEMENTAL FIGURES

Supplemental Figure S1. *dHDAC6^{KO}* enhances the rough eye phenotype caused by proteasome impairment. Scanning electron microscopy images of fly eyes with *GMR-GAL4*-driven expression of DTS7 in wild-type (A and B), homozygous *dHDAC6^{KO}* background (C), or with homozygous *dHDAC6^{KO}* plus *dHDAC6* transgene (D). The flies were reared at 25°C (A) or 28°C (B-D). Lower panels show high magnification of the ommatidia. Genotypes: *DTS7* flies are *w;GMR-GAL4/+;UAS-Prosbeta2^{1}/+*. *dHDAC6^{KO};DTS7* flies are *w,dHDAC6^{KO};GMR-GAL4/+;UAS-Prosbeta2^{1}/+*. *dHDAC6^{KO};dHDAC6;DTS7* flies are *w,dHDAC6^{KO};GMR-GAL4/+;UAS-Prosbeta2^{1}/UAS-dHDAC6*.

Supplemental Figure S2. *dHDAC6^{KO}* flies are not sensitive to oxidative stress or heat stress. (A) The survival rate of adult flies treated with 20 mM paraquat. (B) The percentage of third instar larvae survived to adulthood after heat shock at 39°C for 45min with (PT) or without (NO PT) pretreatment at 35°C for 30 min. Statistics were analyzed with Student's *t* test and no statistical difference between wild-type and *dHDAC6^{KO}* flies is detected.

Supplemental Figure S3. TH-GAL4 driving GFP expression highly colocalizes with anti-TH signals. Confocal images show DA neuron clusters in the dorsomedial (DM, arrow heads), posteriomedial (PM, arrows) and dorsolateral (DL_1, circles) regions of the posterior brain labeled with anti-GFP (A), anti-TH (B), and colocalization of the signals (C). Scale bar represents 50 μm. (D) The numbers of DA neurons labeled with anti-GFP and anti-TH as indicated were analyzed by Student's *t* test and presented as mean ± SEM (n=10) with * for $p<0.05$, ** for $p<0.01$, and *** for $p<0.001$. Genotype: *w; UAS-GFP/+; TH-GAL4/+*.

Supplemental Figure S4. Over-expression of *dHDAC6* rescues α-synuclein-induced dopaminergic neuron loss. (A) Confocal images of dopaminergic neurons in the dorsomedial (DM, arrow heads), posteriomedial (PM, arrows) and dorsolateral (DL_1, circles) regions immunostained with anti-GFP antibody on 20-day-old fly brains. (B) Quantitative graphs showing DA neuron numbers of the DM, PM and DL_1 clusters in flies with different genotypes as indicated on the right. Statistics were analyzed with Student's *t* test and presented as mean ± SEM (n>20): * $p<0.05$, ** $p<0.01$, and *** $p<0.001$. Scale bar represents 50 μm. Genotypes: *control* flies are *w;UAS-mCD8::GFP/+;TH-GAL4/+*. *α-syn* flies are *w;UAS-mCD8::GFP/+;TH-GAL4,UAS-α-synuclein/+*. *α-syn;dHDAC6* flies are *w;UAS-mCD8::GFP/UAS-dHDAC6;TH-GAL4,UAS-α-synuclein/+*.

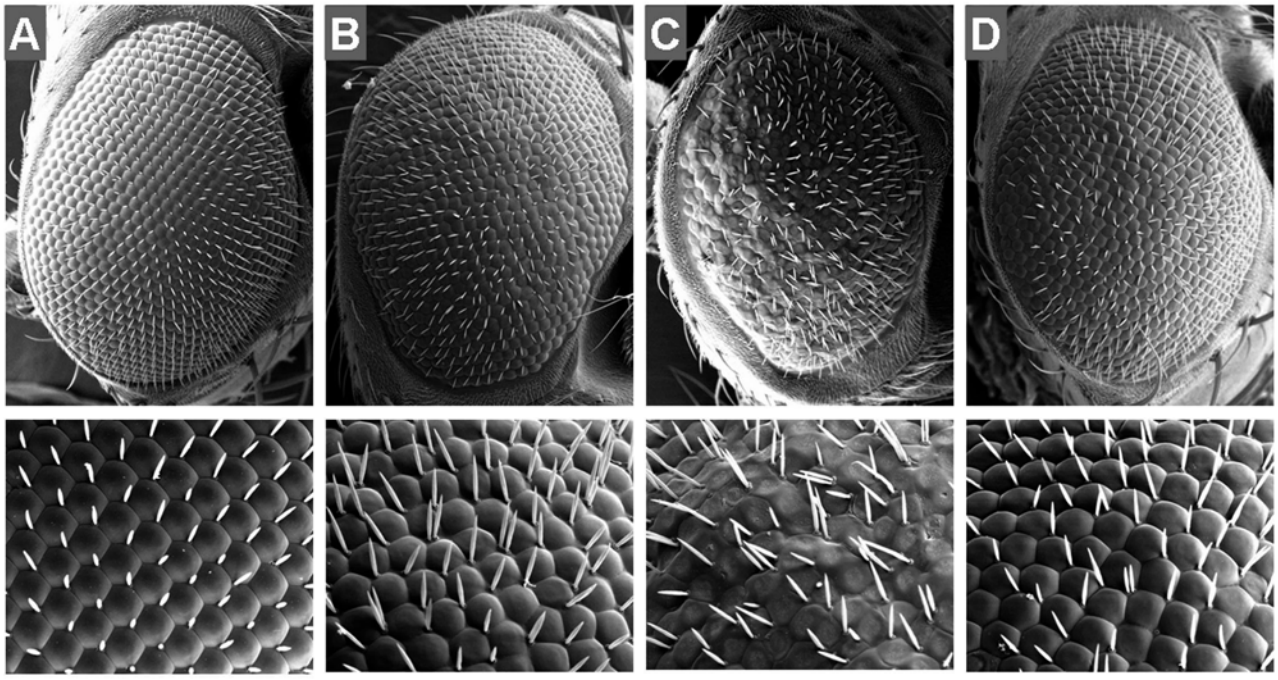
Supplemental Figure S5. *dHDAC6^{KO}; α-syn* flies show shortened lifespan. The lifespan profile shows the survival rate of *yw* (squares), *dHDAC6^{KO}* (circles), *α-syn* (diamonds) and *dHDAC6^{KO}; α-syn* (triangles) flies. Totally 200 adult flies of each genotype were collected. The flies were transferred to new vials with regular food

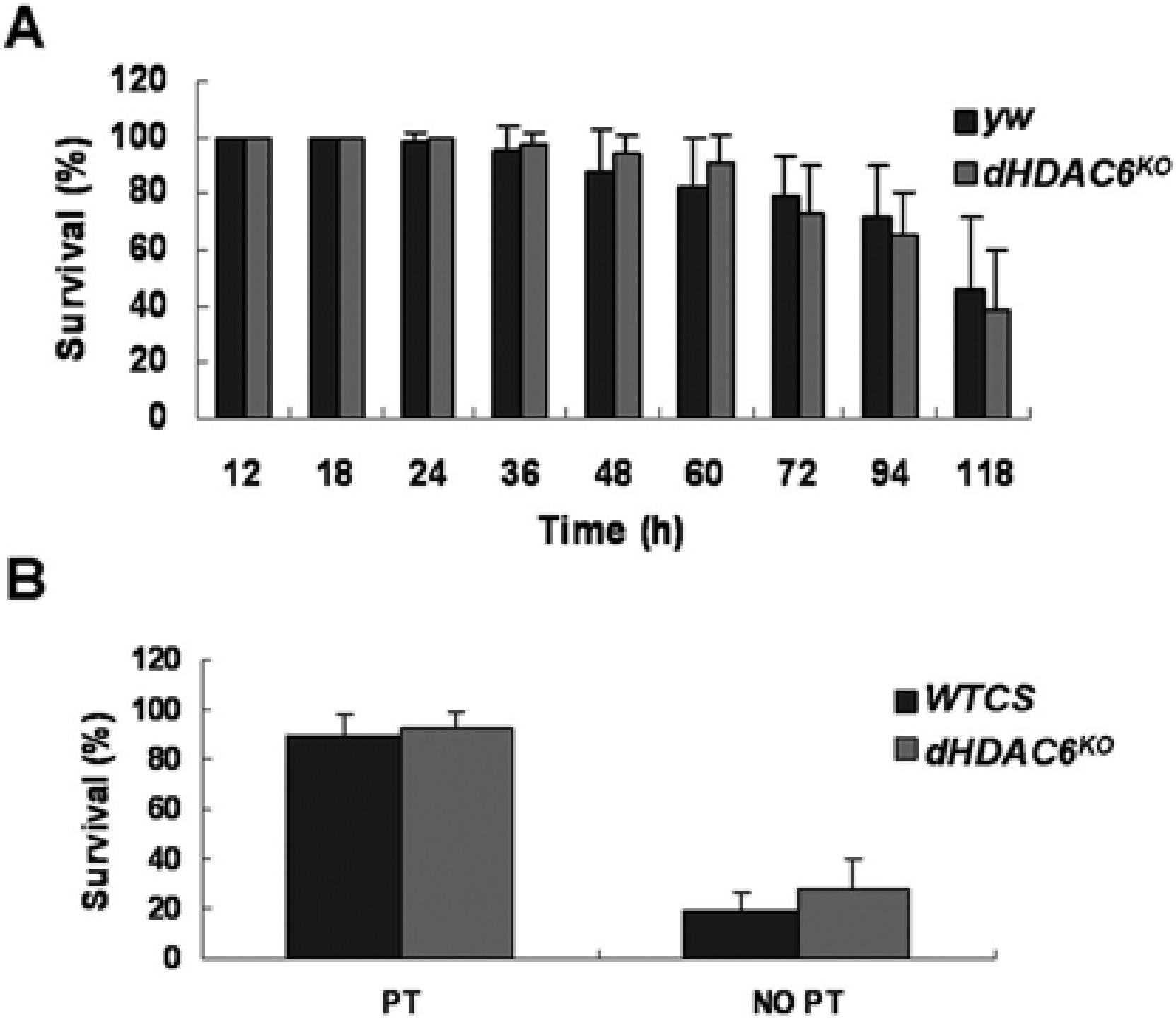
everyday or every other day, and the number of dead flies in each vial was counted everyday. The percentage of flies alive at each time point was depicted. The genotypes of *α-syn* and *dHDAC6^{KO};α-syn* flies are *w;TH-GAL4/UAS-α-synuclein* and *w,dHDAC6^{KO};TH-GAL4/UAS-α-synuclein* respectively.

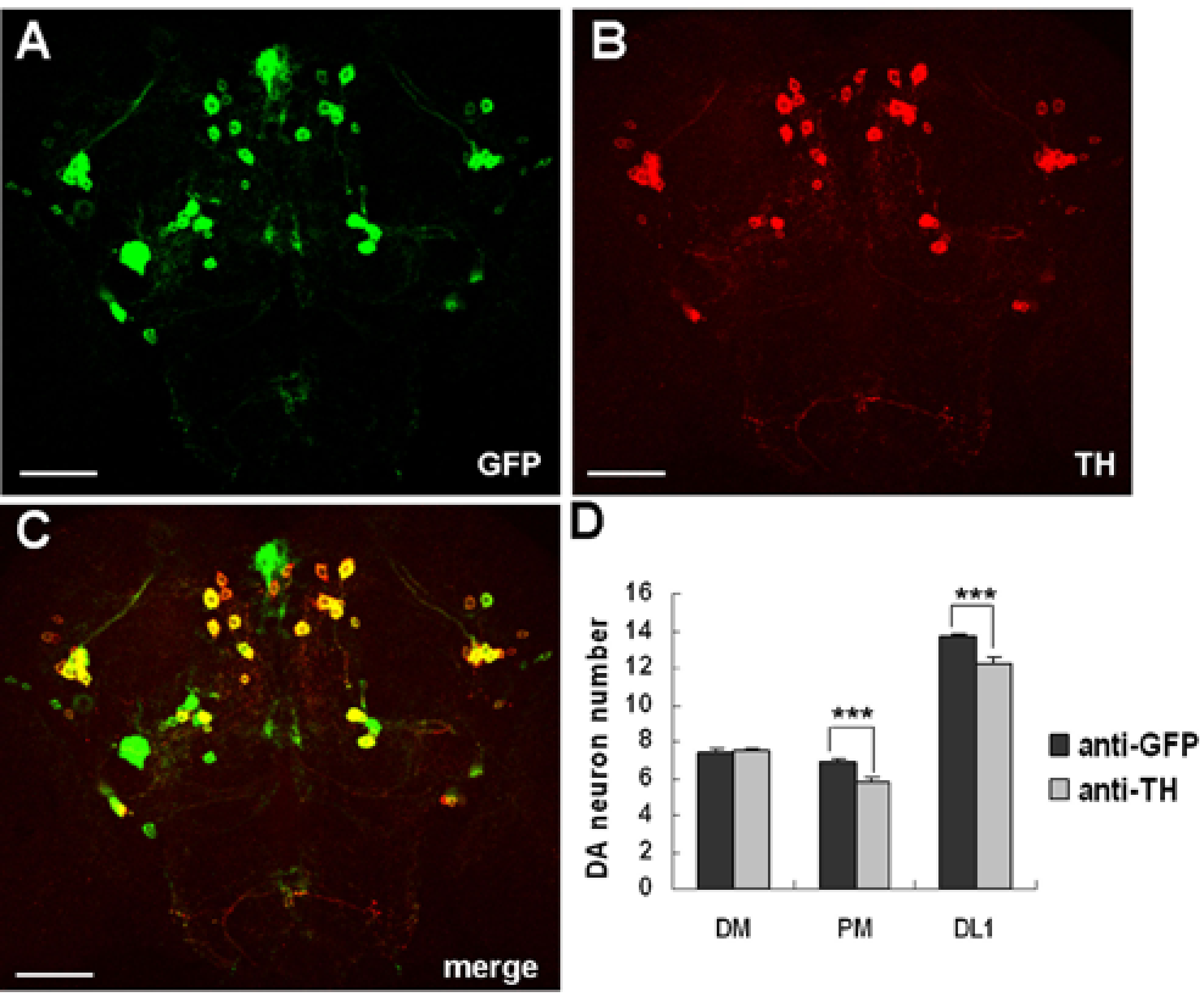
Supplemental Figure S6. Homozygous *dHDAC6^{KO}* enhances neurodegeneration caused by over-expression of glutamine expanded human SCA3. Targeted expression of human MJDtr-Q78 in the fly eyes of wild-type (A) or *dHDAC6^{KO}* background (B and C) shows cell death and pigment loss. Weaker phenotype (majority) is shown in (B), and stronger phenotype (minority) is shown in (C). Genotypes: fly in A is *w;GMR-GAL4/+;UAS-MJDtr-Q78/+*. Flies in B and C are *w,dHDAC6^{KO};GMR-GAL4/+;UAS-MJDtr-Q78/+*.

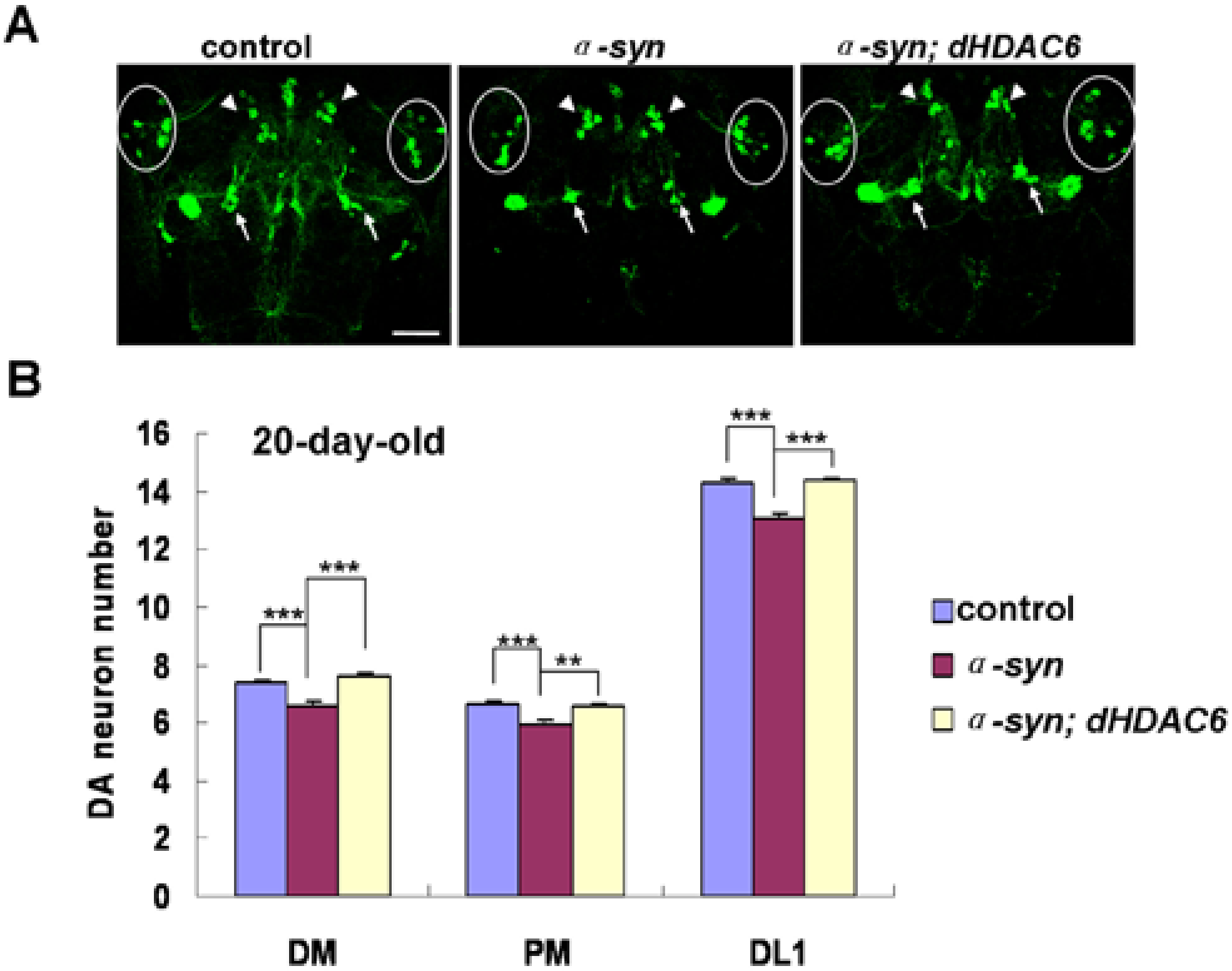
Supplemental Figure S7. *Hsp70* transgene partially rescues the *dHDAC6^{KO}*-induced defects of inclusion formation. Whole-mount immunostaining with anti-α-synuclein antibody shows LB-like inclusions in the DM region of the brains in the flies *dHDAC6^{KO};α-syn* (A) and *dHDAC6^{KO};α-syn* plus *UAS-Hsp70* transgene (B). C and D show the numbers of total inclusions (C) and large inclusions that are larger than 1 μm (D) at 20-day-old with the genotypes as indicated. Data were analyzed by Student's *t* test and presented as mean ± SEM (n>5) with * for p<0.05, ** for p<0.01, and *** for p<0.001. Scale bar represents 40 μm. Genotypes: fly in A is *w,dHDAC6^{KO};TH-GAL4/UAS-α-synuclein*. Fly in B is *w,dHDAC6^{KO};UAS-Hsp70/+;TH-GAL4,UAS-α-synuclein/+*.

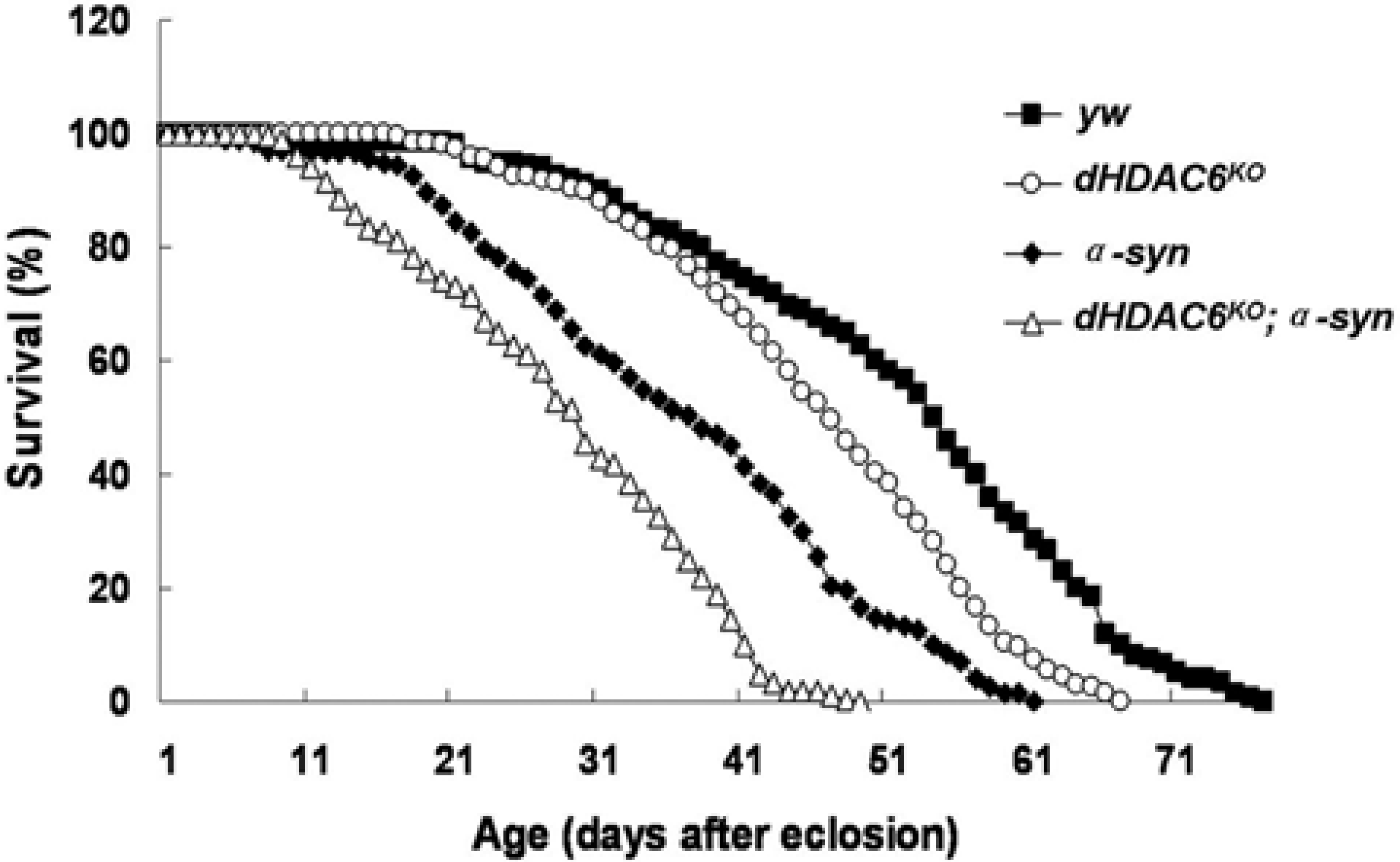
Supplemental Figure S8. A11 antibody recognizes oligomeric α-synuclein but not monomeric α-synuclein, as assayed by co-immunoprecipitation. (A) Protein extracts for immunoprecipitation were prepared from the *w; TH-GAL4/UAS-α-synuclein* fly brains at the age of 1 day, 10 days and 20 days respectively. For each IP sample, protein lysates were extracted from the same amount of fly brains. The co-immunoprecipitation samples were run on the SDS-PAGE and immunoblotted with anti-α-synuclein antibody. Input: 20% of the extracts that were used for immunoprecipitation. (B) Recombinant α-synuclein was expressed in BL21 cells and was purified by chromatography of the supernatant after osmotic shock on a Q-Sepharose fast-flow column as described previously (Huang *et al.*, 2006). For co-immunoprecipitation, about 0.5μg α-synuclein was incubated with agarose beads coupled with A11 antibody or IgG (control). The SDS-10% PAGE of the co-immunoprecipitation samples was immunoblotted with anti-α-synuclein antibody. Input: 10% of the purified protein that were used for immunoprecipitation.

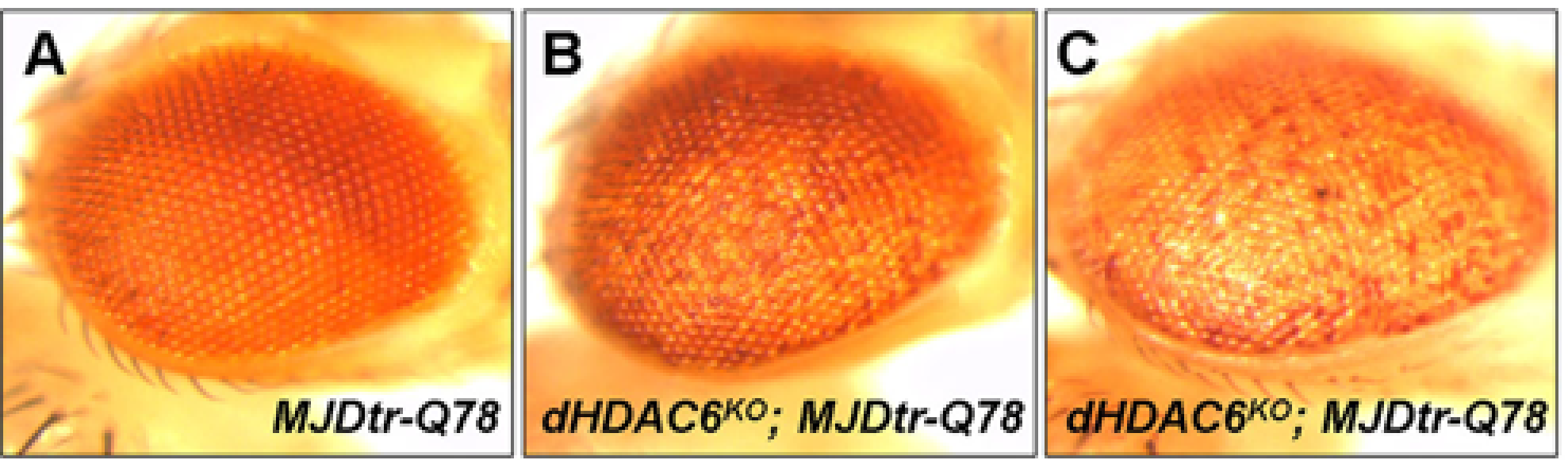

DTS7, 25 ℃
DTS7, 28 ℃
dHDAC6KO
DTS7, 28 ℃
dHDAC6KO;dHDAC6
DTS7, 28 ℃
A
B
C
D

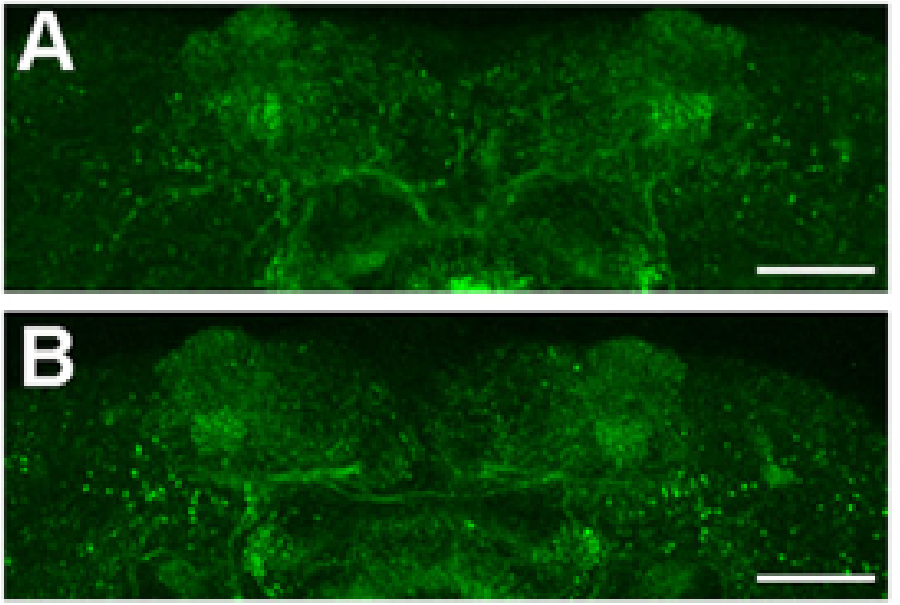

A
120
100
80
60
40
20
0
Survival (%)
yw
dHDAC6KO
12
18
24
36
48
60
72
94
118
Time (h)
B
120
100
80
60
40
20
0
Survival (%)
WTCS
dHDAC6KO
PT
NO PT

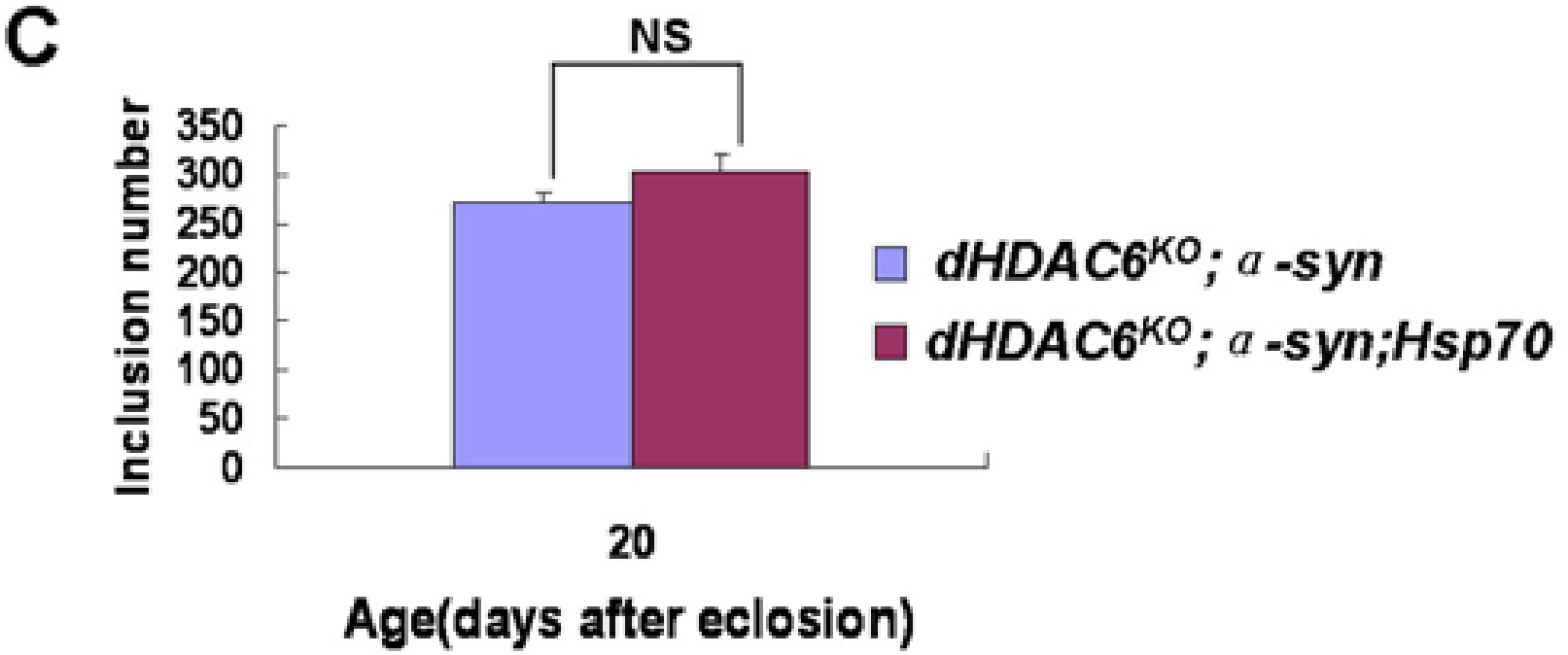

A
GFP
B
TH
C
merge
D
DA neuron number
16
14
12
10
8
6
4
2
0
***
***
DM
PM
DL1
anti-GFP
anti-TH

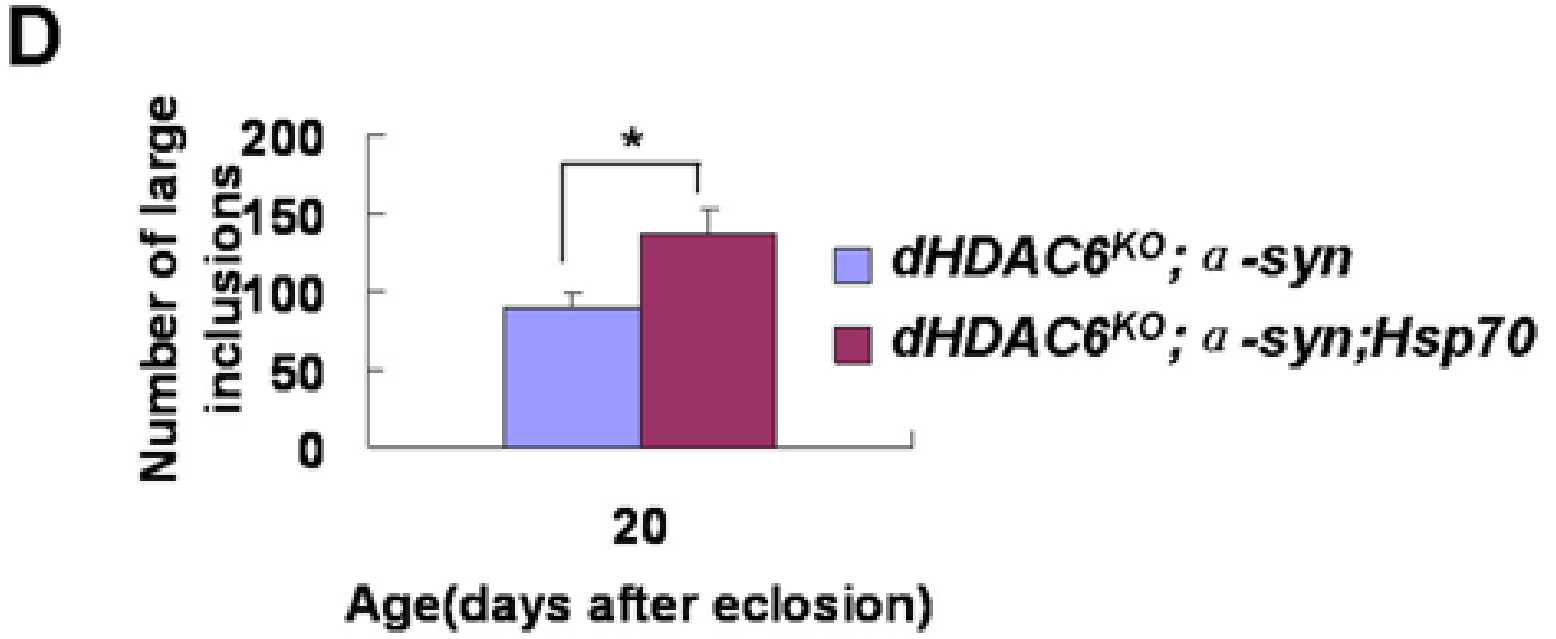

A
control
α-syn
α-syn; dHDAC6
B
20-day-old
DA neuron number
16
14
12
10
8
6
4
2
0
***
***
***
***
**
DM
PM
DL1
control
α-syn
α-syn; dHDAC6

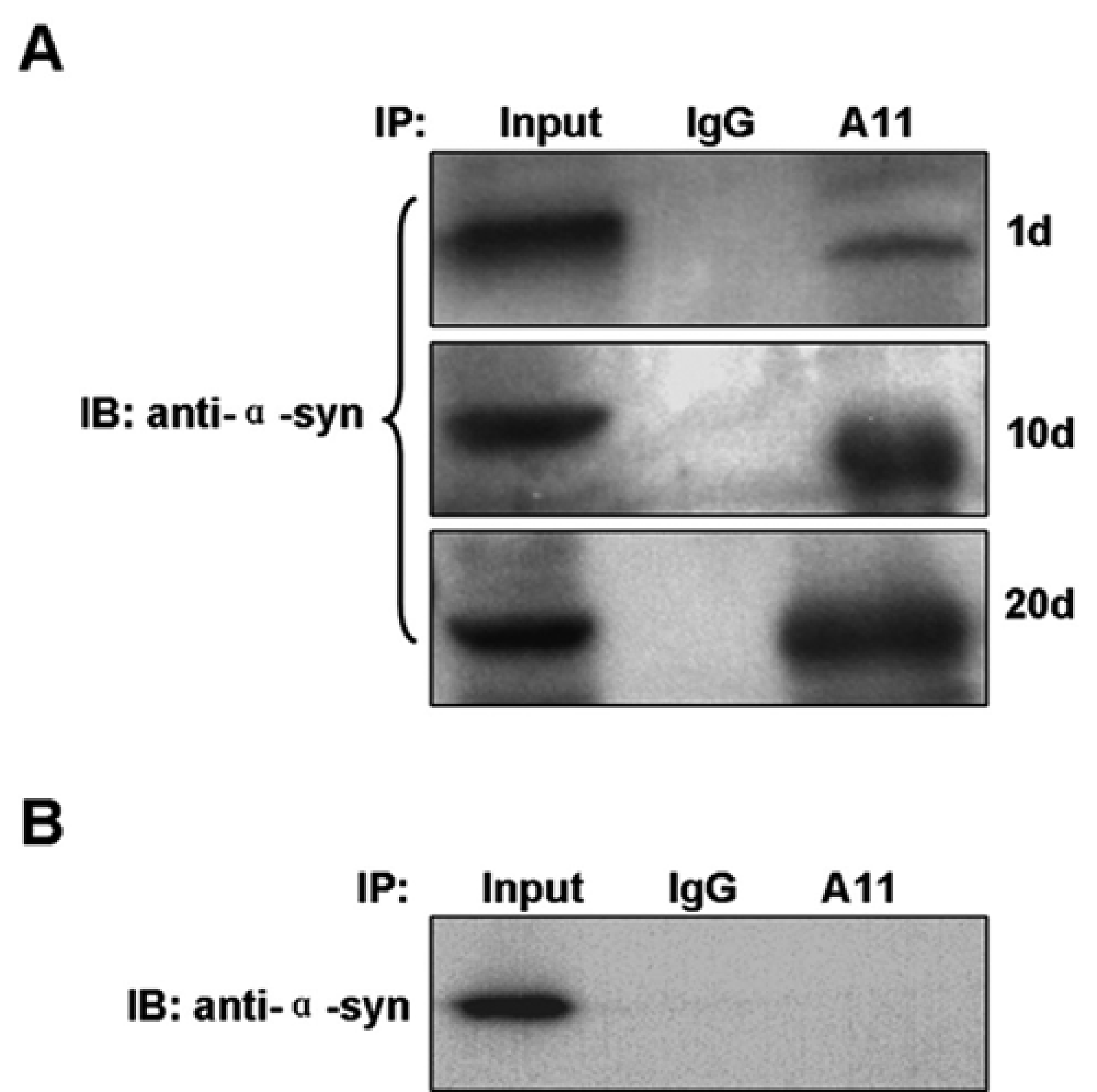

120
100
80
60
40
20
0
Survival (%)
1
11
21
31
41
51
61
71
Age (days after eclosion)
yw
dHDAC6KO
α-syn
dHDAC6KO; α-syn


A
MJDtr-Q78
B
dHDAC6KO; MJDtr-Q78
C
dHDAC6KO; MJDtr-Q78


A
B


C
NS
Inclusion number
350
300
250
200
150
100
50
0
dHDAC6KO; α -syn
dHDAC6KO; α -syn;Hsp70
20
Age(days after eclosion)


D
Number of large inclusions
200
150
100
50
0
*
dHDAC6KO; α -syn
dHDAC6KO; α -syn;Hsp70
20
Age(days after eclosion)


A
IP:
Input
IgG
A11
1d
IB: anti-α -syn
10d
20d
B
IP:
Input
IgG
A11
IB: anti-α -syn