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Supplemental information

Phosphatidylserine synthase plays an essential role in glia and affects development, as well as the maintenance of neuronal function

Ye-Jin Park, Sungkyung Kim, Hyeon-Pyo Shim, Jae H. Park, Gyunghee Lee, Tae-Yeop Kim, Min-Cue Jo, Ah-Young Kwon, Mihwa Lee, Seongjae Lee, Jiwon Yeo, Hyung-Lok Chung, Hugo J. Bellen, Seung-Hae Kwon, and Sang-Hak Jeon

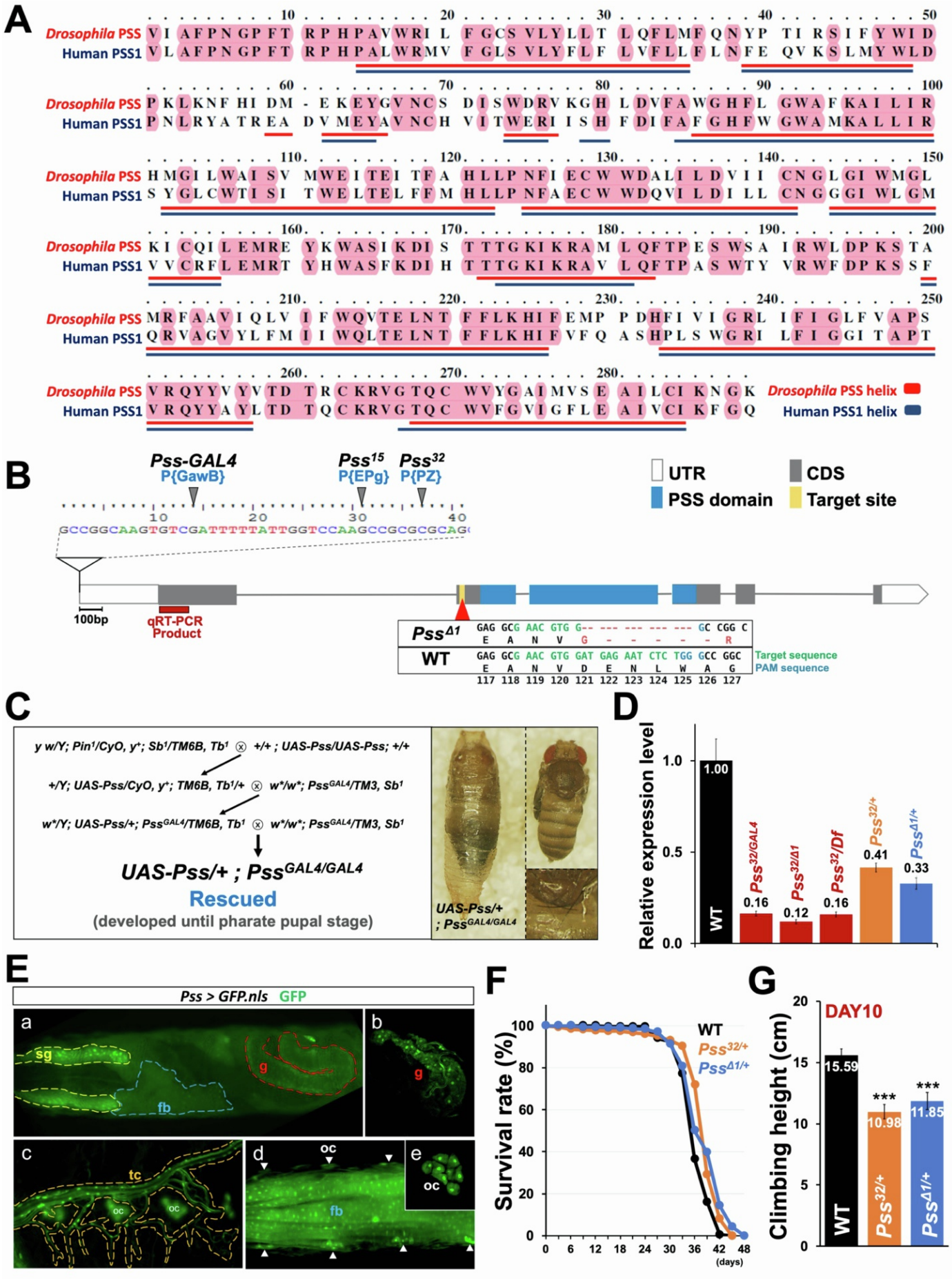
A
Drosophila PSS
Human PSS1
Drosophila PSS helix
Human PSS1 helix
B
Pss-GAL4
P{GawB}
Pss15
P{EPg}
Pss32
P{PZ}
UTR
CDS
PSS domain
Target site
100bp
qRT-PCR Product
Pss Δ1
WT
Target sequence
PAM sequence
C
y w/Y; Pin1/CyO, y+; Sb1/TM6B, Tb1 ⊗ +/+ ; UAS-Pss/UAS-Pss; +/+
+/Y; UAS-Pss/CyO, y+; TM6B, Tb1/+ ⊗ w*/w*; PssGAL4/TM3, Sb1
w*/Y; UAS-Pss/+; PssGAL4/TM6B, Tb1 ⊗ w*/w*; PssGAL4/TM3, Sb1
UAS-Pss/+ ; PssGAL4/GAL4
Rescued
(developed until pharate pupal stage)
UAS-Pss/+ ; PssGAL4/GAL4
D
Relative expression level
WT
Pss32/GAL4
Pss32/Δ1
Pss32/Df
Pss32/+
PssΔ1/+
1.00
0.16
0.12
0.16
0.41
0.33
E
Pss > GFP.nls GFP
sg
fb
g
tc
oc
F
Survival rate (%)
(days)
WT
Pss32/+
PssΔ1/+
G
Climbing height (cm)
DAY10
15.59
10.98
11.85
***

Figure S1. Related to Figures 1 and 2

(A) Comparison of *Drosophila* PSS with human PSS1 in the PSS domain. The alignment of the protein sequences was performed with PRALINE. Identical residues are highlighted in red. Protein regions implicated in helix formation are underlined in blue for human PSS1 and in red for *Drosophila* PSS.

(B) *Pss* mutant alleles. The predicted PSS domains are shown as blue boxes within the coding sequence (CDS).

(C) Genetic crossing schemes for the rescue of the homozygous *Pss* mutant by a *GAL4* enhancer trap line (Pss^{GAL4}) and *UAS-Pss*. The embryonic lethality of $Pss^{GAL4/\,GAL4}$ was rescued by a single copy of *UAS-Pss*. However, the rescued mutants die as pharate adults.

(D) qRT-PCR showing *Pss* expression levels in *Pss trans*-heterozygous or heterozygous flies when compared with wild-type flies (WT). Bars represent max and min values.

(E) *Pss* is also expressed in some other tissues such as salivary gland (sg), fat body (fb), gut (g), trachea (tc), and oenocytes (oc) besides the nervous system.

(F) The lifespan of $Pss^{32/+}$ (n=300) and $Pss^{\Delta 1/+}$ (n=300) flies are not reduced compared to wild-type flies (n=200) (Log-rank test: $p<0.0001$).

(G) Both $Pss^{32/+}$ flies and $Pss^{\Delta 1/+}$ climb less than similarly aged wild-type flies. n=100.

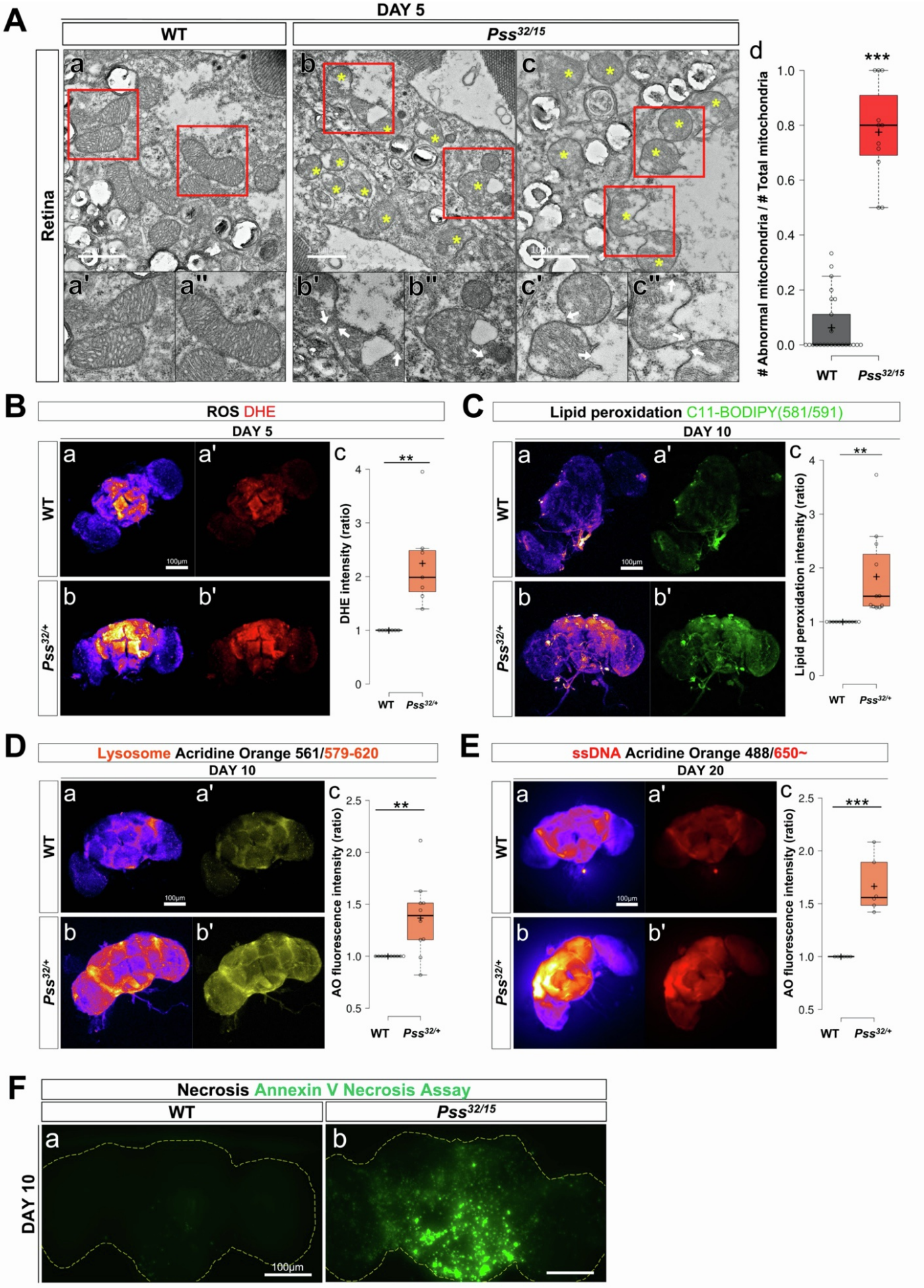
A
DAY 5
WT
Pss32/15
Retina
a
a'
a''
b
b'
b''
c
c'
c''
d
***
Abnormal mitochondria / # Total mitochondria
1.0
0.8
0.6
0.4
0.2
0.0
WT
Pss32/15
B
ROS DHE
DAY 5
WT
Pss32/+
100μm
c
**
DHE intensity (ratio)
4
3
2
1
0
WT
Pss32/+
C
Lipid peroxidation C11-BODIPY(581/591)
DAY 10
WT
Pss32/+
100μm
c
**
Lipid peroxidation intensity (ratio)
4
3
2
1
0
WT
Pss32/+
D
Lysosome Acridine Orange 561/579-620
DAY 10
WT
Pss32/+
100μm
c
**
AO fluorescence intensity (ratio)
2.5
2.0
1.5
1.0
0.5
WT
Pss32/+
E
ssDNA Acridine Orange 488/650~
DAY 20
WT
Pss32/+
100μm
c
***
AO fluorescence intensity (ratio)
2.5
2.0
1.5
1.0
0.5
WT
Pss32/+
F
Necrosis Annexin V Necrosis Assay
WT
Pss32/15
DAY 10
a
b
100μm

Figure S2. Related to Figures 3 and 4

(A) TEM images of mitochondria in the retina of 5-day-old **(a)** WT and **(b-c)** *Pss*$^{32/15}$ adults. **(d)** Quantification of abnormal mitochondria.

(B-E) ROS, Lipid peroxidation, Lysosome, ssDNA in *Pss* heterozygous mutant flies (WT vs. *Pss*$^{32/+}$). ***p < 0.01; ****p < 0.001*

(F) Necrosis assay in 10-day-old *Pss trans*-heterozygous mutant flies (WT vs. *Pss*$^{32/15}$)

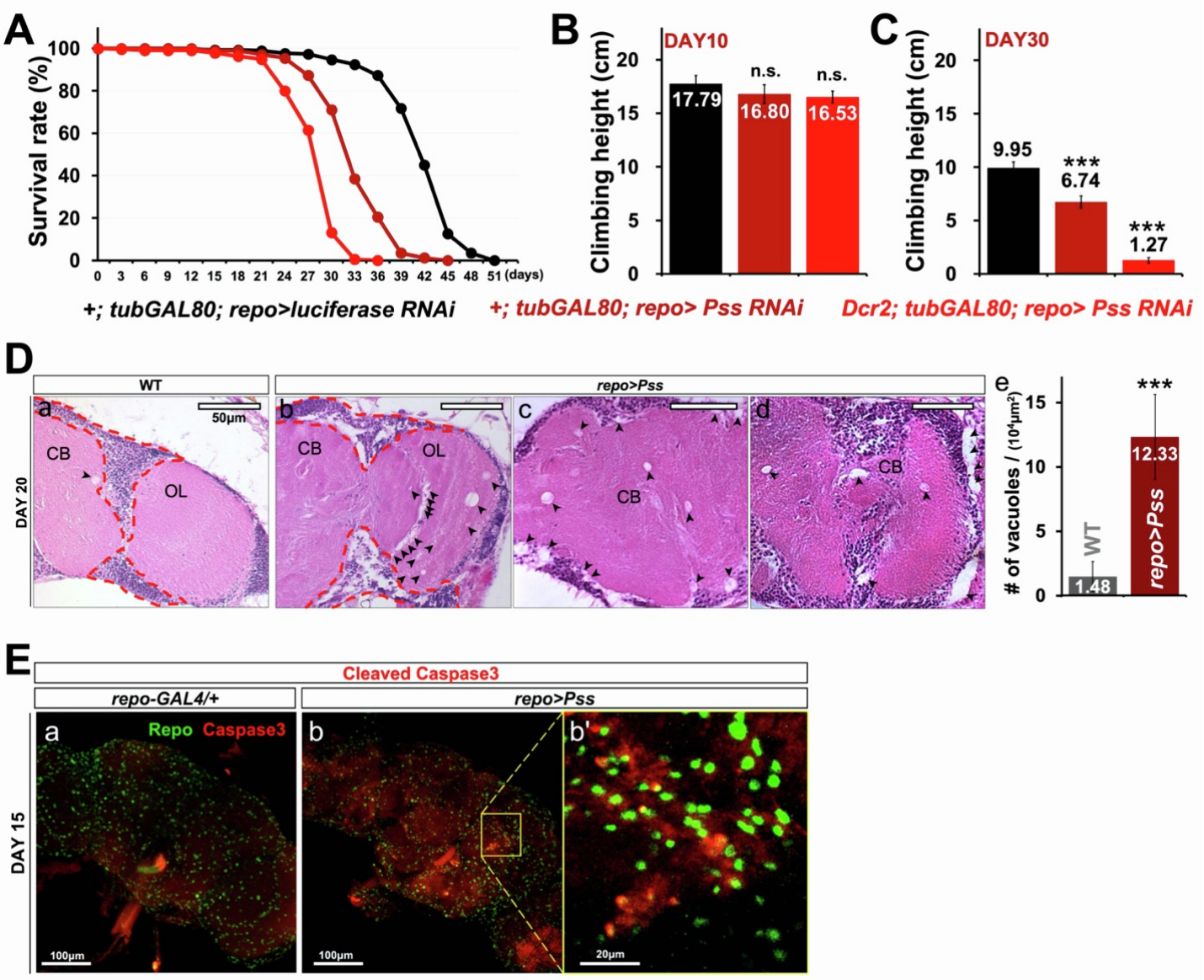

Figure S3. Related to Figure 5

(A-C) Temporal regulation of Pan-glial *Pss* knockdown after eclosion using temperature-sensitive GAL80^{ts} by sifting the flies from 22°C to 29°C. **(A)** *+; tubGAL80; repo>Pss RNAi* flies (n=172) and *Dcr2; tubGAL80; repo>Pss RNAi* flies (n=238) show decreased life span when compared to the control flies (n=265) (Log-rank test, $p<0.0001$). **(B)** In climbing assay, both mutant flies do not show any significant difference when compared to the control flies (n=67) at day 10. *+; tubGAL80; repo>Pss RNAi* flies (n=56) and *Dcr2; tubGAL80; repo>Pss RNAi* flies (n=93). **(C)** However, mutant flies show severe climbing defects compared to controls (n=72) when aged for 30 days. *+; tubGAL80; repo>Pss RNAi* flies (n=68) and *Dcr2; tubGAL80; repo>Pss RNAi* flies (n=43). Bars represent the mean ± SEM (***$p < 0.001$; n.s., not significant). Numbers within/above bars indicate mean values.

(D) Detection of vacuoles in the brains of 20-day-old WT and *repo>Pss* flies. Statistically significant more vacuoles (indicated by arrowheads) are observed in r*epo>Pss* flies than in WT flies. Scale bar, 50 μm. **(E)** Cleaved caspase 3 signals in the brain of the *Repo>Pss* mutant. **(b)** *repo>Pss* flies show stronger and broader caspase 3 fluorescence (red) in the brain than **(a)** control *repo/+* flies, and co-staining with the anti-Repo antibody (green) confirms that cell death is taking place in glia. **(b')** The yellow box in b is shown in higher magnification. Scale bars are indicated in each box.