Mitochondrial fission regulates germ cell differentiation by suppressing ROS-mediated activation of Epidermal Growth Factor Signaling in the *Drosophila* larval testis

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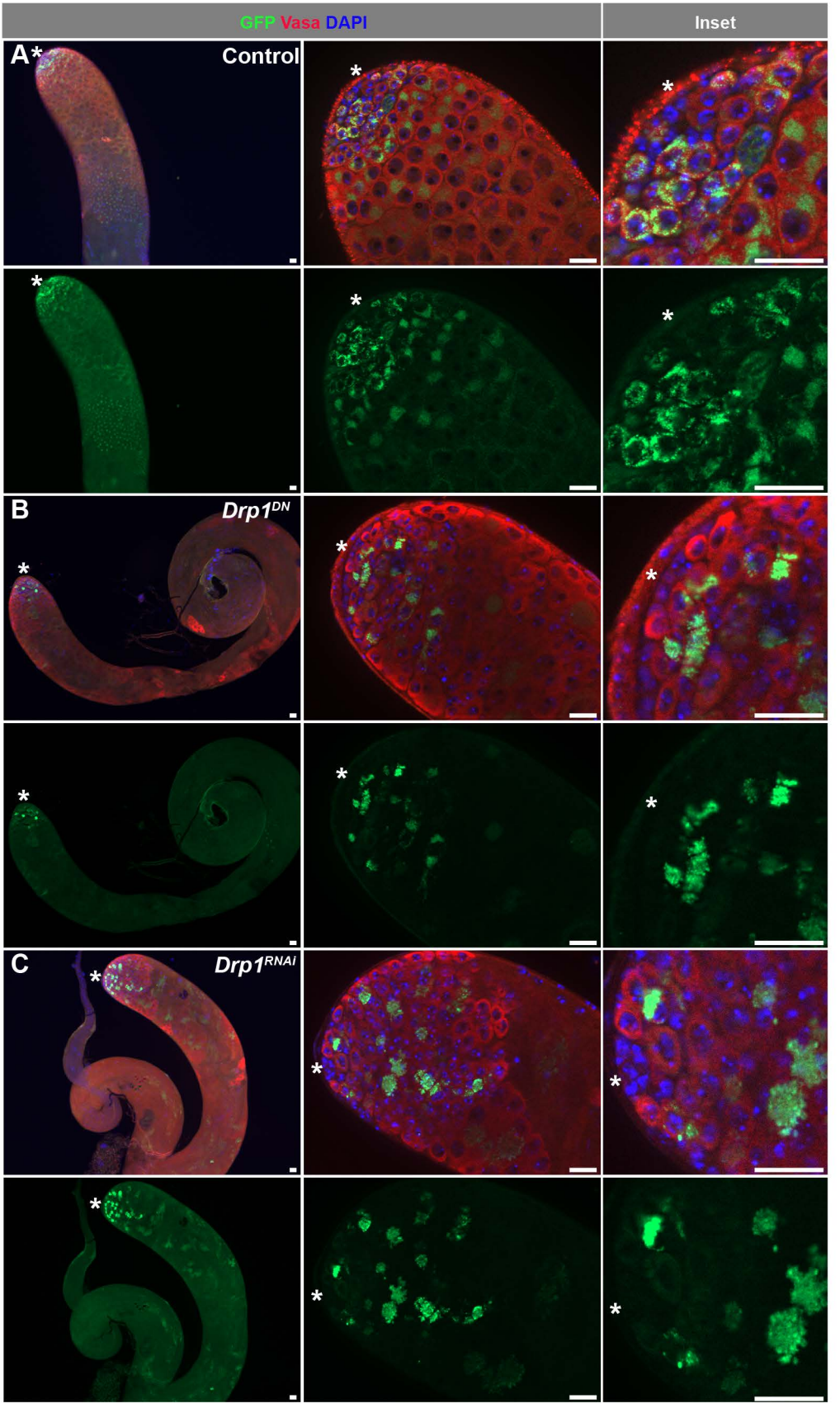
Supplemental Figure Legends

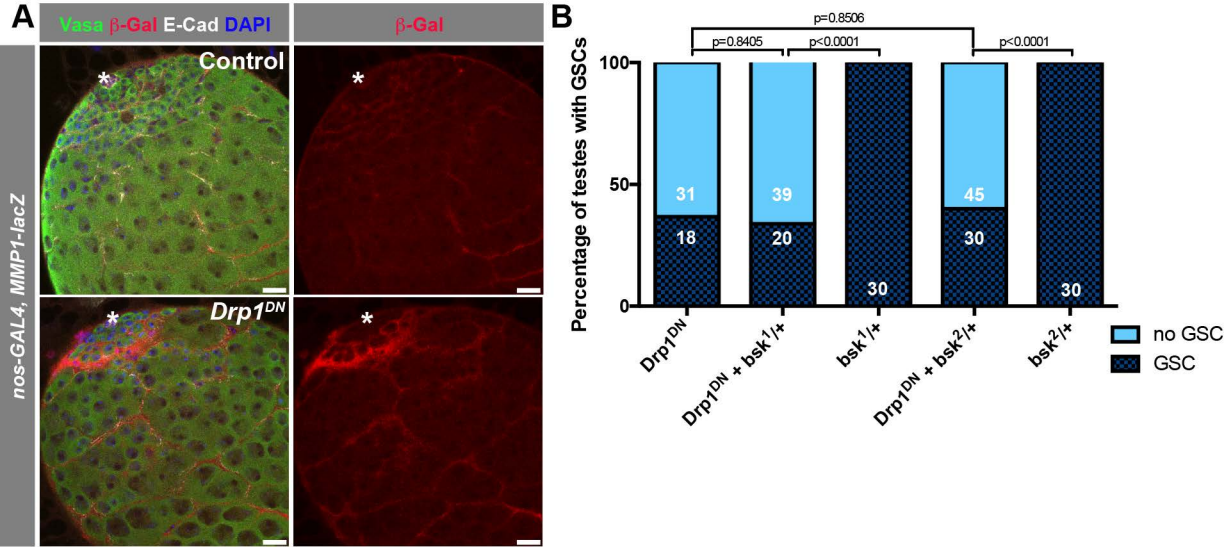
Figure S1. Depletion or inhibition of Drp1 results in hyperfused mitochondria

A-C. Representative images (>20 samples acquired from 3 biological replicates) of testes from 1do animals expressing GFP^{mito} in GSCs and early spermatogonia under the driver *nanosGAL4:VP16*. Mitochondrial fission was inhibited by expression of $Drp1^{DN}$ (B) or $Drp1^{RNAi}$ (C). Full genotypes: (A) w^{1118}*/Y; UAS-*GFP^{mito}*/+; nosGAL4:VP16/+* (B) w^{1118}*/Y; UAS-*GFP^{mito}*/+; nosGAL4:VP16/UAS-*$Drp1^{DN}$ (C) w^{1118}*/Y; UAS-*GFP^{mito}*/+; nosGAL4:VP16/UAS-*$Drp1^{RNAi}$. Asterisk (*) represents the hub; scale bar, 20μm.

Figure S2. JNK activation did not contribute to the GSC loss caused by inhibition of Drp1.

A. Images of testes from animals harboring the *MMP1-lacZ* transgene, a readout for the activation of the JNK pathway. Note the somatic activation of MMP1 in *nanosGAL4:VP16>*$Drp1^{DN}$ animals. **B.** Representation of the percentage of testes with at least one GSC present at the niche/no GSC present in 1do animals. Number of testes in analyzed each category displayed on the graph in white. Two-sided fisher's exact used. In B, asterisk (*) represents the hub; scale bar, 20μm. Individual images representative of >20 samples acquired from 3 biological replicates.


GFP Vasa DAPI
Inset
A
Control
B
Drp1DN
C
Drp1RNAi
nos-GAL4, UAS-mitoGFP


A
Vasa β-Gal E-Cad DAPI
β-Gal
Control
Drp1DN
nos-GAL4, MMP1-lacZ
B
p=0.8506
p=0.8405
p<0.0001
p<0.0001
Percentage of testes with GSCs
100
50
0
31
18
39
20
30
45
30
30
Drp1DN
Drp1DN + bsk1/+
bsk1/+
Drp1DN + bsk2/+
bsk2/+
no GSC
GSC