Supplementary table.

Table S1. Oligonucleotides used as primers for generating dsRNAs.

Gene	Forward primer (5'→3')	Reverse primer (5'→3')
GFP	aattaaccctcactaaagggatggtgagcaagggcgagga	aattaaccctcactaaagggcttgtacagctcgtccatgc
SPS1/SelD	aattaaccctcactaaagggatgagctacgccgctgatg	aattaaccctcactaaagggagttcatcgcccggtggta
Dare	aattaaccctcactaaaggggctgtggatgtggctaggat	aattaaccctcactaaagggcagaactggtgtccagagca
CG15093	aattaaccctcactaaaggggtcaaggccgtcctagagtg	aattaaccctcactaaagggcgctgaacttctccttcttca
Debcl	aattaaccctcactaaagggtacgcgcacgatgact	aattaaccctcactaaagggcatatatgcgcctgcagaga
Pepck	aattaaccctcactaaagggtatcgatgaagcctgggaag	aattaaccctcactaaagggtgtctacgcgggaagatagc
Adk3	aattaaccctcactaaagggaaaatctttcgcgctgtgat	aattaaccctcactaaagggcgttgaggaaaagctccatc
CG31075	aattaaccctcactaaagggctcctctgactgcccttcac	aattaaccctcactaaagggaagatctcctcctgggcaat
GS1	aattaaccctcactaaagggttgtcctgtgcgacacctac	aattaaccctcactaaagggccggtcttcgaggtatcctt
l(2)01810	aattaaccctcactaaagggaacggatgacgattctgagg	aattaaccctcactaaagggatgtagaagccccaatcgtg
CG16888	aattaaccctcactaaagggcgaatcctcgccaagaagta	aattaaccctcactaaagggagcttcttcagtcgctgctc
CG3085	aattaaccctcactaaaggggggagaagctcaatcgtctgg	aattaaccctcactaaagggttcagtgcatccgtcttgtc
CG8745	aattaaccctcactaaagggctgagcaaaacggagaccat	aattaaccctcactaaagggagcgtaccgcatcgtagact
CG31337	aattaaccctcactaaagggtttgtgctgcgaactcaaag	aattaaccctcactaaagggcacaagaaccaactcgagca
CG32625	aattaaccctcactaaaggggacgaattagctcgctgtcc	aattaaccctcactaaagggttcggatcatatgtcgaaacc
Alp23b	aattaaccctcactaaagggtacgctcgcactagcaagaa	aattaaccctcactaaaggggcttcaggatccagttgctc
Tsp42Ed	aattaaccctcactaaagggttatcaccttcggctccatc	aattaaccctcactaaagggtggtctggacgatcttctcc

Table S2. Oligonucleotides used as primers for RT-PCR or in generating probes for northern or Southern analyses

Gene	Forward primer (5'→3')	Reverse primer (5'→3')
SPS1/SelD	tactaggccacgctcaaa	gccggttacaactgaatg
SPS2	ttggtttactgggtcacg	gcgataaccaaactcgat
Cytb	ggacgaggaatttattacggttc	gttgaatatgggcaggtgttac
ND5	tgtgaataatagccccagcaca	tagggtgagatggtttaggact
COI	gctggaattgctcatggtgga	tccaacggtaaatatatgatgagc
RP49	cagtcggatcgatatgcta	aatctccttgcgcttctt

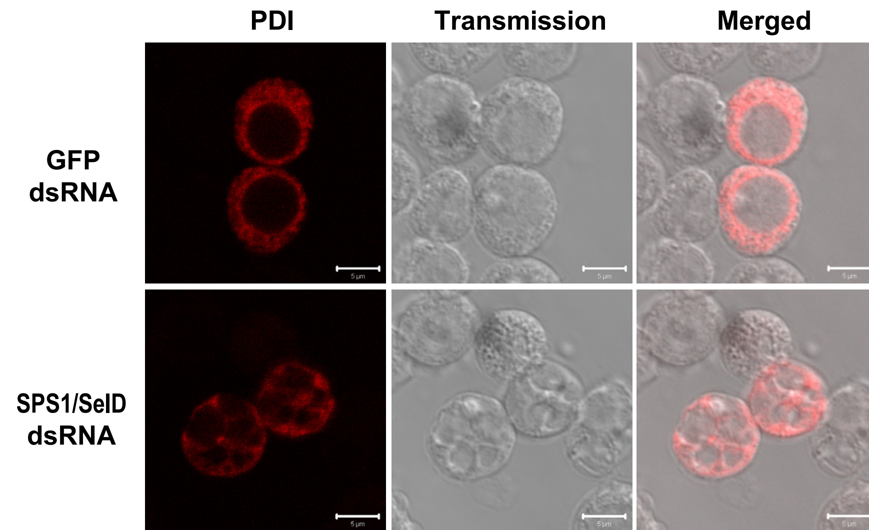
Table S3. Oligonucleotides used as primers for constructing vectors

Gene	Forward primer (5'→3')	Reverse primer (5'→3')
GS1	aagcttgctagccaccatggcactacgcgtggcagg	aagcttggatcctcgttgagcaggcaggtgc
L(2)01810	aagcttgctagccaccatgacgcaacagccacaatg	aagcttggatccgagctctgctccagaccac
HA	gatccctacccatacgatgttccagattacgcttaagc	ggccgcttaagcgtaatctggaacatcgtatgggtagg

Supplementary Figures

Fig. S1

A



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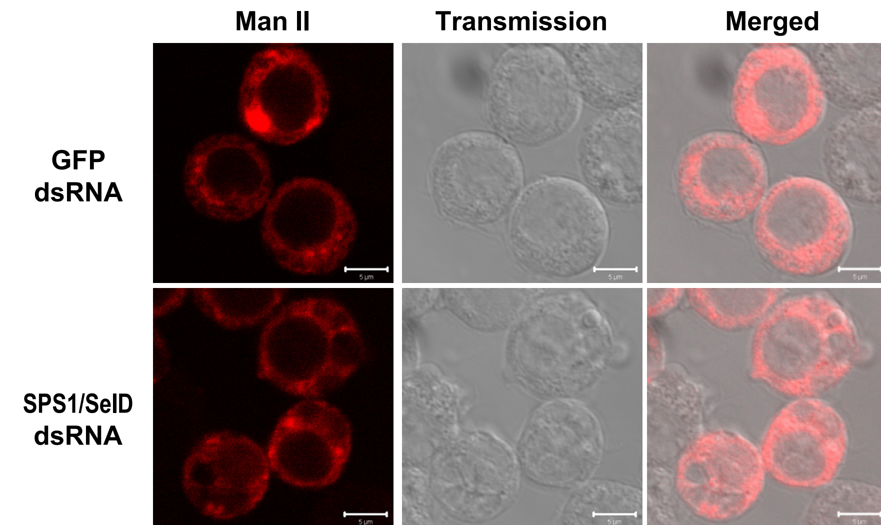

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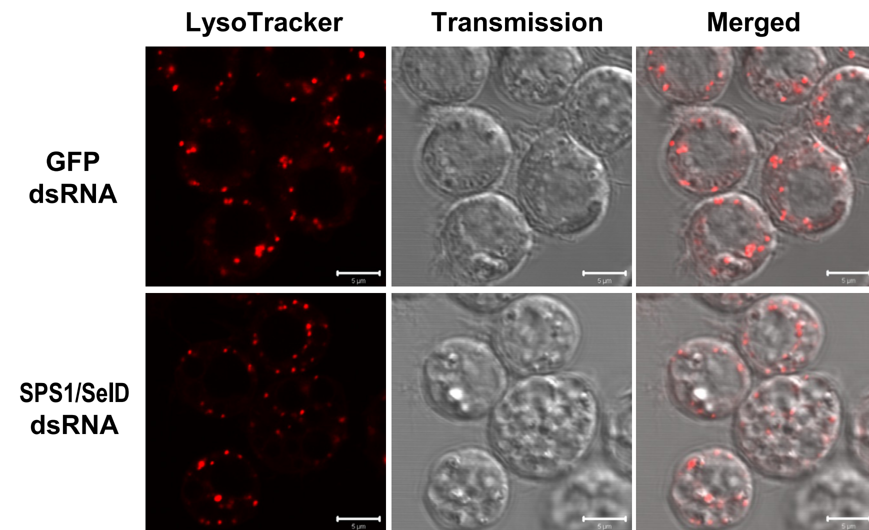

Fig. S2

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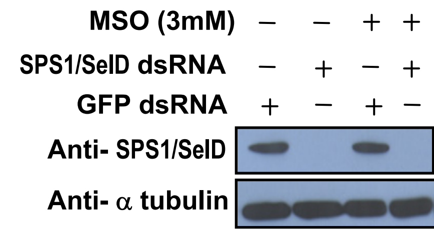

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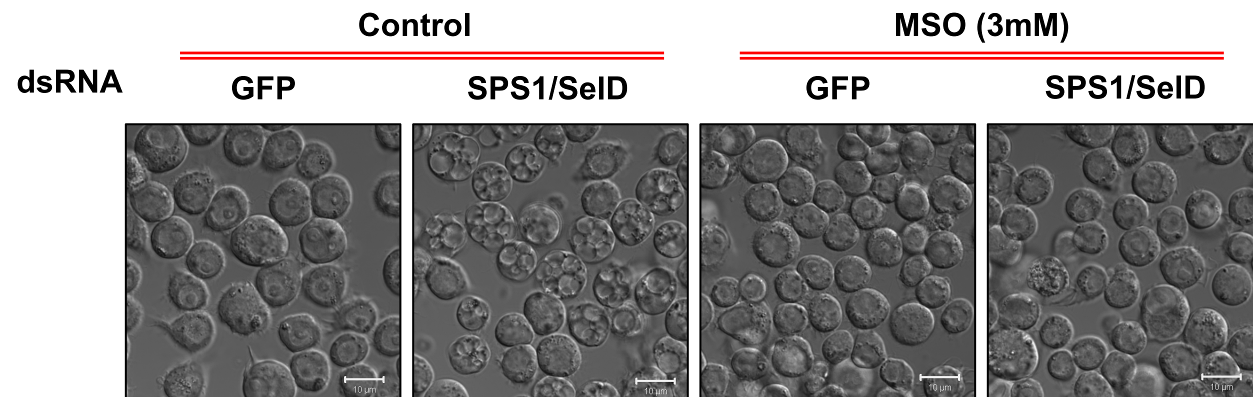

Fig. S3

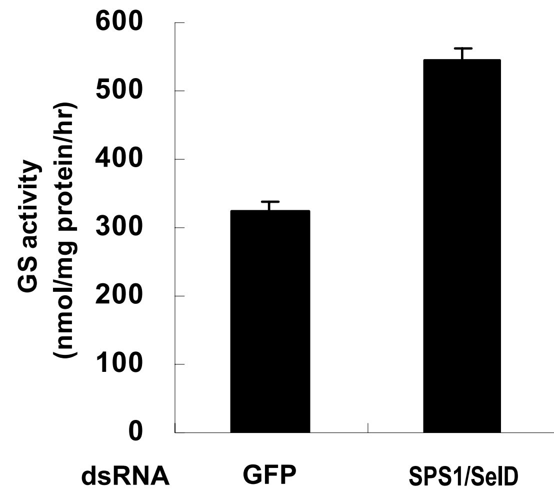

Fig. S4

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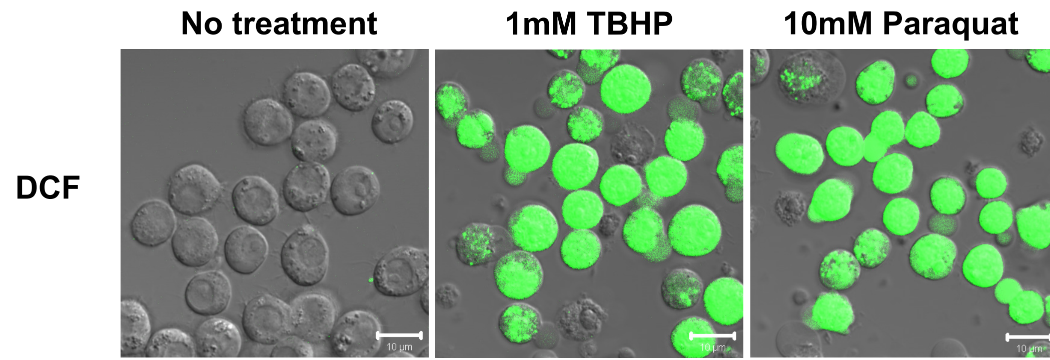

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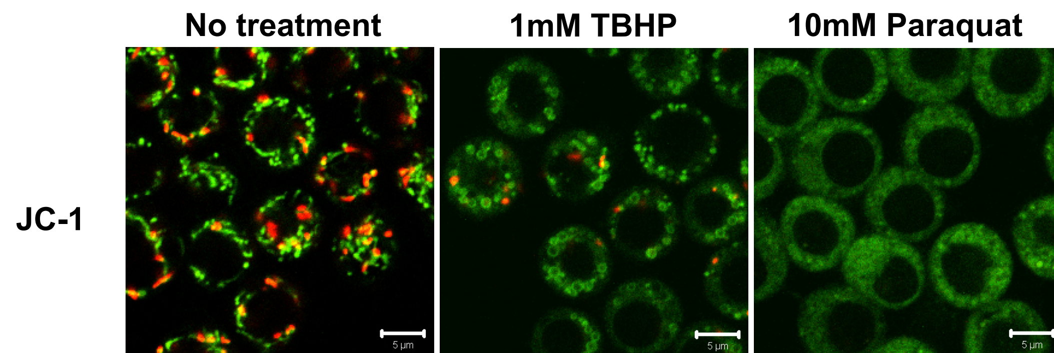

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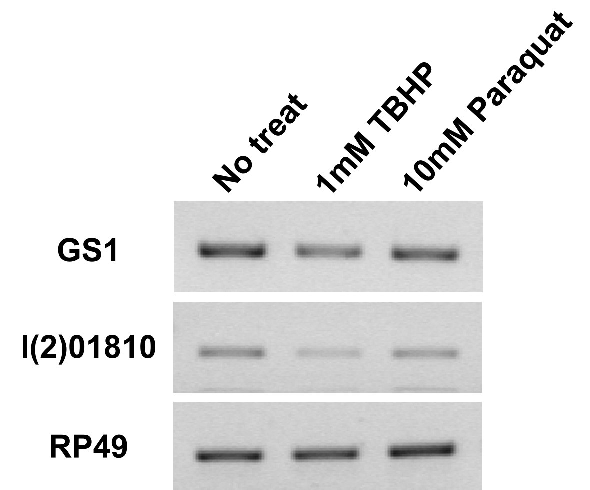

Fig. S5

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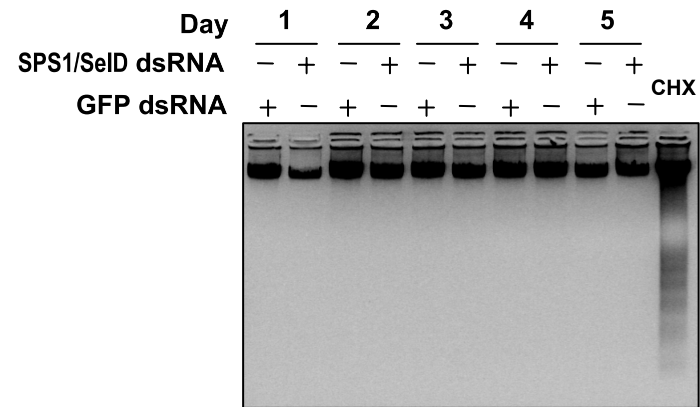

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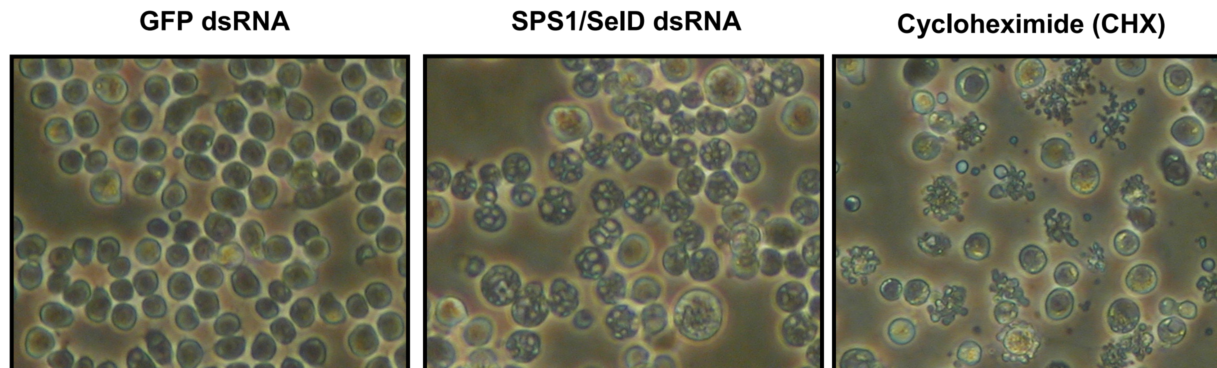
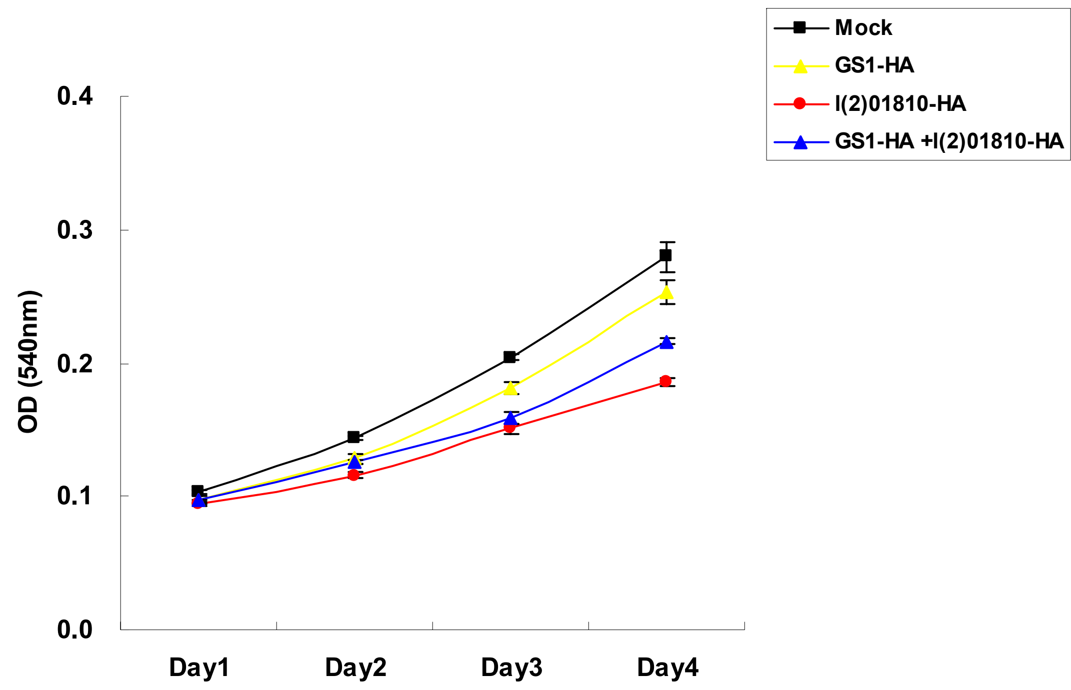


Fig. S6



Supplementary figure legends

Fig. S1. Staining of endoplasmic reticulum (ER), Golgi and lysosome. To stain ER (A panel) and Golgi (B panel), *Drosophila* SL2 cells were transfected with pAcPDIDsRed, which encodes PDI-red fluorescence protein (RFP) and pAc(ΔC)ManDsRed which encodes mannosidase-RFP fusion proteins, respectively, dsRNA of SPS1/SelD was then added into culture media and the cells were observed with a confocal microscope four days after dsRNA transfection as described in Experimental Procedures. (C) Four days after dsRNA transfection, LysoTracker Red was added to the culture medium to stain lysosome. After staining, images were observed with a confocal microscope. Scale bars represent 5 μm in all images. As shown in all panels, vacuole-like globular structures (VLGSs) did not overlap with ER, Golgi or lysome.

Fig. S2. Inhibition of megamitochondria formation by methionine sulfoximine (MSO). After *Drosophila* SL2 cells were transfected with either SPS1/SelD dsRNA or GFP dsRNA, the cells were then incubated in the presence or absence of 3 mM MSO for four days and either an extract prepared for assessing SPS1/SelD levels by Western blotting (A) or analyzed for megamitochondria formation (B) as described in Experimental Procedures. In (A), the effect of MSO on SPS1/SelD levels was determined in extracts of transfected cells using anti-SPS1/SelD antibody as indicated. α-tubulin was used as a loading control. In (B), the effect of MSO on megamitochondria formation was determined by observing cells under a confocal microscope. Scale bars represent 10 μm.

Fig. S3. Glutamine synthetase activity in SPS1/SelD knockdown cells. Following transfection with GFP or SPS1/SelD dsRNA, 6×10^7 cells were harvested and mitochondria isolated as described (supplementary reference 1), the mitochondria lysed with 0.5% Triton-X100, protein measured in the lysed extract by the method of Bradford (supplementary reference 2) and glutamine synthetase (GS) activity measured by the radioisotopic method of Prusiner and Milner (supplementary reference 3). Labeled glutamine was measured with a Tri-Carb 2900TR liquid scintillation analyzer (Perkin Elmer). GS activity is expressed in nanomoles of glutamine formed per mg of protein per hour. Experiments were performed in triplicate and error bars denote the standard deviation from the mean of three independent experiments.

Fig. S4. Effect of ROS generation on megamitochondria formation and GS1 and l(2)01810 expression. (A) To induce ROS, cultured normal were treated with 1 mM tert-butyl hydroperoxide (TBHP) or 10 mM paraquat for 12 hrs. The cells were stained with 10 μM H_2DCFDA (A) or with 1 μg/ml JC-1 (B) and observed under a confocal microscope. Scale bars represent 5 μm. (C) Measurement of mRNA levels GS1 and l(2)01810. Total RNAs were isolated from each group of cells and cDNAs generated from 3 μg of total RNA, and RT-PCR was performed using 150 ng of cDNA as template. After 27 cycles of amplification, DNAs were separated on 2% agrose gel. rp49 was used as an internal control.

Fig. S5. DNA fragmentation and cell blebbing in SPS1/SelD knockdown cells. (A) DNA fragmentation assay. dsRNAs of GFP or SPS1/SelD were added into the culture media, the cells harvested each day until day 5 after dsRNA addition, total DNAs prepared and analyzed on an agarose gel as described in Experimental Procedures. To provide a positive control, cells were treated with 20 μg/ml of cycloheximide (CHX) for 24 hrs. (B) Examination of cell blebbing after SPS1/SelD knockdown. Seven days after SPS1/SelD knockdown, cell morphology was examined under an inverted microscope at ×200 of magnification. No blebbing was observed in the SPS1/SelD knockdown cells.

Fig. S6. Effect of GS1 and l(2)01810 over-expression on cell growth. Growth rates of *Drosophila* SL2 cells in which GS1 and 1(2)01810 were over-expressed were determined by MTT assay as described in Experimental Procedures. The cells were transfected with plasmids encoding GFP (Mock), GS1-HA (yellow triangle), l(20)1810 (red circle) or co-transfected with

plasmids encoding GS1 and l(2)01810 (blue triangle). Cells were measured for MTT contents each day until day 5 as described in Experimental Procedures. Experiments were performed in triplicate and error bars denote the standard deviation from the mean of three independent experiments.

Supplementary references

1. Teranish, M., Karbowski, M., Kurono, C., Nishizawa, Y., Usukura, J., Soji, T., and Wakabayashi, T. (1999) *Arch. Biochem. Biophys*. **366**, 157-167
2. Bradford, M. M. (1976) *Anal. Biochem*. **72**, 248-254
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