

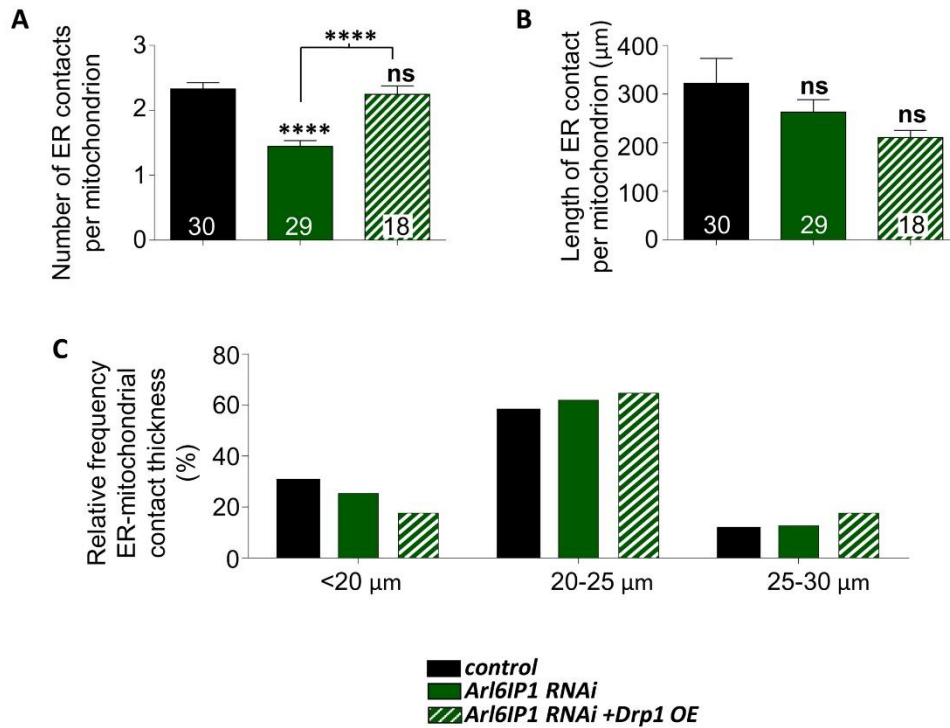


Figure S1. Analysis of ER-mitochondrial contacts in *Arl6IP1* knockdown *Drosophila*. Further analysis of electro micrographs of larvae as described in Figure 2. Graphs show quantification of number of ER contacts per mitochondrion (A), total length of ER-mitochondrial contacts per mitochondrion (B) and frequency distribution of the thickness of ER-mitochondrial contacts (E) in the three genotypes studied. Data are expressed as means $\pm$ SEM ($n = 18-30$ cells from three independent experiments) and values significantly different from control were determined by one-way ANOVA and Tukey's multiple comparisons test (****, $P < 0.0001$; ns, $P > 0.05$).

Table S1. Statistical comparison of climbing assays of male flies generated by crossing *nSyb-GAL4* flies to *w¹¹¹⁸* (control), *UAS-Arl6IP1 RNAi* (*Arl6IP1 RNAi*), *UAS-Arl6IP1 RNAi.UAS-Drp1^{WT}* (*Arl6IP1 RNAi + Drp1^{WT}*) or *UAS-Arl6IP1 RNAi.UAS-Drp1^{K38A}* (*Arl6IP1 RNAi + Drp1^{K38A}*).

Tukey's multiple comparisons test	P value summary
Day 3	
control vs. <i>Arl6IP1 RNAi</i> (GD)	ns
control vs. <i>Arl6IP1 RNAi</i> (GD) + <i>Drp1^{WT}</i>	ns
control vs. <i>Arl6IP1 RNAi</i> (GD) + <i>Drp1^{K38A}</i>	*
<i>Arl6IP1 RNAi</i> (GD) vs. <i>Arl6IP1 RNAi</i> (GD) + <i>Drp1^{WT}</i>	ns
<i>Arl6IP1 RNAi</i> (GD) vs. <i>Arl6IP1 RNAi</i> (GD) + <i>Drp1^{K38A}</i>	ns
<i>Arl6IP1 RNAi</i> (GD) + <i>Drp1^{WT}</i> vs <i>Arl6IP1 RNAi</i> (GD) + <i>Drp1^{K38A}</i>	ns
Day 9	
control vs. <i>Arl6IP1 RNAi</i> (GD)	ns
control vs. <i>Arl6IP1 RNAi</i> (GD) + <i>Drp1^{WT}</i>	ns
control vs. <i>Arl6IP1 RNAi</i> (GD) + <i>Drp1^{K38A}</i>	****
<i>Arl6IP1 RNAi</i> (GD) vs. <i>Arl6IP1 RNAi</i> (GD) + <i>Drp1^{WT}</i>	ns
<i>Arl6IP1 RNAi</i> (GD) vs. <i>Arl6IP1 RNAi</i> (GD) + <i>Drp1^{K38A}</i>	****
<i>Arl6IP1 RNAi</i> (GD) + <i>Drp1^{WT}</i> vs <i>Arl6IP1 RNAi</i> (GD) + <i>Drp1^{K38A}</i>	****

Day 18	
control vs. <i>Arl6IP1</i> RNAi (GD)	***
control vs. <i>Arl6IP1</i> RNAi (GD) + Drp1 ^{WT}	ns
control vs. <i>Arl6IP1</i> RNAi (GD) + Drp1 ^{K38A}	****
<i>Arl6IP1</i> RNAi (GD) vs. <i>Arl6IP1</i> RNAi (GD) + Drp1 ^{WT}	*
<i>Arl6IP1</i> RNAi (GD) vs. <i>Arl6IP1</i> RNAi (GD) + Drp1 ^{K38A}	**
<i>Arl6IP1</i> RNAi (GD) + Drp1 ^{WT} vs <i>Arl6IP1</i> RNAi (GD) + Drp1 ^{K38A}	****
Day 24	
control vs. <i>Arl6IP1</i> RNAi (GD)	****
control vs. <i>Arl6IP1</i> RNAi (GD) + Drp1 ^{WT}	ns
control vs. <i>Arl6IP1</i> RNAi (GD) + Drp1 ^{K38A}	****
<i>Arl6IP1</i> RNAi (GD) vs. <i>Arl6IP1</i> RNAi (GD) + Drp1 ^{WT}	***
<i>Arl6IP1</i> RNAi (GD) vs. <i>Arl6IP1</i> RNAi (GD) + Drp1 ^{K38A}	****
<i>Arl6IP1</i> RNAi (GD) + Drp1 ^{WT} vs <i>Arl6IP1</i> RNAi (GD) + Drp1 ^{K38A}	****
Day 30	
control vs. <i>Arl6IP1</i> RNAi (GD)	****
control vs. <i>Arl6IP1</i> RNAi (GD) + Drp1 ^{WT}	*
control vs. <i>Arl6IP1</i> RNAi (GD) + Drp1 ^{K38A}	****
<i>Arl6IP1</i> RNAi (GD) vs. <i>Arl6IP1</i> RNAi (GD) + Drp1 ^{WT}	ns
<i>Arl6IP1</i> RNAi (GD) vs. <i>Arl6IP1</i> RNAi (GD) + Drp1 ^{K38A}	****
<i>Arl6IP1</i> RNAi (GD) + Drp1 ^{WT} vs <i>Arl6IP1</i> RNAi (GD) + Drp1 ^{K38A}	****
Day 36	
control vs. <i>Arl6IP1</i> RNAi (GD)	****
control vs. <i>Arl6IP1</i> RNAi (GD) + Drp1 ^{WT}	**
control vs. <i>Arl6IP1</i> RNAi (GD) + Drp1 ^{K38A}	****
<i>Arl6IP1</i> RNAi (GD) vs. <i>Arl6IP1</i> RNAi (GD) + Drp1 ^{WT}	ns
<i>Arl6IP1</i> RNAi (GD) vs. <i>Arl6IP1</i> RNAi (GD) + Drp1 ^{K38A}	****
<i>Arl6IP1</i> RNAi (GD) + Drp1 ^{WT} vs <i>Arl6IP1</i> RNAi (GD) + Drp1 ^{K38A}	****

Table S2. Statistical comparison of climbing assays of male flies generated by crossing *nSyb-GAL4* flies to *w¹¹¹⁸* (control), UAS-*Arl6IP1* RNAi (*Arl6IP1* RNAi), UAS-*Arl6IP1* RNAi.UAS-*Drp1^{WT}* (*Arl6IP1* RNAi + *Drp1^{WT}*) or UAS-*Arl6IP1* RNAi.UAS-*Drp1^{K38A}* (*Arl6IP1* RNAi + *Drp1^{K38A}*).

Tukey's multiple comparisons test	P value summary
Day 3	
control vs. <i>Arl6IP1</i> RNAi (KK)	ns
control vs. <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{WT}	ns
control vs. <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{K38A}	ns
<i>Arl6IP1</i> RNAi (KK) vs. <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{WT}	ns
<i>Arl6IP1</i> RNAi (KK) vs. <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{K38A}	ns
<i>Arl6IP1</i> RNAi (KK) + Drp1 ^{WT} vs <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{K38A}	ns
Day 9	
control vs. <i>Arl6IP1</i> RNAi (KK)	ns
control vs. <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{WT}	ns
control vs. <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{K38A}	*

<i>Arl6IP1</i> RNAi (KK) vs. <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{WT}	ns
<i>Arl6IP1</i> RNAi (KK) vs. <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{K38A}	ns
<i>Arl6IP1</i> RNAi (KK) + Drp1 ^{WT} vs <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{K38A}	*
Day 18	
control vs. <i>Arl6IP1</i> RNAi (KK)	*
control vs. <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{WT}	ns
control vs. <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{K38A}	****
<i>Arl6IP1</i> RNAi (KK) vs. <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{WT}	*
<i>Arl6IP1</i> RNAi (KK) vs. <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{K38A}	ns
<i>Arl6IP1</i> RNAi (KK) + Drp1 ^{WT} vs <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{K38A}	****
Day 24	
control vs. <i>Arl6IP1</i> RNAi (KK)	**
control vs. <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{WT}	ns
control vs. <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{K38A}	****
<i>Arl6IP1</i> RNAi (KK) vs. <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{WT}	*
<i>Arl6IP1</i> RNAi (KK) vs. <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{K38A}	ns
<i>Arl6IP1</i> RNAi (KK) + Drp1 ^{WT} vs <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{K38A}	****
Day 30	
control vs. <i>Arl6IP1</i> RNAi (KK)	*
control vs. <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{WT}	ns
control vs. <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{K38A}	****
<i>Arl6IP1</i> RNAi (KK) vs. <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{WT}	ns
<i>Arl6IP1</i> RNAi (KK) vs. <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{K38A}	*
<i>Arl6IP1</i> RNAi (KK) + Drp1 ^{WT} vs <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{K38A}	****
Day 36	
control vs. <i>Arl6IP1</i> RNAi (KK)	ns
control vs. <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{WT}	ns
control vs. <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{K38A}	****
<i>Arl6IP1</i> RNAi (KK) vs. <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{WT}	ns
<i>Arl6IP1</i> RNAi (KK) vs. <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{K38A}	****
<i>Arl6IP1</i> RNAi (KK) + Drp1 ^{WT} vs <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{K38A}	****

Table S3. *n* numbers and median lifespan for survival assays of male flies generated as in Tables S1 and S2.

Genotype	<i>n</i> number	median life span
control (GD)	61	54
<i>Arl6IP1</i> RNAi (GD)	59	56
<i>Arl6IP1</i> RNAi (GD) + Drp1 ^{WT}	63	56
<i>Arl6IP1</i> RNAi (GD) + Drp1 ^{K38A}	57	54
control (KK)	76	49
<i>Arl6IP1</i> RNAi (KK)	74	65
<i>Arl6IP1</i> RNAi (KK) + Drp1 ^{WT}	74	56
<i>Arl6IP1</i> RNAi (KK) + Drp1 ^{K38A}	68	55.5

Table S4. Statistical comparison of survival assays of male flies generated as in Tables S1 and S2.

Log-Rank (Mantel Cox) test	<i>P</i> value	<i>P</i> value summary
control (GD) vs <i>Arl6IP1</i> RNAi (GD)	0.5556	ns
control (GD) vs <i>Arl6IP1</i> RNAi (GD) + Drp1 ^{WT}	0.4496	ns
control (GD) vs <i>Arl6IP1</i> RNAi (GD) + Drp1 ^{K38A}	0.6898	ns
control (KK) vs <i>Arl6IP1</i> RNAi (KK)	<0.0001	****
control (KK) vs <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{WT}	0.266	ns
control (KK) vs <i>Arl6IP1</i> RNAi (KK) + Drp1 ^{K38A}	0.0096	**