		0 :	20 30 20 30) 40 1 2) 51) 51) 60) 60	70 60	80 80	90 90	100 1970
1. NP_003162	MSFSRALLWA	RLPAG ROAG	HRAAICSALRP	HFGPF P GVLG	QVSVLATAS	SASGGSKIPN	TSLFVPL	T V KPQGP S ADO		KNEVKKVL
2. NP_649452		MQNCR	RCI SL TGL <mark>LR</mark> M	TLYLRPSFSI	DLSLRRLHRA	AFLFSRKKPE	TNLSTLFKPV	Q <mark>V</mark> HAYVD <mark>S</mark> E	DVGSELSGKL	KAELLKIL
	11 10	0 1 Ø 1	20 13 20 12	0 140 Ø 130) 15 7 15	0 160 Ø 160) 170 9 160) 180 9 18 0	190 190	200 290
1. NP_003162	DKFYKRKEI	KLGADY <mark>GLD</mark>	ARLFHQAFISF	RNYIMQSHSL 4 124	DVDIHIVLNI 13	ICF <mark>GA</mark> AHADD	FPFFLRHAK	QIFPVLDCKDI 164	LRKISDLRIPP	NWYPDARA
2. NP_649452	NKFTQRREI	S L C N E N GL D	DYLQQQAFGSF	RRFCIEAENL	PVDLHITFSI	ITQGAGHIDD	FPYFLRHAK	TVFPHLDCMDI	DLKKISDLROPA	NWYSNARA
	21	.0 2 10 2	20 23 20 22	0 240 Ø 230	0 25 Ø 25	0 260 Ø 260	270 270 260	280 280	290 290	300 290
1. NP_003162	MORKIIFHSO 19	PTNSGKTYH 4 2	AIQKYFSAKSC	VYCGPLKLLA	HEIFEKSNAA 4 23	GVPCDLVTGE	ERVTVQPNGK	OASHVSCTVEN	ICSVITPYEVAV 274	IDEIQMIR 284
2. NP_649452	ITRKIVFHAC	GP TN SG KTY H	AMERYLSAKT	VYCGPLKLLA	TEVYNKANEF	GTPCDLVTGE	ERKFGISESL	PANHVACTVEN	ITSVNTPYEVAV	IDEIQ <mark>QIR</mark>
	31 30	.0 3 NØ 3	20 33 20 32	0 340 Ø 330	0 35 Ø 34	0 360 Ø 360) 370 380) 380 380	390 390	400 390
1. NP_003162	DPARGWAWT	ALLGLCAPE	VHLCGEPAAID	LVMELMY TTG	EEVEVRDYK 4 33	LTPISVLDHA	LESLDNLRPG	DCIVCFSKNDI	YSVSROIEIRG	LESAVIYG
2. NP_649452	DPQRGWAWTF	RAFLGLIADE	VHVCGEPGALD	LLQKICETTG	EIVEVRLYDF	LTELTVENTA	LGSLDNIVPG	DCIVCFSKIDI	YUVSREIEARG	KEVAVIYG
	4] 40	.0 4 00 4	20 43 20 42	0 440 Ø 430	0 45 Ø 45	0 460 Ø 460) 470 7 460	0 480 0 480	490 49 0	500 9 0 0
1. NP_003162	SLPPGTKLAC	AKKFNDPND	PCKILVATDAI	GMGLNLSIRR	IIFYSLIKPS	INEKGERELE	PITTSQALQI	AGRAGRESSEI	KEGEVTTMNHE 474	DLSLLKET 484
2. NP_649452	GLPPGTKLAC	AAKFNDPAN	SCKVMVATDAI	GMGLNLSIRR	IIFYSLIKPS	MNERGEREID	TISVSSALQI	AGRAGRFRIQ	E H G Y VT AFKSE	DLQTLQRI
	51 50	0 5 Ø 5	20 53 20 52	0 540 Ø 530	0 55 7 54	0 560 0 560) 570 9 506) 580) 586	590 590	600 590
1. NP_003162	LKRPVDPIRA 49	AGLHPTADQ	IEMFAYHLPDA 04 51	TLSNLIDIFV 4 524	DFSQVDGQ-X	FVCNMDDFKF 4 542	SAELIQHIPL 554	SLRVRYVFCT 564	PINKKOPFVCS	SLLQFARQ 584
2.NP_649452	LARTPEPIKO	AGLHPTADQ	IELYAYHLPSS	SLSNLMDIFV	NLCTVDDSL	FMCNIEDFKF	LAEMIQHVAL	PLRARYVFCC	PINRKMPFVCS	MFLKVARQ
	61	.0 6)0 6	20 63 20 62	0 640 6 630	0 65 6 64	0 660) 670 9 666	0 680 680	690 690	700 696
1. NP_003162	YSRNEP TFA	WLRRYIKNE 4 6	LLP PK N I K DL 04 61	DLEAVHDVLD 4 624	LYLWLSYRFM 4 63	IDMFPDASLIR 4 644	DLQKELDGII 4 654	ODGVHNITKL 664	KMSETHKLLN 667	EGFPSCSÓ 676
2. NP_649452	Y SRNEP T F	FIKKNCG <mark>NP</mark>	FKL PK T ILDL	HLEAVFDVMD	LYLWLSYRFM	IDEFPEAAY VR	DAQKELDEII	QQGVFQITRLI	K <u>N</u> I	E A SQ D G E T
	71 70	.0 7)6 7	20 73 26 72	0 740 6 730	0 75 6 74	0 760 0 760	5 770 5 766	780 780 780	790 796	800 800
1. NP_003162	SRLSGTLKSÇ	ARRTRGTKA 681	LGSKATEPPS 689 69	DAGELSLASR 3 70	LVQQGLLTP	MLKQLE <u>KEW</u> M 3 723	T QQ TE HNK E K 73	TESGTHPKGTF 743	RRKKEPDSD 753	763
2. NP_649452	SNYA	IRRITHVK-	EPRLE	SLSRGRLTER	LAQGLLTP	MLSELRKEWD	A <mark>QQ</mark> LGK S NSQ	SNENSE <mark>P</mark> VVNS	DD E DNYSGIGR	KTRKKRRK

Clemente et al Figure S1

Figure S1. The mitochondrial helicase SUV3 is conserved between *Drosophila melanogaster* and humans. (A) ClustalW alignment of the human protein SUPV31L (NP_003162, top) and its *Dm* ortholog DmSUV3 (NP_649452, bottom).



Clemente et al. Figure S2

Figure S2. Increased mRNA stability in *dmsuv3* knockdown and P-element insertion larvae. (A) Q-PCR analysis of mtDNA steady-state levels in control (w;;), heterozygous P-element insertion (w;;P{EPgy2}CG9791^{EY12505}/TM6B-GFP) and homozygous P-element insertion (w;;P{EPgy2}CG9791^{EY12505}) larvae at 3 days ael. (B) qRT-PCR of mitochondrial mRNAs in control, heterozygous P-element insertion and homozygous P-element insertion larvae at 3 days ael. RP49 transcript was used as endogenous control. All data are represented as mean +/- SEM. (* p<0.05, ***p<0.001, n=5). (C) Northern blot analysis of the steady-state levels of mitochondrial mRNAs and rRNAs in *dmsuv3* KD (w;UAS-*dmsuv3*-RNAi/+;daGAL4/+) and control (w;UAS-dmsuv3-RNAi/+; and w;;daGAL4/+) larvae at 5 days ael.



Clemente et al Figure S3

Figure S3. tRNA maturation in *dmsuv3* deficient larvae. A. Northern blot analysis of the aminoacylation status of mitochondrial tRNAs in dmsuv3 KD (w;UAS-*dmsuv3*RNAi/+;daGAL4/+) and control (w;UAS-dmsuv3RNAi/+; and w;;daGAL4/+) larvae at 5 days ael. 5S rRNA is shown as a loading control B. Northern blot analysis of mitochondrial tRNA-Val, tRNA-Cys and tRNA-Tyr in control (w;;), heterozygous insertion (w;;P{EPgy2}CG9791^{EY12505}/TM6B-GFP) and homozygous P-element P-element insertion (w;;P{EPgy2}CG9791^{EY12505}) larvae at 3 days ael. 5S rRNA was used as a loading control.



Figure S4. Loss of DmSUV3 leads to altered processing of non-transcript flanked mitochondrial tRNAs. (A) Schematic representation of the end-labeled oligonucleotide probes (black arrows) and Northern blot experiments against tRNA-Gln and its 5' and 3' flanking regions in control (w;UAS-*dmsuv3*-RNAi/+; and w;;daGAL4/+) and *dmsuv3* KD (w;UAS-*dmsuv3*-RNAi/+;daGAL4/+) larvae at 5 days ael. **(B)** Schematic representation of the end-labeled oligonucleotide probes and Northern blot experiments against tRNA-Val (left panel), tRNA-Gly (middle panel) and tRNA-Phe (right panel) in KD and control larvae at 5 days ael. **(C)** Northern blot analysis of 5S rRNA, used as a loading control. **(D)** qRT-PCR of mitochondrial tRNA-Gln, tRNA-Ile and tRNA-Met junctions in KD and control larvae at 5 days ael. RP49 transcript was used as endogenous control. **(E)** qRT-PCR of mitochondrial tRNA-Gly, COX3 and ND3 junctions in KD and control larvae at 5 days ael. RP49 transcript was used as endogenous control. All data are represented as mean \pm SEM. (* p<0.05, ** p<0.01, *** p<0.001, n=5).



Clemente et al Figure S5

Figure S5. Loss of DmSUV3 leads to altered processing of non-transcript flanked mitochondrial tRNAs. (A) the Northern blot experiments in figure Overexposure of shown 6C in dmsuv3 KD (w;UAS-dmsuv3-RNAi/+;daGAL4/+) and control (w;UAS-dmsuv3-RNAi/+; and w;;daGAL4/+) larvae at 5 days ael. (B) qRT-PCR of the mitochondrial anti-tRNA-Cys-COX1 and ND2-COX1 containing transcripts in KD and control larvae at 5 days ael. (C) Schematic representation of the end-labeled oligonucleotide probes (black arrows) and single-stranded RNA probes (dashed arrows) used in Northern blot experiments. (D) Northern blot experiments against tRNA-Phe and ND5 (left panel) and tRNA-Val (right panel) in control and dmsuv3 KD larvae at 5 days ael. (E) qRT-PCR of the mitochondrial tRNA-Phe-ND5 junction in KD and control larvae at 5 days ael. RP49 transcript was used as endogenous control. (F) qRT-PCR of the mitochondrial tRNA-Val-16S junction in KD and control larvae at 5 days ael. RP49 transcript was used as endogenous control. All data are represented as mean ± SEM. (*** p<0.001, n=5).