Calcium independent phospholipase A_2-VIA affects female but not male fertility in *Drosophila melanogaster*, with altered mitochondrial distribution in the developing female germ cells

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Introduction:

- *PLA2G6/PARK14/iPLA$_2$-VIA* encodes a calcium independent phospholipase A_2 that hydrolyzes glycerophospholipids into free fatty acids and lysophospholipids. By doing so, it regulates membrane phospholipid composition and signaling pathways that can affect neuronal function, fertility, inflammation, metabolism, and apoptosis (Review Ramanadham et al., 2015; Abi Nahed et al., 2015; Beck et al., 2011; Turk et al., 2019).
- Human *iPLA$_2$-VIA* mutations are associated with neurodegenerative and locomotor disorders, including inherited dystonia-parkinsonism, in which neuroaxonal dystrophy is correlated with mitochondrial degeneration (Beck et al., 2011; Paisan-Ruiz et al., 2012). However, the underlying mechanisms are not clear.

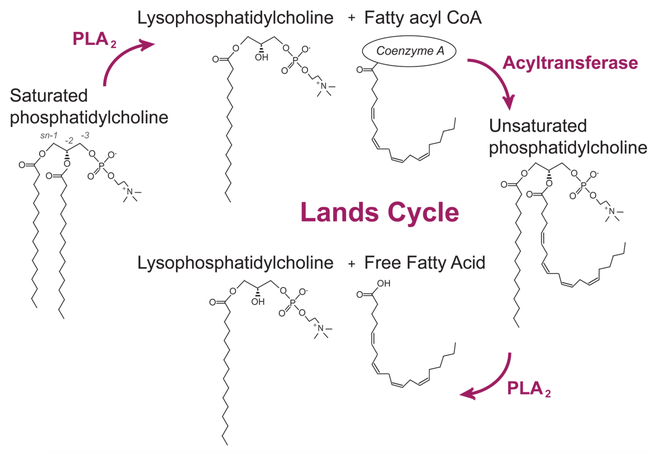


Fig 1. Role of PLA_2 in phospholipid metabolism.

- The human and *D. melanogaster* iPLA$_2$-VIA proteins share high homology, with 8 ankyrin (Ank) repeats, one oxyanion hole (Oxy), a catalytic site (Cat), a catalytic aspartate residue (Cat D), and a 1-9-14 calmodulin binding motif (CaM). Thus, we use the fly model to investigate the cellular and molecular activities of iPLA$_2$-VIA.

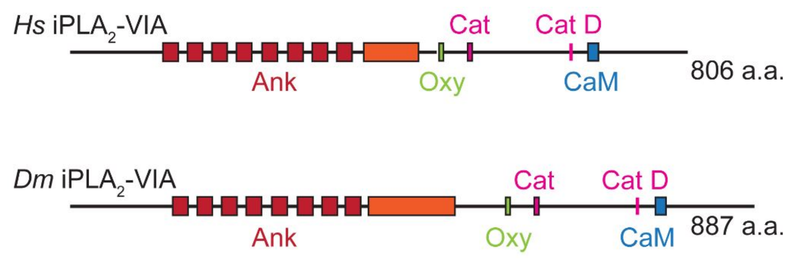


Fig 2. The human (*Hs*) and *D. melanogaster* (*Dm*) iPLA$_2$-VIA proteins share high homology in their conserved domains, motifs and catalytic residues.

Result 1: *iPLA$_2$-VIA* null mutants or knockdown flies show age dependent locomotor defects, which can be rescued.

- We made a null mutant, called *iPLA$_2$-VIA$^{\Delta 23}$*, by excising the P-element EY5103.
- The 1.4 kb deletion (verified by sequencing) includes the transcription start site and predicted translation start codons.
- RT-PCR verified the absence of full length transcript in the homozygous mutant whole fly lysate.
- The homozygous and hemizygous null mutants are viable at room temperature.

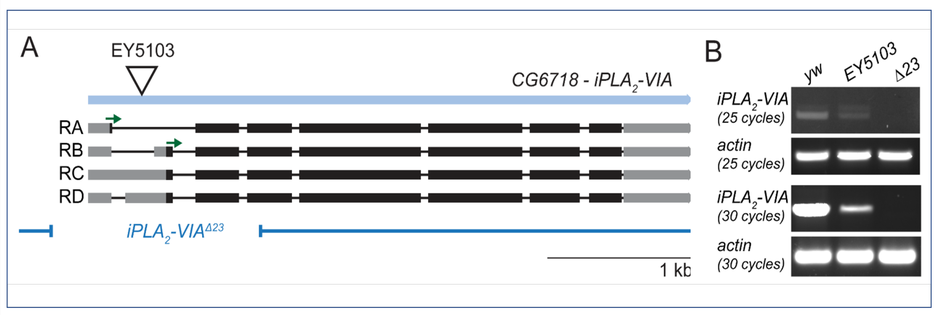


Fig 3. (A) Breakpoints indicated by the dark blue lines show the 1.4 kb deletion in *iPLA$_2$-VIA* gene. (B) Absence of a full length transcript was verified in the mutant (Δ23) whole adult flies by RT-PCR.

- Homozygous mutant *iPLA$_2$-VIA$^{\Delta 23}$* (A) and whole body *(tubulin-GAL4)* knockdown male and female flies (D, E) show reduced climbing ability with age compared to control flies (room temp). Locomotor defects become highly significant in older flies (light bars).
- This locomotor defect is rescued when the full length *iPLA$_2$-VIA* cDNA is overexpressed in all tissues in the null mutant background (B, C, dark bars, 26^{o}C).
- Overexpressing a catalytically inactive ("SA") cDNA also significantly rescues the locomotor defects (B, C, yellow bars).
- These results suggest that iPLA$_2$-VIA prevents age dependent motor decline partially independently of its catalytic activity.
- Knockdown in neurons (*elav-GAL4*) or muscles (*DJ667-GAL4*) phenocopies the defect (F-I), indicating a role in multiple tissues.

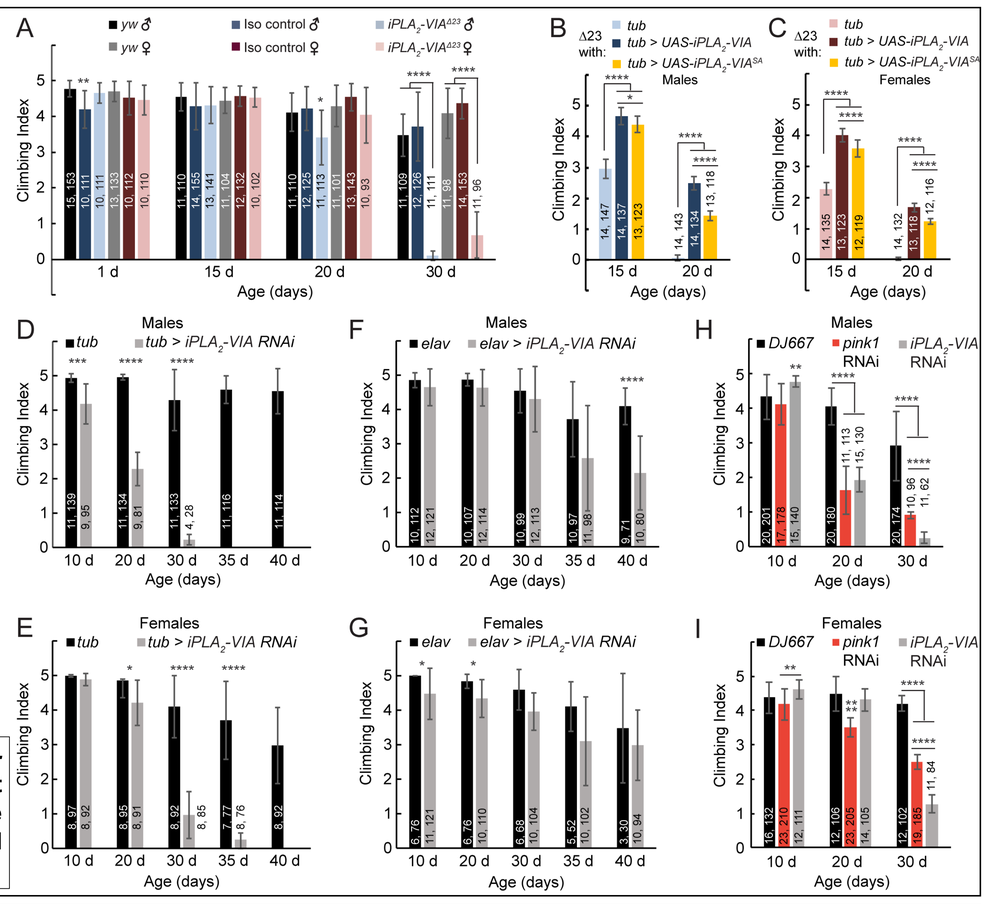
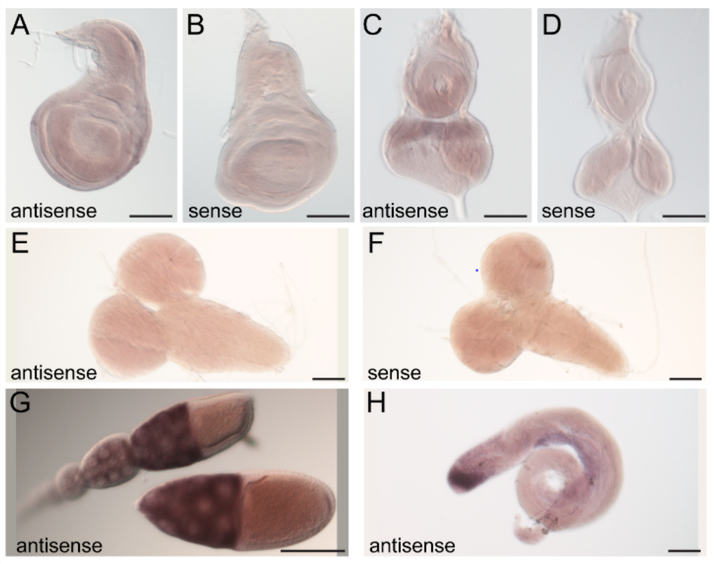


Fig 4. *iPLA$_2$-VIA$^{\Delta 23}$* homozygous flies (A) and *iPLA$_2$-VIA* knockdown flies (D-I) show age dependent reduced climbing ability. Locomotor defects can be partially rescued by wild-type or catalytic dead ("SA") cDNA (B, C).

Result 2: The *iPLA$_2$-VIA* mRNA is highly expressed in the adult germline.



- In situ hybridization to endogenous *iPLA$_2$-VIA* mRNA (purple) in wild-type animals shows weak ubiquitous expression in wing imaginal discs (A) and eye-antennal discs (C). Expression is negligible in larval brains (E). Strong expression is seen in both female (G) and male (H) adult germlines. Sense probes (B, D, F) were used as controls. Scale bars: 100 μm.
- Although it is not likely to contribute significantly during adult tissue development from larval tissues, the results suggest a role in fertility.

Fig 5. *iPLA$_2$-VIA* mRNA expression is weak in imaginal tissues and larval brain but strong in adult germlines.

Result 3: *iPLA$_2$-VIA* mutation reduces female but not male fertility.

- Young (<1 week old) *iPLA$_2$-VIA$^{\Delta 23}$* homozygous (A) or hemizygous (B, *iPLA$_2$-VIA$^{\Delta 23}$/Df(3L)BSC282*) mutant females show significantly reduced fertility compared to isogenic controls.
- Egg laying by *iPLA$_2$-VIA$^{\Delta 23}$* mutants is reduced significantly compared to isogenic controls in young (C, one week old) and aged (D, 18-19 day old) females.
- *iPLA$_2$-VIA$^{\Delta 23}$* males do not show fertility defects (data not shown).

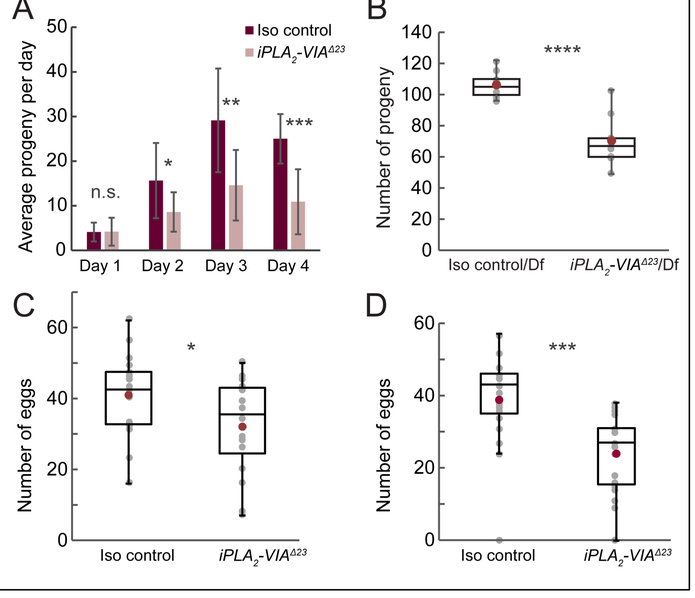
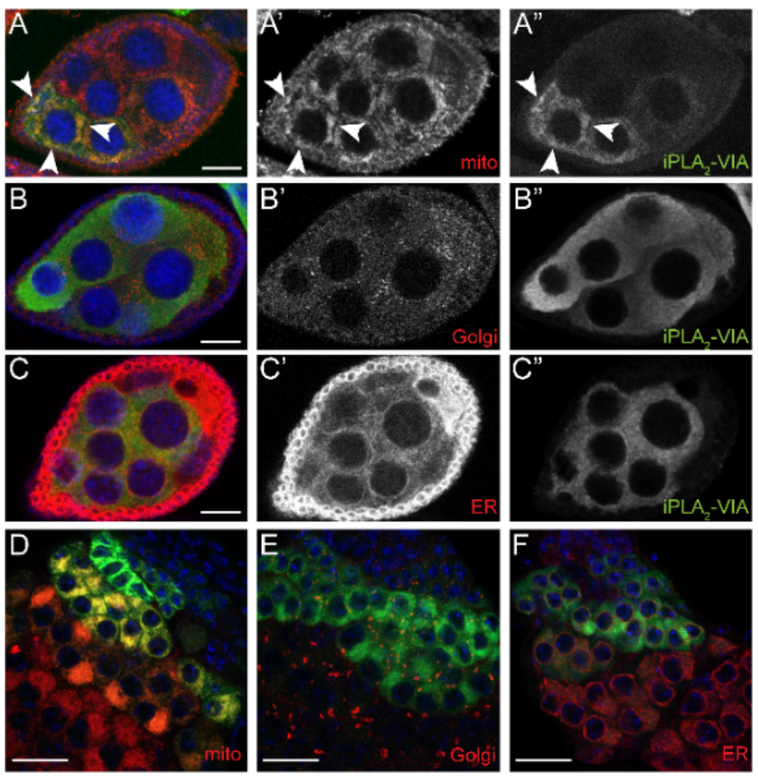


Fig 6. *iPLA$_2$-VIA* mutation reduces female fertility.

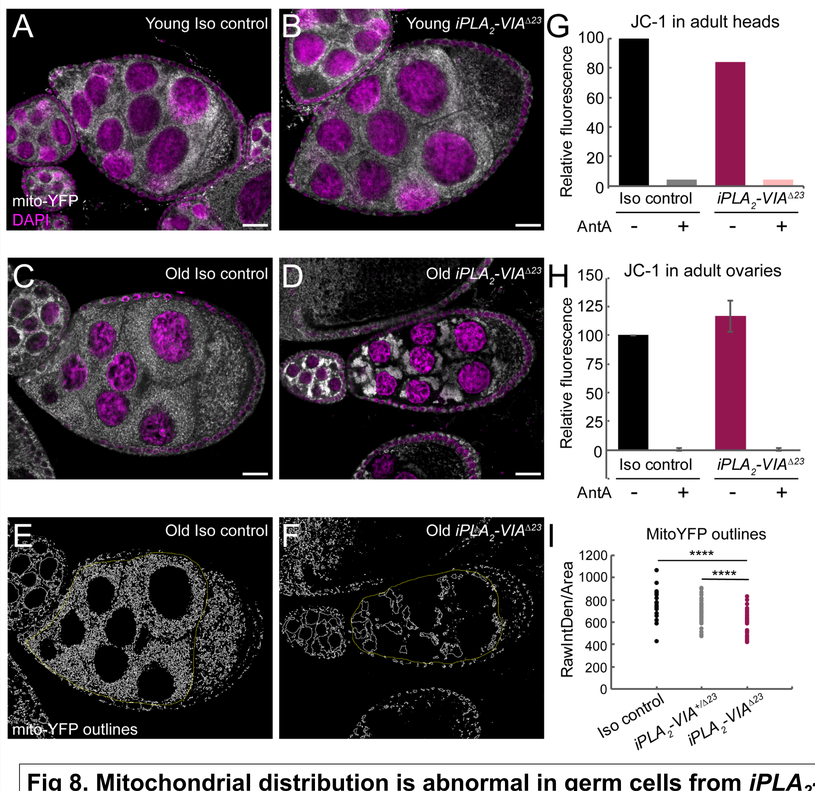
Result 4: iPLA$_2$-VIA protein localizes to mitochondria in adult germ cells.



- In the female germline, iPLA$_2$-VIA-HA (green, grayscale shown in A"-C", expressed with *NGT40-GAL4*) colocalizes with a mitochondrial marker (A, red, Psqh-mito-EYFP, arrowheads) but not with Golgi (B, red, anti-Golgin84) and ER (C, red, anti-Calnexin99A) markers. Individual channels for mitochondria, Golgi, and ER shown in A', B', C', respectively.
- In the male germline (D-F), iPLA$_2$-VIA-HA (green, expressed with *bam-GAL4-VP16*) also colocalizes more strongly with a mitochondrial marker (D, red, UAS-mCherry-mitoOMM) than Golgi (E, red) or ER (F, red) markers. Scale bars: 20 μm.
- The data suggest that iPLA$_2$-VIA affects fertility possibly by a mitochondria-related process.

Fig 7. iPLA$_2$-VIA-HA protein localizes to mitochondria of female and male germ cells.

Result 5: *iPLA$_2$-VIA$^{\Delta 23}$* female germ cells show irregular distribution of mitochondria.



- We used Psqh-mito-EYFP to observe mitochondrial distribution in female germlines from young and old *iPLA$_2$-VIA$^{\Delta 23}$* mutants and controls (A-D). Scale bars: 20 μm.
- We developed a method using ImageJ to quantify mitochondrial distribution within the area of the germline nurse cells (E-F, I).
- Mitochondria are significantly clumpy in germ cells from aged *iPLA$_2$-VIA$^{\Delta 23}$* female flies compared to controls (I, 21 days old) but no such difference is observed in young flies.
- We used a plate-based fluorometric assay with JC-1 dye to measure mitochondrial potential in heads and ovaries isolated from 28 day old control and *iPLA$_2$-VIA$^{\Delta 23}$* female flies (G, H, ± Antimycin A, mitochondrial poison).
- Mitochondrial potential is reduced in heads but not in ovaries of *iPLA$_2$-VIA$^{\Delta 23}$* female flies compared to controls (G, H).

Fig 8. Mitochondrial distribution is abnormal in germ cells from *iPLA$_2$-VIA* mutant females. Mitochondrial potential is normal in germ cells but reduced in heads from *iPLA$_2$-VIA* mutant females.

Conclusions:

1. A null mutation in *iPLA$_2$-VIA* causes severe loss of locomotor activity in aging flies, as in neurodegenerative disorders associated with mutations in the human ortholog.
2. Expressing either full length wild-type or a catalytic dead *iPLA$_2$-VIA* cDNA ubiquitously in the null mutant partially rescues the locomotor activity, suggesting that iPLA$_2$-VIA has both catalytic and non-catalytic functions during aging.
3. Knocking down *iPLA$_2$-VIA* in either neurons or muscles phenocopies the locomotor defect of the mutant, indicating functions in multiple tissues during aging.
4. iPLA$_2$-VIA is strongly localized to mitochondria of both male and female adult germ cells. Loss of *iPLA$_2$-VIA* affects only female fertility.
5. Mitochondrial distribution is abnormal in germ cells of aged *iPLA$_2$-VIA* mutant females, with no apparent perturbation of mitochondrial potential. Thus, the mechanism and the effects of such phenotype on female fertility in the *iPLA$_2$-VIA* mutant flies requires further investigation.

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