Electronic Supplementary Information

Spectroscopic features of Perylenediimide probe for sensing amyloid fibrils: *In vivo* imaging of Aβ-aggregates in *Drosophila* model organism

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Note S1

Synthesis and Characterization of L-Phe-PDI:

Synthesis of N,N'-bis-(L)-phenylalanyl perylenebisimide: The PDI derivatives were synthesised through condensation of perylene tetracarboxylic acid dianhydride with L(+)-phenyl alanine in molten imidazole. Perylene-3,4,9,10-tetracarbozylic acid dianhydride (0.196g, 0.5mmol, 1eq.) and L–phenyl alanine (0.198g, 1.2mmol, 1.4eq.) was mixed thoroughly in powdered imidazole (10g) in a 100ml round bottom flask. Then the mixture was stirred at 140°C for 1h in an oil bath and cooled to room temperature. Hydrochloric acid (1M, 200ml) was added to the solid mass with continuous shaking leading to the precipitation of solid. The resultant mixture was stirred at room temperature for 30 mins and the precipitate was filtered and washed with distilled water until the filtrate is neutral to pH paper. The precipitate was dried under suction to yield the L-Phe-PDI derivative as red solid in 97% yield. The product was characterised by ^{1}HNMR (Fig.S1A) and the chiral configuration was reaffirmed by CD measurement (Fig.S1B).

^{1}H-NMR (THF-d_8, 500MHz, 298K): δ3.64 (d, 2H *J*=5Hz), 3.71 (d, 2H *J*=5Hz), 6.06 (dd, 2H, $J_{1,2}$=5Hz), 7.00 (t, 2H, *J*=5Hz), 7.09 (t, 4H, *J*=5Hz), 7.24 (d, d, 4H *J*=5Hz), 8.34-8.39 (broad, 8H, PDI-core protons) and 10.86 (bs, -COOH) (Fig.S1A)

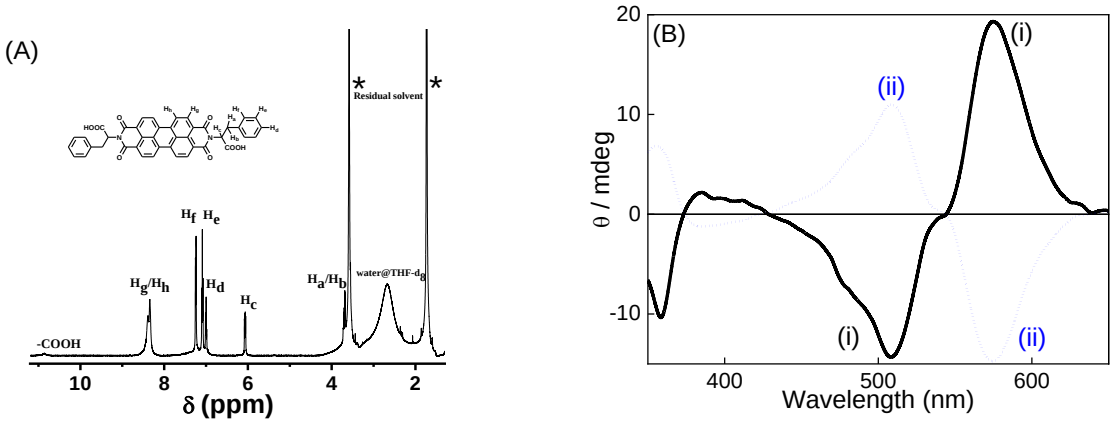


Fig. S1. (A) ^{1}H NMR Spectrum of L-Phe-PDI recorded in THF-d_8 solvent. **(B)** CD spectrum of L-Phe-PDI (i) recorded at pH 7.5, tris buffer. The CD spectrum of D-Phe-PDI (ii) is displayed for comparison and to afirm the chirality of the L-Phe-PDI used in this study.

Note S2

Treatment of L-Phe-PDI dye to 3rd instar larva. Third instar larva was taken out and washed with PBS to remove extra food particle. Then the larva was taken in an Eppendorf tube and L-Phe-PDI dye (10mM) was added. This treatment was done for 2 hours.

DAPI staining. DAPI binds to dsDNA. Guts of the L-Phe-PDI-treated larvae were dissected out and fixed on 4% PFA for 45 mins in room temperature. Afterwards larvae were washed with PBS for 2 times, 5mins in each wash. Then the larvae were washed with PBST for 2 times, 15mins in each wash. Then DAPI staining was done for 5mins, then the extra dye is washed out with the help of PBS and the gut was fixed in 20% glycerol. Afterwards images were taken under confocal microscopy (Leica TCS SP8, CLSM).[1]

DCFH-DA staining. DCFH-DA staining was used for the detection of ROS. The guts of the treated larvae were dissected out and fixed on 4% PFA for 45mins at room temperature, then washed with PBS for 2 times, 5mins in each wash. Next it was washed with PBST for 2 times, 15mins each, and then DCFH-DA staining was done for 35mins. Afterwards the extra dye was washed out with the help of PBS and the guts were fixed in 20% glycerol. Then confocal microscopy (Leica TCS SP8, CLSM) was used to visualize the larval gut.[1]

Transverse section of DAPI and phalloidin staining of gut. To examine the impact of L-Phe-PDI on the gut, gut was dissected and fixed with 4% PFA for overnight. Then the guts were washed with PBS and stained with phalloidin and DAPI. Phalloidin was stained for 45 minutes that binds to actin while DAPI stained for 5 minutes that binds to the nucleus. Once the gut was stained it was dissected transversely and mounted with 20% glycerol.[1-3] The gut was visualized under confocal microscope (Leica TCS SP8, CLSM).

Hematoxylin and Eosin (H&E) staining 3rd instar larval mid gut. Twenty 3rd instar larval mid guts of control and the L-Phe-PDI treated Drosophila larvae were dissected out and washed in 1X PBS. After that the tissues were fixed in Bouin's fixative. Alcohol gradations were prepared and tissues were transferred for the paraffin embedding. 5 µm thick transverse sections of the mid guts were obtained and imaged under a bright field microscope (Olympus ix 71).[3]

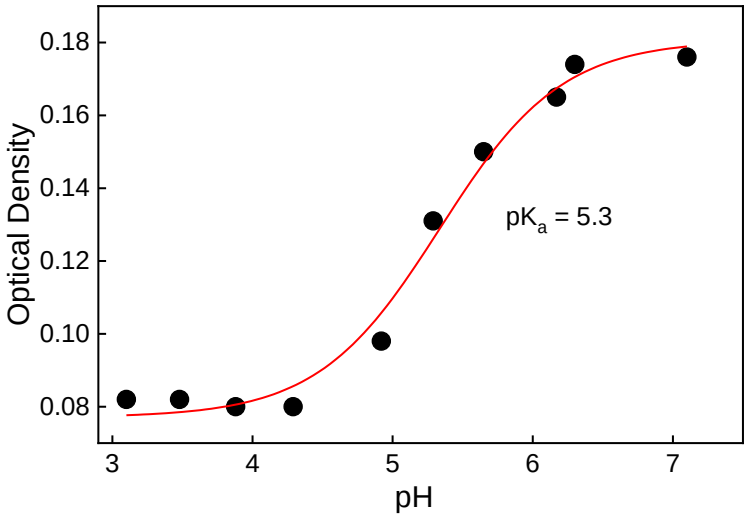


Fig. S2. Variation of optical density of L-Phe-PDI at 550 nm at different pH values of the solution.

Aggregation of L-Phe-PDI in water at pH 2

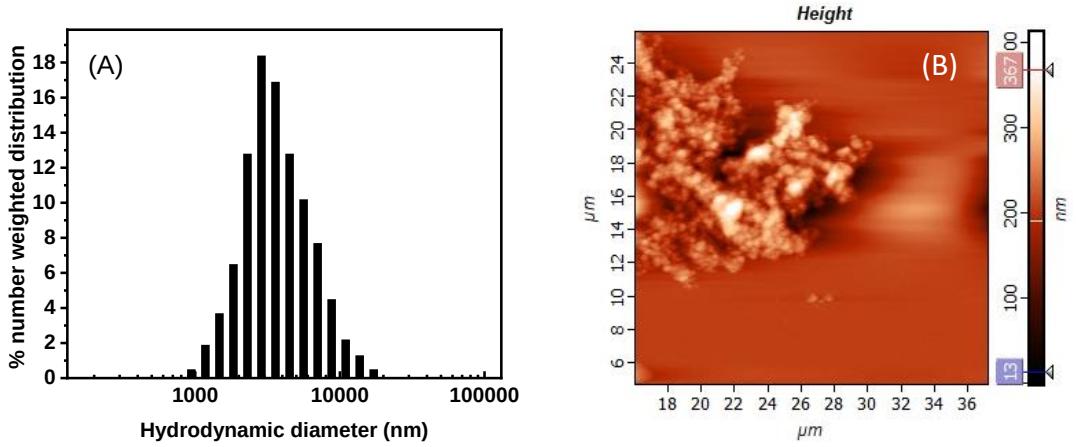


Fig. S3. (**A**) Dynamic Light Scattering data showing the aggregates (average size >2.5 µm) present in the aqueous solution of the L-Phe-PDI at acidic pH. (**B**) represents the AFM image recorded for the sample A drop casted on mica surface.

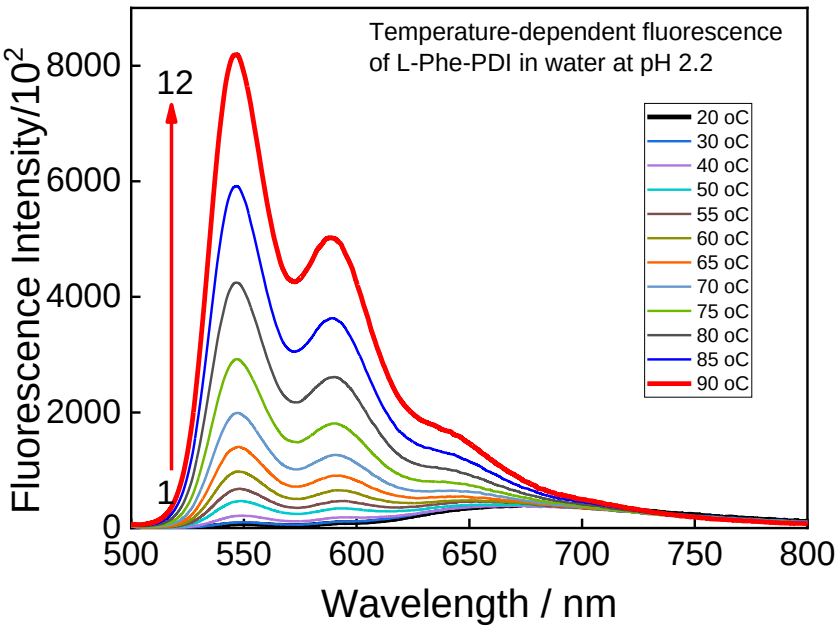


Fig. S4. Temperature-dependent fluorescence behavior of L-Phe-PDI in water at pH 2.2.

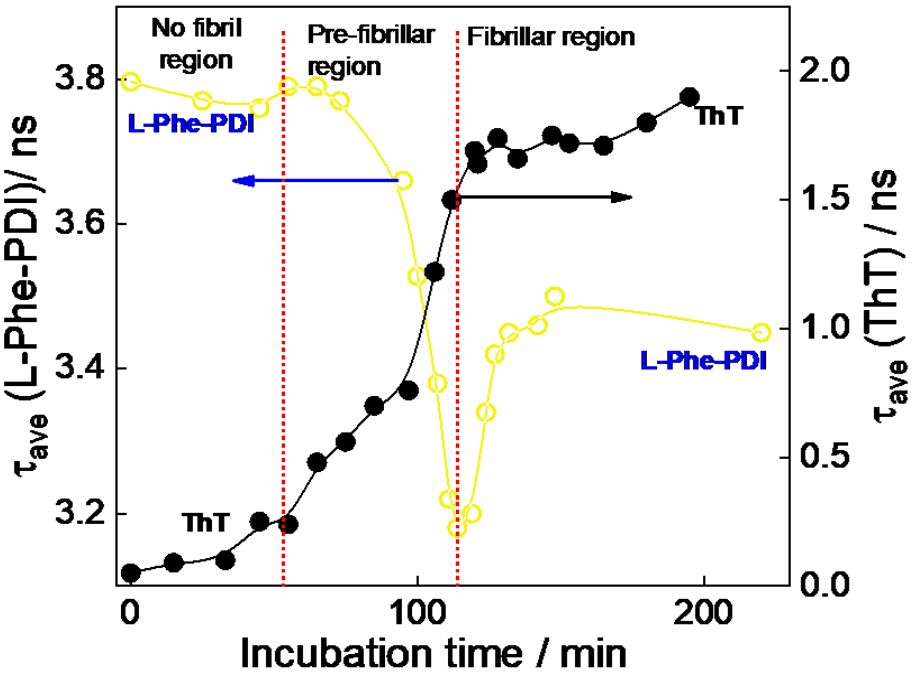
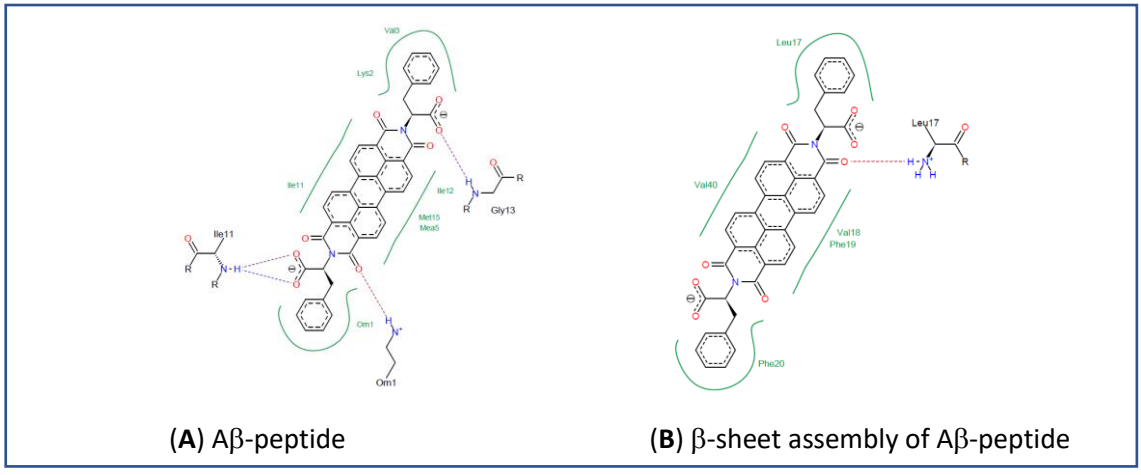


Fig. S5. Variation in the average fluorescence lifetime of L-Phe-PDI and ThT dyes with incubation time.

Note S3

Molecular docking studies: The structure of L-Phe-PDI is docked on the crystal structure of amyloid β-peptide (Aβ42) monomer and β-sheet forms (PDB file number 6SZF and 2BEG) using LeadIT 2.1.3-FlexS (BioSolveIT, GmbH, Germany) docking software. Before docking the structure of the ligand was optimized with Gaussian-92 software. The water molecules and any metal residues present in the protein structures were removed in the docking software and ligand was docked as dianion. While in case of monomer all amino acids of the Aβ42 monomer was considered the three chains namely A, B, and C of the for the β-sheet structure was considered for docking. The interaction between ligand and amino acid residues was analysed by combining both enthalpy- and entropy-based approaches. The favoured pose of the ligand on the protein was evaluated by the scoring method followed by calculation of binding free energy using the in-built HYDE program of the docking software.

Initial docking studies to evaluate the interaction of L-Phe-PDI with amyloid β-peptide monomer and β-sheet forms of Aβ42 peptide revealed strong interaction of L-Phe-PDI with the amino acid residues. As shown in the **Fig. S6**, the isoleucine (Ile11) and glycine (Gly13) residues of the Aβ42 interacts electrostatically with the deprotonated carboxylate groups of the ligand while the phenyl group and the PDI core resides in the hydrophobic environment of lysine (Lys2), valine (Val3) and methionine (Met15) amino acid residues. On the other hand, in case of the β-sheet assembly of Aβ42 peptide the ligand resides mainly in the hydrophobic region created by the phenyl alanine, valine and leucine amino acid residues of the peptide assembly along with the electrostatic interaction of protonated leucine (Leu17) residue with the imide carbonyl group of the ligand (Fig.S6B).



(**A**) Aβ-peptide (**B**) β-sheet assembly of Aβ-peptide

Fig. S6. Representative image of the nature of interaction of ligand L-Phe-PDI with amino acids present in the binding site of monomeric and β-sheet assembly of Aβ-peptide evaluated from docking studies

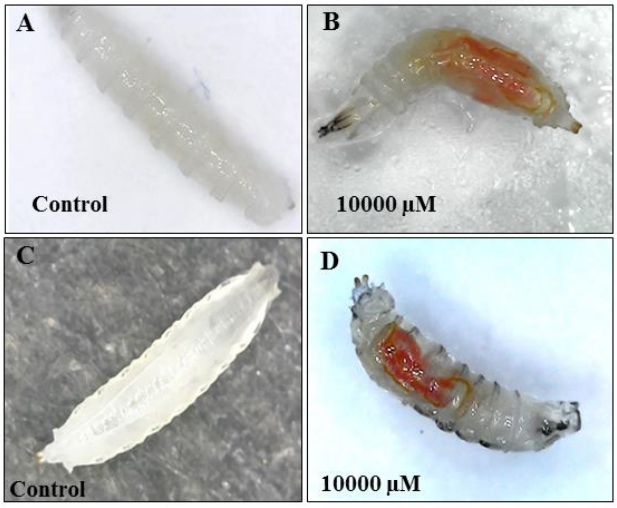


Fig. S7. Larval gut phenotype observation (A-B) and Trypan blue assay (C-D). **(**A) is the control image of the third instar larva which shows a transparent gut. (B) The larva taken from L-Phe-PDI (10 mM) treated food vial. (C) Control larva after trypan blue staining (D) L-Phe-PDI treated larva after trypan blue staining.

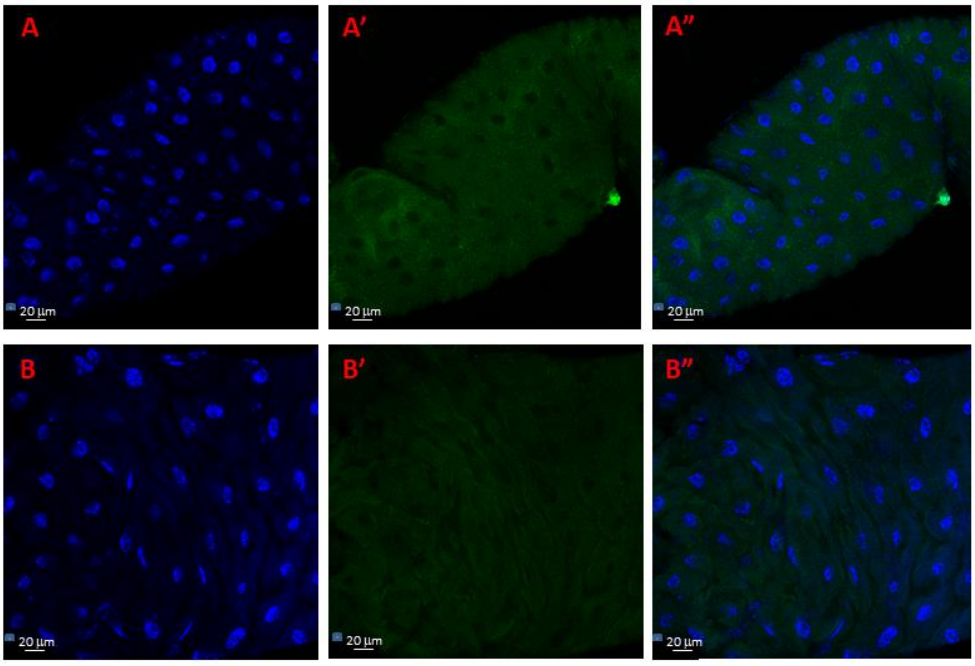


Fig. S8. (**A**) DAPI staining of control larva, (**A'**) DCFH-DA staining of control larva, (**A''**) merged.
(**B**) DAPI staining of L-Phe-PDI treated larva, (**B'**) DCFH-DA staining of L-Phe-PDI treated larva, (**B''**) merged.

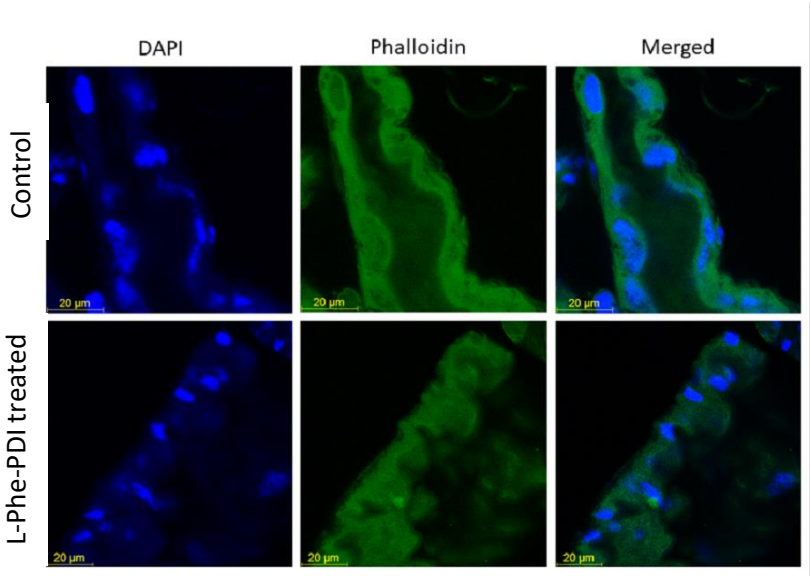


Fig. S9. Transverse Section of Gut of Drosophila (For Green, Alexa Fluor Excitation: ~561nm, Emission: ~405nm; Blue, DAPI).

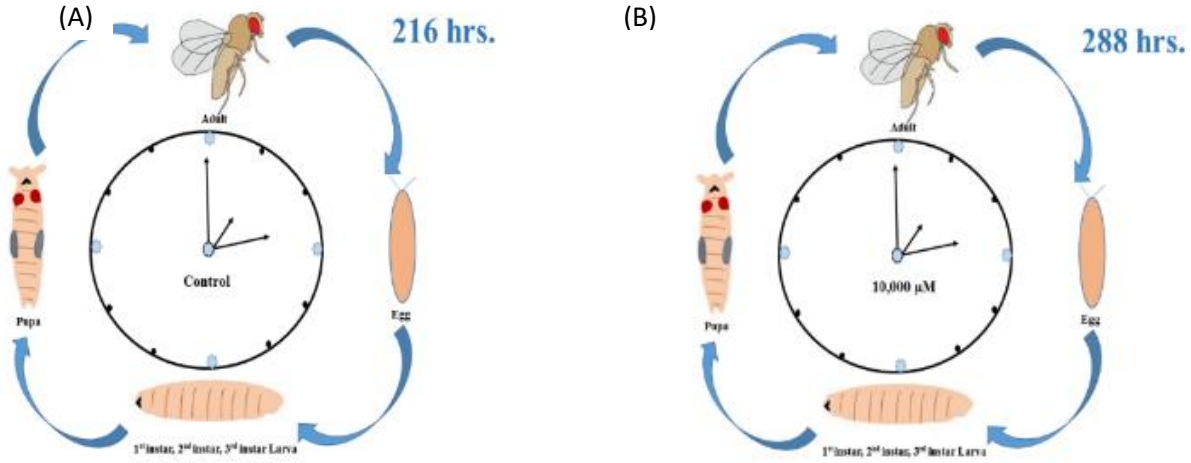


Fig. S10. (**A**) is the developmental cycle of the control flies which generally take 216 hrs. for fertilization of the egg to the conversion of an adult fly. Whereas (**B**) is the developmental cycle of the 10 mM L-Phe-PDI dye treated flies which shows delay in the developmental cycle. The cycle takes total of 288 hrs to produce a mature adult fly from the fertilized egg.

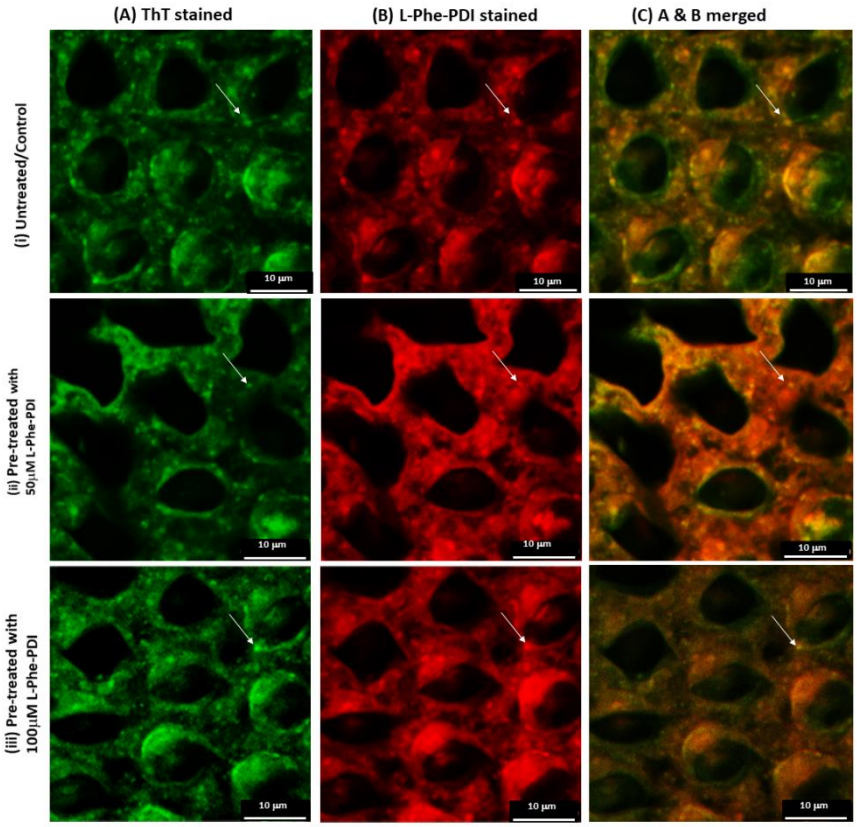


Fig. S11. ThT-(L-Phe-PDI) double staining (**A-B**). (A) and (B) are the ThT- (L-Phe-PDI) dye staining images and (C) is the colocalization of (A) and (B). (i) from untreated fly, (ii) treated with 50μM L-Phe-PDI, (iii) treated with 100μM L-Phe-PDI.

References

1. S. Priyadarsini, S. Mukherjee and M. Mishra, Methodology to Detect the Abnormality of Drosophila Gut by Various Staining Techniques, Fundamental Approaches to Screen Abnormalities in Drosophila, **2020**, 51-64.

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