



B		C	
Pupal stage Shift (25°→18°)	IFM analyzed post eclosion	Pupal stage Shift (18°→25°)	IFM analysed post eclosion
Less than 50 hrs APF	Muscle fibre normal	Less than 50* hrs APF	Degeneration of muscle fibre
50-65 hrs APF	Muscle fibre normal	50-65* hrs APF	Degeneration of muscle fibre
60-75 hrs APF	Muscle fibre normal	60-75* hrs APF	Degeneration of muscle fibre
80-90 hrs APF	Hypercontracted muscle fibre	80-90* hrs APF	Muscle fibre normal

Figure S3. Muscle hypercontraction in *fliH* starts at late puparium stages. (A-A''') *Act88F-GFP* line was used to track the timing of IFM degeneration when genetically brought in the *fliH* background at 25°. Rupturing of the IFM is visible in adult flies post eclosion. Arrows point to DLMs, which are still intact at pupal stage, whereas arrowheads indicate site of muscle rupture in adult thorax. (B and C) Temperature shift assay to track exact pupal developmental stage in which IFM hypercontraction starts (details in supplementary materials and methods). (B) Temperature shift down experiment demonstrates that muscle degeneration in *fliH* mutant flies grown at 25°, starts after 75 hrs APF. (C) Temperature shift up assay supports the fact that degeneration of muscle fibre in *fliH* starts after 75 hrs APF. Asterisk denotes corrected development at 18° to match the development at 25° since pupal development is slow at 18°.