Supplementary Online Material:

We compared the *heph²* and *heph^{03429}* mutants for gene expression profiles. There was little overlap between the differentially expressed genes in the *heph²* and *heph^{03429}* analyses. There were 63 differentially expressed genes shared between the two analyses (Fig. S1A), which is about the number of genes that would be expected by chance. Further, none of the alternative isoform candidates overlapped. This possibility reflects how the two mutants result in developmental phenotypes – embryonic phenotype and adult phenotypes in testes. Nonetheless, the more general gene ontology comparison of differentially expressed genes showed some similarity, for example, the profile of stress response genes, extracellular and signal peptide-related genes, and peptidase genes (Fig. S1B). Further experiments will be needed to understand the biological significance of these observations.
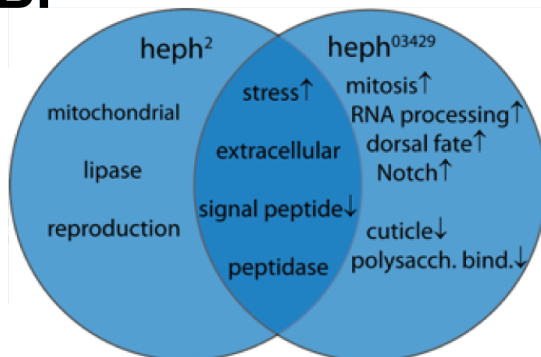
A.
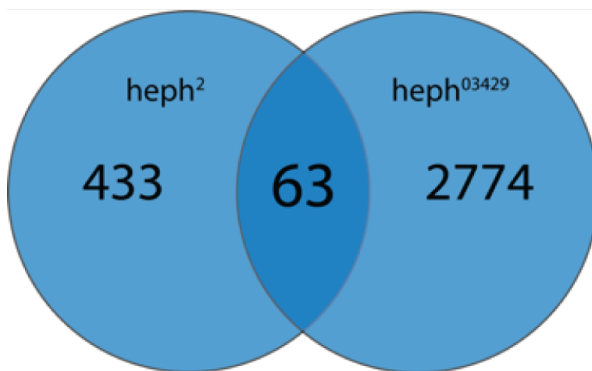
B.

Figure S1. A) Venn diagram of the number of differentially expressed genes in the *heph²* and *heph^{03429}* analyses. There are 63 differentially expressed genes in common between the *heph²* and *heph^{03429}* analyses, changing in the same direction. **B)** Venn diagram of overlapping gene ontologies. Arrows denote whether genes in ontology group were mostly up or down-regulated. If no arrow, the genes in the group were mixed.

Supplementary Table 1. Primers used for validation of differential expression in the *heph²* mutant.

Gene / isoform	Forward primer	Reverse primer
RpS15	TCTACAACGGCAAGGACTTCGG	CGTTTCATTTATTCACGCATCA
Mlc1	CCCATCTACTCACAGGTCAAGAAG	ATGGTTGTCGCTAAAAGTTTGTTG
Tequila 3' end	GCAGAATGTCCATCCTAAGTTGTG	ATCCAAGTAATCATGTGCAGAGAG
Tequila 5' end	TTCCCATTTTGGCCGATCACGTAT	AGCAAGCTCTCATTGATTTTCTCG
Prm	CGTCTGCACGAATTGGAACTGGAG	CATTGAGCTGCTCGATCTCGATCG
Act88	CGCACAACAATTATCATCCAACTC	CACGACTCTTACGATTAGTTCTTC
Vm34Ca	ATAAGGAGGATAAGATGCTCGGCT	TTGATCAGGGGTTAGTGAAGTCTG
CG11598	TCCTGAGGAACTTGATCAACTACA	CTCCTTTGCTGTCGAACTATCATC
CG11162	CGCAGTCCGGGGATTATCTG	GGGTGAAGATGCCCGCATAG
Hsp70bc	AGGAGATGAGCACGGGCAAG	CCTGCTCCACGGACTGCTTT
Tpnc4	GCAAACTGGCAGCTCGCTTT	TACCGGATCCATCCGCATCA
CG42245	CCGCAGGACCAGGAGGACTA	CGTCAGATCCGCTCGCTTTT