PHLDA1 Does Not Contribute Directly to Heat Shock-Induced Apoptosis of Spermatocytes

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Figure 1. Phlda1 transcript levels analyzed by RT-PCR in mouse organs after heat shock performed in vivo and indicated recovery time. C, control, physiological temperature; HS, heat shock. Hspa1 was used as transcript level control for the heat shock response, Gapdh, Actb – as transcript level controls for loading.
Figure 2. Specificity of anti-PHLDA1 antibodies: mouse monoclonal (Santa Cruz, sc-23866; Ab1) and rabbit polyclonal (Novus #NBP1-84969, Ab2). (a) PHLDA1 expression was reduced by sgRNA/Cas9 (kd, PHLDA1 knockdown; wt, wild type) in mouse HECa10 cells and analyzed by western blot. (b) Expression of PHLDA1 analyzed by western blot in the mouse testes during postnatal development and NIH3T3 cells transiently transfected with a vector coding for EGFP/PHLDA1 fusion protein. ACTB was used as a loading control. Both antibodies recognized the reduced level of PHLDA1 protein after PHLDA1 sgRNA knockdown and recognized EGFP/PHLDA1 fusion protein after its overexpression. Using Ab1, a higher background is likely on mouse tissues (IgG heavy and light chains were stained in western blot). In addition, a few other protein bands were detected by both antibodies (marked by asterisks); however, these bands would not be observed if cells with a high level of PHLDA1 expression (e.g., mouse hepatocytes) were analyzed using a shorter time of western blot exposure (not shown). (c) Detection of the PHLDA1 protein using Ab1 (green) and Ab2 (red) by immunofluorescence in HECa10 cells cultured in vitro (cells were fixed with 10% buffered formalin or 4% PFA for 10-15 minutes). Negative controls were performed for specific labeling by omitting the primary antibody (-Ab). DNA was stained with DAPI (blue). Scale bar – 50 μm. Both antibodies recognized endogenous PHLDA1 protein in cytoplasm and nuclei, which resembled the cellular distribution of EGFP/PHLDA1 fusion protein (Figure 3 in the manuscript), however using the mouse Ab1, a background is possible on mouse cells (even when Mouse on Mouse Kit is used for detection).
Table 1. Characteristics of primers used in RT-PCR analyses.

| Gene Symbol | NCBI Reference Sequence | Primers Sequences | Product Length [bp] |
|-------------|-------------------------|-------------------|---------------------|
| Dazl        | NM_010021.5             | F: tgaagttgtacccaggaagt<br>R: ccccttgagaatggttagca | 261                 |
| Hspa1a      | NM_010479.2             | F: ccacctcagagacaaggaag<br>R: cgtttaccccgggtgacac | 699                 |
| Hspa1b      | NM_010478.2             | F: ccatccagaagacaaggaag<br>R: gtattaccggcagtcac | 699                 |
| Hspa2       | NM_008301.4             | F: agggcccacacttgaggaag<br>R: gtagctgcagtttcgctga | 344                 |
| HSF1        | NM_005526.2             | F: ccagcaacagaaagttgcga<br>R: gagctcattcttgtccagcc | 325 in mutant HSF1 |
| Pgk2        | NM_031190.2             | F: ggccttcagcaacatgttaat<br>R: aggacttgccggaaaacctga | 228                 |
| Phlda1      | NM_009344.3             | F: caacagcttcaactctacc<br>R: ggtctgtcacaaggtgatga | 394                 |
| Gapdh       | NM_008084.3             | F: tgtgtgaagcaggcatctgag<br>R: cagttgtgcacccacctgt | 203                 |
| Actb        | NM_007393               | F: ggacttcgagcaagagattg<br>R: agcaactgtgctgctag | 234                 |
| Hnrnpk      | NM_001301341.1          | F: tgggttagcgtgtgatgaa<br>R: aataggcttgccagatcgc | 151                 |