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Mass Spectrometry-Based Substrate Identification and Genetic Validation Reveal the Functional Role of *Drosophila* Protein Tyrosine Phosphatase DPTP61F

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Recent biochemical and genetic approaches have clearly defined the functional role of critical components in tyrosine phosphorylation-dependent signal transduction. These signaling modulators often exhibit evolutionarily conserved functions across various species. It has been proposed that if protein tyrosine kinases (PTKs), protein tyrosine phosphatases (PTPs) and thousands of their substrates could be identified and characterized, it would significantly advance our understanding of the underlying mechanisms that control animal development and physiological homeostasis. The fruit fly *Drosophila melanogester* has been used extensively as a model organism for investigating the developmental processes but the state of its tyrosine phosphorylation is poorly characterized. In the current study, we used advanced mass spectrometry (MS)-based shotgun analyses to profile the tyrosine phosphoproteome of *Drosophila* S2 cells. Using immunoaffinity isolation of the phosphotyrosine (pTyr) ubproteome from cells treated with pervanadate followed by enrichment of phosphopeptides, we identified 562 non-redundant pTyr sites in 245 proteins. Both this pre-defined pTyr proteome subset and the total cell lysates were then used as sample sources to identify potential substrates of dPTP61F, the smallest member in terms of amino acid number and molecular weight in the *Drosophila* PTP family and the ortholog of human PTP1B and T Cell-PTP, by substrate trapping. In total, 20 unique proteins were found to be specifically associated with the trapping mutant form of dPTP61F, eluted by vanadate (VO_4^{3-}), and identified by MS analyses. Interestingly, several potential substrates were previously

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identified as components of SCAR/WAVE complex, which may work in coordination to control actin dynamics. To validate the results of MS-based substrate identification and to further illustrate the functional role of dPTP61F in regulating actin action, genetic approaches were applied in *Drosophila*. Our data clearly demonstrate that dPTP61F plays a central role in counteracting PTK-mediated signaling pathways in regulating actin reorganization and remodeling through tyrosine dephosphorylation of critical components of SCAR/WAVE complex during *Drosophila* development.

