Supplementary Figure 1. The most significant motifs of phosphosites in 6 Drosophila species are similar.

Supplementary Figure 2. Phosphosites were aligned across the Drosophila and mosquito species.  27,358 aligned sites that are covered by our mass-spec data of the 6 Drosophila species (bolded) were selected and a propensity score combining experimentally determined sites from other species with the sequence-based phosphosite predictions was assigned to each phospho-acceptor site that is not covered by the data but is likely missed due to the incomplete coverage of the data (Studer et al., 2016).

Supplementary Figure 3a. Correlation of propensity score and observed phosphorylation in Drosophila melanogaster proteomics data  
11,691 phosphosites in Drosophila melanogaster were assigned a propensity score, based on their likelihood of phosphorylation in other Drosophila species. The propensity score correlates with observation of phosphorylation for individual sites in three independent Drosophila melanogaster proteomic datasets.

Supplementary Figure 3b. The frequency of phosphorylation among six Drosophila species with reported data for orthologous human proteins  
Phosphosites identified in Drosophila were aligned to the corresponding amino acid of the human ortholog, considering a sliding window of five amino acids surrounding the identified phosphosite. The probability of the corresponding phospho-acceptor site having been observed as phosphorylated in human data correlates with the degree of sequence similarity.

Supplementary Figure 3c. False negative rate estimation for coverage of the six Drosophila phosphoproteomes  
An estimation of missed phosphorylations (false negative rate) was determined for each of the Drosophila species by considering those phosphosites identical among all the six species (considering eleven amino acid peptides comprising the phosphosite plus five amino acids upstream and downstream) and for which phosphorylation was observed in at least two species. The false negative rate is the reciprocal of the number of observed phosphorylations in an individual species divided by the number derived above.

Supplementary Figure 4.  Evolutionary relationships among Drosophila melanogaster kinases  
The core of the plot illustrates the phylogenetic relationships between Drosophila melanogaster kinases estimated by total sequence similarity. The outer circle reflects the presence of orthologs in other species.  
a. CMGC - CDK, MAPK, GSK3 and CLK family  
b. CAMK - Calmodulin/Calcium regulated kinases family  
c. AGC - Protein Kinase A, G, and C family (PKA, PKC, PKG)  
d. STE - Homologs of the yeast STE7, STE11 and STE20 genes family  
e. Atypical kinase family  
f. CK1 - Casein Kinase 1 family  
g. RGC - Receptor Guanylate Cyclases family  
Supplementary Figure 5. Statistics of phosphosites in iProteinDB: percentage of phosphorylated serine, threonine and tyrosine residues for human, mouse, rat and Drosophila melanogaster

Supplementary Table 1: Summary of our phosphoproteomics datasets of 6 Drosophila Species

Supplementary Table 2: Phosphosites that are 100% identical between human and Drosophila melanogaster

Supplementary Table 3: Phospho-acceptor residue that are conserved among Drosophila but changed or absent in human despite the surrounding sequences being 100% identical