Supplemental Fig. 1 Detailed experimental workflow for the acquisition of the quantitative phosphoproteome with phosphatase inhibitor cocktail treatment. • phosphate group, peptides titanspere (TiO2).

Light
Arg “0”, Lys “0”
untreated

Heavy
Arg “10”, Lys “8”
15 min incubation with phosphatase inhibitor cocktail

1:1 cell mix and whole cell lysis

In-solution digestion with lysine-C and trypsin

Multiple phosphopeptide enrichment steps by TiO2 chromatography

Fractionation by strong cation exchange chromatography

Phosphopeptide enrichment by TiO2 chromatography

Peptide identification and quantitation by LC-MS/MS
Supplemental Fig. 2 Histogram of log2-transformed normalized ratios of pS/pT sites (A) and pY sites (B) upon phosphatase inhibitor cocktail treatment.
Supplemental Fig. 3 Detailed experimental workflow for the acquisition of the quantitative phosphoproteome and proteome in response to Ptp61F deletion by RNA interference. • phosphate group, Peptides, * titanspere (TiO2).
Supplemental Fig. 4 Overlap of phosphoproteins encoding genes (A) and their corresponding high confidence phosphorylation sites (B) of Bodenmiller et al. (2007), Zhai et al. (2008) and our SL2 phosphoproteome dataset.
Supplemental Fig. 5 Matching sequence motifs of the SL2 phosphoproteome (left panel) and the HeLa phosphoproteome identified upon EGF stimulus (Olsen et al., 2006) (right panel) applying Motif-X analysis (Schwartz and Gygi, 2005). Kinases of matching kinase motifs are indicated.
Supplemental Fig. 6 Proportion of phosphosites and their non-phosphorylated counterparts that are located in loop and coil regions (A). Average accessibility of phosphorylation sites and their non-phosphorylated counterparts as calculated by SABLE (B).
Supplemental Fig. 7 Gene transcripts (left) and identified phosphorylated gene transcripts (right) localized on fly chromosomes.
Supplemental Fig. 8 Proportion of phosphorylated (red) and non-phosphorylated (blue) fly proteins that have orthologs in given species.
Supplemental Fig. 9 Aligned upregulated phosphotyrosine responder using Needle.