

897 **Figure S1. PPKL localizes to the nucleus.** A. Western blot of protein samples after  
898 cytoplasmic and nuclear fractionation. Anti-HA was used to detect HA-tagged PPKL. Anti-eIF2 $\alpha$   
899 and anti-histoneH3 were used as controls to detect eIF2 $\alpha$ , a cytoplasmic protein, and histone  
900 H3, a nuclear protein. B. ImageJ was used to quantify the relative intensity of the bands in the  
901 two portions labeled by the same antibody. Fisher exact test was used to compare the ratios of  
902 cytoplasmic/nuclear of PPKL was significantly different from that of the control eIF2 $\alpha$ .

903 **Figure S2. Fusion of AID to the C-terminus of PPKL reduced its expression.** A. Western  
904 blot of protein samples isolated from PPKL<sup>HA</sup> and PPKL<sup>AID</sup> parasites. Anti-HA was used to detect  
905 PPKL-3xHA and PPKL-AID-3xHA. The protein Sag1 was used as a loading control. B. The

906 quantification of the Western blot in panel A reveals the relative expression levels of PPKL-AID-  
907 3xHA normalized to Sag1 and PPKL-3xHA.

908 **Figure S3. PPKL-TurboID validation.** A. Localization of PPKL-TurboID-3xHA in intracellular  
909 parasites assessed by IFA. B. Western blot showing biotinylated proteins extracted from  
910 PPKL<sup>TurboID</sup> parasites treated with or without D-biotin. Detection was achieved using  
911 Streptavidin-Conjugated Horseradish Peroxidase.

928 **Supplementary datasets**

929 **Supplementary Dataset 1.** Proteins immunoprecipitated with TgPPKL and identified by LC-  
930 MS/MS. The cutoff of fold change is  $PPKL.3xHA /Control \geq 2$ .

931 **Supplementary Dataset 2.** List of proteins biotinylated by the PPKL-TurboID fusion. For each  
932 repeat, the fold change cutoff was  $PPKL-TurboID/Control \geq 2$ . The list of PPKL neighboring  
933 proteins was selected based on the following criteria: in combination with two replicates, 1)  
934 identified in both replicates; 2) a total of 10 or more peptides were identified between the two  
935 replicates; 2) the fold change was equal to or larger than 3.5.

936 **Supplementary Dataset 3.** Listed are phosphopeptides identified in PPKL<sup>AID</sup> parasites treated  
937 with auxin or ethanol for 6 h by phosphoproteomics analysis. The sheet "PeptideGroups"  
938 contains all phospho-peptides identified in parasites and host cells. *Toxoplasma*  
939 phosphopeptides that were significantly (p-value  $\leq 0.05$ ) increased or decreased in auxin-  
940 treated parasites are listed in the sheets titled "Toxo Increased" and "Toxo Decreased". The  
941 phosphopeptides that were increased or decreased by more than two-fold in phosphorylation  
942 are listed in "6h Increased FC  $>2$ " and "6h Decreased FC  $< 0.5$ ". The phosphopeptides that  
943 were from the proteins identified by TurboID analysis are listed in "Increase overlap with  
944 TurboID" and "Decrease overlap with TurboID". Proteins in Fig. 8A have been listed in the sheet  
945 "Proteins of Fig. 8A". Those proteins that are PPKL neighboring proteins identified by TurboID  
946 analysis were highlighted.

947 **Supplementary Dataset 4.** Listed are phosphopeptides identified in PPKL<sup>AID</sup> parasites treated  
948 with auxin for 1 and 3 h or ethanol for 1 h. The sheet 'PeptideGroups' lists all phosphopeptides  
949 identified in parasites and host cells. The phosphopeptides identified in parasites are shown in  
950 the sheet "Toxo peptides". The phosphopeptides that are more/less abundant in 1 or 3 h auxin-  
951 treated parasites were filtered via specific fold changes and are shown in corresponding sheets.

952 Proteins of Fig. 8A have listed in the sheet "Proteins of Fig. 8A". Proteins identified as putative

953 PPKL neighboring proteins by TurboID are highlighted.

954 **Supplementary Dataset 5.** List of primers used in this study.

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