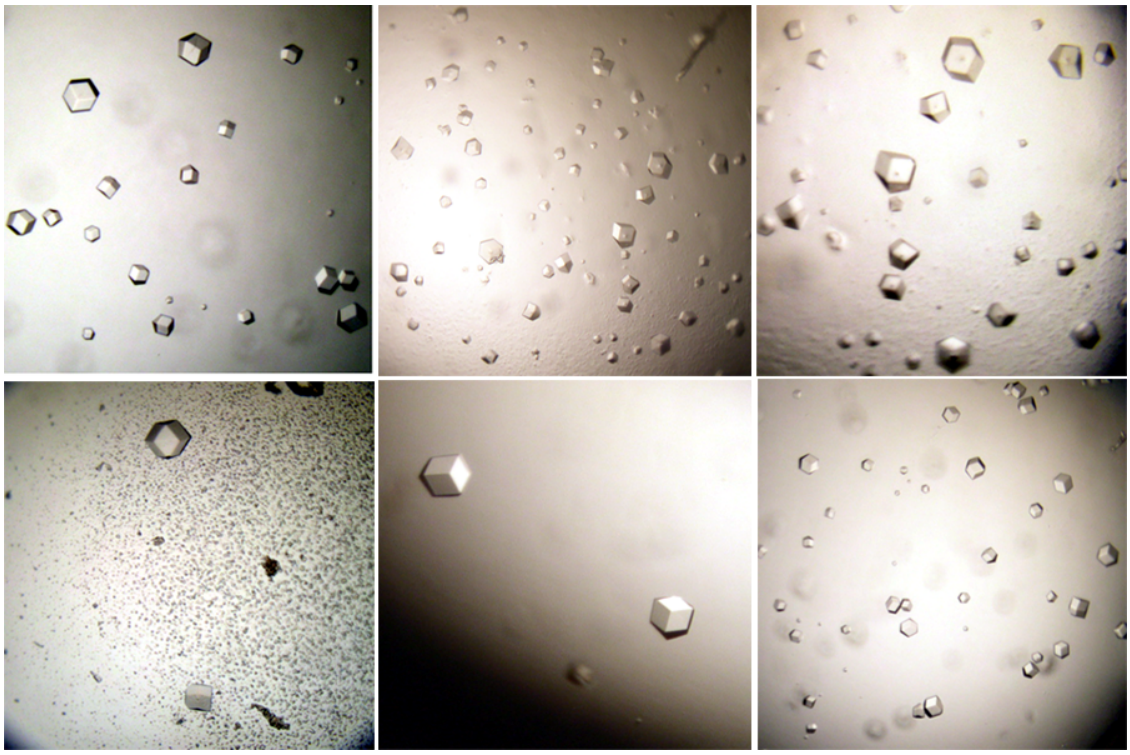
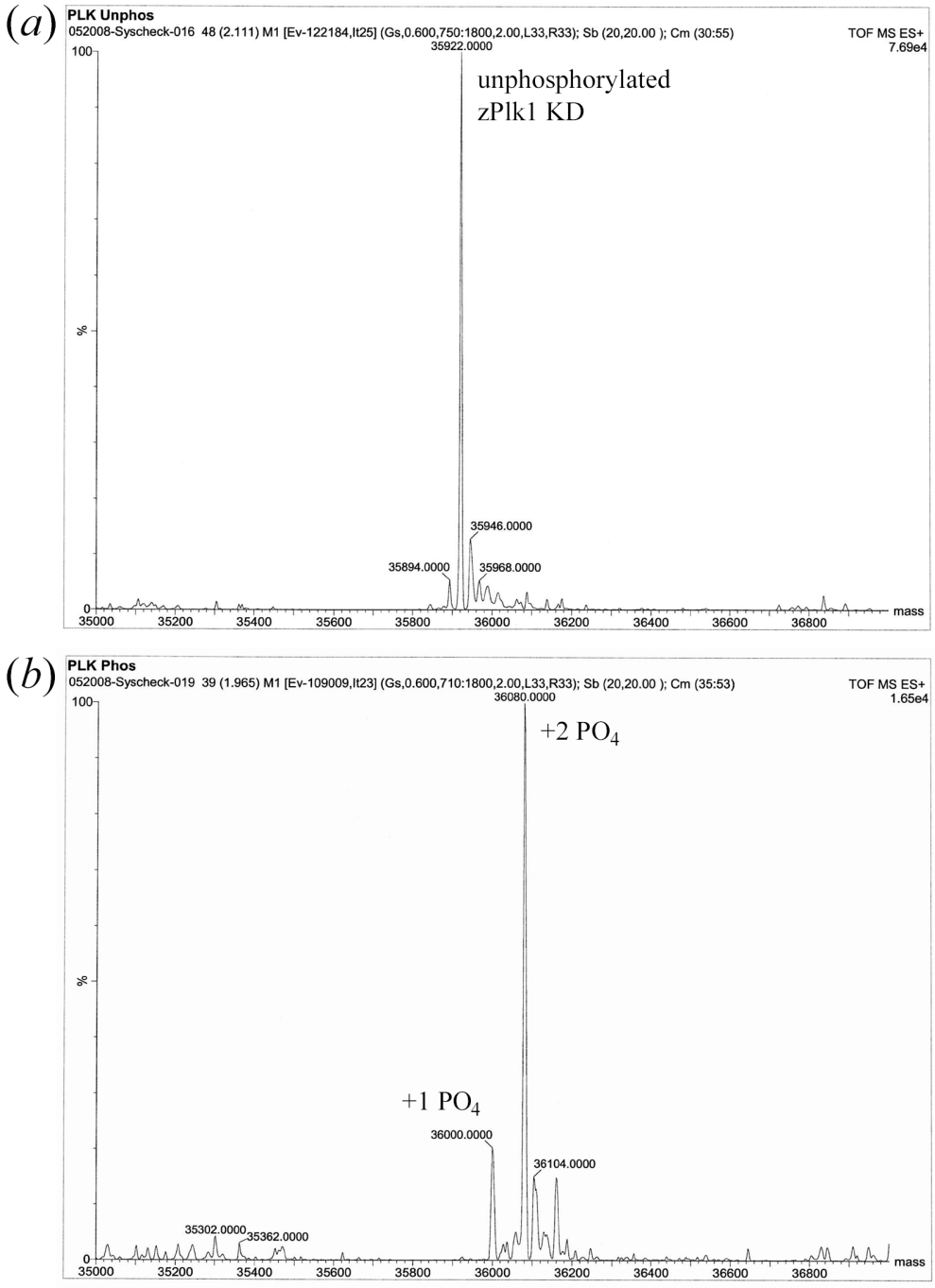
Supplementary Table 1 Conservation between zebrafish and human Plk1 polypeptide chain fragments.

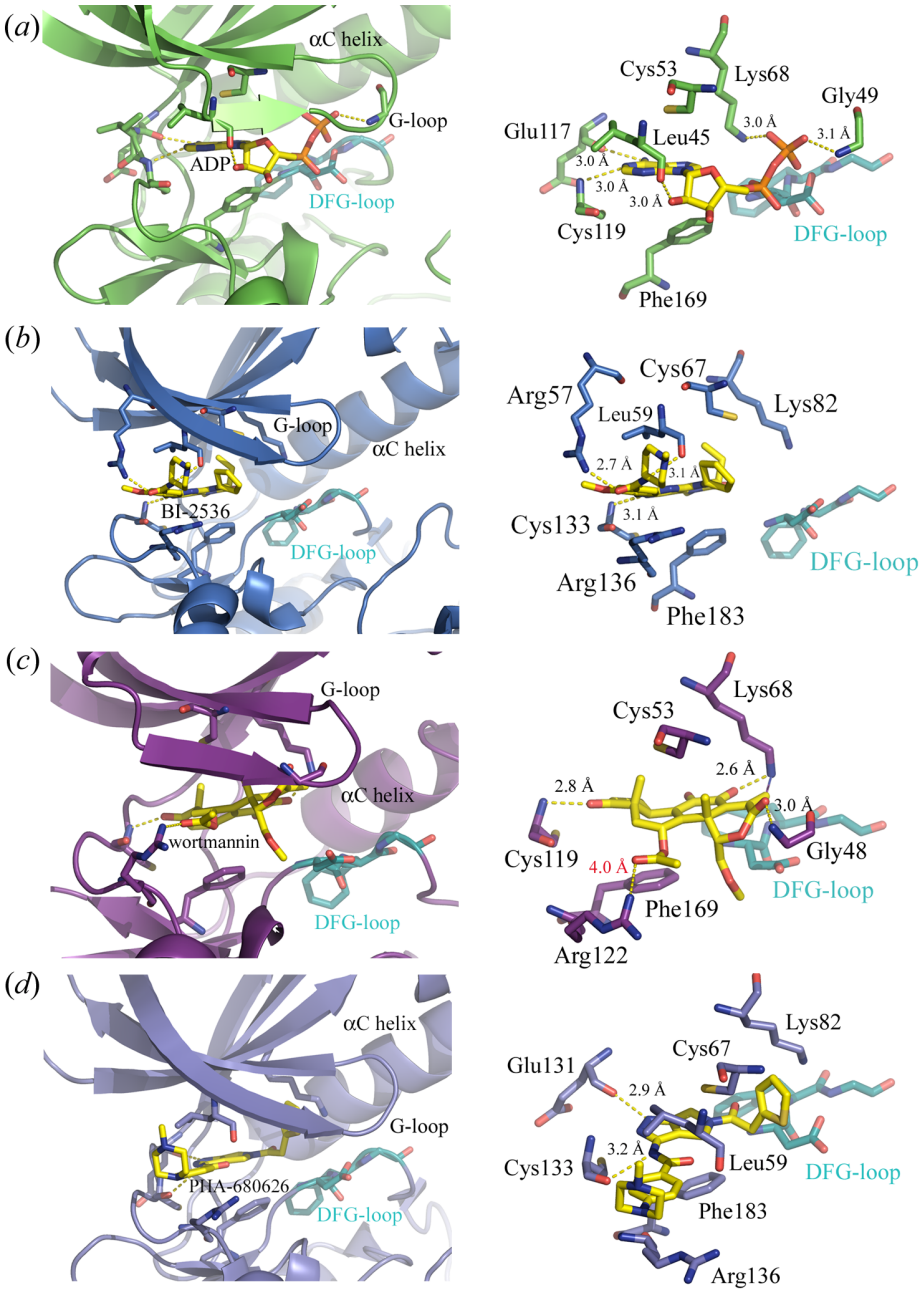
zPlk1 residue numbers	hPlk1 residue numbers	Number of identical residues	Identity (%)	Number of conserved residues	Conservation (%)	Number of residues in gaps	Gaps (%)
Entire protein							
3-589	26-598	440/587	74	518/587	88	14/587	2
Catalytic (kinase) domain							
24-312	38-326	233/289	80	272/289	94	0/289	0
N-terminal small lobe of the kinase domain							
24-94	38-108	57/71	80	69/71	97	0/71	0
C-terminal large lobe of the kinase domain							
98-312	112-326	176/215	81	202/215	93	0/215	0
Polo-box sequence motif							
362-589	371-598	180/228	78	209/228	91	0/228	0



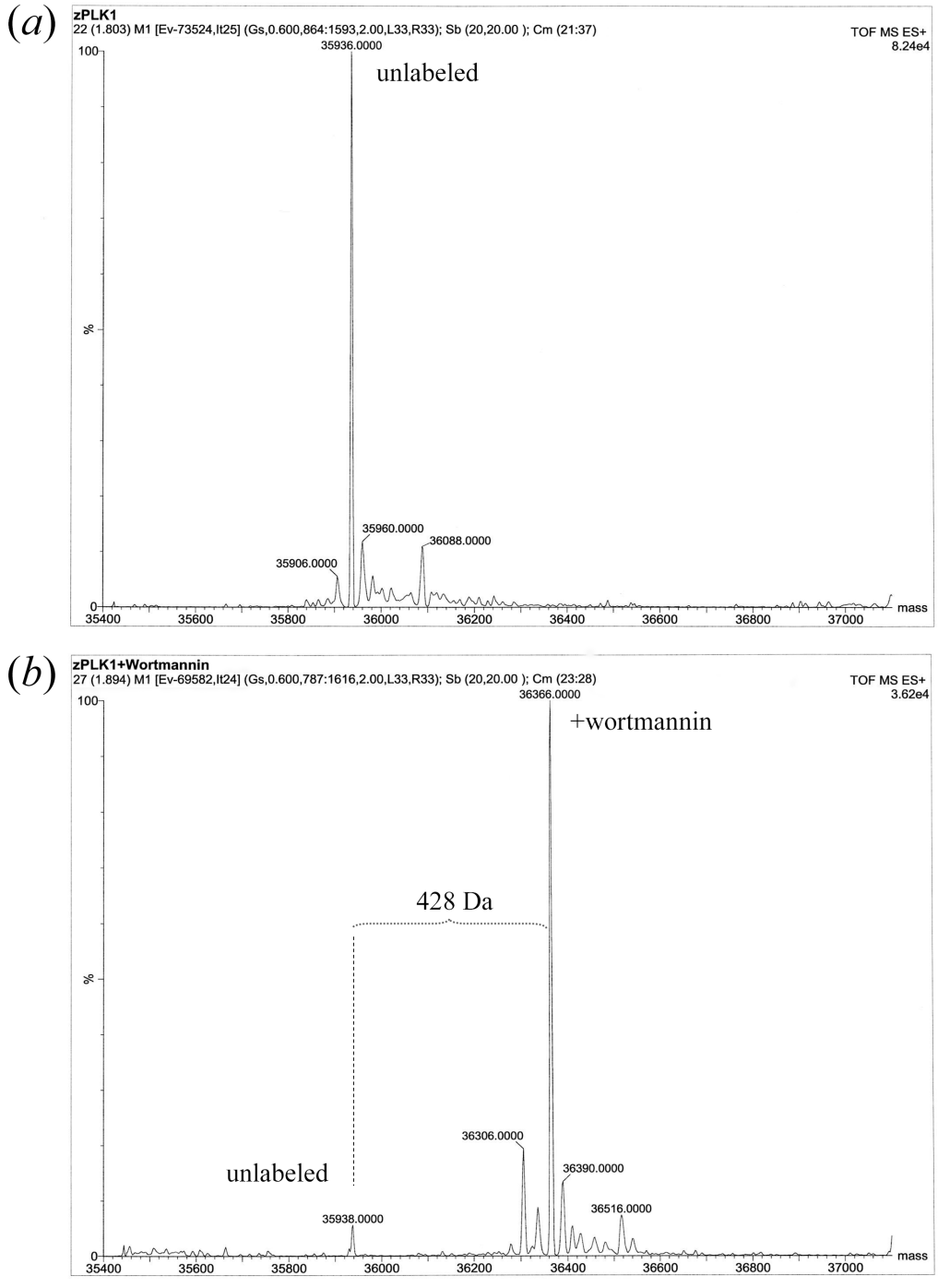
Supplementary Figure 1 Crystals of zPlk1. Each panel represents crystals of the different constructs grown in the presence of various ligands under the crystallization conditions described in this study. All crystals display cubic morphology. The protein variants crystallized in the same *I*23 space group with nearly identical unit cell dimensions irrespective of the presence and identity of ligands as well as crystallization conditions.



Supplementary Figure 2 Analysis of autophosphorylation in zPlk1 by electrospray mass spectrometry. (*a*) Unphosphorylated wildtype zPlk1 KD residues 1-312. (*b*) Protein modified covalently by two phosphate groups. Approximately 90% of all protein has been modified by two phosphate groups, and 10% by one phosphate group. Unphosphorylated protein could not be detected.



Supplementary Figure 3 Binding of ADP and small-molecule inhibitors in the active site of Plk1. Each panel on the left shows secondary structural elements surrounding the active site. Each panel on the right displays key direct H-bonding interactions between the small molecule and protein atoms. (*a*) zPlk1 in complex with ADP (PDB ID code 3d5w). (*b*) hPlk1 in complex with BI-2536 (PDB ID code 2rku). (*c*) zPlk1 with wortmannin covalently bound to catalytic Lys68 (PDB ID code 3d5x). (*d*) hPlk1 in complex with PHA-680626 (PDB ID code 2owb).



Supplementary Figure 4 Mass-spectrometric confirmation of covalent modification of unphosphorylated zPlk1 KD by wortmannin. (*a*) Unlabeled protein. (*b*) Protein modified covalently by one molecule of wortmannin.