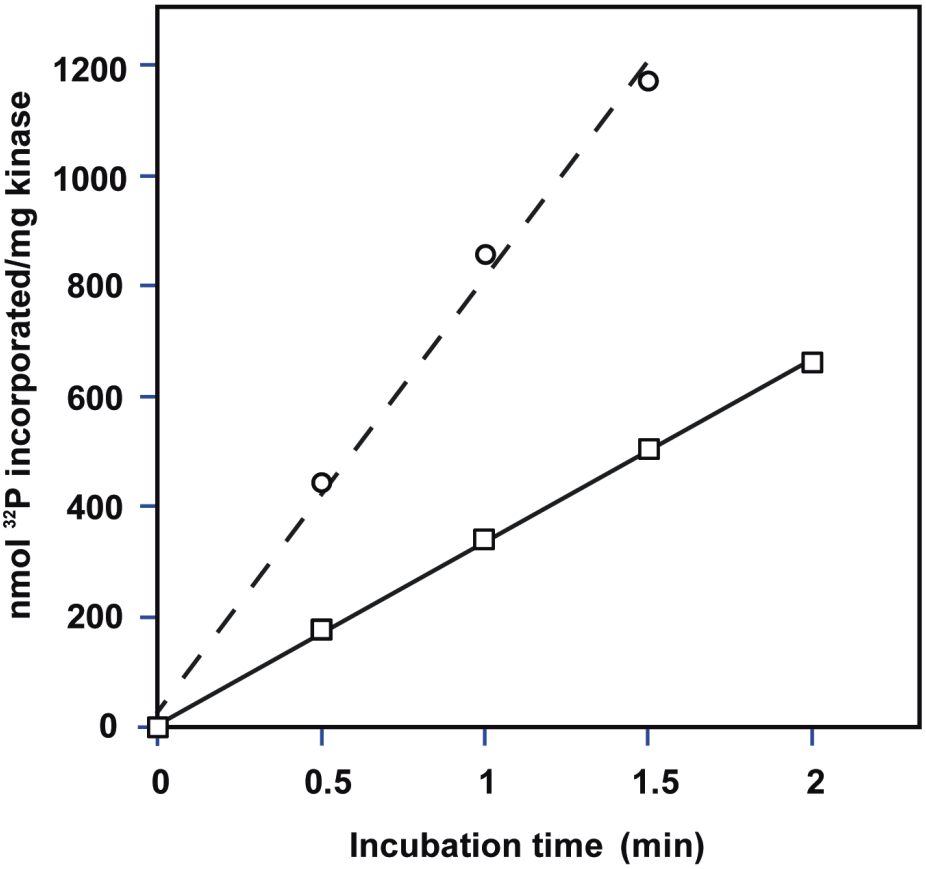
Supplementary Data

Fig. S1. Linearity of PDK activities as functions of the incubation time and kinase concentrations. Activities of different SUMO-PDK isoforms were assayed at 23°C in 20 mM Tris-HCl buffer, 10 mM KCl, 2 mM $MgCl_2$ and different concentrations of E1p (30 μM for PDK1 to 3 and 20 μM for PDK4) using the microcentrifuge-based method described in Experimental Procedures. The phosphorylation reaction was initiated by the addition of 0.5 mM [γ-^{32}P]ATP (specific activity 200-300 cpm/pmol), and terminated by introducing 20% TCA and 50 mM sodium pyrophosphate to the reaction mixture. *A*. Time course of PDK2 and PDK4 activities. The reaction mixture contained 0.2 μM PDK2 or 0.1 μM PDK4. Kinase activities are expressed as nmol ^{32}P incorporated/mg kinase. Each point is the average of triplicate assays. □, PDK2; ○, PDK4. *B*. PDK activities measured with increasing kinase concentrations. The assays were carried out for 1 min with indicated concentration of PDK1, PDK2, PDK3 or PDK4 in the absence and presence of 1.2 μM E2p/E3BP core. Kinase activities are expressed as pmol ^{32}P incorporated/min.

Fig. S2. Structure of the DW-motif in subunit A of PDK4. *A*. The refined (2*Fo-Fc*) electron density depicts the DW-motif of subunit A (brown) bound to the N-terminal domain of subunit B (green). Residues from subunit A and subunit B are shown as ball-and-stick and stick models, respectively. The density is countered at the 1-σ level. *B*. The refined electron (2*Fo-Fc*) density of the bound ADP and water molecules in the PDK4 structure determined using crystals grown in the HEPES buffer. The density is countered at a 1-σ level.

Figure S1

A


nmol ^{32}P incorporated/mg kinase
1200
1000
800
600
400
200
0
0
0.5
1
1.5
2
Incubation time (min)

B

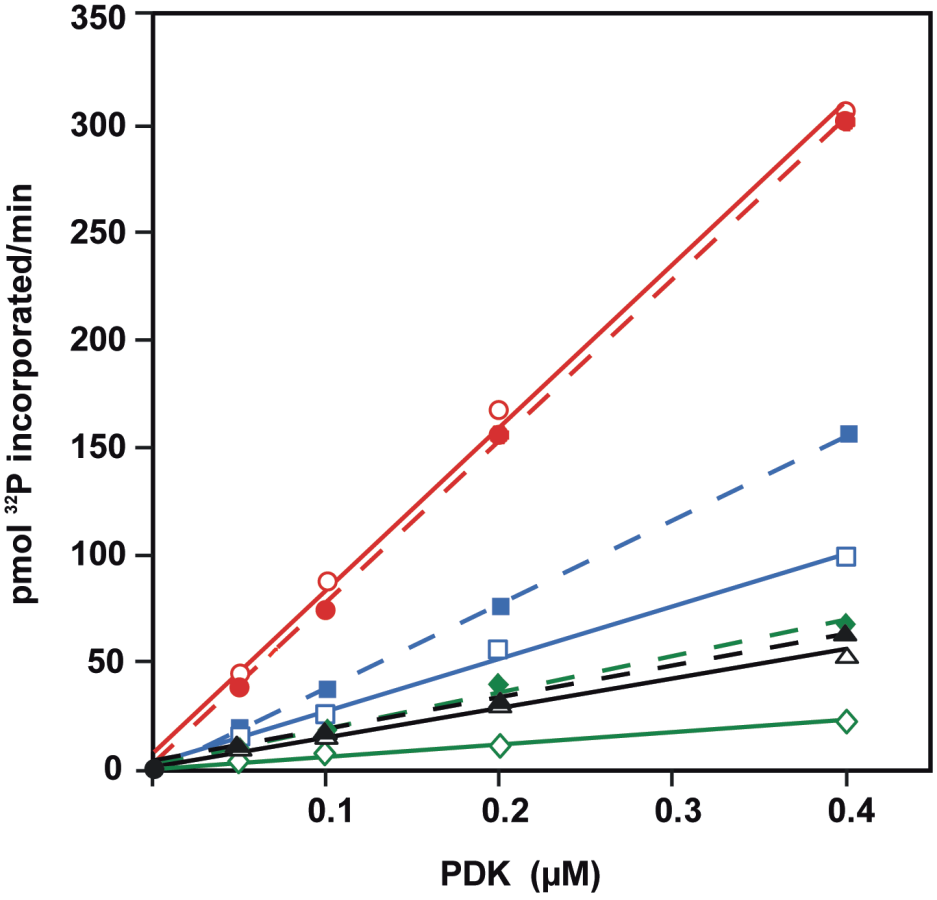
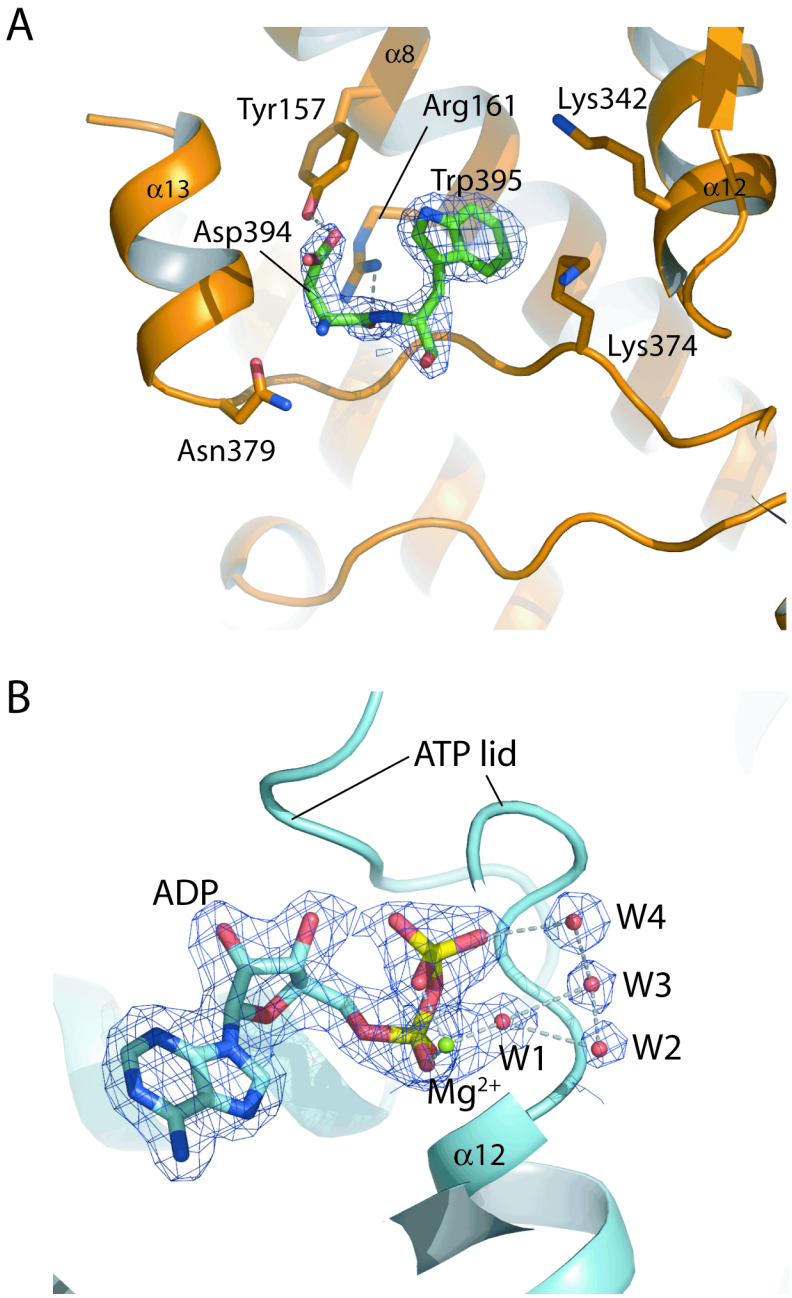

pmol ^{32}P incorporated/min
350
300
250
200
150
100
50
0
0.1
0.2
0.3
0.4
PDK (μM)
PDK1
PDK1 + E2P/E3BP
PDK2
PDK2 + E2P/E3BP
PDK3
PDK3 + E2P/E3BP
PDK4
PDK4 + E2P/E3BP

Figure S2


A
α8
Tyr157
Arg161
Lys342
α13
Trp395
α12
Asp394
Lys374
Asn379
B
ATP lid
ADP
W4
W3
W2
W1
Mg2+
α12