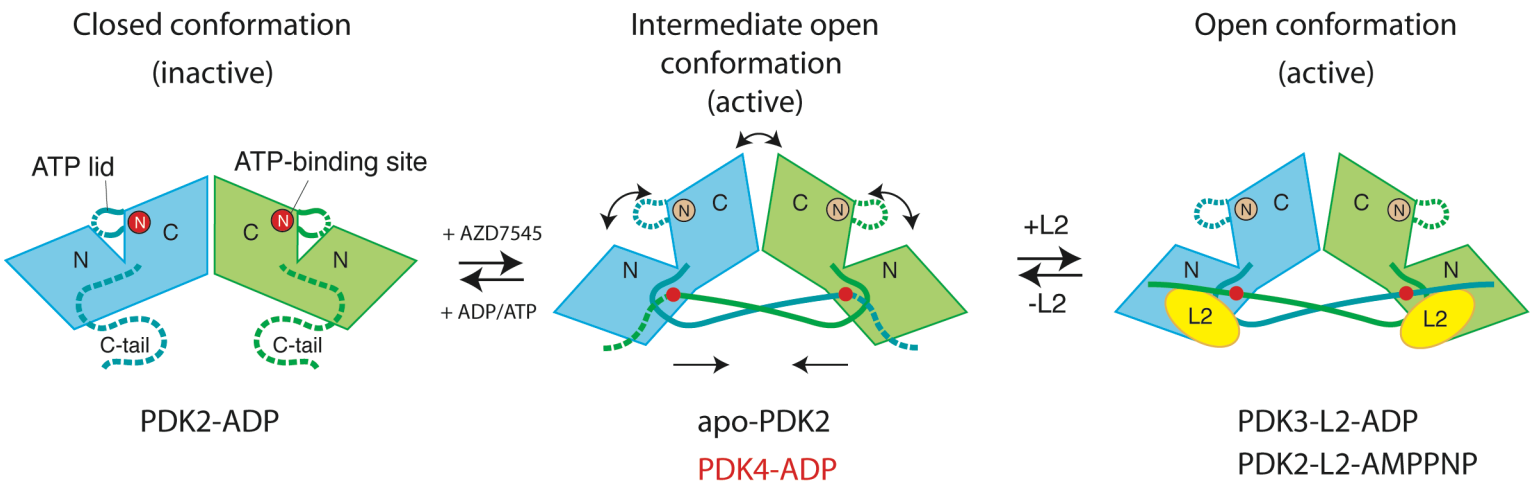
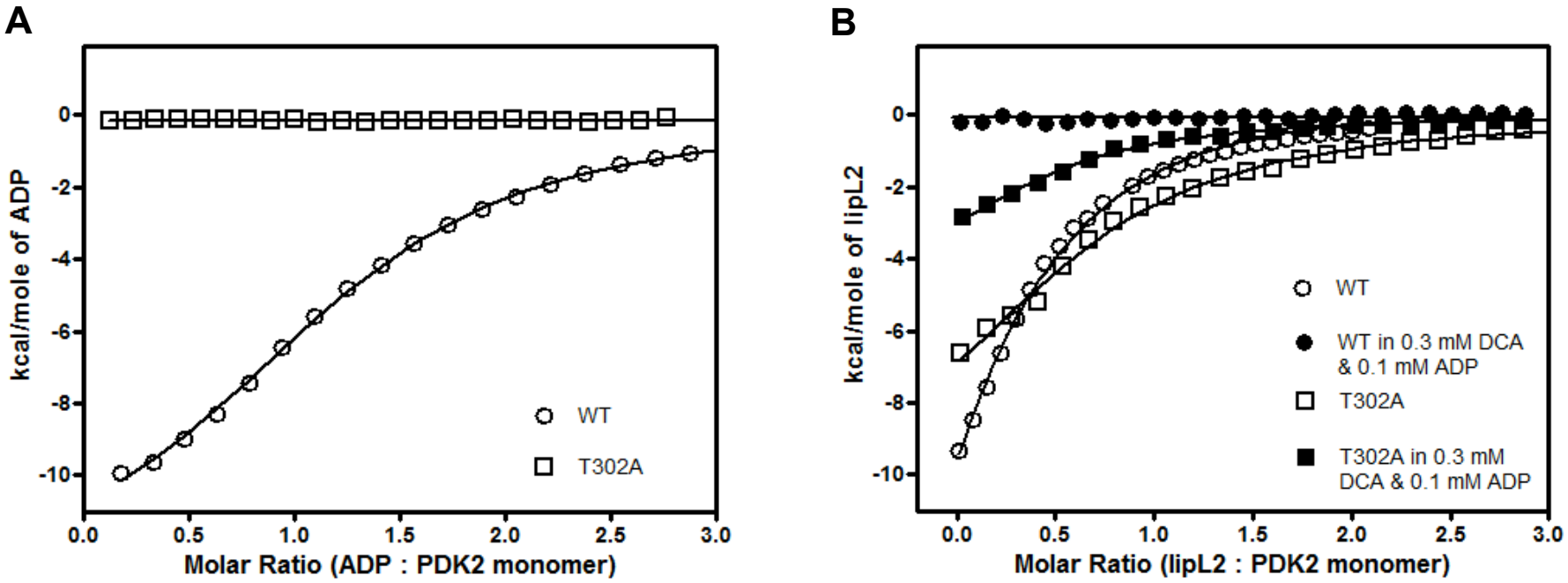


Supplemental Figure S1. **Allosteric models for equilibrium between different conformational states in PDK isoforms.** The inactive closed conformation with disordered C-terminal tails and closed active-site clefts are observed in the rat PDK2-ADP structure (left model) (PDB code: 1JM6) (20). The active "intermediate open" conformation (middle model) with partially ordered C-terminal cross-tails and open active-site clefts is present in human apo-PDK2 (2BTZ) (21) and the PDK4-ADP (2ZKJ and 3D2R) (18) structures. The active open conformation (right model) with fully ordered C-terminal cross-tails and open active-site clefts is present in the human apo-PDK3-L2 (1Y8N) (19) and PDK2-L2-(AMP-PNP) (3CRL) (48) structures. Basal activity of a PDK isoform is determined by the equilibrium between the closed and the intermediate open conformations. The presence of adenine nucleotides ADP/ATP shifts the equilibrium toward the closed conformation (21). The dihydrolipoamide mimetic AZD7545 favors the intermediate open conformation (22). Red spheres, the conserved DW (Asp-Trp)-motif anchoring sites; Letters N and C, the N-terminal and C-terminal domains, respectively, which form the active-site cleft; Circled letter N in red and orange colors, high-affinity and low-affinity nucleotide-binding sites, respectively; Solid and dotted lines, ordered and disordered loop conformations, respectively. This figure is adapted from (18).



Supplemental Figure S2. **Binding affinities of wild-type and T302A variant PDK2 for ADP and lipL2 determined by ITC.** *(A)* The 150 μM concentration of ADP in the syringe was injected into the reaction cell containing 15 μM SUMO-PDK2 (based on the monomer) at 15^0C. One-site binding model of the Origin 7 program was used to fit the binding isotherms. The wild-type PDK2 shows K_d of 7.43 mM for ADP. The T302A mutant exhibits non-measurable ADP binding. *(B)* WT-PDK2, WT-PDK2 in 0.3 mM DCA and 0.1 mM ADP, T302A-PDK2, and T302A-PDK2 in 0.3 mM DCA and 0.1 mM ADP were titrated with lipL2 in the absence or presence of indicated ligands. Wild-type PDK2 show K_d of 14.4 μM for lipL2, and non-measurable lipL2 binding in the presence of DCA and ADP. In contrast, the T302A variant exhibits similar binding affinities for lipL2 either in the absence (K_d = 13.8 μM, ΔH = -12.3 kcal) or presence (K_d = 12.7 μM, ΔH = -6.0 kcal) of DCA and ADP.