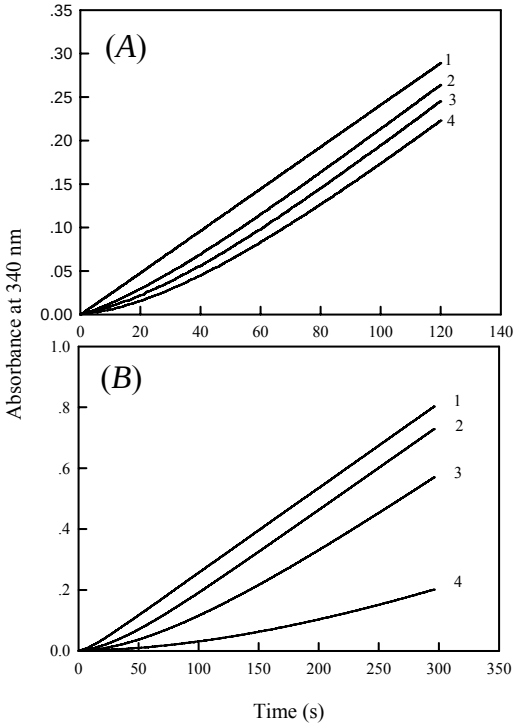
SUPPLEMENTAL LEGENDS

Supplemental Figure 1. Effect of the levels of coupling enzymes (LDH and PK) on the utilization of ADP resulting from p38α/pTpY ATPase activity. (A) Effect of the levels of LDH on the coupled enzyme system. The reaction mixture contained the kinase assay buffer (50 mM MOPS，pH 7.0, 100 mM NaCl, 0.1 mM EDTA, 10 mM $MgCl_2$, 0.2 mM NADH, 1.0 mM PEP), 1 mM ATP , 15 units/mL PK and different concentrations of LDH at 25°C. The reaction was initiated by adding 1.8μM p38α/pTpY, and the absorbance at 340 nm was monitored. The concentrations of LDH were: *curve 1*, 7 units/mL; *curve 2*, 2 units/mL; *curve 3*, 1 units/mL; *curve 4*, 0.3 units/mL. (B) Effect of the levels of PK on the coupled enzyme system. The reaction mixture contained the kinase assay buffer, 1 mM ATP, 20 units/mL LDH and different concentrations of PK at 25°C.. The reaction was initiated by adding 1.8μM p38α/pTpY, and the absorbance at 340 nm was monitored. The concentrations of PK were: *curve 1*, 12 units/mL; *curve 2*, 8 units/mL; *curve 3*, 4 units/mL; *curve 4*, 1 units/mL.

Supplemental Figure 2. The time-dependence absorption profiles for sequential additions of different reagents of the MESG assay system at 25°C. The initial mixture contains the standard phosphatase assay buffer, and 1.0 μM bisphosphorylated p38α. The absorbance at 360 nm was measured following the addition of individual reagents (as indicated) to the reaction mixture: (A) 186 nM MKP5C408S and 186 nM MKP5. (B) 186 nM MKP5 and 0.1 mg/mL purine nucleotide phosphorylase (PNPase). (C) 670 nM PP2CαD239N and 670 nM PP2Cα. (D) 670 nM PP2Cα and 0.1 mg/mL PNPase. (E) 470 nM HePTPC270S and 470 nM HePTP. (F) 470 nM HePTP and 0.1 mg/mL PNPase.

Supplemental Figure 1



Supplemental Figure 2