

Concentration of metal ions (µM)

3						
	Activation of		Mg ²⁺ /ATP	Mn ²⁺ /ATP	Mg ²⁺ /ATP	Mn ²⁺ /ATP
	PP2Ai-PTPA		(100/0.3 μM)	(100/0.3 μM)	(900/9 μ M)	(900/9 μ M)
	pThr (23°C)	K _{cat} (min⁻¹)	305±21	116±3	248±30	127±7
		K _m (μM)	459±52	174±11	500±95	292±29
		K _{cat} /K _m (min ⁻¹ μM ⁻¹)	0.66	0.67	0.50	0.43
	pNPP (37°C)	K _{cat} (min⁻¹)	7.6±0.2	40±1	54±3	37±2
		K _m (μΜ)	227±27	469±52	752±94	589±93
		K _{cat} /K _m (min⁻¹μM⁻¹)	0.03	0.09	0.07	0.06
		e specificity toward r versus pNPP	20	7	7	7

Fig. S7. Examination of tyrosyl phosphatase activity of PP2A associated with PTPAmediated activation. (A) Increase of phosphatase activity toward pNPP, a chemical mimic of phospho-Tyr, associated with activation of PP2A(Mn)i-PTPA by increasing concentration of Mg²⁺ and Mn²⁺ in the presence and absence of ATP. PP2Ac was purified in the presence of Mn²⁺ prior to inactivation by PPi. The ability of Mg²⁺ to increase tyrosyl phosphatase activity depends on the presence of ATP, while Mn²⁺ could increase this activity independent of ATP. The metal ion-specific ATP-dependence for increase of tyrosyl phosphatase activity is similar to activation of phosphoserine/threonine phosphatase activity (Figure 4C). (B) Summary of enzyme kinetics of PP2A-PTPA activated by low and high concentrations of Mg²⁺/ATP or Mn²⁺/ATP. PP2Ai-PTPA was generated as in Figure 4C. Briefly, the PP2Ac in the PP2Ai-PTPA had been exchanged for Zn²⁺ before inactivation by PPi. The enzyme activated by Mg²⁺/ATP gave different level of specificity depending on the concentration of Mg^{2+}/ATP . The complex activated by Mn^{2+}/ATP gave lower specificity regardless of free metal concentration. Experiments were performed in triplicate and repeated three times; representative results are shown in mean ± SEM.