## **Supplementary Data**

Supplementary Figure 1. βγ-methylene ATP as a selective probe of recombinant eNPP1 expressed in HEK293 cells

A: intact HEK293 cells stably transfected with GFP (HEK-GFP) were pulsed with 100 nM exogenous ATP in the absence ( $\blacksquare$ ) or presence ( $\square$ ) of 300 μM β,γ-methylene ATP (MeATP); ( $\bullet$ ) cell free control in the presence of exogenous ATP. *B*: intact HEK293 cells stably transfected with human NPP1 (HEK-NPP1) were pulsed with 100 nM exogenous ATP in the absence ( $\blacksquare$ ) or presence ( $\square$ ) of 300 μM MeATP; ( $\bullet$ ) cell free control in the presence of exogenous ATP; *Insert*, western blot of native HEK293 (HEK), HEK-NPP1, and HEK-GFP cell extracts probed for NPP1 (top), GFP (middle), and actin (bottom). Data represent the average  $\pm$  range from representative experiments repeated three times using  $10^6$  cells suspended in 200 μl BSS at 23°C. *C*, *D*: HPLC chromatograms of extracellular media from intact HEK-GFP cells or HEK-NPP1 cells pulsed with 20 μM exogenous ε-ATP and incubated for the indicated times.

## Supplementary Figure 2. Levamisole-sensitive alkaline phosphatase activity in L cells and cultured VSMC

Alkaline phosphatase activity, measured by pNPP hydrolysis, in lysates of VSMC ( $\square$ ) or two-day cAMP-differentiated (1 mM CPT-cAMP) L-cells ( $\blacksquare$ ). Inset illustrates the activity in VSMC lysates using an expanded Y-axis. Whole cell lysates were assayed in the absence or presence of 5 mM levamisole (Lev). Data represent the means  $\pm$  SE from four separate experiments with each experiment performed in duplicate.