

Supplemental figures and legends

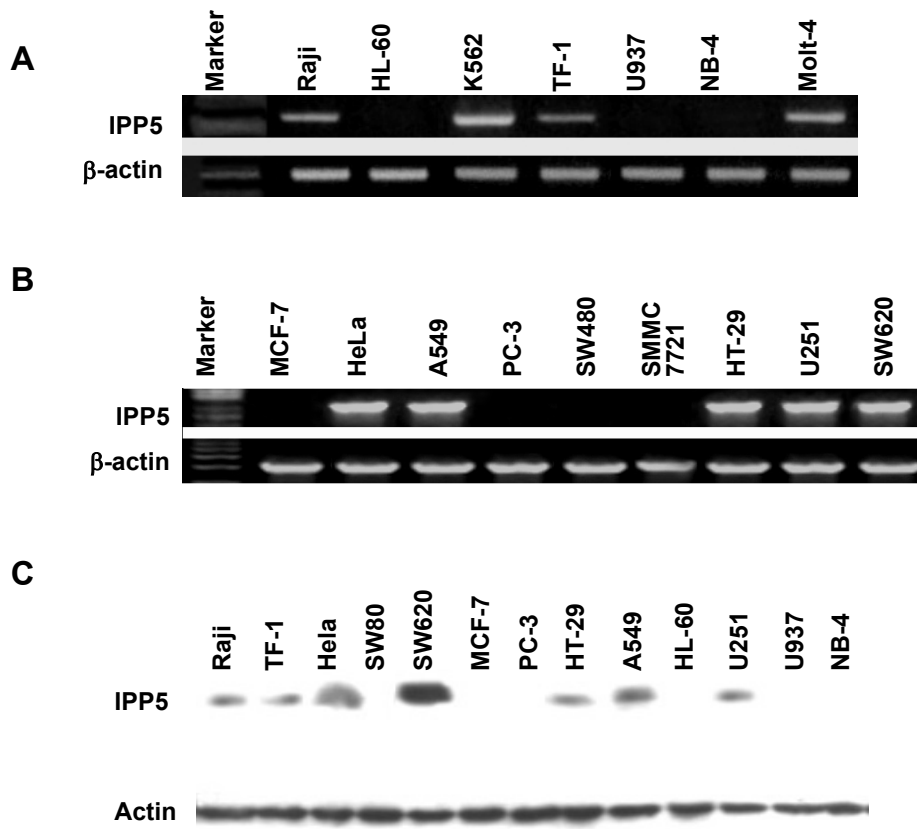
Fig. S1. Sequence alignment of PP1 regulatory subunits in the vicinity of the (R/K)(V/I)XF motif. Human IPP5 contain two conserved motifs of PP1 inhibitory subunits, including KIQF in position on 8th-11th amino acids and Thr-40.

Fig. S2. Expression pattern of IPP5 in cell lines. RT-PCR analysis of IPP5 mRNA expression in (A) human haematological tumor cells, (B) solid tumor cell lines. RT-PCR with IPP5 and human β -actin-specific primers was performed for 30 and 23 cycles, respectively. (C) Lysates of tumor cell lines were analyzed by Western blot using anti-IPP5 antibody.

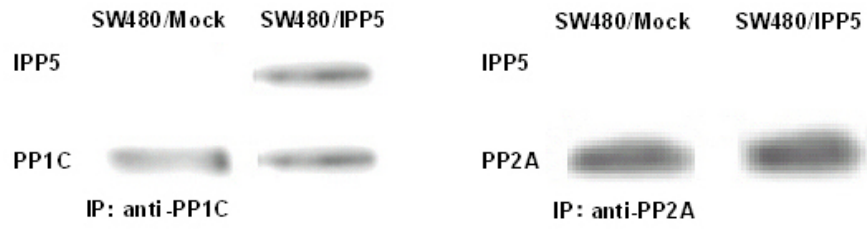
Fig. S3. Specific association of IPP5 with PP1 catalytic subunit but not PP2A. (A) IPP5 interacts with PP1, but not PP2A. Cell lysates of IPP5-B-stably transfected SW480 cells were pre-cleared with protein A-Sepharose beads, and immunoprecipitation (IP) performed using anti-PP2A antibody or anti-PP1 antibody which cross-linked to protein-A Sepharose beads. The immunoprecipitates were then subjected to Western blots with the indicated antibodies. (B) IPP5 has no effect on PP2A phosphatase activity. Cell lysates of stably transfected SW480 cells were pre-cleared with protein A-Sepharose beads (Sigma), and immunoprecipitation performed using anti-PP2A antibody cross-linked to protein-A Sepharose beads. Samples were subjected to PP2A phosphatase activity analysis using the non-radioactive Serine/Threonine phosphatase Assay System.

Fig. S4. More significant proliferation of IPP5-overexpressing SW480 cells stimulated with isoproterenol. The stable transfectants of SW480 cells were serum-starved for 24 hr, then stimulated with 100 μ mol/L isoproterenol which was used to elevate intracellular cAMP, in complete RPMI 1640 medium for 48 hr. The proliferation of the transfectants was detected by [³H] thymidine incorporation. *, $p < 0.05$ versus mock.

PP1-binding subunit	(R/K) (V/I)x F motif	Residues
G _M -subunit	S G G R R V S F A D N	61 - 71
G _L -subunit	K V K K R V S F A D N	57 - 67
G _L -related protein	Q A K K R V V F A D S	80 - 90
M ₁₁₀ subunit	R Q K T K V K F D D G	31 - 41
p53BP2	A H G M R V K F N P L	794 - 804
IPP-1	N S P R K I Q F T V P	5 - 15
DARPP32	K D R K K I Q F S V P	4 - 14
IPP-5	N S P K K I Q F A V P	4 - 14
NIPP-1	R K N S R V T F S E D	196 - 206
splicing factor PSF	G R Q L R V R F A T H	12 - 22



A



B

