Supplementary Experimental Procedures

Plasmid construction— pHM6-wt-p27^{Kip1}, pc5FLAG-wt-Pim-1, and pHM6-wt-Pim-1 plasmids were established in our laboratory (1). To generate an EGFP-tagged p27^{Kip1} carboxy-terminal peptide, the coding sequence of T198wt (corresponding to amino acids188–198) was inserted into pEGFP-C2 vector using a PCR-based insertion method. The EGFP-tagged mutant p27^{Kip1} peptides, in which both Arg^{194} and Arg^{196} were substituted by Asp (T198mu) or Thr^{198} was substituted by Ala, Asp or Glu (T198A, T198D or T198E, respectively) were generated using QuickChange site–directed mutagenesis kit (Stratagene) with pEGFP-C2-T198wt as the template.

Design and synthesis of carboxy-terminal p27^{Kip1} peptides— Because arginine-rich peptides are efficiently translocated through the cell membrane (2), carboxy-terminal p27^{Kip1} T198wt (KKPGLRRRQT), p27^{Kip1} T198mu (KKPGLDRDQT), and Thr^{198}-phosphorylated p27^{Kip1} (T198phospho; KKPGLRRRQpT; pT represents phosphorylated Thr) were conjugated with eight arginine residues (GGGRRRRRRRRGC) to form GGGRRRRRRRGC-KKPGLRRRQT, GGGRRRRRRGC-KKPGLDRDQT, and GGGRRRRRRGC-KKPGLRRRQpT respectively. These peptides and their FITC-labeled peptides were chemically synthesized (Toray Research Center, Tokyo, Japan). The purity of the peptides as determined by reversed-phase high-performance liquid chromatography analysis was >95%.

Small interfering RNA design and transfection— Two small interfering RNA (siRNA) constructs were designed from the human *pim1* sequence. The coding strands of the siRNA were CCAUGGAAGUGGUCCUGCUGAAGA A (*pim1-1*; directed to 623–648) and GUAUGAUAUGGUGUGUGGAGAUAU U (*pim1-2*; directed to 1059–1084). Non-silencing control siRNA was purchased from Invitrogen.

Recombinant protein purification— BL21 Star (DE3) pLysS *Escherichia coli* (Invitrogen) containing a pET28a plasmid encoding human Pim-1 (amino acids 14–313) was cultured with shaking in LB medium. The overnight culture was diluted 100× with fresh LB medium. The culture was incubated at 20°C with shaking until an OD_{600} of 0.5 was obtained, and then for an additional 4 h with 1 mM isopropyl–β-D-thiogalactopyranoside at 20°C with shaking. Cells were harvested and lysed in lysis buffer (20 mM Tris-HCl (pH 7.6), 150 mM NaCl, 20% glycerol, 1 mg/ml lysozyme, 1 mM DTT, and 10 mM imidazole) using the freeze-thaw method.

After ultracentrifugation, the supernatant fraction was loaded onto a HisTrap FF crude column (GE Healthcare), which was washed with washing buffer (50 mM NaH_2PO_4 (pH 8.0), 300 mM NaCl, 1 mM DTT, 10% glycerol, and 20 mM imidazole) and then eluted with a linear imidazole gradient using 50 mM NaH_2PO_4 (pH 8.0), 300 mM NaCl, 1 mM DTT, 10% glycerol, and 500 mM imidazole as the elution buffer. Fractions of interest were pooled and dialyzed against 50 mM NaH_2PO_4 (pH 8.0), 100 mM NaCl, 1 mM DTT, and 10% glycerol. The sample was further loaded onto a MonoQ 10/100 GL column (GE Healthcare), which was washed with washing buffer (20 mM Tris-HCl (pH 7.6), 5 mM DTT, and 10% glycerol) and then eluted with a linear NaCl gradient using 20 mM Tris-HCl (pH7.6), 5 mM DTT, 10% glycerol, and 1 M NaCl as the elution buffer. Fractions of interest were pooled and further purified by gel filtration on a Superdex 75 pg column (GE Healthcare) with 20 mM Tris-HCl (pH 7.6), 200 mM NaCl, 5 mM DTT, and 10% glycerol as the buffer. The sample was concentrated in an Amicon Ultra unit (Millipore).

Preparation and crystallization of Pim-1-p27^{Kip1} peptide (T198wt peptide) complex and data collection— The p27^{kip1} peptide (T198wt) used for crystallization with Pim-1 was synthesized as described below. Pim-1 (10 mg/ml) was mixed with a 1.5-fold molar excess of p27^{kip1} peptide (T198wt), 5 mM AMP-PNP, and 5 mM $MgCl_2$. The protein was crystallized using the hanging drop vapor diffusion method at 4°C. Diffraction quality crystals of Pim-1 complexed with the p27^{kip1} peptide (T198wt) were obtained by mixing equal volumes of the protein solution with a reservoir solution containing 0.6 M sodium acetate trihydrate, and 0.1 M imidazole buffer at pH 6.2. Single crystals were coated with the reservoir solution containing 35% glycerol, mounted using a nylon loop (Hampton Research) and flash-cooled in the cold stream of the goniometer. Data were collected at 100 K with a wavelength of 0.9700 Å at BL26B2, SPring-8, Harima. Diffraction data were processed with the HKL2000 program (3).

Structure determination and model refinement— The structure of Pim-1-AMP-PNP in the presence of T198wt was determined by molecular replacement using the coordinate of the AMP-PNP bound form of Pim-1 (PDBID: 1XYT) as a search model, using the program MOLREP (CCP4). The model was corrected iteratively using O (4) and refined using the Crystallography & NMR system (CNS) (5). Refinement statistics are presented in Table S1. The quality of the model was inspected using the program PROCHECK (6). Graphic figures were created using the program

PyMOL (DeLano Scientific).

Surface Plasmon Resonance (SPR) analysis— Biosensor analyses were performed on a BIACORE T100 system (GE Healthcare). Recombinant Pim-1 dissolved in 25 mM HEPES, 5 mM DTT, pH 6.0, was covalently attached to a CM5 sensor chip (GE Healthcare) by the amine coupling method according to the manufacturer's instructions. Final levels of immobilization were about 2000 response units. Analyses were performed at 25 °C using 10 mM HEPES, pH 7.4, 200 mM NaCl, 2.5 mM $MgCl_2$, 200 μM AMP-PNP, 1 mM DTT, 5% DMSO, and 0.05% surfactant P20 as a driving buffer at a flow rate of 30 μl/min. All experiments were carried out at 25 °C at a constant flow rate of 30 μl/min driving buffer. The analyte (FITC-labeled R8-T198wt, R8-T198phospho, and R8-T198mu peptides) diluted in driving buffer were passed over the immobilized Pim-1 for 2 min followed by passing over the surface with driving buffer alone for 2 min. Six concentrations of analyte (15.63, 31.25, 62.5, 125, 250, and 500 nM) were passed over the chip. Sensorgrams were fit by global analysis using Biacore T100 evaluation software. Equilibrium dissociation constant (K_D) was determined from the steady-state affinity model.

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References

1. Morishita, D., Katayama, R., Sekimizu, K., Tsuruo, T., and Fujita, N. (2008) *Cancer Res* **68**, 5076-5085
2. Yang, L., Mashima, T., Sato, S., Mochizuki, M., Sakamoto, H., Yamori, T., Oh-Hara, T., and Tsuruo, T. (2003) *Cancer Res* **63**, 831-837
3. Otwinowski, Z., and Minor, W. (1997) *Methods Enzymol* **276**, 307-326
4. Jones, T. A., Zou, J. Y., Cowan, S. W., and Kjeldgaard, M. (1991) *Acta Crystallographica* **47**, 110-119
5. Brünger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J. S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T., and Warren, G. L. (1998) *Acta Crystallogr D Biol Crystallogr* **54**, 905-921
6. Laskowski, R. A., Macarthur, M. W., Moss, D. S., and Thornton, J. M. (1993) *J Appl Crystallogr* **26**, 283-291