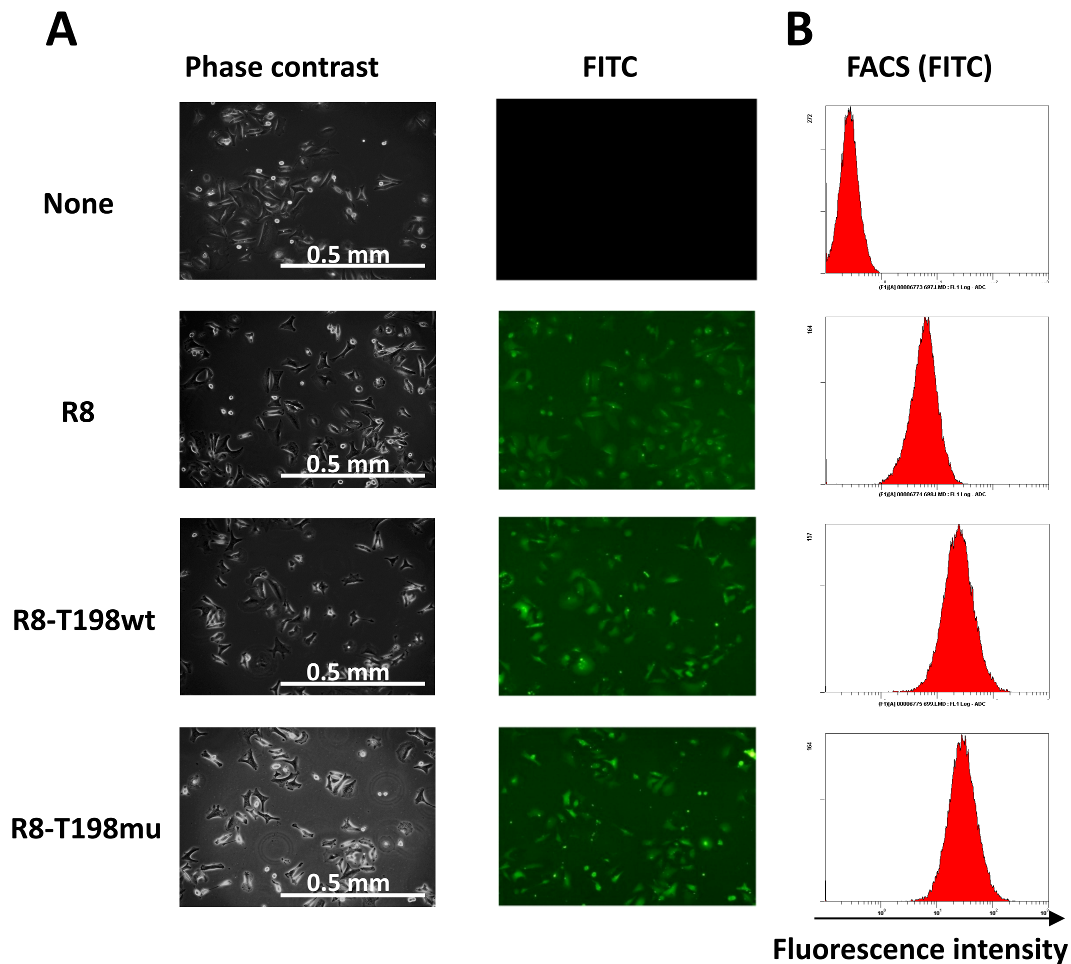
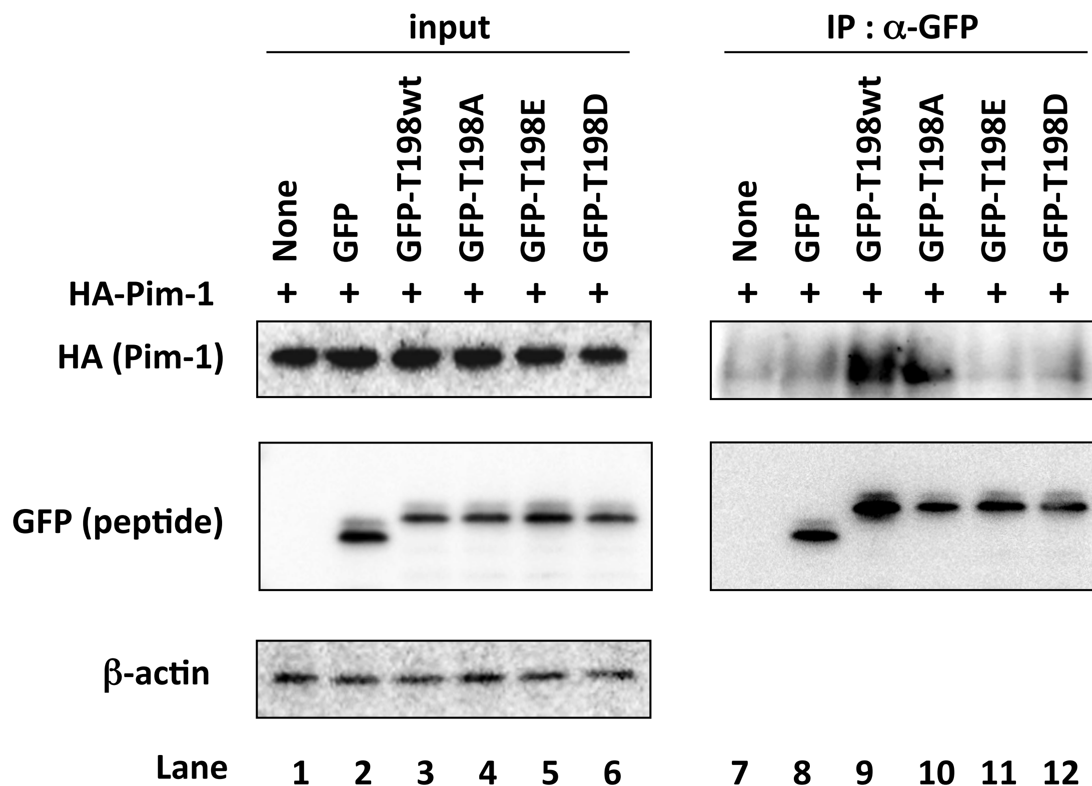


Supplementary Figure S1.

A. Du145 cells were incubated with 20 μM FITC-labeled cell-permeable R8, R8-T198wt or R8-T198mu peptide or without any peptide (None) for 4 h. After washing, specimens were viewed with both phase contrast and fluorescence (FITC) images.

B. Internalization of FITC-labeled R8, R8-T198wt or R8-T198mu peptide was confirmed by performing flow cytometric analysis.

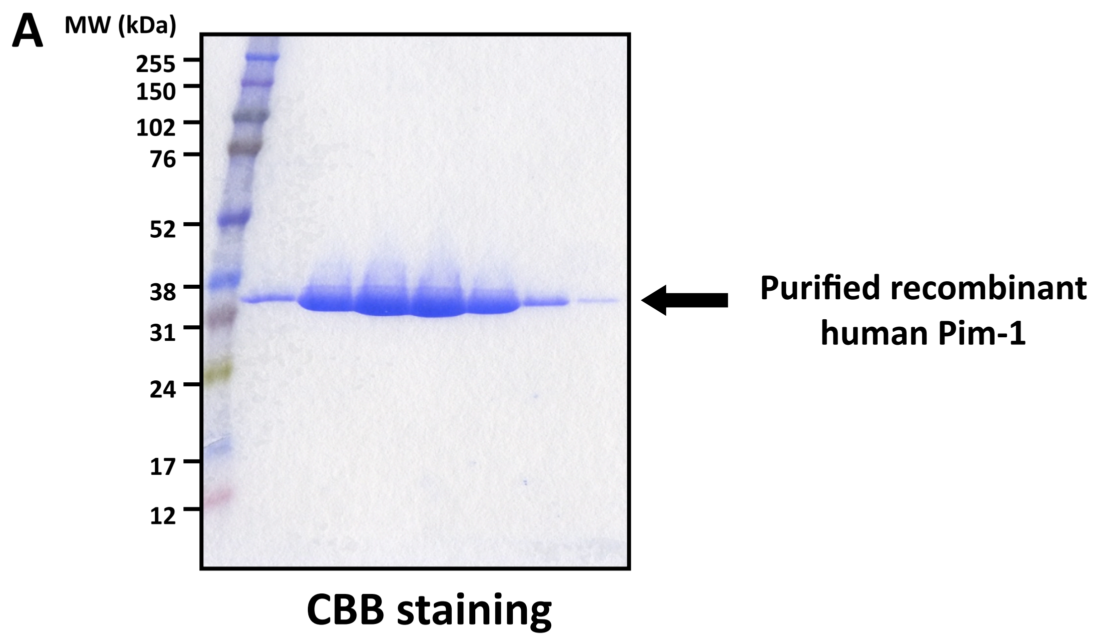
Supplementary Figure S1

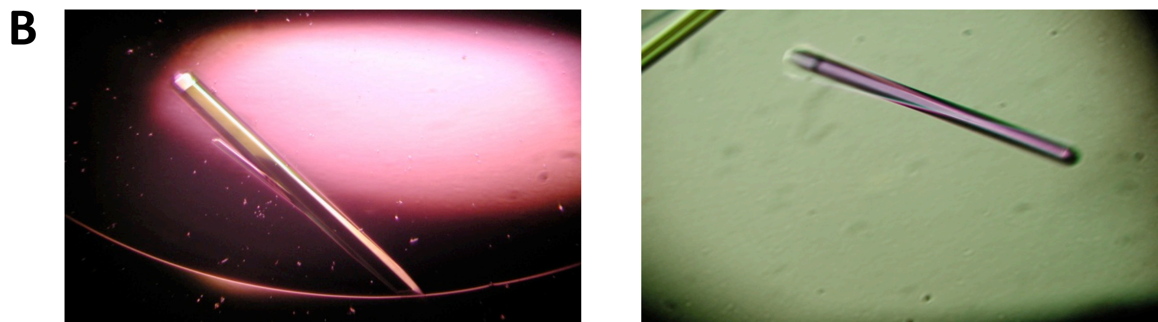


Supplementary Figure S2.

HEK293T cells were not transfected (None) or were transfected with empty pEGFP-C2 vector (GFP), pEGFP-C2-T198wt (GFP-T198wt) , pEGFP-C2-T198A (GFP-T198A), pEGFP-C2-T198E (GFP-T198E) or pEGFP-C2-T198D (GFP-T198D) together with the pHM6–Pim-1 plasmid (+). After transfection for 24 h, cells were lysed, and the GFP-tagged peptides were immunoprecipitated from the cell lysates with protein A–agarose-conjugated anti-GFP antibody. The immunoprecipitated proteins (IP) and cell lysates (input) were subjected to immunoblot analysis with the indicated antibodies.

Supplementary Figure S2

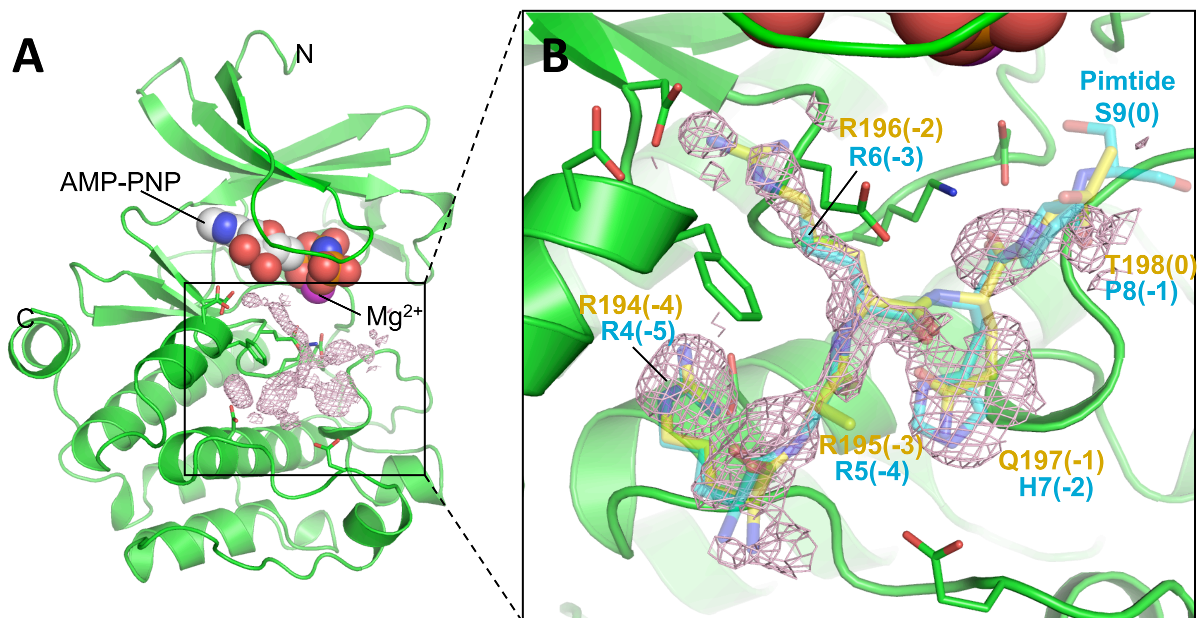




Supplementary Figure S3.

A. Purified recombinant human Pim-1 (residues 14-313) was electrophoresed and stained with CBB.
B. Photomicrograph of crystals of Pim-1 in complex with carboxy-terminal $p27^{Kip1}$ peptide (T198wt peptide). Large needle-like crystals were appeared.

Supplementary Figure S3

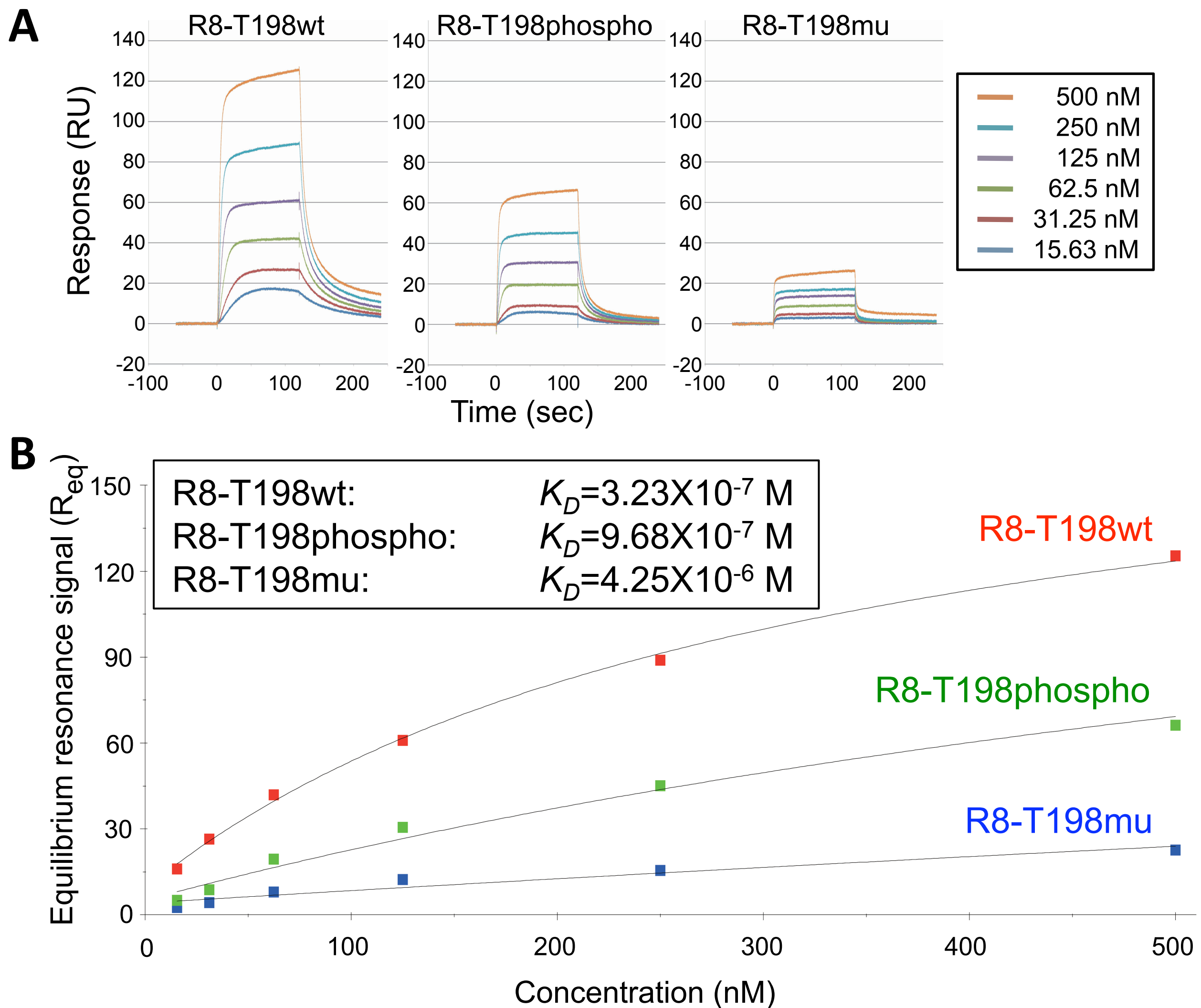


Supplementary Figure S4.

A. Structural overview of Pim-1 co-crystallized with the carboxy-terminal human p27^{Kip1} (T198wt) peptide. Bound AMP-PNP and magnesium ion are shown in *sphere representation*. The 2*Fo*-*Fc* electron density map for the substrate-binding site is contoured at 0.8 σ.

B. Enlarged view of substrate-binding site with superimposition of pimtide bound to Pim-1 (cyan, PDB ID: 2BZK) and a hypothetical model of the T198wt peptide (yellow). Substrate-binding residues of Pim-1 are represented by green stick models.

Supplementary Figure S4

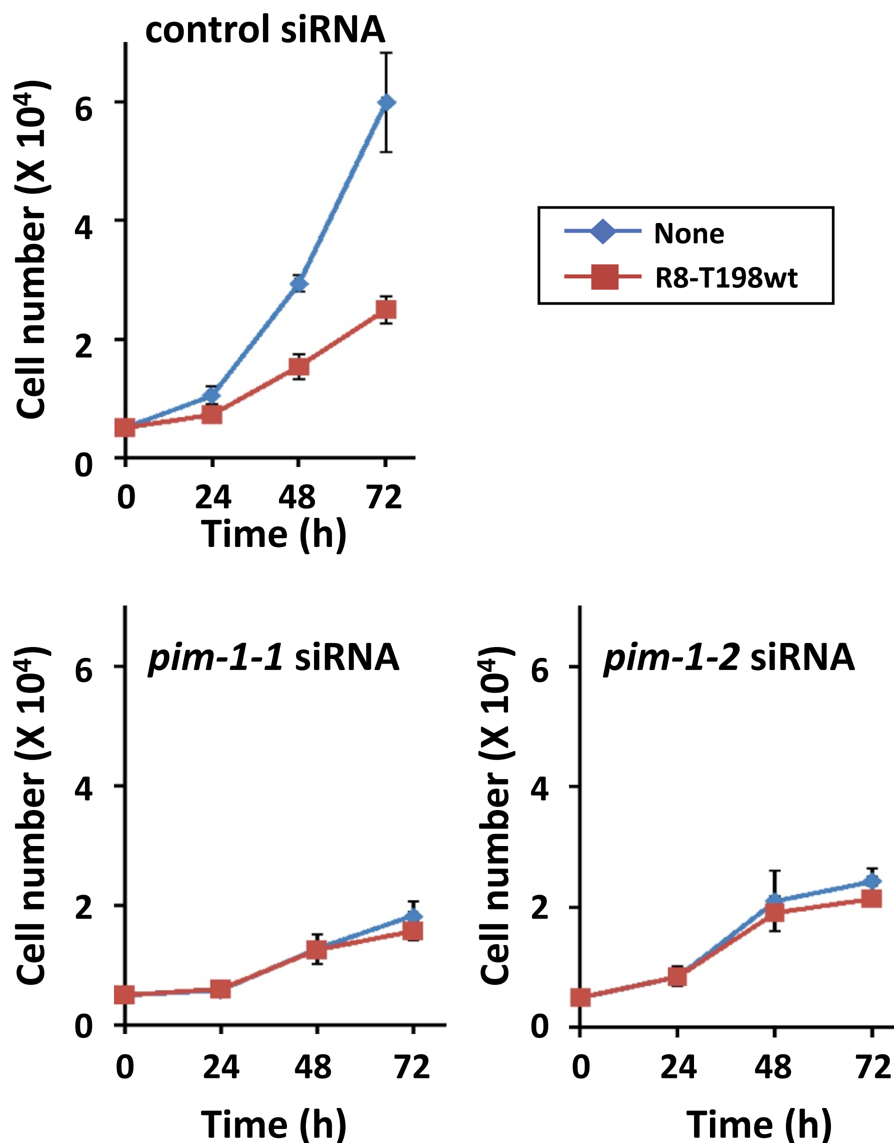


Supplementary Figure S5.

A. Interaction between analyte (FITC-labeled R8-T198wt, R8-T198phospho, and R8-T198mu peptides) and recombinant Pim-1 monitored in real time by Biacore T100. Increasing concentrations of analyte (15.63-500 nM) were passed over a sensor chip with immobilized Pim-1 for 120 s (association phase) before the flow was switched to buffer alone for another 120 s (dissociation phase).

B. Equilibrium response signal (R_{eq}) were plotted against peptide concentration. Using global fitting, equilibrium dissociation constant (K_D) was determined from the steady-state affinity model. Repeated experiments gave similar K_D value.

Supplementary Figure S5



Supplementary Figure S6.

Du145 cells were transfected with control, *pim-1-1* or *pim-1-2* siRNA using the LipofectAMINE RNAi MAX. After transfection for 24 h, cells were incubated with vehicle (None) or 10 µM FITC-labeled R8-T198wt peptide. At the indicated time points, viable cell number was counted by trypan blue dye exclusion method.

Supplementary Figure S6