Supplemental Material

Selective blockade of cancer cell proliferation and anchorage-independent growth by Plk1 activity–dependent suicidal inhibition of its polo-box domain

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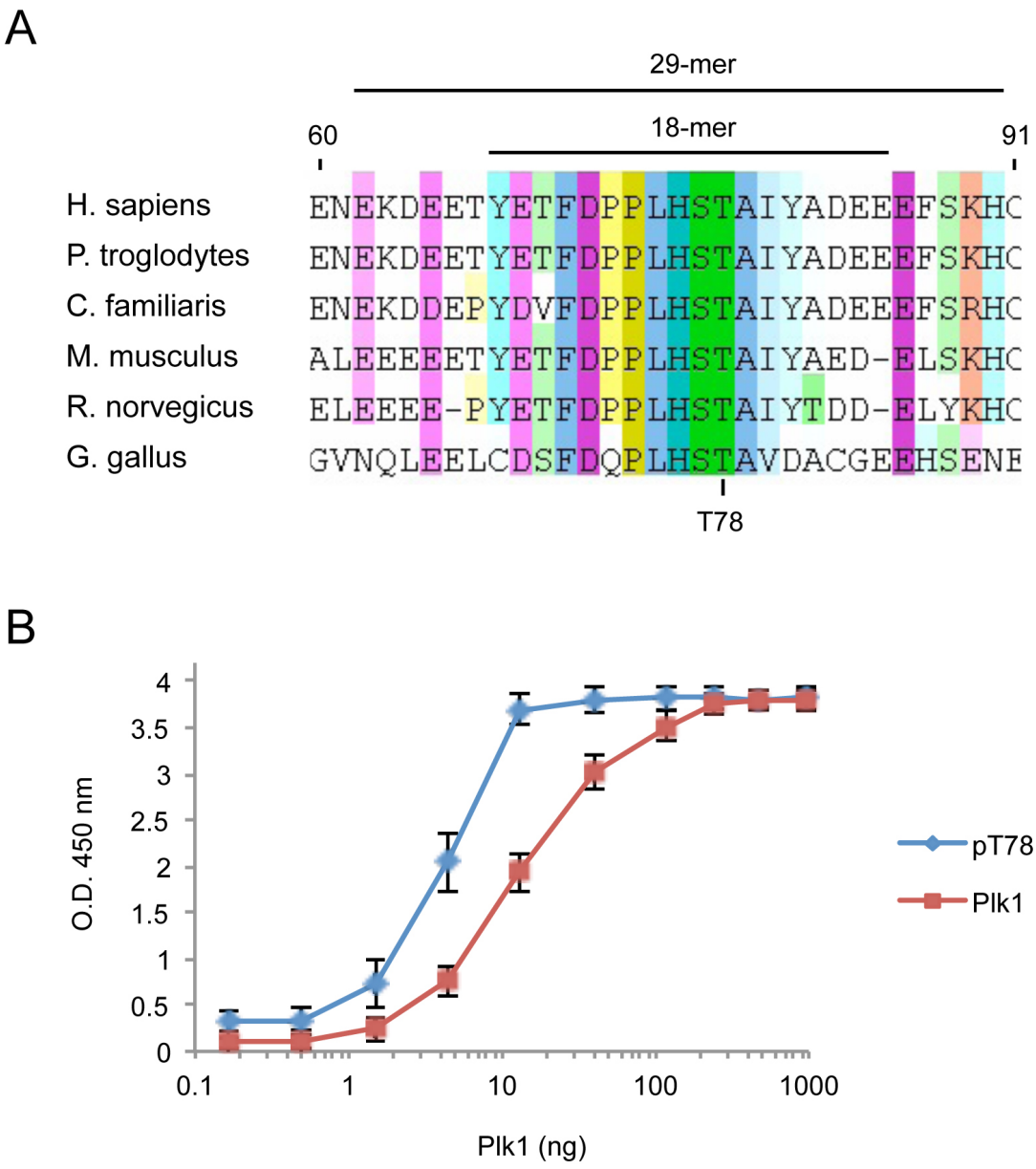

Figure S1. Plk1-dependent generation of the p-T78 epitope and induction of the Plk1-PBIPtide interaction. **(A)** Sequence alignment of the T78 region among PBIP1 orthologs and the PBIPtides (29-mer and 18-mer) used in this study are shown. Numbers indicate the residues in human PBIP1. Colors indicate conserved residues. **(B)** Plk1 generates and binds to PBIPtide p-T78 in a concentration-dependent manner. Various amounts of

recombinant Plk1 [1] purified from Sf9cells were incubated with GST-PBIPtide [2] coated on ELISA wells. The level of the p-T78 epitope generated and the amount of Plk1 bound to the p-T78 epitope were quantified by using anti-p-T78 and anti-Plk1 antibodies, as described previously [2]. Bars, standard deviation.

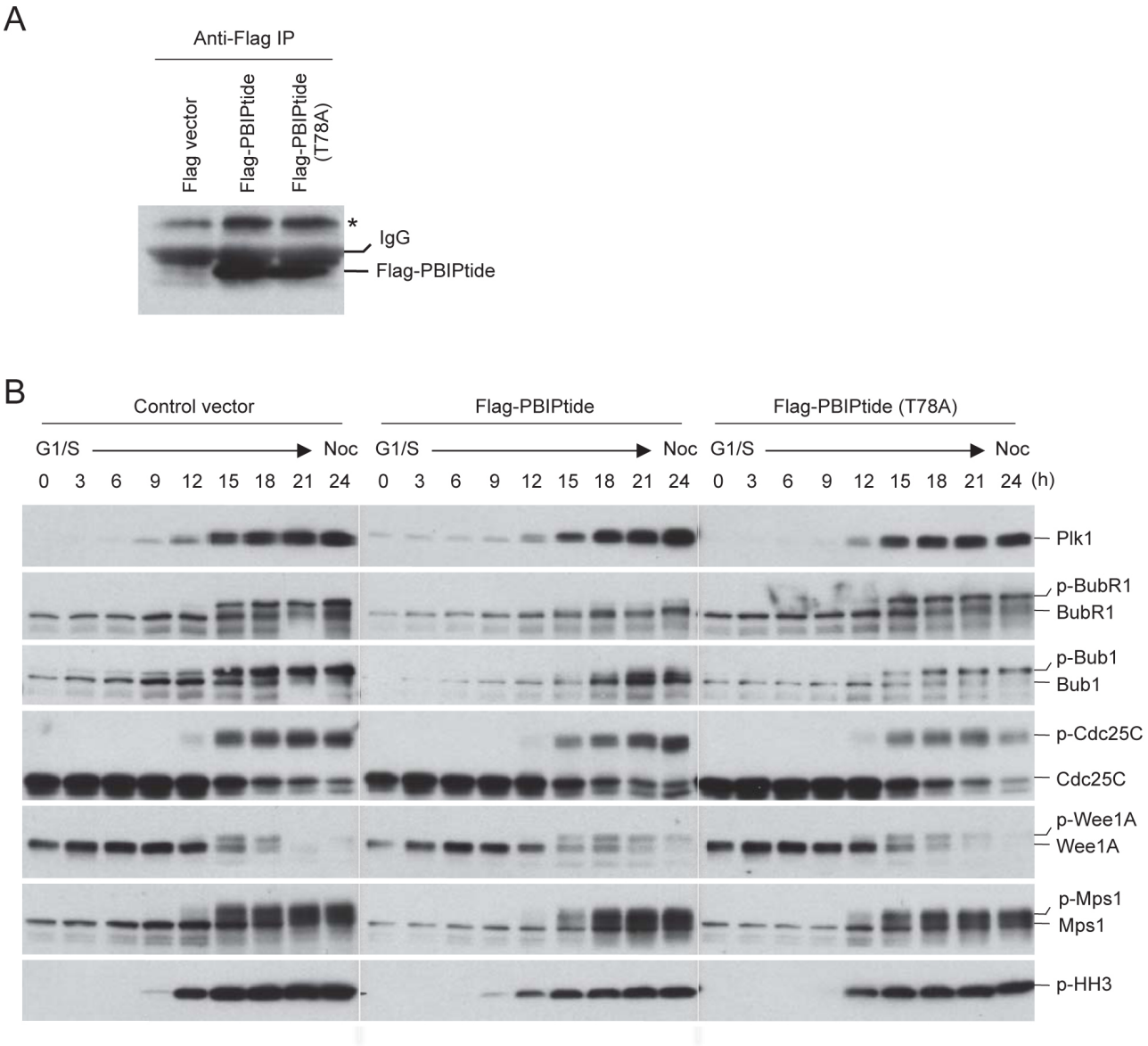

Figure S2. Flag-fused PBIPtide induces improper BubR1 phosphorylation in HeLa cells. (**A**) HeLa cells were infected with lentiviruses expressing the indicated Flag-tagged constructs. To detect the expressed ligand, Flag-fused ligands were immunoprecipitated and immunoblotted. Asterisk, a cross-reacting protein. (**B**) The same cells in (**A**) were arrested by a double thymidine block (G1/S) and released into medium containing nocodazole (Noc) to trap the cells in mitosis. Immunoblotting analyses were performed using the samples harvested at the indicated time points after G1/S release. p-histone H3 (p-HH3) was used to indirectly assess cells entering mitosis.

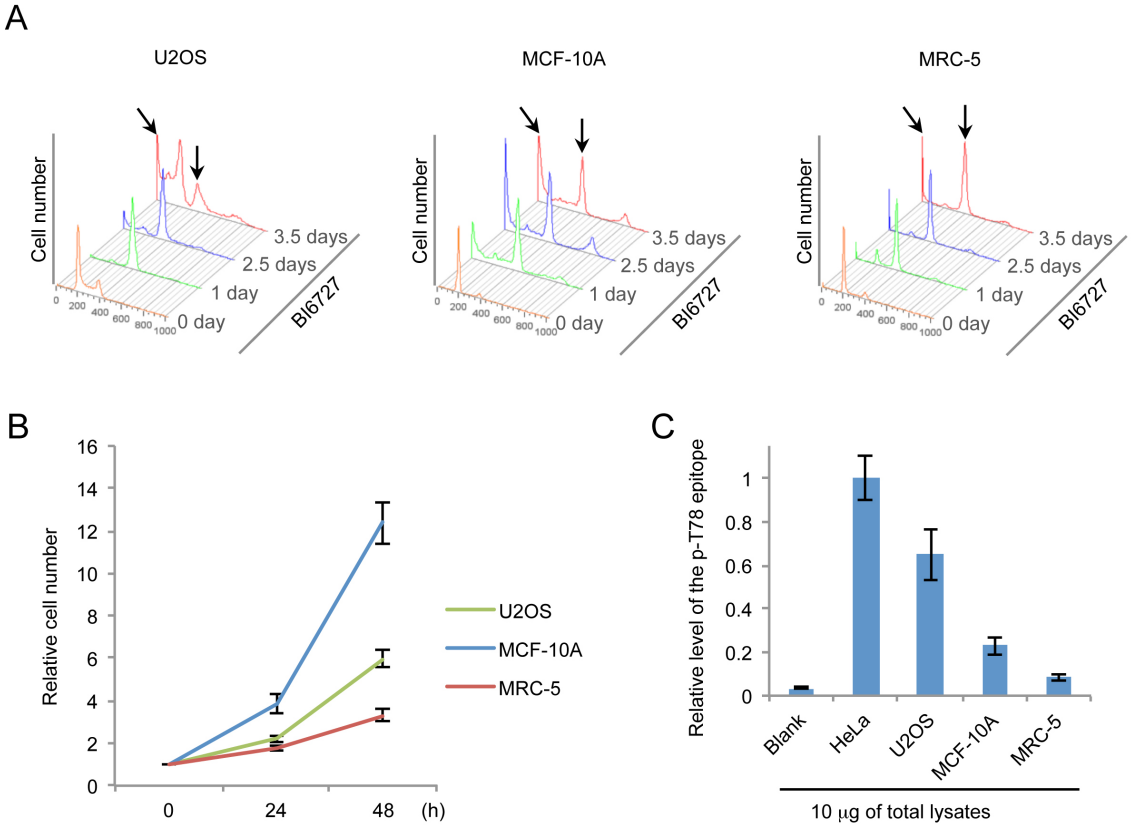

Figure S3. Growth rates and intracellular Plk1 activities for U2OS, MCF-10A, and MRC-5 cells and the effect of BI6727 treatment. **(A)** Cells were cultured asynchronously and treated with 100 nM of BI6727, a Plk1-specific inhibitor [3]. At the indicated time point after treatment, cells were harvested and subjected to flow cytometry analysis. Arrows indicate an apoptotic sub-G1 population (slanted) and a population arrested in G2/M with 4N DNA content (vertical). **(B)** Triplicate samples of asynchronously growing cells were quantified at the indicated time points. Doubling time was calculated using the "Least Squares Fitting—Exponential" method [4]. Bars, standard deviation. **(C)** Total lysates (10 μg) prepared asynchronously growing cells were used to carry out an ELISA-based Plk1 activity assay using immobilized GST-PBIPtide as substrates. After reaction, the level of the p-T78 epitope (a reaction product of Plk1 kinase activity) was determined by using anti-p-T78 antibody and HRP-conjugated secondary antibody, as described previously [2]. Quantification was performed from three independent experiments. Bars, standard deviation.

References:

1. Lee KS, Yuan Y-L, Kuriyama R, Erikson RL. Plk is an M-phase-specific protein kinase and interacts with a kinesin-like protein, CHO1/MKLP-1. Mol Cell Biol 1995; 15:7143-51.

2. Park J-E, Li L, Park J, Knecht R, Strebhardt K, Yuspa SH, et al. Direct quantification of polo-like kinase 1 activity in cells and tissues using a highly sensitive and specific ELISA assay. Proc Natl Acad Sci USA 2009; 106:1725-30.

3. Rudolph D, Steegmaier M, Hoffmann M, Grauert M, Baum A, Quant J, et al. BI 6727, A Polo-like Kinase Inhibitor with Improved Pharmacokinetic Profile and Broad Antitumor Activity. Clin Cancer Res 2009; 15:3094-102.

4. Weisstein EW. Least Squares Fitting--Exponential. MathWorld--A Wolfram Web Resource http://mathworld.wolfram.com/LeastSquaresFittingExponential.html.