Materials and methods

Microinjection to synchronized cells

Hela cells were synchronized at G1/S transition by a double thymidine block. In brief, cells were treated with 2.5 mg/ml thymidine (Sigma) for 17 h and thymidine was removed for 9 h. 2.5 mg/ml thymidine was re-added to the cells for 15 h. Cells were released by removing thymidine for 0 h (G1/S), 4 h (S) and 11 h (G2/M). Flag-p27 was microinjected into the Hela cytoplasm and incubated for 30 min at 37 ºC. BrdU (50 □g/ml) was added to the culture medium 30 min before injection. Subcellular localization of Flag-p27 was detected by indirect immunofluorescence using anti-Flag antibody and Alexa 546 labeled secondary antibody. After re-fixation and treatment with 4 N HCl, the incorporation of BrdU was detected by anti-BrdU antibody (BioRad) and Alexa 488 labeled secondary antibody (Molecular Probe).

Figure legend

Fig. S2. Cell cycle-independent nuclear translocation of exogenously injected p27.

Hela cells were synchronized at G1/S by a double thymidine block and released. Flag-p27 was injected into the Hela cytoplasm at indicted phase. After 30 min, cells were fixed and subcellular localization of p27 was detected by anti-Flag antibody. Incorporation of BrdU was detected by anti-BrdU antibody.

Fig. S2 Sekimoto et al.