Supplementary materials

Results and Discussion

It is well known that in certain cases, Asp or Glu can mimic the phosphorylated state of serine and threonine residues. Indeed, S10D or S10E of p27 acted in the same manner as phosphorylated residues (Rodier et al., 2001; Ishida et al., 2002). Thus, we changed Thr157 of full-length p27 to Asp (Flag-p27 T157D) to assess the phosphorylated form. Although Liang et al showed that nuclear localization of p27 was impaired when YFP-fused p27 T157D and His-tagged p27 T157D were used for transfection and an in vitro transport assay, respectively (Liang et al., 2002), Flag-p27 T157D was translocated into the nucleus as effectively as wild type, as evidenced by transfection (Fig. S1A) and microinjection of recombinant proteins (data not shown), and Flag-p27 T157D bound to importin □3 and □5 to a similar extent as wild type (Fig. S1B). Therefore Asp did not mimic the phosphorylated state of Thr157 in our assay. These results support our view that phosphorylation, but not a negative charge is critical for the binding of 14-3-3 to Thr157-phosphorylated p27.

Materials and methods

Transfection and solution binding assay

Site directed mutagenesis to Thr157 was performed using QuikChange

Mutagenesis kit (Stratagene). pcDNA-Flag-p27 (wild, T157A or T157D) was transfected to Hela cells and subcellular localization of Flag-p27 were detected by indirect immunofluorescence using anti-Flag-antibody.

Solution binding assays were performed as described in the main text.

References

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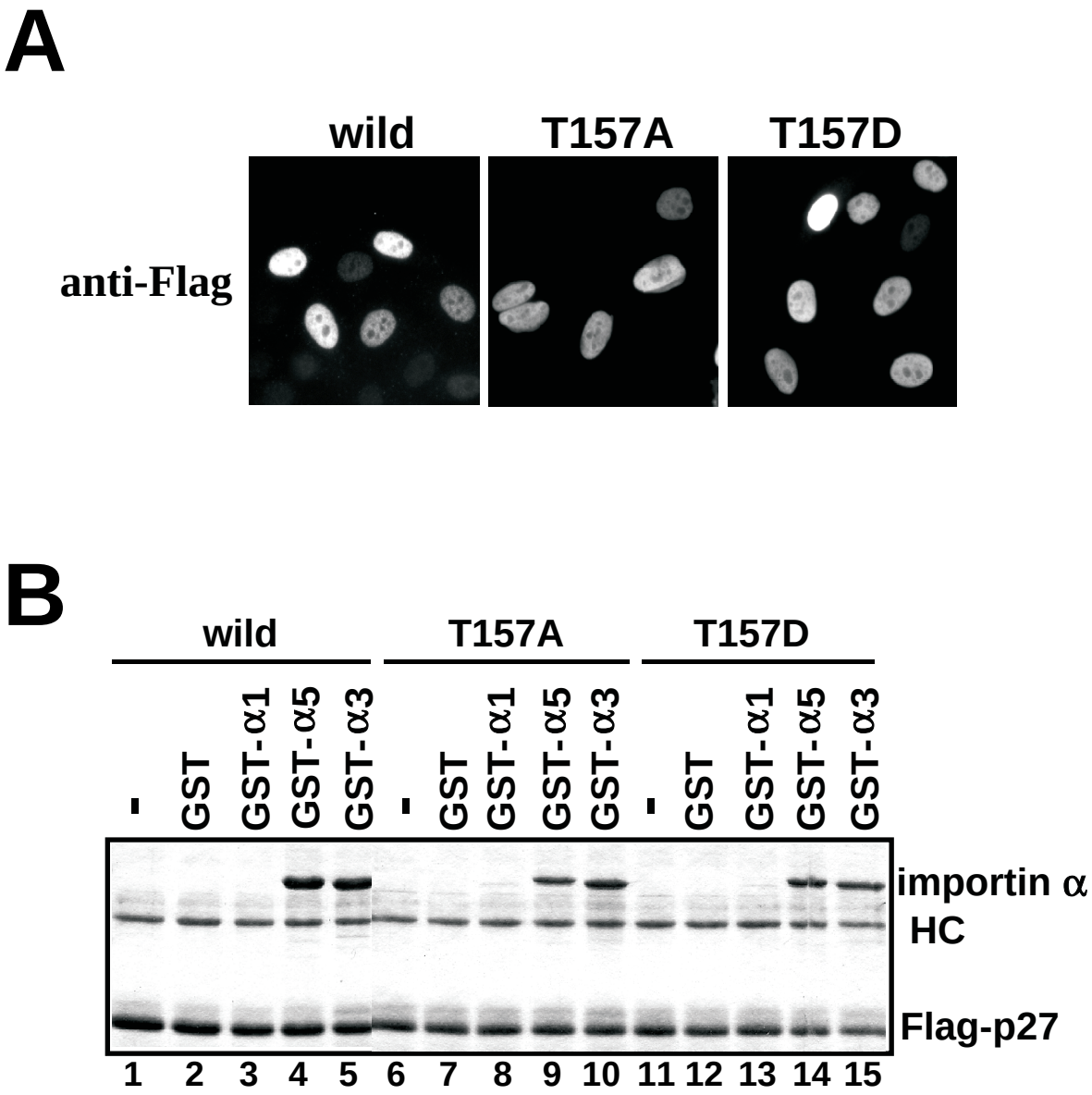
Liang, J., Zubovitz, J., Petrocelli, T., Kotchetkov, R., Connor, M.K., Han, K., Lee, J.H., Ciarallo, S., Catzavelos, C., Beniston, R., Franssen, E. and Slingerland, J.M. (2002) PKB/Akt phosphorylates p27, impairs nuclear import of p27 and opposes p27-mediated G1 arrest. *Nat Med*, **8**, 1153-1160.

Rodier, G., Montagnoli, A., Di Marcotullio, L., Coulombe, P., Draetta, G.F., Pagano, M. and Meloche, S. (2001) p27 cytoplasmic localization is regulated by phosphorylation on Ser10 and is not a prerequisite for its proteolysis. *EMBO J*, **20**, 6672-6682.

Figure legend

Fig. S1. Substitution of Thr157 to Asp did not affect the nuclear import of p27

(A) Nuclear import of mutant p27s. Hela cells were transfected with pcDNA-Flag-p27 (wild, T157D or T157A) and subcellular localization of p27 was detected by anti-Flag antibody. (B) Solution binding assay. Purified recombinant Flag-p27 (wild, T157D or T157A) was incubated with GST or GST-importin s and bound proteins were analyzed as Fig. 3A.


A
wild
T157A
T157D
anti-Flag
B
wild
T157A
T157D
-
GST
GST-α1
GST-α5
GST-α3
importin α
HC
Flag-p27
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15