Supporting information

The p25 subunit of the dynactin complex plays a dual role in cargo binding and dynactin regulation

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This supporting information file contains 4 Supplemental figures.

Figure S1. Sequence alignment of p27 proteins. The first four amino acids of all the beta-strands are highlighted with gray color. Residues in the hydrophobic ridge pointing to the surface of LβH are boxed with red color (1). Note that the hydrophobic residue Isoleucine (I) at position 6 (boxed with blue color) in human or mouse p27 is replaced by Lysine (K) in *A. nidulans* p27 (1). The alignment was done using CLUSTALW. Residues that are identical (*), strongly similar (:) or weakly similar (.) are indicated below the sequences.

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                                                                      1
A.nidulans       MDIKPPLSSYSSSQHLRPPPHHRPSASPASAQTQAQSAGAPRIPAKTTADPSAVIAEQTY 60
Human            --------------------------------------MAEKTQKSVKIAPGAVVCVESE 22
Mouse            --------------------------------------MAEKTQKSVKIAPGAVVCVESE 22
                                                        * :   ...  *.**:. ::

                              2                    3
A.nidulans       FCGPYSISIGRGTVIHPRVRICATEGPIRIGDGCIISEKSVIGTYPPPAAPADSTSSGSG 120
Human            IRG--DVTIGPRTVIHPKARIIAEAGPIVIGEGNLIEEQALIINAYPDNITPDTEDP--- 77
Mouse            IRG--DVTIGPRTVIHPKARIIAEAGPIIIGEGNLIEEQALIINAHPDNIIPDTEDT--- 77
                 : *  .::**  *****:.** *  *** **:* :*.*:::* .  *    .*: ..

                               4                 5                 6
A.nidulans       GSGEEKSIVISNNVIIGLQAVVHPGARVHSFAVVDNQAVIGRGVDVGGHAKVCARCEIFQ 180
Human            ---EPKPMIIGTNNVFEVGCYSQA-MKMGDNNVIESKAYVGRNVILTSGCIIGACCNLNT 133
Mouse            ---EPKPMIIGTNNVFEVGCHSQA-MKMGDNNVIESKAYVGRNVILTSGCIIGACCSLNT 133
                    * *.::*..* :: : .  :.  :: .  *::.:* :**.* : . . : * *.:

                         7
A.nidulans       GARVKEWSVVWGGGKGTGLKTRMKAQKKVVSPFAMGEKGFEGVLEGKAIEDARLVAVKRE 240
Human            FEVIPENTVIYGADCLRRVQTERPQPQTLQLDFLMK------ILP-------NYHHLKKT 180
Mouse            FEAIPENTVIYGADCLRRVQTERPQPQTLQLDFLMK------ILP-------NYHHLKKT 180
                    : * :*::*..    ::*.    :.:   * *       :*        .   :*:

A.nidulans       RDALSRLIVGKKRG 254
Human            MKGSSTPVKN---- 190
Mouse            MKGSSTPVKN---- 190
                  .. *  : .
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Figure S2. PCR verification of site-specific integration of the Δp27 construct into the p27 locus in *A. nidulans* genome. (A) A diagram showing the Δp27 linear construct with the *AfpyrG* (*pyrG* from *A. fumigatus*) marker flanked by the 5' and 3' flanking sequences of the p27 gene. Homologous recombination events occurred between this construct and the wild-type genome (wild type) are indicated by crosses. The resulting Δp27 locus is shown at the bottom. The positions of the primers, 27N3 and 27C, used for PCR analyses are indicated by arrows, and the sizes of the predicted PCR products are indicated. (B) A DNA gel image showing the PCR products amplified with the same pair of primers 27N3 and 27C. The wild type product is missing in the Δp27 mutant, and instead, a larger-sized product appears as predicted. Sequencing analysis of the product from the Δp27 mutant further confirms that the gene encoding p27 is indeed deleted and replaced with the selective marker *AfpyrG*.

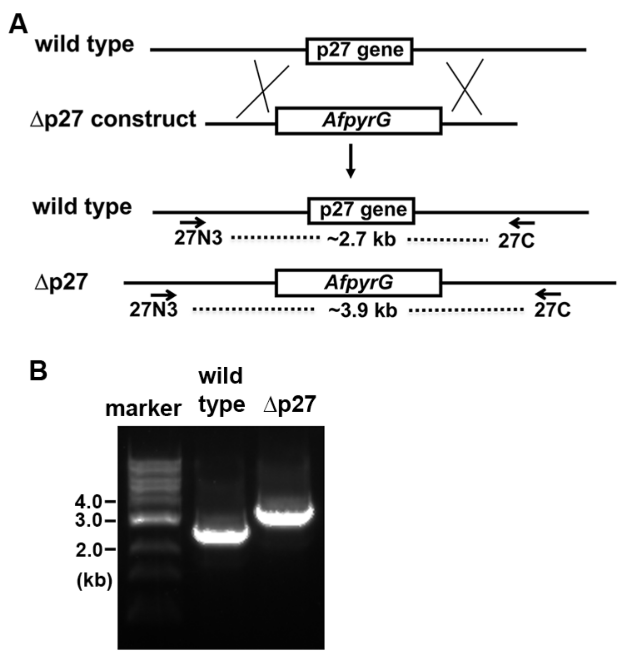


Figure S3. PCR verification of site-specific integration of the p25$^{\Delta L1}$-GFP construct into the p25 locus in *A. nidulans* genome. (A) A diagram showing three different p25 alleles in the p25 locus: wild type, p25-GFP and p25$^{\Delta L1}$-GFP. The positions of the primers, Q1 and GFP-5R, used for PCR analyses are indicated by arrows, and the sizes of the predicted PCR products are indicated. Note that sequence of the primer GFP-5R is not present in wild-type genome. (B) A DNA gel image showing the expected PCR products amplified with the same pair of primers, Q1 and GFP-5R. Names of the samples are indicated on the right. The p25-GFP allele has been published previously (2). Sequencing analysis of the product from the p25$^{\Delta L1}$-GFP mutant further confirms that the p25$^{\Delta L1}$-GFP mutant allele has indeed replaced the wild-type allele in the p25 locus.

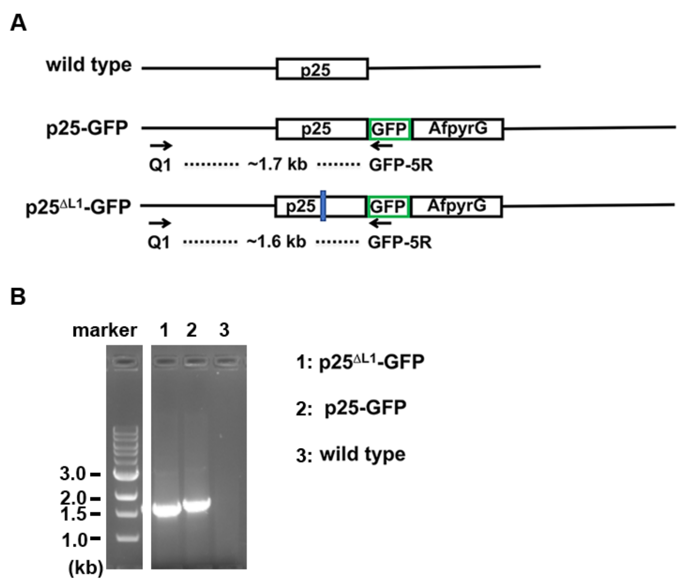
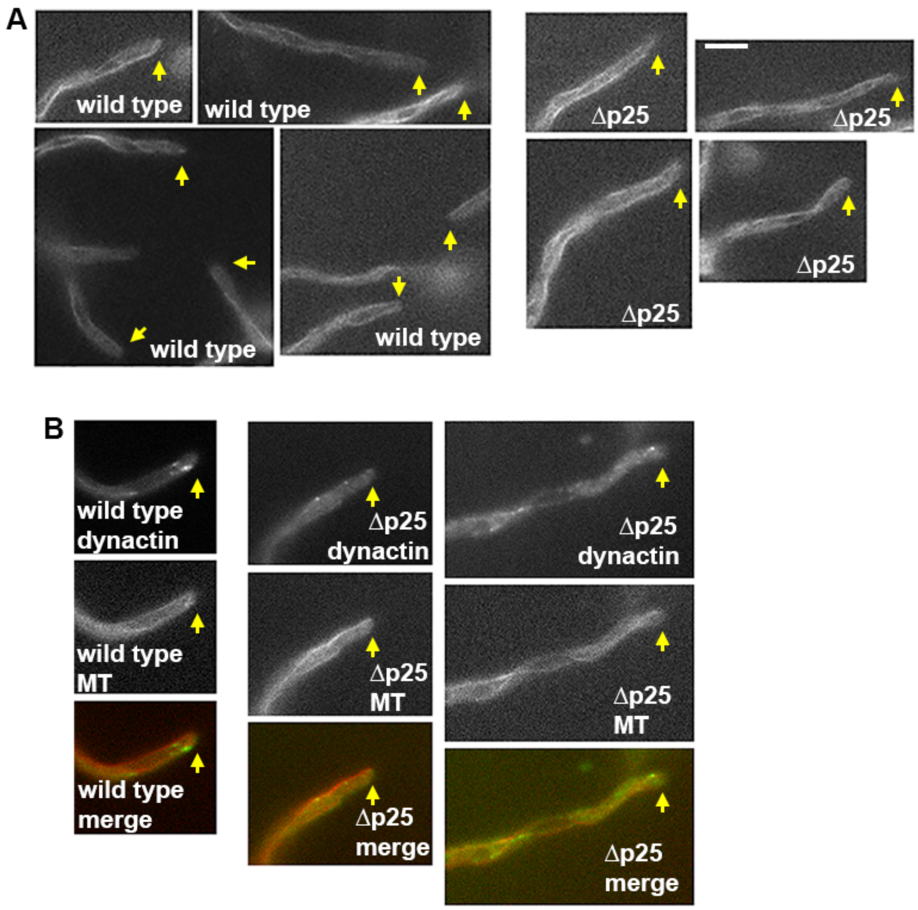


Figure S4. Microtubule (MT) organization and dynactin localization in wild type and the Δp25 mutant. (A) MTs labeled by CFP-TubA in wild type and the Δp25 mutant. The images indicate that the MT organization is normal in the Δp25 mutant. (B) Localization of dynactin labeled by p150-GFP in wild type and the Δp25 mutant. MT is pseudo-colored red and dynactin is green in merged images. Yellow arrows indicate positions of hyphal tips. Bar, 5 μm.



References

1. Yeh, T. Y., Kowalska, A. K., Scipioni, B. R., Cheong, F. K., Zheng, M., Derewenda, U., Derewenda, Z. S., and Schroer, T. A. (2013) Dynactin helps target Polo-like kinase 1 to kinetochores via its left-handed beta-helical p27 subunit. *The EMBO journal* **32**, 1023-1035
2. Zhang, J., Yao, X., Fischer, L., Abenza, J. F., Penalva, M. A., and Xiang, X. (2011) The p25 subunit of the dynactin complex is required for dynein-early endosome interaction. *The Journal of cell biology* **193**, 1245-1255