



### Supplementary figure 5

Co-immunoprecipitation analysis implies that Cdk5 does not interact with nest-314 (left panel). As nestin is completely incorporated into filaments and hence insoluble (Fig 5 A) most of nest-314 is lost during centrifugation and preclearing of the lysate prior to immunoprecipitation. In fact very little nest-314 is detected in the INPUT (data not shown). Lysate denotes whole cell lysate prior to centrifugation. In order to further verify that Cdk5 does not interact with nest-314 we performed an intermediate filament pelleting assay and analysed the ability of nest-314 filaments to sequester Cdk5. Due to their insolubility intermediate filaments are pelleted after extraction in detergent buffers. Possible interaction between the nestin fragment nest-314 and Cdk5 was assayed by fractionation of control nest-314 overexpressing cells and analyzing the ability of the filamentous nest-314 to sequester Cdk5 to the pellet fraction. Triton X-100 buffer (20 mM Hepes, pH 7.6, 100 mM NaCl, 5 mM MgCl<sub>2</sub>, 5 mM EGTA, 1% Triton X-100, 1 mM PMSF, protease inhibitors) was added to the culture dishes, cells were detached and collected, homogenized on ice, and centrifuged for 15 min at 10,000 × *g* (4 °C) to pellet IFs. Volumes of the pellet and supernatant were adjusted with Laemmli Sample buffer and the samples were sonicated and boiled for analysis by western blotting. As Cdk5 is not sequestered by the nest-314 filaments we conclude that nest-314 and Cdk5 does not interact (right panel).