Supplementary Figure 1: Expression of various proteins in the primary hippocampal neurons. (A-E) Specific antibodies were used to detect the expression of importin α1, importin α3, importin β1, E47 and NeuroD1 in NIH3T3 cells, mouse ES cells and rat primary hippocampal neurons. Asterisks indicate the band for each protein according to their molecular weights. (F) GAPDH antibody was used as loading control.

Supplementary Figure 2: Localization of flag-Δ134-161 in comparison with flag-NeuroD1. NIH3T3 cells were transfected with flag-NeuroD1 and flag-Δ134-161 and 20 hours post-transfection, cells were fixed and immunostained using anti-flag.

Supplementary Figure 3: The small GTPase Ran is involved in nuclear import of NeuroD1. (A-H) NeuroD1-GFP was cytoplasmically microinjected with or without RanQ69L-GTP into HeLa and NIH3T3 cells. Microinjected NeuroD1-GFP efficiently translocated into the nuclei of HeLa (A) and NIH3T3 cells (E). Co-injection of RanQ69L-GTP with NeuroD1-GFP resulted in the cytoplasmic retention of NeuroD1-GFP (C, G). Alexa568-labeled anti-rat IgG was used as an injection marker (B, D, F and H). Live cell imaging was carried out 1 hr post-injection.

Supplementary Figure 4: GST pull-down assay. Glutathione sepharose prebound with NeuroD1-GST was incubated with the clear HeLa cell cytosol, with or without RanQ69L-GTP for 4 hours at 4°C. After extensive washing, the bound proteins were eluted by sample buffer, resolved by SDS-PAGE and immunoblotted using anti-RanBP5 (Santa cruz) (A) and anti-RanBP7 (Novous) (B).

Supplementary Figure 5: In vitro nuclear import assay using importin β1 with or without importin α.

Supplementary Figure 6: Protein identification. The upper panel (a) shows MALDI-TOF spectrum of peptide fragment eluted from silver-stained gel. The lower panel (b) shows Mascot database search performed by peptide mass fingerprinting.