# Supplementary Information Titles

**Journal:** Nature Neuroscience

<table>
<thead>
<tr>
<th>Article Title:</th>
<th>Wnt-mediated activation of NeuroD1 and retroelements during adult neurogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corresponding Author:</td>
<td>Tomoko Kuwabara</td>
</tr>
</tbody>
</table>

### Supplementary Item & Number (add rows as necessary)

<table>
<thead>
<tr>
<th>Title or Caption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunohistochemical analysis of Wnt3a in adult hippocampus.</td>
</tr>
<tr>
<td>The activity of β-catenin shRNA.</td>
</tr>
<tr>
<td>Schematic representation of retrovirus-based <em>in vivo</em> analysis to determine the role of β-catenin at the single cell level.</td>
</tr>
<tr>
<td>Immunohistochemical analysis of the DCX+ cells in β-catenin floxed mice</td>
</tr>
<tr>
<td>Immunohistochemical analysis of the TUJ1+ cells in β-catenin cKO mice.</td>
</tr>
<tr>
<td>Lineage tracing Sox2+ NSCs in β-catenin cKO mice with neuronal markers.</td>
</tr>
<tr>
<td>Supplementary Figure 7</td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>Supplementary Figure 8</td>
</tr>
<tr>
<td>Supplementary Figure 9</td>
</tr>
<tr>
<td>Supplementary Figure 10</td>
</tr>
<tr>
<td>Supplementary Figure 11</td>
</tr>
<tr>
<td>Supplementary Figure 12</td>
</tr>
<tr>
<td>Supplementary Figure 13</td>
</tr>
<tr>
<td>Supplementary Figure 14</td>
</tr>
<tr>
<td>Supplementary Figure 15</td>
</tr>
<tr>
<td>Supplementary Table 1</td>
</tr>
<tr>
<td>Supplementary Table 2</td>
</tr>
<tr>
<td>Supplementary Table 3</td>
</tr>
</tbody>
</table>

**SUPPLEMENTARY FIGURES**

Wnt-mediated activation of NeuroD1 and retroelements during adult neurogenesis

Tomoko Kuwabara, Jenny Hsieh, Alysson Muotri, Gene Yeo, Masaki Warashina, Dieter Chichung Lie, and Lynne Moore, Kinichi Nakashima, Makoto Asashima and Fred H. Gage
Supplemental Figure 1.

Immunohistochemical analysis of Wnt3a in adult hippocampus.

Immunohistochemical analysis of adult hippocampal DG by using antibodies against Wnt3a (red) and astrocyte-specific markers. Astrocytes expressing S100β (green) also produced Wnt3a protein [merged image with DAPI (blue) in left]. Astrocytes expressing GFAP (middle) also produced Wnt3a protein (left, and merged image, right). Double positive cells (GFAP+Wnt3a+) are indicated by white arrows.
**Supplemental Figure 2.** The activity of β-catenin shRNA. The knock-down effect of shRNA on β-catenin protein was confirmed by Western blot (left panels). The knock-down of β-catenin also caused the decrease in L1 ORF2 transcription, as confirmed by RT-PCR (right panels).

**Supplemental Figure 3**
Supplemental Fig. 3. Schematic representation of retrovirus-based *in vivo* analysis to determine the role of β-catenin at the single cell level. Retrovirus containing Sox2 promoter-driven Cre/GFP (Sox2^{Cre}GFP) was injected into the DG of mice with a conditional ‘floxed’ allele of β-catenin reporter mice (β-catenin^{floxed}/ROSA26-EGFP; β-catenin cKO). Two weeks after the retrovirus injection into β-catenin cKO mice, BrdU (colored in blue) was injected once a day for two weeks.

Supplemental Fig. 4

Supplemental Figure 4. Immunohistochemical analysis of the DCX+ cells in β-catenin floxed mice.
Sox2^{Cre}GFP retrovirus was injected into the DG of control (left) or β-catenin cKO mice (right). Immunohistochemical analysis of the DCX+ (red), GFP+ cells (green) and DAPI (blue) in both animal groups is shown. The GFP+ cells co-localizing with DCX in the control animal are indicated by white arrows (left panels).
Supplemental Figure 5. Immunohistochemical analysis of the TUJ1+ cells in β-catenin cKO mice.

Immunohistochemical analysis of the TUJ1+ (red), GFP+ cells (green) and DAPI (blue) in both control and β-catenin cKO mice is shown. GFP+TUJ1+ cells in the control animals are indicated by white arrows (left panels).
Supplemental Fig. 6. Lineage tracing Sox2+ NSCs in β-catenin cKO mice with neuronal markers.

Quantification of GFP+ cells expressing various neuronal markers (DCX, TUJ1 and Prox-1) in DG of control and β-catenin cKO mice. Numbers (a) and percentage (b) of GFP+ cells in the DG of control mice (white bars) and β-catenin cKO mice (black bars) are shown.
Supplemental Fig. 7

(a) Immunohistochemical analysis of the β-catenin shRNA-GFP+ cells (green) and mature neuronal cells (NeuN; magenta) is shown in left panel. The proportion of the cells double positive for NeuroD1 and GFP against total GFP+ cells is plotted for each set of injection experiments (Control GFP and β-catenin shRNA-GFP). (b) Triple immunohistochemistry of NeuroD1, shRNA-GFP and BrdU is shown. NeuroD1+ cells did not co-localize with shRNA-GFP (green arrows) but well co-localized well with BrdU (white arrows). (c) Control-EGFP+ cell was determined to be NeuroD1+ (red) and BrdU+ (blue, in the left panel). Triple staining of the control GFP+ cell with NeuroD1 and DAPI (blue) is also shown in the right panel.

Supplemental Fig. 7. Infection of LV β-catenin shRNA in vivo. (a) Immunohistochemical analysis of the β-catenin shRNA-GFP+ cells (green) and mature neuronal cells (NeuN; magenta) is shown in left panel. The proportion of the cells double positive for NeuroD1 and GFP against total GFP+ cells is plotted for each set of injection experiments (Control GFP and β-catenin shRNA-GFP). (b) Triple immunohistochemistry of NeuroD1, shRNA-GFP and BrdU is shown. NeuroD1+ cells did not co-localize with shRNA-GFP (green arrows) but well co-localized well with BrdU (white arrows). (c) Control-EGFP+ cell was determined to be NeuroD1+ (red) and BrdU+ (blue, in the left panel). Triple staining of the control GFP+ cell with NeuroD1 and DAPI (blue) is also shown in the right panel.
Supplemental Fig. 8

a. LV β-catenin shRNA

b. LV β-catenin shRNA

c. LV Control GFP
Supplemental Fig. 8. The β-catenin shRNA-GFP+ and control-GFP+ cells co-localized with Sox2. (a) Immunohistochemical analysis of the β-catenin shRNA-GFP+ cells (green) and neural stem cells (Sox2; red) is shown. The shRNA-GFP cells co-localize with Sox2 (indicated by white arrows). (b) Triple immunohistochemistry of Sox2 (red), shRNA-GFP (green) and BrdU (blue) is shown in the left panel. Triple staining of same region by Sox2 (red), shRNA-GFP (green) and DAPI (blue) is also shown in the right panel. (c) Control LV-GFP injections resulted in many GFP+ cells in the granule cell layer of DG. Some of those GFP+ cells were determined to be Sox2+ (white arrows) and BrdU+ (blue arrows).

Supplemental Fig. 9

Supplemental Fig. 9. The shRNA-GFP cells co-localized with GFAP and the radial glial cell marker Nestin. (a) The immunohistochemistry of GFAP (red), shRNA-GFP (green) and DAPI (blue) is shown. The shRNA-GFP cells co-localize with GFAP, and the co-localizing cell (in white square region) is magnified in right panels. (b) The immunohistochemistry of Nestin (red), shRNA-GFP (green) and DAPI (blue) is shown. The shRNA-GFP cells co-localizing with Nestin (in white square region) is magnified in right panels.
Supplemental Fig. 10. Immunohistochemical analysis of apoptotic cells labeled by active caspase 3 in β-catenin cKO mice.

Representative image of AC3+ cells (red), GFP+ cells (green) and DAPI (blue) in β-catenin cKO mice (left). GFP+ cell (green) was determined to be AC3+ (red, left panels) and Sox2+ (blue, the right panel). Triple staining of the GFP+ cell with AC3+ and Sox2 (blue) is shown in the right panel.
Supplemental Fig. 11. Loss-of-function experiments of NeuroD1 by using siRNA with and without Wnt3a. Adult hippocampal NSCs were cultured under the introduction of NeuroD1 siRNA in vitro, with or without Wnt3a (50 ng/mL) for two days. Immunostaining of the neuron marker Map2AB (red) was performed and the percentage of Map2AB+ cells is shown in bottom of each panel.
Supplemental Fig. 12. **NeuroD1 is necessary in Wnt3a-mediated neuronal differentiation.** Adult neurosphere culture from ND1 cKO mice. Immunostaining of the neuron marker TUJ1 (red) was performed on neurosphere cultures expressing lentivirus GFP (green) or Cre-GFP (green). Wnt3a ligand or DMSO was added to the spheres for 2 days.

Supplemental Figure 13
Supplemental Figure 13. **In vivo** L1-based promoter activity.

The 5’ UTR of L1-driven GFP showed neurogenic area-specific expression. GFP+ cells (green) also express the neuron-specific β-III tubulin isoform, recognized by the TUJ1 antibody (red).

Supplemental Figure 14

**Supplemental Fig. 14. The expression of RUNX3 in NeuN+ mature neuron.** Immunohistochemical analysis of adult hippocampal DG by using antibodies against RUNX3 (red) and NeuN (green). Co-staining with RUNX3 (red) and NeuroD1 (cyan) is also shown in bottom panels. *Some LINE-GFP expressions were observed within the differentiated neurons deeper [NeuroD1(-)] in the granule cell layer (Fig. 8a), and they were determined to be NeuN+ (Fig. 8c). Since previously it was determined that RUNX3 increased L1 expression and retrotransposition (HeLa and 143B cells[42]), these data suggest that L1-based promoters retain to be active not only in the early neurogenic stage but also in mature neurons, probably by RUNX3. The role of RUNX3 in adult
neurogenesis may likely be in later stages rather than in the initial steps of L1 expression during the neurogenic stage, when NeuroD1 is expressed (Fig. 1) and regulated by Wnt/β-catenin signaling.

Supplemental Figure 15. Wnt-mediated regulatory mechanism to simultaneously coordinate activation of NeuroD1 and L1. Schematic representation of Sox/LEF
site-mediated transcriptional regulation during adult neurogenesis. Overlapping Sox/LEF binding site is shown in green box. L1 retroelements embedded in mammalian genome work as bi-directional promoters (red triangles under L1s), suggesting that they may actively influence nearby genomic transcription status actively during adult hippocampal neurogenesis.

### Supplemental Table 1. L1 elements proximal to the transcriptional start site of known protein-coding genes in human genome.

| A) Gene ID (GenBank accession number) | B) Gene name | C) hg17 (human version 17) | D) chromosome | E) start coordinate of L1 | F) end coordinate of L1 | G-I) family of L1 | J- TCF and SOX2 sites a|b|c separated by colons "::"

| a = location in L1 of the site, | b = site motif | c = which transcription factor (TF) (Sox or TCF/LEF) |
Nature Neuroscience: doi:10.1038/nn.2360
Supplemental Table 2. L1 elements proximal to the transcriptional start site of known protein-coding genes in mouse genome.
A) Gene ID (GenBank accession number).
B) Gene name
C) mm5 (mouse version 5)
D) chromosome
E) start coordinate of L1
F) end coordinate of L1
G-I) family of L1
J-) TCF and SOX2 sites. a|b|c separated by colons ":"  
a = location in L1 of the site,  
b = site motif  
c = which TF (Sox or TCF/LEF)
Supplemental Table 3. L1 elements proximal to the transcriptional start site of known protein-coding genes in rat genome.

A) Gene ID (GenBank accession number).
B) Gene name
C) rn3 (rat version 3)
D) chromosome
E) start coordinate of L1
F) end coordinate of L1
G-I) family of L1
J-) TCF and SOX2 sites. a|b|c separated by colons ":"

a = location in L1 of the site,
b = site motif
c = which TF (Sox or TCF/LEF)