

1 **Material and Method**

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3 **Plasmid constructs** - QuikChange Multi Mutagenesis Kit (Stratagene, West Cedar, Tx) were uti-
4 lized according to, the manufacturer's instructions. HA tagged protein GSK-3 β (WT), 83stop,
5 131stop, or 201stop mutants were generated with the mutagenic primers 83stop :(5' - TCA GGA
6 GAA CTG GTC TAG ATC AAG AAA GTA - 3', down 5' - caa TAC TTT CTT GAT CTA
7 GAC CAG TTC TCC - 3'), 131stop : (up 5' - CTT AAT CTG GTG TAG GAC TAT GTT CCG
8 GAA - 3', down 5' - CGG AAC ATA GTC CTA CAC CAG ATT AAG ATA - 3'), 201stop :
9 (up 5' - GGA AGT GCA AAG TAG CTG GTC CGA GGA GAA - 3', down 5' - TCC TCG GAC
10 CTA CTT TGC ACT TCC AAA- 3'). GST-tagged recombinant proteins for GSK-3 β , GSK-3 β
11 PY mutants, 83stop, 131stop, or 201stop mutants were purified from *Escherichia coli* BL21
12 (DE3), after performing PCR. All constructions were confirmed by DNA sequencing.

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14 **Short interfering RNA** -The short interfering RNA (siRNA) duplexes specific for Kap β 2 (nu-
15 cleotides 1041–1063) r(GAGGCUGAGCGGCCUGAUGGCU)d(TT) and
16 r(AGCCAUCAGGCCGCUCAGCCUC)d(TT) and control inverted duplexes
17 r(UCGGUAGUCCGGCGAGUCGGAG)d(TT) and
18 r(CUCCGACUCGCCGACUACCGA)d(TT) were synthesized by Bioneer; (Daejen, Korea).
19 HEK293 cells were transfected by using Oligofectamine (Invitrogen).

20

1 **Supplement Fig. 1**

2 A) Co-immunoprecipitation with HA-GSK 3 β deletion mutant or WT with of Kap β 2 in
3 HEK293 cell. After immunoprecipitating with the rabbit HA antibody, each immunoprecipitant
4 was immunoblotted with either mouse Kap β 2 (upper lane) or mouse GSK-3 β antibody (bottom
5 lane).

6 B) Glutathione S-transferase (GST)-GSK 3 β fusion proteins and pull-down assays.

7 GSK-3 β deletion mutant or WT in pGEX-5X-1 expressed in *Escherichia coli* BL21. GST- GSK
8 3 β fusion proteins bound to glutathione-Sepharose were equilibrated in PBS buffer containing
9 0.1% Triton X-100 and 1 mM CaCl₂ or 2 mM EGTA. Incubation with the total cell lysate of
10 HEK293 cell was followed by three washes with the appropriate buffers, and the bound proteins
11 were eluted with sample buffer, subjected to SDS gel-electrophoresis, and immunoblotted with
12 either mouse Kap β 2 (upper lane) or mouse GSK 3 β antibody (bottom lane).

13

14 **Supplement Fig. 2**

15 The kinase activity of GSK-3 β and its subcellular localization.

16 A) After transfection with HA-GSK-3 β WT, Y216A, Y265A, or Y117A mutant, the cell lystae
17 was immunoprecipitated with the rabbit HA antibody. The immunoprecipitant was
18 immunoblotted with either mouse pY216 Ab (upper lane) or mouse GSK-3 β Ab (bottom lane).

19 B) The subcellular localization of HA-GSK-3 β WT, Y117A , Y216A, or Y265A. The green color
20 represent GSK-3 β , and the red color indicate the endogenous Kap β 2 in HEK293 cells.

21

22 **Supplement Fig. 3**

23 The inhibition of Karyopherin β 2 with its specific siRNA blockades the import of GSK-3 β into
24 the nucleus.

25 A) The confocal microscopy of HA-GSK-3 β WT with the endogenous karyopherin β 2.

26 B) The confocal microscopy of HA-GSK-3 β Y117A mutant with the endogenous Karyopherin
27 β 2.

28 C) The confocal microscopy of HA-GSK-3 β WT with the treatment of Karyopherin β 2 siRNA.

29

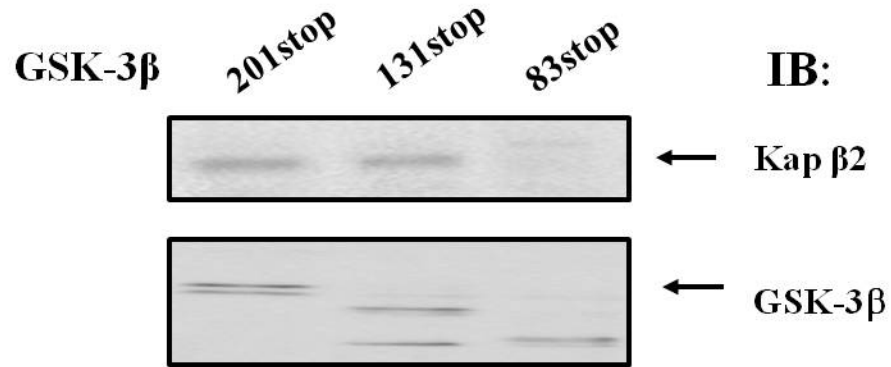
30 **Supplement Fig. 4**

31 The confocal results of GSK-3 β WT, R113A, Y117A, and Kap β 2.

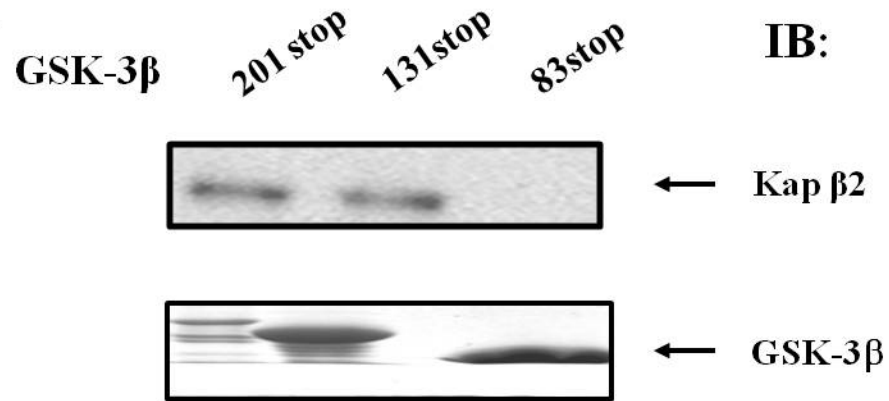
- 1 A) In order to show the subcellular localization of the endogenous GSK-3 β and Kap β 2 alone.
- 2 GSK-3 β the confocal result was provided.
- 3 B) The confocal results of GSK-3 β (WT, R113A, Y117A) with HOECHST. The R113A, Y117A
- 4 mutants were not observed in the nuclei, while WT was detected in the nuclear.

Supplement Fig. 1

A) Co immunoprecipitation

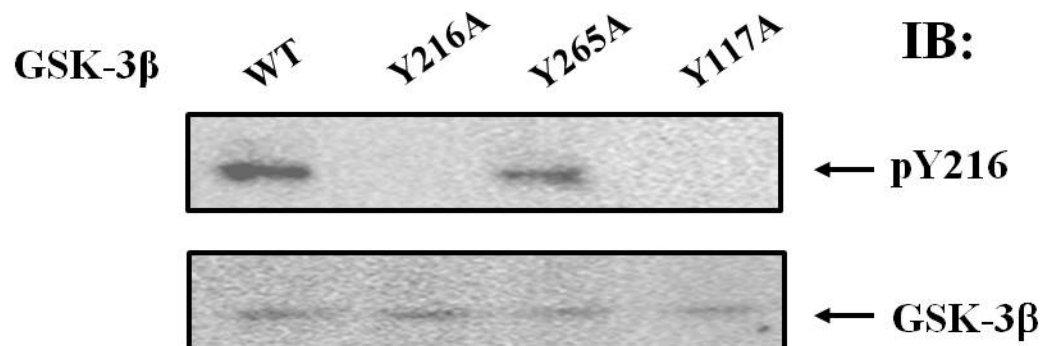


B) Pull down



Supplement Fig. 2

A)



B)

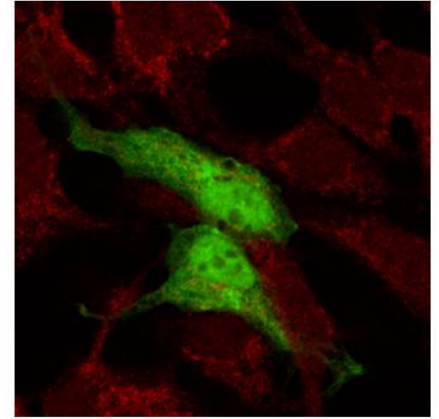
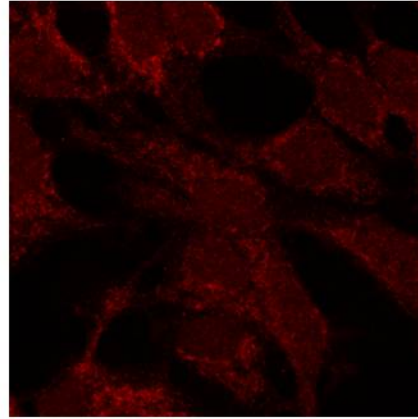
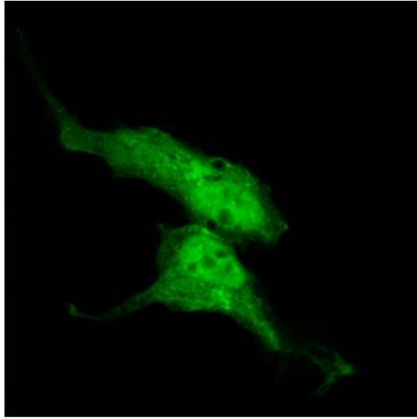
GSK-3 β

WT Y216A Y265A Y117A

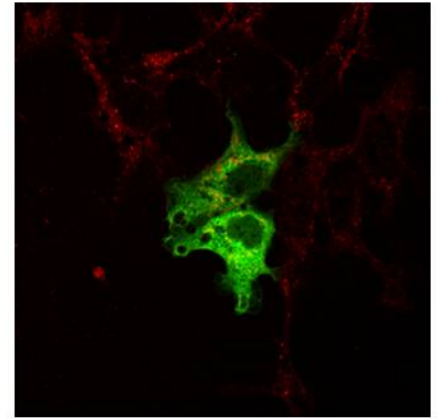
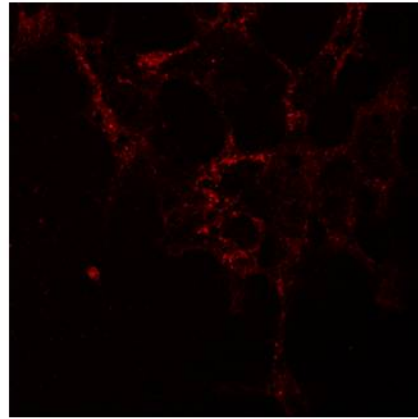
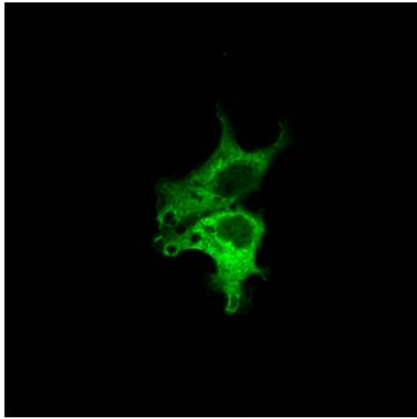


Supplement Fig. 3

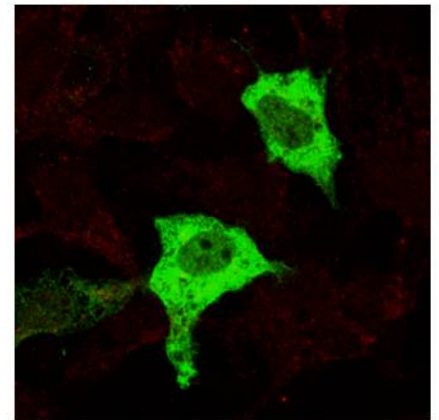
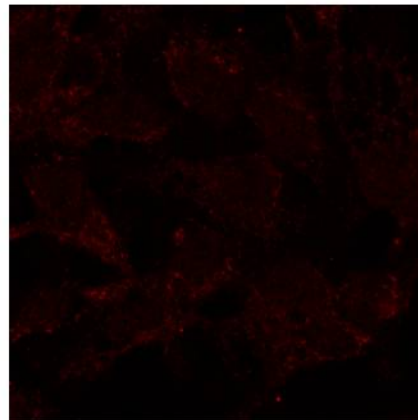
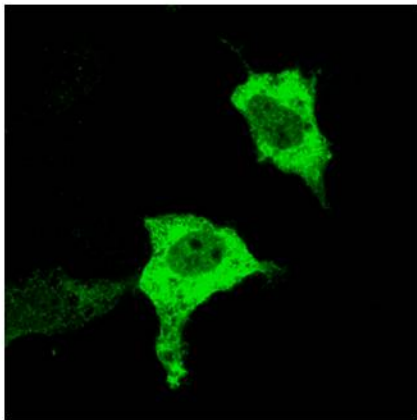
A)



B)



C)



Supplement Fig. 4

