Supplementary information

GSK3β regulates a novel β-Catenin degradation pathway via the GID complex in Wnt signaling.

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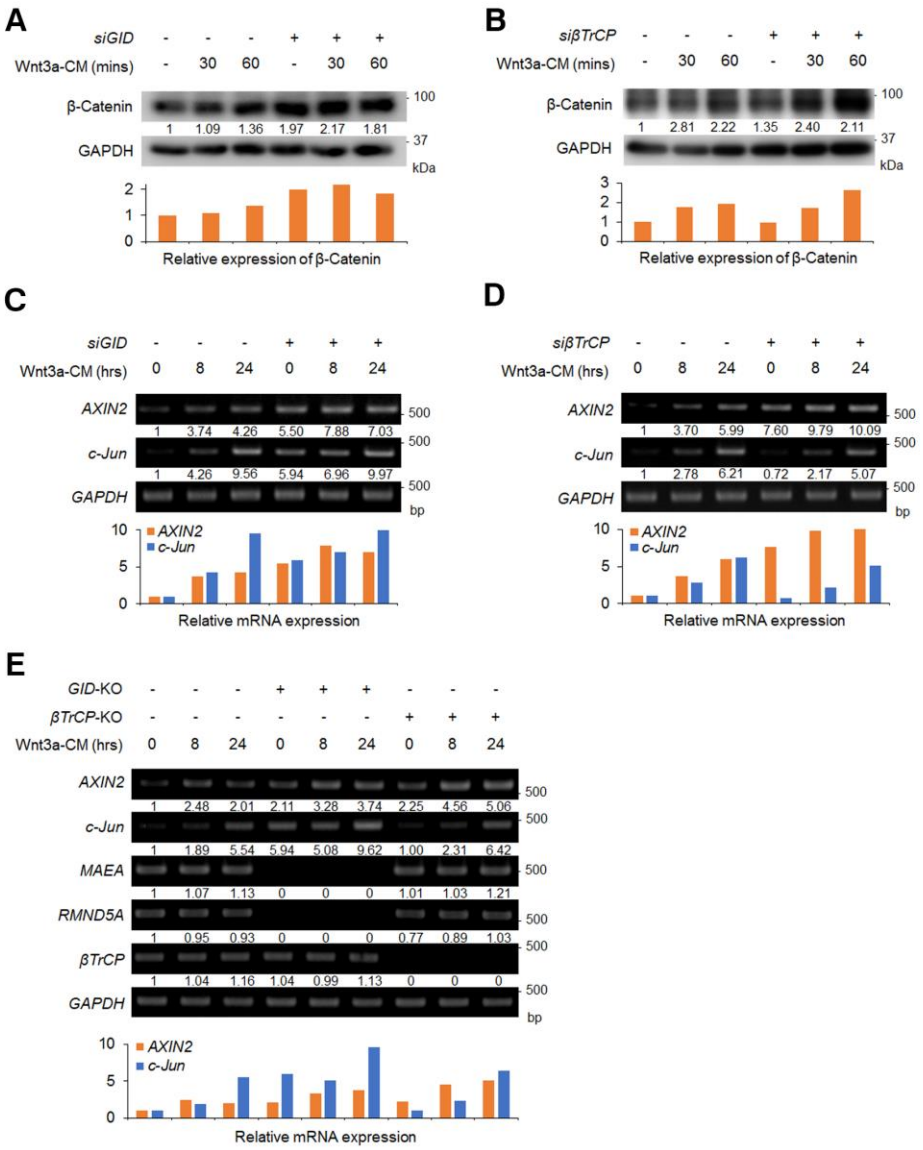
Supplementary Figure 1

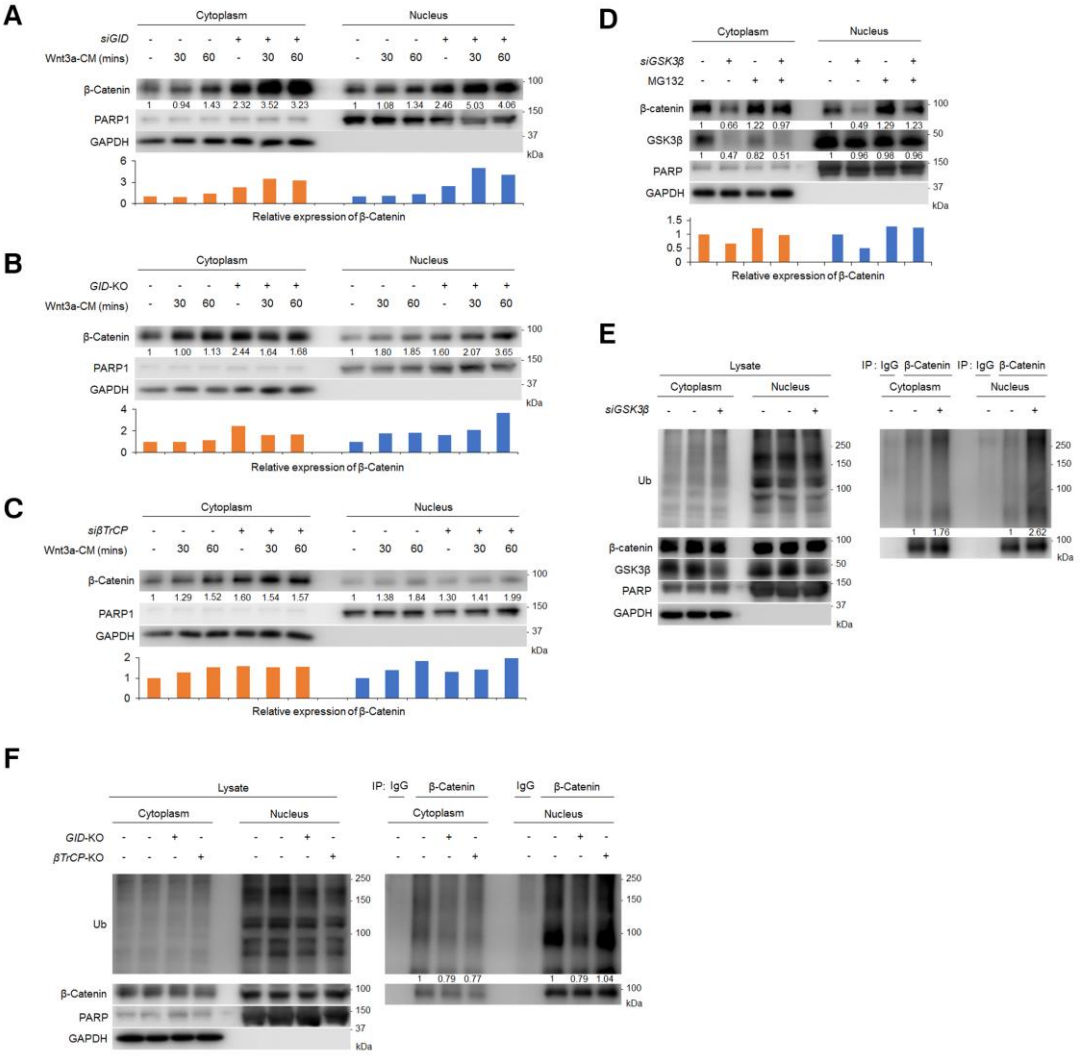


Supplementary Figure 1. Expression of β-Catenin and Wnt target genes in *GID* genes- or *βTrCP* genes-suppressed cells.

A and **B**, Immunoblotting of β-Catenin in HEK293T cells after transfection of MAEA and RMND5A siRNA (*siGID*, A) or βTRCP and FBXW11 siRNA (*siβTrCP*, B) for 48h and treatment of Wnt3a-CM for indicated times. **C** and **D**, RT-PCR analysis of Wnt target genes after transfection of *siGID* or *siβTrCP* for 24h and Wnt3a-CM for indicated times.

E, Expression of Wnt target genes in *MAEA*- and *RMND5A*-knockout (*GID*-KO) or *βTrCP*-knockout (*βTrCP*-KO) HEK293T cells after treatment of Wnt3a-CM.

Supplementary Figure 2

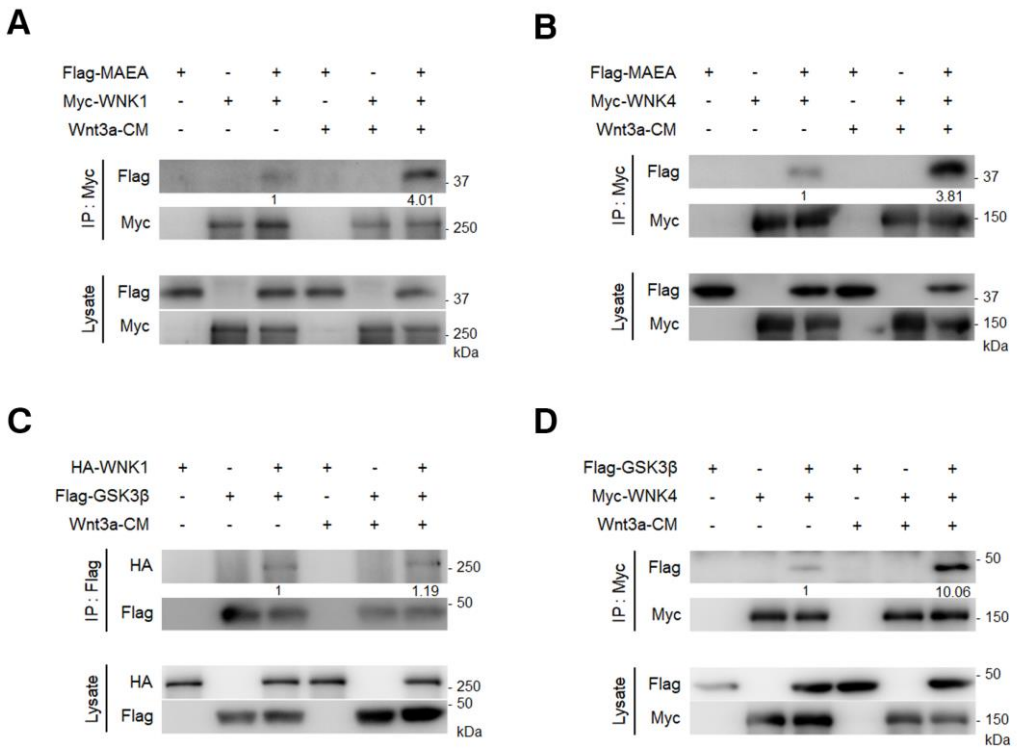


Supplementary Figure 2. Expression and ubiquitination of β-Catenin in cytoplasm and nucleus of *GID* genes- or *βTrCP* genes-suppressed cells.

A , Western blot analysis of β-Catenin in cytoplasm and nucleus of HEK293T cells after transfection of MAEA and RMND5A siRNA (*siGID*) for 48h and treatment of Wnt3a-CM for indicated times. **B**, Immunoblotting of β-Catenin in cytoplasm and nucleus of *GID*-KO cells with Wnt3a-CM stimulation. **C**, Western blot analysis of β-Catenin in cytoplasm and nucleus of HEK293T cells after transfection of βTRCP and FBXW11 siRNA (*siβTrCP*) for 48h and treatment of Wnt3a-CM for indicated times. **D** and **E**,

Expression (D) and ubiquitination (E) of β-Catenin in cytoplasm and nucleus after treatment GSK3β siRNA for 48h and MG132 (20mM) for 8h. **F**, Ubiquitination levels of endogenous β-Catenin in cytoplasm and nucleus of *GID*-KO or βTrCP-KO cells after treatment MG132 (50mM) for 4h.

Supplementary Figure 3



Supplementary Figure 3. Interaction of WNK1 and WNK4 with MAEA and GSK3β. **A** and **B**, Binding assay of WNK1 (A) or WNK4 (B) to MAEA after treatment of Wnt3a-CM for 4h. **C** and **D**, Interaction between GSK3β and WNK1 (C) or WNK4 (D) after treatment of Wnt3a-CM for 4h.