Supplementary files

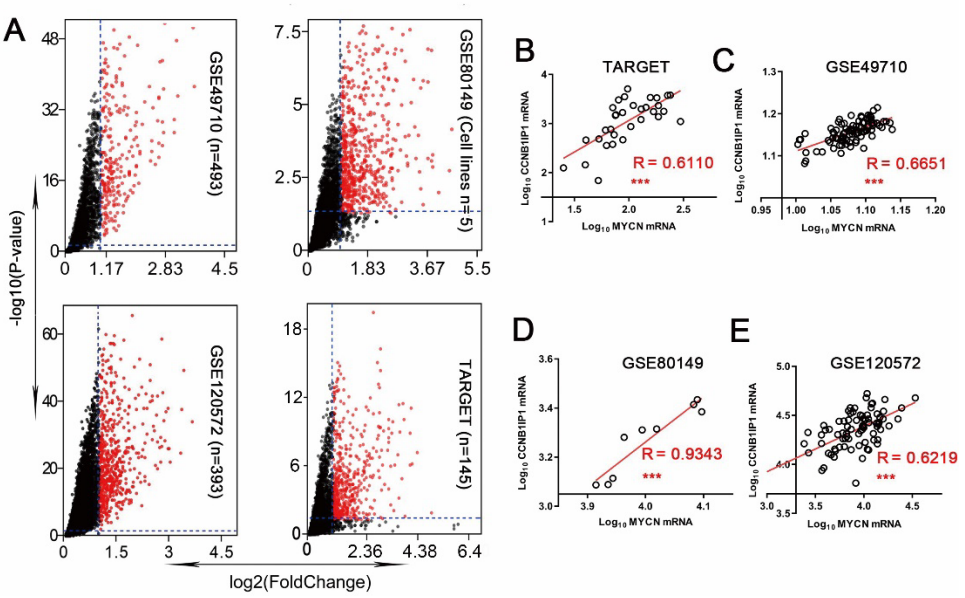

Figure S1. Differential gene expression and correlation analysis between MYCN-AM and NA NB samples and cells. (A) Differential genes between MYCN-AM and NA NB samples or cell lines from TARGET, GEO (GSE49710, GSE80149, GSE120572) datasets (log2FC > 1, $P < 0.01$). (B-E) Correlation between CCNB1IP1 and MYCN transcription levels from four different datasets.

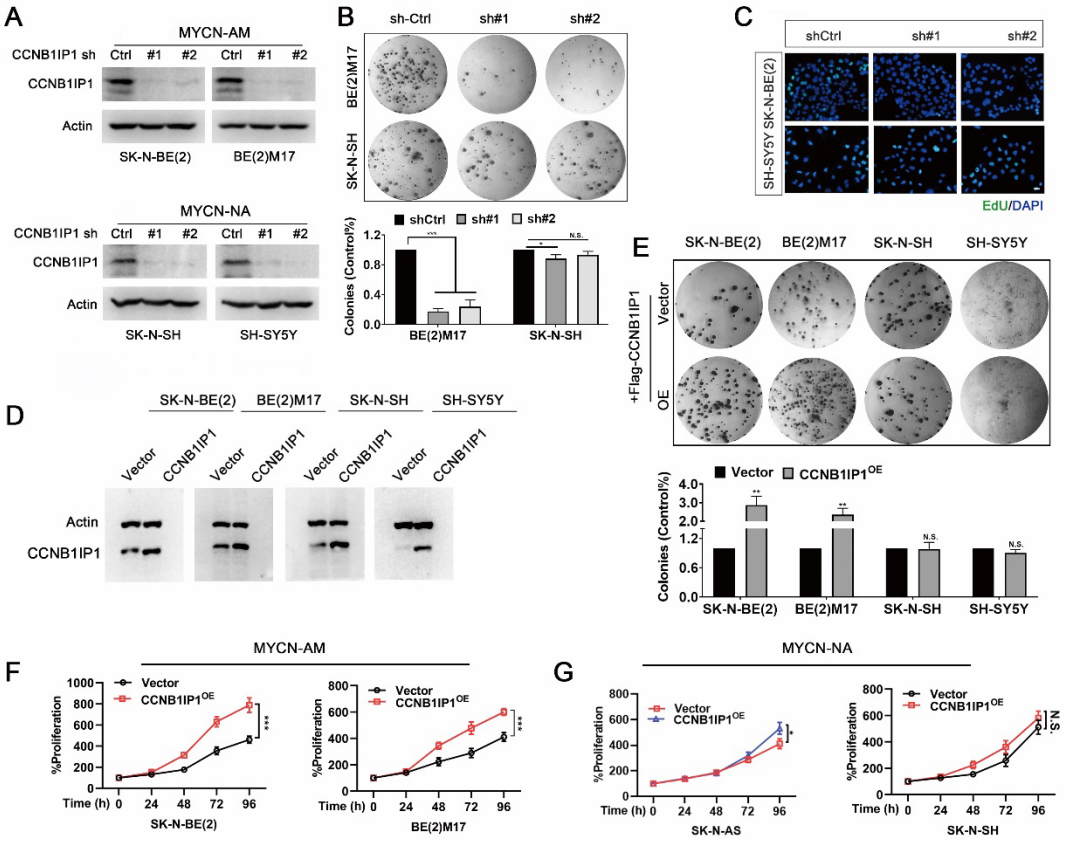

Figure S2. Knockdown or overexpression of CCNB1IP1 selectively inhibited and promoted the proliferation and growth of MYCN-AM NB cells, respectively. Two shRNAs targeting CCNB1IP1 with different sequences were transfected into NB cells (SK-N-BE(2), BE(2)M17, SK-N-SH and SH-SY5Y cells) for 48h. (A) IB analysis of CCNB1IP1 protein levels was performed to detect the efficiency of shRNA. (B) Colony-formation assay of NB cells with CCNB1IP1 knockdown. (C) EdU incorporation assay. Scale bar-10μM. Empty vector or vectors encoding CCNB1IP1 were transfected into SK-N-BE(2), BE(2)M17, SK-N-SH and SH-SY5Y cells for 48h. (D) IB analysis was performed to detect the CCNB1IP1 protein expression level. (E) Colony-formation assay. (F and G) MTT assay was performed in NB cells overexpressed with CCNB1IP1. B, E, F and G, Data represent the mean ± SD of at least three independent experiments (N.S., no significant differences; $^{*}P<0.05$; $^{**}P<0.01$ and $^{***}P<0.001$).

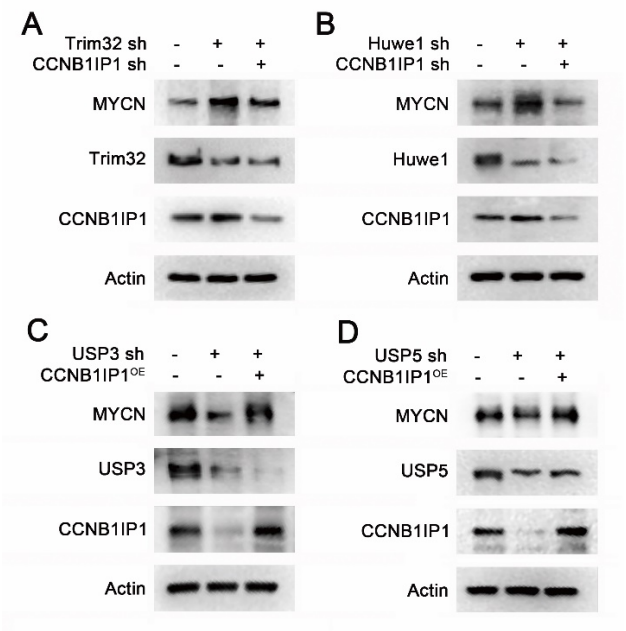

Figure S3. CCNB1IP1 affects MYCN protein expression in a non-dependent manner on Trim32, Huwe1, USP3 and USP5. IB analysis of Trim32, Huwe1, USP3, USP5, MYCN and CCNB1IP1 protein expression. BE(2)M17 cell infected with shTrim32 (A) shHuwe1 (B) alone or together with shCCNB1IP1 or infected with shUSP3 (C) shUSP5 (D) alone or together with CCNB1IP1 overexpression plasmid. All experiments were repeated at least three times.

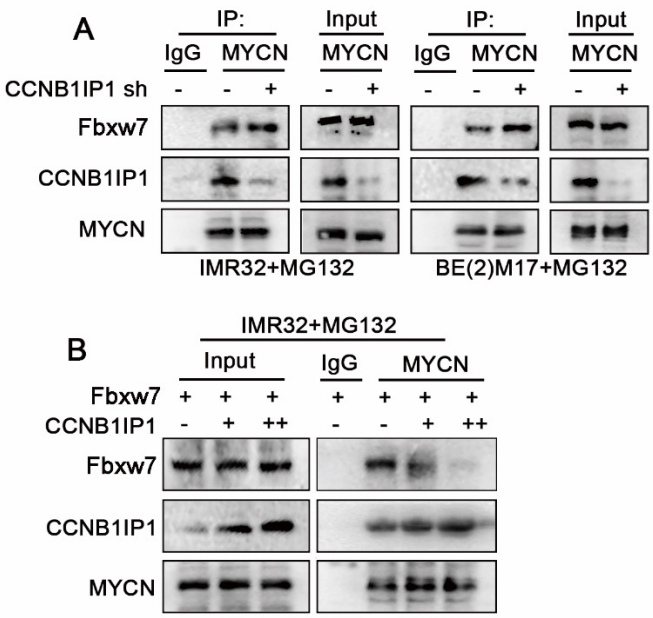

Figure S4. CCNB1IP1 competing with Fbxw7 for MYCN binding. IP assay was performed to detect the interference of knockdown of (A) or exogenously expressed (B) CCNB1IP1 with the interaction between Fbxw7 and MYCN. IP assay was performed using anti-MYCN antibody and IgG was used as a negative control. All experiments were repeated at least three times.

Table S1. The primer sequences for qRT-PCR.

Gene	Forward primer (5′ - 3′)	Reverse primer (5′ - 3′)
β-Actin	CCTGGCACCCAGCACAAT	GGGCCGGACTCGTCATAC
CCNB1IP1	ACTCAGCAAATACAAAGCAAGG	GCCTTCATGGTTAGCAATAGTG
MYCN	ATGAAGAGGAAGAAATCGACGT	CTTTATCTTCTTCTGTGGGGGT

Table S2. The primer sequences for ChIP-qPCR.

Fragments	Forward primer (5′ - 3′)	Reverse primer (5′ - 3′)
ND	GGACACGAAGTGAGACCCTGTTTC	CCTCACATCGCCCTCCAGTTAAAG
PD	CAAGGCGTGGCAGGAAATCA	TTAATCCTCATTAGAAGTGGGGCCT

Table S3. The shRNA sequences.

ShFbxw7	CCGGTTCAACAAGAACTTCGTAATTCTCGAGAATTACGAAGTTCTT
ShTrim32	GTCCAATAGTCAAGTGGTAGAGGAGCAGA
ShHuwe1	CGACGAGAACTAGCACAGAAT
ShUSP3	AGTTTATCCGATCCAGCTT
ShUSP5	CGAGGAGAAGTTTGAATTA