Counts

FL1

FSC

Counts

Dip G1: 45.99 %
Dip G2: 14.02 %
Dip S: 39.99 %

Dip G1: 46.26 %
Dip G2: 13.57 %
Dip S: 40.17 %

Dip G1: 28.16 %
Dip G2: 44.29 %
Dip S: 27.55 %

Total BiFC- BiFC+

FIGURE S2 Ding et al., 2013

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Thymidine release into DMSO

Thymidine release into iCRT

Thymidine release into iCRT then DMSO

Thymidine release into DMSO then iCRT

Asynchronous Thymidine release

24 h  20 h  18 h  16 h  14 h  12 h  10 h  8 h  5 h  0 h

Asyn DMSO iCRT iCRT DMSO iCRT DMSO iCRT DMSO iCRT

A1  A2  A3  A4

Release after Thymidine block

6 h  8 h  10 h  12 h  14.5 h

DAPI  β-tub  p-H3  Merge

Caspase-3/7 Activity (fold change)

0  20  40  60  80  100

DOX  -  +  -  +
iCRT  -  -  +  +

Caspase-3/7 Activity (fold change)

NG 60  -  +  -  +
iCRT  -  -  +  +

Caspase-3/7 Activity (fold change)

130 KDa  17 KDa  43 KDa

DOX  -  +  -  +
iCRT  -  -  +  +

Caspase-3/7 Activity (fold change)

NG 60  -  +  -  +
iCRT  -  -  +  +

Caspase-3/7 Activity (fold change)

130 KDa  17 KDa  43 KDa

DOX  -  +  -  +
iCRT  -  -  +  +

Caspase-3/7 Activity (fold change)

NG 60  -  +  -  +
iCRT  -  -  +  +

Caspase-3/7 Activity (fold change)
Supplementary Figure legends

Fig. S1. Visualization of the β-catenin/TCF complex by BiFC.

A. Top: Schematic of the BiFC biosensor. The BiFC biosensor comprises β-catenin (yellow) and TCF3 (red) proteins, each was fused with a half of Venus protein (VC and VN) or YFP protein (YC and YN) at their C- and N-terminus, respectively. Bottom: Schematic of the BiFC constructs. FLAG or Myc tag was added at their N-terminus for protein detection. A C-terminal deleted β-catenin (β-catenin ΔC) was used in all cases to minimize the Wnt activity when these constructs were over-expressed.

B. Top: Wnt-responsive TOP-FLASH reporter assay in HEK293T cells transfected with BiFC components. Five or ten ng plasmid DNA per well in 96 well plate were transfected, respectively. Bottom: The expression levels of the BiFC components in these samples were determined by Western blot. FLAG antibody was used to detect FLAG-β-cateninΔC-VC/YC and Myc antibody was used to detect Myc-VN/YN-TCF3. GAPDH was used as a loading control.

C. BiFC signals (upper panels) or GFP signals (lower panels) in HEK293T cells after co-transfection with the indicated plasmids. Fluorescence and phase-contrast merged fluorescence images are shown. Bar = 100 μm.

D. Schematic of the constructs for the doxycycline-repressible BiFC stable lines. The pBI vector allows the simultaneous regulation of both β-cat-VC and VN-TCF3 by one central, bidirectional TRE (Tet responsive element). The internal ribosome entry site (IRES) permits the translation of two open reading frames from one messenger RNA. These constructs were introduced into HeLa-tTA cells to produce stable cell lines. A doxycycline-repressible Venus stable line was also established as a control.

E. Western blot analysis of the expression levels of the biosensor components and Venus in doxycycline-repressible BiFC and Venus stable cell lines. Withdraw of Dox (doxycycline) induced the expression of the proteins. The relative expression level was compared in lower panel, which indicated that the β-cat-VC (*) was at a similar
level to endogenous β-catenin (arrow). Since the C-terminal domain of β-catenin was deleted, it is still slightly smaller than the wild type, even though it is fused to half of the Venus protein (VC).

**Fig. S2. The β-catenin/TCF BiFC signal accumulates during the S-G2 phases.**
Analysis of the DNA content profile and YFP signal intensity of BiFC-YFP stable cells by flow cytometry. The BiFC-YFP signal could only be detected after incubation for 4 h at 30°C. The cells were then fixed by paraformaldehyde, stained with propidium iodide and analyzed by FACS. The DNA content profiles of the total, BiFC-positive, and -negative gated populations are shown.

**Fig. S3. Identification of β-catenin/TCF target genes during the S-G2 phases.**
A, B. Effects on Wnt target genes expression in S-G2 phase HCT116 cells upon Wnt inhibition by (A) iCRT or (B) aStAx. HCT116 cells were synchronized and treated as in Fig. 3B. Forty two genes in the cell death and cell cycle categories were analyzed using real-time PCR. The results are the average of triplicate data; the SD and p-values were calculated in a two-tailed student t-test.
C. Wnt-responsive TOP-FLASH reporter assay in HCT116 cells with the indicated treatments. iCRT or vehicle was introduced 24 h before cell lysis and detection.
D. Wnt-responsive TOP-FLASH reporter assay in HCT116 cells to compare the inhibitory effects of aStAx and iCRT. iCRT, aStAx, or vehicle was introduced 24 h before cell lysis and detection.
E. MTT assay in HCT116 cells to compare the inhibitory effect of aStAx and iCRT on cell proliferation. The cells were treated with DMSO, iCRT (50 µM) or aStAx (20 µM) for 4 days and the MTT assay was performed every day. The results presented are the mean ± SD from quintuplicate samples.
F. Chromatin immunoprecipitation assays in G2- or G1-enriched HCT116 cells (5 h and 12.5 h after release from the thymidine block, using TCF4 or a mouse IgG antibodies as indicated. Promoter regions of AXIN2, CCND1 and AURKA were tested
and the promoter of ACTB (β-actin) was used as a control. All graphs show the average with SD (n = 3). The cell cycle status is also shown.

**Fig. S4. β-catenin/TCF complex-mediated target gene expression ensures G2/M progression and cell survival.**

A. Delay of cell cycle progression by iCRT treatment. Synchronized HCT116 cells were released into medium containing either DMSO (control) or 75 μM iCRT. (A1) DMSO; (A2) iCRT; (A3) iCRT for the first 8 h and then replaced by DMSO containing medium; (A4) DMSO for the first 9 h and then replaced by iCRT containing medium. Cells were harvested at the indicated time points and their cellular DNA content was determined by propidium iodide staining and flow cytometry. Asyn, asynchronous cells.

B. HCT116 cells after release from a thymidine block were treated with DMSO or iCRT for the indicated time periods and then fixed and stained with p-H3 antibody (green) or DAPI (blue). Bar = 100 µm.

C. Closer views of representative iCRT-treated HCT116 cells at different stages of mitosis. Cells were stained with DAPI (blue), p-H3 (green) or β-tubulin (β-tub, red). Bar = 10 µm.

D. Caspase-3/7 activation in response to Wnt inhibition by iCRT. HCT116 cells were incubated with DMSO, iCRT or DOX (Doxorubicin, as a positive control) for 24 h. Effects on Caspase-3/7 activity were assessed using the Apo-ONE homogeneous Caspase-3/7 assay. Results show the fold increase of Caspase-3/7 activity relative to the DMSO-treated cells. Each data point represents the mean ± SD of the experiment performed in triplicate.

E, F. Sensitivity of iCRT-treated (50 μM) HCT116 cells to Doxorubicin-triggered (DOX, 1 μM) apoptosis. Cells were treated for 24 h. Apoptosis was detected by measurement of (E) Caspase-3/7 activity and (F) cleavage of Caspase-3 and PARP1 by Western blot. The arrow indicates the cleaved PARP1.

G, H. Sensitivity of iCRT-treated (50 μM) HCT116 cells to NG 60-triggered (7.5 μM) apoptosis. Cells were treated for 24 h. Apoptosis was detected by measurement of (G)
caspase-3/7 activity and (H) cleavage of Caspase-3 and PARP1 by Western blot. The arrow indicates the cleaved PARP1.

**Movie 1**: Example 1 of a dynamic β-catenin/TCF-BiFC signal during the cell cycle. HeLa cells expressing the BiFC biosensor were traced for 40 h. Images were recorded every 30 min by a high content screening platform (HCS, ArrayScan VTI 700, ThermoFisher Cellomics).

**Movie 2**: Example 2 of a dynamic β-catenin/TCF-BiFC signal during the cell cycle. HeLa cells expressing the BiFC biosensor were traced for 40 h. Images were recorded by HCS platform.

**Movie 3**: HeLa cells expressing the BiFC biosensor were released from a thymidine block and then traced for 26 h. Images were recorded every 20 min by a Nikon Ti-E A1Rsi inverted confocal microscope (Nikon Instruments).

**Movie 4**: Example 1 of a Venus fluorescence signal during the cell cycle. HeLa cells expressing the Venus fluorescence proteins were traced for 40 h. Images were recorded by HCS platform.

**Movie 5**: Example 2 of a Venus fluorescence signal during the cell cycle. HeLa cells expressing the Venus fluorescence proteins were traced for 40 h. Images were recorded by HCS platform.
Movie 5.

Tables S1 to S7.

Download Supplementary Tables