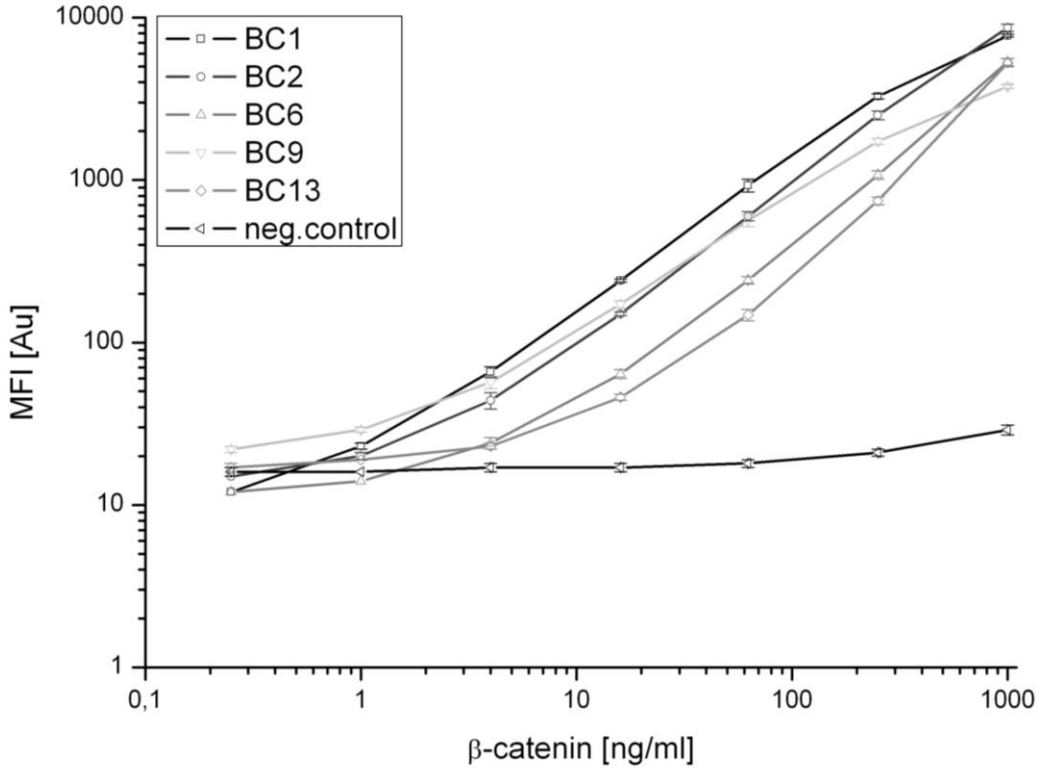
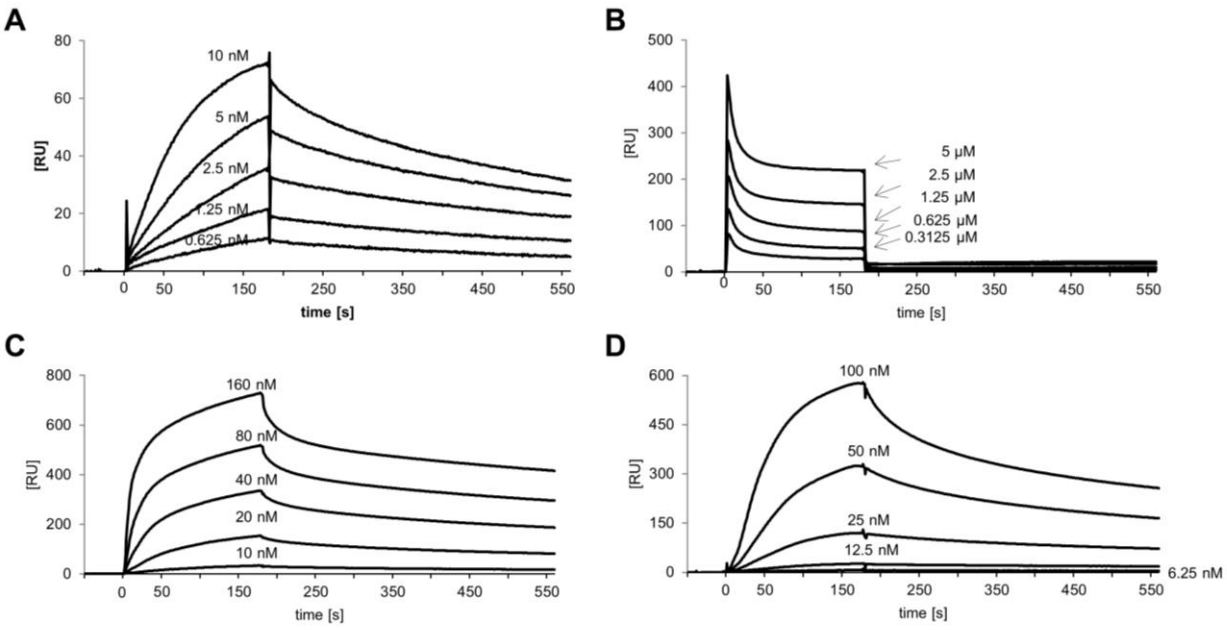
Supplementary information



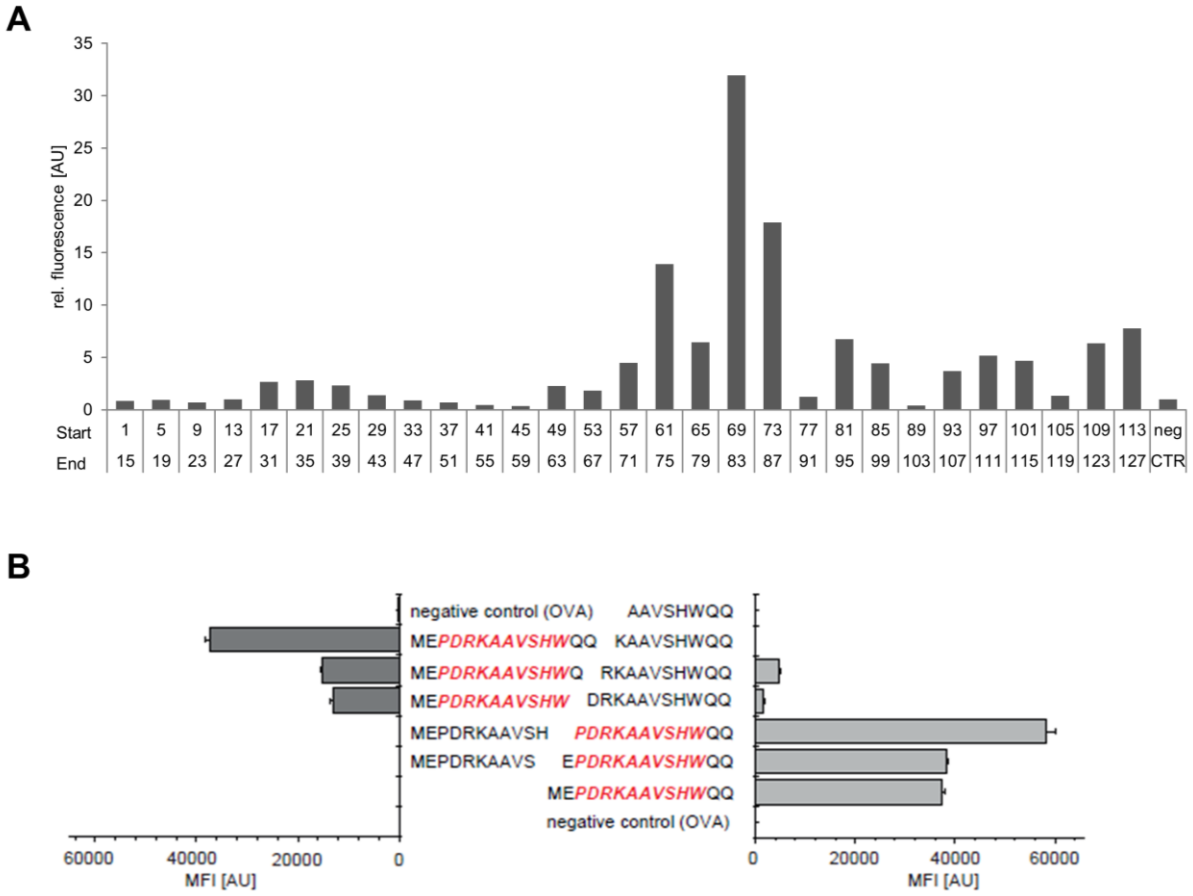
suppl. Figure 1

β-catenin-specific nanobodies show high binding sensitivities. Nbs were covalently immobilized on microspheres and incubated with serial dilutions of β-catenin ranging from 0.2 ng/ml to 1 µg/ml. Bound protein was detected with an anti-β-catenin antibody. Shown are mean signal intensities of three independent replicates ± stds.



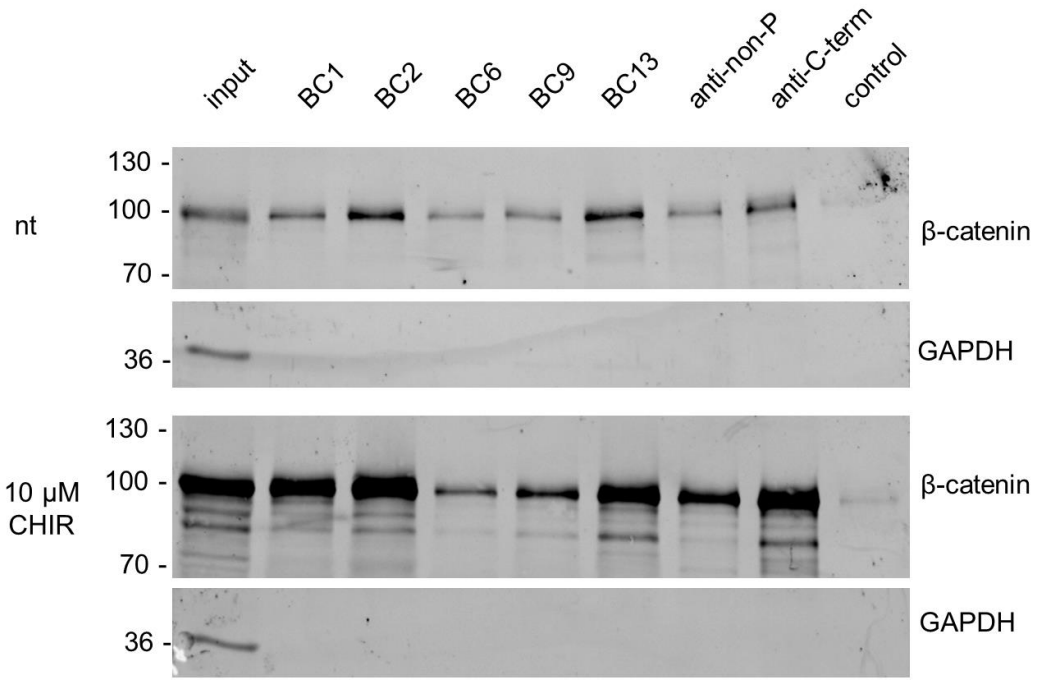
suppl. Figure 2

Affinity measurements of β-catenin-specific nanobodies. Nbs were used for surface plasmon resonance spectroscopy (SPR) measurements against immobilized β-catenin as described in the result section and Figure 2. SPR sensograms of BC2 (**A**), BC6 (**B**), BC9 (**C**) and BC13 (**D**) with indicated Nb concentrations are shown. The Nb injection time was 180 s, followed by a dissociation time of 300 s. The data was evaluated using the software Bia evaluation 4.1 and the 1:1 Langmuir binding model.



suppl. Figure 3

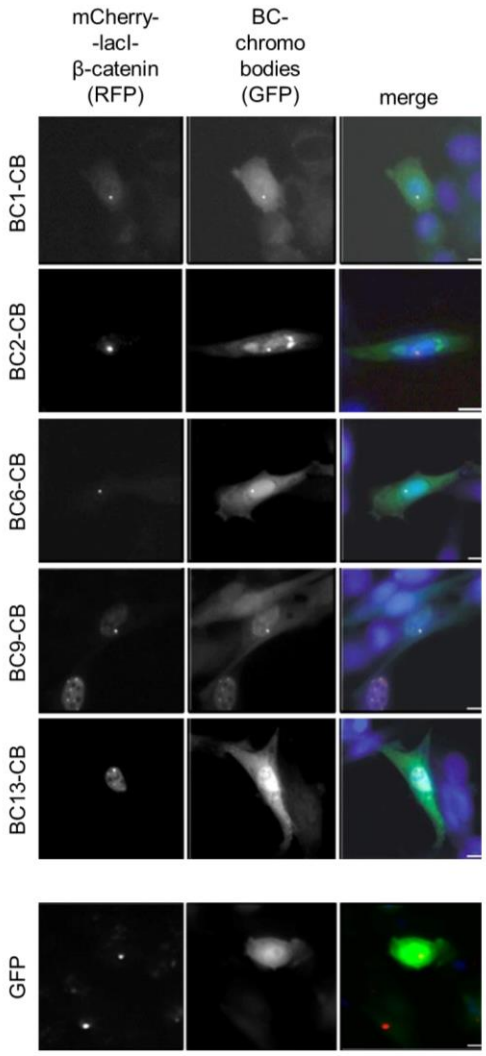
Nanobodies bind distinct epitopes at the N-terminus of β-catenin. (**A**) Identification of the epitope for BC1 using a peptide screen. 29 overlapping 15-mer peptides covering the N-terminus from aa 1-127 of β-catenin were immobilized on microspheres with varying IDs per peptide and incubated with biotinylated BC1. Peptide-bound BC1 nanobodies were detected with streptavidin-phycoerythrin (PE). The Myc-peptide (EKLISEEDL) was used as negative control. (**B**) Determination of the minimal epitope of BC2. Incubation of serial N- and C-terminally truncated peptides covering aa 14 - 27 of β-catenin with biotinylated BC2. Peptide-bound BC2 nanobodies were detected with streptavidin-phycoerythrin (PE). Aminoacid residues comprising the minimal epitope of BC2 are highlighted in red. Shown are mean signal intensities of three independent replicates ± stds.



suppl. Figure 4

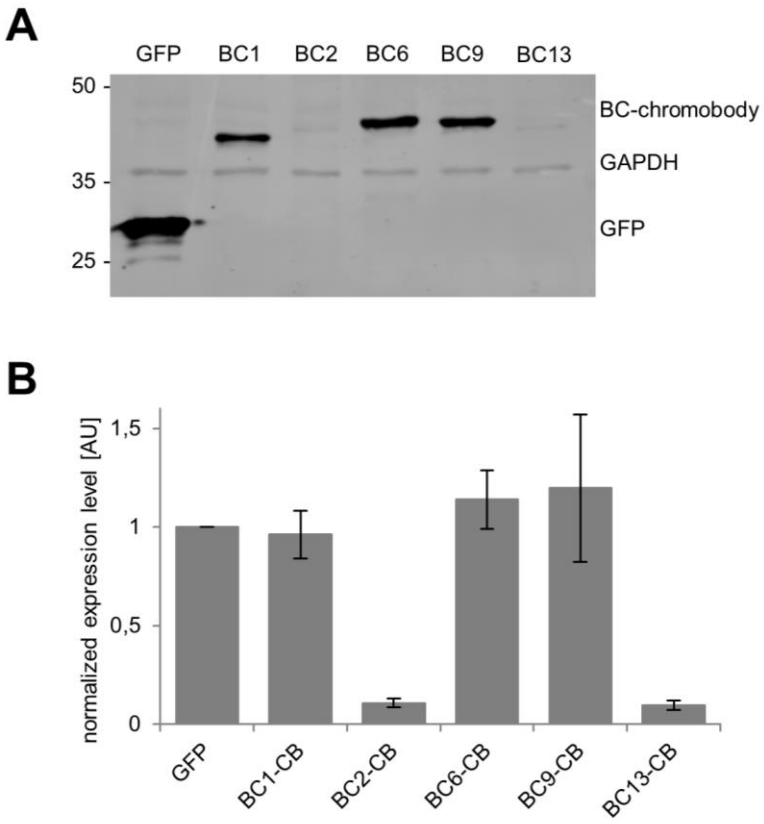
Nanobodies bind endogenous β-catenin comparable to conventional antibodies.

HEK293T cells were either left untreated (nt) or were incubated with CHIR (GSK3β-inhibitor) for 24 h. Cells were lysed under native conditions and membrane-bound fraction was depleted using Concanavalin A (ConA)-beads. Depleted supernatants were adjusted to 2 mg/ml and incubated with equal amounts of immobilized Nbs. For the precipitation using conventional antibodies an anti-non-phospho antibody (anti-non-P) or an antibody directed against the C-terminus of β-catenin (anti-C-term) was used. Both antibodies were immobilized on Protein A/G sepharose. Input and bound fractions were subjected to SDS-PAGE followed by immunoblot analysis using antibodies specific for β-catenin and GAPDH. *Input*: 0.5% of ConA-depleted supernatant; *BC1 – BC13*: 10% of bound fraction of nanobodies as indicated; *anti-non-P and anti-C-term*: 10% of antibody bound fraction; *control*: 10 % of bound fraction of a non-related nanobody. Shown are representative blots of three independent experiments.



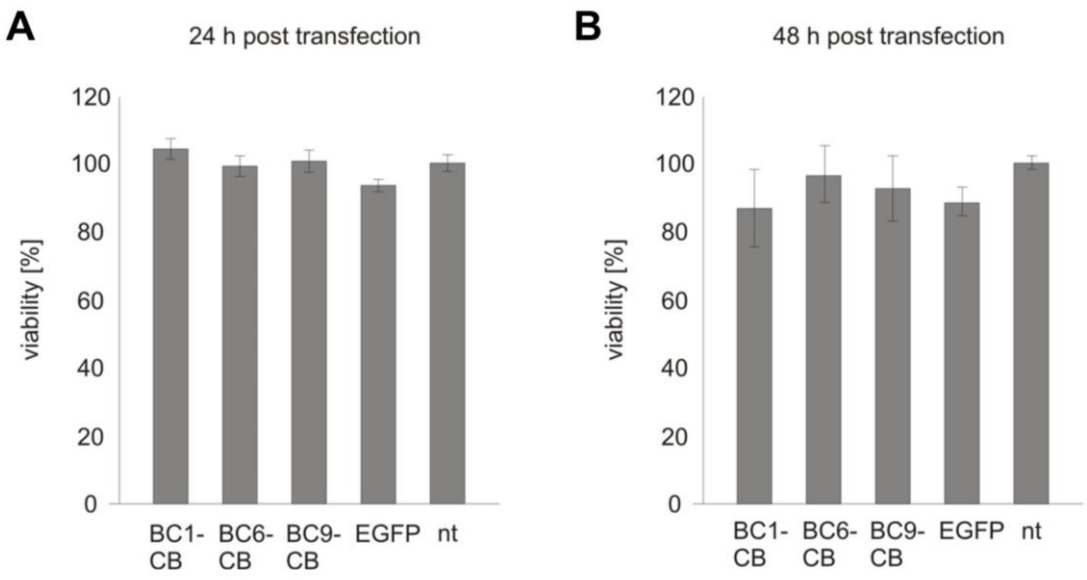
suppl. Figure 5

BC-chromobodies bind β-catenin within living cells in a Fluorescent Two-hybrid assay. Transgenic BHK (baby hamster kidney) cells containing a lac operator array were co-transfected with expression constructs coding for mCherry-lacI-β-catenin and the GFP-tagged chromobodies BC1,-2,-6,-9,-13 or EGFP as negative control. Upon expression the red fluorescent β-catenin construct localizes at the lac operator array and thus becomes visible as precise red spots within the nucleus. Mediated by the intracellular interaction, β-catenin-specific chromobodies (BC-chromobodies) are recruited to these spots and a co-localization of mCherry and GFP fluorescence is visible in an image overlay. In cells expressing GFP in combination with mCherry-lacI-β-catenin no fluorescence co-localization is observable. Shown are representative images. Scale bar: 10 µM.



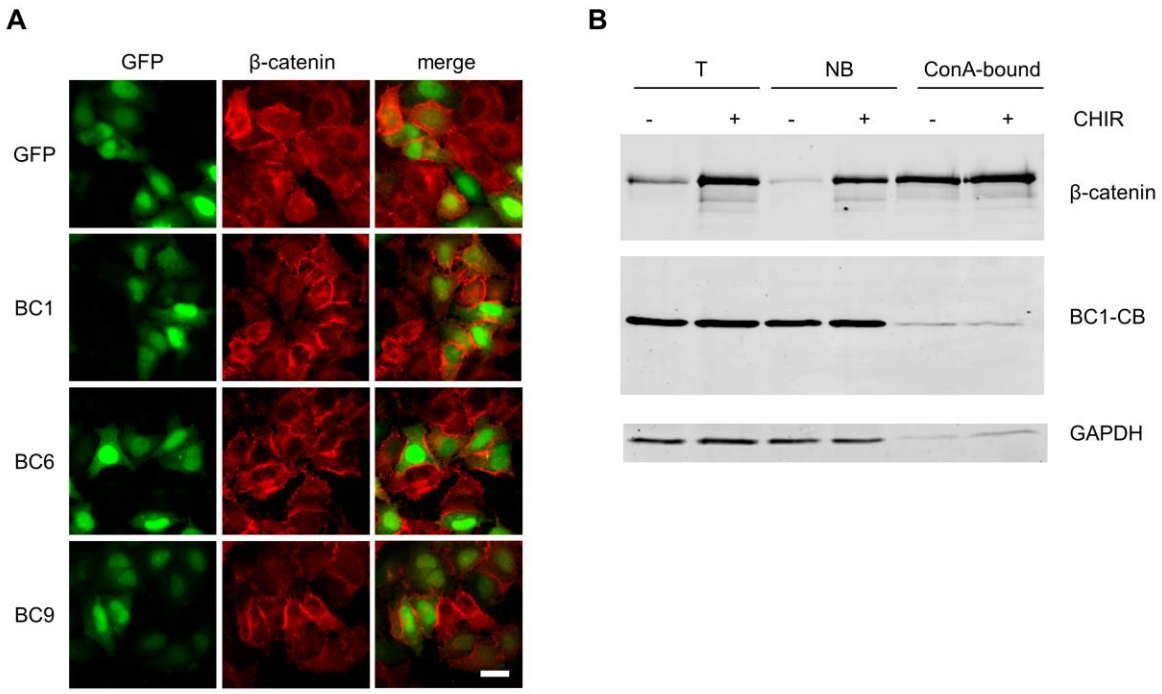
suppl. Figure 6

BC-chromobodies show different intracellular protein levels. (**A**) Immunoblot analysis of soluble protein fraction of HEK293T cells expressing GFP or the BC-chromobodies. Shown are the antibody signals for GFP (chromobody) and GAPDH (control). (**B**) Densitometric evaluation of immunoblot analysis. Immunoblot signals of GFP and BC-chromobodies were normalized to GAPDH. GFP expression was set to one and ratios of BC-chromobodies in comparison to GFP are shown. Shown are mean signals of three independent replicates ± stds.



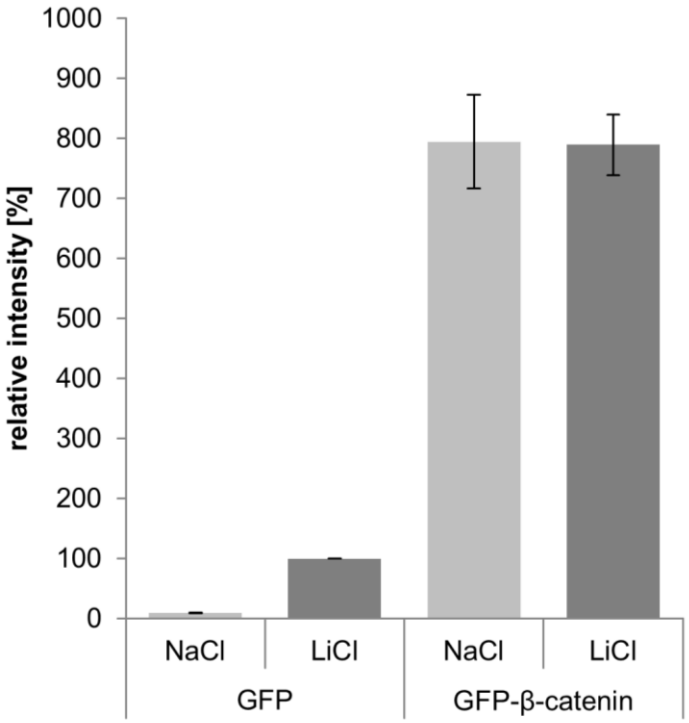
suppl. Figure 7:

BC-chromobodies are not cytotoxic. Resazurin assay of HeLa cells expressing BC1-, BC6- or BC9-chromobodies or EGFP control. Depicted viability values represent relative mean fluorescence intensities (± stds) determined for transfection triplicates after 24 h (**A**) and 48 h (**B**). Viability of untreated cells was set to 100 %.



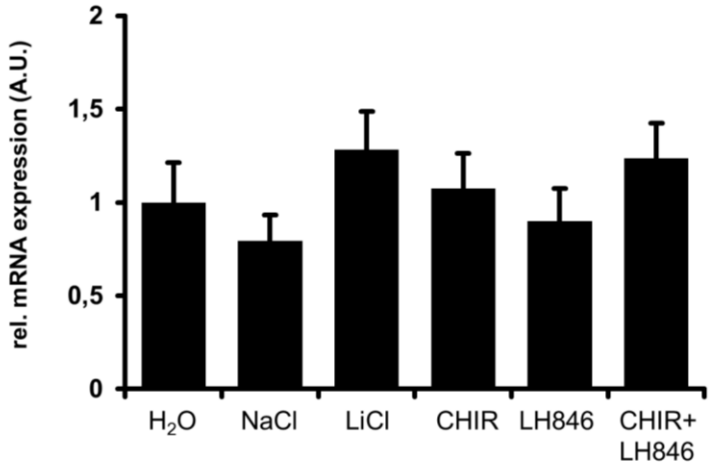
suppl. Figure 8:

BC-chromobodies do not recognize membrane-associated β-catenin. (**A**) HeLa cells transiently expressing indicated GFP-tagged BC-chromobodies (left panel) were fixed and immunostained with an anti-β-catenin antibody, H102 (middle panel). Scale bar: 25 µm. (**B**) HEK293T stably expressing the BC1-chromobody (BC1-CB) cells were either left untreated (-) or were incubated with 10 µM CHIR (+). E-cadherin-associated β-catenin was precipitated using ConA beads. From each sample 0.5 % of the total lysate (T), 0.5 % of the non-bound fraction (NB) and the 10% of the ConA-bound fraction (ConA-bound) were analyzed by SDS-PAGE and immunoblotting using an anti-β-catenin antibody (upper panel), an anti-tagGFP antibody (mid panel) or GAPDH (lower panel).



suppl. Figure 9

Expression of GFP-β-catenin induces transcriptional activity. HEK293T cells were either transfected with GFP or GFP-β-catenin in combination with reporter constructs containing TCF-Promoter-luciferase-reporter sites (TOP-flash) or a corresponding control construct with mutated TCF-binding sites (FOP-flash). Reporter activity of NaCl-treated cells is shown in light grey bars and LiCl-induced reporter activity is shown in dark grey bars. All values are normalized to mean luminescence values of LiCl-treated GFP control. Reporter induction after 24 h upon LiCl treatment is shown. Columns represent the results of three independent experiments ± stds.



suppl. Figure 10

Compound treatment does not affect mRNA levels of BC1 chromobody. HeLa_BC1-GFP cells were incubated for 24 h with H_2O or NaCl as controls or compounds as indicated. The BC1-CB mRNA expression level was quantified by qRT-PCR analysis and normalized to GAPDH mRNA. Columns represent mean mRNA expression ± stds of four independent replicates.