Supplementary Figure Legends

Supplementary Figure 1. PB does not directly influence interactions of β-catenin with its binding partners. Recombinant fusion proteins GST-TCF4, GST-ECT, and GST-ICAT were incubated with recombinant β-catenin in the presence of increasing concentrations of PB and bound β-catenin was assayed. Mean ±SD (n≥3) are shown.

Supplementary Figure 2. Basal and LiCl (15 mM)-induced activities from the 8x β-catenin/TCF-driven Supertopflash (STF) reporter vector are efficiently inhibited in 70.4 cells by incubation with 20 µM iCRT3, a model inhibitor of the pathway, for 24h. Cell treatment with 3 mM PB decreases basal as well as LiCl-induced STF reporter activities in a similar manner. Mean ±SD (n≥3 independent experiments; each experiment performed in triplicates or quadruplicates) are shown. Statistical significance (Student’s t-test) is indicated by asterisks: *, p<0.05; **, p<0.01; ***, p<0.001.

Supplementary Figure 3. Regulation of β-catenin signaling by PB is independent of CAR. (A) Expression of mRNAs related to CAR-mediated signal transduction was measured in 70.4 cells by real-time RT-PCR in the absence or presence of 3 mM PB, and compared to normal mouse liver (set to 100%). Expression of Car mRNA and its model target genes Cyp2b10 and Cyp2c is barely or not detectable. Similarly, the mRNA encoding Cx32, a protein involved in tumorigenicity of CAR activators, is barely detectable. Only the CAR binding partner RXRα is detectable at the mRNA level in meaningful amounts. (B) Treatment of 70.4 cells with 10 µM of the CAR activator TCPOBOP (TCP) did not mimic the activity of PB on basal or LiCl (15 mM)-induced activities from the 8x β-catenin/TCF-driven Supertopflash (STF) reporter vector. Mean ±SD (n≥3 independent experiments; reporter assays: each experiment performed in triplicates or quadruplicates) are shown. Statistical significance (Student’s t-test) is indicated by asterisks: *, p<0.05; **, p<0.01; ***, p<0.001. Statistical significance was not calculated for mRNA analyses due to the fact that expression of the
respective genes in 70.4 cells was compared to a single reference sample (mRNA pool from fresh mouse liver).

Supplementary Figure 4. Basal and LiCl (15 mM)-induced activities from the 8x β-catenin/TCF-driven Supertopflash (STF) reporter vector are inhibited by treatment with 3 mM PB for 24h in serum-free medium (left panel). Treatment of cells with 20 µM γ-aminobutyric acid (GABA) did not mimic the PB effect (right panel). Mean ±SD (n≥3 independent experiments; each experiment performed in triplicates or quadruplicates) are shown. Statistical significance (Student’s t-test) is indicated by asterisks: *, p<0.05; **, p<0.01; ***, p<0.001.
Supplemental Figure 2

Relative luciferase activity

Ctr  PB  iCRT3  LiCl  LiCl +PB  LiCl iCRT3

***  ***  ***  ***

0  2  4  6  8  10  12  14  16  18
Supplemental Figure 3

(A) Relative mRNA expression in %

(B) Relative luciferase activity

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Supplemental Figure 4

A

Relative luciferase activity

Ctr  PB  LiCl  LiCl+PB

B

Relative luciferase activity

Ctr  GABA  LiCl  LiCl+GABA

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