# Regulation of PTEN by CK2 and Notch1 in primary T-cell acute lymphoblastic leukemia: rationale for combined use of CK2- and γ-secretase inhibitors

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## Supplementary information

The cycling protocol for *NOTCH1* amplifications consisted of 1 cycle at 94°C for 3 min, followed by 10 cycles of annealing at decreasing temperatures from 61°C to 57°C (1°C decrease every two cycles) for 30 s, extension at 72°C for 1:45 min and denaturation at 95°C for 30 s. Subsequently, 30 cycles were performed with annealing at 56°C for 30 s. A final extension step for 5 min at 72°C was included. PCR reactions contained 50ng of DNA or 0.5 µl cDNA, 10 pmol of each primer, 2 mM MgCl2, 5% DMSO, 1X PCR Buffer (Biotools), and 1.5 U of Taq DNA Polimerase (Biotools). The PCR cycling protocol for *PTEN*, *HES1*, and *ABL* consisted of 10 min at 95°C, followed by 40 cycles of 58°C for 30 s, 72°C for 40 s, 95°C for 15 s, and a final extension step for 5 min at 72°C. For MYC a two-step cycling protocol was used consisting of 10 min at 95°C, followed by 40 cycles of annealing/extension at 65°C for 1 min and 95°C for 15 s, and a final extension step for 5 min at 72°C. A standard melting-curve cycle was used to check the quality of amplification, such as no primer dimer being formed during PCR. Fluorescence was measured at 72°C.

**Online Supplementary Table S1.** Primers used for *NOTCH1* sequencing and RQ-PCR analysis of *HES1*, *MYC*, *PTEN*, and *ABL*.

| Primer | Primer sequence (5'-→ 3') | Reference |
|---|---|---|
| **Primers for PCR and sequencing of *NOTCH1*** | | |
| Notch2627F | GGACTGTGCGGAGCATGTA | 1 |
| Notch2627R | CAGCCCACGAAGAACAGAA | 1 |
| Notch26F | CAGTGGGCTGGAGGCA | 1 |
| Notch26R | GGGGATTGACCGTGGG | 1 |
| Notch27F | GGTGGGTATCTGGGATGAGC | 1 |
| Ex27R743 | GAAAAGCCCTACCCCAACTC | 2 |
| Notch34TF | TTTGAATGGTCAATGCGAGTG | 1 |
| Notch34PR | AAAAAGGCTCCTCTGGTCG | 1 |
| Ex34PestF | CCTCACCTGGTGCAGACC | 2 |
| Ex34PestR | AAATTAAAATCCTCGTTCTTATTTTG | 2 |
| Notch34PF | AGCCAGCAAACATCCAGC | 1 |
| **Primers for RQ-PCR** | | |
| HES1-F | TGGAAATGACAGTGAAGCACCT | 3 |
| HES1-R | GTTCATGCACTCGCTGAAGC | 3 |
| MYC-F | CTTCTCTCCGTCCTCGGATTCT | 4 |
| MYC-R | GAAGGTGATCCAGACTCTGACCTT | 4 |
| PTEN-F | GCTACCTGTTAAAGAATCATCTGG | 1 |
| PTEN-R | CATGAACTTGTCTTCCCGT | 5 |
| ABL-F | TGGAGATAACACTCTAAGCATAACTAAAGGT | 6 |
| ABL-R | GATGTAGTTGCTTGGGACCCA | 6 |

## References

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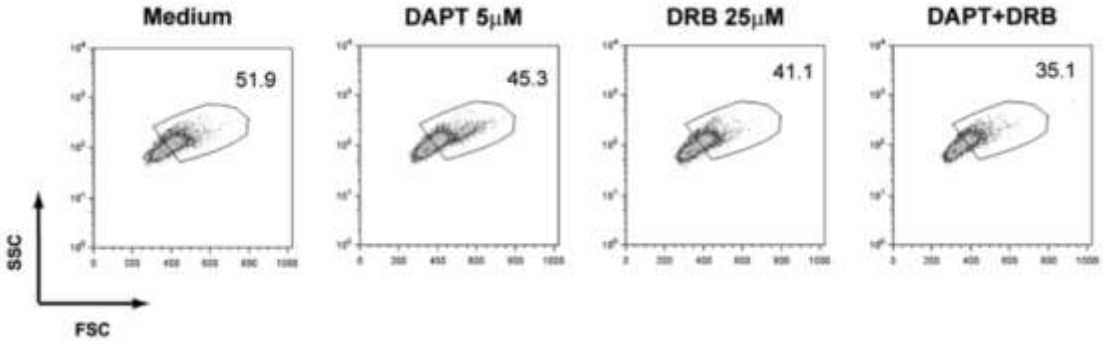
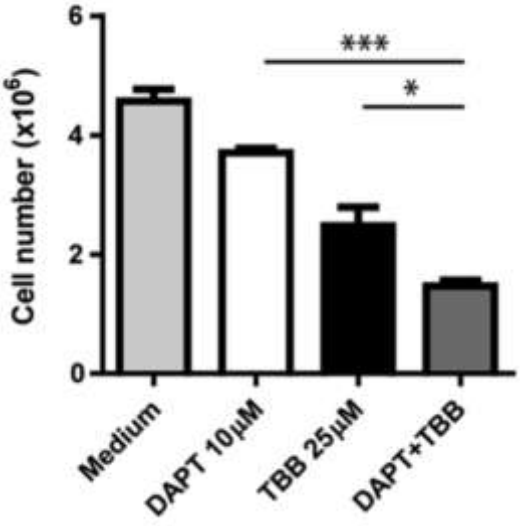
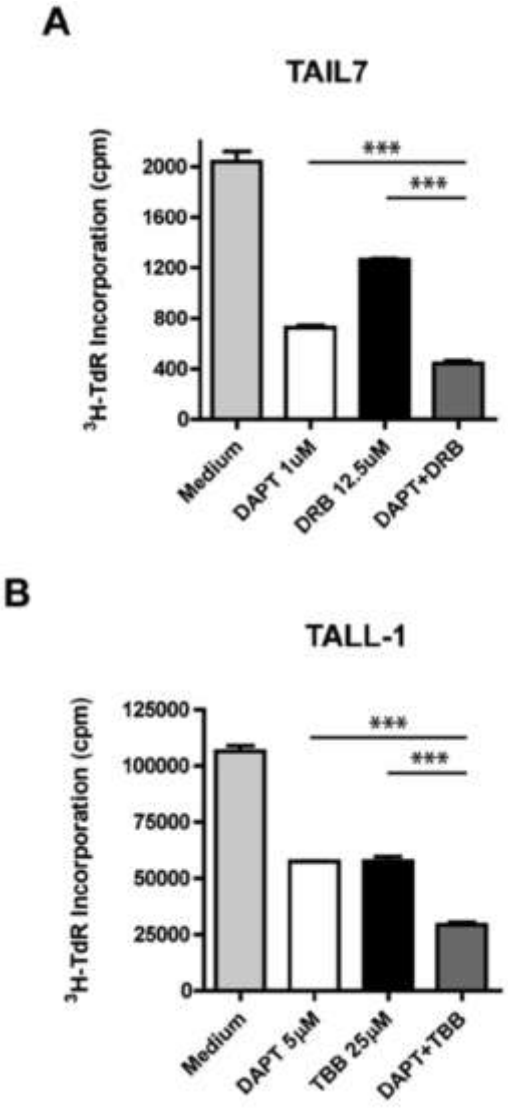



Online Supplementary Figure S1. Cooperative effects of GS- and CK2 inhibitors in decreasing the size of TAIL7 cells. TAIL7 cells were cultured in the indicated conditions for 72h and cell size was determined by FSC x SSC discrimination by flow cytometry analysis.




Online Supplementary Figure S2. Cooperative effects of GS- and CK2 inhibitors in decreasing HPB-ALL cell numbers. HPB-ALL cells were cultured in triplicates for four days in the indicated conditions and number of viable cells was assessed by trypan blue exclusion using a hemocytometer. *$P$<0.05; ***$P$<0.001.




Online Supplementary Figure S3. Cooperative effects of GS- and CK2 inhibitors in decreasing TAIL7 and TALL-1 cell proliferation. TAIL7 (A) and TALL-1 (B) cells were cultured in triplicates for seven days in the indicated conditions and proliferation was evaluated by assessing DNA synthesis by $^3$H-thymidine incorporation. ***$P$<0.001.