Endonuclease and redox activities of human apurinic/apyrimidinic endonuclease 1 have distinctive and essential functions in IgA class switch recombination

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Running title: The redox function of APE1 is important for CSR

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SUPPORTING INFORMATION

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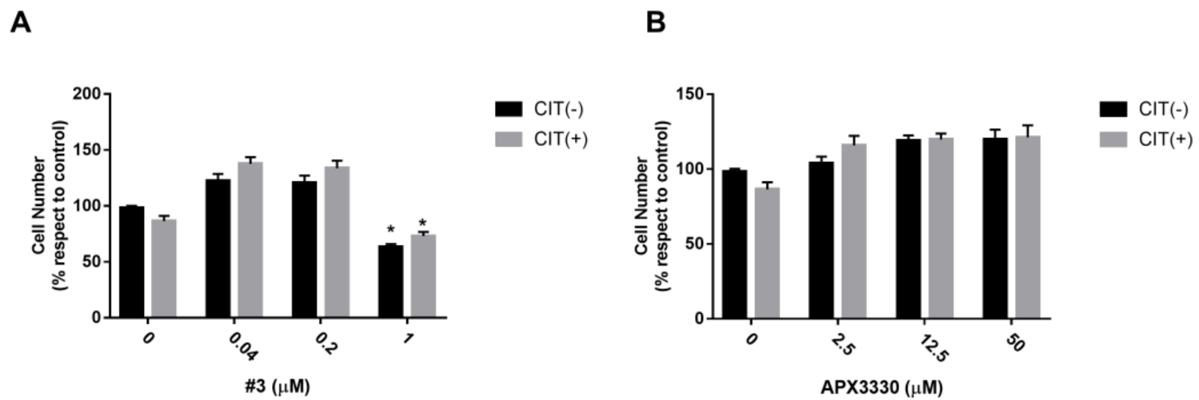
Supplementary Figure legend

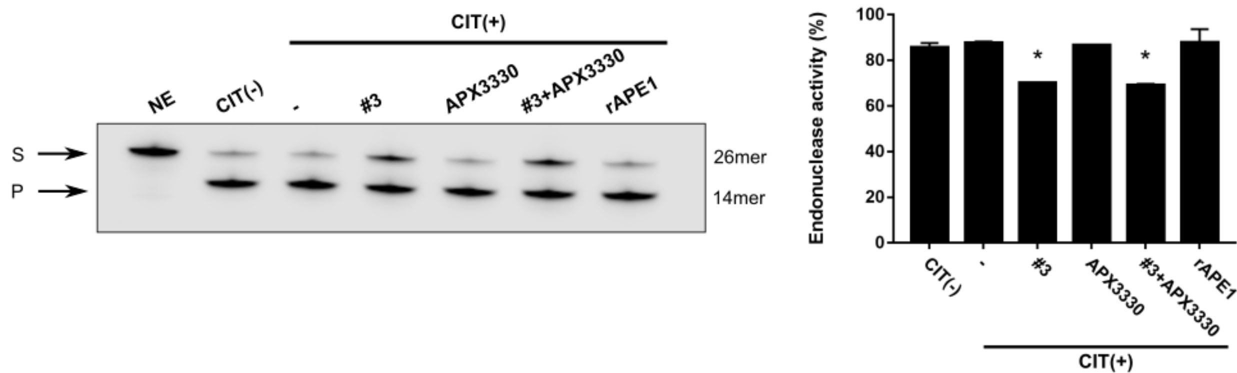
Supplementary Figure S1



Supplementary Figure S1. Effect of APE1 inhibitors on CH12F3 cell viability.
Toxicity effects of APE1 inhibitors on CH12F3 growth as determined by the MTS assay. CH12F3 cells under CIT(-) and CIT(+) conditions were treated with increasing concentrations of #3 (A) and APX3330 (B) for 72 h and cell viability was estimated by the MTS colorimetric assay. Histograms reported means ± SD of three independent experiments. Asterisks represent a significant difference with respect to non-treated cells. * $p < 0.05$

Supplementary Figure S2

A



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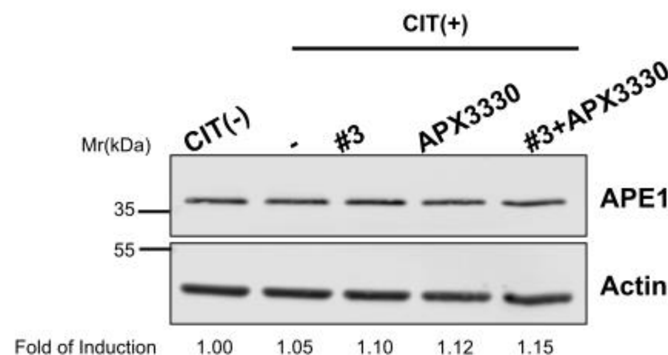
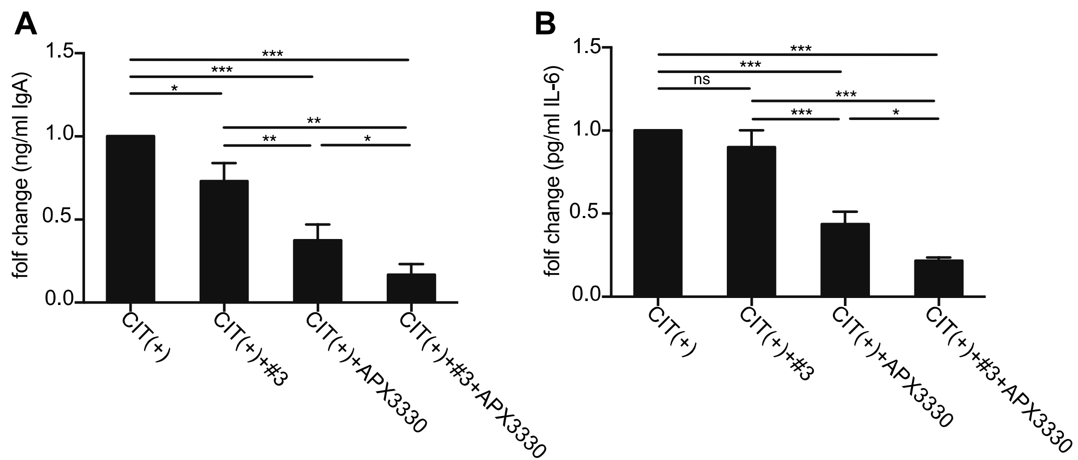


Supplementary Figure S2. **Effectiveness of APE1 inhibitors on APE1 activity and expression.**

A) APE1 endonuclease activity was measured *in vitro* on total cell extracts from CH12F3 cells under CIT(-) or CIT(+) conditions and treated with 0.2 µM compound #3, 50 µM APX3330 and combined treatment for 24 h, as indicated in Methods section. Histogram reports endonuclease activity expressed as percentage conversion of an AP site-containing a 26-mer oligonucleotide DNA substrate (S) to the incised 14-mer product (P). A representative image of a denaturing polyacrylamide gel analysis of the enzymatic reactions is shown, reporting the nucleotide length of substrate and incised product. NE, no cell extract; NT, resting cells. Asterisks represent a significant difference with respect to NT. * $p < 0.05$.

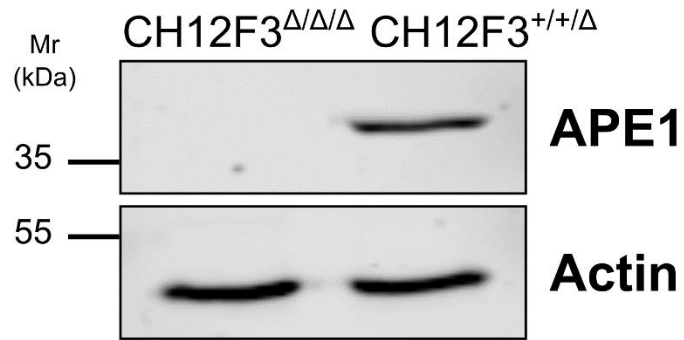
B) APE1 protein level evaluated in CH12F3 cells under CIT(-) or CIT(+) conditions and treated with 0.2 µM compound #3, 50 µM APX3330 and combined treatment for 24 h. Representative Western blotting analyses of total cell extracts probed with APE1 antibody are shown. Actin was used as loading control. Normalized APE1 protein level in respect to resting cells is reported under each relative lane.

Supplementary Figure S3



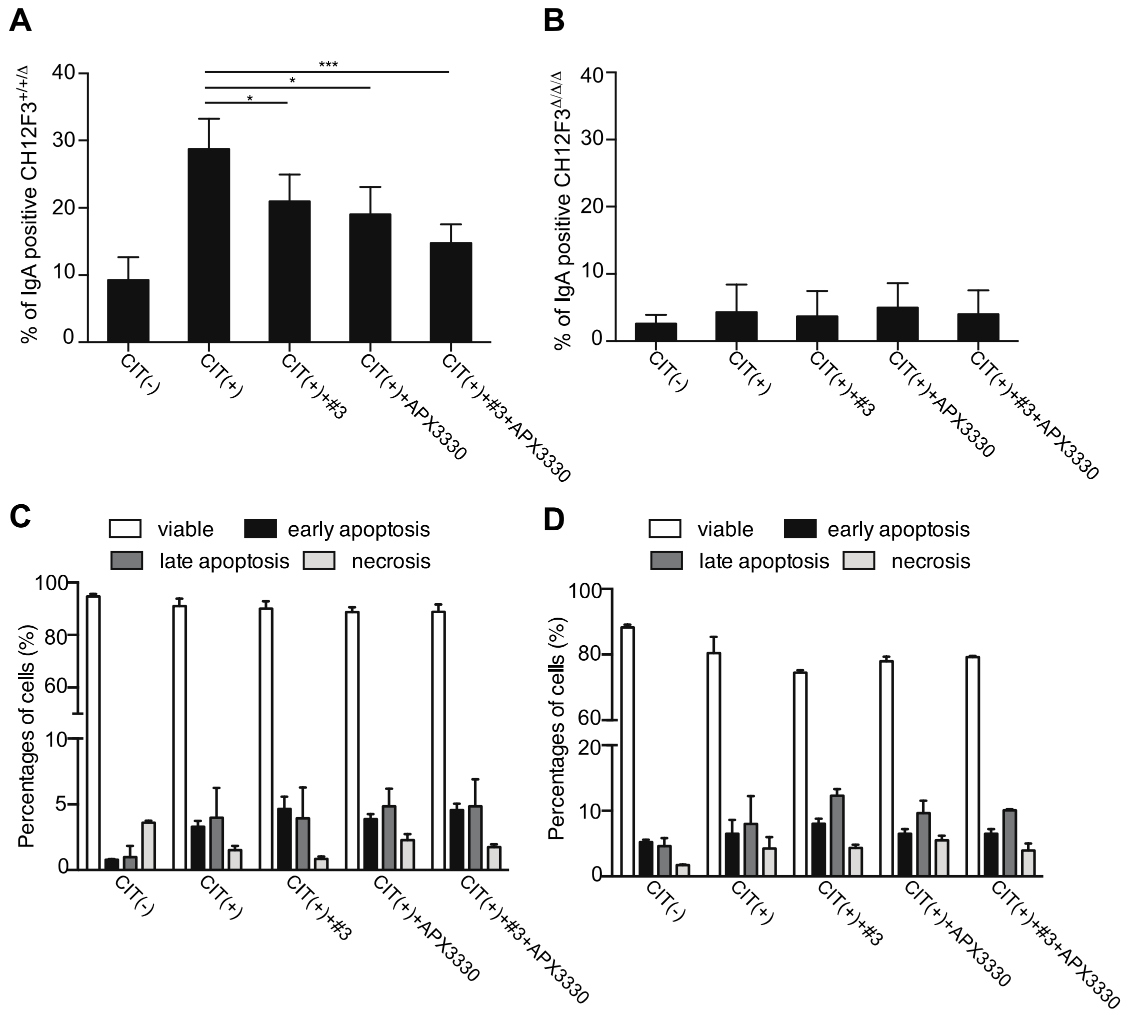
Supplementary Figure S3. **Fold changes of IgA and IL-6 release in cell supernatants.** CH12F3 cells were cultured under CIT(-) or CIT(+) conditions in presence or absence of 0.2 µM compound #3, 50 µM APX3330 and combined treatment and the amounts of IgA (A) and IL-6 (B) released in the supernatants were detected by ELISA assays after 72h. Data are represented as fold change of IgA and IL-6 detection relative to cells undergoing CSR in absence of inhibitors and are representative of three independent experiments. *$p<0.05$, **$p<0.005$, ***$p<0.001$, ns, not significant.

Supplementary Figure S4



Supplementary Figure S4. No APE1 expression in CH12F3 *APE1*-null cells.
Western blot analysis of APE1 protein level in cells containing two (CH12F3 APE1$^{+/+/\Delta}$) or zero (CH12F3 APE1$^{\Delta/\Delta/\Delta}$) copies of APE1, respectively. Actin was used as loading control.

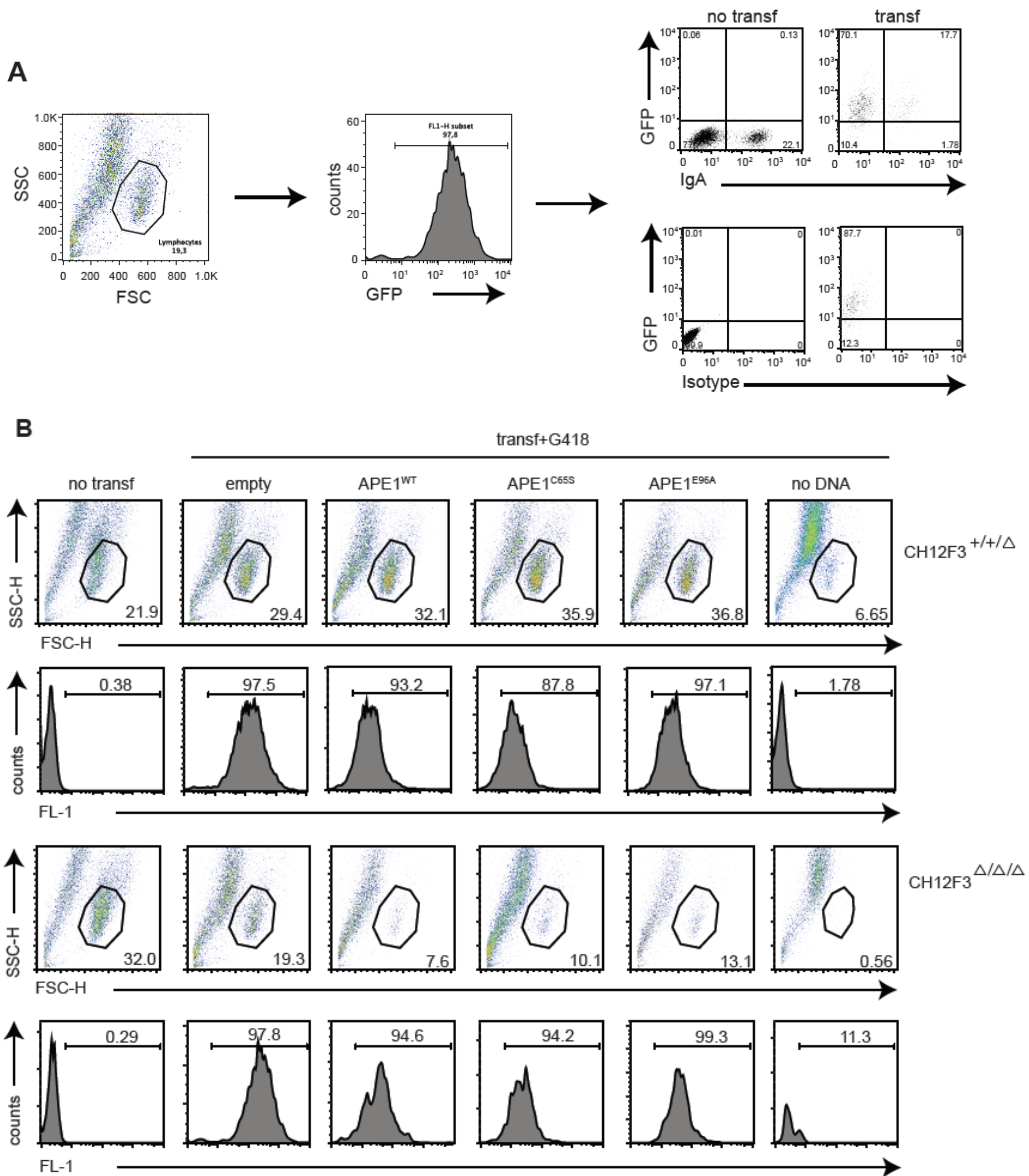
Supplementary Figure S5

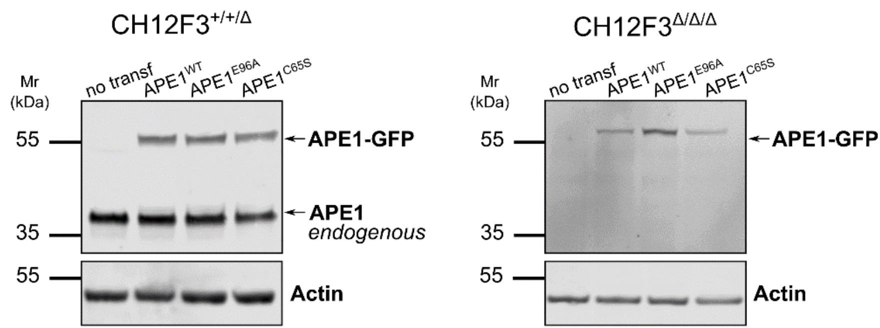


Supplementary Figure S5. Effect of APX3330 and #3 on class switch recombination of CH12F3 APE1 $^{+/+/\Delta}$ and CH12F3 APE1 $^{\Delta/\Delta/\Delta}$ cells.

Percentages of CD19/IgA double-positive CH12F3 APE1 $^{+/+/\Delta}$ (A) and CH12F3 APE1 $^{\Delta/\Delta/\Delta}$ (B) cells under CIT(-) or CIT(+) conditions and in presence of 50 μM APX3330, 0.2μM #3 or both. Data are representative of 3 independent experiments. *$p<0.05$, ***$p<0.001$. Percentages of viable, early apoptotic, late apoptotic and necrotic CH12F3 APE1 $^{+/+/\Delta}$ (C) and CH12F3 APE1 $^{\Delta/\Delta/\Delta}$ (D) cells measured after 72h of culture under resting condition or undergoing CSR in presence of indicated inhibitors. Data are representative of 2 independent experiments performed in triplicate.

Supplementary Figure S6





Supplementary Figure S6. Gate strategy and efficiency of transfection.

Gate strategy for the identification of GFP and IgA double positive transfected cells (A). Viability and transfection efficiency of CH12F3 APE1 $^{+/+/\Delta}$ and CH12F3 APE1 $^{\Delta/\Delta/\Delta}$ cells under G418 selection (B), gated as in panel (A). Western blot analysis of CH12F3 APE1 $^{+/+/\Delta}$ and CH12F3 APE1 $^{\Delta/\Delta/\Delta}$ cells transfected with APE1-expressing plasmids (C).