

## **Supplementary Figure legends.**

### **Supplementary Figure S1. Treatment with CpG-ODN sensitizes CLL cells to NAE**

**inhibition.** CLL cells were co-cultured with CD40L-expressing stroma in the presence or not of 1.5  $\mu$ M CpG-ODN. After 48 hours, cells were treated with the indicated doses of MLN4924 or vehicle control for 24 hours. (A) Apoptosis within the CD19<sup>+</sup> subset of cells (N=6) was determined by Annexin V and 7-AAD staining. Data are mean $\pm$ SEM. \*\* -  $p < 0.01$  compared to control conditions. (B) Cells were lysed and subjected to immunoblotting. A representative image from 1 of 4 experiments is shown.

### **Supplementary Figure S2. NAE inhibition in PBMCs.**

PBMC's isolated from healthy donors were co-cultured with CD40L-expressing stroma and 25 ng/mL Il-21 for 72 hours. Cells were treated with 0.25 - 1  $\mu$ M MLN4924 or vehicle control for 24 h. (A) Apoptosis within the CD19<sup>+</sup> and CD3<sup>+</sup> subset of cells (N=6) was determined by Annexin V and 7-AAD staining. Data are mean $\pm$ SE. \*\* -  $p < 0.01$  compared to control conditions. (B) Cells were lysed and subjected to immunoblotting. A representative image from 1 of 4 experiments is shown. Right lane is a CLL sample treated similarly.

### **Supplementary Figure S3. Targeting NAE does not induce Cdt1, p21 or p27 accumulation**

**in peripheral blood CLL cells cultured off stroma.** CLL cells were incubated with 0.05, 0.25 or 0.5  $\mu$ M MLN4924 or vehicle control for 2 - 24 hours. Whole-cell protein lysates were subjected to immunoblotting. A representative image from 1 of 3 experiments is shown. As a reference, cells were also treated with 0.25  $\mu$ M MLN4924 for 8 hours on CD40L-expressing stroma (right lane - +Ctrl).

**Supplementary Figure S4. Genetic knockdown of Cdt1 counters MLN4924-mediated DNA damage in CLL cells.** CLL cells (N=6) were transfected with the individual siRNA's against Cdt1 or control siRNA using Amaxa program X-05. Immediately after nucleofection, cells were cultured in the presence of 25 ng/mL IL-21 for 72 hours. Thereafter, cells were treated with 1  $\mu$ M MLN4924 or vehicle control for 6 hours and immunostained with phospho-RPA antibodies. Nuclei were counterstained with 4,6-diamino-2-phenylindole. A representative case is shown.