**Cell Titer Glo Assay**

For the growth assays, cells were plated in 96-well plates at 5000 cells/well and treated with inhibitors the indicated compound concentrations. On Day 5, cells were lysed with CellTiter-Glo® Luminescent Cell Viability Assay reagent (Promega) and luminescence was read using the Envision plate reader. Percent cell growth was calculated relative to DMSO treated cells.

**Clonogenic Assay**

Cells were seeded at 10000 cells per well in a 48-well plate, in methycellulose medium (HSC-001; Stem Cell Technologies). A stock of 1.44% methylcellulose was made in medium and diluted to a final concentration of 1.17% by the addition of cell suspension. Colonies were stained with Hoechst 33342 after 14 days and visualized using the Chem-Genius imaging system.

**BrdU Assay**

Cell proliferation was also measured by BrdU incorporation (Roche). Cells were plated in poly-D-lysine-coated 96-well plates (Beckton Dickson) at 10000 cells/well and treated with inhibitor at concentrations ranging from 120nM to 10uM (3-fold dilutions). On Day 3, cells were pulsed with BrdU for 2 hours and fixed, followed by immunodetection of incorporated BrdU label. Colorimetric detection was performed using the Spectramax plate reader. Percent growth was calculated as described above.

**TaqMan mRNA Expression**

Cells were plated in 6-well plates in 2ml media at $10^5$ cells/ml, then treated with DMSO or inhibitors for 16h. In vivo tumor samples were harvested and snap frozen in liquid nitrogen. Tissue samples were homogenized and lysed in RLT buffer with Reagent DX using the TissueLyser (Qiagen). Total RNA was harvested using the RNeasy kit (Qiagen) and cDNA was made from 1ug total RNA using High Capacity cDNA kit (ABI). Taqman probes from ABI were used to determine the amount of mRNA expression for IL-6 and IL-10 relative to the endogenous control gene (TBP).

**Gene Expression analysis**

Gene expression profiling was performed for HBL1 cells after treatment with DMSO, the PKC inhibitor Sotrastaurin (STN) or the IKKb inhibitor MLN120B for the indicated timepoints (6, 12, 24 or 48 h). Gene expression was measured using whole-genome Agilent 4x44K gene expression arrays (Agilent Technologies) following the manufacturer’s protocol. Signals from DMSO-treated HBL-1 cells (labeled with Cy3) were compared to signals from the respective MLN120B and AEB071-treated cells (labeled with Cy5). A gene was selected as an NF-κB target gene in HBL-1 cells if MLN120B decreased the expression of the gene by at least 50% at three time points. This gene signature was subsequently applied to the gene expression data following treatment with PKC inhibitor AEB071. Additionally, we applied a previously developed NF-kB target gene signature (NF-kB_all_OCILy3_Ly10 signature) (21) using those genes, which were significantly inhibited by MLN120B (p<0.05).
**NF-κB nuclear translocation and IL6/IL10 secretion assay.**

40μg of protein from total cell lysate were loaded onto 96-well Trans-AM ELISA plates containing immobilized NF-κB consensus binding site (Active Motif). Assays were performed according to the manufacturer’s instructions. IL-6 and IL-10 secretion levels in cell supernatants were determined by the Quantikine colorimetric ELISA kits (R & D Systems) according to the manufacturer’s instructions. Cells were washed with fresh media immediately prior to compound treatment, and supernatants were collected 48 hours post-treatment.

**CaspaseGlo 3/7 Assay**

Caspase 3/7 activity was measured by CaspaseGlo (Promega). Cells were plated at a density of 10000 cells/well in 96-well plates and treated with inhibitor at concentrations of 1μM and 5μM. CaspaseGlo reagent was added to lyse cells and detect Caspase 3/7 activity at designated time points. Caspase 3/7 activity is reported as the fold change relative to DMSO treated cells.  

**Cell cycle FACs**

To determine cell cycle distribution, cells were washed with phosphate-buffered saline (PBS) and fixed in 70% ethanol at 4 °C over night. Cells were washed twice in PBS and then treated with 200 μg/ml ribonuclease A and 33 μg/ml of propidium for 15 min at 37 °C. Cell cycle distribution was assessed by flow cytometry (FACS).

**Tumor xenografts**

Mice were maintained and handled in accordance with Novartis Biomedical Research Animal Care and Use Committee protocols and regulations. 6-8 week old female SCID-beige mice were purchased from Charles River Labs (Wilmington MA) and housed in temperature and humidity-controlled vivarium with a 12 h light cycle and provided food and water ad libitum. Mice were implanted subcutaneously with 10x10⁶ TMD8 cells in 50% matrigel (BD Biosciences, #354234) in the right dorsal axillary region. Treatment was initiated when tumor volume reached an average size of 160 mm² (21 days post tumor implantation). Sotrastaurin solution was prepared weekly and dosed orally on a tid schedule. Tumor volume was determined by twice weekly digital calipering and calculated using the formula: Length x Width² /2. T/C percentage was calculated as the mean change in tumor volumes of treated animals divided by the mean change in tumor volumes of vehicle animals multiplied by 100. Data were expressed as mean ± SEM, and differences were considered statistically significant at p < 0.05 by Student t-test.
Supplementary Figure 1, Naylor et al.

A  Sotrastaurin (STN)

PKC biochemical IC50 (nM)  Selectivity
\[\begin{array}{cccccc}
\alpha & \beta & \gamma & \epsilon & \eta & \theta \\
2.1 & 2.0 & 2.3 & 6.2 & 6.1 & 1.0 \\
\end{array}\]

SYK  BTK

\[\begin{array}{cc}
\text{SYK} & \text{BTK} \\
>10000 & >10000 \\
\end{array}\]

B  BHA536

PKC biochemical IC50 (nM)  Selectivity
\[\begin{array}{cccccc}
\alpha & \beta & \gamma & \epsilon & \eta & \theta \\
4.6 & 10.3 & 75.3 & 525 & 1000 & 225 \\
\end{array}\]

SYK  BTK

\[\begin{array}{cc}
\text{SYK} & \text{BTK} \\
10000 & >10000 \\
\end{array}\]
Supplementary Figure 3, Naylor et al.

A) R406 (SYK)

B) Enzastaurin

C) Velcade (Bortezomib)
Supplementary Figure 4, Naylor et al.

A

HBL1 (Y196F)

GTA$\rightarrow$GAA

B

TMD8 (Y196H)

GTA$\rightarrow$GTG
Supplementary Figure 7, Naylor et al.

![Graph showing IL-10 secretion in different cell lines with DMSO and Sotrastaurin (5μM) conditions.](image)
Supplementary Figure 8, Naylor et al.
Supplementary Figure 11, Naylor et al.

A

BrdU assay (48h post treatment)
Supplementary Figure Legends:

Supplementary Figure 1: Chemical structure and PKC isoform selectivity data for sotrastaurin (STN) and BHA536. Chemical structures are shown on the left and the table displays the biochemical IC$_{50}$ values for the inhibition of classical ($\alpha,\beta$) and novel ($\delta,\epsilon,\eta,\theta$) PKC isoforms, and selectivity against SYK and BTK in biochemical assays. (A) the pan-PKC inhibitor sotrastaurin, and (B) the $\alpha/\beta$ selective inhibitor BHA536.

Supplementary Figure 2: B-cell receptor signaling pathway. Schematic depiction of the BCR signaling pathway to illustrate the epistatic relationship between CD79A/B, SYK, BTK, PKC$\beta$, and CARD11. For example, CD79A/B mutations (marked by star) occur upstream of PKC$\beta$, whereas CARD11 (mutation marked by star) functions downstream of PKC$\beta$.

Supplementary Figure 3: Evaluation of additional small molecule inhibitors on growth of lymphoma cell line panel. The growth assays were performed as described in Figure 1. CD79 mutant ABC DLBCL cell lines (blue; HBL1, TMD8), ABC DLBCL cell lines with downstream mutations (green; OCI-LY3, U2932, SU-DHL2) and GC DLBCL cell lines (red; SU-DHL4, DB) were treated for 5 days with serial dilutions of (A) the SYK inhibitor R406, (B) the PKC inhibitor Enzastaurin, or (C) the proteasome inhibitor Velcade (Bortezomib). Relative cell growth was measured by Cell Titer Glo and normalized to DMSO treated cells. The growth curves represent the averages of at least 3 independent experiments.
Supplementary Figure 4: Confirmation of CD79B mutations in HBL1 and TMD8 cells.
Sequencing of genomic DNA confirmed mutations in CD79B (as described in Davis et al. 2010) in HBL1 and TMD8 cells, which result in codon changes in Y196F and Y196H, respectively. Note that the sequencing trace is shown in the reverse direction (3’ to 5’).

Supplementary Figure 5: Sotrastaurin inhibits growth in a clonogenic assay in CD79 mutant, but not CARD11 mutant ABC DLBCL. Methylcellulose assay was performed as described in Materials and Methods. Colonies were stained with Hoechst 33342 after 14 days of compound treatment and visualized using the Chem-Genius imaging system. HBL1 (top), TMD8 (middle) or OCI-LY3 (bottom).

Supplementary Figure 6: Sotrastaurin (STN) downregulates NF-κB target genes. The heatmap (A) displays genes from a previously developed NF-κB target gene signature, which includes genes that were downregulated in expression following treatment of the ABC DLBCL cell lines OCI-Ly3 and OCI-Ly10 with the IKKβ inhibitor MLN120B or MLX105 (an older version of the same class of inhibitor) as measured on Lymphochip cDNA microarrays (NF-κB_all_OCILy3_Ly10signature(http://lymphochip.nih.gov/cgi-bin/signaturedb/signatureDB_DisplayGenes.cgi?signatureID=83). The figure depicts those NF-κB signature genes that were significantly downregulated in the IKKβ inhibitor treated HBL1 cells (P<0.05; paired t-test). Also shown are the expression levels of these NF-κB signature
genes in HBL1 cells treated with the PKC inhibitor sotrastaurin (5μM). (B) The NF-κB target genes were averaged for each sample and graphed at the bottom (error bars depict standard error of the mean). The significance of the decrease in the NF-kB signature average in each treated sample is also shown (paired t-test). (C) Gene expression changes of IL6 and IL10 in response to sotrastaurin (STN) treatment were assessed by microarrays and depicted according to the color scale shown.

**Supplementary Figure 7: Sotrastaurin inhibits IL10 secretion in CD79 mutant but not CARD11 mutant ABC DLBCL cells.** IL-10 secretion was determined 48 hours after compound treatment with the Quantikine ELISA assay using cell supernatant from treated cells.

**Supplementary Figure 8: NF-κB activation by CARD11-L244P is not inhibited by sotrastaurin.** 293 cells carrying a stably integrated NF-κB-Luciferase reporter were engineered to express oncogenic CARD11-L244P. Cells were treated with 100ng/ml of Dox and 5μM Sotrastaurin or DMSO for 8 hours before the addition of 50ng/ml of PMA and 1μg/ml of Ionomycin (where indicated). Luciferase activity was measured 24 hours after Dox induction. NF-κB reporter activity induced by PMA/ionomycin, which activates several PKC isoforms, was strongly inhibited by sotrastaurin. In contrast, CARD11-L244P induced NF-κB reporter activity was not inhibited by Sotrastaurin, indicating that this mutant does not require PKC for NF-κB pathway activation.
Supplementary Figure 9:  Sotrastaurin resistance induced by CARD11-L244P expression requires the CARD domain of CARD11. HBL1 cells were stably transduced with constructs that express activated CARD11-L244P (CARD11*) or the same construct lacking the N-terminal CARD domain (CARD11*-dCARD) under the control of the UBC promoter. (A) Parental HBL1 and engineered HBL1-CARD11 mutant cells were treated for 5 days with the PKC inhibitor sotrastaurin (STN) (0-5μM). Relative cell growth was determined using the Cell Titer Glo assay and is expressed as a percentage of DMSO-treated cells (averages of 3 independent experiments and error bars denote standard deviation).  (B) Parental HBL1 and engineered HBL1-CARD11 mutant cells were treated for 5 days with the IKKβ inhibitor AFN700. The experiment was performed as described in Figure 4C. (C) TMD8 cells were transduced with cDNAs encoding mutant CARD11-L244P or a control GFP vector. CARD11-L244P rescues TMD8 cells from Sotrastaurin induced toxicity.

Supplementary Figure 10:  Sotrastaurin induces a dose-dependent G1 arrest and/or cell death in HBL1 and TMD8 cells. Sotrastaurin (STN) treatment induces G1 arrest in (A) TMD8 and (B) HBL1 in a dose-dependent manner. Cells were treated with the indicated concentrations of STN for 48 hours, fixed, stained with PI, and analyzed by FACS. The FACS profiles in this figure were used to calculate the cell cycle distribution shown in Figures 5A-B. (C) Cell cycle distribution of TMD8 (left) and HBL1 (right) treated with 5μM STN for 24, 48 or 72 hours.

Supplementary Figure 11:  STN caused a dose-dependent inhibition of S-phase entry in HBL1 and TMD8 cells. Cell cycle progression (S-phase entry) was measured using BrdU
incorporation (Roche). CD79 mutant ABC DLBCL cell lines TMD8 (blue), and HBL1 (green) were treated for 48 hours with serial dilutions of the PKC inhibitor Sotrastaurin. S-phase entry was measured by BrdU incorporation (2 hour labeling, followed by immunodetection of incorporated BrdU label) and normalized to DMSO treated cells. The data points represent the average of at least 2 independent experiments.

**Supplementary Figure 12:** STN treatment only induces a modest increase in Caspase 3/7 activity in HBL1 and TMD8, but induces PARP cleavage at later time points in TMD8 cells. Caspase 3/7 activity was measured by CaspaseGlo (Promega). Cells were plated at a density of 10,000 cells/well in 96-well plates and treated with DMSO, 1uM or 5 uM STN, or 10uM Camptothecin. CaspaseGlo reagent was added to lyse cells and detect Caspase 3/7 activity at (A) 6 hours and (B) 24 hours. Caspase 3/7 activity is graphed as fold change relative to DMSO treated cells. (C) HBL1 and TMD8 cells were treated with 5μM STN and total protein lysates were harvested at the indicated time points. Lysates were separated by SDS page and immunoblotted with antibodies to PARP and GAPDH (loading control).