Supplementary Figure Legends

Supplementary Figure S1: Dose response experiments with the IRAK1/4 inhibitor (IRAKi) in CLL cells. A-B, Primary CLL PBMCs were pre-treated with or without various doses of IRAKi for one hour followed by stimulation of the TLR for five hours with CpG (1μM). **A,** Representative expression of IRAK1 and GAPDH loading control as determined by western blot analysis. **B,** Representative expression of pSTAT3(Y705), total STAT3, and γ-tubulin **l**oading control as determined by western blot analysis. **C,** Primary CLL PBMCs were pre-treated with or without various doses of IRAKi for one hour followed by stimulation of the TLR for 24 hours with CpG (1μM). Median (±IQR) MFI of CLL cells expressing the cell surface marker CD69 as determined by flow cytometry (n=3). All comparisons by paired Student t-test; **P*<0.05 and ***P*<0.01.

Supplementary Figure S2: TLR signaling is active in lymph node-resident CLL cells. Proximity ligand assay displaying representative interactions between MYD88- pIκBα (top panels) and IRAK1- pIκBα (bottom panels) for two patients. Green represents the CD20 counterstain indicating a B cell, Blue represents DAPI identifying the nucleus and, and Red puncta identify an interaction between the two probes indicated (representing active signaling). Red arrows indicate representative positive cells and yellow arrows indicated representative negative cells.

Supplementary Figure S3: Flow Cytometry gating strategy. Representative images of gating strategy showing all cells, singlets, barcoded patients, individual patient B Cells, and select histograms of alexa-488 stained cells including pNF-κB(P65(S529)), pSTAT3(Y705), pSTAT1(S727), and pSTAT3(S727). Orange represents isotype control, red represents peripheral blood cells and blue represents lymph node cells.

Supplementary Figure S4: Early activation of STAT3 leads to early production of serum IL-10. Primary CLL PBMCs (n=4) were treated with or without 1μM CpG for five hours. Colors correspond to indicated patients. Representative expression of pSTAT3(Y705), total STAT3, and γ-Tubulin loading control as determined by western blot analysis are shown (top panel). IL-10 production for the same four patients at the corresponding time points is shown in the bottom panel.

Supplementary Figure S5: TLR signaling induces similar levels of IRAK1 degradation and STAT signaling *in vitro* with CpG stimulation in primary CLL PBMCs. A-B, Primary CLL PBMCs were treated with or without 1μM CpG for five hours. Colors correspond to indicated patients. **A,** pSTAT3(Y705) in CLL cells as determined by flow cytometry. Each bar represents one patient. **B,** Representative expression of IRAK1, pSTAT3(Y705), total STAT3, and GAPDH loading control as determined by western blot analysis for the corresponding three patients from panel S2A, identified by color.

Supplementary Figure S6: Upstream IRAK1 degradation and downstream phosphorylation of STAT3 and IκBα are inversely related. A-C, Primary CLL PBMCs were treated with or without 1μM CpG for five hours. Representative immunblots showing the change in **A,** IRAK1 and pSTAT3(Y705), **B,** pSTAT3 and pIκBα and **C,** IRAK1 and pIκBα, the corresponding total protein and either γ-Tubulin or GapdH loading control is shown.

Supplementary Figure S7: CpG induced TLR signaling promotes cell survival and activates CLL cells *in vitro*. A-B, Primary CLL PBMCs were treated with or without 1μM CpG for five hours. **A,** mean (±SEM) MFI of CLL cells expressing the cell surface markers CD40, CD54, CD69, and CD86 as determined by flow cytometry (n=16). **B,** Min to max box and whisker plot of the % of viable CLL cells as determined by flow cytometry (n=16). All comparisons by Wilcoxon matched-pairs signed rank test; ****P<0.0001.

Supplementary Figure S8: Inhibiting induced TLR activation downregulates expression of activation markers and prevents the survival advantage in CLL cells. A-B, Primary CLL PBMCs were pre-treated with or without 1μM ibrutinib or 10μM IRAKi for one hour followed by five hours of CpG stimulation (1μM). **A,** Mean (±SEM) % change compared to CpG stimulated controls in CD54 or CD69 MFI in CLL cells as determined by flow cytometry (n=14). **B,** Mean (±SEM) % change in viable CLL cells compared to CpG stimulated controls as determined by flow cytometry (n=14). Asterisks indicate comparisons made to CpG stimulated control, brackets show comparisons between treatments. All comparisons by Wilcoxon matched-pairs signed rank test; **P<0.01 and ***P<0.001.

Supplementary Figure S9: αIgM stimulation induces activation of BCR pathway signaling. **A-C,** Primary CLL PBMCs were incubated for 5.75 hours followed by 15 minutes of αIgM stimulation (20µg/mL). MFI in CLL cells was determined by flow cytometry for **A,** pBTK(Y223), **B,** pPLCg2(Y759) and **C,** pERK(T202/Y204). Each line represents one patient (n=11). Comparisons by Wilcoxon matched-pairs signed rank test.

Supplementary Figure S10: Ibrutinib inhibits both BCR and TLR signaling in activated CLL cells harvested from the lymph node. A-B, Primary CLL lymph node (LN) resident multinucleated cells were treated with or without 1µM ibrutinib (IB) or 10µM IRAKi for 30 minutes. Shown is the mean (±SEM) % change compared to untreated LN controls in MFI of **A,** TLR signaling molecules, pSTAT3(Y705), pSTAT1(S727), and pSTAT3(S727) and **B,** BCR signaling molecules pBTK(Y223), pPLCγ2(Y759), and pERK(T202/Y204) in CLL cells as determined by flow cytometry (n=6). Asterisks indicate comparisons made to untreated LN control, brackets show comparisons between IB and IRAKi. All comparisons by Wilcoxon matched-pairs signed rank test; *P<0.05.