**Supplemental Information- Figures & Legends (Supplemental Tables loaded separately)**

**Supplemental Figures & ALL supplemental Legends**



**Figure S1. Epigenetic Ibrutinib Resistance in ABC DLBCL lines, related to Figure 1.**

1. Cartoon depiction of the genetic ibrutinib resistance mutants from IR clones as detected by targeted sequencing of BTK and PLCG2. **B.** Alleles of *BTK* and *PLCG2* were cloned into retroviral vectors bearing a co-expressed LYT2 marker that could be monitored by flow cytometry. ABC DLBCL lines were transduced and treated with DMSO or 10nM ibrutinib for one week. Competitive outgrowth was measured by measuring the ratio of LYT2+ (allele+) to un-transduced, allele-negative cells in each culture is shown, error bars = s.e.m. **C.** As in B. with GFP replacing the LYT2 marker. In addition, single cell clones *without* mutation, as determined by whole-exome sequencing, were selected from IR pools and marked by transduction with a GFP expression vector and mixed with parental HBL1 cells. Competitive outgrowth was monitored over 12 days in the presence (top) or absence (bottom) of 10nM ibrutinib, error bars = s.e.m. **D.** HBL1 ibrutibib-sensitive parent al cells or and IR clone with a *PLCG2*R665W mutation were treated with ibrutinib (10nM) for the indicated times. After 7 day culture without ibrutinib, cells were re-challenged with ibrutinib (10nM) or DMSO, as indicated and viable cells were enumerated after 4 days by flow cytometry (calcein+, EtBr-). Viability data were normalized to the counts of cells in each DMSO culture. **E.** Average expression of genes from the ibrutinib response (IBRx) signature (Table S1C) plotted over a time course of acute drug treatment (1-24 hours), in cells with naturally evolved genetic resistance mutations in PLCG2, and in single cell clones from IR pools with no detectable resistance mutations as determined by whole-exome sequencing. Significance determined by ANOVA (\*\* p<0.001, \*\*\*\* p<0.00001, error bars = s.e.m.). **F.** Live cells counted by flow cytometry from cultures of 2 ABC DLBCL cell lines, as well as IR clones and IR pools of those lines with and without serial treatment with the HDAC inhibitor givinostat (50nM, 5 days, HDACi). Cell numbers are normalized to DMSO-treated vehicle controls (error bars = s.e.m.).

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**Figure S2. Tracking the evolution of Ibrutinib Resistance phenotypes in ABC DLBCL lines, related to Figure 2.**

1. 100,000 TMD8 ABC DLBCL cells were transduced at an MOI of 1:3 such that each cell received one unique barcode. Cells were expanded, a starting sample of 1 million cells was taken for NGS DNA sequencing to determine the initial clonal distribution of barcodes, and the remaining culture was split and challenged with increasing concentrations of ibrutinib (0.1nM week1 to 2nM week5), or with DMSO alone. DNA was harvested at week5 and subjected to NGS sequencing for identification and counting of barcodes from each condition. The depletion or enrichment of each barcode was calculated, compared to the starting population, and binned with the number of each barcode/bin shown with the resistance phenotype (sensitive, persistent, resistant). **B.** scRNA-Seq t-SNE plots of the gene expression behavior of HBL1 cells with expressed barcodes (EBC) representing each phenotype (sensitive (S), persistent (P), resistant (R)) from Fig. 2C. Each cell (barcode) is shown in gene expression space from the DMSO-treated culture (gray) vs. the ibrutinib-treated culture (blue). **C.** Summary of gene expression signature enrichment (Table S2E) from EBC scRNA-seq in TMD8, comparing of genes differentially expressed in persistent (light pink) vs. resistant (dark pink) cells. Representative genes upregulated in each population are shown.

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**Figure S3. RAC2 protein interactions are a marker of epigenetic ibrutinib resistance, related to Figure 6.**

1. Proximity ligation assay (PLA, red puncta) for interaction between RAC2 and PLCG2 (upper panels) or RAC2 and IgM (lower panels) in TMD8 parent and an IR pool. Nuclei are stained blue (DAPI) and cell membranes are green (wheat germ agluttinin Alexa488).

**Supplemental Table Legends (for Excel Tables, uploaded separately)**

**Supplemental Table 1. Epigenetic Ibrutinib Resistance in ABC DLBCL lines, relates to Fig1/FigS1**

1. Summary of mutations detected by whole exome sequencing in short-term (6 weeks in drug) and long-term (>1 year in drug) IR pools. Genes with mutations >10% allele frequency are shown for the TMD8 ABC DLBCL cell line. **B.** As in A. for the HBL1 ABC DLBCL cell line. **C.** The ibrutinib-response signature (IBRx) of genes down regulated (log2 <-0.4) by acute treatment (10nM, 24hrs) of the HBL1 and TMD8 ABC DLBCL lines. **D.** RNA-SEQ expression (log2 normalized FPKM relative to parent line) of IBRx signature genes in acutely treated parental cells, IR pools and genetic mutants. IB – 10nM ibrutinib, SCC- single cell clone. **E.** Enrichment of previously-defined gene expression signatures (see text) within the set of ibrutinib signature (IBRx) genes. **F.** Log2 FPKM RNA-SEQ expression normalized to the untreated HBL1 parent cell line of IBRx signature genes in IR pools, IR genetic mutant clones and single cell clones without resistance mutations.

**Supplemental Table 2. Tracking the evolution of ibrutinib resistance phenotypes in ABC DLBCL lines, relates to Fig2/FigS2**

1. NGS counts of unique cell barcodes and statistics from HBL1 (Fig. 2B) and TMD8 (Fig. S2A) treated as described in the figure legends. **B.** Comparison of scRNA-seq gene expression (from 10x Genomics Loupe program) from populations in the HBL1 expressed barcode (EBC) experiment: sensitive cells (S), persistent cells (P) in DMSO, resistant (R) cells in DMSO, persistent cells after ibrutinib selection (P\_IBRx), resistant cells after ibrutinib selection (R\_IBRx). **C.** Lists of genes differentially expressed in various populations from the HBL1 EBC scRNA-seq experiment (log2 +/-0.4). **D.** Gene expression signature enrichment on gene sets from C. (p-value <0.01, enrichment > 2). **E.** Genes higher in persistent or resistant TMD8 cells, and gene expression signature enrichment from a similar EBC experiment in TMD8 as in (B.). **F.** scRNA-seq gene expression measured in sensitive parental ABC DLBCL lines and 3 independently-derived IR pools of each. **G.** Overlapping sets of genes upregulated in IR pools vs. parents from (F.) and persistent vs. resistant cells from (B.).

**Supplemental Table 3. Epigenetic retuning of oncogenic signaling in ibrutinib resistance, relates to Fig3**

**A.** Summary statistics of ATAC-SEQ data identifying regions of open chromatin (ROC) from the HBL1 parental line, genetic resistance mutant (PLCG2 R665W), and IR pools, and the same samples treated for two days with a combination of 5-AZAC/HDACi (Fig.1). Data are normalized to the parental HBL1 control (see Methods) **B.** Coordinates, statistics, and associated genes (2 nearest ~ +/- 100kb as determined by GREAT analysis) for regions of open chromatin in HBL1 samples from Fig. 3A. Log2 +/- 0.30 of the ROC signal count was used as the metric for determining changes in ROC accessibility among samples. **C.** RSAT (<http://rsat01.biologie.ens.fr/rsa-tools/peak-motifs_form.cgi>) was used to identify transcription factor motifs enriched in all regions of open chromatin that were well-measured (log2 SUM count >5) in the parental HBL1 line and those higher (log2 SUM of counts >= 0.3) in IR pools than the parent line or genetic resistance mutant. The top ENCODE motifs with e-values < 0.01 for motifs of 6, 7, and 8 bp length are displayed for each group of ROCs. Note the TCF3/4 motifs differ between parent (CACGTG) IR pools (CACCTG). **D.** TCF4-related gene lists: 1- Genes with TCF4 ChIP-Seq peaks in parental HBL1 cells. 2- Genes with regions of open chromatin more accessible in HBL1 IR pools than parent and the PLCG2 resistance mutant with a TCF4 DNA-binding motif (RSAT) 3- intersection of 1 and 2. 4- Genes from 3 whose open chromatin regions and TCF4 ChIP-seq peaks overlap by at least 50bp (assessed by GALAXY) and have at least one TCF binding motif (RSAT). 5-Genes relevant to ABC DLBCL pathophysiology and their neighbors. 6- Intersection of 4 and 5.

**Supplemental Table 4.** **Altered dependencies in ibrutinib-resistant ABC DLBCL, relates to Fig4**

Summary of CRISPR ‘drop-out’ screens comparing ibrutinib-sensitive parent lines to IR pools. Average log2 CRISPR screen score (CSS) was calculated for each gene targeted by guide RNAs in the ‘Brunello’ sgRNA library and compared between populations.

**Supplemental Table 5.** **RAC2 as a mediator of epigenetic ibrutinib resistance, relates to Fig5**

**A.** RNA-Seq of gene expression in TMD8 parental and IR pool cells bearing inducible shRNAs against RAC2 or a control transcript (CTRL) measured at days 0 and 4 post-induction; log2 differential (shRAC2-shCTRL) normalized FPKM values are reported. **B.** Gene expression signature enrichment analysis of genes downregulated specifically in the TMD8 IR pool by RAC2 knockdown at day 4 post shRNA induction (log2 shRAC2-shCTRL <= -0.5).

**Supplemental Table 6. RAC2 protein interactions facilitate and are a marker of epigenetic ibrutinib resistance, relates to Fig6, FigS3**

**A.** RAC2 interacting proteins from quantitative SILAC mass spectrometry analysis of proteins pulled down with streptavidin from cells expressing RAC2bioID2 (3 ABC DLBCL parental lines (TMD8 n=2, HBL1 n=1) and 4 IR pools (TMD8 n=2, HBL1n=2)). Average log2 of normalized enrichment values are shown vs. cells expressing BioID2 only (positive value = increased interaction). **B.** The RAC2 interactome organized by STRING analysis showing known or inferred RAC2 interacting proteins. **C.** Characteristics of CLL patients and quantitation of RAC2/PLCG2 puncta per IgM+ cell for each CLL patient category (n=4/category, from patients pre-treatment (preRx), during treatment with acalabrutinib while still manifesting lymphocytosis (persistent disease, PERS), or on ibrutinib at the time of progressive disease, PD). **D.** As C. for each category and for matched samples from the same patients.

**Supplemental Table 7. Targeting epigenetic resistance to BTK inhibitor treatment in ABC DLBCL, relates to Fig7**

1. Summary of toxicity data (Maximum response, MAXRESP, uM) from high-throughput drug screening of ABC DLBCL parent lines and independently-derived IR pools using the MIPE library of ~2400 therapeutic and experimental compounds. **B.** Summary statistics for specific drugs/drug classes that impinge on ABC DLBCL survival pathways.