**SUPPLEMENTARY FIGURE LEGENDS**

**Figure S1. Different ABL TKIs demonstrate potency differences in relative cellular and biochemical target inhibition. (A)** Summary of inhibition of cellular proliferation and GST-ABL kinase autophosphorylation by ABL TKIs. K562 and LAMA cells were incubated continuously in the presence of graded concentrations of each of the indicated inhibitors and proliferation was measured by methanethiosulfonate-based assay at 72 h. Cellular $IC_{50}$ values are shown for each inhibitor and cell line, along with the fold over $IC_{50}$ values for each of the primary, physiologically relevant concentrations of the inhibitor used in subsequent washout studies. Autophosphorylation data for GST-ABL kinase assays (full-length) was compiled from the indicated previously published studies, quantitated by densitometry, normalized for protein expression, and analyzed for $IC_{50}$ concentrations of each inhibitor using Graphpad Prism software. **(B)** Inhibition of BCR-ABL tyrosine phosphorylation by ABL TKIs in CML cells. K562 and LAMA cells were incubated overnight in the presence of graded concentrations of the indicated inhibitors, lysed, and subjected to SDS-PAGE analysis. Immunoblotting was performed using an anti-phosphotyrosine antibody for detection of BCR-ABL phosphorylation (pBCR-ABL) and an anti-actin antibody as a loading control. pBCR-ABL bands were quanititated by densitometry, normalizing to the corresponding actin levels, and $IC_{50}$ values for inhibition of BCR-ABL phosphorylation were calculated using Graphpad Prism software.

**Figure S2. Incomplete restoration of BCR-ABL signaling activity following washout of dasatinib or ponatinib tracks with intracellular drug retention and commitment to apoptosis in LAMA cells. (A)** Levels of apoptosis in LAMA cells following continuous or acute exposure to dasatinib and ponatinib. Cells were incubated in the presence of the indicated inhibitor concentrations for 2 h, and samples were collected just prior to washout and immediately following standard and expanded washout. Annexin V-positivity was measured at 72 h after the start of the experiment, and bars represent the mean of three replicates ± S.E.M. **(B)** Residual levels of dasatinib and ponatinib detected post washout in isolated intracellular and media fractions. After acute (2 h) exposure to the indicated inhibitor concentrations,

LAMA cells were collected immediately following standard and expanded washout. Cellular and media fractions were isolated by centrifugation, and cells were washed in PBS and subjected to hypotonic lysis on ice. Clarified intracellular lysate and culture media samples were analyzed for levels of inhibitor by LC/MS/MS and results are reported as the mean ng/$10^6$ cells and nM in media, respectively, of three replicate experiments ± S.E.M. Data labels of "n.d." indicate "not detected"; values preceded by a "<" symbol indicate detection of a low level peak, but below the lower limit of quantitation (LLOQ). **(C)** Phosflow FACS analysis of BCR-ABL signaling in LAMA cells following washout of dasatinib or ponatinib. LAMA cells were incubated alone or in the presence of 10 and 100 nM dasatinib or ponatinib for 2 h, subjected to standard and expanded washout, and collected at the indicated timepoints after washout. Cells were fixed, permeabilized, and stained using Alexa647-pCrkL and Alexa488-pSTAT5 conjugated antibodies. Results are displayed, for comparison purposes, as the overlaid signal peak traces of isotype control, untreated cells, and each indicated timepoint post washout. Vertical, black dashed lines highlight the peak signal in untreated LAMA cells for reference.

**Figure S3. Incomplete restoration of BCR-ABL signaling activity following washout of nilotinib, imatinib, or DCC-2036 tracks with intracellular drug retention and commitment to apoptosis in LAMA cells. (A)** Levels of apoptosis in LAMA cells following continuous or acute exposure to nilotinib, imatinib, and DCC-2036. Cells were incubated in the presence of the indicated inhibitor concentrations for 2 h, and samples were collected just prior to washout and immediately following standard and expanded washout. Annexin V-positivity was measured at 72 h after the start of the experiment, and bars represent the mean of three replicates ± S.E.M. **(B)** Residual levels of nilotinib, imatinib, and DCC-2036 detected post-washout in isolated intracellular and media fractions. After acute (2 h) exposure to the indicated inhibitor concentrations, LAMA cells were collected immediately following standard and extra washes. Cellular and media fractions were isolated by centrifugation, and cells were washed in PBS and subjected to hypotonic lysis on ice. Clarified intracellular lysate and culture media samples were analyzed for levels of inhibitor by LC/MS/MS and results are reported as the mean ng/$10^6$ cells and nM in media,

respectively, of three replicate experiments ± S.E.M. Data labels of "n.d." indicate "not detected"; values preceded by a "<" symbol indicate detection of a low level peak, but below the lower limit of quantitation (LLOQ). **(C)** Phosflow FACS analysis of BCR-ABL signaling in LAMA cells following washout of nilotinib, imatinib, or DCC-2036. LAMA cells were incubated alone or in the presence of 500 and 5000 nM nilotinib, imatinib, or DCC-2036 for 2 h, subjected to standard and expanded washout, and collected at the indicated timepoints after washout. Cells were fixed, permeabilized, and stained using Alexa647-pCrkL and Alexa488-pSTAT5 conjugated antibodies. Results are displayed, for comparison purposes, as the overlaid signal peak traces of isotype control, untreated cells, and each indicated timepoint post washout. Vertical, black dashed lines highlight the peak signal in untreated LAMA cells for reference.

**Figure S4. BCR-ABL signaling is partially attenuated in conditions of continuous low concentrations of ABL TKIs that induce apoptosis. (A)** Survey of levels of apoptosis induced by continuous low concentrations of ABL TKIs in culture media. To establish the lowest concentration of each inhibitor capable of inducing substantial apoptosis levels, K562 cells were exposed to graded concentrations of dasatinib, ponatinib, nilotinib, imatinib, or DCC-2036 inhibitors continuously for 72 h, at which time annexin V-positivity was measured. Bars represent the mean of three replicates ± S.E.M. (B) Phosflow FACS analysis of BCR-ABL signaling in K562 cells following continuous exposure to ABL TKIs. K562 cells were incubated alone or in the presence of graded concentrations of dasatinib, ponatinib, nilotinib, imatinib, or DCC-2036 for 24 h, at which time cells were fixed, permeabilized, and stained using Alexa647-pCrkL and Alexa488-pSTAT5 conjugated antibodies. Results are displayed, for comparison purposes, as the overlaid signal peak traces of isotype control, untreated cells, and each indicated inhibitor concentration. Vertical, black dashed lines highlight the peak signal in untreated K562 cells for reference.

**Figure S5. Commitment to apoptosis in primary CML cells tracks with intracellular retention of ABL TKIs.** Mononuclear cells from two patients with newly diagnosed CML (12/061 and 11/442) were

incubated for 4 h in the presence of the indicated concentrations of each of five ABL TKIs. Due to insufficient number of cells available, only the standard washout protocol was performed for sample 11/442. Cells were collected just prior to washout and immediately following standard washout (and expanded washout for sample 12/061 only) and analyzed for **(A)** apoptosis, **(B)** intracellular inhibitor levels, and **(C)** residual levels of inhibitor in the culture media. Apoptosis was measured by annexin V-positivity at 72 h after the start of the experiment, and results represent the mean of three replicates ± S.E.M. Cellular and media fractions were isolated by centrifugation, and cells were washed in PBS and subjected to hypotonic lysis on ice. Clarified intracellular lysate and culture media samples were analyzed for levels of ABL kinase inhibitors by LC/MS/MS and results are reported as ng/$10^6$ cells and nM in media, respectively. Data labels of "n.d." indicate "not detected"; values preceded by a "<" symbol indicate detection of a low level peak, but below the lower limit of quantitation (LLOQ).

**Figure S6. Intracellular retention of dasatinib, nilotinib, and imatinib is reduced following washout in primary CML cells harboring the resistant BCR-ABL$^{T315I}$ mutant.** Mononuclear cells from a CML patient (07/103) in myeloid blast crisis (M-BC) expressing the BCR-ABL$^{T315I}$ mutant were incubated for 4 h in the presence of the indicated concentrations of each of five ABL TKIs. Due to insufficient number of cells available, only the standard washes protocol was performed. Cells were collected just prior to washout and immediately following standard washes and analyzed for **(A)** intracellular inhibitor levels and **(B)** residual levels of inhibitor in the culture media. Cellular and media fractions were isolated by centrifugation, and cells were washed in PBS and subjected to hypotonic lysis on ice. Clarified intracellular lysate and culture media samples were analyzed for levels of ABL TKIs by LC/MS/MS and results are reported as ng/$10^6$ cells and nM in media, respectively. Data labels of "n.d." indicate "not detected"; values preceded by a "<" symbol indicate detection of a low level peak, but below the lower limit of quantitation (LLOQ).