Supplementary Information:

Development of a protease-resistant reporter to quantify BCR-ABL activity in intact cells.

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# Table of Contents

**Supplementary Figures**

Supplemental Figure S1 ............................................................................................................ S-3  
Supplemental Figure S2 .......................................................................................................... S-4  
Supplemental Figure S3 ......................................................................................................... S-5  
Supplemental Figure S4 ......................................................................................................... S-6  
Supplemental Figure S5 ......................................................................................................... S-7  
Supplemental Figure S6 ......................................................................................................... S-8  

**References** ........................................................................................................................ S-9
SUPPLEMENTAL FIGURES

Supplemental Figure S1: Chemical structures of non-native amino acids incorporated into the peptides.
Supplemental Figure S2: Select electropherograms of peptide VII-A (A), VII-B (B), and VII-C (C) incubated for 60 min in a Baf/BCR-ABL cytosolic lysate. The asterisk marks the migration time of the intact parent peptide and unlabeled peaks were assumed to be proteolytic degradation products. The electrophoretic buffer was 100 mM tris, 100 mM tricine, pH 8.1. (D) The percentage of the intact, full length peptide was measured over time for the series VII peptides. (E) The percentage of peptide phosphorylation by purified Abl-1 kinase was measured over time for the series VII peptides. The symbols are defined as follows: black closed square (peptide III-67B), red open circle (peptide V-48B), blue open diamond (peptide VII-A), green closed circle (peptide VII-B), and grey closed triangle (peptide VII-C). Figure adapted from A. Proctor’s dissertation.
Supplemental Figure S3: Select electropherograms of peptide VIII-A (A), VIII-B (B), and VIII-C (C) incubated for 60 min in a Baf/BCR-ABL cytosolic lysate. The asterisk marks the migration time of the intact parent peptide and unlabeled peaks are assumed to be proteolytic degradation products. The electrophoretic buffer utilized was 100 mM tris, 100 mM tricine, pH 8.1. Figure adapted from A. Proctor’s dissertation.¹
Supplemental Figure S4: Electropherograms of peptide VIII-B incubated in a Baf/BCR-ABL cytosolic lysate for 0 (A), 30 (B), or 60 (C) min. The electrophoretic buffer utilized was 100 mM tris, 100 mM tricine, pH 8.1. (D) Percentage of intact VIII-B and peptide fragments formed over time relative to the total starting amount of VIII-B. The symbols are defined as follows: black closed square (peptide xii), red open circle (peptide xi), blue closed triangle (peptide ix), pink open triangle (peptide v), purple closed circle (peptide ii). (E) The uppercase letters are the single amino acid abbreviations for peptide VIII-B’s sequence, with Sarc the abbreviation for sarcosine, and MePh the abbreviation for N-methyl phenylalanine. The roman numerals indicate the cleavage locations that generated the indicated peptide fragment. Figure adapted from A. Proctor’s dissertation.
Supplemental Figure S5: Properties of peptide X-A in Baf/BCR-ABL cytosolic lysates with and without imatinib. Degradation (A) and phosphorylation (B) over time of peptide X-A in Baf/BCR-ABL cytosolic lysates with pervanadate phosphatase inhibitor. Symbols are defined as follows: open square (no imatinib), open circle (with imatinib).
Supplemental Figure S6: Phosphorylation of peptide X-A by purified Abl-1 in the presence of tyrosine kinase inhibitors. Symbols are defined as follows: closed black square, DMSO control; closed red circle, imatinib; closed blue triangle, dasatinib; open pink triangle, masitinib; closed green diamond, sunitinib.
REFERENCES