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Supporting Information

Detection of Early Abl Kinase Activation after Ionizing Radiation by Using a Peptide Biosensor

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cbic_201100763_sm_miscellaneous_information.pdf

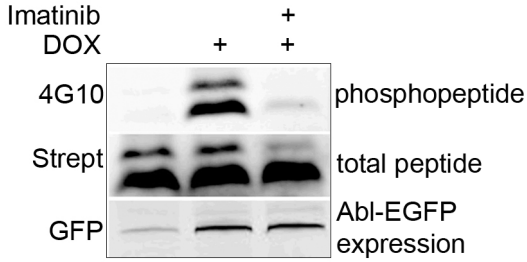

Figure S1. A peptide-based biosensor monitors conditionally induced Abl kinase activity in live cells. Abl-WT-EGFP cells were seeded at 1.2 $\times 10^6$ cells per well in a 6-well plate. Cells were allowed to adhere overnight then treated with doxycycline (DOX) (2 μM) for 18 h to induce the expression of the Abl-WT-EGFP protein. The Abl biosensor peptide (25 μM) was added and cells were incubated at 37 ^{o}C for 30 min with or without imatinib pre-treatment (20 min, 20 μM). Lysates were run on SDS-PAGE (200 ug/lane, 4-12% Bis-Tris gel), and biotinylated peptide (streptavidin-Dylight 680), phosphotyrosine (anti-phosphotyrosine antibody 4G10) and Abl-WT-EGFP (anti-GFP) were detected with immunoblotting using a LiCor two-color infrared imaging system. While the biosensor peptide signal for the phosphorylated (4G10) form is typically not very prominent from the basal, non-IR-induced activity (as shown in Figure 2 in the manuscript), the higher amount of total protein loaded (200 μg vs. 100 μg) allowed the detection of this basal phosphorylation and its inhibition by imatinib. Without overexpression of the kinase (lane 1), little phosphorylation is seen after 30 min incubation—indicating that the peptide is not appreciably phosphorylated in untreated HEK293 cells—however upon induction of the Abl-WT-EGFP expression the signal for phosphotyrosine, which overlaps with the signal for streptavidin, increases (lane 2). This signal is diminished with imatinib treatment (lane 3), which should inhibit the Abl kinase and prevent phosphorylation of the peptide biosensor.

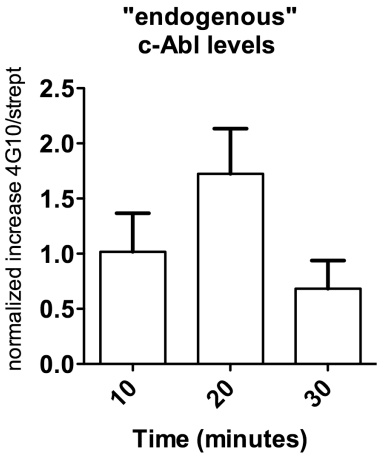

Figure S2. Phosphorylation of the peptide after IR by 'endogenous' levels of kinase. 4G10 and streptavidin band data from triplicate samples not treated with doxycycline, with and without IR treatment (as shown in Figure 2A, representative lanes 1, 2, 5, 6, 9 and 10) were integrated as described in the legend for Figure 2 and plotted to show a slight change in biosensor phosphorylation observed over time, however this difference was not significant ($p = 0.17$, measured by one-way ANOVA). Additionally, this difference cannot be confidently ascribed to the activation of Abl kinase and any increases seen may be the result of off-target phosphorylation of the biosensor by other tyrosine kinases in the cell.

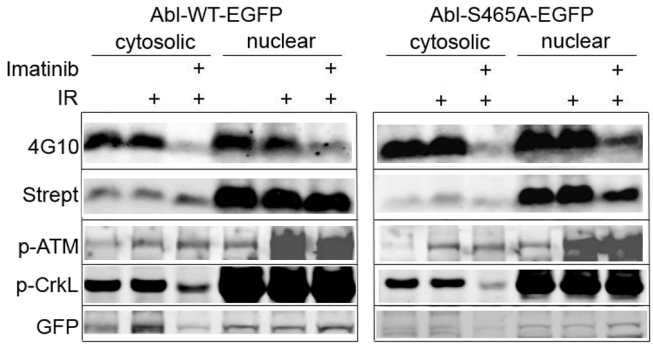

Figure S3. Analysis of cytoplasmic and nuclear fractions from cells treated with the Abl biosensor peptide. Cells were cultured as described in the materials and methods, and Abl-WT-EGFP or Abl-S465A-EGFP expression was induced with doxycycline for 18h. Cells were pre-incubated with the biosensor peptide for 6 min, then treated with IR and harvested at 10 min post-IR. The cell contents were fractionated using the Nuclear Extract Kit from ActiveMotif according to the manufacturers instructions. 100 μg of each fraction was denatured with NuPAGE Laemmli protein gel loading buffer (Invitrogen) and run on a 4–12% Bis–Tris NuPAGE gel (Invitrogen). Proteins were transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA, USA) for 1 h at 100V and 4 °C. Membranes were blocked in 5% milk/TBS-T (Tris-buffered saline containing Tween 20) for 1 h at room temperature and cut into two segments: >15 kDa (containing most endogenous proteins) and <15 kDa (containing biosensor peptide). The >15-kDa segment was analyzed with immunoblotting using different antibodies: GFP (1:1000, rabbit, Invitrogen)), pATM (S1981, 1:1000, mouse, Abcam), and pCrkl (Y207, 1:1000, rabbit, Abcam). The <15-kDa segment was analyzed with immunoblotting using a cocktail of anti-phosphotyrosine antibody 4G10 (1:1000, mouse, Millipore) and streptavidin labeled with DyLight 680 (1:1000, Rockland Immunochemical) and incubated overnight at 4 °C.Membranes were washed (3 × 5 min, TBS-T), visualized by incubating for 1 h at room temperature with goat-derived secondary antibodies (1:10,000 for anti-mouse and anti-rabbit and 1:20,000 for anti-rat in 5% milk/TBS-T) tagged with IRDye 680 or 800 (LI-COR Biosciences) and scanned for both dyes simultaneously using a LI-COR Odyssey infrared scanner.