SUPPLEMENTAL MATERIAL

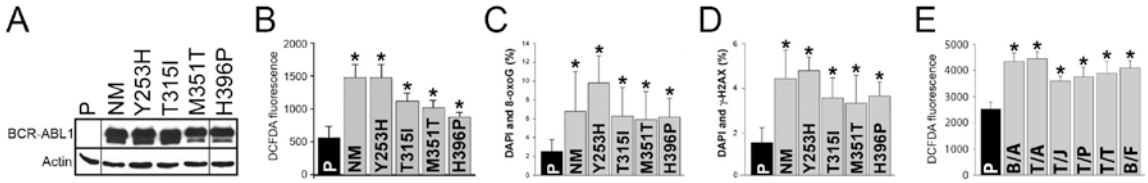

Figure S1. TKI-resistant BCR-ABL1 mutants and OTKs induce ROS and oxidative DNA damage. (**A**) Western analysis of 32Dcl3 parental cells (P) and cells expressing non-mutated BCR-ABL1 kinase (NM) or the indicated TKI-resistant BCR-ABL1 kinase domain mutants (P-loop - Y253H, the site of a hydrogen bond with imatinib - T315I, activation loop hinge - M351T, and activation loop - H396P). (**B**) ROS were measured with DCFDA and oxidative DNA damage was assessed by (**C**) 8-oxoG and (**D**) γ-H2AX immunofluorescence in P, NM and TKI-resistant clones. (**E**) ROS were detected using DCFDA in 32Dcl3 cells (P) and clones expressing BCR-ABL1 (B/A), TEL-ABL1 (T/A), TEL-JAK2 (T/J), TEL-PDGFβR (T/P), TEL-TRKC(L) (T/T) and BCR-FGFR1 (B/F). Results represent mean values of triplicate measurements/cell line ± SD, *p < 0.05 in comparison to parental cells (P).

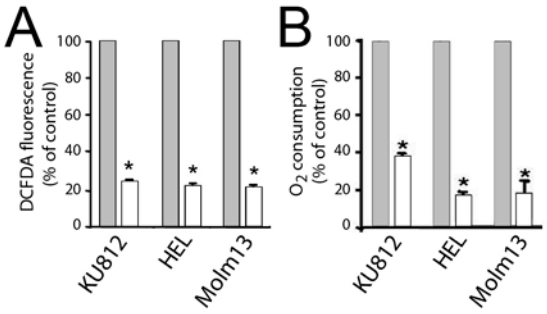

Figure S2. MRC complex III inhibitors reduces ROS and oxygen consumption in BCR-ABL1-positive CML cells, FLT3(ITD)-positive acute myeloid leukemia (AML) cells and JAK2(V617F)-positive polycythemia vera (PV) cells. BCR-ABL1 -positive KU812 cells, FLT3(ITD)-positive Molm13 cells and JAK2(V617F)-positive HEL cells were untreated (grey bars) or treated with 1μM myxothiazol (white bars). (**A**) ROS were measured with DCFDA. (**B**) Oxygen consumption was measured as described before [1]. Results represent mean values of triplicate measurements/cell line ± SD, *p < 0.05 in comparison to untreated cells (P).

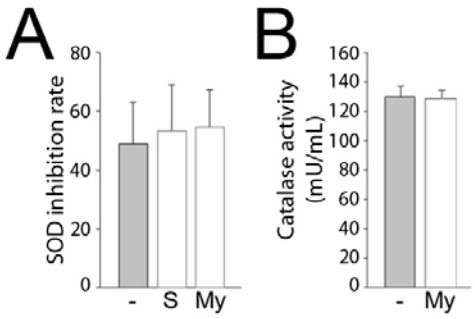

Figure S3. MRC complex III inhibitors do not affect the superoxide dismutase (SOD) and catalase activities. (**A**) SOD activity was assessed using a SOD determination kit (Fluka). (**B**) Catalase activity was measured using a Catalase Assay Kit (Abcam). Results represent mean values of triplicate measurements/cell line ± SD.

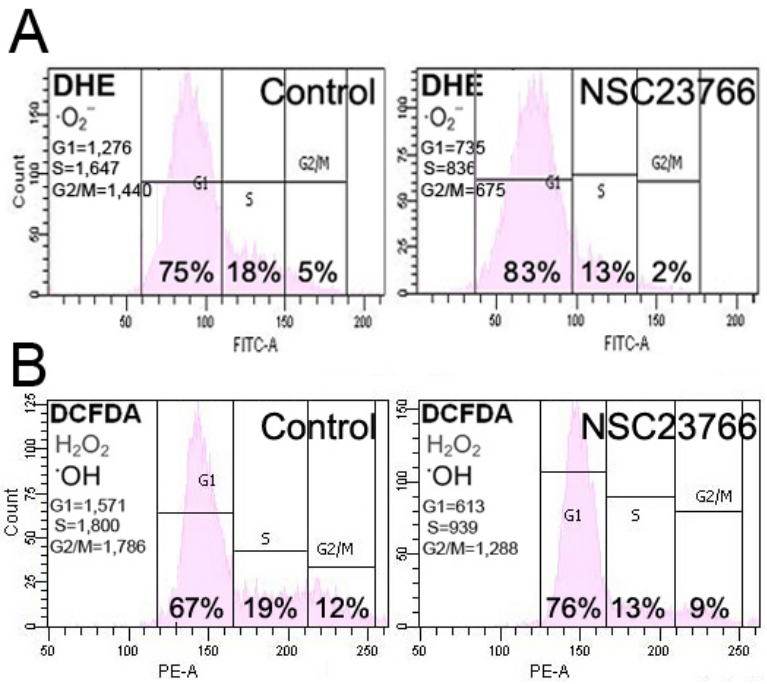

Figure S4. Rac inhibitor NSC23766 reduced ROS in G1, S and G2/M. CD34$^+$ CML-CP cells in medium supplemented with growth factors were incubated with 25 μM NSC23766, or left untreated (Control). ROS were detected by DHE (**A**) and DCFDA (**B**) in G1, S and G2/M phase determined by (**A**) Vybrant DyeCycle Green and (**B**) Vybrant DyeCycle Orange live cell staining (Invitrogen/Molecular Probes). ROS measurements are at the left sides, and percentages of cells in cell cycle phases are indicated at the bottom. Representative results of 2-3 experiments are shown.

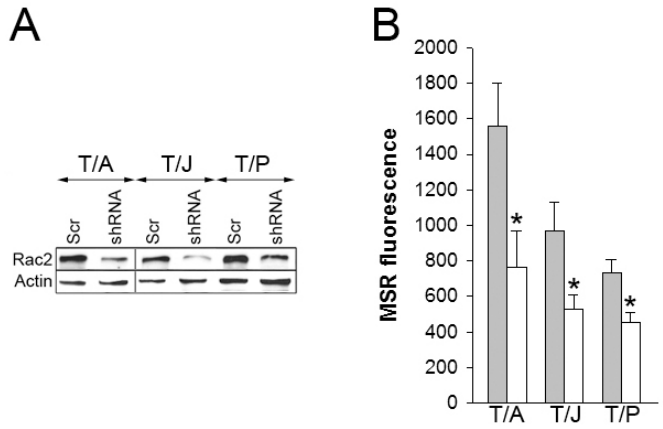

Figure S5. Expression of Rac2 shRNA inhibited mitochondrial $\cdot O_2^-$ in leukemia cells expressing OTKs. 32Dcl3 cells expressing TEL-ABL1 (T/A), TEL-JAK2 (T/J) and TEL-PDGFβR (T/P) were transfected with pGFP-V-RS retroviral vector encoding Rac2-specific shRNA (shRNA, white bars) or scrambled RNA (Scr, grey bars). (**A**) Western analysis showing the expression of Rac2 in GFP+ cells, actin served as loading control. (**B**) Mitochondrial $\cdot O_2^-$ was measured with MSR in GFP+ cells; results represent mean values of 3 experiments ± SD, ***** $p < 0.05$ in comparison to scrambled RNA.

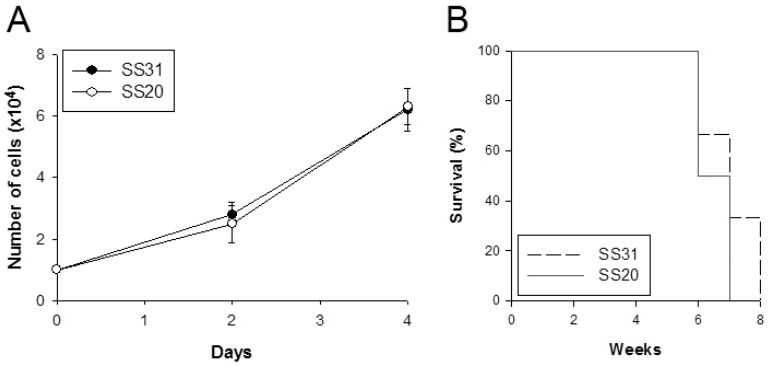

Figure S6. **SS31 did not affect the proliferation and leukemogenic capability of BCR-ABL1 –muBMCs.** (**A**) BCR-ABL1 –muBMCs were cultured in the presence of SS20 and SS31 as described in the main text. Living cells were scored in Trypan blue. Results represent mean values of 3 measurements ± SD. (**B**) Survival of the SCID mice injected with BCR-ABL1 –muBMCs and treated with SS20 and SS31 as described in Figure 7E (5 mice/group).

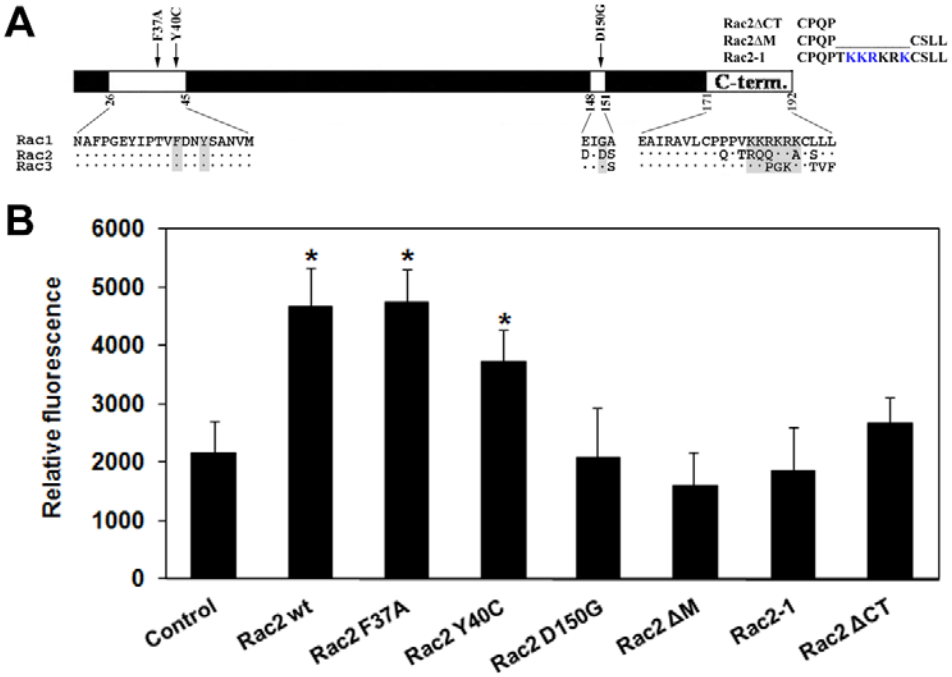

Figure S7. **Rac2 unique D150 and the C-terminal polybasic domain play a key role in the induction of ROS in BCR-ABL1-positive leukemia cells.** (**A**) A comparison of Rac1, 2, and 3 functional domains; dots indicate sites where amino acid residues are identical to the corresponding wild-type Rac1 and the differences are indicated. Amino acid (aa) substitutions in Rac2 used in the experiment are indicated; these marked in blue in Rac2-1 mutant are from Rac1. (**B**) BCR-ABL1 –positive *Rac2*$^{-/-}$ bone marrow cells were reconstituted with the empty bicistronic retroviral construct MIEG3 (Control) and with the constructs encoding for Rac2(wt) and indicated mutants [2,3]. ROS were measured in GFP+ cells with DHE. Rac2(F37A) and Rac2(Y40C) switch I domain mutants[4] stimulate ROS to the same extend as Rac2(wt). However, Rac2(D150G) mutant in the unique Rac2 aa 148-151 motif and Rac2 mutants disrupting its specific C-terminal polybasic motif (Rac2ΔCT, Rac2ΔM, Rac2-1) failed to restore ROS levels. Results represent mean values of triplicate measurements/sample ± SD; *p<0.01.

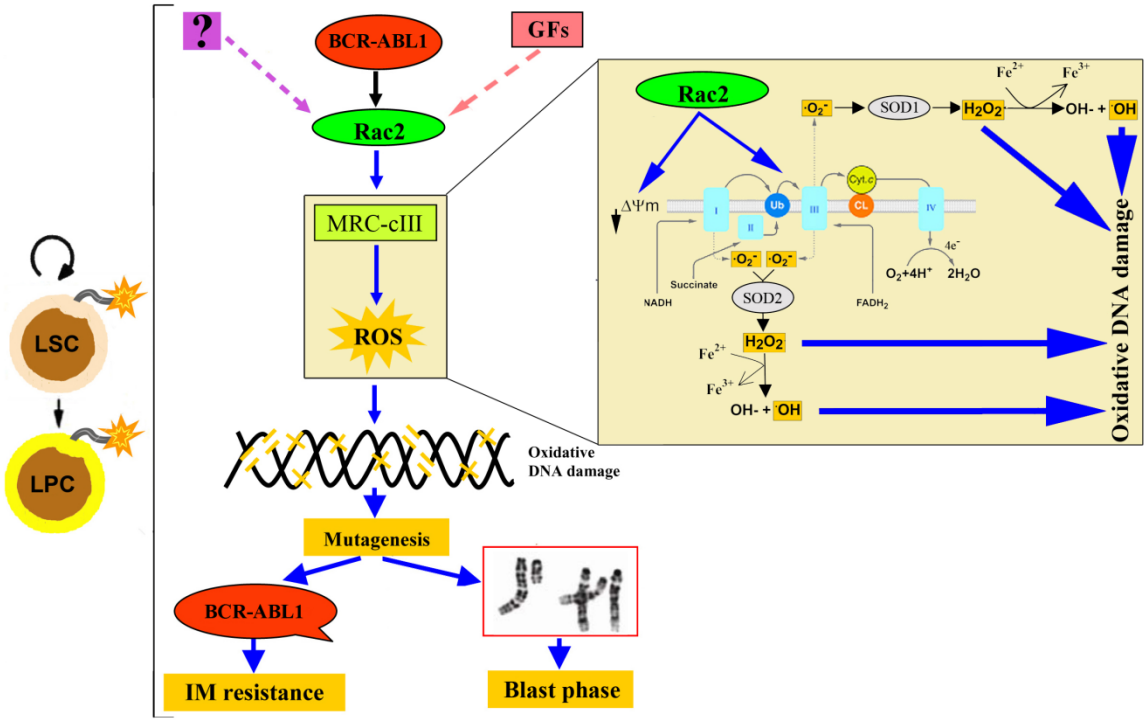

Figure S8. A model illustrating the role of Rac2 - MRC-cIII pathway in genomic instability in CML. BCR-ABL1 kinase and other factors (e.g., growth factors = GFs; and mechanisms responsible for the appearance of t(9;22)) activate Rac2, which reduces mitochondrial membrane potential ($\Delta\Psi_m$) and affects electron flow to MRC-cIII to enhance the production of superoxide radical anion ($\bullet O_2^-$) in LSCs and LPCs. Mitochondrial SOD2 and mainly cytoplasmic SOD1 transform $\bullet O_2^-$ to hydrogen peroxide (H_2O_2), which may be converted by iron (Fe^{2+})-driven cleavage of H_2O_2 in a Fenton reaction to generate the highly reactive hydroxyl radical (•OH). High levels of ROS in LSCs and LPCs cause an excessive DNA damage. Unfaithful and inefficient repair of numerous oxidative DNA lesions induces mutagenesis responsible for the accumulation of point mutations in BCR-ABL1 kinase encoding imatinib (IM) resistance, and chromosomal aberrations contributing to blast phase progression. Therefore, LSCs and/or LPCs can be considered as "ticking time-bombs" accumulating additional genetic aberrations and eventually "exploding" to cause the disease relapse and/or malignant progression.[5]

1. Reddy MM, Fernandes MS, Salgia R, Levine RL, Griffin JD, Sattler M. NADPH oxidases regulate cell growth and migration in myeloid cells transformed by oncogenic tyrosine kinases. *Leukemia*. 2011;25(2):281-289.
2. Tao W, Filippi MD, Bailey JR, et al. The TRQQKRP motif located near the C-terminus of Rac2 is essential for Rac2 biologic functions and intracellular localization. *Blood*. 2002;100(5):1679-1688.
3. Filippi MD, Harris CE, Meller J, Gu Y, Zheng Y, Williams DA. Localization of Rac2 via the C terminus and aspartic acid 150 specifies superoxide generation, actin polarity and chemotaxis in neutrophils. *Nat Immunol*. 2004;5(7):744-751.
4. Carstanjen D, Yamauchi A, Koornneef A, et al. Rac2 regulates neutrophil chemotaxis, superoxide production, and myeloid colony formation through multiple distinct effector pathways. *J Immunol*. 2005;174(8):4613-4620.
5. Skorski T. Chronic myeloid leukemia cells refractory/resistant to tyrosine kinase inhibitors are genetically unstable and may cause relapse and malignant progression to the terminal disease state. *Leuk Lymphoma*. 2011;52 Suppl 123-29.