**Supplementary information**

# Targeting Oxidative Phosphorylation with a Novel Thiophene Carboxamide Increases the Efficacy of Imatinib against Leukemic Stem Cells in Chronic Myeloid Leukemia

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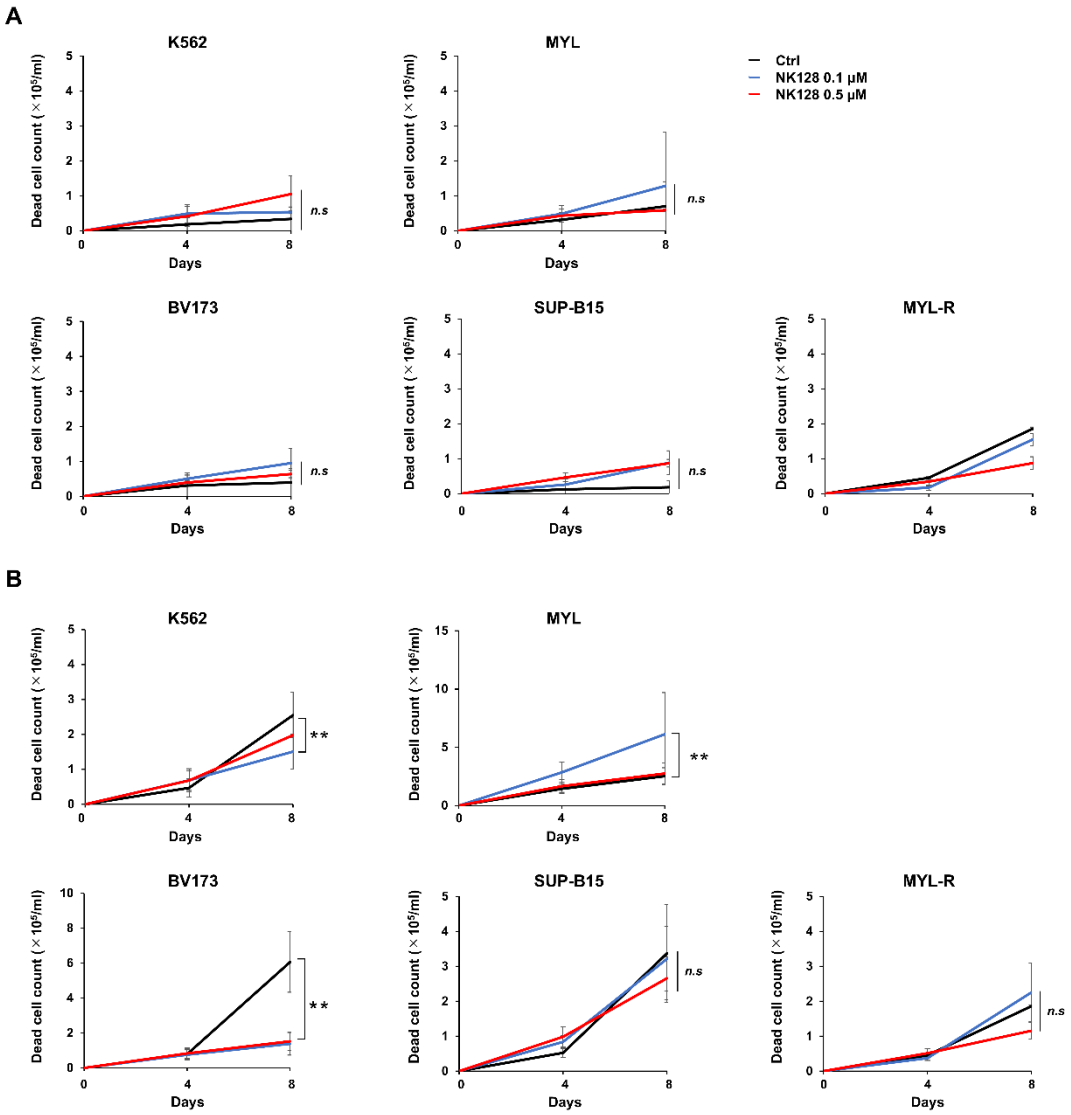
**Supplementary methods**

**Determination of the IC50 for imatinib**

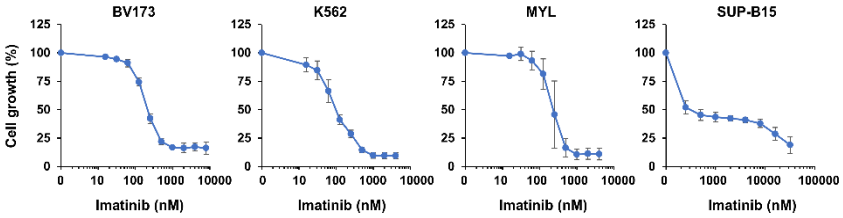
The IC50 for imatinib was determined using a Cell Counting Kit-8 (CCK-8) (Dojindo Molecular Technology, Kumamoto, Japan) according to the manufacturer's instructions 72 hours after treatment of CML cell lines with imatinib.

**Limiting dilution analysis using a primary CML mouse model**

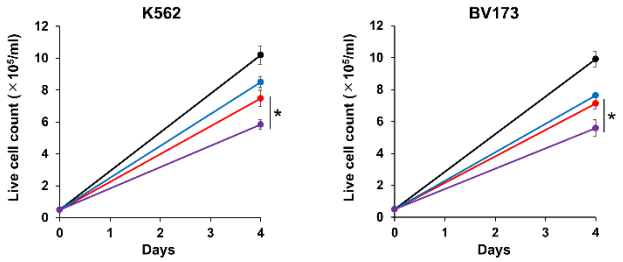
Recipient C57BL/6 mice (8 weeks old, female) were lethally irradiated (9.5 Gy), and then a mixture of GFP+ MIG-BCR-ABL1 retrovirally transduced mouse bone marrow (BM) cells ($2 \times 10^4$ cells/mouse) and whole BM cells ($2 \times 10^5$ cells/mouse, for radioprotection) were intravenously injected. BM cells were isolated from recipient mice treated daily with vehicle, IM (100 mg/kg, gavage), and NK-128 (10 mg/kg, intraperitoneally) for 10 days and then transplanted into sub-lethally irradiated secondary recipient mice to perform limiting dilution assay; three different numbers of GFP+ cells ($2 \times 10^6$, $1 \times 10^6$, and $5 \times 10^5$ cells/mouse) from each group were transplanted. The survival of mice was monitored.




**Supplementary Fig. S1. Cancer cell death induced by NK-128 monotherapy and combination therapy with imatinib. A.** CML and $Ph^+$ALL cell lines were treated with NK-128 for 8 days. The number of dead cells was counted by trypan blue staining. **B.** CML and $Ph^+$ALL cell lines were treated with imatinib (blue) or a combination of imatinib and 0.1 μM NK-128 (red). The concentration of imatinib was 0.25 μM for K562, 0.2 μM for MYL, 0.1 μM for BV173, 0.4 μM for SUP-B15, and 1 μM for MYL-R. The number of dead cells was counted by trypan blue staining. (** $p < 0.01$, n.s. not significant).

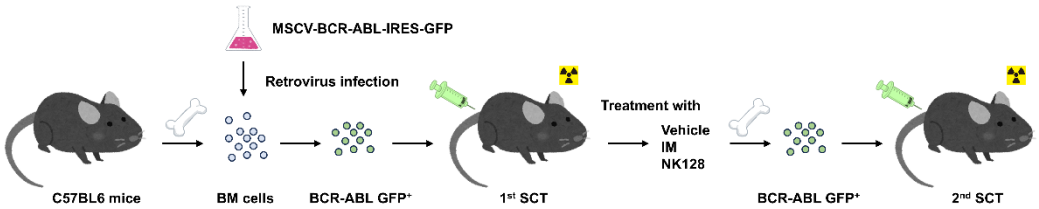



**Supplementary Fig. S2. Susceptibility of CML and Ph$^+$ALL cell lines to imatinib.** CML and Ph$^+$ALL cell lines were treated with imatinib for 4 days. Cell growth was accessed using CCK-8 reagent. The absorbance of non-treated cells was defined as 100%. The results are expressed as the mean of three independent experiments ± SD.




**Supplementary Fig. S3. Combination treatment with NK-128 and oligomycin inhibits proliferation of CML cell lines.** K562 and BV173 cells were treated with 0.1 μM NK-128 (red), oligomycin (0.25 nM for BV173 and 0.5 nM for K562, blue) or the combination (purple) for 4 days. The number of live cells was counted by trypan blue staining (* $p < 0.05$).

A




B

Survival at 60 days after 2nd SCT

| Group | # of cells | Survival |
|---|---|---|
| Vehicle | $1x10^6$ | 0/4 |
| | $5x10^5$ | 3/5 |
| IM | $2x10^6$ | 0/5 |
| | $1x10^6$ | 0/6 |
| | $5x10^5$ | 3/5 |
| NK-128 | $1x10^6$ | 5/6 |
| | $5x10^5$ | 6/6 |

**Supplementary Fig. S4. NK-128 impairs the maintenance of tumor stemness in the CML mouse model. A.** Schema of the CML mouse model. Bone marrow (BM) cells transfected with a retroviral vector containing BCR-ABL1 and GFP were transplanted into primary recipient mice. Recipient mice were treated daily with vehicle (1% DMSO, intraperitoneally), NK-128 (10 mg/kg, intraperitoneally), or IM (100 mg/kg, gavage) for 10 days. BM cells were harvested, and transplanted to secondary recipient mice to perform limiting dilution assay; three different numbers of GFP+ cells from each group were transplanted. **B.** Survival of mice at 60 days after secondary transplantation.