## Supplemental Table 1 Mutations of PRPS2 in relapse childhood ALL samples

| Sample ID | Immunophenotype | Mutation type | Position | Allele change | Amino <br> acid <br> change | Alle frequency(\%) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| ALL-114 | B | Nonsyn. | $\begin{gathered} \text { ChrX: } \\ 12,809,680 \end{gathered}$ | $\mathrm{C}>\mathrm{A}$ | R22S ${ }^{\text {a }}$ | 21 |
| ALL-120 | B | Nonsyn. | $\begin{gathered} \text { ChrX: } \\ 12,827,354 \end{gathered}$ | $\mathrm{G}>\mathrm{T}$ | S106I ${ }^{\text {a }}$ | 19 |
| ALL-127 | T | Nonsyn. | $\begin{gathered} \text { ChrX: } \\ 12,828,244 \end{gathered}$ | $\mathrm{C}>\mathrm{G}$ | P173R ${ }^{\text {a }}$ | 31 |
| ALL-177 | B | Nonsyn. | $\begin{gathered} \text { ChrX: } \\ 12,817,345 \end{gathered}$ | $\mathrm{G}>\mathrm{A}$ | V48M ${ }^{\text {b }}$ | 28 |
| ALL-219 | B | Nonsyn. | $\begin{gathered} \text { ChrX: } \\ 12,827,437 \end{gathered}$ | $\mathrm{G}>\mathrm{A}$ | A134T ${ }^{\text {b }}$ | 24 |
| ALL-219 | B | Nonsyn. | $\begin{gathered} \text { ChrX: } \\ 12,828,249 \end{gathered}$ | $\mathrm{G}>\mathrm{A}$ | A175T ${ }^{\text {b }}$ | 47 |
| ALL-247 | T | Nonsyn. | $\begin{gathered} \mathrm{Chr}_{\mathrm{X}} \\ 12,828,243 \end{gathered}$ | $\mathrm{CC}>\mathrm{TA}$ | $\mathrm{P} 173 \mathrm{Y}^{\mathrm{b}}$ | 39.2 |

Note: a, from Li et al., 2015 [17]; b, from Li et al., 2020 [16]. Nonsyn, nonsynonymous.

Supplemental Table 2 Detection of PRPS2 mutations in matched samples obtained at diagnosis, remission, and relapse from ALL-219 and ALL-247 patients by ultradeep sequencing (mean, 250,000 reads).

| Sample ID | Immunophenotype | Gene | Amino acid change | Diagnosis <br> blast ratio <br> (\%) | VAF(\%) | Remission blast ratio (\%) | VAF(\%) | Relapse <br> blast ratio (\%) | VAF(\%) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| ALL-219 | B | Nonsyn. | A134T | 69.2 | 0 | <0.01 | 0 | 85.6 | 24 |
| ALL-219 | B | Nonsyn. | A175T | 69.2 | 0 | <0.01 | 0 | 85.6 | 47 |
| ALL-247 | T | Nonsyn. | P173Y | 72.7 | 0 | <0.01 | 0 | 89.1 | 39.2 |

Note: Nonsyn, nonsynonymous. VAF, variant allele frequency.


Supplementary Figure 1 Effects of PRPS2 mutations on ALL chemotherapy drug treatment. A, Cell viability analysis. Reh cells expressing PRPS2 wild type (WT), mutants, or empty vector (EV) were treated with increasing concentrations of ALL chemotherapy drugs, methotrexate (MTX), L-asparaginase (L-ASP), daunorubicin (DNR), or cytosine arabinoside (Ara-C), respectively. B, Cell proliferation assay. Data represent the mean $\pm$ SD. $P$ values were calculated using two-tailed Student's $t$-tests.

A


B


C



Supplementary Figure 2 Effects of functional PRPS2 mutations on the treatment of 6mercaptopurine ( $6-\mathrm{MP}$ ) and 6 -thioguanine ( $6-\mathrm{TG}$ ). A, WB of ectopic expression of PRPS2 mutations in SUP-B15, Jurkat, and Vocb6 cells. B, Viability of cells with empty vector (EV), PRPS2 wild type (WT), or mutations treated with 6-MP or 6-TG. C, Early apoptosis analysis. Cells were treated with $10 \mu \mathrm{~g} / \mathrm{ml} 6-\mathrm{MP}$ for 48 h . Data in $\mathbf{A}$ to $\mathbf{C}$ represent the mean $\pm$ SD. ${ }^{*} P<0.05$. ${ }^{* *} P<0.01$. $P$ values were calculated using two-tailed Student's $t$ tests.

ID: ALL-127 with PRPS2 P173R mutation


ID: ALL-247 with PRPS2P173Y


ID: ALL-219 with PRPS2A134T and A175T mutations

| Treatment | 6-TG |  | 6-TG |  | 6-TG |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  | Wwil |  | WWW |  | Wwis |
| Elapsed days Day0 | 19 | 98 | 197 | 227 | 29.-. 256 |
| Tumor burden $69.2 \%$ | <0.01\% | <0.01\% | <0.01\% | $<0.01 \%$ | 56.3\% |

ID: ALL-114 with PRPS2R22S mutation


ID: ALL-120 with PRPS2S106I mutation


ID: ALL-177 with PRPS2 V48 mutation


Supplementary Figure 3 Clinical 6-TG and/or 6-MP treatment schemes of ALL patients with PRPS2 mutations.

A
$\stackrel{1}{M P N I V L E S G S S H Q D L S Q R V A D R L G L E L G K V V T K K E S N Q E T S V E I G E S V R G E D V Y} \cdot \stackrel{-20}{-10}$
hPRPS2 MPNIVLESGSSHQDLSQRVADRLGLELGKVVTKKESNQETSVEIGESVRGEDVY hPRPS2 IIQSGCGEINDNLME $\dot{\operatorname{Li}} \mathrm{IM} M N A C K I A S S S R V T A V I \dot{P} C \hat{G} P Y A R Q D K K D K V G E S R A$ hPRPSI IVQSGCGEINDNLMELLIMINACKIASASRVTAVIPCEPYARQDKKDK. . SRA

3AA loop


hPRPS2 AEWKNCIIVSPDAGGAKRVTSIADRLNVE FALIHKERKKANEVDRMVLVGDVKD

hPRPS1 RVAILVDDMADTCGTICHAADKLISAGATRVYAILTHGIFSGPAISRINNACFE
$\cdot 280 \cdot 290 \quad \cdot 300 \quad \cdot 310 \quad .320$
hPRPS2 AVVVTNTIPQEDKMKHCTKIQVIDISMILAEAIRRTHNGESVSYLESHVPL hPRPS1 AVVVTNTIPQEDKMKHCSKIQVIDISMILAEAIRRTHNGESVSYLESHVPI

B


Supplementary Figure 4 The sequence alignments of human PRPS1 and PRPS2 and the location of loop 98-DKKDKSRAPISAK-110 of PRPS1 in PRPS1 hexamer. A, The sequence alignments of human PRPS1 and PRPS2. Red color shows the 3AA (V103-G104-E105) loop and the P173 residue of PRPS2. B, Six PRPS1 monomer (marked as PRPS1-1 to PRPS1-6) forms a compact hexamer. Crystal structure of PRPS1 (PDB: 3EFH). The loop of 98 -DKKDKSRAPISAK-110 was marked with orange square.

