Sample ID	Immuno- phenotype	Mutation type	Position	Allele change	Amino acid change	Alle frequency(%)
ALL-114	В	Nonsyn.	ChrX: 12,809,680	C >A	R22Sª	21
ALL-120	В	Nonsyn.	ChrX: 12,827,354	G>T	S106Iª	19
ALL-127	Т	Nonsyn.	ChrX: 12,828,244	C>G	P173Rª	31
ALL-177	В	Nonsyn.	ChrX: 12,817,345	G >A	V48M <sup>b</sup>	28
ALL-219	В	Nonsyn.	ChrX: 12,827,437	G>A	A134Tb	24
ALL-219	В	Nonsyn.	ChrX: 12,828,249	G>A	A175T⁵	47
ALL-247	Т	Nonsyn.	Chr <sub>X:</sub> 12,828,243	CC>TA	P173Y♭	39.2

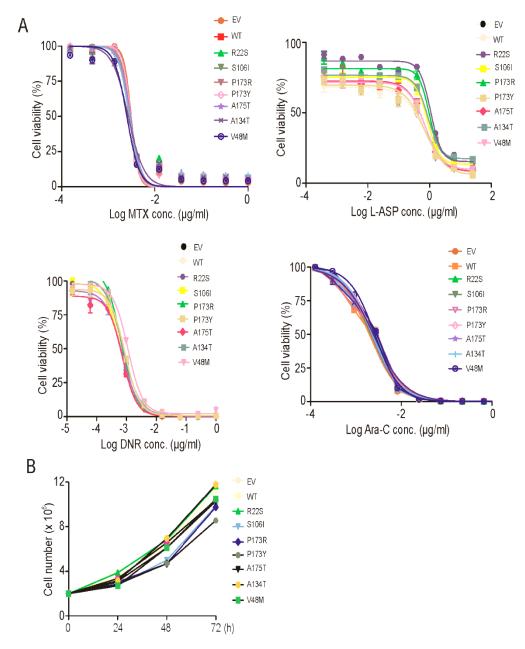
# Supplemental Table 1 Mutations of *PRPS2* in relapse childhood ALL samples

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 ultra-deep sequencing (mean, 250,000 reads).

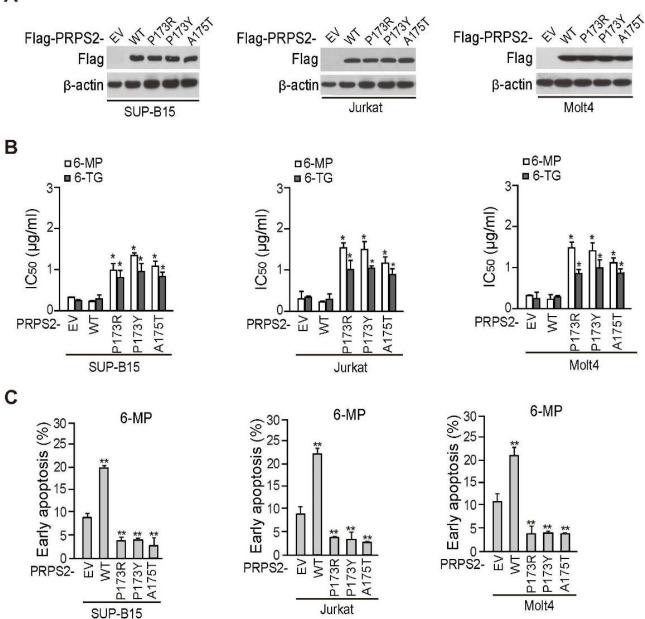
Sample ID	Immuno- phenotype	Gene	Amino acid change	Diagnosis blast ratio (%)	VAF(%)	Remission blast ratio (%)	VAF(%)	Relapse blast ratio (%)	VAF(%)
ALL-219	В	Nonsyn.	A134T	69.2	0	<0.01	0	85.6	24
ALL-219	В	Nonsyn.	A175T	69.2	0	<0.01	0	85.6	47
ALL-247	т	Nonsyn.	P173Y	72.7	0	<0.01	0	89.1	39.2

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.

Α



**Supplementary Figure 2** Effects of functional *PRPS2* mutations on the treatment of 6mercaptopurine (6-MP) and 6-thioguanine (6-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 µg/ml 6-MP for 48 h. Data in **A** to **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

	6-TG	6-TG	6-TG	6-TG
Treatment	*******	*****	****	******
Elapsed days Day0 (34)	95 <sup>(98)</sup> 222	(132) 354	(128) 482	<u>(125)</u> 607
Tumor burden 95.2% <0.	.01% 1.3%	4.4%	<0.01%	6 34.4%

#### ID: ALL-247 with PRPS2P173Y

Treatment	6-MP	6-MP	6-MP	6-MP
Elapsed days Day0(18)	<b>28</b> <sup>(58)</sup> 77	<sup>(89)</sup> <b>1</b> 67 <sup>(68)</sup>	<b>235</b> <sup>(153)</sup> <b>388</b> <sup>(5)</sup>	<sup>1)</sup> 439 880
Tumor burden 69.2%	<0.01% <0.019	6 <0.01%	<0.01% <0.01%	<0.01% 89.08%

ID: ALL-219 with PRPS2A134T and A175T mutations						
	6-TG		6-TG	6-TG		
Treatment	100000		*****	******		
Elapsed days Day0(18)	19 <u>(79)</u> 98	. <sup>(99)</sup> <b>1</b> 97				

Tumor burden	69.2%	<0.01%	<0.01%	<0.01%	<0.01%	56.3%

## ID: ALL-114 with PRPS2R22S mutation

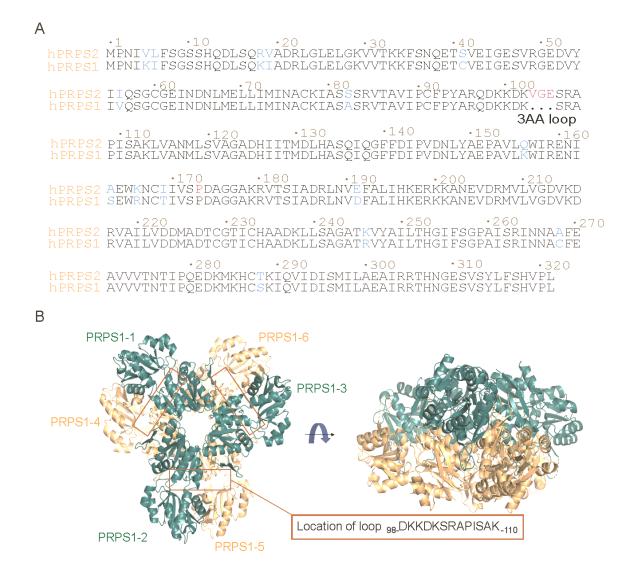
		6-TG	6-TG		6-MP	6-MP	6-MP			
Treatment		******	****		****	******	*****			
Elapsed days	Day0 (18) 19- (25)	) <b>35</b> <sup>(11)</sup> 55	(55) 111 -	<u>(47)</u> <b>158</b>	(154)	(184) 496	(177) <b>673</b>	(184) <b>85</b> 7 (346)	1203(35)	3
Tumor burden	90.8% 2.8%	<0.01% 0.4	7% <0.01%	3.6%	<0.01%	<0.4%	6.0%	<0.01%	6.0% 92%	

ID: ALL-120 with PRPS2S106I mutation						
	6-TG	6-TG	6-TG			
Treatment	******	*****	444444			
Elapsed days Day0 (18	) <b>19</b> - <sup>(11)</sup> - <b>30</b> - <sup>(25)</sup>	55(74) 129	<sup>(35)</sup> 164 <sup>(20)</sup> 184 <sup>(25)</sup>	<b>209</b> <sup>(61)</sup> <b>2</b> 70		
Tumor burden 52%	11% <0.01%	0.4% 0.8%	<0.01% 4.4%	0.4% 93.2%		

## ID: ALL-177 with PRPS2V48 mutation

	6-TG 6-	G 6-TG	6-TG End of	6-TG 6-TG
Treatment	₩₩₩ ₩	***	treatment	4486444 44864444
Elapsed days Day0(18) 1928		51 <sup>(114)</sup> -365 <sup>(114)</sup> -490	(112) 602-(112) 713-(112)	88 - <sup>(112)</sup> 923 - <sup>(92)</sup> 1015 - <sup>(26)</sup> 1041 <sup>(91)</sup> 1132
Tumor burden 92.40% 0.1% <0.0	)1% <0.01% <0	01% <0.01% <0.019	% <0.01% <0.01% 99	20% <0.01% <0.01% 90.4%

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



**Supplementary Figure 4** The sequence alignments of human PRPS1 and PRPS2 and the location of loop <sub>98-</sub>DKKDKSRAPISAK<sub>-110</sub> 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 <sub>98-</sub>DKKDKSRAPISAK<sub>-110</sub> was marked with orange square.