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

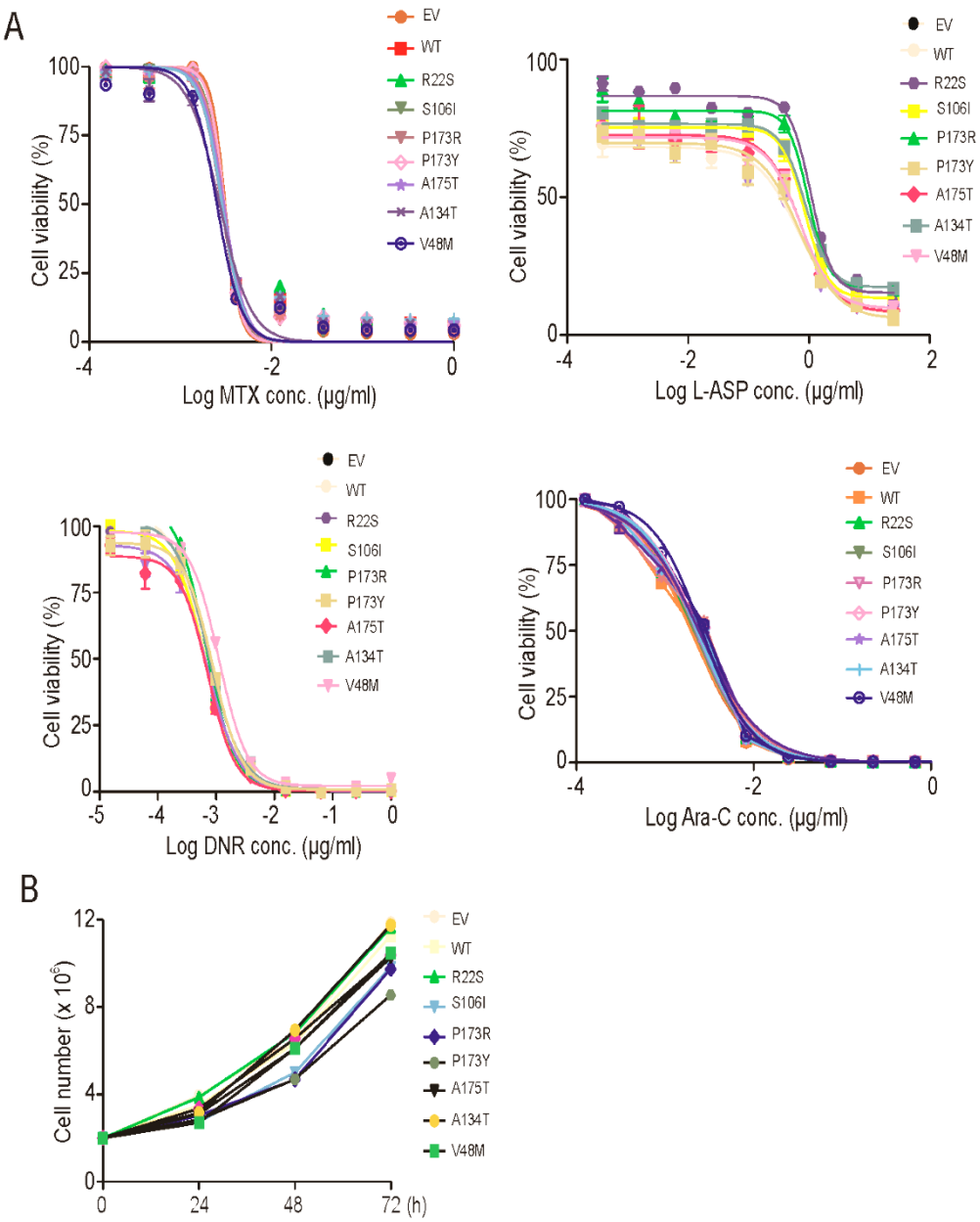
Sample ID	Immuno-phenotype	Mutation type	Position	Allele change	Amino acid change	Alle frequency(%)
ALL-114	B	Nonsyn.	ChrX: 12,809,680	C >A	R22S[a]	21
ALL-120	B	Nonsyn.	ChrX: 12,827,354	G>T	S106I[a]	19
ALL-127	T	Nonsyn.	ChrX: 12,828,244	C>G	P173R[a]	31
ALL-177	B	Nonsyn.	ChrX: 12,817,345	G >A	V48M[b]	28
ALL-219	B	Nonsyn.	ChrX: 12,827,437	G>A	A134T[b]	24
ALL-219	B	Nonsyn.	ChrX: 12,828,249	G>A	A175T[b]	47
ALL-247	T	Nonsyn.	ChrX: 12,828,243	CC>TA	P173Y[b]	39.2

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

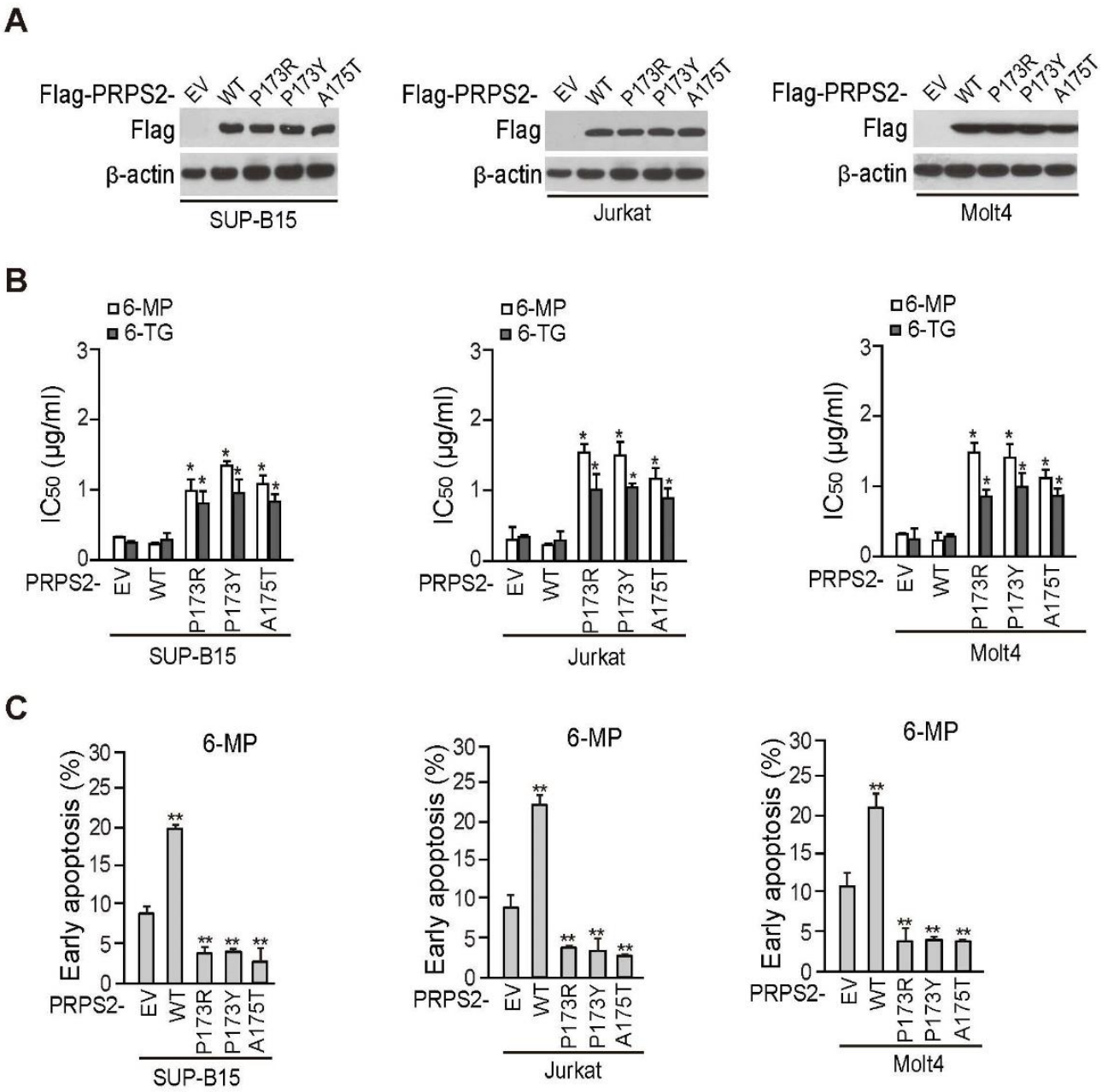
Supplemental Table 2 Detection of *PRPS2* mutations in matched samples obtained at diagnosis, remission, and relapse from ALL-219 and ALL-247 patients by ultra-deep sequencing (mean, 250,000 reads).

Sample ID	Immuno-phenotype	Gene	Amino acid change	Diagnosis blast ratio (%)	VAF(%)	Remission blast ratio (%)	VAF(%)	Relapse blast ratio (%)	VAF(%)
ALL-219	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

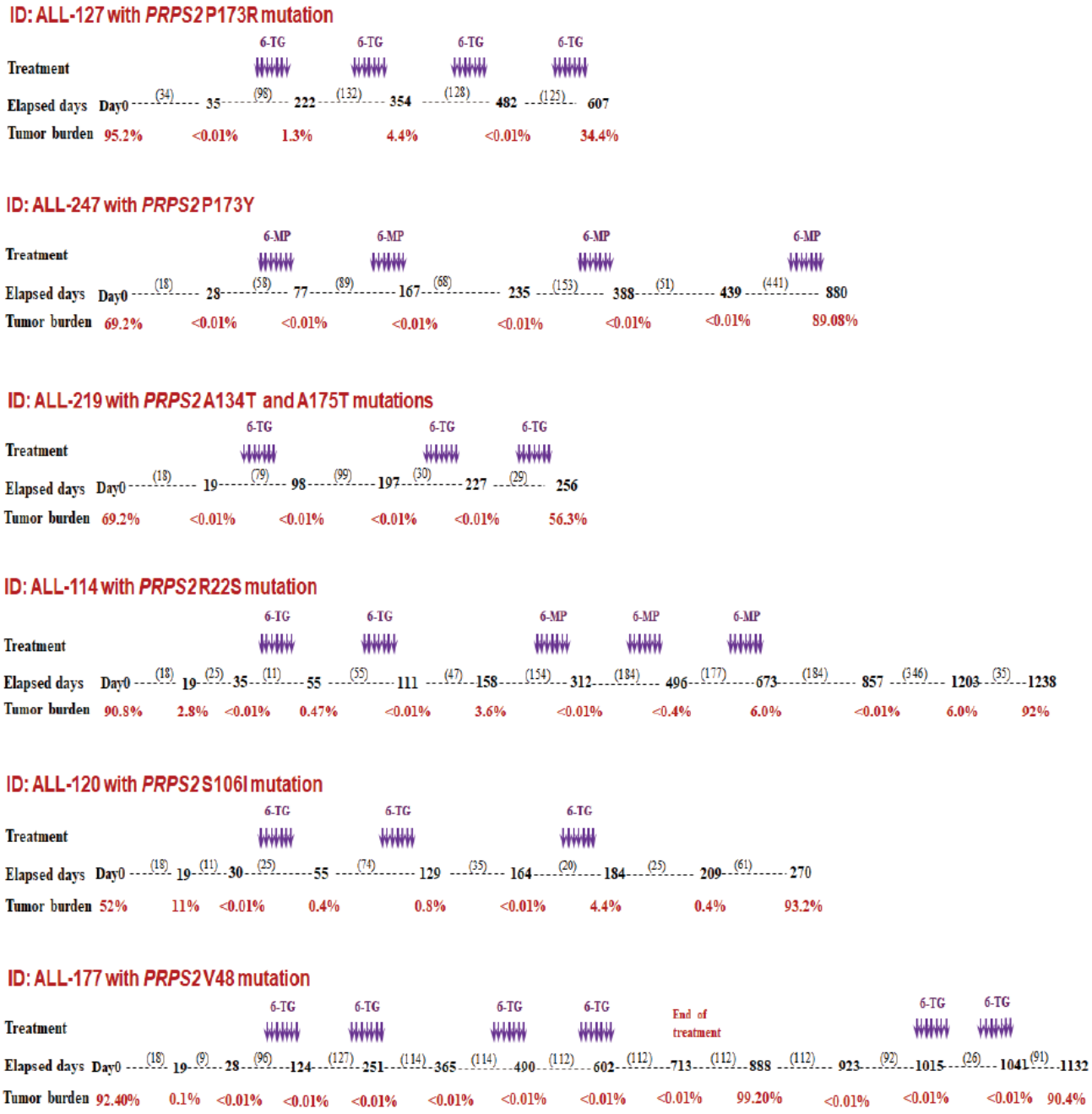
Note: Nonsyn, nonsynonymous. VAF, variant allele frequency.



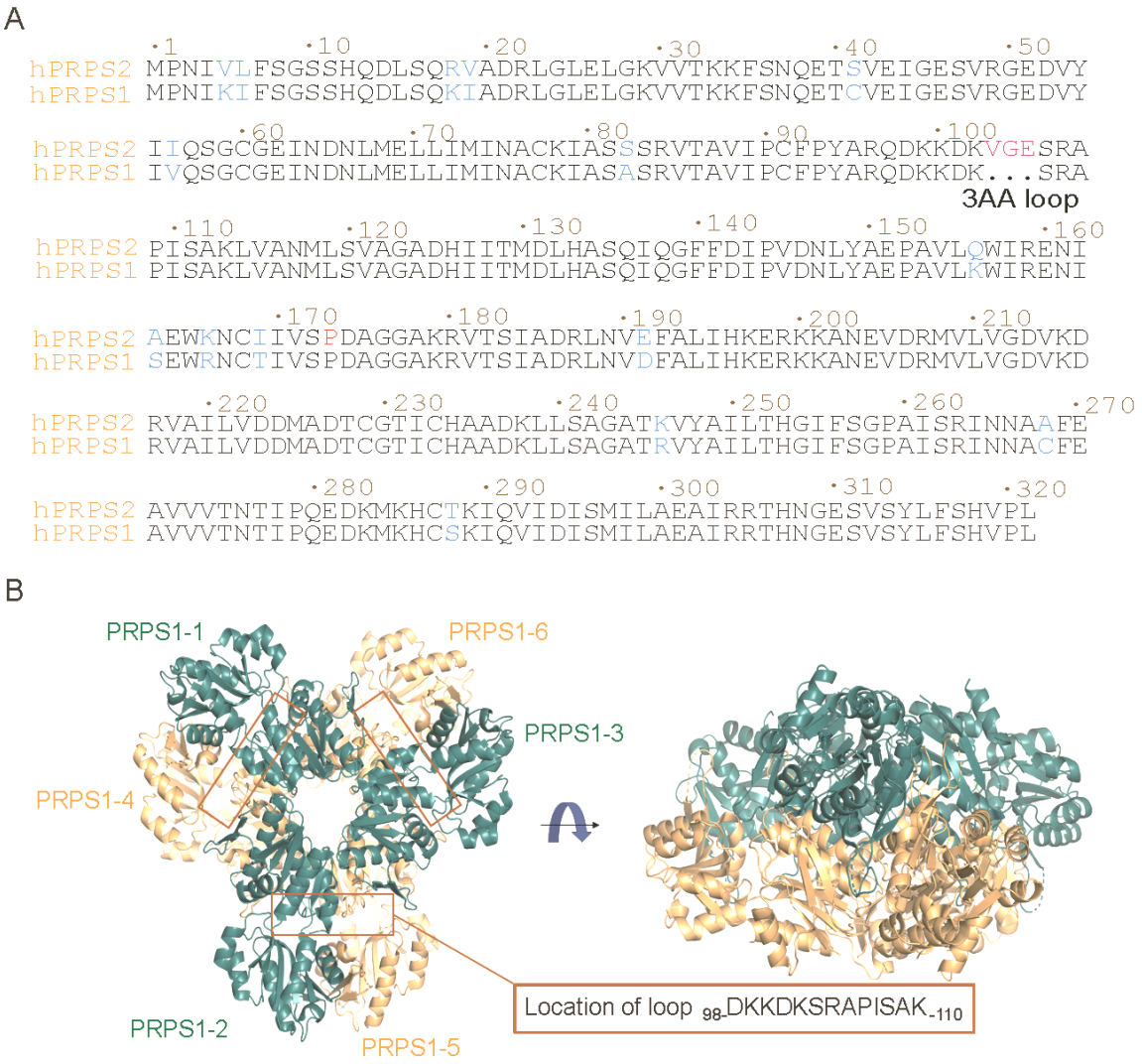
Supplementary Figure 1 Effects of *PRPS2* mutations on ALL chemotherapy drug treatment. **A,** Cell viability analysis. Reh cells expressing PRPS2 wild type (WT), mutants, or empty vector (EV) were treated with increasing concentrations of ALL chemotherapy drugs, methotrexate (MTX), L-asparaginase (L-ASP),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



Supplementary Figure 2 Effects of functional *PRPS2* mutations on the treatment of 6-mercaptopurine (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 ± SD.*$P < 0.05$. **$P < 0.01$. *P* values were calculated using two-tailed Student's *t*-tests.



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 $_{98}$-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.