**Supplementary Method**

**Droplet digital PCR (ddPCR) experiments**

*Identifying the diplotype of cases with NUDT15 c.36_37insGGAGTC and c.415C>T*

As *NUDT15* c.36_37insGGAGTC and c.415C>T often coexist, we attempted to determine whether the diplotype was heterozygous (both variants occurring on the same allele) or compound heterozygous (variants occurring on different alleles) using droplet digital PCR (ddPCR). We designed primers including exon1 to exon3 of *NUDT15* and specific probes for *NUDT15* wild-type sequences corresponding to the sequences of *NUDT15* c.36_37insGGAGTC (exon1) and c.415C>T (exon3), respectively. Each probe was labeled by different fluorescent dyes (FAM and HEX). Furthermore, we designed a DNA oligo corresponding to c.36_37insGGAGTC as a blocker to prevent the wild type probe from misannealing within the mutation sequence. Each probe and the blocker oligo included locked nucleic acids (LNA, indicated by "+" before the base) to increase specificity: common forward primer, 5'- ATGACGGCCAGCGCACAG -3', common reverse primer, 5'-TCCCACCAGATGGTTCAGAT -3', wild-type specific probe of exon1, CAGGAGTCGGAGTCGGAGTCG+T+GG FAM_IowaBlack, wild-

type specific probe of exon3, ACAA+C+G+CAGTCCC HEX_IowaBlack, mutation specific blocker oligo of exon1, AGTCGGAGTCGGAGTCG+G+AGT+C.

ddPCR experiments were performed using the QX-200 Droplet Generator (Biorad). For all assembled 20 µL PCR reaction mixtures, we included ddPCR Supermix for Probes (No dUTP; Bio-Rad®), primers at a final concentration of 1 µM, probes at a final concentration of 0.25 µM, and the blocker oligo at a final concentration of 2.5 µM. For the analysis of the patients, we loaded complementary DNA (cDNA) produced from RNA extracted from peripheral blood during complete remission for all reactions. Thermal cycling conditions were 95°C for 10 minutes (1 cycle), 94°C for 30 seconds (ramp rate 2°C/ second, 40 cycles), 60°C for 1 minute (ramp rate 2°C/second, 40 cycles), 98°C for 10 minutes (1 cycle), and 4°C hold. The emulsion was achieved using a QX-200 Droplet Generator and read by a QX-200 Droplet Reader, and the analysis of the experimental results was performed using the Bio-Rad QuantaSoft Analysis Pro software version 1.0. Each assay was performed in three wells and the results were merged.

First, we confirmed that the heterozygotes showed a single population of double positive droplets. We used template vectors in this analysis to show populations of separate single positive droplets in the compound heterozygotes. Thereafter, we analyzed cDNA from the patient with mutations in both exons. Finally, we confirmed that all the patients with *NUDT15* c.36_37insGGAGTC and c.415C>T were heterozygous.

**Supplementary Table 1. Patient characteristics**

| Characteristics | Number of patients n (%) | |
|---|---|---|
| All patients | 138 | (100) |
| Gender | | |
| Male (%) | 88 | (63.7) |
| Median age, months (range) | 60 | (5-238) |
| Diagnosis | | |
| BCP-ALL (%)* | 100 | (72.5) |
| T-ALL (%) | 25 | (181) |
| LBL (%) | 13 | (9.4) |
| NCI risk | | |
| SR | 77 | (55.8) |
| HR | 44 | (31.9) |
| *NUDT15* genotype | | |
| No variant | 100 | (72.5) |
| Heterozygous variants | | |
| c.36_37insGGAGTC and c.415C>T | 14 | (10.1) |
| c.52G>A and c.415C>T | 1 | (0.7) |
| c.36_37insGGAGTC | 1 | (0.7) |
| c.52G>A | 7 | (5.1) |
| c.415C>T | 12 | (8.7) |
| Homozygous variants | | |
| c.415C>T | 3 | (2.2) |
| *TPMT* genotype | | |
| No variant | 135 | (97.8) |
| c.719A>G heterozygous variant | 3 | (2.2) |

*BCP-ALL included 1 case of mixed phenotype acute leukemia (MPAL).

BCP-ALL, B-cell precursor ALL; LBL, lymphoblastic lymphoma; NCI, National cancer institute; SR, standard risk; HR, high risk;

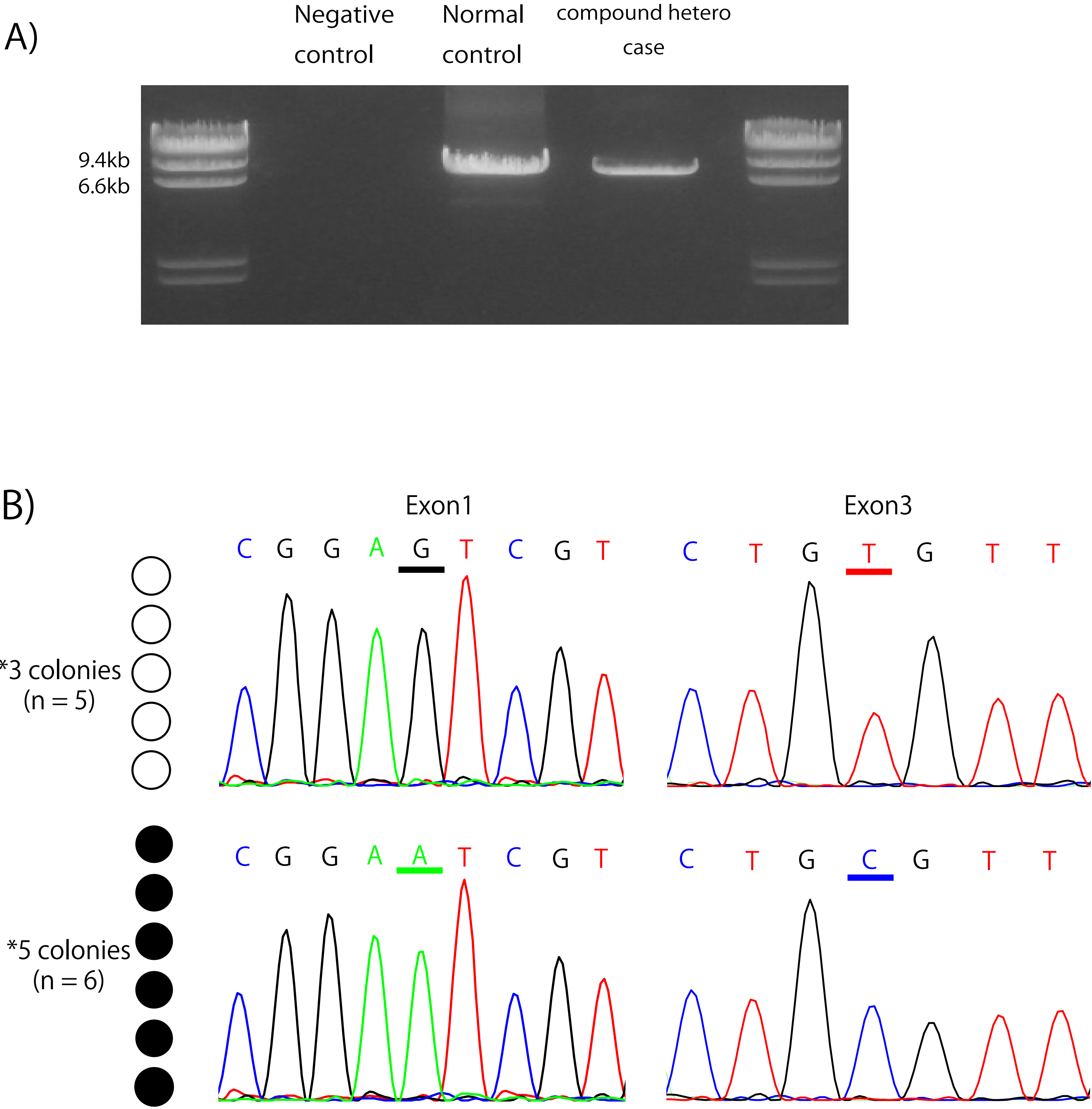
## Supplementary Table 2. Primer sequences and PCR condition

| Primer sets | Forward primer sequence (5' to 3') | Reverse primer sequence (5' to 3') |
|---|---|---|
| ***NUDT15*** | | |
| **Genotyping** | | |
| exon 1 | CAAAGCACAACTGTAAGCGACT | GAAAGACCCAGCTAGCAAAGAC |
| exon 3 | AAGCAAATGCAAAGCATCAC | GGCTGAAAGA GTGGGGGATA |
| **cDNA PCR** | ATGACGGCCAGCGCACAG | CTAGAGATGATTTCCTTTGTATCCCAC |
| **genomic cloning** | ACTGAGGATCCGACGCATTACGCACCGCGGA CAGCTGGAGA | ACGTGCATGCCCCAGACCACACTTTGACAACCACTG CTCTAGAC |
| ***TPMT* genotyping** | | |
| c.280G>C | | |
| G (wilde type) | GTATGATTTTATGCAGGTTTG | TAAATAGGAACCATCGGACAC |
| C (mutant) | GTATGATTTTATGCAGGTTTC | |
| c.460G>A | AGGCAGCTAGGGAAAAAGAAAGGTG | CAAGCCTTATAGCCTTACACCCAGG |
| c.719A>G | GAGACAGAGTTTCACCATCTTGG | CAGGCTTTAGCATAATTTTCAATTCCTC |

**NUDT15 cDNA PCR:** PCR was performed using AmpliTaq Gold 360 Master mix. The PCR conditions were following: an initial denaturation at 95 °C for 10 min, followed by 40 cycles of amplification (30 s at 95 °C, 30 s at 60 °C, and 60 s at 72 °C), and a final step of 72 °C for 7 min.

**NUDT15 whole genome region cloning**: PCR was performed using PrimeSTAR GXL DNA Polymerase. The PCR conditions were following: an initial denaturation at 98 °C for 1 min, followed by 35 cycles of amplification (10 s at 98 °C, 9 min at 68 °C).The PCR product was restricted by BamHI and SphI and was subcloned to pUC19 cloning vector.

# Supplementary Figure 1

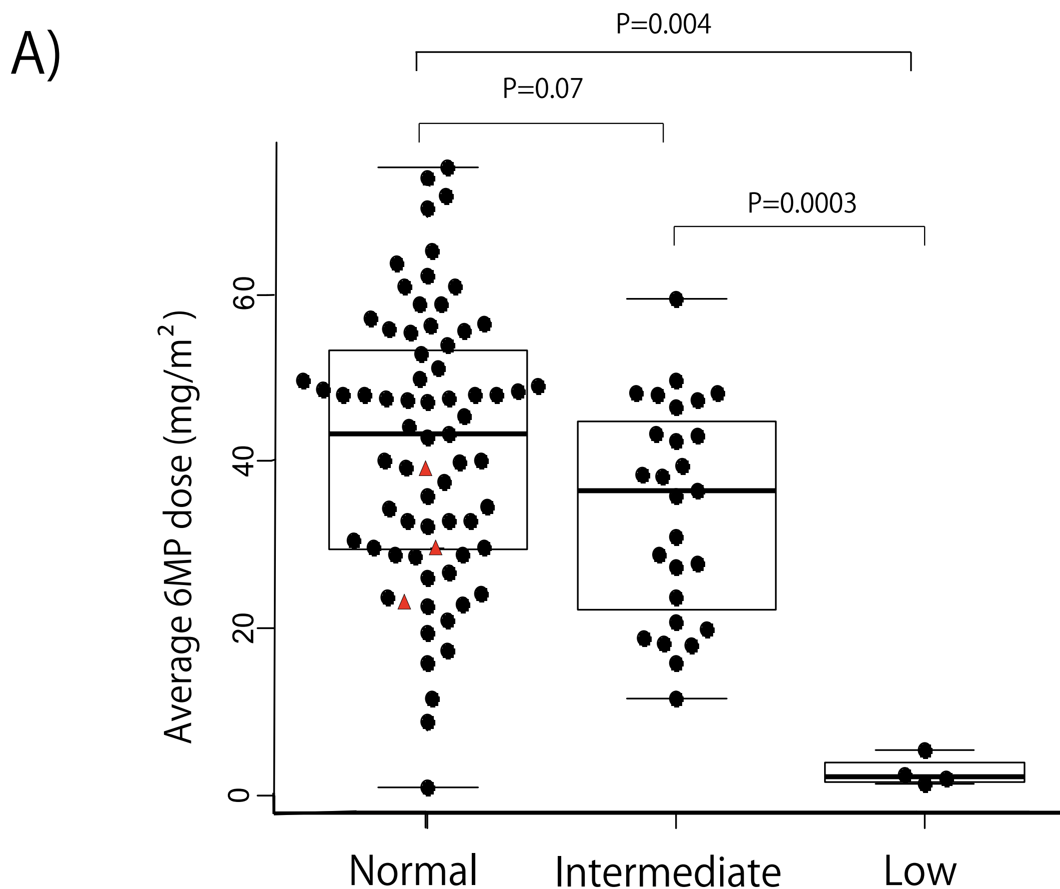


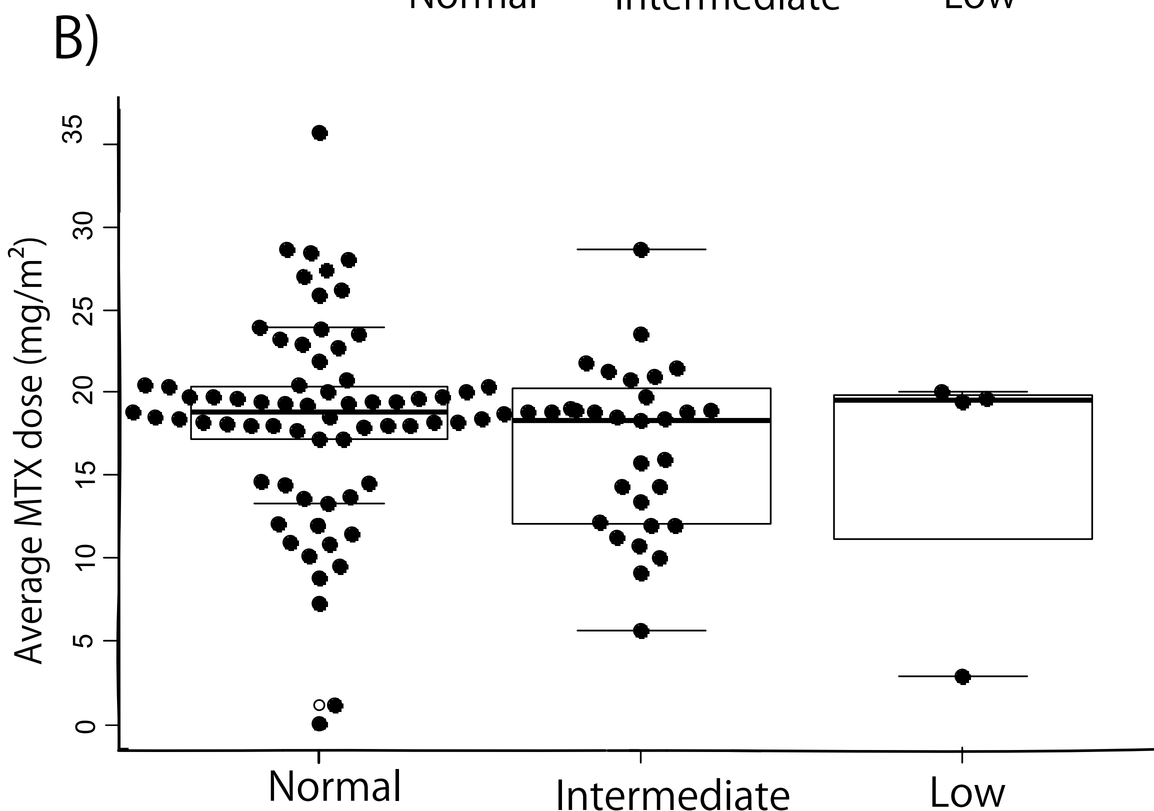

Supplementary Figure 1

Analysis of compound heterozygous case by PCR of genomic NUDT15 and cloning

A) PCR was performed using PrimeSTAR GXL DNA Polymerase. The PCR conditions were following: an initial denaturation at 98 ° C for 1 min, followed by 35 cycles of amplification (10 s at 98 $^0$C, 9 min at 68 $^0$C )

B) The PCR product was restricted by BamHI and SphI and was subcloned to pUC19 cloning vector. We collected eleven colonies, extracted plasmids and confirmed the sequence of c.52 G>A and c.415 C>T region by Sanger sequence.




Supplementary Figure 2

Effect of diplotype of NUDT15 on Average 6MP and MTX dose

A) Average dose of 6MP B) Average dose of MTX. 6MP and MTX dose. Dose of 6MP and MTX were adjusted by body surface area and were defined as the stable dose at least 6 months. Each boxes included the data between 25 percentile and 75 percentile . Horizontal bar indicates median dose of 6MP and MTX. Normal, intermediate and Low group were respectively defined as wild type/wild type, wild type/variant and variant/variant according to diplotype analysis. 6MP dose was significantly lower in the low group than in the normal and intermediate group. However, MTX dose was not different among the three groups.