# Benchmarking miRNA Reference Genes in B-Cell Precursor Acute Lymphoblastic Leukemia

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**Supplementary Table 1 CT values of PDX ALL samples and ALL cell lines of the identification cohort.**

| | | RNU6 | miR-103a-3p | 5sRNA | RNU1A1 | SNORD44 | miR-532-5p |
|---|---|---|---|---|---|---|---|
| **Cell lines** | **KOPN-8** | 24.35 | 23.72 | 17.88 | 20.25 | 21.45 | 28.88 |
| | **UoCB6** | 24.62 | 23.63 | 17.82 | 20.41 | 21.24 | 28.75 |
| | **EU3** | 24.86 | 23.13 | 18.31 | 20.47 | 20.91 | 28.12 |
| | **NALM-6** | 23.32 | 23.57 | 16.06 | 18.01 | 20.96 | 27.9 |
| | **RS4;11** | 23.61 | 22.34 | 18.3 | 20.39 | 21.33 | 29.53 |
| | **RCH-ACV** | 23.75 | 22.62 | 17.51 | 18.92 | 21.19 | 29.1 |
| | **MHH-CALL2** | 22.93 | 23.86 | 17.27 | 18.92 | 21.34 | 29.63 |
| | **HAL01** | 22.81 | 22.75 | 17.07 | 20.02 | 22.53 | 28.88 |
| | **REH** | 23.8 | 22.62 | 16.69 | 19.55 | 21.13 | 30.53 |
| **Spleen** | **X001** | 24.7 | 24.53 | 18.37 | 21.18 | 22.68 | 29.18 |
| | **X002** | 24.24 | 22.18 | 18.15 | 19.99 | 21.9 | 27 |
| | **X003** | 24.84 | 23.68 | 13.77 | 19.4 | 23.38 | 30.17 |
| | **X004** | 30.51 | 25.09 | 11.95 | 21.6 | 26.62 | 30.12 |
| | **X005** | 20.75 | 23.1 | 12 | 15.95 | 21.53 | 28.46 |
| | **X006** | 21.58 | 23.29 | 15.85 | 19.37 | 20.31 | 29.74 |
| | **X007** | 21.35 | 23.61 | 15.54 | 17.53 | 20.61 | 28.47 |
| | **X008** | 20.86 | 24.23 | 20.4 | 16.62 | 21.19 | 28.1 |
| | **X009** | 26.23 | 22.09 | 18.61 | 22.77 | 23.16 | 28.42 |
| | **X010** | 26.06 | 23.63 | 14.06 | 18.22 | 23.75 | 26.84 |
| | **X011** | 17.86 | 22.2 | 11.78 | 14.04 | 19.42 | 29.19 |
| | **X012** | 26.61 | 25.3 | 21.72 | 23.68 | 24.36 | 30.35 |
| | **X013** | 25.14 | 23.67 | 11.99 | 19.13 | 24.62 | 28.75 |
| | **X014** | 22.71 | 23.26 | 14.72 | 18.93 | 22.19 | 28.78 |
| | **X015** | 26.06 | 23.19 | 12.93 | 19.23 | 24.99 | 27.94 |
| | **X016** | 21.85 | 23.69 | 14.09 | 17.64 | 21.07 | 29.11 |
| | **X017** | 23.44 | 25.48 | 15.26 | 18.96 | 21.2 | 29.54 |
| | **X018** | 23.76 | 25.35 | 17.54 | 20.3 | 21.94 | 30.29 |
| | **X019** | 19.84 | 23.16 | 14.02 | 15.62 | 22.99 | 26.13 |
| | **X020** | 23.62 | 24.82 | 11.81 | 18.34 | 24.48 | 29.5 |
| | **X021** | 23.03 | 24.54 | 14.05 | 18.61 | 20.98 | 28.34 |
| | **X022** | 24.37 | 23.55 | 17.45 | 21.22 | 20.93 | 28.11 |
| **BM** | **X001** | 25.99 | 24.15 | 15.84 | 20.88 | 22.43 | 28.84 |
| | **X002** | 24.91 | 22.13 | 19.17 | 20.67 | 22.33 | 27.56 |
| | **X003** | 28.81 | 24.09 | 12.76 | 18.7 | 24.58 | 29.66 |
| | **X004** | 28.4 | 26.28 | 11.34 | 19.98 | 27.4 | 31.23 |
| | **X005** | 24.72 | 24.76 | 10.64 | 16.93 | 26.57 | 30.14 |
| | **X006** | 19.69 | 24.18 | 16.87 | 19.93 | 18.95 | 31.22 |
| | **X007** | 24.72 | 22.95 | 14.75 | 18.99 | 23.12 | 28.43 |
| | **X008** | 23.74 | 18.41 | 18.07 | 20.52 | 21.56 | 27.75 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **X009** | 26.35 | 22.5 | 20.34 | 22.65 | 22.93 | 28.46 |
| | **X010** | 26 | 23.04 | 12.07 | 16.42 | 27.42 | 27.66 |
| | **X011** | 27.49 | 24.11 | 14.63 | 17.97 | 22.05 | 29.83 |
| | **X012** | 22.96 | 25.99 | 21.48 | 25.27 | 26.68 | 31.3 |
| | **X013** | 26.15 | 24.47 | 14.24 | 18.5 | 26.34 | 29.16 |
| | **X014** | 23.86 | 23.67 | 12.54 | 19.13 | 24.78 | 29.93 |
| | **X015** | 24.17 | 23.2 | 14.84 | 18.69 | 25.65 | 27.93 |
| | **X016** | 25.6 | 23.44 | 11.05 | 19.19 | 23.89 | 28.86 |
| | **X017** | 21.74 | 25.82 | 17.33 | 20.12 | 20.44 | 30.1 |
| | **X018** | 23.23 | 25.45 | 17.72 | 17.86 | 21.93 | 29.57 |
| | **X019** | 22.37 | 21.96 | 15.19 | 17.68 | 21.63 | 26.1 |
| | **X020** | 27.22 | 26.79 | 11.48 | 19.74 | 31.19 | 31.55 |
| | **X021** | 21.53 | 24.06 | 15.69 | 19.27 | 20.31 | 27.89 |
| | **X022** | 27.46 | 26.02 | 16.82 | 24.97 | 26.64 | 29.18 |
| **CNS** | **X001** | 25.13 | 23.8 | 17.64 | 20.89 | 21.8 | 28.72 |
| | **X002** | 24.54 | 22.53 | 19.1 | 21 | 21.65 | 27.37 |
| | **X003** | 23.86 | 24.15 | 13.18 | 17.75 | 21.95 | 30.26 |
| | **X004** | 26.57 | 26.11 | 12.77 | 19.64 | 24.3 | 29.77 |
| | **X005** | 23.79 | 23.75 | 15.46 | 19.52 | 21.48 | 29.22 |
| | **X006** | 19.8 | 23.65 | 15.71 | 18.51 | 21.13 | 29.53 |
| | **X007** | 22.22 | 22.97 | 16.89 | 18.6 | 21.05 | 28.57 |
| | **X008** | 23.74 | 23.09 | 17.65 | 18.22 | 22.36 | 27.7 |
| | **X009** | 26.44 | 22.98 | 18.56 | 22.81 | 22.71 | 29.33 |
| | **X010** | 24.44 | 22.87 | 16.25 | 19.14 | 22.09 | 26.62 |
| | **X011** | 22.39 | 23.53 | 15.59 | 17.77 | 20.82 | 29.84 |
| | **X012** | 26.41 | 25.61 | 23.67 | 25.64 | 24.93 | 30.48 |
| **Mean CT values** | | **24.15** | **23.76** | **15.85** | **19.52** | **22.79** | **28.98** |

PDX: patient-derived xenograft; ALL: acute lymphoblastic leukemia; BM: bone marrow; CNS: central nervous system.

**Supplementary Table 2 CT values of PBMCs from healthy controls of the identification cohort.**

| | | RNU6 | miR-103a-3p | 5sRNA | RNU1A1 | SNORD44 | miR-532-5p |
|---|---|---|---|---|---|---|---|
| **Healthy Controls** | **HC1** | 19.47 | 20.8 | 13.95 | 15.95 | 19.27 | 25.79 |
| | **HC2** | 22.61 | 20.32 | 16.6 | 19.18 | 19.69 | 26.79 |
| | **HC3** | 19.05 | 19.62 | 14.13 | 16.82 | 18.5 | 25.21 |
| | **HC4** | 21.96 | 18.84 | 15.63 | 18.67 | 18.63 | 24.06 |
| | **HC5** | 21.97 | 23.85 | 18.94 | 15.88 | 20.07 | 23.49 |
| | **HC6** | 23.61 | 19.97 | 15.22 | 16.56 | 21.73 | 24.36 |
| **Mean CT value** | | **21.45** | **20.57** | **15.75** | **17.18** | **19.65** | **24.95** |

PBMC: peripheral blood mononuclear cells; HC: healthy control.

**Supplementary Table 3 CT values of PDX ALL samples of the validation cohort.**

| | | RNU6 | miR-103a-3p | 5sRNA | RNU1A1 | SNORD44 | miR-532-5p |
|---|---|---|---|---|---|---|---|
| Spleen | Validation X001 | 15.33 | 22.00 | 10.59 | 12.66 | 19.85 | 32.22 |
| | Validation X002 | 17.27 | 24.56 | 10.30 | 12.84 | 20.93 | 33.26 |
| | Validation X003 | 16.45 | 22.18 | 10.34 | 12.26 | 21.75 | 31.29 |
| | Validation X004 | 15.70 | 22.97 | 10.19 | 11.89 | 20.14 | 30.62 |
| | Validation X005 | 16.56 | 22.91 | 10.58 | 12.87 | 21.80 | 31.96 |
| | Validation X006 | 17.78 | 24.04 | 11.40 | 14.36 | 22.93 | 32.45 |
| | Validation X007 | 15.52 | 22.17 | 10.98 | 11.91 | 20.79 | 29.85 |
| | Validation X008 | 18.60 | 24.83 | 11.01 | 13.83 | 23.70 | 33.13 |
| | Validation X009 | 17.89 | 23.71 | 11.52 | 14.06 | 23.27 | 30.44 |
| | Validation X010 | 15.46 | 23.96 | 10.63 | 12.17 | 20.48 | 34.55 |
| | Validation X011 | 21.35 | 23.34 | 12.57 | 16.59 | 23.06 | 30.69 |
| | Validation X012 | 24.05 | 24.50 | 12.89 | 19.12 | 23.96 | 32.51 |
| BM | Validation X001 | 14.66 | 22.20 | 10.13 | 11.56 | 20.04 | 32.78 |
| | Validation X002 | 16.72 | 23.78 | 10.26 | 12.19 | 21.01 | 31.72 |
| | Validation X003 | 19.59 | 23.49 | 10.58 | 14.53 | 24.11 | 32.53 |
| | Validation X004 | 17.71 | 24.29 | 10.82 | 13.55 | 22.64 | 32.73 |
| | Validation X006 | 17.17 | 24.48 | 11.91 | 14.09 | 22.71 | 32.70 |
| | Validation X007 | 14.64 | 21.43 | 10.19 | 10.76 | 19.74 | 29.85 |
| | Validation X008 | 18.16 | 24.01 | 11.63 | 14.18 | 22.10 | 32.11 |
| | Validation X009 | 22.22 | 25.08 | 10.97 | 16.68 | 29.12 | 32.04 |
| | Validation X010 | 16.58 | 24.11 | 11.34 | 13.27 | 21.48 | 32.19 |
| | Validation X011 | 19.73 | 22.68 | 12.08 | 14.22 | 22.22 | 30.66 |
| | Validation X012 | 23.73 | 24.47 | 12.84 | 19.37 | 23.14 | 32.67 |
| | Validation X013 | 23.70 | 24.65 | 10.69 | 16.66 | 25.69 | 32.49 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **CNS** | **Validation X004** | 15.63 | 24.15 | 10.37 | 12.54 | 20.59 | 32.14 |
| | **Validation X006** | 17.03 | 23.87 | 12.12 | 14.05 | 22.18 | 31.83 |
| | **Validation X007** | 15.65 | 22.48 | 11.25 | 12.68 | 20.78 | 31.25 |
| | **Validation X008** | 16.65 | 23.94 | 10.71 | 12.33 | 21.64 | 32.71 |
| | **Validation X011** | 20.42 | 23.47 | 12.61 | 17.66 | 22.46 | 30.88 |
| | **Validation X012** | 21.87 | 23.84 | 14.01 | 18.75 | 22.11 | 32.38 |
| | **Validation X013** | 21.16 | 23.62 | 10.93 | 15.59 | 23.79 | 31.15 |
| **Mean CT values** | | **18.23** | **23.59** | **11.24** | **14.17** | **22.27** | **31.93** |

PDX: patient-derived xenograft; ALL: acute lymphoblastic leukemia; BM: bone marrow; CNS: central nervous system.

**Supplementary Table 4 Mean of ∆CT values and STD of PDX ALL samples of the validation cohort.**

| | Mean | STD | Mean STD |
|---|---|---|---|
| RNU6 vs miR-103a-3p | -5.36 | 2.43 | |
| RNU6 vs 5sRNA | 6.99 | 2.32 | |
| RNU6 vs RNU1A1 | 4.06 | 1.01 | |
| RNU6 vs SNORD44 | -4.04 | 1.83 | |
| RNU6 vs miR-532-5p | -13.70 | 2.92 | 2.10 |
| miR-103a-3p vs RNU6 | 5.36 | 2.43 | |
| miR-103a-3p vs 5sRNA | 12.35 | 1.15 | |
| miR-103a-3p vs RNU1A1 | 9.42 | 2.00 | |
| miR-103a-3p vs SNORD44 | 1.32 | 1.52 | |
| miR-103a-3p vs miR-532-5p | -8.34 | 0.91 | 1.60 |
| 5sRNA vs RNU6 | -6.99 | 2.32 | |
| 5sRNA vs miR-103a-3p | -12.35 | 1.15 | |
| 5sRNA vs RNU1A1 | -2.93 | 1.64 | |
| 5sRNA vs SNORD44 | -11.03 | 1.90 | |
| 5sRNA vs miR-532-5p | -20.69 | 1.47 | 1.69 |
| RNU1A1 vs RNU6 | -4.06 | 1.01 | |
| RNU1A1 vs miR-103a-3p | -9.42 | 2.00 | |
| RNU1A1 vs 5sRNA | 2.93 | 1.64 | |
| RNU1A1 vs SNORD44 | -8.10 | 1.83 | |
| RNU1A1 vs miR-532-5p | -17.76 | 2.44 | 1.78 |
| SNORD44 vs RNU6 | 4.04 | 1.83 | |
| SNORD44 vs miR-103a-3p | -1.32 | 1.52 | |
| SNORD44 vs 5sRNA | 11.03 | 1.90 | |
| SNORD44 vs RNU1A1 | 8.10 | 1.83 | |
| SNORD44 vs miR-532-5p | -9.66 | 2.08 | 1.83 |
| miR-532-5p vs RNU6 | 13.70 | 2.92 | |
| miR-532-5p vs miR-103a-3p | 8.34 | 0.91 | |
| miR-532-5p vs 5sRNA | 20.69 | 1.47 | |
| miR-532-5p vs RNU1A1 | 17.76 | 2.44 | |
| miR-532-5p vs SNORD44 | 9.66 | 2.08 | 1.96 |

PDX: patient-derived xenograft; ALL: acute lymphoblastic leukemia; STD: standard deviation.

**Supplementary Table 5 CT values of PBMCs from healthy controls of the validation cohort.**

| | | RNU6 | miR-103a-3p | 5sRNA | RNU1A1 | SNORD44 | miR-532-5p |
|---|---|---|---|---|---|---|---|
| **Healthy Controls** | **Validation HC1** | 19.62 | 22.49 | 12.84 | 16.27 | 19.11 | 29.46 |
| | **Validation HC2** | 21.08 | 23.04 | 13.79 | 17.35 | 20.58 | 29.90 |
| | **Validation HC3** | 19.14 | 24.07 | 11.84 | 14.67 | 19.84 | 31.80 |
| | **Validation HC4** | 18.72 | 22.21 | 11.81 | 14.56 | 19.69 | 29.45 |
| | **Validation HC5** | 20.83 | 21.89 | 13.30 | 16.91 | 19.81 | 28.73 |
| | **Validation HC6** | 19.58 | 21.63 | 12.61 | 15.36 | 19.82 | 28.79 |
| **Mean CT value** | | **19.83** | **22.56** | **12.70** | **15.85** | **19.81** | **29.69** |

PBMC: peripheral blood mononuclear cells; HC: healthy control.

**Supplementary Table 6 Mean of ∆CT values and STD of PBMCs derived from HCs of the validation cohort.**

| | Mean | Std. Deviation | Mean STD |
|---|---|---|---|
| RNU6 vs miR-103a-3p | -2.728 | 1.36 | 0.90 |
| RNU6 vs 5sRNA | 7.13 | 0.2867 | |
| RNU6 vs RNU1A1 | 3.98 | 0.3964 | |
| RNU6 vs SNORD44 | 0.02167 | 0.7805 | |
| RNU6 vs miR-532-5p | -9.857 | 1.668 | |
| miR-103a-3p vs RNU6 | 2.728 | 1.36 | 1.10 |
| miR-103a-3p vs 5sRNA | 9.857 | 1.315 | |
| miR-103a-3p vs RNU1A1 | 6.708 | 1.586 | |
| miR-103a-3p vs SNORD44 | 2.748 | 0.9001 | |
| miR-103a-3p vs miR-532-5p | -7.13 | 0.3314 | |
| 5sRNA vs RNU6 | -7.13 | 0.2867 | 0.87 |
| 5sRNA vs miR-103a-3p | -9.857 | 1.315 | |
| 5sRNA vs RNU1A1 | -3.152 | 0.4164 | |
| 5sRNA vs SNORD44 | -7.108 | 0.7169 | |
| 5sRNA vs miR-532-5p | -16.99 | 1.629 | |
| RNU1A1 vs RNU6 | -3.98 | 0.3964 | 1.08 |
| RNU1A1 vs miR-103a-3p | -6.708 | 1.586 | |
| RNU1A1 vs 5sRNA | 3.152 | 0.4164 | |
| RNU1A1 vs SNORD44 | -3.957 | 1.093 | |
| RNU1A1 vs miR-532-5p | -13.84 | 1.91 | |
| SNORD44 vs RNU6 | -0.02167 | 0.7805 | 0.93 |
| SNORD44 vs miR-103a-3p | -2.748 | 0.9001 | |
| SNORD44 vs 5sRNA | 7.108 | 0.7169 | |
| SNORD44 vs RNU1A1 | 3.957 | 1.093 | |
| SNORD44 vs miR-532-5p | -9.882 | 1.151 | |
| miR-532-5p vs RNU6 | 9.857 | 1.668 | 1.34 |
| miR-532-5p vs miR-103a-3p | 7.13 | 0.3314 | |
| miR-532-5p vs 5sRNA | 16.99 | 1.629 | |
| miR-532-5p vs RNU1A1 | 13.84 | 1.91 | |
| miR-532-5p vs SNORD44 | 9.882 | 1.151 | |

PBMC: peripheral blood mononuclear cells; HC: healthy control; STD: standard deviation.

**Supplementary Table 7 RefFinder ranking of PDX ALL samples and HCs of the validation cohort.**

| | Rank | | | | |
|---|---|---|---|---|---|
| **miRNA reference** | **RefFinder** | **Normfinder [Stability Value]** | **BestKeeper [STD]** | **geNorm [Stability Value]** | **ΔCT [Means of STD]** |
| miR-103a-3p | 1 | 0.917 | 0.848 | 0.952* | 1.722 |
| 5sRNA | 2 | 1.153 | 0.905 | 1.7 | 1.882 |
| miR-532-5p | 3 | 1.801 | 1.106 | 0.952* | 2.152 |
| SNORD44 | 4 | 1.491 | 1.484 | 1.473 | 2.067 |
| RNU1A1 | 5 | 1.404 | 1.808 | 1.895 | 1.936 |
| RNU6 | 6 | 1.824 | 2.224 | 1.99 | 2.18 |

*Best performance in data normalization using miR-103a-3p and miR-532-5p in combination according to the geNorm algorithm. PDX: patient-derived xenograft; ALL: acute lymphoblastic leukemia; HCs: healthy controls; STD: standard deviation.

**Supplementary Table 8 CT values of primary patient material and corresponding BM-derived PDX ALL sample.**

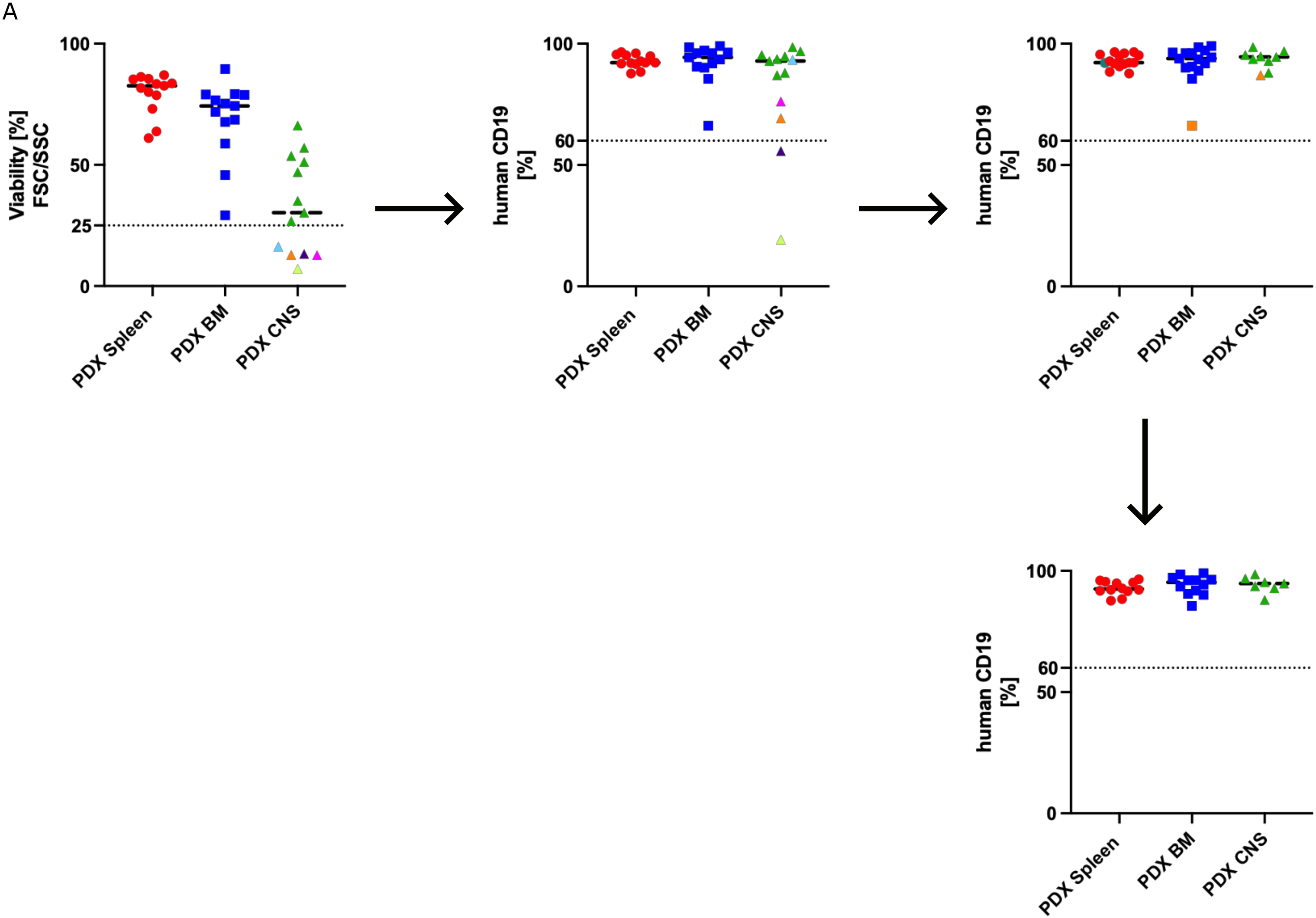
| Patient | PDX | Patient | PDX | Patient | PDX | Patient | PDX |
|---|---|---|---|---|---|---|---|
| | | miR-532-5p | | miR103a-3p | | RNU6 | |
| P1 | X002 | 31.94 | 27.56 | 23.89 | 22.13 | 17.61 | 24.91 |
| P2 | X005 | 36.89 | 30.14 | 26.87 | 24.76 | 20.13 | 24.72 |
| P3 | X006 | 36.29 | 31.22 | 27.18 | 24.18 | 19.33 | 19.69 |
| P4 | X007 | 36.71 | 28.43 | 29.88 | 22.95 | 20.72 | 24.72 |
| P5 | X008 | 31.32 | 27.75 | 23.67 | 18.41 | 20.53 | 23.74 |
| P6 | X009 | 34.60 | 28.46 | 25.34 | 22.50 | 21.61 | 26.35 |
| P7 | X011 | 31.76 | 29.83 | 22.91 | 24.11 | 18.25 | 27.49 |
| P8 | X013 | 31.49 | 29.16 | 23.28 | 24.47 | 18.49 | 26.15 |
| P9 | X015 | 31.24 | 27.93 | 23.64 | 23.20 | 18.29 | 24.17 |
| P10 | X016 | 37.57 | 28.86 | 26.92 | 23.44 | 21.19 | 25.6 |
| P11 | X018 | 35.50 | 29.57 | 25.81 | 25.45 | 20.07 | 23.23 |
| P12 | X020 | 29.32 | 31.55 | 22.23 | 26.79 | 16.21 | 27.22 |
| P13 | X021 | 28.92 | 27.89 | 22.26 | 24.06 | 18.42 | 21.53 |
| P14 | X022 | 31.28 | 29.18 | 25.79 | 26.02 | 27.18 | 27.46 |
| P15 | Validation X001 | 33.41 | 32.78 | 23.86 | 22.20 | 18.72 | 14.66 |
| P16 | Validation X002 | 31.04 | 31.72 | 23.35 | 23.78 | 17.06 | 16.72 |
| P17 | Validation X004 | 32.78 | 32.73 | 25.97 | 24.29 | 24.19 | 17.71 |
| P18 | Validation X006 | 30.66 | 32.70 | 22.82 | 24.48 | 18.55 | 17.17 |
| P19 | Validation X008 | 34.69 | 32.11 | 24.46 | 24.01 | 20.53 | 18.16 |
| P20 | Validation X010 | 31.40 | 32.19 | 23.86 | 24.11 | 20.68 | 16.58 |
| P21 | Validation X011 | 30.61 | 30.66 | 22.75 | 22.68 | 19.29 | 19.73 |
| P22 | Validation X012 | 32.82 | 32.67 | 23.47 | 24.47 | 16.27 | 23.73 |
| P23 | Validation X013 | 30.34 | 32.49 | 22.29 | 24.65 | 18.54 | 23.70 |

| Patient | PDX | Patient | PDX | Patient | PDX | Patient | PDX |
|---|---|---|---|---|---|---|---|
| | | 5sRNA | | RNU1A1 | | SNORD44 | |
| P1 | X002 | 12.23 | 19.17 | 14.09 | 20.67 | 21.67 | 22.33 |
| P2 | X005 | 16.46 | 10.64 | 18.59 | 16.93 | 20.75 | 26.57 |
| P3 | X006 | 14.59 | 16.87 | 15.85 | 19.93 | 20.04 | 18.95 |
| P4 | X007 | 16.50 | 14.75 | 18.13 | 18.99 | 21.49 | 23.12 |
| P5 | X008 | 12.48 | 18.07 | 17.28 | 20.52 | 22.15 | 21.56 |
| P6 | X009 | 15.55 | 20.34 | 19.22 | 22.65 | 22.72 | 22.93 |
| P7 | X011 | 11.66 | 14.63 | 13.85 | 17.97 | 19.92 | 22.05 |
| P8 | X013 | 13.22 | 14.24 | 15.45 | 18.5 | 20.81 | 26.34 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| P9 | X015 | 12.61 | 14.84 | 14.58 | 18.69 | 20.83 | 25.65 |
| P10 | X016 | 17.49 | 11.05 | 19.84 | 19.19 | 20.41 | 23.89 |
| P11 | X018 | 18.44 | 17.72 | 17.55 | 17.86 | 20.99 | 21.93 |
| P12 | X020 | 10.58 | 11.48 | 13.27 | 19.74 | 18.46 | 31.19 |
| P13 | X021 | 13.87 | 15.69 | 17.57 | 19.27 | 19.56 | 20.31 |
| P14 | X022 | 11.83 | 16.82 | 19.52 | 24.97 | 26.48 | 26.64 |
| P15 | Validation X001 | 12.96 | 10.13 | 15.06 | 11.56 | 21.04 | 20.04 |
| P16 | Validation X002 | 11.72 | 10.26 | 13.77 | 12.19 | 19.42 | 21.01 |
| P17 | Validation X004 | 11.53 | 10.82 | 18.74 | 13.55 | 23.75 | 22.64 |
| P18 | Validation X006 | 12.21 | 11.91 | 14.94 | 14.09 | 20.58 | 22.71 |
| P19 | Validation X008 | 12.30 | 11.63 | 16.02 | 14.18 | 23.01 | 22.10 |
| P20 | Validation X010 | 13.04 | 11.34 | 16.99 | 13.27 | 22.12 | 21.48 |
| P21 | Validation X011 | 12.35 | 12.08 | 15.22 | 14.22 | 20.89 | 22.22 |
| P22 | Validation X012 | 9.91 | 12.84 | 11.69 | 19.37 | 19.92 | 23.14 |
| P23 | Validation X013 | 11.99 | 10.69 | 14.84 | 16.66 | 19.97 | 25.69 |

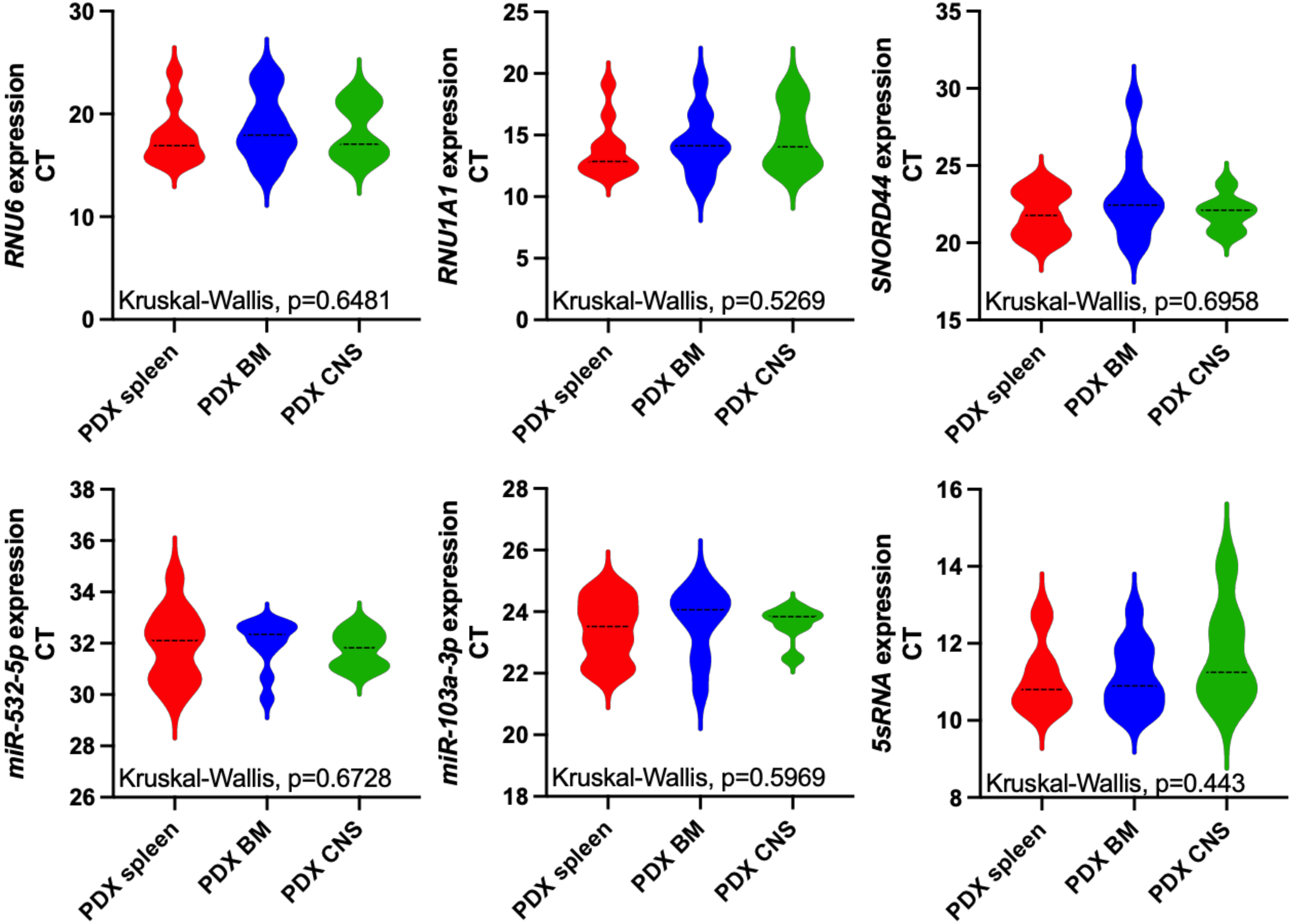
PDX: Patient-derived xenograft; ALL: acute lymphoblastic leukemia; BM: bone marrow.

# Supplementary Figure 1



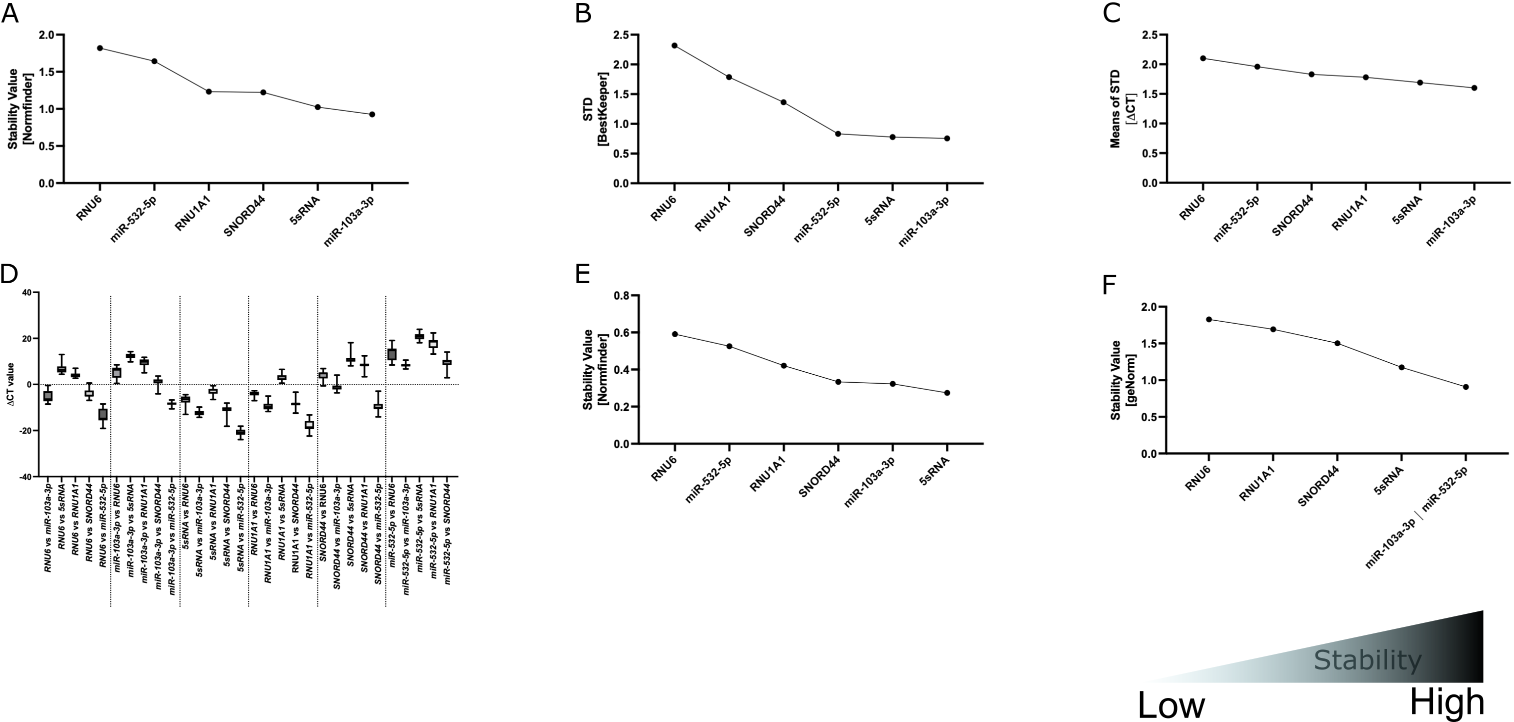
**Supplementary Figure 1 PDX sample selection of the validation cohort.** 13 PDX ALL samples (>25% viability and >60% human CD19; same coloring of excluded samples) were chosen for RNA extraction. One spleen- (green), BM- (brown), and CNS-derived (brown) sample each were excluded due to poor RNA quality (below right), leaving 12 BM-, and spleen-, and 7 CNS-derived ALL samples for further analyses. PDX: patient-derived xenograft; ALL: acute lymphoblastic leukemia; FSC/SSC: forward scatter/side scatter; BM: bone marrow; CNS: central nervous system.

**Supplementary Figure 2**



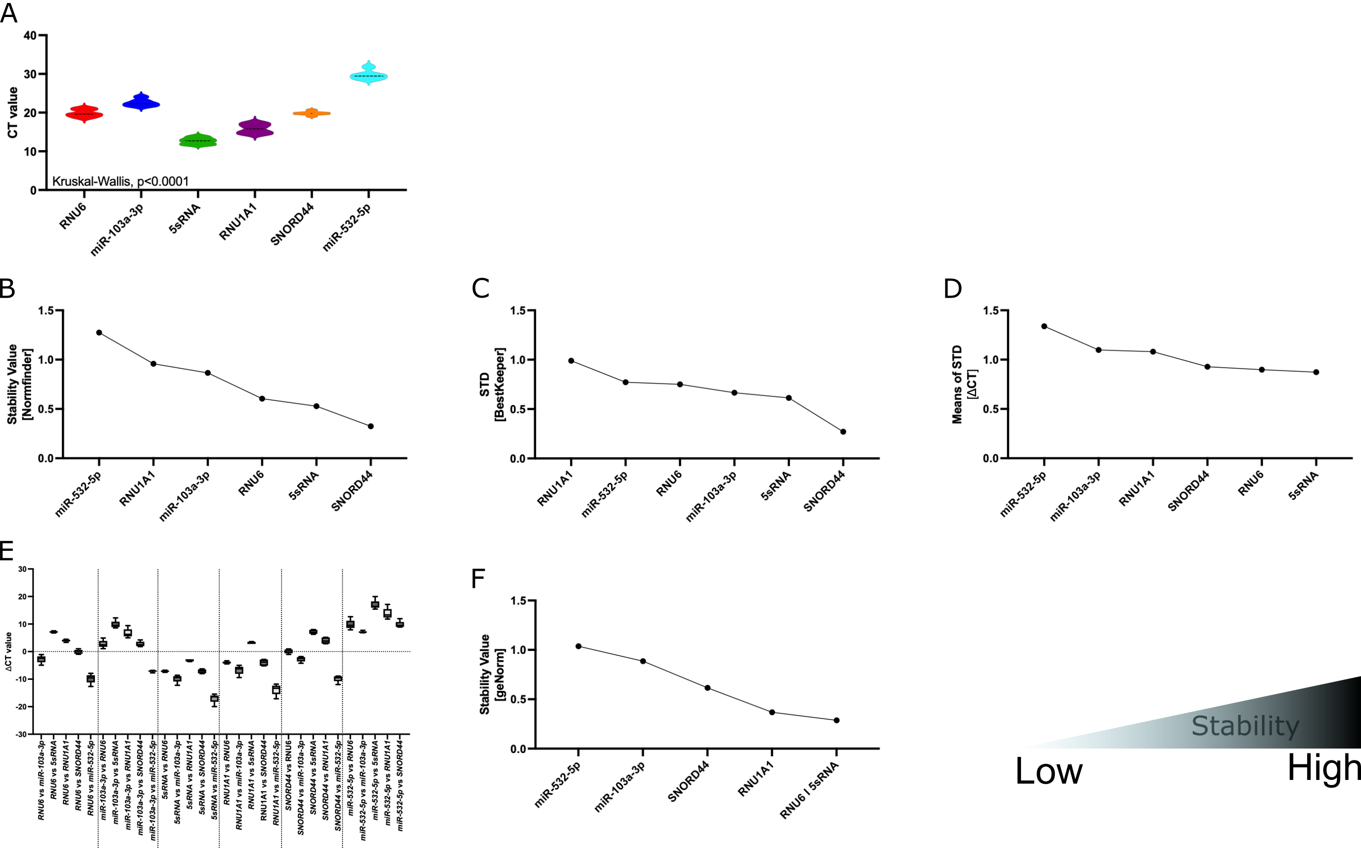
**Supplementary Figure 2 Expression of miRNA references in PDX ALL specimens of the validation cohort.** The expression of RNU6, RNU1A1, SNORD44, miR-532-5p, miR-103a-3p and 5sRNA were assessed in PDX ALL samples derived from spleen (n=12), BM (n=12), or CNS (n=7) of the validation cohort. The Kruskal-Wallis test was used to determine whether the median CT values of miRNA reference controls differ across ALL sample groups. PDX: patient-derived xenograft; ALL: acute lymphoblastic leukemia; BM: bone marrow; CNS: central nervous system.

# Supplementary Figure 3



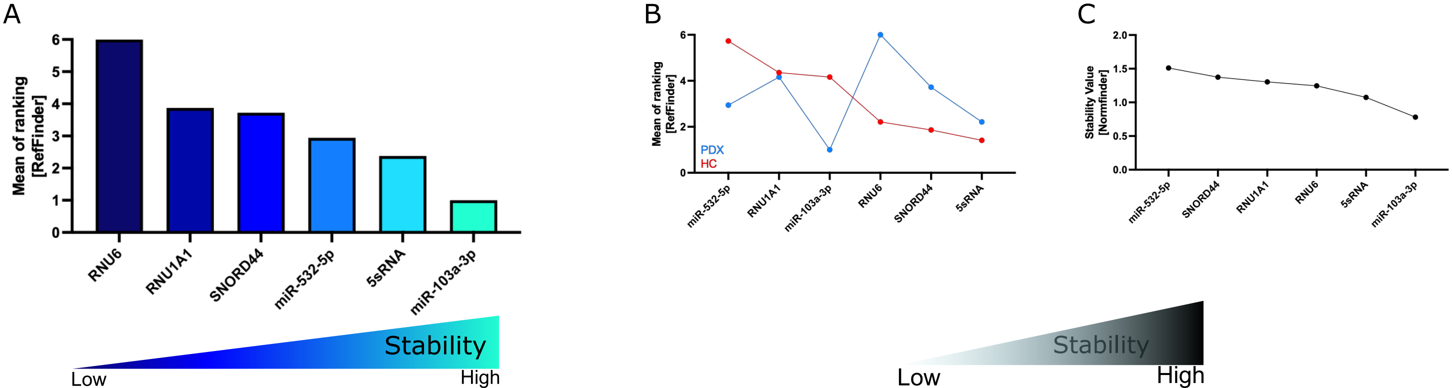
**Supplementary Figure 3 Stability of miRNA references in PDX ALL samples of the validation cohort.** The Normfinder (A; samples clustered in 1 group), BestKeeper (B), ∆CT (C and D), Normfinder applying grouping to the validation cohort (3 groups: spleen, BM, CNS; E), and geNorm algorithm (F) were used to assess reference stability within the validation cohort. For A-C, E, and F: decreasing values indicate increasing stability. E: ∆CT calculations based on CT[reference 1] – CT[reference 2]; mean CT values with min to max whiskers are shown. PDX: patient-derived xenograft; ALL: acute lymphoblastic leukemia; BM: bone marrow, CNS: central nervous system.

# Supplementary Figure 4



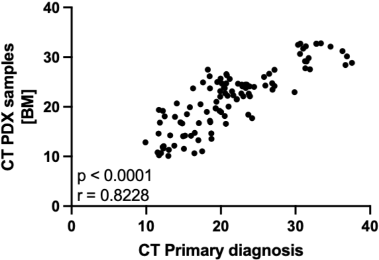
**Supplementary Figure 4 Expression and stability of miRNA references in healthy controls of the validation cohort.** (A) CT values of miRNA references within the validation cohort of healthy controls. The Normfinder (B), BestKeeper (C), ∆CT (D and E), or geNorm algorithm (F) were applied to assess gene stability. For A-D and F: decreasing values indicate increasing stability. E: ∆CT calculations based on CT[reference 1] – CT[reference 2]; mean CT values with min to max whiskers are shown.

# Supplementary Figure 5



**Supplementary Figure 5 Influence of sample type on miRNA reference stability in the validation cohort.** (A) RefFinder values (mean of ranking; according to the Normfinder, BestKeeper, geNorm, and ΔCT algorithms) of the combined cohort of HCs and PDX ALL samples of the validation cohort (PDX ALL samples including spleen, BM, and CNS). (B) RefFinder values of PDX ALL specimens and HCs are depicted in Figure 10 and Figure 11 in a combined graph. (C) Normfinder results applying grouping (HCs and PDX ALL specimens) to the combined samples of the validation cohort. Decreasing values indicate increasing stability. BCP ALL: B-cell precursor acute lymphoblastic leukemia; PDX: patient-derived xenograft; HC: healthy control.

# Supplementary Figure 6



**Supplementary Figure 6 Correlation of miRNA reference expression between PDX ALL samples and primary patient material.** Primary patient material used to establish the mouse models was available for 23 of the 35 PDX ALL samples analyzed in this study. MiRNA reference expression of these samples was assessed and correlated to the expression levels of corresponding PDX ALL samples derived from BM. Spearman correlation. PDX: patient-derived xenograft; ALL: acute lymphoblastic leukemia; BM: bone marrow.