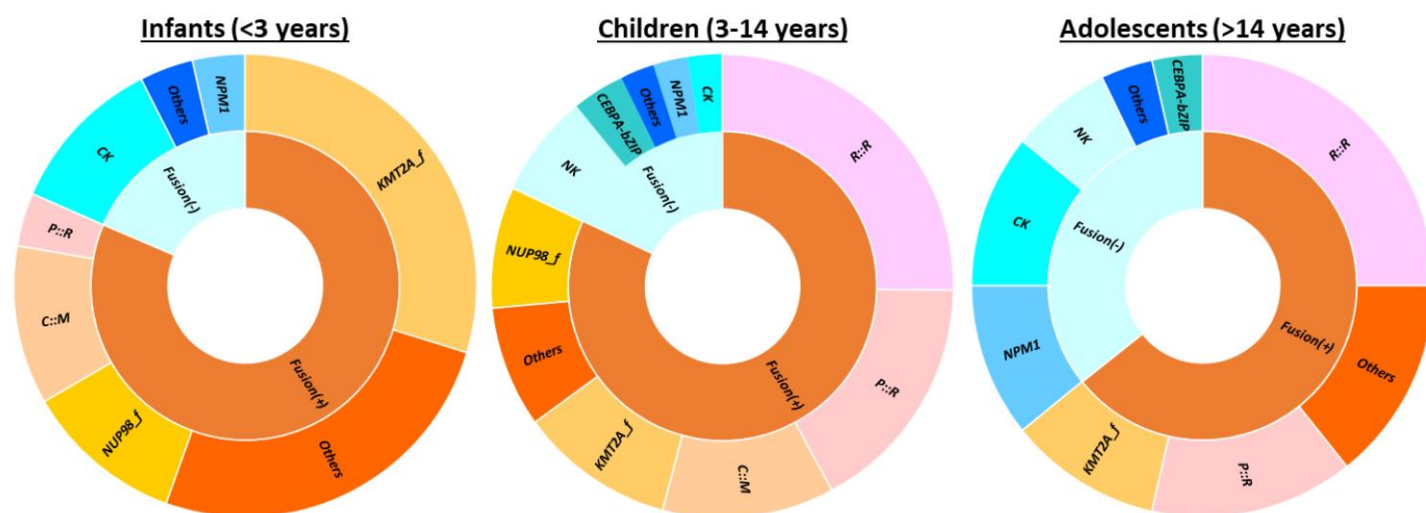
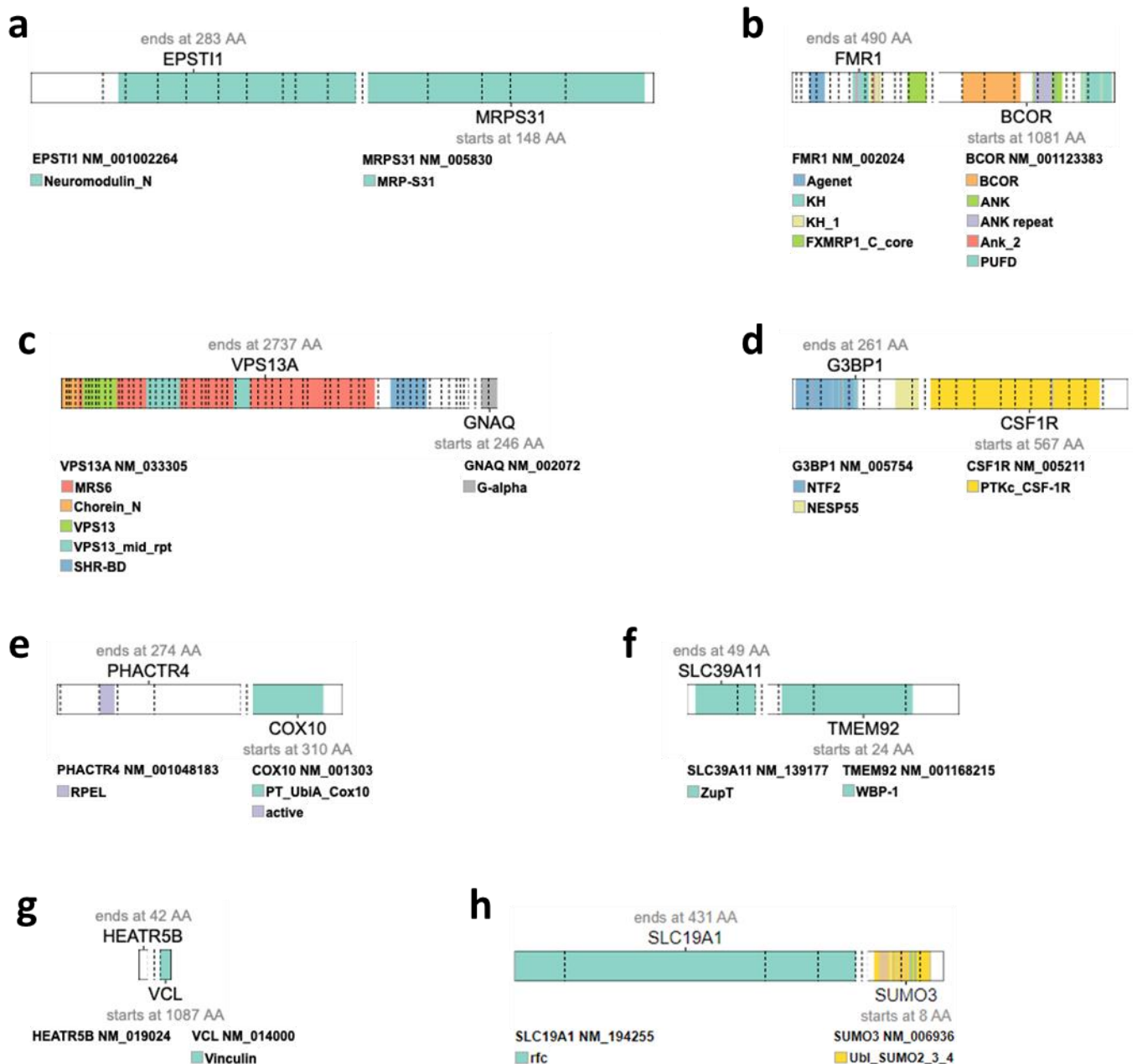


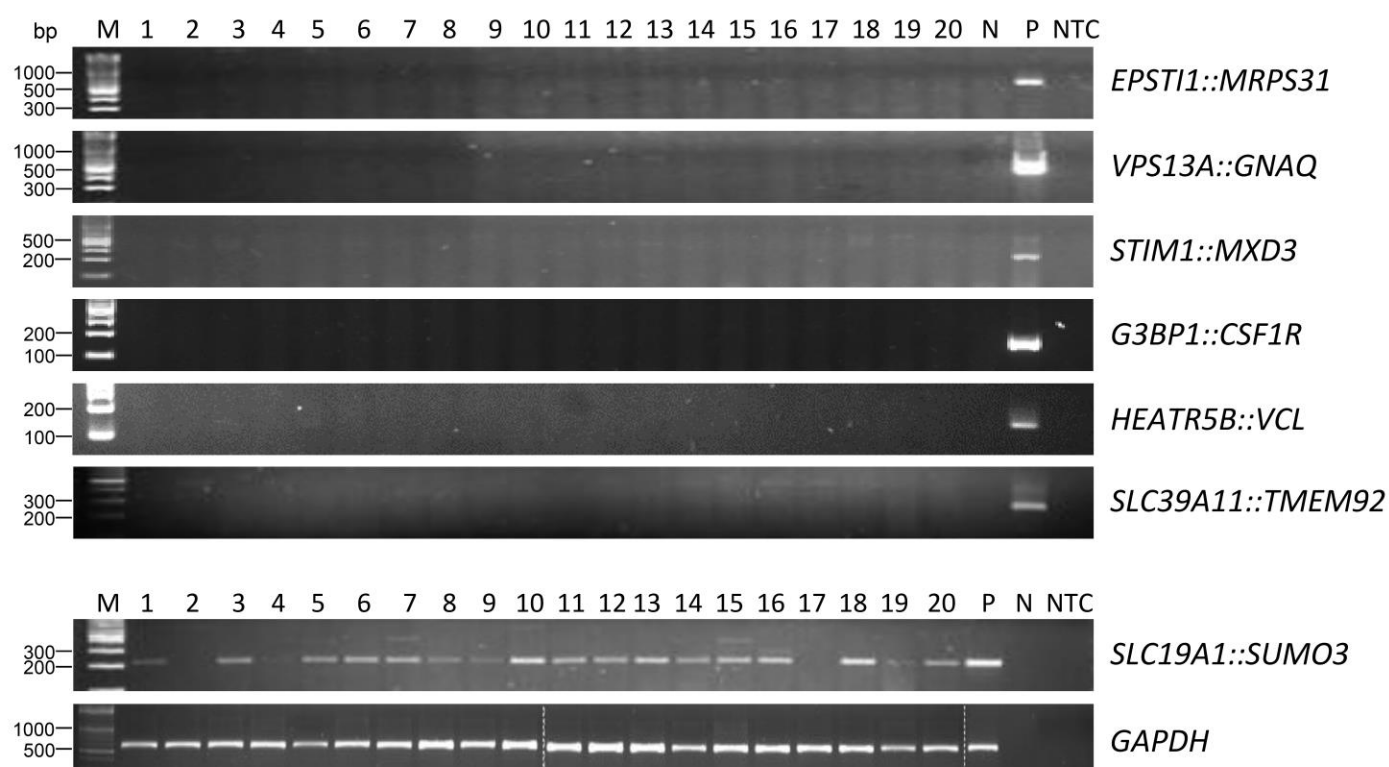


|           | Molecular/cytogenetic features | No. of infants (n=27) | %   | No. of children (n=83) | %   | No. of adolescents (n=28) | %   | P-value | Adjusted P-value |
|-----------|--------------------------------|-----------------------|-----|------------------------|-----|---------------------------|-----|---------|------------------|
| Fusion(-) | CK                             | 3                     | 11% | 2                      | 2%  | 3                         | 11% | 0.062   | 0.347            |
|           | CEBPA-bZIP                     | 0                     | 0%  | 3                      | 4%  | 1                         | 4%  | 0.822   | 0.904            |
|           | NPM1                           | 1                     | 4%  | 2                      | 2%  | 3                         | 11% | 0.186   | 0.347            |
|           | NK                             | 0                     | 0%  | 6                      | 7%  | 2                         | 7%  | 0.463   | 0.566            |
|           | Others                         | 1                     | 4%  | 2                      | 2%  | 1                         | 4%  | 1.000   | 1.000            |
| Fusion(+) | R::R                           | 0                     | 0%  | 21                     | 25% | 7                         | 25% | 0.004   | <b>0.047</b>     |
|           | P::R                           | 1                     | 4%  | 14                     | 17% | 4                         | 14% | 0.252   | 0.347            |
|           | C::M                           | 3                     | 11% | 10                     | 12% | 0                         | 0%  | 0.133   | 0.347            |
|           | KMT2A_f                        | 8                     | 30% | 9                      | 11% | 3                         | 11% | 0.061   | 0.338            |
|           | NUP98_f                        | 3                     | 11% | 7                      | 8%  | 0                         | 0%  | 0.174   | 0.347            |
|           | Others                         | 7                     | 26% | 7                      | 8%  | 4                         | 14% | 0.065   | 0.347            |

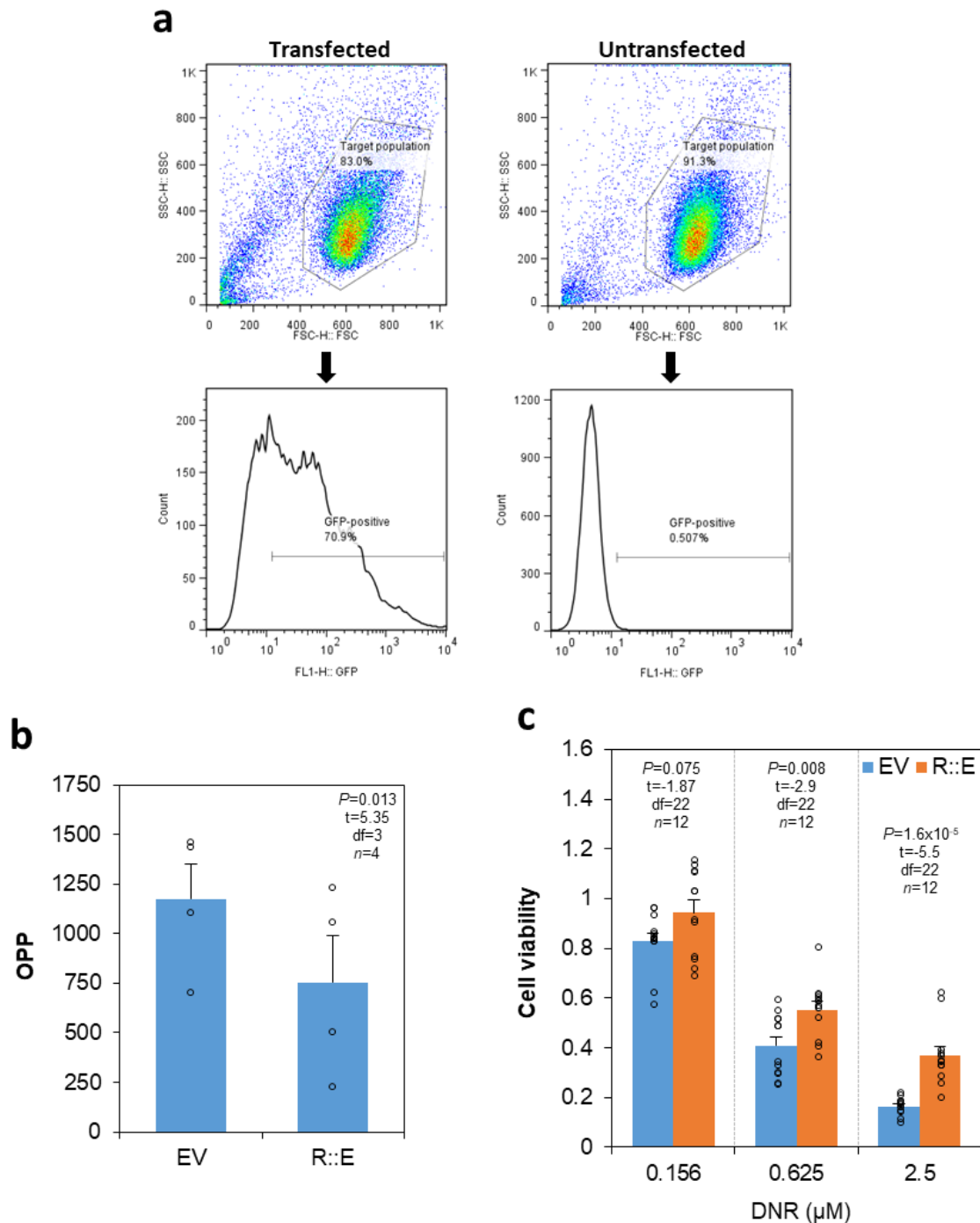
**Supplementary Fig. 1. Distribution of FGs across age groups.** Pediatric AML patients were stratified into three age groups (infants, children and adolescents) as previously described in the TARGET-AML study.<sup>1</sup> Only the 138 patients with suitable materials for FG studies were included in this analysis. The table below shows the number of patients with different molecular/cytogenetic features in each age group. *P*-values were calculated by the Fisher's exact test and corrected for multiple comparisons using the Benjamini-Hochberg method. CK, complex karyotype; CEBPA-bZIP, CEBPA-basic leucine zipper; NK, normal karyotype; R::R, RUNX1::RUNX1T1; P::R, PML::RARA; C::M, CBFB::MYH11; KMT2A\_f, KMT2A fusions; NUP98\_f, NUP98 fusions.



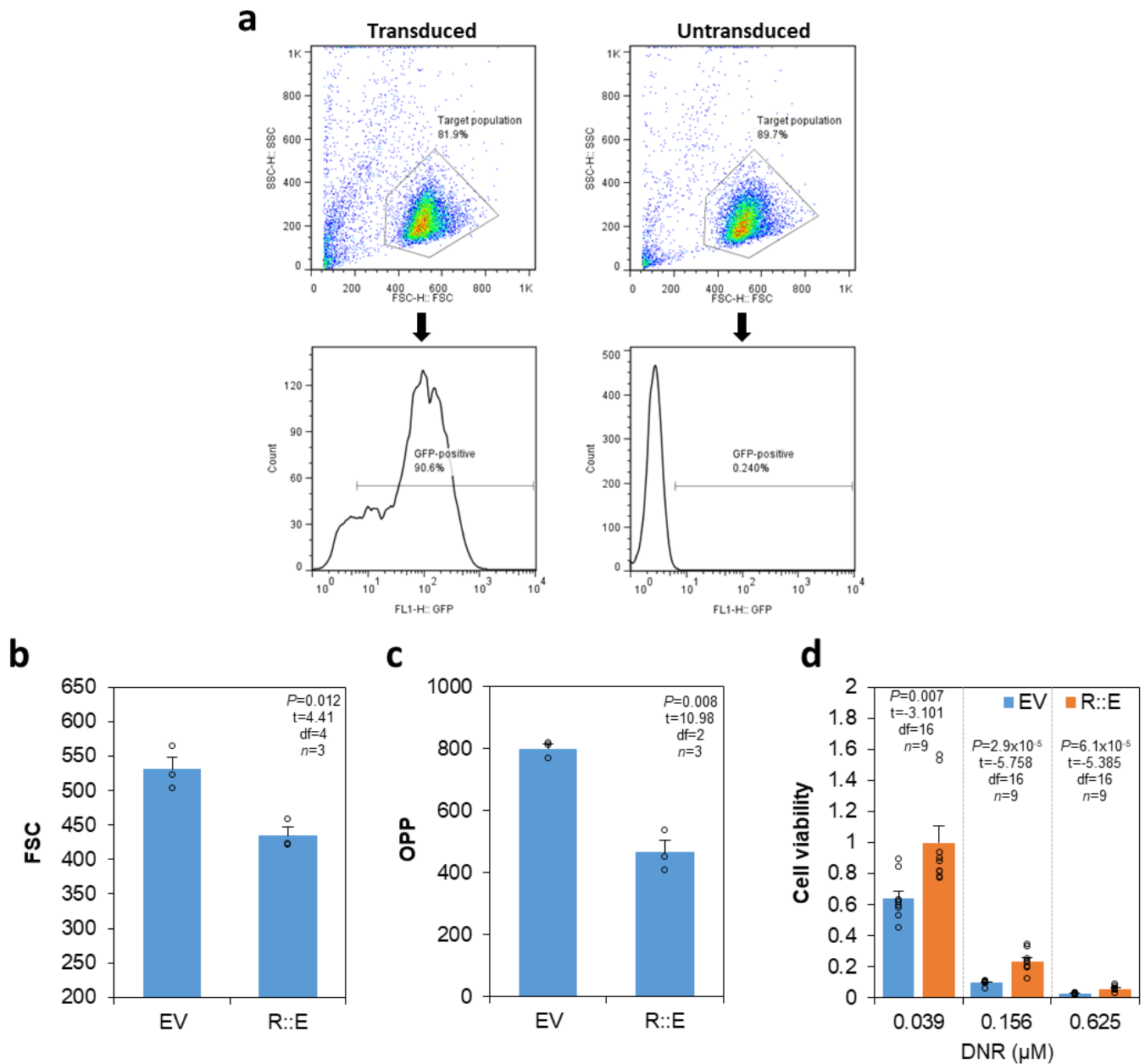
**Supplementary Fig. 2. Novel in-frame FGs identified in this study.** Schematic diagrams of FGs generated by ProteinPaint.<sup>2</sup> **a** EPSTI1::MRPS31. **b** FMR1::BCOR. **c** VPS13A::GNAQ. **d** G3BP1::CSF1R. **e** PHACTR4::COX10. **f** SLC39A11::TMEM92. **g** HEATR5B::VCL. **h** SLC19A1::SUMO3. Protein domains present in the fusion proteins are shown. The novel STIM1::MXD3, STIM1::F12 and RUNX1::ERG are shown in Fig. 1d and 2a.



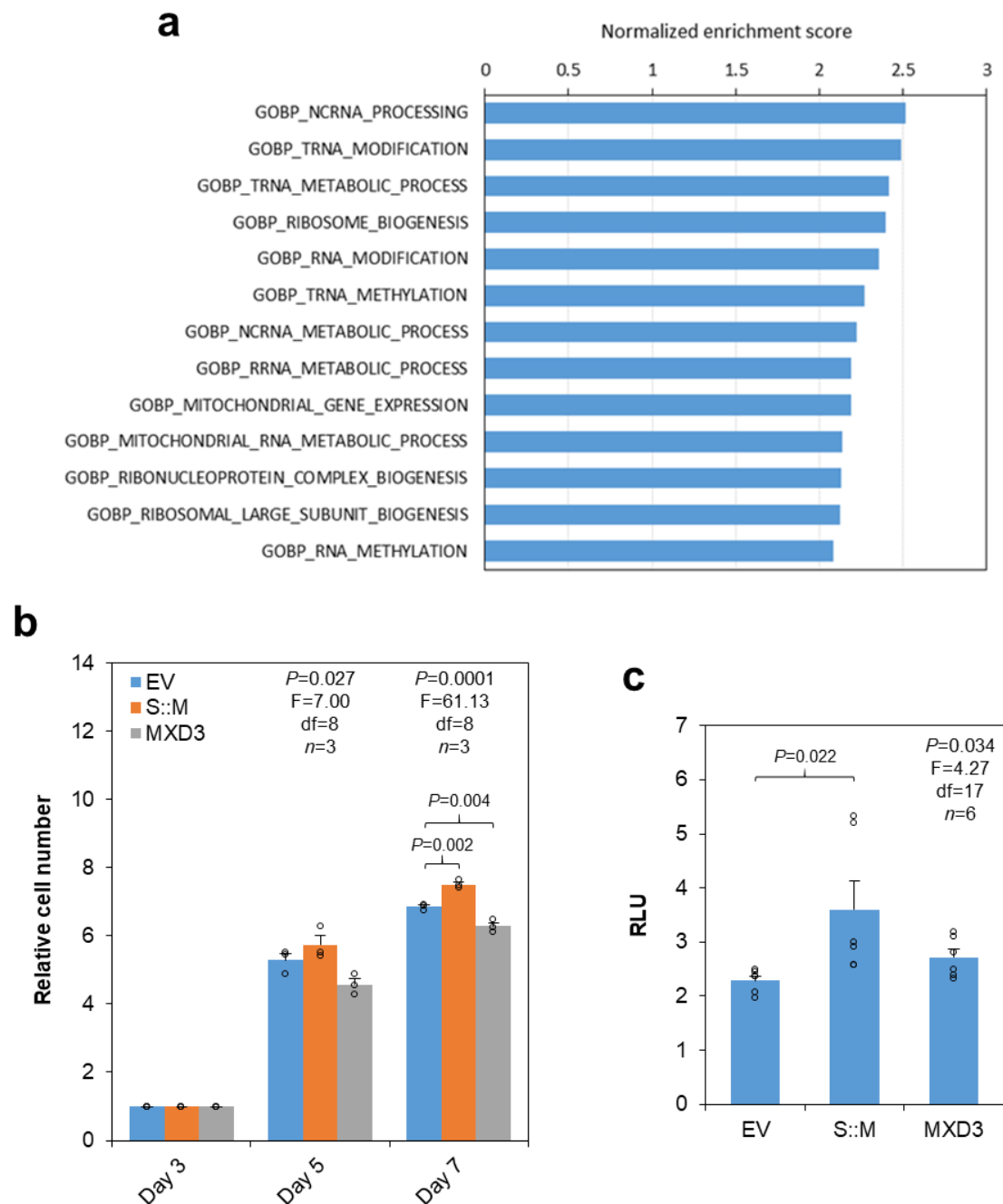
**Supplementary Fig. 3. Expression of novel FGs in normocellular bone marrow samples.** Representative RT-PCR results of seven novel FGs in 20 normocellular bone marrow samples (1-20) from individuals without a prior hematological malignancy. *GAPDH* served as the internal control. Dotted lines indicate merged lanes from the same gel. Lanes were merged to show the 20 bone marrow samples with adequate RNA quality as indicated by noticeable expression of *GAPDH*. M, size marker; N, no reverse transcriptase control; P, the patient sample positive for the FG; NTC, no template control.



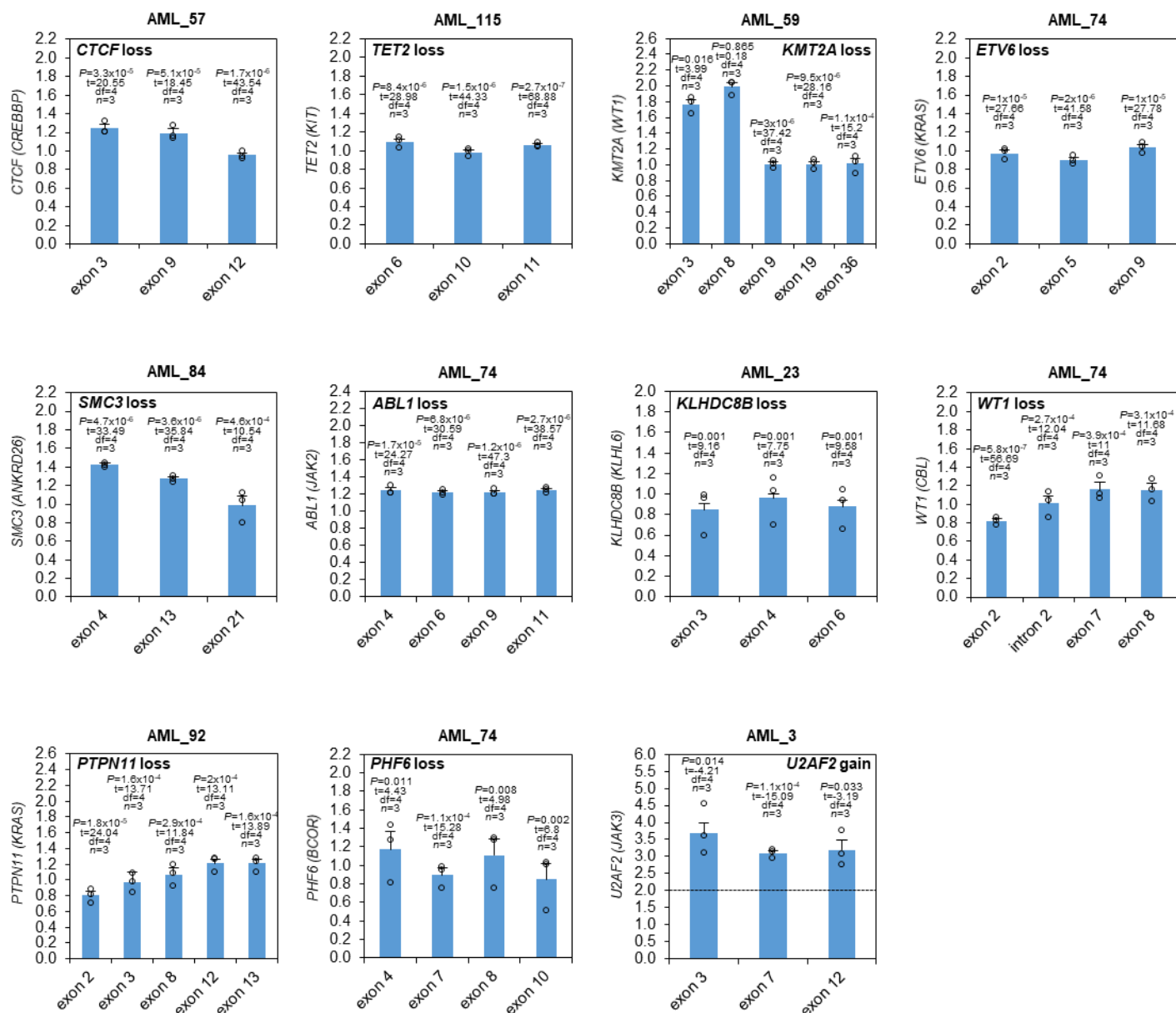
**Supplementary Fig. 4. Overexpression of RUNX1::ERG inhibited protein synthesis and conferred chemotherapy resistance.** K562 cells were transfected with LeGO-iG2-RUNX1::ERG (R::E) or the empty LeGO-iG2 (EV). **a** Gating strategy for flow cytometric analysis of transfected K562 cells. Target cell population was first gated, and green fluorescent protein (GFP)-positive cells were identified by parallel analysis of untransfected cells. The GFP-positive (transfected) cells were then analysed for the desired functional properties. **b** Incorporation of O-propargyl-puromycin (OPP) (median fluorescence intensity) into newly translated proteins in the transfected cells was measured after 72 hours of the transfection. **c** After 72 hours of transfection, cells were treated with the indicated concentrations of daunorubicin (DNR) for 48 hours and viability was measured with the CellTiter-Glo assay. Cell viability was relative to the vehicle-treated control. In panels **b** and **c**, data are expressed as mean  $\pm$  SE from four independent experiments. The number of values used to calculate the statistics (*t* test) in each group is indicated.



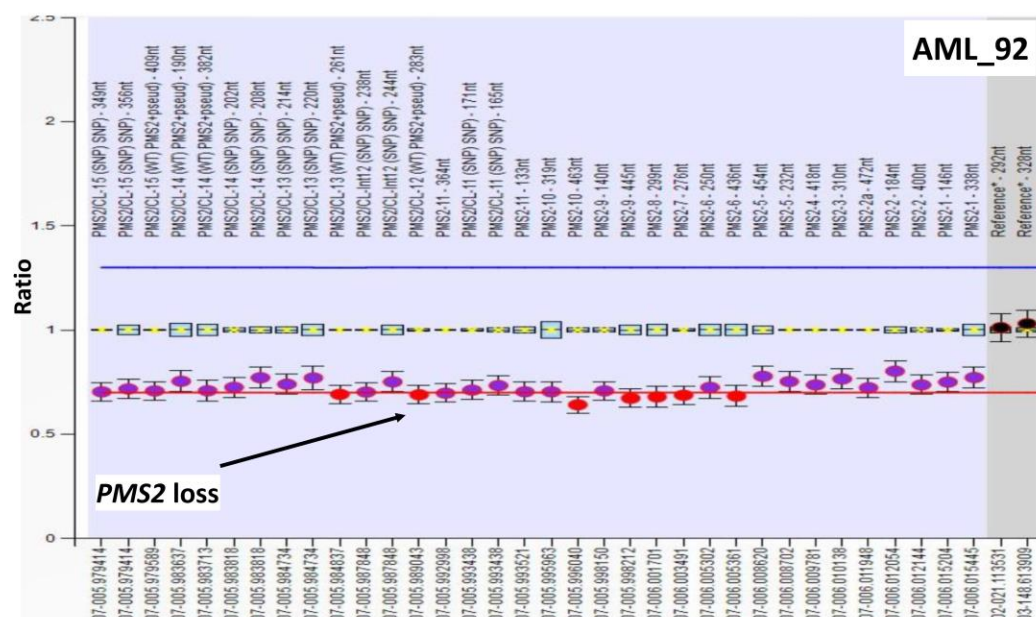
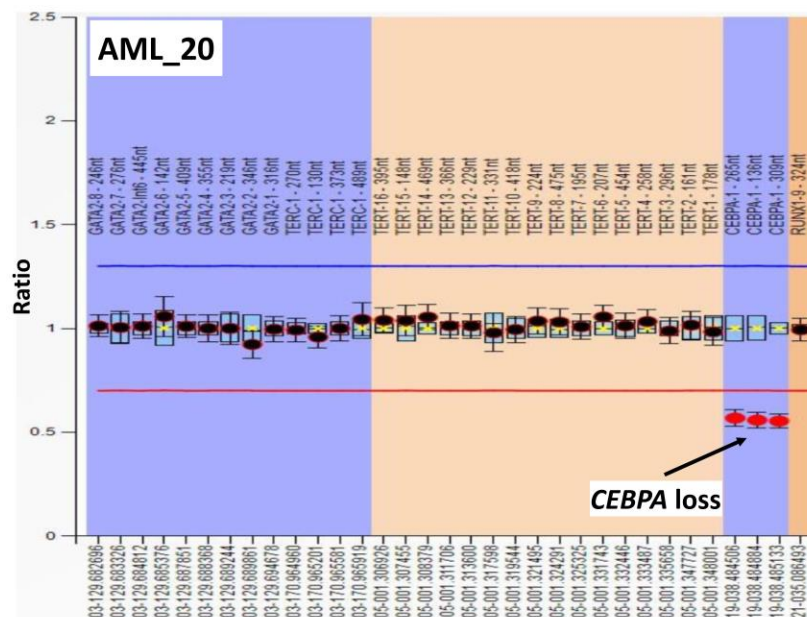
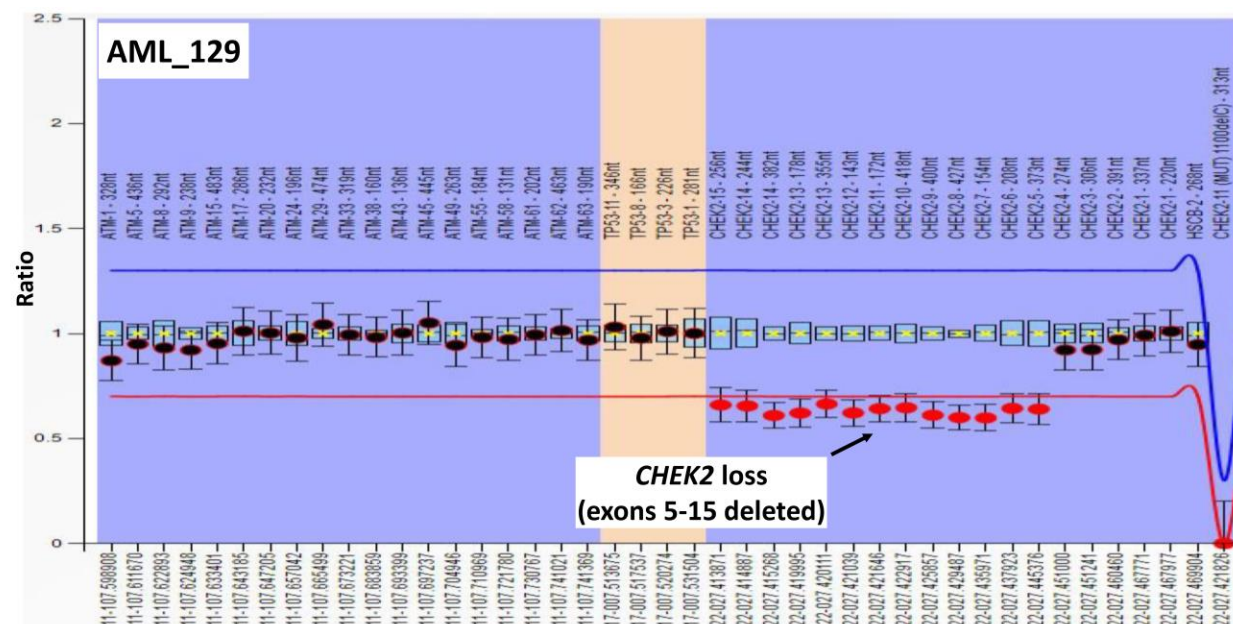
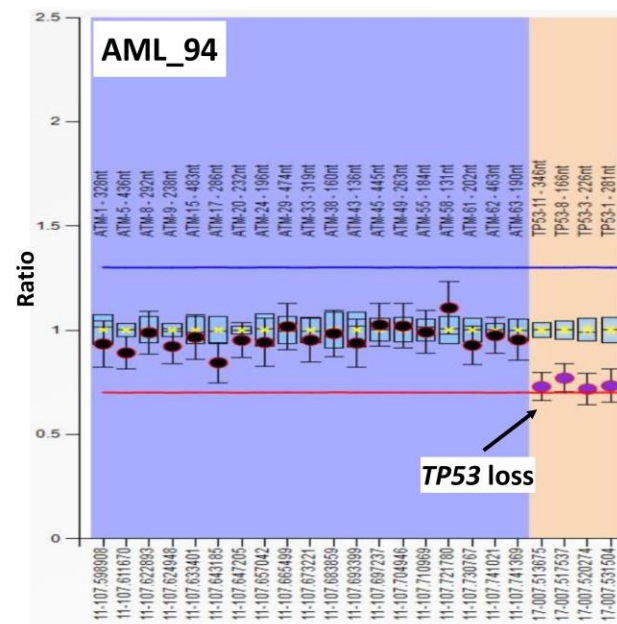
**Supplementary Fig. 5. Effects of RUNX1::ERG overexpression on U937 myeloid leukemia cells.** U937 cells were transduced with RUNX1::ERG (R::E) or the empty vector (EV) lentivirus at a multiplicity of infection of 4. **a** Gating strategy for flow cytometric analysis of transduced U937 cells. Target cell population was first gated, and green fluorescent protein (GFP)-positive cells were identified by parallel analysis of untransduced cells. The GFP-positive (transduced) cells were then analysed for the desired functional properties. Cell size (**b**) and incorporation of O-propargyl-puromycin (OPP) into newly translated proteins (**c**) in the transduced cells were measured after 96 hours of the transduction. The median fluorescence intensity of forward size scatter (FSC) and OPP signal are shown. **d** After 96 hours of transduction, cells were treated with the indicated concentrations of daunorubicin (DNR) for 48 hours and viability was measured with the CellTiter-Glo assay. Cell viability was relative to the vehicle-treated control. In panels **b-d**, data are expressed as mean  $\pm$  SE from three independent experiments. The number of values used to calculate the statistics (*t* test) in each group is indicated.



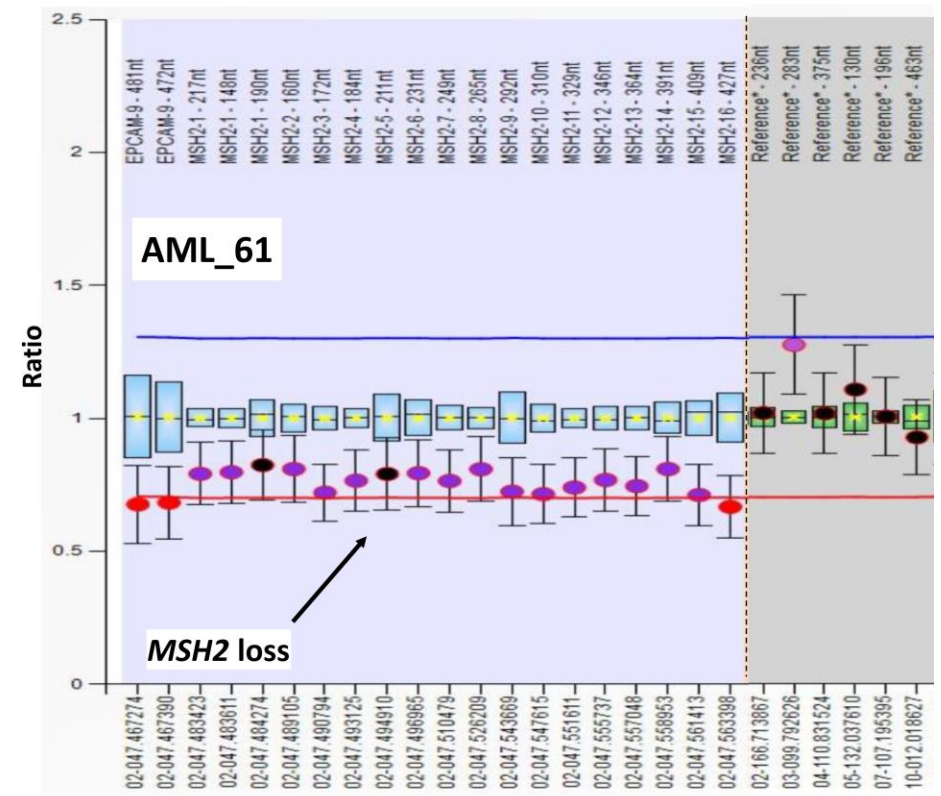
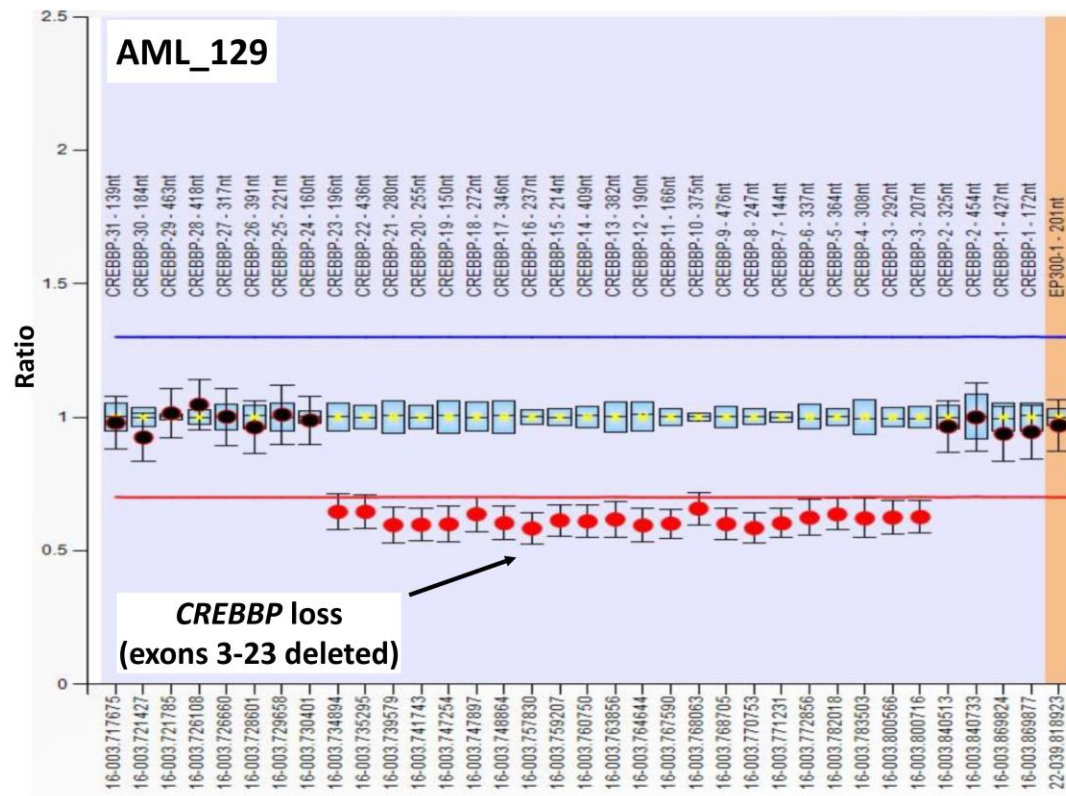
**Supplementary Fig. 6. Differential biological properties of STIM1::MXD3.** **a** GSEA comparing K562 cells transfected with pCMV-HA plasmid expressing STIM1::MXD3, MXD3 or the empty vector (EV). Two independent transfections and transcriptome sequencing were performed for each of the group. Pre-ranked gene lists were generated by comparing STIM1::MXD3 or MXD3 with the EV group, and the Gene Ontology biological process gene sets were analysed. Gene sets that were significantly (FDR<0.05 and FWER-adjusted  $P$ -value<0.05) associated with STIM1::MXD3 but not MXD3 are shown. **b** Effects of STIM1::MXD3 (S::M) and MXD3 overexpression on K562 cell proliferation as assessed by trypan blue cell counting. Cell number was relative to the 72-hour post-transfection time point. **c** Effects of STIM1::MXD3 and MXD3 overexpression on K562 cell viability as determined by CellTiter-Glo assays. Luminescence signal was measured 7 days post-transfection and relative to the 72-hour post-transfection time point. RLU, relative luminescence. Data in **b** and **c** are expressed as mean  $\pm$  SE from 3 independent experiments. The number of values used to calculate the statistics (One-way ANOVA followed by Dunnett's test) in each group is indicated.



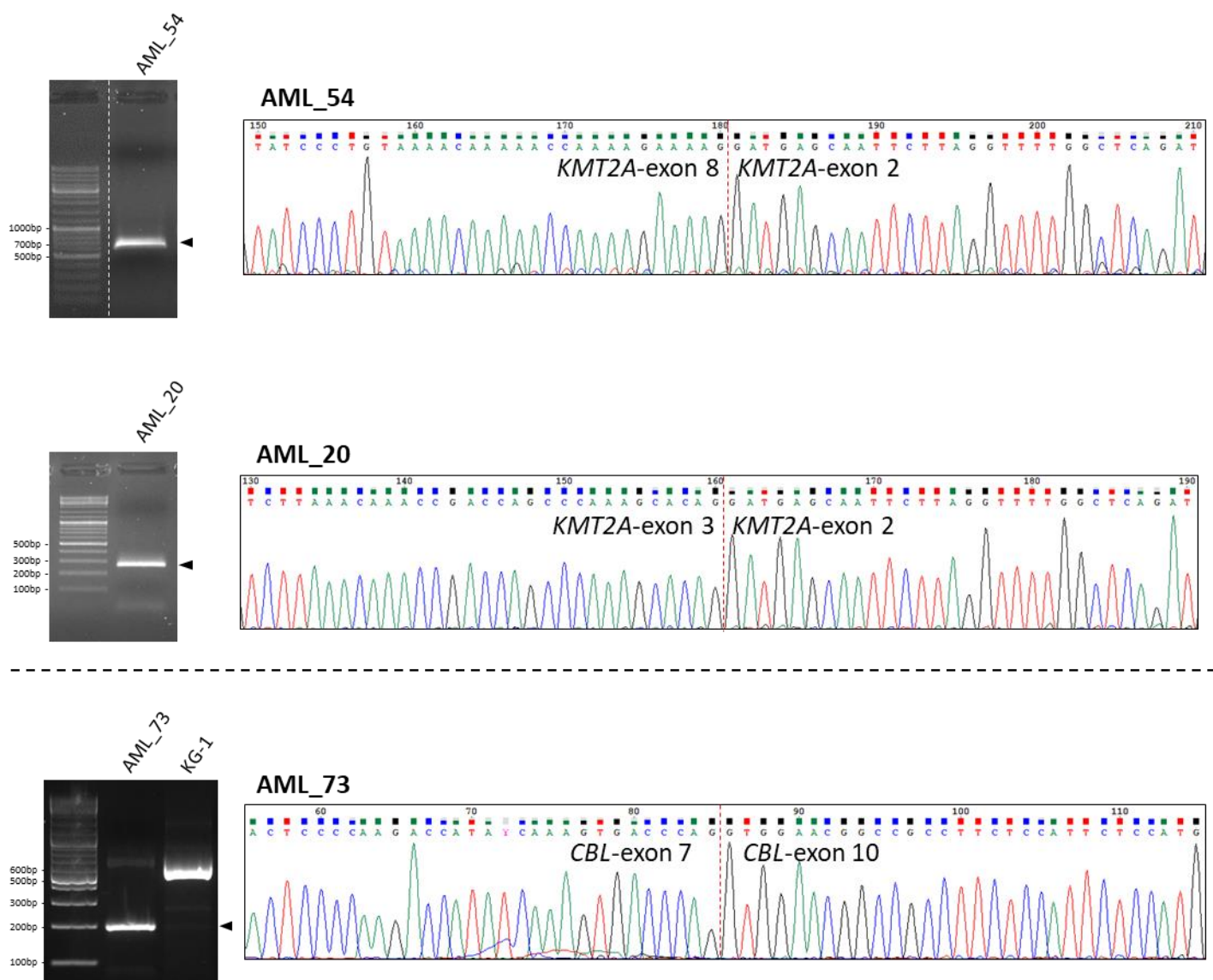
**Supplementary Fig. 7. Validation of copy number alterations by qPCR.** At least three different genomic regions of the involved genes were analysed and the results were normalized to another gene region (*bracketed*) of the same chromosome without copy number changes. Gene copy numbers were compared to those obtained from normal peripheral blood samples ( $n=3$ ) used in quandico/CNVkit analysis. All qPCR measurements were performed in triplicates. Data are expressed as mean  $\pm$  SE and the  $t$  test was used for statistical analysis. Representative results are shown.



Supplementary Fig. 8 continued

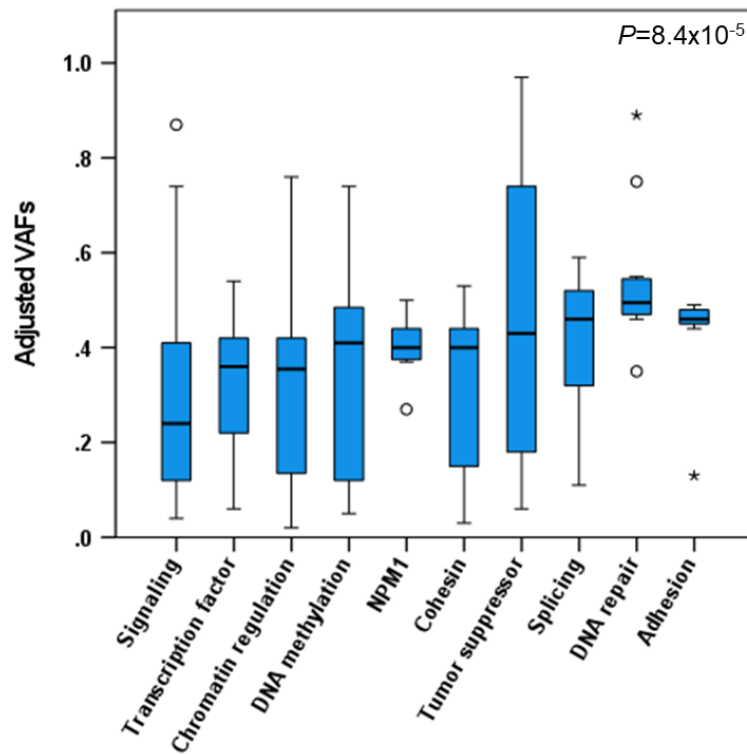


**Supplementary Fig. 8. Validation of copy number alterations by MLPA.** Representative MLPA results showing focal gene deletions in different patient samples. Exon numbers and the normalized probe ratios are shown above the charts and on the y-axis, respectively. Red dots indicate losses, while purple dots indicate significantly decreased signals compared to the reference control samples ( $n=3$ ). Black dots indicate no copy number changes. Error bars indicate the 95% confidence intervals of the probes' ratio.



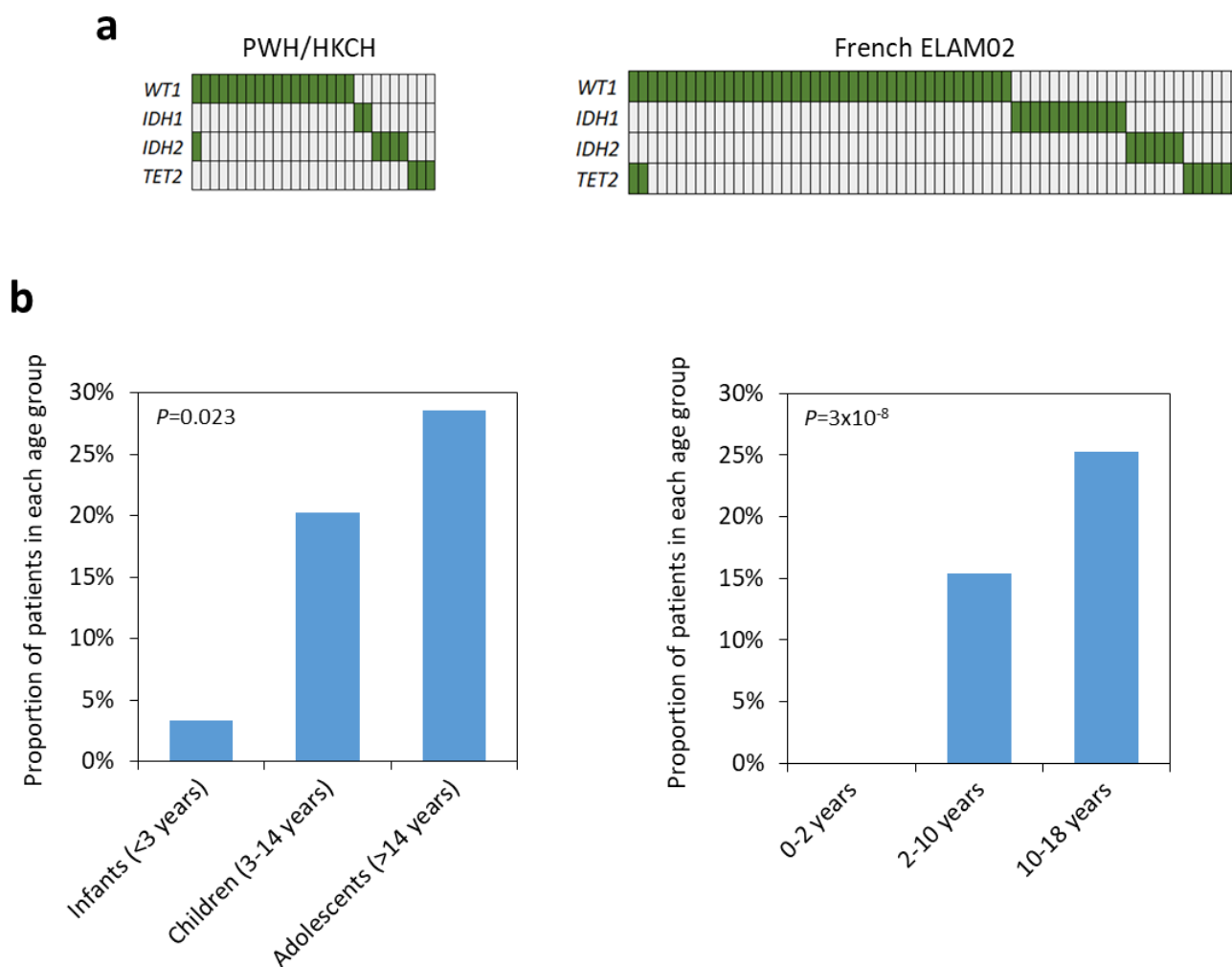
**Supplementary Fig. 9. Validation of *KMT2A* partial tandem duplications (PTDs) and *CBL* intragenic deletion.** *KMT2A*-PTDs and *CBL* deletion were validated by RT-PCR, followed by Sanger sequencing. A novel PTD involving only exons 2-3 was identified in AML\_20. Deletion of *CBL* exons 8-9 was detected in AML\_73 but not the negative control KG-1 cell line (wild-type PCR product of 532 bp). Arrowheads indicate the mutant PCR products. The white dotted line indicates merged lanes from the same gel. Lanes were merged to show the patient sample (AML\_54) among others with *KMT2A*-PTD.

| Functional pathways  | Mutated genes                                                                                                                                 |
|----------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|
| Signaling            | BCR, BRAF, CBL, CBLB, CSF1R, CSF3R, DNM2, EGFR, FLT3, JAK2, JAK3, KDR, KIT, KRAS, MPL, NF1, NOTCH1, NRAS, NTRK3, P2RY2, PDGFRA, PTPN11, STAT3 |
| Transcription factor | BCL6, CEBPA, CUX1, ETV6, GATA1, GATA2, IKZF1, MYC, RUNX1                                                                                      |
| Chromatin regulation | ASXL1, ASXL2, BCOR, BCORL1, CREBBP, DAXX, EZH2, KAT6A, KDM6A, KMT2C, PHF6, SETBP1, SUZ12                                                      |
| DNA methylation      | DNMT3A, IDH1, IDH2, TET2, WT1                                                                                                                 |
| NPM1                 | NPM1                                                                                                                                          |
| Cohesin              | CTCF, RAD21, SMC1A, SMC3, STAG2                                                                                                               |
| Tumor suppressor     | CDKN2A, RB1, TP53                                                                                                                             |
| Splicing             | DDX41, PRPF8, SF1, SF3A1, SF3B1, U2AF1                                                                                                        |
| DNA repair           | ATM, BRCA1, BRCA2, MLH1, MSH2, PMS2, WRN                                                                                                      |
| Adhesion             | GJB3, PCDHB1, RELN                                                                                                                            |
| Others               | ANKRD26, HNRNPK, SRP72, TERT, XPO1                                                                                                            |

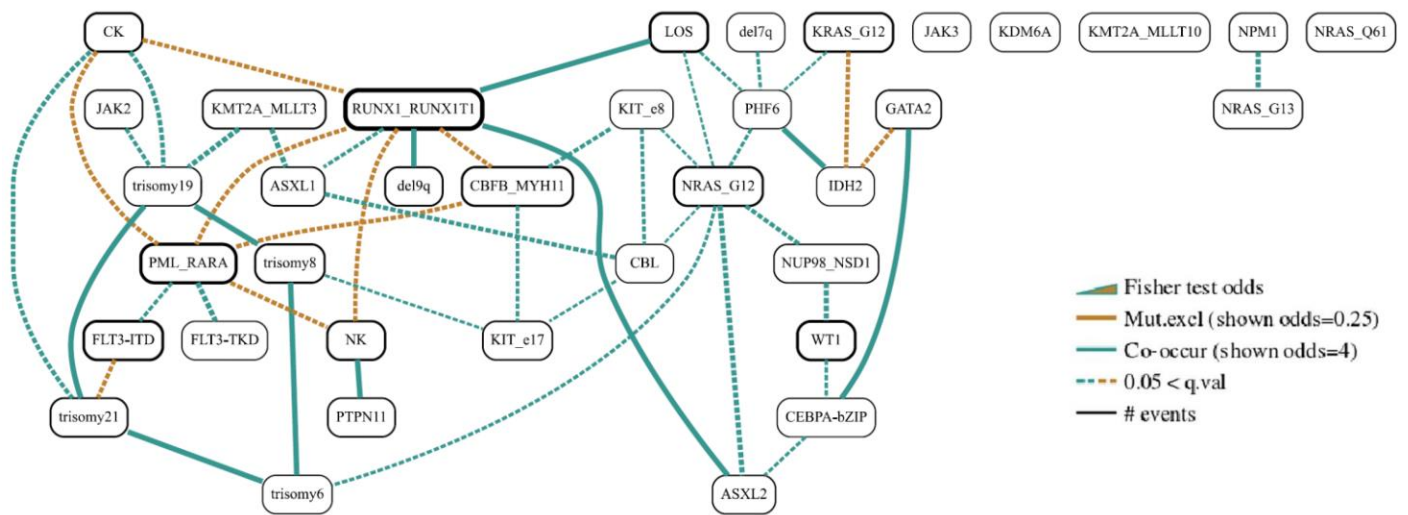


**Supplementary Fig. 10. Variant allele frequencies (VAFs) of genes grouped into different pathways.** *Top*, Mutated genes were grouped into distinct functional pathways. *Bottom*, A boxplot showing the VAFs of genes in different categories in the entire cohort of 147 patients. VAFs were adjusted for local copy number determined by quandico/CNVkit. The boxes represent the lower and upper quartiles, with the lines inside representing the median. The whiskers represent the maximum and minimum excluding outliers and extreme values. Small circles and asterisks represent outliers ( $>1.5 \times$  interquartile range) and extreme values ( $>3 \times$  interquartile range), respectively. *P*-value was calculated by the Kruskal-Wallis test.

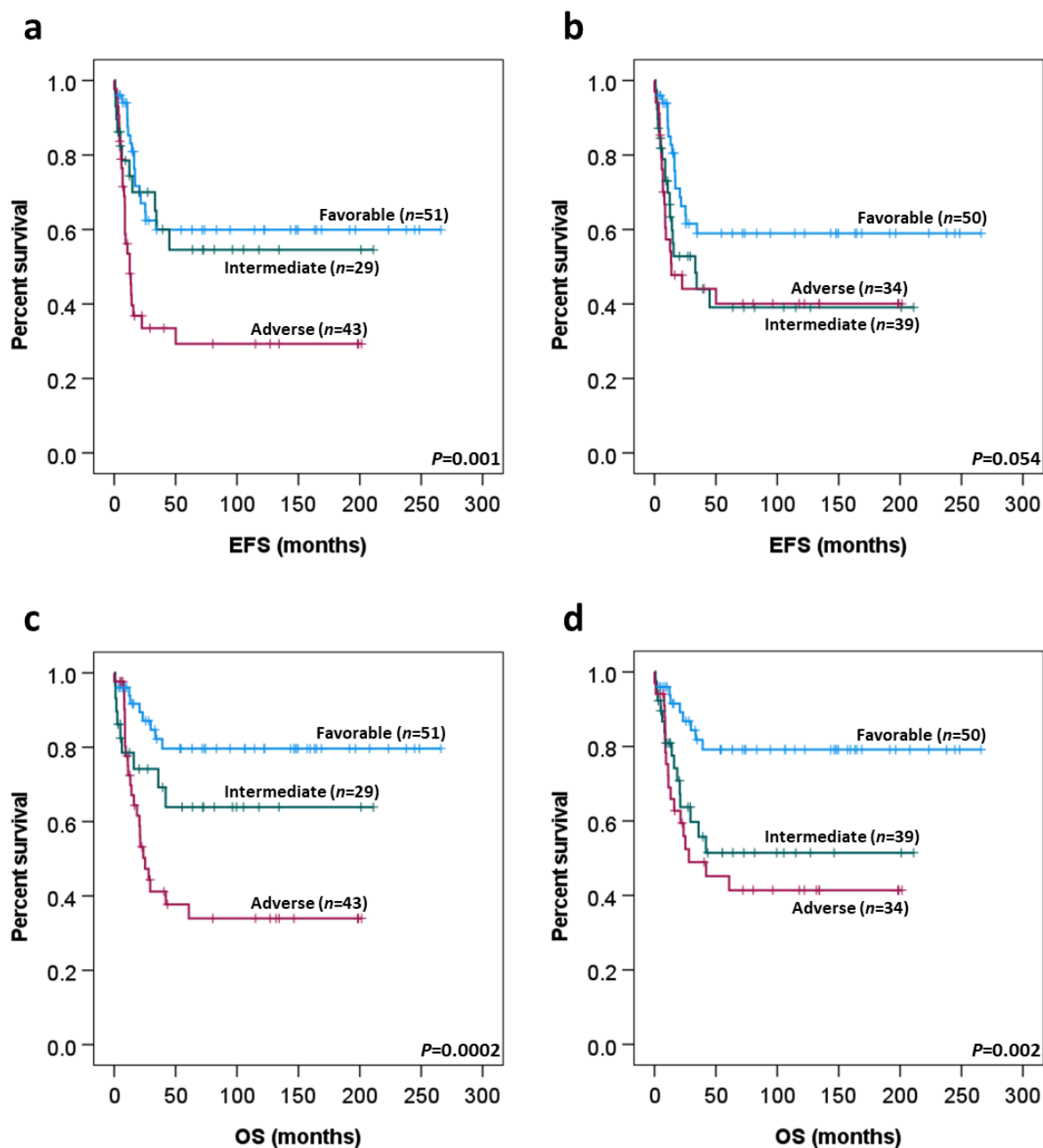




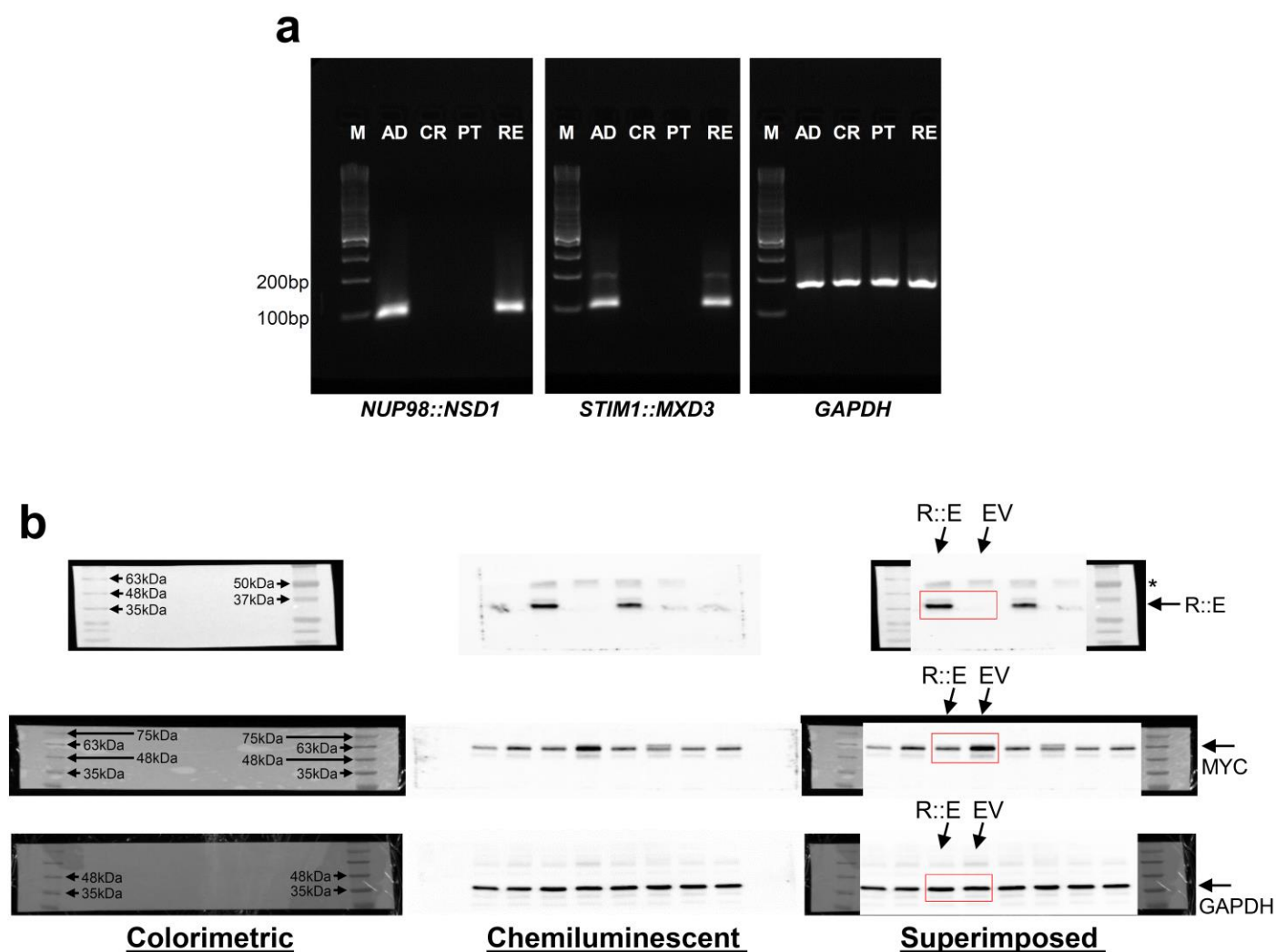
**Supplementary Fig. 12. Mutations of the WIT pathway in pediatric AML.** **a** Largely non-overlapping mutations of *WT1*, *IDH1/2* and *TET2* in the PWH/HKCH (*left*) and French ELAM02 (*right*) cohorts. **b** Age-related disruption of the WIT pathway in the two pediatric AML patient cohorts. *P*-values were calculated by the Fisher's exact test.



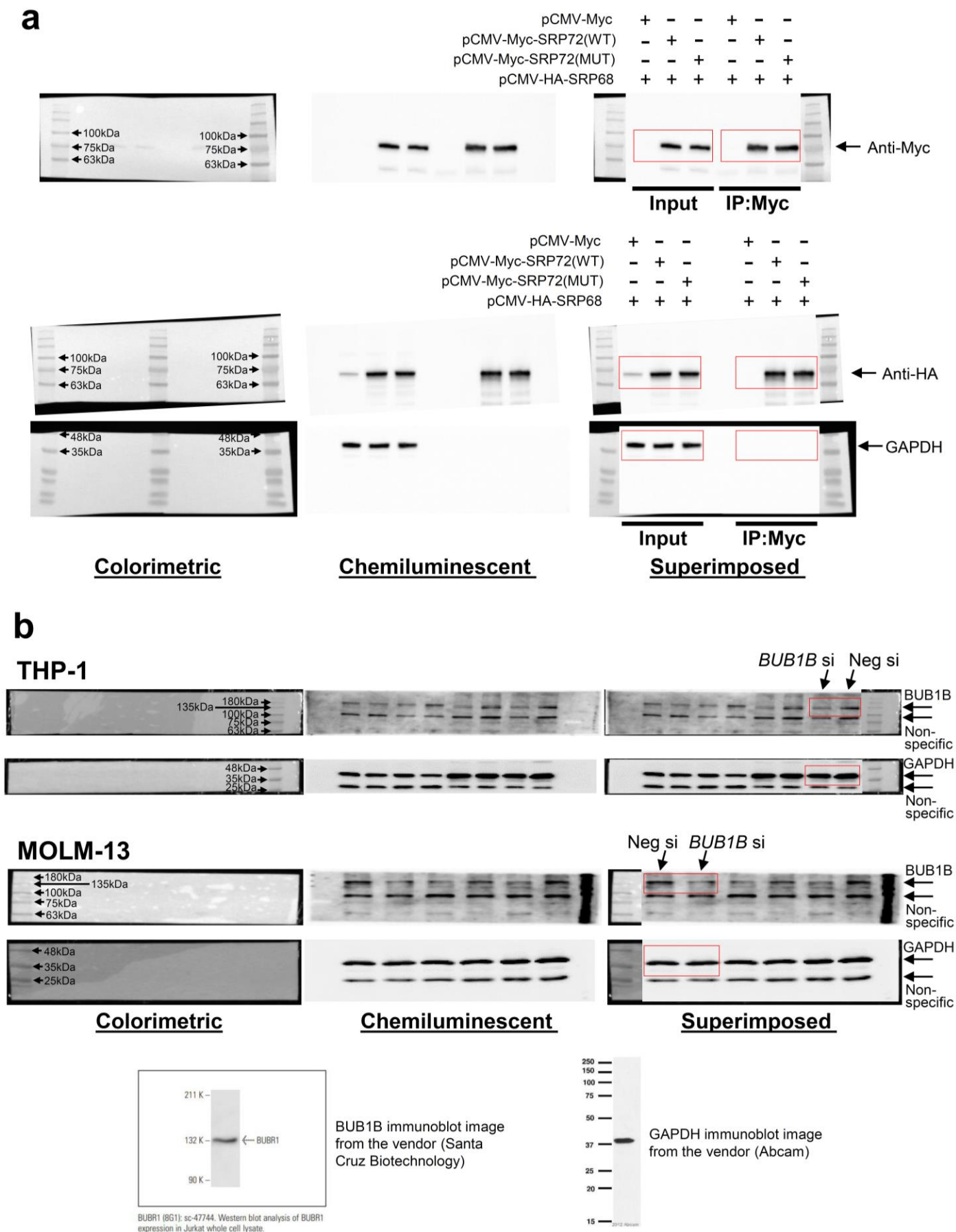
**Supplementary Fig. 13. A Bayesian network showing the complex relationships among cytogenomic changes in pediatric AML patients.** The network was constructed with the GOBNILP software program using the default settings (edge penalty=1).<sup>3</sup> Only those changes occurring in >3% of the entire cohort were included in the analysis. Edges visualised were based on Fisher's exact test corrected for multiple hypothesis testing. LOS, loss of sex chromosome.



**Supplementary Fig. 14. Prognostic risk stratification of pediatric AML patients.** Kaplan-Meier analysis of EFS and OS based on the cytogenomic risk classification described in this study (a, c) or the 2022 ELN risk classification for adult AML patients (b, d).

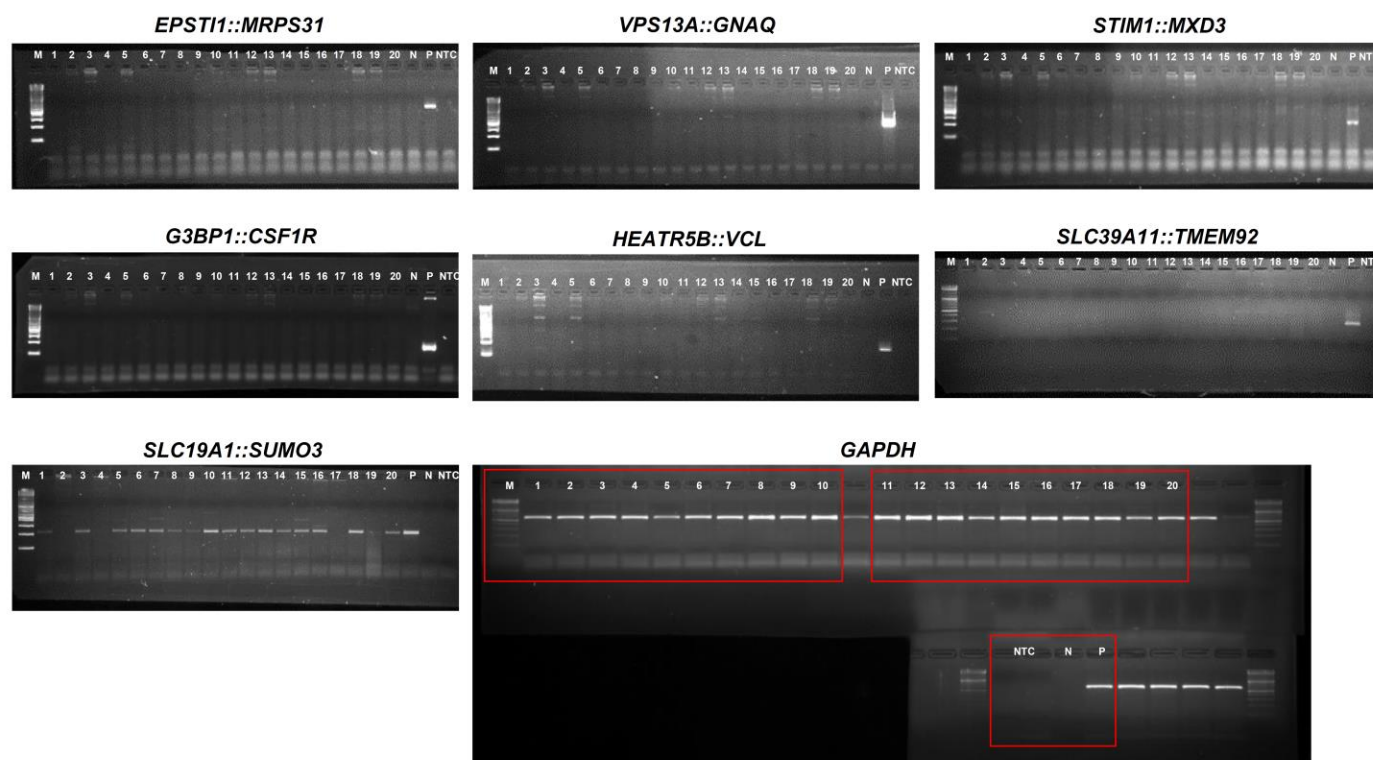


**Supplementary Fig. 15. Uncropped blot and gel images.** The uncropped versions of Fig. 1f (**a**) and Fig. 2f (**b**). For immunoblotting, two images were taken for each blot (colorimetric for ladder and chemiluminescent for bands) and the two images were superimposed in order to determine the molecular weight of the detected proteins. The asterisk in the R::E blot represents the endogenous RUNX1 that was also detected by the anti-AML1 (4334S, Cell Signaling Technology) antibody. Red boxes indicate the final cropped images shown in the main figure.

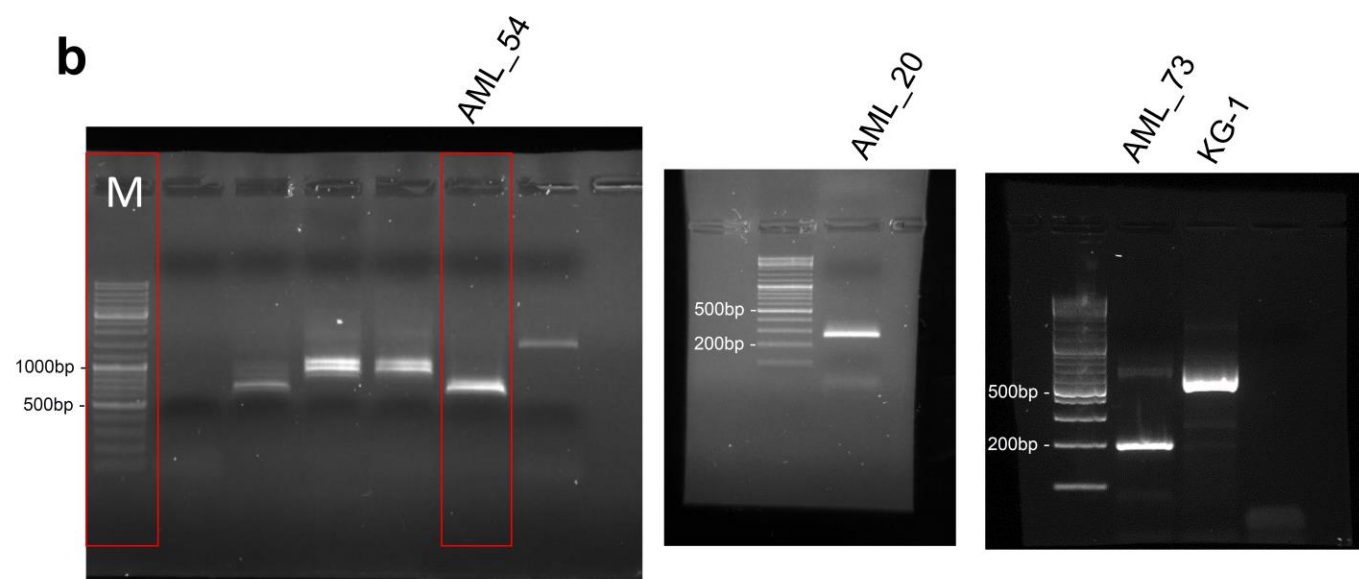


**Supplementary Fig. 16. Uncropped blot images.** The uncropped versions of Fig. 4e (a) and Fig. 6c (b). For immunoblotting, two images were taken for each blot (colorimetric for ladder and chemiluminescent for bands) and the two images were superimposed in order to determine the molecular weight of the detected proteins. Red boxes indicate the final cropped images shown in the main figure.

**a**



**b**



**Supplementary Fig. 17. Uncropped gel images.** The uncropped versions of Supplementary Fig. 3 (a) and Supplementary Fig. 9 (b). Red boxes indicate the merged lanes in the figure.

**Supplementary Table 1. Characteristics and concurrent mutations in patients harboring novel FGs.**

| <b>Sample</b> | <b>Novel FGs</b>                                | <b>Concurrent FGs</b>  | <b>Concurrent mutated genes</b>                        |
|---------------|-------------------------------------------------|------------------------|--------------------------------------------------------|
| AML_103       | <i>RUNX1::ERG</i>                               | /                      | <i>SRP72</i>                                           |
| AML_132       | <i>G3BP1::CSF1R</i> , <i>VPS13A::GNAQ</i>       | /                      | /                                                      |
| AML_96        | <i>EPSTI1::MRPS31</i>                           | /                      | <i>WT1</i>                                             |
| AML_50        | <i>SLC39A11::TMEM92</i> , <i>SLC19A1::SUMO3</i> | /                      | <i>TP53</i>                                            |
| AML_139       | <i>STIM1::F12</i>                               | <i>NUP98::NSD1</i>     | <i>PMS2</i> , <i>NTRK3</i>                             |
| AML_140       | <i>STIM1::F12</i>                               | <i>NUP98::NSD1</i>     | <i>WT1</i> , <i>NRAS</i> , <i>BCL6</i>                 |
| AML_123       | <i>STIM1::MXD3</i>                              | <i>NUP98::NSD1</i>     | <i>CEBPA</i> , <i>WT1</i> , <i>FLT3</i> , <i>P2RY2</i> |
| AML_81        | <i>PHACTR4::COX10</i>                           | <i>NUP98::KDM5A</i>    | /                                                      |
| AML_41        | <i>HEATR5B::VCL</i>                             | <i>KMT2A::ARHGEF12</i> | <i>CUX1</i>                                            |
| AML_145       | <i>FMR1::BCOR</i>                               | <i>MN1::FLI1</i>       | <i>CTCF</i> , <i>MLH1</i>                              |

**Supplementary Table 2. The 141 genes covered in the panel.**

|                |                 |               |               |                |               |                |                |                  |              |
|----------------|-----------------|---------------|---------------|----------------|---------------|----------------|----------------|------------------|--------------|
| <i>ABL1</i>    | <i>BRCA2</i>    | <i>CTCF</i>   | <i>FAS</i>    | <i>JAK1</i>    | <i>LUC7L2</i> | <i>NTRK3</i>   | <i>PTPN11</i>  | <i>SRP72</i>     | <i>U2AF2</i> |
| <i>ADA</i>     | <i>BRINP3</i>   | <i>CUX1</i>   | <i>FBXW7</i>  | <i>JAK2</i>    | <i>MAP2K1</i> | <i>OR13H1</i>  | <i>RAD21</i>   | <i>SRSF2</i>     | <i>WAS</i>   |
| <i>ANKRD26</i> | <i>C17orf97</i> | <i>DAXX</i>   | <i>FLRT2</i>  | <i>JAK3</i>    | <i>MLH1</i>   | <i>OR8B12</i>  | <i>RB1</i>     | <i>STAG2</i>     | <i>WRN</i>   |
| <i>ASXL1</i>   | <i>CALR</i>     | <i>DDX41</i>  | <i>FLT3</i>   | <i>KAT6A</i>   | <i>MPL</i>    | <i>P2RY2</i>   | <i>RELN</i>    | <i>STAT3</i>     | <i>WT1</i>   |
| <i>ASXL2</i>   | <i>CARD11</i>   | <i>DNM2</i>   | <i>GATA1</i>  | <i>KCNA4</i>   | <i>MSH2</i>   | <i>PAX5</i>    | <i>RUNX1</i>   | <i>STXBP2</i>    | <i>XPO1</i>  |
| <i>ATM</i>     | <i>CBL</i>      | <i>DNMT1</i>  | <i>GATA2</i>  | <i>KCNK13</i>  | <i>MSH6</i>   | <i>PCDHB1</i>  | <i>SAXO2</i>   | <i>SUZ12</i>     | <i>ZRSR2</i> |
| <i>ATRX</i>    | <i>CBLB</i>     | <i>DNMT3A</i> | <i>GJB3</i>   | <i>KDM6A</i>   | <i>MYC</i>    | <i>PDGFRA</i>  | <i>SETBP1</i>  | <i>TAL1</i>      |              |
| <i>BCL6</i>    | <i>CBLC</i>     | <i>EED</i>    | <i>GNAS</i>   | <i>KDR</i>     | <i>MYD88</i>  | <i>PHF6</i>    | <i>SF1</i>     | <i>TERC</i>      |              |
| <i>BCOR</i>    | <i>CDKN2A</i>   | <i>EGFR</i>   | <i>HNRNPK</i> | <i>KIT</i>     | <i>NBN</i>    | <i>PML</i>     | <i>SF3A1</i>   | <i>TERT</i>      |              |
| <i>BCORL1</i>  | <i>CEBPA</i>    | <i>ELANE</i>  | <i>HRAS</i>   | <i>KLHDC8B</i> | <i>NF1</i>    | <i>PMS2</i>    | <i>SF3B1</i>   | <i>TET2</i>      |              |
| <i>BCR</i>     | <i>CHEK2</i>    | <i>EP300</i>  | <i>IDH1</i>   | <i>KLHL6</i>   | <i>NOTCH1</i> | <i>PRAMEF2</i> | <i>SH2B3</i>   | <i>TNFRSF13B</i> |              |
| <i>BIRC3</i>   | <i>CREBBP</i>   | <i>ETNK1</i>  | <i>IDH2</i>   | <i>KMT2A</i>   | <i>NPAT</i>   | <i>PRF1</i>    | <i>SH2D1A</i>  | <i>TP53</i>      |              |
| <i>BLM</i>     | <i>CRLF2</i>    | <i>ETV6</i>   | <i>IKZF1</i>  | <i>KMT2C</i>   | <i>NPM1</i>   | <i>PRPF40B</i> | <i>SMARCB1</i> | <i>TPMT</i>      |              |
| <i>BRAF</i>    | <i>CSF1R</i>    | <i>EZH2</i>   | <i>IKZF3</i>  | <i>KRAS</i>    | <i>NRAS</i>   | <i>PRPF8</i>   | <i>SMC1A</i>   | <i>TUBA3C</i>    |              |
| <i>BRCA1</i>   | <i>CSF3R</i>    | <i>FAM47A</i> | <i>IL7R</i>   | <i>LRRC4</i>   | <i>NSD1</i>   | <i>PTEN</i>    | <i>SMC3</i>    | <i>U2AF1</i>     |              |

**Supplementary Table 3. Focal gene-level copy number alterations identified in the present cohort.**

| Sample  | Focal copy number alterations            | Genomic region | Validation |
|---------|------------------------------------------|----------------|------------|
| AML_74  | <i>ABL1</i> loss                         | 9q34.12        | qPCR       |
| AML_93  | <i>BRCA1</i> loss                        | 17q21.31       | MLPA       |
| AML_145 | <i>BRINP3</i> gain                       | 1q31.1         | qPCR       |
| AML_73  | <i>CBL</i> loss (exons 8-9 deleted)      | 11q23.3        | RT-PCR     |
| AML_117 | <i>CDKN2A</i> loss                       | 9p21.3         | MLPA       |
| AML_20  | <i>CEBPA</i> loss*                       | 19q13.11       | MLPA       |
| AML_94  | <i>CHEK2</i> loss                        | 22q12.1        | MLPA       |
| AML_129 | <i>CHEK2</i> loss (exons 5-15 deleted)*  | 22q12.1        | MLPA       |
| AML_129 | <i>CREBBP</i> loss (exons 3-23 deleted)* | 16p13.3        | MLPA       |
| AML_11  | <i>CTCF</i> loss                         | 16q22.1        | qPCR       |
| AML_57  | <i>CTCF</i> loss                         | 16q22.1        | qPCR       |
| AML_35  | <i>ETV6</i> loss                         | 12p13.2        | qPCR       |
| AML_74  | <i>ETV6</i> loss                         | 12p13.2        | qPCR       |
| AML_23  | <i>KLHDC8B</i> loss                      | 3p21.31        | qPCR       |
| AML_59  | <i>KMT2A</i> loss (exons 9-36 deleted)   | 11q23.3        | qPCR       |
| AML_20  | <i>KMT2A</i> -PTD                        | 11q23.3        | RT-PCR     |
| AML_54  | <i>KMT2A</i> -PTD                        | 11q23.3        | RT-PCR     |
| AML_133 | <i>KMT2A</i> -PTD                        | 11q23.3        | RT-PCR     |
| AML_61  | <i>MSH2</i> loss*                        | 2p21           | MLPA       |
| AML_61  | <i>MSH6</i> loss*                        | 2p16.3         | MLPA       |
| AML_92  | <i>MSH6</i> loss (exons 1-3 deleted)     | 2p16.3         | MLPA       |
| AML_74  | <i>PHF6</i> loss                         | Xq26.2         | qPCR       |
| AML_92  | <i>PMS2</i> loss                         | 7p22.1         | MLPA       |
| AML_63  | <i>PTPN11</i> loss                       | 12q24.13       | qPCR       |
| AML_92  | <i>PTPN11</i> loss                       | 12q24.13       | qPCR       |
| AML_94  | <i>PTPN11</i> loss                       | 12q24.13       | qPCR       |
| AML_84  | <i>SMC3</i> loss                         | 10q25.2        | qPCR       |
| AML_115 | <i>TET2</i> loss                         | 4q24           | qPCR       |
| AML_94  | <i>TP53</i> loss                         | 17p13.1        | MLPA       |
| AML_50  | <i>U2AF1</i> gain                        | 21q22.3        | qPCR       |
| AML_3   | <i>U2AF2</i> gain                        | 19q13.42       | qPCR       |
| AML_48  | <i>U2AF2</i> gain                        | 19q13.42       | qPCR       |
| AML_74  | <i>WT1</i> loss                          | 11p13          | qPCR       |

\* Somatic as determined by remission blood sample testing.

Supplementary Table 4. Differential associations of pathway alterations with different molecular/cytogenetic subtypes in pediatric AML.

| Functional pathways  | R:R          |     | C:M          |      | P:R          |     | KMT2A fusions |     | NUP98 fusions |     | Rare fusions |     | NPM1        |     | CEBPA -bZIP |      | NK           |     | CK           |     | Others      |     | P-value* | Adjusted P-value <sup>#</sup> |
|----------------------|--------------|-----|--------------|------|--------------|-----|---------------|-----|---------------|-----|--------------|-----|-------------|-----|-------------|------|--------------|-----|--------------|-----|-------------|-----|----------|-------------------------------|
|                      | (total n=28) |     | (total n=13) |      | (total n=19) |     | (total n=20)  |     | (total n=10)  |     | (total n=10) |     | (total n=7) |     | (total n=5) |      | (total n=13) |     | (total n=13) |     | (total n=9) |     |          |                               |
|                      | n            | %   | n            | %    | n            | %   | n             | %   | n             | %   | n            | %   | n           | %   | n           | %    | n            | %   | n            | %   | n           | %   |          |                               |
| Transcription factor | 2            | 7%  | 2            | 15%  | 1            | 5%  | 7             | 35% | 4             | 40% | 1            | 10% | 2           | 29% | 5           | 100% | 7            | 54% | 3            | 23% | 3           | 33% | 0.0001   | <b>0.001</b>                  |
| Chromatin regulation | 15           | 54% | 0            | 0%   | 2            | 11% | 9             | 45% | 1             | 10% | 2            | 20% | 3           | 43% | 1           | 20%  | 6            | 46% | 2            | 15% | 3           | 33% | 0.003    | <b>0.013</b>                  |
| Signaling            | 25           | 89% | 13           | 100% | 16           | 84% | 14            | 70% | 7             | 70% | 4            | 40% | 4           | 57% | 3           | 60%  | 10           | 77% | 6            | 46% | 5           | 56% | 0.004    | <b>0.013</b>                  |
| Tumor suppressor     | 0            | 0%  | 0            | 0%   | 0            | 0%  | 3             | 15% | 0             | 0%  | 0            | 0%  | 0           | 0%  | 0           | 0%   | 0            | 0%  | 4            | 31% | 1           | 11% | 0.007    | <b>0.016</b>                  |
| DNA methylation      | 5            | 18% | 3            | 23%  | 2            | 11% | 0             | 0%  | 4             | 40% | 1            | 10% | 4           | 57% | 2           | 40%  | 4            | 31% | 1            | 8%  | 2           | 22% | 0.016    | <b>0.029</b>                  |
| Adhesion             | 2            | 7%  | 2            | 15%  | 1            | 5%  | 0             | 0%  | 0             | 0%  | 0            | 0%  | 0           | 0%  | 0           | 0%   | 0            | 0%  | 4            | 31% | 0           | 0%  | 0.082    | 0.122                         |
| Splicing             | 1            | 4%  | 1            | 8%   | 0            | 0%  | 0             | 0%  | 0             | 0%  | 1            | 10% | 1           | 14% | 0           | 0%   | 1            | 8%  | 2            | 15% | 2           | 22% | 0.189    | 0.243                         |
| Cohesin              | 6            | 21% | 2            | 15%  | 1            | 5%  | 1             | 5%  | 0             | 0%  | 2            | 20% | 2           | 29% | 0           | 0%   | 1            | 8%  | 2            | 15% | 0           | 0%  | 0.430    | 0.484                         |
| DNA repair           | 1            | 4%  | 2            | 15%  | 1            | 5%  | 2             | 10% | 2             | 20% | 1            | 10% | 1           | 14% | 0           | 0%   | 2            | 15% | 2            | 15% | 3           | 33% | 0.472    | 0.484                         |

R::R, RUNX1::RUNX1T1 ; C::M, CBFβ::MYH11 ; P::R, PML::RARA ; NK, normal karyotype; CK, complex karyotype.

\* P-values were calculated by the Fisher's exact test.

<sup>#</sup> The Benjamini-Hochberg method was used for adjustment of multiple comparisons.

**Supplementary Table 5. Associations of pathway alterations with age groups.**

| Functional pathways     | Infants (<3 years) |     | Children (3-14 years) |     | Adolescents (>14 years) |     | P-value* | Adjusted P-value# |
|-------------------------|--------------------|-----|-----------------------|-----|-------------------------|-----|----------|-------------------|
|                         | (total n=30)       |     | (total n=89)          |     | (total n=28)            |     |          |                   |
|                         | n                  | %   | n                     | %   | n                       | %   |          |                   |
| Epigenetic <sup>‡</sup> | 4                  | 13% | 46                    | 52% | 17                      | 61% | 0.0001   | <b>0.001</b>      |
| Tumor suppressor        | 1                  | 3%  | 1                     | 1%  | 4                       | 14% | 0.009    | <b>0.042</b>      |
| Adhesion                | 3                  | 10% | 2                     | 2%  | 4                       | 14% | 0.020    | 0.059             |
| Signaling               | 19                 | 63% | 70                    | 79% | 18                      | 64% | 0.137    | 0.308             |
| Transcription factor    | 4                  | 13% | 26                    | 29% | 7                       | 25% | 0.219    | 0.393             |
| Cohesin                 | 3                  | 10% | 8                     | 9%  | 6                       | 21% | 0.221    | 0.618             |
| <i>NPM1</i>             | 1                  | 3%  | 3                     | 3%  | 3                       | 11% | 0.248    | 0.618             |
| Splicing                | 1                  | 3%  | 5                     | 6%  | 3                       | 11% | 0.549    | 0.618             |
| DNA repair              | 3                  | 10% | 12                    | 13% | 2                       | 7%  | 0.768    | 0.768             |

<sup>‡</sup> Chromatin regulation and DNA methylation were combined as epigenetic for the analysis.

\* *P* -values were calculated by the Fisher's exact test.

<sup>#</sup> The Benjamini-Hochberg method was used for correction of multiple comparisons.

**Supplementary Table 6. Comparison of cytogenomic changes in the PWH/HKCH and TARGET-AML cohorts.**

| Cytogenomic alterations | PWH/HKCH cohort (≤18 years) |     | TARGET cohort (≤18 years) |     | P- value* | Adjusted P- value <sup>#</sup> |
|-------------------------|-----------------------------|-----|---------------------------|-----|-----------|--------------------------------|
|                         | (total n=140)               |     | (total n=631)             |     |           |                                |
|                         | n                           | %   | n                         | %   |           |                                |
| FLT3                    | 25                          | 18% | 208                       | 33% | 0.0003    | <b>0.007</b>                   |
| Trisomy 21              | 10                          | 7%  | 12                        | 2%  | 0.003     | <b>0.031</b>                   |
| NRAS                    | 28                          | 20% | 195                       | 31% | 0.010     | 0.079                          |
| PHF6                    | 6                           | 4%  | 9                         | 1%  | 0.039     | 0.232                          |
| KMT2A fusions           | 16                          | 11% | 116                       | 18% | 0.048     | 0.349                          |
| NPM1                    | 7                           | 5%  | 65                        | 10% | 0.054     | 0.349                          |
| -Y                      | 12                          | 9%  | 31                        | 5%  | 0.102     | 0.349                          |
| del(9q)                 | 10                          | 7%  | 26                        | 4%  | 0.125     | 0.375                          |
| ASXL1                   | 9                           | 6%  | 23                        | 4%  | 0.157     | 0.418                          |
| CBFB::MYH11             | 13                          | 9%  | 89                        | 14% | 0.167     | 0.484                          |
| TET2                    | 3                           | 2%  | 32                        | 5%  | 0.177     | 0.484                          |
| ASXL2                   | 8                           | 6%  | 23                        | 4%  | 0.242     | 0.484                          |
| RUNX1::RUNX1T1          | 28                          | 20% | 100                       | 16% | 0.258     | 0.491                          |
| PTPN11                  | 7                           | 5%  | 51                        | 8%  | 0.287     | 0.491                          |
| del(7q)                 | 5                           | 4%  | 13                        | 2%  | 0.347     | 0.555                          |
| CEBPA                   | 7                           | 5%  | 48                        | 8%  | 0.364     | 0.608                          |
| CBL                     | 6                           | 4%  | 19                        | 3%  | 0.431     | 0.608                          |
| GATA2                   | 8                           | 6%  | 29                        | 5%  | 0.519     | 0.691                          |
| KIT                     | 15                          | 11% | 81                        | 13% | 0.572     | 0.723                          |
| KRAS                    | 15                          | 11% | 81                        | 13% | 0.572     | 0.906                          |
| IDH2                    | 5                           | 4%  | 17                        | 3%  | 0.575     | 0.906                          |
| WT1                     | 16                          | 11% | 86                        | 14% | 0.582     | 0.906                          |
| Trisomy 8               | 11                          | 8%  | 54                        | 9%  | 0.868     | 0.906                          |
| -X                      | 5                           | 4%  | 22                        | 3%  | 1.000     | 1.000                          |

\* P-values were calculated by the Fisher's exact test.

<sup>#</sup> The Benjamini-Hochberg method was used for correction of multiple comparisons.

**Supplementary Table 7. Baseline characteristics of the patients treated with the three chemotherapy protocols.**

| Variables                                | Modified UK MRC AML 12<br>(n =46) | %   | NOPHO-AML 2004<br>(n =43) | %   | NOPHO-DBH AML 2012<br>(n =34) | %   | P-value* |
|------------------------------------------|-----------------------------------|-----|---------------------------|-----|-------------------------------|-----|----------|
| Male sex, <i>n</i>                       | 29                                | 63% | 26                        | 60% | 20                            | 59% | 0.946    |
| Age, median (range), years               | 9.8 (0.7-17)                      | /   | 10 (0.3-17)               | /   | 9.4 (0.2-17)                  | /   | 0.814    |
| WBC, median (range), x10 <sup>9</sup> /L | 25.3 (1.4-352)                    | /   | 18.7 (0.7-263.1)          | /   | 23.3 (1.6-218.6)              | /   | 0.437    |
| <b><i>Cytogenomic risk groups</i></b>    |                                   |     |                           |     |                               |     | 0.163    |
| Adverse                                  | 12                                | 26% | 15                        | 35% | 16                            | 47% |          |
| Non-adverse                              | 34                                | 74% | 28                        | 65% | 18                            | 53% |          |

WBC, presentation white blood cell counts.

\* Categorical and continuous variables were analysed by the Fisher's exact and Kruskal-Wallis test, respectively.

**Supplementary Table 8. Univariate analysis of EFS and OS.**

| Variables <sup>#</sup>                 | n  | EFS    |              |                            | OS     |              |                            |
|----------------------------------------|----|--------|--------------|----------------------------|--------|--------------|----------------------------|
|                                        |    | HR     | 95% CI       | P-value                    | HR     | 95% CI       | P-value                    |
| Male sex                               | 75 | 0.712  | 0.421-1.204  | 0.205                      | 0.603  | 0.329-1.105  | 0.102                      |
| Age                                    | /  | 0.997  | 0.951-1.045  | 0.898                      | 0.998  | 0.944-1.055  | 0.944                      |
| Presentation WBC counts                | /  | 1.004  | 1.000-1.007  | <b>0.035</b>               | 1.002  | 0.997-1.006  | 0.437                      |
| Adverse cytogenomic risk*              | 43 | 2.557  | 1.505-4.347  | <b>0.001</b>               | 3.202  | 1.728-5.934  | <b>0.0002</b>              |
| NOPHO-AML 2004                         | 43 | 1.336  | 0.739-2.414  | 0.338                      | 1.553  | 0.772-3.123  | 0.217                      |
| NOPHO-DBH-AML 2012                     | 34 | 1.346  | 0.654-2.774  | 0.42                       | 1.968  | 0.854-4.537  | 0.112                      |
| CR after first induction course        | 92 | 0.482  | 0.275-0.847  | <b>0.011</b>               | 0.321  | 0.172-0.596  | <b>0.0003</b>              |
| SCT at CR1                             | 21 | 0.564  | 0.255-1.246  | 0.157                      | 0.904  | 0.401-2.037  | 0.808                      |
| Number of mutations                    | /  | 0.867  | 0.723-1.039  | 0.121                      | 0.897  | 0.732-1.100  | 0.296                      |
| <b>Genes / distinct mutation types</b> |    |        |              |                            |        |              |                            |
| <i>NRAS</i>                            | 27 | 0.937  | 0.495-1.774  | 0.841                      | 0.918  | 0.439-1.920  | 0.821                      |
| <i>KRAS</i>                            | 14 | 0.594  | 0.237-1.491  | 0.267                      | 0.296  | 0.071-1.224  | 0.093                      |
| <i>PTPN11</i>                          | 9  | 0.415  | 0.101-1.702  | 0.222                      | 0.629  | 0.152-2.604  | 0.522                      |
| <i>JAK2</i>                            | 9  | 0.913  | 0.285-2.923  | 0.878                      | 1.315  | 0.406-4.262  | 0.648                      |
| <i>GATA2</i>                           | 9  | 1.274  | 0.508-3.196  | 0.605                      | 1.811  | 0.711-4.610  | 0.213                      |
| <i>ASXL1</i>                           | 9  | 0.735  | 0.230-2.352  | 0.604                      | 0.315  | 0.043-2.292  | 0.254                      |
| <i>KIT</i> -ex17                       | 8  | 0.939  | 0.339-2.597  | 0.903                      | 0.638  | 0.154-2.642  | 0.535                      |
| <i>ASXL2</i>                           | 8  | 0.671  | 0.210-2.147  | 0.501                      | 0.599  | 0.145-2.481  | 0.48                       |
| <i>CBL</i> <sup>‡</sup>                | 6  | 1.029  | 0.321-3.297  | 0.961                      | 0.422  | 0.058-3.069  | 0.394                      |
| <i>PHF6</i> <sup>‡</sup>               | 6  | 0.235  | 0.033-1.703  | 0.152                      | 0.362  | 0.050-2.635  | 0.316                      |
| <i>KDM6A</i>                           | 6  | 0.967  | 0.302-3.098  | 0.955                      | 0.433  | 0.060-3.150  | 0.408                      |
| <i>NPM1</i>                            | 6  | 0.044  | 0.001-3.841  | 0.171                      | 0.045  | 0.000-10.045 | 0.261                      |
| <i>CEBPA</i> -bZIP                     | 5  | 0.685  | 0.167-2.810  | 0.599                      | 0.949  | 0.229-3.928  | 0.942                      |
| <i>KIT</i> -ex8                        | 5  | 0.54   | 0.075-3.905  | 0.541                      | 0.047  | 0.000-75.569 | 0.417                      |
| <i>JAK3</i>                            | 5  | 1.263  | 0.307-5.187  | 0.746                      | 1.915  | 0.461-7.951  | 0.371                      |
| <i>IDH2</i>                            | 5  | 0.782  | 0.190-3.211  | 0.732                      | 0.557  | 0.077-4.048  | 0.563                      |
| <i>DNM2</i>                            | 4  | 1.411  | 0.440-4.520  | 0.562                      | 0.557  | 0.077-4.054  | 0.564                      |
| <i>CEBPA</i> <sup>‡</sup>              | 5  | 1.801  | 0.562-5.769  | 0.322                      | 2.417  | 0.742-7.874  | 0.143                      |
| <i>KMT2C</i>                           | 4  | 1.372  | 0.333-5.653  | 0.661                      | 0.703  | 0.097-5.124  | 0.728                      |
| <i>TP53</i> <sup>‡</sup>               | 6  | 6.392  | 2.703-15.118 | <b>2.4×10<sup>-5</sup></b> | 8.351  | 3.442-20.261 | <b>2.7×10<sup>-6</sup></b> |
| <i>TP53</i> mutation                   | 3  | 12.056 | 3.509-41.421 | <b>7.7×10<sup>-5</sup></b> | 15.427 | 4.359-54.601 | <b>2.2×10<sup>-5</sup></b> |
| <i>TP53</i> deletion                   | 3  | 4.079  | 1.269-13.115 | <b>0.018</b>               | 5.162  | 1.584-16.825 | <b>0.006</b>               |

WBC, white blood cell; CR, complete remission; SCT, stem cell transplantation; *CEBPA* sm, *CEBPA* single mutation; EFS, event-free survival; OS, overall survival; HR, hazard ratios; 95% CI, 95% confidence interval.

<sup>#</sup> Age, presentation WBC counts and the number of mutations were analysed as continuous variables. The modified UK MRC AML 12 protocol was used as the reference when compared to other protocols.

\* Includes complex karyotype, -7, -5, del(5q), del(12p), *WT1*, *FLT3*-ITD, *DEK*::*NUP214*, *KMT2A*::*AFDN*, *KMT2A*::*MLLT10*, *NUP98* fusions, *FUS*::*ERG* and *CBF2AT3*::*GLIS2*.

<sup>‡</sup> Mutations and deletions of these genes were considered together as the changes could be categorised as functional loss.

Supplementary Table 9. Characteristics of pediatric AML patients with *TP53* alterations.

| Sample  | <i>TP53</i> | Sex | Age | WBC, x10 <sup>9</sup> /L | FAB subtypes | Cytogenetics                                                                                                                                                                                                              | Molecular/cytogenetic subtypes | Ethnicity |
|---------|-------------|-----|-----|--------------------------|--------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------|-----------|
| AML_105 | mutation    | M   | 17  | 6.3                      | M7           | 47,XY,+1,-13,+21[2]/46,idem,del(Y)(q11.23q12),add(3)(q25),-4,-14,+mar1[9]/46,idem,del(Y)(q11.23q12),ins(2;?)(p13;?),add(3)(q25),-4,-14,+mar2[8]/46,XY[1]                                                                  | Complex karyotype              | Chinese   |
| AML_127 | mutation    | M   | 16  | 164.2                    | M5           | 46,X,-Y,-13,+marx2[18]                                                                                                                                                                                                    | Complex karyotype              | Chinese   |
| AML_50  | mutation    | M   | 16  | 0.7                      | M0           | 44,XY,del(1)(p11),-4,del(5)(q13q31),-7,der(14;21)(q10;q10),+mar[20]                                                                                                                                                       | Complex karyotype              | Chinese   |
| AML_108 | deletion    | F   | 16  | 35.7                     | M5           | 46,XX,t(9;11)(p22;q23)[14]/46,idem,i(17)(q10)[6]                                                                                                                                                                          | <i>KMT2A</i> fusions           | Chinese   |
| AML_142 | deletion    | M   | 16  | 34.6                     | M1           | 44~48,XY,add(3)(p21)[6],add(3)(q12)[4],-5[5],add(5)(p15)[2],add(5)(q13)[7],add(6)(q13)[8],-7[11],add(9)(p13)[9],-14[3],-17[9],+1~6mar[cp13]/45,XY,add(3)(p21),add(5)(q13),add(6)(q13),-7,add(9)(p13),-17,+mar[5]/46,XY[1] | Complex karyotype              | Chinese   |
| AML_94  | deletion    | F   | 1   | 5.8                      | Unknown      | 46,XX,t(5;6)(q33;q33)[22]                                                                                                                                                                                                 | Others                         | Chinese   |

WBC, presentation white blood cell counts; FAB, French-American-British.

**Supplementary Table 10. The 56 pediatric AML patients with transcriptome sequencing done for *TP53* pathway analysis.**

| Sample  | <i>TP53</i> status | Molecular/cytogenetic subtypes |
|---------|--------------------|--------------------------------|
| AML_105 | altered            | Complex karyotype              |
| AML_108 | altered            | <i>KMT2A</i> fusions           |
| AML_127 | altered            | Complex karyotype              |
| AML_142 | altered            | Complex karyotype              |
| AML_50  | altered            | Complex karyotype              |
| AML_94  | altered            | Others                         |
| AML_100 | wt                 | <i>RUNX1::RUNX1T1</i>          |
| AML_102 | wt                 | <i>CBFB::MYH11</i>             |
| AML_103 | wt                 | Others                         |
| AML_104 | wt                 | <i>RUNX1::RUNX1T1</i>          |
| AML_106 | wt                 | <i>RUNX1::RUNX1T1</i>          |
| AML_107 | wt                 | Rare fusions                   |
| AML_111 | wt                 | <i>NUP98</i> fusions           |
| AML_112 | wt                 | <i>CBFB::MYH11</i>             |
| AML_113 | wt                 | <i>RUNX1::RUNX1T1</i>          |
| AML_114 | wt                 | Complex karyotype              |
| AML_115 | wt                 | <i>RUNX1::RUNX1T1</i>          |
| AML_116 | wt                 | <i>RUNX1::RUNX1T1</i>          |
| AML_117 | wt                 | <i>KMT2A</i> fusions           |
| AML_118 | wt                 | Normal karyotype               |
| AML_121 | wt                 | Others                         |
| AML_123 | wt                 | <i>NUP98</i> fusions           |
| AML_124 | wt                 | <i>KMT2A</i> fusions           |
| AML_125 | wt                 | <i>RUNX1::RUNX1T1</i>          |
| AML_126 | wt                 | <i>RUNX1::RUNX1T1</i>          |
| AML_129 | wt                 | <i>KMT2A</i> fusions           |
| AML_132 | wt                 | Complex karyotype              |
| AML_133 | wt                 | Normal karyotype               |
| AML_135 | wt                 | <i>KMT2A</i> fusions           |
| AML_136 | wt                 | Others                         |
| AML_137 | wt                 | <i>NPM1</i>                    |
| AML_139 | wt                 | <i>NUP98</i> fusions           |
| AML_140 | wt                 | <i>NUP98</i> fusions           |
| AML_143 | wt                 | <i>KMT2A</i> fusions           |
| AML_144 | wt                 | Rare fusions                   |
| AML_145 | wt                 | Complex karyotype              |
| AML_146 | wt                 | <i>RUNX1::RUNX1T1</i>          |
| AML_147 | wt                 | <i>RUNX1::RUNX1T1</i>          |
| AML_148 | wt                 | <i>KMT2A</i> fusions           |
| AML_20  | wt                 | Normal karyotype               |
| AML_27  | wt                 | <i>NPM1</i>                    |
| AML_35  | wt                 | Complex karyotype              |
| AML_41  | wt                 | <i>KMT2A</i> fusions           |
| AML_44  | wt                 | Complex karyotype              |
| AML_52  | wt                 | Normal karyotype               |
| AML_55  | wt                 | <i>NUP98</i> fusions           |
| AML_69  | wt                 | <i>RUNX1::RUNX1T1</i>          |
| AML_70  | wt                 | Normal karyotype               |
| AML_71  | wt                 | Normal karyotype               |
| AML_79  | wt                 | Others                         |
| AML_81  | wt                 | <i>NUP98</i> fusions           |
| AML_92  | wt                 | Normal karyotype               |
| AML_95  | wt                 | <i>RUNX1::RUNX1T1</i>          |
| AML_96  | wt                 | Normal karyotype               |
| AML_98  | wt                 | <i>CEBPA</i> -bZIP             |
| AML_99  | wt                 | <i>NUP98</i> fusions           |

**Supplementary Table 11. Characteristics of the PWH/HKCH, SCMC and TARGET pediatric AML cohorts. <sup>#</sup>**

|                                 | PWH/HKCH cohort (n=140) |     | SCMC cohort (n=292) |     | TARGET cohort (n=631) |     | P-value*            |                     |                       |
|---------------------------------|-------------------------|-----|---------------------|-----|-----------------------|-----|---------------------|---------------------|-----------------------|
|                                 |                         |     |                     |     |                       |     | PWH/HKCH vs. SCMC   | PWH/HKCH vs. TARGET | SCMC vs. TARGET       |
| Platform for mutation detection | DNA-based               |     | RNA-based           |     | DNA-based             |     |                     |                     |                       |
|                                 | <i>n</i>                | %   | <i>n</i>            | %   | <i>n</i>              | %   |                     |                     |                       |
| Sex                             |                         |     |                     |     |                       |     | 0.675               | 0.024               | 0.016                 |
| Female                          | 53                      | 38% | 117                 | 40% | 307                   | 49% |                     |                     |                       |
| Male                            | 87                      | 62% | 175                 | 60% | 324                   | 51% |                     |                     |                       |
| FAB subtypes                    |                         |     |                     |     |                       |     | 4x10 <sup>-6</sup>  | 0.001               | 1.1x10 <sup>-20</sup> |
| M0/M1                           | 20                      | 14% | 10                  | 3%  | 91                    | 14% |                     |                     |                       |
| M2                              | 33                      | 24% | 110                 | 38% | 146                   | 23% |                     |                     |                       |
| M4                              | 23                      | 16% | 34                  | 12% | 148                   | 23% |                     |                     |                       |
| M5                              | 26                      | 19% | 95                  | 33% | 112                   | 18% |                     |                     |                       |
| M7                              | 14                      | 10% | 40                  | 14% | 15                    | 2%  |                     |                     |                       |
| Age groups                      |                         |     |                     |     |                       |     | 3x10 <sup>-12</sup> | 0.92                | 8.1x10 <sup>-18</sup> |
| <3                              | 28                      | 20% | 107                 | 37% | 118                   | 19% |                     |                     |                       |
| 3-15                            | 84                      | 60% | 181                 | 62% | 380                   | 60% |                     |                     |                       |
| 15-18                           | 28                      | 20% | 4                   | 1%  | 133                   | 21% |                     |                     |                       |

<sup>#</sup> Data of the SCMC and TARGET cohorts were obtained from Liu *et al.* <sup>35</sup> Only Chinese patients in the PWH/HKCH cohort (140 of 147 patients) were included in the comparison.

\* Fisher's exact test was used to compare sex, while chi-square test was used to compare FAB and age group distribution.

### Supplementary References

1. Bolouri, H., et al. The molecular landscape of pediatric acute myeloid leukemia reveals recurrent structural alterations and age-specific mutational interactions. *Nat. Med.* **24**, 103-112 (2018).
2. Zhou, X., et al. Exploring genomic alteration in pediatric cancer using ProteinPaint. *Nat. Genet.* **48**, 4-6 (2016).
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