Derepression of retroelements in acute myeloid leukemia with 3q aberrations

Jagoda Mika,[1] Sophie Ottema,[2] Sandra Kiehlmeier,[1] Sabrina Kruse,[1] Leonie Smeenk,[2] Judith Müller,[1] Sabrina Schweiggert,[1] Carl Herrmann,[3] Mathijs Sanders,[2] Ruud Delwel[2] and Stefan Gröschel[1,4,5]

[1]Molecular Leukemogenesis Group, German Cancer Research Center, Heidelberg, Germany; [2]Department of Hematology, Erasmus University Medical Center, Oncode Institute, Rotterdam, the Netherlands; [3]Health Data Science Unit, Medical Faculty Heidelberg and BioQuant, Heidelberg, Germany; [4]Internal Medicine V, Heidelberg University Hospital, Heidelberg, Germany and [5]Oncology Center Worms, Worms, Germany

Correspondence: STEFAN GROESCHEL - stefan.groeschel@dkfz.de

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Supplementary Information

Supplementary figures

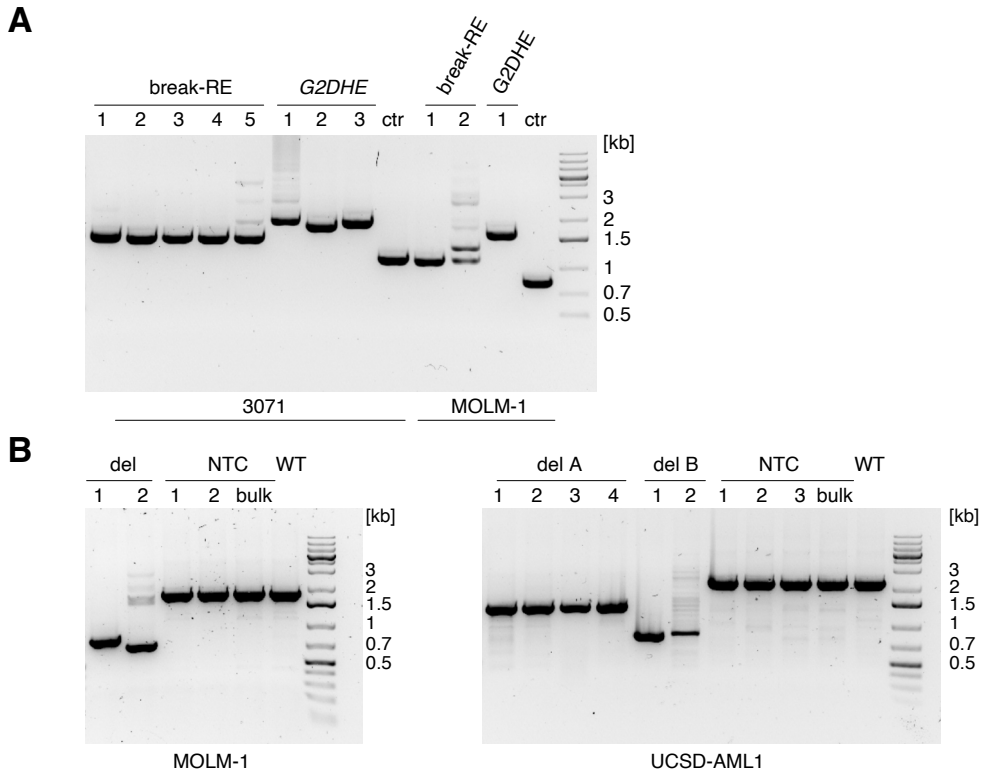
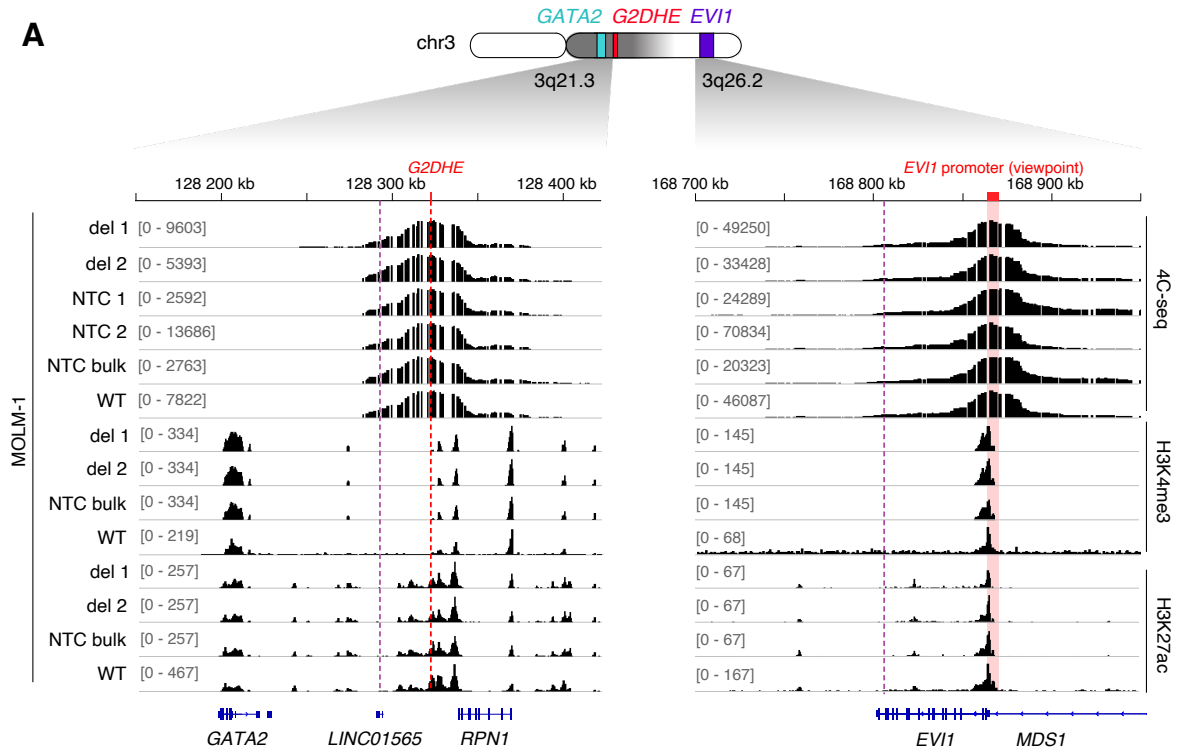


Fig. S1. PCR validation of expanded single clones upon CRISPR-Cas9 targeting. (**A**) PCR spanning the insertion sites in K562 shows successful *knock-in* of desired sequences (break-RE: breakpoint-RE) by the presence of higher-running bands on an agarose gel in respect to the control bands. (**B**) Rearranged allele-specific PCR of the MOLM-1 and UCSD-AML1 deletion clones (del, del A, del B) confirms the presence of desired deletions with smaller amplicons in comparison to control samples (NTC: nontargeting control, WT: wild-type). Number over each lane corresponds to a different single clone within a given condition.


A
GATA2 G2DHE EVI1
chr3
3q21.3
3q26.2
G2DHE
128 200 kb
128 300 kb
128 400 kb
EVI1 promoter (viewpoint)
168 700 kb
168 800 kb
168 900 kb
MOLM-1
del 1 [0 - 9603]
del 2 [0 - 5393]
NTC 1 [0 - 2592]
NTC 2 [0 - 13686]
NTC bulk [0 - 2763]
WT [0 - 7822]
del 1 [0 - 334]
del 2 [0 - 334]
NTC bulk [0 - 334]
WT [0 - 219]
del 1 [0 - 257]
del 2 [0 - 257]
NTC bulk [0 - 257]
WT [0 - 467]
[0 - 49250]
[0 - 33428]
[0 - 24289]
[0 - 70834]
[0 - 20323]
[0 - 46087]
[0 - 145]
[0 - 145]
[0 - 145]
[0 - 68]
[0 - 67]
[0 - 67]
[0 - 67]
[0 - 167]
4C-seq
H3K4me3
H3K27ac
GATA2
LINC01565
RPN1
EVI1
MDS1

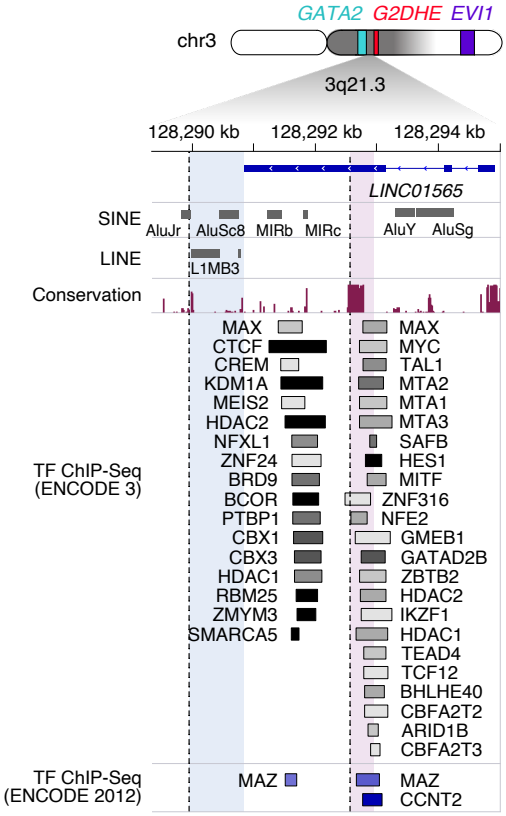

B
GATA2 G2DHE EVI1
chr3
3q21.3
128,290 kb
128,292 kb
128,294 kb
LINC01565
SINE
AluJr
AluSc8
MIRb
MIRc
AluY
AluSg
LINE
L1MB3
Conservation
TF ChIP-Seq (ENCODE 3)
MAX
CTCF
CREM
KDM1A
MEIS2
HDAC2
NFXL1
ZNF24
BRD9
BCOR
PTBP1
CBX1
CBX3
HDAC1
RBM25
ZMYM3
SMARCA5
MAX
MYC
TAL1
MTA2
MTA1
MTA3
SAFB
HES1
MITF
ZNF316
NFE2
GMEB1
GATAD2B
ZBTB2
HDAC2
IKZF1
HDAC1
TEAD4
TCF12
BHLHE40
CBFA2T2
ARID1B
CBFA2T3
TF ChIP-Seq (ENCODE 2012)
MAZ
MAZ
CCNT2

Fig S2. (A) Deletion of the breakpoint-RE affects neither the chromatin landscape nor the chromatin composition in the MOLM-1 deletion clones. 4C-Seq (top six tracks) and H3K4me3 and H3K27ac ChIP-Seq peaks (bottom eight tracks) of selected MOLM-1 deletion (del) and control (NTC, WT) samples showing the 3q21 region around *G2DHE* (left) and the 3q26 region around *EVI1* promoter (right), which was used as a viewpoint in 4C-Seq. Chromosomal breakpoints in MOLM-1 are indicated with purple dashed lines. **(B)** TF binding sites overlap with the deleted 3q21 region in MOLM-1, but not UCSD-AML1. The dashed line indicates the position of the chromosomal breakpoints and the deleted 3q21 fragments are colored (MOLM-1: purple, UCSD-AML1: blue). No overlap of TF binding sites was found at the 3q26 breakpoint region in any of the two cell lines. TF ChIP-Seq data from K562 were taken from ENCODE 3 (grey-black) and ENCODE 2012 (blue) dataset.[1,2] The darkness of the boxes is proportional to the ChIP signal strength.

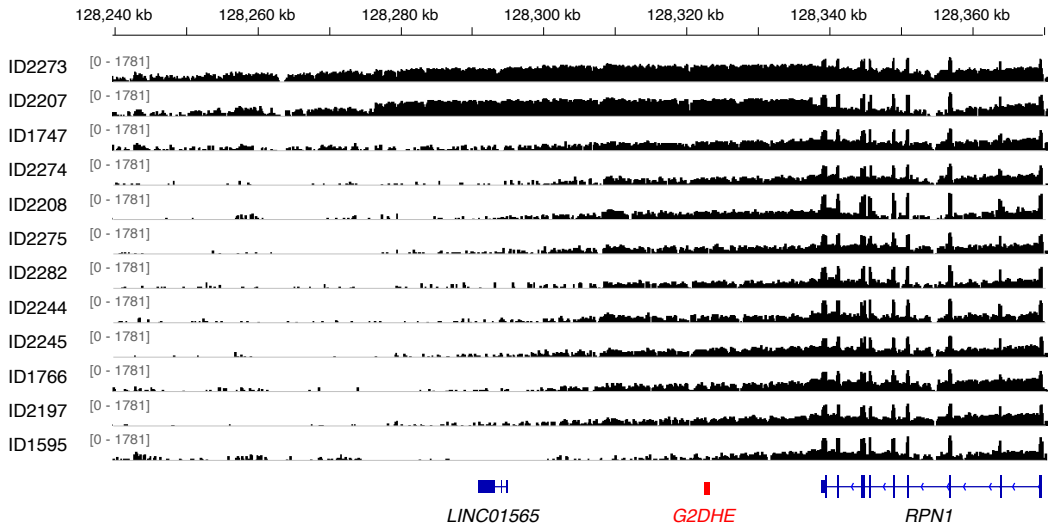


Fig S3. RNA-sequencing tracks from a selection of non 3q-AML patients showing the 3q21.3 region around *G2DHE*. Low-level RNA readthrough spanning the *G2DHE* is observed in these patients. Tracks are shown in a logarithmic scale. Sequencing data has been deposited at the European Genome-phenome Archive (EGA, http://www.ebi.ac.uk/ega/),

which is hosted by the EBI, under accession number EGAS00001004684 (Mulet-Lazaro *et al*., 2021, under review).

Supplementary references

1. Dunham I, Kundaje A, Aldred SF, et al. An integrated encyclopedia of DNA elements in the human genome. Nature 2012;489(7414):57–74.
2. Davis CA, Hitz BC, Sloan CA, et al. The Encyclopedia of DNA elements (ENCODE): Data portal update. Nucleic Acids Res 2018;46(D1):D794–D801.