# A novel *AGGF1-PDGFRβ* fusion in pediatric T-cell acute lymphoblastic leukemia

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*doi:10.3324/haematol.2017.165282*

## SUPPLEMENTAL METHODS

### Cell lines

ALL-SIL and HPB-ALL cell lines were graciously provided by Dr. Robert G. Hawley (The George Washington University, USA) and Dr. Ioannis Aifantis (NYU Langone Medical center, USA) respectively. Cells were cultured in RPMI-1640 medium (ThermoFisher, 11875093) and supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin (ThermoFisher, 15070063), and 1% L-glutamine (ThermoFisher, 25030081).

### Fusion transcript detection

The JAFFA (version 1.09) fusion transcript detection program[1] was used to map sequencing reads to human genome, hg19, and GENCODE version 19 using the command "$ bpipe run JAFFA_direct.groovy *.fastq.gz" for all paired-end RNA-Seq sequencing files in compressed format. ChimeraScan (version 0.4.5a), another fusion transcript detection program, was also used to align reads to the hg19 human reference genome utilizing the UCSC Known Gene annotation file with default parameters.[2]

### Generation of AGGF1-PDGFR$\beta$ cDNA and qPCR

RNA was isolated from the day 114 sample (Figure 1A) using the RNeasy® Mini Kit (Qiagen). Reverse-transcription was performed using the BioRad iScript cDNA synthesis kit using 140 ng total RNA as template. Using the cDNA as a template, Phusion® High-Fidelity DNA Polymerase (New England Biolabs) and primers AGGF1_1152_F and PDGFRβ_2153_R were used to confirm the presence of AGGF1-PDGFRβ (Figure S3B). Sanger Sequencing was performed for further confirmation.

Real-time PCR (qPCR) was conducted on a C1000 instrument (Bio-Rad) using SsoAdvanced™ Universal Probes Supermix, (172-5280) and with PrimePCR™ probe assay (TLX1, human; qHsaCEP0050458), (TLX3, human; qHsaCEP0033078) (Bio-Rad) and GUSB FAM TaqMan™ Probe 20X, (Hs00939627_m1) (ThermoFisher, 4331182). Multiplex PCR reactions were performed in triplicates. RNA was isolated from ALL-SIL and HPB-ALL as

previously described and used to test the efficacy of TLX1 and TLX3 PrimePCR probe assays. qPCR data was analyzed using the ΔΔCt-method normalized to glucuronidase beta.

**Amplification of the AGGF1-PDGFR*β* fragment and cloning into pMSCV-IRES-GFP**

Full-length human AGGF1-PDGFRβ was amplified with Phusion® High-Fidelity DNA Polymerase using overlap extension PCR[3] and inserted into pMSCV-IRES-GFP vector *(Addgene #20672). cDNA was used for PCR amplification of a 2099 nucleotide (nt) region from* the AGGF1 5'-UTR into PDGFRβ using the AGGF1_5'-UTR_F and PDGFRβ_1781_R primers. A subsequent PCR amplification was performed using the 2099 nt amplicon as a template for InF_NotI_AGGF1_F and AGGF1_1602_R primers to complete the 5'-end of AGGF1-PDGFRβ (**Step 1,** Figure S2B). The remaining 1788 nt region was amplified from AGGF1 to the 3'-end of PDGFRβ with the AGGF1_1588_F and InF_HpaI_PDGFRβ_R primers (**Step 2,** Figure S3B). Finally, the 1602 nt and 1788 nt sequences were joined via PCR with InF_NotI_AGGF1_F and InF_HpaI_PDGFRβ_R primers (**Step 3,** Figure S3B) and inserted into pMSCV-IRES-GFP using In-Fusion® HD Cloning system (Clontech).

**RNA-Seq of the Day 75 specimen to independently verify the AGGF1-PDGFRβ fusion location and to facilitate comparative hierarchical clustering analysis with Liu *et al.*[4]**

Total RNA was extracted from 1 million (HPB-ALL and ALL-SIL) or 2 million (primary T-ALL Day 75 specimen) cells using the Norgen Animal Tissue RNA Purification Kit (Norgen Biotek Corporation). Quality control for RNA samples were done on an Agilent Technologies 2200 TapeStation using an RNA ScreenTape assay. Total RNA samples were hybridized with Ribo-Zero Gold to substantially deplete cytoplasmic and mitochondrial rRNA from the samples. Stranded RNA sequencing libraries were prepared as described using the Illumina TruSeq Stranded Total RNA Kit. Purified libraries were qualified on an Agilent Technologies 2200 TapeStation using a D1000 ScreenTape assay. The molarity of adapter-modified molecules was defined by quantitative PCR using the Kapa Biosystems Kapa Library Quant Kit. Individual libraries were normalized to 10 nM and equal volumes were pooled in preparation

for Illumina sequence analysis. Sequencing libraries (25 pM) were chemically denatured and applied to an Illumina HiSeq v4 paired-end flow cell using an Illumina cBot. Hybridized molecules were clonally amplified and annealed to sequencing primers with reagents from an Illumina HiSeq PE Cluster Kit v4-cBot. Following transfer of the flow cell to an Illumina HiSeq 2500 instrument (HCS v2.2.38 and RTA v1.18.61), a 125-cycle paired-end sequence run was performed using HiSeq SBS Kit v4 sequencing reagents. RNA-seq data were mapped using human genome, hg19, with HISAT2.[5] The gene-level read count was generated with the Subread aligner[6] and the featureCounts program[7] for assigning sequence reads to genomic features. The number of fragments per kilobase of transcript per million mapped reads (FPKM) was calculated on the basis of the transcript models in GENCODE version 19. FPKM values from this study and the ones from the 264 T-ALL cases reported in Liu *et al*. (Table S5 RNAseq FPKM)[4] were combined based on the gene list from Liu *et al*. Transcription factor genes were used for cluster analysis by Ward's minimum variance method.

**SUPPLEMENTAL REFERENCES**


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5. Kim D, Langmead B, Salzberg SL. HISAT: a fast spliced aligner with low memory requirements. Nat Methods. 2015;12(4):357-360.

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**SUPPLEMENTAL FIGURE AND TABLE LEGENDS**

**Figure S1.** Identification of the exact location of the AGGF1-PDGF*Rβ* fusion in the Day 75 specimen. The *AGGF1-PDGFRβ* fusion transcript was detected in 125 bp-long paired-end RNA-seq performed on T-cell ALL patient cells (Day 75 specimen) and was not detected in HPB-ALL or ALL-SIL cells. The 5′ gene partner is depicted in blue, and the inverted 3′ gene partner is depicted in red. Fusion transcripts detected by the JAFFA and ChimeraScan programs are consistent with each other. The intergenic chromosomal distance between the fusion partners is denoted in megabase pairs (Mb).

**Figure S2.** Characterization of the chromosomal rearrangements. A. Cytogenetic analysis of diagnostic marrow, identifying chromosomal abnormalities in chromosomes 3 and 5, as indicated by arrows. Metaphase spreads from cultures of bone marrow aspirate were prepared using standard procedures. Images of the G-banded metaphases were captured by Metafer Slide Scanning Platform and karyotype analysis was performed with the Ikaros Software (MetaSystems). The karyotypes were described according to the recommendations of the International Standing Committee on Human Cytogenetic Nomenclature (ISCN 2013: An International System for Human Cytogenetic Nomenclature, Shaffer LG, McGowan-Jordan J, Schmid M (eds), S Karger, Basel 2013). B-E. Single nucleotide polymorphism (SNP) microarray analysis of tumor DNA showed several copy number changes including a 405 kb deletion within 3q21.1 (B), a 4.6 Mb deletion from 5q14.1 to 5q14.2 (C), an approximately 2.5 kb deletion involving exons 9 and 10 of the *PDGFRβ* gene (D), and a 169 kb deletion within 9p21.3 involving the *CDKN2A* gene (E). The red rectangles mark the deletions, blue dots represent the probes in the microarray. Upper section shows Log2 Ratio (copy number probes) and the lower section shows the allele difference (SNP probes). RefSeq genes in the deleted regions as well as the cytogenetic bands and nucleotide coordinates are also shown at the bottom.

**Figure S3.** Schematic showing: A. The location of all primers used for the purpose of amplifying, sequencing, and cloning *AGGF1-PDGFRβ*, B. The method used to identify the presence of the fusion gene, and C. The steps involved in amplifying and cloning the fusion gene for the purpose of expression in Ba/F3 cells.

**Figure S4.** Clustering using Ward's minimum variance method of the selected transcription factors from transcriptome sequencing data from this study (3 specimens) and from 264 specimens of Liu *et al.*[1], with annotation of samples and transcription factor subgrouping according to the subgroup used in the published data of Liu *et al.*[1]

**Table S1**. Clinical response metrics

**Table S2**. Primers used in this study

**Table S3**. FISH and cytogenetic findings before and after dasatinib initiated

**Table S4**. FPKM values extracted from transcriptome data for the T-ALL Day 75 specimen and the HPB-ALL and ALL-SIL cell lines

## Reference List

1. Liu Y, Easton J, Shao Y, et al. The genomic landscape of pediatric and young adult T-lineage acute lymphoblastic leukemia. Nat Genet. 2017;49(8):1211-1218.

Figure S1

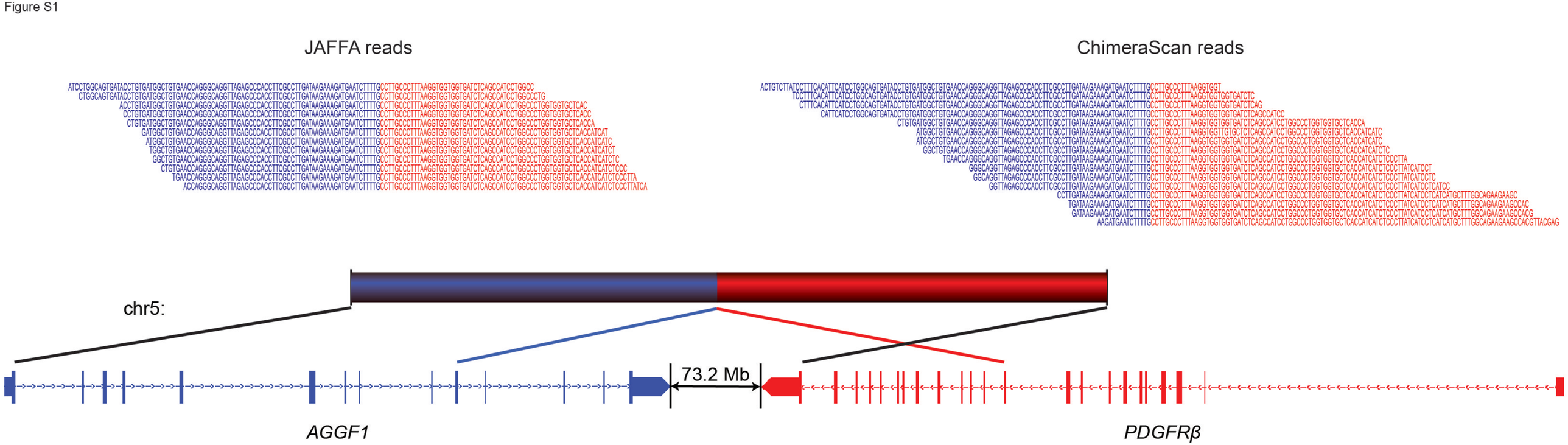
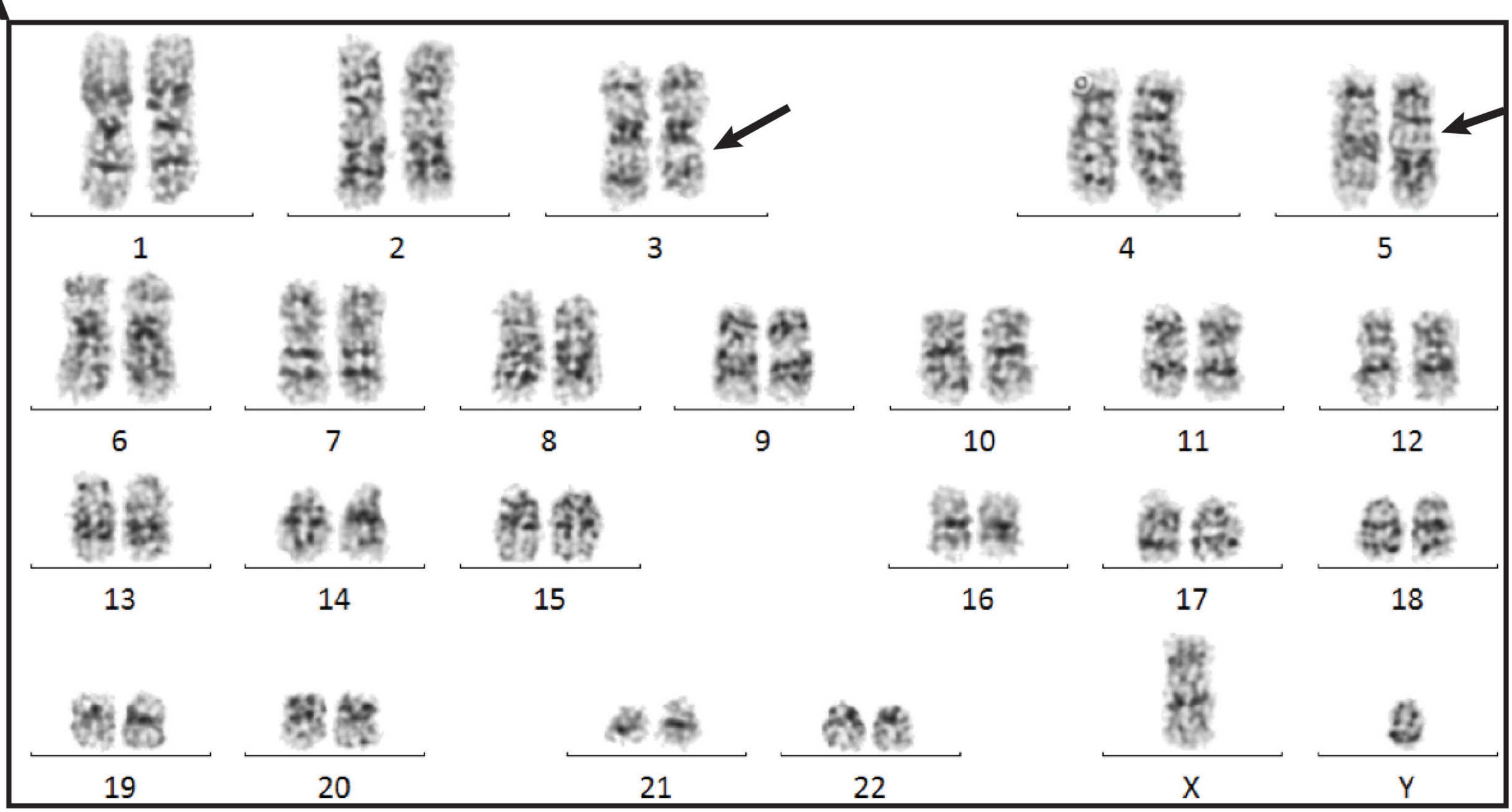


Figure S2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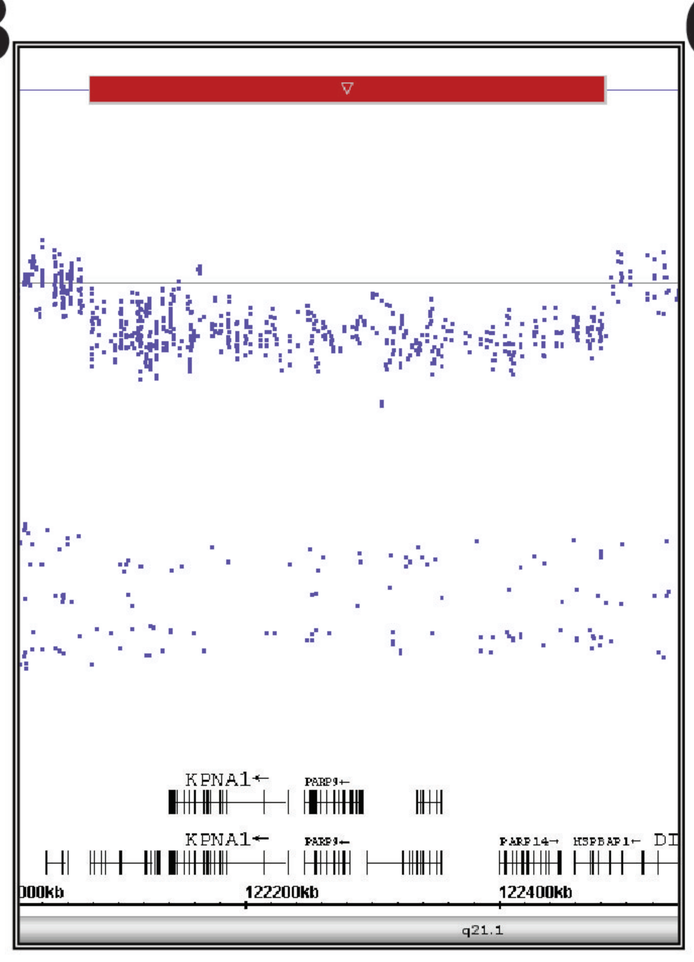
X
Y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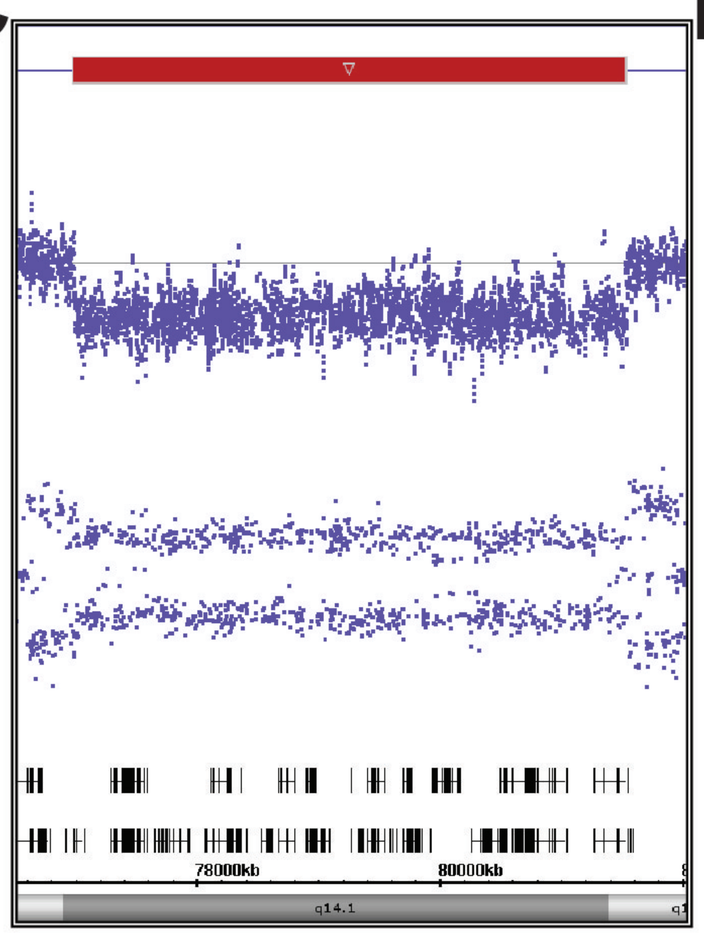
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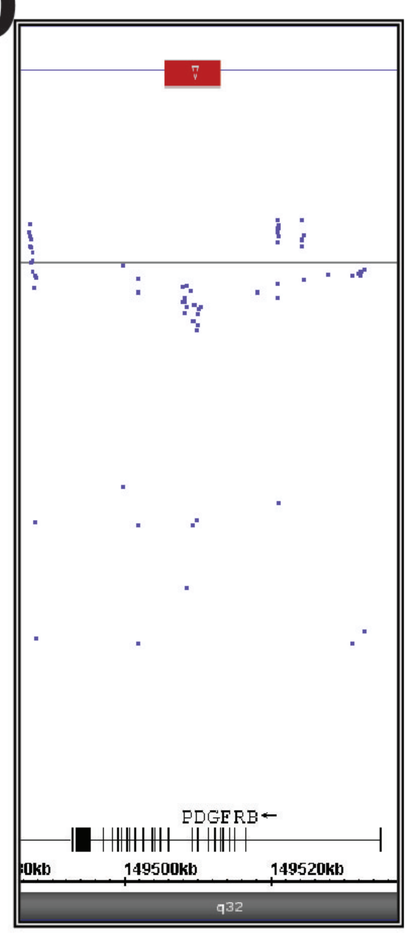
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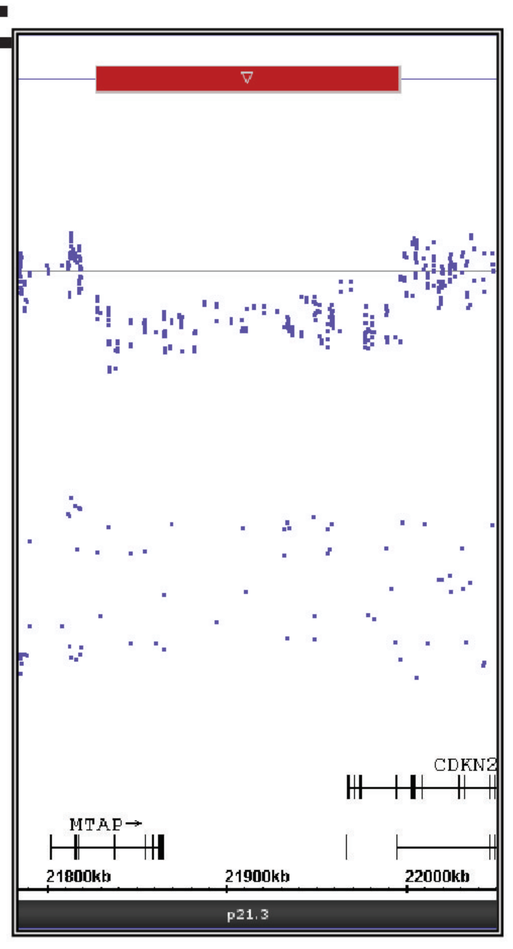
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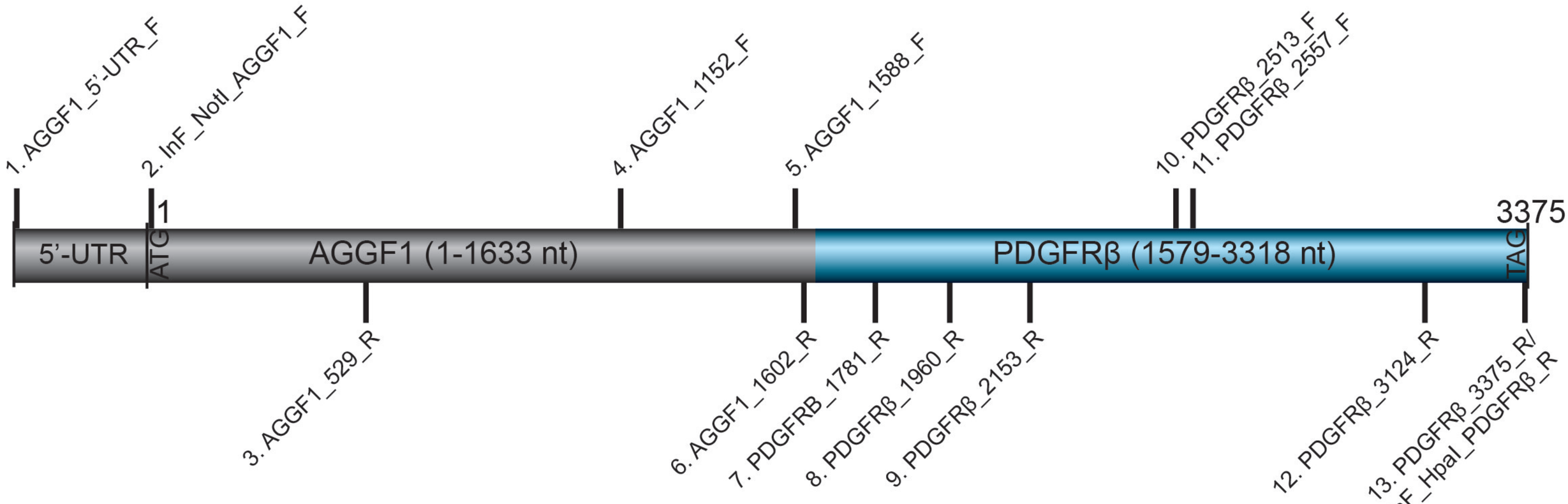
p21.3

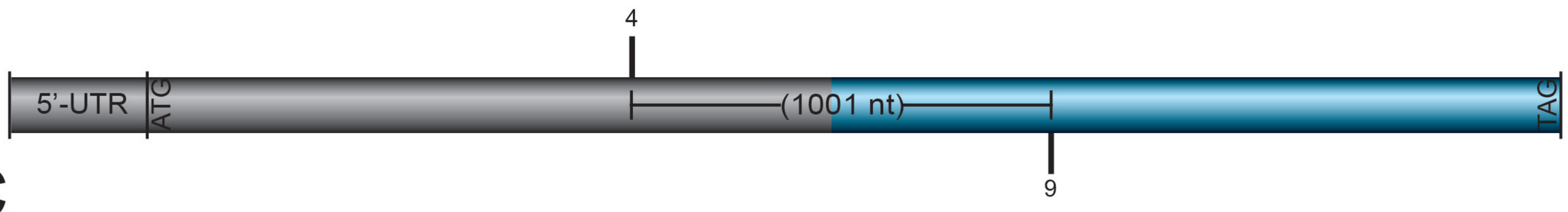
Figure S3

**A** AGGF1-PDGFRβ: Primer locations



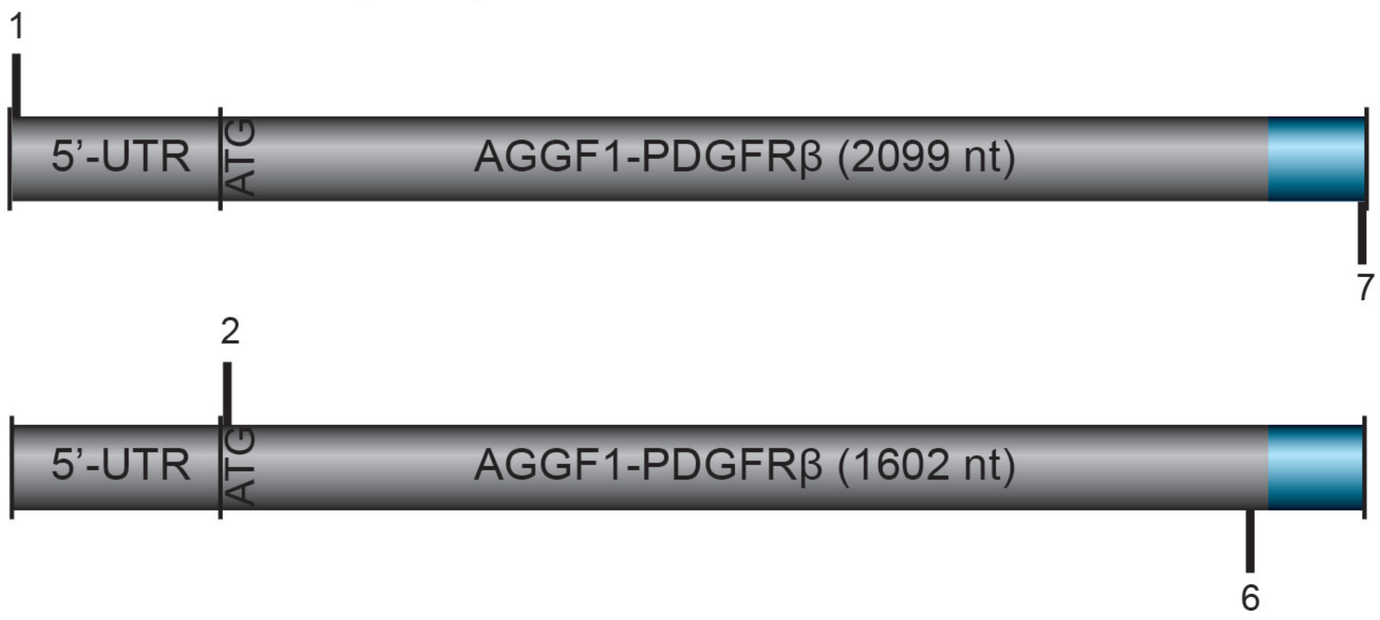

**B**

AGGF1-PDGFRβ: Confirmation of AGGF1-PDGFRβ breakpoint




**C**

AGGF1-PDGFRβ: Step 1




AGGF1-PDGFRβ: Step 2


AGGF1-PDGFRβ: Step 3

: Indicates overlapping sequences (15 nt)

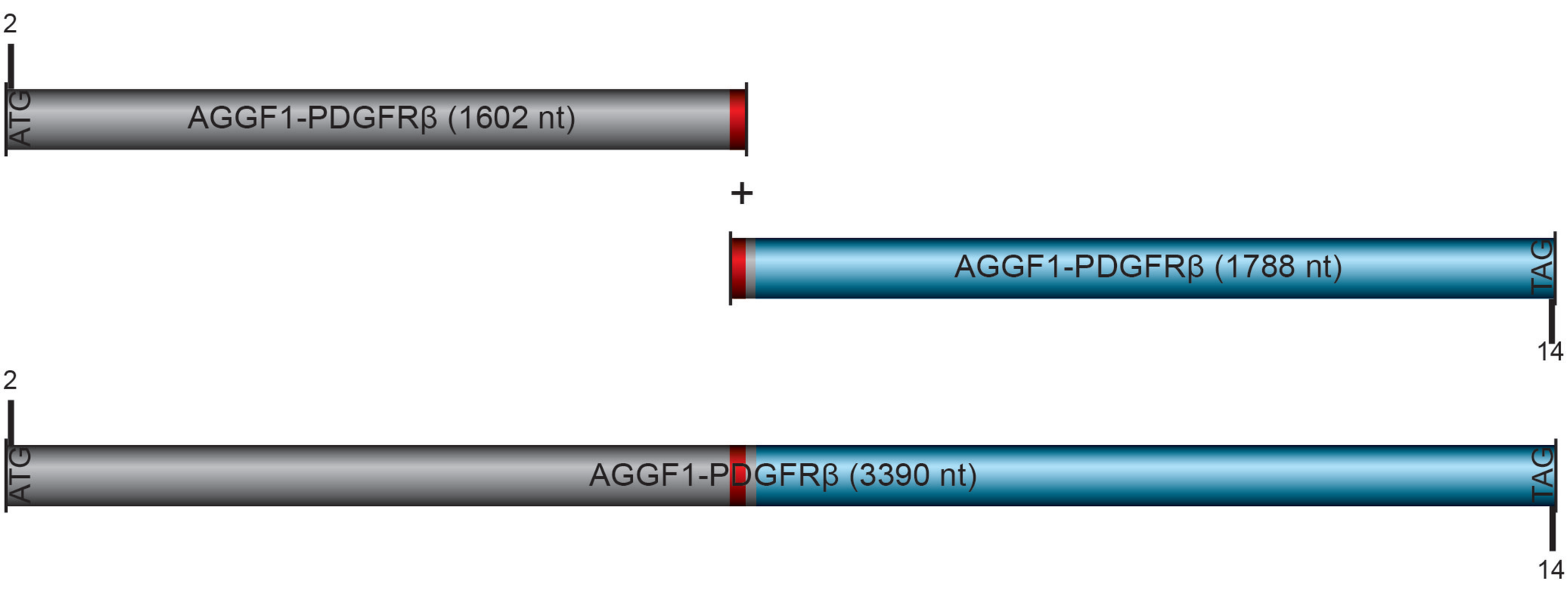


Figure S4

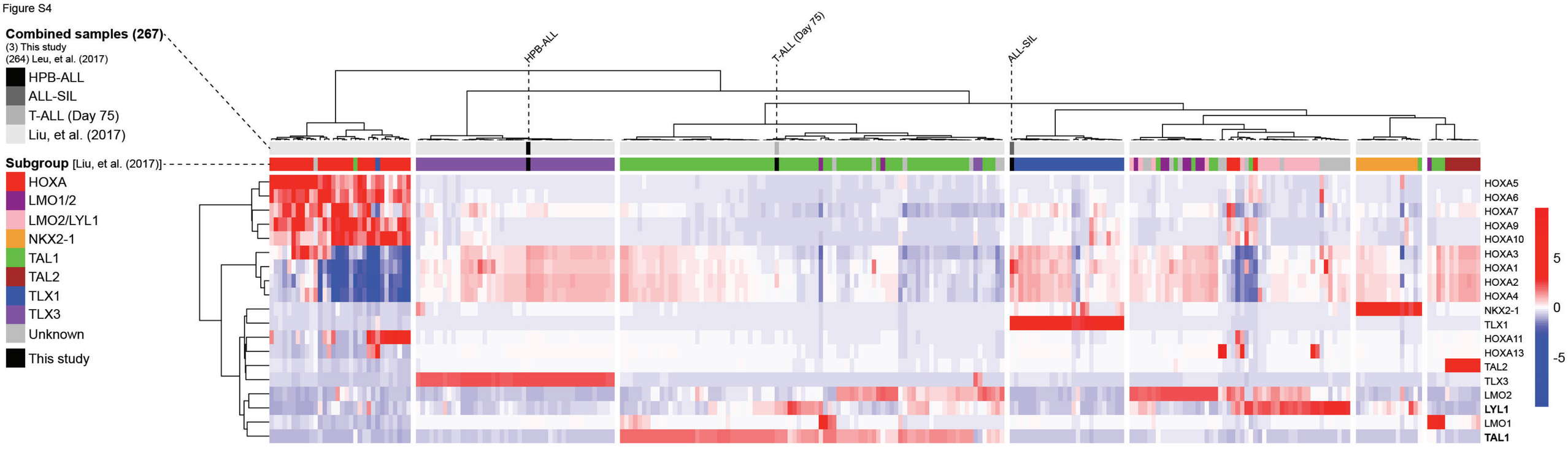

Combined samples (267)
(3) This study
(264) Leu, et al. (2017)
HPB-ALL
ALL-SIL
T-ALL (Day 75)
Liu, et al. (2017)
Subgroup [Liu, et al. (2017)]
HOXA
LMO1/2
LMO2/LYL1
NKX2-1
TAL1
TAL2
TLX1
TLX3
Unknown
This study
HPB-ALL
T-ALL (Day 75)
ALL-SIL
HOXA5
HOXA6
HOXA7
HOXA9
HOXA10
HOXA3
HOXA1
HOXA2
HOXA4
NKX2-1
TLX1
HOXA11
HOXA13
TAL2
TLX3
LMO2
LYL1
LMO1
TAL1
5
0
-5

**Table S1.** Clinical Response Metrics

| Day | Absolute blast count (per μL) | Marrow blast count (%) | CNS status* | Response** | Treatment*** | Treatment Duration |
|---|---|---|---|---|---|---|
| 1 | 548064 | 90 | 2 | Date of diagnosis | VCR, DEX, DAUNO, PEG | Day 1-22 |
| 14 | 4250 | – | – | Best clinical response | | |
| 22 | 12642 | 62 | 1 | PD | CYCLO, ARA-C, MP, VCR, PEG | Day 22-49 |
| 49 | 42 | 16 | 1 | SD | NELARABINE, CYCLO, ETOP | Day 49-75 |
| 75 | 3360 | – | – | PD | HD-ARA-C, ASNASE | Day 75-86 |
| 83 | 858 | 75 | 2 | PD | | |
| 87 | 18250 | – | – | PD | DASATINIB, VCR, DEX, DAUNO, PEG | Day 86-114 |
| 114 | 495 | 76 | 2 | SD | DASATINIB, HD-MTX, CYCLO, HD-ARA-C, DEX, VCR, PEG | Day 114-145 |
| 145 | 114 | 22 | 2 | SD | DASATINIB, VCR, MTX | Day 145-166 |
| 166 | 792 | – | – | PD | – | – |
| 172 | 267873 | – | – | PD | – | – |
| 176 | 454680 | – | – | PD | – | – |
| 180 | – | – | – | Date of death | – | – |

*CNS 1: In cerebral spinal fluid (CSF), absence of blasts on cytospin preparation, regardless of the number of white blood cells (WBCs); CNS 2: In CSF, presence of < 5/μL WBCs and cytospin positive for blasts or ≥ 5/μL WBCs with negative Steinherz Bleyer algorithm.

**PD: Progressive disease; SD: Stable disease.

***VCR: vincristine; DEX: dexamethasone; DAUNO: daunorubicin; PEG: pegaspargase; CYCLO: cyclophosphamide; ARA-C: cytarabine; MP: mercaptopurine; ETOP: etoposide; HD: high dose; ASNASE: asparaginase; MTX: methotrexate. Intrathecal chemotherapy was also delivered throughout each treatment phase.

**Table S2.** Primers used in this study

| No. | Primer name | Direction | Primer sequence | Purpose |
|---|---|---|---|---|
| 1 | AGGF1_5'-UTR_F | Forward | CCTCTGGTTTTCCGACTGCT | PCR of AGGF1 5'-UTR to PDGFRβ; Template for AGGF1 fragment |
| 2 | InF_NotI_AGGF1_F | Forward | AATTAGATCTCTCGAGGCGGCCGCATGGCCTCGGAGGCGCCG | Homology for insertion into pMIG (Red); Addition of NotI restriction site (Blue) |
| 3 | AGGF1_529_R | Reverse | CAGATGCTGGCTCCTGTGAA | Sequencing |
| 4 | AGGF1_1152_F | Forward | AGCCATTACCAGTGAAGGCA | Demonstrate the presence of the breakpoint; Sequencing |
| 5 | AGGF1_1588_F | Forward | CAGGTTAGAGCCCACCTTCG | PCR of AGGF1 to PDGFRβ 3'-end; Homology for joining AGGF1 & PDGFRβ; Sequencing |
| 6 | AGGF1_1602_R | Reverse | GTGGGCTCTAACCTGCCCTGGTTC | PCR AGGF1 for insertion into pMIG; Homology for joining AGGF1 & PDGFRβ |
| 7 | PDGFRβ_1781_R | Reverse | CCGTCAGAGCTCACAGACTC | PCR of AGGF1 5'-UTR to PDGFRβ; Template for AGGF1 fragment |
| 8 | PDGFRβ_1960_R | Reverse | GCATCTTGACGGCCACTTTC | Sequencing |
| 9 | PDGFRβ_2153_R | Reverse | AAGGTGTGTTTGTTGCGGTG | Demonstrate the presence of the breakpoint; Sequencing |
| 10 | PDGFRβ_2513_F | Forward | AGAACTGCGTCCACAGAGAC | Sequencing |
| 11 | PDGFRβ_2557_F | Forward | TGTGAAGGCAAGCTGGTCAA | Sequencing |
| 12 | PDGFRβ_3124_R | Reverse | GGATGATATAGTCGTTGTCACCC | Sequencing |
| 13 | PDGFRβ_3375_R | Reverse | CTACAGGAAGCTATCCTCTGCTTCC | PCR of AGGF1 to PDGFRβ 3'-end |
| 14 | InF_HpaI_PDGFRβ_R | Reverse | GGGGGGGGCGGAATTCGTTAACCTACAGGAAGCTATCCTCTGCTTC | Homology for insertion into pMIG (Red); Addition of HpaI restriction site (Blue) |

**Table S3.** FISH and cytogenetic findings before and after initiation of dasatinib

| Dasatinib | Cell type | PDGFRβ FISH (% positive) | Karyotype analysis |
|---|---|---|---|
| Before treatment | Peripheral blood† | 71.5 | – |
| | Bone marrow | – | 46,XY,add(3)(q21),add(5)(q11)[17]/46,XY[3], *Added material on 5q in 85% of cells* |
| 30 days after treatment | Peripheral blood | – | – |
| | Bone marrow†† | 19.5 | 46,XY,add(3)(q21),add(5)(q11)[2]/46,XY[18], *Added material on 5q in 10% of cells* |
| 60 days after treatment | Peripheral blood | – | – |
| | Bone marrow††† | 8 | ND* |

*ND: Not determined; †66% blasts by morphology; ††76% blasts by morphology; †††22% blasts by morphology.

**Table S4.** FPKM values extracted from transcriptome data for the T-ALL Day 75 specimen and the HPB-ALL and ALL-SIL cell lines

| Gene ID | T-ALL (Day 75) | HPB-ALL | ALL-SIL |
|---|---|---|---|
| TAL1 | **14.8** | 0.0 | 0.0 |
| LYL1 | **8.0** | 0.5 | 0.6 |
| TLX1 | 0.0 | 0.0 | **9.4** |
| TLX3 | 0.1 | **15.5** | 0.0 |
| HOXA1 | 0.1 | 0.0 | 0.4 |
| HOXA2 | 0.2 | 0.0 | 0.1 |
| HOXA3 | 0.0 | 0.0 | 0.0 |
| HOXA4 | 0.0 | 0.0 | 0.0 |
| HOXA5 | 0.0 | 0.0 | 0.0 |
| HOXA6 | 0.0 | 0.0 | 0.0 |
| HOXA7 | 0.0 | 0.0 | 0.0 |
| HOXA9 | 0.1 | 0.0 | 0.0 |
| HOXA10 | 0.0 | 0.0 | 0.0 |
| HOXA11 | 0.1 | 0.0 | 0.0 |
| HOXA13 | 0.1 | 0.0 | 0.0 |
| LMO1 | 0.1 | 0.0 | 0.0 |
| LMO2 | 0.9 | 0.0 | 0.0 |
| NKX2-1 | 0.0 | 0.0 | 0.0 |
| TAL2 | 0.2 | 0.2 | 0.2 |