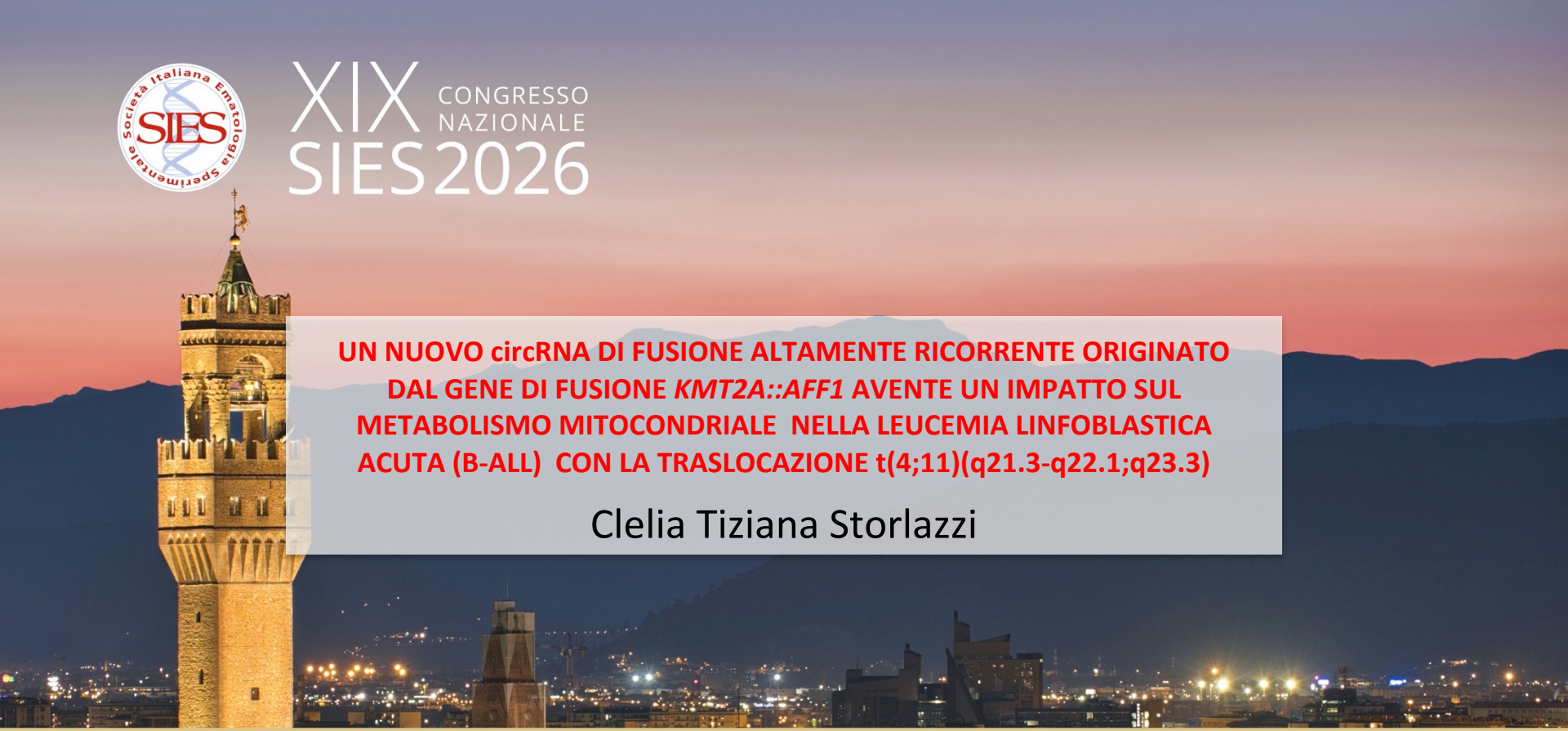


XIX CONGRESSO NAZIONALE SIES 2026

UN NUOVO circRNA DI FUSIONE ALTAMENTE RICORRENTE ORIGINATO DAL GENE DI FUSIONE *KMT2A::AFF1* AVENTE UN IMPATTO SUL METABOLISMO MITOCONDRIALE NELLA LEUCEMIA LINFOBLASTICA ACUTA (B-ALL) CON LA TRASLOCAZIONE t(4;11)(q21.3-q22.1;q23.3)

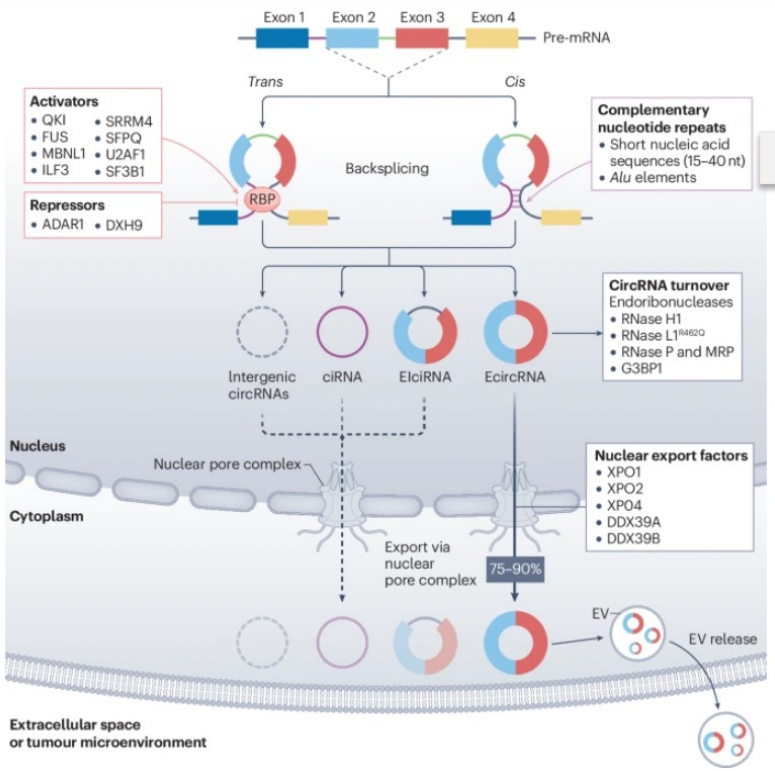
Clelia Tiziana Storlazzi

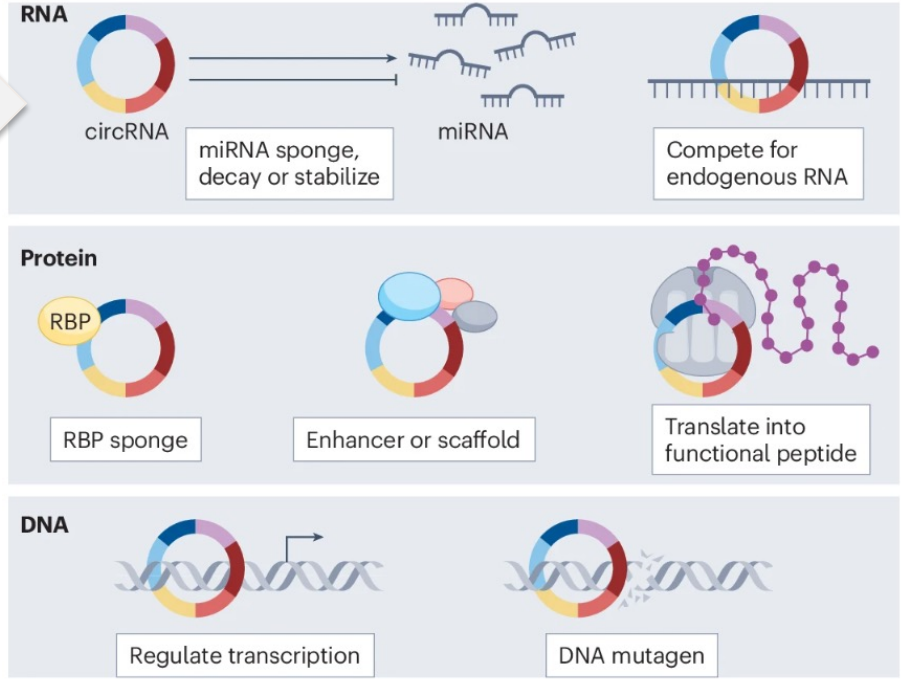
Firenze | 4-6 marzo 2026
Palazzo degli Affari

Disclosures of Clelia Tiziana Storlazzi

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other

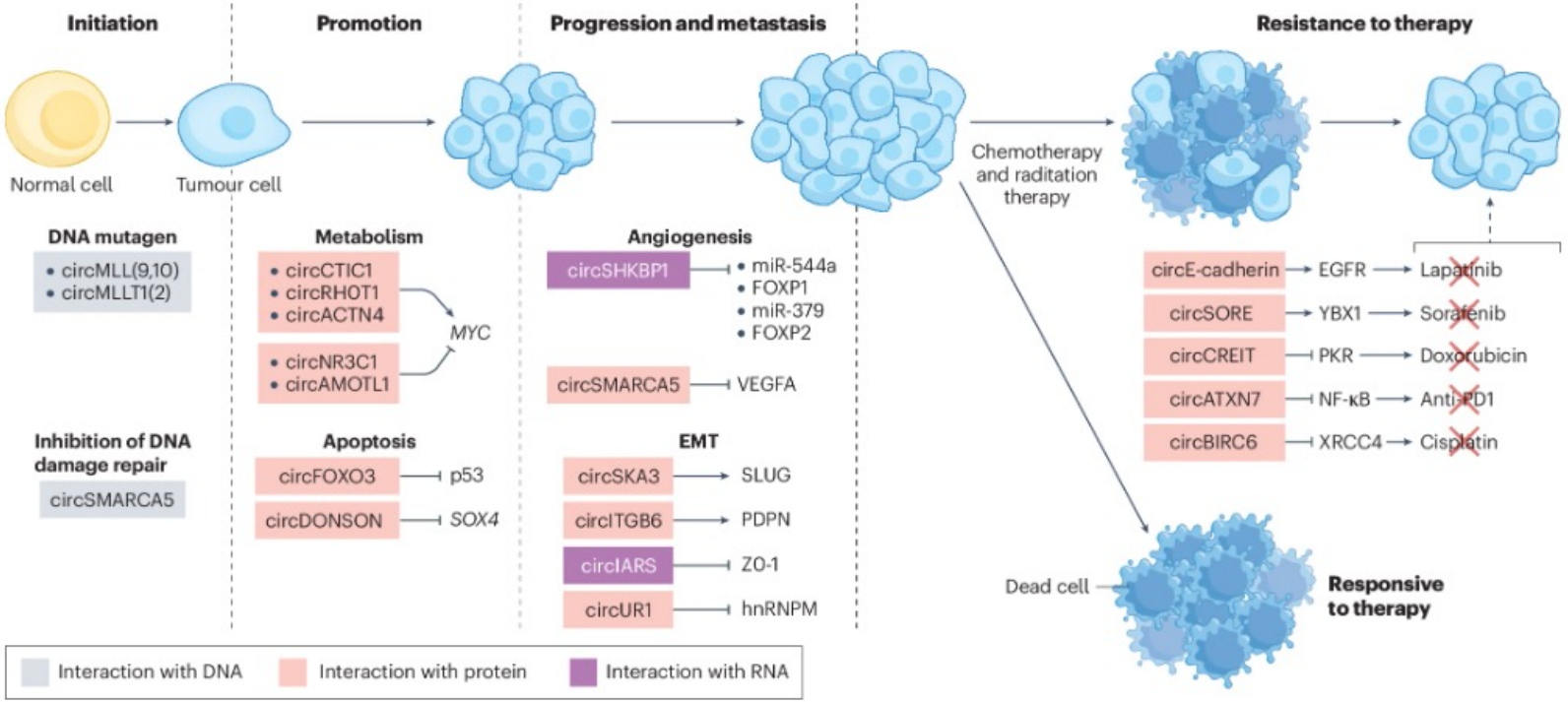
Circular RNA biogenesis and function





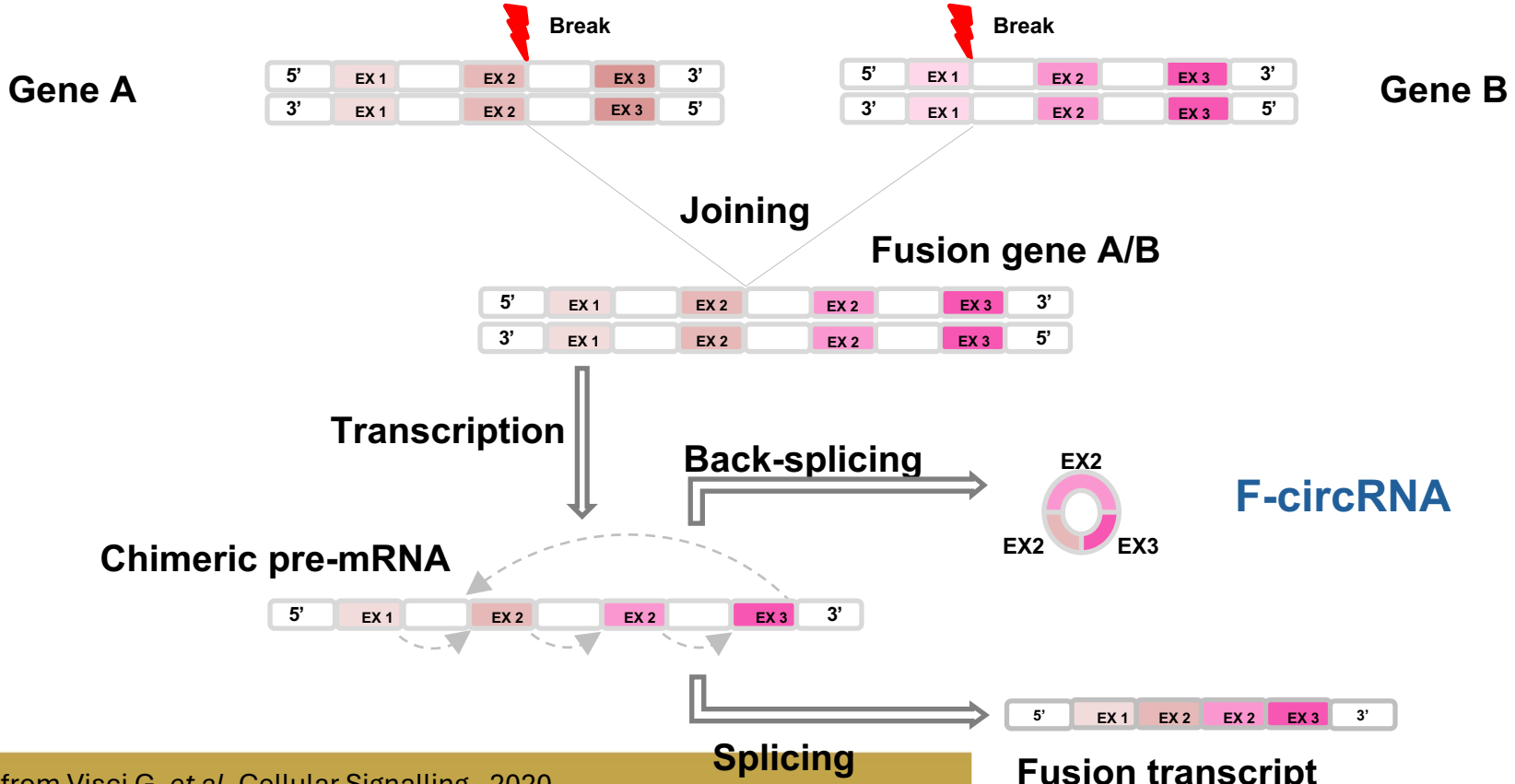
Conn, V.M., et al. *Nat Rev Cancer* 2024

Functional roles of circular RNAs at each stage of oncogenesis.



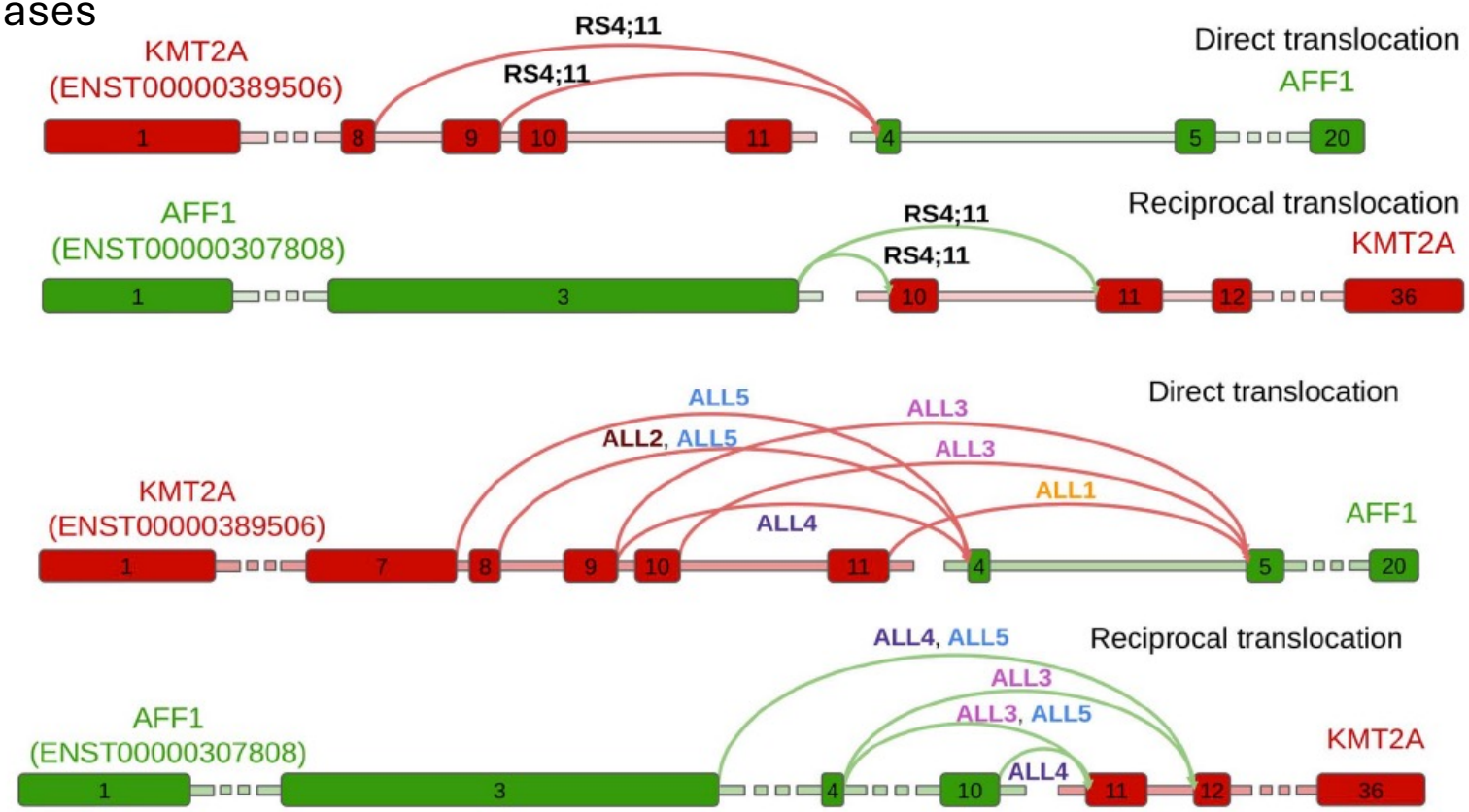
Conn, V.M., et al. *Nat Rev Cancer* 2024

Genomic rearrangement (e.g. Translocation)



Adapted from Visci G, *et al.* Cellular Signalling, 2020

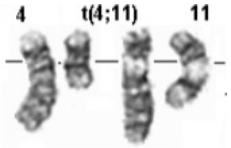
Adult B-cell acute lymphoblastic leukemia (B-ALL) cell line (RS4;11) and pediatric B-ALL cases



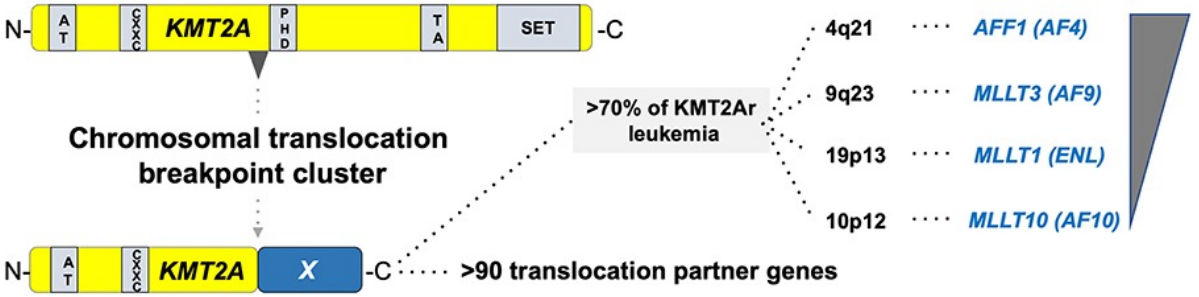
Dal Molin A, *et al.* Brief Bioinform, 2023

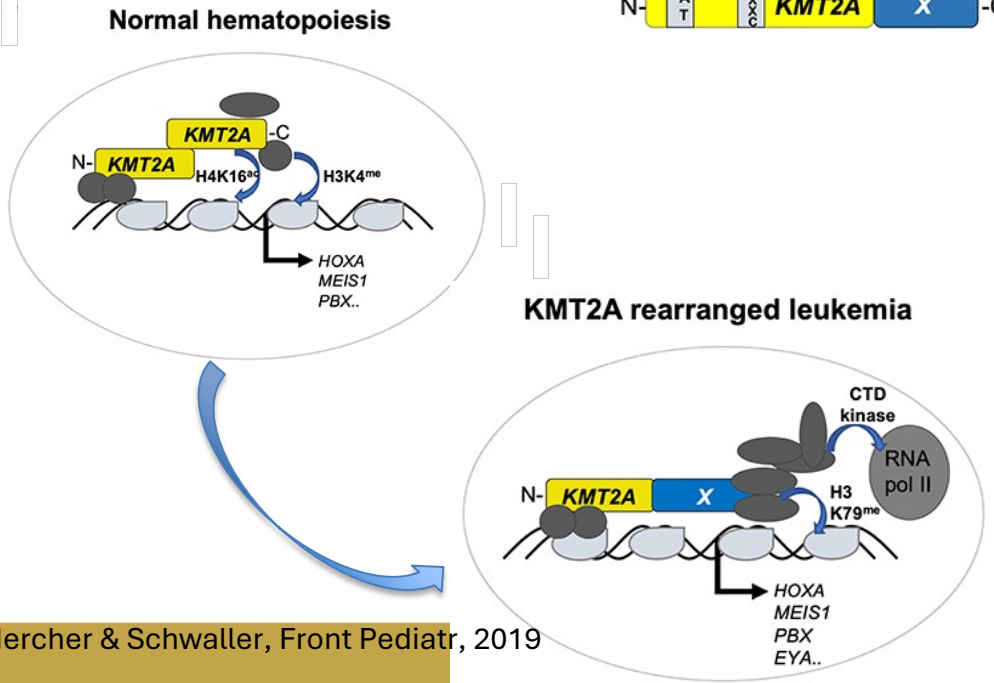
INTRODUCTION

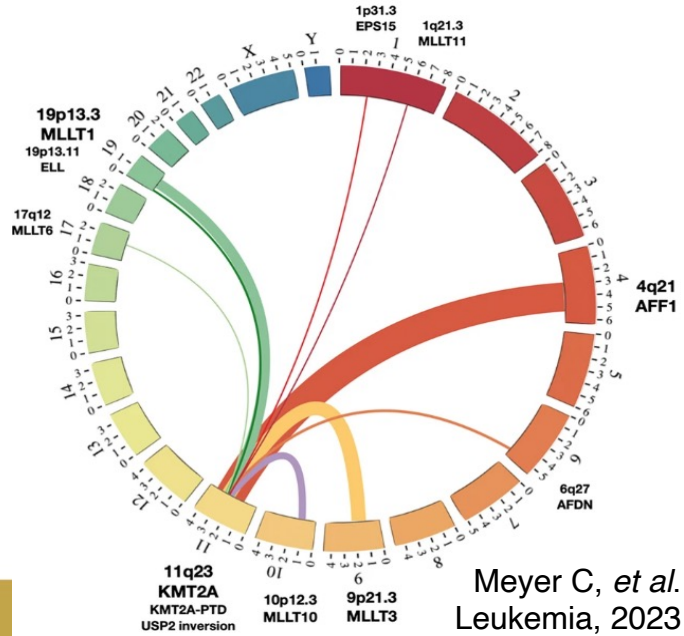
KMT2A::AFF1 fusion gene



t(4;11)(q21.3;q23.3)
KMT2A::AFF1
***AFF1* (4q21)**
***KMT2A* (11q23)**







Mercher & Schwaller, Front Pediatr, 2019

Meyer C, *et al.* Leukemia, 2023

AIM OF THE STUDY

Identification of fusion circRNAs originated by *KMT2A::AFF1* in pediatric and adult B-ALL cell lines and patients and characterization of their potential oncogenic role.

MATERIALS & METHODS

- RT-/RT-qPCR in:

- t(4;11) positive SEM, RS4;11 and ALL PO B-ALL cell lines
- 10 pediatric B-ALL patients with t(4;11) + 2 relapse/20 patients without t(4;11)
- 10 adult B-ALL patients with t(4;11)+1 relapse/3 patients without t(4;11)

- DsiRNA knockdown of the *KMT2A::AFF1* f-circRNA in the SEM cell line.
- Total RNA-seq of KD silenced SEM cells (Illumina, 2x150 bp; DESeq2, v.1.38.3).
- Confocal, Seahorse bioenergetic profiling and electron microscopy
- Cell death analysis (Cell Death Detection ELISA kit)

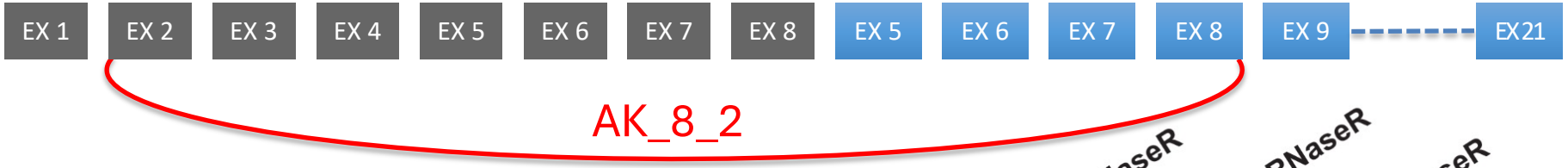
RESULTS

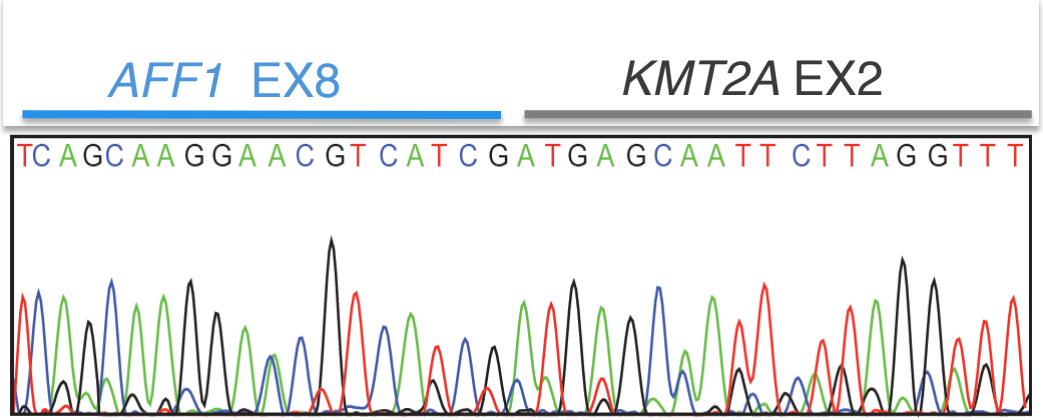
F-circRNA identification

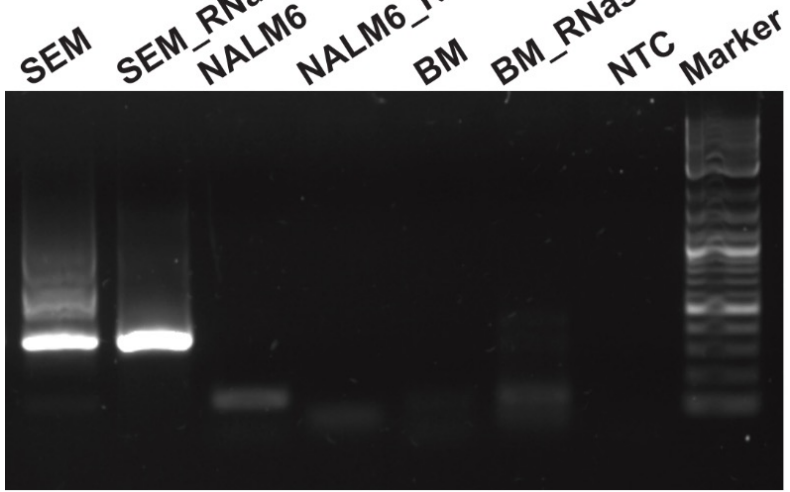
KMT2A
NM_005933.4

374 bp

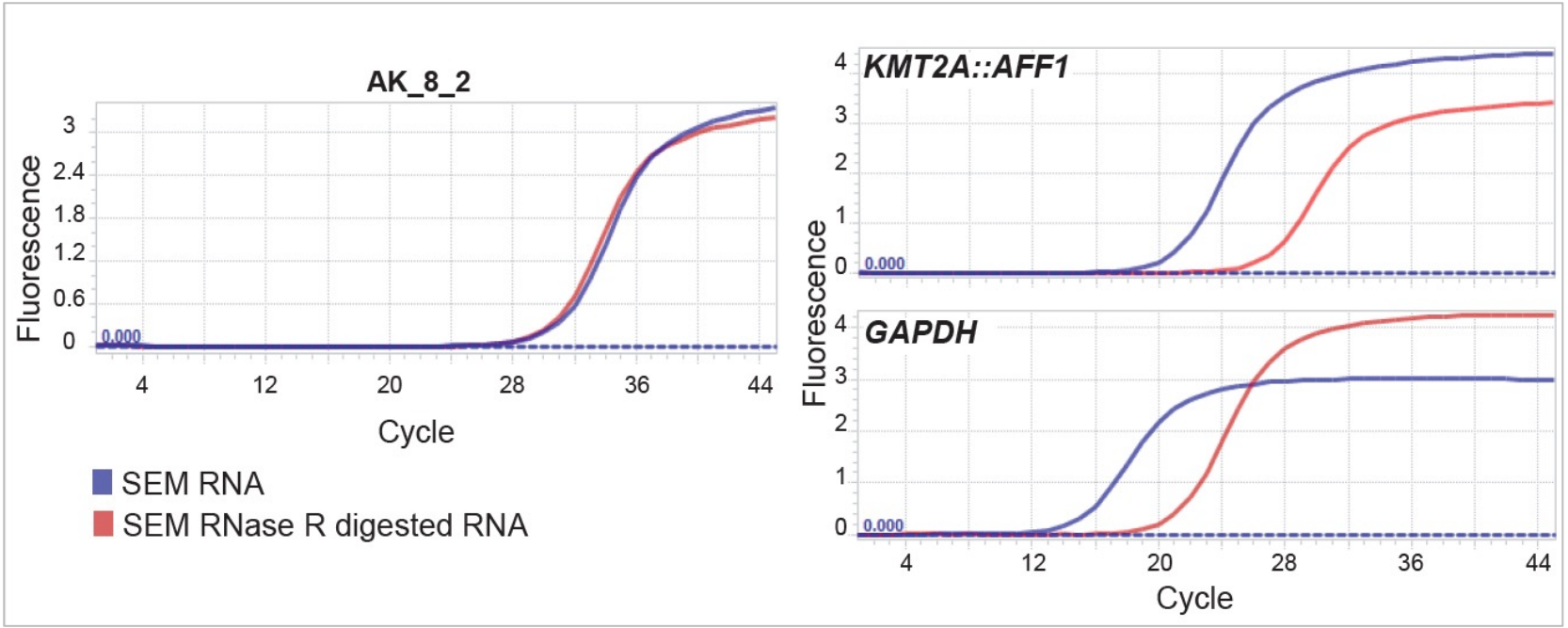
AFF1
NM_001166693.3





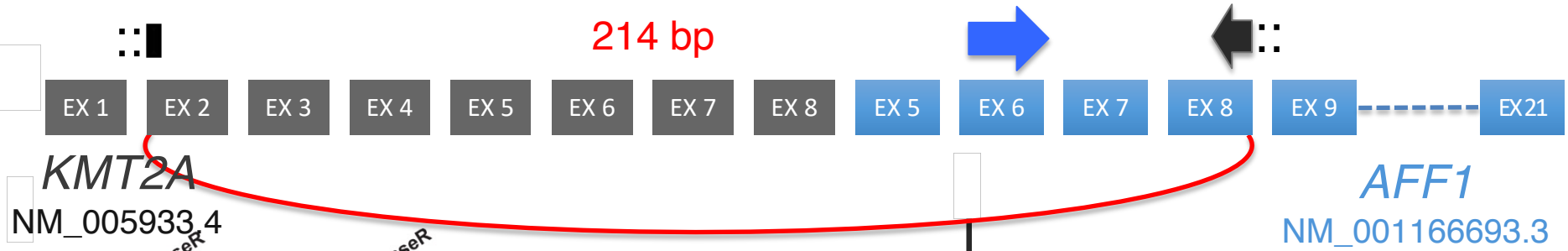


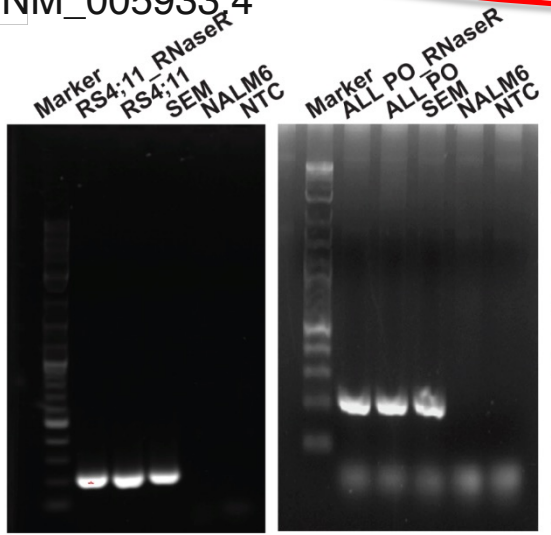
AK_8_2 RT-qPCR analysis on SEM samples RNase R digested/not digested

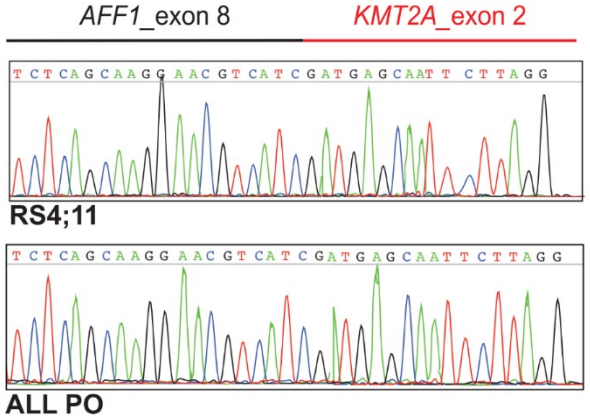


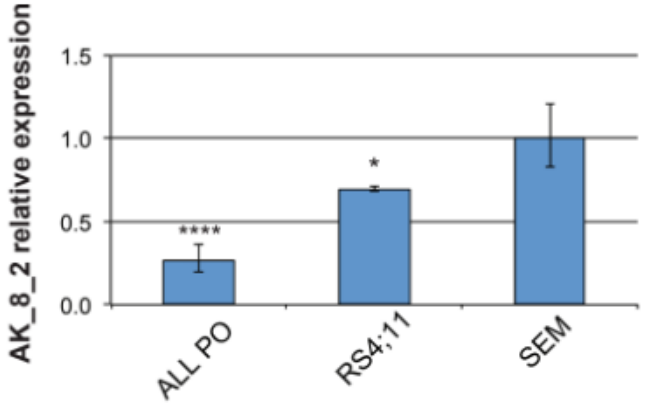
The back-splicing product expression level is the same after RNAse R digestion, differently from *KMT2A::AFF1* linear and *GAPDH* transcripts.

AK_8_2 is expressed in RS4;11 and ALL PO cell lines

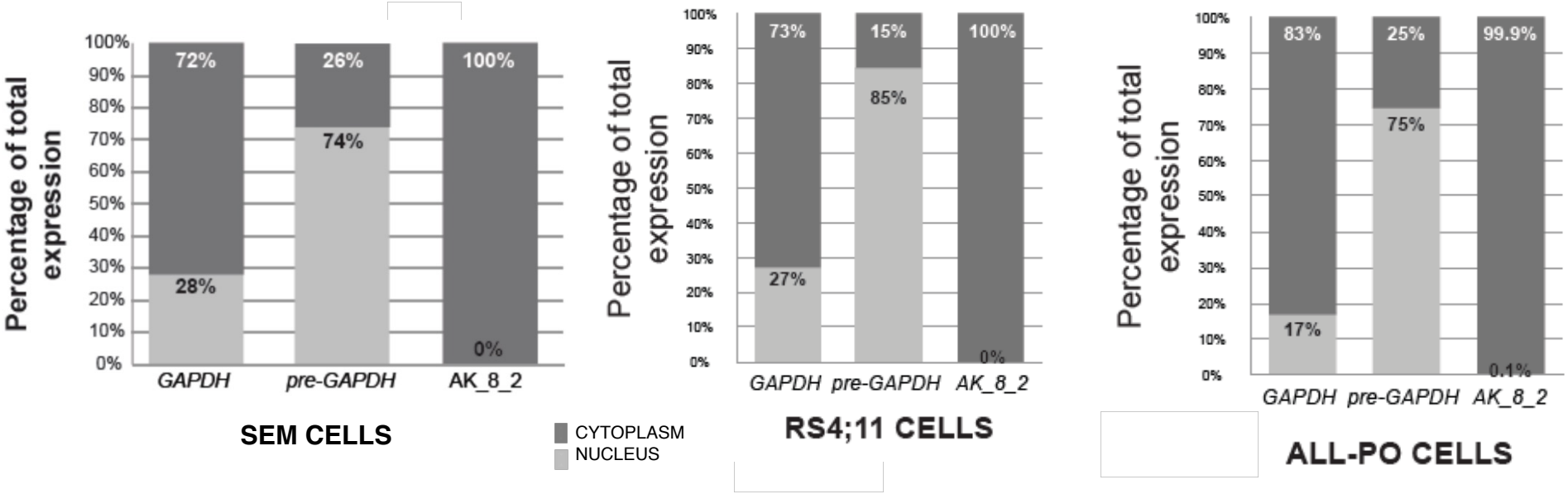






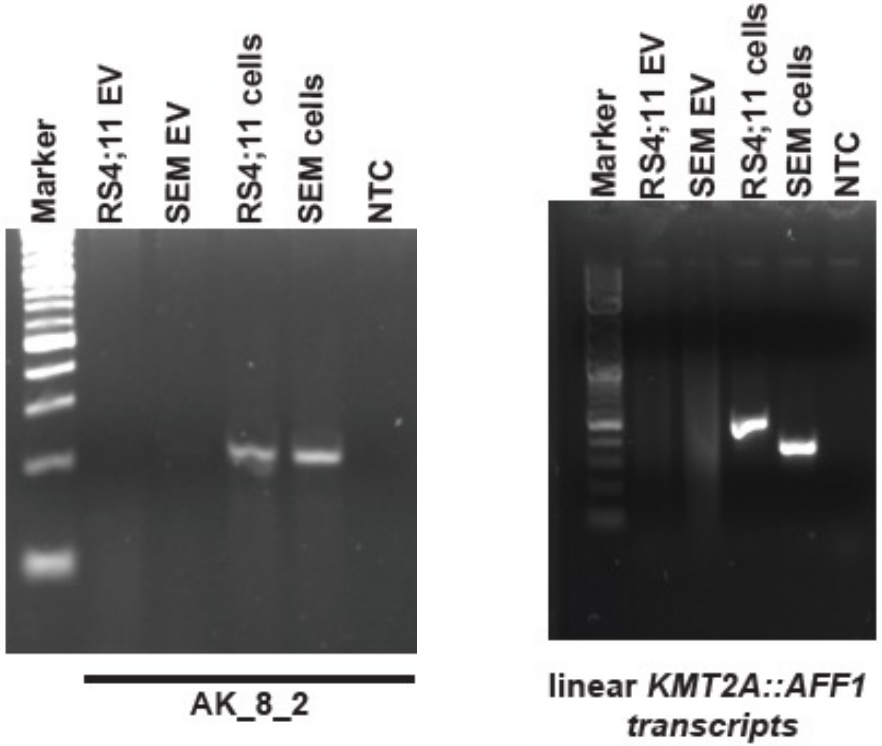


AK_8_2 is expressed in the cytoplasm of t(4;11)+ B-ALL cells

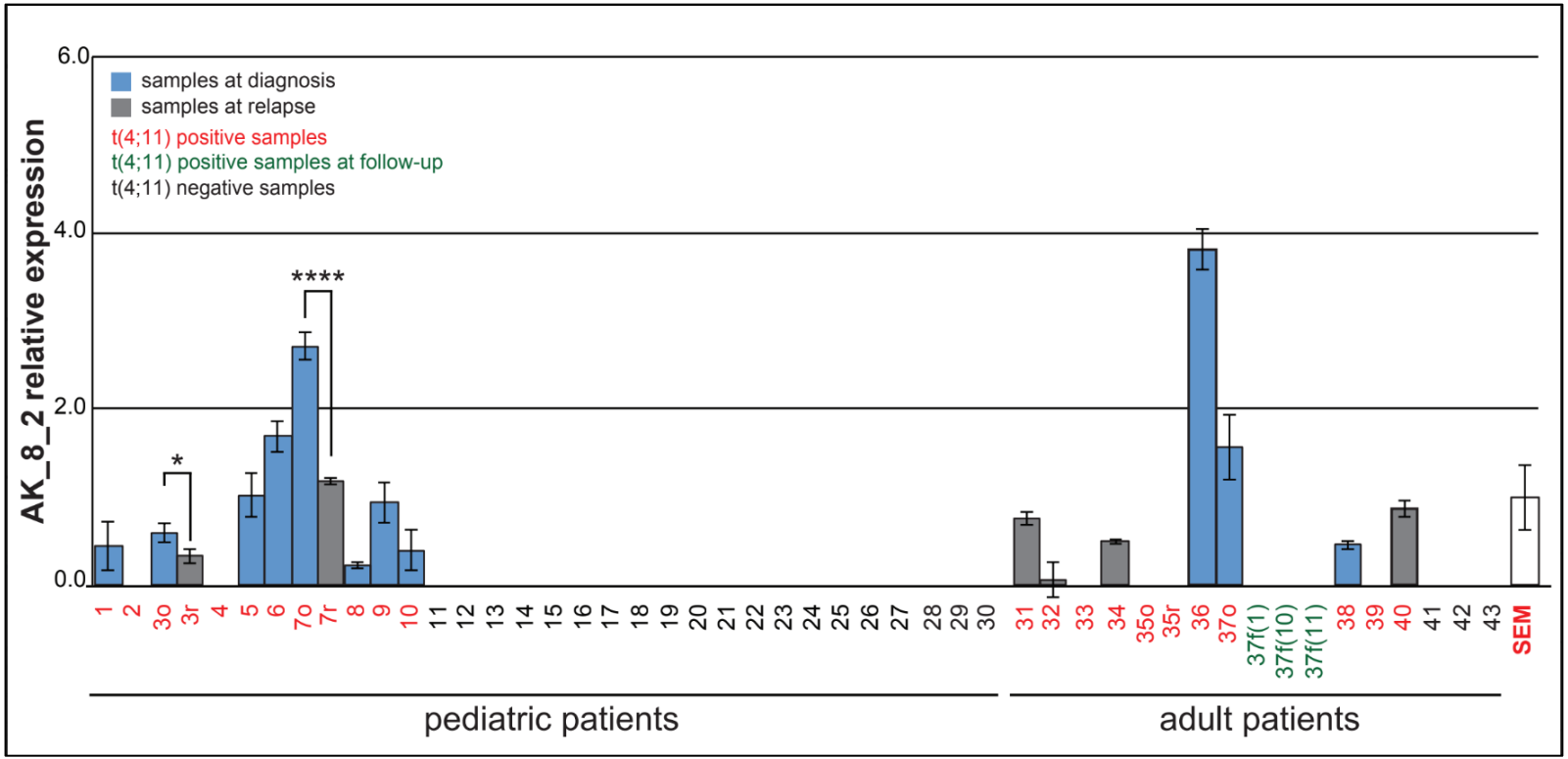


AK_8_2 subcellular localization analysis: RT-qPCR on fractionated RNA (nuclear/cytosolic RNAs) from SEM, RS4;11, and ALL PO cell lines

No secretion of AK_8_2 and linear *KMT2A::AFF1* in EVs of SEM and RS4;11 cell lines



AK_8_2 is specifically expressed in t(4;11) positive B-ALL patients

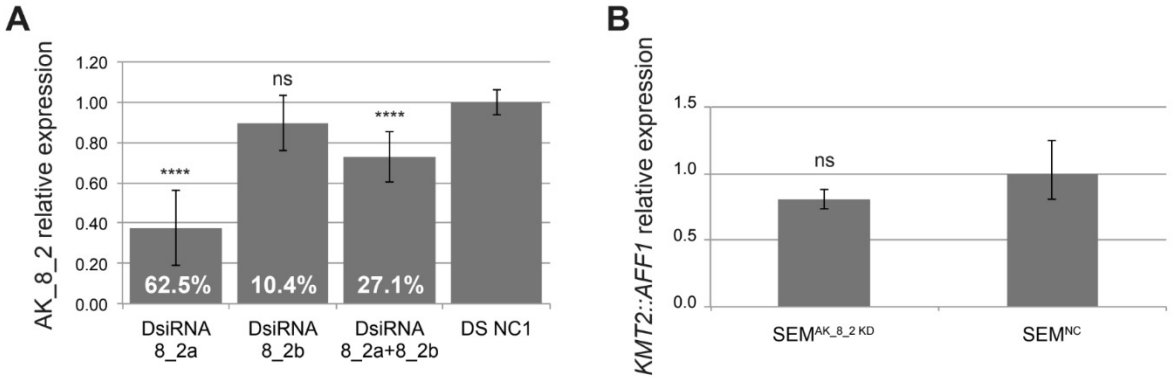


AK_8_2 transient silencing on SEM cell line and RNA-seq of the silenced cells

DsiRNA designed on the AK_8_2 back-splicing junction:

AK_8_2 AATTCTCAGCAAGGAACGTCATCGATGAGCAATTCTTAGGTTTTGG

DsiRNA 8-2a GGAACGTCATCGATGAGCAATTCTT

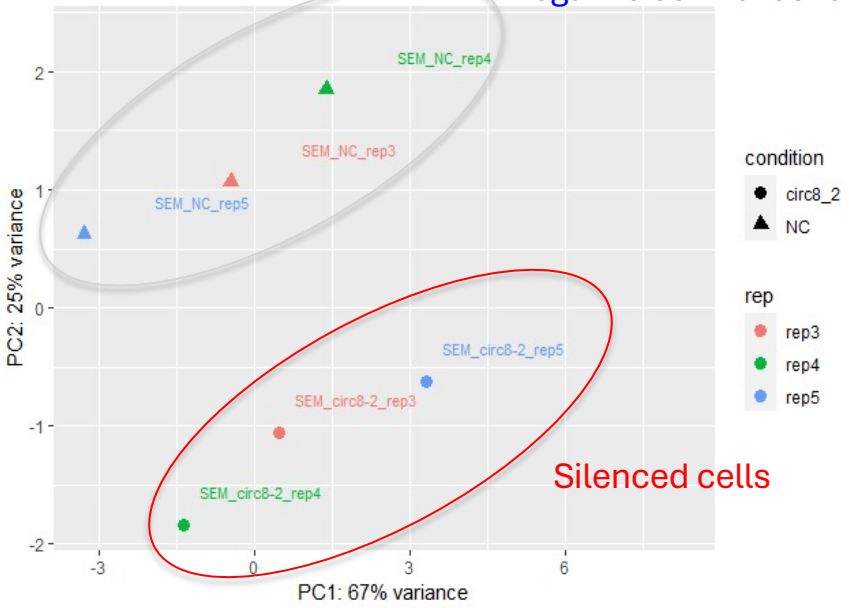


We achieved 62.5% silencing in the SEM cell line.

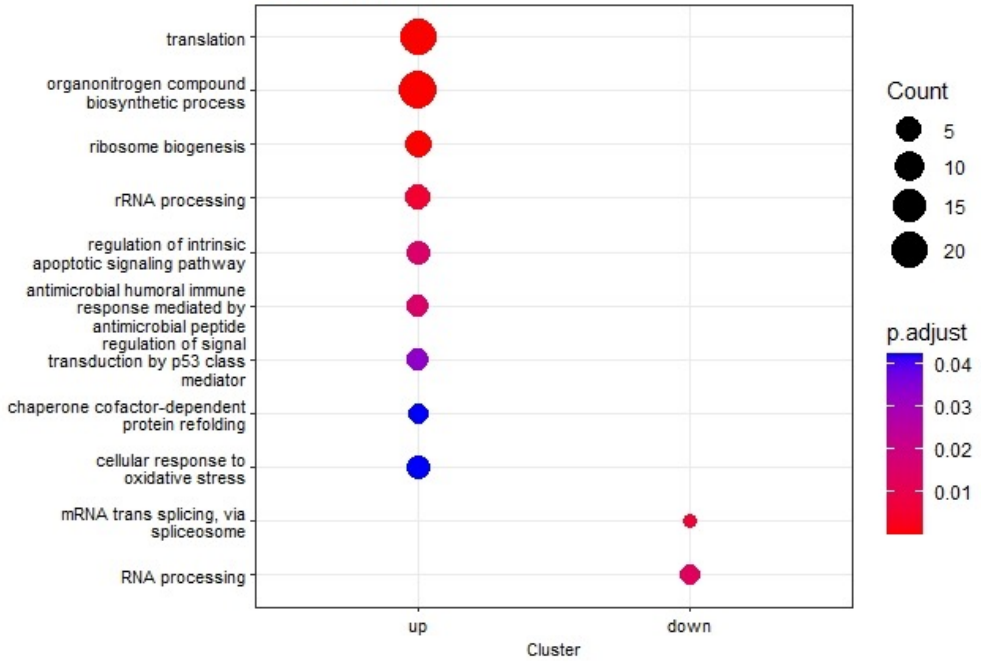
Unpublished data, please do not post

Three biological replicates of silenced AK_8_2 SEM cells and control cells (transfected with the negative control DsiRNA) underwent to total RNA sequencing.

PCA ANALYSIS

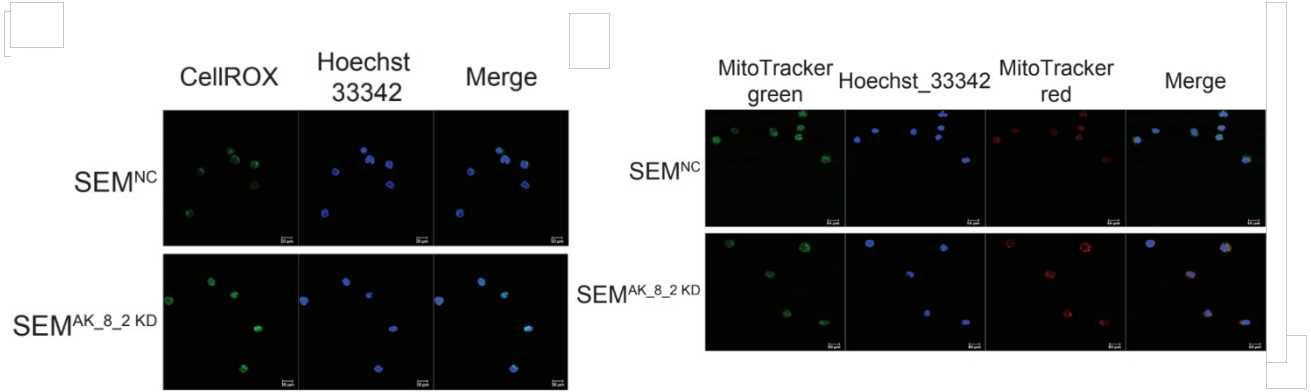


ORA on GO-BP

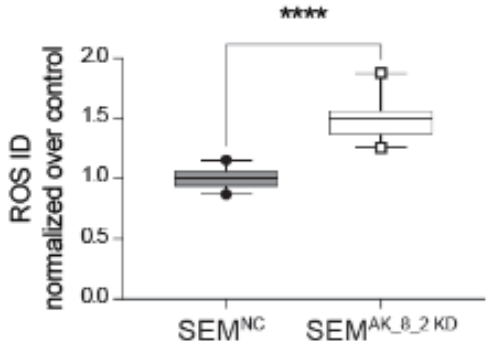


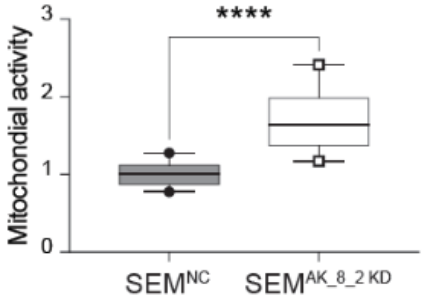
Unpublished data, please do not post

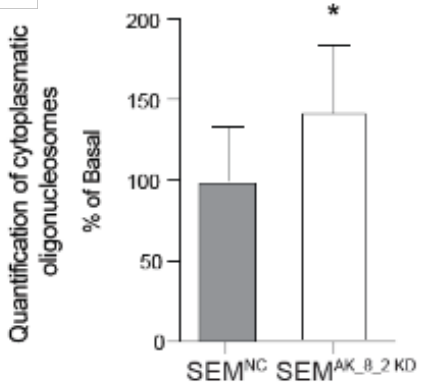
Increased ROS activity and MMP in AK_8_2 KD cells vs. controls.



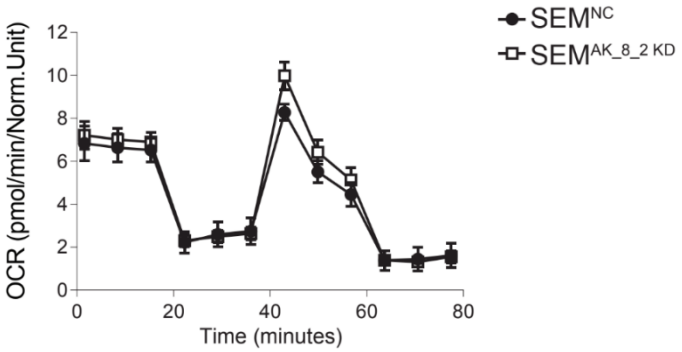
Increased cell death in AK_8_2 KD cells vs. controls.

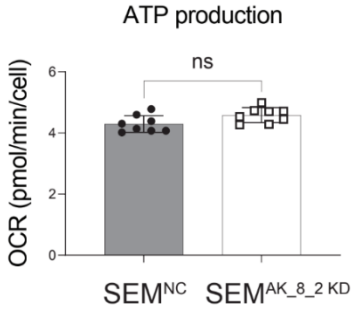


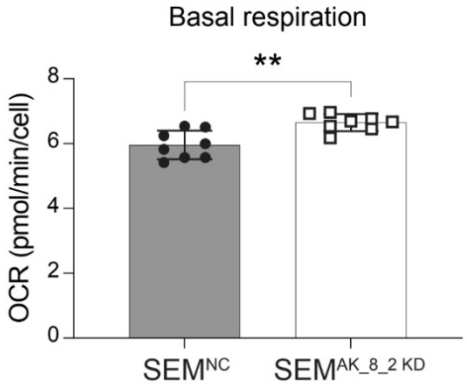


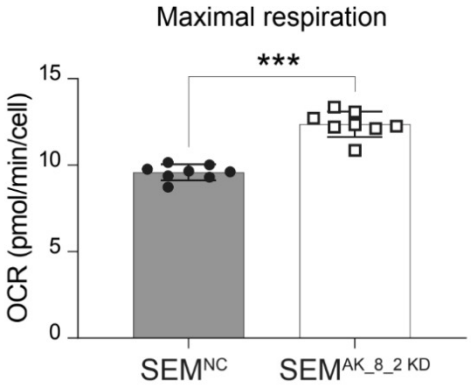


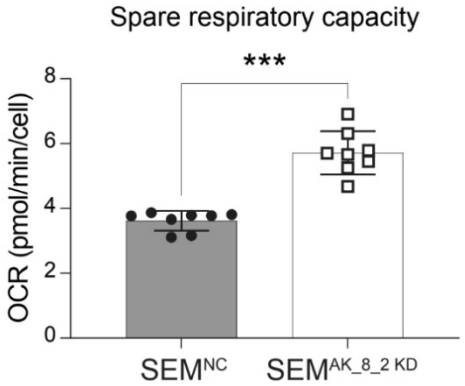
AK_8_2 knockdown in SEM cells affects mitochondrial metabolism







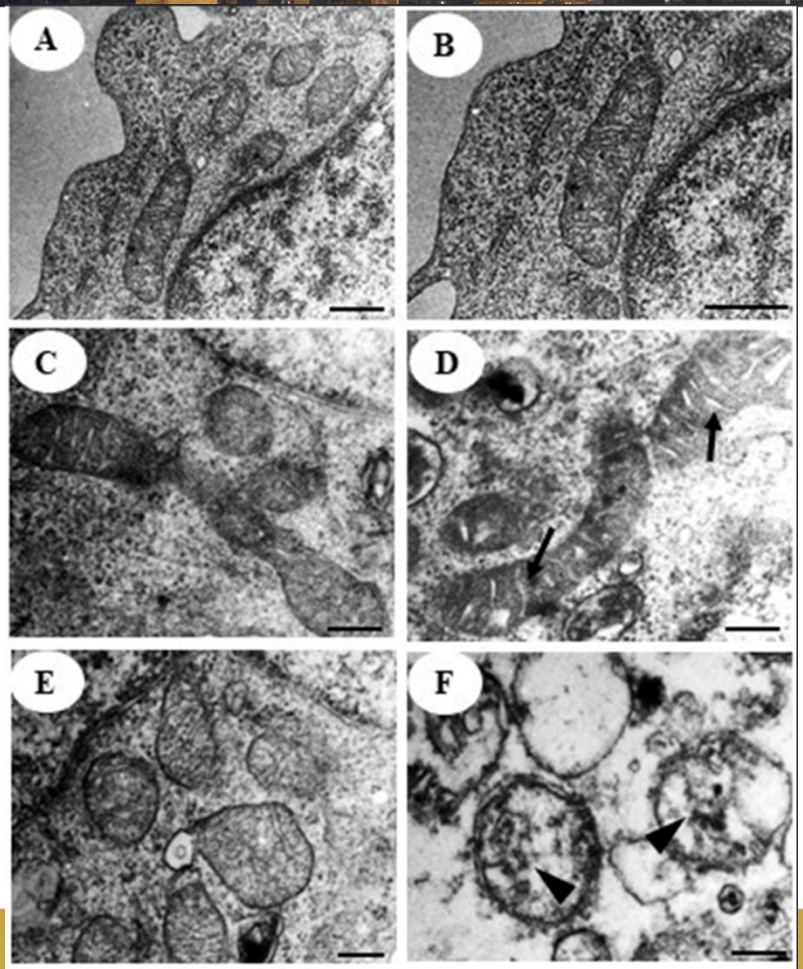




untreated SEM cells

SEM^{NC}

$SEM^{AK\_8\_2\ KD}$



AK_8_2 knockdown induces dysmorphic mitochondrial ultrastructure *in vitro*.

➡ large space between the outer and inner membrane

➤ Swollen mitochondria with fragmented cristae

Unpublished data, please do not post

CONCLUSIONS (1)

- We identified a novel f-circRNA back-splicing junction from the *KMT2A::AFF1* chimeric gene in B-ALL.
- AK_8_2 is not found in cases without the t(4;11) translocation, suggesting its connection with the linear chimera.
- AK_8_2 shows a predominant cytoplasmic localization in SEM cells.
- AK_8_2 is not secreted in EVs, suggesting an intracellular functional role.

CONCLUSIONS (2)

- AK_8_2 silencing affects ROS metabolism and the intrinsic apoptotic signaling pathway.

- AK_8_2 silencing impacts the structure of the mitochondria (with electron and confocal microscopy) and their functions (with Seahorse assays) in silenced and control SEM cells.

- AK_8_2 enables leukemia cells to survive under stressful conditions and to avoid apoptosis induced by high ROS levels or impaired mitochondrial function, thereby conferring a survival advantage

FUTURE PERSPECTIVES

- Increase the size of the pediatric and adult B-ALL patient cohorts.

- Validate the results in RS4;11 and ALL PO KD model vs. controls

Thank you for your attention!

UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

Doron Tolomeo
Santina Venuto
Lucrezia Calamo
Grazia Visci
Angelo Lonoce
Maria Mastrodonato
Daniela Semeraro

istituto tumori Giovanni Paolo II - Bari IRCCS

Amalia Azzariti
Simona Serrati

ISTITUTO ROMAGNOLO PER LO STUDIO DEI TUMORI DINO AMADORI

Anna Ferrari
Giorgia Simonetti

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Giovanni Martinelli

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UNIVERSITÀ DEGLI STUDI DI MILANO BICOCCA

itb

Marco Severgnini
Ingrid Cifola

unipg UNIVERSITÀ DEGLI STUDI DI PERUGIA

Roberta La Starza
Martina Lucarelli

Pediatric Oncology AOUC, Policlinico of Bari

Nicola Santoro

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