UNIVERSITÀ DI PARMA

DIPARTIMENTO DI SCIENZE CHIMICHE, DELLA VITA E DELLA SOSTENIBILITÀ AMBIENTALE

CORSO DI LAUREA MAGISTRALE IN
SCIENZE BIOMOLECOLARI GENOMICHE E CELLULARI

SERCA-Ca^{2+} Modulation Impairs Glucocorticoid Resistance in *NOTCH1*-Mutated T-Cell Acute Lymphoblastic Leukemia

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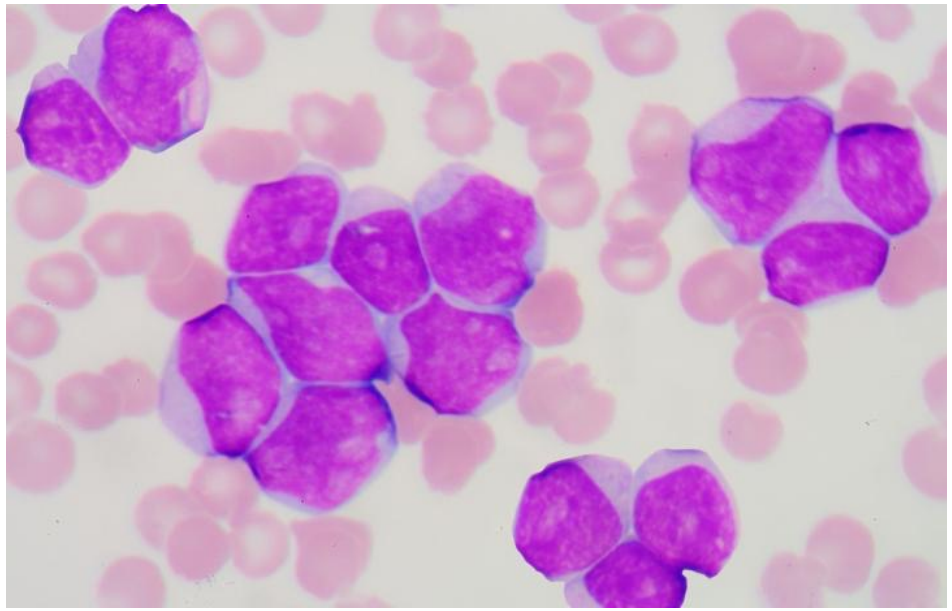
Relatore: Prof. Giovanni Roti

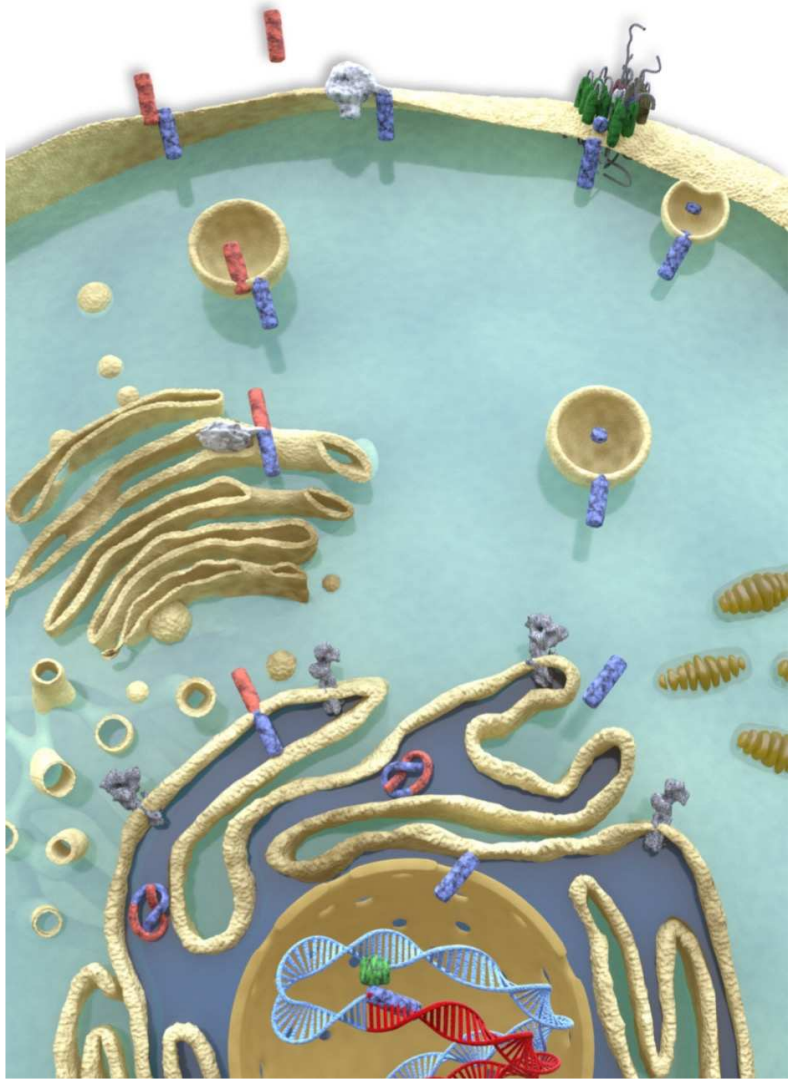
Anno accademico 2021/2022

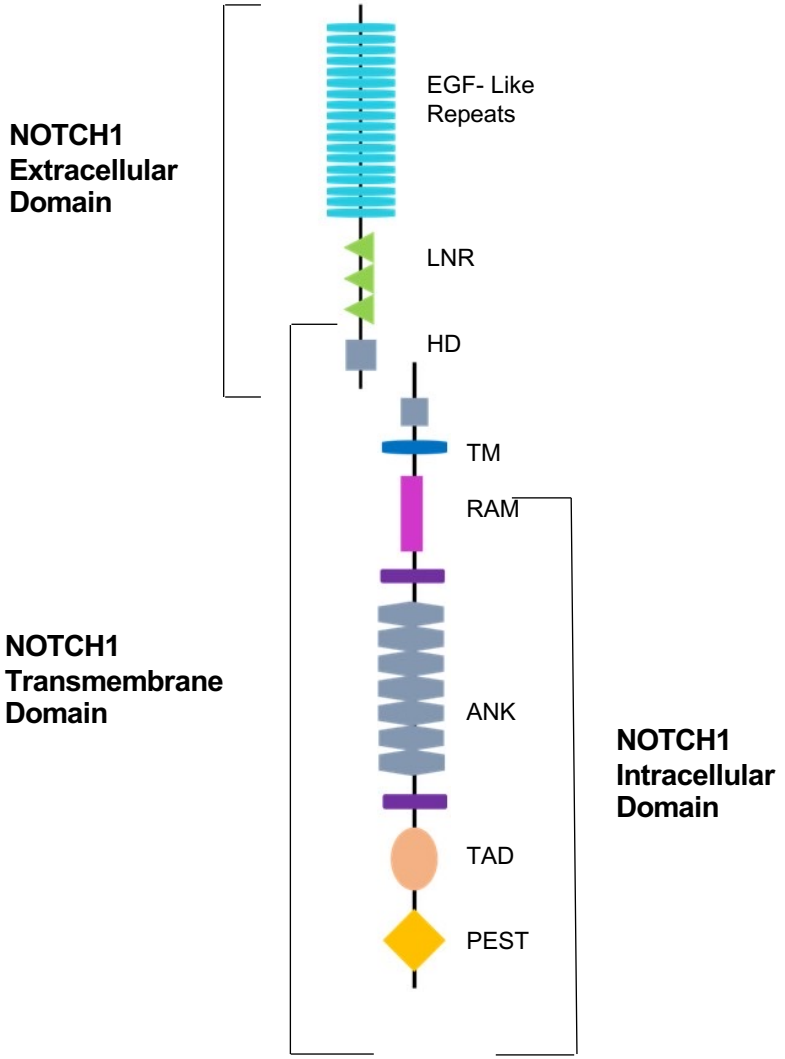
T-Cell Acute Lymphoblastic Leukemia (T-ALL)

- T cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematologic tumor resulting from the malignant transformation of T cell progenitors
- High tumour burden with uncontrolled clonal expansion of malignant lymphoid cells
- It counts for 10–15% of paediatric and 25% of adult acute lymphoblastic leukemia (ALL) cases
- *NOTCH1* mutations are observed in 40% to 70% of childhood and adult T-cell acute lymphoblastic leukemia (T-ALL)



The Notch1 Pathway

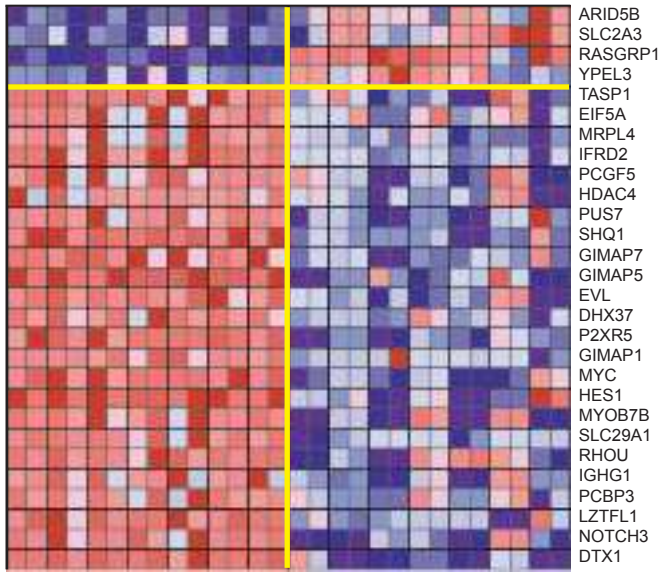




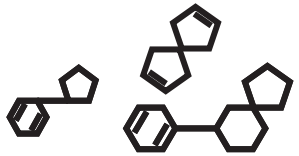
Pagliaro et al. 2021, Journal of Hemat & Oncol

P-Type ATPase-SERCA and NOTCH1

Vehicle *Compound E*

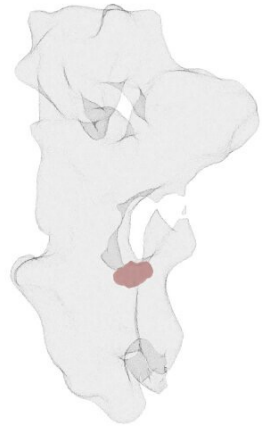


NOTCH1 On *NOTCH1 Off*



GE-HTS small molecules screen

cDNA enhancer activity screen

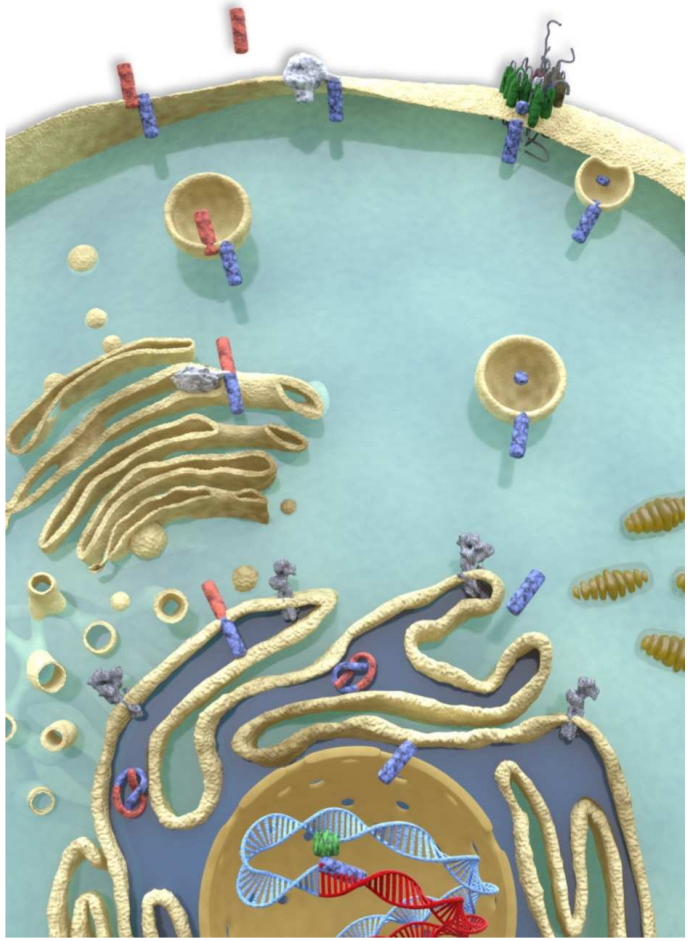


SERCA Inhibitors

Thapsigargin, CPA

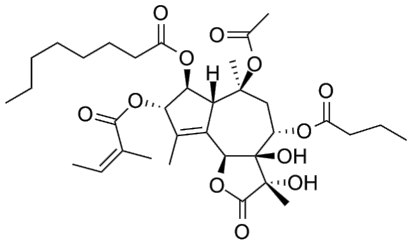
Roti et al. 2013, Cancer Cell

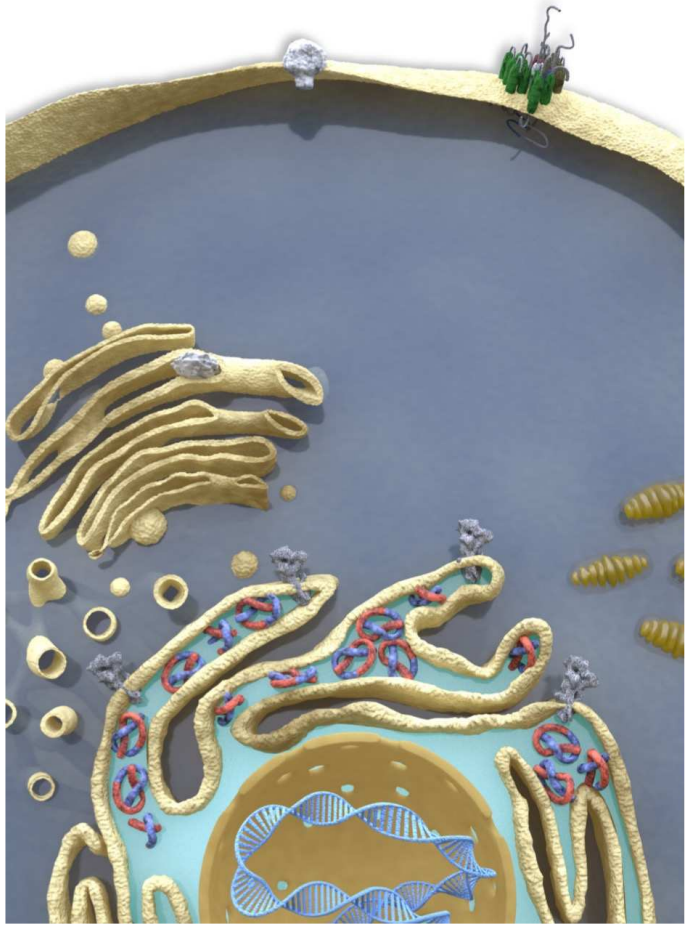
SERCA and NOTCH1



SERCA Inhibitors

Thapsigargin, CPA





Pagliaro et al. 2021, Journal of Hemat & Oncol

Thapsigargin

LIMITATIONS

- Unmodified TG is difficult to administer and deliver systemically without significant nonspecific host toxicity (cardiac toxicity).

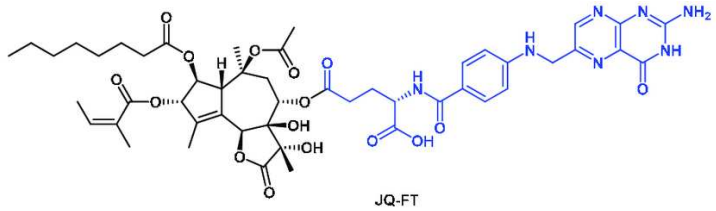
- TG-induced inhibition of SERCA leads to depletion of the ER Ca^{2+} pool. Activates plasma membrane calcium channels, thereby resulting in an influx of extracellular calcium.

SOLUTIONS

- Improve TG delivery (tumor specificity):

- Antibody-based strategy
- Folic acid endocytosis strategy

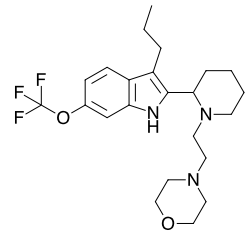
JQ-FT



Roti et al. 2018, JEM

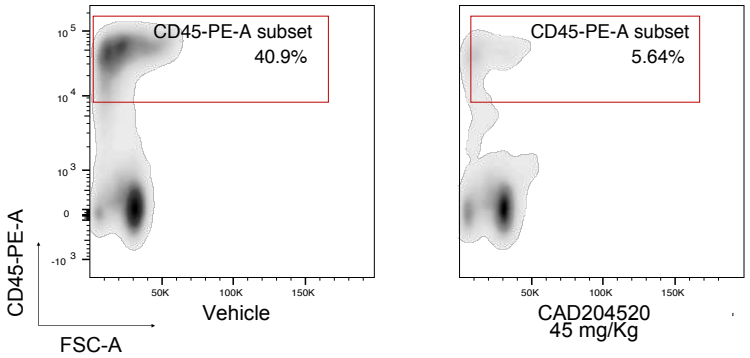
- Generate isoform specific SERCA inhibitors

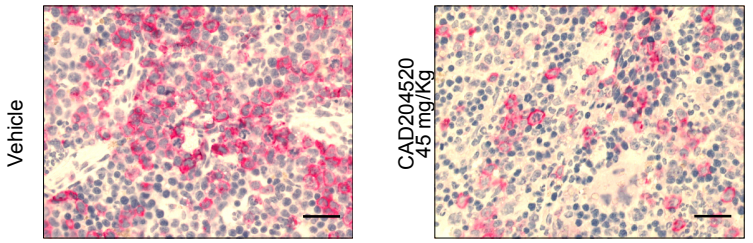
CAD204520

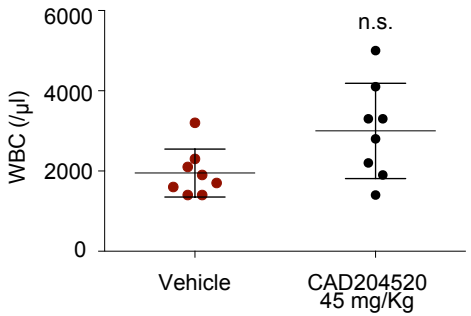


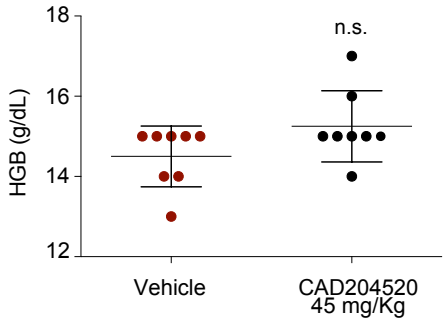
Marchesini et al. 2020, Cell Chem Biol

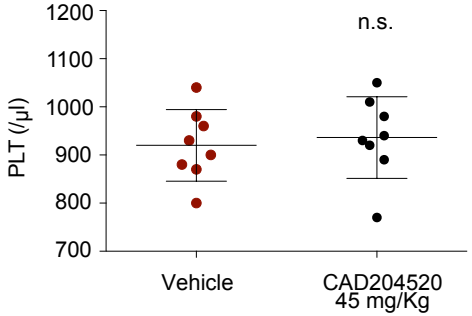
CAD204520 Anti-*NOTCH1* Mutated Leukemia Specific Effects

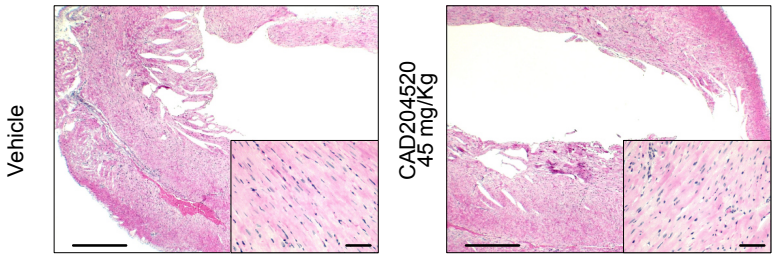


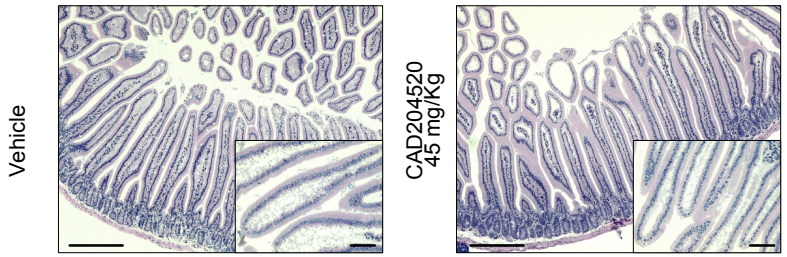






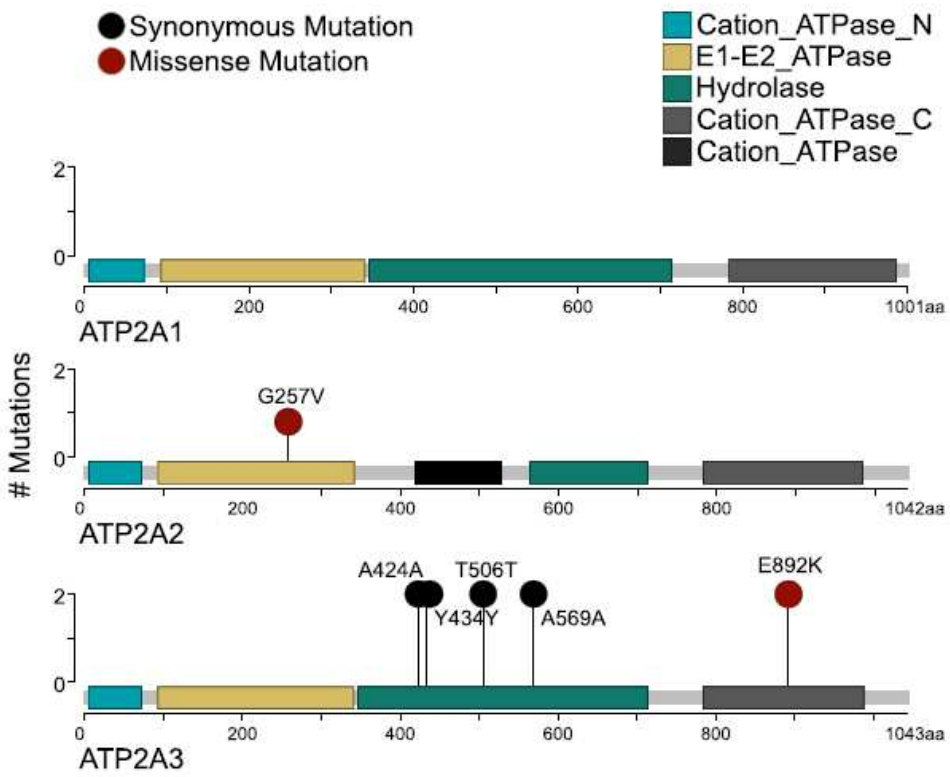


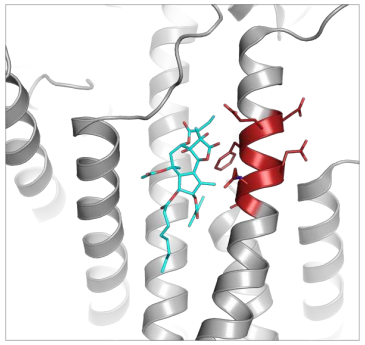


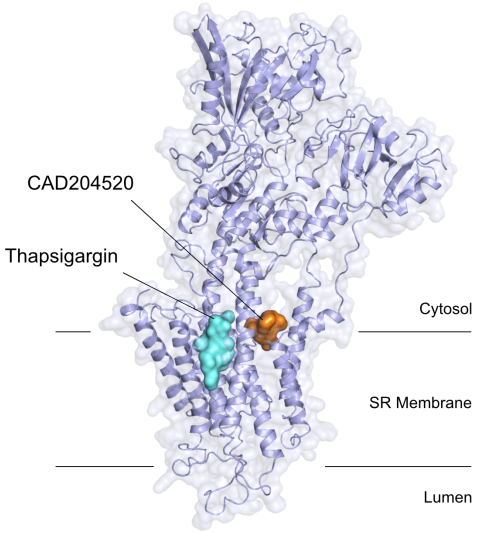


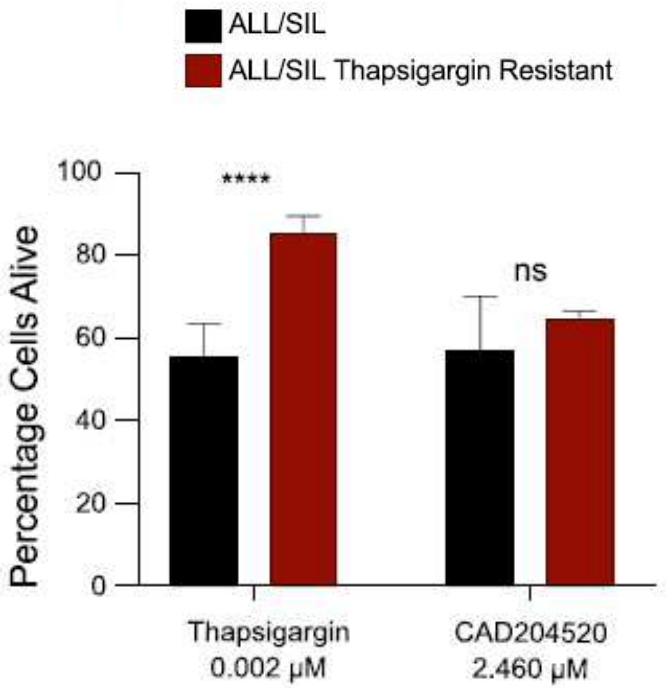
Marchesini et al. Cell Chem Biol, 2020

Generation of *ATP2A2*^{mut} T-ALL Model



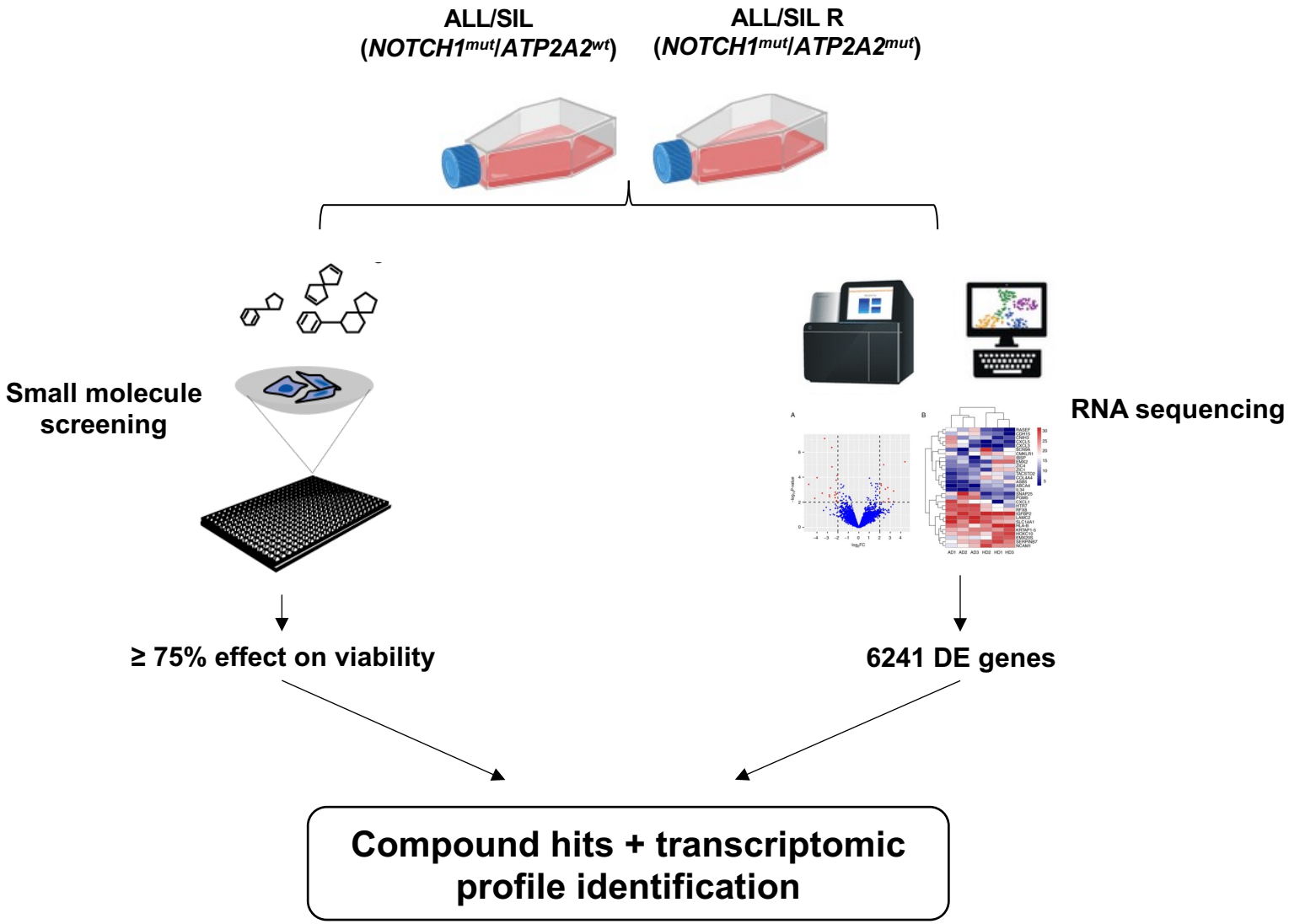




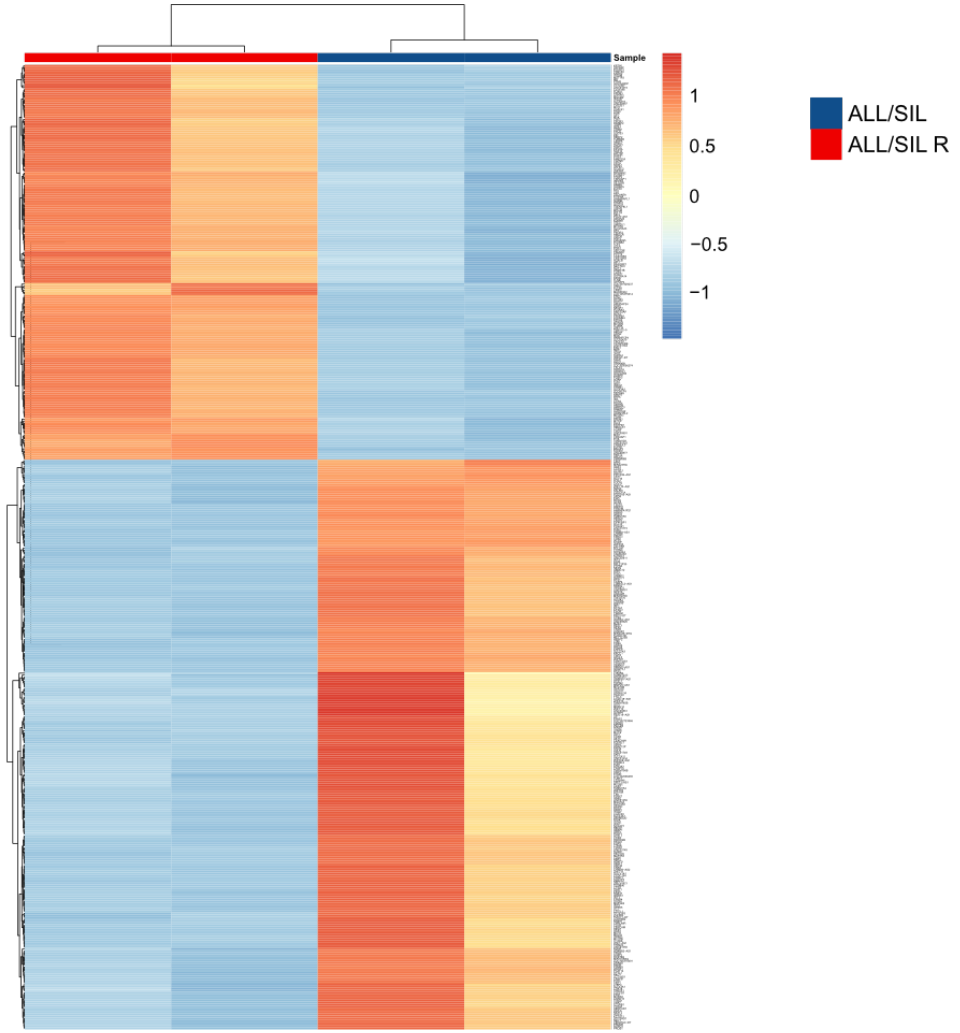


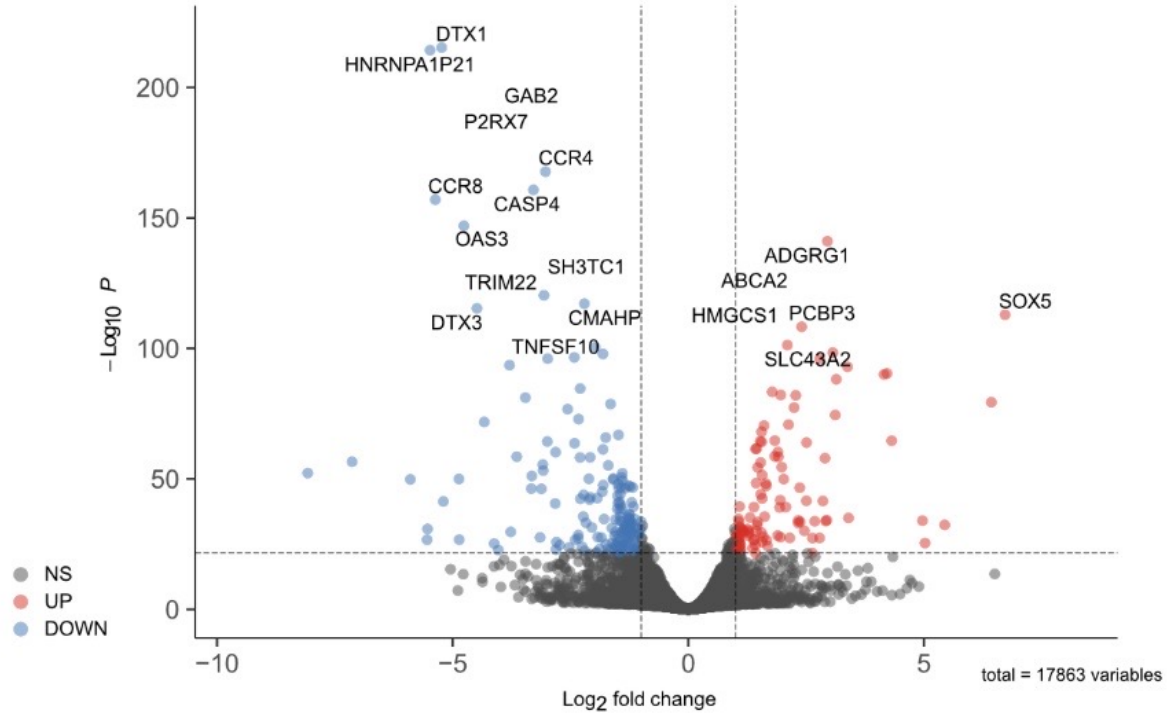
Marchesini et al. Cell Chem Biol, 2020

Intersecting a Chemotranscriptomic Screens to Anticipate SERCA Resistance in T-ALL

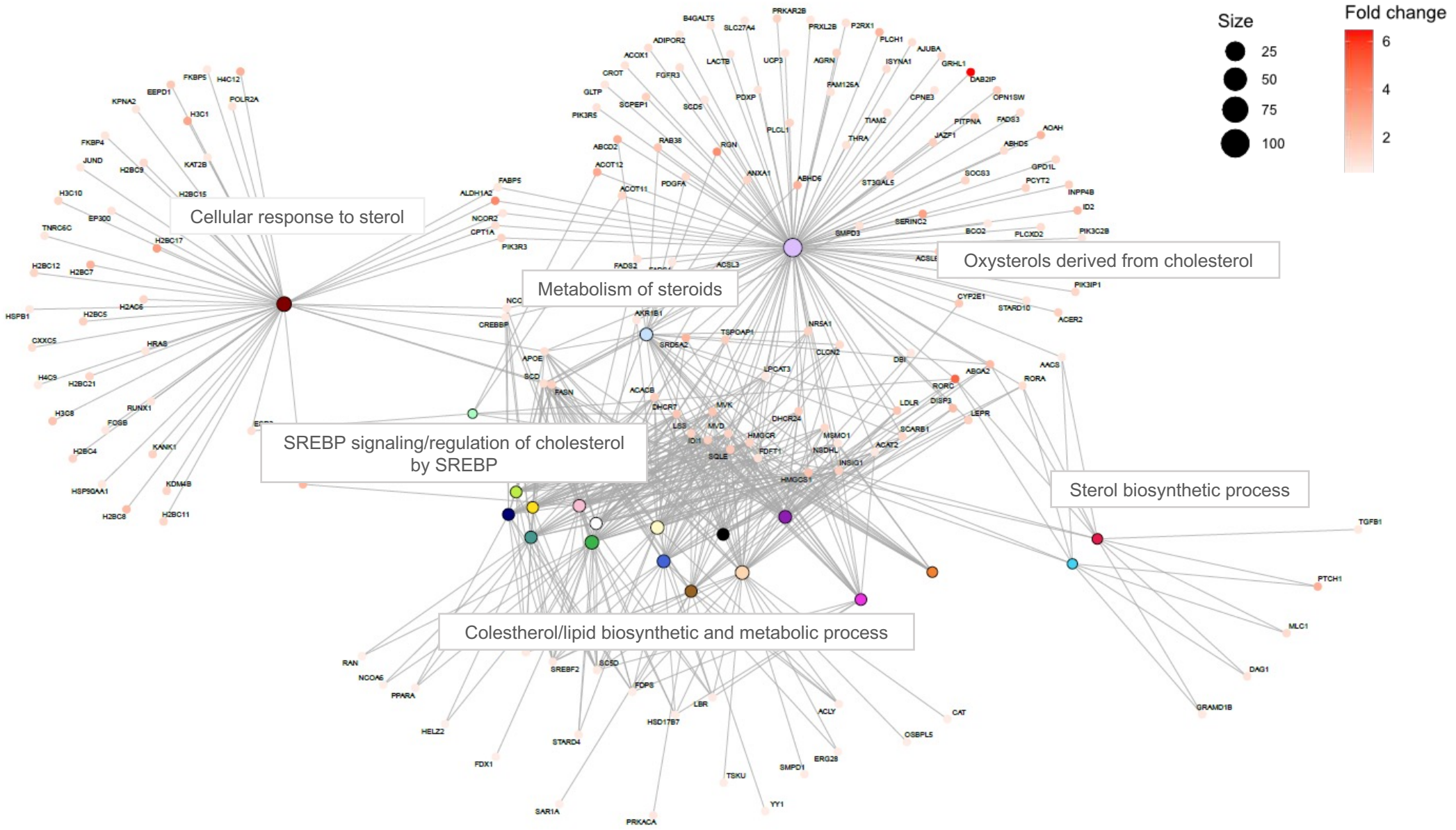


RNAseq Identifies Different Transcriptomic Profiles

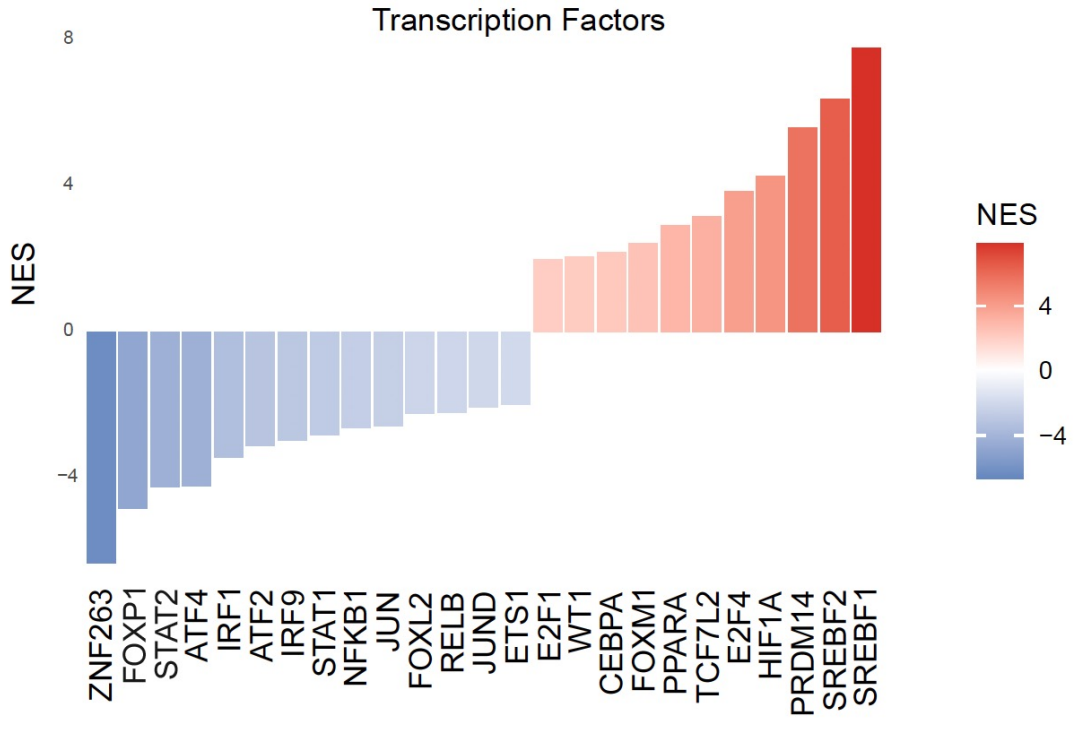


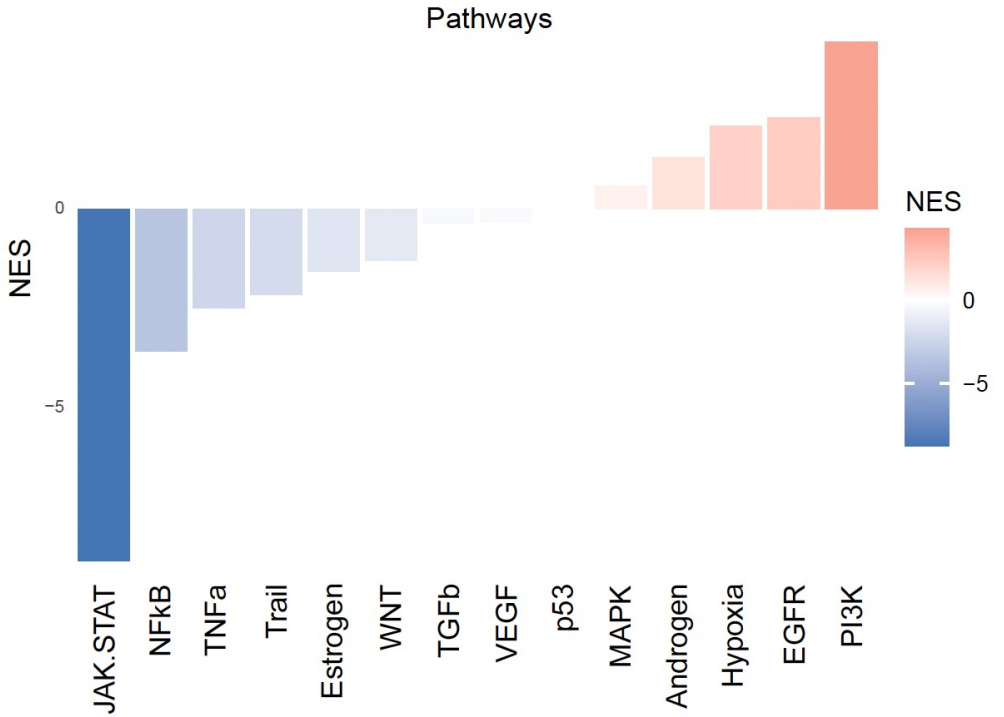


ATP2A2 Mutation Induces Upregulation of Steroid Metabolism

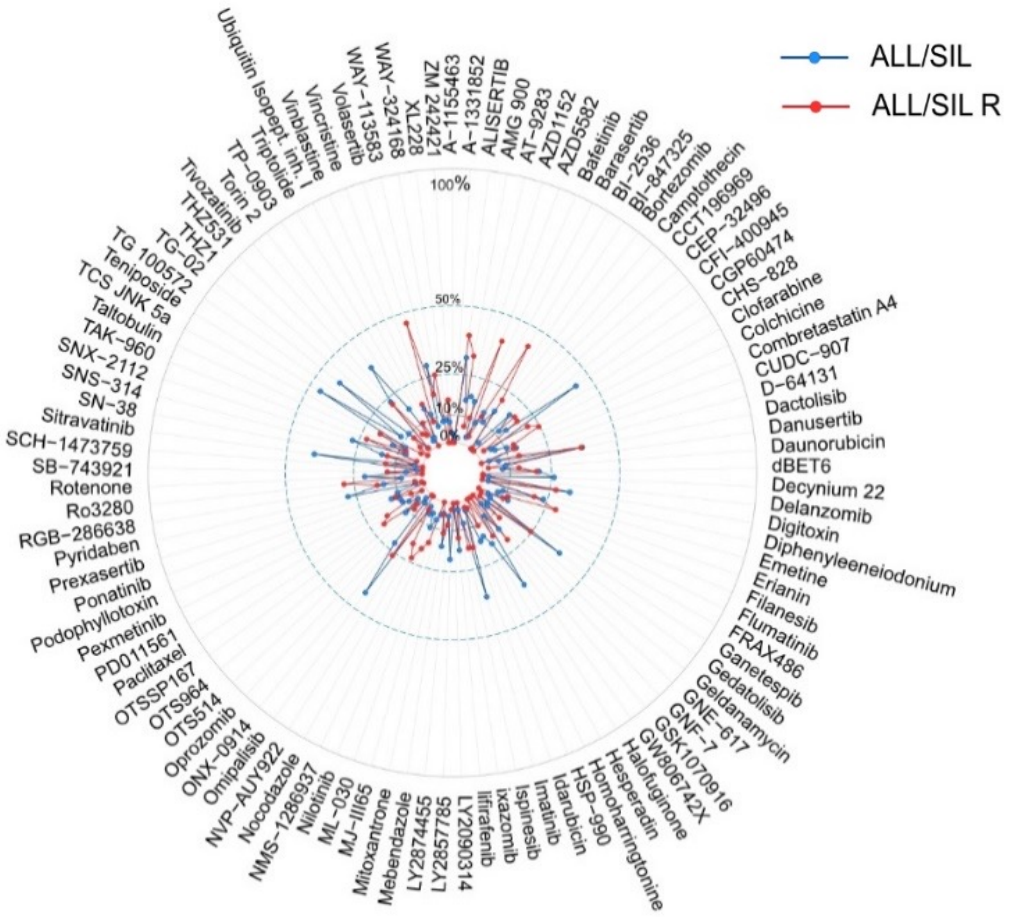


Transcription Factor Analysis Confirm a Role of Sterol Regulation





EU-OPENSCREEN Identifies Different Sensitivity Profiles



- Small molecule library screening in TG-sensitive and resistant cell line (ALL/SIL and ALL/SIL R)
- The screening library is part of the **European Chemical Biology Library (ECBL)**
- 2464 bioactive compounds screened
- The compounds are active against 1039 different targets (654 approved drugs and 368 highly selective probes from the public domain).
- Pathway coverage: cellular response to stress, Immune System, Signal Transduction, Developmental Biology, Gene Expression (Transcription), Hemostasis, Metabolism of proteins, Cell Cycle, Neuronal System

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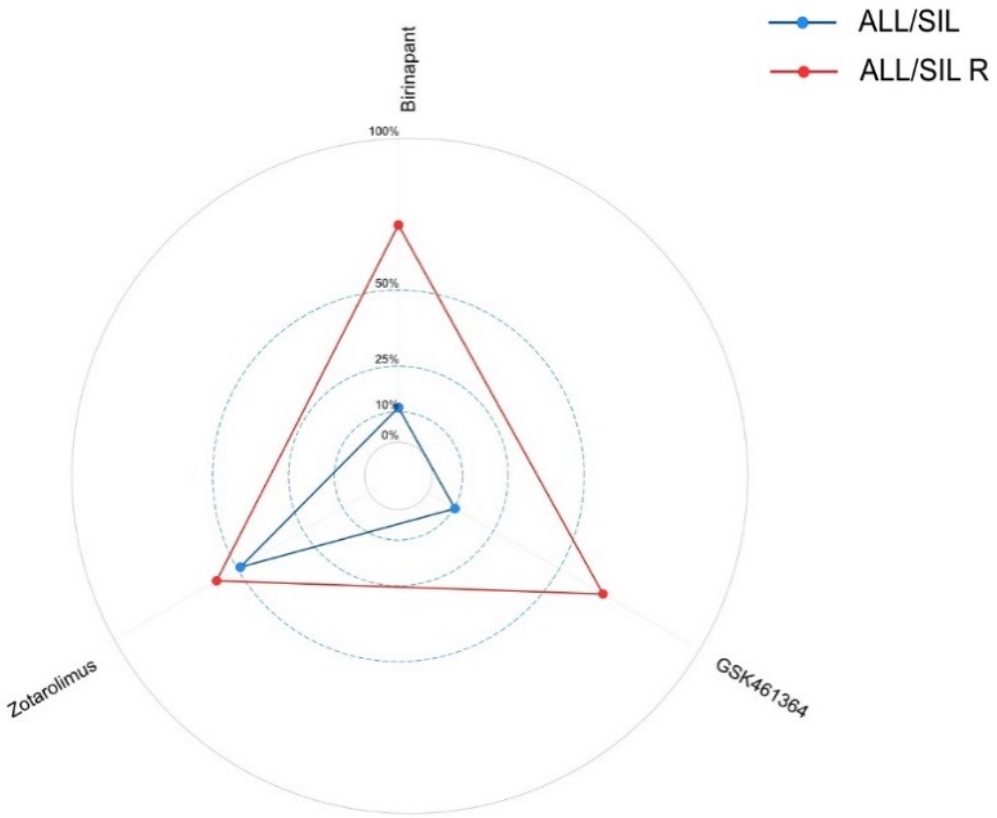

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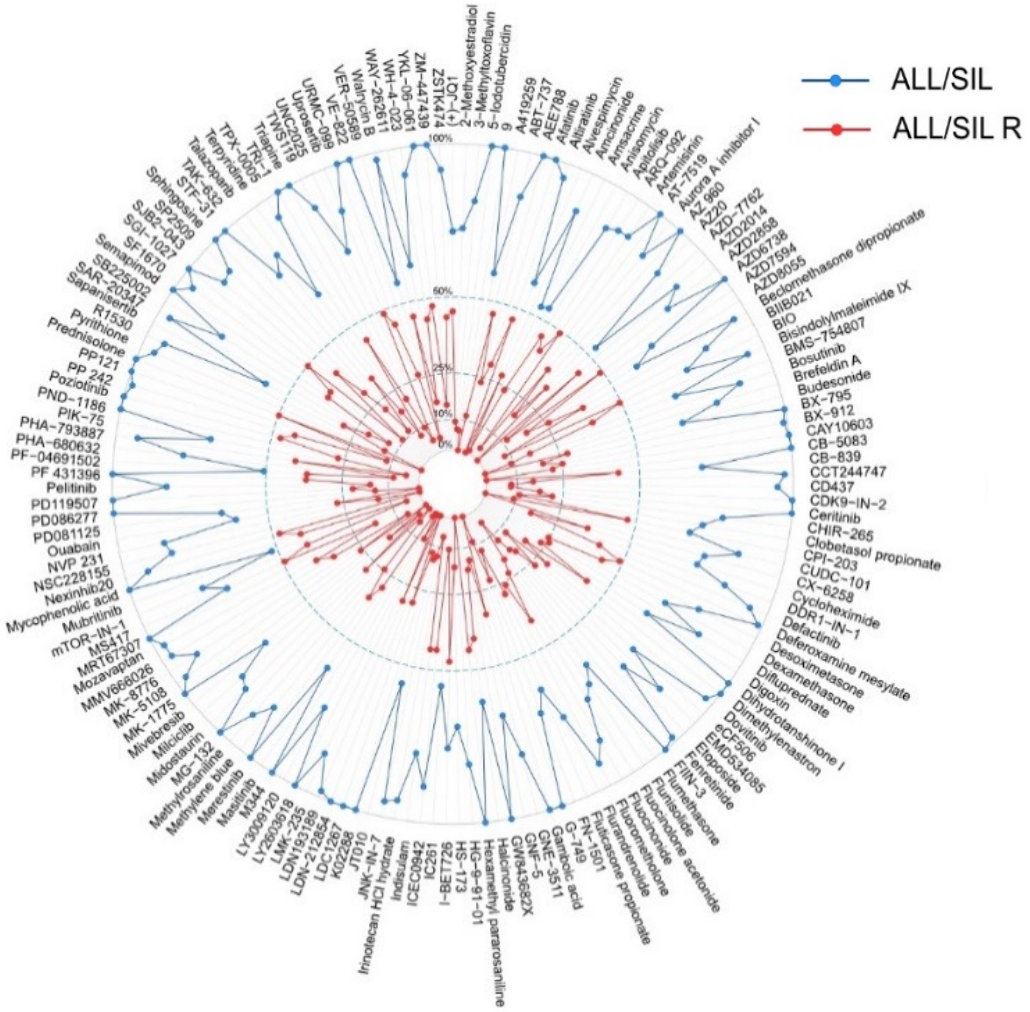

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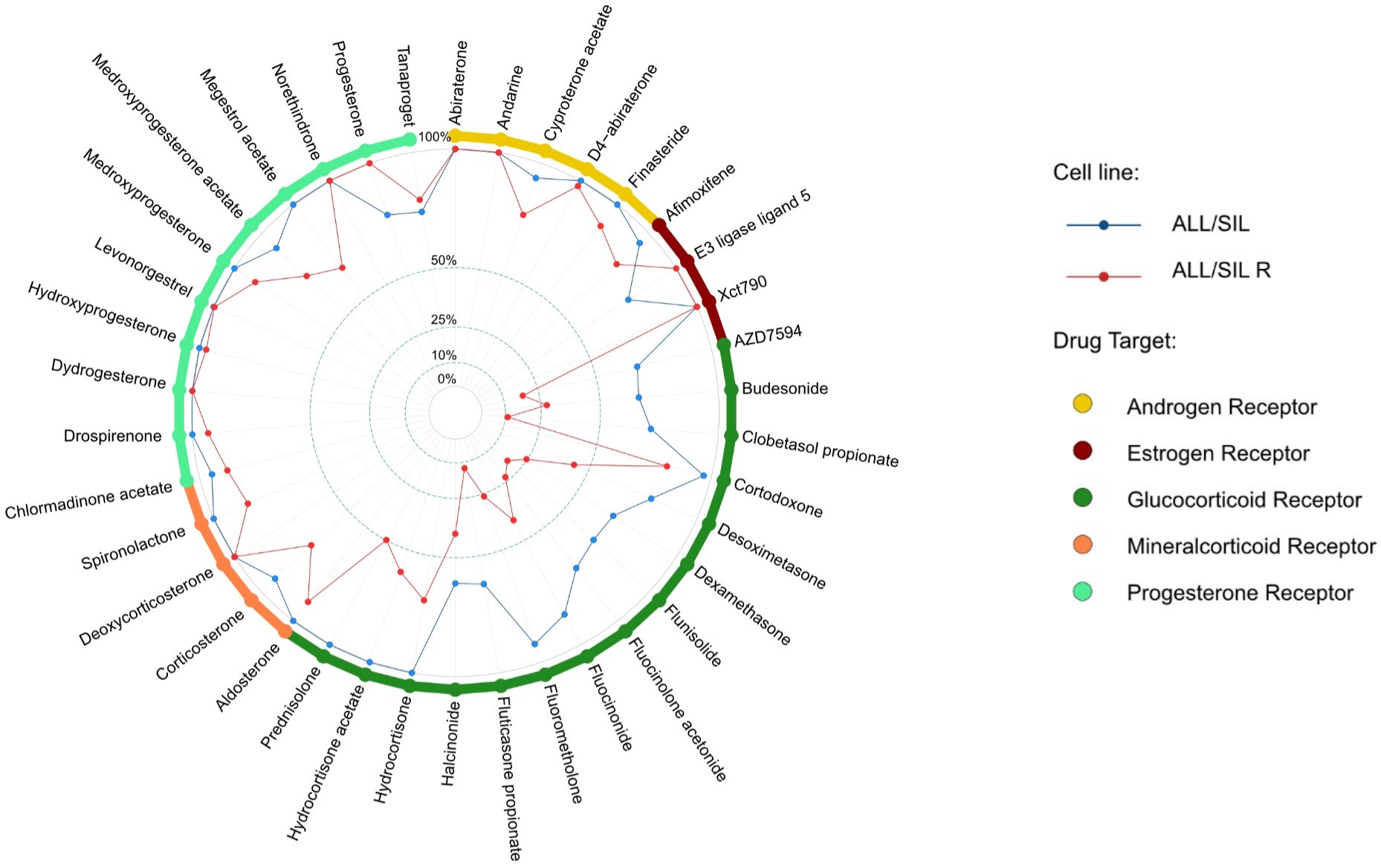
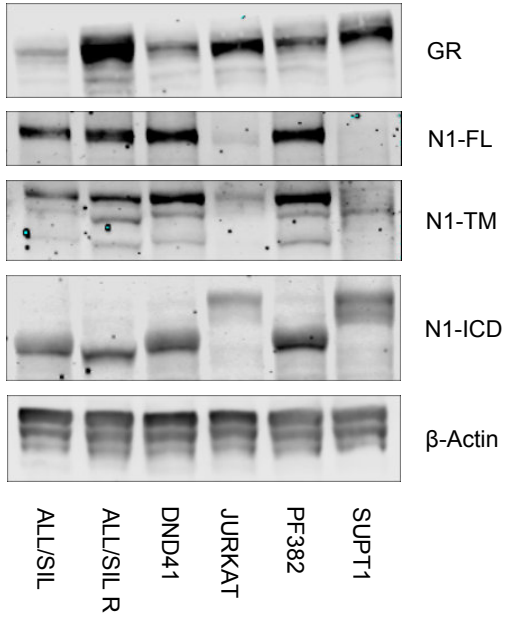
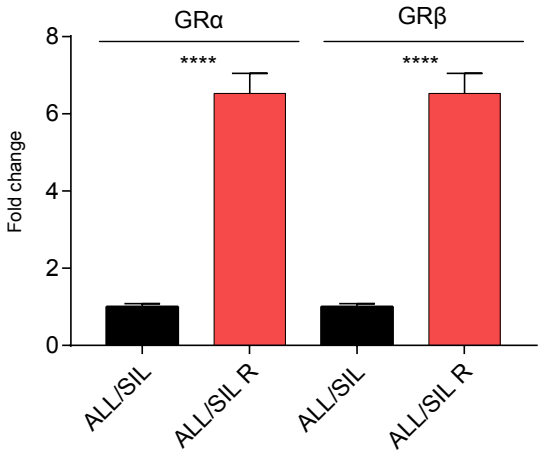
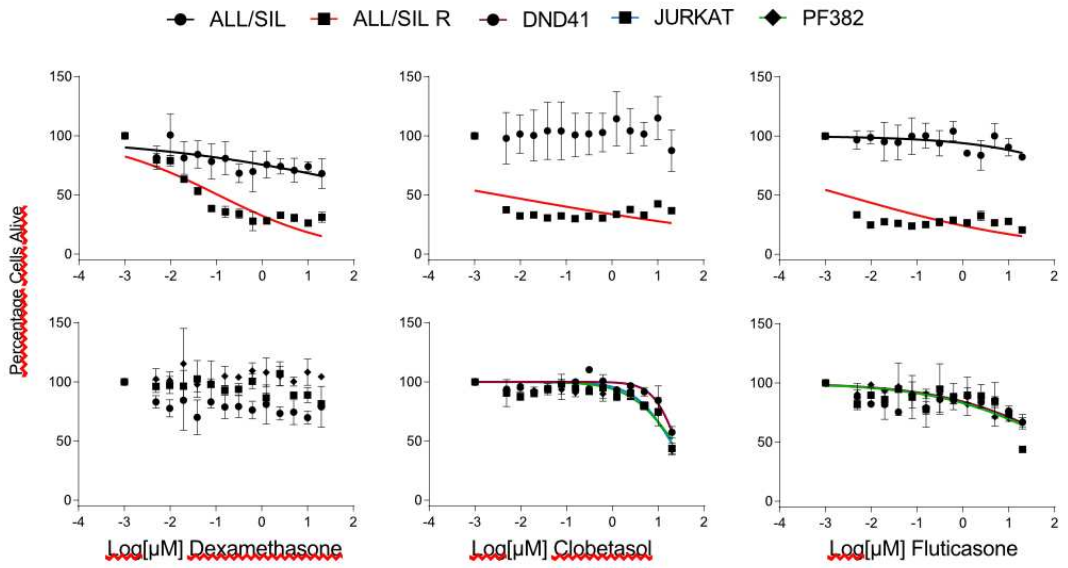
Glucocorticoids are a Specific Active Class in *SERCA*^{mut} T-ALL

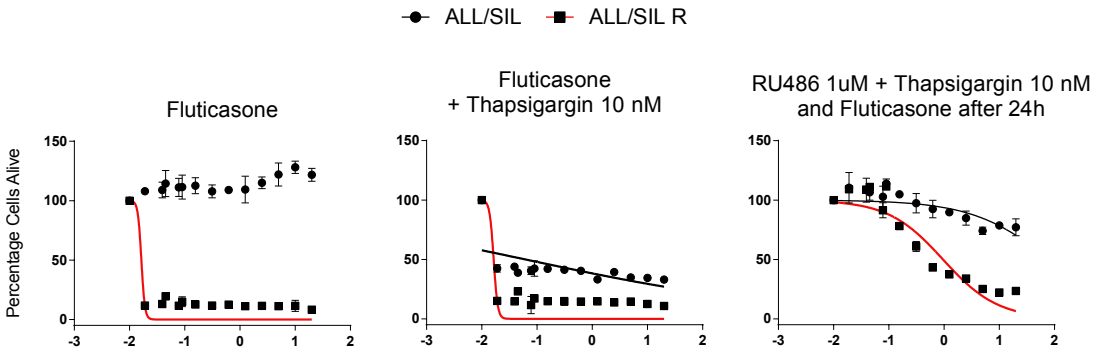


ATP2A2 Mutation Induces Glucocorticoid Sensitivity

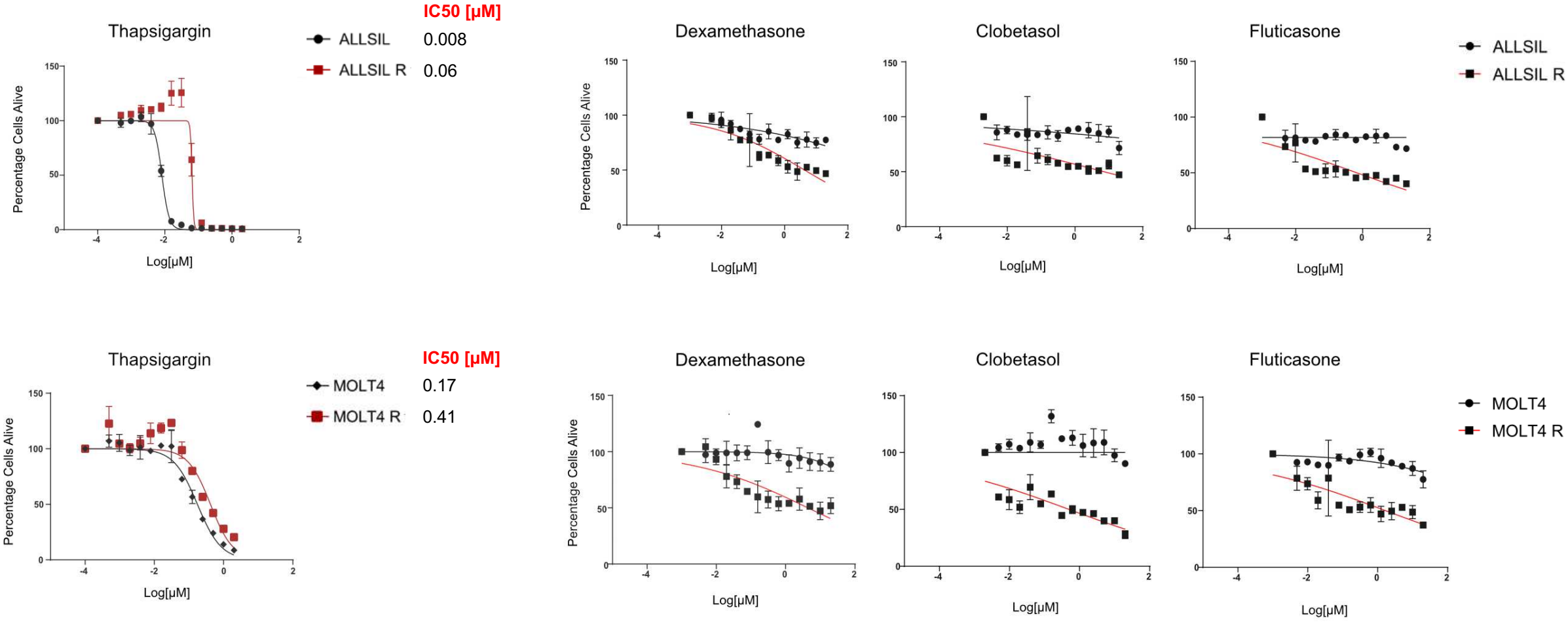




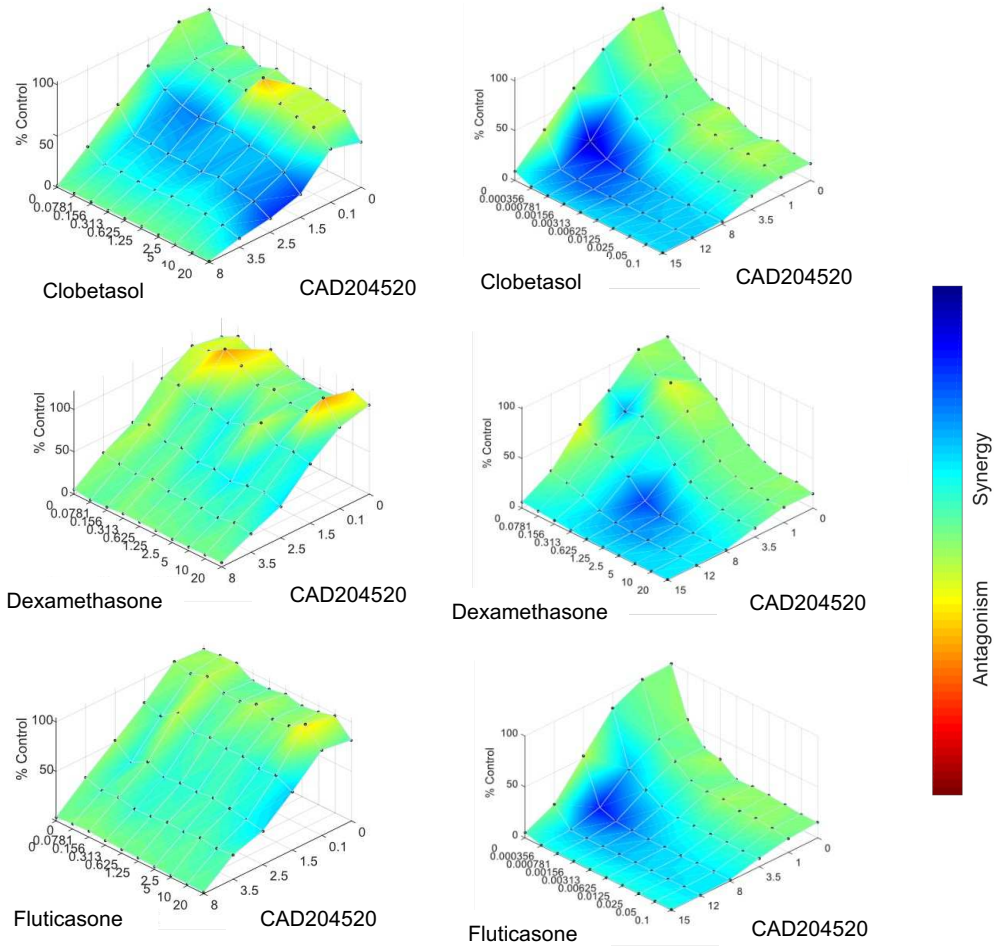




New Thapsigargin-Resistant Cell Line

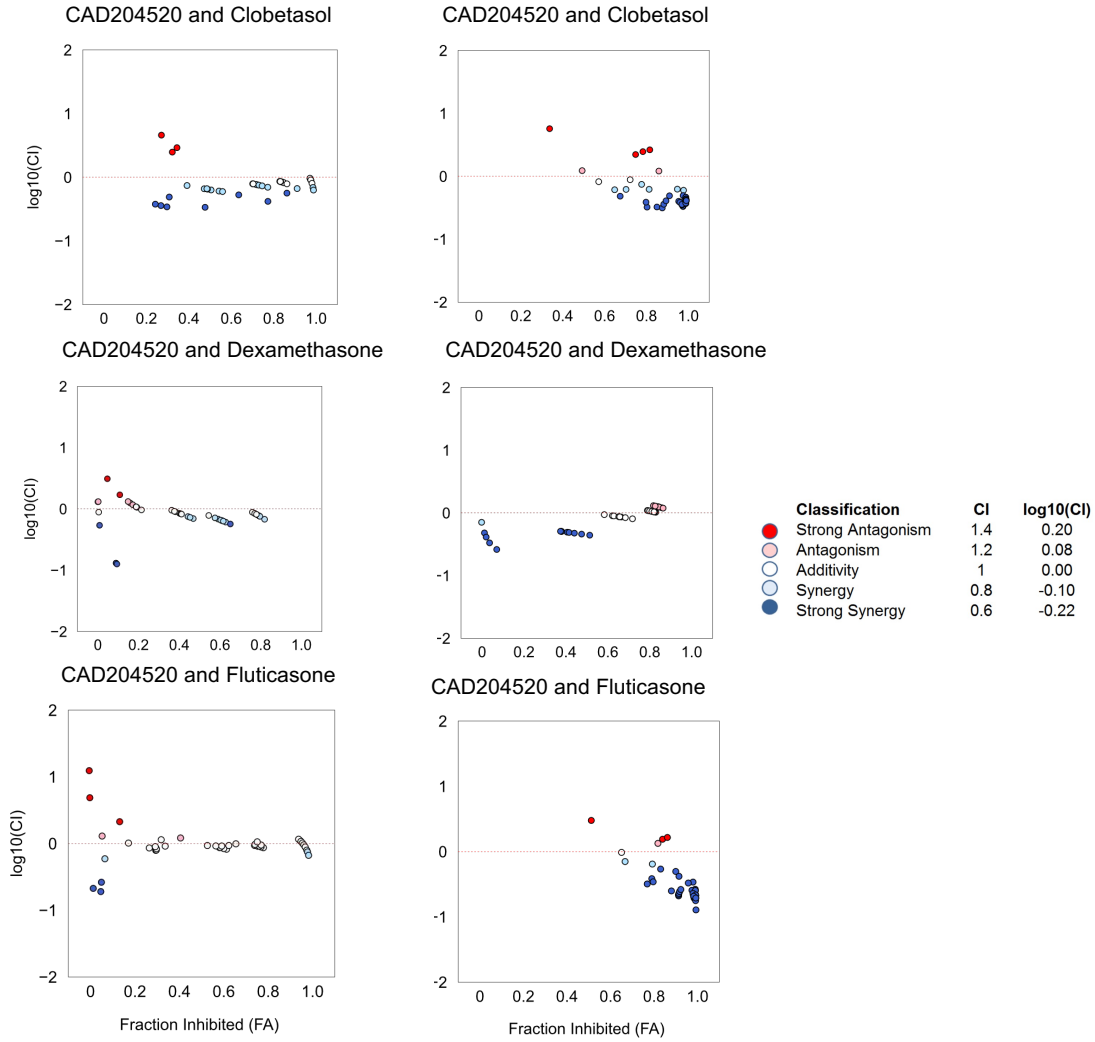


Glucocorticoids Synergize with SERCA Inhibitors *in vitro*



ALL/SIL

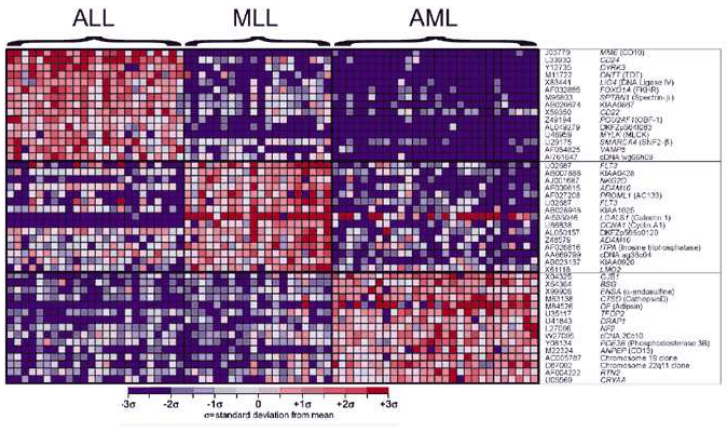
ALL/SIL R

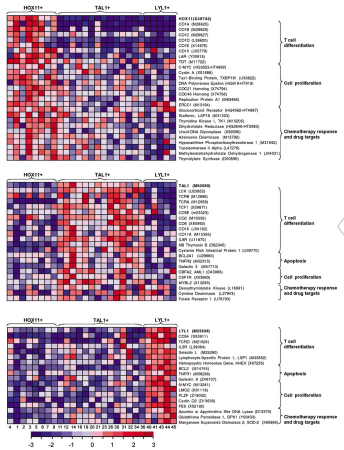


ALL/SIL

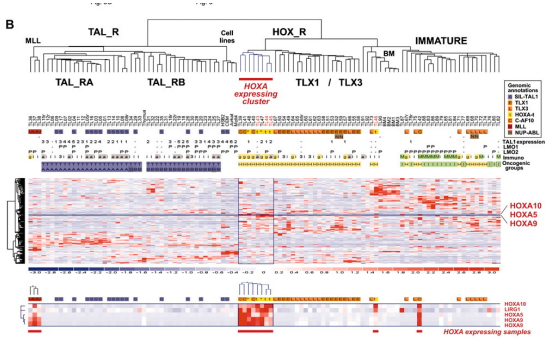
ALL/SIL R

Genes Expression Distinguishes Specific T-ALL Subtypes

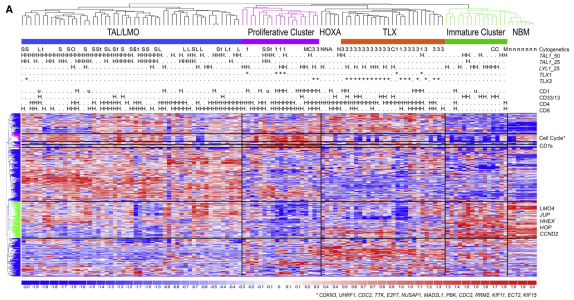




TAL1 and LYL



HOXA related



NKX2-1 and MEF2C

T-ALL a Model of TF Activation

TAL/LMO

TLX1

TLX3

HOXA

NKX2-1/NKX2-1

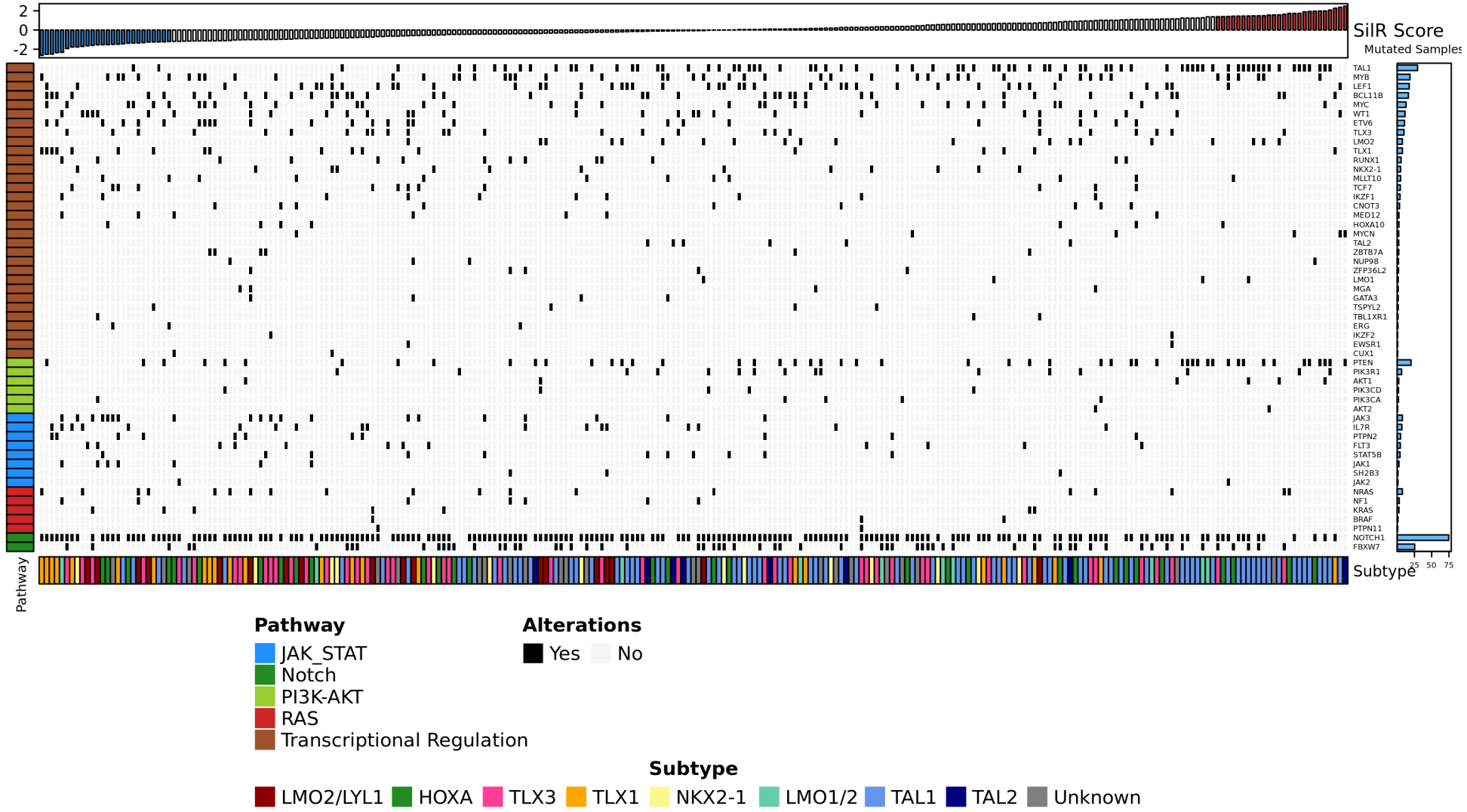
MEF2C

BCL11B-a

Nature Genetics volume 30, 41–47 (2002)

Cancer Cells 2002 (1) 75,87
Cancer Cell 2011, (4) 484-497
Blood 2005 (106), 274-286
Blood 2017;129:1113-1123
Blood. 2021 Sep 2;138(9):773-784

SERCA^{mut} Cell Line Clusters with TAL/LMO Genetic Subgroup



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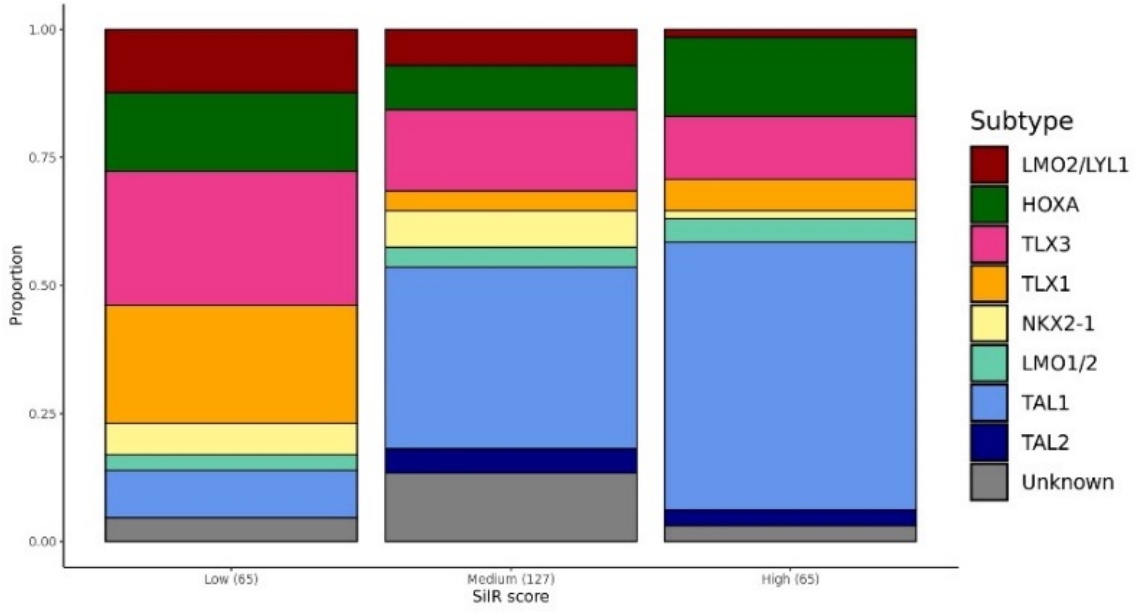

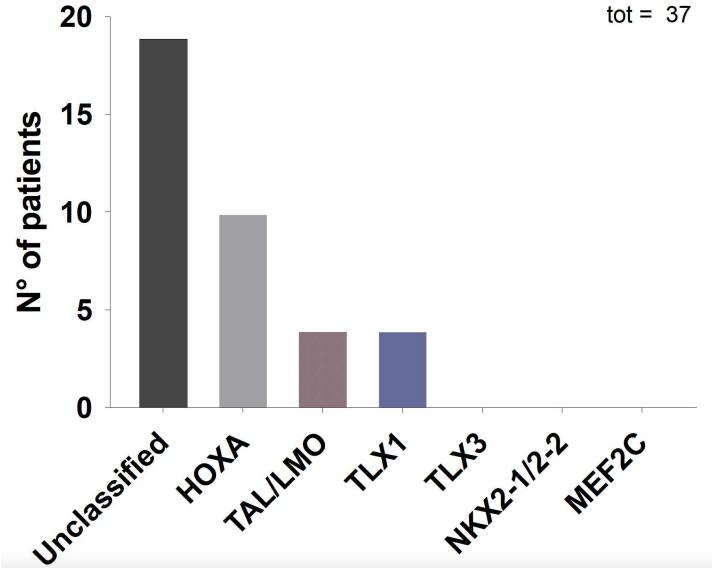

Patient	Sex	Phenotype	CI-FISH	IC50 (µM) / AUC		
				CAD	Dex	CAD+Dex
BM-117-2021	M	Early	STIL::TAL1 CDKN2AB bDEL CASP8AP2-GRIK2-SEC63-FYN del NF1-SUZ12 gain TP53 DEL	8.59 365	NR 295	0.85 263
BM-122-2021	M	Early	SIL::TAL1 NF1 del SUZ2 del	5.59 343	0.27 215	0.06 189
BM-015-2021	M	Mature	TRAD::LMO2 CDKN2AB del GRIK2-CASP8AP2-SEC63-FYN del TP53 mDEL	1.58 304	NR 350	1.94 290
BM-066-2022	F	Pro-T/ETP	TRAD::LMO2; LEF1 del; iso(17q)/TP53del	4.01 336	0.03 165	0.02 153

Conclusions

- Prolonged administration of TG induces the rising of mutations at the ligand binding site (LBD)
- As long as *ATP2A2*^{mut} and wt cell line are syngenic, the LBD mutation induces transcriptomic modifications and generate sensitivity to glucocorticoids
- SERCA inhibition synergize with glucocorticoids in *ATP2A2*^{mut} cell lines
- The R cell lines shows a gene expression profile that is similar to the gene expression pattern of TAL1 T-ALL genotypic subgroup
- The dissection of metabolic pathways could help to define the relationship between SERCA and glucocorticoid metabolism

Acknowledgements

- **Giovanni Roti, M.D.,Ph.D.**
- Luca Pagliaro
- Anna Montanaro
- Federica Vento
- Elisa Cerretani
- Elisa Simoncini
- Anna D'Antuono
- Andrea Gherli
- Lucas Moron Dalla Tor
- Raffaella Zamponi
- Maria Teresa Giaimo
- Bruno Lorusso
- Alessia Ciringione

THEC

TRANSLATIONAL HEMATOLOGY AND CHEMOGENOMICS