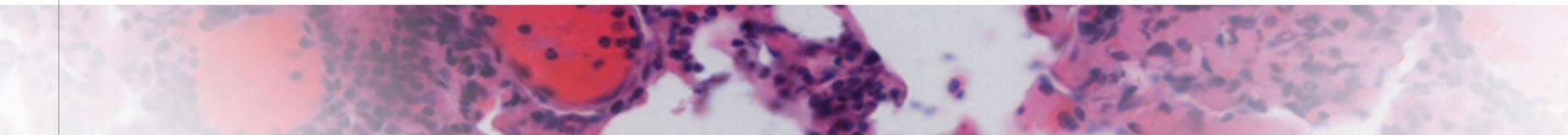




American Society of Hematology
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SELECTIVE STAT3 DEGRADERS DISSECT PERIPHERAL T-CELL LYMPHOMAS VULNERABILITIES EMPOWERING PERSONALIZED REGIMENS

Giuseppina Astone, PhD

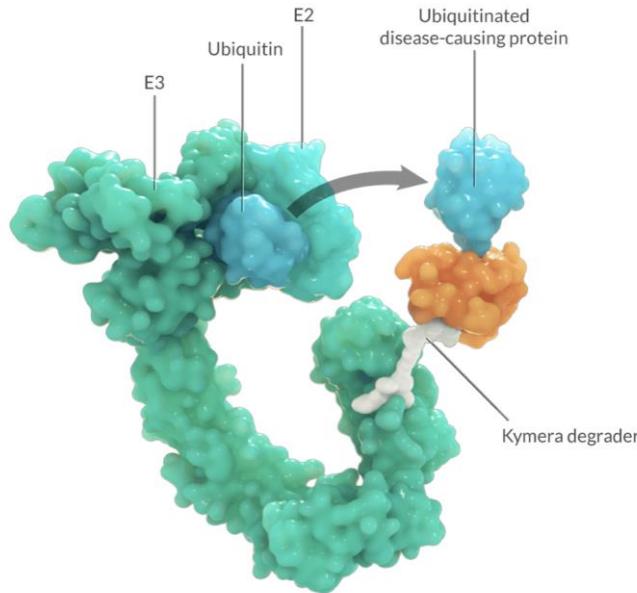
Department of Pathology and Laboratory Medicine, Weill Cornell Medicine, New York

Peripheral T-cell lymphomas (PTCLs)

- Peripheral T-cell lymphomas (PTCLs) comprise a heterogeneous group of aggressive malignancies that are derived from mature T-cells.
- Recurrent aberrations and the deregulated activation of distinct signaling pathways have been mapped and linked to selective subtypes. The JAK/STAT signaling pathway's deregulated activation plays a pathogenetic role in several PTCL, including ALCL subtypes.
- Somatic activating mutation of JAK1/STAT3 (~50% ALK- ALCLs) drives constitutive activation of STAT3 pathway (Crescenzo R et al, 2015).
- STATs regulate the differentiation/phenotype, survival and cell-growth, metabolism, and drug resistance of T-cell lymphomas as well as host immunosuppressive microenvironments.



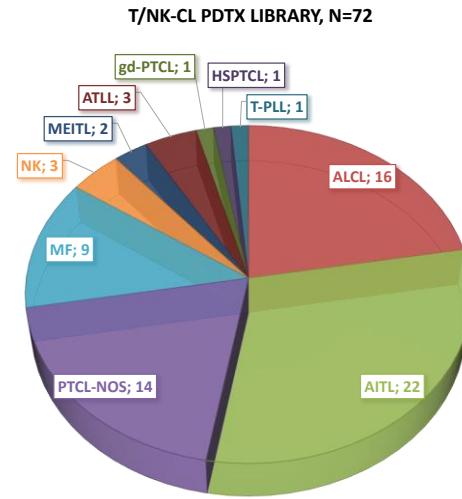
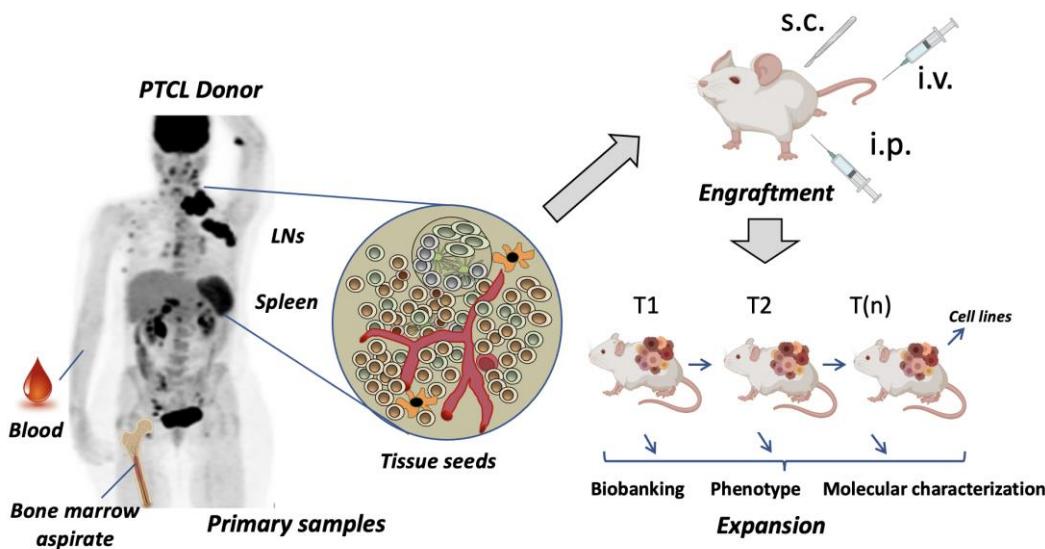
Schematic representation of Interaction of Kymera degraders with E-ligase and target recognition



Targeted protein degradation mediated by heterobifunctional small molecule degraders is a novel therapeutic modality to target difficult-to-drug oncogenic proteins. These molecules bind to both the target protein and an E3 ligase, enabling the formation of a ternary complex which leads to ubiquitination and proteasomal degradation of the target protein.



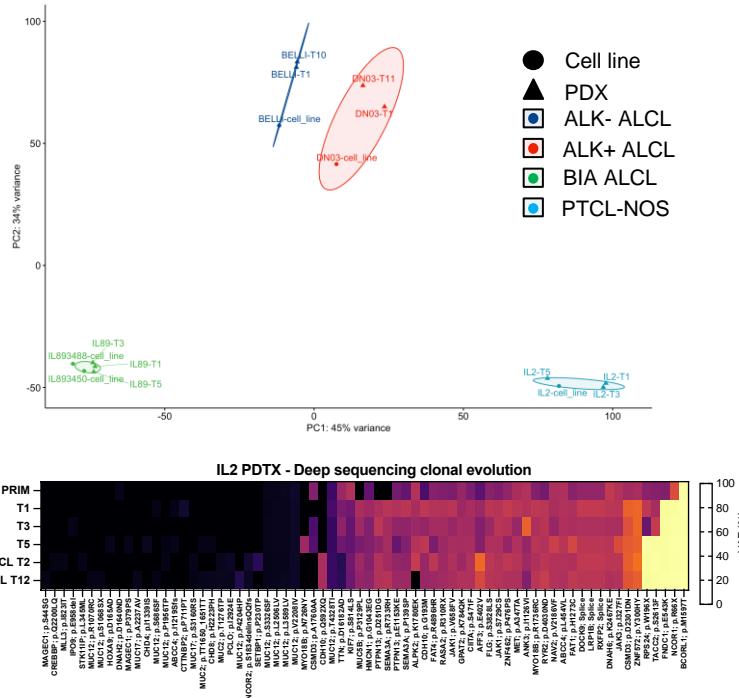
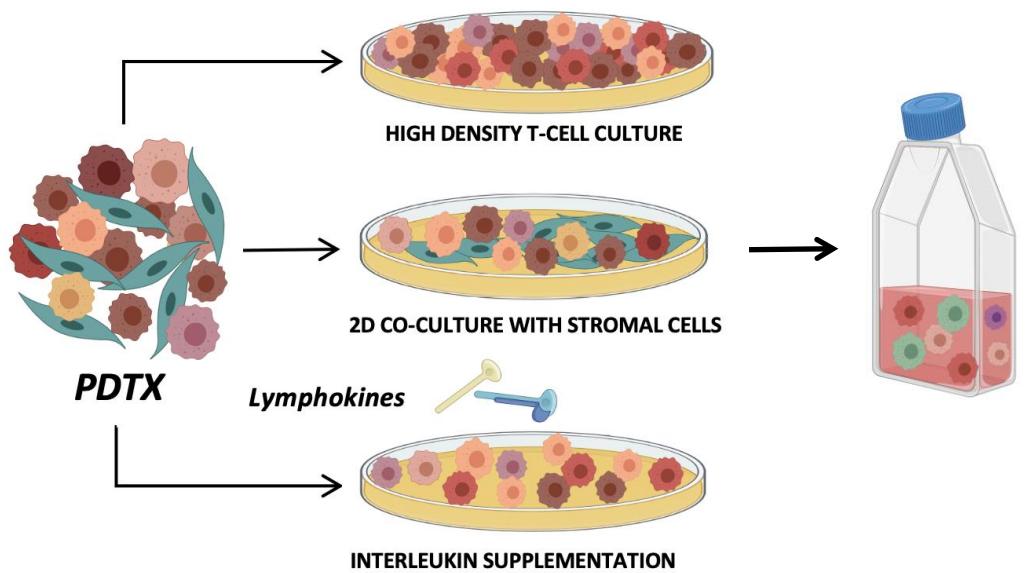
Patient-Derived-Tumor-Xenograft (PDTX) models



- Resemble the pathophysiology of human tumors
- Reproduce somehow the tumor heterogeneity
- Grow within a host environment

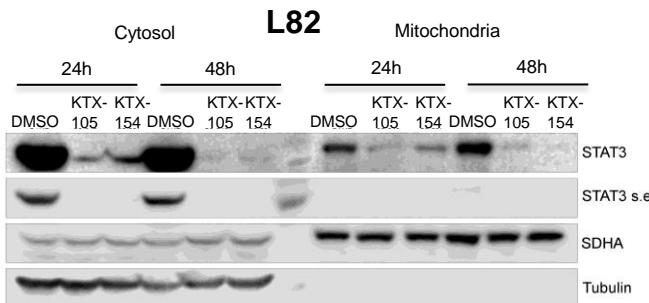
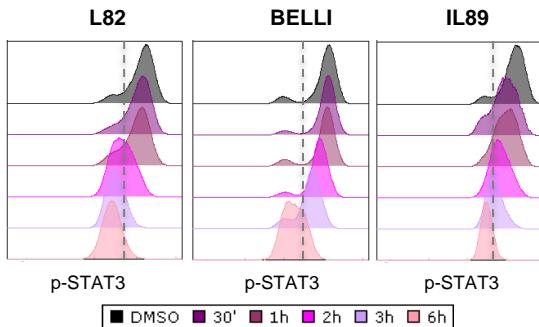


Patient-Derived-Tumor-Xenograft (PDTX) derivative models



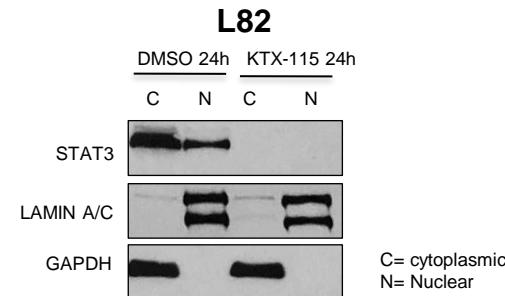
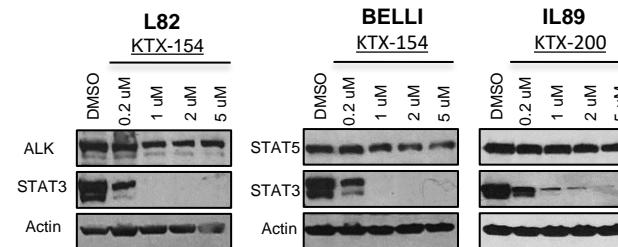
STAT-degraders lead to the rapid and effective down-regulation of STAT3

Flow cytometry determination of p-STAT3 in ALK- and ALK+ ALCL cells exposed to 1 μ M of KTX-115 degrader



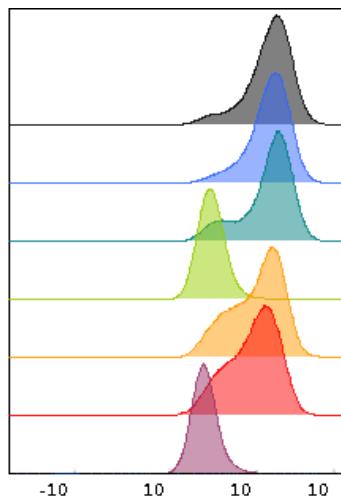
Cytosolic, mitochondrial and nuclear STAT3 downregulation in ALK+ ALCL (L82) exposed to 1 μ M STAT3 degraders

Loss of STAT3 protein expression in ALK- and ALK+ ALCL cells exposed to KTX-154 or KTX-200 degrader (144 h)

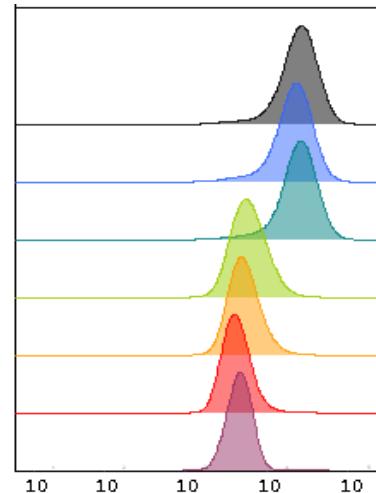


Protein degradation efficiency of different STAT3 degraders

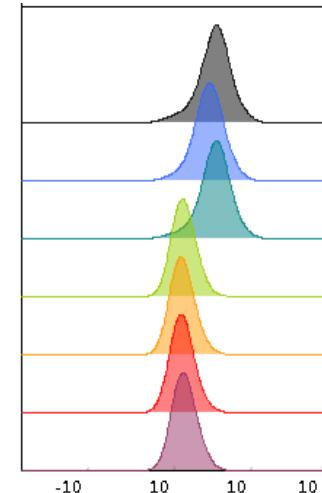
BELL1
(ALK- ALCL: mut Jak1 and Stat3)



IL89
(BIA ALCL: mut Jak1)



IL2
(PTCL: mut Jak1)



■ DMSO ■ KTX-201-IC ■ KTX-105-IC ■ KTX-154 ■ KTX-200 ■ KTX-201 ■ KTX -105

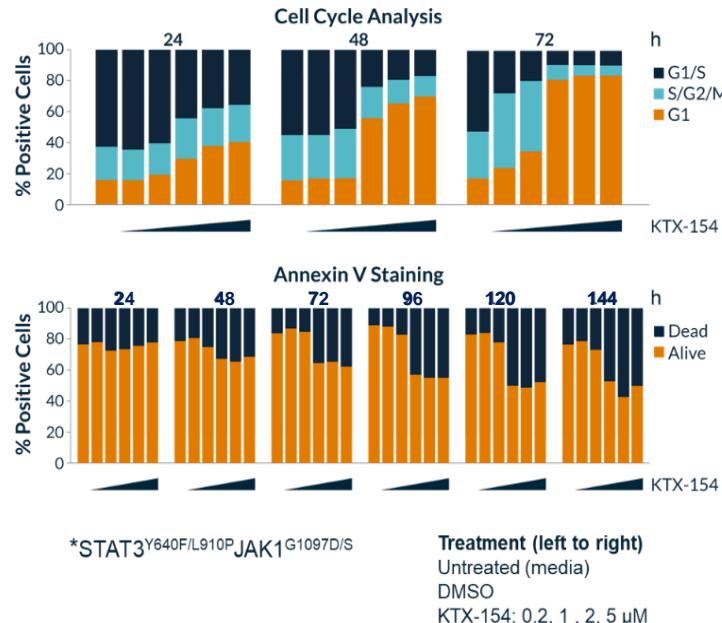
IC= Inactive Control



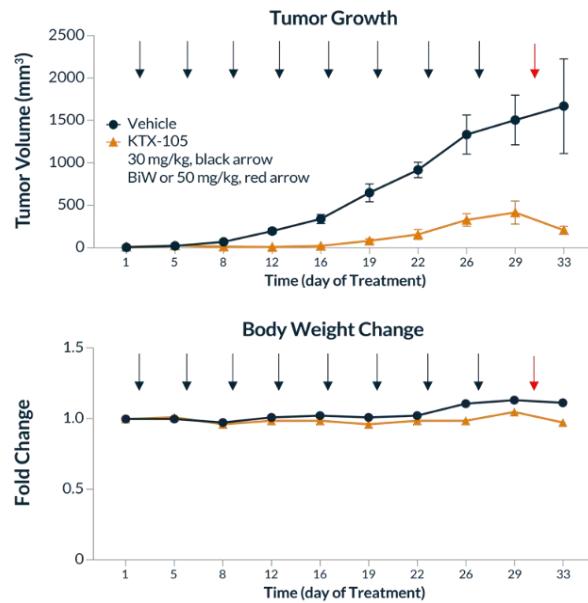
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Effect of STAT3 degraders KTX-154 and KTX-105 on the growth of the BELLi (STAT3^{mt}JAK1^{mt*}) ALK- primary ALCL model in vitro and in vivo

Time and dose-dependent cell cycle arrest and cell death of the BELLi model

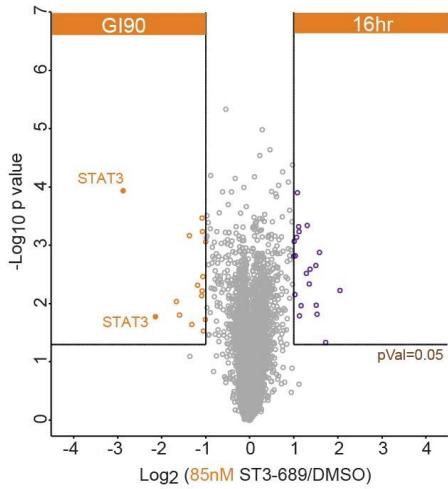


Intermittent dosing of KTX-105 significantly inhibits growth of BELLi xenograft tumors and is tolerated

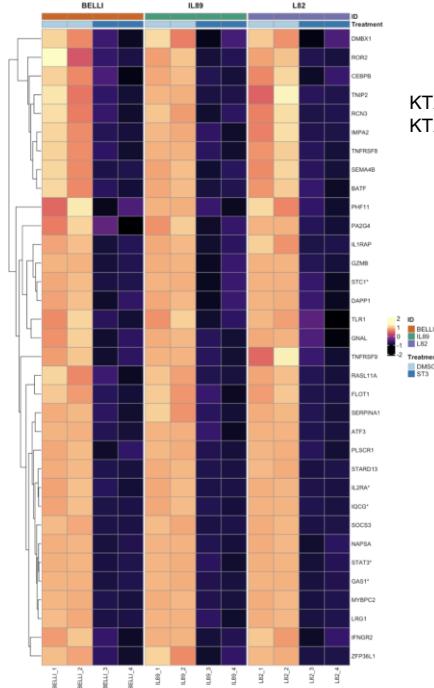


ALCL cells treated with specific STAT3-degraders undergo shared transcriptional changes

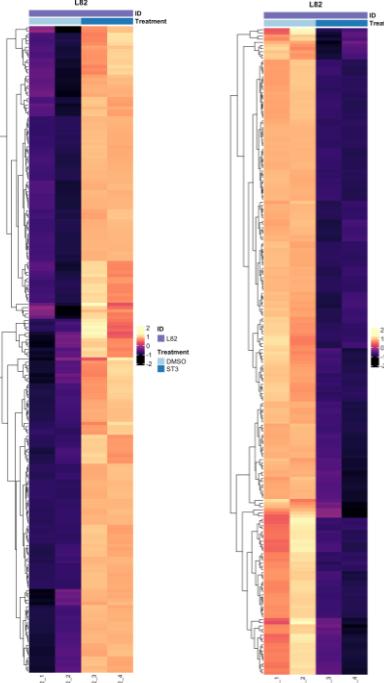
Deep proteomics shows strong reduction of STAT3 in SU-DHL-1 Cells treated with KTX-217 (at 16 h)



Overlapping genes down-regulated in STAT3-degrader (BELLi, IL89 and L82)

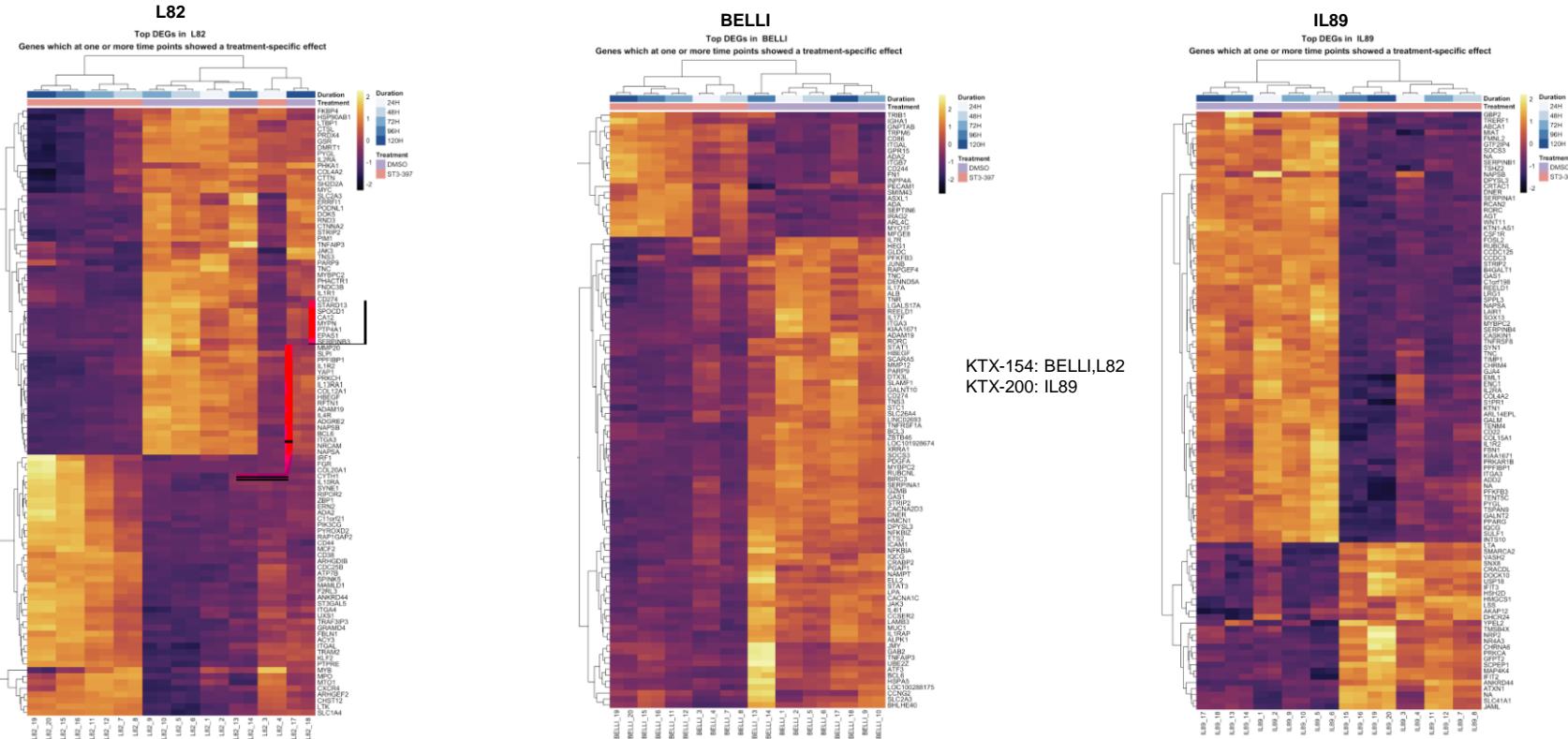


Heatmap of the overlapping upregulated genes (201) and downregulated (206) genes in STAT3- treated cells (L82)

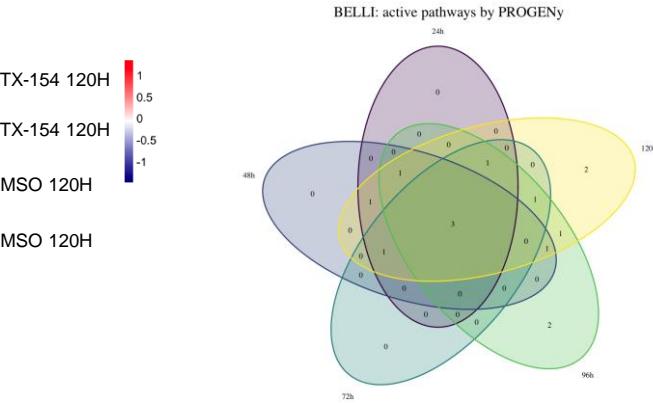
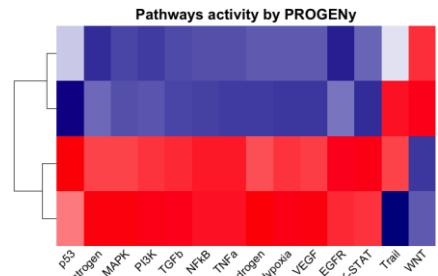
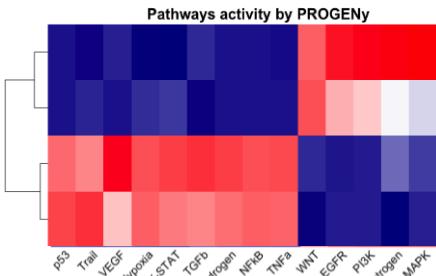
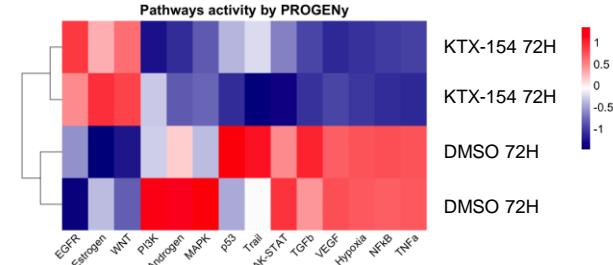
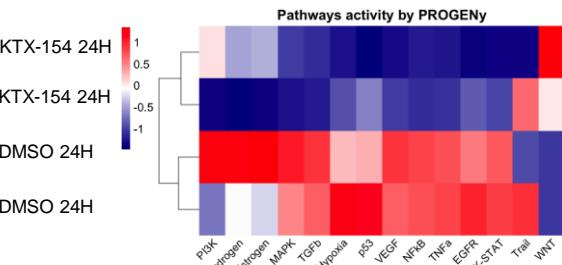
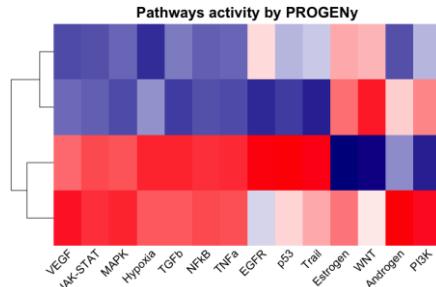


Individual ALCL cell lines display unique transcriptomic changes after STAT3 loss

Genes which at one or more time point (24h,48h,72h,96h,120h) showed a treatment-specific effect

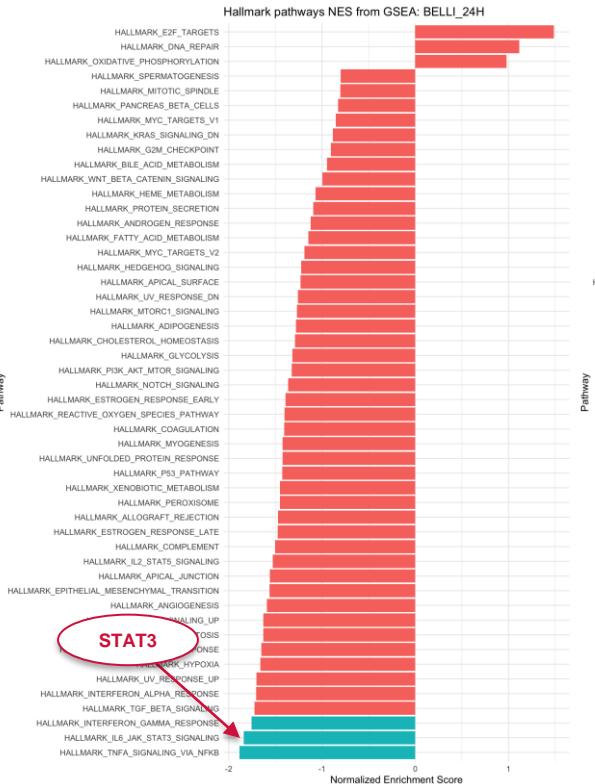


Pathway activity modulation by STAT3 degrader identifies selective changes in BELLi

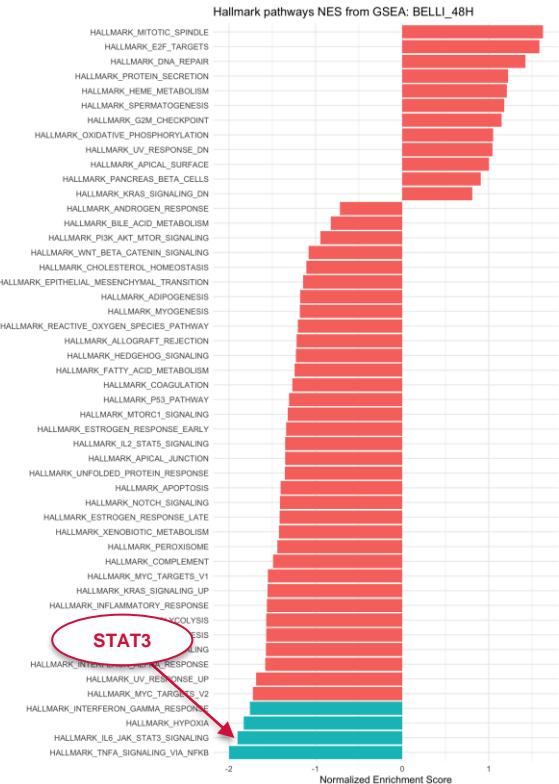


Hallmark pathways in ALK- ALCL (BELLi)

24h



48h

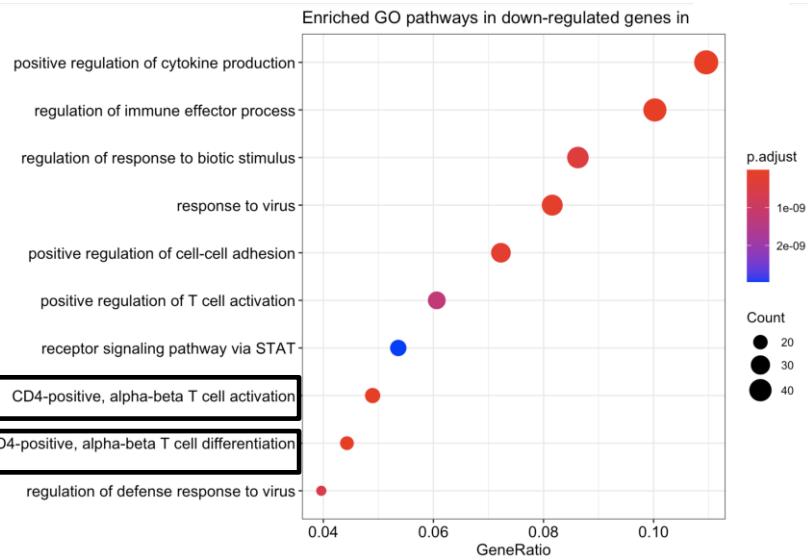


120h

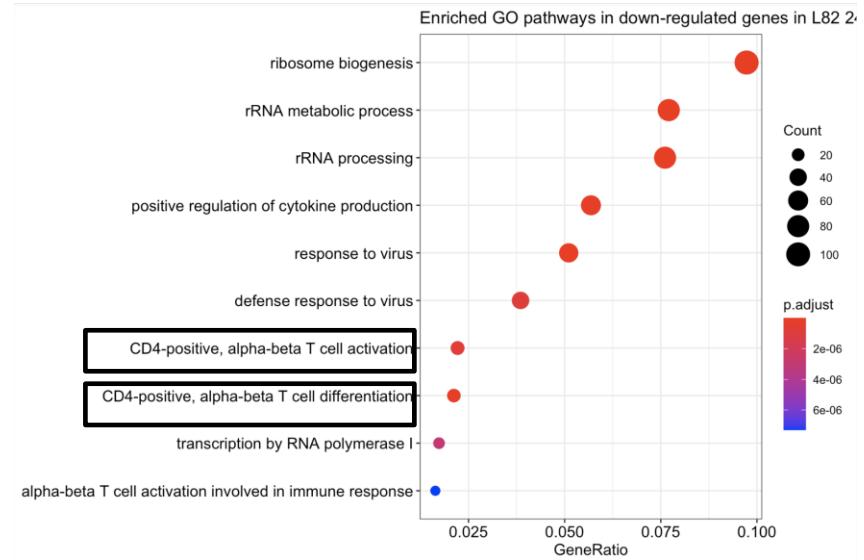


Enriched pathways STAT3 regulated genes

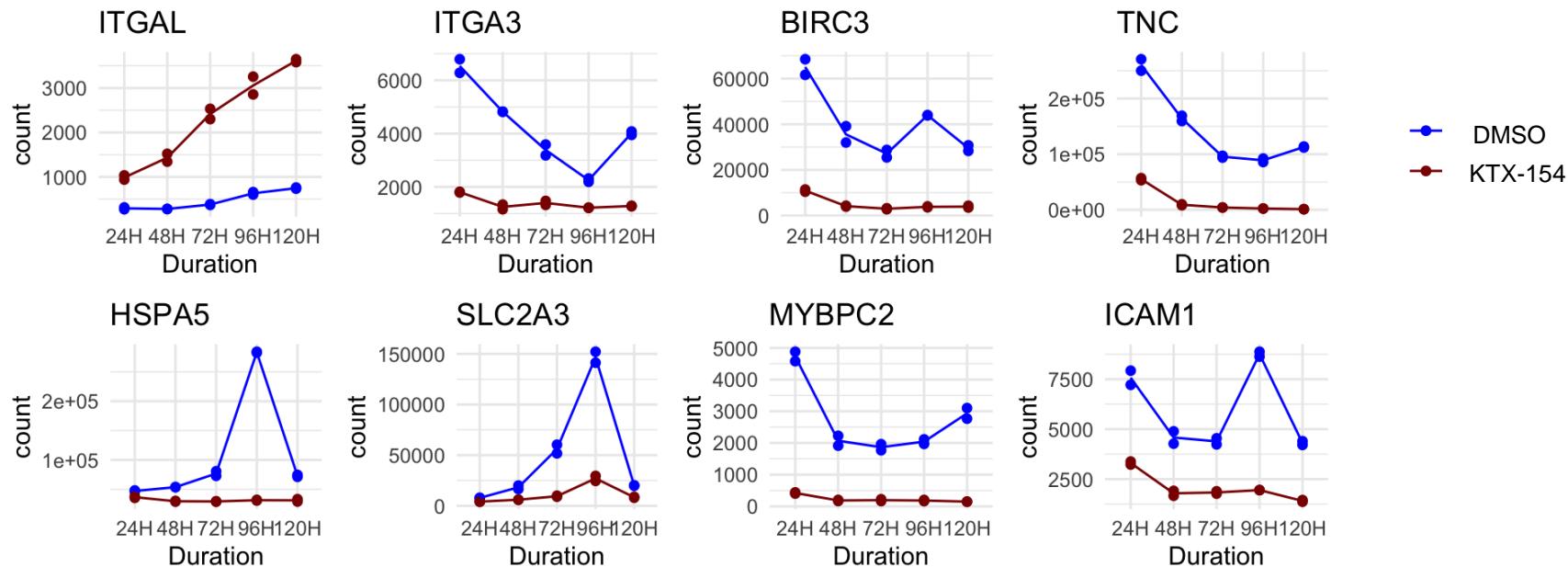
BELLI 24h



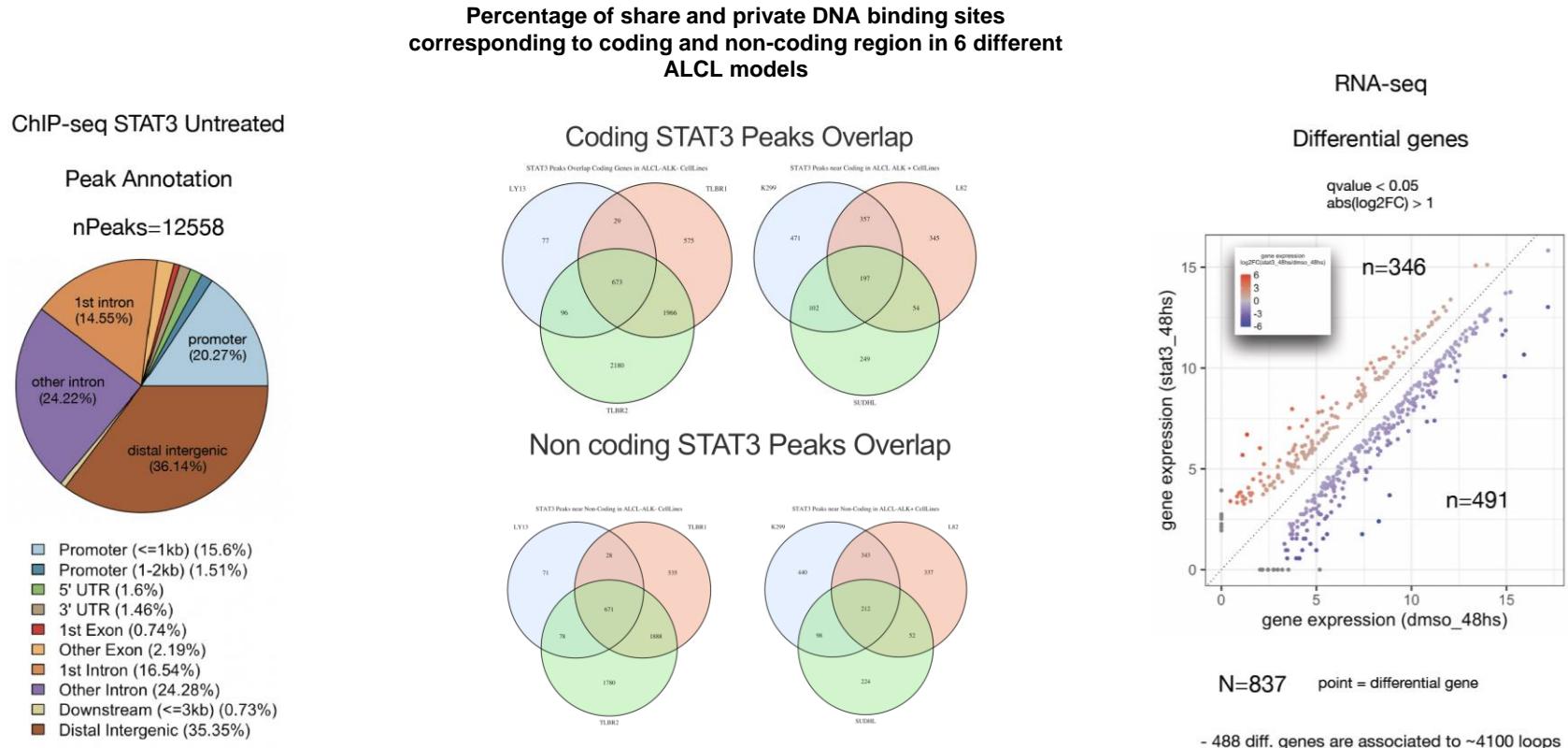
L82 24h



Gene modulation trajectories after STAT3 loss in BELLi

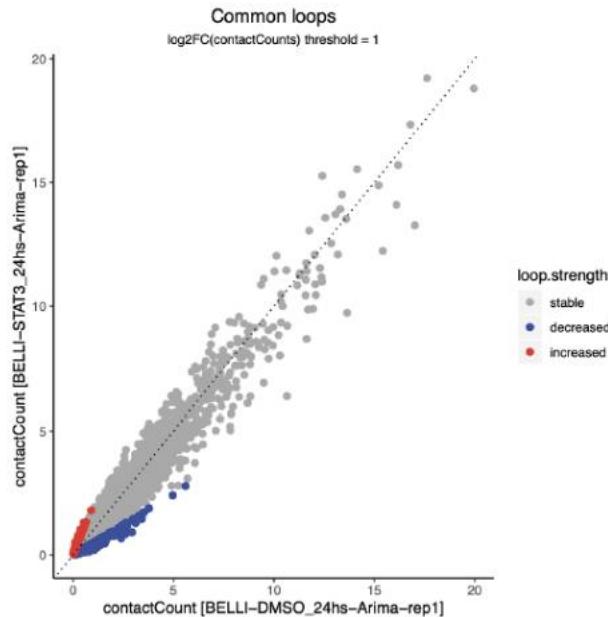


Mapping of STAT3 DNA binding in PTCL/ALCL cells

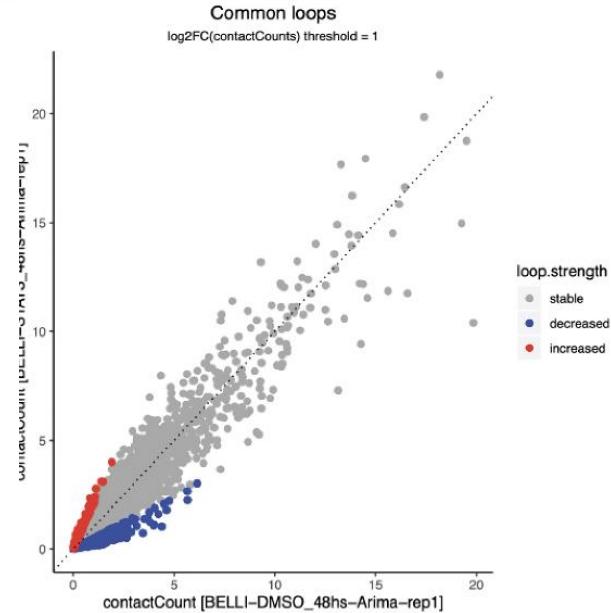


Loss of STAT3 leads to significant chromatin changes

Loops (Belli 24hs - dmso vs stat3)



Loops (Belli 48hs - dmso vs stat3)



RNA-seq - Differential expression by loop group (gene centric)

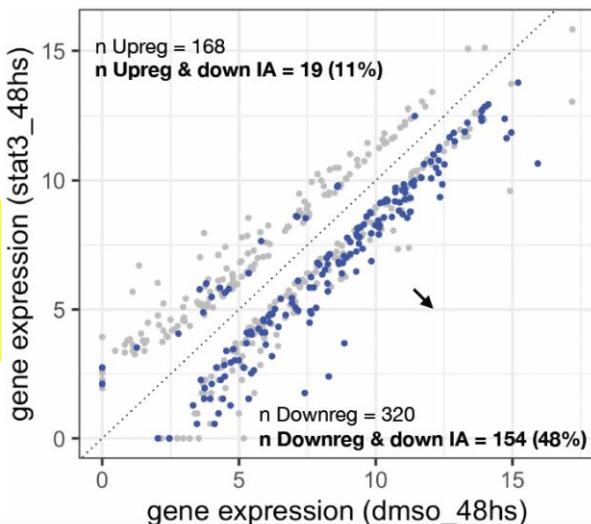
decreased IA loops
(stat3_48hs/dmso_48hs)

IA = interactivity

- decreased IA loops = 0 nDEGs = 315
- decreased IA loops > 0 **nDEGs = 173 (35%)**

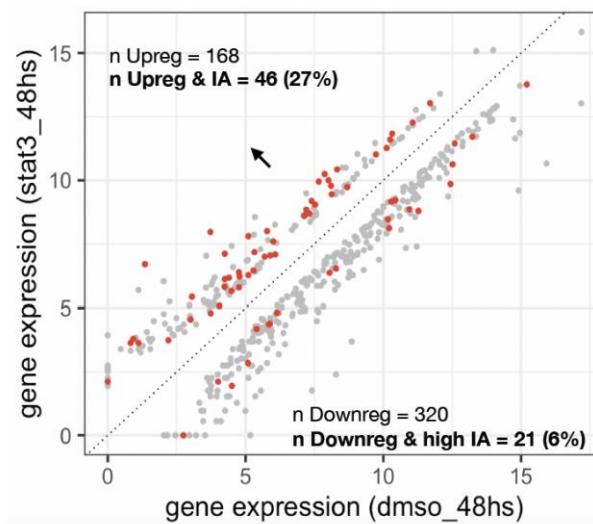
increased IA loops
(stat3_48hs/dmso_48hs)

- increased IA loops = 0 nDEGs = 421
- increased IA loops > 0 **nDEGs = 67 (14%)**



point = differential gene

downregulated genes
are associated to
loops with **decreased**
interactivity

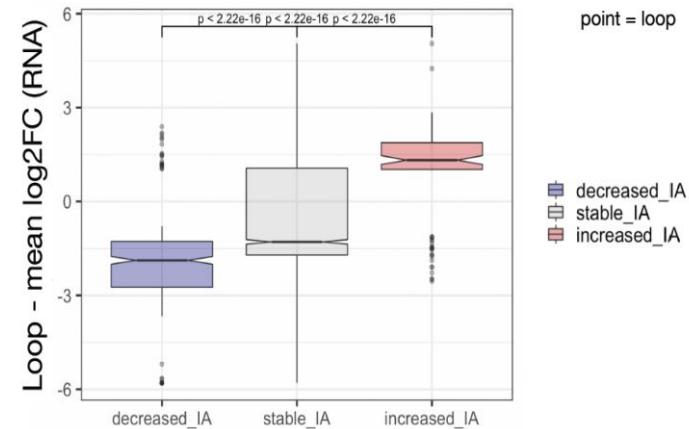
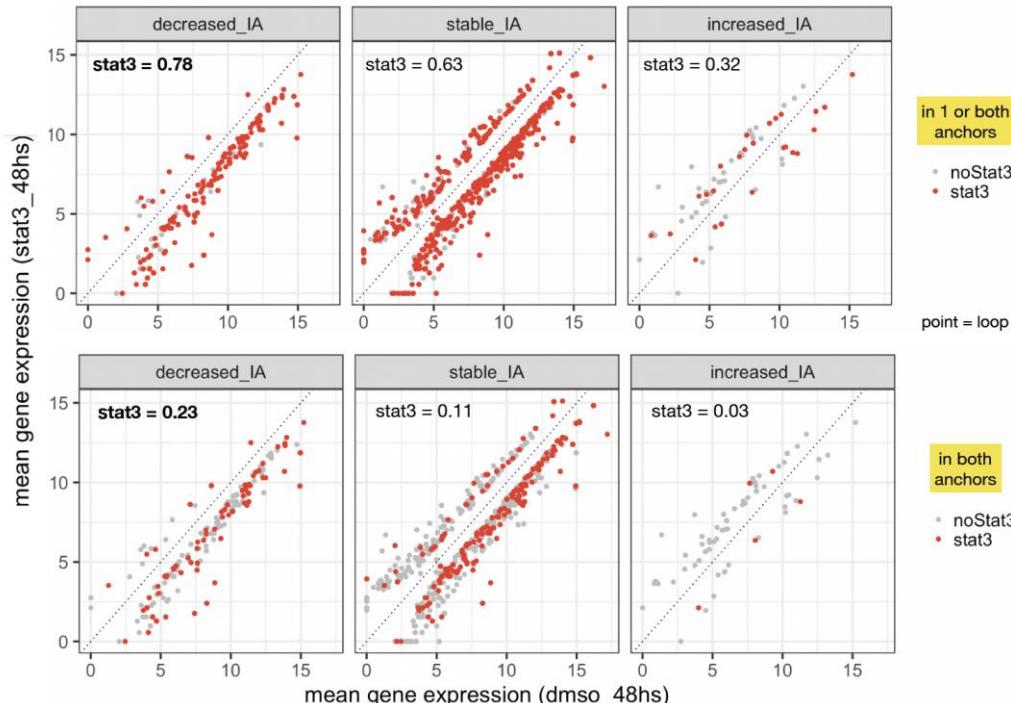


upregulated genes
are associated to
loops with **increased**
interactivity



STAT3 is enriched in loops with decreased interactivity (loop centric)

STAT3 is enriched in loops with decreased interactivity (loop centric)



loops with **decreased interactivity** are associated to **downregulated genes**

loops with **increased interactivity** are associated to **upregulated genes**



Conclusions

- Heterobifunctional STAT3 degraders can potently and selectively target STAT3 leading to growth arrest in primary ALCL cell models.
- STAT3 degraders are powerful tools to define the STAT3 pathogenetic mechanisms and dissect genes/pathways to be targeted for T-cell lymphoma eradication.
- STAT3 degraders will provide the mean to define STAT3 tumor addiction of PTCL and other human cancers
- STAT3 degraders represent powerful tools to dissect the mechanisms of STAT3-mediated gene transcription
- The pre-clinical data generated in PDTX models will provide the rationale for testing STAT3 degraders in the clinic for the treatment of aggressive malignancies including PTCL/ALCL.



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