



IOR
Un istituto
affiliato all'USI

Mechanisms of epigenetic dynamics

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Hematology

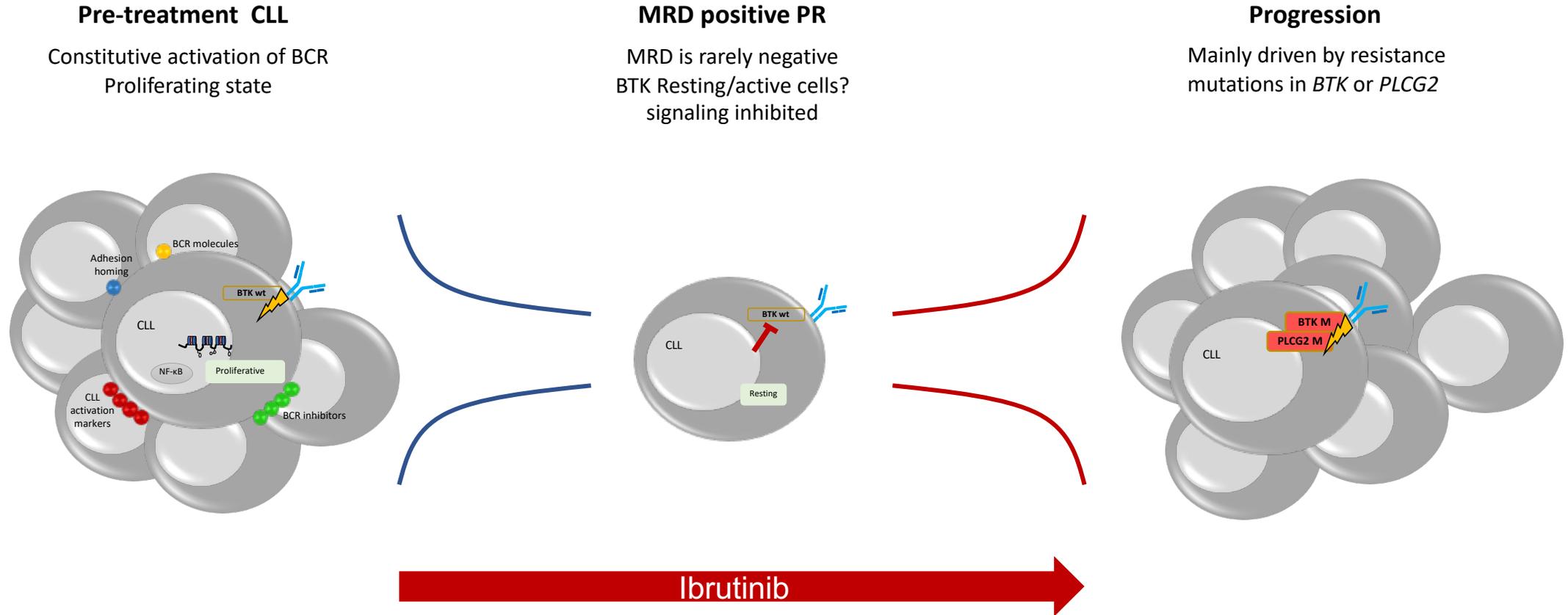
IOSI - Oncology Institute of Southern Switzerland

IOR - Institute of Oncology Research

USI – Università' della Svizzera Italiana

Bellinzona - Switzerland

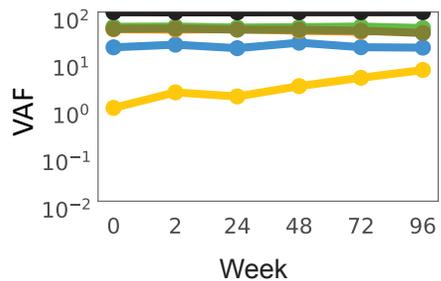
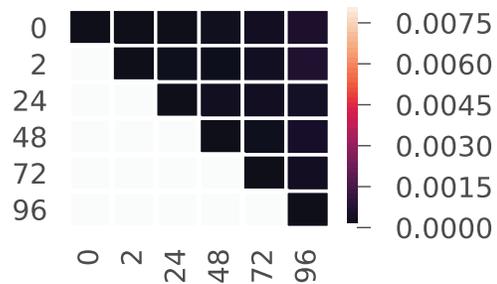
Progression in induced by mutations in *BTK* or *PLCG2* genes



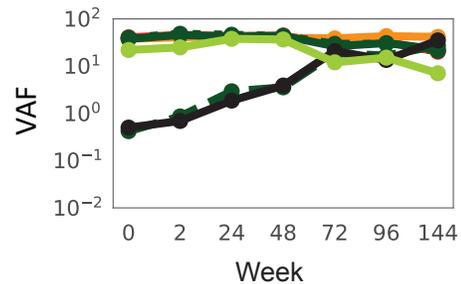
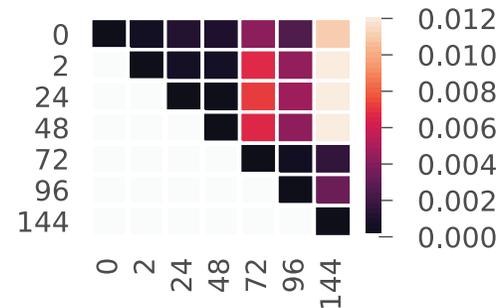
Does MRD genetically evolve?

Clonal evolution is not responsible for MRD survival

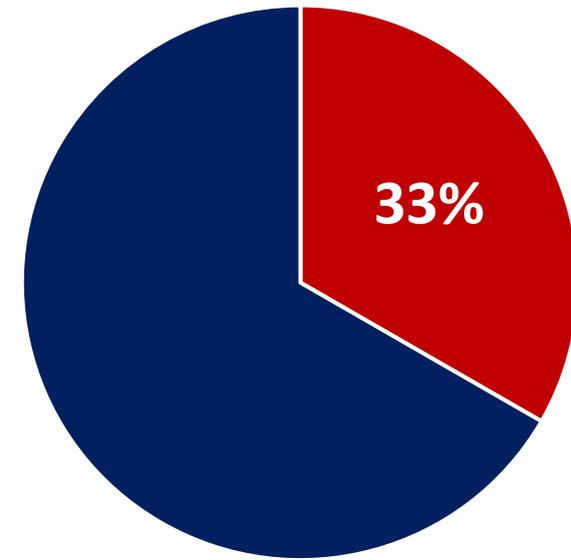
Case 1
Genetic similarity in temporal samples during treatment



Case 2
Changes in genetic similarity with rise of one mutation



■ Clonal evolution
■ No Clonal evolution



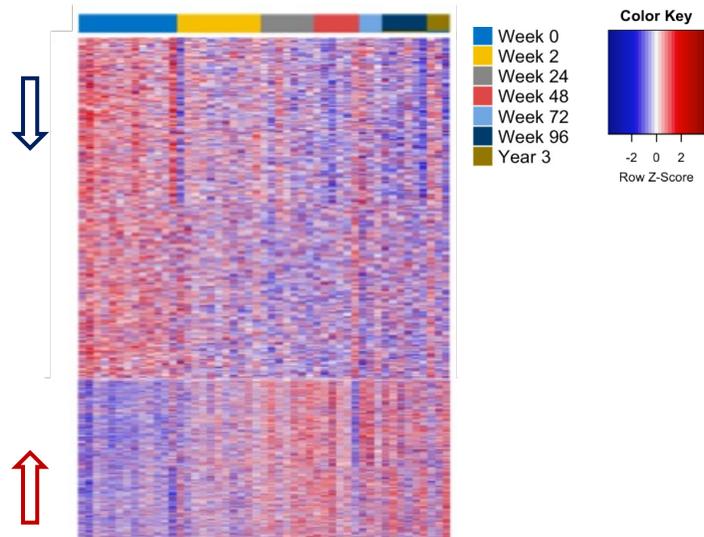
■ Yes ■ No

* No common patterns between patients

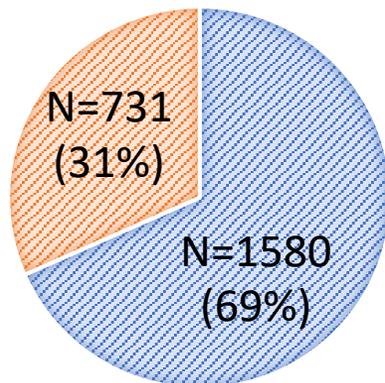
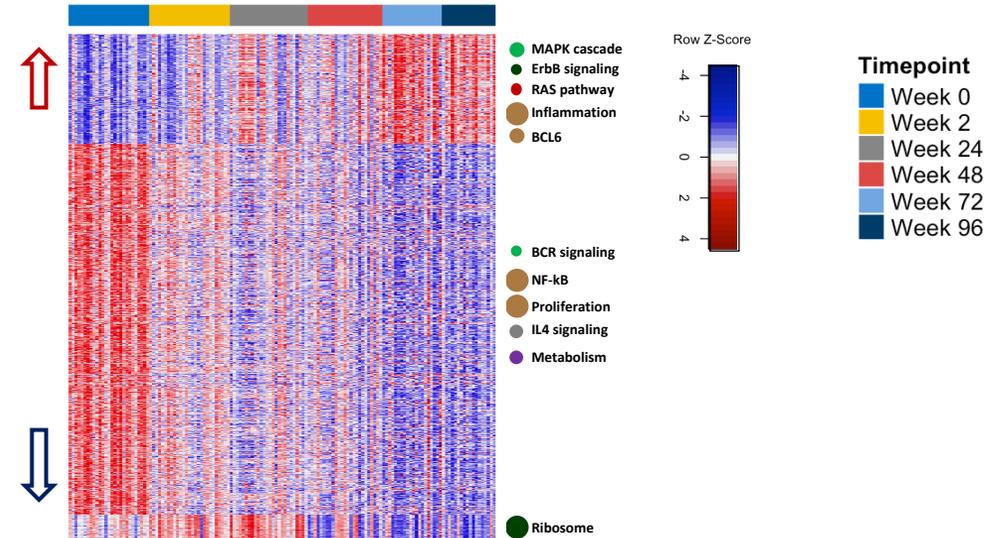
Does MRD epigenetically evolve?

MRD is in a predominantly closed chromatin state

ATAC-seq



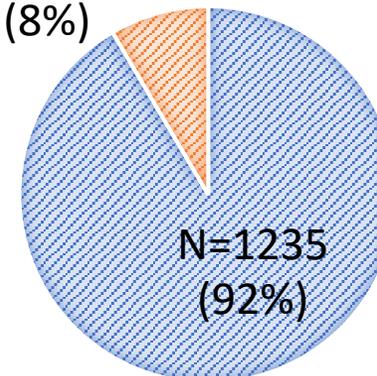
RNA-seq



Genomic regions

- Regions of decreased accessibility
- Regions of increased accessibility

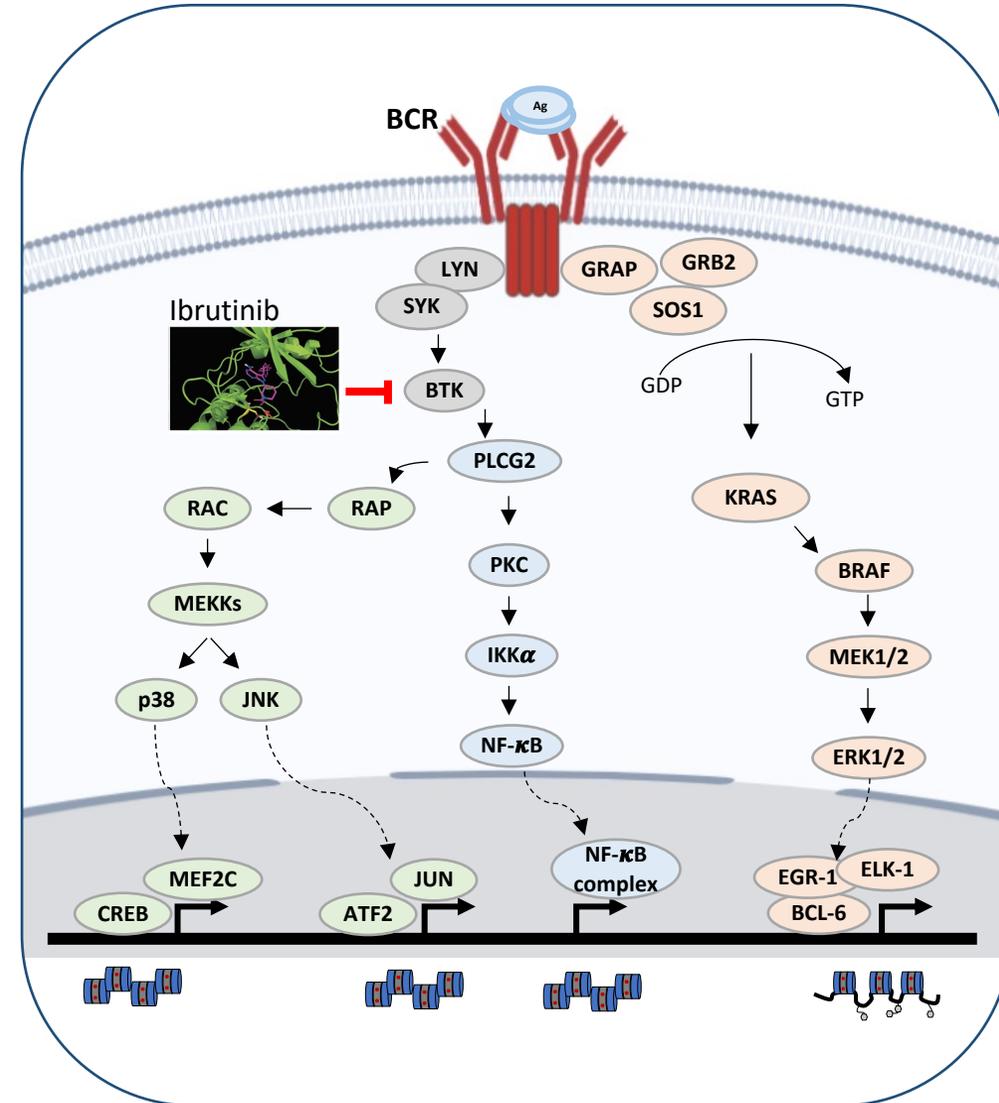
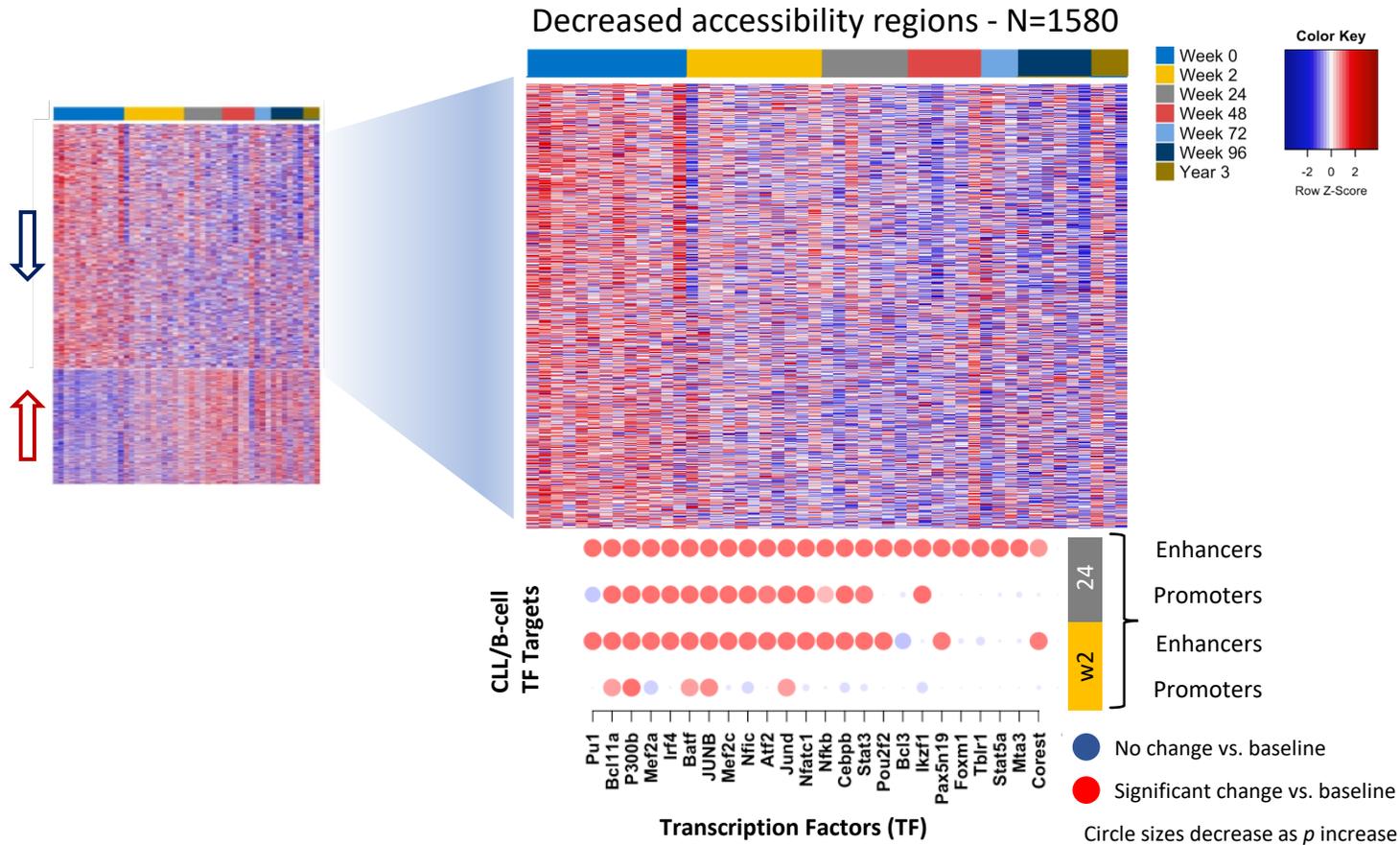
N=114 (8%)



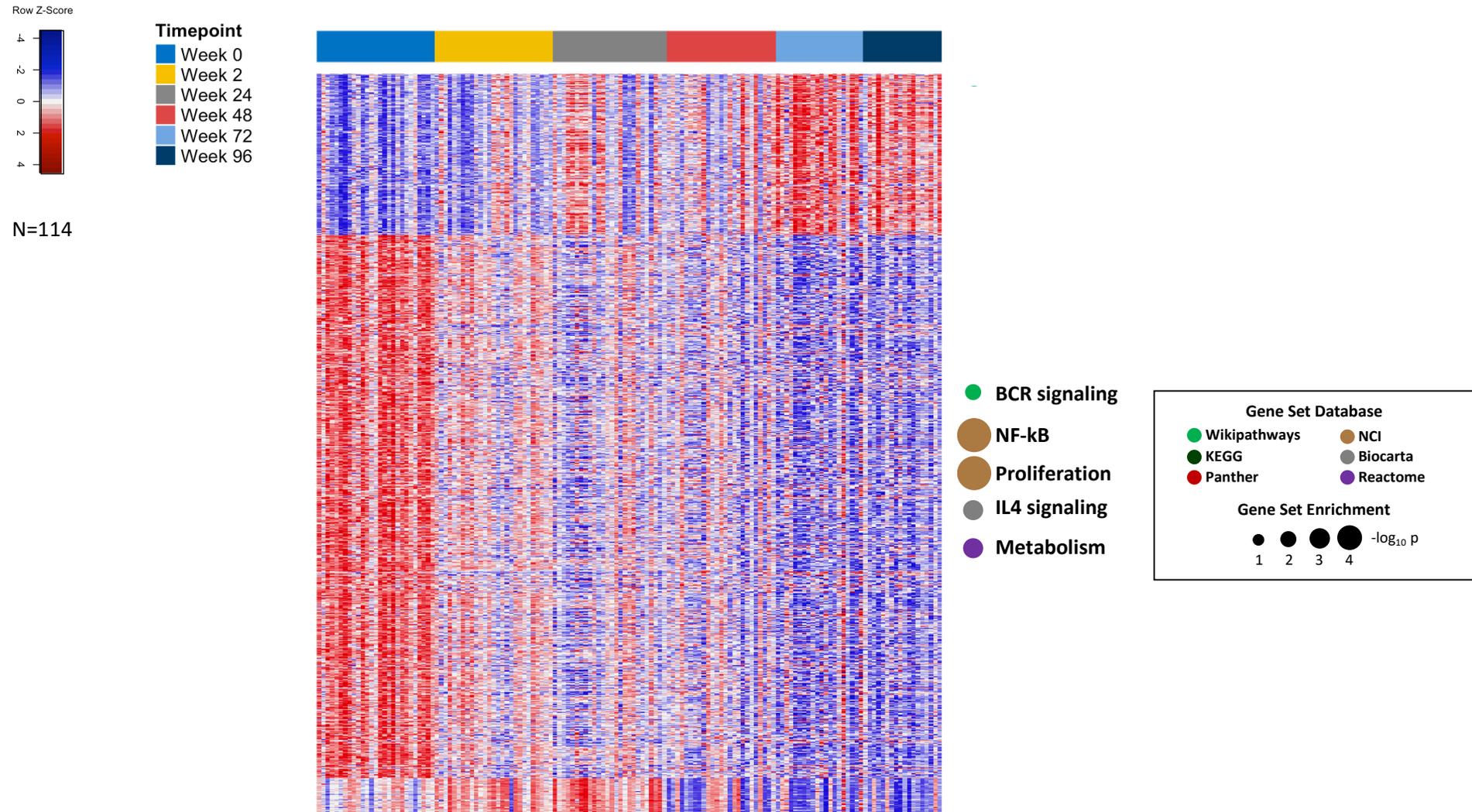
Transcripts

- Transcripts of decreased expression
- Transcripts of increased expression

Transcription factor map of MRD is rewired: NF- κ B, JUN, NFAT targets decrease their accessibility

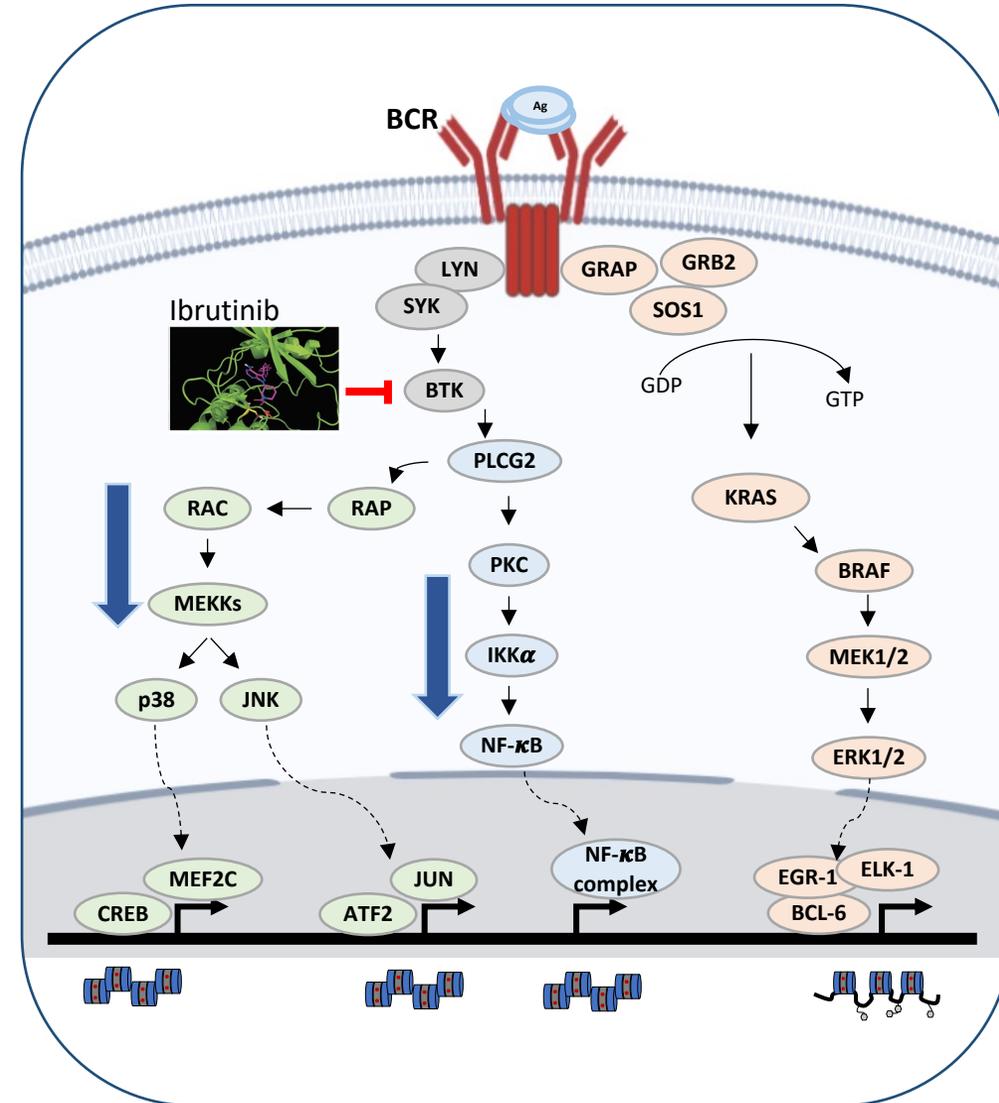
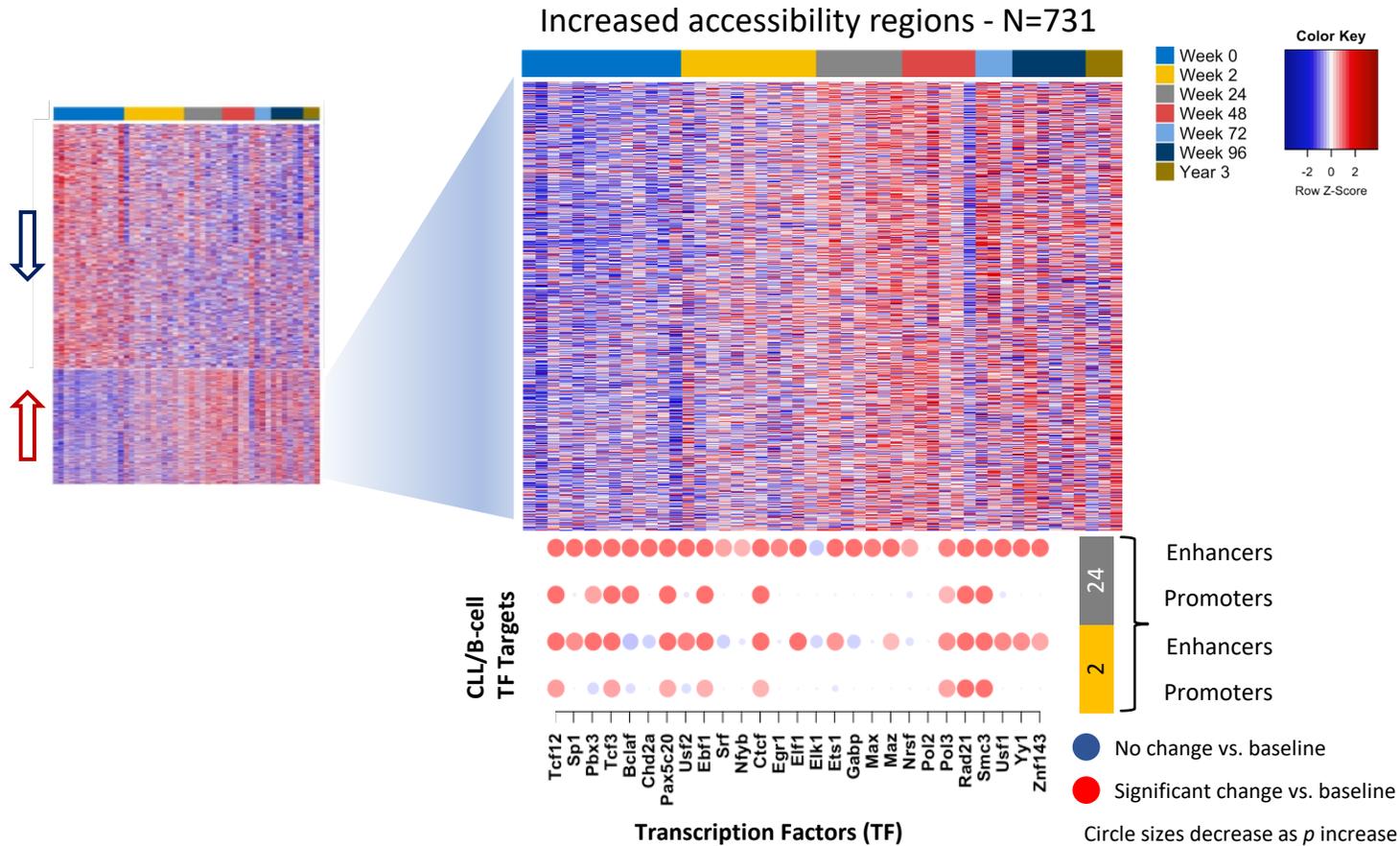


BCR signaling is downregulated under ibrutinib



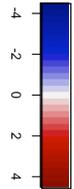
What is the pathway that supports MRD?

Transcription factor map of MRD is rewired: ERK targets increase their accessibility



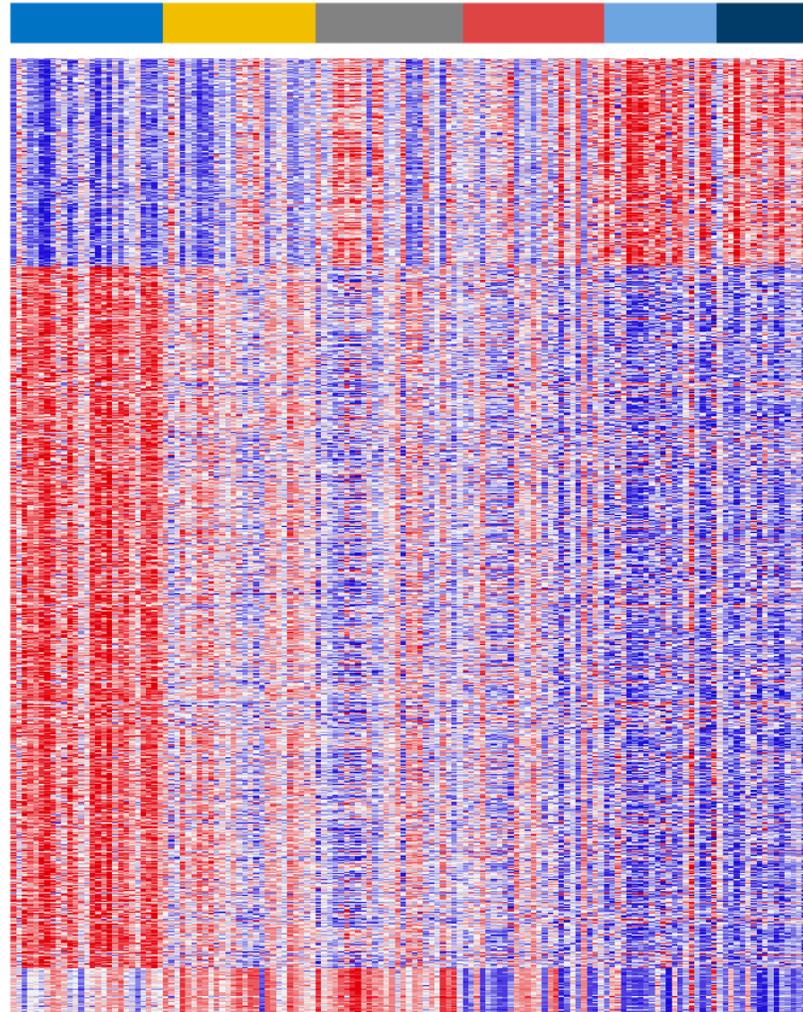
MAPK pathway is upregulated under ibrutinib

Row Z-Score

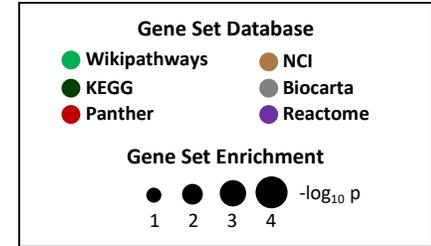


Timepoint

- Week 0
- Week 2
- Week 24
- Week 48
- Week 72
- Week 96



- MAPK cascade
- ErbB signaling
- RAS pathway
- Inflammation
- BCL6 targets

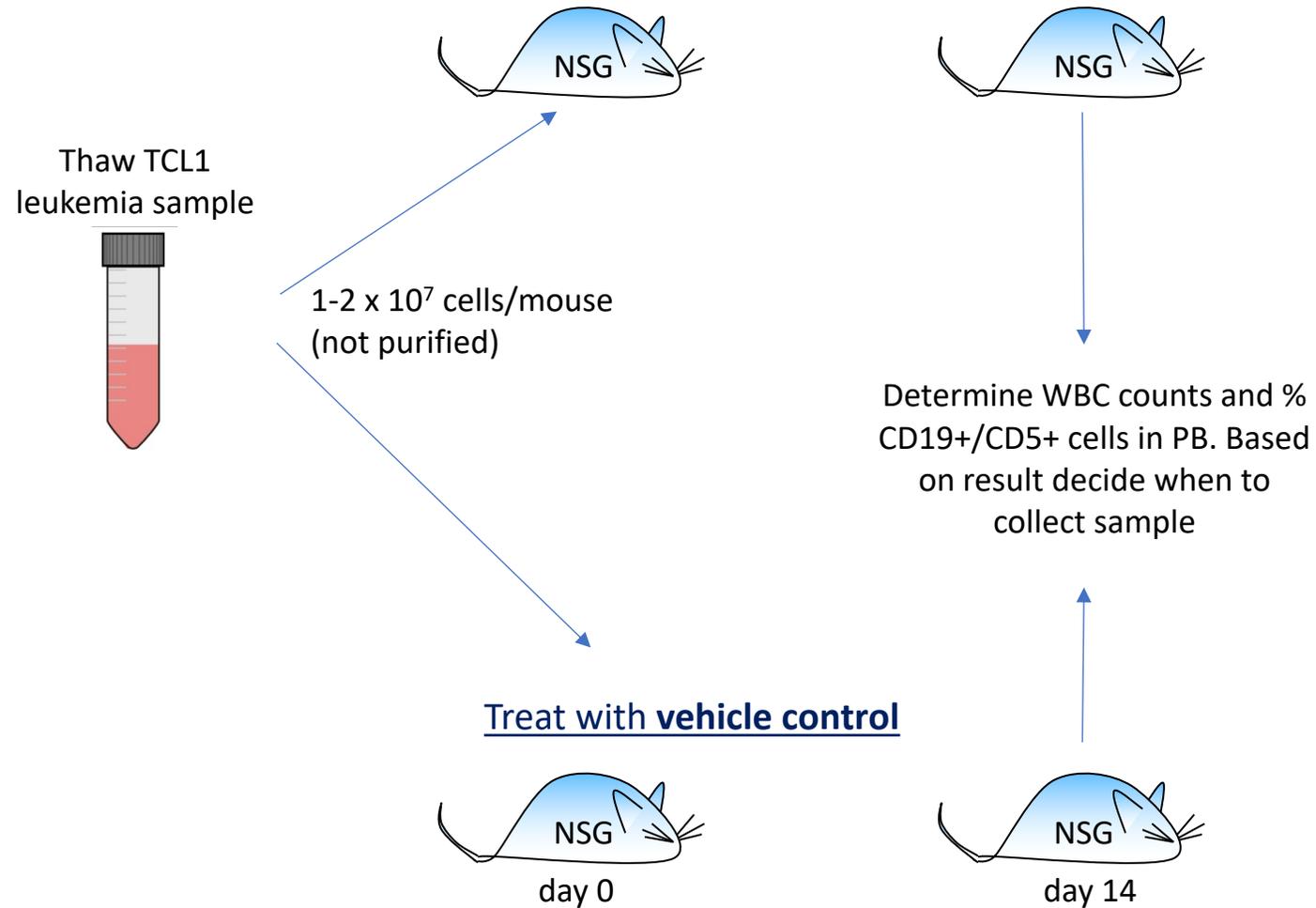


- Ribosome

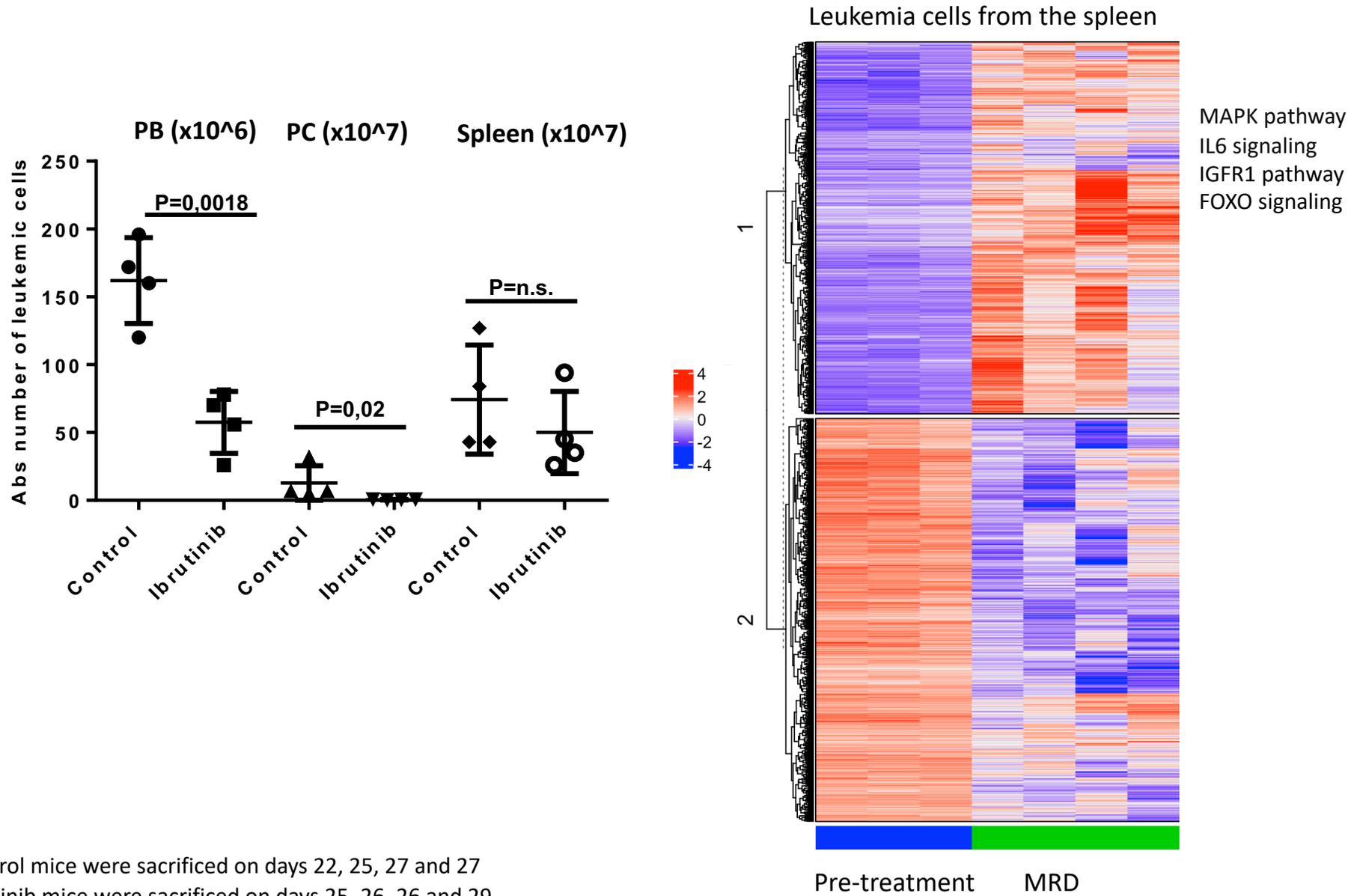
Is RAS-RAF-MEK-ERK active in the tissue compartment?

In vivo modeling of MRD in the tissue

Treat with **Ibrutinib** 25mg/kg/day (Ibrutinib administered in drinking water at conc. 0.16mg/ml)



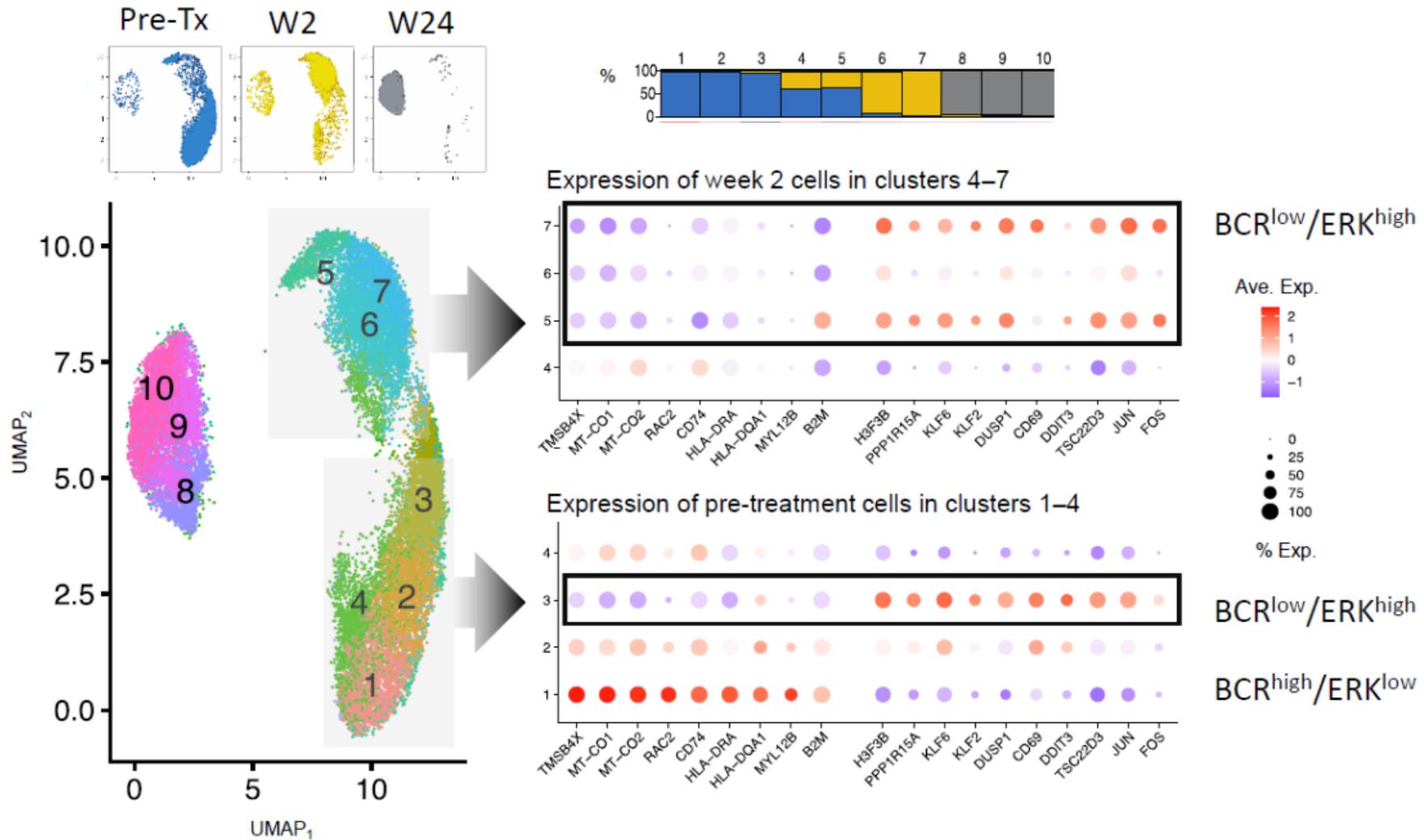
TCL1 leukemia cells persisting in the spleen under ibrutinib have active RAF-RAF-MEK-ERK



Control mice were sacrificed on days 22, 25, 27 and 27
Ibrutinib mice were sacrificed on days 25, 26, 26 and 29

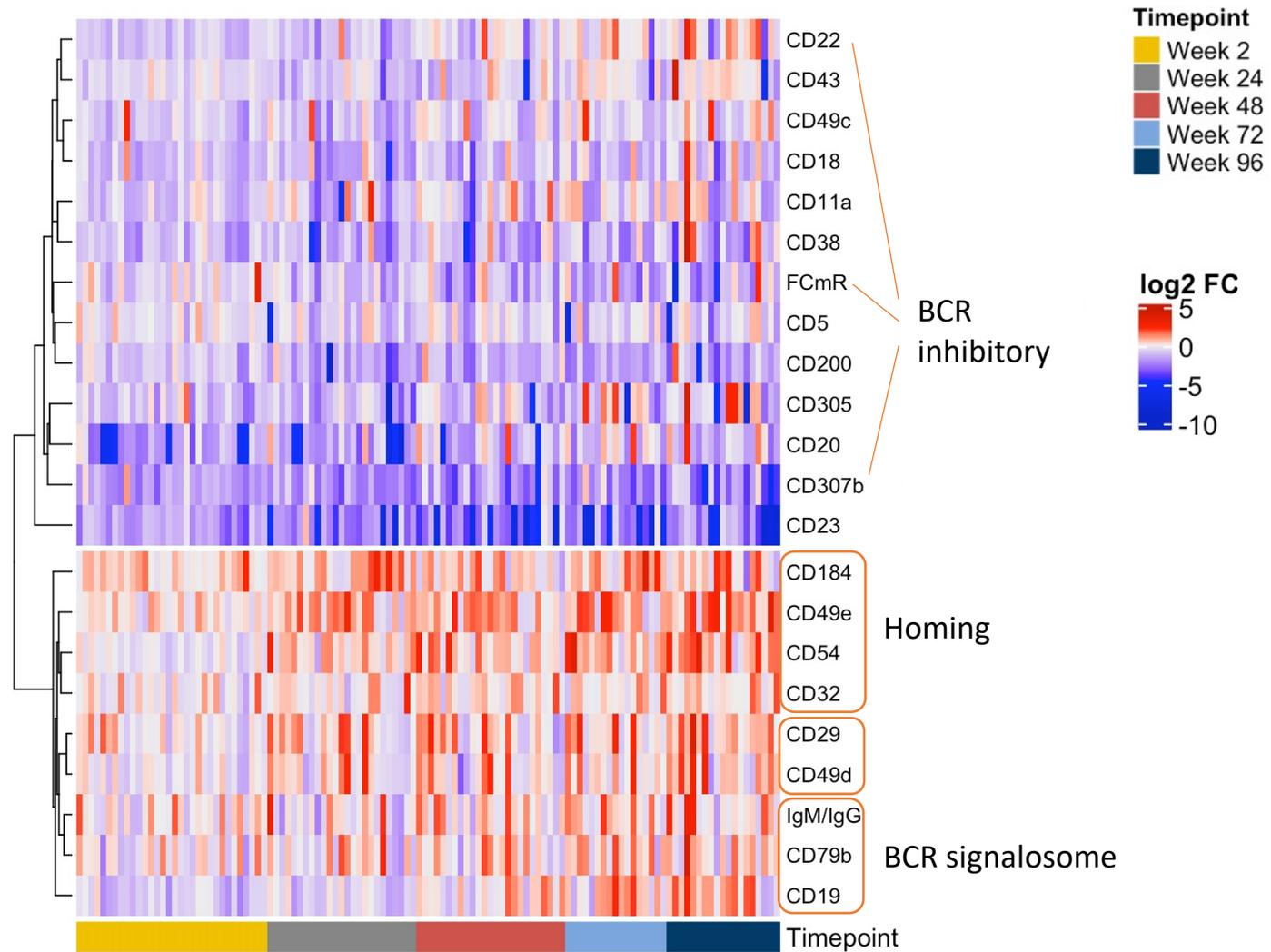
Does BCR^{low}/ERK^{high} cells pre-exist before ibrutinib start?

Single cell RNA-seq reveals a pre-existing BCR^{low}/ERK^{high}

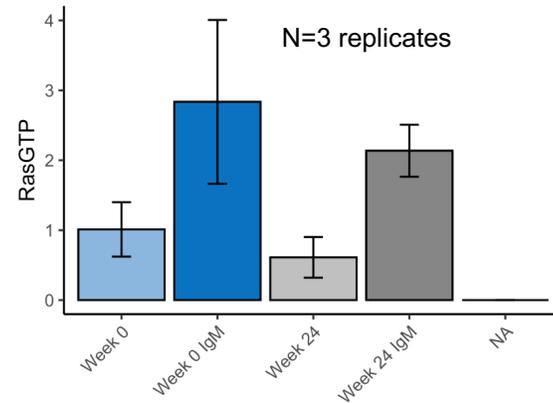
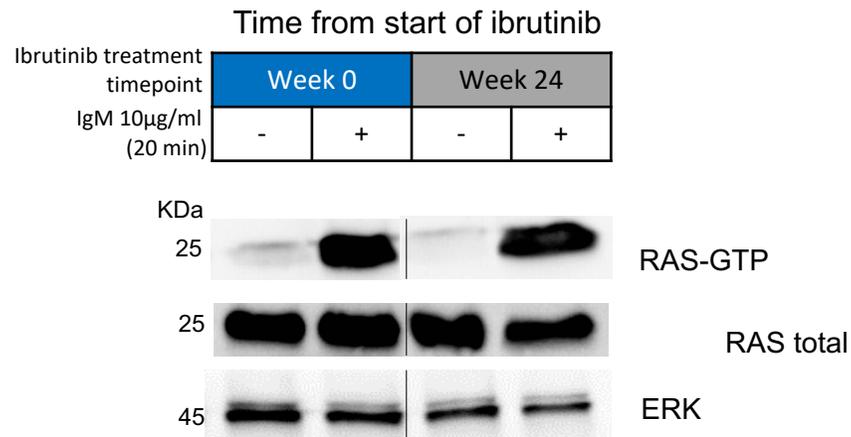
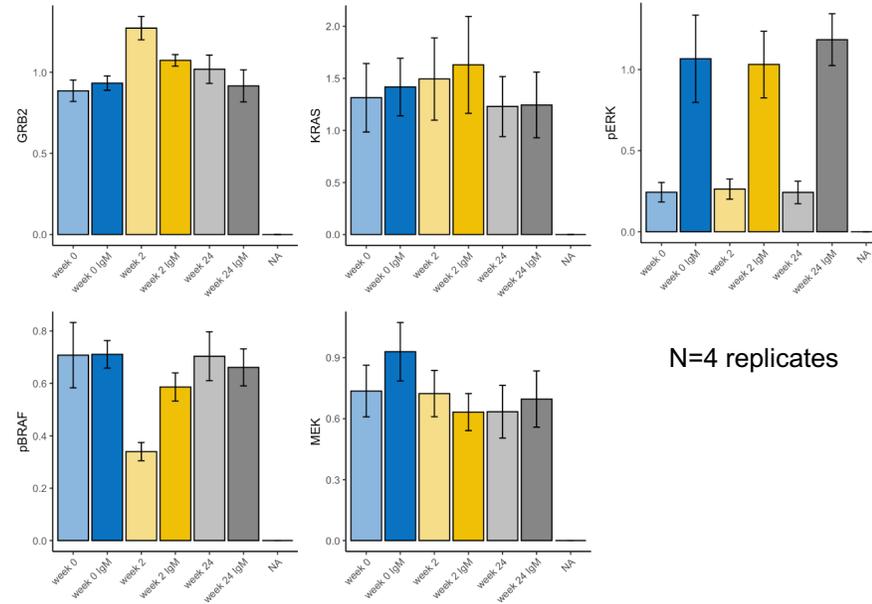
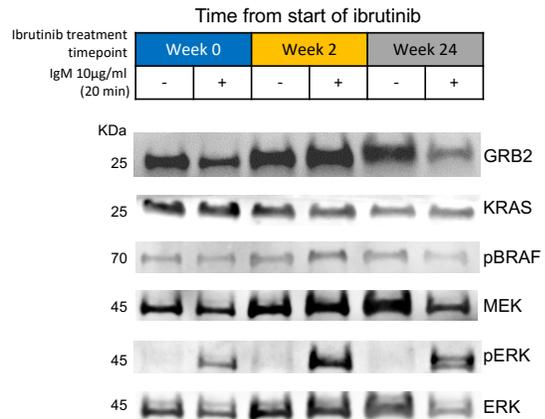


What does activate of RAS-RAF-MEK-ERK?

MRD cells adapt their surface machinery

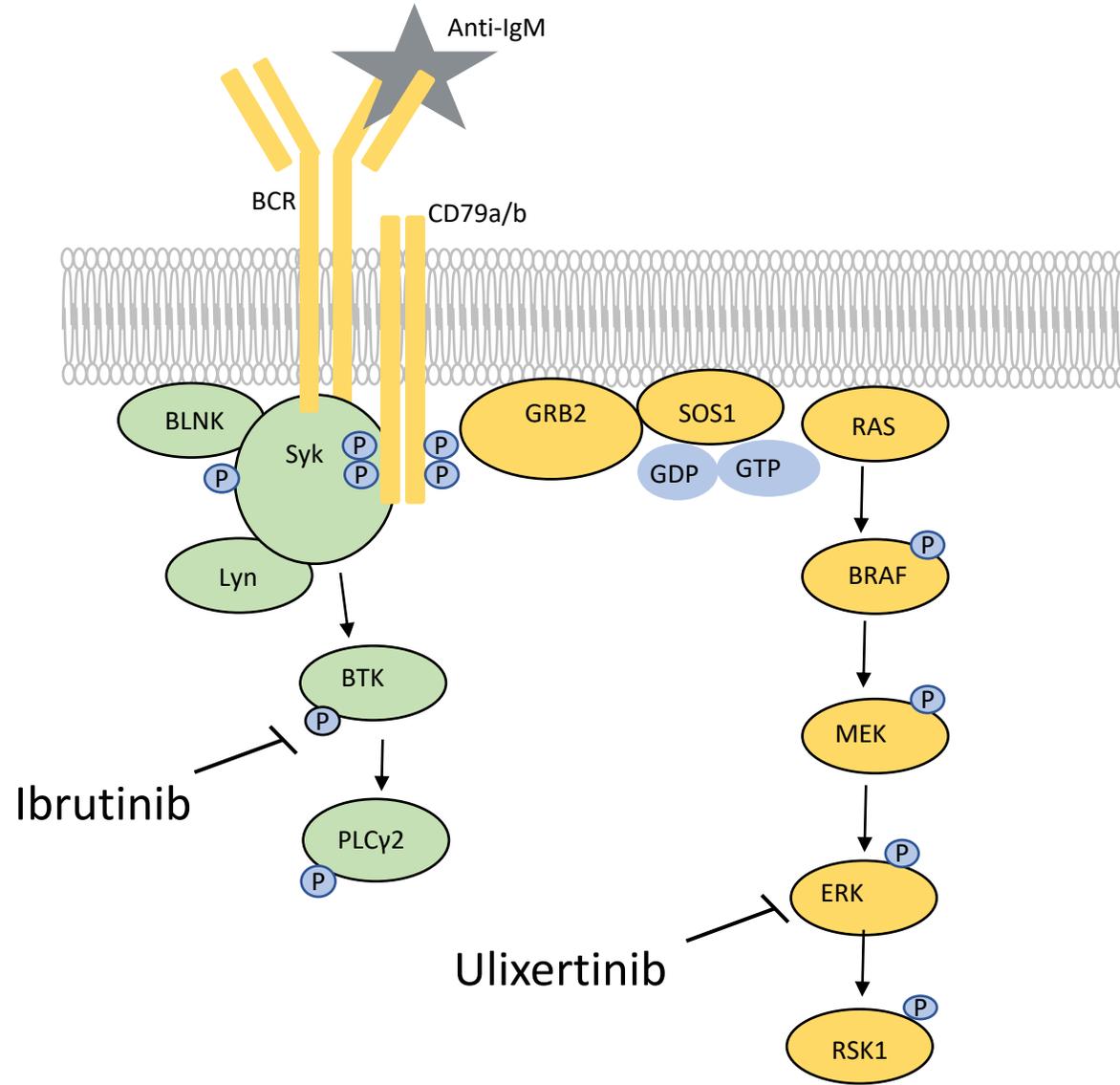


MRD maintains signalling via RAS-RAF-MEK-ERK

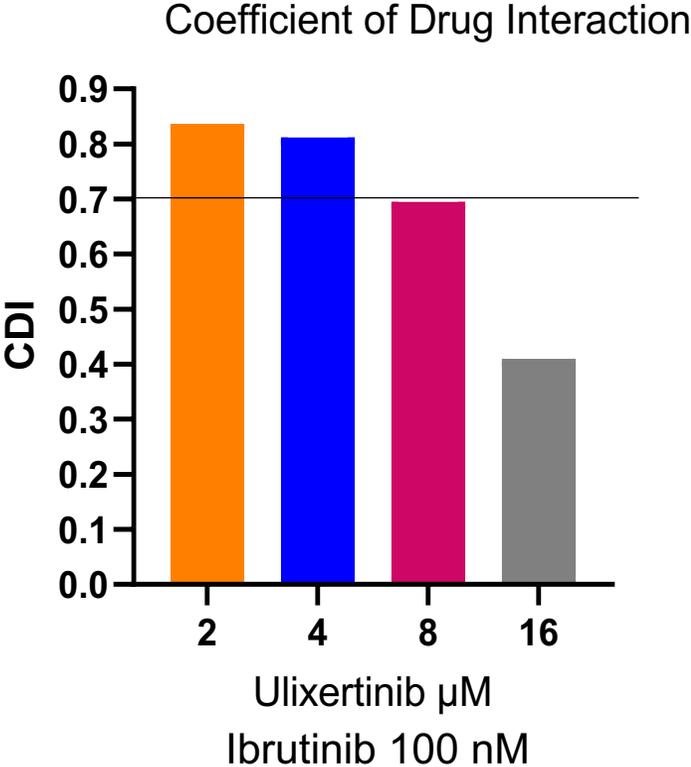
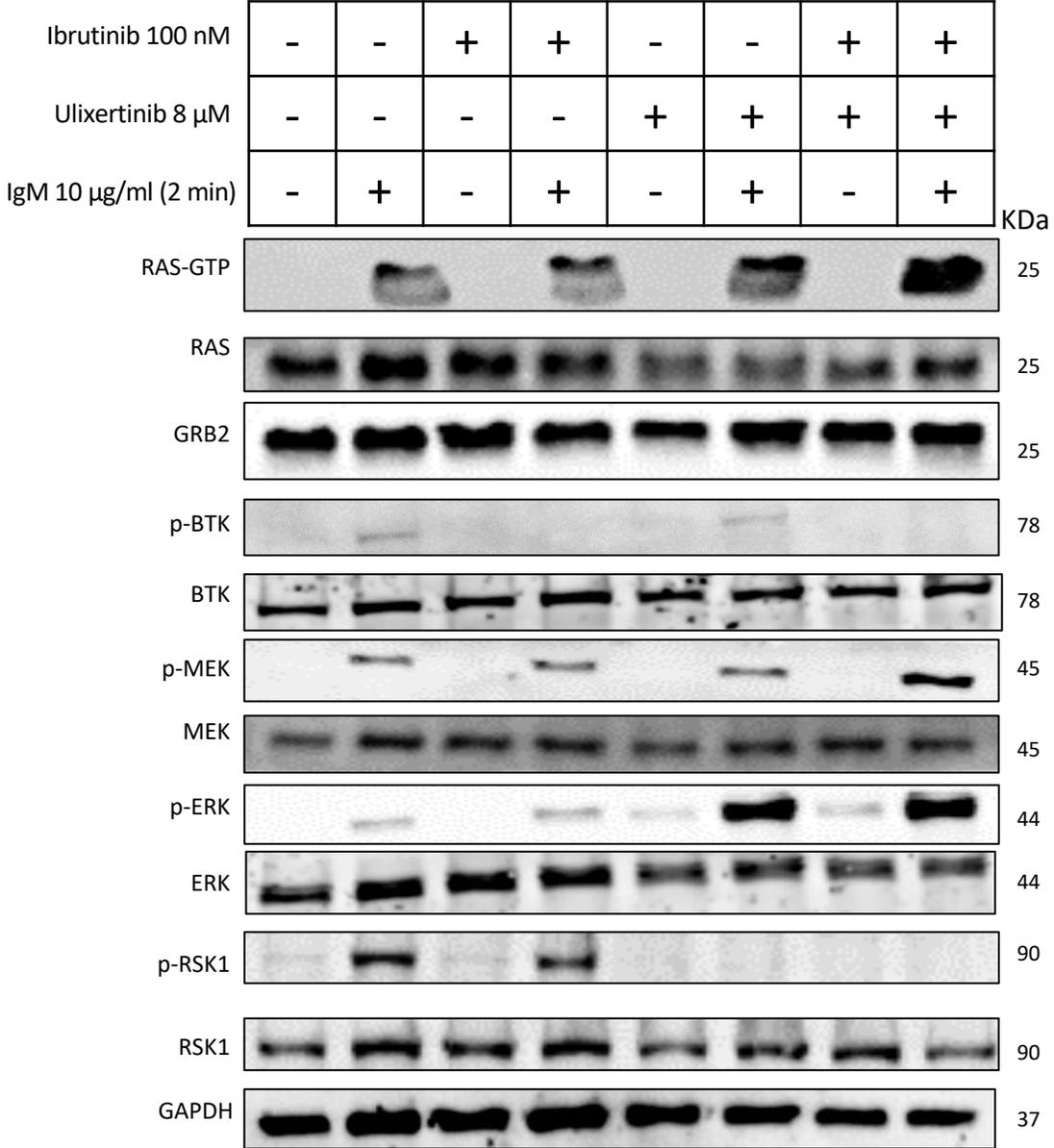


Is RAS-RAF-MEK-ERK a vulnerability of MRD?

Opportunities for pharmacological manipulation of the RAS-RAF-MEK-ERK pathway



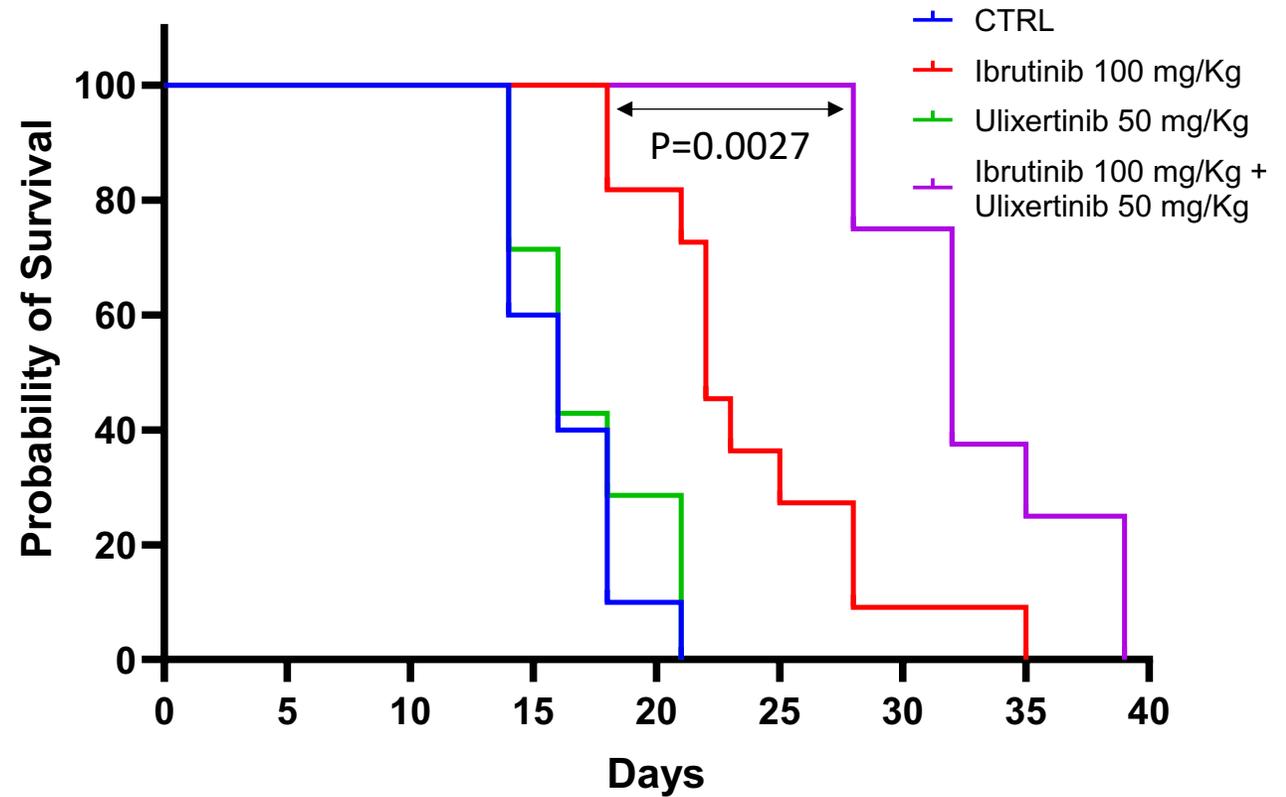
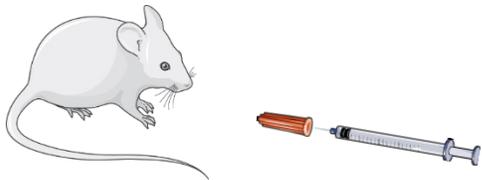
ERK inhibition synergizes with ibrutinib



CDI <1 indicates synergism
 CDI <0.7 indicates a significantly synergistic effect

Ulixertinib in combination with ibrutinib prolongs survival

REC1 xenograft
NOD-SCID mice 6-8 weeks old



Conclusions

- **BTK inhibition results into a large chromatin rewiring**
- **RAS-RAF-MEK-ERK remains active**
- **BCR engagement triggers RAS-RAF-MEK-ERK to sustain MRD viability**
- **Clones BCR^{low}/ERK^{high} pre-exist ibrutinib treatment**
- **RAS-RAF-MEK-ERK is a vulnerability of MRD**