Mechanisms of Response and Resistance to Epigenetic Therapies

Dr. Wilson Miller

7th International Symposium on APL

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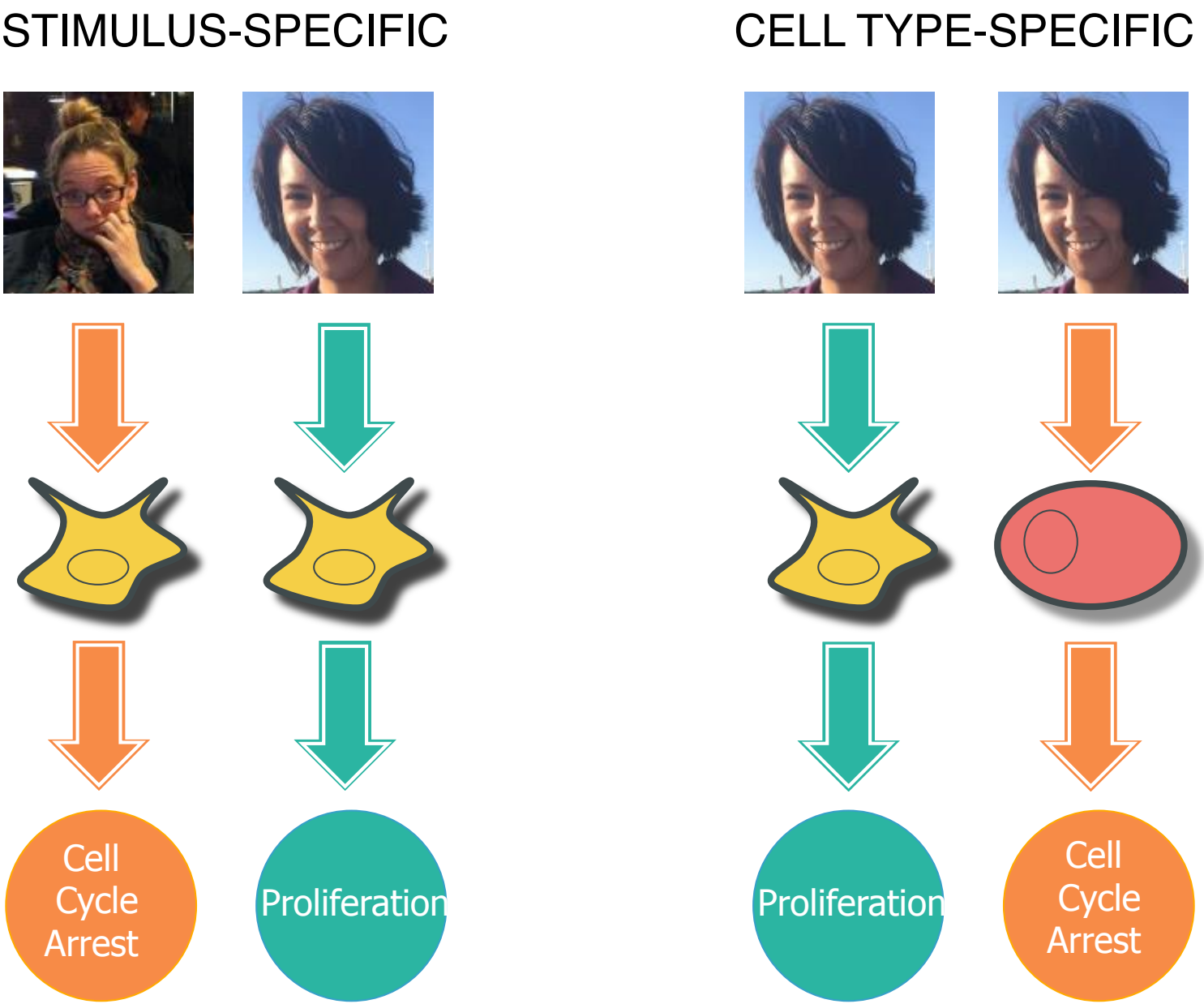
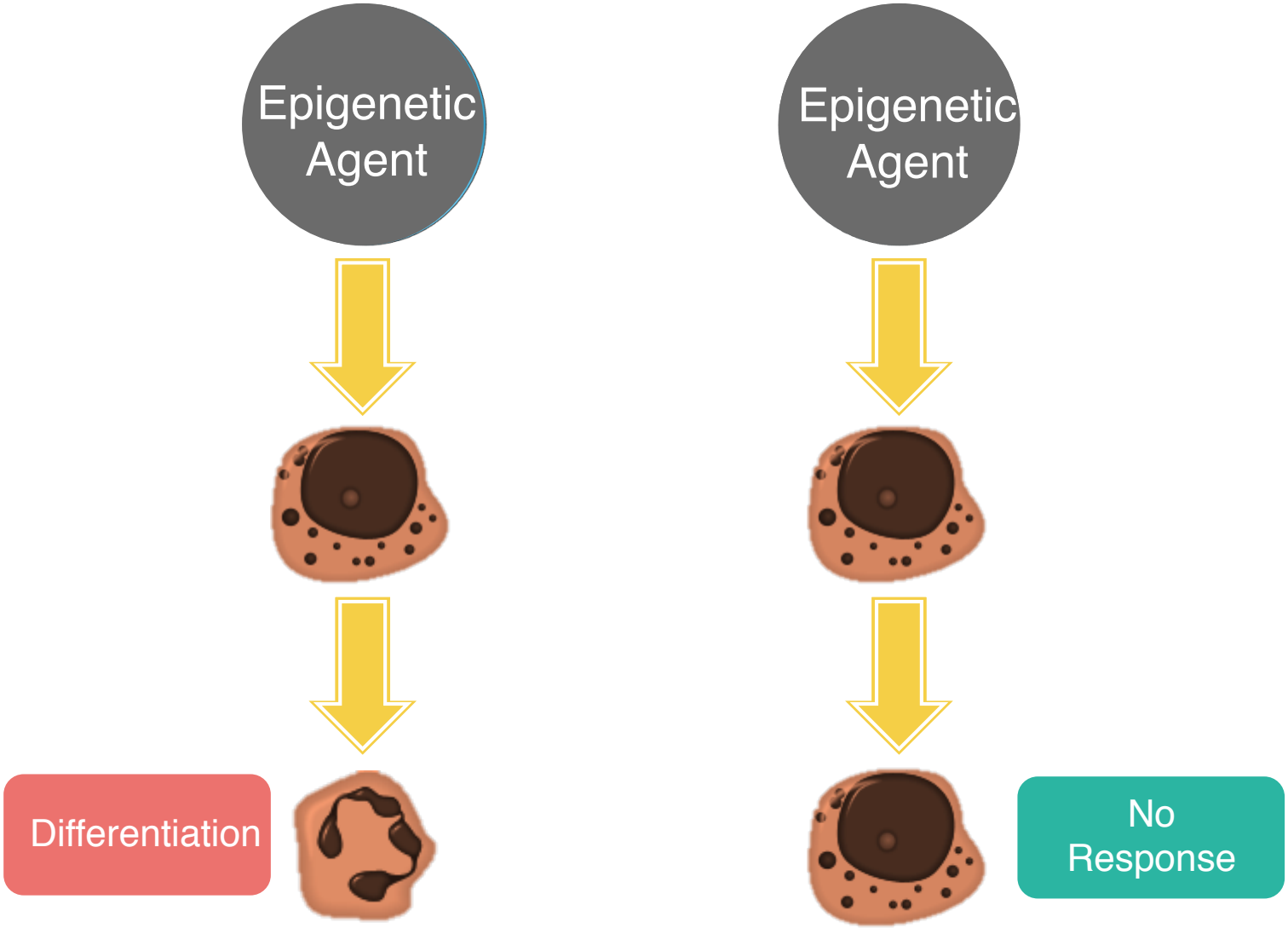
Drug Response Programs are Stimulus & Cell Specific



Response and Resistance to Epigenetic Drugs

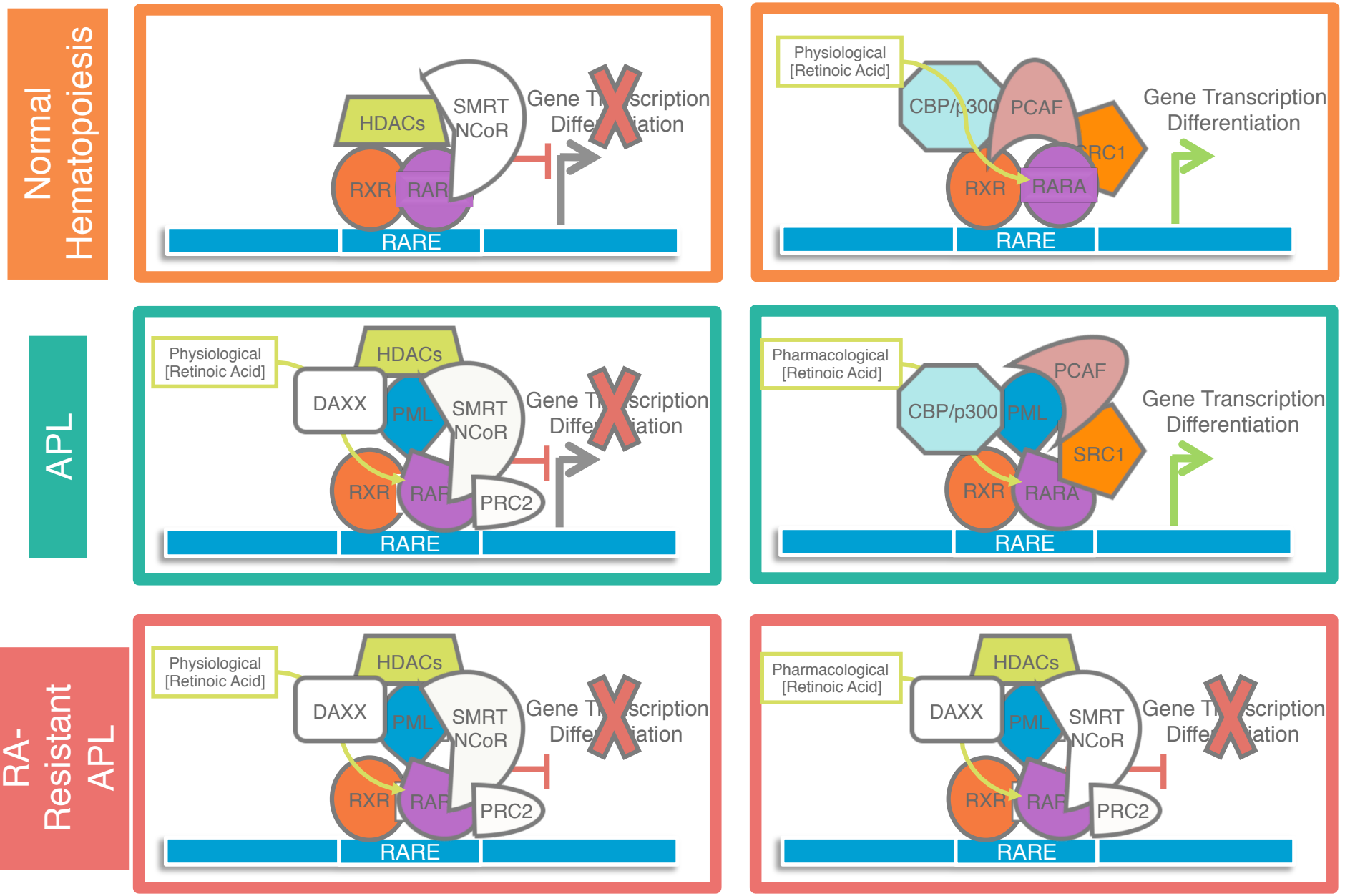


Research Goals

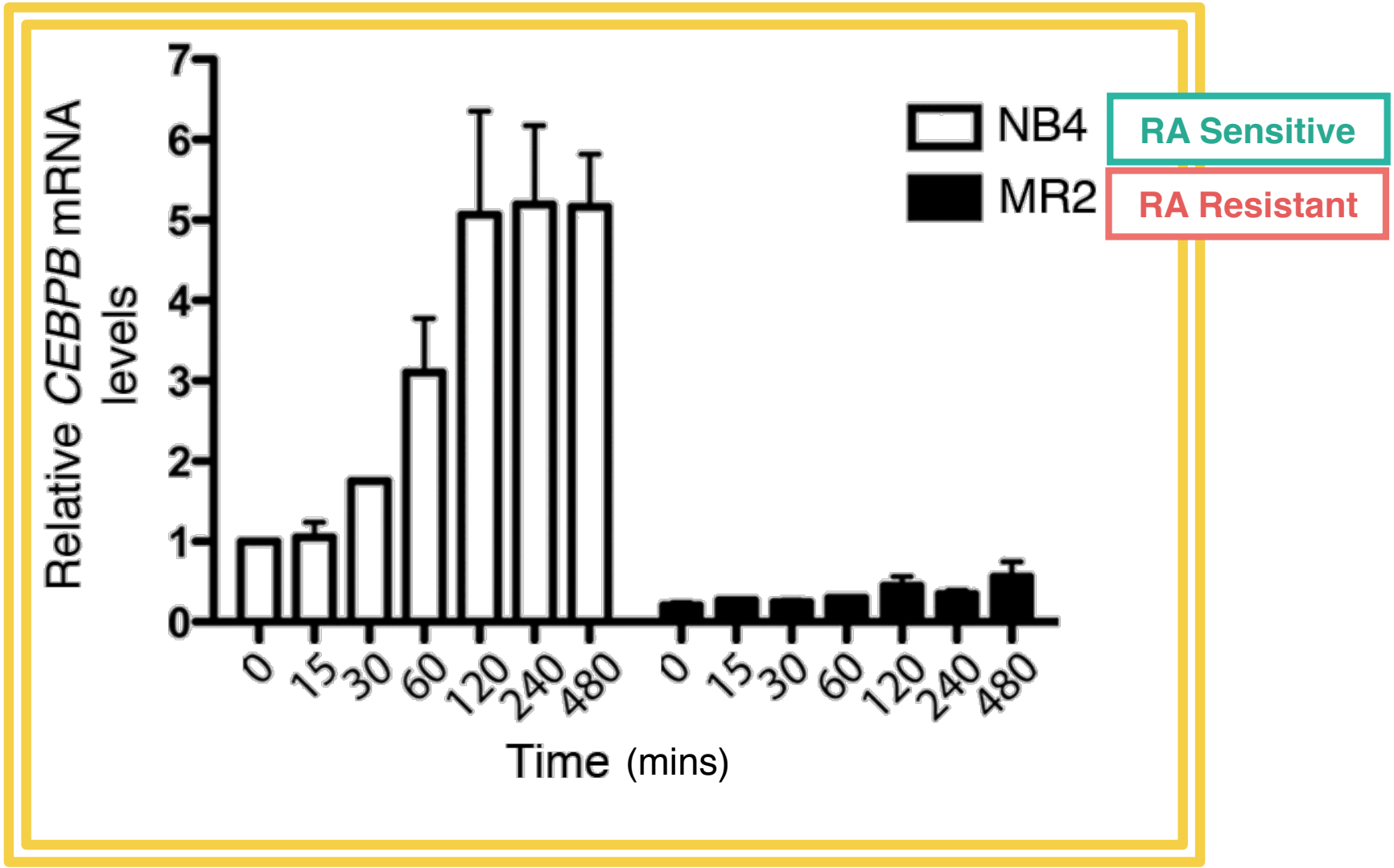
Examine the epigenetic deregulation in hematopoietic malignancies

1. To understand why these diseases respond to these therapies
2. To uncover common mechanisms of resistance
3. To inform on potential combination or second-line therapies

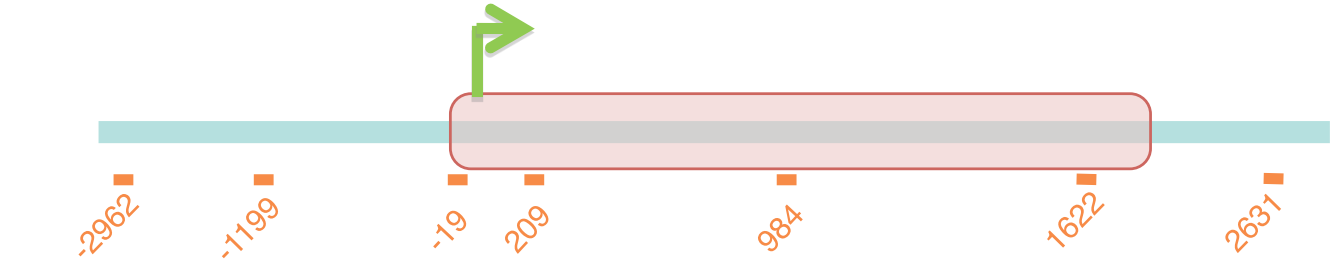
1. RA resistance in APL



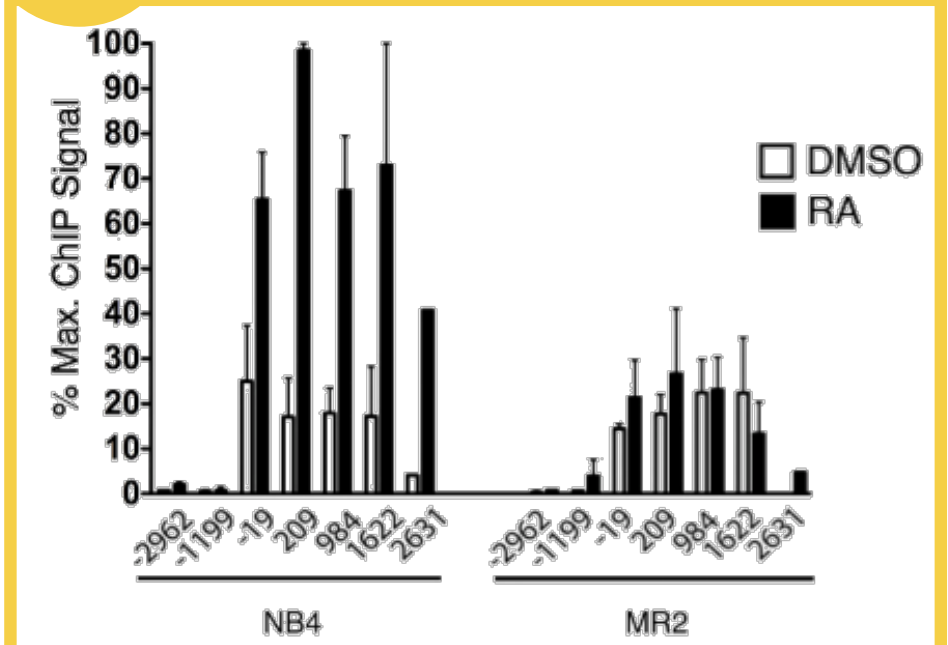
Transcriptional Blockade in Resistant Line



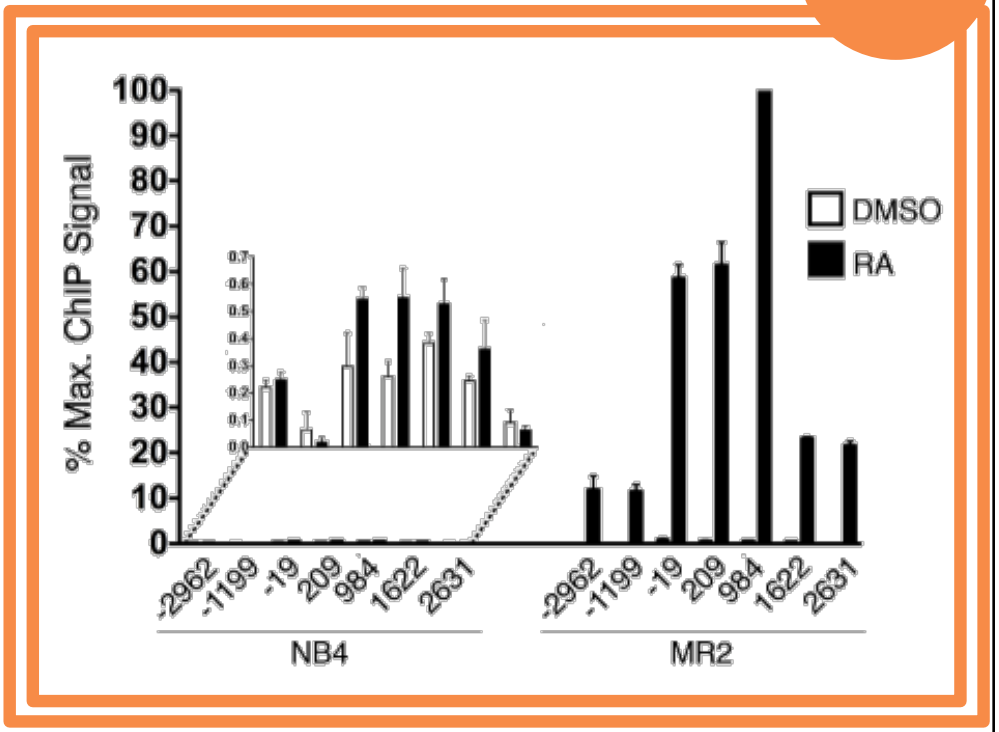
High Resolution ChIP Tiling of *CEBPB* locus



S5P



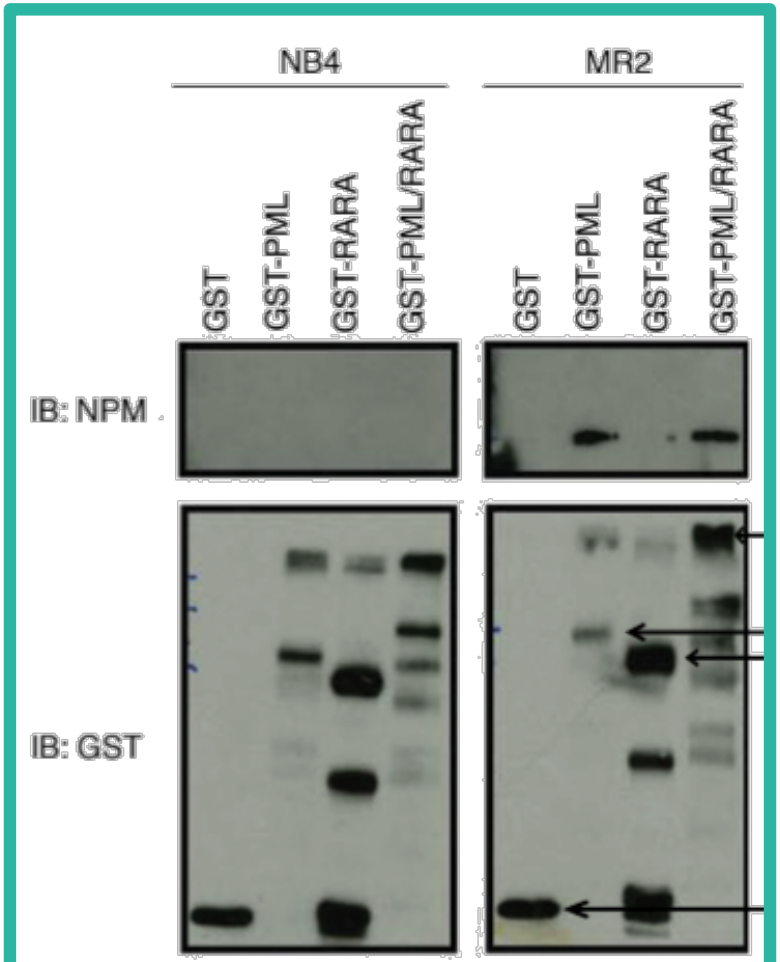
BRG1



Nichol et. al., Cell Reports 2016

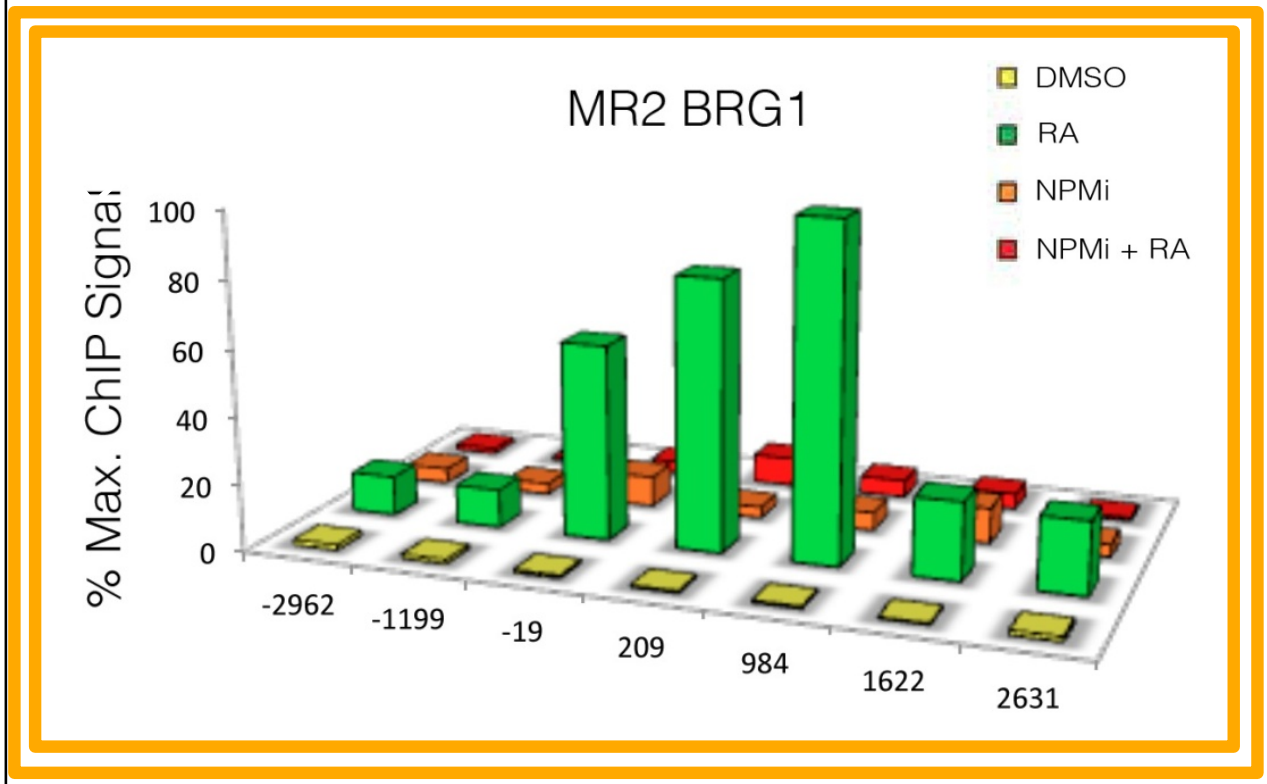
Novel Associations with PML/RARA in Resistant Cells

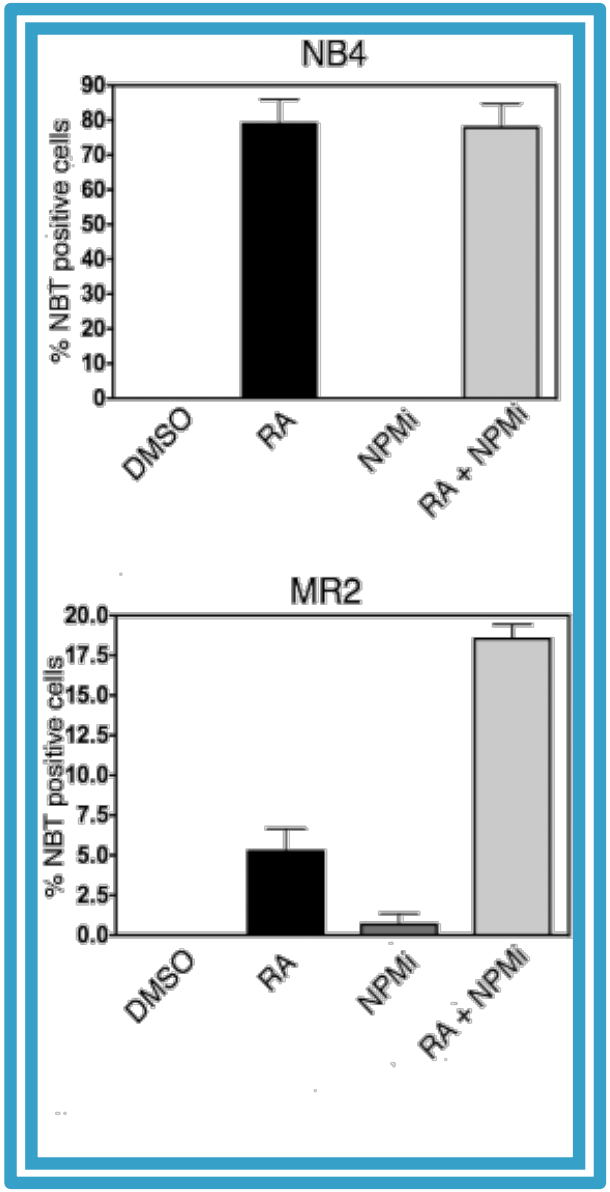
Protein	Mass (Da)	Ion Score
DNA Topoisomerase II Beta	183,548	493
Replication Factor C Subunit 4	40,170	465
Nucleophosmin	31,090	371
Hetereogeneous Nuclear Ribonucleoproteins C1/C2	32,004	304
60S Acidic Ribosomal Protein P0	34,423	106
Histone Deacetylase Complex Subunit SAP130	136,590	82
U5 Small Nuclear Ribonucleoprotein 40 kDa	39,730	68
Hetereogeneous Nuclear Ribonucleoproteins U	89,631	62



Nichol et. al., Cell Reports 2016

NPMi Abrogates BRG1Recruitment & Restores RA Response



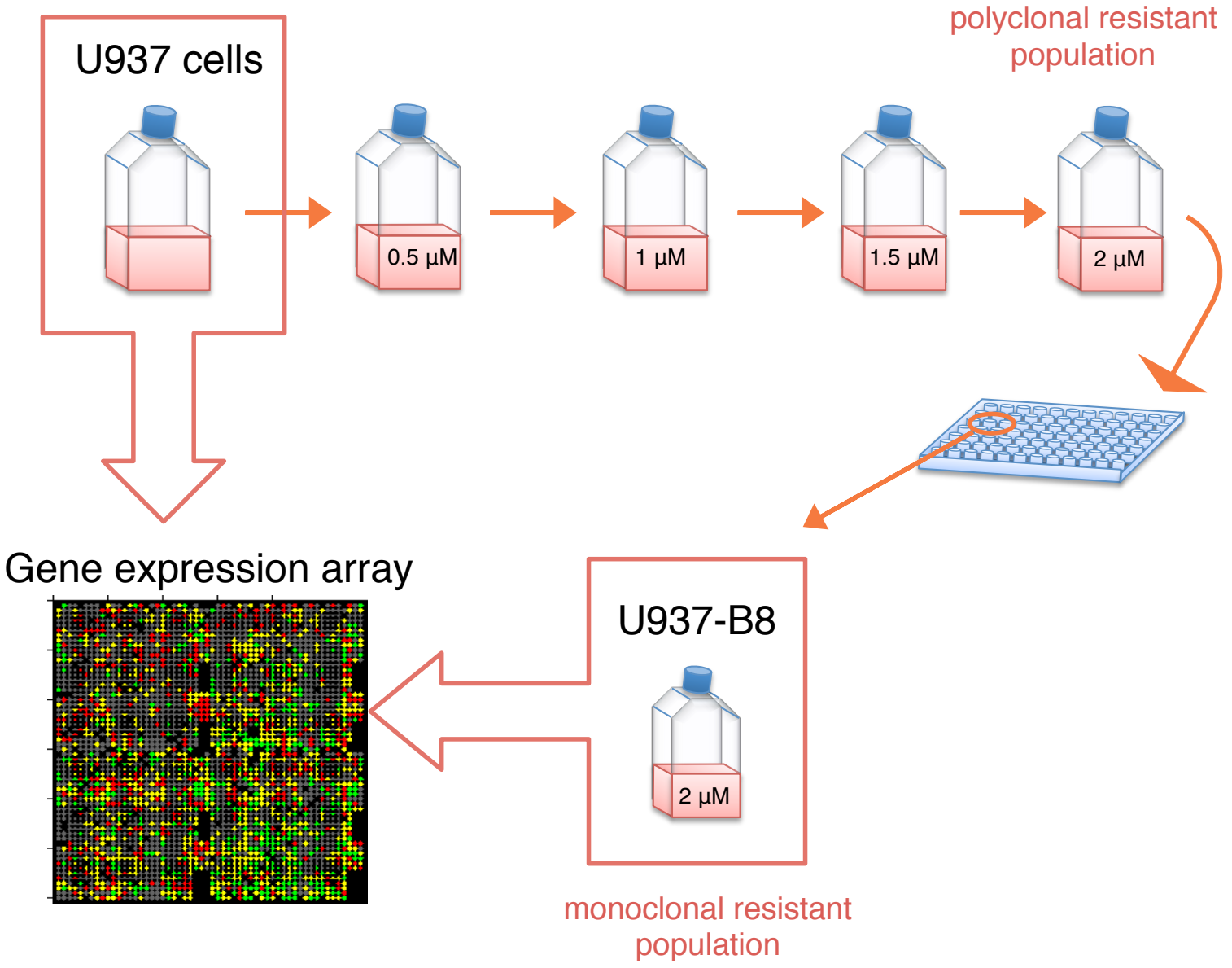


Nichol et. al., Cell Reports 2016

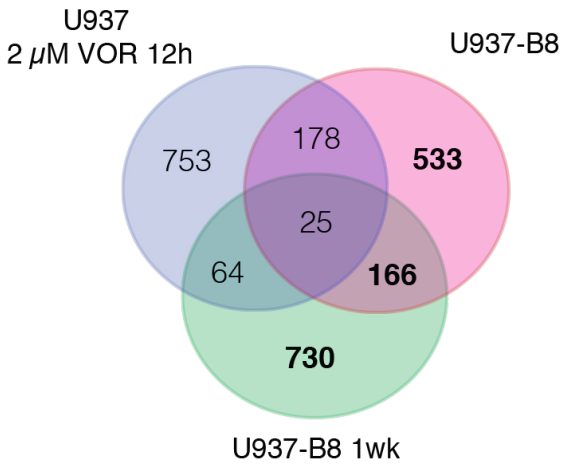
Conclusions: Part 1

- APL is a paradigm for transcriptional deregulation.
- A molecular mechanism of RA resistance involves a novel association of proteins, including NPM, with PML/RARA.
- Research on APL, a rare and mostly curable disease, can still produce interesting new results.

2. HDACi resistance

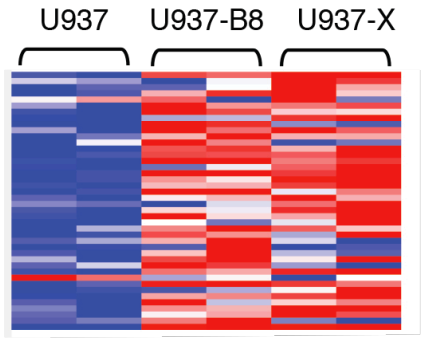


Increased Protein Processing in HDACi-resistant Cells

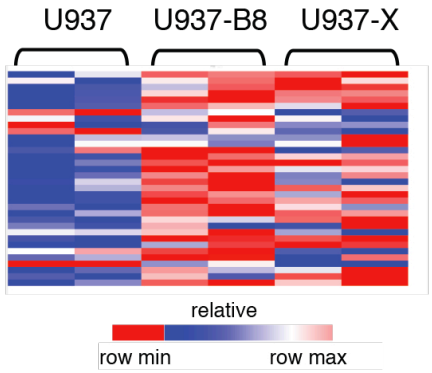


Annotation Cluster	Representative annotation terms	Enrichment score
1	mitochondrial inner membrane	3.75
2	endoplasmic reticulum membrane	3.61
3	lysosome	3.02
4	protein catabolic process	2.56
5	MAPKKK cascade	2.33
6	intracellular protein localization/transport	2.02
7	coenzyme /cofactor binding	1.74
8	vacuolar/lysosomal membrane	1.62
9	cytoplasmic vesicle	1.61
10	Golgi vesicle transport	1.60

GO term:
ER Membrane

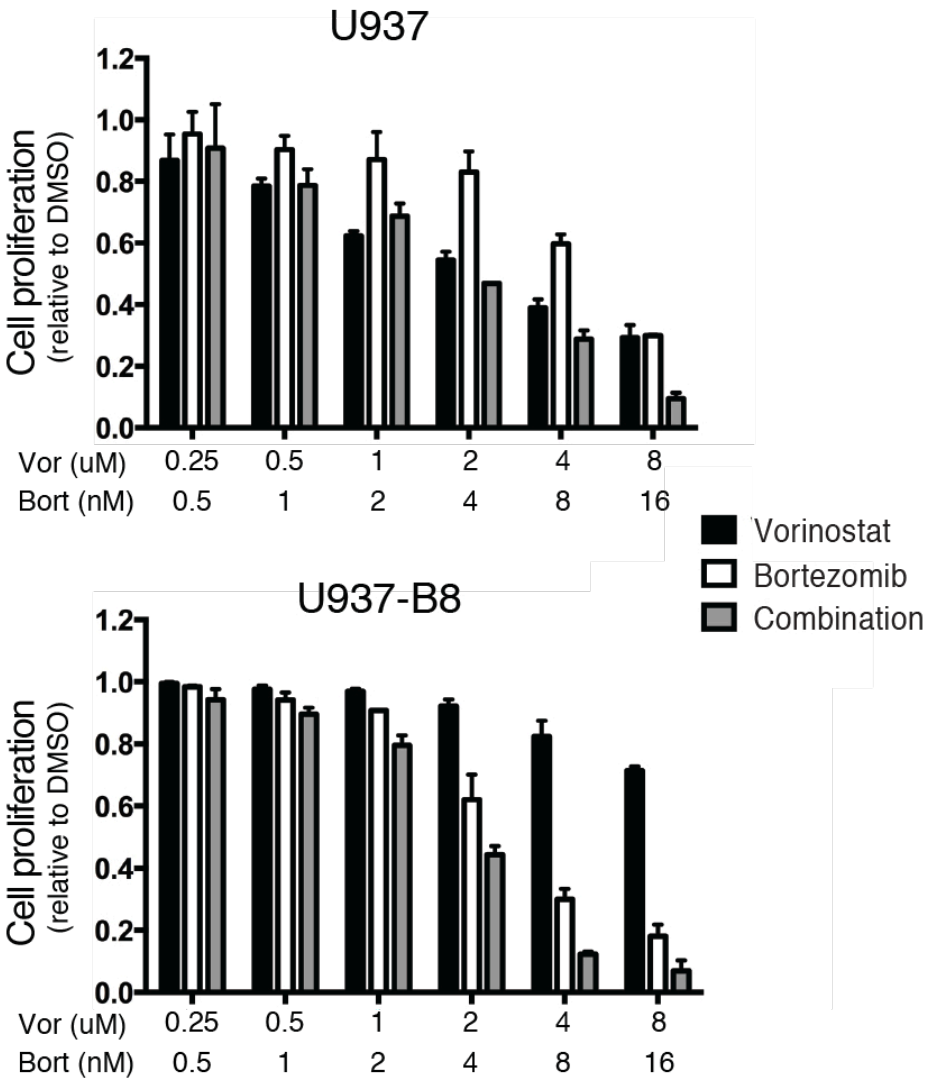


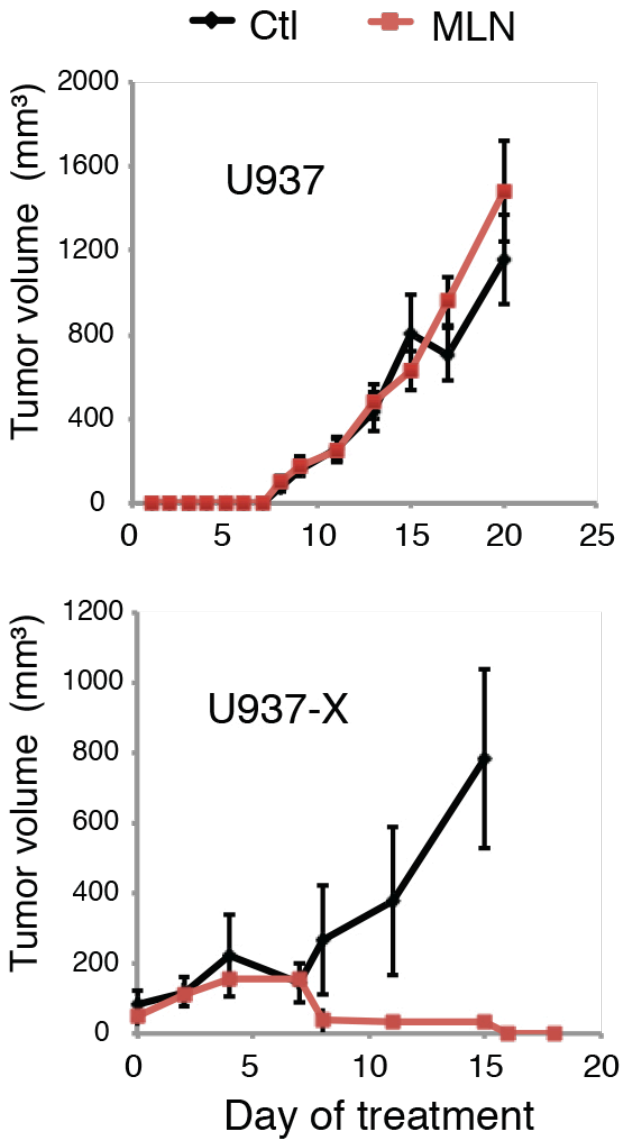
GO term:
Proteasomal catabolic process



Dupéré-Richer et. al., Leuk Lymphoma 2017

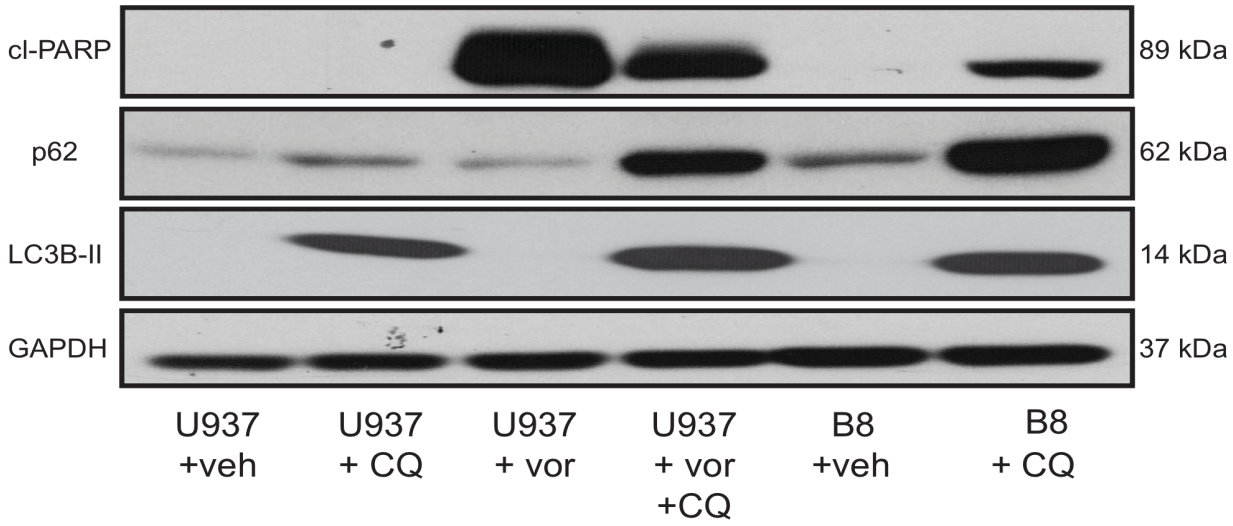
Gene Signature Predicts Response to PROTi

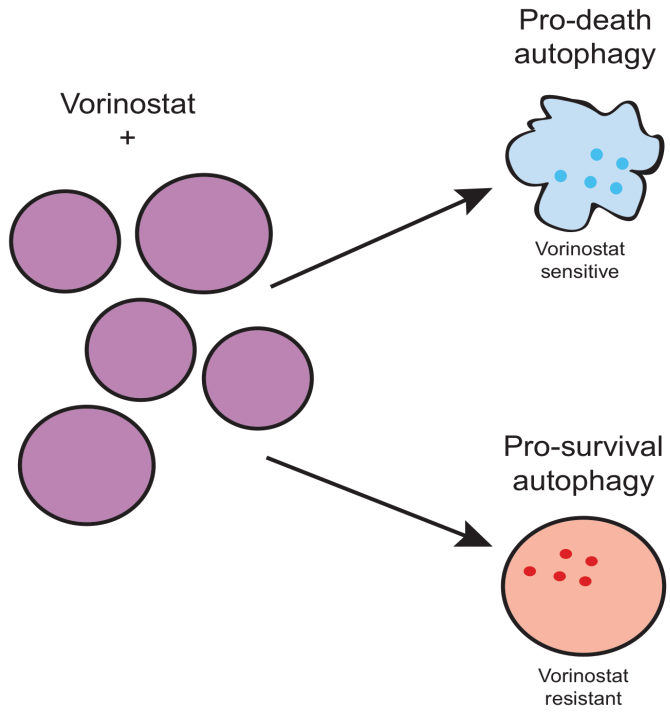




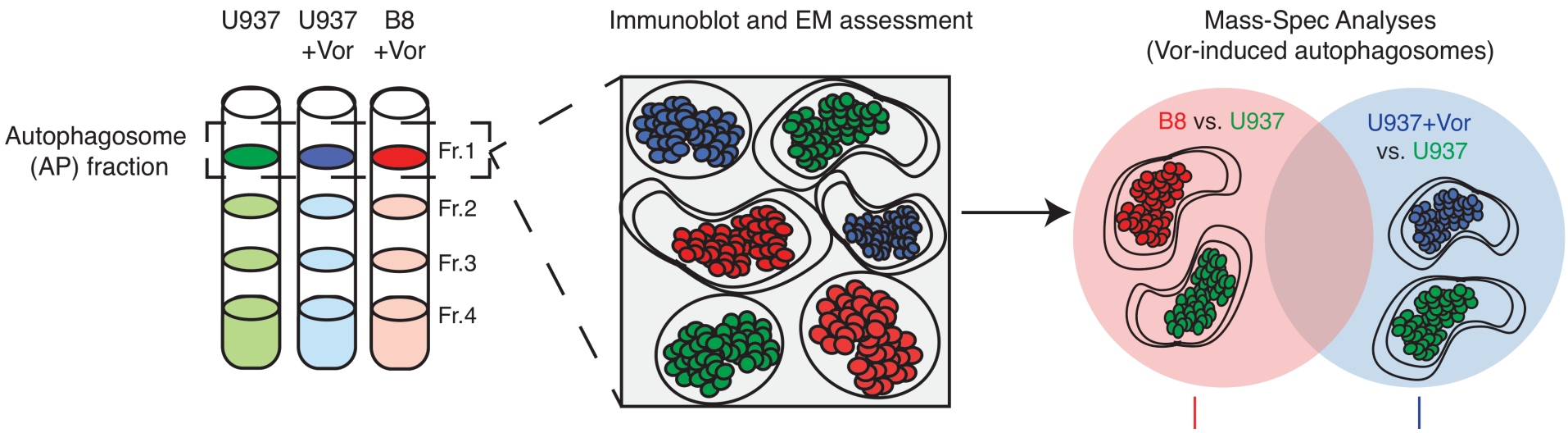
Dupéré-Richer et. al., Leuk Lymphoma 2017

Pro-death versus Pro-survival Autophagy



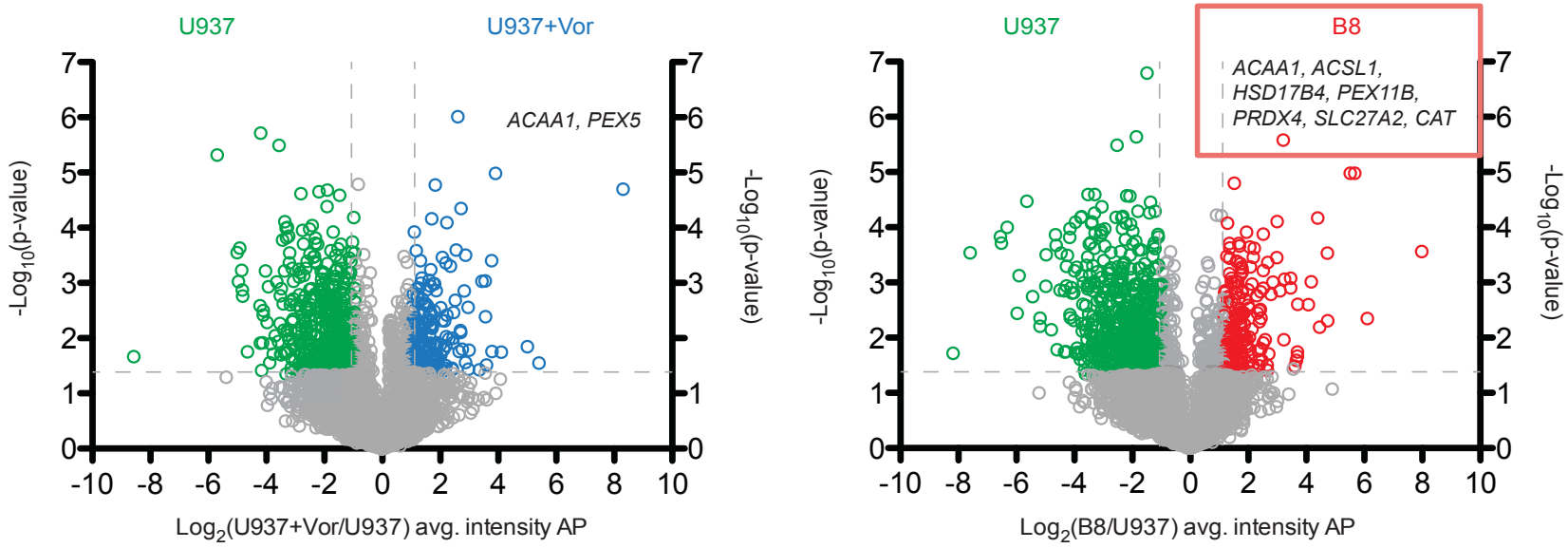


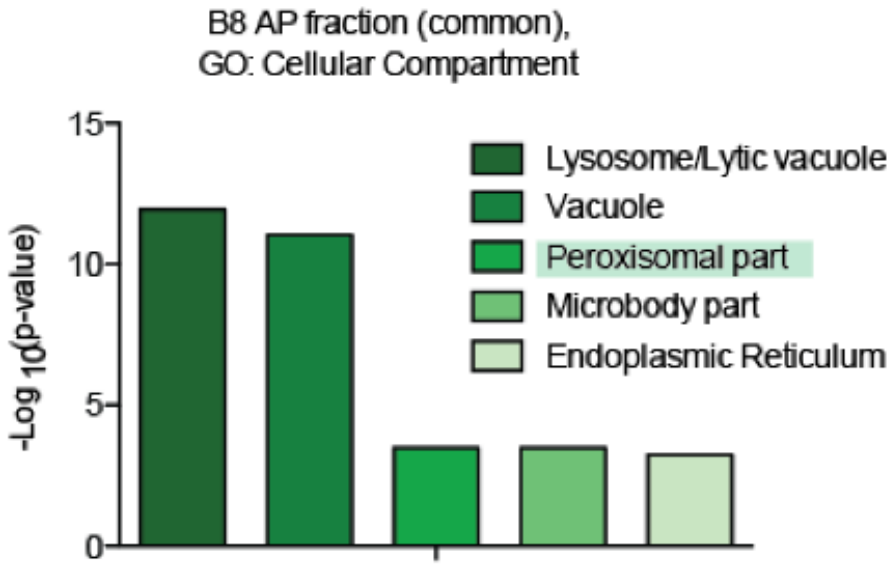
Autophagosome Isolation



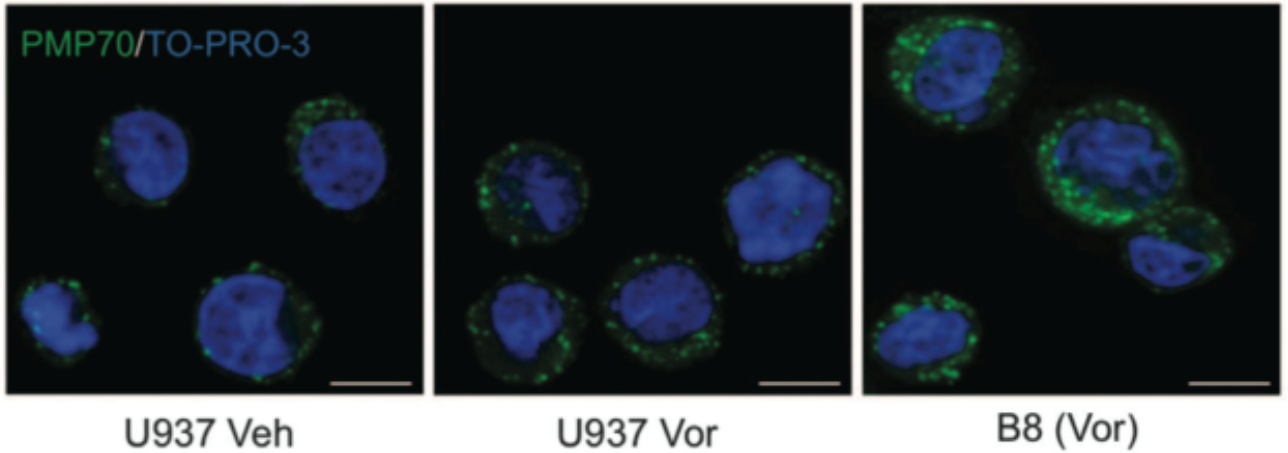
Chang et. al., Oncotarget 2017

Enrichment of Peroxisomal Proteins in Autophagosomes





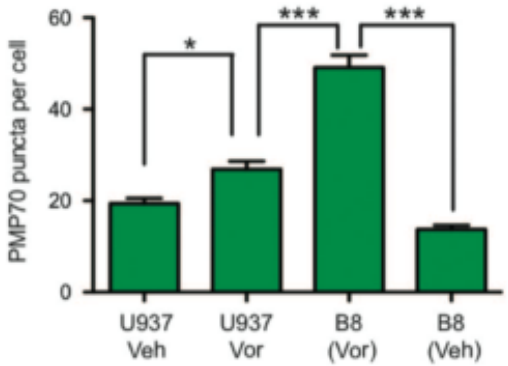
Resistant Cells undergo Apoptosis with Peroxisome KD

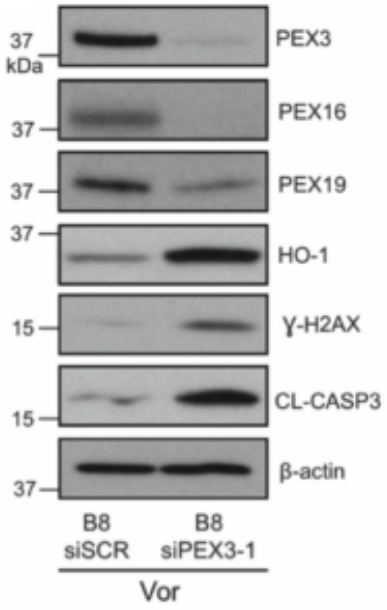


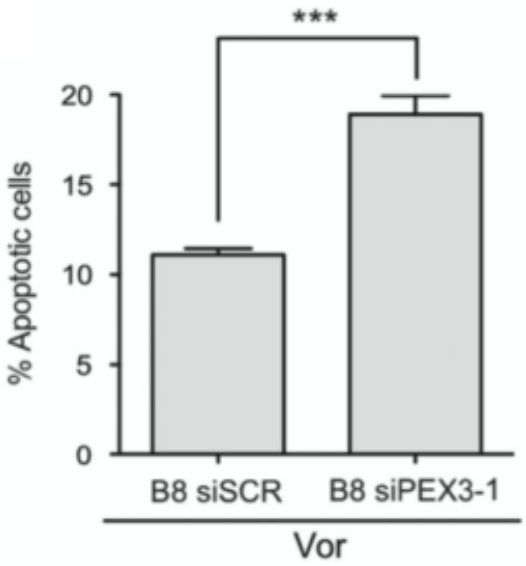
U937 Veh

U937 Vor

B8 (Vor)
(Vor-resistant U937)



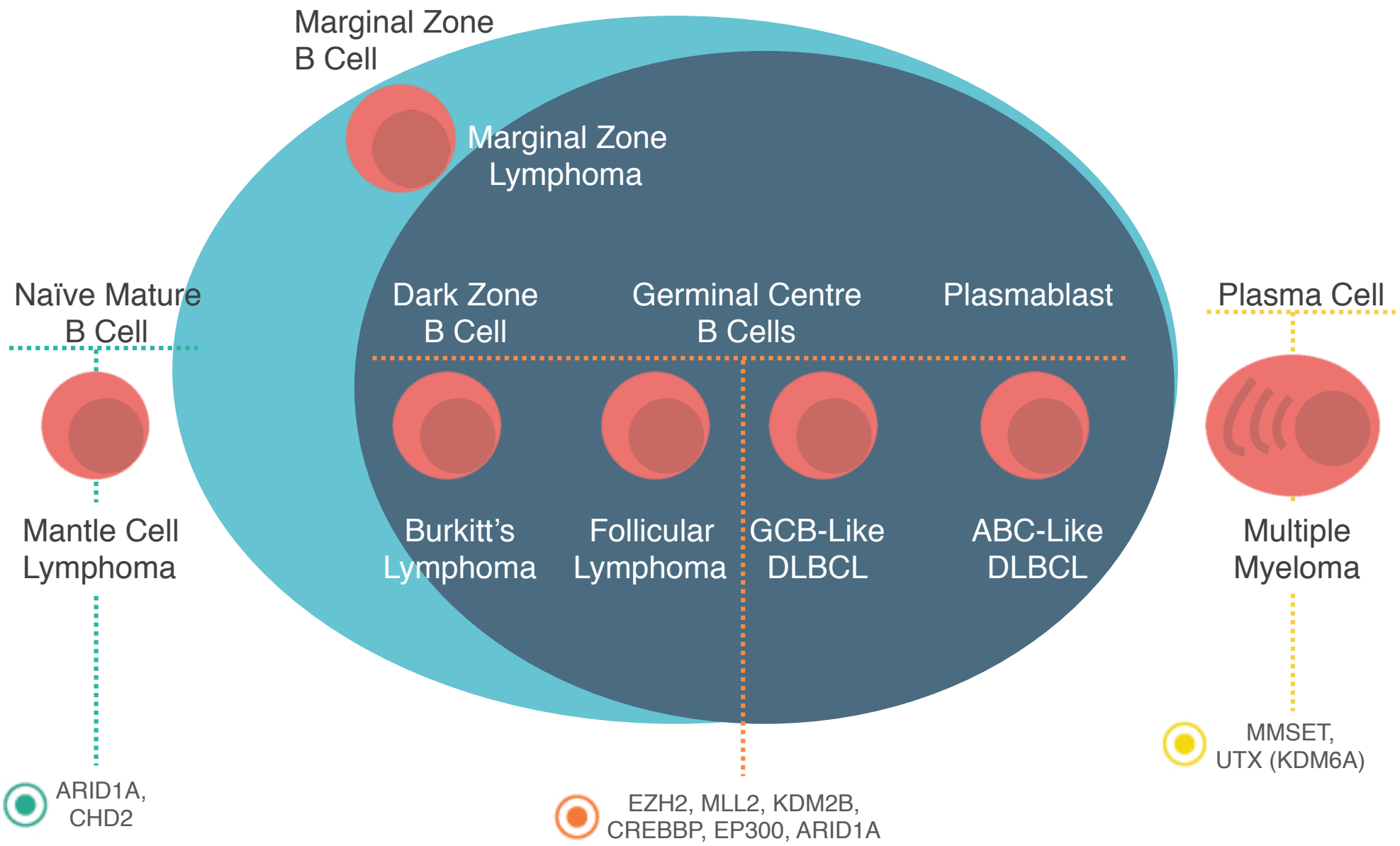




Conclusions: Part 2

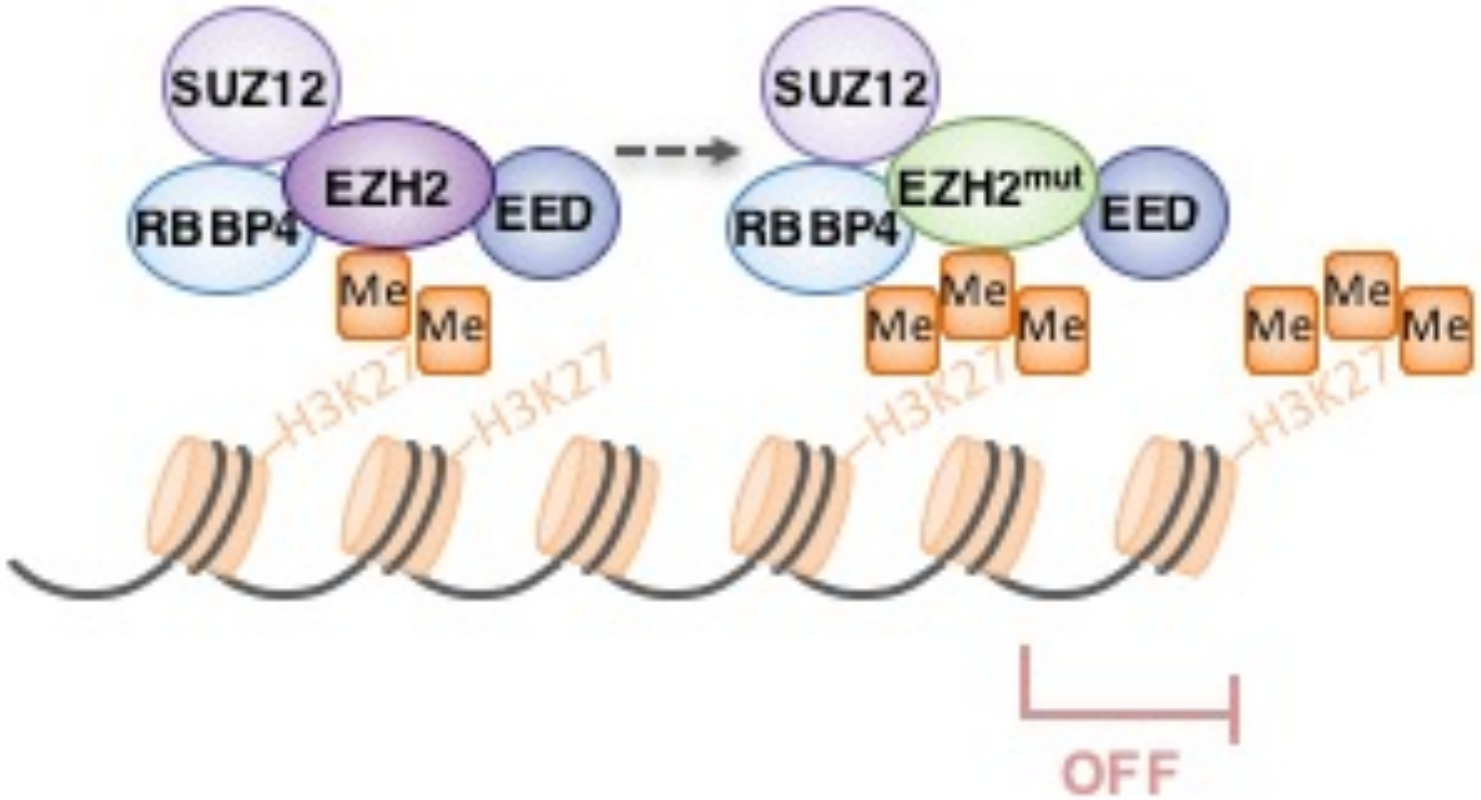
- Analysis of gene expression in HDACi-resistant cells predicted sensitivity to proteasome inhibitors in vivo.
- A molecular mechanism of HDACi resistance involves upregulation of peroxisomal proteins.

3. EZH2i in B-Cell Malignancies

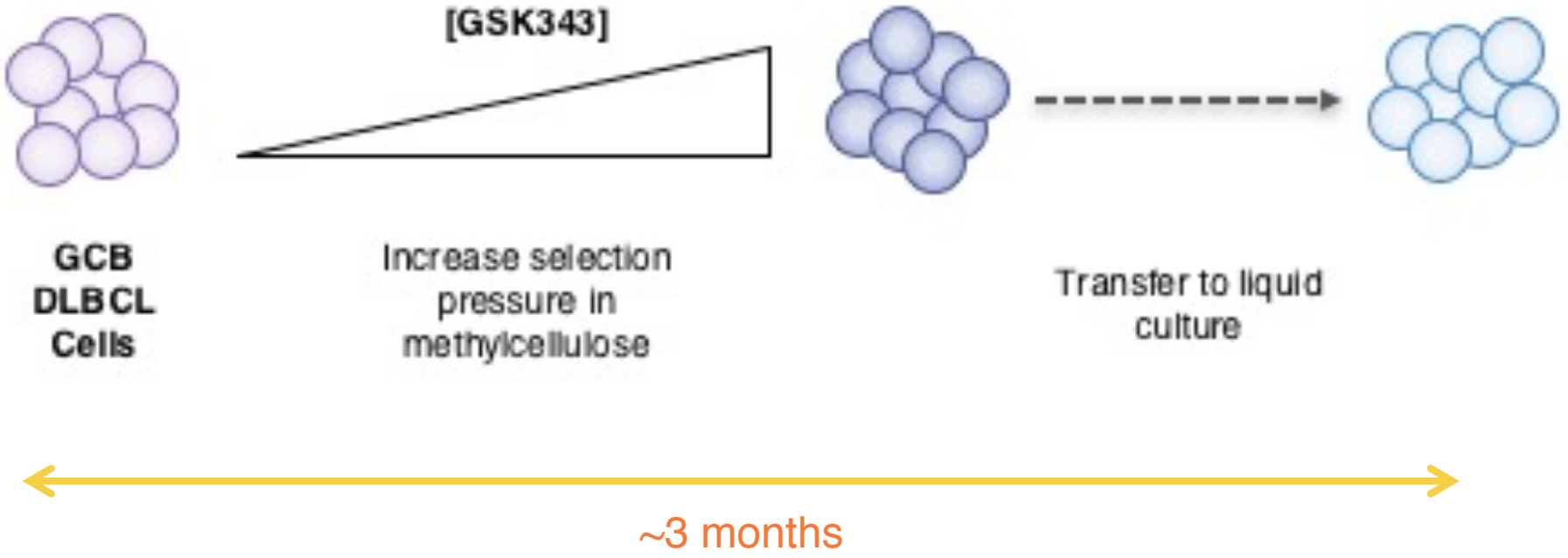


H3K27Me Patterning is Disrupted

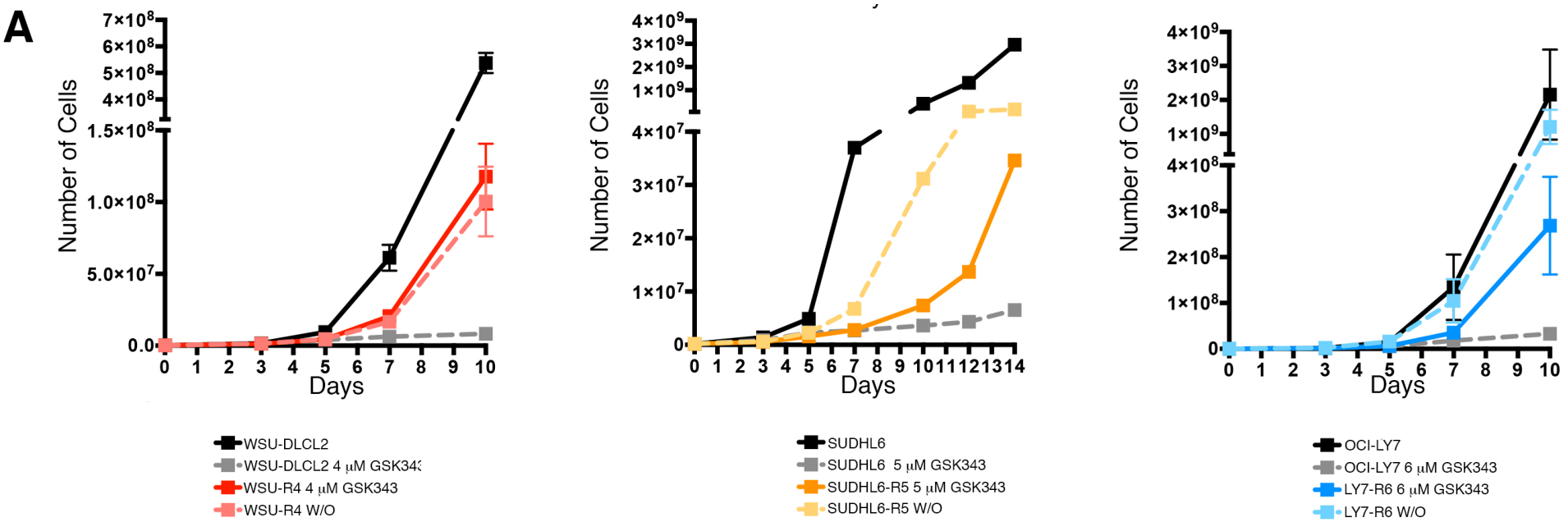
EZH2 Mutations in DLBCL



Establishment of Models of EZH2i Resistance



Resistant to the Growth Inhibitory Effects of EZH2i

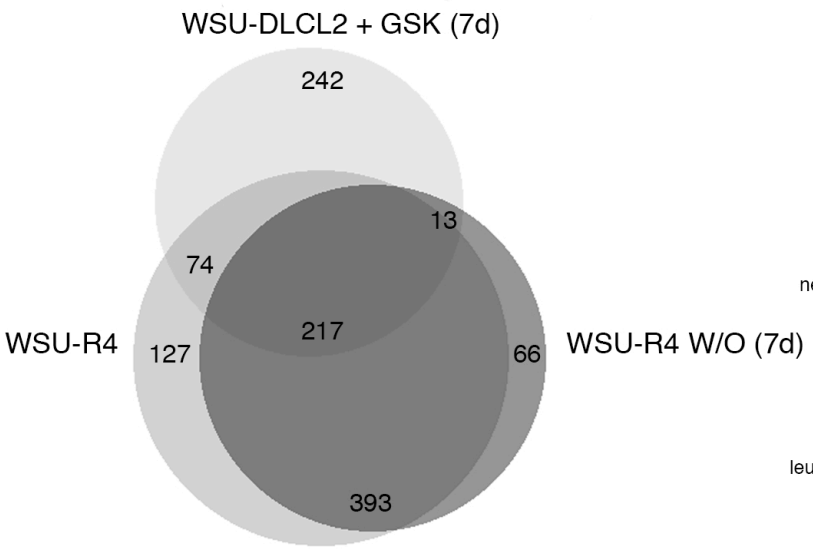


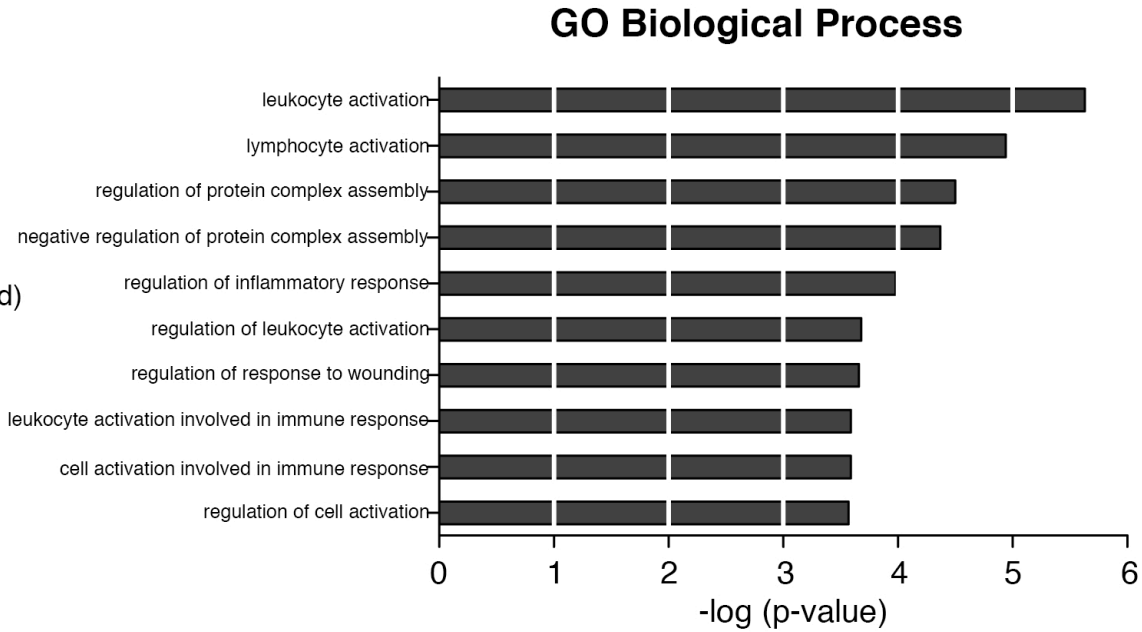
WSU-DLCL2
GCB
MUT EZH2
5-day GI50 = 2.4 uM
Elevated H3K27me^3

SUDHL6
GCB
MUT EZH2
5-day GI50 = 2.3 uM
Elevated H3K27me^3

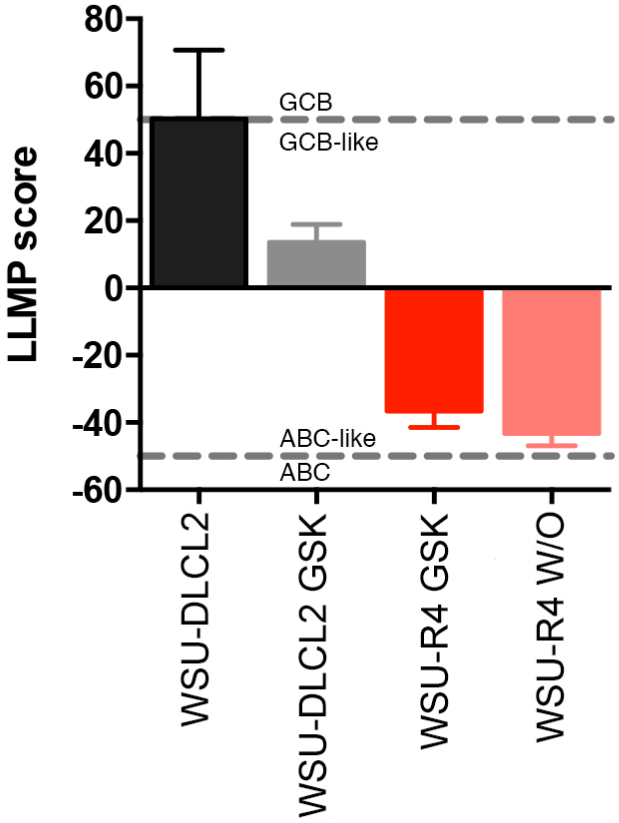
OCI-LY7
GCB
WT EZH2
5-day GI50 = 2.8 uM
translocation MYC-IGH and a TP53 point mutation

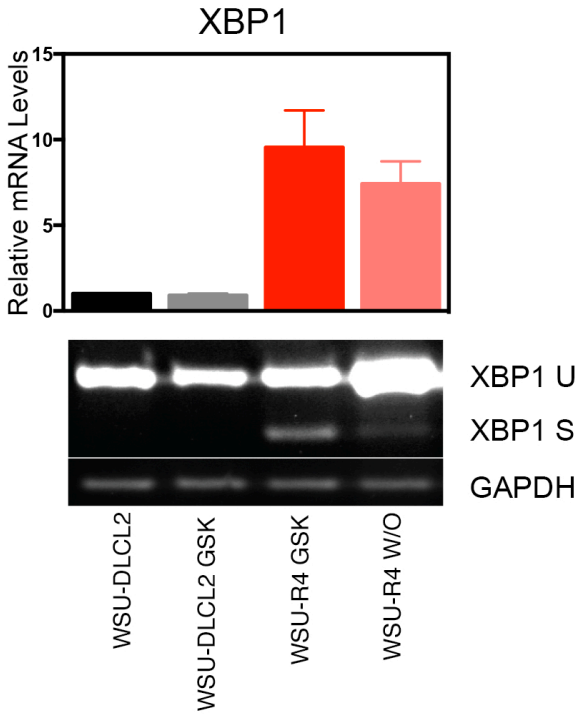
EZH2i-Resistant Clones Have Differentiated

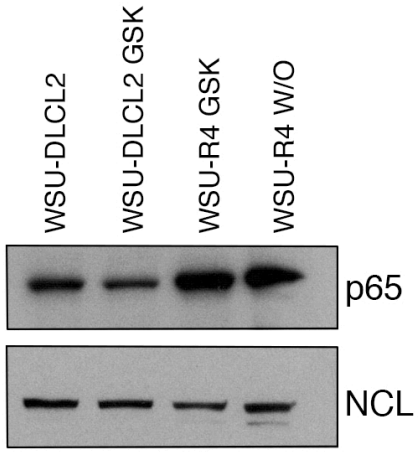




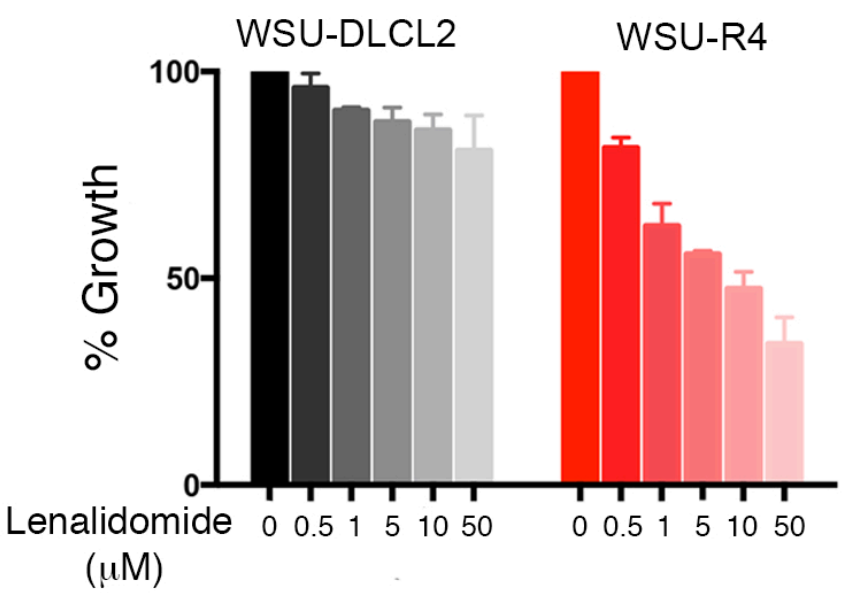
EZH2i-Resistant Clones are Plasmablast-Like

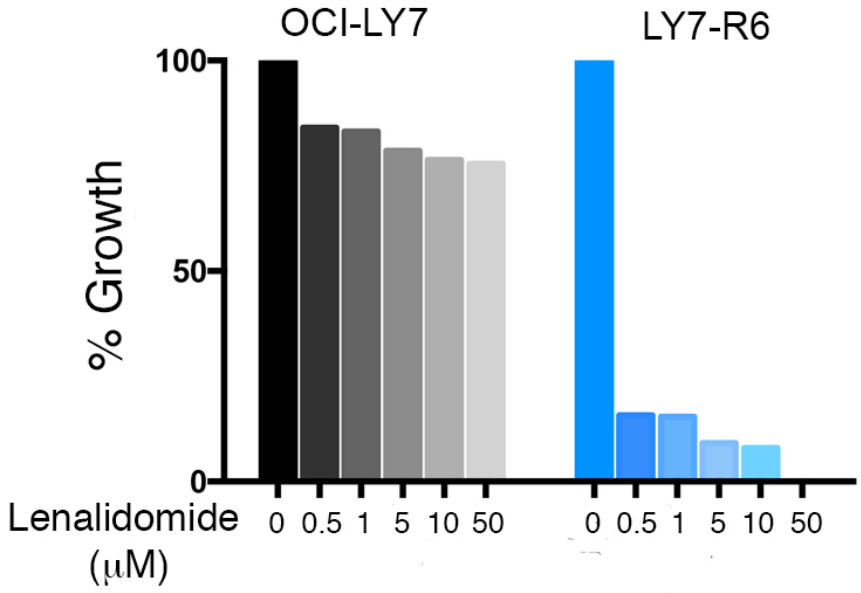


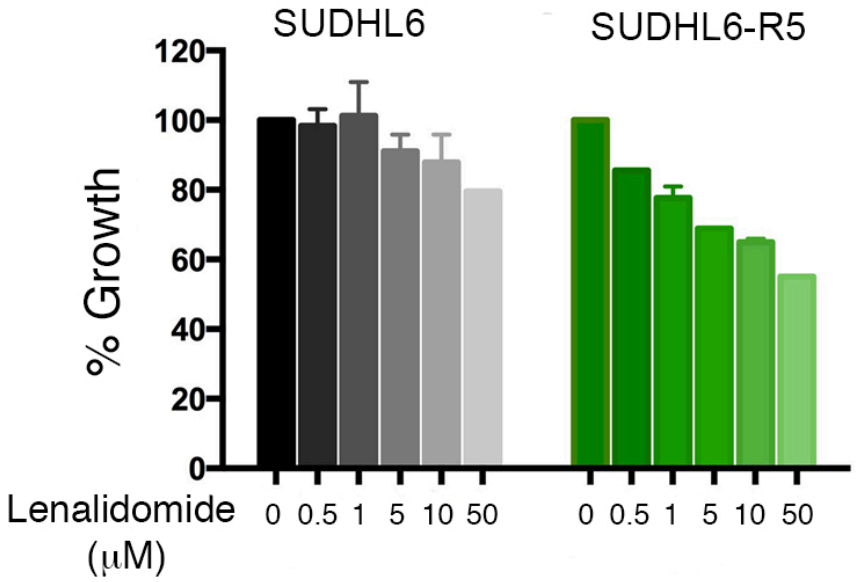




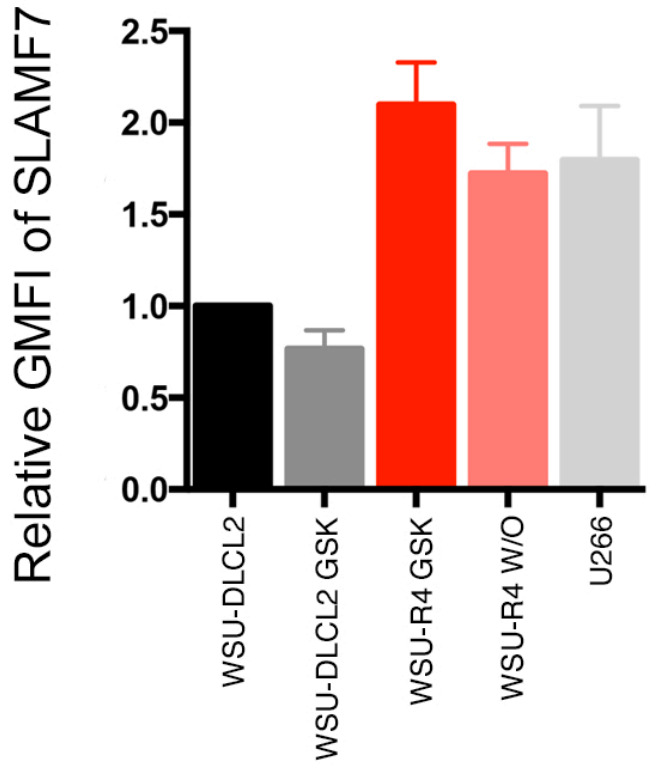
Resistant Clones are More Sensitive to Lenalidomide

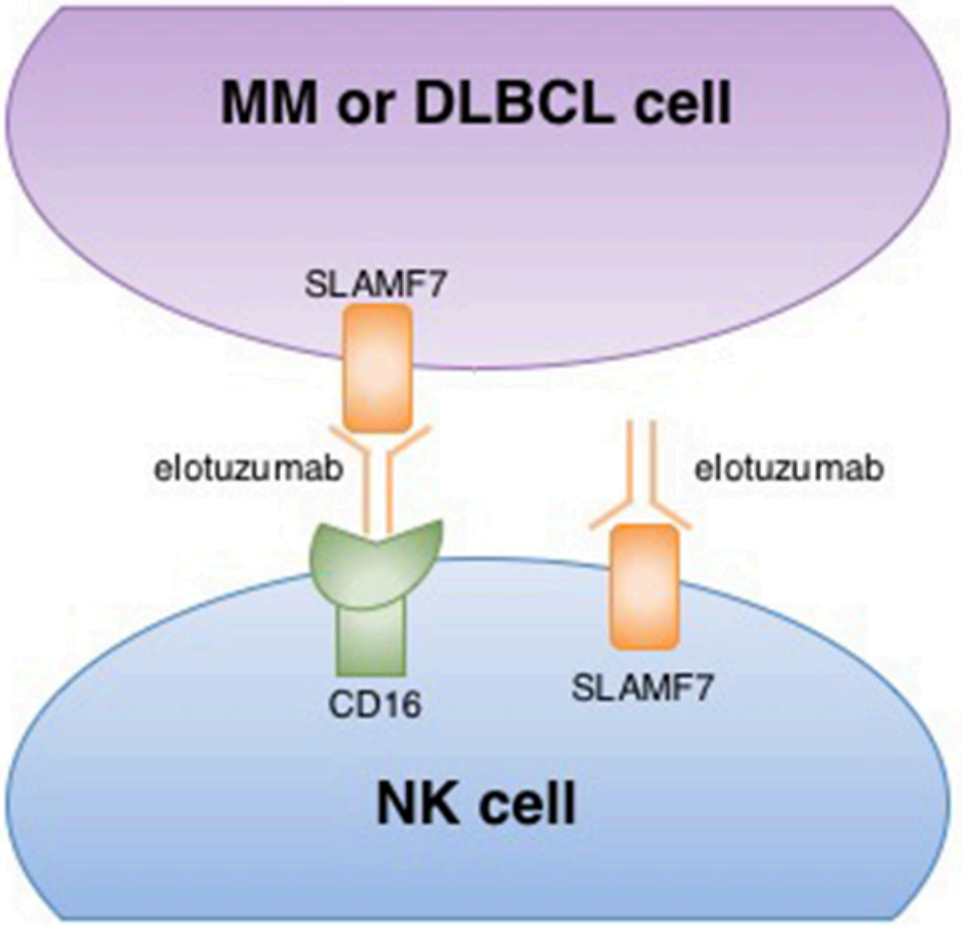






SLAMF7 Increases with Chronic Exposure to EZH2i





Conclusions: Part 3

- DLBCL cells that undergo chronic exposure to EZH2i differentiate to become more plasmablast-like.
- This differentiation is associated with an increased sensitivity to Lenalidomide.
- Increases in SLAMF7 may predict sensitivity to elotuzumab.

Acknowlegements

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