BC CANCER LYMPHOID CANCER RESEARCH
Provincial Health Services Authority

MLLT1 and MLLT3 expression in B-cell lymphomas

Laura Hilton

Table of contents

Background

The University of Montreal team of Javier Di Noia and Noé Seija Desivo have generated mouse and cell line models demonstrating the role of two chromatin readers, MLLT1 and MLLT3, in targeting AID to chromatin to induce somatic hypermutation (SHM). They are looking to orthogonally validate some of their findings in lymphomas:

"Noe will prepare the coordinates/signal of the MLLT1 ChiP seq in RAMOS and SUDHL5 for correlation with SHM load.

"The genes we are interested are all SEC components: the two histone readers (MLLT1 and MLLT3) and three scaffolds (AFF1, AFF3, AFF4) that we find contribute to AID activity. They can form 6 different complexes by combinations of one MLLT and one AFF.

"This current manuscript we may submit in early spring (depending on our competitors timeline too) we would like to know if there is any correlation between *MLLT1* or *MLLT3* with AID expression and SHM load."

They also have some data from TCGA to suggest that higher expression of *MLLT1* in DLBCL is associated with inferior outcomes. Here we will examine whether *MLLT1/3* expression varies across lymphomas and try to correlate it aSHM load.

Procedure

Identify samples

To examine the expression of *MLLT1/3* across lymphomas, we will incorporate BL, DLBCL, HGBCL-DH-BCL2, and FL RNAseq data used in Hilton et al, 2024[1].

Table 1 summarizes the available RNAseq data from different lymphoma types.

Table 1: Summary of available expression data

ICC_class	group	n
BL	BL EBV-	81
BL	BL EBV+	107
DLBCL	DZsig+	38
DLBCL	GCB-DLBCL	91
DLBCL	UNCLASS-DLBCL	18
DLBCL	ABC-DLBCL	79
FL	FL	89
HGBCL-DH-BCL2	HGBCL-DH-BCL2	124

Compare expression across lymphoma types

Figure 1 shows the expression of SEC genes across different lymphoma subtypes. As shown before, *AICDA* expression is high in BL and ABC-DLBCL. Notably *MLLT1* expression is lowest in BL, which would be consistent with the globally lower rates of aSHM observed in BL.

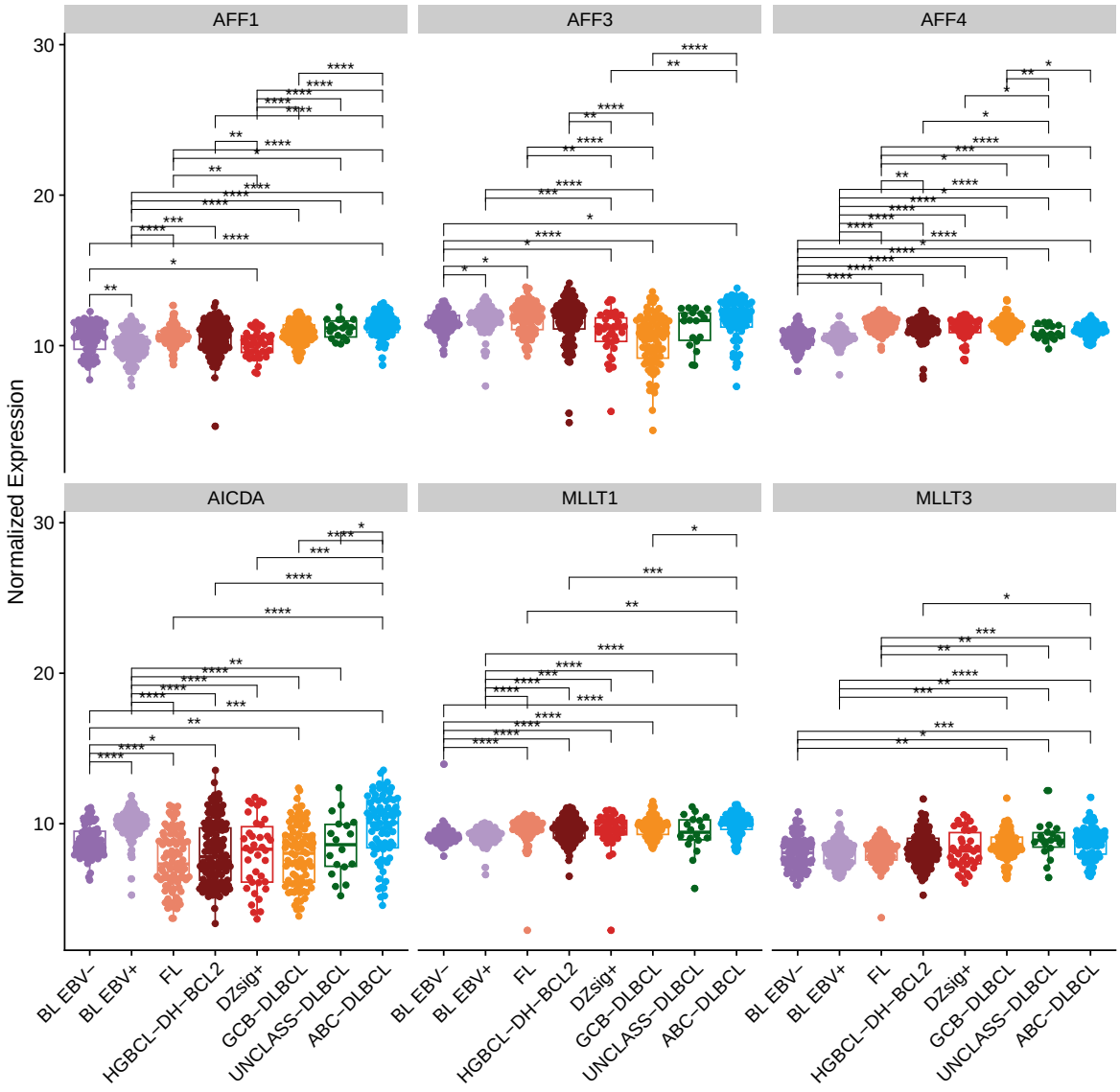

Figure 1: Expression of *AICDA* and SEC complex genes across lymphoma subtypes

Figure 2 shows that there are no strong correlations between *AICDA* expression and that of any other SEC genes.

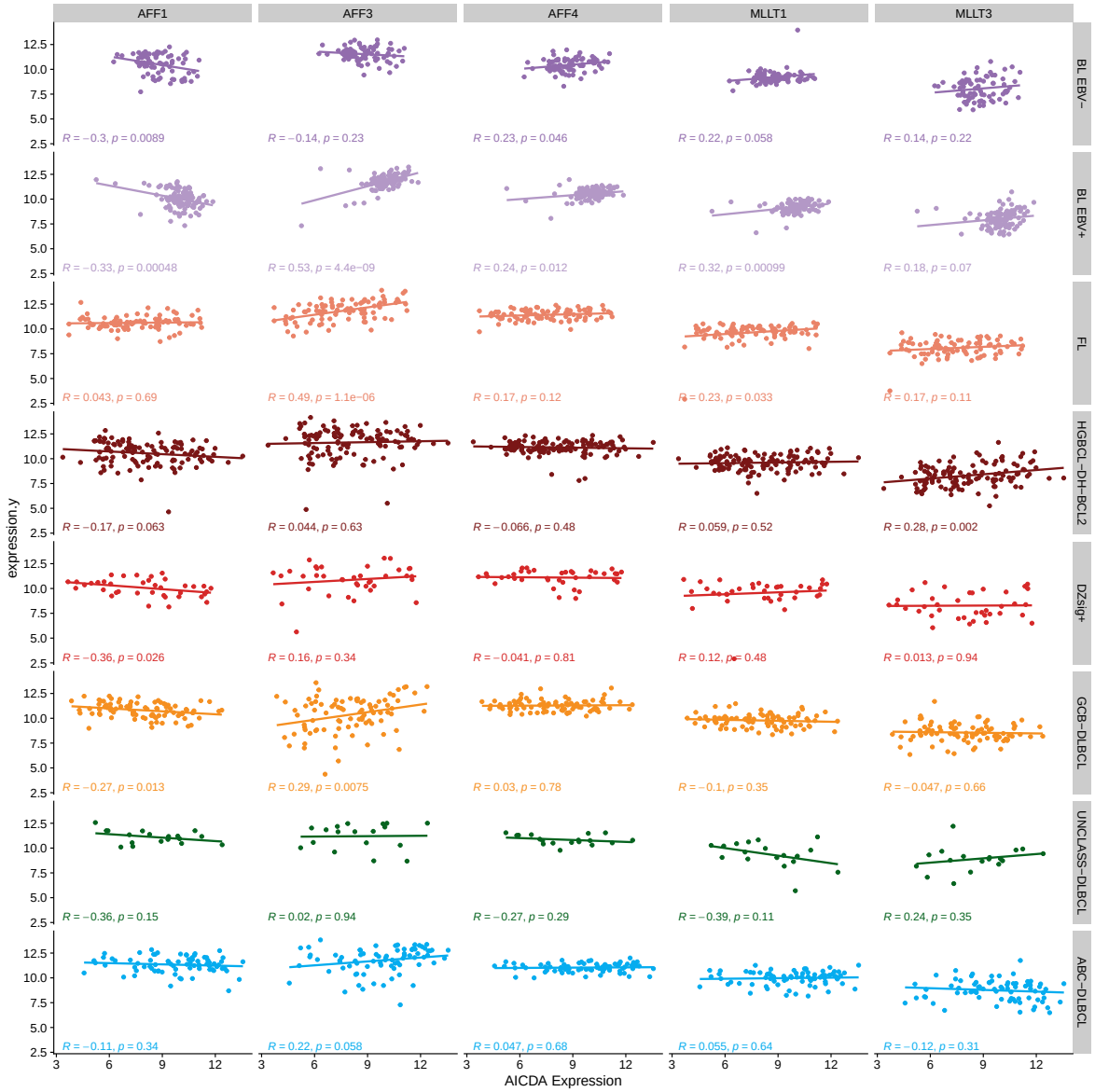

Figure 2: Scatter plot correlating pairwise expression of the SEC genes

Figure 3 shows the expression of each gene across lymphoma entities.

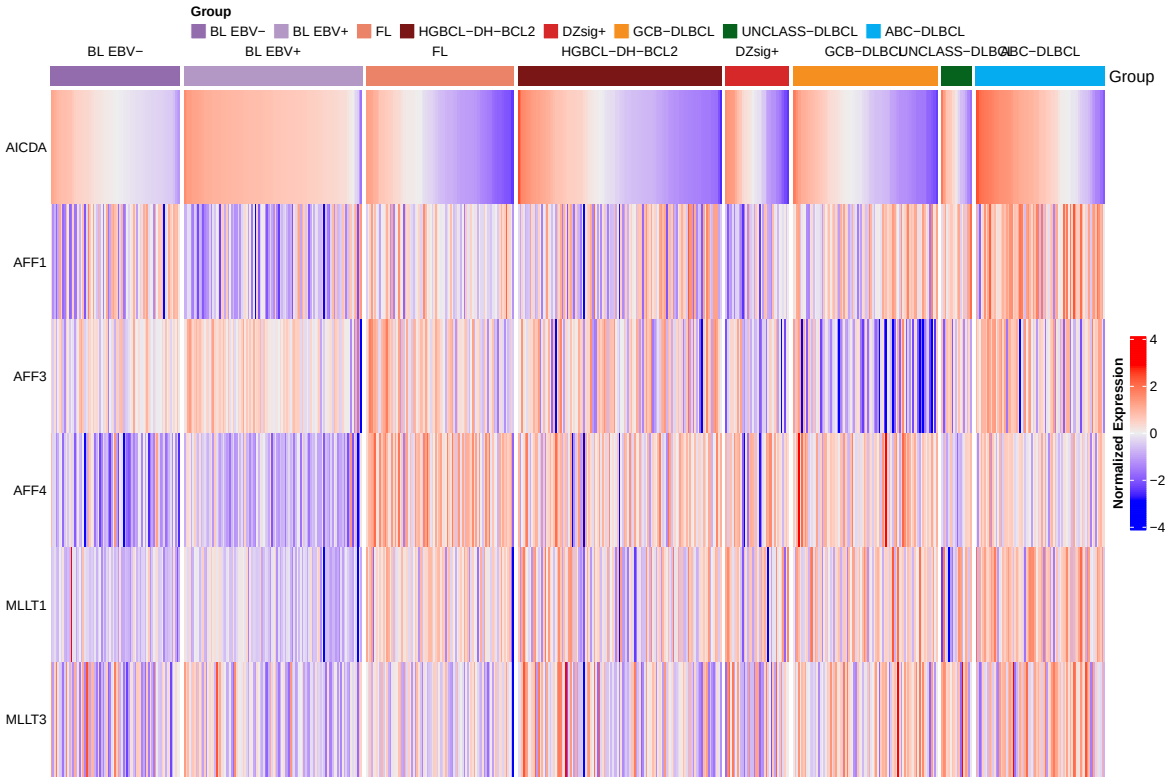

Figure 3: Heatmap of *AICDA* and SEC gene expression ordered on *AICDA* expression

The next version of the heatmap (Figure 4) is clustered. This version emphasizes the heterogeneity of expression across and within different lymphoma types.

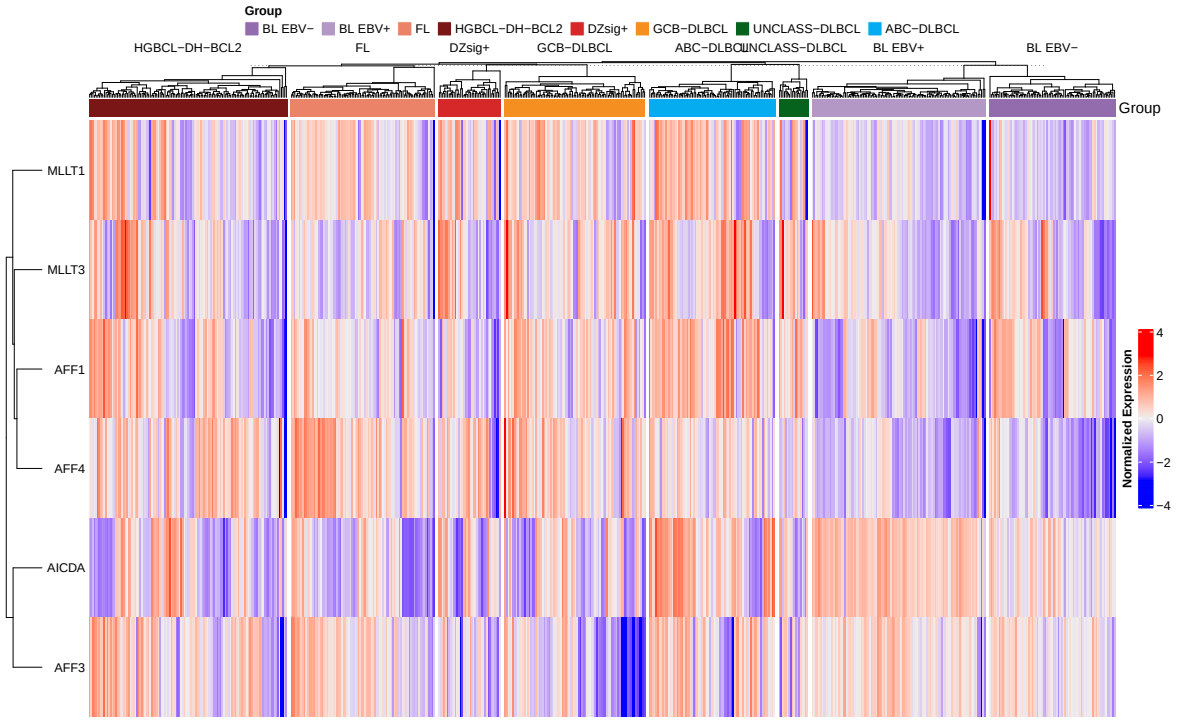

Figure 4: Clustered heatmap of *AICDA* and SEC gene expression

Correlate expression with aSHM and SBS84

Next, we will try to layer on aSHM information for these tumours. For this, I'll obtain SSM status for mutations across aSHM space and layer on mutation signatures using SigMiner. This reduces the number of samples shown on the heatmap because not all samples with RNAseq also have WGS. aSHM regions are defined here. Then, SigMiner will be run to quantify exposure to SBS84 across aSHM space specifically.

The ordered heatmap (Figure 5) does not reveal any major patterns of association between expression of SEC genes and levels of aSHM or SBS84 exposure.

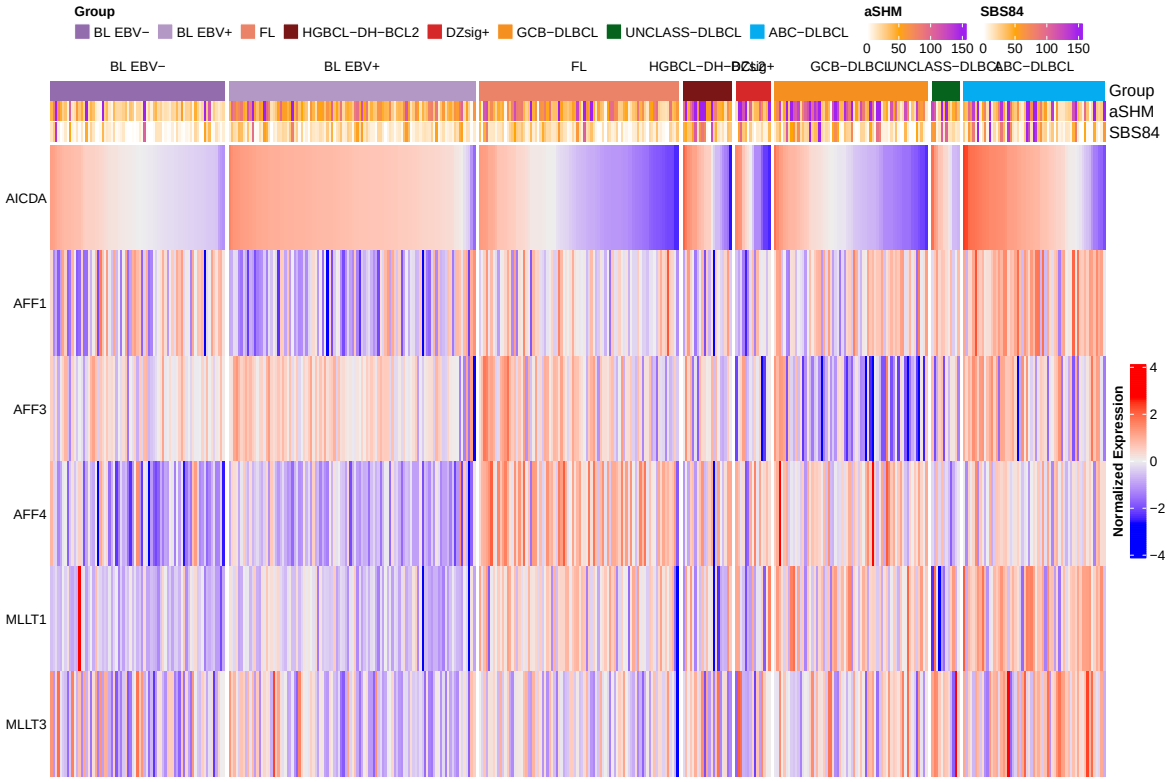

Figure 5: Heatmap of SEC gene expression ordered on *AICDA* expression annotated with aSHM amounts

The clustered heatmap (Figure 6) suggests perhaps some association between levels of *MLLT1* and amount of aSHM in GCB-DLBCL and HGBCL-DH-*BCL2*. Let's explore that further with a correlation plot.

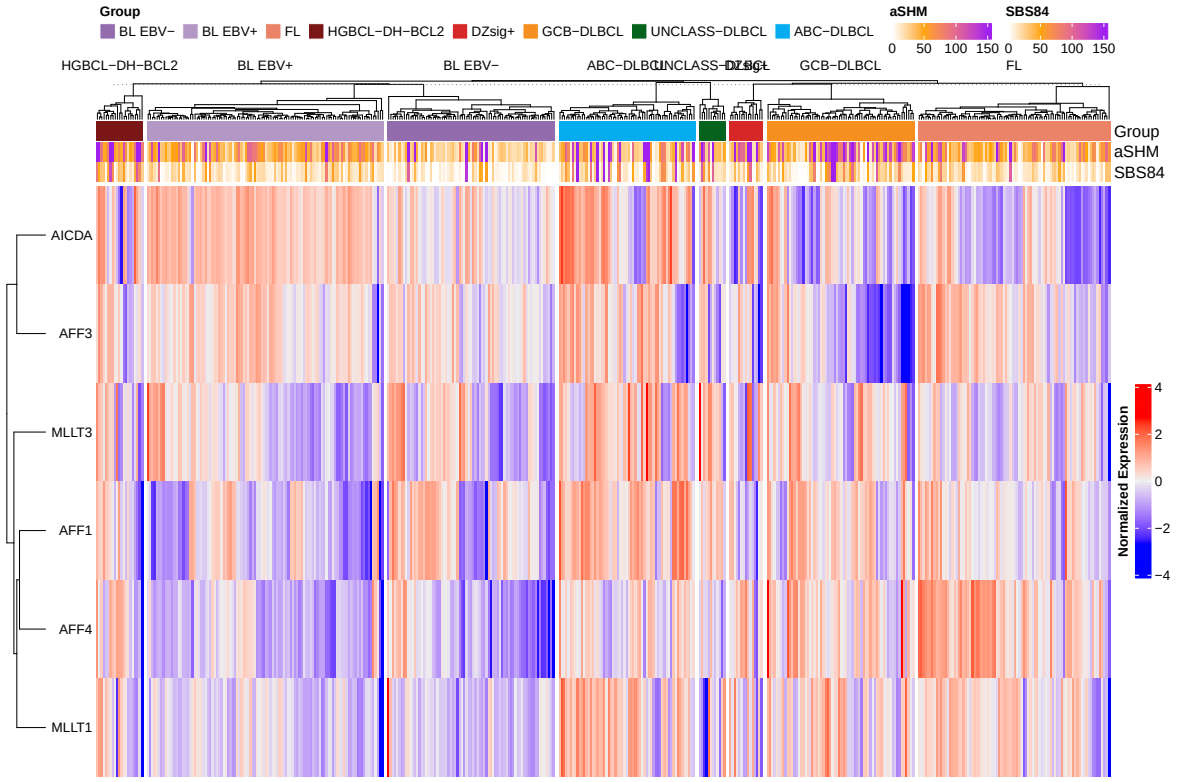

Figure 6: Clustered heatmap of SEC gene expression annotated with aSHM amounts

There aren't any clear linear correlations between the expression of each SEC gene and either total SBS84 (Figure 7) or total aSHM mutation load (Figure 8). However, it's possible that the combination of *MLLT3* with *AICDA* is required to achieve high aSHM. For this, we will bin tumours into combinations of *MLLT3* high/low and *AICDA* high/low. We will set the median expression for each gene within each group to acknowledge the range of expression values across groups.

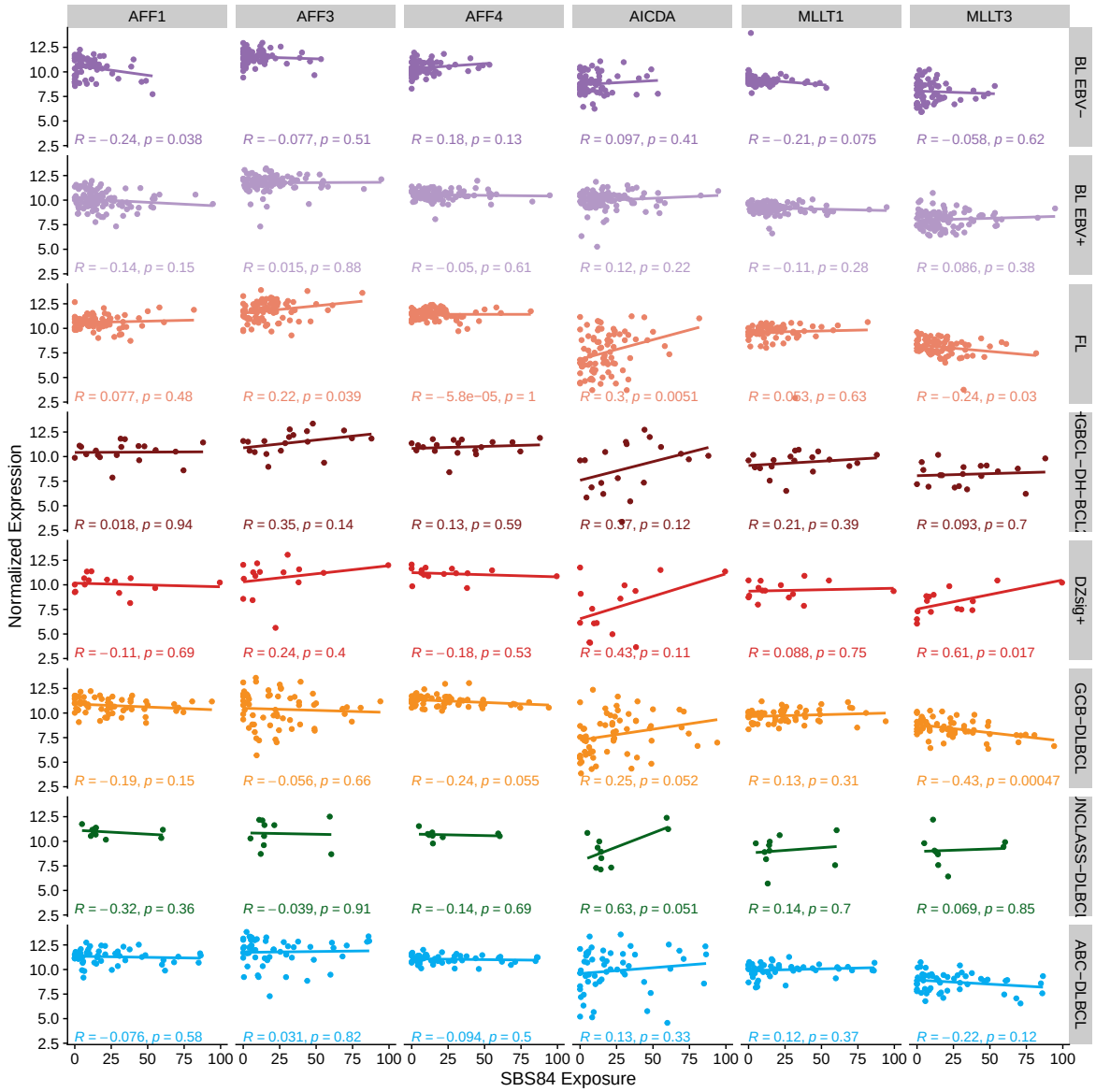

Figure 7: Correlation between SBS84 exposure and SEC genes

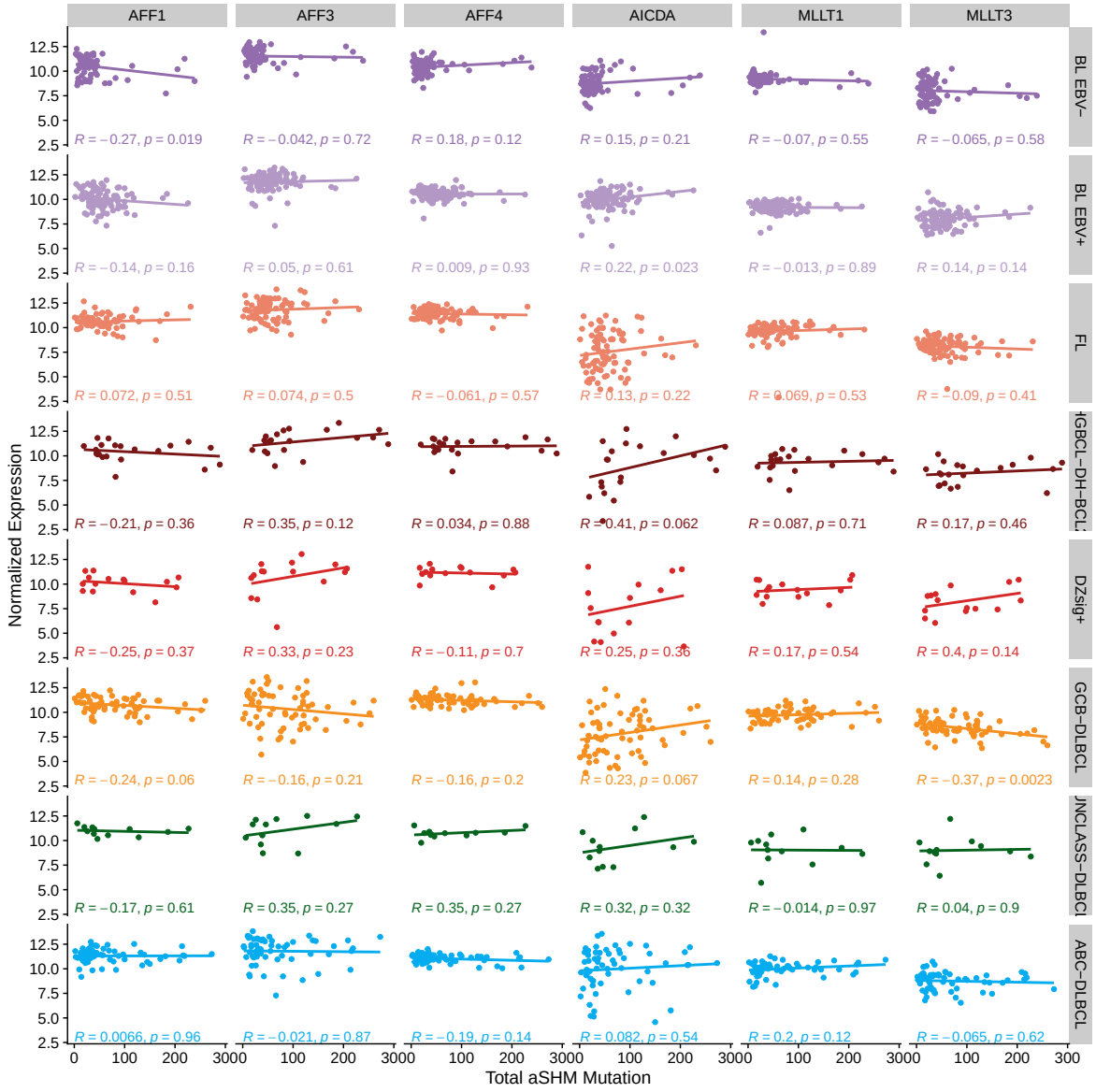

Figure 8: Correlation between total mutations in aSHM regions and SEC genes

There is no significant association between either SBS84 exposure (Figure 9) and *AICDA*/*MLLT3* expression or total aSHM mutations (Figure 10); however, there is a pattern in HGBCL-DH-*BCL2*, DZSig+, and ABC-DLBCL where the cases with the highest aSHM/SBS84 burden are in the group with high *AICDA* and *MLLT3* expression. Let's explore this further with a linear model.

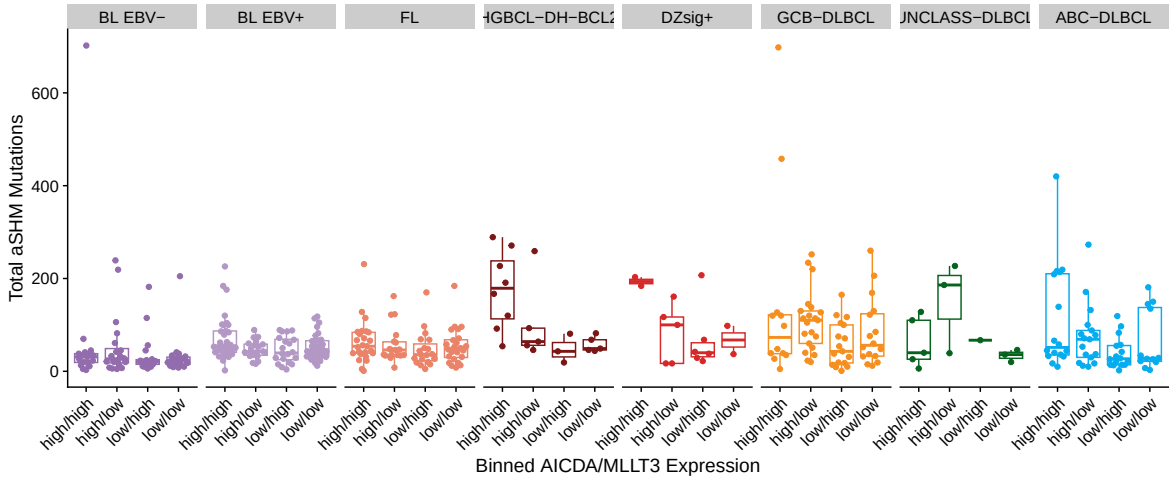

Figure 9: Total aSHM mutations exposure binned by *AICDA* and *MLLT3* expression

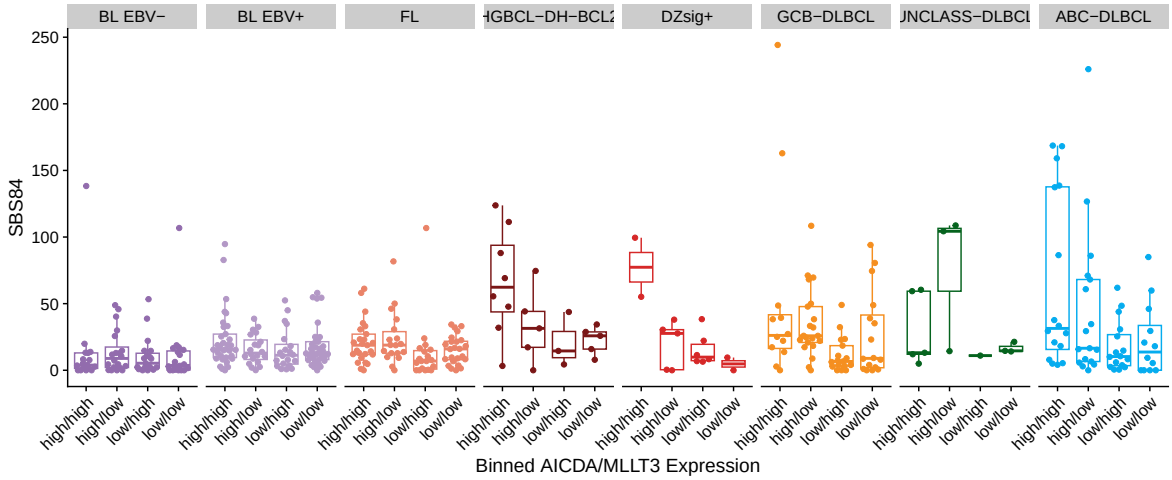

Figure 10: SBS84 exposure binned by *AICDA* and *MLLT3* expression

Table 2 shows an association between *AICDA* expression and aSHM independent of lymphoma type, while there is no significant association with either *MLLT1* or *MLLT3*. A similar pattern appears for SBS84 (Table 3).

Table 2: Linear model of aSHM vs expression of SEC genes

term	estimate	std.error	statistic	p.value
(Intercept)	-51.6067697	50.198456	-1.0280549	0.3045006
groupBL EBV+	0.3839771	10.994614	0.0349241	0.9721564
groupFL	19.2704959	11.743885	1.6408962	0.1015485
groupHGBCL-DH-BCL2	64.5018837	17.603054	3.6642440	0.0002790
groupDZsig+	51.9621885	20.315256	2.5577915	0.0108751
groupGCB-DLBCL	57.4567878	12.523668	4.5878562	0.0000059
groupUNCLASS-DLBCL	26.2380850	22.451985	1.1686310	0.2431983
groupABC-DLBCL	15.4213023	13.152632	1.1724880	0.2416489
AICDA	8.0470718	1.991384	4.0409447	0.0000630
MLLT3	1.2531529	3.600128	0.3480856	0.7279458
MLLT1	1.8362049	4.590965	0.3999606	0.6893837

Table 3: Linear model of SBS84 vs expression of SEC genes

term	estimate	std.error	statistic	p.value
(Intercept)	-28.5407284	20.9840244	-1.3601170	0.1745039
groupBL EBV+	1.3845815	4.5959829	0.3012591	0.7633622
groupFL	10.3497228	4.9091941	2.1082326	0.0355875
groupHGBCL-DH-BCL2	28.5578706	7.3584516	3.8809620	0.0001203
groupDZsig+	15.7893725	8.4922100	1.8592772	0.0636691
groupGCB-DLBCL	21.3389741	5.2351601	4.0760882	0.0000545
groupUNCLASS-DLBCL	22.8361220	9.3854083	2.4331517	0.0153737
groupABC-DLBCL	21.4196413	5.4980804	3.8958399	0.0001134
AICDA	4.0282254	0.8324409	4.8390529	0.0000018
MLLT3	-0.6924579	1.5049304	-0.4601262	0.6456577
MLLT1	1.2043276	1.9191211	0.6275412	0.5306369

Conclusions

These analyses confirm an overall association between *AICDA* expression and levels of aSHM and SBS84 exposure. However, meaningful correlations with *MLLT1* and *MLLT3* expression were not identified. Further analyses of loci bound by these histone readers, as identified by ChIP-seq, may further be used to tease apart subtype-specific patterns of aSHM driven by these proteins.

References

1. Hilton, L. K. *et al.* Motive and opportunity: *MYC* rearrangements in high-grade B-cell lymphoma with *MYC* and *BCL2* rearrangements (an LLMPP study). *Blood* **144**, 525–540 (2024).