

Article

Modification of the Histone Landscape with JAK Inhibition in Myeloproliferative Neoplasms

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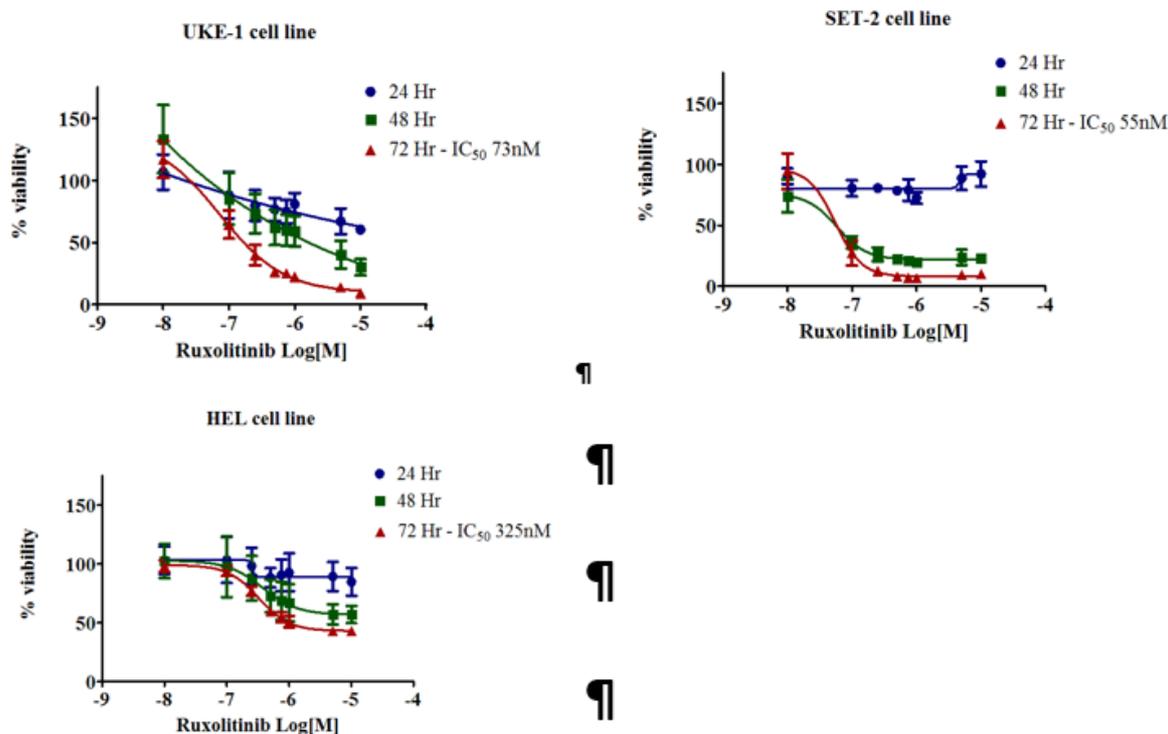
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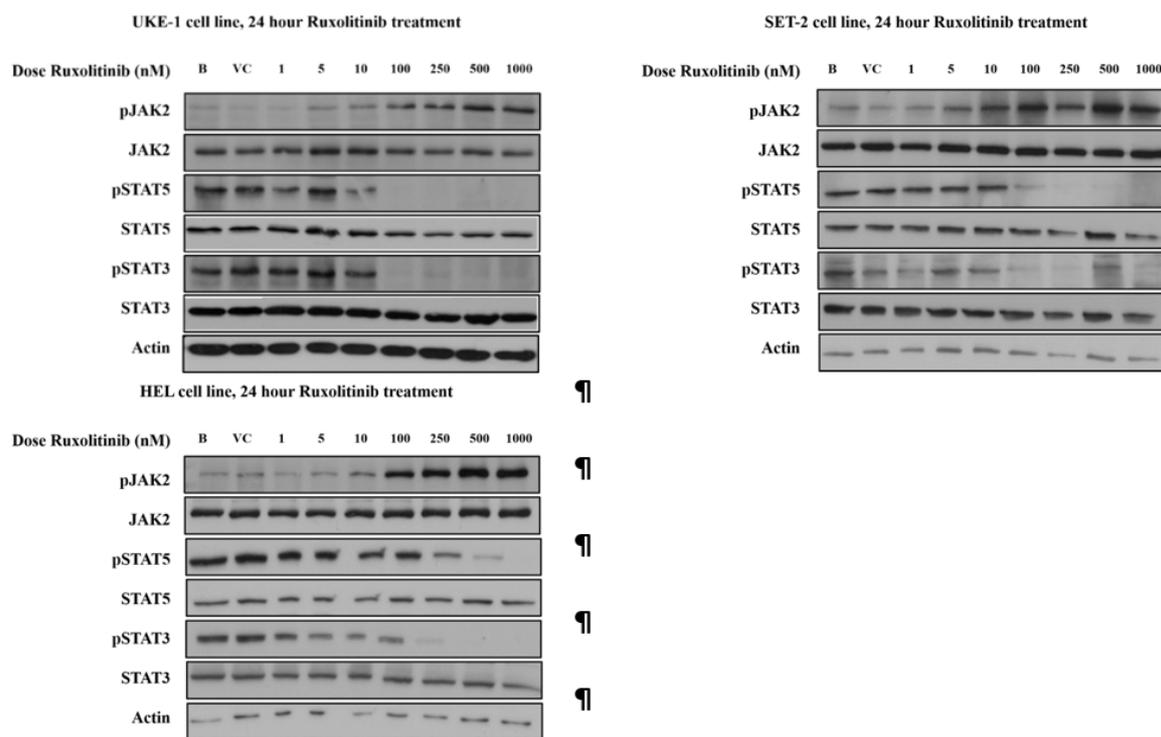
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Supplementary materials



(A)



(B)

Figure 1. (A) UKE-1, SET-2 and HEL cells incubated with doses of Ruxolitinib and cell viability assessed by CTG® after 24, 48 and 72 hours. The IC₅₀ was calculated using GraphPad Prism software. (B) UKE-1, SET-2 and HEL cells treated for 24 hours with doses of Ruxolitinib and protein extracted. Western blotting performed utilizing antibodies directed at the JAK/STAT pathway.

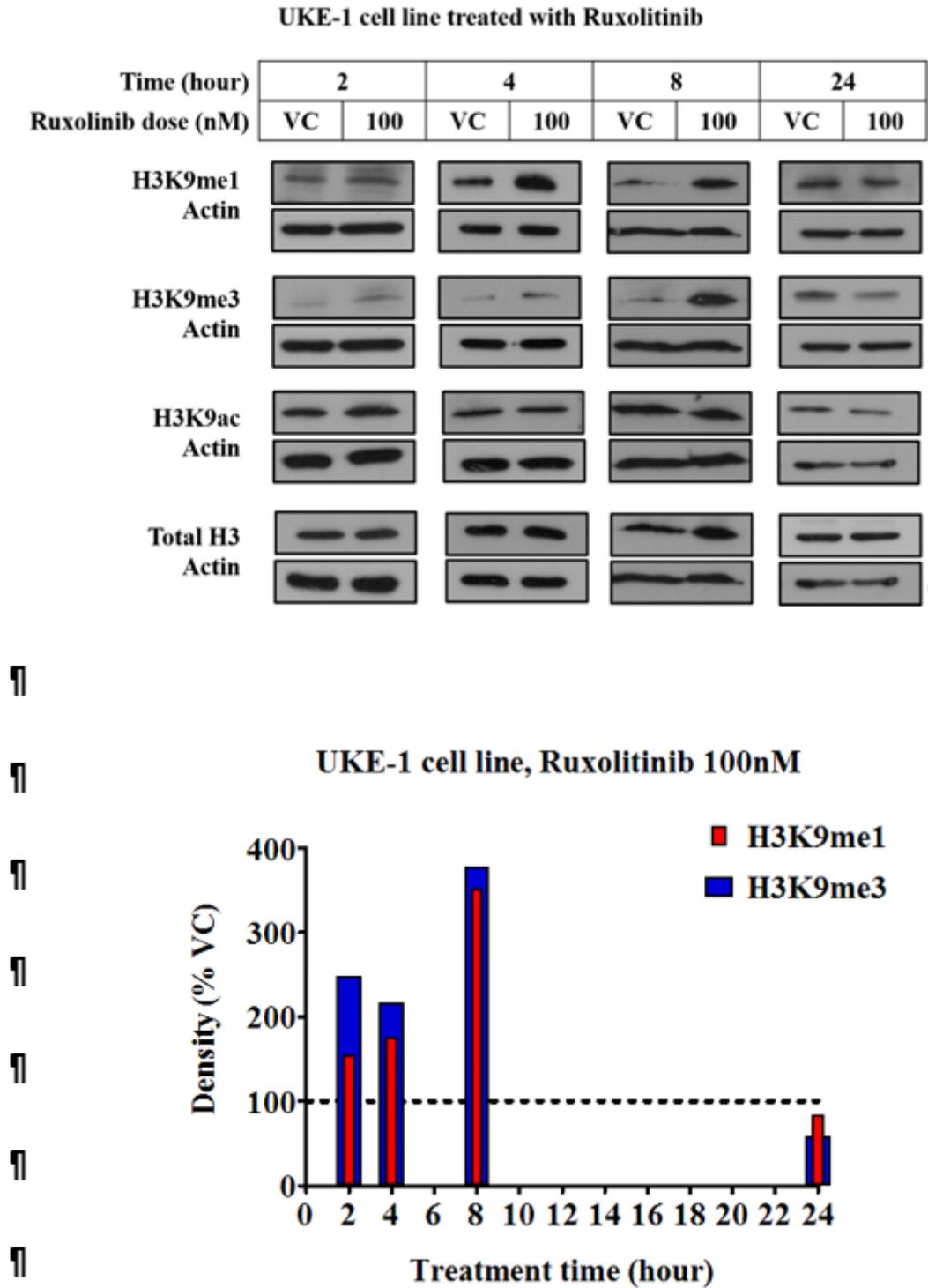
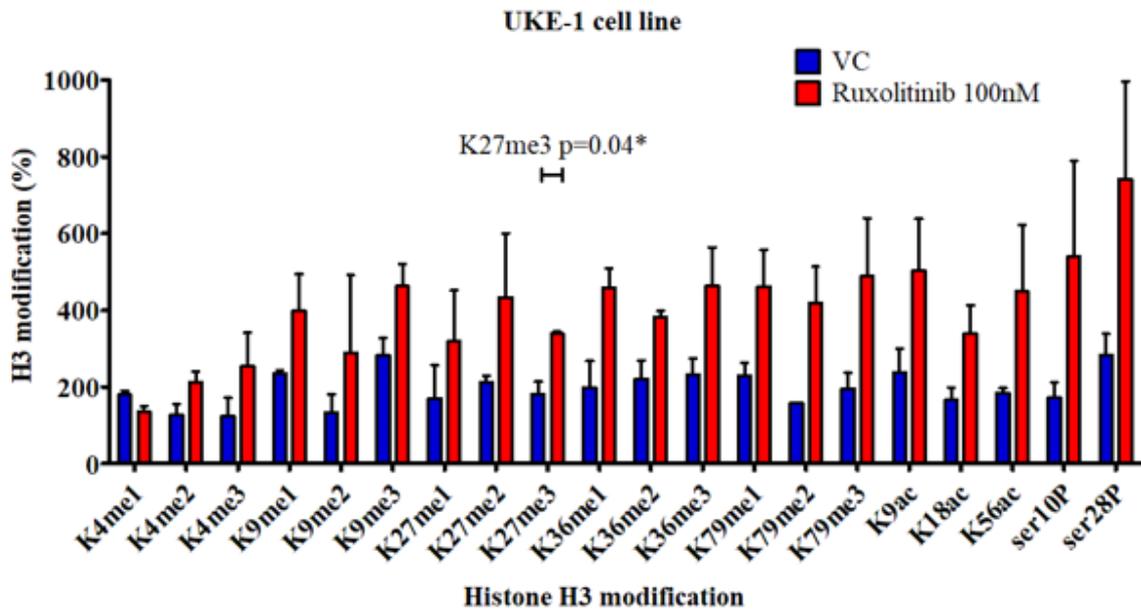
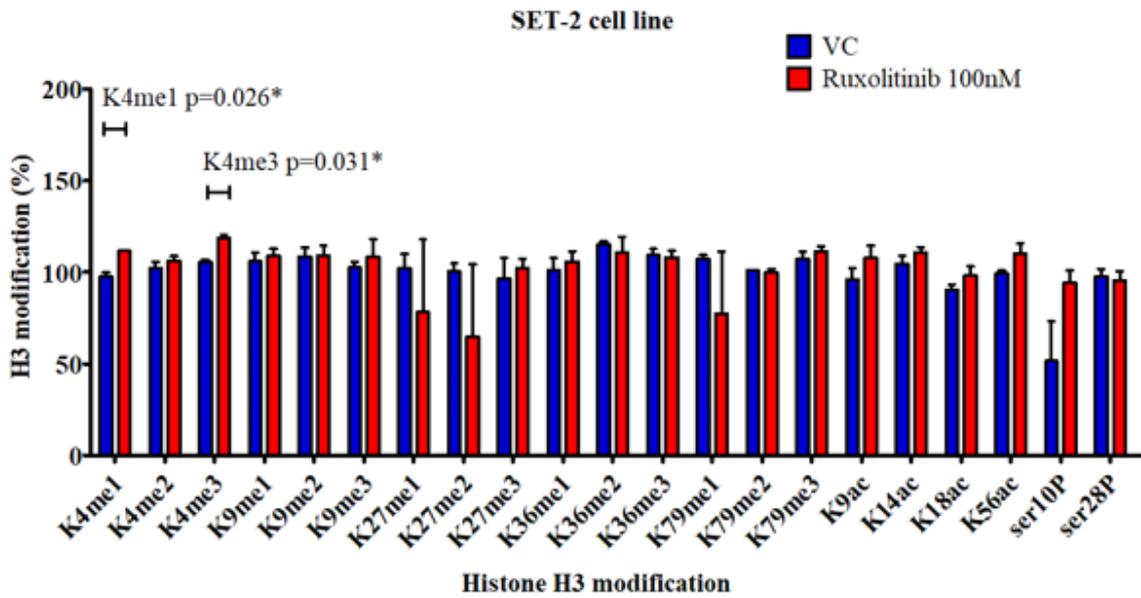


Figure 2. Protein extracted from vehicle control or 100nM Ruxolitinib treated UKE-1 cells at 2, 4, 8 and 24 hours. Western blots performed to look for effects on Histone H3 with densitometry confirming an increase in mono- and tri-methylation at lysine 9 in treated cells. Acetylation levels were slightly reduced by 24 hours.

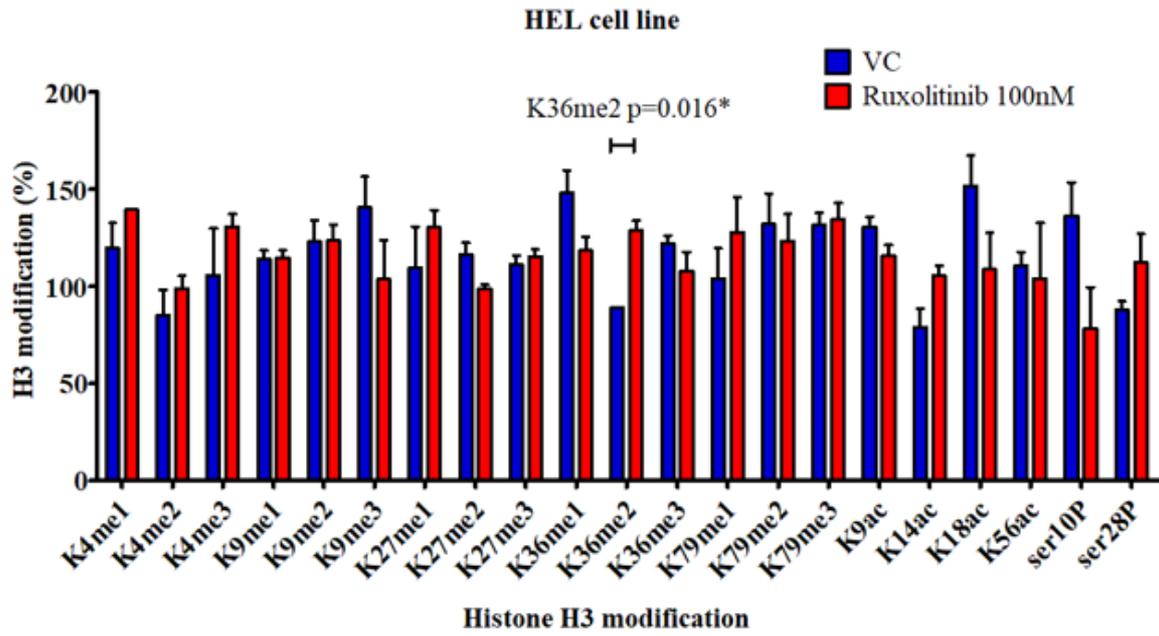


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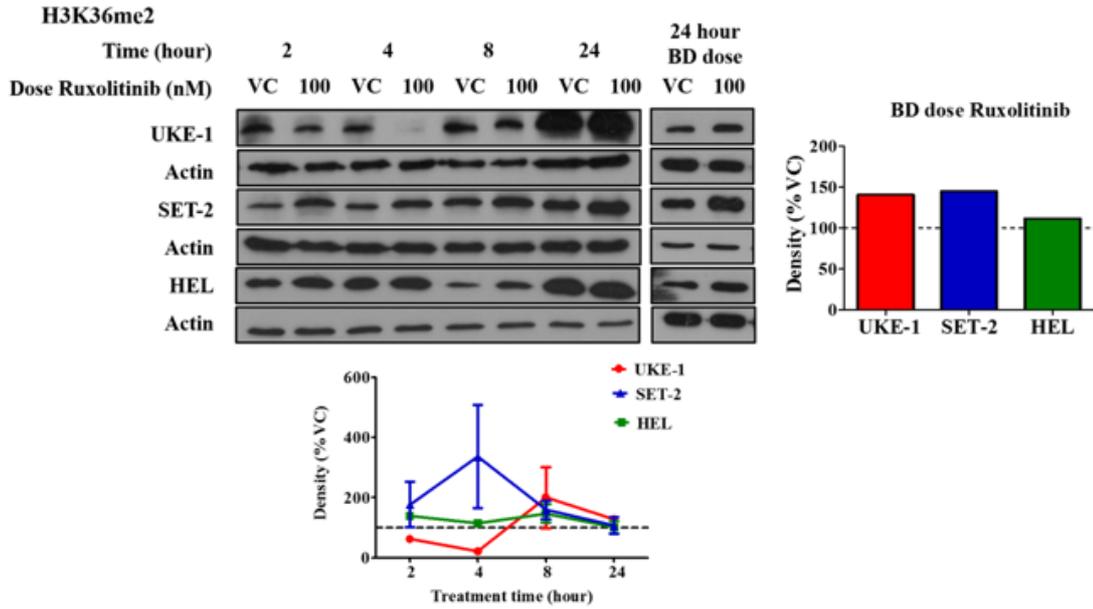


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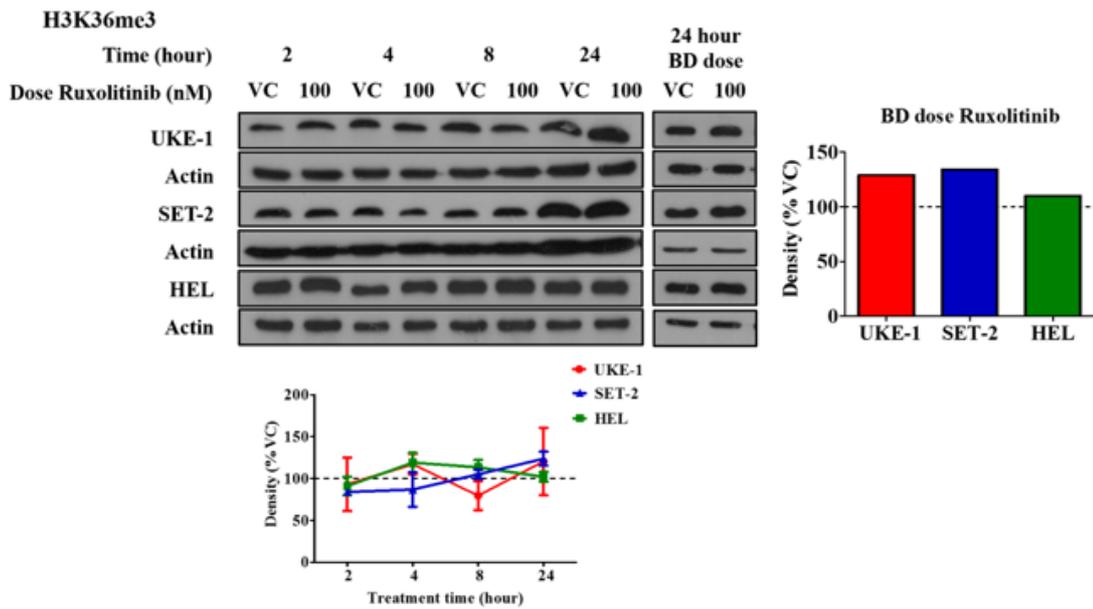


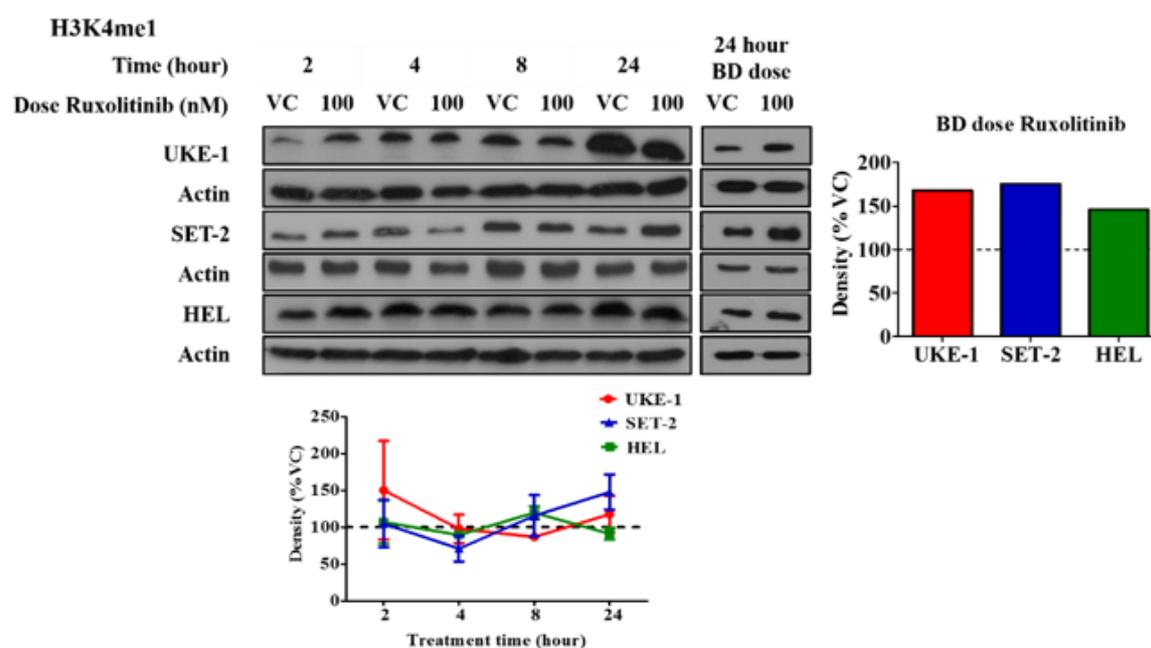
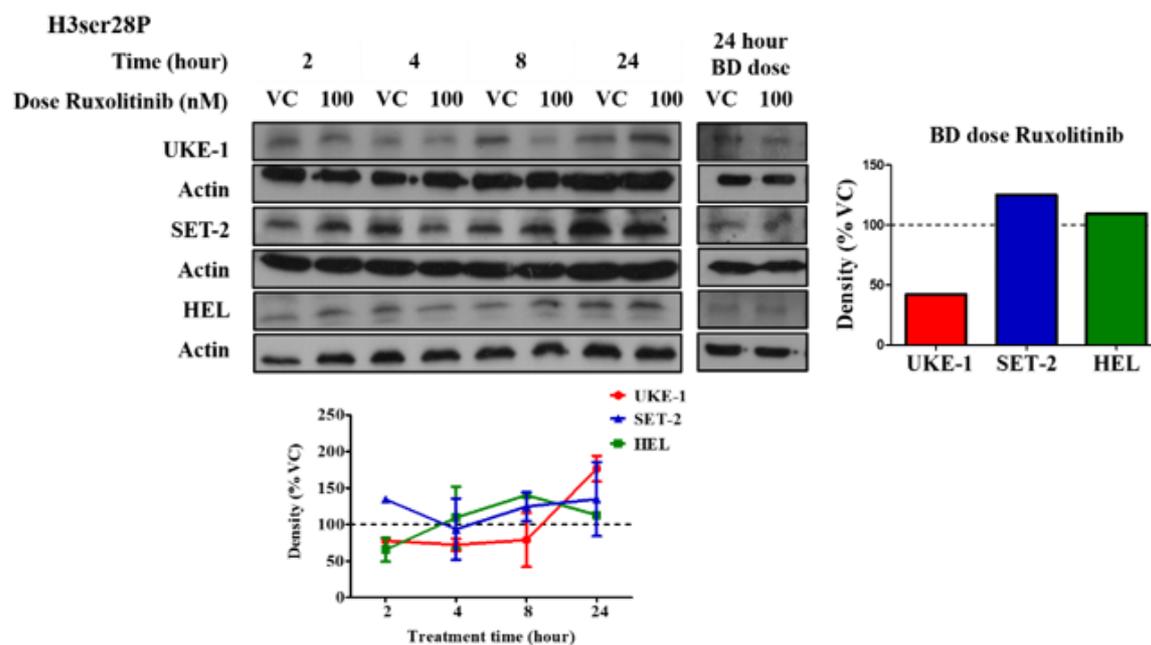
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Figure 3. Quantity of H3 modification as a percentage of total H3 present in UKE-1, SET-2 and HEL cell lines. Experiments repeated in duplicate for each cell line and treatment/control.



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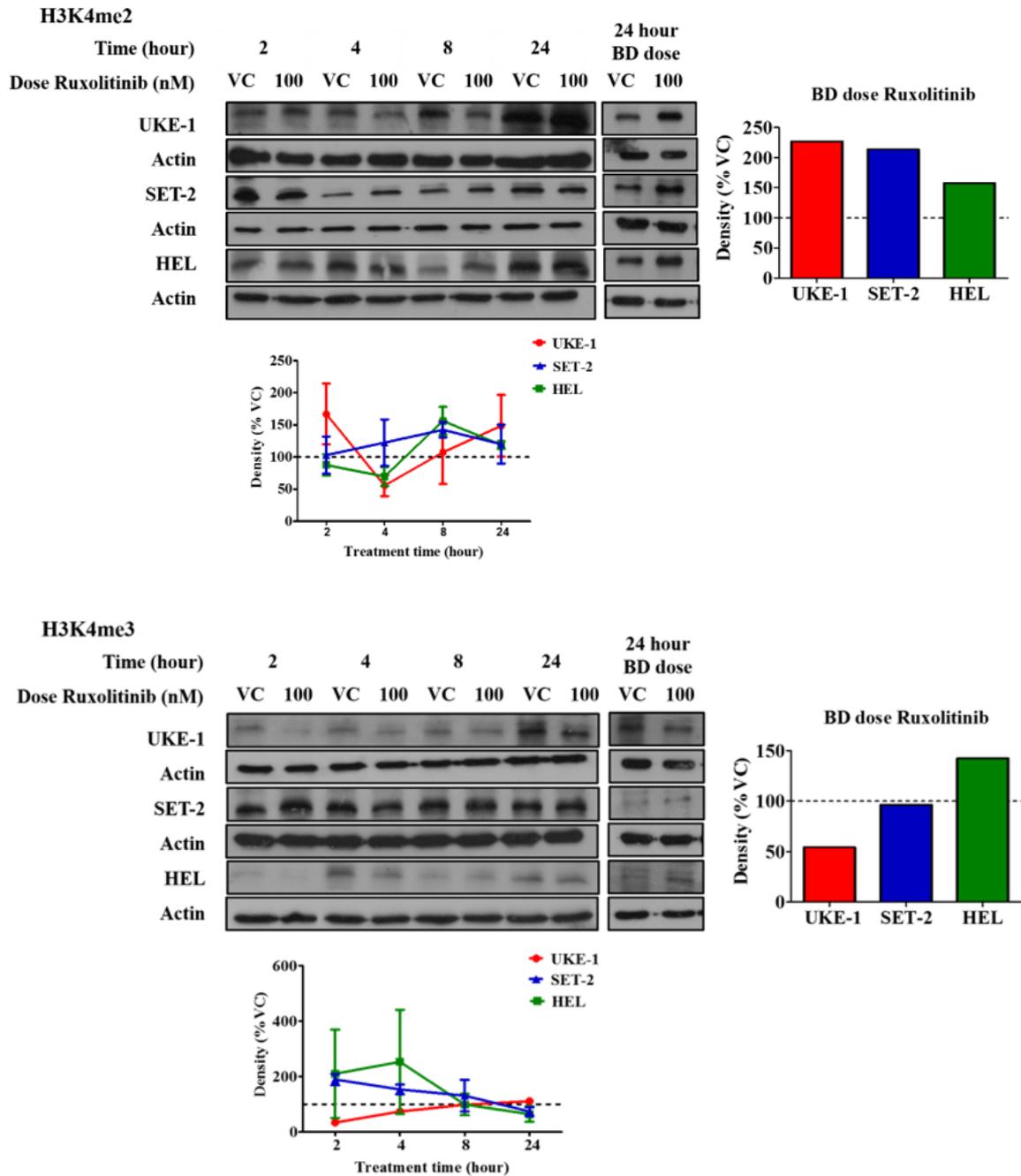
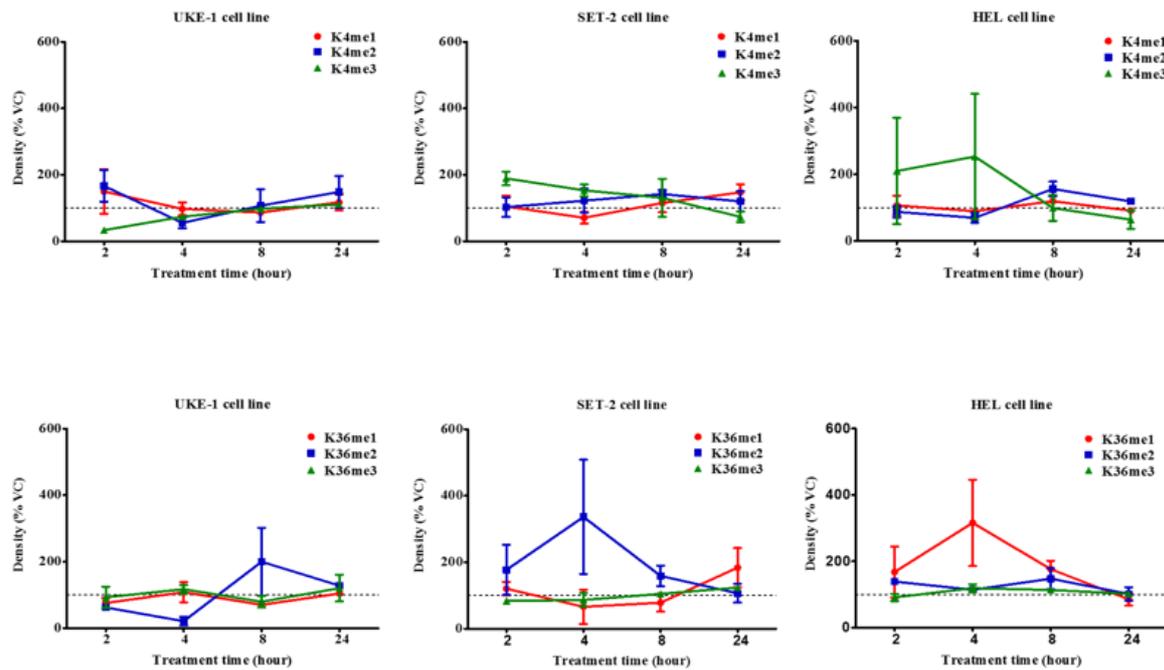


Figure 4. Western blots for H3K36me2, H3K36me3, H3ser28P, H3K4me1, H3K4me2 and H3K4me3 Histone H3 modifications in MPN cell lines (UKE-1, SET-2 and HEL) treated with vehicle control or 100nM Ruxolitinib for 2, 4, 8 or 24 hours (once or twice daily). Densitometry values are normalized for loading control.



Histone H3 validation, correlation of 2 techniques

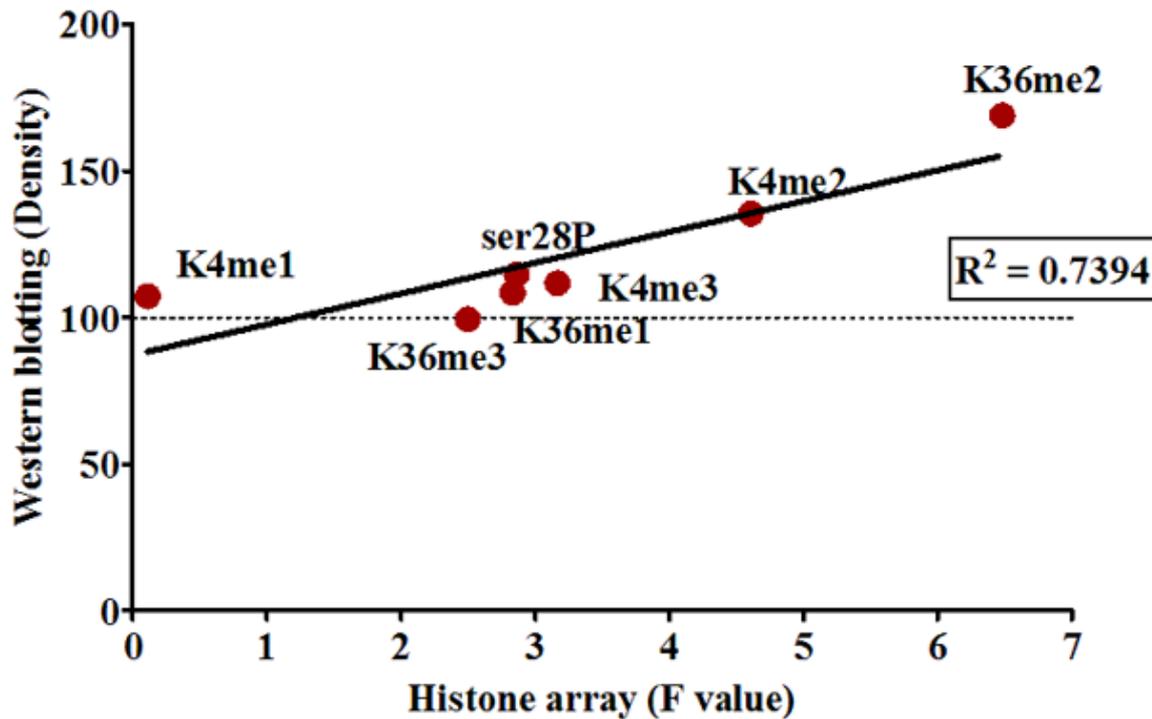


Figure 5. (A) Pattern of Histone H3 modifications in MPN cell lines with varying duration of Ruxolitinib treatment. (B) Densitometry values from western blots correlated with histone array values for each histone H3 modification tested. A positive correlation is seen between the 2 techniques with an R^2 value of 0.74.

Patient number	1		2		3		4		5		6	
	TE	FU										
H3K36me1												
Pan-actin												
Patient number	7		8		9		10		11		12	
	TE	FU										
H3K36me1												
Pan-actin												
Patient number	13		14		15		16		17		18	
	TE	FU										
H3K36me1												
Pan-actin												
Patient number	19		20		21		22		23		24	
	TE	FU										
H3K36me1												
Pan-actin												
Patient number	25		26		27		28		29		30	
	TE	FU										
H3K36me1												
Pan-actin												
Patient number	31		32		33		34		35		36	
	TE	FU										
H3K36me1												
Pan-actin												
Patient number	37		38		39		40		41		42	
	TE	FU										
H3K36me1												
Pan-actin												
Patient number	43		44		45		46		47		48	
	TE	FU										
H3K36me1												
Pan-actin												
Patient number	49		50		51							
	TE	FU	TE	FU	TE	FU						
H3K36me1												
Pan-actin												

Patient number	37		38		39		40		41		42	
	TE	FU										
H3K36me1												
Pan-actin												
Patient number	43		44		45		46		47		48	
	TE	FU										
H3K36me1												
Pan-actin												
Patient number	49		50		51							
	TE	FU	TE	FU	TE	FU						
H3K36me1												
Pan-actin												

Patient number	19		20		21		22		23		24	
	TE	FU										
H3K36me2												
Pan-actin												
Patient number	25		26		27		28		29		30	
	TE	FU										
H3K36me2												
Pan-actin												
Patient number	31		32		33		34		35		36	
	TE	FU										
H3K36me2												
Pan-actin												

Patient number	37		38		39		40		41		42	
	TE	FU										
H3K36me2												
Pan-actin												
Patient number	43		44		45		46		47		48	
	TE	FU										
H3K36me2												
Pan-actin												
Patient number	49		50		51							
	TE	FU	TE	FU	TE	FU						
H3K36me2												
Pan-actin												

Patient number	1		2		3		4		5		6	
Time point	TE	FU										
H3K36me3												
Pan-actin												

Patient number	7		8		9		10		11		12	
Time point	TE	FU										
H3K36me3												
Pan-actin												

Patient number	13		14		15		16		17		18	
Time point	TE	FU										
H3K36me3												
Pan-actin												

Patient number	19		20		21		22		23		24	
Time point	TE	FU										
H3K36me3												
Pan-actin												

Patient number	25		26		27		28		29		30	
Time point	TE	FU										
H3K36me3												
Pan-actin												

Patient number	31		32		33		34		35		36	
Time point	TE	FU										
H3K36me3												
Pan-actin												

Patient number	37		38		39		40		41		42	
Time point	TE	FU										
H3K36me3												
Pan-actin												

Patient number	43		44		45		46		47		48	
Time point	TE	FU										
H3K36me3												
Pan-actin												

Patient number	49		50		51			
Time point	TE	FU	TE	FU	TE	FU		
H3K36me3								
Pan-actin								

Patient number	1		2		3		4		5		6	
	TE	FU										
H3K4me1												
Pan-actin												

Patient number	7		8		9		10		11		12	
	TE	FU										
H3K4me1												
Pan-actin												

Patient number	13		14		15		16		17		18	
	TE	FU										
H3K4me1												
Pan-actin												

Patient number	19		20		21		22		23		24	
	TE	FU										
H3K4me1												
Pan-actin												

Patient number	25		26		27		28		29		30	
	TE	FU										
H3K4me1												
Pan-actin												

Patient number	31		32		33		34		35		36	
	TE	FU										
H3K4me1												
Pan-actin												

Patient number	37		38		39		40		41		42	
	TE	FU										
H3K4me1												
Pan-actin												

Patient number	43		44		45		46		47		48	
	TE	FU										
H3K4me1												
Pan-actin												

Patient number	49		50		51			
	TE	FU	TE	FU	TE	FU		
H3K4me1								
Pan-actin								

Patient number	1		2		3		4		5		6	
Time point	TE	FU										
H3K4me2												
Pan-actin												
Patient number	7		8		9		10		11		12	
Time point	TE	FU										
H3K4me2												
Pan-actin												
Patient number	13		14		15		16		17		18	
Time point	TE	FU										
H3K4me2												
Pan-actin												
Patient number	19		20		21		22		23		24	
Time point	TE	FU										
H3K4me2												
Pan-actin												
Patient number	25		26		27		28		29		30	
Time point	TE	FU										
H3K4me2												
Pan-actin												
Patient number	31		32		33		34		35		36	
Time point	TE	FU										
H3K4me2												
Pan-actin												
Patient number	37		38		39		40		41		42	
Time point	TE	FU										
H3K4me2												
Pan-actin												
Patient number	43		44		45		46		47		48	
Time point	TE	FU										
H3K4me2												
Pan-actin												
Patient number	49		50		51							
Time point	TE	FU	TE	FU	TE	FU						
H3K4me2												
Pan-actin												

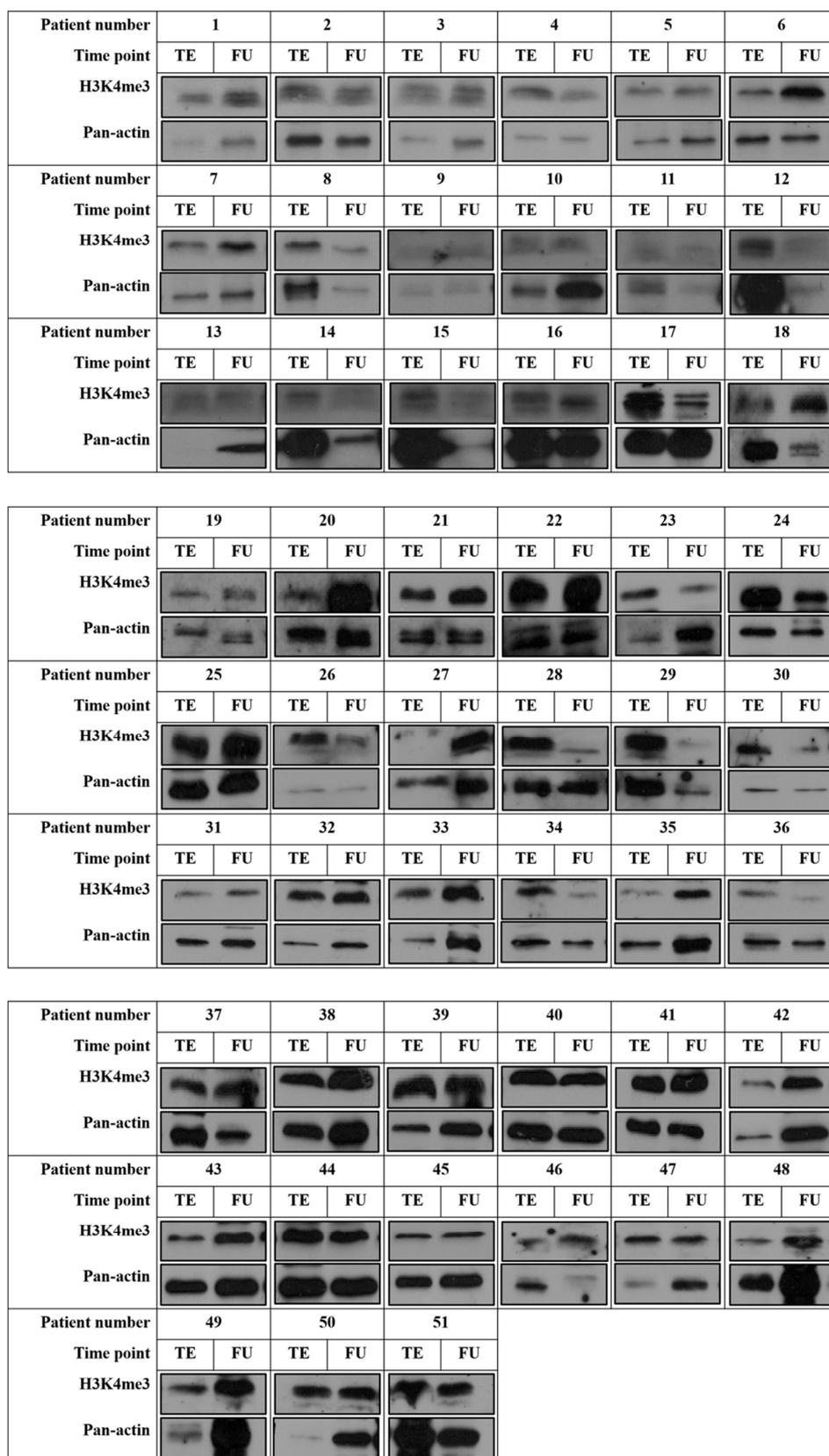
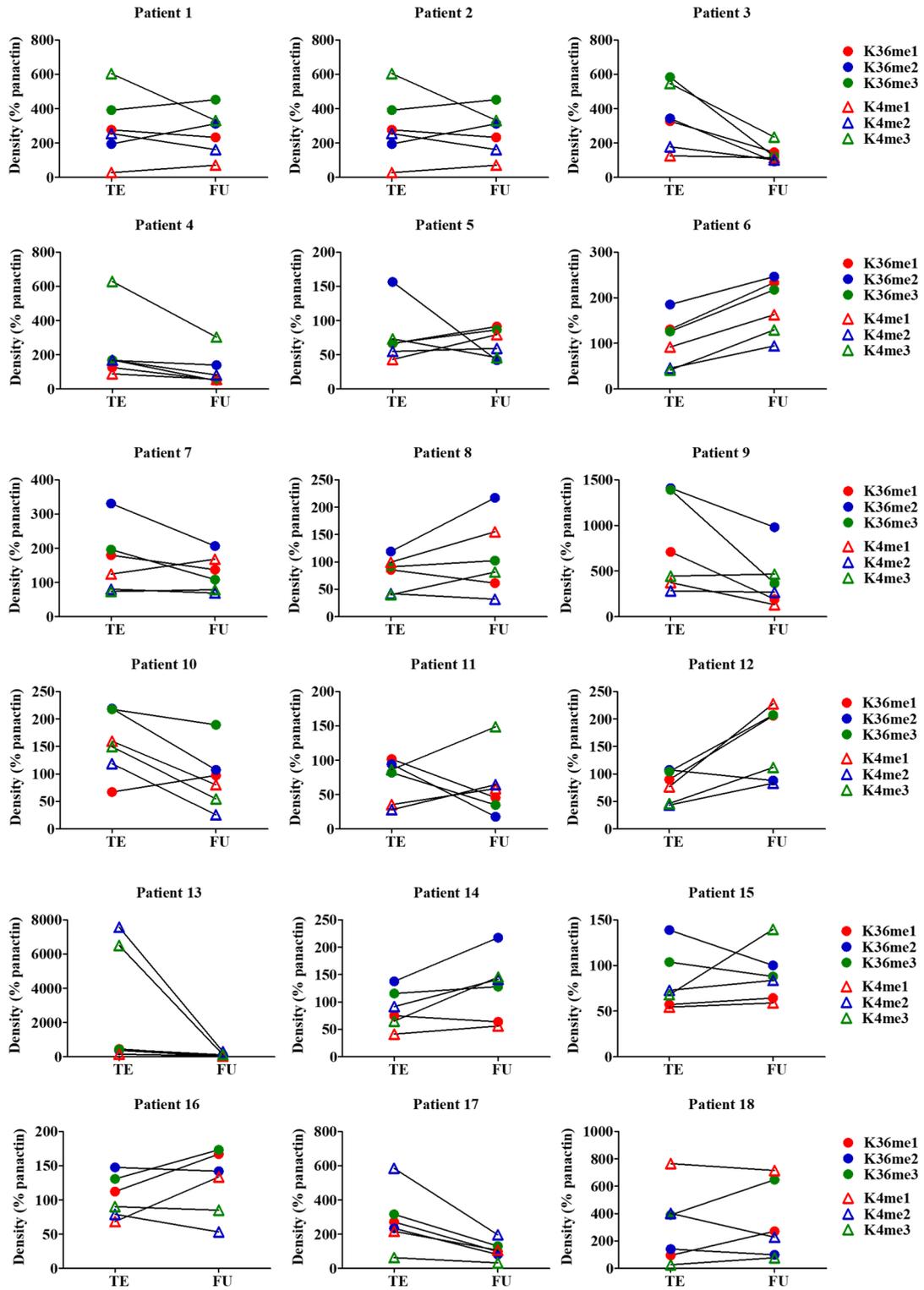
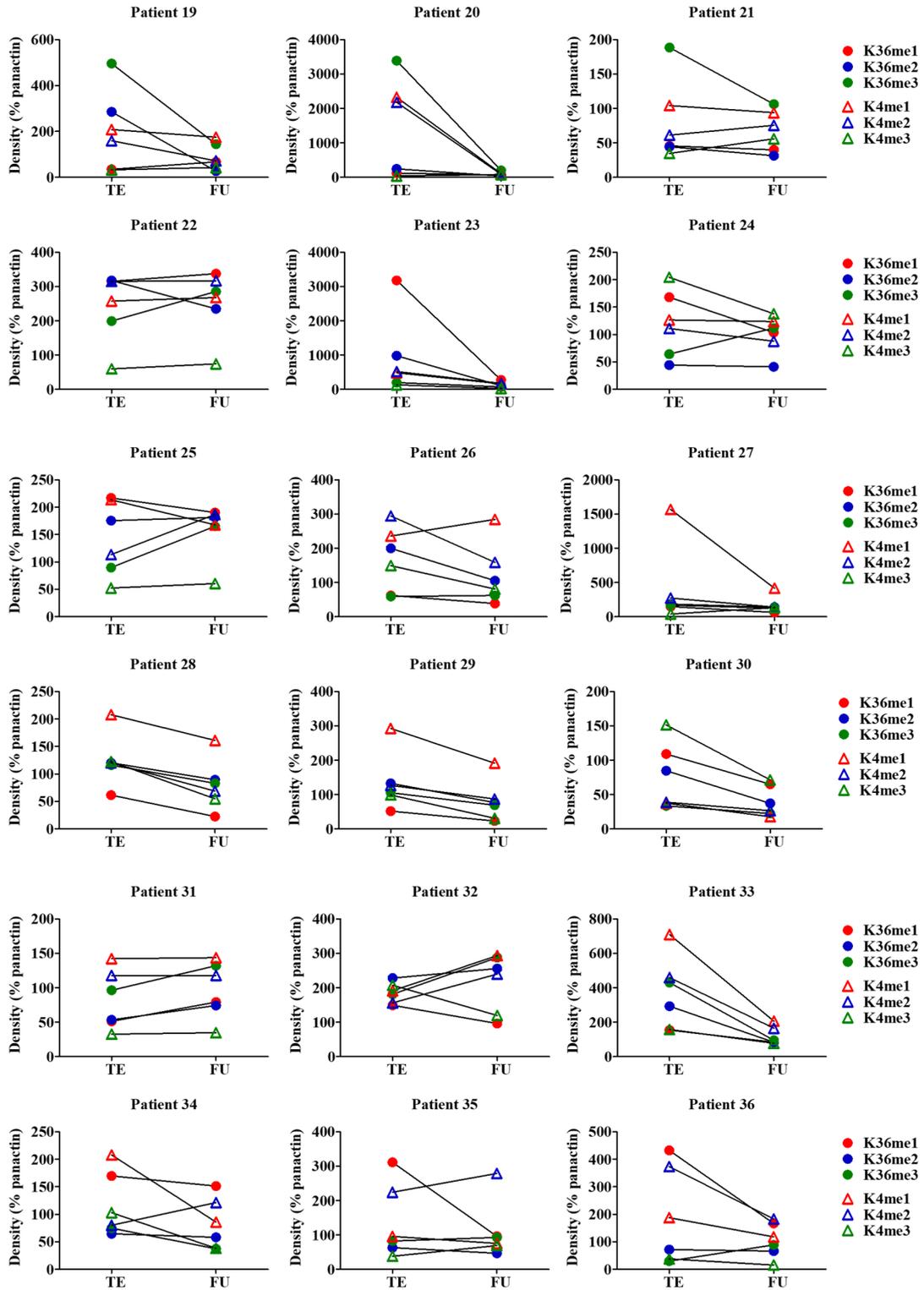


Figure 6. Western blots for mono, di and tri-methylation at lysine 36 and lysine 4 on histone H3 and corresponding pan-actin for all 51 MAJIC patients examined.





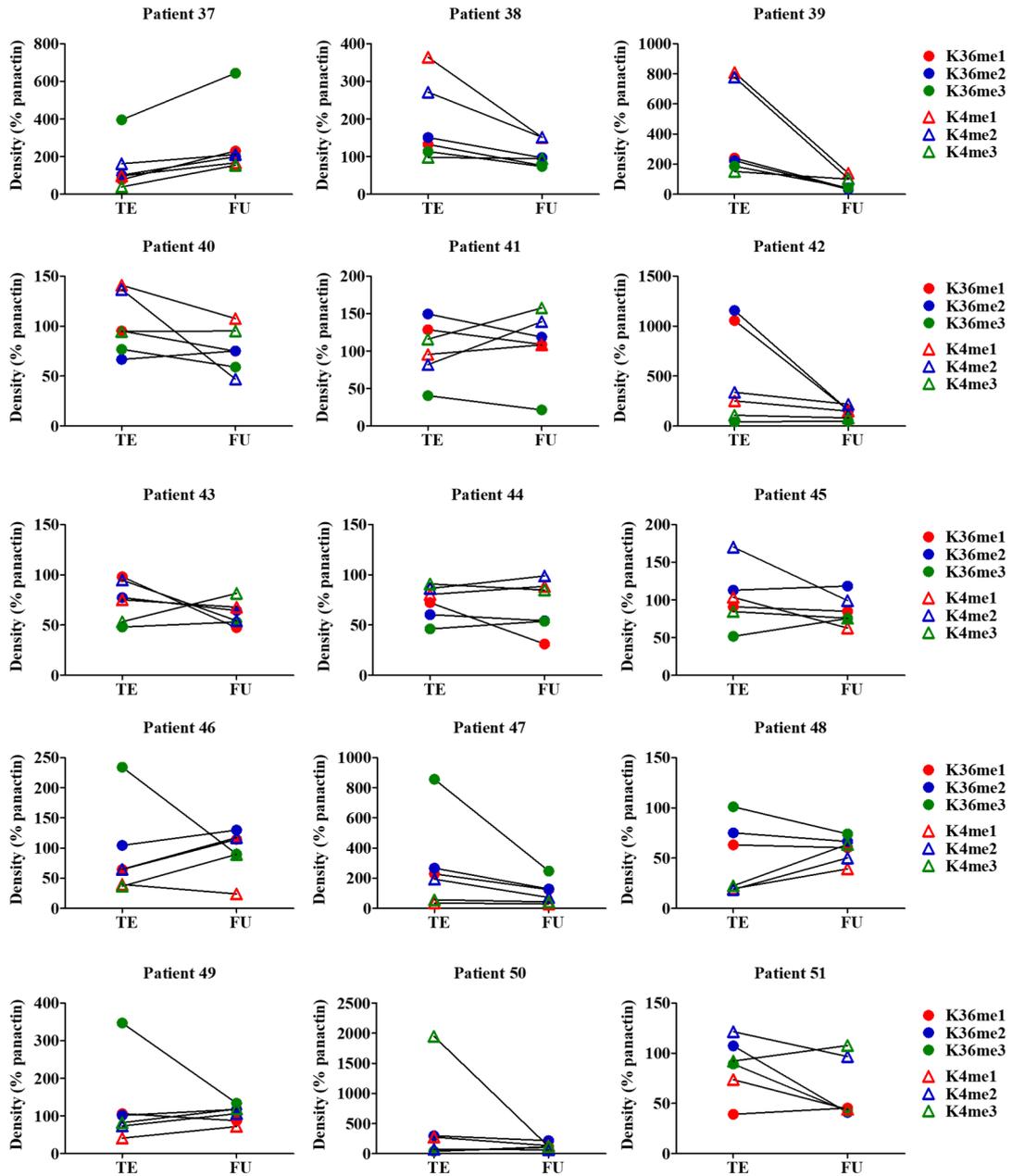


Figure S7: For each patient the normalized densitometry values before and after therapy for each histone mark examine

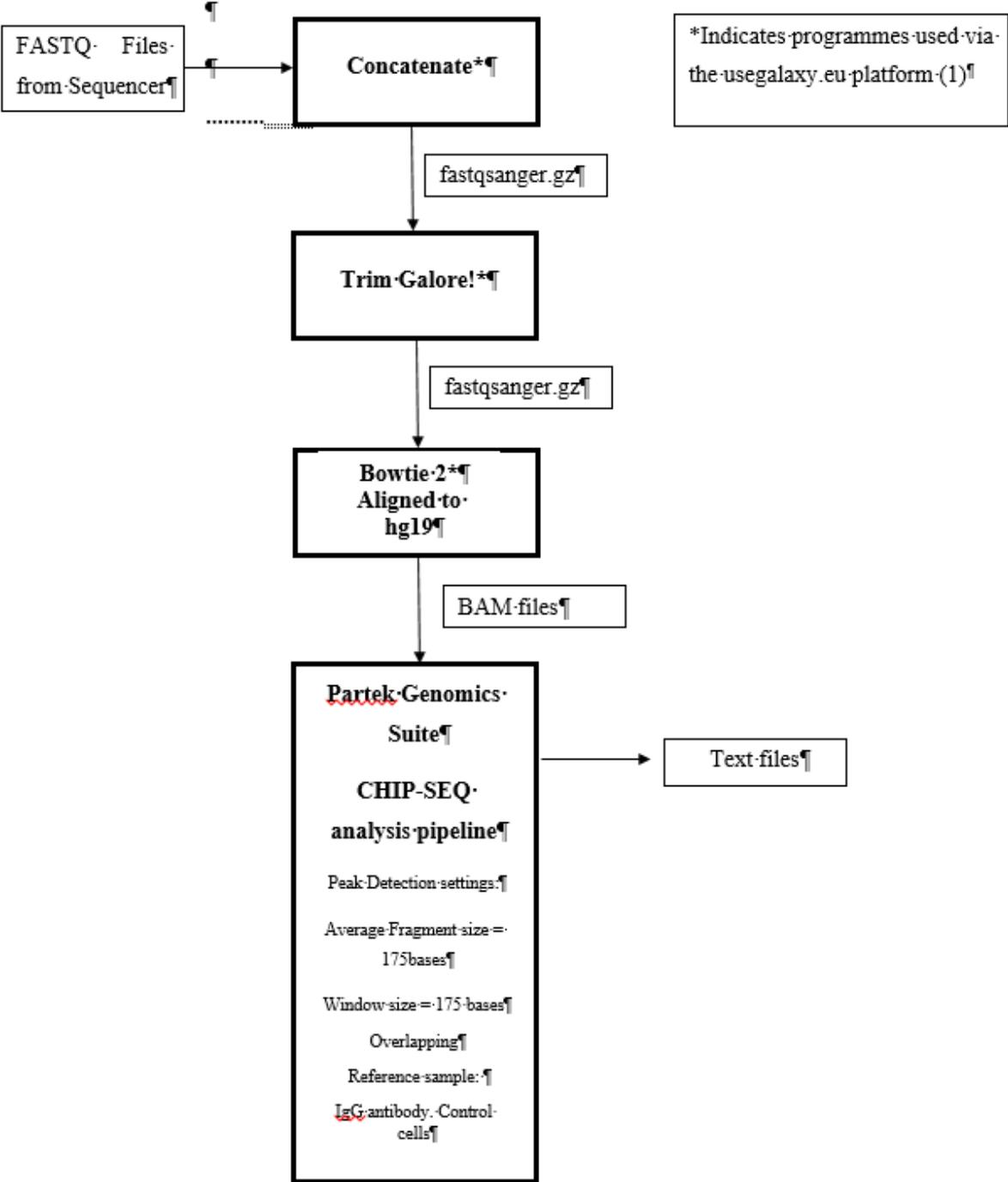


Figure 8. CHIP-seq analysis pipeline.

Table 1. Histone arrays, rank order of *p* values.

Histone modification	UKE-1		SET-2		HEL		All cell lines		UKE-1 + SET-2		UKE-1 + HEL		SET-2 + HEL		Sum of Ranks	Overall rank
	<i>p</i> value	Rank														
H3K4me1	0.148	5	0.026	1	0.263	9	0.745	21	0.328	17	0.468	20	0.021	1	74	12
H3K4me2	0.166	7	0.498	11	0.443	14	0.057	3	0.106	5	0.072	3	0.231	7	50	3
H3K4me3	0.326	17	0.032	2	0.418	12	0.106	4	0.183	14	0.154	8	0.117	3	60	5
H3K9me1	0.230	13	0.671	16	0.954	20	0.139	9	0.154	11	0.162	10	0.644	15	94	17
H3K9me2	0.531	20	0.975	21	0.966	21	0.393	19	0.421	19	0.421	19	0.944	21	140	21
H3K9me3	0.136	4	0.629	13	0.284	10	0.198	13	0.095	4	0.238	13	0.314	11	68	6
H3K27me1	0.447	19	0.613	12	0.461	15	0.342	17	0.441	20	0.275	16	0.939	20	119	20
H3K27me2	0.317	16	0.466	8	0.119	2	0.355	18	0.331	18	0.271	15	0.168	5	82	15
H3K27me3	0.040	1	0.687	18	0.560	16	0.046	2	0.053	1	0.056	2	0.417	12	52	4
H3K36me1	0.092	3	0.637	14	0.160	4	0.123	7	0.079	2	0.145	7	0.243	8	45	2
H3K36me2	0.084	2	0.671	17	0.016	1	0.029	1	0.095	3	0.020	1	0.124	4	29	1
H3K36me3	0.167	8	0.825	20	0.320	11	0.145	10	0.127	7	0.157	9	0.212	6	71	10
H3K79me1	0.150	6	0.472	9	0.423	13	0.130	8	0.194	15	0.078	4	0.883	19	74	13
H3K79me2	0.354	18	0.667	15	0.716	17	0.231	15	0.224	16	0.254	14	0.600	14	109	19
H3K79me3	0.204	10	0.491	10	0.806	18	0.123	6	0.139	9	0.142	6	0.477	13	72	11
H3K9ac	0.218	11	0.323	6	0.193	6	0.147	11	0.134	8	0.185	11	0.855	17	70	7
H3K14ac		21	0.352	7	0.135	3	0.577	20	0.920	21	0.676	21	0.044	2	95	18
H3K18ac	0.171	9	0.298	5	0.218	7	0.240	16	0.106	6	0.295	17	0.276	10	70	8
H3K56ac	0.266	14	0.202	4	0.845	19	0.166	12	0.168	13	0.204	12	0.876	18	92	16
H3ser10P	0.283	15	0.200	3	0.170	5	0.208	14	0.146	10	0.296	18	0.774	16	81	14
H3ser28P	0.220	12	0.783	19	0.252	8	0.122	5	0.159	12	0.132	5	0.246	9	70	9

Table 2. STRING pathway analysis of downregulated genes following ruxolitinib administration to HEL cells.(2).

Term Description	observed gene count	background gene count	false discovery rate
PI3K-Akt signaling pathway	13	348	4.00E-05
Transcriptional misregulation in cancer	9	169	9.92E-05
Jak-STAT signaling pathway	8	160	0.00041
Cytokine-cytokine receptor interaction	9	263	0.0013
Focal adhesion	8	197	0.0013
Inflammatory bowel disease (IBD)	5	62	0.0014
Pathways in cancer	12	515	0.0017
HTLV-I infection	8	250	0.0033
Hippo signaling pathway	6	152	0.0068

Th17 cell differentiation	5	102	0.0068
AGE-RAGE signaling pathway in diabetic complications	5	98	0.0068
TNF signaling pathway	5	108	0.008
Human papillomavirus infection	8	317	0.0094
Proteoglycans in cancer	6	195	0.0154
Measles	5	133	0.0158
Wnt signaling pathway	5	143	0.0174
ECM-receptor interaction	4	81	0.0174
Cell adhesion molecules (CAMs)	5	139	0.0174
Primary immunodeficiency	3	37	0.0174
MAPK signaling pathway	7	293	0.0178
MicroRNAs in cancer	5	149	0.0178
Breast cancer	5	147	0.0178
Hematopoietic cell lineage	4	94	0.0204
Malaria	3	47	0.0229
Insulin resistance	4	107	0.0293
Viral myocarditis	3	56	0.0338
Platelet activation	4	123	0.0435