Supplementary Materials

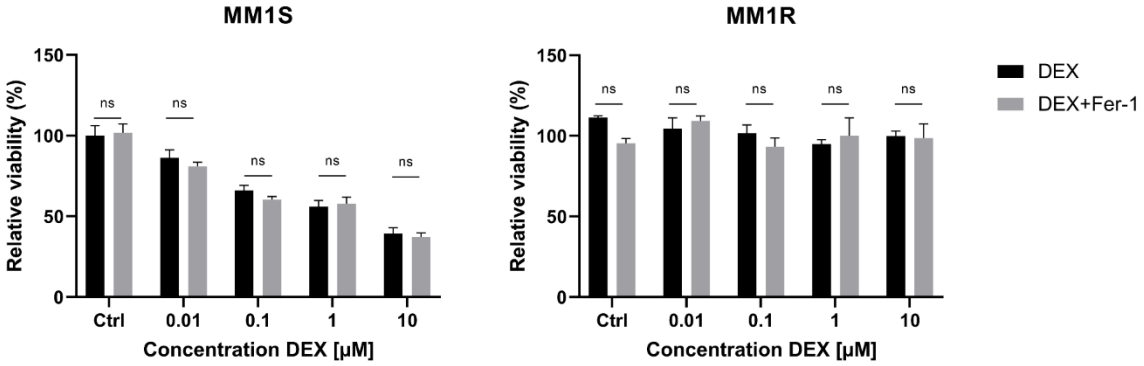


Figure S1. Relative viability of MM1S (left) and MM1R (right) cells upon 24 h exposure to increasing concentrations of dexamethasone (DEX) with or without pre-treatment with ferrostatin-1 (Fer-1). Data are plotted as the mean ± s.d., $n = 3$ biologically independent replicates (ns $p < 0.05$, ANOVA).

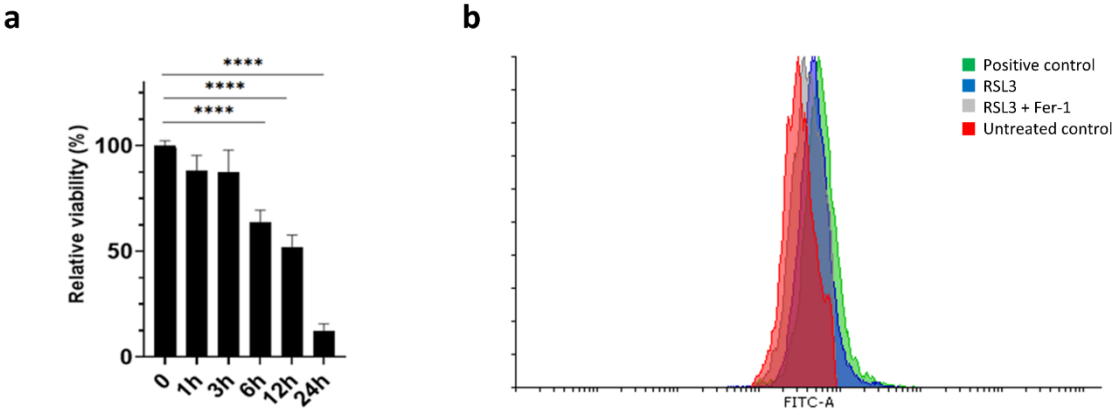


Figure S2. (**a**) Time-dependent relative viability of MM1 cells after 5 µM RSL3 treatment. Data are plotted as the mean ± s.d., $n = 3$ biologically independent replicates (****$p < 0.0001$, ANOVA). (**b**) Flow cytometric analysis of the lipid peroxidation sensor (C11-BODIPY-581/591 dye) on live MM1R cells after 3h treatment with 5µM RSL3 with or without pre-treatment with 2 µM ferrostatin-1 (Fer-1) or 100 µM cumene hydroxide (positive control).

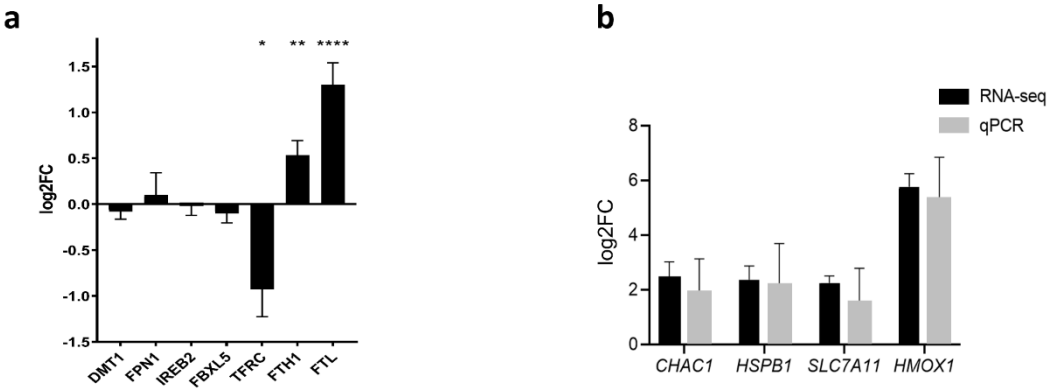


Figure S3. (**a**) LogFC of iron-responsive genes in RSL3-treated MM1 cells versus untreated controls as determined by RNAseq. Data are plotted as the mean logFC ± SEM, n=3 biologically independent replicates per cell line (*$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$, ANOVA). (**b**) Comparison of log2FC of RNAseq and qPCR analysis of 4 ferroptosis-related genes. Data are plotted as the mean ± s.d., $n = 3$ biologically independent replicates per cell line.

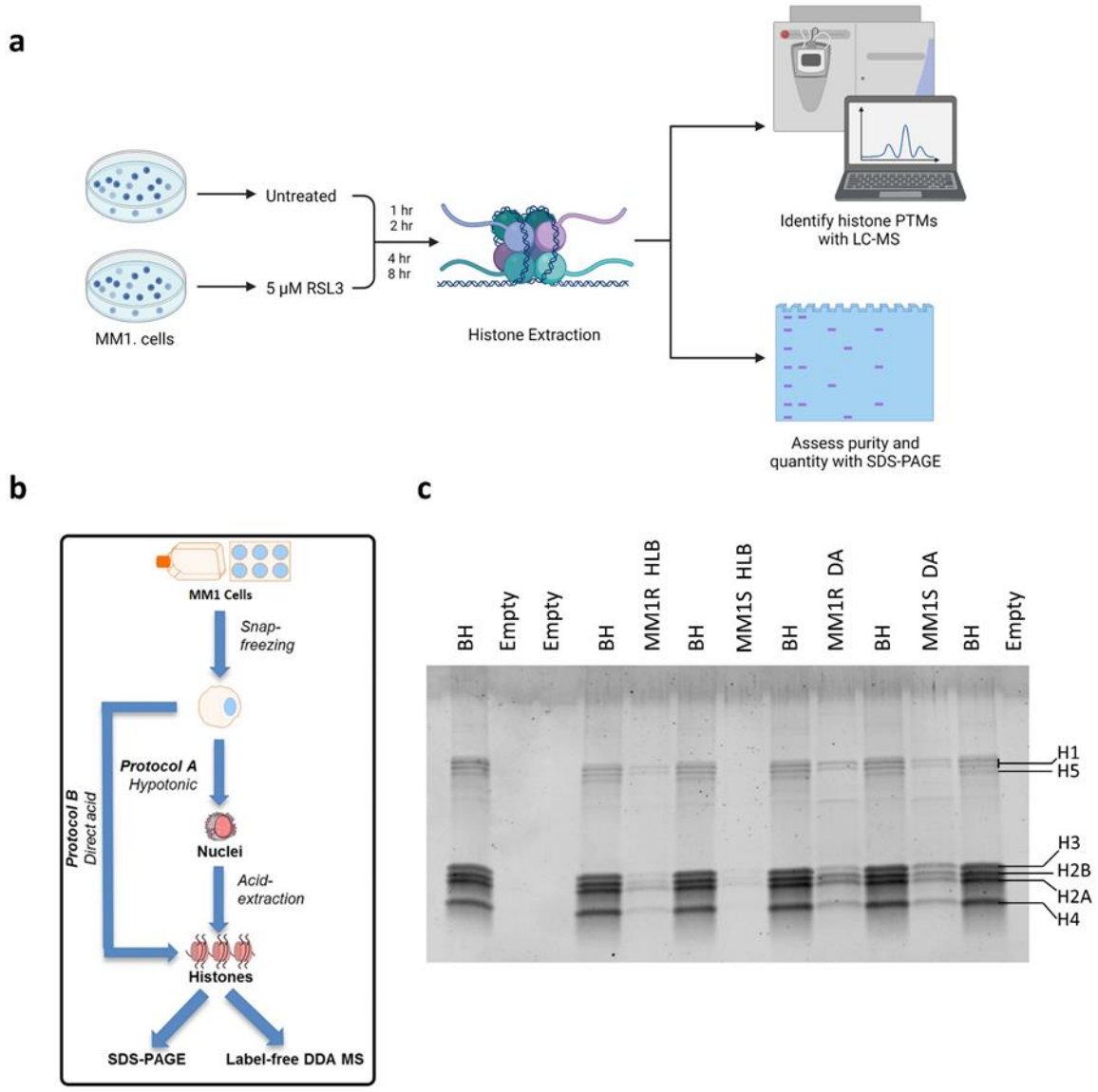


Figure S4. Histone extraction optimization from MM1 cells for LC-MS analysis of post-translational modifications (PTMs). (**a**) Experimental pipeline for identification of histone PTMs with liquid chromatography-mass spectrometry (LC-MS). Prior to LC-MS analysis, purity and quantity of histone extracts is assessed with polyacrylamide gel electrophoresis (PAGE). (**b**) Lysis protocols that were explored for histone extraction from MM1 cells. Adapted from [108]. (**c**) Purity of MM1 histone extracts obtained with either hypotonic lysis buffer (HLB) or direct acid lysis (DA). Extracts from 4 x 10^5 cells were loaded onto a 8–16% gradient gel and compared to a 2 µg bovine histone (BH) commercial standard.

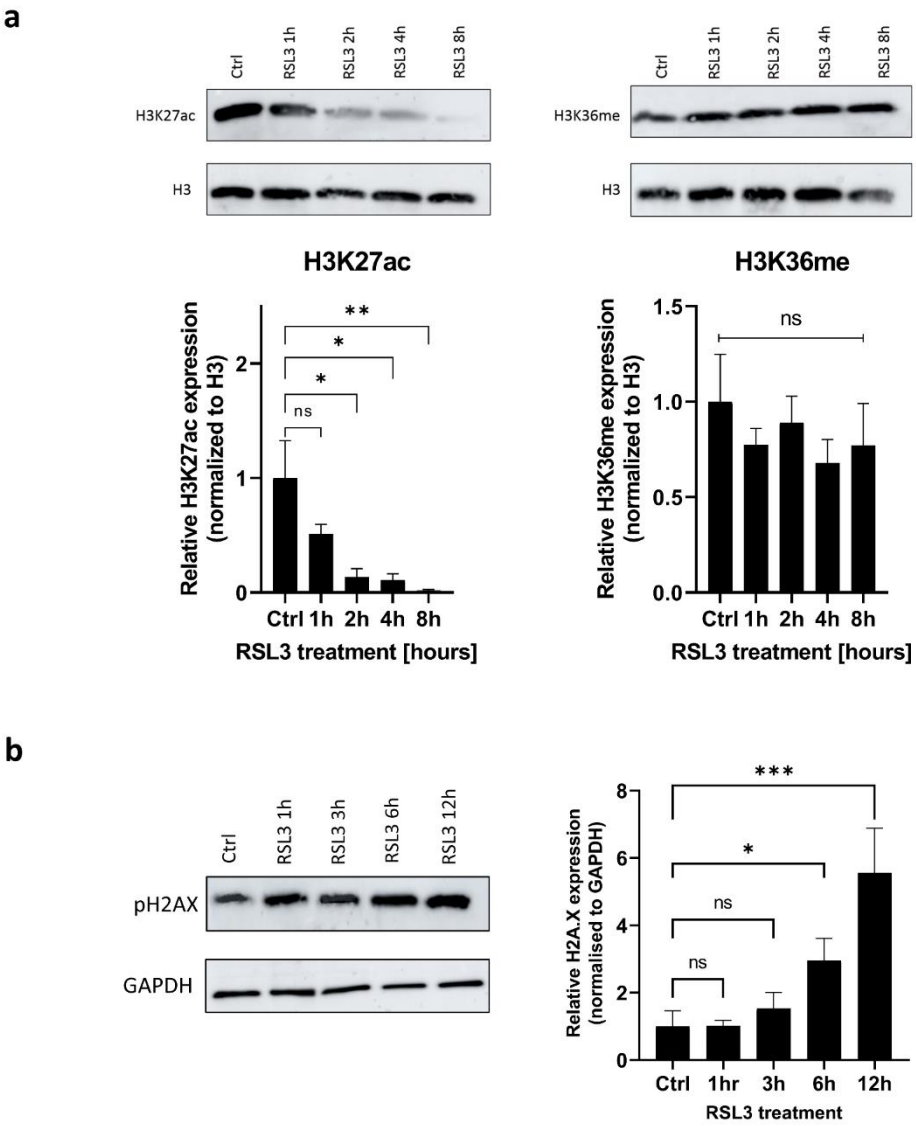
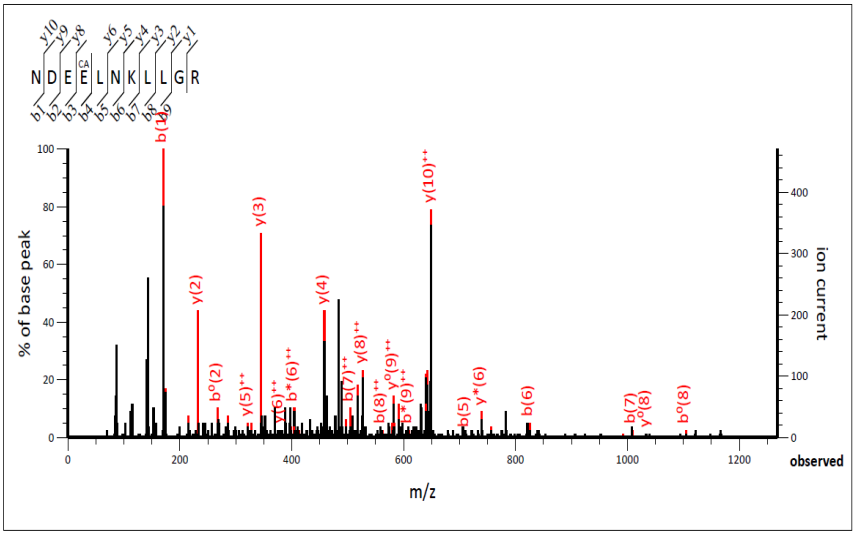


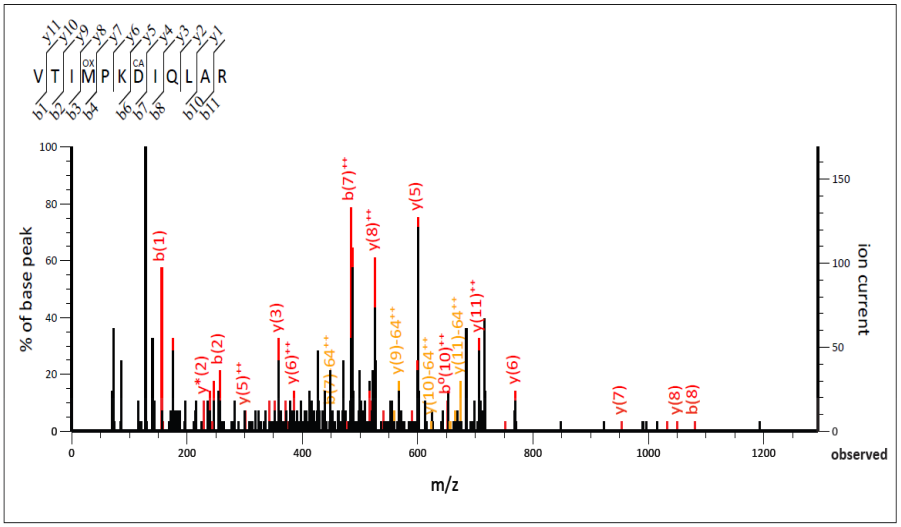
Figure S5. (**a**) Western blot validation of histone proteomics LC-MS data. H3K27ac (left) was significantly upregulated upon RSL3 treatment in MM1 cells while H3K36me3 (right) levels remained unchanged, both in LC-MS and western blot analysis. (**b**) Western blot detection and quantification of pH2AX and GAPDH expression levels after RSL3 treatment in MM1 cells. Data are plotted as the mean ± s.d., $n = 3$ biologically independent replicates. (ns $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ ANOVA).



Monoisotopic mass of neutral peptide Mr(calc): 1465.6500
Fixed modifications: Propionyl (K),Propionyl (N-term) (apply to specified residues or termini only)
Variable modifications:
E4 : Cation:Fe[II] (DE)
Ions Score: 24 **Expect:** 0.0061 (help)

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	171.0764	86.0418	154.0499	77.5286			N							11
2	286.1034	143.5553	269.0768	135.0420	268.0928	134.5500	D	1296.5881	648.7977	1279.5616	640.2844	1278.5776	639.7924	10
3	415.1460	208.0766	398.1194	199.5633	397.1354	199.0713	E	1181.5612	591.2842	1164.5346	582.7710	1163.5506	582.2789	9
4	598.1078	299.5576	581.0813	291.0443	580.0973	290.5523	E	1052.5186	526.7629	1035.4920	518.2497	1034.5080	517.7577	8
5	711.1919	356.0996	694.1654	347.5863	693.1813	347.0943	L	869.5567	435.2820	852.5302	426.7687			7
6	825.2348	413.1211	808.2083	404.6078	807.2243	404.1158	N	756.4726	378.7400	739.4461	370.2267			6
7	1009.3560	505.1816	992.3295	496.6684	991.3454	496.1764	K	642.4297	321.7185	625.4032	313.2052			5
8	1122.4401	561.7237	1105.4135	553.2104	1104.4295	552.7184	L	458.3085	229.6579	441.2820	221.1446			4
9	1235.5241	618.2657	1218.4976	609.7524	1217.5136	609.2604	L	345.2245	173.1159	328.1979	164.6026			3
10	1292.5456	646.7764	1275.5190	638.2632	1274.5350	637.7712	G	232.1404	116.5738	215.1139	108.0606			2
11							R	175.1190	88.0631	158.0924	79.5498			1

(**a**)



Monoisotopic mass of neutral peptide Mr(calc): 1565.7574
Fixed modifications: Propionyl (K),Propionyl (N-term) (apply to specified residues or termini only)
Variable modifications:
M4 : Oxidation (M), with neutral losses 0.0000(shown in table), 63.9983
D7 : Cation:Fe[II] (DE)
Ions Score: 39 **Expect:** 0.00047 (help)

#	b	b^{++}	b*	b^{*++}	b^0	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^0	y^{0++}	#
1	156.1019	78.5546					V							12
2	257.1496	129.0784			239.1390	120.0731	T	1411.6701	706.3387	1394.6435	697.8254	1393.6595	697.3334	11
3	370.2336	185.6205			352.2231	176.6152	I	1310.6224	655.8148	1293.5959	647.3016	1292.6118	646.8096	10
4	517.2690	259.1382			499.2585	250.1329	M	1197.5383	599.2728	1180.5118	590.7595	1179.5278	590.2675	9
5	614.3218	307.6645			596.3112	298.6593	P	1050.5029	525.7551	1033.4764	517.2418	1032.4924	516.7498	8
6	798.4430	399.7251	781.4164	391.2119	780.4324	390.7199	K	953.4502	477.2287	936.4236	468.7155	935.4396	468.2234	7
7	967.3892	484.1982	950.3627	475.6850	949.3787	475.1930	D	769.3290	385.1681	752.3025	376.6549	751.3184	376.1629	6
8	1080.4733	540.7403	1063.4467	532.2270	1062.4627	531.7350	I	600.3828	300.6950	583.3562	292.1817			5
9	1208.5319	604.7696	1191.5053	596.2563	1190.5213	595.7643	Q	487.2987	244.1530	470.2722	235.6397			4
10	1321.6159	661.3116	1304.5894	652.7983	1303.6054	652.3063	L	359.2401	180.1237	342.2136	171.6104			3
11	1392.6530	696.8302	1375.6265	688.3169	1374.6425	687.8249	A	246.1561	123.5817	229.1295	115.0684			2
12							R	175.1190	88.0631	158.0924	79.5498			1

(**b**)

Figure S6. Annotated MS/MS spectra of H3 (**a**) and H2A (**b**) proteins isolated from RSL3-treated MM1 cells. Annotated spectra indicate Fe^{2+} binding to glutamate (E) and aspartate (D) residues of H2A and H3.

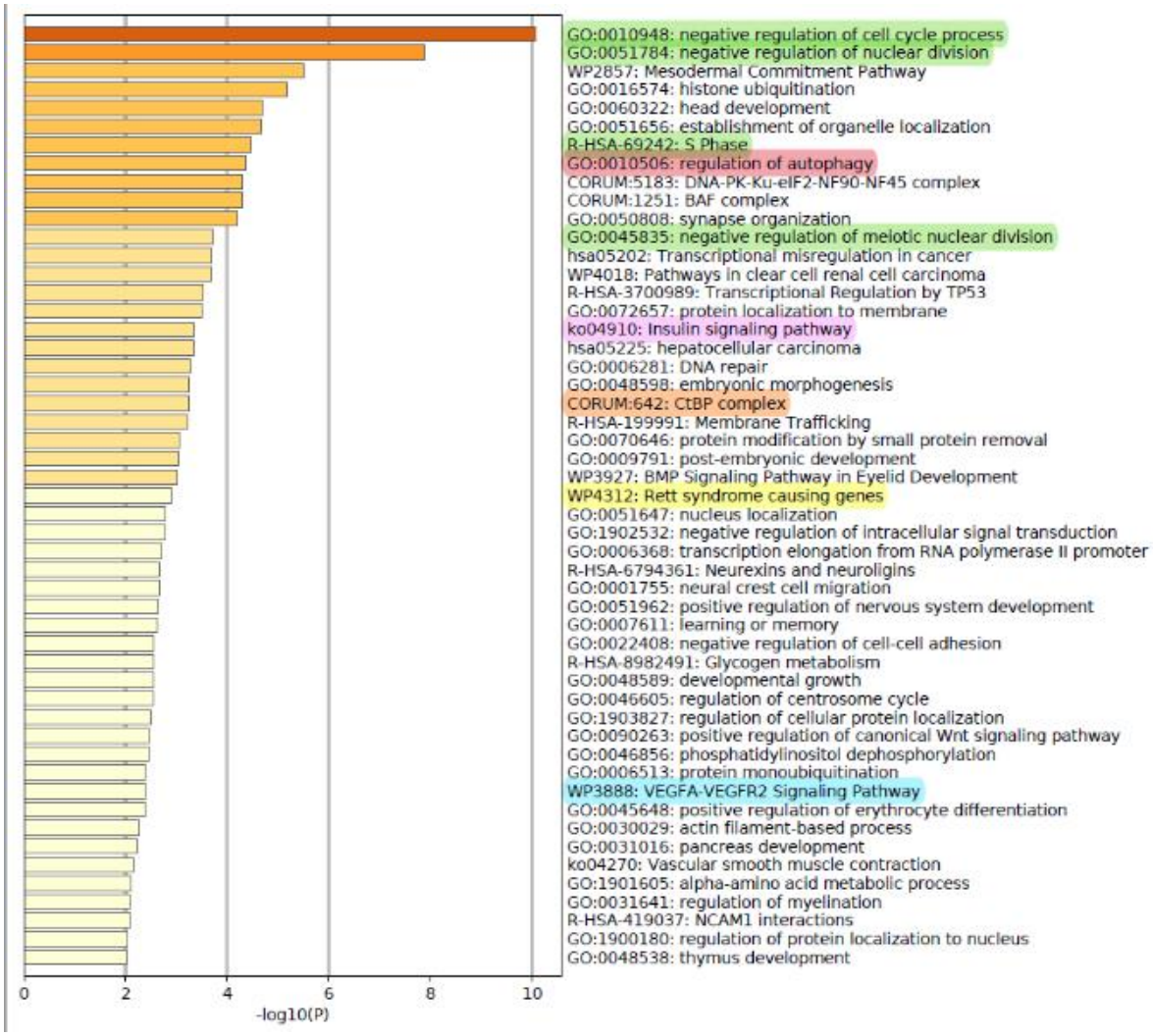


Figure S7. Metascape pathway enrichment of significantly differentially methylated CpG probes (FDR <0.05 & Δβ differences > 0.1) in RSL3-treated **MM1S** cells compared to Ferrostatin-1 pre-treated, RSL3-treated MM1S cells. Terms marked in color are common pathways between MM1S and MM1R cells (see Figure S6).

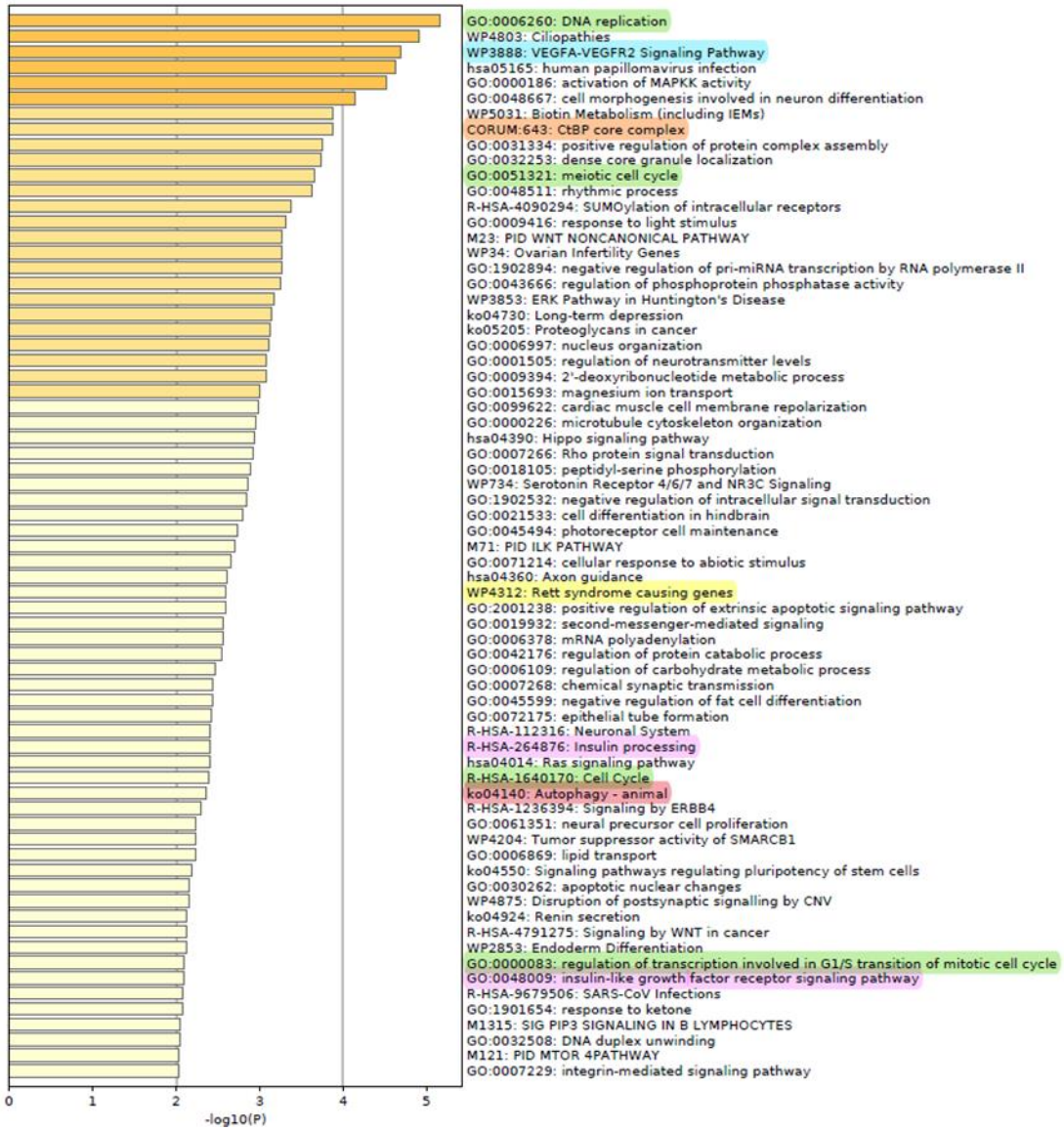


Figure S8: Metascape pathway enrichment of significantly differentially methylated CpG probes (FDR <0.05 & $\Delta\beta$ differences > 0.1) in RSL3-treated MM1R cells compared to Ferrostatin-1 pre-treated, RSL3-treated MM1S cells. Terms marked in color are common pathways between MM1S and MM1R cells (see Figure S5).

Table S1. Overview of significantly altered histone PTMs in RSL3-treated MM1 cells compared to untreated controls.

Histone	PTM	Sequence	Log2FC				*p*-Value			
			1 h	2 h	4 h	8 h	1 h	2 h	4 h	8 h
H2A	E57Fe	VGA-GAPVYMAAVLEYLTAEILELAGNAAR	0,20	0,61	0,69	0,56	$3{,}70 \times 10^{-1}$	$1{,}46 \times 10^{-3}$	$1{,}75 \times 10^{-4}$	$5{,}90 \times 10^{-3}$
	R89Cit	HLQLAIRNDEELNKLLGR	−0,15	−0,63	−0,47	−0,53	$4{,}51 \times 10^{-1}$	$1{,}27 \times 10^{-2}$	$5{,}28 \times 10^{-2}$	$3{,}54 \times 10^{-2}$
	E93Fe	NDEELNKLLGR	0,32	0,63	0,80	0,90	$1{,}21 \times 10^{-1}$	$6{,}33 \times 10^{-4}$	$1{,}93 \times 10^{-5}$	$1{,}84 \times 10^{-4}$
	K96Ac	HLQLAIRNDEELNKLLGK	0,52	2,26	2,86	1,93	$2{,}99 \times 10^{-1}$	$5{,}55 \times 10^{-3}$	$1{,}37 \times 10^{-3}$	$1{,}17 \times 10^{-2}$
	K100Ac	HLQLAIRNDEELNKLLGK	0,52	2,26	2,86	1,93	$2{,}99 \times 10^{-1}$	$5{,}55 \times 10^{-3}$	$1{,}37 \times 10^{-3}$	$1{,}17 \times 10^{-2}$
	K100But	HLQLAIRNDEELNKLLGK	0,52	2,26	2,86	1,93	$2{,}99 \times 10^{-1}$	$5{,}55 \times 10^{-3}$	$1{,}37 \times 10^{-3}$	$1{,}17 \times 10^{-2}$
H2A.Z	K8Ac	AGGKAGKDSGKAKA-KAVSR	0,08	−0,14	−0,23	−0,61	$6{,}84 \times 10^{-1}$	$4{,}78 \times 10^{-1}$	$2{,}78 \times 10^{-1}$	$1{,}68 \times 10^{-2}$
	K14Ac	AGGKAGKDSGKAKA-KAVSR	−0,77	−2,49	−2,32	−2,08	$2{,}29 \times 10^{-1}$	$1{,}36 \times 10^{-2}$	$1{,}61 \times 10^{-2}$	$2{,}81 \times 10^{-2}$
	K16Suc	AGGKAGKDSGKAKA-KAVSR	−0,77	−2,49	−2,32	−2,08	$2{,}29 \times 10^{-1}$	$1{,}36 \times 10^{-2}$	$1{,}61 \times 10^{-2}$	$2{,}81 \times 10^{-2}$
	K16Hib	AGGKAGKDSGKAKA-KAVSR	−0,43	−3,28	−2,15	−2,15	$5{,}66 \times 10^{-1}$	$3{,}18 \times 10^{-2}$	$6{,}74 \times 10^{-2}$	$8{,}32 \times 10^{-2}$
	K102Hib	HLQLAIRGDEELDSLIKATI-AGGGVIPHIHKSLIGKKGQQKTA	−0,08	−0,56	−0,98	−0,84	$8{,}10 \times 10^{-1}$	$1{,}21 \times 10^{-1}$	$1{,}94 \times 10^{-2}$	$4{,}04 \times 10^{-2}$
	K120Cro	HLQLAIRGDEELDSLIKATI-AGGGVIPHIHKSLIGKKGQQKTA	−0,08	−0,56	−0,98	−0,84	$8{,}10 \times 10^{-1}$	$1{,}21 \times 10^{-1}$	$1{,}94 \times 10^{-2}$	$4{,}04 \times 10^{-2}$
	K121Me3	HLQLAIRGDEELDSLIKATI-AGGGVIPHIHKSLIGKKGQQKTA	−0,08	−0,56	−0,98	−0,84	$8{,}10 \times 10^{-1}$	$1{,}21 \times 10^{-1}$	$1{,}94 \times 10^{-2}$	$4{,}04 \times 10^{-2}$
	K121But	HLQLAIRGDEELDSLIKATI-AGGGVIPHIHKSLIGKKGQQKTA	−0,20	−1,55	−0,87	−0,63	$5{,}91 \times 10^{-1}$	$2{,}93 \times 10^{-3}$	$4{,}95 \times 10^{-2}$	$0{,}14 \times 10^{-1}$
	K125Me3	HLQLAIRGDEELDSLIKATI-AGGGVIPHIHKSLIGKKGQQKTA	−0,08	−0,56	−0,98	−0,84	$8{,}10 \times 10^{-1}$	$1{,}21 \times 10^{-1}$	$1{,}94 \times 10^{-2}$	$4{,}04 \times 10^{-2}$
H2A.FY	K33But	YIKKGHPKYR	−0,04	−0,90	−1,32	0,56	$8{,}36 \times 10^{-1}$	$1{,}72 \times 10^{-3}$	$5{,}90 \times 10^{-5}$	$0{,}38 \times 10^{-1}$
	K34Me3	YIKKGHPKYR	−0,04	−0,90	−1,32	0,56	$8{,}36 \times 10^{-1}$	$1{,}72 \times 10^{-3}$	$5{,}90 \times 10^{-5}$	$0{,}38 \times 10^{-1}$
	R79Me	VTPRHILLA-VANDEELNQLLKGVTIASGGVLPNIHPELLAKKR	0,09	0,55	0,62	0,73	$8{,}19 \times 10^{-1}$	$9{,}05 \times 10^{-2}$	$7{,}74 \times 10^{-2}$	$2{,}34 \times 10^{-2}$
H3	K18Ac	KQLATKAAR	−0,61	−5,01	−2,99	−3,30	$4{,}03 \times 10^{-1}$	$1{,}67 \times 10^{-2}$	$3{,}11 \times 10^{-2}$	$3{,}31 \times 10^{-2}$

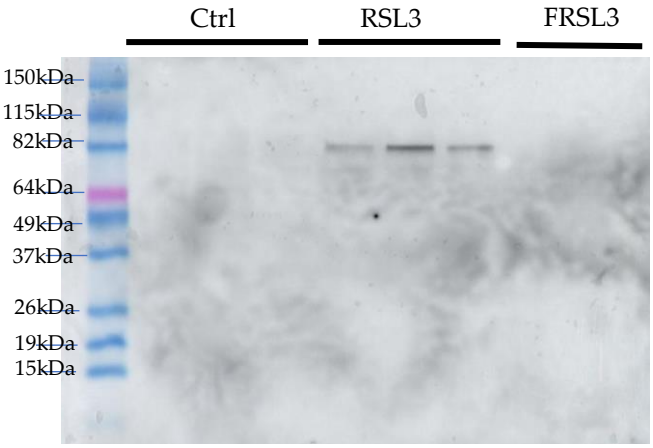
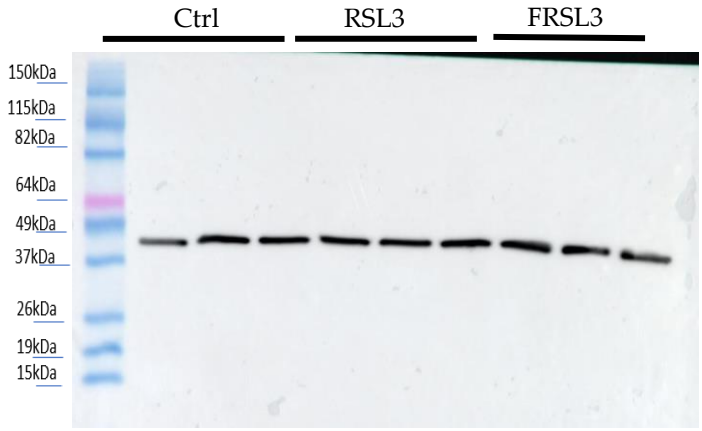
	K27Ac	KSAPATGGVKKPHR	−0,40	−0,56	−0,72	−0,56	$1,38 \times 10^{-1}$	$6,21 \times 10^{-2}$	$2,19 \times 10^{-2}$	$8,62 \times 10^{-2}$
	K37Me3	KSAPATGGVKKPHR	−0,91	−2,79	−2,80	−2,03	$7,09 \times 10^{-2}$	$8,88 \times 10^{-4}$	$9,00 \times 10^{-4}$	$5,21 \times 10^{-3}$
	K37Bu	KSAPSTGGVKKPHR	−0,11	0,19	0,17	0,34	$3,94 \times 10^{-1}$	$1,27 \times 10^{-1}$	$1,91 \times 10^{-1}$	$2,02 \times 10^{-3}$
	R40Cit	KSAPATGGVKKPHR	0,12	0,45	0,31	0,66	$4,46 \times 10^{-1}$	$5,00 \times 10^{-4}$	$6,95 \times 10^{-2}$	$3,99 \times 10^{-5}$
	D77Fe	EIAQDFKTDLR	0,25	0,73	0,78	0,62	$2,73 \times 10^{-1}$	$1,79 \times 10^{-4}$	$6,56 \times 10^{-5}$	$5,31 \times 10^{-3}$
	K79But	EIAQDFKTDLR	0,04	0,25	0,11	0,74	$7,54 \times 10^{-1}$	$4,46 \times 10^{-2}$	$3,66 \times 10^{-1}$	$2,48 \times 10^{-4}$
	K79Me3	EIAQDFKTDLR	−0,27	−0,84	−0,80	-1,07	$4,76 \times 10^{-2}$	$1,14 \times 10^{-6}$	$2,40 \times 10^{-6}$	$1,45 \times 10^{-7}$
	R83Cit	EIAQDFKTDLR	0,01	0,22	−0,08	0,94	$9,72 \times 10^{-1}$	$3,56 \times 10^{-1}$	$7,61 \times 10^{-1}$	$3,99 \times 10^{-3}$
	M97Ox	VTIMPKDIQLAR	0,57	1,43	1,39	1,32	$1,36 \times 10^{-2}$	$2,49 \times 10^{-2}$	$2,46 \times 10^{-2}$	$2,48 \times 10^{-2}$
	D100Fe	VTIMPKDIQLAR	0,56	1,57	2,25	1,38	$1,43 \times 10^{-1}$	$2,83 \times 10^{-1}$	$0,35 \times 10^{-1}$	$0,27 \times 10^{-1}$
H4	R17Me	GKGGKGLGKGGAKRHR	−0,17	0,47	0,02	0,53	$5,09 \times 10^{-1}$	$2,53 \times 10^{-2}$	$9,31 \times 10^{-1}$	$1,02 \times 10^{-2}$
	E63Fe	GVLKVFLENVIR	0,37	0,88	0,96	0,60	$4,50 \times 10^{-1}$	$2,32 \times 10^{-2}$	$1,51 \times 10^{-2}$	$0,18 \times 10^{-1}$
	M84Ox	KTVTAMDVVYALKR	0,12	0,29	0,29	0,28	$2,46 \times 10^{-1}$	$3,92 \times 10^{-3}$	$3,81 \times 10^{-3}$	$7,12 \times 10^{-3}$
	D85Fe	KTVTAMDVVYALKR	0,09	0,57	0,54	0,52	$5,36 \times 10^{-1}$	$1,56 \times 10^{-5}$	$1,23 \times 10^{-4}$	$4,66 \times 10^{-4}$

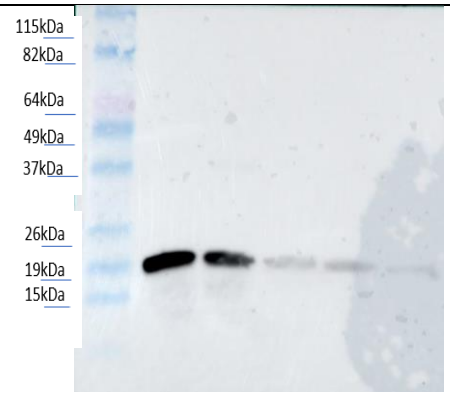
Table S2. Overview of primers used in this study.

Target		Primer Sequence	Gene Accession Number
		qPCR primers	
CHAC1	Forward	ATG CCT GGC CGT GTG G	ENSG00000128965
	Reverse	GCT TAC CTG CTC CCC TTG C	
HSPB1	Forward	AGG ATG GCG TGG TGG AGA T	ENSG00000106211
	Reverse	GAT GTA GCC ATG CTC GTC CTG	
SLC7A11	Forward	CAC ATG CCT CTT CAT GGT TG	ENSG00000151012
	Reverse	AGT GAT GAC GAA GCC AAT CC	
HMOX1	Forward	CCA GCG GGC CAG CAA CAA AGT GC	ENSG00000100292
	Reverse	AAG CCT TCA GTG CCC ACG GTA AGG	
ACTB	Forward	CTG GAA CGG TGA AGG TGA CA	ENSG00000075624
	Reverse	AAG GGA CTTC CTG TAA CAA TGC A	
NR4A2	Forward	GGT CCC TTT TGC CTG TCC A	ENSG00000153234
	Reverse	TGG CTT CAG CCG AGT TAC AG	
		Methylation-specific PCR primers	
SALL3	Forward	GTT TGG GTT TGG TTT TTG TT	ENSG00000263310

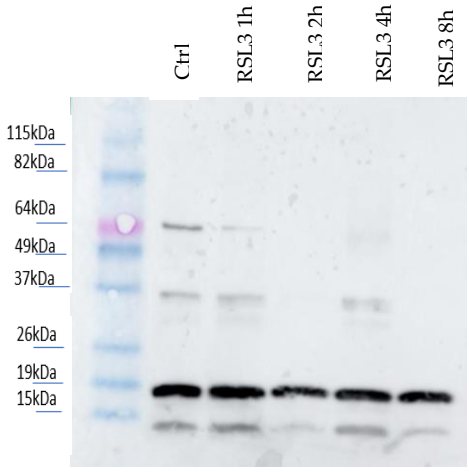
	Reverse	ACC CTT TAC CAA TCT CTT AAC TTT C
		Pyrosequencing primers
LINE-1	Forward	TTT TTT GAG TTA GGT GTG GG
	Reverse	TCT CAC TAA AAA ATA CCA AAC AA
	Sequencing	GGG TGG GAG TGA T

Table S3. Original images western blot analysis.

Figure	**Blot(s) with molecular marker**
7: Western blot detection and quantification of NR4A2 and GAPDH protein expression levels after RSL3 treatment in MM1 cells	α-NR4A2 (70 kDa) and α-GAPDH (37 kDa) *marker:* Benchmark Pre-stained protein standard (Thermo, #10748-010) α-NR4A2  α-GAPDH 
S5a: Western blot detection and quantification of H3K27ac, H3K36me, and H3 protein expression levels after RSL3 treatment in MM1R cells	α-H3K27ac (15 kDa), and α-H3 (11 kDa) *marker:* Precision Plus Protein All Blue prestained protein standard (Bio-Rad, #1610373) α-H3K27ac Ctrl RSL3 1h RSL3 2h RSL3 4h RSL3 8h

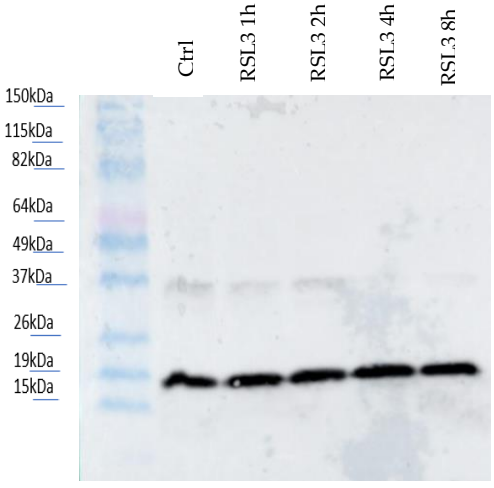


α-H3

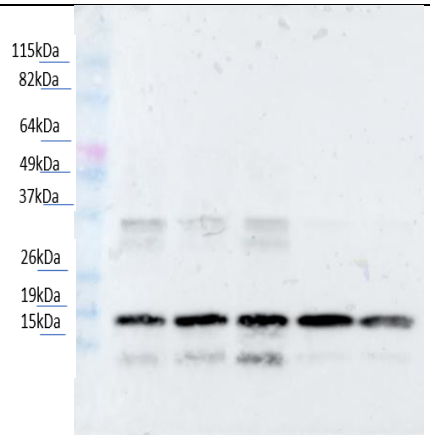
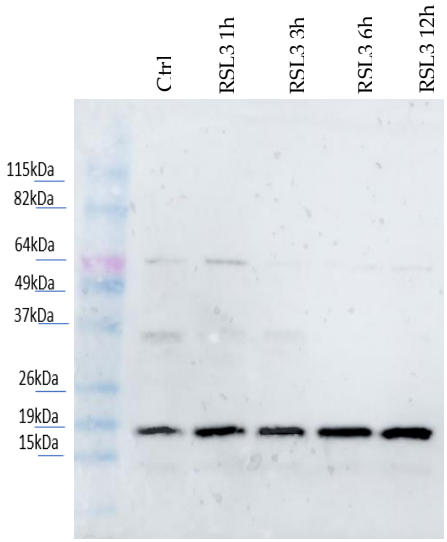


α-H3K36me (full length kDa, and α-H3 (11 kDa)
marker: Precision Plus Protein All Blue prestained protein standard (Bio-Rad, #1610373)

α-H3K36me



α-H3

	
S5b: Western blot detection and quantification of pH2AX and H3 protein expression levels after RSL3 treatment in MM1R cells	α-pH2AX (15 kDa), and α-GAPDH (37 kDa) *marker:* Precision Plus Protein All Blue prestained protein standard (Bio-Rad, #1610373) α-pH2AX  α-GAPDH