Table S1. False discovery rate, number of PTMs identified and sequence coverage in WT and Δ*TXR1* cells

FDR (identity, %)	0.64(0.51)		
FDR (homology, %)	2.36(0.38)		
H3 isoforms	H3	H3.3	H3.4
Sequence Coverage	76%	76%	76%
# Peptides Statistically Quantified*	42	22	N/A
# PTMs Identified	20	10	2
# PTMs Statistically Quantified*	12	6	0

*: number of peptides/PTMs statistically quantified means that those peptides are presented at least twice in replicate wild-type or knockout cells.

Table S2. Summary of valid diagnostic peptides and PTMs quantified in variant H3.3 for *TXR1* mutant.

start	end	Sequence	Modifications	z	WT/¹⁵N WT Mean (SD)	*TXR1*/¹⁵N WT Mean (SD)	p-value*	Final ratio *TXR1*/WT	Normalized *TXR1*/WT
							Protein-level Ratio**		
H3.3					0.78 (0.09)	1.41(0.15)	0.007	1.81	2.53
		Sequence	Modifications	z			Peptide-level Ratios		
9	17	KSTGVKAPR	Pr(N-term,K9,K14)	1	0.57 (0.14)	0.72 (0.06)	0.199274	1.26	2.01
18	26	KQLATKAAR	Pr(N-term, K23)	2	0.64 (0.15)	1.06 (0.07)	0.023676	1.66	2.38
18	26	KQLATKAAR	Ac(K18,K23);Pr(N-term)	2	0.99 (0.03)	0.96 (0.11)	0.677255	0.97	1.35
18	26	KQLATKAAR	Ac(K23); Pr(N-term,K18)	1	2.29 (1.49)	0.8 (0.03)	0.39201	0.35	0.56
				2	1.20 (0.05)	0.96 (0.17)	0.118136	0.8	1.11
18	26	KQLATKAAR	Pr(N-term,K18,K23)	1	0.47 0.13)	0.82 (0.04)	0.033675	1.76	2.5
				2	0.59 (0.14)	1.05 (0.06)	0.020046	1.78	2.53
27	49	KSAPVSGGVKKPHKFRPGTVALR	Pr(N-term,K36,K37,K40)	3	0.83 (0.02)	3.81 (0.1)	0.000174	4.62	6.5
				4	0.84 (0.01)	3.89 (0.03)	1.80E-06	4.66	6.59
27	49	KSAPVSGGVKKPHKFRPGTVALR	Me1(K27);Pr(N-term,K36,K37,K40)	3	0.73 (0.15)	0.2 (0.02)	0.025915	0.27	0.39
27	49	KSAPVSGGVKKPHKFRPGTVALR	Me1(K27);Pr(N-term,K27, K36,K37,K40)	3	0.73 (0.15)	0.22 (0.01)	0.028321	0.3	0.43
27	49	KSAPVSGGVKKPHKFRPGTVALR	Me1(K27);Me1(K36);Pr(N-term, K36,K37,K40)	4	0.55 (0.12)	0.17 (0.09)	0.029351	0.3	0.43
27	49	KSAPVSGGVKKPHKFRPGTVALR	Ac(K37);Me1(K36);Pr(N-term,K36,K40)	3	0.84 (0)	3.86 (0.06)	0.009465	4.6	6.04
				4	0.85 (0.01)	3.89 (0.04)	0.005618	4.58	6.04
53	63	KYQKTTDLLIR	Ac(K56);Pr(N-term)	2	1.25 (0.12)	1 (0.15)	0.093929	0.8	1.11
53	63	KYQKTTDLLIR	Pr(N-term,K56)	2	0.66 (0.14)	1.17 (0.04)	0.018789	1.76	2.49
53	63	KYQKTTDLLIR	Pr(N-term,K1,K56)	2	0.68 (0.13)	1.18 (0.03)	0.01697	1.74	2.77
73	83	DIAMEMKSDIR	Pr(N-term,K79)	2	0.61 (0.19)	1.22 (0.04)	0.027935	2	2.83
116	128	RVTIMTKDLHLAR	Pr(N-term,K122)	2	0.60 (0.25)	1.17 (0.08)	0.047206	1.94	2.77
				3	0.58 (0.23)	1.16 (0.05)	0.042797	2.01	2.87
117	128	VTIMTKDLHLAR	Pr(N-term,K122)	2	0.64 (0.21)	1.27(0.06)	0.027222	1.98	2.8
117	129	VTIMTKDLHLARR	Pr(N-term,K122)	2	0.48 (0.08)	1.11 (0.07)	0.013342	2.33	3.68

* Species with significant p-values (p <0.05) are boxed. The p-value is calculated from peptides which are present at least twice in replicate WT or KO cells. The average CV for peptides from histone H3.3 in WT and *TXR1* knockout cells is 17.9% and 6.3% respectively.

** Protein Ratios are calculated from diagnostic peptides to H3.3.

Table S3. Summary of all PTMs identified in WT and knockout cells

Start	End	Sequence	Modifications	z	Observed	ΔM*	Ion Score	Intensity	RT(min)
H3 major									
Start	End	Sequence	Modifications	z	Observed	ΔM*	Ion Score	Intensity	RT(min)
3	8	TKQTAR	K4Me1	2	387.7275	1	37	9.46E03	41.1
9	17	KSTGAKAPR	K9Ac	2	542.2835	1	29	5.49E04	53.6
9	17	KSTGAKAPR	K14Ac	2	514.2699	0	63	3.51E04	48.4
9	17	KSTGAKAPR	K14Me1	2	556.2968	-3	42	5.96E05	64.6
18	26	KQLASKAAR	K18Ac	2	571.3108	1	47	2.10E05	78.1
18	26	KQLASKAAR	K18Ac, K23Ac	2	556.8246	0	56	3.10E05	74.5
18	26	KQLASKAAR	K23Ac	2	563.8319	-1	57	2.31E05	78.1
18	26	KQLASKAAR	K23Me1	2	557.3122	-1	53	3.60E05	61.9
18	26	KQLASKAAR	K23Me2	2	556.8409	-4	28	8.62E05	71.4
18	26	KQLASKAAR	K23Me3	2	571.3286	0	34	2.37E05	69.9
27	40	KSAPATGGIKKPHR	K27Me1	2	815.476	1	83	8.13E05	70.9
27	40	KSAPATGGIKKPHR	K27Me2	2	822.4858	3	96	2.59E04	63.1
27	40	KSAPATGGIKKPHR	K27Me3	2	829.4924	2	62	2.58E04	64.1
27	40	KSAPATGGIKKPHR	K27Ac	2	829.474	1	74	6.57E05	69.3
27	40	KSAPATGGIKKPHR	K27Me1, K36Me1	2	850.4947	-2	86	3.06E04	77.5
27	40	KSAPATGGIKKPHR	K27Me2, K36Me1	2	840.4605	2	72	5.64E04	67.5
27	40	KSAPATGGIKKPHR	K27Me1, K36Me2	2	829.4922	1	67	1.02E05	72.4
27	40	KSAPATGGIKKPHR	K27Ac, K36Me2	2	815.4771	2	62	1.56E04	62.9
27	49	KSAPATGGIKKPHRFRPGTVALR	K27Me1, R40Me2	3	885.8521	-3	59	3.03E05	82.8
27	49	KSAPATGGIKKPHRFRPGTVALR	K27Me1, K36Me1, R40Me2	3	890.5257	-4	64	1.33E05	83.1
27	40	KSAPATGGIKKPHR	K27Me1, K36Me1, K37Me3	2	843.5031	-4	60	9.49E04	80.8
27	40	KSAPATGGIKKPHR	K36Me1	2	843.4901	2	83	1.46E05	74.3
27	40	KSAPATGGIKKPHR	K36Me2	2	794.4721	3	55	1.23E05	63.3
53	63	KYQKSTDLLIR	K56Me1	2	745.9297	-4	67	1.23E06	84.9
73	83	DIAHEFKAELR	K79Me1	2	727.8858	0	57	1.62E05	105.1
73	83	DIAHEFKAELR	R83Me1	2	727.8863	1	54	1.16E05	107.1
H3.3									
9	17	KSTGVKAPR	K9Ac, K14Ac	2	549.2912	1	45	2.86E05	56.4

9	17	KSTGVKAPR	K14Ac	2	556.2992	1	47	8.09E05	65.6
18	26	KQLATKAAR	K18Ac, K23Ac	2	563.8322	-1	56	6.56E05	78.7
18	26	KQLATKAAR	K23Ac	2	570.8404	0	57	1.39E05	93.2
18	26	KQLATKAAR	K23Me2	2	571.329	1	48	5.44E05	73.6
27	49	KSAPVSGGVKKPHKFRPGTVALR	K27Me1	3	885.8548	2	121	3.09E05	84.9
27	49	KSAPVSGGVKKPHKFRPGTVALR	K27Me2	4	668.1464	1	63	6.54E05	79.2
27	49	KSAPVSGGVKKPHKFRPGTVALR	K36Me1	3	904.5299	1	77	1.89E05	88.9
27	49	KSAPVSGGVKKPHKFRPGTVALR	K36Me2	3	871.8516	2	58	3.46E04	79.5
27	49	KSAPVSGGVKKPHKFRPGTVALR	K27Me1, K36Me1	3	890.5257	0	118	1.33E05	83.1
27	52	KSAPVSGGVKKPHKFRPGTVALREIR	K36Me1, K37Ac	3	1013.9262	2	52	1.37E05	85.0
28	49	SAPVSGGVKKPHKFRPGTVALR	K36Ac, K37Me1	3	838.4809	-3	59	2.65E04	84.8
53	63	KYQKTTDLLIR	K56Ac	2	738.9255	1	74	6.51E04	93.1
H3.4									
27	49	KSAPISGGIKKPHKFRPGTVALR	K36Ac	3	904.5299	1	55	1.89E05	89.0
27	49	KSAPISGGIKKPHKFRPGTVALR	K36Me1	3	895.1992	2	41	2.28E04	87.1

*: Mass error is measured as parts per million (ppm).

Table S4. Full list of all ^{15}N H3 peptide ratios (Heavy vs Light)

H3

Start	End	Sequence	Modifications	z	H/L
9	17	KSTGAKAPR	Ac(K14)	2	0.94451
10	17	STGAKAPR	Ac(K14)	2	0.999426
18	26	KQLASKAAR	Ac(K18,K23)	1	0.992764
18	26	KQLASKAAR	Ac(K23)	2	0.994031
18	26	KQLASKAAR	Me2(K23)	2	0.999974
18	26	KQLASKAAR	Me3(K23)	2	0.998306
19	26	QLASKAAR	Ac(K23)	2	0.97774
27	36	KSAPATGGIK	Ac(K27)	1	0.999981
27	36	KSAPATGGIK	Me2(K27)	2	0.98534
27	36	KSAPATGGIK	Me1(K27)	1	0.999974
27	36	KSAPATGGIK	Me1(K27)	2	0.998732
27	36	KSAPATGGIK	Me3(K27)	2	0.991533
28	36	SAPATGGIK		1	0.98837
27	40	KSAPATGGIKKPHR	Ac(K27),Me2(K36)	2	0.992491
27	40	KSAPATGGIKKPHR	Ac(K27),Me1(K36)	2	0.997828
27	40	KSAPATGGIKKPHR	Ac(K27),Me1(K36)	3	0.98475
27	40	KSAPATGGIKKPHR	Me2(K27),Me1(K36)	2	0.996233
27	40	KSAPATGGIKKPHR	Me1(K27),Me2(K36)	2	0.996233
27	40	KSAPATGGIKKPHR	Me1(K27),Me1(K36)	2	0.995498
27	40	KSAPATGGIKKPHR	Me1(K27),Me1(K36)	3	0.98896
27	40	KSAPATGGIKKPHR	Me1(K36)	2	0.999973
28	40	SAPATGGIKKPHR	Me2(K36)	2	0.991499
28	40	SAPATGGIKKPHR	Me1(K36)	2	0.998617
28	40	SAPATGGIKKPHR	Me1(K36)	3	0.98299
43	49	PGTVALR		1	0.997312
70	83	LVRDIAHEFKAELR		2	0.9886
70	83	LVRDIAHEFKAELR		3	0.99698
73	79	DIAHEFK		1	0.998278
73	79	DIAHEFK		2	0.992109
73	83	DIAHEFKAELR		1	0.999972
73	83	DIAHEFKAELR		2	0.993934
123	128	DMQLAR		1	0.95991
130	135	IRGERF		1	0.997647

H3.3

Start	End	Sequence	Modifications	z	H/L
18	26	KQLATKAAR	Ac(K18,K23)	1	0.99997
18	26	KQLATKAAR	Me2(K23)	2	0.998306
27	36	KSAPVSGGVK	Me2(K27)	1	0.999977
27	36	KSAPVSGGVK	Me2(K27)	2	0.99104
27	36	KSAPVSGGVK	Me1(K27)	1	0.999979
27	36	KSAPVSGGVK	Me1(K27)	2	0.998358
27	36	KSAPVSGGVK		1	0.999959
27	36	KSAPVSGGVK		2	0.999973
27	40	KSAPVSGGVKKPHK	Me1(K27),Me1(K36)	2	0.999706
28	40	SAPVSGGVKKPHK	Me1(K36)	2	0.997124
57	63	TTDLLIR		1	0.997749

| 57 | 63 | TTDLLIR | 2 | 0.997586 |
| 73 | 79 | DIAMEMK | 1 | 0.98676 |

Table S5. Peptides used in PCA analysis of 16 PTM sites in H3 and H3.3

Peptide ID	Modifications	WT	*TXR1*	p-value	*EZL2*	p-value	*TXR1*/WT	*EZL2*/WT
1	H3K4Me1	0.61(0.06)	1.09(0.24)	0.01503	1.36(N/A)	N/A	1.78	2.21
2	H3K14Un	0.68(0.04)	0.61(0.12)	0.022982	0.83(0.02)	0.88193	0.91	1.23
3	H3K14Ac	1.17(0.31)	1.45(0.22)	0.538428	1.22(N/A)	N/A	1.24	1.05
4	H3K18Ac	0.82(0.04)	1.11(0.16)	0.669132	0.88(N/A)	N/A	1.35	1.07
5	H3K18AcK23Ac	0.95(0.05)	1.34(0.19)	0.872236	1.12(0.22)	0.967862	1.41	1.18
6	H3K23Ac	0.83(0.04)	1.11(0.15)	0.685553	0.95(0.13)	0.394129	1.35	1.15
7	H3K23Me1	0.52(0.13)	1.32(0.25)	0.019933	1.55(0.47)	0.006643	2.52	2.97
8	H3K18UnK23Un	1(0.06)	1.11(0.21)	0.126337	1.15(0.08)	0.967352	1.12	1.16
9	H3K27Ac	0.82(0.08)	1.23(0.21)	0.37916	0.94(0.29)	0.759725	1.5	1.15
10	H3K27Me1*	1.12(0.05)	0.21(0.03)	4.17E-05	1.94(0.27)	0.000358	0.18	1.73
11	H3K27Me2	0.81(0.06)	0.46(0.07)	0.000824	0.13(0.01)	0.001446	0.56	0.16
12	H3K27Me3	0.52(0.02)	0.47(0.1)	0.040226	0.1(0.024)	0.000263	0.92	0.2
13	H3K27Me1K36Me1	0.65(0.07)	0.07(0.02)	0.004257	0.78(0.19)	0.864776	0.11	1.2
14	H3K27Me2K36Me1	0.48(0.06)	0.49(0.01)	0.160328	0.50(0.15)	0.606316	0.86	0.94
15	H3K27UnK36Un	1.83(0.38)	5.7(0.91)	0.020405	2.37(0.61)	0.61335	3.12	1.3
16	H3K56Un	1.03(0.12)	1.15(0.16)	0.093877	1(N/A)	N/A	1.11	0.97
17	H3K56Me1	0.55(0.11)	1.37(0.29)	0.019107	0.85(N/A)	N/A	2.49	1.54
18	H3R83Un	0.94(0.2)	1.18(0.17)	0.569269	1.1(0.01)	0.97823	1.26	1.17
19	H3R83Me1	0.96(0.04)	1.12(0.06)	0.675039	1.02(N/A)	N/A	1.17	1.07
20	H3.3K18AcK23Ac	0.82(0.04)	1.11(0.16)	0.677255	0.95(0.14)	0.008598	1.35	1.16
21	H3.3K23Ac	1(0.06)	1.1(0.2)	0.118136	1.16(0.08)	0.25722	1.11	1.16
22	H3.3K18UnK23Un	0.49(0.11)	1.23(0.32)	0.020046	1.59(0.48)	0.006213	2.53	3.26
23	H3.3K56Ac	1.04(0.12)	1.15(0.16)	0.093929	1.21(N/A)	N/A	1.11	1.17

*: The peptide, $_{(2pr/me1)}K^{27}SAPATGGI_{pr}K^{36}{}_{pr}K^{37}PHR$, was used to calculate the ratio of K27Me1 as it was present in both $\Delta TXR1$ and $\Delta EZL2$ cells.

Note: Ratios in ths table were normalized as described in the Materials and Methods. Species with significant p-values (p <0.05) are boxed.

Appendix:
R code for PCA and Biplot analysis:

```
PTM=read.table("H3PTM.txt",header=T)
#PCA analysis
pca.ptm<-princomp(PTM[,-1], scale=TRUE, center=TRUE, cor=F)
summary(pca.ptm)
loadings(pca.ptm)

#Print cummulative proportion of variance explained
cumprop=cumsum(pca.ptm$sdev^2)/sum(pca.ptm$sdev^2)
print(cumprop,digits=2)

#Screeplot
plot(pca.ptm, main="Screeplot of histone PTM data")

# Data projected on the first two PCs
plot(pca.ptm$scores[,1:2],type="n",xlab="1st PC",ylab="2nd PC")
text(pca.ptm$scores[,1:2],rownames(PTM))
title("Projection of PTM data to the subspace spanned by the first 2 PCs")

#Biplot of histone PTM data
biplot(pca.ptm)
title("Biplot of histone PTM data")
```