

Supplementary Figure legends

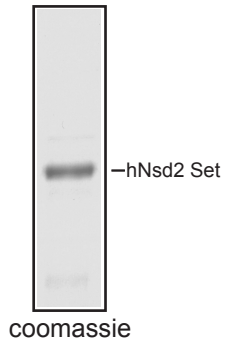
Figure S1. Coomassie blue staining of histone methyltransferases used in this study. (A) Recombinant human NSD2 Set domain; (B) Recombinant human Ash1 Set domain; (C) Reconstituted *Drosophila* PRC2 complex.

Figure S2. Mass spectra of all peptides listed in Fig. 1C.

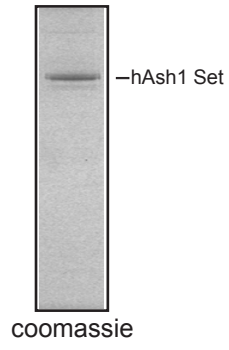
Figure S3. MS/MS spectra of all H3 peptides carrying various methylation status at K27 and K36.

Figure S4. (A) H3Kc36me2 and H3Kc36me3 inhibits PRC2 activity; (B) H3Kc27me3 does not affect HYPB's activity; (C) H3K36A mutation reduces PRC2 activity; (D) The Set domain of Ash1 is more closely related to H3K36 methyltransferases like HYPB or Mes4 than H3K4 specific methyltransferase such as Set1 or Trithorax; (E) H3Kc27me3 does not affect Ash1's activity.

A



B



C

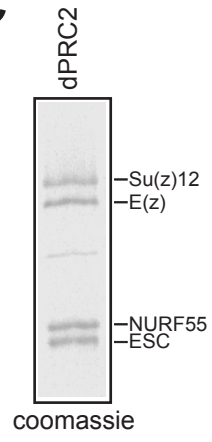


Figure S1

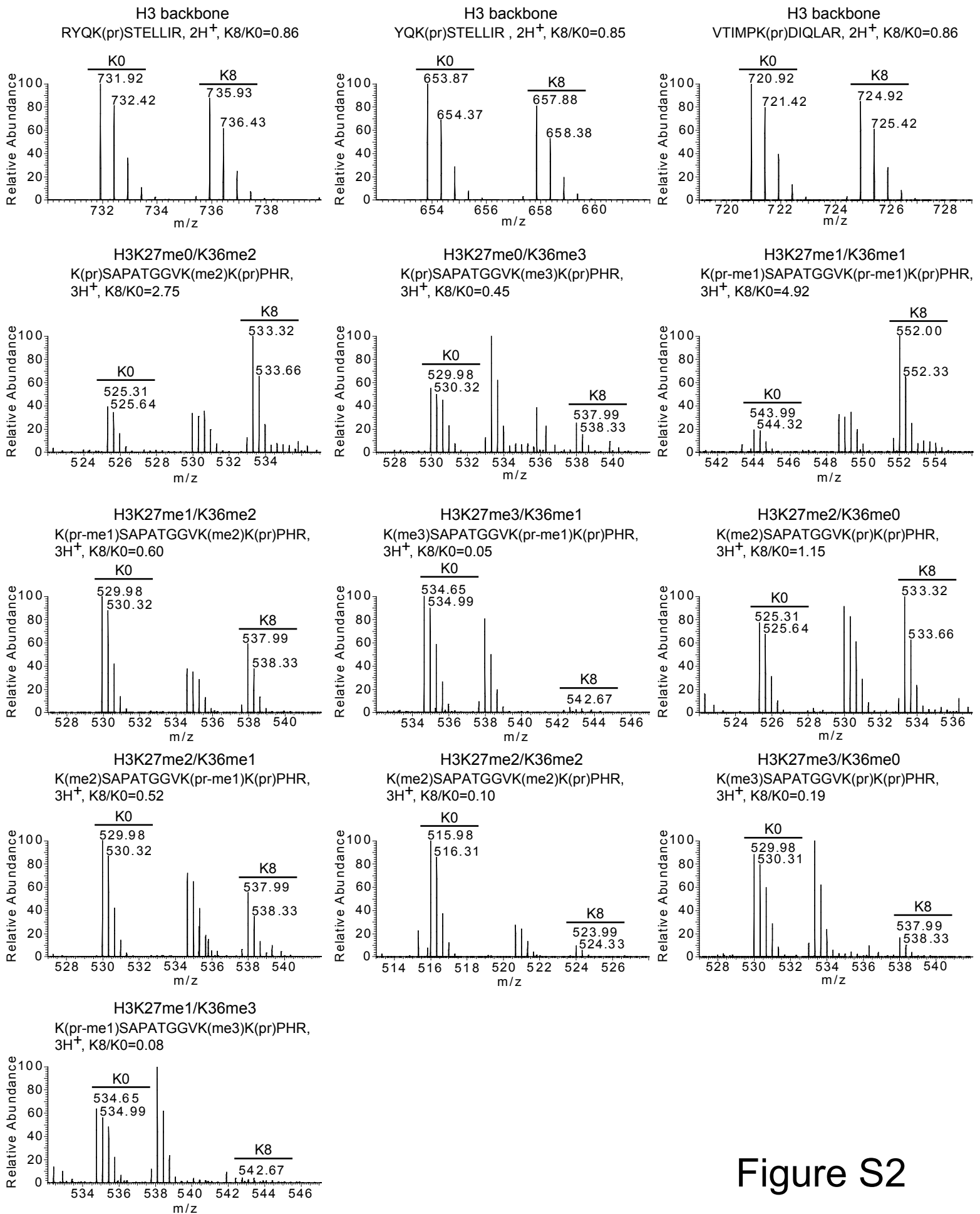
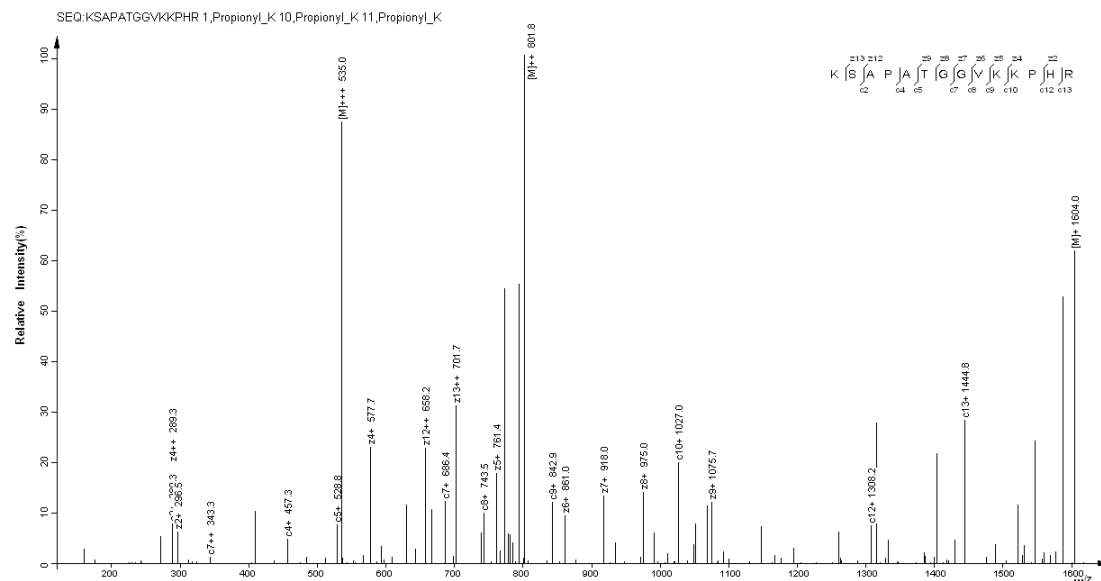
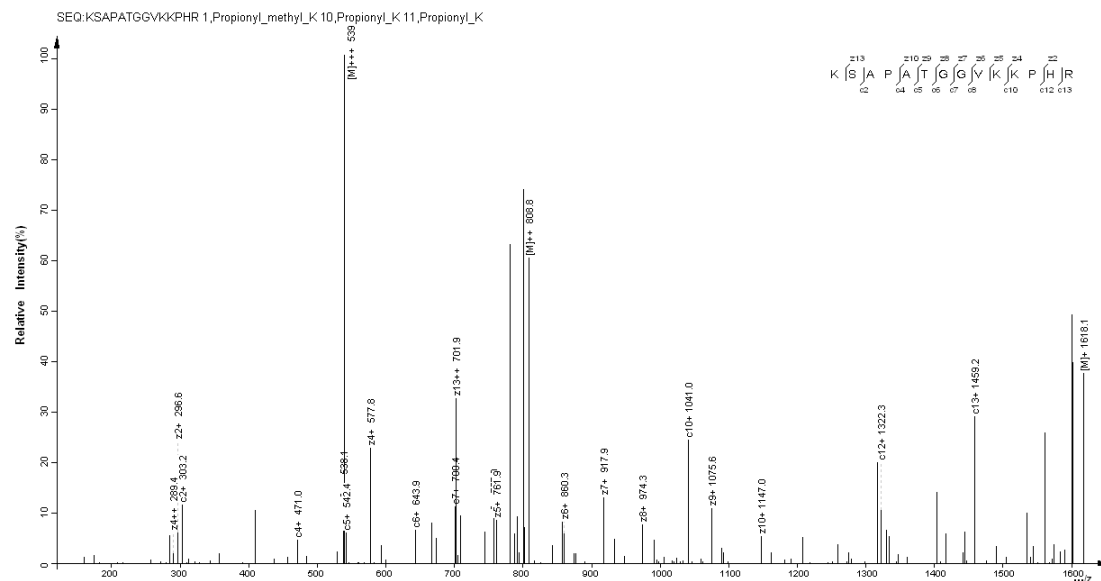


Figure S2

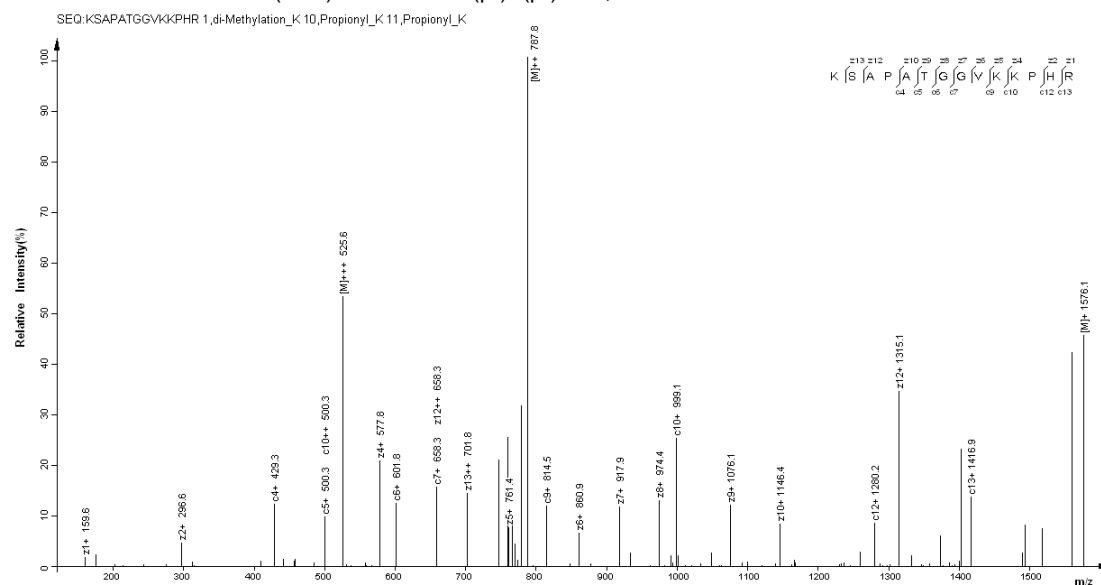
H3K27me0/K36me0: K(pr)SAPATGGVK(pr)K(pr)PHR, 3H⁺



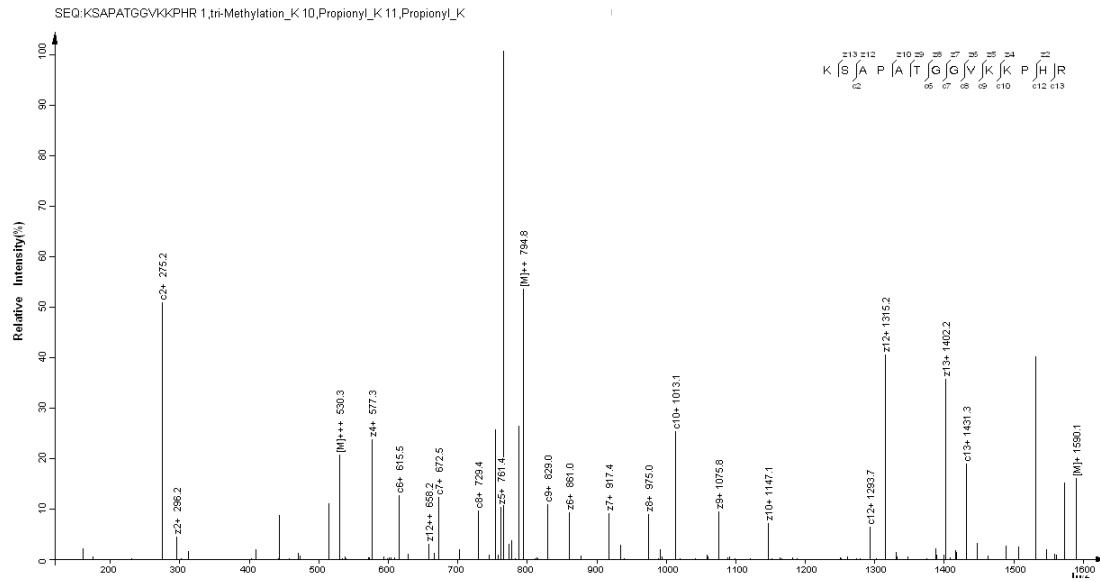
H3K27me1/K36me0: K(pr-me1)SAPATGGVK(pr)K(pr)PHR, 3H⁺



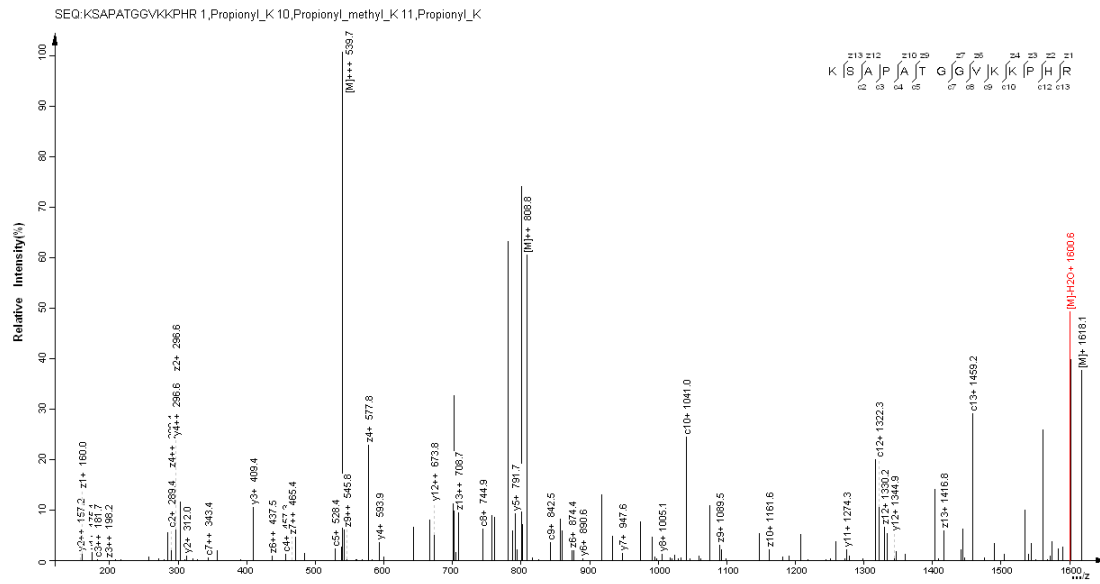
H3K27me2/K36me0: K(me2)SAPATGGVK(pr)K(pr)PHR, 3H⁺



H3K27me3/K36me0: K(me3)SAPATGGVK(pr)K(pr)PHR, 3H⁺



H3K27me0/K36me1: K(pr)SAPATGGVK(pr-me1)K(pr)PHR, 3H⁺



H3K27me1/K36me1: K(pr-me1)SAPATGGVK(pr-me1)K(pr)PHR, 3H⁺

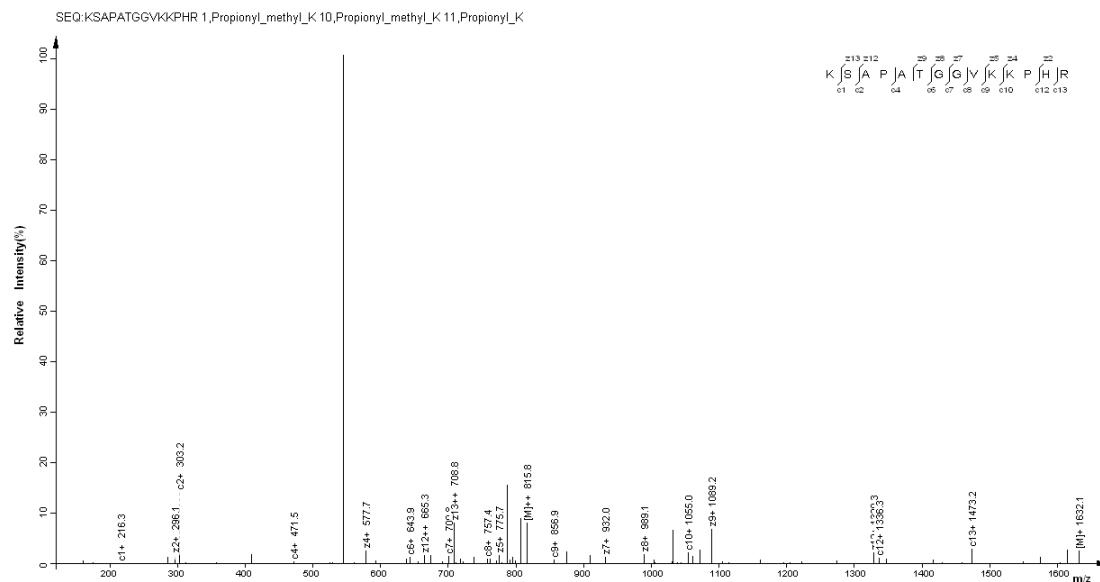
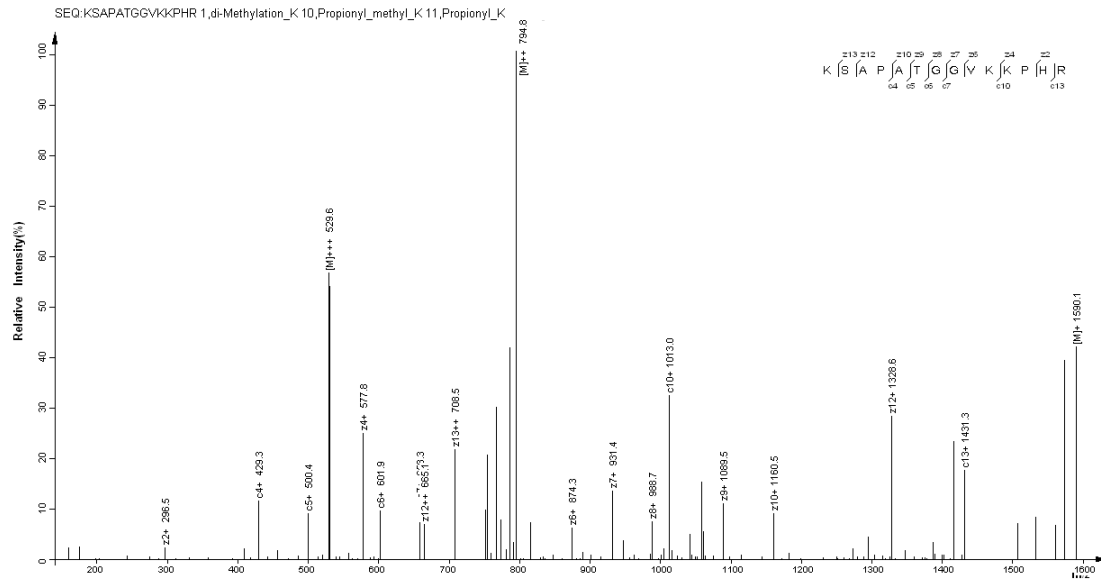
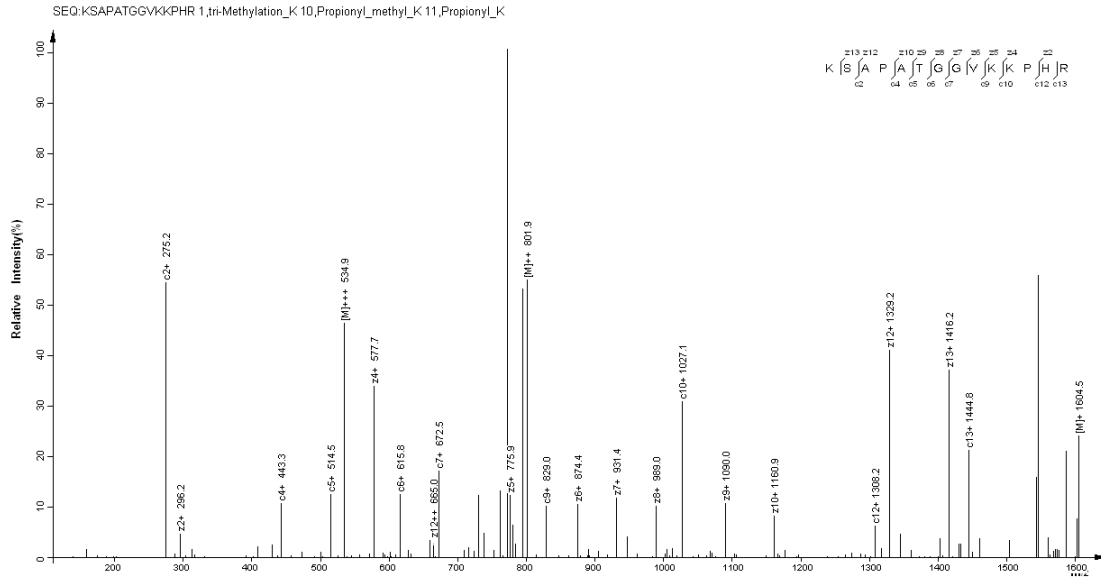


Figure. S3 Page 2

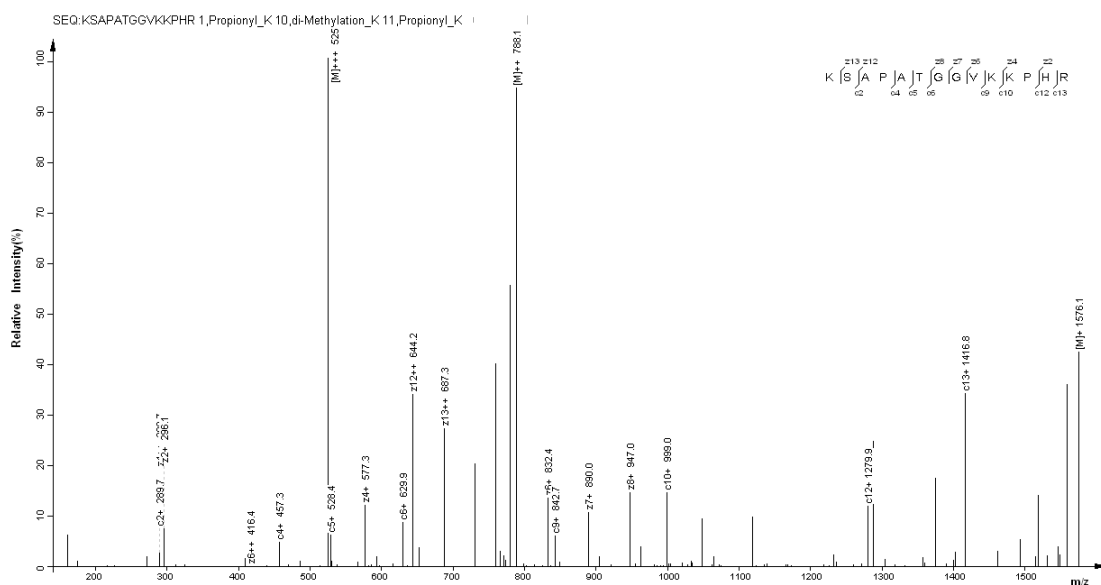
H3K27me2/K36me1: K(me2)SAPATGGVK(pr-me1)K(pr)PHR, 3H⁺



H3K27me3/K36me1: K(me3)SAPATGGVK(pr-me1)K(pr)PHR, 3H⁺

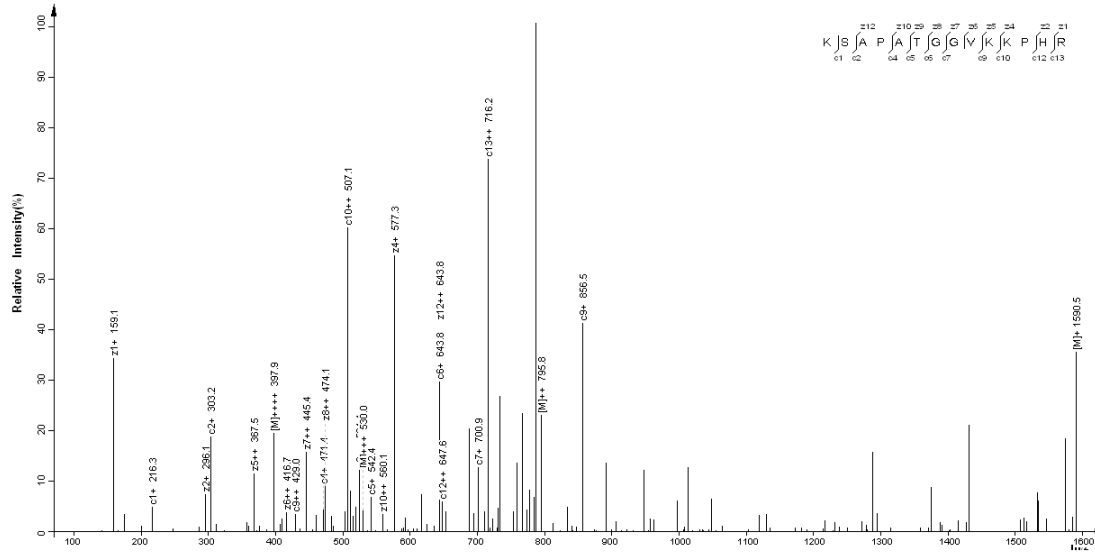


H3K27me0/K36me2: K(pr)SAPATGGVK(me2)K(pr)PHR, 3H⁺



H3K27me1/K36me2: K(pr-me)SAPATGGVK(me2)K(pr)PHR, 3H⁺

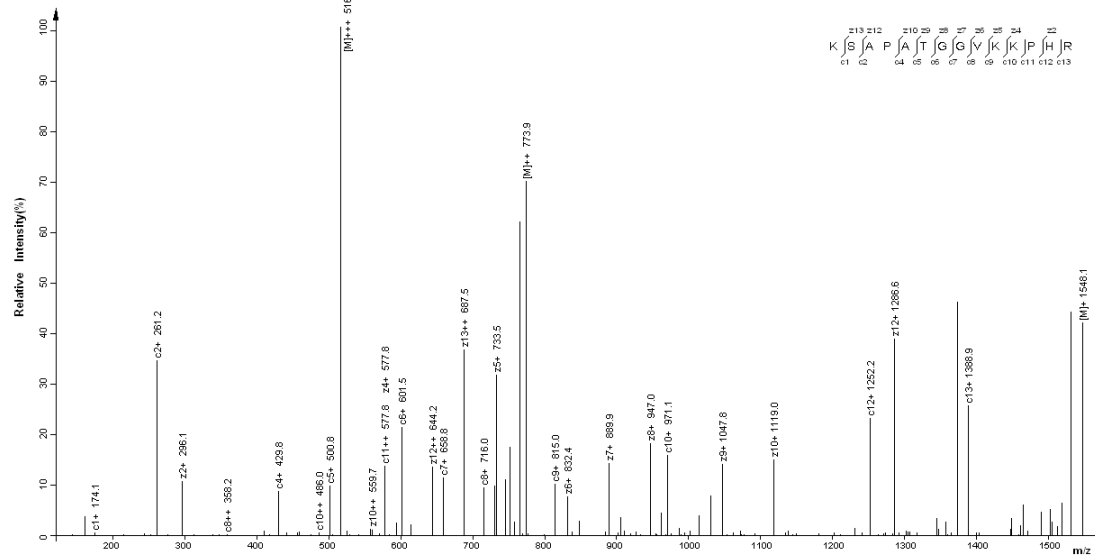
SEQ:KSAPATGGVKKPHR 1,Propionyl_methyl_K 10,di-Methylation_K 11,Propionyl_K



K S A P A T G G V K P H R
 1 2 3 4 5 6 7 8 9 10 11 12 13

H3K27me2/K36me2: K(me2)SAPATGGVK(me2)K(pr)PHR, 3H⁺

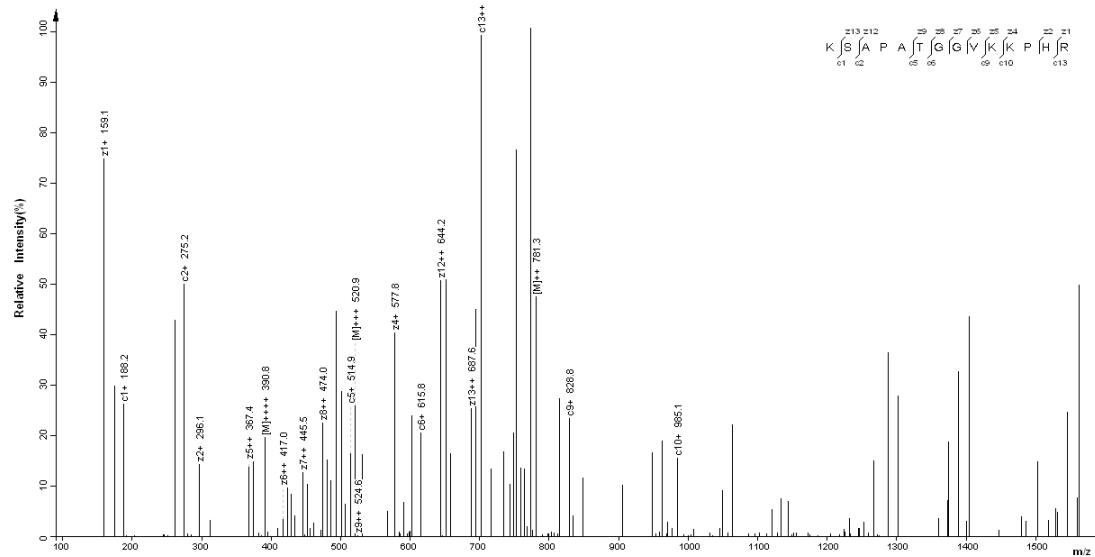
SEQ:KSAPATGGVKKPHR 1,di-Methylation_K 10,di-Methylation_K 11,Propionyl_K



K S A P A T G G V K P H R
 1 2 3 4 5 6 7 8 9 10 11 12 13

H3K27me3/K36me2: K(me3)SAPATGGVK(me2)K(pr)PHR, 3H⁺

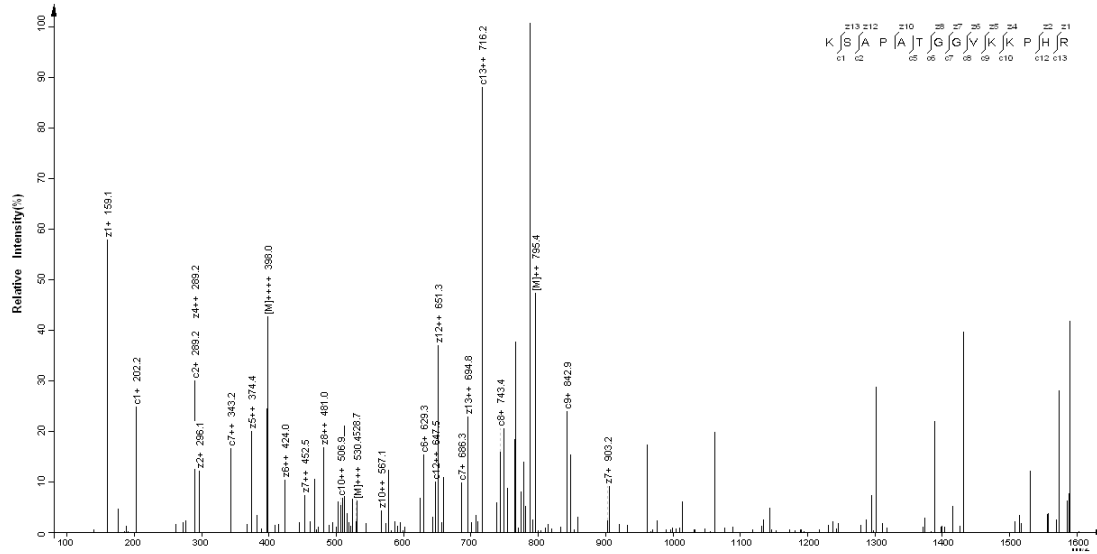
SEQ:KSAPATGGVKKPHR 1,tri-Methylation_K 10,di-Methylation_K 11,Propionyl_K



K S A P A T G G V K P H R
 1 2 3 4 5 6 7 8 9 10 11 12 13

H3K27me0/K36me3: K(pr)SAPATGGVK(me3)K(pr)PHR, 3H⁺

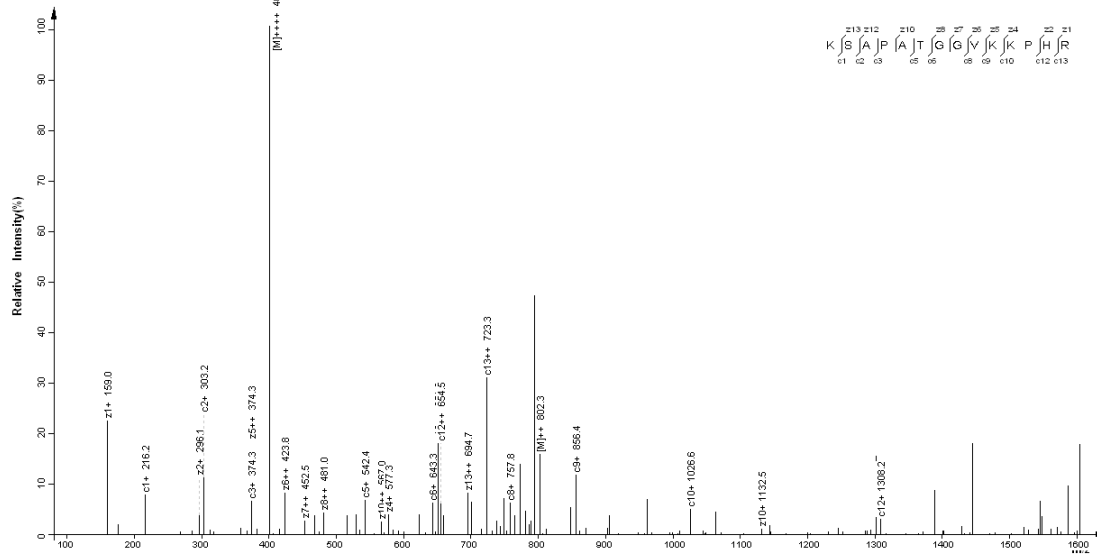
SEQ:K:SAPATGGVK:K:PHR 1,Propionyl_K 10,tri-Methylation_K 11,Propionyl_K



K S A P A T G V K K P H R
 1 2 3 4 5 6 7 8 9 10 11 12 13

H3K27me1/K36me3: K(pr-me1)SAPATGGVK(me3)K(pr)PHR, 3H⁺

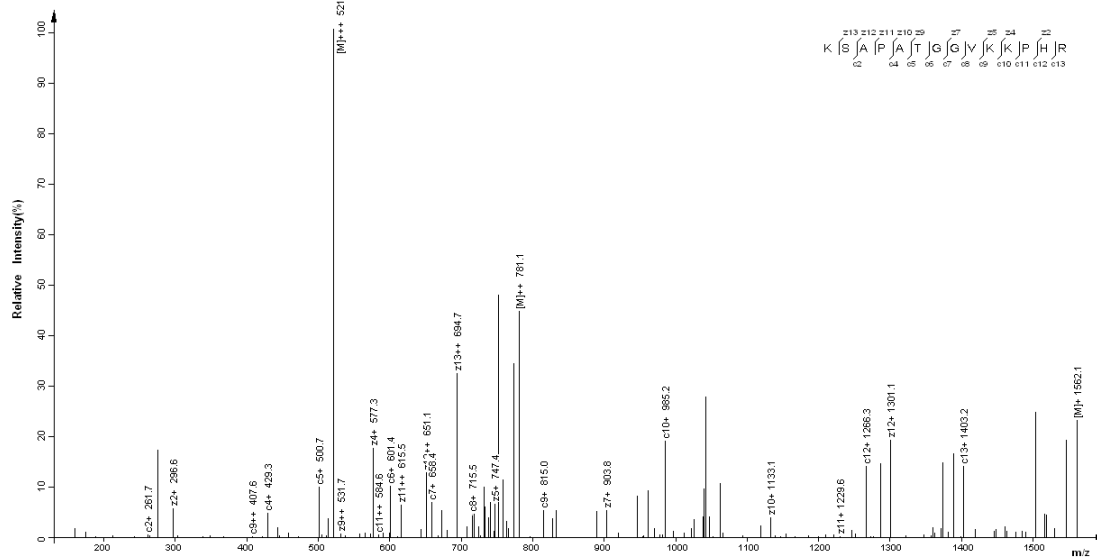
SEQ:K:SAPATGGVK:K:PHR 1,Propionyl_methyl_K 10,tri-Methylation_K 11,Propionyl_K



K S A P A T G V K K P H R
 1 2 3 4 5 6 7 8 9 10 11 12 13

H3K27me2/K36me3: K(me2)SAPATGGVK(me3)K(pr)PHR, 3H⁺

SEQ:K:SAPATGGVK:K:PHR 1,di-Methylation_K 10,tri-Methylation_K 11,Propionyl_K



K S A P A T G V K K P H R
 1 2 3 4 5 6 7 8 9 10 11 12 13

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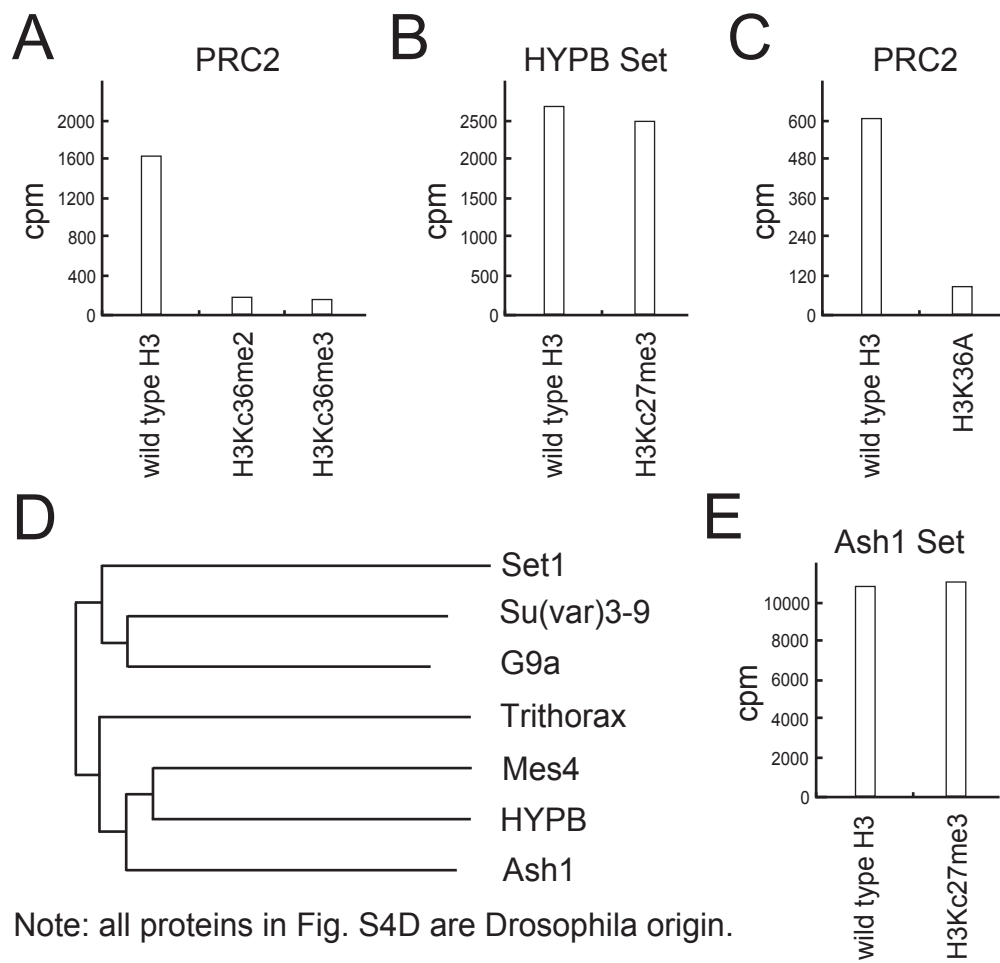


Figure. S4