SETD4 regulates cell quiescence and catalyzes the trimethylation of H4K20 during diapause formation of *Artemia*

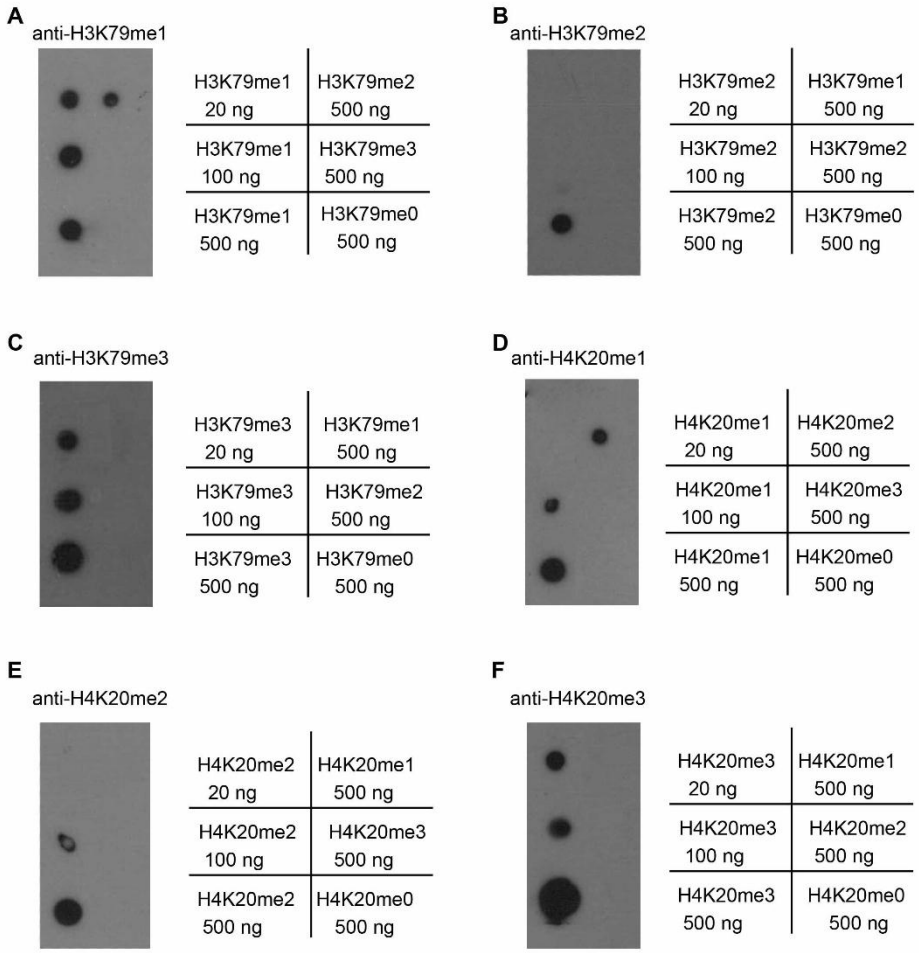
Li Dai,[a] Sen Ye,[a] Hua-Wei Li,[b] Dian-Fu Chen,[a] Hong-Liang Wang,[a] Sheng-Nan Jia,[a] Cheng Lin,[a] Jin-Shu Yang,[a] Fan Yang,[a] Hiromichi Nagasawa,[a,c] Wei-Jun Yang[a,#]

College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang 310058, China[a]; School of Basic Medical Sciences, Zhejiang Chinese Medical University, Hangzhou, Zhejiang 310053, China[b]; Department of Biological Chemistry, The University of Tokyo, Yayoi, Bunkyo, Tokyo 113-8657, Japan[c]

Address correspondence to Wei-Jun Yang [a,#], w_jyang@ zju.edu.cn.

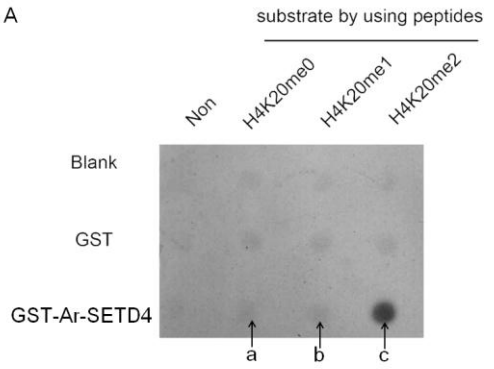
Running title: SETD4 catalyzes the trimethylation of H4K20

Supplemental Material

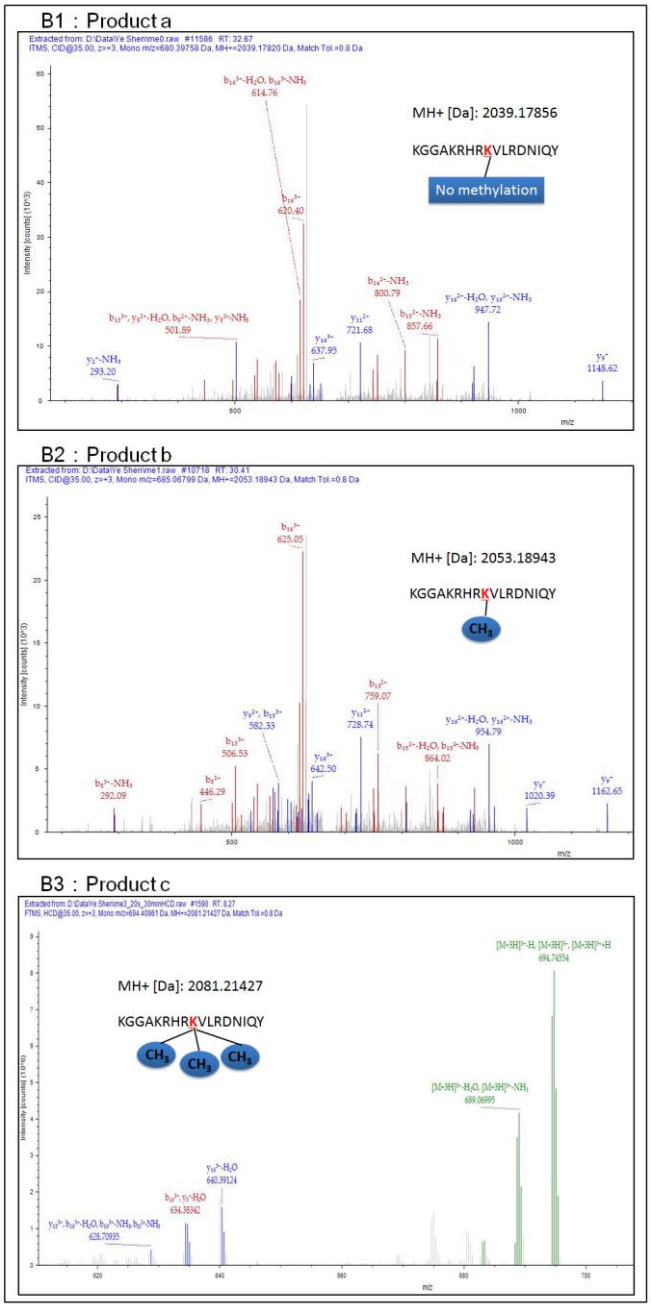


Supplemental Figure S1. Dot blot analysis on the specificity of anti-H3K79me1, anti-H3K79me2, anti-H3K79me3, anti-H4K20me1, anti-H4K20me2 and anti-H4K20me3 antibodies. The peptides of histone H3 (VREIAQDF**K**^{79}TDLRFQSSAV) with various methylation states on K79 (H3K79me0, H3K79me1, H3K79me2, and H3K79me3) and histone H4 (KGGAKRHR**K**^{20}VLRDNIQY) with various methylation states on K20 (H4K20me0, H4K20me1, H4K20me2, and H4K20me3) were synthesized (GL Biochem (Shanghai) LTD., Shanghai, China). The corresponding peptides with serial dilutions (20 ng, 100 ng, 500 ng) were dotted on a PVDF membrane and blotted separately with anti-H3K79me1, anti-H3K79me2, anti-H3K79me3, anti-H4K20me1, anti-H4K20me2, or anti-H4K20me3 antibodies.

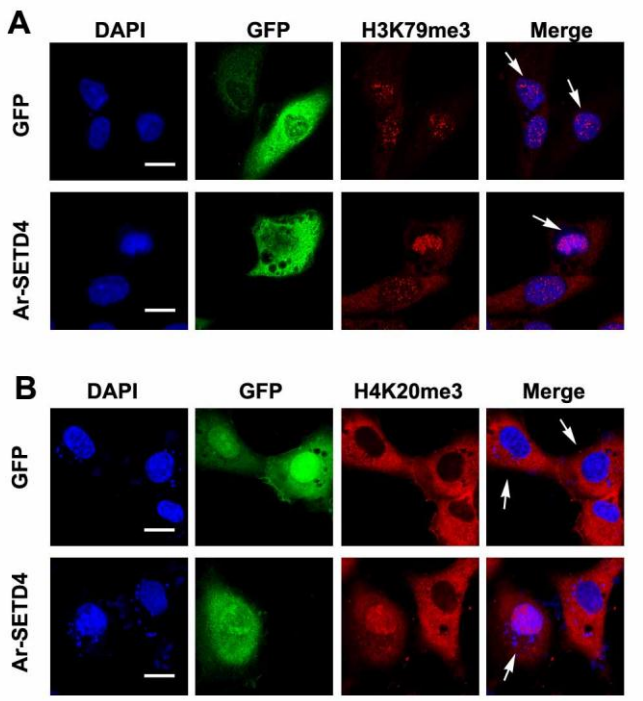
A


substrate by using peptides
Non
H4K20me0
H4K20me1
H4K20me2
Blank
GST
GST-Ar-SETD4
a
b
c

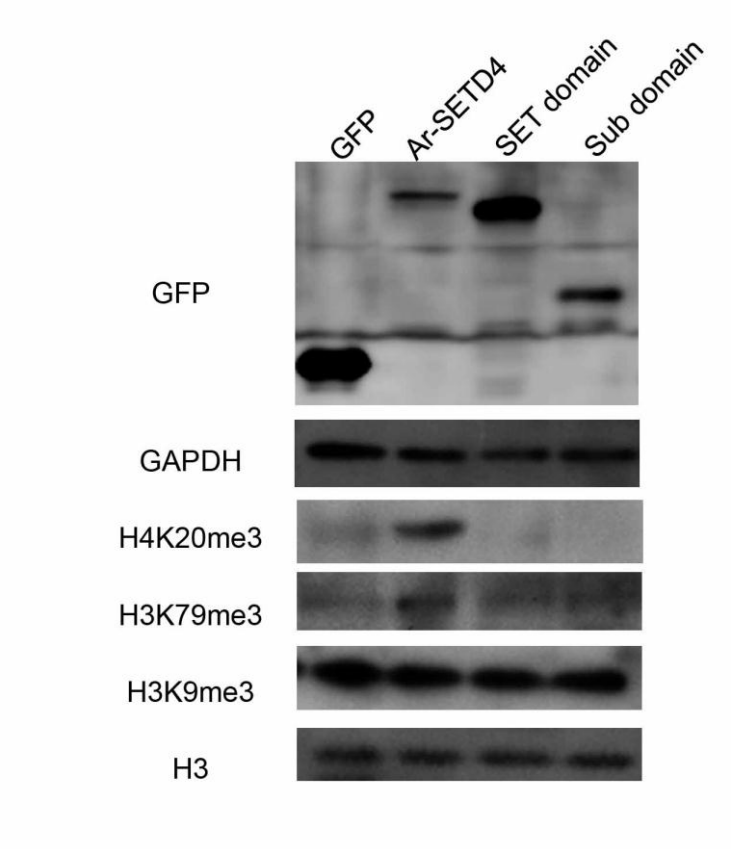
B


B1 : Product a
MH+ [Da]: 2039.17856
KGGAKRHRKVLRDNIQY
No methylation
B2 : Product b
MH+ [Da]: 2053.18943
KGGAKRHRKVLRDNIQY
CH3
B3 : Product c
MH+ [Da]: 2081.21427
KGGAKRHRKVLRDNIQY
CH3
CH3
CH3

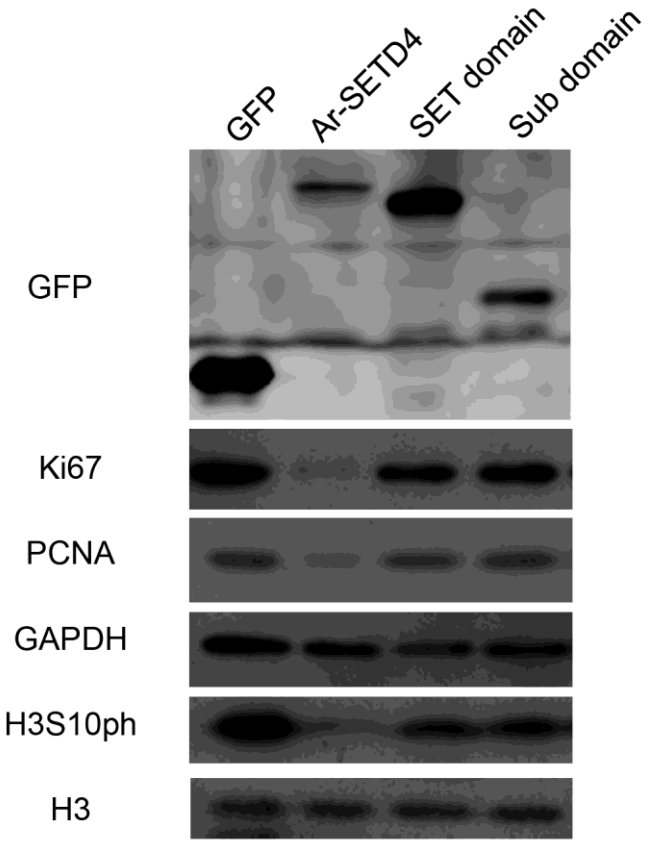
Supplemental Figure S2. Identification of H4K20 trimethylated by Ar-SETD4. A: Dot blot analysis of the products of In vitro methyltransferase assay. Synthesized peptides of H4K20me0, H4K20me1, H4K20me2 and no peptide were used as substrates and catalyzed by GST-Ar-SETD4, GST and blank. The methylation of products deduced from H4K20me0 (a), H4K20me1 (b) and H4K20me2 (c) were examined by anti-H4K20me3 antibody. B: Mass spectrometry analysis of the products of In vitro methyltransferase assay. The methylations of each product (a, b and c) were identified by mass spectrometry analysis (B1, B2 and B3). The X-axis of a mass spectrum represents the mass-to-charge ratio (m/z) and the Y-axis represents the signal intensity of the ions. The calculate MH+ of the product a is 2039.17856 Da (B1), the product b is 2053.18943 Da (B2) and the product c is 2081.21427 Da (B3), which were analyzed by Seguest HT search engine configured with Proteome Discoverer 1.4 workflow (Thermo Fischer Scientific, Bremen, Germany). The calculate MH+ of the product c is 2081.21427 Da and increase 14.02259 Da than 2067.19168 Da of H4K20me2 (the substrate). The results indicated that Ar-SEDT4 catalyzed H4K20me2 into H3K20me3 specifically.



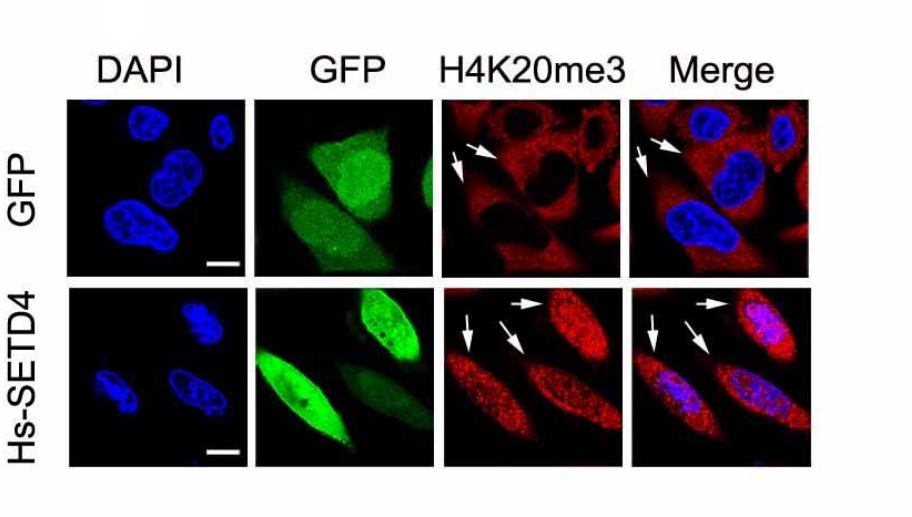
Supplemental Figure S3. Ar-SETD4 mediates the trimethylation of H3K79 and H4K20 in HT1080 cells. HT1080 cells were transfected with pEGFP-C1 (GFP) and pEGFP-C1-Ar-SETD4 (Ar-SETD4) plasmids, and then fixed with methanol (for anti-H3K79me3) or 4% paraformaldehyde (for anti-H4K20me3). Immunofluorescent signals of H3K79me3 (A) and H4K20me3 (B) were detected (one of twenty fields was shown representatively). Note: the signals of anti-H4K20me3 located in the cytoplasm were non-specific. The white arrows indicated the cells with GFP or GFP-Ar-SETD4 overexpression. Green, signals of GFP and Ar-SETD4. Red, H3K79me3 and H4K20me3 detected by Alexa 594-conjugated secondary antibody, respectively. Blue, nuclei counterstained with DAPI. Merge, blue and red signals were merged. Scale bars, 20 μm.



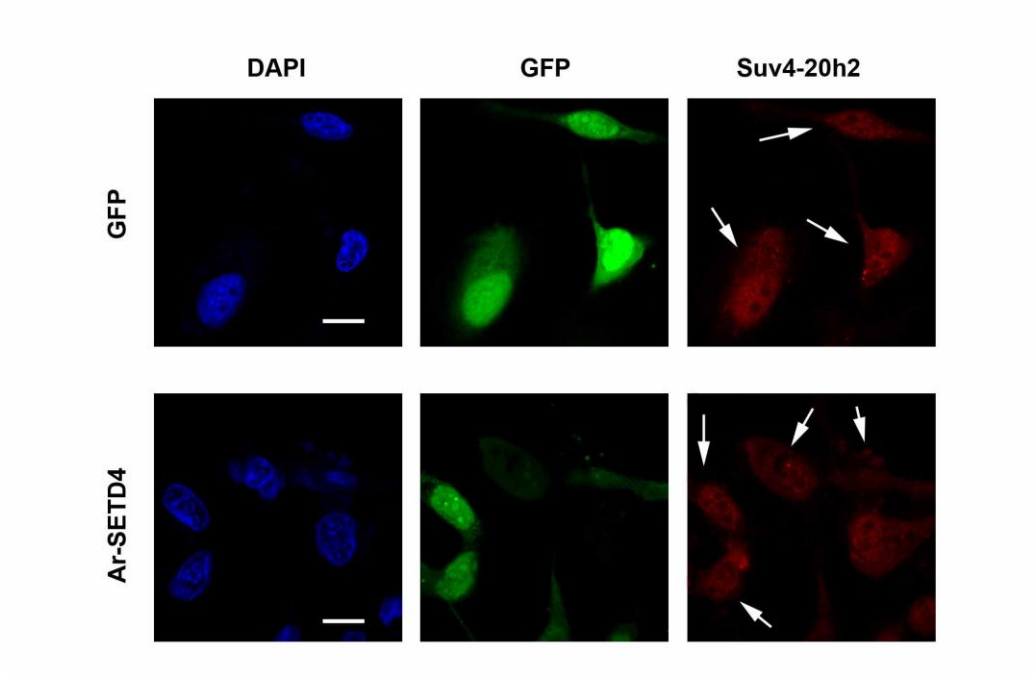
Supplemental Figure S4. Western blot analysis of the levels of H420me3, H3K79me3, and H3K9me3 after Ar-SETD4 and its mutants (SET domain and Sub domain) overexpression in MKN45 cells. GAPDH and H3 were used as loading controls.



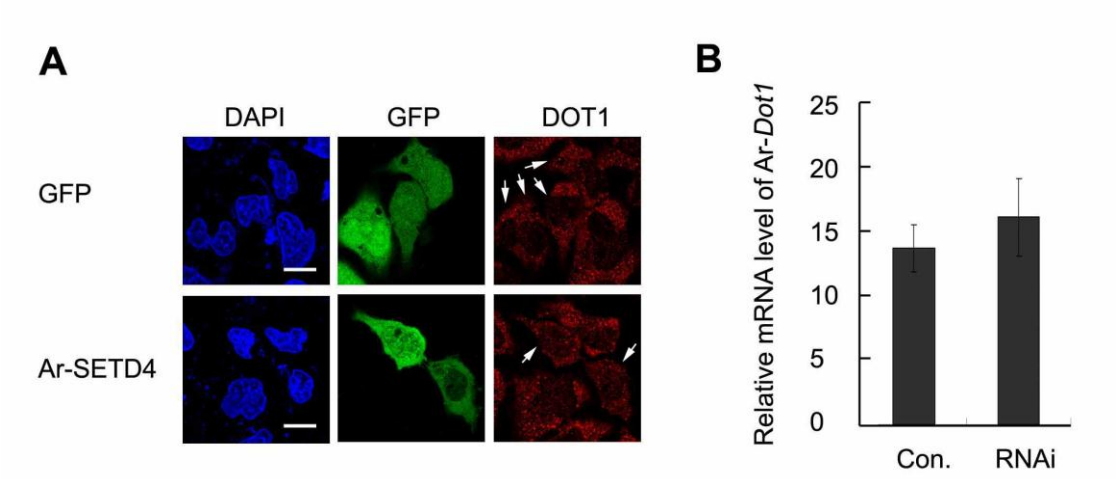
Supplemental Figure S5. Western blot analysis of the cell proliferation marker Ki67, PCNA and H3S10ph after Ar-SETD4 and its mutants (SET domain and Sub domain) overexpression. GAPDH and H3 was used as loading controls.



Supplemental Figure S6. Hs-SETD4 catalyzed the trimethylation of H4K20 in MKN45 cells. MKN45 cells were transfected with pEGFP-C1 (GFP) and pEGFP-C1-Hs-SETD4 (Ar-SETD4) plasmids, and then fixed with 4% paraformaldehyde. Immunofluorescent signals of H4K20me3 was detected (one of twenty fields was shown representatively). Note: the signals of anti-H4K20me3 located in the cytoplasm were non-specific. Green, signals of GFP and GFP-Hs-SETD4. Red, H4K20me3 detected by Alexa 594-conjugated secondary antibody. Blue, nuclei counterstained with DAPI. Merge, blue and red signals were merged. Scale bars, 20 μm.



Supplementary Figure S7. Suv4-20h2 didn't response to SETD4 overexpression in MKN45 cells. MKN45 cells were transfected with pEGFP-C1 (GFP) or pEGFP-C1-Ar-SETD4 (Ar-SETD4) plasmid, and then fixed with 4% paraformaldehyde. Immunofluorescent signals of Suv4-20h2 were detected. The white arrows indicated the cells with GFP or Ar-SETD4 overexpression. Green, signals of GFP and GFP-Ar-SETD4. Red, Suv4-20h2 detected by Alexa 594-conjugated secondary antibody. Blue, nuclei counterstained with DAPI. Scale bars, 20 μm.



Supplementary Figure S8. DOT1 didn't response to SETD4 overexpression in MKN45 cells and SETD4 knockdown in *Artemia*. (A)MKN45 cells were transfected with pEGFP-C1 (GFP) or pEGFP-C1-Ar-SETD4 (Ar-SETD4) plasmid, and then fixed with 4% paraformaldehyde. Immunofluorescent signals of DOT1 were detected. The white arrows indicated the cells with GFP or Ar-SETD4 overexpression. Green, signals of GFP and GFP-Ar-SETD4. Red, DOT1 detected by Alexa 594-conjugated secondary antibody. Blue, nuclei counterstained with DAPI. Scale bars, 20 μm. (B) Real-time quantitative PCR analysis of the expression level of the Ar-*Dot1* mRNA in the embryos produced by the female treated with GFP-specific (Con.) or Ar-SETD4-specific RNAi (RNAi). The mRNA amounts were normalized to those of tubulin mRNA. Data are represented as the mean ± S.D. of replicates (n = 10).