Supplementary Information for

Mechanistic insight into the recognition of single-stranded and double-stranded DNA substrates by ABH2 and ABH3

Baoen Chen, Hongchuan Liu, Xiaoxu Sun, and Cai-Guang Yang*

Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Shanghai 201203, China

Corresponding author:

*Cai-Guang Yang,

Fax: 86-21-50807088;

Tel: 86-21-50806029;

E-mail: yangcg@mail.shcnc.ac.cn

Key words: oxidative demethylation; ABH2; ABH3; structure-activity; cross-linking

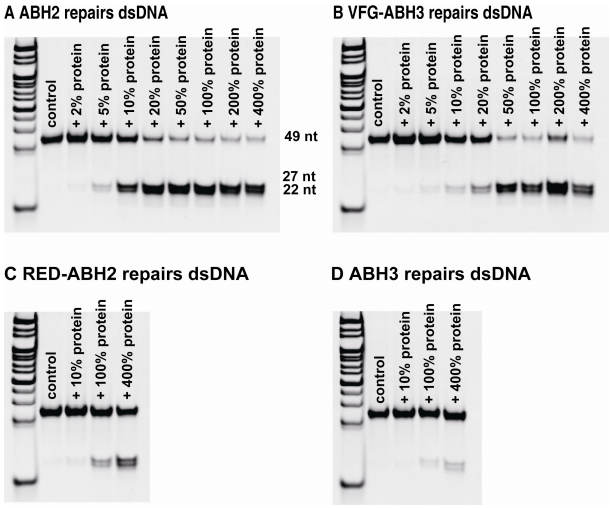

Fig. S1. 1-meA containing dsDNA demethylation by wild-type and swapped ABH2 and ABH3. 100 pmole 1-meA containing dsDNA was incubated with varying amount of full-length proteins at 4 ^{o}C for 1 hour. After 1 hour incubation with *Dpn*II at 37 ^{o}C, the dsDNA sample was subjected to 20% non-reducing PAGE analysis. The high mobility bands are the 22 and 27-mer dsDNA product of *Dpn*II digestion, and the low mobility band is 1-meA containing 49-mer dsDNA substrate. The protein quantity is shown in each lane. PAGE gel analysis pictures are shown for 1-meA repair by wtABH2 (*A*), VFG-ABH3 (*B*), RED-ABH2 (*C*) and wtABH3 (*D*) in dsDNA, respectively.

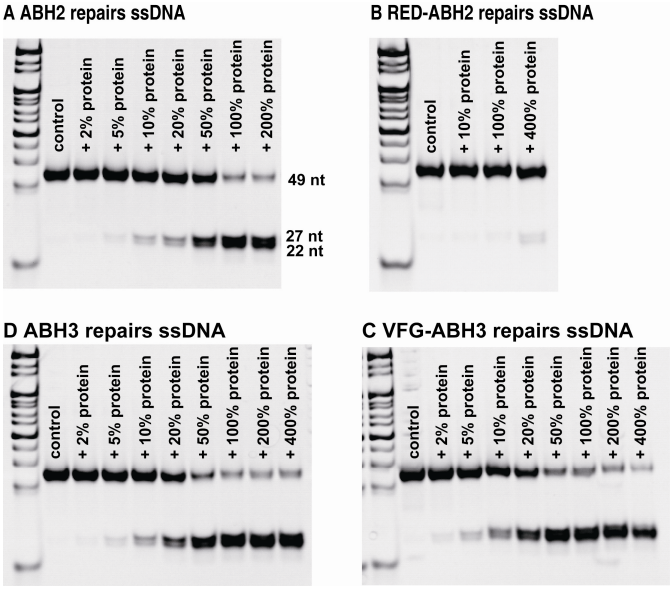

Fig. S2. 1-meA containing ssDNA demethylation by wild-type and swapped ABH2 and ABH3. 100 pmole 1-meA containing ssDNA was incubated in the presence of full-length proteins in varying amount. The ssDNA was then annealed with slightly excess amounts of complementary strand to generate dsDNA for *Dpn*II enzyme digestion. After 1 hour incubation with *Dpn*II at 37 ^{o}C, the dsDNA sample was subjected to 20% non-reducing PAGE analysis. The high mobility bands are *Dpn*II digestion products, 22 and 27-mer dsDNA respectively, and the low mobility band is 1-meA containing 49-mer dsDNA substrate. The protein quantity is shown in each lane. PAGE gel analysis pictures are shown for 1-meA repair by wtABH2 (*A*), RED-ABH2 (*B*), wtABH3 (*C*) and VFG-ABH3 (*D*) in ssDNA, respectively.

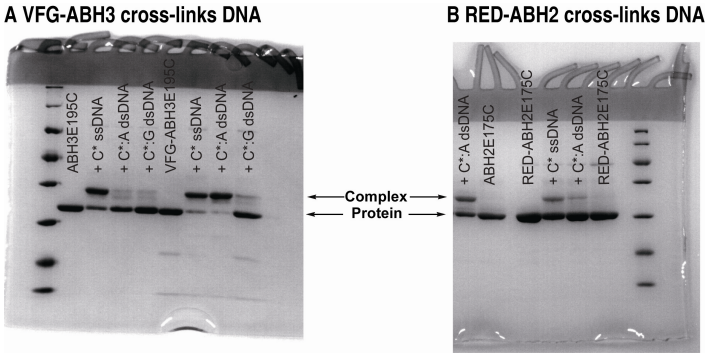

Fig. S3. SDS-PAGE image of swapped ABH2 and ABH3 cross-linking with different DNA. (A) Coomassie blue stained non-reducing SDS-PAGE analysis of the cross-linking reaction between DNA and ABH3 variations. The high mobility band is free protein and the low mobility band is the covalently linked protein-DNA complex. (B) SDS-PAGE analysis of the cross-linking reaction between C*:A-containing dsDNA with ABH2 and RED-ABH2 in protein active site.