

## Supporting information

### **Fusion of the N-terminal domain of bacterial Enzyme I facilitates recombinant expression and purification of the human RNA demethylases FTO and Alkbh5**

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**Nucleotide sequence of the EIN fusion construct used in the present work.** Blocks are connected together as indicated in Figure 2 (see main text).

**NcoI recognition site**

5'-CCATGG-3'

**Spacer**

5'-GCAGCAGC-3'

**Histidine tag**

5'-CATCATCATCATCACCAT-3'

**Spacer**

5'-AGCAGC-3'

**EIN tag**

5'-ATTTTCAGGCATTTTAGCATCCCCGGGTATCGCTTTTCGGTAAAGCTCTGCTTCTGAAAGAA  
GACGAAATTGTCATTGACCGGAAAAAATTTCTGCCGACCAGGTTGATCAGGAAGTTGAAC  
GTTTTCTGAGCGGTCTGTTGCAAGGCATCAGCCCAGCTGGAAACGATCAAACGAAAGCTG  
GTGAAACGTTTCGGTGAAGAAAAAGAAGCCATCTTTGAAGGGCATATTATGCTGCTCGAAGA  
TGAGGAGCTGGAGCAGGAAATCATAGCCCTGATTAAGATAAGCACATGACAGCTGACGC  
AGCTGCTCATGAAGTTATCGAAGGTCAGGCTTCTGCCCTGGAAGAGCTGGATGATGAATAC  
CTGAAAGAACGTGCGGCTGACGTACGTGATATCGGTAAGCGCCTGCTGCGCAACATCCTG  
GGCCTGAAGATTATCGACCTGAGCGCCATTGAGGATGAAGTCATTCTGGTTGCCGCTGAC  
CTGACGCCGTCCGAAACCGCACAGCTGAACCTGAAGAAGGTGCTGGGTTTTCATCACCGAC  
GCGGGTGGCCGTACTTCCCACACCTCTATCATGGCGCGTTCTCTGGAACCTACCTGCTATC  
GTGGGTACCGGTAGCGTCACCTCTCAGGTGAAAAATGACGACTATCTGATTCTGGATGCC  
GTAAATAATCAGGTTTACGTCAATCCAACCAACGAAGTTATTGATAAAATGCGCGCTGTTCA  
GGAGCAAGTGGCTTCTGAA-3'

**TEV recognition sequence**

5'-CATAGCAGCGAAAATCTGTATTTTCAGAGC-3'

**NdeI recognition site**

5'-CATATG-3'

**Alkbh5**

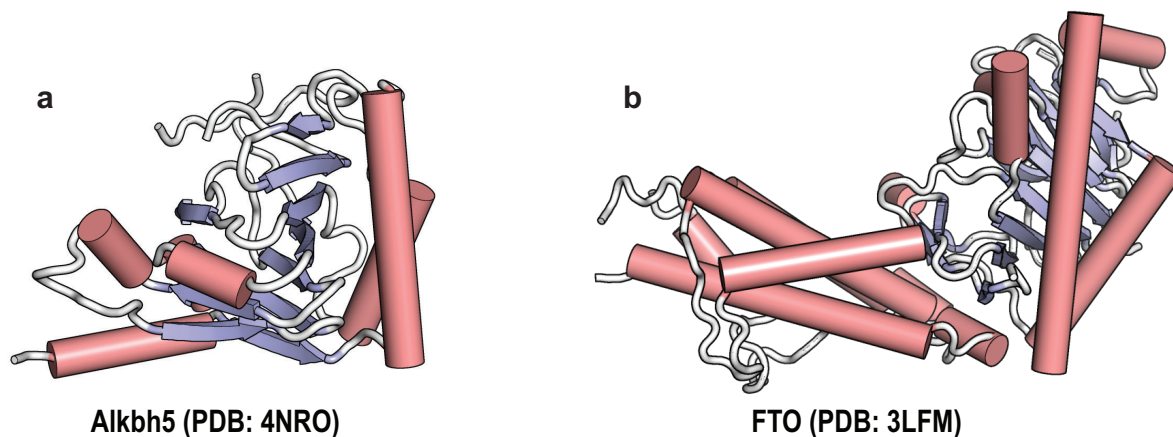
5'-CCGGAACGTAGCGATTATGAAGAACAGCAGCTGCAGAAAGAAGAAGAAGCACGTAAAG  
TTAAAAGCGGTATTCGTCAGATGCGTCTGTTTAGCCAGGATGAATGTGCAAAAATTGAAGC  
CCGTATTGATGAAGTTGTTAGCCGTGCAGAAAAAGGCCTGTATAATGAACATACCGTTGAT  
CGTGCACCGCTGCGCAATAAATACTTTTTTGGTGAAGGTTATACCTACGGTGCACAGCTGC  
AAAAACGTGGTCCGGGTCAAGAACGTCTGTATCCGCCTGGTGTGTTGATGAAATTCGGAA  
ATGGGTTTCATCAGCTGGTTATTCAGAACTGGTTGAACATCGTGTATTCCGGAAGGTTTTG  
TTAATAGCGCAGTGATCAATGATTATCAGCCTGGTGGTTGTATTGTGAGCCATGTTGATCC  
GATTCATATTTTTGAACGTCCGATTGTTAGCGTGAGCTTTTTTAGCGATAGCGCACTGTGTT  
TTGGTTGCAAATTTTCAGTTTTAAACCGATTCTGTGTTAGCGAACCGGTTCTGAGCCTGCCGGT  
TCGTGTTGGTAGCGTTACCGTTCTGAGCGGTTATGCAGCAGATGAAATTACCCATTGTATT  
CGTCCGCAGGATATCAAAGAACGTTCGTGCAGTTATTATTCTGCGTAAAACCCGTCTGGATG  
CACCGCGTCTGTAA -3'

**FTO**

5'-ACCCCGAAAGACGACGAATTTTACCAGCAATGGCAACTGAAGTACCCGAAACTGATCCT  
GCGTGAAGCATCGAGCGTGAGCGAAGAAGTTC AAGAAGCATTCTGAC  
CCTGCATAAGCACGGCTGCCTGTTCCGTGATCTGGTGCGCATT CAGGGTAAAGACCTGCT  
GACCCCGTTAGCCGATTCTGATCGGCAACCCGGGTTGTACGTATAAGTACCTGAATACC  
CGCCTGTTTACGGTCCCGTGGCCGGTGAAAGGCTCTAACATTAAGCACACCGAAGCGGAA  
ATCGCGGCCGCATGCGAAACGTTCCCTGAAACTGAATGATTATCTGCAGATTGAAACCATCC  
AAGCTCTGGAAGAAGTGGCTGCGAAAGAAAAGGCTAACGAAGATGCGGTCCCGCTGTGTA  
TGTCAGCGGACTTTCCGCGTGTGGGCATGGGTAGCTCTTATAATGGCCAAGATGAAGTTG  
ACATCAAATCGCGCGCCGCATACAACGTCACGCTGCTGAATTTTATGGACCCGCAGAAAAT  
GCCGTATCTGAAGGAAGAACCGTACTTCGGCATGGGTAAAATGGCGGTGTCATGGCATCA  
CGATGAAAACCTGGTTGACCGTTCGGCTGTGGCGGTTTATTCATACTCGTGCGAAGGTCC  
GGAAGAAGAAAGTGAAGATGACTCCCATCTGGAAGGCCGCGATCCGGACATTTGGCACGT  
GGGTTTTAAAATTAGCTGGGATATTGAAACCCCGGGTCTGGCAATCCCGCTGCATCAGGGT  
GACTGCTATTTTATGCTGGATGACCTGAATGCCACCCATCAGCACTGTGTTCTGGCAGGCA  
GCCAACCGCGTTTCAGTTCACGCACCGCGTCGCCGAATGTTCTACCGGTACGCTGGATT  
ACATTCTGCAGCGTTGCCAACTGGCACTGCAGAACGTTTGTGATGACGTCGATAATGATGA  
CGTCAGCCTGAAATCTTTTGAACCGGCCGTGCTGAAGCAGGGCGAAGAAATCCATAACGA  
AGTTGAATTCGAATGGCTGCGCCAGTTTTGGTTCCAAGGTAATCGTTATCGCAAATGCACC  
GATTGGTGGTGTGTCAGCCGATGGCCCAACTGGAAGCACTGTGGAAAAAGATGGAAGGCGTG  
ACGAACGCAGTTCTGCATGAAGTCAAACGTGAAGGTCTGCCGGTGGAAACAGCGCAACGAA  
ATTCTGACCGCCATCCTGGCAAGTCTGACGGCTCGTCAGAATCTGCGTCGCGAATGGCAC  
GCGCGCTGCCAATCCCGTATTGCTCGCACCCCTGCCGGCGGATCAGAAACCGGAATGTCGT  
CCGTA CTGGGAAAAGGATGACGCCAGCATGCCGCTGCCGTTTGACCTGACCGACATCGTC  
TCTGAACTGCGCGGCCAACTGCTGGAAGCGAAACCGTAA -3'

**BamHI recognition site**

5'-GGATCC-3'



**Figure S1.** Crystal structures of (a) human Alkbh5 (PDB code: 4NRO)<sup>1</sup> and (b) FTO (PDB code: 3LFM)<sup>2</sup>.

### References

1. Feng, C.; Liu, Y.; Wang, G.; Deng, Z.; Zhang, Q.; Wu, W.; Tong, Y.; Cheng, C.; Chen, Z., Crystal structures of the human RNA demethylase Alkbh5 reveal basis for substrate recognition. *J Biol Chem* **2014**, *289* (17), 11571-83.
2. Han, Z.; Niu, T.; Chang, J.; Lei, X.; Zhao, M.; Wang, Q.; Cheng, W.; Wang, J.; Feng, Y.; Chai, J., Crystal structure of the FTO protein reveals basis for its substrate specificity. *Nature* **2010**, *464* (7292), 1205-9.