JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Communication
pubs.acs.org/JACS

Fluorescein Derivatives as Bifunctional Molecules for the Simultaneous Inhibiting and Labeling of FTO Protein

Tianlu Wang,[†,‡] Tingting Hong,[†,‡] Yue Huang,[‡,§] Haomiao Su,[†] Fan Wu,[†] Yi Chen,[†] Lai Wei,[†] Wei Huang,[†] Xiaoluan Hua,[†] Yu Xia,[†] Jinglei Xu,[†] Jianhua Gan,[⊥] Bifeng Yuan,[†] Yuqi Feng,[†] Xiaolian Zhang,[#] Cai-Guang Yang,[*,§] and Xiang Zhou[*,†]

[†]College of Chemistry and Molecular Sciences, Institute of Advanced Studies and [#]School of Medicine, Wuhan University, Wuhan, Hubei 430072, P. R. China

[§]State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China

[⊥]School of Life Sciences, Fudan University, Shanghai 200433, China

Supporting Information

ABSTRACT: The FTO protein is unequivocally reported to play a critical role in human obesity and in the regulation of cellular levels of m^{6}A modification, which makes FTO a significant and worthy subject of study. Here, we identified that fluorescein derivatives can selectively inhibit FTO demethylation, and the mechanisms behind these activities were elucidated after we determined the X-ray crystal structures of FTO/fluorescein and FTO/5-aminofluorescein. Furthermore, these inhibitors can also be applied to the direct labeling and enrichment of FTO protein combined with photoaffinity labeling assay.

As a prevalent post-transcriptional modification in mRNA for higher eukaryotes,[1] m^{6}A (N$_6$-methyladenosine) was found to be enriched primarily near stop codons and in 3′UTRs.[2] The recent discoveries of a m^{6}A methyltransferase tricomplex (METTL3−METTL14−WTAP)[3] and two demethylases (FTO and ALKBH5)[4,5] revealed new insights into the dynamic regulation of m^{6}A methylation on gene expression. Together with the m$_6$A-binding protein (YTHDF2 and YTHDF1),[6] researchers have found that perturbation of the activities of these enzymes may result in the disturbance of the translation status and RNA metabolism, which further highlights the fundamental physiological roles of m^{6}A in biology and diseases.[7]

Initially, the fat mass and obesity-associated (FTO) protein was known to be involved in human obesity and energy homeostasis.[8] As a member of the AlkB dioxygenase family, FTO was identified to be the first RNA demethylase that catalyzes the demethylation of m^{6}A in an Fe (II)- and 2-oxoglutarate-dependent manner.[5,9,10] Later, m^{6}A demethylation was also found to be linked to adipogenesis through FTO-dependent m^{6}A demethylation.[11] Recently, some studies aiming at exploiting small molecules as functional probes for studying FTO have been reported.[12] These small molecules exhibit good inhibitory activities on m^{6}A demethylation *in vitro* and *in vivo*. Among them meclofenamic acid (MA) was identified as a highly selective inhibitor of FTO,[12c] and dihydroxyfuran sulfonamides were demonstrated with anticonvulsant activity other than inhibiting FTO demethylation.[12e] However, to our knowledge, no compounds equipped with the ability to simultaneously selectively inhibit and label FTO have been developed to date.

Inspired by the previous work,[12c] we tried to screen potent FTO inhibitors from many fluorescent molecules having structures similar to MA. To our delight, we surprisingly found that fluorescein and some of its derivatives could be utilized as bifunctional molecules for selectively inhibiting FTO demethylation and directly labeling FTO protein at the same time.

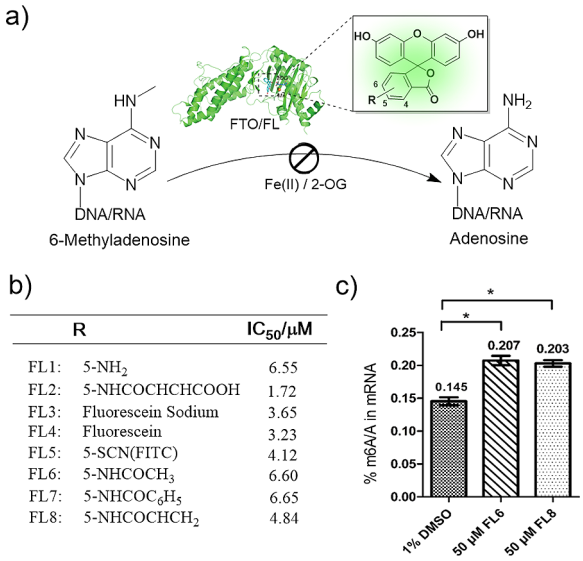
Recently, activity-based protein profiling (ABPP) has been widely implemented to monitor enzyme activity directly in native biological systems through designing active-site-directed covalent probes to specifically perturb target enzymes.[13] A few photoreactive groups are employed in ABPP to covalently label interacting proteins, such as aryl azides, diazirines, and benzophenones.[14] Here, through introducing a diazirine group to fluorescein derivatives, we can further provide a platform for the downstream biochemical analyses of FTO, such as *in situ* FTO labeling within the native cellular environment and FTO enrichment for subsequent proteome-wide identification.

We initially designed and synthesized 11 fluorescein derivatives, which were named as FL1−11. The preliminary inhibition evaluation of these compounds was performed via a LC-MS assay (Figure S1) and the robust restriction endonuclease digestion assay (Figures S2a and S2c). Our results revealed that 100 μM of compounds FL1−8 could completely abolish the demethylation of FTO *in vitro*, while FL9−11 displayed poor or no inhibitory activity under the same condition. Moreover, these fluorescein analogues were shown to be inactive against ALKBH5 demethylation even at 100 μM (Figure S2b), which demonstrated that FL1−8 displayed high selectivity toward FTO over ALKBH5 *in vitro*.

To further investigate the structure−activity relationships of these inhibitors, we precisely validated their FTO inhibitory activities through IC$_{50}$ values measurement using a LC-MS based assay (Figures 1b and S3). The results revealed that all eight fluorescein analogues showed good inhibitory activities toward FTO, with IC$_{50}$ values between 1.0 and 7.0 μM. In addition, the

Received: June 28, 2015
Published: October 12, 2015

DOI: 10.1021/jacs.5b06690
J. Am. Chem. Soc. 2015, 137, 13736−13739



b)

R		IC_{50}/μM
FL1:	5-NH_2	6.55
FL2:	5-NHCOCHCHCOOH	1.72
FL3:	Fluorescein Sodium	3.65
FL4:	Fluorescein	3.23
FL5:	5-SCN(FITC)	4.12
FL6:	5-$NHCOCH_3$	6.60
FL7:	5-$NHCOC_6H_5$	6.65
FL8:	5-$NHCOCHCH_2$	4.84

Figure 1. Fluorescein derivatives as potent inhibitors against FTO demethylation. (a) Scheme of demethylation of m^6A to A by FTO, which can be selectively inhibited by fluorescein derivatives. (b) IC_{50} values of FL1−8 against FTO demethylation. (c) The cellular levels of m^6A in mRNA were increased when the HeLa cells were treated with 50 μM FL6 or FL8. *P < 0.01. Error bar, mean ± SEM, n = 3.

amide derivatives of 5-aminofluorescein presented equal or even higher inhibitory activities compared with 5-aminofluorescein, which offers a new perspective for further structural optimization.

Among these inhibitors, FL6 and FL8 were chosen to modulate the level of m^6A in the mRNA of living cells because of their better cell permeability. First, a MTT assay was performed to find that >95% of the cells remained viable after treatment with 20−150 μM FL6 and FL8 (Figure S4). Using DMSO-treated HeLa cells as control, we could observe a significant increase in the level of m^6A modification in mRNA for the cells that were treated with 50 μM FL6 or FL8. These results revealed that these two fluorescein derivatives could efficiently inhibit FTO demethylation inside live cells (Figure 1c).

Next, in order to investigate the inhibition mechanism of these fluorescein analogs, differential scanning fluorometry[15] was first carried out (Table S1). We observed that the presence of 80-fold compounds FL1−8 could significantly increase the melting temperature (T_m) of the FTO protein, which revealed that these inhibitors could directly bind to FTO *in vitro*. In contrast, FL9−11 minimally altered the T_m values of the FTO protein, which is consistent with their poor inhibitory activities against FTO *in vitro*.

In addition, we also performed aggregation tests in order to rule out the possibility of these fluorescent analogues inhibiting FTO protein through aggregate-based mechanism.[16] Although we observed a slight decrease in IC_{50} values of FL2 and FL4 against FTO demethylation after preincubation, the results of both dynamic light scattering and detergent-based assay confirmed that these fluorescein derivatives did not act as promiscuous inhibitors (Figure S5 and Tables S2−S4). Then by increasing the concentrations of ferrous ion or 2-oxoglutarate (2-OG), we found that even the presence of 2 mM ferrous ion or 2-OG had little impact on the inhibitory activity of fluorescein, which thus excludes the possibility of fluorescein acting as a ferrous ion chelator or mimicking 2-OG to inhibit FTO demethylation (Figure S6).

In order to gain insight into the inhibitory mode of action from the atomic level, we determined the X-ray crystal structures of FTO/fluoresceins complexes. The structures were solved by molecular replacement and refined to a resolution of 2.15 Å for FTO/FL4 and 2.45 Å for FTO/FL1, respectively (Figure S7 and Table S5). The atomic coordinates and structure factors have been deposited in the RCSB Protein Data Bank under accession ID code 4ZS3 (FTO/FL1) and 4ZS2 (FTO/FL4). In the structural complexes, we observed that the esters were hydrolyzed to carboxyl acids[17] (Figure 2a,d), which form hydrogen bonding

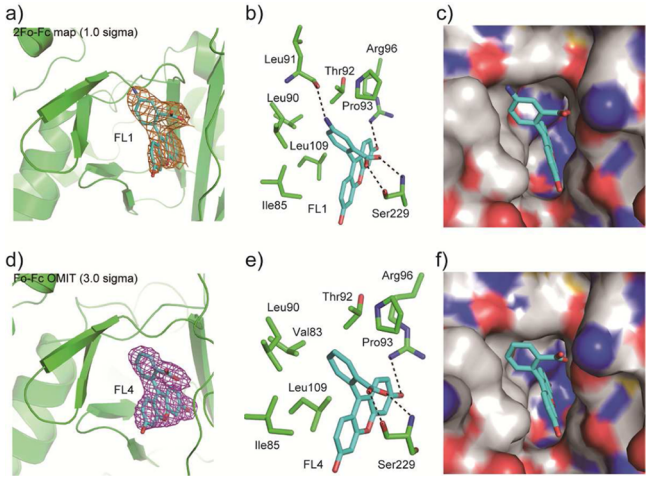


Figure 2. Structural complexes of inhibitors bound to the FTO protein. (a) The 2Fo − Fc map contoured to 1.0 sigma confirms that FL1 indeed binds to FTO. The FTO protein is depicted as a cartoon and colored in green, the inhibitor FL1 is depicted in stick and colored in cyan, and the map is colored in orange. (b) Interaction networks between the FTO protein and FL1 in the active site pocket. The environmental residues involved in the interactions are shown as sticks. Dark dotted lines indicate the hydrogen bonds. (c) Surface view showing the vacancy to hold inhibitor FL1. (d) The m|Fo| − D|Fc| map was calculated with the PHENIX program suite after removing the inhibitor from the complex model and subsequent simulated annealing. The OMIT map density contoured to 3.0 sigma is colored in magenta. (e) Close view of the interaction between FTO and FL4 in the binding pocket. (f) Surface potential view of the pocket in FTO for binding to FL4.

with Ser 229 (Figure 2b,e). The phenyl ring is surrounded by the side chains of adjacent residues Val83, Ile85, Leu90, Pro93, and Leu109, providing extensive hydrophobic interactions to stabilize the inhibitor binding (Figures 2b,e and S9a). This hydrophobic nucleotide recognition lid (NRL) motif contributes to the highly selective inhibition of FTO over ALKBH5 by fluorescein (Figure S2b).[18] In addition, it is applied to explain why compounds FL9−11 failed to inhibit FTO (Figure S1). The bulky substituent on either the 5- or 6-position of the phenyl ring would severely hinder the binding to FTO (Figures 2 and S9). Overall, quite similar to MA for FTO-binding (Figure S8a), fluorescein inhibitors partially occupy the nucleotide binding site (Figure S8b) and do not disturb the cofactor 2-OG or metal ion binding to FTO (Figure S8c). Therefore, these fluorescein inhibitors are not acting as an adenine mimetic or stacking with active site residues which are involved in nucleotide recognition. It is worthy to note that the 5-amino group in FL1 forms an extra hydrogen bonding with the backbone carbonyl of Leu 91 (Figure 2b). Generally, the additional interaction would contribute to an enhanced binding. However, this hydrophilic amino substituent is close to the hydrophobic NRL motif (Figure S9b,c), which appears to be less

DOI: 10.1021/jacs.5b06690
J. Am. Chem. Soc. 2015, 137, 13736−13739

favorable for the FTO/FL1 interaction, explaining why FL1 displays weaker inhibitory activity than FL4 (Figure 1b).

Furthermore, these two structures clearly reveal that the 4-position in the phenyl ring of fluorescein is fully exposed to the solvent. Therefore, we anticipate that a proper substituent on this site would minimally disturb the binding to FTO. Thus, we synthesized 4-aminofluorescein (FL12) and found a slight, but not statistically significant, decrease in its IC_{50} value (4.37 μM) compared with that of 5-aminofluorescein (FL1) (Figure S18 and Table S6). In summary, this investigation of the structure–activity relationships showed good agreement between the observed activity of the inhibitors and the structural feature of FTO/FL interactions.

Due to the fluorescence properties of these inhibitors,[19] they were further designed for the labeling of FTO. Considering that the photoaffinity labeling was successfully demonstrated by the capture of the binding proteins in a distance-dependent manner, particularly under native cellular conditions,[20] we introduced diazirine into FL2 as a photoreactive group for the purpose of FTO labeling and enrichment (Figure 3a).

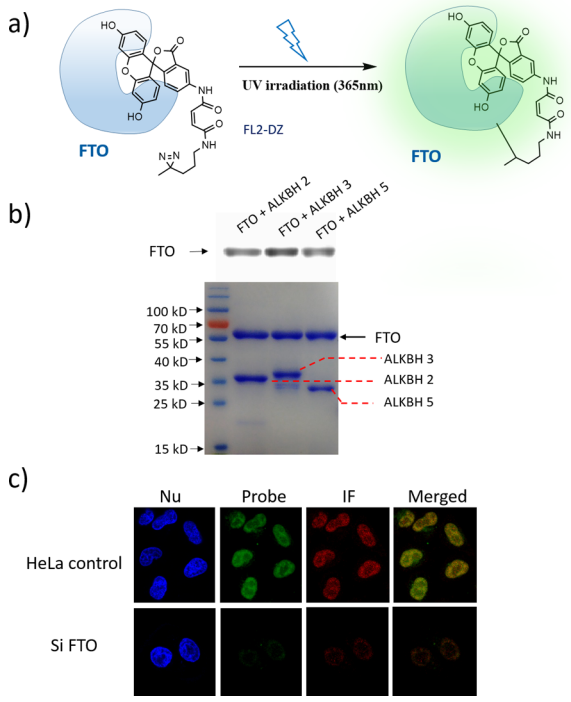


Figure 3. Selective labeling of FTO using probe FL2-DZ. (a) Scheme of the photoaffinity labeling of FTO demethylase by FL2-DZ under UV irradiation. (b) Selective labeling of FTO over other human AlkB enzymes. (c) *In situ* labeling of the FTO in HeLa cells or siRNA FTO HeLa cells with the FL2-DZ probe (20 μM).

The inhibitory activity of FL2-DZ was first measured, and minor change in its IC_{50} value (4.49 μM) was observed compared with FL2 due to the small size of a diazirine substitution (Figure S10). Nonspecific cross-linking between BSA and FL2-DZ can be remarkably suppressed with increasing salt concentrations and completely disappeared under 0.4 M PBS and 0.5 M NaCl (Figures S11 and S12). The selective photoaffinity labeling of FTO by FL2-DZ was further confirmed by comparison with other AlkB enzymes. A mixture of FTO and ALKBH5 was treated with FL2-DZ under the conditions described above, followed by UV irradiation (λ = 365 nm, 20 min) and SDS-PAGE analysis. As shown in Figure 3b, no labeling band corresponding to ALKBH5 was observed. The same results were also obtained with two other human AlkB demethylases, ALKBH2 and ALKBH3[21,22] (Figure 3b). This extraordinary selectivity of FL2-DZ for FTO demonstrates its prospective application in the identification of FTO in living cells.

Because of its excellent cell permeability and biochemical activities, the FL2-DZ probe was added to the medium of HeLa cells to demonstrate its bioimaging capacity for the labeling of endogenous FTO demethylase *in situ*. After being covalently cross-linked to its targeted proteins by UV irradiation, we observed strong green fluorescence throughout the nucleus, which was well colocalized with the red signals obtained from the immunofluorescence (IF) results using anti-FTO (Figure 3c). We then transfected the HeLa cells with FTO siRNA to silence the *fto* gene (Figure S13a), and a significant decrease in the green signals was observed, which is consistent with the red signals corresponding to the IF results (Figure 3c). As expected, HeLa cells with stable overexpression of wide-type FTO showed a notable increase in both green fluorescence and red signals (Figures S13b and S15). The tracking of FTO using the FL2-DZ probe was further demonstrated in living cells that were exposed to oxidative stress, heat shock, or knockdown of METTL3 (Figures S14 and S16).

Lastly, by using a small terminal alkyne as the reporter, we further applied this specific FL1-DZ-alkyne probe to the identification of cellular FTO by pull-down and Western blotting through the click chemistry with biotin-N_3 (Figure 4a). The newly designed FL1-DZ-alkyne probe was first detected for its biological activities through IC_{50} measurements (Figure 4b). Next, the selectivity and sensitivity of the probe were further verified through the enrichment of low-abundance FTO in the diluted

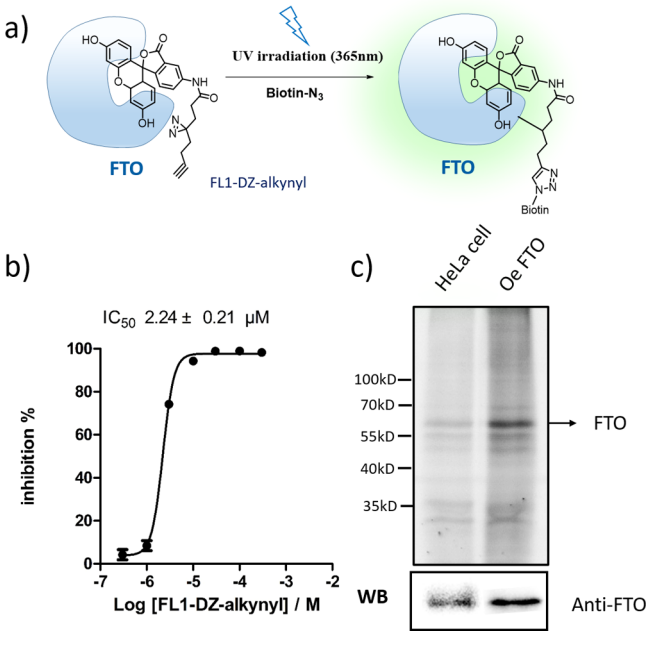


Figure 4. Affinity labeling and enrichment of FTO in cell lysates using FL1-DZ-alkynyl probe. (a) Scheme of the pull-down of FTO by using FL1-DZ-alkynyl. (b) The inhibition curves of FL1-DZ-alkynyl against FTO demethylation. Error bar, mean ± SEM, n = 3. (c) In-gel fluorescence scanning of the proteins enriched by FL1-DZ-alkynyl from HeLa cells and stable cell lines that overexpress FTO; the protein was further verified through Western blotting (bottom).

DOI: 10.1021/jacs.5b06690
J. Am. Chem. Soc. 2015, 137, 13736–13739

HeLa cell lysates (Figure S17b). Then, the FL1-DZ-alkyne probe was successfully applied to the identification of the cellular FTO in cell lysates. For comparison, we performed the labeling of both endogenous FTO in HeLa cells and recombinant FTO overexpressed in stable cell lines. The captured proteins were enriched by strepavidin-agarose beads and separated by SDS-PAGE. The in-gel fluorescence scanning showed a clear labeled protein band for both cell lysates that matches the expected molecular weight of FTO (Figure 4c, the upper gel), which was further identified through Western blotting (Figure 4c, the bottom gel).

In summary, we have explored fluorescein and its derivatives as bifunctional molecules for selectively inhibiting FTO demethylation and specifically photoaffinity labeling of FTO. We also provide molecular insights to elucidate the structure−activity relationships of these fluorescent inhibitors through crystallography and pave the way for the structural optimization of FTO demethylation inhibitors. Besides, this strategy realized the downstream biochemical analysis of FTO, such as visualization of the FTO protein in living cells and FTO enrichment. We anticipate that this potential useful tool can facilitate the study of the roles of FTO in m^6A-mediated epigenetic processes. Finally, it is worthy to note that when fluorescein and some of its derivatives are applied to imaging studies to investigate some cellular biochemistry, their potent ability to inhibit FTO demethylation should also be taken into account.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b06690.

Experiment methods, synthesis and characterization data, IC_{50} measurement, thermal shifts of FTO protein, photoaffinity labeling, Western blot quantification of FTO (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*xzhou@whu.edu.cn

*yangcg@simm.ac.cn

Author Contributions

‡These authors contributed equally.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the 973 Program (2012CB720600, 2012CB720603, and 2012CB720604 to X.Z.; 2015CB910603 to C.-G.Y.), the National Science Foundation of China (21432008, 91413109, and 91213302 to X.Z.; 21372237 and 91313303 to C.-G.Y.). We also thank all beamline staff at the BL19U of the Shanghai Synchrotron Radiation Facility and Y. Zhu and Dr. X. Chen (Peking University) for offering us linker L2.

■ REFERENCES

(1) (a) Fu, Y.; Dominissini, D.; Rechavi, G.; He, C. *Nat. Rev. Genet.* **2014**, *15*, 293. (b) Meyer, K. D.; Jaffrey, S. R. *Nat. Rev. Mol. Cell Biol.* **2014**, *15*, 313. (c) Niu, Y.; Zhao, X.; Wu, Y. S.; Li, M. M.; Wang, X. J.; Yang, Y. G. *Genomics, Proteomics Bioinf.* **2013**, *11*, 8.

(2) (a) Dominissini, D.; Moshitch-Moshkovitz, S.; Schwartz, S.; Salmon-Divon, M.; Ungar, L.; Osenberg, S.; Cesarkas, K.; Jacob-Hirsch, J.; Amariglio, N.; Kupiec, M.; Sorek, R.; Rechavi, G. *Nature* **2012**, *485*, 201. (b) Meyer, K. D.; Saletore, Y.; Zumbo, P.; Elemento, O.; Mason, C. E.; Jaffrey, S. R. *Cell* **2012**, *149*, 1635.

(3) (a) Liu, J.; Yue, Y.; Han, D.; Wang, X.; Fu, Y.; Zhang, L.; Jia, G.; Yu, M.; Lu, Z.; Deng, X.; Dai, Q.; Chen, W.; He, C. *Nat. Chem. Biol.* **2014**, *10*, 93. (b) Ping, X. L.; Sun, B. F.; Wang, L.; Xiao, W.; Yang, X.; Wang, W. J.; Adhikari, S.; Shi, Y.; Lv, Y.; Chen, Y. S.; Zhao, X.; Li, A.; Yang, Y.; Dahal, U.; Lou, X. M.; Liu, X.; Huang, J.; Yuan, W. P.; Zhu, Y. F.; Cheng, T.; Zhao, Y. L.; Wang, X.; Danielsen, J. M. R.; Liu, F.; Yang, Y. G. *Cell Res.* **2014**, *24*, 177. (c) Wang, Y.; Li, Y.; Toth, J. I.; Petroski, M. D.; Zhang, Z.; Zhao, J. C. *Nat. Cell Biol.* **2014**, *16*, 191.

(4) Zheng, G.; Dahl, J. A.; Niu, Y.; Fedorcsak, P.; Huang, C. M.; Li, C. J.; Vagbo, C. B.; Shi, Y.; Wang, W. L.; Song, S. H.; Lu, Z.; Bosmans, R. P.; Dai, Q.; Hao, Y. J.; Yang, X.; Zhao, W. M.; Tong, W. M.; Wang, X. J.; Bogdan, F.; Furu, K.; Fu, Y.; Jia, G.; Zhao, X.; Liu, J.; Krokan, H. E.; Klungland, A.; Yang, Y. G.; He, C. *Mol. Cell* **2013**, *49*, 18.

(5) Jia, G.; Fu, Y.; Zhao, X.; Dai, Q.; Zheng, G.; Yang, Y.; Yi, C.; Lindahl, T.; Pan, T.; Yang, Y. G.; He, C. *Nat. Chem. Biol.* **2011**, *7*, 885.

(6) (a) Wang, X.; Lu, Z.; Gomez, A.; Hon, G. C.; Yue, Y.; Han, D.; Fu, Y.; Parisien, M.; Jia, G.; Ren, B.; Pan, T.; He, C. *Nature* **2014**, *505*, 117. (b) Wang, X.; Zhao, B. S.; Roundtree, L. A.; Lu, Z.; Han, D.; Ma, H.; Weng, X.; Shi, H.; He, C. *Cell* **2015**, *161*, 1388.

(7) Lee, M.; Kim, B.; Kim, V. N. *Cell* **2014**, *158*, 980.

(8) (a) Church, C.; Moir, L.; McMurray, F.; Girard, C.; Banks, G. T.; Teboul, L.; Wells, S.; Bruning, J. C.; Nolan, P. M.; Ashcroft, F. M.; Cox, R. D. *Nat. Genet.* **2010**, *42*, 1086. (b) Fischer, J.; Koch, L.; Emmerling, C.; Vierkotten, J.; Peters, T.; Bruning, J. C.; Ruther, U. *Nature* **2009**, *458*, 894.

(9) Han, Z.; Niu, T.; Chang, J.; Lei, X.; Zhao, M.; Wang, Q.; Cheng, W.; Wang, J.; Feng, Y.; Chai, J. *Nature* **2010**, *464*, 1205.

(10) Falnes, P. O.; Johansen, R. F.; Seeberg, E. *Nature* **2002**, *419*, 178.

(11) Zhao, X.; Yang, Y.; Sun, B. F.; Shi, Y.; Yang, X.; Xiao, W.; Hao, Y. J.; Ping, X. L.; Chen, Y. S.; Wang, W. J.; Jin, K. X.; Wang, X.; Huang, C. M.; Fu, Y.; Ge, X. M.; Song, S. H.; Jeong, H. S.; Yanagisawa, H.; Niu, Y.; Jia, G. F.; Wu, W.; Tong, W. M.; Okamoto, A.; He, C.; Rendtlew, D. J.; Wang, X. J.; Yang, Y. G. *Cell Res.* **2014**, *24*, 1403.

(12) (a) Chen, B.; Ye, F.; Yu, L.; Jia, G.; Huang, X.; Zhang, X.; Peng, S.; Chen, K.; Wang, M.; Gong, S.; Zhang, R.; Yin, J.; Li, H.; Yang, Y.; Liu, H.; Zhang, J.; Zhang, H.; Zhang, A.; Jiang, H.; Luo, C.; Yang, C. G. *J. Am. Chem. Soc.* **2012**, *134*, 17963. (b) Toh, J. D. W.; Sun, L.; Lau, L. Z. M.; Tan, J.; Low, J. J. A.; Tang, C. W. Q.; Cheong, E. J. Y.; Tan, M. J. H.; Chen, Y.; Hong, W.; Gao, Y.; Woon, E. C. Y. *Chemical. Science.* **2015**, *6*, 112. (c) Huang, Y.; Yan, J.; Li, Q.; Li, J.; Gong, S.; Zhou, H.; Gan, J.; Jiang, H.; Jia, G.; Luo, C.; Yang, C. *Nucleic Acids Res.* **2015**, *43*, 373. (d) Aik, W.; Demetriades, M.; Hamdan, M. K. K.; Bagg, E. A. L.; Yeoh, K. K.; Lejeune, C.; Zhang, Z.; McDonough, M. A.; Schofield, C. J. *J. Med. Chem.* **2013**, *56*, 3680. (e) Zheng, G.; Cox, T.; Tribbey, L.; Wang, G. Z.; Iacoban, P.; Booher, M. E.; Gabriel, J. G.; Zhou, L.; Bae, N.; Rowles, J.; He, C.; Olsen, M. J. *ACS Chem. Neurosci.* **2014**, *5*, 658.

(13) Niphakis, M. J.; Cravatt, B. F. *Annu. Rev. Biochem.* **2014**, *83*, 341.

(14) (a) David, A.; Steer, D.; Bregant, S.; Devel, L.; Makaritis, A.; Beau, F.; Yiotakis, A.; Dive, V. *Angew. Chem., Int. Ed.* **2007**, *46*, 3275. (b) Shigdel, U. K.; Zhang, J.; Chuan, He. *Angew. Chem., Int. Ed.* **2008**, *47*, 90. (c) Shi, H.; Liu, K.; Xu, A.; Yao, S. Q. *Chem. Commun.* **2009**, 5030.

(15) Niesen, F. H.; Berglund, H.; Vedadi, M. *Nat. Protoc.* **2007**, *2*, 2212.

(16) (a) McGovern, S. L.; Caselli, E.; Grigorieff, N.; Shoichet, B. K. *J. Med. Chem.* **2002**, *45*, 1712. (b) Feng, B. Y.; Shoichet, B. K. *Nat. Protoc.* **2006**, *1*, 550.

(17) Klonis, N.; Sawyer, W. H. *J. Fluoresc.* **1996**, *6*, 147.

(18) Aik, W.; Scotti, J. S.; Choi, H.; Gong, L.; Demetriades, M.; Schofield, C. J.; McDonough, M. A. *Nucleic Acids Res.* **2014**, *42*, 4741.

(19) (a) Lane, B. C.; Cohen-Gadol, A. A. *Curr. Drug Discovery Technol.* **2013**, *10*, 160. (b) Zhang, X. F.; Zhang, J.; Liu, L. *J. Fluoresc.* **2014**, *24*, 819.

(20) Li, Z.; Hao, P.; Li, L.; Tan, C. Y.; Cheng, X.; Chen, G. Y.; Sze, S. K.; Shen, H. M.; Yao, S. Q. *Angew. Chem., Int. Ed.* **2013**, *52*, 8551.

(21) Chen, B.; Liu, H.; Sun, X.; Yang, C. G. *Mol. BioSyst.* **2010**, *6*, 2143.

(22) Ringvoll, J.; Nordstrand, L. M.; Vagbo, C. B.; Talstad, V.; Reite, K.; Aas, P. A.; Lauritzen, K. H.; Liabakk, N. B.; Bjork, A.; Doughty, R. W.; Falnes, P. O.; Krokan, H. E.; Klungland, A. *EMBO J.* **2006**, *25*, 2189.

DOI: 10.1021/jacs.5b06690
J. Am. Chem. Soc. 2015, 137, 13736−13739