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A covalently bound inhibitor triggers EZH2 degradation through CHIP-mediated ubiquitination

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Reporting Checklist For Life Sciences Articles (Rev. July 2015)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ^2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

Please ensure that the answers to the following questions are reported in the manuscript itself. We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

In the pink boxes below, provide the page number(s) of the manuscript draft or figure legend(s) where the information can be located. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable).

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B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	N/A
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	N/A
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No animals were excluded from the analysis
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	The animals were randomly distributed to each group.
For animal studies, include a statement about randomization even if no randomization was used.	After 5 days, the tumor sizes were determined using micrometer calipers, and the nude mice with similar tumor volumes (eliminating mice with tumors that were too large or too small) were then randomly divided into 3 groups (10 nude mice per group)
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	No
4.b. For animal studies, include a statement about blinding even if no blinding was done	The animal studies were performed under blinding condition.
5. For every figure, are statistical tests justified as appropriate?	Yes
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes
Is there an estimate of variation within each group of data?	Yes
Is the variance similar between the groups that are being statistically compared?	Yes

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	The antibodies that were used in this study were as follows: mouse monoclonal antibody against EZH2 (612667, BD Science); rabbit polyclonal antibody against SUZ-12 (20366-1-AP, Proteintech, USA); mouse monoclonal antibody against β-actin (ab6276, Abcam, USA); rabbit polyclonal antibody against H3K27Me3 (07-449, Millipore, USA); rabbit polyclonal antibody against H3K9Me3 (05-1242, Millipore, USA); rabbit polyclonal antibody against H3 histone (AH433, Beyotime, China); rabbit polyclonal antibody against EED (16818-1-AP, Proteintech); rabbit polyclonal antibody against BMI-1 (10832-1-AP, Proteintech); rabbit polyclonal antibody against PARP (AP102, Beyotime, China); rabbit polyclonal antibody against caspase-8 (13423-1-AP, Proteintech, USA); rabbit polyclonal antibody against caspase-3 (19677-1-AP, Proteintech, USA); rabbit polyclonal antibody against Bcl-2 (12789-1-AP, Proteintech, USA); rabbit polyclonal antibody against Bax (50599-2-Ig, Proteintech, USA); the rabbit polyclonal antibody against ubiquitin (1646-1, Epitomics, USA); mouse monoclonal antibody against Smurf1 (sc-100616, Santa Cruz, USA); rabbit polyclonal antibody against Smurf2 (sc-25511, Santa Cruz, USA); rabbit monoclonal antibody against Bim (2933, Cell Signal, USA); and mouse monoclonal antibody against Flag (F1804, Sigma, USA); Methyl-Histone H3 Antibody Sampler Kit (9847, Cell Signal, USA); Tri-Methyl Histone H3 Antibody Sampler Kit (9783, Cell Signal, USA); Rabbit polyclonal antibodies against H4K20 methylation (ab9051, ab9052, ab9053, Abcam, USA); Rabbit polyclonal antibodies against EZH1(A5818, Abclonal, China).

7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	The human head and neck cancer cell lines UMSCC-12 and SCC-25 as well as breast cancer cell lines MDA-MB-231, MDA-MB-468, and MCF-7 and their drug-resistant variant MCF-7/ADM were obtained from the American Type Culture Collection. The human head and neck cell lines HN-4, HN 6, HN-12, HN-13, HN-30, Cal-27, KB, and KB/VCR; the hepatocyte carcinoma cell line SMMC-7721; and the cervical cancer cell line HeLa were obtained from NIH. These cells were maintained in Dulbecco's minimum essential medium (Invitrogen) that was supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. MV4-11, RS4-11, Reh, Daudi, Pfeiffer and KE-37 were obtained from the American Type Culture Collection and cultured with RPMI-1640 medium and 10% fetal bovine serum (Invitrogen). The indicated cell lines were incubated in a humidified atmosphere with 5% CO2 at 37°C.

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	Animal experiments Male BALB/C nude mice, aged 30-35 days and weighing 18-22 g, were used for the animal experiments. The mice were maintained in autoclaved filter-top micro-isolator cages with autoclaved water and sterile food ad libitum. The cages were kept in an isolator unit that was provided with filtered air. To generate the results for Fig 5, thirty mice were subcutaneously inoculated with injections of 1×106 cells/nude mice. After 5 days, the tumor sizes were determined using micrometer calipers, and the nude mice with similar tumor volumes (eliminating mice with tumors that were too large or too small) were then randomly divided into 3 groups (10 nude mice per group): the saline tumor control group (negative control group); the oral gavage group with the oral administration of GNA002 100 mg/kg/day group; and the intraperitoneal injection (i.p.) group with intro injection of cisplatin 5 mg/kg/week group. At the end of 4 weeks, the nude mice were sacrificed, and the tumor xenografts were excised and measured. Tumor volume (TV) was calculated using the following formula: TV (mm3)=d2×D/2, where d and D are the shortest and the longest diameters, respectively. The entire experimental procedure was performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (revised 1996). In addition, the animals were weighed twice per week and monitored for mortality throughout the experimental period to assess treatment toxicity. Pharmacokinetic study
	Adult male ICR mice were fasted overnight prior to drug administration. GNA002 was administered as a single dose of 12 mg/kg by tail vain injection or oral gavage. At pre-dose and at 0.083, 0.25, 0.5, 1, 2, 4, 8, and 24 hours post-dose, blood was collected from three male mice and immediately processed for plasma by centrifugation for 10 minutes at 3,000 ×g. The resulting plasma was frozen on dry ice, and the samples were stored at -80°C until analysis. Proper measures were taken to minimize pain and discomfort experienced by the mice. The experimental procedures were in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (revised 1996).
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	The experiments were performed in compliance with ethical regulations and the protocols were approved by the institute's committee.
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	We confirm compliance with the recommended ARRIVE guidelines.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	N/A
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	N/A
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	N/A
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	N/A
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17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	N/A

F- Data Accessibility

18. Provide accession codes for deposited data. See author guidelines, under 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	N/A
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	N/A
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21. As far as possible, primary and referenced data should be formally cited in a Data Availability section. Please state whether you have included this section. Examples: **Primary Data** Wetmore KM, Deutschbauer AM, Price MN, Arkin AP (2012). Comparison of gene expression and mutant fitness in Shewanella oneidensis MR-1. Gene Expression Omnibus GSE39462 **Referenced Data** Huang J, Brown AF, Lei M (2012). Crystal structure of the TRBD domain of TERT and the CR4/5 of TR. Protein Data Bank 4O26 AP-MS analysis of human histone deacetylase interactions in CEM-T cells (2013). PRIDE PXD000208	N/A
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G- Dual use research of concern

23. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	N/A