Local generation of fumarate promotes DNA repair through inhibition of histone H3 demethylation

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Histone methylation regulates DNA repair. However, the mechanisms that underlie the regulation of histone methylation during this repair remain to be further defined. Here, we show that exposure to ionizing radiation induces DNA-PK-dependent phosphorylation of nuclear fumarase at Thr 236, which leads to an interaction between fumarase and the histone variant H2A.Z at DNA double-strand break (DSB) regions. Locally generated fumarate inhibits KDM2B histone demethylase activity, resulting in enhanced dimethylation of histone H3 Lys 36; in turn, this increases the accumulation of the Ku70-containing DNA-PK at DSB regions for non-homologous end-joining DNA repair and cell survival. These findings reveal a feedback mechanism that underlies DNA-PK regulation by chromatin-associated fumarase and an instrumental function of fumarase in regulating histone H3 methylation and DNA repair.

DNA DSBs are one of the most deleterious forms of DNA damage. Higher eukaryotic cells primarily repair DSBs by one of two genetically separable pathways, homologous recombination (HR) or non-homologous end-joining (NHEJ). HR operates in dividing cells in late S/G2 phase, as it requires homologous sister chromatid sequences as the template for mediating faithful repair. In contrast, NHEJ repairs broken ends, with little or no need for sequence homology; it can function in both dividing and non-dividing cells and can occur in all cell-cycle stages. In NHEJ, DSBs are recognized by the DNA-dependent protein kinase (DNA-PK) holoenzyme, which consists of the DNA end-binding heterodimer Ku70–Ku80 and the catalytic subunit DNA-PKcs. This binding results in activation of DNA-PKcs and the subsequent recruitment and activation of end-processing enzymes, polymerases and DNA ligase IV.

DSB repair requires chromatin and nucleosome remodelling, which creates open, relaxed chromatin domains. The histone variant H2A.Z is exchanged onto nucleosomes at DSBs, which promotes specific patterns of histone modification and reorganization of the chromatin architecture. H2A.Z exchange at DSBs is required for the acetylation and ubiquitylation of histones, loading the BRCA1 complex during HR DNA repair, and loading Ku70–Ku80 for NHEJ DNA repair. Among the post-translational modifications of histone, histone methylation is instrumental for DNA repair. Histone methylation mediates localization of 53BP1 to DSBs during HR repair. During NHEJ, ionizing radiation was found to primarily induce dimethylation at Lys 36 of histone H3 H3K36me2; this process was regulated by recruiting the DNA repair protein Metnase (also known as SETMAR), a SET histone methylase domain-containing protein, to DSBs. H3K36me2 near DSBs can be counteracted by expression of the KDM2 histone demethylase. H3K36 dimethylation improved the association of early DNA repair components, including Ku70, with DSBs. Although H2A.Z exchange and histone H3 methylation play a vital role in NHEJ, how these events are regulated is unknown.

Metabolic enzymes can possess non-metabolic functions in response to extracellular stimuli, as reported by our research group and others. Fumarase (FH) catalyses the reversible hydration and dehydration of fumarate to malate in the tricarboxylic acid cycle to facilitate a transition step in the production of energy in the form of NADH. In the cytosol, FH metabolizes fumarate, which is a by-product of the urea cycle and amino-acid catabolism. FH translocates from the cytosol into the nucleus on DNA damage. FH knockdown increases the sensitivity of cells to DNA damage, which can be complemented by high concentrations of fumarate but not by the expression of a catalytically inactive FH mutant. These findings indicate that the enzymatic activity of FH and its product,
Figure 1 H2A.Z-regulated recruitment of FH to DSB regions promotes the accumulation of Ku70 at DSB regions. In a–h, immunoblotting (a–d) and ChIP (e–h) analyses were performed using the indicated antibodies. In a–e, data represent 1 out of 3 experiments. (a) U2OS cells synchronized by thymidine double block (2 mM, upper left panel) underwent no release (upper right panel, G1 phase) or release for 2 h (bottom left panel, S phase) or 6 h (bottom right panel, G2 phase). These cells were then exposed to ionizing radiation (IR; 10 Gy) and collected 1 h after exposure. Chromatin extracts were prepared. CENP-A was used as a control for chromatin-associated proteins. (b) Thymidine double block-synchronized U2OS cells expressing Flag–FH were exposed to ionizing radiation (10 Gy) and collected 1 h after exposure. Chromatin extracts subjected to immunoprecipitation with an anti-FH antibody were analysed by Coomassie brilliant blue staining and immunoblotting analyses. (c,d) Thymidine double block-synchronized U2OS cells, with or without H2A.Z shRNA (c) or FH shRNA (d) expression, were exposed to ionizing radiation (10 Gy) and collected 1 h after exposure. Chromatin extracts and total cell lysates were prepared. (e–h) U2OS cells expressing the DR-GFP reporter, with or without H2A.Z shRNA, were transfected with a vector expressing I-SceI. ChIP analyses with the indicated antibodies were performed at the indicated time points (e.g., h) or 30 h (f) after I-SceI transfection. The indicated primers covering a range of distances from the cutting open site (f) or F1/R1 (e.g., h) primers were used for the PCR. Control primers were selected against a specific region of chromosome 12. The y axis shows the value of the I-SceI-induced fold increase of specific protein binding (the IP value was normalized to the input). The data represent the mean ± s.d. (n = 3 independent experiments). ∗ represents P < 0.01 between the cells expressing control shRNA and the cells expressing the indicated shRNA. Unprocessed original scans of blots/gels are shown in Supplementary Fig. 8.
We stably expressed Flag–FH in U2OS cells. Immunoprecipitation of chromatin–associated proteins with an anti–Flag antibody followed by mass spectrometry (Supplementary Fig. 1c) and immunoblotting analyses (Fig. 1b) showed that exposure to ionizing radiation induced a marked increase in the association of FH with histones, and more histone variant H2A.Z than conventional H2A was detected in the immunoprecipitates. H2A.Z depletion in U2OS cells largely reduced the ionizing-radiation-induced chromatin association of FH, Ku70 and Ku80 (Fig. 1c). In contrast, FH depletion reduced the amount of chromatin–associated Ku70 and Ku80 but not H2A.Z (Fig. 1d).

To support this finding, we expressed the I-SceI restriction enzyme to create DSBs in U2OS cells that contained an integrated DR-GFP reporter gene with a unique I-SceI cutting site. A chromatin immunoprecipitation (ChIP) assay with antibodies against H2A.Z and FH showed that both H2A.Z and FH bound to the DNA adjacent to the I-SceI cutting site (Fig. 1e,f). In addition, H2A.Z depletion blocked the recruitment of FH to DSB regions (Fig. 1f, bottom left panel), whereas FH depletion had no significant effect on H2A.Z recruitment.
to DSBs (Fig. 1f, bottom right panel). Time-course studies showed that H2A.Z (Fig. 1g) and FH (Fig. 1h) depletion moderately affected the initial binding of Ku70 but led to dampened enrichment of Ku70 at the I-SceI cutting site. These results suggest that H2A.Z promotes the binding of FH to DSB regions, which is required for the DNA-PK complex accumulation at DNA damage areas.

**DNA-PK phosphorylates FH at Thr 236**

To determine the relationship between FH and the Ku70–Ku80-containing DNA-PK complex, we irradiated U2OS cells after DNA-PK inhibitor NU7441 treatment or Ku70 depletion. Figure 2a shows that Ku70 depletion and DNA-PK inhibition blocked the ionizing-radiation-induced binding of FH to chromatin, indicating an essential role for DNA-PK activity in this binding. In line with this result, an in vitro protein phosphorylation assay showed that purified, activated DNA-PK phosphorylated purified recombinant FH; this phosphorylation was detected by immunoblotting analyses only of specific protein binding (the IP value was normalized to the input). The data represent the mean ± s.d. (n=3 independent experiments). (c, d) DR-GFP-expressing U2OS cells with depleted endogenous FH and reconstituted expression of the indicated FH proteins were transfected with or without a vector expressing I-SceI. PCR analyses for NHEJ were performed 42 h after transfection. A representative image of PCR products digested by I-SceI and BcgI is shown (left panel). The data represent the mean ± s.d. (n=3 independent experiments, right panel). ∗represents P < 0.05 between the FH-depleted cells, with or without WT rFH(N) expression (c), and between the cells expressing WT rFH(N) and rFH(N) T236A (d). Unprocessed original scans of blots are shown in Supplementary Fig. 8.

Next we mutated Scansite analysis-identified potential DNA-PK-phosphorylated residues and found that only the mutation of evolutionarily conserved Thr 236 abolished DNA-PK-mediated FH phosphorylation, as demonstrated by autoradiography and immunoblotting analysis with a specific FH pThr-236 antibody (Fig. 2b and Supplementary Fig. 2b). FH Thr 236 phosphorylation was also detected in ionizing-radiation-treated U2OS cells (Fig. 2c) and blocked by pretreating the cells with NU7441 DNA-PK inhibitor but not KU55933 ATM inhibitor (Supplementary Fig. 2c). These results indicate that DNA-PK phosphorylates FH at Thr 236 in vitro and in vivo.

Cell fractionation analyses showed that the amount of nuclear FH was much lower than that of cytosolic FH, and ionizing radiation exposure enhanced both nuclear and chromatin-associated FH (Supplementary Fig. 2d, left panel). Immunoblotting analyses of the nuclear extract with an anti-FH antibody after immunodepletion with an anti-FH pThr-236 antibody showed that about 23% of nuclear FH

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**Figure 3** DNA-PK-phosphorylated FH promotes the DNA-PK complex accumulation at DSB regions and NHEJ. (a) Thymidine double block-synchronized U2OS cells with depleted endogenous FH and reconstituted expression of the indicated FH proteins were exposed to ionizing radiation (IR; 10 Gy) and collected 1 h after exposure. Chromatin extracts or total cell lysates were prepared. (b) DR-GFP-expressing U2OS cells with depleted endogenous FH and reconstituted expression of the indicated FH proteins were transfected with or without a vector expressing I-SceI. ChIP analyses with antibodies against FH (left panel) or Ku70 (right panel) and F1/R1 primers for the PCR were performed at the indicated time points after I-SceI transfection. The y axis shows the value of the I-SceI-induced fold increase with an anti-phospho-threonine antibody (Supplementary Fig. 2a). The data represent the mean ± s.d. (n=3 independent experiments). (c, d) DR-GFP-expressing U2OS cells with depleted endogenous FH and reconstituted expression of the indicated FH proteins were transfected with or without a vector expressing I-SceI. PCR analyses for NHEJ were performed 42 h after transfection. A representative image of PCR products digested by I-SceI and BcgI is shown (left panel). The data represent the mean ± s.d. (n=3 independent experiments, right panel). ∗represents P < 0.05 between the FH-depleted cells, with or without WT rFH(N) expression (c), and between the cells expressing WT rFH(N) and rFH(N) T236A (d). Unprocessed original scans of blots are shown in Supplementary Fig. 8.

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was phosphorylated (Supplementary Fig. 2d, right panel), and most phosphorylated FH was associated with chromatin on ionizing radiation exposure (Supplementary Fig. 2d, left panel). In line with these findings, exposure to ionizing radiation enhanced the co-localization of γH2AX with FH pThr-236 (Fig. 2d), and FH T236A expression did not affect ionizing-radiation-induced γH2AX or ATM pSer-1981 phosphorylation. The latter conclusion was supported by ChIP analyses, which showed that DNA-PK-mediated FH phosphorylation is involved in NHEJ and does not affect ATM-regulated γH2AX or HR-dependent DNA repair; they also suggest that ionizing radiation exposure induces the recruitment of FH specifically to the DNA damage regions. The latter conclusion was supported by ChIP analyses, which showed that DNA-PK-mediated FH phosphorylation is involved in NHEJ and does not affect ATM-regulated γH2AX or HR-dependent DNA repair; they also suggest that ionizing radiation exposure induces the recruitment of FH specifically to the DNA damage regions.
phosphorylated FH Thr 236 was primarily located in the region within 0.5 kilobases (kb) of the I-SceI cutting site in U2OS cells (Fig. 2e). Consistent with a previous report \(^1\), FH depletion did not affect the initial stage of H2AX accumulation but affected the duration of γH2AX accumulation on exposure to ionizing radiation (Supplementary Fig. 2f, bottom panel), suggesting that FH participates in DNA repair at a late stage in an FH pThr-236-independent manner.

**DNA-PK-phosphorylated FH promotes the DNA-PK complex accumulation at DSB regions and NHEJ**

To determine whether ionizing-radiation-induced FH Thr-236 phosphorylation is dependent on mitochondrial localization of FH, we depleted endogenous FH and reconstituted the expression of RNai-resistant rFH(N), in which the 21 amino-terminal amino acids containing the mitochondrial localization sequence were deleted \(^{21,22}\), rFH(N) T236A, or full-length rFH (FL; Supplementary Fig. 3a). Immunofluorescence analysis confirmed that rFH(N) and rFH(N) T236A were not localized in mitochondria (data not shown). rFH(N) showed efficient binding to chromatin (Supplementary Fig. 3b) and phosphorylation at Thr 236 compared with its full-length counterpart on exposure to ionizing radiation (Supplementary Fig. 3c), indicating that FH Thr 236 phosphorylation is independent of its mitochondrial localization. In contrast, FH T236A failed to associate with chromatin (Fig. 3a) and bind to the I-SceI cutting site (Fig. 3b, left panel) and reduced Ku70 accumulation at DSB regions (Fig. 3b, right panel). These results indicate that the DNA-PK complex and FH regulate each other and that DNA-PK-dependent FH phosphorylation promotes the accumulation of FH and the DNA-PK complex at DSB regions.

To determine the role of FH Thr 236 phosphorylation in DNA repair, we used a PCR strategy and a DR-GFP system to analyse NHEJ repair and HR, respectively (Supplementary Fig. 3d)\(^{19,20}\). The NHEJ-regulated repair of I-SceI-induced DSBs in the GFP locus...
Figure 6  Fumarate produced by chromatin-associated FH inhibits KDM2B-mediated H3K36me2 demethylation. In a,b,e,f,i, immunoblotting was performed with the indicated antibodies. Data represent 1 out of 3 experiments. In c–h, j, the data represent the mean ± s.d. (n = 3 independent experiments). In c,d,f–h, * represents P < 0.05 between the indicated samples and the counterparts without adding monoethyl fumarate. (a,b) Thymidine double block-synchronized GSC11 cells with depleted FH and reconstituted expression of the indicated FH proteins were exposed to ionizing radiation (IR; 10 Gy) and collected 1 h after exposure. Chromatin extracts were prepared at the indicated time points after exposure to ionizing radiation. (c,d,g,h,j) DR-GFP-expressing U2OS cells with depleted endogenous FH and reconstituted expression of the indicated FH proteins (c,g, j left panel) or with depleted endogenous H2A.Z and reconstituted expression of the indicated H2A.Z proteins (d,h, j right panel) expressed or did not express KDM2B shRNA and/or I-Scel (c,d, j). (c,d) ChIP analyses were performed with antibodies against H3K36me2 and Ku70. (g,h) The cells were incubated with or without the indicated concentration of monoethyl fumarate for 20 h after I-Scel transfection. ChIP analyses were performed with an anti-H3K36me2 antibody. (j) An NHEJ analysis was performed 42 h after I-Scel transfection. A representative image of PCR products digested by I-Scel and BcgI is shown. * represents P < 0.01 between the indicated samples and the counterparts without KDM2B shRNA. (e) U2OS cells with depleted FH and reconstituted expression of the indicated FH proteins were expressed with the indicated H3 proteins. ChIP analyses with an anti-Ku70 antibody were performed 30 h after I-Scel transfection. # denotes no statistical significance between the indicated samples and the WT counterparts. (f) DR-GFP-expressing U2OS cells with or without expression of Flag-KDM2B were incubated with or without the indicated concentration of monoethyl fumarate for 20 h after I-Scel transfection. ChIP analyses were performed with an anti-H3K36me2 antibody. (i) An NHEJ analysis was performed 42 h after I-Scel transfection. A representative image of PCR products digested by I-Scel and BcgI is shown. * represents P < 0.01 between the indicated samples and the counterparts without KDM2B shRNA.

resulted in a 0.65-kb PCR fragment that was resistant to both I-Scel and BcgI digestion (Supplementary Fig. 3e). As expected, depletion of Ku70 (Supplementary Fig. 3e) and FH (Fig. 3c) largely dampened NHEJ-regulated repair, and this defect induced by FH depletion was rescued by reconstituted expression of rFH(N) but not FH(N)T236A (Fig. 3c,d) whereas FH depletion and reconstituted expression of rFH(N) and FH(N)T236A had no effect on the efficiency of DSB production by I-Scel (Supplementary Figs 3f,g). In contrast, reconstituted expression of FH(N) and FH(N)T236A did not affect the HR-regulated repair of the I-Scel-induced DSB, which was represented by HR-restored GFP expression (Supplementary Fig. 3h). These results indicate that DNA-PK-regulated FH...
pThr-236 plays an instrumental role in NHEJ but not HR-mediated DNA repair.

The binding of FH pThr-236 to H2A.Z promotes the DNA-PK complex accumulation at DSB regions and NHEJ

H2A.Z mediates the association between FH and chromatin on DNA damage (Fig. 1 c,f, bottom left panel). To determine the relationship between H2A.Z and FH Thr 236 phosphorylation, we pretreated U2OS cells with NU7441, treated immunoprecipitated FH with calf intestinal alkaline phosphatase (CIP), or expressed FH(N) T236A. We demonstrated that DNA-PK inhibition, CIP treatment (Fig. 4 a), and FH(N) T236A expression (Fig. 4 b) blocked ionizing-radiation-induced interaction between FH and H2A.Z. Of note, purified WT His–FH failed to bind to purified H2A.Z (Fig. 4 c). However, the inclusion of DNA-PK enabled WT His–FH but not His–FH T236A to bind to H2A.Z (Fig. 4 c, left panel). These results indicate that DNA-PK-mediated FH phosphorylation promotes the binding of FH to H2A.Z. Consistent with this finding, purified phosphorylation-mimic FH T236D mutant directly bound to H2A.Z in vitro (Fig. 4 c, right panel). In response to DNA damage, FH T236D largely increased its association with chromatin (Fig. 4 d) and accumulation at DSB regions (Fig. 4 e) and this increase was blocked by Ku70 depletion. In addition, ionizing-radiation-induced binding of FH T236D to H2A.Z was also markedly impaired by Ku70 depletion (Fig. 4 f). These results suggest that the DNA-PK complex, in addition to recruiting and phosphorylating FH at DSB regions, alters the local chromatin structure and makes H2A.Z accessible for interaction with phosphorylated FH.

The M6 cassette that encompasses amino acids 89–100 of H2A.Z specifically interacts with other proteins. The main differences between the M6 cassettes of H2A.Z and H2A, which had limited binding to FH (Fig. 1 b), reside in the small αC helix (ref. 23). Replacing DSLI in the αC helix of H2A.Z with the corresponding NKLLG in H2A showed that the purified H2A.Z(NKLLG) mutant lost its binding to purified FH in vitro (Fig. 4 g). In addition, the Flag–H2A.Z(NKLLG) mutant failed to bind to FH in endogenous

Figure 7 Fumarate produced by chromatin-associated FH promotes cell survival. In a, b, immunoblotting analyses were performed with the indicated antibodies. Data represent 1 out of 3 experiments. In c, d, cell viability was examined 12 h after exposure to ionizing radiation (IR) by using clonogenic assay. The data represent the mean ± s.d. (n = 3 independent experiments). (a) Thymidine double block-synchronized U87 cells with depleted endogenous FH and reconstituted expression of FH WT or T236A were exposed to ionizing radiation at the indicated doses and collected at 1 h after exposure (left panel) or were exposed to ionizing radiation (10 Gy) and collected at the indicated time points (right panel). Chromatin extracts were prepared. (b) Thymidine double block-synchronized U87 cells with depleted endogenous FH and reconstituted expression of the indicated FH proteins were exposed to ionizing radiation (10 Gy) and collected at 1 h after exposure. Nuclear fractions and chromatin extracts were prepared. (c) U2OS cells with depleted endogenous FH and reconstituted expression of FH proteins were exposed to ionizing radiation at the indicated doses. (d) U2OS cells with depleted endogenous FH and reconstituted expression of the indicated FH proteins (left panel) or with depleted endogenous H2A.Z and reconstituted expression of the indicated H2A.Z proteins (right panel) were transfected with or without a vector expressing KDM2B shRNA and exposed to ionizing radiation (6 Gy). Unprocessed original scans of blots are shown in Supplementary Fig. 8.
H2A.Z-depleted U2OS cells on exposure to ionizing radiation (Fig. 4h and Supplementary Fig. 4a). Furthermore, expression of the H2A.Z (NKLLG) mutant, which accumulated at DSB regions like its WT counterpart (Supplementary Fig. 4b), largely reduced its binding of FH (Fig. 4i, top panel; Supplementary Fig. 4b) and Ku70 (Fig. 4i, bottom panel) at DSB regions. Analyses of NHEJ showed that H2A.Z depletion (Fig. 4j) or reconstituted expression of rH2A.Z (NKLLG; Fig. 4k), which did not affect the efficiency of DSB production by I-SceI (Supplementary Fig. 4c), inhibited DSB repair. These results indicate that the interaction between H2A.Z and FH pThr-236 is critical for the binding of FH and the DNA-PK complex at DSB regions and for NHEJ-mediated DNA repair.

**Fumarate produced by chromatin-associated FH promotes NHEJ**

To determine whether FH activity is involved in NHEJ repair, we examined the chromatin-associated FH activity in converting malate to fumarate. As shown in Fig. 5a, ionizing radiation exposure enhanced comparable chromatin-associated activity between FLAG-tagged WT FH(N) and FH(N) T236D mutant, which was in line with the increased enrichment of FH in chromatin (Figs 1a and 4d). In contrast, the catalytically inactive FH(N) R233H mutant, which was phosphorylated and recruited to chromatin (Supplementary Fig. 5a, left panel), exhibited little activity, and FH(N) T236A, which had similar activity to its WT counterpart and FH(N) R233H, which retained its ability to co-immunoprecipitate with H2A.Z (Supplementary Fig. 5a, right panel) and bind it in vitro (Supplementary Fig. 5c), was able to translocate to I-SceI cutting regions (Fig. 5b); however, it acted like FH T236A, reducing Ku70 accumulation at the DSB regions (Fig. 5c) and blocking the repair of I-SceI-induced DSB by NHEJ (Fig. 5d). These results indicate that FH activity is required for DSB repair by NHEJ.

FH-depletion-damped DNA repair can be partially rescued by high concentrations of fumarate, indicating the important role of fumarate in this process. Enzymatically active FH T236A failed to mediate DSB repair, suggesting that local accumulated fumarate in DNA damage regions is critical for DNA repair. This assumption was supported by the addition of various amounts of exogenous monoethyl fumarate into the cell medium, which resulted in accordingly increased concentrations of intracellular fumarate (up to 15-fold; Supplementary Fig. 5d). Figure 5c shows a partial rescue dependent on fumarate dosage of Ku70 accumulation at DSB regions and restoration of NHEJ repair (Fig. 5d) in U2OS cells expressing FH R233H or FH T236A mutants. In contrast, the addition of exogenous malate in a high dosage, which cannot be converted into fumarate in the DNA damage regions by FH R233H or FH T236A, did not exert any effect on Ku70 binding at DSB regions for NHEJ repair (Supplementary Fig. 5e) or NHEJ repair (Supplementary Fig. 5f). Intriguingly, reconstituted expression of inactive FH(N) R233H, but not of its WT counterpart or enzyme activity-intact FH(N) T236A, in endogenous FH-depleted cells increased total cellular fumarate production, as did FH depletion (Supplementary Fig. 5g). Compared with FH(N) T236A expression, expression of FH(N) R233H resulted in less dampened Ku70 accumulation at DSB regions (Fig. 5c) and DNA

**Figure 8** A schematic model showing that DNA-PK-mediated phosphorylation of fumarase promotes DNA repair by local fumarate-inhibited histone H3 demethylation. Ionizing radiation (IR) induces DNA-PK-mediated phosphorylation of FH Thr 236, leading to the binding of FH to H2A.Z adjacent to DSBs. Local FH-produced fumarate inhibits KDM2B, which results in enhanced H3K36me2 and accumulation of DNA-PK at DSBs for NHEJ.

**Fumarate produced by chromatin-associated FH inhibits KDM2B-mediated H3K36me2 demethylation**

Fumarate can inhibit α-ketoglutarate (αKG)-dependent dioxygenases, altering histone methylation. H3K36 dimethylation improves the association of Ku70 at DSBs (ref. 10). As expected, H3K36me2, but not H3K9me2, H3K9me3 or H3K27me2, was markedly increased at the DSBs induced by I-SceI expression (Supplementary Fig. 6a). In addition, H3K36me2 primarily accumulated in the region within 0.5 kb of the I-SceI cutting site (Supplementary Fig. 6b),
and expression of the H3K36R mutant largely compromised the recruitment of Ku70 to I-SceI cutting sites (Supplementary Fig. 6c). To determine whether chromatin-associated FH regulates H3K36me2 and the subsequent Ku70 accumulation at DSB regions, we performed immunoblotting analyses and showed that reconstituted expression (Supplementary Fig. 6d) of rFH(N) T236A (Fig. 6a) or H2A.Z (NKLLG; Fig. 6b) largely inhibited ionizing-radiation-induced chromatin-associated H3K36me2. Consistent with these findings, reconstituted expression of rFH T236A, rFH R233H (Fig. 6c, left panel) and H2A.Z (NKLLG; Fig. 6d, left panel) notably suppressed H3K36me2 at the DSB regions. In addition, rFH T236A or rFH R233H expression did not further reduce H3K36R-suppressed Ku70 accumulation at DSB regions (Fig. 6e). These results indicate that chromatin-associated FH regulates H3K36me2 and the subsequent accumulation of the DNA-PK complex at DSB regions.

KDM2B preferentially demethylates H3K36me2 (ref. 12). KDM2B depletion (Supplementary Fig. 6e) rescued rFH(N) R233H-, rFH(N) T236A- and H2A.Z(NKLLG)-suppressed H3K36me2 at DSB regions (Fig. 6c,d, left panel) and Ku70 accumulation at DSB regions (Fig. 6c,d, right panel). Notably, expression of these mutants did not affect the localization of KDM2B at the DSB regions (Supplementary Fig. 6f). These results strongly suggest that chromatin-associated FH regulates H3K36me2 by inhibiting KDM2B.

To determine the involvement of fumarate in KDM2B-mediated H3K36me2 demethylation, we overexpressed KDM2B and added exogenous monoethyl fumarate to the cell medium. Figure 6f shows that ectopic expression of KDM2B reduced H3K36me2 levels at the DSB regions; this suppression was counteracted by exogenous fumarate in a dosage-dependent manner. In addition, exogenous fumarate partially rescued the suppression of H3K36me2 at the DSB regions induced by the expression of FH T236A, FH R233H (Fig. 6g) or H2A.Z (NKLLG; Fig. 6h). Furthermore, mixing purified KDM2B with chromatin extracted from WT FH-expressing GSC11 cells demethylated ionizing-radiation-induced H3K36me2 (Fig. 6i). However, this demethylation was inhibited by adding malate in a dosage-dependent manner (Fig. 6i), and this demethylation inhibition was abrogated by inclusion of αKG (Supplementary Fig. 6g). In sharp contrast, malate did not affect H3K36me2 levels in cells expressing rFH R233H that lost its ability to convert malate to fumarate (Fig. 6i).

We next determined the effect of FH-regulated H3K36me2 on NHEJ-mediated DSB repair. As shown in Fig. 6j, KDM2B depletion in U2OS rescued impaired DSB repair in cells expressing rFH T236A, rFH R233H (left panel) and H2A.Z (NKLLG; right panel). These results indicate that FH-produced fumarate promotes NHEJ-dependent DNA repair by inhibiting KDM2B-mediated demethylation at DSB regions.

**Fumarate produced by chromatin-associated FH promotes cell survival**

We found that ionizing-radiation-induced and FH phosphorylation-regulated H3K36me2 occurred in both U2OS and GSC11 cells. To determine whether this finding also applied to other cell lines, we deleted FH or H2A.Z and reconstituted the expression of rFH(N) T236A (Supplementary Fig. 7a), H2A.Z (NKLLG; Supplementary Fig. 7b), or their WT counterparts in U87 GBM cells, HeLa cervical cancer cells, and A549 non-small lung cancer cells. In contrast to the WT protein expression, rFH T236A (Fig. 7a and Supplementary Fig. 7c) and H2A.Z (NKLLG; Fig. 7b and Supplementary Fig. 7d) expression largely reduced ionizing-radiation-enhanced chromatin-associated FH pThr-236 and H3K36me2. These results indicate that FH-phosphorylation-regulated H3K36me2 occurs in different types of cancer cell.

Successful DNA repair prohibits DNA damage-induced cell death. Clonogenic assay showed that exposure to ionizing radiation enhanced the death rate of U2OS cells expressing rFH T236A (Fig. 7c) or H2A.Z (NKLLG; Fig. 7d, right panel) compared with that of cells with expression of their WT counterparts; this enhanced cell death by the mutant protein expression was blocked by KDM2B depletion (Fig. 7d and Supplementary Fig. 7e). Similar results were obtained through trypan blue analyses of different types of cancer cell (Supplementary Fig. 7f,g). These results indicate that FH phosphorylation regulates NHEJ by antagonizing H3 demethylation and promotes cell survival in response to exposure to ionizing radiation.

**DISCUSSION**

Metabolic enzymes execute their metabolic functions in cytosol and mitochondria. However, in response to extracellular stimuli, these enzymes, which include PKM2, FH and MATII (a methionine adenosyltransferase isozyme), possess functions that are not directly linked to metabolism regulation when they alter their subcellular localization12–15. In this report, we demonstrated that exposure to ionizing radiation results in the binding of FH to H2A.Z at DSB regions in a manner that is dependent on DNA-PK-mediated FHThr236 phosphorylation. FH pThr-236, which dissociated from DNA-PK, interacted with the adjacent αC helix of H2A.Z, leading to DSB-region-enriched FH and the subsequent fumarate production in this region. The locally accumulated FH inhibited KDM2B activity at DSB regions and enhanced H3K36me2, which in turn promoted the accumulation of the DNA-PK complex at DSB regions for subsequent NHEJ-mediated DNA repair and cell survival (Fig. 8). The results of this study underscore the instrumental role of chromatin-associated FH in ionizing-radiation-induced DSB repair.

HR, which requires complete histone eviction adjacent to the DSBs, regulates DNA repair during S–G2 phase5. In our study, FH increased its association with chromatin after exposure to ionizing radiation. In addition, DNA-PK, which is active at the early stage of the NHEJ process, phosphorylates FH Thr 236. Importantly, FH T236A expression, which had little effect on HR-regulated DSB repair, blocked H3K36me2 at DSB regions and impaired the function of NHEJ. These results revealed that FH Thr 236 phosphorylation plays an important role in NHEJ-mediated DNA repair in a cell-cycle-dependent manner. As FH is also involved in regulating γH2AX (ref. 15), which was not affected by FH T236A expression (data not shown), these findings suggest that FH participates in HR repair independently of the DNA-PK-mediated FH phosphorylation.

FH Thr 236 is rapidly phosphorylated by DNA-PK at the early phase of NHEJ in response to ionizing radiation exposure. FH depletion or FH T236A expression had less effect in the early phase than in the late stage on the binding of the DNA-PK complex to the DNA open end. These results suggest that FH Thr 236 prevents the dissociation of DNA-PK from chromatin and are in...
line with the previous observation that H3K36me2 in DSB regions decreased the rates of dissociation of DNA-PK from chromatin. These findings imply that accumulated local fumarate production by FH and enhanced H3K36me2 at DNA damage regions stabilizes the repair components of NHEJ at the DSBs. Our studies reveal a feedback mechanism for regulating DNA-PK and thus NHEJ-mediated DSB repair through DNA-PK-mediated FH Thr236 phosphorylation, fumarate-dependent H3K36me2, and the subsequent maintenance of the DNA-PK complex at the DSB regions for recruiting other DNA repair components.

Chromatin-localized metabolite biosynthesis plays an important role in nucleosome and chromatin modification. For instance, protein acetyltransferases require acetyl-CoA as a donor of acetyl groups in poly(ADP-ribosyl)ation reactions. Poly(ADP-ribose) polymerases require acetyl-CoA as a donor of acetyl groups in regulating histone H3 methylation and DNA repair. These findings imply that accumulated local fumarate production by FH and enhanced H3K36me2 at DNA damage regions stabilize the repair components of NHEJ at the DSBs. Our studies reveal a feedback mechanism for regulating DNA-PK and thus NHEJ-mediated DSB repair components.

Note: Supplementary Information is available in the online version of the paper.

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AUTHOR CONTRIBUTIONS
This study was conceived by Z.L.; Z.L. and Y.J. designed the study; Y.J., X.Q., J.S., Y.W., X.L., R.L. and Y.X. performed experiments; Q.C., G.P. and S.-Y.L. provided reagents and conceptual advice; Z.L. wrote the paper with comments from all authors.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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METHODS

Cell culture and synchronization. U2OS cells (ATCC) were maintained in McCoy’s 5A medium supplemented with 10% fetal bovine serum (FBS). U87, HeLa and AS49 cells (ATCC) were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% bovine cell serum (HyClone). Human primary GSC11 cells (M.D. Anderson Cancer Center) were maintained in DMEM/F-12 50/50 supplemented with B27, EGF (10 ng ml⁻¹) and basic fibroblast growth factor (10 ng ml⁻¹).

Cells (30–40% in confluence) were washed twice with phosphate-buffered saline (PBS), treated with 2 mM thymidine for 17 h, washed twice with PBS again, released in complete medium containing 10 μM deoxyuridine for 9 h, treated with 2 mM thymidine for 17 h, and released in complete medium with 10 μM deoxyuridine before being assayed.

Immunoprecipitation and immunoblotting analysis. Proteins were extracted from cultured cells using a modified buffer, followed by immunoprecipitation and immunoblotting with the corresponding antibodies, as described previously²⁹.

Chromatin extract isolation. Cells (2 × 10⁶) were washed with PBS and resuspended in 200 μl of solution A (10 mM HEPES [pH 7.9], 10 mM KCl, 1.5 mM MgCl₂, 0.34 M sucrose, 10% glycerol, 1 mM diithiothreitol, 10 mM NaF, 1 mM Na₂VO₃, and protease inhibitors). Triton X-100 was added to a final concentration of 0.1%, and the cells were incubated for 5 min on ice, followed by low-speed centrifugation (4 min at 1,300 g at 4°C) to separate the cytoplasmic proteins from the nuclei. The isolated nuclei were then lysed in 200 μl of solution B (5 mM ethylenediaminetetraacetic acid, 0.2 mM EDTA, 1 mM diithiothreitol, and protease inhibitors). Insoluble chromatin was collected by centrifugation (4 min at 1,700 g at 4°C), washed once with solution B, and centrifuged at high speed (10,000g) for 1 min. The chromatin pellet was resuspended in Laemmli buffer and sonicated for 15 s. For immunoprecipitation and GST pulldown assays, the chromatin pellet was subjected to sonication and suspended with micrococcal nuclease (MNase) buffer (20 mM Tris-HCl, 1 mM CaCl₂, 2 mM MgCl₂, 0.1 mM KCl, 0.1% Triton X-100, 0.3 mM sucrose, 1 mM diithiothreitol, 1 mM benzamidine, 0.2 mM phenylmethylsulphonyl fluoride, 1 mM STZ (pH 7.9), and 0.5 μg ml⁻¹ ethidium bromide). The samples were incubated with 50 units of micrococcal nuclease for 30 min at room temperature. The reaction was stopped by adding 5 mM EDTA and 5 mM EDTA. After centrifugation at 3,000g for 30 min at 4°C, the supernatant was passed through a WGA agarose column (Vector). The flow-through fraction was mixed with anti-Flag M2 agarose beads and rotated for 12 h at 4°C. Finally, the chromatin extract was washed three times with buffer (20 mM Tris-HCl, 0.2 mM EDTA, 5 mM MgCl₂, 0.1 mM KCl, 0.05% NP-40, 10% glycerol, 1 mM diithiothreitol, 1 mM benzamidine, 0.2 mM phenylmethylsulphonyl fluoride and 1 mM STZ (pH 7.9)).

ChIP assay. A ChIP assay was performed using an Upstate Biotechnology kit, as described previously²⁹. Quantitative real-time PCR was used to measure the amount of bound DNA, and the value of enrichment was calculated according to the relative amount of input and the ratio to IgG. The specific primers used in PCR for the sequences that were 0.2 kb, 0.4 kb and 0.6 kb distant from I-SceI-induced DSBs were 5'-GATCGACGACGAGACGACCC-3' (forward) and 5'-GAA CAGCTTCTTCGGCCTTG-3' (reverse), 5'-TTATTGTTGCTGTCTCTACA TT-3' (forward) and 5'-TGCTCTGATGCTCTGGCACT-3' (reverse), and 5'-TACATCCATGGGCGCTGCTG-3' (forward) and 5'-AGCTTTTACAGGCC GCGCTTCA-3' (reverse), respectively.

Clonogenic cell survival assay. The indicated cells (5 × 10⁴) were plated in 100-mm dishes and treated with various doses of ionizing radiation (0, 2, 4, and 6 Gy). Cells were then re-cultured for 10 days and stained with 0.5% crystal violet in methanol to determine colony-formation efficiency. The cluster of staining cells was considered a colony at more than 50 cells.

DNA constructs and mutagenesis. The Flag-tag of FH in the pcDNA6 vector is located at the carboxy terminus of FH. Flag–FH(N) with deletion of 21 N-terminal amino acids was constructed by PCR reactions. pcDNA 3.1/hygro (+) FH T236A and H2AZ(NKLLG) were created using the QuikChange site-directed mutagenesis kit (Stratagene). pGIPZ human FH shRNA was generated using the 5'-GGGATAATGTGTGTTATATG-3' oligonucleotide. pGIPZ human H2AZ shRNA was generated with the oligonucleotides 5'-TCTAGAGCAACGACTGATG-3' and 5'-TACATCTGTTGCTGACTCCA-3'. pGIPZ human KDM2B shRNA was generated with the oligonucleotide 5'-GCTTCCACCTGATGTGTTAA-3'. Two pGIPZ human Ku70 shRNAs were generated with the oligonucleotides 5'-GACACAGGGGAGAATAT A-3' and 5'-TCACGTGTACACTGAGA-3'. The pGIPZ controls were generated in 50 mM ammonium bicarbonate buffer containing RapiGest (Waters Corp). The sample was heated to 95°C for 10 min and allowed to cool down before 100 ng of sequencing-grade modified trypsin (Promega) was added. The digestion proceeded overnight at 37°C and was analysed by LC-MS/MS using an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific).

Proteins were identified by comparing the fragment spectra against those in the SwissProt protein database (EBI) using Mascot v.2.3 (Matrix Science) and Sequest v.1.20 via Proteome Discoverer v.1.3 (Thermo Fisher Scientific) software.
with control oligonucleotide 5′-GCTTCTAACCGGAGGTCTT-3′ or 5′-GCCGAAAGGGTCCAGCTTA-3′.

DNA-PK kinase assay. Purified DNA-PK complexes were purchased from Promega. This complex is composed of an approximately 460-kDa catalytic subunit and heterodimeric DNA-binding subunits, including Ku80 and Ku70. Kinase reactions were performed as described previously. In brief, the purified DNA-PK complex was incubated with FH (100 ng) in kinase buffer with 50 mM HEPES (pH 7.5), 1 mM dithiothreitol, 0.1 mM EDTA, 0.2 mM EGTA, 10 mM MgCl₂, 0.1 M KCl, 80 μg ml⁻¹ bovine serum albumin, 10 μg ml⁻¹ linear double-stranded DNA, and 40 Ci ml⁻¹ [γ-³²P]ATP in 30 μl at 37°C for 1 h. DNA-PK was added last. The reaction mixtures were incubated at 30°C for 10 min and terminated by adding sodium dodecyl sulphate–polyacrylamide gel electrophoresis loading buffer and heating to 100°C.

NHEJ repair analysis and PCR analysis of I-SceI-induced DSBs. The genomic DNA of cells with or without I-SceI expression was extracted. PCR was performed as previously described using primers that flanked the DSB site and PCR products were digested with I-SceI and BcgI (ref. 19). After gel electrophoresis of PCR products, the intensity of the enzyme-resistant and enzyme-cleaved fragments were measured using NIH IMAGE software. To assay the cutting efficiency of I-SceI in cells, genomic DNA was extracted and adjusted to equal concentrations.

Semiquantitative PCR was carried out using the same primers for NHEJ analysis and PCR analysis of I-SceI-induced DSBs. The genomic DNA of cells with or without I-SceI expression was extracted. PCR was performed as previously described using primers that flanked the DSB site and PCR products were digested with I-SceI and BcgI (ref. 19). After gel electrophoresis of PCR products, the intensity of the enzyme-resistant and enzyme-cleaved fragments were measured using NIH IMAGE software. To assay the cutting efficiency of I-SceI in cells, genomic DNA was extracted and adjusted to equal concentrations.

HR repair analysis. U2OS cells containing a single copy of the HR repair reporter substrate DR-GFP were used. GFP-expressing plasmid (pEGFP-C1) was used for transfection efficiency control. Seventy-two hours after transfection of a vector expressing DR-GFP, cells were re-seeded and transfected with mock or pCBASce plasmid. Forty-eight hours later, a flow cytometry analysis was performed to detect GFP-positive cells using a FACS Calibur apparatus with Cellquest software (Becton Dickinson) at the MD Anderson Flow Cytometry Facility. To induce chromatin relaxation, cells were incubated for 16 h in sodium butyrate (3 mM) or trichostatin A (200 ng ml⁻¹) before being analysed by flow cytometry.

Flow cytometry analysis. Cells (1 × 10⁶) were fixed in 70% ethanol on ice for 3 h, spun down, and incubated for 1 h at 37°C in PBS with DNase-free RNase A (100 μg ml⁻¹) and propidium iodide (50 μg ml⁻¹). Cells were then analysed by fluorescence-activated cell sorting.

Recombinant protein purification. WT and mutant His–FH and GST–H2A.Z were expressed in bacteria and purified, as described previously.

Enzyme activity assay. To measure FH activity, we collected chromatin extract from cells 1 h after exposure to ionizing radiation (10 Gy). Immunoprecipitated Flag–FH proteins were eluted using a Flag peptide. Eluted proteins were added to an enzyme assay buffer (50 mM malate and 10 mM potassium phosphate at pH 7.5), and the absorbance at OD₅₅₀ was recorded.

Fumarate measurement. Relative fumarate concentrations in tumour cells were measured using the Fumarate Detection Kit purchased from Abcam.

Statistical analysis. Statistical analysis was conducted with the two-tailed unpaired Student’s t-test. All data represent the mean ± s.d. of three independent experiments.
Author Correction: Local generation of fumarate promotes DNA repair through inhibition of histone H3 demethylation

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Correction to: Nature Cell Biology https://doi.org/10.1038/ncb3209, published online 3 August 2015.

In the version of this Article originally published, the authors mistakenly used the same images for three different time points in Fig. 2d: images for 24 h post-IR WT were also used for 0 h post-IR WT (top row, left panels) and for 1 h post-IR, T236A mutant (2nd row from the top, right panels). The correct images from these experiments have now been inserted in the figure in all online versions of the Article. In addition, results from this experiment and from two additional independent experiments have been uploaded to Figshare and the sentence 'Additional repeats of this experiment have been uploaded to Figshare28.' has been added to the figure legend to highlight this fact. The Figshare doi has been added as reference 28. The new reference and corrected Fig. 2d are shown below.

28. Jiang, Y. et al. FH pT236-gammaH2AX-1-2-3.tif. Figshare https://doi.org/10.6084/m9.figshare.5867907 (2018).

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**Supplementary Figure 1** IR induces the association of FH with chromatin. 

**a**, U2OS cells synchronized by thymidine double block (2 mM) underwent no release (G1 phase) or release for 2 h (S phase) or 6 h (G2 phase). These cells were then exposed to IR (10 Gy) and analyzed by an immunoblotting assay with the indicated antibodies and flow cytometry. Data represent one out of 3 experiments. 

**b**, GSC11 cells that were synchronized by thymidine double block (2 mM) were not released (G1 phase) or were released for 2 h (S phase) or 6 h (G2 phase). These cells then underwent IR (10 Gy) and were harvested at the indicated time points. Chromatin extracts were prepared. CENP-A was used as a control for chromatin-associated proteins. Immunoblotting analyses were performed with the indicated antibodies. Data represent one out of 3 experiments. 

**c**, Thymidine double block-synchronized U2OS cells expressing Flag-FH were exposed to IR (10 Gy). Chromatin extracts were subjected to immunoprecipitation with an anti-FH antibody and analyzed by mass spectrometry. FH-interacting histone proteins in the cells with or without IR treatment identified by MS analysis were shown.

**List of FH-interacting Histone proteins in cells with or without IR treatment identified by MS analysis.**

| Peptide hits | Protein name |
|--------------|--------------|
| IR - | + |
| 2 | 12 | H3.3 |
| 1 | 6 | H2A.Z |
| 3 | 8 | H2B1C |
| 0 | 3 | H2B1B |
| 1 | 2 | H2AH |
| 1 | 4 | H4 |
Supplementary Figure 2 DNA-PK phosphorylates FH at T236. Data represent one out of 3 experiments unless specifically indicated. 

a, In vitro phosphorylation analyses were performed by mixing the purified DNA-PK complex with bacterially purified His-FH in the presence of $[^{32}P]ATP$. Immunoblotting analyses were performed with the indicated antibodies.

b, The specificity of this FH pT236 antibody was validated using a specific phosphorylation-blocking peptide. In vitro phosphorylation analyses were performed by mixing the purified DNA-PK complex with bacterially purified His-FH. Immunoblotting analyses were performed with the indicated antibodies in the presence or absence of a phospho-specific blocking peptide for the FH pT236 antibody.

c, Thymidine double block-synchronized U2OS cells, pretreated with or without NU7441 (1 μM) or KU55933 (10 μM) underwent IR (10 Gy). Immunoblotting analyses were performed with the indicated antibodies.

d, U2OS cells were harvested 1 h after IR (10 Gy). The cytosolic and nuclear fractions and chromatin-associated proteins were prepared. The FH expression in each fraction was quantified and normalized by actin levels in harvested cells (left panel). The nuclear fractions of IR-treated cells were immuno-depleted by normal IgG or an anti-FH pT236 antibody and immunoblotted with the indicated antibodies (right panel). CE: chromatin extract; NE: nuclear extract; Cy: cytosol.

e, U2OS cells with depleted endogenous FH and reconstituted expression of the indicated FH proteins were irradiated with IR (3 Gy). Immunofluorescence analyses with the indicated antibodies were performed at 1 h after IR. Bar, 10 μM.

f, U2OS cells with or without depleted endogenous FH and reconstituted expression of the indicated FH proteins were irradiated with IR (10 Gy) and harvested at the indicated time points. Immunoblotting analyses were performed with the indicated antibodies.
Supplementary Figure 3 DNA-PK-phosphorylated FH promotes the DNA-PK complex accumulation at DSB regions and NHEJ. a, b, c, Immunoblotting analyses were performed with the indicated antibodies. a, U2OS cells were expressed with a vector for control shRNA or FH shRNA and reconstituted with expression of rFH (N) and rFH (N) T236A (left panel) or rFH at full length (right panel). rFH (FL) underwent a N-terminal cleavage in mitochondria and was redistributed in mitochondria and cytosol. b, c, Thymidine double block-synchronized U2OS cells with depleted endogenous FH and reconstituted expression of the indicated FH proteins were exposed to IR (10 Gy) and harvested 1 h after IR. The chromatin extracts were prepared. d, A schematic of NHEJ and HR repair at the DR-GFP locus, which is described in Material and Method section. e, DR-GFP-expressed U2OS cells with depleted Ku70 expressed or did not express I-SceI. PCR analyses for NHEJ were performed 42 h after transfection. A representative image of PCR products digested by I-SceI and BcgI is shown (left panel). The data represent the mean ± SD (n=3 independent experiments, right panel). * stands for P < 0.05 between the cells with or without expressing Ku70 shRNA. f, A schematic of the efficiency of I-SceI-dependent cleavage at the DR-GFP locus, which is described in Material and Method section. g, h, DR-GFP-expressed U2OS cells with FH depletion and with or without reconstituted expression of WT rFH (N) or rFH (N) T236A expressed or did not express I-SceI. (g) PCR analyses for I-SceI-uncut ScgGFP were performed 42 h after transfection, as described in Supplementary Figure 2f. Representative images of PCR products were shown (left panel). The data represent the mean ± SD (n=3 independent experiments, right panel). Comparable amount of 0.65 Kb PCR products suggested that the efficiency of DSB production by I-Sce1 in each cell line is similar to each other. (h) Flow cytometry analyses for HR were performed 42 h after transfection (left panel). The data represent the mean ± SD (n=3 independent experiments, right panel). # stands for no statistical significance between expression of WT rFH(N) and rFH(N) T236A.
Supplementary Figure 4  FH binds to H2A.Z.  

**a**, U2OS cells were expressed with a vector for control shRNA or H2A.Z shRNA and reconstituted with expression of WT rH2A.Z or rH2A.Z (NKLLG). Immunoblotting analyses were performed with the indicated antibodies. Data represent one out of 3 experiments.  

**b**, DR-GFP-expressing U2OS cells with depleted H2A.Z and reconstituted expression of the indicated H2A.Z proteins were transfected with a vector that expressed I-SceI. ChIP analyses were performed with an anti-Flag antibody at the indicated time points after I-SceI transfection. Data represent one out of 3 experiments.  

**c**, DR-GFP-expressing U2OS cells with H2A.Z depletion and with or without reconstituted expression of WT rH2A.Z or rH2A.Z (NKLLG) were transfected with a vector with or without expressing I-SceI. PCR analyses for I-SceI-uncut ScgGFP were performed 42 h after transfection, as described in Supplementary Figure 2f. Representative images of PCR products were shown (left panel). The data represent the mean ± SD (n=3 independent experiments, right panel). Comparable amount of 0.65 Kb PCR products suggested that the efficiency of DSB production by I-SceI in each cell line is similar to each other.
Supplementary Figure 5 Fumarate produced by chromatin-associated FH promotes NHEJ. 

a, Chromatin extracts of thymidine double block-synchronized U2OS cells with depleted endogenous FH and reconstituted expression of the indicated FH proteins were collected 1 h after IR. Chromatin extracts were subjected to immunoblotting (left panel) or immunoprecipitation analyses (right panel) with the indicated antibodies. Data represent one out of 3 experiments. 

b, U2OS cells were expressed with the indicated FH-Flag proteins. FH-Flag proteins were immunoprecipitated and the relative FH activity was measured. The data represent the mean ± SD (n=3 independent experiments). 

c, Immobilized, purified GST-H2A.Z protein was mixed with the indicated purified His-FH proteins, with or without DNA-PK. A GST pull-down assay was performed. Immunoblotting analyses were performed with the indicated antibodies. Data represent one out of 3 experiments. 

d, U2OS cells were incubated with the indicated concentrations of monoethyl-fumarate. The relative abundance of intracellular fumarate was measured. The data represent the mean ± SD (n=3 independent experiments). 

e, DR-GFP-expressing U2OS cells with depleted FH and reconstituted expression of the indicated FH proteins were incubated with malate (5 mM) for 20 h after I-SceI transfection. ChIP analyses were performed with an anti-Ku70 antibody. The data represent the mean ± SD (n=3 independent experiments). 

f, DR-GFP-expressing U2OS cells with depleted FH and reconstituted expression of the indicated FH proteins were incubated with malate (5 mM) for 20 h after I-SceI transfection. An NHEJ analysis was performed. A representative image of the PCR products digested by I-SceI and BglI is presented. The data represent the mean ± SD (n=3 independent experiments). 

g, U2OS cells, with or without FH depletion, were reconstituted with expression of the indicated FH proteins before being harvested. The relative abundance of intracellular fumarate was measured. The data represent the mean ± SD (n=3 independent experiments).
Supplementary Figure 6  Fumarate produced by chromatin-associated FH inhibits KDM2B-mediated demethylation at DSB regions.  a, c, f, The data represent the mean ± SD (n=3 independent experiments). d, e, g, Data represent one out of 3 experiments. a, b, U2OS cells were transfected with or without a vector expressing I-SceI. (a) ChIP analyses with antibodies for H3K36Me2, H3K9me2, H3K9me3, and H3K27me2 were performed at the indicated time points after I-SceI transfection. (b) ChIP analyses with the indicated histone H3 methylation antibodies were performed 20 h after I-SceI transfection. The primers described in Fig. 1f were used for the PCR. Control primers were selected against a specific region of chromosome 12. The y-axis stands for the value of I-SceI-induced fold increase of binding of the methylated H3 (the IP value was normalized to the input). c, U2OS cells were expressed with the indicated H3 proteins. ChIP analyses with an anti-Ku70 antibody were performed at the indicated time points after I-SceI transfection. Immunoblotting analyses were performed with the indicated antibodies. d, GSC11 cells were expressed with a vector for control shRNA, FH shRNA (left panel), H2A.Z shRNA (right panel) and were reconstituted with the indicated FH or H2A.Z protein expression. Immunoblotting analyses were performed with the indicated antibodies. e, U2OS cells were expressed with a vector for control shRNA or KDM2B shRNA. Immunoblotting analyses were performed with the indicated antibodies. f, U2OS cells with depleted FH and reconstituted expression of the indicated FH proteins or with depleted H2A.Z and reconstituted expression of the indicated H2A.Z proteins were transfected with a vector expressing I-SceI. ChIP analyses with an anti-KDM2B antibody were performed 30 h after I-SceI transfection. # stands for no statistical significance between the indicated samples and the WT counterparts. g, GSC11 cells with depleted endogenous KDM2B were exposed to IR (10 Gy) and harvested 1 h after IR. Chromatin extracts were incubated with malate (2.5 mM) in the presence or absence of αKG (50 μM or 2.5 mM) for 30 min, followed by incubation with KDM2B.
Supplementary Figure 7 Fumarate produced by chromatin-associated FH promotes cell survival. a–t. Data represent one out of 3 experiments unless specifically indicated. a. The indicated cells were expressed with a vector for control shRNA or FH shRNA and reconstituted with the indicated FH proteins. Immunoblotting analyses were performed with the indicated antibodies. b. The indicated cells were expressed with a vector for control shRNA or H2A.Z shRNA and reconstituted with the indicated H2A.Z proteins. Immunoblotting analyses were performed with the indicated antibodies. c, d. Thymidine double block-synchronized HeLa and A549 cells with depleted endogenous FH and reconstituted expression of the indicated FH proteins (c) or with depleted endogenous H2A.Z and reconstituted expression of the indicated H2A.Z proteins (d) were exposed to IR (10 Gy) and harvested after 1 h. Chromatin extracts were prepared. Immunoblotting analyses were performed with the indicated antibodies. e. The indicated cells were expressed with a vector for control shRNA or KDM2B shRNA. Immunoblotting analyses were performed with the indicated antibodies. f, g. The indicated synchronized cells with depleted endogenous FH and reconstituted expression of the indicated FH proteins (f) or with depleted endogenous H2A.Z and reconstituted expression of the indicated H2A.Z proteins (g) were transfected with or without a vector expressing KDM2B shRNA and exposed to IR (10 Gy). Cell viability was measured 12 h after IR by using the trypan blue assay. The data represent the mean ± SD (n=3 independent experiments).
Supplementary Figure 8 Uncropped Western blots
Supplementary Figure 8 continued
Supplementary Figure 8 continued
Supplementary Figure 8 continued
Fig. 6

Fig. 6a left panel

WB: H3K36Me2

WB: H3

Fig. 6a right panel

WB: H3K36Me2

WB: H3K36Me2

Fig. 6h

WB: H3K36Me2

WB: FH

WB: His-KDM2B

WB: H3

Supplementary Figure 8 continued
Supplementary Figure 8 continued

**Fig. 7**

**Fig. 7a left panel**

- WB: FH pT236
- WB: actin

**Fig. 7a right panel**

- WB: FH pT236
- WB: actin

**Fig. 7b**

- WB: FH pT236
- WB: H3K36Me2
- WB: H3
Fig. S2

Supplementary Figure 8 continued