The nucleoskeleton protein IFFO1 immobilizes broken DNA and suppresses chromosome translocation during tumorigenesis

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Supplementary Figure 1

Sequence alignment of IFFO1 homologues.

IFFO1 orthologues were identified using the BLASTP algorithm, which searches the NR database maintained by NCBI. Human (hu, *Homo sapiens* AAI10388.1), mouse (mu, *Mus musculus* Q8BXL9.1), chicken (ch, *Gallus gallus* XP_015149162.1), frog (xp, *Xenopus laevis* XP_002941733.1) and zebrafish (fish, *Danio rerio* XP_690165.5) orthologues are shown. The identical and conserved amino acids are highlighted with red letters on a yellow background and dark blue letters on a blue background, respectively, weakly similar amino acids are highlighted on a green background, and green letters indicate blocks of similar amino acids. The red boxes indicate the 4 coiled-coil motifs of the intermediate filament family protein. The blue box (NLS) indicates a potential nuclear localization signal. The residues mutated in m1-5 and N73D/R85H (see below in Supplementary Fig. 2l, m) are marked with asterisks.
Supplementary Figure 2

Mapping the interacting regions of IFFO1 with XRCC4 and Lamin A/C.

a, Immunoblotting showing that the Superose 6 fractionation profile of IFFO1 overlaps with those of XRCC4. The fractions corresponding to molecular weight standards are indicated at the top. Inputs of some proteins were cut from the right side of the same gel. b, d Schematic representations of the different IFFO1 (b) and XRCC4 (d) deletion mutants (left) and their ability to coimmunoprecipitate with XRCC4 and IFFO1 (right), respectively. FL, full length. (c, e) Immunoprecipitation to assess whether the various deletion mutants of IFFO1 (c) and XRCC4 (e) co-purified with XRCC4 and IFFO1, respectively. NA, not available. f, Immunoblot showing the IPs of Flag-tagged Lamin A and Lamin C. g, i, Schematic representations of the different Lamin A (g) and IFFO1 (i) deletion mutants (left) and their ability to interact with IFFO1 and Lamin A (right), respectively. FL, full length. h, j, Yeast two-hybrid to assess whether the various mutants of Lamin A (h) and IFFO1 (j) interacted with IFFO1 and Lamin A, respectively. k, Schematic representations of mutations of IFFO1 in cancers. Vertical axis presents the mutation frequency of the indicated residues. l, GST-pulldown to assess whether the recurrent mutations of IFFO1 affect its interaction with Lamin A. The 1A motif of IFFO1 and 2B motif of Lamin A were fused with GST-tag and MBP-tag, respectively, and expressed in E.coli for pulldown assays. The pulldown proteins were analyzed by Coomassie Blue-stained SDS-PAGE gels. m, Immunoblotting to show the immunoprecipitates of FLAG-IFFO1 wild-type and mutants using HEK293 cells. All immunoblots, gels and yeast two-hybrid are representative of three independent experiments; unprocessed scans of immunoblots are shown in Supplementary Fig.9.
Supplementary Figure 3

Generation of IFFO1 and LMNA knockout DT40 cells.

a, Schematic representation of the chicken IFFO1 and targeted genomic DNA. The regions containing exon1-7 (marked by red) of the gene are replaced by blasticidine and histidinol resistant genes. The two regions between two pairs of dotted lines were used as arms of the knock-out constructs. Arrows indicated targeted location of primers for genomic PCR. b, Genome-PCR analysis to show that IFFO1 gene is undetectable in the respective knockout DT40 cells. XLF was included as a positive control. c, Immunoblotting to show that IFFO1 protein is absent in the knockout cells. d, Sensitivity assay of the IFFO1−/− DT40 cells to various DSB-induced agents. Bars represent mean ± SD; n = 3 independent experiments. e, Schematic representation of the chicken LMNA and targeted genomic DNA. The regions containing exon2-7 (marked by red) of the gene are replaced by blasticidine, histidinol and puromycin resistant genes. The two regions between two pairs of dotted lines were used as arms of the knock-out constructs. Arrows indicated targeted locations of primers for genomic PCR. f, Genome-PCR analysis to show that LMNA gene is undetectable in the respective knockout DT40 cells. XRCC4 was included as a positive control. g, Sensitivity assay of the LMNA−/−/− DT40 cells to various DSB-induced agents. Bars represent mean ± SD; n = 3 independent experiments. All immunoblots and gels are representative of three independent experiments; unprocessed scans of immunoblots are shown in Supplementary Fig.9. Statistical source data including precise P values are shown in Supplementary Table 5.
Ectopically expressing XLF or PAXX could not suppress the mobility of the broken ends in the IFFO1<sup>−/−</sup> cells.

**a,** Immunoblotting show the expression level of IFFO1 in the IFFO1<sup>−/−</sup> cells transfected with vary versions of IFFO1. **b, c,** Immunoblottings show the knockdown efficiency of XRCC4 (b) and Lamin A/C (c). Two shRNAs were used for XRCC4 (shX4<sub>1/2</sub>) and Lamin A/C (shLM<sub>1/2</sub>), respectively. **d,** Immunoblotting show the expression level of XLF and PAXX in the IFFO1<sup>−/−</sup> cells. **e,** MSD of the mCherry-53BP1 foci in the IFFO1<sup>−/−</sup> cells transfected with XLF or PAXX. Means with SEMs of every time point are showed. The numbers of traces pooled from two independent experiments are indicated. All immunoblots are representative of three independent experiments; unprocessed scans of immunoblots are shown in Supplementary Fig.9. Statistical source data including precise P values are shown in Supplementary Table 5.
**Supplementary Figure 5**

Lig3 promotes chromosome translocation in the *IFFO1*<sup>−/−</sup> cells.

**a**, Schematic of the pDVG94 plasmid used to examine the efficiency of aNHEJ. This figure was adapted from Verkaik NS, et.al (Eur J Immunol. 2002). **b**, The indicated cell lines were treated with or without 10 μM of the DNA-PKcs inhibitor, NU7441, for 4 hr, and then transfected with EcoRV- and AfeI-linearized pDVG94. Cells were allowed 24 hr to repair the linearized template, then plasmids were extracted, and the region spanning the cut site was amplified by PCR, followed by digestion with BstXI. The restriction enzyme products were then analyzed by PAGE gel. **c**, Chromosome translocation of the *IFFO1*<sup>−/−</sup> cells transfected with Lig3 or Lig4 shRNA. Bars represent means with SEM of 4 independent experiments. Two-tailed t-tests were used for the analysis. ***, P<0.01; ns, P>0.05. Western blotting at right panel showed the protein levels. All immunoblots and gels are representative of three independent experiments; unprocessed scans of immunoblots are shown in Supplementary Fig.9.
Supplementary Figure 6

IFFO1 expression is downregulated by its promoter methylation in multiple tumor types.

a, The correlation between the IFFO1 expression and its methylation at promoter. Correlation Coefficient (R) and P Value (P) were calculated using Pearson Correlation; n = 16 cancer types. b, Relative expression in tumor vs. normal tissue in multiple tumor types. Gene expression data in TCGA were accessed through The UCSC Cancer Genomics Browser (https://genome-cancer.ucsc.edu). c, Heatmaps of DNA methylation at IFFO1 promoter in different cell lines. Methylation of 18 CpG sites in chr.12: 6,556,011-6,556,233 (GRCh38.p12) were determined. Red and cyan squares denote methylated and non-methylated CpG sites, respectively. White squares denote CpG sites, which were not covered by sequencing. d, mRNA of IFFO1 was measured by qPCR. GAPDH mRNA was measured as a control. Means with SD from n = 3 independent experiments were present. Two-tailed t-tests were used for the analysis. e, Correlation between IFFO1 expression and its promoter methylation in these cell lines examined. Analysis was performed using Pearson Correlation; n= 9 cell lines. f, IFFO1 mRNA level after azacytidine treatment. The EKVX cells were treated with azacytidine for 3 days before harvest. Means with SD from n = 3 independent experiments were present. Two-tailed t-tests were used for the analysis. Statistical source data including precise P values are shown in Supplementary Table 5.
Supplementary Figure 7

**IFFO1 expression negatively correlates with the frequency of gene fusion in multiple cancer types.**

a, The frequency of inter-chromosome translocation in multiple cancer types. The data were gained from the literature by Yang et al., 2013. OV, ovarian serous cystadenocarcinoma; GBM, glioblastoma multiforme; MM, multiple myeloma. b, Plot shows the correlation between the IFFO1 expression and the frequency of inter-chromosome translocation. Correlation Coefficient (R) and P Value (P<sub>R</sub>) were calculated using Pearson Correlation; n = 7 cancer types. c, Graph showing the average numbers of kinase fusions per sample in the multiple tumor types described by Stransky et al. 2014. d, Plot shows the correlation between the kinase fusion and the inter-chromosome translocation. Correlation Coefficient (R) and P Value (P<sub>R</sub>) were calculated using Pearson Correlation; n = 9 cancer types. e, Plots show the correlation between gene expression and the frequency of the kinase fusion in multiple tumor types, including UCEC, BLCA, LUSC, COAD, READ, LUAD, PRAD, BRCA, THCA, HNSC, LIHC, KIRP and KIRC. Correlation Coefficient (R) and P Value (P<sub>R</sub>) were calculated using Pearson Correlation; n = 13 cancer types. f, h, The frequency of kinase fusion (f) and total gene fusion (h) in multiple cancer types. The data were gained from the literature by Gao et al., 2018. g, i, Plots show the correlation between the inter-chromosome translocation with the frequency of kinase fusion (g) and total gene fusion (i). Correlation Coefficient (R) and P Value (P<sub>R</sub>) were calculated using Pearson Correlation; n = 9 cancer types. Statistical source data including precise P values are shown in Supplementary Table 5.
Supplementary Figure 8

The expression and methylation levels of *IFFO1* and *LMNA* in multiple cancer cell lines from The Cancer Cell Line Encyclopedia (CCLE).

a-c. The expression (a) and methylation (b) levels of *IFFO1* gene and their correlation (c) in CCLE. Correlation Coefficient (R) and P Value (P_R) were calculated using Pearson Correlation; n = 834 cell lines (c). Sample sizes in (a, b) were indicated in the brackets. d-f, The expression (d) and methylation (e) levels of *LMNA* gene and their correlation (f) in CCLE. Correlation Coefficient (R) and P Value (P_R) were calculated using Pearson Correlation; n = 234 cell lines (f). Sample sizes in (d, e) were indicated in the brackets. **g**, Plot of expressions of IFFO1 and LMNA in cancer cell lines of CCLE. Red and blue boxes indicate IFFO1-positive LMNA-negative and IFFO1-negative LMNA-positive cell lines, respectively. Most IFFO1-positive LMNA-negative cells belong hematological malignancies. All the CCLE data and analysis were accessed through the website (https://portals.broadinstitute.org/ccle). Statistical source data including precise P values are shown in Supplementary Table 5.
Supplementary Figure 9
Uncropped images from gels and blots.
Uncropped images from gels and blots, continued.
Supplementary Figure 9
Uncropped images from gels and blots, continued.
Supplementary Figure 2a

Uncropped images from gels and blots, continued.

Asterisks indicate the inputs

Supplementary Figure 9

Uncropped images from gels and blots, continued.

Asterisks indicate the inputs
Supplementary Figure 2f

Supplementary Figure 2h

Supplementary Figure 2j

Supplementary Figure 2l

Supplementary Figure 2m

Supplementary Figure 9

Uncropped images from gels and blots, continued.
Supplementary Figure 9
Uncropped images from gels and blots, continued.
Supplementary Figure 9

Uncropped images from gels and blots, continued.
Supplementary Table 1. Proteins identified by mass spectrometry in the indicated immunoprecipitates
Supplementary Table 2. Data collection and refinement statistics
Supplementary Table 3. Tumor types fitted with the standards from TCGA
Supplementary Table 4. Antibodies
Supplementary Table 5. Statistical source data
Supplementary Note 1. Interactions in the three interfaces of IFFO1-XRCC4 complex.
Interactions in the three interfaces of IFFO1-XRCC4 complex

The electrostatic interactions in region I are shown in Supplementary Fig. 2e, including interaction formed by residues Asp494, Glu504, Lys509, Gln515, Asp513 and Arg520 in IFFO1 and Lys187, Arg179, Glu173, Lys169, Arg161 and Glu163 in stalks of XRCC4, respectively. In the interaction region II (Supplementary Fig. 2f), residues Met516, Cys519, Ile523 from IFFO1 and Val158, Phe162, Cys165 from XRCC4 constitute a hydrophobic cavity, thus tightly tethering the four molecules together. The third interaction region (region III) was mediated by hydrophobic interaction between the tail of XRCC4 and shaft of IFFO (Supplementary Fig. 2g). Residues Ile484, Leu488, Ala491, Met495 and Tyr502 in IFFO1 and Leu198, Leu194, Ile191, Leu184 and Phe180 in XRCC4 are important in the hydrophobic interface.