SUPPLEMENTARY INFORMATION

Extended Materials and methods

Transfections

Transfections of SH-SY5Y cells with the Luciferase-reporter constructs were done 24 h before irradiation following a standard Lipofectamine 3000 protocol (Invitrogen). SiRNA knockdown was performed using Lipofectamine RNAiMAX transfection reagent (Invitrogen). The siRNA sequences against *PRNP* (GCCGAGUAAGCCAAAAACC), c-*JUN* (CAUGCUCUGUUUCAGGAU) and *JNK* (AAAAAGAAUGUCCUACCUUCU) were synthetized by Eurogentec, a control siRNA duplex (SR-CL000-005-Eurogentec) was used as negative control. Cells were grown for 24 h before being transfected and allowed to grow for 48-72h before irradiation.

Clonogenic assay

According to cell lines and doses of irradiation, different quantities of SiRNA transfected cells (500-2000 for SH-SY5Y cells; for 1000-2000 LoVo cells; for 4000-24000 MDA-MB-134 cells) were seeded into 6-well plates and allowed to adhere for 12h. Irradiation was then performed in presence or absence of 5Z and cells were further cultured for 12 days (SH-SY5Y), 16 days (LoVo) or 24-27 days (MDA-MB-134). Then cells were fixed by 4% paraformaldehyde for 30 minutes, washed with PBS and stained with methylene blue. Colonies with more than 30 cells were scored as survivors. The plating efficiency (PE) was calculated by dividing the number of survivors by the number of plated cells at 0 Gy. Surviving fraction (SF) at each dose was determined as the number of survivors divided by the product of the PE and the number of plated cells at the corresponding dose. Clonogenic survival curves were fitted to the linear quadratic model and compared through the extra-sum-of-squares F test using GraphPad Prism.

Western blot analysis

The cell extracts were obtained by sonication of cell pellets in 20mM Tris-HCl, pH 7.5, 250mM NaCl, 1mM ethylenediaminetetraacetic acid and protease inhibitors. The homogenate was centrifuged at 20 000 x g for 30 min and aliquots of the supernatant were stored at −80°C for biochemical assays. Detection of IKKβ and phospho-IKKα/β was done on cytoplasmic extracts obtained using a subcellular

protein fractionation kit (ThermoFisher). Proteins from cell extracts were resolved by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting was carried out using standard procedures. Primary antibodies against c-Jun (#9165), phospho c-Jun (#3270), JNK (#9252), phospho JNK (#9255), IKKβ (#2678), phospho-IKKα/β (#2078) were from Cell Signaling. Vinculin (ab18058-Abcam) detection was used as a control for protein loading. SpectraDye secondary antibodies were from Advansta. Western blot for PrPC was performed as described [4] and quantification analysis was conducted using ImageJ software.

Reverse transcription and QPCR

Total RNA was prepared from frozen cell pellets using RNeasy Plus micro kit (Qiagen) and quantified by Nanodrop spectrophotometer. For the first strand cDNA synthesis at least 100 ng of total RNA was reverse-transcribed using SuperScriptVILO cDNA synthesis kit (Invitrogen). Quantitative PCRs were performed with a StepOnePlus system (Applied Biosystems) by using the iTaq Universal Probes Supermix (Biorad) for Taqman probe system. Probes used are *PRNP* Hs01920617_s1 and *RPLP0* Hs99999902-m1. The mean expression of *RPLP0* (large ribosomal protein gene) was used to normalize the expression of *PRNP* gene.

PrPc expression analysis

PrPc expression was assessed at the protein level by western blot[4] and by flow cytometry[11] using a BD FACSLSRII flow cytometer (BD Biosciences). Flow cytometric data were analyzed with FlowJo software.

***PRNP* promoter constructs studies**

A *PRNP* promoter sequence associated with the exon 1 (-1960 bp, +125bp) was cloned in the pET28A vector (Genecust). The different constructs harboring sequential deletions of the *PRNP* promoter and exon 1 were obtained by PCR and cloned in the pGL4.14 vector (#E6691-Promega). They were named according to the position from the transcription start site (TSS): pGL-1521 (-1521bp from TSS), pGL-

572 and pGL-196. Deletion of each binding site and mutagenesis of the AP-1site (TGACTCA to TAAATGA) in the PGL-196 plasmid were performed by PCR using overlapping primers (Supplementary Table1). SH-SY5Y cells were co-transfected 24h before irradiation using a standard Lipofectamine 3000 protocol (Invitrogen) with the Renilla luciferase reference control plasmid pGL4.74 vector (#E6921-Promega), and one of the engineered *PRNP* promoter constructs. Luciferase activity was then measured using the Dual-Glo® Luciferase Assay System (Promega) with a Clariostar (Labtech) plate reader.

Generation of CRISPR KO PrP SH-SY5Y cells

To perform CRISPR-Cas9 experiment, RNA guide sequence[21] to drive SpCas9 to *HsPRNP* gene has been cloned in pLentiCRISPRv2 plasmid (#52961-Addgene). 10^5 cells were incubated with lentiviruses for 72 h and selected with puromycine (2.5 μg/ml). CRISPR CTL and *PRNP* KO cells were FACS-sorted (BD Influx™, BD Biosciences) after PrPc staining as described[11] with minor modifications (blocking step in 2.5% goat serum before primary antibody incubation and incubation with anti-PRP antibody at 1:300). Gating strategy for cell sorting is shown in Supplementary Fig. 1e.

PrPC overexpression

The open reading frame MmPrnp1-254 obtained from Origene (NM-011170) was cloned in the polycistronic pIRES2-AcGFP1 plasmid (Clontech). LoVo cells were transfected either with an empty pIRES2-AcGFP1 plasmid (EV) or with the pIRES2-MmPrnp1-254-AcGFP1 plasmid (PRP) using Lipofectamine 3000 (Invitrogen). Ectopic expression of PrPC was checked by flow cytometry as described [11].

Twenty-four hours after transfection with either EV and PRP plasmids, cells were subjected to a single dose of 16 Gy (γ-rays) and living cells were counted 4 days treatment using Trypan Blue staining and Countess™ II automated cell counter (Life technologies).

CRISPR interference assay

CRISPR interference assay was performed according to Huang *et al.*[22] using pHR-SFFV-dcas9-BFP (Addgene #46910) and pgRNA humanized-mCherry (Addgene #44248). For targeting AP-1

element in the *HsPRNP* promoter, the guide RNA sequence (Supplementary Table 1) was determined using CRISPOR webtool (http://crispor.tefor.net/). The gRNA sequence was cloned under the U6 promoter in pgRNAh plasmid digested by *Bst*XI + *Xho*I using single-strand annealing cloning strategy. SH-SY5Y cells were transduced with gRNA AP-1-mCherry and dcas9-BFP lentiviruses (MOI=1) for 72 h. Cells expressing both mCherry and BFP or BFP only were sorted using BD Influx™ and amplified for experiments.

ChIP-qPCR assay

ChIP-qPCR assay was performed on crosslinked chromatin obtained from SH-SY5Y cells 4 hours after irradiation. Chromatin immuno-precipitation was made using 2 μg of c-Jun antibody (Cell Signaling Technology 60A8 #9165). Antibody-protein-DNA complexes were isolated by immunoprecipitation with protein G beads (Dynabeads™ M-280 Sheep anti-rabbit IgG, 11204D, Invitrogen). DNA extraction was performed by phenol/chloroform and isopropanol based-technique and used as a template in PCR. The primers used for AP1-PRNP were F, 5'-ACTGATGCAAGTGTTCAAGCG-3' and R, 5'-GCCGGCCGAGGTTTAAGTT-3'. As a negative control we used *Lgr5* intron 3 that contains no c-Jun binding site (Aguilera C, Nakagawa K, Sancho R, Chakraborty A, Hendrich B, Behrens A. *Nature*. 2011 **469**: 231-235), F, 5′-TCTGCCTCAGGCTTACATGGA-3′; R, 5′-CACAAGAATTCTGCAGCACATTT-3.

Quantification and statistical analysis

Data are presented as mean ± SEM. All experiments were repeated at least three times independently. Statistical significance was determined using the Mann-Whitney U-test or using the 2 ways ANOVA. Statistical analyses were carried out using GraphPad Prism. ***, $p<0.001$; **, $p<0.01$; *, $p<0.05$.