

Extensive resection of newly replicated DNA in irradiated HPV-positive HNSCC cell lines

Christian S Betz^{1,2}; Jasmin Burmester^{1,2}; Malte Kriegs²; Sabrina Christiansen^{1,2}; Kai Rothkamm²; Thorsten Rieckmann^{1,2}

1 Department for Otorhinolaryngology and Head and Neck Surgery, 2 Laboratory for Radiobiology; University Medical Center Hamburg-Eppendorf (UKE)

Introduction

HPV+ HNSCC cell lines are characterized by an enhanced cellular radiation sensitivity based on a defect in DNA double-strand break repair (1). Especially a defect in homologous recombination (HR) has repeatedly been suggested (2-5). Apart from its role in DNA repair, the HR machinery is also critically involved in the protection and restart of stalled replication forks. Therefore, we performed comparative studies on DNA replication in a panel of 5 HPV- and 6 HPV+ HNSCC cell lines using the DNA fiber assay to assess replication on the molecular level.

Since the phenotype of extensive resection of nascent replication tracks has been linked to the activation of the cGAS/STING immune response pathway (6), we further explored a possible association in our cells.

Conclusion

- The majority of HPV-positive cell lines, in contrast to HPV-negative, reduced elongation speed upon radiation and demonstrated extensive, resection of newly replicated DNA.
- The resection is dependent on the function of the nuclease Mre11 and may indicate a defect in the HR machinery.
- The cGAS/STING pathway of innate immunity is non-functional in the majority of HPV+ HNSCC cell lines but not in all strains as assessed by nuclear translocation of IRF3 after transfection with calf thymus DNA.
- Upon irradiation only the HPV33+ cell line UT-SCC-45 but not the HPV16+ HNSCC strains demonstrate IRF3 translocation.
- Therefore, a functional role of the cGAS/STING innate immune pathway in the generally enhanced radiation sensitivity of HPV+ HNSCC is highly unlikely. To what extent the pathway may play a role in tumors caused by other high-risk types than HPV16 remains to be determined.

References

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- (6) Bhattacharya et al. Nucleic Acids Res. 2017 May 5;45(8):4590-4605.
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Results

1. DNA fiber assay

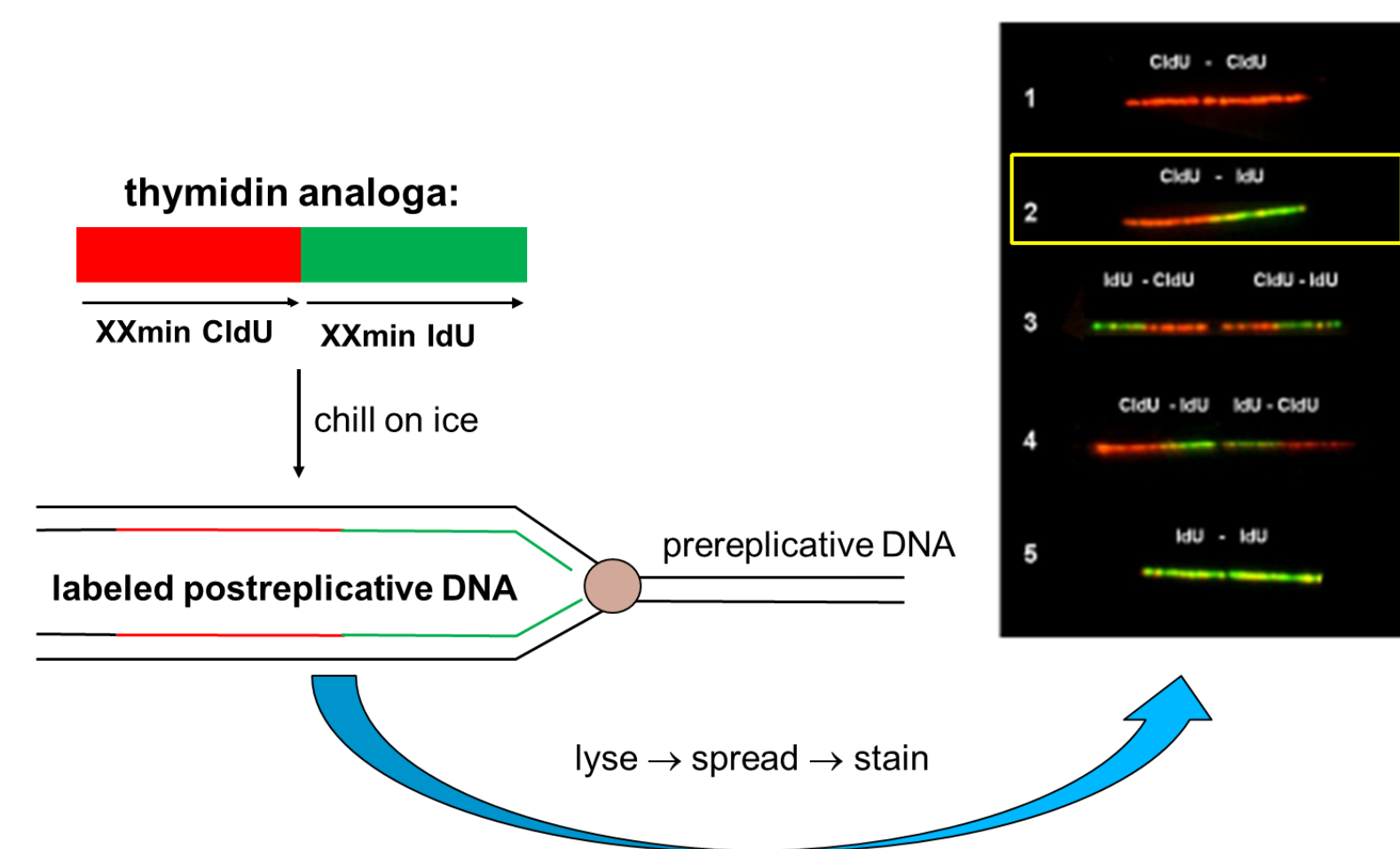
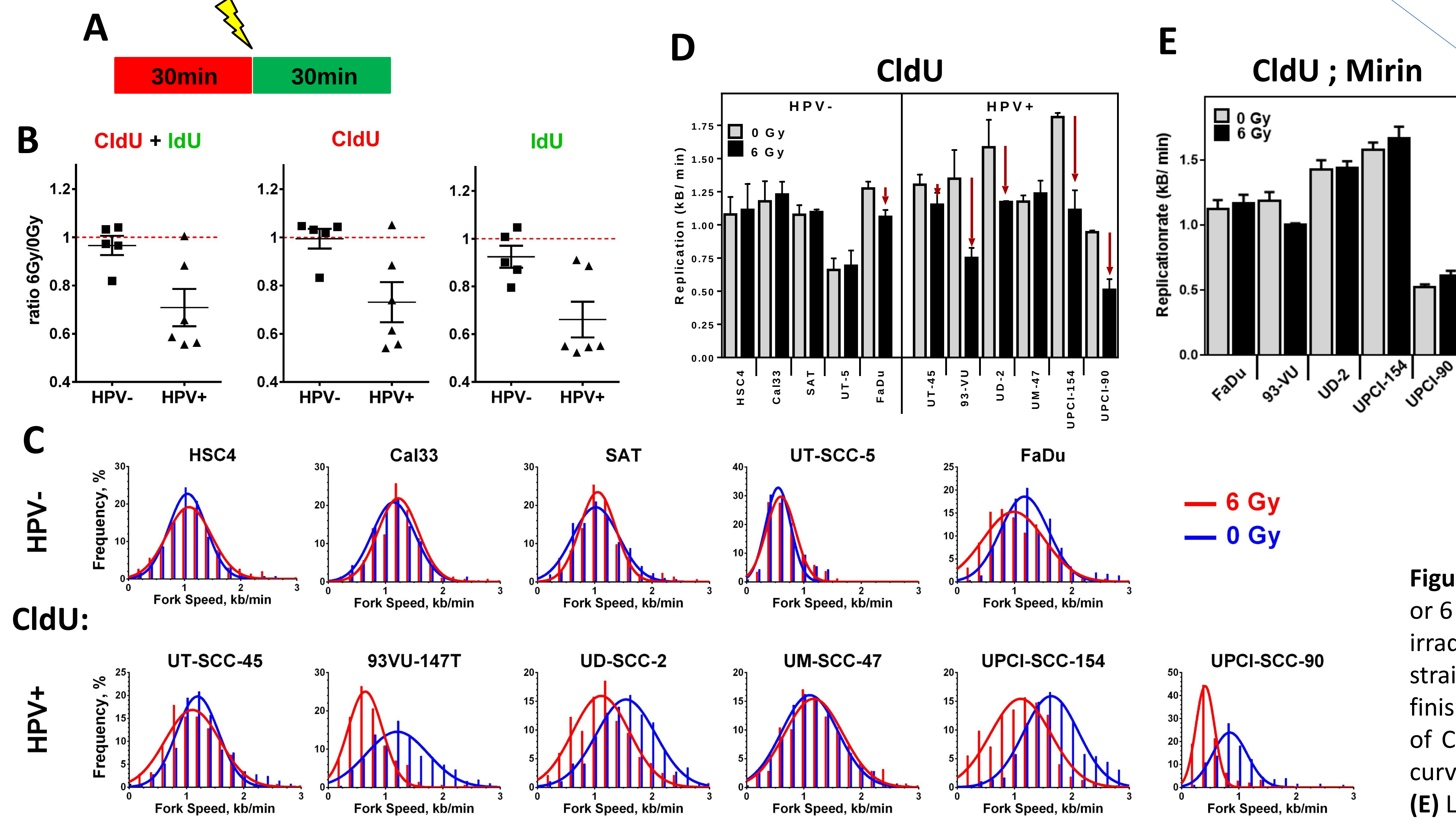


Figure 1. DNA fiber assay. Cells are subsequently pulse labeled with 2 thymidin analogs, washed with cold PBS and immediately chilled on ice. After cell lysis the DNA is spread on microscope slides and afterwards the incorporated thymidin analogs are stained for immunofluorescence microscopy. Single molecule DNA fibers can be analysed for track length (structure 2, elongating forks) and the frequency of further replication structures.

In the following, track length are depicted as average replication rate in kb/min calculated using an established factor.

2. Radiation-induced resection of nascent replication tracks



3. HPV+ HNSCC cells and the cGAS/STING pathway

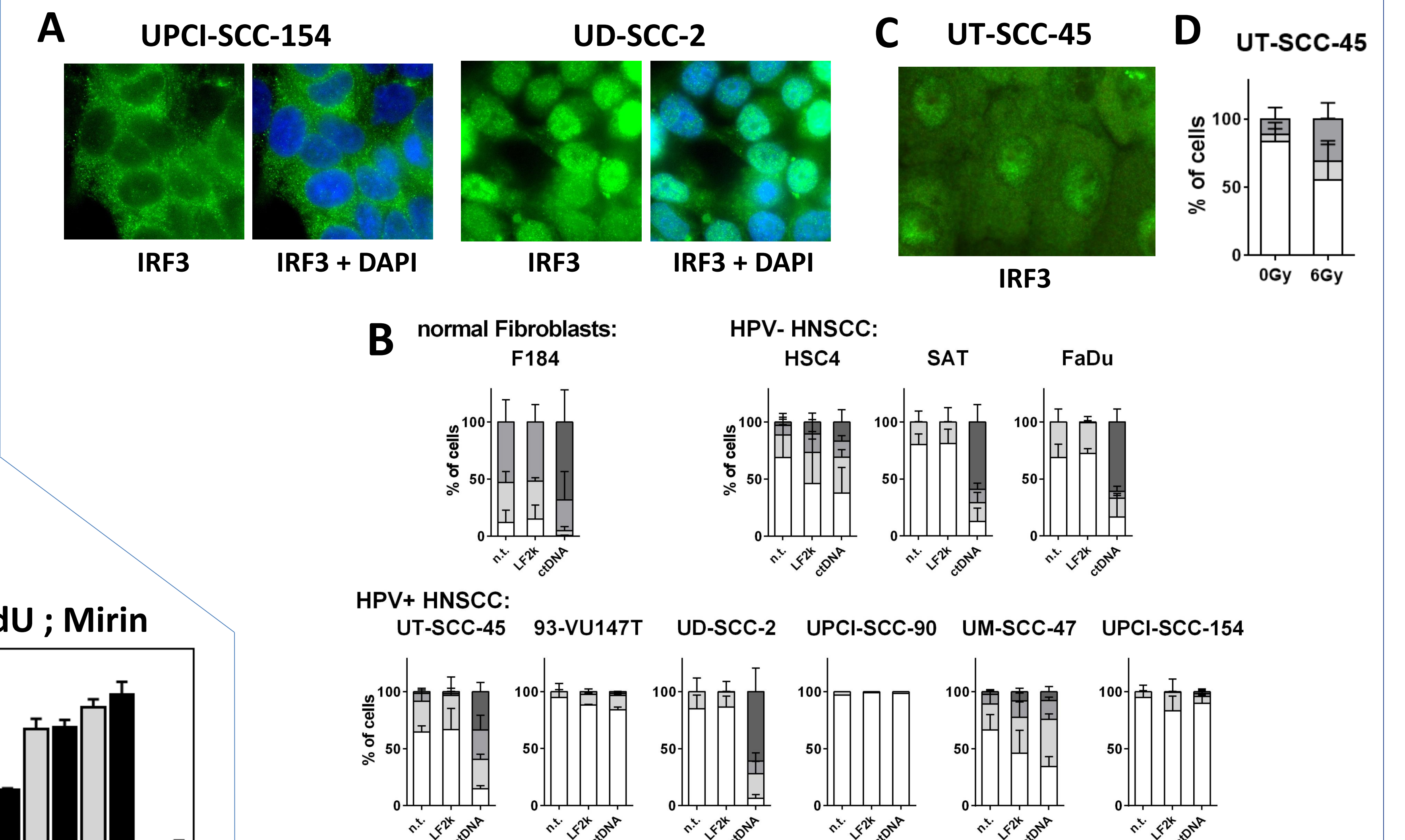


Figure 3. cGAS/STING pathway. While HPV+ cells have been described to be devoid of a functional cGAS/STING response (7,8) a minority of HPV+ HNSCC cell lines responds to transfection with external (calf thymus) DNA with nuclear translocation of the Interferon regulatory factor 3 (IRF3), a hallmark of the cGAS/STING response. **(A)** Microscopic examples after transfection (UD-SCC2 responsive) and **(B)** quantification (white: cytoplasmic; light, darker and dark grey: partly, mostly and solely nuclear IRF3). **(C,D)** Upon radiation with 6 Gy only UT-SCC-45 (positive for HPV33, not HPV16) showed some nuclear translocation of IRF3. n.t.= not treated, LF2k = Lipofectamine2000 only

Figure 2. Resection of nascent replication tracks. **(A)** Experimental setup. Cells are irradiated with 0 or 6 Gy between subsequent 30 min CldU & IdU pulse labels. **(B)** Fiber length ratio of irradiated/non-irradiated cells. Values below 1 indicate shorter labels after irradiation. In HPV-negative cell lines 1/5 strains (FaDu) demonstrates a shortening of replication tracks incl. CldU labels that were already finished at the time of radiation. In HPV+ cell lines 4/6 show a profound and 1/6 a slight shortening of CldU tracks. **(C)** CldU track frequency distribution of individual cell lines. A left-shift of the red curve indicates shorter CldU-labelled tracks. **(D)** Average CldU fiber shortening in individual cell lines. **(E)** Lack of CldU fiber shortening upon inhibition of the nuclease Mre11 through mirin (50µM).