Repair and DNA strand-specific transcriptional toxicity of *cis-syn* cyclobutane TT dimers in an active gene

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Background

Bulky DNA modifications block transcription when present in the transcribed DNA strand (TS) of the gene's transcribed region, by stalling RNA polymerase II (Pol II). Following transcription blockage, cells activate transcription-coupled nucleotide excision repair (TC-NER), mediated by the specific CSB and CSA proteins. TC-NER counteracts transcription blockage through the removal of the Pol II-stalling lesion. On the contrary, damage in the non-transcribed strand (NTS) is not expected to block transcription, since it does not interact with elongating Pol II. Such damage is thus believed to be repaired exclusively via the global genome (GG-) NER, which is initiated by DDB2 and XPC protein complexes. However, systematic investigations of transcription blockage and repair of damage in the NTS are lacking.

Scope and aims

We assessed transcription inhibition by structurally-defined synthetic DNA modifications at selected positions in the TS and NTS of an active gene. The spectrum of tested modifications included the major UV photoproduct, *cis-syn* cyclobutane TT dimer (TT dimer).

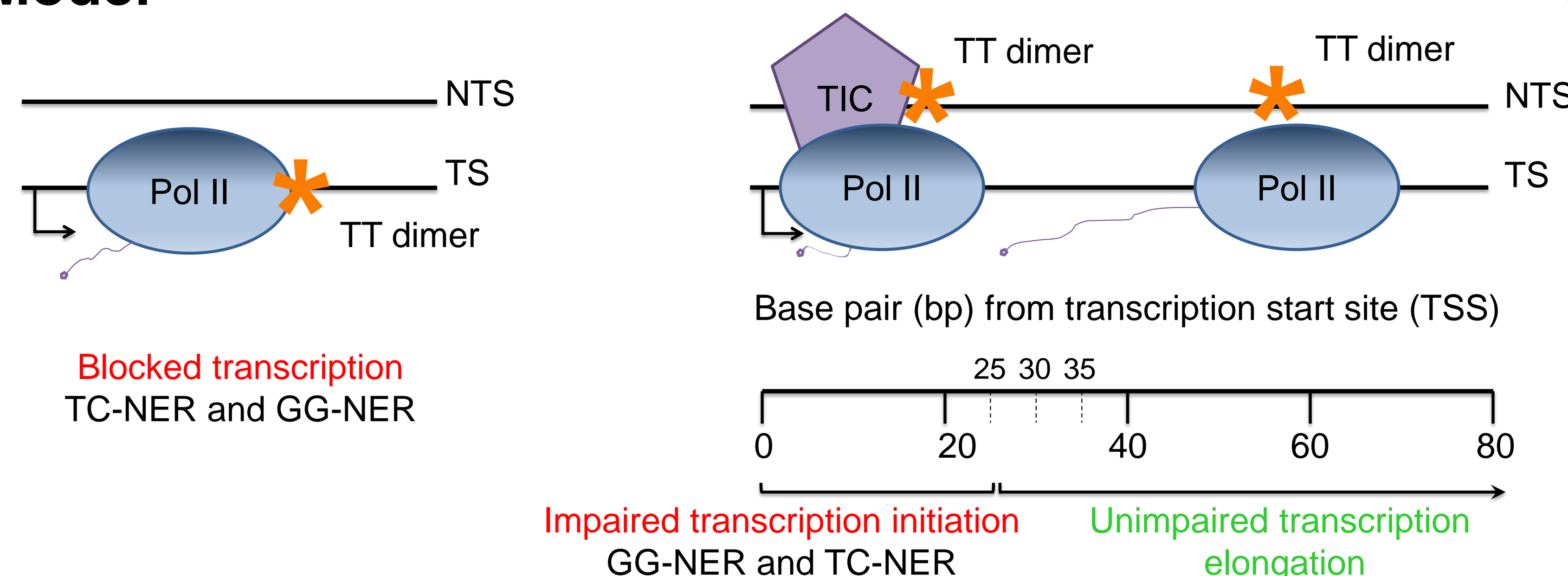
The aim of current research was to investigate potential interference between damage present in the NTS and transcription. The specific questions were:

- Should the lesion be present in the TS in order to cause transcription blockage?
- Should the lesion interact directly with Pol II in order to cause the blockage?
- Is TC-NER confined to the TS?

Key findings and conclusions

1. ***Cis-syn* cyclobutane TT dimers can inhibit the transcription when placed in the NTS (result 1)**
2. **TT dimers in NTS impair transcription only when present within the promoter proximal region (up to 25 base pair from transcription start site) (result 2)**
3. **Both TC-NER and GG-NER repair transcription-blocking TT dimers in the NTS (results 3 and 4)**
4. **We conclude that transcription inhibition by TT dimers in the NTS is likely due to an interaction with a component of the transcription initiation complex (TIC)**

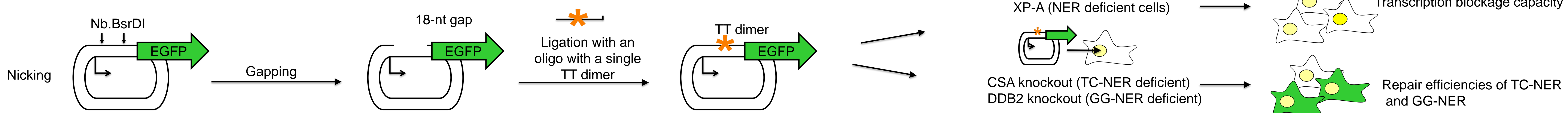
Model



Experimental approach

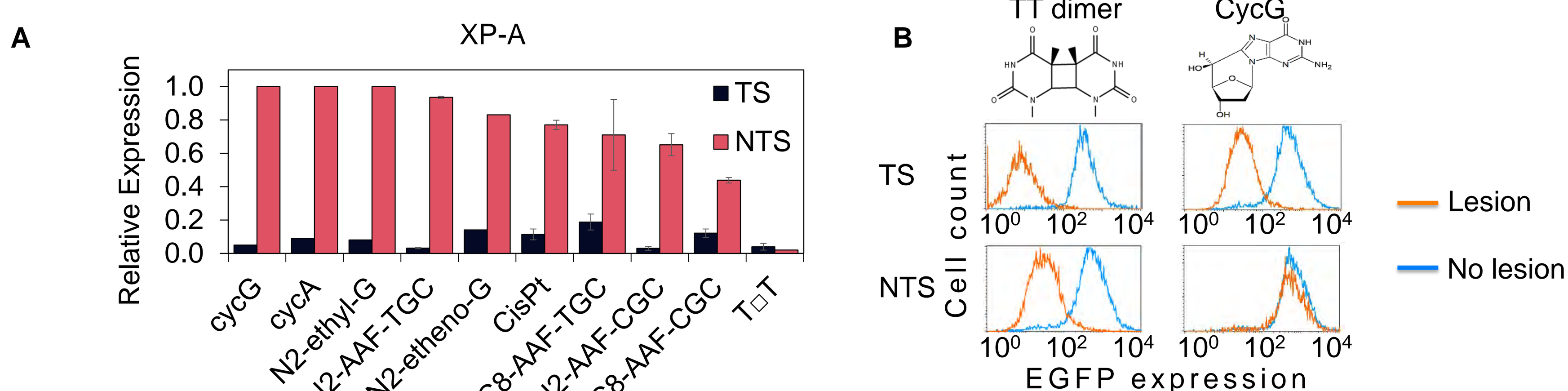
Reporter assay to measure strand-specific transcription blockage by synthetic TT dimers and their repair:

1. We double nicked our EGFP reporters in the 5′-untranslated region (5′-UTR) of either the TS or the NTS using a strand-specific restriction enzyme (Nb.BsrDI)
2. We gapped our reporters using a synthetic oligonucleotide sequence complementary to the nicked fragment
3. Gapped plasmids were then re-ligated with oligonucleotides harboring one synthetic TT dimer. Control constructs ligated to synthetic oligonucleotides with no modifications were prepared in parallel
4. Host cell reactivation (HCR) assay was performed: our constructs were transfected into cells with different NER statuses to assess transcription blockage capacities or repair of TT dimers
5. Finally, EGFP expression was analyzed 24 hours after transfection using flow cytometry



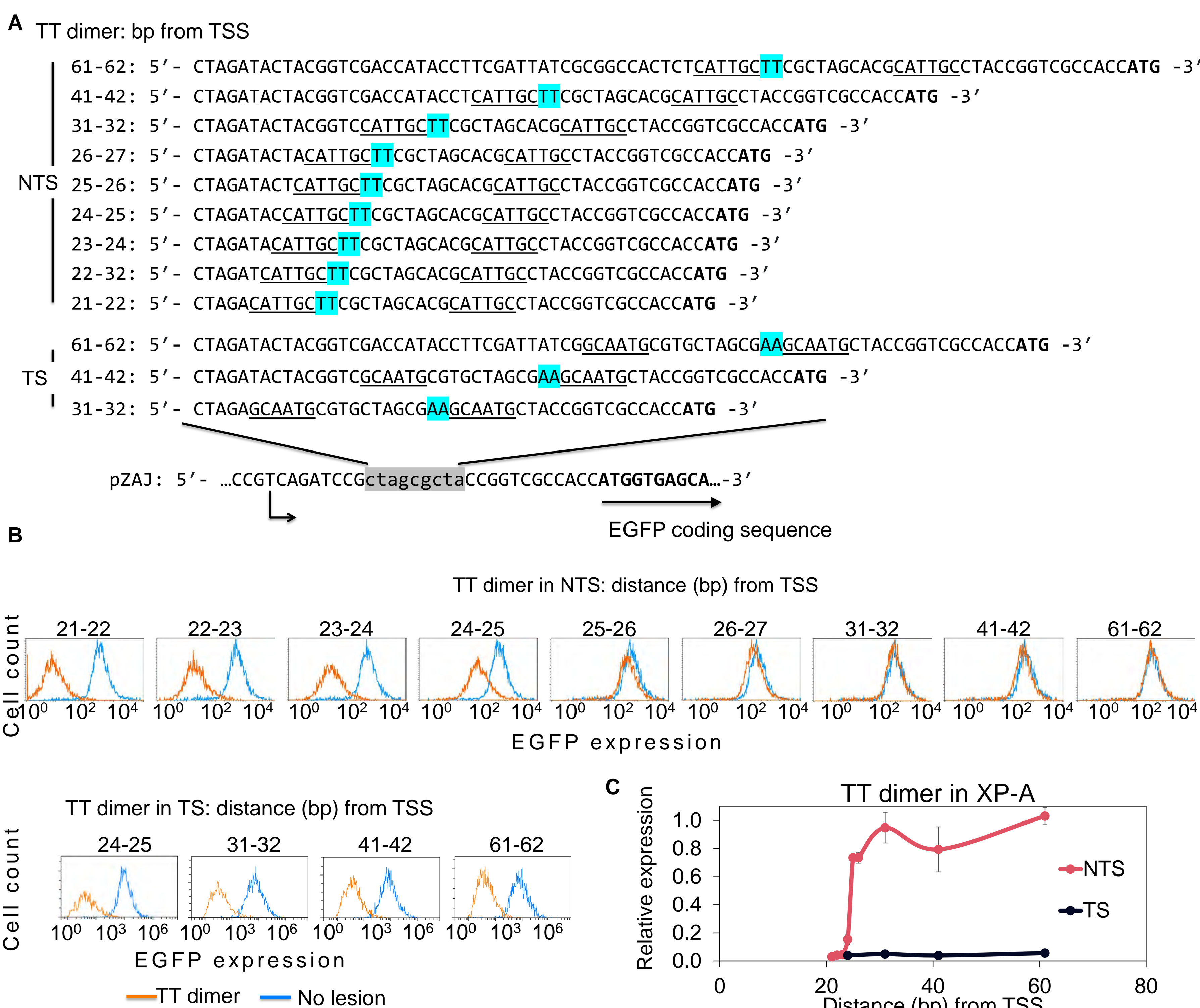
Results

1. TT dimers impair transcription in both TS and NTS



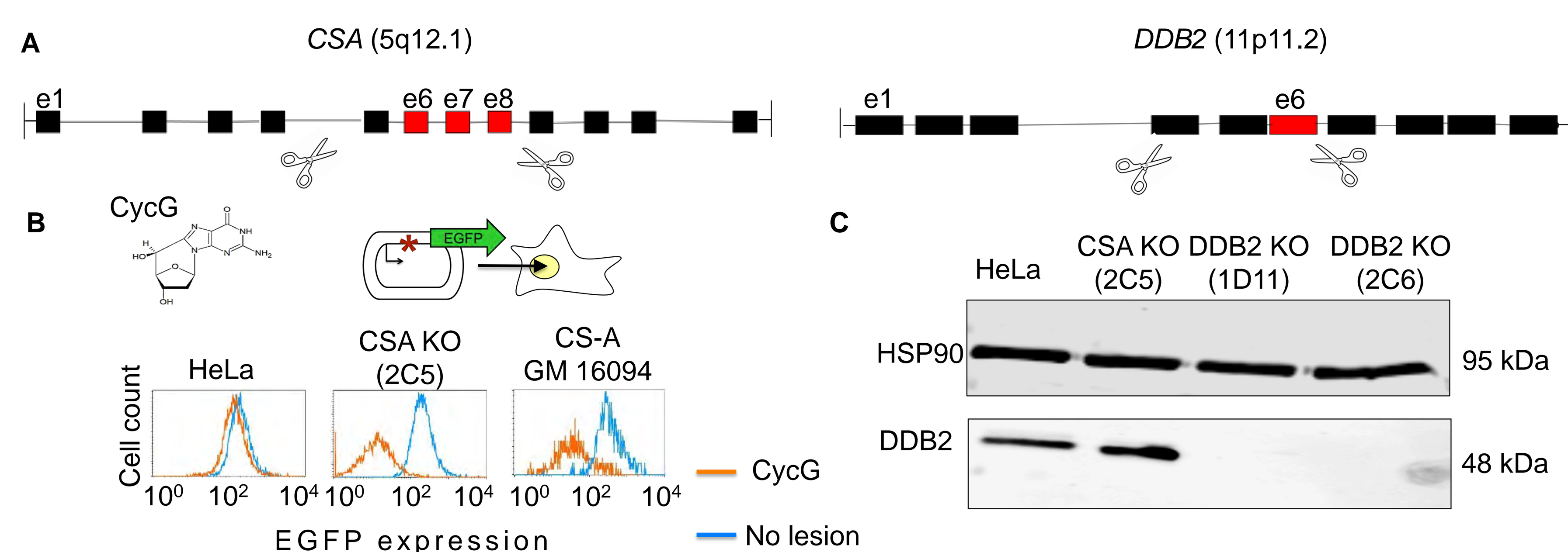
(A) NER deficient XP-A cells were transfected with EGFP constructs harboring single DNA modifications in the TS or the NTS of the 5′-UTR. EGFP expression was measured 24 hours after transfection and normalized to expression from cells transfected with control constructs having no lesions. TT dimer was the only modification to completely inhibit the transcription when placed in both strands. (B) Overlaid distribution plots of XP-A cell counts against EGFP expression after transfection with constructs harboring a modification (cyclobutane TT dimer or *(5′S)*-5′,8-cyclo-guanine (CycG)), or no lesion as a control. TT dimer caused transcription inhibition in both strands, while CycG inhibited the transcription only when placed in the TS.

2. TT dimers in NTS inhibit the transcription only when placed at a close distance to the transcription start site



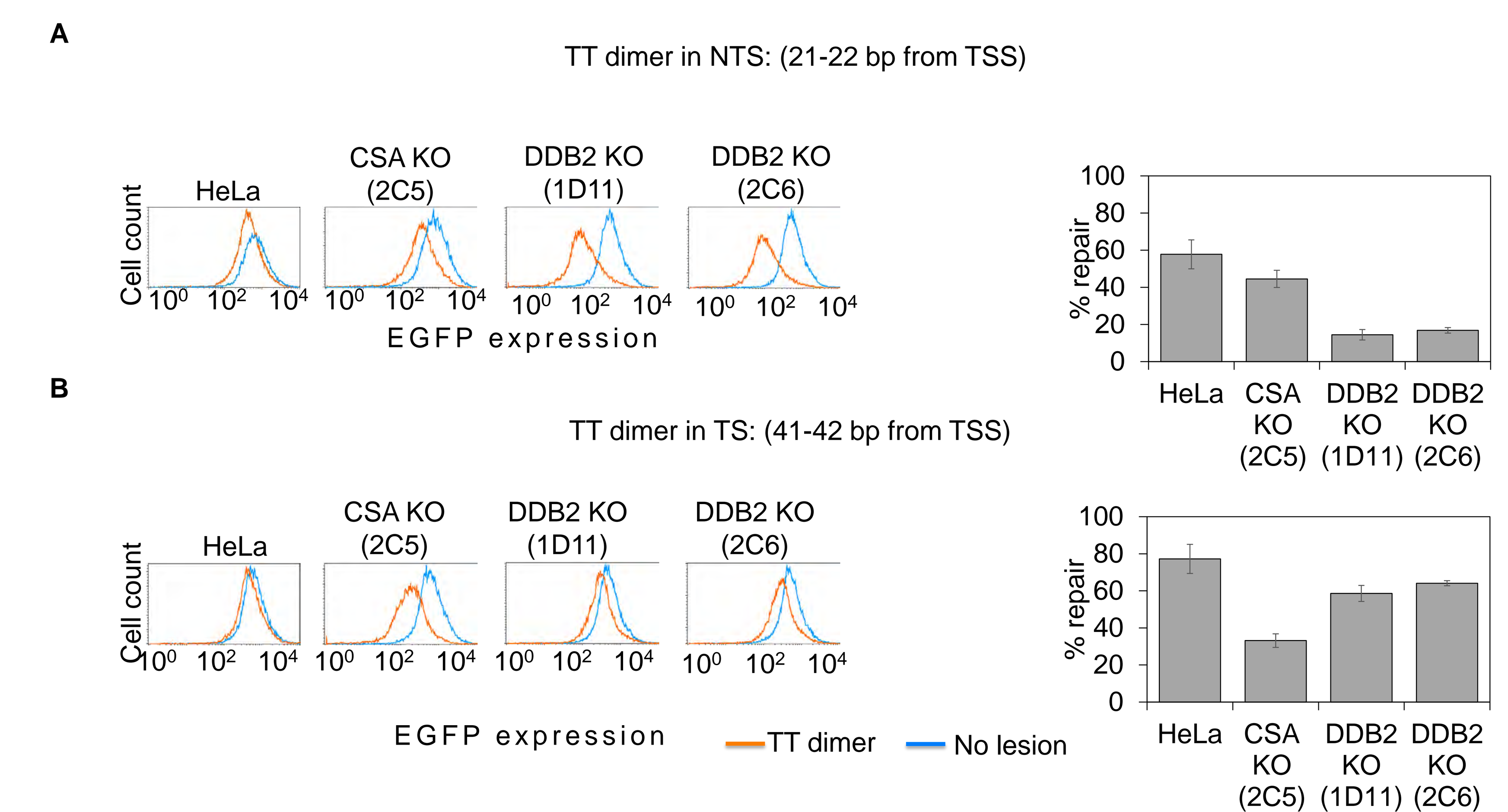
(A) Sequence fragments of newly-cloned plasmids used to insert TT dimers at increased distances from the TSS. The target TT-dinucleotide is highlighted in cyan and BsrDI recognition site is underlined. The TSS is shown as a broken arrow and the EGFP coding sequence is in bold (annotated in the native pZAJ plasmid). (B) Overlaid distribution plots of XP-A cell counts against EGFP expression. XP-A cells were transfected with different constructs harboring TT dimers at increased distance from TSS in both TS and NTS. In the NTS, TT dimers impaired the transcription when placed up to 25 bp from the TSS. Afterwards, the transcription was restored by at least 70%. (C) A comprehensive graph representing the correlation between TT dimer position and EGFP expression in XP-A cells.

3. Generation of isogenic cell lines lacking TC-NER and GG-NER via CRISPR-Cas knockout (KO) system



(A) Targeting of *CSA* and *DDB2* genes in HeLa using CRISPR-Cas. Exons (e) coding for critical sites in both proteins (responsible for binding with DDB1) are shown in red color. For both genes, deletion of critical exons was accomplished using one pair of sgRNAs flanking the critical regions (shown as scissors in genes schemes). (B) Verifying the absence of TC-NER in *CSA* KO (2C5 clone) via HCR using CycG. CycG is a transcription-blocking modification that is repaired solely through TC-NER. Overlaid distribution plots showed no transcription reactivation (no repair) of CycG in CSA KO (2C5 clone) or in fibroblasts that carry a null mutation in the *CSA* gene (GM 16094). (C) Validation of *DDB2* KO in HeLa at the protein level using western blot analysis. HSP90 was used a loading control. 1D11 and 2C6 clones showed no expression of the DDB2 protein.

4. Both TC-NER and GG-NER contribute to TT dimers repair in both DNA strands



HCR assay in HeLa, *CSA* knockout and *DDB2* knockout cell lines. (A) HCR results (overlaid distribution plots and a bar graph) of the mentioned cell lines after transfection with vectors harboring TT dimer in the NTS at a distance of 21-22 bp from TSS. Results showed 45% transcription reactivation (repair of TT dimer) in *CSA* KO cells that have only the GG-NER active. 20% repair by TC-NER was detected in *DDB2* KO cells. (B) HCR results of the mentioned cell lines after transfection with vectors harboring TT dimer present in the TS at a distance of 41-42 bp from TSS. Both TC-and GG-NER repaired TT dimers in the TS (60% repair in *DDB2* KO vs 30% repair in *CSA* KO cells).

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