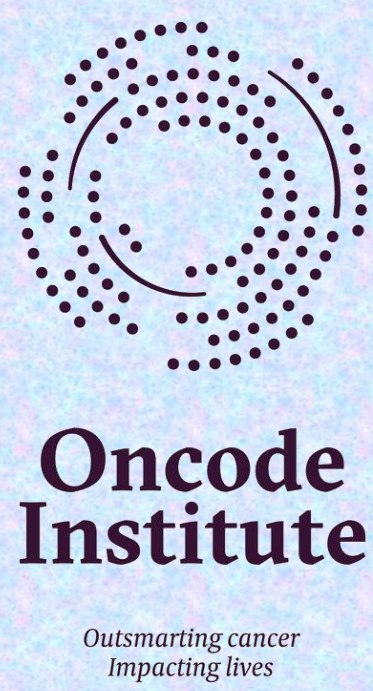# Exploring the determinants of lamina interaction throughout DNA repair

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Abstract:

Double-stranded breaks (DSBs) are one of the most deleterious forms of DNA damage that occur in mammalian cells. Unrepaired DSBs lead to mutations and genome instability which can potentially lead to cell death. Lamina-associated domains (LADs) are chromatin regions in contact with the nuclear lamina which is a fibrillar network covering the inner part of the nuclear envelope. LADs tend to be enriched for the histone modifications H3K9me2, H3K9me3, and H3K27me3 and are often inaccessible within the cell. LADs often also have low transcription rates and are supposed to help with the organization of the genome. DSBs can be induced by ionizing irradiation or endonucleases such as CRISPR/Cas9. Upon DSB induction, many proteins are recruited to the surroundings of the lesion and repair the DSB within a few hours. These proteins induce extensive changes at chromatin and cellular levels which halt cells until the DSB is repaired. Some recent preliminary data have shown that repair in LADs tends to be slower and relies more on mutagenic repair mechanisms than other chromatin contexts. However, the way DNA repair proteins access and repair DSBs in LADs is poorly understood. We used pA-DamID to study LADs which allows us to study the dynamics of LADs during a DSB or a detachment from the nuclear lamina occurs. In the same setting, H3K9me2, H3K9me3, H3K27me3, and γ-H2AX were also profiled using pA-DamID. For the H3K9me2 and H3K9me3 histone modifications, a local loss of signal was observed while H3K27me3 remained unchanged. When profiling γ-H2AX in the same setting, a huge increase across a large proportion of the genome was visible. To explore the determinants of the detachment from the nuclear lamina after induction of a DSB, we performed pA-DamID in the same experimental setup as previously, but with the introduction of three different DNA-repair inhibitors. In the results, we show that when DNApkcs is inhibited the detachment becomes larger. When ATM (Ataxia-telangiectasia mutated) was inhibited, we observed a smaller detachment while the inhibition of ATR (ATM and Rad3-related) did not seem to have any effect. Based on the increase in γ-H2AX after induction of a DSB and the fact that when ATM is inhibited less detachment was observed, we expected γ-H2AX to be involved in the detachment from the nuclear lamina. To test whether γ-H2AX plays a role in the detachment from the nuclear lamina after induction of a DSB, we induced a knockout of the H2AFX gene in the RPE-1 cell line with CRISPR/Cas9 and screened the clones with PCR, Sanger sequencing, and western blot. After the screen, we obtained three H2AX knockout clones on which pA-DamID was performed after induction of a DSB. In the results, we show that the detachment was smaller in the H2AX knockout RPE-1 clones compared to wild-type RPE-1 clones. This hints at a role for γ-H2AX in the detachment from the nuclear lamina.

Background:

Lamina Associated Domains (LADs)

- Lamina-associated domains are parts of the genome that are associated with the lamina (~ 30% genome), the inner membrane of the nucleus

- LADs are big domains that range from 10kb–10Mb (average 500 kb)

- LADs are enriched with histon modifications H3K9Me2, H3K9Me3 and H3K27Me3

- Supposed to help organize the chromatin and tend to have repressive functions
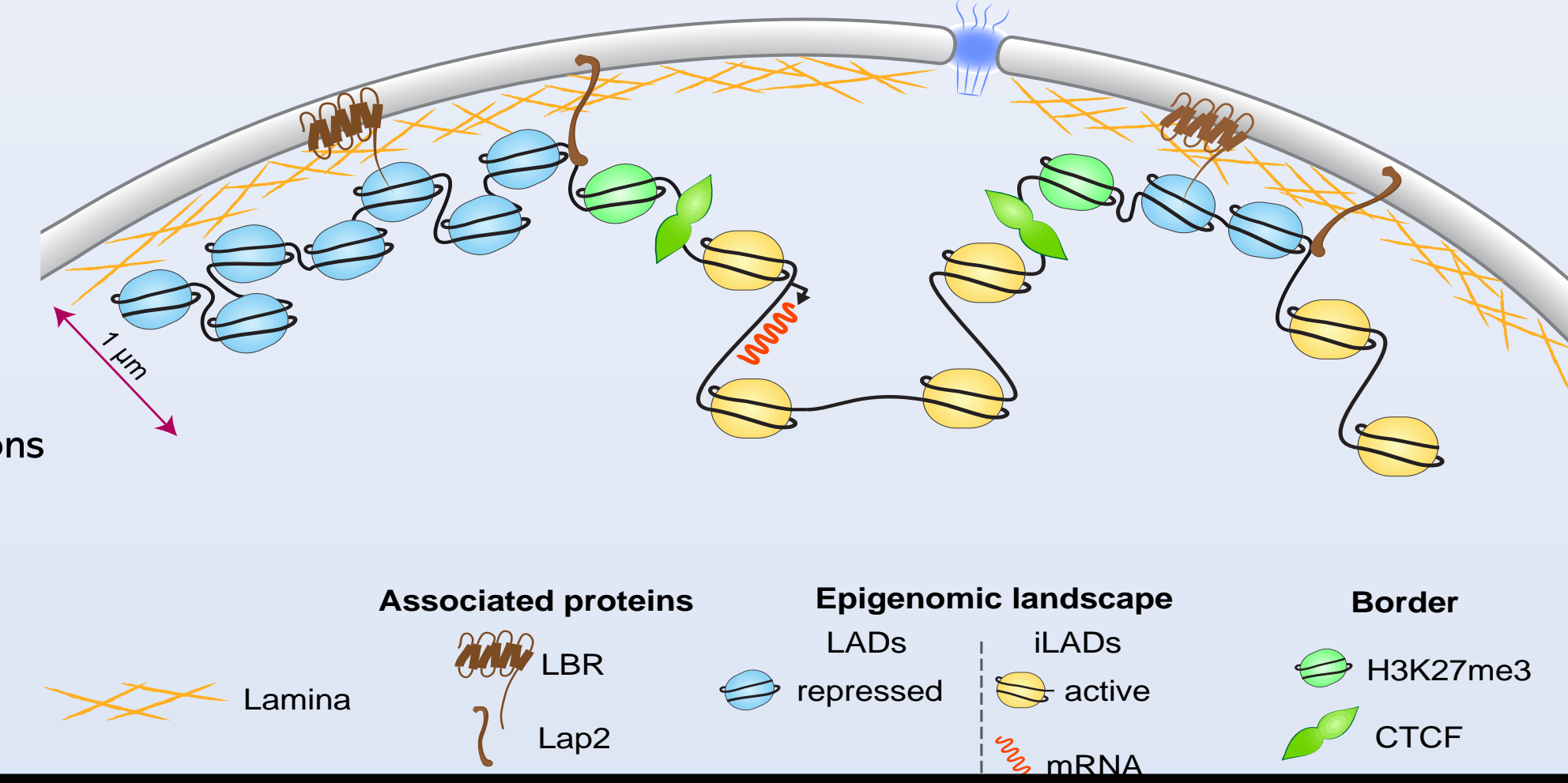
Result A: Detachment from the nuclear and widespread γ-H2AX induction occurred 10 hours after induction of a DSB using CRISPR/Cas9 in the RPE-1 cell line
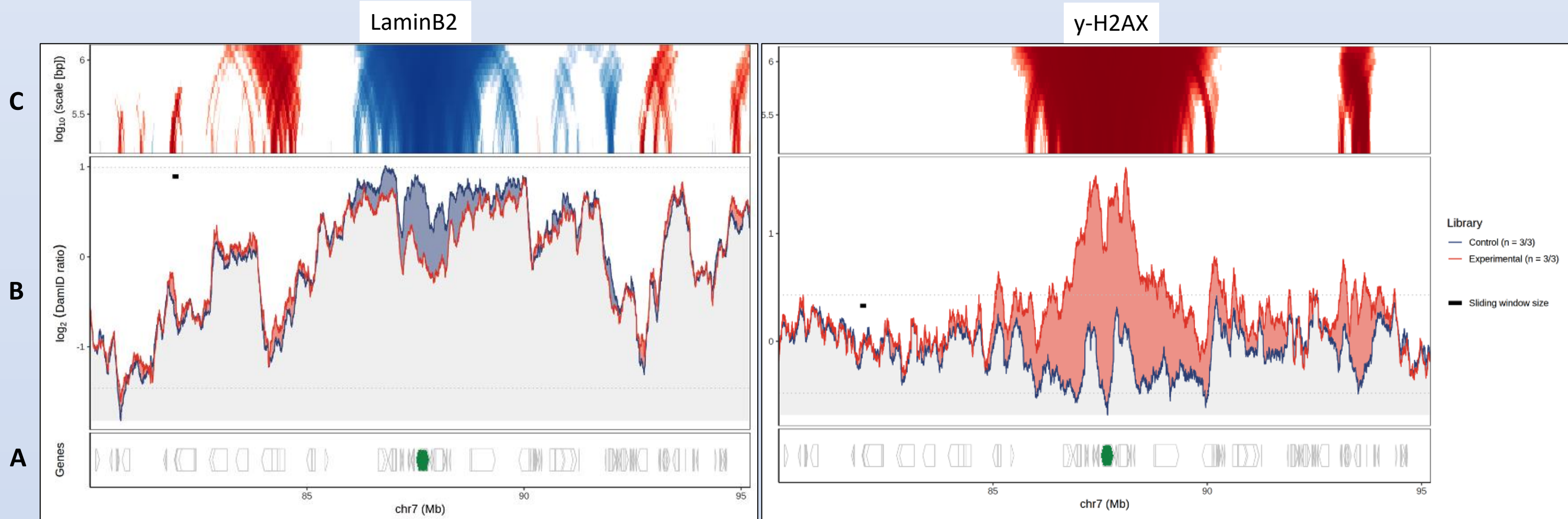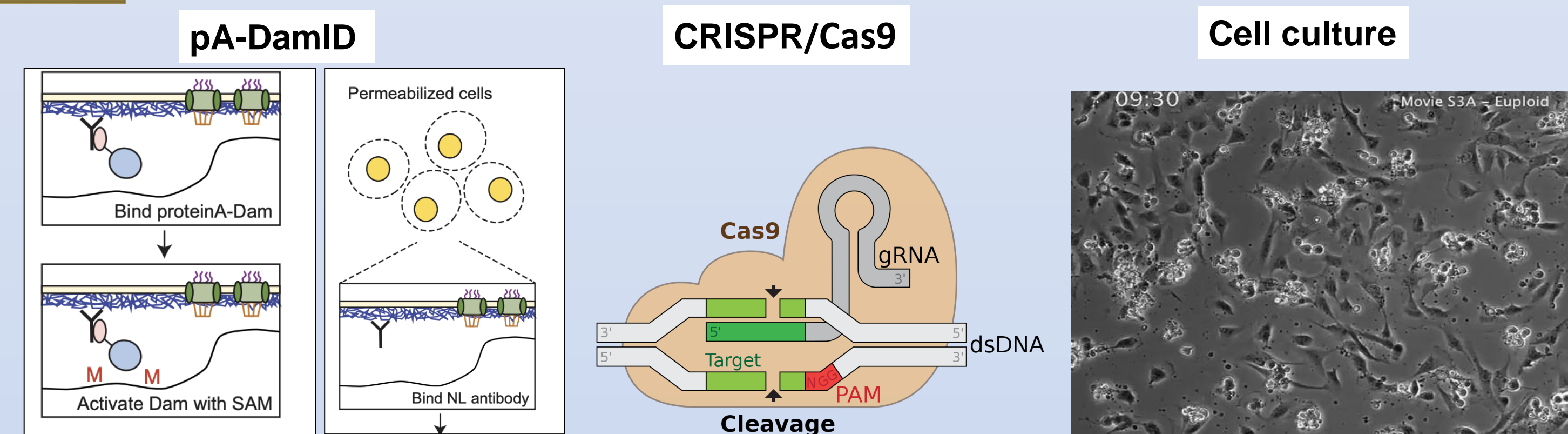
Figure 1. pA-DamID tracks generated with the pA-DamID pipeline using data from 3 experimental replicates. Left track shows the detachment from the nuclear lamina 10h after induction of a DSB (LaminB2). Right track shows the γ-H2AX signal, widespread increase in γ-H2AX signal is visible after induction of a break. Panel A shows the position on the genome with the located genes. In section B the pA-DamID tracks are shown, the red line is the experimental track in which a DSB is induced and is harvested for pA-DamID 10h later. The blue line is the control in which no DSB is induced. The green line is the cute site. Panel C is the domainogram that visualizes the differences between the control and experimental.

Goals:

☐ Determining the impact of a Double-stranded DNA break on Lamina-associated domains

☐ Determining the main cause of the relocalization of the Lamina Associated Domains following the Double-Stranded Breaks

Methods:

Result B: Inducing a DSB in a LAD with the inhibiton of ATM shrinks the detachment, indicating a role for ATM signaling in the detachment from the nuclear lamina
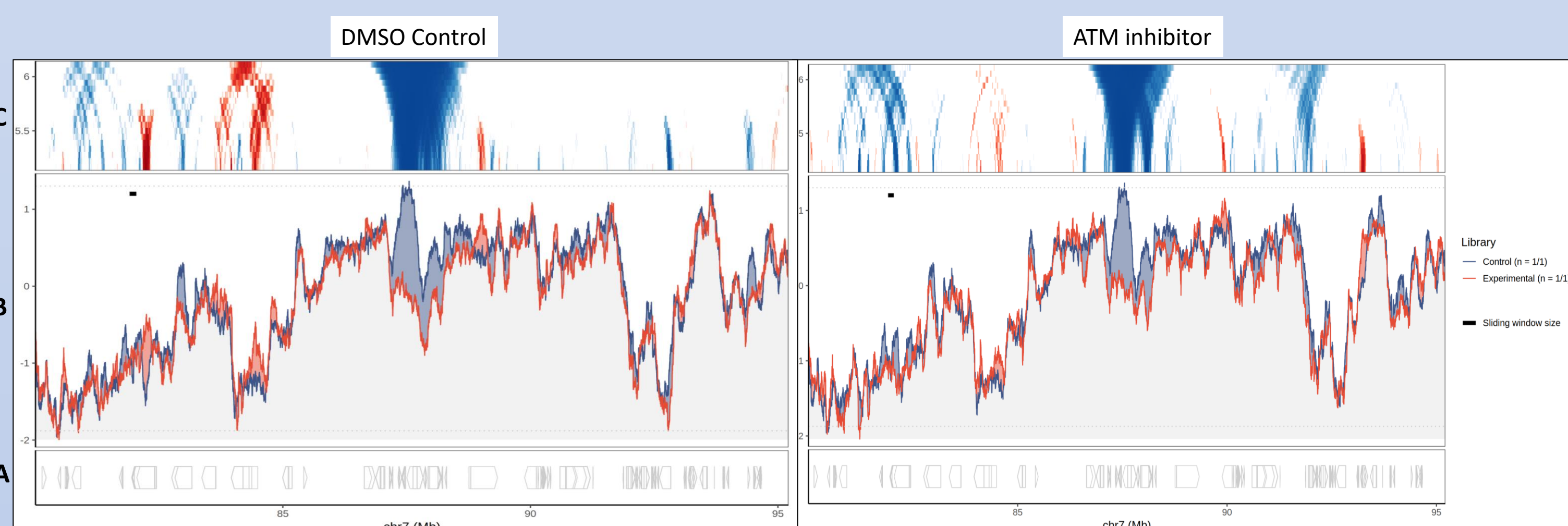
Figure 2. pA-DamID tracks generated with the pA-DamID pipeline using data from 1 experimental replicate. Left track shows the detachment from the nuclear lamina 10h after induction of a DSB (LaminB2) in normal conditions. The right track shows the detachment from the nuclear lamina 10h after induction of a DSB (LaminB2) with ATM inhibitor. Smaller detachment is visible when ATM is inhibited. Panel A shows the position on the genome with the located genes. In section B the pA-DamID tracks are shown, the red line is the experimental track in which a DSB is induced and is harvested for pA-DamID 10h later. The blue line is the control in which no DSB is induced. The green line is the cute site. Panel C is the domainogram that visualizes the differences between the control and experimental.

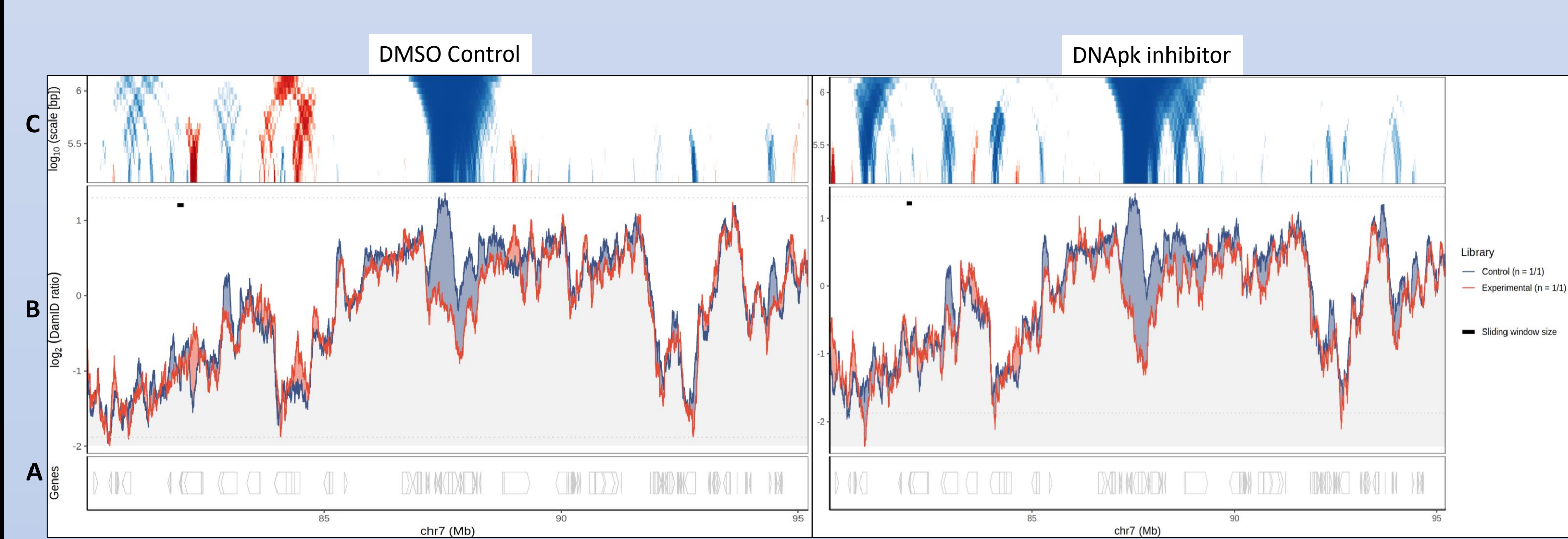## Result C: Inducing a DSB in a LAD with the inhibitor of DNApk enlarges the detachment

Figure 3. pA-DamID tracks generated with the pA-DamID pipeline using data from 1 experimental replicate. Left track shows the detachment from the nuclear lamina 10h after induction of a DSB (LaminB2) in normal conditions. The right track shows the detachment from the nuclear lamina 10h after induction of a DSB (LaminB2) with DNApk inhibitor. Larger detachment is visible when DNApk is inhibited. Panel A shows the position on the genome with the located genes. In section B the pA-DamID tracks are shown, the red line is the experimental track in which a DSB is induced and is harvested for pA-DamID 10h later. The blue line is the control in which no DSB is induced. The green line is the cute site. Panel C is the domainogram that visualizes the differences between the control and experimental.

Result D: Induction of knockout in the H2AFX gene in RPE-1 cell line using CRISPR/Cas9 and confirming the knock out by using TIDE, Westernblot and pA-DamID experiments
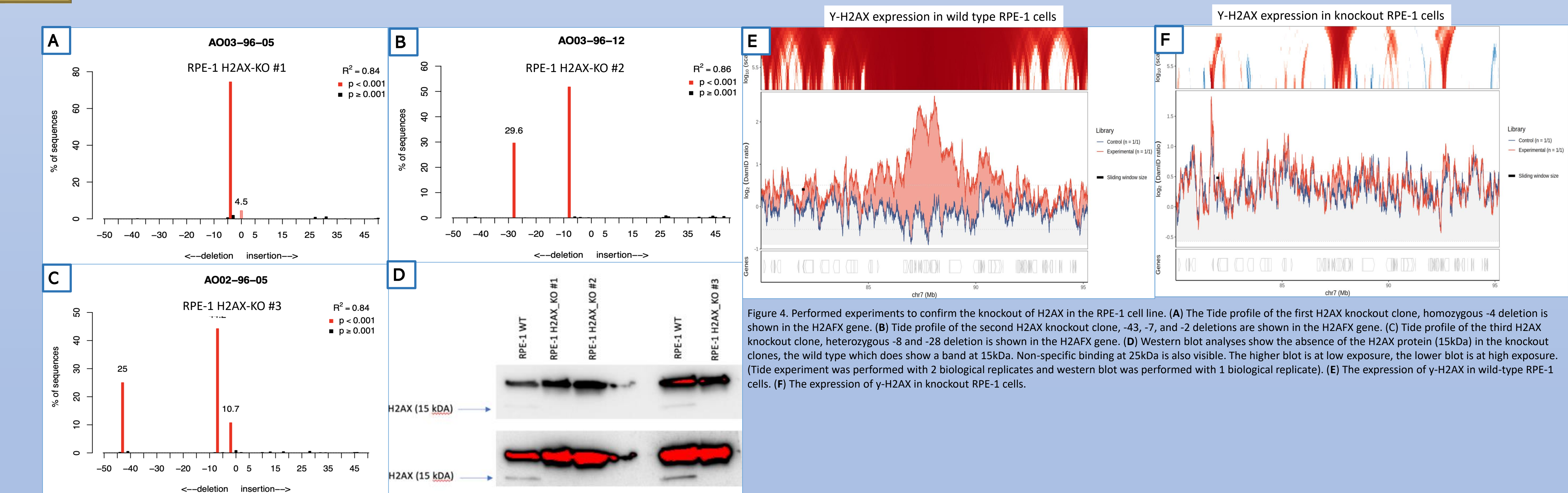
Figure 4. Performed experiments to confirm the knockout of H2AX in the RPE-1 cell line. (A) The Tide profile of the first H2AX knockout clone, homozygous -4 deletion is shown in the H2AFX gene. (B) Tide profile of the second H2AX knockout clone, -43, -7, and -2 deletions are shown in the H2AFX gene. (C) Tide profile of the third H2AX knockout clone, heterozygous -8 and -28 deletion is shown in the H2AFX gene. (D) Western blot analyses show the absence of the H2AX protein (15kDa) in the knockout clones, the wild type which does show a band at 15kDa. Non-specific binding at 25kDa is also visible. The higher blot is at low exposure, the lower blot is at high exposure. (Western blot experiment was performed with 2 biological replicates and western blot was performed with 1 biological replicate). (E) The expression of γ-H2AX in wild-type RPE-1 cells. (F) The expression of γ-H2AX in knockout RPE-1 cells.

Discussion

In general, the impact of a double-stranded break on DNA is well studied, but the impact of a double-stranded break on the organization of lamina-associated domains and the determinants of the potential relocalization of the LADs remains unknown. Here, we show that 10 hours after induction of DSB within a LAD, widespread detachment from the nuclear lamina occurs. At the same time, we also profiled γ-H2AX which showed a drastic increase surrounding the break site. ATM has been described to be responsible for the phosphorylation of γ-H2AX. Based on the increase in γ-H2AX and the fact that in the results, less detachment was observed when ATM was inhibited, we believed that γ-H2AX could play a role in the detachment from the nuclear lamina. To investigate this hypothesis, we induced a knockout by CRISPR/Cas9 of the H2AFX in RPE-1 cells and performed pA-DamID on these cells under the same circumstances (10h timepoint). We showed three confirmed knockout clones by PCR, Sanger sequencing and western blot, RPE-1 H2AX_KO #2 shows within the TIDE profile three peaks which represents a trisomy. Since the RPE-1 cell line is diploid, this result was unexpected. This could come from an accumulated mutation during passaging, however duplicating a whole (or part of) a chromosome seems unlikely. The most likely explanation for this could be that we had a colony of two clones (one heterozygous and one homozygous) and that it was pure luck that both were clones with an off-frame mutation. The western blot experiment was sufficient to confirm the knockout H2AX in the clones, but the results were not clear. The loading control indicated that the loading of samples was not even, the bands required a high intensity to become visible and a significantly non-specific big band is visible at 25kDa. This could be from the fact that our primary antibody (anti-H2AX) was binding to another protein, or it could be that our secondary antibody was binding to another protein. The rather one seems unlikely, so a follow-up experiment on this would be to induce a double-stranded break in the RPE-1 cells and harvest them after 10h to perform a western blot. This will induce γ-H2AX expression in wild-type conditions but in the clones, it should not. The western blot needs therefore to be done with an antibody targeting γ-H2AX. The anti-γ-H2AX is more accessible and more used and tested in comparison to the anti-H2AX antibody. In the pA-DamID experiment on the H2AX clones, we pooled the knockout clones to make a polyclonal mix, the same was done with the wild-type clones and then pA-DamID was performed. This was done to lower the number of samples, even though this could influence the result. In the results we observe smaller detachment in the H2AX knockout clones, indicating a role for H2AX in the detachment from the nuclear lamina.

Conclusion

This study aimed to get insight into the impact of double-stranded break on lamina-associated domains and the determinants of the relocalization of the LADs. We have demonstrated that a detachment from the nuclear lamina occurred 10h after induction of a DSB in LADs. We also have shown that 10h after induction of a DSB, a significant increase in γ-H2AX occurred. While the histone modifications H3K9me2 and H3K9me3 seemed to get lost locally. Based on the increase in γ-H2AX after induction of a DSB and the fact that when ATM is inhibited less detachment was observed, we expected γ-H2AX to be involved in the detachment from the nuclear lamina. We compared the detachment between wild-type RPE-1 and H2AX knockout RPE-1 and showed a smaller detachment in the knockout cells. This indicates a role for γ-H2AX in the detachment from the nuclear lamina. In summary, the impact of a DSB on a LAD has been profiled, and γ-H2AX has been identified as a possible determinant of the relocalization of the LADs. More follow-up experiments are needed to clarify whether γ-H2AX has a direct effect or whether γ-H2AX recruits an important factor responsible for the relocalization of the LADs.

Result E: γ-H2AX is possibly involved in the detachment from the nuclear lamina, less detachment is observed in H2AX knockout RPE-1 cells
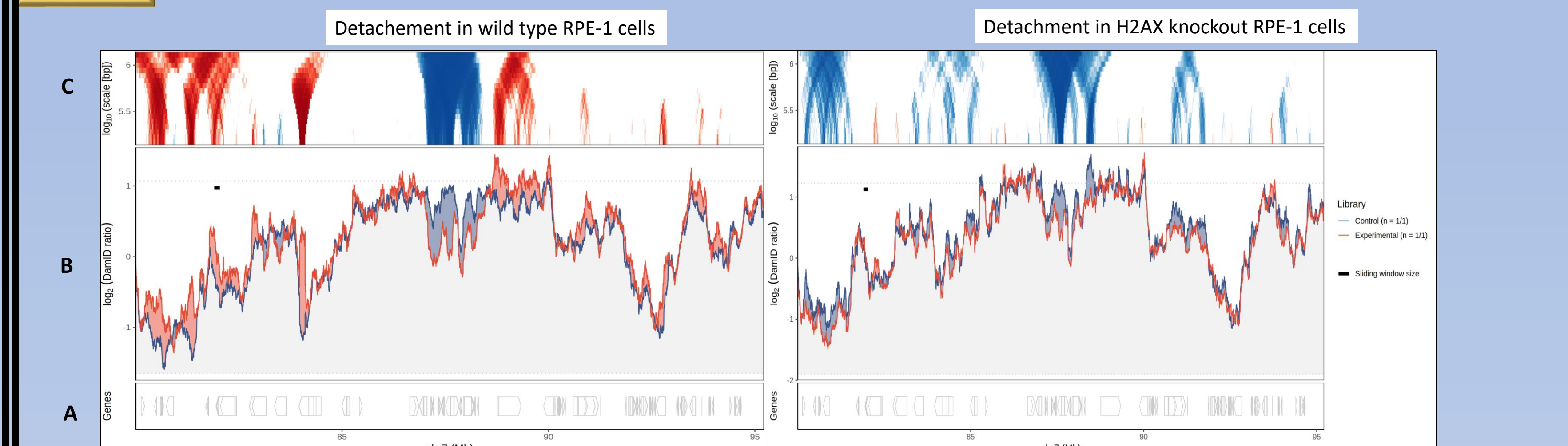
Figure 5. pA-DamID tracks generated with the pA-DamID pipeline using data from 1 experimental replicate. The left track shows the detachment from the nuclear lamina 10h after induction of a DSB (LaminB2) in wild-type RPE-1 cells. The right track shows the detachment from the nuclear lamina 10h after induction of a DSB (LaminB2) in H2AX knockout RPE-1 cells. A smaller detachment is visible in the knockout RPE-1 cells. Panel A shows the position on the genome with the located genes. In section B the pA-DamID tracks are shown, the red line is the experimental track in which a DSB is induced and is harvested for pA-DamID 10h later. The blue line is the control in which no DSB is induced. The green line is the cute site. Panel C is the domainogram that visualizes the differences between the control and experimental.

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