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Supplemental Information

Structural and Dynamical Signatures of Local DNA Damage in Live Cells

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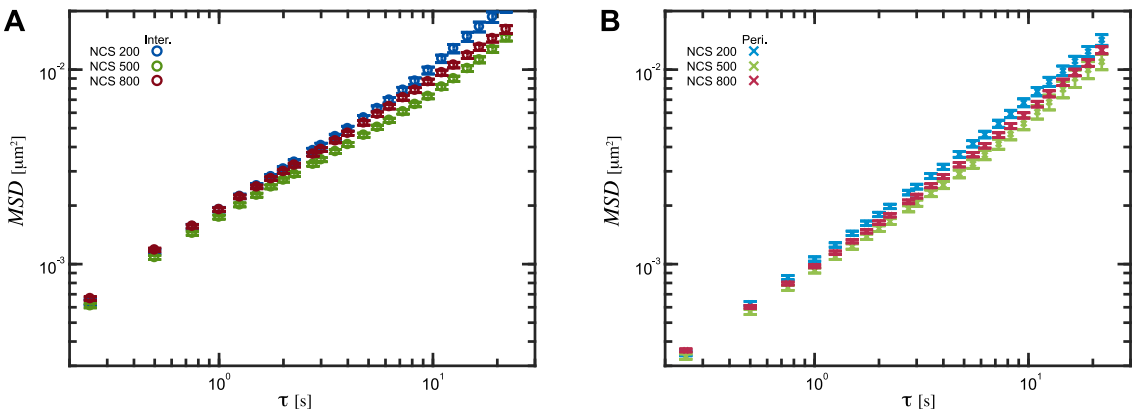
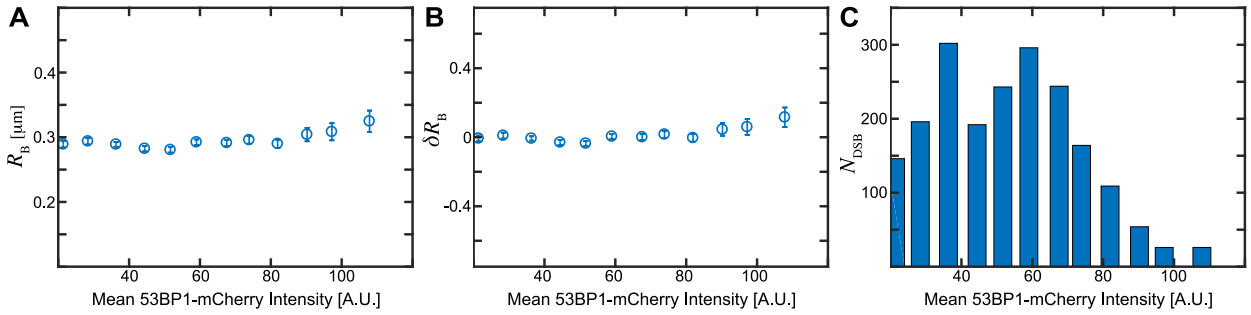
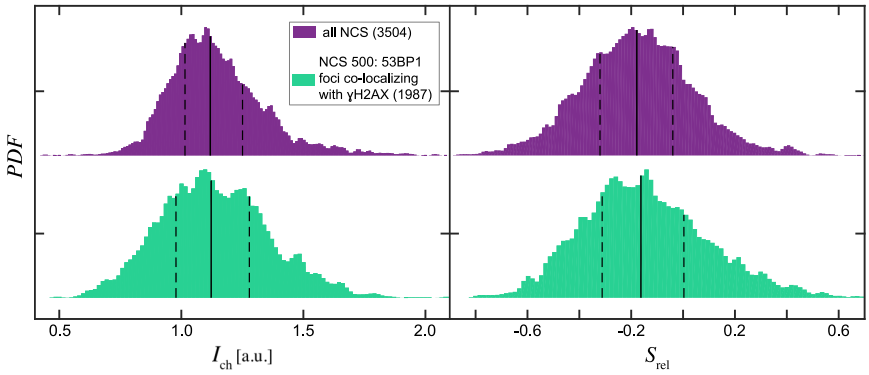


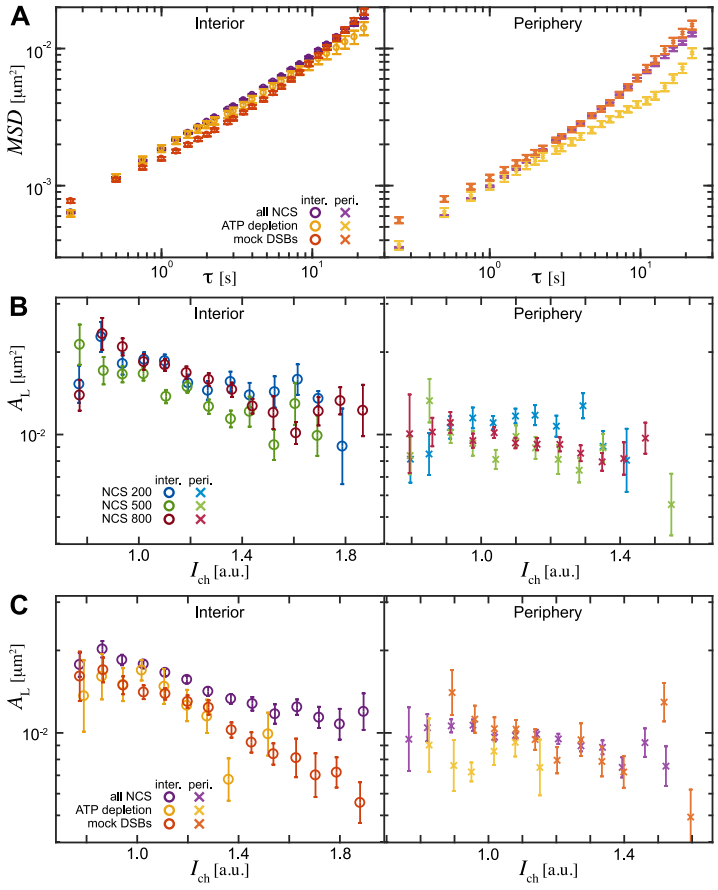
Supplementary Figure 1: DSB dynamics as a function of the DSB position and NCS concentration. (***A***) Average *MSD* for the interior DSBs from Fig. 2E plotted with error bars (standard error). (***B***) Average *MSD* for the periphery DSBs from Fig. 2E plotted with error bars (standard error).



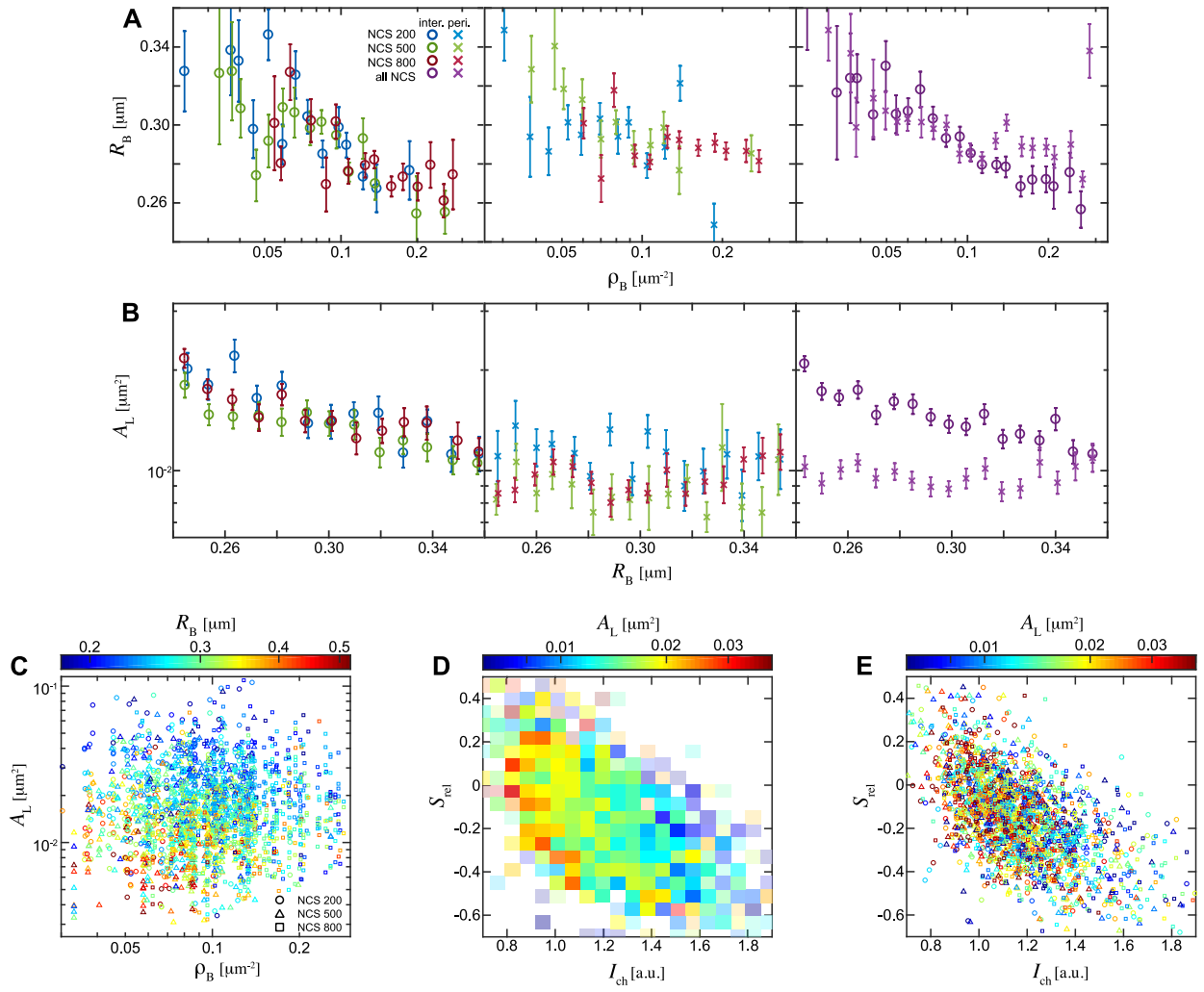
Supplementary Figure 2: The 53BP1 focus size vs. the 53BP1-mCherry expression. (***A***) Size of a DSB focus, R_{B}, as a function of the mean 53BP1-mCherry intensity in the cell nucleus measured for 2052 interior DSBs in 356 cell nuclei. (***B***) Relative change of R_{B}, δR_{B}, as a function of the mean 53BP1-mCherry intensity across the cell nucleus, where $\delta R_{\mathrm{B}} = \left(R_{\mathrm{B}} - \langle R_{\mathrm{B}} \rangle\right) / \langle R_{\mathrm{B}} \rangle$. (***C***) Histogram depicting the number of DSBs evaluated at a given mean 53BP1-mCherry intensity. No correlation was found between the 53BP1 focus size and the 53BP1-mCherry expression. Error bars shown are standard errors.



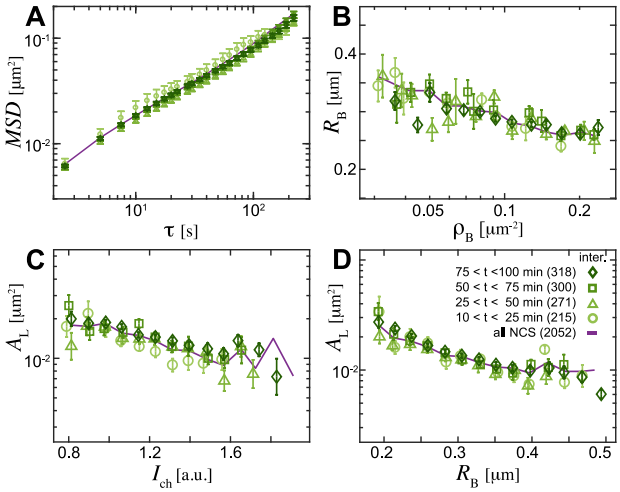
Supplementary Figure 3: Unique chromatin compaction profile at DSBs. Distributions of I_{ch} and S_{rel} including both the interior and periphery 53BP1 foci from NCS-treated nuclei from Fig. 4 (*purple*) and for the 53BP1 foci co-localizing with γH2AX from Fig. 1D (*green*).



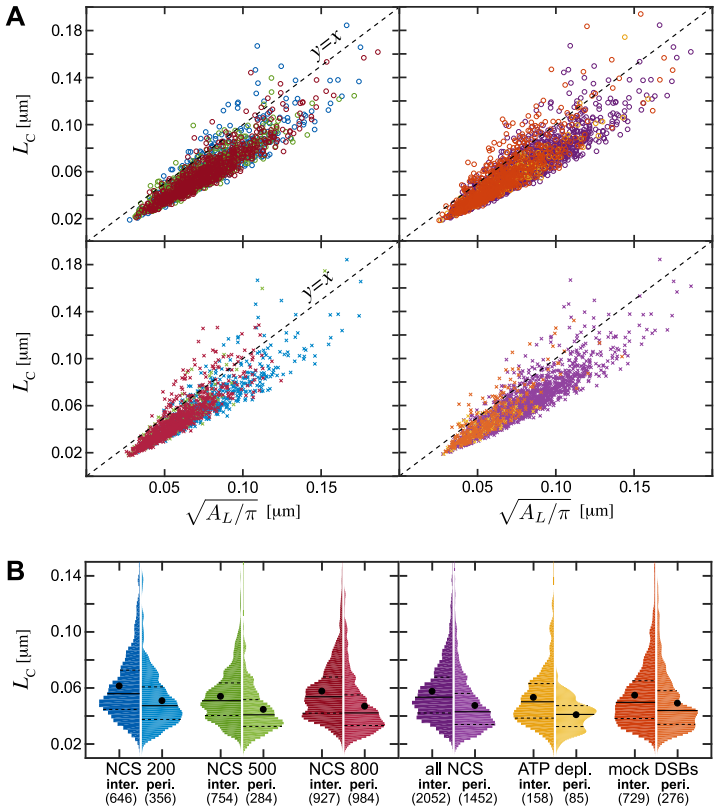
Supplementary Figure 4: Negative control for the DSB dynamics. (***A***) Average *MSD* from Fig. 4D plotted with error bars (standard error). (***B***) Relationship between the long-term DSB mobility (A_L) and the local chromatin compaction (I_{ch}) for three NCS concentrations plotted with error bars (standard error). (***C***) Relationship between the long-term DSB mobility (A_L) and the local chromatin compaction (I_{ch}) from Fig. 4E with plotted with error bars (standard error).



Supplementary Figure 5: Universal behavior of the DSB dynamics. (***A***) Relationship between the DSB focus size (R_B) and nuclear DSB density (ρ_B) from Fig. 5A plotted with error bars (standard error). (***B***) Relationship between the long-term DSB mobility (A_L) and the DSB focus size (R_B) from Fig. 5B plotted with error bars (standard error). (***C***) R_B as a function of A_L and ρ_B for each analyzed DSB in the nuclear interior across all NCS concentrations: NCS 200 (*circles*), NCS 500 (*triangles*) and NCS 800 (*squares*). (***D***) Average value of A_L as a function of S_{rel} and I_{ch} for analyzed DSBs in the nuclear interior across all NCS concentrations. Boxes with greater transparency present average over fewer DSBs. (***E***) A_L as a function of S_{rel} and I_{ch} for each analyzed DSB in the nuclear interior across all NCS concentrations: NCS 200 (*circles*), NCS 500 (*triangles*) and NCS 800 (*squares*).



Supplementary Figure 6: DSB dynamics as a function of time upon DNA damage induction. (***A***) Average *MSD* for the interior DSBs analyzed during 10 - 25 min (*circles*, N_{DSB} = 215, N_{Cells} = 42), 25 - 50 (*triangles*, N_{DSB} = 271, N_{Cells} = 47), 50 - 75 min (*squares*, N_{DSB} = 300, N_{Cells} = 49) and 75 - 100 min (*diamonds*, N_{DSB} = 318, N_{Cells} = 54) after NCS 500ng/mL addition. *Purple line* (N_{DSB} = 2052, N_{Cells} = 356) represents data from Fig. 5C collected at 60 min mark. (***B***) R_{B} as a function of ρ_B for DSBs analyzed at different times. (***C***) A_{L} as a function of I_{ch} for DSBs analyzed at different times. (***D***) A_{L} as a function of R_{B} for DSBs analyzed at different times. Error bars shown are standard errors.



Supplementary Figure 7: Comparison of the long-term mobility, A_L, and the length of constraint, L_C, for a DSB focus trajectory. (***A***) L_C as a function $\sqrt{A_L/\pi}$ shows linear dependence suggesting that the two measures are equivalent. The length of constraint, L_C, is defined as the standard deviation of the centroid position of a DSB focus with respect to its temporal average [35]. (***B***) L_C distributions calculated for all individual DSB trajectories presented in the DSB populations from Figs. 2 and 5.

Supplementary Table 1: Summary of the characteristics (mean ± SE) of the DSB dynamics for the interior and periphery DSBs at 3 different NCS concentrations.

	NCS 200		NCS 500		NCS 800	
N_{Cell} *(total)*	107		121		131	
ρ_B $\left[\#/\mu m^2\right]$	0.077 ±0.003		0.084 ±0.003		0.145 ±0.005	
	inter.	*peri.*	*inter.*	*peri.*	*inter.*	*peri.*
N_{Cell}	107	99	121	100	128	131
N_{DSB}	610	336	682	254	760	862
A_{s} $\left[\mu m^2\right]$	0.00064 ±0.00002	0.00035 ±0.00001	0.00061 ±0.00002	0.00034 ±0.00001	0.00067 ±0.00001	0.00036 ±0.00001
α	0.675 ±0.008	0.717 ±0.012	0.607 ±0.007	0.625 ±0.015	0.634 ±0.007	0.652 ±0.008
A_{L} $\left[\mu m^2\right]$	0.0207 ±0.0006	0.0126 ±0.0004	0.0171 ±0.0004	0.0103 ±0.0004	0.0195 ±0.0005	0.0112 ±0.0002
R_{B} $\left[\mu m\right]$	0.301 ±0.003	0.296 ±0.003	0.295 ±0.003	0.298 ±0.003	0.280 ±0.002	0.289 ±0.002
S_{rel}	-0.142 ±0.009	-0.162 ±0.013	-0.134 ±0.008	-0.168 ±0.015	-0.176 ±0.008	-0.240 ±0.007
I_{ch}	1.158 ±0.009	1.088 ±0.008	1.144 ±0.008	1.095 ±0.012	1.209 ±0.008	1.118 ±0.005

N_{Cell} - number of cells analyzed
N_{DSB} - number of DSBs analyzed
ρ_B - number of DSBs per unit area
A_{s} - area explored by a DSB in 250 ms
α - exponent obtained from fitting the *MSD* to $f(\tau) = C + B\tau^{\alpha}$
A_{L} - area explored by a DSB in 25 s
R_{B} - radius of a 53BP1 focus
S_{rel} - slope of a linear fit to radially averaged H2B-GFP intensity around a DSB
I_{ch} - normalized H2B-GFP intensity at the DSB centroid

Supplementary Table 2: Summary of the p-values (Kolmogorov-Smirnov test) for all measured physical quantities in the nuclear interior and periphery across 3 different NCS concentrations.

	NCS 200 vs NCS 500 inter vs inter	NCS 200 vs NCS 800 inter vs inter	NCS 500 vs NCS 800 inter vs inter
A_s	4.2×10^{-1}	4.4×10^{-5}	1.7×10^{-3}
α	3.7×10^{-7}	1.8×10^{-3}	1.9×10^{-2}
A_L	3.5×10^{-3}	4.9×10^{-1}	7.0×10^{-4}
R_B	2.5×10^{-1}	1.0×10^{-7}	4.8×10^{-4}
S_{rel}	9.5×10^{-1}	4.1×10^{-3}	3.6×10^{-3}
I_{ch}	5.9×10^{-1}	9.6×10^{-5}	3.9×10^{-5}
	NCS 200 vs. NCS 200 peri vs inter	NCS 500 vs. NCS 500 peri vs inter	NCS 800 vs. NCS 800 peri vs inter
A_s	0	0	0
α	1.1×10^{-3}	2.2×10^{-2}	4.0×10^{-3}
A_L	0	0	0
R_B	6.0×10^{-3}	7.1×10^{-4}	6.3×10^{-9}
S_{rel}	4.8×10^{-2}	2.0×10^{-2}	1.8×10^{-8}
I_{ch}	6.7×10^{-7}	3.2×10^{-3}	0
	NCS 200 vs NCS 500 peri vs peri	NCS 200 vs NCS 800 peri vs peri	NCS 500 vs NCS 800 peri vs peri
A_s	2.2×10^{-1}	8.2×10^{-1}	4.1×10^{-2}
α	1.1×10^{-4}	8.0×10^{-4}	1.6×10^{-1}
A_L	3.8×10^{-6}	2.1×10^{-6}	3.4×10^{-1}
R_B	1.8×10^{-1}	3.5×10^{-2}	6.3×10^{-4}
S_{rel}	4.2×10^{-1}	1.4×10^{-6}	1.3×10^{-4}
I_{ch}	3.5×10^{-1}	1.8×10^{-2}	4.2×10^{-3}

Supplementary Table 3: Summary of the characteristics (mean ± SE) of DSB dynamics for the interior and periphery DSBs for the negative control (mock DSBs) and upon ATP depletion.

	All NCS		ATP dep		Mock DSB	
N_{Cell} *(total)*	359		38		83	
	inter.	*peri.*	*inter.*	*peri.*	*inter.*	*peri.*
N_{Cells}	356	330	37	29	83	74
N_{DSB}	2052	1452	158	85	729	276
A_{s} $\left[\mu m^2\right]$	0.00064 ± 0.00001	0.00035 ± 0.00001	0.00063 ± 0.00003	0.00037 ± 0.00003	0.00077 ± 0.00002	0.00056 ± 0.00003
α	0.637 ±0.004	0.662 ±0.006	0.580 ±0.013	0.572 ±0.019	0.587 ±0.009	0.584 ±0.015
A_{L} $\left[\mu m^2\right]$	0.0190 ±0.0003	0.0113 ±0.0002	0.0171 ±0.0010	0.0092 ±0.0005	0.0151 ±0.0004	0.0115 ±0.0005
S_{rel}	-0.152 ±0.005	-0.209 ±0.006	-0.088 ±0.016	-0.074 ±0.024	-0.125 ±0.009	-0.173 ±0.016
I_{ch}	1.172 ±0.005	1.107 ±0.004	1.094 ±0.015	1.014 ±0.014	1.200 ±0.009	1.132 ±0.010

N_{Cell} - number of cells analyzed
N_{DSB} - number of DSBs analyzed
A_{s} - area explored by a DSB in 250 ms
α - exponent obtained from fitting the *MSD* to $f(\tau) = C + B\tau^{\alpha}$
A_{L} - area explored by a DSB in 25 s
S_{rel} - slope of a linear fit to radially averaged H2B-GFP intensity around a DSB
I_{ch} - normalized H2B-GFP intensity at the DSB centroid

Supplementary Table 4: Summary of the *p*-values (Kolmogorov Smirnov test) for all measured physical quantities for the interior and periphery DSBs for the negative control (mock DSBs) and upon ATP depletion.

	all NCS vs ATP dep inter vs inter	all NCS vs Mock DSB inter vs inter	ATP dep vs Mock DSB inter vs inter
A_s	4.9×10^{-1}	2.2×10^{-6}	2.2×10^{-2}
α	1.6×10^{-3}	0	9.0×10^{-2}
A_L	4.4×10^{-2}	0	6.2×10^{-2}
S_{rel}	1.4×10^{-3}	2.1×10^{-1}	1.9×10^{-2}
I_{ch}	1.9×10^{-6}	1.1×10^{-1}	1.5×10^{-7}
	all NCS vs all NCS peri vs inter	ATP dep vs ATP dep peri vs inter	Mock DSB vs Mock DSB peri vs inter
A_s	0	3.8×10^{-8}	5.8×10^{-6}
α	1.1×10^{-7}	6.9×10^{-1}	5.1×10^{-1}
A_L	0	1.8×10^{-8}	5.3×10^{-7}
S_{rel}	0	4.4×10^{-1}	1.2×10^{-3}
I_{ch}	0	2.0×10^{-3}	7.1×10^{-5}
	all NCS vs ATP dep peri vs peri	all NCS vs Mock DSB peri vs peri	ATP dep vs Mock DSB peri vs peri
A_s	2.0×10^{-1}	0	4.2×10^{-4}
α	8.9×10^{-6}	1.7×10^{-8}	1.2×10^{-1}
A_L	3.4×10^{-2}	4.5×10^{-1}	8.2×10^{-2}
S_{rel}	3.2×10^{-7}	3.1×10^{-1}	2.0×10^{-4}
I_{ch}	1.1×10^{-7}	1.0×10^{-1}	1.6×10^{-8}