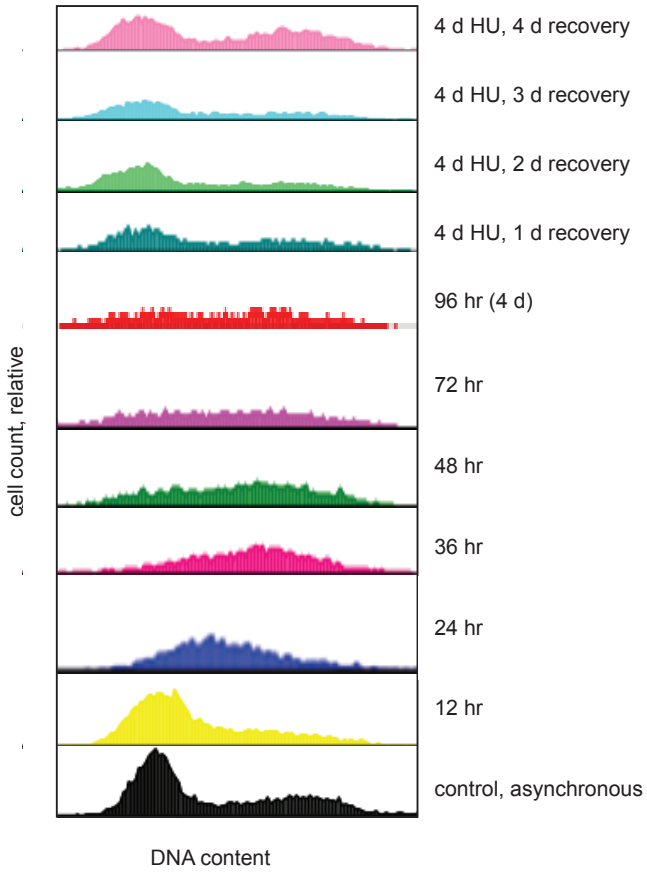
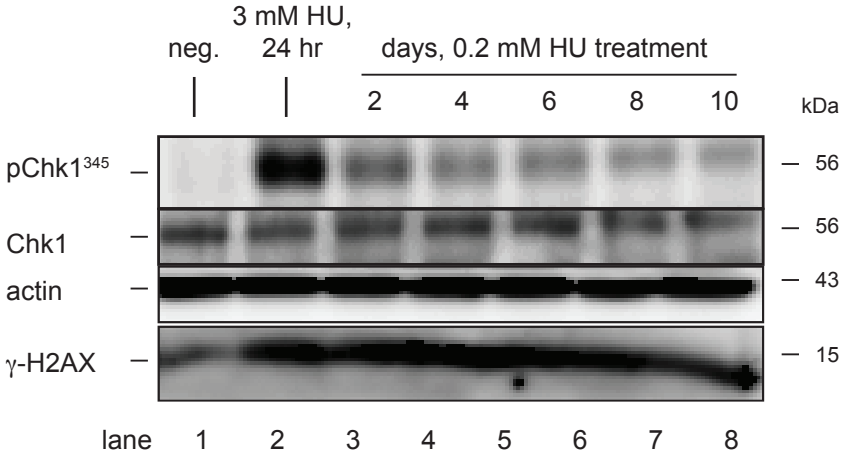
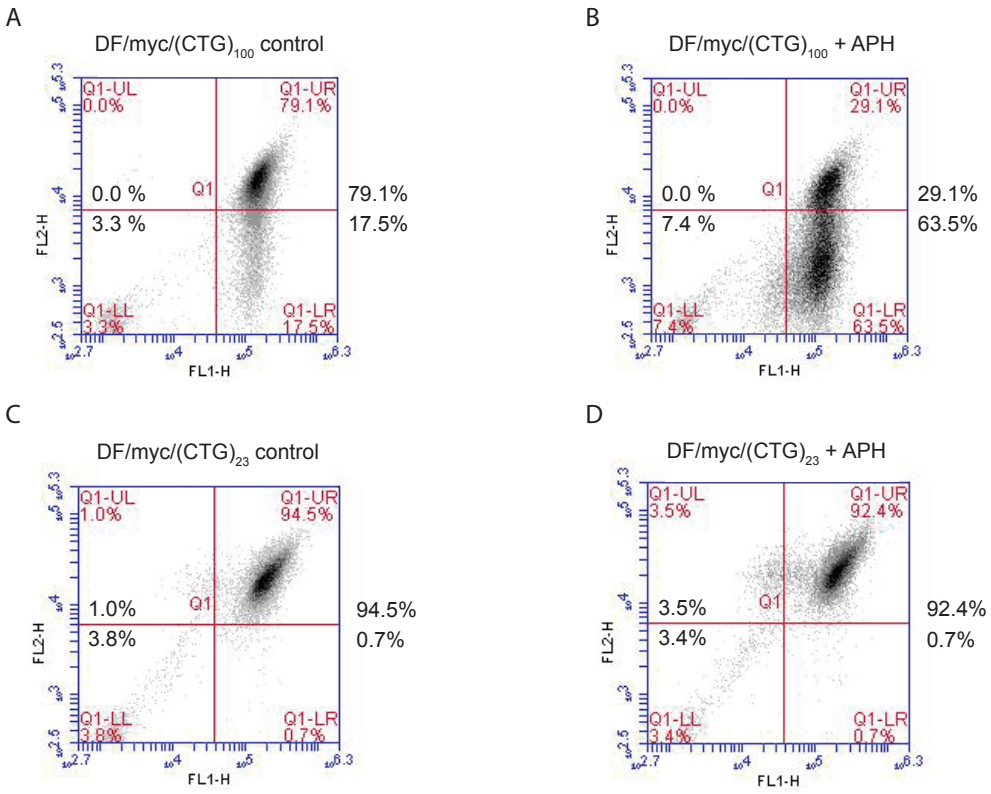
A



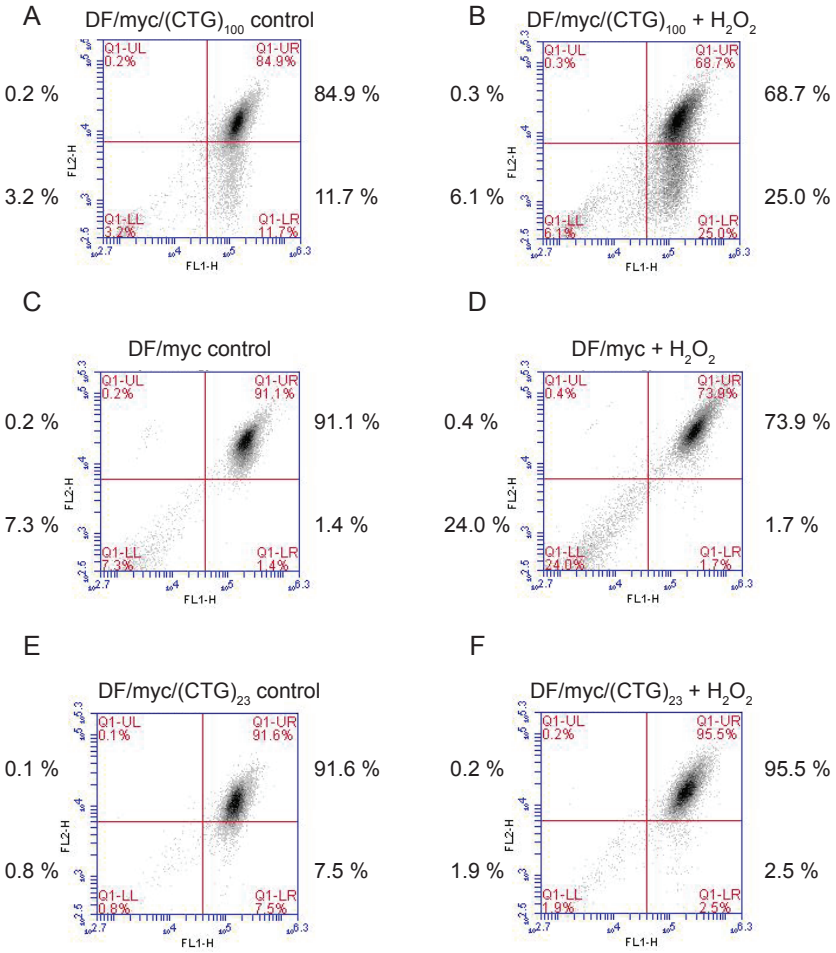
B



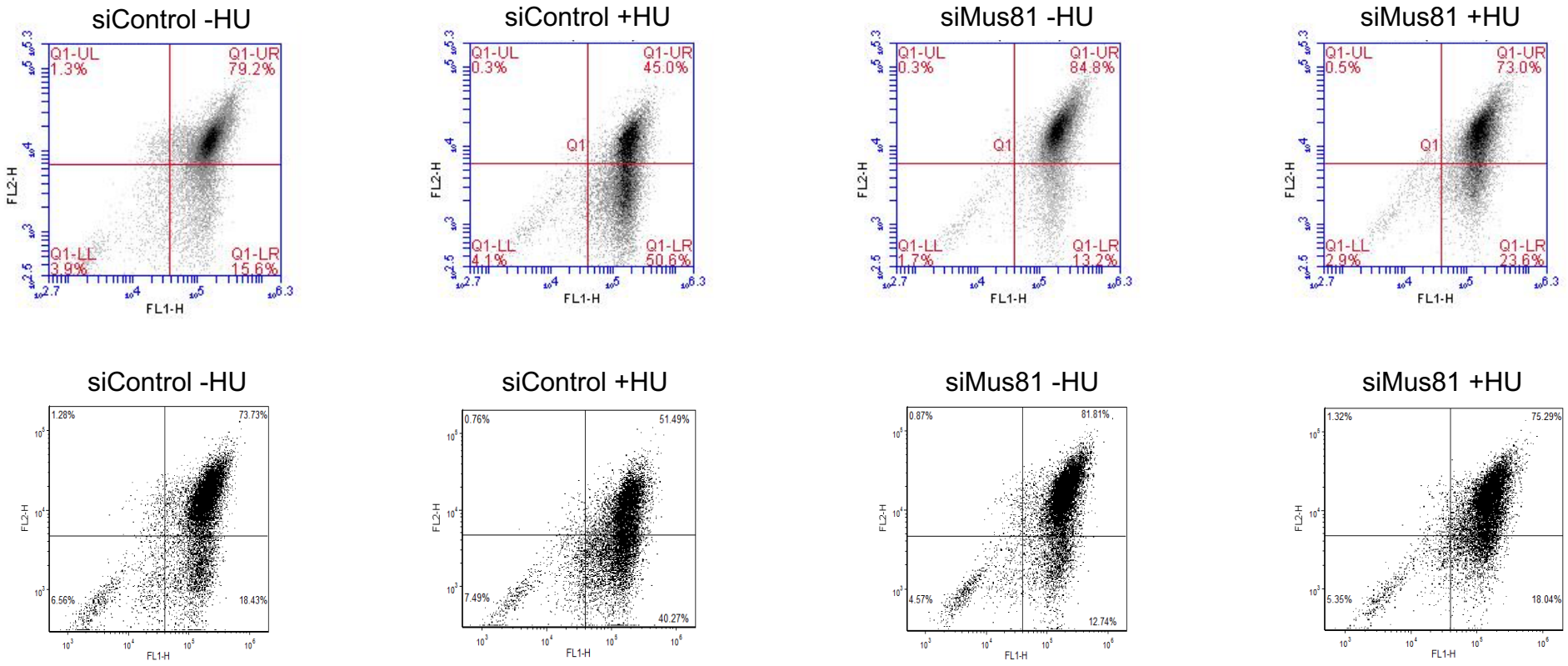
Supplementary Figure 1. Cell responses to HU treatment. (A) Cell cycle analysis by flow cytometry using DRAQ7 DNA dye 162. Cell aliquots were taken at the indicated times of HU treatment. (B) Western blot analysis of replication stress marker proteins. Lane 1, neg,, negative control, untreated cells. Lanes 2-8, HU treated cells. Whole cell extracts were probed for the indicated proteins after western blotting.



Supplementary Figure 2. Aphidicolin induces $(CTG/CAG)_{100}$ DSBs. (A) Untreated DF/myc$(CTG)_{100}$ cells; (B) DF/myc$(CTG)_{100}$ cells treated with low dose aphidicolin (APH, 0.2 uM, 48 hr) and allowed to recover for 8 days. (C) Untreated DF/myc$(CTG)_{23}$ cells; (D) DF/myc$(CTG)_{23}$ cells treated with low dose aphidicolin (APH, 0.2 uM, 48 hr) and allowed to recover for 8 days.

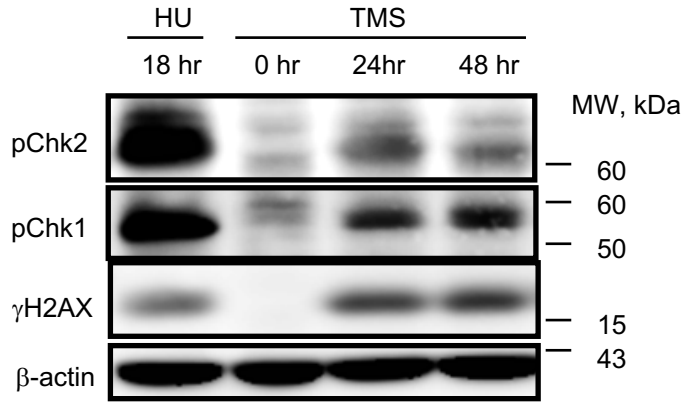


Supplementary Figure 3: $(CTG)_{100}$ repeats cause DNA double strand breaks under oxidative stress. (A) Untreated DF/myc$(CTG)_{100}$ cells; (B) DF/myc$(CTG)_{100}$ cells treated with hydrogen peroxide (Methods); (C) Untreated DF/myc cells; (D) DF/myc cells treated with hydrogen peroxide; (E) Untreated DF/myc$(CTG)_{23}$ cells; (F) DF/myc$(CTG)_{23}$ cells treated with hydrogen peroxide.

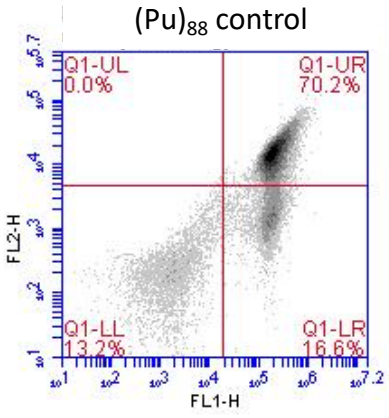


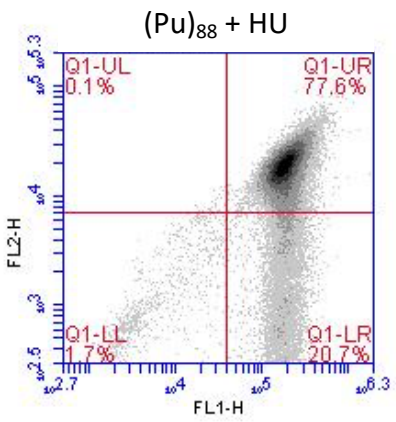
Supplementary Figure 4: Mus81 knockdown decreases replication-dependent DSBs. (A)-(D), (E)-(G) are replicates of the Mus81 knockdown experiments shown in Figure 4.

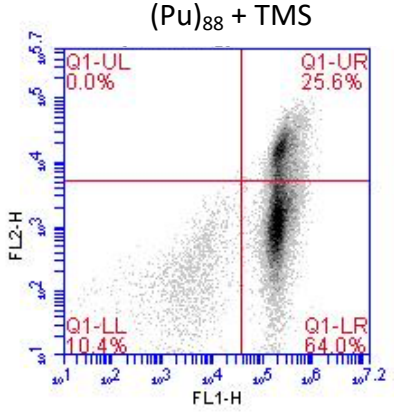
Supplementary Figure 5

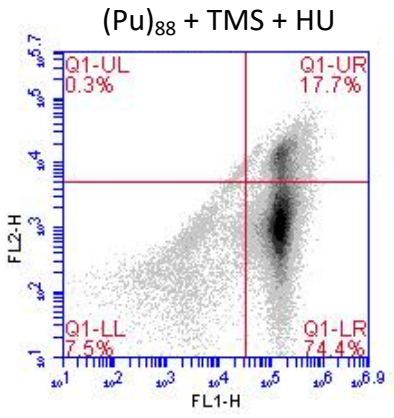


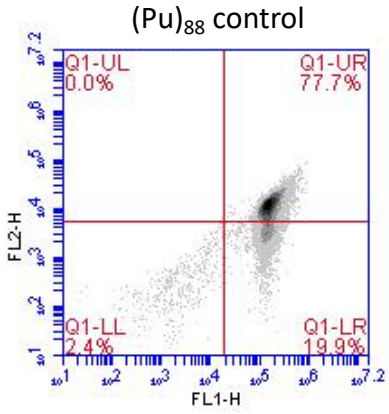
Supplementary Figure 5: Phosphorylation of DNA damage response proteins in cells treated with telomestatin. $(CTG)_{100}$ cells were treated with 2 mM HU (positive control) or 0.5 uM TMS for the indicated times, electrophoresed and blotted to probe for phospho-Chk1, phospho-Chk2, γH2AX and β-actin (loading control).

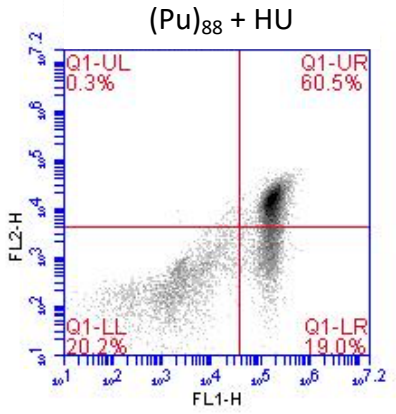

(Pu)88 control
Q1-UL
0.0%
Q1-UR
70.2%
Q1-LL
13.2%
Q1-LR
16.6%
FL2-H
FL1-H

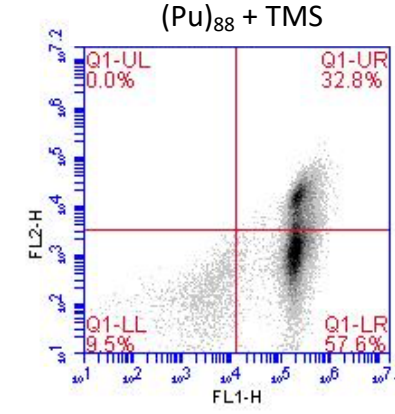

(Pu)88 + HU
Q1-UL
0.1%
Q1-UR
77.6%
Q1-LL
1.7%
Q1-LR
20.7%
FL2-H
FL1-H


(Pu)88 + TMS
Q1-UL
0.0%
Q1-UR
25.6%
Q1-LL
10.4%
Q1-LR
64.0%
FL2-H
FL1-H


(Pu)88 + TMS + HU
Q1-UL
0.3%
Q1-UR
17.7%
Q1-LL
7.5%
Q1-LR
74.4%
FL2-H
FL1-H


(Pu)88 control
Q1-UL
0.0%
Q1-UR
77.7%
Q1-LL
2.4%
Q1-LR
19.9%
FL2-H
FL1-H


(Pu)88 + HU
Q1-UL
0.3%
Q1-UR
60.5%
Q1-LL
20.2%
Q1-LR
19.0%
FL2-H
FL1-H


(Pu)88 + TMS
Q1-UL
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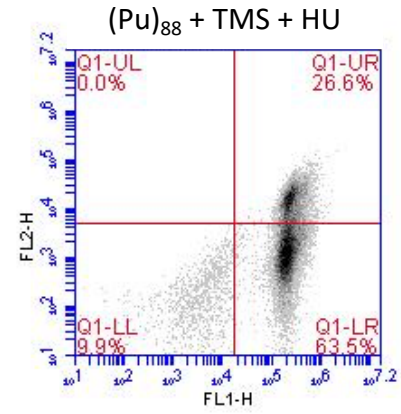
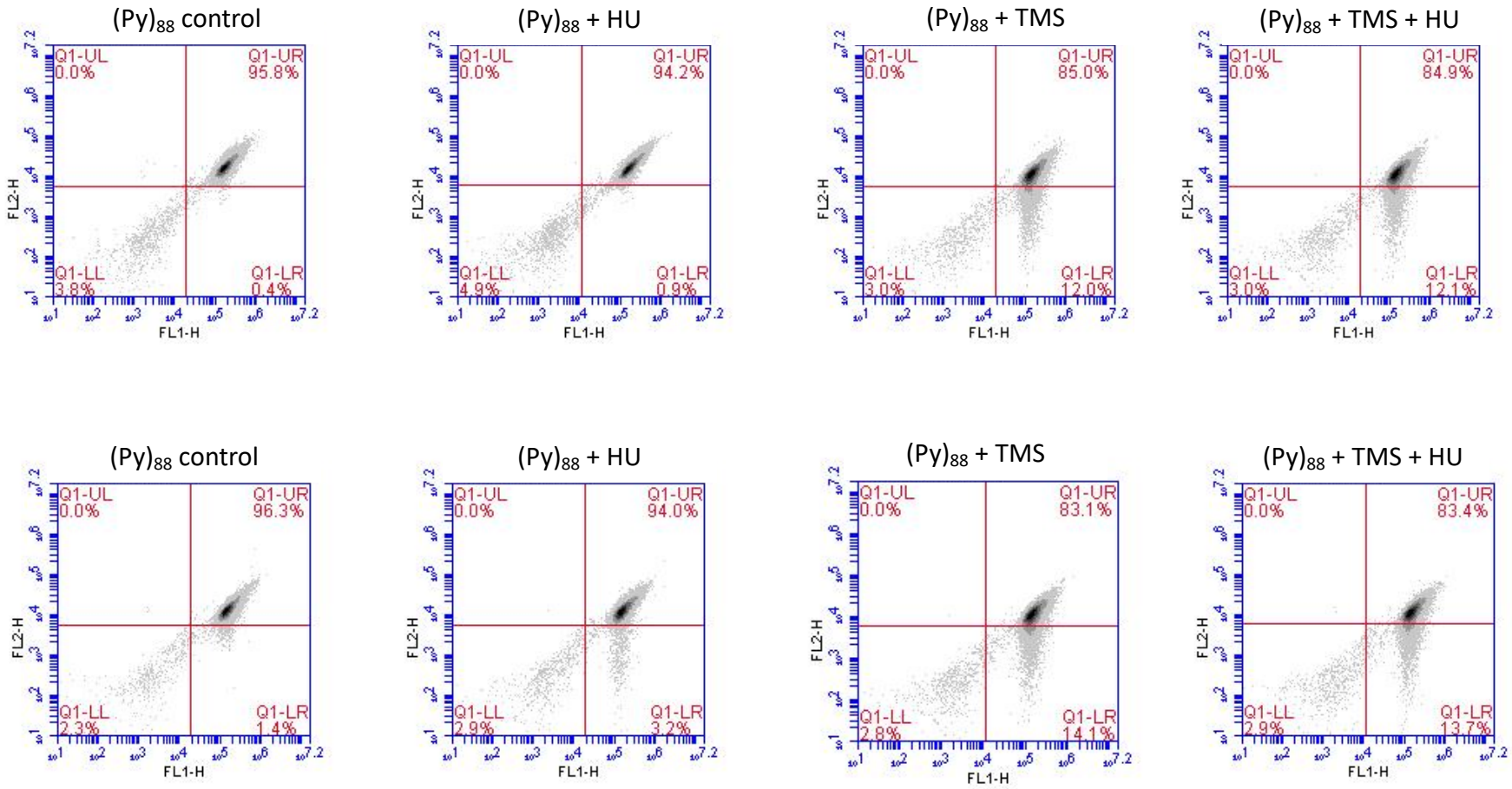
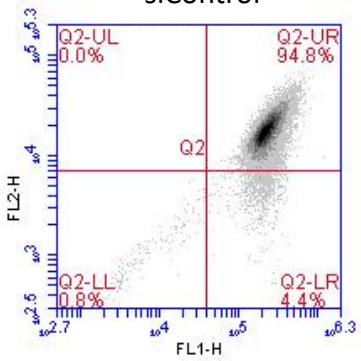
Q1-UR
32.8%
Q1-LL
9.5%
Q1-LR
57.6%
FL2-H
FL1-H


(Pu)88 + TMS + HU
Q1-UL
0.0%
Q1-UR
26.6%
Q1-LL
9.9%
Q1-LR
63.5%
FL2-H
FL1-H

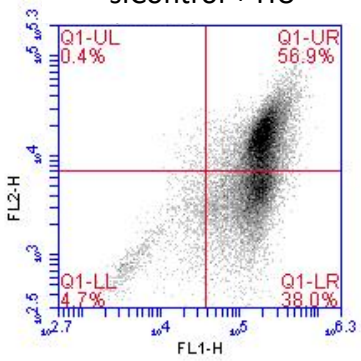


Supplementary Figure 6: $(Pu/Py)_{88}$ replication polarity affects replication-dependent DSBs. Replicates of the experiments shown in Figure 5.

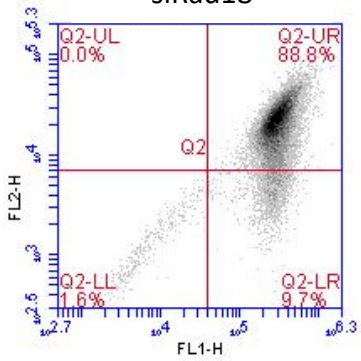
siControl



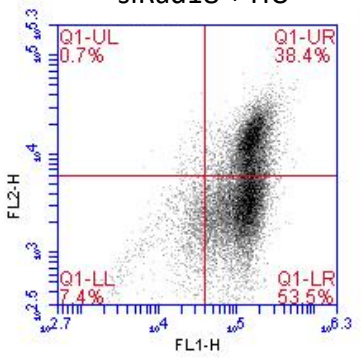
siControl + HU



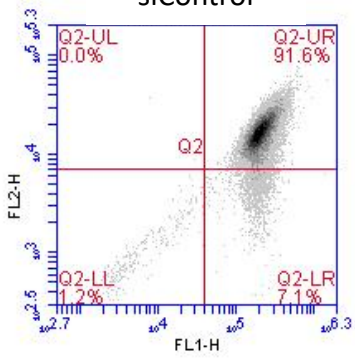
siRad18



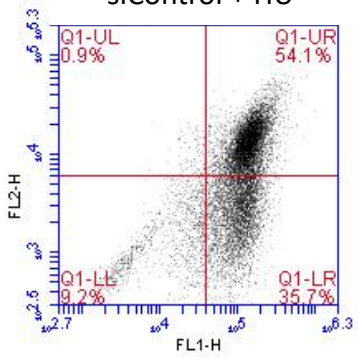
siRad18 + HU



siControl



siControl + HU



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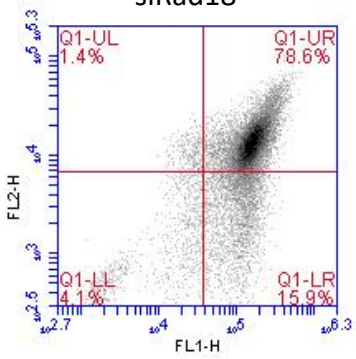

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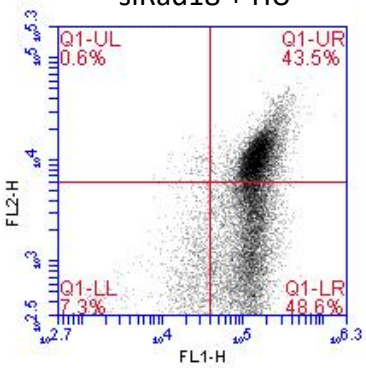

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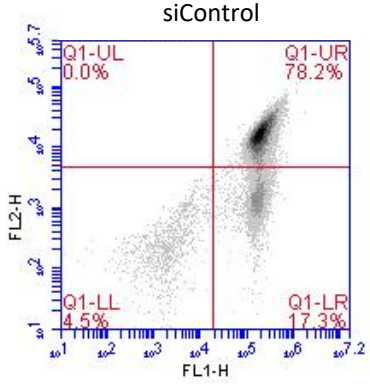
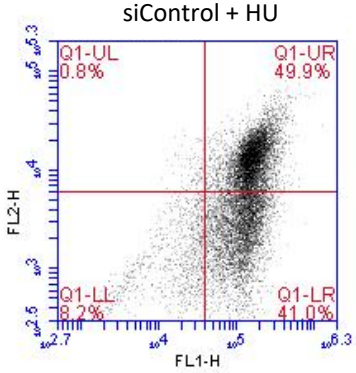
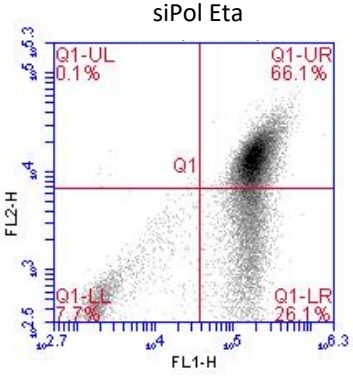


siControl + HU



siPol Eta



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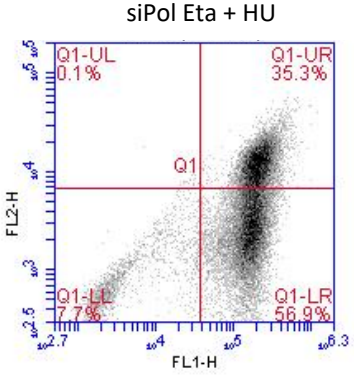

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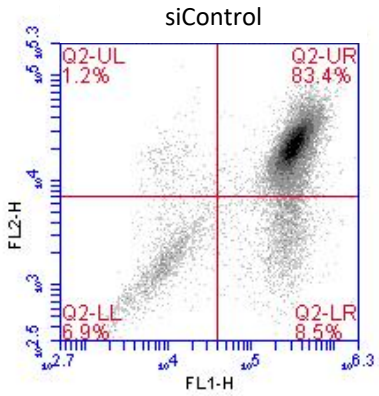

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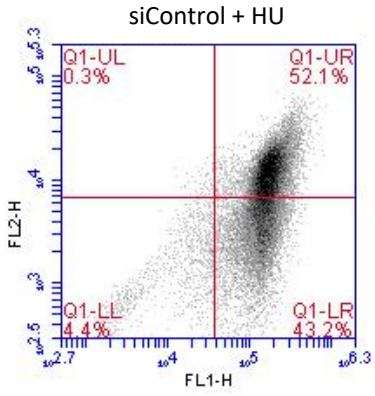

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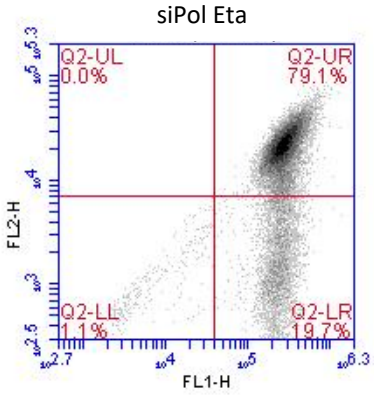
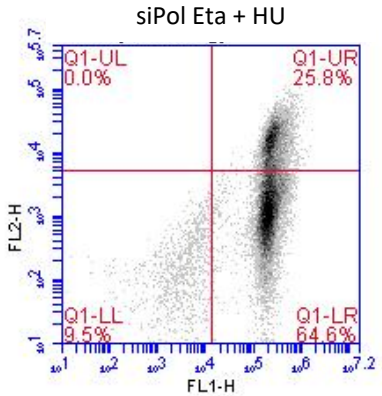
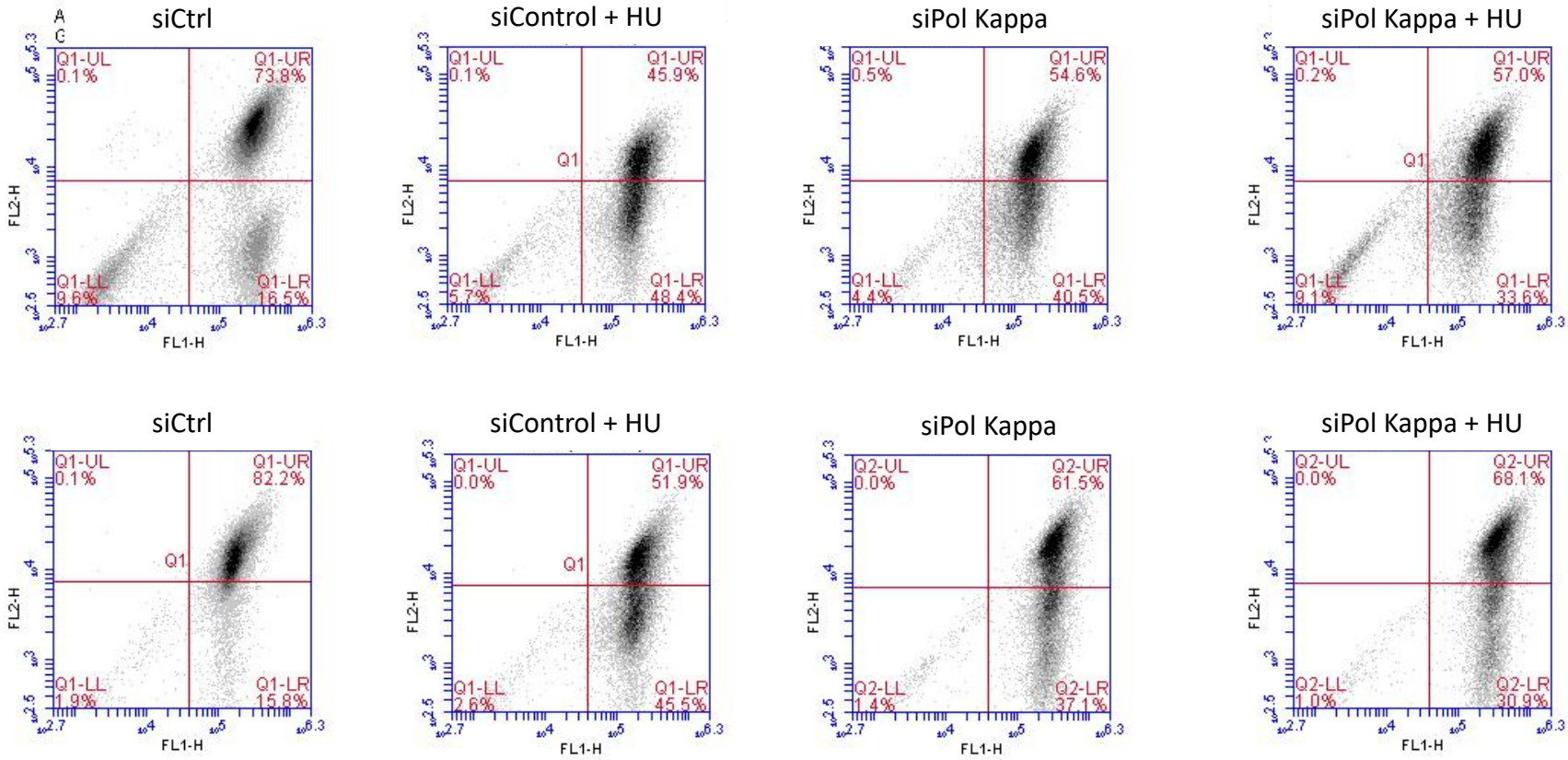


siPol Eta + HU





Supplementary Figure 7: Translesion synthesis pathways affect $(CTG/CAG)_{100}$ stability. Replicates of the experiments shown in Figure 7.