Drosopoulos et al., http://www.jcb.org/cgi/content/full/jcb.201410061/DC1

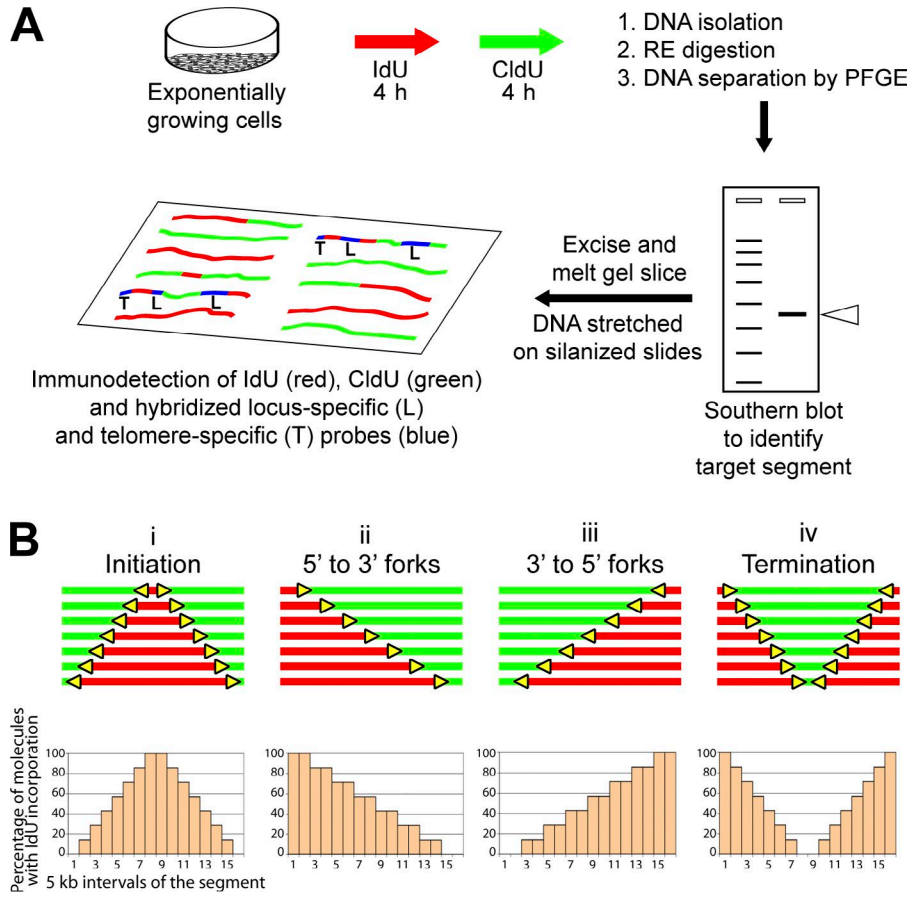

Figure S1. **SMARD.** (A) Exponentially growing cells are labeled by pulsing with IdU for 4 h followed by CldU for 4 h. Labeled cells were embedded in agarose, lysed, and deproteinized to isolate genomic DNA. The genomic DNA is digested with a rare-cutting restriction endonuclease to obtain unique large molecules, which are separated by pulsed field gel electrophoresis to enrich by size for the particular segments of interest. Southern blotting was performed to identify the target segment within the gel (arrowhead), which is excised. The gel slice containing the segment of interest is melted and the enriched DNA in the melted gel solution stretched on silanized glass slides. Indirect IF with antibodies against the halogenated nucleosides is used to identify regions where IdU (red) or CldU (green) were incorporated into the DNA. Immunodetected biotinylated FISH probes (blue) was used to identify the target molecules (Drosopoulos et al., 2012). (B) Images of individual molecules are aligned to produce a composite profile of replication. Characteristic patterns in the aligned molecule images identify specific replication events. Alignments displaying molecules of increasing red stain from one end indicate replication forks progressing in a single direction through the segment (ii and iii). Initiation events are observed as a red tract flanked on both sides by green (i), whereas a green tract flanked on both sides by red (iv) indicates a termination event. Molecule alignments can be graphically represented as histograms of percentage IdU incorporation for each 5 kb of a segment. Specific replication events produce characteristic features in these replication profiles. Locations of the centers of initiation sites are indicated by peaks (i) while termination events are seen as valleys (iv). Replication progressing through the segment primarily in one direction (5′ to 3′ or 3′ to 5′ relative to the polarity of the segment upper strand) from an external origin is observed as a progressive decrease in the percentage of IdU incorporation from one end to the other across the segment (x axis; ii and iii).

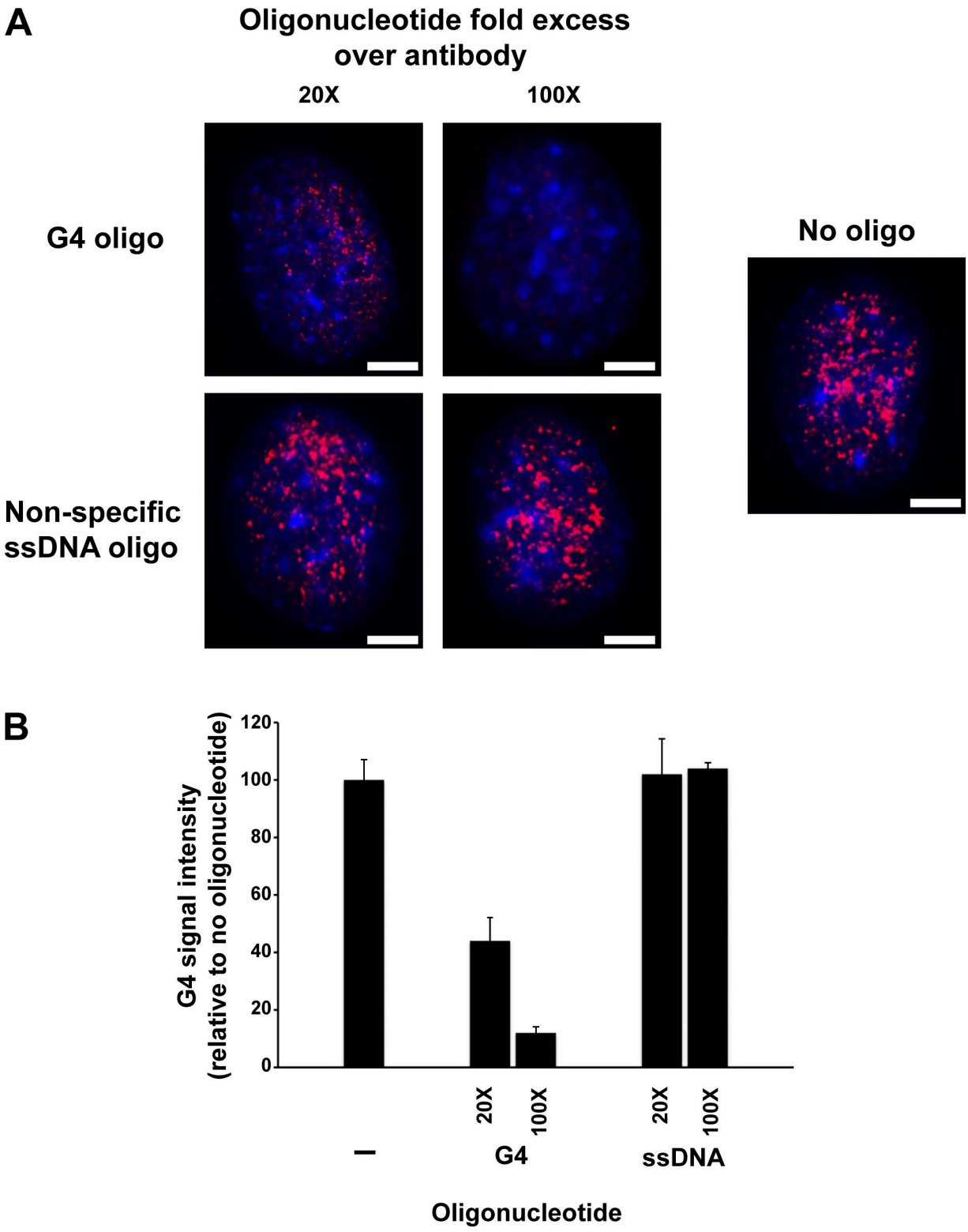

Figure S2. **BG4 antibody immunostaining is blocked by a prefolded G4 oligonucleotide.** To evaluate the in vivo specificity of the BG4 antibody, blocking experiments were performed using prefolded G4 oligonucleotides. (A) BG4 antibody was preincubated for 1 h with the indicated molar excess of prefolded G4 DNA oligonucleotide (5′-AGGGAGGGCGCTGGGAGGAGGG-3′) or single-stranded non–G4-forming DNA oligonucleotide (5′-GGCATAGTGCGT-GGGCG-3′) or no oligonucleotide (control) before antibody use in immunostaining. IF analysis was then performed on BLM-proficient ($BLM^{+/-}$) cells. BG4 staining is shown in red, with the nuclei counterstained with DAPI (blue). Bars, 5 µm. (B) Quantification of G4 signal intensity. Mean nuclear G4 signal intensity (red signal) is shown relative to no oligonucleotide control (–) signal intensity (set to 100). 40–65 cells per condition were analyzed. Bars represent mean values with standard deviations (error bars) of duplicate experiments.

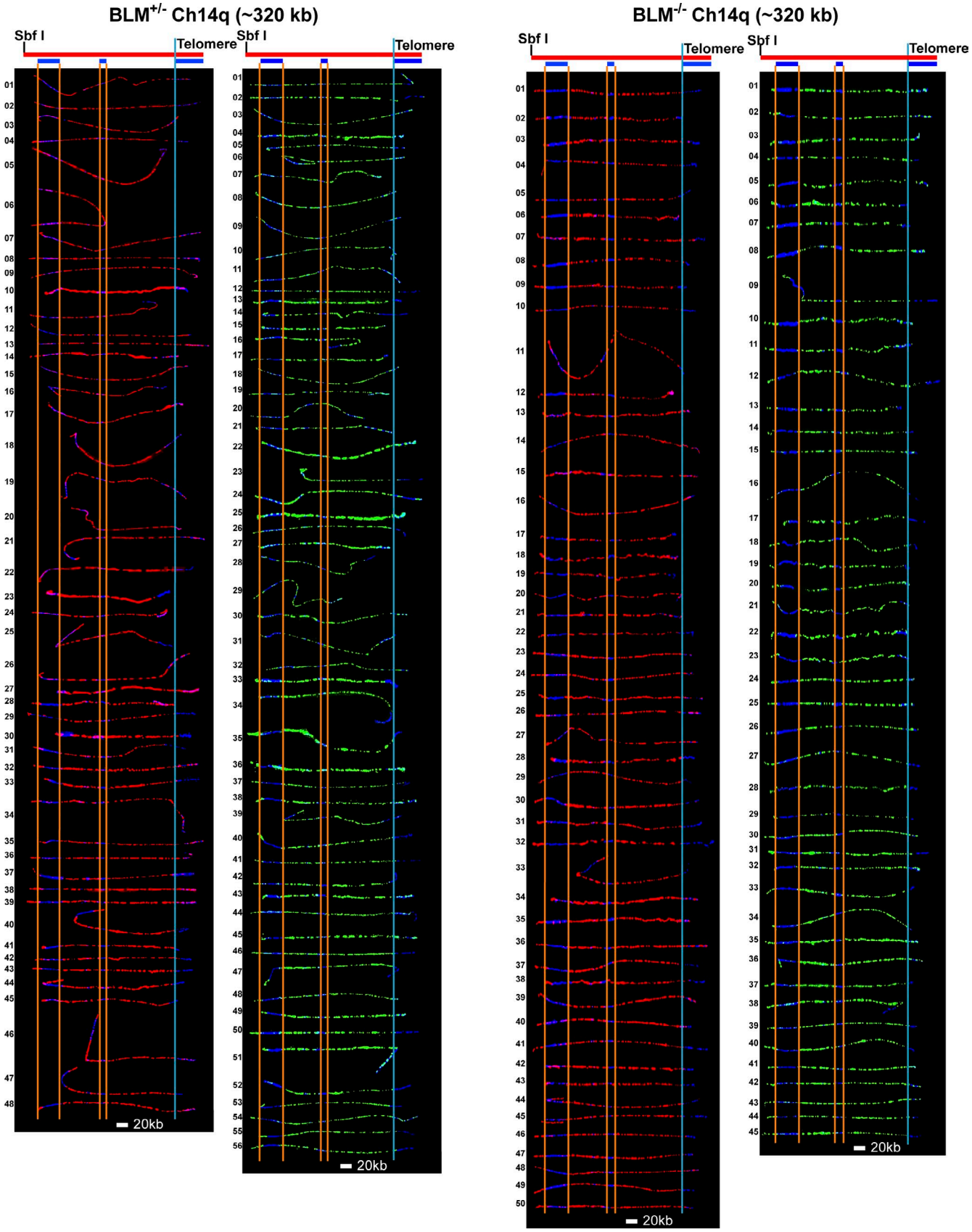

Figure S3. **Ch14q telomere molecules fully substituted with IdU (red) or CldU (green) from BLM-proficient and BLM-deficient cells.** SMARD of replicated molecules from BLM-proficient (left) and BLM-deficient (right) cells is shown. A map of the 14q locus is depicted above the molecule images, with the positions of the telomeric PNA and FISH probes (blue bars below) used to identify and orient the molecules indicated. 48 red molecules and 56 green molecules were collected from BLM-proficient cells. 50 red molecules and 45 green molecules were collected from BLM-deficient cells.

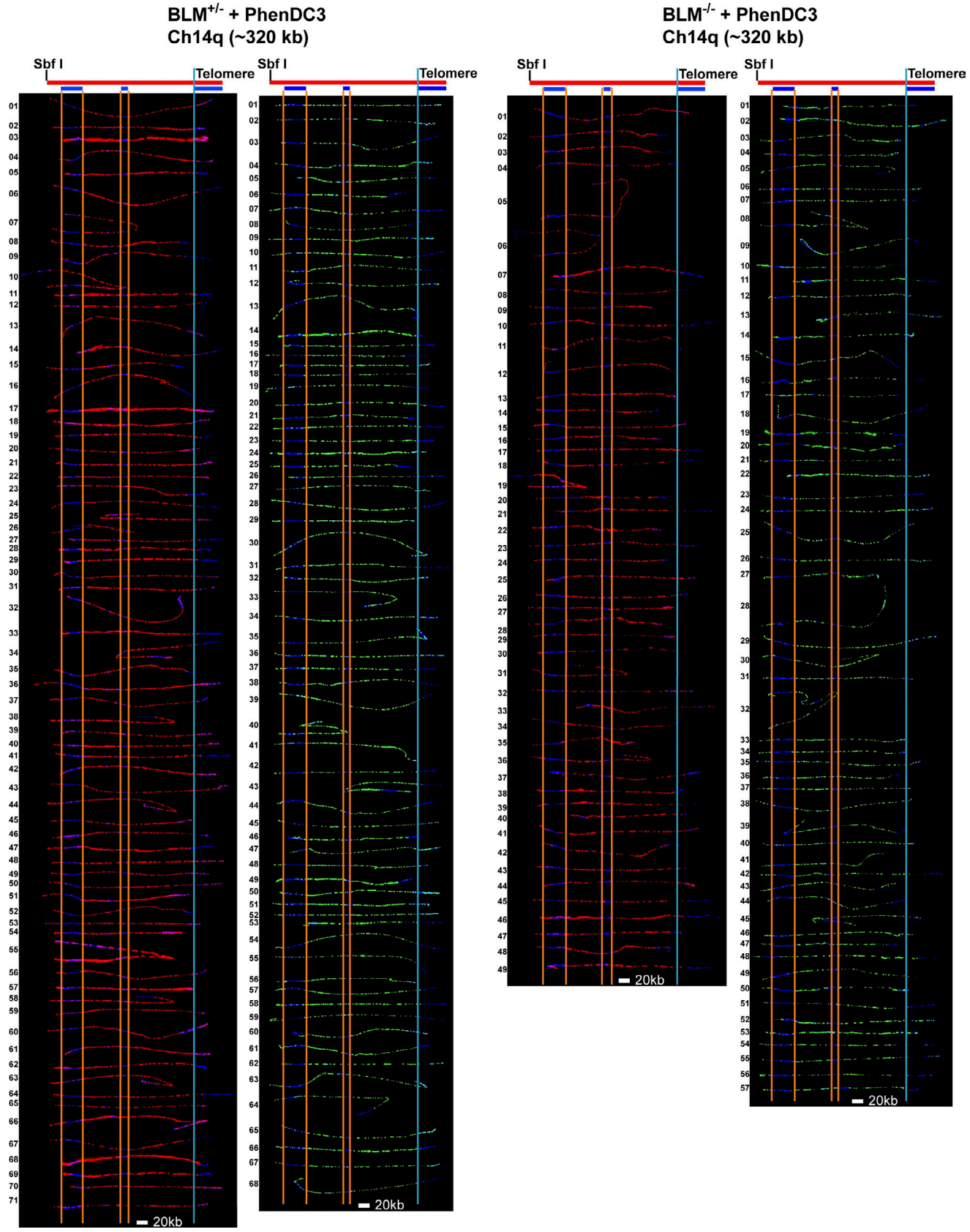

Figure S4. **Ch14q telomere molecules fully substituted with IdU (red) or CIdU (green) from PhenDC3-treated BLM-proficient and BLM-deficient cells.** SMARD of replicated molecules from BLM-proficient (left) and BLM-deficient (right) cells treated with PhenDC3 (as described in the legend for Fig. 4) is shown. 71 red molecules and 68 green molecules were collected from BLM-proficient cells. 49 red molecules and 57 green molecules were collected from BLM-deficient cells.

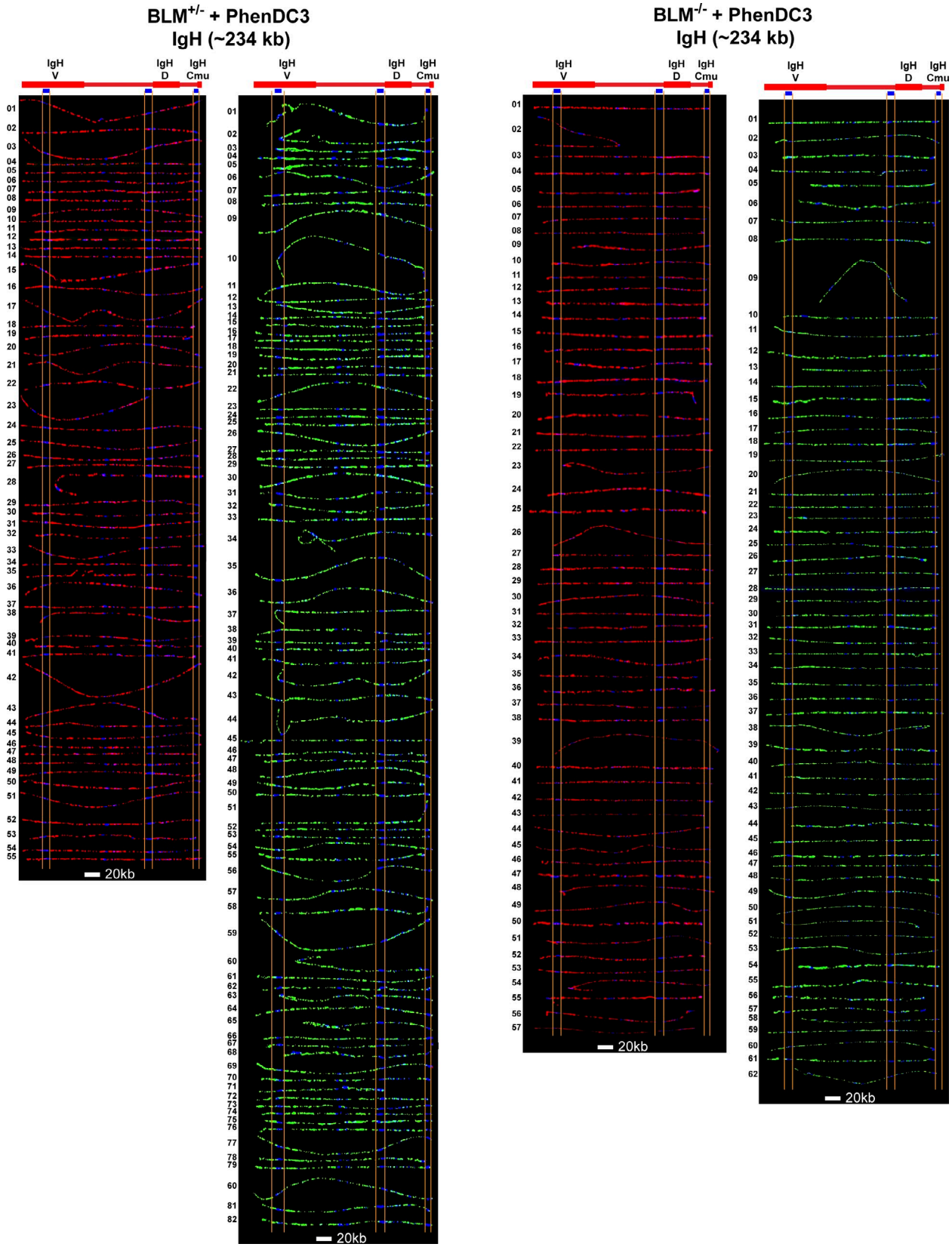

Figure S5. **Igh segment molecules fully substituted with IdU (red) or CIdU (green) from PhenDC3-treated BLM-proficient and BLM-deficient cells.** SMARD of replicated molecules from BLM-proficient (left) and BLM-deficient (right) cells treated with PhenDC3 (as described in the legend for Fig. 4) is shown. A map of the Igh locus is depicted above the molecule images, with the positions of the FISH probes (blue bars below) used to identify and orient the molecules indicated. 55 red molecules and 82 green molecules were collected from BLM-proficient cells. 57 red molecules and 62 green molecules were collected from BLM-deficient cells.

Table S1. **G4 sequence motifs**

Sequence	PQS[a]	Density
		PQS/kb
Telomere (30 kb)	955	31.8
Ch14q subtelomere (268 kb)	122	0.46
Igh Ch 12 (234 kb)	95	0.4
Ch1 nt 4000001–4250000	77	0.31
Ch3 nt 9000001–9250000	132	0.53
Ch5 nt 14000001–14250000	64	0.26
Ch7 nt 21000001–21250000	107	0.43
Ch9 nt 27000001–27250000	114	0.46
Ch11 nt 33000001–33250000	187	0.75
Ch13 nt 39000001–39250000	93	0.37
Ch15 nt 45000001–45250000	89	0.36
Ch17 nt 51000001–51250000	100	0.4
Ch19 nt 57000001–57250000	144	0.58

G4 sequence motifs, referred to as potential G4-forming sequences (PQS), were identified according to the folding rule d($G_{3+}N_{1-7}G_{3+}N_{1-7}G_{3+}N_{1-7}G_{3+}$) described by Huppert and Balasubramanian (2005). The number of PQS was determined for the DNA segments studied here and 10 randomly chosen chromosomal segments for comparison (map coordinates according to GRCm38.p2 reference assembly).
[a]PQS were mapped using QGRS Mapper based on the folding rule with settings for runs of three or more Gs with 1- to 18-nt loops between runs (Kikin et al., 2006). PQS is sum of the number of non-overlapping PQS determined for each strand of the duplex.

References

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Huppert, J.L., and S. Balasubramanian. 2005. Prevalence of quadruplexes in the human genome. *Nucleic Acids Res.* 33:2908–2916. http://dx.doi.org/10.1093/nar/gki609

Kikin, O., L. D'Antonio, and P.S. Bagga. 2006. QGRS Mapper: a web-based server for predicting G-quadruplexes in nucleotide sequences. *Nucleic Acids Res.* 34(Web Server):W676–W682. http://dx.doi.org/10.1093/nar/gkl253