Supplementary Materials

Fig. 1. Addition of UP1 increases protection of 3' G-rich overhang on a model template. (A) The overhang protection assay was performed on pBSK-Rep4 containing an ~ 450 nt 3' $(TTAGGG)_n$ overhang. Indicated amounts of UP1 and gp32 were used. In the absence of UP1, there was some smear below the 450 overhang signal, indicating some incomplete protection. Addition of UP1 decreased the signal in the low MW smear and increased the signal representing the overhang. The mean overhang size was calculated as described in Materials and Methods. (B) Excess amounts of gp32 alone caused a shift of overhang signal towards high MW. The overhang protection assay was performed on pBSK-Rep4 using the indicated amount of gp32. It is possible that excessive amounts of gp32 produce strand displacement.

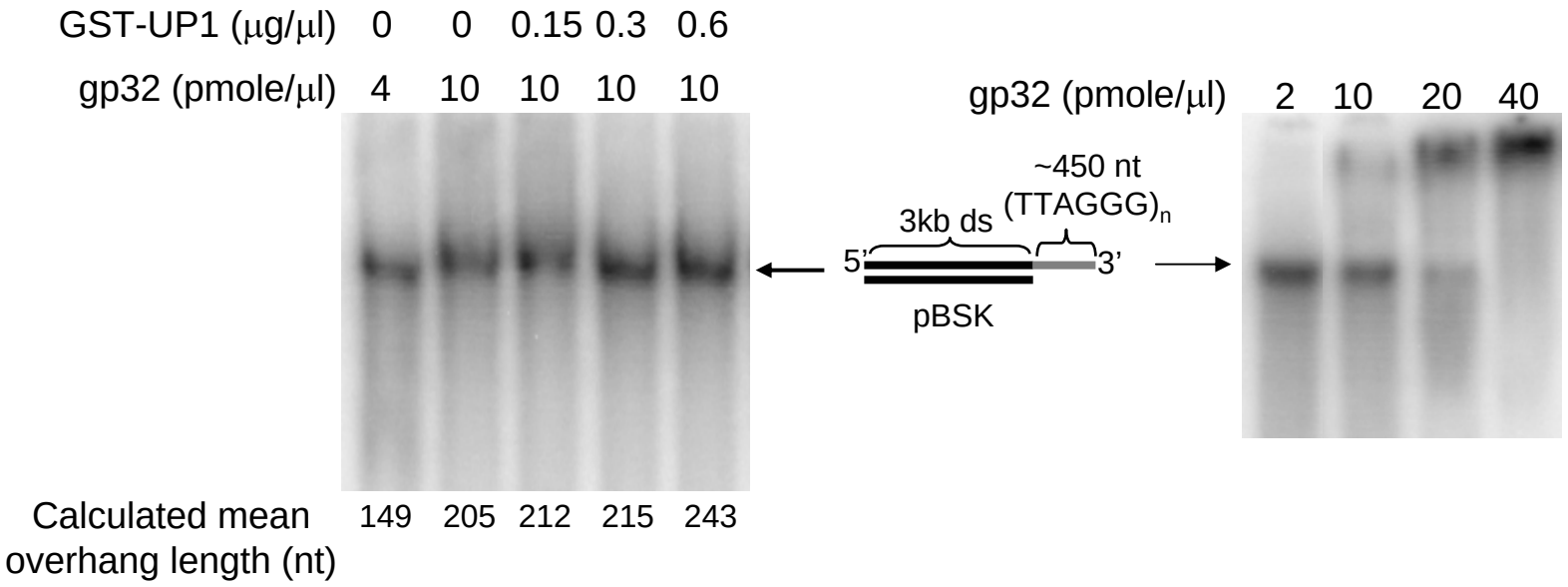
Fig. 2. The overhang protection assay is specific. Different sizes of G-rich oligos were subjected to the overhang protection assay with or without gp32/UP1 protection and then hybridized to either C-rich probe (C) or G-rich probe (G). In the absence of gp32/UP1, no signals were observed above background (lane 1). Hybridization of protected oligos to G-rich probe (lane 4) showed no signals above background while strong bands observed when hybridized to C-rich probe (lane 3). The "ann" lanes (lanes 2,6,7,8) indicates that the same amount of oligos used for overhang protection assay were annealed directly to the indicated probe in 1X gp32 protection buffer for overhang at r.t., without being subjected to the overhang protection assay. One 54 nt long C-rich oligo was used to hybridize to G-rich probe (lane 7) or C-rich probe (lane 8) to demonstrate the specificity

of probe. Hela DNA was also subjected to the overhang protection assay in the presence (lanes 9, 11, 12) and absence (lane 10) of gp32/UP1 and then hybridized to C-rich or G-rich probes. Lane 11 is a sample that was treated with *Exo* I to remove the 3' overhang prior to the overhang protection assay. Only G-rich but not C-rich overhangs were observed.

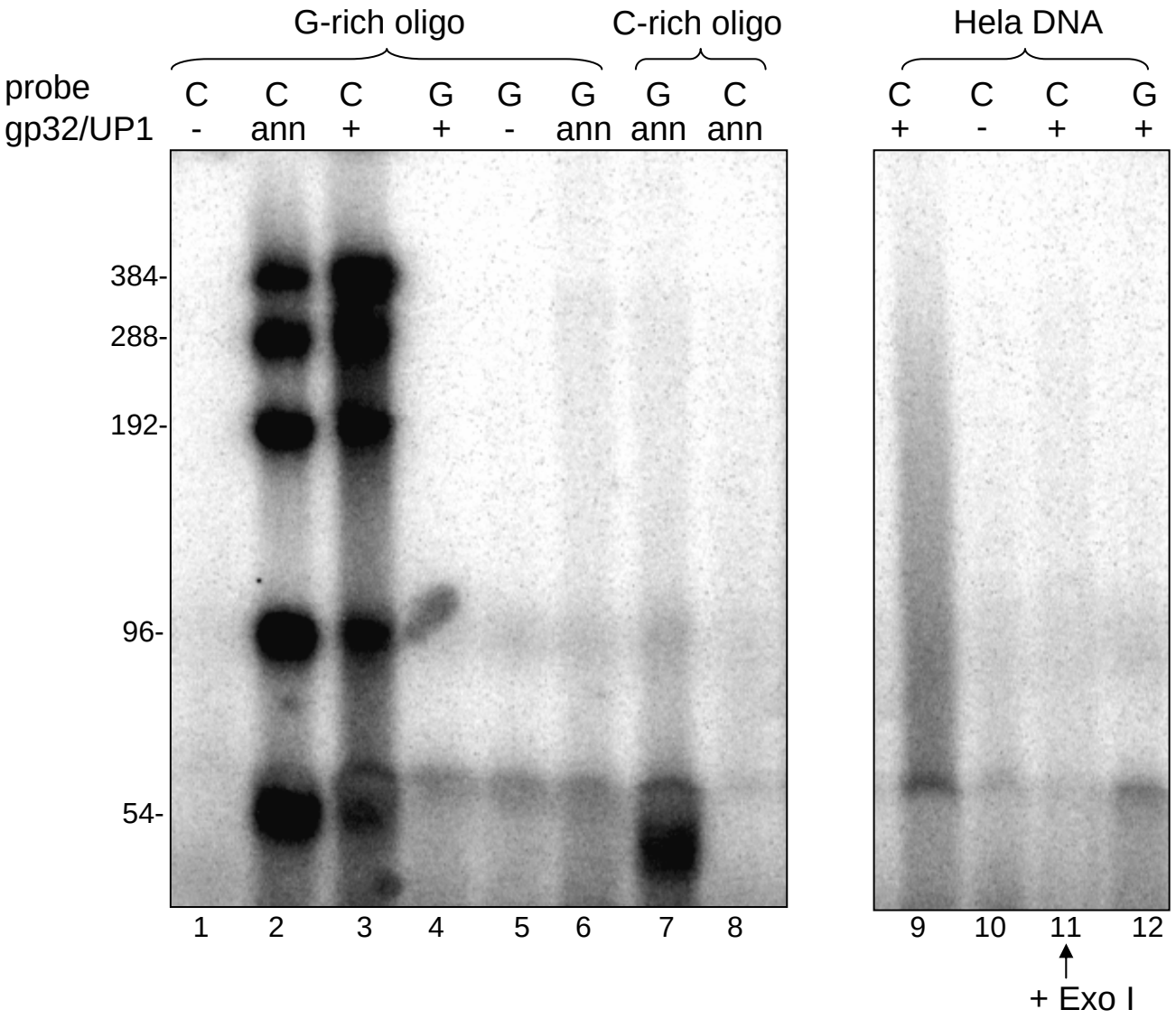
Fig. 3. Artifacts in the overhang protection assay. In the assay, we detected large amount of signals below 45 nt. When we treated the genomic DNA with T7 exonuclease (T7 exo), which resects the C-rich strand from 5'→3' and makes the G-rich overhang longer, the intensity of these low MW signals did not change while the bulk of overhang signals above 45 nt moved to large molecular weight positions. Interestingly, these signals are sensitive to Exo I, which removes overhang signals. Currently, we do not understand the nature of these signals, but they might represent cross-hybridization of the probe to short overhangs present at broken ends of the enormous excess of genomic DNA or small (possibly G-rich) RNA fragments surviving RNase A digestion (RNase A digests at 3' of ss C's and U's).

A

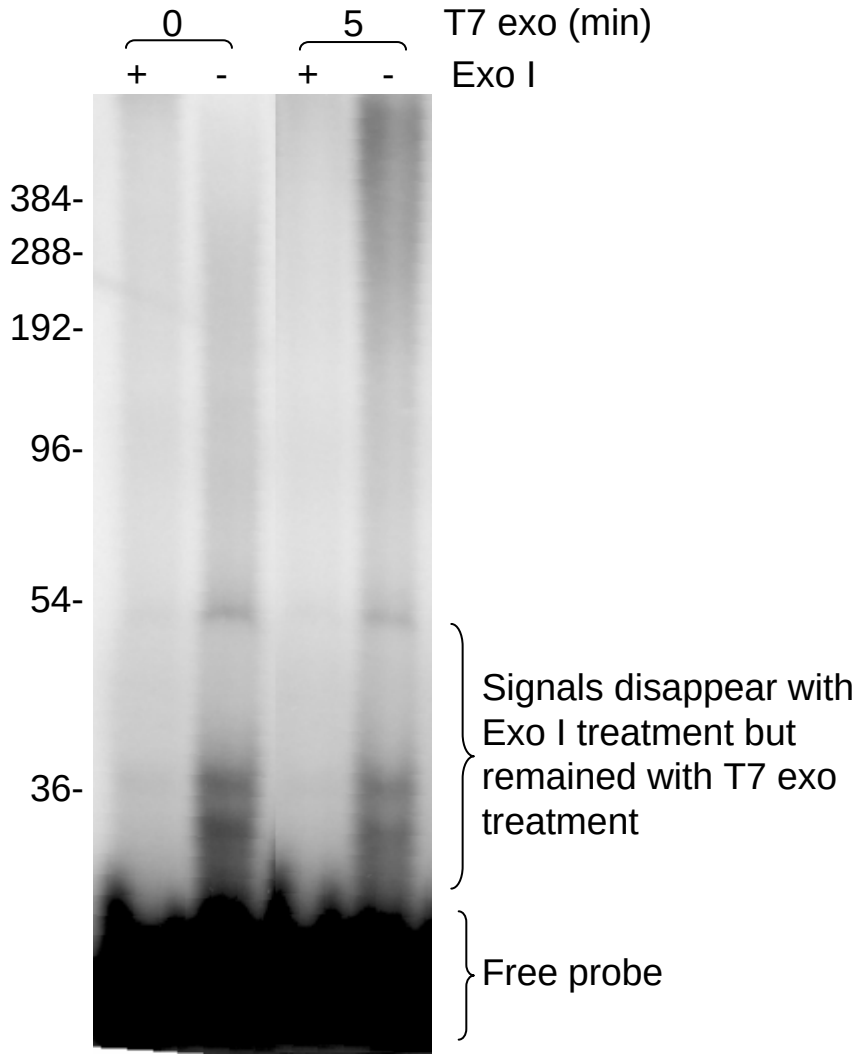
B



Supplemental Fig.1. Chai et al.



Supplemental Fig. 2. Chai et al.



Supplemental Fig. 3. Chai et al.