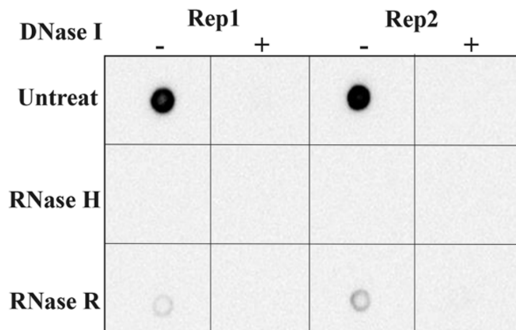
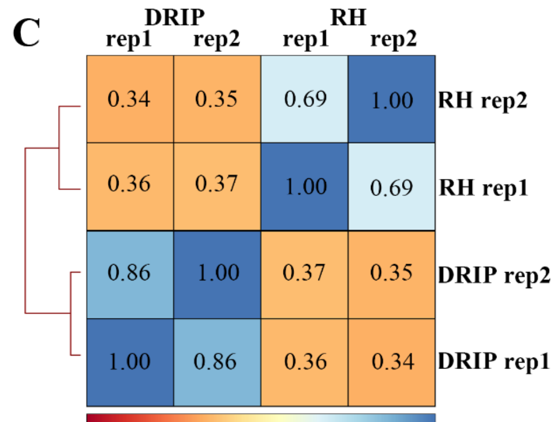
A

Samples	Biological replicates	Clean read	Mappable read	Mappable rate	Unique mappable read	Unique mappable rate
DRIP rep1	1	190,147,266	178,975,199	94.12%	112,051,878	58.93%
DRIP rep2	2	200,289,952	194,112,405	96.92%	118,957,771	59.39%
RH rep1	1	98,759,725	90,841,055	91.98%	63,119,172	63.77%
RH rep2	2	69,749,975	64,713,205	92.78%	44,681,101	64.06%
Input	1	177,644,909	166,668,099	93.82%	95,801,073	53.93%

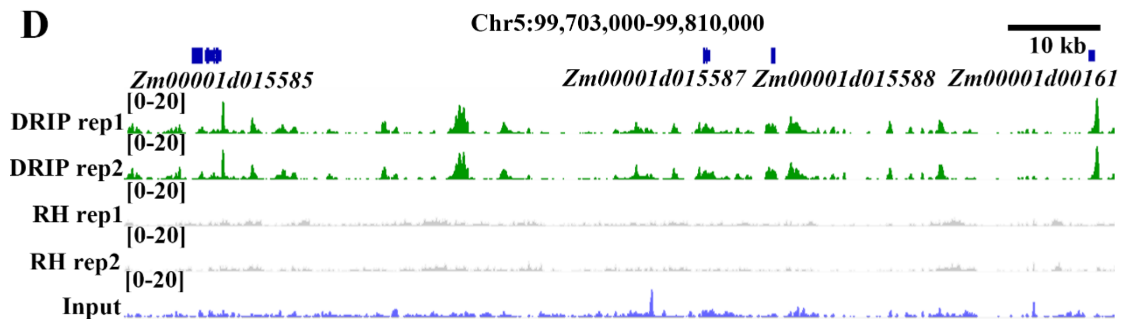
B



C



D



Supplemental Fig S1. Basic information of the ssDRIP-seq data.

(A) Basic information of sequencing data of two replications (DRIP rep1, 2), two negative controls (RNase H rep1, 2) and one input. The input data was derived from the DNA after restriction endonuclease digestion without anti-S9.6 antibody incubation. After data processing, we obtained more than 100 million read pairs that uniquely mapped to the maize B73_RefGen_v4, representing about 15-fold whole-genome coverage for each sample, except for the negative RNase H controls. (B) Dot blotting assay. Genomic DNA (50 ng) treated with or without RNase H (which degrades RNA in DNA: RNA hybrids) and RNase R (exonuclease that degrades linear RNA, but not circular RNA) was loaded onto an an Hybond™-N+ membrane before it was probed with anti-S9.6 antibody (1:10,000 dilution). Maize genomic DNA incubated with the anti-S9.6 antibody exhibited

strong R-loop signals. Pre-treatment with RNase H ablated the signal, confirming the signals are R-loop-specific. Pre-treatment with RNase R ablated the most of signals, indicating that circRNAs may also mediate R-loops formation in maize. (C) and (D) The correlation between two independent biological replicates. (C) Spearman's correlation of read densities (the bin size as 10 bp) was calculated between two independent biological replicates using the deepTools software. (D) Snapshot of the ssDRIP-seq data between two biological replicates.