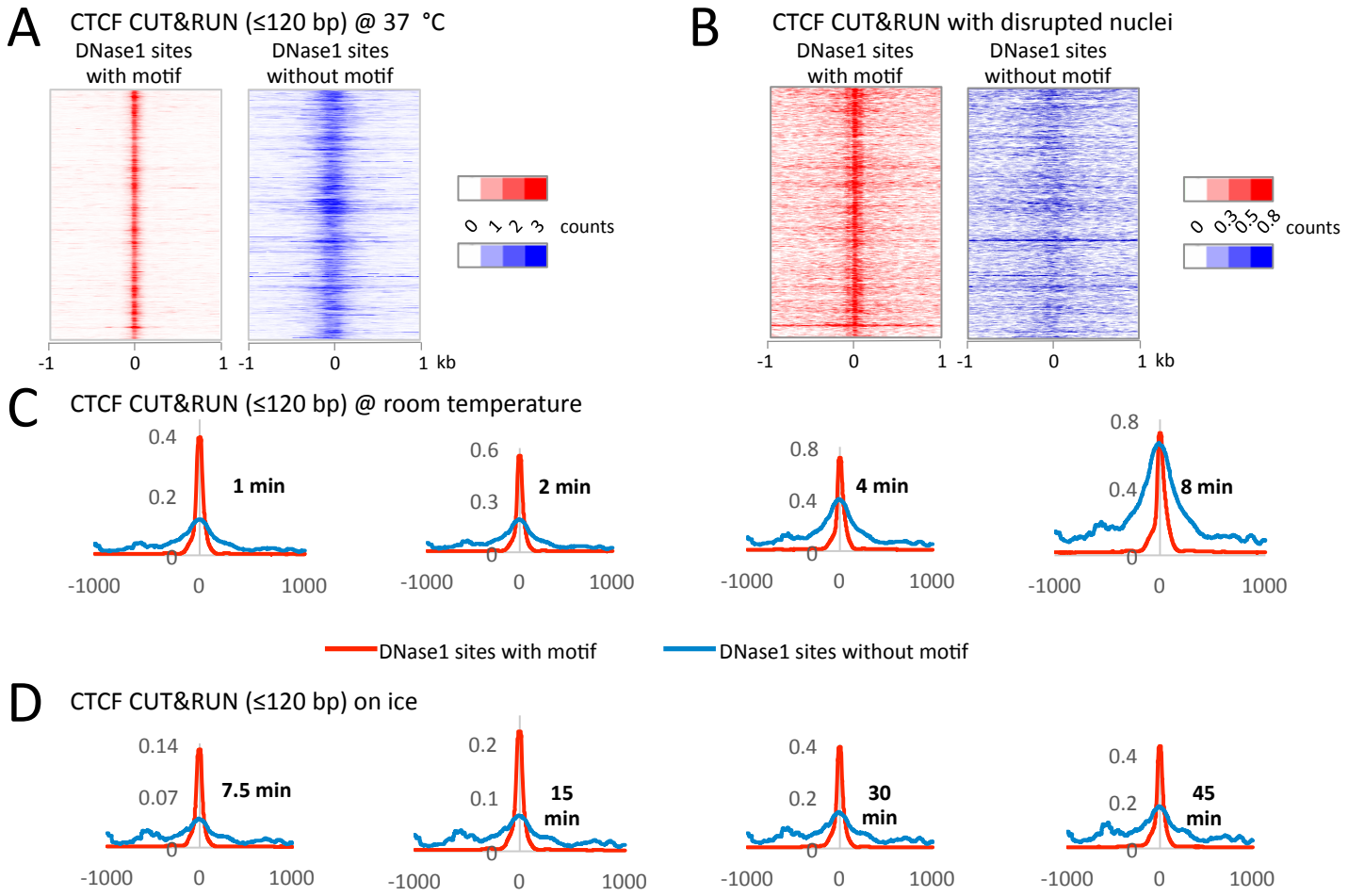

Figure 6–figure supplement 2. CUT&RUN has low background when performed on ice.
During protocol optimization, we performed the cleavage reactions over a range of temperatures. (A) We initially used 37 °C as is often done for MNase reactions. Careful analysis of the data, however, showed that despite clearly mapping CTCF at its true sites with a low density genome-wide background, we also had a specific background at random DNase1 sites. We rationalized that specific background arose from the liberated chromatin complexes that are still bound by Protein A-MNase diffusing around the nucleus and cutting accessible regions of chromatin. (B) To test this hypothesis, after the CTCF antibody and Protein A-MNase had bound *in situ*, we disrupted the nuclear envelope with limited sonication to release the chromatin into the large reaction volume. When CUT&RUN was performed under disrupted conditions, we no longer observed this specific background. (C) We therefore tried to limit the diffusion of these chromatin complexes by performing the cleavage reaction at room temperature. We observed that the signal-to-noise ratio started low, but increased over time and by 8 minutes the noise was indistinguishable from the signal. (D) However, by keeping the reaction on ice the signal-to-noise ratio was low and independent of time. Therefore, by controlling the temperature for the cleavage reaction, we can robustly maintain a low background.