



**Supplementary Figure 5** Comparison of DNA cleavage of consensus substrates with RSSs containing a G→A mutation at nonamer position 2. For each reaction, <sup>32</sup>P-labeled 12RSS (consensus or mutant, as indicated above the lanes) was incubated with RAG1/2 and HMGB1 with or without a 5-fold molar excess of the unlabeled partner 23RSS, which contained the same mutation (denoted by m) or the consensus nonamer sequence (denoted by c), in a reaction buffer containing 1.5 mM MgCl<sub>2</sub>. Samples were resolved on denaturing polyacrylamide gels. The positions of the nicked product, hairpin product, and input substrate are indicated with diagrams. Graphical representation of the cleavage products formed over time is shown in the right panel. Similar results were obtained in repeat experiments (data not shown).