Supplementary Information for Park et al., 2012 (NAR)

Supplementary Figures 1~6

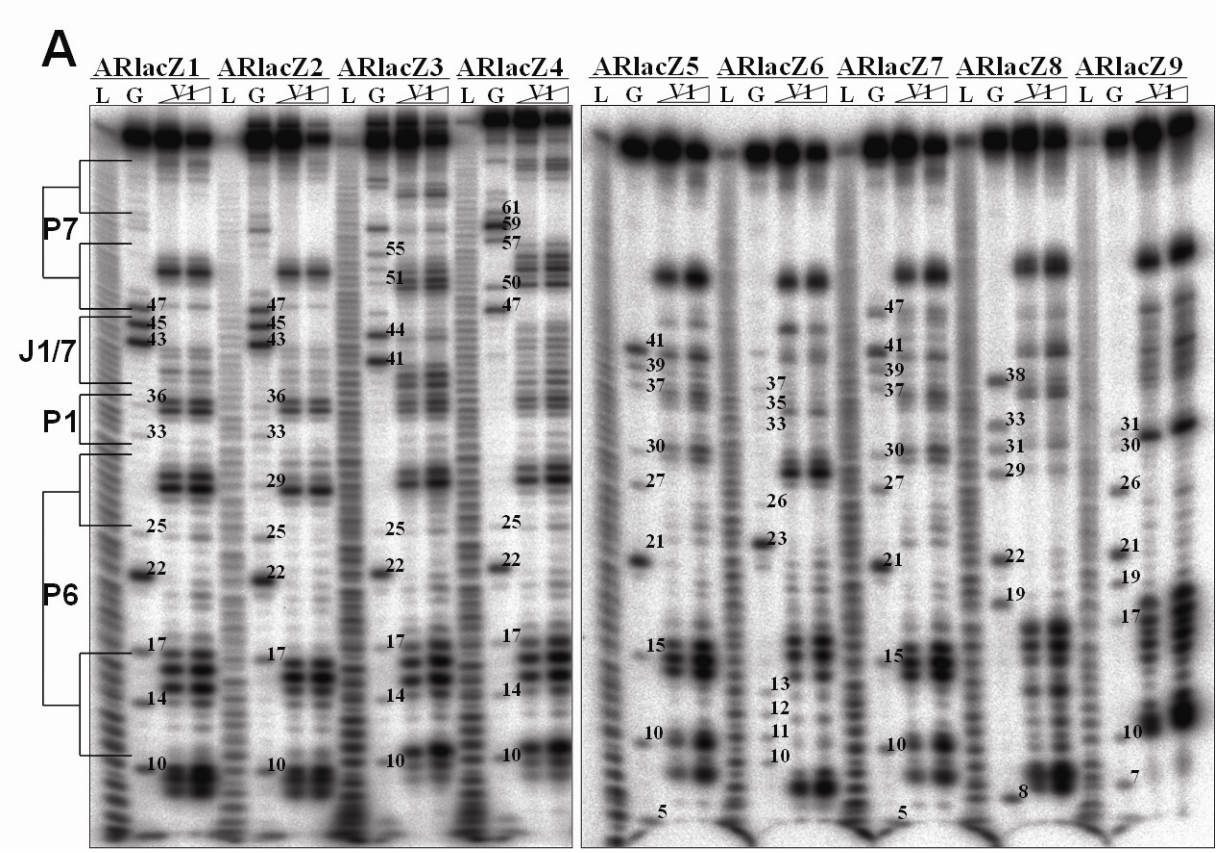

Figure S1. RNase V1 cleavages of afsRNAs. (A) ^{32}P-labeled RNA (20 nM) was partially digested with RNase V1 (0.0025 and 0.01 U). Alkaline ladders and RNase T1 ladder are shown in lanes L and G, respectively. The positions of RNase T1-cleaved G residues and the regions corresponding to possible structural domains of afsRNAs are marked.

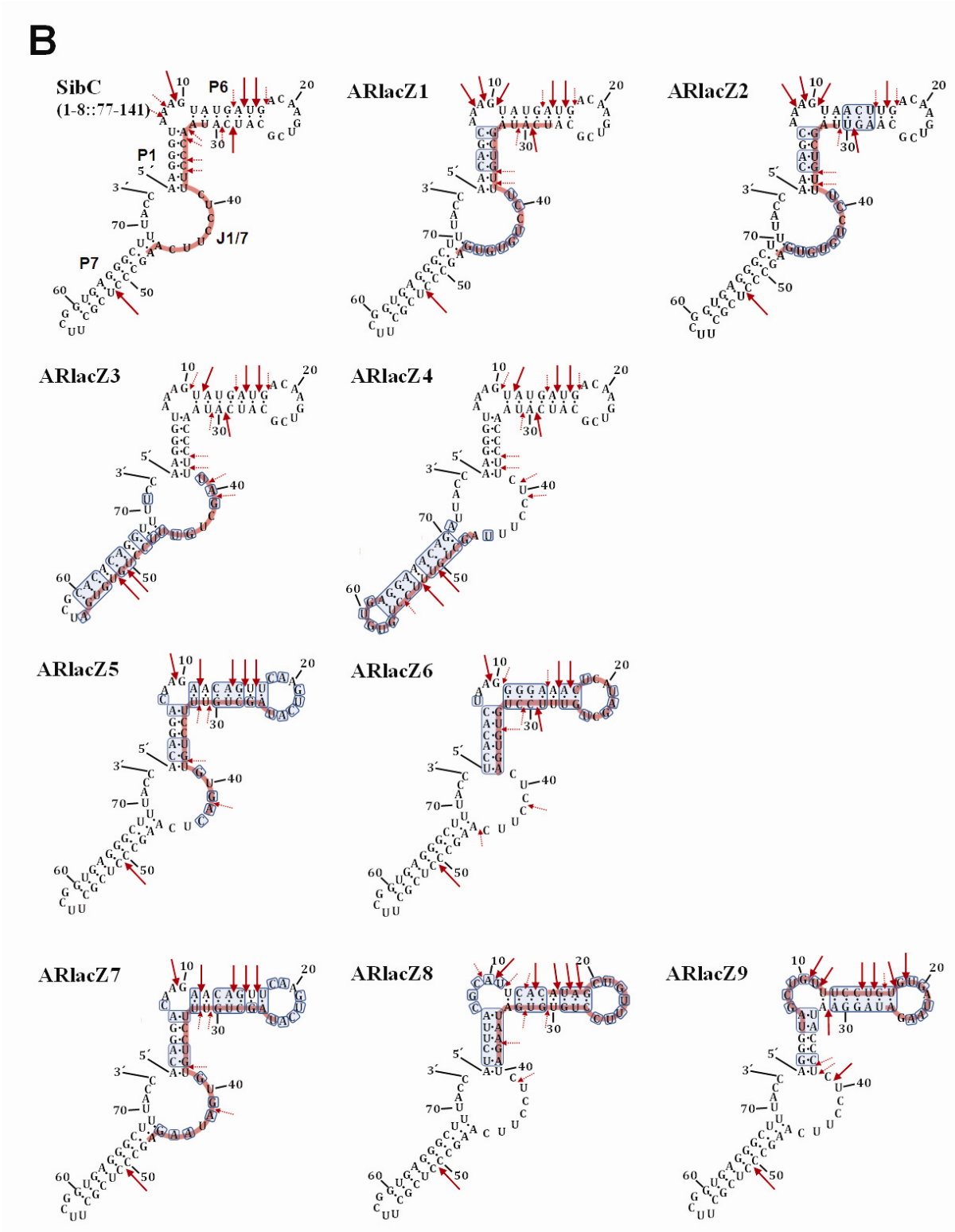

Figure S1. (Continued) (B) RNase V1 cleavage sites are shown in the secondary structure predicted using Mfold. The cleavage levels are indicated with different arrows.

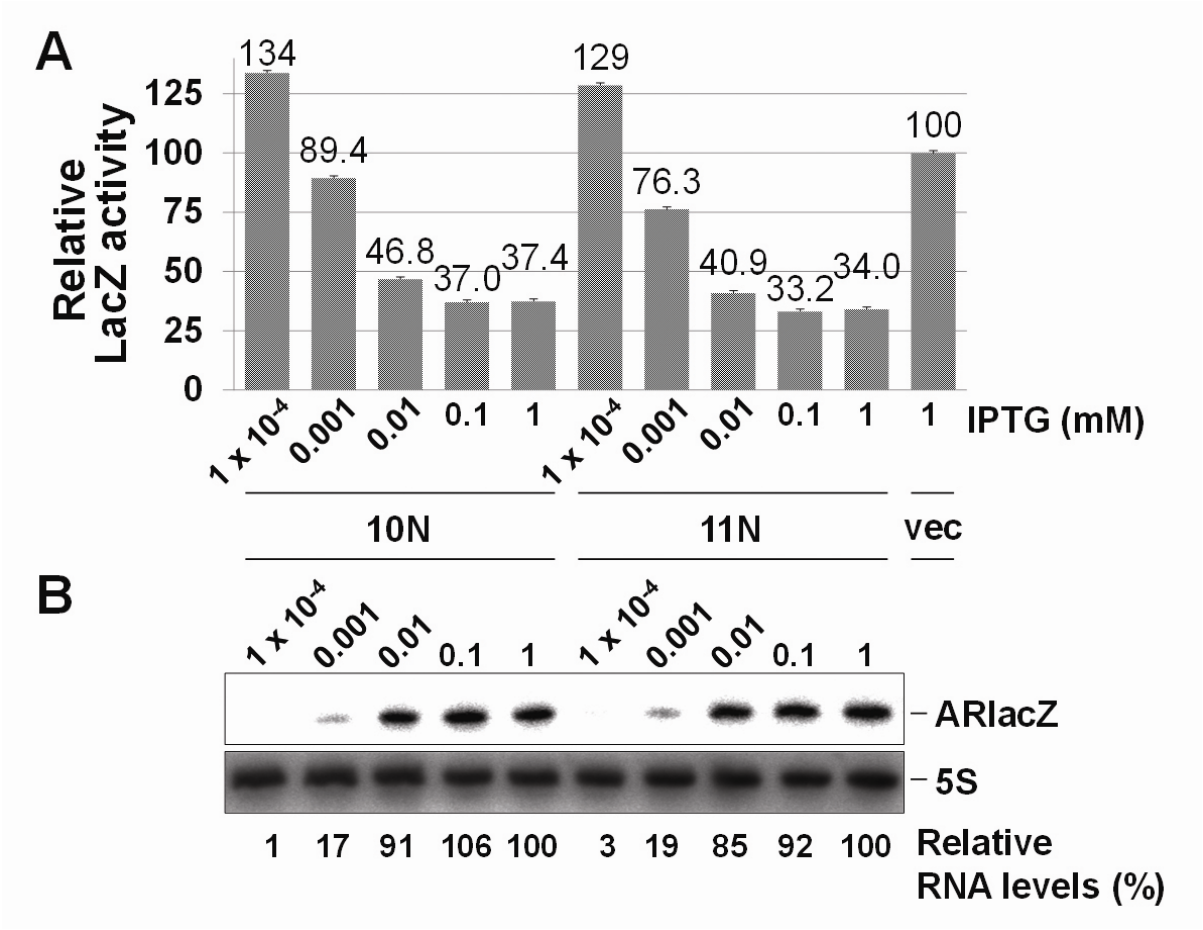

Figure S2. Dosage-dependent effectiveness of afsRNAs on gene silencing. Cells containing the ARlacZ10N or ARlacZ11N-expressing plasmid were treated with different concentrations of IPTG. Gene silencing effects (A) and cellular levels of afsRNAs (B) were analyzed as for Fig. 3.

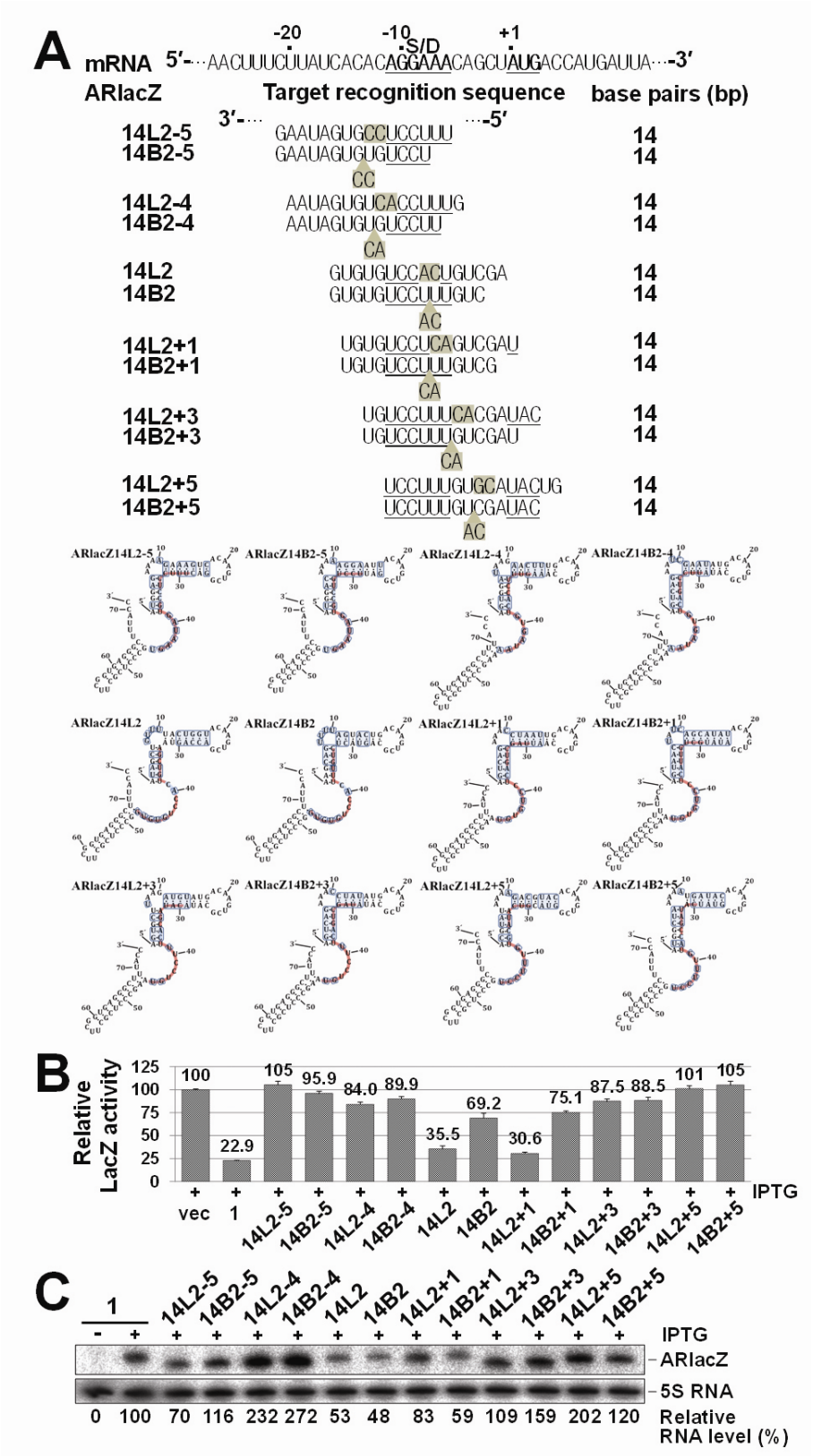

Figure S3. Effects of the position of target sequences on gene silencing by afsRNAs carrying mismatched target recognition sequences. A series of afsRNAs able to form 8+6 base-paring via a 2-base mismatch were analyzed as for Fig. 8. ARlacZ1 was used as a control for evaluation of RNA expression levels. ARlacZ1 was used as a control for evaluation of gene silencing effects and RNA expression levels.

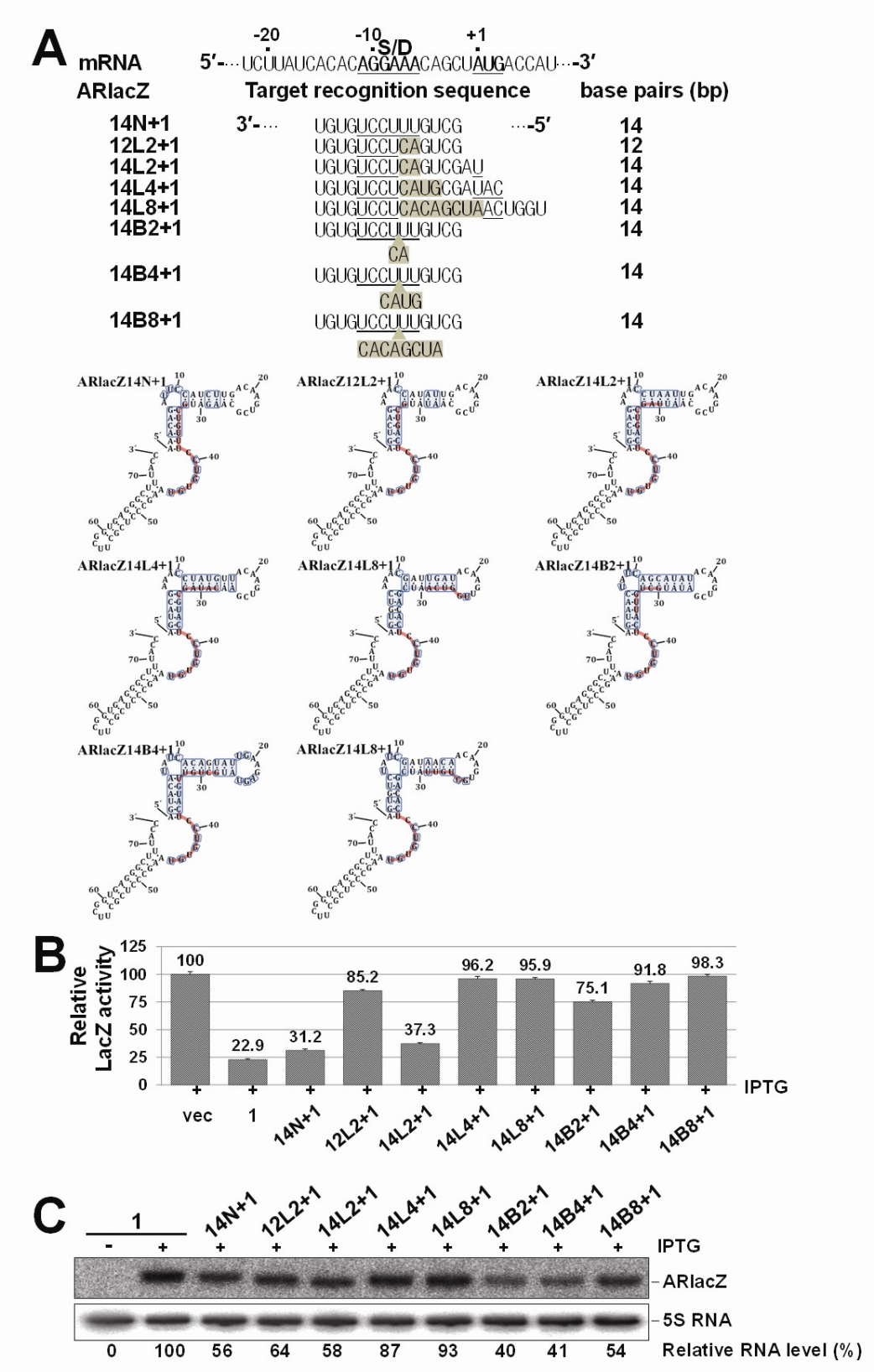

Figure S4. Reexamination of effects of mismatched target recognition sequences on gene silencing by targeting different region of mRNA. ARlacZ12L2+1, ARlacZ14L2+1, ARlacZ14L4+1, and ARlacZ14L8+1 capable of forming internal loops with mRNA and ARlacZ14B2+1, ARlacZ14B4+1, and ARlacZ14B8+ capable of forming bulges were analyzed as for Fig. 8.

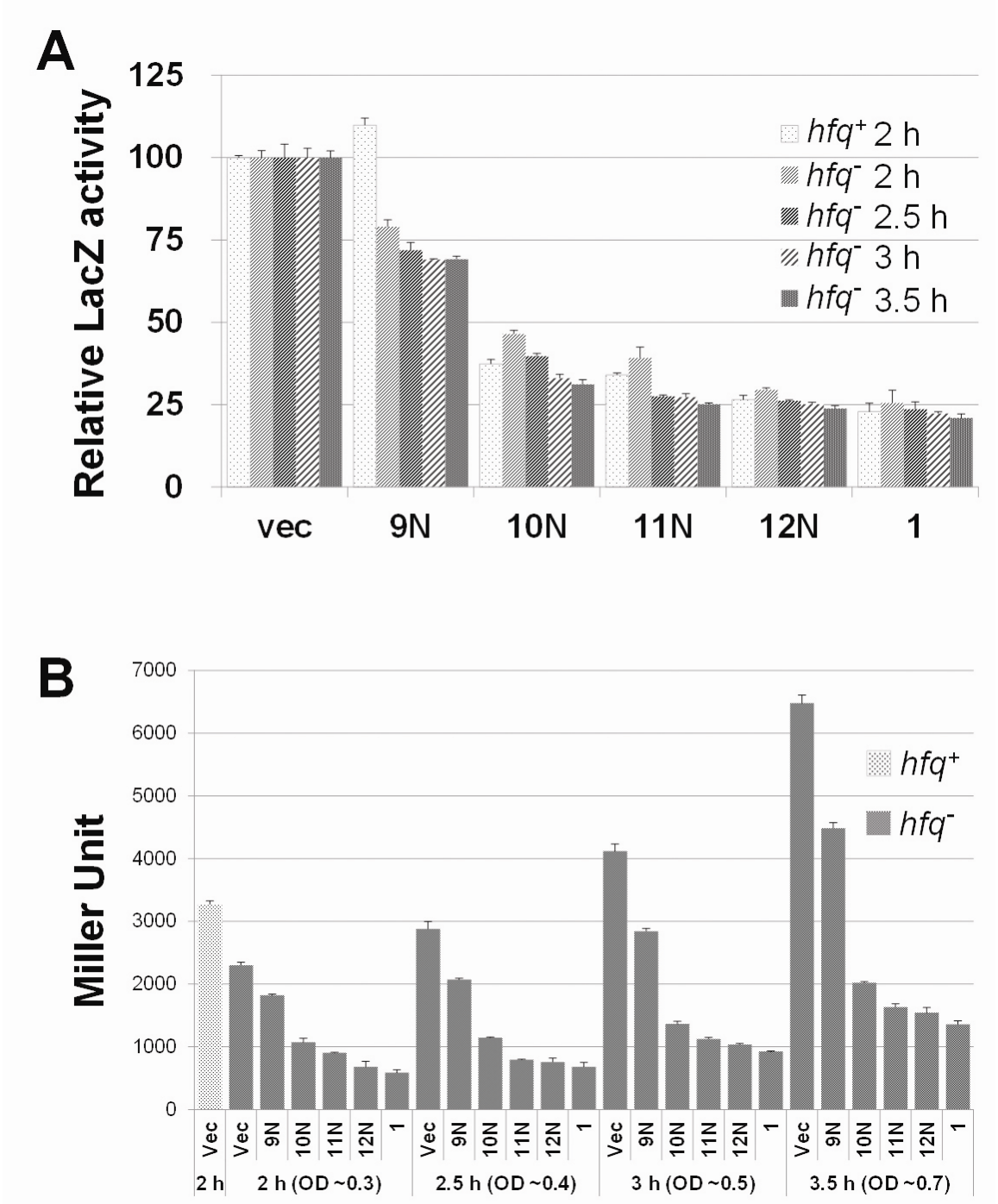

Figure S5. Gene silencing effects of afsRNA in *hfq*$^-$ cells at different growing times. Cells expressing afsRNAs were grown at 37°C for 2, 2.5, 3, and 3.5 h in the presence of 1 mM IPTG. (A) Relative β-galactosidase activities of cells expressing afsRNAs are shown. β-Galactosidase activities were expressed relative to that of cells containing the vector and treated with 1 mM IPTG. The indicated values are calculated from at least three independent experiments. (B) Actual β-galactosidase activities as Miller units were shown. Note that the actual β-galactosidase activity of the vector control in *hfq*$^+$ cells grown for 2 h became comparable to that of *hfq*$^-$ cells grown for more than 2.5 h. Vec, vector control.

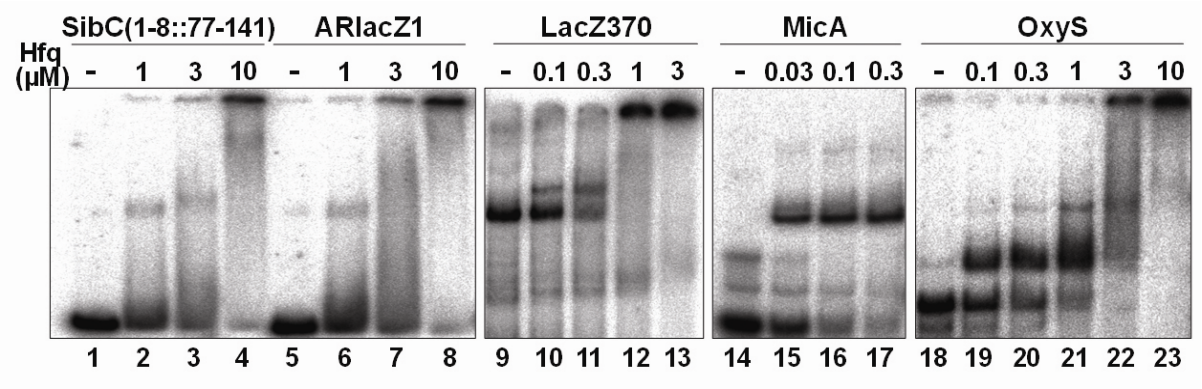

Figure S6. Affinity of sRNAs and LacZ370 to Hfq. RNAs were labeled with ^{32}P and subjected to gel mobility shift assay. The labeled RNA of 20 nM was incubated for 20 min in the absence (*lanes 1, 5, 9, 14, and 18*) of protein or presence (*lanes 2–4, 6-8, 10-13, 25-17, and 19-23*) of increasing amounts of Hfq protein (0.03-10 μM).