## **Supporting Information**

## Soler Bistué et al. 10.1073/pnas.0906529106

## SI Text

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**RNA-Binding Assays.** The 5'-end-labeled EGSs (10 pmol/1  $\mu$ M) were incubated 2 h at 25 °C in 10  $\mu$ L STE buffer with 0, 5, 10, 50, or 100 pmol/0, 0.5, 1, 5, or 10  $\mu$ M aac(6')-*Ib* mRNA and

analyzed on a 5% native GTG-PAGE as described in Sarno R, et al. (2003) Inhibition of aminoglycoside 6'-*N*-acetyltransferase type Ib-mediated amikacin resistance by antisense oligode-oxynucleotides. *Antimicrob Agents Chemother* 47:3296–3304.



Fig. S1. Binding of unsubstituted EGSC3 (RNA), LNA1, and LNA2 to *aac (6)-Ib* mRNA. The 5'-end-labeled EGSs and *aac (6)-Ib* mRNA were incubated as described in *SI Text* and analyzed in native polyacrylamide gel electrophoresis.

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0:1 1:2 1:1 5:1 10:1 0:1 1:2 1:1 5:1 10:1



Fig. S2. Binding of EGSC3, LNA2, LNA9, and LNA10 to aac (6)-Ib mRNA. The 5'-end-labeled EGSs and aac (6)-Ib mRNA were incubated at the EGS:mRNA ratios indicated in the figure as described in *SI Text* and then analyzed in native polyacrylamide gel electrophoresis.

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Fig. S3. Activities of LNA/DNA co-oligomer EGSs. The gel is identical to that shown in Fig. 2*B* but the electrophoresis was carried out for only 20 min to allow all molecular weight products to stay inside the gel. The band at *ca*. 90 nucleotides is present in all radioactively labeled mRNA samples.

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Fig. S4. Time course of EGSC3 decay upon exposure to bacterial lysates. <sup>32</sup>P-labeled EGSC3 was incubated in the presence of cell extracts obtained by soft sonication for the indicated times and analyzed by 15% PAGE.

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**Fig. S5.** Accumulation of [ $^{32}P$ ] LNA9 in *E. coli DH5* $\alpha$  (open squares) or *E. coli AS19* (open triangles). Exponentially grown cells (OD<sub>600</sub> 0.6) were washed twice with ice-cold modified Tanaka buffer. Cells were incubated in the presence of 50  $\mu$ M of [ $^{32}P$ ] LNA9. Aliquots were taken at different time points and filtered through Millipore 0.45- $\mu$ M filters. Filters were washed twice with 1 mL cold PBS buffer and radioactivity was measured with a liquid scintillation counter.

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Fig. S6. Colony forming units of *E. coli* AS19(pFC9) exposed to Ak after pretreatment with different concentrations of LNA9 or LNA10. Results are expressed as log of mean ± SD of colony forming units per milliliter (log CFU/mL).

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