

Resch *et al.* Supplementary Figure S1

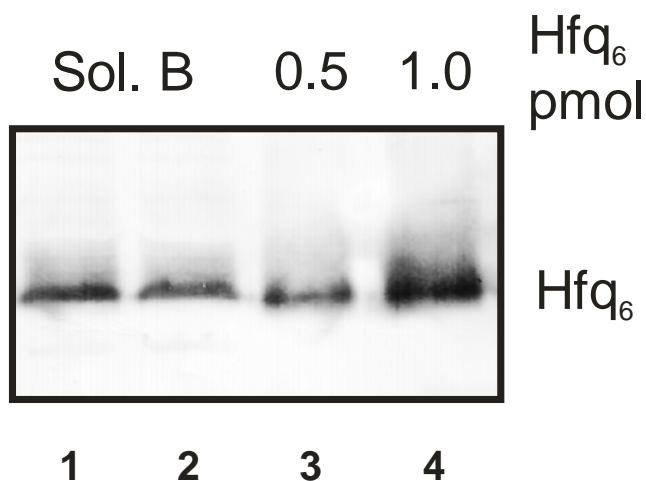


Fig. S1. The PURESYSYSTEM contains Hfq as determined by Western-blot analysis with anti-Hfq antibodies. To test whether the PURESYSYSTEM (PURESYSYSTEM classic II standard, PURE2030C; Wako Chemicals GmbH, Neuss, Germany) contains Hfq protein, we performed a western-blot analysis with anti-Hfq antibodies. Briefly, 1 μ l of two different vials of Sol. B were each adjusted to 10 μ l with water and 2x Laemmli buffer, and boiled for 5 min. before loading on a 10% SDS-polyacrylamide gel and blotting to a nitrocellulose membrane. The blots were blocked with 5% dry milk in TBS buffer, and then probed with antibodies against Hfq (Pineda, Berlin). The antibody-antigen complexes were visualized with alkaline-phosphatase conjugated secondary antibodies and BCIP/NBT solutions following standard procedures. The Hfq levels in Sol. B of the PURESYSYSTEM were assessed by comparing the intensities of the concomitantly loaded purified hexameric Hfq proteins. Lane 1 and 2, different Sol. B, lane 3 and 4, 0.5 pmol and 1 pmol purified Hfq₆ protein, respectively. The position of the hexameric Hfq (Hfq₆) protein is indicated. Only the relevant sections of the western-blot are shown.

Conclusion: The PURESYSYSTEM (PURESYSYSTEM classic II standard, PURE2030C) contains approximately 0.5 to 1 pmol Hfq₆ per microliter solution B.