## **Supporting Information**

## Azpurua et al. 10.1073/pnas.1313473110



Fig. S1. Naked mole-rat (NMR) ribosomal RNA (rRNA) shows an unusual pattern with cleaved 28S rRNA. (A) NMR break pattern is seen independent of extraction method used or whether RNA is extracted from frozen tissues or cell culture. (B) RNA extracted from two additional nonmodel rodents does not show 28S rRNA cleavage. The RNA was separated on native 1% gel.



Fig. S2. Bioinformatics analysis of the NMR 28S rRNA. (A) Flowchart of divergence analysis of the cleavage sites. (B) Alignment of the fragments covering the 5' end cleavage site.

## **Firefly Luciferase Mutations**

| Point Mutants           |           |
|-------------------------|-----------|
| 1 <sup>st</sup> – K529E | AAA 🗲 GAA |
| 2 <sup>nd</sup> – K529I | AAA 🗲 AAA |
| 3 <sup>rd</sup> – K529N | AAA 🗲 ATC |

Frameshift Mutants -G at 244 nucleotide +G at 244 nucleotide

| Premature | Stop | Mutar | nt |     |
|-----------|------|-------|----|-----|
| C81STOP   |      | CGA   | •  | TGA |

Fig. S3. Mutations generated in the firefly luciferase reporters to measure translation fidelity.

Table S1. Sequence conservation at the cleavage sites

| 5' end cleavage site |   |    |       | 3' end cleavage site |   |       |   |       |    |    |   |       |
|----------------------|---|----|-------|----------------------|---|-------|---|-------|----|----|---|-------|
| WGS                  | A | С  | G     | Т                    | Ν | Total | A | С     | G  | Т  | Ν | Total |
| Kim et al.*          | 0 | 0  | 128   | 0                    | 0 | 128   | 0 | 58    | 0  | 0  | 0 | 58    |
| $Broad^{\dagger}$    | 8 | 18 | 6,782 | 12                   | 3 | 6,823 | 5 | 3,855 | 37 | 10 | 2 | 3,909 |

The G and C-terminal nucleotides at the cleavage sites are highly conserved. The low frequency of polymorphic reads is consistent with Illumina sequencing errors. WGS, whole genome sequence.

<sup>†</sup>NMR sequence by the Broad Institute (http://www.ncbi.nlm.nih.gov/nuccore/AHKG00000000).

1. Kim EB, et al. (2011) Genome sequencing reveals insights into physiology and longevity of the naked mole rat. Nature 479(7372):223-227.

<sup>\*</sup>NMR genome sequence published by Kim et al. (1).

| Primer         | Sequence (5′→3′)               | Specificity  |  |  |
|----------------|--------------------------------|--------------|--|--|
| 28S-5P2        | GGCGACCCGCTGAATTTAAGCATATTAGTC | NMR 285 rRNA |  |  |
| 28S-P6-Forward | GCCGTACCCATATCCGCAGCAG         | NMR 28S rRNA |  |  |
| 28S-P7-Reverse | GCCCTTAGAGCCAATCCTTATCCCGAA    | NMR 28S rRNA |  |  |
| SP3-Forward    | GACAGTGCCAGGTGGGGAGTTTGAC      | NMR 28S rRNA |  |  |
| 28S-3P         | ACAAACCCTTGTGTCGAGGGCTG        | NMR 285 rRNA |  |  |

Table S2. List of oligonucleotide primers used for 3' RACE and 5' RACE of NMR 28S ribosomal RNA cleavage point

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