The *in silico* identification of potential members of the Ded1/DDX3 subfamily of DEAD-box RNA helicases from the protozoan parasite *Leishmania infantum* and their analyses in yeast.

Molka Mokdadi, Yosser Zina Abdelkrim, Josette Banroques, Emmeline Huvelle, Rafeh Oualha, Hilal Yeter-Alat, Ikram Guizani, Mourad Barhoumi*, and N. Kyle Tanner*

### Supplementary Table S1. Oligonucleotides used in this study

| Constructs* | Sequence (5’—3’)* |
|-------------|------------------|
| LINF08-1 (SpeI-Ndel–SalI/XhoI) | GCCTATCTAGTCATATGCCTAAGGGGC |
| LINF08-1_up | GCCTATCTAGTCATATGCCTAAGGGGC |
| LINF08-1_low | GCCTATCTAGTCATATGCCTAAGGGGC |
| LINF08-2 (SpeI-Ndel–SalI/XhoI) | GCCTATCTAGTCATATGCCTAAGGGGC |
| LINF08-2_up | GCCTATCTAGTCATATGCCTAAGGGGC |
| LINF08-2_low | GCCTATCTAGTCATATGCCTAAGGGGC |
| LINF08-2syn (SpeI-Ndel–XhoI) | GCCTATCTAGTCATATGCCTAAGGGGC |
| LINF08-2_low2 | GCCTATCTAGTCATATGCCTAAGGGGC |
| LINF32 (SpeI-Ndel–XhoI) | GCCTATCTAGTCATATGCCTAAGGGGC |
| LINJ.32_up | GCCTATCTAGTCATATGCCTAAGGGGC |
| LINJ.32_low | GCCTATCTAGTCATATGCCTAAGGGGC |
| TRYP08 (XbaI-Ndel–SalI/XhoI) | GCCTATCTAGTCATATGCCTAAGGGGC |
| Tb427-08_up | GCCTATCTAGTCATATGCCTAAGGGGC |
| Tb427-08_low | GCCTATCTAGTCATATGCCTAAGGGGC |
| TRYP32 (SpeI-Ndel–XhoI) | GCCTATCTAGTCATATGCCTAAGGGGC |
| Tb427_32_up | GCCTATCTAGTCATATGCCTAAGGGGC |
| Tb427_32_low | GCCTATCTAGTCATATGCCTAAGGGGC |
| TRYP35 (SpeI-Ndel–SalI) | GCCTATCTAGTCATATGCCTAAGGGGC |
| Tb427_35_up | GCCTATCTAGTCATATGCCTAAGGGGC |
| Tb427_35_low | GCCTATCTAGTCATATGCCTAAGGGGC |
| LINF08-2-GAT (SpeI-Ndel–XhoI) | GCCTATCTAGTCATATGCCTAAGGGGC |
| LINJ.08_up3 | GCCTATCTAGTCATATGCCTAAGGGGC |
| LINJ.08_low2 | GCCTATCTAGTCATATGCCTAAGGGGC |
| LINJ.08_GAT_up | GCCTATCTAGTCATATGCCTAAGGGGC |
| LINJ.08_GAT_low | GCCTATCTAGTCATATGCCTAAGGGGC |
| LINF08-1-GAT (SpeI-Ndel) | GCCTATCTAGTCATATGCCTAAGGGGC |
| LINJ.08_up4 | GCCTATCTAGTCATATGCCTAAGGGGC |
| LINF32-GAT (SpeI-Ndel–XhoI) | GCCTATCTAGTCATATGCCTAAGGGGC |
| LINJ.32_up2 | GCCTATCTAGTCATATGCCTAAGGGGC |
| LINJ.32_low2 | GCCTATCTAGTCATATGCCTAAGGGGC |
| LINJ.32_GAT_up | GCCTATCTAGTCATATGCCTAAGGGGC |
| LINJ.32_GAT_low | GCCTATCTAGTCATATGCCTAAGGGGC |
| LINF35-GAT (SpeI-Ndel–XhoI) | GCCTATCTAGTCATATGCCTAAGGGGC |
| LINJ.35_up2 | GCCTATCTAGTCATATGCCTAAGGGGC |
| LINJ.35_low2 | GCCTATCTAGTCATATGCCTAAGGGGC |
| LINJ.35_GAT_up | GCCTATCTAGTCATATGCCTAAGGGGC |
| LINJ.35_GAT_low | GCCTATCTAGTCATATGCCTAAGGGGC |

### Chimera constructs

| Ded1-5’-pUC18_fwd (SpeI-Ndel) | GCCTATCTAGTCATATGCCTAAGGGGC |
| Ded1-3’-pUC18_rev (XhoI) | GCCTATCTAGTCATATGCCTAAGGGGC |
| Ded1-5’-pUC18_fwd2 (XbaI-Ndel) | GCCTATCTAGTCATATGCCTAAGGGGC |
| Ded1-3’-pUC18_rev2 (SalI) | GCCTATCTAGTCATATGCCTAAGGGGC |
| Ded1-DDX3-Ded1 | GCCTATCTAGTCATATGCCTAAGGGGC |
Regions of hybridization are shown underlined, restriction sites are in bold and mutations are in lowercase.

**Supplementary Table S2. Constructs used in this study**

| Name              | Description                               | Source |
|-------------------|-------------------------------------------|--------|
| pUC18             | BlueScript (AM)                           |        |
| ADH-2HA_p415      | 2HA, ADH/CYC1 (LEU2/CEN)                  | [1]    |
| ADH-2HA_p424      | 2HA, ADH/CYC1 (TRP2/µ)                    | [1]    |
| GPD-2HA_p424      | 2HA, GPD/CYC1 (TRP2/µ)                    | This study |
| ADH-2HA-DED1_p415 | 2HA-DED1, ADH/CYC1 (LEU2/CEN)             | [1]    |
| ADH-2HA-DED1_p424 | 2HA-DED1, ADH/CYC1 (TRP2/µ)               | [1]    |
| ADH-2HA-DBP1_p424 | 2HA-DBP1, ADH/CYC1 (TRP2/µ)               | [1]    |
| ADH-2HA-DDX2_p424 | 2HA-DDX3, ADH/CYC1 (TRP2/µ)               | [2]    |
| ADH-2HA-TIF1_p424 | 2HA-TIF1, ADH/CYC1 (TRP2/µ)               | [1]    |
| ADH-2HA-FAL1_p424 | 2HA-FAL1, ADH/CYC1 (TRP2/µ)               | [1]    |
| ADH-2HA-DBP2_p424 | 2HA-DBP2, ADH/CYC1 (TRP2/µ)               | [1]    |
| LINF08L_B.S.      | LINF08L, BlueScript (AM)                  | This study |
| LINF08S_B.S.      | LINF08S, BlueScript (AM)                  | This study |
| LINF32_B.S.       | LINF32, BlueScript (AM)                   | This study |
| LINF35_B.S.       | LINF35, BlueScript (AM)                   | This study |
| ADH-2HA-LINF08L_p415 | 2HA-LINF08L, ADH/CYC1 (LEU2/CEN)    | This study |
| ADH-2HA-LINF08S_p415 | 2HA-LINF08S, ADH/CYC1 (LEU2/CEN) | This study |
| ADH-2HA-LINF32_p415 | 2HA-LINF32, ADH/CYC1 (LEU2/CEN) | This study |
| ADH-2HA-LINF35_p415 | 2HA-LINF35, ADH/CYC1 (LEU2/CEN) | This study |
| ADH-2HA-LINF08L_p424 | 2HA-LINF08L, ADH/CYC1 (TRP2/µ) | This study |
| ADH-2HA-LINF08S_p424 | 2HA-LINF08S, ADH/CYC1 (TRP2/µ) | This study |
| ADH-2HA-LINF32_p424 | 2HA-LINF32, ADH/CYC1 (TRP2/µ) | This study |
| ADH-2HA-LINF35_p424 | 2HA-LINF35, ADH/CYC1 (TRP2/µ) | This study |
1. Tanner, N.K., Cordin, O., Banroques, J., Doere, M. and Linder, P. (2003) The Q motif: a newly identified motif in DEAD box helicases may regulate ATP binding and hydrolysis. Mol Cell, 11, 127-138. 10.1016/s1097-2765(03)00006-6

2. Senissar, M., Le Saux, A., Belgareh-Touze, N., Adam, C., Banroques, J. and Tanner, N.K. (2014) The DEAD-box helicase Ded1 from yeast is an mRNP cap-associated protein that shuttles between the cytoplasm and nucleus. Nucleic Acids Res, 42, 10005-10022. 10.1093/nar/gku584
**Supplementary Figure S1.** Phylogenetic tree of LINF and Ded1/DDX3 proteins. A neighbor-joining tree is shown without distance corrections and with cladogram branch lengths to facilitate viewing. The distances are as shown. (A) Core sequences consisting of the amino-terminal, isolated, aromatic group to the end of motif VI. (B) Flanking sequences consisting of the fused amino- and carboxyl-terminal sequences and excluding the cores sequences used in A.

![Supplementary Figure S1](image1.png)

**Supplementary Figure S2.** Complementation of synthetic LINF genes optimized for expression in yeast. The yeast strain ded1::HIS was transformed with the indicated genes and grown in SD-TRP medium. Cultures were then serial diluted and spotted on SD plates containing 5-FOA. Plates were incubated for 6 days at 18°C and for 3 days at 30°C and 36°C. The isolated colonies that grew with the LINF genes contained the DED1 gene, and hence no LINF-specific complementation was detected.
Supplementary Figure S3. Expression of synthetic *LINF* genes optimized for yeast. The HA-tagged proteins in the p424 plasmid were expressed off the *ADH* promoter in the W303 yeast strain. (A) The proteins from the extracted cells were separated on an 10% SDS-PAGE, the separated proteins transferred to nitrocellulose membranes and then visualized with IgG specific to the HA tag or PGK1. (B) The quantified values of the gels shown in (A). Variations in loading were adjusted relative to the PGK1, and then the values were normalized relative to the expression of HA-Ded1.
Supplementary Figure S4. Complementation of the yeast ded1::HIS strain. The chimeras with the LINF and TRYP catalytic cores and the Ded1 flanking sequences are shown bracketed. They contained the RecA-like catalytic cores of the indicated LINF and TRYP proteins and the amino- and carboxyl-terminal sequences of yeast Ded1. The plates are the same as those shown in Figure 4 except for the chimeras. Plates were incubated 7 days at 30°C and 36°C, and for 10 days at 18°C. The large isolated colonies in the LINF and TRYP lanes contained the DED1 plasmid, which is most apparent at 18°C.