

Candidate Folded-1 was identified as differentially expressed under PV and NK infections (Figure SF1); in addition, a secondary structure with an adjusted minimum free energy (aMFE) of -32.20 was predicted. The putative hairpin and mature sequence are presented in table ST1. The “mature” sequence was validated using northern blotting (Figure SF2). The low throughput validation confirmed the presence of one abundant variant (sharp band on the northern blot) and confirmed the size. Subsequent advances in the annotation of *A. fumigatus* isolates, but not in the *A. fumigatus* 293 strain, indicate that it is likely that folded1 is an rRNA fragment.

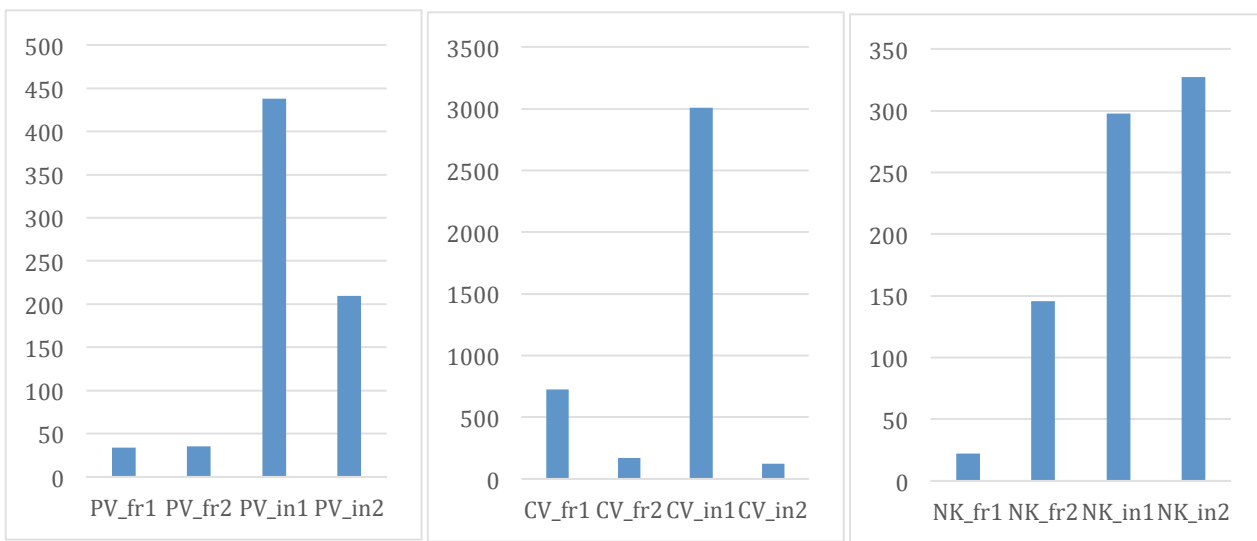


Figure SF1. Normalized expression levels of Folded-1 in the PV, CV and NK virus-free and virus-infected samples. On the y axis we represent the normalized expression levels, in linear scale; on the x-axis we represent the samples.

Name	Hairpin sequence	Mature sequence	Probe sequence (5'→ 3')	Differentially expressed in	Folding
FOLDED-1	ACCCGGTCATTTAGAGG AAGTAAAAGTCGTAACA AGGTTTCCGTAGGTGAA CCTGCGGAAGGATCATT ACCGAGTGAGGGCCCTC TGGGTCCAACC	CCGAGTGAGGGCCCTCTGGGTCCAACC	GGTTGGACCCAGAGGGCCCTCACTGG	CV, NK, PV infected	<p>MFE: -32.30</p>

Table ST1. Characteristics of the Folded-1 sequence such as the hairpin and putative “mature” sequence and the secondary structure corresponding to the minimum free energy. On the secondary structure, we indicate the location of the “mature” sequence in red.

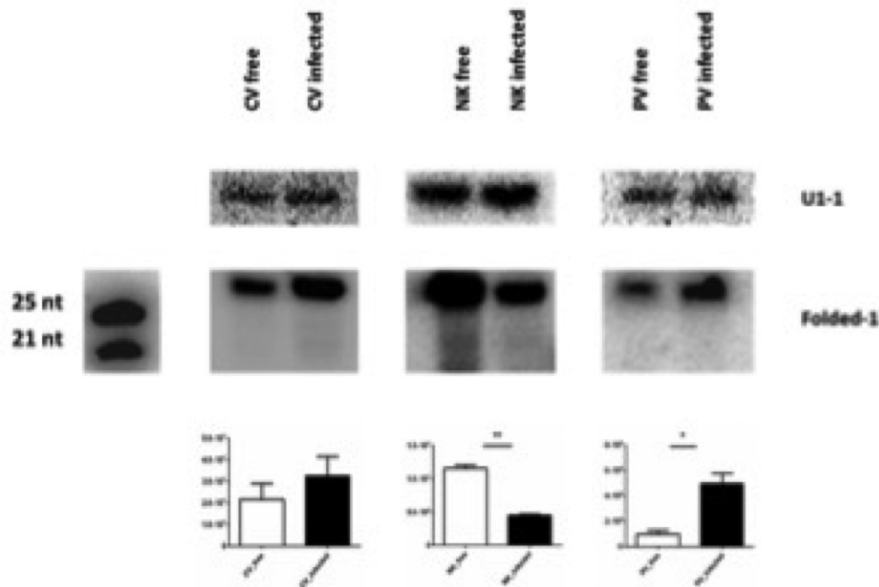


Figure SF2. Quantitative northern blot analysis of Folded-1. The CV, NK and PV correspond to *Aspergillus fumigatus* chrysovirus (AfuCV), a strain of *Aspergillus fumigatus* tetramycovirus-1 (AfuTmV-1) and *Aspergillus fumigatus* partitivirus-1 (AfuPV-1), respectively. Individual lanes loaded with 4 μ g sRNA were blotted and onto membranes and hybridised at 37°C overnight with P^{32} labelled oligonucleotides designed to detect miRNA-like candidate species. *A. fumigatus* U1-1 small nuclear RNA was used as a loading control throughout and the miRNA bands were normalized using the integrity of the U1-1 bands. Relative expression levels were plotted using the GraphPad Prism 6.0 software and *P* values were estimated using an unpaired t-test with Welch’s correction. Error bars were calculated using the standard error values. *P* values less than 0.05 were accepted as statistically significantly different and are shown with an asterisk (** indicates $P \leq 0.01$; $n=3$).

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Aspergillus fumigatus strain B173 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

Sequence ID: [KX090352.1](#) Length: 1210 Number of Matches: 1

Related Information

Range 1: 602 to 697 GenBank Graphics ▾ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
178 bits(96)	1e-44	96/96(100%)	0/96(0%)	Plus/Plus
Query 1	ACCCGGTCATTTAGAGGAAGTAAAAGTCGTAACAGGTTTCCTAGGTGAACCTGCCGAA	60		
Sbjct 602	ACCCGGTCATTTAGAGGAAGTAAAAGTCGTAACAGGTTTCCTAGGTGAACCTGCCGAA	661		
Query 61	GGATCATTACCGAGTGAGGCCCTCTGGGTCCAACC	96		
Sbjct 662	GGATCATTACCGAGTGAGGCCCTCTGGGTCCAACC	697		

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Aspergillus fumigatus strain S48 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

Sequence ID: [KX090348.1](#) Length: 693 Number of Matches: 1

Related Information

Range 1: 110 to 205 GenBank Graphics ▾ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
178 bits(96)	1e-44	96/96(100%)	0/96(0%)	Plus/Plus
Query 1	ACCCGGTCATTTAGAGGAAGTAAAAGTCGTAACAGGTTTCCTAGGTGAACCTGCCGAA	60		
Sbjct 110	ACCCGGTCATTTAGAGGAAGTAAAAGTCGTAACAGGTTTCCTAGGTGAACCTGCCGAA	169		
Query 61	GGATCATTACCGAGTGAGGCCCTCTGGGTCCAACC	96		
Sbjct 170	GGATCATTACCGAGTGAGGCCCTCTGGGTCCAACC	205		

Figure SF3. BLAST result indicating that the folded-1 sequence is similar (plus/plus) with an internal transcribed spacer for the 5.8S ribosomal RNA gene in *A. fumigatus*, strain B173 and strain S48 (annotated in December 2016).

Based on this evidence, we conclude that Folded-1 is highly likely an rRNA-derived read which is DE under virus infection.