Supplementary Figure 1 - PCR analysis and detection of MS2 loop integration and marker excision.

A. Verification of \textit{loxP::Sphis5\textsuperscript{+}::loxP::MS2L} integration into the \textit{ASH1} locus. Integration of the \textit{loxP::Sphis5\textsuperscript{+}::loxP::MS2L} cassette after transformation into wild-type yeast was verified by PCR with the reverse oligonucleotide (R) used originally to amplify the insertion cassette and a forward oligonucleotide (F) complementary to the coding region (5\textsuperscript{`} to the predicted site of integration at the \textit{ASH1} locus). These primers were used to amplify genomic DNA derived from wild-type (WT) control cells or cells transformed with the \textit{ASH1::loxP::Sphis5\textsuperscript{+}::loxP::MS2L::ASH1\textsuperscript{5'UTR}} fragment (\textit{ASH1INT}). The PCR product obtained from the transformed strain had a mobility \textasciitilde2.2kb in agarose gels, which corresponds to the 12 MS2 loops and \textit{S. pombe his5\textsuperscript{+}} marker. This was verified by DNA sequencing. No fragment was amplified from genomic DNA derived from the control cells or from the negative control lacking DNA (No DNA). M = DNA mobility markers.

B. Verification of \textit{Sphis5\textsuperscript{+}::loxP} marker excision and \textit{ASH1::loxP::MS2L::ASH1\textsuperscript{5'UTR}} expression. After \textit{cre} recombinase induction and selection on medium containing histidine, genomic DNA and total RNA were extracted from both wild-type control cells (WT) and the \textit{ASH1::loxP::MS2L-ASH1\textsuperscript{5'UTR}} integrated strain (\textit{ASH1INT}). Amplification of genomic DNA (genomic DNA) was performed using forward (F) and reverse (R) oligonucleotides complementary to the coding region (5\textsuperscript{`} to the site of insertion) and 3'UTR (3\textsuperscript{`} to the site of insertion), respectively. The mobility of the PCR product obtained from the integrated strain was \textasciitilde790bp larger than that obtained from the wild-type strain. No product was obtained from the control reaction lacking DNA (No DNA). Reverse transcription (RT)-PCR of total RNA obtained from the integrated strain, using the same oligonucleotides, also yielded a fragment \textasciitilde790bp larger than that obtained from total RNA derived from wild-type control cells. DNA sequencing revealed that the 12 MS2 loops are present in the mRNA transcribed from \textit{ASH1::loxP::MS2L-ASH1\textsuperscript{5'UTR}} cells. PCR performed on total RNA (\textit{i.e.} non-reverse transcribed) yielded
no products, indicating that there was no DNA contamination in the total RNA preparation.