Supplementary Data

Supplementary Table

TABLE S1. Intracellular polyamine concentrations in the *spe1 spe2 paa1 fms1* deletant strain growing in the presence of 100 μM putrescine, 10 μM spermidine or spermine[a]

Polyamine	**Treatment**		
	Putrescine	**Spermidine**	**Spermine**
Putrescine (mM)	0.031 ± 0.006	ND[b]	ND[b]
Spermidine (mM)	ND[b]	6.34 ± 0.20	ND[b]
Spermine (mM)	ND[b]	ND[b]	0.59 ± 0.02

[a] Analysed by high-pressure liquid chromatography (HPLC)

[b] ND, not detectable by HPLC (detection limit was 0.02 mM).

Supplementary Figures

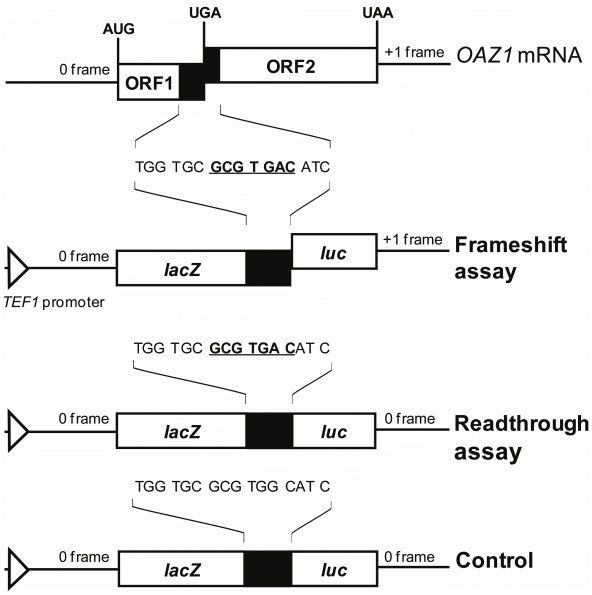

Figure S1 Yeast dicistronic constructs to assay ribosomal frameshifting and readthrough at the antizyme frameshift site. Yeast antizyme, Oaz1, is synthesized via a +1 ribosomal frameshift at the GCG-UGA site (indicated in bold and underlined). Frameshift and readthrough reporter plasmids, pAC98T-OAZ1-FS and pAC98T-OAZ1-RT respectively, and the in-frame control plasmid, pAC98T-OAZ1-Cont, are represented. The *OAZ1* sequences were cloned between translationally-fused β-galactosidase and firefly luciferase ORFs. *TEF1* promoter and reporter genes (*lacZ* and *luc*) are indicated.

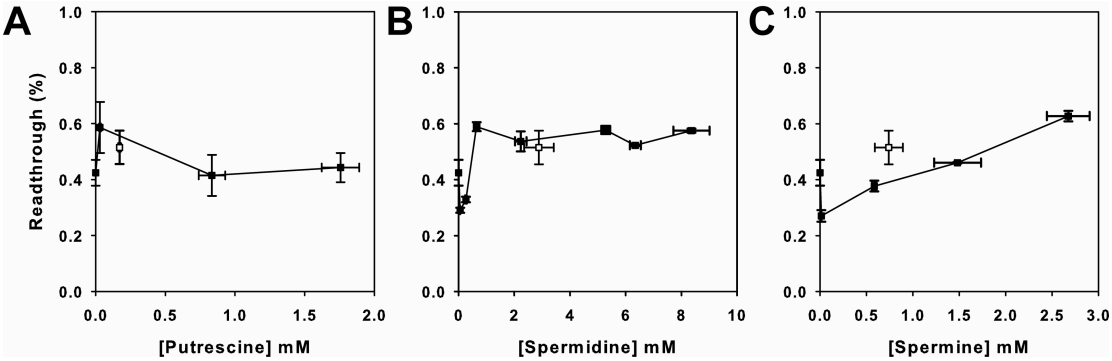

Figure S2 Effect of putrescine, spermidine and spermine on readthrough at the antizyme frameshift site. Average percent readthrough frequencies (filled squares), measured using dicistronic assays, are plotted versus intracellular polyamine intracellular concentrations in the *spe1 spe2 paa1 fms1* deletant strain. (**A**) Putrescine effects on readthrough. The highest putrescine concentration used contained 0.16 mM contaminating spermidine, and was therefore not used in the curve fitting process. (**B**) The effect of intracellular spermidine on readthrough frequencies (**C**) The effect of intracellular spermine on readthrough frequencies. The highest concentration contained 0.2 mM contaminating spermidine. For reference, the wild-type strain BY4741 readthrough frequency (open squares) is represented on all graphs. Error bars are plotted (horizontal and vertical) for all points, and indicate standard deviations for three independent transformants, analysed in triplicate.

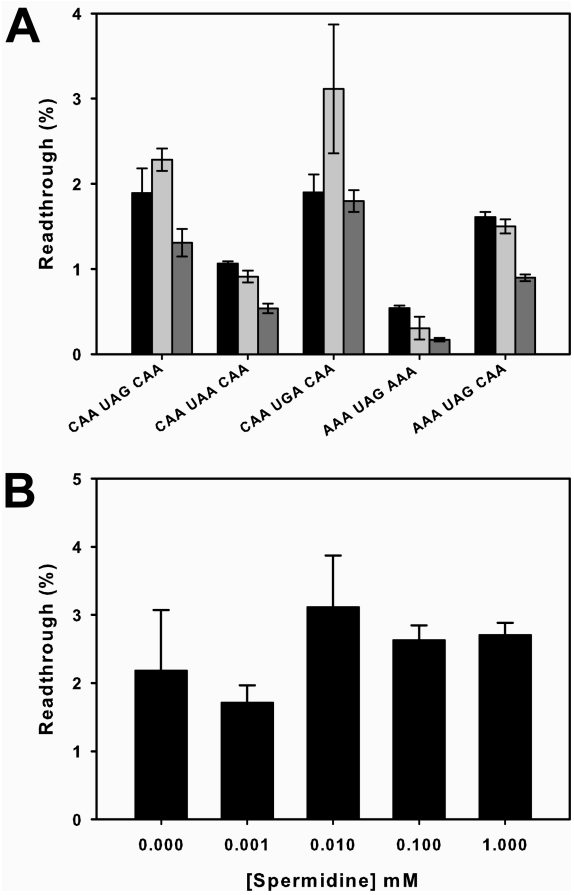

Figure S3 Polyamines do not stimulate readthrough of stop codons in a wide range of nucleotide contexts. Polyamine effects on stop codon readthrough in different contexts were assayed by transforming the quadruple mutant yeast strain with several variants of the pAC98 as described {{150 Williams,I. 2004;}}. pAC98 carries different stop codons (underlined) in diverse contexts (pJR5; CAA UAG CAA, pJR10; CAA UAA CAA, pJR11; CAA UGA CAA, pJR12; AAA UAG AAA, pJR13; AAA UAG CAA, and the control, pJR7; CAA CAG CAA, {{150 Williams,I. 2004;}} each cloned in between translationally-fused *lacZ* and firefly luciferase ORFs. Yeast cultures were grown in the presence of different polyamines and stop codon readthrough frequencies were measured using a dicistronic reporter vector containing a variety of different stop codons placed in different 5' and 3' nucleotide contexts indicated beneath each bar. (**A**) Readthrough frequencies obtained after growth in medium containing 0, 10^{-3} mM spermidine (Spd), and 10^{-2} mM spermine (Spm). (**B**) Readthrough frequencies obtained after growth in medium containing 0, 10^{-3}, 10^{-2}, 10^{-1}, and 1 mM spermidine. Stop codon readthrough used: CAA UGA CAA (pJR11). Error bars indicate +/- standard deviation (n=3).