A

original clone

clone D2

clone D6

clone C5

Construct

CAT expression (%)

0   50   100   150

B

Clone A   clone D2   clone D6

CAT mRNA ▶

rRNA

original clone

clone D2

clone D6

clone C5

Construct

Fold change

0   1   2

Figure S1

Upper panel. Relative CAT expression of four independent cell lines transfected with the ΔALD construct. Clone A ("Original clone') was used in all experiments in this study to provide a comparator for the relative expression of the various *ESAG9-EQ* 3'UTR constructs. Protein expression is indicated in the left hand panel, with mean ± standard error of two experimental replicates being shown. RNA expression is shown in the right hand panel, with ethidium bromide staining of the rRNA providing a loading control.

Lower panel. Relative CAT protein expression from the various ΔALD cell lines after exposure to cell permeable cAMP. In all cases a small (~1.3 fold) increase in CAT protein expression was observed. Mean ± standard error of experimental replicates are shown ($n = 2$, except original clone where $n = 4$).

A — ESAG9 EQ FL 3'UTR

B — 350nt deletion

$\Delta G^\circ = -29.40$

C — 300nt deletion

$\Delta G^\circ = -16.30$

$\Delta G^\circ = -49.00$
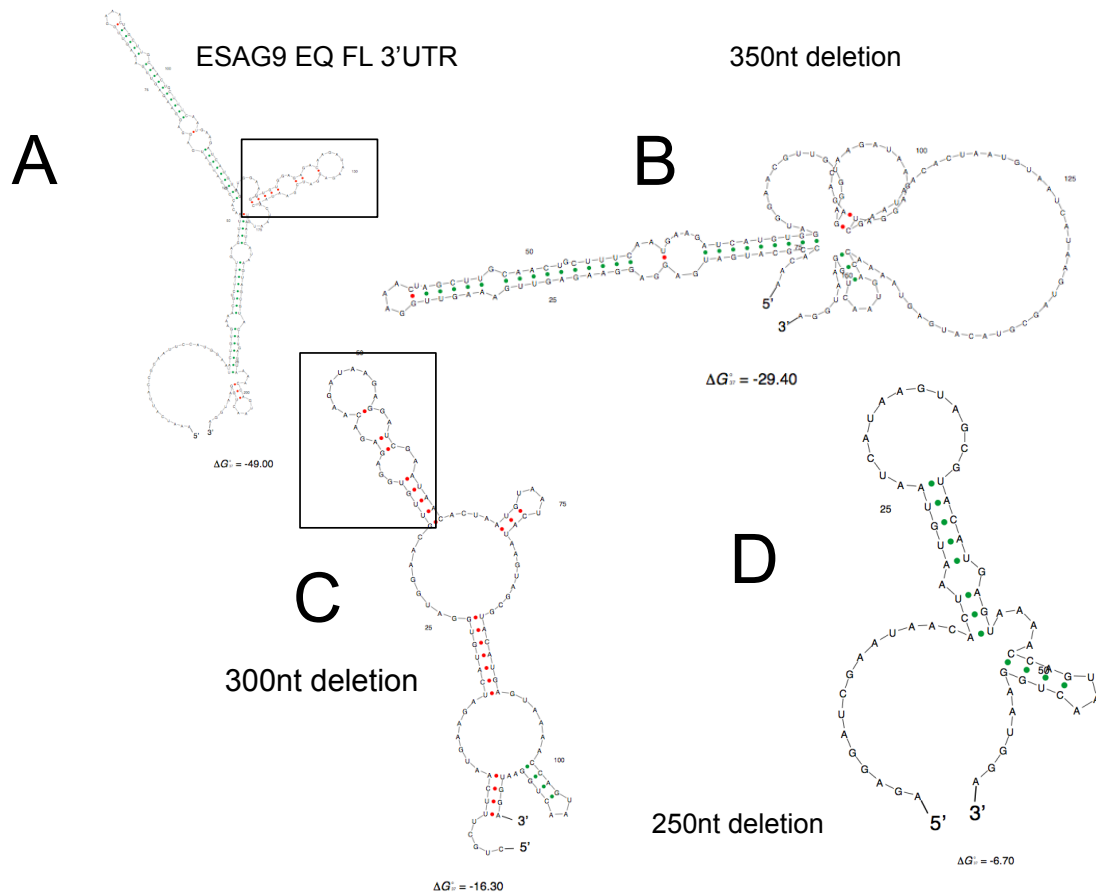
D — 250nt deletion

$\Delta G^\circ = -6.70$

Figure S2

Sfold predicted structures for the intact *ESAG9-EQ* 3'UTR or for deletions of it used in this analysis. The predicted structure associated with the 'regulatory element' characterised in this study is boxed in the 'full length' ('ESAG9-EQ FL 3'UTR') and 300nt 3'UTR deletion. The 250nt and 350nt deletion constructs are not predicted to form the structure.

Table S1. Prediction of the probability of secondary structure formation among different ESAG9 3'UTRs using UNAfold (Markham and Zuker, 2005) within the SSE package (Simmonds, 2012). Details of the analysis are provided within the 'Materials and Methods'. Yellow highlighting shows MFED and Z-score values predictive of a secondary structure in the sequence. Grey shading shows mutant or anti-sense ESAG9-EQ sequences without support for secondary structure, contrasting with the sense intact version.

Sequence	Orientation	Divergence	Native MFE	Control MFE	Control SD	MFED	Z-score
ESAG9 EQ	Sense	0.219	-50.1	-42.04	4.0102	0.1917	-2.0099
ESAG9 K9	Sense	0.23	-48.9	-49.283	4.1139	-0.0078	0.0931
ESAG9 k69	Sense	0.239	-49.2	-44.872	4.3029	0.0965	-1.0058
Tb927.1.5080	Sense	0.227	-59.9	-54.951	3.774	0.0901	-1.3114
Tb927.3.5790	Sense	0.23	-54.8	-54.29	3.9161	0.0094	-0.1302
Tb927.9.7370	Sense	0.229	-36.5	-39.771	3.7807	-0.0822	0.8651
ESAG9u	Sense	0.229	-46	-46.834	3.9262	-0.0178	0.2124
ESAG9c	Sense	0.231	-46.9	-48.613	3.9979	-0.0352	0.4285
ESAG9 EQ	A/Sense	0.219	-30.4	-32.357	3.222	-0.0605	0.6074
ESAG9 K9	A/Sense	0.23	-40.9	-39.953	3.4965	0.0237	-0.2708
ESAG9 k69	A/Sense	0.239	-54.4	-47.299	3.9103	0.1501	-1.8161
Tb927.1.5080	A/Sense	0.227	-49.2	-44.987	3.9318	0.0937	-1.0716
Tb927.3.5790	A/Sense	0.23	-37.9	-41.393	3.3743	-0.0844	1.0351
Tb927.9.7370	A/Sense	0.229	-42.8	-40.804	3.2919	0.0489	-0.6062
ESAG9u	A/Sense	0.229	-28.4	-32.838	3.3341	-0.1352	1.3312
ESAG9c	A/Sense	0.231	-35.9	-39.132	3.3805	-0.0826	0.956
ESAG9 EQ ΔE	Sense	0.553	-39.1	-37.587	3.3569	0.0403	-0.4507
ESAG9 EQ ΔE	A/Sense	0.551	-27.2	-28.888	3.1709	-0.0584	0.5323