Supplementary information for:

Overexpression of the *Aspergillus nidulans* histone 4 acetyltransferase EsaA increases activation secondary metabolite production

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Table S1: Primers used for strain construction

Sequence	**Primer Name**	**Purpose**
TTTGCGGCCGCCCGCTAGACAAGTCGAGGAAGCCG	esaA 3’ flank rev NotI	*OE::esaA* construct
CCATGAGAGTCTCGGACGCCCATTGTGATGTCTGCTCAAGCGGGGTA	gpdA(p) 3' esaA	*OE::esaA* construct
CCCGCTTGAGCAGACATCACAATGGGCGTCCGAGACTCTCATGGCGAGG	esaA 5' for	*OE::esaA* construct
AAGGCTTGCGGCCGCTGCGTTGGT	gpdF NotI	*OE::esaA* construct
GGCCTGTTGTGCTCGATATTGATGCTCTGATG	esaA 5' flank for	*ΔesaA* construct
CAAGCTATCGATACCTCGACTCTAGTCAGTAGAAAATAGAGGATAAACCAC	esaA 5' flank rev	*ΔesaA* construct
GTGGTTTATCCTCTATTTTCTACTGACTAGAGTCGAGGTATCGATAGCTTG	A. p. pyrG 5'	*ΔesaA* construct
TACCACTACTGAATCAACGAGAGGAAAATAAGCAATTCGACAATCGGAGAGGCTGC	A. p. pyrG 3'	*ΔesaA* construct
GCAGCCTCTCCGATTGTCGAATTGCTTATTTTCCTCTCGTTGATTCAGTAGTGGTA	esaA 3' flank for	*ΔesaA* construct
CAGAGCGTTGAGCCTTTCTGGATTGGTTAC	esaA 3' flank rev	*ΔesaA* construct
TTTGAATTCATGGGCGTCCGAGACTCTCATGGCG	esaA cDNA 5' EcoRI	Yeast complementation construct
TTTGAATTCTCACCAATTCCATGTTCGACTGGACGCAG	esaA 3' EcoRI	Yeast complementation construct
TTTGAATTCTTGTCTTCTAGCTGCTTCAGACTCC	ESA1(p) 5' EcoRI	Yeast complementation construct
CCATGAGAGTCTCGGACGCCCATATGTCTGACTCCTCAGAAGCC	ESA1(p) 3’ esaA cDNA	Yeast complementation construct
GAAAAAATAACACGGCCCTTGAGGCGATATGTCTGACTCCTCAGAAGCCACTGTA	ESA1(p) w esaA UTR 3'	Yeast complementation construct
TACAGTGGCTTCTGAGGAGTCAGACATATCGCCTCAAGGGCCGTGTTATTTTTTC	esaA w esaA UTR 5'	Yeast complementation construct
GCGTCCAAATATCGTGCCTCTCC	gpdAp int 5’	*OE::esaA* confirmation
TCTCTGCTTTTCTTAACGCTCCATCCTACA	esaA int 3’	*OE::esaA* confirmation

GACTCTGCAGTGCGAGATGCGC GGG	AN10956 5' ext	*ΔesaA* confirmation
TATTCCATCAGCTACTGAACAA CTTCTAC	pyrG 3' int	*ΔesaA* confirmation

Table S1. Primers used for strain construction and confirmation. Restriction enzyme sites are underlined.

Table S2: Primers used for qPCR

Sequence	**Primer Name**
GCTTGATGGTACGATGCTGCG	acvA(p) F
CTGAGGGGATATCGCTTGAG	acvA(p) R
GATATTTGCATATGATACAGGCCCGCATTG	aflR(p) F
AACCCTTGAAACCCATAGCCAGTAAAG	aflR(p) R
CGACGAGTGTGGTCGGCTC	ipnA(p) F
CGGTGGTAACGGAAGGATCC	ipnA(p) R
CAGCTTCCAGCCATGATTAAG	orsA(p) F
GCACTGCTGTTCTATTGCC	orsA(p) R
GTGAGTCCAGAGTATCCCAG	orsD(p) F
AATGAGAAGAATGCCAAGCC	orsD(p) R
CCTGATGTAGGATTAGGATAGAATG	stcO(p) F
CCTGATGTAGGATTAGGATAGAATG	stcO(p) F
AAGGCTGGGCTGAAGATTATCGAG	stcO(p) R
AAGGCTGGGCTGAAGATTATCGAG	stcO(p) R
CGTCAGCGTGTAATATGAAC	tdiA(p) F
CCACGTCACCACTTTCAACTC	actA F
GACGGAGTATTTGCGCTCAG	actA R
AGTCTATTGTCGGATTCTTCGTC	acvA F
CGTCTCGAATTCTTTGTCC	acvA R
GACAGCACCATCACCAACAAC	aflR F
GAGTGACGATAGGTCGGTGG	aflR R
CCGACGGCACCAAACTGAG	ipnA F
GCTCTGCATTAACCCATTTCAC	ipnA R
GCTGACCTGGTACTTCTGACC	orsA F
CTATCCCATCCGCTGACGTG	orsA R
GATGTCGATTTGGAAGAGCAG	orsD F
CATCAACCATCTCTGCTGGAG	orsD R
CCACTGACATGAAGGACAAAGAA	stcO F
TTCTCTGCCATATCTGTAGTCC	stcO R
GCTCAGGGCAGCCAAGTACC	tdiA F
CCAGAAGGACAGACCAGTAC	tdiA R
GACTCTACGTGGATTACAGCC	tdiB F
GTCGCTGAGACGGCCTCC	tdiB R
CCGATGGATACTTGACCGAAG	tubA F
CACGAGCGTAGTTGTTTGAGG	tubA R

Table S2. Primers used for qPCR analysis.

Figure S1: Alignment of *A. nidulans* EsaA and homologs

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A.n. EsaA       MGVRDSHGEAAGTPDPVEKGIATLNTIRIGVKAMVHKDGALRKAEILSIKQRKDGLAFYVHYVDFNKRLDEWVASSRLDL         80
S.c. ESA1       -MSHDGKEEPG-----IAKKINSVDDIIIKCQCWVQKNDEERLAEILSINTRKAPPKFYVHYVNYNKRLDEWITTDRINL         74
Sch.p. Mst1     -MSNDVDDESK-----IETKSYEAKDIVYKSKVFAFKDGEYRKAEILMIQKRTRGVVYYVHYNDYNKRLDEWITIDNIDL         74
Consensus       -MSXDXXXEXX-----IEKKIXXXXDIXIKXKXXVXKDGEXRKAEILSIXXRKXGXXFYVHYVDYNKRLDEWITXDRIDL

A.n. EsaA       SQEVEWPQPEKPEKKKSGPAKAPSKNKRVRAGSRDVSATPDTLTGKNTNVGKAQRPSKAGGKENRGDETPADLSMLASEA        160
S.c. ESA1       DKEVLYP---KL-KATDEDNKK---QKKKKATN-------------TSETPQDSLQDGVDGFSREN-----------TDV        123
Sch.p. Mst1     SKGIEYPPPEKP-KKAHGKGKS---SKRPKAVDRRRSITAPSKTEPSTPSTEKPEPSTPSGESDHG-----------SNA        139
Consensus       SKEVEYPXPEKP-KKXXGXXKX---XKRXKAXXRXXSXTXXXXTXXXTXXXXXXXPSXXXGXSXXG-----------SXA

A.n. EsaA       VSADGTPKAVSEDIDMMDASFTDAKEIKEEERALGLMSREEEIEKLRTSGSMTQNPTEVHRVRNLDRLQMGKYDIEPWYF        240
S.c. ESA1       MDLDN----------------LNVQGIKDEN-----ISHEDEIKKLRTSGSMTQNPHEVARVRNLNRIIMGKYEIEPWYF        182
Sch.p. Mst1     GNESLP---------------LLEEDHKPES-----LSKEQEVERLRFSGSMVQNPHEIARIRNINKICIGDHEIEPWYF        199
Consensus       XXXDXX---------------LXXXXIKXEX-----XSXEXEIEKLRTSGSMTQNPHEVARVRNLNRIXMGKYEIEPWYF

A.n. EsaA       SPYPASFSDAEVVYIDEFCLSYFDNKRAFERHRTKCTLTHPPGNEIYRDDNISFFEVDGRRQRTWCRNLCLLSKLFLDHK        320
S.c. ESA1       SPYPIELTDEDFIYIDDFTLQYFGSKKQYERYRKKCTLRHPPGNEIYRDDYVSFFEIDGRKQRTWCRNLCLLSKLFLDHK        262
Sch.p. Mst1     SPYPKEFSEVDIVYICSFCFCYYGSERQFQRHREKCTLQHPPGNEIYRDDYISFFEIDGRKQRTWCRNICLLSKLFLDHK        279
Consensus       SPYPXEFSDXDXVYIDXFCLXYFGSKRQFERHRXKCTLXHPPGNEIYRDDYISFFEIDGRKQRTWCRNLCLLSKLFLDHK

A.n. EsaA       TLYYDVDPFLFYCMCTRDETGCHLVGYFSKEKESGEGYNLACILTLPQYQRRGYGRLLISFSYELSKREGKVGSPEKPLS        400
S.c. ESA1       TLYYDVDPFLFYCMTRRDELGHHLVGYFSKEKESADGYNVACILTLPQYQRMGYGKLLIEFSYELSKKENKVGSPEKPLS        342
Sch.p. Mst1     MLYYDVDPFLFYCMCRRDEYGCHLVGYFSKEKESSENYNLACILTLPQYQRHGYGKLLIQFSYELTKREHKHGSPEKPLS        359
Consensus       TLYYDVDPFLFYCMCRRDEXGCHLVGYFSKEKESXEGYNLACILTLPQYQRXGYGKLLIXFSYELSKREXKVGSPEKPLS

A.n. EsaA       DLGLLGYRQYWRETLVEILLDSGRETVSENELAMLTSMTEKDVHETLVTFKMLRYNKGQWIIVLTDEVIEERNKRLEKEK        480
S.c. ESA1       DLGLLSYRAYWSDTLITLLVEHQKEITID-EISSMTSMTTTDILHTAKTLNILRYYKGQHIIFLNED-ILDRYNRLKAKK        420
Sch.p. Mst1     DLGLISYRAYWAEQIINLVLGMRTETTID-ELANKTSMTTNDVLHTLQALNMLKYYKGQFIICISDG-IEQQYERLKNKK        437
Consensus       DLGLLSYRAYWXETLIXLLLXXXXETTID-ELAXXTSMTTXDVLHTLXTLNMLRYYKGQXIIXLXDX-IEXRYXRLKXKK

A.n. EsaA       IKGSRKIDPARLQ-WKPPVFTASSRTWNW                                                        508
S.c. ESA1       R---RTIDPNRLI-WKPPVFTASQLRFAW                                                        445
Sch.p. Mst1     R---RRINGDLLADWQPPVFHPSQLRFGW                                                        463
Consensus       R---RXIDPXRLX-WKPPVFTASQLRFXW
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Figure S1. Alignment of *A. nidulans* EsaA with previously characterized fungal orthologs. EsaA has 65% protein identity with *Saccharomyces cerevisiae* ESA1 and 53% identity with *Schizosaccharomyces pombe* Mst1. *A.n. Aspergillus nidulans, S.c. Saccharomyces cerevisiae, Sch.p. Schizosaccharomyces pombe*.

Figure S2: Confirmation of *OE::esaA* strain

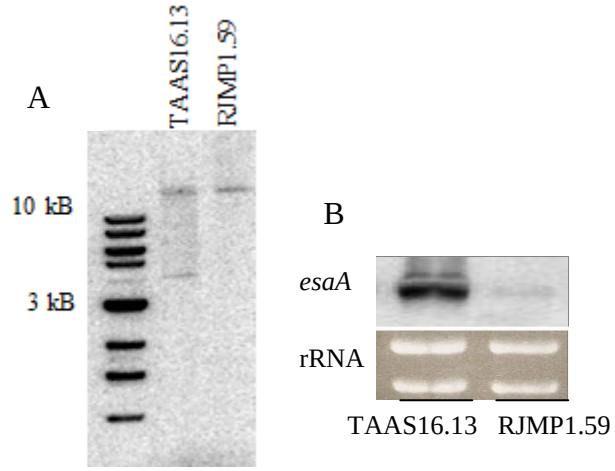


Figure S2. A. Southern blots confirming targeted integration of the OE::*esaA* construct to the *pyroA* locus. A NotI digest drops out the *gpdA(p)::esaA* at approximately 4 kB. As this construct is targeted to the *pyroA* locus, the native *esaA* copy is also still apparent as a large band around 93 kB. B. Northern blot confirms greatly increased expression of the *esaA* gene.

Figure S3: Confirmation of *esaA* deletion

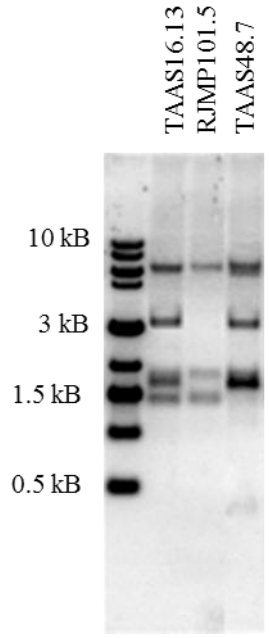
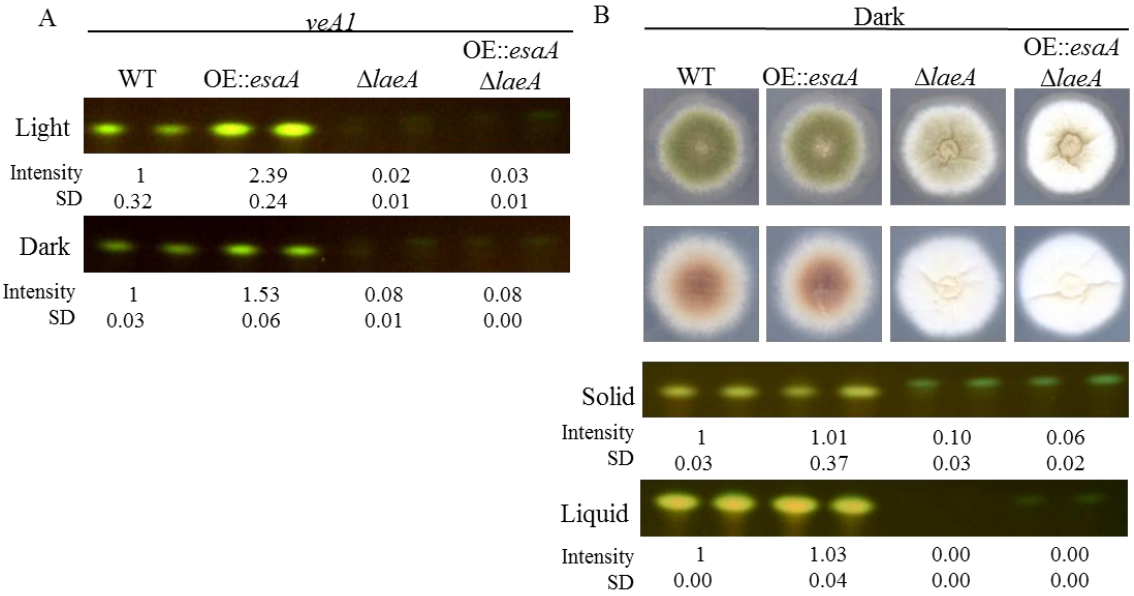


Figure S3. A. Southern blot confirming deletion of the native *esaA* allele in an OE::*esaA* background. ClaI digest was used to distinguish between native and ectopic copies of *esaA*. Expected sizes: WT (RJMP101.19) – 6395 bp, 1852 bp, 1458 bp; OE::*esaA* (TAAS16.13) – 3121 bp, 1695 bp; OE::*esaA ΔesaA* (TAAS48.7) – 6213 bp, 3121 bp, 1852 bp, 1695 bp, 411 bp.

Figure S4: Additional *OE::esaA* phenotypes



C

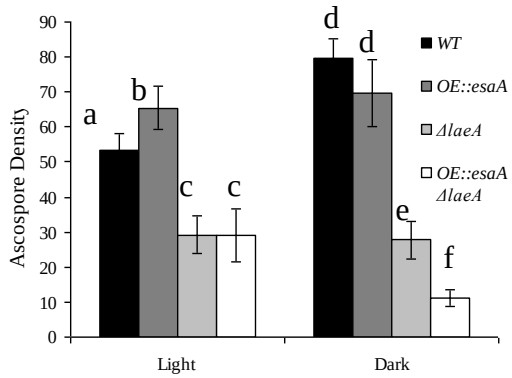


Figure S4. A. TLC of extracts from cultures from solid GMM after 3 days under light and dark conditions in a *veA1* background. OE::*esaA* increased ST regardless of illumination, but is unable to restore *ΔlaeA* defects. WT – RDIT2.3; *OE::esaA* – RAAS22.135; *ΔlaeA* – RJW46.4; *OE::esaA ΔlaeA* – RAAS22.100. Shown is the band corresponding to sterigmatocystin (ST). B. Under dark conditions, where SM production and sexual development are favored, OE::*esaA* does not significantly increased ST production. C. Sexual spore counts after 84 hours incubation. The OE::*esaA* strain has a mild increase in the number of sexual spores at early timepoints in the light. Incubation for longer period or in the dark abrogates this increase. Statistical analysis was performed on four replicate cultures using JMP9 Software (Version 9) to analyze data according to the Tukey-Kramer multiple comparison test with a P value < 0.05.

Figure S5: Schematic of SM clusters

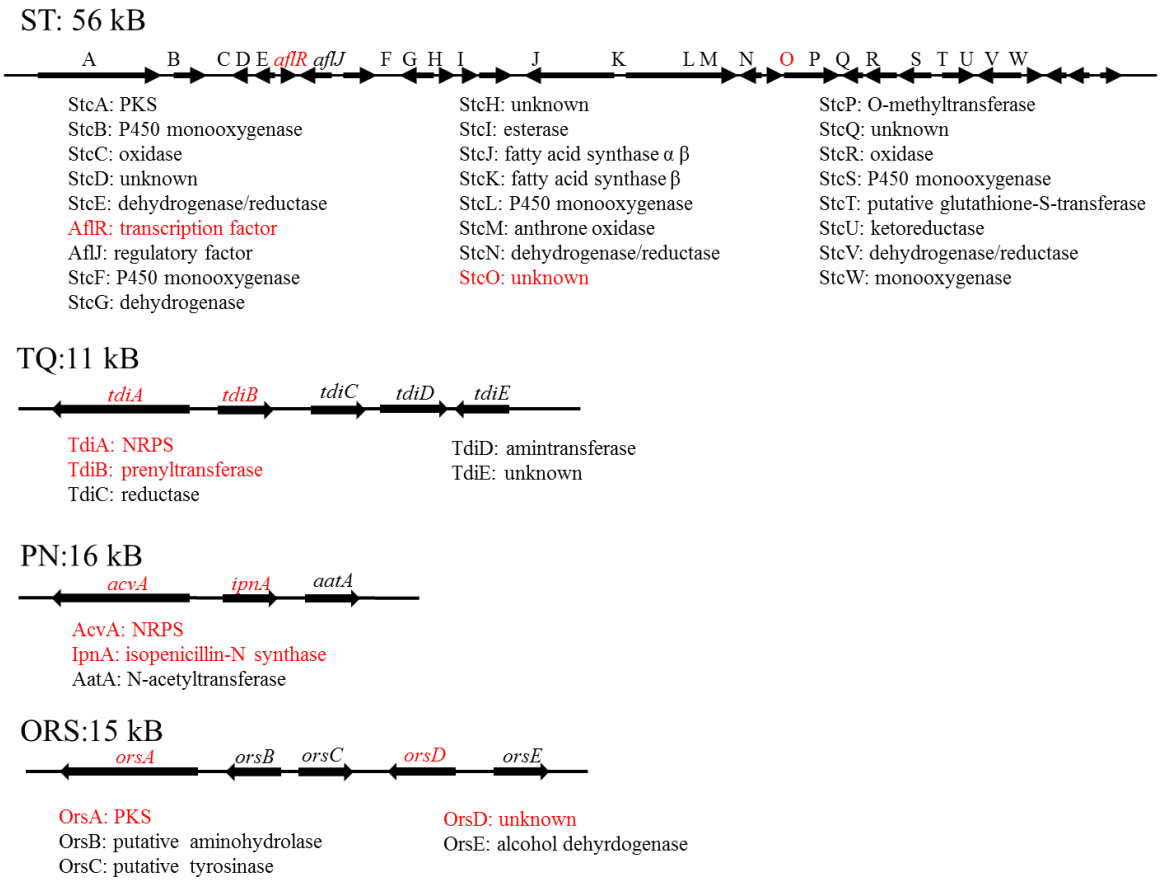


Figure S5. Schematic representation of the gene clusters examined in this study. Gene products and approximate cluster sizes are provided. Genes examined with qRT-PCR and ChIP are depicted in red.

Figure S6: *OE::esaA* is unable to suppress telomere position effect growth defects

A B

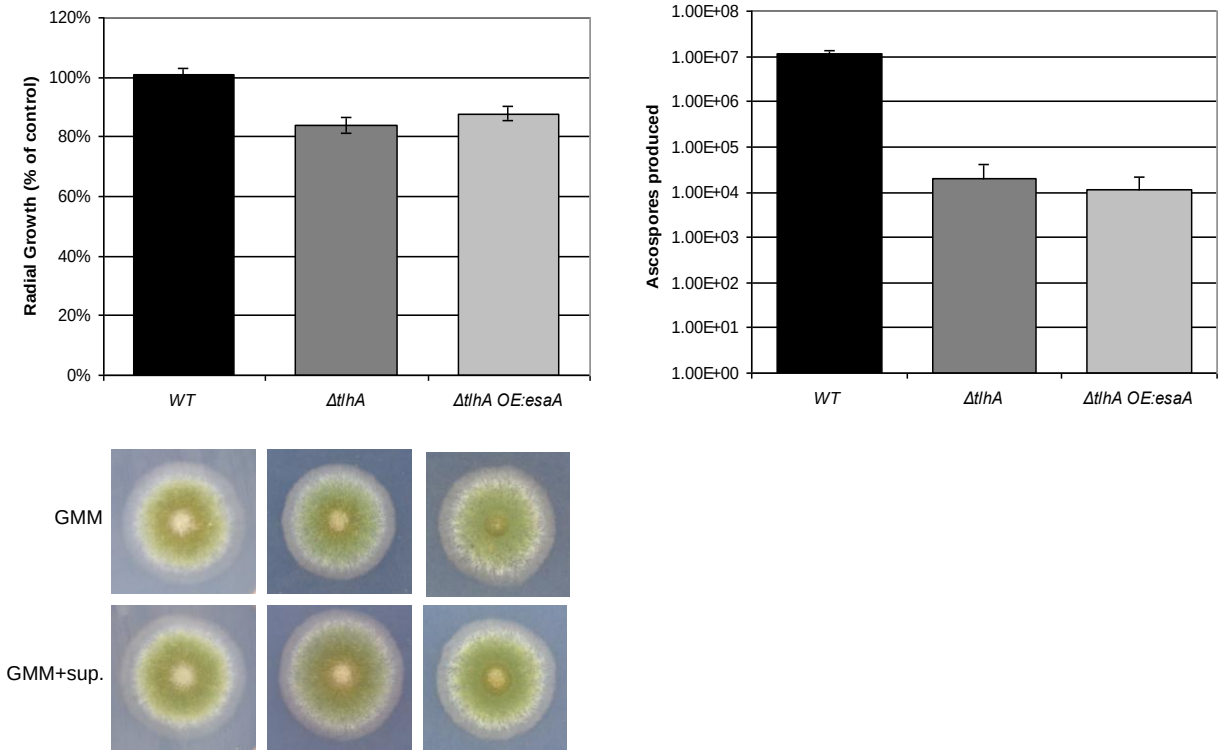


Figure S6. Introduction of *OE::esaA* is unable to restore normal growth production to a telomere position effect mutant, Δ*tlhA::pyrG*. Improper expression of the *pyrG* impairs growth of the Δ*tlhA* mutant, which can be remediated by supplementing the medium with the appropriate pyrimidines. Radial growth of the OE::*esaA* Δ*tlhA* strain is identical to that of the Δ*tlhA* single mutant, indicating no restoration of the expression of the auxotrophic marker used for the disruption. B. Ascospore production is also not restored in the double mutant. Strains used are WT – RJMP103.5, Δ*tlhA* – RJMP116.3, *OE::esaA* Δ*tlhA* – RAAS141.4. Supplement is 5 mM uracil and 5 mM uridine.