# Supplementary Information

# Non-canonical chromatin-based functions for the threonine metabolic pathway

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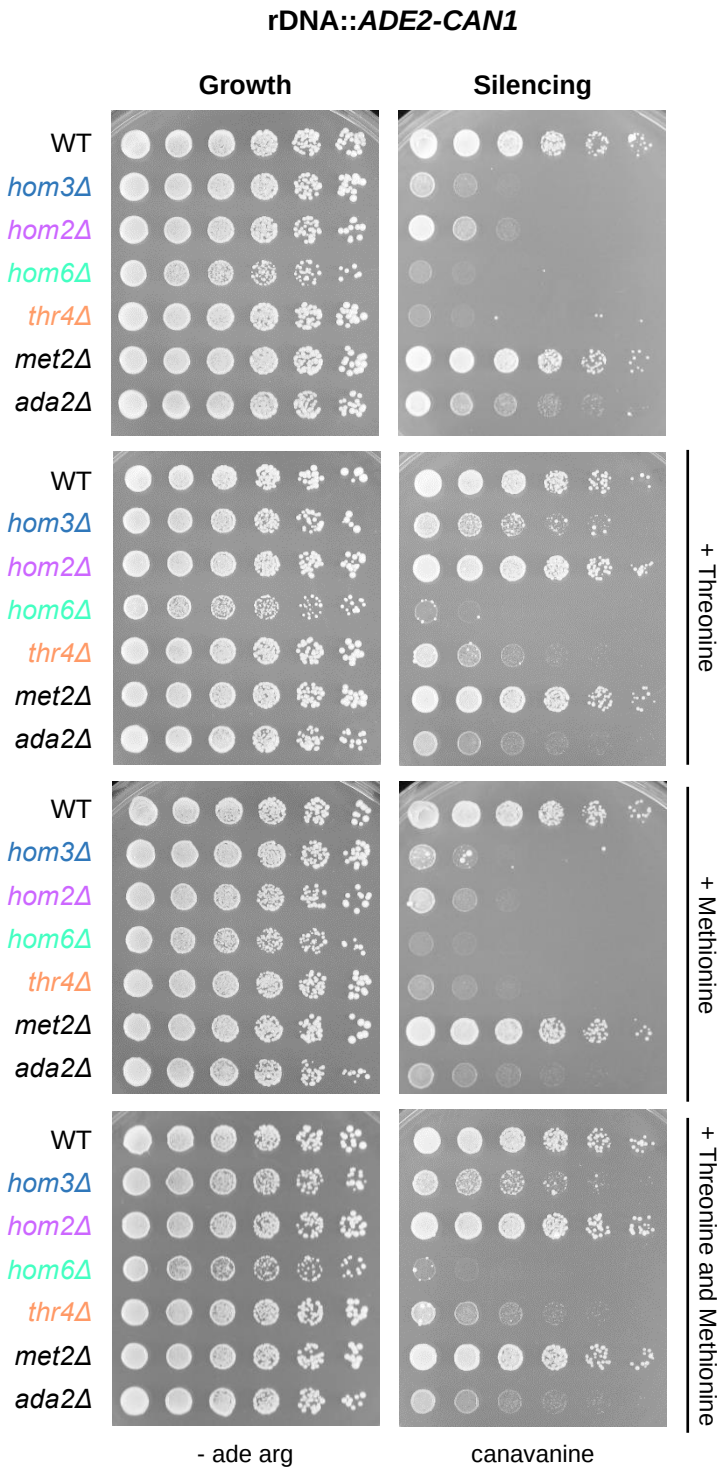


**Figure S1.** Methionine supplementation does not affect rDNA silencing.
SC -ade arg and SC -ade arg 8 µg/mL canavanine plates were top-coated with excess threonine, methionine, or a combination of threonine and methionine and assayed for defects in rDNA silencing. Methionine supplementation does not rescue the rDNA silencing defects of any strain, including *hom3Δ*, *hom2Δ,* and *thr4Δ.* Strains presented as in Figure 2. Imaged after a 3-day incubation.

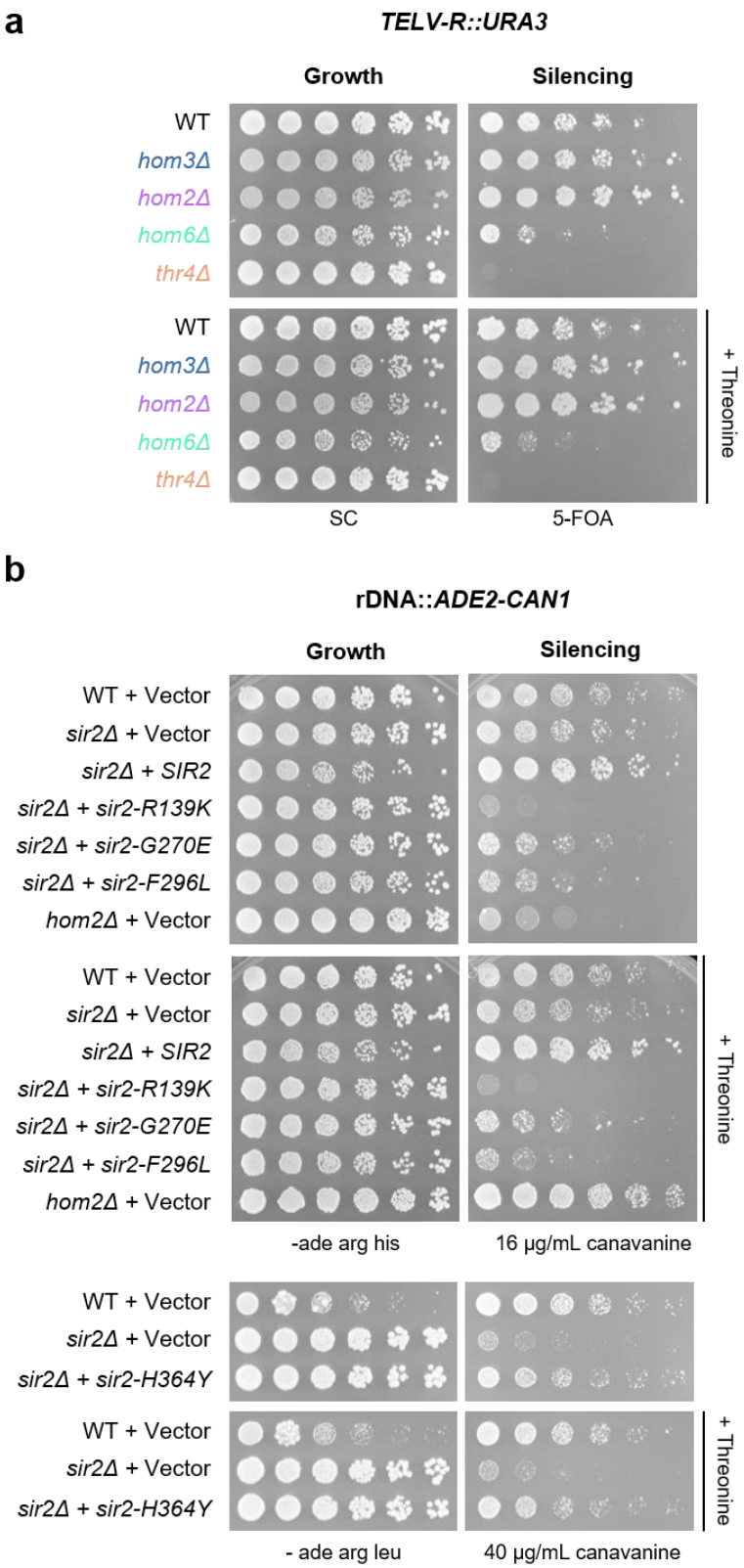
a
TELV-R::URA3
Growth
Silencing
WT
hom3Δ
hom2Δ
hom6Δ
thr4Δ
WT
hom3Δ
hom2Δ
hom6Δ
thr4Δ
+ Threonine
SC
5-FOA
b
rDNA::ADE2-CAN1
Growth
Silencing
WT + Vector
sir2Δ + Vector
sir2Δ + SIR2
sir2Δ + sir2-R139K
sir2Δ + sir2-G270E
sir2Δ + sir2-F296L
hom2Δ + Vector
WT + Vector
sir2Δ + Vector
sir2Δ + SIR2
sir2Δ + sir2-R139K
sir2Δ + sir2-G270E
sir2Δ + sir2-F296L
hom2Δ + Vector
+ Threonine
-ade arg his
16 µg/mL canavanine
WT + Vector
sir2Δ + Vector
sir2Δ + sir2-H364Y
WT + Vector
sir2Δ + Vector
sir2Δ + sir2-H364Y
+ Threonine
- ade arg leu
40 µg/mL canavanine

**Figure S2.** Threonine supplementation specifically rescues threonine biosynthetic pathway rDNA silencing phenotypes.

(a) Threonine supplementation does not improve defects in telomeric silencing. Strains contain a *URA3* reporter gene at the right arm of telomere V, where it is epigenetically silenced. Decreased growth on 5-FOA is indicative of a defect in *URA3* silencing. *hom6Δ* and *thr4Δ* exhibit defects in telomeric silencing that are not rescued by threonine supplementation. Images were taken after a 3-day incubation. Strains Plated: WT (LPY23157), *hom3Δ* (LPY16903), *hom2Δ (*LPY16034), *hom6Δ* (LPY23065), *thr4Δ* (LPY23341).

(b) Supplementation with threonine does not rescue rDNA silencing defects of *sir2* hypomorphic alleles. Cells were transformed with either vector (pLP 60 or pLP400), a *SIR2* plasmid (pLP285), or a plasmid with mutant alleles of *SIR2* (pLP1102, pLP1110, pLP1112, pLP1976). Imaged after a 3-day incubation. Strains: WT (LPY23157), sir2*Δ* (LPY5013), *hom2Δ* (LPY16020).

**Table S1.** Yeast Strains

Except where noted, strains were constructed during the course of this study. Strains LPY5013-LPY23386 are of the W303 background and LPY6495-LPY23320 are of the BY background.

| Strain | Genotype |
|---|---|
| LPY5013 | *MATα W303 sir2Δ::TRP1 rDNA::ADE2-CAN1* |
| LPY11674 | *MATα W303 ada2Δ::kanMX rDNA::ADE2-CAN1* |
| LPY13435 | *MATα W303 gcn5Δ::natMX* |
| LPY16020 | *MATα W303 hom2Δ::kanMX rDNA::ADE2-CAN1* |
| LPY16034 | *MATα W303 hom2Δ::kanMX TELV-R::URA3* |
| LPY16903 | *MATα W303 hom3Δ::kanMX TELV-R::URA3* |
| LPY16907 | *MATα W303 hom3Δ::kanMX rDNA::ADE2-CAN1* |
| LPY23065 | *MATα W303 hom6Δ::kanMX hmrΔE::TRP1 rDNA::ADE2-CAN1 TELV-R::URA3* |
| LPY23157 | *MATα W303 hmrΔE::TRP1 rDNA::ADE2-CAN1 TELV-R::URA3* |
| LPY23250 | *MATα W303 hom6-E208L hmrΔE::TRP1 rDNA::ADE2-CAN1* |
| LPY23274 | *MATα W303 hom6-D219L hmrΔE::TRP1 rDNA::ADE2-CAN1* |
| LPY23330 | *MATα W303 met2Δ::kanMX hmrΔE::TRP1 rDNA::ADE2-CAN1 TELV-R::URA3* |
| LPY23341 | *MATα W303 thr4Δ::kanMX hmrΔE::TRP1 rDNA::ADE2-CAN1 TELV-R::URA3* |
| LPY23380 | *MATα W303 fob1Δ::kanMX hmrΔE::TRP1 rDNA::ADE2-CAN1 TELV-R::URA3* |
| LPY23381 | *MATα W303 fob1Δ::kanMX hom3Δ::kanMX hmrΔE::TRP1 rDNA::ADE2-CAN1 TELV-R::URA3* |
| LPY23382 | *MATα W303 fob1Δ::kanMX hom6Δ::kanMX hmrΔE::TRP1 rDNA::ADE2-CAN1 TELV-R::URA3* |
| LPY23384 | *MATα W303 fob1Δ::kanMX hom2Δ::kanMX rDNA::ADE2-CAN1* |
| LPY23386 | *MATα W303 fob1Δ::kanMX thr4Δ::kanMX hmrΔE::TRP1 rDNA::ADE2-CAN1 TELV-R::URA3* |
| LPY6495 | *MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* |
| LPY23282 | *MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 xrs2Δ::kanMX* |
| LPY23285 | *MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 mre11Δ::kanMX* |

| | |
|---|---|
| LPY23292 | *MAT*a *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 hom6Δ::kanMX* |
| LPY23296 | *MAT*a *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 hom6Δ::kanMX mre11Δ::kanMX* |
| LPY23297 | *MAT*a *his3Δ1 leu2Δ0 lys2Δ0 met15Δ0 ura3Δ0 hom6Δ::kanMX xrs2Δ::kanMX* |
| LPY23320 | *MAT*a *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 sir2Δ::kanMX* |

**Table S2.** Plasmids

| Plasmid | Description | Marker |
|---|---|---|
| pLP60* | pRS313 | *HIS3* |
| pLP270** | pRS423 | *HIS3* |
| pLP285*** | pRS313-*SIR2* | *HIS3* |
| pLP400* | pRS315 | *LEU2* |
| pLP891 | pRS423-*SIR2* | *HIS3* |
| pLP1102*** | pRS313-*sir2-R139K* | *HIS3* |
| pLP1110*** | pRS313-*sir2-G270E* | *HIS3* |
| pLP1112*** | pRS313-*sir2-F296L* | *HIS3* |
| pLP1976**** | pRS315-*sir2-H364Y* | *LEU2* |
| pLP2628 | pRS315-*HOM6* | *LEU2* |
| pLP2794 | *pRS313-HOM6* | *HIS3* |
| pLP3075 | pRS316-*HOM6* | *URA3* |
| pLP3510 | pML104-*HOM6* gRNA | *URA3* |
| pLP3515 | pRS423-*THR4* | *HIS3* |
| pLP3542 | pRS313-*hom6-E208L* | *HIS3* |
| pLP3546 | pRS313-*hom6-D219L* | *HIS3* |

* Sikorski, R. S. & Hieter, P. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. *Genetics* 122, 19-27 (1989).
****** Christianson, T. W., Sikorski, R. S., Dante, M., Shero, J. H. & Hieter, P. Multifunctional yeast high-copy-number shuttle vectors. *Gene* 110, 119-122 (1992).
*** Garcia, S. N. & Pillus, L. A unique class of conditional *sir2* mutants displays distinct silencing defects in *Saccharomyces cerevisiae. Genetics* 162, 721-736 (2002).
****Tanny, J. C., Dowd, G. J., Huang, J., Hilz, H. & Moazed, D. An enzymatic activity in the yeast Sir2 protein that is essential for gene silencing. *Cell* 99, 735-745 (1999).

**Table S3.** Oligonucleotides

| Oligo Number | Name | Sequence (5’ → 3’) |
|---|---|---|
| oLP1516 | HOM2_5_KO | CGACGGAGAAGAAGGAGAC |
| oLP1517 | HOM2_3_KO | CTTGGGTCAGCGAGAGAATTAC |
| oLP1604 | MET2_5KO | CAGCTGCGTCCAATAGATGAG |
| oLP1606 | HOM3_5KO | CTTTCCGTACGCAGTCTTCTC |
| oLP1607 | HOM3_3KO | GTCAGTGATGGGGATTTGC |
| oLP1769 | hom6_5KOnew | CAATAACGCACATGGTGG |
| oLP1770 | hom6_3KOnew | GCCCCATGACATGGATGAG |
| oLP2493 | MET2_3KO_V2 | CGGTAACTCGTGTGCTCTCATTC |
| oLP2496 | THR4_5KO_V2 | GTCTTCCAAGCAAAACGAGC |
| oLP2497 | THR4_3KO_V2 | GGTTCCTTACTCTCGCAATC |
| oLP2516 | THR4SpeI_F | CGATACTAGTTGCATCCAAGAGAAACGCTG |
| oLP2517 | THR4ClaI_R | GGCATCGTTAGTGTATGCACATCCTG |
| oLP2531 | MRE11KO_JKC_F | GTCAGAGTTCACAAGCAAGCC |
| oLP2532 | MRE11KO_JKC_R | CGAACAAAAGAGCAAAGGCTGG |
| oLP2533 | XRS2KO_F | CGTAAAGTTGTTAACTACGC |
| oLP2534 | XRS2KO_R | AGCAACCTGTAGTGCTTC |
| oLP2535 | hom6_E208L_HDRF | GGGTTATACTTTACCAGATCCAAGAGATGATTTGAATGGG TTGG |
| oLP2536 | hom6_E208L_HDRR | GAGATTCAACTTCCACACCAGATATCCTACCAACAATGGT AACTTTTCTAGCAACATCCAACCCATTCAAATCATC |
| oLP2537 | hom6_D219L_HDRF_v2 | GGGTTATACTGAACCAGATCCAAGAGATGATTTGAATGGG TTGTTGGTTGCTAGAAAAG |
| oLP2538 | hom6_D219L_HDRR_v2 | GAGATTCAACTTCCACACCAGATATCCTACCAACAATGGT AACTTTTCTAGCAACCAAC |
| oLP2541 | HOM6-XhoI-F | AAAGCACTCGAGACCCTACTTC |
| oLP2542 | HOM6-SacI-R | CTGCTTCGAGCTCTTCATTAG |
| oLP2547* | BUD23F-QPCR | GACCATGTGTGGTGTGGTTT |

| | | |
|---|---|---|
| oLP2548* | BUD23R-QPCR | CCCATATCCTGCAACATCAA |
| oLP2549* | 25SF-QPCR | GAATCCATATCCAGGTTCCG |
| oLP2550* | 25SR-QPCR | GACGTGGGTTAGTCGATCCT |
| oLP2551 | FOB1KO-F | GATCGAGGTTTCCAGGAAGAG |
| oLP2552 | FOB1KO-R | CGGGCAAGATCATATTATCCAAGTTG |
| oLP2559 | HOM6-intseqR | GATAGAGTACCAGAGAAGATACC |

* Jack, C. V. et al. Regulation of ribosomal DNA amplification by the TOR pathway. *Proc Natl Acad Sci U S A* 112, 9674-9679 (2015).