

Supplementary Information for

Interspecies analysis of MYC targets identifies tRNA synthetases as mediators of growth and survival in MYC-overexpressing cells

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Supplemental Materials and Methods

qPCR primers

alpha-tubulin, fwd: CAACCAGATGGTCAAGTGCG
alpha-tubulin, rev: ACGTCCTTGGGCACAACATC

GAPDH, fwd: CCAATGTCTCCGTTGTGGA
GAPDH, rev: TCGGTGTAGCCCAGGATT

white, fwd: AGGCAGCAAACACCCATCTG
white, rev: CCCGAAGCCCTGGTTAATG

surf6, fwd: GAGATCGTGAAATGTCTGCGG
surf6, rev: GGGCACTTTGTGAATGGTCAG

CG7993, fwd: CAGGAAACCAAAAACCCGCAA
CG7993, rev: TCCGTCCAAAACAGCATTGT

CG14230, fwd: TTGCACGGCGAAAAGCTAAGA
CG14230, rev: TCATCCTCTCCGAATACCCTG

CG8368, fwd: GAAAATCCGCAATTACGATGCTG
CG8368, rev: GCCGACAACCTGATTGTA CTGAT

CG12050, fwd: CGATGGAAGATTCATGTTTCGTGC
CG12050, rev: GGACTCGGGTAAGTTCGCC

Fib, fwd: CTCCGTTGAGACCAATGGC
Fib, rev: ACATGCGAGACTGTCGTTCC

Nop56, fwd: TCGAACCTGTATGTGCTGTACG
Nop56, rev: GAAAGGTGCAAATCCAGCCAG

CG8858, fwd: TGATCCTCAAATGAACTCGCC
CG8858, rev: GCCTCTACGGGTATTTGCACA

Nopp140, fwd: AGCTCCTGCACTAACAAACGG
Nopp140, rev: CCTTCTTGCCGACAGCCTT

Aats-tyr, fwd: CCATGTCGAAGATTGCCGATT
Aats-tyr, rev: TTGTCCAGATAGGCGTGGAGA

eIF3-S9, fwd: GAAGCTGAAGTTGGTCATCAACA
eIF3-S9, rev: TCTGGCCTGCTTGTACTCCA

CG9791, fwd: ATCAGCCTAACGGGCCTTTTG
CG9791, rev: GACAAGTTCGTCTCCGGTTTT

Rrp45, fwd: AGTGACCTGCGAAATGGGAC
Rrp45, rev: ACACCGCCAAGATATACGTTCA

CG1463, fwd: TGTCCCAAATGCGAGGTGAAA
CG1463, rev: CTCAGCGGTACGAACTTGGT

sesB, fwd: GCCAACGTCATCAGATACTTCC
sesB, rev: AAAGCACAGAGAGGTAGCACC

Nmt, fwd: GGCTTCAGTGTCTGCGAATG
Nmt, rev: TGTGGACCAAATGCAAACCTCT

CG10426, fwd: ATACGATGTCAGTTTCGCCAAG
CG10426, rev: GCTTTTGAGGGTGCTCCCATT

path, fwd: GGGACAATCCGCATCCAACAA
path, rev: GGAGCACATGAAGGCAAATGG

mRpl49, fwd: ATCCCGCGAAGTTCAGGAG
mRpl49, rev: CCGGATGGATATTTTGGCTTCTC

Rab39, fwd: CGCCGAGTTATCCGATCCC
Rab39, rev: TTCCGGTAGTAGGACTTGGTG

CG6767, fwd: TCCACTCCAGAATGCCGAAC
CG6767, rev: GACTATCCGCTGGGCCAAAT

ced-6, fwd: TTCAGCTTCATTGCCAAGACG
ced-6, rev: CATCGCCATTTCGTTTGCAT

CG4611, fwd: TTATTCTGCCTGCAACTTGGTC
CG4611, rev: AAACCGGCTTGTTGATCCGT

ps, fwd: ACGCCTGCAAGATGATCCTC
ps, rev: AGCCGGTCGGATTAAAGTTGG

Gfat2, fwd: GCAGCGATCCGATGAGGATAA
Gfat2, rev: GCCGAAAGTG TAGCCAGGA

rhea, fwd: ATGGTGGAAGAGTCAGTGGC
rhea, rev: TCGTTTCGTGTTGCAAGAAG

pyx, fwd: TCGGGGAGAAACCGAGCTA
pyx, rev: CGCAAGGCTCATAAGGAAGAA

CG11523, fwd: CCACTTGCTGCGTTCAGGT
CG11523, rev: CTGTCCCTCGTCTATGGTGTCTG

CapG, fwd: TGGGACACGAGTCCTTTTCG
CapG, rev: GCCATGTTTGC GTTCTCATTG

bcgn, fwd: AGCCCAAGACGCTGGTTTT
bcgn, rev: TCCGCGTAAAGCTCCAAATCC

Vps36, fwd: CTCACCACACACCGACTGTTT
Vps36, rev: GAGGCAGTAGTCTCTTCGCTG

crn, fwd: AATCTCCGCAAGAACCGCAT
crn, rev: GTTCCGGTGCTCATTGTCC

YARS, fwd: ACCTACACAGCTTACGTGGACC
YARS, rev: CCAGCAACTTGTTTCAGTGCAC

AARS, fwd: GTGAAGGTGGATGACAGCAGTG
AARS, rev: CCACTTTCAGGTCACCGTAGATG

DARS, fwd: GCCTGAGGCAGAAGGAGAAGAG
DARS, rev: ATGGCAGATGCCAGACTGGAGA

RARS, fwd: GAAACAGTGCGCCTCATGGATC
RARS, rev: AGCCATACGCAACGGATGTCTG

TARS, fwd: CTTCAACAGCCGACTCTGGATG
TARS, rev: GGCAGTTCTCGCCAGGACCTT

IARS, fwd: CCTCTTTGGACAACCGCCTTTC
IARS, rev: GGCATCAGCACCATACTTCTGG

EPRS, fwd: TTGACCCAGTGGCTCCACGATA
EPRS, rev: CACAGGCTTCAAGCCAACCTCA

Fly Stocks:

Dmef2-Gal4 (BDSC: 25756), *UAS-Myc* (BDSC: 9674 and 9675),

UAS-RNAi (TRiP collection at BDSC): 33644, 51455, 36707, 51820, 25962, 38953, 50651, 26211, 26230, 36728, 54834, 42530, 34889, 31055, 34838, 43298, 31373, 52902, 28592, 65030, 29619, 60121, 38340, 25864, 34594, 34725, 31556, 34615, 32380, 32343, 34975, 26779, 50683, 31402, 35761, 42513, 28775, 29535, 32889, 31334, 31924, 31752, 32976, 31762, 50733, 62316, 34827, 38385, 38941, 31766, 34830, 32920, 28685, 35765, 28296, 31175, 32880, 33373, 42553, 28889, 40934, 26214, 34740, 51849, 42542, 26216, 34037, 41701, 34971, 33685, 27549, 28594, 28931, 31141, 36662, 38535, 25783, 25784, 36123, 33686, 42935, 35484, 33694, 51021, 33382, 52980, 34580, 51892, 40863, 26212, 38290, 35439, 65087, 34349, 33426, 27516, 53311, 38962, 51836, 31297, 53995, 25953, 31769, 42635, 32931, 32999, 33913, 28950, 58202, 34894, 28004, 31230, 36661, 64027, 33996, 43545, 36921, 28513, 34645, 28387, 33915, 33914, 34563, 34016, 28914, 41696, 27489, 34378, 34394, 28326, 41966, 32490, 32901, 26290, 50727, 38286, 42528, 28317, 42606, 55327, 34886, 58176

UAS-RNAi (VDRC collection): 18182, 37726, 5527, 39572, 36504, 9129, 25590, 30465, 25510, 44679, 16003, 8464, 48241, 45385, 46794, 42976, 24813, 30046, 46902, 38793, 32621, 13793, 32810, 20415, 8767, 7433, 16550, 40232, 45840, 29439, 41790, 24709, 26524, 21461, 27352, 38133, 35112, 5229, 22326, 9134, 35325, 28246, 44649, 35507, 6932, 12759, 37336, 13331, 19636, 30485, 31436, 22612, 26651, 25101, 17187, 7518, 27482, 38243, 12548, 47790, 28132,

19097, 9507, 19855, 28215, 28019, 35314, 45582, 23075, 20824, 10675, 25976, 20529, 10461, 37933, 23017, 10614, 19150, 26413, 52457, 37863, 10374, 25140, 21814, 16061, 46667, 21053, 31340, 20336, 35988, 41096, 40541, 22924, 32163, 16846, 11521, 39776, 41928, 23949, 34994

UAS-RNAi (NIG collection): 11188R-2, 30170R-2, 17054R-2, 11804R-1, 10286R-1, 11523R-4, 13890R-1, 14230R-1, 1463R-2, 14641R-1, 15547R-3, 1607R-3, 17018R-1, 2225R-1, 2889R-2, 6572R-3, 32104R-1, 4611R-1, 4646R-4, 6071R-2, 6767R-1, 6928R-1, 7339R-1, 7739R-3 , 8858R-4, 1044R-2, 8357R-2, 11166R-3, 31731R-3, 18374R-1, 5688R-1, 17530R-2, 4647R-1, 7436R-1, 1553R-1, 13849R-3 , 3424R-2, 17725R-2, 2488R-1, 7487R-3, 9606R-1, 4510R-2, 9791R-2 , 10496R-1, 4561R-2, HMJ21228, HMJ21046, HMJ21018

Flies were reared at 27°C and fed with ‘standard’ food composed of 16.5 g/L yeast, 9.5 g/L soy flour, 71 g/L cornmeal, 5.5 g/L agar, 5.5 g/L malt, 7.5% corn syrup, 0.4% propionic acid, and 1.3% Tegosept.

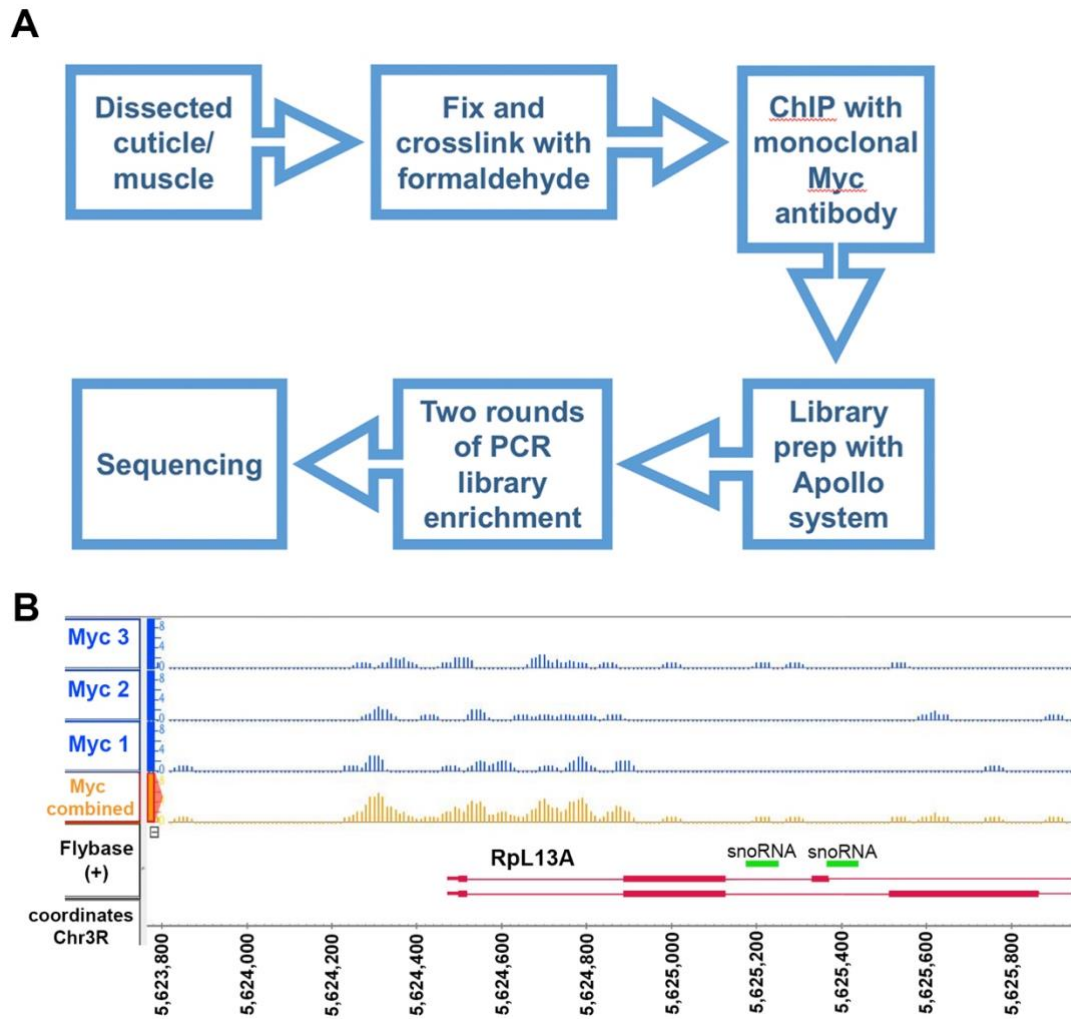


Figure S1. Myc ChIP-seq procedure and peak calling

(A) Outline of Myc Chromatin immunoprecipitation-high throughput sequencing (ChIP-seq) protocol. (B) ChIP-seq tracks for Myc at the *Rpl13A* gene locus. Uniquely mapped, duplication removed reads from 3 replicates (blue) from WT 3rd instar larval muscles were pooled together to generate finalized peaks (orange).

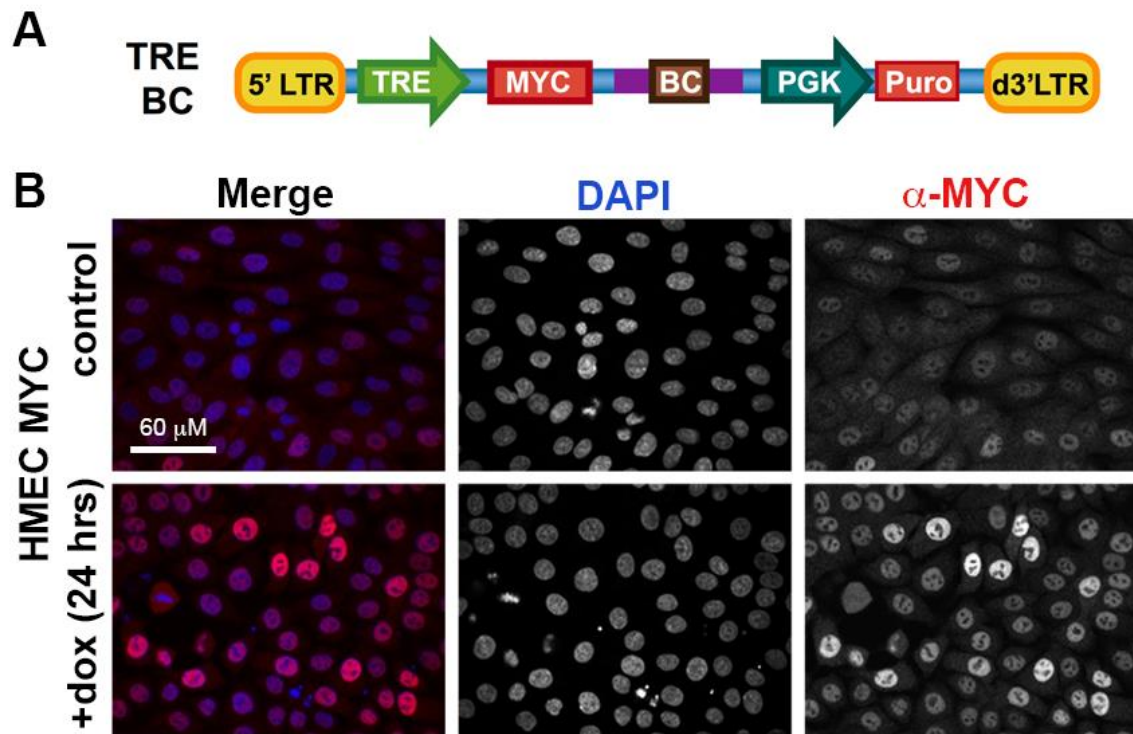


Figure S2. Inducible MYC expression in Human Mammary Epithelial Cells (HMECs)

(A) Schematic of inducible MYC vector stably transduced into HMECs. Cells had previously been transduced with a lentivirus carrying the reverse tetracycline transactivator (rtTA). LTR – Long Terminal Repeat; TRE – tetracycline responsive element promoter; BC – Barcode; PGK – phosphoglycerate kinase 1 promoter; Puro – puromycin resistance. (B) HMEC MYC cells +/- dox (10ng/ml) for 24 hrs. Cells stained with DAPI (blue) and α -MYC (red). Note the increased MYC expression in dox-treated cells compared to untreated control.

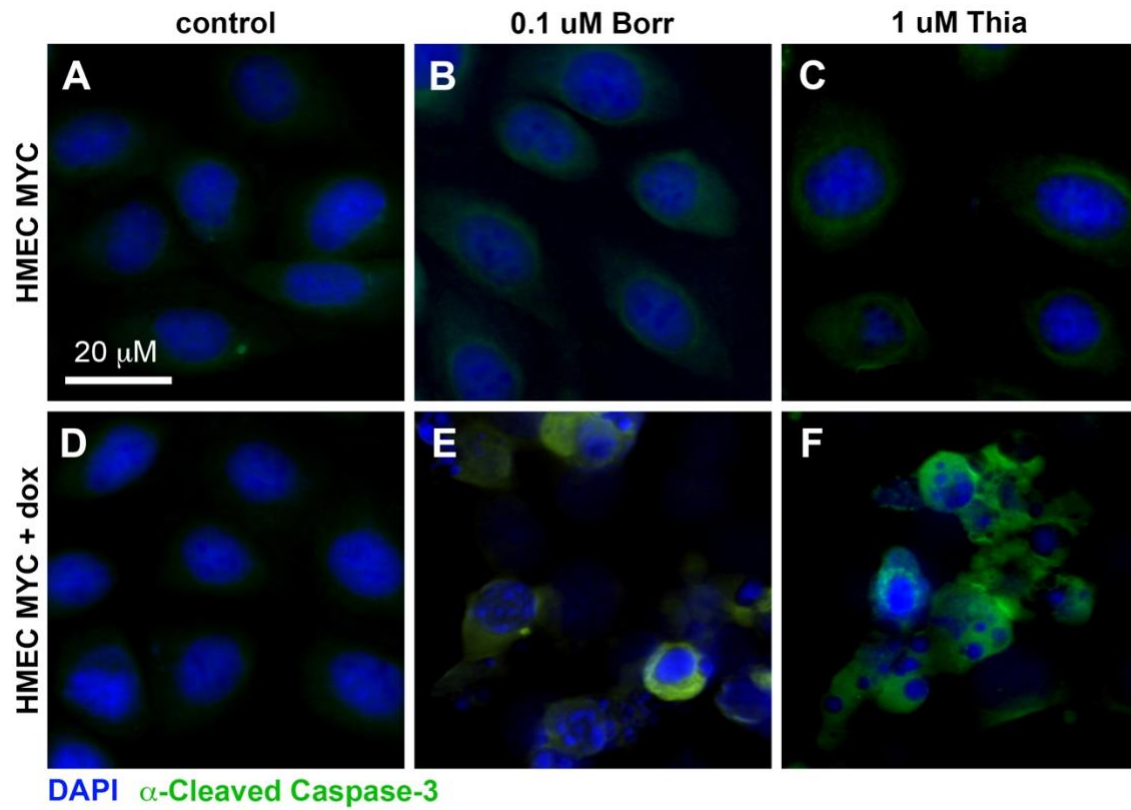


Figure S3. aaRS inhibitors trigger apoptosis in HMEC-MYC cells.

(A-C) Control and drug treated uninduced HMEC-MYC cells are viable. Borrelidin or Thiaisoleucine treatment triggers cell death of dox-induced HMEC-MYC, but not control HMEC-MYC cells. Cells stained with DAPI (blue) and α -Cleaved Caspase-3 (green).

Dataset S1: FPKM from RNA-seq of S2R+ cells 0 to 180 minutes after insulin stimulation (20 min intervals, 3 samples each time point).

Dataset S2: Overlap of insulin temporally differentially expressed genes and nucleolus regulator genes

Dataset S3: Screen of RNAi lines targeting 163 insulin responsive nucleolar regulator genes

Dataset S4: Nucleolar size resulting from gene knockdown in larval muscle.

Dataset S5: Myc ChIP-Seq genes within 5kb and 3kb of peak.

Dataset S6: Overlap of insulin/nucleolus gene set with expression data