

Supplemental Information

Inactivation of *Minar2* in Mice Hyperactivates mTOR Signaling and Results in Obesity

Saran Lotfollahzadeh¹, Chaoshuang Xia², Razie Amraei³, Ning Hua⁴, Konstantin V. Kandror⁵, Stephen R Farmer⁵, Wenyi Wei⁶, Catherine E Costello^{2,5*}, Vipul Chitalia^{1,7,8*}, Nader Rahimi^{3*}

¹Renal Section, Department of Medicine, Boston University Chobanian & Avedisian School of Medicine, Boston, MA, USA. ²Center for Biomedical Mass Spectrometry, Boston University Chobanian & Avedisian School of Medicine, Boston. ³Department of Pathology and Laboratory Medicine, Boston University Chobanian & Avedisian School of Medicine, Boston, MA, USA. ⁴Biomed Research Center, Boston University Chobanian & Avedisian School of Medicine. ⁵Department of Biochemistry, Boston University Chobanian & Avedisian School of Medicine, Boston, MA, USA. ⁶Department of Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, 02215 USA. ⁷Veterans Affairs Boston Healthcare System, Boston MA, USA. ⁸Institute of Medical Engineering and Sciences, Massachusetts Institute of Technology, Cambridge, MA, USA.

Key words: MINAR2, mTOR, mTORC1, RAPTOR, obesity

***Co-corresponding Authors:**

Nader Rahimi

Email: nrahimi@bu.edu

Catherine E Costello

Email: cecmsms@bu.edu

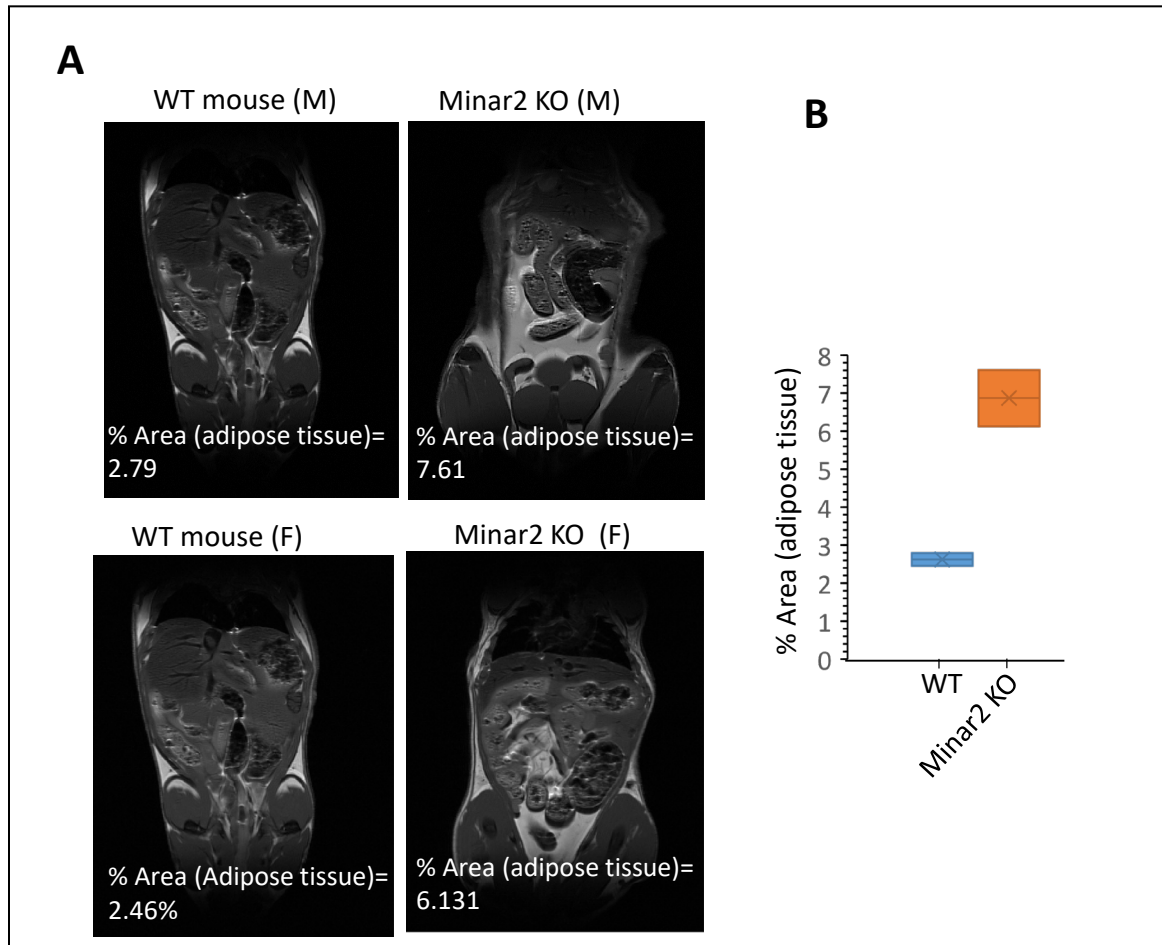
Vipul Chitalia

Email: vichital@bu.edu

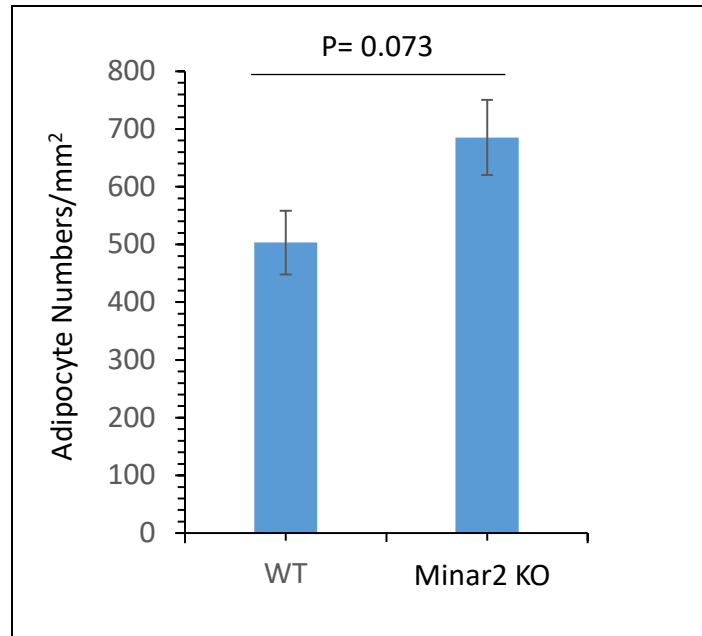
Acknowledgment and Funding:

This work was supported in part through a grant from Boston University School of Medicine Genome Science Institute (to C.E.C. and N.R.), the Center of Cross-Organ Vascular Pathology, DOM, BU (VC) and NIH grants R24 GM134210, S10 OD021728, S10 OD010724 (C.E.C.).

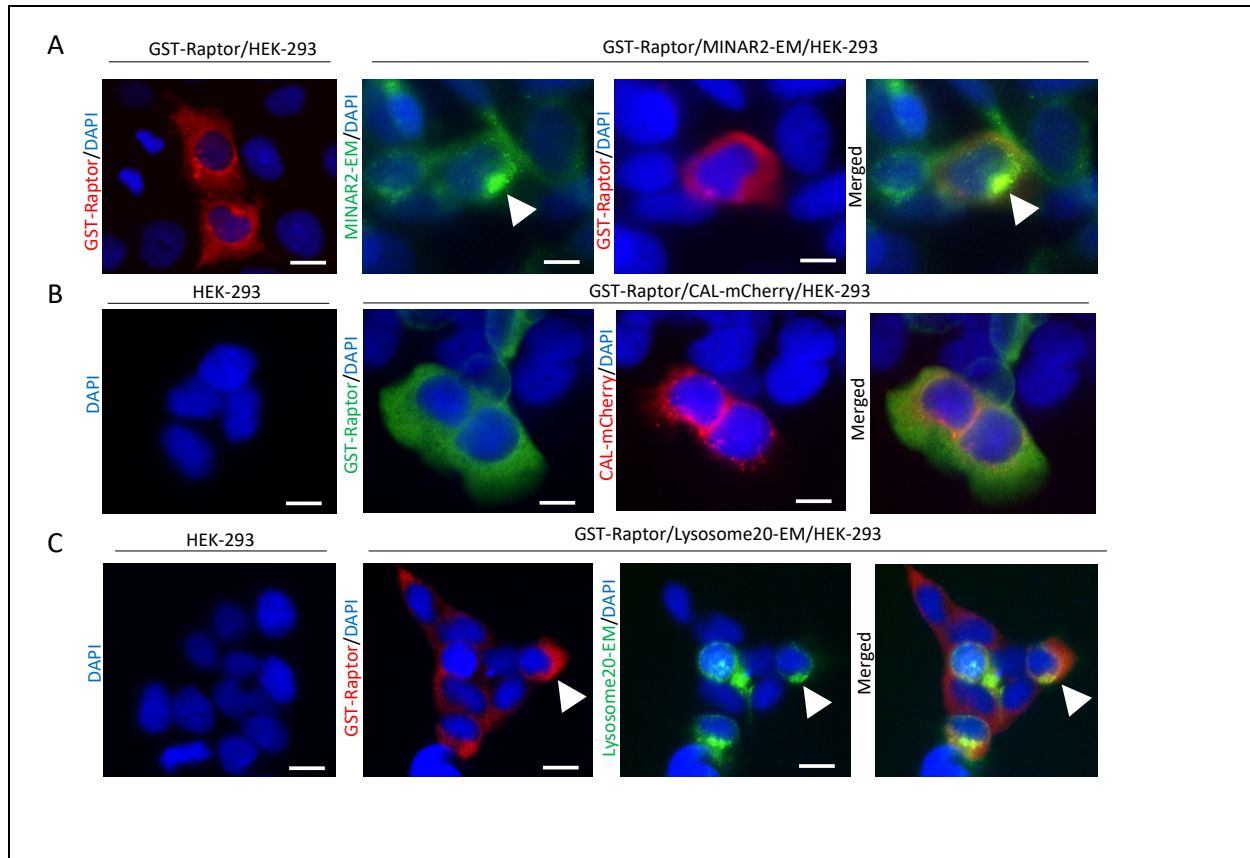
S. Figure 1. MRI analysis shows *Minar2* KO mice have increase fat mass. (A) MRI analysis of *Minar2* KO and sex- and age-matched control WT mice. F, female, M, Male. (B) The graph is the quantification of adipose tissue (6 weeks old, n=4/group, 2 males and 2 females/group) of MRI analysis.



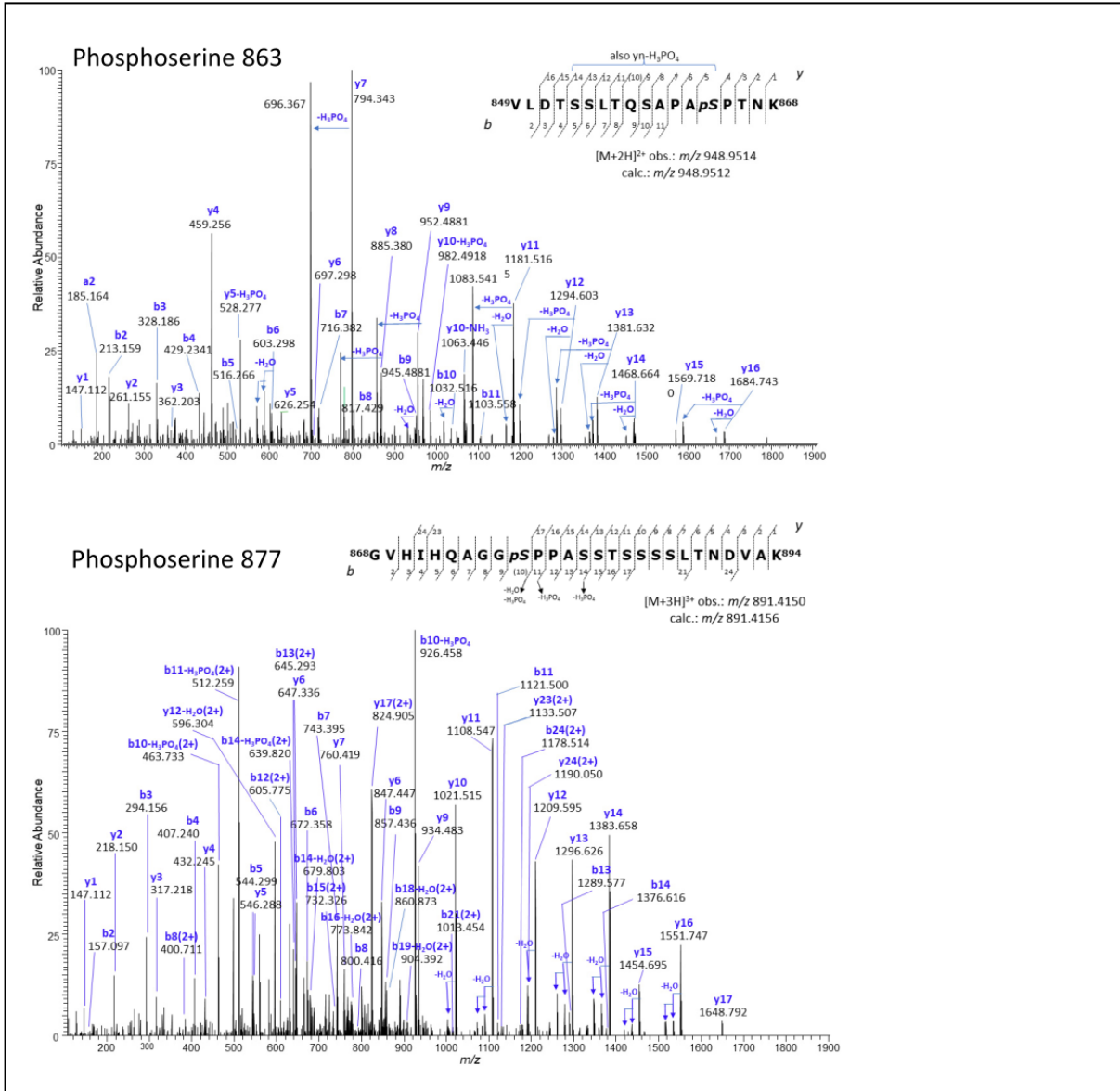
S. Figure 2. Adipocyte cell numbers are not altered in Minar2 KO mice. The number of adipocytes from H&E slides were calculated by Image J software. The graph is representative of three randomly but non-overlapping areas per slide, mean \pm SEM, $n=3$). $P>0.05$ is considered not significant.



S. Figure 3. Raptor co-localizes with Minar2. (A) HEK-293 cells expressing GST-Raptor alone or GST-Raptor with Minar2-emerald (EM) were stained anti-GST antibody. Image magnification 50 μ M. (B) HEK-293 cells or HEK-293 cells expressing GST-Raptor with calreticulin (CAL) mCherry. Image magnification 50 μ M. (C) HEK-293 cells or HEK-293 cells co-expressing GST-Raptor and Lysosome20-emerald (EM). Image magnification 50 μ M.



S. Figure 4. Raptor is phosphorylated on Ser 863 and Ser877. Higher energy collisional dissociation (HCD) tandem mass spectra of Raptor tryptic phosphor-peptides that contain the S863 and S877 phosphorylation sites. NanoUPLC retention times and fragmentation patterns were matched.



S. Figure 5. *Minar2* cDNA and vector sequence information. *Minar2* sequence is highlighted in yellow. Myc tag sequence is underlined. Emerald sequence (795bp) is highlighted in green.

>Minar2-pQCXIP

```
tccgcgttacataacttacggtaaatggcccgcctggctgaccgcccacgacccccgccattgacgtcaataatgacgtatggt
cccatagtaacgccaatagggactttccattgacgtcaatgggtggagtatttacggtaaacctgcccactggcagtagcatcaagg
tatcatatgccaagtacgccccctattgacgtcaatgacggtaaatggcccgcctggcattatgcccagtagcatgaccttatggga
ctttcctacttggcagtagcatctacgtatttagtcatcgctattaccatgggtgatgagggttttggcagtagcatcaatgggcgtggat
agcgggttgactcacgggattttccaagtctccacccattgacgtcaatgggagtttggtttggcaccaaaatcaacgggacttt
ccaaaatgtcgtaacaaactccgccccattgacgcaaatggggcgttagcgtgtacggggaggtctatataagcagagctcaata
aaagagcccacaaccctcactcggcgccagcttccgatagactgctgcgccgggtaccggtattcccaataaagcctcttg
ctgtttgcatccgaatcgtgggtctcgtgttccctgggaggggtctcctctgagtgattgactaccacgacgggggtctttcattt
gggggctcgtccggattttggagaccctcaccaggaccaccgcccaccgggaggttaagctggccagcaacttatctgtgtg
ctgtccgatgttctgattgtttagtattgctttagcctcgtctgtagttagttagtaactagctgtatctggcggaccgg
tgggtggaactgacgagttctgaacaccggccgcaaccctgggagacgtcccagggactttgggggcccgtttttgtggccgacct
gaggaagggagtgatgtggaatccgaccccgtcaggatattgtggttctggtaggagacgagaacctaaaacagttcccgccctccg
tctgaatttttgccttcgggtttggaaccgaagccgcgctctgtctgctgcagcgtgcagcatcgttctgtgtgtctctgtct
gtgtttctgtatattgtctgaaaattagggccagactgttaccactcccttaagtttgaccttaggtcactggaaagatgtcagcgcg
atcgtcacaaaccagtcggtagatgtcaagaagacagcttgggttacctctgtctcgcagaatggccaacctttaacgtcggatg
ccgcgagacggcaccctttaaccgagacctcaccacaggttaagatcaaggtctttcacctggcccgcagtaggacccagaccg
gtccccacatcgtgacctgggaagccttggcctttgacccccctcctgggtcaagccctttgtacacccctaagcctccgcctcc
tcttccctccatccgccccgtctctcccccttgaacctcctcgttcgacccccgcctcgatcctccctttatccagccctcactcctt
ctctaggcgcgggaattgaagatctgggggatcgatcctctagagtcogttacataacttacggtaaatggcccgcctggctgacc
gcccacgacccccgcccattgacgtcaataatgacgtatgttcccatagtaacgccaatagggactttccattgacgtcaatggg
tggagtatttacggtaaacctgcccacttggcagtagcatcaagtgatcatatgccaagtacgccccctattgacgtcaatgacggg
aaatggcccgcctggcattatgcccagtagcatgacctatgggactttcctacttggcagtagcatctacgtatttagtcatcgtat
taccatgggtgatgagggttttggcagtagcatcaatgggcgtgaaatagcgggtttagctcacggggatttccaagctccacccccattg
acgtcaatgggagtttgttttggcaccaaaatcaacgggactttccaaaatgtcgttaacaactccgccccattgacgcaaatgggc
ggtaggcgtgtacgtgggaggtctatataagcagagctcgttttagtgaaccgtcagatcgccctggagacgccatccacgctgtttt
gacctccatagaagacaccgggaccgatccagcctccgcccggcgggaacgggtgcatggaaacgtgcaggaattgatccgcccggccg
cATGGATCTCTCTGTTTGGCAAATAACAACCATCTCGACAAATTCCTGCAGCTTGACGTAAGTCTTTAACGAGGAGCTCAGCCC
TCCTTCAGGCCAGCCTGGTGAGGTTCCGGGTGGAATATCTGCTGCACAACACTGGCAAAACCTTGTCTACTCACAGAGGGAA
AAGAAGAATATTGCTGCTCAACGAATTAGGGGATCCAGTGCAGACAGCCTTGTCACTGCTGATAGCCCCCACCATCCATGTCATC
AGTTATGAAGAATAACCCACTCTATGGTGACCTAAGTTTGGAGGAAGCTATGGAAGAAAGAAAAAGAACCCTCATGGACCATTG
AGGAATATGACAAACATTCCCTGCACACAAACCTCTCTGGACATCTGAAGGAAAACTCTAATGACCTGCGGTTTGGTTGGGAGAC
ATGTACTCTCCAGGTTTGGACACTTTATTGAAAAAGGAAGAGAAAACAAGAGAAGCATTCAAAATTCCTGCTGATGGGTCTGATTTT
ACTTGTGCTTATCTCCATCTTGGTTACCATAGTGACTATCATTACTTTTTTACCAGGATCCAGGGGAACAAAACTCATCTCAGAA
GAGGATCTGTGAAAATTTTCgaattccgcccctctcctccccccccctaactgtaactggccgaagccgcttggaaataagccggtg
tgcgtttgtctatattgttattttccaccatattgcccgtcttttggcaattgtgagggcccggaacctggccctgtcttcttgacga
gcattccttaggggtcttccccctcgcgcaaggaatgcaaggtctgttgaattgtcgtgaaggaagcaggttctctggaagctctct
tgaagacaaaacacgtctgtagcgcacctttgcaagcagcggccccccacctggcgcaggtgctctgcggccaaaagccacg
tgtataagatcacacctgcaaagggcggcacaaccccagtgccacgttgtgagttggatagttgtggaagagtcataatggctctcct
caagcgtattcaacaaggggctgaaggtgcccagaaggtaccccattgtatgggatctgatctgggcctcgtgacatgcttt
acatgtgttttagtcgaggttaaaaaacgtctagggccccccgaaccacggggacgtggttttcccttgaaaaaacagatgataagct
tgccacaaccacaaaggagacgaccttccatgaccgagtagcaagccccaggtgctcctcgcaccgccgacgagctccccggggcc
gtacgcaacctcgcgcgcgcttgcggactaccccgcacgcgccaacccgtcgaccggaccgccaacatcgagcgggtcaccga
gctgcaagaactcttctcaccgcgctcgggctcgacatcggcaaggtgtgggtcgcggagcagcggcgcgctggtgggggacca
cgccggagagcgtcgaagcggggcggtgttgcggagatcggcccgcgcatggccgagttgagcgggtccccggtggcgcgcgag
caacagatggaagggcctcctggcgcgcgaccggcccaaggagcccgcgtggttctggccaccgtcggcgtctcgcggcaaccag
gcaagggctcgggcagcgcgctcgtcctccccgagtgaggcggccgagcgcgcggggtgcccgccttccctggagacctccgcg
ccccgcaacctccccctctacgagcggctcggcttaccgtcaccgcgcagctcgagtgcccgaaggaccgcgcgacctgtgcatg
accgcaagcccgggtcctgacgcccgcacacgacccgcagcggcccgaaccgaaaggagcgcacgacccccatggctccgacccaag
ccgacccggcgccccggcagccccgcacccgccccggagcccaccgactctagtcgagggctgcagcggctgcagagggccagtagggcagt
gcgaactgctccaaaggcactcaaggctttccgagagcactagctgactccatcgagccagtgtagagataagcttatcgat
tagtccaatttgtttaaagacaggatatacgtggtccaggctctagttttagctcaacaatataccagctgaagcctatagagtag
gagccatagataaaaaaaagattttatatttagtctccagaaaaaggggggaatgaaagaccccacctgtaggtttggcaagctagc
ttaagtaacgccattttgcaaggcatggaaaaatacataactgagaatagagaagttcagatcaaggtcaggaacagatggaacag
ggtcgacctagagaaccatcagatgtttccagggtgcccgaaggacctgaaatgacctgtgccttatttgaactaaccaatcag
ttcgtctctcgtctctgttgcgcgcttctgctccccgagctcaataaaagagcccacaacccctcactcggggcgccagctctcc
gattgactgagtcgccccgggtaccctgtatgttccaataaacctcttgcagttgcatcogacttgtggtctcgtctccttgggag
ggtctcctctgagtgattgactaccctcagcgggggtctttcatttgggggctcgtccgggatccgggagaccctgccaggggac
caccgacccaccaccgggaggttaagctggctgcctcgcgcttccggtgatgacggtgaaaacctctgacacatgcagctccccga
```


ATGACCGACCAAGCGACGCCAACCTGCCATCACGAGATTTTCGATTCACCCGCGCCTTCTATGAAAAGTTGGGCTTCGGAATCGT
TTTCCGGGACGCCGCTGGATGATCCTCCAGCGCGGGGATCTCATGCTGGAGTTCTTCGCCACCCCGGGCTCGATCCCTCGCGA
GTTGGTTCAGCTGCTGCTGAGGCTGGACGACCTCGCGGAGTTTACCAGGAGTGCAAAATCCGTCGGCATCCAGGAAACCAGCAGC
GGCTATCCGCGCATCCATGCCCCGAACCTGCAGGAGTGGGGAGGCACGATGGCCGCTTTGGTTCGAGGCGGATCCGGCCATTAGCCA
TATTATTCATTGGTTATATAGCATAAATCAATATTGGCTATTGGCCATTGCATACGTTGTATCCATATCATAAATATGTACATTTAT
ATTGGCTCATGTCCAACATTACCGCCATGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCA
TAGCCATATATGGAGTTCGCGTTACATAACTTACGGTAAATGGCCGCTGGCTGACCGCCCAACGACCCCGCCATTGACGT
CAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCAC
TTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCA
GTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTCGTATTAGTCATCGCTATTACCATGGTGTAGCGGTTTTGGCAGT
ACATCAATGGGCGTGGATAGCGGTTGACTCAGCGGGATTTCAGTAAGTCTCCACCCATTGACGTCAATGGGAGTTGTTTTGGCAG
CAAAATCAACGGGACTTTCCAAAATGTCGTAACAACCTCGCCCATTTGACGCAAAATGGGCGGTAGGCACTGTACGTTGGAGGTTCTA
TATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACCCGGGACG
ATCCAGCTCCGCGGCCCGTTAACGCGGCCG**ATGGATCTCTCTGTTTTGCCAAATAACAACCATCCTGACAAATTCCTGCAGT**
TGACGTAAAGTCTTTAACGAGGAGCTCAGCCCTCCTCAGGCCAGCTGGTGGGTTTCCGGGTGAAAATTATCCTGCTGCACAAAC
ACTGGCAAAACCTTGCTACTCACAGAGGGAAAAGAATATTGCTGCTCAACGAATTAGGGGATCCAGTGCAGACAGCCTTGTC
ACTGCTGATAGCCCCCACCATCCATGTATCAGTTATGAAGAATAACCCACTCTATGGTACCTAAGTTTGGAGGAAGCTATGGA
AGAAAGAAAAAGAACCCCTCATGGACCATTTGAGGAATATGACAAACATTTCCCTGCACACAAACCTCTCTGGACATCTGAAGGAAA
ATCCTAATGACCTGCGGTTTTGGTTGGGAGACATGTACACTCCAGGTTTTGACACTTTATTGAAAAAGGAAAGAGAAAACAAGAGAAG
CATTCAAAATTCCTGTCGTATGGGCTGATTTTACTTGTGCTTATCTCCATCTGGTTACCATAGTACTATCATTACTTTTTTTCAC
CAAGCTTGCATCCGGCGGTAGCGCGGTGGCAGCGGACCGGTGCCACCATGGTGAGCAAGGGCGAGGAGCTGTTACCCGGGGTGG
TGCCATCCTGGTTCGAGCTGGACGGCGACGTAACCGGCCACAAGTTACAGCTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGC
AAGCTGACCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCACCCTCGTGACCACCTTGACCTACGGCGTGCA
GTGCTTCGCCCGCTACCCGACCACATGAAGCAGCAGCACTTCTCAAGTCCGCCATGCCCGAAGGCTACCCGAGGCGCACCA
TCTTCTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCTTGGTGAACCCGATCGAGCTGAAG
GGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACACAACAGCCACAAGGTCTATATCACCGCCGA
CAAGCAGAAGAACGGCATCAAGGTGAACCTCAAGACCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCCTACCAGC
AGAACACCCCATCGCGACGGCCCGTGTGCTGCTGCCGACAACCCTACCTGAGCACCCAGTCCAAGCTGAGCAAAGACCCCAAC
GAGAAGCGCGATCACATGGTCTGCTGGAGTTCGTGACCGCCCGCGGGATCCTCTCGGCATGGACGAGCTGTACAAGTAAGCGGC
CGCGACTCTAGATCATAATCAGCCATTGA**GTTCGACAAAATAAAAGATTTTATTTAGTCTCCAGAAAAAGGGGGGAATGAAAGACC**
ACCTGTAGTTTGGCAAGCTAGCTTAAGTAACGCCATTTTGCAGGCATGGAAAAATACATAACTGAGAATGAGAAGTTCCAGATC
AAGTTCAGGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCTGCCCCGGCTCAGGGCCAAGAA
CAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCC
CAGATGCGGTCCAGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCTGTGCCCT
ATTTGAACATAACCAATCAGTTTCGCTTCTCGCTTCTGTTTCGCGCGTCTGCTCCCCGAGCTCAATAAAAGAGCCACAACCCCTCA
CTCGGGGCGCCAGTCTCCGATTGACTGAGTTCGCCCCGGTACCCTGTATCCAATAAACCCCTCTTGACAGTTGCATCCGACTTGTGG
TCTCGCTGTTTCTGGGAGGCTCCTCTGAGTGATTGACTACCCGTACGCGGGGCTTTTCAATTTGGGGGCTCGTCCGGGATCGG
GAGACCCCTGCCAGGACCCGACCCACCCACCCGAGGAGGTAAGCTGGCTGCCCTGCGCGTTTTCCGTTGATGACGTTACAAAACCTCT
GACACATGCAGCTCCCGGAGACGGTACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCGCTCAGGGCGCGTACGCGGGT
GTTGGCGGGTGTGGGGGCGAGCCATGACCCAGTACAGTACGATAGCGGAGTGTATACTGGCTTAACATGCGGCATCAGAGAGA
TTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCTCTTCCGCTTCG
CTACTGACTCGCTCGCTCGGTCGTTCCGGTGCAGCGAGCGGATCAGCTCAAAAGGCGGTAATACGGTTATCCACAGAATC
AGGGGATAACGCAGGAAAGAATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTGTGCTGGCGTTTTCCA
TAGGCTCCGCCCCCTGACGAGCATCAAAAAATCGAGCTCAAGTACAGAGTGGCGAAACCCGACAGGACTATAAAGATACAGG
CGTTTTCCCTGGAAGCTCCTCTGCGCTCTCCTGTTCCGACCTGCTCCGCTTACCAGTACCTGCTCCGCTTCCCTTCGGTA
AGCGTGGCGTTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTTCCGCTCCAAGCTGGGCTGTGTGCACGAACC
CCCCGTTACGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGAAGACACGACTTATCGCCACTGGCAG
CAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGTACAGAGTCTTGAAGTGGTGGCCTAATACGGCTACACT
AGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAC
CACCGCTGGTAGCGGTGGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTT
CTACGGGCTGACGCTCAGTGGAAACGAAAACCTCAGTCAAGGATTTTGGTTCATGAGATATCAAAAAGGATCTTACCTAGATC
CTTTTAAATTAATAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCCTAATCAGTGA
GGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCCATCAGTGTGCTGACTCCCGCTCGTGTAGATAACTACGATACGGGAGGGCT
TACCATCTGGCCCCAGTGTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACAGCCAGCCGAA
GGGCCGAGCGCAGAAGTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTTGTTGCCGGGAAGCTAGAGTAAGTAGTCCG
CAGTTAATAGTTTGGCAACGTTGTTGCCATTGCTGCAGGCATCGTGGTGTACGCTCGTTCGTTTGGTATGGCTTCAATCAGCTCC
GTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCCGATCGTTGCTAG
AGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAAATTCCTTACTGTATGCCATCCGTAAGATGCTTTTCT
GTGACTGGTGTAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGGCCGGCGTCAACACGGGATAA
TACCGCGCCACATAGCAGAACTTTAAAAGTGTCTCATCATTGAAAAAGCTTCTTCGGGGCGAAAACCTCAAGGATCTTACCGCTGT
TGAGATCCAGTTTCGATGTAACCCACTCGTGCACCCAACTGATCTTACGATCTTTTACTTTTACCAGCGTTTTCTGGGTGAGCAAAA
ACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGGCGACACGAAAATGTTGAATACTCATACTCTTCTTTTTTCAATATTATG
AAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAACAATAAGGGTTCCGCGCACAT

TTCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAAATAGGCGTATCAGAGGCCCTT
CGTCTCAAGAATTCATACCAGATCACCGAAAACGTCTCCAAATGTGTCCCCCTCACACTCCCAAATTCGCGGGCTTCTGCCTC
TTAGACCACTCTACCCTATTCCCCACACTCACCGGAGCCAAAGCCGCGGCCCTCCGTTTCTTTGCT