

High Throughput Compound Screening in Neuronal Cells Identifies Statins as Activators of Ataxin 3 Expression

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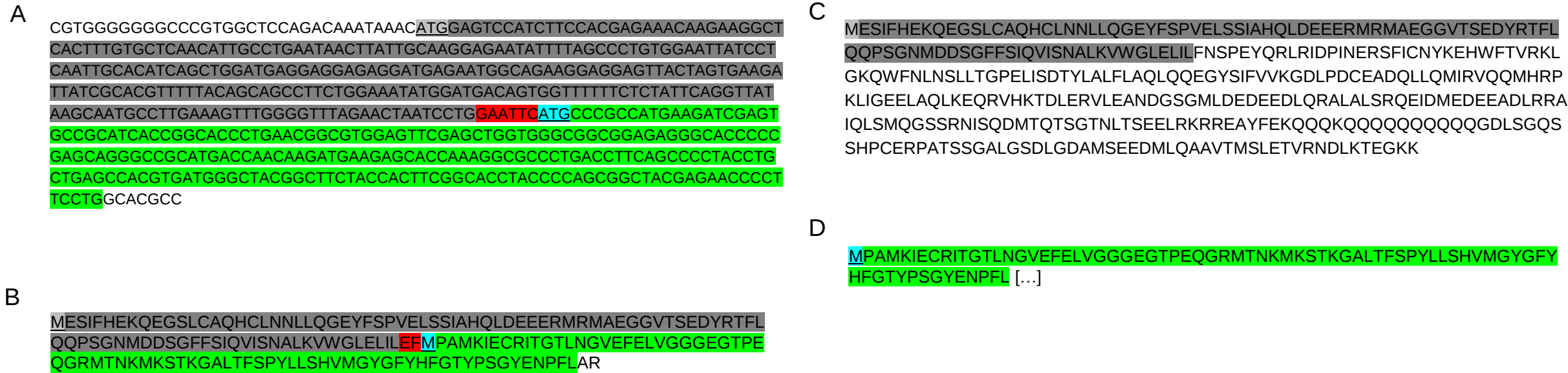
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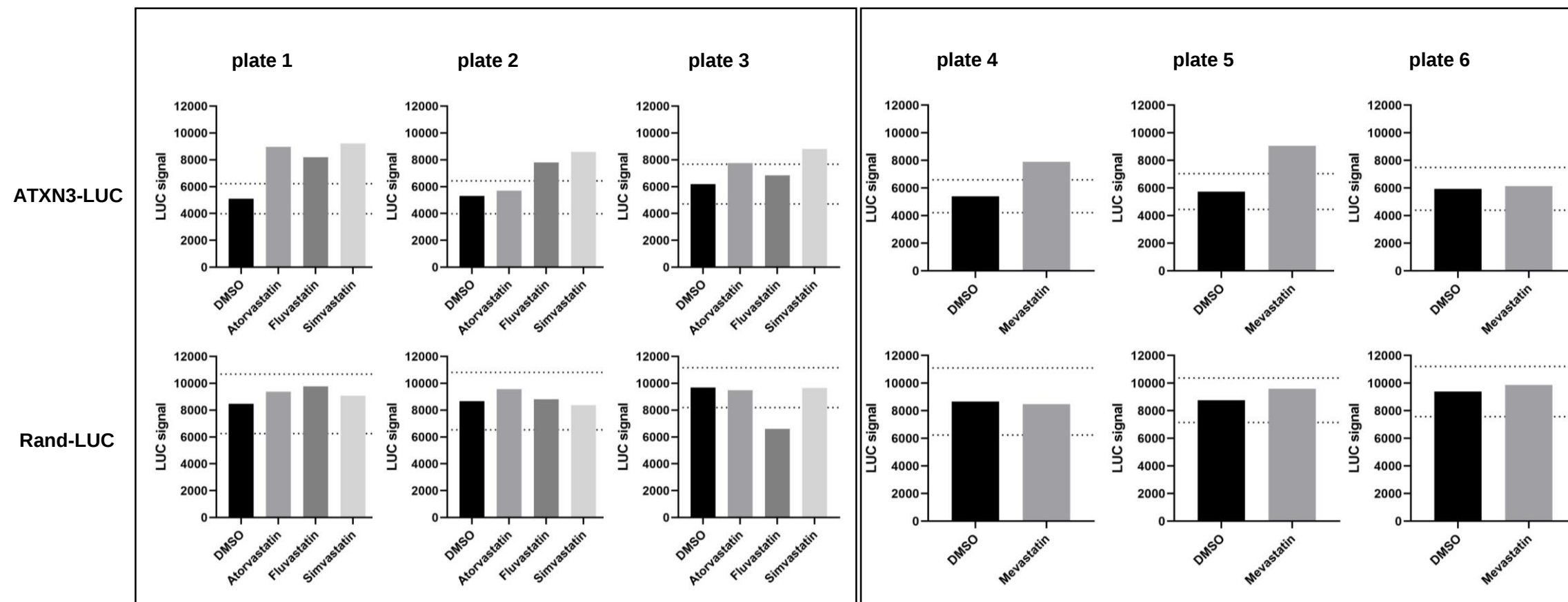
⁴ shared last authorship

Supplementary Figure S1



Supplementary Figure S1. Confirmation of in-frame ATXN3-Exon4-GFP-luciferase (LUC) genomic fusion. A) cDNA sequence of ATXN3-Exon4-GFP-LUC genomic fusion. To confirm the in-frame insertion of GFP-T2A-LUC into ATXN3 Exon4, mRNA of the screening cell line was extracted, transcribed into cDNA and amplified with primers as follows: MJD-27 (upstream start codon ATXN3) CGTGGGGGGCCGTTGGCTCCAGACAAA and HR150 GFP RV (within the GFP cassette) TTCTCGATGCGGGTGTGG. B) *In-silico* translation of the sequenced cDNA (A) via expasy translator (<https://web.expasy.org/translate/>). C) Sequence was compared to uniprot ATXN3 protein sequence (<https://www.uniprot.org/uniprotkb/P54252/entry>) and D) to *in-silico* translated GFP sequence from the HR150 plasmid. Light grey underlined: start codon ATXN3, grey: coding sequence (CDS) ATXN3 Exon1 to half of Exon4; red: EcoRI site; turquoise underlined: start codon GFP cassette; green: GFP sequence (partial).

Supplementary Figure S2



Supplementary Figure S2. Luciferase signal data (LUC signal) from the primary luciferase assay in the screening (ATXN3-LUC) and control cell line (Rand-LUC). Each compound was measured in biological triplicates. To exclude plate specific effects, all compounds were spotted in three independent 384-well plates, respectively. The dotted lines represent the three-fold standard deviation (3x SD) of the median of DMSO controls per plate. Compounds that increased LUC signal above 3x SD were considered as effective. Efficacy 0 = no effect observed; 1 = in 1/3 plates effective; 2 = 2/3 and 3 = 3/3 effective. LUC signals were represented as absolute values. Atorvastatin, Fluvastatin and Simvastatin were spotted on the same plate. DMSO controls per plate n = 58. Summary of efficacy see in Supplementary Figure (S3).

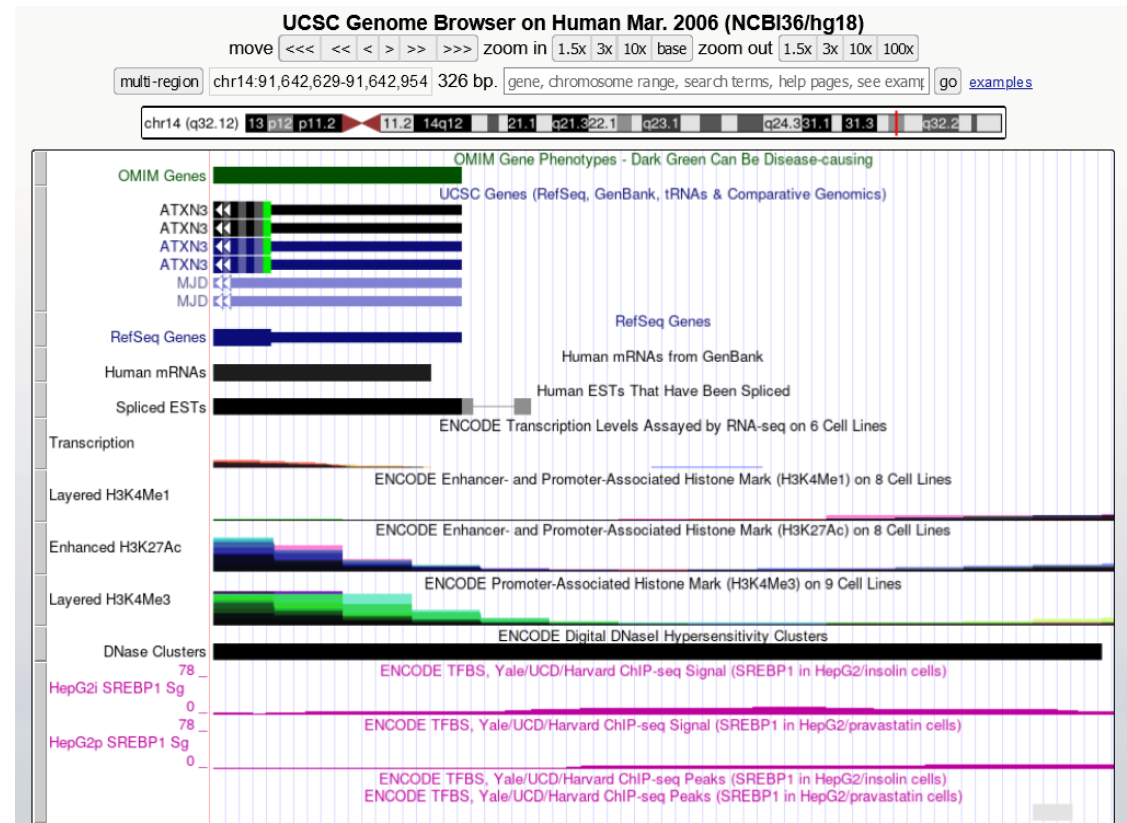
Supplementary Figure S3

Efficacy	Name	Solubility	Trade name
3	Simvastatin	Lipophilic	Zocor
2	Fluvastatin	Lipophilic	Lescol
2	Atorvastatin	Lipophilic	Lipitor
2	Mevastatin	Lipophilic	%
0	Lovastatin	Lipophilic	Mevacor/Altoprev
0	Pitavastatin	Lipophilic	Livalo
0	Pravastatin	Hydrophilic	Pravachol
0	Rosuvastatin	Hydrophilic	Crestor

Supplementary Figure S3. ATXN3-GFP-LUC reporter cell line-based high throughput screening of 2,640 bioactive compounds, including FDA approved drugs identifies statins as modulators of ATXN3 expression. List of Statins included in the screening library and sorted according to their efficacy. Initial screening was performed in three rounds of single treatments normalized to controls of respective plates (Supplementary Figures S2 A, B). Efficacy of 3 represents LUC signal increase above a threshold of the 3xSD from controls in 3/3 experiments. Efficacy 0 = no effect observed.

Supplementary Figure S4

A



B

ENCODE TFBS, Yale/UCD/Harvard ChIP-seq Signal (SREBP1 in HepG2/pravastatin cells).
CTCGTGGAAGATGGACTCCATGTTTATTTGTCTGGAGCCAACGGCCCCACGCCGAAC
CACCCCCTCCAGCTCCGCCCCCTGCCCTCTCCTCCGCCACCCGCCCTCCCCGG
CCCGCCCCGGTACCCGCCACGTGCCGCACGCAGACCAATACCCGTGAGGATGC
GAGTGTCTGTAAGCGAAGAGCGAGTGAACGCCGGGACACGTGACCTTGGTAGCGC
GTCCTCCTAGCTTCTGTTAGCGGGCCTTCTGGGGGATGCTACATCGCCACCTGCTGTC
TGGAGGATCTTCTCCGCTTCCACCATTTCTATGGATTCT

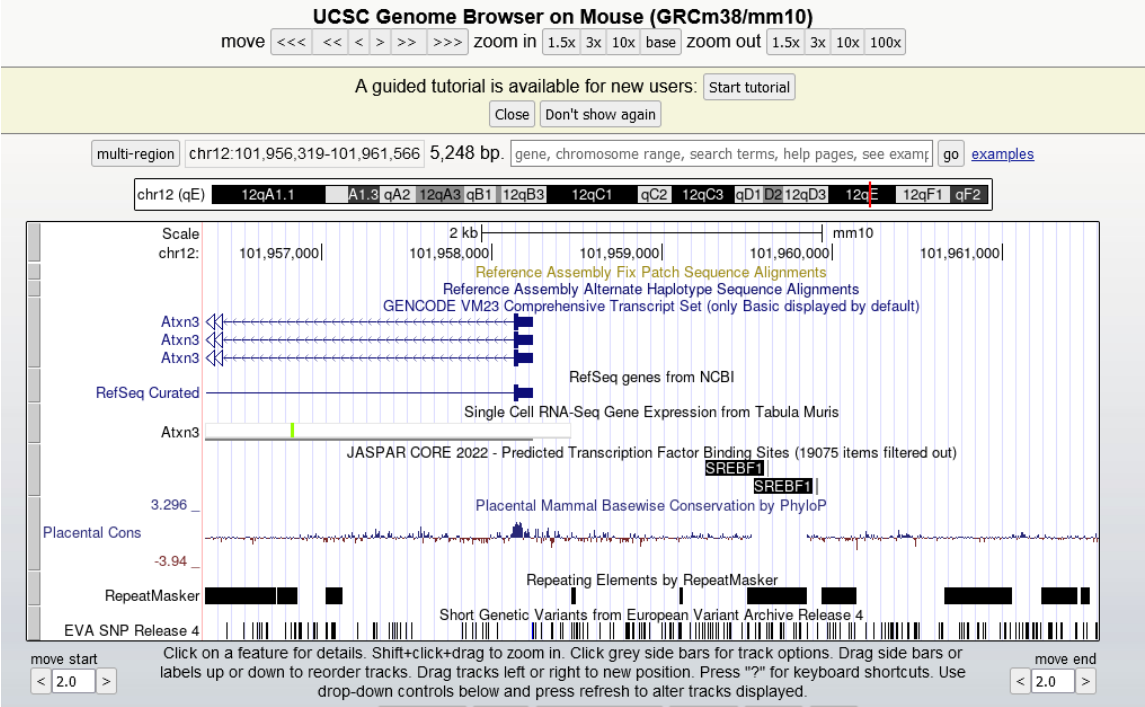
C

ATXN3 Promotor (NCBI: NG_008198.2).

CCTTAACCTCTCCGTGCCTCGGTTTCTCATGTGTATGAATAACATCAACACCTACATCAAAGTTT
GCTGTATTAAATTTGATAATATATGCAAAGCATTAGAAAAGTGCTAGCTCATAGAAAGCCTTAT
GTAAATATTAACATCATTTTTTTCTTTTTTGGGGTGGTGGGGGAGGGGTTTCGCTTTTGTGGC
CAGGCTGGCGTGCAATGGCACGATCTCGGCTCACCACAACCTCGGCCTCCCGGGTTCAAGCGA
TTCTCCTGCCTCAGCCTCCCGAGTACCTGGGATTACAGGCATGCACCACCACGCCCGGGTAATT
TTGTATTTTTAGTGGAGACGGGGTTTCTCCATGTTGGTCAGGCTGATCTCAAACCTCCCGACCTCA
GGTGATCCGCCCCGCTCGGCCTCCCAAAGTGCTGGGATTACAAGCGTGAAGCACCTCGCCCGG
CCTAGCTATCATTTTTATACAAGTGCTGGGTTTGGGAGAATGTAATGATGGCTTTTTTCTTACTAA
ACTTTCAGTGCCAGGAGGAGGAGAAAGAAAGTAAATAGTTATATGAACACAGTAGAAAGTCAAAGT
GGAAAAACAAAAGAACATAGAACCCAGGTGAGCGGTCCAGACCTCCCCCAGAAACCTAAGAAAT
CCATAGAAATGGGTGGGAAGCGGAGAAGATCCTCCAGACAGCAGGTGGCGATGTAGCATCCCC
CAGAAGGCCCGCTAACAGAAGCTAGGAGGACGCGCTACCAAGGTCACGTGTCCCCGGCGTTCA
CTCGCTCTTCGCTTCACGACACTCGCATCCCTACCGGGTGATTGGTCTGCTGCGGCACGTGGGC
GGGGTACCGGGGGCGGGCCGGGGAGGGGGCGGGGTGGGCGGAGGAGAGGGGGCAGGGGGCGGA
GCTGGAGGGGGTGGTTCCGGCGTGGGGGCCGTTGGCTCCAGACAAATAAACATGAGATCCATCT
TCCACGAGAAA

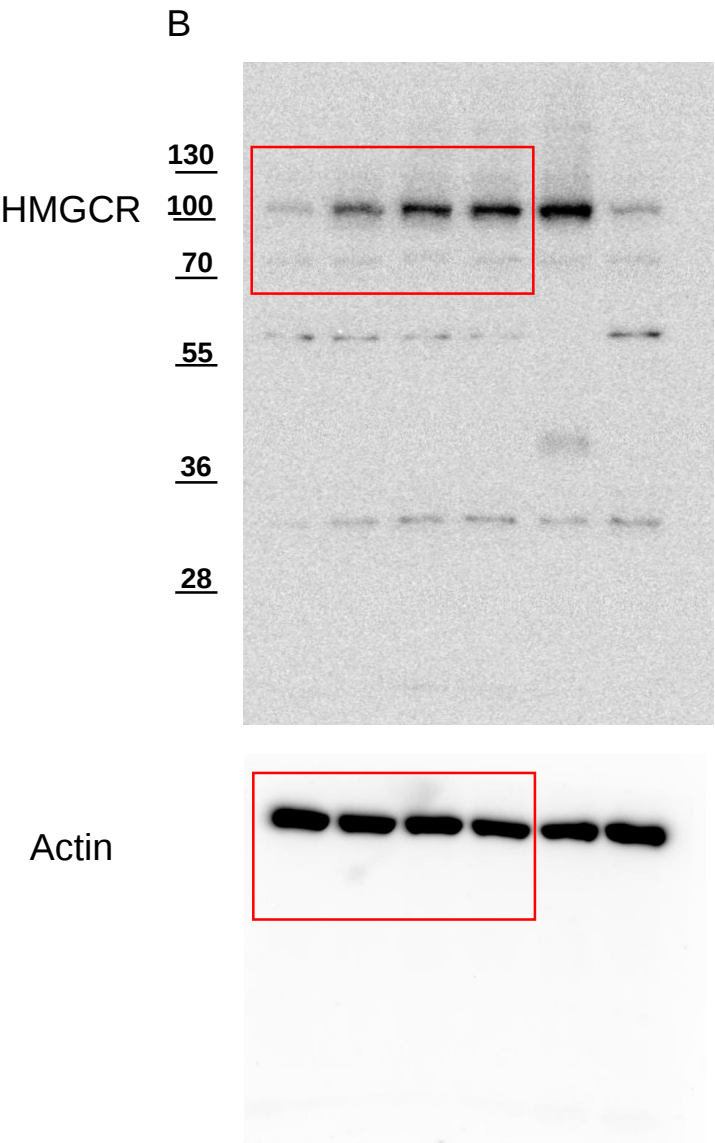
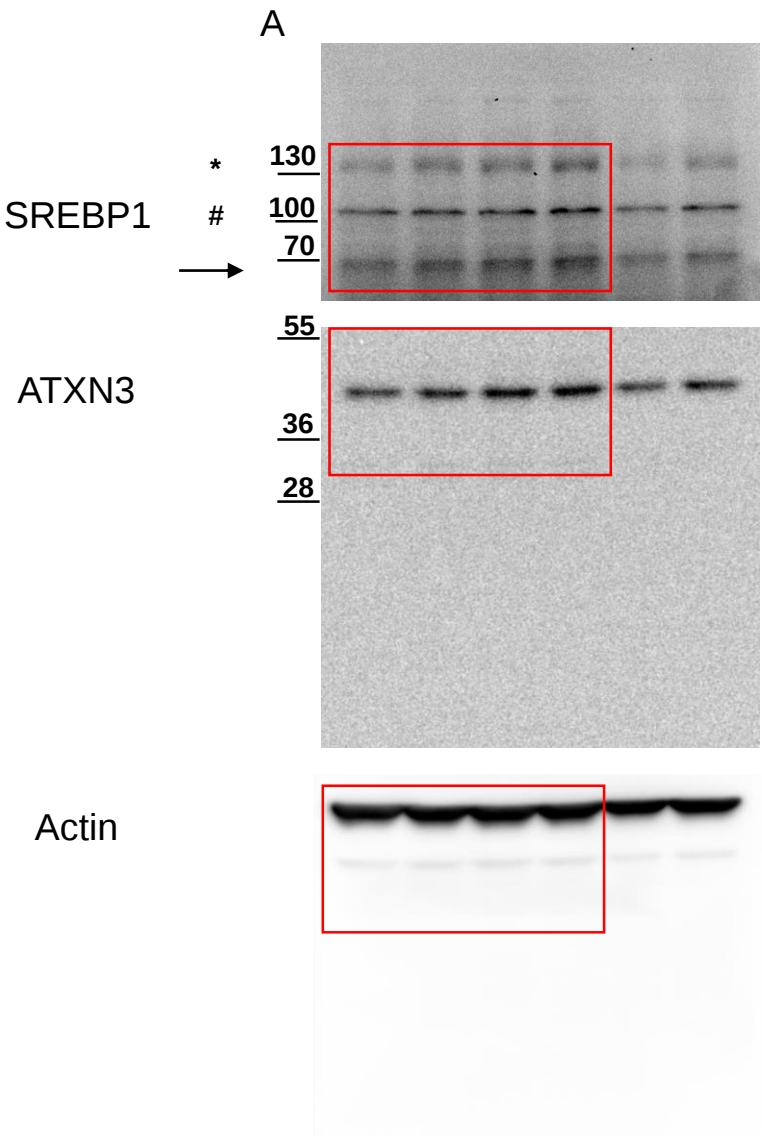
Supplementary Figure 4. Detailed information about retrieving SREBP1 binding motif at the ATXN3 promotor. We used the motifmap database (<https://motifmap.ics.uci.edu/>) to find putative binding of SREBP1 to ATXN3 promotor by using the “gene search” function. We selected the species alignment “Human (hg18 multiz28way_placental)” and searched for ATXN3 gene (NM_001127696). We screened the transcription factor (TF) entry list for the ATXN3 promotor and found SREBP1. Further data were retrieved from the UCSC genome browser via a direct link from motifmap (by choosing SREBP1). A) Screenshot from UCSC genome browser shows SREBP1 Chromatin immunoprecipitation (ChIP)-sequencing (seq) signal data, generated by ENCODE TFBS, Yale/UCD/Harvard, at the ATXN3 promotor (http://genome.ucsc.edu/cgi-bin/hgTracks?db=hg18&lastVirtModeType=default&lastVirtModeExtraState=&virtModeType=default&virtMode=0&nonVirtPosition=&position=chr14%3A91642596%2D91643084&hgid=1662301856_SE1yh97ifny0lfha2aJvwt1dE5a3). B) The DNA sequence of ChIP-seq data were obtained by selecting SREBP1 (http://genome.ucsc.edu/cgi-bin/hgc?hgid=1662301856_SE1yh97ifny0lfha2aJvwt1dE5a3&g=htcGetDna2&table=&i=mixed&l=91642628&r=91642954&getDnaPos=chr14%3A91%2C642%2C62991%2C642%2C954&db=hg18&hgSeq.cdsExon=1&hgSeq.padding5=0&hgSeq.padding3=0&hgSeq.casing=upper&boolshad.hgSeq.maskRepeats=0&hgSeq.repMasking=lower&boolshad.hgSeq.revComp=0&submit=get+DNA). C) Putative ATXN3 promotor sequence. Red: promotor; grey: ATXN3 exon1; light grey: ATXN3 start codon; pink: start and end of ChIP-seq data alignment; yellow: qPCR primer for ChIP-qPCR experiments to amplify a part the ATXN3 promotor region of SREBP1 ChIP-seq data.

Supplementary Figure S5



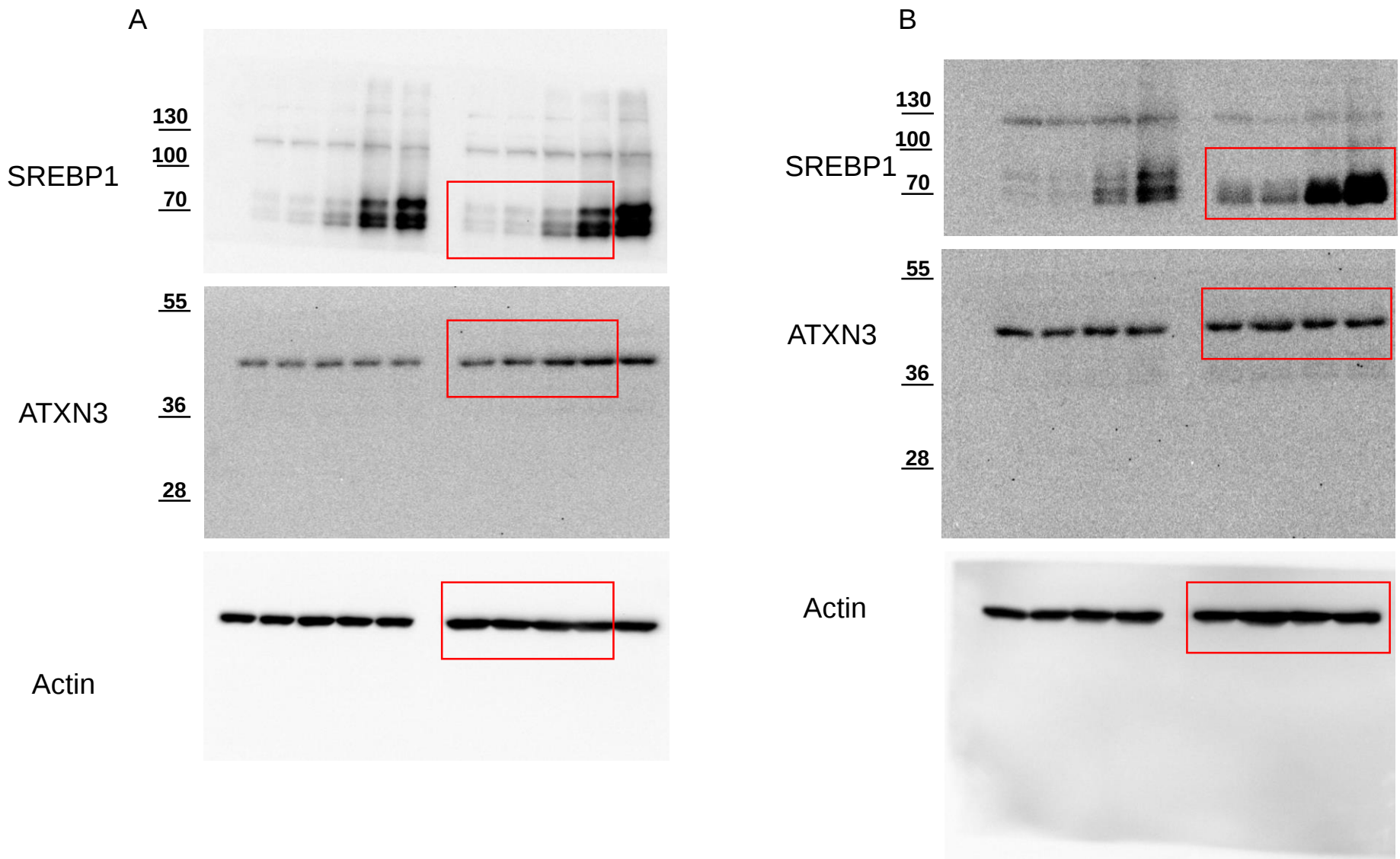
Supplementary Figure S5. Screenshot of the UCSC genome browser on Mouse. Predicted SREBF1 binding sites in murine *ATXN3* promotor by JASPAR CORE 2022 (https://genome.ucsc.edu/cgi-bin/hgTracks?db=mm10&lastVirtModeType=default&lastVirtModeExtraState=&virtModeType=default&virtMode=0&nonVirtPosition=&position=chr12%3A101956319%2D101961566&hgid=1665753116_8Akmet9F5RUbYTMomIMNQa7jijRd).

Supplementary Figure S6



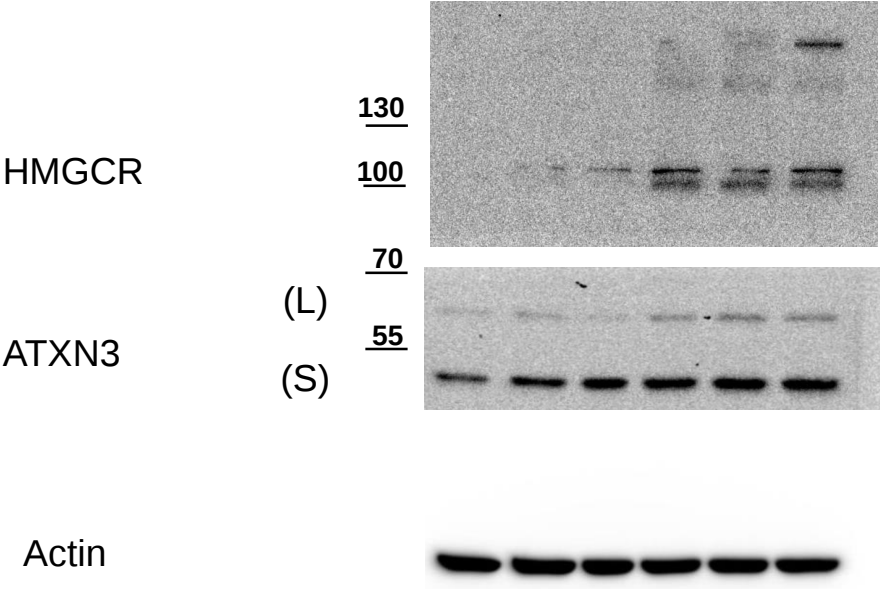
Supplementary Figure S6. Full-size Western Blot of Figure 2 C. A) Membrane was cut at 55 kDa before incubation with the indicated antibodies. Asterix: unprocessed SREBP1, hash: unspecific band, arrow: activated SREBP1 (mSREBP1). B) HMGCR and Actin Western Blot. Membrane was cut at 55 kDa after HMGCR development. The lower part was incubated with Actin antibody. Red boxed lanes were used for Figure 2 C.

Supplementary Figure S7



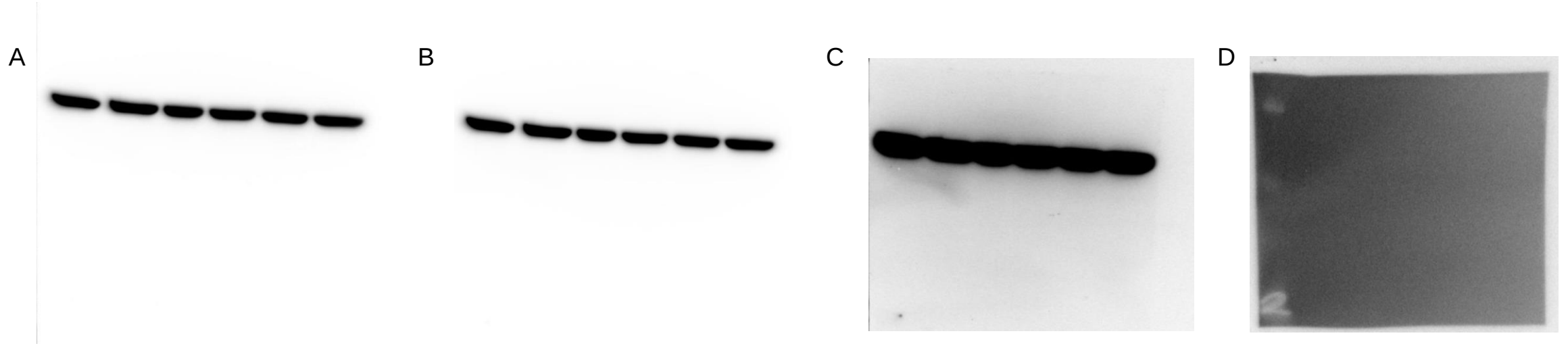
Supplementary Figure S7. Full-size Western Blot of Figure 3. Membranes were cut at 55 kDa to incubate with the indicated antibodies. A) Overexpressed SREBP1a (red boxed lanes) and B) SREBP1c (red boxed lanes) were shown in Fig. 3 A and B, respectively. Red boxed lanes were used for Figure 3.

Supplementary Figure S8



Supplementary Figure S8. Full-size Western Blot of Figure 4. Membrane was cut at ~70 kDa to incubate with the HMGCR, ATXN3 and Actin, respectively. L) Long ATXN3 allele and S) short allele. Several exposure images of Actin Western Blot see Supplementary Figure S9.

Supplementary Figure S9



Supplementary Figure S9. Several exposure images of Actin Western Blot of Fig. 4. and imaged membrane. A) to C) several exposure images of Actin Western Blot of Fig 4. D) Image of the membrane from Actin Western blot of Fig. 4).