SUPPLEMENTAL INFORMATION

Antisense RNA Controls LRP1 Sense Transcript Expression Through Interaction With a Chromatin-Associated Protein, HMGB2

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Figure S1

(Related to Figure 1)

A)

Mouse *Lrp1*-AS

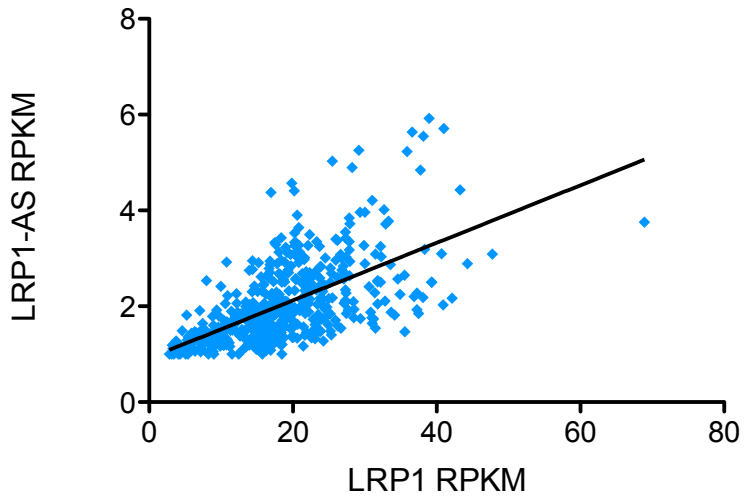
CCTTCATTCTCCAGAGAACTAACTATAGAACAGCAGCCCCTGTGCAG I
CACGAGAGGGGATCTGCACCCAGCCATGCGTGAGTCCAGCCTGTGACA
CCCTTCCTCACGCTTAGACAGCAAAGTTGCCTCGGAAGAGAAGAGAAGC
CTGCATGGGAATGGCCAGCACATCCTAAATGCTCCAGTGGCCCGTGGTT
CGTCCCTTCGTCTCATTGACTGCCACAGACAGGAAGTAGGCTCAGGGAC
TTGGCACCTACCCAACAGCAGGACGTCCTTTCTGGCCATACTCCTGAGG
GTAACAAAATCACATGGAAGCCCAAAGCAAGCCAGGCTCAGGCTCCTCT
GCCCTCTGCTACTTAACAATGTCCGTCCTTCCCCAGCCCCCCTGCAGAT
GCTTCTGTATGGGAAAGCCCCTCTGCATCTAATGACACTCTGCTTTCAAA
GACGGGACAGTCCCTGGTCTCTGGAGAGTGACCATTCGTGGCCTTCTCA
GTTGACACTTCTCCGCTGAGGCATCCCTTAGCCCTGAACCAGAAATGAAA
GAGCCGGCTCAGAGTGAAAAGGAAGAATAGCCATCAATCTGCTCCTGTG
TGCAAGGAGCACAGACCTGGTCTCAGACTCTGCCCGTCTCCCCCGCTTC
CCTGCCCTCTGAGTGACTCACGGTGCAGGCTGAGGGAGATGTTGATGGT
ATGCTCATCCACAAAGCCCTTCAGGCCAGGCATCCGGGCACACTTGAGC
TGTGTCTGGGCAGCACTGTCCCCAACGTGCACCCAGCATACGGTCTCAT
TGGCATAACTGAAGTCCATGGCCGTGGTTTGTCGGGTGCTGGTGGGTGT
GATGGTAGACACTTGGGCCCCACTCAGGTACGTAGCTAGGATGTTCTGA
GAGTTGGCAATCAGTAGCACTGGCGGCCGATCTACTGGCTCTGGGGATG
GGACAGAGAAAGGGCAGTTGGGACTTGTAACCGTTGAGGCGACTATTCA
CTGGGGAGCCGCATTGCAGGTCCCCGGCTCTGTGGTCACTGGCAGACC
CACTCACCATTCTTGGCCTTGCAGGAGCGGTTGTCCGGTTGCAGCAGGT
AGCCTTCAACACAGCCACATGTGAAGGAGCCATCTGTGTTGGTGCAAAG
CTGGCTGCAGGTGCCATACACGGAACACTCGTCAAAATCTGCCCAGAGA
GAAAAGGAAGAGGAGATGTTCATTCTAGTGTAGCACAGGTGCCCCGAGA
CACCAGCAGACAACTGGCGGAATCGATTACAGTTCAGAAGGTGCAAACC
CACTGCCCGGCAAACAGTTTTGGGATTTTCTGGGTCATGCGCACTGACA
GTGATTTGGAAAACATTTCTTGGATAACAGATGGGGCAGGTAGAGAATAC
AATGATGAATTTGAAAA

Human *LRP1*-AS

TGCACCCCTGCTGCATGAGGCTCAGCCCCAGGGGTCCAACAGAGGTCA
CAGTCACAGCACAGAATGGAAGGCTTGCAGGGAGGGCTGCCCTGGAGG
GCAGAGTGAAAGCAGGGGAAAGCTGCAGCCTTTCTTCTTCGTCCTCCTT
GGGGAGCTAATTACAGGCCAGCAGCCACTGTGCAG I
GAGCGGTTATCCGGCTGCAGGAGGTATCCTTCAACACAGCCACATATGA
AGGAGCCGTCTGTGTTGGTGCATAGCTGGCTGCAGGTGCCGTACACTGA

GCACTCATCAAAATCTGCCGAGGAAAGGGCAAGAATGTGGTCATTTCCA
GTGTGACAAGGGCAACGAAGTGGGTGATCATCAGACATGATCAACATGT
CCATCCTTACAGCTACCTTGAAGGACAGTTCGGCAGAATCTATTACAGCA
TGAAATGTGCAAACCTTAACACCCAGCAAGTCCAGTCTTGGAATCCACTC
TAGAGAAACACTCGTGTACATATCACTCATCCACGCAGGGAAGATTTATT
GAGTCCCTACTATGGGTCAGGTGCCAGGGGTGCATCAGTGAACTTTAAA
ATAGACCCACATTGCTGTCCTCATGAAACTTATATTCAGATAATAAATGAA
ATATAGTTACTATAATAA

B)



Pearson r	0.6115
95% confidence interval	0.5549 to 0.6625
P value (two-tailed)	< 0.0001
P value summary	****
Is the correlation significant? (alpha=0.05)	Yes
R square	0.3740

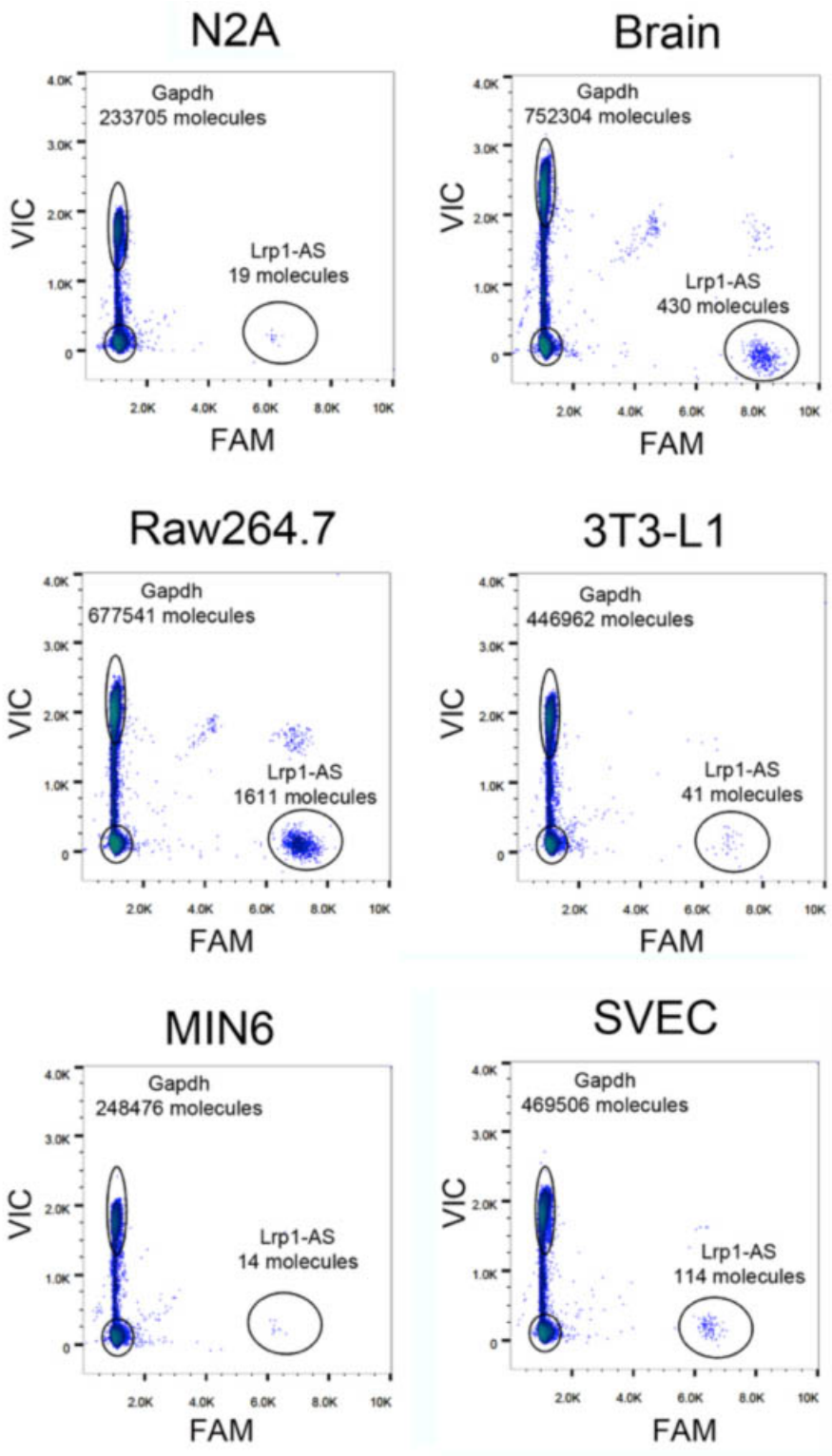
Figure S1.

A) Sequences of mouse *Lrp1*-AS and human *LRP1*-AS. The potential overlap regions with mouse Lrp1 or human LRP1 are highlighted in red.

B) Expression correlation of LRP1 and LRP1-AS in the developing human brain. Expression correlation analysis of LRP1 and LRP1-AS in RNAseq data from the Developmental Transcriptome project of the BrainSpan atlas.

Figure S2

A


N2A
Gapdh
233705 molecules
Lrp1-AS
19 molecules
Brain
Gapdh
752304 molecules
Lrp1-AS
430 molecules
Raw264.7
Gapdh
677541 molecules
Lrp1-AS
1611 molecules
3T3-L1
Gapdh
446962 molecules
Lrp1-AS
41 molecules
MIN6
Gapdh
248476 molecules
Lrp1-AS
14 molecules
SVEC
Gapdh
469506 molecules
Lrp1-AS
114 molecules
VIC
FAM
4.0K
3.0K
2.0K
1.0K
0
2.0K
4.0K
6.0K
8.0K
10K

B

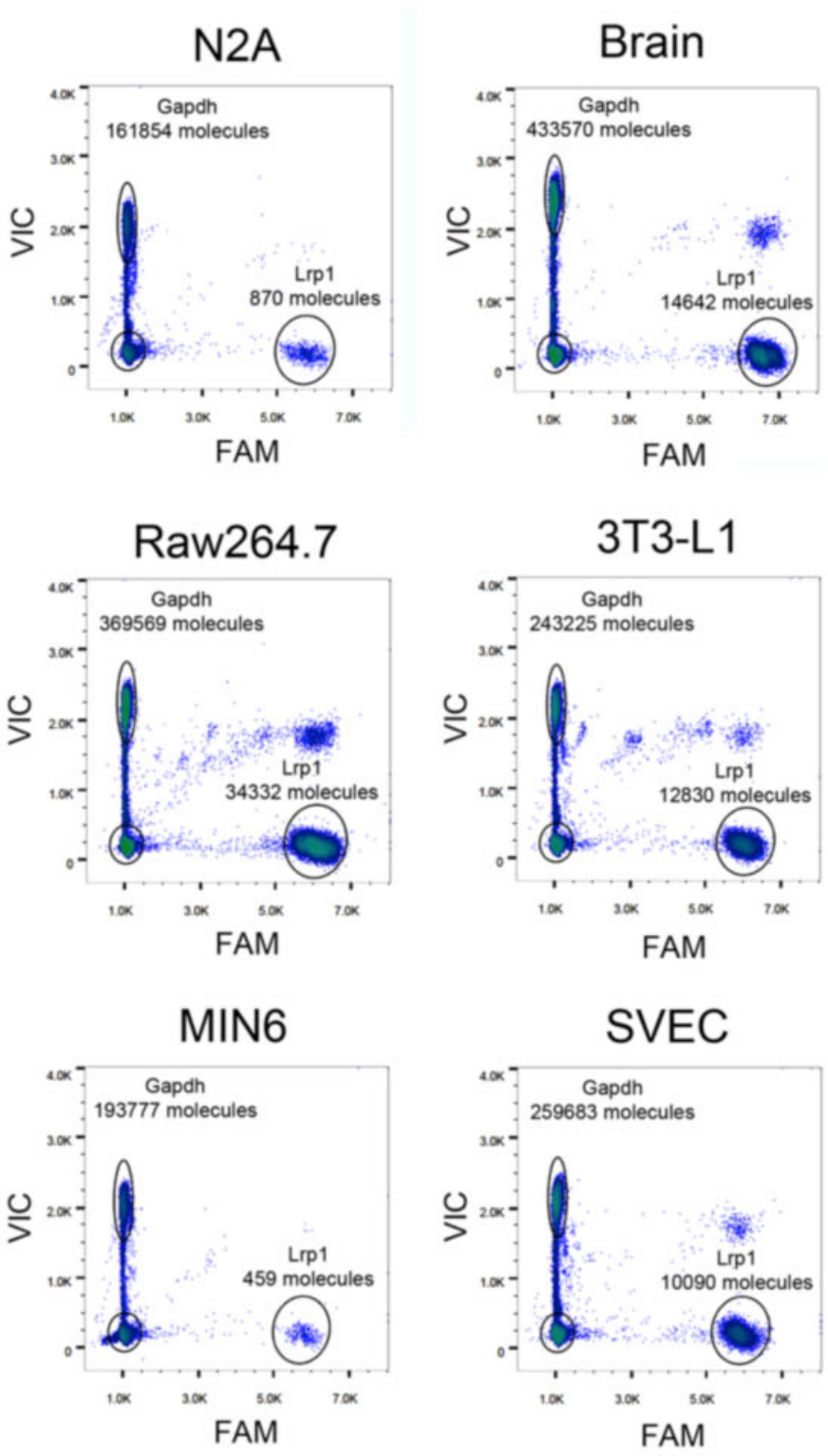

N2A
Gapdh
161854 molecules
Lrp1
870 molecules
Brain
Gapdh
433570 molecules
Lrp1
14642 molecules
Raw264.7
Gapdh
369569 molecules
Lrp1
34332 molecules
3T3-L1
Gapdh
243225 molecules
Lrp1
12830 molecules
MIN6
Gapdh
193777 molecules
Lrp1
459 molecules
SVEC
Gapdh
259683 molecules
Lrp1
10090 molecules
VIC
FAM
4.0K
3.0K
2.0K
1.0K
0
1.0K
3.0K
5.0K
7.0K

Figure S2. Lrp1-AS and Lrp1 expression in mouse cell lines.

A) Digital PCR (dPCR) analysis of FAM-labeled Lrp1-AS and VIC-labeled Gapdh expression in mouse brain tissue and different mouse cell lines. N2A neuroblastoma, Raw264.7 macrophages, 3T3-L1 embryonic fibroblast, MIN6 pancreatic beta cells, SVEC endothelial.

B) Digital PCR (dPCR) analysis of FAM-labeled Lrp1 and VIC-labeled Gapdh expression in mouse brain tissue and different mouse cell lines. N2A neuroblastoma, Raw264.7 macrophages, 3T3-L1 embryonic fibroblast, MIN6 pancreatic beta cells, SVEC endothelial.

Figure S3

(Related to Figure 3)

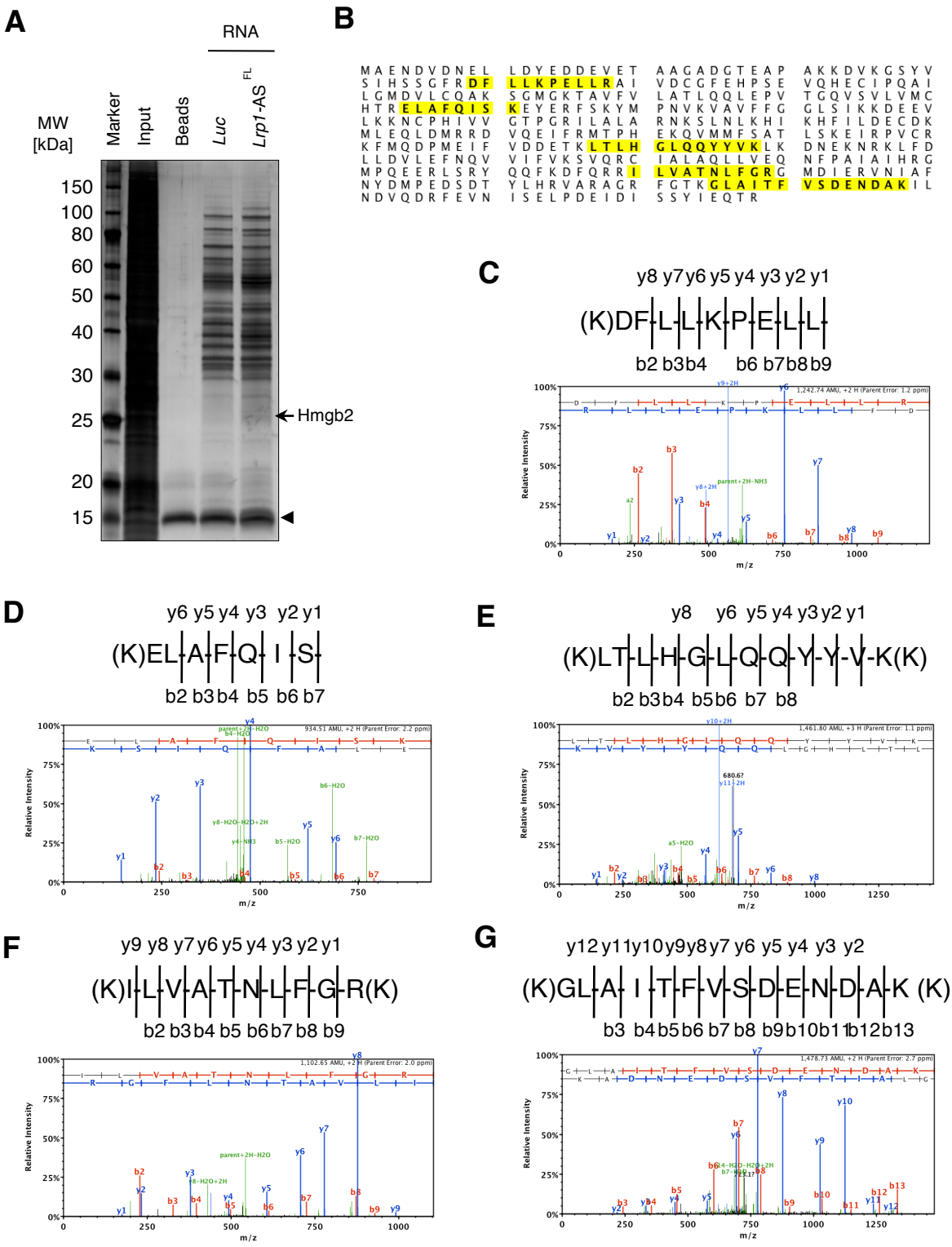


Figure S3. Identification of Hmgb2 bound to *Lrp1*-AS

(A) RNase-assisted RNA chromatography on full-length *Lrp1*-AS FL in RAW264.7 nuclear extracts, visualized by silver staining (*upper*). Luciferase (*Luc*) RNA chromatogram was used as a negative control. Protein band (*arrow*) corresponds to Hmgb2, which was enriched in the *Lrp1*-AS RNA chromatogram. The *arrowhead* indicates a band corresponding to RNase A/T1/V1.

(B) Mass spectrometry analysis identifies five peptides (highlighted in yellow) in the total protein sequence of Hmgb2

(C-G) Mass spectrum of each peptide shown in (B)

Figure S4

(Related to Figure 4)

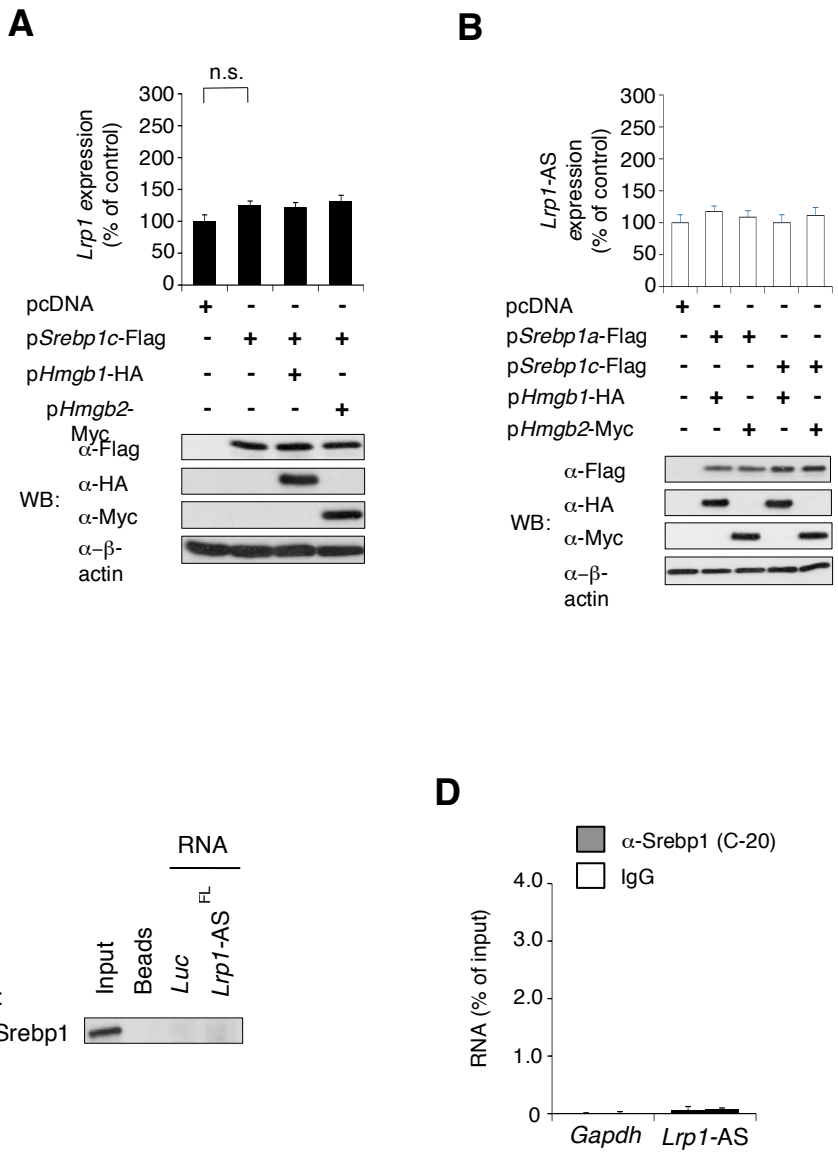


Figure S4.

(A) *Lrp1* levels after overexpression of mature Srebp1c-Flag and Hmgb1-HA/2-Myc in RAW264.7 cells (*upper*). (B) *Lrp1*-AS levels after overexpression of mature Srebp1a/c-Flag and Hmgb1-HA/2-Myc in RAW264.7 cells (*upper*). Expression of exogenous proteins was monitored by Western Blotting (WB) with the indicated antibodies (*lower*), and an antibody to β-actin was used as

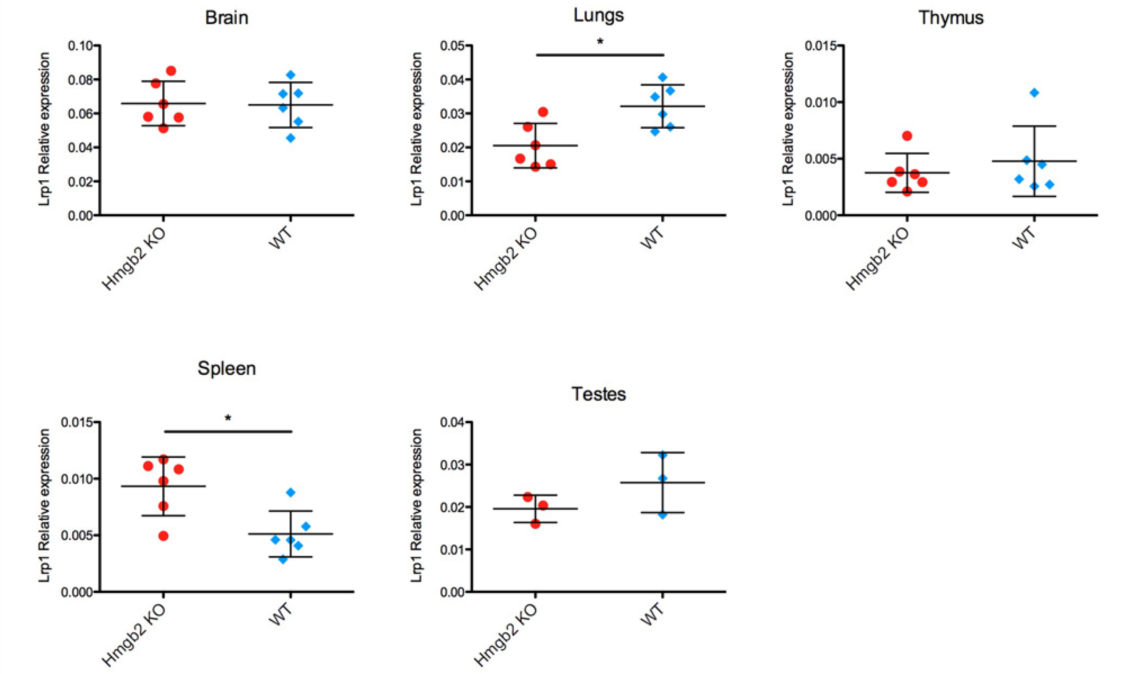
loading control in (A) and (B).

(C) RNase-assisted RNA chromatography on full-length *Lrp1*-AS FL in RAW264.7 nuclear extracts, followed by Western blotting (WB) of the corresponding RNA chromatograms with Srebp1 antibody. (D) RNA Immunoprecipitation with control IgG or specific antibodies against Srebp1 from RAW264.7 lysates. Co-precipitated RNAs were detected by qRT-PCR using primer pairs for *Lrp1*-AS or *Gapdh*.

Figure S5

(Related to Figure 4)

A



B

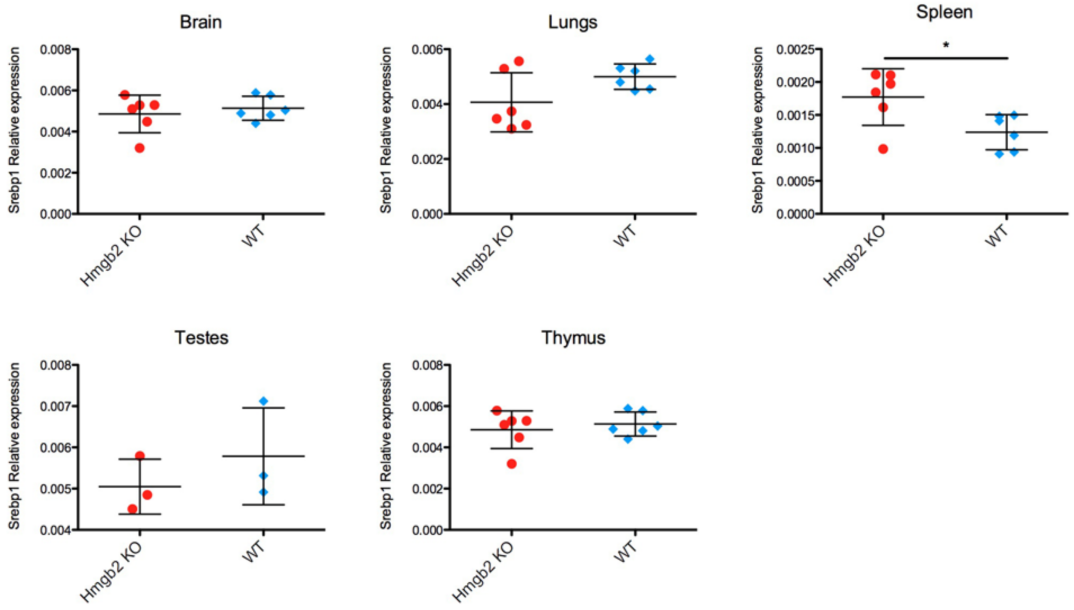


Figure S5.

qRT-PCR expression analysis of Lrp1 (A) and Srebp1 (B) expression in Hmgb2 KO and wild type (WT) mice. *$P < 0.05$

Figure S6

(Related to Figure 5)

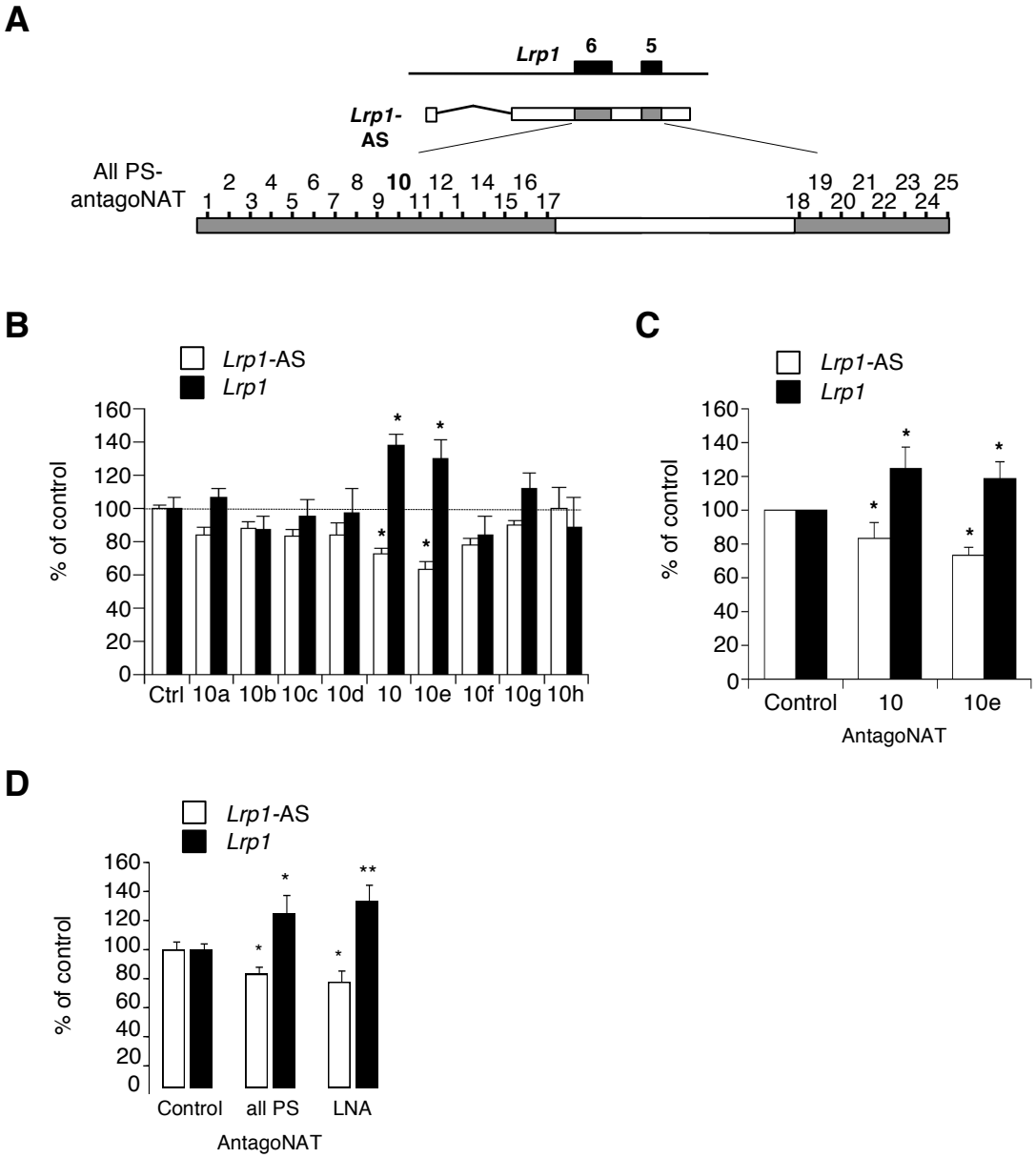


Figure S6.

(A) Schematic presentation of antagoNATs covering the overlap region of *Lrp1*-AS. (B) *Lrp1* and *Lrp1*-AS levels after transfection of Control or Specific antagoNATs against *Lrp1*-AS, which were adjunct to antagoNAT10. Control

(Ctrl) antagoNAT has a sequence with no homology to any gene. (C) *Lrp1* and *Lrp1*-AS levels after transfection of antagoNAT10 or 10e against *Lrp1*-AS. (D) *Lrp1* and *Lrp1*-AS levels after transfection of either all PS- or locked nucleic acid (LNA)-enhanced antagoNAT10 against *Lrp1*-AS. Mean ± s.d. (n = 3 replicates) are shown in all bar graphs. $^{*}P < 0.05$, $^{**}P < 0.01$ determined by one-way ANOVA.