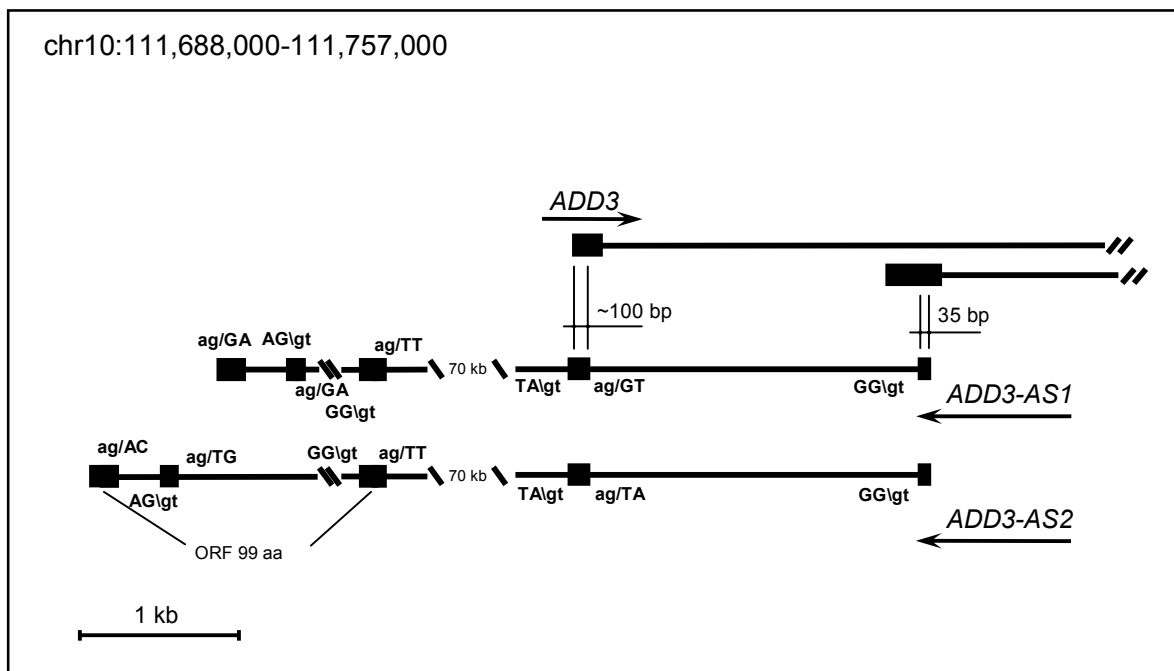
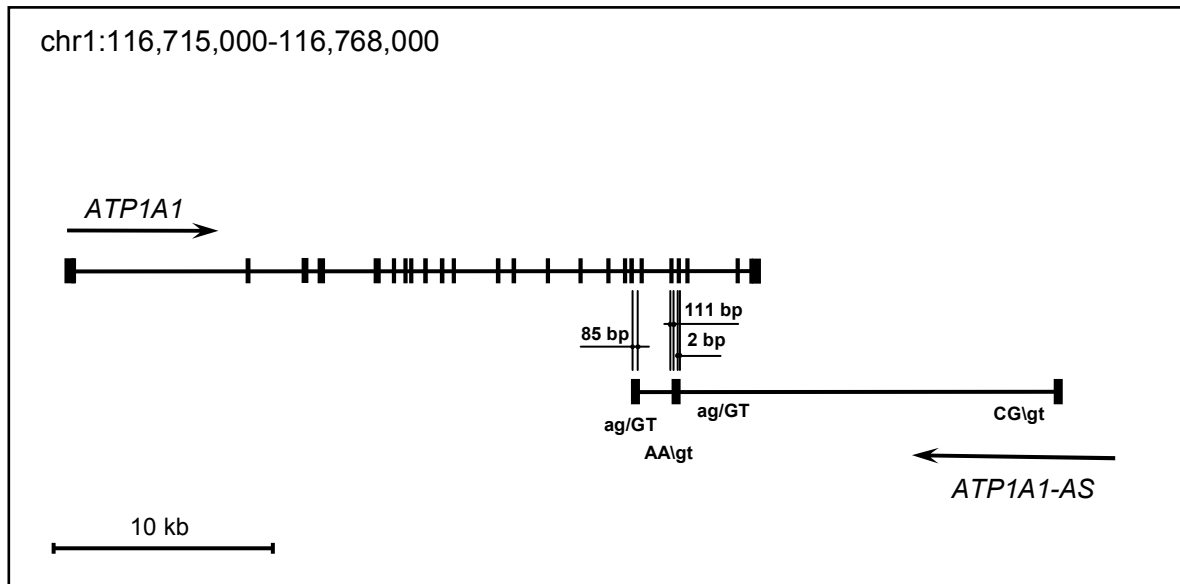
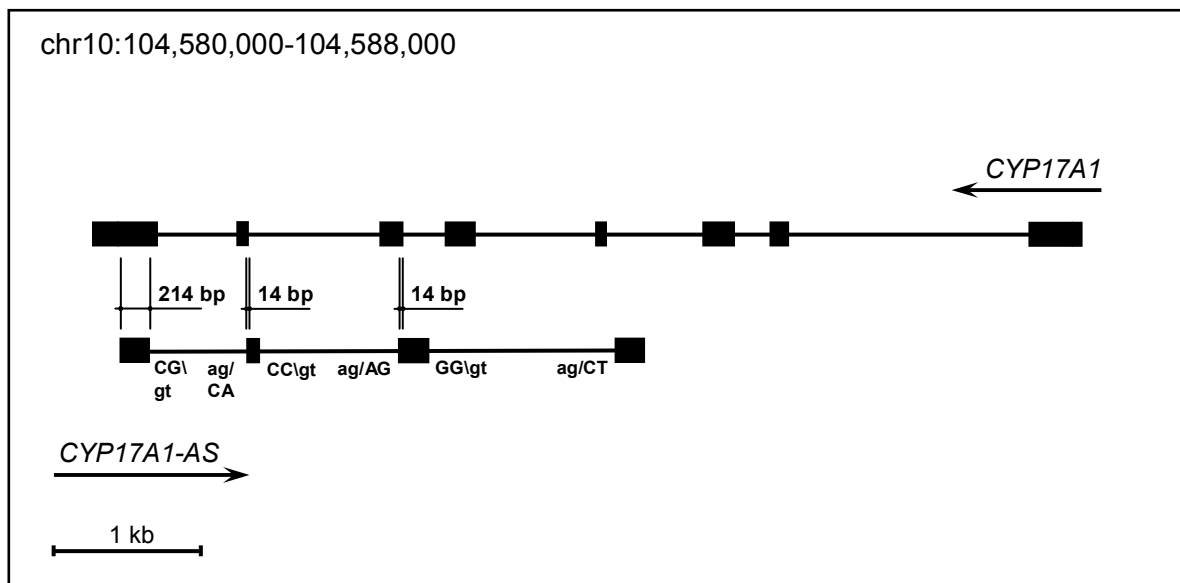


## Additional file 2

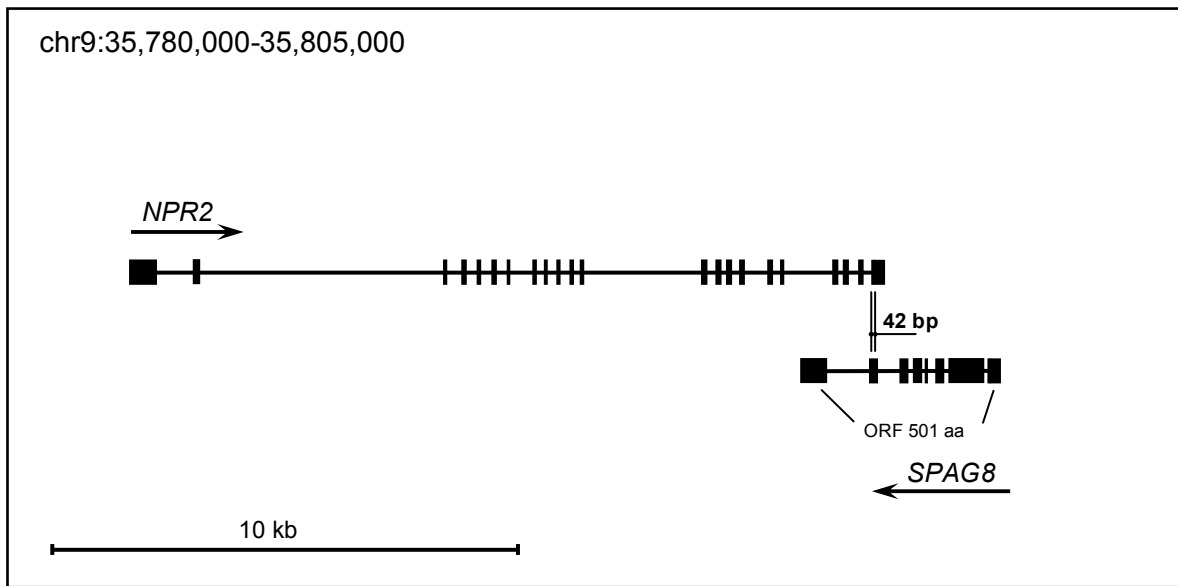
**Figure S1.** Genomic structure of six natural antisense transcripts that are associated with hypertension candidate genes in human. The structure of *NPPA* and *NPPA-AS* is shown on Figure 1A in the main text. Chromosomal position according to Human Mar. 2006 (hg18) assembly is shown. Arrows indicate the direction of transcription. Antisense transcripts are identified by appendix “-AS”, except for antisense partners for *NPR2* and *ACSM3*, that are indicated according to GenBank annotations as *SPAG8* and *EXOD1*, respectively. In case where alternative splicing isoforms could be predicted from ESTs, they are numbered and shown separately. Overlap between sense and antisense exons is indicated in basepairs. Nucleotide sequence at the splice sites of antisense transcripts is shown (except for *SPAG8* and *EXOD1*), with uppercase letters indicating exonic and lowercase letters indicating intronic sequence.

**A**

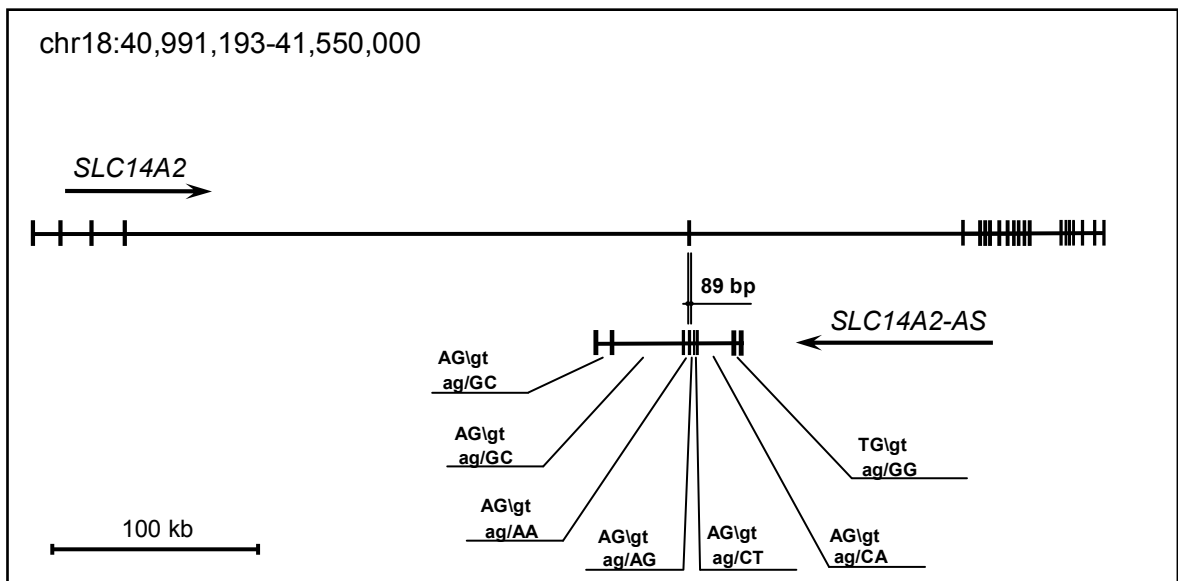


**B****C**

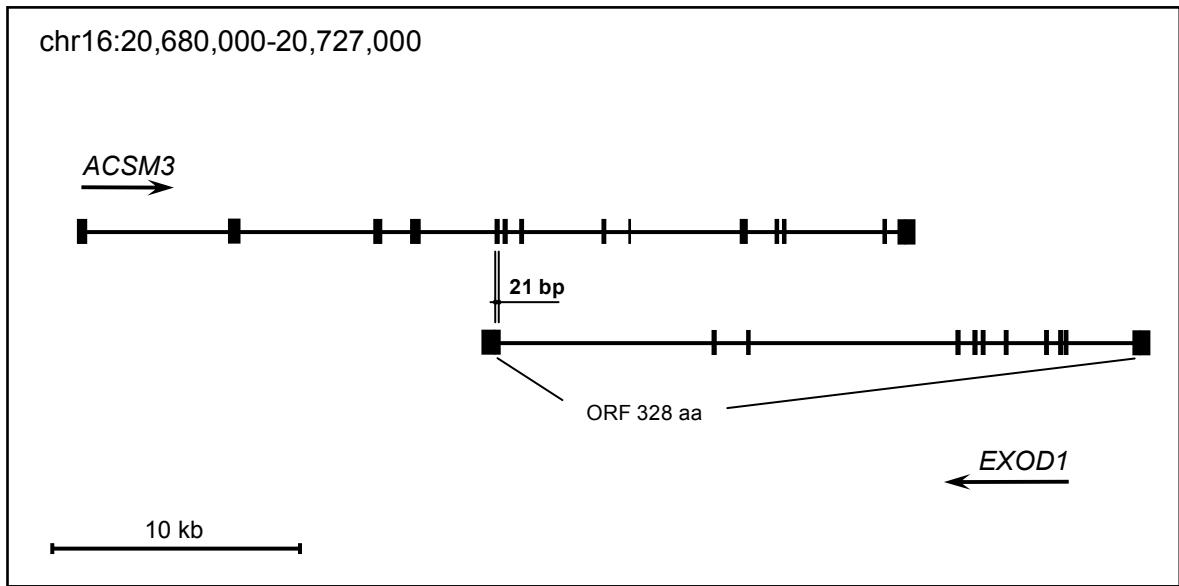
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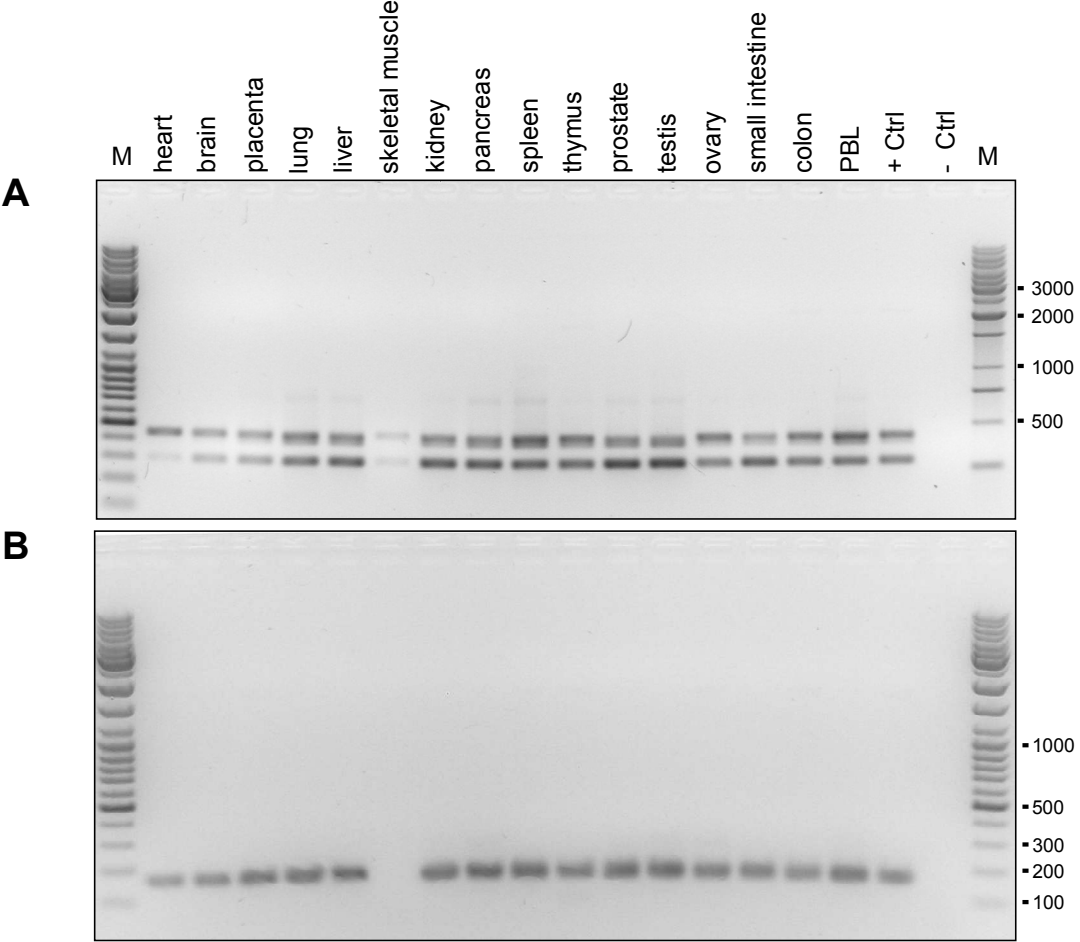
**E**



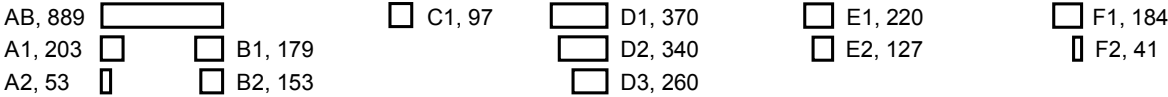
**F**



**Figure S2.** Detection of *NPPA-AS* expression using oligonucleotides NPPA-AS-F1 and NPPA-AS-R1 (A), NPPA-AS-F1 and NPPA-AS-R2 (B). Location of primers is shown in Figure 1. Primer sequences are provided in Table S2, Additional file 1. Expected products of 296, 439 bp (panel A) and 156 (panel B) were observed on the agarose gel. No unspliced products were detected, excluding the possibility that intron-containing *NPPA* form in Figure 1 derived from genomic DNA contamination.



**Figure S3.** The length (in nucleotides) of *NPPA-AS* alternatively spliced exons from Figure 1B. Each exon is labeled using a combination of letters and numbers. Exact coordinates and sequence of exons is shown in Figure S4.



**Figure S4.** Nucleotide sequence of *NPPA-AS* exons. Nucleotide positions are indicated according to Human March 2006 (hg18) assembly. In addition to exonic sequence (uppercase), ten nucleotides of flanking intronic sequence (lowercase) for each exon is included (except for first and last exon, where the PCR primers were located).

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>NPPA-AS_exon-AB_889bp (chr1:11822963-11823861)
ATTCCCAGTC ACCCACTCAC TCAGAAAGCC GGGAGTCATC GGACACCTTG 11823012
CTGGTCAGAG GTCCTGGGGG TGGTTTTGAA CCATCAGAGC TTGGACTTTT 11823062
CTGACTTCCC CAGCAAGGAT CTTCCCCTT CCTGCTCCCT GTGTTCCCAC 11823112
CCTCCAGTGT TGGCACAGGC CCACCCCTGG CTCCACCAGA GCCAGAAGCA 11823162
GAGGTAGAAT CAGGCGGGCC CCGGGCTGCA CTCCGAGCAG TGTTCTCTGGC 11823212
CATCTTTGCT ACTTTCCTAG AGAACCCGGC TGTTGCCTTA AATGTGTGAG 11823262
AGGGACTTGG CCAAGGCAAA AGCTGGGGAG ATGCCAGTGA CAACATACAG 11823312
TTCATGACTA GGTTTAGGAA TTGGGCACTG AGAAAATTCT CAATATTTCA 11823362
GAGAGTCCTT CCCTTATTTG GGA CTCTTAA CACGGTATCC TCGCTAGTTG 11823412
GTTTTAAGGG AAACACTCTG CTCCTGGGTG TGAGCAGAGG CTCTGGTCTT 11823462
GCCCTGTGGT TTGACTCTCC TTAGAACCAC CGCCCACCAG AAACATAAAG 11823512
GATTAATAATC AACTAATAA CCCCTGGATG GTCAATCTGA TAATAGGATC 11823562
AGATTTACGT CTACCCTAAT TCTTAACATT GCAGCTTTCT CTCCATCTGC 11823612
AGATTATTCC CAGTCTCCCA GTAACACGTT TCTACCCAGA TCCTTTTTC 11823662
TTTCCTTAAG TTTTGATCTC CGTCTTCCTG ATGAAGCAGG CAGAGCTCAG 11823712
AGGATCTTGG CATCACCCAC CAAAGTTAGC TGAAAGCAGG GCACTCCTGG 11823762
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GTAGAAAGGA AGCACTTCTG AGCCAGTGAC CACTGAAAGg tatgtgcta

>NPPA-AS_exon-A1_203bp (chr1:11822953-11823175)
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CTGACTTCCC CAGCAAGGAT CTTCCCCTT CCTGCTCCCT GTGTTCCCAC 11823112
CCTCCAGTGT TGGCACAGGC CCACCCCTGG CTCCACCAGA GCCAGAAGCA 11823162
GAGgtagaat cag

>NPPA-AS_exon-A2_53bp (chr1:11822953-11823025)
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ATTCCCAGTC ACCCACTCAC TCAGAAAGCC GGGAGTCATC GGACACCTTG 11823012
CTGgtcagag gtc

>NPPA-AS_exon-B1_179bp (chr1:11823663-11823861)
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TTTTGATCTC CGTCTTCCTG ATGAAGCAGG CAGAGCTCAG AGGATCTTGG 11823722
CATCACCCAC CAAAGTTAGC TGAAAGCAGG GCACTCCTGG ATAAAGCAGC 11823772
TTCACTCAAC TCTGGGGAAT GCTACCATTT TTTTTCAAA GTAGAAAGGA 11823822
AGCACTTCTG AGCCAGTGAC CACTGAAAGg tatgtgcta

>NPPA-AS_exon-B2_153bp (chr1:11823689-11823861)
cctgatgaag 11823698
CAGGCAGAGC TCAGAGGATC TTGGCATCAC CCACCAAAGT TAGCTGAAAG 11823748
CAGGGCACTC CTGGATAAAG CAGCTTCACT CAACTCTGGG GAATGCTACC 11823798
ATTTTTTTTC CAAAGTAGAA AGGAAGCACT TCTGAGCCAG TGACCACTGA 11823848
AAGgtatgtg cta

>NPPA-AS_exon-C1_97bp (chr1:11825155-11825271)
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AAACCAGCCT TTTCAGCATC TCACCCATTA GCAGCCCAT CACCCAGTGA 11825214
TCAGTCGCCT CAGTAAAGCA GATCTGTGGA TGGGGAGCCT ACGGGTGgta 11825264
agaagtg

>NPPA-AS_exon-D1_370bp (chr1:11826338-11826727)

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 TGGTGTTTTAG GAAGTTGCC CACACACAGA TTGCCCGAT GTCCAACCAG 11826497  
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 TTCTGTTGTT GCCATCGGCC ATTGCTTTTT GTTCGCTTGC TTTTGGTTTT 11826597  
 GCAAGAAGAG CGGCCTCTGT CTCTGATCTG CTTCAAATCA TCATTCCATC 11826647  
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 TTGCCCGAT GTCCAACCAG AAGAAGTGAA ACTGCTGCTG GGTCTGGAGA 11826527  
 GGTGAAGACC CGTGGCCAGC TTCTGTTGTT GCCATCGGCC ATTGCTTTTT 11826577  
 GTTCGCTTGC TTTTGGTTTT GCAAGAAGAG CGGCCTCTGT CTCTGATCTG 11826627  
 CTTCAAATCA TCATTCCATC AGTGACAGAA GTGGCTGTTC CATCAGTGGT 11826677  
 CGCAGCCAGT TCAGCTCCTG CATCCATCCC CAAGTGTTCT gtaagtccca

>NPPA-AS\_exon-D3\_260bp (chr1:11826448-11826727)

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 GCCATCGGCC ATTGCTTTTT GTTCGCTTGC TTTTGGTTTT GCAAGAAGAG 11826607  
 CGGCCTCTGT CTCTGATCTG CTTCAAATCA TCATTCCATC AGTGACAGAA 11826657  
 GTGGCTGTTC CATCAGTGGT CGCAGCCAGT TCAGCTCCTG CATCCATCCC 11826707  
 CAAGTGTTCT gtaagtccca

>NPPA-AS\_exon-E1\_220bp (chr1:11828181-11828420)

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 TGAAAGGAAG AAGAAAACT CAGCATTCTT TCCTCTGACA AAGAGTAAAA 11828290  
 CGACAAGGAA TATCGGCCTG AATTCTCTTC CCAAGAAGAA AGAAAGCACA 11828340  
 CCAACGCAGG CATTGTCTCT CTGTCCATGG TGCTGAAGTT TATTCACTTT 11828390  
 CAAACCACTT TCAGTAACAG gtgaggttct

>NPPA-AS\_exon-E2\_127bp (chr1:11828274-11828420)

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 AAGCACACCA ACGCAGGCAT TTGTCTTCTG TCCATGGTGC TGAAGTTTAT 11828383  
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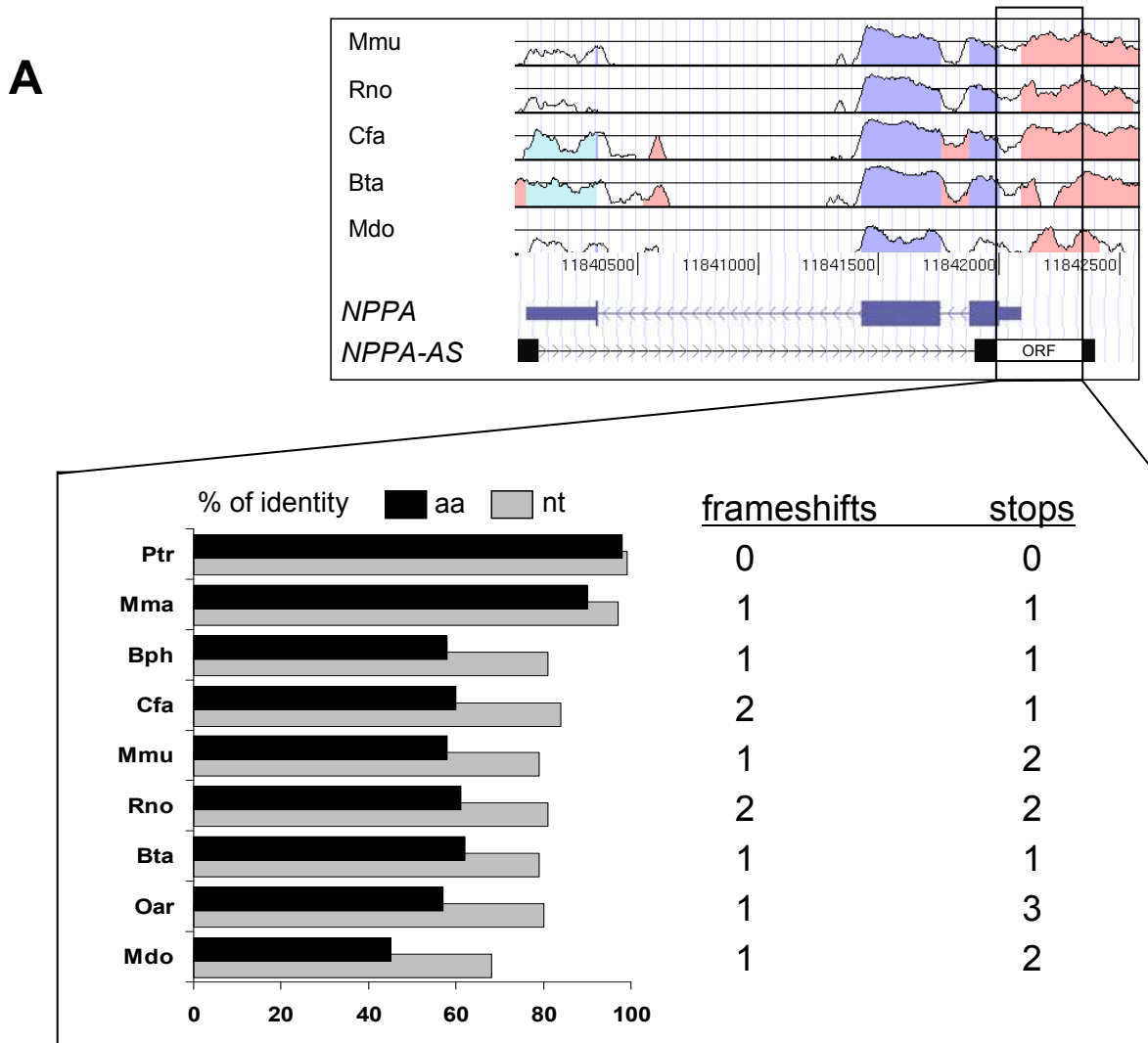
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 CAGACTGCAC CCGCTTTCCT GGCCCTACCT TGAAATCCAT CAGGTCTGCG 11830227  
 TTGGACACGG CATTGTACAT GGGATTAGCT CTGG

>NPPA-AS\_exon-F2\_41bp (chr1:11830211-11830261)

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**Figure S5.** Analysis of *NPPA-AS* ORF. (A) The predicted ORF in human (121 aa) is disrupted by several frameshift mutations and stop codons in different mammalian species. In all cases, degree of identity is higher at the nucleotide than at the amino acid level, indicating the nonconserved nature of the ORF. On top, VISTA conservation plot is shown for mouse, rat, dog, cow and opossum. Below, the percent of identity, the number of frameshifts and stop codons interrupting the predicted ORF is shown for following organisms: Ptr, *Pan troglodytes*; Mma, *Macaca mulatta*; Bph, *Balaenoptera physalus* (fin whale); Cfa, *Canis familiaris*; Mmu, *Mus musculus*; Rno, *Rattus norvegicus*; Bta, *Bos taurus*; Oar, *Ovis aries*; Mdo, *Monodelphis domestica* (gray short-tailed opossum). (B) Predicted amino acid sequence of a putative protein encoded by the *NPPA-AS* ORF.



**Figure S6.** Multiple sequence alignment of human *NPPA-AS* ORF (Figure S5) with respective regions from different mammalian species. Alignment was performed using ClustalW2 (<http://www.ebi.ac.uk/Tools/clustalw2/index.html>). Asterisks indicate nucleotide positions that are identical in all species. Hsa, *Homo sapiens*; Ptr, *Pan troglodytes* (chimpanzee); Mma, *Macaca mulatta* (rhesus macaque); Cja, *Callithrix jacchus* (marmoset); Bph, *Balaenoptera physalus* (fin whale); Mmu, *Mus musculus* (mouse); Fca, *Felis catus* (cat); Cfa, *Canis familiaris* (dog); Eca, *Equus caballus* (horse); Bta, *Bos taurus* (cow).

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Hsa   ATGCTGGCGTTCG-TCAAGGAGCAATCCACTGCTTGCTGCTCTGTCTCTCCCCTCTGGTTC 59
Ptr   ATGCTGGCGTTCG-TCAAGGAGCAATCCACTGCTTGCTGCTCTGTCTCTCCCCTCTGGTTC 59
Mma   ATGCTGGCGTAG-TCAAGGAGCAATCCACTGCTTGCTGCTCTGTCTCTCCCCTCTGGTTC 59
Cja   ATGCTGGTGTAG-TCAAGGAGCGATCGACTGCTTGCTGCTCTGTCTCTCCCCTCTGGTTC 59
Bph   ATGCTGGCGTTCGGTCAAGGGG-----CGGTCTGGTGTCTGCTGCTCTTCTCTC--TC 51
Mmu   ATGCTGGCGTGGGTTCGGGACGATCTGATGTTTGCTG-TCTCTGC--CCTCTCTGTTT 57
Fca   ATGATGGCGTTCGGTCAAGGAG-----TGTTCTGATGCTTGCTG---CCTCTCTG--TC 48
Cfa   ATGCTGGCGTTCGGTCAAGGAG-----TGCCCGGTGCTTGCTG---CTCCCTG--TC 48
Eca   ATGCTGGCGTTCGGTCAAGGAG-----GTCTGGTGTCTGCTGCTCTTCCCCTC--TC 49
Bta   ATGCTGGCGTTCGGTCAAGGAATGGT---CGGTCTGGTGTCTGCTGCTCTCCCCTG--TC 55
***  ***  *   *  **  **                               *  **  *   *   *  *  **  *

Hsa   CTCTCTGGTTCCCCTCTCTTGGCCTACGTCTGTCCCTGTCTCCCAGCTGCCAGTGCCGC 119
Ptr   CTCTCTGGTTCCCCTCTCTTGGCCTATGTCTGTCCCTGTCTCCCAGCTGCCAGTGCCCTC 119
Mma   CTCTCTGGTTCCCCTCTCTTGGCCTACGTCCGTCCCTGTCTCCCAGCTGCCAGTGCCCTC 119
Cja   CTCTCTAGTTCCCCTCTCTTGGCCTACGTCCGTCCCTGTCTCCAACTGCCAGCACCTC 119
Bph   CTCTCTGCTTTCCACCCCTCGGCCTCCCTCCGTCTCCGTCTCCCAGCTGCCCTGCGCCTC 111
Mmu   CTTTCT---CTCTCTCTCTCAGCTTTTGTCCGTCACTGTCTCCTAGCTGCC-AGCATCTC 113
Fca   CTCTC-----TTCTCTCAGCCTCCCTCCGCCT--GTCTCCCAGCTGCCCTGCGCCTC 98
Cfa   CTCTCG--CCTCCCTCTCTCAGCCTCCGTCTGCCTCTGTCTCCCAGCTGCCCTGCGCCTC 106
Eca   CTCCCTGGTTTCCCCTCTCTTGGCGTTTCGTCTGTCTCTGTCTCCCAGCTGCCCTGCGCCTC 109
Bta   CTCTTTGGCCTTTTGTGTTGGCCTTTGTCCGTCTCTGTCTCCCAGCTGCCCTGAGCCTC 115
**   *                               **  *   **  *  *   *  *  *  *  *  *

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Ptr   CTCTTTTTTATAGCCC-CCCAGCTCTCCAAGCAGCGA-----GGAGAGACAGAACCCTCC 172
Mma   CTCTTTTTTATAGCCC-CCCAGCTCTCCAAGCAGCGA-----GGAGAGACAGAACCCTCC 172
Cja   CTCTTTTTTATAGTCTCCCAACTCTTCAAGCAGCGA-----GGAAAAGATAGAACCCTCC 173
Bph   CTCTTTTTTATAGCCC-CTTGGCTCTCTGAGAGCACA-----AGAGGGACGGAACCCTCC 164
Mmu   CCGTTTTTATAGCCC-CCCAGCTCTTTGAGAAGGCA-----GGGGGGCTAGAACCCTCC 166
Fca   CTCTTTTTTATAGCCC-CCCAGCTCTCCAAGAGCGA-----AGAGGGACGGAACCCTCC 151
Cfa   CTCTTTTTTATAGCCC-CCCGGCTCTCCAAGAGCACA-----AGAGGGACGGAACCCTCC 159
Eca   CTCTTTTTTATAGCCC-CCCAGCTCTCCGAGAACGCA-----AGAGGGATGGAACCCTCC 162
Bta   CTCTTTTTTATAGCCC-CCTGGCTCTCTGGGAGCTCGTCCGGGAGAGGGACGGAACCCTCC 174
*   *****  **  *   *****  *   *                               *   *****

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Mma   CCATTCTGTCACTTGCAAGTGATAAAGCTGCCAAGTGAGAAGCCAGCAGGAGATGCCCTTT 232
Cja   CCATTCTGTCACTTGCAAGTGATAAAGCTGCCAAGTGAGAAGCCAGCAGGAGATGCCCTTT 233
Bph   CCATTCTGTCACTTGCAAGTGATAAAGCTGCCAAGTGAGAAGCCAGCAGGAGATGCCCTTT 224
Mmu   CCATTCTGTCACTTGCAAGTGATAAAGCTGCCAAGTGAGAAGCCAGCAGGAGATGCCCTTT 226
Fca   CCATTCTGTCACTTGCAAGTGATAAAGCTGCCAAGTGAGAAGCCAGCAGGAGATGCCCTTT 211
Cfa   CCATTCTGTCACTTGCAAGTGATAAAGCTGCCAAGTGAGAAGCCAGCAGGAGATGCCCTTT 218
Eca   CCATTCTGTCACTTGCAAGTGATAAAGCTGCCAAGTGAGAAGCCAGCAGGAGATGCCCTTT 222
Bta   CCATTCTGTCACTTGCAAGTGATAAAGCTGCCAAGTGAGAAGCCAGCAGGAGATGCCCTTT 234
*****  *****  *   ***  *****  **  *   *****

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Ptr TAAAGTTATCA-TCCCGCAGGGCTTGTACAGCCCCCTCTCTGCCTTGGGCAGCCCCAACA 291  
Mma TAAAGTTATCA-TCCCGCAGGGCTTGTACAGCCCTC--TGCCTTGGGCAGCCCCAACA 289  
Cja TAAAGTTATCA-TCCACAGGGCTTGTACAGCCCCCTC--TGCCTCGGGCAGCCCCAACA 290  
Bph TAAAGTTATCAGTCCAGCC-AGCTCGCCACAGCCCC-T-CCGCCTTGAGCAGCTCCGGCA 281  
Mmu TAAAGTTATCAGTTCGGCAAAGCTTGTACGGCCCCCTT--TGCCTTGAGCAGCTCCACTC 284  
Fca TAAAGCTATCAGCCCAGCAGAGCCTGTCACTGCCCCCT-CCACCTTGAGCAGCCCCAACA 270  
Cfa TAAAGCTATCAGGCCCCGAGAGCCTGTCACTACCCC-T-CCACCTTGAGCAGCCC-AACA 275  
Eca TAAAGTTATCAGTCCAGCGGAACCTGTACAGCCCC-T-CCGCCTTGAGCAGCCCCAACA 280  
Bta TAAAGTTATCAGTCCAGCC-TGCTTGTACAGCCCC-T-CTGCCTTGAGCAGCCCCGACA 291  
\*\*\*\*\* \*\* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \*

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Ptr GCCCCC-----CAGACCCTCAGCTGCAAGAGCCGCATTCTTACTGATTTGACTCAAGAG 345  
Mma GCCCCC-----CAGACCCTCGGCTGCAAGAGCCGCATTCTTGCTGATTTGACTCAAGAG 343  
Cja GCCCCC-----CAGACCCTCAGCTGCAAGAGCTGAATTCTTGCTGATTTGACTCAAGAG 344  
Bph AGCCC-----GGGACCCTCAGCTGCAGGAGCCACATTCTTGTTGATTGGCTCAAGAG 334  
Mmu ACCCCCCTCCCCCAGACCCTCAGCTGCAAGAGTCACATTCTTGCTGATTTGCCTCAAGAG 344  
Fca ACCCC-----AGACCCTCAGCTGCAAGAGCCACATTCTTGTTGATTTGCCTCAAGAG 322  
Cfa GCCCC-----GGACCCTCAGCGCAAGAGCCACATTCTTGTTGATTTGCCTCAAGAG 327  
Eca ACCTCC-----GGGACCCTCGGCTGCAAGAGCCACATTCTTGTTGATTTGCCTCAAGAG 334  
Bta ACCCCC-----AGGACCCTCAGCTGCAGGAGCCACATTCTTGTTGATTTGCCTCAAGAG 345  
\* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \*

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Bta GTTCCCACTTCAAAGGTGTGA 366  
\* \* \* \* \* \*