Supplementary Materials

Antisense regulation of atrial natriuretic peptide expression

Celik et al

Supplementary Figures

Supplementary Tables

Supplementary Figures

Supplementary Figure 1

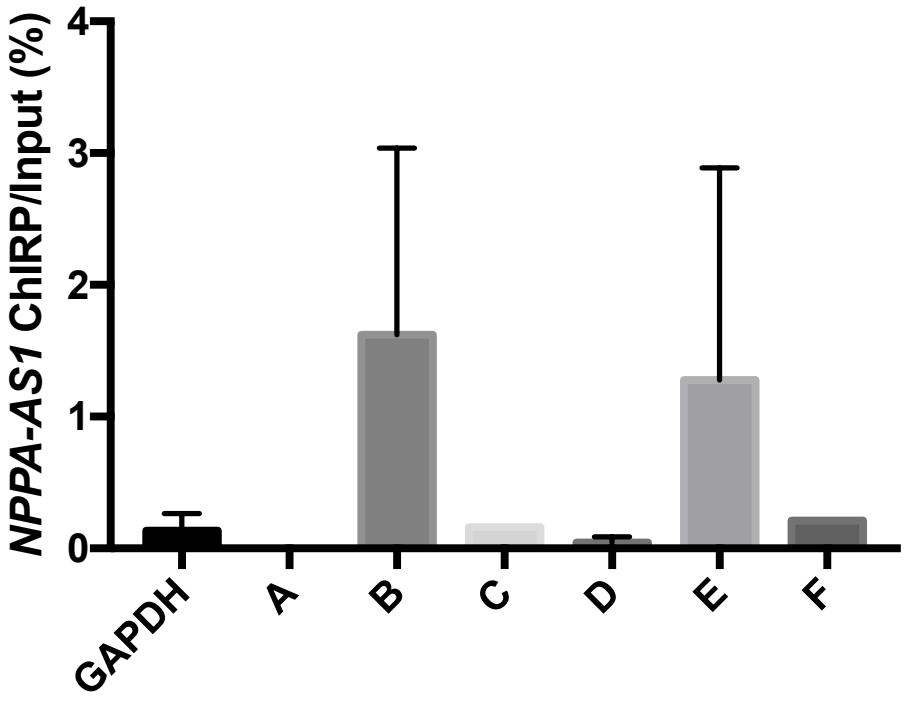

Figure S1. *NPPA-AS1* ChIRP of human ventricular tissue. qRT-PCR analysis of human ventricular DNA co-precipitated with probes specific for NPPA-AS1, n=2. Primers specific for six regions in the NPPA promoter (A-E) and a region of the GAPDH promoter were used to quantify the co-precipitated DNA. Mean and standard deviation is indicated.

Supplementary Figure 2

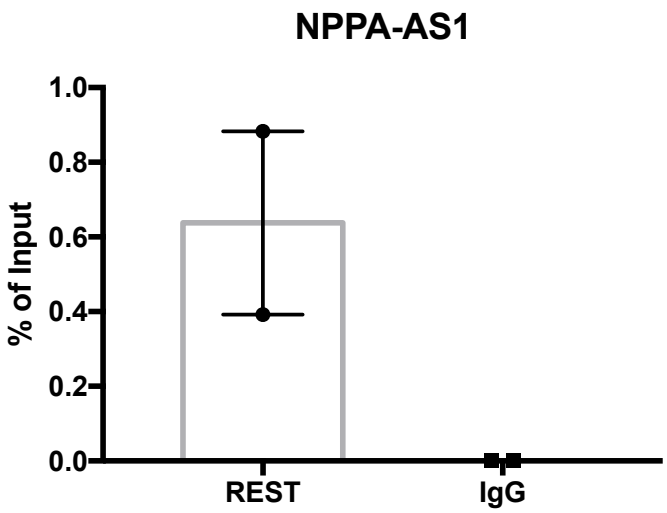

Figure S2. Quantification of *NPPA-AS1* in RNA immunoprecipitated with a REST antibody. qRT-PCR analysis of RNA immunoprecipitated with either REST or IgG control antibody. N=2 d)

Supplementary Figure 3

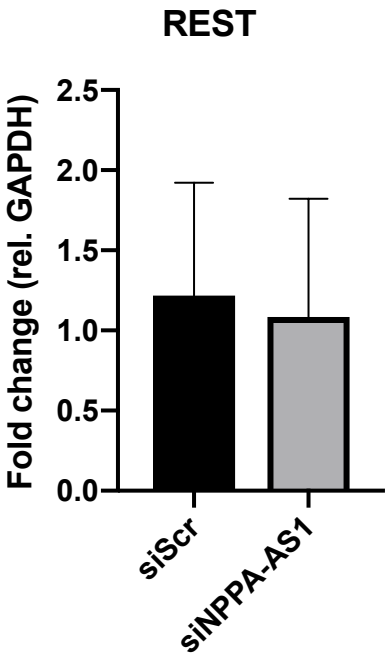

Figure S3. qRT-PCR quantification of *REST* gene expression in iPS-CM transfected with siRNA specific for *NPPA-AS1*. Results are expressed relative to *GAPDH* and normalized to the mean of the control group. Results are based on two separate experiments with three replicates each. Mean and standard deviation is shown.

Supplementary Figure 4

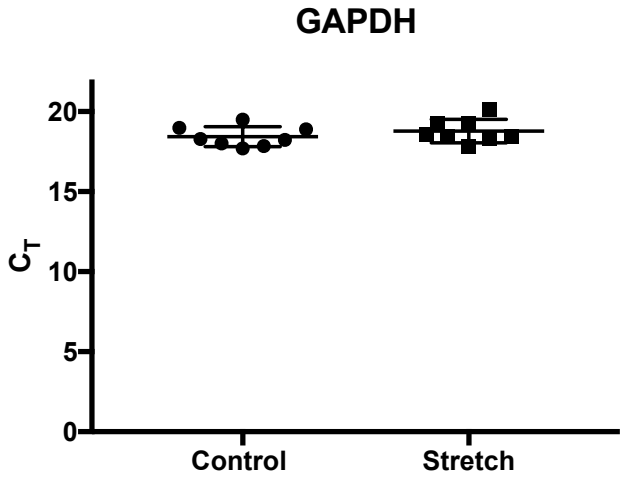

Figure S4. Effect of stretch on *GAPDH* expression. qRT-PCR C_T-values for GAPDH from iPS-CM subjected to 48 hours of stretch or unstretched cells. Data comes from two separate experiments with 4 replicates in each group.

Supplementary Figure 5

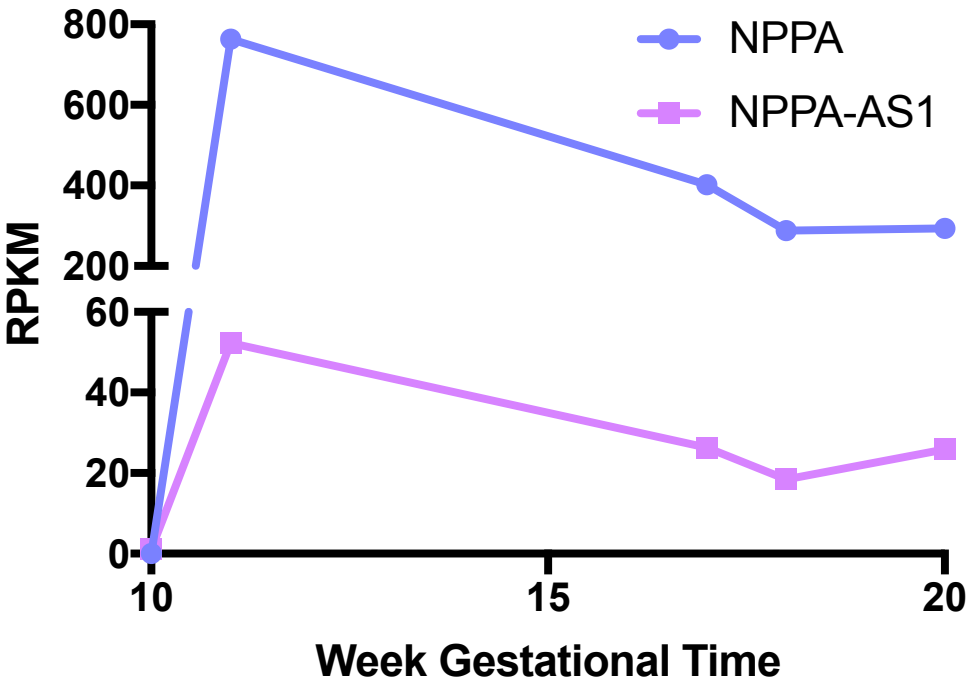

Figure S5. RNA-Seq of human fetal cardiac tissue at different gestational time points (extracted from Gene Expression Omnibus #GSE64283). Fetal tissue was collected at gestational week 10, 11, 17, 18 and 20 and sequenced using Illumina TruSeq Stranded Total RNA with Ribo-Zero Gold sample prep kit. RPKM: Reads per Kilobase Million.

Supplementary Figure 6

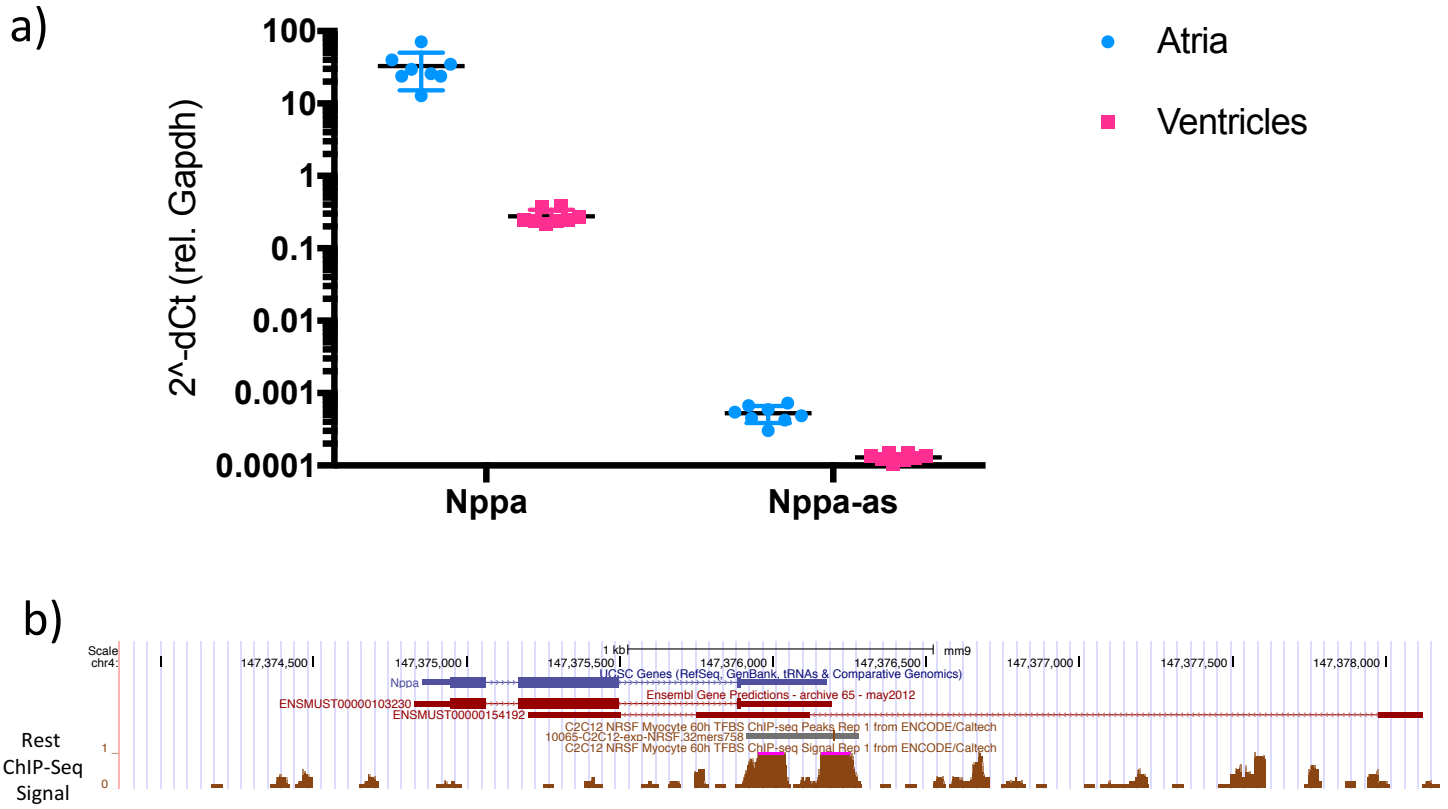

Figure S6. a) Expression of Nppa and Nppa-as in mouse atria and ventricles. RNA was extracted from atria and ventricles of mouse hearts (n=8) and qRT-PCR was used to assess the relative levels of *Nppa* and *Nppa-as.*Results are expressed relative to Gapdh. Mean and standard deviation is shown. b) Overview of the Nppa locus including ENCODE/Caltech Rest ChIP-Seq data from the mouse myocyte cell line C2C12.

Supplementary Figure 7

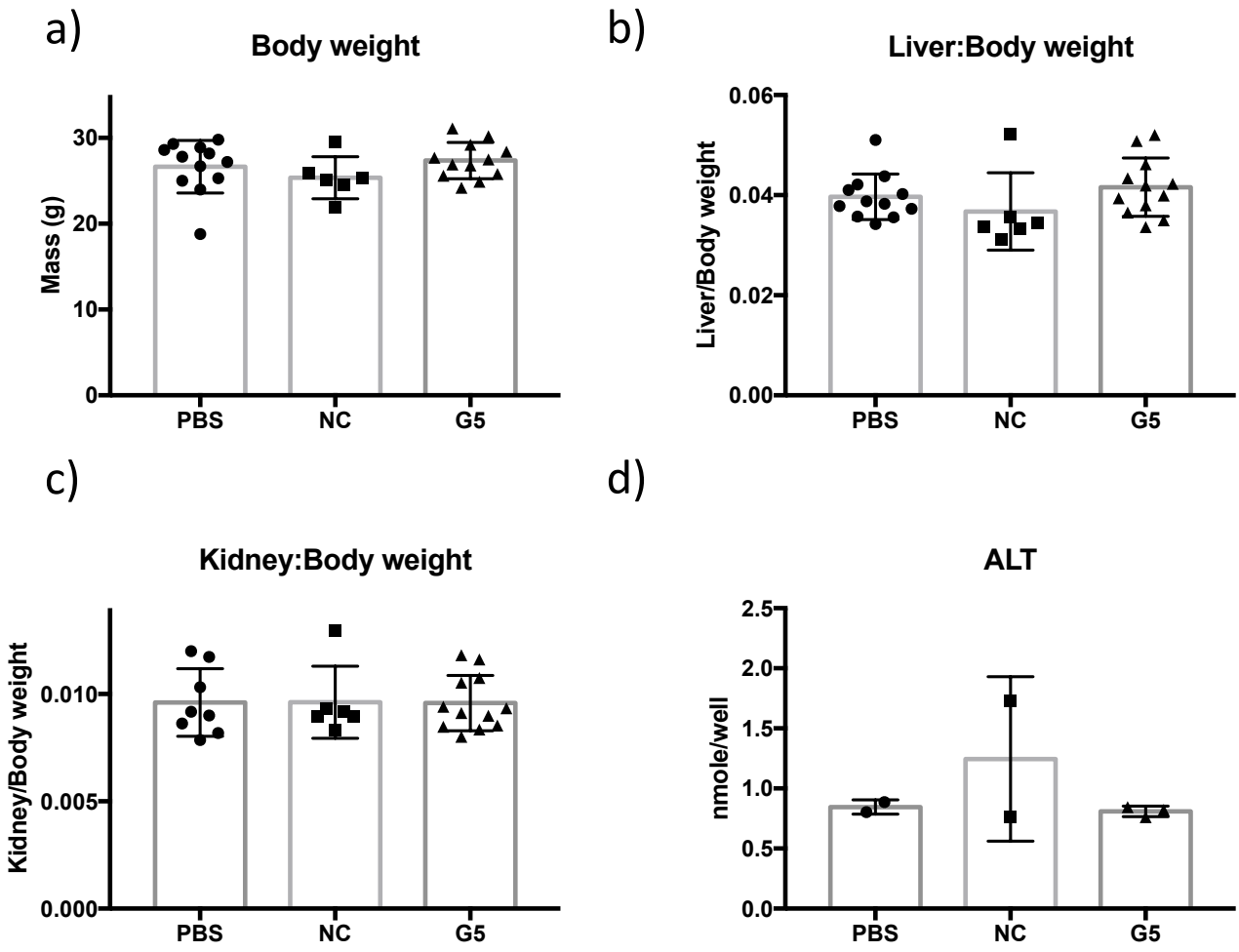

Figure S7. Assessment of toxicity of GapmeR injections. Mice were injected subcutaneously with PBS or 25 mg/kg of G5 or negative control Gapmer. 48 hours after injection, body weight (a), liver (b) and kidney (c) weight was recorded. Plasma alanine aminotransferase (d) was analyzed in a subset of animals.

Supplementary Figure 8

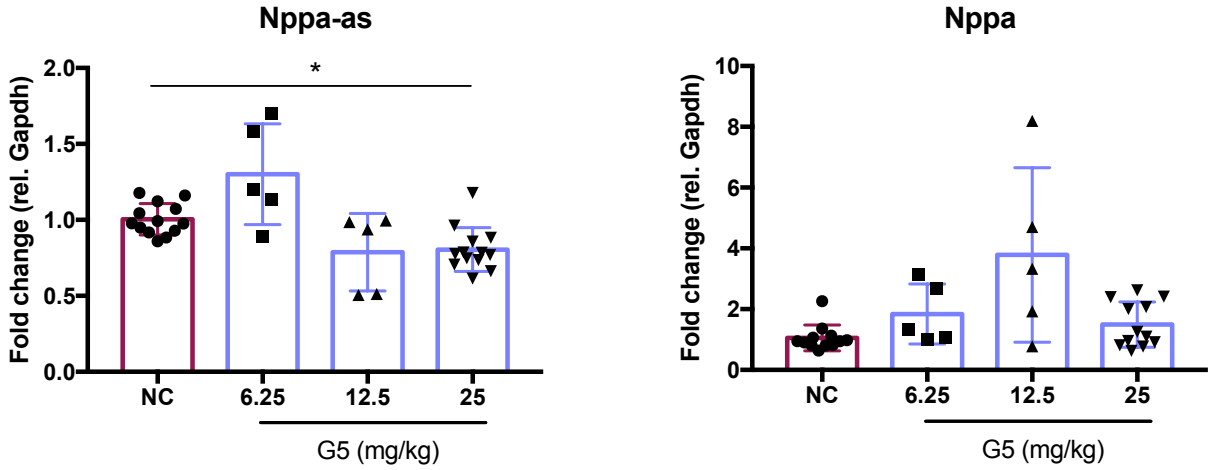

Figure S8. *Nppa and Nppa-as expression in GapmeR-injected mice. Nppa* and *Nppa-as* expression levels in ventricular tissue of mice injected intraperitoneally with different doses of GapmeR5 (G5) or 25 mg/kg negative control (NC) for 48h. Results are expressed relative to *Gapdh* and normalized to the mean of the negative control group (n=5-13). Mean and standard deviation is shown. Kruskal-Wallis was used to test the difference between animals treated with negative control and each of the G5 doses, as well as within G5 treatment groups. *p<0.05 after adjustment for multiple comparisons using Dunn's test.

Supplementary Tables

Supplementary Table 1. Primer and probe sequences

***NPPA-AS1* FISH Probes**

Probe ID	*Sequence*
NPPA-AS_1	agcaaggtgtccgatgactc
NPPA-AS_2	tctgatggttcaaaaccacc
NPPA-AS_3	ggggaagtcagaaaagtcca
NPPA-AS_4	caggaagtgggaagatcctt
NPPA-AS_5	tgattctacctctgcttctg
NPPA-AS_6	aaagatggccaggaacactg
NPPA-AS_7	ccgggttctctaggaaagta
NPPA-AS_8	aagtccctctcacacattta
NPPA-AS_9	tgtatgttgtcactggcatc
NPPA-AS_10	attcctaaacctagtcatga
NPPA-AS_11	ttgagaattttctcagtgcc
NPPA-AS_12	gtcccaaataagggaaggac
NPPA-AS_13	tagcgaggataccgtgttaa
NPPA-AS_14	gagtgtttcccttaaaacca
NPPA-AS_15	aaaccacagggcaagaccag
NPPA-AS_16	ggcggtggttctaaggagag
NPPA-AS_17	tatcagattgaccatccagg
NPPA-AS_18	ggtagacgtaaatctgatcc
NPPA-AS_19	gcagatggagagaaagctgc
NPPA-AS_20	gttactgggagactgggaat
NPPA-AS_21	gcttcatcaggaagacggag
NPPA-AS_22	tgatgccaagatcctctgag
NPPA-AS_23	ctgctttcagctaactttgg
NPPA-AS_24	agagttgagtgaagctgctt
NPPA-AS_25	gctcagaagtgcttcctttc
NPPA-AS_26	tgttcatctttcagtggtca
NPPA-AS_27	tgctagcaggaggagatgaa
NPPA-AS_28	caatctgtgtgtggggcaac
NPPA-AS_29	agcagatcagagacagaggc
NPPA-AS_30	tgatggaacagccacttctg
NPPA-AS_31	atggatgcaggagctgaact
NPPA-AS_32	attccactcagaacacttgg
NPPA-AS_33	ccttgtcgttttactctttg
NPPA-AS_34	ggaagagaattcaggccgat
NPPA-AS_35	aagacaaatgcctgcgttgg
NPPA-AS_36	gctgttactgaaagtggttt
NPPA-AS_37	cttgatttcctccaagtcag
NPPA-AS_38	aaggtagggccaggaaagcg
NPPA-AS_39	caacgcagacctgatggatt
NPPA-AS_40	agagctaatcccatgtacaa

NPPA-AS1 ChIRP Probes

Probe ID	Sequence
NPPA-AS1_1	gctctgatggttcaaaacca
NPPA-AS1_2	caaagatggccaggaacact
NPPA-AS1_3	cctagtcatgaactgtatgt
NPPA-AS1_4	gagtgtttcccttaaaacca
NPPA-AS1_5	gaccatccaggggttattag
NPPA-AS1_6	agaaacgtgttactgggaga
NPPA-AS1_7	ctttcagctaactttggtgg
NPPA-AS1_8	tcatctttcagtggtcactg
NPPA-AS1_9	caatctgtgtgtggggcaac
NPPA-AS1_10	cagatcagagacagaggccg
NPPA-AS1_11	aattccactcagaacacttg
NPPA-AS1_12	ggaagagaattcaggccgat
NPPA-AS1_13	cttgatttcctccaagtcag
NPPA-AS1_14	agagctaatcccatgtacaa

Ribonuclease protection assay primers

Primer ID	*Sequence*
Overlapping FWD	gacacggcattgtacatggg
Overlapping REV	agcagtggattgctccttga
Non-overlapping FWD	gggcagatcgatcagaggag
Non-overlapping REV	tagaagatgaggtcgtgccc

Site-directed mutagenesis primers	
Primer ID	*Sequence*
dREST_FWD	ggagggtcgcgggggacatggaag
dREST_REV	gaggacgcagccaatttcatttcttccgtt
dREST_Seq_FWD	gtcattctattctggggg
dREST_Seq_REV	ttgttctcggtgggcttggc

Supplemental Table 1. Primers and probe sequences. Continued.

Primers for ChIRP and ChIP-qPCR

Primer ID	*Sequence*
Region A FWD	gagagacagaaccctcccc
Region A REV	caagccctgcgggatgataa
Region B FWD	gttatcatcccgcagggctt
Region B REV	acacctttgaagtgggagcc
Region C FWD	tgactcaagaggctcccact
Region C REV	acggcggtgagataaccaag
Region D FWD	aaacagagggtgacgcttgg
Region D REV	tatttggaggccctgacagc
Region E FWD	ctctccatggtcaggcgaaa
Region E REV	tggagagaggatgcttgtgc
Region F FWD	agaggacgcagccaatttca
Region F REV	tcctccatcggtcaagttgc
GAPDH Promoter FWD	cgggattgtctgccctaattat
GAPDH Promoter REV	gcacggaaggtcacgatgt
NPPA-AS1 Promoter FWD	tcaccgttgtctgtttctccc
NPPA-AS1 Promoter REV	caatggccgatggcaacaac
NPPA-AS1 5' FWD	gcttctgttgttgccatcgg
NPPA-AS1 5' REV	cagaaaatgcccctgggact
NPPA-AS1 GB1 FWD	ggcgaggaagtcaccatcaa
NPPA-AS1 GB1 REV	ccaggtcaccaagccagata
NPPA-AS1 GB2 FWD	gggcagatcgatcagaggag
NPPA-AS1 GB2 REV	tgaggtttatccctttccctgt
NPPA-AS1 3' FWD	gagagacagaaccctcccca
NPPA-AS1 3' REV	acggcggtgagataaccaag