Supplementary Information:

Figure Supp. 1: Predicted RNA Structures of miRBase listed sno-miRNAs and all C/D box sno-miRNA candidates tested in this study

The snoRNA sequences with putative gene regulatory function were subjected to folding predictions using RNAfold. In (A) the set of miRBase listed sno-miRNAs is diplayed, the well-characterized and uniform H/ACA box snoRNAs to the right, the structurally very heterogeneous C/D box sno-miRNAs to the left. Panel (B) summarizes the calculated structures of the C/D sno-miRNAs, which were efficient in gene silencing in at least one of the cell types used. The fold-predictions reach from no or only short hairpins (e.g. U83a) to weakly base-paired hairpins (e.g. U27) to stable stems (e.g. U15a, HBII-142). The sno-miRNAs that displayed no gene-silencing activity are given in (C). The ratio of unstructured sRNAs is higher when compared to (B), but still stably folded hairpins can be identified (e.g. U59b or HBI-43).

Figure Supp. 2: Overexpression of full length box C/D snoRNAs does not increase effects of miRNA-activities

Full length box C/D snoRNA sequences were cloned into the polymerase III (H1) promotor vector pSUPER (Oligoengine) and transfected into HeLa cells for constitutive expression. Cotransfection of psiCheck2 vectors containing sno-miRNA specific target sites was utilized to determine sno-miRNA-activities. pSUPER vectors expressing HBII-336, snR39b, U60, U20 or U74 were cotransfected with the specific psiCheck2 vectors containing target sites for the corresponding sno-miRNAs or a non-cognate sequence (NC). A psiCheck vector with a target site for has-miR-23 served as control for gene silencing activities and effects of the pSUPER constructs. Plasmids were cotransfected in a 1:4 ratio (psiCheck:pSUPER) to increase the chance of snoRNA transcription in the presence of the reporter plasmid. Increased levels of the snoRNA did not

increase the miRNA-activity of any snoRNA. The only measurable effect was a mild toxicity reducing in cell death of approximately 25% of the HeLa cells. Expression of snoRNAs did not affect the RNAi machinery. Gene silencing activities of the endogenous miRNA-23 were not or only slightly altered in presence of snoRNA-pSUPER plasmids. pSTUFFER is a pSUPER vector with a non-silencing insert in the transcribed region.

Supplemental Materials:

snoRNA oligonucleotides for reporter gene assays and Northern blots.

(Given are the insert sequences for the reporter gene insertion (pSicheck2). In bold is indicated which RNA strand is was identified as a putative sno-miRNA. Probes for Northern blot analysis were the complementary DNA oligonucleotides.)

U-44 CCTGGATGATGATAAGCAAA

5` GGCC**CCTGGATGATGATAAGCAAA** 3` (F)
5` TCGATTTGCTTATCATCATCCAGG 3` (R)

HBI-43 TCACAAAGATGAGTGGTGAA

5` GGCC**TCACAAAGATGAGTGGTGAA** 3` (F)
5` TCGATTCACCACTCATCTTTGTGA 3` (R)

U-59B TTTGCTGAAGCCAGATGCAA

5` GGCC**TTTGCTGAAGCCAGATGCAA** 3` (F)
5` TCGATTGCATCTGGCTTCAGCAAA 3` (R)

ACA45 AAAGGTAGATAGAACAGGTC

5` GGCC**AAAGGTAGATAGAACAGGTC** 3` (F)
5` TCGAGACCTGTTCTATCTACCTTT 3` (R)

U45AC **GTTTAGCTCTAGAATTACTT** (XbaI site)

5` GGCC**GTTTAGCTCTAGAATTACTT** 3` (F)
5` TCGAAAGTAATTCTAGAGCTAAAC 3` (R)

U48 **ATGATGACCCCAGGTAACTC**

5` GGCC**ATGATGACCCCAGGTAACTC** 3` (F)
5` TCGAGAGTTACCTGGGGTCATCAT 3` (R)

U-27 **TCAAGTGATGTCATCTTACT**

5` GGCC**TCAAGTGATGTCATCTTACT** 3` (F)
5` TCGAAGTAAGATGACATCACTTGA 3` (R)

U-51 **AGTTCGTGATGGATTTGCTT**

5` GGCC**AGTTCGTGATGGATTTGCTT** 3` (F)
5` TCGAAAGCAAATCCATCACGAACT 3` (R)

U-74 **AGTAATGATGAATGCCAACC**

5` GGCC**AGTAATGATGAATGCCAACC** 3` (F)
5` TCGAGGTTGGCATTCATCATTACT 3` (R)

HBII-142 **ACACCATGATGGAACTGAGG**

5` GGCC**ACACCATGATGGAACTGAGG** 3` (F)
5` TCGACCTCAGTTCCATCATGGTGT 3` (R)

U-15a **TTCGATGAAGAGATGATGAC**

5` GGCC**TTCGATGAAGAGATGATGAC** 3` (F)
5` TCGAGTCATCATCTCTTCATCGAA 3` (R)

U3-4 **AGGGAGAGAACGCGGTCTGA**

5` GGCC**AGGGAGAGAACGCGGTCTGA** 3` (F)
5` TCGATCAGACCGCGTTCTCTCCCT 3` (R)

snR39B AGTGAAATGATGGCAATCAT

5` GGCC**AGTGAAATGATGGCAATCAT** 3` (F)
5` TCGAATGATTGCCATCATTTCACT 3` (R)

U83A GTTCGTTGATGAGGCTCAGA

5` GGCC**GTTCGTTGATGAGGCTCAGA** 3` (F)
5` TCGATCTGAGCCTCATCAACGAAC 3` (R)

U78 ATGTAGACAAAGGTAACACT

5` GGCC**ATGTAGACAAAGGTAACACT** 3` (F)
5` TCGAAGTGTTACCTTTGTCTACAT 3` (R)

HBII-336 GGCCAAGGATGAGAACTCTA

5` GGCC**GGCCAAGGATGAGAACTCTA** 3` (F)
5` TCGATAGAGTTCTCATCCTTGGCC 3` (R)

U-21 CTGAATGATGATATCCCACT (EcoRV site)

5` GGCC**CTGAATGATGATATCCCACT** 3` (F)
5` TCGAAGTGGGATATCATCATTCAG 3` (R)

HBII-429 ACTGCCATGAGGAAACTGCC

5` GGCC**ACTGCCATGAGGAAACTGCC** 3` (F)
5` TCGAGGCAGTTTCCTCATGGCAGT 3` (R)

U3-1 AAGTTTCTCTGAACGTGTAG

5` GGCC**AAGTTTCTCTGAACGTGTAG** 3` (F)
5` TCGACTACACGTTCAGAGAAACTT 3` (R)

U3-2 ACCACGAGGAAGAGAGGTAG

5` GGCC**ACCACGAGGAAGAGAGGTAG** 3` (F)
5` TCGACTACCTCTCTTCCTCGTGGT 3` (R)

U3-3 GGAGAGAACGCGGTCTGAGT

5` GGCC**GGAGAGAACGCGGTCTGAGT** 3` (F)
5` TCGAACTCAGACCGCGTTCTCTCC 3` (R)

neg. control CACGTACGCGGAATACTTCGAAA

5` GGCC**CACGTACGCGGAATACTTCGAAA** 3` (F)
5` TCGATTTCGAAGTATTCCGCGTACGTG 3` (R)

Figure S1A

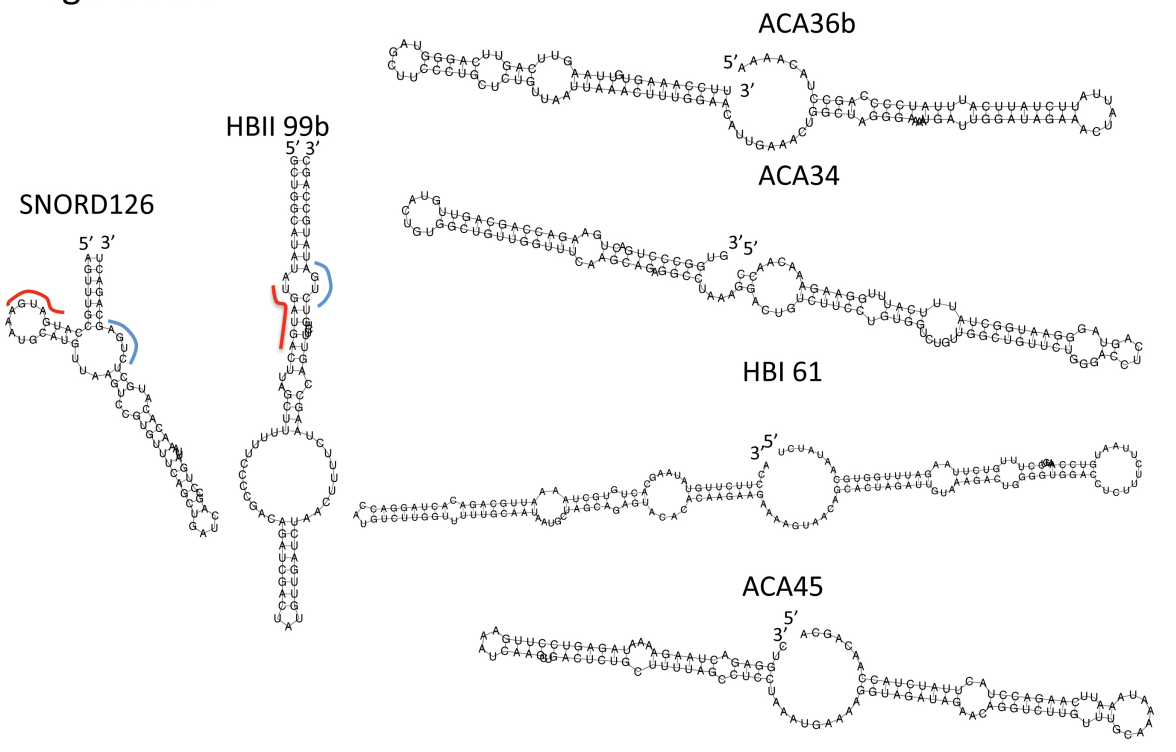

Figure S1B

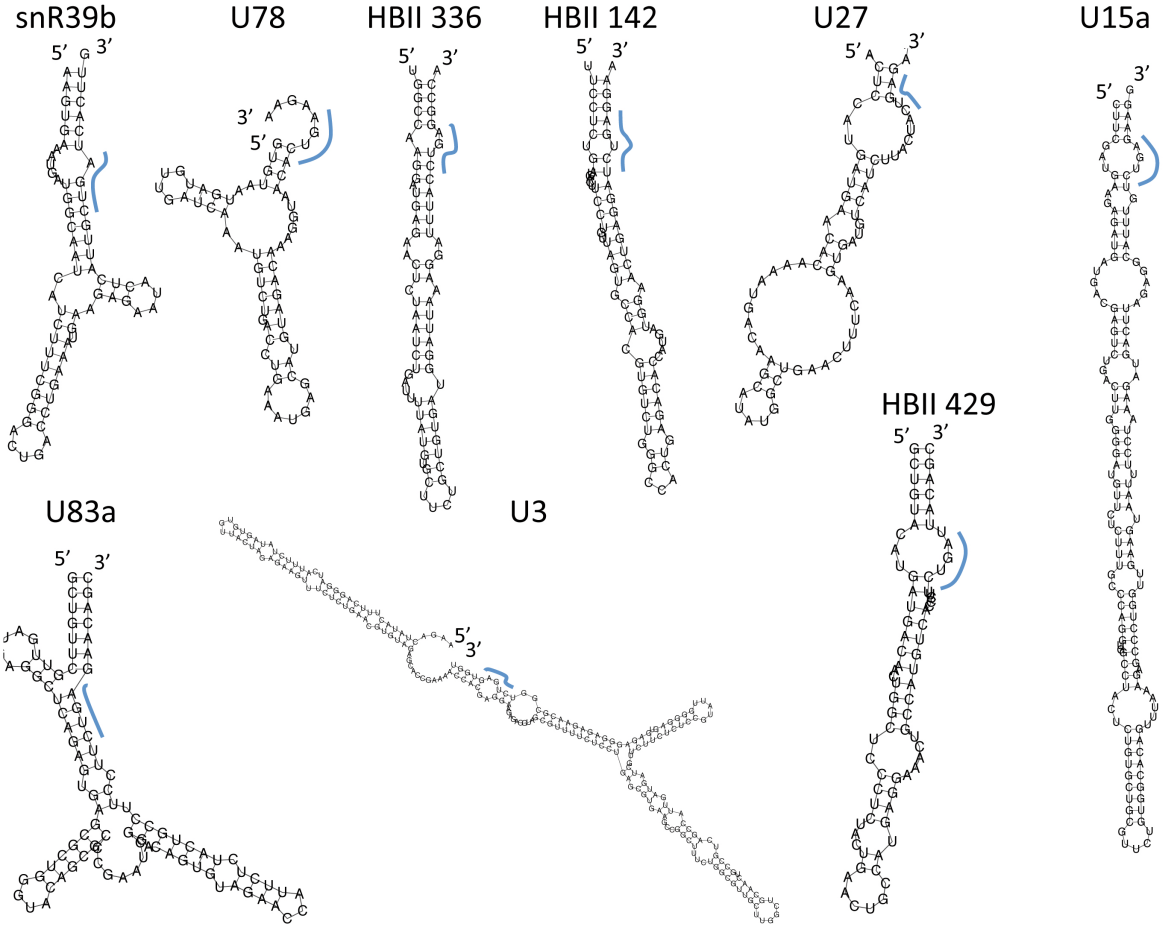

Figure S1C

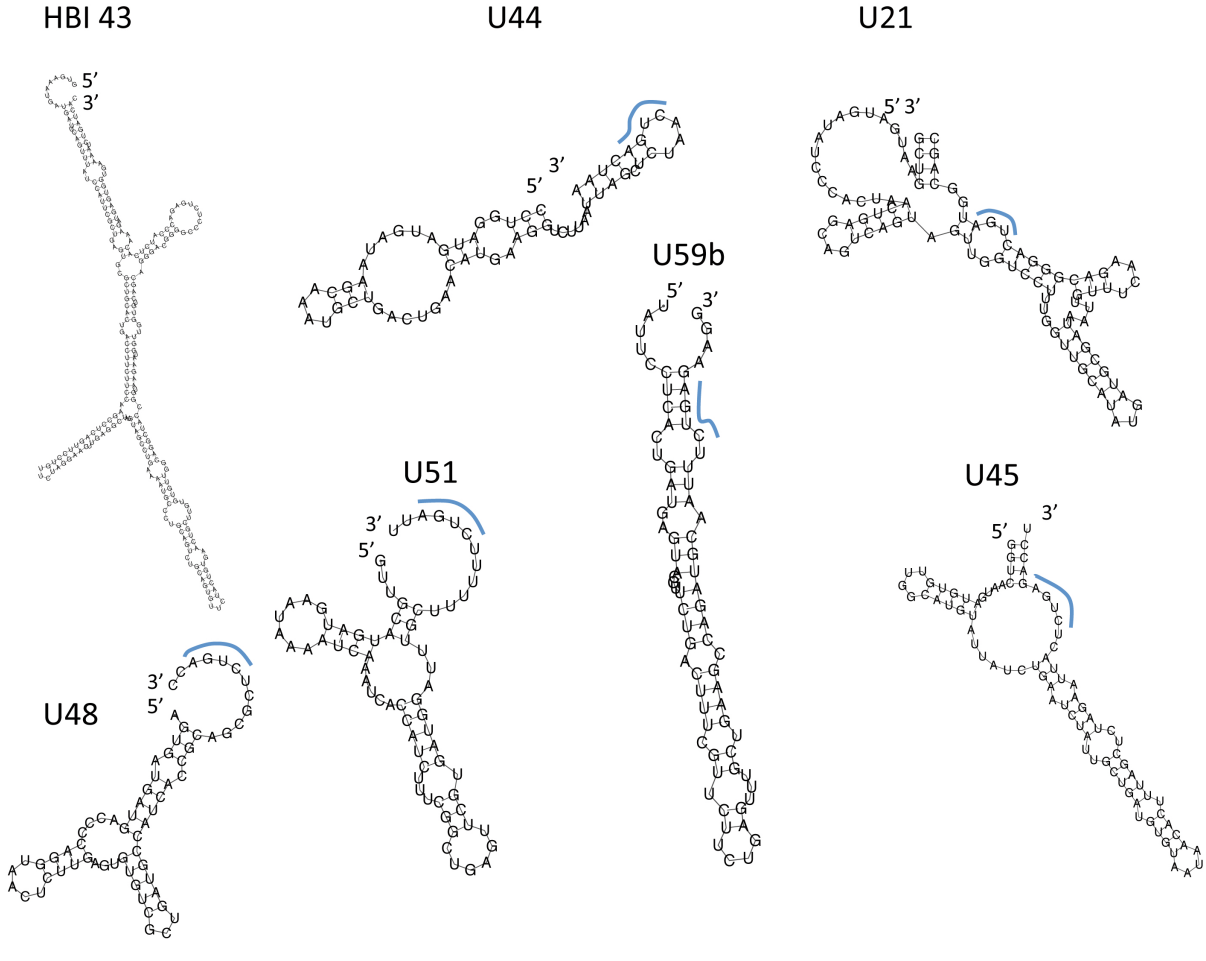

Figure S2

Overexpression of full length box C/D snoRNAs from a pol III promotor in human HeLa cells