

# Supplementary Materials: Regulation of the U3-, U8-, and U13snoRNA Expression by the DEAD Box Proteins Ddx5/Ddx17 with Consequences for Cell Proliferation and Survival

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## Supplementary Tables

**Table S1.** Primers used for construction of plasmids.

| Constructs                  | Forward Primer 5'-3'                              | Reverse Primer 5'-3'                                   |
|-----------------------------|---------------------------------------------------|--------------------------------------------------------|
| pCMVDdx5-myc                | CGAATTCGGTCGACCATGTCCGG<br>TTATTCGAGTGA           | CGCTCGAGCTATTGGAATATCC<br>TGTGG                        |
| pGEM-U3                     | GGCCTCGAGAAGACTATACITTT<br>CAGGGATCAT             | GCCCTCGAGACCA CTCAGACCGC<br>GTTCTCTCCC TCTCA           |
| pGEM-U13                    | GCCCTCGAGATCCTTTTGAGTT<br>CATGAGCGTGAGATT         | CGGAAGCTTGGTCAGACGGGTA<br>ATGTGCCACGT                  |
| pGEM-GAPDH                  | GCCCTCGAGATGGGAAGGTGA<br>AGGTCGGAGTC              | GCGGATCCTTACTCCTTGGAGGC<br>CATGTGGG                    |
| pGL3-U3-luc                 | GGCCCTCGAGCTGCAGCTTTGAA<br>CTCAGCCAGCTTCCCTG      | GGCCCATGGAACGGCCTCCTCG<br>CACACCTCTGCTAGG              |
| pGL3-U8-luc                 | GGCCCTCGAGCCACTTGCCTTGG<br>TAGTGCCCTTCCAGCTG      | GGCCCATGGACAGACAAACAGC<br>CGACATTCTGCACTCAGTG          |
| pGL3-U13-luc                | GGCCCTCGAGCTGACTGCAGCAC<br>CAGAAGG                | GGCCCATGGCATCTTATAGTTGA<br>CATTAAAGGAGTGC              |
| pGL3-SV40-luc               | GGCCCTCGAGCAGTTAAGGGTGT<br>GGAAAGTCCCCAG          | GGCCCATGGCTGGAATAGCTC<br>AGAGGCCGAGGCG                 |
| pcDNAU3                     | GGCCTCGAGCTAATTAAGACTAT<br>ACTTTCAG               | GGCCTCGAGCCCGAGATCGCGC<br>GCCACTG                      |
| pcDNAU8                     | GGCCTCGAGCTAACTATCGTCAG<br>GTGGGATAATCCTT         | CTTGGCCTCGAGAATCAGACAG<br>GAGCAATCAGGGTG               |
| pcDNAU13                    | GGCCTCGAGCTAACTATCCTTTT<br>GTAGTTCATGAGCGTGATGATT | CTTGGCCTCGAGGGTCAGACGG<br>GTAATGTGCCACGT               |
| pIRESneo- FLAG/HA-Ago2Y529E | GGCAAGACGCCCCGTGGAGGCCG<br>AGGTCAAGCG             | CGCTTGACCTCGGCCTCCACGGG<br>CGTCTTGCC                   |
| pCIneo-EGFP-3'-UTR-AKAP9    | CAACTCCGGATAAGCCAGAAAG<br>CAGATAGAAGGAGTTTG       | ACCTGCCCCCGGCCGCGTCTG<br>ATCATTTCTAATTCATGATTACA GCCGG |
| pFUW-U3-luc                 | GCCTTAATTAAGTCTGGGCGGAG<br>GGAGGAAAGGG            | TAGCAGATCTAACGGCCTCCTCG<br>CACACCT                     |
| pFUW-U8-luc                 | GCCTTAATTAACCACTTGCCTTG<br>GTAGTGCCCTTCCAGCTG     | GGCGGATCCACAGACAAACAGC<br>CGACATTCTGCACTCAGTG          |

**Table S2.** Used small interfering RNAs (siRNAs).

| siRNA        | Target Sequence 5'-3' |
|--------------|-----------------------|
| Ddx5/Ddx17si | AAGGCTAGATGTGGAAGATGT |
| U3si         | CTGAACGTGTAGAGCACCGAA |
| U14si        | ATTGGTTGCCAGACATTCGCA |

**Table S3.** Primers used for chromatin immunoprecipitation (ChIP).

| Promoter           | Forward Primer 5'-3'      | Reverse Primer 5'-3'                 |
|--------------------|---------------------------|--------------------------------------|
| U3snoRNA promoter  | GCCTGGGCTGGTGTGCAACT CATA | AAGAGGTTTAAAACCGGGGG<br>GGGGGGCATGCT |
| U8snoRNA promoter  | CGTCTTGAGTCTGGGATTAT CCGC | GATACCAAGTATCTTACGGTC<br>TGCAGG      |
| U13snoRNA promoter | GTCTTGAGGAGGGGGCGA GT     | TGTAAGACTCTTTTACTCCCT<br>GAGAGTCC    |

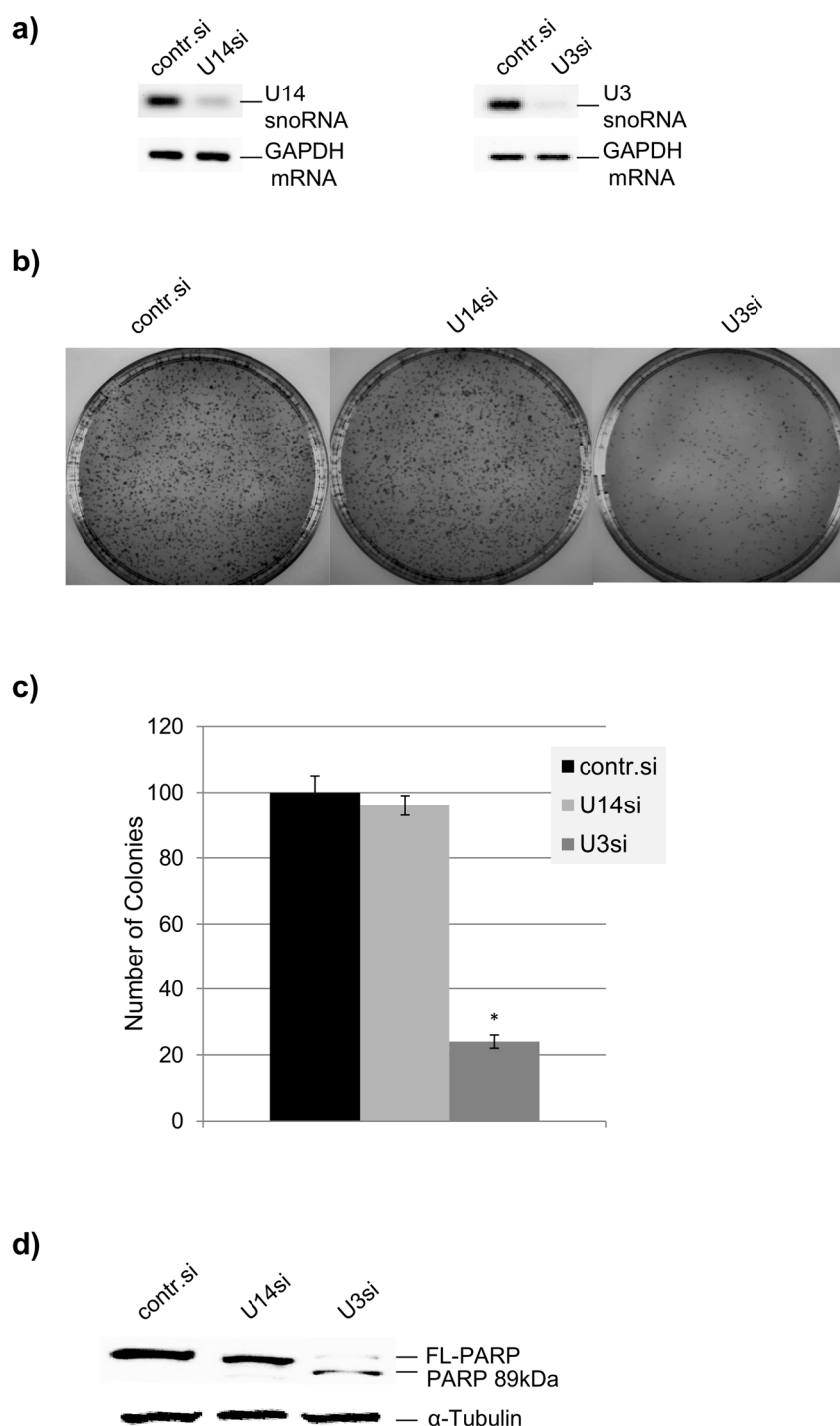
**Table S4.** Putative targets of U3snoRNA segments functioning as microRNA (miRNA). To capture potential miRNA regions in U3snoRNA, its sequence was split into 202 segments, 23 nucleotides in length and moving along the sequence with one-nucleotide shift. Results of the DIANA-T Analysis with a threshold value above 19 are shown.

| Sequence of U3snoRNA Segments | Target with Scores > 19.0                           |
|-------------------------------|-----------------------------------------------------|
| UUAAGACUAUACUUUCAGGGAUC       | SOX6/(5) 27.55<br>DCP2 23.16                        |
| GACUAUACUUUCAGGGAUCAUUU       | ZFPM2 = Fog2 interagiert mit p300                   |
| ACUAUACUUUCAGGGAUCAUUUC       | ZFPM2 36,8                                          |
| AUACUUUCAGGGAUCAUUUCUAU       | VGLL3 colon carcinoma related protein 23,08         |
| CUUUCAGGGAUCAUUUCUAUAGU       | CREB 3L3 (CREB-H)33                                 |
| UUUCAGGGAUCAUUUCUAUAGUG       | CREB 3L3 (CREB-H)32                                 |
| AUCAUUUCUAUAGUGUGUUACUA       | Dystonin (DST) 22/AKAP 19                           |
| UCAUUUCUAUAGUGUGUUACUAG       | AKAP9 22,9/(DST)                                    |
| CAUUUCUAUAGUGUGUUACUAGA       | AKAP9 21,5/KLF12 24.19                              |
| AUUUCUAUAGUGUGUUACUAGAG       | KLF12 21.68                                         |
| UAGUGUGUUACUAGAGAAGUUUC       | Integrin- $\beta$ -binding protein 20               |
| AGUGUGUUACUAGAGAAGUUUCU       | Integrin- $\beta$ -binding protein 39               |
| GUGUGUUACUAGAGAAGUUUCUC       | Integrin- $\beta$ -binding protein                  |
| UAGAGAAGUUUCUCUGAACGUGU       | NTRK3 23                                            |
| AGAAGUUUCUCUGAACGUGUAGA       | EVC (DWF1) Ellis-van Creveld syndrome<br>protein 33 |
| GAAGUUUCUCUGAACGUGUAGAG       | EVC (DWF1) 46<br>EVC (DWF1) 44                      |
| AAGUUUCUCUGAACGUGUAGAGC       | Microtubule-actin crosslinking factor 1<br>MACF1 24 |
| AGUUUCUCUGAACGUGUAGAGCA       | EVC (DWF1) 40 + div                                 |
| GUUUCUCUGAACGUGUAGAGCAC       | EVC (DWF1) 32 + div + AKAP9 26/MACF1 25             |
| UUUCUCUGAACGUGUAGAGCACC       | DST AKAP9 MACF1                                     |
| UUCUCUGAACGUGUAGAGCACCG       | MACF1 22                                            |
| UCUCUGAACGUGUAGAGCACCGA       | MACF1 19                                            |
| AGCACCGAAAACACGAGGAAGA        | Cadherin 23 21.6                                    |
| GAAAACACGAGGAAGAGAGGUA        | GABA receptor 24 ROD                                |
| AAAACACGAGGAAGAGAGGUAG        | ROD regulator of differentiation                    |
| CGAGGAAGAGAGGUAGCGUUUUC       | PolyA ribose polymerase PARP11                      |
| GAGGAAGAGAGGUAGCGUUUUCU       | PARP11                                              |
| AGGAAGAGAGGUAGCGUUUUCUC       | PARP11                                              |
| GGAAGAGAGGUAGCGUUUUCUCC       | PARP11                                              |
| GAAGAGAGGUAGCGUUUUCUCCU       | PARP11 26                                           |
| AAGAGAGGUAGCGUUUUCUCCUG       | Actin binding LIM protein                           |
| AGAGAGGUAGCGUUUUCUCCUGA       |                                                     |
| GAGAGGUAGCGUUUUCUCCUGAG       |                                                     |
| AGAGGUAGCGUUUUCUCCUGAGC       | extrem                                              |

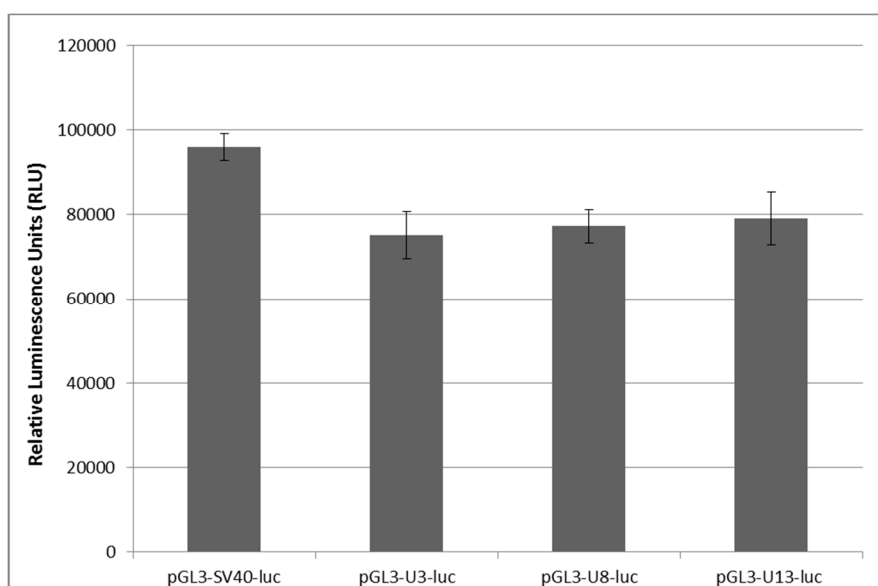
|                         |                                            |
|-------------------------|--------------------------------------------|
| GAGGUAGCGUUUUCUCCUGAGCG | extrem                                     |
| AGGUAGCGUUUUCUCCUGAGCGU | Fibrillin                                  |
| GUUUUCUCCUGAGCGUGAAGCCG | DST                                        |
| UUUUCUCCUGAGCGUGAAGCCCG | AKAP9/MACF1/onecut2                        |
| UUUCUCCUGAGCGUGAAGCCGGC | Pericentrin 32 MACF1                       |
| UUCUCCUGAGCGUGAAGCCGGCU | DST                                        |
| UCUCCUGAGCGUGAAGCCGGCUU | Importin 9 22 import of ribosomal proteins |
| UGAGCGUGAAGCCGGCUUUCUGG | SV2 $\beta$ 33                             |
| GAGCGUGAAGCCGGCUUUCUGGC | Laminin SV2 receptor                       |
| GGCUUUCUGGCGUUGCUUGGCUG | MACF1 26.14 AKAP9 23                       |
|                         | CDK6 22.5                                  |
| GCUUUCUGGCGUUGCUUGGCUGC | AKAP9 24 DST 32.25                         |
| CUUUCUGGCGUUGCUUGGCUGCA | MACF1 26.6 CDK6                            |
| CUGCAACUGCCGUCAGCCAUUGA | DST MACF1 AKAP9                            |
| UGCAACUGCCGUCAGCCAUUGAU | NTRK3                                      |
| GCAACUGCCGUCAGCCAUUGAUG | NTRK3                                      |
| CAACUGCCGUCAGCCAUUGAUGA | NTRK3                                      |
| CGUUCUUCUCUCCGUUUGGGGA  | NTRK3                                      |
| GUUCUUCUCUCCGUUUGGGGAG  | AKAP9 20.5 MDN1 19.11                      |
| UUCUUCUCUCCGUUUGGGGAGU  | AKAP9 49.83                                |
| UCUUCUCUCCGUUUGGGGAGUG  | AKAP9 44.17 eIF4G3                         |
| CUUCUCUCCGUUUGGGGAGUGA  | AKAP9 eIF4G3                               |
| UUCUCUCCGUUUGGGGAGUGAG  | div. + AKAP20                              |
| CCGUUUGGGGAGUGAGAGGGAG  | div. zinc finger protein                   |
| UUGGGGAGUGAGAGGGAGAGAAC | UBE2E3 UBE2E4P                             |
| UGGGGAGUGAGAGGGAGAGAACG | ubiquitin conjugating enzymes              |
| GGGGAGUGAGAGGGAGAGAACGC |                                            |
| GGGAGUGAGAGGGAGAGAACGCG | GNG13                                      |
| AGUGAGAGGGAGAGAACGCGGUC | GNG13                                      |
| GUGAGAGGGAGAGAACGCGGUCU | BOK32                                      |
| UGAGAGGGAGAGAACGCGGUCUG | BOK32                                      |
| AGAGGGAGAGAACGCGGUCUGAG | GRWD1 glutamate rich WD repeat containing  |
| GAGGGAGAGAACGCGGUCUGAGU | protein A                                  |
| AGGGAGAGAACGCGGUCUGAGUG | NADH                                       |
| GGAGAGAACGCGGUCUGAGUGGU | NADH                                       |
|                         | PAX2                                       |

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## Supplementary Figures



**Figure S1.** Indication of apoptosis by U3snoRNA knockdown. HeLa cells were transfected with control small interfering RNA (siRNA) (contr.si), U14snoRNAsiRNA (U14si), or U3snoRNAsiRNA (U3si) and harvested 72 h thereafter. (a) Demonstration of the U14- and U3snoRNA knockdown. 1  $\mu$ g of total RNA was analyzed by reverse transcription polymerase chain reaction (RT-PCR) with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA serving as a loading control; (b) An overview of the outcome of a colony forming assay performed with cells transfected with control siRNA (contr.si), U14snoRNAsiRNA (U14si), or U3snoRNAsiRNA (U3si); (c) Quantitative analysis of the colony forming assay (mean  $\pm$  standard deviation (SD);  $n = 3$ ; \*  $p$  value  $< 0.05$ ); (d) Western blot analysis of poly(ADP-ribose) polymerase (FL-PARP) cleavage into the 89 kDa fragment (PARP 89 kDa) after small nucleolar RNA (snoRNA) knockdown.  $\alpha$ -Tubulin served as a loading control.



**Figure S2.** Promoter activity of the isolated U3-, U8-, and U13snoRNA gene promoters in comparison to that of the SV40 early region. For details of the constructs see Figure 2a. Expression of firefly luciferase is expressed as luminescence units (mean  $\pm$  SD;  $n = 3$ ).