# Short loop-targeting oligoribonucleotides antagonize Lin28 and enable pre-let-7 processing and suppression of cell growth in let-7 deficient cancer cells.

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## Supplementary Materials

Table S1: Sequences of oligonucleotides used in the study, masses calculated and masses observed.

| Oligoribonucleotide name | Sequence 5’ -> 3’ | Type | Mass calc.[g/mol] | Mass obs [g/mol] |
|---|---|---|---|---|
| Let-7a-2 total *complementary* + primer + restriction sites | CCTCCACTTCAGCCAGGACTCGAGTTTTCATTTTGAAGGGGCCTCACCGAGTGGGGGCATCATCAAAAACTTT*AACTATACAACCTACTACCTCA*GGAGTCCCCTCACCTCCTCTAAGGTTGGGCAGGGTGACCCTGAAGTGAGCACAGCCTAGGGCTGAGCTGGGGACCTGGTGCGGCCGCTGAGTCTTCGGACCTCGC | DNA | - | - |
| Let-7a-2 total *complementary mutated* + primer + restriction sites | CCTCCACTTCAGCCAGGACTCGAGTTTTCATTTTGAAGGGGCCTCACCGAGTGGGGGCATCATCAAAAACTTT*AACTATACAACCTACGAACGCA*GGAGTCCCCTCACCTCCTCTAAGGTTGGGCAGGGTGACCCTGAAGTGAGCACAGCCTAGGGCTGAGCTGGGGACCTGGTGCGGCCGCTGAGTCTTCGGACCTCGC | DNA | - | - |
| Pre-let-7a-2 T7 DNA template | GGAAAGCTAGGAGGCTGTACAGTTATCTCCCTTGATGTAATTCTAAACTATACAACCTACTACCTCCTATAGTGAGTCGTATTAGGATCC | DNA | - | - |
| T7 DNA Polymerase Promotor | GGATCCTAATACGACTCACTATAG | DNA | - | - |
| siRNA lin28b5’ | AAAUCCUUCCAUGAAUAGUTT | RNA/DNA | 6852 | - |
| siRNA lin28b3’ | ACUAUUCAUGGAAGGAUUUTT | RNA/DNA | 6909 | - |
| siRen 5’ | GAGCGAAGAGGGCGAGAAAUU | RNA | 6901.31 | 6902.5 |
| siRen 3’ | UUUCUCGCCCUCUUCGCUCUU | RNA | 6436.78 | 6436.2 |
| Pre-let-7a-2 | UGAGGUAGUAGGUUGUAUAGUUUAGAAUUACAUCAAGGGAGAUAACUGUACAGCCUCCUAGCUUUCC | RNA | 21'502.9 | 21'503.3 |
| Pre-mir-18a + Biotin(X) | UAAGGUGCAUCUAGUGCAGAUAGUGAAGUAGAUUAGCAUCUACUGCCCUAAGUGCUCCUUCUGGX | RNA | - | - |
| Pre-mir-122 + Biotin (X) | UGGAGUGUGACAAUGGUGUUUGUGUCUAAACUAUCAAACGCCAUUAUCACACUAAAUAX | RNA | - | - |
| Pre-let-7a-2 truncated + linker + Biotin(X) | AGGUUGUAUAGUUUAGAAUUACAUCAAGGGAGAUAACUGUACAGCCUCTTTTTTUX | RNA/DNA | 18'039.3 | 18'039.6 |
| Lcon | AUGUAGUUCCCUC | 2’-OMe RNA | 4'220.8 | 4'220.1 |
| L35-09 | AUCUCCCUU | 2’-OMe RNA | 2'838.9 | 2'838.3 |
| L33-11 | AUCUCCCUUGA | 2’-OMe RNA | 3'541.4 | 3'540.8 |
| L31-13 | AUCUCCCUUGAUG | 2’-OMe RNA | 4'220.8 | 4'220.1 |
| L31-09 | CCCUUGAUG | 2’-OMe RNA | 2'918.0 | 2'917.2 |
| L30-14 | AUCUCCCUUGAUGU | 2’-OMe RNA | 4'541.0 | 4'540.3 |
| L30-13 | UCUCCCUUGAUGU | 2’-OMe RNA | 4'197.8 | 4'197.1 |
| L29-13 | CUCCCUUGAUGUA | 2’-OMe RNA | 4'220.8 | 4'220.2 |
| L29-13 + Cy5-label | CUCCCUUGAUGUA | 2’-OMe RNA | 4833.61 | 4832.8 |
| L29-09 | CUUGAUGUA | 2’-OMe RNA | 2'943.0 | 2'942.4 |
| General primer 1 forward | CCTCCACTTCAGCCAGGA | DNA | - | - |
| General primer 1 revers | GCGAGGTCCGAAGACTCA | DNA | - | - |

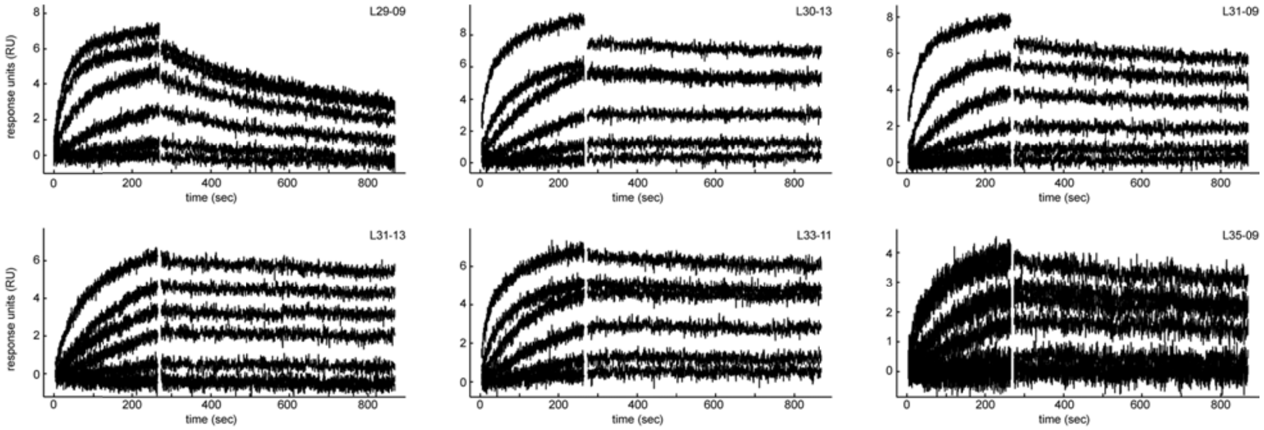

Fig. S1: Looptomirs assayed for binding to truncated biotinylated pre-let-7a-2 bound to the surface of a streptavidin-coated biosensor by surface plasmon resonance (SPR) spectroscopy.

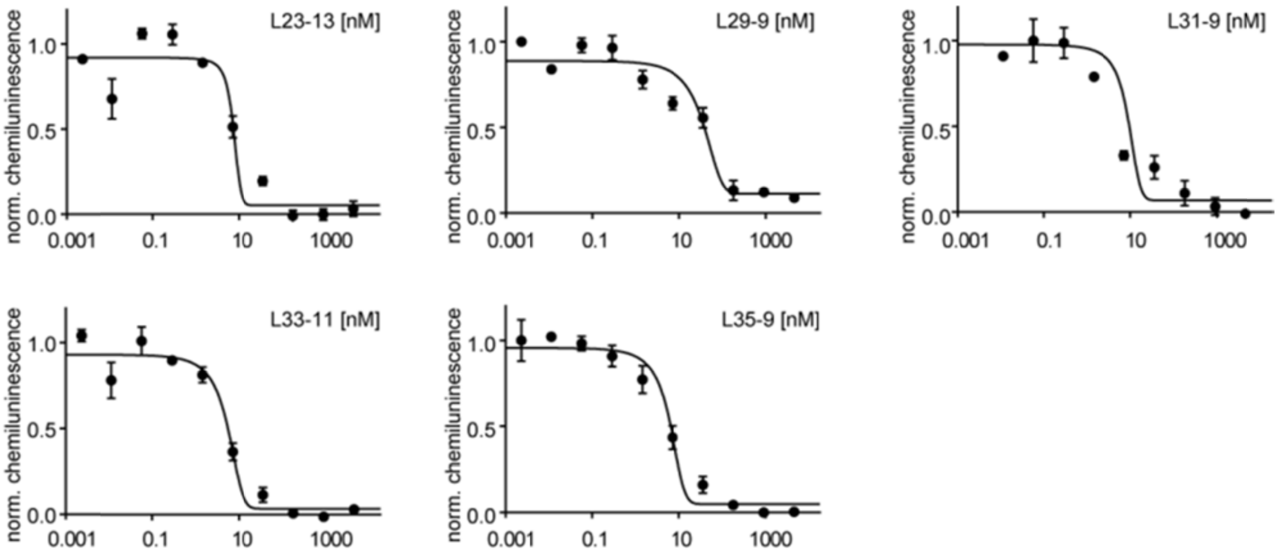

Fig. S2: Inhibition by looptomirs of Lin28A binding to immobilized pre-let-7a-2 (see Fig. 1 in main document).

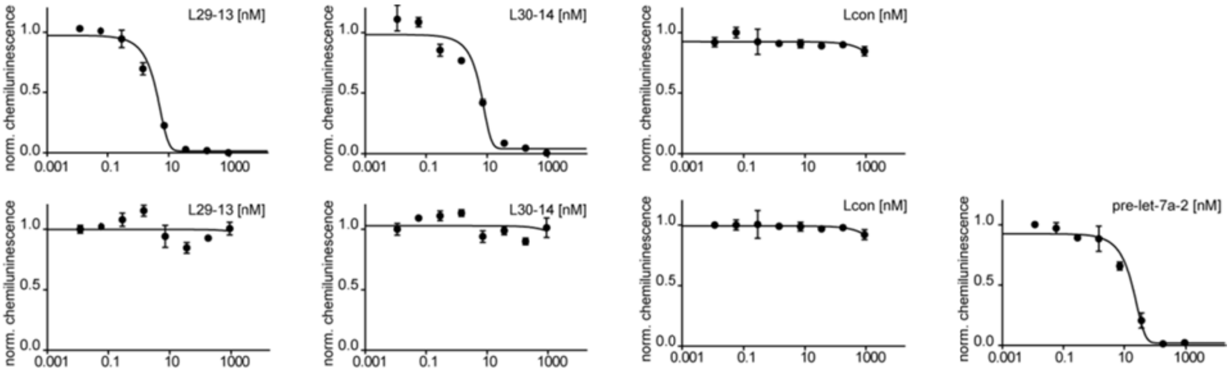

Fig. S3 Binding curves of looptomirs inhibiting Lin28 binding (upper panel) but not hnRNPA1 (lower panel) to pre-let-7a-2. The binding curves of the two proteins were assed in parallel.

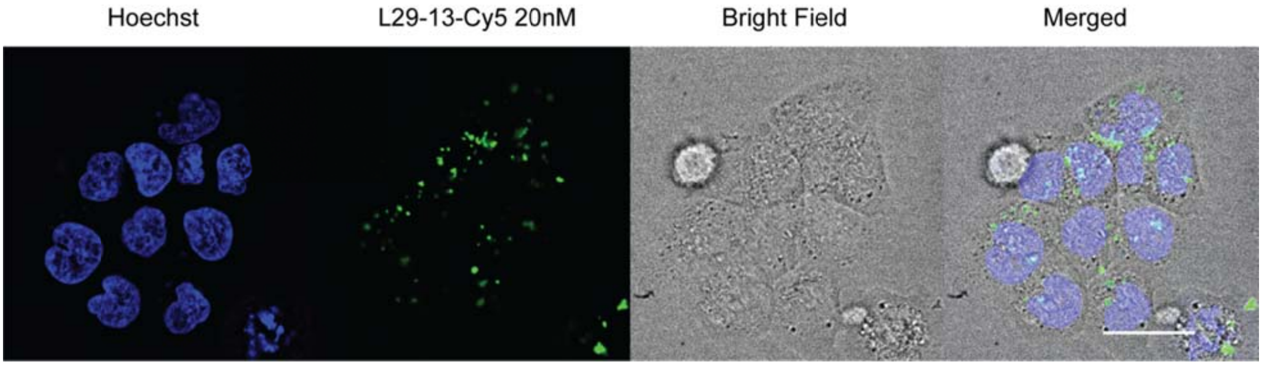

Figure S4: Cellular localization of looptomirs. Huh7 cells were transfected with Cy5-labelled **L29-13** (Table S1) at 20 nM concentrations under standard conditions (See Methods). **L29-13** accumulates in the nucleus and the cytoplasm 12 h post transfection as shown by live fluorescence images of **L29-13** (in green). Scale bar indicate 15 µm; picture post processing details are given in Materials and Methods.

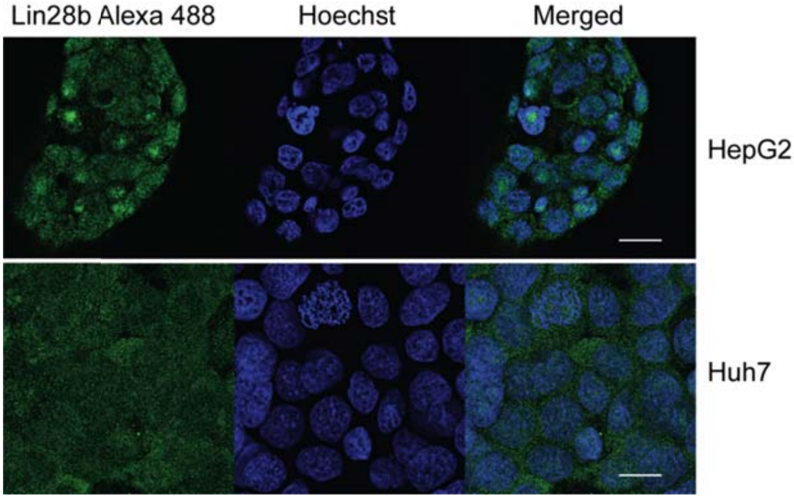

Figure S5: Detection of endogenous Lin28B (in green) by immunofluorescence in HepG2 and Huh7 cells; scale bars indicate 15 µm. Picture post processing details are given in Materials and Methods.

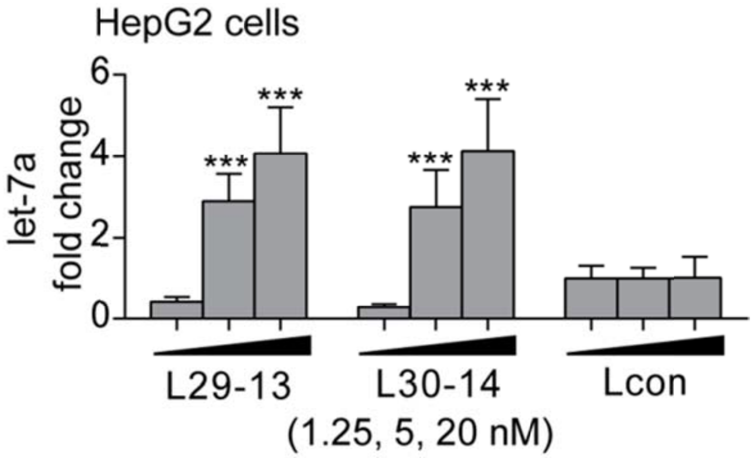

Figure S6: Endogenous levels of mature let-7a 48 h after transfections of **L29-13**, **L30-14** and **L$_{\mathbf{con}}$** into liver HepG2 cells, measured by TaqMan RT-qPCR (normalized to **L$_{\mathbf{con}}$**).

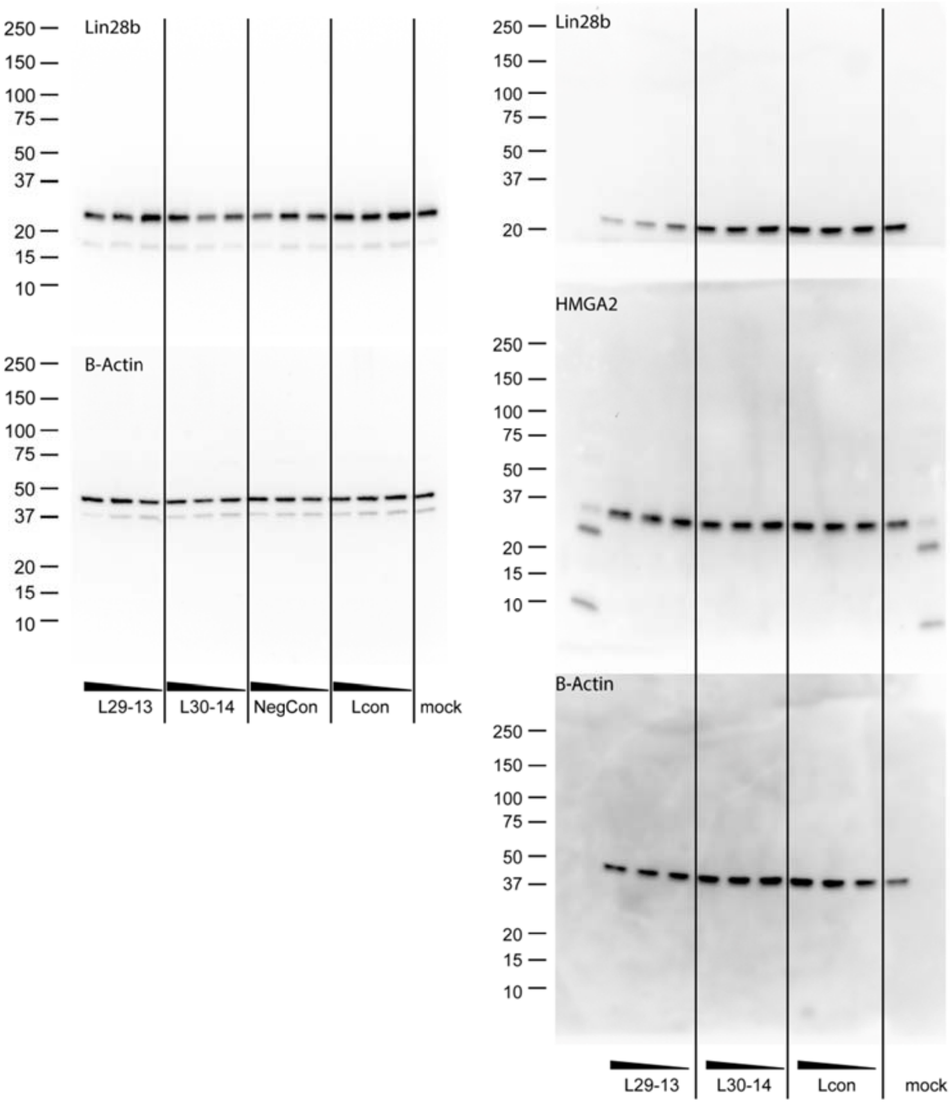

Figure S7 Uncropped Western blot membranes showing Lin28B and B-Actin detection in HepG2 cell lysate and Lin28B, HMGA2 and B-Actin detection in Huh7 cell lysate.

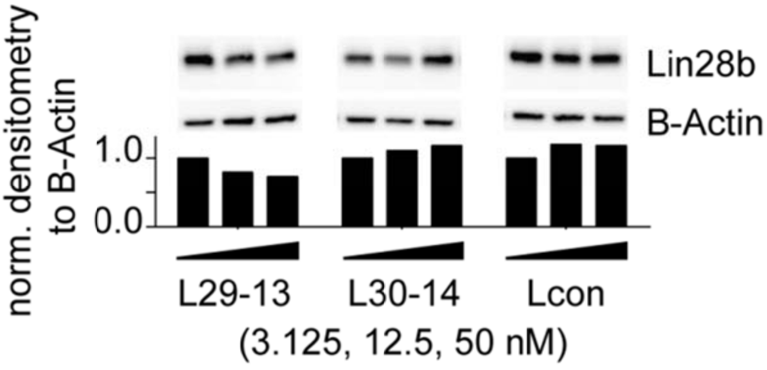

Figure S8: Western blot showing levels of let-7 target protein Lin28B after treatment of HepG2 cells with looptomirs. Uncropped pictures are shown in Fig. S7

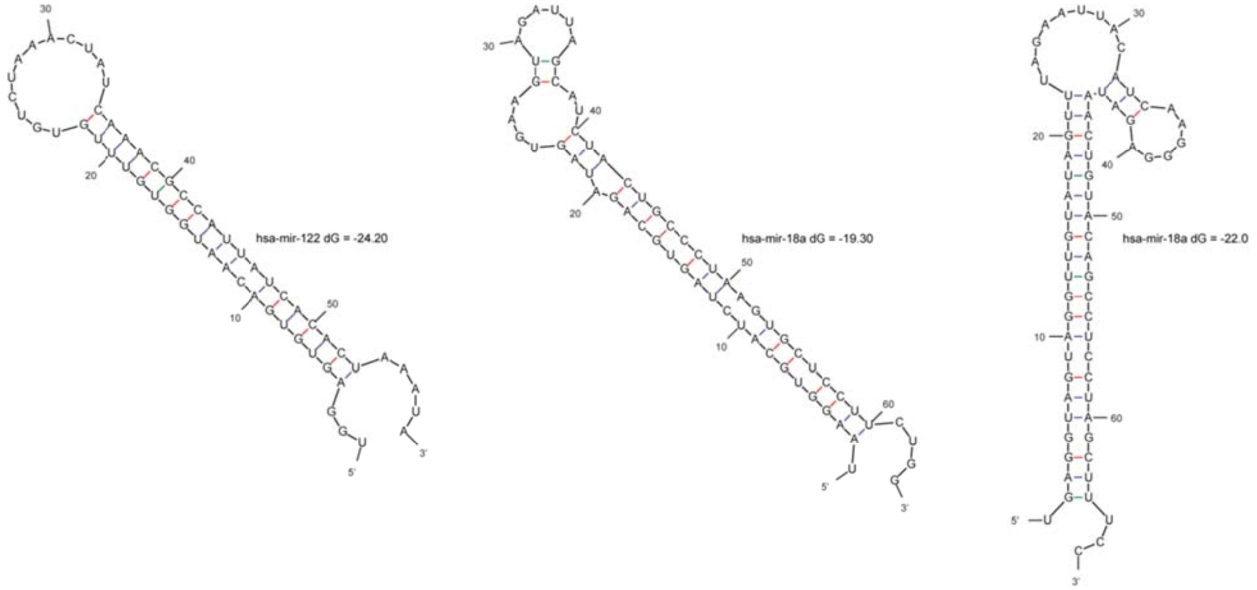

Fig. S9: Secondary structures of pre-miR-18a, pre-mir-122 and pre-let-7a-2 as predicted by Mfold (Waugh et al., 2002; Zuker, 2003; Zuker and Jacobson, 1998)).

## Literature

Waugh, A., Gendron, P., Altman, R., Brown, J.W., Case, D., Gautheret, D., Harvey, S.C., Leontis, N., Westbrook, J., Westhof, E., *et al.* (2002). RNAML: a standard syntax for exchanging RNA information. RNA *8*, 707-717.

Zuker, M. (2003). Mfold web server for nucleic acid folding and hybridization prediction. Nucleic Acids Res *31*, 3406-3415.

Zuker, M., and Jacobson, A.B. (1998). Using reliability information to annotate RNA secondary structures. RNA *4*, 669-679.