Supplementary Data

Table S1. Screening set of pre-miRNAs. Sequences were retrieved from miRBase or published data. The oligoribonucleotides were chemically synthesized in biotinylated and non-biotinylated forms. BioTEG-Q is a biotin derivative with a tetraethyleneglycol type linker. See Material and Methods section for details.

Designation	Sequence (in 5' to 3' direction)
hsa-let-7a-1	UGAGGUAGUAGGUUGUAUAGUUUUAGGGUCACACCCACCACUGGGAGAUAACUAUACAAUCUACUGUCUUUC[BioTEG-Q]
hsa-let-7a-2	UGAGGUAGUAGGUUGUAUAGUUUAGAAUUACAUCAAGGGAGAUAACUGUACAGCCUCCUAGCUUUCC[BioTEG-Q]
hsa-let-7c	UGAGGUAGUAGGUUGUAUGGUUUAGAGUUACACCCUGGGAGUUAACUGUACAACCUUCUAGCUUUCC[BioTEG-Q]
hsa-let-7d	AGAGGUAGUAGGUUGCAUAGUUUUAGGGCAGGGAUUUUGCCCACAAGGAGGUAACUAUACGACCUGCUGCCUUUC[BioTEG-Q]
hsa-let-7f-1	UGAGGUAGUAGAUUGUAUAGUUGUGGGGUAGUGAUUUUACCCUGUUCAGGAGAUAACUAUACAAUCUAUUGCCUUCCC[BioTEG-Q]
hsa-let-7f-2	UGAGGUAGUAGAUUGUAUAGUUUUAGGGUCAUACCCCAUCUUGGAGAUAACUAUACAGUCUACUGUCUUUCC[BioTEG-Q]
hsa-let-7g	UGAGGUAGUAGUUUGUACAGUUUGAGGGUCUAUGAUACCACCCGGUACAGGAGAUAACUGUACAGGCCACUGCCUUGC[BioTEG-Q]
hsa-let-7i	UGAGGUAGUAGUUUGUGCUGUUGGUCGGGUUGUGACAUUGCCCGCUGUGGAGAUAACUGCGCAAGCUACUGCCUUGCU[BioTEG-Q]
hsa-mir-101-1	CAGUUAUCACAGUGCUGAUGCUGUCUAUUCUAAAGGUACAGUACUGUGAUAACUGAA[BioTEG-Q]
hsa-mir-101-2	CGGUUAUCAUGGUACCGAUGCUGUAUAUCUGAAAGGUACAGUACUGUGAUAACUGAA[BioTEG-Q]
hsa-mir-103-1	UCGGCUUCUUUACAGUGCUGCCUUGUUGCAUAUGGAUCAAGCAGCAUUGUACAGGGCUAUGA[BioTEG-Q]
hsa-mir-106b	UAAAGUGCUGACAGUGCAGAUAGUGGUCCUCUCCGUGCUACCGCACUGUGGGUACUUGCUGC[BioTEG-Q]
hsa-mir-107	UCAGCUUCUUUACAGUGUUGCCUUGUGGCAUGGAGUUCAAGCAGCAUUGUACAGGGCUAUCA[BioTEG-Q]
hsa-mir-10a	UACCCUGUAGAUCCGAAUUUGUGUAAGGAAUUUUGUGGUCACAAAUUCGUAUCUAGGGGAAUA[BioTEG-Q]
hsa-mir-10b	UACCCUGUAGAACCGAAUUUGUGUGGUAUCCGUAUAGUCACAGAUUCGAUUCUAGGGGAAUA[BioTEG-Q]
hsa-mir-1-2	ACAUACUUCUUUAUGUACCCAUAUGAACAUACAAUGCUAUGGAAUGUAAAGAAGUAUGUAU[BioTEG-Q]
hsa-mir-125b-1	UCCCUGAGACCCUAACUUGUGAUGUUUACCGUUUAAAUCCACGGGUUAGGCUCUUGGGAGCU[BioTEG-Q]
hsa-mir-128-1	CGGGGCCGUAGCACUGUCUGAGAGGUUUACAUUUCUCACAGUGAACCGGUCUCUUU[BioTEG-Q]
hsa-mir-133a-1	GCUGGUAAAAUGGAACCAAAUCGCCUCUUCAAUGGAUUUGGUCCCCUUCAACCAGCUG[BioTEG-Q]
hsa-mir-134	UGUGACUGGUUGACCAGAGGGGCAUGCACUGUGUUCACCCUGUGGGCCACCUAGUCACCAA[BioTEG-Q]
hsa-mir-135a-2	UAUGGCUUUUUAUUCCUAUGUGAUAGUAAUAAAGUCUCAUGUAGGGAUGGAAGCCAUGAA[BioTEG-Q]
hsa-mir-136	ACUCCAUUUGUUUUGAUGAUGGAUUCUUAUGCUCCAUCAUCGUCUCAAAUGAGUCU[BioTEG-Q]
hsa-mir-137	ACGGGUAUUCUUGGGUGGAUAAUACGGAUUACGUUGUUAUUGCUUAAGAAUACGCGUAG[BioTEG-Q]
hsa-mir-138-2	AGCUGGUGUUGUGAAUCAGGCCGACGAGCAGCGCAUCCUCUUACCCGGCUAUUUCACGACACCAGGGUU[BioTEG-Q]
hsa-mir-140	CAGUGGUUUUACCCUAUGGUAGGUUACGUCAUGCUGUUCUACCACAGGGUAGAACCACGG[BioTEG-Q]
hsa-mir-142	CAUAAAGUAGAAAGCACUACUAACAGCACUGGAGGGUGUAGUGUUUCCUACUUUAUGGA[BioTEG-Q]
hsa-mir-147b	GUGGAAACAUUUCUGCACAAACUAGAUUCUGGACACCAGUGUGCGGAAAUGCUUCUGCUA[BioTEG-Q]
hsa-mir-148a	AAAGUUCUGAGACACUCCGACUCUGAGUAUGAUAGAAGUCAGUGCACUACAGAACUUUGU[BioTEG-Q]
hsa-mir-153-2	UCAUUUUUGUGAUGUUGCAGCUAGUAAUAUGAGCCCAGUUGCAUAGUCACAAAAGUGAUC[BioTEG-Q]
hsa-mir-15a	UAGCAGCACAUAAUGGUUUGUGGAUUUUGAAAAGGUGCAGGCCAUAUUGUGCUGCCUCA[BioTEG-Q]
hsa-mir-16-1	UAGCAGCACGUAAAUAUUGGCGUUAAGAUUCUAAAAUUAUCUCCAGUAUUAACUGUGCUGCUGAA[BioTEG-Q]
hsa-mir-181a-2	AACAUUCAACGCUGUCGGUGAGUUUGGGAUUUGAAAAAACCACUGACCGUUGACUGUACC[BioTEG-Q]
hsa-mir-181b-1	AACAUUCAUUGCUGUCGGUGGGUUGAACUGUGUGGACAAGCUCACUGAACAAUGAAUGCAAC[BioTEG-Q]
hsa-mir-181c	AACAUUCAACCUGUCGGUGAGUUUGGGCAGCUCAGGCAAACCAUCGACCGUUGAGUGGAC[BioTEG-Q]
hsa-mir-18a	UAAGGUGCAUCUAGUGCAGAUAGUGAAGUAGAUUAGCAUCUACUGCCCUAAGUGCUCCUUCUGG[BioTEG-Q]
hsa-mir-18b	UAAGGUGCAUCUAGUGCAGUUAGUGAAGCAGCUUAGAAUCUACUGCCCUAAAUGCCCCUUCUGGC[BioTEG-Q]
hsa-mir-190	UGAUAUGUUUGAUAUAUUAGGUUGUUAUUUAAUCCAACUAUAUAUCAAACAUAUUCCU[BioTEG-Q]
hsa-mir-193b	CGGGGUUUUGAGGGCGAGAUGAGUUUAUGUUUUAUCCAACUGGCCCUCAAAGUCCCGCU[BioTEG-Q]
hsa-mir-196b	UAGGUAGUUUCCUGUUGUUGGGAUCCACCUUUCUCUCGACAGCACGACACUGCCUUCA[BioTEG-Q]
hsa-mir-19a	AGUUUUGCAUAGUUGCACUACAAGAAGAAUGUAGUUGUGCAAAUCUAUGCAAAACUGA[BioTEG-Q]
hsa-mir-204	UUCCCUUUGUCAUCCUAUGCCUGAGAAUAUAUGAAGGAGGCUGGGAAGGCAAAGGGACGU[BioTEG-Q]
hsa-mir-20b	CAAAGUGCUCAUAGUGCAGGUAGUUUUGGCAUGACUCUACUGUAGUAUGGGCACUUCCAG[BioTEG-Q]
hsa-mir-21	UAGCUUAUCAGACUGAUGUUGACUGUUGAAUCUCAUGGCAACACCAGUCGAUGGGCUGU[BioTEG-Q]
hsa-mir-214	UGCCUGUCUACACUUGCUGUGCAGAACAUCCGCUCACCUGUACAGCAGGCACAGACAGGCAGU[BioTEG-Q]
hsa-mir-22	AGUUCUUCAGUGGCAAGCUUUAUGUCCUGACCCAGCUAAAGCUGCCAGUUGAAGAACUGU[BioTEG-Q]

hsa-mir-224	CAAGUCACUAGUGGUUCCGUUUAGUAGAUGAUUGUGCAUUGUUUCAAAAUGGUGCCCUAGUGACUACA[BioTEG-Q]
hsa-mir-24-1	UGCCUACUGAGCUGAUAUCAGUUCUCAUUUUACACACUGGCUCAGUUCAGCAGGAACAG[BioTEG-Q]
hsa-mir-25	AGGCGGAGACUUGGGCAAUUGCUGGACGCUGCCCUGGGCAUUGCACUUGUCUCGGUCUGA[BioTEG-Q]
hsa-mir-28	AAGGAGCUCACAGUCUAUUGAGUUACCUUUCUGACUUUCCCACUAGAUUGUGAGCUCCUGGA[BioTEG-Q]
hsa-mir-299	UGGUUUACCGUCCCACAUACAUUUUGAAUAUGUAUGUGGGAUGGUAAACCGCUU[BioTEG-Q]
hsa-mir-29a	ACUGAUUUCUUUUGGUGUUCAGAGUCAAUAUAAUUUUCUAGCACCAUCUGAAAUCGGUUA[BioTEG-Q]
hsa-mir-29b-1	CUGGUUUCAUAUGGUGGUUUAGAUUUAAAUAGUGAUUGUCUAGCACCAUUUGAAAUCAGUGUU[BioTEG-Q]
hsa-mir-29b-2	CUGGUUUCACAUGGUGGCUUAGAUUUUUCCAUCUUUGUAUCUAGCACCAUUUGAAAUCAGUGUU[BioTEG-Q]
hsa-mir-29c	UGACCGAUUUCUCCUGGUGUUCAGAGUCUGUUUUUGUCUAGCACCAUUUGAAAUCGGUUAUG[BioTEG-Q]
hsa-mir-302a	ACUUAAACGUGGAUGUACUUGCUUUGAAACUAAAGAAGUAAGUGCUUCCAUGUUUUGGUGA[BioTEG-Q]
hsa-mir-302c	UUUAACAUGGGGGUACCUGCUGUGUGAAACAAAAGUAAGUGCUUCCAUGUUUCAGUGG[BioTEG-Q]
hsa-mir-30a	UGUAAACAUCCUCGACUGGAAGCUGUGAAGCCACAGAUGGGCUUUCAGUCGGAUGUUUGCAGC[BioTEG-Q]
hsa-mir-30c-1	UGUAAACAUCCUACACUCUCAGCUGUGAGCUCAAGGUGGCUGGGAGAGGGUUGUUUACUCC[BioTEG-Q]
hsa-mir-30c-2	UGUAAACAUCCUACACUCUCAGCUGUGGAAAGUAAGAAAGCUGGGAGAAGGCUGUUUACUCU[BioTEG-Q]
hsa-mir-31	AGGCAAGAUGCUGGCAUAGCUGUUGAACUGGGAACCUGCUAUGCCAACAUAUUGCCAU[BioTEG-Q]
hsa-mir-32	UAUUGCACAUUACUAAGUUGCAUGUUGUCACGGCCUCAAUGCAAUUUAGUGUGUGUGAUAUUU[BioTEG-Q]
hsa-mir-323	AGGUGGUCCGUGGCGCGUUCGCUUUAUUUAUGGCGCACAUUACACGGUCGACCUCU[BioTEG-Q]
hsa-mir-331	CUAGGUAUGGUCCCAGGGAUCCCAGAUCAAACCAGGCCCCUGGGCCUAUCCUAGAA[BioTEG-Q]
hsa-mir-34b	UAGGCAGUGUCAUUAGCUGAUUGUACUGUGGUGGUUACAAUCACUAACUCCACUGCCAUCA[BioTEG-Q]
hsa-mir-34c	AGGCAGUGUAGUUAGCUGAUUGCUAAUAGUACCAAUCACUAACCACACGGCCAGG[BioTEG-Q]
hsa-mir-361	UUAUCAGAAUCUCCAGGGGUACUUUAUAAUUUCAAAAAGUCCCCCAGGUGUGAUUCUGAUUU[BioTEG-Q]
hsa-mir-365-1	GAGGGACUUUUGGGGGCAGAUGUGUUUCCAUUCCACUAUCAUAAUGCCCCUAAAAAUCCUUAU[BioTEG-Q]
hsa-mir-370	CAGGUCACGUCUCUGCAGUUACACAGCUCACGAGUGCCUGCUGGGGUGGAACCUGGU[BioTEG-Q]
hsa-mir-373	ACUCAAAAUGGGGGCGCUUUCCUUUUUGUCUGUACUGGGAAGUGCUUCGAUUUUGGGGUGU[BioTEG-Q]
hsa-mir-375	GCGACGAGCCCCUCGCACAAACCGGACCUGAGCGUUUUGUUCGUUCGGCUCGCGUGA[BioTEG-Q]
hsa-mir-376a-1	GUAGAUUCUCCUUCUAUGAGUACAUUAUUUAUGAUUAAUCAUAGAGGAAAAUCCACGU[BioTEG-Q]
hsa-mir-377	AGAGGUUGCCCUUGGUGAAUUCGCUUUAUUUAUGUUGAAUCACACAAAGGCAACUUUUGU[BioTEG-Q]
hsa-mir-378	CUCCUGACUCCAGGUCCUGUGUGUUACCUAGAAAUAGCACUGGACUUGGAGUCAGAAGGC[BioTEG-Q]
hsa-mir-379	UGGUAGACUAUGGAACGUAGGCGUUAUGAUUUCUGACCUAUGUAACAUGGUCCACUAACU[BioTEG-Q]
hsa-mir-382	GAAGUUGUUCGUGGUGGAUUCGCUUUACUUAUGACGAAUCAUUCACGGACAACACUUUU[BioTEG-Q]
hsa-mir-383	AGAUCAGAAGGUGAUUGUGGCUUUGGGUGGAUAUUAAUCAGCCACAGCACUGCCUGGUCAGA[BioTEG-Q]
hsa-mir-411	UAGUAGACCGUAUAGCGUACGCUUUAUCUGUGACGUAUGUAACACGGUCCACUAACC[BioTEG-Q]
hsa-mir-423	UGAGGGGCAGAGAGCGAGACUUUUCUAUUUUCCAAAAGCUCGGUCUGAGGCCCCUCAGU[BioTEG-Q]
hsa-mir-424	CAGCAGCAAUUCAUGUUUUGAAGUGUUCUAAAUGGUUCAAAACGUGAGGCGCUGCUAUA[BioTEG-Q]
hsa-mir-425	AAUGACACGAUCACUCCCGUUGAGUGGGCACCCGAGAAGCCAUCGGGAAUGUCGUGUCCGCCC[BioTEG-Q]
hsa-mir-452	CUUACAACUGUUUGCAGAGGAAACUGAGACUUUGUAACUAUGUCUCAGUCUCAUCUGCAAAGAAGUAAGUG[BioTEG-Q]
hsa-mir-495	GAAGUUGCCCAUGUUAUUUUCGCUUUAUAUGUGACGAAACAAACAUGGUGCACUUCUU[BioTEG-Q]
hsa-mir-497	CAGCAGCACACUGUGGUUUGUACGGCACUGUGGCCACGUCCAAACCACACUGUGGUGUUAGA[BioTEG-Q]
hsa-mir-539	GGAGAAAUUAUCCUUGGUGUGUUCGCUUUAUUUAUGAUGAAUCAUACAAGGACAAUUUCUUUUU[BioTEG-Q]
hsa-mir-582	UUACAGUUGUUCAACCAGUUACUAAUCUAACUAAUUGUAACUGGUUGAACAACUGAACC[BioTEG-Q]
hsa-mir-592	UUGUGUCAAUAUGCGAUGAUGUGUUGUGAUGGCACAGCGUCAUCACGUGGUGACGCAACA[BioTEG-Q]
hsa-mir-604	GCUUGACCUUCCACGCUCUCGUGUCCACUAGCAGGCAGGUUUUCUGACACAGGCUGCGGAAUUCAGGAC[BioTEG-Q]
hsa-mir-628	AUGCUGACAUAUUUACUAGAGGGUAAAAUUAAUAACCUUCUAGUAAGAGUGGCAGUCGA[BioTEG-Q]
hsa-mir-7-1	UGGAAGACUAGUGAUUUUGUUGUUUUUAGAUAACUAAAUCGACAACAAAUCACAGUCUGCCAUA[BioTEG-Q]
hsa-mir-873	GCAGGAACUUGUGAGUCUCCUAUUGAAAAUGAACAGGAGACUGAUGAGUUCCCGGGA[BioTEG-Q]
hsa-mir-874	GGCCCCACGCACCAGGGUAAGAGAGACUCUCGCUUCCUGCCCUGGCCCGAGGGACCGA[BioTEG-Q]
hsa-mir-876	UGGAUUUCUUUGUGAAUCACCAUAUCUAAGCUAAUGUGGUGGUGGUUUACAAAGUAAUUCAUA[BioTEG-Q]
hsa-mir-9-1	UCUUUGGUUAUCUAGCUGUAUGAGUGGUGUGGAGUCUUCAUAAAGCUAGAUAACCGAAAGU[BioTEG-Q]
hsa-mir-9-2	UCUUUGGUUAUCUAGCUGUAUGAGUGUAUUGGUCUUCAUAAAGCUAGAUAACCGAAAGU[BioTEG-Q]
hsa-mir-96	UUUGGCACUAGCACAUUUUUGCUUGUGUCUCUCCGCUCUGAGCAAUCAUGUGCAGUGCCAAUAUG[BioTEG-Q]

Table S2. Sequences of forward (F) and reverse (R) PCR primers used for determination of miRNA precursors.

Name	Sequence (all in 5' to 3' direction)
let7a1-pre-F	GGTAGTAGGTTGTATAGTTTTAGGGTCA
let7a1-pre-R	AGATTGTATAGTTATCTCCCAGTGGT
let7a2-pre-F	GGTTGTATAGTTTAGAATTACATCAAGG
let7a2-pre-R	GAAAGCTAGGAGGCTGTACAGTTA
let7c-pre-F	GAGGTAGTAGGTTGTATGGTTTAGAGT
let7c-pre-R	GGAAAGCTAGAAGGTTGTACAGTT
let7d-pre-F	TTTAGGGCAGGGATTTTGC
let7d-pre-R	AGAAAGGCAGCAGGTCGTAT
let7f-1-pre-F	GATTGTATAGTTGTGGGGTAGTGA
let7f-1-pre-R	GCAATAGATTGTATAGTTATCTCCTGA
let7f-2-pre-F	TGAGGTAGTAGATTGTATAGTTTTAGGG
let7f-2-pre-R	GTAGACTGTATAGTTATCTCCAAGATGG
let7g-pre-F	CAGTTTGAGGGTCTATGATACCAC
let7g-pre-R	GCAAGGCAGTGGCCTGTA
let7i-pre-F	CTGTTGGTCGGGTTGTGAC
let7i-pre-R	AGCTTGCGCAGTTATCTCCA
mir18a-pre-F	GGTGCATCTAGTGCAGATAGTGA
mir18a-pre-R	GAAGGAGCACTTAGGGCAGT
mir107-pre-F	TCTCTGCTTTCAGCTTCTTTACAGT
mir107-pre-R	GTACAATGCTGCTTGAACTCCAT

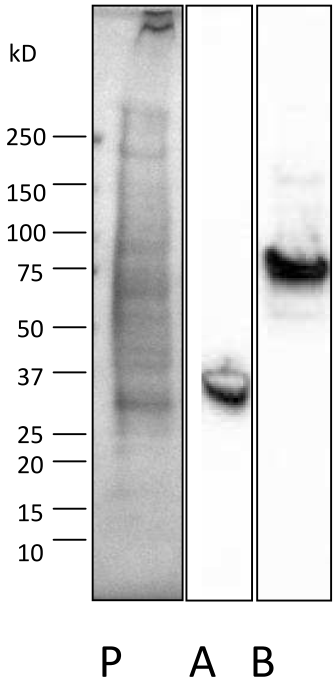

Figure S1. Western blots of Hela lysate developed with antibodies against hnRNP A1, clone 3B10 (lane **A**) and against KSRP (polyclonal antibody, lane **B**). Molecular weight standards and a Ponceau stained lane (**P**) are shown. Lane A contained 5 µg and lanes **B** and **P** 50 µg of total lysate.

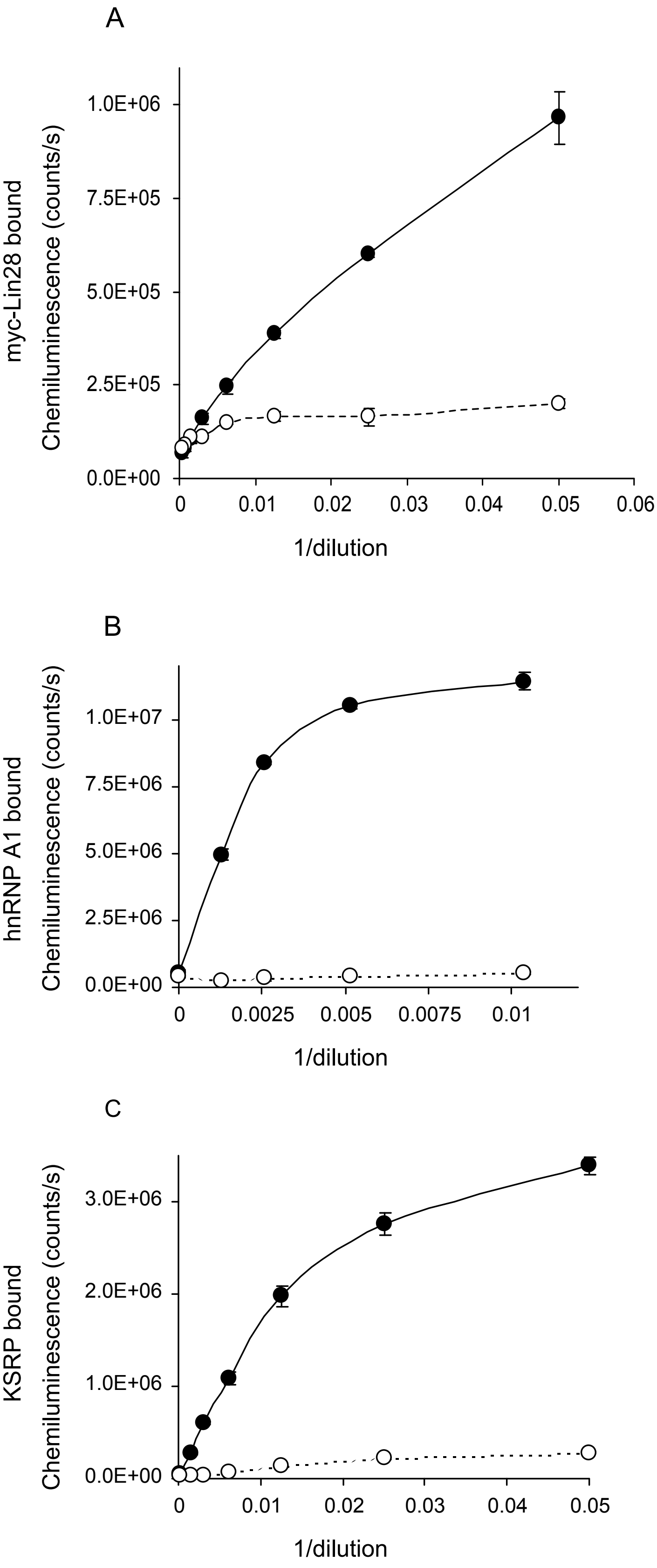

Figure S2. Examples of lysate titration curves. (**A**) Lin28 binding to biotinylated pre-let-7a-2. Source of Lin28 were lysates of HEK293T cells transfected with myc-Lin28 plasmid; detection with a myc-specific monoclonal antibody. (**B**) hnRNP A1 binding to biotinylated hnRNP A1 consensus sequence in Hela cell lysates with detection by an hnRNP A1 specific monoclonal antibody. (**C**) KSRP binding to biotinylated pre-mir-1-2 in Hela cell lysates with detection by a polyclonal antibody againt KSRP. Filled symbols refer to signals of wells coated with streptavidin and biotinylated RNAs; open symbols represent signals of wells coated with strepavidin only. No backgrounds subtracted. Error bars are standard deviations (N=2-4).

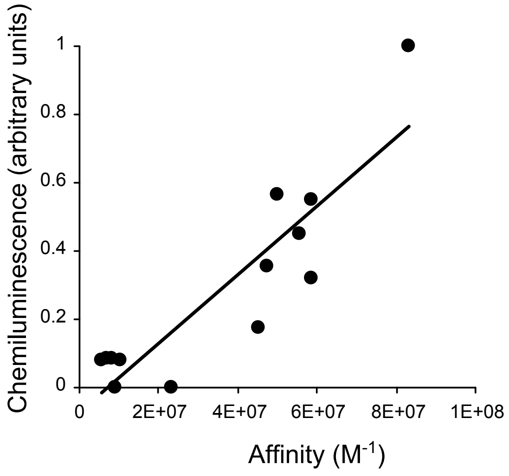

Figure S3. Correlation between signals of hnRNP A1 binding to immobilized biotinylated pre-miRNAs and affinity determined by competition assay. Data are taken from Figures 2C and 3. Affinity = 1/Kd. Kd-values that could not be determined (> 150 nM) were omitted from the analysis. The line indicates the linear least square regression line; R^2=0.795 (Pearson correlation coefficient)..