Supplementary Information

A small-molecule inhibitor of Lin28

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Supplementary Information

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Supplementary Methods

Synthesis of labeled oligoribonucleotides

Chemicals were purchased from Aldrich and TCI and Maybridge and phosphoramidites from Thermo Fisher. The activator 5-benzylthiotetrazole was purchased from Biosolve. Oligoribonucleotides were synthesized with regular 2'-*O*-TBDMS-phosphoramidites on a 50 nmol scale. 2'-*O*-Me-RNA were prepared under similar conditions with corresponding 2'-*O*-Me-phosphoramidites (not subjected to HF.Et3N treatment as described below). All oligonucleotides were synthesized with a MM12 synthesizer from Bio Automation Inc. on 1000 Å UnyLinker CPG from ChemGenes with a coupling time for phosphoramidites of 2 x 90 s. The oligoribonucleotides were purified DMT-on and DMT-off by RP-HPLC. The isolated synthesis product was dried in a SpeedVac and treated for 1 h with 40% aq. acetic acid at room temperature. After drying in a SpeedVac, the oligoribonucleotide was dissolved in 200 µL of water and purified DMT-off by RP-HPLC. The isolated product was dried in a SpeedVac and diluted with water to 20 µM concentration. Mass and purity (>95%) was confirmed by LCMS (Figure S1).

Purification of the oligonucleotides was carried out on an Agilent 1200 series preparative HPLC on a Waters XBridge OST C-18 column, 10 x 50 mm, 2.5 µm at 65 °C. Solvents of elution were: Solvent A, 0.1M aqueous triethylamine/acetic acid, pH 8.0; solvent B: 100 % MeOH with a flow-rate of 5 mL/min. Gradient for the DMT-on purification: 5 % to 100 % solvent B over 10 min. Gradient for the DMT-off purification: 5 % to 60 % solvent B over 7 min then to 90% for 2 min. Collected fractions containing the product were dried in a miVac duo SpeedVac from Genevac. The oligonucleotides were analyzed by LC-MS (Agilent 1200/6130 system) on a Waters Acquity OST C-18 column, 2.1 x 50 mm, 1.7 µM, 65 °C. Solvent A: 0.4M HFIP, 15 mM triethylamine; solvent B: MeOH. Gradient: 5-80 % B in 10 min with a flow-rate: 0.3 mL/min.

Optimization of the FRET acceptor by spectrofluorometry

The FRET assay contains N-terminally EGFP-tagged Lin28B as donor and a truncated pre-let-7a-2 as acceptor, which is labeled with Cy3 fluorescent dye or BHQ-1 quencher (Figure 1b). Assays were carried out on a spectrofluorometer (PTI, Edison New Jersey) in a 500µL cuvette. Briefly, EGFP-Lin28B lysate diluted with binding buffer (1:10) was mixed with various concentrations of labeled pre-let-7a-2 (0 nM, 0.313 nM, 1.25 nM, 5 nM, 20 nM and 80 nM) individually. Solutions were incubated for 30 min and their fluorescence spectra were acquired between 475-600 nm with excitation of the sample at 465 nm. The excitation wavelength was chosen to minimize excitation of Cy3. FRET efficiency was determined as:

$E = 1 - I_{Donor+Acceptor} / I_{Donor}$, where $I_{Donor+Acceptor}$ is EGFP fluorescent signal intensity (at its maximum: 507 nm) of solutions containing GFP-tagged Lin28B with various concentrations of labeled pre-let-7a-2, and IDonor is fluorescent signal intensity of a solution containing only GFP-tagged Lin28B. For Cy3-let7 acceptors, correction from Cy3 spectral bleedthrough was carried out by subtracting the background signal from Cy3 in buffer with excitation at 465nm. For BHQ acceptors, signal correction was not necessary. Each single experiment was carried out at least in duplicates with different batches of EGFP-Lin28B lysate, with adjusted concentration to allow similar initial fluorescence.

Cell cultures and transfections

HEK 293-T cells and Huh-7 cells (ATCC) were cultured as monolayers in DMEM GlutaMAXTM-I (31966-021, Gibco®, Life Technologies, Carlsbad) supplemented with 10% of FBS (fetal bovine serum). Stable EGFP-Lin28B HEK 293-T cells were cultured as monolayers in DMEM GlutaMAXTM-I (31966-021, Gibco®, Life Technologies, Carlsbad) supplemented with 10% of FBS (fetal bovine serum) and 500 µg/ml Geneticin G418 (10131-035, Life Technologies, Carlsbad). Transfections were performed according to the manufacturer's protocol with Oligofectamine 2000 (12252-011, Invitrogen, Life Technologies, Carlsbad) for siRNAs and JetPEI (101-10, Polyplus transfections, Illkirch) was used for plasmid DNA. For cellular treatment the small molecules were dissolved in DMSO resulting in a maximum 1% DMSO content in the cell growth media.

HEK 293 T stable cell line

160’000 HEK 293 T cells were seeded per well in 6 well plates and transfected with 320 ng pEGFP-C2-Lin28B plasmid according to the experimental setup with the reagents described above and cells were allowed to recover for 48h. To start selection, cell growth medium was changed by adding selective medium DMEM GlutaMAXTM-I containing 0.5 mg/ml Geneticin (G-418 Sulphate, 108321-42-2, Gibco®, Life Technologies, Carlsbad) and cells were reseeded in 6 cm diameter dishes. Geneticin concentration of medium was increased two days later to 1 mg/ml and antibiotics containing media was replaced every second day for two further weeks. Subsequently, selective antibiotic concentration in the medium was decreased to 0.5 mg/ml and positive clones were selected by fluorescent microscopy transferring positive clones to individual wells of a 96 well plate. Antibiotic selection was maintained for one further week. Finally, larger colonies of individual clones were analyzed for expression levels of EGFP-Lin28B by performing the FRET assay described previously in this text.

PsiCHECK-2 reporter constructs

The target sequences were amplified from synthetic DNA using oligonucleotides (Table S2) from Microsynth (Balgach) and cloned into the NotI and XhoI restriction sites of psiCHECK-2 plasmid (C8021, Promega, Fitchburg). The primers used for cloning as well as the inserted sequences in the psiCHECK2 vector are reported in table S2. Further cloning and purification procedure was done as described previously in [1].

pEGFP-C2 Lin28B plasmid

pCMV6-XL4 plasmid containing cDNA from Lin28B was commercially obtained (Homo sapiens lin-28 homolog B, SC300636, OriGene Technologies, Rockville). The PCR product of Lin28B was subcloned into pEGFP-C2 vector (GenBank Accession 57606, Catalog 6083-1, Clontech Laboratories former BD Biosciences Clontech, Mountain View) at the SacI and SacII sites. Forward and reverse primers used for Lin28B sequence amplification are reported in table S2. Bacterial Transformation, PCR purification and Miniprep was performed as described earlier.

qRT-PCR

Total RNA was extracted using the RNeasy kit (74104, Qiagen, Venlo). TaqMan® qRT-PCR was performed using standard reagents from Life Technologies (TaqMan® MicroRNA

Assays: hsa-let-7a: 000377, hsa-let-7c: 000379, hsa-let-7e: 002406, hsa-let-7f: 000382, hsa-let-7g: 002282, hsa-mir-15a: 000369, RNU44: 001094). The RT was performed using the TaqMan® primers from MicroRNA Assays and the TaqMan® MicroRNA Reverse Transcription Kit (4366596, Life technologies, Carlsbad) with 20 ng total RNA. The PCR was performed in a LightCycler 480 instrument (Roche, Penzberg) with GoTaq® Probe qPCR Master mix (A6102, Promega, Fitchburg) according to the manufacturer's protocol. Each reaction was carried out in four technical replicates. Ct values were calculated for each and averaged.

SYBR Green qRT-PCR was performed with total RNA extracted in the same way as described above. RT was performed with the High-Capacity cDNA Reverse Transcription Kit (4374967, Life technologies, Carlsbad) and oligo-dT (C1101, Promega, Fitchburg). The SYBR Green PCR was performed in a LightCycler 480 instrument (Roche, Penzberg) with FastStart Universal SYBR Green Master (Rox) (04913914001, Roche, Penzberg). Each reaction was carried out in three technical replicates and the corresponding Ct values were calculated for each and averaged.

mESC treatments

E14 mESC line (192/Ola background) (Hooper et al, 1987) was cultured in Dulbecco’s Modified Eagle Media (DMEM) (Sigma), containing 15% of fetal bovine serum (FBS; Life technologies) tested for optimal growth of mESCs, 100 U/mL LIF (Millipore), 0.1 mM 2-ß-mercaptoethanol (Life technologies) and 1% Penicillin/Streptomycin (Gibco), on 0.2% gelatin-coated support in absence of feeder cells. The culture medium was changed daily. All cells were grown at 37°C in 8% CO2. The ligant: N-methyl-N-[3-(3-methyl[1,2,4]triazolo[4,3-b]pyridazin-6-yl)phenyl]acetamide (1632) was dissolved in DMSO and added to the cells for 48h, 3 days or 6 days to a final concentration of 20 µM or 60µM. A fresh dilution was supplemented every day to the fresh medium. Pictures of treated cells were acquired using a Nikon inverted microscope coupled to a CCD camera.

Statistics

Each experiment was repeated as indicated. All statistical analyses were performed by ANOVA using Dunnett’s post-test, comparing against the lowest dose in each group or the negative control treatment. (*) $P < 0.05$; (**) $P < 0.01$; (***) $P < 0.001$. All statistics were run with GraphPad. For data in Fig. 2b column statistics were calculated using a confidence interval (CI) of the mean corresponding to 95%. GraphPad Prism 6 was used to run statistics.

SSMD calculation for assay quality assessment

Using uniformly minimal-variance unbiased estimate (UMVUE), SSMD is

$$\hat{\beta} = \frac{\bar{X}_P - \bar{X}_N}{\sqrt{\frac{2}{n_P + n_N - 3.5}}\left((n_P - 1)s_P^2 + (n_N - 1)s_P^2\right)}$$

Where n_P and n_N are 3 each (number of pre-let-7 positive control readouts and number of pre-miR-101 readouts on the plate reader). $\bar{X}_P$ and s_P indicate the sample mean and the standard deviation of the pre-let-7 samples and $\bar{X}_N$ and s_N represents the sample mean and the standard deviation of pre-miR-101 samples.

The assay performance was judged based on criteria as published in Zhang *et al.*.

SSMD* calculation for hit selection

Data from the pilot screen and the full HTS (without replicates) were analyzed using SSMD* and UMVUE, which is recommended for HTS hit selection for screens performed without replicates[2]:

$$SSMD^* = \frac{Y_i - \tilde{Y}_N}{\frac{2}{K}(n_N - 1)MAD_N}$$

$$K = n_N - 2.48$$

$$MAD = 1.4826\,median\,(|y_i - median(y)|)$$

where $\tilde{Y}_N$ is the measured signal of the wells only containing EGFP-Lin28B, n_N = 4 and Y_i indicates the measured signal of each well.

For primary hit selection, upper and lower SSMD thresholds of 133% and 66%, respectively were used.

Optimization of the plate reader technical parameters

We chose to adapt our assay to a 384-wells format with a final volume of 19 µL and to perform the measurement on a monochromator plate reader (Tecan Infinite M1000 PRO). Technical parameters of the plate reader were optimized by monitoring SSMD with various concentrations of 19B-let7. Briefly, EGFP-Lin28B lysate (1:10) was mixed with various concentrations of labeled pre-let-7a-2 (0 nM, 0.313 nM, 1.25 nM, 5 nM, 20 nM and 80 nM) individually. Solutions were incubated for 30 min and their fluorescence spectra were acquired at 507 nm (5 nm bandwidth) after excitation of the sample at 485 nm (5 nm bandwidth).

Plates: Perkin Elmer, ProxiPlate™ #**6008260**

Mode: Fluorescence Top Reading

Flash frequency: 100 Hz

Number of flashes: 20

Integration time: 20 µs

Z’ position: calculated individually for each plate from positive control in A1

Excitation wavelength: 485 nm

Excitation bandwidth: 5 nm

Emission wavelength: 507 nm

Emission bandwidth: 5 nm

Assessment of HTS assay quality by pilot experiment

Plates for the pilot experiment were pipetted by the Tecan Aquarius™ 96 in a 384 well plate format. Measurements were acquired on a monochromator plate reader Tecan Infinite®M1000 Pro.

In 380 wells were pipetted 4 µL of a 23.75 nM solution of 19B-let7. In these 380 wells, 376 wells were added 0.76 µL of a DMSO solution and 4 wells 0.76 µL of a 20 µM solution of L29-13[3]. After an incubation time of 30 min, 14 µL of pure EGFP-Lin28B lysate was added to the 384 wells. After 30 min incubation, samples were measured on a monochromator plate reader with the aforementioned parameters. SSMD* values were calculated using Excel.

Selected hit re-synthesis

Selected hits were synthesized for evaluation. Synthetic protocols were not optimized for chemical yields.

- *ID1036 (CAS 105189-44-4)*

ID1036 was prepared by subsequent esterification and amidation of 3,5-dibromo-L-tyrosine following the procedure of Crowe et al.[4].

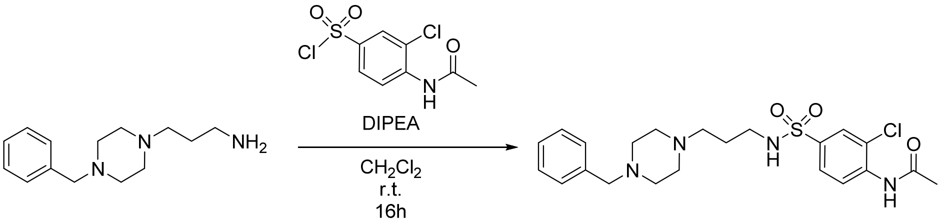
^{1}H NMR (400 MHz, CD_3Cl_3) δ=7.17 (s, 2H), 6.85 (br, 1H), 5.87 (s, 1H), 4.80 (dd, J = 12.7, 5.6Hz, 1H), 3.82 (s, 3H), 3.16-3.03 (m, 2H). ESI-MS: positive mode 449.2 ([M+H]+). Calc.: 449.0.

ID1632 (CAS 108825-65-6)

ID1632 was purchased from Maybridge

- *ID4019 (CAS 651714-49-7)*

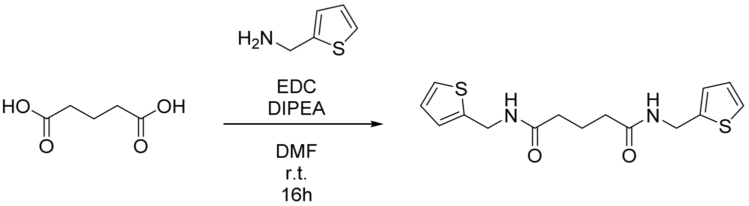
ID 4019 (CAS 651714-49-7) was prepared by condensation of 3-(4-benzyl-piperazin-1-yl)propylamine) to 4-actemido-3-chlorobenzenesulfonyl chloride.



To a solution of 3-(4-benzyl-piperazin-1-yl)propylamine) (200mg, 0.86 mmol, 1 equiv.) in dry dichloromethane (10 mL) at room temperature was successively added *N,N*-diisopropylamine (0.25 mL, 1.29 mmol, 1.5 equiv.) and 4-actemido-3-chlorobenzenesulfonyl chloride (254mg, 0.95 mmol, 1.1 equiv.). After overnight stirring, water (1 mL) was added and the reaction mixture was partitioned between dichloromethane (50 mL) and water (25 mL). After separation, the aqueous layer was extracted twice with dichloromethane (25 mL). The combined organic layers were washed once with water (25 mL) and brine (25 mL) and dried over Na_2SO_4. Purification by flash chromatography up to 7% MeOH in dichloromethane afforded the desired compound (47 mg, 0.118 mmol, 12%). ^{1}H NMR (400 MHz, $CDCl_3$) δ=8.59 (d, J = 8.7Hz, 1H), 7.88 (d, J = 2.0Hz, 1H), 7.80 (s, 1H), 7.72 (dd, J = 8.7, 2.0Hz, 1H), 7.33 (d, J = 4.3Hz, 4H), 7.28-7.23 (m, 1H), 3.06 (d, J = 5.6Hz, 2H), 2.47 (br, 10H), 2.29 (s, 3H), 1.66-1.60 (m, 2H). ESI-MS: positive mode 464.9 ([M+H]+). Calc.: 464.2.

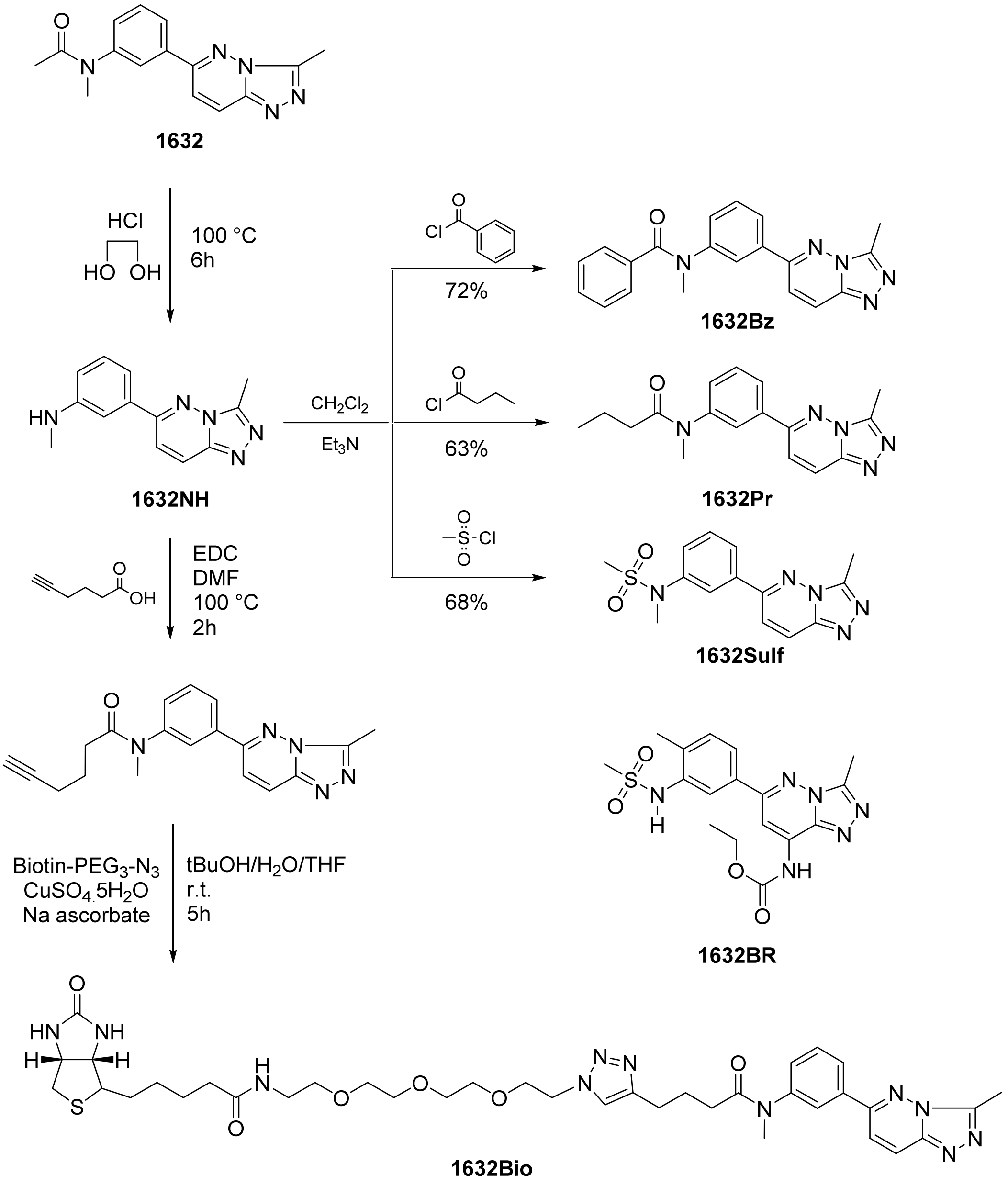
- *ID4256 (CAS 546118-73-4)*

ID4256 was prepared by coupling between glutaric acid and thiophene-2-methylamine.



To a solution of glutaric acid (250mg, 1.89 mmol, 1 equiv.) in dry *N,N*-dimethylformamide (5 mL) at room temperature was successively added *N,N*-diisopropylamine (0.56 mL, 4.16 mmol, 2.2 equiv.), thiophene-2-methylamine (640mg, 5.68mmol, 3 equiv.) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (800mg, 4.15 mmol, 2.2 equiv.). After overnight stirring, the crude was evaporated to dryness, dissolved in dichloromethane (150 mL), and washed three times with water. The organic phase was evaporated to dryness, coevaporated with methanol (2x 15mL). Purification by flash chromatography up to 5% MeOH in dichloromethane afforded the desired compound (280 mg, 0.68 mmol, 46%). ^{1}H NMR (400 MHz, CD_3OD) δ=7.26 (dd, J = 5.1, 1.2Hz, 2H), 6.97-6.95 (m, 2H), 6.92 (dd, J = 5.1, 3.5Hz, 2H), 4.52 (s, 4H), 2.24 (t, J = 7.5Hz, 4H), 1.92 (quint., J = 7.5Hz, 2H). ESI-MS: positive mode 322.9 ([M+H]+). Calc.: 322.1.

Synthesis of 1632 derivatives



- *1632NH*

N-methyl-N-[3-(3-methyl[1,2,4]triazolo[4,3-b]pyridazin-6-yl)phenyl]acetamide 1632 (200mg, 0.71 mmol, 1 equiv.) was dissolved in 0.75 mL of ethylene glycol and 0.25 mL of 12N HCl. After 4h at 100 °C, the crude solution was cooled to room temperature and neutralized by addition of $NaHCO_3$. After evaporation of the volatiles, the crude was filtered on a short pad of silica gel and eluted with 10% MeOH in dichloromethane. After evaporation of the volatiles, N-methyl-N-[3-(3-methyl[1,2,4]triazolo[4,3-b]pyridazin-6-yl]benzenamine (160mg, 0.67 mmol, 94%, CAS 108810-87-3) was obtained as a yellow amorphous solid. ^1H NMR (400 MHz, $CDCl_3$) δ=8.02 (d, J = 9.7 Hz, 1H), 7.44 (d, J = 9.7 Hz, 1H), 7.27 (t, J = 7.9 Hz, 1H), 7.20-7.13 (m, 2H), 6.71 (dd, J = 8.0, 1.7 Hz, 1H), 2.86 (s, 3H), 2.79 (s, 3H). ESI-MS: positive mode 240.0 ([M+H]+). Calc.: 239.1.

- *1632Bz*

To a solution of 1632NH (80 mg, 0.335 mmol) in dichloromethane (5 mL) were successively added triethylamine (90 µL, 0.67 mmol, 2 equiv.) and benzoyl chloride (59 µL, 0.50 mmol, 1.5 equiv.). The reaction was stirred at room temperature for 2h and evaporated to dryness. The crude was purified by flash chromatography with a gradient up to 4% MeOH in ethyl acetate to get 1632Phenyl (82 mg, 0.24 mmol, 72%) as a slightly brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ=8.09 (d, J = 9.7 Hz, 1H), 7.77-7.74 (m, 1H), 7.66 (t, J = 1.7 Hz, 1H), 7.44 (t, J = 7.9 Hz, 1H), 7.38-7.35 (m, 2H), 7.33-7.19 (m, 5H), 3.59 (s, 3H), 2.85 (s, 3H). ESI-HRMS calculated for C20 H18 N5 O positive mode ([M+H]+) 344.1512. Calc.: 344.1506.

- *1632Pr*

To a solution of 1632NH (80 mg, 0.335 mmol) in dichloromethane (5 mL) were successively added triethylamine (90 µL, 0.67 mmol, 2 equiv.) and butyryl chloride (52 µL, 0.50 mmol, 1.5 equiv.). The reaction was stirred at room temperature for 2h and evaporated to dryness. The crude was purified by flash chromatography with a gradient up to 6% MeOH in ethyl acetate to get 1632Pr (65 mg, 0.21 mmol, 63%) as an amorphous white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ=8.16 (d, J = 9.7 Hz, 1H), 7.95 (d, J = 7.5 Hz, 1H), 7.86 (t, J = 1.8 Hz, 1H), 7.63-7.55 (m, 2H), 7.39-7.35 (m, 1H), 3.32 (s, 3H), 2.86 (s, 3H), 2.18-2.02 (m, 2H), 1.69-1.55 (m, 2H), 0.88-0.80 (m, 3H). ESI-HRMS calculated for C17 H20 N5 O positive mode ([M+H]+) 310.1666. Calc.: 310.1662.

- *1632Sulf*

To a solution of 1632NH (80 mg, 0.335 mmol) in dichloromethane (5 mL) were successively added triethylamine (90 µL, 0.67 mmol, 2 equiv.) and mesyl chloride (39 µL, 0.50 mmol, 1.5 equiv.). The reaction was stirred at room temperature for 2h and evaporated to dryness. The crude was purified by flash chromatography with a gradient up to 5% MeOH in a (1:1) ethyl acetate/dichloromethane mixture to get 1632Sulfo (72 mg, 0.23 mmol, 68%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ=8.17 (d, J = 9.7 Hz, 1H), 8.06 (t, J = 1.08 Hz, 1H), 7.94 (td, J = 7.6, 1.3 Hz, 1H), 7.63-7.54 (m, 3H), 3.44 (s, 3H), 2.93 (s, 3H), 2.90 (s, 3H). ESI-HRMS calculated for C14 H16 N5 O2 S positive mode ([M+H]+) 318.1021. Calc.: 318.1019.

- *1632Bio (biotin-PEG3 conjugate)*

Biotinylated ID1632Bio was obtained click conjugation with biotin-PEG3-azide (875770-34-6).

N-methyl-N-[3-(3-methyl[1,2,4]triazolo[4,3-b]pyridazin-6-yl]benzenamine (150 mg, 0.63 mmol, 1 equiv.) was dissolved in dry DMF. Triethylamine (271 µL, 1.88 mmol, 3 equiv.), hexynoic acid (210 mg, 1.88 mmol, 3 equiv.) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (360mg, 1.88 mmol, 3 equiv.) were subsequently added. After 2h stirring at 100 °C, the crude solution was evaporated to dryness and purified by flash chromatography up to 3% MeOH in dichloromethane to get N-methyl-N-[3-(3-methyl-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)phenyl]hex-5-ynamide (120 mg, 0.36 mmol, 57%). ^{1}H NMR (400 MHz, $CDCl_3$) δ=8.14 (d, J = 9.7 Hz, 1H), 7.91 (d, J = 7.2 Hz, 1H), 7.82 (t, J = 1.7 Hz, 1H), 7.56 (t, J = 7.8 Hz, 1H), 7.51 (d, J = 9.7 Hz, 1H), 7.34 (dd, J = 7.9, 1.0 Hz, 1H), 3.28 (s, 3H), 2.82 (s, 3H), 2.45 (t, J = 7.4 Hz, 1H), 2.31-2.06 (m, 4H), 1.83-1.76 (m, 2H). ESI-MS:

positive mode 333.5 ([M+H]+). Calc.: 333.16. To a solution of N-methyl-N-[3-(3-methyl-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)phenyl]hex-5-ynamide (15 mg, 0.045 mmol, 1 equiv.) in 1.5 mL of a tBuOH, H_2O and THF mixture (1:1:1) was subsequently added biotin-PEG3-azide (20 mg, 0.045 mmol, 1 equiv.), $CuSO_4.5H_2O$ (3.3 mg, 0.012 mmol, 0.3 equiv.) and sodium ascorbate (5.4 mg, 0.027 mmol, 0.6 equiv.). After 4h at room temperature, volatiles were evaporated and the crude purified by flash chromatography up to 12% MeOH in dichloromethane to get 1632Bio (25 mg, 0.32 mmol, 71%). ESI-HRMS calculated for C37 H52 N11 O6 S positive mode ([M+H]+) 778.3814. Calc.: 778.3817.

Competition ELISA with immobilized 1632

White microtiter plates (96-well plates, NUNC, Maxisorp) were coated for 24 h with streptavidin (2µg/ml in PBS) and blocked with a 1% solution of a gelatin derivative (Top Block, Lubio Science) in 25 mM HEPES, 0.05% Tween 20 pH 7 overnight. After washing with water (used for all subsequent washing steps), chemically biotinylated small molecule 1632Bio and biotin as negative control were allowed to bind to the surface for 3 h at a concentration of 2.5nM in 25mM HEPES pH 7. Meanwhile, varying concentration of non-biotinylated 1632 small molecules were incubated with a constant dilution (1/100) HeLa cell lysates with overexpressed c-Myc LIN28A in binding buffer containing 300mM NaCl, 25mM HEPES pH 7.2, 10µM ZnCl2, 1% Top-Block, 0.05% Tween 20. These mixtures, prepared in polypropylene 96-well plates (NUNC, cat. No 732-2620) and were incubated at 4°C for 2 h. The 1632Bio coated white microtiter plate was washed with cold water to minimize temperature dependent edge effects. 50µl protein lysate/small molecule mixtures were transferred to the white microtiter plates. After 1 h incubation at 4°C, the plate was emptied (without washing), and exposed to a 50µl of fixation solution (0.5% formaldehyde in PBS) for 5 min. The plate bound c-Myc LIN28 was measured by an antibody specific for the Myc tag (BML-SA294, clone 9E10, Enzo Life Sciences) at 0.01µg/ml in 150mM NaCl, 25mM HEPES pH 7.2, 10µM $ZnCl_2$, 1% Top-Block, 0.05% Tween 20 with an incubation for 1 h at room temperature. Bound primary antibody was detected by a secondary peroxidase conjugated anti-mouse IgG antibody (074-1806, KPL, Gaitherburg), with 1:3000 dilution for 1 h at room temperature. Peroxidase activity was measured in a microtiter plate reader (Mithras 940, Berthold) using a chemiluminescent substrate (BM reagent, Roche Applied Science, cat no. 11582). Inhibition data were fitted to the 4-parameter logistic equation described for the RNA-ELISA method.

Capture of affinity purified c-Myc-LIN28A by 1632Bio

White microtiter plates (96-well plates, NUNC, Maxisorp) were coated for 24 h with streptavidin (2µg/ml in PBS) and blocked with a 1% solution of a gelation derivative (Top Block, Lubio Science) in 25 mM HEPES, 0.05% Tween 20 pH 7 at overnight. After washing with water (used for all subsequent washing steps), chemically biotinylated small molecule 1632Bio and biotin as negative control were allowed to bind to the surface for 3 h at a concentration of 2.5nM in 25mM HEPES pH 7. The 1632Bio pre-coated white microtiter plate was washed with cold water to minimize temperature dependent edge effects. Increasing dilutions of 50µl of purified c-Myc tagged LIN28A, c-Myc tagged MBNL1 or mock purified protein (1:3, 1:6, 1:12, 1:24, 1:48, 1:96, 1:192, 1:384, 1:768) in a buffer containing 300mM NaCl, 25mM HEPES pH 7.2, 10µM $ZnCl_2$, 1% Top-Block, 0.05% Tween 20 were pipetted to the microtiter plate and incubated for 2 h at 4°C. The plate was fixed emptied and fixed with formaldehyde as described earlier in the text. The c-Myc purified protein bound to the small molecule/biotin was detected by the antibody against c-Myc as described in completion ELISA. The binding data were fitted to the 4-parameter logistic equation described for the RNA-ELISA.

Affinity purification of c-Myc tagged Proteins and Immunoblotting

HeLa cells were cultured without antibiotics, all c-Myc tagged constructs (pCMV-myc-Lin28A, pTRE2pur-Myc-MBNL1) were transfected into HeLa cells by Lipofectamine 2000 (Invitrogen, cat. no. 11668-027) following manufacture's protocol. Control mock transfection was performed by using Lipofectamine 2000 only. 48 h after transfection, cells were washed twice with PBS then lysed in 4°C with HEPES buffer pH 7.2 (150mM NaCl, 25mM HEPES pH 7.2, 10µM $ZnCl_2$, 0.05% Tween 20) and complete mini EDTA-free protease inhibitor cocktail (Roche, cat. no. 11873580001). The lysate were then sonicated for 30 seconds with 20% amplitude by using a sonicator (Vibra Cell, VCX130) followed by centrifugations at 10,000 g for 10 minutes. The supernatants were collected and protein concentrations were determined by colorimetric assay by using Pierce BCA protein assay kit (Thermo Scientific, cat no 23225). The affinity column purification of c-Myc tagged proteins were performed by Pierce c-Myc-Tag IP/Co-IP kit (Thermo Scientific, Cat. no. 23620) by following manufacture's protocol. Protein samples were added to Laemmli buffer + β-mercapto-ethanol and denatured by heating at 95°C for 5 minutes. 15-30 µg of total protein per sample were resolved on 10-12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose blotting membrane (GE Healthcare Life Sciences, cat. no. 10600008). After transfer nitrocellulose membranes were blocked for 1 h at room temperature with 5% non-fat milk in Tris-buffered saline/Tween 20 (TBS-T). Next the membranes were incubated overnight with previously described Primary mouse c-Myc monoclonal antibody (BML-SA294, clone 9E10, Enzo Life Sciences) (1:1000 dilutions). After washing three times for 30 min with TBS-T, membranes were incubated for 1 h with corresponding secondary anti-mouse IgG antibody (074-1806, KPL, Gaitherburg). Bands were visualized with horseradish peroxidase (HRP) conjugated antibodies against mouse IgG using Biorad-Chemidoc imaging system by following manufacture's protocol.

Northern blotting

The RNA was extracted from the cell pellets using TRIzol reagent (Life technologies) according to the manufacturer's instructions. The total RNA (20-40µg) was resuspended in 50% formamide, heated at 95 °C for 5 min and loaded on a 17.5% Acrylamide/Bis 19 :1, 7M Urea denaturing gel. The electrophoresis was performed in TBE 0.5X buffer at 100V for 3-4 h. The samples were transferred on a neutral nylon membrane Hybond-NX (GE Healthcare), using a Trans-Blot® SD semi-dry transfer devise (Biorad) for 1 h at 100 mA. The RNA was chemically cross-linked on the membrane using EDC reagent (Pall G.S and Hamilton J. 2008) and hybridized at 42 °C with the radiolabelled probes overnight using PerfectHyb™ Plus Hybridization Buffer (Sigma). The membrane was washed 3 times with SSC 2X, 0.1% SDS solution at 42 °C and exposed for 5 days with a phosphor Imaging Plate (Fujifilm) and revealed on a Typhoon FLA 7000 scanner. The DNA oligonucleotide probes (30 pmol) were radiolabelled on their 5' end using a T4 polynucleotide kinase and 50 µCi of gamma-

[32P]ATP (>3000 Ci/ mmol) . The Let-7 probe consists on a mixture of 4 DNA oligonucleotides antisense to mmu-Let-7a (AACTATACAACCTACTACCTCA), mmu-Let-7g (AACTGTACAAACTACTACCTCA), mmu-Let-7f (AACTATACAATCTACTACCTCA) and mmu-Let7-e (AACTATACAACCTCCTACCTCA) miRNAs. The U6 probe is an oligonucleotide antisense to a region the U6 RNA (GCAGGGGCCATGCTAATCTTCTCTGTATCG). The intensity of the signal was quantified using the ImageJ-Fiji software.

Western blotting

The proteins were extracted from the same samples as the RNA using the TRIzol reagent (Life technologies) according to the manufacturer's instructions. The protein extracts (20 µg) were run on a classical 15% SDS-PAGE and transferred on a nitrocellulose blotting-membrane 0.22 µm (GE Healthcare) using the Trans-Blot® SD semi-dry transfer devise (Biorad). The proteins were detected using the anti-lin28a antibody (#8706; cell Signaling technology) and the anti-alpha-tubulin antibody (Sigma, T6199) at respectively 1:2000 and 1:10000 dilutions. The western blots were revealed using the Clarity Western ECL substrate (Biorad) on a ChemiDoc MP imager (Biorad). The intensity of the signal was quantified using the ImageJ-Fiji software.

Selectivity of 1632: activity against commonly assayed receptors and a kinase

Compound 1632 was evaluated in a "safety" screen at 40 µM concentration performed as a service at Eurofins Cerep (STUDY NUMBER 100025099), against a panel of seven commonly assayed receptors (Adrenergic b1, Dopamine D1, Muscarinic M1, Opiod k (KOP), Vasopressin V1a, Ca2+ channel , potassium Kv channel) and one kinase (Lck kinase). In each experiment, the respective reference antagonist/agonist was tested head to head with 1632, and the data were compared with historical values determined at Eurofins. Compound binding was calculated as a percentage inhibition of the binding of a radioactively labeled ligand specific for each target. Results showing an inhibition or stimulation higher than 50% are considered to represent significant effects of the test compounds. Results showing an inhibition (or stimulation) lower than 25% are not considered significant and mostly attributable to variability of the signal around the control level. Compound 1632 was inactive in all assays.

Dicer *in vitro* assay

Dicer *in vitro* assays were carried out according to Leuschner PJ and Martinez J[5]. Frozen pellets of mouse embryonic stem cells were lysed using 600 µl lysis buffer (30 mM Hepes, pH 7.4, 100 mM KCl, 5 mM MgCl2, 10% glycerol, 0.5 mM DTT, 1x ROCHE cOmplete Mini EDTA-free protease inhibitor and 0.2% NP40) and incubated 5 min at room temperature followed by 10 min at 4 °C on a platform rocker. The lysate was transferred to a tube and cell debris removed by centrifugation (5 min at 10000xg at 4 °C). The supernatant was saved, diluted to approximately 10 mg/ml protein and stored at -80 °C or used directly for processing reactions. Pre-miRNAs were synthesized on a MerMade 12 synthesizer (Bioautomation Corporation). We used phosphoramidites (Thermo Fisher Scientific) and UnySupport controlled-pore glass 500 Å solid support (CPG; Glen Research). 10 pmol of pre-miRNA were labelled using 1.5 µl of [γ-32P]-ATP 6000Ci/mmol (Perkin-Elmer) and 1U PNK

(ROCHE) in 10 µl total reaction volume according to manufacturer's protocol. Deactivated labelling reactions were diluted to 25 µl using water and purified using illustra MicroSpin G-25 Columns (GE Healthcare). The eluted RNA was heated to 95°C and slowly cooled to room temperature in order to achieve uniform, unimolecular annealing of the hairpins. In vitro processing reactions were carried out using 2 µl of 100 nM labelled RNA, 6 µl of 3x processing buffer (300 mM KCl, 15 mM MgCl2, 1.5 mM DTT, 3 mM ATP and 0.6 mM GTP), 4-6 µl of lysates and filled up to 18 µl total reaction volume with water. Small molecule 1632 or DMSO was added resulting in final concentrations between 1 and 4 mM. The reactions were incubated for 2 h at 35 °C and heat inactivated 5 min at 95 °C before separation of products was performed using denaturing 12 % PA gels on a Protean II xi gel system (Bio-Rad). The gel was placed on top of a phosphor-screen and left overnight at -80°C for exposition. Screens were measured on a Typhoon FLA7000 PhosphorImager (GE healthcare) and densitometrical analysis carried out using ImageJ (Rasband WS, NIH).

Cell proliferation, clonogenic and tumor-sphere assays

Cell growth was evaluated using the sulforhodamine B (SRB) assay. Clonogenic and tumor-sphere assays were performed as previously described[6]. In tumor-sphere forming assays cells were seeded at a density of 1x103 cells/ml in 6-well plates coated with poly-HEMA and incubated compound 1632 (5, 10, 25, 50 and 100 µM). Tumor-spheres were stained with MTT, fixed with 10% paraformaldehyde and counted under a microscope.

BROMOscan assay

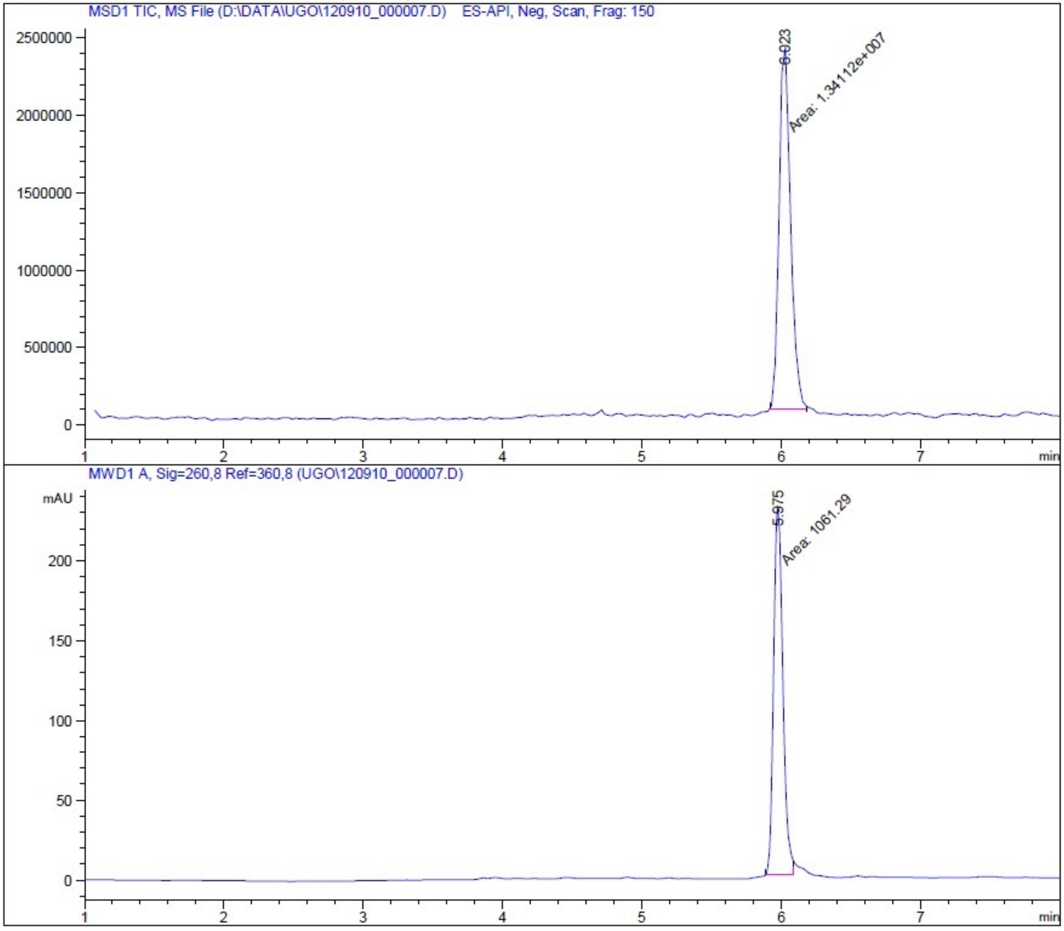
BROMOscan technology is a competition experiment that uses an immobilized ligand and a DNA-tagged bromodomain protein[7]. Compounds that bind to the bromodomain of interest will prevent binding of the bromodomain to the immobilized ligand. The amount of captured bromodomain is quantified by qPCR, and the dissociation constants are calculated with a standard dose-response curve.

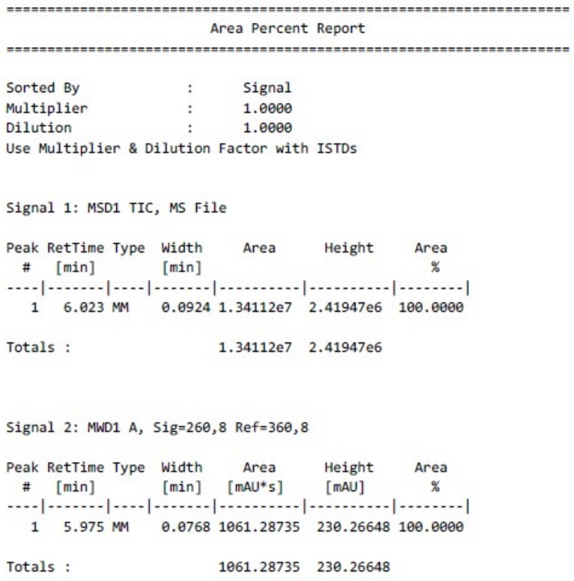
Supplementary Figures

LCMS chromatograms of labeled pre-miRNA (Fig S1)

10B-let7

```
Additional Info : Peak(s) manually integrated
```





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                        Area Percent Report
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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: MSD1 TIC, MS File

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]                           %
----|-------|----|-------|----------|----------|--------|
   1   6.023 MM    0.0924 1.34112e7  2.41947e6  100.0000

Totals :                  1.34112e7  2.41947e6


Signal 2: MWD1 A, Sig=260,8 Ref=360,8

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   5.975 MM    0.0768 1061.28735  230.26648 100.0000

Totals :                  1061.28735  230.26648
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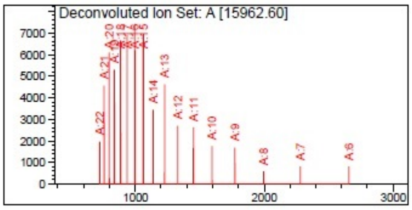
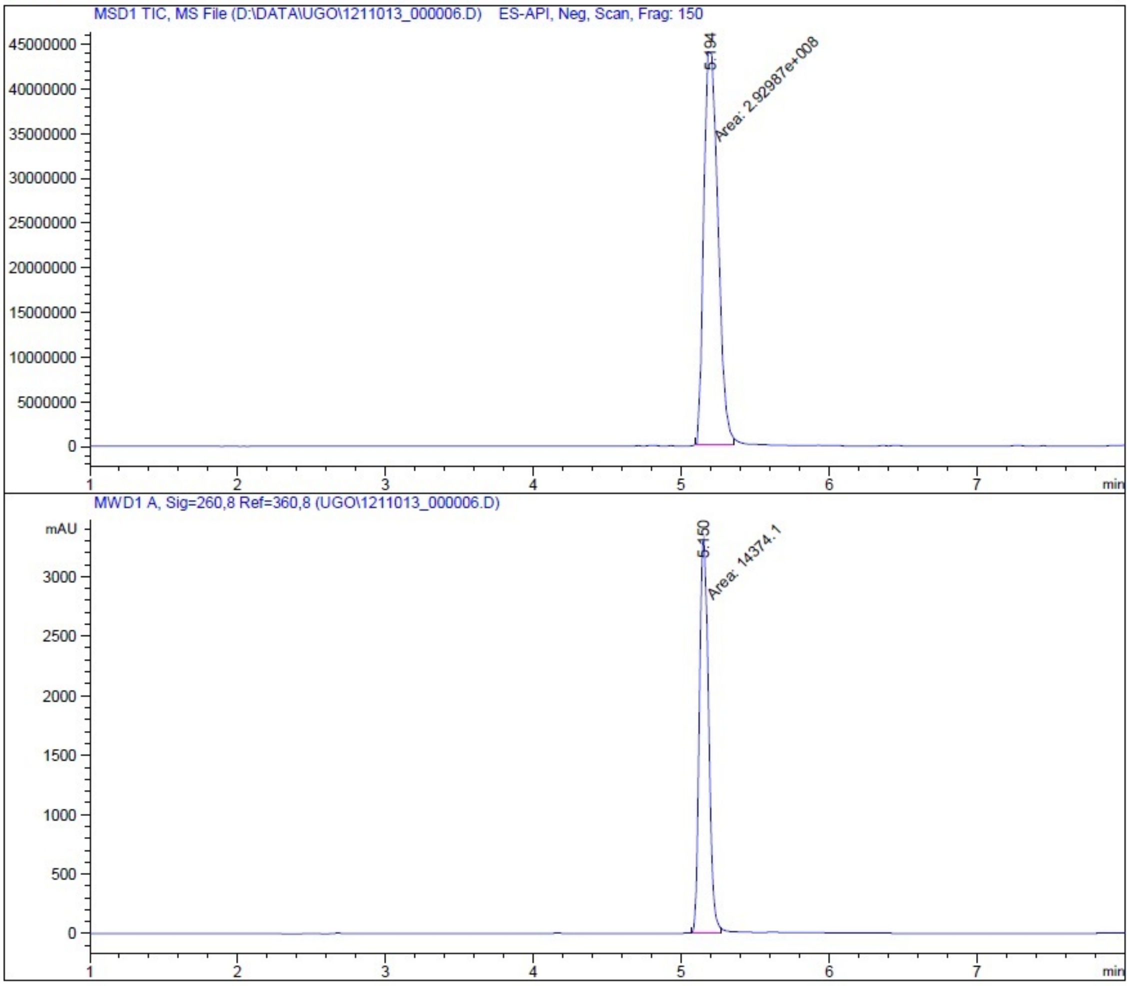


```
Component  Molecular      Absolute
            Weight        Abundance
   A        15962.60         59950
```

19Cy3-let7

```
Additional Info : Peak(s) manually integrated
```



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                         Area Percent Report
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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: MSD1 TIC, MS File

Peak RetTime Type  Width      Area      Height     Area
  #   [min]        [min]                            %
----|-------|----|-------|----------|----------|--------|
   1   5.194 MM    0.1054 2.92987e8  4.63213e7  100.0000

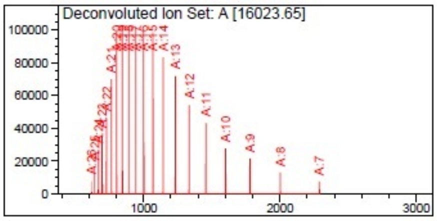
Totals :                  2.92987e8  4.63213e7



Signal 2: MWD1 A, Sig=260,8 Ref=360,8

Peak RetTime Type  Width      Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   5.150 MM    0.0722 1.43741e4  3320.40015 100.0000

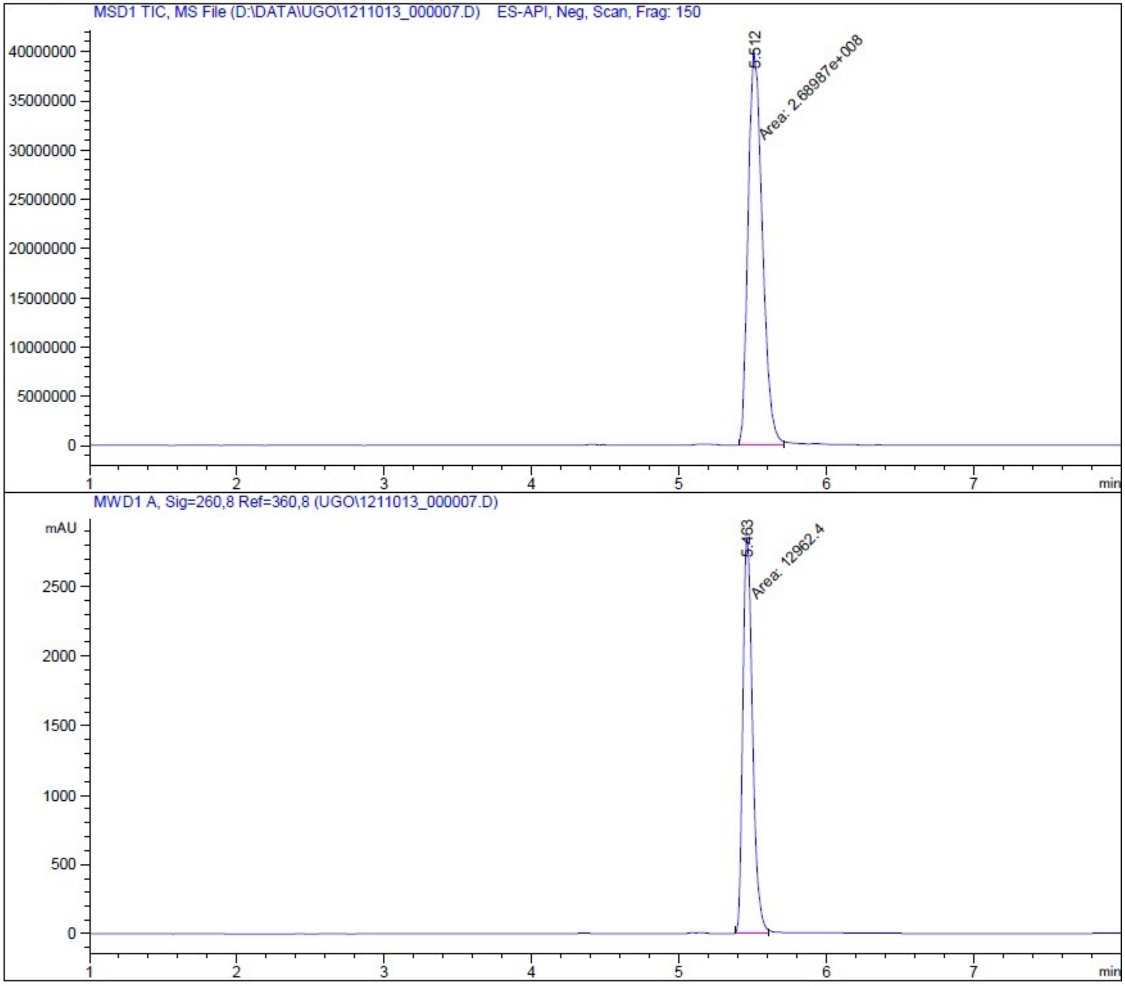
Totals :                  1.43741e4  3320.40015
```



```
Component  Molecular       Absolute    Relative
            Weight         Abundance   Abundanc
    A      16023.65         1099882     100.00
```

19B-let7

```
Additional Info : Peak(s) manually integrated
```



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                         Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: MSD1 TIC, MS File

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]                           %
----|-------|----|-------|----------|----------|--------|
   1   5.512 MM    0.1093 2.68987e8  4.10225e7  100.0000

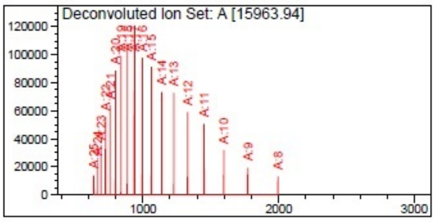
Totals :                  2.68987e8  4.10225e7



Signal 2: MWD1 A, Sig=260,8 Ref=360,8

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   5.463 MM    0.0757 1.29624e4  2853.91870 100.0000

Totals :                  1.29624e4  2853.91870
```



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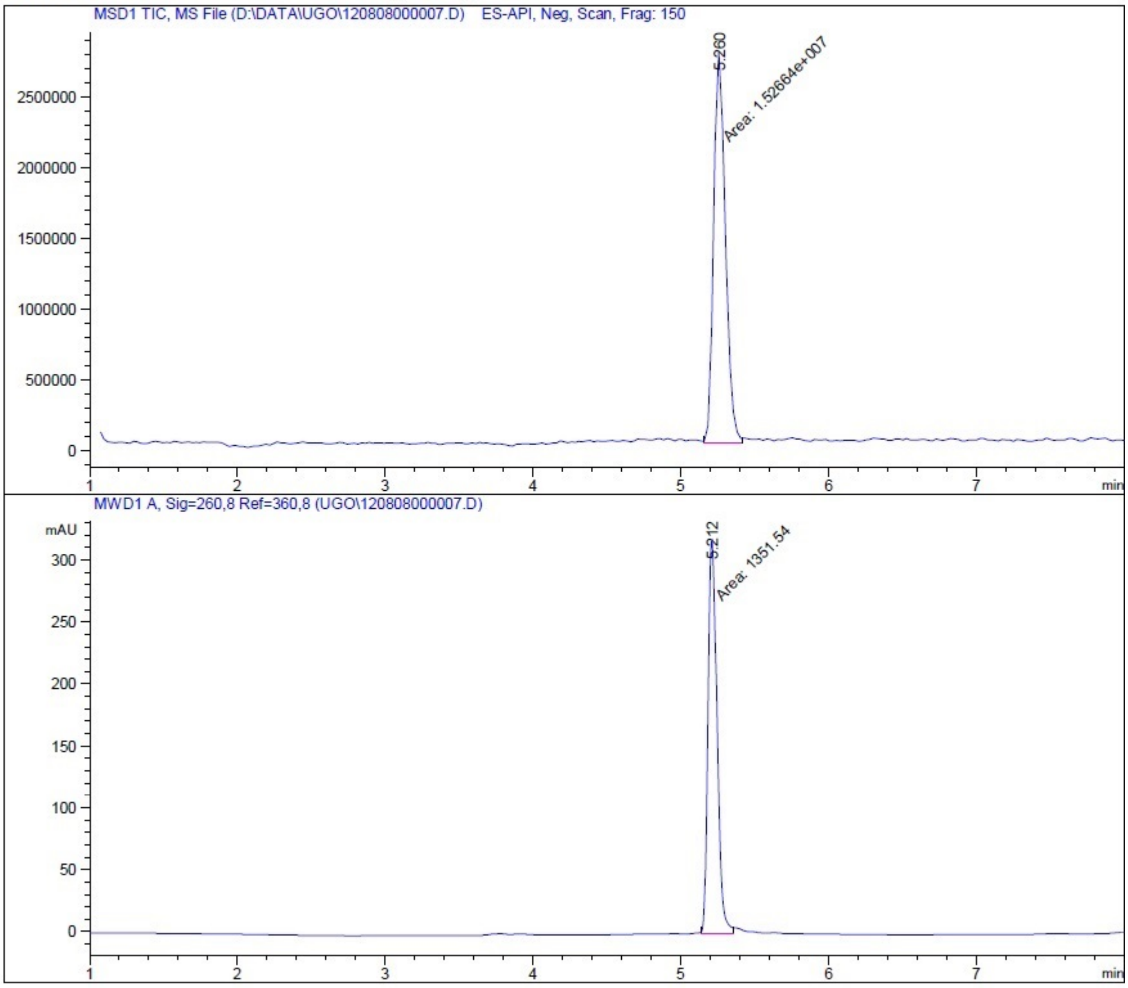
Component  Molecular         Absolute
             Weight          Abundance
    A      15963.94           1090351
```

34Cy3-let7

```
Additional Info : Peak(s) manually integrated
```



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                     Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: MSD1 TIC, MS File

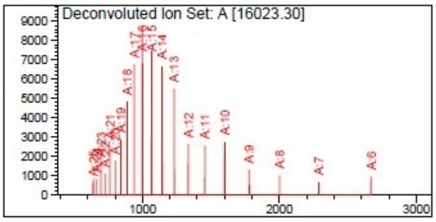
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]                           %
----|-------|----|-------|----------|----------|--------|
   1   5.260 MM    0.0901 1.52664e7  2.82372e6 100.0000

Totals :                  1.52664e7  2.82372e6


Signal 2: MWD1 A, Sig=260,8 Ref=360,8

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   5.212 MM    0.0706 1351.53687  319.03378 100.0000

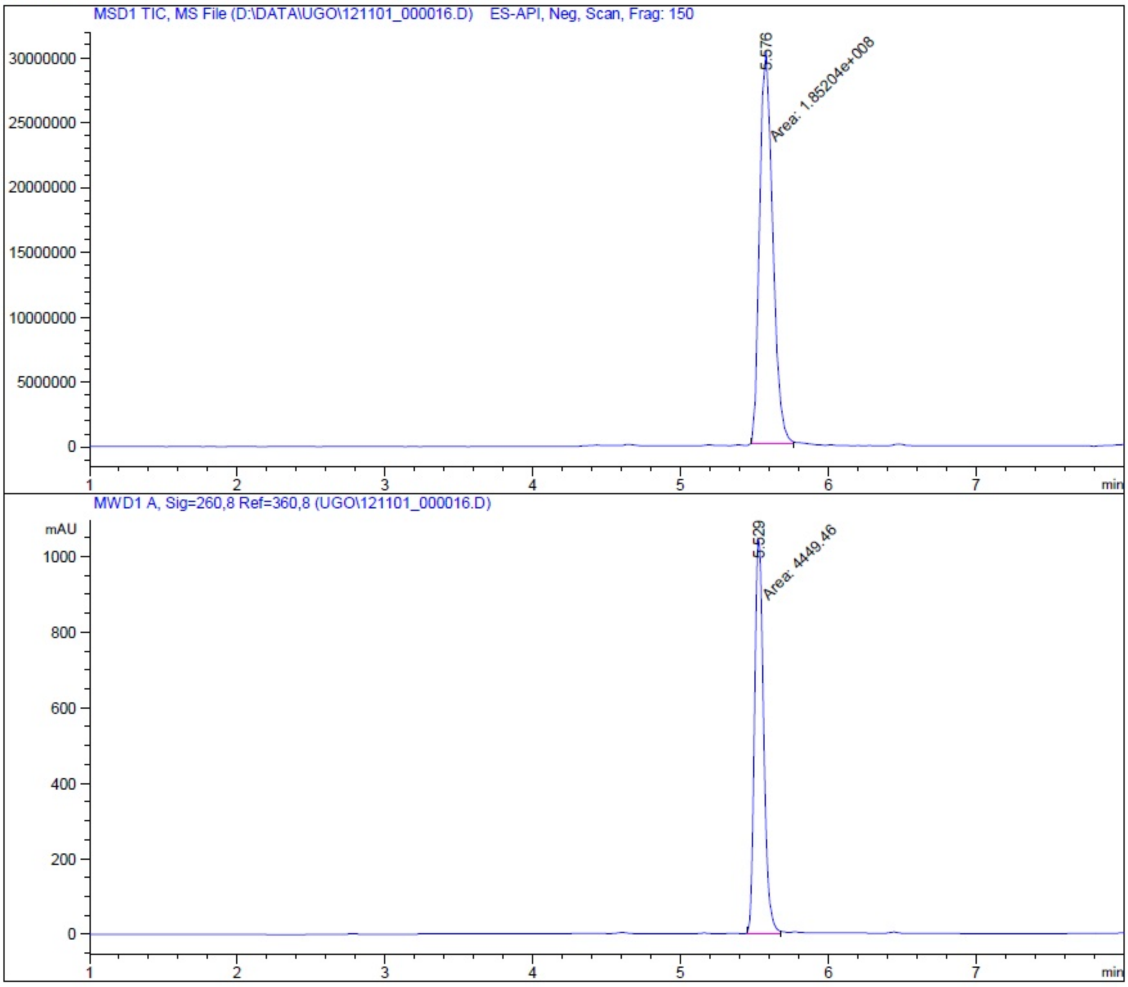
Totals :                  1351.53687  319.03378
```



```
Component  Molecular      Absolute
            Weight        Abundance
    A      16023.30         58697
```

34B-let7

```
Additional Info : Peak(s) manually integrated
```



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                         Area Percent Report
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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: MSD1 TIC, MS File

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]                          %
----|-------|----|-------|----------|----------|--------|
   1   5.576 MM    0.1003 1.85204e8  3.07854e7  100.0000

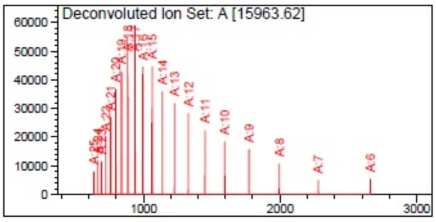
Totals :                  1.85204e8  3.07854e7



Signal 2: MWD1 A, Sig=260,8 Ref=360,8

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]      %
----|-------|----|-------|----------|----------|--------|
   1   5.529 MM    0.0708 4449.46387 1047.91870 100.0000

Totals :                  4449.46387 1047.91870
```



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Component  Molecular       Absolute
             Weight        Abundance
    A       15963.62         522900
```

57Cy3-let7

```
Additional Info : Peak(s) manually integrated
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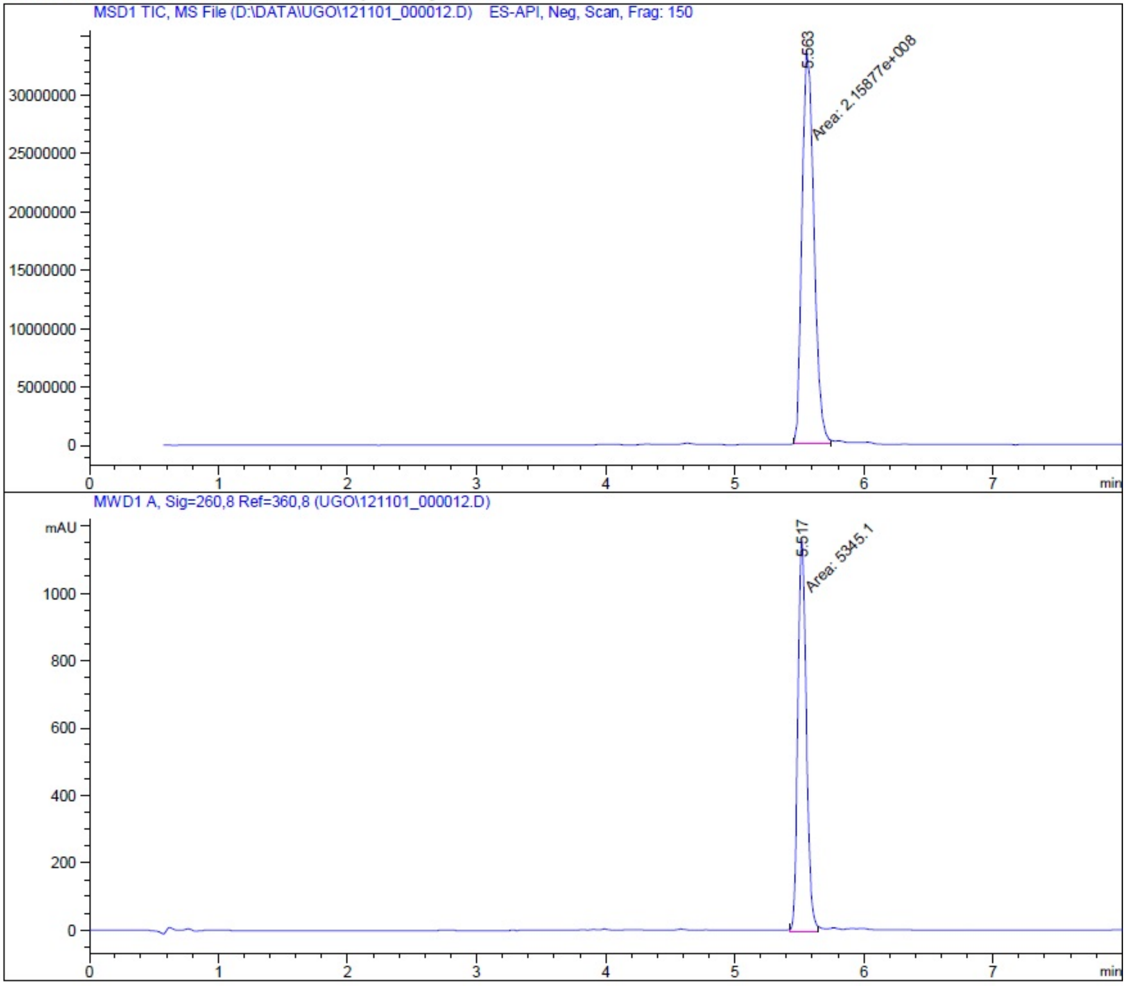

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                        Area Percent Report
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Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: MSD1 TIC, MS File

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]                           %
----|-------|----|-------|----------|----------|--------|
   1   5.563 MM    0.1038 2.15877e8  3.46653e7  100.0000

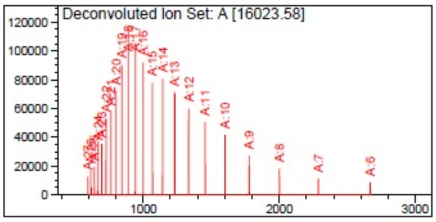
Totals :                  2.15877e8  3.46653e7



Signal 2: MWD1 A, Sig=260,8 Ref=360,8

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   5.517 MM    0.0761 5345.10010 1170.80981 100.0000

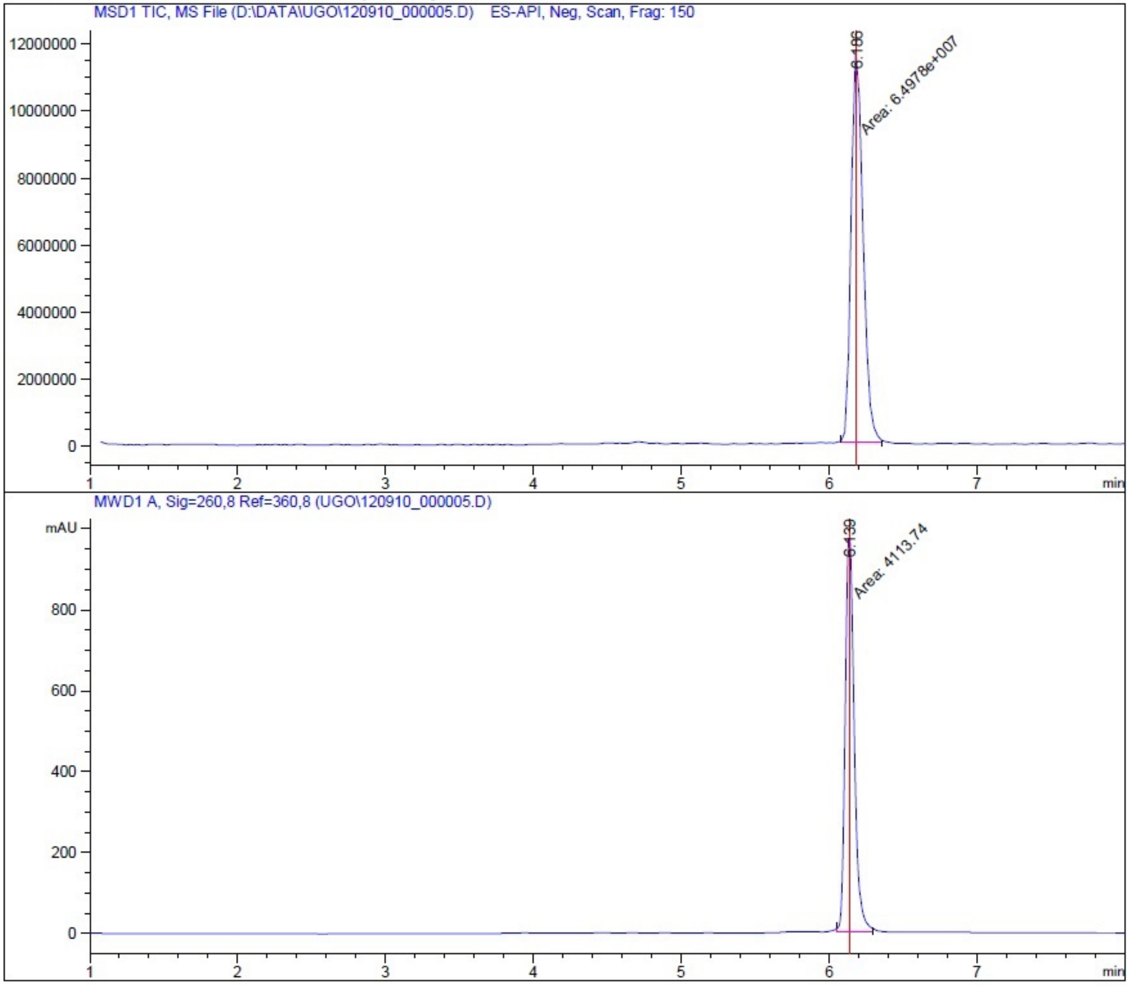
Totals :                  5345.10010 1170.80981
```



```
Component  Molecular      Absolute
            Weight        Abundance
    A       16023.58       1135895
```

10-19Cy3-let7

```
Additional Info : Peak(s) manually integrated
```



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                         Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: MSD1 TIC, MS File

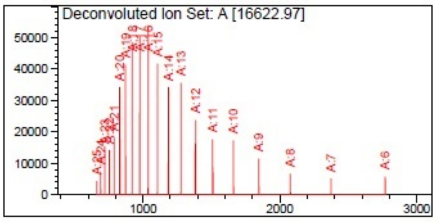
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]                           %
----|-------|----|-------|----------|----------|--------|
   1   6.186 MM    0.0899 6.49780e7  1.20515e7  100.0000

Totals :                  6.49780e7  1.20515e7


Signal 2: MWD1 A, Sig=260,8 Ref=360,8

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   6.139 MM    0.0699 4113.74121  981.51654 100.0000

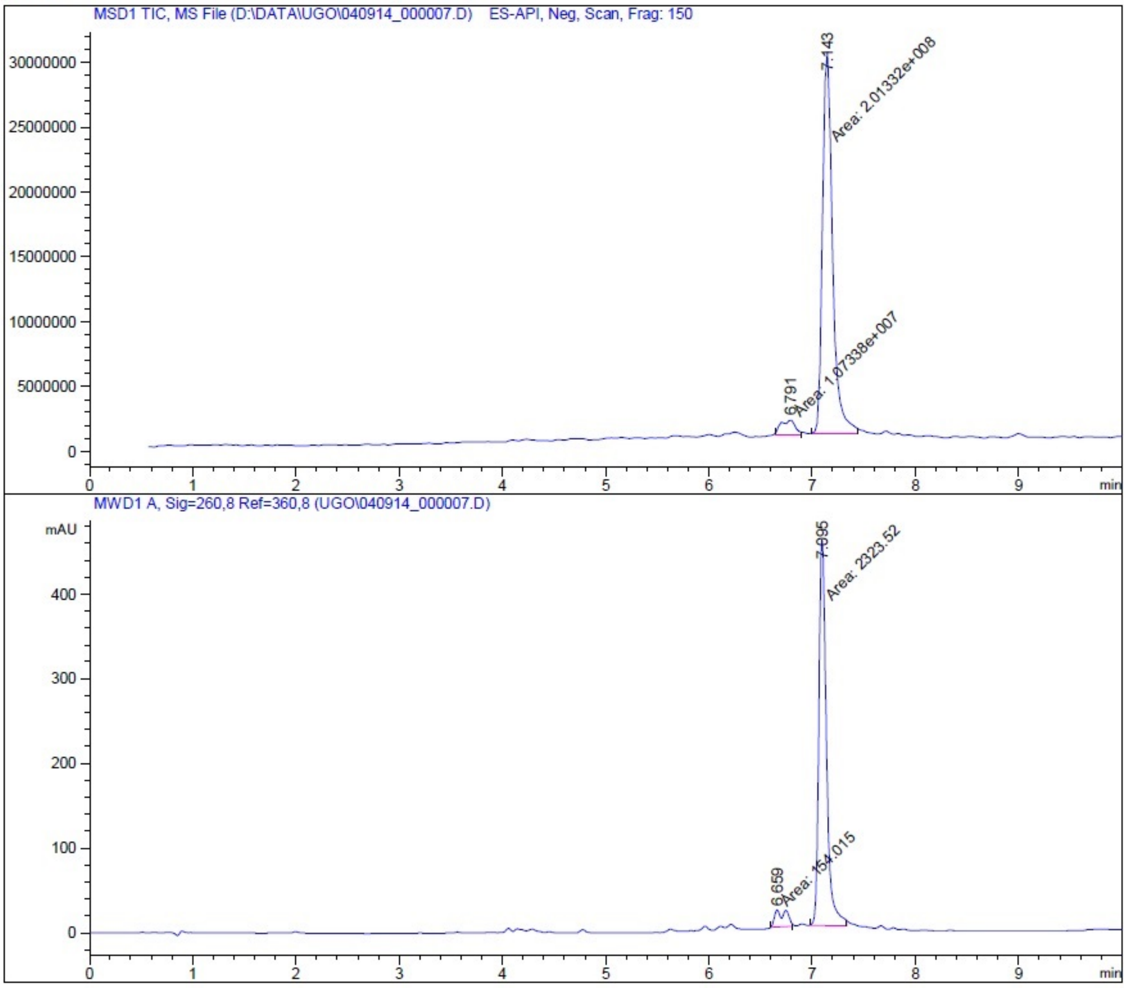
Totals :                  4113.74121  981.51654
```



```
Component  Molecular       Absolute
            Weight         Abundance
    A      16622.97         462593
```

10-19B-let7

```
Additional Info : Peak(s) manually integrated
```



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                         Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: MSD1 TIC, MS File

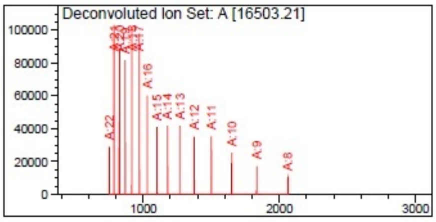
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]                           %
----|-------|----|-------|----------|----------|--------|
   1   6.791 MM    0.1565 1.07338e7  1.14305e6   5.0616
   2   7.143 MM    0.1113 2.01332e8  3.01575e7  94.9384

Totals :                  2.12066e8  3.13006e7


Signal 2: MWD1 A, Sig=260,8 Ref=360,8

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   6.659 MM    0.1282  154.01476   20.02979   6.2165
   2   7.095 MM    0.0846 2323.52002  457.79669  93.7835

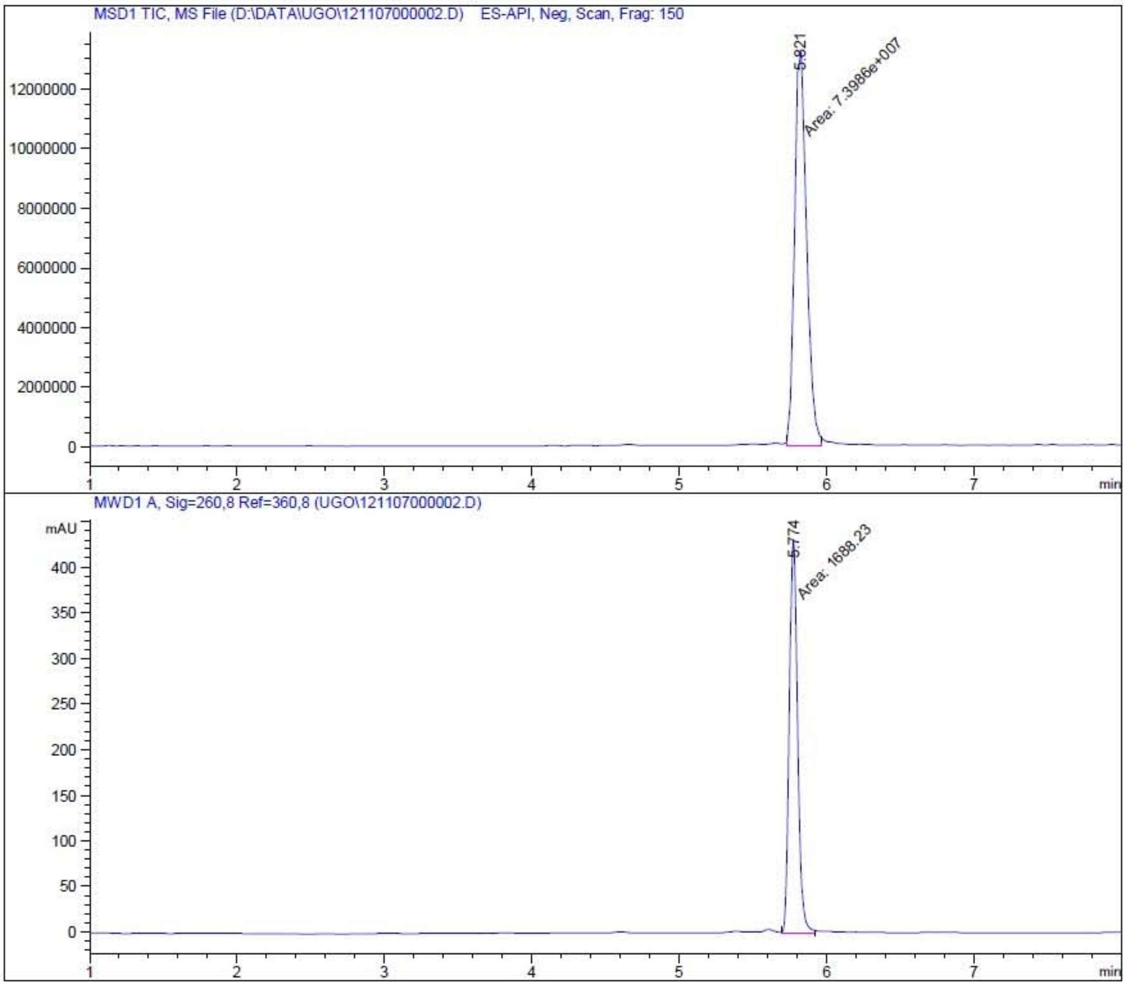
Totals :                  2477.53477  477.82649
```



```
Component  Molecular      Absolute
            Weight        Abundance
    A      16503.21        772287
```

10-34Cy3-let7

```
Additional Info : Peak(s) manually integrated
```



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=====================================================================
                       Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: MSD1 TIC, MS File

Peak RetTime Type  Width      Area       Height      Area
  #   [min]        [min]                              %
----|-------|----|-------|----------|----------|--------|
   1   5.821 MM    0.0899 7.39860e7  1.37148e7  100.0000

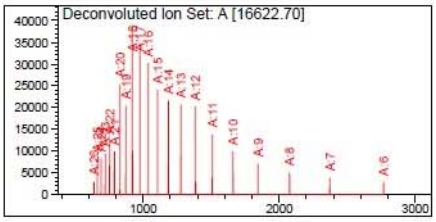
Totals :                  7.39860e7  1.37148e7



Signal 2: MWD1 A, Sig=260,8 Ref=360,8

Peak RetTime Type  Width      Area       Height      Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   5.774 MM    0.0650 1688.22925  432.91113 100.0000

Totals :                  1688.22925  432.91113
```



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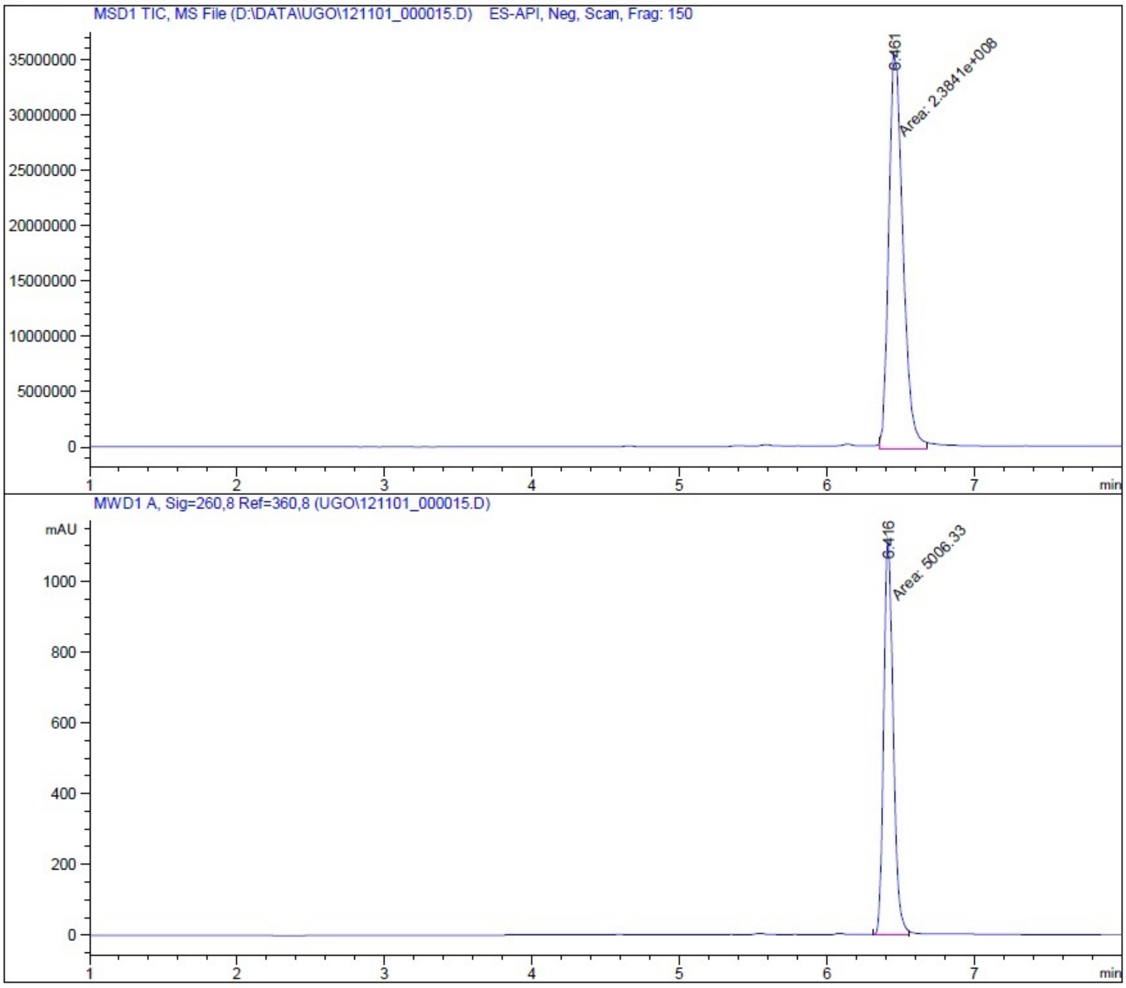
Component  Molecular      Absolute
            Weight        Abundance
   A        16622.70        304037
```

10-34B-let7

```
Additional Info : Peak(s) manually integrated
```



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=====================================================================
                         Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: MSD1 TIC, MS File

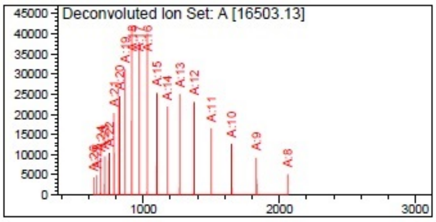
Peak RetTime Type  Width      Area      Height     Area
  #   [min]        [min]                             %
----|-------|----|-------|----------|----------|--------|
   1   6.461 MM    0.1070 2.38410e8  3.71426e7  100.0000

Totals :                  2.38410e8  3.71426e7


Signal 2: MWD1 A, Sig=260,8 Ref=360,8

Peak RetTime Type  Width      Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   6.416 MM    0.0746 5006.32959 1118.32629 100.0000

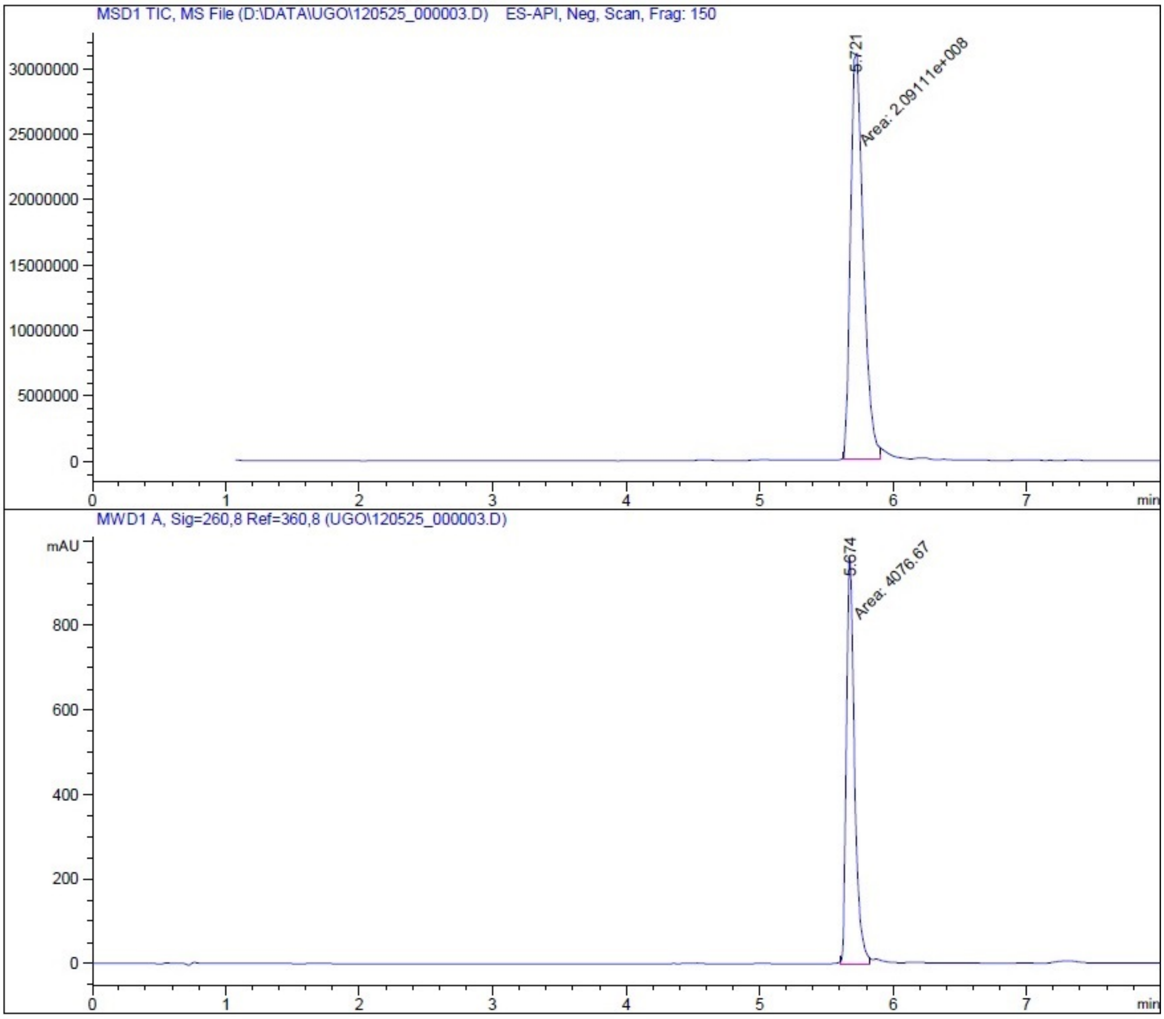
Totals :                  5006.32959 1118.32629
```



```
Component  Molecular      Absolute
             Weight       Abundance
    A       16503.13        356809
```

1Cy3-miR32

```
Additional Info : Peak(s) manually integrated
```



```
=====================================================================
                         Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: MSD1 TIC, MS File

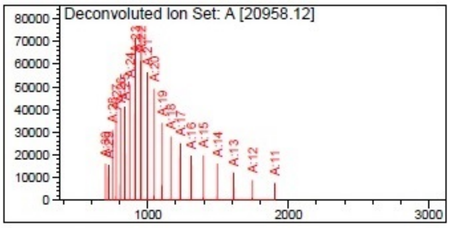
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]                           %
----|-------|----|-------|----------|----------|--------|
   1   5.721 MM    0.1085 2.09111e8  3.21209e7  100.0000

Totals :                  2.09111e8  3.21209e7


Signal 2: MWD1 A, Sig=260,8 Ref=360,8

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]  [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   5.674 MM    0.0700 4076.67090  970.28418 100.0000

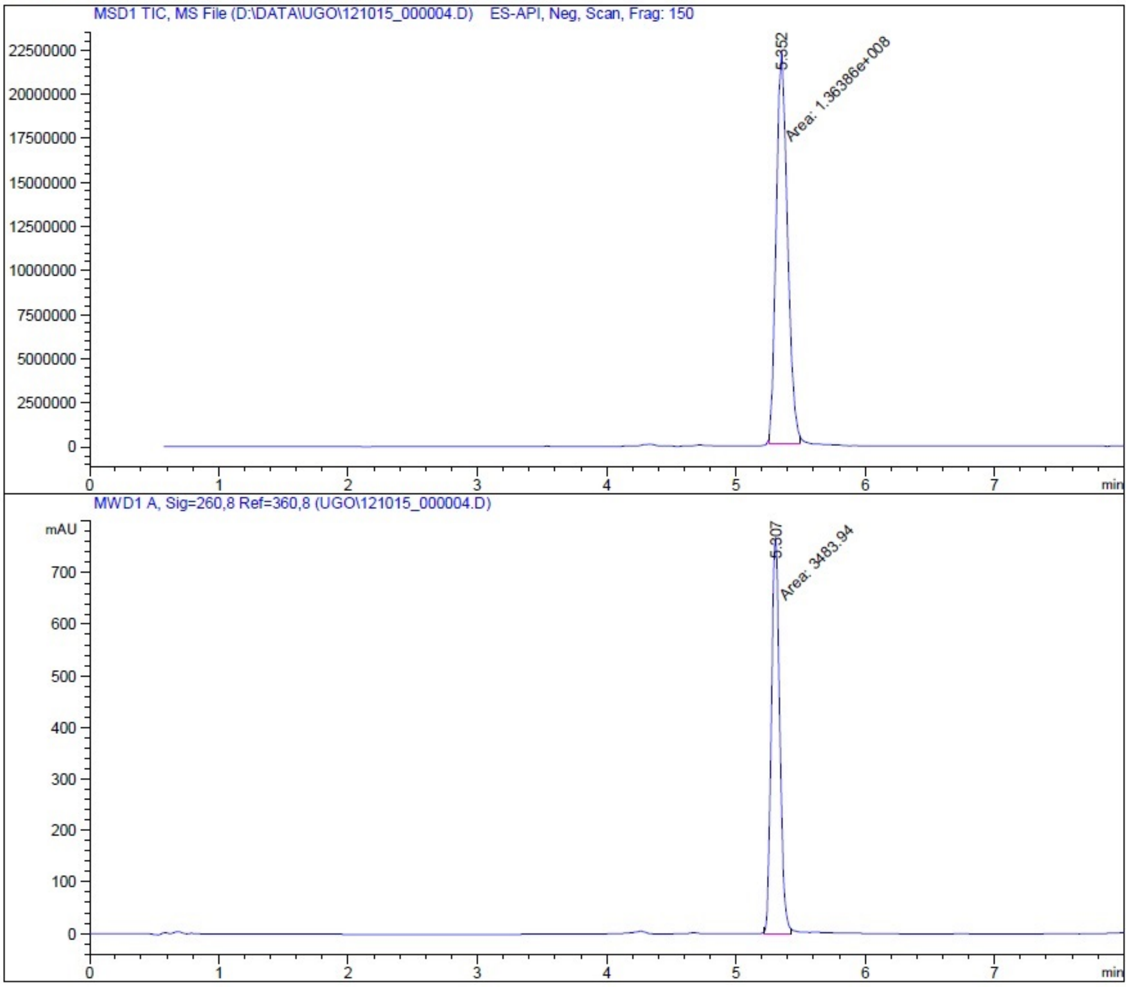
Totals :                  4076.67090  970.28418
```



```
Component  Molecular      Absolute
            Weight        Abundance
    A       20958.12        627124
```

1Cy3-miR101

```
Additional Info : Peak(s) manually integrated
```



```
=====================================================================
                         Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: MSD1 TIC, MS File

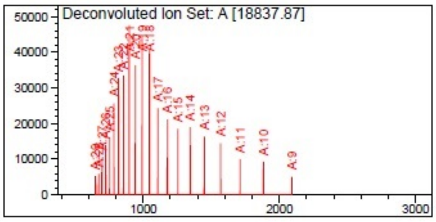
Peak RetTime Type  Width      Area       Height      Area
  #   [min]        [min]                              %
----|-------|----|-------|----------|----------|--------|
   1   5.352 MM     0.1004 1.36386e8  2.26416e7  100.0000

Totals :                    1.36386e8  2.26416e7


Signal 2: MWD1 A, Sig=260,8 Ref=360,8

Peak RetTime Type  Width      Area       Height      Area
  #   [min]        [min]    [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   5.307 MM     0.0757 3483.93530  766.99707 100.0000

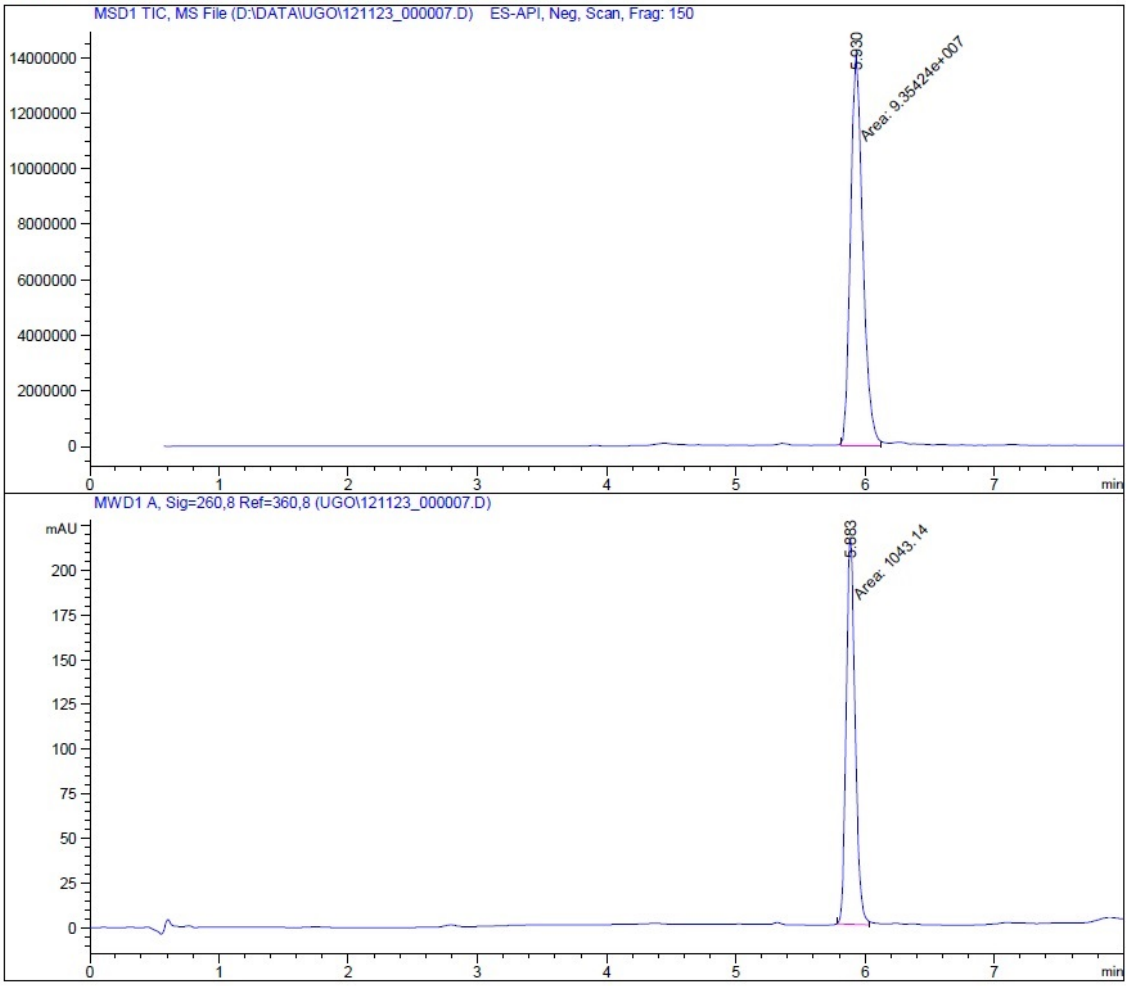
Totals :                    3483.93530  766.99707
```



```
Component  Molecular      Absolute
            Weight        Abundance
    A       18837.87        438297
```

1B-miR101

```
Additional Info : Peak(s) manually integrated
```



```
=====================================================================
                          Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: MSD1 TIC, MS File

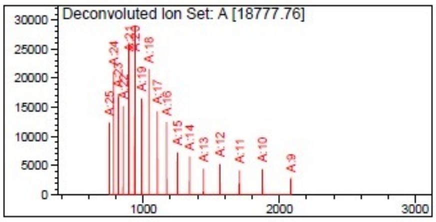
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]                            %
----|-------|----|-------|----------|----------|--------|
   1   5.930 MM     0.1082 9.35424e7  1.44132e7 100.0000

Totals :                   9.35424e7  1.44132e7


Signal 2: MWD1 A, Sig=260,8 Ref=360,8

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   5.883 MM     0.0806 1043.13721  215.81125 100.0000

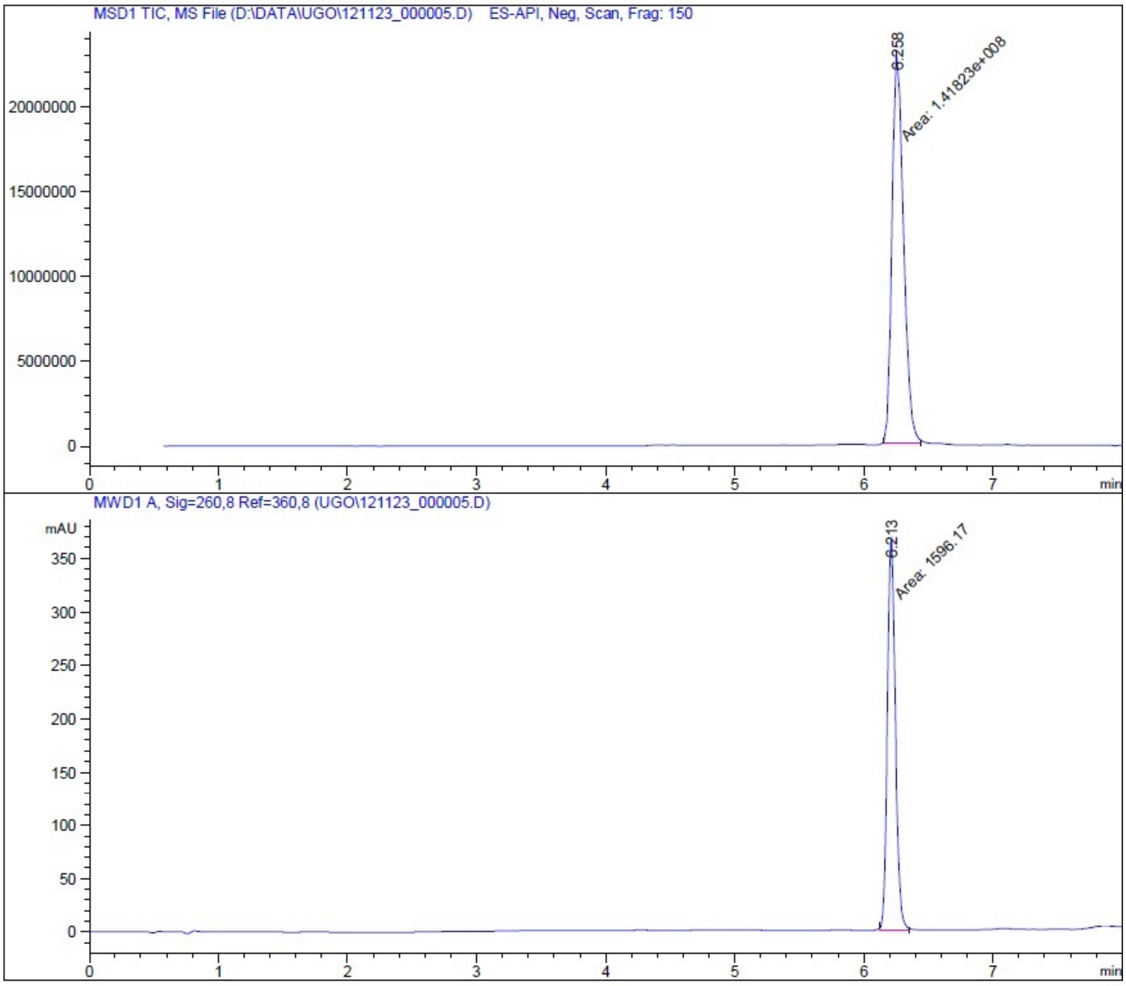
Totals :                   1043.13721  215.81125
```



```
Component  Molecular       Absolute
            Weight         Abundance
    A       18777.76        210736
```

1-8Cy3-miR101

```
Additional Info : Peak(s) manually integrated
```



```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: MSD1 TIC, MS File

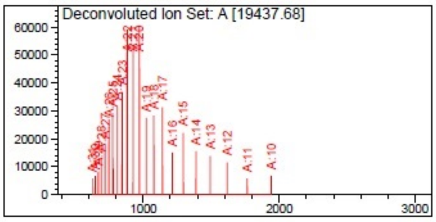
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]                           %
----|-------|----|-------|----------|----------|--------|
   1   6.258 MM    0.1001 1.41823e8  2.36203e7 100.0000

Totals :                  1.41823e8  2.36203e7


Signal 2: MWD1 A, Sig=260,8 Ref=360,8

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   6.213 MM    0.0722 1596.16980  368.36450 100.0000

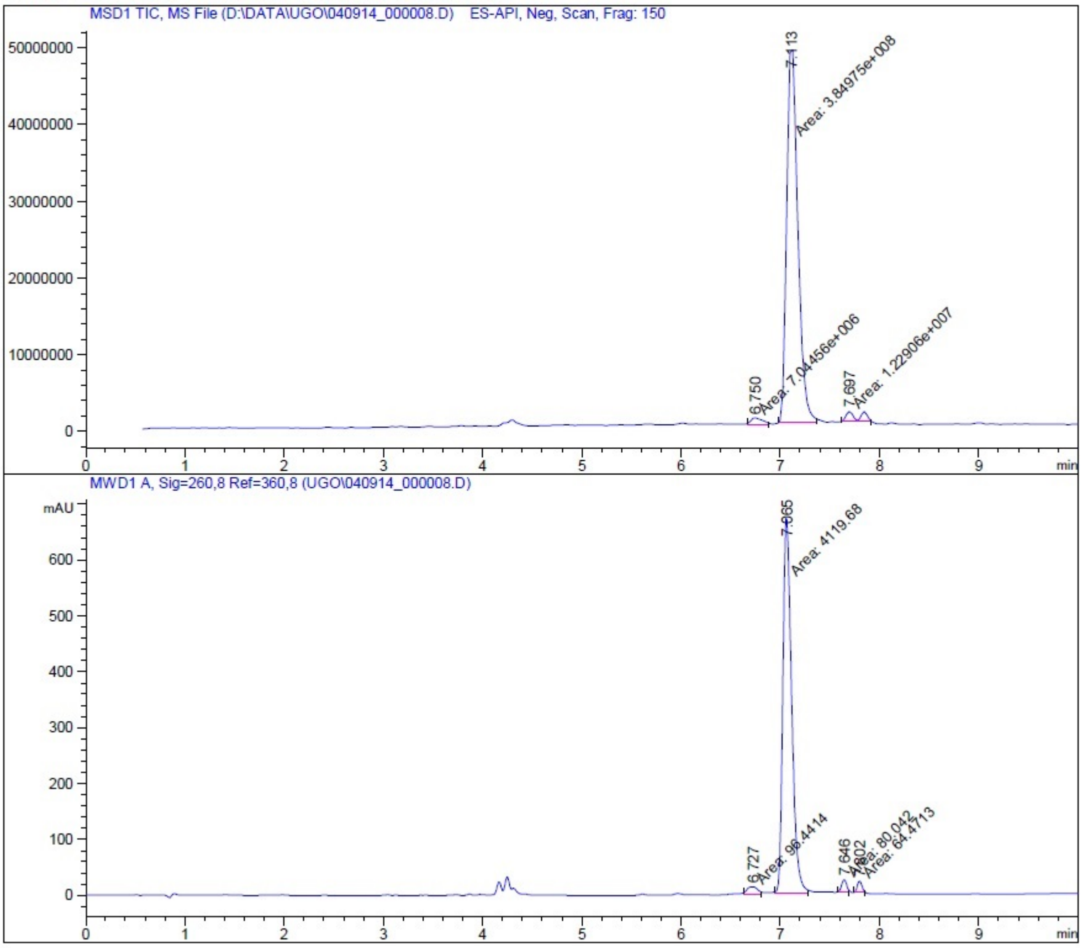
Totals :                  1596.16980  368.36450
```



```
Component  Molecular      Absolute
             Weight       Abundance
    A       19437.68       502778
```

1-8B-miR101

```
Additional Info : Peak(s) manually integrated
```



```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: MSD1 TIC, MS File

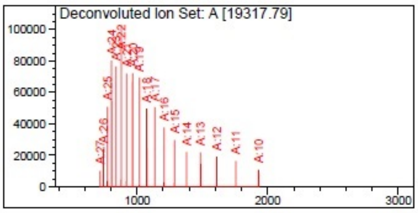
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]                           %
----|-------|----|-------|----------|----------|--------|
   1   6.750 MM    0.1364 7.04456e6  8.60647e5   1.7424
   2   7.113 MM    0.1284 3.84975e8  4.99851e7  95.2177
   3   7.697 MM    0.1524 1.22906e7  1.34454e6   3.0399

Totals :                  4.04310e8  5.21903e7


Signal 2: MWD1 A, Sig=260,8 Ref=360,8

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   6.727 MM    0.1245   96.44141   12.90719   2.2116
   2   7.065 MM    0.1020 4119.67920  673.11963  94.4743
   3   7.646 MM    0.0590   80.04203   22.62934   1.8356
   4   7.802 MM    0.0525   64.47128   20.46119   1.4785

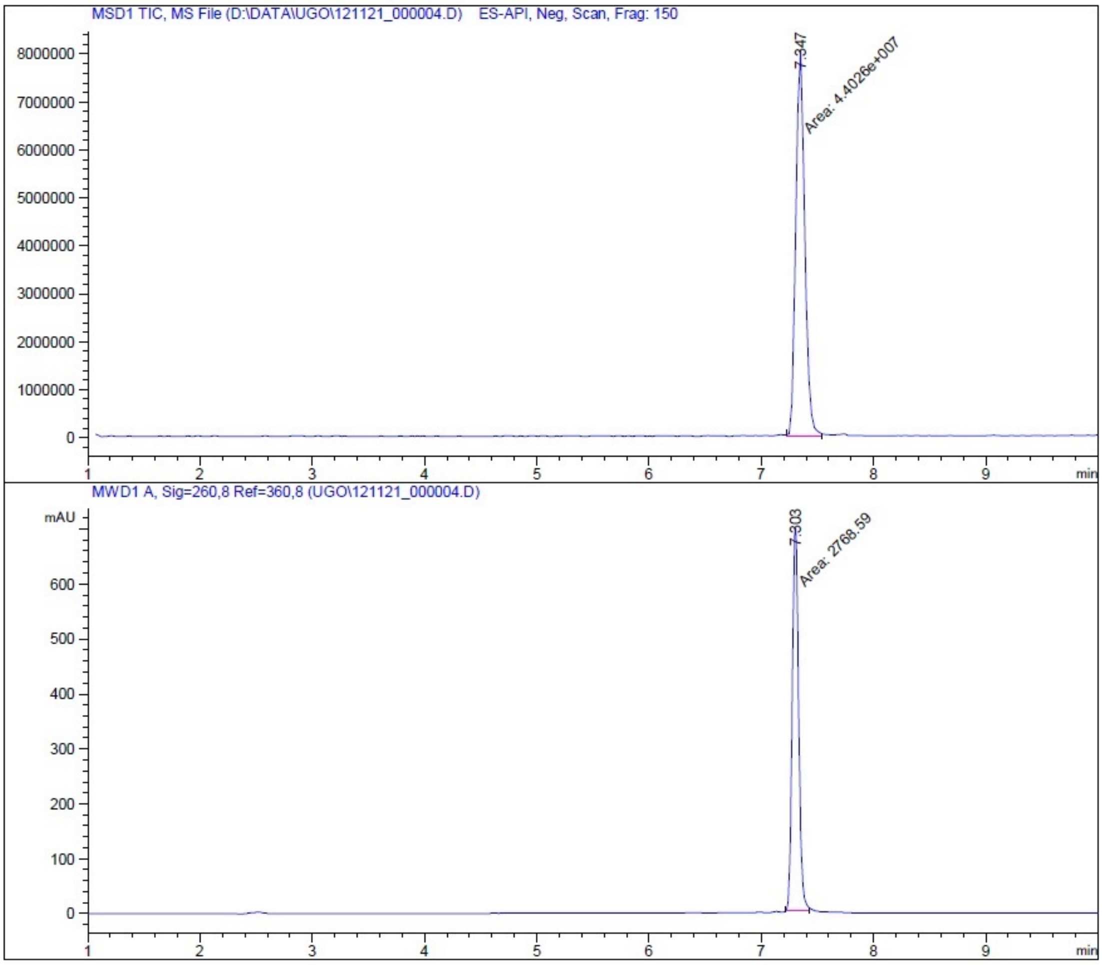
Totals :                  4360.63393  729.11736
```

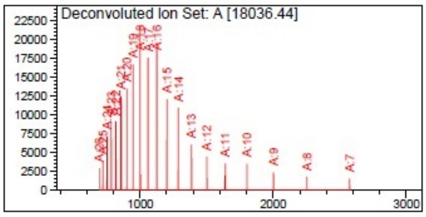


```
Component  Molecular      Absolute
            Weight        Abundance
    A       19317.79        786256
```

3’-biotin-let7a

```
Additional Info : Peak(s) manually integrated
```





```
Component  Molecular        Absolute
            Weight          Abundance
    A       18036.44          165934
```

```
=====================================================================
                         Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: MSD1 TIC, MS File

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]                            %
----|-------|----|-------|----------|----------|--------|
   1   7.347 MM    0.0890 4.40260e7  8.24123e6  100.0000

Totals :                  4.40260e7  8.24123e6


Signal 2: MWD1 A, Sig=260,8 Ref=360,8

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   7.303 MM    0.0660 2768.59375  699.56787 100.0000

Totals :                  2768.59375  699.56787
```

Figure S1. Reverse-phase high performance liquid chromatography mass spectrometry (LCMS) chromatograms of labeled pre-miRNAs.

Optimization of the FRET acceptor on spectrofluorometer (Fig S2)

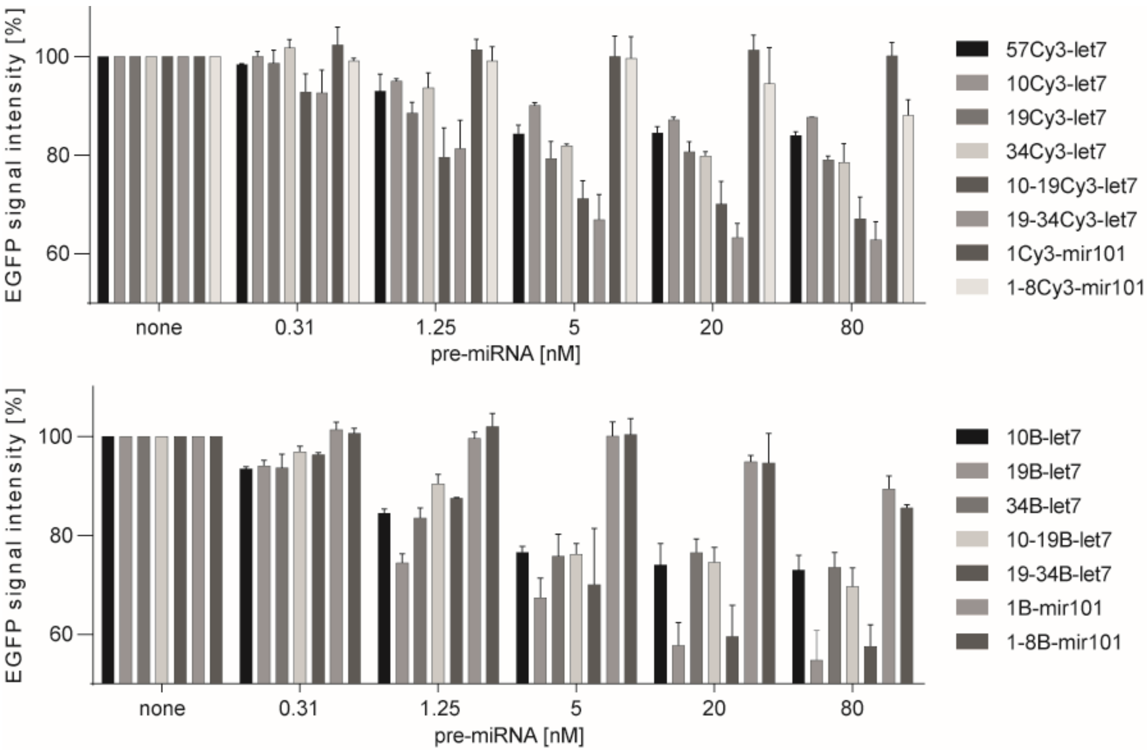


Figure S2: Evaluation of pre-let-7a-2 labeled at various positions with Cy3 or BHQ-1. FRET was determined by measuring fluorescence intensity of EGFP-Lin28 relative to samples without addition of RNA.

Absorption spectra of 19B-let7 and fluorescence spectra of EGFP-Lin28B (Fig S3).

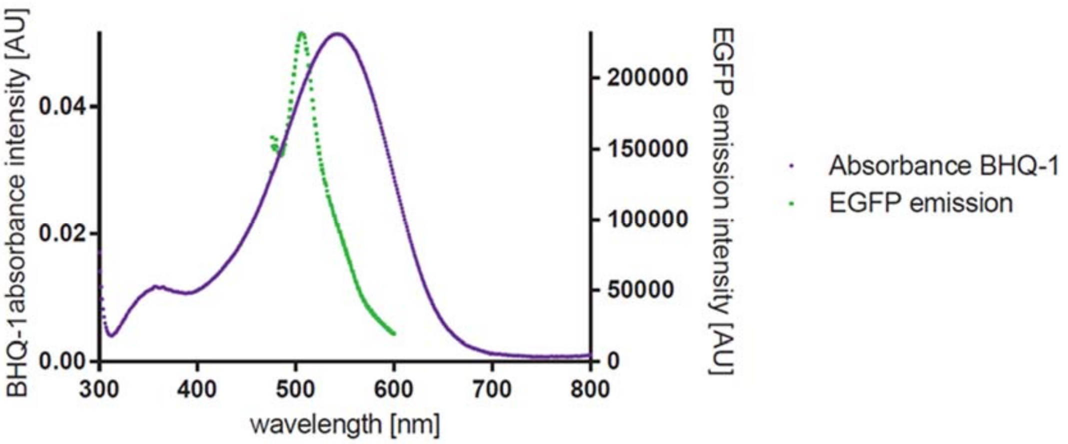


Figure S3. Absorption spectrum of 19B-let7 in water (purple) and fluorescence spectrum of EGFP-Lin28B in binding buffer (green). Absorption was measured in a Perkin Elmer Lambda 35 UV/VIS spectrometer; fluorescence was measured on a Photon Technology International (PTI) spectrofluorometer with excitation at 465 nm and correction for binding buffer signals. Binding buffer was 300 mM NaCl, 25 mM HEPES pH 7.2, 10 µM $ZnCl_2$, 1% Top-Block, 0.05% Tween 20.

Pilot FRET experiment using SSMD* (Fig S4a)

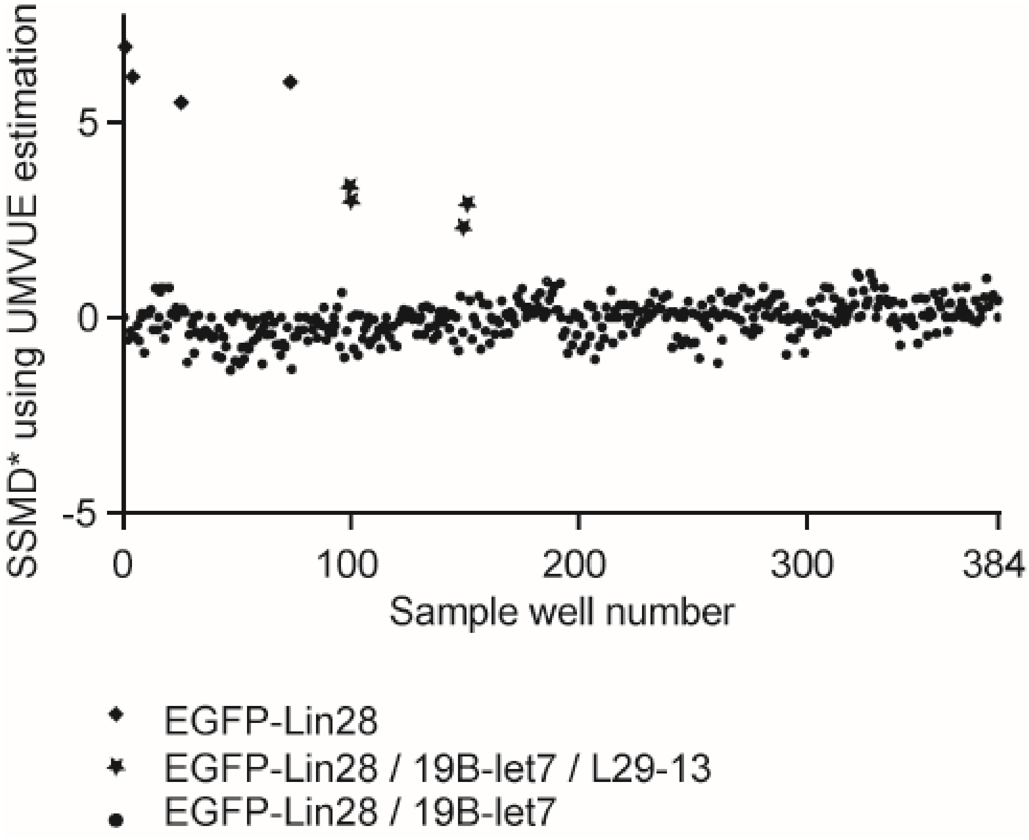
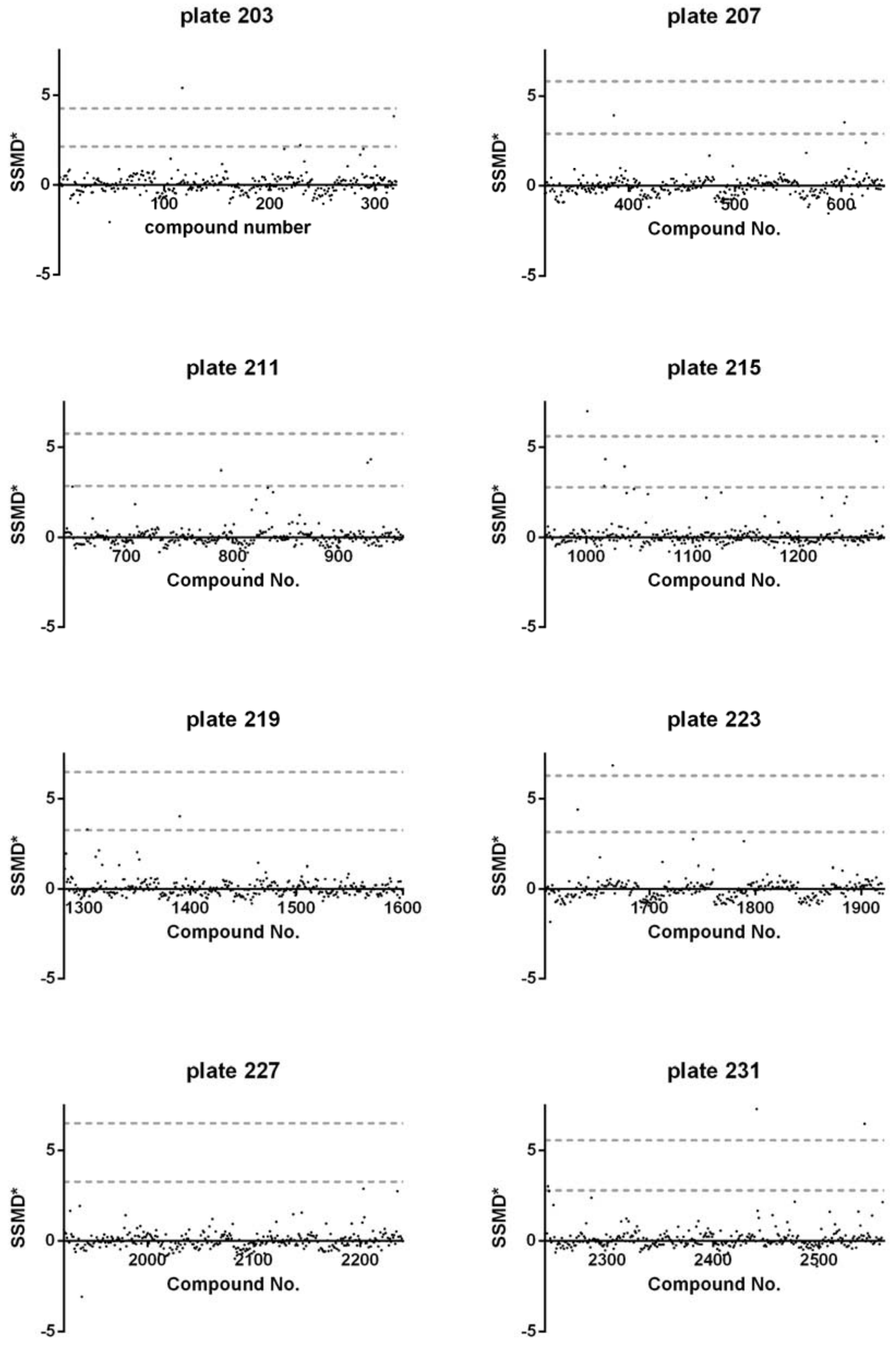


Figure S4a. Pilot-screen conducted by measuring EGFP in 376 wells containing 19B-let7/EGFP-Lin28B (black dots), four wells containing EGFP-Lin28B alone (diamonds) and four wells containing 19B-let-7/EGFP-Lin28B/L29-13 (stars).

Plate-to-plate analysis of high throughput screening data using SSMD* (Fig S4b)



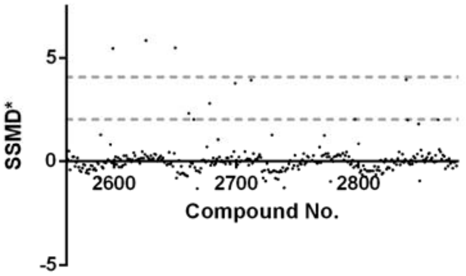


plate 235

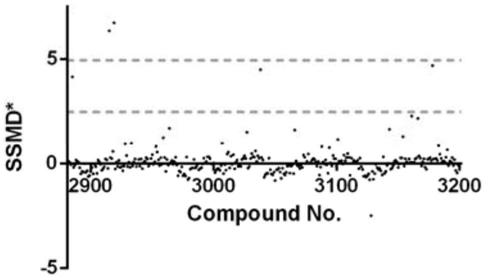


plate 239

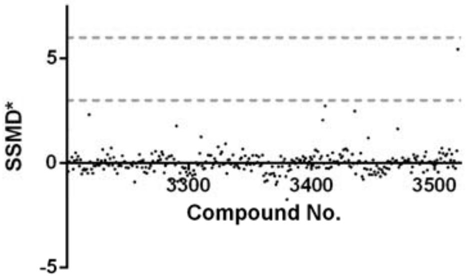


plate 243

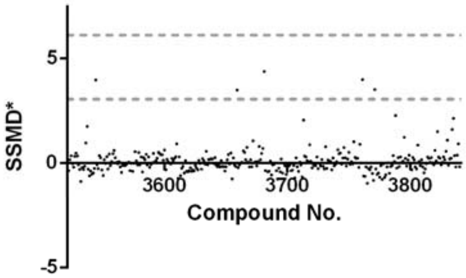


plate 247

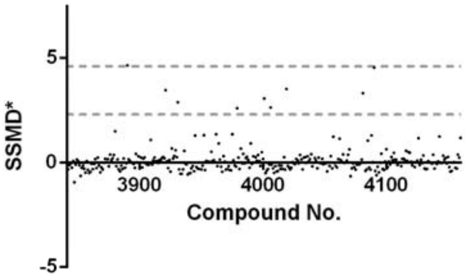


plate 251

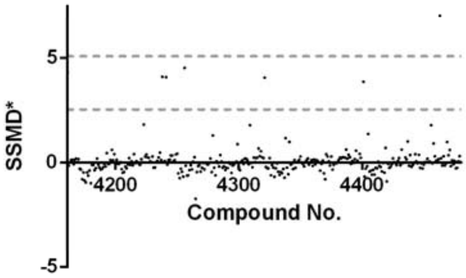
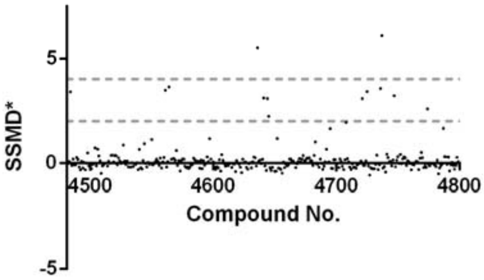


plate 255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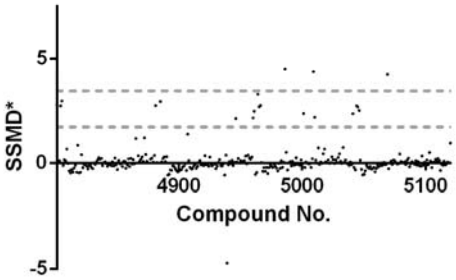
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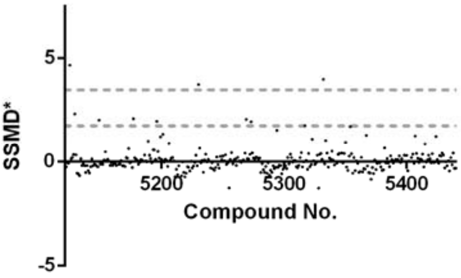
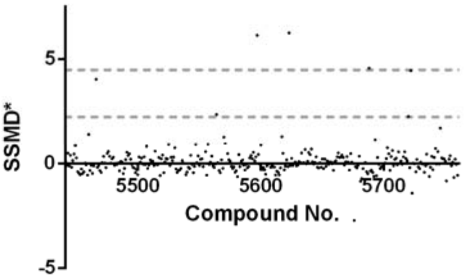


plate 267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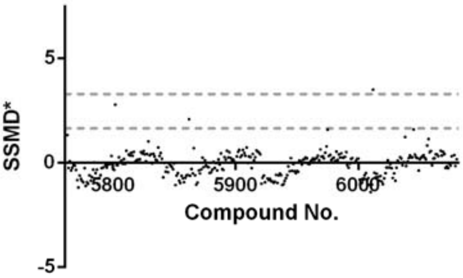


plate 275

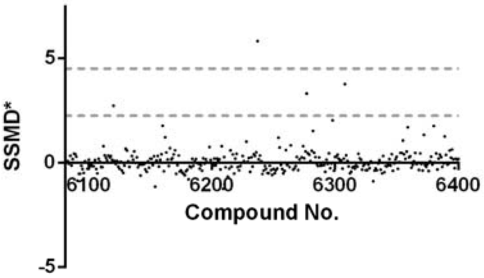


plate 279

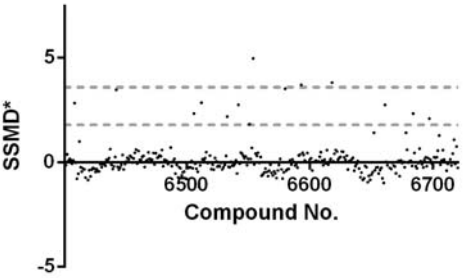


plate 283

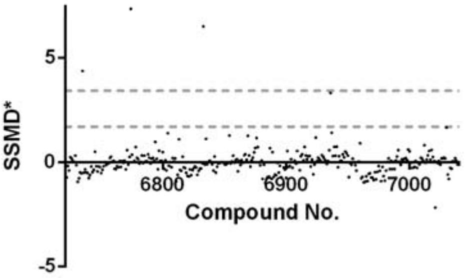


plate 287

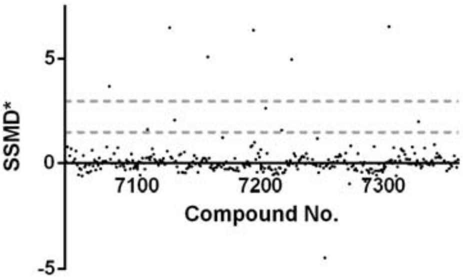


plate 291

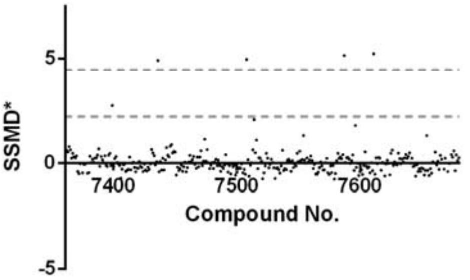


plate 295

plate 267

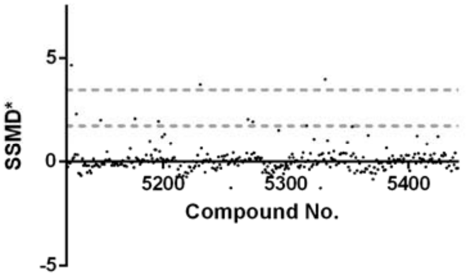


plate 271

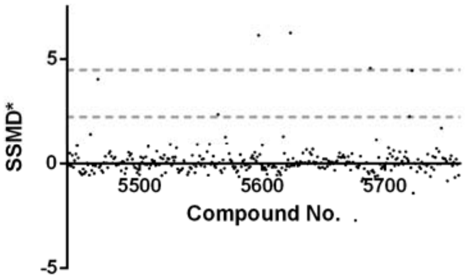


plate 275

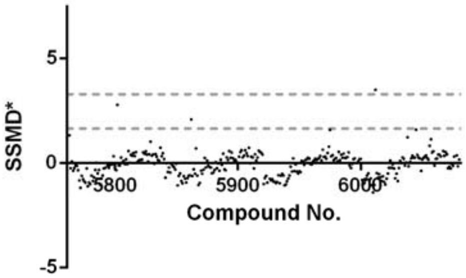


plate 279

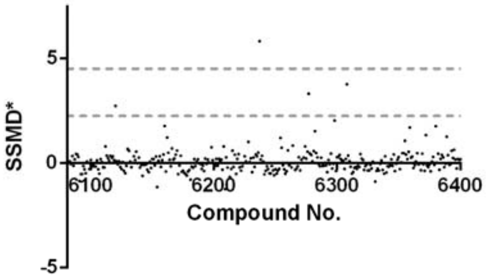


plate 283

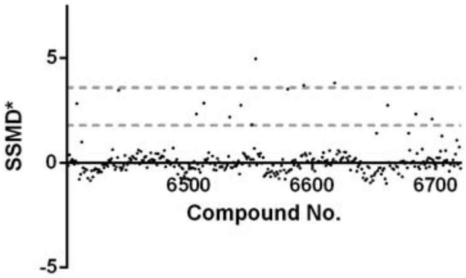


plate 287

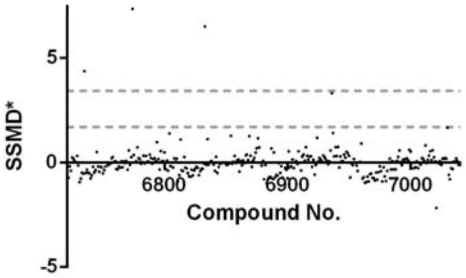


plate 291

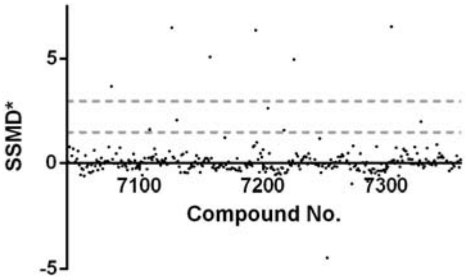
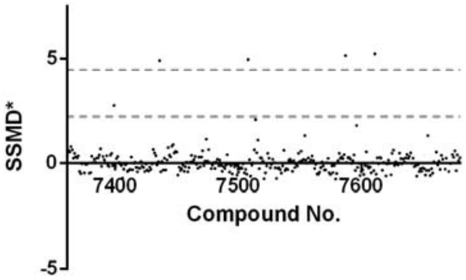


plate 295



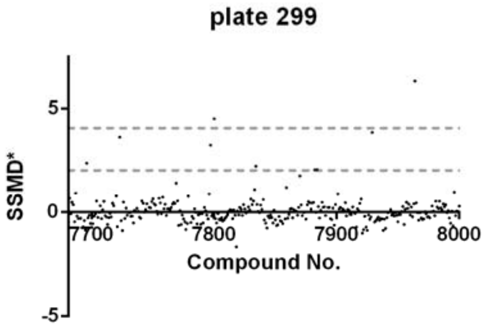


plate 299

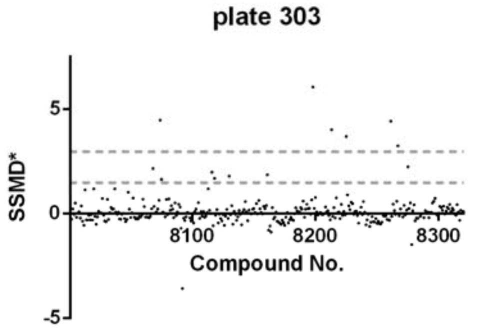


plate 303

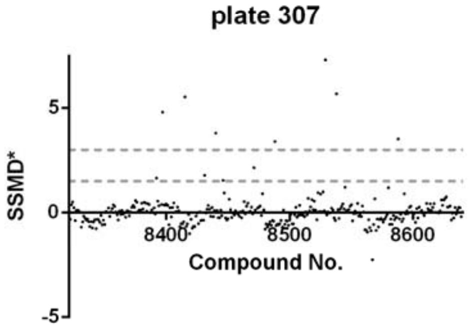


plate 307

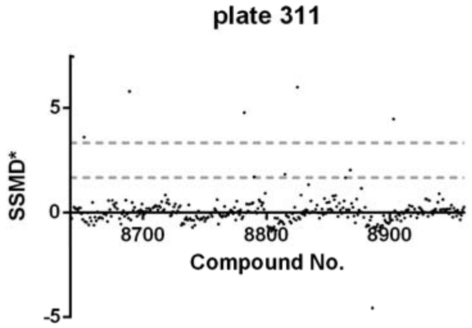
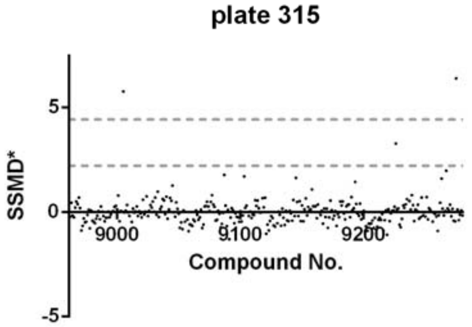


plate 311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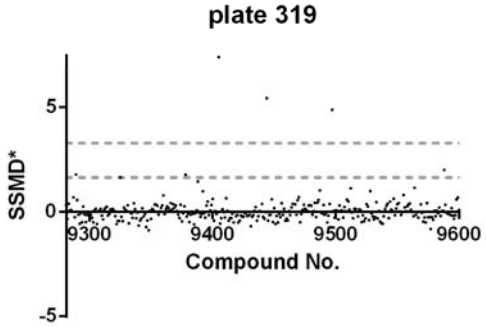
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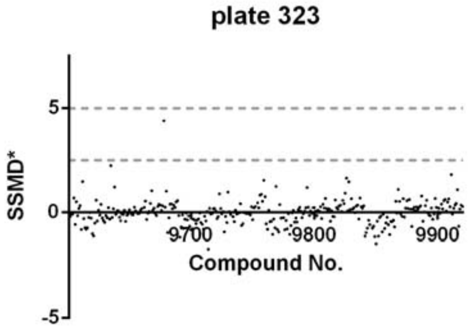
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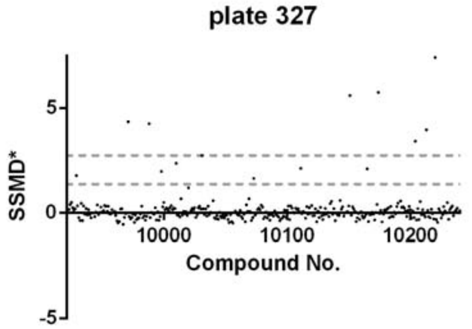


plate 327

plate 335

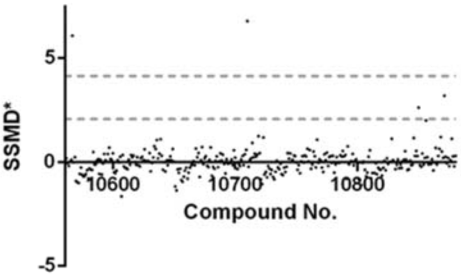
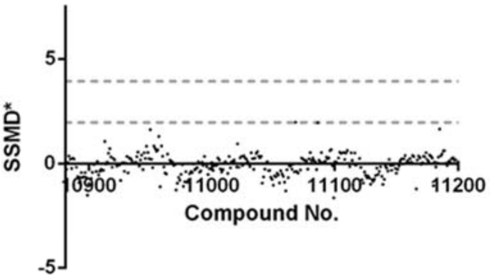


plate 339



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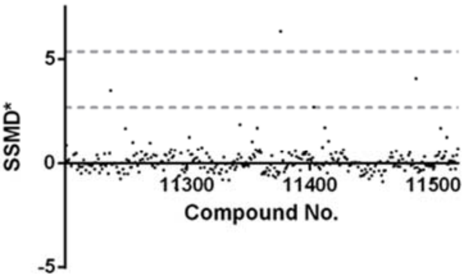

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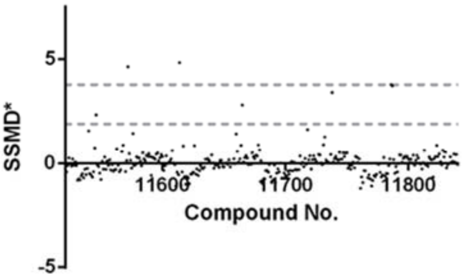

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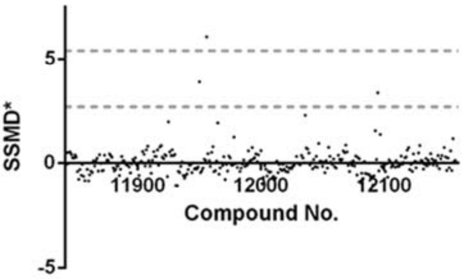

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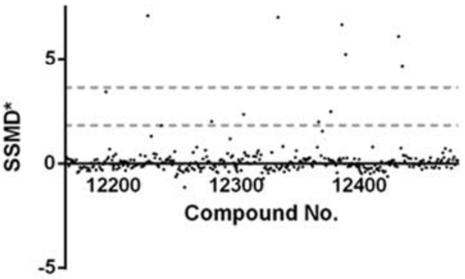

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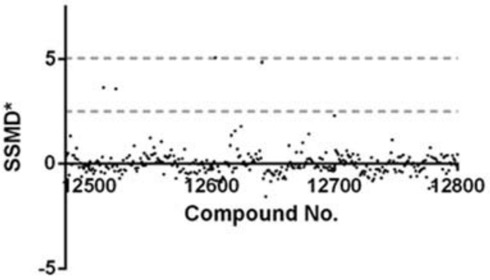

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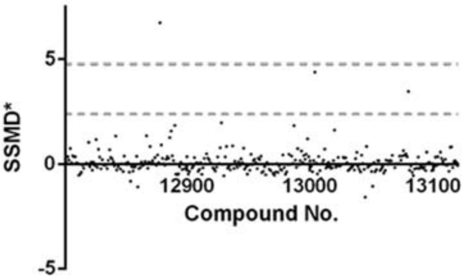

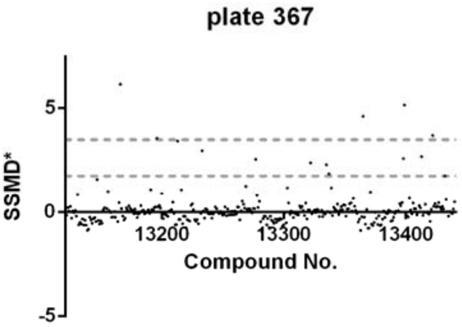
plate 367
SSMD*
5
0
-5
13200
13300
13400
Compound No.

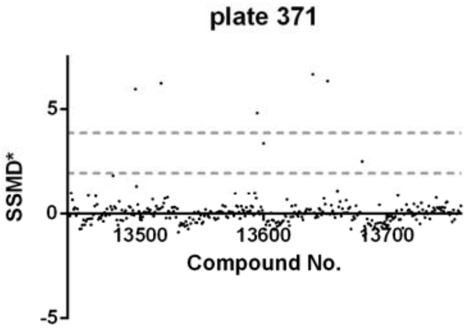
plate 371
SSMD*
5
0
-5
13500
13600
13700
Compound No.

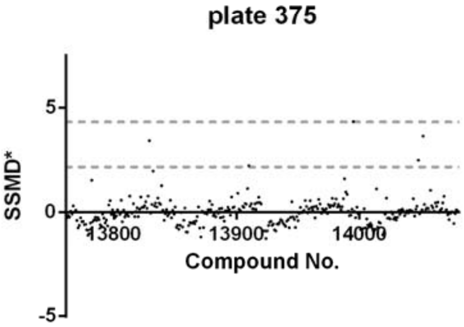
plate 375
SSMD*
5
0
-5
13800
13900
14000
Compound No.

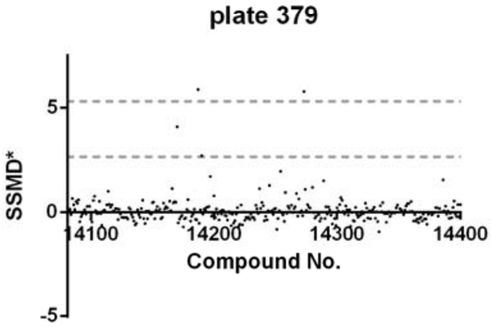
plate 379
SSMD*
5
0
-5
14100
14200
14300
14400
Compound No.

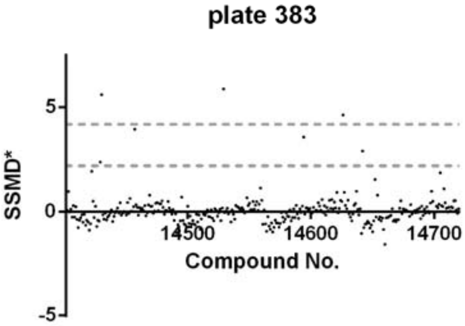
plate 383
SSMD*
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0
-5
14500
14600
14700
Compound No.

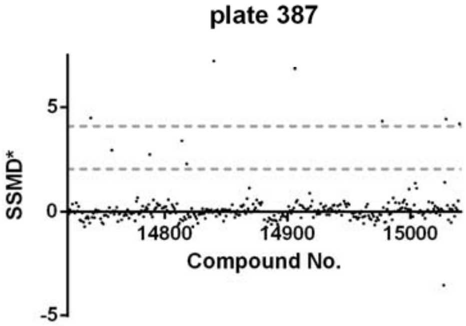


plate 387
SSMD*
5
0
-5
14800
14900
15000
Compound No.

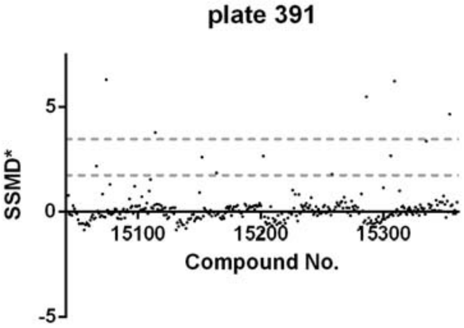


plate 391
SSMD*
5
0
-5
15100
15200
15300
Compound No.

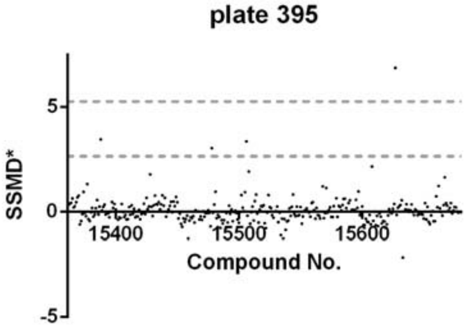


plate 395
SSMD*
5
0
-5
15400
15500
15600
Compound No.

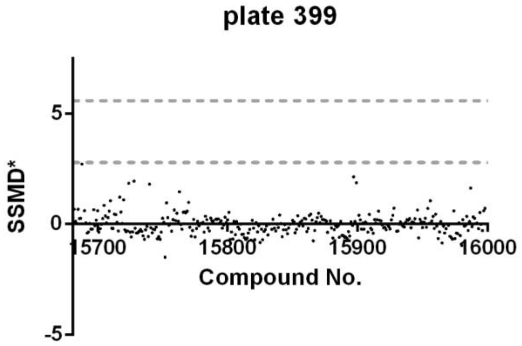


Figure S4b. Data of 16’000 compounds from the FRET HTS screen (without replicates) shown plate-by-plate. Lower grey dotted line indicates the lower threshold set for compound selection: > 66% of SSMD* of the baseline reference (EGFP-Lin28B/DMSO) values in each respective plate. Upper gray dotted line indicates the upper threshold set for compound selection (< 133%).

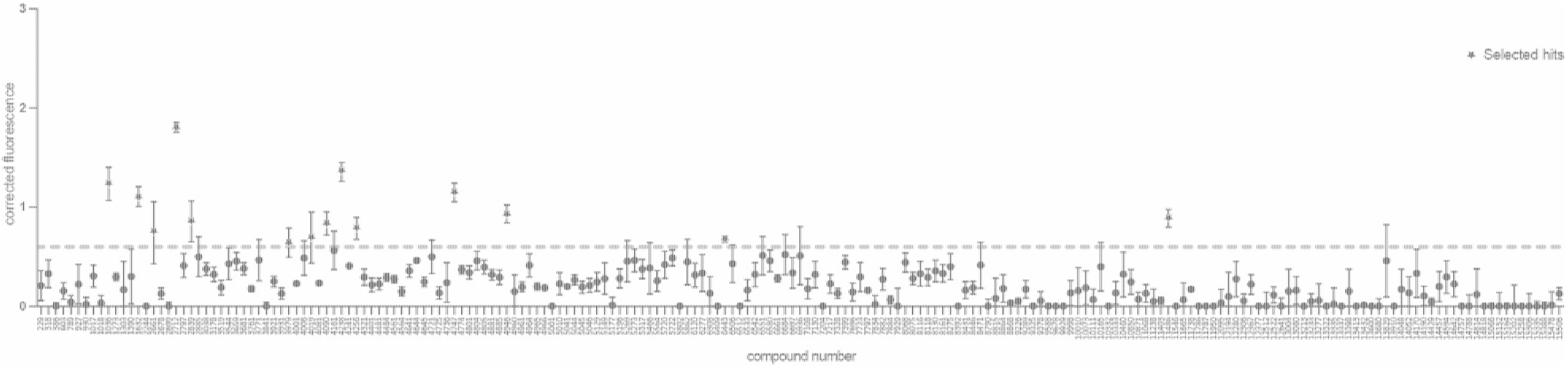

3
2
1
0
corrected fluorescence
compound number
★ Selected hits

Figure S5a (previous page). Full data set (selected hits are shown in Fig 2b of the main manuscript) from the confirmatory screen of 203 hits selected from the primary HTS, corrected for compound self-fluorescence. Values represent triplicates. Stars indicate hits selected for follow up in cellular assays (above dotted line: top 7%). Error bars indicate ± 1 SD (n=3).

Re-screening of the 14 confirmed hits from the primary screen (Fig S5b).

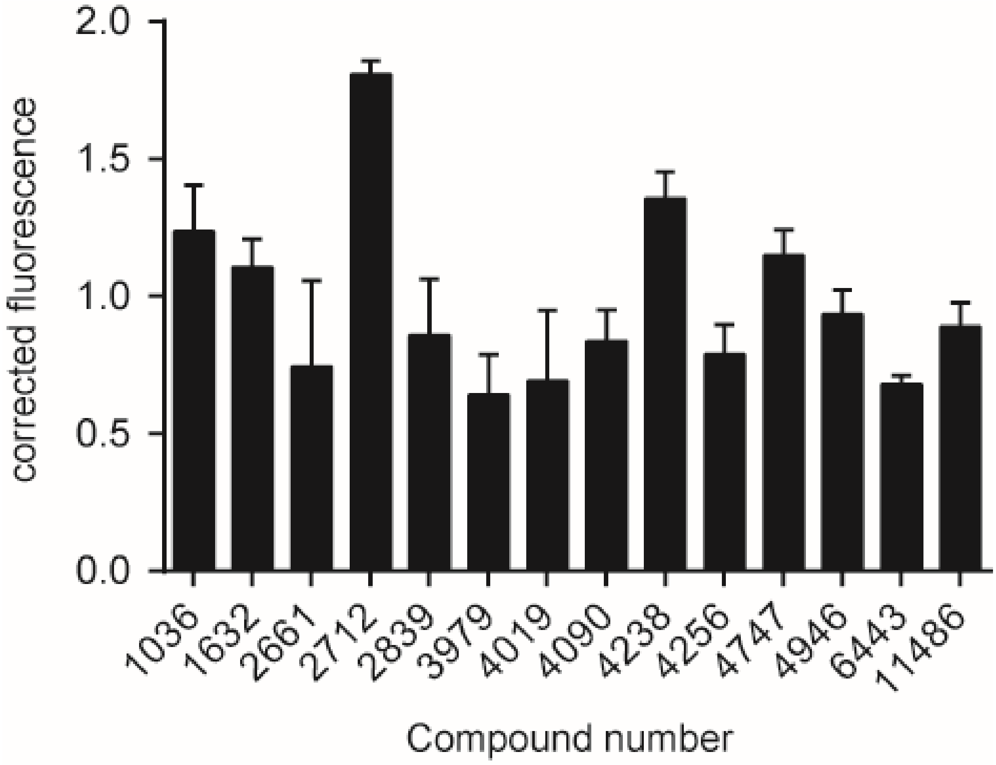


Figure S5b. Data from 14 confirmed compounds from the re-screen of 203 hits chosen from the primary HTS, corrected for compound self-fluorescence. Values represent triplicates. Error bars indicate ± 1 SD (n=3).

Luciferase reporter activity upon treatment of Huh7 cells with hit compounds (Fig S6a).

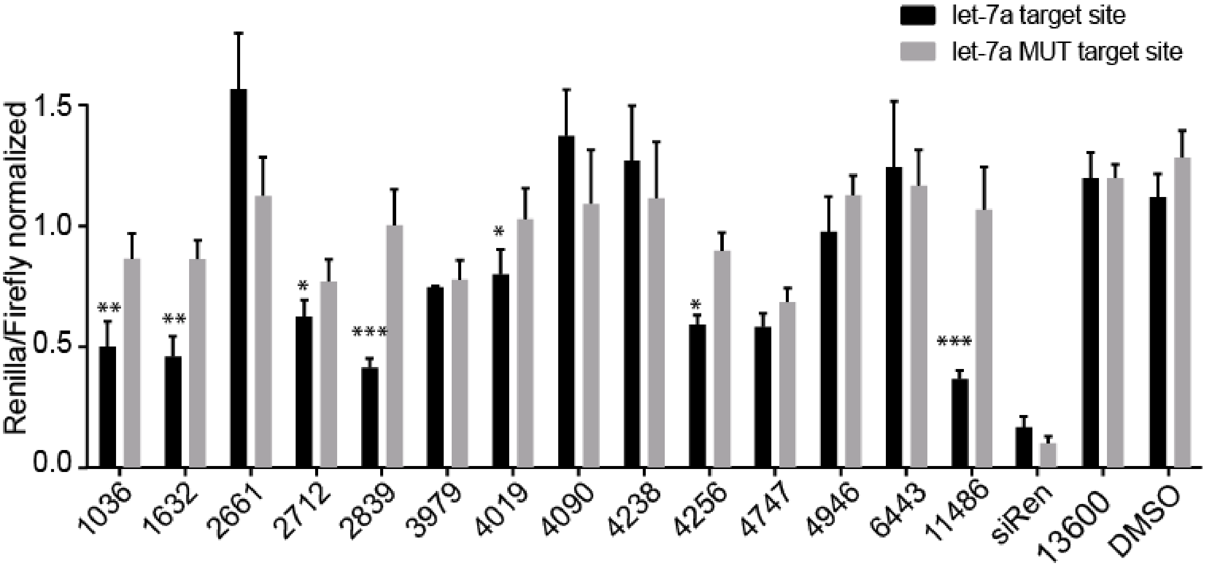


Figure S6a. Huh7 cells express measurable levels of Lin28B and show an active Lin28-mediated suppression of let-7[3]. A conventional Renilla luciferase let-7 reporter bearing four let-7 target sites from HMGA2 was used to assess endogenous let-7 functional activity and a mutated vector as a negative control (Table S2)[3]. Hit compounds and controls in aqueous DMSO (30 μM) were co-administered with let-7 dual-luciferase vectors (wild-type and mutated control) into Huh7 cells. Black bars: a vector containing four let-7 target sites from *HMGA2* (Table S2); grey bars: the same construct mutated at 3 positions in each seed-targeting sequence.

Dose-dependent luciferase reporter activity upon treatment of Huh7 cells with follow-up hit compounds (Fig S6b).

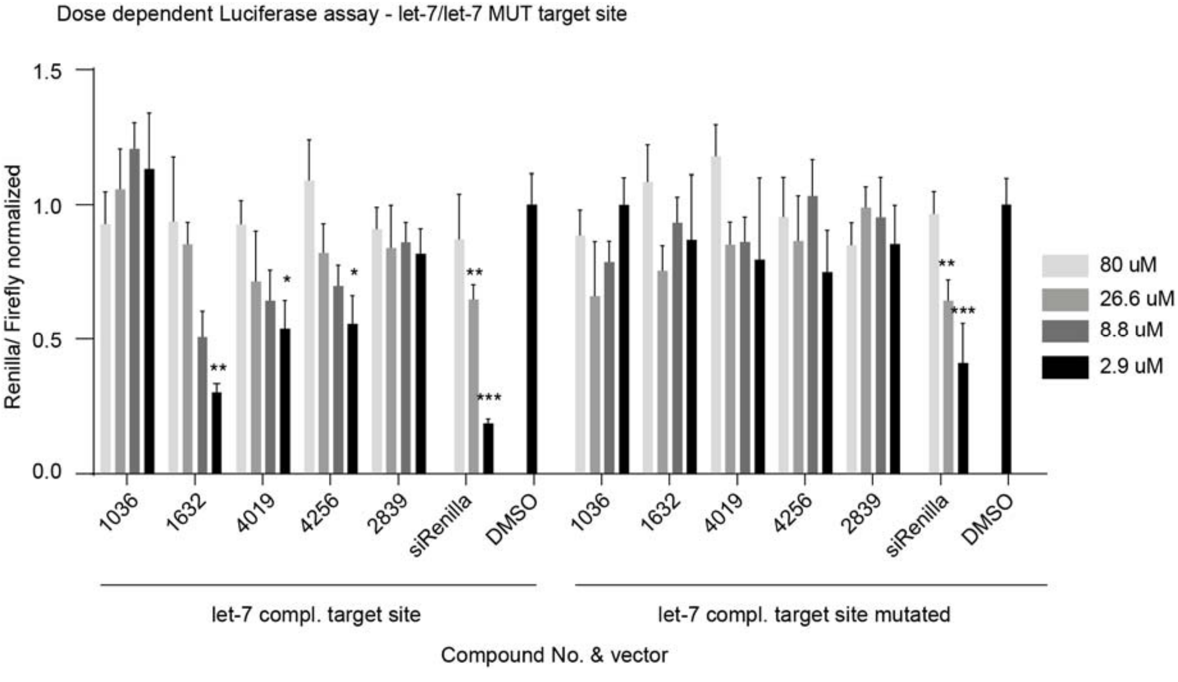


Figure S6b. Concentration-dependent effects of selected hits compounds 1036, 1632, 4019, 4256 (reordered or resynthesized) and negative control 2839 on luciferase target vector and mutated target vector, normalized to DMSO treatment. Experiment was performed in triplicates. Statistical analysis was performed by ANOVA using Dunnett's post-test, comparing against the negative control treatment DMSO for each dose. (*) $P < 0.05$; (**) $P < 0.01$; (***) $P < 0.001$. Statistics were run with GraphPad.

Endogenous let-7 levels upon treatment of Huh7 cells with hit compounds (Fig S7).

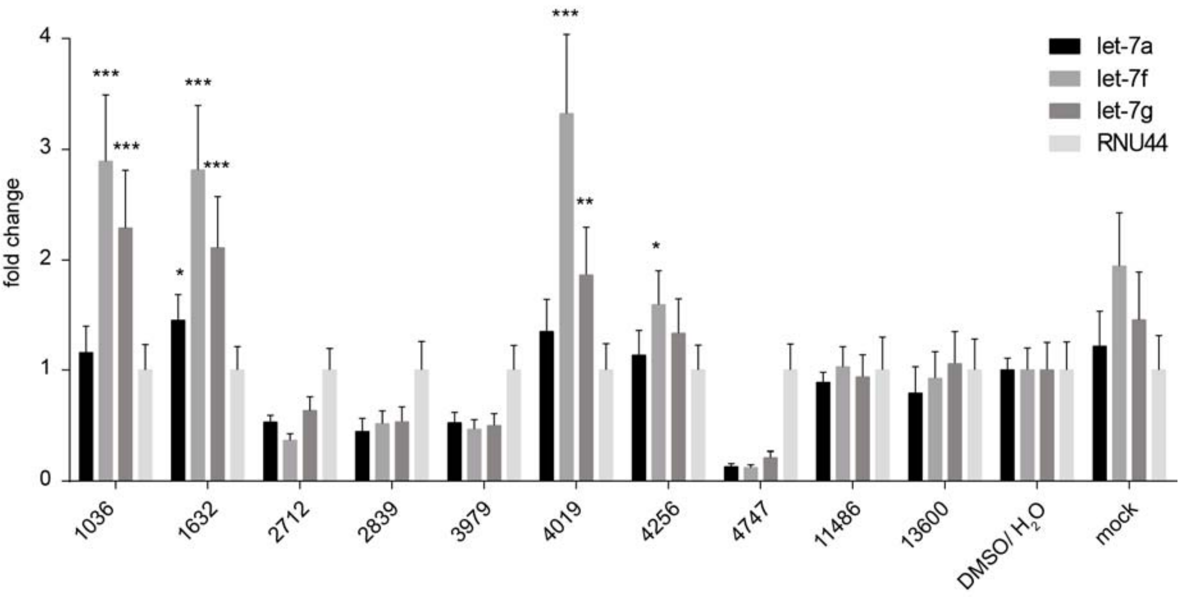


Figure S7. Endogenous levels of mature let-7a, let-7g, and let-7f, 48h after treatment with nine selected compounds and negative control (13600) in Huh7 cells at 60 µM concentration, measured by Taqman RT-qPCR. Levels are normalized to DMSO treatment and snoRNA RNU44. Experiment was repeated three times. Statistical analysis was performed by ANOVA using Dunnett's post-test, comparing against the negative control treatment DMSO/H2O. (*) $P < 0.05$; (**) $P < 0.01$; (***) $P < 0.001$. Statistics were run with GraphPad.

Inhibitory effects of selected compounds on Lin28/pre-let-7 measured by RNA ELISA (Fig S8).

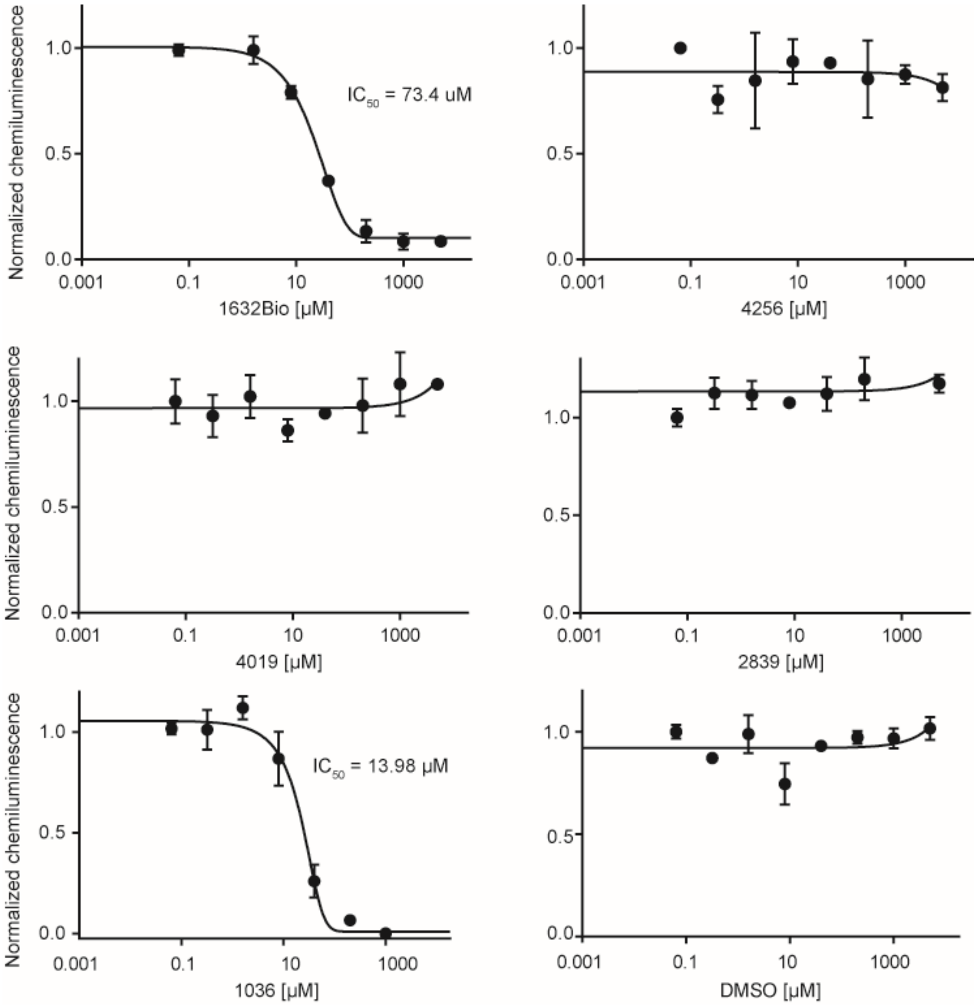
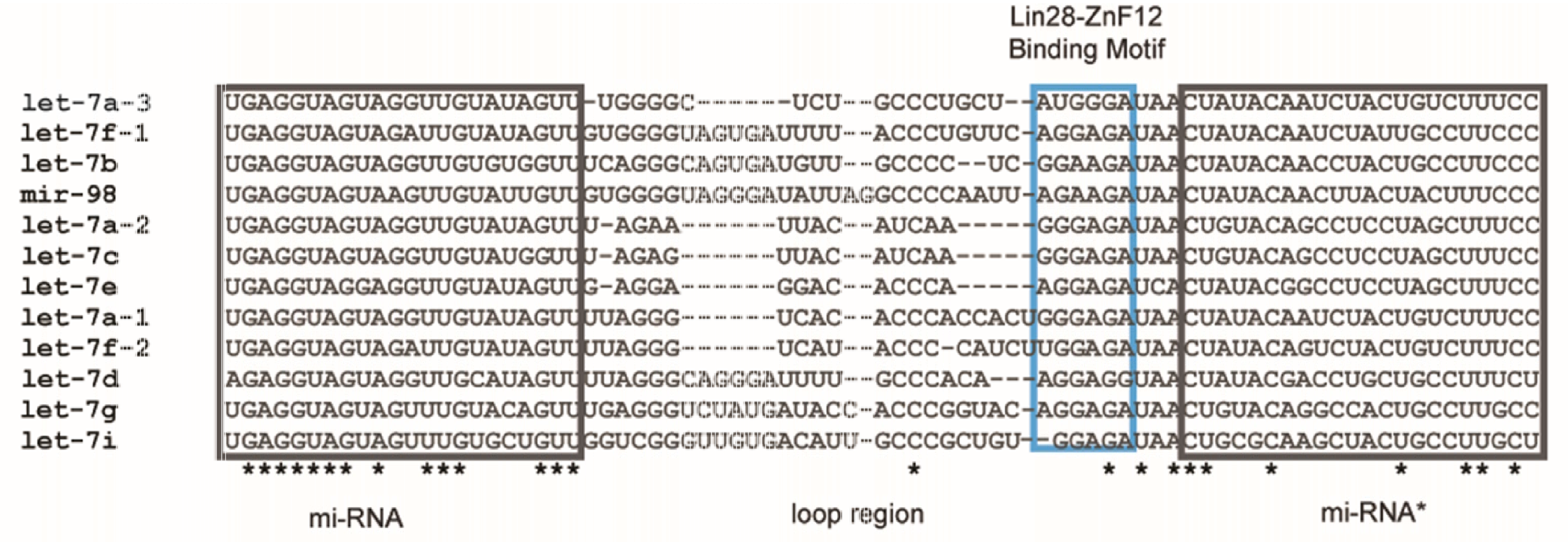


Figure S8. Binding inhibition curves of small molecules 4256, 4019, 2839, 1036, biotin-tagged 1632 (1632Bio) and DMSO to pre-let-7a-2 by RNA-based ELISA. Compounds were pre-incubated with HeLa cell lysates containing Myc-tagged Lin28A and added to wells coated with truncated pre-let-7a-2. The fraction of bound Lin28A was determined by immunostaining of its Myc-tag. No binding inhibition was detectable for 4019, 4256, the negative control 2839 and DMSO whereas the binding affinity (IC_{50}) of compound 1036 was 13.98 µM. We verified that the modified 1632Bio was able to antagonize EGFP-Lin28B/pre-let-7a-2 similarly to the parent 1632, i.e. that the biotinylated linker did not interfere with inhibition. We measured an attenuated IC50 of 73.4 µM, approximately nine-fold weaker than that of the parent compound (1632) (Fig. 3d). Experiment was done in triplicates. Error bars indicate +/- 1 SD.

Sequences of the precursors of let-7s (Fig S9).



```
let-7a-3  UGAGGUAGUAGGUUGUAUAGUU-UGGGGC------UCU--GCCCUGCU--AUGGGAUAACUAUACAAUCUACUGUCUUUCC
let-7f-1  UGAGGUAGUAGAUUGUAUAGUUGUGGGGUAGUGAUUUU--ACCCUGUUC-AGGAGAUAACUAUACAAUCUAUUGCCUUCCC
let-7b    UGAGGUAGUAGGUUGUGUGGUUUCAGGGCAGUGAUGUU--GCCCC--UC-GGAAGAUAACUAUACAACCUACUGCCUUCCC
mir-98    UGAGGUAGUAAGUUGUAUUGUUGUGGGGUAGGGAUAUUAGGCCCCAAUU-AGAAGAUAACUAUACAACUUACUACUUUCCC
let-7a-2  UGAGGUAGUAGGUUGUAUAGUUU-AGAA------UUAC--AUCAA-----GGGAGAUAACUGUACAGCCUCCUAGCUUUCC
let-7c    UGAGGUAGUAGGUUGUAUGGUUU-AGAG------UUAC--AUCAA-----GGGAGAUAACUGUACAGCCUCCUAGCUUUCC
let-7e    UGAGGUAGGAGGUUGUAUAGUUG-AGGA------GGAC--ACCCA-----AGGAGAUCACUAUACGGCCUCCUAGCUUUCC
let-7a-1  UGAGGUAGUAGGUUGUAUAGUUUUAGGG------UCAC--ACCCACCACUGGGAGAUAACUAUACAAUCUACUGUCUUUCC
let-7f-2  UGAGGUAGUAGAUUGUAUAGUUUUAGGG------UCAU--ACCC-CAUCUUGGAGAUAACUAUACAGUCUACUGUCUUUCC
let-7d    AGAGGUAGUAGGUUGCAUAGUUUUAGGGCAGGGAUUUU--GCCCACA---AGGAGGUAACUAUACGACCUGCUGCCUUUCU
let-7g    UGAGGUAGUAGUUUGUACAGUUUGAGGGUCUAUGAUACC-ACCCGGUAC-AGGAGAUAACUGUACAGGCCACUGCCUUGCC
let-7i    UGAGGUAGUAGUUUGUGCUGUUGGUCGGGUUGUGACAUU-GCCCGCUGU--GGAGAUAACUGCGCAAGCUACUGCCUUGCU
           ******* *  ***    ***                    *           * * ***   *       *   ** *
```

Binding of immobilized 1632 to purified Myc-Lin28A and Myc-Lin28A in cell lysates (Fig S10).

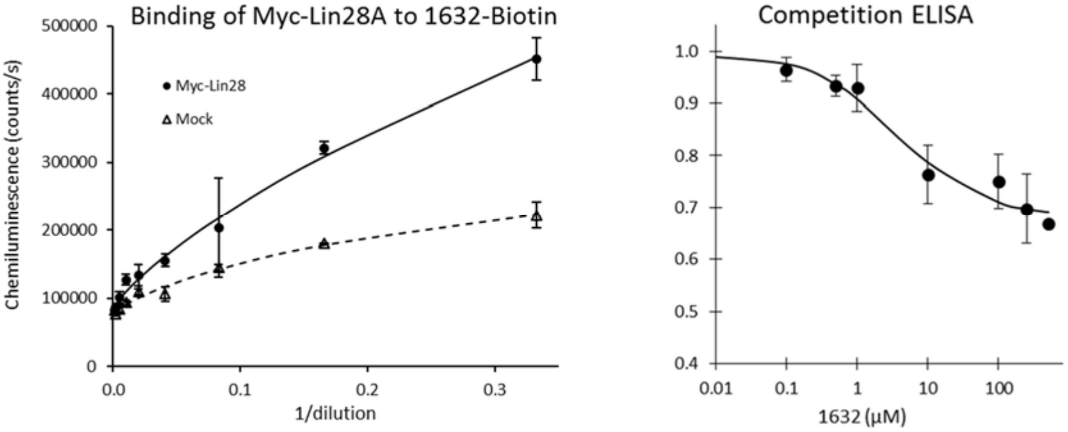


Figure S10. (a) Left panel: Binding of affinity purified Myc-Lin28A fusion protein by surface immobilized 1632 (1632Bio). Myc tagged Lin28A was obtained from plasmid transfected in HeLa cells. Mock represents a control containing purified proteins from mock transfected Hela cells. (b) Right panel: Inhibition of Myc-Lin28A binding to plate bound 1632Bio by 1632. Lysates of Myc-Lin28A plasmid transfected Hela cells were used as source of the protein. Error bars indicate standard deviations (n=3 and 2, for left and right panel respectively). Captured Myc-Lin28A was measured by a Myc-specific detection system.

Murine ESCs treated with 1632 compound. (Fig S11)

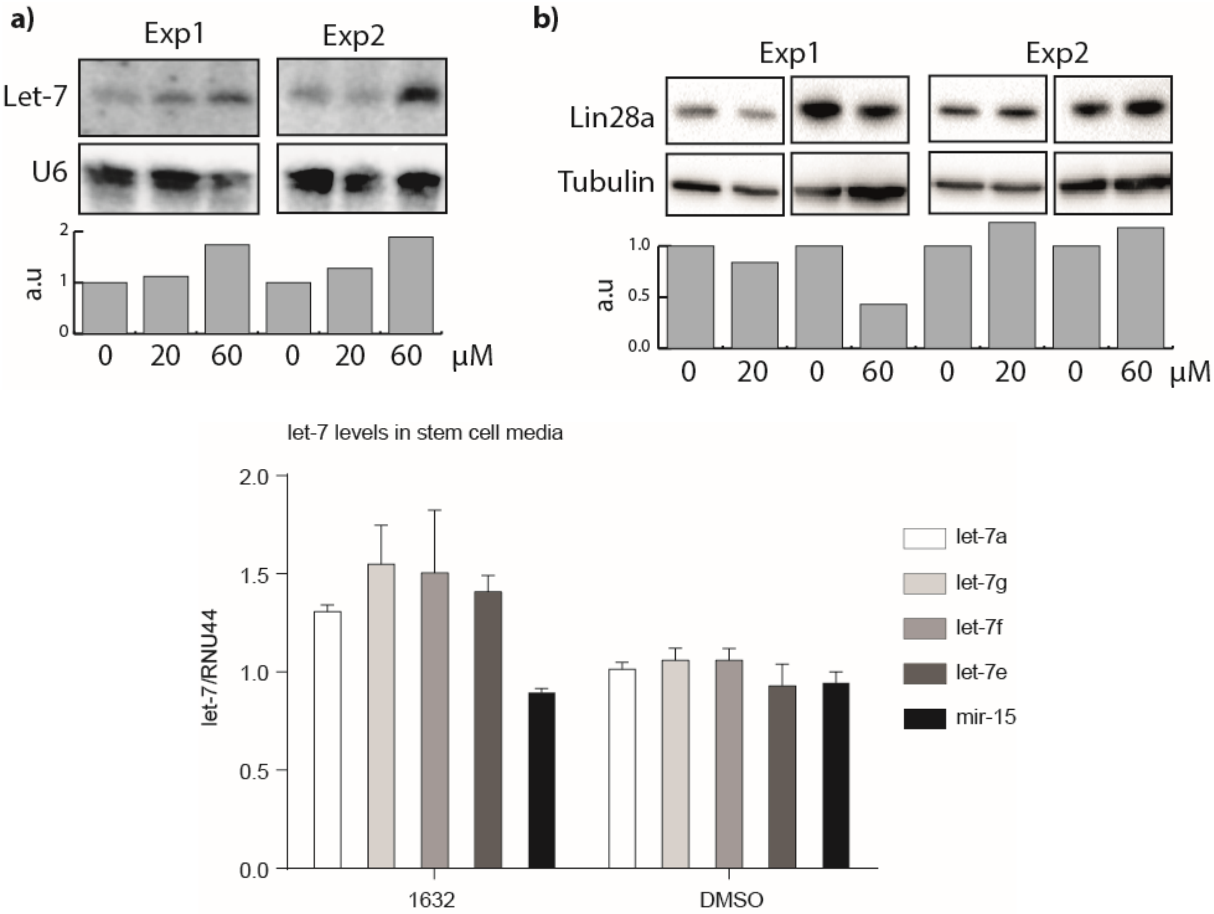


Figure S11. (a) The levels of let-7 miRNAs (let7a, let7g, let7e and let7e) by northern blot and (b), levels of Lin28a protein by western blot after 3 days of treatment with 0, 20 or 60 µM of 1632 compound. Two independent experiments (Exp1 and Exp2) are represented. The protein and miRNA levels are normalized respectively to U6 and to Tubulin. (a.u = arbitrary unit). (c) Let-7 levels after treatment with 1632 after 48 h measured by RT-qPCR. Error bars indicate ± 1 SEM (n=3).

In vitro Dicer assay with compound 1632 (Fig S12)

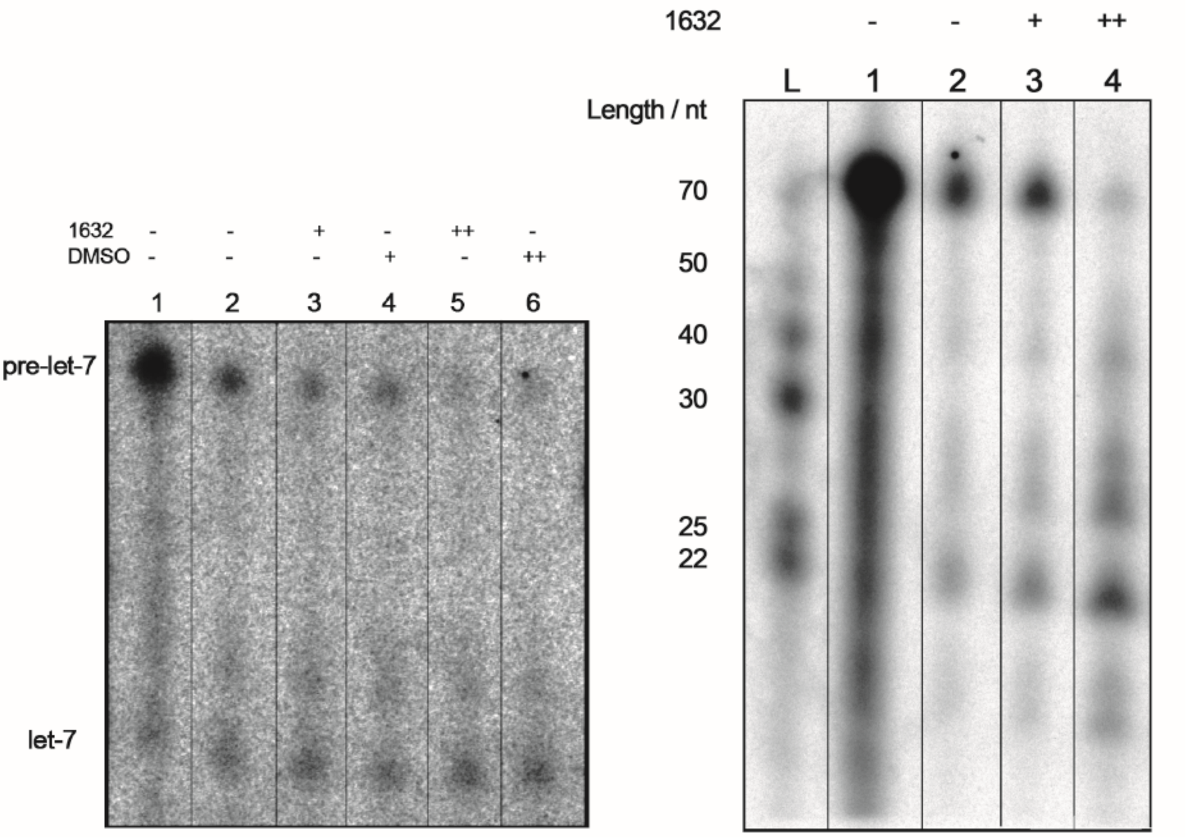


Figure S12. *In vitro* assays with compound 1632. Two independent replicates are shown. Assays are conducted in the presence of 1 mM or 4 mM 1632 and controls (see Supplementary methods). Lane 1 contains contains the reaction mixes without lysate.

Treatment of cancer cells with compound 1632 (Fig S13)

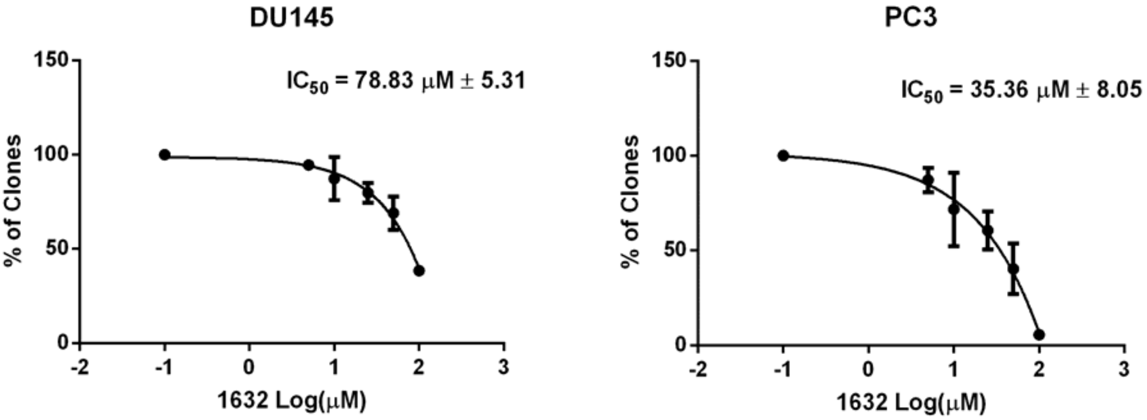


Figure S13a. Clonogenic activity DU145 and PC3 cancer cells treated with 1632. Cells were seeded at low density (200 cells/well in 24-well-plate) and incubated with compound 1632 (5, 10, 25, 50 and 100 μM). Colonies were fixed with 10% (w/v) trichloroacetic acid, stained with sulforhodamine B and counted using ImageJ. Data (mean ± SEM of 3 experiments) are presented as percentage of colony number relative to DMSO-treated cells. The IC50 values are mean ± SEM of the 3 experiments.

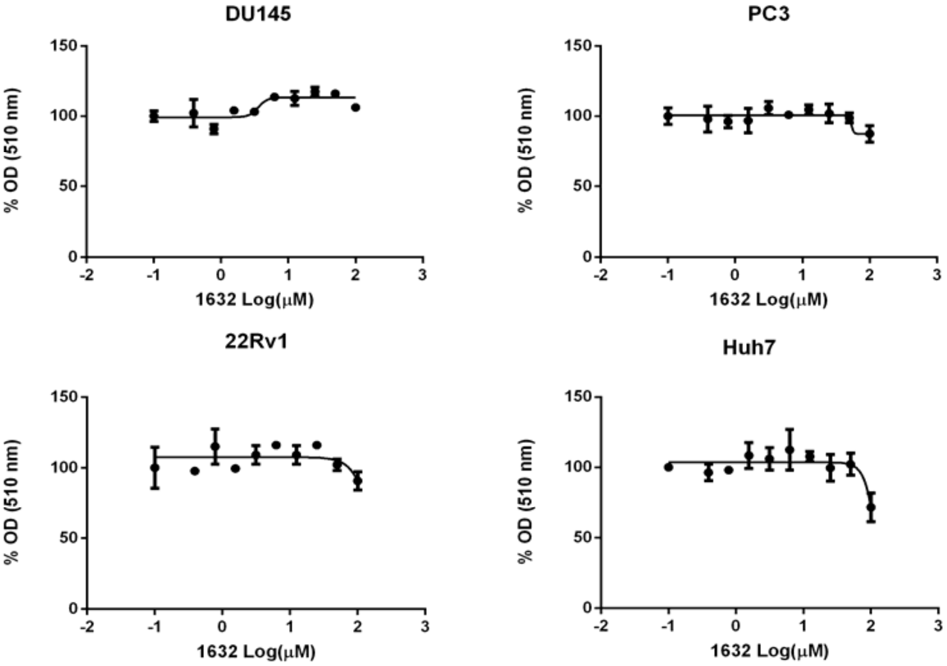
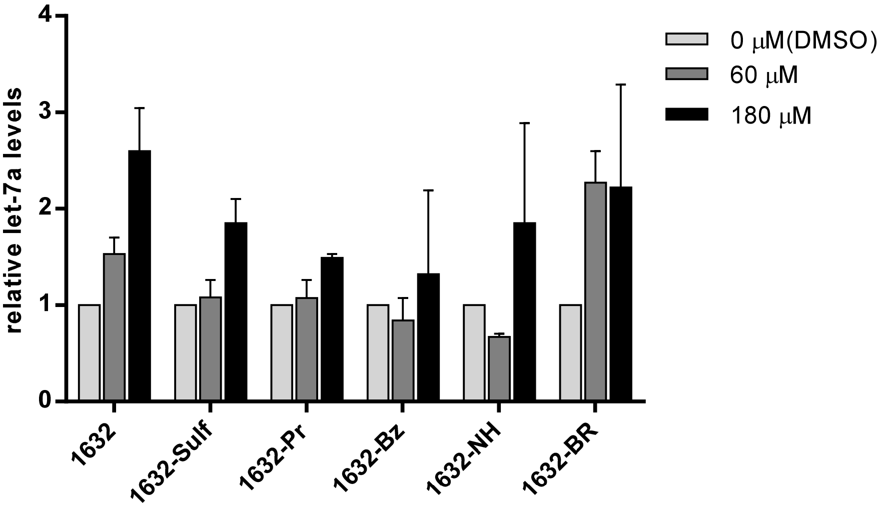


Figure S13b. Proliferation of cancer cells exposed to compound 1632. Cells were seeded in 96 well-plates (2000 cells/well) and incubated with compound 1632 (5, 10, 25, 50 and 100 μM) for 72 h. The cells were fixed with 10% (w/v) trichloroacetic acid, stained with sulforhodamine B and their density measured in a microplate reader at 510 nM. Data (mean ± SD) are expressed as percentage of cell number relative to DMSO-treated cells.

Evaluation of 1632 derivatives in qPCR assay (Fig S14)



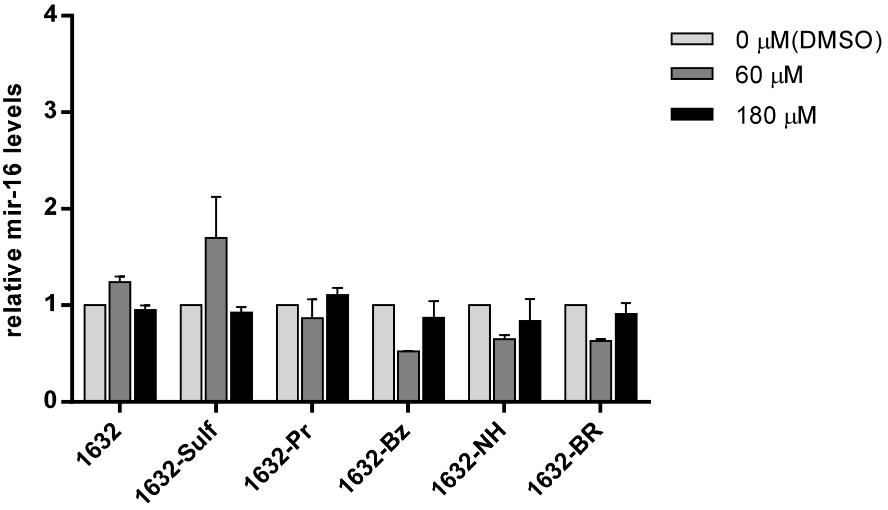


Figure S14. Determination of cellular levels of mature let-7a and mir-16 with 1632 and derivatives in Huh-7 cells. MiR-16 was used as a control, 48 h after treatment. Error bars indicate SD (n=2).

Dose-response curves for compound 1632 for CREBBP and BRD4 at DiscoverX (Fig S15)

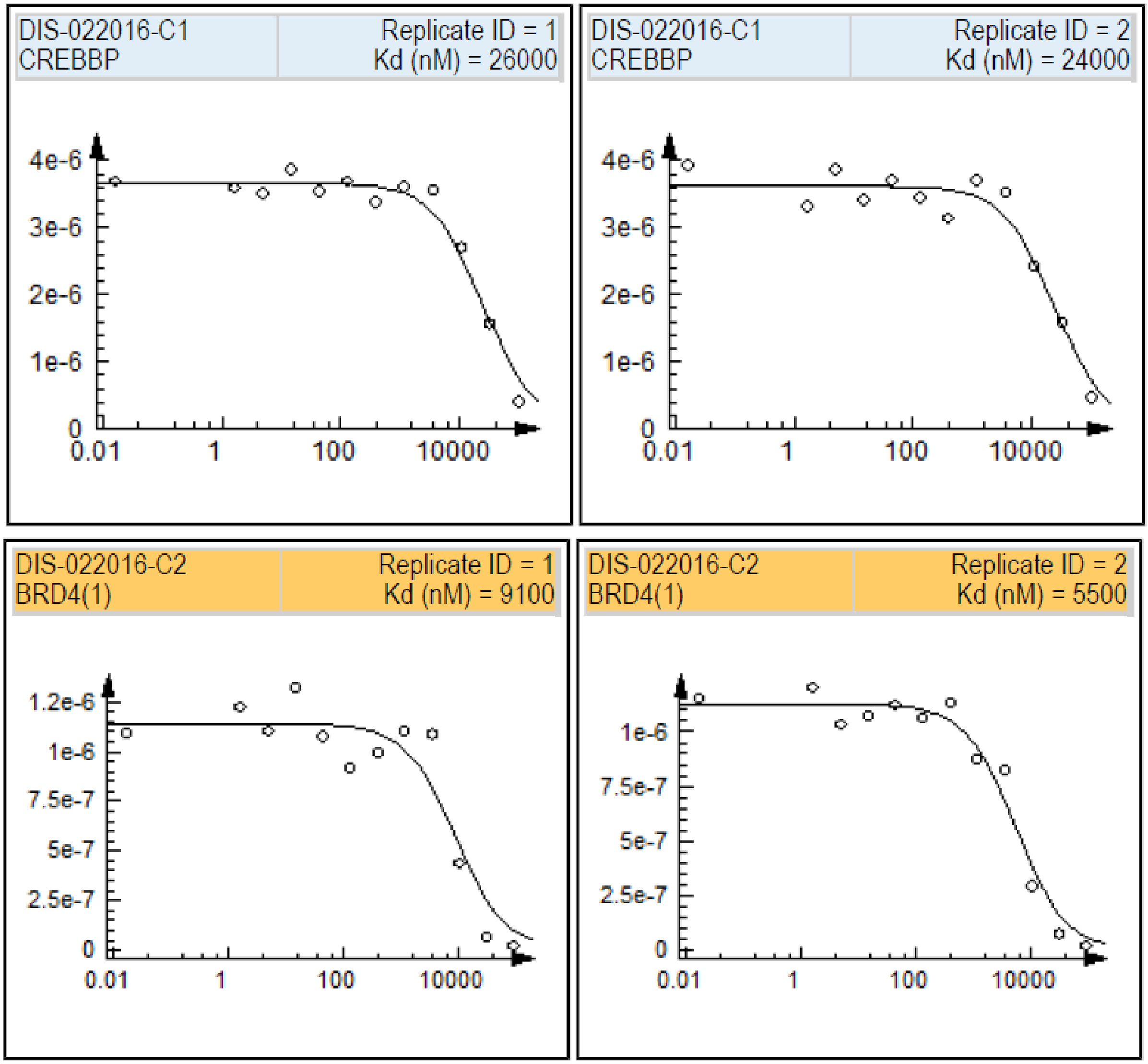


Figure S15. Dose-response curves in duplicates for compound 1632 tested for binding to CREBBP and BRD4 using the competition binding assay at DiscoveRx.

Supplementary Tables

RNA sequences (Table S1)

Sequences of RNA oligonucleotides used in the study; masses calculated and masses observed.

Name*	Corresponding Sequence 5'- to -3'	Type	Label	Mass calc. [g/mol]	Mass found [g/mol]
Pre-let-7a-2	UGAGGUAGUAGGUUGUAUAGUUUAGAAUUACAUCAAGGGAGAUAACUGUACAGCCUCCUAGCUUUCC	RNA	/	21502.9	21503.3
3'-biotin-let7a	AGGUUGUAUAGUUUAGAAUUACAUCAAGGGAGAUAACUGUACAGCCUCTTTTTTC	RNA	biotin	18037.7	18036.4
10Cy3-let7	AGGUUGUAUAGUUUAGAAUUACAUCAAGGGAGAUAACUGUACAGCCUC	RNA	Cy3	16024.3	16023.6
10B-let7	AGGUUGUAUAGUUUAGAAUUACAUCAAGGGAGAUAACUGUACAGCCUC	RNA	BHQ-1	15964.3	15962.6
19Cy3-let7	AGGUUGUAUAGUUUAGAAUUACAUCAAGGGAGAUAACUGUACAGCCUC	RNA	Cy3	16024.3	16023.9
19B-let7	AGGUUGUAUAGUUUAGAAUUACAUCAAGGGAGAUAACUGUACAGCCUC	RNA	BHQ-1	15964.3	15963.9
34Cy3-let7	AGGUUGUAUAGUUUAGAAUUACAUCAAGGGAGAUAACUGUACAGCCUC	RNA	Cy3	16024.3	16023.3
34B-let7	AGGUUGUAUAGUUUAGAAUUACAUCAAGGGAGAUAACUGUACAGCCUC	RNA	BHQ-1	15964.3	15963.2
57Cy3-let7	AGGUUGUAUAGUUUAGAAUUACAUCAAGGGAGAUAACUGUACAGCCUC	RNA	Cy3	16024.3	16023.6
10-19Cy3-let7	AGGUUGUAUAGUUUAGAAUUACAUCAAGGGAGAUAACUGUACAGCCUC	RNA	Cy3	16623.3	16623.0
10-19B-let7	AGGUUGUAUAGUUUAGAAUUACAUCAAGGGAGAUAACUGUACAGCCUC	RNA	BHQ-1	16503.3	16503.2
10-34Cy3-let7	AGGUUGUAUAGUUUAGAAUUACAUCAAGGGAGAUAACUGUACAGCCUC	RNA	Cy3	16623.3	16622.7
10-34B-let7	AGGUUGUAUAGUUUAGAAUUACAUCAAGGGAGAUAACUGUACAGCCUC	RNA	BHQ-1	16503.3	16503.1
1Cy3-miR32	CUAUUGCACAUUACUAAGUUGCAUAUUGUCACGGCCUCAAUGCAAUUUAGUGUGUGUGAUAUUU	RNA	Cy3	20958.1	20958.1
1Cy3-miR101	CAGUUAUCACAGUGCUGAUGCUGUCUAUUCUAAAGGUACAGUACUGUGAUAACUGAA	RNA	Cy3	18839.0	18837.9
1B-miR101	CAGUUAUCACAGUGCUGAUGCUGUCUAUUCUAAAGGUACAGUACUGUGAUAACUGAA	RNA	BHQ-1	18779.0	18777.8
1-8Cy3-miR101	CAGUUAUCACAGUGCUGAUGCUGUCUAUUCUAAAGGUACAGUACUGUGAUAACUGAA	RNA	Cy3	19438.0	19437.7
1-8B-miR101	CAGUUAUCACAGUGCUGAUGCUGUCUAUUCUAAAGGUACAGUACUGUGAUAACUGAA	RNA	BHQ-1	19318.0	19317.8
L29-13	CUCCCUUGAUGUA	2'-OMe RNA	/	4'220.8	4'220.2
siRen (5p)	GAGCGAAGAGGGCGAGAAAUU	RNA	/	6901.3	6902.5
siRen (3p)	UUUCUCGCCCUCUUCGCUCUU	RNA	/	6436.8	6436.2

*: Numerical pre-fixes refer to nucleotide positions on the pre-miRNA according to miRBase; B refers to BHQ-1; Underlined nucleotides represent labeling positions.

DNA sequences (Table S2)

Table S2: Sequences of DNA oligonucleotides used in the study.

Name	Corresponding Sequence 5'- to -3'
Four let-7 *target (seed) sites from HMGA2* + primer + restriction sites	CCTCCACTTCAGCCAGGACTCGAGGGGGCGCCAACGTTCGATTT*CTACCTCA*GCAGCAGTTGTCCCCACTACTCAATA*CTACCTC*TGAATGTTACGGACTAATTGACTTGCAAAGAC*CTACCTC*CAGACTTCAAACAATCAAAACACACTA*CTACCTC*TTAAGTCCCAGTATACCTCATTTGCGGCCGCTGAGTCTTCGGACCTCGC
Four mutated let-7 target sites from HMGA2 *complementary mutated* + primer + restriction sites	CCTCCACTTCAGCCAGGACTCGAGGGGGCGCCAACGTTCGATTTC*GAACGC*AGCAGCAGTTGTCCCCACTACTCAATA*CGAACGC*TGAATGTTACGGACTAATTGACTTGCAAAGA*CCGAACGC*CAGACTTCAAACAATCAAAACACACTA*CGAACGC*TTAAGTCCCAGTAGAACGCATTTGCGGCCGCTGAGTCTTCGGACCTCGC
General primer 1 (reverse)	GCGAGGTCCGAAGACTCA
Lin28B amplification primer forward + SacI restriction site	TCG AGC TCAATGGCCGAAGGCGGGGCTA
Lin28B amplification primer reverse+ SacII restriction site	ATC CGC GGG TTA TGT CTT TTT CCT TTT TTG AAC TGA AGG CCC C
General primer 1 forward	CCTCCACTTCAGCCAGGA
Mouse Pou5f1 forward	CAACTCCCGAGGAGTCCCA
Mouse Pou5f1 reverse	CTGGGTGTACCCCAAGGTGA
Mouse Rex1 forward	ATAAAACCGCCCTGAGGAAG
Mouse Rex1 reverse	AGTTTCGAGCTCTCCGTGAA
Mouse Sox2 forward	CACAGATGCAACCGATGCA
Mouse Sox2 reverse	GGTGCCCTGCTGCGAGTA
Mouse Stella forward	AAAGTCGACCCAATGAAGGA
Mouse Stelle reverse	ACACCGGGGTTTAGGGTTAG
Mouse Lin28 forward	ATCCCGACTTTGTCAGATGG
Mouse Lin28 reverse	AAGGCCAACCAGGAAAAGTT
Mouse Rrm2 forward	CCGAGTCGGAAAGTAAAGCG
Mouse Rrm2 reverse	ATGGGAAAGACAACGAAGCG
Mouse HMGA2 forward	CAGCCCAGAAGAAAGCAGAG
Mouse HMGA2 reverse	TTGTGGCCATTTCCTAGGTC
Mouse Nestin forward	CTGCAGGCCACTGAAAAGTT
Mouse Nestin reverse	TTCCAGGATCTGAGCGATCT
Mouse Dnmt3b forward	AAGCCCATGCAATGATCTCTCT
Mouse Dnmt3b reverse	AGCCTTCCTGTGCCCTCATA

1632 Selectivity: Activity versus commonly-assayed receptors and kinases (Table S3)

Table S3: Data from testing 1632 against a panel of receptors and enzymes.

Target	Assay	% Inhibition of Control Specific Binding		
		1st	2nd	**Mean**[1]
Adrenergic b1 *(h)*	agonist radioligand	3.2	11.0	**7.1**
Dopamine D1	antagonist radioligand	-0.4	-3.5	**-1.9**
Muscarinic M1	antagonist radioligand	9.4	15.5	**12.5**
Opiod k (KOP)	agonist radioligand	-8.9	-7.9	**-8.4**
Vasopressin V1a	agonist radioligand	14.1	17.3	**15.7**
Ca2+ channel Dihydropyridine	antagonist radioligand	-3.1	-2.3	**-2.7**
K+ channel K_V channel	antagonist radioligand	-3.2	-6.5	**-4.9**
Lck kinase	enzyme	6.7	0.6	**3.6**

[1]. See Supplementary Methods for a description and conclusions from the measurements.

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