Supplementary information, Data S1 Materials and Methods

Plasmid construction

Total RNA was extracted from 293T cells using TRI Reagent (Sigma) and cDNA synthesized by Reverse Transcription PCR using TaqMan® Reverse Transcription Reagents with oligo(dT) primers (Applied Biosystems). The human METTL3 gene (Gene ID: 56339) open reading frame encoding the full-length protein was amplified and subcloned into pCMV-Myc (Sigma) vector using the following primers: forward (Sal I): 5'-GCGTCGACATGTCGGACACGTGGAGCTC-3', reverse (Not I): 5'-CCGGCGGCCGCCTATAAATTCTTAGGTTTAGAG-3'; and also into S-protein/FLAG/SBP (streptavidin-bindign protein) triple-tagged destination vector [1] with the following primers: forward (Sal I): 5'-ATTGTCGACACATGTCGGACACGTGGAGCTC-3', reverse (Hind III): 5'-CCCAAGCTTCTATAAATTCTTAGGTTTAGAG-3'. The METTL3 gene was also subcloned into pGEX-5x-2 (GE healthcare) to generate GST-METTL3 construct using the following primers: forward (Xma I): 5'-TCCCCCCGGGATGTCG GACACGTGGAGCTC-3', reverse (Not I): 5'-CCGGCGGCCGCCTATAAATTCTTAGGTTTAG-3'. The human WTAP gene (Gene ID: 9589) open reading frame encoding the full-length protein was subcloned into p3XFLAG-CMV-14 (Sigma) using the following primers: forward (Xho I) 5'-ATACTCGAGATGACCAACGAAGAACCTCTTC-3', reverse: (Not I) 5'-ATAAGAATGCGGCCGCTTACAAAACTGAACCCTGTAC-3'. Deletion mutants (N-terminal fragment 1-200 aa and C-terminal fragment 201aa-396 aa) were generated by PCR from 3xFlag-WTAP. The WTAP gene was also subcloned into pProEX-HTb (Invitrogen) to generate 6XHis-WTAP construct using the following primers: forward (Not I): 5'-TATAATGCGGCCGCAATGACCAACGAAGAACCTCTTC-3', reverse (Xho I): 5'- CCGCTCGAGTTACAAAACTGAACCCTGTACATTT-3'. The human METTL14 gene (Gene ID: 57721) open reading frame encoding the full-length protein was amplified using the following primers: forward (Xho I): 5'-CCGCTCGAGATGGATAGCCGCTTGCAGGAGATC-3', reverse (Not I): 5'-ATAAGAATGCGGCCGCTTATCGAGGTGGAAAGCCACCTC-3' and subcloned into pcDNA3-HA (Invitrogen) vector. Zebrafish WTAP(full-length, N-terminal and C-terminal) were amplified from zebrafish cDNA library by PCR and sequentially subcloned into the pCS2 (Invitrogen) to generate pCS2-WTAP constructs

by using the primers: forward (EcoR I)：TATTAT GAATTC ATG GAC TAC AAG

GAC GAC GAC GAT AAG ATG ACT AAC GAG GAG CCA, reverse (XbaI) : TATTAT TCTAGA TTACAAAACAGACCCTGCAGAGG; For the N-terminal: forward (EcoR I): TATTAT GAATTC ATG GAC TAC AAG GAC GAC GAC GAT AAG ATG ACT AAC GAG GAG CCA, reverse (XbaI): TATTAT TCTAGA TAATCCAGCTGAATGATGAAGTCA; for the C-terminal: forward (EcoR I): TATTAT GAATTC ATG GAC TAC AAG GAC GAC GAC GAT AAGGAGGAGGTGGAGGGCATGCAG, reverse (XbaI) : TATTAT TCTAGA

TTACAAAACAGACCCTGCAGAGG, All the above DNA constructs were verified by DNA sequencing.

Plasmid transfection and RNA interference

10 μg plasmids and 30 μl polyethylenimine (Polysciences), were mixed in serum- and antibiotic-free DMEM. After 15 minutes, the mixture was introduced into the cells cultured in 10 cm dish. After 4 hours medium of the culture was replaced with fresh medium and cells were harvested after 48 hrs incubation for immunoprecipiation.

siRNA duplexes (GenePharma, China) were transfected into cells at a final concentration of 50 nM by using Lipofectamine RNAiMAX™ (Invitrogen) as the manufacturer's instructions. The following siRNA were synthesized (GenePharma, China) and used in the study: METTTL3 #1: 5'-GCACAUCCUACUCUUGUAA-3', METTTL3 #2: 5'-GGAGAUCCUAGAGCUAUUA-3', METTTL3 #3: 5'-GACUGCUCUUUCCUUAAUA -3'. WTAP #1: 5'-CACAGAUCUUAACUCUAAU -3', WTAP #2: 5'-GGGAAAACAUCCUUGUAAU-3', WTAP #3: 5'-GACCCAGCGAUCAACUUGU-3', METTTL14 #1: 5'-GCUAAAGGAUGAGUUAAU -3', METTTL14 #2: 5'-GGACUUGGGAUGAUAUUAU -3', METTTL14 #3: 5'-GGACUUCAUUCAUGCUAAU -3'. Scrambled siRNA (siCTRL): 5'-UUCUCCGACGUGUCACGU-3'; Scrambled siRNA (siCTRL): 5'-UUCUCCGACGUGUCACGU-3'. For immunofluorescence experiments the above siRNAs labeled with FAM (Fluorescein amidite) as indicated were synthesized by GenePharma, China.

Cell culture and antibodies

Human HeLa and 293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Sigma) at 37 °C in a humidified incubator with 5% CO_2(v/v). The following antibodies were used in the study: mouse-anti-flag (Sigma, WB,1:5000; IF:1:500), rabbit-anti-flag (Sigma, WB,1:5000; IF:1:500), rabbit-anti-Myc (Abcam, WB, 1:5000; IF:1:500), rabbit-anti-HA(Clontech, WB,1:5000), rabbit-anti-METTL3 (Abcam,1:1000), mouse-anti-METTL3 (Abnova, WB,1:1000; IF,1:500), rabbit-anti-WTAP (Atlas, WB, 1:1000), rabbit-anti-METTL14 (Atlas, WB, 1:1000; IF:1:500), mouse-anti-WTAP (Santa Cruz, WB, 1:1000; IF:1:500); anti-m6A antibody (Synaptic Systems,1:2000), anti-rabbit IgG-HRP(Dakocytomation,1:5000), anti-mouse IgG-HRP(Dakocytomation,1:5000), anti-mouse IgG - Cy3(Sigma,1:500), anti-rabbit IgG -Cy3(Sigma,1:500), anti-mouse IgG-FITC(Sigma,1:500), anti-rabbit IgG-FITC(Sigma,1:500), anti-GST-HRP(Abcam,1:1000), anti-6xhis tag HRP(Abcam,1:1000), mouse-anti-beta-tubulin (Sigma,1:5000), mouse-anti-gama-actin (Santa Cruz,1:1000), mouse-anti-SC35 (Sigma,1:1000).

Tandem Affinity Purification and Mass spectrometry

293T cells were transiently transfected with triply-tagged METTTL3 plasmid (SFB-METTL3) and after 40 hrs lysed with NETN buffer on ice for 30 min. Cell debris was removed by centrifugation and the crude lysates were incubated with streptavidin-Sepharose beads (Amersham Biosciences) for 1hr at 4°C. The bead-bound proteins were eluted twice with 1 mg/ml biotin (Sigma) for 1 hr at 4 °C. The eluates were further incubated with S-protein-agarose (Novagen) for 1 hr at 4°C and washed 3 times with NETN buffer. The proteins bound to S-protein-agarose beads were subjected to SDS-PAGE and visualized by Coomassie Blue staining. For mass spectrometry, METTL3 specific band was cut from the gel, bleached with 50 mM NH_4HCO_3 / acetonitrile = 1:1 solution(37°C, 5min) and dehydrated with acetonitrile. Then, 10 mM DTT was added and incubated at 56 °C for 1hr to break the disulfide bond. After cooling down to room temperature, 55mM IAM (2-iodoacetamide) was added to prevent the restoration of disulfide bond and kept in dark for 45 minutes. Proteins were trypsinized into peptides incubating with 0.1 μg/μl Trypsin at 37°C, overnight, and further digestion was stopped by adding 1% TFA (trifluoroacetate). Samples thus obtained were subjected to analysis by Q Exactive mass spectrometer (Thermo Fisher Scientific). Peptides were loaded onto a pre-column (75 μm ID packed to 8cm with 10μm-C18 resin) and separated on an analytical 75 μm ID column packed to 11cm with 3 μm-C18 resin (pre-column: ODS-AQ 12 17 nM S-10 μm (YMC Co., LTD.), analytical column: Luna 3 μm 100 Å resin (Phenomenex)) with anacetonitrile gradient from 0% to 25% in 60 min and 25% to 80% in another 7 min at a flow rate of 200nl per minute. Spectra were acquired in a data-dependent mode: the 10 most intense ions of +2, +3, or +4 charge from each full scan (R = 70,000) were isolated for HCD MS2 (R = 17,500) at NEC 27 with a dynamic exclusion time of 150 seconds. For peptide identification, the MS2 spectra were searched against an SwissProt human database (forward + reversed sequences) using 15 ppm peptide mass tolerance and 20mmu fragment mass tolerance. The data files have been uploaded to http://www.peptideatlas.org with the access number: PASS00296.

Co-immunoprecipitation and Immunoblotting

Cells transfected with plasmids and/or siRNA as indicated were lysed with NETN buffer (20 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1mM EDTA, 0.5% Nonidet P-40) containing 1mM NaF, 1mM PMSF, 1mM Na_3VO_4, and 1mM beta-glycerophosphate, and rotated at 4°C for 30 min. Cell debris was removed by centrifugation and the soluble fractions were collected and incubated with Myc- (Sigma) or Flag- (Sigma) or HA- (Sigma) or protein A (incubated with WTAP antibody in advance) IP beads, for at least 4 hrs at 4°C. The immunoprecipitates were then washed 3 times with NETN buffer and boiled in 2xSDS loading buffer. Samples were resolved on

SDS-PAGE and transferred to polyvinylidene difluoride membranes, and immunoblotting was carried out with antibodies as indicated.

For Figures 1A, 1D, 1E, 1F, S2A, S2D, S2E, and S1C, 1/10-1/15 of the IP(50 μl in total) was loaded and 1% (Figures 1A, 1D, S2A, S2D, S2E) and 4% (Figures 1E, 1F and S2B) of the WCL lysate(2mg in total) was loaded as input, respectively. For Figure 1B, 6% of WCL and 1/8 of IP samples were loaded.

GST pull down assay

The human METTL3 gene was subcloned into pGEX-5X-2 expression plasmid with GST-tag and the human WTAP gene was subcloned into pProEX-HTb expression plasmid with His tag. Then recombinant GST-METTL3 and His-WTAP proteins were induced to express in E.coli strain BL21(DE3) and purified by FPLC using Bio-Scale Mini Profinity GST cartridge (Biorad) and Bio-Scale Mini Profinity IMAC cartridge (Biorad) as described in the commercial instruction manual. His-WTAP proteins were incubated with glutathione sepharose (GE Healthcare) to be pre-cleared in NETN buffer (100mM NaCl, 1mM EDTA, 20mM Tris-HCl pH 7.4, 0.5% NP-40). Then pre-cleared His-WTAP was mixed with GST or GST-METTL3 protein and incubated overnight at 4°C with equal amount of glutathione sepharose beads. After washing the beads five times with NETN buffer, proteins bound to the beads were analyzed by 8% SDS-PAGE followed by immunoblotting with HRP-conjugated anti-His and anti-GST antibodies (Abcam).

Immunofluorescence

For immunoflorescence staining, HeLa cells grown on coverslips were transfected with siRNAs(50nM) or DNA constructs as indicated for about 4 hrs, and 24 hrs later fixed with 4% paraformaldehyde on ice followed by permeabilization with 0.1% Triton X-100 and 0.05% NP-40 on ice. After pre-blocking with 5% non-fat dried milk in TBST, coverslips were first incubated with primary antibody for 1hr, then with fluorescent dye-conjugated secondary antibody for half an hour and then mounted with DAPI containing mounting medium (Vector Laboratories, Burlingame, CA) and then visualized under immunofluorescence confocal microscope Leica TCS SP5 (Leica). The method used for Immunofluorescence staining following RNase A treatment was adapted from Mayer et al. and Sytnikova et al. In brief, cells grown on coverslips were permeabilized with 0.05% Triton X-100 in 20 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, 0.5 mM EDTA, and 25% glycerol; washed twice with PBS; and treated for 7 min with RNase A (1mg/ml)(diluted in PBS) in 37℃. After washing, cells were fixed with cold methanol (15 min) and stained with the indicated antibodies following the blocking procedure, and then the immunofluorescent images were digitally recorded by using confocal microscope [2].

Apoptosis assay

HeLa cells were treated with control, METTL3 or WTAP siRNA for 48h, and apoptotic cells were detected by staining 1x106 cells with annexin-PI using the Annexin V-FITC & PI Apoptosis Detection Kit (Jiamay Biotech) and analyzed by FACSAria III flow cytometer using FACSDiva software 6.0 (Becton-Dickinson).

m6A Dot blot Assay

Total RNA was extracted from siRNAs treated 293T cells or Morpholinos-treated Zebrafish embryos as indicated, then mRNA was isolated by Dynabeads® mRNA Purification Kit (Ambion Dynabeads® mRNA Purification Kit #61006), 200 ng and 100 ng mRNA was hybridized onto Amersham Hybond-N+ membranes using Bio-Dot®Microfiltration Apparatus (GE Healthcare, Catalog Number 170-6545) following manufacturer's instruction. The dot blot was detected after baking at 80°C for 30 min using anti-m6A antibody (Synaptic Systems). Signal density of m6A on the spots was quantified using Gel-Pro Analyzer 4 (Media Cybernetics, Inc. USA). To ensure equal spotting of mRNA on to the membrane, the same blot was stained with 0.02% methylene blue in 0.3M sodium acetate.

PAR-CLIP

293T cells overexpressed Myc-METTL3 and Flag-WTAP were labeled with 200 μM 4-SU (Sigma T4509) for 16 hrs, then applied for crosslinking with 0.4 J/cm^2 of 365nm UV light in a crosslinker BLX-E365 (Vilber) as described previously[2]. In brief, irradiated cells were lysed with NP-40 lysis buffer on ice for 30 minutes. Cell debris was removed by centrifugation and the crude lysates were incubated with Myc beads (Sigma E6654) and Flag (Sigma A2220), respectively for 2 hrs at 4°C. After immunoprecipitation, protein-RNA coupled beads were treated with CIP (NEB M0290). A minor part of beads were left for biotin labeling as the instruction of RNA 3' end biotinylation kit (Thermo 20160) for further detection, while the major part were treated with PNK (NEB M0201) and non-radioactive ATP (NEB P0756). After running on 4%-12% NuPAGE gel (Invitrogen NP0321B0X), the protein-RNA complexes were transferred to PVDF membrane. Biotin-labeled RNA were detected and visualized following the instruction of the chemiluminescent nuclei acid kit (Thermo 89880). Non-labeled RNA-protein complexes were cut out from the membrane corresponding to the size of Myc-METTL3 and Flag-WTAP visualized. The membrane was cut into small pieces and placed into a microtube. The protein was digested by proteinase K (Roche 03115828001) in PK buffer (100mM pH7.4 Tris-HCl, 50mM NaCl, 10mM EDTA) for 20min at 37°C, followed by adding PKurea buffer (100mM pH7.4 Tris-HCl, 50mM NaCl, 10mM EDTA, 7M Urea) and incubating for another 20min. RNAs were isolated from the solution with phenol-chloroform, then subjected to the small RNA library construction.

The reads from the METTL3 and WTAP deep sequencing library were stripped of the adaptor sequence with the Cutadapt software[3]. Reads that were less than 20 nt in

length or contained an ambiguous nucleotide were discarded. The remaining reads were aligned to the human genome (hg19), with up to two mismatches allowed, by the Bowtie algorithm[4]. Mapped locations were only reported for those with the minimum number of observed mismatches for each read. Clusters were obtained by PARalyzer [5] (http://www.genome.duke.edu/labs/ohler/research/PARalyzer/) with the default parameters. In PARalyzer, overlapping reads were grouped together for further analysis. A group must contain at least five reads with conversions at two or more locations. Separate kernel density estimates were calculated across all positions based on read counts both with and without T to C conversions, as long as there was a minimum read depth of 5 at a position to ensure a robust density estimate. Then, a single-linkage clustering of the sequence reads was performed, with two reads being placed in the same cluster if they overlapped by at least one nucleotide in their genomic mappings. Clusters were defined as regions for which the conversion density was greater than the nonconversion density for at least 5 consecutive nucleotides, and extended until the group read depth fell below 5[5-7]. Each cluster was then annotated relative to a known transcript, was determined based on the Ensembl v72 database. If a cluster mapped to a location representing multiple categories, it was reported to belong to the category based on the following order of preference: 3 UTR, coding sequence, 5 UTR, intron, noncoding RNA, and intergenic. The cluster data was analysed by HOMER software[8] to identify the binding motif. All clusters mapping to mRNA and not overlapping repetitive regions were used to do the motif discovery.

RNA-seq

293T cells were treated with METTL3, WTAP or Control siRNAs for 48h and total RNA was isolated using RNAzol (Molecular Research Center, Inc., USA). Poly(A) RNA from 1 μg total RNA was used to generate the cDNA library according to TruSeq RNA Sample Prep Kit protocol, which was then sequenced using the HiSeq 2000 system (Illumina Inc.). RNA-seq reads of 101 bp for each sample were aligned against the human genome (hg19) references with TopHat2 [9]. Two mismatches were allowed at the maximum and only uniquely mapped reads with mapping quality larger than or equal to 20 were kept for the subsequent analysis for 6 sample respectively. The number of reads mapped to each of the Ensembl genes (release 72) was counted using the HTSeq python package [10], with the ‘union’ overlap resolution mode, and -stranded =no.

For each sample, RPKM was computed as the number of reads which map per kilobase of exon model per million mapped reads for each gene. Differentially expressed genes between control, METTL3 or WTAP deficient cells samples were determined using the R-package DEGseq with the method MARS (MA-plot-based method with random sampling model), p value cutoff = 0.001[11]. Gene Ontology (GO) analysis of DEGs were performed using DAVID (http://david.abcc. ncifcrf.gov/) [12, 13]. GO terms with p value of less than 0.05 were determined to be statistically significant. Enrichment map of DEGs was constructed by Cytoscape 2.8.3 installed with the Enrichment Map plugin and the parameter is that: $p < 0.005$, FDR $q < 0.05$,

overlap cutoff > 0.5 [14].The isoform expression level was calculated using Cufflinks software (version 2.0.2)[15] with default parameters.

Alternative splicing analysis

Based on the TopHat2 results of RNA-Seq data for METTL3, WTAP or Control siRNAs-treated samples aligned against the human genome (hg19) references, Cufflinks v2.0.2 were applied to calculate the reads count and FPKM for each isoform with –G parameter which means that quantification against reference transcript annotation. Meanwhile, Scripture, one method for transcriptome reconstruction that relies solely on RNA-Seq reads and an assembled genome to build a transcriptome ab initio were applied to calculate the isoform number for each genes in 6 samples [16]. If isoform number for one gene in METTL3-deficient cells or WTAP-deficient cells was more or less than that in the control in duplicate RNA-Seq transcript, these genes may be with alternative splicing event in the treatment.

Meanwhile, exon-exon junctions (EEJs) methods applied in previous study [17] was used to analyze alternative splicing event. Information on intron-exon structures was extracted from Ensembl annotations (release 72) for human (hg19) genomes. From the resulting datasets, a Bowtie library of non-redundant EEJ sequences was generated for human by combining every possible (forward combination) of splicing donor and acceptor within each gene and all the RNA-seq reads were aligned against this library with Bowtie 0.12.7 software [4]. To the exon-exon junctions (EEJs) with reads mapping, according to their continuous or discrete exon number, they can be divided into constitutive or alternative splicing exons. All the WTAP/METTL3 clusters were assigned to these constitutive or alternative splicing exons.

Spatially correlation of WTAP/METTL3 clusters with m6A sites

Dominissini et al. and Meyer et al used different methods to determine m6A sites (Computed Winscore >2 (4× enrichment) + filtering +MACS Dominissini et al. and Fisher's exact test of IP read enrichment Meyer et al.) [18, 19]. To obtain the best coverage, we download their m6A data and used both methods in our analysis of m6A sites [18, 19]. Furthermore, considering that the alignment method has a significant impact on the number of peaks identified [20], when aligning to the hg19 genome in the alignment step, we chose to use Tophat2. We then used MACS [21] and Fisher test [18, 19] methods to determine m6A peaks. The peaks identified in our dataset were then combined with the previously published m6A sites and duplicates were removed [18, 19].

This pooled set of m6A sites were used in the analysis of a potential spatial overlap between WTAP/METTL3 clusters and m6A sites. We used the BEDTools' closestBed to calculate the distance between WTAP/METTL3 clusters and m6A sites. As control, we generated control clusters with BEDTools' shuffleBed to randomly

shuffle regions of the same size as the clusters throughout gene regions [22].

Zebra fish strains and husbandry, Morpholinos and constructs

Zebrafish embryos were obtained by natural spawning of adult Tubingen strain. Embryos were raised and maintained in system water at 28.5℃ and staged as described[23]. The following antisense morpholino oligonucleotides were synthesized by Gene Tools, LLC and prepared as 1mM stock solution with ddH_2O: WTAPMO: AGGTTCTTCATTGGTCATTCTGATC; METTL3MO: GGATGTGACTCCATGTGTCCGACAT. The morpholinos (2.5ng-8ng) were injected alone or in combination into embryos from one-cell to four-cell stage.

Zebrafish WTAP and METTL3 were amplified from zebrafish cDNA library by PCR using the following primers: WTAP-F: 5'-aacttccccttcaacagcc-3', WTAP-R: 5'-agcaaatgaacagtacagaacc-3'; METTL3-F: 5'-cagcaaagcaaaaaggtgag-3', METTL3-2-R:5'-aaaggtgaatgccgtccag-3' and subcloned into pGEM-T vector for antisense RNA probe synthesis. The 5’ UTR and a portion of the coding sequence of zebrafish WTAP and METTL3 were amplified using the following primers: WTAP-MO-F (HindIII):5'-TATTATAAGCTTATCTCACATTGGAAGACATAC, WTAP-MO-R (SacII): 5'-TATTATCCGCGGAGAAGTGCCTTGGGTTTGGTT; METTL3-MO-F (HindIII):5'-TATTATAAGCTTTACTGTTTGTTTATTTGCTGT-3', METTL3-MO-R(SacII):5'-TATTATCCGCGGCTCTTCTTTGGTTCCATAATCAC A-3' and subcloned into vector pEGFP-N1 (Clontech) in-frame to generate pWTAP-GFP or pMETTL3-GFP construct for checking morpholino efficiency. For overexpression experiments in zebrafish, the full-length (1-423 aa), N-terminal (1-212 aa), and C-terminal (213-423 aa) of zebrafish WTAP gene were cloned into pCS2+ vector and the corresponding mRNAs were synthesized using the mMessage mMachine SP6 kit according to the provider’s protocol (Ambion, Austin, USA)[24].

Whole-mount in situ hybridization and TUNEL assay

Whole-mount in situ hybridization (WISH) with zebrafish embryos was performed using ZF-A4 in situ hybridization machine (Zfand, China) with probes including *WTAP, METTL3, myod, gooscoid* and *no tail* as described previously [25].

TUNEL assay was performed as described previously [24]. In brief, embryos were fixed in 4 % paraformaldehyde overnight at 4 °C and dehydrated with methanol at -20°C at least 2 hours. After rehydration, the embryos were permeabilized with Proteinase K (10 μg/ml) for 10 min at room temperature and refixed in 4% PFA for 30 min. After washing 3 times with PBST for 5 minutes each, embryos were incubated with 90 μl of labeling solution plus 10 μl of enzyme solution (In Situ Cell Death Detection Kit, Fluorescein; Roche) at room temperature for 6 hours or at 4°C overnight. Embryos were washed 3 times with PBST for 5 minutes each and the images were examined by confocal microscopy.

References

1 Maddika S, Sy SM, Chen J. Functional interaction between Chfr and Kif22 controls genomic stability. *J Biol Chem* 2009; **284**:12998-13003.

2 Hafner M, Landthaler M, Burger L *et al.* Transcriptome-wide identification of RNA-binding protein and microRNA target sites by PAR-CLIP. *Cell* 2010; **141**:129-141.

3 M M. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J 2011, 17:10-12. 2011.

4 Langmead B, Trapnell C, Pop M, Salzberg SL. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol* 2009; **10**:R25.

5 Corcoran DL, Georgiev S, Mukherjee N *et al.* PARalyzer: definition of RNA binding sites from PAR-CLIP short-read sequence data. *Genome Biol* 2011; **12**:R79.

6 Hongay CF, Orr-Weaver TL. Drosophila Inducer of MEiosis 4 (IME4) is required for Notch signaling during oogenesis. *P Natl Acad Sci USA* 2011; **108**:14855-14860.

7 Mukherjee N, Corcoran DL, Nusbaum JD *et al.* Integrative regulatory mapping indicates that the RNA-binding protein HuR couples pre-mRNA processing and mRNA stability. *Mol Cel* 2011; **43**:327-339.

8 Heinz S, Benner C, Spann N *et al.* Simple Combinations of Lineage-Determining Transcription Factors Prime cis-Regulatory Elements Required for Macrophage and B Cell Identities. *Mol Cel* 2010; **38**:576-589.

9 Trapnell C, Pachter L, Salzberg SL. TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics (Oxford, England)* 2009; **25**:1105-1111.

10 Anders S. HTSeq: analysing high-throughput sequencing data with Python.http://www-huber.embl.de/users/anders/HTSeq/doc/overview.html . 2010.

11 Wang L, Feng Z, Wang X, Wang X, Zhang X. DEGseq: an R package for identifying differentially expressed genes from RNA-seq data. *Bioinformatics (Oxford, England)* 2010; **26**:136-138.

12 Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* 2009; **4**:44-57.

13 Huang da W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res* 2009; **37**:1-13.

14 Shannon P, Markiel A, Ozier O *et al.* Cytoscape: A software environment for integrated models of biomolecular interaction networks. *Genome Res* 2003; **13**:2498-2504.

15 Trapnell C, Williams BA, Pertea G *et al.* Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nature Biotechnol* 2010; **28**:511-515.

16 Guttman M, Garber M, Levin JZ *et al.* Ab initio reconstruction of cell type-specific transcriptomes in mouse reveals the conserved multi-exonic structure of lincRNAs. *Nature Biotechnol* 2010; **28**:503-510.
17 Han H, Irimia M, Ross PJ *et al.* MBNL proteins repress ES-cell-specific alternative splicing and reprogramming. *Nature* 2013; **498**:241-+.
18 Dominissini D, Moshitch-Moshkovitz S, Schwartz S *et al.* Topology of the human and mouse m(6)A RNA methylomes revealed by m(6)A-seq. *Nature* 2012; **485**:201-U284.
19 Meyer KD, Saletore Y, Zumbo P *et al.* Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons. *Cell* 2012; **149**:1635-1646.
20 Saletore Y, Meyer K, Korlach J *et al.* The birth of the Epitranscriptome: deciphering the function of RNA modifications. *Genome Biol* 2012; **13**:175.
21 Zhang Y, Liu T, Meyer CA *et al.* Model-based Analysis of ChIP-Seq (MACS). *Genome Biol* 2008; **9**.
22 Quinlan AR, Hall IM. BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics (Oxford, England)* 2010; **26**:841-842.
23 Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF. Stages of Embryonic-Development of the Zebrafish. *Dev Dynam* 1995; **203**:253-310.
24 Wang L, Zhang PP, Wei YL *et al.* A blood flow-dependent klf2a-NO signaling cascade is required for stabilization of hematopoietic stem cell programming in zebrafish embryos. *Blood* 2011; **118**:4102-4110.
25 Liu F, Patient R. Genome-Wide Analysis of the Zebrafish ETS Family Identifies Three Genes Required for Hemangioblast Differentiation or Angiogenesis. *Circ Res* 2008; **103**:1147-U1194.