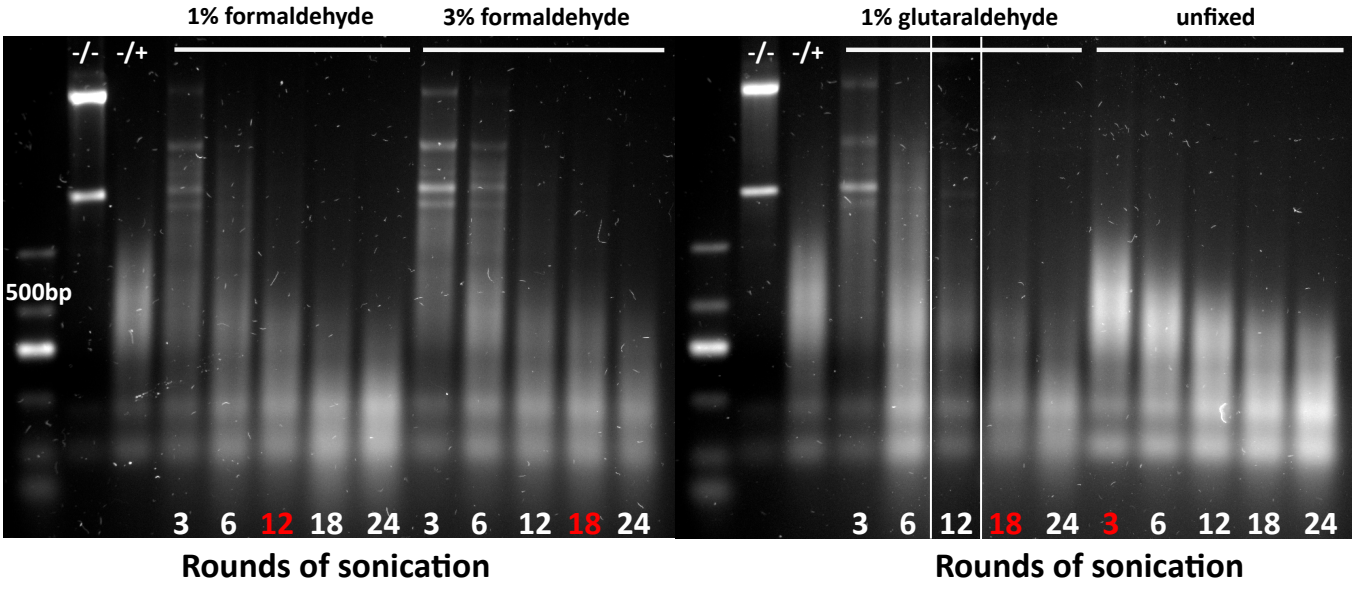
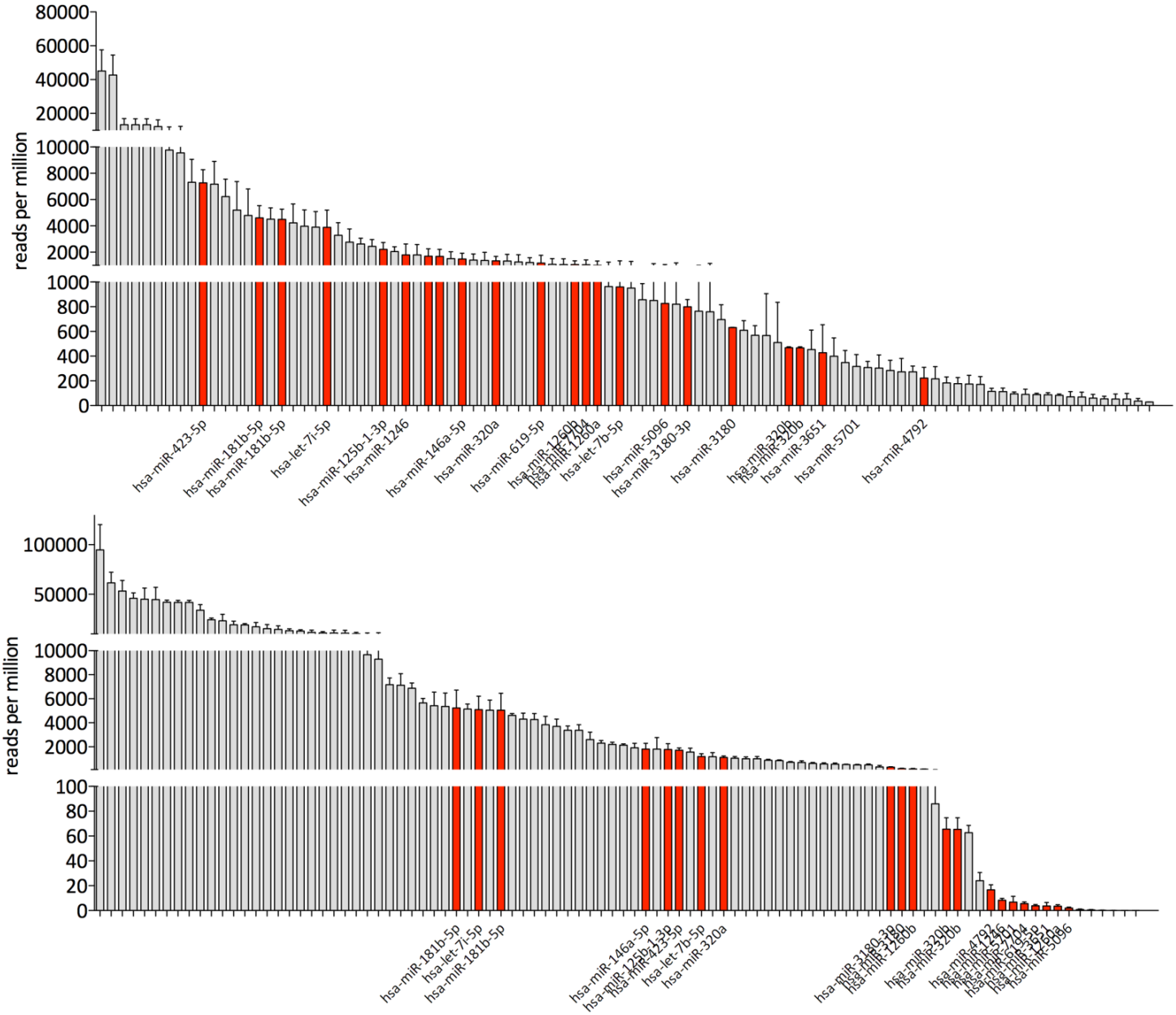
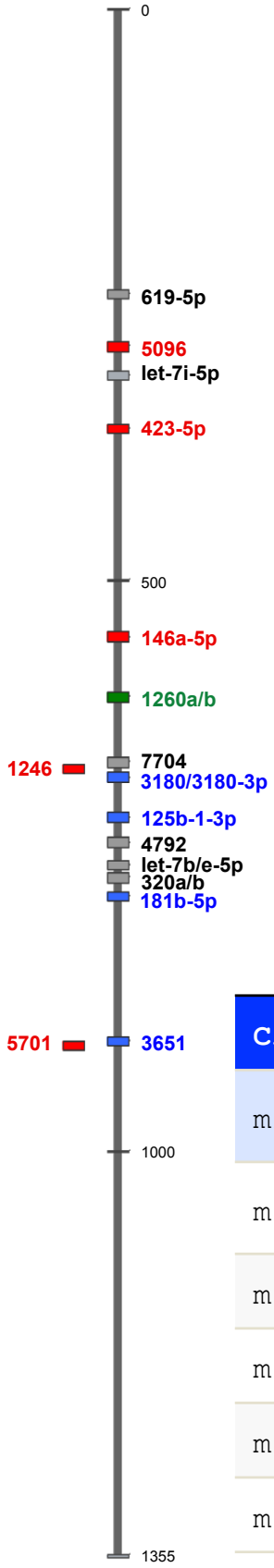


-/- = unfixed/unsonicated
-/+ = unfixed/sonicated

Supplementary Figure S1. Optimization of the sonication step.
Unfixed and cross-linked A375 cells were sonicated at 70% of amplitude for increasing number of rounds (1 round = 30'' pulse + 45'' cool down on ice). Total RNA was then extracted and visualized on agarose gel. For the subsequent steps of protocol optimization, the number of rounds that lead to the production of RNA fragments of 200-1000nt was chosen: 1% formaldehyde = 12 rounds; 3% formaldehyde = 18 rounds; 1% glutaraldehyde = 18 rounds; unfixed cells = 3 rounds.



Supplementary Figure S2. Ranking of the 94 microRNAs selected as described in Fig. 2A.
The ranking according to expression levels in the small RNA-seq of captured microRNAs **(*upper*)** and in the small RNA-seq of A375 cells **(*lower*)** is reported.
The 20 microRNAs selected for experimental validation are highlighted in red. They all show a scrolling of at least 15 positions up in the captures compared to the microRNA profile. For miR-181b-5p, the ranking of both miR-181b-5p-1 and 2 is reported. Analogously, for miR-320b, the ranking of both miR-320b-1 and 2 is reported.



Class I microRNAs	
microRNA	MFE (kcal/mol)
miR-146a-5p	-22.2
miR-423-5p	-27.4
miR-1246	-22.2
miR-5701	-19.7

Class II microRNAs	
microRNA	MFE (kcal/mol)
miR-125b-1-3p	-26.8
miR-181b-5p	-26.9
miR-3180	-33.3
miR-3180-3p	-37.2
miR-3651	-24.3

ClassIIIa/b microRNAs	
microRNA	MFE (kcal/mol)
miR-320a	-28.2
miR-320b	-28.5
miR-619-5p	-25.0
miR-1260a	-23.2
miR-1260b	-25.4
miR-4792	-24.3
miR-5096	-23.7
miR-7704	-23.7
let-7b-5p	-25.3
let-7e-5p	-24.2
let-7i-5p	-24.3

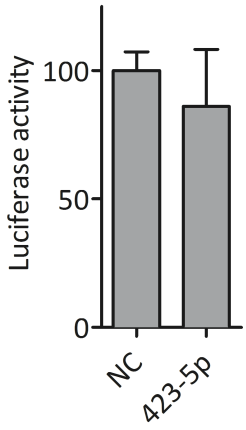
Supplementary Figure S3. Predicted MREs of the 20 top-scoring microRNAs identified by miR-CATCHv2.0.
(*left*) Position of microRNA Recognition Elements (MREs) along the X1 3'UTR, according to RNAhybrid prediction algorithm (https://omictools.com/rnahybrid-tool). **(*right*)** Tables reporting the binding energies of the 20 top-scoring microRNAs, divided by Classes. MFE: Minimal Free Energy.

Class I microRNAs		
miRNA	position	
hsa-miR-146a-5p	522	target 5' U UUUCUAU UGCGU GC G 3' GCCC GUGG GUUCAG CUU UGGG UACC UAAGUC GAG miRNA 3' U U AA U 5'
hsa-miR-423-5p	345	target 5' G CAGUCAA U A 3' AAAGUCU GC CUUUGCCC CU UUUCAGA CG GAGACGGG GA miRNA 3' G A GU 5'
hsa-miR-1246	652	target 5' U AC C 3' CCUG CUCCGGGGGUCU GGAC GAGGUUUUUAGG miRNA 3' UAA 5'
hsa-miR-5701	886	target 5' C ACAAAAUGCC U 3' CAGGGC GUGGCAGUA GUCUUG CACUGUUAU miRNA 3' UUA U 5'

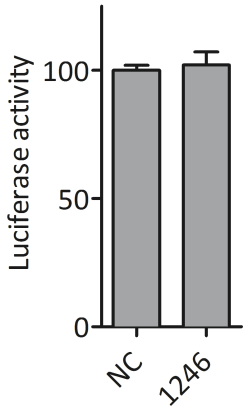
Supplementary Figure S4. Pairing of Class I microRNAs with the corresponding MREs.

Class I microRNAs

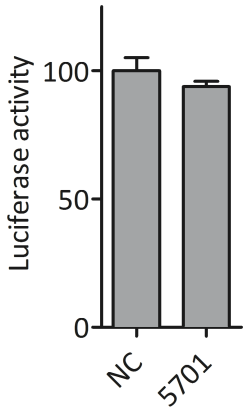
```
miR-423-5p  3’UUUCAGAGCGAGAGACGGG GAGU 5’
                              |||||:||
Target        GCCAGTCAACTTTGCCCTTCTAACC
Deleted target GCCAGTCAACTT--------TAACC
```




```
miR-1246      3’  GGACGAGGUUUUUAGGUAA 5’
                    ::||::::  |:|
Target 1246/3180(-3p) CTTCCTGACCTCCGGGGTCTCTGGC
Deleted target        CTTCCTGACCTCCGGGGTC------
```

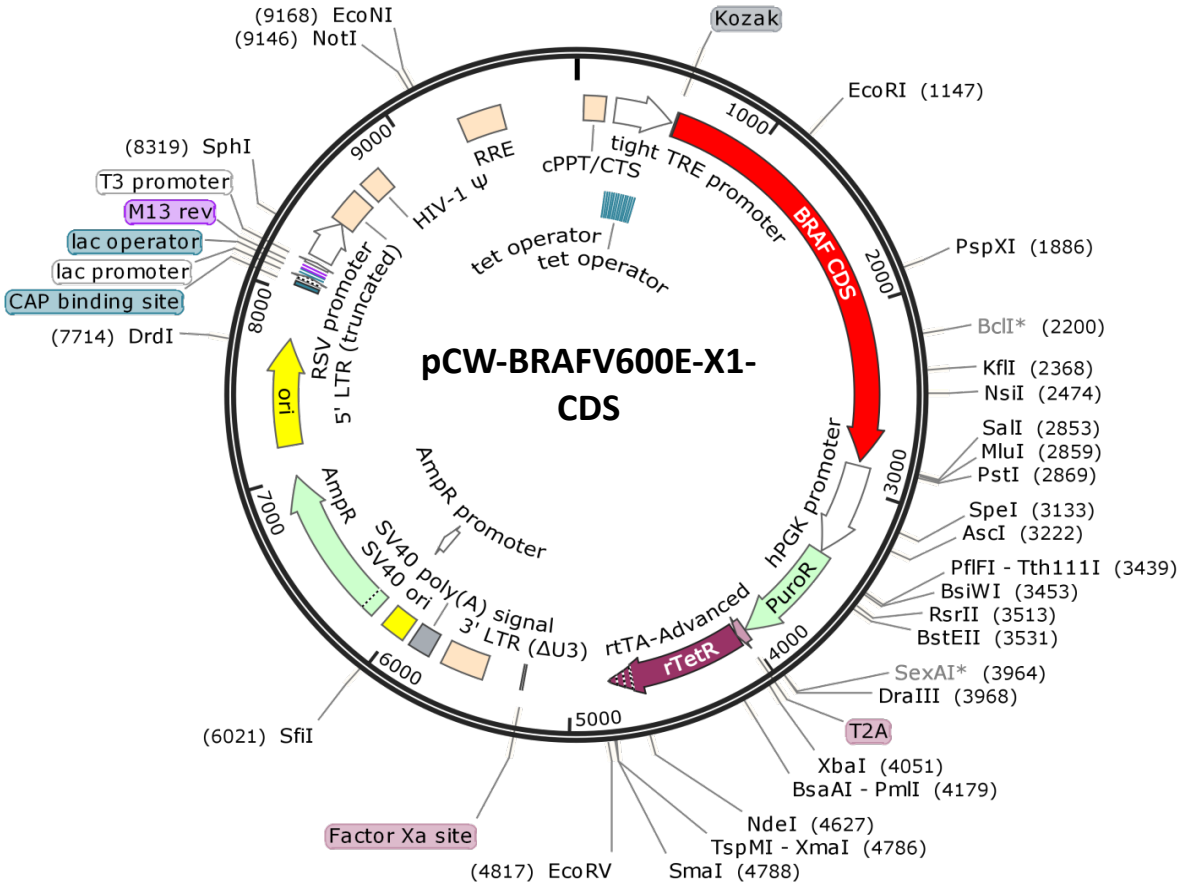



```
miR-5701      3' UUAGUCUUGCACUGUUAUU 5'
                           ||||:||:|
Target 3651/5701 AGGGCACAAAATGCCGTGGCAGTATTCTAA
Deleted target   AGGGCACAAAAT--------AGTATTCTAA
```

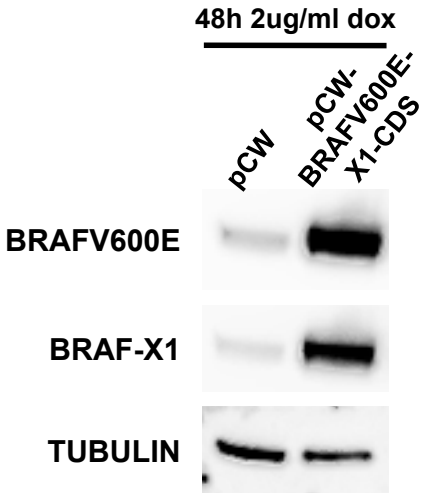


Supplementary Figure S5. Luciferase constructs in which the MREs of Class I microRNAs have been mutagenized.
Contrary to the wt constructs, the mutant constructs no longer respond to the transfection of the appropriate si-miRNA with a decrease in luciferase activity. The graphs represent the mean ± SEM of 3 independent experiments.

A



B



Supplementary Figure S6. pCW-BRAFV600E-X1-CDS lentiviral vector for the inducible overexpression of BRAFV600E-X1 CDS.
A. Vector map. The coding sequence (CDS) of BRAFV600E-X1 is shown in red.
B. Immunoblot detection of BRAFV600E (upper) and BRAF-X1 (middle) in A375 cells that were first infected with pCW empty vector (left) or pCW-BRAFV600E-X1-CDS vector (right) and then treated for 48h with 2ug/ml doxycycline (dox).

A

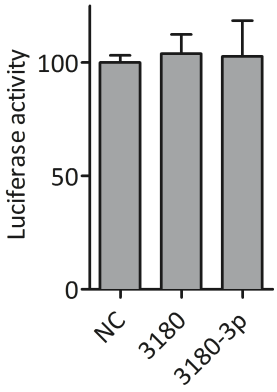
Class II microRNAs		
miRNA	position	
hsa-miR-125b-1-3p	688	target 5' U A GGUCU A 3' GCUC CUGAGG GUCUAACC CGAG GGUUCU CGGAUUGG miRNA 3' U GCA 5'
hsa-miR-181b-5p	750	target 5' U UC AGAUAA U 3' CUCAUUGG CAGCAAUGA GUGUU GGGUGGCU GUCGUUACU UACAA miRNA 3' U 5'
hsa-miR-3180	658	target 5' C GG U G U 3' CUCCGG G CUCUG CCU GAGGCC C GAGGC GGG miRNA 3' UU G U 5'
hsa-miR-3180-3p	657	target 5' A GG U G U 3' CCUCCGG G CUCUG CCU GGAGGCC C GAGGC GGG miRNA 3' CC UU G U 5'
hsa-miR-3651	882	target 5' U G CACAAAAU U A 3' UGU CCAG G GCCG GGC ACA GGUC C UGGC CCG miRNA 3' AGU U G AUAC 5'

B

miR-3180(-3p) 3’(CCG)GAGGCCUUCGAGGCGGGGU 5’

Target 1246/3180(-3p) CTTCCTGACCTCCGGGGTCTCT GGCCTTTTGT

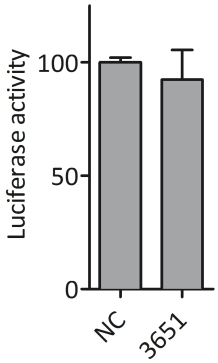
Deleted target CTTCCTGACCTCCGGGGTC--- -----TTTGT



miR-3651 3‘ AGUACAUGGUCGCUGGC CCGAUAC 5'

Target 3651/5701 AGGGCACAAAATGCCGTGGCAGTATTCTAA

Deleted target AGGGCACAAAAT--------AGTATTCTAA

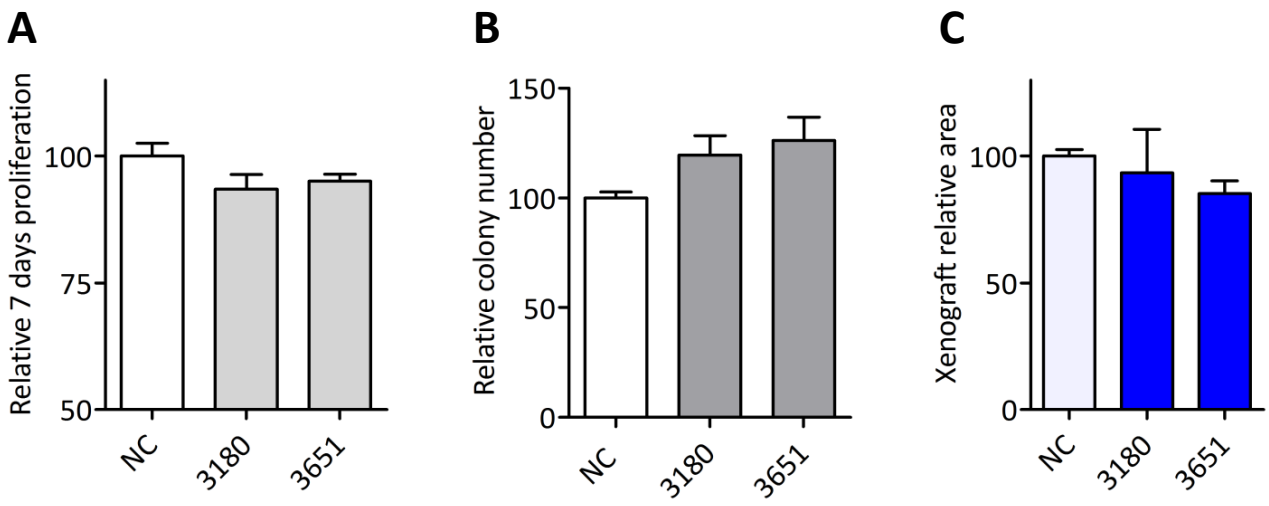


Supplementary Figure S7. MREs of Class II microRNAs.

A. Pairing of Class II microRNAs with the corresponding MREs.

B. Luciferase constructs in which the MREs of Class II microRNAs have been mutagenized. Contrary to the wt constructs, the mutant constructs no longer respond to the transfection of the appropriate si-miRNA with an increase in luciferase activity. Since the MREs of miR-1246 and miR-3180(-3p), as well as those of miR-3651 and miR-5701 are largely overlapping, the same mutant construct was used to test the binding of both members of the same pair.

The graphs represent the mean ± SEM of 3 independent experiments.



Supplementary Figure S8. Biological properties of A375 cells transfected with si-miR-3180 and si-miR-3651.

A-B. Effect of the overexpression of miR-3180 and miR-3651 on the colony forming ability (**A**) and the proliferation (**B**) of A375 melanoma cells. The cells were seeded for the 2 cellular assays at the end of the transfection.

C. Effect of the overexpression of miR-3180 and miR-3651 on the growth of A375-mCherry cells, when xenografted in zebrafish embryos. 48h after the transfection with si-miR-3180 or si-miR-3651, cells were injected in 48 hours post fertilisation embryos and allowed to grow for an additional 48h. At the end of this period, the size of red cell masses was measured.

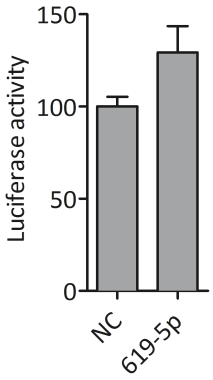
The graphs represent the mean ± SEM of 3 independent experiments.

Class III a/b microRNAs		
miRNA	position	
hsa-miR-320a	739	target 5' A UUCUCAU GG A 3' UUGCCUUCU U UCCAGC AGCGGGAGA G GGGUCG miRNA 3' UU AAAA 5'
hsa-miR-320b	739	target 5' A UUCUCAU GG A 3' UUGCCUUCU U UCCAGC AACGGGAGA G GGGUCG miRNA 3' UU AAAA 5'
hsa-miR-619-5p	230	target 5' A AUA A CCC G 3' GC CA GUCUG AGUCCCAG CG GU CGGAC UUAGGGUC miRNA 3' C A AA G 5'
hsa-miR-1260a	589	target 5' A A U U G 3' UGG GGC GA GUGG GA ACC CCG CU CACC CU miRNA 3' A U C A 5'
hsa-miR-1260b	588	target 5' A A UG AGUA G 3' AUGG GGC AGUGGUG GAU UACC CCG UCACCAC CUA miRNA 3' A C 5'
hsa-miR-4792	718	target 5' G AU G A 3' GCUAG AGUGCU GC CGGUC UCGCGA UG miRNA 3' GC G GC 5'
hsa-miR-5096	278	target 5' U UUUUG C A 3' UCUGACC AUGGU AGGC GGACUGG UACCA UUUG miRNA 3' C UUG C 5'
hsa-miR-7704	638	target 5' A AUCUUCUUCC U G 3' UGCUGC UGACC CCG GCGGCG GCUGG GGC miRNA 3' GUGCA 5'
hsa-let-7b-5p	733	target 5' G UU U UU 3' CACACA GCCU CU UCUCA GUGUGU UGGA GA GGAGU miRNA 3' UUG U U 5'
hsa-let-7e-5p	728	target 5' U GCAC UU U U 3' GCUG ACA GCCUUCU UCUCA UGAU UGU UGGAGGA GGAGU miRNA 3' U A U 5'
hsa-let-7i-5p	297	target 5' A GAUGGAAAG A G 3' GGCAU AAACUGCUGCU CA UCGUG UUUGAUGAUGG GU miRNA 3' UUG A 5'

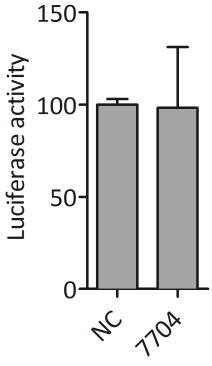
Supplementary Figure S9. Pairing of Class III microRNAs with the corresponding MREs.

Class IIIa/b microRNAs

miR-619-5p 3' CGAGUACGGACAUUAGGGUCG 5'
|:|||||
Target TACAAGTCTGCCCAGTCCCAGGAAGA
Deleted target TACAAGTCTGCCC--------GAAGA



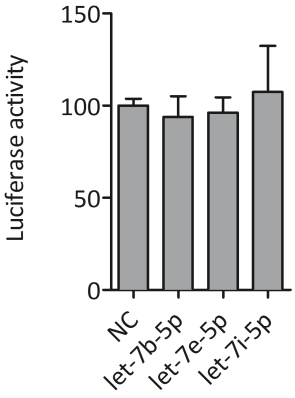
miR-7704 3'GUGCAGCGGCGGCUGG GGC 5'
|||| |||
Target CATCTTCTTCCTGACCTCCGGGGTC
Deleted target CATCTTCTTCCT--------GGGTC



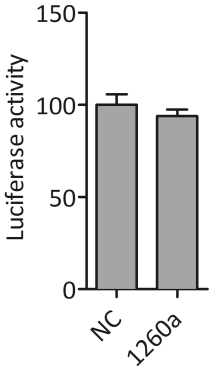
let-7b-5p 3'UUGGUGUGUUUGGAUGAUGGAGU 5'
|| ||||
Target ACACATTGCCTTCTTTCTCATTGGG
Deleted target ACACATTGCCTT--------TTGGG

let-7e-5p 3'UUGAUAUGUUGGAGGAUGGAGU 5'
||| :||||
Target ACACATTGCCTTCTTTCTCATTGGG
Deleted target ACACATTGCCTT--------TTGGG

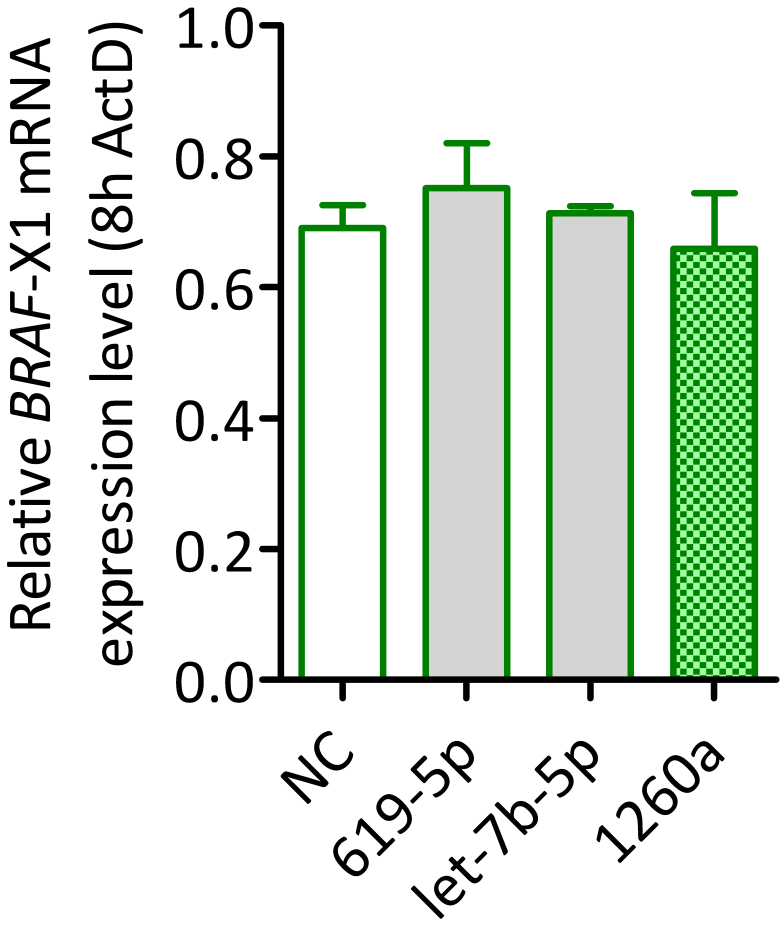
let-7i-5p 3'UUGUCGUGUUUGAUGAUGGAGU 5'
|| :||||
Target ACACATTGCCTTCTTTCTCATTGGG
Deleted target ACACATTGCCTT--------TTGGG



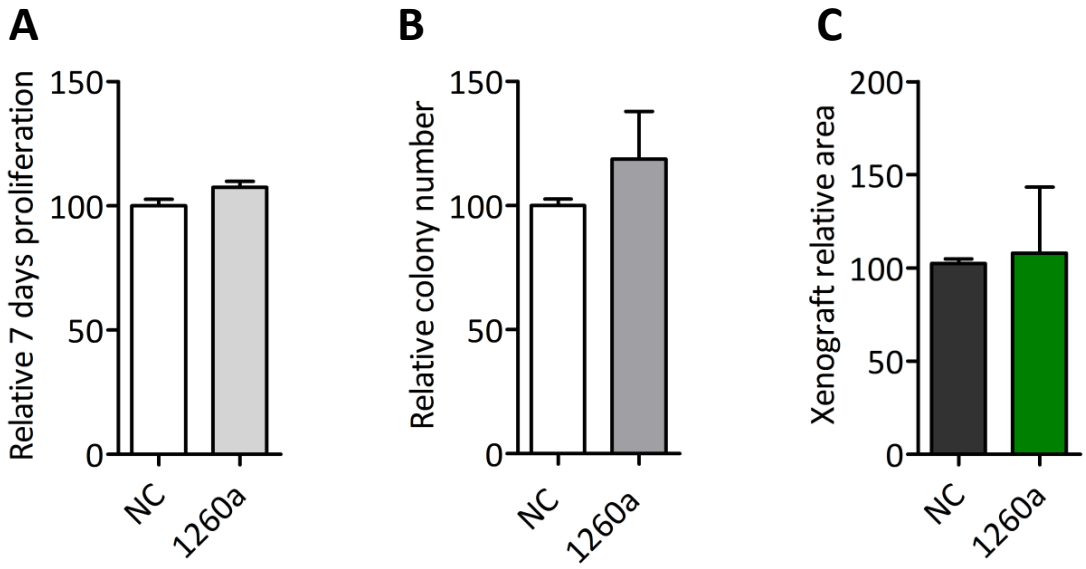
miR-1260a 3' ACCACCGUCUCCACC CUA 5'
|||| ||
Target AAATGGAGGCTGAGTGGTGAGTAGA
Deleted target AAATGGAGGCTG--------GTAGA



Supplementary Figure S10. Luciferase constructs in which the MREs of Class III microRNAs have been mutagenized.
Contrary to the wt constructs, the mutant constructs no longer respond to the transfection of the appropriate si-miRNA with a decrease (Class IIIa) or increase (Class IIIb) in luciferase activity. The graphs represent the mean ± SEM of 3 independent experiments.



Supplementary Figure S11. *BRAF*-X1 RNA stability upon transfection of Class III microRNAs.
Real-time PCR quantification of *BRAF*-X1 mRNA in A375 cells that were first transfected with the indicated siRNAs and 24h later treated with 10ug/ml Actinomycin D (ActD) for 8 hours.

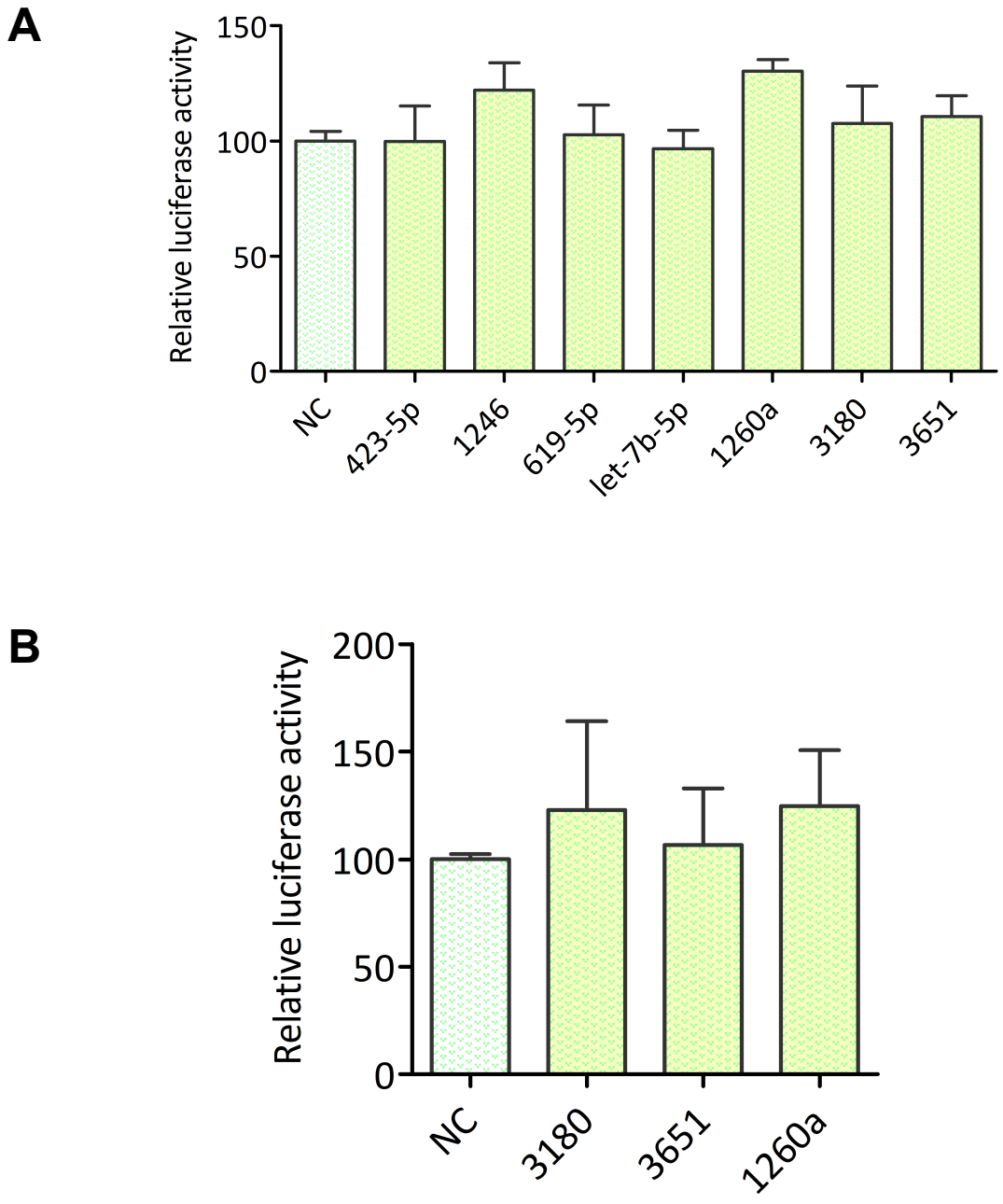


Supplementary Figure S12. Biological properties of A375 cells transfected with si-miR-1260a.

A-B. Effect of the overexpression of miR-1260a on the colony forming ability (**A**) and the proliferation (**B**) of A375 melanoma cells. The cells were seeded for the 2 cellular assays at the end of the transfection.

C. Growth of A375-mCherry cells, when xenografted in zebrafish embryos. 48h after the transfection with miR-1260a, cells were injected in 48 hours post fertilisation embryos and allowed to grow for an additional 48h. At the end of this period, the size of red cell masses was measured.

The graphs represent the mean ± SEM of 3 independent experiments.

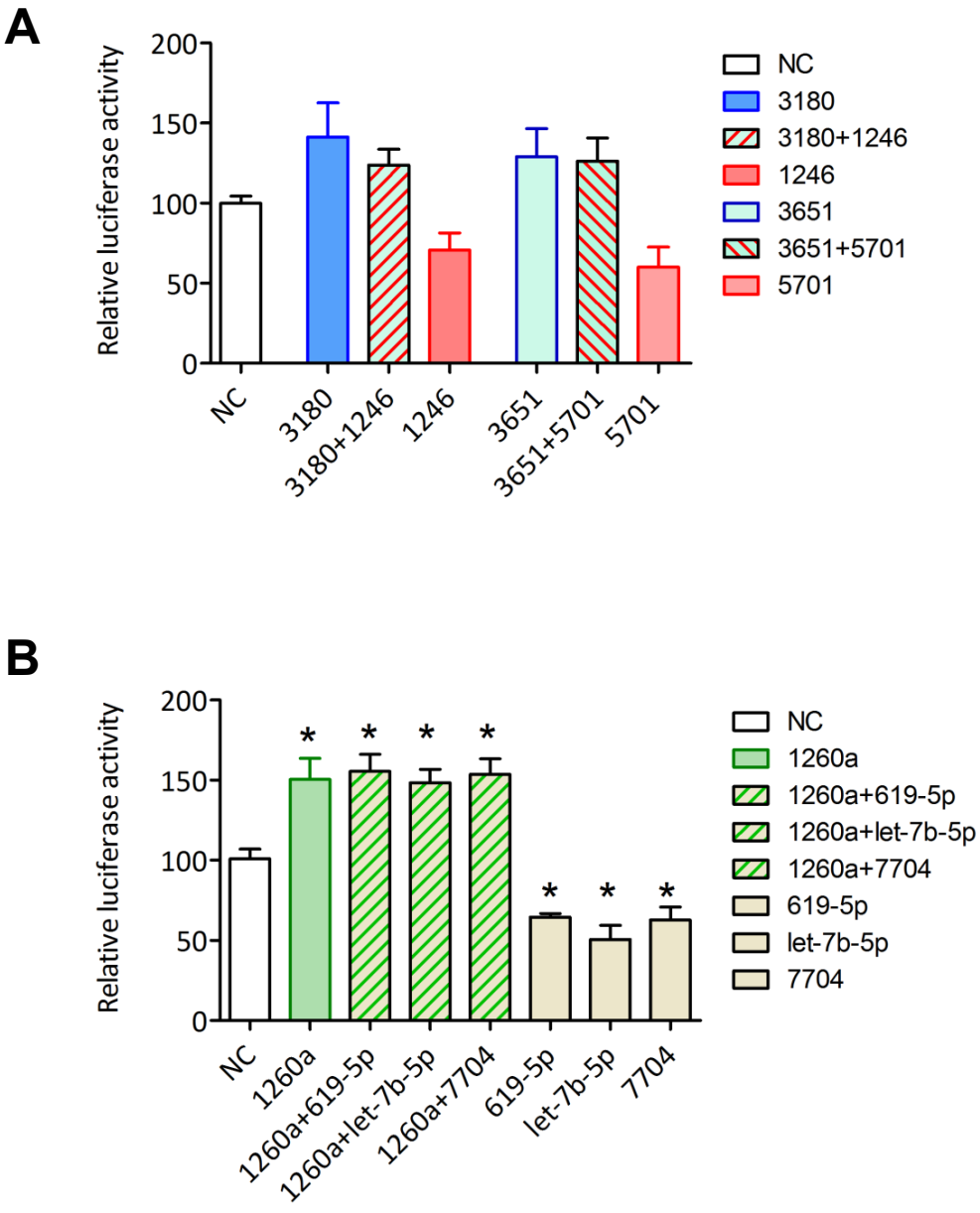


Supplementary Figure S13. Specificity of X1-targeting microRNAs.

A. The Luciferase reporter assay performed in HCT116 Dicer-/- cells using pMIR-ref-3'UTR shows that the indicated microRNAs do not alter Luciferase activity, i.e. do not target the ref isoform of *BRAF* mRNA.

B. The same assay performed with pMIR-MEK1-3'UTR shows that the indicated microRNAs do not target MEK. This results confirms that the increase in pMEK levels observed upon the transfection of si-miR-3180 and the decrease observed upon the transfection of si-miR-3651 and si-miR-1260a are due to changes in phosphorylation, not expression levels.

The graphs represent the mean ± SEM of 3 independent experiments.



Supplementary Figure S14. Competition between X1-targeting microRNAs.

A. Luciferase reporter assay performed in HCT116 Dicer-/- cells using Class II activators and Class I repressors that share overlapping MREs. When present together, the effect of Class II activators (miR-3180 and miR-3651, blue) prevails over the effect of Class I repressors (miR-1246 and miR-5701, respectively, red). These results are consistent with the binding affinities of miR-3180 and miR-5701, which are predicted to be higher than those of miR-1246 and miR-5701, respectively (see minimal free energy values in Supplementary Fig. S3).

B. Luciferase reporter assay performed in HCT116 Dicer-/- cells using Class IIIb activator miR-1260a (green) and Class IIIa repressors miR-619a-5p, 7704, let-7b-5p (black). In spite of similar binding affinities (Supplementary Fig. S3), the activator effect of miR-1260a invariably prevails over the repressor effect of miR-619a-5p, miR-7704 and let-7b-5p.

The graphs represent the mean ± SEM of 3 independent experiments. *p< 0.05.