Trichoderma atroviride hyphal regeneration and conidiation depend on cell signaling process regulated by a microRNA-like RNA

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Supplementary Information

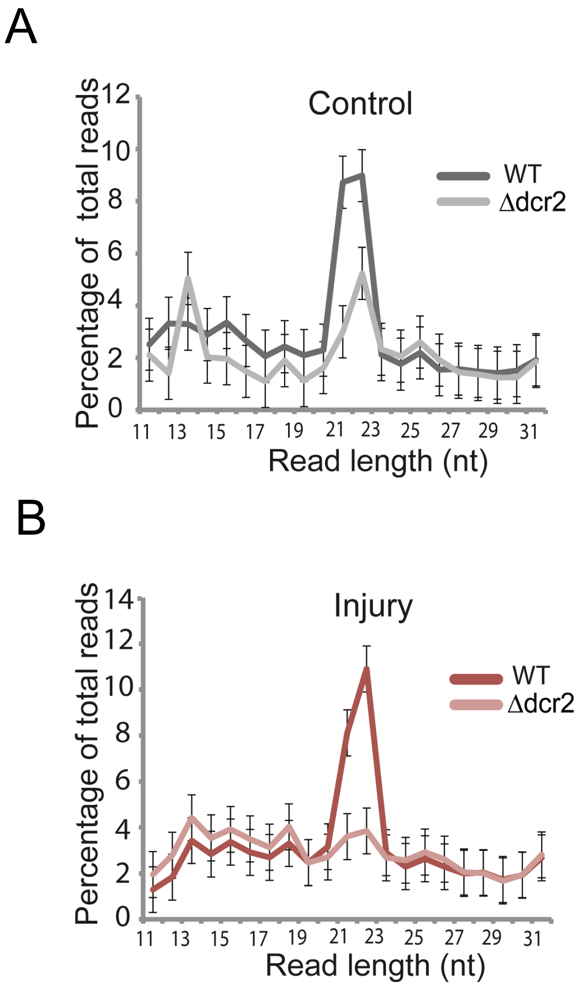
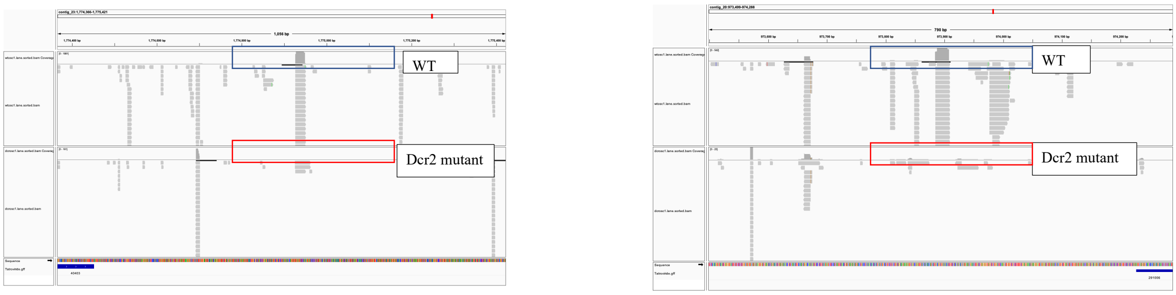


Figure S1. Percentage of total reads of small RNAs of different sizes, both in the control (A) and in response to injury (B), for the Δ*dcr*2 mutant and the WT strain.


Conting_23: milRNA4

Conting_20: milRNA5

Figure S2. Accumulation of reads on the milRNA4 and milRNA5. This image shows the reads from small RNA for the regions that code for milRNA4 and 5. In both cases, only one reads accumulation peak of accumulated reads is evident for the WT strain, with no accumulation of reads not being able to observe reads accumulation in the 5P region (highlighted in blue box). In the Δ*dcr2* mutant, at this scale, it is not possible to observe reads (highlighted in red box).

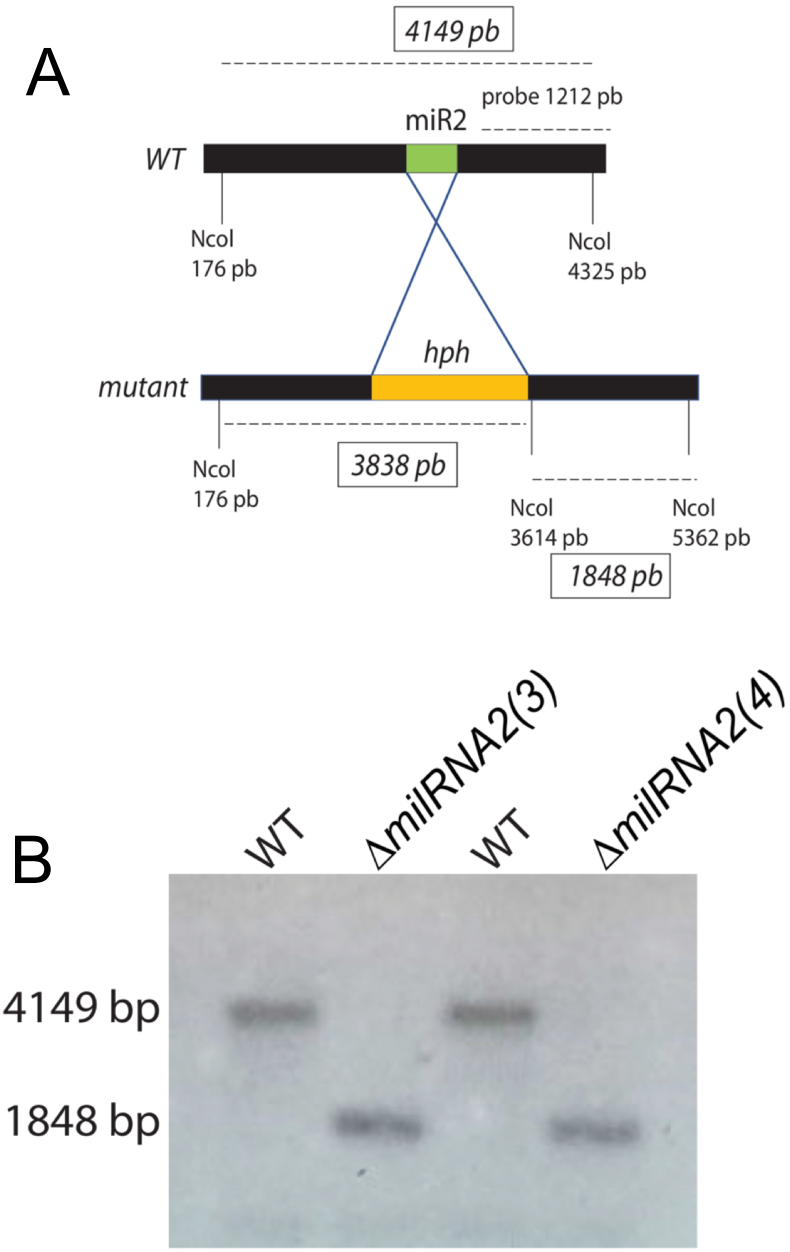


Figure S3. Confirmation of the mutation of the milRNA2 by Southern blot. A) Restriction map pattern with the NcoI enzyme. The 3’ fragment of the construct was used as a probe. B) Restriction pattern obtained for the WT strain and mutants ΔmilRNA2-3 and ΔmilRNA2-4.

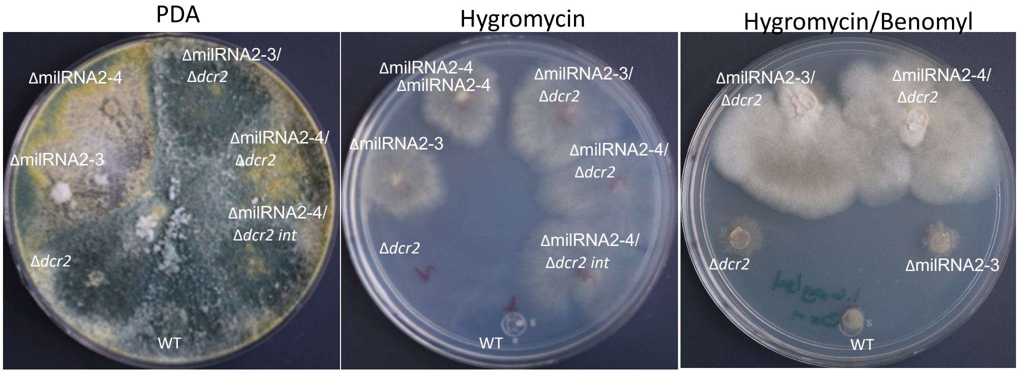


Figure S4. Growth assay of milRNA2 mutants and heterokaryon colonies. The image shows the growth of the mutant strains in PDA, hygromycin, and the combination of hygromycin with benomyl, demonstrating that the fused strains form heterokaryon colonies that are capable of resisting both antibiotics.

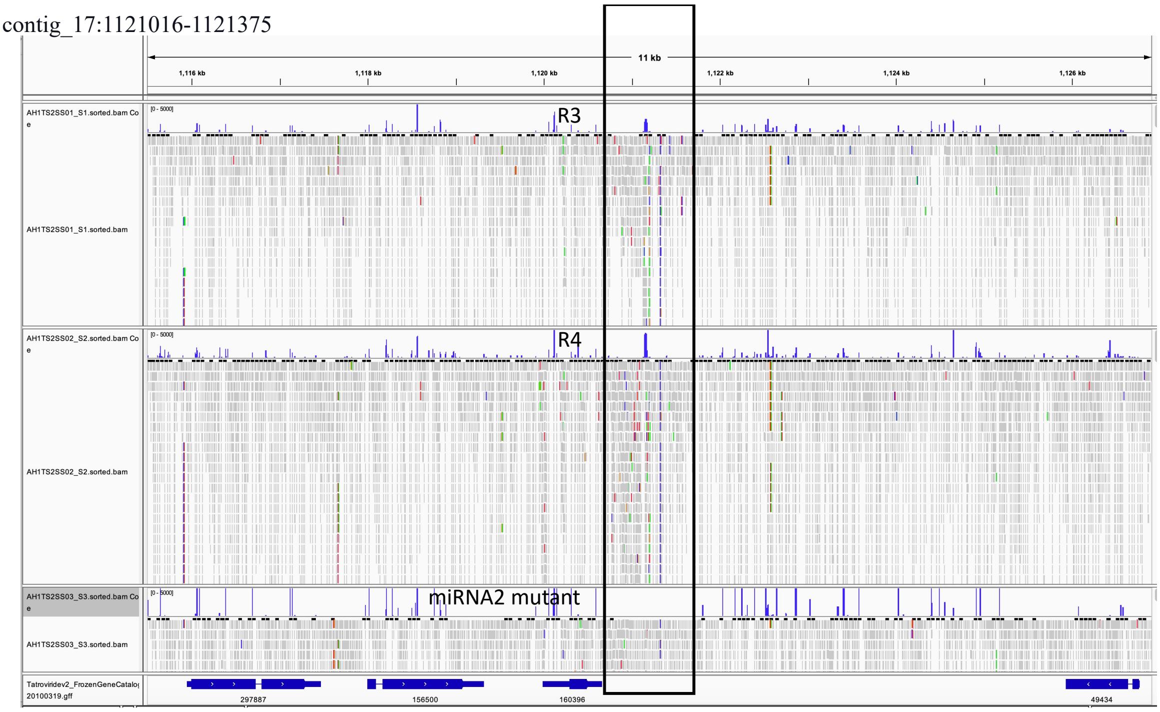


Figure S5. Accumulation of sRNA reads on the genomic region encoding milRNA2. This image shows the sRNA accumulation profile of the ΔmilRNA2-3 mutant and two of the fusion complements (R3 and R4), where the recovery of milRNA2 can be seen (image generated in IGV). The tracks blue ones above the line show the reads accumulation for all strains.

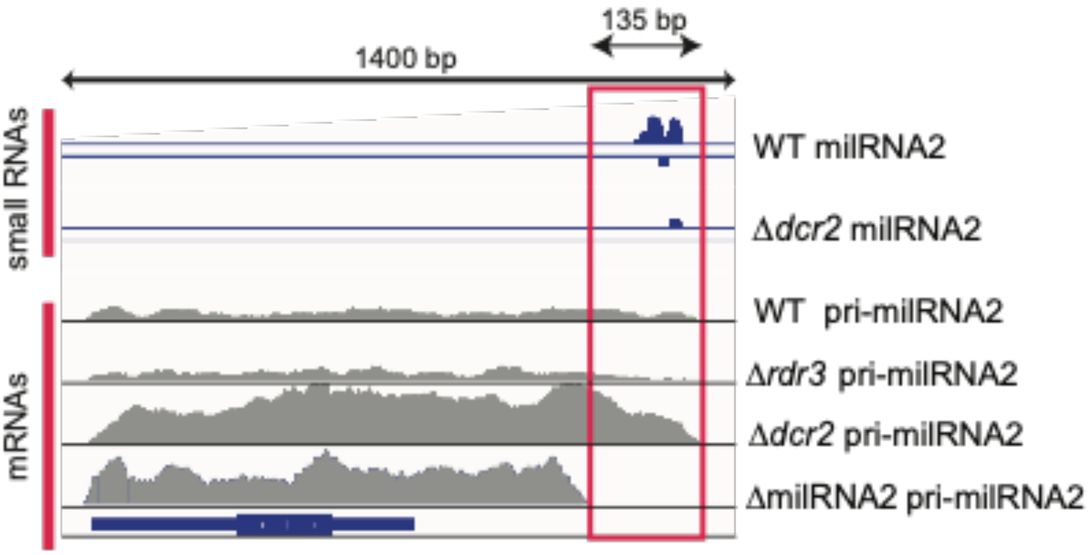


Figure S6. Accumulation of reads on the pri-milRNA2 region. This image shows the reads from small RNA and messenger RNA libraries in the WT, Δ*dcr*2, Δ*rdr*3 and ΔmilRNA2 strains.

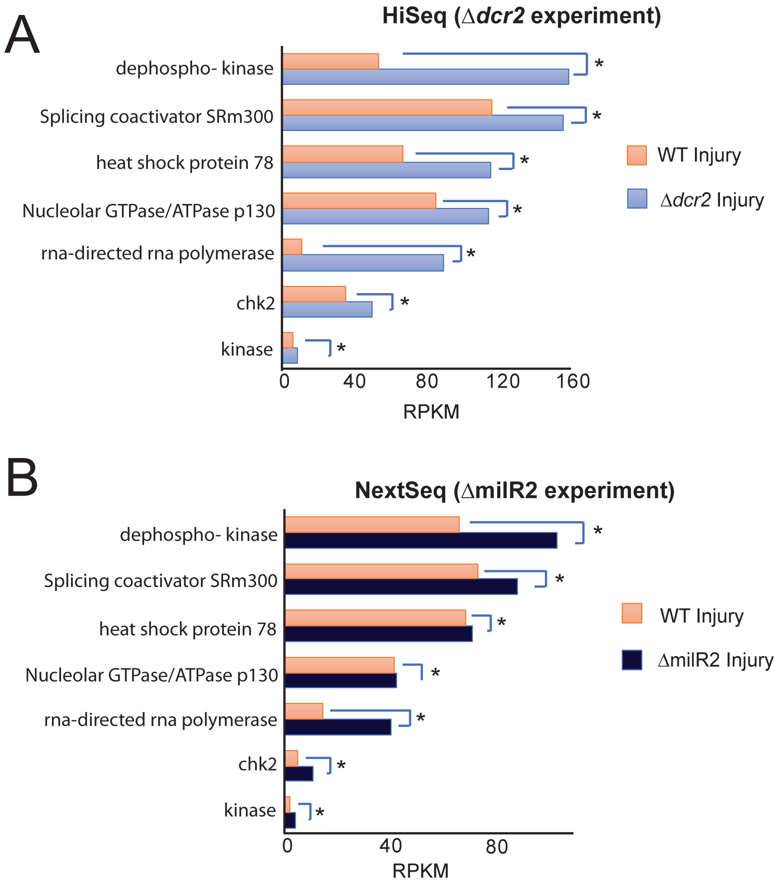
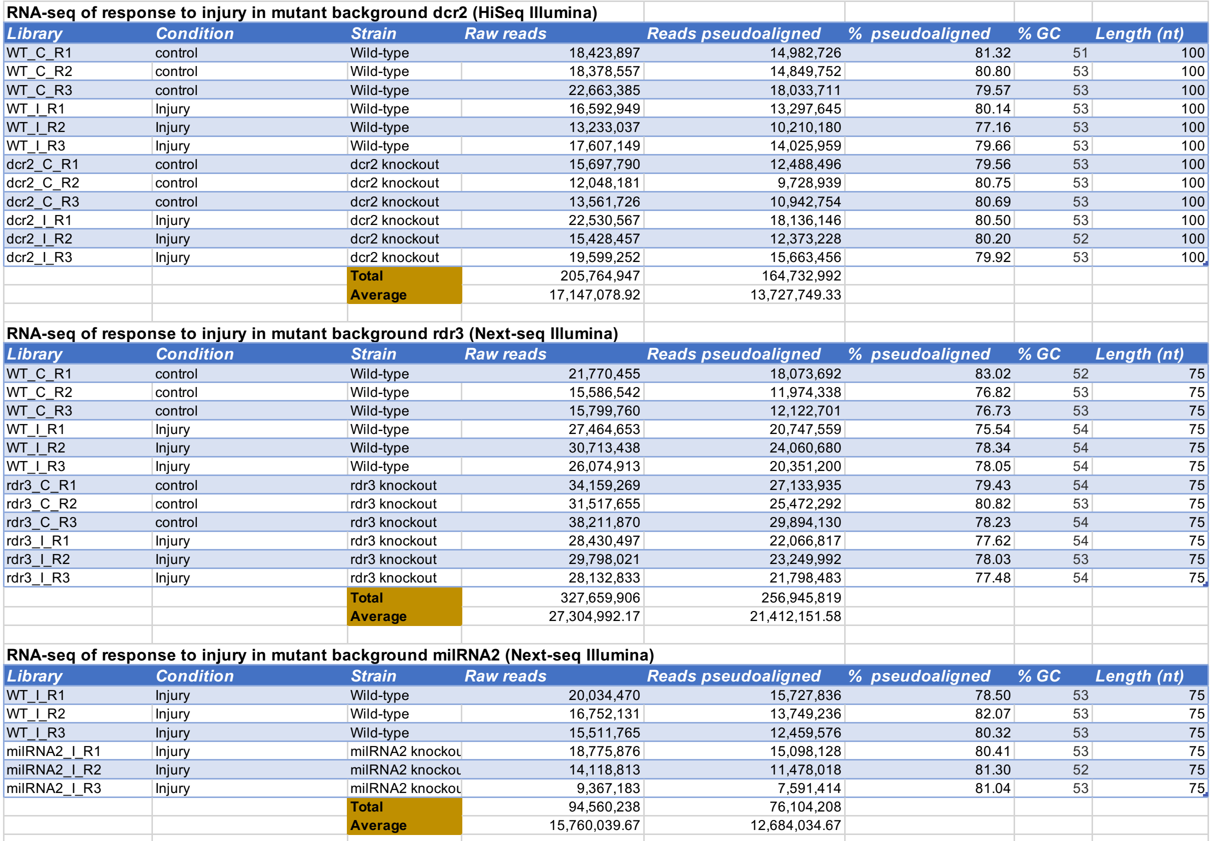


Figure S7. Target genes predicted by TargetFinder. Expression profile of milRNA2-target genes affected by both the mutation of milRNA2 and the *dcr2* gene. These values are expressed in counts per million, given that they were independent sequencing experiments for each strain, the HiSeq (A) and NextSeq (B) data are presented.

Table S1. Sequencing statistics. The table shows the sequencing and alignment statistics for each of the RNAseq libraries used in this work.



RNA-seq of response to injury in mutant background dcr2 (HiSeq Illumina)

Library	Condition	Strain	Raw reads	Reads pseudoaligned	% pseudoaligned	% GC	Length (nt)
WT_C_R1	control	Wild-type	18,423,897	14,982,726	81.32	51	100
WT_C_R2	control	Wild-type	18,378,557	14,849,752	80.80	53	100
WT_C_R3	control	Wild-type	22,663,385	18,033,711	79.57	53	100
WT_I_R1	Injury	Wild-type	16,592,949	13,297,645	80.14	53	100
WT_I_R2	Injury	Wild-type	13,233,037	10,210,180	77.16	53	100
WT_I_R3	Injury	Wild-type	17,607,149	14,025,959	79.66	53	100
dcr2_C_R1	control	dcr2 knockout	15,697,790	12,488,496	79.56	53	100
dcr2_C_R2	control	dcr2 knockout	12,048,181	9,728,939	80.75	53	100
dcr2_C_R3	control	dcr2 knockout	13,561,726	10,942,754	80.69	53	100
dcr2_I_R1	Injury	dcr2 knockout	22,530,567	18,136,146	80.50	53	100
dcr2_I_R2	Injury	dcr2 knockout	15,428,457	12,373,228	80.20	52	100
dcr2_I_R3	Injury	dcr2 knockout	19,599,252	15,663,456	79.92	53	100
		Total	205,764,947	164,732,992			
		Average	17,147,078.92	13,727,749.33			

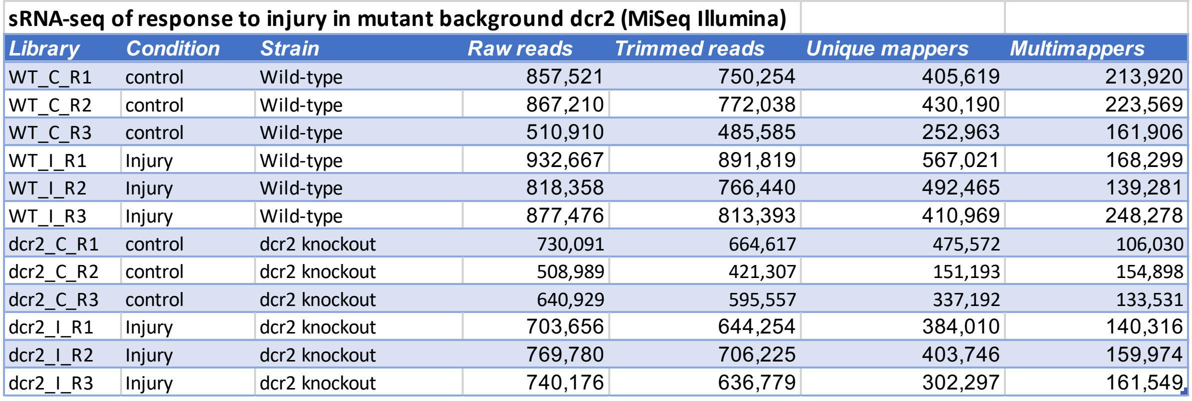
RNA-seq of response to injury in mutant background rdr3 (Next-seq Illumina)

Library	Condition	Strain	Raw reads	Reads pseudoaligned	% pseudoaligned	% GC	Length (nt)
WT_C_R1	control	Wild-type	21,770,455	18,073,692	83.02	52	75
WT_C_R2	control	Wild-type	15,586,542	11,974,338	76.82	53	75
WT_C_R3	control	Wild-type	15,799,760	12,122,701	76.73	53	75
WT_I_R1	Injury	Wild-type	27,464,653	20,747,559	75.54	54	75
WT_I_R2	Injury	Wild-type	30,713,438	24,060,680	78.34	54	75
WT_I_R3	Injury	Wild-type	26,074,913	20,351,200	78.05	54	75
rdr3_C_R1	control	rdr3 knockout	34,159,269	27,133,935	79.43	54	75
rdr3_C_R2	control	rdr3 knockout	31,517,655	25,472,292	80.82	53	75
rdr3_C_R3	control	rdr3 knockout	38,211,870	29,894,130	78.23	54	75
rdr3_I_R1	Injury	rdr3 knockout	28,430,497	22,066,817	77.62	54	75
rdr3_I_R2	Injury	rdr3 knockout	29,798,021	23,249,992	78.03	53	75
rdr3_I_R3	Injury	rdr3 knockout	28,132,833	21,798,483	77.48	54	75
		Total	327,659,906	256,945,819			
		Average	27,304,992.17	21,412,151.58			

RNA-seq of response to injury in mutant background milRNA2 (Next-seq Illumina)

Library	Condition	Strain	Raw reads	Reads pseudoaligned	% pseudoaligned	% GC	Length (nt)
WT_I_R1	Injury	Wild-type	20,034,470	15,727,836	78.50	53	75
WT_I_R2	Injury	Wild-type	16,752,131	13,749,236	82.07	53	75
WT_I_R3	Injury	Wild-type	15,511,765	12,459,576	80.32	53	75
milRNA2_I_R1	Injury	milRNA2 knockou	18,775,876	15,098,128	80.41	53	75
milRNA2_I_R2	Injury	milRNA2 knockou	14,118,813	11,478,018	81.30	52	75
milRNA2_I_R3	Injury	milRNA2 knockou	9,367,183	7,591,414	81.04	53	75
		Total	94,560,238	76,104,208			
		Average	15,760,039.67	12,684,034.67			

Table S2. Sequencing statistics. small RNA-seq of the response to injury in the mutant background *dcr2* (MiSeq Illumina)**.**



sRNA-seq of response to injury in mutant background dcr2 (MiSeq Illumina)

Library	Condition	Strain	Raw reads	Trimmed reads	Unique mappers	Multimappers
WT_C_R1	control	Wild-type	857,521	750,254	405,619	213,920
WT_C_R2	control	Wild-type	867,210	772,038	430,190	223,569
WT_C_R3	control	Wild-type	510,910	485,585	252,963	161,906
WT_I_R1	Injury	Wild-type	932,667	891,819	567,021	168,299
WT_I_R2	Injury	Wild-type	818,358	766,440	492,465	139,281
WT_I_R3	Injury	Wild-type	877,476	813,393	410,969	248,278
dcr2_C_R1	control	dcr2 knockout	730,091	664,617	475,572	106,030
dcr2_C_R2	control	dcr2 knockout	508,989	421,307	151,193	154,898
dcr2_C_R3	control	dcr2 knockout	640,929	595,557	337,192	133,531
dcr2_I_R1	Injury	dcr2 knockout	703,656	644,254	384,010	140,316
dcr2_I_R2	Injury	dcr2 knockout	769,780	706,225	403,746	159,974
dcr2_I_R3	Injury	dcr2 knockout	740,176	636,779	302,297	161,549

Table S3. Primers designed for stem-loop RT-PCR and for the construction used to mutate milRNA2.

Primers for stem-loops		
name	**sequence**	**nts**
miR1RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACgcaagt	50
miR1F	CGCGtgaaaccccggacaa	19
miR2RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACacagat	50
miR2F	CGCGttttgcgatgcccaat	21
miR3RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACataccg	50
miR3F	CGCGtgtgaagctaatcact	20
TRNA1RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTTATCT	50
TRNA1F	GCGCGGTTCAAATCCTCCCCTGG	23
S297535RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTACGCC	50
S297535F	GCGCATTACAACCAAGAGTGA	21
URP	GTGCAGGGTCCGAGGT	16

Primers to perform the mutation by replacement of the milRNA2		
miRNA F tail	CCCAGCACTCGTCCGAGGGCAAAGGAATAGACCAAAGGTGAGTCCGAATC	50
miRNA R tail	CTCCTTCAATATCAGTTAACGTCGATCCTGTCTAATTTTGCGAAGCCC	48
Fnest	AGGTACTTATTCCTCATTGGCC	22
Rnest	GTCCGAAGTATCAGAGCTGC	20