SUPPLEMENTARY TABLES AND FIGURE

Supplementary Table S1. miRNA expression profiling in chordoma

microRNA	*p*-value	Mean Normal	Mean Chordoma
hsa-miR-1	0.001	27231.4	31.3
hsa-miR-206	0.001	16049.0	29.5
hsa-miR-26a	0.003	35790.6	9611.8
hsa-miR-95	0.003	4243.3	18.9
hsa-miR-23a	0.004	21432.1	11497.6
hsa-miR-101	0.004	875.3	20.6
hsa-miR-133b	0.004	15708.8	68.3
hsa-miR-133a	0.004	16094.8	74.7
hsa-miR-1268	0.005	2042.8	134.1
hsa-miR-193a-5p	0.005	283.5	2717.0
hsa-miR-27a	0.006	11084.8	4992.5
hsa-miR-139-5p	0.006	705.4	17.2
hsa-miR-34a	0.008	98.2	711.2
hsa-miR-128	0.009	5141.8	616.2
hsa-miR-320c	0.010	787.1	8221.0
hsa-miR-320a	0.011	905.5	8227.1
hsa-miR-23b	0.011	21411.4	10999.0
hsa-miR-221	0.012	451.9	8061.4
hsa-miR-320b	0.013	617.6	7706.4
hsa-miR-320d	0.013	475.4	6497.5
hsa-miR-199a-5p	0.015	246.1	1817.6
hsa-miR-16	0.015	15115.1	5025.6
hsa-miR-214	0.016	2378.7	10784.6
hsa-miR-30b	0.017	8789.7	1555.8
hsa-miR-1308	0.018	327.4	6861.4
hsa-miR-155	**0.018**	**117.6**	**1359.6**
hsa-miR-99b	0.021	445.6	2868.4
hsa-let-7a	0.022	34643.8	15626.8
hsa-miR-30e	0.026	408.9	55.0
hsa-let-7f	0.028	33505.7	12725.1
hsa-miR-22	0.030	863.8	135.7
hsa-miR-30c	0.030	7559.2	2373.7
hsa-miR-378	0.033	2735.0	159.3
hsa-let-7d	0.033	26905.7	13981.6
hsa-miR-130a	0.033	62.0	367.5
hsa-miR-140-3p	0.034	281.2	17856.1

(*Continued*)

microRNA	*p*-value	Mean Normal	Mean Chordoma
hsa-miR-222	0.036	557.6	6547.4
hsa-miR-126	0.039	9251.9	771.0
hsa-miR-181b	0.043	19.0	1143.1
hsa-let-7i	0.043	7727.4	19842.1
hsa-miR-572	0.002	191.4	8.4
hsa-miR-933	0.003	106.7	13.3
hsa-miR-940	0.003	119.0	31.8
hsa-miR-1228	0.004	34.0	10.8
hsa-miR-130b	0.005	21.0	167.1
hsa-miR-628-3p	0.009	300.9	55.2
hsa-miR-30b	0.011	59.8	15.4
hsa-let-7f-1	0.011	66.8	17.1
hsa-miR-378	0.013	191.8	13.8
hsa-miR-181d	0.015	6.1	103.5
hsa-miR-584	0.017	28.8	84.5
hsa-miR-188-5p	0.018	59.2	12.7
hsa-miR-563	0.018	32.2	7.4
hsa-miR-486-3p	0.019	287.4	34.2
hsa-miR-542-3p	0.020	55.0	8.2
hsa-miR-671-5p	0.020	9.9	73.4
hsa-miR-628-5p	0.023	109.4	20.0
hsa-miR-148b	0.023	27.7	175.7
hsa-miR-484	0.025	30.8	251.7
hsa-miR-125a-3p	0.025	12.6	30.2
hsa-miR-126	0.028	46.0	5.6
hsa-miR-489	0.029	205.4	30.9
hsa-miR-1281	0.033	186.4	26.7
hsa-miR-145	0.035	221.6	48.5
hsa-miR-138	0.038	5.0	110.7
hsa-miR-20b	0.048	333.9	118.6
hsa-miR-886-5p	0.049	5.3	31.9

Supplementary Table S2. Differential expression and regulatory activity of miR-155 in chordoma

miR-155 expression determined by microarray

Mean Normal Expression	Mean Chordoma Expression	*p*-value		
117.6	1359.6	0.018		
miR-155 regulatory activity inferred by target mRNA expression				
Target prediction method (RE-score method)	Normalized RE-score	*p*-value	FDR	
Pictar	−4.24	< 0.01	0.01	
Pita	−4.09	< 0.01	0.02	
miRanda (GSA method)	LS permutation *p*-value	KS permutation *p*-value	Efron-Tibshirani’s GSA test *p*-value	Goeman’s global test *p*-value
	0	0.04	0.2	0.01

miR-155 expression levels were found to be significantly higher in chordoma relative to normal samples by miRNA microarray. mRNA microarray data were used to infer regulatory activity of miR-155 with two distinct computational techniques which include miRNA target predictions with three distinct algorithms (Pictar, Pita, and miRanda). The RE-score method compares the difference in average expression levels of target genes and non-target genes across two phenotypes. A negative RE-score is indicative of higher regulatory activity in chordoma relative to normal tissue. The GSA method compares expression levels of target gene sets (determined by miRanda) between chordoma and normal tissue with several statistical tests implemented in NCI-BRB Arraytools.

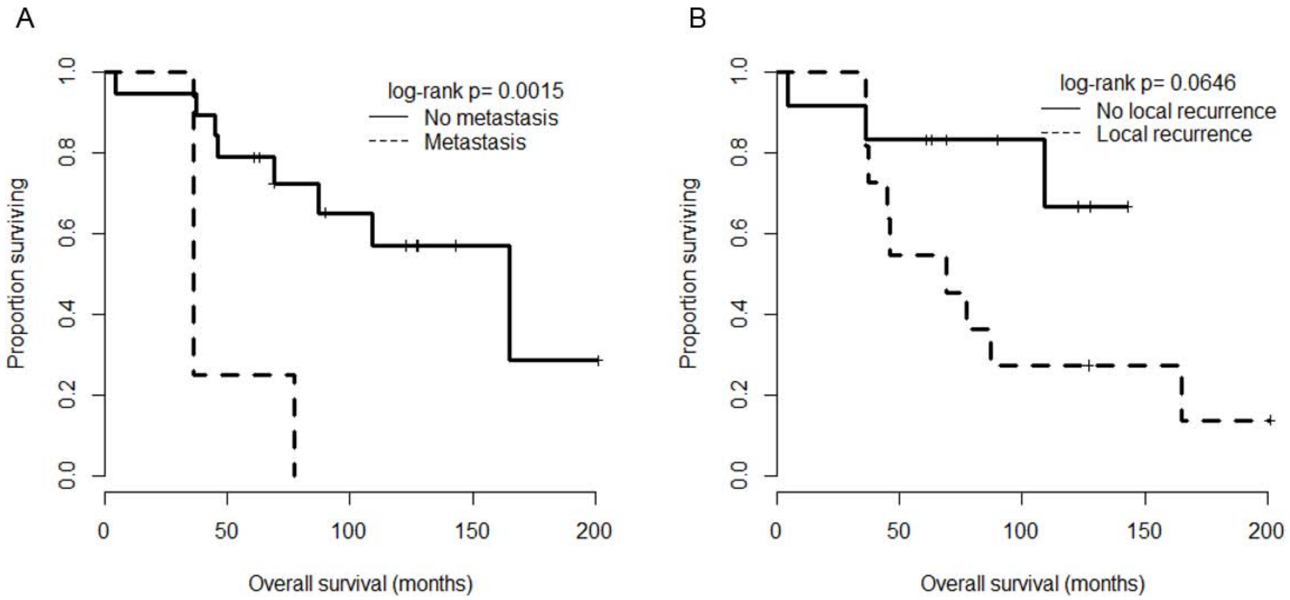
Supplementary Table S3. Correlation between the expression level of miR-155 in clinicopathological features

Clinicopathological features	Total	Average[a] ± SD	*P*-value
Gender			
Male	18	1.30 ± 0.34	0.347
Female	5	1.06 ± 0.55	
Age			
≥ 63	12	1.01 ± 0.59	0.304
< 62	11	1.23 ± 0.41	
Location			
Sacrum	20	1.05 ± 0.48	0.12
Other	3	1.23 ± 0.58	
Stage			
1A + 1B	13	0.87 ± 0.41	0.006[b]
2A + 2B + 3	10	1.43 ± 0.46	
Origin			
Primary origin	12	0.94 ± 0.30	0.088
Recurrent origin	11	1.31 ± 0.64	
Local recurrence			
Overall			
Yes	11	1.11 ± 0.56	0.93
No	12	1.13 ± 0.49	
Primary origin			
Yes	3	0.88 ± 0.10	0.69
No	9	0.96 ± 0.34	
Recurrent origin			
Yes	8	1.19 ± 0.64	0.354
No	3	1.61 ± 0.63	
Metastasis			
Yes	4	1.76 ± 0.52	0.003[b]
No	19	0.98 ± 0.41	

The relationships of quantitative miR-155 expression levels with clinicopathological features of chordoma patients were analyzed by Student's *t*-test.

[a] Log10 transformed

[b] Indicates statistical significance. Statistical significance was defined as a *P*-value of <0.05.



Supplementary Figure S1: Clinical characteristics associated with overall survival. Kaplan-Meier analysis comparing overall survival between chordoma patients with versus without metastatic disease **A.** and with versus without local recurrence **B.**