SUPPLEMENTARY INFORMATION. Figures S1-7. Sequence maps redrawn from JBrowse screen captures for the remaining genes in this study are shown in a single figure. The first panel shows the high throughput tag vector screen for cis-regulatory activity in the marker BAC (cf. Table 1). The BAC insert ~2kb fragments are shown as an overlapping tiling array (back rectangles). Each 2kb fragment was incorporated in a tag vector and 129 of these vectors were injected into fertilized eggs. Transcribed tag DNA and genomically incorporated tag DNA for all vectors were measured simultaneously by Nanostring. The ratio of these values, which indicates the transcriptional activity driven by the sequence in each respective vector normalized to the amount of vector DNA incorporated, are shown at right in the histogram. 2kb fragments that are thus revealed to include active cis-regulatory modules are shown in red. Marginally expressing fragments are shown in purple and light blue. The second and third panels show various features mapped to the reference genome sequence at different approximate scales. The BAC insert is shown in green. The red line depicts the DNA sequence of the active fragment in the BAC insert. Deleted regions are shown in blue below it. Symbols identifying the deletions are the ones used in the test and tables. Vertical bars represent the position and number of ATAC-seq reads mapped. The position of the modified CIS-BP transcription factor binding sites are indicated in light green. Mutated sites are in orange.

Table S1. Coordinates of Hot Pieces and their deletions. The coordinates of the regulatory fragments identified for each gene mapped onto the reference genome assembly (v3.1) posted on Echinobase.org. Similarly the coordinates of the deletions used to show loss of function within the Hot Piece are indicated.

Table S2. qPCR primers. The primers used to measure the expression of each hot piece-tag fusion construct in this study. The primers are taken from the 13-tag set of Nam et al (2010).

Table S3. Microscopic observation of the fragment fusion constructs that expressed above an empirically determined threshold level from the Nanostring nCounter results. The cutoff is indicated for each gene set. For each fragment the transcript copies per construct and the microscopic observation is indicated. The microscopic onservations were divided into 3 classes: 1) spatially correct; only expressed in primary mesenchyme cells; 2) low, the percentage of embryos observed was a minor fraction of the total; 3) negative: no fluorescent expression seen.

Table S1. Coordinates of Hot Pieces and their deletions

Hot Piece ID	Scaffold	Hot Piece Coordinates	Deletion ID	Deletions Coordinates
Colp3a_306	Scaffold559	248133..246240	D1/D2	247883..246534
Arhgap28_504	Scaffold315	:629818..627956	D1 D2	628449..627956 629599..629100
Astacin1_504	Scaffold663	301994..300122	D1/D2	301016..300174
Csrnp2_i10	Scaffold931	191176..193382	D2	192934..193382
Dri_507	Scaffold48	160888..163027	D2 D3	161909.162625 160888..161668
Hypp2998_306	Scaffold198	271304..273701	D2	272044..273023
Mitf_506:	Scaffold743	175745..177823	D1 D2	175746..176099 176300..176599
p58a_5021	Scaffold107	248322..250994	D1 D3	248923..249234 250500..250994

Table S2. qPCR primers

[1]Tag No	Forward	Reverse	Amplicon	Hot piece
Tag 1	agccactcagctccagaaaaa	acggtaattcgctgtgatgcc	165bp	Col3a_306 (A)
Tag 2	agagagccactcagctccag	gtctgccaggtggttttacc	165bp	Arhgap28_504 (B)
Tag 3	agccactcagctccagaaaaa	cgaaatatgccgcctcagg	152bp	Astacin1_504 (C)
Tag 4	agccactcagctccagaaaaa	cacttgttagcgacgatggc	165bp	Csrnp2_i10 (D)
Tag 7	agagagccactcagctccag	ctgcggaccgtcgttatatgg	168bp	Dri_507 (E)
Tag 8	agccactcagctccagaaaa	gcggctttgaatcgtcttcc	161bp	Hypp2998_306 (F)
Tag 9	agtccgctagaagggagagc	agggttcagcacccacac	153bp	Mitf_506 (G)
Tag 10	agccactcagctccagaaaa	agatggctggcgtacgag	163bp	p58a_5021 (H)

Control primers

GFP	gctggccgaccattatcaac	acaaactccagcaggaccat	141bp
UBIQ	cacaggcaagaccatcacac	gagagagtgcgaccatcctc	147bp
FoxA	ccaaccgactccgtatcatc	cgtagctgctcatgctgtgt	160bp

[1]Tag numbers and primers are derived from 13 tag system previously reported (Nam et al, 2010).

Table S3. Microscopic observation of the fragment fusion constructs that expressed above an empirically determined threshold level from the Nanostring nCounter results. The cutoff is indicated for each gene set. For each fragment the transcript copies per construct and the microscopic observation is indicated. The microscopic onservations were divided into 3 classes: 1) spatially correct; only expressed in primary mesenchyme cells; 2) low, the percentage of embryos observed was a minor fraction of the total; 3) negative: no fluorescent expression seen.

		Nanostring	
Gene	Fragment	cDNA/gDNA	Result
Colp3a	Colp3a_306	14.28	spatially correct
	Colp3a_305	2.47	negative
cutoff=1.2	Colp3a_I07	2.04	negative
Arhgap28	Arhgap28_507	167.17	low
	Arhgap28_3010	64.79	negative
	Arhgap28_303	61.04	negative
	Arhgap28_I04	18.93	negative
	Arhgap28_504	17.87	spatially correct
	Arhgap28_3017	14.19	negative
	Arhgap28_302	11.36	negative
	Arhgap28_315	6.53	negative
	Arhgap28_316	5.83	negative
cutoff=3.5	Arhgap28_505	5.58	negative
Astacin1	Astacin1_509	358.02	negative
	Astacin1_504	24.55	spatially correct
	Astacin1_508_N	15.41	negative
cutoff=4	Astacin1_507	4.91	low
Csrnp2	Csrnp2_i03	105.63	ectopic
	Csrnp2_i10	31.37	spatially correct
	Csrnp2_i06	20.47	low
	Csrnp2_i02	16.94	low
cutoff=3.5	Csrnp2_i04	13.11	low
Dri	Dri_514a	26.18	ectopic
	Dri_507a	16.32	spatially correct
	Dri_504	9.02	negative
	Dri_502	5.1	negative
	Dri_301	7.8	negative
cutoff=3.5	Dri_501	8.56	ectopic
Hypp2998	Hypp2998_511	33.12	negative
	Hypp2998_306	20.01	spatially correct
cutoff=2.5	Hypp2998_io1B	7.32	negative

MitF	Mitf_506	4.64	spatially correct
	Mitf_505	3.57	negative
cutoff=2.5	Mitf_514	2.65	negative
p58a	P58a I10	32.73	ectopic
	P58a I09	5.12	negative
cutoff=2.5	p58a 5021	3.52	spatially correct