

Table S1. Previously characterized melanocyte enhancers.

Melanocyte Enhancer	Reference (PMID)	Coordinates (mm9)	EP300 peak	H3K4me1-flanked region
<i>Tyr</i> DRE -15kb	17516925	chr7:94,656,685-94,656,889	Yes	Yes
<i>Tyrp1</i> enhancer	16934245	chr4:80,465,467-80,465,746	Yes	No*
<i>Sox10</i> MCS4	18773071	chr15:79,023,255-79,024,074	Yes	Yes
<i>Sox10</i> MCS5	18773071	chr15:79,031,936-79,032,482	Yes	Yes
<i>Sox10</i> MCS7	18773071	chr15:79,043,282-79,043,579	No**	Yes
<i>Sox10</i> MCS9	18773071	chr15:79,053,865-79,054,530	Yes	Yes

*H3K4me1 peak on 3' side. Repeat element immediately 5' of enhancer prevents sequencing reads from being mapped there, so the presence of H3K4me1 enrichment there cannot be ruled out.

**EP300 peak called by MACS for replicate 2 but not replicate 1, though there is a noticeable spike in ChIP-seq signal at that locus in replicate 1.

Table S2. Results of *in vitro* function assay.

tested sequence	chr	start	end	size	luciferase F*	luciferase R*	F primer	R primer
Putative enhancer 1	chr16	28953120	28953594	474	2.54	0.97	TGAGAGTTGTTGTCATGGTGTC	AGGCATGCCAATCAAATAGG
Putative enhancer 2	chr11	72098039	72098515	476	26.39	1.21	GCAACGGAGTGATTCTACAGC	CTTGGGCTGGAAGGCAGT
Putative enhancer 3	chr1	88899830	88900305	475	1.68	0.20	TTTCTCCAGATTCCATAGC	CCTTGCTGGTACAGATGATT
Putative enhancer 4	chr10	53225309	53225774	465	1.22	0.49	ATAGCCCTTGCGTGAAGTAC	CCCTTTGGATAAAATGTAGCTTTG
Putative enhancer 5	chr1	84854191	84854637	446	4.71	5.93	TGGACTGGGAGATTACAGC	TCCAAGGGCTATAGGGGTAA
Putative enhancer 6	chr6	91509319	91509792	473	1.82	3.79	GCTGTGCTTACCACTCCA	TTCTAGCTAGGTGACAAATACAAGG
Putative enhancer 7	chr14	68528144	68528593	449	5.77	9.46	AAACAGCCAAGGGCACTTT	GGGTGGGAGGATCATGTAAA
Putative enhancer 8	chr13	98019440	98019932	492	26.55	17.27	ACTGAGAGCCCTGTGGAATG	CAAAATCTTCTCTCGCCTTG
Putative enhancer 9	chr19	23424751	23424521	470	28.61	13.53	TGGAAAAACAACCTAATTCTTTGAGA	CTCCAGGCTTAACCAACTC
Putative enhancer 10	chr1	155411758	155412237	479	17.24	7.85	CTGACACGGGAATGATAACAA	CCGAGCCCATGTAAAAAGAA
Putative enhancer 11	chr9	95546331	95546801	470	2.26	2.47	TGTGAGGGATGAAGTGGGTA	AATGCACTCGCATGTCTTT
Putative enhancer 12	chr15	11519662	11520127	465	0.46	1.13	GGACTGTGAGCTGGATGAT	AAGCGACTTGCTTGATCAGG
Putative enhancer 13	chr3	19878490	19878960	470	4.86	2.88	CATTGACTCAGGAGCTTTTGC	CACATCAGCCTTAAGCTCAG
Putative enhancer 14	chr6	101899563	101900015	452	69.06	1.25	AAAATGTCTAACTTGTAGATAGTGTGG	GGCTCCAGCAGACTAATGGT
Putative enhancer 15	chr3	121224582	121225047	465	7.10	3.87	CCCTACCTTCCATGATCGAC	GGCCCATATTGGTTCTTTGA
Putative enhancer 16	chr12	54162403	54162870	467	11.58	1.98	TTTTTATTGGCATTGGAGAAA	GGATGGAATCAAACCTAACCCTG
Putative enhancer 17	chr1	51878299	51879175	446	7.15	2.62	CCTACTCGCAAGCCATAAAC	TGCTGTCATGAGCTGATT
Putative enhancer 18	chr10	19944373	19944841	468	2.52	2.41	GCTCTGTGTGAGGGGACTGT	CACCCATTTTGACTGAATCG
Putative enhancer 19	chr11	75962463	75962948	485	6.94	2.79	CCTCAGCTGCTTTTGCTGA	GTTCCCACTGCTTTTGAA
Putative enhancer 20	chr1	136798731	136799197	466	5.13	9.63	TGCTGCCTCTGAATAGTCCA	AAATGAGTAGTGCATCAGAAGTGC
Putative enhancer 21	chr1	108051826	108052288	462	25.03	13.06	AAAAACAGATAAGGAGTCTCCAG	ATTTGAGTTTTAGAAATGATTGT
Putative enhancer 22	chr7	119282193	119282641	448	103.84	1.02	ACACTGTTTGCCCCCTACAT	CAGGCCATGGTAGGAACAG
Putative enhancer 23	chr2	125635277	125635751	474	2.17	3.98	TGGAATCTACTGCTTGAGTGGTG	TGAAGTAATCTGTCTATCTACTTGA
Putative enhancer 24	chr14	32216801	32217268	467	3.67	3.56	GTGACTTGGCCTCCAAGAAG	TCAGCCTCAACTCTTTTCCA
Putative enhancer 25	chr1	60882510	60882993	483	49.26	5.72	AGTACTTCACTATTTCGGAGA	CACCTGGAATCCGAGCATCT
Putative enhancer 26	chr15	59591320	59591792	472	4.85	0.73	ATTTGGCCAGGGAAGAAGTT	AAAAAGGGGTGAGGTCACAT
Putative enhancer 27	chr7	90789690	90790139	449	51.02	7.84	GTTACATCCCATGAAACC	GAGCAGCTGGAAGTGTAGG
Putative enhancer 28	chr9	49808536	49808997	461	3.23	3.89	CCCAACTTGTCTCACCTTA	AGGCTCTCATTGCAATCT
Putative enhancer 29	chr5	93559127	93559578	451	0.96	16.91	GCCCTTGTGAGACTTCTTG	AGCTTGCGCTCCCTACCC
Putative enhancer 30	chr16	23518908	23519391	483	12.96	5.99	TGGGATTGAGAGCTCATAGA	GACATGGCATCTCTTCTTTG
Putative enhancer 31	chr2	26390348	26390808	460	2.14	1.15	CTCTGGGCTCTCGTCTACT	TCTCCTAAGCAGCAGCCAAT
Putative enhancer 32	chr18	5215896	5216296	400	4.27	4.33	AACCAAAGAGACACAGGATGA	CCCCACAATTTTCAGAAAAGG
Putative enhancer 33	chr4	81572482	81572973	491	7.51	0.33	TACTCGGCTTTTGATTCTAGC	AGACAGAGCTTCCCATCTG
Putative enhancer 34	chr18	49263371	49263867	496	15.14	3.91	TTATTCTGGGTCAATGCATCTT	AAGGAAGGAAGCAAAATCCAAA
Putative enhancer 35	chr9	44375544	44375990	446	33.45	18.71	ACAGGCTCTGGGTCTCTTTC	ACATAGCTTCTTTTGTAGTCCAGT
Putative enhancer 36	chr11	104133520	104134002	482	23.03	5.17	GCAGATGTTGGGAAAATGCT	GGAGCCTACAAGGGGAGAAA
Putative enhancer 37	chr5	132121523	132121974	451	6.88	6.65	GGCAGAGCGAATGCTCTAAA	TTACGGAATGCTTCAGTGGTC
Putative enhancer 38	chr19	17836119	17836596	477	2.27	10.94	ACAGAGCAAGGCAATGCAGAT	CTCACCATTGTCTGTTTGG
Putative enhancer 39	chr17	91213077	91213571	494	1.71	8.77	TGGCCTTAGAAGTTGTTTCATCC	GCACTTCAAAGTTATTTCCAGA
Putative enhancer 40	chr3	30026159	30026634	475	19.65	36.95	GCCTTGGCCATGAAATCTTA	GAGTAGTAGAAAAATCGGGTCTG
Putative enhancer 41	chr9	117035915	117036387	472	7.43	1.17	TTATTTATGCCCGATGCT	TGCCAGTTGGGCACTTTAAT
Putative enhancer 42	chr13	28644812	28645265	453	17.36	2.36	CCAAACAATAAACAATAGCAGTTGG	GGACACAGCCAGACTGGTTT
Putative enhancer 43	chr3	62253723	62254198	475	25.80	8.89	TCAAACATCATTTATGAAATCTCCA	CAGATTTACAGACAGTTTTAAACCACA
Putative enhancer 44	chr7	141419475	141419968	493	10.39	15.92	GCTGCCACTCCATCCATAAA	GCAGGGAGAGAGAAAAGCTG
Putative enhancer 45	chr2	12642449	12642922	473	15.96	1.74	AACTATAGACCCTTCAAACTG	AGTCTGCAAGGAGCACCAT
Putative enhancer 46	chr14	63750963	63751434	471	54.69	3.55	GACTCCCTCTGTGTACTACC	AGAAGCCCTTGTGGTTTTC
Putative enhancer 47	chr11	85492012	85492431	419	9.30	6.89	CCAGTCACAGAGGCCAGAAT	CTGGCCATCCACTTTAAACC
Putative enhancer 48	chr13	28782397	28782883	486	5.56	6.36	GACCCACACAAGGAGTGT	GGCTCTCTCTGGCTTCACT
Putative enhancer 49	chr3	38596903	38597369	466	11.31	13.58	GCAGCCAGCTTTCTTGACAT	GCCACTGAGGATCAGAAACG
Putative enhancer 50	chr15	54122652	54123059	407	12.25	4.85	GGAAGAGATTGTGGGCAGA	TTGTGCCACCAGCCTTTG
Negative control 1	chr5	11920958	11921537	579	2.75	0.57	AGGCATCCTTAAAGCCCTCA	CCTGTTCTGCATTGAGGTT
Negative control 2	chr17	14353462	14353880	418	1.66	2.08	AATCAGCACAGACGGGAGTT	AAGAGCAGTCCCATTACAGGA
Negative control 3	chr15	91129629	91130055	426	1.30	1.59	GAGCCAGCTTCCACATAGGA	TCATAGGATTCAGGGCAAG
Negative control 4	chr1	75526164	75526596	432	1.50	0.45	TGAAACCCAAGGAGAACCCAG	GCACAGCATATCGACGTAGC
Negative control 5	chr17	47551488	47551913	425	0.25	0.28	TTTGGCCATCTCCAAATCAT	GGCATGGTGTGTTGAGGAAC
Negative control 6	chr2	144191018	144191465	447	0.61	0.74	GGGTCTGGACAGAACTGGAA	GTCTTAAACTTGCCCATCC
Negative control 7	chr18	86887961	86888363	402	0.52	1.80	TCACAGCAATTAATCCACACA	GCGTGAAGTTAAGGAAATGAGG
Negative control 8	chr12	75230648	75231144	496	0.17	0.19	TGGCCAGCAATAGCTATCAT	GCCCTTCCCTGTCTAACCTC
Negative control 9	chr2	173922131	173922550	419	2.13	0.48	TCTCTGAGTGCCAACTTCTTCA	ATTGCTGTGGGAACCGTAAC
Negative control 10	chr10	121755675	121756136	461	0.37	0.64	TCCCTCATTTGTCTCTTCA	ATGCCGAAGATTGTGCTAC
Predicted enhancer 1	chr13	51324257	51324722	466	24.50	4.16	GACACCGCATGTCTCTCTGA	GCTGCCGACCTACTTAGACG
Predicted enhancer 2	chr10	109525611	109526010	400	8.61	4.58	TTTTTGTTTCTCCTGAACAAAGG	TGAAAAACATTGGCTGTTTG
Predicted enhancer 3	chr4	139243874	139244329	456	3.61	4.83	GTCTCATGCATCCAGGCTA	CTTAGGACGTCCACGCTGAG
Predicted enhancer 4	chr16	45982681	45983140	460	2.93	9.91	GGGAGGGATGGTTAAAGAA	CCACAAAAATATTGAGCTGATT
Predicted enhancer 5	chr2	40782175	40782646	472	1.78	1.46	TTTGGAATTTTGTCTGTTAATAAGG	CCTCCAGCAGTGAATTTCAAG
Predicted enhancer 6	chr4	8914765	8915241	477	3.45	4.28	CTCAGGGCAGAGCTGAAAGT	CTGTGTTAAGGGGTGGCTTC
Predicted enhancer 7	chr5	81555968	81556436	469	2.64	1.60	CAGGATTAATATAAACTGGAGCAG	GAAATCAATTTAACTATTTGGGTTTT
Predicted enhancer 8	chr11	71567256	71567730	475	2.40	35.44	GTTGTGACTGGCTGTGCTGT	CATGTAGATCTGTCAATCACAAATAG
Predicted enhancer 9	chr13	8698977	8699429	453	1.63	1.72	GAACCAAGCTTGTACAGGAG	CAGAGTTGGCCGAATGTCTT
Predicted enhancer 10	chr3	34230781	34231220	440	7.78	7.80	AAAAGCTTTTCCCTTCCAAA	GTGGTGACCTCAACCACAAA
Predicted enhancer 11	chr14	99848359	99848831	473	1.92	12.77	AGATCAGCTCTGCTCCGTGT	TCATGAAAAAGCCACTAAGAAAA
Tyr DRE-15kb	chr7	94656595	94656967	372	41.86	11.45	TGTAGAAGTTCACAATCTGAATAGGA	GAGGCAGAAAGGAGTGAAGTC
Tyrp1 enhancer	chr4	80465391	80465824	433	50.87	17.19	CCTCACCTCTCCCTTCTTCC	CGCAAGCTATAGTCTTATGATTG
Sox10 MCS4	chr15	79023255	79024074	819	7.89	10.97	GGGTCTACTAAGAAGACTCC	GTGCCAGGCAGCAGAGGCTA

*fold increase in expression relative to minimal promoter only

Table S3. Results of *in vivo* functional assay.

tested sequence	orientation	melanocyte expression	# embryos with documented melanocyte expression (3 dpf)	# embryos screened (3 dpf)
putative enhancer 3	F	Y	32	140
putative enhancer 7	R	Y	8*	63
putative enhancer 9	F	Y	19	86
putative enhancer 13	F	Y	24	93
putative enhancer 20	R	N	-	-
putative enhancer 22	F	Y	38	132
putative enhancer 25	F	N**	-	-
putative enhancer 27	F	Y	15	130
putative enhancer 29	R	Y	26	125
putative enhancer 45	F	N	-	-
Predicted enhancer 1	F	Y***	31	145
Predicted enhancer 8	R	Y	14	112
Predicted enhancer 11	R	N	-	-

*15 additional positives documented from earlier injections

**consistent expression observed in ganglia of the peripheral nervous system

***consistent expression observed in CNS and otic vesicle