

Supplementary materials and methods

ChIP washes

After incubating the chromatin with antibody-coated beads overnight at 4° C, the beads were washed three times with 125ul of ice cold Buffer A. All washes were performed in a 4° C cold room as follows: Add buffer, close cap and invert to resuspend beads, incubate on rotating wheel for 5 minutes, inverting by hand and make sure beads remain suspended at 2 minutes and 4 minutes, spin, collect beads on magnetic rack for 2 minutes, remove buffer.

Visualizing ChIP-seq data

To produce coverage pileups, AER and ZPA reads were extended to the predicted fragment size of 300bp using the slopBed utility in bedtools. A BED file encompassing the mm9 genome in 100bp intervals was generated, and overlapping AER, ZPA and input reads were counted in each interval using the bedtools utility coverageBed. The counts were then normalized by dividing by the total number of aligned reads for the respective sample, after which the normalized input signal was subtracted off the normalized AER and ZPA signals. The AER and ZPA signals were then scaled by a factor of 80,925,525 (the # of input reads) to facilitate viewing.

Supplementary Figures

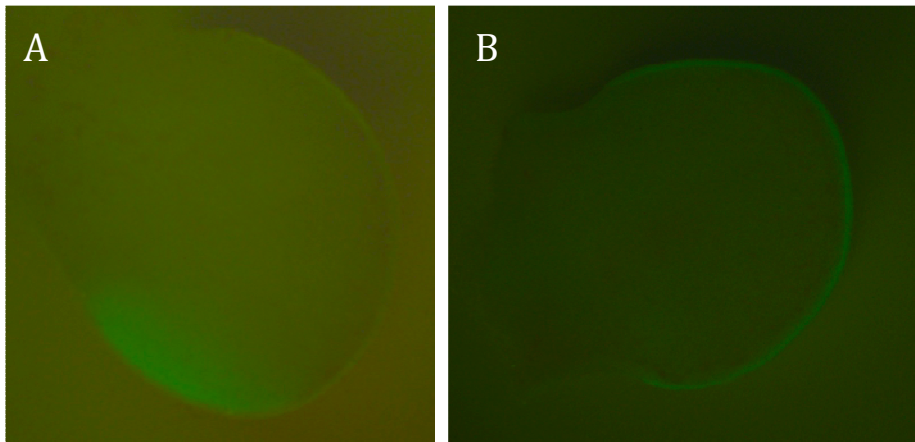


Fig. S1. ZPA- and AER-specific GFP expression. (A) A limb from E11.5 $Shh^{tm1(EGFP/cre)Cjt}$ mice showing ZPA specific expression. (B) An E11.5 limb from a homozygous male mice carrying the Cre transgene ($tg(Msx2-cre)$) crossed to a floxed reporter ($B6.129-GT(ROSA)26Sor^{tm1Joc}/J$) showing specific GFP expression in the AER.

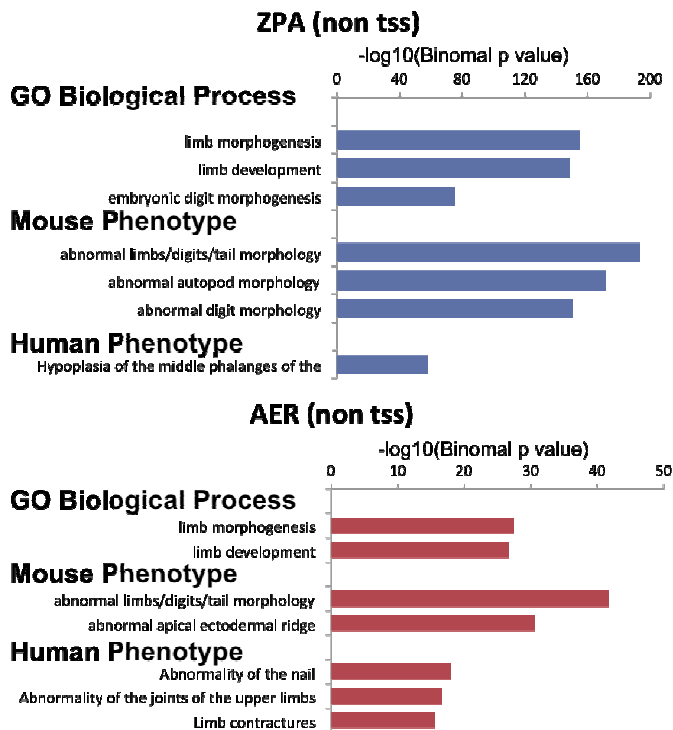


Fig. S2. GREAT results for non-TSS peaks from the ZPA and AER ChIP-seq datasets show GO terms and phenotypes associated with the roles of the ZPA and AER in limb development.

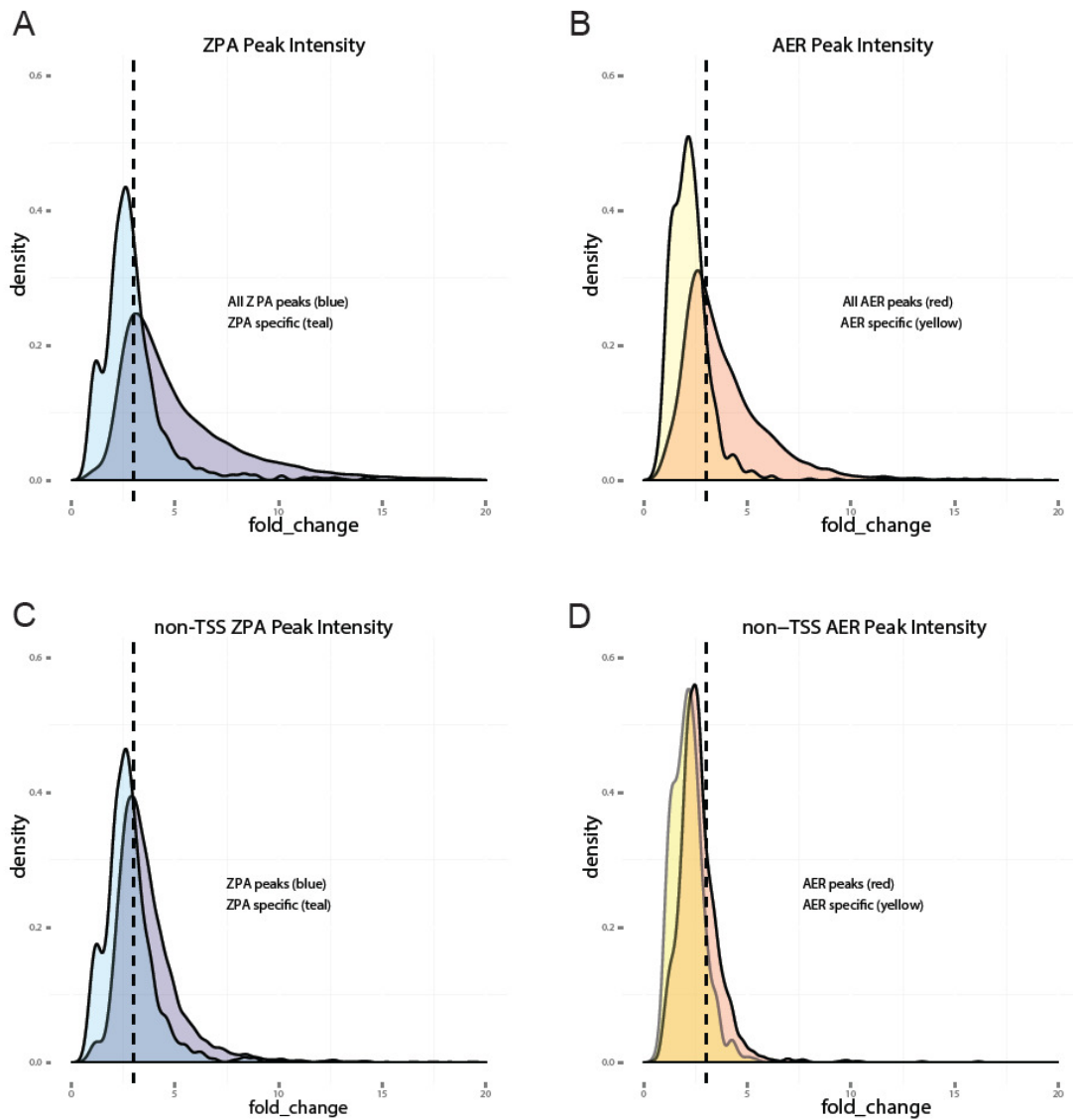


Fig. S3. Peak height and signaling center specificity. The distribution of peak signal intensity shows that the ZPA and AER-specific peaks have a weaker signal in general compared to the entire ZPA and AER sets. (A) The distribution of all ZPA peaks (blue) compared to ZPA specific peaks (peaks with no overlap to whole limb) (teal) shows that the peaks with no overlap are generally weaker. (B) The distribution of all AER peaks (red) compared to AER specific peaks (peaks with no overlap to whole limb) (orange) shows that the peaks with no overlap are generally weaker. (C,D) The same trend is seen when looking only at non-TSS peaks from either data set. This suggests that some of the very strongest enhancer signals are the ones that overlap with whole-limb signal.

Supplementary Tables

Table S1

[Click here to Download Table S1](#)

Table S2

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Table S3. Primers for mouse transgenic assays

Peak	Coordinates (mm9)	PrimerF	PrimerR	Limb gene (location)
AER203	chr1:122510000-122512399	GTAGGAGGAGGCTTCATTGC	CTCCAGCTCGCCATACACTA	En1 (+12136)
AER2292	chr7:136794000-136796399	CCAAGAGCATATCCCATCT	CAGTGATAGACAGCCCTGGA	Fgfr2 (+615122)
AER3723	chr12:119942200-119943599	AGAGAGAGAGAGCACCTCATCAA	GACATCTGGGCTCACTTCCT	Sp8 (-141902)
AER308	chr1:170693400-170694599	GGCCGACCTGTTACTTGTT	GGGGATAGCAGGCTACTCAG	Pbx1 (-331611)
AER4460	chr16:62861400-62862199	TGCCATTTTATTGCAAGTCAG	TCAGCAGATTATTCGTAAGTGG	Arl13b (+15345)
ZPA6706	chr8:60898600-60899199	CATGGTTATTTGTCCACATTTT	GCAATGCTTTTCTTTTGTGG	Hand2 (-1098890)
ZPA12651	chr16:49332600-49333199	TTAAAAATATTGAAAAGGTGGCTA	GCAGTAGACCTTAGGGGAGCA	Ift57 (-366507)
ZPA1563	chr2:93377800-93378599	GCTCGGTACTTGCTCAGGAC	CTCTCCTCCCAAATCCCTTC	Alx4 (-104391)
ZPA2794	chr3:130665200-130665799	TACCAAGCACTTACTACAGGCTACA	ATAGGGGGCGTCTTTTATT	Lef1 (-147889)
ZPA44	chr1:19231200-19231999	CCTGTGGTCTTCTCCTTTG	CATCTGTACCAACCTCT	Tcfap2b (+29465)