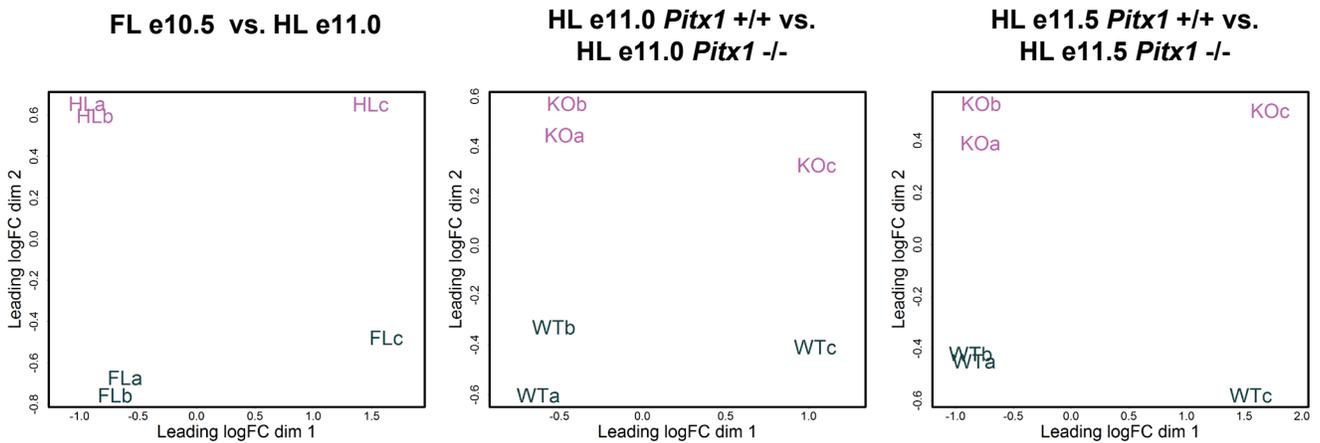


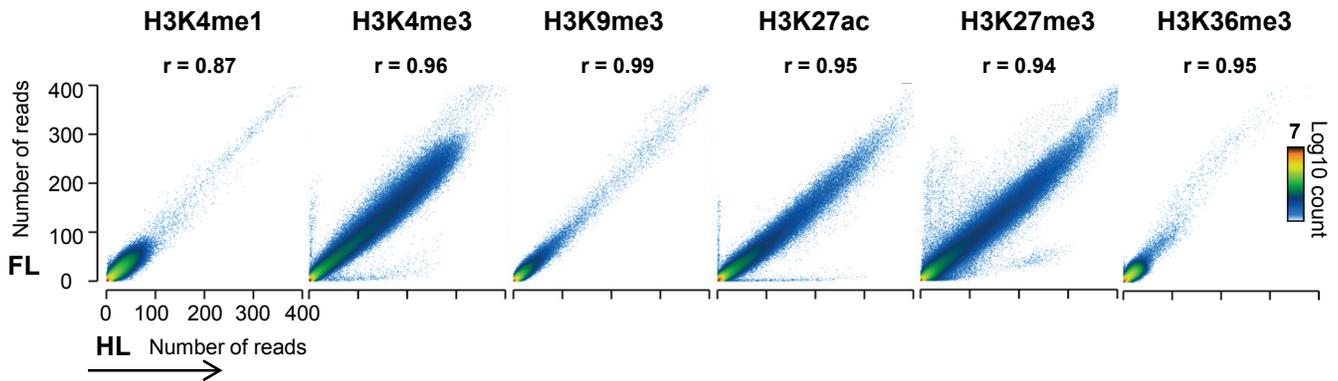
## Supplementary Figures and Tables

**Figure S1**

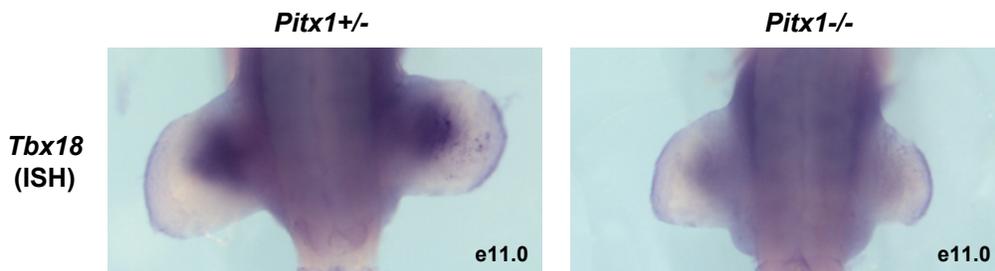
**A Multidimensional Scaling (MDS) plots of expression profiling comparisons**



**B Correlation analysis of stage-matched FL e10.5 and HL e11.0 chromatin mark ChIP-seq datasets**



**C**



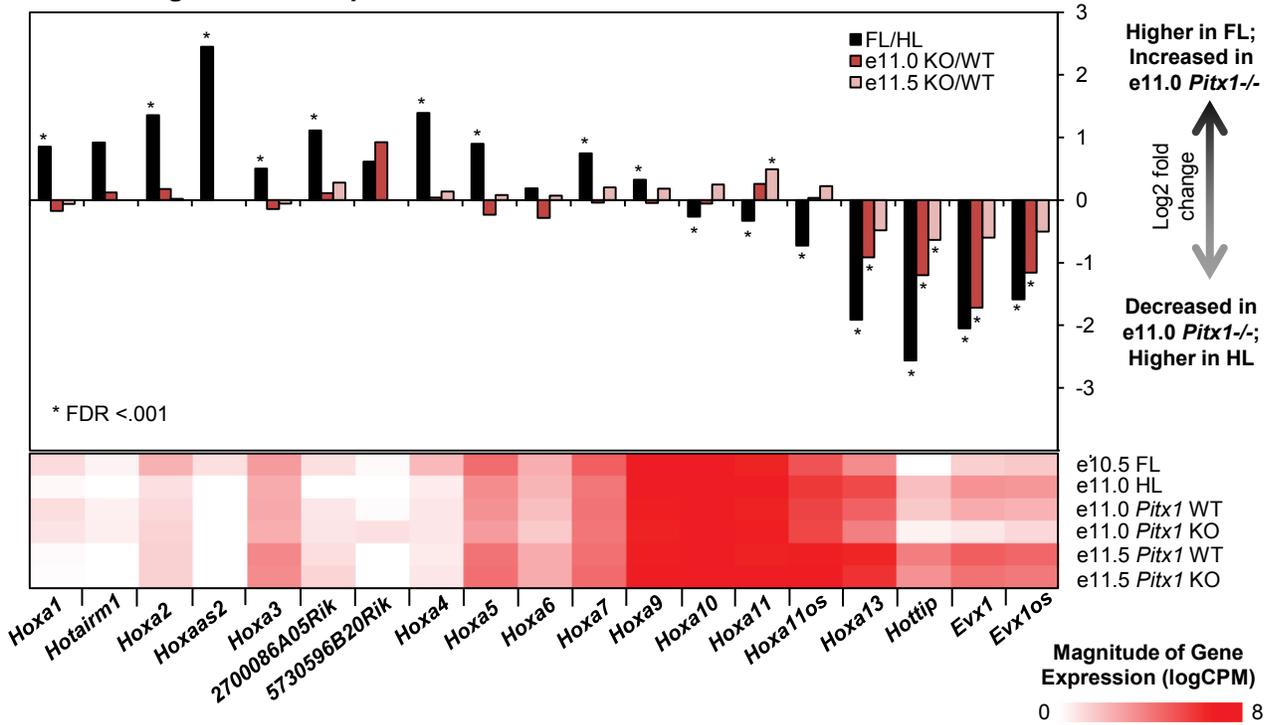
### Supplementary Figure S1

- A) Multidimensional scaling (MDS) plots of expression profiling analyses by RNA-seq.
- B) Correlation analysis of chromatin mark profiles in stage-matched e10.5 FL vs. e11.0 HL. The genome is partitioned into 100 bp bins and the read counts for each mark in each bin are plotted. The Pearson's correlation coefficients are computed using using bins that register more than 50 reads in each sample.
- C) In situ hybridization for *Tbx18* on e11.0 *Pitx1*<sup>+/-</sup> and *Pitx1*<sup>-/-</sup> HLs.

Figure S2

A

Fold-change in Gene Expression at *HoxA* locus



B

Relative enrichment of chromatin marks in FL vs. HL

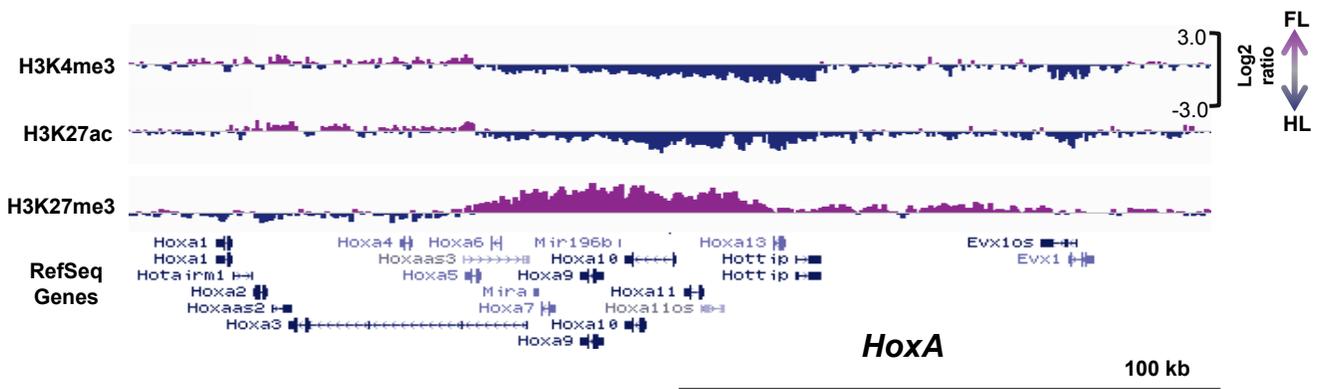
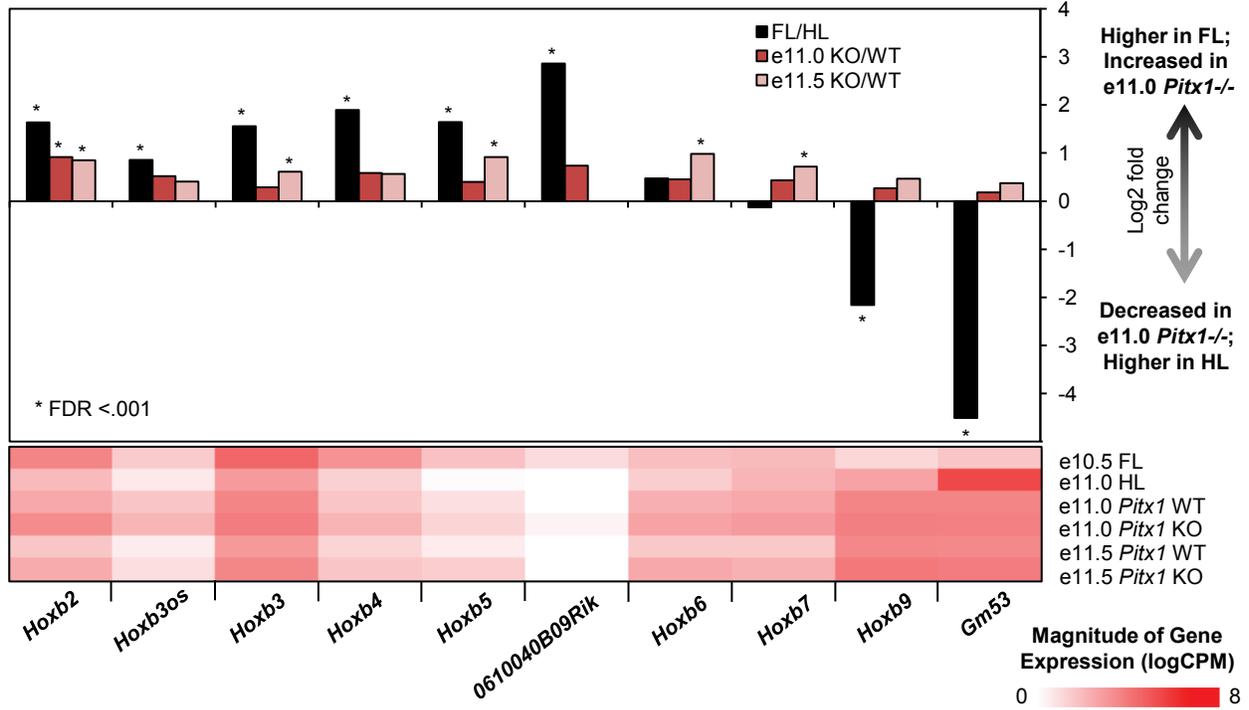




Figure S4

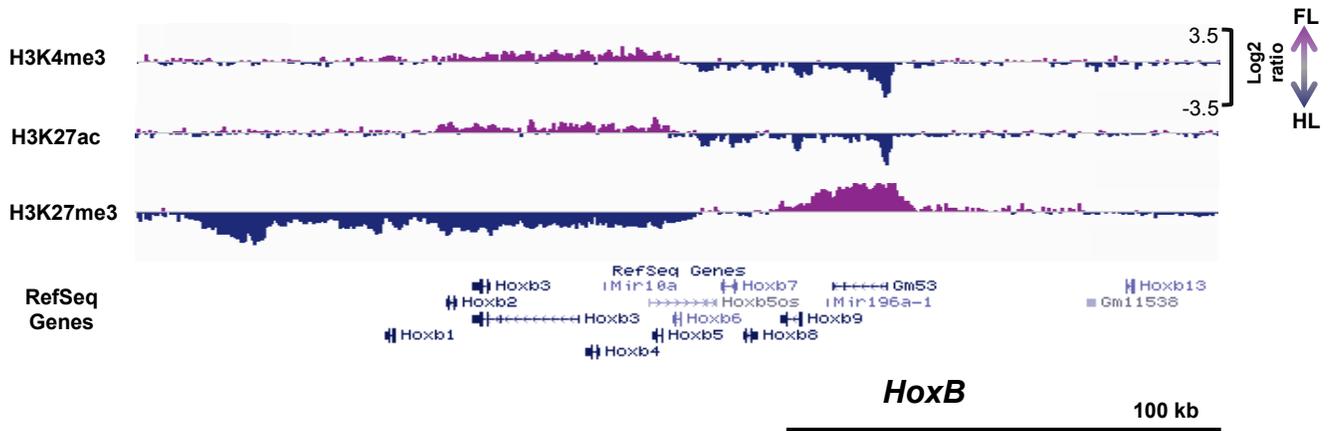
A

Fold-change in Gene Expression at *HoxB* locus



B

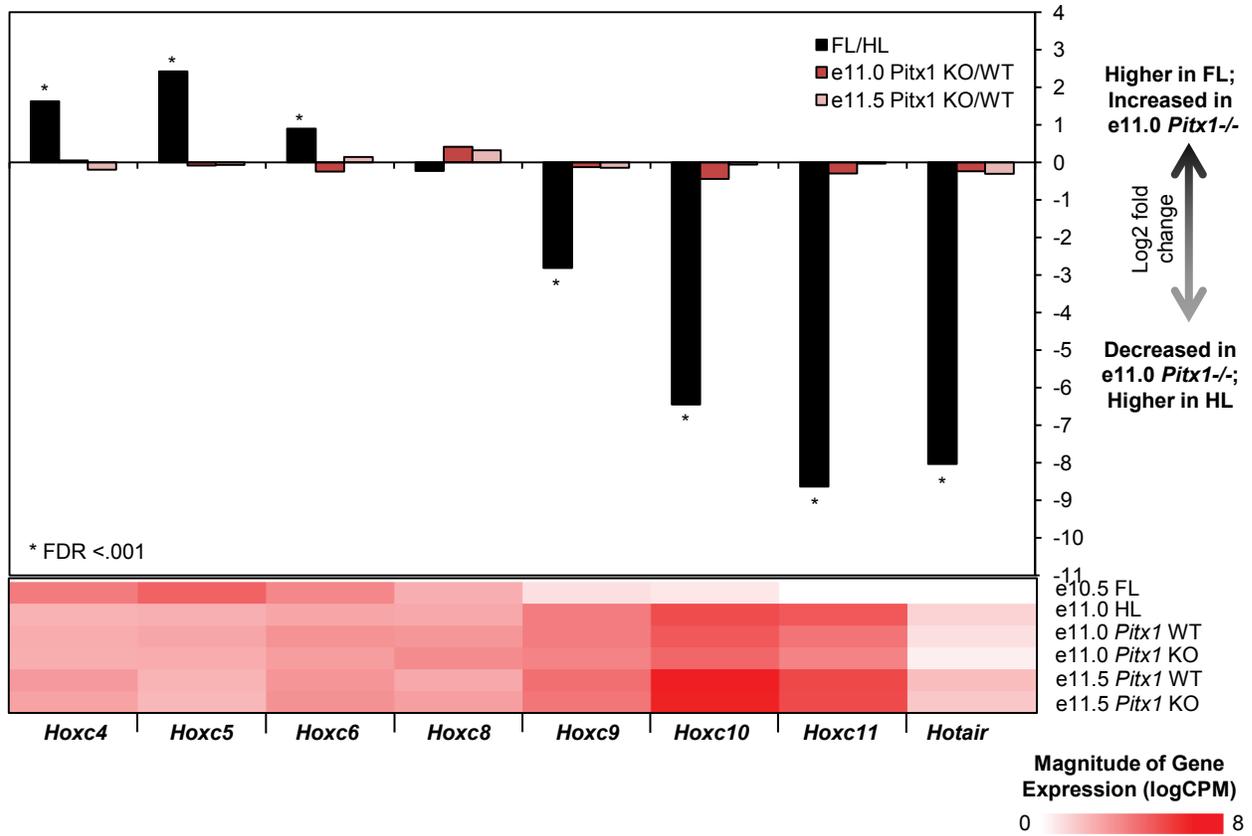
Relative enrichment of chromatin marks in FL vs. HL



**Figure S5**

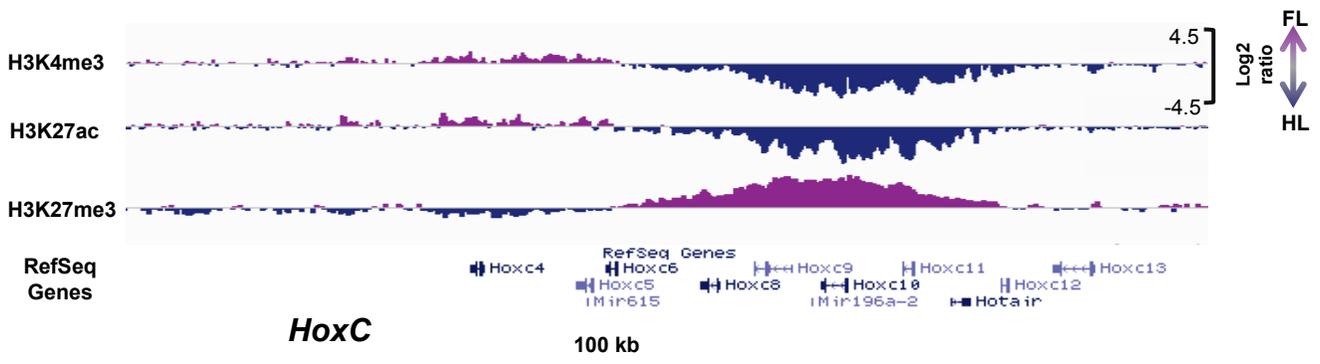
**A**

**Fold-change in Gene Expression at *HoxC* locus**



**B**

**Relative enrichment of chromatin marks in FL vs. HL**



### Supplementary Figures S2-S5

- A) Log<sub>2</sub> fold-changes in *HoxA-D* gene expression in stage-matched FL vs. HL, e11.0 *Pitx1*<sup>-/-</sup> vs. wt HL, and e11.5 *Pitx1*<sup>-/-</sup> vs. wt HL. Below the bar graph of gene expression changes is a heatmap of the magnitude of gene expression in each sample, in log<sub>2</sub> counts per million.
- B) The relative enrichment of chromatin mark profiles for H3K4me3, H3K27ac, and H3K27me3 in stage-matched FL vs. HL at the *HoxA-D* loci. These tracks are the log<sub>2</sub> ratios of HL/FL

## Supplementary Tables S1-S3

Complementary to Figure 2B: Lists of *Pitx1*-dependent genes clustered according to limb-type restricted expression.

**Table S1** HL-enriched, *Pitx1*-dependent genes at e11.0 – 46 genes

Gene ID	Gene Symbol	log2FC FL/HL	log2FC KO/WT	FDR FL/HL	FDR KO/WT
ENSMUSG00000100096	<b>Gm28760</b>	-8.91	-4.04	2.59E-43	7.42E-19
ENSMUSG00000021506	<b>Pitx1</b>	-9.22	-2.48	0.00E+00	1.09E-130
ENSMUSG00000048747	<b>E130114P18Rik</b>	-2.22	-2.22	1.36E-37	3.78E-19
ENSMUSG00000075334	<b>Rprm</b>	-0.88	-1.72	7.30E-05	8.01E-11
ENSMUSG00000086126	<b>5730457N03Rik</b>	-2.05	-1.72	4.10E-14	4.81E-17
ENSMUSG00000072720	<b>Myo18b</b>	-2.08	-1.65	1.28E-15	2.50E-07
ENSMUSG00000030111	<b>A2m</b>	-1.66	-1.28	1.68E-20	1.19E-07
ENSMUSG00000033965	<b>Slc16a2</b>	-0.75	-1.25	1.46E-16	4.01E-17
ENSMUSG00000052854	<b>Nrk</b>	-0.49	-1.23	6.56E-17	3.56E-23
ENSMUSG00000000094	<b>Tbx4</b>	-4.42	-1.21	0.00E+00	3.29E-24
ENSMUSG00000055408	<b>Gm15053</b>	-2.56	-1.20	6.98E-32	7.53E-06
ENSMUSG00000102373	<b>9530018H14Rik</b>	-1.92	-1.16	3.20E-40	2.51E-08
ENSMUSG00000005503	<b>Evx1</b>	-1.59	-1.16	2.08E-16	5.03E-04
ENSMUSG00000028519	<b>Dab1</b>	-0.66	-1.08	8.31E-04	5.79E-06
ENSMUSG000000061353	<b>Cxcl12</b>	-0.65	-1.07	2.53E-19	6.66E-23
ENSMUSG00000053137	<b>Mapk11</b>	-0.46	-1.00	5.14E-06	6.64E-07
ENSMUSG00000038203	<b>Hoxa13</b>	-1.91	-0.92	2.30E-72	2.48E-06
ENSMUSG00000025743	<b>Sdc3</b>	-0.26	-0.87	6.15E-04	2.04E-08
ENSMUSG00000052581	<b>Lrrtm4</b>	-0.67	-0.87	7.98E-05	7.09E-05
ENSMUSG00000029695	<b>Aass</b>	-0.72	-0.86	2.15E-16	2.09E-09
ENSMUSG00000057777	<b>Mab21l2</b>	-0.47	-0.83	4.66E-17	3.89E-10
ENSMUSG00000061524	<b>Zic2</b>	-0.43	-0.81	3.16E-08	1.12E-10
ENSMUSG00000033618	<b>Map3k13</b>	-0.52	-0.79	6.54E-04	1.63E-04
ENSMUSG00000031654	<b>Cbln1</b>	-0.52	-0.77	4.67E-06	2.83E-04
ENSMUSG00000058145	<b>Adamts17</b>	-0.76	-0.76	3.44E-21	3.94E-07
ENSMUSG00000110874	<b>NA</b>	-1.03	-0.75	3.66E-09	4.65E-05
ENSMUSG00000051177	<b>Plcb1</b>	-0.61	-0.72	4.07E-13	3.60E-07
ENSMUSG00000021253	<b>Tgfb3</b>	-0.53	-0.67	3.81E-11	2.37E-06
ENSMUSG00000021136	<b>Smoc1</b>	-0.40	-0.66	2.87E-09	8.96E-05
ENSMUSG00000051515	<b>Fam181b</b>	-0.64	-0.59	6.57E-16	1.42E-05
ENSMUSG00000004040	<b>Stat3</b>	-0.28	-0.58	2.36E-04	7.52E-05
ENSMUSG00000030351	<b>Tspan11</b>	-0.69	-0.58	1.93E-06	7.69E-04
ENSMUSG00000016763	<b>Scube1</b>	-0.27	-0.57	1.24E-04	5.03E-04
ENSMUSG00000021097	<b>Cimn</b>	-0.34	-0.55	1.49E-03	7.81E-04
ENSMUSG00000036523	<b>Greb1</b>	-0.40	-0.54	7.66E-09	2.56E-04
ENSMUSG00000029622	<b>Arpc1b</b>	-0.57	-0.52	1.20E-14	7.46E-07
ENSMUSG00000023079	<b>Gtf2ird1</b>	-0.46	-0.50	4.43E-14	2.38E-06
ENSMUSG00000020044	<b>Timp3</b>	-0.35	-0.45	1.24E-08	2.60E-04
ENSMUSG00000057315	<b>Arhgap24</b>	-0.44	-0.44	4.83E-07	3.05E-04
ENSMUSG00000038193	<b>Hand2</b>	-0.43	-0.44	1.24E-13	2.47E-05
ENSMUSG00000004631	<b>Sgce</b>	-0.41	-0.43	1.10E-07	2.66E-04
ENSMUSG00000029790	<b>Cep41</b>	-0.35	-0.41	2.01E-08	4.03E-05
ENSMUSG00000056947	<b>Mab21l1</b>	-0.53	-0.40	9.42E-14	4.21E-04
ENSMUSG00000030352	<b>Tspan9</b>	-0.24	-0.40	8.79E-04	7.09E-04
ENSMUSG00000027962	<b>Vcam1</b>	-0.53	-0.38	3.96E-20	2.14E-04
ENSMUSG00000054675	<b>Tmem119</b>	-0.32	-0.34	4.06E-06	2.09E-04

Table S2

**A** FL-enriched genes with increased expression in *Pitx1*<sup>-/-</sup> HL – 24 genes

Gene ID	Gene Symbol	log2FC FL/HL	log2FC KO/WT	FDR FL/HL	FDR KO/WT
ENSMUSG00000028023	<b>Pitx2</b>	1.36	1.59	8.42E-26	2.39E-16
ENSMUSG00000053117	<b>E330013P04Rik</b>	0.85	1.24	4.73E-05	8.34E-13
ENSMUSG00000020297	<b>Nsg2</b>	0.89	1.01	2.08E-04	5.07E-06
ENSMUSG00000052504	<b>Epha3</b>	1.52	0.96	1.07E-74	3.86E-07
ENSMUSG00000075588	<b>Hoxb2</b>	1.64	0.92	1.58E-42	4.18E-10
ENSMUSG00000019894	<b>Slc6a15</b>	1.12	0.83	3.94E-07	9.59E-04
ENSMUSG00000046318	<b>Ccbe1</b>	0.72	0.81	4.44E-03	1.58E-04
ENSMUSG00000043969	<b>Emx2</b>	1.03	0.78	2.93E-19	1.95E-12
ENSMUSG00000022309	<b>Angpt1</b>	0.36	0.78	5.10E-03	1.02E-12
ENSMUSG00000033981	<b>Gria2</b>	2.52	0.67	1.70E-68	2.30E-05
ENSMUSG00000041920	<b>Slc16a6</b>	0.69	0.66	2.01E-05	2.39E-04
ENSMUSG00000025592	<b>Dach2</b>	0.41	0.64	7.19E-03	6.73E-04
ENSMUSG00000025856	<b>Pdgfa</b>	0.27	0.58	5.61E-03	1.35E-10
ENSMUSG00000024087	<b>Cyp1b1</b>	1.01	0.56	7.34E-09	7.84E-05
ENSMUSG00000067931	<b>Zfp948</b>	0.35	0.54	3.49E-03	4.22E-07
ENSMUSG00000073988	<b>Ttpa</b>	0.45	0.50	2.95E-05	1.36E-05
ENSMUSG00000025921	<b>Rdh10</b>	0.36	0.49	2.01E-05	3.81E-05
ENSMUSG00000040464	<b>Gtpbp10</b>	0.22	0.49	9.54E-03	2.36E-06
ENSMUSG00000031558	<b>Slit2</b>	0.56	0.47	5.06E-19	8.80E-05
ENSMUSG00000026739	<b>Bmi1</b>	0.28	0.46	5.14E-03	2.44E-07
ENSMUSG00000045103	<b>Dmd</b>	0.65	0.44	4.64E-18	1.42E-04
ENSMUSG00000031299	<b>Pdha1</b>	0.28	0.43	3.04E-05	1.84E-05
ENSMUSG00000074519	<b>Etohi1</b>	0.43	0.42	2.54E-05	8.75E-04
ENSMUSG00000037720	<b>Tmem33</b>	0.27	0.37	1.90E-03	8.55E-05

**B** HL-enriched genes with increased expression in *Pitx1*<sup>-/-</sup> HL – 11 genes

Gene ID	Gene Symbol	log2FC FL/HL	log2FC KO/WT	FDR FL/HL	FDR KO/WT
ENSMUSG00000034009	<b>Rxfp1</b>	-7.66	1.13	3.90E-93	7.09E-26
ENSMUSG00000031636	<b>Pdlim3</b>	-1.87	1.02	6.66E-16	1.51E-12
ENSMUSG00000065232	<b>Gm22973</b>	-0.34	0.91	5.52E-03	6.31E-15
ENSMUSG00000048138	<b>Dmrt2</b>	-0.87	0.90	4.78E-07	2.15E-11
ENSMUSG00000073940	<b>Hbb-bt</b>	-1.57	0.85	3.37E-16	2.03E-04
ENSMUSG00000052305	<b>Hbb-bs</b>	-1.37	0.84	4.24E-16	5.19E-08
ENSMUSG00000032033	<b>Barx2</b>	-0.73	0.64	2.56E-04	1.32E-05
ENSMUSG00000019987	<b>Arg1</b>	-0.49	0.61	2.25E-04	1.39E-06
ENSMUSG00000058258	<b>Idi1</b>	-0.36	0.60	9.23E-03	6.73E-08
ENSMUSG00000042258	<b>Isi1</b>	-3.55	0.56	2.09E-139	4.72E-05
ENSMUSG00000021508	<b>Cxcl14</b>	-0.48	0.40	1.01E-10	8.54E-04

**Table S3** FL-enriched, *Pitx1*-dependent genes at e11.0 – 39 genes

Gene ID	Gene Symbol	log2FC FL/HL	log2FC KO/WT	FDR FL/HL	FDR KO/WT
ENSMUSG00000047793	<b>Sned1</b>	0.75	-1.67	2.20E-12	3.31E-32
ENSMUSG00000039153	<b>Runx2</b>	2.36	-1.65	2.72E-49	2.53E-05
ENSMUSG00000053141	<b>Ptprr1</b>	0.64	-1.60	5.89E-04	8.47E-07
ENSMUSG00000062713	<b>Sim2</b>	1.62	-1.41	6.27E-58	4.38E-05
ENSMUSG00000042607	<b>Asb4</b>	0.40	-1.26	5.21E-06	6.04E-38
ENSMUSG00000004872	<b>Pax3</b>	0.65	-1.25	1.58E-14	3.17E-12
ENSMUSG00000049538	<b>Adamts16</b>	0.72	-1.19	4.44E-06	4.01E-09
ENSMUSG00000025216	<b>Lbx1</b>	0.60	-1.17	1.13E-10	5.08E-05
ENSMUSG00000031285	<b>Dcx</b>	0.44	-1.16	3.30E-06	1.46E-25
ENSMUSG00000047759	<b>Hs3st3a1</b>	0.97	-0.95	2.58E-21	9.71E-05
ENSMUSG00000020218	<b>Wif1</b>	0.51	-0.94	1.23E-06	8.08E-09
ENSMUSG00000032852	<b>Rspo4</b>	1.99	-0.90	5.00E-96	4.98E-05
ENSMUSG00000019230	<b>Lhx9</b>	0.40	-0.80	2.01E-08	1.77E-25
ENSMUSG00000025584	<b>Pde8a</b>	0.68	-0.79	2.32E-06	1.20E-04
ENSMUSG00000030029	<b>Lrig1</b>	0.35	-0.75	3.05E-04	2.32E-08
ENSMUSG00000052951	<b>C130021120Rik</b>	0.22	-0.74	2.08E-03	7.61E-11
ENSMUSG00000038765	<b>Lmx1b</b>	0.24	-0.72	2.07E-03	3.97E-07
ENSMUSG00000038119	<b>Cdon</b>	0.52	-0.71	9.67E-12	2.49E-12
ENSMUSG00000067276	<b>Capn6</b>	0.31	-0.69	2.92E-04	5.87E-11
ENSMUSG00000037138	<b>Aff3</b>	0.40	-0.67	4.70E-09	1.55E-08
ENSMUSG00000042961	<b>Egflam</b>	0.39	-0.66	2.66E-05	1.07E-04
ENSMUSG00000021318	<b>Gli3</b>	0.46	-0.64	1.35E-12	1.32E-06
ENSMUSG00000032035	<b>Ets1</b>	0.19	-0.64	9.22E-03	4.43E-07
ENSMUSG00000070407	<b>Hs3st3b1</b>	0.80	-0.63	7.78E-36	7.14E-07
ENSMUSG00000032332	<b>Col12a1</b>	0.28	-0.55	1.88E-04	2.25E-07
ENSMUSG00000026147	<b>Col9a1</b>	0.48	-0.54	2.01E-11	1.29E-06
ENSMUSG00000096054	<b>Syne1</b>	0.32	-0.54	5.08E-03	5.95E-07
ENSMUSG00000052331	<b>Ankrd44</b>	0.52	-0.50	1.07E-17	1.65E-04
ENSMUSG00000042594	<b>Sh2b3</b>	0.28	-0.49	1.05E-03	8.17E-04
ENSMUSG00000054263	<b>Lifr</b>	0.38	-0.49	7.94E-08	1.59E-04
ENSMUSG00000027833	<b>Shox2</b>	0.21	-0.49	4.35E-03	2.58E-06
ENSMUSG00000022231	<b>Sema5a</b>	0.33	-0.48	5.32E-08	8.49E-04
ENSMUSG00000001300	<b>Efnb2</b>	0.33	-0.47	2.88E-06	7.31E-05
ENSMUSG00000032846	<b>Zswim6</b>	0.22	-0.46	7.34E-03	2.22E-05
ENSMUSG00000063564	<b>Col23a1</b>	0.27	-0.42	1.18E-05	7.46E-05
ENSMUSG00000029231	<b>Pdgfra</b>	0.30	-0.42	7.85E-04	1.82E-04
ENSMUSG00000035245	<b>Eogt</b>	0.25	-0.40	8.21E-04	4.97E-04
ENSMUSG00000047786	<b>Lix1</b>	0.48	-0.39	1.77E-11	1.78E-04
ENSMUSG00000037605	<b>Lphn3</b>	0.43	-0.38	4.55E-10	8.64E-04