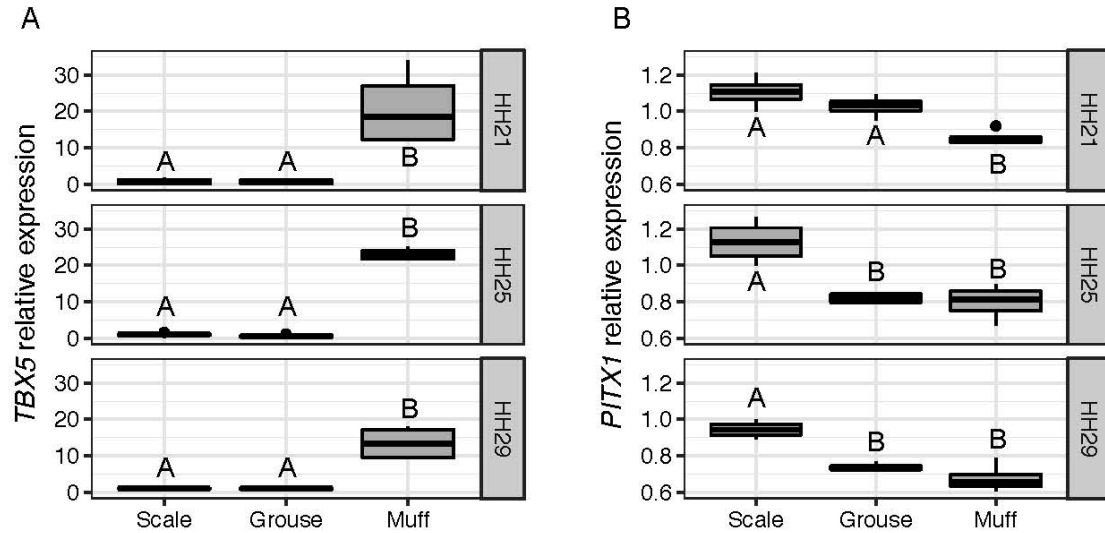
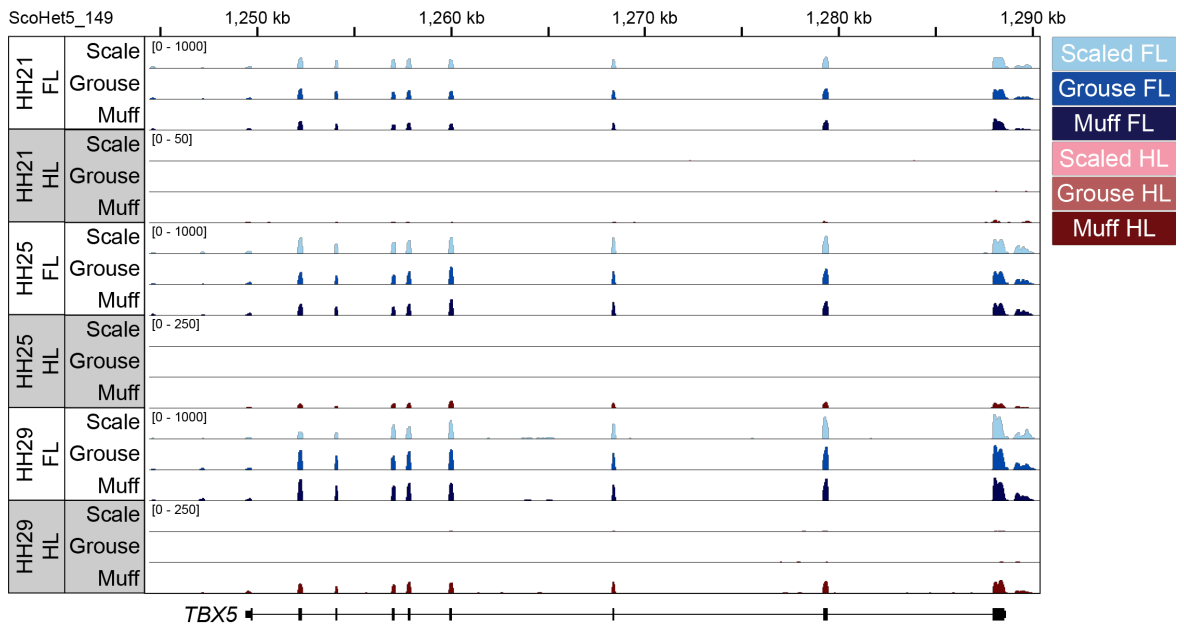


**Supplemental Figure 1. Differentially expressed genes between pigeon FL and scaled HL buds.** (A) Gene-wise hierarchical clustering heat map showing 978 genes differentially expressed between pigeon FL and scaled HL buds at HH21, HH25, and/or HH29. All genes that are significantly differentially expressed at one or more developmental stage are included in heat map. Z score scale represents mean subtracted regularized log transformed read counts. (B) Table summarizing number of significantly differentially expressed genes between FL and scaled HL at HH21, HH25, HH29, or at all stages combined.



**Supplemental Figure 2. Temporal dynamics of *TBX5* and *PITX1* expression in pigeon limb buds revealed by qRT-PCR.** (A-B) qRT-PCR analyses of *TBX5* (A) and *PITX1* (B) expression relative to *ACTB* in HH21, HH25, and HH29 pigeon hindlimb buds. Boxes span 1<sup>st</sup> to 3<sup>rd</sup> quartiles, bars extend to minimum and maximum values, black line denotes median, dot indicates outlier. For scale n=4 sets of hindlimb buds, grouse n=4, muff n=10. Letter above box (A or B) denotes significance group.

A

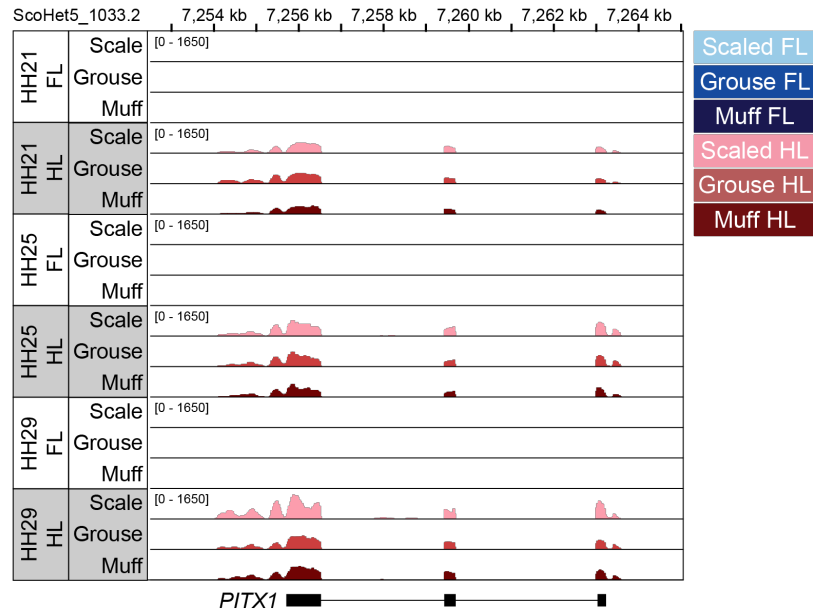


B

	HH21		HH25		HH29	
	Log2FC	padj	Log2FC	padj	Log2FC	padj
FL vs Scale HL	5.59	0.00E+00	5.75	0.00E+00	4.59	3.56E-197
Scale HL vs Grouse HL	-0.13	6.30E-01	-0.08	8.30E-01	-0.95	9.50E-01
Scale HL vs Muff HL	0.87	7.87E-06	2.55	8.13E-110	2.40	2.56E-51
Grouse HL vs Muff HL	1.00	1.95E-07	2.63	7.94E-126	2.45	1.98E-53

**Supplemental Figure 3. Differential *TBX5* expression in pigeon limb bud samples. (A)** Representative RNA-seq tracks for *TBX5* in FL and HL bud samples from scaled, grouse, and muff embryos at HH21, HH25, and HH29. Scale is 0-1000 reads for FL tracks and 0-250 reads for HL tracks. **(B)** Table showing *TBX5* log<sub>2</sub> fold change (Log<sub>2</sub>FC) and corresponding adjusted p-value (padj) for each comparison included in differential expression analyses.

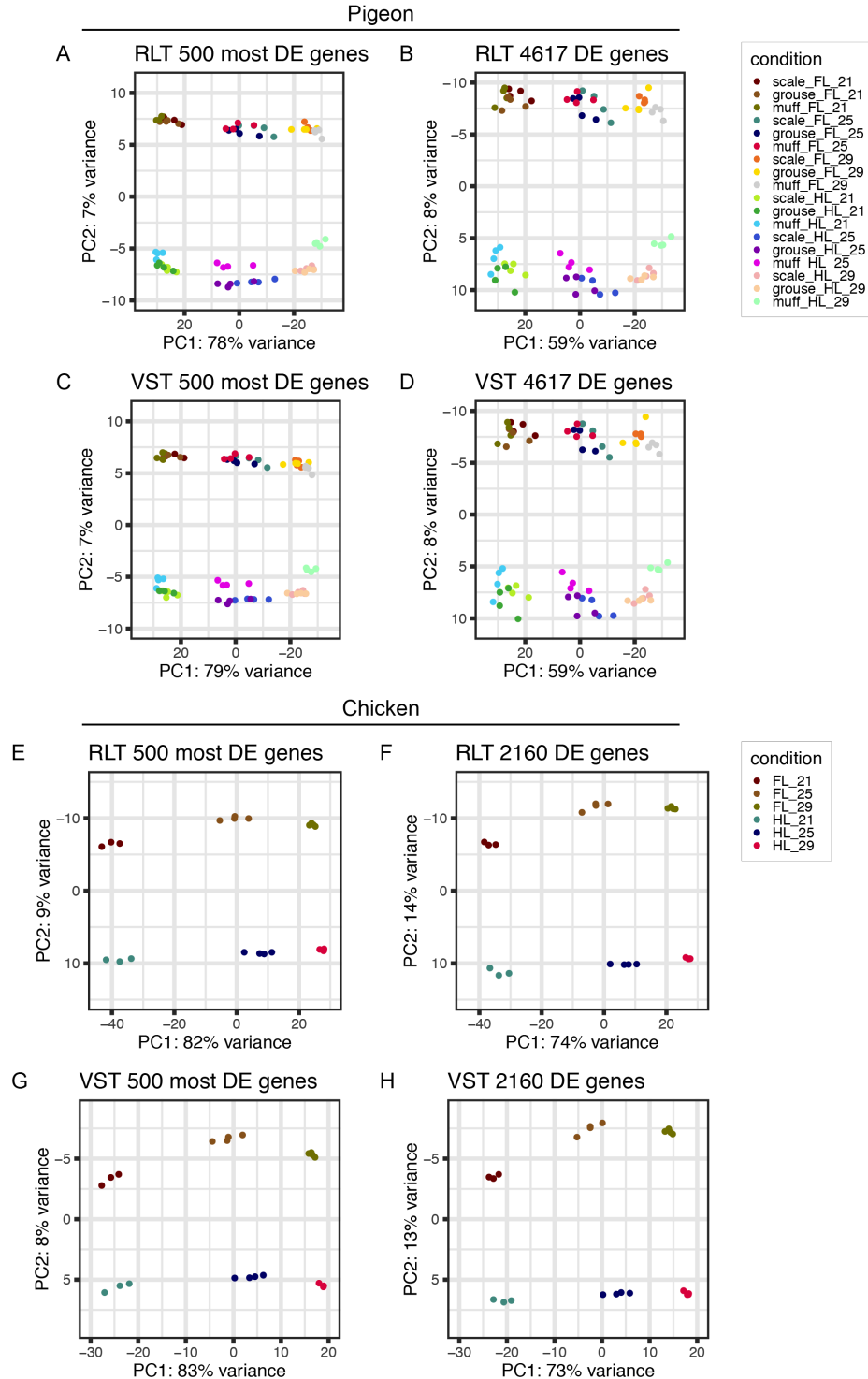
A



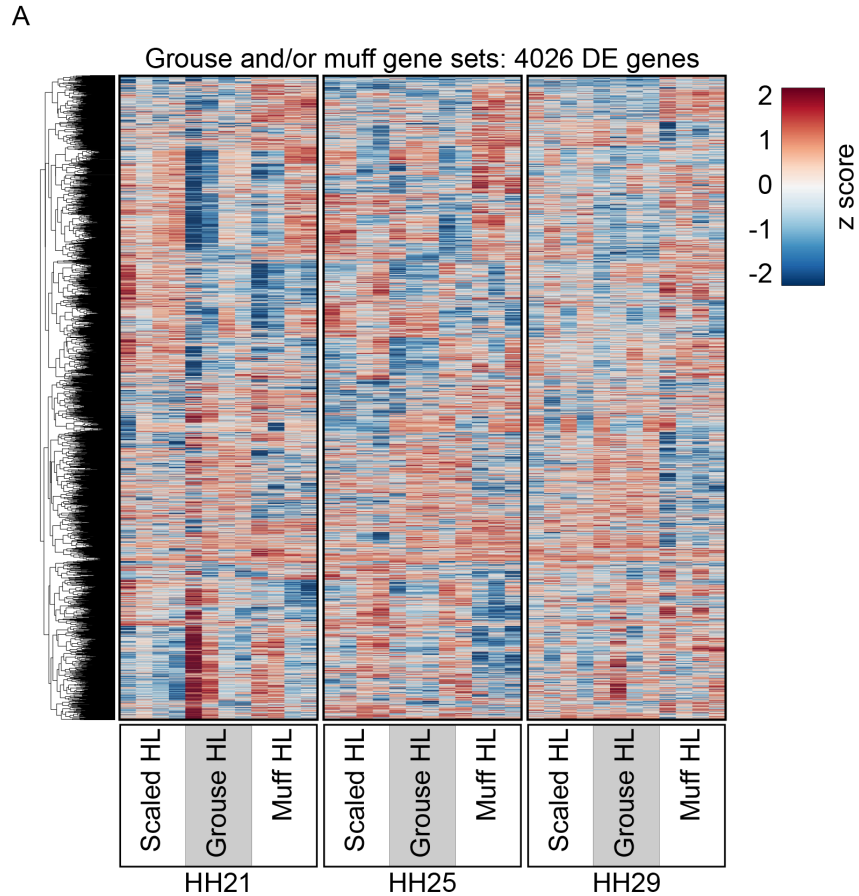
B

	HH21		HH25		HH29	
	Log2FC	padj	Log2FC	padj	Log2FC	padj
FL vs Scale HL	-5.58	0.00E+00	-5.02	0.00E+00	-4.83	0.00E+00
Scale HL vs Grouse HL	-0.06	7.30E-01	-0.29	3.00E-02	-0.30	9.00E-02
Scale HL vs Muff HL	-0.45	3.20E-04	-0.46	1.38E-05	-0.32	2.00E-02
Grouse HL vs Muff HL	-0.40	5.30E-03	-0.18	1.30E-01	0.10	9.40E-01

**Supplemental Figure 4. Differential *PITX1* expression in pigeon limb bud samples. (A)** Representative RNA-seq tracks for *PITX1* in FL and HL bud samples from scaled, grouse, and muff embryos at HH21, HH25, and HH29. Scale is 0-1650 reads for all tracks. **(B)** Table showing *PITX1* log2 fold change (Log2FC) and corresponding adjusted p-value (padj) for each comparison included in differential expression analyses.



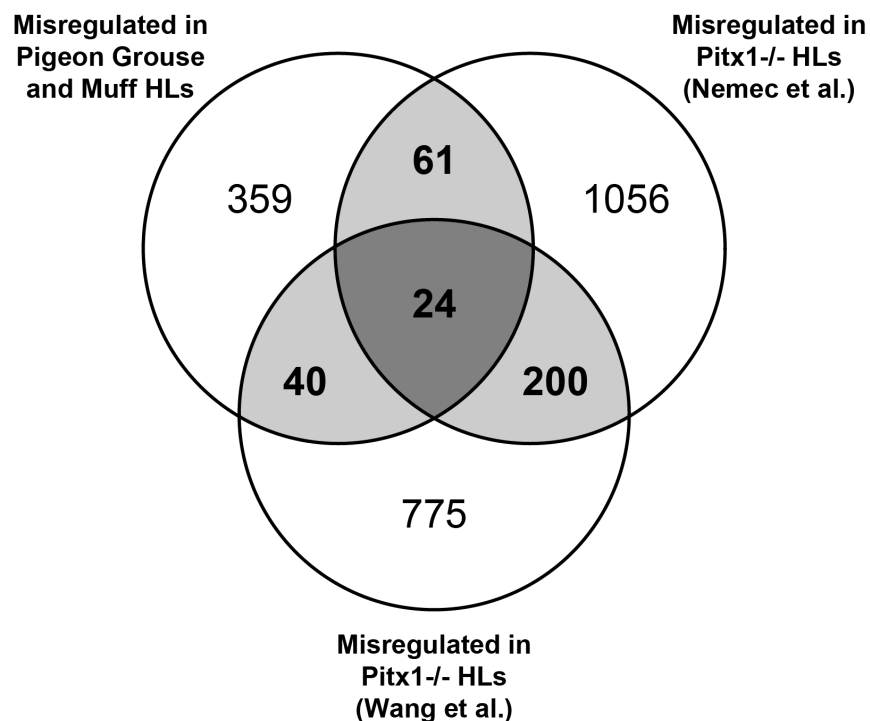
**Supplemental Figure 5. Principal component analyses (PCA) of pigeon and chicken RNA-seq datasets.** Regularized log transformations (RLT: A,B,E,F) or variance stabilized transformations (VST: C,D,G,H) were applied to entire pigeon (A-D) or chicken (E-H) datasets in DESeq2. PCA clustering was performed based on top 500 most differentially expressed (DE) genes (A,C,E,G) or all significantly DE genes (B,D,F,H).



B

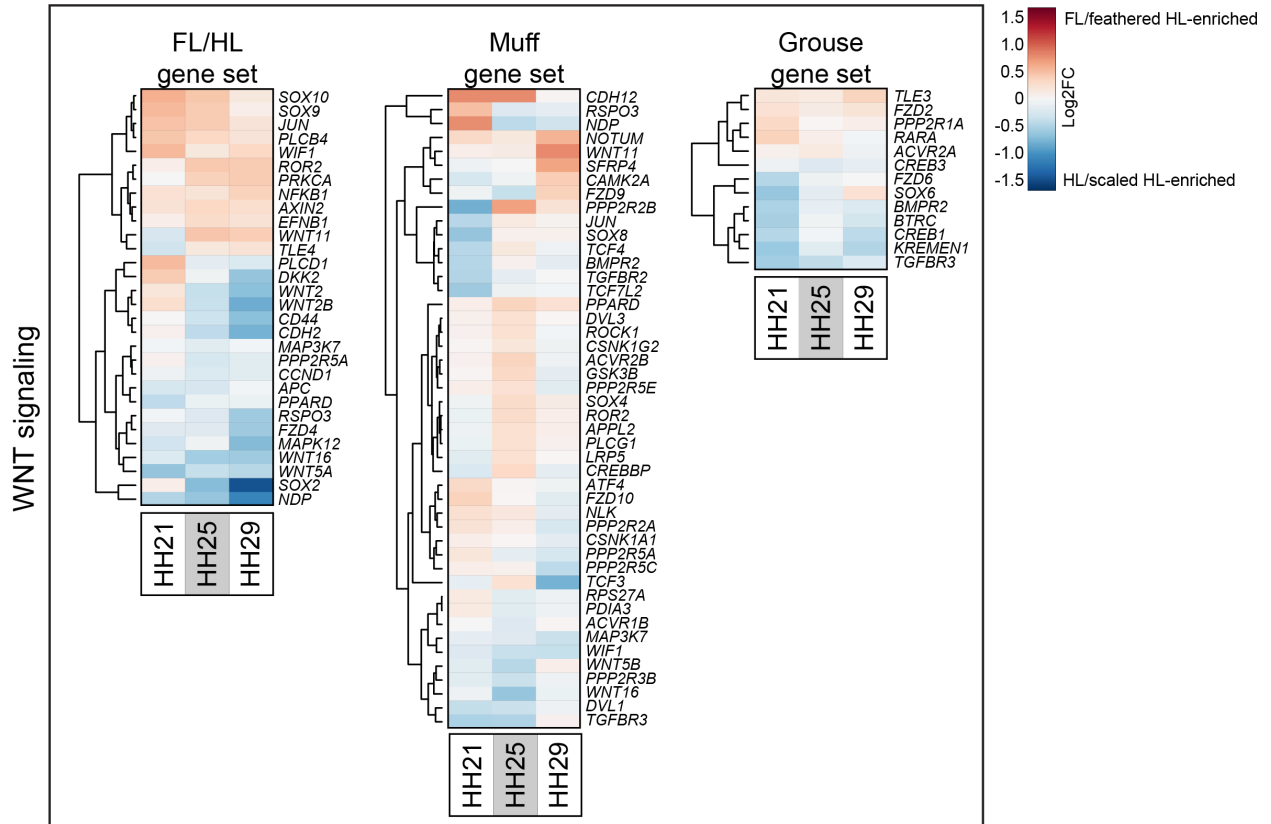
	Grouse gene set	Muff gene set	Grouse and/or muff gene sets
HH21	941	1147	1838
HH25	479	1585	1858
HH29	227	879	1055
All stages	1425	3202	4026

**Supplemental Figure 6. Differentially expressed genes between scaled and feathered pigeon HLs.** (A) Gene-wise hierarchical clustered heat map showing 4026 genes differentially expressed between HL buds from scaled, grouse, and muff embryos at HH21, HH25, and/or HH29. All genes that are significantly differentially expressed in at least one comparison at one or more developmental stage are included in heat map. Z score scale represents mean subtracted regularized log transformed read counts. (B) Table summarizing number of significantly differentially expressed genes between scaled, grouse, and muff HL buds at HH21, HH25, HH29, or at all stages combined.



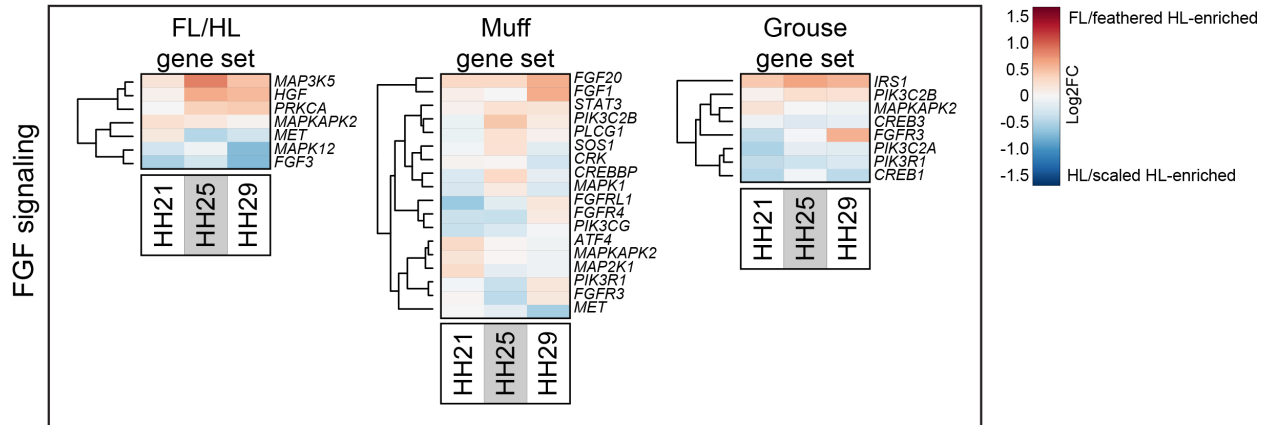
Comparison	# of overlapping genes	Intersection p-value	Odds ratio
Pigeon Grouse and Muff vs. Mouse Pitx1 <sup>-/-</sup> HL (Nemec et al.)	85	1.5e-04	1.6
Pigeon Grouse and Muff vs. Mouse Pitx1 <sup>-/-</sup> HL (Wang et al.)	64	2.2e-03	1.5
Pigeon Grouse and Muff vs. Mouse Pitx1 <sup>-/-</sup> HL (Nemec et al. and Wang et al.)	24	3.7e-05	2.7
Mouse Pitx1 <sup>-/-</sup> HL (Nemec et al.) vs. Mouse Pitx1 <sup>-/-</sup> HL (Wang et al.)	224	2.7e-68	5.0

**Supplemental Figure 7. Comparison of *PITX1*-dependent genes in pigeon and mouse limb buds.** Venn diagram showing overlap of differentially expressed gene sets from pigeon scaled HL vs. grouse HL and pigeon scaled HL vs. muff HL with two published RNA-seq datasets of mouse wild-type vs. *Pitx1*<sup>-/-</sup> HLs (Nemec et al., 2017, Wang et al., 2018). For each gene set overlap, intersection p-value and Odds ratio is presented in table.

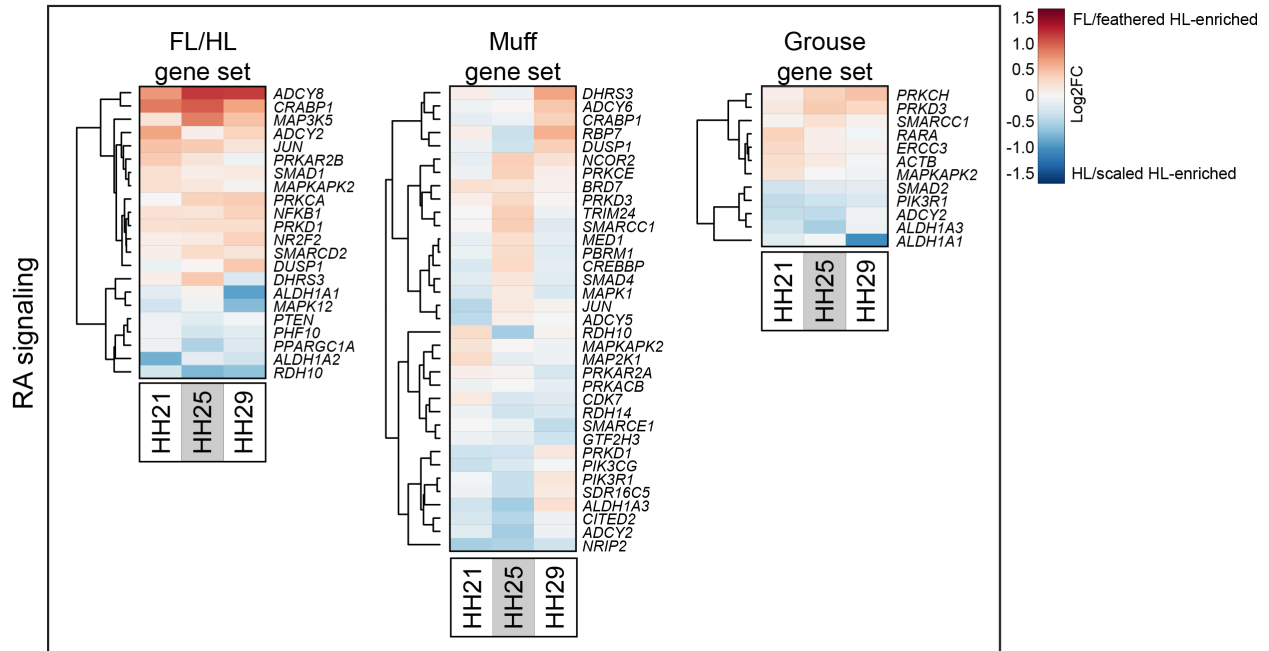


**Supplemental Figure 8. Differentially expressed genes associated with WNT signaling pathway.** Gene-wise hierarchical clustering heat maps of differentially expressed genes associated with WNT signaling in FL/HL, muff, and grouse gene sets. Genes were identified by Ingenuity Pathway Analysis. Scale is Log2FC -1.5 to 1.5.

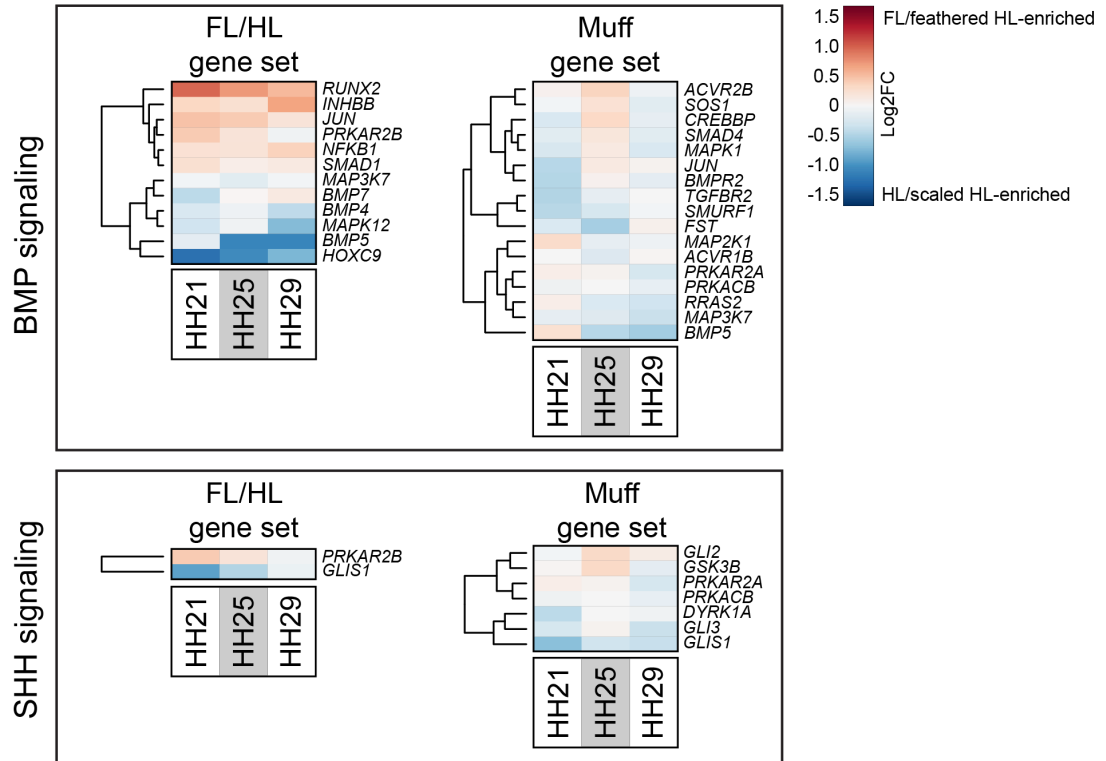




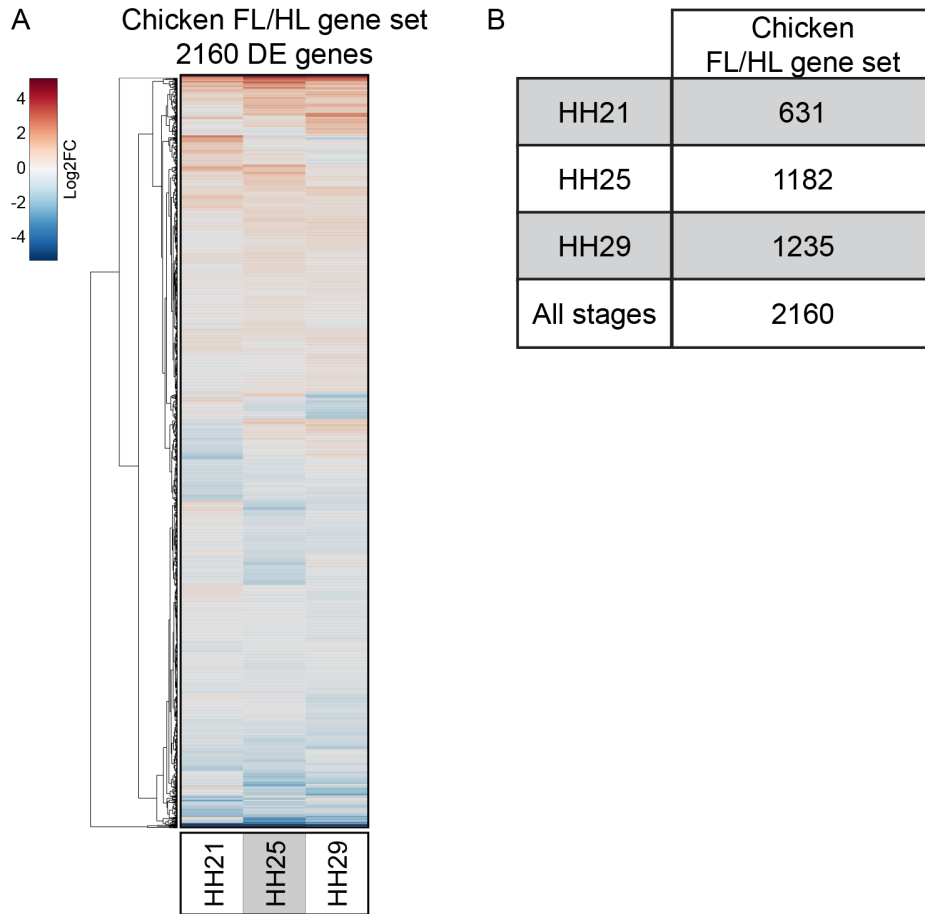
**Supplemental Figure 9. Differentially expressed genes associated with FGF signaling pathway.** Gene-wise hierarchical clustering heat maps of differentially expressed genes associated with FGF signaling in FL/HL, muff, and grouse gene sets. Genes were identified by Ingenuity Pathway Analysis. Scale is Log2FC -1.5 to 1.5.



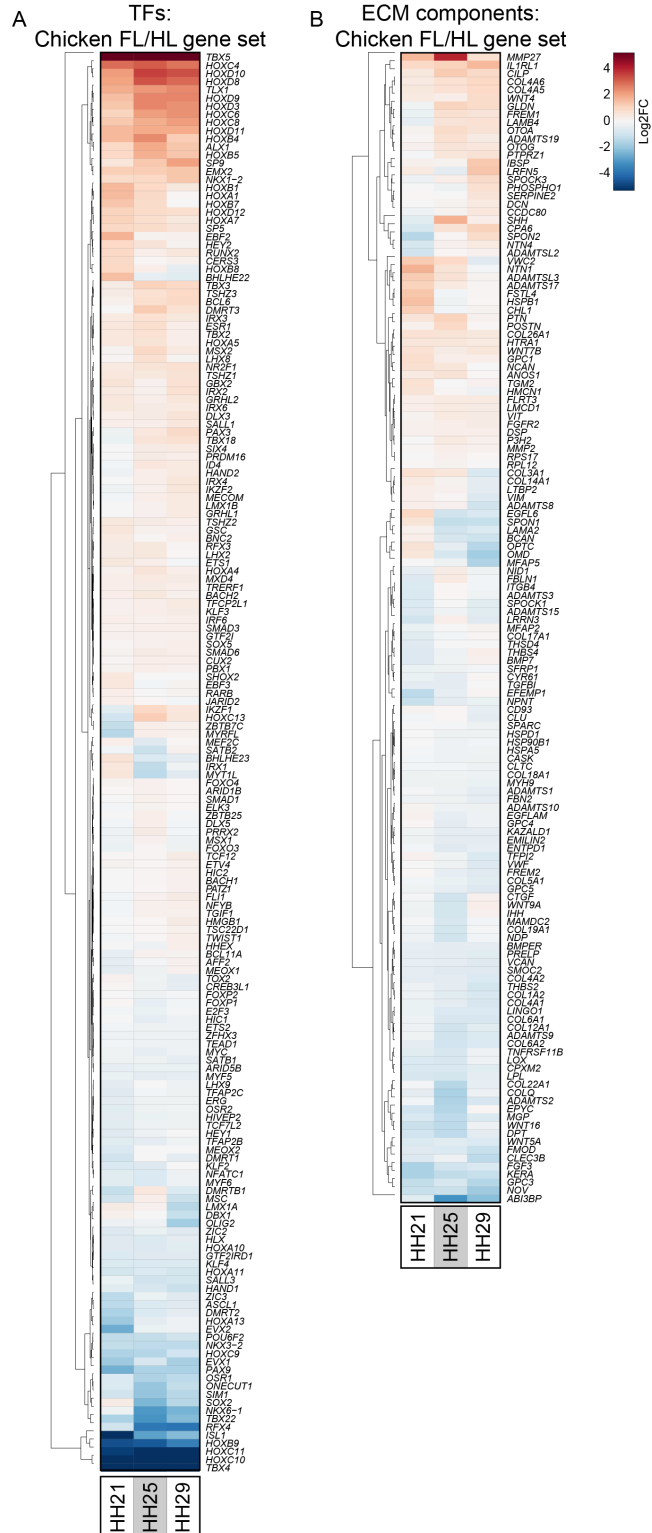
**Supplemental Figure 10. Differentially expressed genes associated with Retinoic Acid (RA) signaling pathway.** Gene-wise hierarchical clustering heat maps of differentially expressed genes associated with RA signaling in FL/HL, muff, and grouse gene sets. Genes were identified by Ingenuity Pathway Analysis. Scale is Log2FC -1.5 to 1.5.



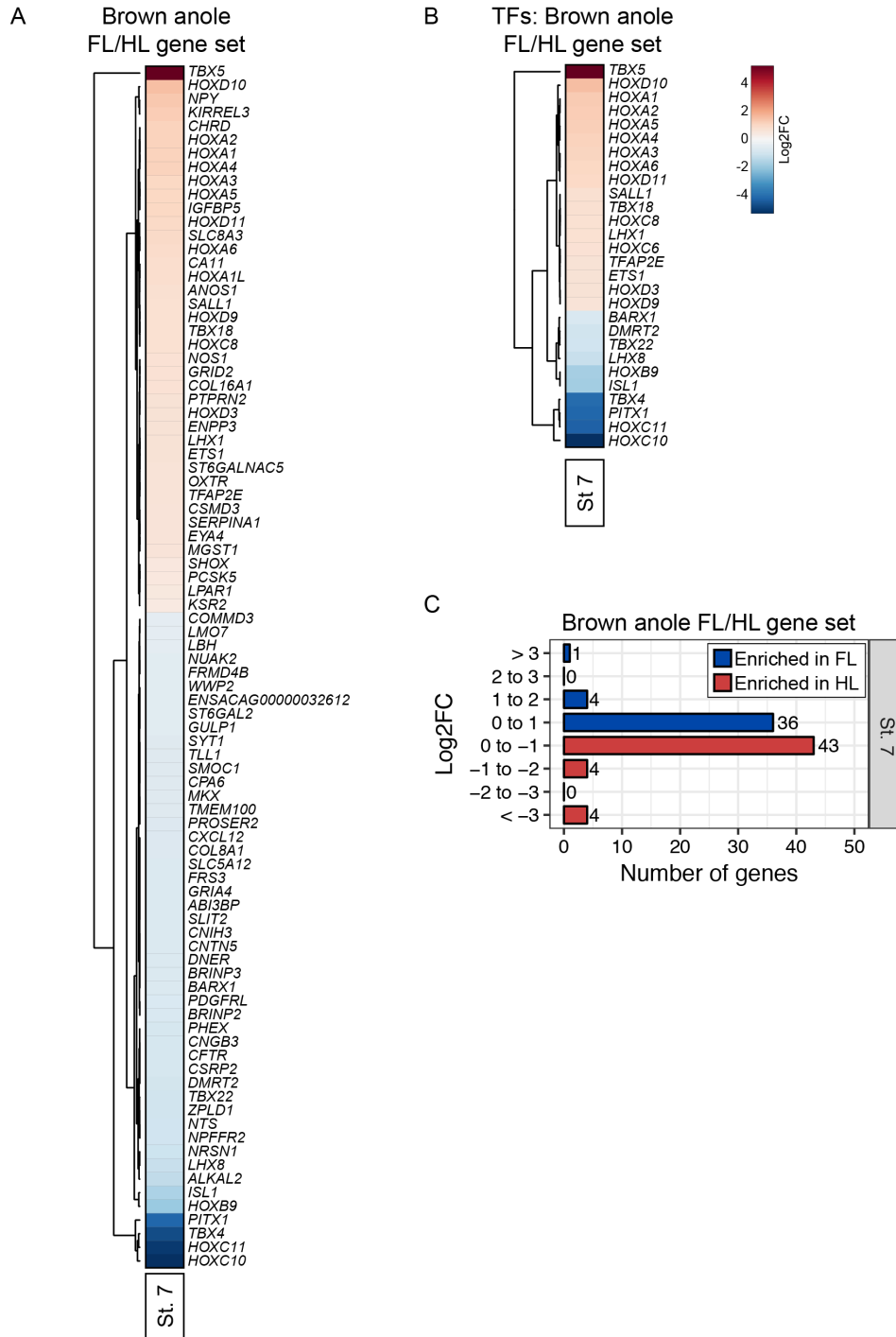
**Supplemental Figure 11. Differentially expressed genes associated with BMP or SHH signaling pathways.** Gene-wise hierarchical clustering heat maps of differentially expressed genes associated with BMP or SHH signaling in FL/HL and muff gene sets. Genes were identified by Ingenuity Pathway Analysis. Scale is Log<sub>2</sub>FC -1.5 to 1.5.



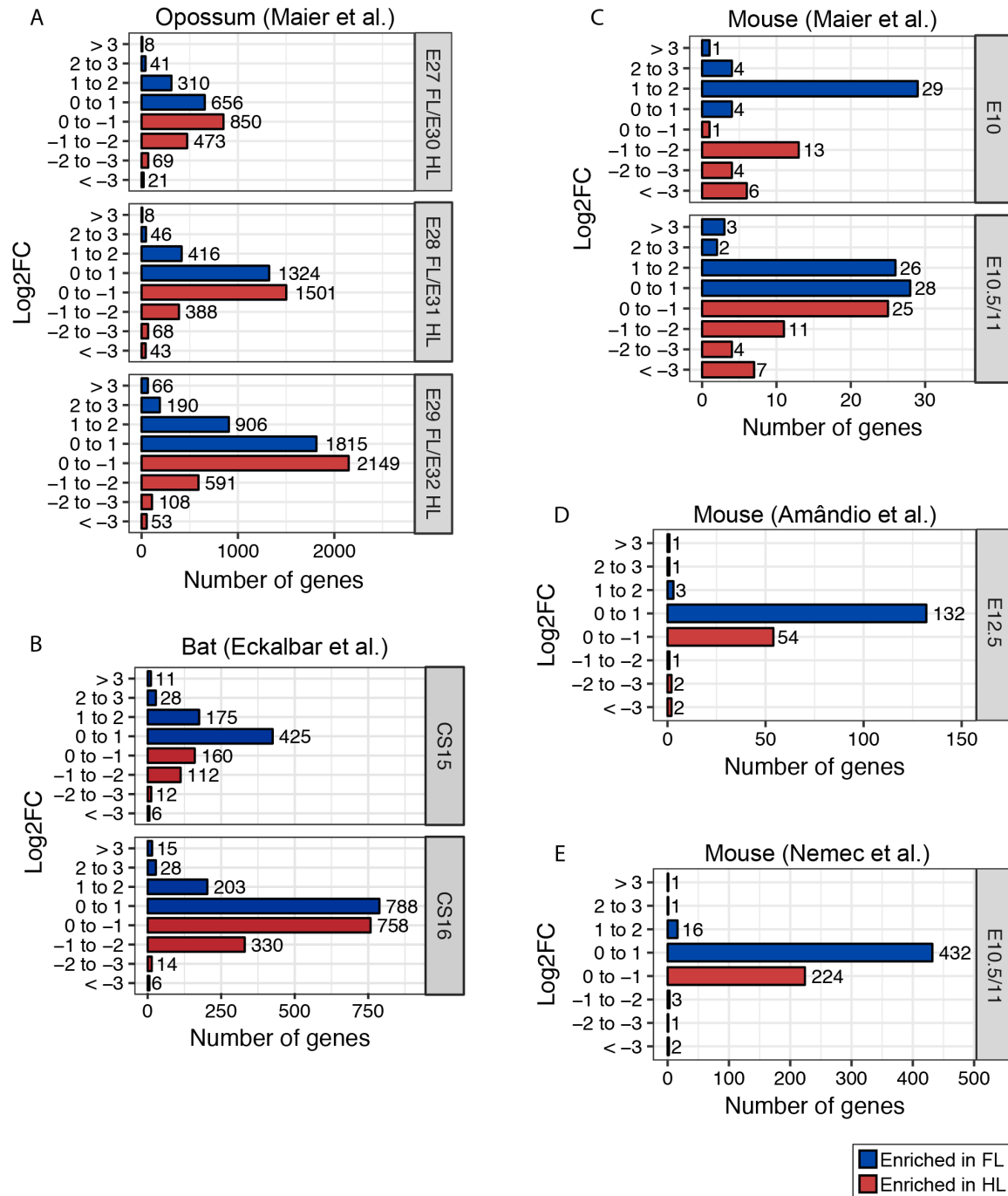
**Supplemental Figure 12. Differentially expressed genes between chicken FL and HL buds.** (A) Gene-wise hierarchical clustering heat map showing 2160 genes differentially expressed between chicken FL and HL buds at HH21, HH25, and/or HH29. All genes that are significantly differentially expressed at one or more developmental stage are included in heat map. Scale is Log<sub>2</sub>FC -5 (HL-enriched) to 5 (FL-enriched). (B) Table summarizing number of significantly differentially expressed genes between chicken FL and HL at HH21, HH25, HH29, or at all stages combined.



**Supplemental Figure 13. TFs and ECM components differentially expressed between chicken FL and HL. (A-B)** Hierarchical gene clustering heat maps of all TFs (A) and ECM components (B) that are differentially expressed between chicken FL and HL buds at HH21, HH25, or HH29. Scale is Log<sub>2</sub>FC -5 (HL-enriched) to 5 (FL-enriched).



**Supplemental Figure 14. Differentially expressed genes between brown anole FL and HL buds.** (A-B) Hierarchical gene clustering heat maps of all genes (A) or TFs (B) that are differentially expressed between FL and HL buds from Stage 7 brown anole embryos. Scale is Log<sub>2</sub>FC -5 (HL-enriched) to 5 (FL-enriched). (C) Number of genes enriched in FL or HL specific manner in Stage 7 brown anole embryos. Genes are grouped based on Log<sub>2</sub>FC from FL vs. HL comparison.



**Supplemental Figure 15. Differentially expressed genes in re-analysis of mammalian FL and HL datasets. (A-E)** Number of genes enriched in FL or HL specific manner identified in opossum (A), bat (B), and mouse (C-E) datasets. Genes are grouped based on Log2FC from FL vs. HL comparisons.