

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure S1: Expression patterns of all 22 TFs. Images of each TF at the developmental stage at which it was analyzed, showing expression of the TF:GFP fusion protein.

Supplemental Figure S2: Correlation between biologically independent replicates. (A) The average log of the signal for each peak was binned into 100nt window, and correlated between replicates. Only peaks with a p value $< .0001$ were included in the analysis. (B) For each factor, the overlap (< 1 nt) of called binding sites between the top 40% of each replicate was determined and graphed.

Supplemental Figure S3: Description of how overlapped peaks were defined. We used a stringent method to filter out unqualified peaks. We first took the peaks found by Peak-seq from the pooled reads of both replicates. These were then compared to the peaks from each replicate. The overlap between the pooled peak and each replicate peak has to be larger than 50% with a minimum peak length of 50bp in each replicate for that peak to be called.

Supplemental Figure S4: Overlapping peaks between replicates permits more robust peak calling. Peaks that were overlapped as described in Supplemental Figure S3 are more resistant to changes in the q value and do not increase in number as the q value decreases (right panel), unlike peaks called by cumulatively combining replicate reads (left panel).

Supplemental Figure S5: Distribution of TF binding among peaks and gene targets. Binding site occupancy by one or more TFs was determined and graphed (upper panel). The overlap of target genes (both coding and noncoding) between TF is shown in the lower panel.

Supplemental Figure S6: Binding distribution over coding and noncoding targets for each factor. Binding distributions were determined as described in the text and in Materials and Methods, for the different classes of gene targets (coding and noncoding) for each factor, and plotted over the distance of 2kb upstream and downstream of the TSS.

Supplemental Figure S7: Assignment of peaks to coding and noncoding gene targets. Numbers of coding (A) and noncoding (B) gene targets are divided into proximal (-500 to +300) and distal (-2000 to -501) subsets relative to the TSS.

Supplemental Figure S8: Validation of target genes bound by Hox TFs. (A) Average enrichment (y-axis) of binding from three technical replicates of ChIP-qPCR was determined for each gene. *fer-1* is a negative control gene. Standard deviation is indicated by the error bars. (B) Fold change (y-axis) in gene expression between control (mock RNAi) and lin-39(RNAi) L3 samples.

Supplemental File S1: Read number for each replicate for each factor

Supplemental File S2: List of binding peak coordinates and other statistics for each factor

Supplemental File S3: List of Highly Occupied Target (HOT) regions

Supplemental File S4: List of called target genes for each factor

Supplemental File S5: Detailed list of called target genes and associated peaks for each factor

Supplemental File S6: Number of non-coding RNA genes targeted by each factor

Supplemental File S7: Detailed GO categories for each factor

Supplemental File S8: Genes associated with GO categories for unique targets of LIN-39, MAB-5, and EGL-5

Supplemental File S9: Gene targets associated with the sequence motifs of LIN-39, MAB-5, and EGL-5

Supplemental Table S1: Detailed genotypes of all 22 strains used for ChIP-seq

Supplemental Table S2: Number of gene targets assigned to a single peak

Supplemental Table S3: Primers used in ChIP-PCR

Supplemental Table S1: Detailed genotypes of all 22 strains used for ChIP-seq.

Factor	Strain name	Genotype	Growth condition
LIN-15B	OP184	unc119(ed3);wglS184(lin-15B::TY1 EGFP FLAG;unc119)	Plate
EGL-27	OP177	unc119(ed3);wglS177(egl-27::TY1 EGFP FLAG;unc119)	Plate
HLH-1	OP64	unc119(ed3);wglS64(hlh-1::TY1 EGFP FLAG;unc119)	Liquid
MDL-1	OP106	unc119(ed3);wglS106(mdl-1::TY1 EGFP FLAG;unc119)	Liquid
SKN-1	OP178	unc119(ed3);wglS178(skn-1::TY1 EGFP FLAG;unc119)	Plate
MEP-1	OP70	unc119(ed3);wglS70(mep-1::TY1 EGFP FLAG C;unc119)	Liquid
BLMP-1	OP109	unc119(ed3);wglS109(blmp-1::TY1 EGFP FLAG;unc119)	Plate
LIN-13	OP51	unc119(ed3);wglS73(lin-13::TY1 EGFP FLAG;unc119)	Liquid
UNC-130	OP77	unc119(ed3);wglS77(unc-130::TY1 EGFP FLAG;unc119)	Liquid
PES-1	OP87	unc119(ed3);wglS87(pes-1::TY1 EGFP FLAG;unc119)	Liquid
PHA-4	OP37	unc119(ed3);wglS37(pha-4::TY1 EGFP FLAG C;unc119)	Liquid
ELT-3	OP75	unc119(ed3);wglS75(elt-3::TY1 EGFP FLAG;unc119)	Liquid
ALR-1	OP200	unc119(ed3);wglS200(alr-1::TY1 EGFP FLAG C;unc119)	Liquid
CEH-14	OP73	unc119(ed3);wglS73(ceh-14::TY1 EGFP FLAG;unc119)	Liquid
LIN-11	OP62	unc119(ed3);wglS62(lin-11::TY1 EGFP FLAG;unc119)	Liquid
MAB-5	OP26	unc119(ed3);wglS26(mab-5::TY1 EGFP FLAG;unc119)	Liquid
EGL-5	OP54	unc119(ed3);wglS54(egl-5::TY1 EGFP FLAG;unc119)	Liquid
LIN-39	OP18	unc119(ed3);wglS18(lin-39::TY1 EGFP FLAG;unc119)	Liquid
CEH-30	OP120	unc119(ed3);wglS120(ceh-30::TY1 EGFP FLAG;unc119)	Plate
GEI-11	OP179	unc119(ed3);wglS179(gei-11::TY1 EGFP FLAG;unc119)	Plate
EOR-1	OP81	unc119(ed3);wglS81(eor-1::TY1 EGFP FLAG;unc119)	Liquid
PQM-1	OP201	unc119(ed3);wglS201(pqm-1::TY1 EGFP FLAG C;unc119)	Plate

Supplemental Table S2: Number of gene targets assigned to each peak

# targets/peak	# Proximal Peaks		# Distal Peaks	
	coding genes	ncRNA genes	coding genes	ncRNA genes
1	21824	2659	12331	447
2	5827	186	1719	43
3	53	14	65	2
>3		1(4)*		1**

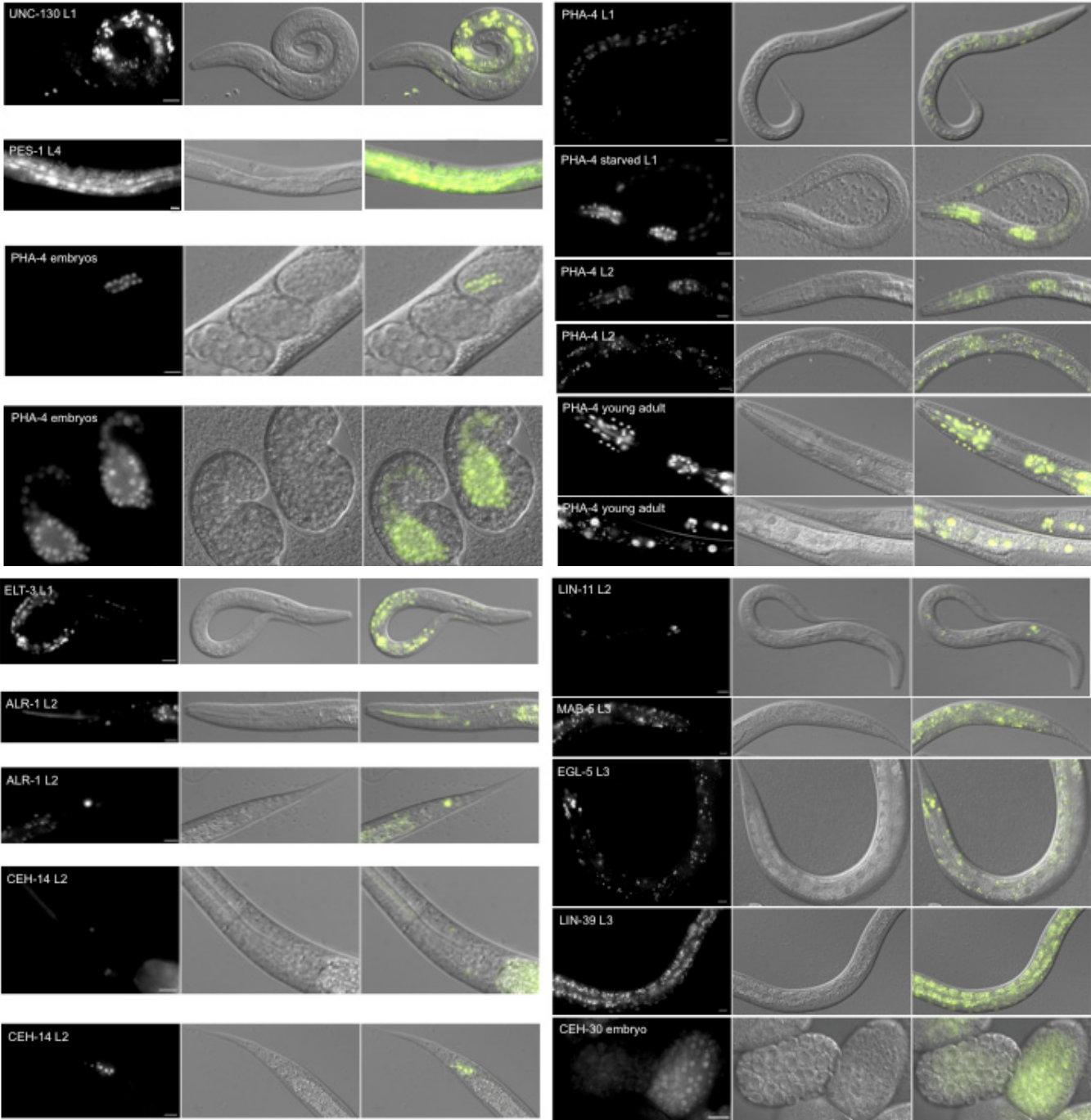
**mir-36*, *mir-37*, *mir-38*, *mir-39* and *mir-40* are adjacent microRNAs associated with a single promoter region bound by four factors

***mir-229*, *mir-64*, *mir-65*, *mir-66* are adjacent microRNAs associated with a single promoter region bound by a single factor

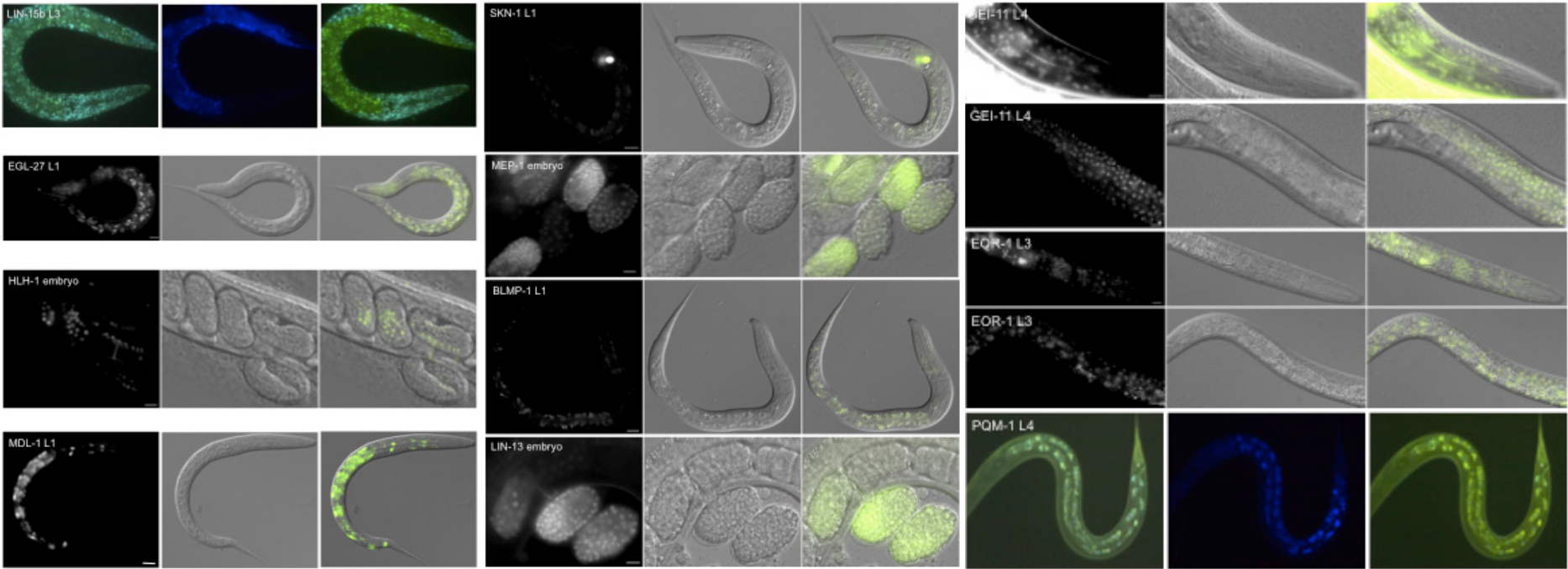
Supplemental Table S3: Primers used in ChIP-qPCR.

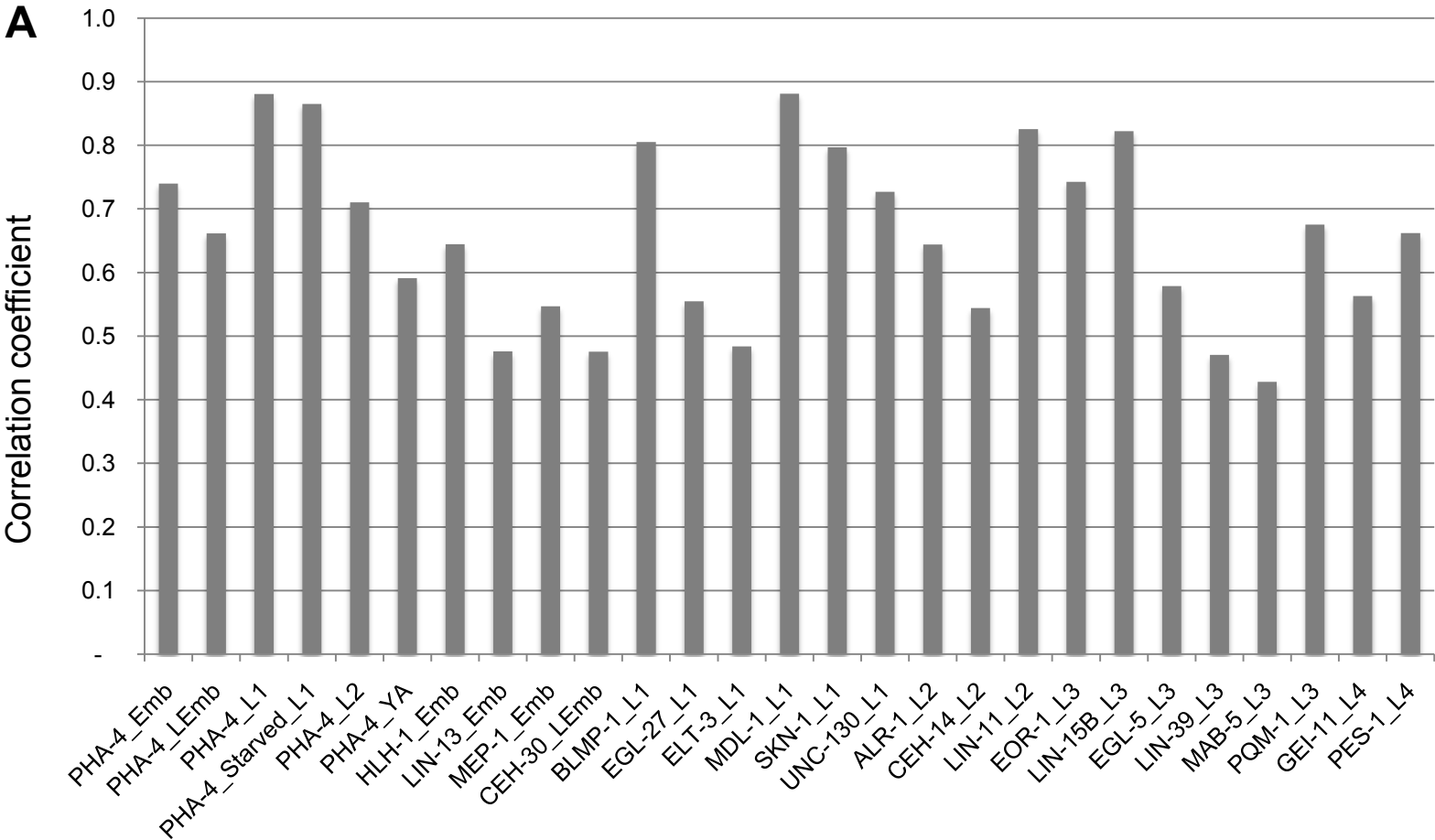
Genes	Primer sequence
<i>mec-3</i>	acaaatcacccgtcaagagc
	tccttacacacactttctagcttca
<i>unc-30</i>	tagacaccgtagcccgtttc
	tgccacccacatatggtaaa
<i>prk-2</i>	gtgggaaacacgtgggatac
	cccctaccatcaatcttca
<i>lin-39</i>	tgtagctctcggtgcatctg
	cgctctcgtcctcatttat
<i>glp-1</i>	cggcgtaaaataggttcaa
	tgcgaaatgtggagaaaaca
<i>emb-4</i>	aaaaccgcaagaaactcgaa
	tctccacacattttccgaca
<i>unc-62</i>	gagttggctggcagagagac
	acgtccctgtgtgtgtgtgt
<i>kin-1</i>	cagagcttttcgctgctctt
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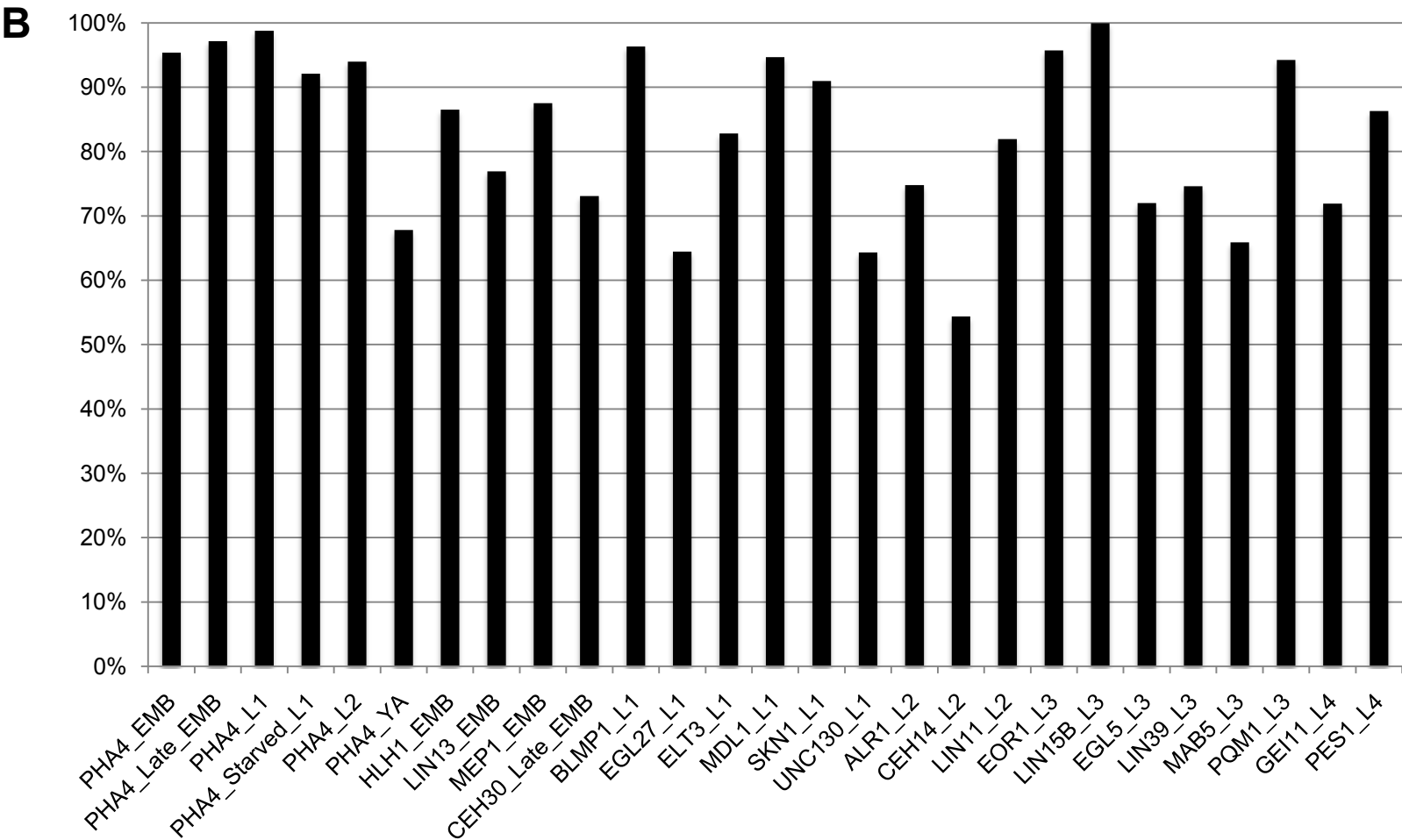
Niu et al., Figure S1



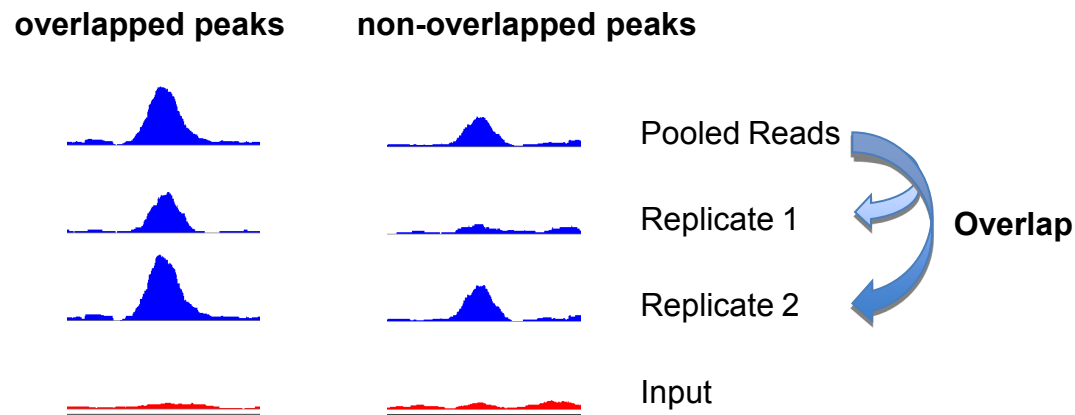
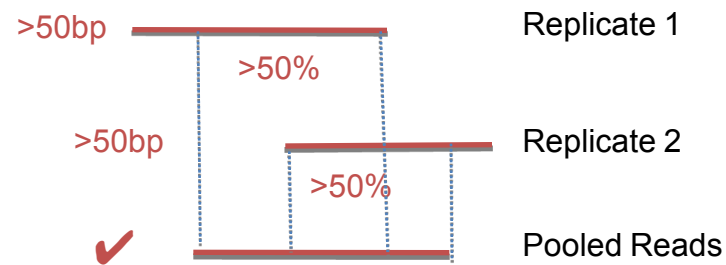
Niu et al., Figure S1



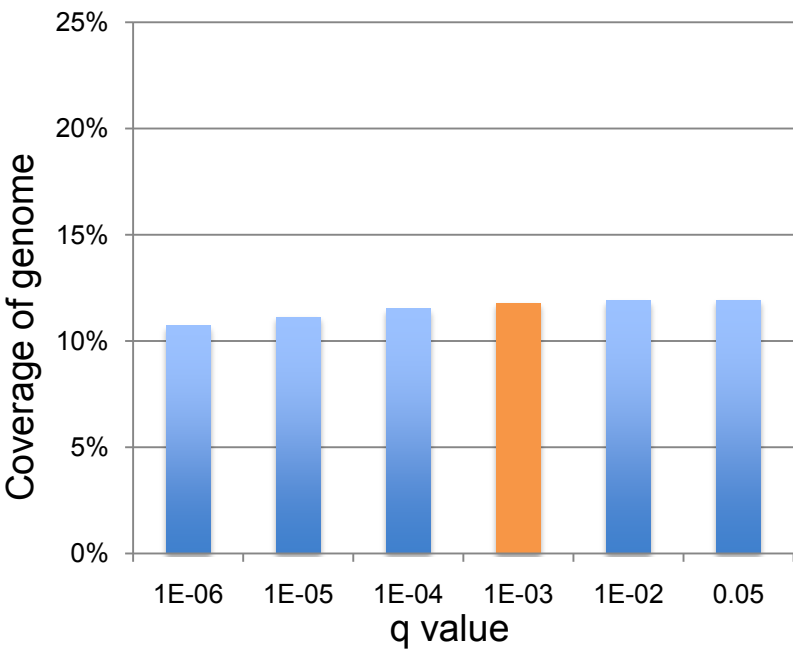
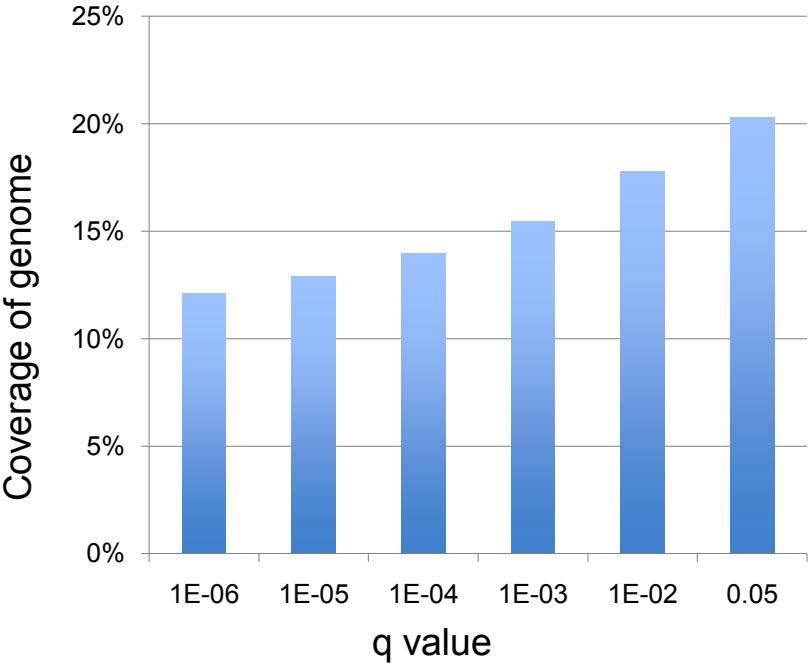




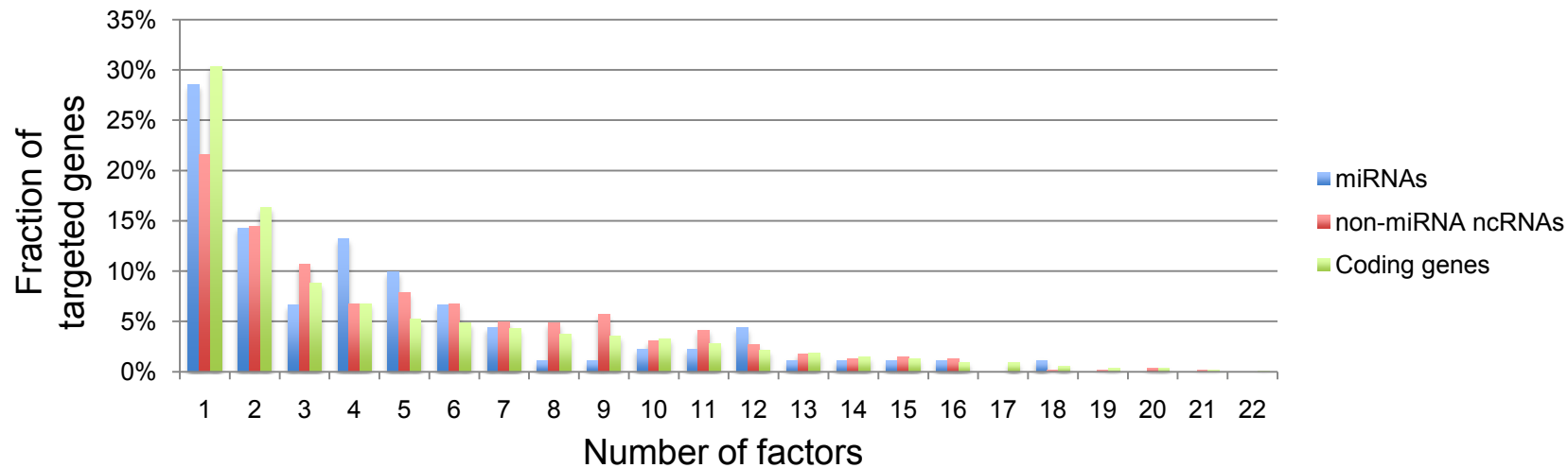
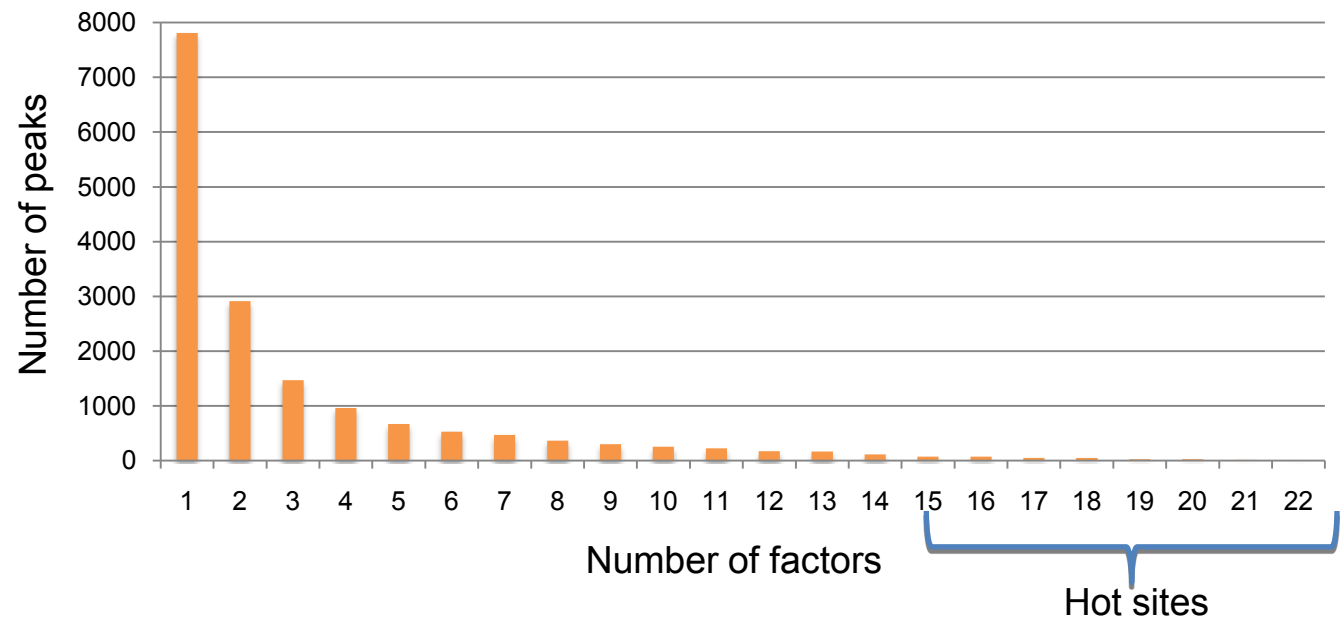
Niu et al., Figure S3



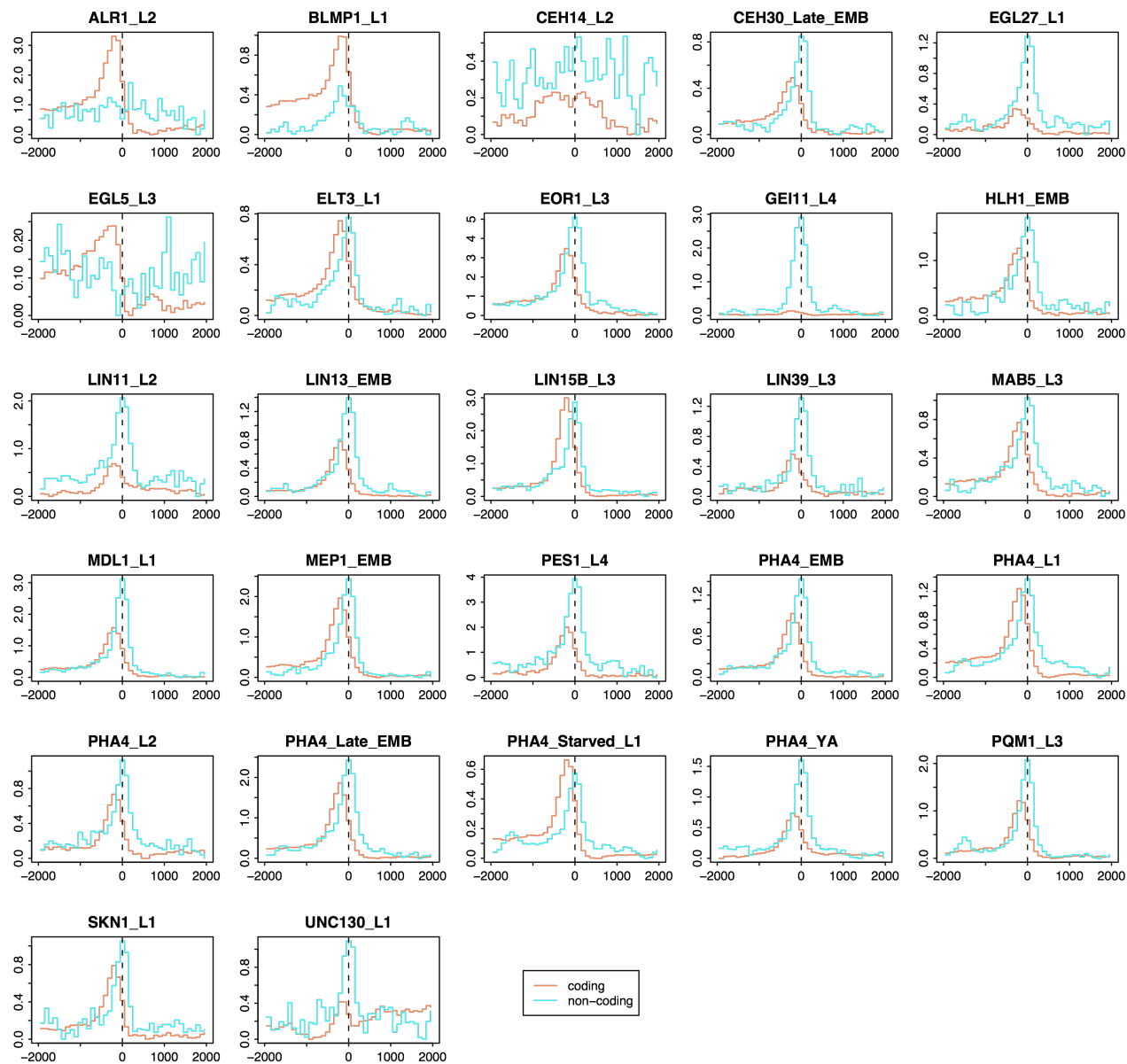
Niu et al., Figure S4

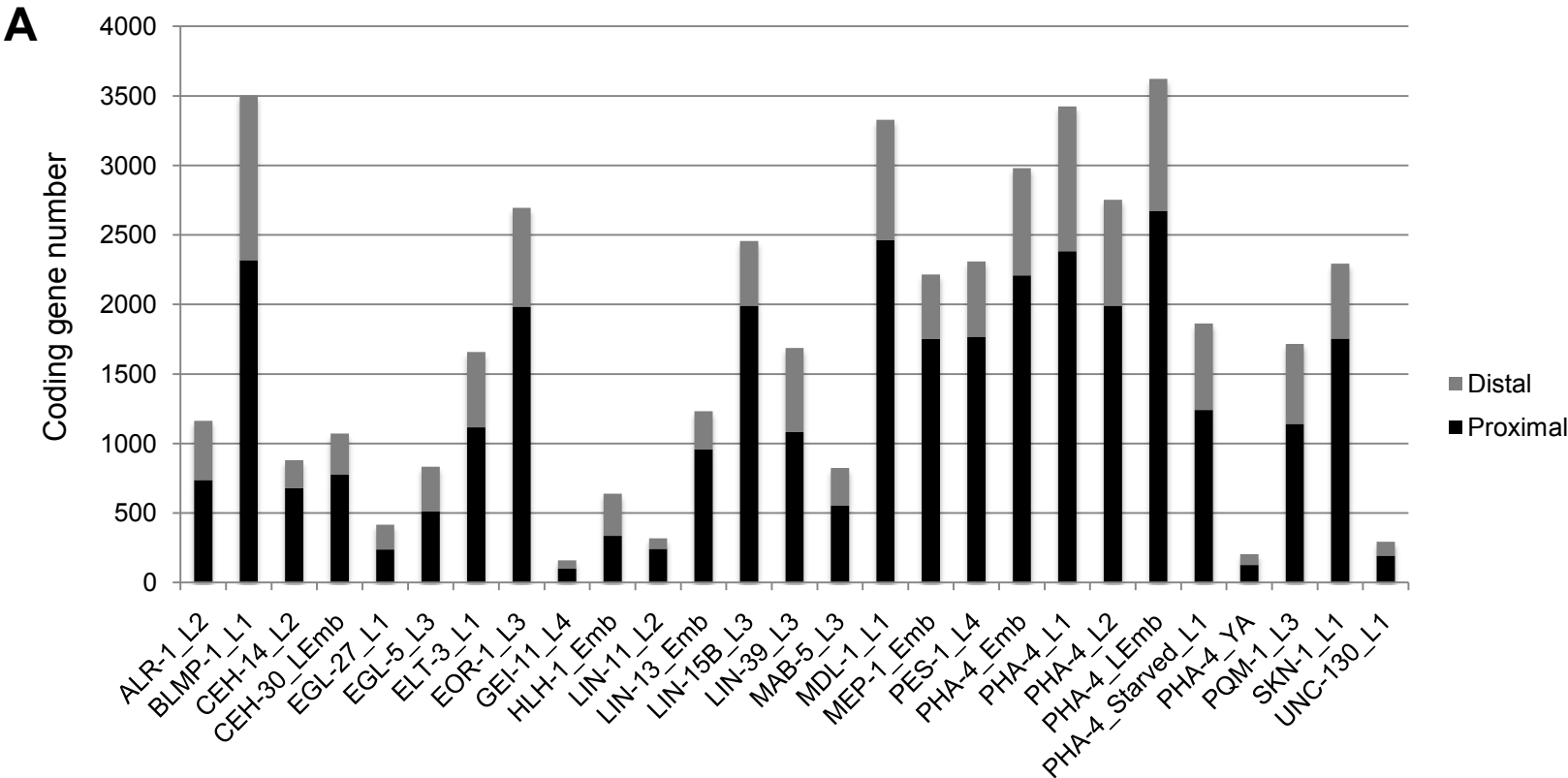


Niu et al., Figure S5

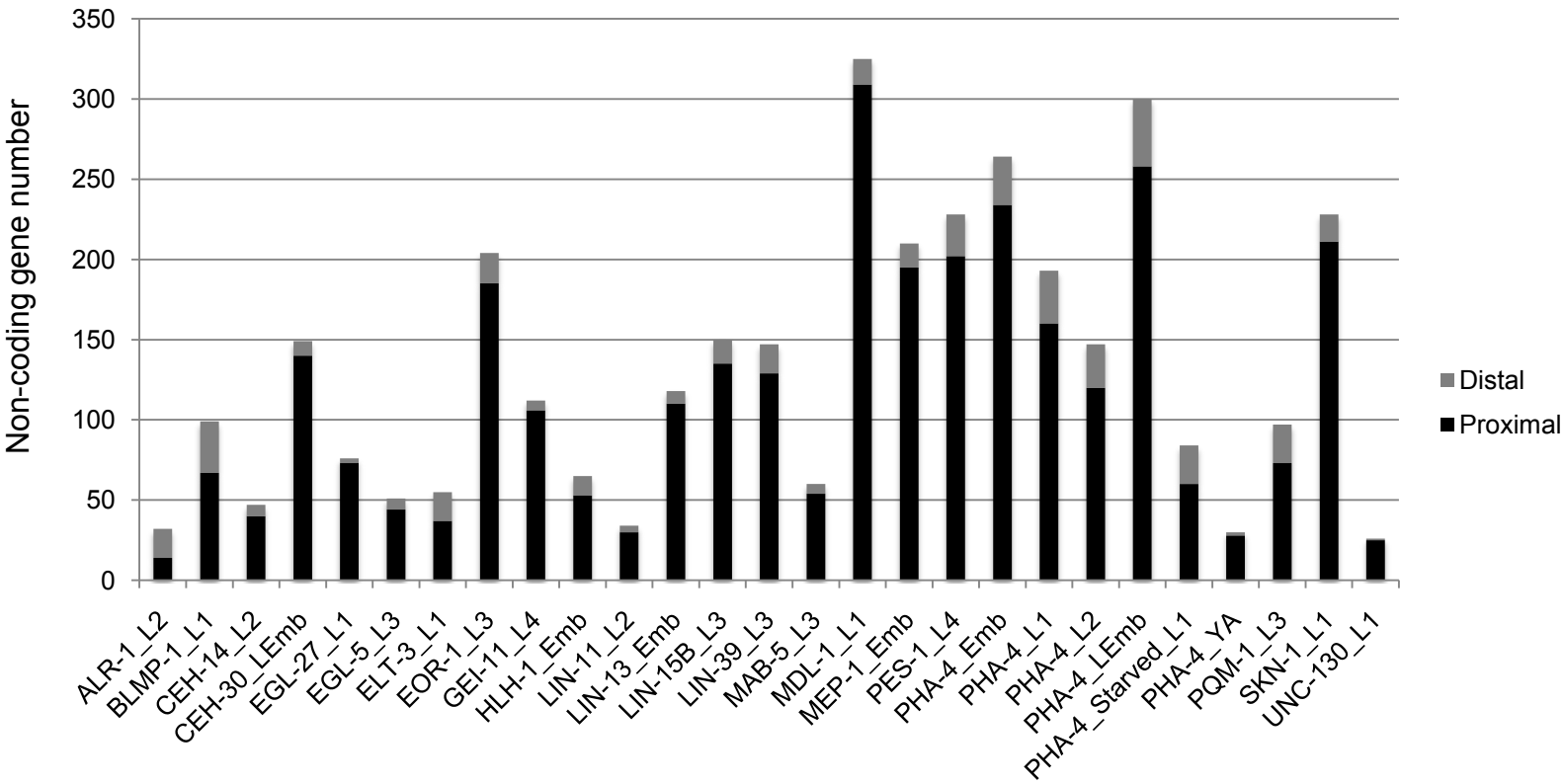


Niu et al., Figure S6





B



Niu et al., Figure S8

