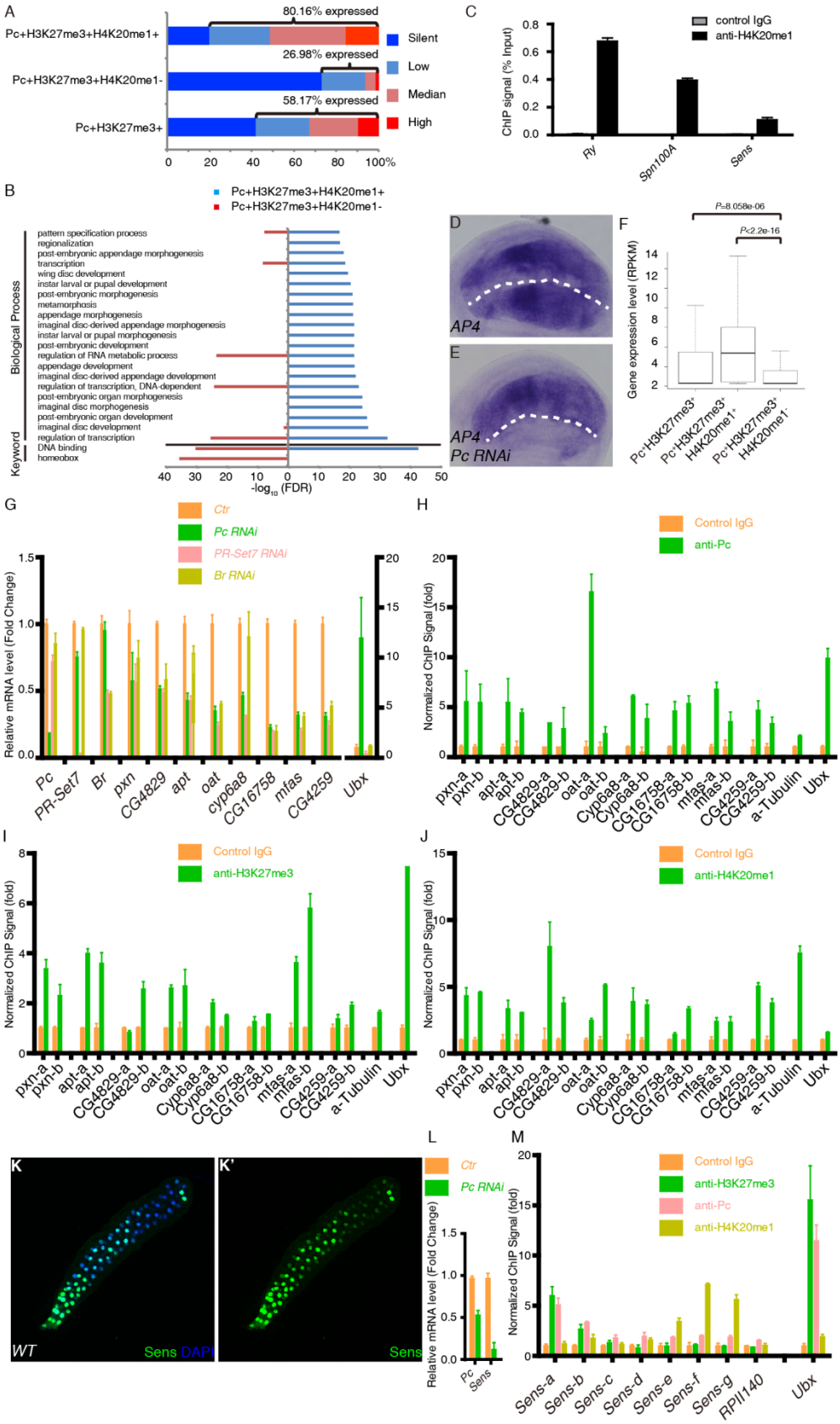
A
80.16% expressed
Pc+H3K27me3+H4K20me1+
26.98% expressed
Pc+H3K27me3+H4K20me1-
58.17% expressed
Pc+H3K27me3+
Silent
Low
Median
High
0 20 40 60 80 100%
C
control IgG
anti-H4K20me1
ChIP signal (% Input)
0.0 0.2 0.4 0.6 0.8
Fly
Spn100A
Sens
B
Pc+H3K27me3+H4K20me1+
Pc+H3K27me3+H4K20me1-
Biological Process
Keyword
pattern specification process
regionalization
post-embryonic appendage morphogenesis
transcription
wing disc development
instar larval or pupal development
post-embryonic morphogenesis
metamorphosis
appendage morphogenesis
imaginal disc-derived appendage morphogenesis
instar larval or pupal morphogenesis
post-embryonic development
regulation of RNA metabolic process
appendage development
imaginal disc-derived appendage development
regulation of transcription, DNA-dependent
post-embryonic organ morphogenesis
imaginal disc morphogenesis
post-embryonic organ development
imaginal disc development
regulation of transcription
DNA binding
homeobox
40 30 20 10 0 10 20 30 40 50
-log10 (FDR)
D
AP4
E
AP4
Pc RNAi
F
P=8.058e-06
P<2.2e-16
Gene expression level (RPKM)
2 4 6 8 10 12 14
Pc+H3K27me3+
Pc+H3K27me3+H4K20me1+
Pc+H3K27me3+H4K20me1-
G
Ctr
Pc RNAi
PR-Set7 RNAi
Br RNAi
Relative mRNA level (Fold Change)
0.0 0.5 1.0 1.5
0 5 10 15 20
Pc
PR-Set7
Br
pxn
CG4829
apt
oat
cyp6a8
CG16758
mfas
CG4259
Ubx
H
Control IgG
anti-Pc
Normalized ChIP Signal (fold)
0 5 10 15 20
pxn-a
pxn-b
apt-a
apt-b
CG4829-a
CG4829-b
oat-a
oat-b
Cyp6a8-a
Cyp6a8-b
CG16758-a
CG16758-b
mfas-a
mfas-b
CG4259-a
CG4259-b
a-Tubulin
Ubx
I
Control IgG
anti-H3K27me3
Normalized ChIP Signal (fold)
0 2 4 6 8
J
Control IgG
anti-H4K20me1
Normalized ChIP Signal (fold)
0 5 10 15
K
WT
Sens DAPI
K'
Sens
L
Ctr
Pc RNAi
Relative mRNA level (Fold Change)
0.0 0.5 1.0 1.5
Pc
Sens
M
Control IgG
anti-H3K27me3
anti-Pc
anti-H4K20me1
Normalized ChIP Signal (fold)
0 5 10 15 20
Sens-a
Sens-b
Sens-c
Sens-d
Sens-e
Sens-f
Sens-g
RPII140
Ubx

Figure S4. Transcriptional regulation and functional analysis of the $Pc^{+}H3K27me3^{+}H4K20me1^{-}$ genes and $Pc^{+}H3K27me3^{+}H4K20me1^{+}$ genes. Related to Figure 4

(A) The histogram showing the ratios of silent genes and expressed genes (with low, median or high levels of expression) in the indicated gene sets.

(B) Gene ontology (GO) analysis of the $Pc^{+}H3K27me3^{+}H4K20me1^{-}$ and $Pc^{+}H3K27me3^{+}H4K20me1^{+}$ genes. The most significant ontologies of $Pc^{+}H3K27me3^{+}H4K20me1^{+}$ genes are DNA binding and development or morphogenesis while that of $Pc^{+}H3K27me3^{+}H4K20me1^{-}$ genes are homeobox and DNA binding, suggesting different roles of these two sets of genes in wing discs.

(C) ChIP-qPCR analysis using control IgG (grey) or anti-H4K20me1 antibody (black) with the *Act5c-Gal4* wing disc at *Ry*, *Spn100A* and *Sens* loci. ChIP signal levels are represented as percentages of input chromatin.

(D-E) In situ hybridization analysis of *AP4* (D) or *AP4;Pc RNAi* (E) wing discs showed that *Spn100A* is expressed ubiquitously in WT (*AP4*) wing pouch (D), and its expression is down-regulated in the dorsal part below the dashed lines where *Pc* is knocked down specifically (E).

(F) Average expression levels of $Pc^{+}H3K27me3^{+}H4K20me1^{+}$ genes are significantly higher than those of $Pc^{+}H3K27me3^{+}H4K20me1^{-}$ genes in S2 cells. ChIP-seq (GSE32756, GSE24521 and GSE41440) and RNA-seq (GSE34390) data were from published references.

(G) RT-qPCR analysis of mRNA levels of indicated genes (a group of $Pc^{+}H3K27me3^{+}H4K20me1^{+}$ genes from Figure S4F) in S2 cells treated with control dsRNA or dsRNA specifically targeting *Pc*, *PR-Set7* or *Br*.

(H-J) ChIP-qPCR assay with S2 cells using anti-Pc (H), anti-H3K27me3 (I), or anti-H4K20me1 (J) antibodies to verify the $Pc^{+}H3K27me3^{+}H4K20me1^{+}$ genes tested in Figure S4G. Most of them are $Pc^{+}H3K27me3^{+}H4K20me1^{+}$ genes.

(K-K') Wild type salivary gland was immunostained with anti-Sens antibody (green) and DAPI (blue). 55 layers were scanned with 5 μm interval, and then merged to generate the final images. Sens expression was detected in every DAPI-positive cell.

(L) RT-qPCR analysis of indicated genes in salivary gland with indicated genotypes.

(M) ChIP-qPCR assay with salivary gland using anti-Pc, anti-H3K27me3 or anti-H4K20me1 antibodies.