Supplementary Figures:

FIG. S1. *Pp*-dependent transcription is induced by AR and androgen in DC2 epididymal cells. A. Schematic diagram of the wild-type (WT) *Pp* construct containing 0.3 kb of 5′ flanking sequence and the *Renilla* luciferase gene downstream (this construct is the same as the wild-type construct in Fig. 2A). B. Luciferase analysis of DC2 epididymal cells transiently transfected with the construct shown in panel A (100 ng), performed and quantified as in Fig. 1B. Average values ± standard error from three experiments done in triplicate are shown.

FIG. S2. AREs responsible for *Rhox5 Pp* transcription in DC1 epididymal cells. Luciferase analysis using the DC1 epididymal cell line transiently transfected with the constructs shown in Fig. 1A and performed as described in the legend of Fig. 1B.

FIG. S3. Expression of *Pp* and *Pp*-activating transcription factors in epididymal cell lines, the MSC1 Sertoli cell line, and the epididymis. Real-time PCR analysis of total cellular RNA from 3 independent samples, performed as described in the Fig. 1E legend. Primer efficiency was taken into account to calculate relative gene expression levels; this was determined by generating a standard curve for each primer by performing qPCR with a 2-fold serial dilution of a plasmid template (the slope of the line was used to calculate primer efficiency). Average mRNA levels (± standard error) are shown relative to the lowest mRNA level, which was set as 1.

FIG. S4. Region-specific expression of *Gata* factor mRNAs and *Pp* mRNA in the adult mouse epididymis. Real-time PCR analysis of total cellular RNA from 3 independent samples from each region, performed and quantified as described in Fig. S3 legend.

FIG. S5. Developmental expression of *Gata* factor mRNAs and *Pp* mRNA in the mouse epididymis. Real-time PCR analysis of total cellular RNA from 3 independent samples from each time point, performed and quantified as described in Fig. S3 legend.

FIG. S6. Overexpression of GATA factors did not significantly upregulate *Pp*-dependent transcription in DC2 epididymal cells. Luciferase analysis using DC2 cells transiently transfected with the wild type *Pp* construct shown in Fig. S1, performed as described in the Fig. S1 legend. The result shown is representative of two independent experiments.

FIG. S7. The developmental expression pattern of AR protein mirrors that of *Ar* mRNA in the mouse epididymis. A representative blot used to generate the histogram in Fig. 3G is shown. Whole cell lysates from mouse epididymides from the indicated time points were electrophoresed, blotted, and probed with antiserum against AR and β-tubulin. The values shown are the ratio of AR protein level divided by β-tubulin protein level, as determined by densitometric analysis. The highest AR protein level was at postnatal day 20 (D20), which was given a value of 1.

FIG. S8. DNMT1 and MeCP2 occupancy at the *Pp* in the caput epididymis during development. ChIP analysis was performed on pooled caput epididymides from mice of the indicated ages using antibodies against DNMT1 and MeCP2 as well as a no-antibody control. The values are calculated using the formula in the "Materials and Methods". A representative plot is shown from three separate qPCR data sets. The signal in the no-antibody control was given a value of 1.

Table S1. Primer Sequences used for the study.

Construct	Primer sequence	Primer	Strategy/ Purpose	Parent vector
Pem-250 (WT-0.3kb)	**F** CGACAAGCTTGTAACTGGGCACCCTAAG **R** ACGCGTCGACACCCTGAATAGGATCAATG	MDA 1598 MDA 1599	Deletion PCR, subcloning	*Pem-124* ***(Wt-0.6 kb)***
Pem-251 (ΔA)	**F** CATCACAGATCTCATTCTGTTCCCG **R** GATTTGCTCACAGGACGTTCCTG	MDA 1614 MDA 1615	Deletion PCR	*Pem-250*
Pem-252 (ΔB)	**F** CCACAGGAACGTCCTGTGAGCAATC **R** GATGTAATGAGACGATGTGCTTGCAAG	MDA 1651 MDA 1652	Deletion PCR	*Pem-250*
Pem-262 (ΔC)	**F** CAGAACTTAGGGTGCCCAGTTAC **R** GCAAGCACATCGTGCTCATTACATC	MDA 1820 MDA 1821	Deletion PCR	*Pem-250*
Pem-267 (mG1)	**F** CATCCCCAAACTGCTCACACTTGTGTACCCCAAAG **R** CTTTGGGGTACACAAGTGTGAGCAGTTTGGGGATG	MDA 1858 MDA 1859	Site-directed mutagenesis	*Pem-250*
Pem-278 (mG3)	**F** CCCATGAACTGTGTCCACTTTGCAAGCACATC **R** CGATGTGCTTGCAAAGTGGACACAGTTCATGGG	MDA 1998 MDA 1999	Site-directed mutagenesis	*Pem-250*
Pem-279 (mG2)	**F** CATCTTGCAAGCACACTGTGCTCATTACATCCCC **R** GGGGATGTAATGAGCACAGTGTGCTTGCAAGATG	MDA 2000 MDA 2001	Site-directed mutagenesis	*Pem-250*
Pem-282 (mG1 &3)	**F** CTTGCAAGCACATCGTTCTAATTACATCCCCAAAC **R** GTTTGGGGATGTAATTAGAACGATGTGCTTGCAAG	MDA 2014 MDA 2015	Site-directed mutagenesis	*Pem-250*
Pem-290 (mG1, 2 &3)	**F** CATCCCCAAACTGCTCACACTTGTGTACCCCAAAG **R** CTTTGGGGTACACAAGTGTGAGCAGTTTGGGGATG	MDA 1858 MDA 1859	Site-directed mutagenesis	*Pem-281*
Pem-270	**F** CATCCCCAAACTGCTCACACTTGTGTACCCCAAAG **R** CTTTGGGGTACACAAGTGTGAGCAGTTTGGGGATG	MDA 1858 MDA 1859	Site-directed mutagenesis	*Pem-214*
Pem-242 (mARE1)	**F** GTTGAGCATCACATATATCATTCTTTTACCGGGGACACCAG **R** CTGGTGTCCCCGGTAAAAGAATGATATATGTGATGCTCAAC	MDA 1307 MDA 1308	Site-directed mutagenesis	*Pem-124 (Wt-0.6 kb)*
Pem-243 (mARE2)	**F** CTGGGCCCACAGTACTGTCCTTTGAGCAAA TCAC **R** GTGATTTGCTCAAAGGACAGTACTGTGGGC CGAG	MDA 1309 MDA 1310	Site-directed mutagenesis	*Pem-124 (Wt-0.6 kb)*
Pem-247 (mARE3)	**F** CTGTGTCCATCTTGATATCAAATCGTTCTAATTACATCCCC **R** GGGGATGTAATTAGAACGATTTGATATCAAGATGGACACAG	MDA 1450 MDA 1451	Site-directed mutagenesis	*Pem-124 (Wt-0.6 kb)*
Pem-248 (mARE4)	**F** GCTGTAACTGGACAACCTAAATTTTGCACACCCAC **R** GTGGGTGTGCAAAATTTAGGTTGTCCAGTTACAGC	MDA 1452 MDA 1453	Site-directed mutagenesis	*Pem-124 (Wt-0.6 kb)*
	F GAGGAGATCCCTGCCAGGT **R** GGTGTCCCCGGGAACAG	MDA 2117 MDA 2118	Amplification of *Rhox5 Pp*	
	F TGTTTATGAATTGTGTTTATTTTGTAAGTA **R** TCTAAACT TAAACCCCTAATATCCC	MDA 1838 MDA 1839	Amplification of BST *Rhox5 Pp*	

FIG. S1A

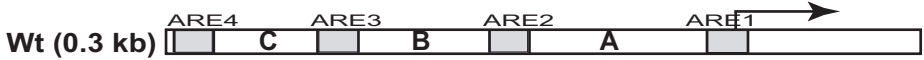

FIG. S1B

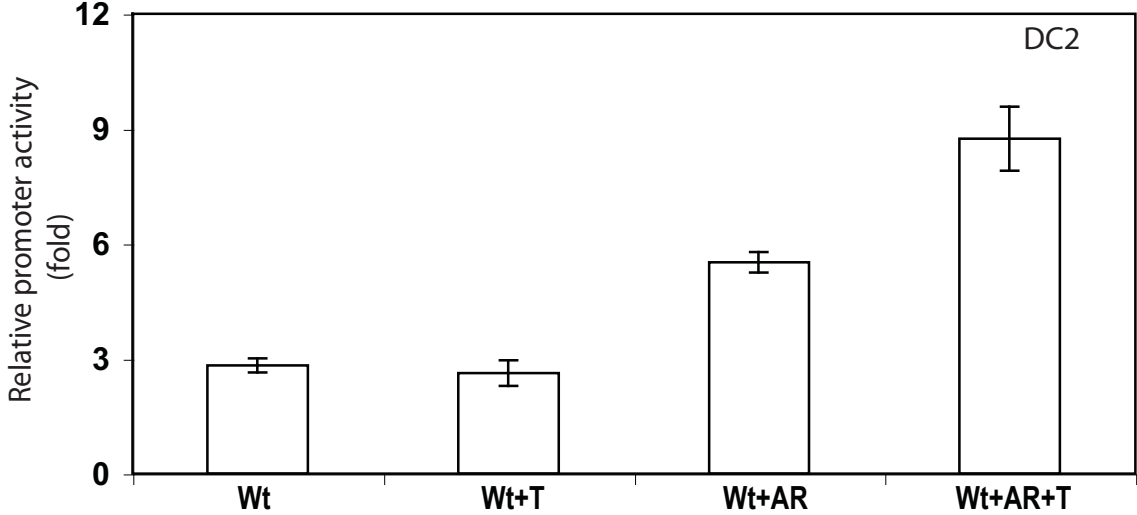

FIG. S2

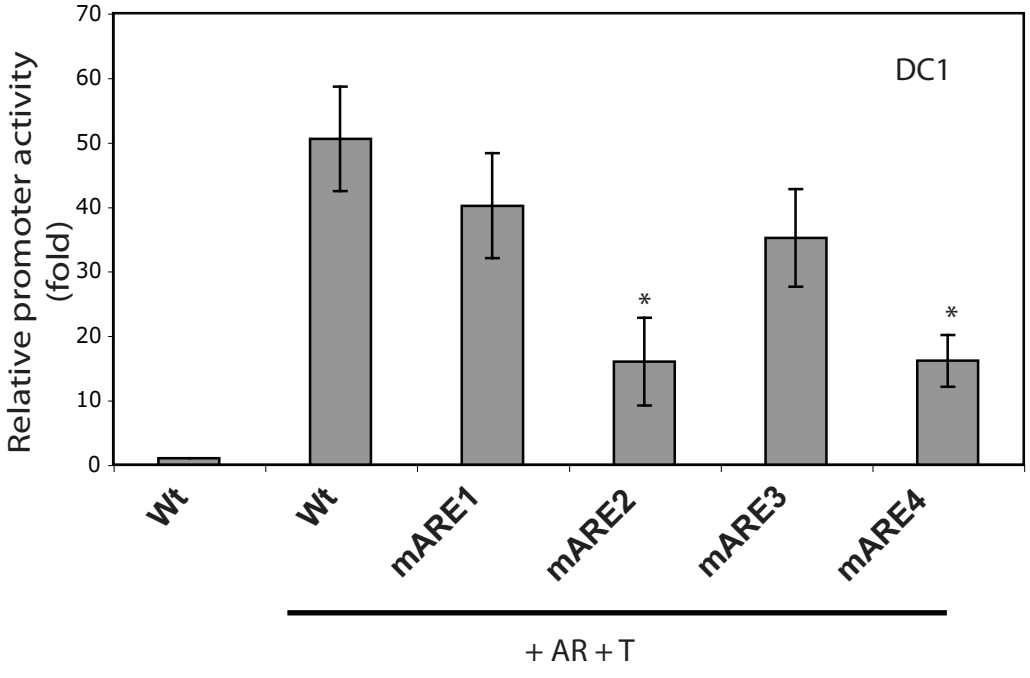
Relative promoter activity (fold)
70
60
50
40
30
20
10
0
DC1
*
*
Wt
Wt
mARE1
mARE2
mARE3
mARE4
+ AR + T

FIG. S3

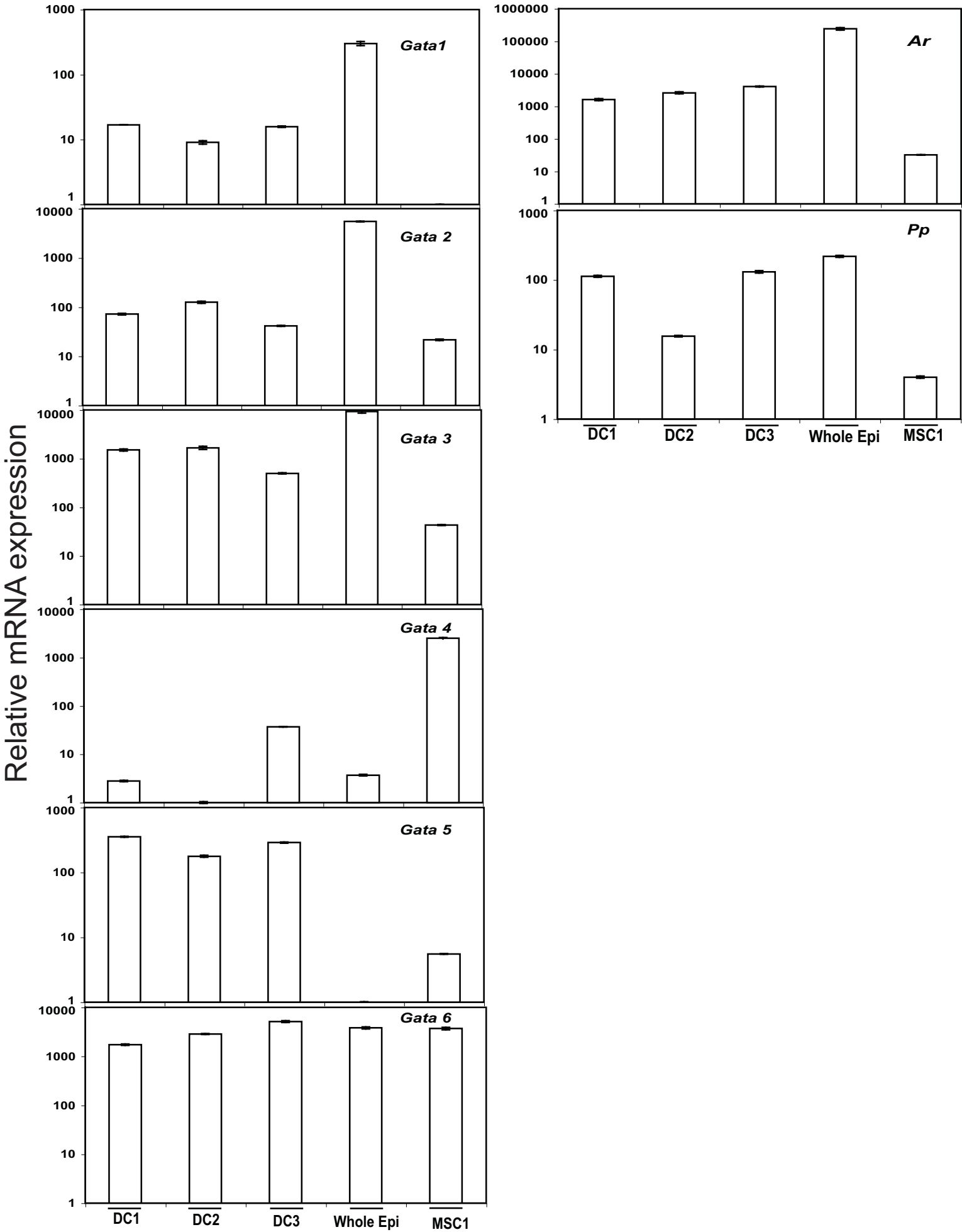
Relative mRNA expression
Gata1
Gata 2
Gata 3
Gata 4
Gata 5
Gata 6
Ar
Pp
1000
100
10
1
10000
1000000
DC1
DC2
DC3
Whole Epi
MSC1

FIG. S4

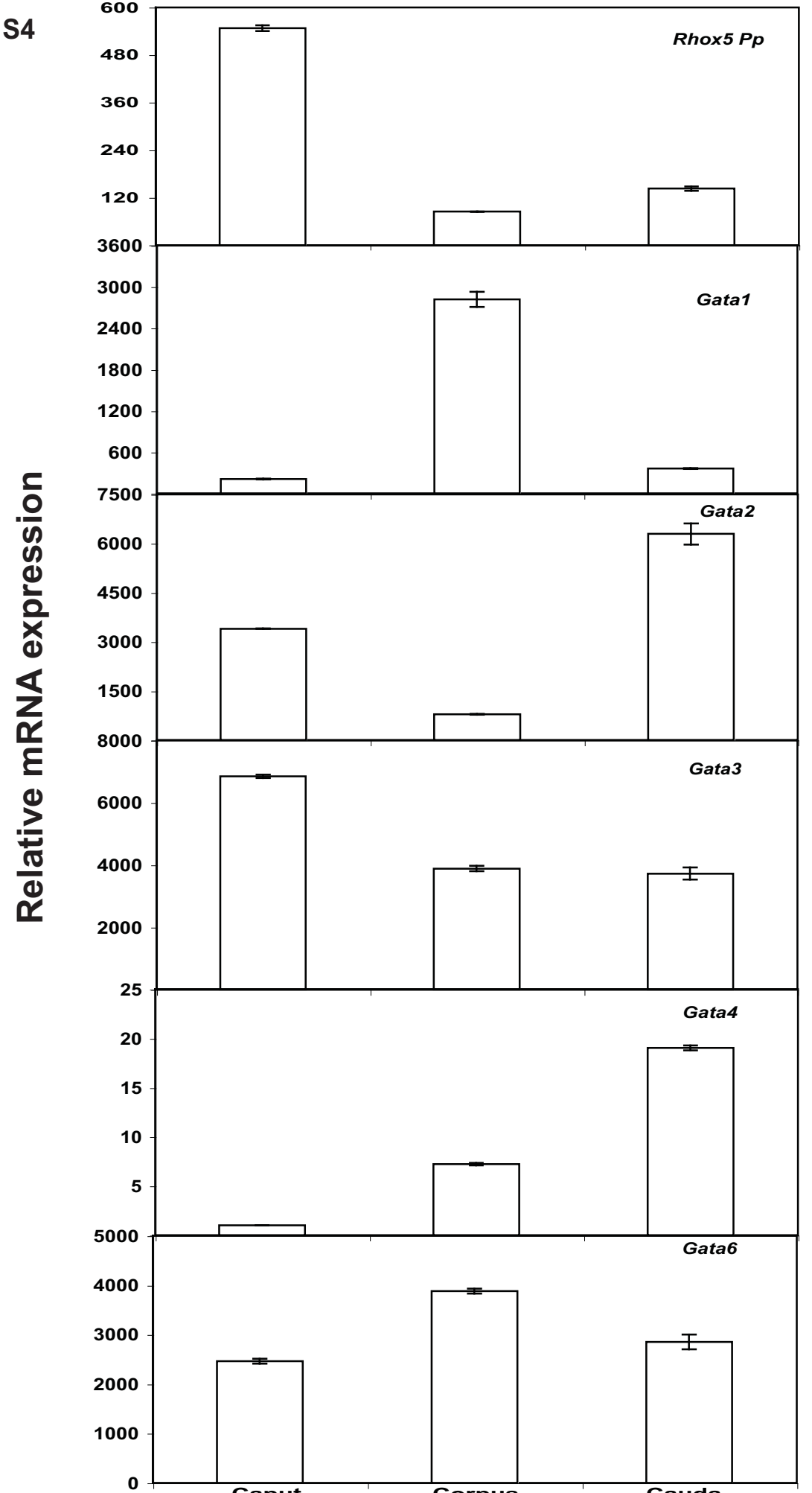
Relative mRNA expression
Rhox5 Pp
600
480
360
240
120
0
Gata1
3600
3000
2400
1800
1200
600
0
Gata2
7500
6000
4500
3000
1500
0
Gata3
8000
6000
4000
2000
0
Gata4
25
20
15
10
5
0
Gata6
5000
4000
3000
2000
1000
0
Caput
Corpus
Cauda

FIG. S5

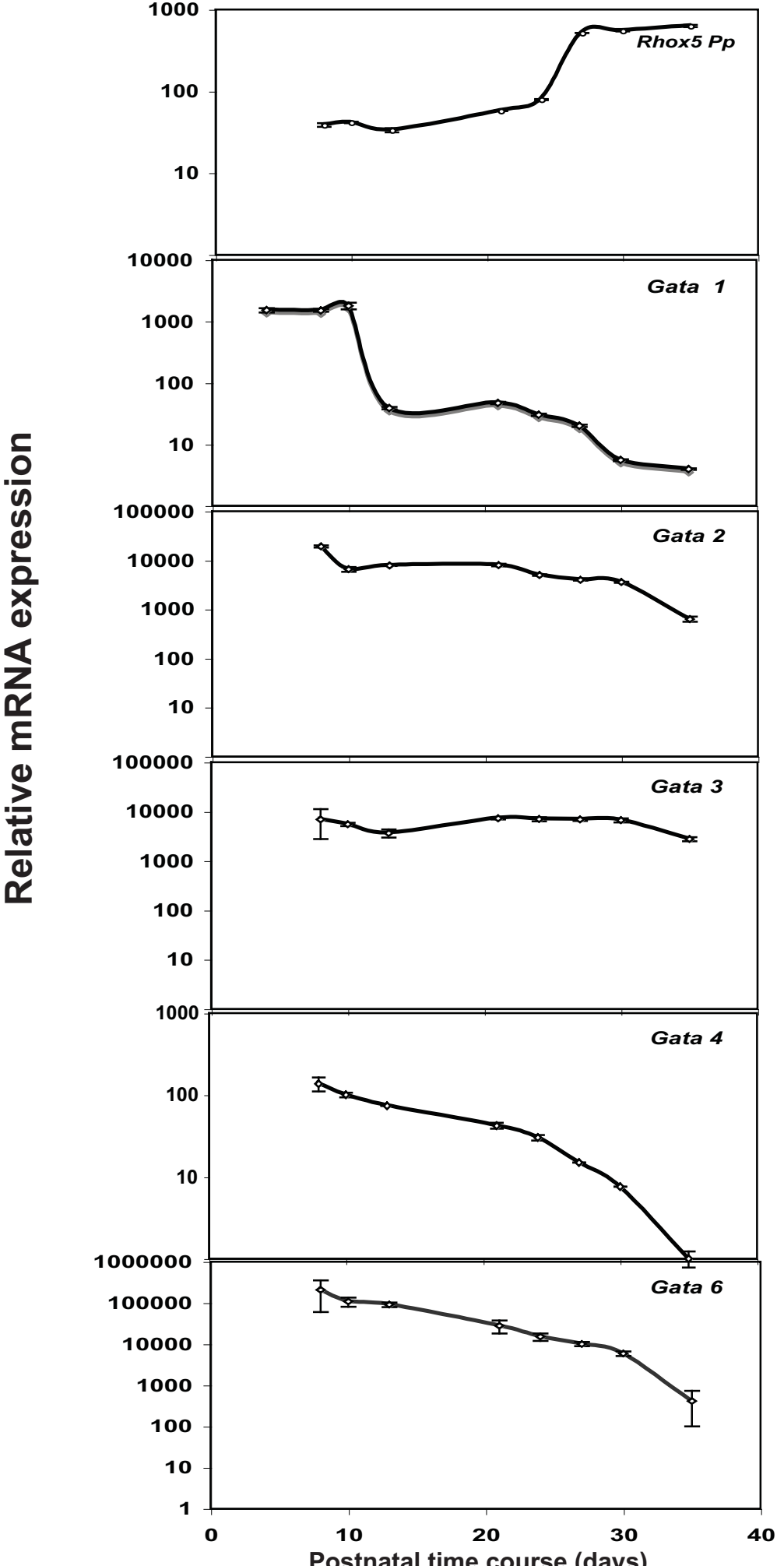
Relative mRNA expression
Rhox5 Pp
Gata 1
Gata 2
Gata 3
Gata 4
Gata 6
1000
100
10
10000
1000
100
10
100000
10000
1000
100
10
100000
10000
1000
100
10
1000
100
10
1000000
100000
10000
1000
100
10
1
0
10
20
30
40
Postnatal time course (days)

FIG. S6

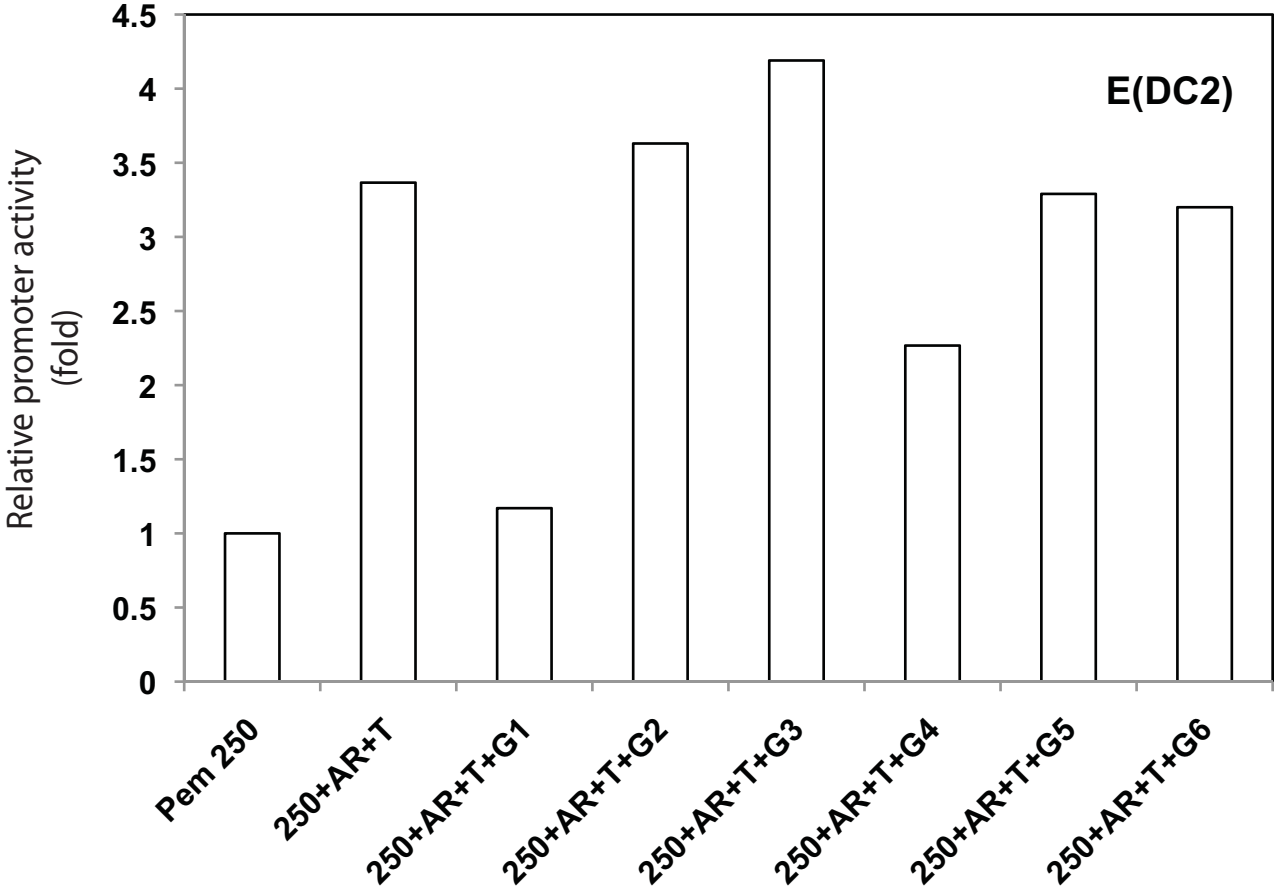
E(DC2)
Relative promoter activity (fold)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Pem 250
250+AR+T
250+AR+T+G1
250+AR+T+G2
250+AR+T+G3
250+AR+T+G4
250+AR+T+G5
250+AR+T+G6

FIG. S7

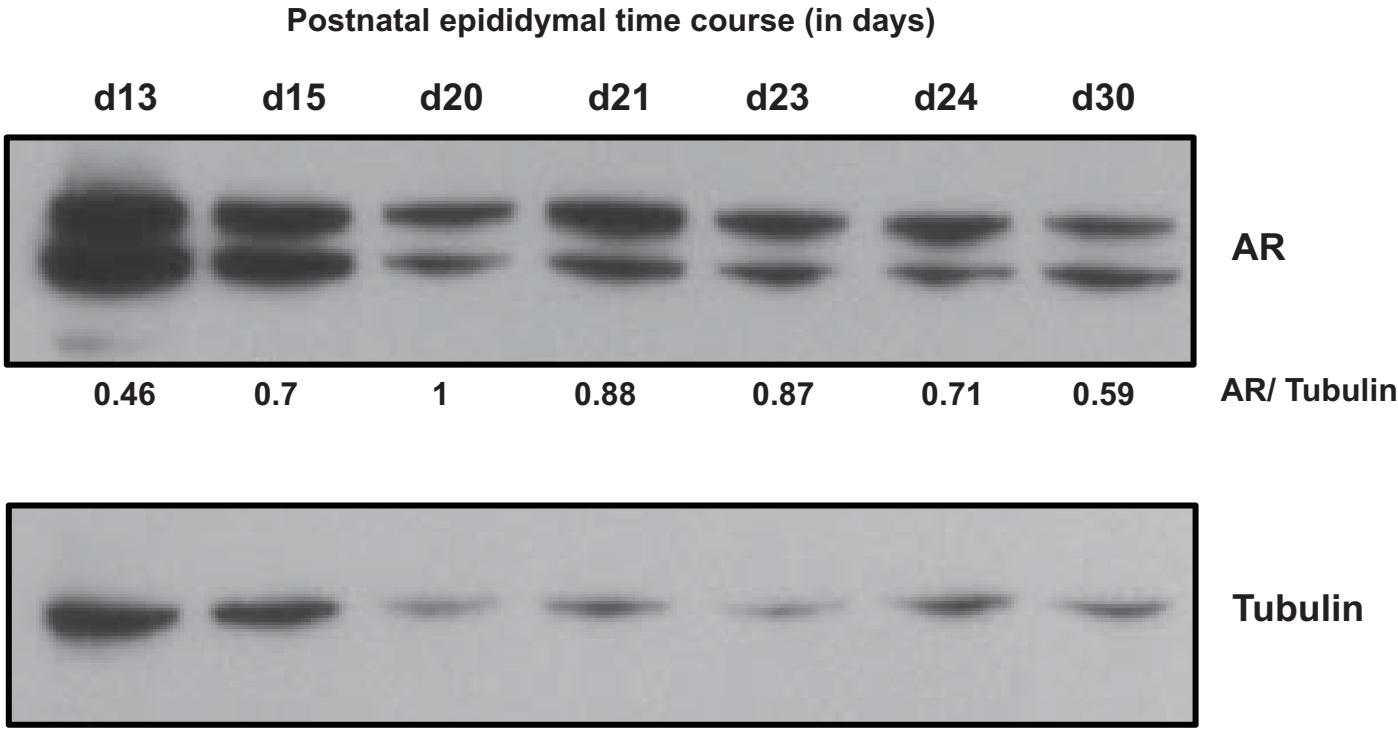
Postnatal epididymal time course (in days)
d13
d15
d20
d21
d23
d24
d30
AR
0.46
0.7
1
0.88
0.87
0.71
0.59
AR/ Tubulin
Tubulin

FIG. S8A

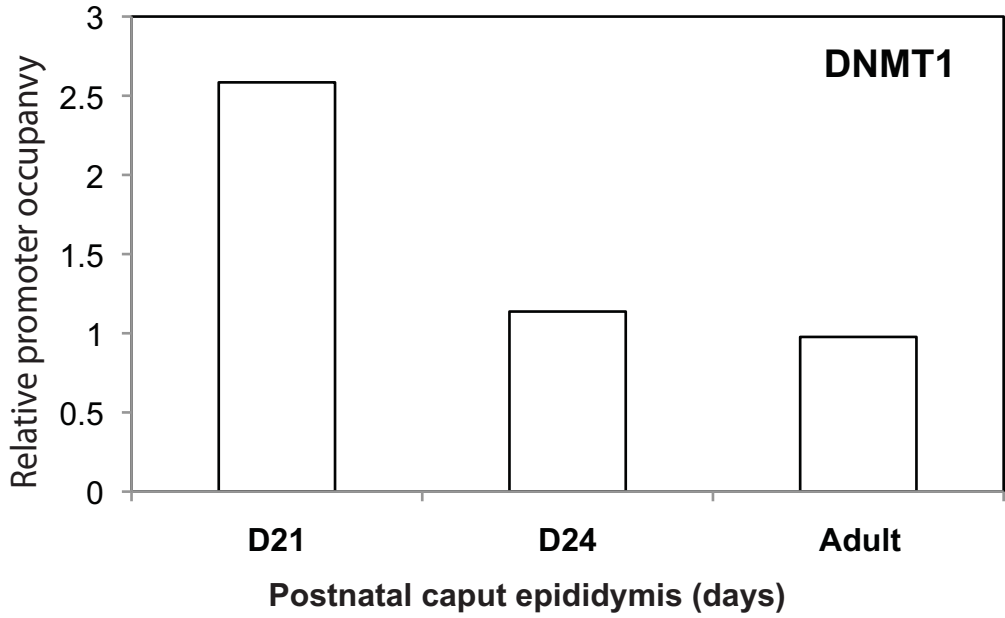

FIG. S8B