

**Table S7: Motifs enriched at functionally distinct classes of sites bound by exogenously introduced PU.1: effects of chromatin states and chromatin developmental dynamics**

A. Motif analysis of Group 5,6 and 7 peaks defined in Fig. 4C. The top five motifs defined by Homer De Novo motif search are shown for each site category. **B.** Motif analysis of exogenous PU1WTHA-occupied non-promoter (distal) peaks in CD25<sup>+</sup> cells, as detected by  $\alpha$ HA ChIP. Sites with quality scores above or below PWM score median are compared for co-enriched motifs, depending on whether they lie in normally inaccessible (PWM median score=9.74) or accessible (PWM median score=8.97) regions in DN3 cells. Note strongly similar pattern of motif preference for endogenous PU.1 at its natural sites (Fig. 1G, Supplementary Fig. S1D). The top five motifs defined by Homer De Novo motif search are shown for each site category.

C. Top five motifs enriched at PU1WTHA  $\alpha$ HA peaks in regions that change dynamically from DN1 to DN3.

Table 7: TOP MOTIFS AT SITES OCCUPIED BY EXOGENOUS PU.1 IN PRO-T CELLS

**A** Fig 4C group 5

Motif	Best match	% of Targets	% of Bg	P-value
AAAGAGGAAGT <sup>S</sup>	<i>SpiB(ETS)</i>	80.9	7.9	1e-20016
GTTTGTCATTT	<i>Cebpa</i>	2.4	0.01	1e-1476
TCCCCCATCCCT	<i>Klf5</i>	2.6	0.06	1e-955
CTAAACACACA	<i>Runx2</i>	26.0	11.9	1e-942
CITATCAGGAGG	<i>Zic</i>	2.9	0.12	1e-844

Fig 4C group 6

Motif	Best match	% of Targets	% of Bg	P-value
AAAGAGGAAGT <sup>S</sup>	<i>SpiB(ETS)</i>	63.2	3.1	1e-8735
GGGTCACTTATA	<i>Nr2f2</i>	9.6	0.3	1e-1381
AATTCTACACACA	<i>Dlx2</i>	8.7	0.2	1e-1365
CTAAAGGTGATA	<i>SD0003.1</i> at AC acceptor	9.2	0.3	1e-1348
CATGATAATATA	<i>Mecom</i>	10.3	0.4	1e-1342

Fig 4C group 7

Motif	Best match	% of Targets	% of Bg	P-value
SACTTCC <sup>S</sup> IST	<i>PU.1(ETS)</i>	65.1	16.3	1e-6469
ATGTGGTTA <sup>S</sup>	<i>Runx1</i>	12.5	4.8	1e-498
GGGGCGGGGC <sup>S</sup>	<i>Sp1</i>	15.2	9.0	1e-217
AAAAGCAGAAC <sup>S</sup>	<i>SPI1</i>	1.3	0.1	1e-213
CCACTAGGGGC <sup>S</sup>	<i>BORIS</i>	1.1	0.3	1e-89

**B**

PU1WTHA closed above PWM median score

Motif	Best match	% of Targets	% of Bg	P-value
AAAGAGGAAGT <sup>S</sup>	<i>SpiB(ETS)</i>	86.5	1.7	1e-24217
SCAAAAAGGAT <sup>S</sup>	<i>Scrt2</i>	9.6	0.2	1e-2204
GTCTCAATTCTT <sup>S</sup>	<i>Sox4</i>	8.2	0.2	1e-1772
TATATATA <sup>S</sup> AG	<i>Six6</i>	7.3	0.2	1e-1592
AIGTATTACCA <sup>S</sup>	<i>Otx</i>	6.3	0.1	1e-1548

PU1WTHA closed below PWM median score

Motif	Best match	% of Targets	% of Bg	P-value
ACTTCCT <sup>S</sup> TTT	<i>Sfp1</i>	84.0	15.3	1e-9022
TGTGGTTA <sup>S</sup>	<i>Runx2</i>	36.5	20.8	1e-506
TCTCGTC <sup>S</sup>	<i>IRC900814</i>	13.3	5.5	1e-335
TGGTTTATACAG <sup>S</sup>	<i>Cpx</i>	1.0	0.11	1e-111
AAACACCTTC <sup>S</sup> C	<i>Foxo1</i>	0.4	0.01	1e-91

PU1WTHA open above PWM median score

Motif	Best match	% of Targets	% of Bg	P-value
AAAGAGGAAGT <sup>S</sup>	<i>PU.1(ETS)</i>	42.6	3.0	1e-2123
ATGTGGTTA <sup>S</sup>	<i>Runx1</i>	17.7	6.8	1e-169
ACACAGCT <sup>S</sup> CC	<i>Ptf1a</i>	23.8	15.1	1e-67
GTGGCGGT <sup>S</sup> TTT	<i>Runx2</i>	22.8	15.1	1e-53
CCACCAAGTG <sup>S</sup> GC	<i>Ctcf</i>	1.8	0.3	1e-48

PU1WTHA open below PWM median score

Motif	Best match	% of Targets	% of Bg	P-value
ACTTCCT <sup>S</sup> TTT	<i>Sfp1</i>	52.7	22.7	1e-538
AAACACCAA <sup>S</sup>	<i>Runx1</i>	31.1	12.2	1e-315
CAC <sup>S</sup> AGCTGCC <sup>S</sup>	<i>Ctcf</i>	2.5	0.4	1e-69
GCACCTGT <sup>S</sup>	<i>E2A</i>	23.1	15.9	1e-45
AGTCAAAGTAAT <sup>S</sup>	<i>Prdm1</i>	0.4	0.01	1e-39

**C**

PU1WTHA in closing regions

Motif	Best match	% of Targets	% of Bg	P-value
AAAAGAGGAAGT <sup>S</sup>	<i>SpiB(ETS)</i>	64.6	2.8	1e-4969
TGTGGTTA <sup>S</sup>	<i>Runx1</i>	26.5	8.9	1e-394
CGGAAGT <sup>S</sup> AA	<i>PU.1-IRF</i>	12.8	2.9	1e-291
AAATCCACAAACC <sup>S</sup>	<i>Cebpa</i>	0.7	0.01	1e-67
AGCAAGTG <sup>S</sup> GC	<i>PU.1(ETS)</i>	2.4	0.5	1e-59

PU1WTHA in opening regions

Motif	Best match	% of Targets	% of Bg	P-value
CACTTCCT <sup>S</sup> TTT	<i>PU.1(ETS)</i>	64.3	5.9	1e-2208
AACCACAA <sup>S</sup>	<i>Runx2</i>	20.7	9.2	1e-112
CCAGGTG <sup>S</sup> AA	<i>Tcf3</i>	30.1	17.0	1e-98
AGAT <sup>S</sup> AAAGS <sup>S</sup>	<i>Tcf3(HMG)*</i>	13.0	5.3	1e-81
GTGATAAGATCA <sup>S</sup>	<i>GATA</i>	0.6	0.01	1e-32

\*Tcf3(HMG) = Tcf7l1

