Legends to Supplementary Figures

Supplementary Figure 1. Characterization of Pax7 EMSA complexes with various probes. A) DNA sequences of probes used in gel retardation assays (EMSA). B,C) Competitive EMSA assays to assess relative affinities of composite HP.E0, PD and H.I2 targets. Complexes formed between the HP.E0 probe and Pax7 from AtT20Pax7 nuclear extracts were competed with the indicated molar excesses of either HP.E0, PD or H.I2 probes. Displacement curves (means ±SD, n=4) for quantitated band intensities (Image Lab, BioRad) were computed with GraphPad Prism. D) EMSA supershift experiments using the Flag2 antibody to supershift Flag-Pax7-containing DNA complexes.

Supplementary Figure 2. Nucleosome positioning relative to Pax7 Pioneer sites was determined by MnaseSeq analyses in AtT20neo and AtT20Pax7 cells. The MnaseSeq heatmaps (right) are sorted according to central read densities (+/-100 bp) and centered (+/- 1000bp) on the by Pax7 peak summit for each subset of sites. For comparison (left), the Pax7 ChIPseq and ATACseq profiles centered on Pax7 binding are shown (data from ref. 4). Only the Pioneered subset of sites shows a different pattern in AtT-Pax7 cells compared to the Neo control, revealing recruitment to nucleosome-occupied sites and nucleosome depletion after Pax7 binding.

Supplementary Figure 3. Characterization of Pax7 mutants within the PD (G43A and R56L) and HD (S264E) domains. A) Western blot analysis for expression of Pax7 and three mutants in AtT20 cells expressing N-terminally flagged Pax7 proteins. PCNA Western blot is used as loading control. B) EMSA using the indicated DNA probes incubated with nuclear extracts from AtT20 cells expressing the indicated Pax7 and mutants. C) Transcriptional activity of Pax7 and its DBD mutants assessed by cotransfection of indicated expression plasmids together with a luciferase reporter plasmid driven by six copies of the HP.E0 composite or by the PC2 gene enhancer. Luciferase activity was assessed as previously (5) in extracts of AtT20 cells prepared 48 hours post-transfection with PEI25K. D) Genomic recruitment (Flag-Pax7 ChIPseq) and ATACseq profiles at the Pax7 Pioneered sites compared to the same sites in AtT20 cells expressing the three Pax7 DBD mutants.

Supplementary Figure 4. Alignment of mouse Pax7, human PAX3 and human PAX6 sequences showing structural features of PD (green) and HD (blue) DNA binding domains. Sequences (Accession numbers P47239.2, P23760.2, P26367.2) were aligned using NCBI Blast 2. Amino acids legend: Black = all different ; Blue = 2/3 identical ; Red = 3/3 identical, Upper case = corresponding in number ; lower case = specific to one protein. The numbering used in indicated previous publications is shown together with positions in mouse Pax7 used in present work.

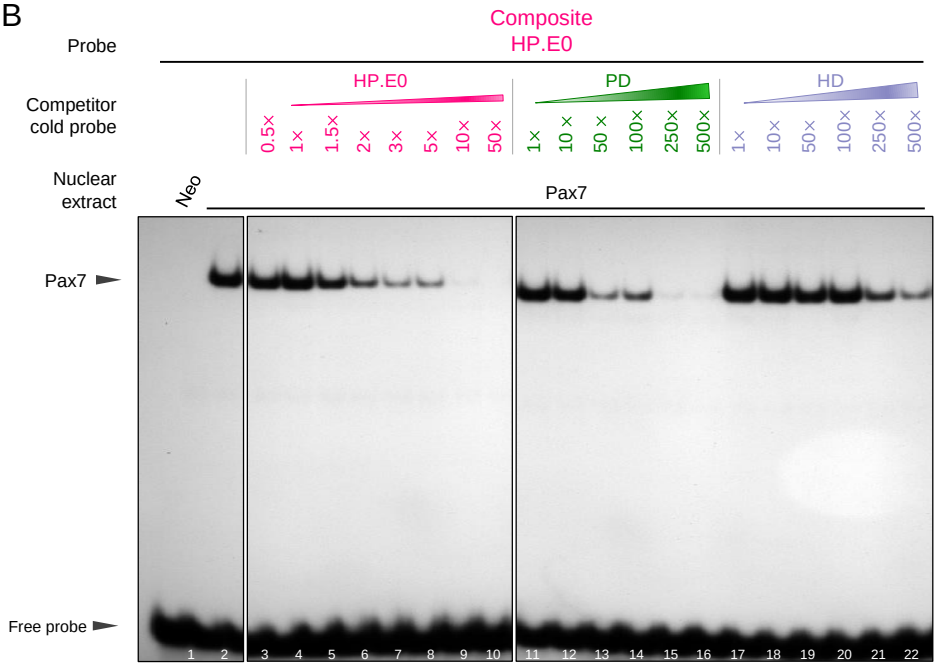
Supplementary Figure 5. Representative loci for genomic recruitment (Flag-Pax7 ChIPseq) and ATACseq for wildtype Pax7 and three DBD mutants as indicated. Two representative loci are shown for each subset of peaks in the Constitutive, Activated and Pioneered categories. Examples are shown for peaks containing multiple motifs (A), or only one Composite (B), PD (C) or HD (D) motif.

Supplementary Figure 6. Pax7 target genes. A) Time courses of mRNA accumulation determined by RNAseq analyses (5) showing rapid responses for genes regulated through “Pax7 Activated” enhancers compared to the slower responses of “Pioneered” target genes. B) A unique example, the *Oacyl* gene, of Pax7 pioneer action at promoter rather than enhancer sequences. Gene browser views of the *Oacyl* locus show the indicated ChIPseq and ATACseq analyses performed in AtT20Neo, AtT20Pax7 WT or mutant cells.

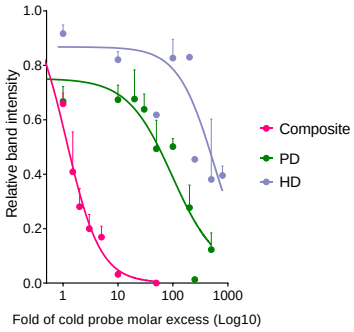
A

Probe sequences	
Composite HP.E0	TCGACGCAGAT**CCGTGACTAAT**GTTCCGACCTCGA
PD (mutated HD)	TCGACGCAGAT**CCGTGAC***GCCA*GTTCCGACCTCGA
HD (mutated PD)	TCGACGCAGAT*CGTCCGC***TAAT**TCCAACGACCTCGA
H.I-2	TCGACCAGAT*CGTCCGC***TAATTA**CCAACGACCTCGA
H.I2	TCGACTCGTCCGC**TAATTGATTA**TCCAACGACCTCGA

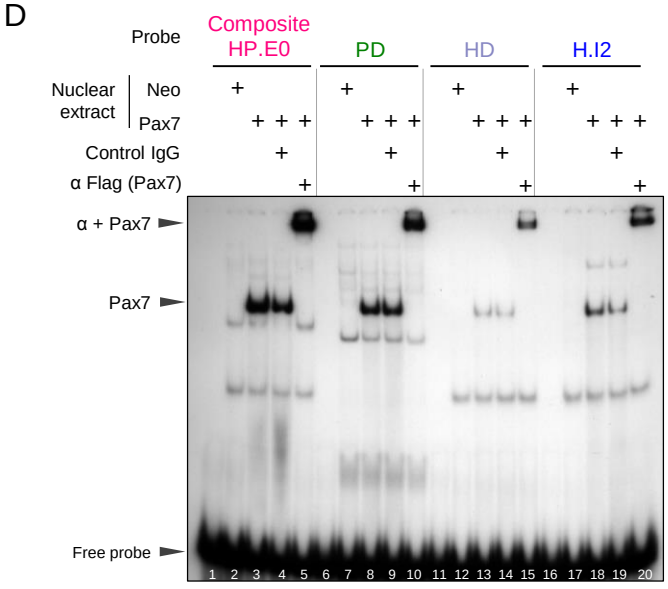
B



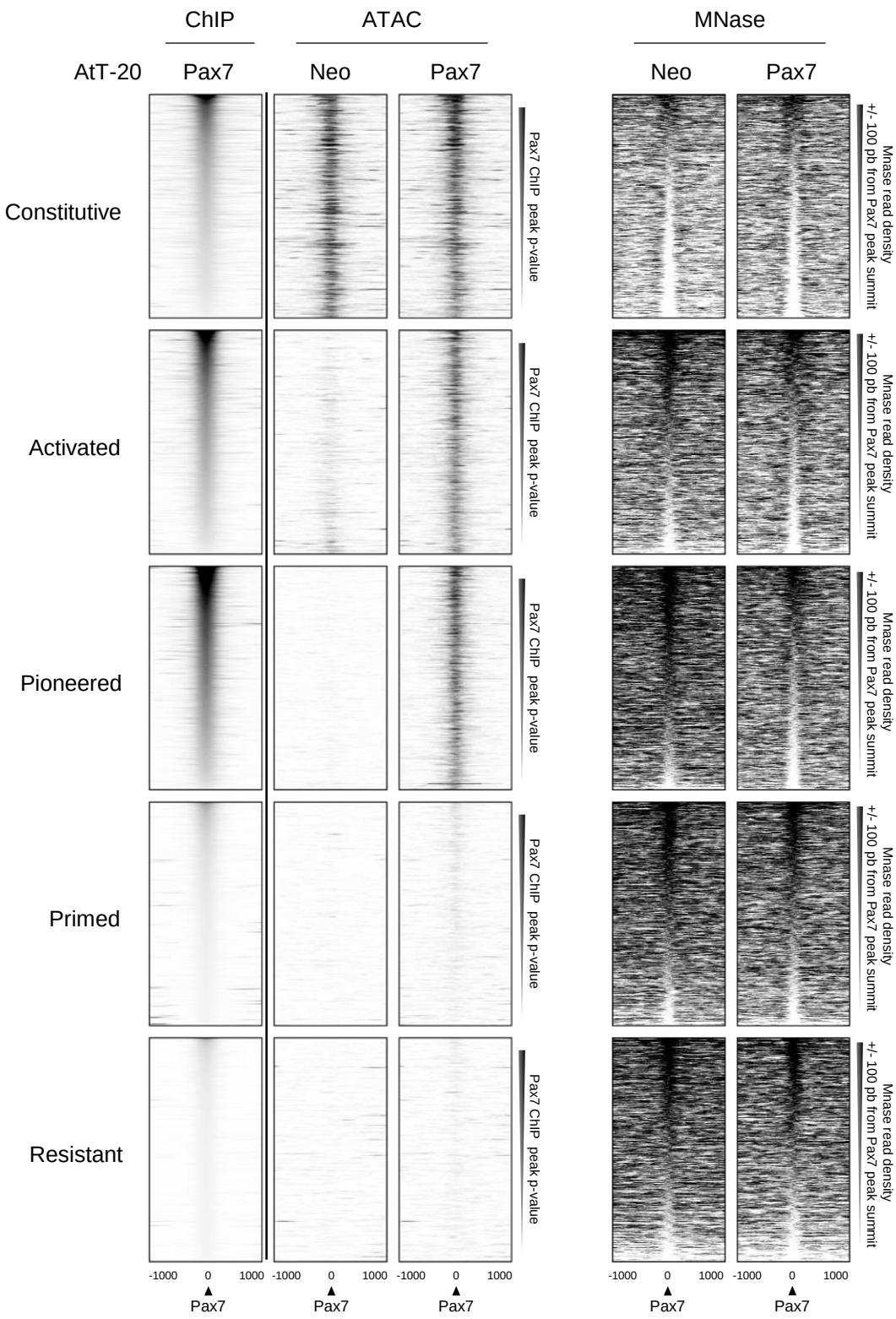
C

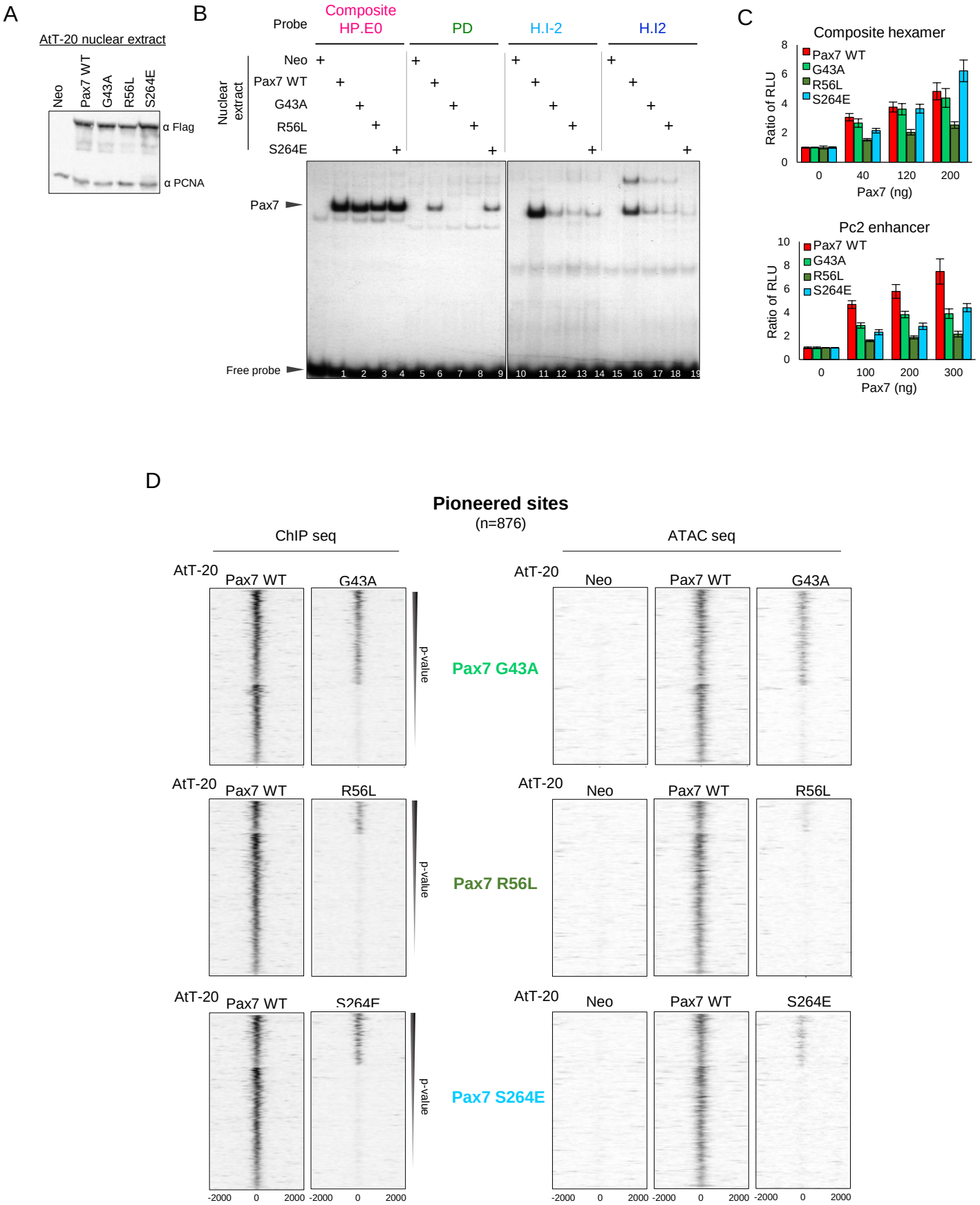


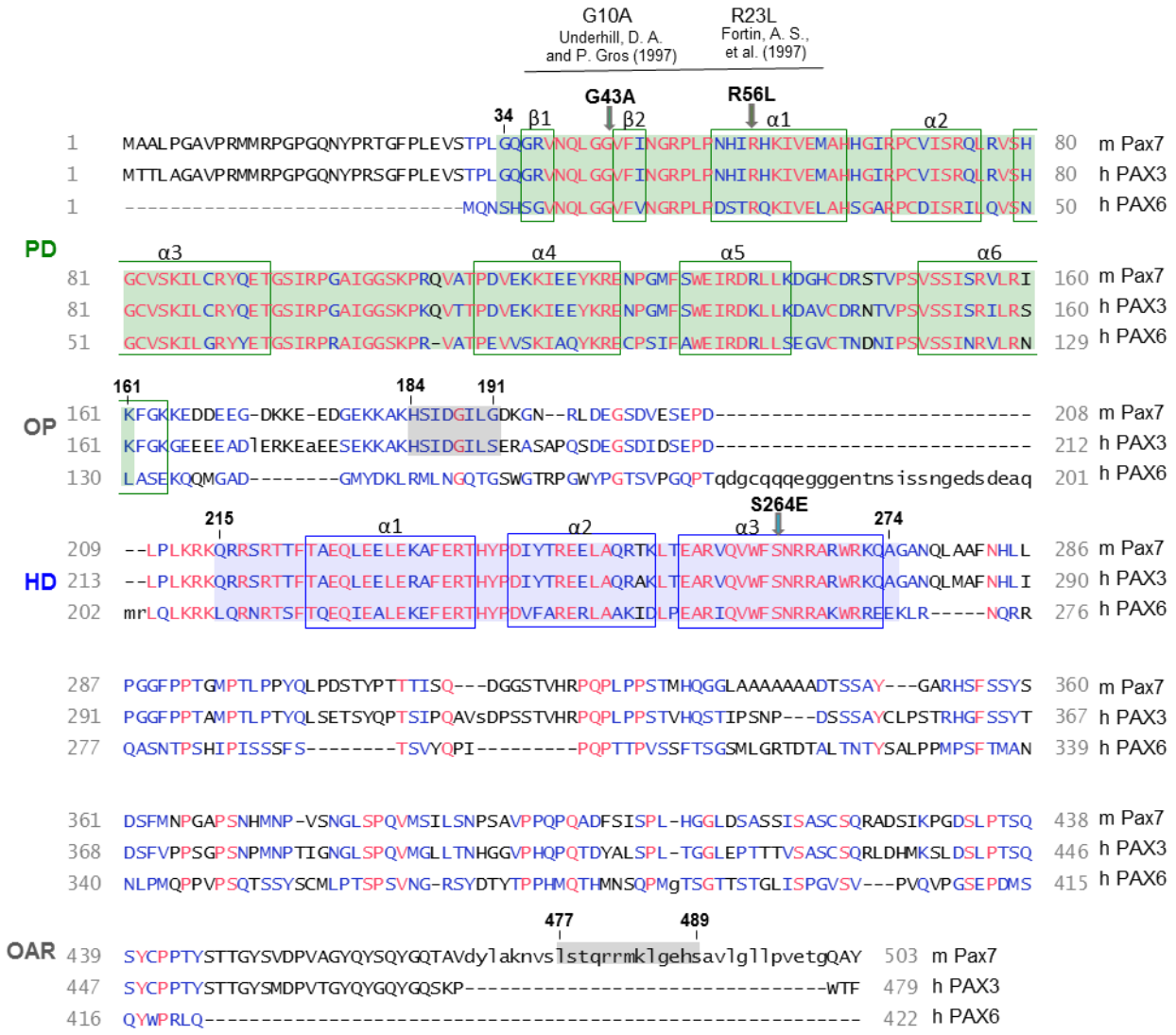
D



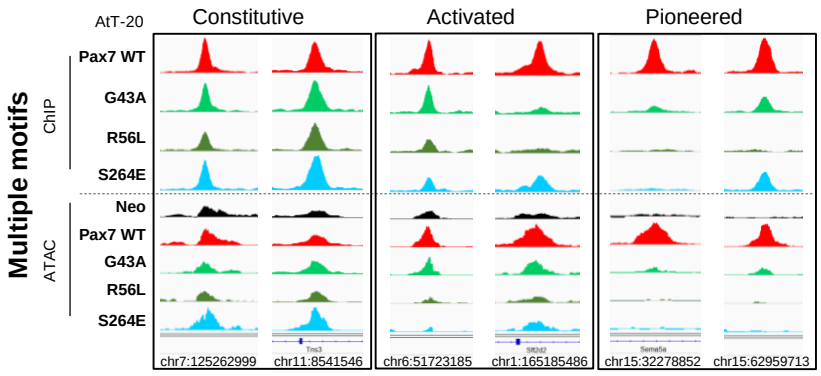
A


ChIP
ATAC
MNase
AtT-20
Pax7
Neo
Pax7
Neo
Pax7
Constitutive
Activated
Pioneered
Primed
Resistant
Pax7 ChIP peak p-value
Mnase read density +/- 100 pb from Pax7 peak summit
-1000
0
1000
Pax7

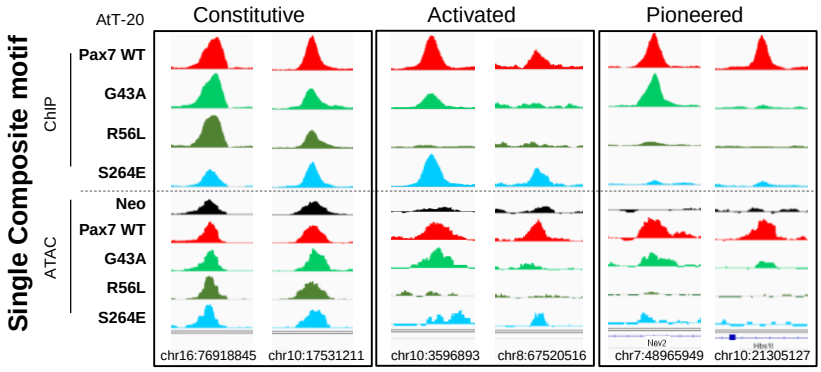

A
AtT-20 nuclear extract
Neo
Pax7 WT
G43A
R56L
S264E
α Flag
α PCNA
B
Probe
Composite HP.E0
PD
H.I-2
H.I2
Nuclear extract
Neo
Pax7 WT
G43A
R56L
S264E
Pax7
Free probe
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
C
Composite hexamer
Pax7 WT
G43A
R56L
S264E
Ratio of RLU
Pax7 (ng)
Pc2 enhancer
D
Pioneered sites
(n=876)
ChIP seq
ATAC seq
AtT-20
Pax7 WT
G43A
Neo
Pax7 G43A
R56L
Pax7 R56L
S264E
Pax7 S264E
p-value
-2000
0
2000


G10A
Underhill, D. A. and P. Gros (1997)
R23L
Fortin, A. S., et al. (1997)
G43A
R56L
34
β1
β2
α1
α2
PD
α3
α4
α5
α6
OP
161
184
191
HD
215
S264E
274
OAR
477
489
m Pax7
h PAX3
h PAX6

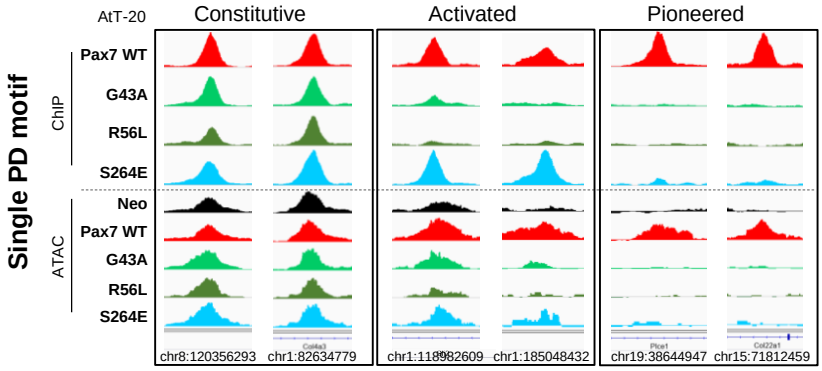
A


Multiple motifs
AtT-20
Constitutive
Activated
Pioneered
ChIP
ATAC
Pax7 WT
G43A
R56L
S264E
Neo
chr7:125262999
chr11:8541546
chr6:51723185
chr1:165185486
chr15:32278852
chr15:62959713

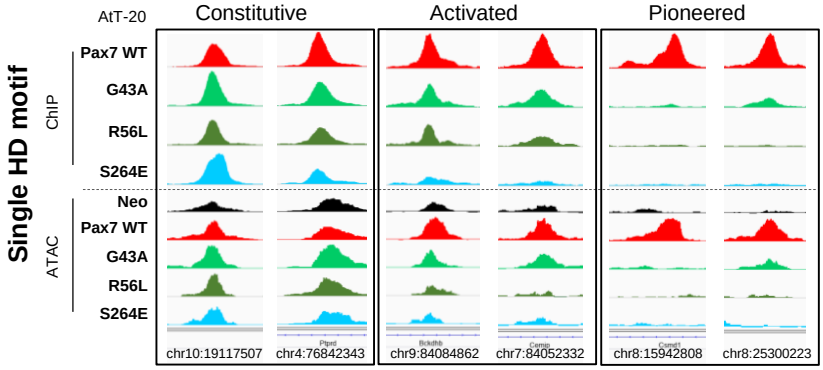
B


Single Composite motif
AtT-20
Constitutive
Activated
Pioneered
ChIP
ATAC
Pax7 WT
G43A
R56L
S264E
Neo
chr16:76918845
chr10:17531211
chr10:3596893
chr8:67520516
chr7:48965949
chr10:21305127

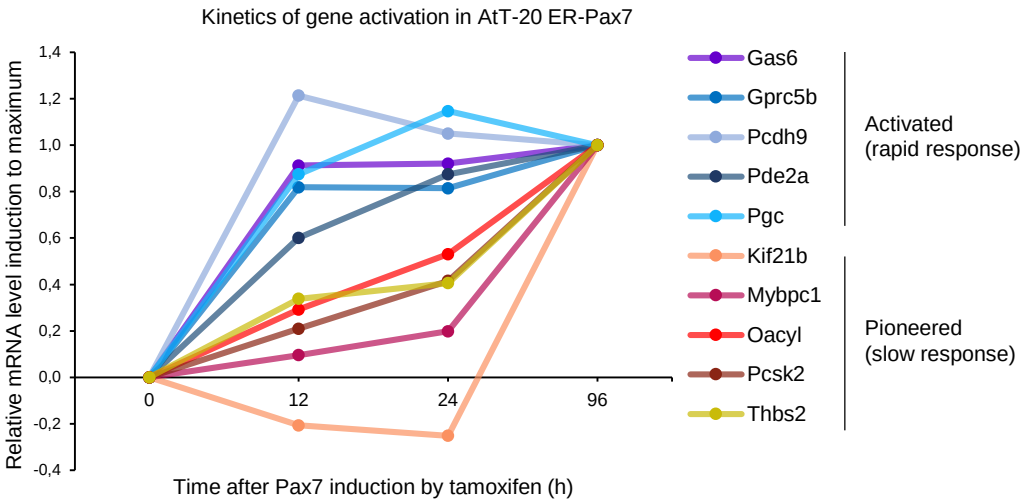
C


Single PD motif
AtT-20
Constitutive
Activated
Pioneered
ChIP
ATAC
Pax7 WT
G43A
R56L
S264E
Neo
chr8:120356293
chr1:82634779
chr1:185048432
chr19:38644947
chr15:71812459

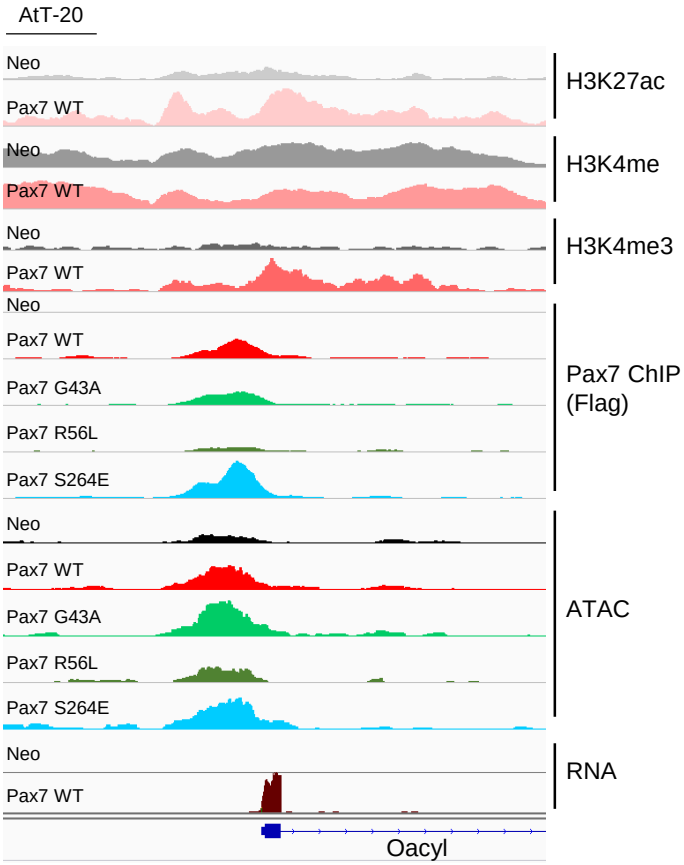
D


Single HD motif
AtT-20
Constitutive
Activated
Pioneered
ChIP
ATAC
Pax7 WT
G43A
R56L
S264E
Neo
chr10:19117507
chr4:76842343
chr9:84084862
chr7:84052332
chr8:15942808
chr8:25300223

A


Kinetics of gene activation in AtT-20 ER-Pax7
Relative mRNA level induction to maximum
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
-0,2
-0,4
0
12
24
96
Time after Pax7 induction by tamoxifen (h)
Gas6
Gprc5b
Pcdh9
Pde2a
Pgc
Kif21b
Mybpc1
Oacyl
Pcsk2
Thbs2
Activated
(rapid response)
Pioneered
(slow response)

B


AtT-20
Neo
Pax7 WT
H3K27ac
Neo
Pax7 WT
H3K4me
Neo
Pax7 WT
H3K4me3
Neo
Pax7 WT
Pax7 G43A
Pax7 R56L
Pax7 S264E
Pax7 ChIP
(Flag)
Neo
Pax7 WT
Pax7 G43A
Pax7 R56L
Pax7 S264E
ATAC
Neo
Pax7 WT
RNA
Oacyl

Supplementary Table 1. Sequences of oligonucleotide primers

Gene symbol	Sequence accession number	Amplicon length	Primer sequence	Location
Gapdh	NM_008084.3	110	TGCAGTGGCAAAGTGGAGAT	Exon 2
			ACTGTGCCGTTGAATTTGCC	Exon 2
Gas6	NM_019521.2	107	GCCTCTATCACCTGAATCT	Exon 11
			CCAGTTCCAACTCCTCAT	Exon 12
Gprc5	NM_022420.2	122	CACCGTTCAGAAGCAATG	Exon 3
			GGAACTTAAGGTCTTTCACC	Exon 4
Pcdh9	NM_001081377.3	150	CAGCCACAGGACGAATTCTAC	Exon 4
			ACCCAAAACCAGACACTCTTG	Exon 5
Pde2a	NM_001143848.2	161	GAGAACCAGGAGGTCATT	Exon 18-19
			CGGTATTGGGCTTCATTC	Exon 20
Pgc	NM_025973.3	168	TGCCCAGTACCTGAATGA	Exon 7
			CCTTCCTCCTGGATGATGTA	Exon 8-9
Kif21b	NM_001039472.2	161	AGAACCAGTCTCGCTATG	Exon 15
			CTTCTTGAGCTGAGCAATC	Exon 16
Mybpc1	NM_175418.5	200	ACGAGGTCCGAATCTTTG	Exon 19
			ATACTCTAGCACATAGCCATC	Exon 20
Oacyl	NM_177028.3	181	GTCCAGATATGTTTGCTTCC	Exon 4
			CTGTTGCTCTCCATGTTTC	Exon 5
Pcsk2	NM_008792.4	123	TGACAAGTGGCCTTTCAT	Exon 12
			ATCAGGGTCCATTCCTTC	Exon 12
Thbs2	NM_011581.3	183	CACTCAAAGTGGTAAACTCC	Exon 19
			CTCTCATGTAGCCTGTCTTA	Exon 20-21

Supplementary Table 2. Summary of high throughput sequencing datasets and experimental conditions

Experiment	Cell line	Number of cells	Sequencing depth (read number)	Matching control for peak calling	Antibody reference	Antibody quantity (µg)	Replicate number
ATAC-seq	AtT-20 Neo	1,00E+05	95251912	Input AtT Pax7	N/A	N/A	2
ATAC-seq	AtT-20 Pax7	1,00E+05	82583056	Input AtT Pax7	N/A	N/A	2
ATAC-seq	AtT-20 Pax7 G43A	1,00E+05	32502682	Input AtT Pax7	N/A	N/A	1
ATAC-seq	AtT-20 Pax7 R56L	1,00E+05	31497109	Input AtT Pax7	N/A	N/A	1
ATAC-seq	AtT-20 Pax7 S264E	1,00E+05	26377490	Input AtT Pax7	N/A	N/A	1
ChIP-seq	AtT-20 Pax7	1,00E+07	77949054	AtT-20 Neo	Flag M2 Sigma	6	1
ChIP-seq	AtT-20 Pax7 G43A	1,00E+07	94880640	AtT-20 Neo	Flag M2 Sigma	6	1
ChIP-seq	AtT-20 Pax7 R56L	1,00E+07	72825980	AtT-20 Neo	Flag M2 Sigma	6	1
ChIP-seq	AtT-20 Pax7 S264E	1,00E+07	102208451	AtT-20 Neo	Flag M2 Sigma	6	1
MNAse-seq	AtT-20 Neo	3,00E+06	220458865	N/A	N/A	N/A	2
MNAse-seq	AtT-20 Pax7	3,00E+06	229205088	N/A	N/A	N/A	2

Supplementary Table 3. ChIP and ATAC-seq thresholds to categorize Pax7 peaks

	Pax7	H3K4me1		p300		ATAC		SMC1
	AtT-20 Pax7	AtT-20 Neo	AtT-20 Pax7	AtT-20 Neo	AtT-20 Pax7	AtT-20 Neo	AtT-20 Pax7	AtT-20 Neo
Constitutive n = 13 126	PEAK AT p ≤ 1e-5	PEAK AT p ≤ 1e-5	PEAK AT p ≤ 1e-5	PEAK AT p ≤ 1e-5	PEAK AT p ≤ 1e-5	PEAK AT p ≤ 1e-5	PEAK AT p ≤ 1e-5	N/A
Activated n = 733	PEAK AT p ≤ 1e-5	PEAK AT p ≤ 1e-5	PEAK AT p ≤ 1e-5	NO PEAK AT p ≤ 1e-3	PEAK AT p ≤ 1e-5	N/A	PEAK AT p ≤ 1e-5	N/A
Pioneered n = 876	PEAK AT p ≤ 1e-5	NO PEAK AT p ≤ 1e-3	PEAK AT p ≤ 1e-5	NO PEAK AT p ≤ 1e-3	PEAK AT p ≤ 1e-5	NO PEAK AT p ≤ 1e-3	PEAK AT p ≤ 1e-5	N/A
Primed n = 5 352	PEAK AT p ≤ 1e-5	NO PEAK AT p ≤ 1e-3	PEAK AT p ≤ 1e-5	NO PEAK AT p ≤ 1e-3	NO PEAK AT p ≤ 1e-3	NO PEAK AT p ≤ 1e-3	N/A	N/A
Resistant n = 8 541	PEAK AT p ≤ 1e-5	NO PEAK AT p ≤ 1e-3	NO PEAK AT p ≤ 1e-3	NO PEAK AT p ≤ 1e-3	NO PEAK AT p ≤ 1e-3	NO PEAK AT p ≤ 1e-3	NO PEAK AT p ≤ 1e-3	NO PEAK AT p ≤ 1e-3