Pre-patterning of differentiation-driven nuclear lamin A/C-associated chromatin domains by GlcNAcylated histone H2B

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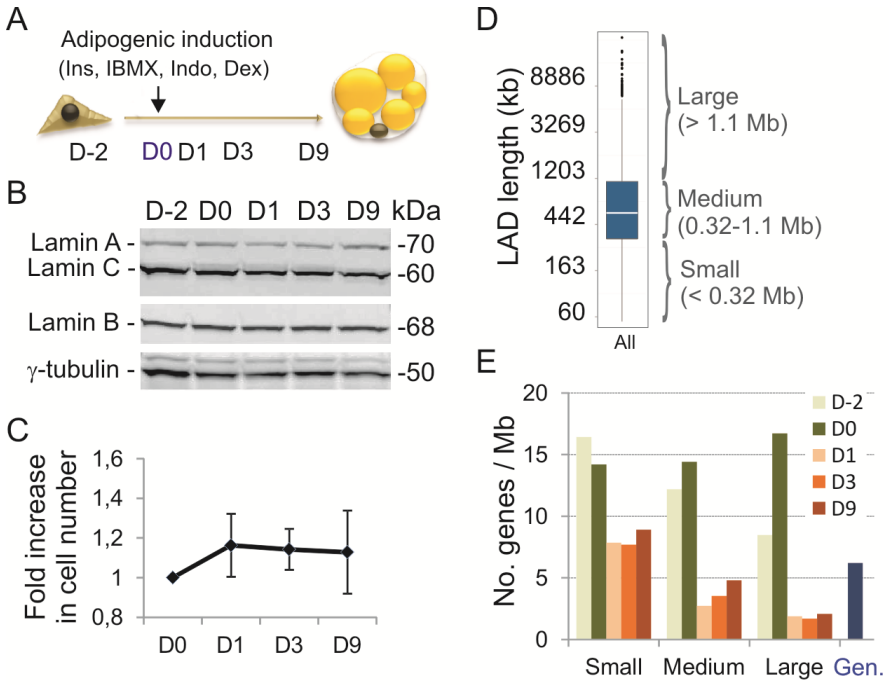
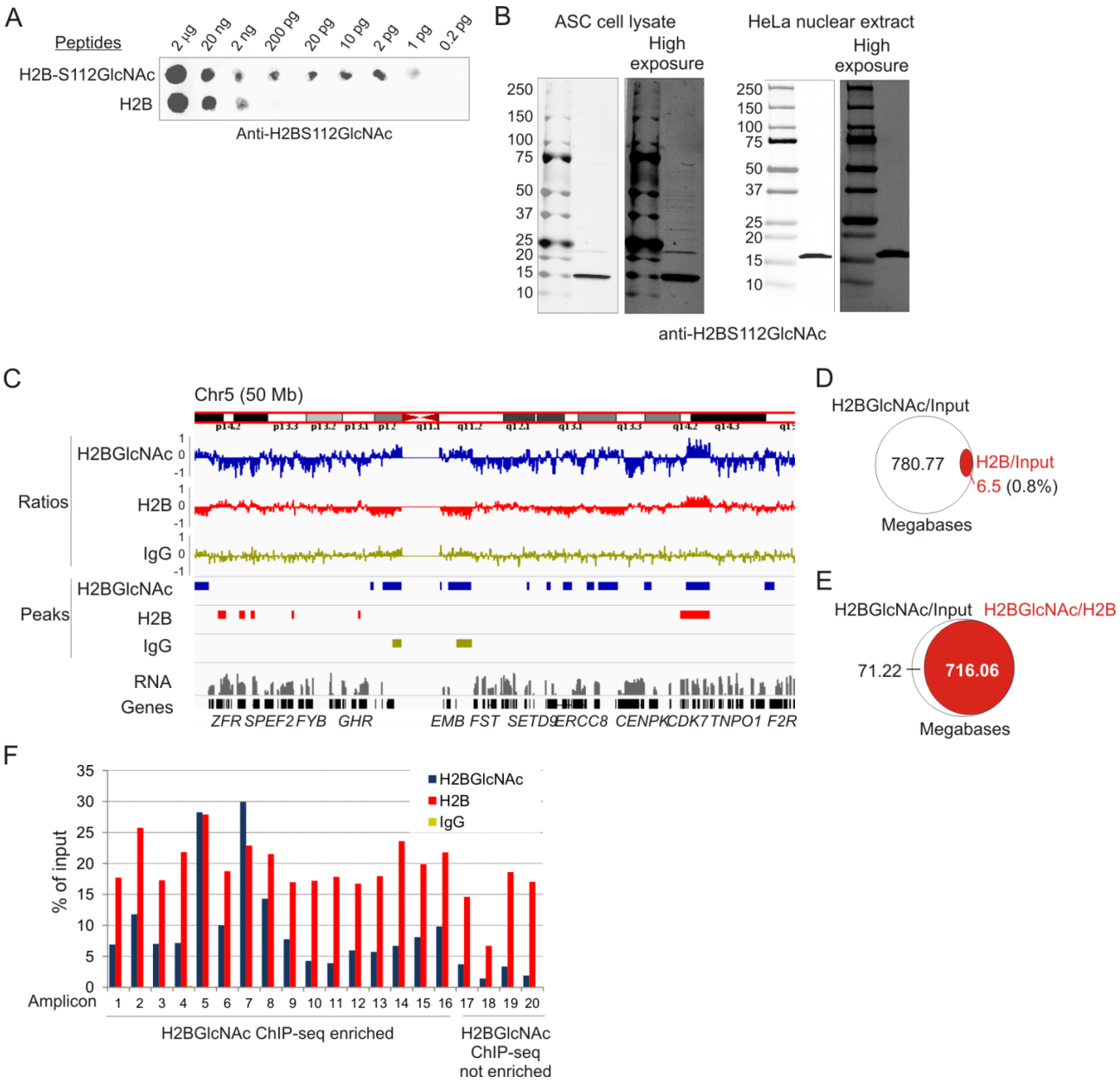
Supplemental Information

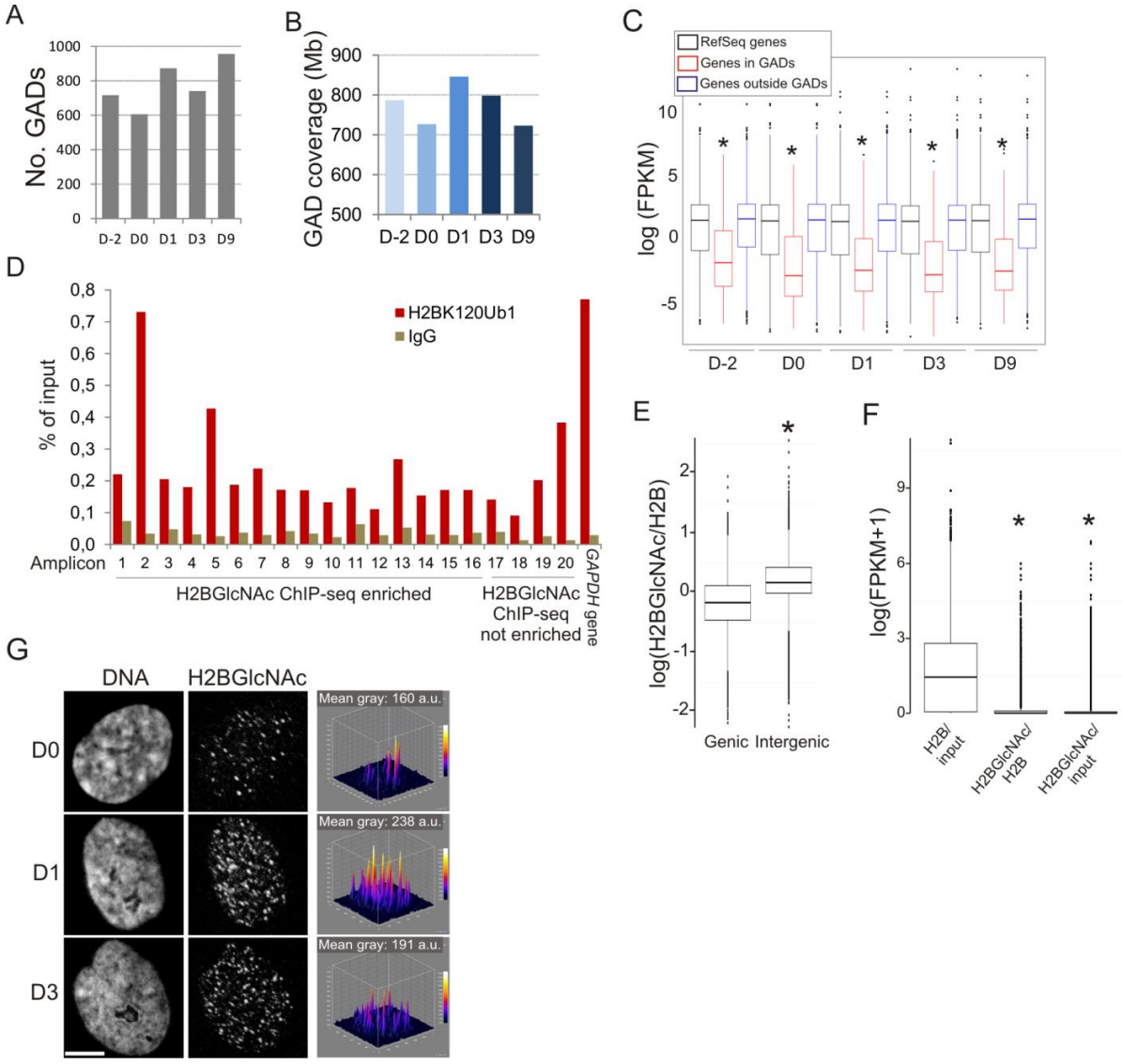


Supplemental Figure 1. Genic composition of lamin A/C LADs during adipogenic differentiation. (A) *In vitro* adipogenic differentiation design and cell collection time points (days). D0 is the time of adipogenic induction. (B) Western blots of lamin A/C and B1 during differentiation, with γ-tubulin as loading control (representative of 3 experiments). (C) Fold increase in cell numbers from D0 to D9 (mean ± SD of 5 experiments). (D) Median length of LADs at all differentiation stages confounded, and partitioning into small, medium and large domains based on quantile size distribution. (E) Gene density in small, medium and large LADs at each differentiation stage, and in the whole genome (Gen.).

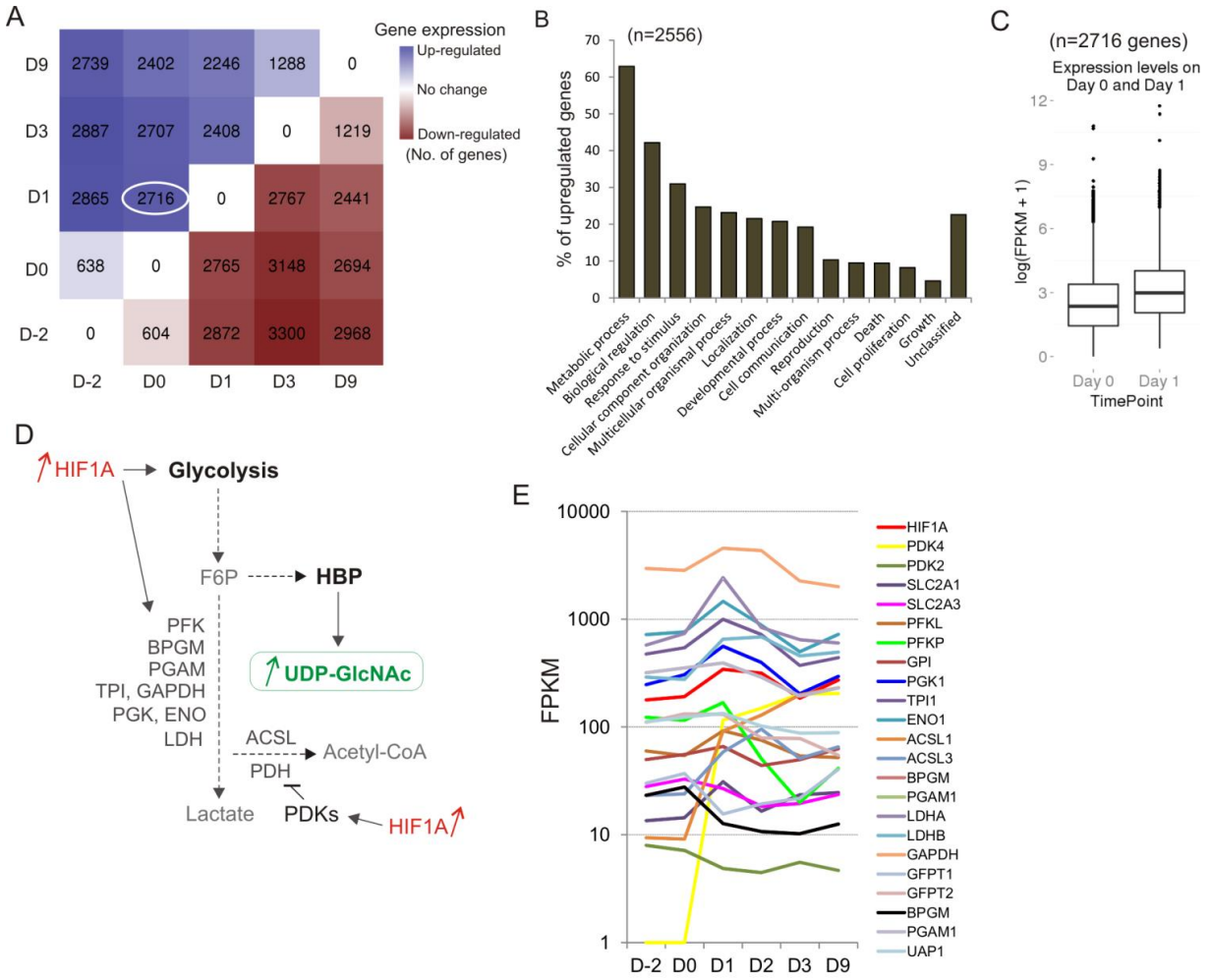


Supplemental Figure 2. Specificity of the anti-H2BS112GlcNAc antibody. (A) Immunoreactivity of anti-H2BS112GlcNAc antibodies on H2BS112GlcNAc and corresponding unmodified H2B peptides (KHAVS^{112}EGTK) immobilized on nitrocellulose. (B) Western blots of H2BS112GlcNAc for an ASC cell lysate (left) and HeLa nuclear extract (right). Overexposed blots are also shown. (C) Browser view of ChIP-seq enrichment profiles and peaks of H2BS112GlcNAc (H2BGlcNAc), total H2B and of a control IgG (ASCs on D-2). (D) Venn diagram analysis of peaks of total H2B overlapping with peaks of H2BGlcNAc (red; in Mb). (E) Venn diagram analysis of overlapping peaks of H2BGlcNAc detected from ratios of (H2BGlcNAc / H2B) (red) and peaks of H2BGlcNAc detected from ratios of (H2BGlcNAc / input). In (C-E), peaks were detected using EDD. (F) ChIP-qPCR analysis of

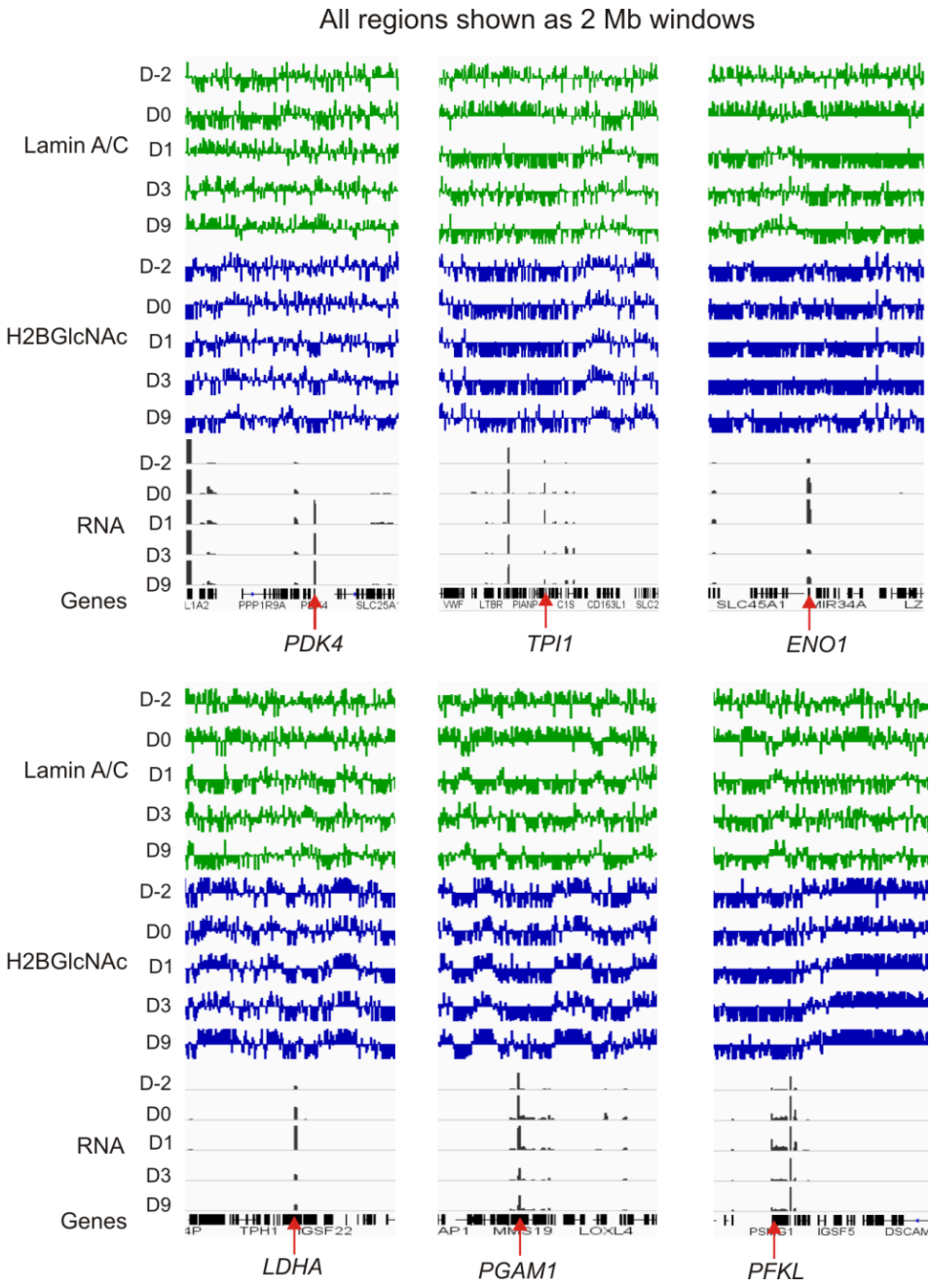
H2BS112GlcNAc and pan-H2B in 20 loci, of which 16 were found by ChIP-seq to be enriched in H2BGlcNAc and 4 not. An unspecific IgG was used in negative control ChIPs. Genomic location of PCR amplicons is shown in Supplemental Table 1.



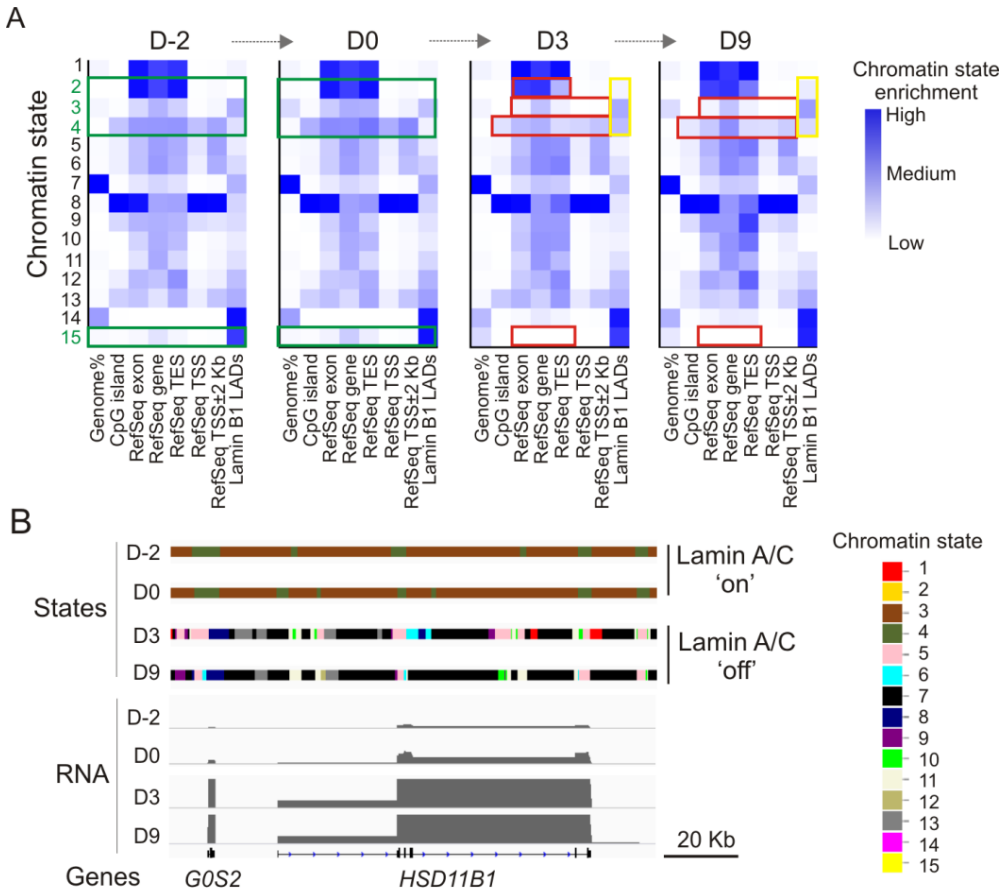
Supplemental Figure 3. Genes located in H2B GlcNAcylated domains (GADs) are repressed. (A) Number of GADs at each adipogenic differentiation stage. (B) GAD coverage throughout differentiation. (C) Median expression level of genes (FKPM > 0) within GADs, outside GADs, and for all RefSeq genes at each differentiation time point; *$P < 10^{-5}$, t-test with Bonferroni correction. (D) ChIP-qPCR analysis of H2BK120ub1 in D-2 ASCs for the 20 loci examined for H2BGlcNAc enrichment (see Supplementary Fig. 2F). (E) Ratio of H2BGlcNAc/H2B is higher in intergenic regions compared to genes ($P = 2.2x10^{-16}$; Wilcoxon test). Size of intergenic regions was set from 10 Kb to 1 Mb. (F) Gene expression level is lower in H2BGlcNAc domains identified either by ratios of H2BGlcNAc/H2B or H2BGlcNAc/input. (G) Confocal immunofluorescence analysis of H2BGlcNAc in ASC nuclei on D0, D1 and D3, and quantification of H2BGlcNAc signal intensities (gray level scale: 100-1300); DNA stained with DAPI; bar, 10 µm.



Supplemental Figure 4. Differential gene expression and lamin A/C enrichment at the D0-D1 transition of the adipogenic program. (A) Numbers of differentially up- and down-regulated genes (fold change > 2, $\alpha < 0.05$) between differentiation time points. (B) GOSlim analysis of gene ontology enrichment distribution for genes upregulated at the D0-D1 transition; 2556 genes out of 2716 total upregulated genes (see A) were associated with enriched GO terms. Note the majority of genes implicated in metabolic processes. (C) D0 and D1 median expression levels of genes upregulated on D1. This shows that these genes are already expressed on D0, consistent with their localization outside GADs (see main text). (D) Simplified representation of the glycolytic pathway and the HBP, with genes examined. (E) mRNA levels of *HIF1A* and HIF1A protein target genes involved in glycolysis, and of pyruvate dehydrogenase kinase (*PDK4*) which inhibits mitochondrial pyruvate oxidation (RNA-seq data).



Supplemental Figure 5. Browser views of extended 2 Mb regions around *PDK4* and indicated glycolytic genes. Zoom-in views on these genes are shown in Figure 4F. Lamin A/C and H2BGlcNAc log ratios are shown (-0.4 to +0.4 range) along with RNA-seq profiles (FPKM) at each differentiation time point.



C

Permutation t-test P-values on chromatin states for indicated genomic regions (D3 vs D0 time points)

	CpG island	Gene	TES	TSS	TSS+2kb
State 2	1.84E-130	1.04E-32	2.17E-137	4.26E-20	1.75E-99
State 3	1.70E-152	3.58E-139	3.73E-139	3.50E-163	7.06E-178
State 4	2.56E-180	5.02E-164	1.91E-160	2.24E-198	2.31E-179
State 15	6.80E-69	1.03E-72	2.02E-63	2.30E-63	2.57E-66

Supplemental Figure 6. Relationship between chromatin states and lamin A/C LADs during adipogenic differentiation. (A) Heat maps of the relative abundance of the 15 chromatin states (y axis) in pre-defined genomic regions (x axis) on D-2, D0, D3 and D9 of differentiation. Chromatin state composition is shown in Figure 6A. D0 and D3 data are also shown in Figure 6B. (B) Chromatin state transitions from D-2 to D9 exemplified for the activated *G0S2/HSD1B1* adipogenic locus. This example illustrates a prototypical lamin A/C 'off' scenario shown on D3. Here, chromatin states (see Fig. 6A) are ascribed an arbitrary color. mRNA levels are shown (RNA-seq; scale 0-500 FPKM). (C) P-values from permutation t-tests of the significance of enrichment level of lamin A/C-containing states (2, 3, 4, 15) for indicated pre-defined genomic regions between D0 and D3.

Supplemental Table 1. ChIP-qPCR primers and genomic location of amplicons

Amplicon	Chromosome	Forward primer (F) Reverse primer (R)	Genomic position (nt)
1	Chr4	F: TGCTGAGATCAGGACGATGA R: GGTACATTCTCCAAACGCCT	137018662-137018783
2	Chr11	F: TGTACCCCTGAGACAATGCG R: TCCCCCATCCTTTGTCAGGA	93904617-93904756
3	Chr3	F: GTCAAAGCCTGGCAGGAATC R: AGACAGGTGCTGAGGGTAAC	80679378-80679526
4	Chr1	F: ATTCAGTGGTCAGGGGGACT R: CTATGGCTTGGGAGGAACCC	193726147-193726246
5	Chr19	F: CCTATCCATCCATCACGCCC R: GAGAACGTAGCACCCGTAGG	1817420-1817568
6	Chr6	F: TCCCTGCCAGTATTTGTGTCC R: AGGCACATACCTCTGTTGGC	97311409-97311531
7	Chr10	F: TCCCAGCTGCAATGATGGAG R: TTACTGCTGCTTCTCCTGC	133379136-133379252
8	Chr12	F: TTGCCGGATTCCATTTGCAC R: CCCCAGCTTTAGAGGGTGTT	130607905-130608000
9	Chr 1	F: ACCTGCTTATATTTGGACCCATCA R:CACTCGGGGATAGCATTGGA	74795144-74795244
10	Chr 3	F: CCTGGGAACATAACAGCAATCT R: TTGGTCATGTCATTGAGGGTGC	84675114-84675194
11	Chr 4	F: ACTGAAATCATGCAGACAGGTCA R: CTAGTCTGCTTTTCTTCAGGCAA	171783280-171783374
12	Chr 4	F: CTCATTAACGCAGGCCCTACT R: ATCCTGTGTTCCTACACACACATT	93453065-93453135
13	Chr 8	F: GAAAGCTGGGACAGCTATGTT R: CTAGCCATGGGATGACTGTTCTT	84868271-84868385
14	Chr 9	F: AAGTATTCCGAGACACAGCATCA R: TTAGCTACTCTCGCCAATGTGA	10865295-10865365
15	Chr 11	F: AAGTAAGAGGAATATTAGAGGACCC R: AGCCAGTGATTTGAGAAGCCT	55762254-55762353
16	Chr 21	F: TGGAGAGACATTCATGGGTCC R: GTGGTGATACTGGTCATTCGTG	22671495-22671609
17	Chr 21	F: TAAGGGACCTCAACGTTTCCAT R: TGCCTACCTTAGGATGGTCC	24852033-24852106
18	Chr1	F:TTTGTGAAGGGAAGGGAGCG R:GCACTGCCATTTTACCCAGC	27022114-27022219
19	Chr2	F: GAAATGTTTCTGCGCGGGAC R:TGCCTCATTACACTGTCGGG	66667523-66667607
20	Chr9	F: AAGTCAAAGGAGCCGTCGAT R: CTGTCCAGCCGCCTAACAA	113799779-113799889
GAPDH gene	Chr12	F: GGCTCCCACCTTTCTCATCC R: GGCCATCCACAGTCTTCTGG	

Supplemental Table 2. Lists of differentially expressed genes at the D0/D1 and D1/D3 transitions, and associated enriched Gene Ontology terms

See Excel file ‘Table S2.xls’