

SUPPLEMENTARY METHODS

Chromatin Immuno-Precipitation (ChIP). Cells were crosslinked for 10 minutes at 37°C with 1% formaldehyde added to the medium. They were collected and lysed in a buffer containing 25 mM Tris-Cl pH8.0, 1.5 mM MgCl₂, 10 mM KCl, 0.5% NP40, 1 mM DTT, 0.5 mM PMSF. Nuclei were collected, resuspended in 50 mM Tris-Cl pH8.0, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium desoxycholate, 0.1% SDS and disrupted by sonication using a Bioruptor sonicator (Diagenode), under conditions producing DNA fragments ranging from 1 to 3 kb in length. Chromatin was immuno-precipitated using various antibodies (Supplementary Table S1) and 10⁷ cells were used for one ChIP. Crosslinking was reversed at 65°C overnight. After RNase treatment (100 µg/ml) for one hour at 37°C and incubation with proteinase K (100 µg/ml) for 2 hours at 42°C, immunoprecipitated DNA was purified by phenol/chloroform extraction and analyzed by quantitative PCR or by microarray hybridization (ChIP-on-chips).

Real time quantitative PCR. Quantifications were carried out using the 7500 Real time PCR system (Applied Biosystem) with the SYBR green PCR Master Mix (Applied Biosystem). All quantifications were done in duplicate. Primers used are listed in Supplementary Table S2. Immunoprecipitated DNA was analyzed using 8 appropriate primer couples distributed along the AMPD2 locus (primer set #2-9). RNA levels were normalized to that of a reporter gene (GNAI3 alpha - primer set #1) whose expression is not modified under TSA treatment.

Microarray hybridization. Microarray design was adapted from (Sasaki et al, 2006). Seventy-three probes of 500 bp covering 170 kb along the AMPD2 locus were amplified using specific primers (sequences are available upon request). After purification, the PCR products were freeze-dried and spotting was contracted out to Capacités (Nantes, France). For hybridization on microarrays, the immunoprecipitated DNA or 0.1% of genomic DNA from the chromatin extract was amplified by random primer extension followed by PCR amplification with incorporation of amino-allyl dUTP for subsequent dye coupling with Cy3 or Cy5. Hybridization at 63°C and washes were done under standard conditions (<http://derisilab.ucsp.edu>). Array slides were scanned using a 4000B scanner (Axon Instruments) and images were analyzed using the Genepix Pro 5.0 software.

Statistical analyses. The R environment was used for all the analyses (Team RDC, 2006). Tables, graphics and R code can be addressed on request. Statistical significances were set to $p \leq 0.05$. Two-tailed tests were systematically used. Type I errors were not controlled by any procedure of correction (exploratory statistical analyses). Results were summarized in the Supplementary Table S3. WSR: Wilcoxon signed rank. MWW: Mann-Whitney-Wilcoxon. NS: No difference could be detected. Normal approximation and continuity correction were applied for the WSR, MWW and χ^2 tests. The Fisher exact test was carried out when the Cochran rule was not respected for the χ^2 test. Tests are too conservative to be carried out in figures 4D and 4E. A Spearman test with a significant p-value and a positive r value indicates that the two compared distributions are correlated, meaning that their curve vary in the same manner along the horizontal axe. In figure 1, the WSR was use in a second step to determine whether one distribution is significantly over the other one, along the vertical axe, or not. The figure 3 gives example of such distributions.

SUPPLEMENTARY REFERENCES

Sasaki T, Ramanathan S, Okuno Y, Kumagai C, Shaikh SS, Gilbert DM (2006) The Chinese hamster dihydrofolate reductase replication origin decision point follows activation of transcription and suppresses initiation of replication within transcription units. *Mol Cell Biol* **26**(3): 1051-1062

Team RDC. (2006) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Fig. S1. Map of the AMPD2 locus in Chinese hamster cells and organization of the repeats in amplified cells.

(A) On scale map of the locus. Genes are represented by grey rectangles with arrows indicating promoter location and direction of transcription. Replication origins are symbolized by red boxes which width reflects the uncertainty in the precise location of the origin (Anglana et al, 2003). (B) Derivation of mutants amplified for the AMPD2 gene. Cell lines 422 and 474 were selected from GMA32 cells for their resistance to coformycin (an inhibitor of the AMPD2). The physical maps of the AMPD2 locus in line GMA32 and in amplified cell lines are represented (black arrows: genes; vertical dash line: palindrome centre). Gene copy number is indicated for each amplified cell line.

Supplementary Fig. S2. Acetylation patterns on specific single positions of histone H3 and H4 along the AMPD2 locus correlate neither with the localization nor with the efficiency of replication origins.

ChIP experiments were performed with antibodies that recognize specific single acetylated residues on histone H3 (A) or histone H4 (B) (Supplementary Table S1) and analysed by quantitative PCR. For each cell line (422, 474 and 474AU), acetylation level was normalized to the median level along the locus (arbitrary units). No data were obtained with primer set #3 for H4K5, H4K12 and H4K16 in 422 cells. On the schematic representation of the locus, symbols are as in figure 1A.

Supplementary Fig. S3. Histone acetylation patterns along the AMPD2 locus are similar in parental and amplified cell lines.

ChIP experiment were performed on GMA32 cell line with anti-acetyl histone H3 (A) or anti-acetyl histone H4 (B) antibodies (Supplementary Table S1) and analyzed by quantitative PCR. In each case, acetylation level was normalized to the median level along the locus (arbitrary units). Symbols are as in figure 1C.

Supplementary Fig. S4. TSA treatment affects pyrimidic deoxyribonucleotide biosynthesis in cells 422.

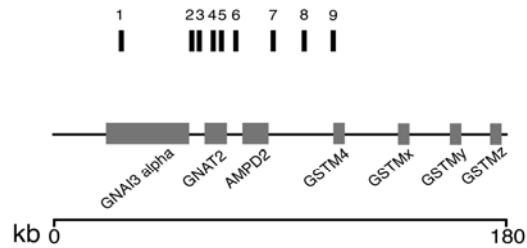
(A) TS expression after TSA treatment was analysed by Western blotting. PCNA was used as a loading control. Note that antibodies specific to isoform 1 of CTP synthetase are not available. (B, C) Cells were asynchronously growing or were synchronized in S phase by treatment with lovastatin (see Methods) followed by an incubation in normal medium for 14 h. Cellular extracts were prepared at 0 h (ctl) or after 6 h or 24 h (asynchronous cells only) of TSA treatment. Pools of dTTP and dCTP pools were measured by an enzymatic assay. For asynchronous cells, errors bars represent standard error of the mean of two experiments.

Supplementary Table S1. Antibodies used for ChIP experiments

Target	Acetylated positions recognized by the antibodies	Provider	Code
H3		Abcam	ab1791
acetyl H3 acetyl H4	H3K9, H3K14 H4K5, H4K8, H4K12, H4K16	Upstate Upstate	06-599 06-598
acetyl H3K9 acetyl H3K14 acetyl H3K18 acetyl H3K23 acetyl H3K27 acetyl H3K56	H3K9 H3K14 H3K18 H3K23 H3K27 H3K56	Abcam Upstate Abcam Upstate Upstate Upstate	ab12178 07-353 ab1191 07-355 07-360 07-677
acetyl H4K5 acetyl H4K8 acetyl H4K12 acetyl H4K16 acetyl H4K91	H4K5 H4K8 H4K12 H4K16 H4K91	Abcam Upstate Abcam Upstate Abcam	ab51997 07-328 ab1761 07-329 ab4627

Supplementary Table S2. Primers for quantitative PCR

Primers used for real time quantitative PCR were designed using Primer Express software 3.1.0 (Applied Biosystem). The positions of the 1 to 9 PCR products are indicated on the map of the AMPD2 locus. F : forward ; R : reverse



1	F	CATGGGCTGCACGTTGAG
	R	GTTGCGGTCAATCATCTTGCT
2	F	CGGCAACTCACAACCGTCTATA
	R	ACTTGTACCTGGTGTCCACAGAAG
3	F	GCTGTCCCTGCTGGAATCTG
	R	AGGTGCCACAGCAGCAATG
4	F	GACTCACATGCTTTCTCCAGTGA
	R	TGCCCCCTGGGTTTTTACTA
5	F	AAAATCAGGTCCTTTGGAGCTAAG
	R	GGACCAAGGTGTGGGAGTCA
6	F	TGGGATTTATTTTGTTCACTATTGCA
	R	ACCTGTAATAGCTCCCAGACTTAGAAA
7	F	CCCCAGGTCCATGATGTATTCT
	R	TGCACAGGAGGCACAAGGT
8	F	CCCCTCTGGCAATTCTGTTC
	R	CAGCCCTGGGTTTGTAGGTAAC
9	F	CTTTCAGTCGTCCTTAGGGTTTCTA
	R	CCACCGAATCAAATGTTTTTCCT
CTP synthetase	F	CCTCACATCACAGATGCAATCC
	R	TCCAAGCCATCTTCATCTACAGGTA
Thymidylate synthase *	F	TGGCACCCCTGTCGGTGTT
	R	TGTGAGCAGAGGAAATTCATCT

* The coding sequence for Chinese hamster thymidylate synthase is not available. Primers were designed from the rat sequence.

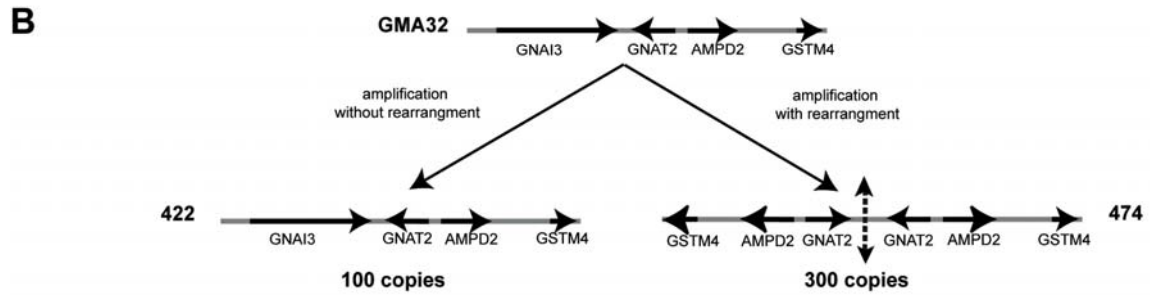
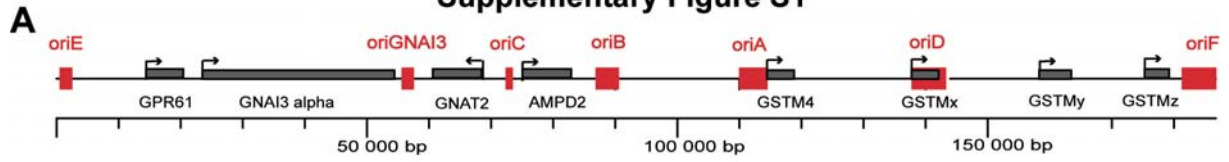
Supplementary Table S3. Statistical analyses

Figure	Comparison	Alternative hypothesis *	Sample size	Test (correlation value)	p-value
1a	474 and 474AU	Correlation between mean values (identical curve shape)	n = 8	Spearman (r = 0.81)	0.02
		Difference between medians of mean values	n = 8	WSR	NS
	474 and 422	Correlation between mean values (identical curve shape)	n = 8	Spearman (r = 0.86)	0.01
		Difference between medians of mean values	n = 8	WSR	NS
	oriGNAI3 and ori(A, B, C)	Difference between medians of acetylation values in oriGNAI3 and ori(A, B, C)	n _{GNAI3} = 17 n _{A,B,C} = 27	MWW	0.004
	oriA and ori(GNAI3, B, C)	Difference between medians of acetylation values in oriA and ori(GNAI3, B, C)	n _A = 9 n _{GNAI3,B,C} = 35	MWW	5e-6
1b	474 and 474AU	Correlation between mean values (identical curve shape)	n = 8	Spearman (r = 0.86)	0.02
		Difference between medians of mean values	n = 8	WSR	NS
	474 and 422	Correlation between mean values (identical curve shape)	n = 8	Spearman (r = 0.88)	0.007
		Difference between medians of mean values	n = 8	WSR	NS
	oriGNAI3 and ori(A, B, C)	Difference between medians of acetylation values in oriGNAI3 and ori(A, B, C)	n _{GNAI3} = 17 n _{A,B,C} = 27	MWW	5e-6
	oriA and ori(GNAI3, B, C)	Difference between medians of acetylation values in oriA and ori(GNAI3, B, C)	n _A = 9 n _{GNAI3,B,C} = 35	MWW	6e-6
2	474 fig2a and 474AU fig2c	Correlation between values (identical curve shape)	n = 57	Spearman (r = 0.83)	1e-15
	474 fig2b and 474AU fig2d	Correlation between values (identical curve shape)	n = 57	Spearman (r = 0.94)	< 2e-16
	474 fig2a and 422 fig2i	Correlation between values (identical curve shape)	n = 57	Spearman (r = 0.89)	< 2e-16
	474 fig2b and 422 fig2j	Correlation between values (identical curve shape)	n = 57	Spearman (r = 0.70)	2e-9
	474 fig2a and 474/G1 fig2e	Correlation between values (identical curve shape)	n = 57	Spearman (r = 0.88)	< 2e-16
	474 fig2b and 474/G1 fig2f	Correlation between values (identical curve shape)	n = 57	Spearman (r = 0.83)	9e-16

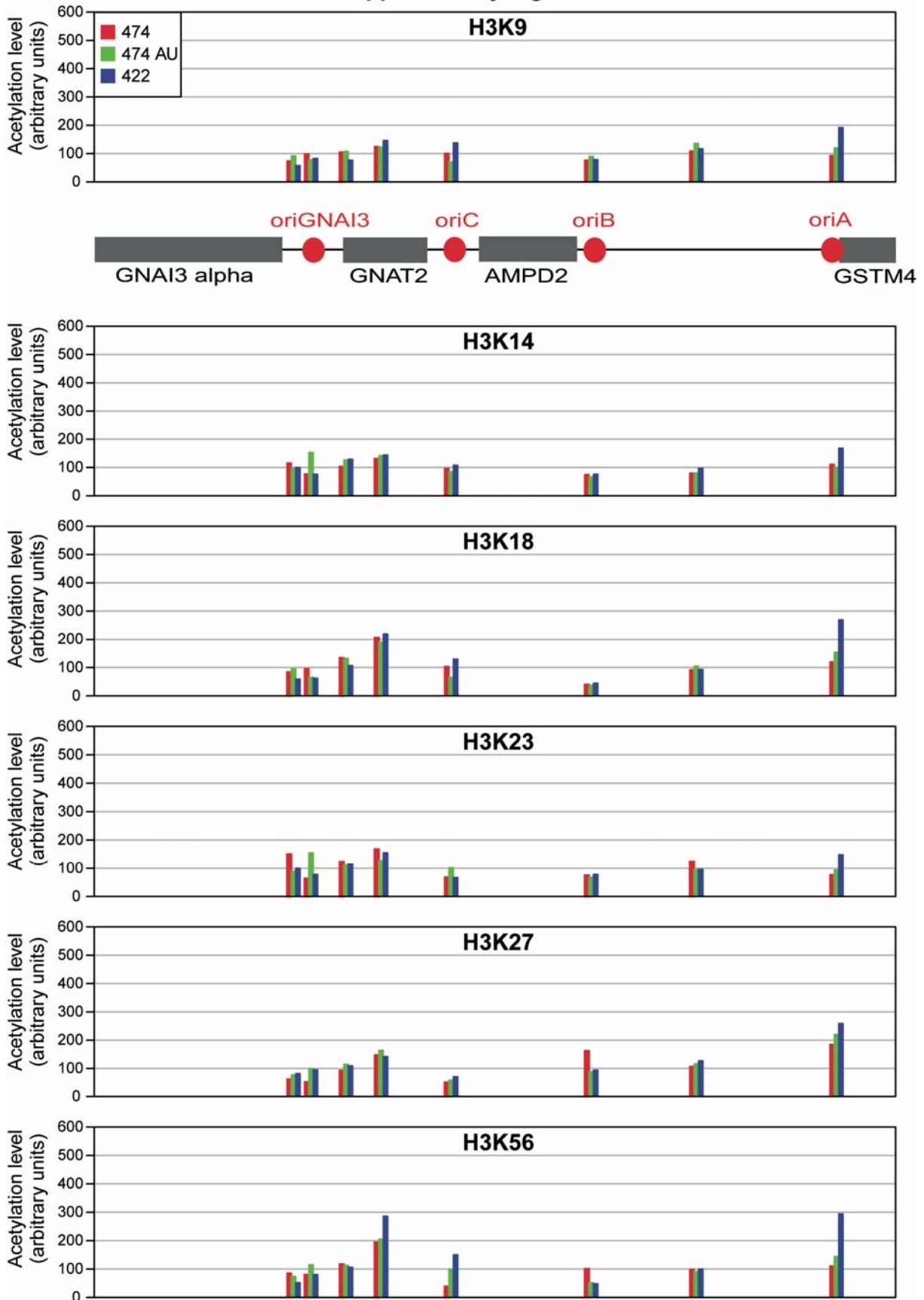
2	474AU fig2c and 474AU/G1 fig2g	Correlation between values (identical curve shape)	n = 57	Spearman (r = 0.77)	2e-12
	474AU fig2d and 474AU/G1 fig2h	Correlation between values (identical curve shape)	n = 57	Spearman (r = 0.59)	1e-6
3b	Ctl and 24h	Difference between medians of mean values	n = 8	WSR	0.008
3c	Ctl and 24h	Difference between medians of mean values	n = 8	WSR	0.02
4a	-CT and +CT at 0h	Difference between proportions of initiation	n _{-CT} = 309 n _{+CT} = 455	χ^2	NS
	-CT and +CT at 24h	Difference between proportions of initiation	n _{-CT} = 98 n _{+CT} = 425	χ^2	0.01
	0h and 24h in +CT	Difference between proportions of initiation	n _{0h} = 84 n _{24h} = 110	χ^2	NS
4b	Ctl and TSA 6h	Difference between distributions of proportions	n _{Ctl} = 28 n _{TSA6h} = 83	Fisher exact	0.007
	Ctl and TSA 6h + CT	Difference between distributions of proportions	n _{Ctl} = 28 n _{TSA6h+CT} = 51	Fisher exact	NS
4c	-CT and +CT at 0h	Difference between fork speed means	n _{-CT} = 98 n _{+CT} = 117	MWW	NS
	-CT and +CT at 24h	Difference between fork speed means	n _{-CT} = 53 n _{+CT} = 120	MWW	< 2e-16
	0h and 24h in +CT	Difference between fork speed means	n _{0h} = 117 n _{24h} = 173	MWW	0.007

* Statistical analyses are described in Supplementary Methods.

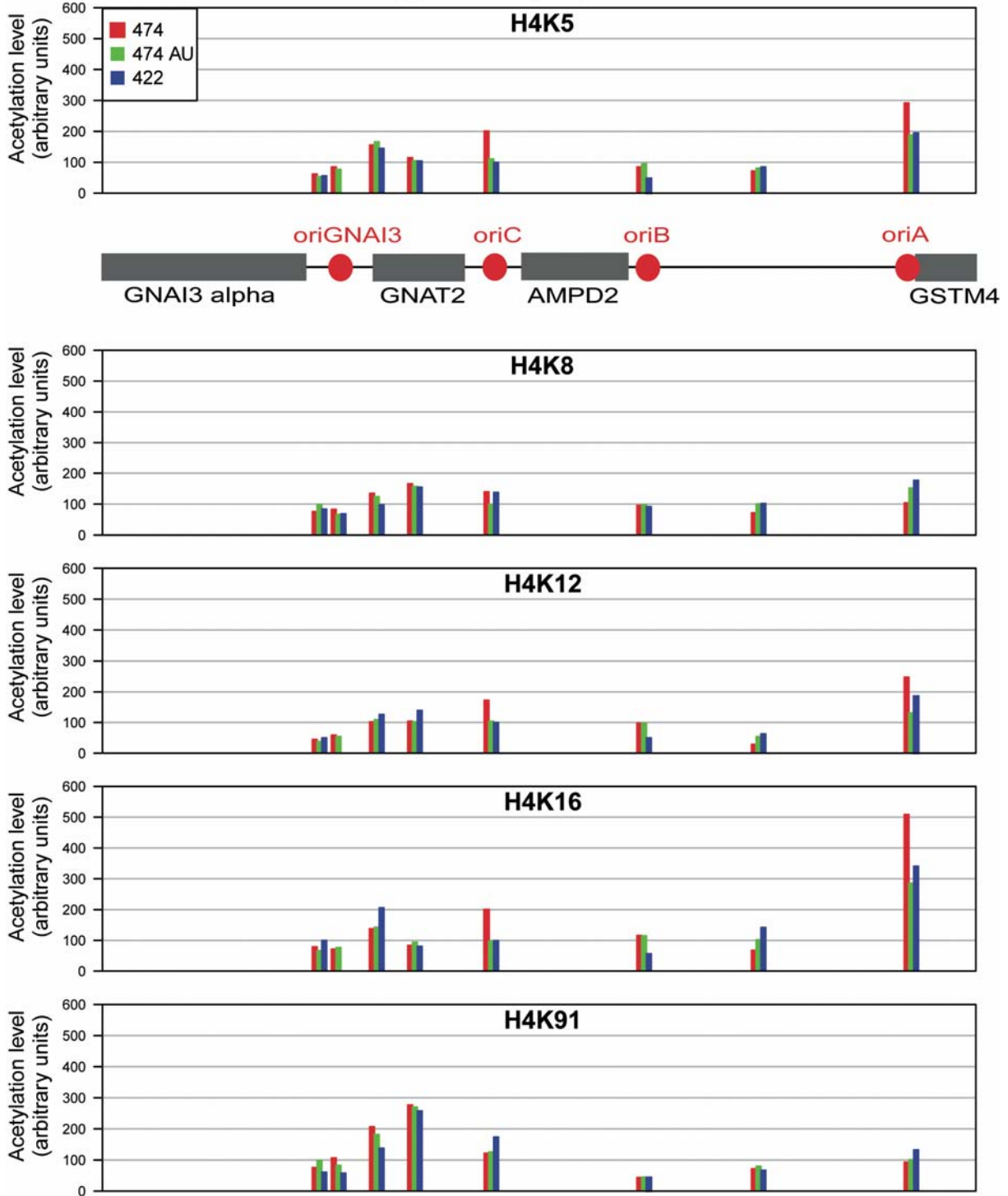
Supplementary Figure S1



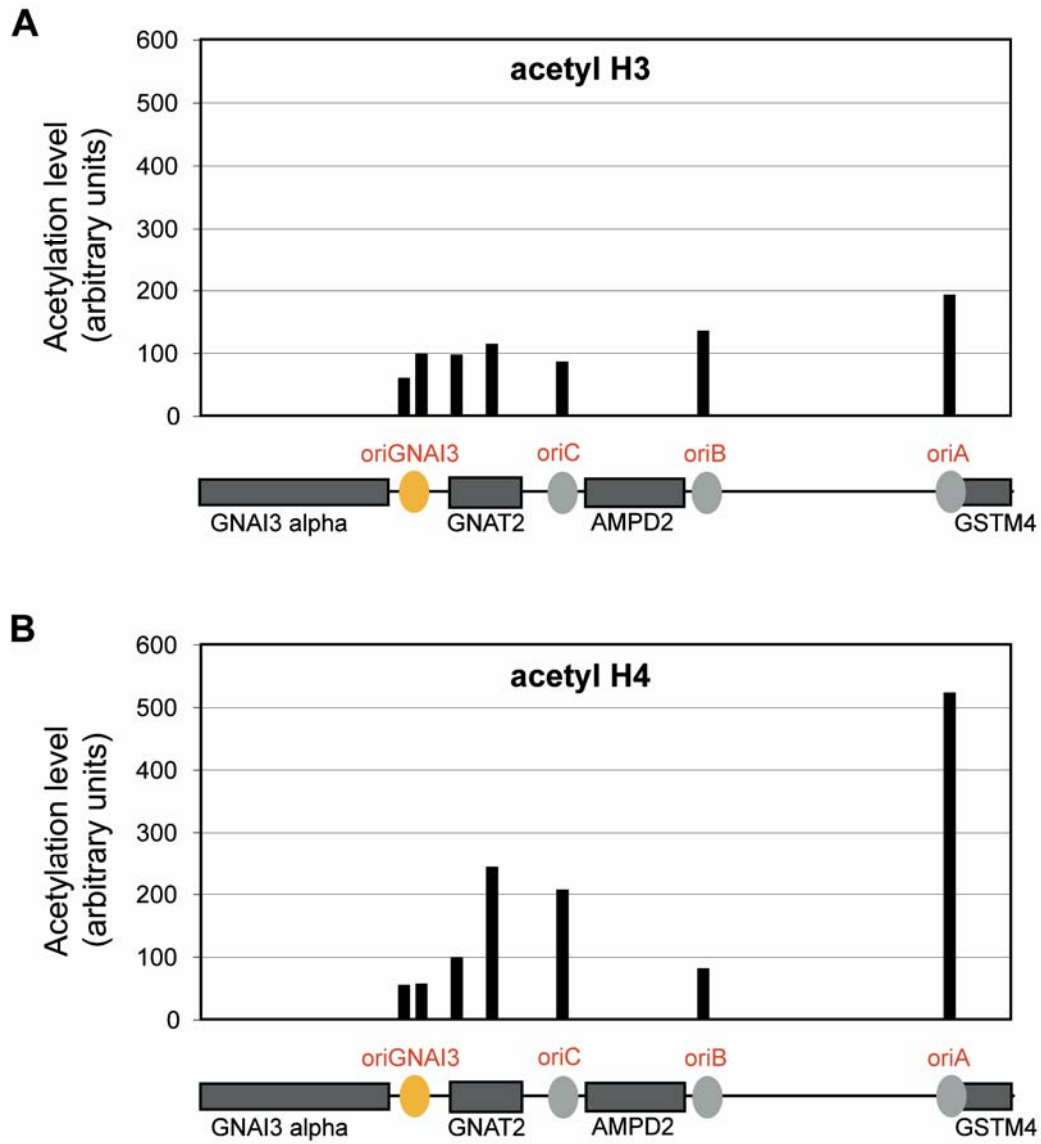
Supplementary Figure S2A



Supplementary Figure S2B



Supplementary Figure S3



Supplementary Figure S4

