Resetting the Yeast Epigenome with Human Nucleosomes

Graphical Abstract

Highlights

- At most, 1 in $10^7$ yeast survive with fully human nucleosomes, but they rapidly evolve
- Nucleosome positioning and nucleosome repeat length are not influenced by histone type
- Human nucleosomes reduce RNA, delay environmental adaptation, and remodel slowly
- A system for assessing histone variants is demonstrated with H3.1 and H3.3 nucleosomes

Authors

David M. Truong, Jef D. Boeke

Correspondence
davemtruong@gmail.com (D.M.T.), jef.boeke@nyumc.org (J.D.B.)

In Brief

Yeast can survive with human histones, providing insight into the regulation of nucleosome positioning and remodeling.
Resetting the Yeast Epigenome with Human Nucleosomes

David M. Truong1,* and Jef D. Boeke1,2,*

1Institute for Systems Genetics, Department of Biochemistry and Molecular Pharmacology, NYU Langone Health, New York, NY 10016, USA
2Lead Contact
*Correspondence: davemtruong@gmail.com (D.M.T.), jef.boeke@nyumc.org (J.D.B.)
https://doi.org/10.1016/j.cell.2017.10.043

SUMMARY

Humans and yeast are separated by a billion years of evolution, yet their conserved histones retain central roles in gene regulation. Here, we “reset” yeast to use core human nucleosomes in lieu of their own (a rare event taking 20 days), which initially only worked with variant H3.1. The cells adapt by acquiring suppressor mutations in cell-division genes or by acquiring certain aneuploid states. Converting five histone residues to their yeast counterparts restored robust growth. We reveal that humanized nucleosomes are positioned according to endogenous yeast DNA sequence and chromatin-remodeling network, as judged by a yeast-like nucleosome repeat length. However, human nucleosomes have higher DNA occupancy, globally reduce RNA content, and slow adaptation to new conditions by delaying chromatin remodeling. These humanized yeasts (including H3.3) pose fundamental new questions about how chromatin is linked to many cell processes and provide a platform to study histone variants via yeast epigenome reprogramming.

INTRODUCTION

Because they serve as a central interface for hundreds of other proteins, histones are among the most conserved genes in eukaryotes. They serve central cellular roles by regulating genome access, DNA compaction, transcription, replication, and repair (Talbert and Henikoff, 2017). Whereas higher eukaryotes evolved myriad histone variants with specialized functions, Saccharomyces cerevisiae (budding yeast) encodes but a few, a simplicity that has facilitated many fundamental discoveries in chromatin biology (Rando and Winston, 2012). However, this begs the question: why do budding yeast have such streamlined chromatin compared to humans, and do differences in histone sequences reflect functional divergence (Figure 1A)? Might the simple yeast serve as a “chassis” for understanding how histone variants exert control over cellular transcription (Figures 1B and 1C)?

Here, we asked a simple but far-reaching question: to what extent can S. cerevisiae utilize the core histones from humans? Thus far, only single yeast genes have been individually humanized (Hamza et al., 2015; Kachroo et al., 2015; Laurent et al., 2016; Osborn and Miller, 2007), but not a whole protein complex or one of such central importance. These studies could reveal intrinsically different properties between yeast nucleosomes and those from humans. We initially suspected two possible outcomes for our study: (1) that human histones would fully complement in place of yeast histones because of their high conservation, suggesting that histone sequence divergence provides only minor functional differences; or (2) that human histones in yeast would very poorly complement or even fail entirely, suggesting that the divergent residues are highly optimized for each species and serve specialized or novel functions. Our results are consistent with the latter.

The humanized yeast had pronounced delays in adapting to new environmental conditions, likely due to slowed remodeling of human core nucleosomes. In addition, our results suggest that human core nucleosomes may have evolved to occupy DNA more tenaciously, as we observed reduced RNA content and greater DNA occupancy by microccocal nuclease sequencing (MNase-seq). Our results also suggest that yeast may maintain human chromatin even when given access to native yeast histones. This may represent a type of chromatin “memory,” whereby cells partition and reproduce parental chromatin to new daughter cells. Finally, we show that our system can be used to generate histone variant H3.3 nucleosomes in addition to H3.1. Thus, while the species-specific coevolution of histones and their associated protein networks is extensive, it is nonetheless possible to reprogram the genome of at least one organism to accept histones of a very distant relative.

RESULTS

S. cerevisiae Can Subsist on Fully Human Core Histones

S. cerevisiae cells possess a relatively simple repertoire of histones. The core nucleosome, the four histones H3, H4, H2A, and H2B, comprise duplicate copies in the genome (each with divergent promoters and terminators), for a total of 8 histone copies (Eriksson et al., 2012) (Figures 1B, 1C, and S1). Additionally, there are three histone variants, H2A.Z, CENPA, and H1 (HTZ1, CSE4, and HHO1, respectively).

We constructed three histone deletion strains in which (1) both genomic copies of H3/H4 are deleted, (2) both copies of H2A/H2B are deleted, or (3) all 8 core histones are deleted, and then we provided the missing histone genes on URA3 plasmids (Figure S1). We then used a plasmid-shuffle approach (i.e., yeast versus human histones on plasmids) to quickly eliminate the...
native yeast histones in favor of their human counterparts using 5-fluoroorotic acid (5-FOA) counter-selection (Boeke et al., 1987) (Figures 1C and S1). The two plasmids, containing either human or yeast histone genes, are expressed from different sets of endogenous histone promoters and terminators to eliminate recombination. Like the parental cells powered by the 4 plasmid-borne yeast histone genes, the humanized strains have 4 plasmid-borne human histone genes. Previous work has shown that such single-copy histone plasmid strains lead to robust growth (Eriksson et al., 2012).

First, we determined the relative “humanization frequencies” (i.e., humanized colonies per cell plated) for individual or pairs of human histones. Human histone H4 (hH4) had the highest humanization frequency (fast growth, 20%), followed by hH2B (slow growth, 20%), hH2A (slow growth, 10^{-2}), and finally either hH3.1 or hH3.3 (very slow growth, 10^{-4} and 10^{-5}, respectively) (Figure 1D). Combining hH4 with either hH3.1 or hH3.3 also produced very slow growth (frequencies of 10^{-4} and 10^{-5}, respectively), whereas combining hH2A with hH2B led to slow growth but a modest humanization frequency of 10^{-3}. Surprisingly, the H3.3 variant, which is more “yeast-like” than H3.1 and associated with active chromatin in mammalian systems, performed worse than H3.1 in these assays (Ahmad and Henikoff, 2002).

For both human histone combinations, we confirmed the loss of yeast histones using both PCRTag analysis (Mitchell et al., 2015), which uses PCR to discriminate between sequence differences of yeast and human histones, and Sanger-sequencing-recovered plasmids (Figures S1D and S1E).

We then attempted to exchange all four histone genes simultaneously (Figure 1D). An “isogenic-WT” strain (yDT67) that replaces one native yeast histone plasmid with a plasmid containing the other set of 4 native histone genes “shuffled” readily (Figures 1D and S1), yet neither plasmid encoding fully human core nucleosomes containing either hH3.1 or hH3.3 produced colonies within 9 days. However, upon plating at least 10^7 cells and waiting 20 days, we did finally see colonies representing humanized yeast, but only for the hH3.1 plasmid (Figure 1E). These humanized yeast colonies were confirmed via PCRTag analysis and sequencing of extracted plasmids (Figure 1F), which showed no mutations in the human histones. These humanized colonies are unlikely to contain residual yeast histones, as “old” histones turn over by at least 2-fold per cell division (Anunziato, 2005). Since each haploid cell contains ~67,000 nucleosomes (Brogaard et al., 2012) and a small yeast colony contains at least 10^7 cells, those cells underwent at least 23 cell divisions. Therefore, the cells contain on average no more than 0.01
original yeast nucleosomes, a calculation assuming no loss of histones by degradation.

To date, we have only identified 8 such confirmed direct humanization events ("yHs" series; Figure S1F). Increased (high-copy plasmid) or decreased (genomic integration) human nucleosome gene copy number did not enhance humanization frequency (Table S3). The yHs cells grow on both synthetic complete (SC) and yeast complete (YPD) medium at 30°C and 25°C, but not at higher temperatures (37°C), mate, and grow to various degrees on media that enhance defects in DNA replication, DNA repair, and vacuole formation (Figures S2A and S2B). Finally, each of the humanized strains possessed different rates of substantially slower-than-normal growth and frequently produced larger and faster-growing colonies over time (Figures 1E and S1H). These observations are consistent with the accumulation of suppressors. A second factor reducing the humanization frequency is that a substantial proportion of the humanized cells in a population are unable to form a living colony.

**Bypass of Cell-Division Genes Promotes Growth with Human Nucleosomes**

We performed evolution experiments on seven yHs lineages to determine how and to what extent yeast cells adapt to "live with" core human nucleosomes. The seven lineages were selected by serially diluting and subculturing liquid stationary phase cultures for 5 cycles (Figure 2A). The two fastest growing lineages did not improve further through 10 cycles, suggesting they hit a fitness plateau early (Table S4). The evolved pools and isolates outperformed the pre-evolved strains on solid media and doubled 33% more rapidly in liquid culture (Figures 2B and S2C). We then performed whole-genome sequencing (WGS) on 32 such humanized yeasts.

Pulsed-field gel electrophoresis of whole chromosomes from each yHs lineage showed normal chromosome size (Figure S2D). However, WGS revealed recurrent aneuploidy of specific chromosomes (Figures 2D and S2E; Table S2). The human-histone plasmid copy number was no more than 2-fold higher than that in the parental strain yDT51. The majority of aneuploidies may be a detrimental consequence of human nucleosomes, as aneuploidies typically reduce fitness (Sheltzer et al., 2011), but our frequently recurring aneuploidies (Figure 2D) were consistent with other studies (Pavelka et al., 2010), which consider them possible reservoirs for positive selection. Chromosome number was often unstable during lineage evolution, as fractional differences (e.g., 1.5-fold chromosome 1 [chr1]) were not due to underlying diploidy that then gave rise to triploidy, indicating potentially variable levels of aneuploidy at the population level. Only the evolved isolates yHs4-evo and yHs7-evo showed no sign of aneuploidy based on coverage; yHs7-evo carried but a single mutation in the gene DAD1, which controls microtubule force at the centromere (Sanchez-Perez et al., 2005). By contrast, yHs5 and its evolved progeny had higher levels of aneuploidy, perhaps due to a subtle mutation in the gene SCC4, a cohesin loader (Lopez-Serrà et al., 2014).

All humanized strains were either missing segments of mtDNA (p+) or showed complete loss of mtDNA (p−), except for the lineages from yHs5 (p−). We investigated whether mtDNA loss alone might explain the slow growth rates, but we found that isogenic-WT p− cells (with yeast histones) grow faster than all humanized lines, and moreover, the p+ yHs5-evo isolate was not the fastest-growing isolate (Table S2).

We identified 36 mutations in or near genes among the 8 initial isolates and their evolved derivatives (Figure 1C, Tables S1 and S2), and 22 unique mutations appeared likely to affect gene function based on alterations to protein sequences. We constructed an interaction network from these 22 mutations using the STRING algorithm (Szklarczyk et al., 2015) (Figure 2E). The enrichment of
gene ontology (GO) terms in this network was non-random, as the genes clustered in 4 processes: chromosome segregation, cytoskeleton, cell-cycle progression, and genes affecting RNA metabolism. These first 3 processes collectively affect mitotic cell division (Lew and Reed, 1995). The interaction network identifies the master cyclin-dependent kinase (CDK) CDC28, which is critical for cell-cycle progression, as a central node coordinating most of the suppressors, as it phosphorylates numerous types of cyclins that control major cell processes (Lew and Reed, 1995).

By using the YeastRACT algorithm, which searches for transcriptional regulators by tracking their putative consensus sequences in potential target genes (Teixeira et al., 2014), we further found that four transcription factors (Ace2, Sfp1p, Ste12, and Tec1p), which are also critical for cell-cycle progression, were commonly represented among the promoters of the genes hit by the 22 suppressor mutations (Figure 2F; Table S1). Therefore, mutations in genes that affect cell division may suppress defects arising from human histones, possibly by circumventing cellular checkpoints triggered by aberrant chromatin properties. These results suggest it is easier to evolve the surrounding gene network to accommodate new functions than the gene itself.

Specific Residues in Termini of Human Histones H3 and H2A Limit Yeast Growth

We were surprised to not identify any mutations within the human histone genes themselves. Converting the C-terminal residues of H3 back to the yeast sequence enhances complementation (McBurney et al., 2016), and some yeast-specific residues cause lethality when mutated to alanine (Dai et al., 2008; Nakamichi et al., 2008). We systematically swapped residues from human to yeast across histones hH3, hH4, and hH2A in order to identify important species-specific regions (Figures S3 and S4), but not on hH2B, as it complemented relatively well.

Swapping back three residues in the C terminus of hH3 enhances the humanization frequency (Figure S3A), consistent with a recent study (McBurney et al., 2016), whereas swapping back the lethal residues provided no benefit. Although hH4 already worked well, we identified two residues in its C terminus that enhanced humanization (Figure S3B). Only two swap-back residues in hH3 (hH3KK; human → yeast P121K and Q125K) were required for complementation when combined with completely human H4 (Figure S3C), although there appear to be differences between hH3.1 and hH3.3 in this regard. A possible explanation for the two hH3 swap-back residues may be that in yeast H3, the two lysine swap-back residues are ubiquitylated by Rtt101Mms1 to weaken affinity for Asf1 (Han et al., 2013). Moreover, mutations in H3 of K121R/K125R cannot be ubiquitylated and thus lead to reduced release from Asf1, restricting transfer to other histone chaperones. Therefore, swapping back these two residues restored sites for ubiquitylation.

Swapping back three broad regions in hH2A enhanced complementation in combination with fully human hH2B, the N terminus, the C terminus, and a region from residues 19 to 42 (Figure S4). Further analyses narrowed the essential residues to three residues each in the N terminus and C terminus. Combining all six of these residues significantly enhanced the humanization frequency and growth rate of the yeast (Figure S4D). Intriguingly, the mammalian lineage-specific N-terminal arginine residues, when inserted into yeast H2A, have been shown to increase chromosome compaction (Macadangdang et al., 2014). The C-terminal portion, which is exposed on the nucleosome face (White et al., 2001), may interact with histone chaperones (e.g., NAP1) analogous to the H3/H4 interaction with Asf1.

We combined the three terminal regions (hH3.1KK, hH2AΝ, and hH2ΑC) into human nucleosomes as various “swap-back strains” (Figure 3). As expected, combining all three swap-back regions enhanced humanization (8-residue swap-back strain yDT98) to 10⁻² in only 3 days (Figure 3B). However, the swap-back strain with only the two C-terminal regions (hH3.1KK and hH2ΑC; 5-residue swap-back strain yDT97) grew as fast as the 8-residue swap-back version (yDT98), and both of these strains grew nearly as fast as our isogenic-WT strain (yDT67) in 3 days. The 5-residue swap-back strain (yDT97) was used for further studies.

**Figure 3. Specific Residues in the C Termini of Histones H3 and H2A Limit Yeast Growth**

(A) Maps of swap-back residues that enhance human histone utilization identified in Figures S3 and S4. Two residues in the Cterminus of human histone H3 (hH3) and three swap-back residues each in the N terminus or C terminus of hH2A improved the complementation frequency and growth rate in conjunction with their respective human histone counterpart (i.e., hH4 and hH2B, respectively).

(B) Systematic combinations of swap-back residues in hH3 and hH2A, along with fully human hH4 and hH2B, show that eight swap-back residues promote the highest rates of complementation.

(C) Colony growth rate analyses shows that the 5-residue swap-back strain (yDT97) grows as well as the 8-residue swap-back strain (yDT98). Both swap-back strains grow at rates closer to isogenic-WT yeast (yDT67) and better than the fastest-growing completely humanized isolate (yHs5-evo). All further swap-back strain experiments refer to yDT97.
Nucleosome Organization Is Specified by the Chromatin Remodeling Network

To evaluate the organization of human nucleosomes on the yeast genome, we performed MNase digestion titrations and MNase-seq on evolved and swap-back strains using “high” and “low” enzyme concentrations (Figures 4 and S5) to reveal possible differences in nucleosome accessibility.

Unexpectedly, the nucleosome repeat length (NRL) of yeast chromatin built using human nucleosomes was identical to the NRL in isogenic-WT yeast and is substantially shorter than that for human HeLa cells (Figure 4A). The di-nucleosome length (200–300 bp) from low-concentration MNase-seq confirms a short mean NRL (Figure S5B). These data indicate that the NRL in humans is not an intrinsic property of human core nucleosomes but is likely specified by nucleosome remodelers, the genomic sequence itself (Segal and Widom, 2009), or some combination of these factors.

To our surprise, the numbers of nucleosomes with altered positioning or fuzziness (movement) was no different than that of isogenic-WT “noise” (Chen et al., 2013). However, there are substantial occupancy differences (Figure 4B). These include some major differences found in the −1 nucleosomes of many genes (see below). Nevertheless, these results suggest that nucleosome positioning is determined less by the type of nucleosome and much more by the underlying DNA sequences and the network of chromatin remodelers for a given species.

As suggested by the chromosomal segregation suppressor mutations identified earlier (Figure 2E), we find that human nucleosomes lead to depletion of centromeric nucleosomes relative to the surrounding nucleosomes, perhaps due to conflict with the yeast centromeric H3 variant CSE4 (Figures 4C and S5D). Relative to the neighboring nucleosomes, depletion was greatest for centromeres on aneuploid chromosomes observed earlier by WGS (Figures 2D and 4C). Strain yHs5-evo, which had the highest levels of aneuploidy, had greater depletion at centromeric nucleosomes, whereas strain yHs7-evo, which has normal chromosome numbers and carries a relatively subtle missense mutation (E50D) in the essential gene DAD1, has slightly better positioning (Figure 4C).

Finally, all 275 tRNA genes had depleted sequence coverage in their gene bodies compared to wild-type (WT) (Figure 4D). Unlike RNAP2 genes, tRNAs possess an “internal control region”; thus, the depleted regions could represent a loss of RNAP3 and accessory factors (Acker et al., 2013), or nucleosome depletion coupled to RNAP3 transcription elongation. In fact, substantially elevated tRNA levels were observed in RNA from yHs cells (Figure S6A), perhaps suggesting that human nucleosomes are less stably bound to tRNA sequences. However, as yeast tRNAs are already highly expressed and mostly devoid of nucleosomes, this could instead indicate that tRNA levels are normal and that it is mRNAs that are highly repressed by human nucleosomes, thus altering the tRNA/mRNA ratio.

Figure 4. Human Nucleosome Organization in Yeast

(A) MNase digestions reveal that human nucleosomes produce the same NRL as yeast nucleosomes compared to the longer length of human nucleosomes in HeLa cells. Red arrows indicate position of the tri-nucleosome. bp indicates base pair size of the DNA ladder (L).

(B) Table of high (2 U/mL) MNase-seq nucleosome dynamics between humanized to WT yeast and WT experiment 1 to WT experiment 2 (“noise”). Occupancy and fuzziness changes use a strict false discovery rate cutoff of 0.05 (p < 10⁻⁵) and additional parameters in STAR Methods.

(C) High MNase-seq read counts at centromeric regions plotted for chromosomes that were normal or aneuploid in Figure 2D. RCPM, read counts per million mapped reads.

(D) High MNase-seq read counts for all 275 tRNA genes comparing humanized versus WT strains showing depletion of either RNAP3 or nucleosomes.
explained by substantially altered cell numbers per A<sub>600</sub> or reduced cell viability as determined by vital staining (Figures S6C and S6D). Humanized whole-cell extracts had similar bulk protein yields to isogenic-WT, but the SDS-page gel stained with Coomassie shows numerous proteins with reduced levels, consistent with reduced RNA (Figure S6E), whereas other presumably highly stable proteins are relatively unaffected. Immunoblots using antibodies more specific for human H3 and H4 show greater signal for humanized strains (Figure S6B). Finally, both H3K4 trimethylation and H3K36 trimethylation signals were similar to the isogenic-WT strain, as these modifications are in histone regions conserved between yeast and humans. This suggests that low mRNA levels are not due to changes in these histone modifications.

We performed mRNA-seq on all 7 pre-evolved yHs strains to understand how human nucleosomes affect gene expression (Figure 5C). We used equal amounts of RNA for library preparation, despite the fact that mRNA levels per cell were drastically reduced in the humanized strains. The histone genes themselves are expressed at generally similar levels to isogenic-WT cells (Figure S6F). We identified 1,046 differentially expressed genes (evenly distributed between up- and downregulation) common to the 7 independent humanized lines. The GO terms predominantly comprise metabolism, potentially explaining some of the slow growth defects (Figure 5C). Furthermore, the most upregulated genes could be explained by paradoxical telomeric desilencing, perhaps due to poor interaction with silencing factors such as Sir proteins (Figure S6G), and most severely, Telomere III L. However, we saw no other general pattern of differential expression that explained the overall reduction in mRNA or growth properties.

Thus, the reduced RNA content and slowed growth might reflect differences in nucleosome dynamics (Chen et al., 2013) and could indicate a fundamentally repressive property of human histones and/or their relative inability to interact with yeast chromatin remodelers. To better understand this effect, we mapped the MNase-seq reads across the transcription start sites (TSSs) of the top 1,500 genes by expression and the bottom 1,500 genes by expression. NDR, nucleosome-depleted region; RCPM (and color key), read counts per million mapped reads. (E) Repressed genes with highly occupied –1 nucleosomes from MNase-seq.

**Figure 5. Human Nucleosomes Are More Repressive**

(A) Pre-evolved yHs strains have reduced levels of bulk total RNA (6- to 8-fold), as well as the evolved and swap-back strains (2- to 3-fold). Data represent mean ± SEM of 3 biological replicates.

(B) Acid-extracted histones from strains analyzed for equal loading by Coomassie staining and then immunoblotted using different H3 and H4 antibodies.

(C) Volcano plot of differentially expressed genes shared among all 7 pre-evolved yHs strains compared to isogenic-WT yeast. 1,046 genes had a q-value < 0.05.

(D) Heatmaps and average profiles of high concentration MNase-seq reads aligned around the transcription start sites (TSSs) of the top 1,500 genes by expression and the bottom 1,500 genes by expression. NDR, nucleosome-depleted region; RCPM (and color key), read counts per million mapped reads.

(E) Repressed genes with highly occupied –1 nucleosomes from MNase-seq.
expressed yeast genes in isogenic-WT yeast (and even in repressed genes poised for induction) contains the TATA box. Here, we show examples of 3 inducible genes that are repressed in the presence of glucose: GAL10, GAL1, and HXT16. In the humanized strains, but not the isogenic-WT control, these genes contain an occupied −1 nucleosome. Because these genes are “off,” they are unlikely to be actively interacting with chromatin remodelers. The gene HXT16, for instance, has zero RNA transcripts by mRNA sequencing (mRNA-seq) (Figure 5C).

**Human Nucleosomes Delay Adaptation to New Transcriptional Programs in Yeast**

The humanized cells were larger in size on average and produced a greater range in cell sizes (Figures 6A and S7). By micro-manipulating single cells onto YPD plates, we found no growth difference between large and small cells (Figure S7E).

However, un budded cells (G1) were less likely to continue to grow than budding cells, although they all mostly remained intact after several days of monitoring. Surprisingly, a high fraction of single cells grew for a number of cell divisions before arresting as a population (i.e., arrested before reaching the size of a visible colony). This might indicate an inability to regulate cell-size control due to less permissive chromatin, and indeed, cell-size-regulating genes such as a suppressor gene identified earlier (WHI4)—themselves cell-cycle regulated—have highly occupied −1 and −2 nucleosomes specific to the humanized cells (Figure 6B).

We then assessed how readily the cells adjust to new phases of the cell cycle, a process based on an elaborate transcriptional program that requires extensive chromatin remodeling. Using both budding and flow cytometry of log-phase cells, we observed reduced cell division, as only 40%–60% of humanized cells reach the G2/M phase compared to ~90% of isogenic-WT cells (Figure S7B). More importantly, the lagging-phase cultures of the swap-back strain display a prolonged S phase, indicative of a delay in adjusting to log-phase growth (Figures 6C and S7D). This could result from an inability to accumulate new histones onto nascent DNA or an inability to remodel and remove appropriately chromatin-bound factors. In this case too, cell-cycle genes (e.g., CLB1) also have highly occupied −1 and −2 nucleosomes in humanized cells (Figure 6B).

More intriguingly, we often observed that the humanized cells had difficulty adapting to new environments (e.g., colony to liquid culture). This, along with the above observations, led us to hypothesize that human nucleosomes slow chromatin remodeling to new transcriptional programs. Consistent with this...
Initially, we could only generate fully human core nucleosomes with the major histone variant H3.1, but not with H3.3. We reasoned that using a strain containing the dad1-E50D suppressor, which had normal chromosome coverage and good re-humanization efficiency, might enable us to do so. We re-engineered this mutation into the histone shuffle strain yDT51 using starvation, WT chromatin remodeled at PHO5 rapidly and was essentially nucleosome-free by 5 hr. In contrast, both humanized strains remained highly occupied even after 24 hr of phosphate starvation, and the −1 nucleosome remained more occupied at 24 hr than the WT did at 1 hr. Taken together, these results are consistent with the hypothesis that human nucleosomes delay adaptation to new transcriptional programs primarily by delayed remodeling of human nucleosomes.

Suppressor Mutations and Human Chromatin “Memory” Enhance Humanization Frequency

The numerous suppressor mutations identified earlier (Figure 2) may counteract the various defects observed in yeast with human nucleosomes. If the suppressors make human histones more tolerable, they would be predicted to enhance the “humanization frequency.” To determine this, we reintroduced the native yeast histone plasmid into 13 humanized suppressor strains (Figure 7A, black dots) and allowed mitotic loss of the human histone plasmid, thus reverting these cells to native yeast chromatin, whereupon their growth properties improve. These lines were used for dual-plasmid histone shuffling as before by reintroducing the human histone plasmid. The humanization frequencies, 10- to 100-fold greater than for non-suppressor ρ0 or ρ− strains, confirms that the identified suppressors enhance tolerance of human nucleosomes. Furthermore, humanized colonies appeared as early as 12 days instead of the 20 originally required. These frequencies are high enough to support histone shuffling using any histone variant from potentially any species.

While the suppressor mutations improved the humanization frequency going from native yeast chromatin to fully human chromatin, we also contemplated how readily human chromatin resists “invasion” by native yeast histones. If the humanization frequency improves in this scenario relative to the above, this might suggest maintenance of human chromatin and a preference for reincorporation of nucleosomes of their own type. To test this hypothesis, we reintroduced the native yeast histones into the fully humanized suppressor strains and allowed for single colonies containing both types of chromatin to grow for ~26 cell division generations (Figure 7A, red dots). This is a suitable time frame for native yeast chromatin to completely outcompete human chromatin, if not for “parental” nucleosome maintenance. As in the above experiment, growth properties improved to WT levels, indicating that yeast chromatin reoccupies and predominates the yeast genome. However, upon performing 5-FOA plasmid shuffling to remove the native yeast histones, the humanization frequencies reached 10−5−10−3, and cells appeared as early as 7 days. This suggests human histones were still maintained on DNA, even when given access to native yeast histones.

Introduction of Histone Variant H3.3 Nucleosomes in Yeast

Initially, we could only generate fully human core nucleosomes with the major histone variant H3.1, but not with H3.3. We reasoned that using a strain containing the dad1-E50D suppressor, which had normal chromosome coverage and good re-humanization efficiency, might enable us to do so. We re-engineered this mutation into the histone shuffle strain yDT51 using

hypothesis, using a GAL1-promoter-driven EGFP as a proxy for switching to the galactose utilization transcriptome using the remodeling the structure of chromatin (RSC) complex (Fober et al., 2010), we showed that cells with human nucleosomes had a pronounced delay in transcriptional response to galactose as the sole carbon source (Figure 6D). This delay could be directly related to the strongly positioned −1 nucleosome in humanized cells (Figure 5E). Moreover, the delayed kinetics are similar for the fully humanized strain and for the swap-back strain. As the swap-back strain presumably interacts more robustly with certain histone chaperones, this suggests the additional sequence variations between human and yeast histones alter certain histone chaperones, this suggests the additional properties of histones.

To definitively show that human nucleosomes delay chromatin remodeling, we assessed chromatin accessibility at the highly inducible promoter using a nucleosome scanning assay (NuSA) time course upon phosphate starvation (Small et al., 2014). Importantly, the PHO5 promoter relies on multiple transcriptional regulators (Musladin et al., 2014) and functions independently of replication (Schmid et al., 1992); thus, its remodeling should not be confounded by cell-cycle alterations. As with others, this promoter also features a highly occupied −1 nucleosome in the humanized cells (Figure 6E). Furthermore, we compared humanized H3.1 core nucleosomes as well as newly generated H3.3 core nucleosome strains (Figure 7; see section below) to WT cells (Figure 6E). As expected upon phosphate...
CRISPR/Cas9 in order to retain mitochondrial DNA, which the earlier strain lacked. Indeed, using this new “clean” suppressor strain, we were able to easily produce fully humanized strains with H3.3 (as well as H3.1), which we confirmed by both PCR-tag analysis and sequencing of the extracted plasmids (Figure 7B). However, the hH3.1-containing plasmid still produced humanized colonies at a higher frequency. Thus, the hH3.1 gene paradoxically seems to work better in yeast than does hH3.3 in the context of a fully humanized nucleosome, just as it does in the earlier experiments where we substituted individual human histone genes and pairs of human histone genes. This experiment sets the stage for a comprehensive analysis of human histone variants in the yeast system.

**DISCUSSION**

Because histones are some of the most conserved genes among eukaryotes, it was surprising that fully human nucleosomes so rarely led to bona fide humanized yeast. This speaks to the centrality of nucleosomes in regulating diverse cellular processes, including transcription and chromosome structure and movement. Cumulatively, our data suggest that human histones in yeast are deposited less efficiently, possibly due in part to sequence incompatibilities mapping to only 5 residues in the C termini of H3 and H2A. When they do get deposited, the human histones lead to greater gene repression via less accessible NDRs, a strongly positioned –1 nucleosome covering the TATA box, variations in chromatin, delayed environmental adaptation resulting from slowed chromatin remodeling, and depleted centromeres that possibly limit kinetochore assembly. The sum of these effects seemingly leads to partial cell-cycle arrest in G1 and a slower S phase, which suppressor mutations in these same pathways partially alleviate.

Converting only 5 residues (2 in H3 and 3 in H2A) promoted relatively robust utilization of almost fully human nucleosomes in the form of swap-back strains. In the case of hH3, the incompatibility with yeast may be attributed to an absence of two lysines required for ubiquitylation in yeast (Han et al., 2013). Human H2A may also poorly interact with yeast histone-interacting factors. However, this finding is still somewhat surprising, as numerous other residues differing between yeast and humans might be expected to have more important roles, such as the many modified residues. As just one example, histone H3 position 42 is a lysine in yeast but an arginine in humans (Hyland et al., 2011). Numerous other positions differ (Figure 1A); thus, the relative inability to interact and modify histones at these different sites poses a serious question about the cumulative role of histone modifications during cell growth.

Furthermore, our data suggest that histone sequences do not contribute to NRL, as the NRL of yeast with human histones remained yeast-like. In higher eukaryotes, longer linker length is partially attributed to linker histone H1 (Fan et al., 2005; Woodcock et al., 2006), but in yeast, it has been shown that expressing the human H1.2 linker histone had no effect on the NRL (Panday and Grove, 2016). In humans, the NRL ranges from ~178 to 205 bp, depending on histone modification state (Valouev et al., 2011), with activation marks having the shortest NRL. Thus, the underlying DNA sequence (Segal and Widom, 2009) and/or the proteins that interact with histones, such as lsw1a (Krietenstein et al., 2016), are more likely to specify this property.

The highly positioned –1 nucleosomes and reduced RNA suggest that human nucleosomes may have evolved to occupy DNA more readily than yeast nucleosomes. Specifically, they had highly occupied –1 nucleosomes even in completely repressed genes (Figure 5E) that presumably are not interacting with remodelers. Thus, human nucleosomes may bind DNA more tightly, as they can remain in place for years, if not for decades, in a terminally differentiated state (Toyama et al., 2013), whereas yeast nucleosomes are lost during aging (Hu et al., 2014). Earlier studies on *in vitro* reconstitution of yeast and mammalian nucleosomes suggested that mammalian nucleosomes bind DNA more readily (80% chromatin assembly) and that yeast nucleosomes are comparatively unstable (~16% assembly) (Lee et al., 1982). Furthermore, it has long been known that *in vitro* transcription using human chromatin (or any higher eukaryote chromatin) inhibits transcription (Konesky and Layboum, 2007; Lorch et al., 1987), but a recent study using native yeast chromatin showed enhanced *in vitro* transcription compared to naked DNA (Nagai et al., 2017). Our study may provide a means to reconcile these opposing observations. Given that yeast genes are generally expressed (Rando and Winston, 2012) and human genes repress the majority of the genome in virtually all cell types (Talbert and Henikoff, 2017; Thurman et al., 2012), this might indicate an evolutionary basis for histone sequence divergence. Yeast, which must readily adapt to new environments, evolved highly dynamic histones, that retain bifunctional characteristics of histone variants found in humans (Rando and Winston, 2012). For instance, yeast H3 acts as both an H3.1 (replication-dependent) and H3.3 (replication-independent) variant, while yeast H2A acts as both an H2A.1 and H2A.X (DNA-damage) variant (Eriksson et al., 2012). In contrast, human cells must retain transcriptional states corresponding to cellular type. Thus, specialized histone variants with higher DNA affinity and stronger gene repression may enable multicellular organisms to generate and “lock in” more diverse transcriptional landscapes (Buschbeck and Hake, 2017).

In addition, we found that human nucleosomes had substantially delayed chromatin remodeling, likely because they are less easily removed or relocated by the yeast chromatin remodelers, which did not coevolve with these histone sequences. These effects are likely dominant to differences in the physical properties between different histone variants. However, these highly delayed kinetics, especially at the –1 nucleosome compared to the –2 nucleosome, are slower than those observed when using strains with multiple remodeler deletions (Musladin et al., 2014), which seems inconsistent with incompatibility with chromatin remodelers being the sole explanation. Thus, this argues for additional effects owing to differences in yeast versus human nucleosomes. Moreover, the hH3.3-containing nucleosomes did not remodel any more readily than did those containing hH3.1, which is surprising given that hH3.3 is considered more unstable and is enriched at enhancers, promoters, and termination sites of active genes as well as sites of DNA repair (Talbert and Henikoff, 2017). However, similarly high turnover for both hH3.3 and hH3.1 for active genes at enhancers has recently been observed (Deaton et al., 2016) and...
suggests that nucleosome disruption does not particularly favor any histone variant. Thus, observations about hH3.3 enrichment at active genes may have more to do with its availability due to transcription throughout the cell cycle, whereas hH3.1 is only expressed during S phase.

Our study suggests a type of chromatin partitioning memory, as yeast with preexisting human chromatin more readily lost re-introduced native yeast histones (Figure 7). Although we used suppressor mutants for these experiments, which could influence this result, the consistency of this effect among the many different types of suppressors suggests a more general phenomenon. We interpret this result to suggest that preexisting human chromatin might help maintain chromatin of its own kind, at least regionally—a type of chromatin memory and trans-generational inheritance—thus predisposing some small fraction of cells to not fully revert to native yeast chromatin even after many cellular divisions. These results are surprising, because with few exceptions, yeast do not have protein machinery dedicated to maintenance of different histone variants, let alone for human histones. While our observations of higher DNA occupancy and delayed remodeling also might explain this phenomenon, the rescued growth upon native yeast histone introduction indicates a strong preference for yeast chromatin, as expected. Thus, in our model (Figure 7C), nucleosomes prefer their own type, thus seeding and maintaining similar chromatin domains. Therefore, different histone types or nucleosome compositions are less likely to invade and outcompete this “parental” chromatin during the partitioning of chromatin during cellular division. This may occur in all cells or perhaps in a smaller fraction of “older” cells that retain more of the earlier deposited human chromatin. How cells determine which histone subtype or modification state is deposited on each daughter DNA strand in the replication fork remains an open question (Lai and Pugh, 2017). Based on our results, the restoration of nucleosomes to the parental strand and inheritance on the daughter strand may occur as a type of “semiconservative replication” of chromatin, whereby both parent and daughter strands retain a portion of the ancestral nucleosomes (human) and then may simply preferentially attract the same type of new neighboring nucleosomes.

More generally, humanizing the chromatin of budding yeast provides new avenues to study fundamental properties of nucleosomes. We have explored some long-standing questions about histone variants, including how they alter the dynamics of the genome at the structural and transcriptional level (Talbert and Henikoff, 2017), how they associate into different compositions of nucleosomes (Bernstein and Hake, 2006), and how they are partitioned and repositioned from cell to cell across generations (Budhavarapu et al., 2013; Campos et al., 2014). These questions remain fundamental, as many human cells are reprogrammed and differentiate using a plethora of histone variants. Thus, introducing foreign histones from distant species into the simple yeast genome may help address these and many other fundamental questions about histone function.

**STAR+METHODS**

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **CONTACT FOR REAGENT AND RESOURCE SHARING**
- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
  - Strains, plasmids, and media
- **METHOD DETAILS**
  - Dual-plasmid histone shuffle assay
  - Plasmid isolation from yeast cells
  - PCR tag analysis
  - Pulsed-field gel electrophoresis
  - Mating and sporulation tests
  - Microscopy
  - Cell counting and viability
  - Cell-cycle analysis using sytox green
  - Flow cytometry of GAL1-eGFP induction
  - “Re-humanization” of suppressor mutants
  - Protein analysis and Western Blotting
  - Growth assay on various types of solid media
  - Whole genome sequencing
  - MNase-digestions and MNase-sequencing
  - RNA extraction and mRNA-seq
  - Identification of small RNAs
  - Nucleosome scanning assay (NuSA) of the PHO5 promoter
- **QUANTIFICATION AND STATISTICAL ANALYSIS**
  - Microscopy and flow cytometry statistical analysis
  - WGS analysis and variant calling
  - Nucleosome occupancy and positioning analysis
  - Differential gene expression analysis
  - NuSA qPCR analysis
- **DATA AND SOFTWARE AVAILABILITY**

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes seven figures and seven tables and can be found with this article online at https://doi.org/10.1016/j.cell.2017.10.043.

**AUTHOR CONTRIBUTIONS**

Conceptualization, Funding, Methodology, Writing – Review & Editing, D.M.T. and J.D.B.; Investigation, Formal Analysis, Writing – Original Draft, D.M.T.; Supervision, Resources, J.D.B.

**ACKNOWLEDGMENTS**

We thank Junbiao Dai for plasmids and strains and Michael Shen for the Gal1-EGFP plasmid. We thank Leslie Mitchell, Liam Holt, and Karim-Jean Armache for comments on the manuscript. This work was supported by NIH (NIGMS) fellowship F32GM116411 to D.M.T. J.D.B. is a founder and director of Neo-chromosome and serves as a scientific adviser to Modern Meadow, Recombinetics, and Sample6. These arrangements are reviewed and managed by the committee on conflict of interest at NYULMC. The topic of this paper is the subject of a patent application.
REFERENCES


# STAR★METHODS

## KEY RESOURCES TABLE

<table>
<thead>
<tr>
<th>REAGENT or RESOURCE</th>
<th>SOURCE</th>
<th>IDENTIFIER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibodies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>human histone H3 (mouse monoclonal)</td>
<td>Abcam</td>
<td>Cat#ab24834</td>
</tr>
<tr>
<td>human histone H4 (rabbit polyclonal)</td>
<td>Abcam</td>
<td>Cat#ab10158</td>
</tr>
<tr>
<td>histone H3 K4 trimethyl (mouse monoclonal)</td>
<td>Abcam</td>
<td>Cat#ab1012</td>
</tr>
<tr>
<td>histone H3 K36 trimethyl (rabbit polyclonal)</td>
<td>Abcam</td>
<td>Cat#ab9050</td>
</tr>
<tr>
<td>IRDye 800CW goat anti-mouse</td>
<td>Li-Cor</td>
<td>Cat#926-68071</td>
</tr>
<tr>
<td>IRDye 680RD goat anti-rabbit</td>
<td>Li-Cor</td>
<td>Cat#926-32210</td>
</tr>
<tr>
<td>Chemicals, Peptides, and Recombinant Proteins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CircLigase</td>
<td>Epicenter</td>
<td>Cat#CL4111K</td>
</tr>
<tr>
<td>TGIRT-III reverse transcriptase</td>
<td>InGex</td>
<td>Cat#TGIRT10</td>
</tr>
<tr>
<td>Yeast Nitrogen Broth without phosphate</td>
<td>MP biomedicals</td>
<td>Cat#114027812</td>
</tr>
<tr>
<td>Micrococcal Nuclease</td>
<td>Sigma-Aldrich</td>
<td>Cat#N5386</td>
</tr>
<tr>
<td>Zymolase 20T</td>
<td>US Biological</td>
<td>Cat#Z1000</td>
</tr>
<tr>
<td>Sytox Green</td>
<td>Thermo-Fisher</td>
<td>Cat#S7020</td>
</tr>
<tr>
<td>Deposited Data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All High-throughput sequencing data are listed in Table S7 and available in the SRA database.</td>
<td>SRA database</td>
<td>Bioproject: PRJNA379735</td>
</tr>
<tr>
<td>Experimental Models: Organisms/Strains</td>
<td></td>
<td></td>
</tr>
<tr>
<td>yDT51 (parental shuffle strain)</td>
<td>This work</td>
<td>N/A</td>
</tr>
<tr>
<td>yDT172 (dad1 shuffle strain)</td>
<td>This work</td>
<td>N/A</td>
</tr>
<tr>
<td>All Saccharomyces cerevisiae strains used in this study are listed in Table S5.</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Oligonucleotides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primers are listed in STAR Methods and Table S7.</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Recombinant DNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasmids are listed in Table S6.</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Software and Algorithms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BWA (version 0.7.7)</td>
<td>Durbin Lab</td>
<td><a href="http://bio-bwa.sourceforge.net/">http://bio-bwa.sourceforge.net/</a></td>
</tr>
<tr>
<td>GATK (version 3.7)</td>
<td>Broad Institute</td>
<td><a href="https://software.broadinstitute.org/gatk/">https://software.broadinstitute.org/gatk/</a></td>
</tr>
<tr>
<td>Trimmomatic (version 0.36)</td>
<td>Usadel Lab</td>
<td><a href="http://www.usadellab.org/cms/?page=trimmomatic">http://www.usadellab.org/cms/?page=trimmomatic</a></td>
</tr>
<tr>
<td>FastQC (version 0.11.4)</td>
<td>Babraham Institute</td>
<td><a href="https://www.bioinformatics.babraham.ac.uk/projects/fastqc/">https://www.bioinformatics.babraham.ac.uk/projects/fastqc/</a></td>
</tr>
<tr>
<td>Samtools (version 1.3.1)</td>
<td>Durbin Lab</td>
<td><a href="http://samtools.sourceforge.net/">http://samtools.sourceforge.net/</a></td>
</tr>
<tr>
<td>NGS Plot (version 2.61)</td>
<td>Shen Lab</td>
<td><a href="https://github.com/shenlab-sina/ngsplot">https://github.com/shenlab-sina/ngsplot</a></td>
</tr>
<tr>
<td>Danpos2 (version 2.2.2)</td>
<td>Chen Lab</td>
<td><a href="https://sites.google.com/site/danposdoc/">https://sites.google.com/site/danposdoc/</a></td>
</tr>
<tr>
<td>Panther</td>
<td>Gene Ontology Phylogenetic Annotation Project</td>
<td><a href="http://pantherdb.org/">http://pantherdb.org/</a></td>
</tr>
<tr>
<td>Yeastract</td>
<td>Teixeira Lab</td>
<td><a href="http://www.yeastract.com/">http://www.yeastract.com/</a></td>
</tr>
<tr>
<td>String</td>
<td>Swiss Institute of Bioinformatics</td>
<td><a href="https://string-db.org/">https://string-db.org/</a></td>
</tr>
<tr>
<td>Kallisto (version 0.43.0)</td>
<td>Pachter Lab</td>
<td><a href="https://pachterlab.github.io/kallisto/">https://pachterlab.github.io/kallisto/</a></td>
</tr>
<tr>
<td>Sleuth</td>
<td>Pachter Lab</td>
<td><a href="http://pachterlab.github.io/sleuth/">http://pachterlab.github.io/sleuth/</a></td>
</tr>
<tr>
<td>R studio (version 3.3.1)</td>
<td>R studio</td>
<td><a href="https://www.rstudio.com/">https://www.rstudio.com/</a></td>
</tr>
<tr>
<td>IGV viewer</td>
<td>Broad Institute</td>
<td><a href="http://software.broadinstitute.org/software/igv/">http://software.broadinstitute.org/software/igv/</a></td>
</tr>
<tr>
<td>ggplot2</td>
<td>Hadley Wickham</td>
<td><a href="http://ggplot2.org/">http://ggplot2.org/</a></td>
</tr>
</tbody>
</table>

Cell 171, 1508–1519.e1–e6, December 14, 2017
CONTACT FOR REAGENT AND RESOURCE SHARING

Requests for materials should be addressed to the Lead Contact, Jef Boeke (Jef.Boeke@nyumc.org).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Strains, plasmids, and media

All yeast strains used in this study were S288C derived and haploid MATα, except as indicated in Table S5 (complete list). Yeast to human complementation studies of histones H3 or H4 alone or in combination, were performed in strain yDT17. Strain yDT17 was generated by replacing the HHT1-HHF1 locus with NatMX4 by one-step PCR recombination, reintroducing HHT1-HHF1 on a URA3 containing pRS416 plasmid, and then replacing the HHT2-HHF2 locus with HygMX4. Experiments involving H2A or H2B alone or in combination used strain yDT30. Strain yDT30 was generated by replacing the HTA2-HTB2 locus with HygMX4, transformation with pRS416-HTA2-HTB2, and then deleting the HTA1-HTB1 locus as above with KanMX4. Analysis of all four histones shuffle was performed in strain yDT51. yDT51 was generated similarly to the above, but contains plasmid yDT83 (pRS416-HTA2-HTB2-HHT1-HHF1). The antibiotic markers (KanMX4, NatMX4, HygMX4) and the His3MX4 cassette used in replacing the four loci were reclamed by deleting these open reading frames using the CRISPR/Cas9 system (DiCarlo et al., 2013) at the positions indicated with red arrows in Figure S1A.

Human histone genes were codon-optimized for yeast and synthesized by Epoch Biolabs. All cloning was performed by Gibson Assembly. Swap-back residue (human to yeast) histone variants were generated either by gene synthesis or site-directed mutagenesis. A complete list of available strains and plasmids are in Tables S5 and S6.

METHOD DETAILS

Dual-plasmid histone shuffle assay

Shuffle strains (yDT17, yDT30, or yDT51) (Figures 1, 3, S1, S3, and S4), which already contains a set of yeast histones on a URA3 plasmid, were transformed by a standard Lithium Acetate protocol with a TRP1 human histone plasmid, which uses the endogenous promoters/terminators from the other yeast histone set. Colonies were selected for 3 days at 30°C on SC–Ura–Trp plates. Single colonies were picked and grown up overnight at 30°C in 2 mL of SC–Trp. Spot assays (as indicated) were diluted 10-fold from overnight cultures (A600 of ~10) and spotted on both SC–Trp and SC–Trp+S-FOA plates. Shuffle assays for fully human nucleosomes using strain yDT51, were done as above, except 400 μl of overnight culture were spread onto a 10-cm SC–Trp+S-FOA plate (25 mL) and incubated at 30°C for up to 20 days in a sealed Tupper-ware container.

Plasmid isolation from yeast cells

Cells were harvested from 5 mL SC–Trp overnight culture and re-suspended in 600 μl of water and glass beads. Cells were vortexed for 10 min to disrupt cells. Plasmids were then isolated by alkaline lysis using the Zymo Zyppy miniprep kit and eluted in 20 μl of water.

PCRtag analysis

Crude genomic DNA was generated using a SDS/Lithium Acetate method (Løoke et al., 2011). Comparative PCRtag analysis (Figures 1 and S1) was performed using 0.5 μl of crude gDNA in a 20 μl GoTaQGreen Hot Start Polymerase reaction (Promega) containing 400 mM of each primer (Table S7). Reactions were run as follows: 95°C/5 min, followed by 35 cycles of (95°C/30 s, 62°C/30 s, 72°C/30 s) followed by a 72°C/2 min extension. A 10 μl aliquot was run on a 1% agarose/TTE gel.

Pulsed-field gel electrophoresis

Intact chromosomal DNA plugs were prepared as described elsewhere (Hage and Houseley, 2013). Chromosome identity was inferred from the known molecular karyotype of parental cells (yDT51) itself derived from S288C that was run on the same gel. Samples were run on a 1.0% agarose gel in 0.5x TBE for 24 h at 14°C on a CHEF apparatus (Figure S2). The voltage was 6 V/cm, at an angle of 120° and 60-120 s switch time ramped over 24 h.

Mating and sporulation tests

Mating tester lawns (his1 strains 17/17 MATα or 17/14 MATα) were replica plated to YPD plates (Figure S2). A large amount of humanized strains (His1) were then smeared onto the replica plate to form rectangles, and then incubated overnight at 30°C. Plates were then replica plated onto synthetic defined (SD) plates and incubated overnight at 30°C. The diploids were sporulated for 7 days as previously described (Dai et al., 2008).

Microscopy

All yeast were grown to an A600 of 0.5-0.9 in SC–Trp liquid media, and imaged under phase-contrast conditions at 100X magnification using a Nikon Eclipse Ti microscope. Cellular diameters (Figures 6 and S7) were measure from 4 images each, comprising a total of 50 single cells. Violin plots and boxplots were generated using the R-package ggplot2.
Cell counting and viability
Cells were manually counted using a hemacytometer with Trypan blue vital dye under a microscope (Figure S6C). Cell viability was also measured by incubating cells in 1 μM Sytox Green solution in PBS, and counting number of fluorescent cells (dead) by flow cytometry on a BD Accuri C6 flow cytometer (Figure S6D). Coulter counting was performed using a Millipore Scepter by diluting log phase cultures 1:100 in PBS, and then taking up cells according to manufacturer’s recommendations. Micromanipulation of single cells was performed using a Singer MSM 400 onto YPD plates.

Cell-cycle analysis using sytox green
Yeasts were grown to log-phase unless otherwise indicated in SC–Trp. Lag-phase for yDT67 (WT) took 45 min, whereas yDT97 (swap-back) took 2 h. Briefly, 10^7 cells were fixed overnight in 70% EtOH. Cells were incubated in 500 μL of 2 mg/ml RNaseA solution for 2 h at 37°C. Then, 25 μL of 20 mg/ml proteinase K solution was added, and cells incubated for 45 min at 37°C. Cells were washed and then stored in 1 mL 50 mM Tris pH 7.5. 50 μL of cells were re-suspended in 1 mL of 1 μM solution of Sytox Green (Thermofisher), and then 10,000 events were analyzed by flow cytometry on a BD Accuri C6 flow cytometer (Figures 6 and S7).

Flow cytometry of GAL1-eGFP induction
Strains as indicated were transformed with plasmid pAV115-GAL-GFP, and selected on SC–Leu+2% glucose plates at 30°C. Single colonies were grown overnight at 30°C in SC–Leu+2% glucose. Cells were washed once in PBS, and then sub-cultured into SC–Leu+2% galactose+1% raffinose media and incubated at 30°C. For the times indicated, 25 μL of cells were diluted into 0.2 mL PBS and 10,000 events were analyzed by flow cytometry on a BD Accuri C6 flow cytometer (Figure 6).

“Re-humanization” of suppressor mutants
Humanized lineages were re-transformed with native yeast histone plasmid pDT83 (URA3) using standard Lithium Acetate transformation, and selected on SC–URA–Trp plates for 4 days at 30°C. To determine the “human histone memory,” single colonies were grown overnight in 2 mL SC–Trp and directly used in dual-plasmid histone shuffle as described above (Figure 7). To determine the “re-humanization” rate of suppressor mutations, single colonies from the above re-transformed strains were grown in SC–Ura for multiple sub-cultures to allow mitotic loss of the TRP1 human histone plasmid pDT109. Cells were replica plated onto SC–Ura and SC–Trp to identify those containing only the native yeast histones. These strains were then used for another round of dual-histone plasmid shuffle as described above.

Protein analysis and Western Blotting
Whole-cell extracts were generated by resuspending 10^6 log-phase yeast cells in 400 μL 0.15 M NaOH and 0.5 mM dithiothreitol (DTT), and incubated for 10 min on ice. Cells were pelleted at top speed for 10 min at 4°C, and re-suspended in 65 μL lysis buffer (20 mM HEPES pH 7.4, 0.1% Tween20, 2 mM MgCl2, 300 mM NaCl, 0.5 mM DTT, and 1 mM Roche Complete protease inhibitor) and an equal volume of 0.5 mm glass beads. Mixture was vortexed at top speed for 10 min in the cold room. Subsequently, 25 μL of NuPAGE (4X) LDS Sample buffer and 10 μL beta-mercaptoethanol was added, and the mixture was heated at >95°C for 10 min. The debris was pelleted and the supernatant was run on a 12% Bis-Tris SDS acrylamide gel and stained with Coomassie blue (Figure S6E).

Acid extracted histones were generated by first resuspending 5 × 10^6 log-phase yeast in spheroblasting buffer (1.2M Sorbitol, 100 mM potassium phosphate pH 7.5, 1 mM CaCl2, and 0.5 mM δ-mercaptoethanol) containing Zymolase 20T (40 units/ml) and incubating for 20 min at 37°C. Spheroplasts were gently spun down at 3000 rpm for 3 min, and then re-suspended in 1 mL of 0.5 M HCl/10% glycerol with glass beads on ice for 30 min. Cells were vortexed at top speed for 1 min every 5 min and kept on ice. Mixture was spun at 16,000 x g for 10 min and the supernatant was added to 8 volumes of acetone and left at –20°C overnight. The following day, mixture was pelleted for 5 min at 16,000 x g, the solution poured off and the pellet was air-dried.

Pellet was resuspended in 130 μL water, and then 50 μL NuPAGE (4X) LDS sample buffer and 20 μL beta-mercaptoethanol was added, and the mixture was heated at >95°C for 10 min. Supernatant was run on a 12% Bis-Tris SDS acrylamide gel and stained with Coomassie blue, or directly used for western blotting (Figure S5B).

Protein samples run on 12% Bis-Tris SDS acrylamide gel were transferred to membrane (Millipore, Immobilon-FL) using the BioRad Trans-Blot Turbo system according to manufacturer’s recommendations. Membranes were blocked for 1.5 h at room temperature in 1:1 Tris-buffered saline (TBS)/Odyssey blocking buffer (Li-Cor). Blocking buffer was removed and membrane re-suspended in primary buffer overnight at 4°C containing 1:1 TBS + 0.05% Tween-20 (TBST)/Odyssey, and the following antibodies used at 1:2,000 dilution: human H3 (abcam ab24834), H3K4me3 (abcam ab1012), H3K36me3 (abcam ab9050), human H4 (abcam ab10158). The following day, membrane was washed 5 times for 5 min each in TBST/Odyssey, re-suspended in secondary antibody buffer TBST/Odyssey/0.01% SDS with 1:20,000 dilution of both IRDye 800 goat anti-mouse and IRDye 680 goat anti-rabbit (Li-Cor) for 1.5 h at room temperature. Secondary was washed 5 times for 5 min each in TBST/Odyssey and then imaged using dual channels on a Li-Cor Odyssey CLx machine.

Growth assay on various types of solid media
Cultures were normalized to an A_600 of 10 and serially diluted in 10-fold increments in water and plated onto each type of medium. The following drugs and conditions (Figure S2) were mixed into YPD+ 2% glucose+ 2% agar: benomyl (15 μg/ml; microtubule inhibitor),
camptothecin (0.5 μg/ml; topoisomerase inhibitor), hydroxyurea (0.2 M; defective DNA replication), NaOH (pH 9.0; vacuole formation defects), HCl (pH 4.0; vacuole formation defects), and methyl methanosulfate (MMS 0.05%; defective DNA repair). Galactose plates were prepared in SC media+ 1% raffinose+ 2% galactose.

Whole genome sequencing
Genomic DNA was isolated using Norgen Biotek’s Fungal/Yeast Genomic DNA isolation kit, which included a spheroblasting step and bead-beating step. At least 1 μg of genomic DNA was used for Illumina library preparation using the Kapa Truseq library prep, and we routinely multiplexed 30 yeast genomes on a single HiSeq 4000 or 2500 lane.

MNase-digestions and MNase-sequencing
Biological triplicate yeast colonies were each grown at 30°C to an A600 of ~0.9 in 100 mL of SC–Trp media. Cultures were crosslinked with 1% formaldehyde for 15 min at 25°C, and then quenched with 125 mM glycine. Cultures were washed twice in water, and pellets were then stored at ~80°C. To perform MNase digestions, cells were first spheroplasted by suspending pellets in 4 mL spheroplasting buffer (1.2M Sorbitol, 100 mM potassium phosphate pH 7.5, 1 mM CaCl2, and 0.5 mM β-mercaptoethanol) containing Zymolase 20T (40 units/ml) and incubated for 20 min at 37°C. Spheroplasts were gently washed twice with spheroplasting buffer, and then re-suspended in 1 mL digestion buffer (1M Sorbitol, 50 mM NaCl, 10 mM Tris-HCL (pH 7.4), 5 mM MgCl2, 1 mM CaCl2, 0.5 mM spermidine, 0.075% NP-40, and 1 mM β-mercaptoethanol). Samples were split into 500 μl aliquots equivalent to 50 mL culture each. To each sample, micrococcal nuclease (Sigma: NS386) was added to a final concentration for high digestion (2 units/ml) or low digestion (0.2 units/ml) or as specified in Figure S5. Digestions proceeded at 37°C for 45 min. Reactions were quenched with 16.6 μL of 0.5 M EDTA. Crosslinks were reversed in 0.5% SDS and 0.5 mg/ml proteinase K, by incubating at 37°C for 1h, followed by 65°C for 2 h. Nucleic acid was extracted with phenol/chloroform twice, followed by chloroform. Nucleic acid was precipitated by adding 50 μl sodium acetate (3M, pH 5.2), an equal volume of isopropanol, and spinning for 20 min at 16,000 x g. Pellets were washed once with 70% EtOH, and then resuspended in 50 μL TE buffer containing 6 kUnit of RNase A, and incubated for 30 min at 37°C. DNA was then purified using a Zymo DNA clean and concentrator, and eluted in 20 μl. MNase digested fragment DNA was measured by Qubit, and assessed on a 1.5% agarose TTE gel. At least 200 ng of DNA (PCR-free or minimal PCR of 2-3 cycles) for each replicate was used to generate a library for paired-end sequencing on an Illumina Hiseq 4000 (Table S7).

RNA extraction and mRNA-seq
Strains were subjected to poly(a+) RNA-seq analysis with at least three biological replicates (Figure 5; Table S7). Briefly, each biological replicate was grown at 30°C in 5 mL SC–Trp to an A600 between 0.6-0.8 from an initial starting A600 of 0.15. Cultures were spun down, quenched in 100% EtOH, and then stored at ~80°C. Total RNA was then extracted by a modified hot-phenol method as follows. Pellets were re-suspended in RNA extraction buffer (100 mM EDTA pH 8, 100 mM NaCl, 50 mM Tris-HCL pH 8, 0.25% SDS) and then an equal volume of acid phenol/chloroform (pH 4.5) was added. The mixture was incubated at 70°C for 1 h with intermittent vortexing. Mixture was spun down, and the aqueous fraction was re-extracted with an additional round of acid phenol/chloroform, and then chloroform. The aqueous fraction was mixed with an equal volume of isopropanol, and then spun at ~16,000 x g for 20 min. Pellet was washed once with 70% EtOH, and then re-suspended in 30 μL TE buffer. At least 1 μg of RNA for each biological replicate was then used for poly(a+) selection, cDNA synthesis, and then Illumina library preparation. Libraries were sequenced as single-end reads on a HiSeq 2500 (Table S7).

Identification of small RNAs
Sequencing of tRNAs was adapted from (Zheng et al., 2015) (Figure S6B). Briefly, a template-primer substrate (Integrated DNA Technologies) consisting of a 30-nt RNA oligo (5’-AGAUCGGAAGCGCACACGUCUAGUCCUACA/3SpC3/-3) annealed to a complementary 31-nt DNA oligo (5’-TGTAGACTAGCCTGTCCTTCCGATCTN-3), facilitates templates switching to any RNA species using a single nucleotide overhang (N). 2 μg total RNA from humanized yeasts was mixed with 100 nM template-primer and 500 nM TGIRT-III reverse-transcriptase (InGex), in reaction medium (450 mM NaCl, 5 mM MgCl2, 20 mM Tris-HCL pH 7.5, and 5 mM DTT) for 30 min at room temperature. Reverse transcription was initiated by added dNTP to 1 mM and incubating the reaction for 45 min at 60°C. Reactions were terminated by adding 5 M NaOH to a final concentration of 0.25 M, incubating at 95°C for 3 min, and then neutralizing with 5 M HCl. Product was purified using a DNA clean and concentrator kit (Zymo). Purified cDNA product was then circularized with CircLigase (Epicenter) according to manufacturer’s recommendations. After circularization, product was then re-purified using a DNA clean and concentrator. DNA product was then PCR amplified using Phusion polymerase using primers (For - AGATCGGAAGAGGACACCG; Rev - TGTAGACTAGCGCCTGCT) for 25 standard cycles. The major gel product – an ~120 nt band corresponding to tRNA – was then gel extracted from a 1.5% agarose gel. DNA product was then cloned into a Zero Blunt TOPO cloning kit (Thermo Fisher), and individual colonies were Sanger sequenced.

Nucleosome scanning assay (NuSA) of the PH05 promoter
PH05 nucleosome scanning assay time-course was adapted from (Small et al., 2014) (Figure 6E). Isogenic strains (yDT197 – > WT, yDT180 – > hH3.1-core, yDT186 – > hH3.3-core) as indicated, were grown as two biological replicates in 50 mL SC medium (containing phosphate) with 2% glucose to an A600 of 1-1.3. Cultures were washed twice with 50 mL water to remove residual phosphate.
To induce remodeling at the PHO5 promoter, cultures were then re-suspended in 60 mL SC medium without phosphate (Yeast Nitrogen Broth w/o phosphate (MP biomedicals, sku 114027812), 100 mg/L NaCl, amino acids, and glucose) to an A600 of 1, and then incubated at 30°C with shaking. Time points as indicated were treated as “MNase digestions” as described earlier except in a 10-fold reduced scale (e.g., 10 mL culture, 400 μL spheroblasting media, etc). Time-point cultures (10 mL) were normalized to an A600 of 1 for each time point. Processed MNase digested DNA was analyzed by qPCR using primers listed in Table S7 in a Roche LightCycler 1536 real-time PCR machine using LightCycler 1536 DNA Green Master (Roche) in technical triplicate.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

**Microscopy and flow cytometry statistical analysis**

Cellular diameters (Figure S7) were measured from 4 images each, comprising a total of 50 single cells for each data point, using the Nikon NIS Elements image analysis software (version 4.6). Violin plots and boxplots were generated using the R-package ggplot2. F-tests and t tests showing significance in size distribution and size variance were calculated using excel. Flow cytometry histogram distributions (Figures 6, S6, and S7) were calculated automatically from 10,000 single cell counts per data point using the BD Accuri C6 Sampler software.

**WGS analysis and variant calling**

Paired-end FASTQ files (Table S7; SRA database) were aligned with the following pipeline. First, adapters, reads shorter than 50 bp, and poor quality reads near ends, were removed using Trimmomatic. Data quality was assessed using FastQC. Processed reads were aligned to a custom genome reference (yDT51H.fa) using Burrows Wheeler aligner (BWA) mem algorithm, and Sam files were converted to sorted Bam files using Samtools. Variants were called by adapting the GATK “best practices” pipeline using Haplotype caller, custom scripts, and manually verified on the IGV viewer (Figure 2; Tables S1 and S2). Variants were identical using Samtools “mpileup.” Read counts for each chromosome were determined from WGS Bam files using Bedtools “genome coverage.” Chromosome copy number was then calculated by generating boxplots in R using ggplot2 (Figure 2 and Table S2). Networks for suppressor mutants were generated by uploading genes into the String online server (Szklarczyk et al., 2015) (Figure 2E). Transcription factors predicted to control gene sets were identified using the Yeastact server (Teixeira et al., 2014) (Figure 2F). GO terms were identified using the Panther database (Mi et al., 2016). Of the 37 mutations identified (Tables S1 and S2), 6 synonymous mutations were considered “innocuous” based on their similar codon usage.

**Nucleosome occupancy and positioning analysis**

MNase-seq FASTQ reads (Table S7; SRA database) were processed using Trimmomatic, FastQC, and then aligned to the sacCer3 reference genome using BWA-mem, and then converted to a sorted Bam file using samtools. Custom bed files corresponding to the top and bottom 1500 genes, centromere regions, and tRNA regions were used to align MNase reads using Ngs.plot (Shen et al., 2014) to regions as specified (Figures 4, 5, S5, and S6H). Fragment lengths were obtained from Sam files and plotted using ggplot2 (Figure S5C). Nucleosome dynamics were analyzed using DANPOS2 (Chen et al., 2013) (Figures 4 and S5E). Variance in fragment distributions (e.g., more/less mononucleosomes and dinucleosomes) between WT and humanized, was accounted for by using the options–mifrsz 50 and–mafrsz 500. Custom awk scripts were used to process the data to reduce erroneously called and altered nucleosomes as based on comparing MNase-seq data from WT experiment 1 against WT experiment 2 (“noise”). Nucleosome shifts passed the threshold when both nucleosome comparisons had aligned reads >300 and when shifts were greater than 70 bp. Nucleosome occupancies required that at least one nucleosome comparison have an aligned read count >300, and the False Discovery Rate (FDR) was lower than 0.05 with a p < 10^{-6}. Fuzzy nucleosomes required that both nucleosome comparisons have read counts >300 and an FDR of < 0.05.

**Differential gene expression analysis**

Humanized strains (yHs1-7) and WT each had biological triplicate sequencing runs (Figure 5C; Table S7; and SRA database). Illumina barcodes and quality trimming was performed using Trimmomatic, and then library integrity assessed using FastQC. Reads were then aligned to all known S288C RNA transcripts in a process referred to as pseudo-alignment using Kallisto (Bray et al., 2016). Data analysis was performed in R using the Sleuth package (Pimentel et al., 2017) and ggplot2. Beta values (natural log2 with technical variation removed) was converted to log2 fold change using the formula LOG(POWER(2.71828, Beta), 2) in Excel. Volcano plots were generated using the Wald test. GO-terms were generated using the Panther database (Mi et al., 2016). Differential expression across chromosomes was generated by ranking genes and log2 values by chromosome location and then plotting the results.

**NuSA qPCR analysis**

The qPCR data values were analyzed by absolute quantification as follows (Figure 6E). Lightcycler Cq values (technical triplicate) were exported to an excel table. Standard curves to determine absolute copy number were generated for each primer-set (Table S7) from 10-fold serial dilutions of yeast gDNA buffered in 10 ng/μl herring sperm DNA. Serial dilutions were converted to absolute copy number in log10 values. Cq values from standard curves were plotted against copy number to generate intercept values and slope values in excel (i.e., y = (slope)x+intercept) for each unique primer set. Absolute copy numbers for each value were then
calculated using the formula $10^{((Cq\text{-intercept})/\text{slope})}$ for each primer-set. To determine % nucleosome occupancy, PHO5 qPCR absolute copy numbers were normalized to qPCR absolute copy numbers at the +1 nucleosome of the gene HXT16, a glucose repressed gene that has zero read counts by mRNA-seq (Figure 5), and does not remodel under glucose rich conditions.

**DATA AND SOFTWARE AVAILABILITY**

The accession number for the high-throughput sequencing data reported in this paper is Bioproject: PRJNA379735, and listed in Table S7.
Figure S1. Construction of Yeast with Completely Human Nucleosomes: yHs, Related to Figure 1

(A) Map of histone genomic locations in yeast. Triangles show histone pairs deleted in (C). Red arrows indicate CRISPR/Cas9 deletion junctions. Different shades of green show the divergent histone promoters.

(B) Diagram of main histone plasmids used in this study for the dual-plasmid histone shuffle. Note the different promoters/terminators used shown in different shades of green.

(C) The three histone deletion strains used for humanization studies in Figure 1D, and stabilizing plasmids as indicated.

(D) PCR tag confirmation of yeast containing human histones H3.1/H3.3 and H4 (hH3.1/hH3.3 and hH4).

(E) PCR tag confirmation of yeast containing human histones H2A and H2B (hH2A and hH2B).

(F) PCR tag confirmation of the 8 yeast with completely human nucleosomes with the names “yHs” for “Yeast Homo Sapiens.”

(G) Colony growth rates for various “WT” versions of yeast that contain different complements of native yeast histone plasmids.

(H) Demonstration of how rapidly “yHs” yeast accumulate suppressors and evolve toward faster growth.
Figure S2. Growth Rates of yHs Strains and Chromosomal Aneuploidy, Related to Figures 1 and 2
(A) Growth of yHs1-7 on the following drugs and conditions: SC–Trp + 2% glucose, SC + 1% raffinose and 2% galactose (respiration), YPD + 2% glucose, YPD + 2% glucose + either: HCl (pH 4.0; vacuole formation defects), NaOH (pH 9.0; vacuole formation defects), Benomyl (15 μg/ml; microtubule inhibitor), Methyl methanesulphate (MMS 0.05%; defective DNA repair), Camptothecin (0.5 μg/ml; topoisomerase inhibitor), and Hydroxyurea (0.2 M; defective DNA replication).
(B) Mating tests of yHs1-7 with wild-type yeast strains. Mated diploids were sporulation competent.
(C) Growth comparison of yHs1-7 from original colony isolates, maintenance strains (yHs-m), and evolved strains (yHsC5) on solid medium for 3 and 7 d using 10-fold serial dilutions. Cells were normalized to an A600 of 10.
(D) None of the eight yHs lineages show gross chromosomal abnormalities (deletions or insertions) as analyzed by pulsed-field gel electrophoresis.
(E) Examples of chromosomal aneuploidies for 3 yHs lineages, including yHs7 (aneuploid) and yHs7-evo, which showed no aneuploidies and acquired a mutation in the gene DAD1.
Figure S3. Identification of Residues in Human H3 and H4, when Swapped Back to Yeast, Improve Humanization Frequency, Related to Figure 3

(A) Systematic mapping of human to yeast residues in hH3.3 using 5-FOA plasmid shuffling. The right-hand shows maps of the tested mutants, with black-arrows indicating positions swapped-back to yeast. Each plasmid was tested in strain yDT17, which contains deletions of both H3/H4 loci and is stabilized with a URA3-CEN plasmid containing the HHT1-HHF1 locus. Yeast are spotted in 10-fold serial dilutions. Versions labeled hH3.1-C and hH3.3-C were shown to complement well in yeast (McBurney et al., 2016).

(B) Systematic mapping of human to yeast residues in histone H4. Swap-back residues in hH4 were tested as described in (A) also in strain yDT17.

(C) Combination of different hH3 swap-back strains with completely human H4. When combined with hH4, two swap-back residues (P121K and Q125K) are optimal for hH3.1, whereas three are optimal for hH3.3.
Figure S4. Identification of Swap-Back Residues in Human H2A that Improve Humanization Frequency, Related to Figure 3
(A) hH2A was partitioned into 6 regions, and each region was swapped-back to yeast to test complementation frequency using 5-FOA plasmid shuffling in strain yDT30.
(B) Regions 1, 2, and 4 were partitioned into further systematic swap-backs.
(C) Complementation assays of swap-back strains from (B).
(D) Three swap-back residues each in the N terminus (hH2AN) or C terminus (hH2AC) of hH2A enhanced humanization frequency and growth rates in combination with hH2B. The combination of all six swap-back residues (hH2ANC) is optimal.
Figure S5. MNase Digestions and MNase-Seq of Humanized Yeast, Related to Figure 4

(A) Representative DNA fragments of high (2 units) and low (0.2 units) chromatin MNase digestions used for MNase-sequencing run on a 1% agarose gel. Experiment 1 was performed in biological triplicate and experiment 2 was performed once. All samples from same strain had similar profiles. “M” refers the DNA marker.

(B) Full MNase-titration digestion agarose gel shown in Figure 4A. Red arrows indicate position of the tri-nucleosome, which differs only in the human cell line nucleosome digest. HeLa cells were digested at higher concentrations for a shorter duration and with sonication. “L” refers the DNA marker and “bp” indicates base-pair size.

(C) Fragment length histogram from the low and high MNase-seq reads.

(D) Low MNase-seq read counts at centromeric regions, plotted for chromosomes that were normal or aneuploid in Figure 2D. RCPM refers to read counts per million mapped reads.

(E) Table of Low (0.2 units/ml) MNase-seq nucleosome dynamics between humanized to WT yeast, and WT experiment 1 to WT experiment 2 (‘‘noise’’). Occupancy and fuzziness changes use a strict False Discovery Rate cut-off of 0.05 (p < 10^−5) and additional parameters in STAR Methods.
Figure S6. Humanized Yeast RNA and Protein Levels, Related to Figure 5

(A) Total RNA from humanized cells have a similar rRNA to mRNA pattern and ratios as WT cells, although most have elevated tRNA expression. Because tRNA levels are so elevated, mRNA levels are likely lower than indicated in Figure 5A.

(B) The tRNAs, grouped by amino acid, were identified by reverse-transcribing humanized total RNA from (A), and then sequencing the TOPO-cloned cDNA product.

(C) Reduced RNA content is not due to reduced cell numbers per A600, as yHs cells possess identical or even higher numbers of viable cells (>10^7 cells or A600).

Cells were measured using both coulter counting (Millipore Scepter) and hemocytometer microscopic counting. Viability was determined by counting number of cells that exclude Trypan blue staining. Bars show standard deviation of 2 replicates.

(D) Percentage of non-viable cells (cell viability) determined by Sytox green uptake into dead cells and measured by flow cytometry.

(E) Whole-protein extracts of indicated strains run on 12% SDS-bis-Tris acrylamide gel and stained with Coomassie blue. Protein yields were similar on a per cell basis, and each lane has 50 μg total protein loaded. Proteins < 25 kDa (e.g., histones) appear reduced in abundance.

(F) Boxplots showing histone expression from mRNA-seq based on 3 biological replicates.

(G) mRNA-seq log2 fold change for every gene across each chromosome aggregated for yHs1-7, showing that some telomeres (e.g., TEL-1L, −1R, −3L are de-silenced. The q-value is the False Discovery Rate adjusted p value.

(H) Heatmaps and average profiles of low concentration MNase-seq reads aligned around the transcription start sites (TSS) ± 500 bp of the top and bottom 1500 genes. RCPM refers to read counts per million mapped reads.
Figure S7. Yeast with Human Nucleosomes Have Larger and Less Regulated Cell Sizes and Arrest in G1, Related to Figure 6

(A) Image stills of yDT67 (WT) yeast compared to humanized strains.

(B) Percent of cells in either the unbudded or budded state from phase-contrast microscopy images. Bars are standard error of the mean from 4 separate images.

(C) Violin plots with boxplots inside showing size distributions of the indicated strains for various states of budding. Plots are based on ~50 cells measured from four separate microscopy images. F-tests measure significance of whether two populations have different size distributions. Two-tailed t tests measure significance of difference in average cell size.

(D) Cell-cycle analysis based on DNA content. Cells were stained with sytox green, and DNA content was measured by flow cytometry. Each plot shows 10,000 cells in log-phase growth, except where indicated.

(E) Micromanipulation of single-cells for growth. Most cells remained intact (black underline). Cells with white circles grew for a few cell-divisions and then arrested.