**DATA ACCESS**

Sequencing files generated for this study are in the process of submission on GEO.

Reference files used to generate figures for this paper can be accessed at:

<https://github.com/CEGRcode/Gallego_2019>

All data files were de-duplicated

Most of the analyses were performed on **Scriptmanager v.012**, which can be downloaded at:

<https://github.com/CEGRcode/scriptmanager>

**Figure 7A**

* Deduplicated BAM files were converted to TAB format using **BAM to scIDX** (BAM format converter tab) function of Script Manager v0.12.
* Read 1
* TAB files were shifted by 6 bp using the script **shift\_tags.py**.
* Shifted files were normalized to a 30 bp window centered at NFR dyad (15bp upstream and 15 bp downstream of NFR dyad) of all genes using the script **normalization\_script.py** and reference file **NFR\_dyad\_invivo\_sacCer3\_gff.gff** (included in the link above).
* Normalized files were mapped to nucleosome plus one dyad reference files (.gff) using the script **map\_shifted\_tags\_to\_ref.py**.
* Composite plots were generated using the script **Composite\_plots\_Vinesh\_v2.py**.

**Figure S6A**

* ChIP-exo 5’ tags of TAP-tagged strains were NCIS normalized relative to the no tag negative control, and mapped to the TSSs of indicated gene classes.
* **Tag Pileup** function of Scriptmanager v0.12
* Load BED files – TSS-TES\_midpoint\_all\_gene\_class\_sorted\_genelength\_2000bp.bed
* Load BAM files
* Read 1, Strands – combined, tag shift – 6bp, bin size – 1 bp
* Sliding window – 3 bp
* Output file format : CDT
* **Calculate Scaling Factor**
* Load BAM files
* Load Blacklist Filter : YEP\_Blacklist\_181026.bed
* NCIS
* **Apply Scaling Factor**
* Load TAB files – load CDT files generated above
* Scaling factor – input scaling factors obtained above
* **Figure generation**
* Heatmaps were generated by loading scaled CDT files
* Contrast 3.0

**Figure S6B**

* Datasets were mapped according to expression (Yassour et al., 2009).
* **Tag Pileup**
* Load BED files - top15percenthighlyexpressed\_2000bp.bed and bottom15percent\_lowlyexpressed\_2000bp.bed
* Load BAM files
* Read 1, combined strands, tag shift – 6bp, bin – 1bp,
* Set tags to be equal, sliding window – 3 bp
* Composite plots were generated using Prism 7 software.

**Figure S6C**

* Calculating H2BK123ub density per nucleosome in gene bodies of WT and various Lge1 mutants
* each nucleosome is assumed to be 167 bp including linker on each side (10bp + 147bp + 10 bp).
* Boundaries were determined based on composites generated in **Figure 7**.
* PlusOne boundaries : -83 to +83 bp
* PlusTwo boundaries : +83 to +250 bp
* PlusThree boundaries : +250 to +417 bp
* Tags were summed for each nucleosome and ratios were calculated

**Figure S6D**

* Biological replicates were compared using **BAM Genome Correlation** function of Scriptmanager v0.12
* Window – 500 bp