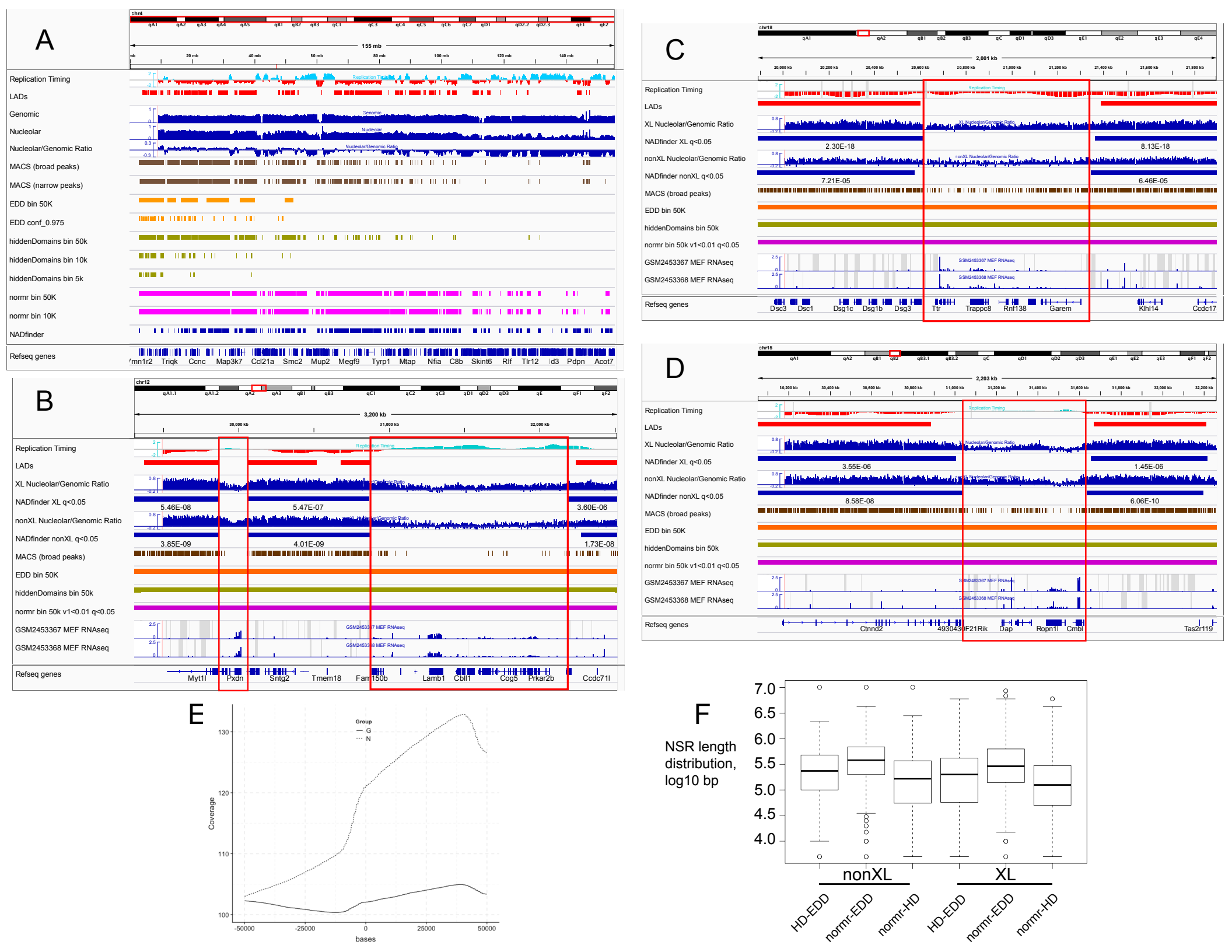
A
chr4
155 mb
Replication Timing
LADs
Genomic
Nucleolar
Nucleolar/Genomic Ratio
MACS (broad peaks)
MACS (narrow peaks)
EDD bin 50K
EDD conf_0.975
hiddenDomains bin 50k
hiddenDomains bin 10k
hiddenDomains bin 5k
normr bin 50K
normr bin 10K
NADfinder
Refseq genes
Mmn1r2 Triqk Ccnc Map3k7 Ccl21a Smc2 Mup2 Megf9 Tyrp1 Mtap Nfia C8b Skint6 Rlf Tlr12 Id3 Pdpn Acot7
B
chr12
3,200 kb
Replication Timing
LADs
XL Nucleolar/Genomic Ratio
NADfinder XL q<0.05
5.46E-08
5.47E-07
3.60E-06
nonXL Nucleolar/Genomic Ratio
NADfinder nonXL q<0.05
3.85E-09
4.01E-09
1.73E-08
MACS (broad peaks)
EDD bin 50K
hiddenDomains bin 50k
normr bin 50k v1<0.01 q<0.05
GSM2453367 MEF RNAseq
GSM2453368 MEF RNAseq
Refseq genes
Myt1l Pxdn Sntg2 Tmem18 Farp1 150b Lamb1 Cbll1 Cog5 Prkar2b Ccdc71l
C
chr18
2,001 kb
Replication Timing
LADs
XL Nucleolar/Genomic Ratio
NADfinder XL q<0.05
2.30E-18
8.13E-18
nonXL Nucleolar/Genomic Ratio
NADfinder nonXL q<0.05
7.21E-05
6.46E-05
MACS (broad peaks)
EDD bin 50K
hiddenDomains bin 50k
normr bin 50k v1<0.01 q<0.05
GSM2453367 MEF RNAseq
GSM2453368 MEF RNAseq
Refseq genes
Dsc3 Dsc1 Dsg1c Dsg1b Dsg3 Ttr Trappc8 Rnf138 Garem Klhl14 Ccdc17
D
chr15
2,203 kb
Replication Timing
LADs
XL Nucleolar/Genomic Ratio
NADfinder XL q<0.05
3.55E-06
1.45E-06
nonXL Nucleolar/Genomic Ratio
NADfinder nonXL q<0.05
8.58E-08
6.06E-10
MACS (broad peaks)
EDD bin 50K
hiddenDomains bin 50k
normr bin 50k v1<0.01 q<0.05
GSM2453367 MEF RNAseq
GSM2453368 MEF RNAseq
Refseq genes
Ctnnd2 4930430F21Rik Dap Ropn1l Cmbl Tas2r119
E
Group
G
N
Coverage
bases
F
NSR length distribution, log10 bp
nonXL
XL
HD-EDD
normr-EDD
normr-HD
HD-EDD
normr-EDD
normr-HD

Supplemental Figure S3. Additional comparisons of different bioinformatic analyses of NAD-seq data.

A. Chromosome 4 is shown in its entirety. In addition to the tracks shown in Figure 1C-D, we compared additional settings for each bioinformatics program tested. For *MACS*, we compared broad and narrow peak settings (Zhang et al., PMID: 18798982; Feng et al. PMID: 22936215). For *EDD*, we compared 50 Kb binning with and without filtering at a 0.975 confidence threshold (Lund et al., PMID:24782521). For *hiddenDomains*, we compared binning at 5, 10, and 50 Kb (Starmer et al., PMID: 27009150). For *normr*, we compared binning at 10 and 50 Kb (Kinkley et al., PMID: 27530917, Helmuth et al., bioRxiv 082263). This panel shows that peak distributions for Chromosome 4 are similar to those for Chromosome 5 as depicted in Fig. 1D. That is, unlike *MACS* and *EDD*, *NADfinder* was capable of identifying peaks in the region distal from the centromere (on the right).

B. A 3.2 Mb region from Chr12qA2-3 is shown, analyzed using the same tracks as in Figure 1E. This region contains two "NAD Splitting Region (NSR)" segments (red boxes) that fall between NADs identified by *NADfinder,* but were assigned to the same large peaks in the *normr*, *EDD* and *hiddenDomains* software. Note that genes within these regions are actively transcribed.

C. As in panel B, showing a ~2 Mb region from Chr18qA2.

D. As in panel B, showing a ~2.2 Mb region from Chr15qB2.

E. Boundary analysis of sequencing reads (normalized to 1x depth coverage) at the borders of NADs. The x-axis displays the distance from the NAD boundary (at 0) in nucleotides. Negative values on the x-axis are within the non-NAD region, and positive values are within the NAD. The y-axis shows the average genome-wide coverage value across three biological replicates. The values from the three Genomic DNA (G, solid line) and Nucleolar (N, dashed line) non-crosslinked samples are shown.

F. The length distributions of "NAD Splitting Regions (NSRs)". NSRs are regions between NADs determined by *NADfinder* that fall within large contiguous peaks determined by the indicated two other software programs (see Supplemental Methods). NSRs from the XL and nonXL NAD datasets were analyzed separately. In all cases, the length distributions were similar and of the same magnitude as the size of NADs themselves (Fig. 2D).