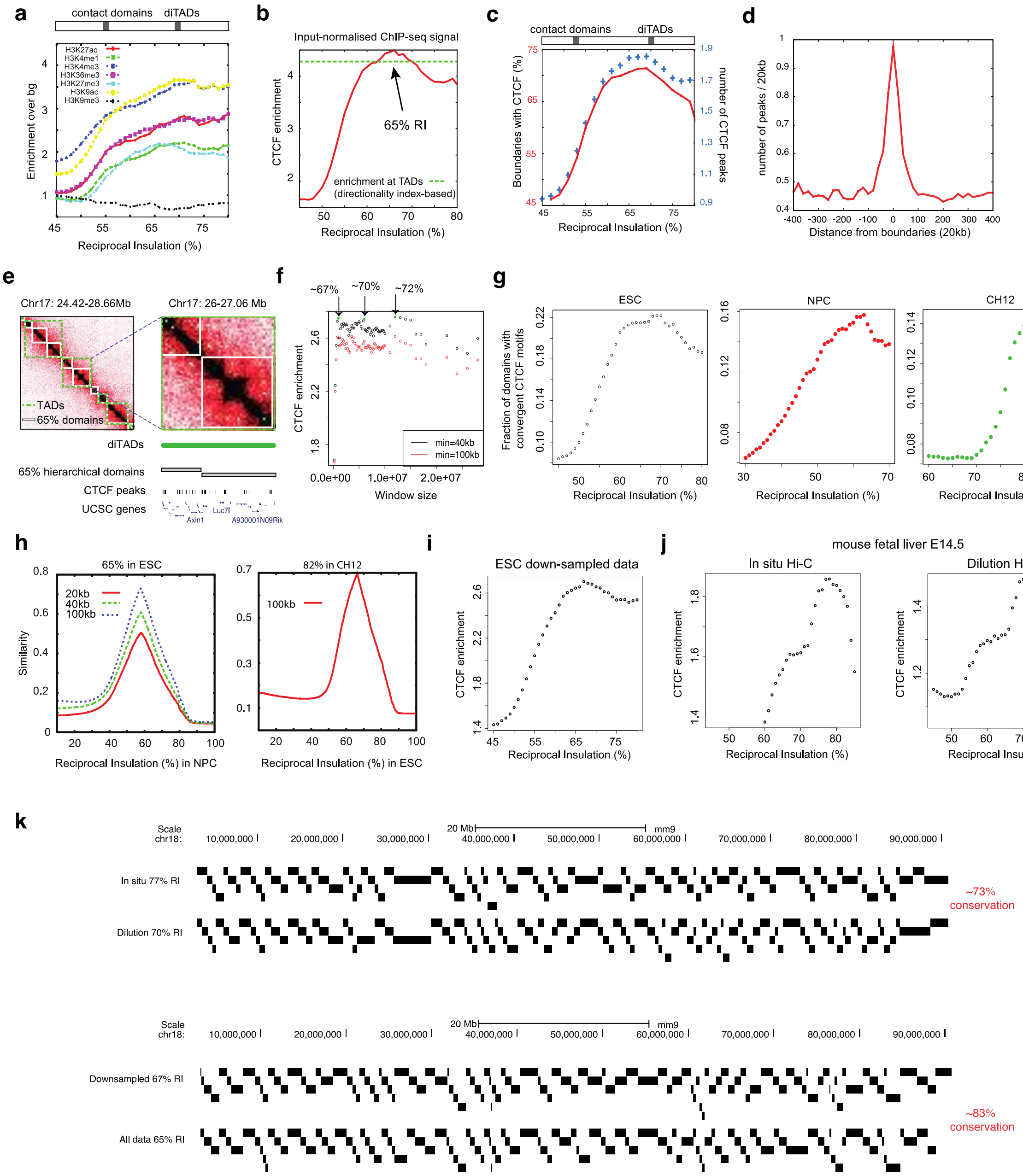

Supplemental Figure S2.

a. Enrichment of histone modification at domains boundaries as a function of RI. All active marks reach maximal enrichment at TAD level. H3K9me3 is depleted everywhere in the hierarchy and in particular at the scale of TADs.

b. Enrichment in CTCF input-normalised ChIP-seq signal at the boundaries as a function of RI. Enrichment is maximum at ~65% RI and slightly higher than diTADs (green line).

c. The number of boundaries that contain at least one CTCF peak, and the number of CTCF peaks per boundary are both maximized around 65% RI.

d. Meta-boundary profile showing CTCF peak abundance in the genomic neighborhood of domains boundaries at 65% RI. Genomic coordinates were aligned to the position of boundaries.

e. Domains at 65% often split a single TAD into smaller domains.

f. CTCF enrichment at the boundaries of domains detected by the directionality index algorithm using different combinations of the ‘window size’ and ‘minimum size’ parameters, which determine the maximal genomic distance used in the computation of the directionality index and the minimum domain size, respectively. Arrows indicate examples of domains where CTCF enrichment is maximal and the corresponding RI value with maximal boundary overlap.

g. Fraction of domains with convergent CTCF motifs at the boundaries as a function of RI. At 65% RI, according to our criterion (see Methods), ~22% of domains possess convergent CTCF sites in ESC (left panel). At ~82% RI ~14% of domains possess convergent CTCF sites in CH12 (right panel).

h. Fraction of conserved boundaries between domains defined in ESCs and either NPCs (left) or CH12 (right). In all cases, maximal conservation of boundaries occurs at the insulation value where maximal enrichment of CTCF at boundaries was found in the various cell types.

i. CTCF enrichment analysis at the domain boundaries as a function of RI, performed on 2X down-sampled dataset in ESC. Maximal enrichment occurs at 67% (cfr. **Figure 2a**).

j. CTCF enrichment analysis at domain boundaries as a function of RI performed on *in situ* (left) and *dilution* (right) Hi-C libraries from mouse E14.5 fetal liver cells (Nagano 2015). Maximal enrichment occurs at different RI for the two protocols (77% for *in situ* and 70% for *dilution* Hi-C).

k. Examples of domains at the scale where CTCF enrichment is maximal. Upper panel shows the domains for *in situ* (77% RI) versus *dilution* (70%) protocols in fetal liver cells. Lower panel shows the domains for the full ESC dataset versus the down-sampled dataset.