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SUPPLEMENTAL FIGURE 1. The majority of Rif1 fails to be solubilised by standard protocols. (**A**) Schematic representation of the position of the putative PP1 docking motifs in mouse Rif1 protein. (**B**) Only a minor fraction of total Rif1 can be solubilised by medium salt (0.4 M NaCl) extraction from ESCs nuclei[46]. (**C, D**) Rif1 from ESCs is completely refractory to solubilisation through nuclease-mediated chromatin digest. (**C**) Ethidium bromide-stained agarose gel showing different degrees of chromatin fragmentation obtained by treatment with different nucleases: micrococcal nuclease (MNase) for the indicated times; DNAse I (D); RNAse A (R); Benzonase (B) for 2 h. (**D**) Protein fraction obtained by the chromatin treated with a combination of DNAse I and RNAse A. Although no visible DNA is left, Rif1 is not released in the soluble fraction. (**E**) About 50% of Rif1 is released in the soluble fraction upon 3 cycles of snap-freezing in liquid nitrogen and thawing of nuclei isolated from both ESCs and mouse embryonic fibroblasts (MEFs). (**F**) Coomassie blue-stained SDS-PAGE showing the amount of GST-Rif1 fragments bound onto glutathione Sepharose beads. (**G**) Western blot of the samples shown in E, probed with anti-GST antibody. (**H**) Normalised NMR intensity ratio of the spectrum of CRI with PP1 (I) and without (I^0) corresponding to the two NMR spectra shown in Fig. 2D.

SUPPLEMENTAL TABLE 1. (**A**) List of all proteins interacting with Rif1, showing the single as well as the averaged LFQ intensities. (**B**) Subset of lower-confidence interacting protein: although peptides are present in at least two out of the three Rif1$^{FH/+}$ ESC lines, no corresponding peptide is present in the negative controls. In order to calculate the ratio of LFQs, the 0 values have been replaced with 1, driving therefore the ratios to artificially high values. (**C**) List of high-confidence nuclear Rif1 partners, with an enrichment cut-off ≥ 1.5 above the negative control.

A

21 HEAT repeats | I | II | III
1   1000   2418

KIAF | SILK | RVSF | DNA binding | CRIII
2090   2418

B

	Sol	Ins
anti-Rif1		
anti-Smc1		

C

0.5 1 2 h
MNase

D R D+R B

D

	Sol	Ins
anti-Rif1		
anti-Smc1		

D+R

E

	MEFs		ESCs	
	Sol	Ins	Sol	Ins
anti-Rif1				

F

HEAT GST CRI+II-III CRI CRII CRIII CRI+II CRII+III

G

CRI+II+III CRI CRII CRIII CRI+II CRII+III HEAT

anti-GST

H

I/I⁰ vs Sequence

I/I_0 plotted against Sequence (2100–2180)