Supporting Information

Rubio *et al.* 10.1073/pnas.0801273105

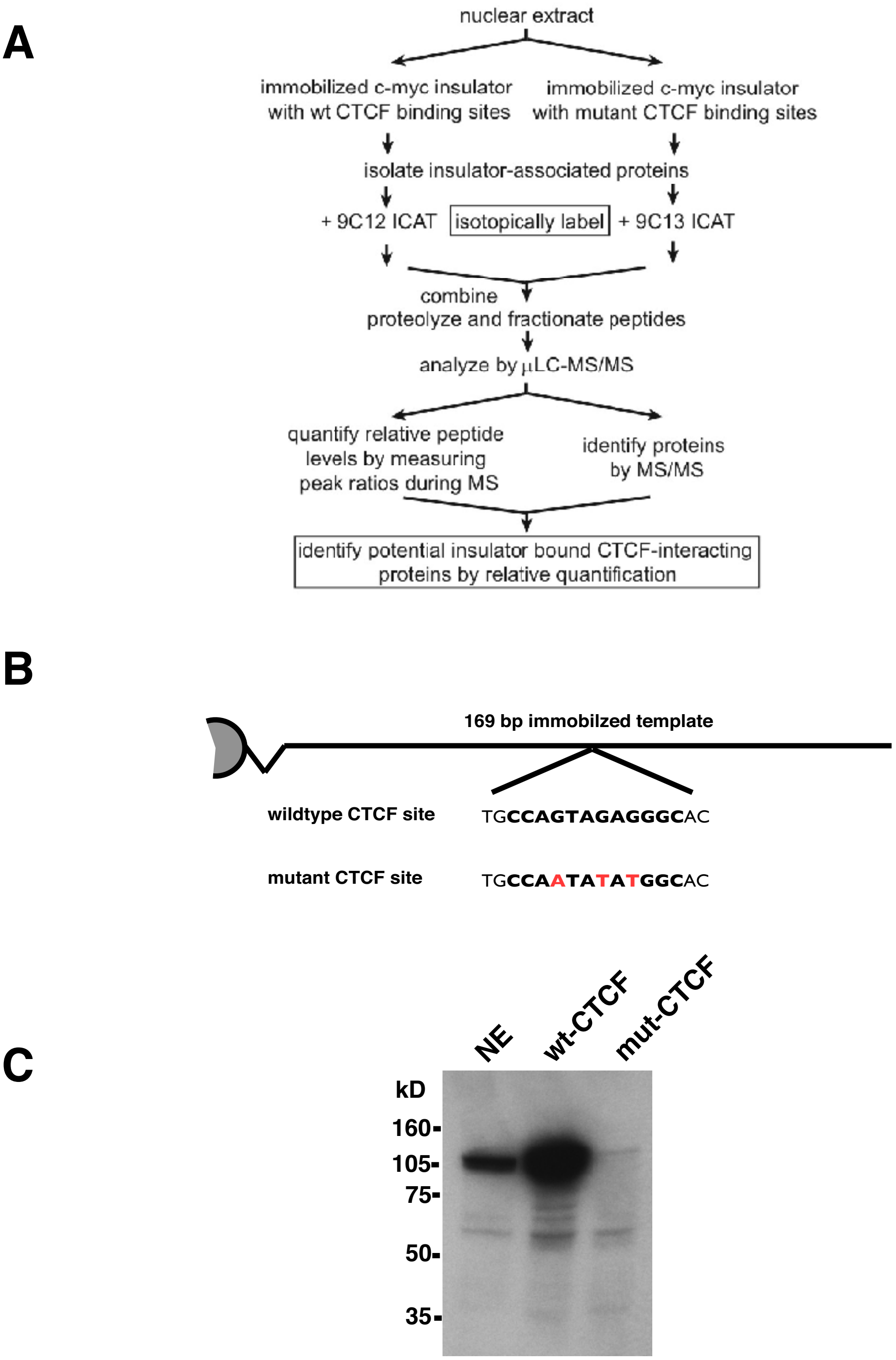


Fig. S1. ICAT mass spectrometry to identify proteins interacting with template-bound CTCF. (*A*) Flow diagram of quantitative proteomics analysis of specific CTCF complex components. (*B*) CTCF wild-type and mutant sequences derived from the human c-myc insulator element MINE were immobilized to magnetic beads. (*C*) Western analysis of CTCF protein enriched by the wildtype and mutant templates (wt-CTCF, mut-CTCF). NE, CTCF detected anti-CTCF antibodies in crude nuclear extract.

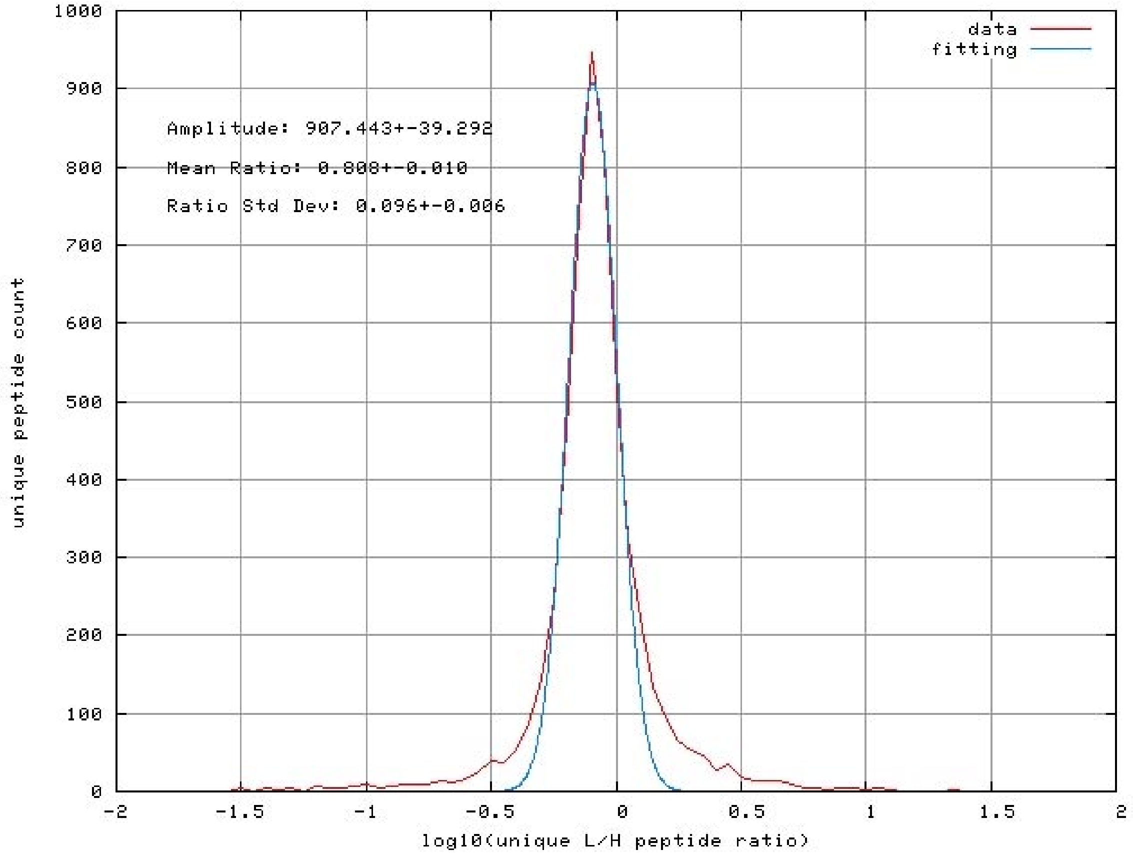


Fig. S2. Distribution of light/heavy unique peptide ratios in the quantitative MS analysis of CTCF-dependent protein interactions. The red line represents the original data, and the blue line is the fitting curve of a normal distribution over the main peak.

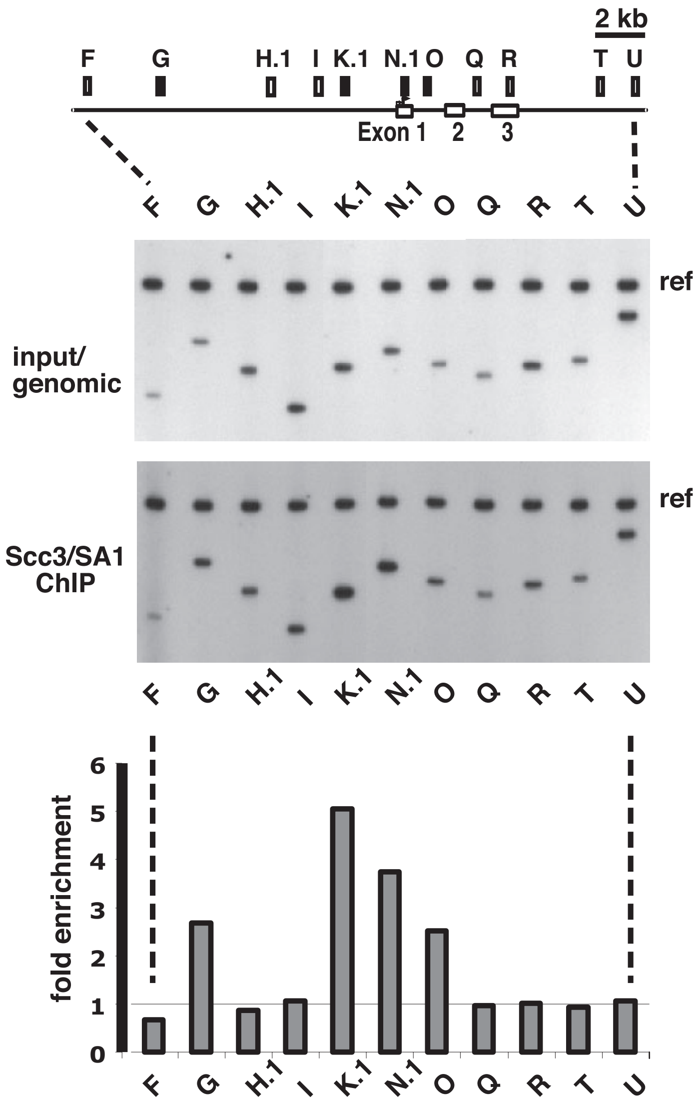


Fig. S3. Scc3/SA1 associates with genomic CTCF-binding regions. Chromatin immunoprecipitation experiments using anti-Scc3/SA1 antibodies reveal association of Scc3/SA1 with CTCF-binding sites. (*A*) Scheme of the human c-myc locus with three exons. Positions of amplicons F through U are shown by vertical bars. Filled bars indicate regions known to bind CTCF, open bars indicate regions that are not associated with CTCF. (*B*) Duplex PCR analysis of DNA recovered from chromatin immunoprecipitations with anti-SA1 antibodies using primers sets F through U and primers specific for the β-globin gene promoter as an internal reference (ref). (*C*) Quantification of enrichments of amplicons F through U relative to the internal reference.

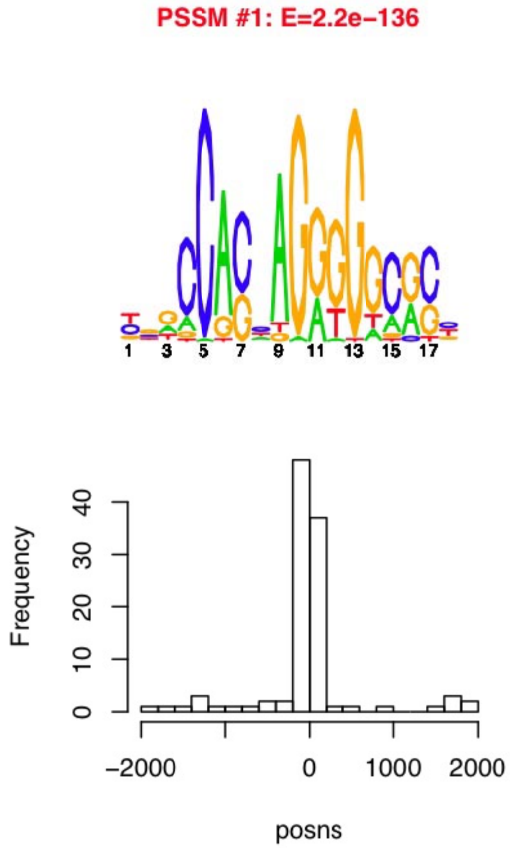


Fig. S4. Detection of the CTCF motif in a subset of sequences identified by ChIP-Chip. (*A*) Sequence logo representation of Position Specific Scoring Matrix (PSSM) identified by MEME. (*B*) Frequency histogram of distances of motifs from the center of ChIP-Chip hits.

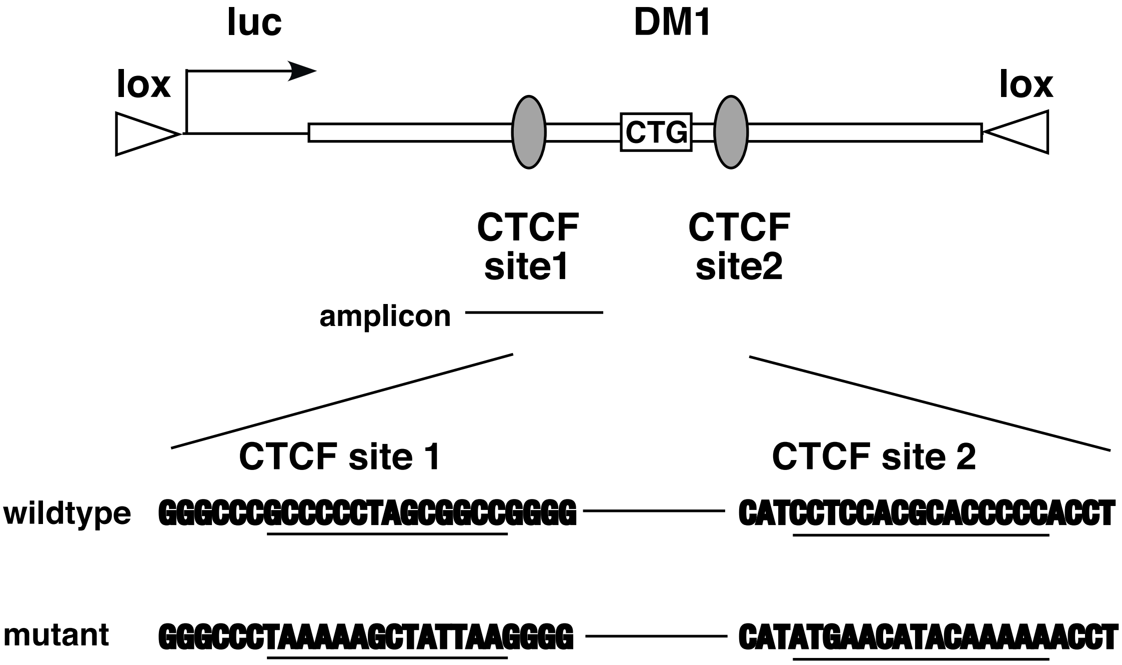
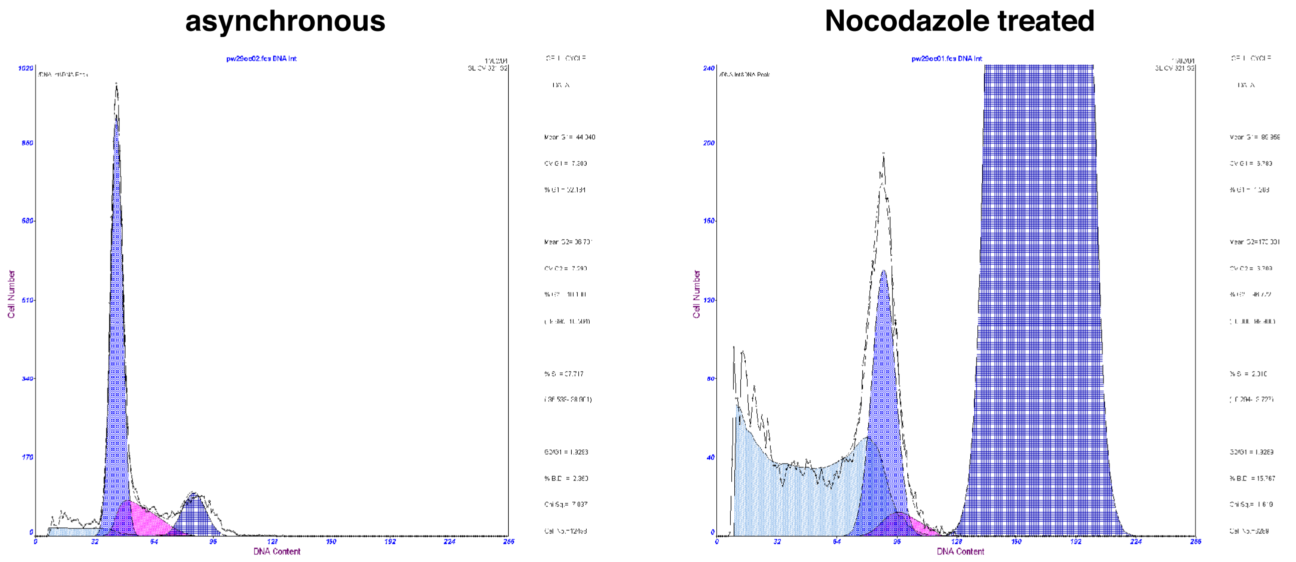


Fig. S5. Structure and CTCF-binding sequences of the human myotonic dystrophy locus DM1 integrated into murine 3T3 cells by Recombination Mediated Cassette Exchange [Cho DH, *et al.* (2005) Antisense transcription and heterochromatin at the DM1 CTG repeats are constrained by CTCF. *Mol Cell* 20: 483–489]. Sequence motifs for CTCF (sites 1 and 2) are separated by 203 bp. Amplification of the region indicated by a black bar (amplicon) in ChIP assays detects occupancy of both sites. Underlined sequences were mutated to abolish binding of CTCF in the mutant sequences of sites 1 and 2.



asynchronous

Nocodazole treated

Fig. S6. Detection of CTCF-associated genomic regions in cycling/asynchronously growing (CTCF-Cyc) and mitotic (CTCF-Mit) HBL100 cells. Asynchronously cycling HBL100 cells were treated with 1 μM nocodazole (Sigma) for 24 h. Cells were then harvested. To determine the distribution of cell cycle stages in untreated and nocodazole-treated cell pools, an aliquot of each preparation was fixed/labeled with DAPI fixation solution (10 μg/ml 4,6-diamidino-2-phenylindole and 0.1% nonidet P-40 detergent in Tris-buffered saline, pH 7.4). DNA content quantitation and cell cycle analysis were performed as described [Rabinovitch *et al.* (1989) *Lab Invest* 60:65]. Briefly, the cell suspension was triturated with a 26-gauge needle and analyzed using a high-speed Influx flow cytometer and Spigot software (Cytopeia) with UV excitation and DAPI emission collected with a 450–40 UV filter. Cell cycle data were analyzed using WinCycle software (Phoenix Flow Systems). The remaining cells were subsequently prepared for chromatin immunoprecipitation. A complete list of all 70 sites that are detected within the ENCODE regions both in cycling and mitotic cells is shown in Table S4.

Table S1. Chromosomal positions, relative intensity, and *P* values of 147 CTCF peaks identified in ChIP-Chip experiments using anti-CTCF antibodies and chromatin prepared from HBL100 cells

Chromosome	Coordinate	Intensity	*P* value
chr2	118288408	5.874429373	0.000
chr2	118309792	2.144833241	0.005
chr2	118477362	3.014167416	0.001
chr2	220149789	3.657752989	0.001
chr2	220200075	2.658218481	0.002
chr2	220211709	1.917243609	0.009
chr2	220232167	5.642631994	0.000
chr2	220332883	6.183224783	0.000
chr2	220427697	2.016111815	0.007
chr2	234402223	2.757001183	0.002
chr5	56232615	1.954003354	0.009
chr5	131371688	2.631531513	0.003
chr5	131428474	4.23783699	0.001
chr5	131602012	3.657703234	0.001
chr5	131636457	2.534630787	0.003
chr5	131750526	2.630632782	0.003
chr5	131783352	3.478291475	0.001
chr5	131890350	2.599806809	0.003
chr5	131890560	7.072892009	0.000
chr5	132184019	3.713460218	0.001
chr5	132229690	2.429856089	0.003
chr5	141908686	2.820953727	0.002
chr5	142015599	2.349638739	0.004
chr5	142073220	9.632074173	0.000
chr5	142077445	3.650085477	0.001
chr5	142240463	1.896395591	0.009
chr6	41500477	3.13879439	0.001
chr6	41539073	2.290811557	0.004
chr6	41667885	5.242354443	0.000
chr6	41678845	2.174150879	0.005
chr6	41758025	2.645180439	0.003
chr6	41759473	2.568727736	0.003
chr6	41811497	2.049282397	0.006
chr6	41862983	4.717446684	0.000
chr6	41863383	4.269413868	0.001
chr6	73896511	5.847889638	0.000
chr6	108541515	2.334676852	0.004
chr6	132642648	2.208674974	0.005
chr7	26949399	2.658869951	0.002
chr7	26956660	3.372889024	0.001
chr7	26956944	2.736828411	0.002
chr7	26973971	4.324661858	0.000
chr7	26992743	2.775304139	0.002
chr7	27023455	4.233059111	0.001
chr7	89485725	2.073798818	0.006
chr7	115414478	3.051099487	0.001
chr7	115760406	2.672261003	0.002
chr7	116164566	2.992800817	0.001
chr7	126487961	2.325384129	0.004
chr7	126822987	2.652736571	0.002
chr8	118943615	2.445747324	0.003
chr9	128848780	3.07032369	0.001
chr9	128945160	2.831807492	0.002
chr9	128973802	6.337059749	0.000
chr9	128981206	3.185824212	0.001
chr9	128984896	3.523970971	0.001
chr9	128985130	2.560341456	0.003
chr9	129016020	3.400143552	0.001
chr11	1815703	2.244803485	0.005
chr11	1931598	2.655970453	0.002
chr11	1980082	2.782060436	0.002
chr11	1980352	1.893236344	0.009

Chromosome	Coordinate	Intensity	*P* value
chr11	2129368	4.546737625	0.000
chr11	2129803	2.393598581	0.003
chr11	2130008	3.770876382	0.001
chr11	2134503	3.246008353	0.001
chr11	2164630	2.244276785	0.005
chr11	2172792	3.253113348	0.001
chr11	2283337	3.416092102	0.001
chr11	5578338	2.176712243	0.005
chr11	64013933	6.76979182	0.000
chr11	64175154	3.24574888	0.001
chr11	64177249	3.274483969	0.001
chr11	64177459	3.118632636	0.001
chr11	64212461	2.015285472	0.007
chr11	64212701	5.628987147	0.000
chr11	64267201	1.946996614	0.009
chr11	64291959	4.8720669	0.000
chr11	64311789	2.861076445	0.002
chr11	64412233	4.63518868	0.000
chr11	64418599	3.263255871	0.001
chr11	116017634	1.900254248	0.009
chr11	116067754	4.201662919	0.001
chr11	116108806	3.78714867	0.001
chr11	116167304	2.559046217	0.003
chr11	131063096	2.108523994	0.006
chr11	131069986	2.905633285	0.002
chr12	38787753	3.238483196	0.001
chr13	29585348	2.7143973	0.002
chr13	29587448	4.034156823	0.001
chr13	112520659	2.107411143	0.006
chr13	112732062	2.326781522	0.004
chr13	112737470	2.457809296	0.003
chr14	52949530	2.031719896	0.007
chr14	52968924	2.408243057	0.003
chr14	98856276	1.933925394	0.009
chr14	98925600	3.020331317	0.001
chr14	98929492	4.198927707	0.001
chr15	41594155	2.698635406	0.002
chr15	41669861	5.949616141	0.000
chr15	41769695	6.058055905	0.000
chr15	41855153	2.039086133	0.007
chr15	41857069	3.848107189	0.001
chr15	41961599	2.429522684	0.003
chr15	41983451	3.560229773	0.001
chr16	55755	4.526460248	0.000
chr16	97209	5.005499382	0.000
chr16	107839	3.530421454	0.001
chr16	171750	1.964405883	0.008
chr16	352341	2.274586779	0.004
chr16	477903	5.734102717	0.000
chr18	59754619	2.554009891	0.003
chr19	59037385	2.75618046	0.002
chr19	59101907	2.82850388	0.002
chr19	59102147	3.7238491	0.001
chr20	33320707	3.534422658	0.001
chr20	33355187	5.12228439	0.000
chr20	33515119	2.172540464	0.005
chr20	33574606	2.05177949	0.006
chr21	33234751	2.507167277	0.003
chr21	33421629	5.600152153	0.000
chr21	33655591	4.613258444	0.000
chr21	34189133	3.441218988	0.001
chr21	34196113	1.912141968	0.009
chr21	34218389	3.483195186	0.001
chr21	39432271	2.510598306	0.003
chr21	39496589	2.22987916	0.005

Chromosome	Coordinate	Intensity	*P* value
chr22	30281924	2.339207366	0.004
chr22	30691508	3.839854175	0.001
chr22	31099384	3.971228763	0.001
chr22	31249716	4.756783674	0.000
chr22	31517654	5.272319662	0.000
chr22	31721526	2.976522175	0.001
chr22	31739054	2.957423559	0.002
chrX	152733957	3.009325505	0.001
chrX	152739699	2.223198656	0.005
chrX	152739969	2.171398468	0.005
chrX	152800003	2.395622709	0.003
chrX	153122674	4.971529195	0.000
chrX	153148194	1.968302757	0.008
chrX	153319176	1.8868729	0.009
chrX	153327160	4.834800248	0.000
chrX	153367231	2.676749206	0.002
chrX	153413186	3.388400745	0.001
chrX	153421097	2.724700617	0.002
chrX	153540967	2.675242106	0.002
chrX	153849586	3.384497013	0.001

Data generated using the NimbleGen ENCODE array. Coordinates indicate the center of the peaks and are given in NCBI build 35 (UCSC hg17).

Table S2. Chromosomal positions (UCSC hg17), relative intensity, and *P* values of Scc3/SA1 peaks identified in ChIP-Chip experiments using anti-Scc3/SA1 antibodies and chromatin prepared from HBL100 cells

Chromosome	Coordinate	Intensity	*P* value
chr2	118215234	0.84400772	0.009
chr2	118477302	1.62311614	0.000
chr2	220544166	0.90840148	0.006
chr2	234667504	1.87051592	0.000
chr5	131372228	1.09124307	0.001
chr5	131749180	1.78165431	0.000
chr5	131783352	0.88631755	0.007
chr5	141908716	1.1160577	0.001
chr5	142077474	0.93412494	0.004
chr6	41667854	1.13750936	0.001
chr6	73896510	1.19846958	0.000
chr6	73947626	1.12727368	0.001
chr6	132344498	0.86799406	0.008
chr7	26949398	1.95060883	0.000
chr7	26956958	0.88325156	0.007
chr7	27009302	1.53306407	0.000
chr7	89620788	1.02909957	0.001
chr7	115823182	0.87294098	0.007
chr7	116164596	0.94317461	0.004
chr7	116949900	1.06397079	0.001
chr7	116950710	0.90470966	0.006
chr9	128973772	2.38146577	0.000
chr11	1977502	0.84711982	0.009
chr11	2129832	1.56303939	0.000
chr11	4893236	0.98476297	0.002
chr11	5358718	1.09288776	0.001
chr11	5529783	0.8628868	0.008
chr11	5686106	1.17856233	0.000
chr11	64138124	1.00852241	0.002
chr11	64157800	0.93877481	0.004
chr11	116255960	0.98181487	0.002
chr11	130615552	0.8372373	0.010
chr13	29819620	0.87076253	0.007
chr13	112737590	1.0428934	0.001
chr14	98780448	1.00568405	0.002
chr15	41952138	0.98876922	0.002
chr15	41983660	1.04806572	0.001
chr19	59310952	1.63747169	0.000
chr20	33515088	1.18426526	0.000
chr21	33421598	1.83928176	0.000
chr21	34177146	0.87299996	0.007

Data generated using the NimbleGen ENCODE array. Coordinates indicate the center of the peaks and are given in NCBI build 35 (UCSC hg17).

Table S3. Colocalization of CTCF and Scc3/SA1 sites within ENCODE in HBL100 cells

Chromosome	CTCF coord.	CTCF intensity	CTCF *P* value	Scc3/SA1 coord.	Scc3/SA1 intensity	Scc3/SA1 *P* value	Distance
chr5	131783352	3.48	0.001	131783352	0.89	0.007	0
chr6	73896511	5.85	0.000	73896510	1.20	0.000	1
chr7	26949399	2.66	0.002	26949398	1.95	0.000	1
chr7	26956944	2.74	0.002	26956958	0.88	0.007	14
chr5	142077445	3.65	0.001	142077474	0.93	0.004	29
chr11	2129803	2.39	0.003	2129832	1.56	0.000	29
chr9	128973802	6.34	0.000	128973772	2.38	0.000	30
chr7	116164566	2.99	0.001	116164596	0.94	0.004	30
chr5	141908686	2.82	0.002	141908716	1.12	0.001	30
chr21	33421629	5.60	0.000	33421598	1.84	0.000	31
chr6	41667885	5.24	0.000	41667854	1.14	0.001	31
chr20	33515119	2.17	0.005	33515088	1.18	0.000	31
chr2	118477362	3.01	0.001	118477302	1.62	0.000	60
chr13	112737470	2.46	0.003	112737590	1.04	0.001	120
chr11	2130008	3.77	0.001	2129832	1.56	0.000	176
chr15	41983451	3.56	0.001	41983660	1.05	0.001	209
chr7	26956660	3.37	0.001	26956958	0.88	0.007	298
chr11	2129368	4.55	0.000	2129832	1.56	0.000	464
chr5	131371688	2.63	0.003	131372228	1.09	0.001	540
chr5	131750526	2.63	0.003	131749180	1.78	0.000	1346

"Distance" indicates the distance of the CTCF peak to the nearest Scc3/SA1 peak. Coordinates are given in NCBI build 35 (UCSC hg17). Highlighted rows indicate Scc3/SA1-associated regions for which more than one CTCF-binding site was assigned.

Table S4. Chromosomal sites within the ENCODE regions that were associated with CTCF both in asynchronously growing and mitotic cells.

	Coord. asynch	Intens.asynch	*P* value asynch	Coord. mitot	Intens. mitot	*P* value mitot
chr2	118288408	5.874429373	0.000382673	118288438	4.47590558	8.15E-05
chr2	220149789	3.657752989	0.000918414	220149609	2.77078166	0.000489197
chr2	220232167	5.642631994	0.000382673	220232167	4.0083502	8.15E-05
chr2	220332883	6.183224783	0.000306138	220332821	2.54599463	0.000896861
chr2	220332883	6.183224783	0.000306138	220334783	2.66696729	0.00057073
chr2	220427697	2.016111815	0.007041176	220427667	1.92791947	0.003913575
chr5	131371688	2.631531513	0.002602174	131371628	2.90828211	0.000489197
chr5	131428474	4.23783699	0.000535742	131428504	1.87607413	0.004402772
chr5	131602012	3.657703234	0.000918414	131602102	3.25486342	8.15E-05
chr5	131636457	2.534630787	0.002678708	131636544	2.63239161	0.00057073
chr5	131783352	3.478291475	0.000994949	131783412	1.66688761	0.009294741
chr5	132184019	3.713460218	0.000765345	132184019	2.2504651	0.001712189
chr5	142073220	9.632074173	7.65E-05	142073265	6.35524784	8.15E-05
chr5	142077445	3.650085477	0.000918414	142077475	2.51625609	0.001059927
chr6	41500477	3.13879439	0.000994949	41500357	1.71290029	0.008316347
chr6	41667885	5.242354443	0.000459207	41667885	5.36194567	8.15E-05
chr6	41678845	2.174150879	0.004974744	41678845	1.69275591	0.008887077
chr6	41678845	2.174150879	0.004974744	41677795	1.89398565	0.004321239
chr6	41862983	4.717446684	0.000459207	41862998	2.75883352	0.000489197
chr6	41863383	4.269413868	0.000535742	41863383	2.40430193	0.001467591
chr6	73896511	5.847889638	0.000382673	73896511	2.38010565	0.001467591
chr7	26973971	4.324661858	0.000459207	26973971	3.19101879	0.000244598
chr7	26992743	2.775304139	0.002142966	26992803	1.83620901	0.005299633
chr7	116164566	2.992800817	0.001224552	116164566	3.95061871	8.15E-05
chr7	126487961	2.325384129	0.003826726	126486225	1.71262381	0.008316347
chr9	128981206	3.185824212	0.000994949	128981266	2.82636636	0.000489197
chr9	129016020	3.400143552	0.000994949	129016050	2.02774974	0.003098247
chr11	1980082	2.782060436	0.002142966	1980382	1.69499399	0.008724011
chr11	1980352	1.893236344	0.009260677	1980382	1.69499399	0.008724011
chr11	2129368	4.546737625	0.000459207	2129353	3.22403423	8.15E-05
chr11	2129803	2.393598581	0.003290984	2130008	2.98019512	0.000407664
chr11	2130008	3.770876382	0.000765345	2130008	2.98019512	0.000407664
chr11	2134503	3.246008353	0.000994949	2134533	2.72256782	0.000489197
chr11	2283337	3.416092102	0.000994949	2283337	3.13502824	0.000326131
chr11	64013933	6.76979182	0.000229604	64014023	1.71937824	0.008153282
chr11	64175154	3.24574888	0.000994949	64175124	3.07267425	0.000326131
chr11	64291959	4.8720669	0.000459207	64291899	3.18885564	0.000244598
chr11	64291959	4.8720669	0.000459207	64292319	1.84384376	0.005136567
chr11	64412233	4.63518868	0.000459207	64412233	4.930201	8.15E-05
chr11	64418599	3.263255871	0.000994949	64418629	1.82291661	0.005544232
chr11	116017634	1.900254248	0.009260677	116017601	2.13243274	0.002364452
chr11	116067754	4.201662919	0.000688811	116068292	2.1360818	0.002364452
chr11	116108806	3.78714867	0.000765345	116108866	3.76532064	8.15E-05
chr11	116108806	3.78714867	0.000765345	116107786	2.31439422	0.001467591
chr11	116167304	2.559046217	0.002678708	116167420	2.44460906	0.001386058
chr11	131069986	2.905633285	0.001836828	131069926	3.55157807	8.15E-05
chr11	131069986	2.905633285	0.001836828	131071391	2.20135225	0.00203832
chr15	41669861	5.949616141	0.000306138	41669891	3.56016472	8.15E-05
chr15	41769695	6.058055905	0.000306138	41769755	3.71486524	8.15E-05
chr15	41855153	2.039086133	0.006658503	41855813	1.76001819	0.007501019
chr15	41857069	3.848107189	0.000765345	41855813	1.76001819	0.007501019
chr15	41983451	3.560229773	0.000918414	41983511	2.44815942	0.001386058
chr16	97209	5.005499382	0.000459207	97149	3.7759144	8.15E-05
chr16	107839	3.530421454	0.000918414	107839	2.41265174	0.001467591
chr16	477903	5.734102717	0.000382673	477903	1.64593652	0.009783938
chr18	59754619	2.554009891	0.002678708	59754679	2.39521204	0.001467591
chr18	59754619	2.554009891	0.002678708	59755364	2.32337051	0.001467591
chr19	59101907	2.82850388	0.002142966	59102147	4.23568068	8.15E-05
chr19	59102147	3.7238491	0.000765345	59102147	4.23568068	8.15E-05
chr20	33320707	3.534422658	0.000918414	33320707	2.54822968	0.000896861
chr20	33355187	5.12228439	0.000459207	33355142	4.16667121	8.15E-05
chr20	33574606	2.05177949	0.006352365	33574666	2.49817771	0.001141459
chr21	33421629	5.600152153	0.000382673	33421749	2.29553862	0.001549124

	Coord. asynch	Intens.asynch	*P* value asynch	Coord. mitot	Intens. mitot	*P* value mitot
chr21	33655591	4.613258444	0.000459207	33655531	2.41998693	0.001467591
chr21	34218389	3.483195186	0.000994949	34218479	3.79165518	8.15E-05
chr22	30691508	3.839854175	0.000765345	30691508	1.99544755	0.003261313
chr22	31099384	3.971228763	0.000765345	31099354	2.95974811	0.000407664
chr22	31517654	5.272319662	0.000459207	31517624	3.90993461	8.15E-05
chrX	153122674	4.971529195	0.000459207	153122659	2.33527061	0.001467591
chrX	153122674	4.971529195	0.000459207	153123079	3.13040943	0.000326131
chrX	153148194	1.968302757	0.008418797	153148024	2.11652508	0.002527517
chrX	153367231	2.676749206	0.00237257	153367381	1.67180581	0.009050143
chrX	153413186	3.388400745	0.000994949	153413137	3.05350893	0.000326131
chrX	153849586	3.384497013	0.000994949	153849616	2.18847706	0.00203832

Coordinates represent the chromosomal coordinates (NCBI build 35; hg17) of the center of the peaks detected in asynchronously growing (coordin. asynch) and mitotic cells (coordin.mitot.). The relative intensities and *P* values of individual peaks are also indicated.