

Supplementary information

Ectopically tethered CP190 induces large-scale chromatin decondensation

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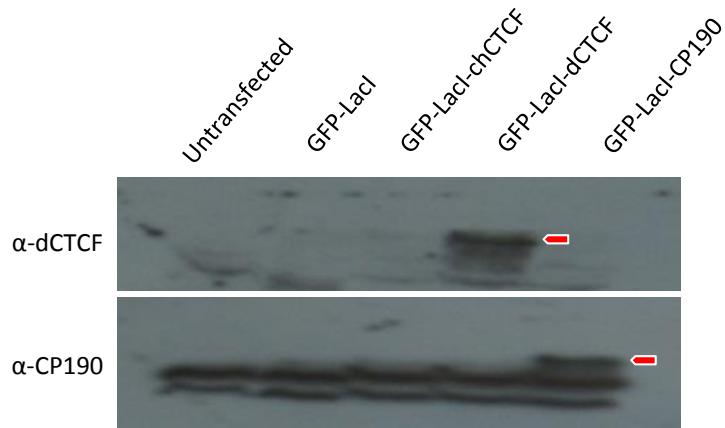
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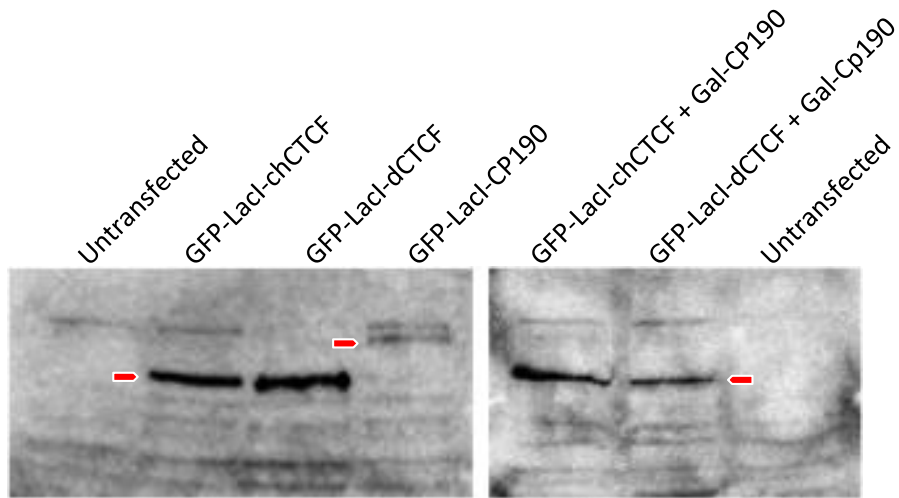
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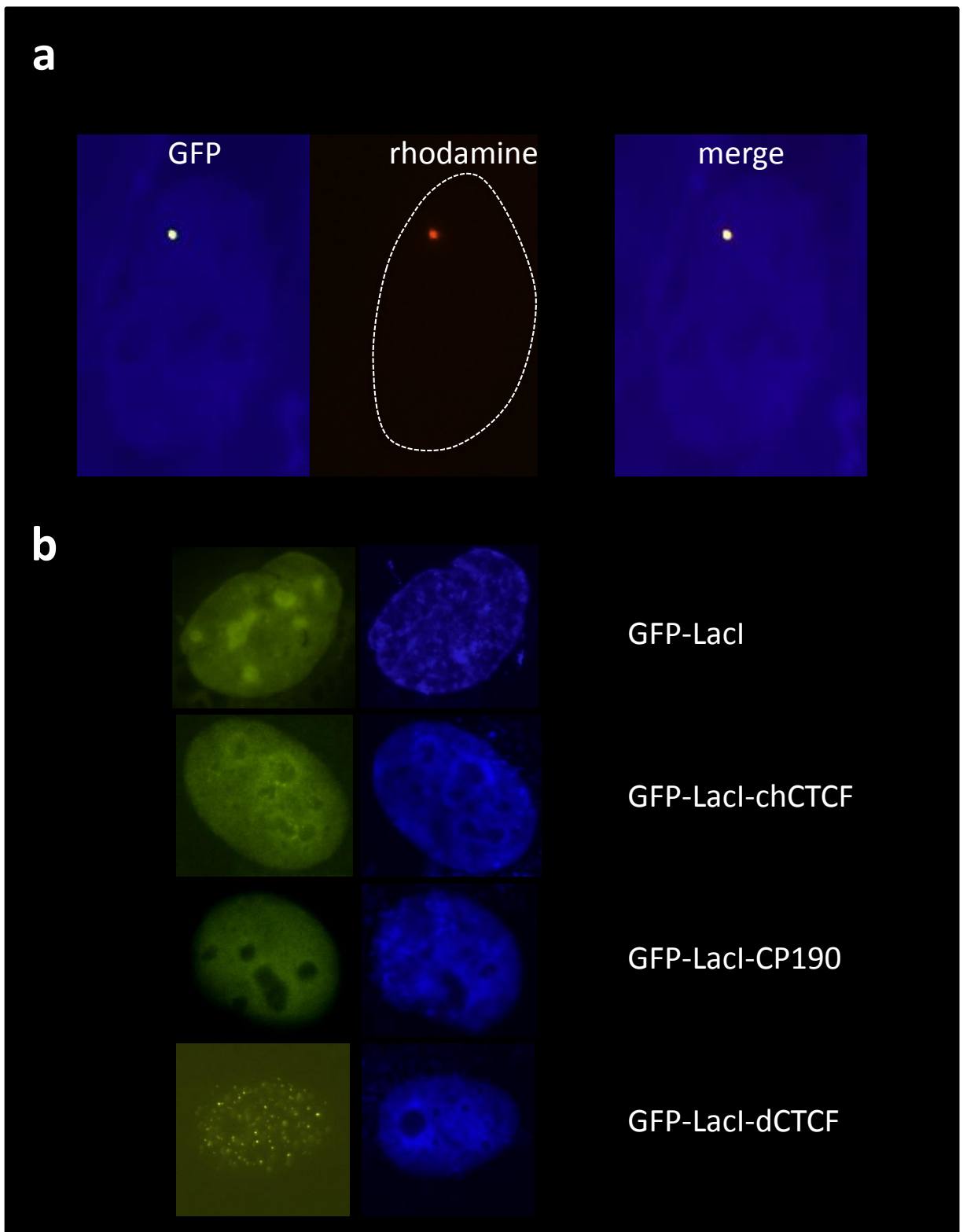
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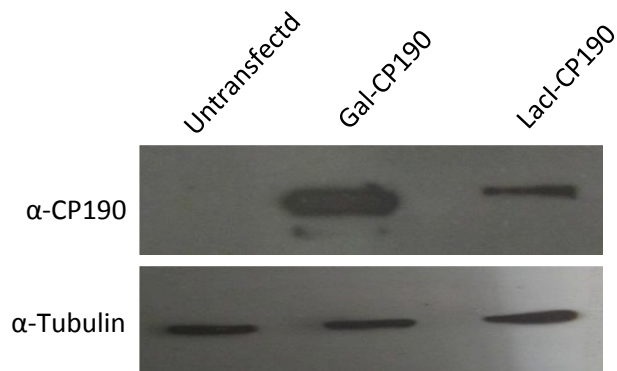
Supplementary figure S1. Verification of the fusion constructs by western blotting. Indicated vectors were transiently transfected into HEK293T cells and dCTCF and CP190 proteins were probed with antibodies as indicated in the figure. The dCTCF antibody does not cross-react with vertebrate CTCF. Specific bands are marked (CP190, ~ 170 KD; dCTCF, ~ 130 KD) .



Supplementary figure S2. Expression levels between different constructs. The indicated constructs were transiently transfected in HEK 293T cells and the blot was probed with anti-GFP antibody. Specific bands are marked. The left and the right part were run under the same experimental conditions.



Supplementary figure S3. The single LacO repeat cluster in the F42B8 nucleus is identified by specific LacI-binding. **A.** GFP-LacI-dCTCF marks the same spot as cherry-LacI. F42B8 cells were co-transfected with both expression vectors, cells were fixed and stained with Hoechst stain. **B.** U2OS cells in the absence of an integrated LacO repeat cluster are not marked by a single spot after expression of the fusion constructs. Transfected constructs are indicated.



Supplementary figure S4. Verification of the expression construct Gal4-CP190 by western-blotting. The indicated constructs were transiently transfected in HEK 293T cells and the blot was probed with anti-CP190 and anti-Tubulin antibody. GFP-LacI-CP190 transfected cells were used as positive control.

Supplementary table 1a. LacO array measurements in mammalian F42B8 cells upon tethering of insulator proteins. Array size was measured by Velocity software and values are in μm^2 .

GFP-LacI	LacI+ GalCP190	dCTCF	dCTCF+ GalCP190	chCTCF	chCTCF+ GalCP190	CP190	CP190+ GalCP190
0.036686	0.0707512	0.0393062	0.335413	0.18867	0.31707	1.27352	0.576492
0.039306	0.115298	0.102196	0.455952	0.31445	0.414026	1.43337	1.02458
0.039306	0.149364	0.102196	0.463814	0.31707	0.482157	1.66134	1.17395
0.086474	0.151984	0.196531	0.497879	0.508361	0.487397	1.72685	1.22111
0.110057	0.154605	0.204392	0.508361	0.516222	0.487397	1.76354	1.62204
0.12578	0.178188	0.269903	0.69179	0.605316	0.597455	1.78712	1.72947
0.136262	0.180809	0.269903	0.702272	0.644622	0.618418	1.84477	1.75044
0.138882	0.225356	0.275144	0.741578	0.731096	0.689169	1.84739	1.81857
0.151984	0.225356	0.275144	0.786125	0.770402	0.767782	2.15922	1.82381
0.154605	0.230597	0.296107	0.809709	0.770402	0.780884	2.31121	1.90504
0.170327	0.243699	0.298727	0.849015	0.791366	0.793986	2.37148	2.23521
0.183429	0.280385	0.31183	0.974795	0.825431	1.04293	2.37672	2.36362
0.19129	0.306589	0.31445	0.980036	0.872599	1.10582	2.77502	2.43961
0.193911	0.306589	0.330172	1.36524	0.911905	1.48316	3.36986	2.48153
0.196531	0.332793	0.345895	1.49102	0.925007	1.58273	3.62928	2.80909
0.212254	0.348515	0.348515	1.57225	0.94597	1.70589	3.6581	3.03968
0.225356	0.356377	0.364238	1.59059	0.948591	1.73996	3.74457	3.43799
0.233217	0.372099	0.448091	1.63776	0.961693	1.92601	3.77864	3.47205
0.25418	0.416646	0.453332	1.85263	1.01672	2.20639	4.01186	3.74982
0.259421	0.479536	0.455952		1.11106	2.85625	4.12191	5.53432

Supplementary table 1b. LacO array measurements in *Drosophila* S2 cells upon tethering of insulator proteins. Array size was measured by Velocity software and values are in μm^2 .

GFP-LacI	GFP-LacI-dCTCF	GFP-LacI-CP190
0.06551	0.275144	0.63152
0.015723	0.172947	0.545047
0.020963	0.162466	0.298727
0.055029	0.238458	0.327552
0.057649	0.0576492	0.332793
0.146743	0.0838533	0.201772
0.086474	0.120539	0.421887
0.044547	0.102196	0.662965
0.049788	0.180809	0.37996
0.083853	0.225356	0.319691
0.073372	0.24894	0.154605
0.070751	0.432369	0.180809
0.06027	0.227976	0.259421
0.102196	0.138882	0.429748
0.073372	0.366858	0.327552
0.267282	0.267282	0.306589
0.175568	0.398303	1.08485
0.06289	0.290866	0.322311
0.12316	0.0340654	0.369479
0.06551	0.24894	0.141502
0.256801	0.167707	0.230597
0.112678	0.12316	0.241078
0.138882	0.104817	0.267282
0.157225	0.193911	0.518842
0.24894	0.238458	0.31707
0.068131	0.220115	0.12316
0.034065	0.110057	0.12578
0.023584	0.427128	0.104817
0.18867	0.243699	0.605316
0.091715	0.209633	0.31707
0.146743	0.432369	0.238458
0.081233	0.25418	0.725855
0.081233	0.437609	0.762541
0.083853	0.267282	0.461193
0.036686	0.351136	0.330172
0.057649	0.0419267	0.290866

0.102196	0.324932	0.395683
0.133641	0.411405	0.285625
0.083853	0.138882	0.872599
0.041927	0.154605	0.348515
0.06289	0.136262	0.524083
0.070751	0.301348	0.280385
0.120539	0.272523	0.246319
0.117919	0.322311	0.414026
0.1284	0.24894	0.269903
0.225356	0.12316	0.235837
0.096955	0.209633	0.395683
0.416646	0.154605	0.366858
0.353756	0.193911	0.298727
0.217495	0.212254	0.429748
0.241078	0.31707	0.264662
0.212254		0.233217
0.183429		0.327552
0.120539		0.720614
0.102196		
0.078613		