Kagami et al., http://www.jcb.org/cgi/content/full/jcb.201308172/DC1

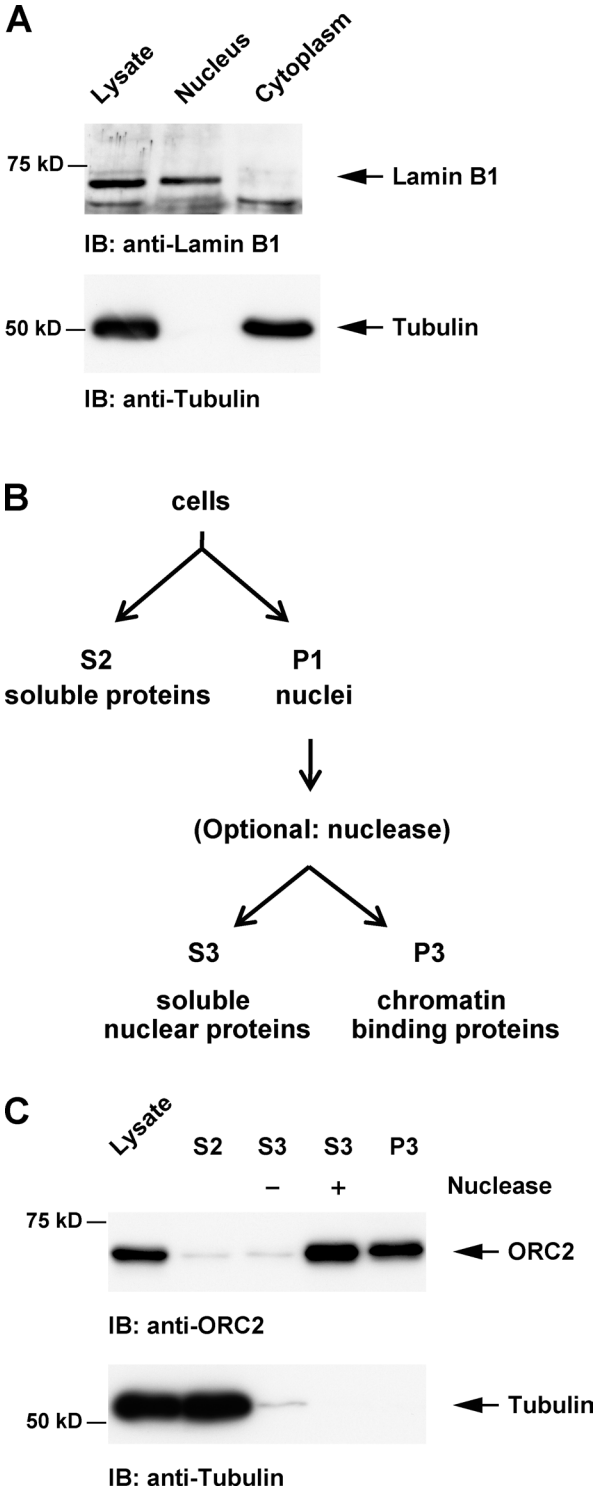


Figure S1. **Purity of subcellular fractionations.** (A) Nuclear or cytoplasmic lysates from HeLa cells were subjected to immunoblotting with anti–lamin B1 or anti-tubulin. (B) Scheme of the chromosome fractionation described in the Materials and methods. (C) HeLa cells were subjected to the chromosome fractionation. These fractions were subjected to immunoblot analysis with anti-ORC2 or anti-tubulin.

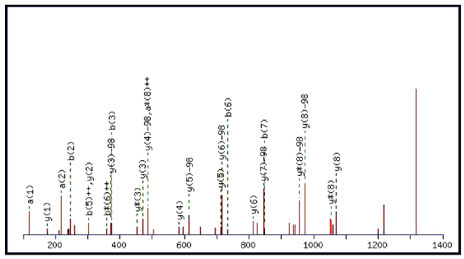
A

Peptide View

MS/MS Fragmentation of FVQETELSQR
Found in CNDH2_HUMAN, Condensin-2 complex subunit H2 OS=Homosapiens GN=NCAPH2 PE=1 SV=1

Match to Query 5126: 1315.571996 from(658.793274,2+)
Title:7945
Data file 121004-Kagami-Flag-CAPH2-GFP-Mps1-WT.txt

Click mouse within plot area to zoom in by factor of two about that point
Or, Plot from 100 to 1400 Da Full range
Label all possible matches ○ Label matches used for scoring ◉



Monoisotopic mass of neutral peptide Mr (calc): 1315.5809
Variable modifications:
S8 :Phospho (ST), with neutral losses 97.9769(shown in table),0.0000
Ions Score:65 **Expect:**7.5e-005
Matches: 25/144 fragment ions using 31 most intense peaks (help)

#	a	a^{++}	a*	a^{*++}	b	b^{++}	b*	b^{*++}	Seq.	y	y^{++}	y*	$*^{++}$	#
1	120.0808	60.5440			148.0757	74.5415			F					10
2	219.1492	110.0782			247.1441	124.0757			V	1071.5429	536.2751	1054.5164	527.7618	9
3	347.2078	174.1075	330.1812	165.5942	375.2027	188.1050	358.1761	179.5917	Q	972.4745	486.7409	955.4480	478.2276	8
4	476.2504	238.6288	459.2238	230.1155	504.2453	252.6263	487.2187	244.1130	E	844.4159	422.7116	827.3894	414.1983	7
5	577.2980	289.1527	560.2715	280.6394	605.2930	303.1501	588.2664	294.6368	T	715.3733	358.1903	698.3468	349.6770	6
6	706.3406	353.6740	689.3141	345.1607	734.3355	367.6714	717.3090	359.1581	E	614.3256	307.6665	597.2991	299.1532	5
7	819.4247	410.2160	802.3981	401.7027	847.4196	424.2134	830.3931	415.7002	L	485.2831	243.1452	468.2565	234.6319	4
8	888.4462	444.7267	871.4196	436.2134	916.4411	458.7242	899.4145	450.2109	S	372.1990	186.6031	355.1724	178.0899	3
9	1016.5047	508.7560	999.4782	500.2427	1044.4997	522.7535	1027.4731	514.2402	Q	303.1775	152.0924	286.1510	143.5791	2
10									R	175.1190	88.0631	158.0924	79.5498	1

B

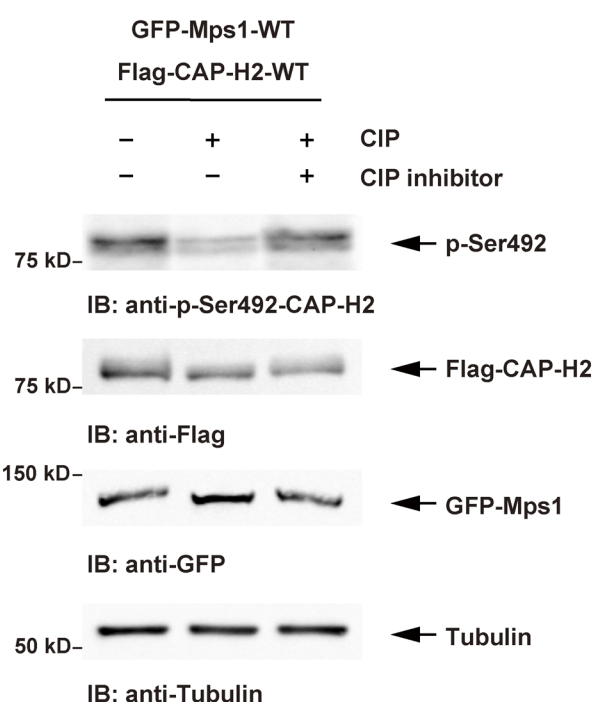


Figure S2. **Mps1 phosphorylates Ser492 of CAP-H2.** (A) The tandem mass spectrometry spectra (top) and peptide fragmentation table (bottom) of the 658.79 m/z (45.59 min) peak are shown. (B) 293 cells were cotransfected with GFP-Mps1-WT and Flag-CAP-H2-WT. Cell lysates were incubated with calf intestinal alkaline phosphatase (CIP) and/or CIP inhibitors and subjected to immunoblot analysis.

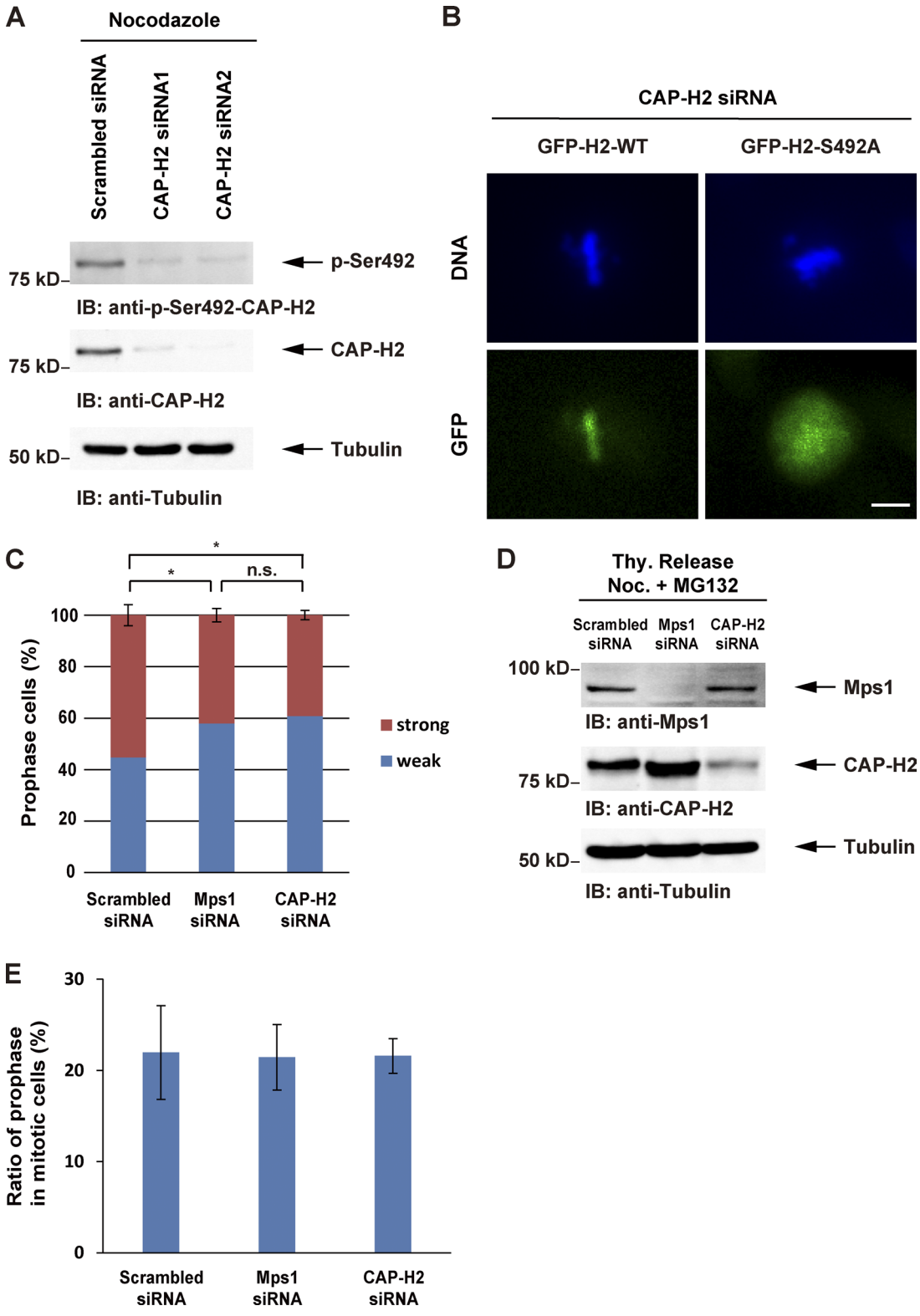


Figure S3. **Ser492 phosphorylation during mitosis.** (A) HeLa cells were transfected with the indicated siRNAs followed by nocodazole treatment. Whole-cell lysates were analyzed by immunoblotting. (B) WT or S492A cell lines were transfected with CAP-H2 siRNA. DNA was stained by Hoechst 33342. The images of living cells were shown. Bar, 10 μm. (C) HeLa cells were synchronized by double thymidine block and transfected with indicated siRNAs. Cells were then released into the medium containing nocodazole. At 9 h after release, cells were treated with MG132 for 30 min and fixed. The percentage of each category, as defined in Fig. 5 A, was calculated. Data represent the mean ± SD from three independent experiments (*, $P < 0.05$; n.s., not significant). (D) The efficiency of protein depletion by siRNAs in C was analyzed by immunoblotting with the indicated antibodies. (E) Ratio of prophase in mitotic cells in C was calculated. Data represent the mean ± SD from three independent experiments.