Supplementary Fig. 1

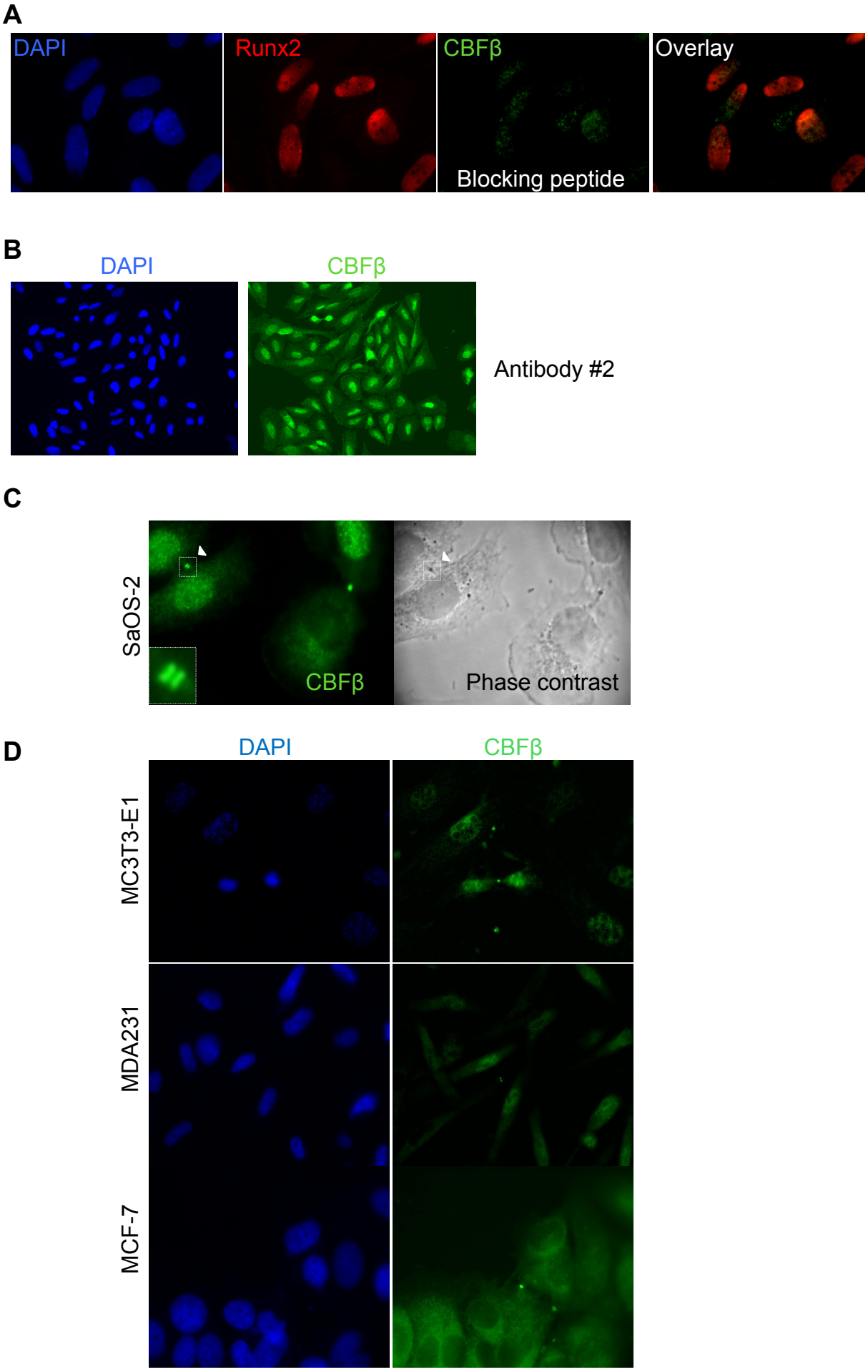
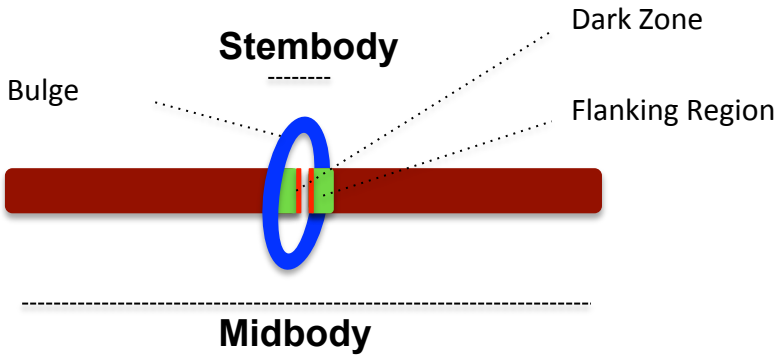


Fig. S1. CBFβ antibody specificity and CBFβ association with the midbody in different cell lines. (A) Analysis of CBFβ antibody specificity (ab33516) by peptide competition. Both RUNX2 and CBFβ antibodies were simultaneously incubated with human CBFβ peptide (ab41799, Abcam) in SaOS-2 cells. CBFβ antibody staining exhibits background levels when incubated with CBFβ peptide whereas RUNX2 labeling remained unchanged. (B) Staining for CBFβ in SaOS-2 cells labeled with CBFβ antibody A303-549A. Both ab33516 (see Fig 1A) and A303-549A CBFβ antibodies exhibited similar patterns of staining. (C) SaOS-2 cells were labeled for CBFβ. Midbody (see inset) is also evident under phase contrast microscopy. (D) Pre-osteoblastic mouse MC3T3-E1 and human breast cancer cell lines MDA-231 and MCF-7 also exhibit CBFβ localization in the midbody.

Supplementary Fig. 2

A



B

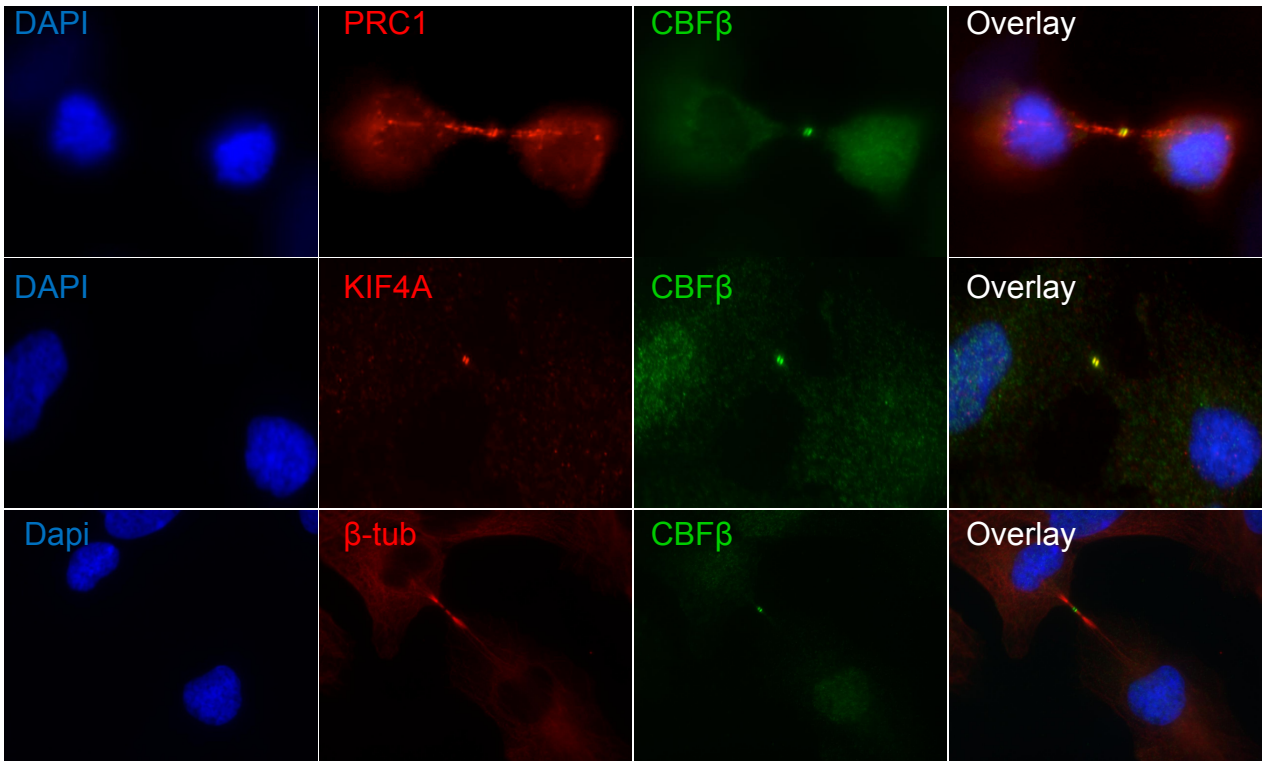
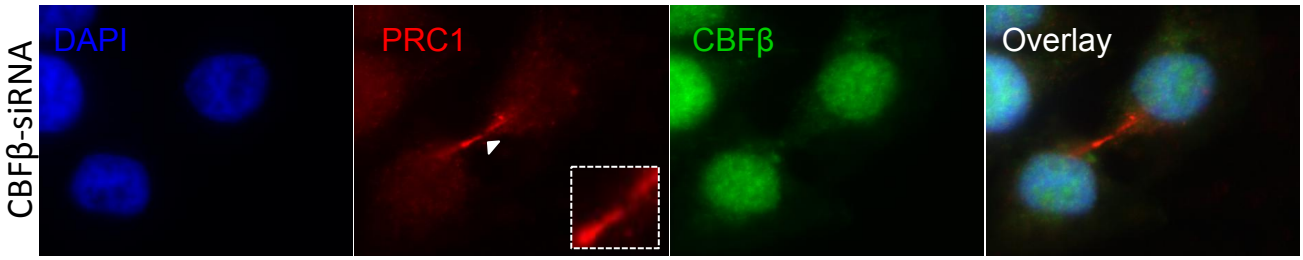


Fig. S2. Midbody Representation and labeling with midbody markers. (A) Diagrammatic representation of the midbody. The stembody comprised of the dark zone, flanking zone and bulge are represented by red, green and blue, respectively. Tubulin localization is represented by purple color. (B) Fixed cells were labeled for midbody markers by immunofluorescence. Full images corresponding to cropped insets from Fig 2C.

Supplementary Fig. 3

A



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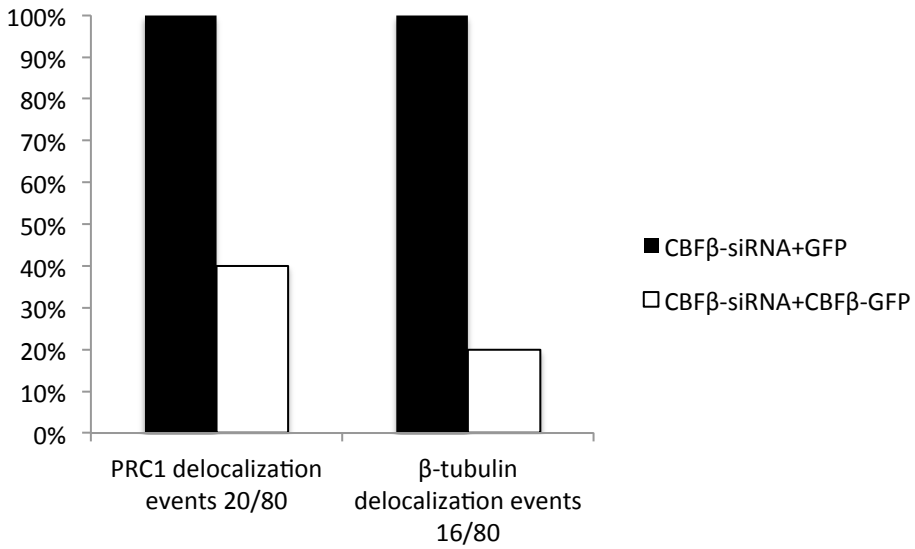

Fig. S3. Depletion of CBFβ causes defects in the midbody structure. (A) Cells transfected with CBFβ-siRNA lose CBFβ staining in the midbody and exhibit altered distribution of PRC1 (see normal PRC1 staining in Fig 2C). PRC1 staining is absent from parallel plate of the midbody. (B) Cells undergoing cytokinesis (n=80) were analyzed for delocalization of β-tubulin or PRC1 in CBFβ-siRNA cells transfected with a GFP plasmid or CBFβ-GFP plasmid. CBFβ-GFP expression rescued delocalization events induced by CBFβ-siRNA in comparison with CBFβ-siRNA treated cells expressing GFP only. Delocalization events are presented as percentile.

A

Interactant GeneCard	External ID(s)	Interactant GeneCard	External ID(s)
RUNX1	Q011962, 3, ENSP000003003054	PDIA3	P301013
CHGB	P050602, 3	PRKACB	P226943
Asun	Q9NVM91, 3	RAB2A	P610193
MAP2	P111371, 3	RARS	P541363
ASUN	Q9NVM91, 3, ENSP000002611914	RPL35	P427663
MYC	P011063, ENSP000003672074	SLC4A10	Q6U8413
MYOD1	P151723, ENSP000002500034	SPARCL1	Q145153
RUNX2	Q139503, ENSP000003525144	SYT1	P215793
RUNX3	Q137613, ENSP000003434774	FOS	ENSP000003062454
CBFA2T2	O434393, ENSP000002626534	SMAD4	ENSP000003415514
UTP3	Q9NQZ23, ENSP000002548034	IGHA1	ENSP000003749894
APP	P050673	JUNB	ENSP000003033154
C1QBP	Q070213	COPRS	ENSP000003043274
CEBPZ	Q037013	TIE1	ENSP000003615544
COL7A1	Q023883	ELAVL1	ENSP000003852694
DHX36	Q9H2U13	BMI1	ENSP000003658514
DST	Q030013	vif	P125041
GAS7	O608613	--	ENSP000003522624
JUN	P054123	--	ENSP000002993514

B

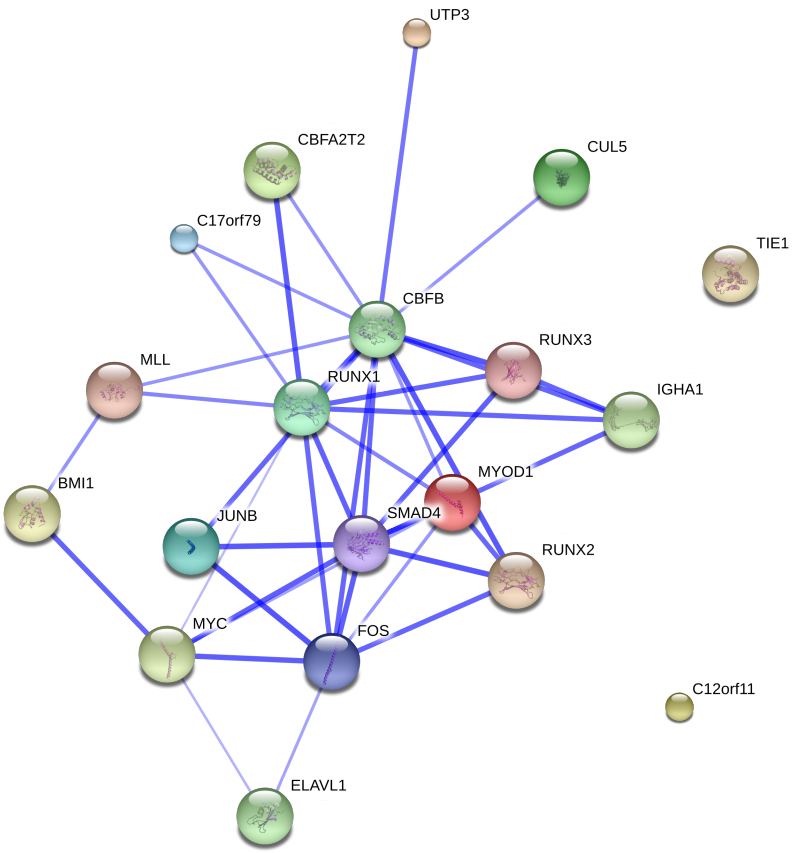

Fig. S4. Reported CBFβ interaction partners. (A) Interaction network for CBFβ using STRING (Search Tool for the Retrieval of Interacting Genes/Proteins). The network was based on "Confidence view", in which stronger associations are represented by thicker lines. The network and functions of the interacting proteins can be retrieved at: http://string-db.org/newstring_cgi/show_network_section.pl?taskId=xMXntWTKLrdH&interactive=yes&advanced_menu=no&network_flavor=confidence.

Table S1. CBFβ interaction partners

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MYC	P011063, ENSP000003672074	SLC4A10	Q6U8413
MYOD1	P151723, ENSP000002500034	SPARCL1	Q145153
RUNX2	Q139503, ENSP000003525144	SYT1	P215793
RUNX3	Q137613, ENSP000003434774	FOS	ENSP000003062454
CBFA2T2	O434393, ENSP000002626534	SMAD4	ENSP000003415514
UTP3	Q9NQZ23, ENSP000002548034	IGHA1	ENSP000003749894
APP	P050673	JUNB	ENSP000003033154
C1QBP	Q070213	COPRS	ENSP000003043274
CEBPZ	Q037013	TIE1	ENSP000003615544
COL7A1	Q023883	ELAVL1	ENSP000003852694
DHX36	Q9H2U13	BMI1	ENSP000003658514
DST	Q030013	vif	P125041
GAS7	O608613	--	ENSP000003522624
JUN	P054123	--	ENSP000002993514