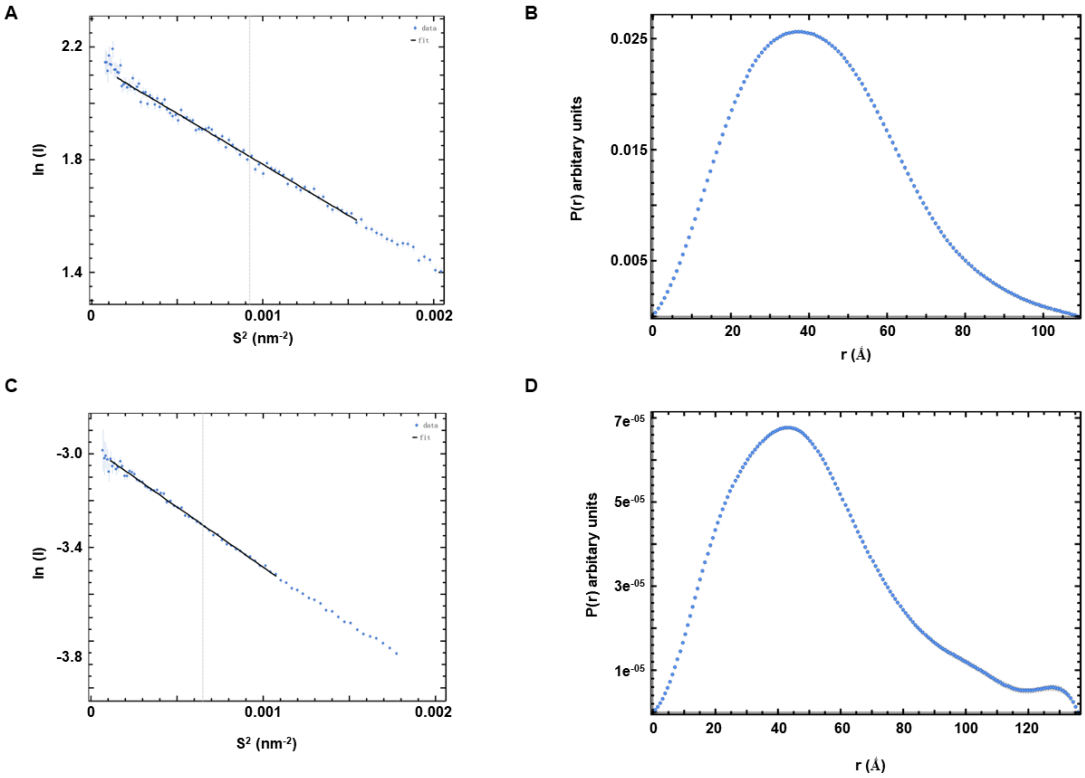
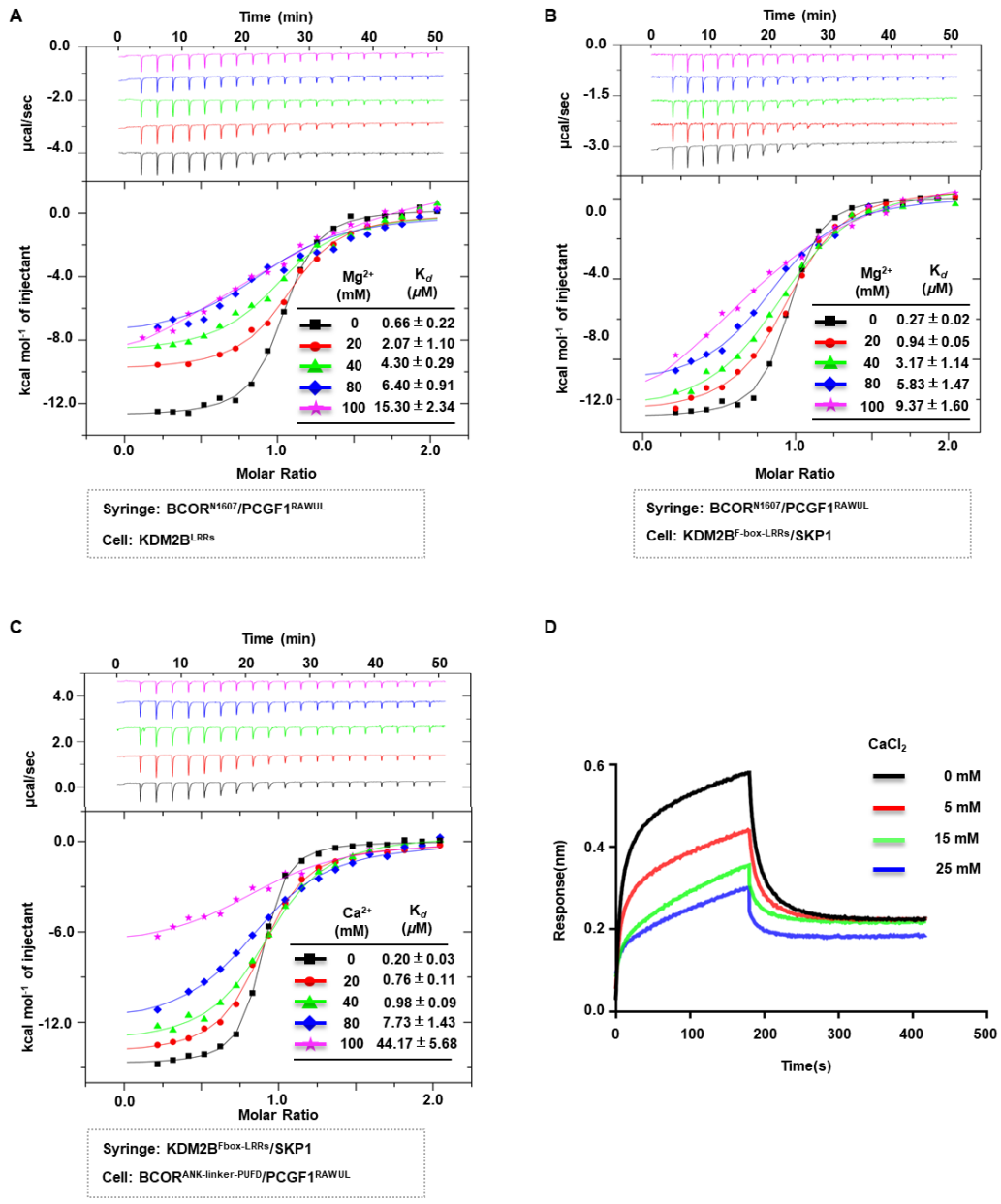
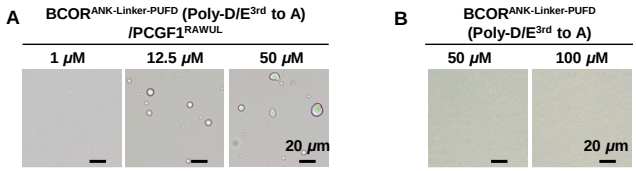


Supplementary Figure 1 SAXS data analysis for BCOR/PCGF1/KDM2B/SKP1 tetramer. (A) Guinier plots for BCOR^{1607}/PCGF1^{RAWUL}/KDM2B$^{F\text{-}box\text{-}LRRs}$/SKP1. (B) P(r) functions for BCOR^{1607}/PCGF1^{RAWUL}/KDM2B$^{F\text{-}box\text{-}LRRs}$/SKP1. (C) Guinier plots for BCOR$^{ANK\text{-}linker\text{-}PUFD}$/PCGF1^{RAWUL}/KDM2B$^{F\text{-}box\text{-}LRRs}$/SKP1. (D) P(r) functions for BCOR$^{ANK\text{-}linker\text{-}PUFD}$/PCGF1^{RAWUL}/KDM2B$^{F\text{-}box\text{-}LRRs}$/SKP1.

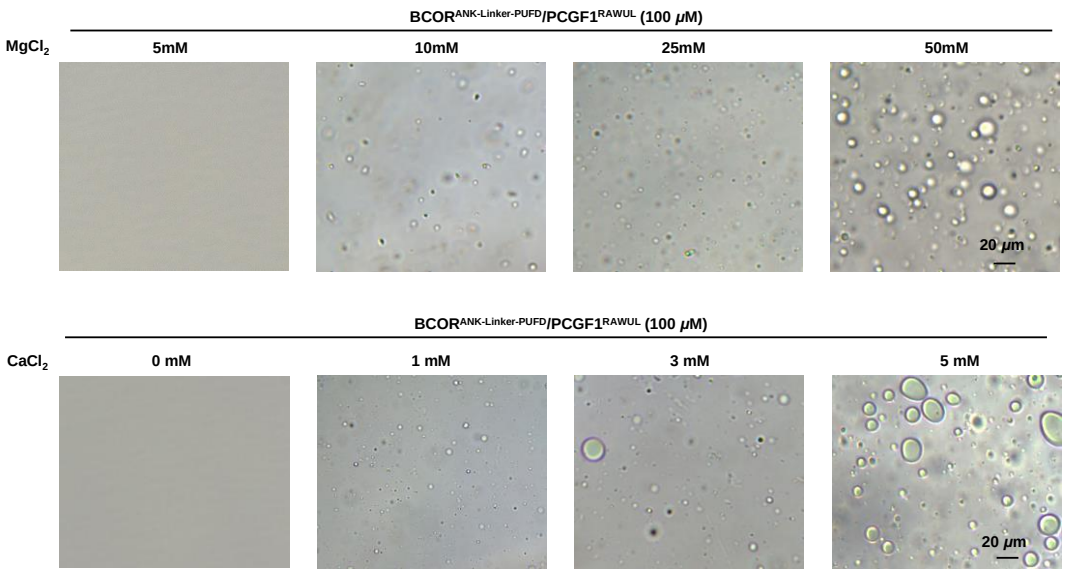


Supplementary Figure 2. Effect of magnesiumion or calcium on BCOR binding to KDM2B. (A-B) Binding affinity between BCOR^{N1607}/PCGF1^{RAWUL} and KDM2B^{LRRs} (A) or KDM2B$^{F\text{-box-LRRs}}$/SKP1 dimer (B) is determined using ITC in the presence of $MgCl_2$ at indicated concentration. $MgCl_2$ at indicated concentration was placed at both of cell and syringe. (C) Binding affinity between BCOR$^{ANK\text{-linker-PUFD}}$/PCGF1^{RAWUL} and KDM2B$^{F\text{-box-LRRs}}$/SKP1 is determined using ITC in the presence of $CaCl_2$ at indicated concentration. $CaCl_2$ at indicated concentration was placed at both of cell and syringe. For (A-C), The K_d values are shown as mean ± SD for triplicate experiments. (D) Effect of calcium on BCOR$^{ANK\text{-linker-PUFD}}$/PCGF1^{RAWUL} binding to KDM2B$^{F\text{-box-LRRs}}$/SKP1 is determined using BLI assay. BCOR$^{ANK\text{-linker-PUFD}}$/PCGF1^{RAWUL} dimer was labeled with biotin and immobilized onto a

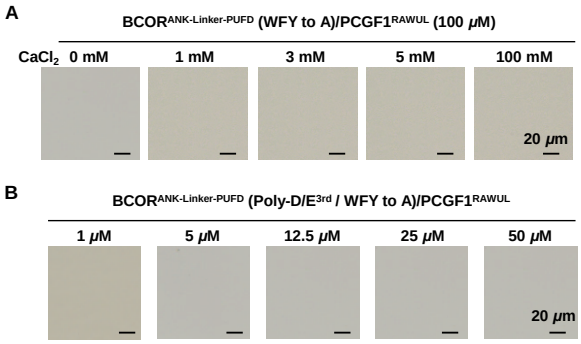
streptavidin biosensor. KDM2B$^{F\text{-}box\text{-}LRRs}$/SKP1 acts as analyte in the buffer containing $CaCl_2$ at indicated concentration.



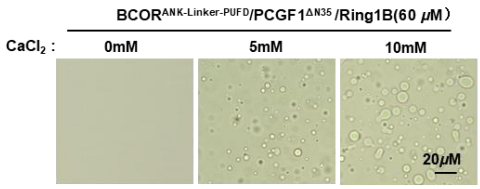
Supplementary Figure 3 LLPS assay of $BCOR^{ANK\text{-}linker\text{-}PUFD}$ (Poly-D/E^{3rd} to A)/$PCGF1^{RAWUL}$ dimer (A), or $BCOR^{ANK\text{-}linker\text{-}PUFD}$ (Poly-D/E^{3rd} to A) (B).



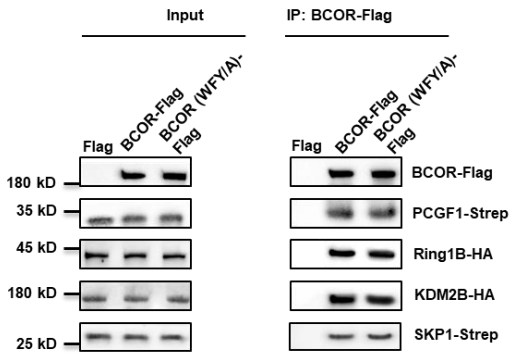
Supplementary Figure 4. Effect of $MgCl_2$ and $CaCl_2$ on inducing phase separation of $BCOR^{ANK\text{-}linker\text{-}PUFD}/PCGF1^{RAWUL}$ hetero-dimer with concentration of 100 μM



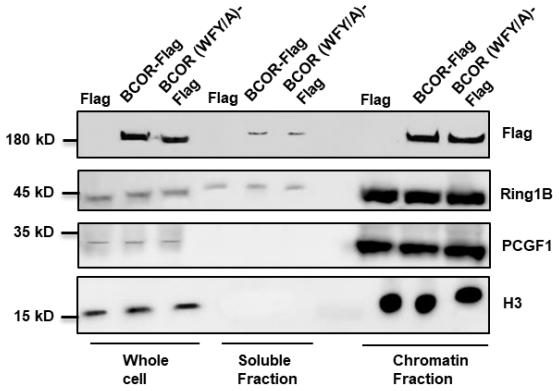
Supplementary Figure 5. The importance of aromatic residues on linker of BCOR for $CaCl_2$ induced phase separation of $BCOR^{ANK\text{-}linker\text{-}PUFD}$/$PCGF1^{RAWUL}$ (A), and phase separation of $BCOR^{ANK\text{-}linker\text{-}PUFD}$(Poly-D/$E^{3rd}$ to A)/$PCGF1^{RAWUL}$ (B).



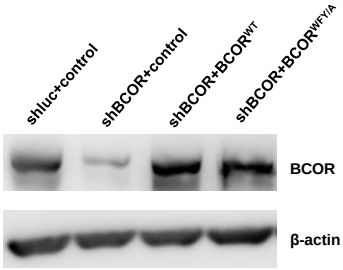
Supplementary Figure 6. Calcium induced LLPS of $BCOR^{ANK\text{-}Linker\text{-}PUFD}$/$PCGF1^{\Delta N35}$ /Ring1B



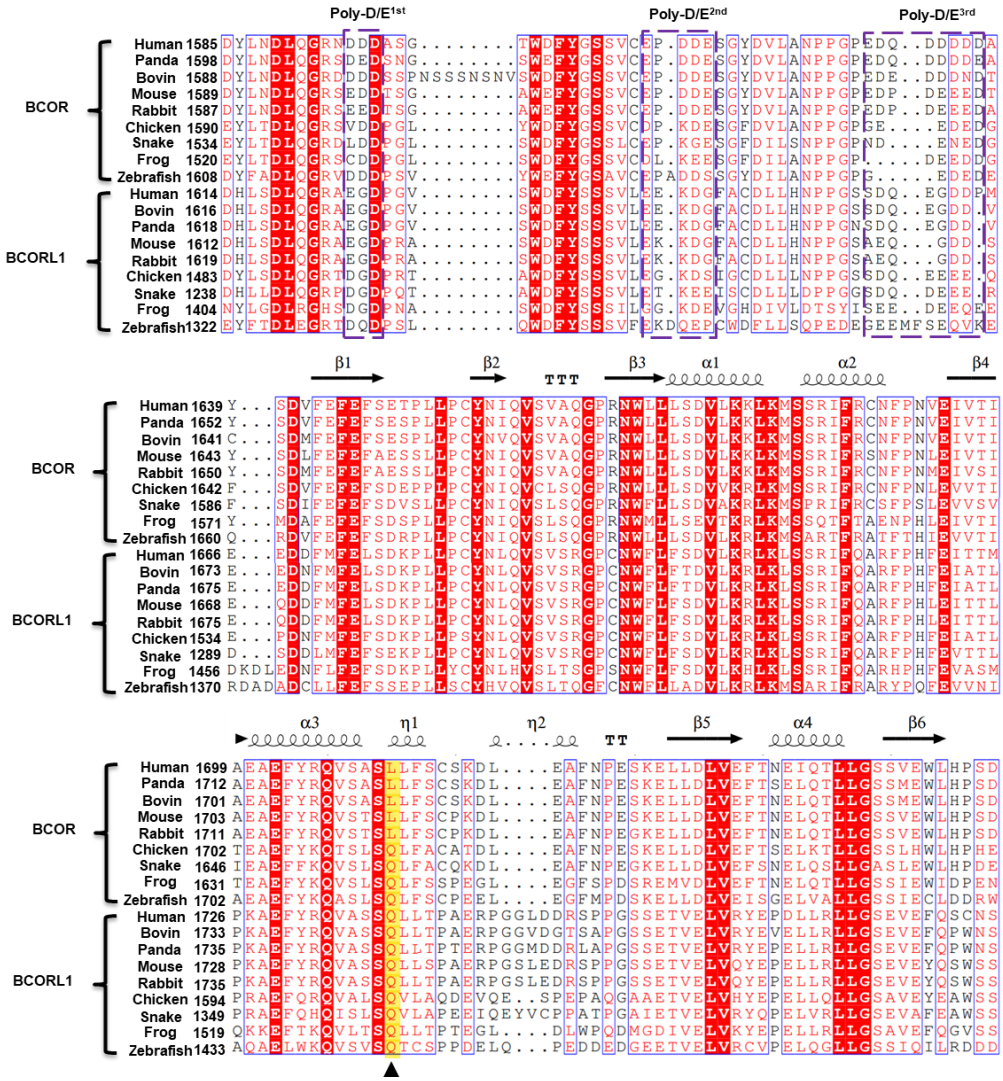
Supplementary Figure 7. The importance of aromatic residues (WFY) on linker for BCOR binding to KDM2B/SKP1 is assessed using Co-IP assay. Expressing plasmids for BCOR (wild or mutant), PCGF1, KDM2B or SKP1, were co-transfected into HEK293T cells. Co-IP was performed with anti-Flag magnetic beads, after 48h transfection. The western-blotting data is representative of two independent experiments.



Supplementary Figure 8. The subcellular localizations of BCOR, RING1B and PCGF1 are determined by a biochemical fractionation assay in 293T cells upon transient expression of the Flag-tagged BCOR (wild type or mutant). Whole-cell extracts, the soluble and chromatin-binding fractions are analyzed by western-blotting. The western-blotting data is representative of two independent experiments.



Supplementary Figure 9. Western blot analysis knockdown efficiency of BCOR in HEK293T cells, as well as the level of over-expressed BCOR (wild type or mutant) in HEK293T cells that knockdown endogenous BCOR.



Supplementary Figure 10. Sequence alignment for BCOR and BCORL1 from different species.

Supplementary table 1. Residues cross-linked by BS_3 between KDM2B and PCGF1

KDM2B	PCGF1	Score
K1111	K234	3
K1304	K244	6
K1304	K255	3
K1304	K180	4
K1304	K184	3
K1139	K180	3
K1139	K190	8
K1139	K159	3
K1334	K255	3
K1334	K184	5

Supplementary table 2. Residues cross-linked by BS_3 between KDM2B and BCOR

KDM2B	BCOR	Score
K1187	K1674	11
K1187	K1568	7
K1304	K1674	11
K1304	K1711	4

Supplementary table 3. Residues cross-linked by EDC between SKP1 and BCOR

SKP1	BCOR	Score
K78	D1555	4
K80	D1522	5
K121	E1484	4
K128	E1518	3
K142	D1555	3

Supplementary table 4. SAXS sample details and parameters

	BCOR^{N1607}/PCGF1^{RAWUL}/ KDM2B$^{F\text{-}box\text{-}LRRs}$/SKP1	**BCOR$^{ANK\text{-}linker\text{-}PUFD}$/PCGF1^{RAWUL}/ KDM2B$^{F\text{-}box\text{-}LRRs}$/SKP1**
Data Collection Parameters		
SEC-SAXS column	Superdex 200 Increase 10/300GL	
Loading concentration	10 mg/ml	
Flow rate	0.5 mL/min	
Solvent	20 mM Tris-HCl pH 7.5, 150 mM NaCl	
Structural Parameters		
From Guinier fit		
Rg (Å)	32.90	39.25
From *P(r)*		
Rg (Å)_	33.42	41.33
Dmax (Å)	109	136
Molecular Mass Determination		
MW (kDa) from Q(p)	73.17	105.83
MW (kDa) from V(c)	71.97	101.45
Estimate MW (kDa)	68.78	91.18
***Ab initio* analysis**		
χ^2 (GASBOR)	1.060	1.005
Rigid-body modeling Validation		
χ^2 (CRYSOL)	1.04	1.73