The internal interaction in RBBP5 regulates assembly and activity of MLL1 methyltransferase complex

¹Jianming Han, ¹Tingting Li, ¹Yanjing Li, ²Muchun Li, ¹Xiaoman Wang, ^{3,4}Chao Peng, ^{3,4}Chen Su, ³Na Li, ³Yiwen Li, ¹Ying Xu, ^{1,2,4}Yong Chen

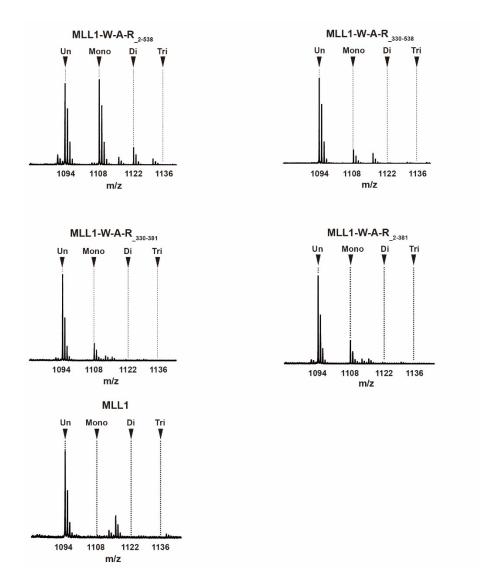
¹State Key Laboratory of Molecular Biology, National Center for Protein Science Shanghai, CAS Center for Excellence in Molecular Cell Science, Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences; University of Chinese Academy of Sciences, 333 Haike Road, Shanghai 201210, China.

²School of Life Science and Technology, Shanghai Tech University, 100 Haike Road, Shanghai 201210, P. R. China

³National Facility for Protein Science in Shanghai, Zhangjiang Lab, Shanghai, 201210, China.

⁴Shanghai Science Research Center, Chinese Academy of Sciences, Shanghai, 201204, China.

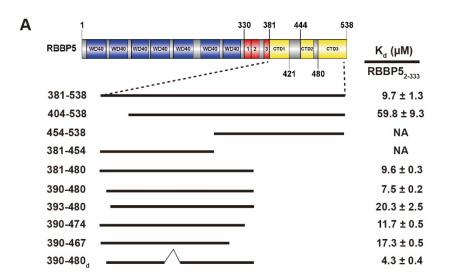
Correspondence should be addressed to Y.C. (yongchen@sibcb.ac.cn)



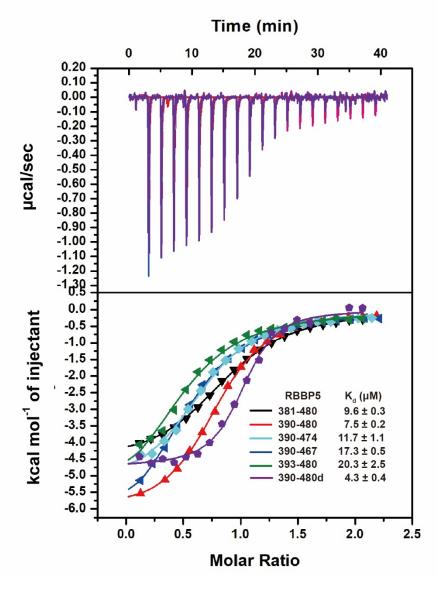
Supplementary Figure 1. Representative MALDI-TOF spectra after 1-hour reaction for MLL1 complexes assembled with different RBBP5 fragments. MLL1 complex with full-length RBBP5 (RBBP5 ₂₋₅₃₈) is more active than others, as reflected by the higher mono-methylated H3 peak. The peaks for unmodified (Un) and mono-, di-, tri-methylated products are labeled.

			В		
Protein1(site)-Protein2(site)	#Spec-Total	Best Score	Protein1(site)-Protein2(site)	#Spec-Total	Best Score
MLL1(3828)-RBBP5(338)	32	8.61E-29	RBBP5(149)-RBBP5(172)	23	1.31E-29
MLL1(3825)-RBBP5(338)	7	1.32E-29	RBBP5(150)-RBBP5(172)	32	1.43E-23
MLL1(3829)-RBBP5(288)	18	1.86E-25	RBBP5(148)-RBBP5(172)	15	3.34E-13
MLL1(3828)-RBBP5(340)	8	6.06E-23	RBBP5(209)-RBBP5(338)	3	1.10E-27
MLL1(3828)-RBBP5(349)	1	1.59E-02	RBBP5(149)-RBBP5(174)	13	1.11E-20
MLL1(3828)-RBBP5(344)	1	5.01E-02	RBBP5(150)-RBBP5(174)	15	1.42E-20
MLL1(3870)-RBBP5(338)	1	8.16E-03	RBBP5(148)-RBBP5(174)	10	1.80E-17
MLL1(3825)-RBBP5(340)	1	3.45E-10	RBBP5(164)-RBBP5(179)	5	2.10E-17
MLL1(3825)-RBBP5(341)	2	6.62E-05	RBBP5(152)-RBBP5(172)	4	9.47E-14
MLL1(3799)-RBBP5(209)	1	6.17E-02	MLL1(3873)-MLL1(3949)	7	1.22E-11
ASH2L(311)-RBBP5(338)	31	6.42E-19	RBBP5(174)-RBBP5(236)	1	3.94E-08
MLL1(3796)-RBBP5(209)	1	6.92E-08	ASH2L(299)-ASH2L(310)	7	1.02E-07
MLL1(3824)-RBBP5(338)	2	3.12E-05	RBBP5(209)-RBBP5(340)	2	1.86E-07
MLL1(3824)-RBBP5(340)	1	6.00E-04	WDR5(27)-WDR5(313)	2	4.02E-07
MLL1(3828)-RBBP5(341)	5	2.17E-21	RBBP5(236)-RBBP5(474)	1	1.70E-05
ASH2L(311)-RBBP5(344)	3	3.01E-16	MLL1(3796)-MLL1(3933)	2	3.43E-05
ASH2L(311)-RBBP5(341)	4	3.95E-15	WDR5(1)-WDR5(292)	2	3.82E-05
ASH2L(311)-RBBP5(340)	7	2.65E-12	RBBP5(129)-RBBP5(466)	2	7.02E-05
RBBP5(410)-WDR5(120)	16	4.67E-10	MLL1(3797)-MLL1(3933)	2	1.94E-04
RBBP5(412)-WDR5(120)	4	1.99E-08	RBBP5(209)-RBBP5(344)	1	2.44E-04
RBBP5(407)-WDR5(120)	12	8.03E-08	RBBP5(129)-RBBP5(150)	1	1.69E-03
RBBP5(407)-WDR5(112)	1	2.45E-05	RBBP5(209)-RBBP5(341)	2	2.33E-03
RBBP5(407)-WDR5(123)	13	2.61E-07	MLL1(3799)-MLL1(3933)	1	7.00E-02
RBBP5(407)-WDR5(159)	21	4.26E-08	RBBP5(99)-RBBP5(449)	1	7.63E-02
RBBP5(407)-WDR5(162)	1	4.26E-08	MLL1(3795)-MLL1(3828)	1	8.86E-02
RBBP5(407)-WDR5(247)	1	4.12E-03	RBBP5(236)-RBBP5(174)	3	2.38E-06
RBBP5(413)-WDR5(120)	4	4.12E-03 1.05E-04	RBBP5(238)-RBBP5(481)	1	5.41E-05
			RBBP5(238)-RBBP5(482)	3	1.99E-15
RBBP5(450)-WDR5(58)	2	4.53E-08	RBBP5(238)-RBBP5(488)	2	2.50E-02
MLL1(3828)-RBBP5(280)	1	2.04E-05	RBBP5(240)-RBBP5(482)	2	2.05E-04
MLL1(3878)-WDR5(211)	5	4.85E-08	RBBP5(495)-RBBP5(238)	2	2.23E-02
MLL1(3828)-WDR5(211)	4	4.59E-05	RBBP5(513)-RBBP5(505)	3	1.19E-08
MLL1(3828)-WDR5(212)	1	4.00E-13	RBBP5(536)-RBBP5(493)	1	8.01E-02
RBBP5(238)-WDR5(162)	1	5.18E-02	RBBP5(536)-RBBP5(517)	1	3.73E-06
WDR5(58)-RBBP5(505)	5	3.49E-07	RBBP5(536)-RBBP5(482)	5	9.66E-10
WDR5(211)-MLL1(3825)	3	6.86E-07	RBBP5(536)-RBBP5(505)	5	1.25E-06
WDR5(211)-RBBP5(505)	2	1.09E-03	RBBP5(536)-RBBP5(488)	2	1.75E-05
MLL1(3878)-WDR5(212)	1	7.09E-15	RBBP5(338)-RBBP5(209)	1	2.49E-06

Supplementary Figure 2. Crosslinking mass spectrometry (CX-MS) analysis of the $MLL1_{3754-3969}$ -WDR5₂₃₋₃₃₄-ASH2L_{FL}-RBBP5_{FL} (M1WAR) complex. The purified M1WAR complex was crosslinked by EDC (1-ethyl-3-[3-imethylaminopropyl] carbodiimide hydrochloride) coupled with Sulfo-NHS and analyzed by MS. This analysis identified 37 pairs of inter-molecular interaction (A) and 38 pairs of intra-molecular interaction (B) with a pLink2 score less than 0.01.

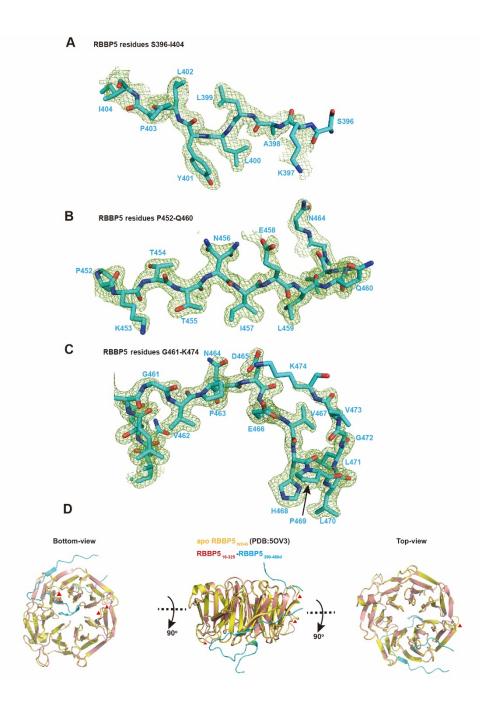






Supplementary Figure 3. Mapping interaction fragments between CTD and WD40 of RBBP5.

A. Schematic summary of different RBBP5 C-terminal constructs tested for binding to RBBP5₂₋₃₃₃ by isothermal titration calorimetry (ITC) assays. NA stands for no heat change during injections. 390-480d represents RBBP5₃₉₀₋₄₈₀ with a loop deletion from 422 to 443. **B.** Representative ITC data for the binding of different RBBP5 C-terminal constructs to RBBP5₂₋₃₃₃. The upper panel is the heat change upon titration of RBBP5 C-terminal construct into RBBP5₂₋₃₃₃, and the lower panel is the binding isotherm profile. The assay buffer is 300 mM NaCl, 25 mM Tris-HCl, pH 8.0. The dissociation constants (K_d) and the reported fitting errors were determined from the representative ITC curves by data fitting using one-site binding model.



Supplementary Figure 4. The crystal structure of RBBP5_{WD40}-RBBP5_{CTD}.

A. The Fo-Fc omit map contoured at the 3 δ level is shown for RBBP5_{CTD1} (residues S396-I404).

B. The Fo-Fc omit map contoured at the 3 δ level is shown for the N-terminal part of RBBP5_{CTD2} (residues P452-Q460).

C. The Fo-Fc omit map contoured at the 3 δ level is shown for the C-terminal part of RBBP5_{CTD2} (residues G461-K474).

D. Superimposition of apo WD40 propeller of RBBP5 (PDB: 5OV3) and RBBP5₁₀₋₃₂₅-RBBP5_{390-480d} complex shows that there is no significant conformational change on WD40 propeller except for some loops contacting RBBP5_{CTD} (labeled with red triangles).



		RBBP5(H.sapiens)
		RBBP5(P.troglodytes)
	te	RBBP5 (C.lupus)
S	ra	RBBP5 (B.taurus)
na	e	RBBP5 (M.musculus)
Animals	t	RBBP5(R.norvegicus)
Ā	2	RBBP5(G.gallus)
		RBBP5(X.tropicalis)
		RBBP5(D.rerio)
		RBBP5(D.melanogaster)
		RBBP5(A.gambiae)
s		RBBP5(C.elegans)
ŧ		Swd1(A.thaliana)
Plants		Swd1(0.sativa)
		Swd1 (P.oryzae)
		Swdl (N.crassa)
g		Swd1(M.thermophila)
Fungi		Swd1(S.cerevisiae)
<u> </u>		Swd1(K.lactis)
		Swd1(E.gossypii)
		Swd1 (S.pombe)

WD7

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RBBP5(H.sapiens) RBBP5(F.troglodytes) RBBP5(F.troglodytes) RBBP5(F.taurus) RBBP5(F.taurus) RBBP5(M.musculus) RBBP5(M.musculus) RBBP5(G.aplus) RBBP5(G.aplus) RBBP5(J.rerio) RBBP5(J.sapies) Swd1(A.thaliana) Swd1(A.thaliana) Swd1(R.toryzae) Swd1(K.locatis) Swd1(K.corevisiae) Swd1(K.toris) Swd1(K.toris) Swd1(K.toris) Swd1(K.toris) Swd1(K.jopmbe)

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Animals Vertebrate

Plants

Fungi

-MNLELLESFGQNYPEEADGTLDCIS-MALTCTFN -MNLELLESFGQNYPEEADGTLDCIS-MALTCTFN	RWGTLLAVGCNDGRIVIWDFLTRGIAKIISAHIHPVCSLCWSRDGHKLVSASTDNIVSQWDVLSG-DCDQRFRFPSPIL	111
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	RWGTLLAVGCNDGRIVIWDFLTRGIAKIISAHIHPVCSLCWSRDGHKLVSASTDNIVSQWDVLSG-DCDQRFRFPSPIL	111
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WD3	WD4	
β2		
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	LPVDDDSDLNVVASFDRRGEYIYTGNAKGKILVLKTDSQDLVASFRVTTG	
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KVQFHPRNDNKFLVCPMRYAAILVDVG-GKHEC-	LPLDNLEIVASFKIVLG	193
FAMFHPRNDNKAIVLOVNKOPTVEOFSPRIOTV-	LANDTPGSSDESASCVSYDRKGKYIIAGTGKGKLLIYNAETLKCVAWCKQN	196
	LPVSVPEMPDVLAPPQRSKCPESNPPFSPAAACFNKCGDLVYIGNSKGEILIVDYKSVRVLALVSAS	
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GAELHPMHHHQFAAALFEEQPMLVDVKESAQGAN	PVEVRHILPSVEKRSDDGADTPTKEKHAKEDARHMTTAIVYTATGEHLLAGTTKGRLNIIDATTHKIIYSEK-I	218
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MCRVIAGCPCPECTARVVEERMALLVDFSA	ADVRE VEDPA EDAABDRGNTLIVTCLIBERADVVI ITGTSKGWVI TYRLLADGGF THAIHSEF - I - I FKHKYLPTNFD I DEN YSDRRNRSKHVILVSFHPSGEY I LSGTSKGWFHVI DAST TKIRSSHR - I WD 6 	212 214 211 205 287 287 287 287 287 287 287 287 287 287
MCRVIAGCPCPECTARVVEERMALLVDFSA	ADVRE VEDPA EDAABDRGNTLIVTCLIBERADVVI ITGTSKGWVI TYRLLADGGF THAIHSEF - I - I FKHKYLPTNFD I DEN YSDRRNRSKHVILVSFHPSGEY I LSGTSKGWFHVI DAST TKIRSSHR - I WD 6 	212 214 211 205 287 287 287 287 287 287 287 287 287 287
MCRVIAGPOPECIARVVERWALLVDFSA SASHPHKINTFVASLLDESPQLIVDFSA WD5 TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREIL TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREIL TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREIL TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREIL TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREIL TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREI TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREIL TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREI TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREI TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREI TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREI SSNTAKSIEPARKOSCFLINTADRIIRVYDGREI SSNTAKSIEPARKOSCFLINTADRIIRVYDGREI SSNTAKSIEPARKOSCFLINTADRIIRVYDGREI SSNTAKSIEPARKOSCFLINTADRIIRVYDGREI SSNTAKSIEPARKOSCFLINTADRIIRVYDGREI -GAPAVORIVYSENARVITNEBORTIRVENILPA GAPAVORIVYSENARVITNSBORTIRVENILPA STSNIIAKSIEPARKALINCSDRIIRVENILPA	ADVRE VEDPA EDAAEDRGNTLYTCLHERAQUVITGTSKGWVITKLLADGGF FALHSEF - I I PKHKYLPTNPDIDEN YSDRRNRSKHVILVSFHPSGEVILGGTSKGWFHVIDAST TKIRSSHR - I WD G WD G TC	212 214 211 205 287 287 287 287 287 287 287 287 287 287
MCRVIAGPOPECIARVVERWALLVDFSA SASHPHKINTFVASLLDESPQLIVDFSA WD5 TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREIL TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREIL TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREIL TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREIL TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREIL TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREI TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREIL TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREI TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREI TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREI TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREI SSNTAKSIEPARKOSCFLINTADRIIRVYDGREI SSNTAKSIEPARKOSCFLINTADRIIRVYDGREI SSNTAKSIEPARKOSCFLINTADRIIRVYDGREI SSNTAKSIEPARKOSCFLINTADRIIRVYDGREI SSNTAKSIEPARKOSCFLINTADRIIRVYDGREI -GAPAVORIVYSENARVITNEBORTIRVENILPA GAPAVORIVYSENARVITNSBORTIRVENILPA STSNIIAKSIEPARKALINCSDRIIRVENILPA	ADVRE VEDPA EDAAEDRGNTLYTCLHERAQUVITGTSKGWVITKLLADGGF FALHSEF - I I PKHKYLPTNPDIDEN YSDRRNRSKHVILVSFHPSGEVILGGTSKGWFHVIDAST TKIRSSHR - I WD G WD G TC	212 214 211 205 287 287 287 287 287 287 287 287 287 287
MCRVIAGPOPECIARVVERWALLVDFSA SASHPHKINTFVASLLDESPQLIVDFSA WD5 TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREIL TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREIL TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREIL TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREIL TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREIL TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREI TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREIL TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREI TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREI TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREI TSNTTAIKSIEPARKOSCFLINTADRIIRVYDGREI SSNTAKSIEPARKOSCFLINTADRIIRVYDGREI SSNTAKSIEPARKOSCFLINTADRIIRVYDGREI SSNTAKSIEPARKOSCFLINTADRIIRVYDGREI SSNTAKSIEPARKOSCFLINTADRIIRVYDGREI SSNTAKSIEPARKOSCFLINTADRIIRVYDGREI -GAPAVORIVYSENARVITNEBORTIRVENILPA GAPAVORIVYSENARVITNSBORTIRVENILPA STSNIIAKSIEPARKALINCSDRIIRVENILPA	ADVRE VEDPA EDAAEDRGNTLYTCLHERAQUVITGTSKGWVITKLLADGGF FALHSEF - I I PKHKYLPTNPDIDEN YSDRRNRSKHVILVSFHPSGEVILGGTSKGWFHVIDAST TKIRSSHR - I WD G WD G TC	212 214 211 205 287 287 287 287 287 287 287 287 287 287
MCRVIAGCPCPECTRVVEERMALLVDFSA	ADVRE VEDPA EDAABDRGNTLIVTCLIBERADVVI ITGSISKOWVI TYRLLADGGF IHAHSEF - I I FKHKYLPTNFD I DEN YSDRRNRSKHVTLVSFHPSGEY I LSGTSKOWFHVI DAST TKIRSSHR - I WD 6 TC	212 214 211 205 287 287 287 287 287 287 287 287 287 287
MCRVIAGCPCPECTRVVEERMALLVDFSA	ADVRE VEDPA EDAABDRGNTLIVTCLIBERADVVI ITGSISKOWVI TYRLLADGGF IHAHSEF - I I FKHKYLPTNFD I DEN YSDRRNRSKHVTLVSFHPSGEY I LSGTSKOWFHVI DAST TKIRSSHR - I WD 6 TC	212 214 211 205 287 287 287 287 287 287 287 287 287 287
MCRVIAGCPCPECTRVVEERMALLVDFSA	ADVRE VEDPA EDAAEDRGNTLYTCLHERAQUVITGTSKGWVITKLLADGGF FALHSEF - I I PKHKYLPTNPDIDEN YSDRRNRSKHVILVSFHPSGEVILGGTSKGWFHVIDAST TKIRSSHR - I WD G WD G TC	212 214 211 205 287 287 287 287 287 287 287 287 287 287
MCRVLAGQPERCIATVVERRMALIVDFSA	ADVRE VEDPA EDAABDRGNTLIVTCLIBERADVVI ITGSISKOWVI TYRLLADGGF IHAHSEF - I I FKHKYLPTNFD I DEN YSDRRNRSKHVTLVSFHPSGEY I LSGTSKOWFHVI DAST TKIRSSHR - I WD 6 TC	212 214 211 205 287 287 287 287 287 287 287 287 287 287
MCRVIAGCPCPECTRVVEERMALLVDFSA	ADVRE VEDPA EDAABDRGNTLIVTCLIBERADVVI ITGSISKOWVI TYRLLADGGF IHAHSEF - I I FKHKYLPTNFD I DEN YSDRRNRSKHVTLVSFHPSGEY I LSGTSKOWFHVI DAST TKIRSSHR - I WD 6 TC	212 214 211 205 287 287 287 287 287 287 287 287 287 287
MCRULAGPOPCLATVVEERNALLVDFSAG SASLHPHKINTFVASLLDESPQLIIVDDG SASLHPHKINTFVASLLDESPQLIIVDDG UD5 TSNTTAIKSIEPARKGSCFLINTADRIIRVTGREIL TSNTTAIKSIEPARKGSCFLINTADRIIRVTGREIL TSNTTAIKSIEPARKGSCFLINTADRIIRVTGREIL TSNTTAIKSIEPARKGSCFLINTADRIIRVTGREIL TSNTTAIKSIEPARKGSCFLINTADRIIRVTGREIL TSNTTAIKSIEPARKGSCFLINTADRIIRVTGREIL TSNTTAIKSIEPARKGSCFLINTADRIIRVTGREIL TSNTTAIKSIEPARKGSCFLINTADRIIRVTGREIL TSNTTAIKSIEPARKGSCFLINTADRIIRVTGREIL TSNTTAIKSIEPARKGSCFLINTADRIIRVTGREIL TSNTTAIKSIEPARKGSCFLINTADRIIRVTGREIL TSNTTAIKSIEPARKGSCFLINTADRIIRVTGREIL TSNTTAIKSIEPARKGSCFLINTADRIIRVTGREIL TVQIRGIVTYBRGREILUTSNDRIVRVTGSKEIL GGTVKNIVFIRDAGULINSNDRIIRVTNILLEV TSSNIKKIIVFSGREILVINSNDRIIRVTNILLEV TSSNIKKIIVFSGREILVINSNDRIIRVTSILDI TSNIKIIVFSGREILVINSNDRIIRVTSILDI SNIKIKIVFSCREILVINSDRIIRVSFISIDD SNIKIKIVFSCREILVINSDRIIRVSFISIDD SNIKIKIVFSCREILVINSDRIIRVSFISIDD SNIKIKIVFSCREILVINSDRIIRVSFISIDD SNIKIKIVFSCREILVINSDRIIRVSFISIDD SNIKIKIVFSCREILVINSDRIIRVSFISIDD	ADVREVEDPA	212 214 211 205 287 287 287 287 287 287 287 287 287 287
MCRVLAGPOPRCLATVVEERWALLVDFSA SASLHPHKINTFVASLLDESPQLIIVDDG WD5 TSNTTAIKSIEFARKOSCFLINTADRIIKVVDGREIL TSNTTAIKSIEFARKOSCFLINTADRIIKVVDGREIL TSNTTAIKSIEFARKOSCFLINTADRIIKVVDGREIL TSNTTAIKSIEFARKOSCFLINTADRIIKVVDGREIL TSNTTAIKSIEFARKOSCFLINTADRIIKVVDGREIL TSNTTAIKSIEFARKOSCFLINTADRIIKVVDGREIL TSNTTAIKSIEFARKOSCFLINTADRIIKVVDGREIL TSNTTAIKSIEFARKOSCFLINTADRIIKVVDGREIL TSNTTAIKSIEFARKOSCFLINTADRIIKVVDGREIL TSNTTAIKSIEFARKOSCFLINTADRIIKVVDGREIL TSNTTAIKSIEFARKOSCFLINTADRIIKVVDGREIL TSNTTAIKSIEFARKOSCFLINTADRIIKVVDGREIL TSNTTAIKSIEFARKOSCFLINTADRIIKVVDGREIL TSNTTAIKSIEFARKOSCFLINTADRIIKVVDGREIL TSNTTAIKSIEFARKASCFLINTADRIIKVVDGREIL TSNTTAIKSIEFARKASCFLINTADRIIKVVDGREIL TSNTTAIKSIEFARKASCFLINTADRIIKVVDGREIL TSNTTAIKSIEFARKASCFLINTADRIIKVVDGREIL TSNTTAIKSIEFARKASCFLINTADRIIKVVDGREIL TSNTTAIKSIEFARKASCFLINTADRIIKVVDGREIL TSNTTAIKSIEFARKASCFLINTADRIIKVVDGREIL TSNTTAIKSIEFARKASCFLINTADRIIKVVDGREIL TSNTTAIKSIEFARKASCFLINTADRIIKVVDGREIL TSNTYTIKIEFARKASCFLINTADRIIKVVDGREIL TSNTYAIKSIEFARKASCFLINTADRIIKVVDGREIL TSNTYTIKIFFARKENTINTIDVIKVVDSKUV TOQIIKOLIVYSKORTIKITANSOPTIRCYLVDSKUV SOSIKKIIVPSRNQULIKINSNOPTIRCYLVDSLOP SOSIKKIISISPAGKLINSSOPTIRCYLVSIQD ANGUITIKKIEVAPVGERMAINCSDPTIRCYLVSIQD- CALVITTIKLITESGKELLVNAQQRIRITFHVPN AGGVITIKLITESGKELLVNAQQRIRITFHVPN	ADVREVEDPAEDAAEDRGNTLIVTCLHERAQUVVITGTSKGWVVITKLLADGGF	212 214 211 205 287 287 287 287 287 287 287 287 287 287
MCRVIAGOPPRCIATVVERWALLVDFSA SASLHPHKINTFVASLLDESPQLIVDFSA WD5 TSNTTAIKSIEFARKSGEFLINTADRIIRVYDGREIL TSNTTAIKSIEFARKSGEFLINTADRIIRVYDGREICH SASIKIEFARKSERTITSTERVYTERVYTIN SASIKIENTERSGERLINTSBERTIRTYNTERVYTERVE SASIKIENTERSGERLINTSBERTIRTYNTERVE SASIKIEFARKSERLINTSBERTIRTYNTERVE SASIKIENTERSGERLINTSBERTIRTYNTERVE SASIKIENTERSGERLINTSBERTERVERTERVENTERVE SASIKIERSERKERSERTERVERTERVERTERVENTERVERTERVE	ADVREVEDPA	212 214 211 205 287 287 287 287 287 287 287 287 287 287

 RBBP5(H. sapiens)
 KILHGTRGELLLDVAMHPVRPIIASIS--SGVVSIMAQNQVENNSAFAPOFKELDENVEY ERESEFDIEDEDKSEFEQTGADAAEDEEVDVTSVDPIAAFCS 388

 RBBP5(C. lupus)
 KILHGTRGELLLDVAMHPVRPIIASIS--SGVVSIMAQNQVENNSAFAPOFKELDENVEY ERESEFDIEDEDKSEFEQTGADAAEDEEVDVTSVDPIAAFCS 388

 RBBP5(B. turus)
 KILHGTRGELLLDVAMHPVRPIIASIS--SGVVSIMAQNQVENNSAFAPOFKELDENVEY ERESEFDIEDEDKSEFEQTGADAAEDEEVDTSVDPIAAFCS 388

 RBBP5(B. turus)
 KILHGTRGELLDVAMHPVRPIIASIS--SGVVSIMAQNQVENNSAFAPOFKELDENVEY ERESEFDIEDEDKSEFEQTGADAAEDEEVDTSVDPIAAFCS 388

 RBBP5(B. turus)
 KILHGTRGELLDVAMHPVRPIIASIS--SGVVSIMAQNQVENNSAFAPOFKELDENVEY ERESEFDIEDEDKSEFEQTGADAAEDEEVDTSVDPIAAFCS 388

 RBBP5(B. turus)
 KILHGTRGELLDVAMHPVRPIIASIS--SGVVSIMAQNVENNSAFAPOFKELDENVEY ERESEFDIEDEDKSEFEQTGADAAEDEEVDTSVDPIAAFCS 388

 RBBP5(G. turus)
 KILHGTRGELLDVAMHPVRPIIASIS--SGVVSIMAQNVENNSAFAPOFKELDENVEY ERESEFDIEDEDKSEFEQTGADAAEDEEVDTSVDPIAAFCS 388

 RBBP5(C. turus)
 KILHGTRGELLDVAMHPVRPIIASIS--SGVVSIMAQNVENNSAFAPOFKELDENVEY ERESEFDIEDEDKSEFEQTGADAAEDEEVDTSVDPIAAFCS 388

 RBBP5(C. turus)
 KILHGTRGELLDVAMHPVRPIIASIS--SGVVSIMAQNVENNSAFAPOFKELDENVEY ERESEFDIEDEDKSEFEQTGADAAEDEEVDTSVDPIAAFCS 388

 RBBP5(C. turus)
 KILHGTRGELLDVAMHPVRPIIASIS--SGVVSIMAQNVENNSAFAPOFKELDENVEY ERESEFDIEDEDKSEFEQTGADAAEDEEVDTSVDYENTSVPLANGS 388

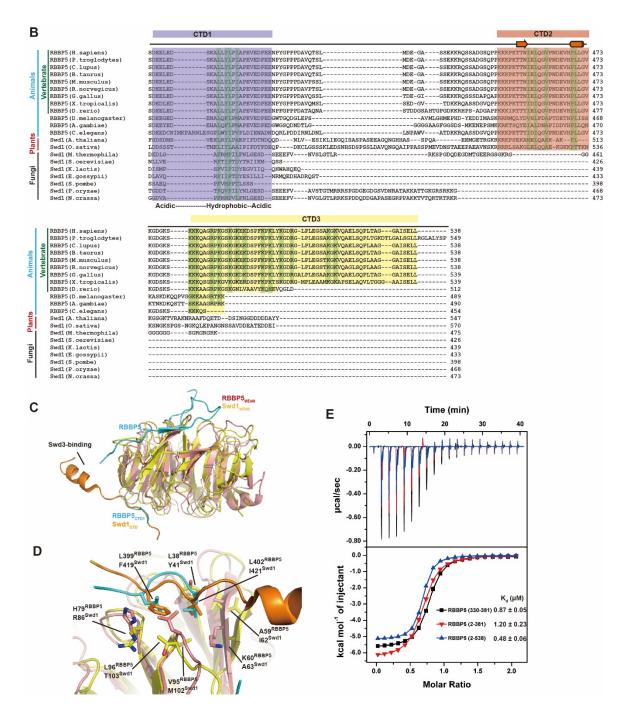
 RBBP5(C. turus)
 KILHGTRGELLDVAMHPVRPIIASIS--SGVVSIMAQNVENNSAFAPOFKELDENVEY ERESEFDIEDEDKSEFUTSKOPKOTOLDVINTSKAFAPOFKELDENVEY ERESEFDIEDEDKSEFUTSKOPKOTOLDVINTSKAFAPOFKELDENVEY ERESEFDIEDEKSEFUTSKOPKOTOLDVINTSKAFAPOFKELDENVEY ERESEFDIEDEKSKEFUTSKOPKOTOLDVINTSKAFAPOFKELDENVEY ERESEFDIE

Conserved arginine ring on the top surface Conserved basic residues on the bottom surface

ASH2L binding motif

WDR5 binding motif

Activation segment



Supplementary Figure 5. Comparison of RBBP5 from different species.

A. Sequence alignment of RBBP5 orthologs from yeast, plant, and animal species. RBBP5 WD40 propeller and WDRP region are shown. Secondary structure assignments based on the hRBBP5 and ySwd1 structures are shown as cylinders (α -helices) and arrows (β strands)

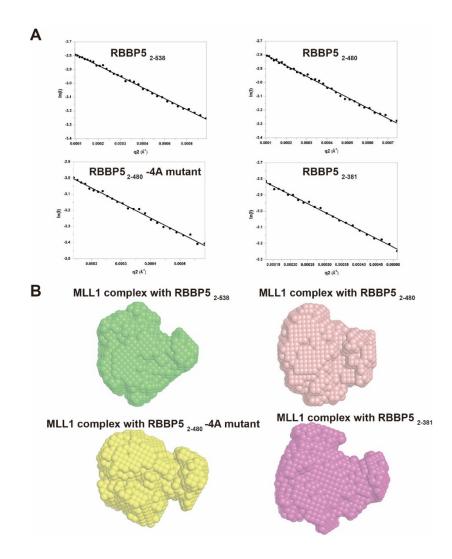
above the sequences. Conserved arginine residues (arginine-ring) at the top surface of WD40 propeller are highlighted in green. The lysine/arginine residues at the bottom of WD40 propeller are highlighted in cyan. These basic residues are conserved in animal species but not in yeast and plant species.

B. Sequence alignment of CTD regions of RBBP5 orthologs from yeast, plant and animal species. The conserved hydrophobic residues in CTD1 and CTD2 are highlighted. The conserved basic residues in CTD3 are also highlighted.

C. Structure superimposition of Swd1 (PDB: 6CHG) and hRBBP5 based on the WD40 propeller. The CTD from Swd1 has an extra helix extension involved in Swd3 binding. RBBP5_{WD40}, red; RBBP5_{CTD1}, cyan; Swd1_{WD40}, yellow; Swd1_{CTD1}, orange.

D. The relatively conserved interface between $RBBP5_{WD40}$ and $RBBP5_{CTD1}$. The structural equivalent residues in RBBP5 and Swd1 are indicated.

E. ITC analysis of the RBBP5-WDR5 interaction in the buffer of 150 mM NaCl, 25 mM Tris-HCl, pH8.0. The dissociation constants (K_d) and the reported fitting errors were determined from the representative ITC curves by data fitting using one-site binding model.



Supplementary Figure 6. Small-angle X-ray scattering analyses of MLL1-WDR5-RBBP5-ASH2L complex.

A. The Guinier plots of the scattering data show a linear fit at low *q*. SAXS data shown were from 2 mg/mL MLL1-WDR5-ASH2L-RBBP5 complexes. MLL1 contains residues 3754-3969, WDR5 contains residues 23-334, and ASH2L is full-length. RBBP5 used are indicated in each panel.

B. The *ab initio* solution structures of MLL1-WDR5-ASH2L-RBBP5 complexes with different RBBP5 constructs.

Supplementary Table 1. Summary of SAXS analyses of MLL1₃₇₅₄₋₃₇₆₉-WDR5₂₃₋₃₃₄-ASH2L_{FL}-RBBP5 complexes assembled with different RBBP5 fragments.

(a) Sample details

	2-381	2-480	2-480-4A*	2-538	
Organism	Homo sapiens				
Source (Catalogue No. or reference)		E.coli es	spressed		
	Q15291(2-381)	Q15291(2-480)	Q15291(2-480- 4A*)	Q15291(2-538)	
UniProt sequence ID (residues in	Q03164 (3754- 3969)	Q03164 (3754- 3969)	Q03164 (3754- 3969)	Q03164 (3754- 3969)	
construct)	P61964 (23-334)	P61964 (23-334)	P61964 (23-334)	P61964 (23-334	
	Q9UBL3-3 (1- 534)	Q9UBL3-3 (1- 534)	Q9UBL3-3 (1- 534)	Q9UBL3-3 (1- 534)	
Extinction coefficient ϵ (wavelength and units) (A280, M ⁻¹ cm ⁻¹)	234130	236690	236690	237970	
Molecular mass <i>M</i> from chemical composition (KDa)	161.8	172.3	172.1	178.4	
Concentration (mg/ml)	0.5-2	0.5-2	0.5-2	0.5-2	
Solvent composition and source	300 mM NaCl, 25 mM Tris pH 8.0, 4% glycerol, 1 mM TCEP				
(b) SAS data collection parameters					
Source, instrument and description or	BL19U2 at SSRF with Pilatus 1M (DECTRIS Ltd)				
reference	(DECTRIS Ltd)				
Wavelength (Å)	(DECTRIS Ltd) 0.9184				
	· · · · ·	V), 2.415m			
Wavelength (Å) Beam geometry (size, sample-to-detector	0.9184	V), 2.415m			
Wavelength (Å) Beam geometry (size, sample-to-detector distance)	0.9184 340μm x 60μm (H x 0.008-0.47	V), 2.415m attering with 1 mm pure	e H2O		
Wavelength (Å) Beam geometry (size, sample-to-detector distance) q-measurement range (Å-1 or nm-1)	0.9184 340μm x 60μm (H x 0.008-0.47 Comparison with sca			he 2D images	
Wavelength (Å) Beam geometry (size, sample-to-detector distance) <i>q</i> -measurement range (Å-1 or nm-1) Absolute scaling method	0.9184 340µm x 60µm (H x 0.008-0.47 Comparison with sca Take silver behenate SAXS data were coll	attering with 1 mm pure	nask, then normalize t	-	
 Wavelength (Å) Beam geometry (size, sample-to-detector distance) <i>q</i>-measurement range (Å-1 or nm-1) Absolute scaling method Basis for normalization to constant counts Method for monitoring radiation damage, 	0.9184 340µm x 60µm (H x 0.008-0.47 Comparison with sca Take silver behenate SAXS data were coll	attering with 1 mm pure as standard to set the r lected as continuous se apared to monitor the ra	nask, then normalize t	-	
 Wavelength (Å) Beam geometry (size, sample-to-detector distance) <i>q</i>-measurement range (Å-1 or nm-1) Absolute scaling method Basis for normalization to constant counts Method for monitoring radiation damage, X-ray dose where relevant 	0.9184 340µm x 60µm (H x 0.008-0.47 Comparison with sca Take silver behenate SAXS data were coll the passes were com	attering with 1 mm pure as standard to set the r lected as continuous se apared to monitor the ra	nask, then normalize t	-	
 Wavelength (Å) Beam geometry (size, sample-to-detector distance) <i>q</i>-measurement range (Å-1 or nm-1) Absolute scaling method Basis for normalization to constant counts Method for monitoring radiation damage, X-ray dose where relevant Exposure time, number of exposures 	0.9184 340µm x 60µm (H x 0.008-0.47 Comparison with sca Take silver behenate SAXS data were coll the passes were corr 1 s per frame, total 2 10	attering with 1 mm pure as standard to set the r lected as continuous se apared to monitor the ra 0 frames	nask, then normalize t	-	

	using PRIMUS (ATSAS 2.8.0)
Extinction coefficient estimate	http://protcalc.sourceforge.net/
Basic analyses: Guinier, P(r), Vp	PRIMUS (ATSAS 2.8.0)
Shape/bead modelling	DAMMIF and DAMAVER

Molecular graphics

(d) Structural parameters

	2-381	2-480	2-480-4A*	2-538
Guinier Analysis				
<i>I</i> (0) (cm-1)	0.069 ± 0.00032	0.067±0.00023	0.058±0.00021	0.068±0.00018
Rg (Å)	56.69±1.07	49.83±0.62	53.46±0.82	49.93±0.52
<i>q</i> -range (Å-1)	0.0092-0.0226	0.0097-0.0257	0.0097-0.0241	0.0097-0.0257
Quality-of-fit parameter (fidelity)	0.91	0.79	0.95	0.92
<i>M</i> (KDa) from <i>I</i> (0) (ratio to expected value)	171.6 (1.06)	160.8 (0.93)	164.4 (0.96)	193.5 (1.08)
<i>P</i> (r) analysis				
<i>I</i> (0) (cm-1)	0.0682	0.066	0.0574	0.0673
Rg (Å)	57.07	49.65	53.88	50.16
dmax (Å)	185.5	152.5	172	155
<i>q</i> -range (Å-1)	0.0092-0.1223	0.0097-0.0.1290	0.0097-0.1316	0.0097-0.1475
Quality-of-fit parameter (Total estimate from GNOM)	0.91	0.94	0.93	0.95
<i>M</i> (KDa) from <i>I</i> (0) (ratio to expected value)	169.6 (1.05)	158.4 (0.92)	162.7 (0.95)	191.5 (1.07)
Porod volume estimate (Å^3)	360000	256000	313000	282000
(e) Shape modelling results (a complete part	nel for each method)			
q-range for fitting(Å-1)	0.0092-0.3000	0.0097-0.3000	0.0097-0.3000	0.0097-0.3000
Symmetry/anisotropy assumptions	<i>P</i> 1	<i>P</i> 1	<i>P</i> 1	<i>P</i> 1
Ambiguity measure(s)	Filteriing of averaged <i>ab initio</i> models	Filteriing of averaged <i>ab initio</i> models	Filteriing of averaged <i>ab initio</i> models	Filteriing of averaged <i>ab initi</i> models
χ^{2} value	1.187	1.36	1.339	1.582
NSD value betten clusters	1.753	1.683	1.778	1.585
Model volume (nm ³)	151.5	167.1	115	159.6
Model precision/resolution (nm)	2.799	2.707	2.833	2.653
(f) Data and model deposition IDs				
SASDBD	SASDGD4	SASDGE4	SASDGF4	SASDGG4
(https://www.sasbdb.org/)				

* 4A stands for L399A/L400A/I457A/L459A mutation of RBBP5.