

Supplemental information

PRISMA and BiOJD disclose

**a motifs-based interactome of the intrinsically
disordered transcription factor C/EBP α**

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Position (Human) CEBPA P42	Position (rat) CEBPA P42	PTM Type	PTM included PRISMA screen	Known PTM/Reference (Human)	Known PTM/Reference (Rat)	known PTM features	BioID detected	phenotype
R12	R12	methylation	me2sym, me2asym				me, me2	
S21	S21	phos	phos	PMID:20101026,	PMID:26515730, 16807249	phos	phos	disrupts granulocytic differentiation
R35	R35	methylation	me2sym, me2asym	PMID:31015230		me		increased cyclin D1 expression and proliferation
K86	K86	acetylation methylation	ac,me2					
K90	K90	acetylation methylation	ac,me2					
K92	K92	acetylation methylation	ac,me2					
R142	R140	methylation	me2sym, me2asym				me	
R149	R147	methylation	me2sym, me2asym					
R156	R154	methylation	me2sym, me2asym	PMID:31015230		me, me2	me	increased cyclin D1 expression and proliferation
K161	K159	methylation acetylation ubiquitination sumoylation	ac, me2	PMID:19608861 PMID:21890473	https://doi.org/10.18452/17281 PMID:22902405 PMID:23639777	me ac ub sumo		interaction with HDAC3
R165	R163	citrullination methylation	citr, me2sym, me2asym		https://doi.org/10.18452/17281	citr		
K171	K169	acetylation methylation	ac, me2					
S190	S193	phos	phos	PMID:27619993, 2615376, 15107404		phos		growth inhibition
T226	T222	phos	phos	PMID:16600022	PMID:26153766 , 27619993 , 16600022	phos		regulation of lipogenic and gluconeogenic gene expression
T230	T226	phos	phos	PMID:16600022	PMID:16600022	phos		regulation of lipogenic and gluconeogenic gene expression
S234	S230	phos	phos	PMID:20101026	PMID:17290224, 10567568	phos		
K254	K250	acetylation methylation	ac,me2					
R264	R260	methylation	me2sym, m2asym					
S266	G262	phos	phos	PMID:25159151		phos		
K352	K352	acetylation methylation	ac,me2					

Table S1: CEBPA PTMs included in PRISMA, related to Figure 1

#	sequence	unmodified sequence	region	AA start	AA end	modification	number interactors
1	MESADFYEAEP RPPM	MESADFYEAEP RPPM	CR2	1	15		284
2	M-Nterm.ac-ESADFYEAEP RPPM	MESADFYEAEP RPPM	CR2	1	15	('1', 'N-term ac')	265
3	MESADFYEAEP R-me2_sym-PPM	MESADFYEAEP RPPM	CR2	1	15	('12', 'me2_sym')	231
4	MESADFYEAEP R-me2_asym-PPM	MESADFYEAEP RPPM	CR2	1	15	('12', 'me2_asym')	223
5	EAEPRPPMSSHLQSP	EAEPRPPMSSHLQSP		8	22		1
6	EAEPR-me2_sym-PPMSSHLQSP	EAEPRPPMSSHLQSP		8	22	('12', 'me2_sym')	2
7	EAEPRPPMSSHLQS-phos-P	EAEPRPPMSSHLQSP		8	22	('21', 'phos')	6
8	EAEPR-me2_asym-PPMSSHLQSP	EAEPRPPMSSHLQSP		8	22	('12', 'me2_asym')	14
9	MSSHLQSPPHAPSSA	MSSHLQSPPHAPSSA		15	29		0
10	MSSHLQS-phos-PPHAPSSA	MSSHLQSPPHAPSSA		15	29	('21', 'phos')	0
11	PPHAPSSAAAFGFPRG	PPHAPSSAAAFGFPRG		22	36		0
12	PPHAPSSAAAFGFPR-me2_sym-G	PPHAPSSAAAFGFPRG		22	36	('35', 'me2_sym')	1
13	PPHAPSSAAAFGFPR-me2_asym-G	PPHAPSSAAAFGFPRG		22	36	('35', 'me2_asym')	0
14	AAFGFPRGAGPAQPP	AAFGFPRGAGPAQPP		29	43		14
15	AAFGFPR-me2_sym-GAGPAQPP	AAFGFPRGAGPAQPP		29	43	('35', 'me2_sym')	17
16	AAFGFPR-me2_asym-GAGPAQPP	AAFGFPRGAGPAQPP		29	43	('35', 'me2_asym')	15
17	GAGPAQPAPPAAPE	GAGPAQPAPPAAPE		36	50		1
18	PAPPAAPEPLGGICE	PAPPAAPEPLGGICE	CR3	43	57		1
19	EPLGGICEHETSIDI	EPLGGICEHETSIDI	CR3	50	64		48
20	EHETSIDISAYIDPA	EHETSIDISAYIDPA	CR3	57	71		106
21	ISAYIDPAAFNDEFL	ISAYIDPAAFNDEFL	CR3	64	78		296
22	AAFNDEFLADLFQHS	AAFNDEFLADLFQHS	CR4	71	85		301
23	LADLFQHSRQEQEKAK	LADLFQHSRQEQEKAK		78	92		0
24	LADLFQHSRQEQEK-ac-AK	LADLFQHSRQEQEKAK		78	92	('90', 'ac')	8
25	LADLFQHSR-me2_asym-QQEKAK	LADLFQHSRQEQEKAK		78	92	('86', 'me2_asym')	0
26	LADLFQHSR-me2_sym-QQEKAK	LADLFQHSRQEQEKAK		78	92	('86', 'me2_sym')	1
27	LADLFQHSRQEQEK-me2-AK	LADLFQHSRQEQEKAK		78	92	('90', 'me2')	0
28	SRQEQEKAKAAVGPTG	SRQEQEKAKAAVGPTG		85	99		0
29	SRQEQEKAK-me2-AAVGPTG	SRQEQEKAKAAVGPTG		85	99	('92', 'me2')	0
30	SR-me2_asym-QQEKAKAAVGPTG	SRQEQEKAKAAVGPTG		85	99	('86', 'me2_asym')	0
31	SR-me2_sym-QQEKAKAAVGPTG	SRQEQEKAKAAVGPTG		85	99	('86', 'me2_sym')	0
32	SRQEQEK-ac-AKAAVGPTG	SRQEQEKAKAAVGPTG		85	99	('90', 'ac')	0
33	SRQEQEKAK-ac-AAVGPTG	SRQEQEKAKAAVGPTG		85	99	('92', 'ac')	0
34	SRQEQEK-me2-AKAAVGPTG	SRQEQEKAKAAVGPTG		85	99	('90', 'me2')	0
35	K-ac-AAVGPTGGGGGGDF	KAAGVPTGGGGGGDF		92	106	('92', 'ac')	11
36	GGGGGGDFDYPGAPA	GGGGGGDFDYPGAPA	CR5	99	113		33
37	FDYPGAPAGPGGAVM	FDYPGAPAGPGGAVM		106	120		7
38	AGPGGAVMPGGAHGP	AGPGGAVMPGGAHGP		113	127		5
39	MPGGAHPPPGYGCA	MPGGAHPPPGYGCA		120	134		14
40	M-Nterm.ac-PGGAHPPPGYGCA	MPGGAHPPPGYGCA		120	134	('120', 'N-term ac')	8
41	PPPGYGCAAGYLDG	PPPGYGCAAGYLDG	CR1L	127	141		16
42	AAAGYLDGRLEPLYE	AAAGYLDGRLEPLYE	CR1L	134	148		172
43	AAAGYLDGR-me2_asym-LEPLYE	AAAGYLDGRLEPLYE	CR1L	134	148	('142', 'me2_asym')	205
44	AAAGYLDGR-me2_sym-LEPLYE	AAAGYLDGRLEPLYE	CR1L	134	148	('142', 'me2_sym')	271
45	GRLEPLYERVGAPAL	GRLEPLYERVGAPAL	CR1L	141	155		6
46	GR-me2_asym-LEPLYERVGAPAL	GRLEPLYERVGAPAL	CR1L	141	155	('142', 'me2_asym')	9
47	GR-me2_sym-LEPLYERVGAPAL	GRLEPLYERVGAPAL	CR1L	141	155	('142', 'me2_sym')	2
48	GRLEPLYER-me2_asym-VGAPAL	GRLEPLYERVGAPAL	CR1L	141	155	('149', 'me2_asym')	5
49	GRLEPLYER-me2_sym-VGAPAL	GRLEPLYERVGAPAL	CR1L	141	155	('149', 'me2_sym')	1
50	ERVGAPALRPLVIKQ	ERVGAPALRPLVIKQ		148	162		0
51	ER-me2_asym-VGAPALRPLVIKQ	ERVGAPALRPLVIKQ		148	162	('149', 'me2_asym')	1
52	ERVGAPALR-me2_sym-PLVIKQ	ERVGAPALRPLVIKQ		148	162	('156', 'me2_sym')	3
53	ERVGAPALR-me2_asym-PLVIKQ	ERVGAPALRPLVIKQ		148	162	('156', 'me2_asym')	1
54	ERVGAPALRPLVIK-me2-Q	ERVGAPALRPLVIKQ		148	162	('161', 'me2')	0
55	ERVGAPALRPLVIK-ac-Q	ERVGAPALRPLVIKQ		148	162	('161', 'ac')	1
56	LRPLVIKQEPREDE	LRPLVIKQEPREDE	CR6	155	169		8
57	LR-me2_sym-PLVIKQEPREDE	LRPLVIKQEPREDE	CR6	155	169	('156', 'me2_sym')	31
58	LR-me2_asym-PLVIKQEPREDE	LRPLVIKQEPREDE	CR6	155	169	('156', 'me2_asym')	19
59	LRPLVIKQEP R-me2_sym-EEDE	LRPLVIKQEPREDE	CR6	155	169	('164', 'me2_sym')	26
60	LRPLVIK-me2-QEPREDE	LRPLVIKQEPREDE	CR6	155	169	('161', 'me2')	16

61	LRPLVIK-ac-QEPREED	LRPLVIKQEPREED	CR6	155	169	('161', 'ac')	47
62	LRPLVIKQEP-me2_asym-EED	LRPLVIKQEPREED	CR6	155	169	('164', 'me2_asym')	12
63	LRPLVIKQEP-citr-EED	LRPLVIKQEPREED	CR6	155	169	('164', 'citr')	53
64	QEPREDEAK-me2-QLALA	QEPREDEAKQLALA		162	176	('171', 'me2')	26
65	QEPREDEAK-ac-QLALA	QEPREDEAKQLALA		162	176	('171', 'ac')	41
66	QEP-me2_asym-EDEAKQLALA	QEPREDEAKQLALA		162	176	('164', 'me2_asym')	6
67	QEP-citr-EDEAKQLALA	QEPREDEAKQLALA		162	176	('164', 'citr')	26
68	EAKQLALAGLFPYQP	EAKQLALAGLFPYQP		169	183		2
69	EAK-ac-QLALAGLFPYQP	EAKQLALAGLFPYQP		169	183	('171', 'ac')	8
70	EAK-me2-QLALAGLFPYQP	EAKQLALAGLFPYQP		169	183	('171', 'me2')	1
71	AGLFPYQPPPPPPS	AGLFPYQPPPPPPS		176	190		37
72	PPPPPPSHPHPHPP	PPPPPPSHPHPHPP		183	197		7
73	PPPPPPS-phos-HPHPHP	PPPPPPSHPHPHPP		183	197	('190', 'phos')	1
74	SHPHPHPPAHLAAP	SHPHPHPPAHLAAP		190	204		0
75	S-phos-HPHPHPAHLAAP	SHPHPHPPAHLAAP		190	204	('190', 'phos')	0
76	PPAHLAAPHLQFQIA	PPAHLAAPHLQFQIA	CR7	197	211		2
77	PHLQFQIAHCGQTM	PHLQFQIAHCGQTM	CR7	204	218		0
78	AHCGQTMHLQPGHP	AHCGQTMHLQPGHP	CR7	211	225		0
79	MHLQPGHPTPPPTPV	MHLQPGHPTPPPTPV	CR7	218	232		1
80	MHLQPGHPT-phos-PPPTPV	MHLQPGHPTPPPTPV	CR7	218	232	('226', 'phos')	2
81	MHLQPGHPTPPPT-phos-PV	MHLQPGHPTPPPTPV	CR7	218	232	('230', 'phos')	4
82	PTPPPTVPSPHPAP	PTPPPTVPSPHPAP	CR7	225	239		2
83	PT-phos-PPPTVPSPHPAP	PTPPPTVPSPHPAP	CR7	225	239	('226', 'phos')	0
84	PTPPPT-phos-PVSPHPAP	PTPPPTVPSPHPAP	CR7	225	239	('230', 'phos')	1
85	PTPPPTVPS-phos-PHPAP	PTPPPTVPSPHPAP	CR7	225	239	('234', 'phos')	0
86	VPSHPAPALGAAGL	VPSHPAPALGAAGL		232	246		1
87	VPS-phos-PHPAPALGAAGL	VPSHPAPALGAAGL		232	246	('234', 'phos')	0
88	PALGAAGLPGPGSAL	PALGAAGLPGPGSAL		239	253		1
89	LPGPGSALKGLGAAH	LPGPGSALKGLGAAH		246	260		0
90	LPGPGSALK-ac-GLGAAH	LPGPGSALKGLGAAH		246	260	('254', 'ac')	0
91	LPGPGSALK-me2-GLGAAH	LPGPGSALKGLGAAH		246	260	('254', 'me2')	0
92	LKGLGAAHPDLRASG	LKGLGAAHPDLRASG		253	267		0
93	LK-me2-GLGAAHPDLRASG	LKGLGAAHPDLRASG		253	267	('254', 'me2')	0
94	LK-ac-GLGAAHPDLRASG	LKGLGAAHPDLRASG		253	267	('254', 'ac')	0
95	LKGLGAAHPDLRAS-phos-G	LKGLGAAHPDLRASG		253	267	('266', 'phos')	0
96	LKGLGAAHPDLR-me2_sym-ASG	LKGLGAAHPDLRASG		253	267	('264', 'me2_sym')	5
97	LKGLGAAHPDLR-me2_asym-ASG	LKGLGAAHPDLRASG		253	267	('264', 'me2_asym')	0
98	HPDLRASGGSGAGKA	HPDLRASGGSGAGKA		260	274		0
99	HPDLR-me2_asym-ASGGSGAGKA	HPDLRASGGSGAGKA		260	274	('264', 'me2_asym')	0
100	HPDLRAS-phos-GSGAGKA	HPDLRASGGSGAGKA		260	274	('266', 'phos')	0
101	HPDLR-me2_sym-ASGGSGAGKA	HPDLRASGGSGAGKA		260	274	('264', 'me2_sym')	0
102	GGSGAGKAKKSVDKN	GGSGAGKAKKSVDKN	BZIP	267	281		0
103	AKKSVDKNSNEYRVR	AKKSVDKNSNEYRVR	BZIP	274	288		0
104	NSNEYRVRERNNIA	NSNEYRVRERNNIA	BZIP	281	295		5
105	RRERNNIAVRKSRDK	RRERNNIAVRKSRDK	BZIP	288	302		126
106	AVRKSVDKAKQRNVE	AVRKSVDKAKQRNVE	BZIP	295	309		243
107	KAKQRNVEVETQQVLE	KAKQRNVEVETQQVLE	BZIP	302	316		1
108	ETQQVLELTSNDNR	ETQQVLELTSNDNR	BZIP	309	323		2
109	ELTSDNDRLRKRVEQ	ELTSDNDRLRKRVEQ	BZIP	316	330		1
110	RLRKRVEQLSRELDT	RLRKRVEQLSRELDT	BZIP	323	337		1
111	QLSRELDTLRGIFRQ	QLSRELDTLRGIFRQ	BZIP	330	344		1
112	TLRGIFRQLPESSLV	TLRGIFRQLPESSLV	BZIP	337	351		1
113	QLPESSLVKAMGNCA	QLPESSLVKAMGNCA		344	358		0
114	QLPESSLVK-me2-AMGNCA	QLPESSLVKAMGNCA		344	358	('352', 'me2')	0

Table S2: CEBPA peptides screened for interactions with PRISMA, related to Figure 1 and STAR methods

List of peptides included in the PRISMA screen. Membership of peptide sequence to CEBPA conserved region (CR) is indicated and CR sequences (Leutz et al., 2011) are highlighted in color. BZIP = basic leucine zipper

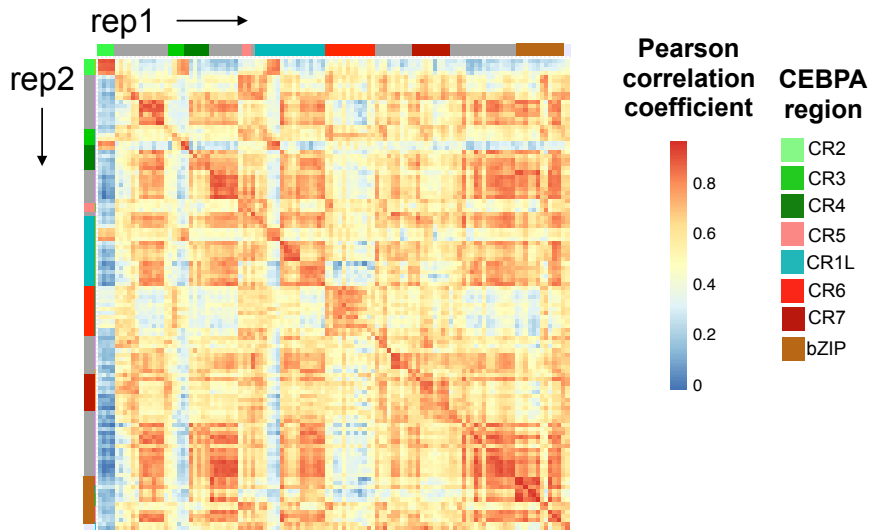


Figure S1: Pearson correlation matrix of PRISMA replicates, Related to Figure 1

Pearson correlation of log₂ LFQ values in each PRISMA spot (114 spots). Replicate 1 is plotted against replicate 2. Annotation bars indicates conserved regions of CEBPA.

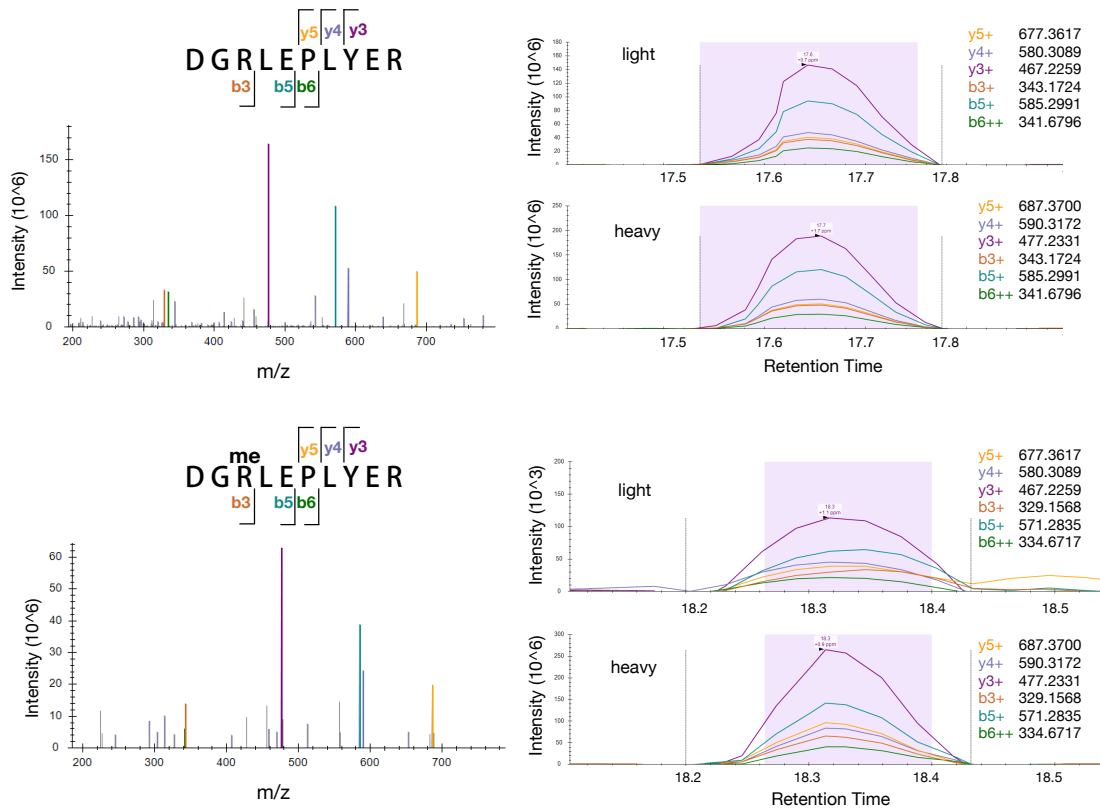


Figure S2: CEBPA is methylated at R142, Related to Figure 1 and 3

Parallel reaction monitoring (PRM) of unmodified (top) and methylated (bottom) peptides spanning R142. A heavy peptide standard isotopically labeled at the C-terminal arginine was used to confirm the identity of the peptide. Left: MS2 spectrum of heavy peptide standard; right: elution profile of MS2 fragments of light and heavy peptides. PRM data was analyzed with Skyline

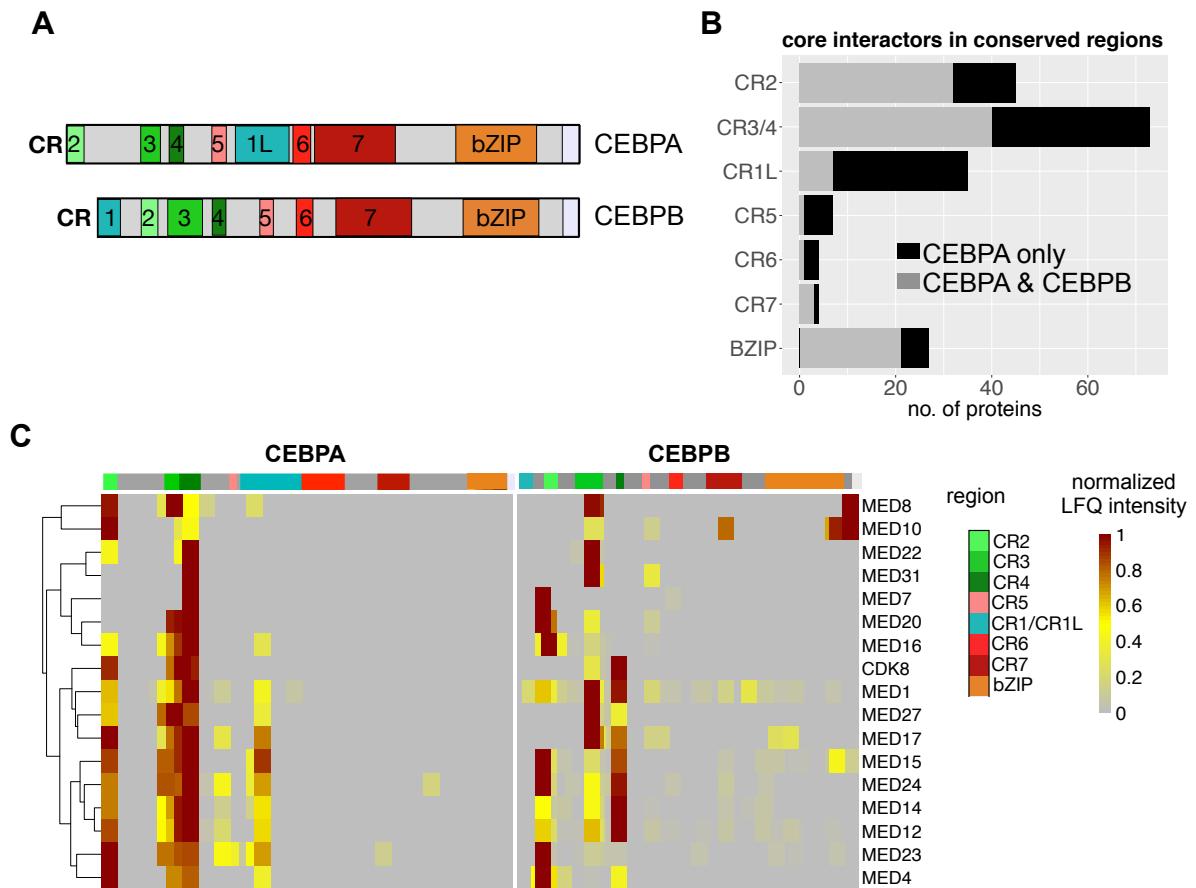


Figure S3: CEBPA and CEBPB share interactors in homologous regions, Related to Figure 2

A: Shared homology of conserved regions in CEBPA and CEBPB. B: Number of high-confidence CEBPA interactors per conserved region in CEBPA as detected by PRISMA and BioID (black bars). Grey bars represent interactors that were also identified as interactors in homologues regions in CEBPB (Dittmar et al., 2019). C: Extracted PRISMA binding profiles of Mediator complex subunits binding to CEBPA and CEBPB (right). Annotation bar on top indicates conserved regions

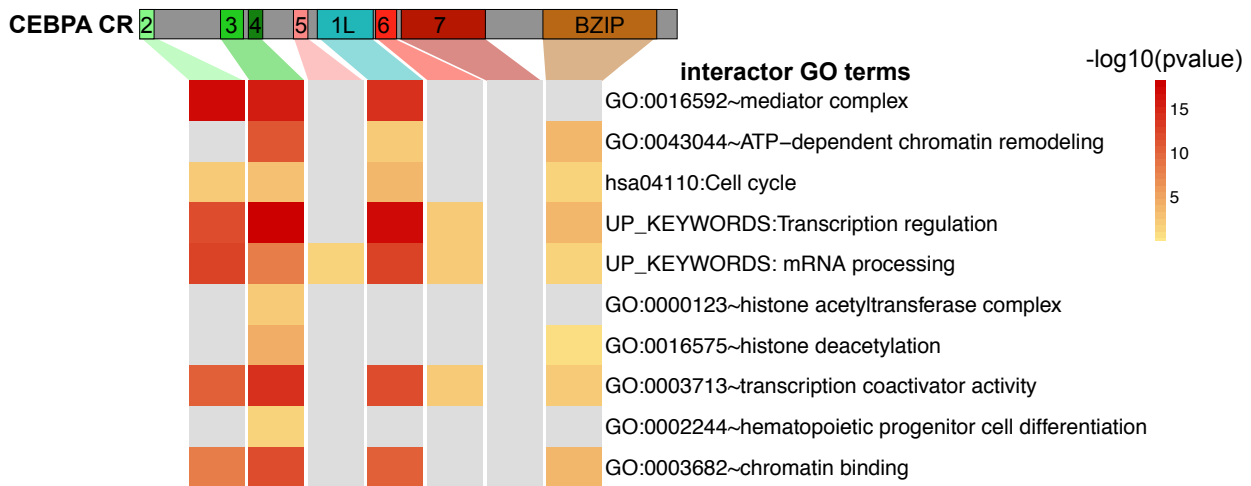


Figure S4: GO term enrichment of mapped CEBPA interactors, Related to Figure 2

PRISMA CEBPA interactors confirmed by BioID were subjected to GO term and domain analysis using the DAVID tool. Informative significant GO terms (p value < 0.05) are displayed. Grey indicates no significant enrichment. No GO terms were significantly enriched in CR7 binders. A complete list of all significant GO terms and domains is available in Table S5.

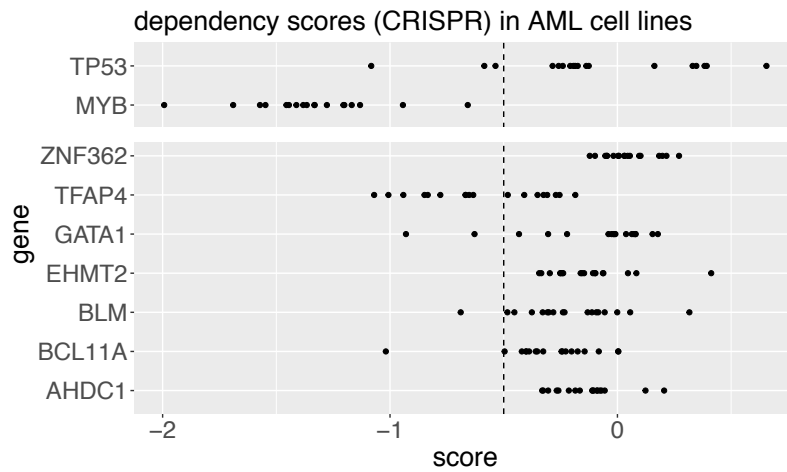


Figure S5: CEBPA P30 specific interactors may represent therapeutic targets in AML, related to Figure 4

Dependency scores from CRISPR knockout screens in AML cell lines extracted from the DepMap portal. Scores of P30 specific interactors are displayed. Known tumor-suppressor P53 and oncogene MYB are plotted on top as a reference.