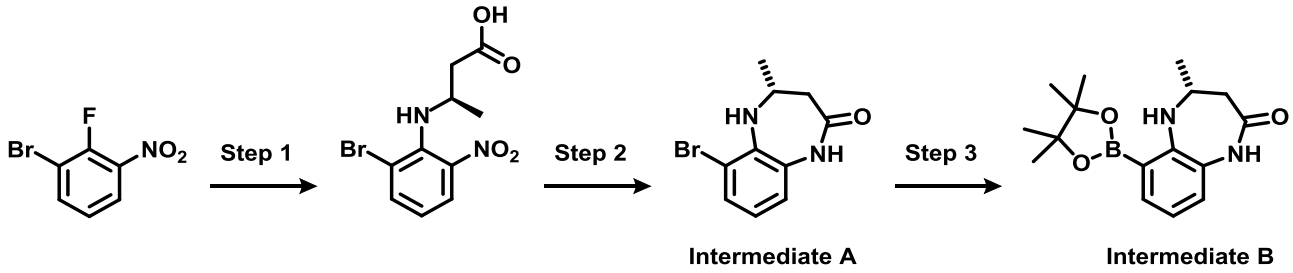
SUPPLEMENTAL DATA

EXPERIMENTAL PROCEDURES

Chemical Synthesis.

CPI703, CPI644, CPI644-(-), and CPI571 were prepared from **Intermediate A** and **Intermediate B**, which were synthesized according to the following scheme:

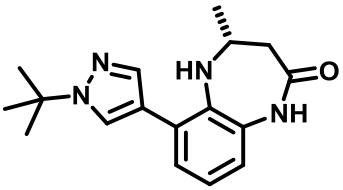


Step 1: To a solution of 1-bromo-2-fluoro-3-nitrobenzene (3.0 g, 13.64 mmol) in dimethylformamide (50 mL), N-ethyl-N-isopropylpropan-2-amine (5.3 g, 40.91 mmol) and (*R*)-3-aminobutanoic acid (1.7 g, 16.36 mmol) were added. The resulting mixture was heated to 80 °C for 10 h. After cooling the reaction mixture to room temperature, water (30 mL) was added, and the mixture was acidified with HCl (1N) to pH 6 and then extracted with ethyl acetate three times. The combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo to give (*R*)-3-((2-bromo-6-nitrophenyl)amino)butanoic acid (3.7 g, 90%) as a yellow solid.

Step 2: To a solution of (*R*)-3-((2-bromo-6-nitrophenyl)amino)butanoic acid (7.5 g, 24.74 mmol) in acetic acid (50 mL) was added iron powder (7.0 g, 0.125 mol). The mixture was heated to 100 °C for 1 h. After cooling the reaction to room temperature, the reaction mixture was filtered, and the filtrate was concentrated in vacuo. Water (30 mL) was added and the mixture was extracted three times with ethyl acetate. The combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified by silica gel chromatography (eluting with a gradient of petroleum ether/ ethyl acetate) to give (*R*)-6-bromo-4-methyl-4,5-dihydro-1H-benzo[b][1,4]diazepin-2(3H)-one, **Intermediate A**, (3.2 g, 51%) as a white solid. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 9.63 (s, 1H), 7.26 (d, J = 8.0 Hz, 1H), 6.87 (d, J = 7.6 Hz, 1H), 6.71 – 6.67 (m, 1H), 4.58 (s, 1H), 3.98 – 3.97 (m, 1H), 2.40 – 2.41 (m, 1H), 2.21 – 2.18 (m, 1H), 1.20 (d, J = 6.0 Hz, 3H).

Step 3: To a solution of (*R*)-6-bromo-4-methyl-4,5-dihydro-1H-benzo[b][1,4]diazepin-2(3H)-one (1.6 g, 6.27 mmol) in dioxane (25 mL) was added 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bis(1,3,2-dioxaborolane) (2.3 g, 9.41 mmol), potassium acetate (1.8 g, 18.82 mmol) and [1,1′-bis(diphenylphosphino)ferrocene] dichloropalladium(II) (0.5 g, 0.68 mmol). The mixture was heated to 100 °C for 16 h under a nitrogen atmosphere. After cooling the reaction to room temperature, the mixture was filtered and concentrated in vacuo to give (*R*)-4-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,4,5-tetrahydro-2H-benzo[b][1,4]diazepin-2-one, **Intermediate B**, (1.8 g, crude) as a brown solid that was used without further purification.

CPI703 (*R*)-6-(1-(tert-butyl)-1H-pyrazol-4-yl)-4-methyl-1,3,4,5-tetrahydro-2H-benzo[b][1,4]diazepin-2-one



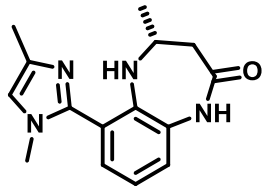
A disposable tube was charged with (**Intermediate A**) (25.5 mg, 0.1 mmol), methanesulfonato(2-dicyclohexylphosphino-2',4',6'-tri-i-propyl-1,1'-biphenyl)(2'-amino-1,1'-biphenyl-2-yl)palladium(II)

(8.46 mg, 10.00 µmol), 1-tert-butyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (50.0 mg, 0.200 mmol), and a stirbar before being evacuated and purged three times with nitrogen. Acetonitrile (1 mL) and sodium hydroxide (1N, aqueous) (200 µL, 0.200 mmol) were added. After stirring at 90 °C for 2 h, the mixture was concentrated with Celite before purification by silica gel chromatography (eluting with a gradient of hexanes/ ethyl acetate). Lyophilization from dioxane provided the title compound as a white amorphous solid. Yield not determined.

LRMS M/Z $(M+H)^+$: 299

^{1}H NMR: (400 MHz, DMSO-d_6) δ 9.50 (s, 1H), 8.00 (s, 1H), 7.60 (s, 1H), 7.00 (dd, *J* = 6.1, 3.0 Hz, 1H), 6.78 - 6.88 (m, 2H), 4.03 (d, *J* = 2.7 Hz, 1H), 3.92 - 4.00 (m, 1H), 2.53 (d, *J* = 4.9 Hz, 1H), 2.20 (d, *J* = 6.0 Hz, 1H), 1.51 - 1.61 (m, 9H), 1.17 (d, *J* = 6.2 Hz, 3H).

CPI571 (*R*)-6-(1,4-dimethyl-1H-imidazol-2-yl)-4-methyl-1,3,4,5-tetrahydro-2H-benzo[b][1,4]diazepin-2-one

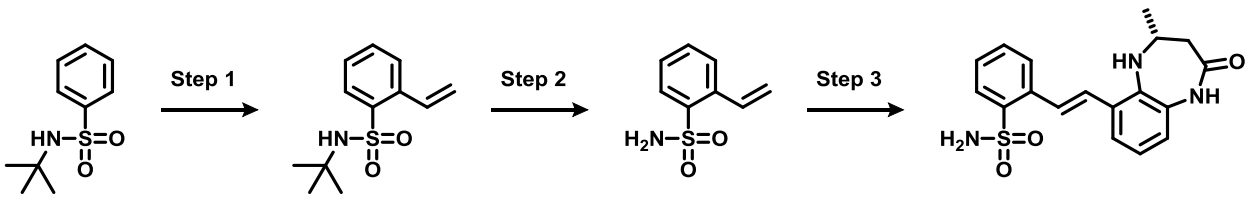


A mixture of (*R*)-4-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-4,5- dihydro-1H-benzo[b][1,4]diazepin-2(3H)-one (100 mg, 0.33 mmol), 2-bromo- 1,4-dimethyl-1H-imidazole (87 mg, 0.50 mmol), cesium carbonate (323 mg, 0.99 mmol) and Pd(dppf)Cl_2 (24 mg, 0.03 mmol) in 5:1 dioxane:water (3 mL) was stirred in a microwave reactor at 110 °C for 30 min. The resulting mixture was filtered and concentrated in vacuo to yield a residue that was purified by HPLC (eluting with a gradient of acetonitrile/ ammonium hydroxide) to give the title product (30 mg, 34%) as a white solid.

LRMS M/Z $(M+H)^+$: 271

^{1}H NMR: (400 MHz, CD_3OD) δ 7.13 – 7.08 (m, 2H), 7.02 – 6.95 (m, 2H), 4.01 – 3.97 (m, 1H), 3.58 (s, 3H), 2.61 – 2.56 (m, 1H), 2.34 – 2.31 (m, 1H), 2.24 (s, 3H), 1.10 (d, J = 6.0 Hz, 3H).

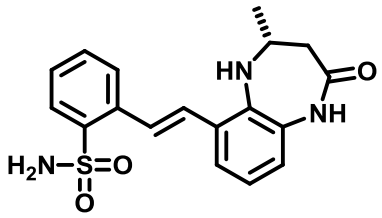
CPI644 was prepared using Intermediate A according to the following scheme:



Step 1: A disposable tube was charged with N-(tert-butyl)benzenesulfonamide (3.1994 g, 15 mmol) and a stirbar before being evacuated and purged with nitrogen three times. Tetrahydrofuran (60 mL) was added, and the solution was cooled to 0 °C for the dropwise addition of butyllithium (15 mL, 37.5 mmol). The reaction was stirred at 0 °C for 15 min before the dropwise addition of N,N-dimethylformamide (3.4843 mL, 45 mmol). It was further stirred at 0 °C 15 min before the addition of methyltriphenylphosphonium bromide (6.4303 g, 18 mmol). Again, the reaction was stirred at 0 °C for 15 min before the addition of sodium 2-methylpropan-2-olate (2.1623 g, 22.5 mmol). The mixture was warmed to room temperature and stirred for 30 min. This reaction was conducted in duplicate, and the batches were combined for workup and purification. The reaction was quenched into water, extracted three times with ethyl acetate, and concentrated in vacuo with Celite. Purification by silica gel chromatography (eluting with a gradient of hexanes/ ethyl acetate) yielded N-(tert-butyl)-2-vinylbenzenesulfonamide as a white amorphous solid (3.18 g, 44% per reaction). LRMS M/Z $(M+H)^+$: 240

Step 2: A disposable tube was charged with N-(tert-butyl)-2-vinylbenzenesulfonamide (3.88 g, 16.2 mmol) and a stirbar. Dichloromethane (50 mL) was added, followed by 2,2,2-trifluoroacetic acid (6.2073 mL, 81.1 mmol). The mixture was stirred at 50 °C for 24 h before being concentrated in vacuo with Celite. Purification by silica gel chromatography (eluting with a gradient of hexanes/ ethyl acetate) yielded 2-vinylbenzenesulfonamide (1.78 g, 60%). LRMS M/Z $(M+H)^+$: 184

Step 4: (*R*,*E*)-2-(2-(4-methyl-2-oxo-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepin-6-yl)vinyl)benzenesulfonamide (CPI644)

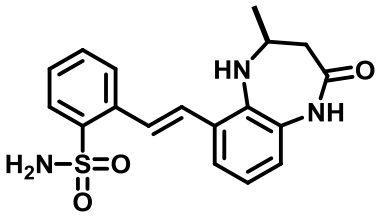


A disposable tube was charged with (**Intermediate A**) (0.5102 g, 2.0 mmol), 2-vinylbenzenesulfonamide (0.4397 g, 2.4 mmol), (0.0652 g, 0.1 mmol), tetrabutylammonium chloride (0.0556 g, 0.2 mmol), and a stirbar before being evacuated and purged with nitrogen three times. Triethylamine (0.4092 mL, 3 mmol) was added, followed by dimethylacetamide (10 mL), and the mixture was stirred at 80 °C 18 h. The reaction was run in triplicate and combined for purification. The mixture was diluted with ethyl acetate, washed three times with brine, and concentrated in vacuo with Celite. Purification twice by silica gel chromatography (eluting with a gradient of hexanes/ ethyl acetate) yielded a solid that was made into a slurry with ethyl acetate, filtered, and washed twice with ethyl acetate to yield the title compound as a yellow/ orange solid (1.58 g).

LC/MS M/Z $(M+H)^+$: 358

^{1}H NMR: (400 MHz, DMSO-d_6) δ 9.49 (s, 1H), 8.08 (d, J = 7.80 Hz, 1H), 7.91 (d, J = 7.80 Hz, 1H), 7.78 (d, J = 15.83 Hz, 1H), 7.63 (t, J = 7.47 Hz, 1H), 7.54 (s, 2H), 7.39 - 7.51 (m, 3H), 6.84 - 6.94 (m, 2H), 5.09 (s, 1H), 3.98 - 4.11 (m, 1H), 2.44 (dd, J = 4.79, 12.82 Hz, 1H), 2.14 (dd, J = 7.13, 12.93 Hz, 1H), 1.26 (d, J = 6.02 Hz, 3H).

CPI644-(-) [(*S*,*E*)-2-(2-(4-methyl-2-oxo-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepin-6-yl)vinyl)benzenesulfonamide] was prepared using the same procedures used for CPI644 but used the enantiomer of **Intermediate A**, (*S*)-6-bromo-4-methyl-4,5-dihydro-1H-benzo[b][1,4]diazepin-2(3H)-one, which was prepared from (*S*)-3-aminobutanoic acid.



LC/MS M/Z $(M+H)^+$: 358

^{1}H NMR: (400 MHz, DMSO-d_6) δ 9.49 (s, 1H), 8.08 (d, J = 7.80 Hz, 1H), 7.91 (d, J = 7.80 Hz, 1H), 7.78 (d, J = 15.83 Hz, 1H), 7.62 (t, J = 7.47 Hz, 1H), 7.54 (s, 2H), 7.38 - 7.51 (m, 3H), 6.86 - 6.94 (m, 2H), 5.09 (s, 1H), 4.05 (d, J = 6.02 Hz, 1H), 2.44 (dd, J = 4.91, 12.93 Hz, 1H), 2.13 (dd, J = 7.25, 12.82 Hz, 1H), 1.26 (d, J = 6.02 Hz, 3H).

ChIP-seq and RNA-seq data analysis. Reads from RNA-seq were mapped to the hg19 version of the human genome using TopHat v1.4.1 with parameters –p 2 --library-type fr-unstranded. The hg19 bowtie genome index was downloaded from ftp://ftp.ccb.jhu.edu/pub/data/bowtie_indexes/. Duplicate read pairs were removed prior to further processing. Cufflinks was run against reference transcriptome Homo_sapiens.GRCh37.73.chr.gtf, obtained from ftp://ftp.ensembl.org/pub/release-73/gtf/homo_sapiens/Homo_sapiens.GRCh37.73.gtf.gz, with parameters --no-effective-length-correction and --library-type fr-unstranded. Failed expression estimate attempts were set to NA and ignored for the rest of the analyses.

Processing of ChIP-seq data. Reads from ChIP-seq were mapped to the hg19 version of the human genome using BWA version 0.7.10. Duplicate reads were removed using samtools' sort and rmdup functions. IGVTools count was run with an extension of 100 and a window size of 25 bp to generate coverage signal in WIG format. The WIG files were scaled to a mean of 1.0 assuming an effective genome size of 2,700,000,000 bases. These normalized WIG files were used to assess average signal in the region (-500, 2000) bases around each transcript start site (TSS). SICER version 1.1 was run on each sample using default parameters. Overlaps of SICER intervals with genomic features were calculated with the help of BEDTools. All SICER intervals within 1000 bp of a transcript start site (TSS) were annotated as TSS, where transcript definition were taken from Ensembl (version GRCh37; http://grch37.ensembl.org/Homo_sapiens/Info/Annotation). To find regions of differential ChIP-seq occupancy genome-wide for the bars shown in figure 6e, we subtracted normalized WIG files, thresholded at -5 and 5, merged intervals within 1000 bases of each other, removed intervals overlapping regions of high background, and removed small intervals. Visualizations of signal along the genome were created using the Integrated Genomics Viewer.

Combined RNA-seq and ChIP-seq data. Figures are based on a combined dataset, CBP_Treg_combined_RNA-seq_ChIP-seq_summary, which contains 20,378 gene TSSs. The average ChIP-seq signal in the region (-500,2000) base pairs around each Ensembl protein-coding TSS is reported, along with the RNA-seq expression. The combined expression and ChIP-seq file was generated by joining the datasets based on the Ensembl ID. Both expression values and TSS interval mean signal are converted into log base 2 after adding 1.0 to the signal. The addition of 1.0 provides a regularizing effect, reducing the noise at the low end. Log fold changes are calculated by subtracting the regularized log2 control from treated values. The ChIP-seq and RNA-seq data are available at the Gene Expression Omnibus with accession nos. GSE70708 and GSE70620, respectively.

FIGURE LEGENDS

Supplementary Figure 1. shRNA-mediated knock-down of *CREBBP* or *EP300* blocks differentiation of human naïve T cells into Tregs. *A,* GFP expression marks transduced cells at day 4, with homogenous and high GFP levels by day 7 post-transduction. *B,* Cell lysates from day 7 transduced cultures were analyzed by western blot (left); the relevant band for either protein is indicated with an arrow. Intervening wells have been cropped out for clarity; all samples were obtained from the same experiment. The loading control, actin, was immunoblotted on the same gel and all slices represent the same blot exposure times. The gel image was digitally scanned, quantified and normalized to actin using Image J (right) (http://imagej.nih.gov/ij/).

Supplementary Figure 2. Visualization in IGV of ChIP-seq signal at *HAVCR2* and *IRF7* loci. Intervals of differential occupancy are shown beneath each pair of treated and control tracks.

TABLES

Supplementary Table 1. Effect of CPI644 in a panel of human kinases. The activity of the indicated kinases were tested in the presence of 1 μM CPI644. Values are represented as percent inhibition.

Kinase	**% inhibition**
Abl	0.9
ACVR1B	2.1
AKT1	2.3
Aurora_B	4.0
CaMKI_delta	5.1
CamKII_alpha	-5.2
CamKIV	-1.0
CDK1/cyclinB	3.1
CDK2/cyclinA	1.7
CDK9/cyclinT1	0.1
CHK1	4.1
CK1_alpha1	-1.2
CK2_alpha1	2.3
CLK1	0.9
CSK	5.0
DAPK1	-0.4
DNA-PK	3.3
DYRK1A	2.1
eEF-2K	2.4
EGFR	2.1
ERK2	3.8
FGFR1	-1.7
Flt3	2.9
GSK3_beta	-3.5
IKK_alpha	-7.1

IKK_beta	-1.0
JAK1	0.0
MAPKAPK2	-0.5
MRCK_alpha	2.3
mTOR	-4.8
NEK1	1.2
NEK6	4.5
p38_delta	5.8
p70S6K	1.8
PAK1	4.2
PDK1(direct)	-3.7
PI3K-A	5.4
PIM1	-0.4
PKA	0.4
PKC_alpha	0.9
PKC_beta1	3.9
PLK2	-0.3
PRKAA1	0.8
Rsk1	4.3
Src	4.4

Supplementary Table 2. shRNA constructs used for lentiviral knockdown of EP300 and CBP in human Tregs.

Gene name	RefSeq	GI#	shRNA name	CDS/UTR	TRCN	mRNA target
EP300	NM_001429.3	189011537	Csh_0001321	CDS	1700	CGGAAACAGTGGCACGAAGAT
EP300	NM_001429.3	189011537	Csh_0001331	CDS	2979	CGGAGGATATTTCAGAGTCTA
EP300	NM_001429.3	189011537	Csh_0001316	UTR	952	CACACACACACACACTTTCTA
CREBBP	NM_004380.2	119943103	Csh_0005501	CDS	3081	GAGCTTCCCAAGTTAAAGAAG
CREBBP	NM_004380.2	119943103	Csh_0005497	UTR	2404	CCTCTTTGGAGTCTGCATCCT
CREBBP	NM_004380.2	119943103	Csh_0001348	CDS	1217	GCCCATTGTGCATCTTCACGA

Supplementary Table 3. List of "Treg-specific genes" (>1.5 fold upregulated in Tregs relative to Th0) inhibited by 4 μM CPI703 (>1.5 fold downregulated upon inhibitor treatment relative to DMSO).

Gene.Symbol	Treg vs Th0 log FC	Treg vs Th0 P	CPI703 vs DMSO log FC	CPI703 vs DMSO P
APOD	1.398175	0.001526	-2.2249	0
CYP2A6	1.16728	0.008191	-1.91183	0.001307
SPRY1	1.013051	0	-1.74557	0
LAG3	0.633365	1.98E-07	-1.64308	0
RASGRP4	1.49655	0	-1.44488	0
CD80	1.396501	4.85E-07	-1.39983	0
LOC283174	1.036572	0.002821	-1.37531	7.59E-07
ANKRD37	0.799335	1.18E-08	-1.35813	0
FOXP3	1.255696	1.15E-11	-1.35658	0
CCL20	2.492866	0	-1.30623	6.56E-12
IL1RN	0.75978	0.020052	-1.27087	1.12E-10
MYO1B	0.671091	0.043032	-1.261	3.37E-07
CHN1	2.677797	0	-1.21887	5.49E-08
IL18RAP	0.713697	0.011831	-1.17326	1.47E-10
C7orf68	0.822096	0	-1.15217	2.05E-08
HSD11B1	0.756644	0.002544	-1.13219	0
BEND5	0.846809	0	-1.10948	0
NRN1	2.030601	0	-1.05672	0
FNBP1L	1.444745	2.00E-13	-1.04512	2.51E-12
PGM2L1	1.019143	0	-1.00095	0
CYB5R2	0.788083	2.22E-09	-0.99787	1.30E-07
LPL	1.750081	5.58E-06	-0.9799	0.001041
PICALM	0.931791	7.69E-11	-0.97349	0.002125

NCR3	0.835091	1.70E-06	-0.97055	0
THBS1	1.237364	0	-0.95702	1.42E-10
RHOB	0.608888	1.61E-06	-0.9397	0
RASGRP3	1.210036	0.000103	-0.93458	2.42E-06
SEMA4A	0.681558	8.17E-05	-0.91762	0.012165
EVI2A	1.326327	1.01E-07	-0.91035	0
DYNLT3	0.733814	0.019624	-0.86797	6.52E-11
BNIP3	0.750815	0	-0.86362	0.003375
ATP5F1	0.795216	0.000605	-0.83619	3.59E-05
RORC	0.854631	0	-0.82155	0
BTLA	0.769452	0.013751	-0.80926	0.001673
TRIM69	0.589379	0.046687	-0.80897	2.80E-14
GLIPR1	1.944902	5.31E-08	-0.80886	2.00E-15
RNF122	0.58761	0.009773	-0.80218	5.81E-10
CD109	0.620912	0.020499	-0.79272	0.00676
CCR4	2.063797	3.50E-13	-0.78808	0.002699
RBMS3	0.804399	0	-0.78477	0
CTLA4	0.842766	4.24E-12	-0.7774	0.000195
AK2	0.619553	0.011956	-0.77515	0.009312
ENOPH1	0.588887	0.041039	-0.76417	0.030507
KLF10	0.664394	3.07E-07	-0.75806	8.63E-09
LTA	0.83988	1.55E-15	-0.75736	1.37E-08
ARL4C	0.899675	0	-0.73405	0.000697
TIAM2	1.466763	0	-0.73095	0.001279
CAMSAP1L1	0.751998	0.002212	-0.72605	0.026612
BARX1	0.622982	0	-0.72169	0
MARCH3	0.994903	6.52E-07	-0.72118	0.000338
CD160	0.84096	0	-0.69666	0
LSP1	0.78166	3.92E-12	-0.68796	0.004779

KLF7	1.038687	2.07E-13	-0.68647	0.008768
GBE1	1.071585	4.76E-05	-0.68085	0.000174
PRKCD	0.64992	0.000221	-0.67651	0.000694
ANK1	0.610913	0	-0.67016	0
HCST	0.927053	0.001564	-0.66931	2.22E-16
WDR75	0.623511	0.007954	-0.66494	0.049981
AMICA1	0.746671	0.017305	-0.6531	0
UPRT	0.714784	0.000771	-0.64505	0.000843
PRSS23	0.981834	7.02E-12	-0.64366	4.79E-13
JUN	0.687877	2.13E-06	-0.64294	0.000566
LAIR2	1.964855	8.72E-06	-0.63417	4.26E-07
GOLGA8A	1.07965	3.16E-07	-0.62766	2.94E-05
SNX33	0.643847	0.003896	-0.6101	0.000146
CRIP1	1.178661	0.007337	-0.60977	0.003298
KDM3A	0.686965	0.000355	-0.60875	0.026511
EVI2B	0.77588	5.17E-10	-0.60656	0
EFNA4	0.630026	0.038964	-0.59248	0.004221

Supplementary Table 4. Data collection and refinement statistics for CBP bound to CPI098.

	CPI098
Data collection	
Space group	H3
Cell dimensions	
a, *b*, *c* (Å)	122.7,122.7,81.17
α, β, γ (°)	90,90,120
Resolution (Å)	25.25-1.65(1.74-1.65)
R_{sym} or R_{merge}	0.093(0.337)
$I / \sigma I$	7.3(3.4)
Completeness (%)	99.9(100.0)
Redundancy	4.7(4.6)
Refinement	
Resolution (Å)	25.25-1.65
No. reflections	52073
R_{work} / R_{free}	24.4/26.9
No. atoms	
Protein	3467
Ligand/ion	56
Water	343
B-factors	
Protein	20.65
Ligand/ion	17.23
Water	24.08
R.m.s. deviations	
Bond lengths (Å)	0.015
Bond angles (°)	1.716

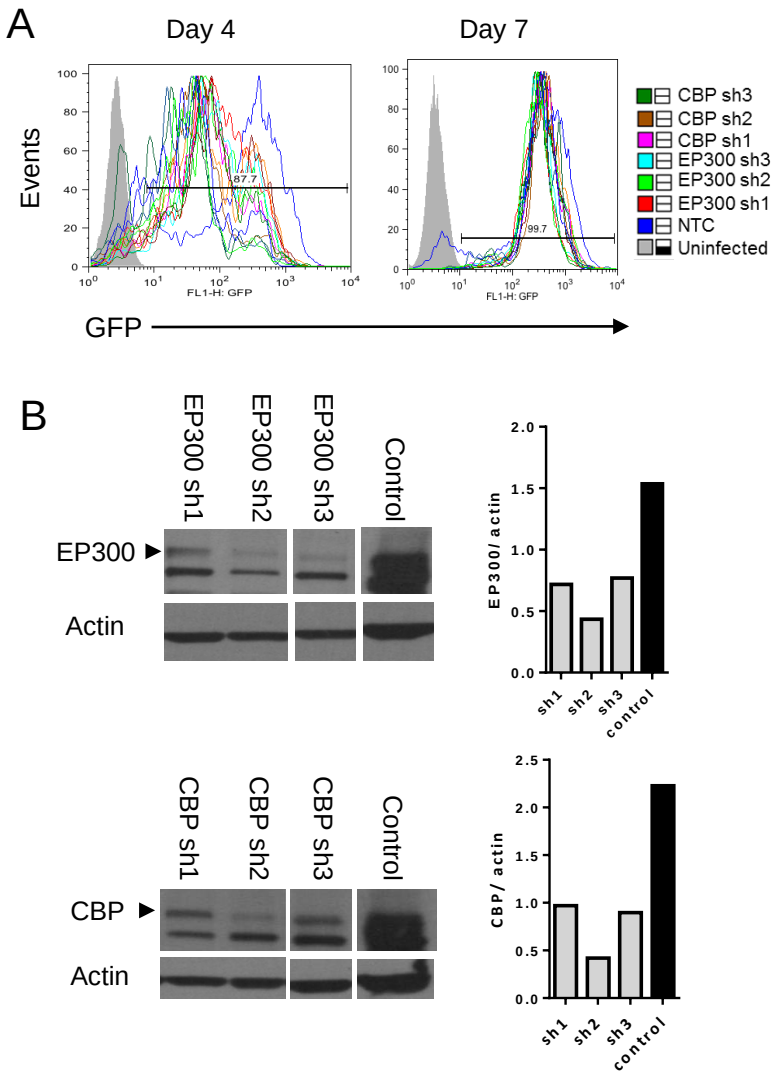
*Values in parentheses are for highest-resolution shell.

Supplementary Table 5. Data collection and refinement statistics for CBP bound to CPI703.

	CPI703
Data collection	
Space group	C2
Cell dimensions	
a, *b*, *c* (Å)	110.6,34.0,117.8
α, β, γ (°)	90,103.9,90
Resolution (Å)	50.00-1.86 (1.92-1.86)
R_{sym} or R_{merge}	0.094(0.680)
$I / \sigma I$	13.5(2.1)
Completeness (%)	99.7(99.0)
Redundancy	3.6(3.5)
Refinement	
Resolution (Å)	50.00-1.86
No. reflections	34687
R_{work} / R_{free}	17.2/22.0
No. atoms	
Protein	2912
Ligand/ion	66
Water	357
B-factors	
Protein	23.84
Ligand/ion	20.01
Water	28.95
R.m.s. deviations	
Bond lengths (Å)	0.021
Bond angles (°)	1.987

*Values in parentheses are for highest-resolution shell.

Supplementary Figure 1



Supplementary Figure 2