HDAC3 HDAC1 HDAC2 HDAC8 HDLP consensus	1 1 1 1 1	MAKTVAYFYD PDVGN MAQTQGTRRKVCYYYD GDVGN MAYSQGGKKVCYYDGDIGN MEEPEEPADSGQSLVPVYIYSPEYVS 	FHYGAGHPMKPHRLAL YYYGOGHPMKPHRIRM YYYGOGHPMKPHRIRM MCDSLAKIPKRASM YRYPKNHPLKIPRVSL *.	THSLVLHY THNLLLNY THNLLLNY VHSLIEAY LLRFLDAM	GLYKKMIVFKPYQA GLYRKMEIYRPHKA GLYRKMEIYRPHKA ALHKQMRIVKPKVA NLIDEKELIKSRPA .**	SQHDMCRE NAEEMTKY TAEEMTKY SMEEMATE TKEELLLE	HSEDYIDF HSDDYIKF HSDEYIKF HDDYLQH HTEDYINT **.
HDAC3 HDAC1 HDAC2 HDAC8 HDLP consensus	70 76 77 79 69 81	LQRVSPTNMQGFTKSLNAFN-VGDDC LRSIRPDNMSEYSKQMQRFN-VGEDC LRSIRPDNMSEYSKQMQRFN-VGEDC LQKVSQEGDDDHPD-SIEYG-LGYDC LMEAERCQCVPKGA-REKYNIGGYEN *	PVFPGLFEFCSRYTGA PVFDGLFEFCQLSTGG PVFDGLFEFCQLSTGG PATEGIFDYAAAIGGA PVSYAMFTGSSLATGS **	SLQGATQL SVASAVKL SVAGAVKI TITAAQCI TVQAIEEF 	NNKICDIAINWAGG NKQQTDIAVNWAGG NRQQTDMAVNWAGG IDGMCKVAINWSGG LKGNVAFNPAGG 	LHHAKK <mark>F</mark> E LHHAKKSE LHHAKKSE WHHAKKDE MHHAFKSR .***.*	ASGFCYVN ASGFCYVN ASGFCYVN ASGFCYIN ANGFCYIN *.****.*
HDAC3 HDAC1 HDAC2 HDAC8 HDLP consensus	149 155 156 157 146 161	DIVIGILELLK-YHPRVLYIDIDIHH DIVLAILELLK-YHORVLYIDIDIHH DIVLAILELLK-YHORVLYIDIDIHH DAVLGILRLRR-KFERILYVDIDIHH PAVGIEYLRKKGFKRILYIDIDAHH ***	GDGVOEAFYLTDRVMT GDGVEEAFYTTDRVMT GDGVEEAFYTTDRVMT GDGVEDAFSFTSKVMT CDGVOEAFYDTDOVFV .*****. **.	VSFHKYGN VSFHKYGE VSFHKYGE VSLHKFSP LSLHQSPE .*.*	YFFPG-TGDMYEVG Y-FPG-TGDLRDIG Y-FPG-TGDLRDIG GFFPG-TGDVSDVG YAFPFEKGFLEEIG . ****	AESGRYYC AGKGKYYA AGKGKYYA LGKGRYYS EGKGK <mark>G</mark> YN **	LNVPLRDG VNYPLRDG VNFPMRDG VNVPIQDG ILNIPLPKG .*.**
HDAC3 HDAC1 HDAC2 HDAC8 HDLP consensus	227 232 233 235 226 241	IDDQSYKHLFQPVINQVVDFYQPTCI IDDESYEAIFKPVMSKVMEMFQPSAV IDDESYGQIFKPIISKVMEMYQPSAV IQDEKYYQICESVLKEVYQAFNPKAV LNDNEFLFALEKSLEIVKEVFEPEVY	VLQCGADSLGCDRLGC VLQCGSDSLSGDRLGC VLQCGADSLSGDRLGC VLQLGADTIAGDPMCS LLQLGTDPLLEDYLSK .**.*.*	FNLSIRGH FNLTIKGH FNLTVKGH FNMTPVGI FNLSNVAF **	GECVEYVKSFNIPL AKCVEFVKSFNLPM AKCVEVVKTFNLPL GKCLKYILQWQLAT LKAFNIVREVFGEG	LVLGGGGY LMLGGGGY LMLGGGGY LILGGGGY VYLGGGGY	TVRNVARC TIRNVARC NLANTARC HPYALARA
HDAC3 HDAC1 HDAC2 HDAC8 HDLP consensus	307 312 313 315 306 321	WTYETSLLVEEAISECLPYSEYFEYF WTYETAVALDTEIPNELPYNDYFEYF WTYETAVALDCEIPNELPYNDYFEYF WTYLTGVILGKTLSSEIPDHEFFTAY WTLIWCELSGREVPEKLNNKAKELLK **	APDFTLHPDVSTR GPDFKLHISPS-N GPDFKLHISPS-N GPDYVLEITPSCR SIDFEEFDDEVDRSYM	IENQNSRQ MTNQNTNE MTNQNTPE PD-RNEPH LETLKDPW	YLDQIRQTIFENLK YLEKIKQRLFENLR YMEKIKQRLFENLR RIQQILNYIKGNLK RGGEVRKEVKDTLE	MLNHAPSV MLPHAPGV MLPHAPGV HVV KAKASS	C-terminus QIHDVPAD QMQAIPED QMQAIPED
HDAC3 HDAC1 HDAC2 HDAC8 HDLP consensus	384 388 389 401	LLTYDRTDEADAEERGPEENYS AIPEESGDEDEDDPDKRISICSS AVHEDSGDEDGEDPDKRISIRAS	RPEAPNEFYDGDHD DKRIACEEEFSDSEEE DKRIACDEEFSDSEDE 	NDK GEGGRKNS GEGGRRNV 	SNFKKAKRVKTEDE ADHKKGAKKARIEE	KEKDPE-E DKKETEDK	ESDVEI KKEVTEEE KTDVKEED
			_		HDAC domain	C-terminus	
HDAC3 HDAC1 HDAC2 HDAC8	464 466	KTKEEKPEAKGVKEEVKLA- KSKDNSGEKTDTKGTKSEQLSNP	В	HDAC3 HDAC1	100% 61.%	100% 22.4%	
consensus	481			HDAC2	61.1%	22,4%	
					00.0%		
				HDAC8	38.6%	l	
				HDLP	28.9%		

Fig. S1. Sequence alignment and homology comparison of Class I HDACs and HDLP. (**A**) Multiple sequence alignment of human Class I HDACs and HDLP. The location of the C-terminal regions of HDAC1, HDAC2 and HDAC3 is indicated by the right angle arrow. Identical amino acids in different HDACs are highlighted in dark blue. Non-identical but conserved amino acids are highlighted in light blue. (**B**) Comparison of sequence homologies of the HDAC domains and the C-terminal regions. The percentages of identical amino acids of HDAC3 with other Class I HDACs or HDLP in HDAC and C-terminal domains are shown. The alignment was performed using the Clustal Omega software (Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J, Thompson JD, Higgins DG. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol. 7:539, 2011).

Figure S1

Α



Fig. S2. Recombinant HDAC3, DAD and HDAC3/DAD complex. The images show Coomassie blue staining of recombinant HDAC3(1-428), HDAC3(1-411), HDAC3(1-391), HDAC3(1-370), HDAC3(1-428)-M1, HDAC3/GFP-DAD complex (Left) and His-DAD (Right). All proteins were purified from baculovirus-infected Sf9 cells.







Fig. S4. Free HDAC3 was treated with Caspase 7 for different times as indicated at the top of the gel image, followed by Western Blot analysis using the HDAC3 N-19 antibody.



Fig. S5. Removal of the C-terminus upon cleavage of free HDAC3 (1-428) by Caspase 7 via anti-FLAG depletion. In the lane marked with "+", free HDAC3 was cleaved with Caspase 7. After cleavage, the reaction mixture was incubated with the anti-FLAG M2 agarose followed by centrifugation to deplete the released C-terminal tail, which is FLAG-tagged. The supernatant was recovered and subject to dot blot analysis using the anti-FLAG antibody, along with other untreated BSA,100 ng HDAC3(1-428) and 10 ng HDAC3(1-428) samples indicated on the top.



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Fig. S6. (A) Trypsin cleaves the N-terminus at K83. N1 and C1 fragments were absent in the trypsin cleavage product of HDAC3-K83A/DAD. **(B) Partial trypsin digestion analyses of FL HDAC3(1-428), HDAC3(1-391) and HDAC3(1-370).** The top panels shows Western blot analysis using N-19 antibody. The bottom panel shows Western blot analysis using anti-FLAG antibody. The experiments were performed and analyzed as in Fig. 2C. The asterisks in the left top panel denote non-specific bands detected by N-19, which were not detected by the anti-FLAG antibody shown at the bottom.

Figure S6



Fig. S7. Wall-eye (or relaxed) stereo images showing that Phe 200 is within contact distance of TSA occupying the substrate pocket. The structure of HDAC3 (PDB# 4A69) was aligned to the structure of TSA/HDLP (PDB# 1C3R) using the UCSF Chimera software. Only the HDAC3 and TSA structures were shown in the figure. Dashed red-colored lines and distances indicate direct contact between the two carbon atoms of the aromatic ring of Phe 200 and C14 and C15 of the side chain of TSA, as detected by the UCSF Chimera software (Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. UCSF Chimera--a visualization system for exploratory research and analysis. J Comput Chem. 25(13):1605-12, 2004).



Fig. S8. Michaelis-Menten analysis of free HDAC3 in the absence and presence of TSA. The reaction was performed as described in the Experimental Procedures. The "cpm" refers to "counts per minute" of the radiolabeled substrate (³H-acetyl-histone) used in the assay. Due to unknown molar concentrations of individually acetylated lysines in the histone octamer, "cpm" was used as part of the unit of the substrate, $K_{\rm m}$ and $V_{\rm max}$. Non-linear fitting was performed to compare different models based on the Michaelis-Menten kinetics using the Graphpad Prism Program (Version 9.2.0). Compared to noncompetitive inhibition and uncompetitive inhibition, competitive inhibition was found to be the best model as indicated by the Goodness of Fit, and its results (± the standard error) and the 95% confidence interval (CI) were shown in the bottom panel. Michaelis-Menten analysis using only the DMSO data showed the same K_{m} and $V_{\rm max}$ as the competitive inhibition. Michaelis-Menten analysis using only the TSA data could not reach stable K_m and V_{max} values due to high apparent K_m (K_m^{app}) in the presence of TSA, which is estimated to be ~ 2152. HDAC3 was used at 40 ng. TSA was added at 10 µM.

Figure S8