Supporting Information for:

One Dalton can make a big difference: Crosstalk between H3R26 citrullination and H3K27 methylation regulates gene expression in ER-positive breast cancer

Kathleen W. Clancy,[1,2] Anna-Maria Russell,[1] Venkataraman Subramanian,[2] Hannah Nguyen,[1] Yuewei Qian,[1] Robert M. Campbell,[1] Paul R. Thompson[2,3*]

[1] Lilly Research Laboratories, Eli Lilly & Company, Indianapolis, IN, 46285

[2] Department of Biochemistry and Pharmacology, University of Massachusetts Medical School, Worcester, MA, 01605

[3] Program in Chemical Biology, UMass Medical School, 364 Plantation Street, Worcester, MA 01605, USA

[*]Author to whom correspondence should be addressed: Paul R. Thompson

paul.thompson@umassmed.edu

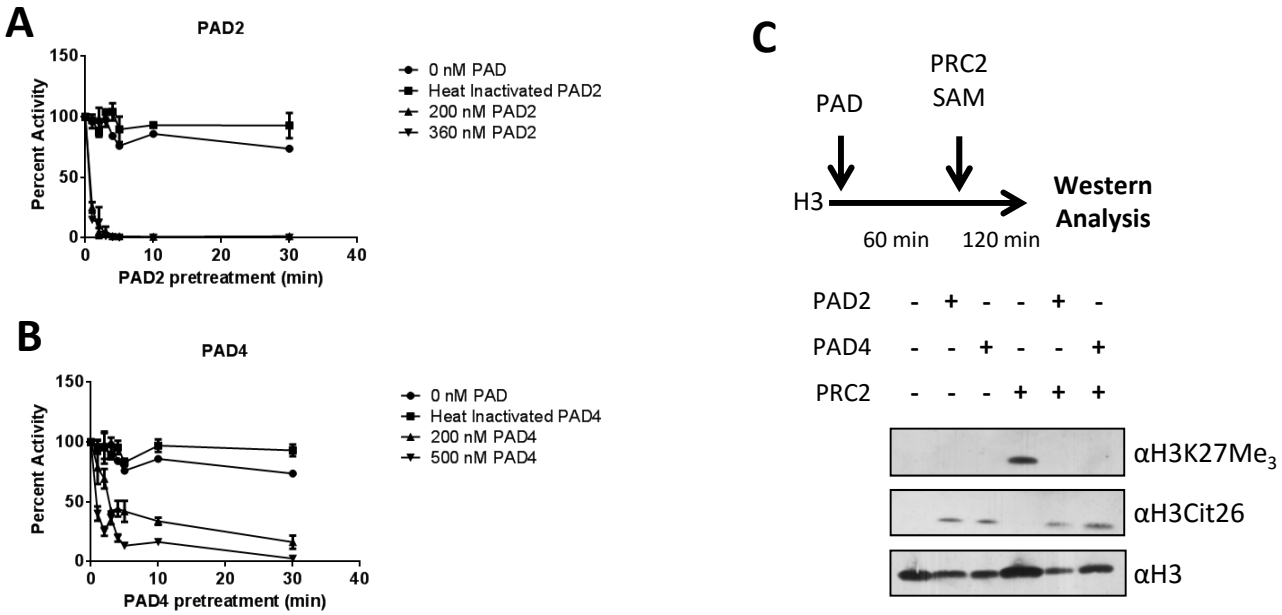

Figure S1: PRC2-catalyzed methylation of Histone H3 is inhibited by incubation with PAD2/4. (A, B) 50 nM PRC2 complex and 1 µM histone H3 were preincubated with varied concentrations of PAD2 (A) or PAD4 (B) before the addition of 15 uM SAM. The methyltransferase reaction was then run for 2 h and analyzed by a ^{14}C radiolabeled PAGE assay. (C) Recombinant histone H3 was tested for methylation of H3K27 by PRC2 complex post-deimination by PAD2 or PAD4.

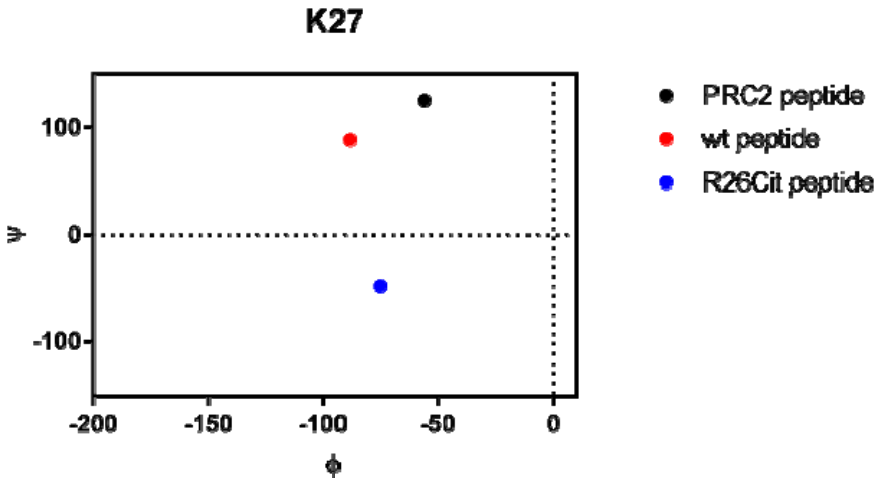

Figure S2: Ramachandran plot of K27 conformation from the peptide substrate in the PRC2 crystal structure (PDB ID 5HYN) with the lowest energy conformers from dynamics analysis of the wt and R26Cit peptides

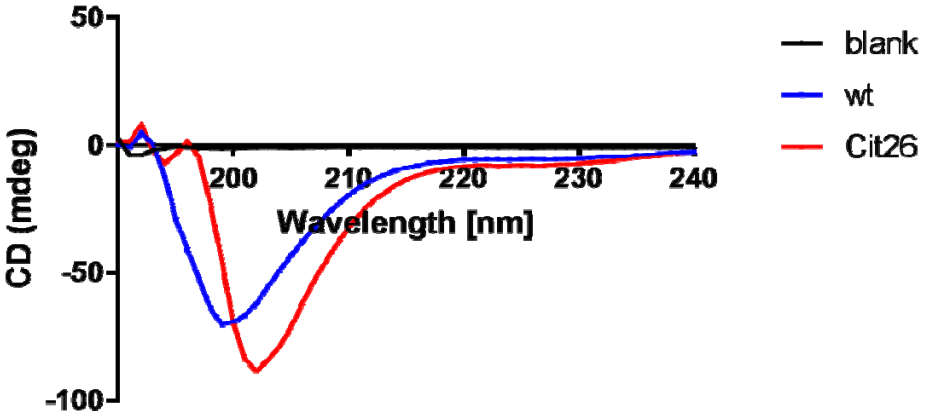

Figure S3: CD Analysis of H3 peptides indicate a stabilization and increased α-helical character in R26Cit peptide compared to wt sequence

Table S1: qPCR Primers (primer sequences can be found at ThermoFisher website)	
Gene	Cat No
cFos	Hs00231528
EGR3	Hs00231780
GDNF	Hs01931883
HSPB8	Hs00205056
KRT13	Hs00357961
LCN2	Hs01008571
MPP3	Hs01115495
PAD2	Hs00247108
PTGES	Hs01115610
RET	Hs01120030
STC2	Hs00175027
TERT	Hs00972650
TFF1	Hs00907239
GAPDH	Hs02758991

Table S2: Kinetic analysis of wt EZH2-catalyzed methylation of H3 substrates

Substrate	K_M (mM)	k_{cat} (min^{-1})	k_{cat}/K_M ($M^{-1}min^{-1}$)	Fold Change
H3	5.7×10^{-4}	1.5 ± 0.07	2.6×10^{6}	-
AcH321-39	0.58 ± 0.1	0.17 ± 0.01	2.9×10^{2}	1
AcH321-39 K27Me1	0.10 ± 0.01	4.2 ± 0.1	4.2×10^{4}	145
AcH321-39 K27Me2	ND	ND	4.1×10^{-1} [a]	1.4×10^{-3}
AcH321-39 K27Me3	ND	ND	BLD[b]	$< 1 \times 10^{-4}$
AcH321-39 K27M	ND	ND	BLD[b]	$< 1 \times 10^{-4}$
AcH321-39 Cit26	ND	ND	5.0×10^{-2} [a]	1.7×10^{-4}
AcH321-39 Cit26K27Me1	ND	ND	1.3×10^{0} [a]	4.5×10^{-3}
AcH321-39 Cit26K27Me2	ND	ND	1.4×10^{-1} [a]	4.8×10^{-4}

[a] The amount of product formation was too low to measure the kinetic parameters for this substrate. An estimation of k_{cat}/K_M was made by dividing the observed rate by the substrate concentration. [b] All readings fell below the limit of detection of the assay.

Table S3: Kinetic analysis of the EZH2(Y641N)-catalyzed methylation of H3 substrates

Substrate	K_M (mM)	k_{cat} (min^{-1})	k_{cat}/K_M ($M^{-1}min^{-1}$)	Fold Change
Histone H3	ND	ND	BLD[b]	< 0.001
AcH321-39	ND	ND	4.0×10^{-1} [a]	1
AcH321-39 K27Me1	0.2 ± 0.06	0.005 ± 0.0004	2.5×10^{1}	6.2
AcH321-39 K27Me2	0.34 ± 0.06	0.41 ± 0.02	1.2×10^{3}	3000
AcH321-39 K27Me3	ND	ND	BLD[b]	$< 1 \times 10^{-4}$
AcH321-39 K27M	ND	ND	BLD[b]	$< 1 \times 10^{-4}$
AcH321-39 Cit26	ND	ND	2.0×10^{-2} [a]	0.05
AcH321-39 Cit26K27Me1	ND	ND	9.0×10^{-2} [a]	0.22
AcH321-39 Cit26K27Me2	ND	ND	1.3×10^{-1} [a]	0.32

[a] The amount of product formation was too low to measure the kinetic parameters for this substrate. An estimation of k_{cat}/K_M was made by dividing the observed rate by the substrate concentration. [b] All readings fell below the limit of detection of the assay.

Table S4: Kinetic analysis of the PAD2-catalyzed deimination of H3 substrates

Substrate	k_{cat}/K_M ($M^{-1}min^{-1}$)	Fold Change
Histone H3	1.2×10^{3} [a, b]	-
AcH321-39	1.2×10^{2} [b]	1
AcH321-39 Cit26	BLD[c]	$< 1 \times 10^{-4}$
AcH321-39 K27Me1	1.3×10^{2} [b]	1.1
AcH321-39 K27Me2	6.0×10^{1} [b]	0.05
AcH321-39 K27Me3	3.6×10^{1} [b]	0.03
AcH321-39 K27M	1.5×10^{2} [b]	1.2

[a] Data from [4] [b]The amount of product formation was too low to measure the kinetic parameters for this substrate. An estimation of k_{cat}/K_M was made by dividing the observed rate by the substrate concentration. [c]All readings fell below the limit of detection of the assay.