Supporting Information for:

Autodeimination of Protein Arginine Deiminase 4 alters protein-protein interactions but not activity

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Supplemental Tables

Table S1: Primers for PAD4 AD substitution mutants

Substitution	Forward Primer
R123K	CTGTGCGCAGACATCACC**AAG**ACCGGCAAAGTGAAGCC
R123Q	CTGTGCGCAGACATCACC**CAG**ACCGGCAAAGTGAAGCCA
R156K	GCTGGTGAACTGTGAC**AAA**GACAATCTCGAATCTTCTGCC
R156Q	CCTGCTGGTGAACTGTGAC**CAA**GACAATCTCGAATCTTCTGCC
R205K	GGTGCTCCACGTGGCC**AAG**TCTGAGATGGAC
R205Q	GCTCCACGTGGCC**CAG**TCTGAGATGGAC
R372K	GGTCTTCGACTCTCCA**AAG**AACAGAGGCCTGAAGG
R372Q	GGTCTTCGACTCTCCA**CAG**AACAGAGGCCTGAAGG
R374K	CGACTCTCCAAGGAAC**AAA**GGCCTGAAGGAG
R374Q	CGACTCTCCAAGGAAC**CAA**GGCCTGAAGGAG
R419K	CCCCAGTCACAGTC**AAG**GGCAAGGAATACCC
R419Q	CCCCAGTCACAGTC**CAG**GGCAAGGAATACCC
R484K	GCCAGCACCCGAC**AAG**AAGGGCTTCCGGC
R484Q	GCCAGCACCCGAC**CAG**AAGGGCTTCCGGC

*Reverse primers were reverse complements to forward primers

Table S2 Steady-state kinetic parameters of PAD4 MCF-7 Cells

Sample	k_{cat} **(s^{-1})**	K_m **(mM)**	k_{cat}/K_m **($s^{-1}M^{-1}$)**
conPAD4, unstimulated	0.34 ± 0.026	0.027 ± 0.0060	13000
conPAD4, stimulated	0.66 ± 0.042	0.028 ± 0.0052	24000
adPAD4, unstimulated	0.40 ± 0.041	0.037 ± 0.0099	11000
adPAD4, stimulated	0.67 ± 0.028	0.027 ± 0.0034	25000

Recombinant conPAD4 or adPAD4 (2 μM) was incubated in the absence or presence of unstimulated or estradiol-stimulated MC7 cell extracts (2 μg) for 1 h at 37 ^{o}C.

Table S3 Citrullinated Arginines

Residue	Sequence	Predicted mass	Observed mass
R123[a]	106-ALLYLTAVEISLCADITRTGK-126	2308.2	2309.1
R609[a]	595-HLGIPKPFGPVINGR-609	1601.9	1602.8
R123[b]	106-ALLYLTAVEISLCADITR-123	2008.1	2011.1
R156[b]	137-TWTWGPCGQGAILLVNCDR-156	2204.0	2205.9
R205[b]	192-DFFTNHTLVLHVAR-205	1669.9	1671.8
R419[c]	395-GPQTGGISGLDSFGNLEVSPPVTVR-419	2483.8	2484.8
R484[b]	462-LYSDWLSVGHVDEFLSFVPAPDR-484	2649.3	2651.1
R639[b]	616-VCSLLEPLGLQCTFINDFFTYHIR-639	2943.3	2945.2

[a]Autodeimination sites determined *in vivo* from enriched endogenous PAD4. [b]Autodeimination sites determined *in vitro* from recombinant PAD4 reconstituted in ^{18}O-labeled H_2O.
[c]Autodeimination site determined *in vitro* from recombinant PAD4 in normal H_2O.

Table S4: Calcium dependence of PAD4 with HDAC1 1-90

	n	$K_{0.5}$ *(μM)*
wild-type	1.5 ± 0.19	500 ± 91
Wild-type + 1 equiv HDAC1 1-90	3.4 ± 0.05	620 ± 3.2
Wild-type + 2 equiv HDAC1 1-90	0.96 ± 0.41	190 ± 90
Wild-type + 3 equiv HDAC1 1-90	1.0 ± 0.14	600 ± 130

Values were obtained by incubating protein (0.2 μM) and BAEE (10 mM) over a range of calcium concentrations (0-10 mM) at 37 ^{o}C for 15 min.

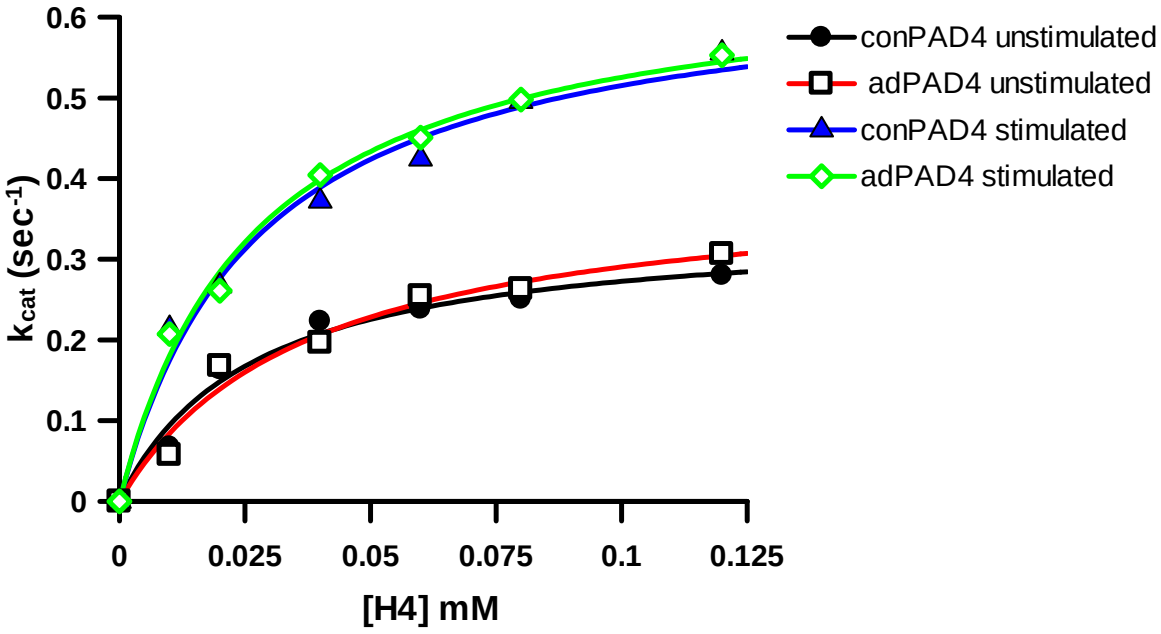

Figure S1: (A) Incubation of autodeiminated PAD4 in unstimulated and estradiol-stimulated MCF7 cell extracts. Recombinant PAD4 (2 μM) was incubated in the absence or presence of 10 mM calcium for 1 h at 37 ^{o}C to generate conPAD4 and adPAD4, respectively. These enzymes were then incubated with unstimulated or estradiol-stimulated MCF7 cell extracts (20 μg) for 30 min at 37 ^{o}C. The enzymatic activity of conPAD4/unstimulated (black, closed circles), adPAD4/unstimulated (red, open squares), conPAD4/stimulated (green, closed triangles), and adPAD4/stimulated (blue, open diamonds) was assayed using 0.2 μM of enzyme and a range of concentrations of histone H4.

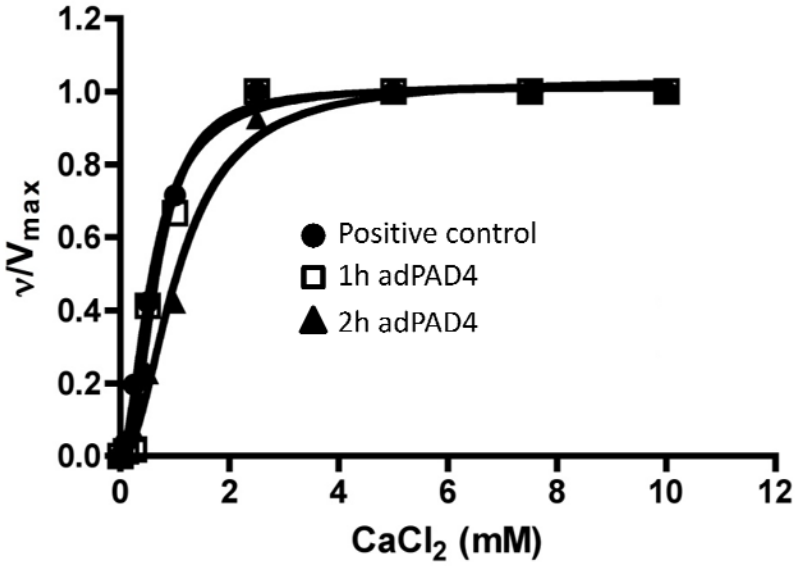

Figure S2: Calcium dependence of adPAD4. Recombinant PAD4 (2 μM) was incubated in the presence of 10 mM calcium for 1 h (open squares) or 2 h (closed triangle) to generate 1 h adPAD4 and 2 h adPAD4, respectively. Autodeiminated PAD4 was then dialyzed overnight to remove calcium, at which point the calcium dependence of 1 h adPAD4 and 2 h adPAD4 were determined using a range of calcium concentrations (0-10 mM) in comparison to non-incubated positive control (closed circles). BAEE was used as the substrate.

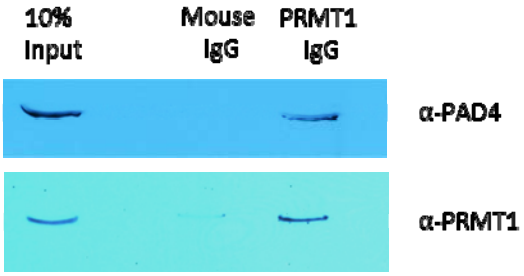

Figure S3: Immunopreciptation of PRMT1 from MCF-7 whole cell extracts (WCE). MCF-7 WCE were incubated with G-sepharose beads crosslinked to either an α-PRMT1 antibody (Abcam, ab7027) or α-mouse IgG. Eluted proteins were probed with either an α-PAD4 (Abcam, ab38772) antibody (*top panel*) or an α-PRMT1 antibody (*bottom panel*).