Supporting Information for:

Calcium Regulates the Nuclear Localization of Protein Arginine Deiminase 2

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Running title: Nuclear Localization of PAD2

Table S1: Materials

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-SOD4	Abcam	cat# ab137131
Anti-histone H3	Abcam	cat# 10799 RRID: AB_470239
Anti-myc	Sigma	cat# C3956; RRID: AB_439680
Goat anti-rabbit IgG Licor IRDye 800CW	Licor	cat# 925-32211; RRID:AB_2651127
Goat anti-rabbit IgG Licor IRDye 680RD	Licor	cat# 925-68071; RRID:AB_2721181
Streptavidin Licor IRDye 800CW	Licor	cat# 925-32230
Streptavidin Licor IRDye680RD	Licor	cat# 926-68079
Anti RAN	Proteintech	cat# 10469-1-AP
Anti ANXA5	Proteintech	cat# 66245-1-lg
Anti rabbit PAD2	Proteintech	cat# 12110-1-AP
Anti mouse PAD2	Proteintech	cat# 66386-1-lg
Chemicals, Peptides, and Recombinant Proteins		
Protein A/G plus agarose beads	Santa Cruz	cat# Sc-2003; RRID: AB_10201400
BAEE	Sigma	cat# B4500
L-citrulline	Sigma	cat# c7629
$CaCl_2$	Sigma	cat# c1016
Fetal bovine serum (FBS)	Sigma	cat# F4135-500ML
Formic acid	Sigma	cat# 06473
Ammonium bicarbonate	Sigma	cat# 09830-500G

L-arginine (R0)	Sigma	cat# A5006
L-lysine (K0)	Sigma	cat# L5501
Ionomycin	Millipore Sigma	cat# 407953
DMEM	Corning	cat# 10-013-cv
Trypsin	Corning	cat# 25-053-CI
Lipofectamine 2000	ThermoFisher	cat# 11668019
Protease inhibitor tablets	ThermoFisher	cat# A32963
[^{13}C/^{15}N]-L-arginine (R10)	Cambridge Isotope Laboratories	cat# CNLM 539
[^{13}C/^{15}N]-L-lysine (K8)	Cambridge Isotope Laboratories	cat# CNLM 291
Dialyzed FBS	Atlanta Biologicals	cat# S12850
Commercial Assays		
DC Protein Assay kit	BioRAD	cat# 5000111
Cell Lines		
HEK293T	ATCC	CRL-3216
HEK293	ATCC	CRL-1573
Oligonucleotides		
Primers for pETRan forward: GACACAGCCGGCCAGGAGAAATTCGGTGGACTG	IDT	
Primers for pETRan reverse: CAGTCCACCGAATTTCTCCTGGCCGGCTGTGTC	IDT	
Primers for pETRan T24N forward: ACTGGAAAAAACACCTTCGTGAAACG	IDT	
Primers for pETRan T24N reverse: ACGTTTCACGAAGGTGTTTTTTCCAGT	IDT	

Primers for BioID2PAD2 forward: GAGTCGAATTCAGGGCACCATGTGCCACCAC	IDT	
Primers for BioID2PAD2 reverse: GACGACGACCTCGAGATGCTGCGCGAG	IDT	
Recombinant DNA		
pET-Ran(Q69L)	Addgene	cat# 42048
myc-BioID2-MCS	Addgene	cat# 74223
pET16B PAD2	(Dreyton et al., 2014)	N/A

Table S3. PAD2 Calcium Dependence with the Substrate BAEE in presence of Ran and ANXA5.

	k_{cat} (s^{-1})	n	$K_{0.5}$ (mM)
PAD2	2.6 ± 0.05	3.1 ± 0.4	0.15 ± 0.01
PAD2 + ANXA5	2.3 ± 0.04	2.8 ± 0.2	0.16 ± 0.007
PAD2 + Ran	2.5 ± 0.1	3.0 ± 0.7	0.16 ± 0.02
PAD2 + Binding buffer[a]	5.0 ± 0.2	2.2 ± 0.39	0.22 ± 0.02
PAD2 + GDP	5.1 ± 0.05	3.5 ± 0.27	0.21 ± 0.005
PAD2 + GTP	4.3 ± 0.2	4.2 ± 2.0	0.20 ± 0.03
PAD2 + Ran Q69L + GTP	5.5 ± 0.1	3.3 ± 0.3	0.43 ± 0.01
PAD2 + Ran T24N + GDP	5.5 ± 0.04	6.9 ± 3.4	0.22 ± 001

[a] Binding buffer: 20 mm Tris-HCl pH 8.0, 50 mm NaCl, 2.5 mM $MgCl_2$, 0.5% NP-40, 1% BSA and 10% (v/v) glycerol). The experiment was carried out in duplicate. Data are represented as mean ± SEM.

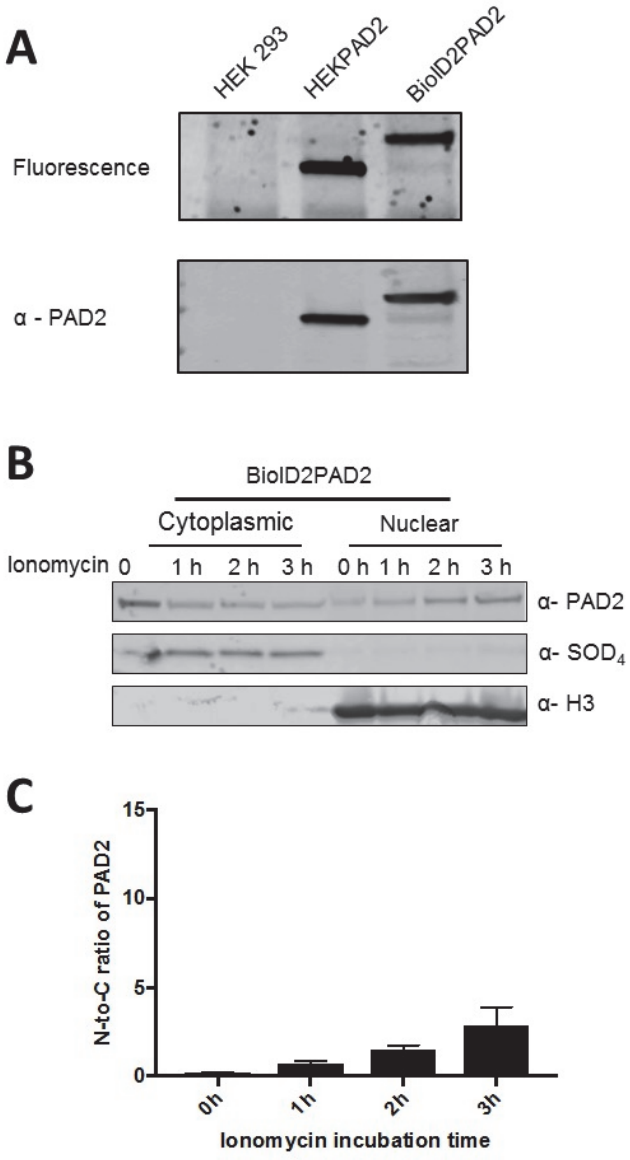

Figure S1. BioID2PAD2 functions similarly to wild type PAD2. (A) RIBFA labeling shows BioID2PAD2 has a comparative activity with wild type PAD2. Equal protein amount was confirmed by western blotting. (B) HEK293 BioID2PAD2 cellular proteins were separated by fractionation methods and examined by western blot. Cleanliness of fractionation was determined with antibodies for Histone H3 (nuclear) and SOD4 (cytoplasmic) protein. (C) The ratio of normalized density of BioID2PAD2 levels in nucleus over those in the cytoplasm is shown on the left of the corresponding western blots (n = 3).

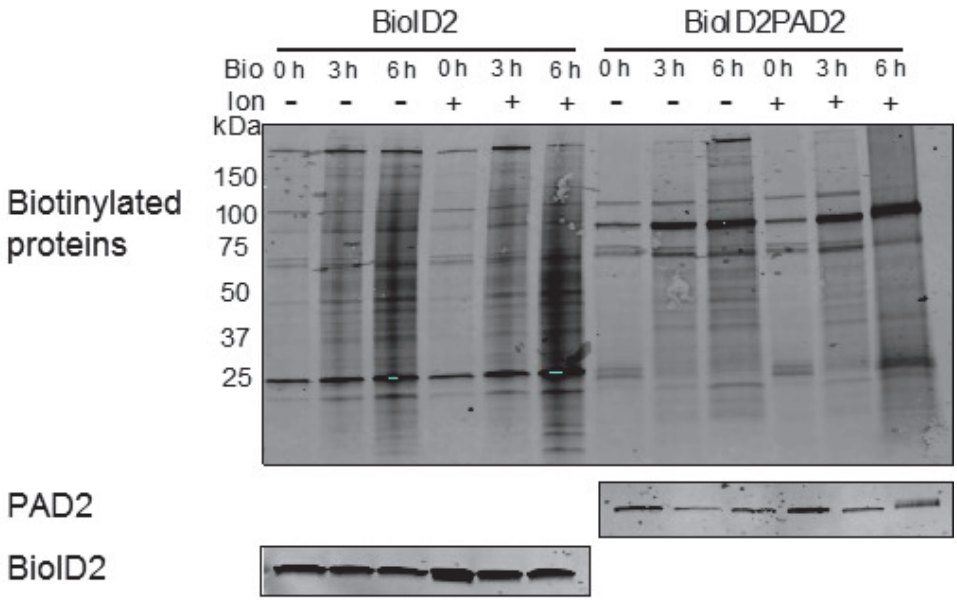

Figure S2. BioID2PAD2 biotinylates a distinct set of proteins in a time- and ionomycin-dependent manner. BioID2 and BioID2PAD2 expressing HEK293 cells were provided with 50 μM biotin for various times plus or minus ionomycin. Biotinylation levels were then assayed by Western blotting. The levels of PAD2 and BioID2 are similar for all conditions (lower panel), while the extent of biotinylation increases with duration of biotin supplementation.

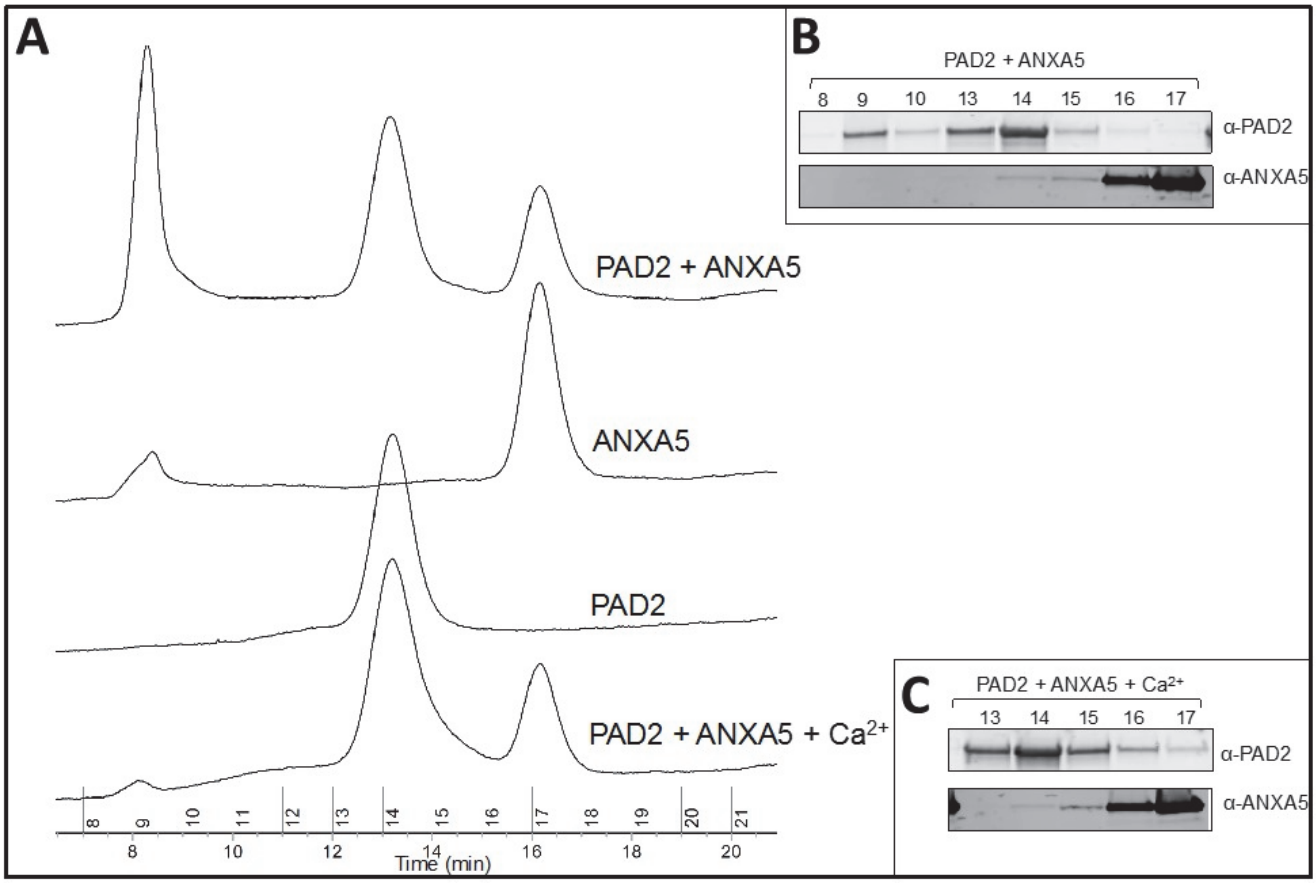

Figure S3. PAD2 quaternary structure changes upon interaction of ANXA5. (A) SEC spectra of PAD2/ANXA5 with or without calcium. Western blot shows proteins in individual fractions of each peak from top spectrum (B) and bottom spectrum (C).

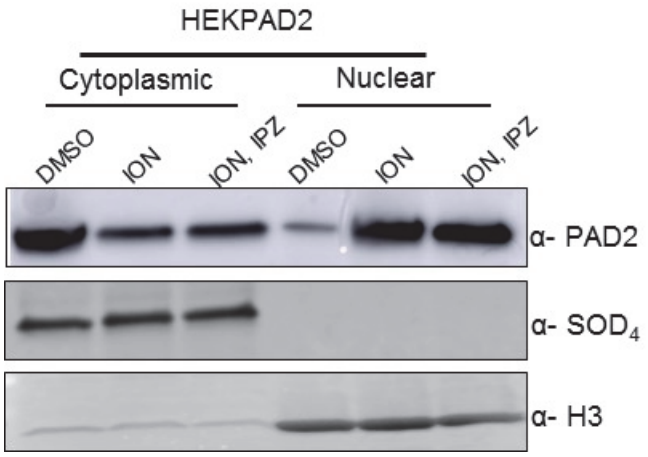

Figure S4. Importazole does not inhibit PAD2 nuclear import. Cellular fractionation of HEKPAD2 cells supplemented with DMSO, ionomycin (ION), or importazole (IPZ).