Supplementary Online Content

Aderibigbe OM, Priel DL, Lee C-CR, et al. Distinct cutaneous manifestations of *PLCG2*-associated antibody deficiency and immune dysregulation. *JAMA Dermatol.* Published online March 11, 2015. doi:10.1001/jamadermatol.2014.5641.

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods[1]

Mutational Analysis of PLCG2

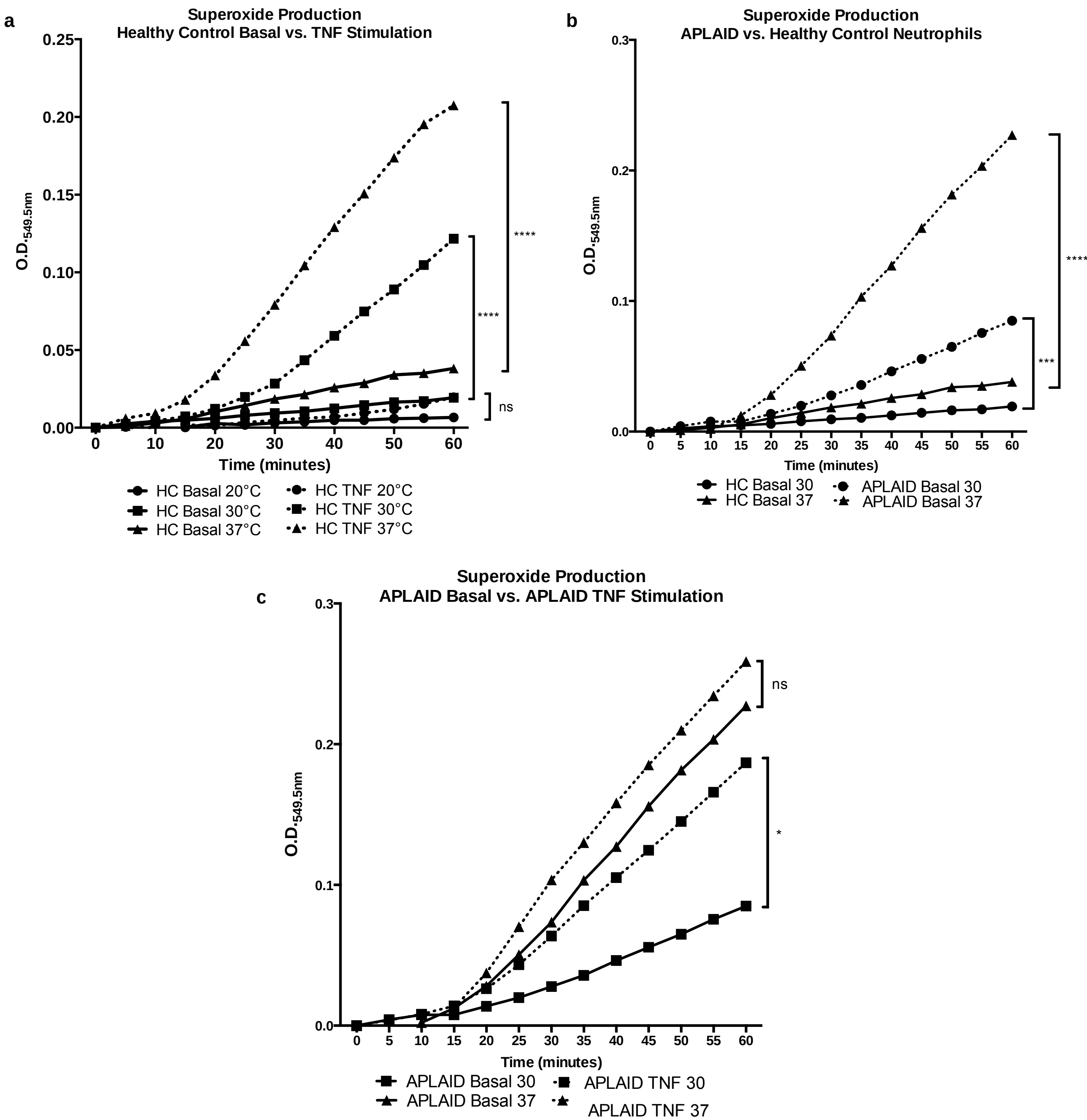
RNA was extracted from peripheral blood mononuclear cells using RNAeasy kits (Qiagen) and complementary DNA (cDNA) was generated by reverse transcription from 1 µg of RNA via SuperScript II Reverse Transcriptase kit (Invitrogen) with random hexamer primers. Overlapping segments of *PLCG2* cDNA were amplified by PCR and the products were directly sequenced using BigDye Terminator chemistry (v3.1, Applied Biosystems) and a capillary sequencer (3130xl Genetic Analyzer, Applied Biosystems). Coding exons and intron-exon junctions of *PLCG2* were amplified by PCR using 50 ng of genomic DNA template, 1 µM of target-specific oligonucleotides and 12.5 µL of AmpliTaq Gold Fast PCR Master Mix (Applied Biosystems) in a final volume of 25 µl. All PCR reactions were carried out according to the manufacturer's published recommendations with using 15 second extension times using the primer pairs listed in eTable 1.

eTable.[1] *PLCG2* PCR Conditions and Primers

Primer target	Forward Primer Sequence	Reverse Primer Sequence
cDNA seg 1	GCCAGCTTCCTGATTTCTCC	TTTAGAGTCAGCTGCCAAGC
cDNA seg 2	AAAGATTTCGAGCGAGCAAA	AGATGGAACTGTTCAAAGCTGA
cDNA seg 3	CATCTTGCCCCTGATCAACT	GTCCACCGCGTCATACTTCT
cDNA seg 4	GATGACACCATGCGTGAAAC	CACTGGGAAGCTCGAGGTAA
cDNA seg 5	ATGGGAAGCCGGTCATCTAC	GGGTATATCCTGGGGCACTT
cDNA seg 6	ATGGAGGACAAGAAGGACGA	AGTGCTGGATGAGGGCATAG
cDNA seg 7	CCCCAATGACTACACCCTGT	ACGAGCTCCACCAGACTCTC
cDNA seg 8	CGACTCCTATGCCATCACCT	GGATGGGAAGTACTGCTGGA
cDNA seg 9	AGCGATGAGCTGAGCTTCTG	CTTGGTTTTGCTGGTTGGTT
cDNA seg 10	GGTTTCAGAGCATCCGAGAG	GACTGTCAGCGTCATCAGGA
cDNA seg 11	GCAGATGAATCACGCATTGT	GCCGCATCTCACAGAAAAC
cDNA seg 12	CGATCCCAACTTTCTTGCTC	GATGGCAGGCTTGAAGAAAA
Exon 1	CGGGTTGCCTCAGTTTCTT	CTGAGCCAGGACGCAGAC
Exon 2	TGCCTTGCCACTAGAACCTT	TGACAAGACAGGAAGGTGGA
Exon 3	GATCCTGTTGGGGAAGGAAG	TCAAAACCATGACCCCAAAT
Exon 4	CAGCATAGGCTTCTCCCATC	GTAGAGCCCACACCGCTACT
Exon 5	TGCACATTCCGTAGGACTCA	CTCCCGACAAGTCATGCTC
Exon 6	GGAACTGATGGGAGCAGCTA	ACGGAACTCAAAGGCAAGC
Exon 7	GCATGCCATTTCTGAAACAA	TGGGTTTCTTAGGAACATGC
Exon 8	CCTTTTGCTTCTTAAGTTTCTGTT	ATCTCTATCCACGCCACAGG
Exon 9	TGGCCAACCTGGTACCTAAC	TCGTTCCATGAAGACAGGTG
Exon 10	GTCTTCGATTGCGACTGGAT	CAGCAAAGTTCTCCAAGGAA
Exon 11	CGTGGGTAACTGAGGTCTGG	CTCCACCTGCTTCACAACAG
Exon 12	ACAACACCCTGAGGTGCAG	TCGAAAGAAAACATGGAGCA
Exon 13	TCTAGTAACTGAACTGGTGTGTGG	TTTGCCTGTCTGTCCATCTG
Exon 14	GCAGATGTGGGGGTTGTG	AGGACATAGAACTTGGTGAACG
Exon 15	GGGAAGAGGCAGATGAAGG	TGGGAACAAGACAGGCGTAT
Exon 16	AACTGGGAAAAGCACATCCA	CAGAGAAGCCACATGGGAAG
Exon 17	TGAGGCTGGCCTCTCTATGT	CCTGCCTGAGCTAGAACCAC
Exon 18	AGGAGCAGAGGGAAGGTTGT	ACCACCAGGCCAGCTTCT
Exon 19	TGGTGCCATTATCTTGTCCTC	GTGAGCCTCCACCTGAAGTT
Exon 20	CCCACTTCTCTAAGGCTGGA	TGCTGAATTCATGAACAGATGA
Exon 21	AAGGAAGCCTAGAACCCTTGTT	AAGTCTTCCCTTGCCCTGTT
Exon 22	GGCCTGACCTTTTCCTTCTT	GACAAAGGGGGTCAGACTTG
Exon 23	CATCAGAATTGAGCCAGCAG	GCAGCACATGGAAAAATTCA
Exon 24	AAACGGTGTGCTTTGGAAAC	AGCCACCTCCCTGTGTAGG
Exon 25	CCATGAGAGAAACAGCTCAGG	TCTGAATCCACCTGGTCTCC
Exon 26	TGTGCAAGAAAGCAAAGTGG	CTCTGCCCCCTCTGAAAATA
Exon 27	AATCTGAGCATCCAGCCATT	CACCACATGGTATCGCTGAC
Exon 28	TTGAACAGCTGCCTCACATT	CAAGACAACCAGCCTCCCTA
Exon 29	TCATCCAGTGTCACTCTAGAACC	CAAAATCCCCCACGAAGA
Exon 30	GGTTGCTAAGGGGATTCACA	CCACCCAATCGGTTTTACAG
Exon 31	CCTATGATCCCGAGGTAGCC	CATGCTTGGGCTAGACAATTC
Exon 32	CTTGTGAGATGCCAGGTTCA	CAACTCAGAGGGCTTTCCAG
Exon 33	TGGAGTCTGCCTCTCTGACA	TCATCCTCCATGGACATCAG

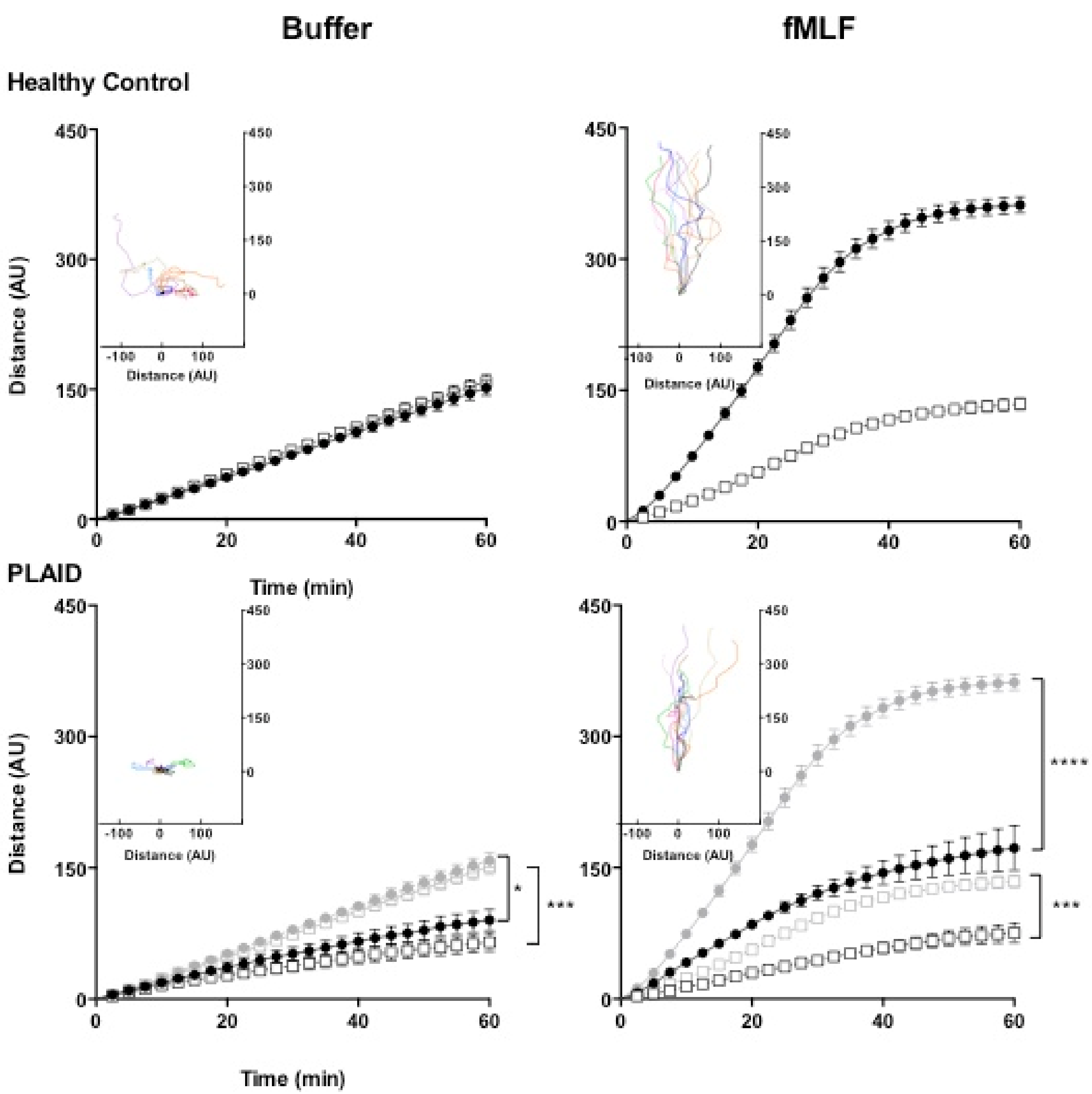
[1] Mutational analysis and PCR data previously described by Ombrello MJ, Remmers EF, Sun G, et al. Cold urticaria, immunodeficiency, and autoimmunity related to PLCG2 deletions. N Engl J Med 2012;366:330-8.

eFigure 1. Neutrophil Superoxide Production



Neutrophil superoxide production. (a) Healthy control neutrophil $O_2^{\bullet-}$ production in response to TNF-α. (b) APLAID neutrophil $O_2^{\bullet-}$ production compared to healthy controls. (c) APLAID neutrophil $O_2^{\bullet-}$ production in response to TNF-α. *$p<0.05$, ***$p<0.001$, ****$p<0.0001$, unpaired t-test.

eFigure 2. Neutrophil Chemotaxis in Presence of Buffer or fMLF



Line graphs represent resolved distance vectors (mean ± SEM) for random migration (open squares) and directed migration (closed circles) from healthy controls (n=32) and PLAID patients (n=6). In the lower graphs, response of healthy control neutrophils has been added in gray for comparison. Inset images represent tracks of 10 individual neutrophils from one patient. $^*p<0.05$, $^{***}p<0.001$, $^{****}p<0.0001$, unpaired t-test