Supplemental material

Ohayon et al., https://doi.org/10.1084/jem.20180371

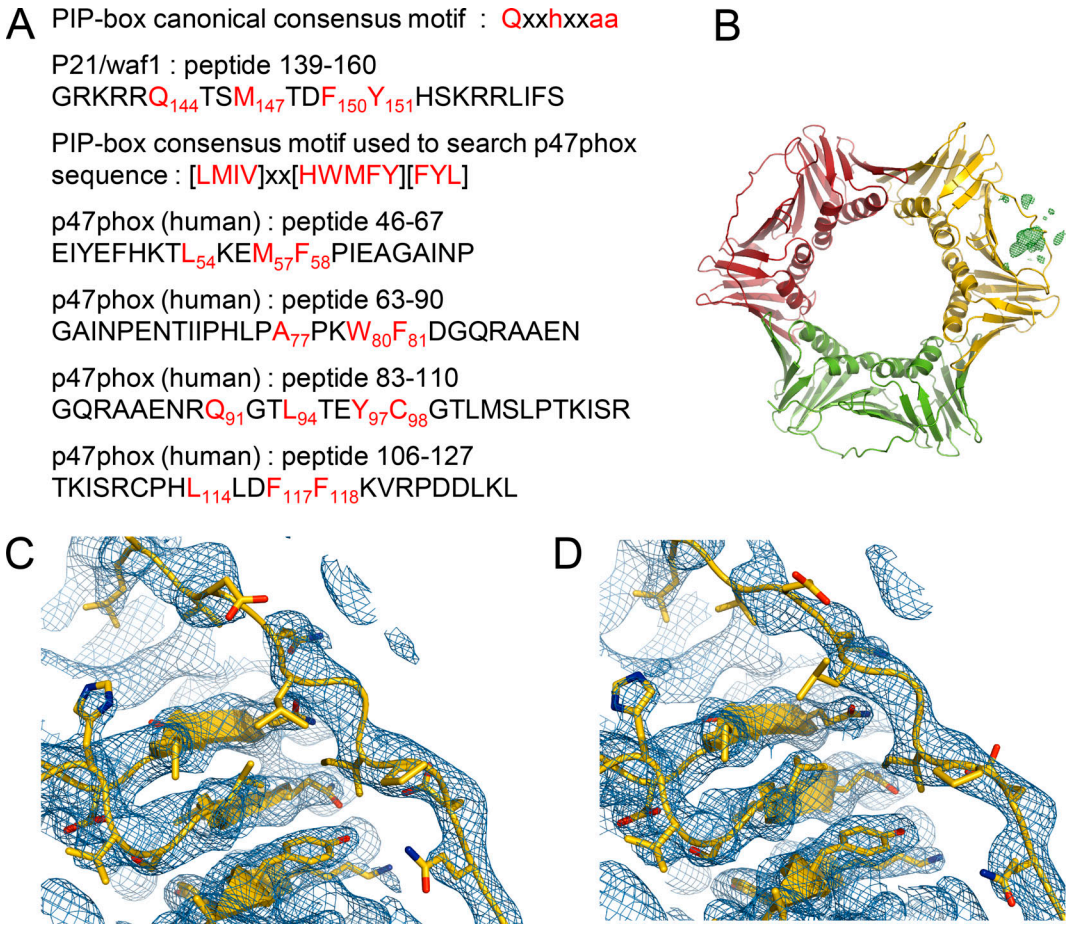

Figure S1. **Structural analysis of the association between PCNA and the p47phox peptides. (A)** Sequences of the putative PIP box on p47phox PX domain and corresponding peptides designed for SPR experiments. **(B–D)** Crystallographic data of PCNA associated with the p47phox peptide. **(B)** Schematic view of trimeric human PCNA refined at 3.23 Å, obtained with human PCNA crystals soaked in a 100-µM p47phox$_{106–127}$ peptide solution for 24 h before data collection. This experiment was performed three times with identical results. The three monomers are shown in green, yellow, and red. The main peak of the residual (F_{obs} − F_{calc}) electron density map, contoured at the +2.8 σ level, is shown in green. **(C)** ($2F_{obs}$ − F_{calc}) electron density map of the 3.23-Å-resolution structure (PDB entry code 6FCN), contoured at the +1 σ level, in the region of the interdomain-connecting loop. **(D)** ($2F_{obs}$ − F_{calc}) electron density map of the 2.80-Å-resolution structure (PDB entry code 6FCM), contoured at the +1 σ level, in the region of the interdomain-connecting loop. Of note, the analysis of the crystal packing shows that there are symmetry-related molecules in the vicinity of the interdomain-connecting loop. These symmetry mates may partially prevent peptide binding. Therefore, the soaking time has been adjusted to optimize peptide binding without affecting the crystal order. The 50 crystals tested differ in size, and also slightly in unit cell parameters (range of 37.3–40.0 Å for a axis, 138.4–140.9 Å for b axis, 164.8–172.1 Å for c axis).

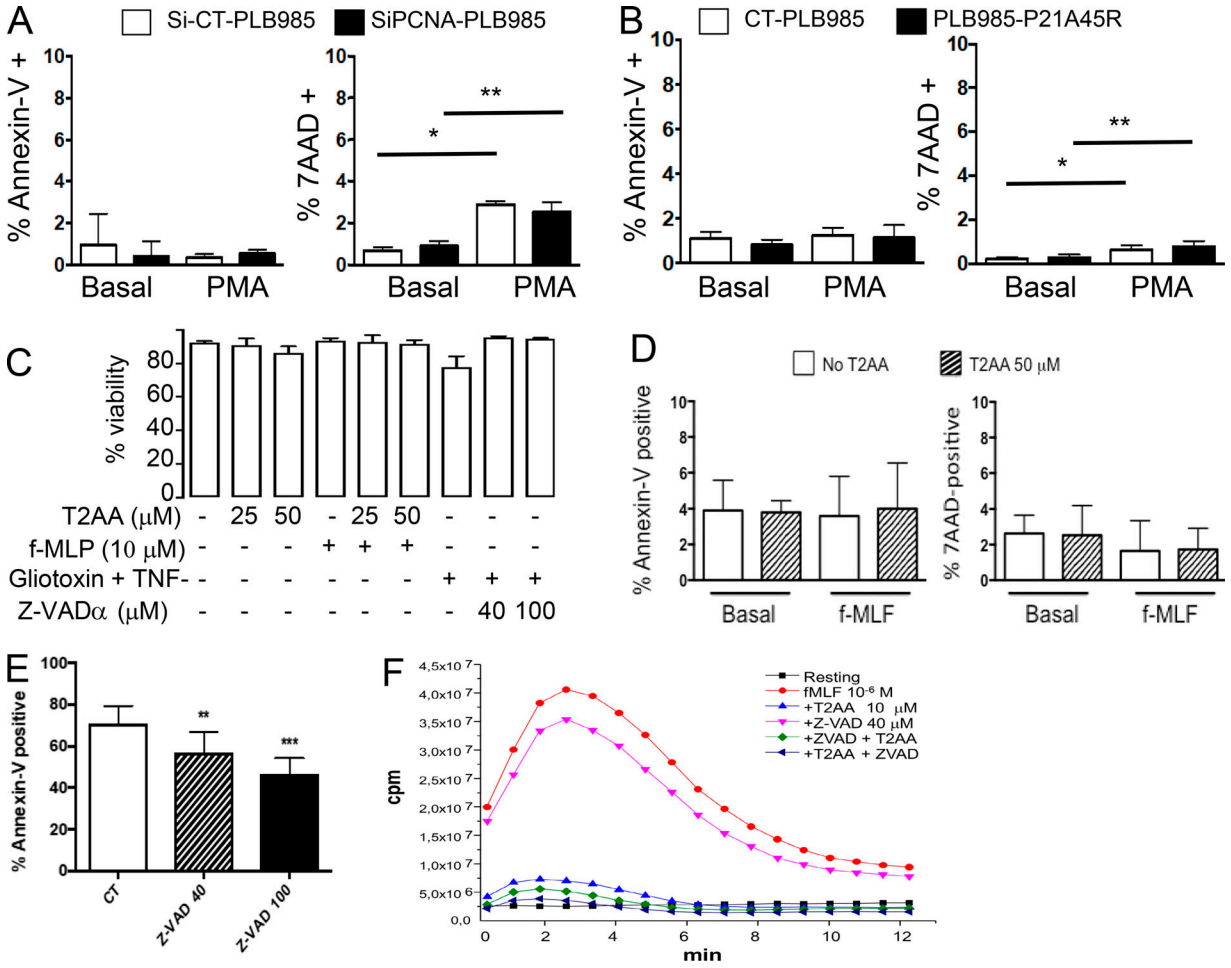

Figure S2. **The decreased oxidative burst triggered by PCNA inhibition observed in differentiated PLB985 cells or in human neutrophils is not due to decreased viability. (A and B)** Flow cytometry analysis of apoptotic and necrotic cells after labeling with Annexin-V and 7-AAD, respectively, in DMF-differentiated PLB985. **(A)** DMF-differentiated PLB985 were transfected by control siRNA or PCNA-siRNA and stimulated by PMA (1 μg/ml) for 1 h. The data are mean ± SEM; *, P < 0.05; **, P < 0.01 (ANOVA; *n* = 4). **(B)** DMF-differentiated PLB985 were transfected with either control plasmid or pcDNA/p21A45R and treated by PMA (1 μg/ml) for 1 h. The data are mean ± SEM; *, P < 0.05; **, P < 0.01 (ANOVA; *n* = 6). **(C and D)** Evaluation of viability in human neutrophils treated with T2AA in the presence or absence of f-MLF (10^{-6} M) for 1 h. Double labeling with Annexin-V and 7-AAD was performed in human isolated neutrophils treated by f-MLF. This time point was chosen because the duration of the measurement of NADPH oxidase activity was 40 min. **(C)** Viable neutrophils are double-negative neutrophils. The data are mean ± SEM; *, P < 0.05 (ANOVA) of four independent experiments with distinct blood donors (*n* = 4). **(D)** The percentage of Annexin-V and 7-AAD neutrophils represent apoptotic and necrotic neutrophils, respectively. The data are mean ± SEM. **(E)** Effect of Z-VAD at 40 and 100 μM on spontaneous apoptosis of human isolated neutrophils incubated for 16 h at 37°C. Apoptosis was evaluated by flow cytometry by the percentage of neutrophils labeled with fluorescent Annexin-V. The data are mean ± SEM; ** P < 0.01 (ANOVA test) of *n* = 6. ***, P < 0.001. **(F)** Effect of the caspase inhibitor Z-VAD on f-MLF–induced NADPH oxidase–dependent oxidant production. Luminol-amplified CL was measured in isolated neutrophils as described in Materials and methods. Isolated neutrophils were preincubated for 15 min with either 10 μM T2AA alone or a combination of T2AA plus Z-VAD (40 μM) before stimulation with f-MLF. The CL was recorded for 15 min, and a representative experiment is shown. This experiment was reproduced with two distinct healthy donors with similar results.

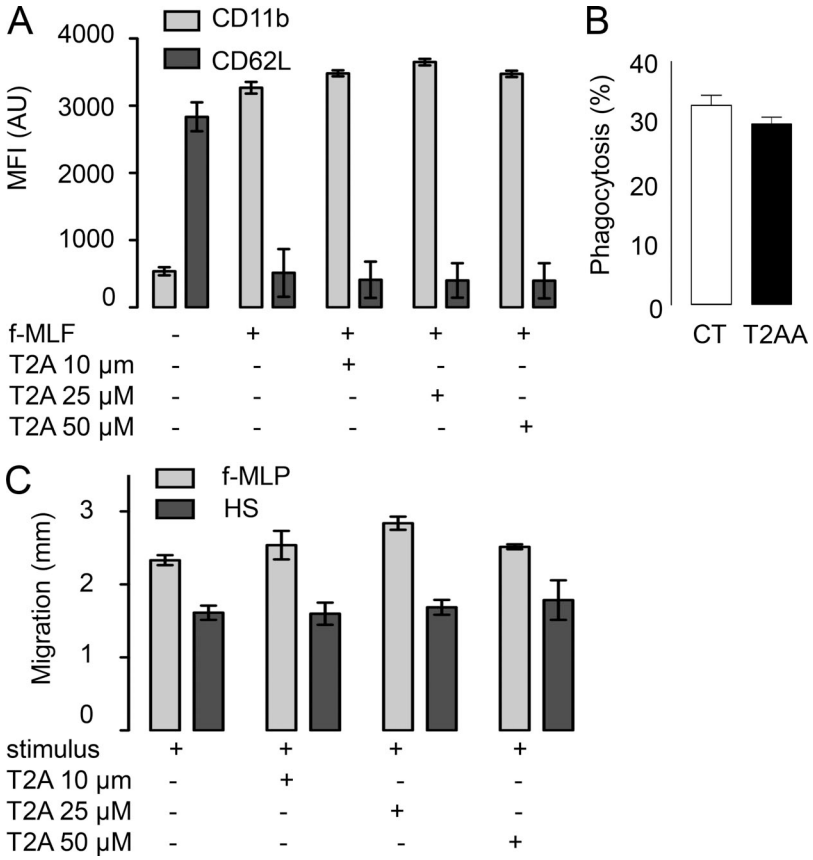

Figure S3. **Effect of T2AA on degranulation, chemotaxis, and phagocytosis in isolated human neutrophils. (A)** Evaluation of degranulation by CD11b and CD62L expression. After 30-min incubation with T2AA followed by 5-min stimulation with f-MLF (10^{-7} M), neutrophils were labeled using anti-CD11b-PE and anti-CD62L-FITC monoclonal antibodies (BD Biosciences) and analyzed by flow cytometry. The data are mean intensity of fluorescence (MFI) ± SEM; $n = 4$ different donors. **(B)** Phagocytosis of fluorescent opsonized *E. coli* by neutrophils treated or not with T2AA (50 mM) performed by flow cytometry using the IngoFlowEx Kit (Proteogenix). The data are mean ± SEM; $n = 4$. **(C)** Evaluation of chemotaxis in the response to f-MLF or C5a using migration on agarose plates as previously described (Nelson et al., 1975; Boudiaf et al., 2016). Briefly, four series of three star shapes were cut in each plate. The central well of each three-well series received 5 µl of suspension containing 10^5 neutrophils preincubated or not with different T2AA concentrations. A pool of human sera (HS) containing C5a fraction or f-MLF (10^{-7} M) was used as stimulus on first and last wells of the three-well series. Plates were incubated for 2 h at 37°C with 5% CO_2. Migration was assessed by measuring distances with an inverted microscope equipped with an ocular micrometer. No migration was observed in the absence of stimulus. The data are migration distances expressed in millimeters ± SEM; $n = 4$.

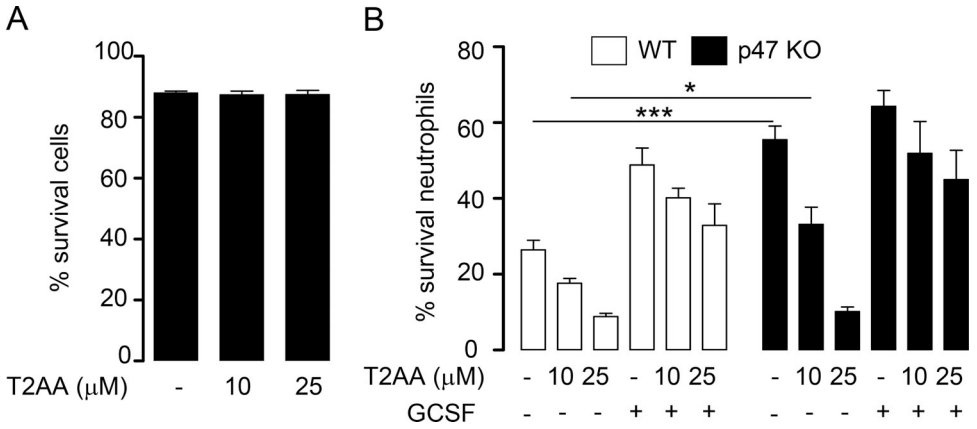

Figure S4. **Effect of T2AA on neutrophil survival isolated from WT and p47phox$^{-/-}$ mice. (A)** Effect of T2AA on bone marrow neutrophil survival. Neutrophils were isolated from bone marrow, and ex vivo spontaneous apoptosis was induced by a 16-h incubation at 37°C with or without T2AA (10–25 μM). Viable Ly6G-positive neutrophils were evaluated by flow cytometry after exclusion of Annexin-V– and 7AAD-labeled neutrophils. The data are mean ± SEM; *n* = 5 mice per group; two independent experiments. **(B)** Effect of T2AA on neutrophil survival isolated from peritoneal lavage in WT or p47phox$^{-/-}$ mice. Neutrophils were isolated from the peritoneal lavage of WT and p47phox$^{-/-}$ mice 4 h after zymosan injection and were cultured as described in A in the presence or absence of G-CSF (50 μM). Using Annexin-V and 7-AAD labeling to exclude apoptotic and necrotic neutrophils, a significant increase was observed in the percentage of surviving neutrophils from p47phox$^{-/-}$ compared with controls. G-CSF promoted survival in both control and p47phox$^{-/-}$ neutrophils, although its effect was more pronounced in controls versus p47phox$^{-/-}$. Nonetheless, T2AA decreased neutrophil survival in a dose-dependent manner in both controls and p47phox$^{-/-}$ mice in the absence or presence of G-CSF, suggesting that (i) T2AA can induce apoptosis even in the presence of G-CSF and (ii) the interaction between PCNA and p47phox was not required for PCNA prosurvival activity. The data are mean ± SEM of 13 mice (for WT) and 8 mice (for p47phox$^{-/-}$) and two independent experiments. *, P < 0.05; ***, P < 0.001, ANOVA.

Table S1. **Crystallographic data collection and refinement statistics**

Data collection statistics	**6FCM**	**6FCN**
Space group	$P2_12_12_1$	$P2_12_12_1$
Cell dimension (Å)		
a	39.18	39.88
b	140.06	140.67
c	169.97	171.77
Resolution (Å)	45.0–2.80 (2.87–2.80)	41.1–3.22 (3.30–3.22)
R_{merge} (%)[a]	4.8 (80.2)	9.6 (170.8)
Completeness (%)	97.0 (92.2)	99.4 (97.8)
I/σ	18.3 (1.62)	14.3 (1.13)
$CC_{1/2}$[b]	99.9 (71.9)	99.9 (59.0)
No. reflections	79,894 (4,753)	93,561 (6,587)
No. unique	23,289 (1,567)	16,369 (1,175)
Refinement statistics		
Protein atoms	5,790	5,784
Water	8	7
Resolution (Å)	45.0–2.80	41.1–3.22
R_{factor} (%)[c]	19.9	21.2
R_{work} (%)[c]	19.5 (35.9)	20.9 (42.5)
R_{free} (%)[c]	27.6 (38.5)	27.2 (42.6)
Rms deviations from ideality		
Bond lengths (Å)	0.008	0.007
Bond angles (°)	1.31	1.233

Values in parentheses refer to the highest-resolution shell.
[a]$R_{merge} = \Sigma_{hkl}\Sigma_i|I_i(hkl) - \langle I(hkl)\rangle|/\Sigma_{hkl}\Sigma_i I_i(hkl)$.
[b]$CC_{1/2}$ is the correlation coefficient of average intensities calculated from two random half of the measurements for each unique reflection.
[c]$R = \Sigma_{hkl}|F_{obs}(hkl) - F_{calc}(hkl)|/\Sigma_{hkl}F_{obs}(hkl)$. R_{factor} is calculated on all reflection, R_{free} on free set reflections (5% of all reflections).

References

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