

Supplementary Figures

Fatty acid desaturation and lipoxygenase pathways support trained immunity

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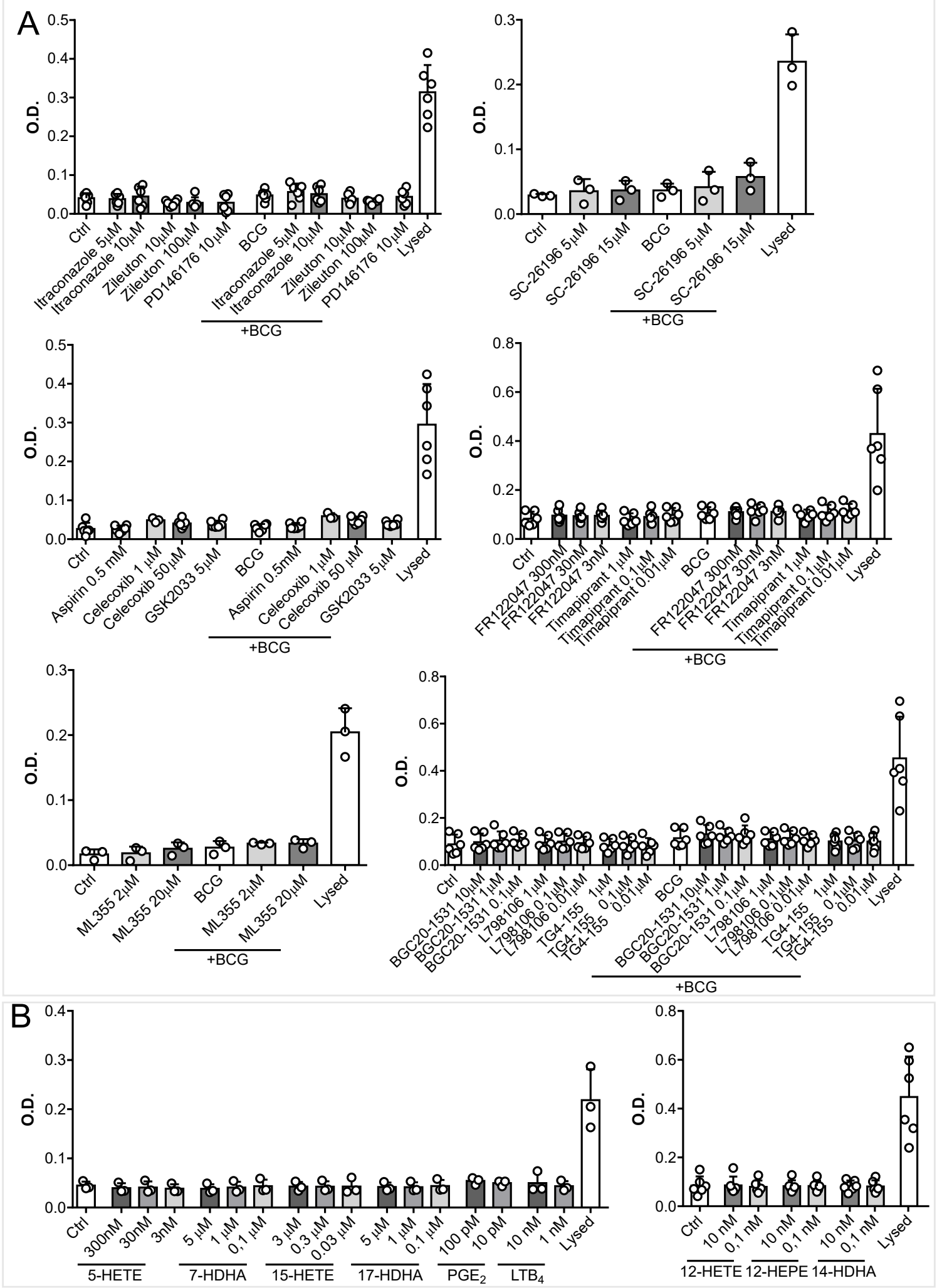
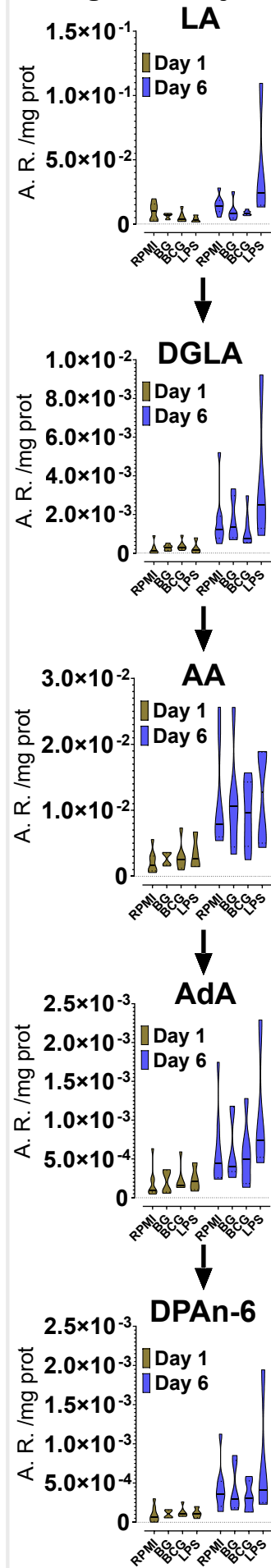


Figure S1. LDH in the conditioned media of monocytes incubated for 24h with (A) Itraconazole, Zileuton, PD146178, SC-26196, Aspirin, Celecoxib, GSK2033, FR122047, Timapiprant, ML355, BGC20-1531, L798106, TG4-155 alone or in combination with BCG (n=3/6 biological replicates pooled from 2 independent experiments, Mean+SD) and (B) 5-HETE, 7-HDHA, 15-HETE, 17-HDHA, LTB₄, PGE₂, 12-HETE, 12-HEPE, 14-HDHA (n=3/6 biological replicates, pooled from 1/2 independent experiments Mean+SD).

Omega - 6 fatty acids



Δ -6 desaturation
Elongation

Δ -5 desaturation

Elongation

Elongation
 Δ -6 desaturation
 β -oxidation

Omega - 3 fatty acids

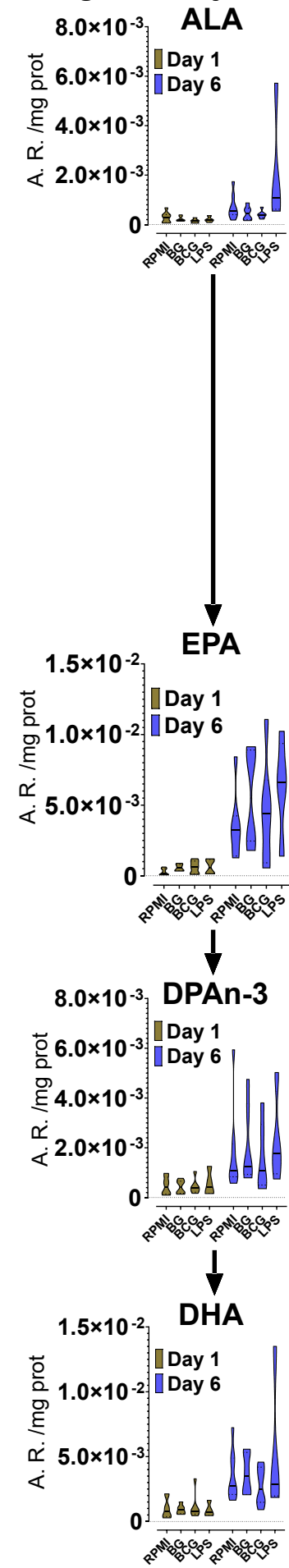


Figure S2. Area ratio (A.R) levels of PUFA corrected for internal standards and protein in the omega-6/3 pathways in stimulated monocytes at 24h with BG, BCG, and LPS and after 6 days of culture (n=4-8 biological replicates), Median and the interquartile region.

Monocytes

RPMI/ BG/ BCG/ LPS

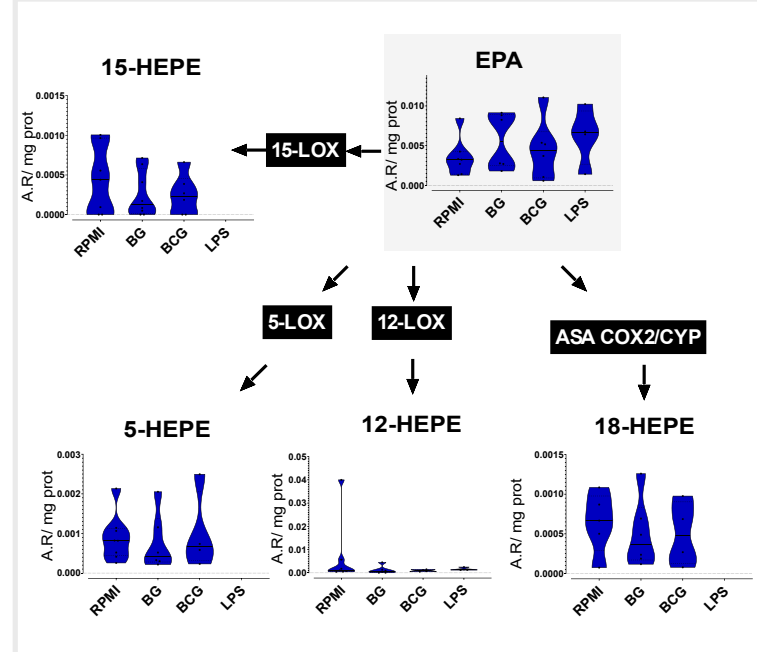
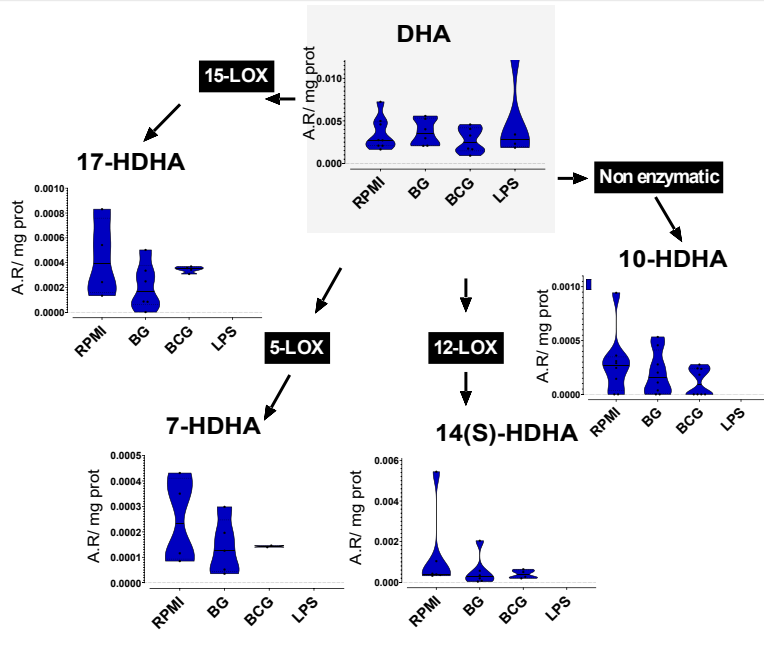
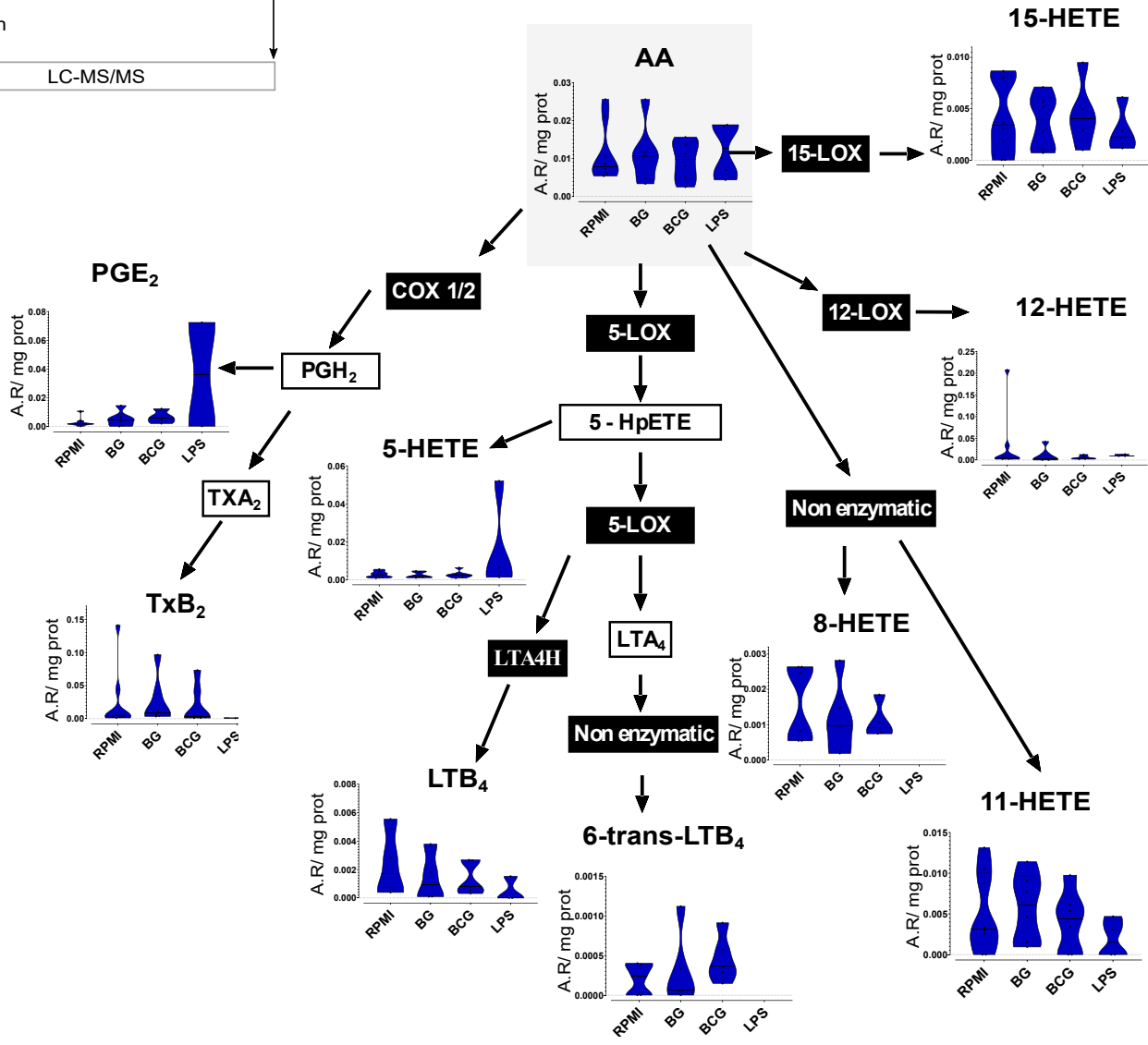
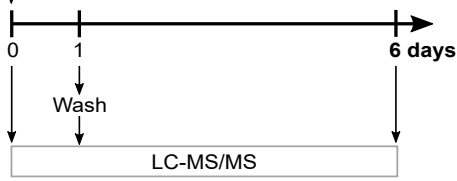


Figure S3. Area ratio (A.R) levels corrected to protein levels of PUFA and oxylipids of macrophages at day 6 of culture after in vitro exposure to BG, BCG or LPS (n=4-8 biological replicates), Median and the interquartile region. Data points below the technical limit of detection are not shown.

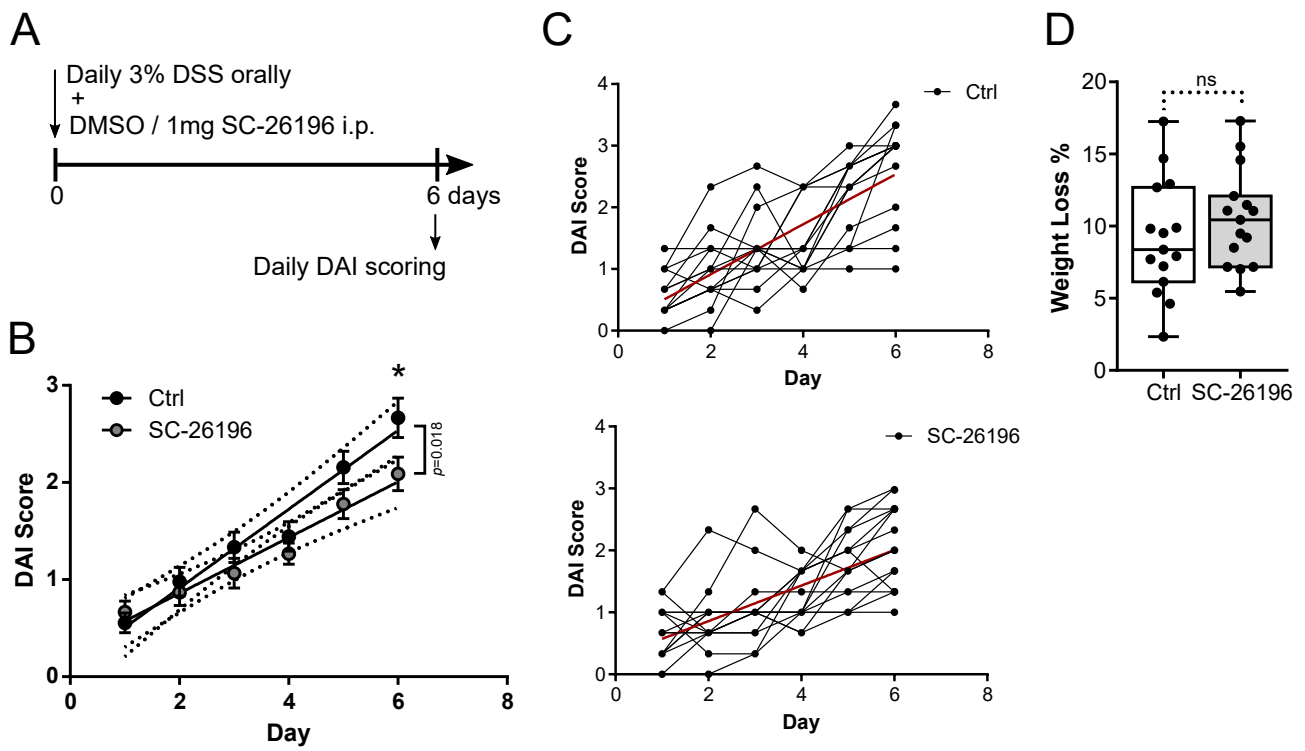


Figure S4. In vivo DSS-induced colitis DAI score and weight loss. (A) Schematic representation of the *in vivo* colitis model used. (B) DAI score (stool consistency, rectal bleeding, and weight loss) of mice with DSS-induced colitis and daily administration of 1 mg SC-26196 or vehicle control. (C) DAI score of individual mice in the vehicle control group (top panel) and in the SC-26196 group (bottom panel), linear regression is shown in red. (D) Weight loss of mice at day 6 (n=15 mice per condition pooled from 2 independent experiments), (A) Mean+SD, (D) Mean, 10-90 percentile and whiskers extend to the most extreme points. Mann-Whitney U test and linear regression analysis. ns: non-significant

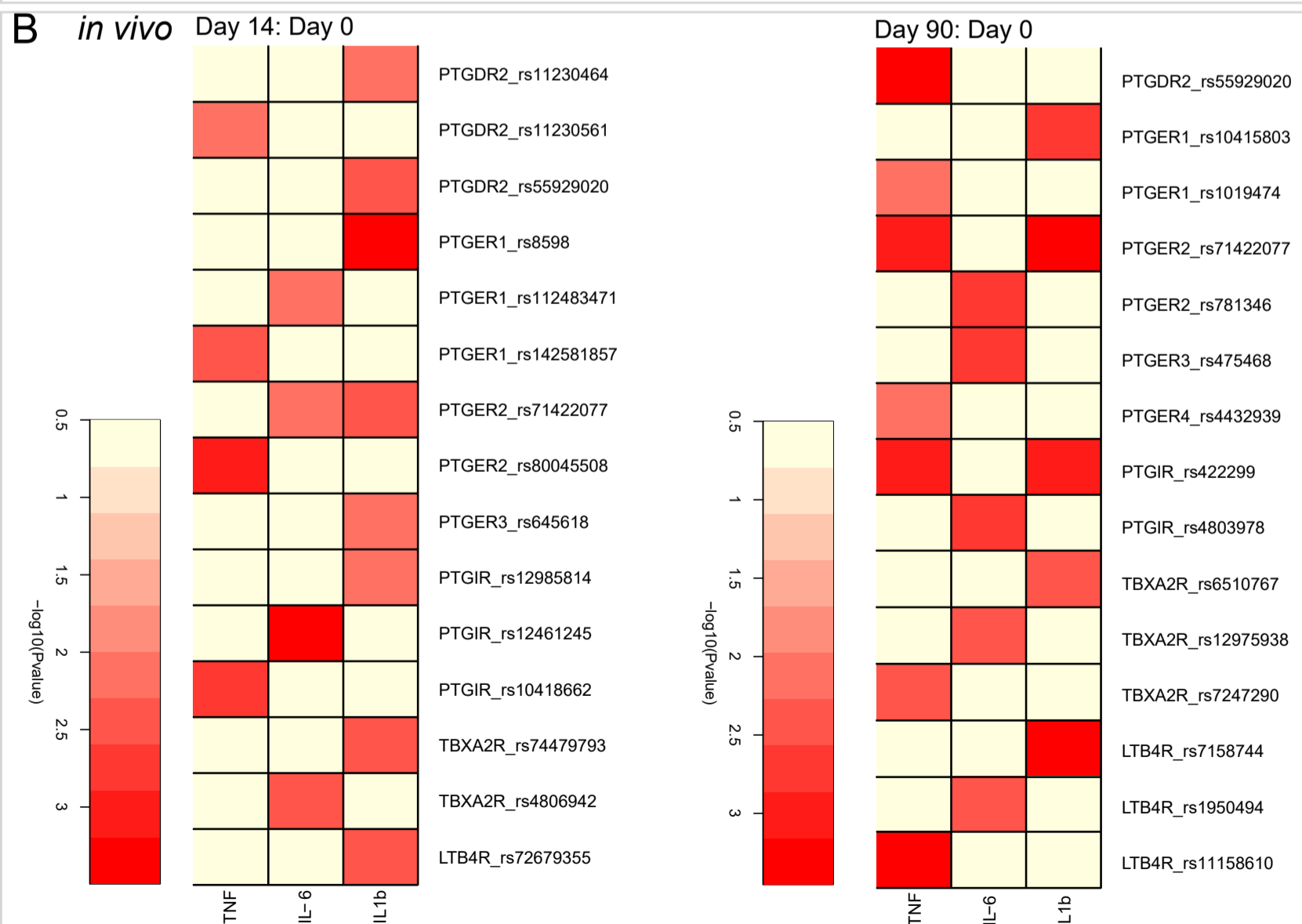
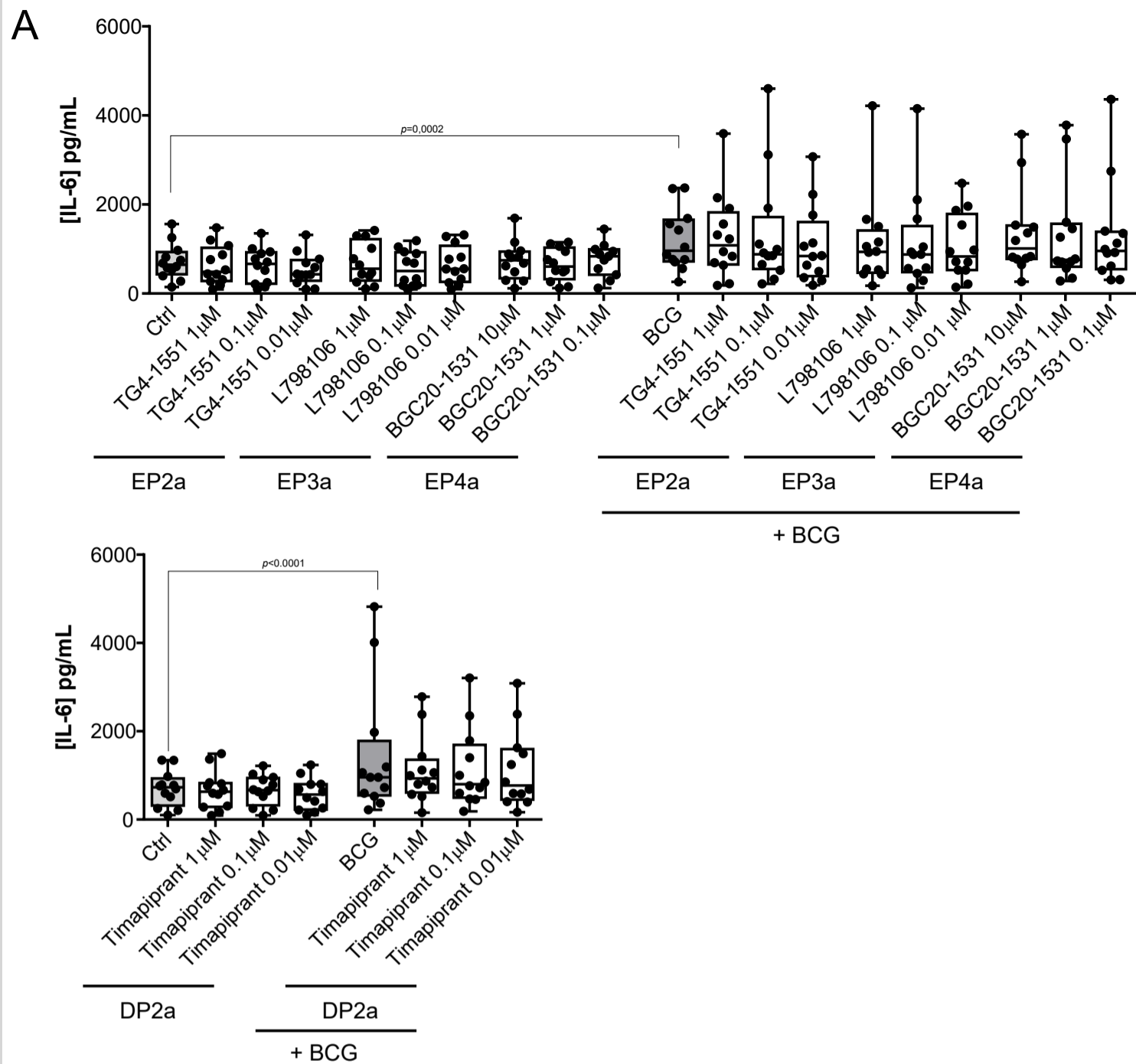
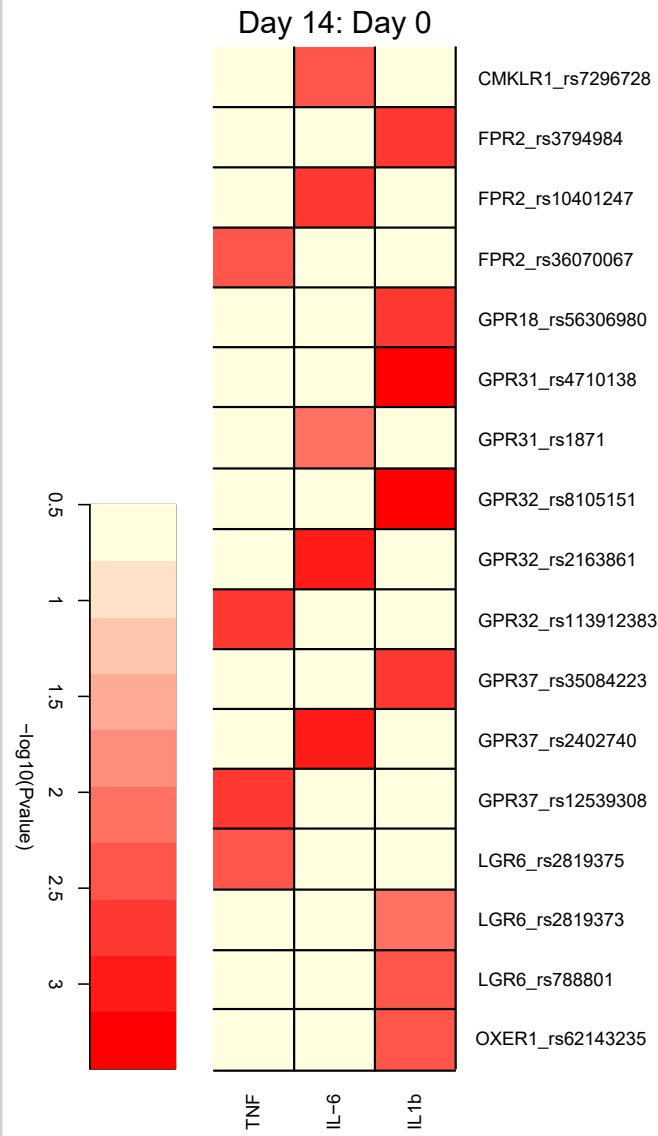


Figure S5. The role of prostanoids receptors in BCG-induced responsiveness. (A) IL-6 by BCG-trained macrophages incubated with (1, 0.1, 0.01 μ M) TG4-155, (1, 0.1, 0.01 μ M) L798106, (10, 1, 0.1 μ M) BGC20-1531 or (1, 0.1, 0.01 μ M) Timapiprant for the first 24 h and restimulated with LPS for another 24 h at day 6 of culture. (n=12 biologic replicates, pooled from 4 independent experiments). Mean, 10-90 percentile and whiskers extend to the most extreme points, two-way ANOVA, Sidak's multiple comparisons test. (B) Heatmaps of the p values of the association between SNPs around *PTGDR2*, *PTGER1*, *PTGER2*, *PTGER3*, *PTGER4*, *PTGIR*, *TBXA2R*, *LTB4R* (+/- 250kb) and the fold change of the production of TNF α , IL-6 and IL-1 β upon BCG vaccination and *S. aureus* *ex vivo* restimulation, 14 days after vaccination (left panel) and 90 days after vaccination (right panel) (n= 278 healthy volunteers).

A
in vivo



B
in vivo

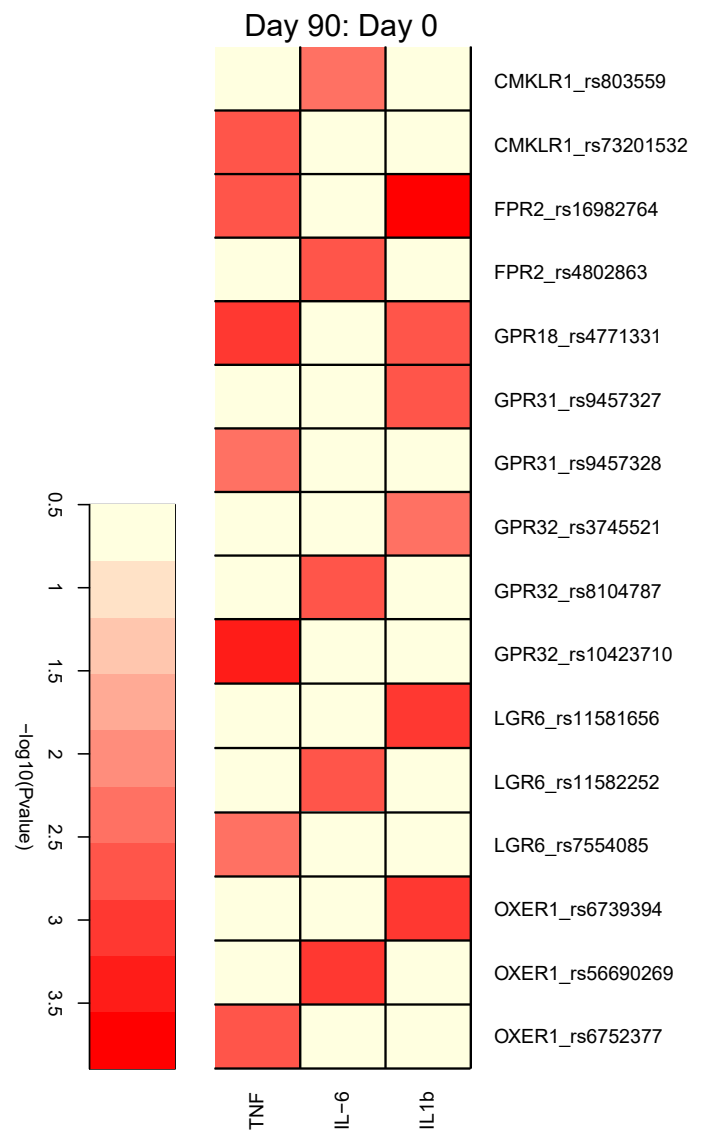


Figure S6. The role of oxylipins receptors in BCG-induced responsiveness. Heatmaps of the p values of the association between SNPs around *CMKLR1*, *FPR1*, *GPR18*, *GPR31*, *GPR32*, *GPR37*, *LGR6*, *OXER1* (+/- 250kb) and the fold change of the production of TNF α , IL-6 and IL-1 β upon BCG vaccination and *S. aureus ex vivo* restimulation, 14 days after vaccination (left panel) and 90 days after vaccination (right panel) (n= 278 healthy volunteers).

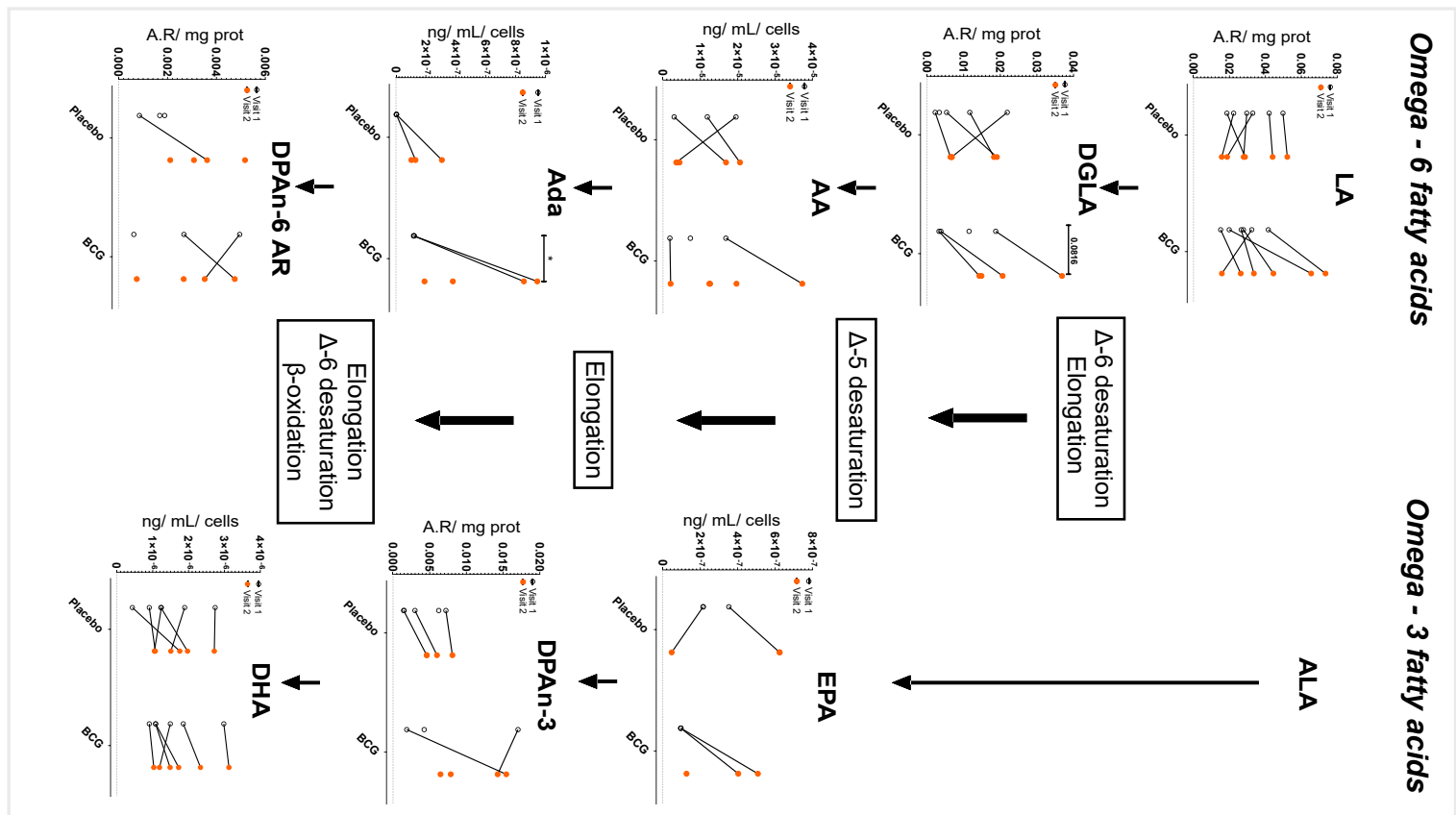
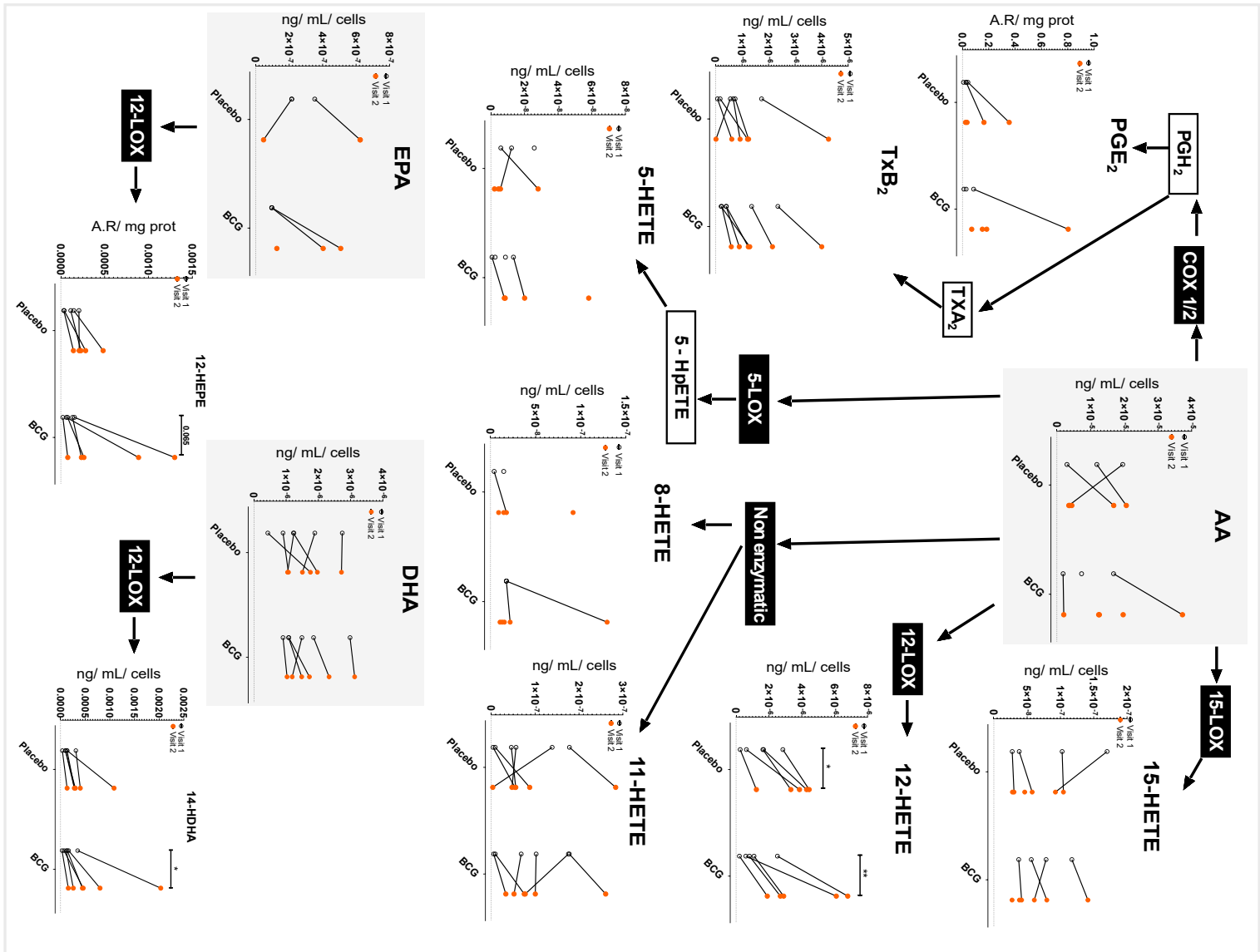


Figure S7. Concentrations (ng/mL) corrected to cell number or Area ratio (A.R) corrected to protein content of PUFA and oxylipids of *in vivo* BCG-trained response in monocytes (n=6 biological replicates), * p ≤ 0.05, ** p ≤ 0.01, two-way ANOVA, Sidak's multiple comparisons test. Data points below the technical limit of detection are not shown. One outlier per group was removed (ROUT test (Q=5%).

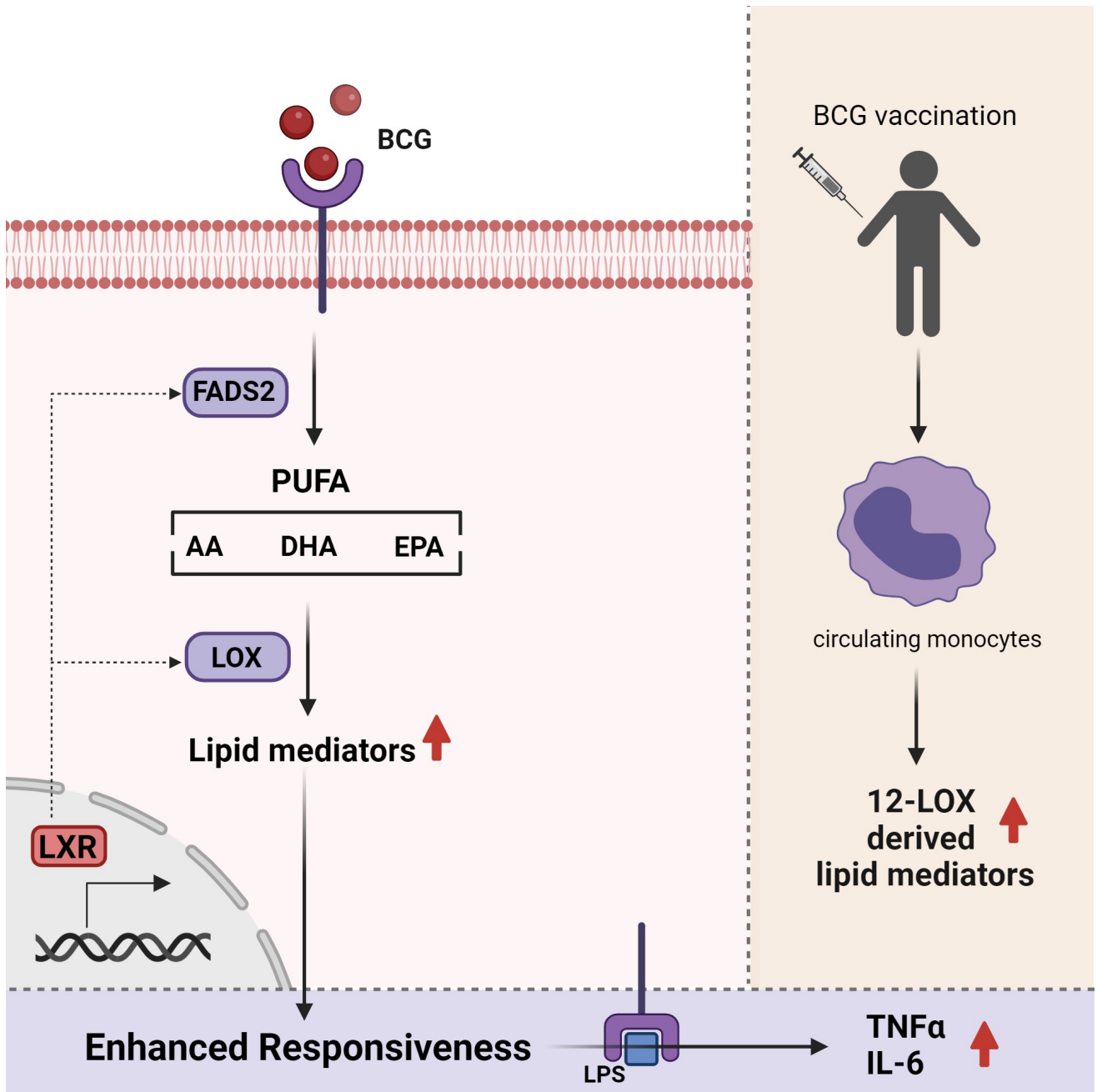


Figure S8. Graphical representation of the key findings of this manuscript. Created with BioRender.com