

## **Title**

**Dietary supplementation with eicosapentaenoic acid inhibits plasma cell differentiation and attenuates lupus autoimmunity**

## **Article type**

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**Figure S1** | Effect of EPA supplementation on body and tissue weights in lupus mice. Body and tissue weights. Four-week-old FVB/N mice were fed CE-2 diet supplemented with 5% (*wt/wt*) palmitate (Pal) or eicosapentaenoic acid (EPA) for 6 weeks. To induce lupus, the mice received imiquimod (IMQ) or vehicle (Veh) treatment 3 times a week during the last 4 weeks. Veh + Pal, n = 8; IMQ + Pal, n = 8; IMQ + EPA, n = 9. Data shown are mean value  $\pm$  SEM of representative experiments at least three times independently. Statistical analysis was performed with Turkey's test. \*p < 0.05; \*\*p < 0.01; ns, not significant.

**Figure S2** | Gating strategy used for immunophenotyping of spleens in **Figure 2**. Spleen from Veh-treated mice fed Pal-supplemented diet, IMQ-treated mice fed Pal-supplemented diet, and IMQ-treated mice fed EPA-supplemented diet were analyzed by flow cytometry.

**Figure S3** | EPA does not affect CD8<sup>+</sup> T or myeloid cellularity in spleen of IMQ-induced lupus mice. Immunophenotyping of spleen from Veh-treated mice fed Pal-supplemented diet, IMQ-treated mice fed Pal-supplemented diet, and IMQ-treated mice fed EPA-supplemented diet by flow cytometry. Gating strategy is available in Figure S2. **(A)** Representative flow cytometry plots (left), percentages (right, top) and cell counts (right, bottom) of myeloid cells in total spleen cells. CD11c<sup>+</sup>MHCII<sup>+</sup> dendritic cells (DC), B220<sup>+</sup>PDCA<sup>+</sup> plasmacytoid DC (pDC), CD11b<sup>+</sup>Ly6G<sup>-</sup> monocytes and CD11b<sup>+</sup>Ly6G<sup>+</sup> neutrophils were analyzed. **(B)** Representative flow cytometry plots (left), percentages (right, top) and cell counts (right, bottom) of CD8<sup>+</sup> T cell subsets in total spleen cells. Total CD3<sup>+</sup>CD8<sup>+</sup> T cells, CD8<sup>+</sup>CD62L<sup>+</sup>CD44<sup>-</sup> naïve T cells and CD8<sup>+</sup>CD62L<sup>-</sup>CD44<sup>+</sup> effector T cells (Teff) were analyzed. Veh + Pal, n = 8; IMQ + Pal, n = 8; IMQ + EPA, n = 8. Data shown are mean value  $\pm$  SEM of representative experiments at least three times independently. Statistical analysis was performed with Dunn's test (A: cell count of DC and Monocyte, B: Teff) or Tukey's test (if not otherwise stated). \*p < 0.05; \*\*p < 0.01; ns, not significant.

**Figure S4** | Effect of EPA supplementation on body and tissue weights in lupus mice. **(A)** Gross morphology of spleen (left) and axillary lymph nodes (right). **(B)** Body and tissue weights. Eight week-old control C57BL/6J<sup>+/+</sup> and C57BL/6J<sup>lpr/lpr</sup> mice were fed CE-2 diet supplemented with 5% (*wt/wt*) Pal or EPA for 16 weeks. Lymph node panel indicates the weight of 2 axillary lymph nodes. B6<sup>+/+</sup> + Pal, n = 9; B6<sup>lpr/lpr</sup> + Pal, n = 7; B6<sup>lpr/lpr</sup> + EPA, n = 8. Data shown are mean value ± SEM of representative experiments at least twice independently. Statistical analysis was performed with Turkey's test. \*p < 0.05; \*\*p < 0.01; ns, not significant.

**Figure S5** | Gating strategy used for immunophenotyping of spleens in **Figure 4**. Spleen from B6<sup>+/+</sup> mice fed Pal-supplemented diet, B6<sup>lpr/lpr</sup> mice fed Pal-supplemented diet, and B6<sup>lpr/lpr</sup> mice fed EPA-supplemented diet were analyzed by flow cytometry.

**Figure S6** | EPA does not affect CD8<sup>+</sup> T or myeloid cellularity in spleen of spontaneous lupus mice. Immunophenotyping of spleen from B6<sup>+/+</sup> mice fed Pal-supplemented diet, B6<sup>lpr/lpr</sup> mice fed Pal-supplemented diet, and B6<sup>lpr/lpr</sup> mice fed EPA-supplemented diet by flow cytometry. Gating strategy is available in Figure S5. **(A)** Representative flow cytometry plots (left), percentages (right, top) and cell counts (right, bottom) of T cell subsets in total spleen cells. Total cells, CD62L<sup>+</sup>CD44<sup>-</sup> naïve T cells and CD62L<sup>-</sup>CD44<sup>+</sup> effector T cells (Teff) in CD4<sup>+</sup> and CD8<sup>+</sup> T cells were analyzed. **(B)** Representative flow cytometry plots (left), percentages (right, top) and cell counts (right, bottom) of myeloid cells in total spleen cells. CD11c<sup>+</sup>MHCII<sup>+</sup> dendritic cells (DC), B220<sup>+</sup>PDCA<sup>+</sup> plasmacytoid DC (pDC), CD11b<sup>+</sup>Ly6G<sup>-</sup> monocytes and CD11b<sup>+</sup>Ly6G<sup>+</sup> neutrophils were analyzed. B6<sup>+/+</sup> + Pal, n = 9; B6<sup>lpr/lpr</sup> + Pal, n = 7; B6<sup>lpr/lpr</sup> + EPA, n = 8. Data shown are mean value ± SEM of representative experiments at least twice independently. Statistical analysis was performed

with Dunn's test (A: Cell count of CD4 Total, Teff, CD8 naïve and Teff, B: population of pDC and Neutrophil, cell count of DC and pDC) or Tukey's test (if not otherwise stated). \*p < 0.05; \*\*p < 0.01; ns, not significant.

**Table S1. List of resources used in this study**

| Antibody   | Source                 | Clone       | Identifier                           |
|--|------------------------|-------------|--------------------------------------|
| Fluorescein (FITC) AffiniPure Goat Anti-Mouse IgG (H+L)                    | Jackson ImmunoResearch |             | Cat#115-095-003<br>RRID : AB_2338589 |
| Fluorescein-Conjugated Goat IgG Fraction To Mouse Complement C3            | MP Biomedicals         |             | Cat#SKU 0855500                      |
| PE/Cy7 anti-mouse CD138 (Syndecan-1) Antibody                              | BioLegend              | 281-2       | Cat#142513<br>RRID : AB_2562197      |
| APC anti-mouse/human CD45R/B220 Antibody                                   | BioLegend              | RA3-6B2     | Cat#103211<br>RRID : AB_312996       |
| Brilliant Violet 510™ anti-mouse/human CD45R/B220 Antibody                 | BioLegend              | RA3-6B2     | Cat#103247<br>RRID : AB_2561394      |
| PE anti-mouse CD95 (Fas) Antibody  | BioLegend              | SA367H8     | Cat#152607<br>RRID : AB_2632903      |
| PerCP/Cyanine5.5 anti-mouse CD23 Antibody                                  | BioLegend              | B3B4        | Cat#101617<br>RRID : AB_2562923      |
| APC/Cy7 anti-mouse CD21/CD35 (CR2/CR1) Antibody                            | BioLegend              | 7E9         | Cat#123417<br>RRID : AB_1953274      |
| APC anti-mouse/human GL7 Antigen (T and B cell Activation Marker) Antibody | BioLegend              | GL7         | Cat#144617<br>RRID : AB_2800674      |
| PE anti-mouse CD3e   | BioLegend              | 145-2C11    | Cat#100308<br>RRID : AB_312673       |
| Brilliant Violet 510™ anti-mouse CD4 Antibody                              | BioLegend              | GK1.5       | Cat#100449<br>RRID : AB_2564587      |
| PE/Cy7 anti-mouse/human CD45R/B220 Antibody                                | BioLegend              | RA3-6B2     | Cat#103221<br>RRID : AB_313004       |
| APC anti-mouse/human CD44 Antibody   | BioLegend              | IM7         | Cat#103011<br>RRID : AB_312962       |
| APC/Cy7 anti-mouse CD62L Antibody  | BioLegend              | MEL-14      | Cat#104427<br>RRID : AB_830798       |
| Brilliant Violet 510™ anti-mouse I-A/I-E Antibody                          | BioLegend              | M5/114.15.2 | Cat#107635<br>RRID : AB_2561397      |
| PE anti-mouse CD317 (BST2, PDCA-1)   | BioLegend              | 9.27E+02    | Cat#127009<br>RRID : AB_1953284      |
| PerCP anti-mouse CD11c Antibody  | BioLegend              | N418        | Cat#117325<br>RRID : AB_893236       |
| PE/Cy7 anti-mouse Ly-6G Antibody   | BioLegend              | 1A8         | Cat#127617<br>RRID : AB_1877262      |
| APC/Cy7 anti-mouse/human CD11b Antibody                                    | BioLegend              | M1/70       | Cat#101225<br>RRID : AB_830641       |

|  |                     |          |                                  |
|--|---------------------|----------|----------------------------------|
| TruStain FcX™ (anti-mouse CD16/32) Antibody  | BioLegend           | 93       | Cat#101320<br>RRID : AB_1574975  |
| PE/Cy7 anti-mouse CD279 (PD-1)               | BioLegend           | 29F.1A12 | Cat#135215<br>RRID : AB_10696422 |
| PE anti-mouse Foxp3                          | TONBO Biosciences   | 3G3      | Cat#50-5773-U025                 |
| APC anti-mouse CD25                          | BioLegend           | 3C7      | Cat#101909<br>RRID : AB_961208   |
| Alexa Fluor® 647 anti-mouse Blimp-1 Antibody | BioLegend           | 5E7      | Cat#150003<br>RRID : AB_2565617  |
| PerCP/cyanine5.5 anti-mouse CD8a Antibody    | BioLegend           | 53-6.7   | Cat#100733<br>RRID : AB_2075239  |
| Cy3 Peanut Agglutinin (PNA)                  | Vector laboratories |          | Cat#CL-1073-1                    |
| Purified anti-mouse CD40 Antibody            | BioLegend           | HM40-3   | Cat#102902<br>RRID : AB_312945   |

| Chemical/Peptide/Recombinant protein  | Source                 | Identifier       |
|---|------------------------|------------------|
| DAPI (used for nuclear staining)  | BioLegend              | Cat#422801       |
| Collagenase Type IV   | Worthington            | Cat#CLS4         |
| DNase I   | Roche Diagnostics      | Cat#11284932001  |
| R848 Resiquimod   | AdipoGen               | Cat#AG-CR1-3582  |
| LPS from E. coli O111:B4  | Sigma-Aldrich          | Cat#L4391-1MG    |
| Recombinant murine IL4  | Pepro Tech             | Cat#214-14       |
| Recombinant murine IFN $\gamma$   | Pepro Tech             | Cat#315-05       |
| Palmitic acid BioXtra, $\geq 99\%$  | Sigma-Aldrich          | Cat#P5585        |
| cis-5,8,11,14,17-Eicosapentaenoic acid sodium salt $\geq 99\%$ (capillary GC) | Sigma-Aldrich          | Cat#E6627-25MG   |
| BESELNA cream 5%  | Mochida Pharmaceutical | Cat#87629        |
| Foxp3/Transcription Factor Staining Buffer Kit                                | TONBO Biosciences      | Cat#TNB-0607-KIT |
| Laurdan   | Cayman Chemical        | Cat#19706        |

| ELISA  | Source                          | Identifier    |
|--|---------------------------------|---------------|
| Mouse Anti-Nuclear Antibodies (ANA) Total Ig ELISA Kit | Alpha Daiagnostic International | Cat#5210      |
| Mouse BAFF/BLyS/TNFSF13B Immunoassay                   | R&D Systems                     | Cat#MBLYS0    |
| LBIS Mouse Anti-dsDNA ELISA Kit                        | FUJIFILM Wako Shibayagi         | Cat#AKRDD-061 |
| Mouse Anti-Histones Ig's ELISA Kit, 96 tests,          | Alpha Daiagnostic               | Cat#5610      |

|                                      |               |                 |
|--------------------------------------|---------------|-----------------|
| Quantitative                         | International |                 |
| Mouse IgG Uncoated ELISA Kit         | Invitrogen    | Cat#88-50400-22 |
| Mouse IgM Uncoated ELISA Kit         | Invitrogen    | Cat#88-50470-22 |
| Mouse IL-12 p70 ELISA Kit            | Invitrogen    | Cat#BMS6004     |
| Mouse IL-6 Quantikine ELISA Kit      | R&D Systems   | Cat#M6000B      |
| Mouse TNF-alpha Quantikine ELISA Kit | R&D Systems   | Cat#MTA00B      |

| Cell separation reagent     | Source          | Identifier      |
|-----------------------------|-----------------|-----------------|
| FcR Blocking Reagent, mouse | Miltenyi Biotec | Cat#130-092-575 |
| B cell Isolation Kit II     | Miltenyi Biotec | Cat#130-090-862 |
| Pan B cell Isolation kit II | Miltenyi Biotec | Cat#130-104-443 |
| CD11c microbeads Ultrapure  | Miltenyi Biotec | Cat#130-108-338 |

| Animal/Cell   | Source          | Identifier   |
|---|-----------------|--------------|
| Mouse: C57BL/6J+/+ females 8 weeks of age             | Japan SLC, Inc  | N/A          |
| Mouse: C57BL/6Jlpr/lpr females 8 weeks of age         | Japan SLC, Inc  | N/A          |
| Mouse: FVB/N females 4 weeks of age                   | CLEA Japan, Inc | N/A          |
| Ba/F3-CL1   | RIKEN BRC       | Cat#RBC4474  |
| Reporter cell: B16-Blue IFN- $\alpha$ / $\beta$ cells | Invivogen       | Cat#bb-ifnt1 |

| Software        | Source                               | Identifier  |
|-----------------|--------------------------------------|---|
| FlowJo software | BD Biosciene                         | <a href="https://www.flowjo.com/">https://www.flowjo.com/</a>     |
| SimFCS software | Laboratory for Fluorescence Dynamics | <a href="http://lfd.uci.edu/globals/">lfd.uci.edu/globals/</a>    |
| Prism9          | GraphPad                             | <a href="https://www.graphpad.com/">https://www.graphpad.com/</a> |



**Table S2. List of primers used to detect mRNAs**

| Name         | Fw/Rv | Sequence                         |
|--------------|-------|----------------------------------|
| <i>36b4</i>  | Fw    | 5'-GGCCCTGCACTCTCGCTTTC-3'       |
|              | Rv    | 5'-TGCCAGGACGCGCTTGT-3'          |
| <i>Baff</i>  | Fw    | 5'-AACAGACGCGCTTTCCAG-3'         |
|              | Rv    | 5'-AGGAGGAGCTGAGAGGTCTACAT-3'    |
| <i>Prdm1</i> | Fw    | 5'-TTCTCTTGAAAAACGTGTGGG-3'      |
|              | Rv    | 5'-GGAGCCGGAGCTAGACTTG-3'        |
| <i>Cd79b</i> | Fw    | 5'-TGTTGGAATCTGCAAATGGA-3'       |
|              | Rv    | 5'-TAGGCTTTGGGTGATCCTTG-3'       |
| <i>Ifnb</i>  | Fw    | 5'-TGGAGCAGCTGAATGGAAAG-3'       |
|              | Rv    | 5'-ATCTCTTGATGGCAAAGGC-3'        |
| <i>Il6</i>   | Fw    | 5'-GCTACCAAACCTGGATATAATCAGGA-3' |
|              | Rv    | 5'-CCAGGTAGCTATGGTACTCCAGAA-3'   |
| <i>Il12b</i> | Fw    | 5'-ACATCTACCGAAGTCCAATGCA-3'     |
|              | Rv    | 5'-GGAATTGTAATAGCGATCCTGAGC-3'   |
| <i>Irf4</i>  | Fw    | 5'-ACCACTGGCAAGGCCCAT-3'         |
|              | Rv    | 5'-GCAGTCTGAGAGCGCCAAG-3'        |
| <i>Tnfa</i>  | Fw    | 5'-ACCCTCACACTCAGATCATCTTC-3'    |
|              | Rv    | 5'-TGGTGGTTTGCTACGACGT-3'        |
| <i>Xbp1</i>  | Fw    | 5'-GAGTCCGCAGCAGGTG-3'           |
|              | Rv    | 5'-GTGTCAGAGTCCATGGGA-3'         |

**Table S3. Composition of experimental diets**

| Product      | Fish meal-free diet<br>(No. 012, CREA<br>Japan) |       | Fish meal-free diet<br>supplemented with<br>5% fatty acid<br>(wt/wt) |       |
|--------------|---|-------|--|-------|
|              | kcal/100g                                       | kcal% | kcal/100g  | kcal% |
| Protein      | 80  | 23    | 76   | 21    |
| Carbohydrate | 222   | 65    | 212  | 58    |
| Fat          | 39  | 11    | 80   | 22    |
| Total        | 341   | 100   | 367  | 100   |