Online Data Supplement:

Lipoxin B_4 promotes the resolution of allergic inflammation in the upper and lower airways of mice

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Methods

Nasal tissue preparation of cell suspensions and histopathology

For extraction of mucosa, the snout/tip of nose, incisor teeth and hard palette was removed to reveal the nasal septum. The septum was gently cut in half and nasal mucosa removed with a 22 gauge needle and placed directly in serum free RPMI (4^oC). Collagenase (0.2 mg/ml) (Sigma Aldrich, Steinheim, Germany) was added (15 min, 37^0C) with gentle shaking. Collagenase activity was stopped by adding 10% FCS and samples were washed with ice cold medium and filtered through a 70µm cell strainer (BD Biosciences, New Jersey) to obtain single cell suspensions.

For preparation of nasal tissue, whole heads were skinned and decalcified in Krajian Decalcifying Solution (JT Baker, Phillipsburg, NJ). Solution was replaced and tissue was checked daily with a 26 gauge needle until bone softened. Tissues were then washed in water (~4-8hrs) and transferred to 70% ethanol and thereafter paraffin embedded sections were prepared and stained with H&E.

Figures:

Fig. S1.

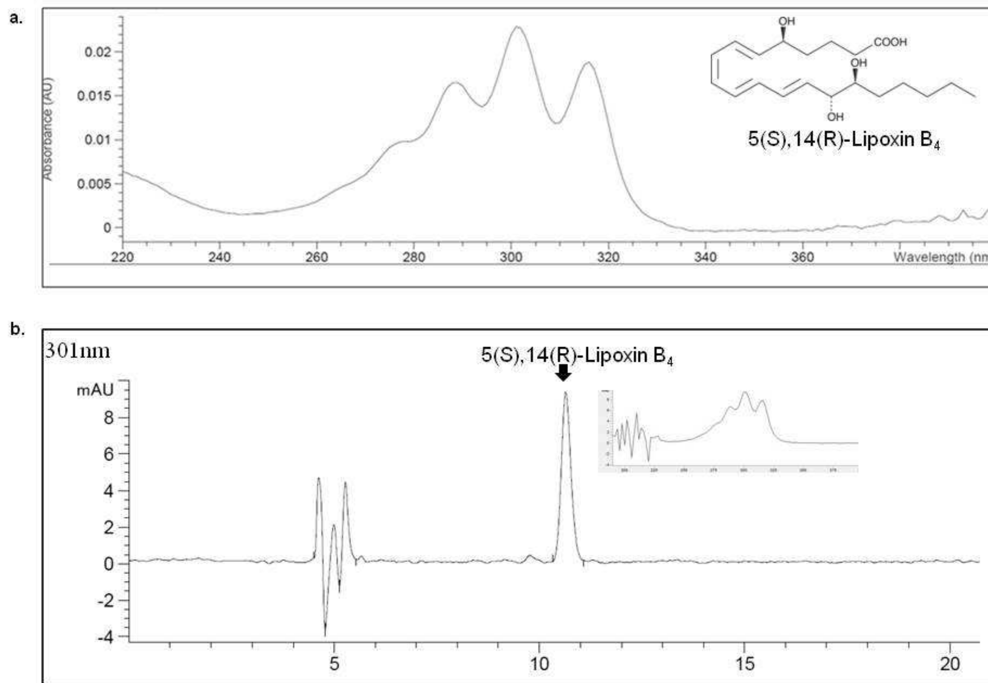

Fig. S1: Validation of LXB_4**.** The LXB_4 integrity was confirmed by **(a)** UV-Vis spectrophotometry to ensure the presence of the diagnostic tetraene chromophore and accurate quantitation and **(b)** HPLC to ensure that only a single peak was present without evidence for isomerization.

Fig. S2

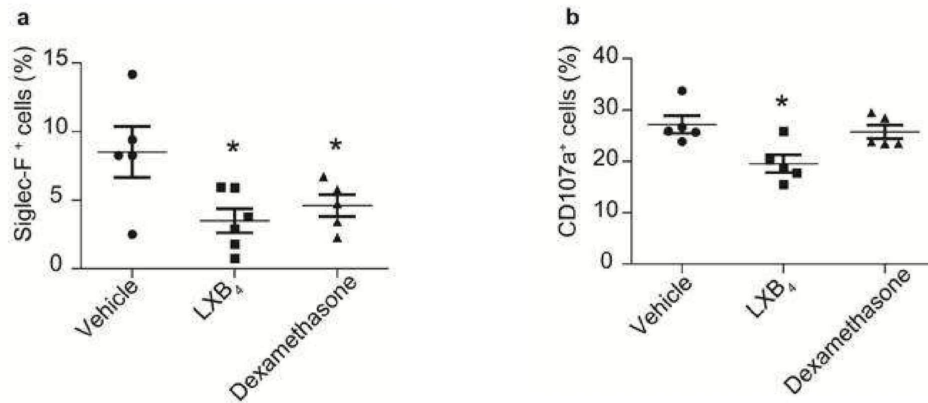

Fig.S2: Flow cytometry staining of eosinophil Siglec-F^+ **(a)** and CD107a^+ **(b)** cells in nasal mucosa.

Fig.S3.

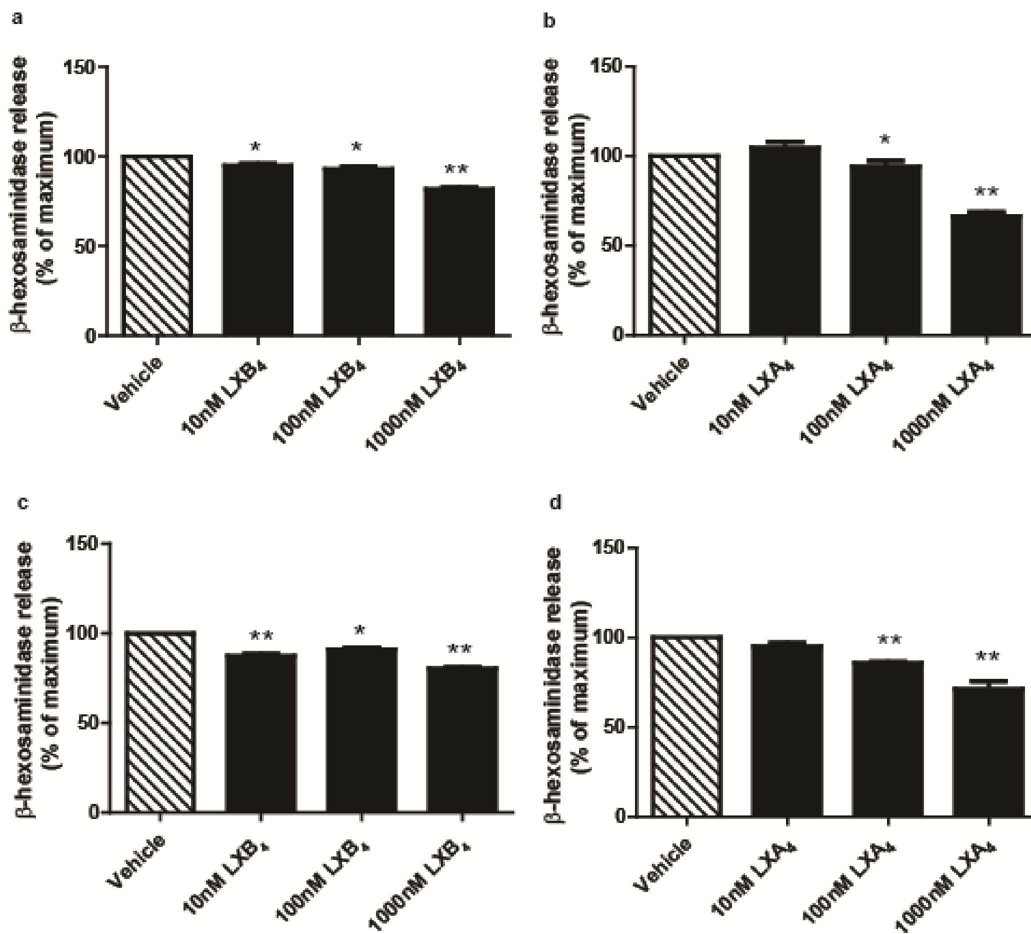

Fig S3: LXB_4 and LXA_4 dose-dependently decrease degranulation of BALB/c and FVB-derived BMMC. BMMC were derived from BALB/C and FVB mice bone marrow, IgE sensitized and activated with goat anti-rat IgG (Fab)2' for 30 mins as described in Methods. As a marker of degranulation, β-hexosaminidase was evaluated. Results are given as percentage of maximum release, where the maximum is the sample activated in the presence of vehicle. Note: The net specific release of vehicle ranged between 35-40% while LXB_4 at the highest concentration decreased this value to 19-33% in the different experiments **(a-b)** β-hexosaminidase release of BALB/c-derived BMMC following incubation with vehicle, or different concentrations of LXB_4 **(a)** or LXA_4 **(b)** **(c-d)** β-hexosaminidase release of FVB-derived BMMC following incubation with vehicle or different concentrations of LXB_4 **(c)** or LXA_4 **(d).** Results are representative of 3 independent experiments performed in triplicates.

Fig. S4.

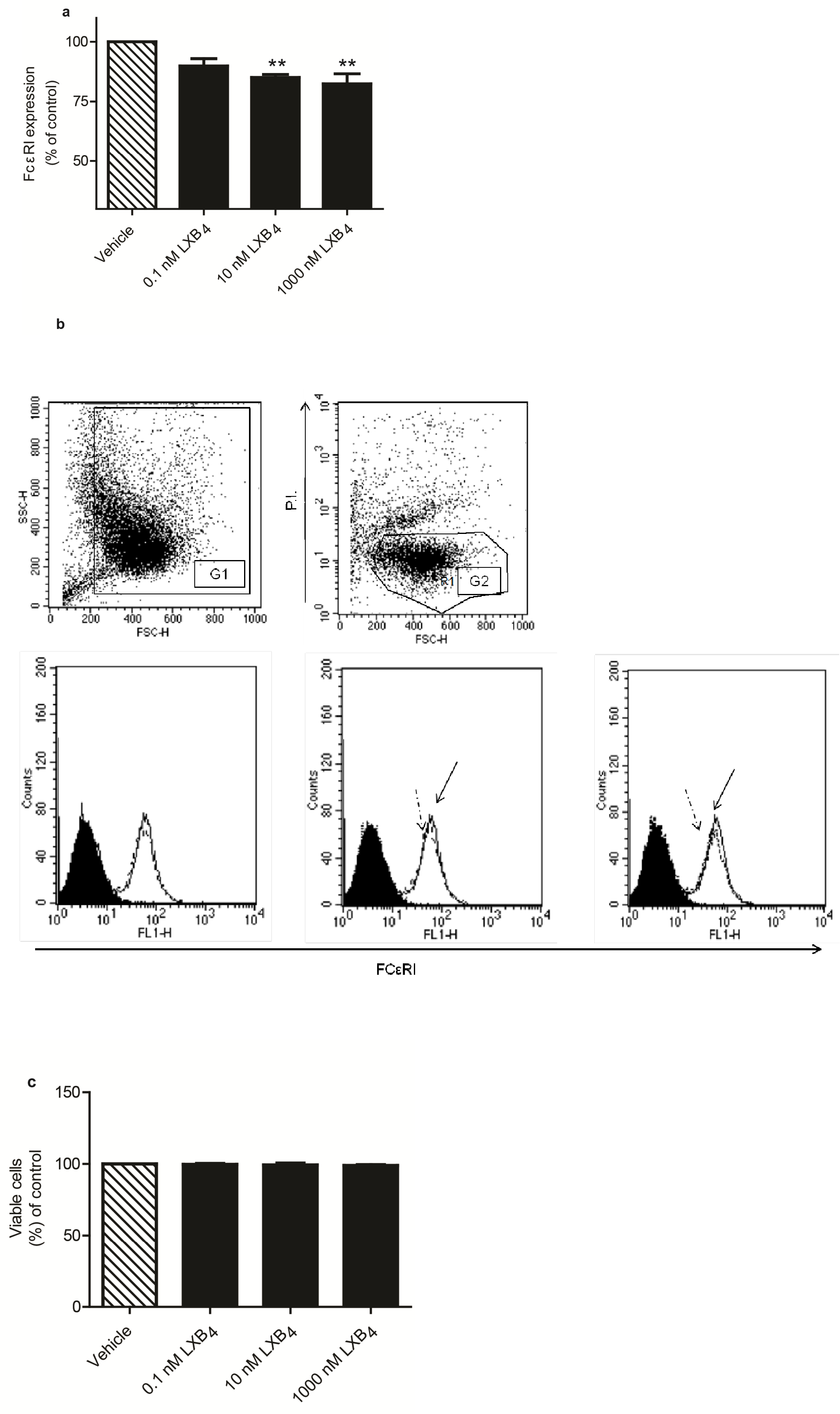

Fig. S4: LXB_4 slightly decreases FCεRI expression without affecting BMMC viability. BMMC from BALB/c mice were incubated for 18 hrs with vehicle at the highest concentration or with different concentrations of LXB_4. **(a)** FCεRI expression (% of control) following incubation with LXB_4. **(b)** representative histogram of FCεRI expression in vehicle (normal line histogram) versus cells incubated with LXB_4 (dashed line histogram). Analysis was performed on live (PI negative-G2) cells of the BMMC population (G1) **(c)** viable cells (% of control) following incubation with LXB_4. Values are normalized to vehicle control, cell viability in each experiment was ~90%. Results are mean $\pm$ SEM of 4 different experiments.