Figure 1

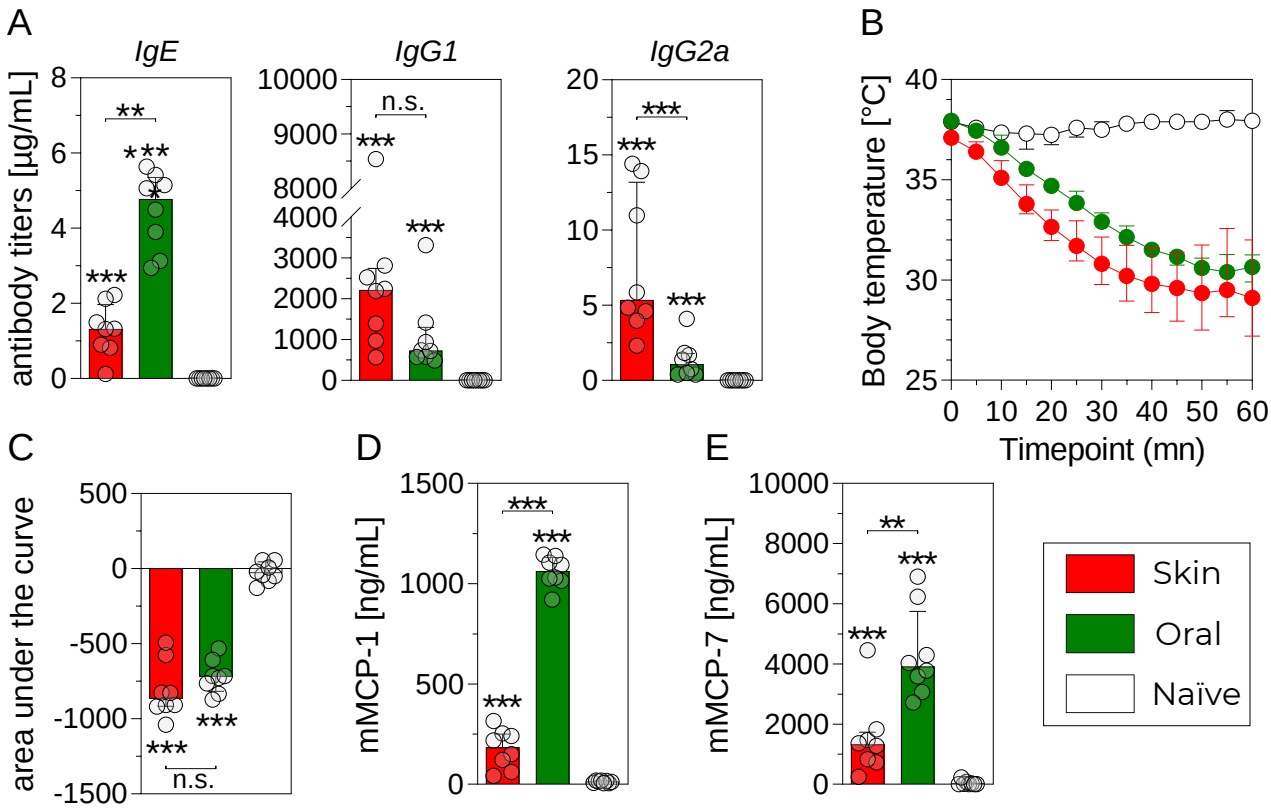

Figure 1: Development and characterization of mouse model of cashew sensitization and anaphylaxis. Mice were sensitized to cashew through skin (in red) or oral (in green) routes. As a negative control, a group of naïve mice (in white) was included. (**A**) Cashew-specific IgE, IgG1 and IgG2a antibody responses were evaluated by ELISA, from blood samples collected one week after the end of the sensitization. Mice were orally challenged to cashew one week after the end of the sensitization. (**B**) Body temperature was measured every 5 minutes following challenge for 60 minutes. (**C**) Area under the curve was calculated for each individual percentage of temperature variation curve using 100 % as a baseline. mMCP-1 (**D**) and mMCP-7 (**E**) concentrations were measured by ELISA from plasma collected immediately after the challenge, (n = 8 per experimental group). Data are median with interquartile range of individual values. P values were determined using the Mann-Whitney unpaired t-test (**, p<0,01; ***, p<0,001; n.s., non-significant). For B, C, D and E panels, the level of significant measured between each sensitized group and the negative control group is indicated above each graph.

Figure 2

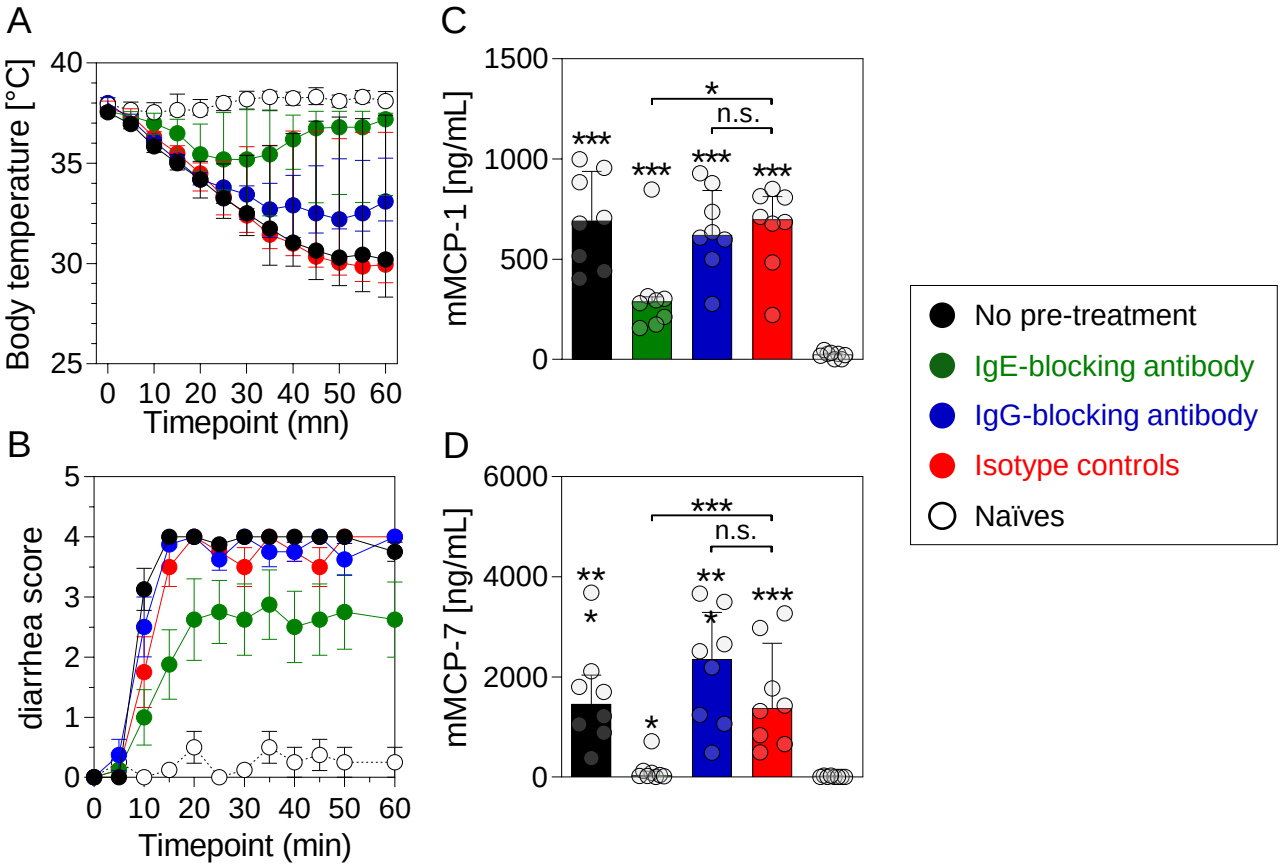

Figure 2: Validation of the oral route of sensitization as a trigger of IgE-mediated anaphylaxis in mice. Mice were orally sensitized to cashew. One week after the end of the sensitization, mice received IgE blocking antibody (clone EM-95, in green), IgG blocking antibody (anti-FcγRII/RIII clone 2.4G2, in blue) or relevant isotype controls (in red). The day after, mice were challenged orally to cashew. (**A**) Body temperature was measured every 5 minutes following challenge for 60 minutes and (**B**) diarrhea occurrence and severity was scored. mMCP-1 (**C**) and mMCP-7 (**D**) concentrations were measured by ELISA from plasma collected immediately after the challenge, (n = 8 per experimental group). Data are median with interquartile range of individual values. P values were determined using the Mann-Whitney unpaired t-test (*, p<0,05; ***, p<0,001; n.s., non-significant). For C and D panels, the level of significant measured between each sensitized group and the negative control group is indicated above each dot plot.

Figure 3

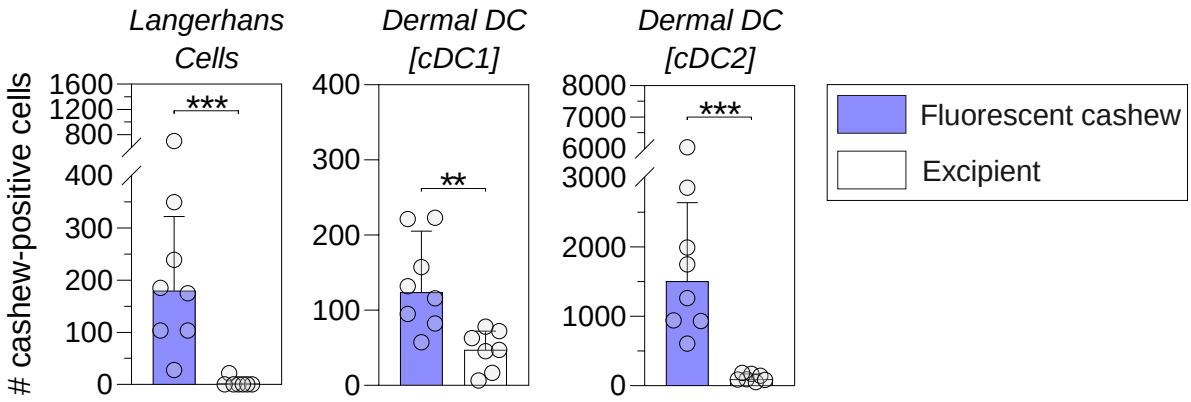

Figure 3: Cashew allergens are efficaciously delivered by epicutaneous patches, leading to allergen capture by skin dendritic cells. Mice were orally sensitized to cashew. One week after the end of the sensitization, mice received a patch loaded with cashew protein extract conjugated to Fluoroprobe-647 (F-647) for 48 hours. As negative controls, mice received a patch containing excipient. Brachial draining lymph nodes were collected, and cells were isolated and labelled for FACS analysis. The absolute number of cashew-positive (F-647-positive) cells was measured among each DC subsets, (n = 7-8 per experimental group). Data are Median and interquartile ranges of individual values. P values were determined according to the Mann-Whitney test (**, $p<0.01$; ***, $p<0.001$).

Figure 4

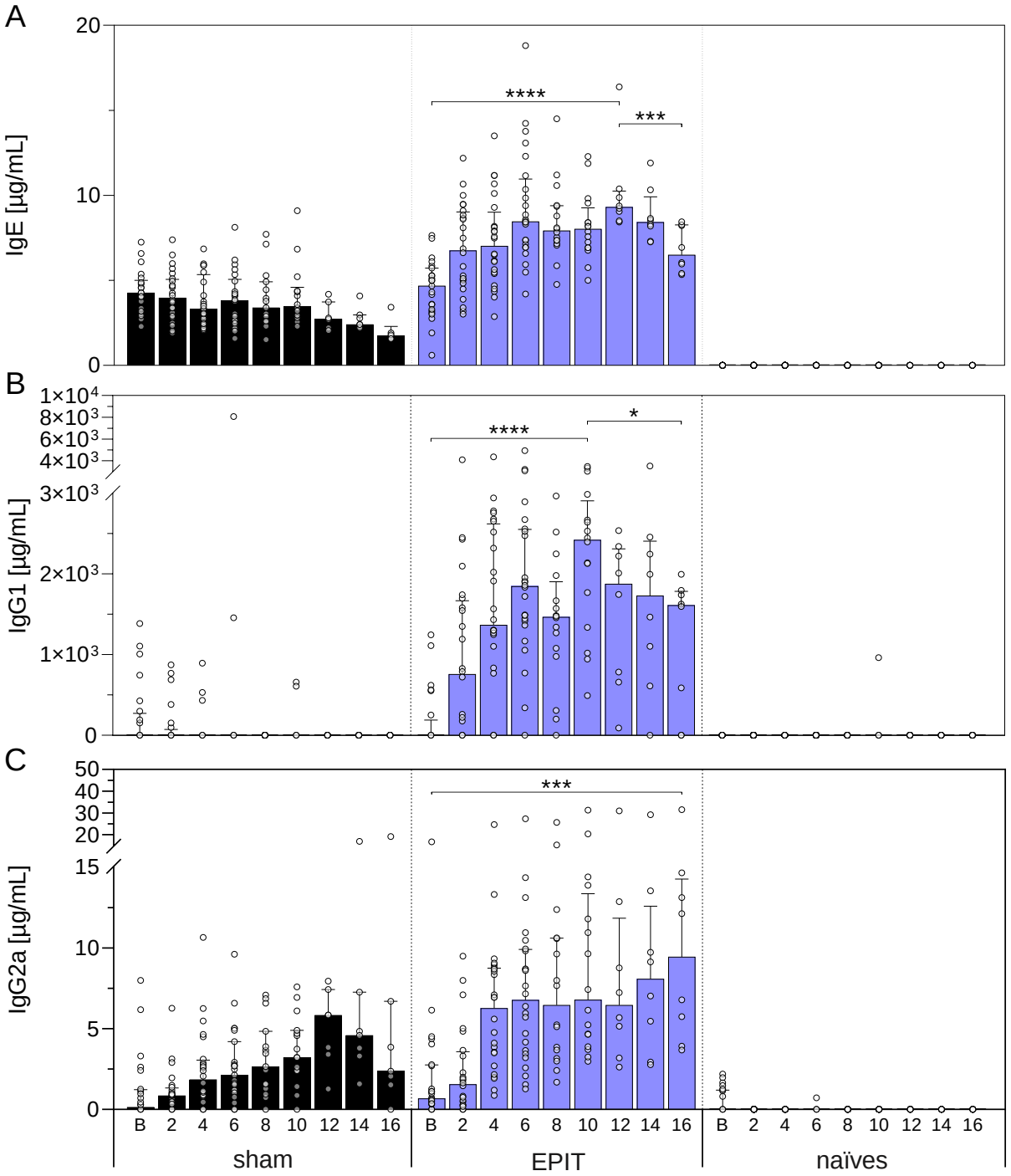

Figure 4: Kinetic modulation of cashew-specific antibody response following EPIT to cashew nut in mice. Mice were orally sensitized to cashew. One week after the end of the sensitization, mice were submitted to EPIT. To that end, mice received cashew patches containing 50 µg of cashew protein extract, once a week for up to 16 weeks (in blue). Patches were applied for 48 hours. As negative controls, mice received patches containing excipient (sham, in black) or were kept untreated (naïves, in white). Blood samples were collected before EPIT (B) or every two weeks during treatment to isolate plasma (weeks 2, 4, 6, 8, 10, 12, 14, 16), as indicated on C panel. Cashew-specific antibody titers were measured from plasma by indirect ELISA (IgE, panel **A**) or direct ELISA (IgG1 and IgG2a, panel **B** and panel **C**, respectively), (n = 8-24 per experimental group – 8 mice of each group were challenged at weeks 8 and 12 and sacrificed). Data are Median and interquartile ranges of individual values.

Figure 5

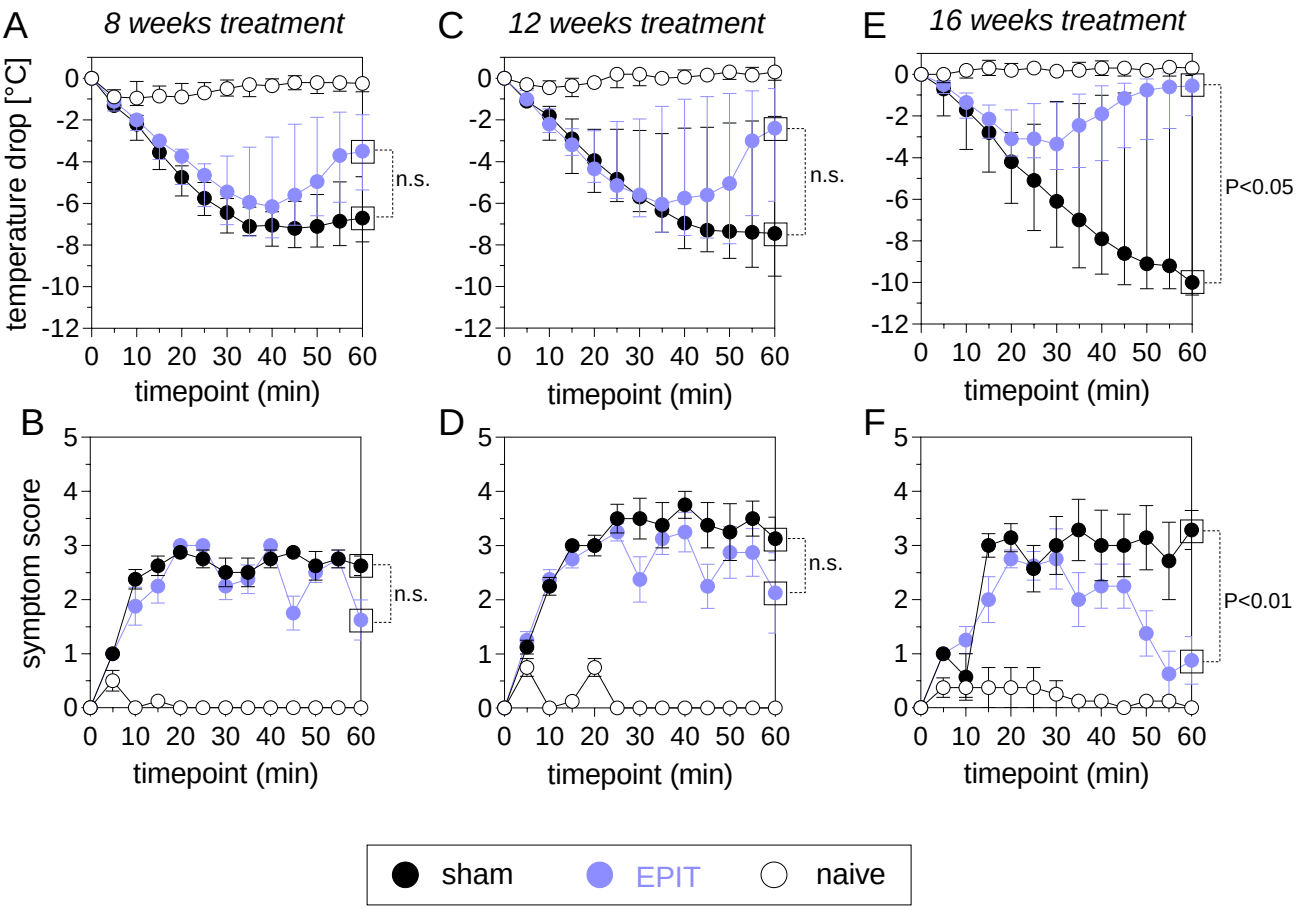

Figure 5: Evaluation of the level of protection afforded by EPIT against anaphylaxis following 8, 12 or 16 weeks of treatment. Mice were orally sensitized to cashew and treated as described in Figure 4. Following 8 (**A**, **B**), 12 (**C**, **D**) or 16 (**E**, **F**) weeks of EPIT, 8 mice of each group were challenged orally to cashew. (**A**, **C**, **E**) Body temperature was measured every 5 minutes following challenge for 60 minutes. Data are Median and interquartile ranges of individual values. (**B**, **D**, **F**) Clinical symptoms were monitored every 5 minutes following challenge for 60 minutes. (n = 8 per experimental group). Data are Mean with SEM of individual values. P values were determined according to the Mann-Whitney test.

Figure 6

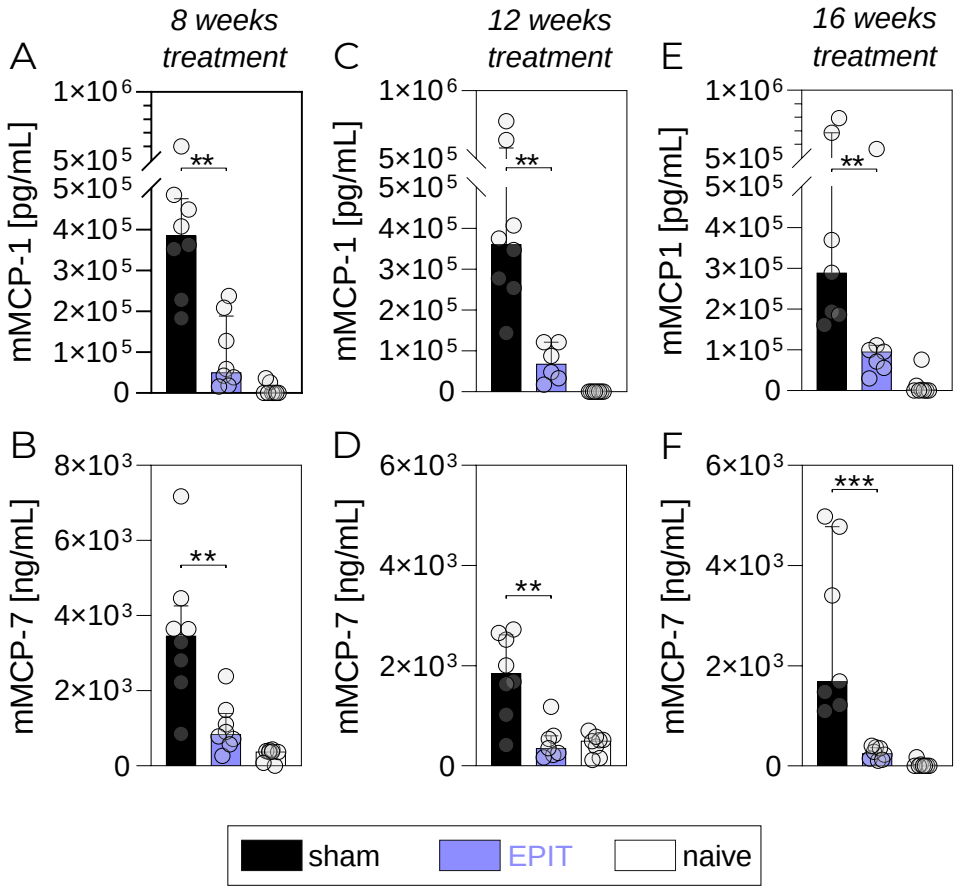

Figure 6: Evaluation of mast-cell activation induced by oral challenge following 8, 12 or 16 weeks of EPIT. Mice were orally sensitized to cashew, treated as described in Figure 4 and finally challenged as described in Figure 5. Blood samples were collected 60 minutes after the challenge to isolate plasma. mMCP-1 (**A**, **C**, **E**) and mMCP-7 (**B**, **D**, **F**) concentrations were measured from plasma by ELISA, (n = 8 per experimental group). Data are median with interquartile range of individual values. P values were determined using the Mann-Whitney unpaired t-test (**, p<0,01; ***, p<0,001).

Figure S1

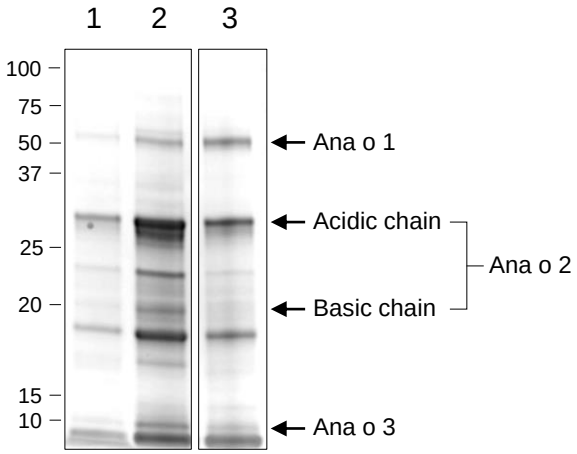

Figure S1: Analysis of on-site prepared cashew protein extracts by SDS PAGE. Protein fraction was extracted from defatted cashew flour as indicated in material and methods. This extract was denatured and analyzed by SDS PAGE (lane 2) in comparison to initial cashew flour (lane 1) and commercial cashew protein extract (Stallergenes Greer, lane 3). The relative molecular mass (kDa) is indicated on the left. The position of major allergens are indicated on the right, based on previous published data [11, 10]. Note that Ana o 2 separates as two distinct bands following denaturation since it is constituted by two subunits (acidic and basic) linked by disulfide bonds.

Figure S2

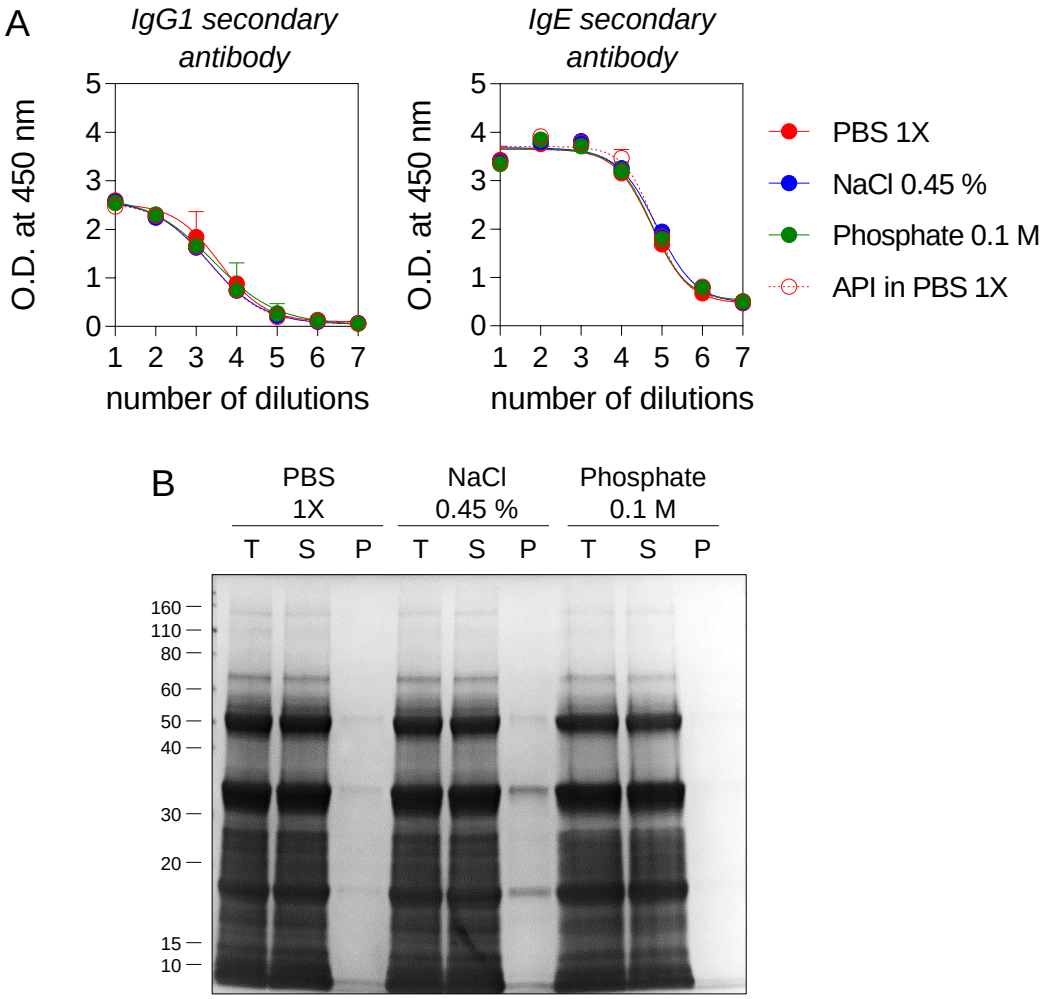

Figure S2: Analysis of cashew protein extracts recovered from patches. Epicutaneous patches were loaded with commercial cashew protein extracts (Stallergenes Greer) in liquid form, by dissolving protein lyophilizate in PBS 1X, NaCl 0.45% or phosphate buffer 0.1 M. Patches were dried for one hour at 30°C. Then, proteins were redissolved from patch backing using distilled water. (**A**) Recovered proteins were used as a coating in ELISA to analyze their capacity to bind to cashew-specific antibodies induced in mice by oral sensitization. HRP-conjugated anti-mouse IgG1 (left panel) or anti-mouse IgE (right panel) were used as secondary antibodies. Optical densities (O.D.) at 450 nm were plotted against plasma dilution, (n = 7 per group). Data are median and range of individual values and non-linear regression curve was obtained using sigmoidal 4PL equation. (**B**) Recovered protein extracts were centrifuged and total extract (before centrifugation, T), supernatant (S) and pellet (P) were denatured and analyzed by SDS PAGE. The relative molecular mass (kDa) is indicated on the left.

Graphical abstract