Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Burks AW, Jones SM, Wood RA, et al. Oral immunotherapy for treatment of egg allergy in children. N Engl J Med 2012;367:233-43. DOI: 10.1056/NEJMoa1200435

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Methods

Skin Prick Testing

Skin prick testing (SPT) with egg extract (Greer Laboratories, Inc., Lenoir, NC) and saline and histamine controls were performed at enrollment and at 10 and 22 months after the initial escalation day. The wheal size was calculated as the average of the largest diameter and the perpendicular midpoint diameter, after subtracting the size of the saline wheal. Endpoint titration skin prick testing was performed at baseline and at 22 months with serial ten-fold dilutions of egg extract. The starting concentration was the standard egg extract 1:20 wt/vol followed by serial dilutions of 1:200, 1:2000, 1:20000, and 1:200000 wt/vol. The endpoint was the lowest concentration of egg extract to result in a wheal of 5 mm greater than the negative control.

IgE Antibody Assay

These were performed in a central laboratory; however, screening IgE antibody assays were performed at local laboratories. There were two subjects whose IgE anti-egg was < 5 kU_A/L, because the entry criteria were based on local laboratory results, which for these two subjects obtained higher levels of IgE anti-egg than the central laboratory.

Basophil Activation Assay

Basophil activation was measured, at various times during treatment, up to Month 22, by CD63 up-regulation by flow cytometry as previously described.[18] Peripheral blood was stimulated with basophil medium alone (medium with 2 ng/mL human IL-3) or the basophil medium with 1, 0.1, 0.01, or 0.001 µg/mL egg white extract

OIT Protocol

While a baseline OFC is generally considered the “gold standard” for confirming a diagnosis of food allergy, this challenge was not performed because the safety of baseline OFC was not fully established at the time the study was initiated.

Epinephrine auto-injectors and instructions on their use were provided to all subjects.

Egg and placebo administration was blinded and identical strategies were used for both. Administration of the egg product is described below. Subjects were instructed to avoid consumption of egg except for the egg in their OIT.

Egg OIT

Initial day escalation. In a clinical research setting, dosing began with 0.1 mg raw egg white powder, followed by an approximate doubling every 30 minutes, up to 50 mg. The maximum tolerated single dose of egg was given in the clinic on the following day and was the starting dose for the build-up phase. Attainment of a minimum dose of 3 mg of egg white powder was required to continue dosing.

Build-up. Subjects ingested a daily dose of egg white powder at home. For subjects whose maximal Day 1 dose was less than 50 mg, doses were doubled every 2 weeks up to 50 mg. After 50 mg, dosing was increased to 75 mg, and then dosing increased by 25% until 2 gm of egg white powder was reached. The maximum time allowed for the build-up phase was 10 months; the dose achieved at 10 months was considered the maintenance dose. Subjects who did not reach 306 mg by 10 months were discontinued from dosing but were included in the endpoint analysis.

*Maintenance***.** After reaching their highest build-up dose (maximum 2 gm), subjects continued this dose daily for at least 2 months before the month 10 OFC and egg OIT subjects continued maintenance dosing through 22 months. Per protocol, subjects not reaching a maintenance dose of 2 gm by 10 months were allowed to escalate to 2 gm after the 10 month OFC.

(Note that 2 gm/day egg white powder is approximately the same as 1.6 gm/day egg white protein; a whole egg is approximately 6-7 gm egg protein.)

Oral Food Challenge (OFC)

The OFC consisted of egg white powder or placebo in gradually increasing doses at 15–30 minute intervals. All subjects underwent a 5 gm DBPC OFC 2 months after reaching the 2 gm (or highest tolerated) dose (at approximately month 10). Egg OIT subjects underwent a second desensitization OFC at a dose of 10 gm at approximately 22 months. The challenge in OIT subjects could be scheduled earlier if egg specific IgE had fallen to <2 kU_A/L in the absence of recent accidental egg exposure with a reaction. Only subjects passing the 22 month OFC were eligible for the 24 month OFC. For subjects who passed the 22 month OFC, all egg consumption, including egg OIT, was discontinued for 4-6 weeks, and the subject then had a 24 month OFC (OFC to assess sustained unresponsiveness). The 24 month OFC consisted of 10 gm egg white OFC followed, 1 hour later, by an open feeding of the equivalent of 1 cooked egg. Placebo subjects had an OFC at 22 months only if their egg-specific IgE was <2 kU_A/L. The OFC was scored as pass or fail. Passing meant subjects successfully consumed the maximum OFC dose of egg white. Failure meant inability to tolerate the maximum dose because of persistent clinically significant symptoms (such as hives, wheezing, vomiting, laryngeal edema); inability to undergo the OFC was considered failure. Persistent symptoms were defined as those that required treatment for resolution or those that worsened over time. Transient symptoms that resolved without treatment did not result in termination of the OFC. Scorers of the OFC were blind to the study group at 10 months.

Additional Information on the Month 10 and Month 22 OFC

During the Month 10 OFC, 33 of 35 tested egg OIT subjects successfully ingested a cumulative dose of at least 1750 mg egg white powder compared with only 1 of 13 placebo recipients; there were no failures between 1000 and 1750 mg. At month 22 all 34 tested egg OIT subjects successfully ingested 1000 mg egg white powder and 31 successfully consumed 2750 mg (an amount that is greater than the maximal daily consumed dose of 2000 mg).

Enrollment and Disposition of Participants

Enrollment and disposition is explained in Figure 1. It should be noted that 136 screened subjects declined participation. The reasons for declining included frequency of visits (at least every 2 weeks for the first 10 months), multiple oral food challenges and multiple blood draws.

Reasons for Withdrawal of Subjects Who Did Not Reach the Month 22 OFC

One placebo subject withdrew after the initial day escalation but before the maintenance phase, because of transportation issues

One placebo subject withdrew just after the initial day escalation because of abdominal pain, difficulty breathing (but without wheezing) and emesis

One egg OIT subject withdrew before month 5.5 because of recurrent intermittent vomiting that required a course of prednisone.

One egg OIT subject withdrew before month 5.5 because of generalized hives and facial flushing, and recurrent allergic symptoms even though the oral dose of egg was reduced.

One egg OIT subject withdrew before month 5.5 because of abdominal pain and mild emesis, which persisted through the first week of dosing.

One egg OIT subject withdrew before month 5.5 because of abdominal pain and nausea, and recurrent allergic symptoms including lip swelling.

One egg OIT subject withdrew before month 5.5 because of anxiety and severe self limitation of food

consumed.

One egg OIT subject withdrew after the 10 month OFC because of wheezing and abdominal pain which persisted even though the oral dose of egg was reduced.

Immunological Correlates of Egg OIT

Skin prick Tests. SPT wheal sizes decreased significantly more from baseline to Month 22 among subjects in the egg OIT group compared with subjects in the placebo group (p=0.02) (Figure E). When positive skin tests were measured with end-point titration, egg OIT subjects had a median 1-log decrease, whereas placebo recipients had no change (p=0.009).

Basophil Activation (CD63 expression). Basophil activation decreased more during therapy in the egg OIT group compared with the placebo group (Figure F) and these differences were statistically significant (0.01 μg/mL, p=0.002; 0.1 μg/mL, p=0.001).

Egg-Specific Serum IgE and IgG4. The median reduction in egg-specific IgE levels from baseline to Month 22 (-5.9 [range: -94.6, 16.6] kU_A/L) for egg OIT was not significantly greater (p=0.06) than that for placebo (-1.9 range [-23.5, 37.1] kU_A/L) (Figure G). In contrast, the median change from baseline to Month 22 in egg-specific IgG4 levels in the egg OIT group (median: 48.5 [range: -0.1, 162.1] kU_A/L, p<0.001) was significantly higher than that for placebo (Figure H).

Supplementary Tables

Table S1. CoFAR Grading System for Allergic Reactions.

Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe	Grade 4 Life threatening	Grade 5 Death
Transient or mild discomforts (< 48 hours), no or minimal medical intervention/therapy required. These symptoms may include pruritus, swelling or rash, abdominal discomfort or other transient symptoms.	Symptoms that produce mild to moderate limitation in activity some assistance may be needed; no or minimal intervention/therapy is required. Hospitalization is possible. These symptoms may include persistent hives, wheezing without dyspnea, abdominal discomfort/ increased vomiting or other symptoms	Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalization is possible. Symptoms may include bronchospasm with dyspnea, severe abdominal pain, throat tightness with hoarseness, transient hypotension among others. Parenteral medication(s) are usually indicated.	Extreme limitation in activity, significant assistance required; significant medical/therapy. Intervention is required; hospitalization is probable. Symptoms may include persistent hypotension and/or hypoxia with resultant decreased level of consciousness associated with collapse and/or incontinence or other life threatening symptoms.	Death

Supplementary Figures

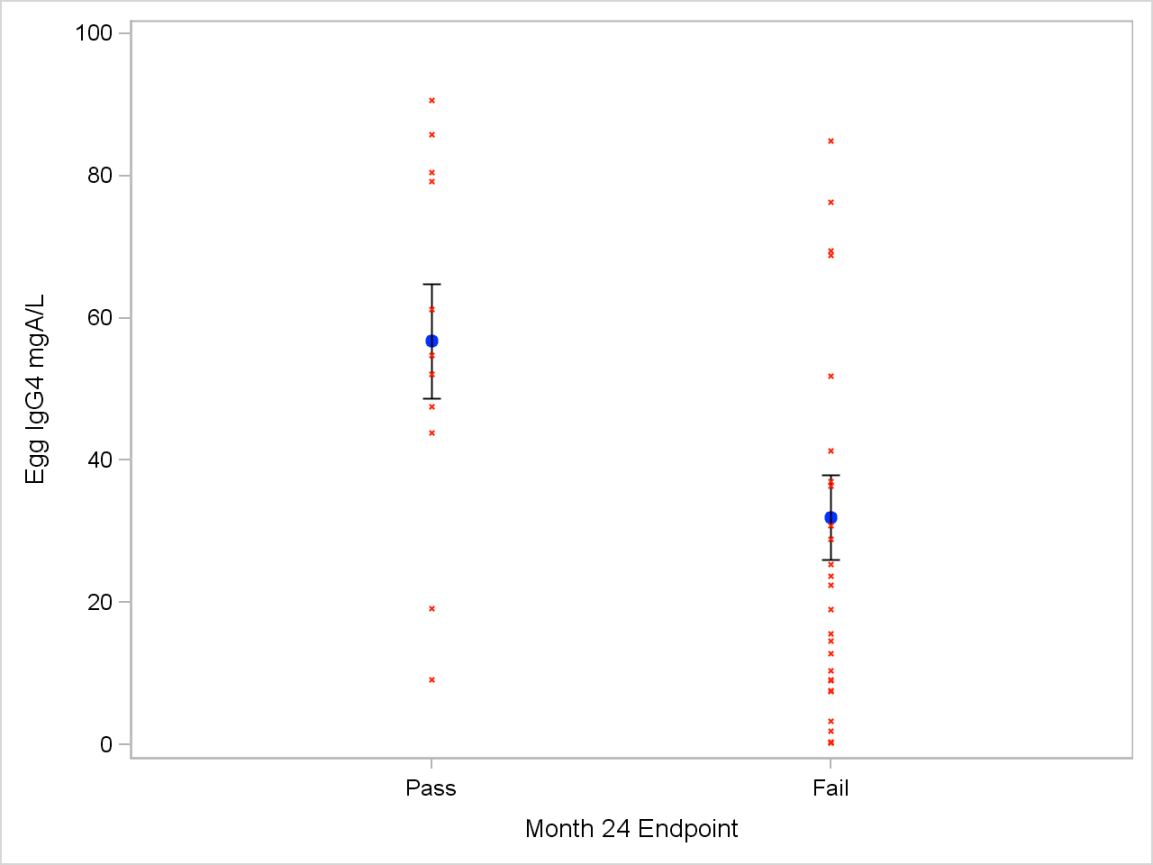

Figure S1. Egg OIT Subjects Month 10 Egg-Specific IgG4 by 24 Month Sustained Unresponsiveness Success vs. Failure. (Additional Statistics in Table 2B)

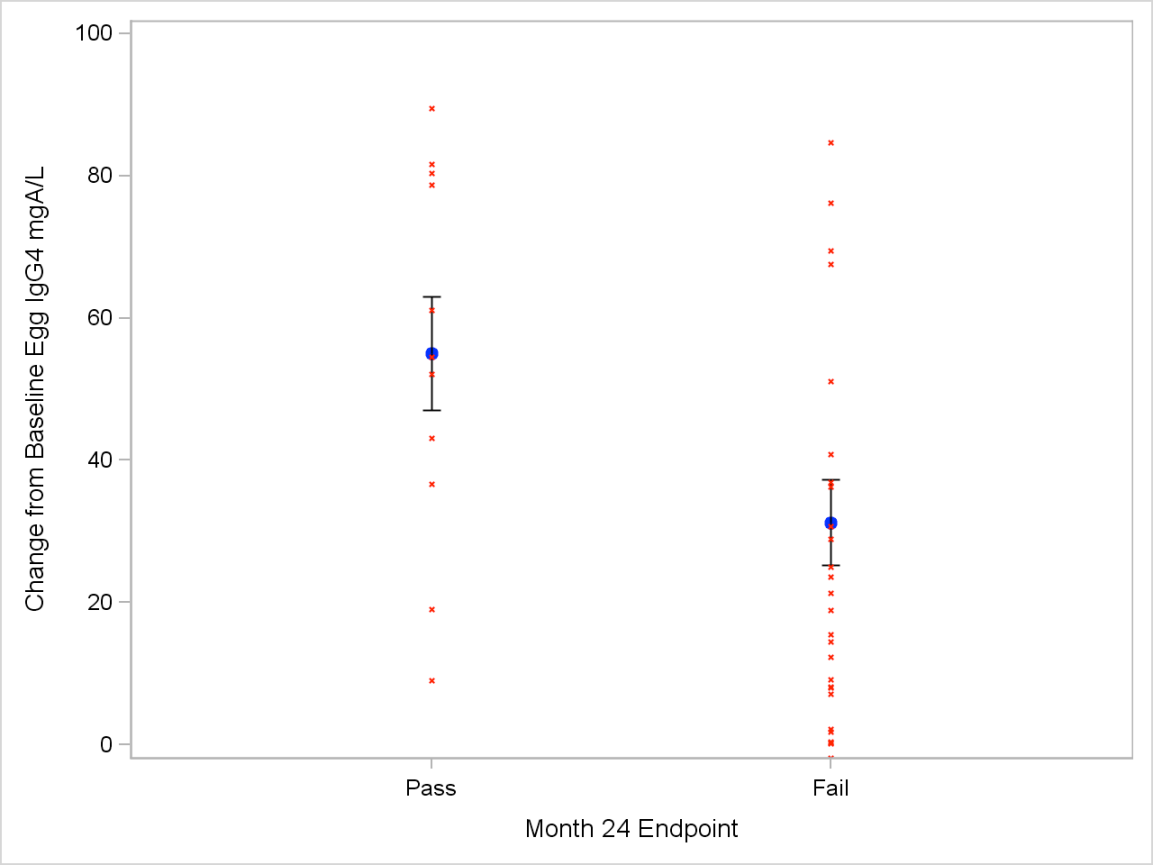

Figure S2. Egg OIT Subjects Month 10 Egg-Specific IgG4 Change From Baseline by 24 Month Sustained Unresponsiveness Success vs. Failure. Medians pass 54.5 mg_A/L vs. failure 21.3 mg_A/L (p=0.02).

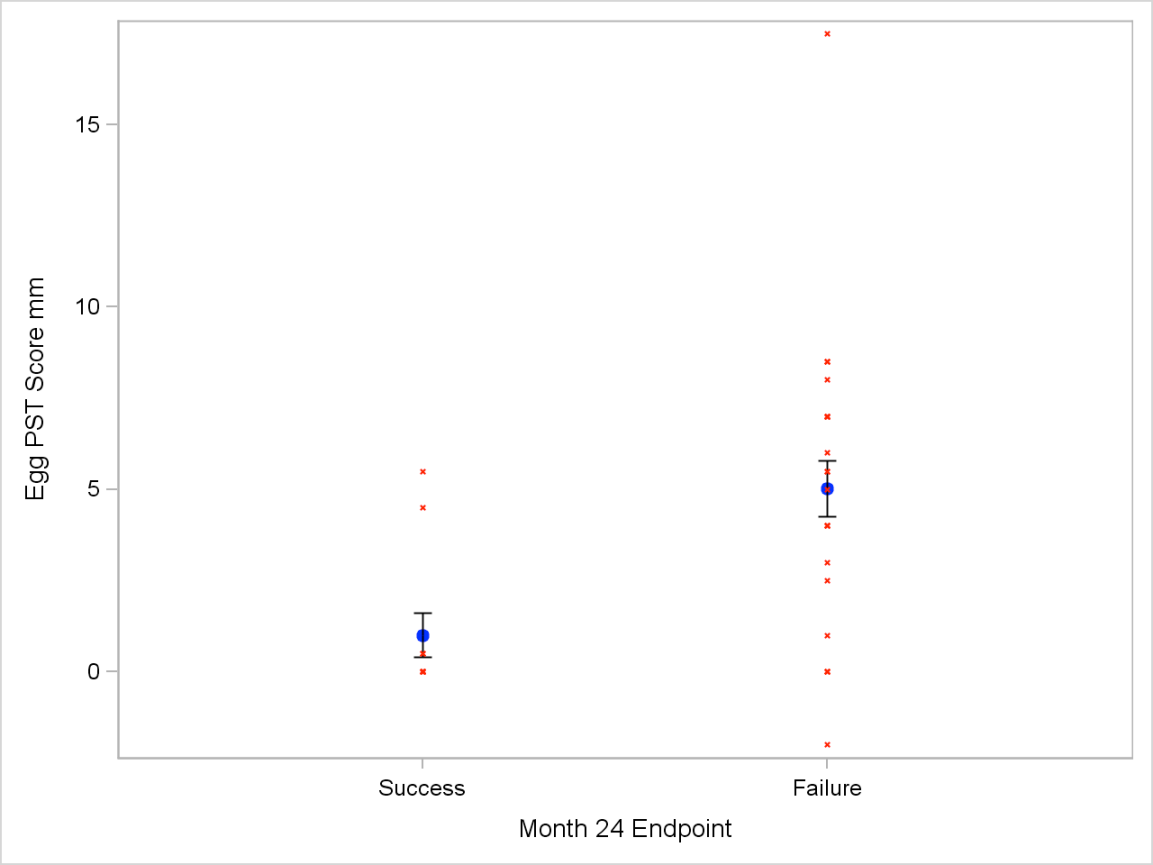

Figure S3. Egg OIT Subjects Month 22 Skin Prick Test Score by 24 Month Sustained Unresponsiveness Success vs. Failure. (Additional Statistics in Table 2B)

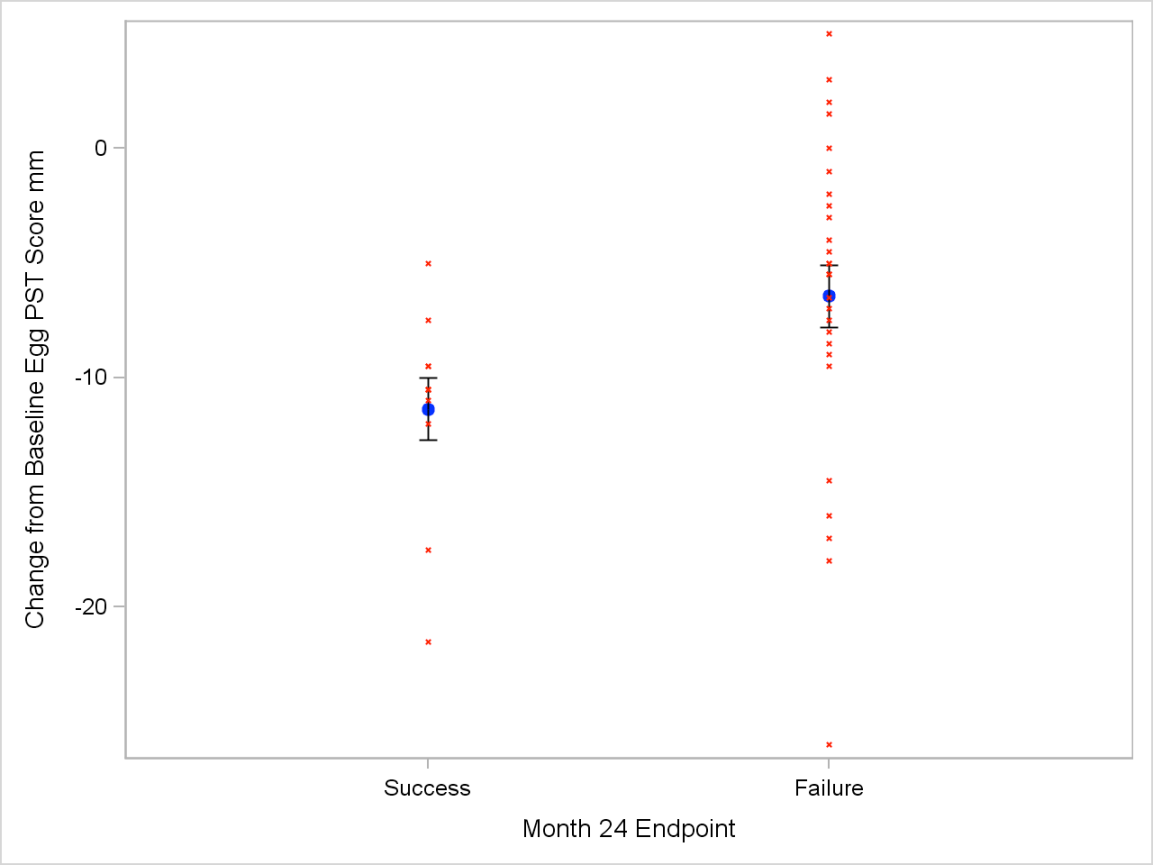

Figure S4. Egg OIT Subjects Month 22 Skin Prick Test Score Change From Baseline by 24 Month Sustained Unresponsiveness Success vs. Failure. Medians pass -10.5 mm vs. failure 5.5 mm (p=0.01).

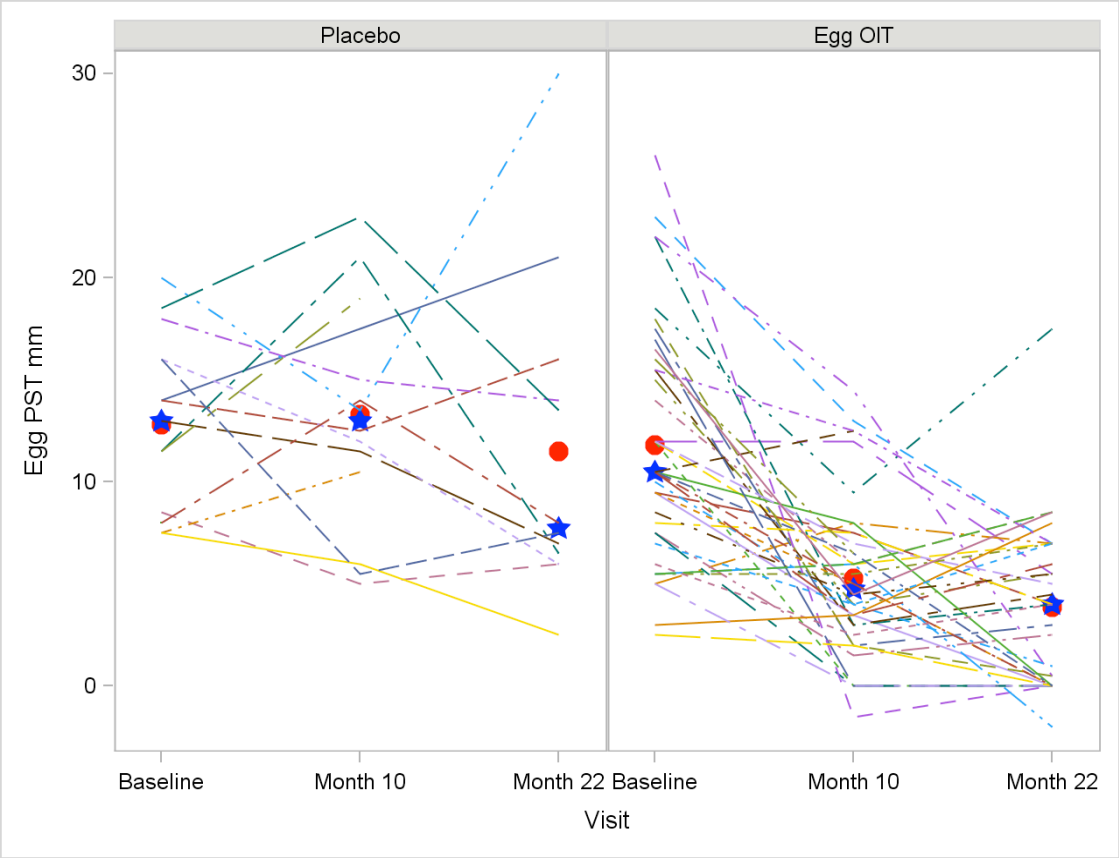

Figure S5. Effect of Egg OIT on Skin Prick Tests. Skin prick test sizes from baseline to month 22 decreased more in subjects undergoing egg OIT than placebo OIT (p=0.02). Circle identifies the mean, star the median.

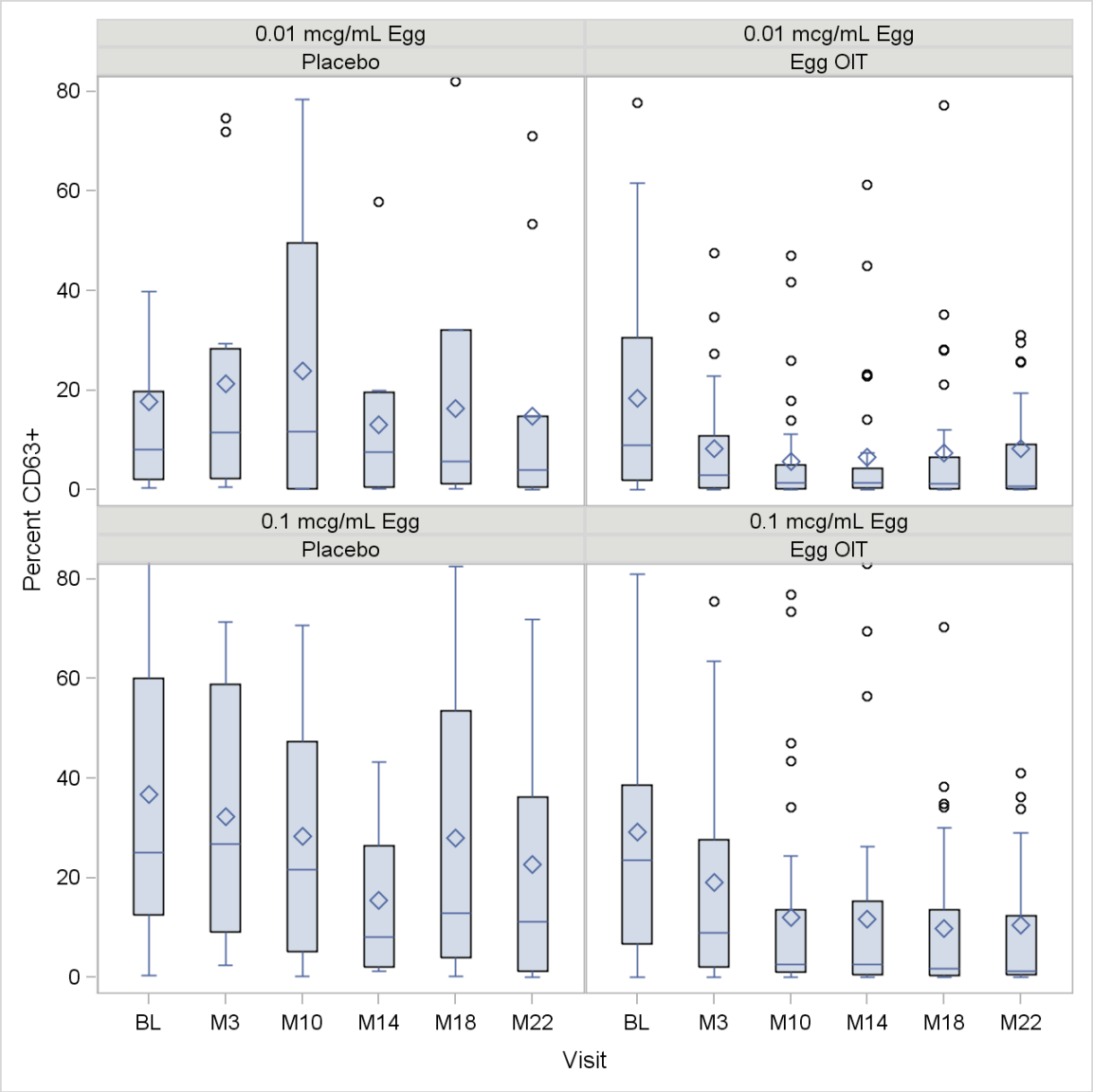

Figure S6. Effect of Egg OIT on Basophil Activation. Left column: placebo OIT. Right column: egg OIT. Cells were stimulated with 0.01 μg/mL egg white extract (top row) or 0.1 μg /mL egg white extract (bottom row). Basophil responsiveness was significantly decreased post baseline in egg OIT subjects versus placebo (0.01μg/mL, Δ=-14.1, p=0.002; 0.1μg/mL, Δ=-17.2, p=0.001).

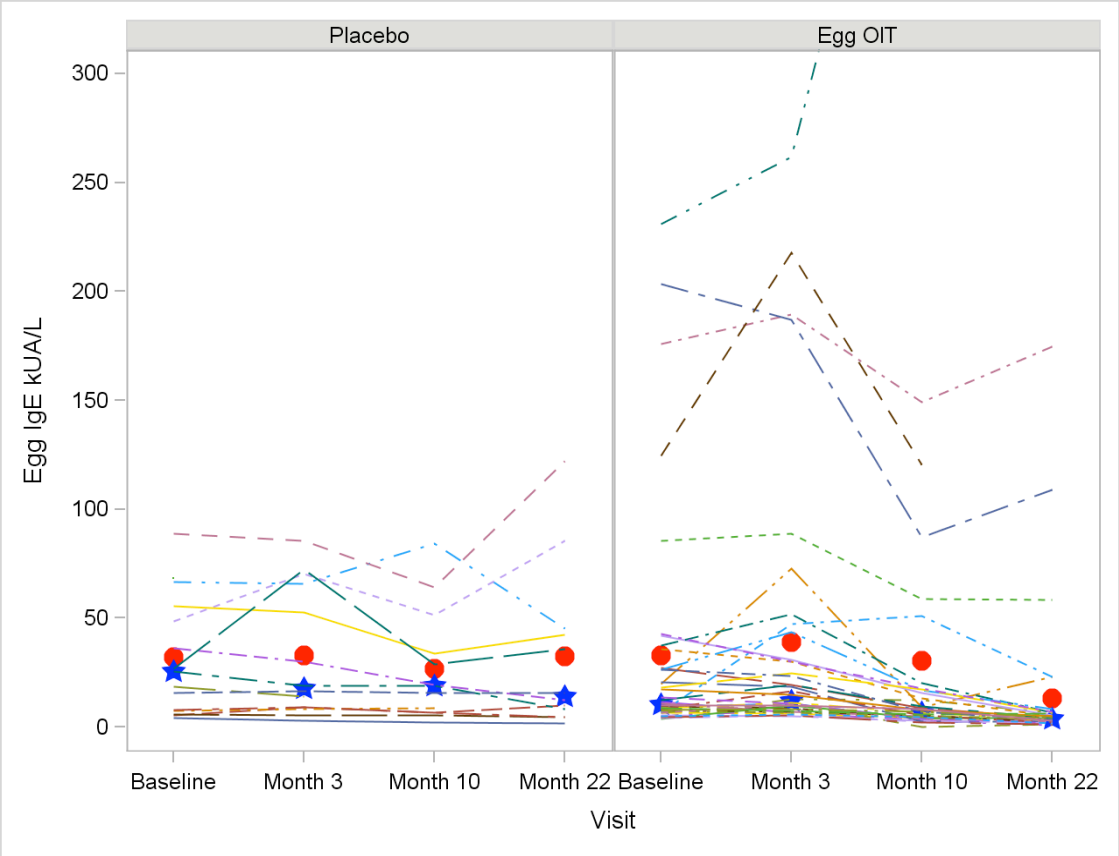

Figure 7S. Change in Serum Levels of Egg-Specific IgE During OIT. Serum levels of egg-specific IgE were measured using the ImmunoCAP^{TM} instrument. At Month 22, egg-specific IgE is lower in the egg OIT group (median=3.8 kU_A/L) than placebo (median=14.0 kU_A/L) ($p<0.001$) although changes from baseline are not different (see text). Circle identifies the mean, star the median.

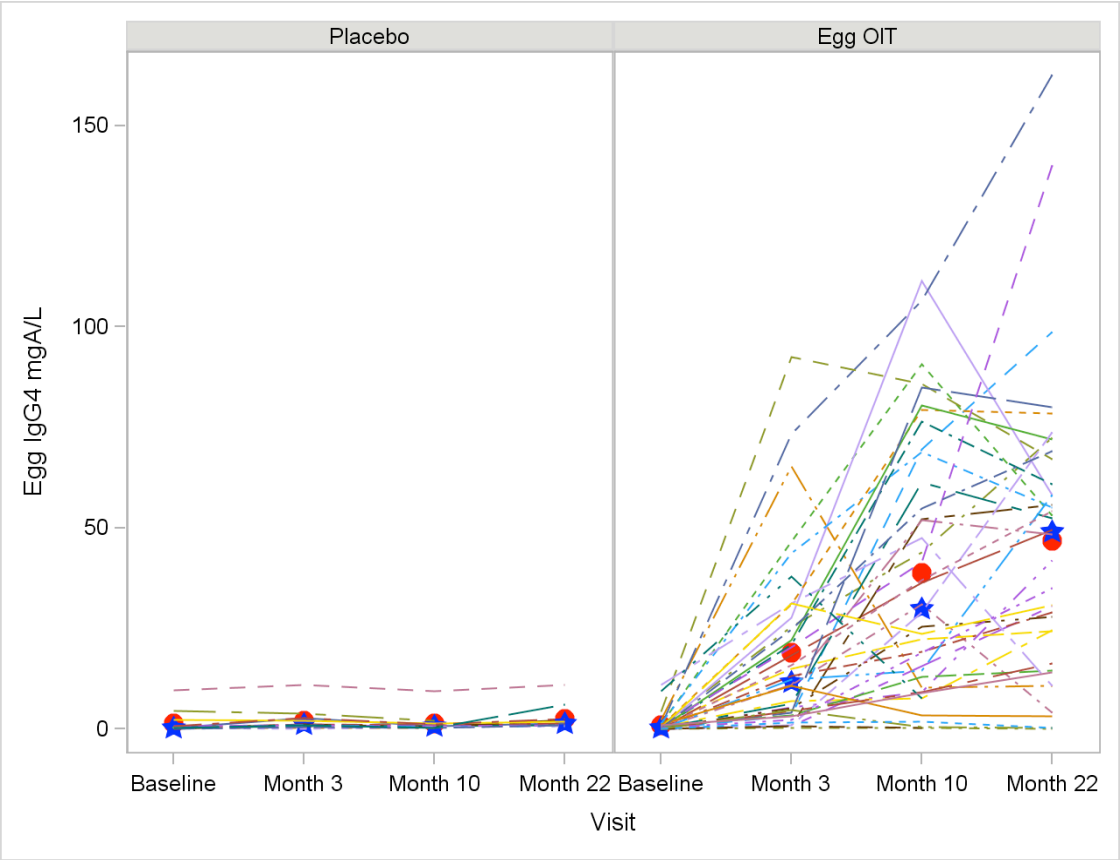

Figure S8. Change in Serum Levels of Egg-Specific IgG4 During OIT. Serum levels of egg-specific IgG4 were measured using the ImmunoCAP^{TM} instrument. Levels were compared from baseline to 3, 10, and 22 months of therapy. Significant increases occurred after egg OIT (*$p < 0.001$) and between treatment groups (*$p < 0.001$). Circle identifies the mean, star the median.