Table E1: Predicted results in placebo group if recommended-to-treat safety and efficacy subgroups received omalizumab[1]

Algorithm for Treating Subgroup	Success Endpoint	Number Placebo Subjects who would be "Treated" Under Algorithm	Success in Placebo Arm	
			Observed	Predicted if subgroup treated
Safety	[2]Mod/Sev < 0.25%	20/26	14/26 = 0.538	24.54/26 = 0.944
Safety	[3]GI < 2%	20/26	16/26 = 0.615	23.54/26 = 0.905
Safety	[4]Any symp except oral < 5%	20/26	10/26 = 0.385	23.54/26 = 0.905
Efficacy	SU	14/27	9/27 = 0.333	15.5/27 = 0.574

[1]Assumes that placebo subjects (theoretically treated with omalizumab) achieved the same success rate as the corresponding subgroups in the omalizumab arm

[2]Percent of MOIT doses that caused moderate to severe symptoms; [3]GI symptoms; or [4]any symptoms other than oropharyngeal symptoms

Fig E2

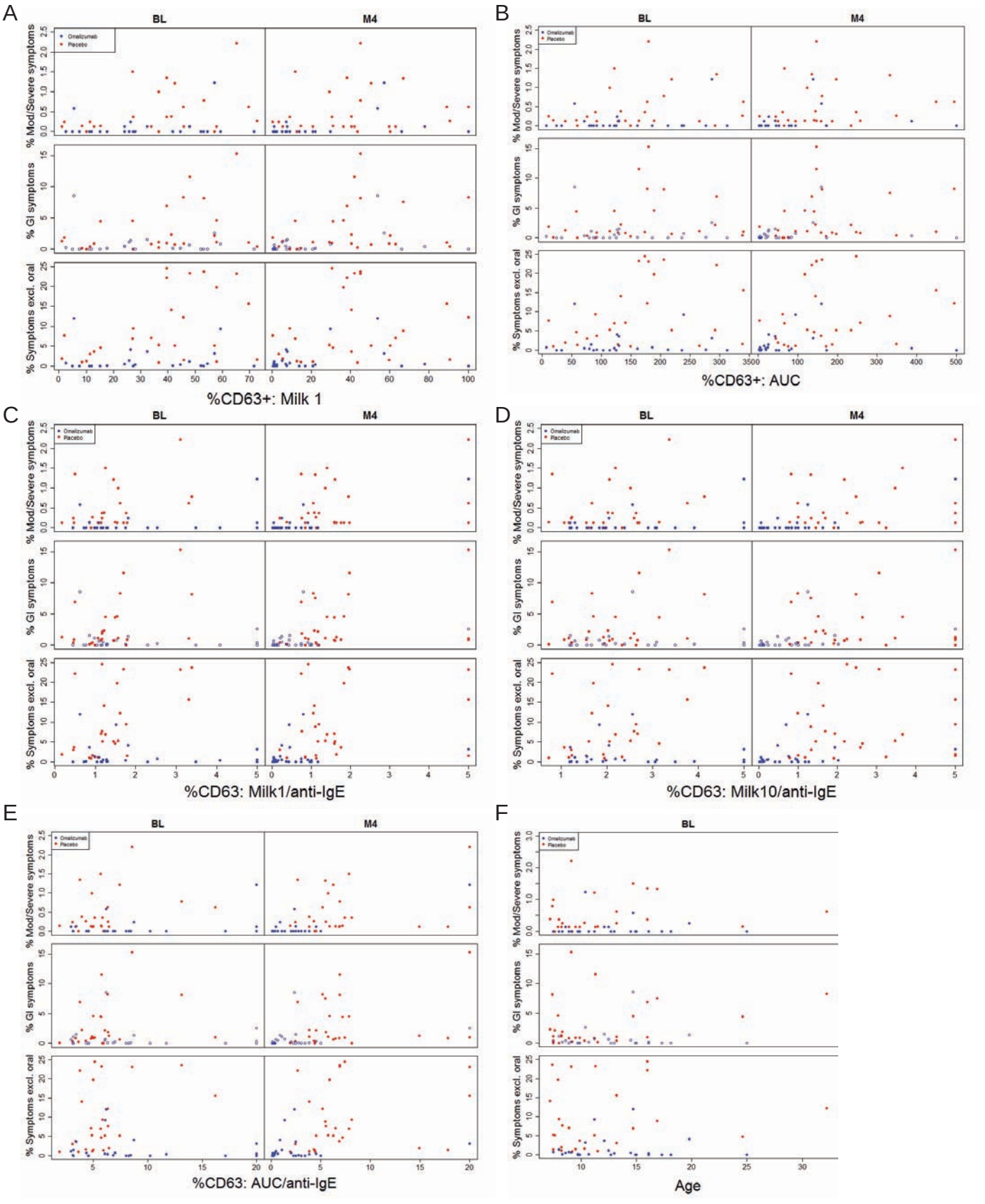
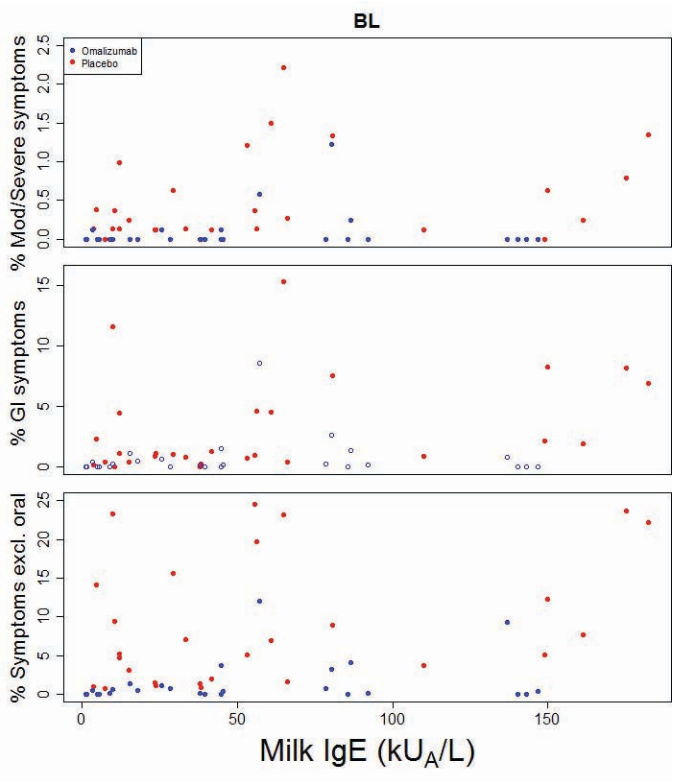
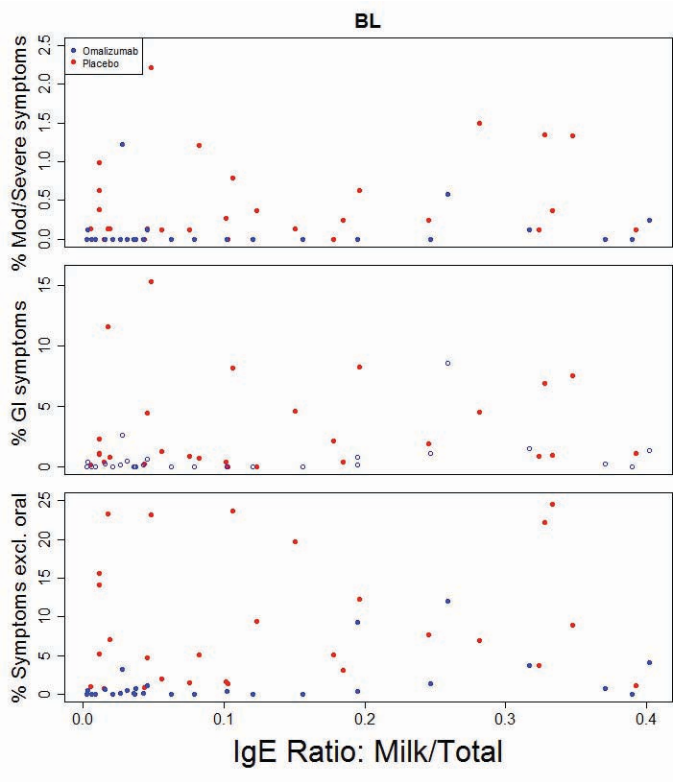

A
B
C
D
E
F
BL
M4
Omalizumab
Placebo
% Mod/Severe symptoms
% GI symptoms
% Symptoms excl. oral
%CD63+: Milk 1
%CD63+: AUC
%CD63: Milk1/anti-IgE
%CD63: Milk10/anti-IgE
%CD63: AUC/anti-IgE
Age

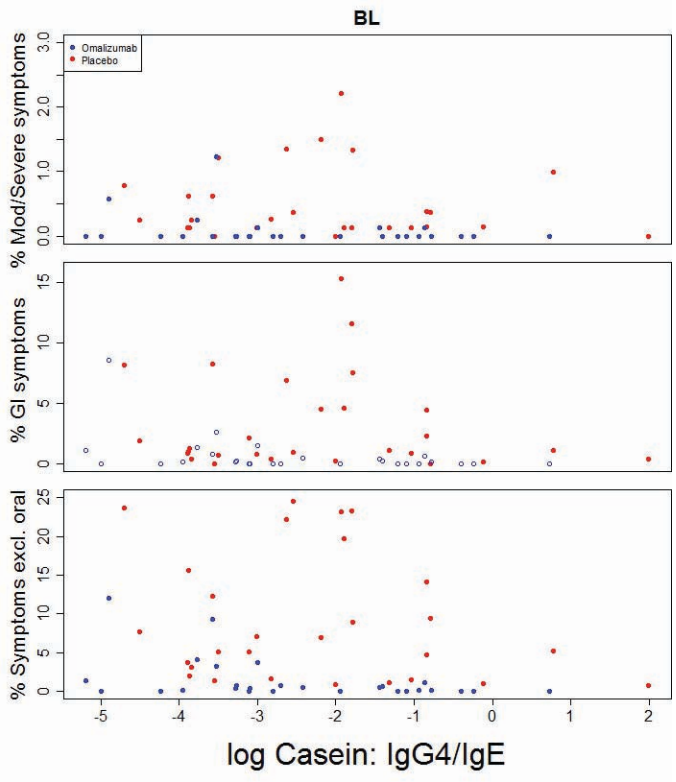
Fig E2 (cont.)

G





H



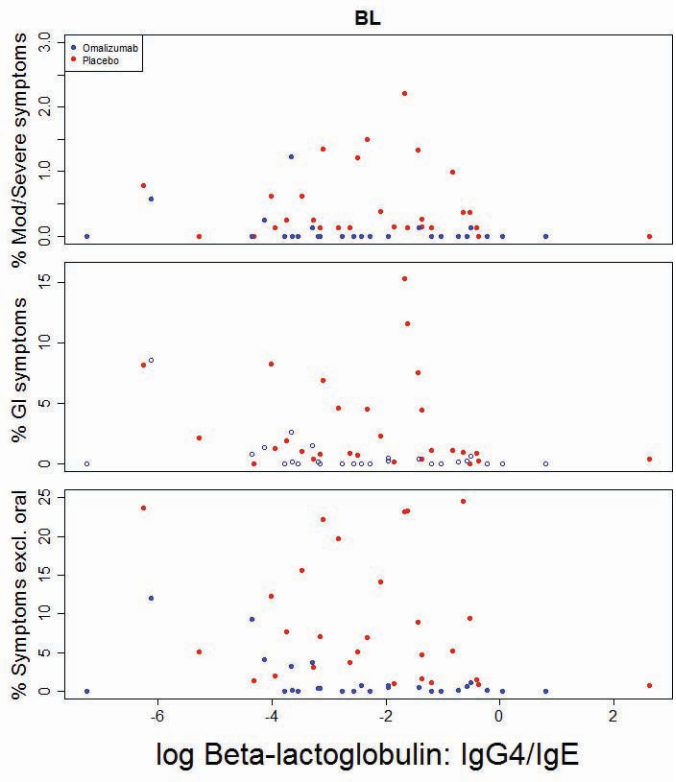


Fig E4

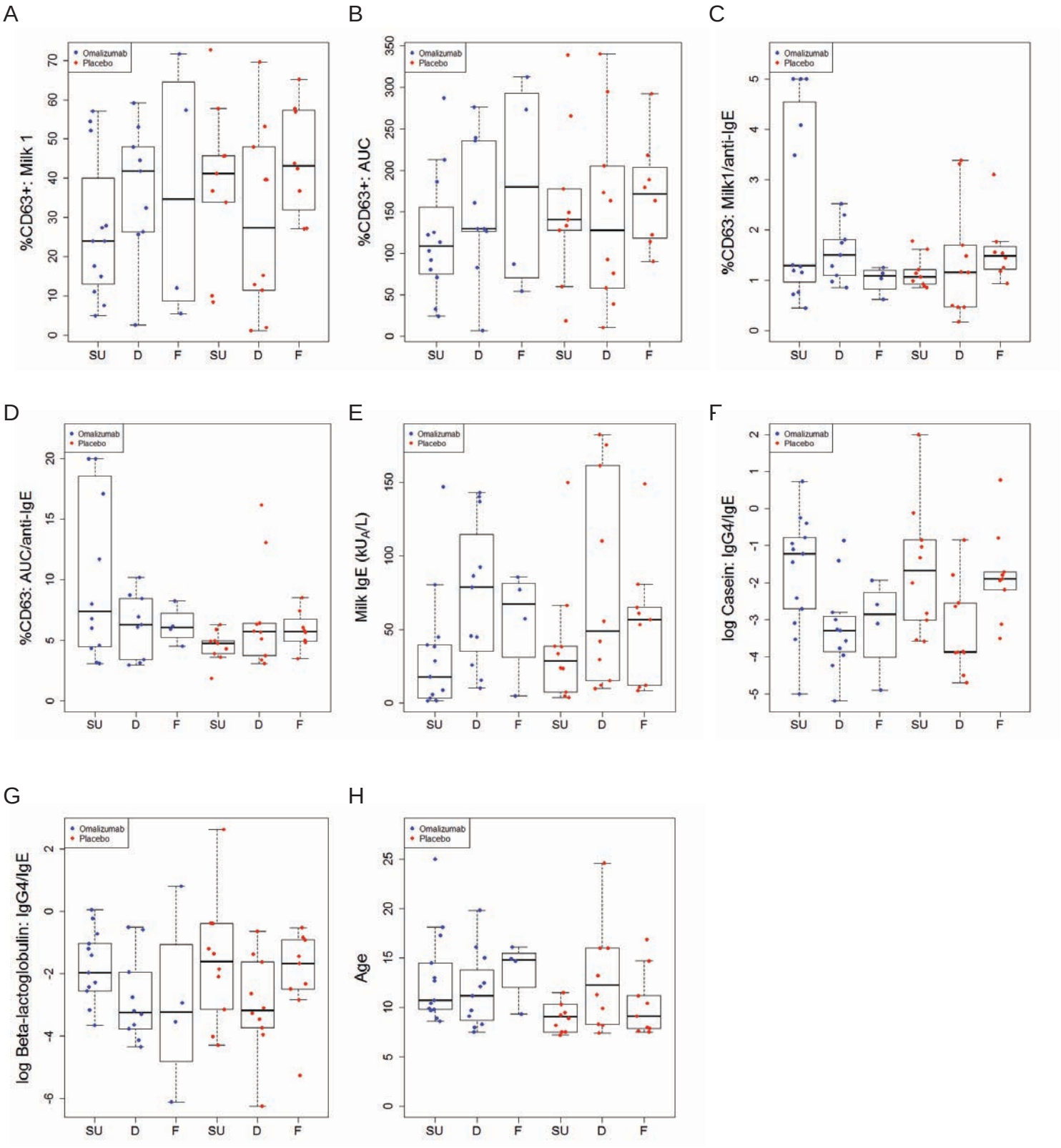

A
B
C
D
E
F
G
H
Omalizumab
Placebo
%CD63+: Milk 1
%CD63+: AUC
%CD63: Milk1/anti-IgE
%CD63: AUC/anti-IgE
Milk IgE (kU$_A$/L)
log Casein: IgG4/IgE
log Beta-lactoglobulin: IgG4/IgE
Age
SU
D
F

Fig E5

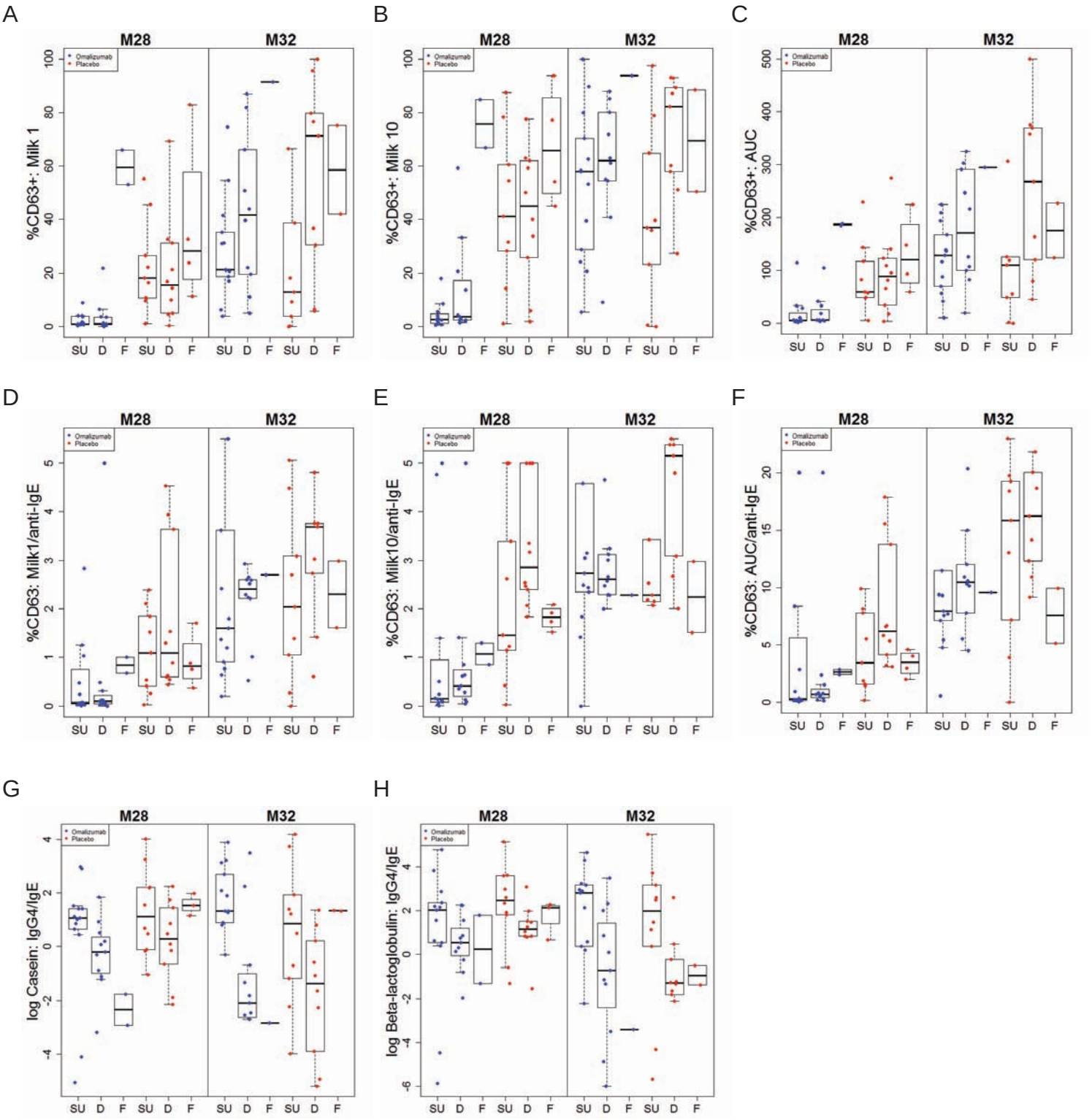

A
B
C
D
E
F
G
H
M28
M32
Omalizumab
Placebo
%CD63+: Milk 1
%CD63+: Milk 10
%CD63+: AUC
%CD63: Milk1/anti-IgE
%CD63: Milk10/anti-IgE
%CD63: AUC/anti-IgE
log Casein: IgG4/IgE
log Beta-lactoglobulin: IgG4/IgE
SU D F SU D F SU D F SU D F

Fig E1

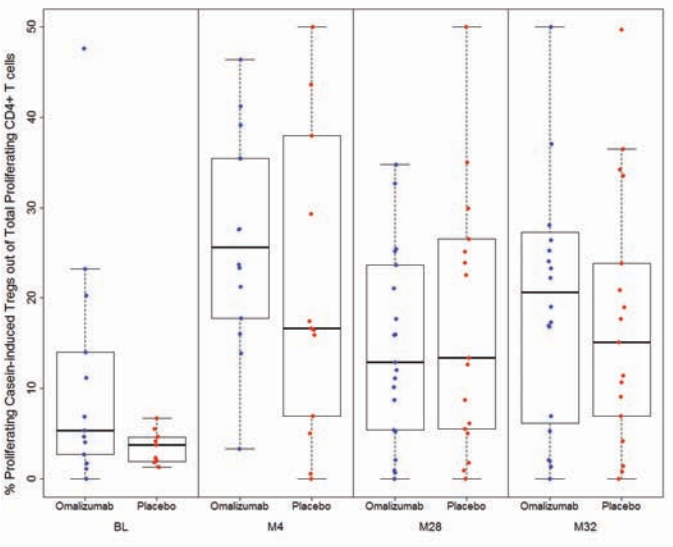
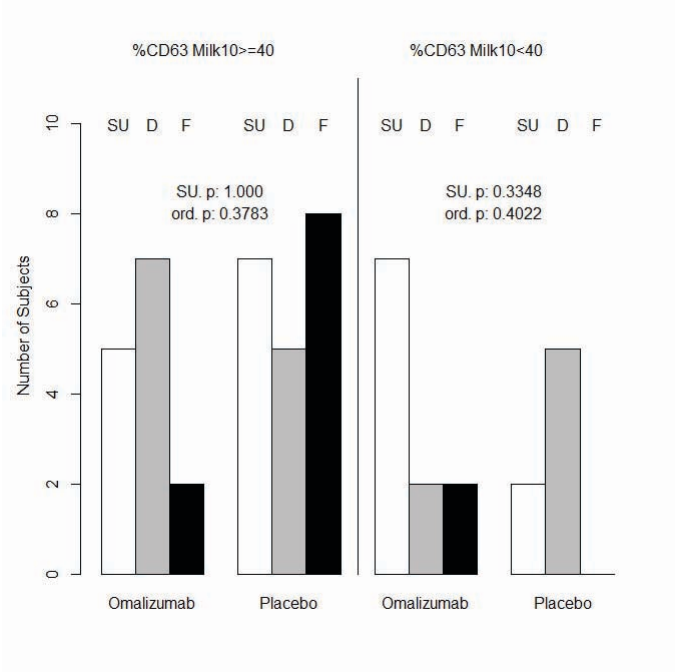

% Proliferating Casein-induced Tregs out of Total Proliferating CD4+ T cells
0
10
20
30
40
50
Omalizumab
Placebo
BL
M4
M28
M32

Fig E3


%CD63 Milk10>=40
%CD63 Milk10<40
SU D F SU D F SU D F SU D F
SU. p: 1.000
ord. p: 0.3783
SU. p: 0.3348
ord. p: 0.4022
Number of Subjects
0 2 4 6 8 10
Omalizumab
Placebo
Omalizumab
Placebo

Online Methods

Basophil Assays

Whole heparinized blood (0.25 mL per condition) was stimulated with anti-IgE (1 µg/mL) (Bethyl Laboratories, Montgomery, TX) or milk allergen (a five-log concentration ranging from 0.001 µg/mL to 10 µg/mL of aqueous extract of non-fat dried milk powder) in complete RPMI medium (Life Technologies, Grand Island, NY) with human recombinant IL-3 (R&D systems, Minneapolis, MN) at 2ng/mL for 30 minutes at 37℃. In addition, control wells were mock-stimulated with medium alone or IL-3 alone. The reaction was stopped by the addition of 2 mM EDTA in PBS, and cells were surface stained with fluorescent conjugated mAbs against CD203c (Beckman Coulter, Brea, CA), CD63, CD123, CD69, CD3, CD14, CD19, CD41, and HLA-DR (BD Biosciences, Franklin Lakes, NJ). Red cells were then lysed and white blood cells were fixed with BD FACS lysis buffer (BD Biosciences). Basophils were defined as $CD123^{+}$, $CD203c^{+}$, $HLA\text{-}DR^{-/lo}$, $CD41a^{-}$, $CD3^{-}$, $CD14^{-}$, $CD19^{-}$ cells and were analyzed for the percentage of cells that were $CD63^{+}$. Cells were analyzed on an LSR II cytometer (BD Biosciences; San Jose, CA) and analysis was performed using the FlowJo software (Tree Star, Inc; Ashland, OR).

At the Mount Sinai and Johns Hopkins sites, blood was collected in EDTA for histamine assays, diluted with normal saline, and subjected to 61% Percoll (Pharmacia Biotech, Piscataway, NJ) density centrifugation. The cell layer generated was aspirated and washed four times in piperazine-N,N′-bis(2-ethanesulfonic acid) (PIPES)/albumin/glucose/EDTA and then again in PIPES/albumin/glucose (PAG). Cells were resuspended in PAG, divided equally, and cultured in a final volume of 50 µL in AIM V medium (Invitrogen Life Technologies, Carlsbad, CA) containing the same panel of stimulants described above, except the culture media did not contain IL-3. After a 45-

minute incubation at 37℃, 1 mL of PAG was added, and the supernatants were frozen at -20℃ until final HR assay. HR was measured in the cell-free culture supernatants by using automated fluorometry. Percentage HR under each condition was calculated relative to total histamine content, which was determined by treating an identical number of cells with perchloric acid (1.6% final). Spontaneous HR was subtracted from values reporting HR in response to milk and anti-IgE.

Treg Assays

PBMCs were labeled with 5 μM CFDA-SE (Fisher Scientific, Pittsburg, PA) and cultured with 50 μg/mL purified milk proteins (α, β, κ caseins, 50 μg/mL each) or media alone in complete serum free AIM-V media supplemented with IL-2 (10 ng/mL; R&D Systems) for 7 days. Cells were harvested, surface stained for CD3, CD4, CD25, and CD127 (BD BioSciences) then fixed, permeabilized, and stained for FoxP3 (clone PCH101, eBioscience; San Diego, CA) according to manufacturer's protocol. Tregs were defined as $CD25^{hi}FoxP3^{hi}$ cells gated from $CD3^{+}$ $CD4^{+}$ $CFSE^{lo}$ $CD25^{+}$ $CD127^{lo/-}$ populations. Minimal to no proliferation was observed in control cultures stimulated with media alone (no casein).

Statistical Analysis

We considered the following baseline biomarkers: %CD63 positive cells at dose 1 and 10 μg/mL and the sum (area under the curve; AUC) of %CD63 following stimulation with the 5 dilutions of milk; these same three variables divided by %CD63 following anti-IgE stimulation; milk IgE; milk-specific IgE divided by total IgE; age at entry into the study; casein-specific IgG4 divided by casein-specific IgE; and beta-lactoglobulin IgG4 divided by beta-lactoglobulin IgE. One key variable, %CD63 at milk dose 10 μg/mL divided by %CD63 to anti-IgE, had three very significant outliers with high values that were

truncated to the value 5 for the purpose of graphics and parametric analyses so that these outliers would not have undue influence. Data on basophil biomarkers (%CD63) were available at all timepoints on 52 of the 57 subjects enrolled, and serologic biomarkers were available on all 57 subjects throughout the study. A few analyses also evaluated post-baseline biomarker values. We considered the following outcomes: clinical (sustained unresponsiveness (SU) plus an ordinal clinical endpoint with SU as the best outcome, desensitized as intermediate, and neither as worst) and symptoms (any symptoms other than oropharyneal, GI symptoms, and moderate-severe symptoms). Moderate to severe reactions were defined as those that led to systemic hives and swelling, respiratory compromise, abdominal pain, repetitive vomiting, hypotension, or change in mental status.

Online Figure Legends

FIG E1 Percent of Foxp3^{hi} CD4^{+} T cells (Tregs) out of total proliferating CD4+ T cells following casein stimulation in omalizumab (blue) or placebo (red) subjects at baseline (BL), M4, M28 or M32. Neither group demonstrated a significant increase in Tregs at any time point studied. Although data on all subjects are represented, statistical analysis was limited to matched pairs between baseline and each of the timepoints studied (n=6, 10, 10 in the omalizumab arm at timepoints M4, M28 and M32 respectively; n=7, 8, 6 in the placebo arm at timepoints M4, M28, and M32 respectively).

FIG E2 Relationship between the percent of MOIT doses that led to symptoms including moderate-severe (Mod/Severe) symptoms, GI symptoms, or any symptom excluding oropharyngeal (Symptoms excl. oral) over the course of MOIT and following biomarkers: the basophil %CD63 expression at milk stimulant concentration 1 µg/mL (**A**; %CD63+: Milk1); AUC (**B**; %CD63+: AUC); ratio of %CD63 expression at milk concentrations 1 (**C**; %CD63 Milk1/anti-IgE) or 10 (**D**;%CD63 Milk10/anti-IgE) µg/mL or AUC (**E**; %CD63 AUC/anti-IgE) over %CD63 anti-IgE all measured at the baseline (BL) or M4 visits. Additional biomarkers included age at study entry (Age; **F**); milk IgE (kU_A/L) and the milk IgE over total IgE ratio (IgE Ratio: Milk/Total) (**G**) as well as log casein IgG4 over casein IgE ratio (log Casein: IgG4/IgE) and log beta-lactoglobulin IgG4 over beta-lactoglobulin IgE ratio (log Beta-lactoglobulin: IgG4/IgE; **H**) all measured at the BL visit. Each point represents an individual subject; omalizumab in blue and placebo in red. Overall, all markers of basophil activation (individually or as a ratio over %CD63 anti-IgE) had a significant positive association with the occurrence of symptoms ($p<0.001$ at M4). Additionally, several parameters were significantly associated with symptoms in the placebo group at baseline, including %CD63 at milk concentration 1 µg/mL/%CD63 anti-

IgE with moderate-severe symptoms and any symptom other than oropharyngeal (p=0.035, p=0.003, respectively); %CD63 at milk concentration 10 µg/mL/%CD63 anti-IgE with any symptom other than oropharyngeal (p=0.007); and AUC/%CD63 anti-IgE with any symptom other than oropharyngeal (p=0.034). Although not significant in the overall population, the log casein IgG4/IgE ratio was negatively associated with any symptoms other than oropharyngeal (p=0.032) and GI symptoms (p=0.034) in the omalizumab group at baseline. Additional significant p-values are indicated in the text.

FIG E3 Number of omalizumab or placebo subjects whose baseline basophil %CD63 expression at milk stimulant concentration 10 µg/mL was greater or less than 40% (%CD63 Milk10>40 or <40, respectively) who achieved sustained unresponsiveness (SU), were desensitized only (D) or failed (F). P-values for a difference in SU (SU. p), and for a difference in ordinal outcomes (SU/D/F; ord. p), are presented.

FIG E4 Basophil %CD63 expression at milk stimulant concentration 1 µg/mL (**A**; %CD63+: Milk 1), AUC (**B**; %CD63+: AUC), ratio of %CD63 expression at milk concentration 1 µg/mL (**C**; %CD63 Milk1/anti-IgE) or AUC (**D**; %CD63 AUC/anti-IgE) over %CD63 anti-IgE, and milk IgE (**E**; kU_A/L), log ratio casein IgG4 over casein IgE (log Casein: IgG4/IgE; **F**), log ratio of beta-lactoglobulin IgG4/beta-lactoglobulin IgE (log Beta-lactoglobulin: IgG4/IgE; **G**), and subject age in years (Age; **H**) all measured at the baseline (BL) visit in omalizumab (blue) or placebo (red) subjects who achieved sustained unresponsiveness (SU), were desensitized only (D) or failed the M28 desensitization OFC (F). Statistically significant p-values are discussed in the text.

FIG E5 Basophil %CD63 expression at milk stimulant concentration 1 (**A**; %CD63+: Milk1) or 10 (**B**; %CD63+: Milk10) µg/mL, AUC (**C**; %CD63+: AUC), ratio of %CD63

expression at milk concentration 1 (**D**; %CD63 Milk1/anti-IgE) or 10 (**E**; %CD63 Milk10/anti-IgE μg/mL) or AUC (**F**; %CD63 AUC/anti-IgE) over %CD63 anti-IgE, casein IgG4/casein IgE ratio (log Casein: igG4/IgE; **G**), and beta-lactoglobulin IgG4/beta-lactoglobulin IgE ratio (log Beta-lactoglobulin: IgG4/IgE; **H**) measured at the indicated visit (M28 or M32) in omalizumab (blue) or placebo (red) subjects who achieved sustained unresponsiveness (SU), were desensitized only (D) or failed the M28 desensitization OFC (F). Statistically significant p-values are discussed in the text.