Appendix A

Supplement to Materials and Methods:

Study subjects

Allergic patients were screened at the Division of Immunology, Allergy and Infectious Diseases, AKH Vienna, the Floridsdorf Allergy Center in Vienna and the outpatient clinic of our institute between September 2013 and July 2015. All study subjects were of Caucasian ethnicity and were enrolled at our outpatient clinic.

Immunoglobulin G Subclasses

Test-specific (Optilite IgG1-4 Kit) normal ranges (95 percentiles in mg/dl) were 382.4 - 928.6 for IgG1, 241.8 - 700.3 for IgG2, 21.8 - 176.1 for IgG3 and 3.9 - 86.4 for IgG4.

TBE-specific IgG subclasses and IgE

Nunc-Maxisorp plates were coated with antigen *TBE TICOVAC-like* (2.4 µg inactivated TBE virus, strain Neudoerfl per 500µl solvent) (Pfizer Corporation Austria GmbH, Vienna, Austria) at 0.16 µg/well in coating buffer (0.1M carbonate-bicarbonate buffer, pH 9.6) overnight at 4°C. Sera obtained before and four weeks after booster were diluted at 1/100 for IgG1, at 1/50 for IgG3, and at 1/20 for IgG4 detection. Dilutions at 1/10 were used for detection of TBE-specific IgE. HRP-conjugated mouse anti-human IgG1 (clone HP6069), mouse anti-human IgG3 (clone HP6047), mouse anti-human IgG4 (clone HP6025, all Thermo Fisher Scientific, Waltham, Massachusetts, USA), and HRP-conjugated mouse anti-human IgE (clone E411 [5H2], Bio-Rad Laboratories Inc., Hercules, California, USA) at 1µg/ml and ABTS substrate were used to measure absorbance. Since standard preparations for TBE-specific IgG subclasses are not available, dilutions of control sera were used to achieve plate-to-plate comparability and results are indicated as optical density (OD).

TBE-specific in-vitro re-stimulation of PBMC

Frozen PBMC were re-established in culture medium RPMI 1640 supplemented with 10% human AB serum (Biochrom, Berlin, Germany) and 2mM L-glutamine, 50µM 2-mercaptoethanol and 0.1 mg/ml gentamycin (all Sigma Aldrich, St. Louis, Missouri, USA). Cells in duplicates were plated in 96-well round-bottom plates at $8x10^5$/well and cultured with *TBE TICOVAC-like* antigen (0.08 µg/well), super-antigen *Staphylococcus* Enterotoxin B (0.2 µg/well) and medium only for cytokine baselines (total culture volume 200µl). Cultures were maintained for 48 hours (37°C, 5% CO_2, 95% humidity); thereafter supernatants were pooled and stored at -20°C until analyses.

Flow-cytometric lymphocyte analyses

PBMC were surface-stained with the monoclonal antibodies anti-human CD19 FITC (clone HIB19), anti-human CD3 PE-Cy5 (clone HIT3a), anti-human CD4 FITC (clone RPA-T4), anti-human CD8 APC (clone RPA-T8), anti-human CD23 PerCP-Cy5.5 (clone EBVCS2), anti-human CD27 PE (clone L128), anti-human CD38 PerCP-Cy5.5 (clone HIT2) (all BD Biosciences, San Jose, California, USA). Regulatory T cells were characterized with anti-human CD3 PE-Cy5 (clone HIT3a), anti-human CD4 FITC (clone RPA-T4), anti-human CD25 PE (clone M-A251), anti-human CD45RA BV510 (clone HI100), (all BD Biosciences, San Jose, California, USA), and intra-cellular anti-human FOXP3-APC (clone PCH101) (eBioscience, now Thermo Fisher Scientific, Waltham, Massachusetts, USA). Foxp3/Transcription Factor Staining Buffer Set (eBioscience) was used for the fix/perm procedure and specific staining was verified with isotype controls.

Supplementary Figure Legends:

Figure S1: *Total IgG1, IgG3, and IgG4 concentrations*

Total IgG1, IgG3, and IgG4 were determined with turbidimetric immunoassay in sera obtained before (d0) and 4 weeks after booster.

Total IgG1, IgG3, and IgG4; Geometric mean (GM) concentrations (mg/dl) with 95% CI

ANOVA with linear contrasts; ***p< 0.001; **p<0.01; *p<0.05

Figure S2: *TBE-specific IgG4 and IgE levels*

TBE-specific IgG4 and IgE were measured with in-house ELISA in sera obtained before (d0) and 4 weeks after booster.

A) TBE-specific IgG4, and B) TBE-specific IgE; OD, line is arithmetic mean

ANOVA with linear contrasts; *p<0.05

Figure S3: *Incidence of local and systemic reactions to intra-muscularly applied TBE booster vaccination.*

A) Incidence of single local and B) single systemic side effects according to gender.

Supplementary Table I: *General study eligibility*

Inclusion criteria:

- adults of both sexes between 18 and 60 years of age
- completed primary TBE vaccination and at least one booster (received at least 2 years prior to enrolment)
- willingness to sign written informed consent form

Exclusion criteria:

- prior TBE infection
- pregnancy and breast feeding
- acute infection on day of inclusion (day 0), body temperature >37,9°C
- concomitant medications: systemic cortisone therapy, chemotherapy, immune-suppressive therapy 4 weeks prior to or during study
- administration of other vaccines 4 weeks before/after TBE vaccination
- planned surgery within 2 weeks before/after TBE vaccination
- start of de-sensitization and the first 4 weeks of allergen dose escalation
- any contraindication to administration of FSME-Immun® vaccine according to manufacturer's instructions
- history of malignant disease(s) within the last 5 years
- autoimmune diseases
- drug addictions
- plasma donors
- receipt of blood transfusions or immuno globulins within 3 month before study entry

Supplementary Table II: *B and T cells as % of total lymphocytes*

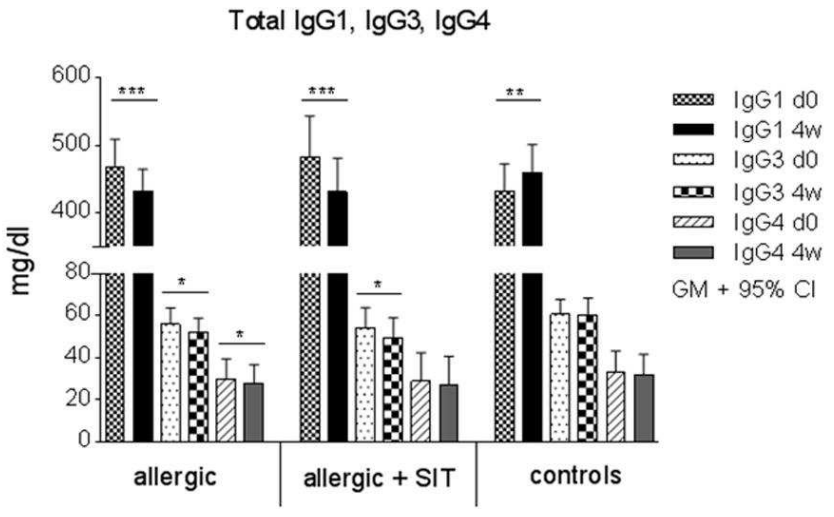
T cells, B cells	CD3		CD19	
as % of total ly	d0	1 week	d0	1 week
allergic	69.32 (66.86 -71.78)	68.67 (66.14 – 71.20)	7.63 (6.67 – 8.57)	7.22 (6.33 – 8.11)
allergic + SIT	73.68 (70.85 – 76.50)*	74.72 (72.37 – 77.07)*	7.01 (6.04 - 7.98)	6.34 (5.34 – 7.34)
controls	68.21 (65.47 – 70.96)	70.59 (68.28 – 72.90)	7.11 (6.34 – 7.89)	6.39 (5.78 – 7.01)
T-helper cells, CTL	**CD4**		**CD8**	
allergic	42.27 (39.91 – 44.63)	42.67 (40.37 – 44.97)	21.53 (19.77 – 23.29)	20.94 (19.25 – 22.63)
allergic + SIT	49.13 (45.47 – 52.42)**	50.46 (47.55 – 53.38)**	20.33 (17.04 – 23.37)	20.65 (17.54 – 23.75)
controls	41.57 (38.71 – 44.43)	42.97 (40.14 – 45.79)	20.56 (18.38 – 22.75)	21.16 (19.03 – 23.30)

PBMC were stained with fluorochrome-labelled mAbs and analyzed on a BD FACS Canto II flow cytometer.

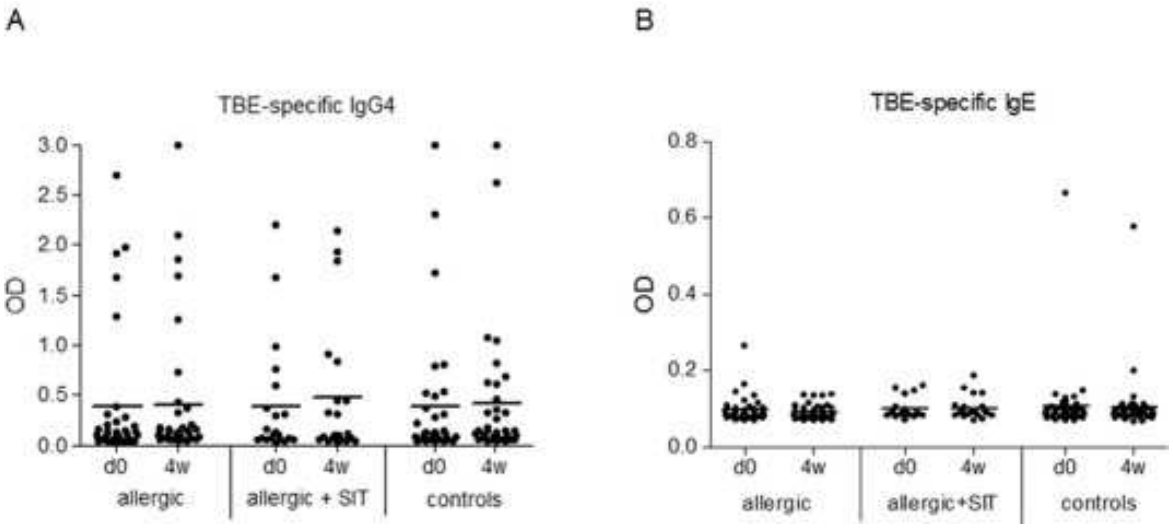
Distributions of CD19+ B cells, CD3+ T cells, CD4+ helper T cells and CD8+ cytotoxic T cells are shown as mean percentages with 95% CI before (d0) and 1 week after booster.

ANOVA with linear contrasts**p<0.01; *p<0.05

Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3