SUPPLEMENTARY APPENDIX

Epitope-specific immunotherapy targeting CD4+ T cells in celiac disease: evaluation in randomized, double-blind, placebo-controlled phase 1 studies

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1. STUDY SITES

1.1 3-dose study

- IDT CMAX (Adelaide, South Australia, Australia
  - Principal Investigator – Assoc. Prof. Jane M Andrews
  - 9 participants randomized to treatment
- ClinSearch, LLC. (Chattanooga, Tennessee, USA)
  - Principal Investigator – Dr Richard Krause
  - 7 participants randomized to treatment
- Clinical Research Institute of Michigan (Chesterfield, Michigan, USA)
  - Principal Investigator – Dr Ronald Fogel
  - 5 participants randomized to treatment
- Wake Research Associates (Raleigh, North Carolina, USA)
  - Principal Investigator – Dr Charles H Barish
  - 4 participants randomized to treatment
- ActivMed Practices & Research (Newington, New Hampshire, USA)
  - Principal Investigator – Dr Roger Epstein
  - 4 participants randomized to treatment
- Q-Pharm Pty Ltd. (Herston, Queensland, Australia)
  - Principal Investigator – Dr A. James Daveson
  - 4 participants randomized to treatment
- Auckland Clinical Studies Ltd. (Auckland, New Zealand)
  - Principal Investigator – Dr Timothy King
  - 4 participants randomized to treatment
- Oklahoma Foundation for Digestive Research (Oklahoma City, Oklahoma, USA)
  - Principal Investigator – Dr Philip B Miner Jr
  - 2 participants randomized to treatment
- Prism Clinical Research (Waconia, Minnesota, USA)
  - Principal Investigator – Dr Timothy Kinney
  - 2 participants randomized to treatment
- Linear Clinical Research (Nedlands, Western Australia, Australia)
  - Principal Investigator – Dr Janakan Krishnarajah
  - 2 participants randomized to treatment

1.2 16-dose study

- Auckland Clinical Studies Ltd. (Auckland, New Zealand)
  - Principal Investigator – Dr Timothy King
  - 15 participants randomized to treatment
- Q-Pharm Pty Ltd. (Herston, Queensland, Australia)
  - Principal Investigator – Dr A. James Daveson
  - 12 participants randomized to treatment
- Linear Clinical Research (Nedlands, Western Australia, Australia)
  - Principal Investigator – Dr Janakan Krishnarajah
  - 6 participants randomized to treatment
- Nucleus Network (Melbourne Victoria, Australia)
  - Principal Investigator – Assoc. Prof. Gregor Brown5 participants randomized to treatment
- Christchurch Clinical Studies Trust Ltd. (Christchurch, New Zealand)
  - Principal Investigator – Dr Chris Wynne
  - 1 participant randomized to treatment

2. STUDY INDEPENDENT ETHICS COMMITTEES

2.1 3-dose study

- Liberty IRB Tracking #12.07.0012
- The University of Okalahoma Institutional Review Board for the Protection of Human Subjects IRB #1370
- Bellbury Human Research Ethics Committee, Application Number 2013-10-553

- Southern Health and Disability Ethics Committee 13/STH/168

2.2 16-dose study

- The Alfred Hospital Ethics Committee, Approval Number 118/12
- Bellbury Human Research Ethics Committee, Application Number 2012-04-735-AA
- Southern Health and Disability Ethics Committees, Ethics Ref. NTY/12/06/049/AM05

3. STUDY ELIGIBILITY CRITERIA

To be eligible to participate, volunteers must have met the following inclusion criteria and none of the exclusion criteria at the first study visit or at the time indicated.

3.1 Inclusion Criteria

1. Patient has signed and understood the informed consent form (ICF) before initiation of any study specific procedures.
2. Patient is between 18 and 70 years old (inclusive).
3. Patient has confirmed "at risk" genotype (HLA-DQ2 and/or DQ8) and has a celiac disease diagnosis consistent with the criteria defined in the National Institutes of Health Consensus Statement 2004 [Department of Health and Human Services, 2004]:
    a. Diagnostic tests should be performed while the patient is on a gluten containing diet.
    b. A serologic antibody test should be positive.
    c. Patients with a positive celiac disease antibody test should undergo small bowel biopsy (those with biopsy-proven dermatitis herpetiformis can be excluded from small bowel biopsy).
        i. Multiple biopsies should be obtained (histologic changes may be focal) and include biopsies from the second portion of the duodenum or beyond.
    d. Some degree of villous atrophy should be observed.
4. Has HLA DQ2·5 genotype (both *DQA1*05* and *DQB1*02*, homozygous or heterozygous)

3.2 Exclusion Criteria

3.2.1 ***At Screening***

1. Patient possesses the genes encoding HLA DQ8 (either *DQA1*03* or *DQB1*0302*).
2. Patient has not been prescribed and/or has not followed a GFD for at least 12 months.
3. Patient has had known gluten exposure within two months prior to Screening.
4. Patient does not have a gluten specific T cell response (measured by IFN-γ release) following the Screening Period gluten challenge.
5. Patient is lactating or pregnant.
6. Patient is premenopausal, unless sterile, or using at least two acceptable birth control methods (Acceptable methods of birth control include tubal ligation, transdermal patch, intrauterine devices/systems, oral, implantable, or injectable contraceptives, sexual abstinence [if allowed by local authorities], double-barrier method, and vasectomized partner).
7. Patient is unable and/or unwilling to comply with study requirements.
8. Patient has had open abdominal surgery within the 12 months prior to Screening. (laparoscopic appendectomy and laparoscopic cholecystectomy within four months of Screening is allowed).
9. Patient has a positive test for human immunodeficiency virus or active hepatitis B or C disease at the time of Screening.
10. Patient has uncontrolled complications of celiac disease or unstable autoimmune disease which, in the opinion of the investigator, would impact the immune response or pose an increased risk to the patient.
11. Patient has uncontrolled peptic ulcer or gastroesophageal reflux disease or dyspepsia. The patient must be on a stable treatment regimen for their peptic ulcer or gastroesophageal reflux disease for two months prior to Screening.
12. Patient has insulin-dependent diabetes.
13. Patient has had treatment with systemic biological agents (e.g., adalimumab, etanercept, infliximab, certolizumab pegol) less than six months prior to Screening.
14. Patient has taken a nonsteroidal anti-inflammatory drug or aspirin within the past seven days prior to Screening. Daily low-dose aspirin therapy (up to 100 mg/day) is permitted.

15. Patient has taken oral corticosteroids within the previous six weeks prior to Screening. Inhaled steroids are acceptable.
16. Patient has taken systemic immunomodulatory agents (e.g., azathioprine, methotrexate) less than 30 days prior to Screening.
17. Patient has received an experimental therapy within 30 days prior to Screening.
18. Patient has been previously exposed to Nexvax2.
19. Patient has a history of clinically confirmed allergy and/or anaphylaxis to wheat, barley, or rye.
20. Patient has any of the following laboratory abnormalities at Screening:
    a. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), or alkaline phosphatase (ALP) >3 × the upper limit of normal (ULN)
    b. Hemoglobin <10 g/dL
    c. Platelet count <100× 109/L
    d. White blood cell count outside the normal range
    e. Thyroid-stimulating hormone outside the normal range
    f. Any other clinically significant abnormal laboratory values, as determined by the investigator
21. Patient is known to be pregnant, has a positive pregnancy test at Screening or Day 1 (Baseline), intends to become pregnant, or is nursing.
22. Patient has a history or presence of any medically significant condition considered by the investigator to have the potential to adversely affect participation in the study and/or interpretation of the study results.
23. Patient has a history of severe allergic reactions (e.g., swelling of the mouth and throat, difficulty breathing, hypotension, or shock) that require medical intervention.
24. Patient has donated blood ≤ 56 days prior to Screening.
25. Patient has a clinically relevant abnormality on electrocardiogram (ECG), as determined by the investigator.
26. Patient has inflammatory bowel disease (defined as ulcerative colitis or Crohn's disease).

3.2.2 ***Prior to randomization***

1. Patient has a positive gluten specific T cell response (measured by IFN-γ release assay) at the end of the Screening Period (**in ascending dose cohorts only)**
2. Patient has Screening small bowel mucosal biopsy histology consistent with a Marsh classification > Marsh 1 (**in biopsy cohorts only)**

4. DOSE ESCALATION CRITERIA

Dose-escalation was evaluated by the following criteria to determine if the study would proceed to the next cohort:

1. There are no more than two patients in a cohort with a local reaction to study drug injection that is > Grade 2.(1)
2. There are no more than two patients in a cohort with an abnormal vital sign (BP, T, HR) that is > Grade 2.(1)
3. There are no more than two patients in a cohort with vomiting, diarrhea, headache fatigue or myalgia that is > Grade 2.(1)
4. There are no more than two patients in a cohort with any symptom recorded in the daily GI symptom diary that reached the following threshold: rated as "severe" or "very severe" for more than 2 days per week of two consecutive weeks and represents at least one level of severity increase for the worst week recorded during the Screening Period for that symptom.
5. There are no more than two patients in a cohort with any symptom recorded in the weekly GI symptom dairy that reached the following threshold: rated as "severe" or "very severe" for more than 2 days per week of two consecutive weeks and represents at least one level of severity increase for the worst week recorded during the Screening Period for that symptom.
6. There are no more than two patients in a cohort with elevations in AST or ALT Grade 2 (≥ 5.1 x ULN) or greater that do not return to normal or near normal (Grade 1) by Day 15. AST and ALT of ≥ Grade 2 triggered a repeat test for confirmation of the value within 48 hours. Escalation to the next dosing

schedule proceeded if the confirmatory lab values indicated a toxicity of < Grade 2. Grading was established according to Guidance for Industry.(1)

7. There are no more than two patients in a cohort with elevations in ALP Grade 2 (≥ 3.1 x ULN) or greater that do not return to normal or near normal (Grade 1) by Study Day 15. ALP of ≥ Grade 2 will trigger a repeat test for confirmation of the value within 48 hours. Escalation to the next dosing schedule may proceed if the confirmatory lab values indicate a toxicity of < Grade 2. Grading will be established according to Guidance for Industry.(1)
8. There are no more than two patients in a cohort with abnormal clinical chemistry laboratory tests, exclusive of alkaline phosphatase (ALP), AST, or ALT, that was ≥ Grade 2, according to Guidance for Industry (Appendix B of protocol). Abnormal clinical chemistry laboratory tests of ≥ Grade 2 triggered a repeat test for confirmation of the value within 48 hours. Escalation to the next dosing schedule proceeded if the confirmatory lab values indicated a toxicity of < Grade 2.
9. There are no more than two patients in a cohort with abnormal hematology laboratory tests (i.e., WBC, Hgb, lymphocyte, neutrophils), that are ≥ Grade 2, according to Guidance for Industry.(1) Abnormal hematology laboratory tests of ≥ Grade 2 triggered a repeat test for confirmation of the value within 48 hours. Escalation to the next dosing schedule proceeded if the confirmatory lab values indicated a toxicity of < Grade 2.
10. There are no more than two patients in a cohort with abnormal urinalysis laboratory tests (i.e., protein, glucose, microscopic blood), that are ≥ Grade 2, according to Guidance for Industry.(1) Abnormal urinalysis laboratory tests of ≥ Grade 2 triggered a repeat test for confirmation of the value within 48 hours. Escalation to the next dosing schedule proceeded if the confirmatory lab values indicate a toxicity of < Grade 2.
11. There are no patients in a cohort with cardiovascular, respiratory, neurological, musculoskeletal, skin, infectious, otolaryngologic, or system toxicities that required ER visit or hospitalization.

5. METHODS

5.1 Development of Nexvax2

To develop Nexvax2, the specificities of polyclonal gluten-specific T cells circulating in blood of HLA-DQ2·5+ celiac patients after feeding them wheat, barley or rye were assessed in quantitative overnight IFN-γ ELISpot assays(2). The peptide composition of Nexvax2 was determined after screening 16,838 unique 12–amino acid oligopeptides in 313 GenBank entries for gliadins, LMW glutenins, and HMW glutenins from Triticum aestivum after wheat challenge, hordeins from Hordeum vulgare after barley challenge, and secalins from Secale cerale after rye challenge. T cell clones raised to the three peptides selected for inclusion in Nexvax2 responded to 61 of the 96 immunoreactive peptides identified from screening a peptide library encompassing 16,838 unique 12–amino acid sequences. T cell responses to these 3 peptides were additive when mixed together and assessed in IFN-γ ELISpot assays using peripheral blood mononuclear cells (PBMC) from CeD donors.

The peptides in Nexvax2, NPL001, NPL002 and NPL003 correspond to partially deamidated germline-encoded sequences in certain wheat α-gliadins, wheat ω-gliadins/barley C-hordeins, or barley B-hordeins with synthetically modified N- and C-termini. In vitro assays performed by the La Jolla Institute of Allergy and Immunology (La Jolla, California, USA) assessed the binding of NPL001, NPL002, and NPL003 to isolated MHC Class II molecules according to established methods(3). Each peptide binds selectively with intermediate affinity to HLA-DQ2·5 (Table S1). The equimolar mixture of peptides in Nexvax2 stimulates concentration-dependent secretion of IFN-γ, and IL-10 by CD4+ T cell clones specific for epitopes in Nexvax2 (Figure S1A-E),(4) attenuated by co-incubation with anti-HLA-DQ, but not anti-HLA-DR (Figure S1F-J).

Conduct of these phase 1 studies were supported by preclinical studies of Nexvax2 investigating pharmacodynamics in HLA-DR3-DQ2·5 transgenic mice, including a related strain that was additionally T-cell receptor transgenic with CD4+ T cells specific for the DQ2·5-glia-α2 epitope present in Nexvax2,(5) toxicology and PK studies in rodents, clinical medicinal chemistry studies, the prior first-in-human study of Nexvax2, and clinical studies of peptide-based therapeutic vaccines.

5.2 Investigational drug product

CS Bio (Menlo Park, California, USA) manufactured NPL001, NPL002, and NPL003. Microtest (Agawam, Massachusetts, USA) formulated and filled vials with a sterile equimolar solution at total peptide concentration 9 mg/mL in sterile USP 0.9% sodium chloride. Placebo and diluent for Nexvax2 in vials was USP 0.9% sodium chloride. Placebo or Nexvax2 150 μg (50 μg of each peptide), or 300 μg in 0.1 mL were administered by 1 mL Luer-Lok™ plastic syringe fitted with a Micro Injection Needle (Becton-Dickinson). Grand River Aseptic Manufacturing (Grand Rapids, Michigan, USA) formulated and filled Soluvia™ syringes (Becton-Dickinson, Franklin Lakes, New Jersey, USA) with Nexvax2 (0.6 mg/mL, 0.9 mg/mL, or 1.5 mg/mL) or placebo.

5.3 Lab procedures for clinical trials

5.3.1 *Safety laboratory pathology assessments*

Laboratory assessments included routine haematology, blood chemistry, and urinalysis performed by Dorevitch Pathology for sites in Australia in New Zealand, and by LabConnect (Johnson City, Tennessee, USA) for sites in the United States.

5.3.2 *Whole blood interferon-γ release assay (IGRA)*

IFN-γ levels in plasma separated from whole blood incubations for IGRA collected during the screening periods of each study were measured by ELISA performed either at ImmusanT, Inc. for samples from sites in the USA, or at the Walter and Eliza Hall Institute (Parkville, Vicotria, Australia) for sites in Australia or New Zealand. After each study was completed, thawed plasma from all whole blood IGRA incubations were re-assessed by IFN-γ ELISA at ImmusanT, which were regarded as the final, reported IGRA data. Briefly, 1 mL of blood was collected into each of three Nil Control Tubes (QuantiFERON®-TB Gold In-Tube, QIAGEN, Hilden, Germany) that had 0.1 mL phosphate buffer saline (PBS) alone, Nexvax2 peptides (each 50 μg/mL), or positive control CEF peptide pool with epitopes derived from cytomegalovirus, Epstein-Barr virus, and influenza (0.1 μg/mL; Mabtech AB, Nacka Strand, Sweden) added, and a QuantiFERON Mitogen Tube with 0.1 mL PBS added. Tubes were incubated at 37°C for 24 h before centrifugation, and IFN-γ in the supernatant was measured by ELISA (Mabtech). To evaluate the magnitude of responses, a stimulation index (SI) was calculated for the average IFN-γ concentrations in the CEF and Nexvax2-peptide incubations divided by the concentration determined for the response to PBS alone. A "positive" response was defined as SI>1.25 and net IFN-γ concentration above PBS control >7.2pg/mL.(6, 7)

5.3.3 *Plasma concentrations of cytokines and chemokines*

Blood was collected into K2 EDTA Vacutainer® tubes, which were immediately placed on wet ice, and then centrifuged at 1100-1300 RCF for 10 minutes within 30 minutes of collection. Plasma was aliquotted and frozen. Cytokines and chemokines were measured in plasma using a 38plex magnetic bead-based assay according the manufacturer's protocol (EMD Millipore Corp., Billerica, MA; Luminex Corporation, Austin, Texas, USA) at ImmusanT, Inc.

5.3.4 *Plasma concentrations of complement components*

Complement levels were measured in plasma collected for cytokine/chemokine assessment by magnetic bead-based assay according the manufacturer's protocol (Milliplex® MAP Human Complement Magnetic Bead Panel 1 and 2) at ImmusanT, Inc.

5.3.5 *Immune cell profiling*

PBMC were prepared at trial sites according to manufacturer's instructions using Ficoll-Paque™ PLUS (Sigma-Aldrich) in SepMate™-50 tubes (STEMCELL Technologies Inc.; Vancouver, BC, Canada), and cryopreserved using CryoStor™ CS10 (STEMCELL Technologies Inc.). Flow cytometry was performed at Duke Center for AIDS Research Flow Cytometry Core Facility (Durham, North Carolina, USA) using pre-mixed labeled antibodies specific for CD3, CD4, CD8, CD45, CD16, CD56, and CD19 according to established protocols(8-11).

5.3.6 ***Pharmacokinetics***

Pharmacokinetics were assessed on the first and last days of dosing. Blood was collected 30 minutes before, and 10, 20, 30, and 45 minutes, and 1, 1.5, 2, 4, and 6 hours after dosing. Blood was collected into K2 EDTA Vacutainer® tubes (Becton-Dickinson), which were centrifuged at 1100-1300 RCF for 10 minutes within 10min of collection. Plasma samples were spiked with isotopically labeled Nexvax2 peptides (50 ng/mL; Pepscan, Lelystad, The Netherlands), extracted using C18 Sep-Pak SPE cartridges, and analyzed by high-performance liquid chromatography with tandem mass spectrometric developed and performed by Blue Stream Laboratories (Woburn, Massachusetts, USA).

5.3.7 ***Celiac disease serology***

Assays for CeD serology in sera collected for anti-Nexvax2 antibodies was performed by Healthscope Pathology (Clayton, Victoria, Australia) using QUANTA Lite® R h-tTG IgA, Gliadin IgA II [DGP], Gliadin IgG II [DGP] kits (INOVA Diagnostics, San Diego, California, USA).

5.3.8 ***Duodenal histology***

The central pathologist (Dorevitch Pathology; Heidelberg VIC, Australia) evaluated biopsies in the screening period to determine eligibility. After the end of each study, the central pathologist masked to the order that biopsies were collected, re-evaluated all biopsies to make a final assessment of modified Marsh type. Histology slides were shipped to the University of Tampere, where villous height to crypt depth (VH:CrD) ratio and intra-epithelial lymphocytes (IEL) density per 100 epithelial cells were measured in well oriented sections.(12)

5.4 Major histocompatibility class II peptide binding

In vitro assays performed by the La Jolla Institute of Allergy and Immunology (La Jolla, California, USA) assessed the binding of NPL001, NPL002, and NPL003 to isolated MHC Class II molecules according to established methods(3).

6. STATISTICAL METHODS FOR POST HOC ANALYSES

6.1 Primary endpoints

Two-tailed Fisher's Exact Test was used to compare (1) number of participants who experienced treatment emergent adverse events in placebo and active arms, and (2) number of Nexvax2-treated participants who experienced severe adverse events stratified by HLA-DQ2.5 homozygosity status.

6.2 Secondary endpoints

Wilcoxon Rank Sum test was used to compare individual and summed daily symptom scores for each participant after dosing relative to pre-dose baseline scores. Wilcoxon Signed-Rank test was used to compare (1) weekly GSRS scores between a treatment week and the baseline week (Table S7), (2) daily symptoms scores between gluten challenge day and placebo challenge day during screening period (Table S8), and (3) change in percentage lymphocytes from day 1 of treatment on other days (Table S9).

6.3 Exploratory endpoints

Villous height to crypt depth (VH:CrD) ratio and intra-epithelial lymphocytes (IEL) density pre- and post-treatment were analyzed by Wilcoxon Signed Rank test (Table S11).

6.4 Pharmacodynamic endpoints

To address the confounding effects of reduced gluten exposure in the post-treatment OGC, an algorithm was developed post hoc to define the populations for post-treatment symptom and pharmacodynamic analysis (Figure S3). Two-tailed Fisher's Exact Test was used to compare (1) number of Nexvax2 and placebo treated participants who finished the post-treatment OGC, and (2) number of Nexvax2 and placebo treated participants who had negative IGRA at day six or eight after commencing post-treatment OGC (Table 3).

7. SUPPLEMENTARY REFERENCES

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Table S1: Nexvax2 composition and binding to HLA-DQ molecules associated with celiac disease

Nexvax2 Peptide	Amino acids	HLA-DQ2·5-restricted T-cell epitopes	9 amino acid epitope sequences	Major Histocompatibility Class II binding affinity (IC50 nM)		
				HLA-DQ2·5	HLA-DQ2·2	HLA-DQ8
NPL001	16	DQ2·5-glia-α1a	PFPQPELPY	Intermediate	Low	Negligible
		DQ2·5-glia-α2	PQPELPYPQ	(109 nM)	(1778 nM)	(>5000 nM)
NPL002	15	DQ2·5-glia-ω1	PFPQPEQPF	Intermediate	Negligible	Negligible
		DQ2·5-glia-ω2	PQPEQPFPW	(87 nM)	(>5000 nM)	(>5000 nM)
NPL003	16	DQ2·5-hor-3	PIPEQPQPY	Intermediate	Low	Negligible
		var DQ2·5-glia-γ5	EQPIPEQPQ	(231 nM)	(1405 nM)	(>5000 nM)

Table S2. Treatment-emergent adverse events that occurred during the treatment period in the 3-dose study

Treatment-Emergent Adverse Events (TEAE)	Treatment-emergent adverse events in 3-dose study						
	1st, 2nd & 3rd	1st	2nd	3rd	4th (biopsy)	4th (biopsy)	All Participants Dosed
	Placebo	Nexvax2	Nexvax2	Nexvax2	Placebo	Nexvax2	
		60µg	90µg	150µg		150µg	
	(N=11)	(N=9)	(N=9)	(N=8)	(N=3)	(N=3)	(N=43)
	Number (%) of participants						
Number of participants with TEAEs	6 (55%)	5 (56%)	7 (78%)	5 (63%)	3 (100%)	1 (33%)	27 (63%)
Number of participants with study drug-related TEAEs	4 (36%)	5 (56%)	6 (67%)	3 (38%)	1 (33%)	1 (33%)	20 (47%)
Number of participants with moderate or severe TEAEs	3 (27%)	3 (33%)	5 (56%)	4 (50%)	1 (33%)	0	16 (37%)
Number of participants with study drug-related, moderate or severe TEAEs	2 (18%)	3 (33%)	5 (56%)	3 (38%)	1 (33%)	0	14 (33%)
Number of participants withdrawn due to TEAEs	0	0	1 (11%)	0	0	0	1 (2%)
Number of participants with a treatment-emergent serious adverse events	1 (9%)	0	0	0	0	0	0
	Number of events						
Number of TEAEs	15	25	65	16	7	1	129
Number of study drug-related TEAEs	10	22	60	11	1	1	105
Number of moderate or severe TEAEs	4	18	47	9	1	0	79
Number of study drug-related, moderate or severe TEAEs	3	15	46	8	1	0	73
Number of TEAEs leading to withdrawal	0	0	1	0	0	0	1
Number of treatment-emergent serious adverse events	4	0	0	0	0	0	0

Table S3. Treatment-emergent adverse events that occurred during the treatment period in the 16-dose study

Treatment-Emergent Adverse Events (TEAE)	Treatment-emergent adverse events in 16-dose study					
	1st & 2nd	1st	2nd	3rd (biopsy)	3rd (biopsy)	All Participants Dosed
	Placebo	Nexvax2	Nexvax2	Placebo	Nexvax2	
		150 µg	300 µg		150 µg	
	(N=7)	(N=8)	(N=10)	(N=7)	(N=7)	(N=39)
	Number (%) of participants					
Number of participants with TEAEs	5 (71%)	6 (75%)	10 (100%)	6 (86%)	5 (71%)	32 (82%)
Number of participants with study drug-related TEAEs	3 (43%)	5 (63%)	9 (90%)	3 (43%)	4 (57%)	24 (62%)
Number of participants with moderate or severe TEAEs	1 (14%)	5 (63%)	8 (80%)	0	2 (29%)	16 (41%)
Number of participants with study drug-related, moderate or severe TEAEs	0	5 (63%)	8 (80%)	0	1 (14%)	14 (36%)
Number of participants withdrawn due to TEAEs	0	0	3 (30%)	0	0	3 (8%)
Number of participants with a treatment-emergent SAE	0	0	1 (10%)	0	0	1 (3%)
	Number of events					
Number of TEAEs	13	21	26	24	18	102
Number of study drug-related TEAEs	5	16	16	5	7	49
Number of moderate or severe TEAEs	1	8	12	0	3	24
Number of study drug-related, moderate or severe TEAEs	0	7	8	0	2	17
Number of TEAEs leading to withdrawal	0	0	3	0	0	3
Number of treatment-emergent serious adverse events	0	0	1	0	0	1

Table S4. Treatment emergent adverse events that occurred during the treatment period in at least 5% of particpants in the 3-dose study.

System Organ Class, Preferred Term	Treatment-emergent adverse events (TEAE)						
	1st, 2nd & 3rd Placebo	1st Nexvax2 60µg	2nd Nexvax2 90µg	3rd Nexvax2 150µg	4th (biopsy) Placebo	4th (biopsy) Nexvax2 150µg	All Particpants Dosed
	(N=11)	(N=9)	(N=9)	(N=8)	(N=3)	(N=3)	(N=43)
Number (%) of particpants with at least 1 TEAE	6 (55%)	5 (56%)	7 (78%)	5 (63%)	3 (100%)	1 (33%)	27 (63%)
[Number of TEAEs]	[15]	[25]	[65]	[16]	[7]	[1]	[129]
Gastrointestinal Disorders	2 (18%)	2 (22%)	5 (56%)	4 (50%)	1 (33%)	0	14 (33%)
	[4]	[9]	[36]	[8]	[3]		[60]
Vomiting	0	2 (22%)	5 (56%)	4 (50%)	0	0	11 (26%)
		[2]	[12]	[6]			[20]
Nausea	1 (9%)	1 (11%)	4 (44%)	2 (25%)	1 (33%)	0	9 (21%)
	[2]	[3]	[9]	[2]	[1]		[17]
Abdominal pain	0	1 (11%)	3 (33%)	0	1 (33%)	0	5 (12%)
		[3]	[5]		[1]		[9]
Abdominal tenderness	0	0	2 (22%)	0	0	0	2 (5%)
			[2]				[2]
Diarrhoea	0	0	2 (22%)	0	0	0	2 (5%)
			[4]				[4]
General Disorders and Administration Site Conditions	2 (18%)	4 (44%)	3 (33%)	1 (13%)	0	0	10 (23%)
	[3]	[10]	[7]	[1]			[21]
Injection site pain	0	3 (33%)	1 (11%)	0	0	0	4 (9%)
	[0]	[8]	[2]				[10]
Fatigue	1 (9%)	0	2 (22%)	0	0	0	3 (7%)
	[1]		[2]				[3]
Infections and Infestations	3 (27%)	1 (11%)	1 (11%)	1 (13%)	2 (67%)	0	8 (19%)
	[3]	[1]	[1]	[1]	[2]		[8]
Urinary tract infection	0	0	1 (11%)	0	1 (33%)	0	2 (5%)
			[1]		[1]		[2]
Viral upper respiratory tract infection	1 (9%)	0	0	1 (13%)	0	0	2 (5%)
	[1]			[1]			[2]
Nervous System Disorders	1 (9%)	1 (11%)	4 (44%)	1 (13%)	0	0	7 (16%)
	[3]	[1]	[8]	[1]			[13]
Headache	0	0	4 (44%)	0	0	0	4 (9%)
			[7]				[7]
Dizziness	0	0	1 (11%)	1 (13%)	0	0	2 (5%)
			[1]	[1]			[2]
Musculoskeletal and Connective Tissue Disorders	0	1 (11%)	2 (22%)	1 (13%)	0	0	4 (9%)
		[1]	[2]	[2]			[5]
Myalgia	0	1 (11%)	1 (11%)	0	0	0	2 (5%)
		[1]	[1]				[2]

Table S5. Treatment emergent adverse events that occurred during the treatment period in at least 5% of particpants in the 16-dose study

System Organ Class, Preferred Term	Treatment-emergent adverse events (TEAE)					
	1st & 2nd	1st	2nd	3rd (biopsy)	3rd (biopsy)	All Particpants Dosed
	Placebo	Nexvax2	Nexvax2	Placebo	Nexvax2	
		150 µg	300 µg		150 µg	
	(N=7)	(N=8)	(N=10)	(N=7)	(N=7)	(N=39)
Number (%) of particpants with at least 1 TEAE	5 (71%)	6 (75%)	10 (100%)	6 (86%)	5 (71%)	32 (82%)
[Number of TEAEs]	[13]	[21]	[26]	[24]	[18]	[102]
Nervous System Disorders	5 (71%)	3 (38%)	5 (50%)	4 (57%)	3 (43%)	20 (51%)
	[7]	[10]	[9]	[6]	[4]	[36]
Headache	3 (43%)	3 (38%)	5 (50%)	3 (43%)	2 (29%)	16 (41%)
	[3]	[9]	[8]	[4]	[2]	[26]
Dizziness	2 (29%)	0	0	1 (14%)	0	3 (8%)
	[4]			[1]		[5]
Migraine	0	1 (13%)	1 (10%)	0	0	2 (5%)
		[1]	[1]			[2]
Lethargy	0	0	0	1 (14%)	1 (14%)	2 (5%)
				[1]	[1]	[2]
Gastrointestinal Disorders	1 (14%)	5 (63%)	6 (60%)	3 (43%)	2 (29%)	17 (44%)
	[1]	[5]	[10]	[5]	[3]	[24]
Vomiting	0	5 (63%)	4 (40%)	1 (14%)	0	10 (26%)
		[5]	[5]	[1]		[11]
Abdominal pain	0	0	1 (10%)	1 (14%)	0	2 (5%)
			[2]	[1]		[3]
Diarrhea	0	0	0	1 (14%)	1 (14%)	2 (5%)
				[1]	[1]	[2]
Dry mouth	0	0	0	1 (14%)	1 (14%)	2 (5%)
				[1]	[1]	[2]
Gastrointestinal disorder	0	0	2 (20%)	0	0	2 (5%)
			[2]			[2]
General Disorders and Administration Site Conditions	1 (14%)	3 (38%)	1 (10%)	1 (14%)	3 (43%)	9 (23%)
	[1]	[3]	[1]	[1]	[4]	[10]
Vessel puncture site haematoma	1 (14%)	0	0	0	1 (14%)	2 (5%)
	[1]				[2]	[3]
Fatigue	0	1 (13%)	0	0	1 (14%)	2 (5%)
		[1]			[1]	[2]
Infections and Infestations	1 (14%)	1 (13%)	1 (10%)	4 (57%)	0	7 (18%)
	[1]	[1]	[1]	[5]		[8]
Upper respiratory tract infection	0	0	0	3 (43%)	0	3 (8%)
				[3]		[3]
Nasopharyngitis	0	0	1 (10%)	1 (14%)	0	2 (5%)
			[1]	[1]		[2]
Pharyngitis	0	1 (13%)	0	1(14%)	0	2 (5%)
		[1]		[1]		[2]
Musculoskeletal and Connective Tissue Disorders	0	0	2 (20%)	1 (14%)	1 (14%)	4 (10%)
			[2]	[2]	[1]	[5]

Back pain	0	0	2 (20%) [2]	1 (14%) [1]	0	3 (8%) [3]
Respiratory, Thoracic and Mediastinal Disorders	0	0	2 (20%) [2]	2 (29%) [4]	0	4 (10%) [6]
Oropharyngeal pain	0	0	1 (10%) [1]	1 (14%) [1]	0	2 (5%) [2]

Table S6. Moderate or severe adverse events, and any occurrence of vomiting in the treatment period

Participant	Treatment	Age	Sex	HLA-DQ2.5 Homozygote	Gluten challenge in screening	Total doses received	Last dose	Onset after last dose	Adverse event	Severity
3-dose study										
S03-01-01	Nexvax2 60 µg	49	F	No	Yes	3	1	5h 20m	Vomiting x1	Mild
S03-01-09	Nexvax2 60 µg	63	F	Homozygote	Yes	3	1	1 day	Worsening of Diabetes Mellitus type 2; Worsening of lower limb edema	Moderate
								3 days	Rash on right forearm "Redness and itching about 5 inch diameter"	Moderate
							2	1 day	Muscle aches both lower limbs	Moderate
							3	0	Worsening of seasonal allergy	Moderate
S03-01-07	Nexvax2 60 µg	36	F	Homozygote	Yes	3	1	0	Burning at Injection Site; Soreness in left arm (near injection site)	Moderate
								2h	Nausea; Abdominal Pain	Severe
								2h 30m	Vomiting	Severe
							2	0	Burning at injection site	Moderate
								3h 30m	Nausea; Abdominal Pain	Moderate
							3	0:00	Constipation	Moderate
								3h 30m	Nausea; Abdominal Pain	Moderate
S03-01-05	Placebo	64	M	Homozygote	Yes	3	1	1 day	Worsening Rash on Right Back (Perivascular Dermatitis)	Moderate
S03-02-13	Nexvax2 90 µg	42	F	Homozygote	Yes	3	1	3h	Nausea; Vomiting	Severe
								3h	Abdominal pain	Moderate
							2	2h	Vomiting	Mild
S03-02-08	Nexvax2 90 µg	29	F	No	Yes	3	1	30m	Flushing; Aggravated Headache; Nausea	Moderate
								2h 45m	Vomiting; Right sided abdominal pain	Moderate
							2	30m	Flushing; Aggravated Headache	Moderate
								2h 30m	Nausea; Vomiting; Right sided abdominal pain	Moderate
							3	30m	Flushing; Aggravated Headache	Moderate
								2h 30m	Nausea; Vomiting; Right sided abdominal pain	Moderate
S03-02-01	Nexvax2 90 µg	58	F	No	Yes	3	1	0	Burning at Injection Site	Moderate
								3h 30m	Nausea; Vomiting; Generalized Weakness	Moderate
							2	2h 45m	Vomiting; Diarrhea; Abdominal Spasms	Severe
								1 day	Muscle aches bilateral legs; Soft Tissue Swelling Left Foot	Moderate
							3	2h 15m	Nausea	Severe
								3h 15m	Vomiting; Diarrhea; Abdominal Pain	Severe
S03-02-07 ❋	Nexvax2 90 µg	62	F	Homozygote	Yes	1	1	2h 30m	Headache	Moderate
								3h	Nausea; Diaphoresis	Moderate
								3h	Dizziness; Adverse drug reaction	Severe
								3h	Lip dysesthesia; Dysphagia (lump in throat upon swallowing)	Moderate
								3h 30m	Abdominal pain & tenderness	Moderate
								4h	Vomiting; Diarrhea	Moderate
								5h 30m	Diffuse arthralgia	Moderate
								7h 30m	Sinus congestion	Severe

								7h 30m	Cough	Moderate
								5 days	Sinus congestion	Moderate
S03-02-10	Nexvax2 90 µg	43	M	Homozygote	Yes	3	1	3h 15m	Vomiting	Moderate
							2	2h 25m	Vomiting	Moderate
							3	2h 20m	Vomiting	Mild
S03-03-03	Nexvax2 150 µg	48	M	No	Yes	3	1	4h 20m	Vomiting	Moderate
							2	3h 10m	Vomiting	Severe
								3h 30m	Clammy skin	Severe
								5h	Shivering	Severe
S03-03-02	Nexvax2 150 µg	18	F	No	Yes	3	1	4h	Vomiting	Moderate
S03-03-12	Nexvax2 150 µg	52	F	No	Yes	3	1	2h 45m	Nausea	Moderate
								3h 15m	Vomiting	Moderate
							3	3h	Vomiting	Moderate
S03-03-09	Nexvax2 150 µg	27	F	No	Yes	3	1	3h	Vomiting	Moderate
S03-03-05	Placebo	49	F	No	Yes	3	1	0	Nausea	Moderate
							2	1 day	Nausea	Moderate
S03-03-11	Placebo	31	F	No	Yes	3	1	1 day	Viral upper respiratory tract infection	Severe
S03-04-06	Placebo	53	F	No	No	3	2	6 days	Insomnia	Moderate
16-dose study										
S16-01-02	Nexvax2 150 µg	64	F	No	Yes	16	3	1 day	Fatigue	Moderate
S16-01-03	Nexvax2 150 µg	51	F	Homozygote	Yes	16	1	2h 45m	Vomiting	Moderate
							3	1 day	Headache	Moderate
S16-01-04	Nexvax2 150 µg	54	F	Homozygote	Yes	16	1	2h 25m	Vomiting	Moderate
S16-01-08	Nexvax2 150 µg	60	F	No	Yes	16	1	3 days	Vomiting	Mild
S16-01-12	Nexvax2 150 µg	40	M	Homozygote	Yes		1	2h 50m	Vomiting	Severe
								4h 15m	Rigors	Severe
S16-01-06	Nexvax2 150 µg	66	F	UNK	Yes	16	1	3h	Vomiting	Moderate
							4	2 days	Headache	Moderate
S16-02-05	Nexvax2 300 µg	64	F	No	Yes	16	9	1 day	Headache	Moderate
S16-02-07 ¶	Nexvax2 300 µg	48	F	No	Yes	1	1	Same day	Vomiting	Moderate
								7h 20m	Headache	Moderate
S16-02-01	Nexvax2 300 µg	28	M	UNK	Yes	16	3	2 days	Headache	Moderate
S16-02-02 □	Nexvax2 300 µg	54	M	No	Yes	1	1	3h 15m	Gastrointestinal reaction to study drug	Moderate
S16-02-03 □	Nexvax2 300 µg	58	F	No	Yes	2	2	3h 50m	Gastrointestinal reaction to study drug	Moderate
S16-02-11	Nexvax2 300 µg	50	F	No	Yes	4	1	4h 5m	Vomiting	Moderate
S16-02-12	Nexvax2 300 µg	55	F	No	Yes	4	1	3h 5m	Vomiting	Moderate
S16-02-13 §	Nexvax2 300 µg	45	M	Homozygote	Yes	1	1	2h 15m	Abdominal Pain	Severe
								2h 15m	Vomiting	Mild
								2 days	Abdominal Pain	Severe
								2 days	Vomiting	Moderate
S16-02-04	Placebo	35	M	No	Yes	15	14	1 day	Vomiting	Moderate
S16-03-02	Nexvax2 150 µg	37	M	Homozygote	No	16	4	7h 30m	Headache	Moderate
S16-03-11	Nexvax2 150 µg	44	F	No	No	16	12	3h	Fatigue	Moderate
								4h 45m	Bilateral thigh muscle pain	Moderate
S16-03-08	Placebo	21	F	No	No	16	14	7h 25m	Vomiting	Mild

 Participant code (SXX-YY-ZZ) refers to the planned total doses in the study, the cohort number (YY), and order of randomization within the cohort (ZZ)

❄ S03-02-07 experienced diarrhea at approximately 3 hours following dosing, felt very faint, severely nauseated, and became very cold and pale. Study treatment was discontinued.

¶ S16-02-07 was discontinued from study treatment as GI symptoms were poorly tolerated.

. S16-02-02 and S16-02-03 had "Gastrointestinal reaction to study drug" including vomiting (MedDRA term of GI disorder) resulting in S16-02-02 discontinuing after 1st dose andand S16-02-03 after 2nd dose

§ S16-02-13 was discontinued after first dose.

Table S7. Weekly GSRS scores

	Weekly gastrointestinal symptom rating scale score					
Study	**3-dose study**					
Cohort/s	**1st, 2nd & 3rd**	**1st**	**2nd**	**3rd**	**4th (biopsy)**	**4th (biopsy)**
Treatment	**Placebo**	**Nexvax2**	**Nexvax2**	**Nexvax2**	**Placebo**	**Nexvax2**
Dose		**60µg**	**90µg**	**150µg**		**150µg**
N	**11**	**9**	**90µg**	**8**	**3**	**3**
Pre-treatment						
Week of gluten challenge	1·91 (1·17)	2·18 (0·56)	2·04 (1·16)	1·82 (0·86)	1·20 (0·24)	1·04 (0·04)
Week of placebo challenge	1·60 (0·53)	1·88 (0·67)	1·98 (0·91)	1·22 (0·41)	1·22 (0·10)	1·13 (0·12)
Last week of screening (baseline)	1·33 (0·41)	1·72 (0·68)	1·59 (0·75)	1·19 (0·23)	1·18 (0·10)	1·04 (0·08)
Treatment						
Treatment Week 1	1·35 (0·41)	1·77 (0·83)	2·36 (1·47) (p = 0·0313)	1·33 (0·35)	1·20 (0·00)	1·11 (0·10)
Treatment Week 2	1·28 (0·35)	1·93 (0·94)	1·75 (0·63)	1·37 (0·37)	1·22 (0·20)	1·03 (0·05)
Study	**16-dose study**					
Cohort/s	**1**	**1**	**2**	**2**	**7**	**7**
Treatment	**Placebo**	**Nexvax2**	**Placebo**	**Nexvax2**	**Placebo**	**Nexvax2**
Dose		**150 µg**		**300 µg**		**150 µg**
N	**4**	**8**	**3**	**10**	**7**	**7**
Pre-treatment						
Week of gluten challenge	2·03 (0·80)	1·65 (0·78)	1·62 (0·56)	1·66 (0·55)	1·30 (0·32)	1·36 (0·30)
Week of placebo challenge	1·62 (0·40)	1·26 (0·31)	1·60 (0·07)	1·45 (0·34)	1·29 (0·22)	1·24 (0·21)
Last week of screening (baseline)	1·58 (0·59)	1·24 (0·38)	1·11 (0·14)	1·29 (0·32)	1·25 (0·25)	1·27 (0·30)
Treatment						
Treatment Week 1	1·75 (0·55)	1·90 (0·99) (p = 0·0313)	1·16 (0·27)	1·77 (0·52) (p = 0·0078)	1·36 (0·26)	1·59 (0·54) (p = 0·0313)
Treatment Week 7	1·25 (0·30)	1·32 (0·41)	1·60 (0·00)	1·17 (0·05)	1·41 (0·35)	1·32 (0·26)

Data are mean (SD). P-value was estimated by Wilcoxon Signed Rank test between a treatment week and the baseline week. Significant values ($p <= 0·05$) are highlighted in red.

Table S8. Daily symptoms diary scores during pre-treatment screening

Cohort	Day	Pain or Discomfort		Hunger Pain		Nausea		Rumbling		Bloating		Diarrhea	
		Gluten Challenge Week	Placebo Challenge Week	Gluten Challenge Week	Placebo Challenge Week	Gluten Challenge Week	Placebo Challenge Week	Gluten Challenge Week	Placebo Challenge Week	Gluten Challenge Week	Placebo Challenge Week	Gluten Challenge Week	Placebo Challenge Week
All screened with OGC (N = 95)	Challenge Day 1	1·84 (1·18) (p = 0·0047)	1·46 (0·74)	1·41 (0·81)	1·42 (0·87)	1·91 (1·68) (p = 0·0011)	1·32 (0·82)	1·74 (0·99) (p = 0·0157)	1·46 (0·80)	2·03 (1·19) (p = 0·0491)	1·76 (1·10)	1·56 (1·21) (p = 0·0224)	1·26 (0·72)
	Challenge Day 2	1·99 (1·26) (p = 0·0326)	1·64 (0·92)	1·36 (0·71)	1·27 (0·68)	1·65 (1·15) (p = 0·0279)	1·34 (0·74)	1·76 (1·04)	1·58 (0·89)	2·44 (1·50) (p = 0·0046)	1·91 (1·19)	1·57 (1·11)	1·32 (0·76)
	Challenge Day 3	1·95 (1·34) (p = 0·0370)	1·62 (1·05)	1·41 (0·88)	1·26 (0·61)	1·81 (1·34) (p = 0·0014)	1·33 (0·75)	1·73 (1·05)	1·52 (0·90)	2·47 (1·52) (p = 0·0005)	1·82 (1·18)	1·55 (1·04)	1·34 (0·83)
	Day 4	1·69 (1·12)	1·57 (1·14)	1·41 (0·81) (p = 0·0192)	1·24 (0·61)	1·40 (0·89)	1·33 (0·98)	1·67 (0·98)	1·47 (0·85)	2·12 (1·34) (p = 0·0014)	1·73 (1·28)	1·52 (0·99)	1·42 (1·04)
	Day 5	1·58 (1·05)	1·44 (0·94)	1·32 (0·73)	1·22 (0·55)	1·29 (0·74)	1·22 (0·62)	1·47 (0·76)	1·45 (0·91)	2·01 (1·30) (p = 0·0012)	1·63 (1·09)	1·52 (1·05)	1·31 (0·84)

Data are mean (SD) of daily symptoms score. Each symptom was scored on a scale of 1 to 7 (1 = no discomfort; 7 = very severe discomfort). P-value was estimated using Wilcoxon Signed Rank test between gluten challenge day and placebo challenge day. Significant values (p <= 0·05) are highlighted in red.

Table S9: Change in % immune cell types in peripheral blood mononuclear cells in particpants receiving Nexvax2 150 µg or placebo

Study		**3-dose study**		**Study**	**16-dose study**			
Cohort/s		**1st, 2nd & 3rd**	**3rd**	**Cohort/s**	**1st & 2nd**	**1st**	**3rd (biopsy)**	**3rd (biopsy)**
Treatment		**Placebo**	**Nexvax2**	**Treatment**	**Placebo**	**Nexvax2**	**Placebo**	**Nexvax2**
Dose			**150 µg**	**Dose**		**150 µg**		**150 µg**
N		**11**	**8**	**N**	**4**	**8**	**7**	**7**
Cell Type	**Day**			**Day**				
CD4+ T cells	Screening day 1	1·60 (5·64)	3·60 (4·41)	Screening day 1	-1·75 (2·17)	1·77 (8·42)	1·59 (5·96)	2·44 (3·45)
	Screening day 13	-0·25 (7·27)	5·02 (4·91)	Screening day 13	-3·63 (5·89)	0·07 (5·23)	-0·69 (5·80)	-0·83 (5·60)
	Day 1 baseline	47·56 (7·95)	50·83 (12·38)	Day 1 baseline	49·67 (8·87)	45·02 (10·22)	48·84 (10·10)	50·80 (5·35)
	Day 8	4·67 (12·52)	1·94 (4·23)	Day 8	2·05 (5·59)	-2·58 (6·24)	0·97 (5·87)	-1·17 (4·18)
	Day 15 EOT	1·03 (8·66)	2·89 (4·81)	Day 25	-1·60 (3·46)	0·65 (5·87)	1·08 (3·71)	-1·94 (4·42)
	Day 28	-0·78 (4·76)	3·43 (2·43)	Day 39	-1·27 (4·34)	-0·25 (5·50)	0·20 (8·15)	-0·97 (3·39)
	Day 47 EOS	4·60 (10·99)	4·88 (3·80)	Day 53 EOT	-0·03 (3·65)	-1·85 (6·29)	0·80 (5·53)	-3·19 (3·59)
	-			Day 66	0·35 (4·02)	-0·95 (4·99)	0·74 (4·22)	-2·86 (5·16)
	-			Day 92 EOS	3·75 (4·42)	-1·87 (5·75)	-2·13 (6·83)	-0·66 (3·48)
CD8+ T cells	Screening day 1	-2·13 (5·92)	-2·51 (4·26)	Screening day 1	-0·37 (3·80)	-3·75 (3·60)	-0·89 (3·20)	-1·74 (2·86)

	Screening day 13	-3·45 (6·63)	-0·80 (2·68)	Screening day 13	-0·12 (2·30)	-1·32 (1·95)	1·51 (1·14)	-0·10 (2·37)
	Day 1 baseline	28·51 (8·02)	24·09 (10·28)	Day 1 baseline	28·90 (12·37)	28·40 (13·61)	26·00 (8·55)	24·21 (6·13)
	Day 8	-3·24 (6·55)	-0·91 (2·14)	Day 8	-1·07 (0·95)	-0·82 (3·34)	1·41 (3·24)	-0·96 (1·99)
	Day 15 EOT	-1·61 (4·56)	-2·04 (5·00)	Day 25	1·92 (1·45)	-1·02 (2·27)	0·77 (1·87)	0·01 (0·99)
	Day 28	0·30 (2·67)	0·11 (3·06)	Day 39	1·65 (1·53)	-0·20 (3·84)	1·19 (3·28)	0·30 (2·71)
	Day 47 EOS	-3·77 (7·36)	-1·97 (4·32)	Day 53 EOT	1·05 (2·22)	-1·57 (4·37)	1·47 (3·39)	0·46 (2·65)
	-			Day 66	-0·23 (1·57)	2·17 (4·37)	0·77 (2·01)	-0·11 (1·46)
	-			Day 92 EOS	-0·90 (2·64)	0·92 (3·43)	1·09 (3·57)	-0·59 (1·40)
B cells	Screening day 1	-0·64 (1·95)	-0·34 (4·60)	Screening day 1	-1·40 (3·76)	-2·57 (4·25)	1·19 (2·09)	0·20 (2·96)
	Screening day 13	2·50 (4·68)	-0·67 (3·75)	Screening day 13	-2·83 (1·76)	-4·13 (5·34)	-0·53 (2·42)	-0·07 (3·39)
	Day 1 baseline	11·60 (5·96)	12·23 (6·56)	Day 1 baseline	10·98 (4·93)	17·43 (6·77)	11·39 (4·32)	11·17 (2·81)
	Day 8	-0·10 (2·38)	-0·57 (3·31)	Day 8	-1·62 (2·49)	-0·15 (5·05)	-1·91 (2·67)	0·06 (3·52)
	Day 15 EOT	0·74 (4·29)	-1·03 (3·24)	Day 25	-2·22 (2·53)	-1·17 (2·46)	-0·48 (1·78)	1·27 (5·19)
	Day 28	-0·05 (3·26)	-2·94 (3·70)	Day 39	-0·98 (1·66)	-2·45 (5·16)	0·49 (1·76)	0·47 (1·69)
	Day 47 EOS	-0·82 (1·07)	-1·55 (4·09)	Day 53 EOT	-2·95 (2·59)	0·67 (4·89)	0·19 (2·59)	0·97 (3·90)
	-			Day 66	-0·63 (1·71)	-5·10 (7·06)	0·10 (1·08)	0·41 (3·48)
	-			Day 92 EOS	-4·10 (3·58)	-3·35 (6·49)	-0·99 (2·46)	0·50 (1·39)

NK cells	Screening day 1	1·60 (3·22)	-0·34 (3·28)	Screening day 1	3·50 (5·64)	3·17 (6·86)	-1·93 (4·12)	0·29 (2·60)
	Screening day 13	1·92 (2·21)	-1·77 (4·95)	Screening day 13	4·45 (4·73)	3·33 (7·71)	-0·23 (4·31)	-0·10 (2·56)
	Day 1 baseline	9·37 (3·71)	10·87 (6·52)	Day 1 baseline	9·88 (4·61)	7·97 (3·38)	11·61 (5·22)	10·19 (2·69)
	Day 8	-0·40 (3·10)	-0·16 (2·34)	Day 8	0·07 (3·42)	2·67 (6·89)	-0·90 (3·30)	1·49 (4·54)
	Day 15 EOT	-0·01 (1·85)	0·16 (2·84)	Day 25	1·27 (2·97)	1·52 (4·98)	-1·70 (1·91)	1·13 (3·95)
	Day 28	0·78 (3·15)	-0·40 (2·75)	Day 39	-0·23 (1·56)	1·35 (3·51)	-1·70 (4·33)	-0·19 (2·03)
	Day 47 EOS	0·68 (3·06)	-0·82 (2·72)	Day 53 EOT	1·50 (2·91)	1·12 (3·56)	-1·93 (3·89)	1·03 (3·20)
	-			Day 66	0·60 (2·89)	2·22 (5·50)	-2·00 (2·42)	1·71 (2·42)
	-			Day 92 EOS	-0·18 (1·77)	4·28 (9·24)	1·11 (3·71)	-0·33 (3·29)

Data are mean (SD) for Day 1 of treatment (shaded in grey), and change in the % of cells from Day 1 of treatment on other days. P-value was estimated by Wilcoxon Sign Rank test between day 1 of treatment and other days. None of the p-values were significant ($p<=0.05$).

Table S10. Celiac disease-specific serology

CeD Serology	Study	3-dose study						16-dose study					
	Cohort/s	**1st, 2nd & 3rd**	**1st**	**2nd**	**3rd**	**4th (biopsy)**	**4th (biopsy)**	**1st**	**1st**	**2nd**	**2nd**	**3rd (biopsy)**	**3rd (biopsy)**
	Treatment	**Placebo**	**Nexvax2**	**Nexvax2**	**Nexvax2**	**Placebo**	**Nexvax2**	**Placebo**	**Nexvax2**	**Placebo**	**Nexvax2**	**Placebo**	**Nexvax2**
	Dose		**60µg**	**90µg**	**150µg**		**150µg**		**150 µg**		**300 µg**		**150 µg**
	Screening OGC	**Yes**	**Yes**	**Yes**	**Yes**	**No**	**No**	**Yes**	**Yes**	**Yes**	**Yes**	**No**	**No**
	N	**11**	**8**	**8**	**8**	**3**	**2**	**4**	**8**	**3**	**8**	**7**	**7**
Tissue transglutaminase (tTG) IgA	Screening day 1	1 (9%)	0 (0%)	0 (0%)	1 (13%)	0 (0%)	0 (0%)	2 (50%)	0 (0%)	0 (0%)	1 (13%)	0 (0%)	1 (14%)
	End of Treatment	2 (18%)	1 (13%)	0 (0%)	2 (25%)	0 (0%)	0 (0%)	2 (50%)	0 (0%)	0 (0%)	1 (13%)	0 (0%)	1 (14%)
Deamidated gliadin peptide (DGP) IgG	Screening day 1	1 (9%)	1 (13%)	0 (0%)	0 (0%)	1 (33%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (14%)
	End of Treatment	4 (36%)	1 (13%)	2 (25%)	2 (25%)	1 (33%)	0 (0%)	2 (50%)	1 (13%)	0 (0%)	1 (13%)	1 (14%)	2 (29%)
Deamidated gliadin peptide (DGP) IgA	Screening day 1	2 (18%)	3 (38%)	2 (25%)	2 (25%)	0 (0%)	0 (0%)	2 (50%)	1 (13%)	0 (0%)	2 (25%)	0 (0%)	2 (29%)
	End of Treatment	4 (36%)	2 (25%)	2 (25%)	2 (25%)	0 (0%)	0 (0%)	2 (50%)	1 (13%)	0 (0%)	2 (25%)	1 (14%)	2 (29%)

Data are n (%) indicating number of participants with elevated serology; Normal serology was defined as: tTG IgA upper level of normal is <4·0; DGP IgG upper level of normal is <20; DGP IgA upper level of normal is <20

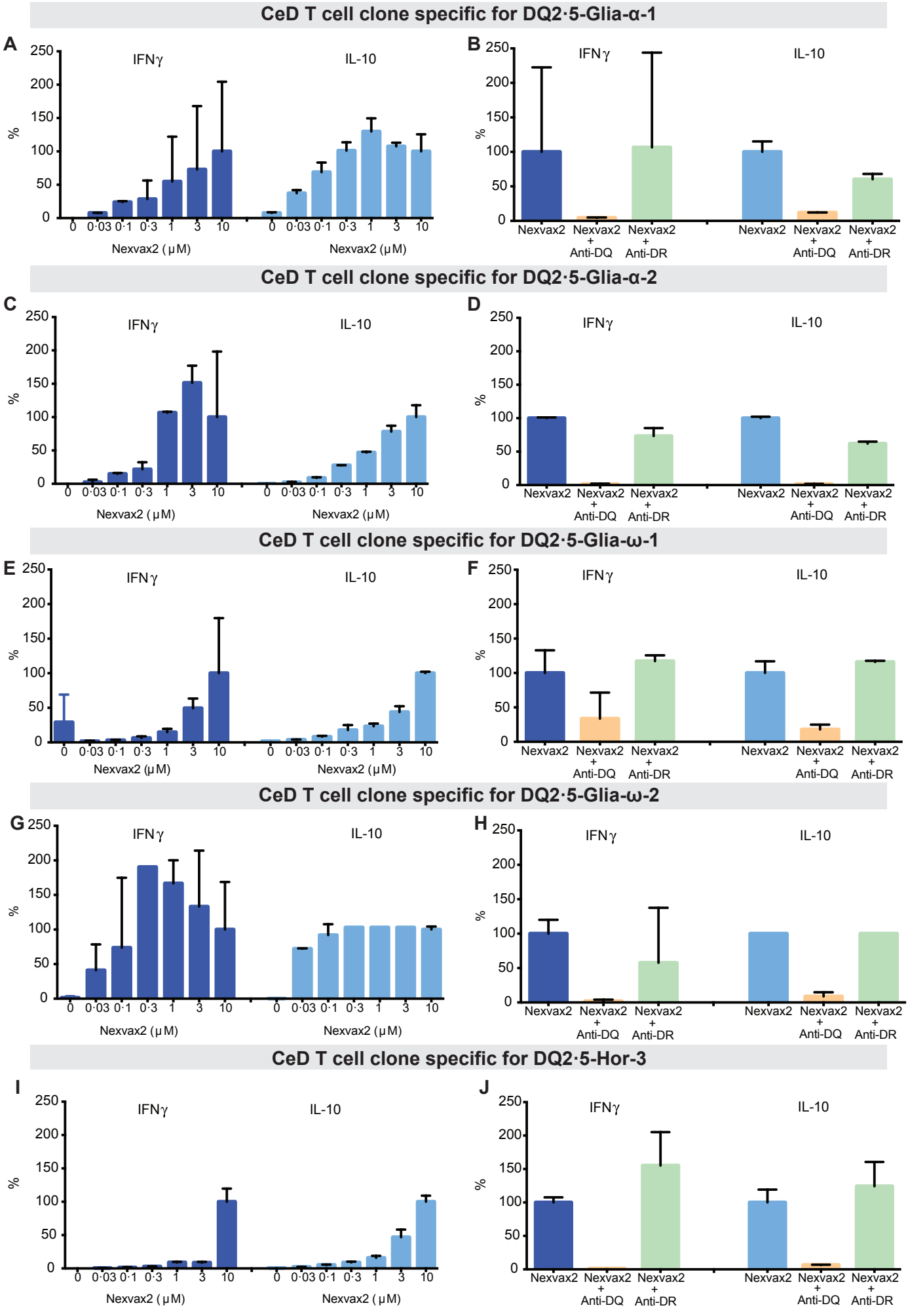
Table S11. Duodenal quantitative histology

Treatment	N	Timing	Sites of biopsy	Modified Marsh types	Villous height to crypt depth ratio	Intraepithelial lymphocyte density
				Median (min-max)	Median (IQR)	Median (IQR)
3-dose study						
Nexvax2 150 µg	2	Screening period	2nd and 3rd parts	0 (0 - 0)	2.04 (0.69)	21 (18)
	2	Post-treatment period		0 (0 - 0)	2.49 (0·67)	21 (10)
	2	Screening period	Bulb and 1st part	0 (0 - 0)	1.52 (0·64)	13 (18)
	2	Post-treatment period		0 (0 - 0)	1.45 (0·01)	17 (8)
Placebo	3	Screening period	2nd and 3rd parts	0 (0 - 1)	1.75 (0.62)	45 (21)
	3	Post-treatment period		1 (1 – 3a)	2.09 (.71)	36 (6)
	3	Screening period	Bulb and 1st part	0 (0 - 1)	1.43 (0·32)	46 (18)
	3	Post-treatment period		0 (0 - 1)	1·53 (0·28)	42 (8)
16-dose study						
Nexvax2 150 µg	7	Screening period	2nd and 3rd parts	0 (0 - 3c)	1·74 (0·54)	46 (24)
	7	Post-treatment period		0 (0 - 3c)	1·56 (0·58)	51 (33)
	7	Screening period	Bulb and 1st part	0 (0 - 3a)	1·44 (0·46)	30 (29)
	7	Post-treatment period		0 (0 - 3a)	1·70 (0·47)	36 (15)
Placebo	7	Screening period	2nd and 3rd parts	0 (0 - 1)	2·10 (0·25)	35 (16)
	7	Post-treatment period		0 (0 - 3b)	1·92 (0·35)	32 (18)
	7	Screening period	Bulb and 1st part	0 (0 - 1)	1·69 (0·48)	28 (13)
	7	Post-treatment period		0 (0 - 3a)	1·65 (0·28)	35 (18)

Table S12. Fold-change in plasma complement cytokines at 6h post-dose

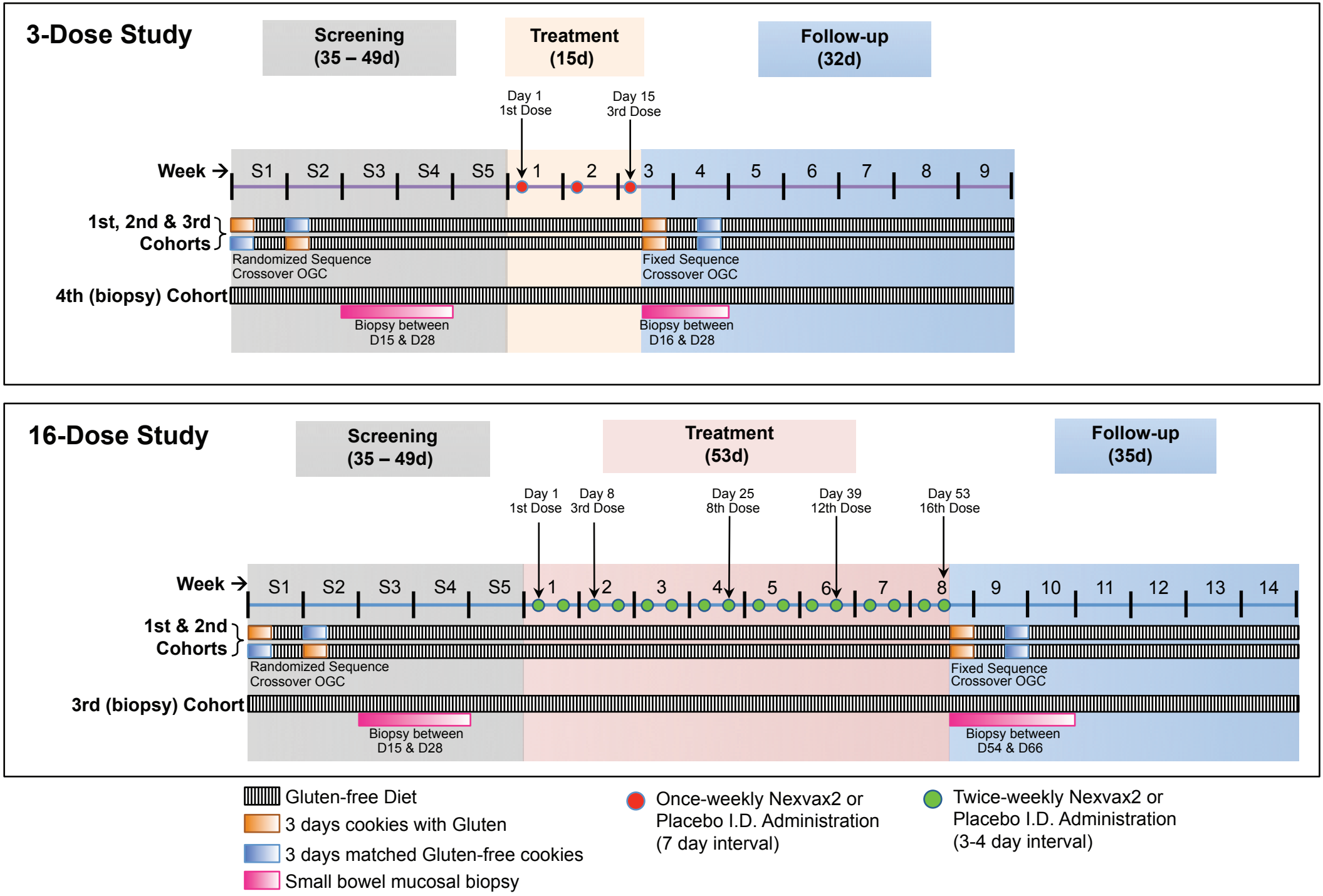
Cohort	1st & 2nd				1st				3rd (biopsy)				3rd (biopsy)			
Treatment	Placebo				Nexvax2 150ug				Placebo				Nexvax2 150ug			
N	7				8				7				7			
Dose	First Dose		Last Dose		First Dose		Last Dose		First Dose		Last Dose		First Dose		Last Dose	
Cytokines	Fold change	P-value	Fold change	P-value	Fold change	P-value	Fold change	P-value	Fold change	P-value	Fold change	P-value	Fold change	P-value	Fold change	P-value
C" C2	0.92 (0.07)	0.2500	0.95 (0.12)	0.8750	0.95 (0.11)	0.6731	0.87 (0.11)	0.4368	0.92 (0.10)	0.2388	0.91 (0.10)	0.4377	0.95 (0.03)	0.2190	0.94 (0.04)	0.4377
C" C4b	1.00 (0.05)	1.0000	0.98 (0.04)	0.8750	0.97 (0.05)	0.5569	0.97 (0.06)	0.7291	0.97 (0.05)	0.5569	0.98 (0.04)	0.8750	0.99 (0.04)	0.8922	0.99 (0.06)	0.8427
C" C5	1.00 (0.04)	0.8922	0.98 (0.05)	0.8427	0.98 (0.05)	0.6496	0.96 (0.10)	0.8750	0.97 (0.07)	0.6158	0.99 (0.06)	0.9681	0.99 (0.04)	0.6158	1.00 (0.03)	0.8750
C" C5a	0.99 (0.03)	0.6496	1.00 (0.06)	0.9800	0.99 (0.03)	0.6250	0.96 (0.06)	0.7291	0.97 (0.04)	0.6158	0.99 (0.02)	0.7158	0.99 (0.04)	0.8922	1.00 (0.02)	1.0000
C" C9	0.99 (0.08)	0.8370	1.02 (0.07)	0.9800	1.05 (0.15)	0.7470	1.04 (0.13)	0.8750	1.03 (0.14)	0.9087	1.01 (0.05)	0.8750	0.96 (0.08)	0.6731	1.05 (0.13)	0.8750
C" FD	0.94 (0.08)	0.3604	0.88 (0.10)	0.7000	0.89 (0.12)	0.2190	0.92 (0.14)	0.7291	0.91 (0.10)	0.2190	0.89 (0.10)	0.4377	0.92 (0.09)	0.2190	0.92 (0.06)	0.4368
MBL	0.98 (0.07)	0.8370	0.97 (0.06)	0.8427	0.94 (0.06)	0.2190	0.92 (0.12)	0.8427	0.95 (0.10)	0.8370	0.96 (0.05)	0.7658	0.99 (0.03)	0.2734	1.00 (0.04)	0.8750
C" FI	1.00 (0.03)	0.8922	0.98 (0.07)	0.8750	0.94 (0.07)	0.2734	0.94 (0.08)	0.4377	0.96 (0.06)	0.2190	0.99 (0.06)	0.8750	0.98 (0.04)	0.6731	0.99 (0.04)	0.8750
C" C1q	0.99 (0.06)	0.7218	0.98 (0.06)	0.8750	0.96 (0.06)	0.4863	0.94 (0.09)	0.7000	0.95 (0.08)	0.2190	0.97 (0.07)	0.7828	0.99 (0.04)	0.8370	0.98 (0.07)	0.8750
C" C3	1.05 (0.26)	1.0000	1.03 (0.41)	1.0000	0.94 (0.15)	0.6496	0.93 (0.17)	0.7291	0.83 (0.18)	0.2190	0.95 (0.18)	0.8427	0.85 (0.13)	0.2500	0.96 (0.21)	0.8333
C" C4	1.01 (0.07)	0.8922	0.95 (0.04)	0.7000	0.95 (0.05)	0.2190	0.96 (0.11)	0.9035	0.96 (0.09)	0.6158	0.99 (0.08)	0.8750	0.98 (0.06)	0.6496	0.99 (0.06)	0.9906
C" FB	1.01 (0.06)	0.9722	0.95 (0.04)	0.7778	0.95 (0.05)	0.2500	0.95 (0.11)	0.8750	0.95 (0.10)	0.5569	0.99 (0.09)	0.9800	0.98 (0.06)	0.6731	1.00 (0.06)	0.9906
C" FH	1.03 (0.08)	0.6731	0.95 (0.04)	0.7000	0.95 (0.06)	0.2190	0.96 (0.10)	0.9035	0.96 (0.09)	0.5569	0.98 (0.09)	0.8427	0.97 (0.07)	0.6496	1.00 (0.07)	0.9906
Properdin	1.01 (0.05)	0.8370	0.96 (0.04)	0.7778	0.98 (0.05)	0.6158	0.95 (0.11)	0.8333	0.96 (0.10)	0.6692	0.98 (0.08)	0.8750	0.99 (0.06)	0.9410	1.00 (0.06)	1.0000

Data are mean (SD) for paired fold change. Paired fold-change was estimate at 6h post-dose compared to pre-dose concentration levels. P-value was estimated by Wilcoxon Sign Rank test of pre-dose and 6h post-dose concentrations. FDR-adjusted p-values, were estimated using Benjamini-Hochberg method.



Supp. Figure S1: Nexvax2 peptides stimulate IFNγ and IL-10 secretion by T-cell clones from HLA-DQ2.5+ CeD donors that are specific for immunodominant, HLA-DQ2.5-restricted gluten epitopes. Cytokine concentrations measured by multiplex bead assay in media after 24 h incubation of T cell clones with HLA-DQ2.5+ B cell lines and equimolar concentrations of the three peptides in Nexvax2. Cytokine levels are represented as percent of concentrations stimulated by Nexvax2 with each **(continued)**

(Supp. Figure S1 continued) constituent peptide at 10 μM. T cell clones were specific for one of five HLA-DQ2.5-restricted gluten epitopes present in Nexvax2 peptides shown in Table S1 (A, C, E, G, and I). Consistent with gluten-reactive T cell clones being activated by Nexvax2 peptides bound to HLA-DQ2.5, cytokine secretion was inhibited by co-incubation with anti-HLA-DQ antibody (clone SPvL3), but not anti-HLA-DR (clone L243) at 10 μg/mL with Nexvax2 peptides at 10 μM (B, D, F, H, J).

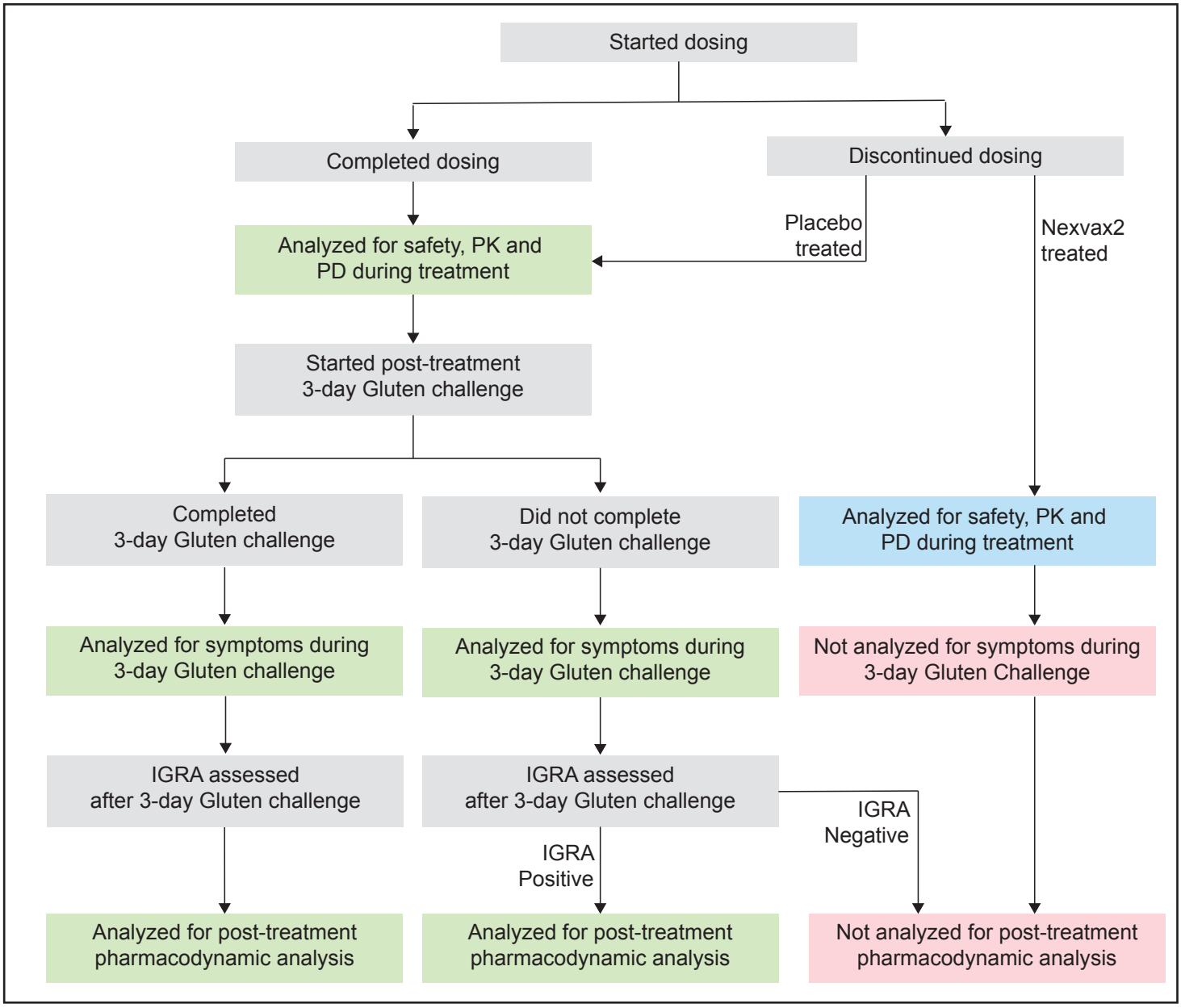


Supp. Figure S2: Study designs

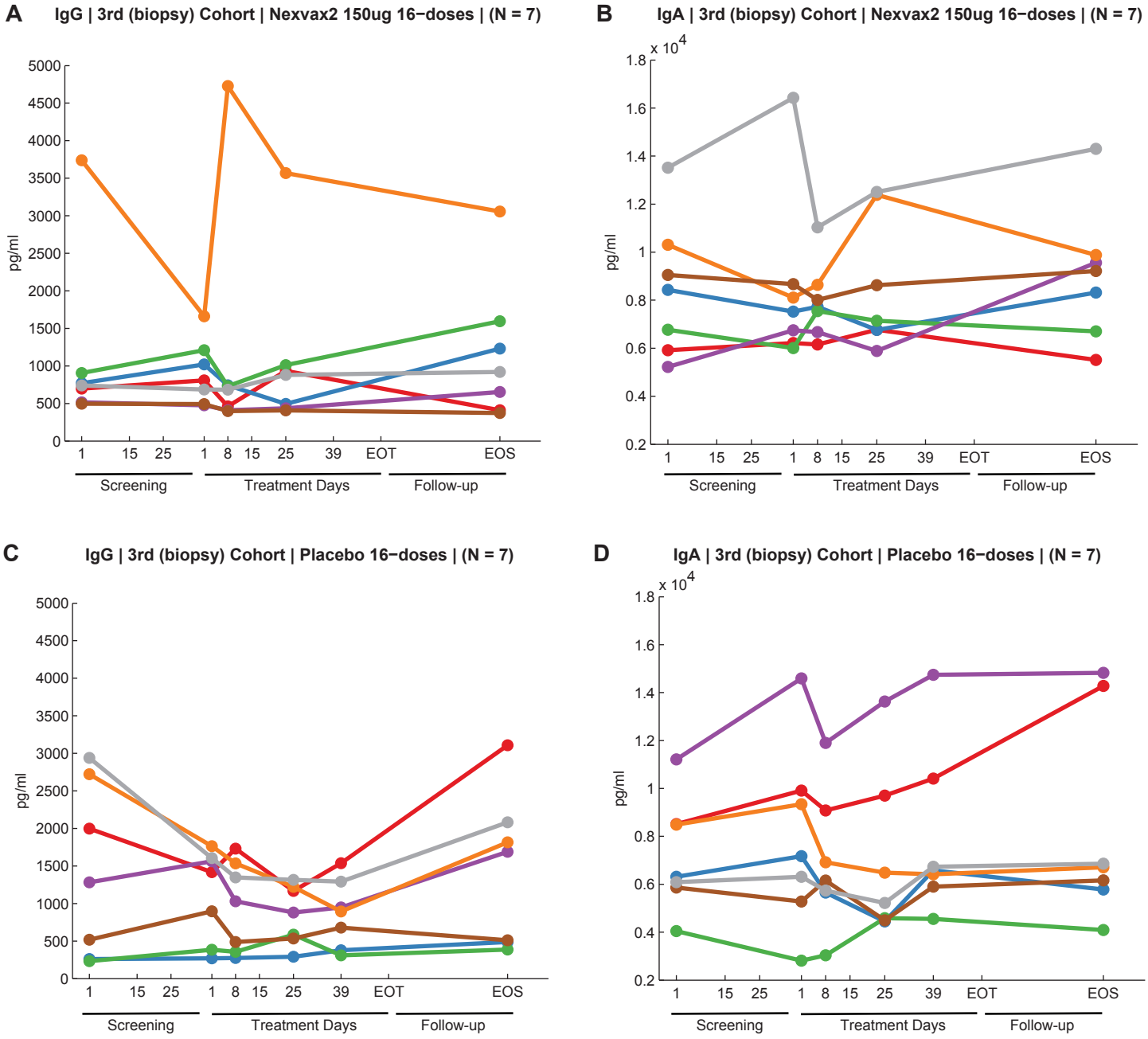
A Gluten cookies

B Placebo cookies

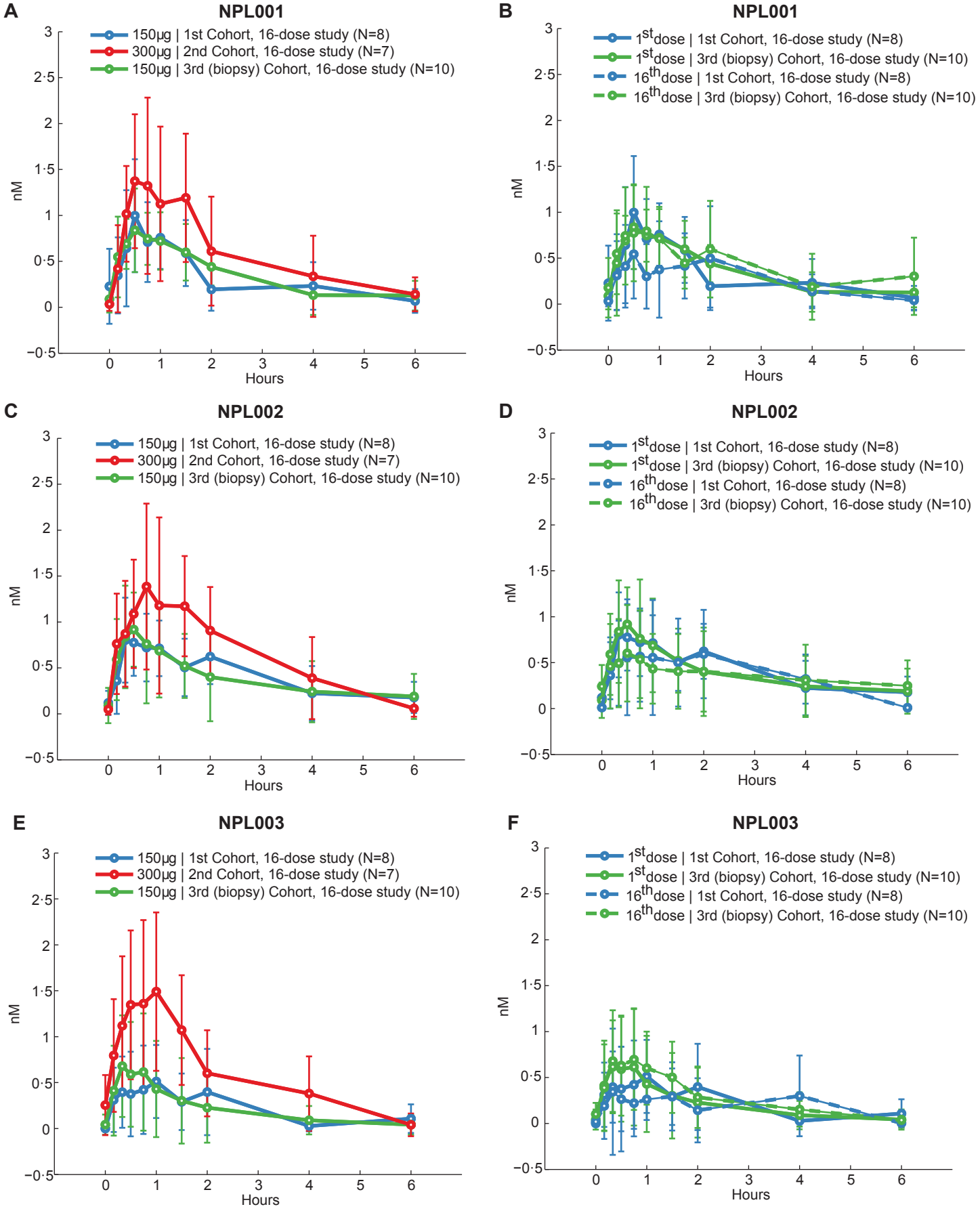
Supp. Figure S3: Cookies used for placebo-controlled, crossover oral gluten challenges. Gluten-containing (A), and matched gluten-free cookies (B) are shown. The gluten-containing cookies were prepared using a mixture of wheat, barley and rye flour providing a total of approximately 3 g gluten according to the Osborne calculation. Gluten-free cookies were matched for their appearance, weight, taste and consistency. Gluten-free cookies had no detectable gluten by R5 ELISA. Participants were advised to eat each cookie slowly over a 1-1.5 hour interval, and eat all three cookies completely each day, and to continue their usual gluten-free diet driven by their individual appetite on these three days.



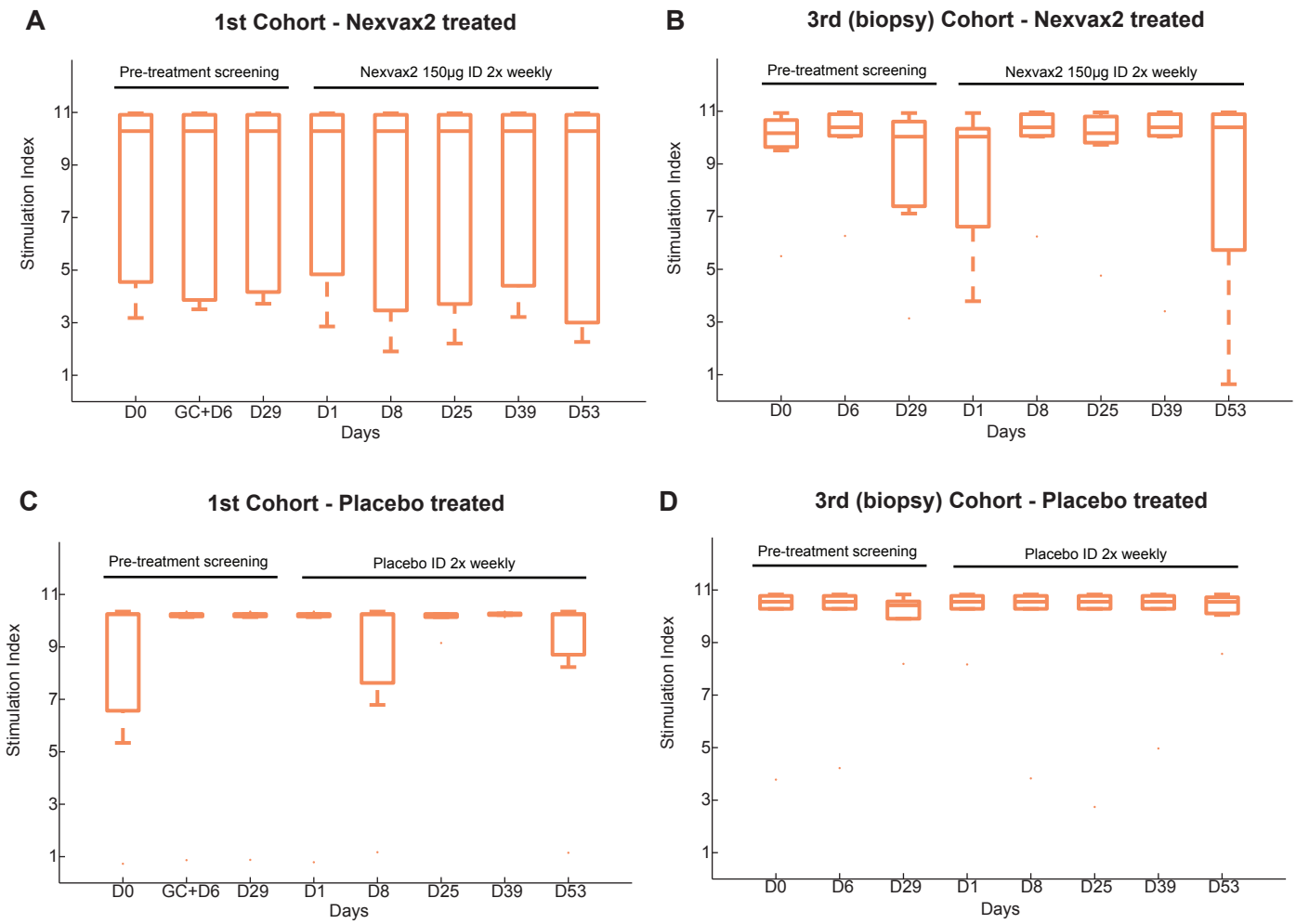
Supp. Figure S4: Participant flow map and data analysis populations for ascending dose cohorts



Supp. Figure S5: Anti-Nexvax2 antibodies. Anti-therapeutic IgG and IgA specific for Nexvax2 peptides were tested by ELISA in serum. IgG and IgA levels are shown for the biopsy cohort in the 16-dose study at Screening day 1, pre-dose on Treatment day 1 and before the 3rd, 8th, 12th dose and at the end-of-study.



Supp. Figure S6: Pharmacokinetics of Nexvax2 peptides in plasma. Mean (±SEM) plasma concentrations of NPL001 (A), NPL002 (C), and NPL003 (E) are shown after the first dose of Nexvax2 150µg (1st Cohort) or 300µg (2nd Cohort) in ascending dose cohorts that had OGC in screening, and in the biopsy cohort (3rd [biopsy] Cohort) in 16-dose Study. Mean (±SEM) plasma concentrations of NPL001 (B), NPL002 (D), and NPL003 (F) after the first and last dose of Nexvax2 150µg for 1st Cohort and 3rd (biopsy) Cohort in 16-dose Study. Measured concentrations of NPL001, NPL002, and NPL003 were below the lower levels of quantitation (2.6 nM, 5.5 nM, and 5.3 nM, respectively), but frequently above the lower levels of detection (0.05 nM, 0.1 nM, and 0.4 nM, respectively).



Supp. Figure S7: CEF activation of T cells ex vivo in blood. Fold increase (stimulation index) in IFNγ release by whole blood (IGRA) incubated for 24h with CEF peptides compared to negative control during screening, and treatment periods in the 16-dose study for participants receiving Nexvax2 150 µg in the 1st cohort (A) that had OGC during screening, and in the 3rd (biopsy) cohort (B), or who received placebo in the 1st cohort (C), and in the 3rd (biopsy) cohort (D). Median with interquartile range are shown. The CEF peptide pool contains MHC Class I epitopes commonly recognized by memory CD8+ T cells specific for cytomegalovirus, Epstein-Barr virus, or influenza antigens.