

Supplementary Material

A component-resolved therapeutic vaccine for cockroach allergy made of Per a 9 and transforming growth factor- β homologue, an immunosuppressive protein of *Brugia malayi*

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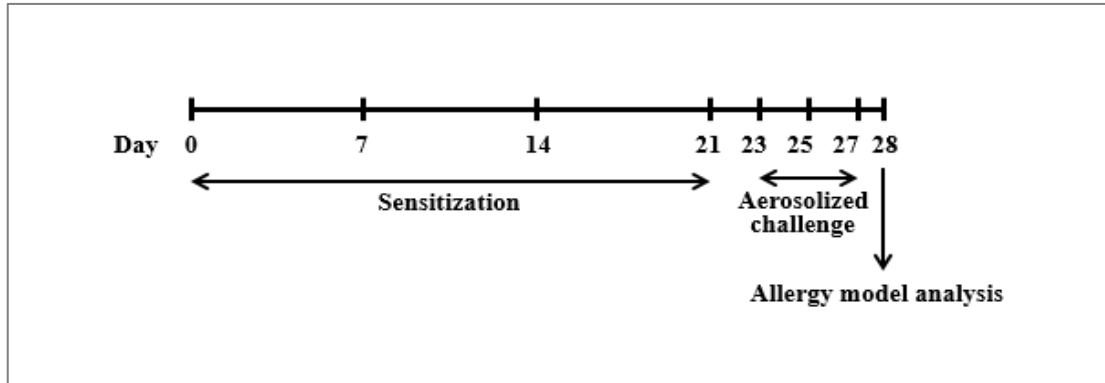
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Supplementary Table 1. Grades and histological features of mouse lung sections stained with hematoxylin and eosin (H&E), periodic acid–Schiff (PAS), and Masson’s trichrome (TRI) dyes.

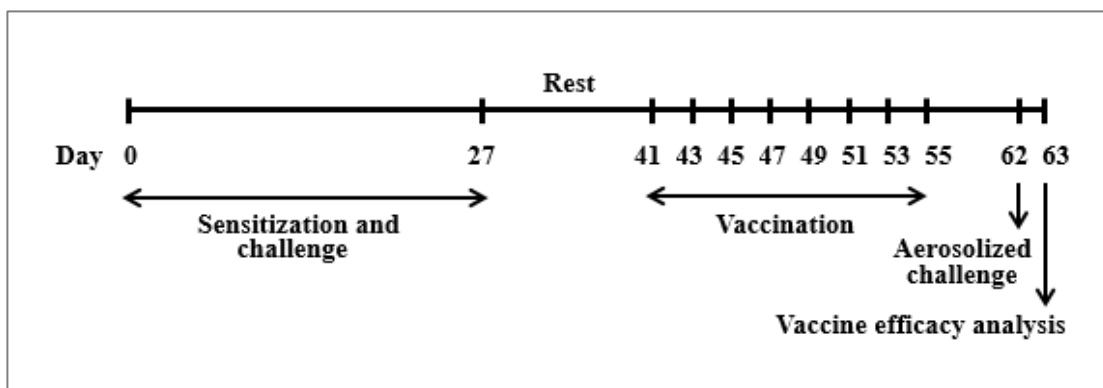
Grade	Histological features revealed by H&E staining
0	Bronchiole with single layer of ciliated epithelial cells, negligible inflammatory cell infiltration into peribronchiolar tissue, submucosa containing normal smooth muscle cells
1	Thickened bronchiole with ciliated pseudo-stratified columnar epithelial cells, few inflammatory cells in the interstitial area, mild degeneration of smooth muscle cells
2	Bronchiolar epithelial hyperplasia, 2–3 layers of inflammatory cells around the bronchiole, degeneration and detachment of smooth muscle in the submucosal area
3	Epithelial hyperplasia and sloughing, intense inflammatory cell infiltration, necrosis of smooth muscle cells and blockade of the airway
Grade	Histological features revealed by PAS staining
0	No goblet cells
1	1–5 goblet cells
2	6–20 goblet cells
3	More than 20 goblet cells
Grade	Histological features revealed by TRI staining
0	Normal collagen content of alveolar septa and thin-walled alveoli
1	Mild collagen deposition and thick fibrosis of the alveolar septa
2	Moderate collagen deposition and continuous fibrosis of the alveolar septa
3	Excessive collagen deposition leading to thickened alveolar septa, compressed alveoli, confluent fibrotic mass, and fibroplasia

Supplementary Table 2. Oligonucleotide primers used in qRT-PCR for monitoring cytokine gene expression.

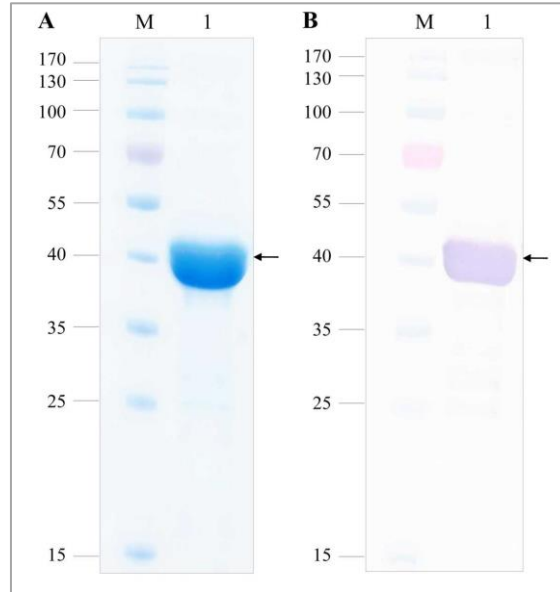
Gene	Primer sequence	Size of PCR product (bp)
<i>IL-4</i>	F: 5'-TCGGCATTTCGAAACGAGGTC-3' R: 5'-GAAAAGCCCCGAAAGAGTCTC-3'	216
<i>IL-5</i>	F: 5'-ATGATCGTGCCTCTGTGCCTGGAGC-3' R: 5'-CTGTTTTTCCTGGAGTAAACTGGGG-3'	242
<i>IL-6</i>	F: 5'-TCCAGTTGCCTTCTTGGGAC-3' R: 5'-GTACTCCAGAAGACCAGAGG-3'	331
<i>IL-13</i>	F: 5'-CGCTGGCGGGTTCTGTGTAG-3' R: 5'-GAGGCTGGAGACCGTAGTGGG-3'	127
<i>TNF-α</i>	F: 5'-CATCTTCTCAAAATTCGAGTGACAA-3' R: 5'-TGGGAGTAGACAAGGTACAACCC-3'	175
<i>IL-12a</i> (p35)	F: 5'-CCACCCTTGCCCTCCTAAAC-3' R: 5'-GTTTTTCTCTGGCCGTCTTCA-3'	132
<i>IL-12b</i> (p40)	F: 5'-GGAAGCACGGCAGCAGAATA-3' R: 5'-AACTTGAGGGAGAAGTAGGAATGG-3'	180
<i>IL-17A</i>	F: 5'-CAGGGAGAGCTTCATCTGTGT-3' R: 5'-GCTGAGCTTTGAGGGATGAT-3'	94
<i>IFN-γ</i>	F: 5'-AACGCTACACACTGCATCTTGG-3' R: 5'-GACTTCAAAGAGTCTGAGG-3'	237
<i>IL-10</i>	F: 5'-CGGGAAGACAATAACTG-3' R: 5'-CATTTCCGATAAGGCTTGG-3'	186
<i>TGF-β</i>	F: 5'-CAAGGGCTACCATGCCAACT-3' R: 5'-AGGGCAAGGACCTTGCTG-3'	84
<i>IL-35</i> (<i>ebi3</i>)	F: 5'-CAATGCCATGCTTCTCGGTAT-3' R: 5'-GGACGTGGATCTGGTGGAGTT-3'	84
<i>β-actin</i>	F: 5'-GGCCAACCGTGAAAAGATGA-3' R: 5'-CACGCTCGGTCAGGATCTTC-3'	251



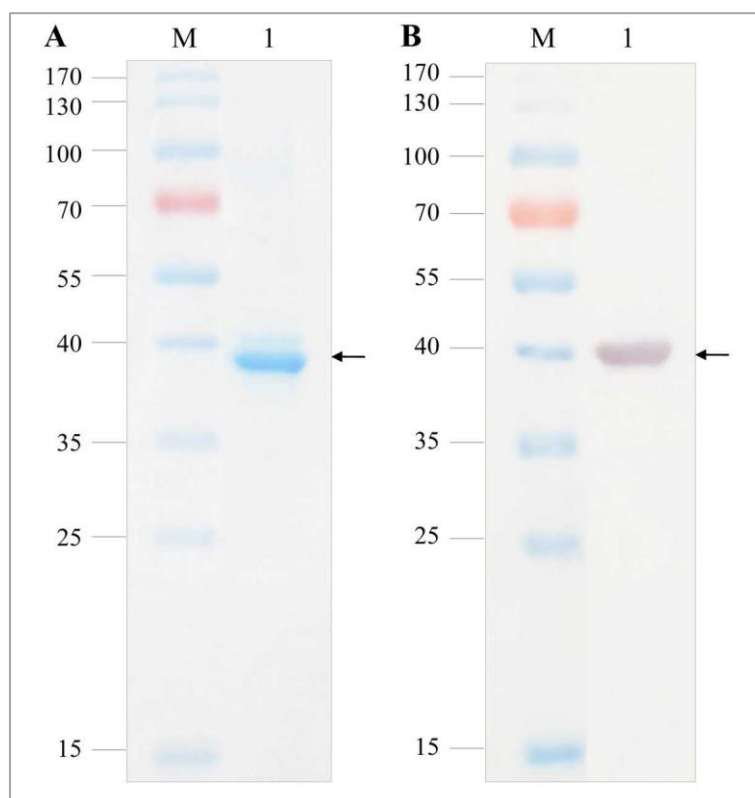
Supplementary Figure 1. Experimental timeline for preparation of allergenized and sham mice.



Supplementary Figure 2. Experimental timeline of vaccination (immunotherapy) of allergic mice, allergen provocation (aerosolized challenge) and vaccine efficacy analysis (determination of the therapeutic efficacies of vaccines after vaccination and CRE-provocation).



Supplementary Figure 3. Recombinant 6×-His tagged-Per a 9. (A) SDS-PAGE-separated purified recombinant 6×-His tagged Per a 9 (38-40 kDa). (B) Western blot pattern of the SDS-PAGE-separated purified recombinant 6×-His tagged-Per a 9 after probing with anti-6x-His antibody. Lanes M, Protein markers; lanes 1, purified recombinant 6×-His tagged Per a 9. Numbers at the left of (A) and (B) are protein masses in kDa.



Supplementary Figure 4. Recombinant TGH. (A) SDS-PAGE-separated pattern of recombinant TGH (~38 kDa) after staining with CBB (lane 1); (B) Western blot pattern of SDS-PAGE-separated recombinant TGH probed with anti-6× His antibody (lane 1). Lanes M of (A) and (B), Protein markers. Numbers at the left of (A) and (B) are protein molecular masses in kDa.