

Expanded View Figures

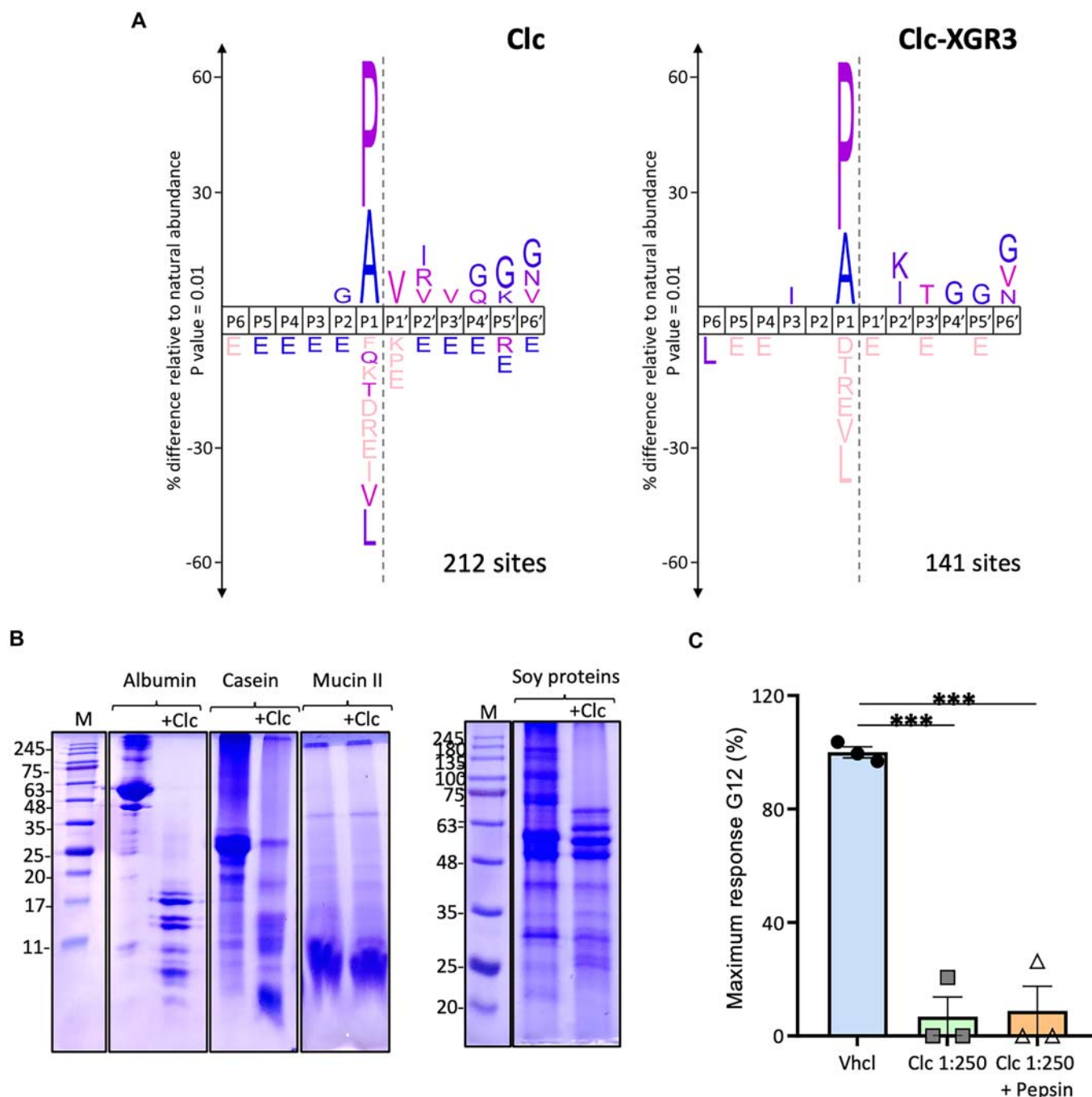


Figure EV1. Specificity profiling of celiacase and mutant Clc-XGR3 using PICS proteomics and activity against other proteins.

(A) The substrate specificities of celiacase (Clc) and Clc multimutant Clc-XGR3 were characterised using a GluC-derived peptide library from *Escherichia coli*. IceLogos visualise statistically significant ($p < 0.01$) amino-acid enrichment (above the x-axis) and depletion (below the x-axis) at non-prime (P_6 - P_1 , left) and prime (P_1' - P_6' , right) positions relative to the cleavage site, based on amino-acid frequencies in the *E. coli* K12 proteome. The data are derived from semi-specific cleavage peptides identified with at least two peptide-spectrum matches, and pink letters indicate amino acids not detected at a given position. (B) SDS-PAGE of albumin, casein, mucin II, and soy proteins after degradation by Clc at a 1:420 enzyme:substrate ratio. (C), ELISA ($n = 3$) of complex-meal digestion products using the G12 mAb pair, comparing Clc (1:250) and Clc (1:250) + Pepsin (0.5 μ M) with control (Vhcl). Data were presented as mean $\pm$ SEM. Statistical analysis via one- or two-way ANOVA: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

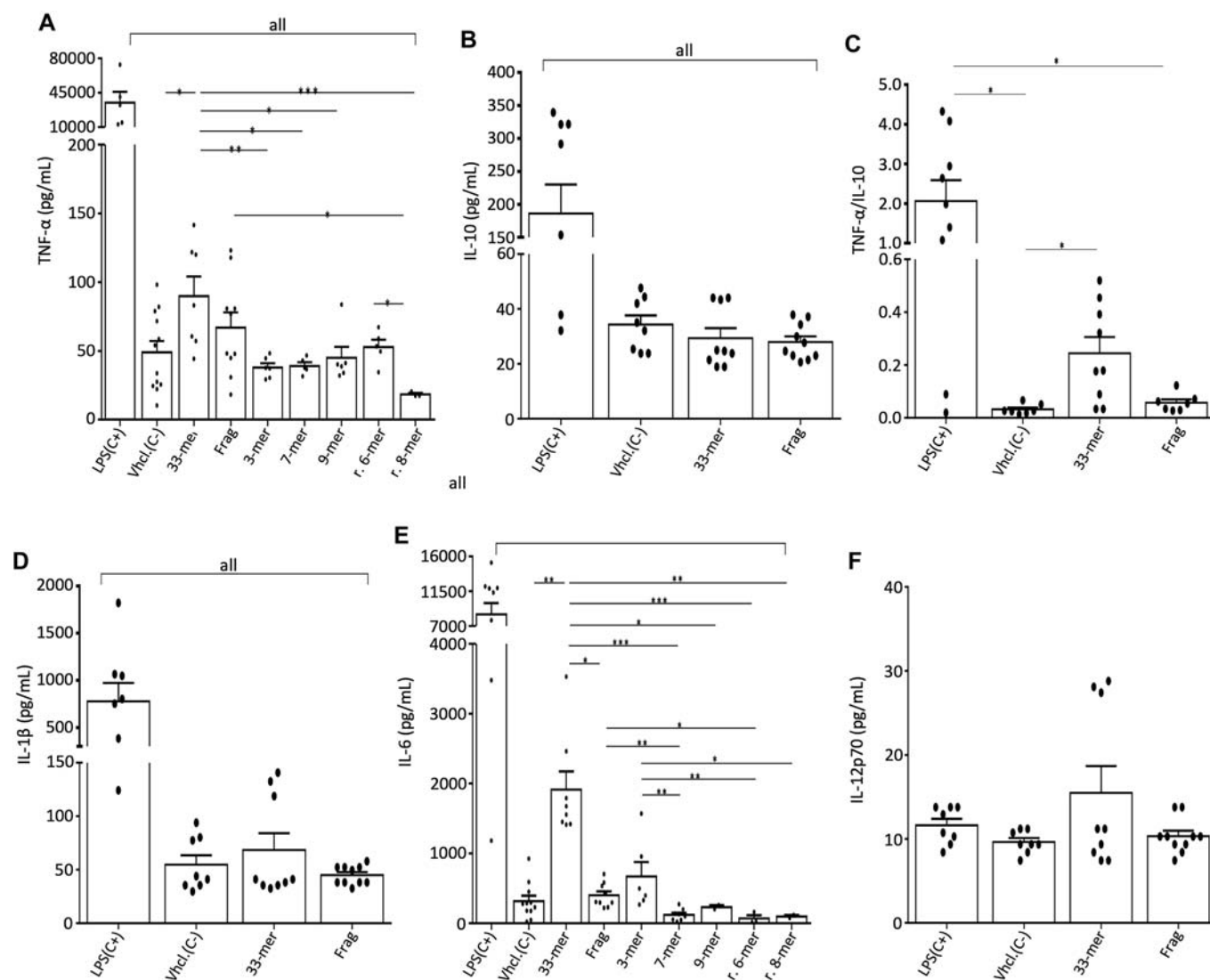
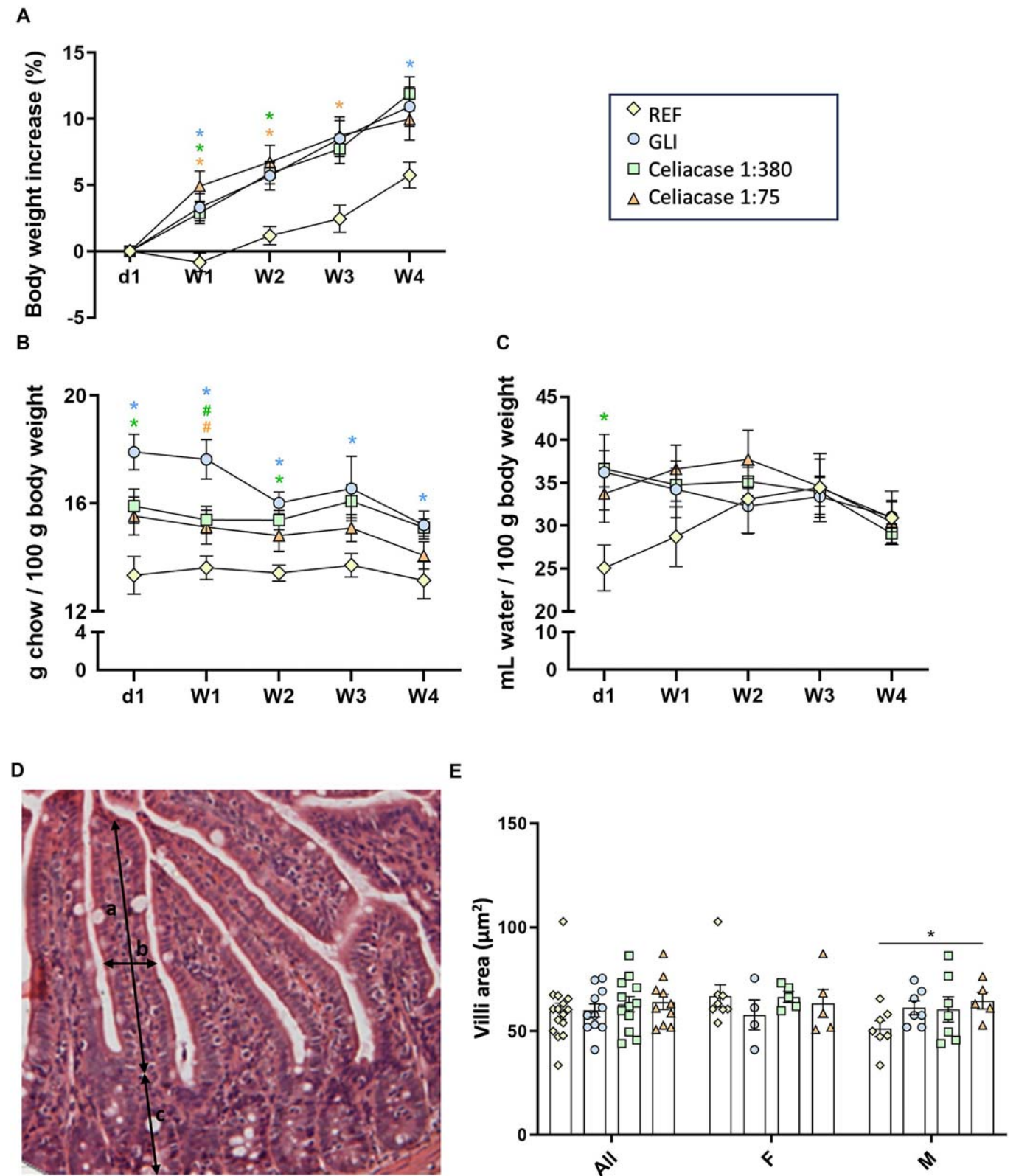


Figure EV2. Immune response of rat macrophages upon stimulation with the 33-mer and its fragments.

(A–F) Assessment of inflammatory cytokine secretion by peritoneal rat macrophages ($n = 24$) following stimulation with the 33-mer, its Clc cleavage fragments (3-mer, 7-mer, 9-mer and combined), or random (r) 6-mer (T A T R G G) and 8-mer (A T L A K V S H) peptides. Only the most significant cytokines are reported. Cytokine levels of TNF- α (A), IL-10 (B), TNF- α /IL-10 ratio (C), IL-1 β (D), IL-6 (E), IL-12p70 (F); all stimuli at 4.25 mM, except (A) at 0.25 mM.



**Figure EV3. Body weight, food and water intake of DQ8-D^d-villin-IL-15tg mice during the ED2 intervention, and post-intervention histological analysis.**

(A–C) Percentage increase in body weight, chow consumption, and water intake at day 1 (d1) and weeks 1–4 (W1–W4) within the 25-day experiment for the REF, GLI; Clc 1:380, and Clc 1:75 groups. Symbol colours indicate significant differences: * $p \leq 0.05$ vs. REF; # $p \leq 0.05$ vs. GLI. (D) Schematic representation of histological measurements: a = villi length, b = villi width, c = crypt depth, $a \times b$ = villi area, b/c = villi length-to-crypt depth ratio. (E) Villi area of the distal small intestine split into the REF, GLI, Clc 1:380 and Clc 1:75 groups. In all cases, $n = 7$ per group and sex, results are expressed as mean $\pm$ SEM for females (F), males (M), and combined (all), * stands for $p \leq 0.05$ vs. REF. Statistical analyses are as described in section “Statistical and data analysis”.