

Figure S1. Participant flow diagram.

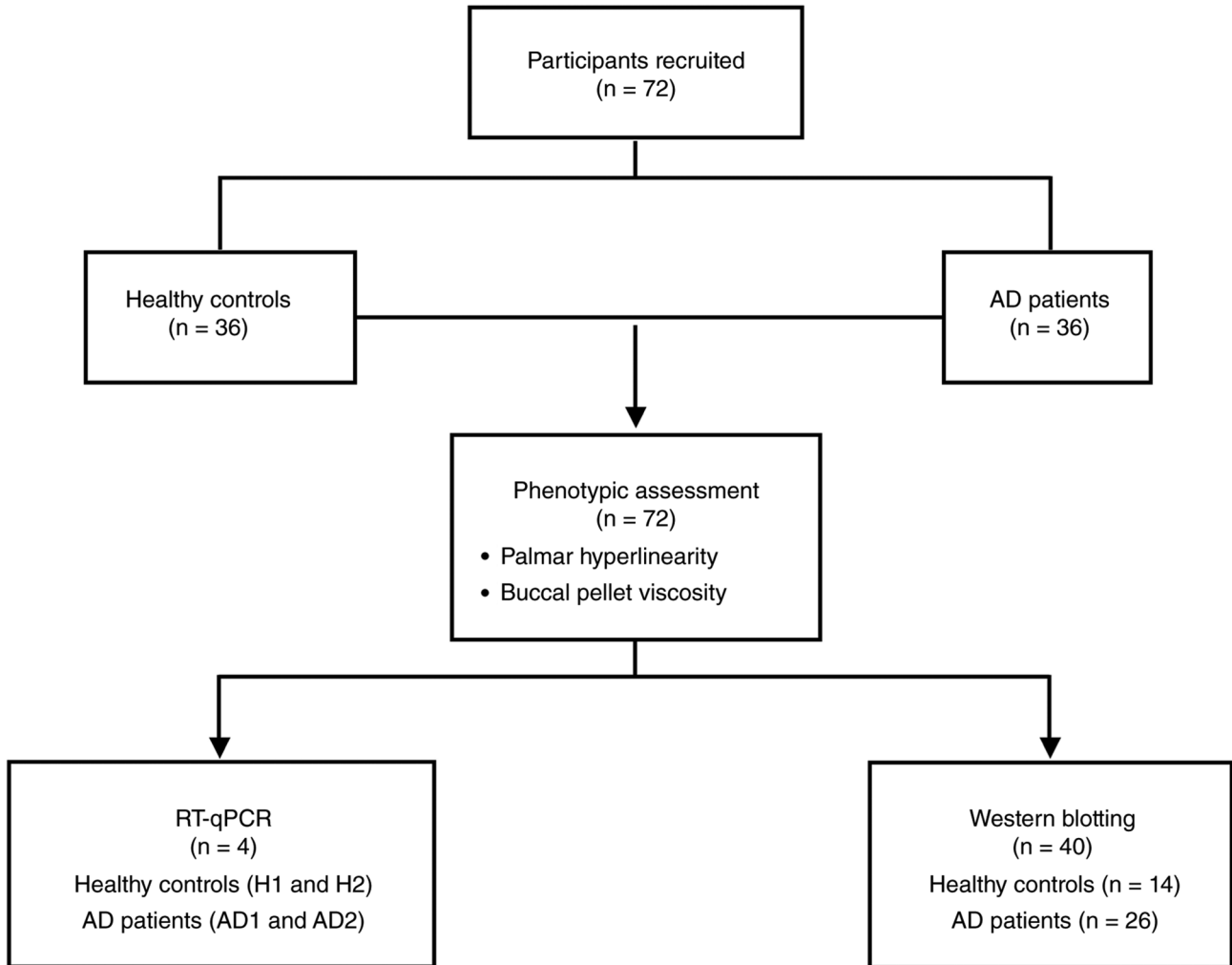


Figure S2. *FLG* gene expression analysis in buccal cells. (A) *FLG* gene expression levels in representative healthy controls (H1 and H2) and patients with AD (AD1 and AD2) across four biological replicates (batch 1-4) collected over different months. Expression levels are normalized to H1 (set as 100%). (B) *FLG* gene expression levels over 4 months, with each time point normalized to H1 (set as 100%). (C) Intra-individual variability of *FLG* gene expression across batches, with values for each participant normalized to their own Batch 1 (set as 100%). (D) Annealing positions of *FLG* primers and validation of primer specificity by sequencing verification of amplified products. Both *FLG* and *GAPDH* primers were sourced from PrimerBank. Data are presented as the mean $\pm$ SD of three technical replicates and are shown descriptively to illustrate temporal and inter-individual variability. No inferential statistical tests were performed. H, healthy control; AD, atopic dermatitis; *FLG*, filaggrin; M, marker.

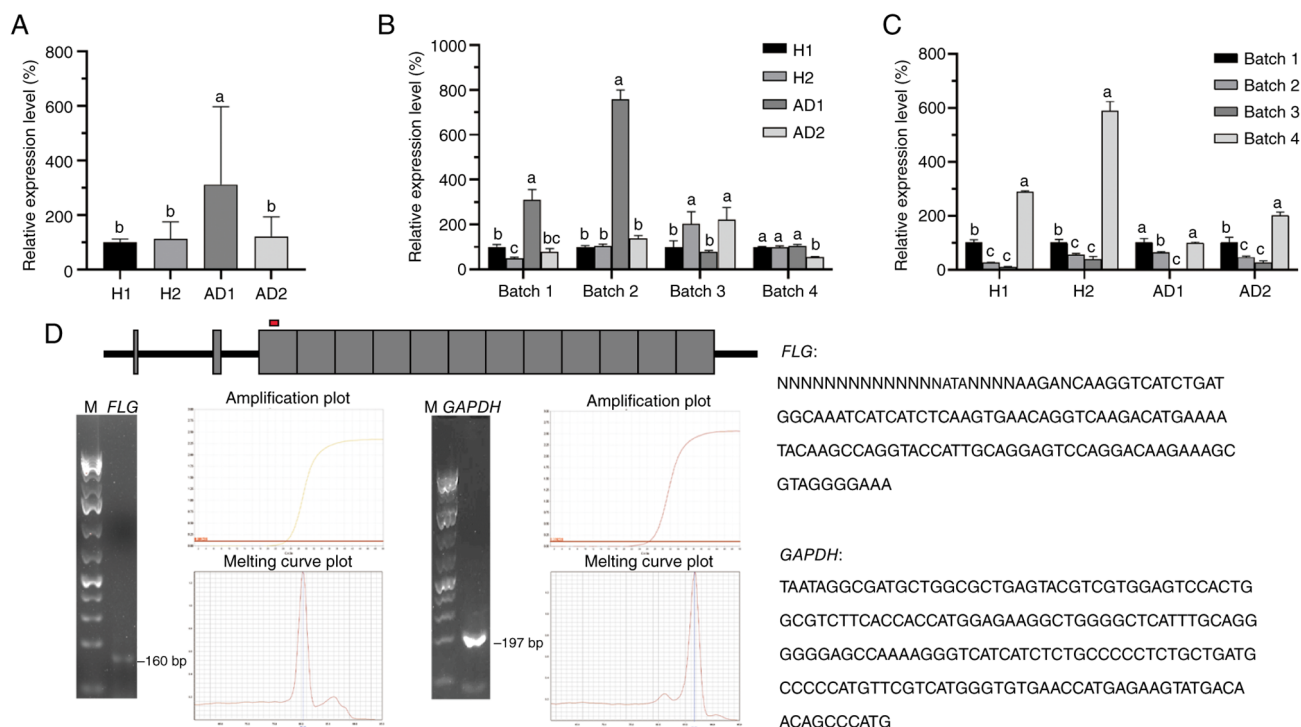


Figure S3. Expression of mucins in buccal cells. Immunoblot analysis of mucins expressed in the oral cavity, including MUC1, MUC4, MUC5B, MUC7, MUC16 and MUC19, in buccal cell samples from healthy controls and patients with AD. The mucin immunoblots and the KRT14 loading control shown here are derived from the same sample set and protein lysates as Fig. 2, and are retained to reflect the original experimental arrangement. Across all examined mucins, expression levels were comparable between groups, with no consistent upregulation in AD samples, indicating that mucin expression is unlikely to explain the increased viscosity of AD buccal cell pellets. MUC, mucin; H, healthy control; AD, atopic dermatitis; KRT14, keratin 14.

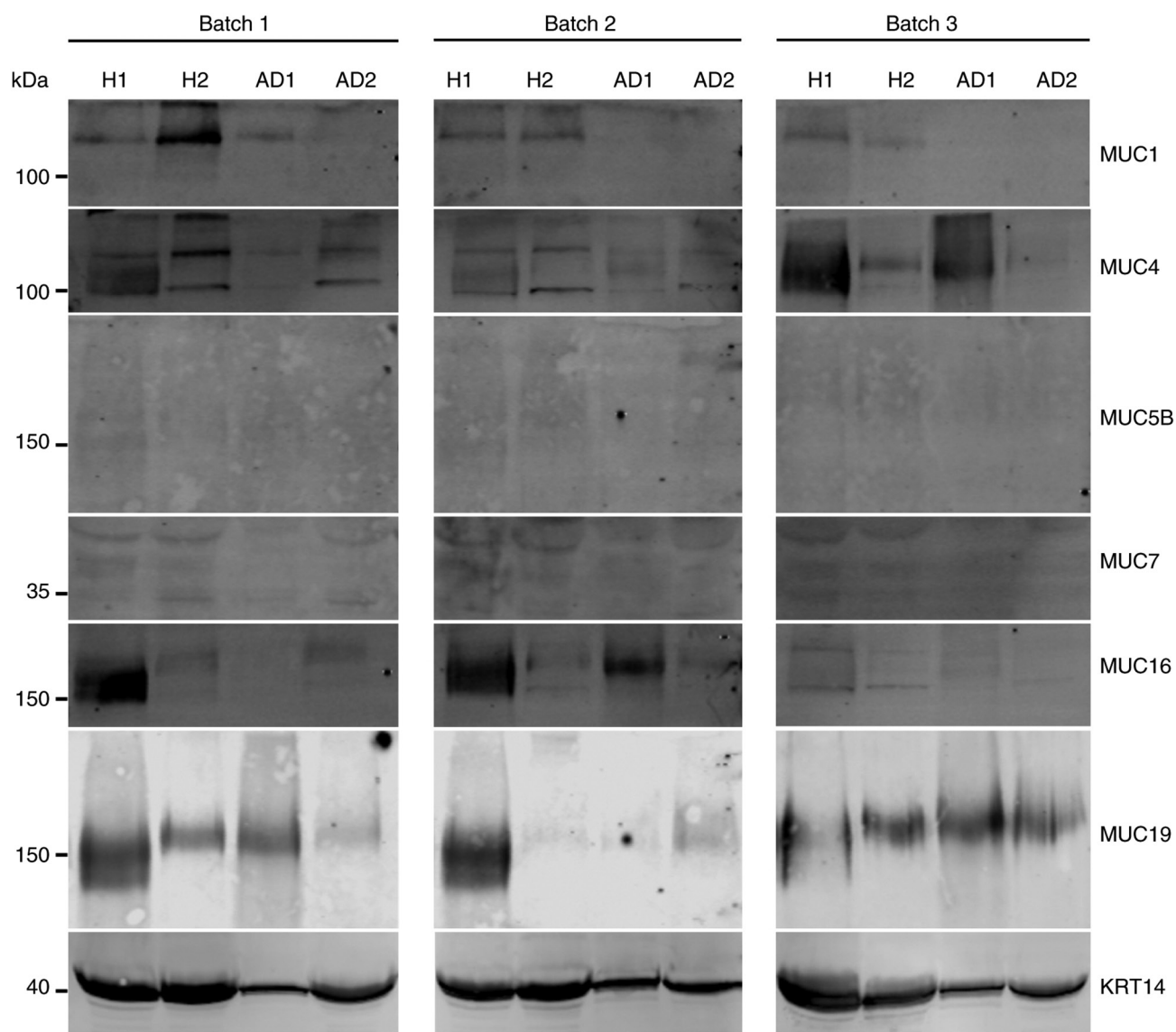


Figure S4. FLG hydrolysis profiles in expanded cohorts: Healthy controls (H3-H14) and patients with AD (AD3-AD26). (A) Mouse anti-FLG monoclonal antibody AKH1 immunoblots. (B) Poly19058 antibody immunoblots. (C) KRT14 was used as the control. Membranes were first probed with AKH1, then Poly19058, and finally stripped and reprobed with KRT14. Red color indicates samples with reduced expression levels of major bands or greater diversity between 20-40 kDa. Additionally, AD3 suffers from both AD and ichthyosis vulgaris, along with a clear PH phenotype (all highlighted). Notably, buccal cells failed to pellet at 7,800 x g. AD4, AD12, AD16 and AD23 have a family history of AD. 'V-21100' indicates viscosity at 21,100 x g. H, healthy control; AD, atopic dermatitis; FLG, filaggrin; PH, palmar hyperlinearity; KRT14, keratin 14.

