Supplementary Table 1: *Primer pair sequences for the analyses of gene expression.*

Gene	Primer pairs	Reference	Cycling conditions
Actb	*fw* 5' AGCTGCGTTTTACACCCTTT 3' *rv* 5' AAGCCATGCCAATGTTGTCT 3'	[Cardoso et al., 2009]	*Pre-Incubation* *2 min 50ºC* *Incubation* *10 min 95ºC* **40 cycles:** *Denaturation* *15 s 95ºC* *Annealing/Extension* 45 s 58ºC + 15 s 60ºC
Cldn2	*fw* 5' GTCATCGCCCATCAGAAGAT 3' *rv* 5' ACTGTTGGACAGGGAACCAG 3'	[Volvolynets et al., 2016]	
Cldn3	*FW 5' CCACTACCAGCAGTCGATGA 3'* *RV 5' CAGCCTGTCTGTCCTCTTCC 3'*	[Corridoni et al., 2012]	
Tjp1	*FW 5' TACCTCTTGAGCCTTGAACTT 3'* *RV 5' ACAGAAATCGTGCTGATGTGC 3'*	[Lozano-Ojalvo et al., 2019]	
Il22	*fw* 5' CATGCAGGAGGTGGTACCTT 3' *rv* 5' CAGACGCAAGCATTTCTCAG 3'	[Lozano-Ojalvo et al., 2019]	
Il6	*fw* 5' TTCCATCCAGTTGCCTTCTTG 3' *rv* 5' GGGAGTGGTATCCTCTGTGAAGTC 3'	[Tordesillas et al., 2014]	
Il4	*fw* 5' CCTCACAGCAACGAAGAACA 3' *rv* 5' ATCGAAAAGCCCGAAAGAGT 3'	[Yang et al., 2009]	
Il13	*fw* 5' CATGGCCTCTGTAACCGCAA 3' *rv* 5' CCTCATTAGAAGGGGCCGTG 3'	[Pérez-Rodriguez et al., 2020]	
Tlr2	*fw 5' TCTGCGACCTAGAAGTGGAA 3'* *rv 5' TGAAGCCCCTACACTCAGAA 3'*	This work	
Tlr4	*fw 5' AATGCCCTATTGGATGGAAA 3'* *rv 5' AGGCCCCAGAGTTTTGTTCT 3'*	[Benedé et al., 2018]	
Tlr5	*fw 5' GGTGTGATCTTCATGGCCAGCCC 3'* *rv 5'CGTCGCTTAAGGAATTCAGTTCCCGG3'*	[Mathur et al., 2012]	
Actb	*fw* 5' AGCTGCGTTTTACACCCTTT 3' *rv* 5' AAGCCATGCCAATGTTGTCT 3'	[Cardoso et al., 2009]	*Pre-Incubation* *2 min 50ºC* *Incubation* *10 min 95ºC* **40 cycles**: *Denaturation* *15 s 95ºC* *Annealing/Extension* *60 s 60ºC*
Il33	*fw* 5' ATTTCCCCGGCAAAGTTCAG 3' *rv* 5' AACGGAGTCTCATGCAGTAGA 3'	[Li et al., 2013]	
Il25	*fw* 5' ACAGGGACTTGAATCGGGTC 3' *rv* 5' TGGTAAAGTGGGACGGAGTTG 3'	[Li et al., 2013]	
Tslp	*fw* 5' AGGCTACCCTGAAACTGAGA 3' *rv* 5' GGAGATTGCATGAAGGAATAC 3'	[Negishi et al., 2012]	
Gata3	*fw* 5' CCTTAAAACTCTTGGCGTCC 3' *rv* 5' AGACACATGTCATCCCTGAG 3'	[Zhang et al., 2013]	
Il9	*fw* 5' GTCCGTCCTTTTCCTGCGAA 3' *rv* 5' TCTGTCTTCATGGTCGGCTT 3'	This work	
IL12p40	*fw 5' AGGTGCGTTCCTCGTAGAGA 3'* *rv 5' AAAGCCAACCAAGCAGAAGA 3'*	[Blazquez and Berin, 2016]	
Jag2	*fw* 5' GGCAAAGAATGCAAAGAAGC 3' *rv* 5' GCTCAGCATTGATGCAGGTA 3'	[Blazquez and Berin, 2016]	
Irf4	*fw* 5' TCCTCGTCCCTTGCTGAAAC 3' *rv* 5' GGGCTTTGGGGCTTCTAGTT 3'	[Pérez-Rodriguez et al., 2020]	
Actb	*fw* 5' AGCTGCGTTTTACACCCTTT 3' *rv* 5' AAGCCATGCCAATGTTGTCT 3'	[Cardoso et al., 2009]	*Pre-Incubation* *2 min 50ºC* *Incubation* *10 min 95ºC* **40 cycles:** *Denaturation* *15 s 95ºC* *Annealing/Extension* *30 s 56ºC + 30 s 58ºC*
Tjp2	*FW 5' TGGGACCGTCGCTTTCTG 3'* *RV 5' CTGTGGCGGGGAGGTTTGA 3'*	This work	
Tnfsf4	*fw* 5' GGGATGCTTCTGTGCTTCATCT 3' *rv* 5' TTTGGATTGGAGGGTCCTTTG 3'	[Mehta et al., 2016]	

fw, forward; rv, reverse

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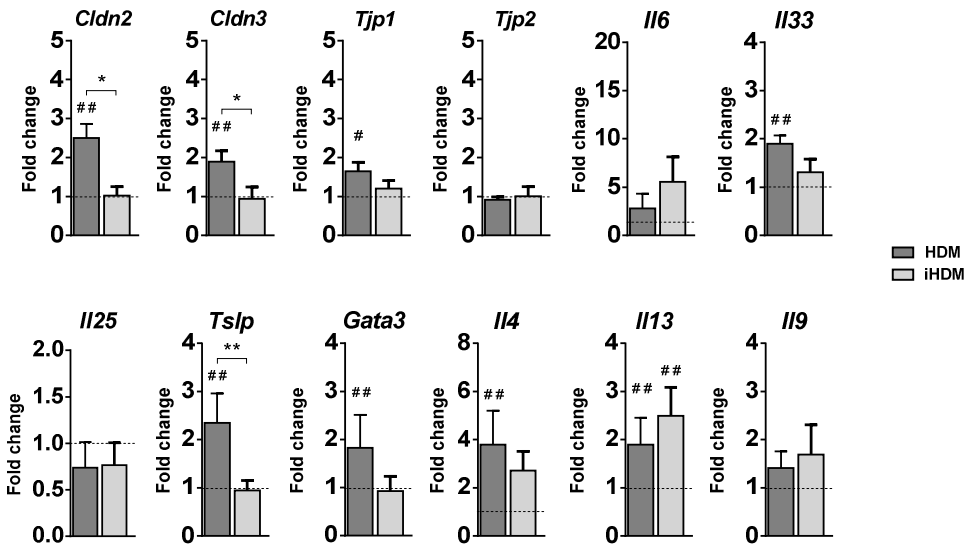
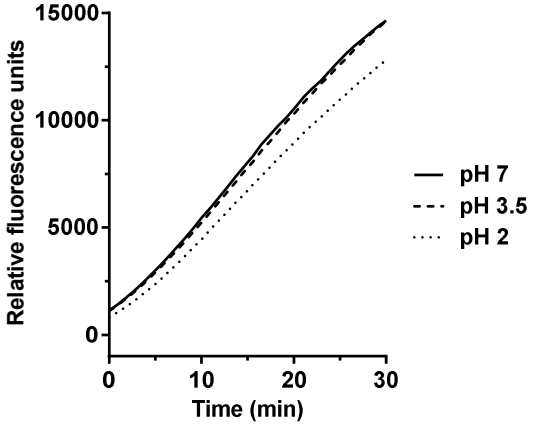
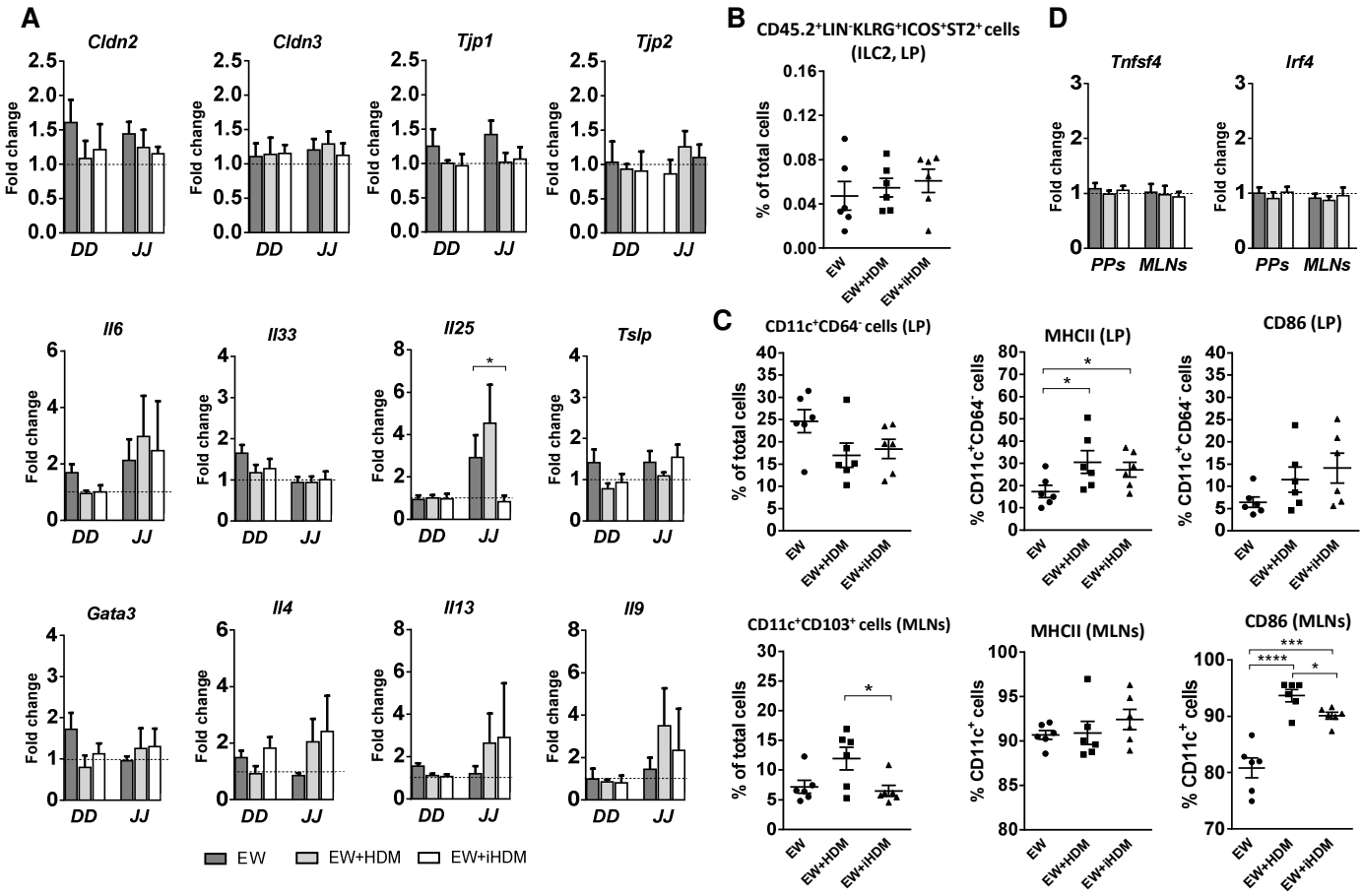


Supplementary Figure 1. Relative gene expression of *Cldn2*, *Cldn3*, *Tjp1*, *Tjp2*, *Il22*, *Il33*, *Il25*, *Tslp*, *Il4*, *Il13*, and *Il9* determined in the duodenum of mice administered intragastrically proteolytically active or inactive house dust mite (respectively HDM and iHDM) for 6 consecutive days. Gene expression was normalized to the reference gene *Actb*. Data are expressed as means ±SEM (n=6). Dashed lines indicate the reference value for mice administered PBS. Pounds and asterisks indicate, respectively, statistically significant differences with respect to mice administered PBS or between both experimental groups. * and # $p<0.05$, ** and ## $p<0.01$.

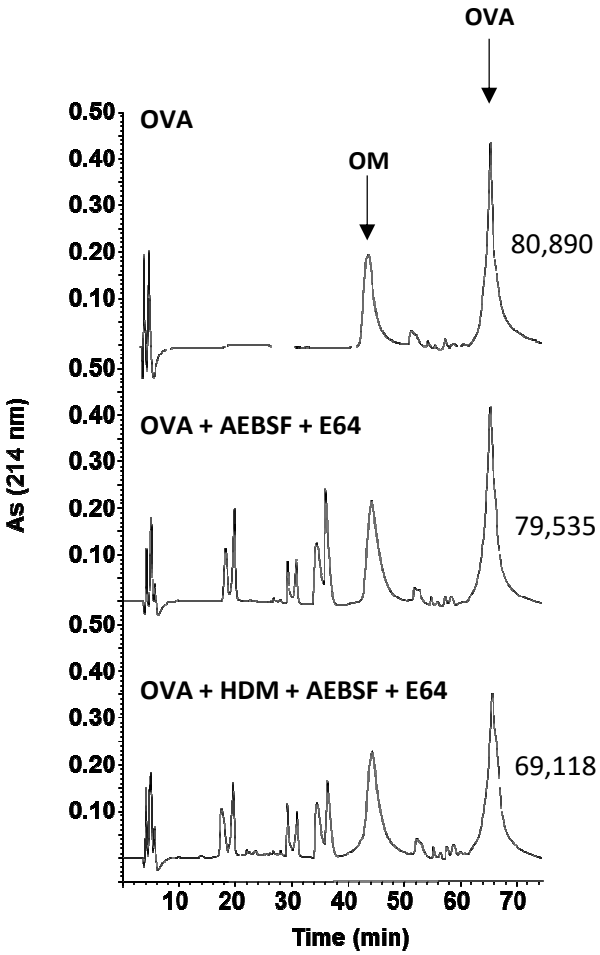


Supplementary Figure 2. Proteolytic activity (expressed as arbitrary fluorescence units) of house dust mite (HDM) at pH 2.0, 3.5 and 7.0.



Supplementary Figure 3. Mice were administered intragastrically egg white (EW) alone or in combination with proteolytically active or inactive house dust mite (respectively EW+HDM and EW+iHDM) for 6 consecutive days. **A** Relative gene expression of *Cldn2*, *Cldn3*, *Tjp1*, *Tjp2*, *Il6*, *Il33*, *Il25*, *Tslp*, *Gata3*, *Il4*, *Il13*, and *Il9* determined in the duodenum (DD) and jejunum (JJ). **B** Group 2 innate lymphoid cells (ILC2s, defined as KLRG1$^+$ICOS$^+$ST2$^+$ cells within the CD45.2$^+$ Lineage$^-$ [CD3$^-$CD45R$^-$CD11b$^-$ TER-119$^-$Ly-G6$^-$CD19$^-$ cells]) in the lamina propria (LP). **C** Dendritic cells (DCs) in the LP (defined by the expression of CD11c$^+$ and CD64$^-$), LP-derived DCs in the mesenteric lymph nodes (MLNs) (defined by the expression of CD11c$^+$ and CD103$^+$), and expression of MHCII and CD86 within DCs. **D** Relative gene expression of *Tnfs4* and *Irf4* determined in Peyer's Patches (PPs) and MLNs). Gene expression was normalized to the reference gene *Actb*. Data are expressed as means ± SEM (n=6). Dashed lines indicate the reference value for mice administered PBS. Asterisks indicate statistically significant differences among groups. * $p<0.05$, ***$p<0.001$, ****$p<0.0001$.

Supplementary Figure 4



Supplementary Figure 4. RP-HPLC analyses of ovalbumin (OVA), as such, and after incubation with house dust mite (HDM) for 24 h at 37 ºC, followed by the addition of the serine protease inhibitor AEBSF and the cysteine protease inhibitor E-64. The effect of the sole addition of inhibitors is also shown for comparison. Ovomucoid impurity (OM). Numbers indicate integration values of OVA peaks (mV*sec).