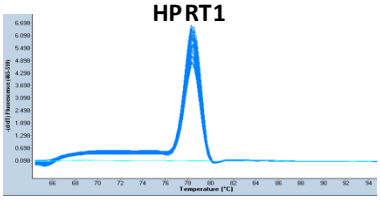
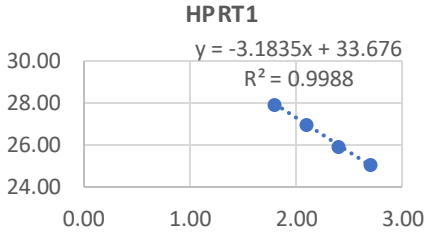
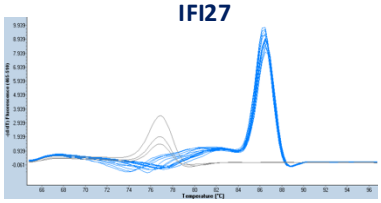
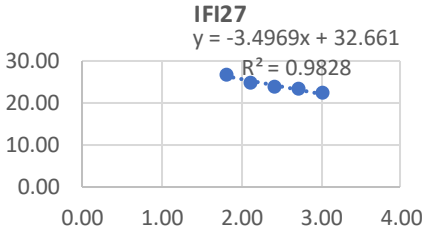
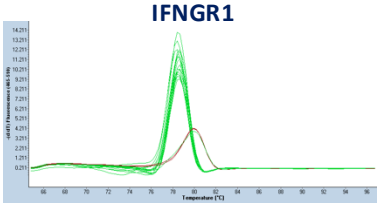
Melting curves and qPCR efficiency

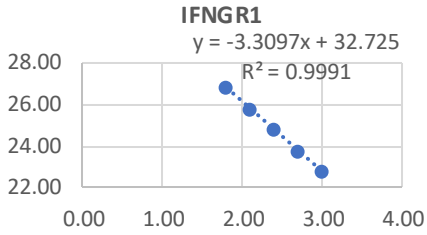


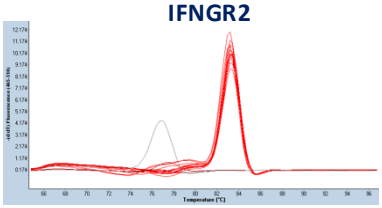


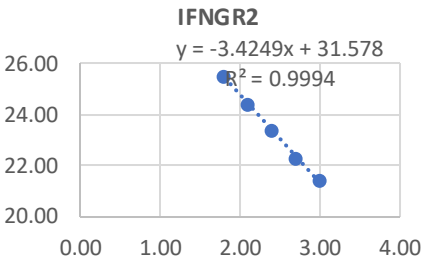


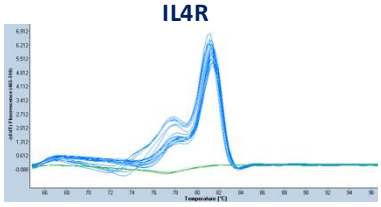


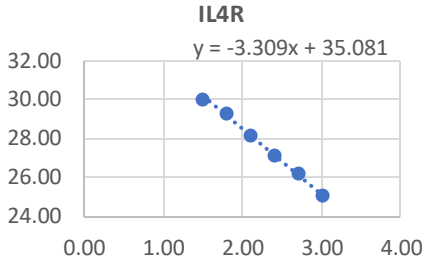


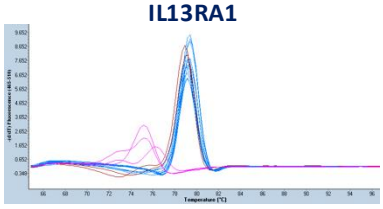


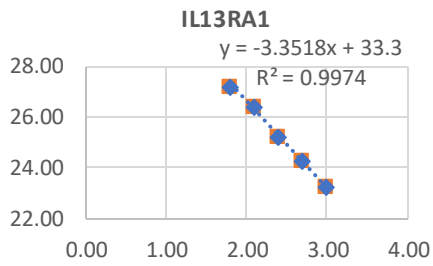


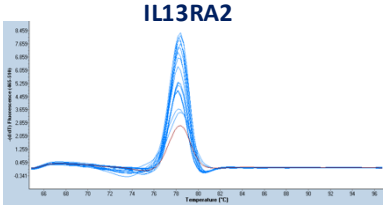


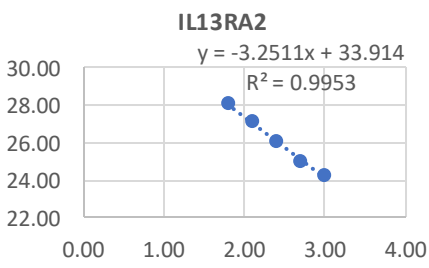


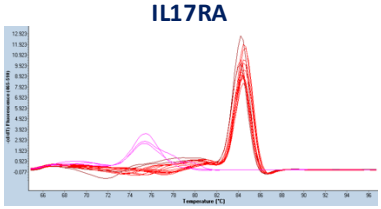


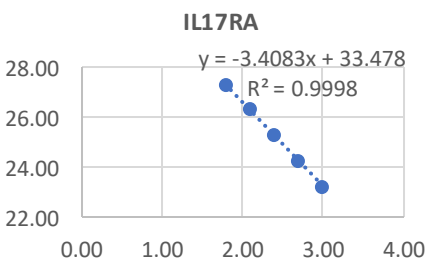


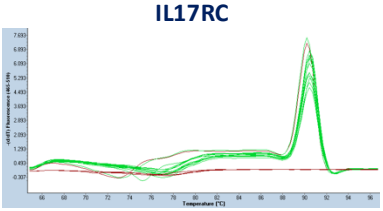


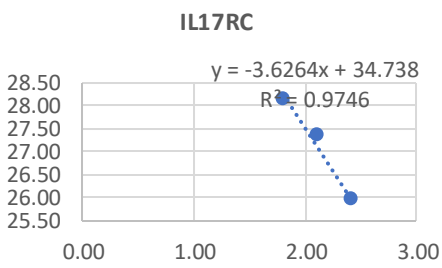












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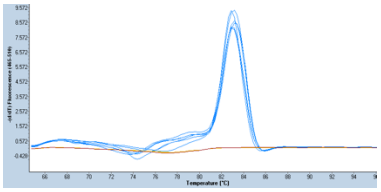

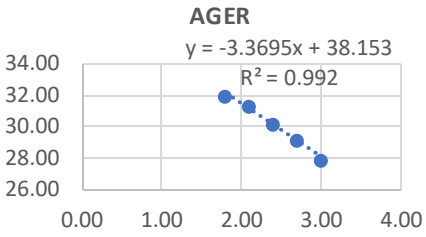

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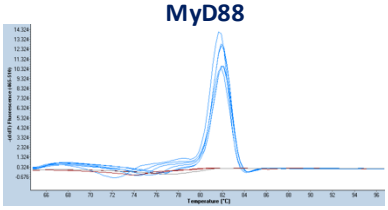

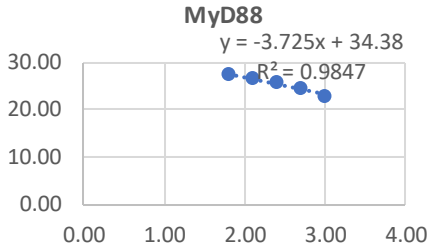

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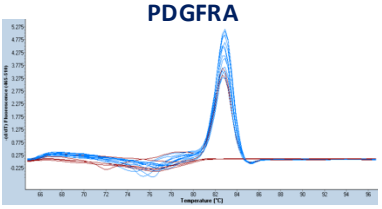

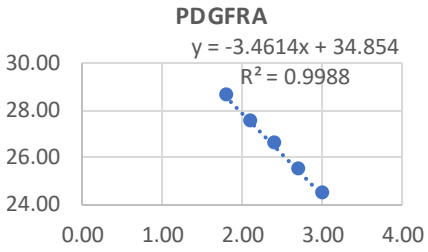

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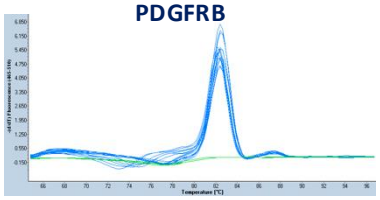

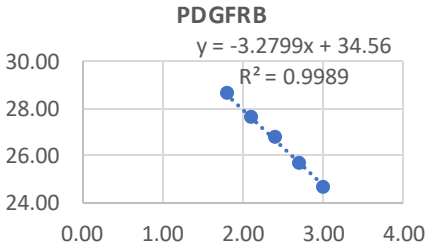

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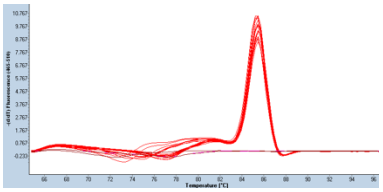

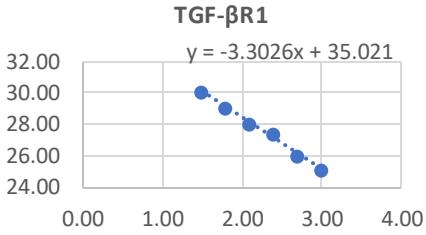

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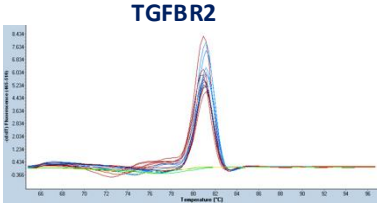

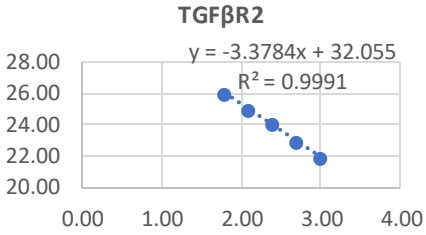

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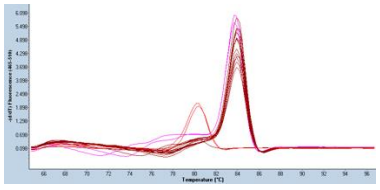

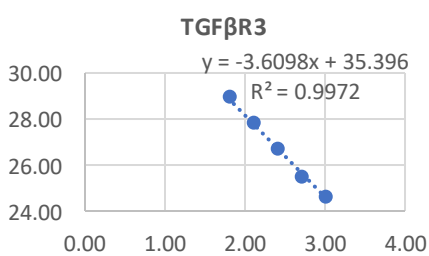

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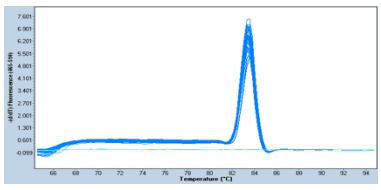

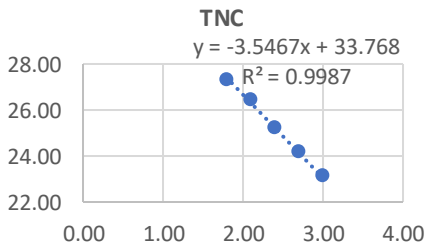

Figure S3. **Melting curves and dynamic ranges of all qPCR reactions**. The dynamic ranges used to determine the qPCR efficiency for each gene are included, as well as the corresponding melting curve. Testing primers for RT-qPCR assays: RNA concentration was determined by spectrophotometry in a Nanodrop, and the samples were treated with DNAse I; Then, 800ng of total RNA was used to synthesize cDNA employing Oligo (dT)18 primers, with the RevertAid H Minus First Strand cDNA Synthesis Kit (ThermoFisher Scientific), following the manufacturer's recommendations. Reactions were run into a Labnet thermocycler (MultiGene Mini, Labnet International, Inc). Finishing the process, 20 µL of water was added to the cDNA mixes, constituting the stock from which five 1:2 serial dilutions were prepared with water. The PCR amplification efficiency for each primer pair design for this study was determined by qPCR assays using the cDNA dilutions as a template. Each reaction contained: 2.0 µL of cDNA, at the corresponding concentration, 7.5 µL of the master mix mixture containing a DNA-intercalating fluorochrome (Maxima SYBR Green/Fluorescein qPCR Master Mix (2X)) (Thermo Scientific), and 0.75 µL of each primer [10 µM] in a final volume of 15.0 µL adjusted with water. For negative controls, the cDNA was substituted by water. The reactions were run in the same plate in triplicates in a LightCycler® 480 Instrument II (Roche Molecular Systems). The thermocycling program consisted of an initial step at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. After the reactions had been finished, a melting curve was generated to identify the melting temperature for each amplicon generated with the corresponding primer pairs. At the end of the runs, an automatized analysis was performed to obtain the CT values[81]. The mean value obtained from each triplicate reaction was correlated with the corresponding cDNA concentration used to get the PCR amplification efficiency for each primer pair. All the primer pairs included in the assays showed efficiencies = 100% (±15%). When a lower efficiency was detected or when more than one amplicon was generated, the primer pair was re-designed for the corresponding molecular target. Detection of mRNA levels of molecular targets in Hypersensitivity Pneumonitis by RT-qPCR assays: as described above, but reactions contained 8.86 ng of cDNA in 1.33 µL, 5.0 µL of the master mix mixture containing a DNA-intercalating fluorochrome (Maxima SYBR Green/Fluorescein qPCR Master Mix (2X)) (Thermo Scientific), and 0.5 µL of each primer [10 µM] in a final volume of 10.0 µL adjusted with water. After obtaining the CT value for each reaction, the mean from triplicates was calculated; then data was analyzed employing the 2-DDCT method[81]. Graphics and statistical analysis were performed with GraphPad Prism 6 (San Diego, CA, USA).