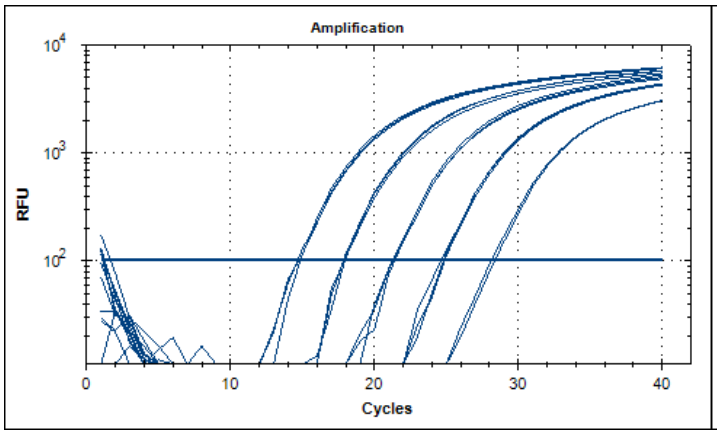
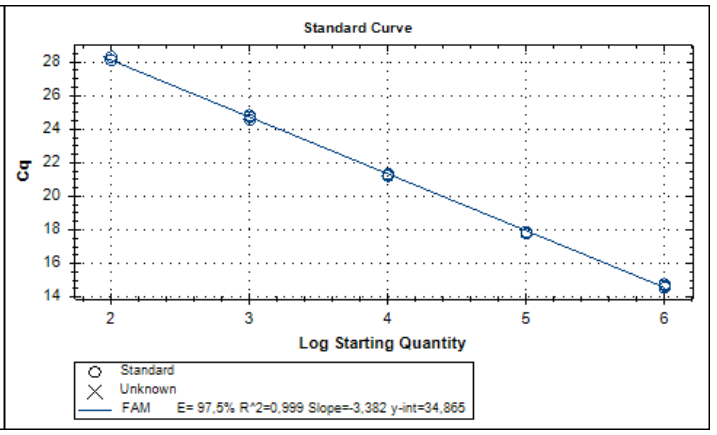
Additional file 3. Primer efficiency calculation for the four T-cell stimulatory epitopes involved in CD and for the four reference genes used to normalize the epitope expression levels.

	Targeted sequence	Slope	R^2	Efficiency (%)
Epitopes	DQ2.5-glia-α1	-3.382	0.999	97.5
	DQ2.5-glia-α2	-3.350	0.999	98.8
	DQ2.5-glia-α3	-3.333	0.999	99.5
	DQ8-glia-α1	-3.412	0.997	96.4
Reference genes [1]	ARF	-3.344	0.994	99.1
	RLI	-3.395	0.998	97.0
	VAS	-3.322	0.998	100.0
	DUF52	-3.347	0.994	99.0

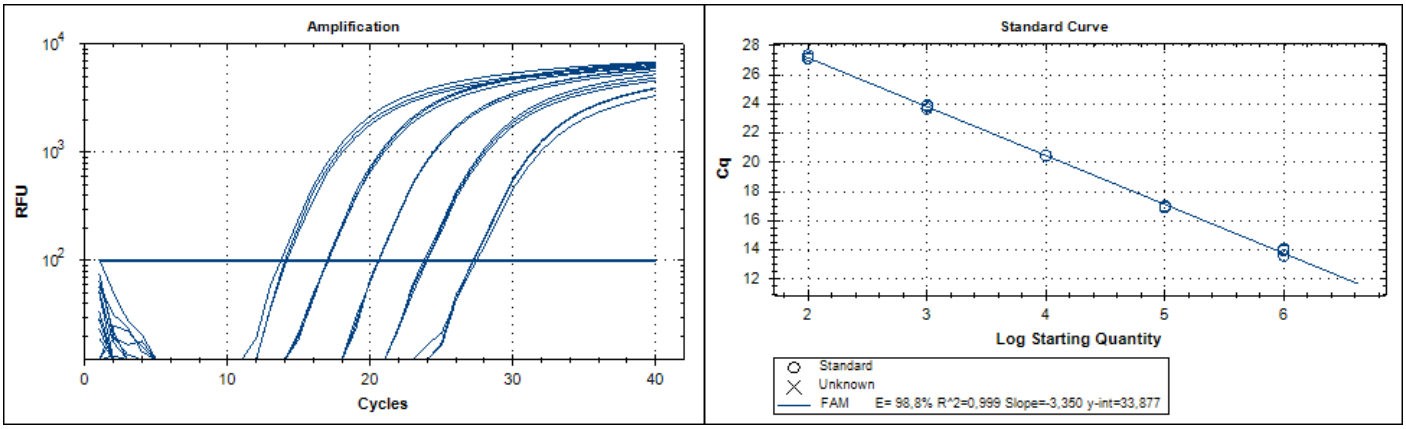
Probes targeting canonical epitopes:

1. DQ2.5-glia-α1

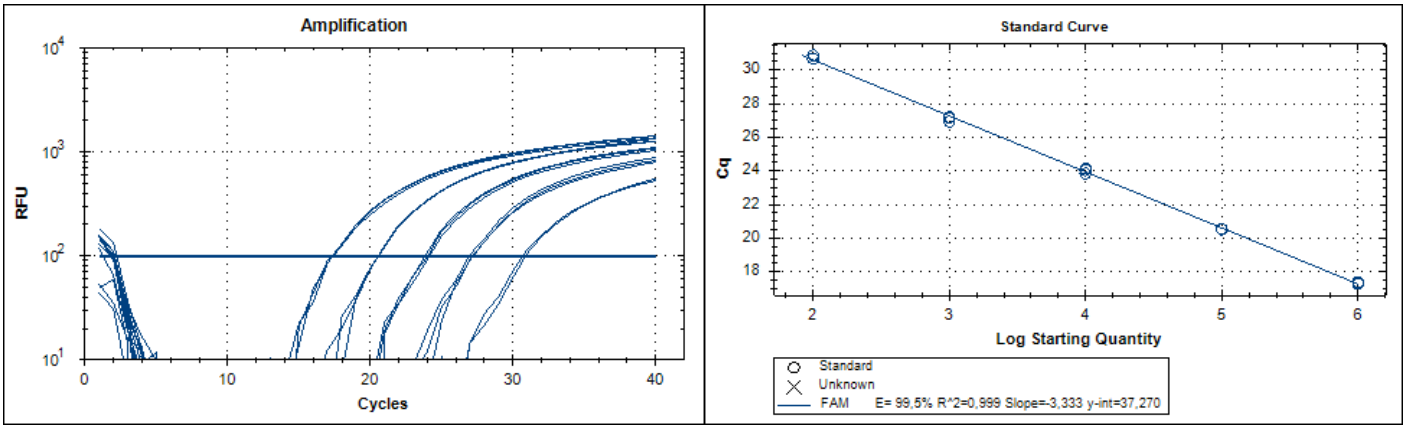




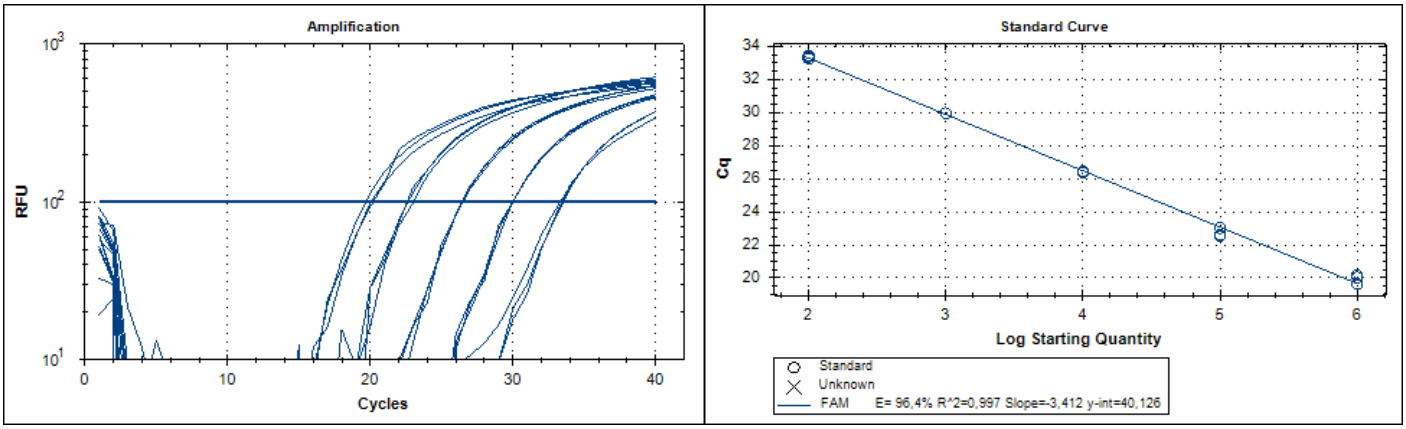
2. DQ2.5-glia-α2



3. DQ2.5-glia-α3

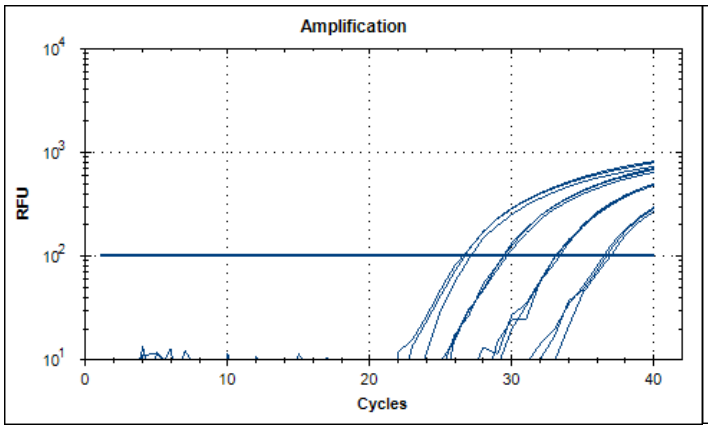


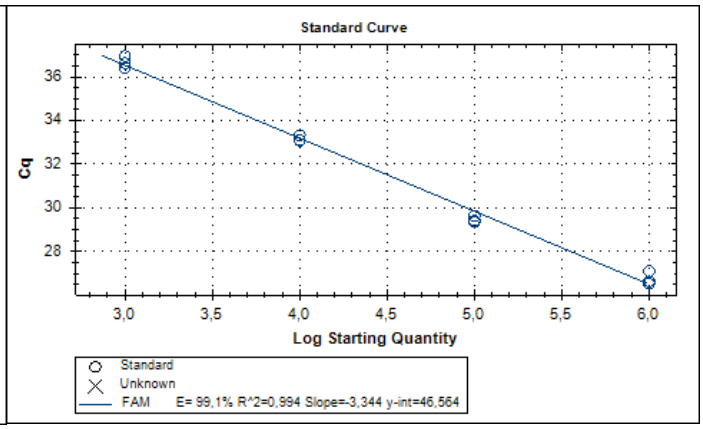
4. DQ8-glia-α1



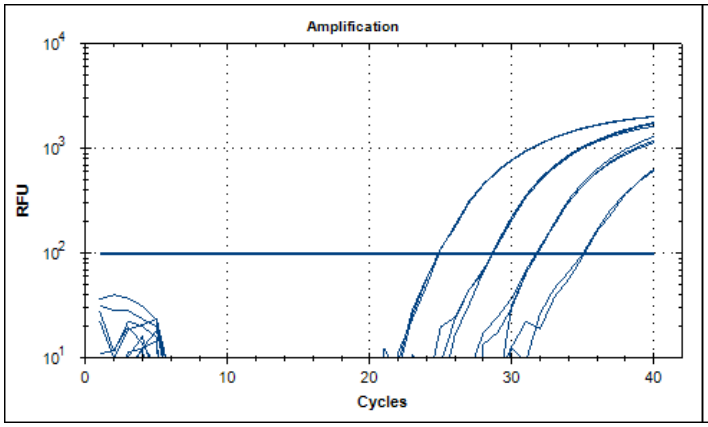
Probes targeting reference genes:

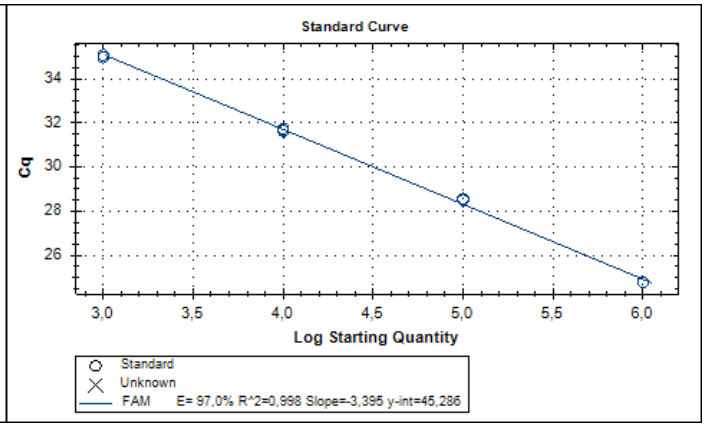
5. ARF



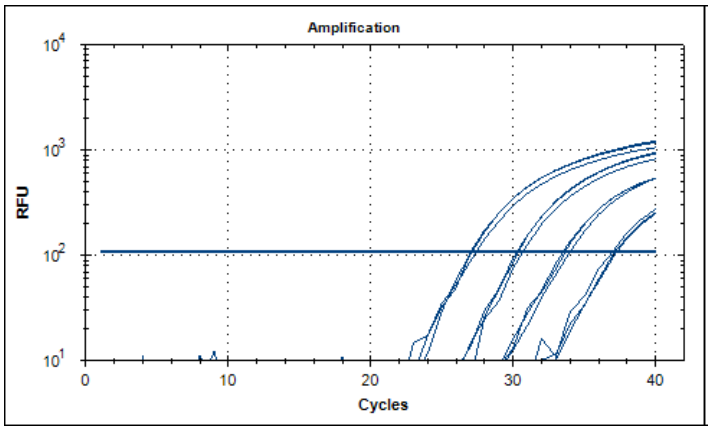


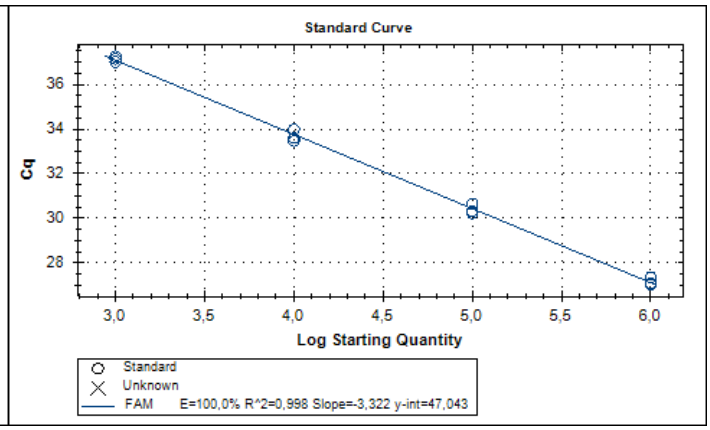
6. RLI



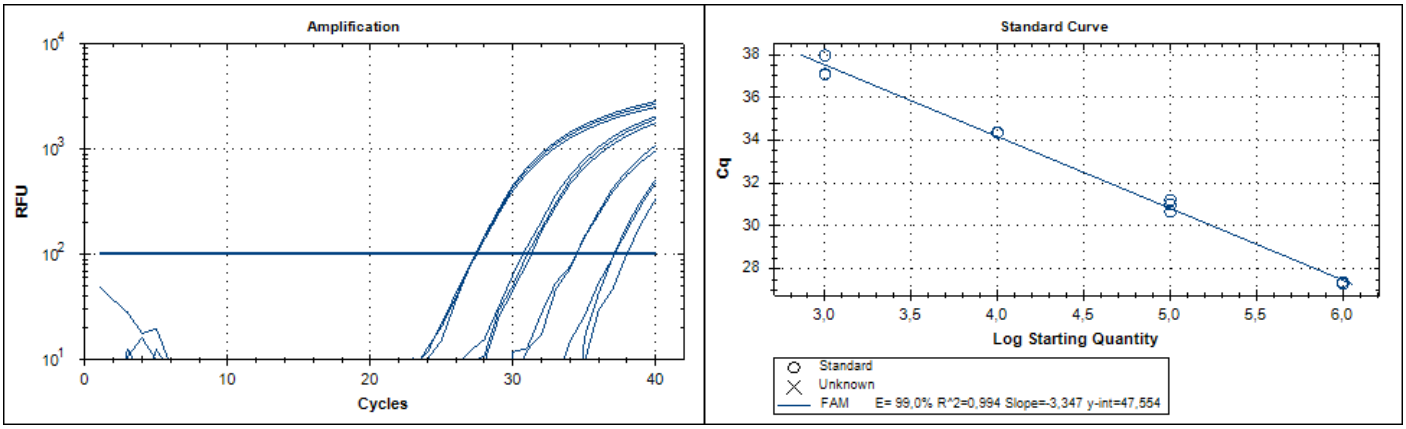


7. VAS





8. DUF52



Each set of primers was validated for its efficiency by carrying out qPCR amplifications with successive 10-fold cDNA dilutions. Calibration curves were drawn by plotting the logarithm of the cDNA concentration on the x-axis and the corresponding C_t values on the y-axis. The efficiency was then calculated based on the curve slope: Efficiency (%) = $[(10^{-1/slope})-1] * 100$.

[1] : ARF: ADP-ribosylation factor, RLI: Similar to RNase L inhibitor-like protein, VAS: Vacuolar ATP synthase 16 kDa proteolipid sub., DUF52: Protein of unknown function [DUF52 family].