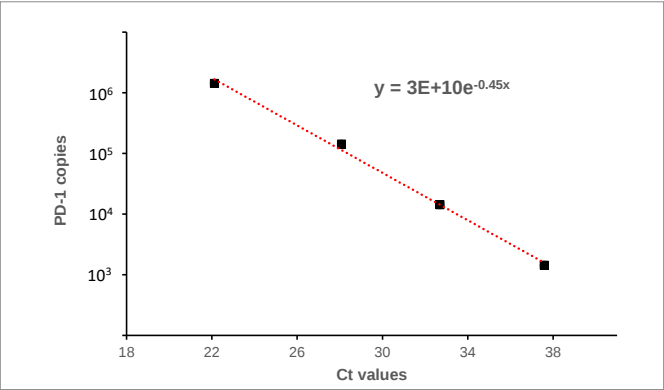


Appendix

Appendix Table S1.....	p. 1
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Appendix Table S1: Approximate calculation of hPD1 mRNA copy number in HEK-293FT-pSB-hPD-1

	PD-1 cDNA copies			
Ct values	1436000	143600	14360	1436
Ct value #1	22.09	28.07	32.73	37.78
Ct value #2	21.94	28.25	32.55	37.26
Ct value #3	22.33	27.90	32.81	37.70
Mean Ct	22.12	28.07	32.70	37.58



Measured Ct values of PD-1 using different concentration of pSB-hPD1 plasmid

	HEK293T-hPD1 (cDNA/0.0125 µg of RNA)		
Doxycycline conc.	1 µg/ml	0.1 µg/ml	0.01 µg/ml
	RPL23A		
Ct value #1	19.96	19.57	19.34
Ct value #2	19.91	19.75	19.46
Ct value #3	20	19.81	19.58
	PD-1		
Ct value #1	31.78	32.79	36.98
Ct value #2	31.67	33.05	36.19
Ct value #3	31.7	32.92	36.85
Mean RPL23A Ct	19.96	19.71	19.46
Norm. factor	0.00	-0.25	-0.50

For each qPCR reaction 0.0125 µg of reverse transcribed RNA was used

	PD-1		
Norm. Ct value #1	31.78	33.04	37.48
Norm. Ct value #2	31.67	33.30	36.69
Norm. Ct value #3	31.70	33.17	37.35

Mean PD-1 Norm. Ct	31.72	33.17	37.17
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	PD-1 cDNA copies in 0.0125 µg RNA		
PD-1 cDNA copies	18995.61	9891.82	1632.66

Using $y = 3E+10e^{-0.45x}$

	PD-1 cDNA copies in 1 µg RNA		
PD-1 cDNA copies	1519648.86	791345.43	130612.45

Original yield was 0.9 µg of RNA from 10⁵ cells

	PD-1 cDNA copies in 1 cell		
Doxycycline conc.	1 µg/ml	0.1 µg/ml	0.01 µg/ml
hPD-1 cDNA copies	17	9	1

Approximate calculation of hPD-1 mRNA copies per cell (HEK-293FT-pSB-hPD-1)

Comparison of results with publicly available measurement of PD1 copies in activated T-cells

Assuming that 1-4 RPKM is ~ 1 copy of RNA per cell
Djebali S et al. Nature. 2012 Sep 6;489(7414):101-8. doi: 10.1038/nature11233. PMID: 22955620; PMCID: PMC3684276.

Experiment #	max. CPM of <i>mPDCD1</i>
GSE176310	68
GSE116697	43
GSE90569	50
GSE122149	22