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Supplemental Information

Lentiviral Vector Production Titer Is Not Limited in HEK293T by Induced Intracellular Innate Immunity

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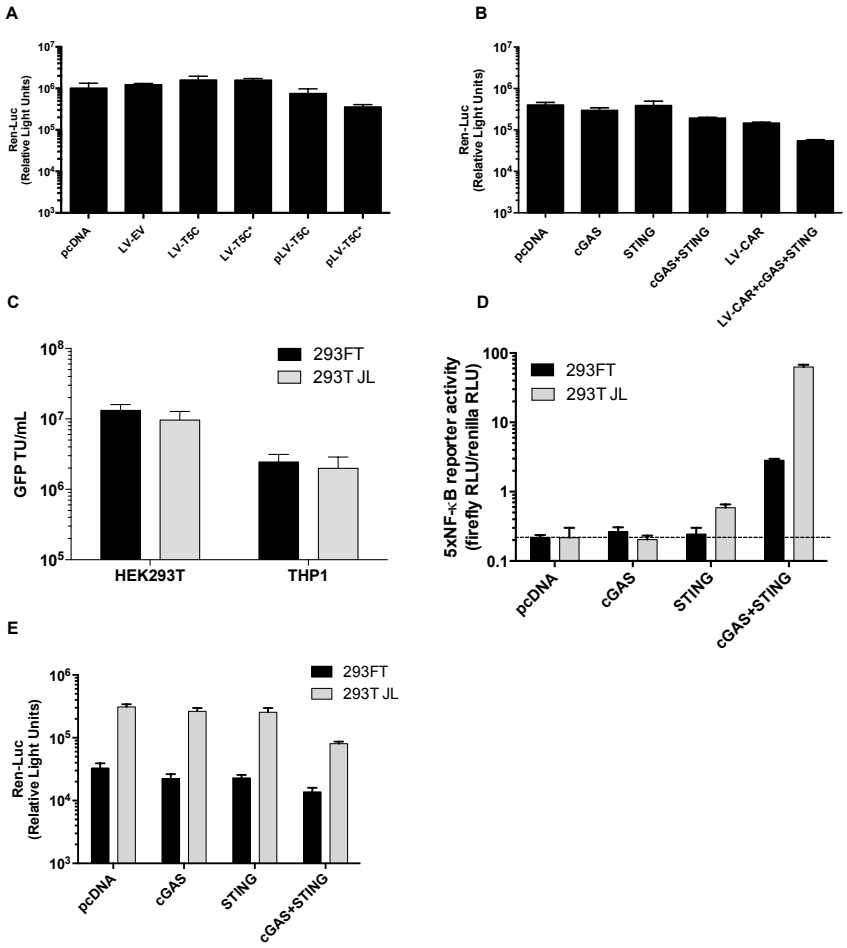

Figure S1. Production of gene therapy LV in HEK 293T triggers NF-κB activation but does not impact LV yield.

(A) *Renilla* luciferase readings for reporter gene assay data presented in Figure 1A (IgK reporter assay). HEK 293T were transfected with firefly luciferase reporter constructs, pRL-TK *Renilla* luciferase and empty pcDNA3 as a control or lentiviral vector constructs as described in Figure 1A. Luciferase activity was measured at 48 h post transfection. Data are presented as mean luminometry readings (relative lights units) ± s.d. **(B)** *Renilla* luciferase readings for reporter gene assay data presented in Figure 1C (IgK reporter assay). HEK 293T were transfected with firefly luciferase reporter constructs, pRL-TK *Renilla* luciferase and empty pcDNA3 as a control or lentiviral vector constructs as described in Figure 1C. Luciferase activity was measured at 48 h post transfection. Data are presented as mean luminometry readings (relative lights units) ± s.d. **(C)** Culture supernatants from HEK 293FT or HEK 293T JL cells that had been transfected to produce a GFP-encoding LV were harvested at 48 h and mean viral titres ± s.d of biological replicates (n = 2) were determined in quadruplicate in HEK 293T and THP-1 cells by enumerating GFP-positive cells. **TU** transducing units. **(D)** HEK 293FT or HEK 293T JL cells were transfected with 5xNF-κB firefly luciferase reporter construct, pRL-TK *Renilla* luciferase and pcDNA-based expression plasmids encoding cGAS and/or STING as shown. Mean reporter activity ± s.d (n = 2) was assessed 48 h later using a dual-luciferase reporter assay and is presented as firefly luciferase relative light units (RLU) divided by *Renilla* luciferase RLU. **(E)** *Renilla* luciferase readings for reporter gene assay data presented in Figure S1D.

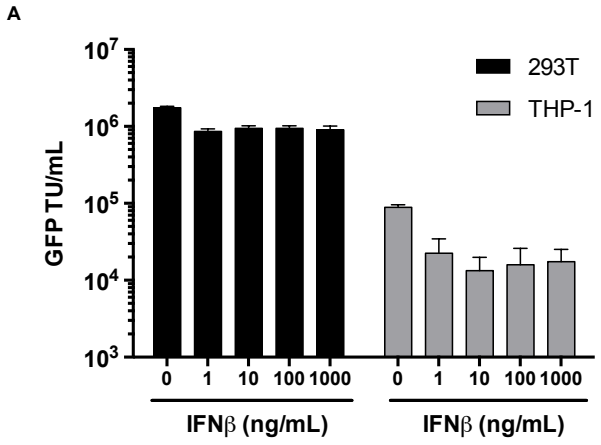

Figure S2. Exogenous IFNβ reduces LV transduction efficiency on monocytic cells but not on HEK 293T or primary T cells.

HEK 293T cells were used to produce GFP-encoding lentiviral vector in the absence or presence of increasing amounts of IFNβ. This experiment simulates the situation in which HEK 293T cells make IFN as a by-product of lentiviral vector production and secrete it into the LV prep. Culture supernatants were harvested at 48 h post transfection and viral titres ± s.d. of biological replicates (*n* = 2) were determined in duplicate by FACS of GFP-positive HEK 293T or THP-1 cells.

Supplementary Table 1. Taqman primers and probes

Primer/Probe	Sequence (5' – 3')	Reference
Psi Fwd	CAGGACTCGGCTTGCTGAAG	Schott et al, 2019[1]
Psi Rev	TCCCCCGCTTAATACTGACG	Schott et al, 2019[1]
Psi Probe	FAM-CGCACGGCAAGAGGCGAGG-TAMRA	Schott et al, 2019[1]
Albumin Fwd	GCTGCTATCTCTTGTGGGCTGT	Schott et al, 2019[1]
Albumin Rev	ACTCATGGGAGCTGCTGGTTC	Schott et al, 2019[1]
Albumin Probe	VIC-CCTGTCATGCCCACACAAATCTCTCC-TAMRA	Schott et al, 2019[1]

Supplementary Table 2. Real time PCR primers

Primer	Sequence (5' – 3')	Reference
hGAPDH Fwd	ACCCAGAAGACTGTGGATGG	Stuart et al, 2016[2]
hGAPDH Rev	TTCTAGACGGCAGGTCAGGT	Stuart et al, 2016[2]
IFNβ Fwd	AGGACAGGATGAACTTTGAC	Gao et al, 2013[3]
IFNβ Rev	TGATAGACATTA GCC AGGAG	Gao et al, 2013[3]
CXCL10 Fwd	TGGCATTCAAGGAGTACCTC	Gao et al, 2013[3]
CXCL10 Rev	TTGTAGCAATGATCTCAACACG	Gao et al, 2013[3]
ISG56 Fwd	CCTCCTTGGGTTCGTCTACA	Jakobsen et al, 2013[4]
ISG56 Rev	GGCTGATATCTGGGTGCCTA	Jakobsen et al, 2013[4]
ISG54 Fwd	CAGCTGAGAATTGCACTGCAA	Jiang et al, 2010[5]
ISG54 Rev	CGTAGGCTGCTCTCCAAGGA	Jiang et al, 2010[5]
TAg Fwd	TGAGGCTACTGCTGACTCTCAACA	Bergsagel et al, 1992[6]
TAg Rev	GCATGACTCAAAAAACTTAGCAATTCTG	Bergsagel et al, 1992[6]

Supplementary Table 3. SV40 Large T antigen cloning primers

Primer	Sequence (5' – 3')	Reference
EcoRI-TAg Fwd	GCATGAATTCATGGATAAAGTTTTAAACAGAGAGG	This study
TAg-NotI Rev	ATGCGGCCGCTTATGTTTCAGGTTCAGGGGG	This study

Supplementary Table 4. shRNA target sequences

shRNA	shRNA target sequence (5' – 3')	Reference
shTAg	TGGGCAACAAACAGTGTAG	This study
shControl	ATGTCTCTGGAAAAGATGT	This study

SUPPLEMENTAL REFERENCES

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