

Supplemental Material to:

**Maria Isaguliants, Olga Smirnova, Alexander V Ivanov,
Athina Kilpelainen, Yulia Kuzmenko, Stefan Petkov,
Anastasia Latanova, Olga Krotova, Gunnel Engström,
Vadim Karpov, Sergey Kochetkov, Britta Wahren, and
Elizaveta Starodubova**

**Oxidative stress induced by HIV-1 reverse transcriptase
modulates the enzyme's performance in gene
immunization**

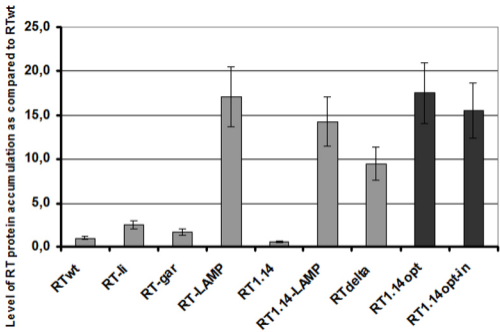
**Human Vaccines & Immunotherapeutics 2013; Vol 9(10)
<http://dx.doi.org/10.4161/hv.25813>**

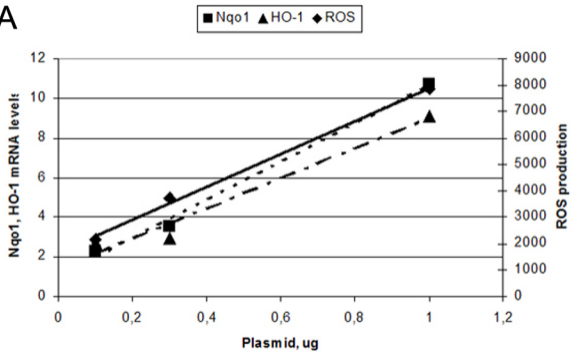
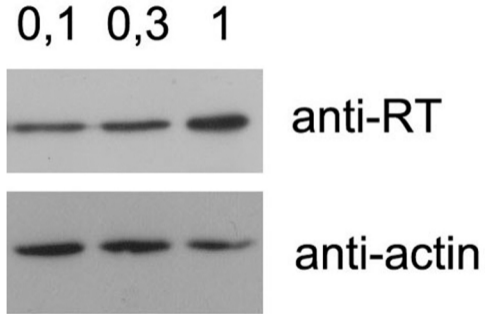
<http://www.landesbioscience.com/journals/vaccines/article/25813>

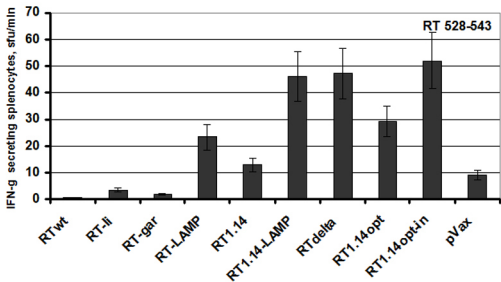
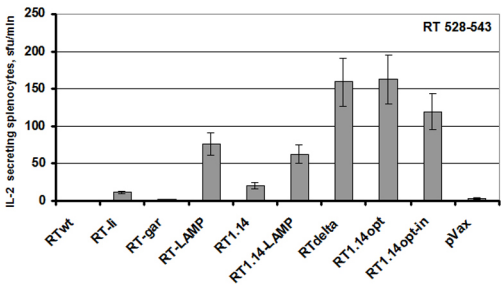
Figure S1. Expression of the panel of RT gene variants in HEK293 cells. Cells were transfected with plasmids encoding wild-type RT (RTwt), multi-drug resistant RT1.14 (RT1.14)²⁸, truncated RT (RTdelta)³¹; chimeras of RT with Invariable chain (RT-Ii)^{34,32}, lysosome associated membrane protein I (RT-LAMP),³⁴ minimal Gly-Ala repeat of EBNA 1 protein of EBV (RT-gar)²⁷ and the chimera of RT1.14 with LAMP-I (RT1.14-LAMP)³⁴ as well as the expression-optimized active and inactivated RT.14 described here (RT1.14opt, RT1.14opt-in). Cells were harvested 48 h post transfection, lysed and the accumulation of RT proteins was assessed by Western blotting.³⁶ Expression levels were normalized to the level of expression of the viral gene encoding the wild-type RT (RTwt). Data set for each gene represent the mean of at least three independent transfection experiments with the standard deviation.

Figure S2. Induction of oxidative stress (ROS) and transcription of genes of phase II detoxifying enzymes human NAD(P)H:quinone oxidoreductase (Nqo1) and heme oxygenase 1 (HO-1) depend on the level of expression of HIV-1 reverse transcriptase (RT). (A) HEK293 cells were transiently transfected with serial dilutions of the plasmid encoding RT1.14opt, starting from 1 µg and monitored for the generation of ROS and for the levels of mRNA HO-1 and Nqo1. (B) Aliquots of lysates were assessed for the expression of RT1.14 by Western blotting. The amount of plasmid encoding RT1.14opt used to transfect cells is indicated above each lane. The upper Western blot panel shows staining with anti-RT and the lower, with anti-actin antibodies, done in order to normalize the probes for the amount of house-keeping cell proteins.

Figure S3. Comparative analysis of cellular immune response against the immunodominant T-cell epitope of RT aa 528-543 induced by the RT gene variants in BALB/c mice, based on published data^{35, 36, 38, 39} and the data obtained in the current work. Results of the Fluorospot assays of IFN-g (A), IL-2 (B) and dual IFN-g/IL-2 production (C).



A**B**

A**B****C**