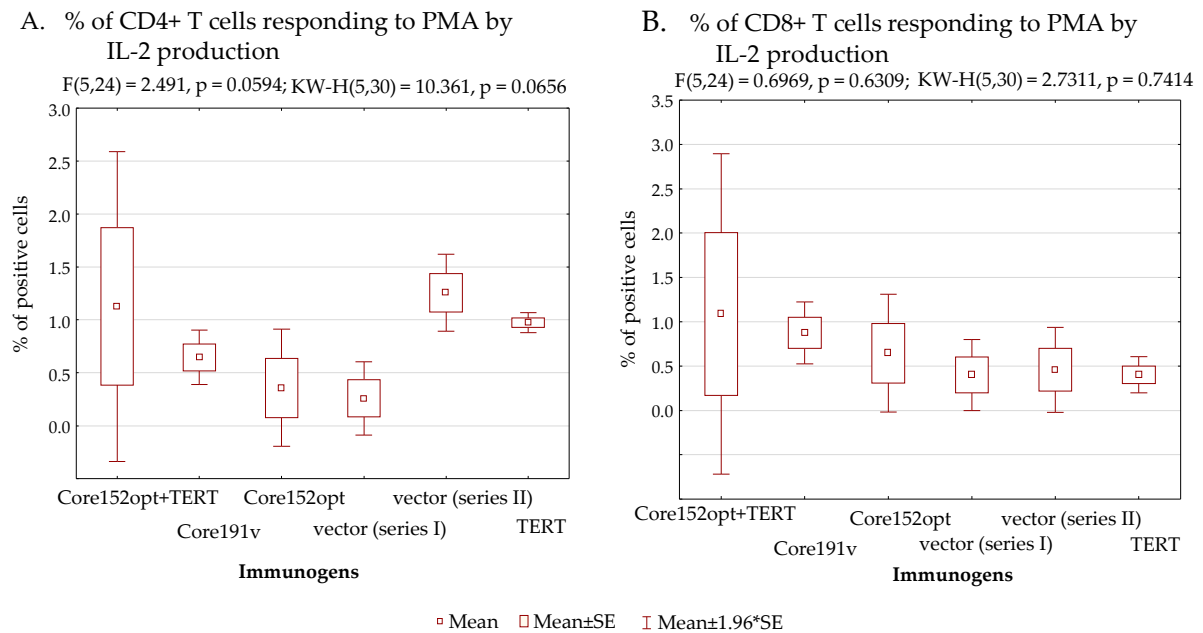
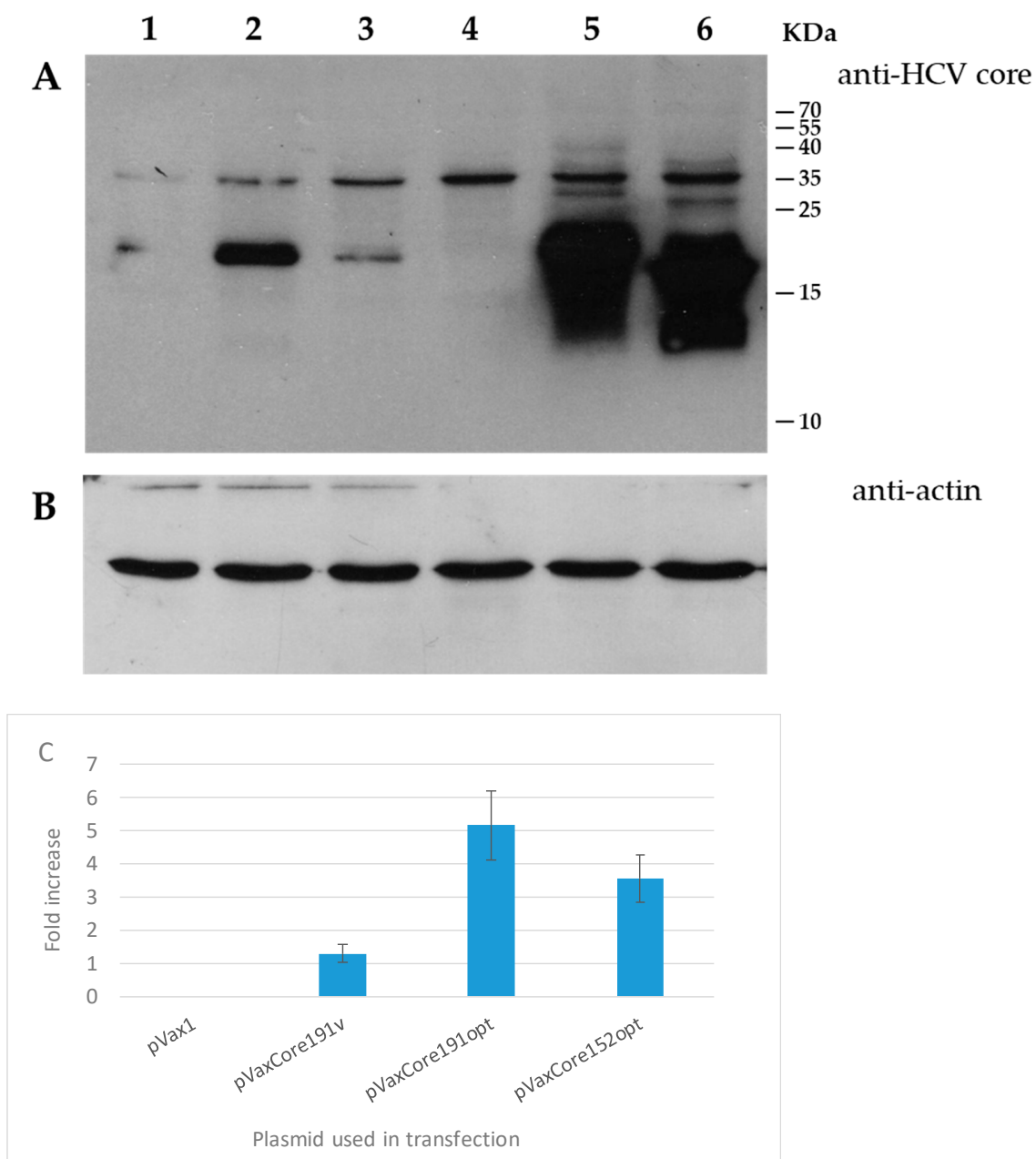


Supplementary Figure S1. Principles of inclusive gating applied in the analysis of specific T-cell response in DNA immunized mice using multiparametric flow cytometry. CD4+ (A, B) and CD8+ (C, D) T cells of mice immunized with pVax1 (A, C) or pVaxCore152opt (B, D) stimulated with pool of peptides derived from HCV core (CORE POOL, Suppl. Table S1). Size of populations

expressing IFN- γ , IFN- γ /IL-2 and IFN- γ /IL-2/TNF- α , based on inclusive principles, is shown as % of the respective CD4+ or CD8+ T cell populations. For immunization details, see Materials and Methods and Table 1.



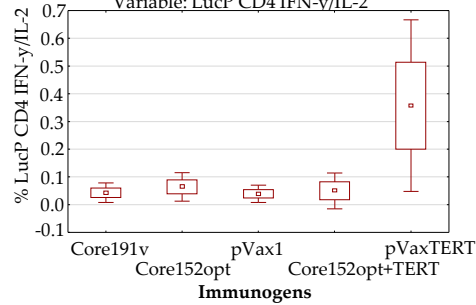
Supplementary Figure S2. Splenocytes of mice in experimental series I and II (Table 1) demonstrate efficient response to stimulation with mitogen(s). In Series I, splenocytes of mice were stimulated with phorbol 12-myristate 13-acetate (PMA; 50 ng/mL), and in Series II, with a mix of PMA and ionomycin (1 μ g/mL). IL-2 positive CD4+ (A) and CD8+ (B) T cells in groups of mice DNA immunized with Core191v, Core152opt, empty vector (series I), TERT, Core152opt+TERT and vector (series 2), in %. $p > 0.05$, Kruskal Wallis test.



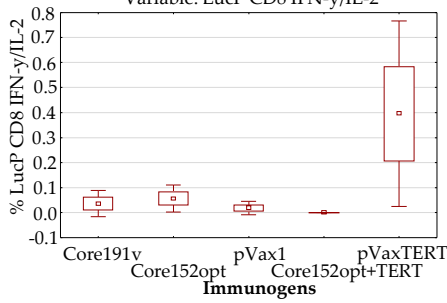
Supplementary Figure S3. Expression of HCV core variants in transiently transfected Huh-7 cells. In brief, Huh-7 cells were transfected with a panel of plasmids pVax1 (lane 1), pVaxCore191v (2), pVaxCore191opt (5), pVaxCore152opt (6) (lanes 3 and 4, transfections with pVaxCore191 plasmid with mutations), for pVaxCore152 in the presence of 5 μ M MG132; 48 h post transfections cells were lysed and subjected to SDS-PAGE with subsequent Western blotting with polyclonal anti-HCV core (A); blots were stripped and re-stained with anti-actin antibodies (B); HCV core expression

normalized to that of actin (C). Figures to the right represent position of the molecular mass marker PageRuler Plus Prestained Protein Ladder (Thermo Scientific). Signals were quantified using ImageJ. Data represent the results of two independent runs, exhibiting 20% deviation.

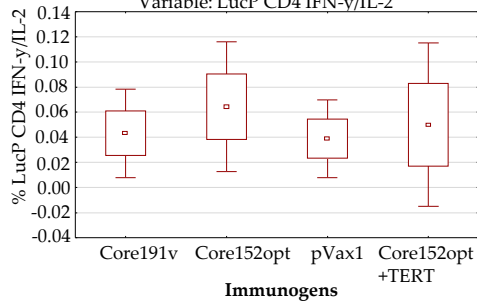
A. % LucP specific IFN- γ /IL-2 secreting CD4+ T cells
LucP CD4 IFN- γ /IL-2: F(4,26) = 5.9469, p = 0.0015;
KW-H(4,31) = 4.9513, p = 0.2923
Variable: LucP CD4 IFN- γ /IL-2



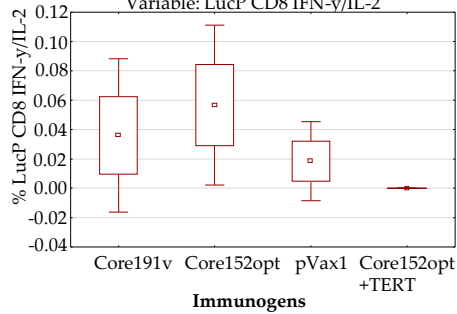
B. % LucP specific IFN- γ /IL-2 secreting CD8+ T cells
LucP CD8 IFN- γ /IL-2: F(4,26) = 6.4385, p = 0.0010;
KW-H(4,31) = 10.7961, p = 0.0290
Variable: LucP CD8 IFN- γ /IL-2



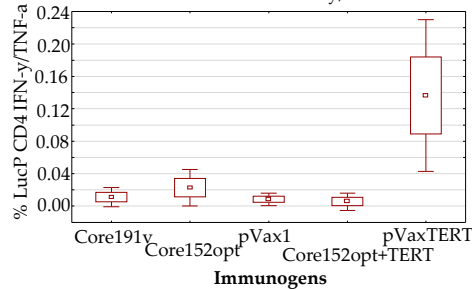
C. No difference in % LucP specific IFN- γ /IL-2 secreting CD4+ T cells
LucP CD4 IFN- γ /IL-2: F(3,23) = 0.2594, p = 0.8538;
KW-H(3,27) = 1.2877, p = 0.7321
Variable: LucP CD4 IFN- γ /IL-2



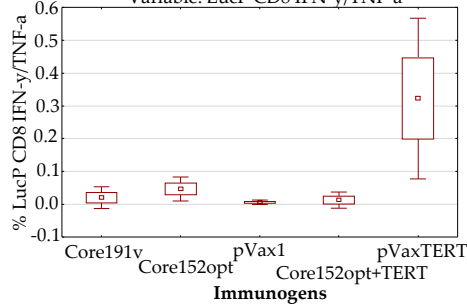
D. No difference in % LucP specific IFN- γ /IL-2 secreting CD8+ T cells (but no secretion in Core152opt+TERT DNA immunized mice)
LucP CD8 IFN- γ /IL-2: F(3,23) = 1.1205, p = 0.3613; KW-H(3,27) = 7.3776, p = 0.0608
Variable: LucP CD8 IFN- γ /IL-2



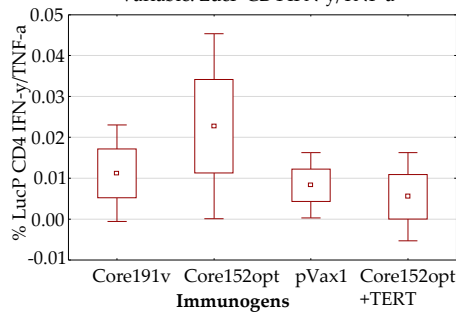
E. % LucP specific IFN- γ /TNF- α secreting CD4+ T cells
LucP CD4 IFN- γ /TNF- α : F(4,26) = 10.7183, p = 0.00003;
KW-H(4,31) = 10.7689, p = 0.0293
Variable: LucP CD4 IFN- γ /TNF- α



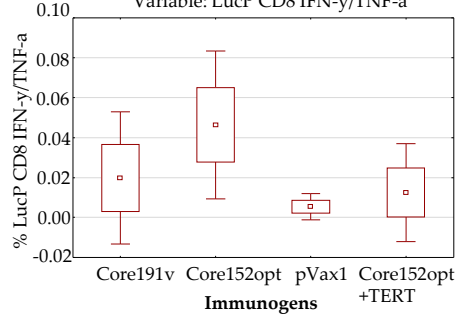
F. % LucP specific IFN- γ /TNF- α secreting CD8+ T cells
LucP CD8 IFN- γ /TNF- α : F(4,26) = 10.5484, p = 0.00003;
KW-H(4,31) = 15.0061, p = 0.0047
Variable: LucP CD8 IFN- γ /TNF- α



G. No difference in % LucP specific IFN- γ /TNF- α secreting CD4+ T cells. LucP CD4 IFN- γ /TNF- α :
F(3,23) = 1.0933, p = 0.3719; KW-H(3,27) = 3.2831, p = 0.3500
Variable: LucP CD4 IFN- γ /TNF- α

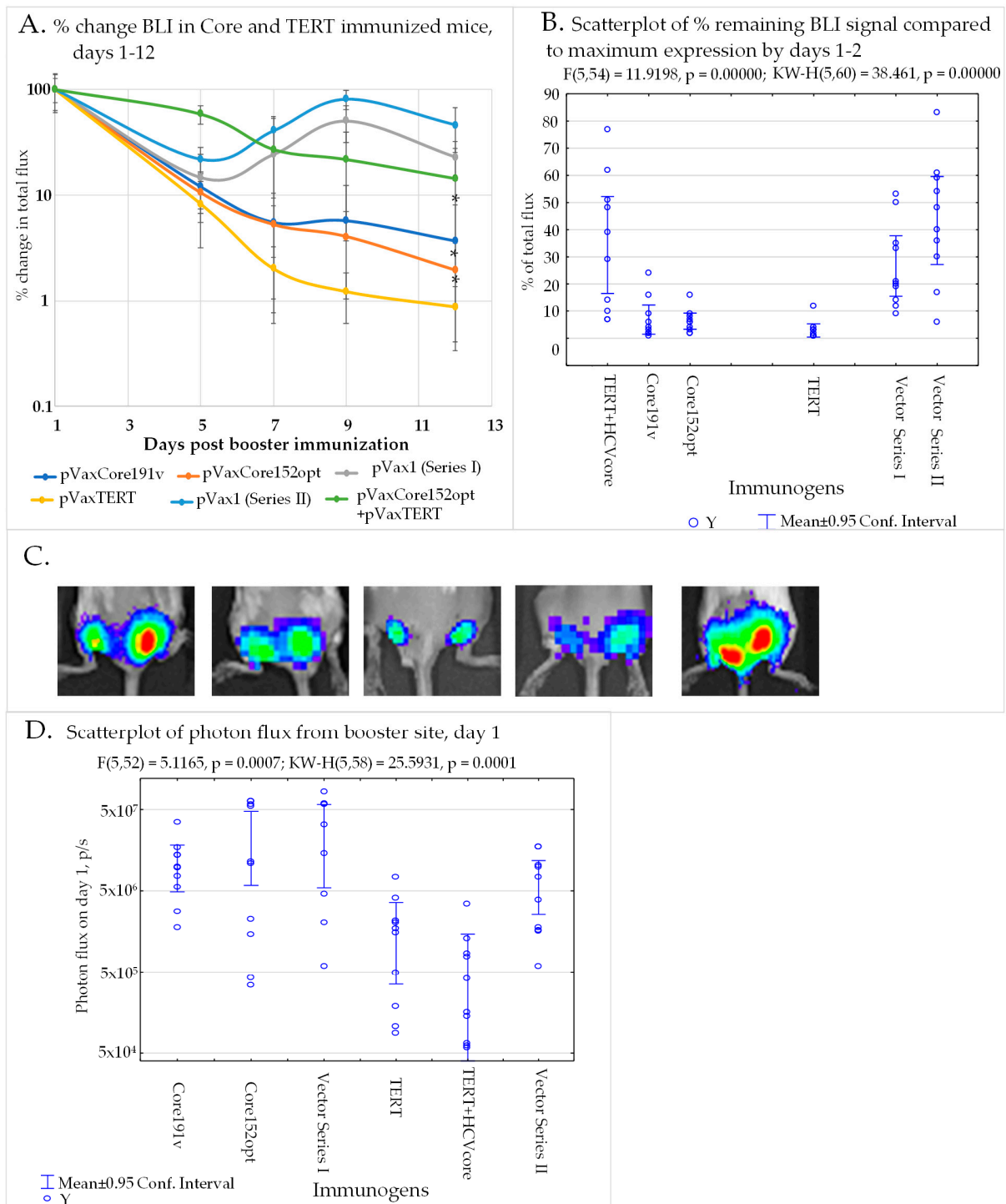


H. No difference in % LucP specific IFN- γ /TNF- α secreting CD8+ T cells (tendency to lower % in pVax group)
LucP CD8 IFN- γ /TNF- α : F(3,23) = 2.8798, p = 0.0579;
KW-H(3,27) = 6.8514, p = 0.0768
Variable: LucP CD8 IFN- γ /TNF- α



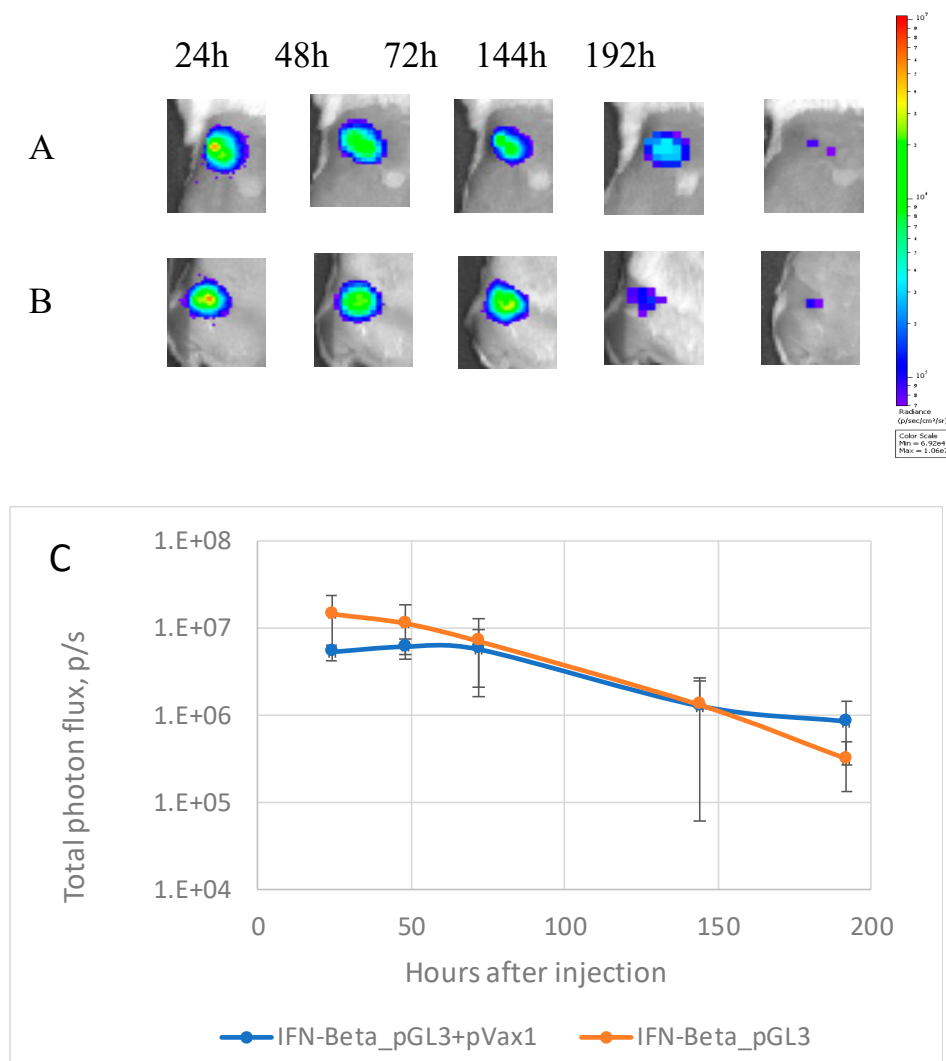
□ Mean □ Mean±SE I Mean±1.96*SE

Supplementary Figure S4. Statistical comparison of T cell response to immunodominant epitope of luciferase LucP in mice DNA immunized with Core191v, Core152opt, TERT, mixture of Core152opt and TERT and empty vector. Data from immunization series I and II; for vector mice represent combined data from series I and II (Table 1). Percent CD4⁺ (A, C) and CD8⁺ T cells (B, D) secreting IFN- γ /IL-2 in all groups (A, B) and in groups excluding TERT immunized mice (C, D); Percent CD4⁺ (E, G) and CD8⁺ T cells (F, H) secreting IFN- γ /TNF- α in all groups (E, F) and in groups excluding TERT immunized mice (G, H); Percent of cytokine secreting CD4⁺ and CD8⁺ T cells is represented by mean \pm standard error (SE). No difference between the groups after exclusion of mice immunized with TERT DNA (C, D, G, H) in Kruskal Wallis test, and in pair-wise comparisons, Mann-Whitney U-test (Statistica Tibco, version 13.5).

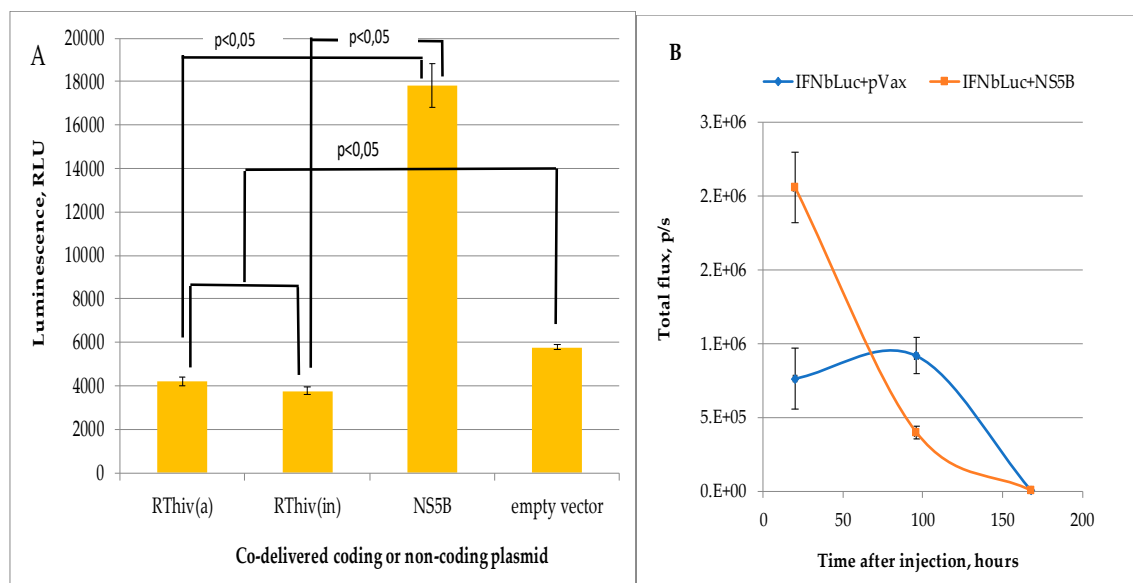


Supplementary Figure S5. Bioluminescence from DNA booster sites in mice receiving HCV core and TERT alone or in a mix. Groups of BALB/c mice ($n=5$) were DNA-immunized with Core191v, Core152opt, TERT, Core152opt and TERT mixture (TERT+Core152opt) and empty vector (Series I and II; Table 1). Change in BLI signal from DNA booster sites from day 1 to 12, in % (A); %

remaining BLI signal by day 7 after the boost compared to maximal expression by days 1-2 (B); Individual mice 24 h after receiving booster injections with pVaxLuc2 mixed with pVaxCore191v (1), pVaxCore152opt (2), pVaxTERT (3), pVaxCore152opt/pVaxTERT (4), pVax1 (5) administered to the left and to the right from the base of the tail (C); Scatterplot of BLI from all injection sites one day after the boost (D). Average \pm STDV per immunization site (A); individual booster sites (B, C); mean \pm error (0.95 confidence interval) per immunization site (D) * - $p < 0,05$ between Core191v, Core152opt, and TERT DNA immunized compared to vector immunized mice starting from day 7, Mann Whitney U-test (A); $p < 0,000001$, % BLI change by day 7, Kruskal-Wallis test (B).



Supplementary Figure S6. Expression in mice of luciferase reporter from the IFN- β promoter. *In vivo* bioluminescent imaging of mice receiving IFN-Beta_pGL3 reporter plasmid (10 mg per site) (A), or IFN-Beta_pGL3 mixed with pVax1 (10 mg of each per site) (B) monitored 24, 48, 72, 144 and 196 h after the injection; Kinetics of the gradual loss of BLI signal, mean \pm STDV (C). Signal intensity in photons/sec is represented as a color scale to the right of the images.



Supplementary Figure S7. Expression of luciferase reporter from the IFN- β promoter *in vitro* (A) and *in vivo* (B). Luminescence signal (in RLU) emitted by the lysates of HEK293 cells co-transfected with IFN-Beta_pGL3 reporter plasmid and pVax1 vector, or plasmid encoding dependent RNA-polymerase of HCV (NS5B), or enzymatically active or inactivated HIV-1 reverse transcriptase, RThiv(a) and RThiv(in), respectively (see Materials and methods for details (A); Total photon flux (mean \pm STDV) from the sites of injection into mice of IFN-Beta_pGL3 reporter mixed with pVax1 vector, or plasmid encoding dependent RNA-polymerase of HCV (NS5B) (10 mg of each per site) registered by *in vivo* BLI of mice (n=2) on days 1, 4 and 7 after the injections (B). * - $p < 0,05$, Mann-Whitney U test.

Supplementary Table S1. Synthetic peptides used in the study.

| Protein | Position, first/last amino acid residue | Amino acid sequence | Abbreviation |
|--------------------|---|-------------------------------------|----------------|
| HCV core | 61-95 | RRQPIPKARRPEGRTWAQPGYPWPLYGNEGMGWAG | CORE POOL |
| | 81-115 | YPWPLYGNEGMGWAGWLLSPRGSRPSWGPNDPRRR | |
| | 101-135 | RGSRPSWGPNDPRRRSRNLGKVIDTLTCGFADLMG | |
| | 141-175 | GAPLGGAARALAHGVRVLEDGVNYATGNLPGCSFS | |
| TERT | 559-584 | QKNRLFFYRKSVWSKLQSIGIRQQL | TERT357 (pool) |
| | 791-815 | SLLHFFLRFVRHSVVKIDGRFYVQ | |
| | 888-917 | KTVVNFPVETGALGGAAPHQLPAHCLFPW | |
| | 845-865 | QQDGLLLRFVDDFLLVTPHL | TERT6 |
| | 901-929 | LGGAAPHQLPAHCLFPWCGLLLDTRTLE | TERT8 |
| Firefly luciferase | 160-168 | GFQSMYTFV | LucP |

Supplementary Table S2. Statistical comparison of populations CD4+ and CD8+ cells responding to stimulation with HCV core peptide pool (Supplementary Table S1) in mice DNA immunized with mixture of Core152opt and TERT (MIX) comparing to vector pVax1 (Mann-Whitney U test w/ continuity correction).

| | Rank Sum (MIX) | Rank Sum (vector) | U | Z | p-value | Z (adjusted) | p-value | Valid N (MIX) | Valid N (vector) | 2*1sided (exact p) |
|---------------------------------------|-------------------|-------------------|----------|----------|----------|--------------|----------|------------------|---------------------|-----------------------|
| CD4 IFN- γ | 36.00000 | 55.00000 | 10.00000 | 1.15728 | 0.247161 | 1.56517 | 0.117545 | 4 | 9 | 0.260140 |
| CD4 IL-2 | 37.00000 | 54.00000 | 9.00000 | 1.31158 | 0.189663 | 2.08528 | 0.037045 | 4 | 9 | 0.198601 |
| CD4 TNF- α | 25.00000 | 66.00000 | 15.00000 | -0.38576 | 0.699676 | -0.47116 | 0.637525 | 4 | 9 | 0.710490 |
| CD4 IFN- γ /IL-2 | 35.00000 | 56.00000 | 11.00000 | 1.00297 | 0.315875 | 1.35648 | 0.174948 | 4 | 9 | 0.330070 |
| CD4 IFN- γ /TNF- α | 40.00000 | 51.00000 | 6.00000 | 1.77449 | 0.075983 | 2.16735 | 0.030209 | 4 | 9 | 0.075524 |
| CD4 IL-2/TNF- α | 35.00000 | 56.00000 | 11.00000 | 1.00297 | 0.315875 | 1.35648 | 0.174948 | 4 | 9 | 0.330070 |
| CD4 IFN- γ /IL-2/TNF- α | 41.00000 | 50.00000 | 5.00000 | 1.92879 | 0.053758 | 2.35581 | 0.018483 | 4 | 9 | 0.050350 |
| CD8 IFN- γ | 32.00000 | 59.00000 | 14.00000 | 0.54006 | 0.589155 | 0.61577 | 0.538050 | 4 | 9 | 0.604196 |
| CD8 IL-2 | 31.00000 | 60.00000 | 15.00000 | 0.38576 | 0.699676 | 0.61332 | 0.539667 | 4 | 9 | 0.710490 |
| CD8 TNF- α | 25.00000 | 66.00000 | 15.00000 | -0.38576 | 0.699676 | -0.47116 | 0.637525 | 4 | 9 | 0.710490 |
| CD8 IFN- γ /IL-2 | 25.00000 | 66.00000 | 15.00000 | -0.38576 | 0.699676 | -0.47116 | 0.637525 | 4 | 9 | 0.710490 |
| CD8 IFN- γ /TNF- α | 28.50000 | 62.50000 | 17.50000 | 0.00000 | 1.000000 | 0.00000 | 1.000000 | 4 | 9 | 0.939860 |
| CD8 IL-2/TNF- α | 26.50000 | 64.50000 | 16.50000 | -0.15430 | 0.877371 | -0.17593 | 0.860347 | 4 | 9 | 0.825175 |
| CD8 IFN- γ /IL-2/TNF- α | 28.00000 | 63.00000 | 18.00000 | 0.07715 | 0.938503 | 0.09423 | 0.924924 | 4 | 9 | 1.000000 |

Supplementary Table S3. Statistical comparison of populations CD4+ and CD8+ cells responding to stimulation with HCV core peptide pool (Supplementary S1) in mice DNA immunized with mixture of Core152opt and TERT (MIX) comparing to Core152opt (Mann-Whitney U test w/ continuity correction).

| | Rank Sum (MIX) | Rank Sum (Core152opt) | U | Z | p-value | Z (adjusted) | p-value | Valid N (MIX) | Valid N (Core152opt) | 2*1sided (exact p) |
|-------------------------------|-------------------|--------------------------|----------|----------|----------|--------------|----------|------------------|-------------------------|-----------------------|
| CD4 IFN- γ | 19.50000 | 25.50000 | 9.500000 | 0.00000 | 1.000000 | 0.00000 | 1.000000 | 4 | 5 | 0.904762 |
| CD4 IL-2 | 10.00000 | 35.00000 | 0.000000 | -2.32702 | 0.019965 | -2.33677 | 0.019452 | 4 | 5 | 0.015873 |
| CD4 TNF-a | 12.50000 | 32.50000 | 2.500000 | -1.71464 | 0.086412 | -1.79089 | 0.073313 | 4 | 5 | 0.063492 |
| CD4 IFN- γ /IL-2 | 13.00000 | 32.00000 | 3.000000 | -1.59217 | 0.111348 | -1.59884 | 0.109856 | 4 | 5 | 0.111111 |
| CD4 IFN- γ /TNF-a | 17.50000 | 27.50000 | 7.500000 | -0.48990 | 0.624206 | -0.49195 | 0.622754 | 4 | 5 | 0.555556 |
| CD4 IL-2/TNF-a | 16.00000 | 29.00000 | 6.000000 | -0.85732 | 0.391268 | -0.86092 | 0.389285 | 4 | 5 | 0.412698 |
| CD4 IFN- γ /IL-2/TNF-a | 14.00000 | 31.00000 | 4.000000 | -1.34722 | 0.177911 | -1.34722 | 0.177911 | 4 | 5 | 0.190476 |
| CD8 IFN- γ | 14.00000 | 31.00000 | 4.000000 | -1.34722 | 0.177911 | -1.35287 | 0.176099 | 4 | 5 | 0.190476 |
| CD8 IL-2 | 11.00000 | 34.00000 | 1.000000 | -2.08207 | 0.037337 | -2.11766 | 0.034205 | 4 | 5 | 0.031746 |
| CD8 TNF-a | 15.00000 | 30.00000 | 5.000000 | -1.10227 | 0.270345 | -1.20748 | 0.227250 | 4 | 5 | 0.285714 |
| CD8 IFN- γ /IL-2 | 10.00000 | 35.00000 | 0.000000 | -2.32702 | 0.019965 | -2.36680 | 0.017943 | 4 | 5 | 0.015873 |
| CD8 IFN- γ /TNF-a | 10.00000 | 35.00000 | 0.000000 | -2.32702 | 0.019965 | -2.36680 | 0.017943 | 4 | 5 | 0.015873 |
| CD8 IL-2/TNF-a | 15.50000 | 29.50000 | 5.500000 | -0.97980 | 0.327188 | -1.07872 | 0.280713 | 4 | 5 | 0.285714 |
| CD8 IFN- γ /IL-2/TNF-a | 10.00000 | 35.00000 | 0.000000 | -2.32702 | 0.019965 | -2.36680 | 0.017943 | 4 | 5 | 0.015873 |

Supplementary Table S4. Statistical comparison of populations CD4+ and CD8+ cells responding to stimulation with TERT357 peptide pool (Supplementary Table S1) in mice DNA immunized with TERT comparing to vector pVax1 (Mann-Whitney U test w/ continuity correction).

| T cell populations | Rank Sum (TERT) | Rank Sum (vector) | U | Z | p-value | Z (adjusted) | p-value | Valid N (TERT) | Valid N (vector) | 2*1sided (exact p) |
|---------------------------------------|--------------------|----------------------|----------|----------|----------|--------------|----------|-------------------|---------------------|-----------------------|
| CD4 IFN- γ | 55.00000 | 50.00000 | 5.00000 | 2.266667 | 0.023411 | 2.269162 | 0.023259 | 5 | 9 | 0.018981 |
| CD4 IL-2 | 57.00000 | 48.00000 | 3.00000 | 2.533333 | 0.011299 | 2.536122 | 0.011209 | 5 | 9 | 0.006993 |
| CD4 TNF- α | 43.00000 | 62.00000 | 17.00000 | 0.666667 | 0.504986 | 0.674116 | 0.500238 | 5 | 9 | 0.518482 |
| CD4 IFN- γ /IL-2 | 59.00000 | 46.00000 | 1.00000 | 2.800000 | 0.005111 | 2.803082 | 0.005062 | 5 | 9 | 0.001998 |
| CD4 IFN- γ /TNF- α | 57.00000 | 48.00000 | 3.00000 | 2.533333 | 0.011299 | 2.547369 | 0.010854 | 5 | 9 | 0.006993 |
| CD4 IL-2/TNF- α | 54.00000 | 51.00000 | 6.00000 | 2.133333 | 0.032898 | 2.142773 | 0.032132 | 5 | 9 | 0.028971 |
| CD4 IFN- γ /IL-2/TNF- α | 58.00000 | 47.00000 | 2.00000 | 2.666667 | 0.007661 | 2.669602 | 0.007595 | 5 | 9 | 0.003996 |
| CD8 IFN- γ | 54.00000 | 51.00000 | 6.00000 | 2.133333 | 0.032898 | 2.138037 | 0.032514 | 5 | 9 | 0.028971 |
| CD8 IL-2 | 56.00000 | 49.00000 | 4.00000 | 2.400000 | 0.016396 | 2.402642 | 0.016278 | 5 | 9 | 0.011988 |
| CD8 TNF- α | 45.00000 | 60.00000 | 15.00000 | 0.933333 | 0.350649 | 0.943762 | 0.345292 | 5 | 9 | 0.363636 |
| CD8 IFN- γ /IL-2 | 58.00000 | 47.00000 | 2.00000 | 2.666667 | 0.007661 | 2.669602 | 0.007595 | 5 | 9 | 0.003996 |
| CD8 IFN- γ /TNF- α | 58.00000 | 47.00000 | 2.00000 | 2.666667 | 0.007661 | 2.678466 | 0.007396 | 5 | 9 | 0.003996 |
| CD8 IL-2/TNF- α | 59.00000 | 46.00000 | 1.00000 | 2.800000 | 0.005111 | 2.812389 | 0.004918 | 5 | 9 | 0.001998 |
| CD8 IFN- γ /IL-2/TNF- α | 58.00000 | 47.00000 | 2.00000 | 2.666667 | 0.007661 | 2.678466 | 0.007396 | 5 | 9 | 0.003996 |

Supplementary Table S5. Statistical comparison of populations CD4+ and CD8+ cells responding to stimulation with TERT357 peptide pool (Supplementary S1) in mice DNA immunized with mixture of Core152opt and TERT (MX) comparing to vector pVax1 (Mann-Whitney U test w/ continuity correction).

| | Rank Sum (MX) | Rank Sum (vector) | U | Z | p-value | Z (adjusted) | p-value | Valid N (MX) | Valid N (vector) | 2*1sided (exact p) |
|-------------------------------|------------------|----------------------|----------|----------|----------|--------------|----------|-----------------|---------------------|-----------------------|
| CD4 IFN- γ | 22.00000 | 69.00000 | 12.00000 | -0.84867 | 0.396066 | -0.85337 | 0.393455 | 4 | 9 | 0.413986 |
| CD4 IL-2 | 23.00000 | 68.00000 | 13.00000 | -0.69437 | 0.487454 | -0.69821 | 0.485045 | 4 | 9 | 0.503497 |
| CD4 TNF-a | 24.00000 | 67.00000 | 14.00000 | -0.54006 | 0.589155 | -0.56806 | 0.569993 | 4 | 9 | 0.604196 |
| CD4 IFN- γ /IL-2 | 23.50000 | 67.50000 | 13.50000 | -0.61721 | 0.537094 | -0.62150 | 0.534273 | 4 | 9 | 0.503497 |
| CD4 IFN- γ /TNF-a | 24.00000 | 67.00000 | 14.00000 | -0.54006 | 0.589155 | -0.55635 | 0.577973 | 4 | 9 | 0.604196 |
| CD4 IL-2/TNF-a | 23.00000 | 68.00000 | 13.00000 | -0.69437 | 0.487454 | -0.71426 | 0.475064 | 4 | 9 | 0.503497 |
| CD4 IFN- γ /IL-2/TNF-a | 24.00000 | 67.00000 | 14.00000 | -0.54006 | 0.589155 | -0.54764 | 0.583942 | 4 | 9 | 0.604196 |
| CD8 IFN- γ | 19.00000 | 72.00000 | 9.00000 | -1.31158 | 0.189663 | -1.31884 | 0.187222 | 4 | 9 | 0.198601 |
| CD8 IL-2 | 24.00000 | 67.00000 | 14.00000 | -0.54006 | 0.589155 | -0.54764 | 0.583942 | 4 | 9 | 0.604196 |
| CD8 TNF-a | 24.00000 | 67.00000 | 14.00000 | -0.54006 | 0.589155 | -0.56806 | 0.569993 | 4 | 9 | 0.604196 |
| CD8 IFN- γ /IL-2 | 20.00000 | 71.00000 | 10.00000 | -1.15728 | 0.247161 | -1.17351 | 0.240593 | 4 | 9 | 0.260140 |
| CD8 IFN- γ /TNF-a | 22.00000 | 69.00000 | 12.00000 | -0.84867 | 0.396066 | -0.87299 | 0.382669 | 4 | 9 | 0.413986 |
| CD8 IL-2/TNF-a | 24.50000 | 66.50000 | 14.50000 | -0.46291 | 0.643429 | -0.47687 | 0.633455 | 4 | 9 | 0.604196 |
| CD8 IFN- γ /IL-2/TNF-a | 22.00000 | 69.00000 | 12.00000 | -0.84867 | 0.396066 | -0.87299 | 0.382669 | 4 | 9 | 0.413986 |

Supplementary Table S6. Statistical comparison of populations CD4+ and CD8+ cells responding to stimulation with TERT6 and TERT8 peptides pool (Supplementary Table S1) in mice DNA immunized with TERT comparing to vector pVax1 (Mann-Whitney U test w/ continuity correction).

| T cell population | Rank Sum (TERT) | Rank Sum (vector) | U | Z | p-value | Z (adjusted) | p-value | Valid N (MIX) | Valid N (vector) | 2*1sided (exact p) |
|---|--------------------|----------------------|----------|-----------|----------|--------------|----------|---------------|---------------------|-----------------------|
| TERT6 CD4 IFN- γ | 48.00000 | 57.00000 | 12.00000 | 1.333333 | 0.182423 | 1.425617 | 0.153980 | 5 | 9 | 0.189810 |
| TERT6 CD4 IL-2 | 48.00000 | 57.00000 | 12.00000 | 1.333333 | 0.182423 | 1.423830 | 0.154497 | 5 | 9 | 0.189810 |
| TERT6 CD4 TNF- α | 35.00000 | 70.00000 | 20.00000 | -0.266667 | 0.789726 | -0.596285 | 0.550985 | 5 | 9 | 0.797203 |
| TERT6 CD4 IFN- γ /IL-2 | 47.50000 | 57.50000 | 12.50000 | 1.266667 | 0.205275 | 1.354336 | 0.175630 | 5 | 9 | 0.189810 |
| TERT6 CD4 IFN- γ /TNF- α | 48.00000 | 57.00000 | 12.00000 | 1.333333 | 0.182423 | 1.423830 | 0.154497 | 5 | 9 | 0.189810 |
| TERT6 CD4 IL-2/TNF- α | 51.50000 | 53.50000 | 8.50000 | 1.800000 | 0.071862 | 1.924583 | 0.054282 | 5 | 9 | 0.059940 |
| TERT6 CD4 IFN- γ /IL-2/TNF- α | 48.50000 | 56.50000 | 11.50000 | 1.400000 | 0.161514 | 1.496898 | 0.134421 | 5 | 9 | 0.146853 |
| TERT6 CD8 IFN- γ | 50.00000 | 55.00000 | 10.00000 | 1.600000 | 0.109600 | 1.708595 | 0.087527 | 5 | 9 | 0.111888 |
| TERT6 CD8 IL-2 | 54.50000 | 50.50000 | 5.50000 | 2.200000 | 0.027808 | 2.436360 | 0.014836 | 5 | 9 | 0.018981 |
| TERT6 CD8 TNF- α | 47.00000 | 58.00000 | 13.00000 | 1.200000 | 0.230140 | 1.503100 | 0.132814 | 5 | 9 | 0.239760 |
| TERT6 CD8 IFN- γ /IL-2 | 54.00000 | 51.00000 | 6.00000 | 2.133333 | 0.032898 | 2.278127 | 0.022720 | 5 | 9 | 0.028971 |
| TERT6 CD8 IFN- γ /TNF- α | 55.00000 | 50.00000 | 5.00000 | 2.266667 | 0.023411 | 2.641624 | 0.008251 | 5 | 9 | 0.018981 |
| TERT6 CD8 IL-2/TNF- α | 55.50000 | 49.50000 | 4.50000 | 2.333333 | 0.019631 | 2.922695 | 0.003470 | 5 | 9 | 0.011988 |
| TERT6 CD8 IFN- γ /IL-2/TNF- α | 55.00000 | 50.00000 | 5.00000 | 2.266667 | 0.023411 | 2.641624 | 0.008251 | 5 | 9 | 0.018981 |
| TERT8 CD4 IFN- γ | 52.50000 | 52.50000 | 7.50000 | 1.93333 | 0.053196 | 1.95714 | 0.050332 | 5 | 9 | 0.041958 |
| TERT8 CD4 IL-2 | 53.00000 | 52.00000 | 7.00000 | 2.00000 | 0.045501 | 2.14653 | 0.031831 | 5 | 9 | 0.041958 |
| TERT8 CD4 TNF- α | 36.00000 | 69.00000 | 21.00000 | -0.13333 | 0.893930 | -0.18553 | 0.852815 | 5 | 9 | 0.898102 |
| TERT8 CD4 IFN- γ /IL-2 | 54.00000 | 51.00000 | 6.00000 | 2.13333 | 0.032898 | 2.28099 | 0.022550 | 5 | 9 | 0.028971 |
| TERT8 CD4 IFN- γ /TNF- α | 52.00000 | 53.00000 | 8.00000 | 1.86667 | 0.061949 | 1.90910 | 0.056251 | 5 | 9 | 0.059940 |
| TERT8 CD4 IL-2/TNF- α | 52.00000 | 53.00000 | 8.00000 | 1.86667 | 0.061949 | 1.99586 | 0.045950 | 5 | 9 | 0.059940 |
| TERT8 CD4 IFN- γ /IL-2/TNF- α | 52.00000 | 53.00000 | 8.00000 | 1.86667 | 0.061949 | 1.99336 | 0.046223 | 5 | 9 | 0.059940 |
| TERT8 CD8 IFN- γ | 51.50000 | 53.50000 | 8.50000 | 1.80000 | 0.071862 | 1.80997 | 0.070301 | 5 | 9 | 0.059940 |
| TERT8 CD8 IL-2 | 43.00000 | 62.00000 | 17.00000 | 0.66667 | 0.504986 | 0.68182 | 0.495353 | 5 | 9 | 0.518482 |
| TERT8 CD8 TNF- α | 29.00000 | 76.00000 | 14.00000 | -1.06667 | 0.286123 | -1.18127 | 0.237498 | 5 | 9 | 0.297702 |
| TERT8 CD8 IFN- γ /IL-2 | 46.50000 | 58.50000 | 13.50000 | 1.13333 | 0.257075 | 1.14600 | 0.251797 | 5 | 9 | 0.239760 |
| TERT8 CD8 IFN- γ /TNF- α | 46.00000 | 59.00000 | 14.00000 | 1.06667 | 0.286123 | 1.09091 | 0.275312 | 5 | 9 | 0.297702 |
| TERT8 CD8 IL-2/TNF- α | 42.50000 | 62.50000 | 17.50000 | 0.60000 | 0.548507 | 0.61014 | 0.541768 | 5 | 9 | 0.518482 |
| TERT8 CD8 IFN- γ /IL-2/TNF- α | 51.00000 | 54.00000 | 9.00000 | 1.73333 | 0.083037 | 1.77273 | 0.076274 | 5 | 9 | 0.082917 |

Supplementary Table S7. Statistical comparison of populations CD4+ and CD8+ cells responding to stimulation with TERT6 and TERT8 peptides pool (Supplementary Table S1) in mice DNA immunized with mixture of Core152opt and TERT (MX) comparing to vector pVax1 (Mann-Whitney U test w/ continuity correction).

| T cell population | Rank Sum (MX) | Rank Sum (vector) | U | Z | p-value | Z (adjusted) | p-value | Valid N (MX) | Valid N (vector) | 2*1sided (exact p) |
|---|---------------|-------------------|----------|-----------|----------|--------------|----------|--------------|------------------|--------------------|
| TERT6 CD4 IFN- γ | 29.00000 | 62.00000 | 17.00000 | 0.07715 | 0.938503 | 0.08797 | 0.929903 | 4 | 9 | 0.939860 |
| TERT6 CD4 IL-2 | 26.00000 | 65.00000 | 16.00000 | -0.23146 | 0.816961 | -0.28270 | 0.777409 | 4 | 9 | 0.825175 |
| TERT6 CD4 TNF- α | 26.00000 | 65.00000 | 16.00000 | -0.23146 | 0.816961 | -0.50000 | 0.617075 | 4 | 9 | 0.825175 |
| TERT6 CD4 IFN- γ /IL-2 | 30.00000 | 61.00000 | 16.00000 | 0.23146 | 0.816961 | 0.26390 | 0.791858 | 4 | 9 | 0.825175 |
| TERT6 CD4 IFN- γ /TNF- α | 26.00000 | 65.00000 | 16.00000 | -0.23146 | 0.816961 | -0.28270 | 0.777409 | 4 | 9 | 0.825175 |
| TERT6 CD4 IL-2/TNF- α | 26.00000 | 65.00000 | 16.00000 | -0.23146 | 0.816961 | -0.28270 | 0.777409 | 4 | 9 | 0.825175 |
| TERT6 CD4 IFN- γ /IL-2/TNF- α | 26.00000 | 65.00000 | 16.00000 | -0.23146 | 0.816961 | -0.28270 | 0.777409 | 4 | 9 | 0.825175 |
| TERT6 CD8 IFN- γ | 28.00000 | 63.00000 | 18.00000 | 0.07715 | 0.938503 | 0.09423 | 0.924924 | 4 | 9 | 1.000000 |
| TERT6 CD8 IL-2 | 29.50000 | 61.50000 | 16.50000 | 0.15430 | 0.877371 | 0.20869 | 0.834691 | 4 | 9 | 0.825175 |
| TERT6 CD8 TNF- α | 31.00000 | 60.00000 | 15.00000 | 0.38576 | 0.699676 | 0.61332 | 0.539667 | 4 | 9 | 0.710490 |
| TERT6 CD8 IFN- γ /IL-2 | 28.00000 | 63.00000 | 18.00000 | -0.07715 | 0.938503 | -0.09423 | 0.924924 | 4 | 9 | 1.000000 |
| TERT6 CD8 IFN- γ /TNF- α | 31.00000 | 60.00000 | 15.00000 | 0.38576 | 0.699676 | 0.61332 | 0.539667 | 4 | 9 | 0.710490 |
| TERT6 CD8 IL-2/TNF- α | 32.50000 | 58.50000 | 13.50000 | 0.61721 | 0.537094 | 1.33333 | 0.182423 | 4 | 9 | 0.503497 |
| TERT6 CD8 IFN- γ /IL-2/TNF- α | 31.00000 | 60.00000 | 15.00000 | 0.38576 | 0.699676 | 0.61332 | 0.539667 | 4 | 9 | 0.710490 |
| TERT8 CD4 IFN- γ | 31.00000 | 60.00000 | 15.00000 | 0.385758 | 0.699676 | 0.397392 | 0.691079 | 4 | 9 | 0.710490 |
| TERT8 CD4 IL-2 | 33.00000 | 58.00000 | 13.00000 | 0.694365 | 0.487454 | 0.791698 | 0.428537 | 4 | 9 | 0.503497 |
| TERT8 CD4 TNF- α | 27.50000 | 63.50000 | 17.50000 | 0.000000 | 1.000000 | 0.000000 | 1.000000 | 4 | 9 | 0.939860 |
| TERT8 CD4 IFN- γ /IL-2 | 33.50000 | 57.50000 | 12.50000 | 0.771517 | 0.440401 | 0.881239 | 0.378189 | 4 | 9 | 0.413986 |
| TERT8 CD4 IFN- γ /TNF- α | 26.50000 | 64.50000 | 16.50000 | -0.154303 | 0.877371 | -0.168018 | 0.866569 | 4 | 9 | 0.825175 |
| TERT8 CD4 IL-2/TNF- α | 28.00000 | 63.00000 | 18.00000 | 0.077152 | 0.938503 | 0.088124 | 0.929778 | 4 | 9 | 1.000000 |
| TERT8 CD4 IFN- γ /IL-2/TNF- α | 34.00000 | 57.00000 | 12.00000 | 0.848668 | 0.396066 | 0.922600 | 0.356216 | 4 | 9 | 0.413986 |
| TERT8 CD8 IFN- γ | 16.00000 | 75.00000 | 6.00000 | -1.774489 | 0.075983 | -1.825344 | 0.067950 | 4 | 9 | 0.075524 |
| TERT8 CD8 IL-2 | 23.00000 | 68.00000 | 13.00000 | -0.694365 | 0.487454 | -0.754854 | 0.450337 | 4 | 9 | 0.503497 |
| TERT8 CD8 TNF- α | 18.00000 | 73.00000 | 8.00000 | -1.465882 | 0.142681 | -1.671362 | 0.094651 | 4 | 9 | 0.148252 |
| TERT8 CD8 IFN- γ /IL-2 | 19.50000 | 71.50000 | 9.50000 | -1.234427 | 0.217045 | -1.298429 | 0.194141 | 4 | 9 | 0.198601 |
| TERT8 CD8 IFN- γ /TNF- α | 21.00000 | 70.00000 | 11.00000 | -1.002972 | 0.315875 | -1.090345 | 0.275562 | 4 | 9 | 0.330070 |
| TERT8 CD8 IL-2/TNF- α | 20.00000 | 71.00000 | 10.00000 | -1.157275 | 0.247161 | -1.258091 | 0.208360 | 4 | 9 | 0.260140 |
| TERT8 CD8 IFN- γ /IL-2/TNF- α | 23.50000 | 67.50000 | 13.50000 | -0.617213 | 0.537094 | -0.703732 | 0.481600 | 4 | 9 | 0.503497 |

Supplementary Table S8. Pair-wise comparison of the strength of photon flux (p/s) reflecting expression of luciferase from the injection sites in mice receiving boosts of plasmids encoding HCV core (Core191v, Core152opt), TERT and mixture of plasmids Core152opt and TERT (Table 1) together with plasmid encoding luciferase, 24 hours post co-delivery. Mann-Whitney test (w/continuity correction); marked tests are significant at $p < 0.05$.

| RANK SUM Group of comparison A | RANK SUM Group of comparison B | U | Z | p-value | Z (adjusted) | p-value | Valid N (Group A) | Valid N (Group B) | 2*1sided (exact p) |
|-----------------------------------|-----------------------------------|----------|-----------|----------|--------------|----------|----------------------|----------------------|-----------------------|
| TERT | TERT+Core152opt | | | | | | | | |
| 128.0000 | 82.00000 | 27.00000 | 1.700840 | 0.088974 | 1.700840 | 0.088974 | 10 | 10 | 0.089210 |
| Core191v | | | | | | | | | |
| 153.0000 | 57.00000 | 2.000000 | 3.590662 | 0.000330 | 3.590662 | 0.000330 | 10 | 10 | 0.000043 |
| Core152opt | | | | | | | | | |
| 144.0000 | 66.00000 | 11.00000 | 2.910326 | 0.003611 | 2.910326 | 0.003611 | 10 | 10 | 0.002089 |
| Vector Series I | | | | | | | | | |
| 111.0000 | 60.00000 | 5.000000 | 3.065405 | 0.002174 | 3.065405 | 0.002174 | 8 | 10 | 0.000868 |
| Vector Series II | | | | | | | | | |
| 148.0000 | 62.00000 | 7.000000 | 3.212698 | 0.001315 | 3.212698 | 0.001315 | 10 | 10 | 0.000487 |
| TERT | Core191v | | | | | | | | |
| 62.00000 | 148.0000 | 7.000000 | -3.21270 | 0.001315 | -3.21270 | 0.001315 | 10 | 10 | 0.000487 |
| | Core152opt | | | | | | | | |
| 77.00000 | 133.0000 | 22.00000 | -2.07880 | 0.037636 | -2.07880 | 0.037636 | 10 | 10 | 0.035463 |
| | Vector Series I | | | | | | | | |
| 66.00000 | 87.00000 | 11.00000 | -2.29337 | 0.021828 | -2.29337 | 0.021828 | 10 | 7 | 0.018511 |
| | Vector Series II | | | | | | | | |
| 78.00000 | 132.0000 | 23.00000 | -2.00321 | 0.045155 | -2.00321 | 0.045155 | 10 | 10 | 0.043257 |
| Vector Series I | Core191v | | | | | | | | |
| 85.00000 | 86.00000 | 30.00000 | -0.844097 | 0.398616 | -0.844097 | 0.398616 | 10 | 8 | 0.408245 |
| Vector Series II | | | | | | | | | |
| 119.0000 | 91.00000 | 36.00000 | 1.020504 | 0.307490 | 1.020504 | 0.307490 | 10 | 10 | 0.314999 |
| Vector Series I | Core152opt | | | | | | | | |
| 89.00000 | 82.00000 | 34.00000 | -0.488688 | 0.625063 | -0.488688 | 0.625063 | 10 | 8 | 0.633438 |
| Vector Series II | | | | | | | | | |
| 116.0000 | 94.00000 | 39.00000 | 0.793725 | 0.427356 | 0.793725 | 0.427356 | 10 | 10 | 0.435872 |