Supplementary methods

Human IFN-ɣ ELISpot assays

IFN-ɣ ELISpot assays were performed directly ex vivo on freshly isolated PBMCs plated in triplicate at 2 × 10^5 PBMCs per well. A positive cut off was previously defined (*12,15*) as 48 SFCs/10^6 PBMCs (mean + 3 SDs) calculated from 74 healthy HCV seronegative volunteers. In addition, a response was only considered positive if this exceeded 3× background in the DMSO control wells. Background controls wells typically contained zero to four SFCs/2 × 10^5 PBMCs.

Concanavalin A (Sigma), FEC (mixed HLA class–I restricted peptides from influenza, Epstein-Barr virus and Cytomegalovirus) (BEI resources) and CMV lysate (Virusys Corp) were included as internal positive controls. Total NS response was calculated by summing responses to all positive pools (NS3p–NS5B II) and corrected for background. Cells were counted using a Muse cell analyzer (Merck Millipore) as previously described (*12,15*).

Mouse IFN-γ ELISpot

IFNγ-ELISpot assay with mouse splenocytes was performed exactly as described (*8*) using antibody pairs obtained from U-CyTech (Utrecht, the Netherlands) and overnight stimulation with immunodominant OVA peptide 257-264 (SIINFEKL) at a final concentration of 2μg/ml.

Human Intracellular cytokine staining (ICS)

ICS was performed on fresh PBMC after 12 hours resting at 37°C in R10 medium. Briefly, PBMC were stimulated using peptides in pool combinations (F+G=NS3, H+I=NS4-NS5A, L+M=NS5B 1μg/ml) or unstimulated (controlled for DMSO) or phorbol 12-myristate 13-acetate (PMA)/ionomycin (50 and 500 ng/ml respectively) for 6hours at 37°C in the presence of GolgiPlug (BD), Golgi Stop (Biolegend) and anti-CD107a. Cells were stained with aqua amine reactive viability dye, were fixed using BD cytofix/cytoperm solution according to manufacturer's instructions and stained with the antibodies listed in table S8.

Cells at least 10,000 viable singlet $CD3^+$ $CD4^+$ or $CD3^+$ $CD8^+$ lymphocyte events were acquired using a BD LSRII-FACS DIVA. All ICS data are corrected for background. Analysis of polyfunctionality was performed using Pestle and SPICE version 5.3.

The Simplified Presentation of Incredibly Complex Evaluations (SPICE) program support analysis of T cell functional profiles. It is a data mining application that analyses large FLOWJO data sets from polychromatic flow cytometry and allows to test for differences between groups of samples

based on the multi-component measurements (*43*). Pestle converts FLOWJO table to adequate form to use into SPICE.

MHC class I pentamer staining

Thawed PBMC (1-2 × 10^6) were incubated with pentamers (previously centrifuged at 14000g 10 min), fixable NIR LIVE/DEAD and antibodies specific for cells surface markers (listed in table S6). For intracellular/intranuclear staining PBMC were first stained with pentamers and surface antibodies, then fixed and permeabilized using Foxp3/Transcription Factor Staining Buffer Set (eBioscience 00-5523-00) for 45 minutes at room temperature, protected from the light, and stained with intracellular/intranuclear antibodies (listed in table S8).

Detection of HCV and ChAd3 Antibodies

HCV Abs were measured at the EOS using the automated clinical Abbott architect immunoassay. ChAd3 neutralising antibody (nAb) titres were assayed as previously described using a secreted alkaline phosphatase (SEAP) assay (*8*).

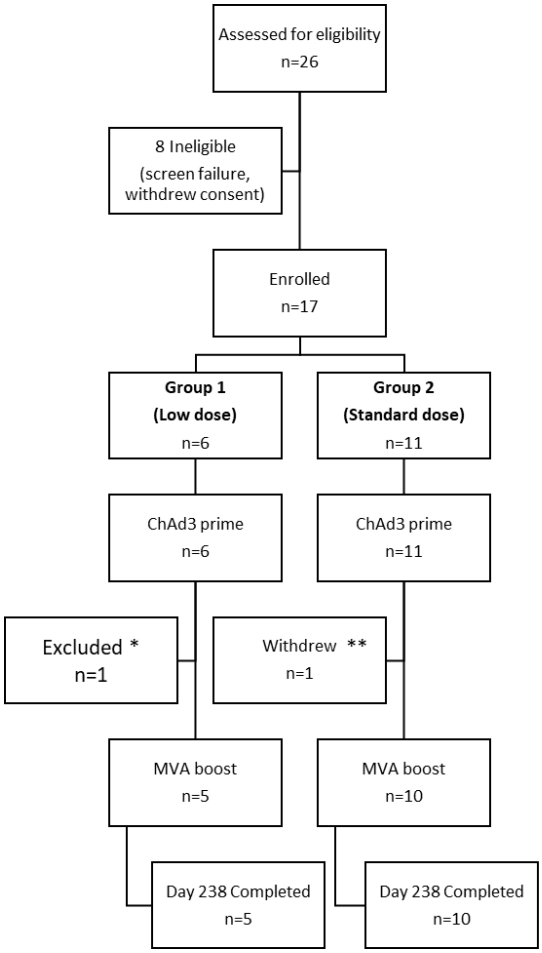

Fig. S1. Flow chart of study participants.

Consort flow diagram showing enrollment and follow-up in the PEACHI 03 trial.

*One volunteer in Group 1 was excluded (after receiving a non-study vaccine) and was replaced with a new volunteer.

**One volunteer in Group 2 withdrew consent (moved from the study area) after receiving the ChAd3-NSmut vaccination and was replaced with a new volunteer.

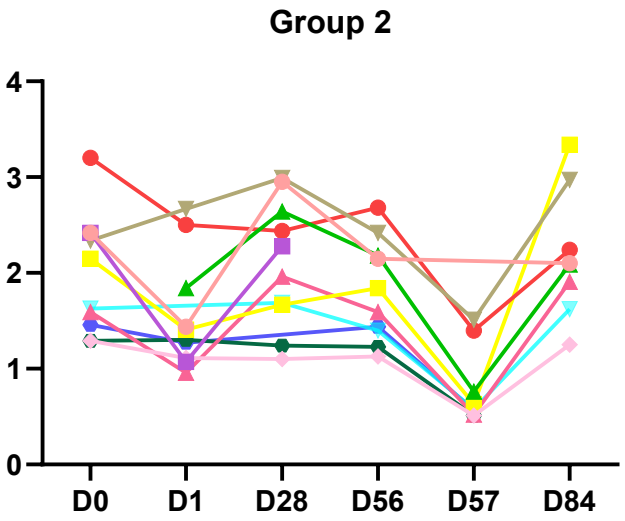

Fig. S2. Lymphocyte count after ChAd3/MVA-hIiNSmut vaccination.

Peripheral blood lymphocyte counts for group 2 (standard dose-ChAd3-hIiNSmut 2.5×10^{10} vp/MVA-hIiNSmut 2×10^{8} pfu, $n = 11$) were measured at different time points at d0, 1, 28, 56, 57 and 84 after hIiNSmut vaccine regimen.

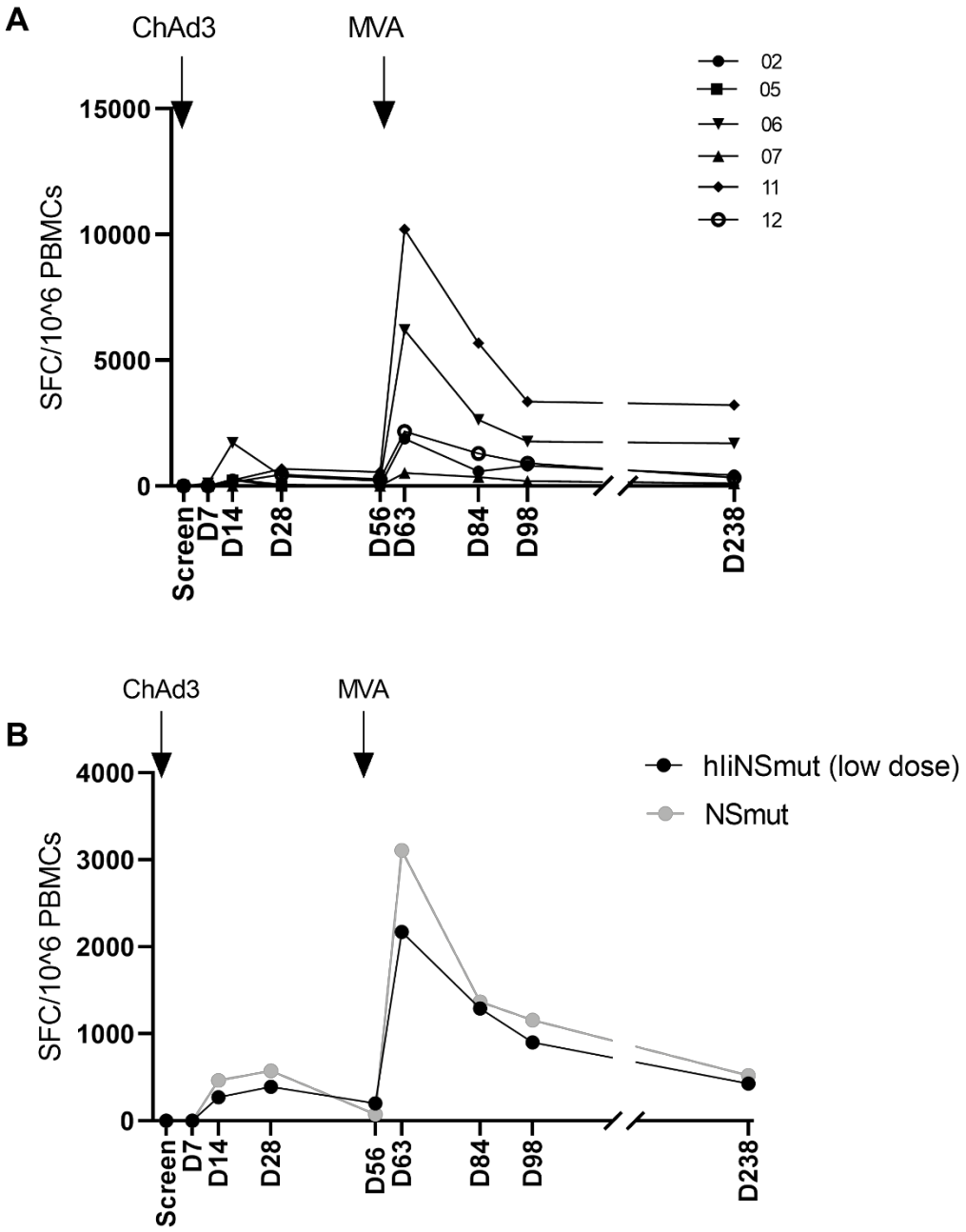

Fig. S3. Magnitude of ChAd3/MVA-hIiNSmut low dose (group 1). The total ex vivo IFN-γ ELISpot response to the NS region of HCV is shown over time (calculated by summing the responses of positive pools corrected for background; see Materials and Methods). (**A**) The kinetics of the response is shown for 6 volunteers who received the prime dose ChAd3-hIiNSmut and 5 that were boosted with MVA-hIiNSmut at low dose (ChAd3-hIiNSmut 5×10^9 vp/MVA-hIiNSmut 5×10^7 pfu). (**B**) Comparisons in ELISpot responses in volunteers receiving ChAd3/MVA-hIiNSmut at low dose (group 1) (black; $n = 5$) or ChAd3/MVA-NSmut (gray; $n = 17$) at standard dose. The median ex vivo IFN-γ ELISpot response to HCV NS is shown. Arrows above graph indicate vaccination timepoints.

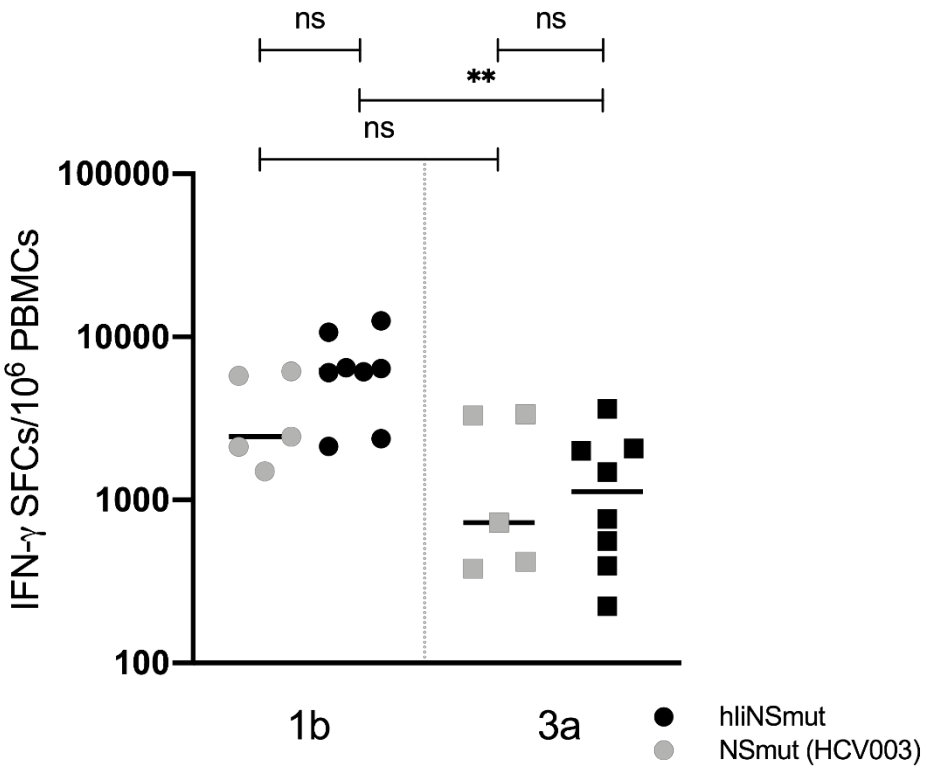

Fig. S4. Cross-reactivity of response in volunteers vaccinated with ChAd3/MVA-NSmut or ChAd3/MVA-hIiNSmut.

Cross-reactivity of T cell response evaluated by IFN-γ ELISpot: total magnitude of T cell responses using peptide pools covering the NS region of HCV genotype 1b (vaccine strain) compared to genotype 3a for volunteers vaccinated with ChAd3/MVA-hIiNSmut (black; $n = 8$) or ChAd3/MVA-NSmut (gray; $n = 5$) (Two-tailed Mann-Whitney test) at peak after boost. Bars shown are median. $^{**}P \leq 0.01$.

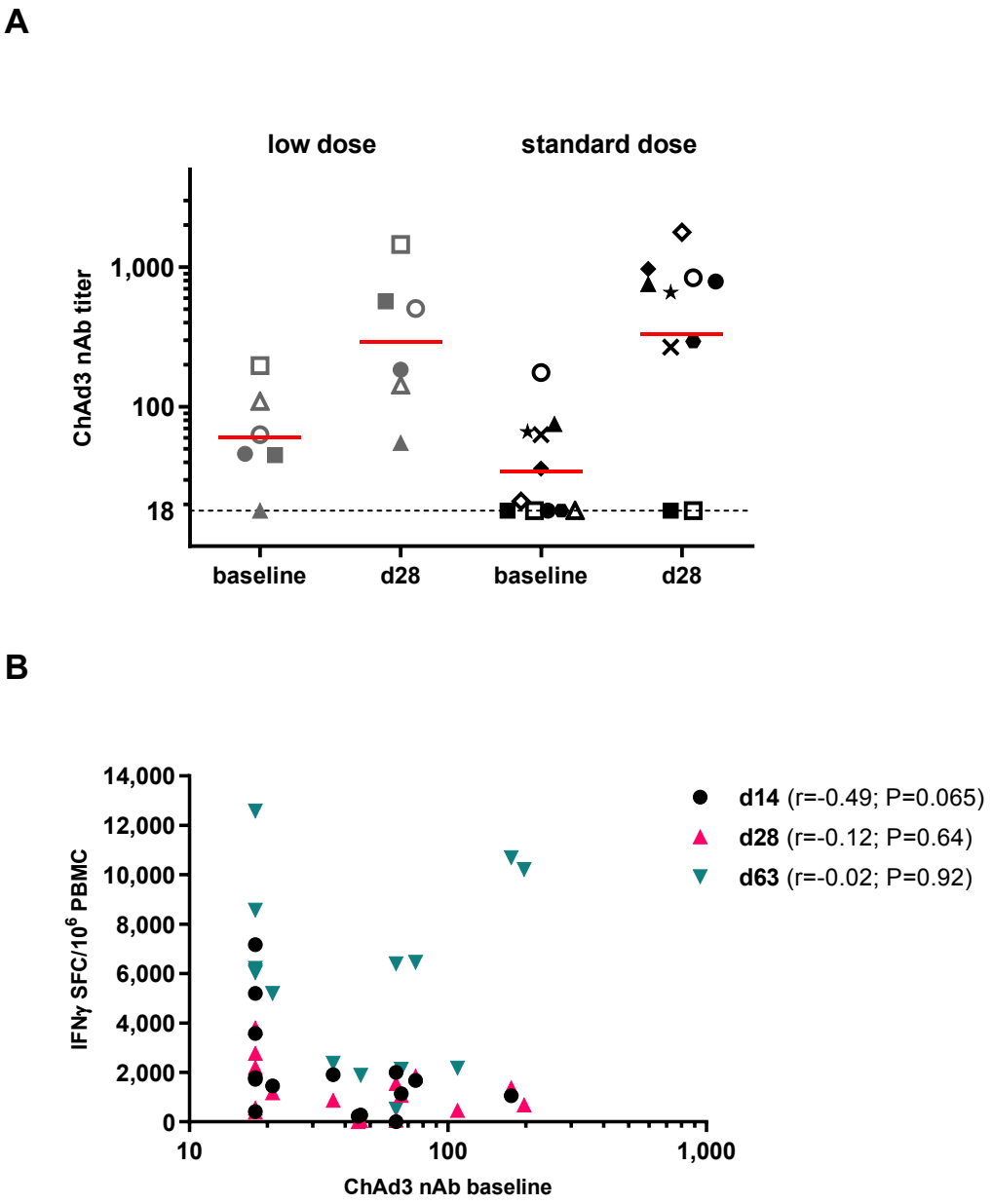

Fig. S5. ChAd3 neutralizing antibody at baseline and at peak after prime after low dose and standard dose of ChAd3/MVA-hIiNSmut.

(**A**) Titers of cross-neutralizing antibodies against ChAd3 in sera of volunteers at baseline and at d28 at low dose (group 1- ChAd3-hIiNSmut 5×10^9 vp/MVA-hIiNSmut 5×10^7 pfu, $n = 6$) and standard dose (group 2- ChAd3-hIiNSmut 2.5×10^{10} vp/MVA-hIiNSmut 2×10^8 pfu, $n = 11$) of hIi vaccine regimen by a secreted alkaline phosphatase (SEAP) assay. (**B**) Correlation between IFN-γ ELISpot response at d14, d28 and d63 and titers of cross-neutralizing antibodies against ChAd3 at baseline.

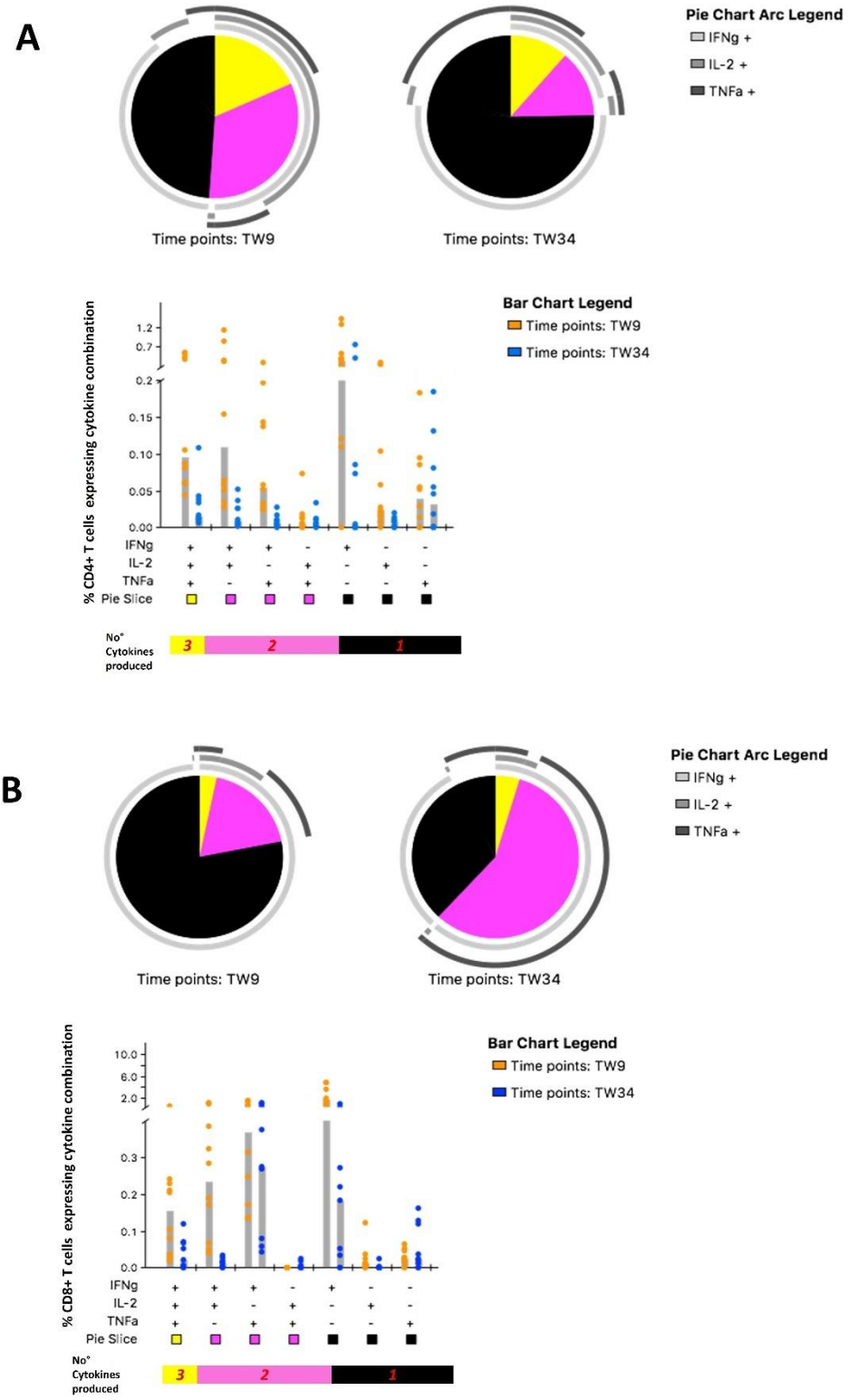

Fig. S6. Polyfunctionality of HCV-specific CD4$^+$ and CD8$^+$ T cells in volunteers receiving ChAd3/MVA-hIiNSmut vaccination.

Dot plots show frequencies of antigen-specific CD4$^+$ (**A**) and CD8$^+$ (**B**) T cell populations defined by parameters shown on the x axis at peak after boost (orange dots) and EOS (blue dots) after stimulation with pools F+G (NS3), H+I (NS4-NS5A) and L+M (NS5B) for 6 hours and analyzed by ICS. Black

bars indicate interquartile ranges. Pie charts show proportions of cytokine-secreting T cells that produce one (black), two (pink) or three (yellow) cytokines measured. Pie arcs show the proportion of cytokine-producing cells that makes a given cytokine. The polyfunctionality analysis was performed using Pestel and SPICE program. Pie base, median.

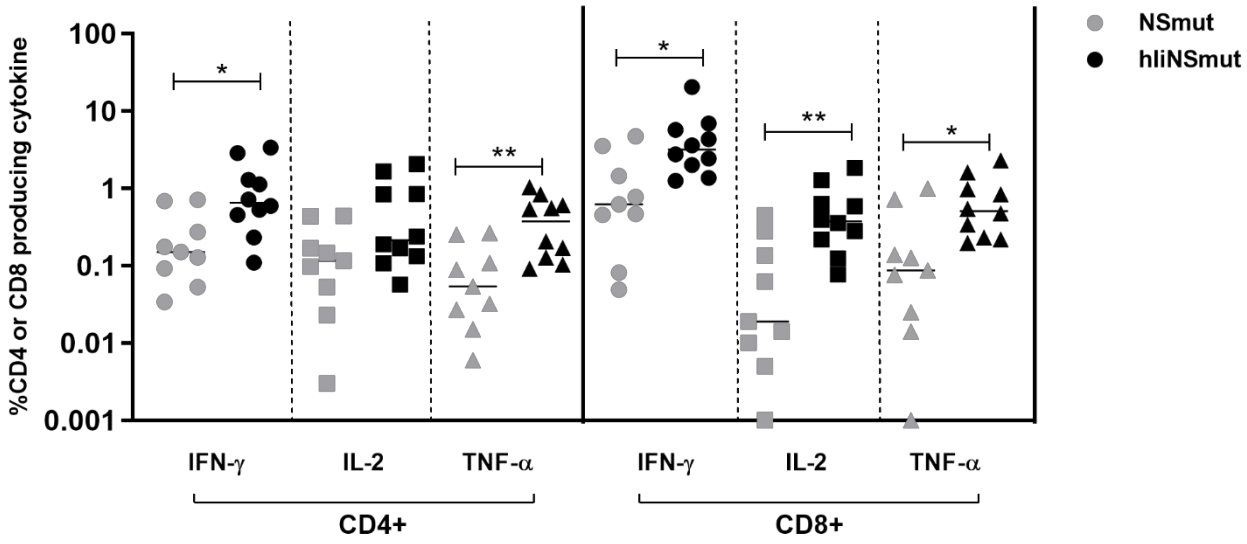

Fig. S7. Comparison of functionality of vaccine-induced T cell responses between ChAd3/MVA-NSmut with and without Ii.

Percentage of total $CD4^+$ and $CD8^+$ T cells producing IFN-ɣ, IL-2 or TNF-α after stimulation with NS3-5 peptide pools at peak after boost using fresh cells in ICS for healthy volunteers vaccinated with ChAd3/MVA-NSmut (gray symbols, $n = 9$) and ChAd3/MVA-hIiNSmut (black symbols, $n = 10$) (Two-tailed Mann-Whitney test $CD4^+$ IFN-ɣ and TNF-α) (Two-tailed Mann-Whitney test $CD8^+$ IFN-ɣ, IL-2 and TNF-α). The summed value is shown after DMSO subtraction. Bars represent median. *$P \leq 0.05$; **$P \leq 0.01$.

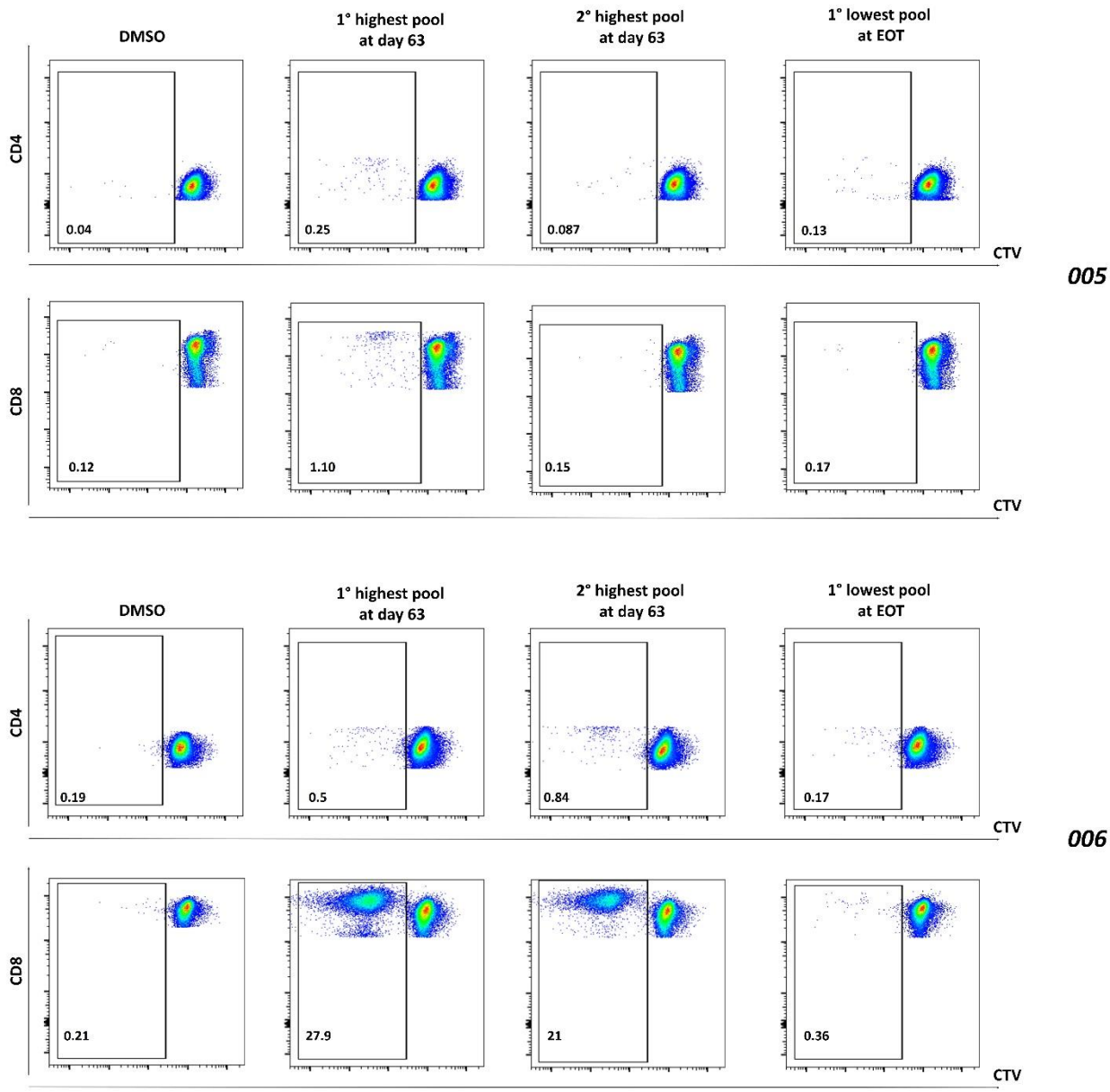

Fig. S8. Gating strategy of proliferated T cells after peptide stimulation.

Example FACS plots for subject at EOS after ChAd3/MVA-NSmut (005) or ChAd3/MVA-hIiNSmut (006) vaccine regimen after stimulation with DMSO (negative control), peptide pools 1 (first highest pool at boost), peptide pools 2 (second highest pool at boost) and peptide pools 3 (lowest pool at EOS that is positive at boost) for $CD4^+$ and $CD8^+$ T cell population using a cell tracer violet (CTV) assay. Cells were gated on singlet lymphocytes, live, $CD3^+$ T cells; $CD4^+$, $CD8^+$ and CTV cell populations are shown.

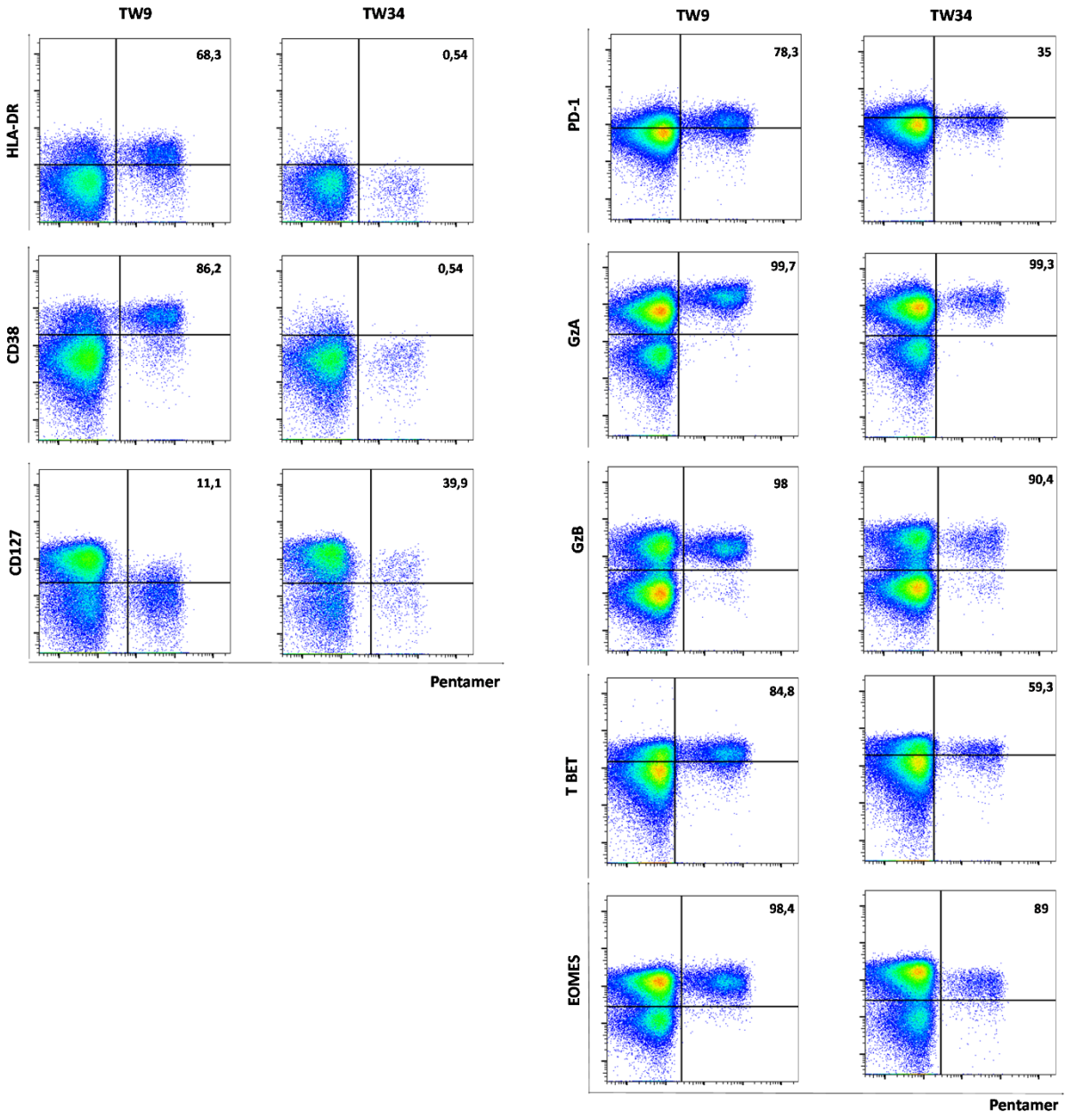

Fig. S9. Gating strategy for MHC class I pentamer$^+$ CD8$^+$ T cells expressing markers.

Example FACS plots from volunteer 002 vaccinated with ChAd3/MVA-hIiNSmut at peak after boost and at EOS are shown for phenotypic markers (Y axis) analyzed against pentamer staining (x axis; HCV NS3 1406-1415, HLA-A*0201). The percentage of the pentamer$^+$ CD8$^+$ T cells that express a given marker is shown (gated using the fluorescence minus one [FMO]).

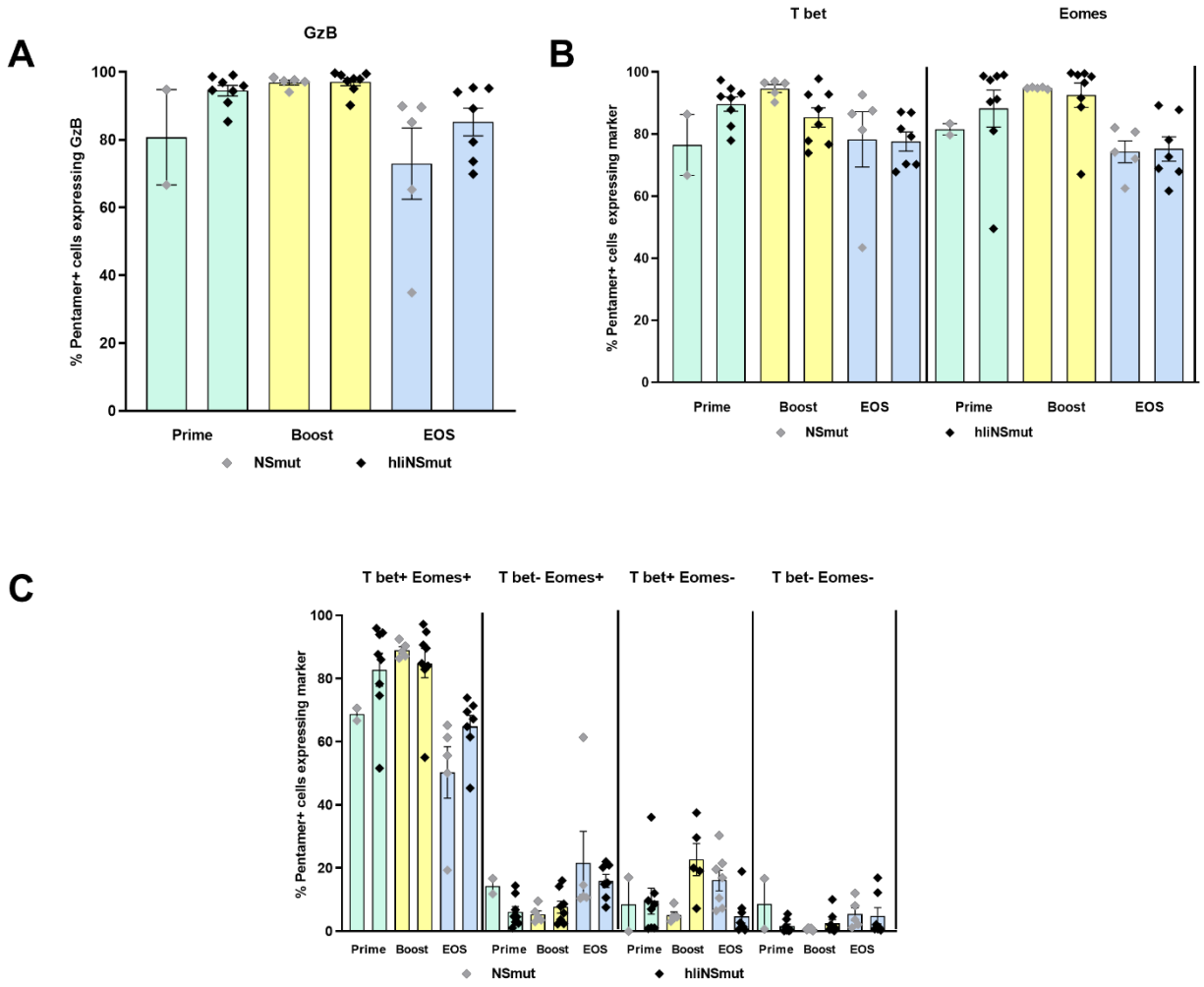

Fig. S10. MHC class I phenotypic analysis of pentamer$^+$ CD8$^+$ T cells after ChAd3/MVA-NSmut vaccine regimen with or without Ii in healthy volunteers.

(**A-B**) The percentage of the pentamer$^+$ cells expressing phenotypic markers GzB, Tbet or Eomes from volunteers vaccinated with ChAd3/MVA-NSmut or ChAd3/MVA-hIiNSmut at peak after prime (green bars, gray $n = 2$; black, $n = 8$) , at peak after boost (yellow bars, gray $n = 5$; black $n = 8$) and at EOS (light blue bars, gray $n = 5$; black $n = 8$). Mean with SEM is shown. (**C**) Co-staining with pentamers and human antibodies against Tbet and Eomes over the course of the study. All pentamer staining and phenotyping performed ex vivo without culture. Mean with SEM is shown.

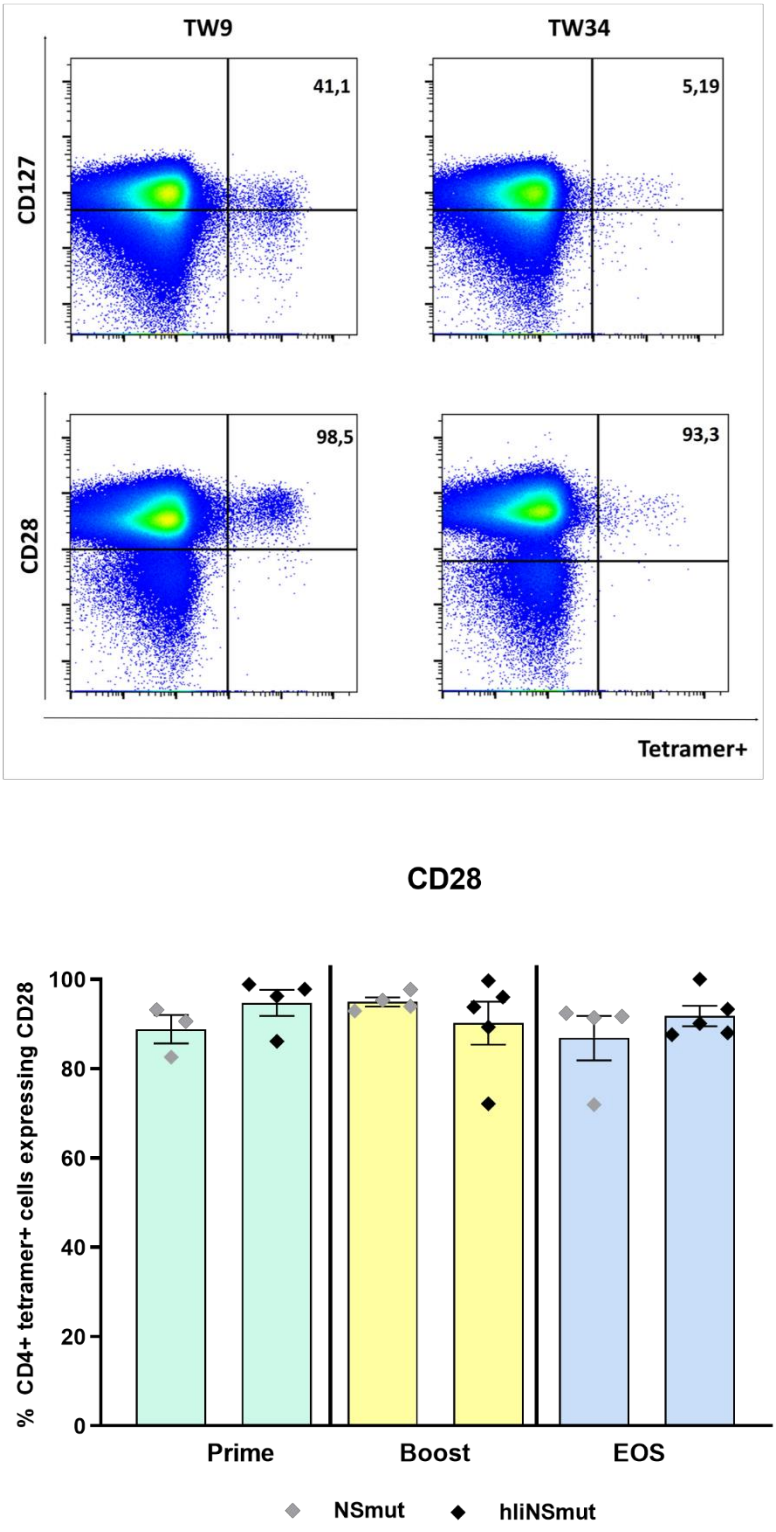

Fig. S11. MHC class II phenotypic analysis after ChAd3/MVA-NSmut vaccine regimen with or without Ii in healthy volunteers.

(**A**) Example FACS plots from volunteer 004 vaccinated with ChAd3-hIiNSmut/MVA-hIiNSmut at peak after boost and at EOS are shown for phenotypic markers (Y axis) analyzed against tetramer staining (x axis; HCV NS4b 1806-1818 TLLFNILGGWVAA, HLA-DRB1*0101). The percentage of

the tetramer^{+} CD4^{+} T cells that express a given marker is shown (gated using the fluorescence minus one [FMO]). (**B**) The percentage of tetramer^{+} cells expressing phenotypic marker CD28 from volunteers vaccinated with ChAd3/MVA-NSmut or ChAd3/MVA-hIiNSmut at peak after prime (green bars, gray $n = 3$; black $n = 4$), at peak after boost (yellow bars, gray $n = 4$; black $n = 5$) and EOS (light blue bars, gray $n = 4$; black $n = 5$). All tetramer staining and phenotyping performed ex vivo without culture.

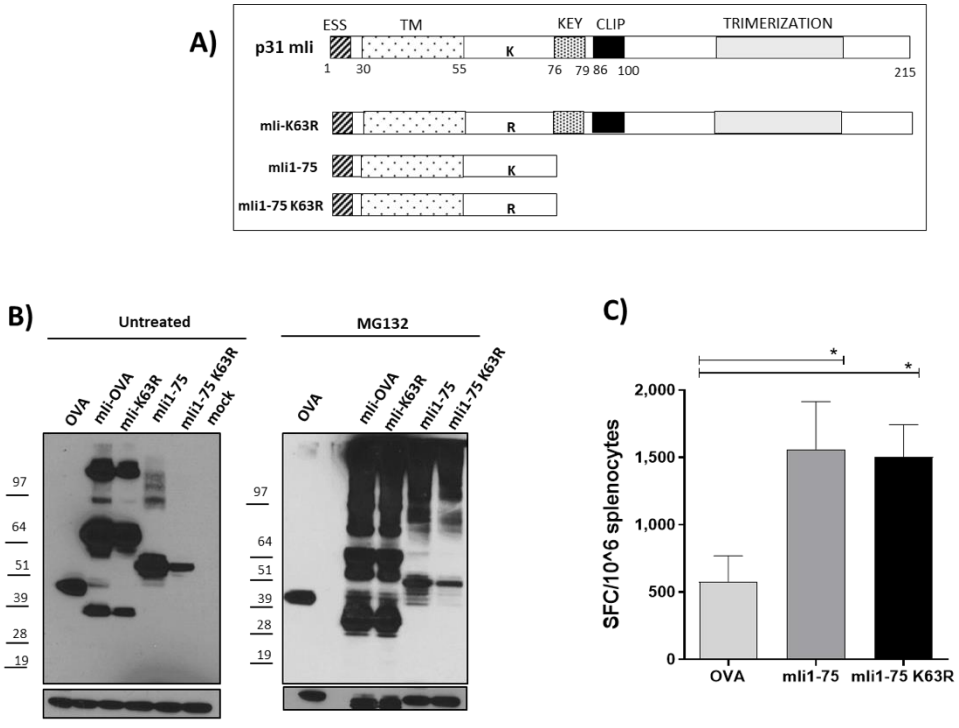

Fig. S12. Ii acts as a degron signal for antigen ubiquitination, degradation, and enhanced immunogenicity.

(**A**) Schematic representation of Ad5 vectors encoding for mIi-OVA fusions. Lysine (K) to arginine (R) mutation was introduced at position 63 by site-directed mutagenesis on mIi full-length mouse invariant chain and mIi1-75 s variant. (**B**) HeLa cells were infected at MOI 100 with Ad5 encoding OVA alone, mIi full-length or mIi1-75 OVA fusions as indicated. K63R constructs were compared with K63 control sequence. After infection, cells were left untreated or incubated with MG132. OVA protein expression was analyzed by Western blotting by anti-HA antibody. GAPDH expression was used as an internal control. The experiments were performed three times. (**C**) C57BL/6 mice were vaccinated with Ad5 viral vectors encoding OVA, mIi1-75 OVA or mIi1-75 OVA K63R, as indicated. Two weeks after a single intramuscular immunization, spleens were harvested to measure IFN-γ-producing T cells by ELISpot assay. Results are expressed as number of IFN-γ-producing T cells per millions of splenocytes Kruskal-Wallis test mIi1-75 OVA K63R versus OVA; mIi1-75 OVA versus OVA). The experiments were performed twice. $*P \leq 0.05$.

Table S1. Study design of ChAd3/MVA-NSmut vaccines with or without hIi.

Study design and dose of hIi and non-hIi vaccination regimens at prime time week (TW) 0 and at boost time week (TW) 8.

hIi vaccines

Study Name	Number of subjects	Priming vaccine (TW0)	Dose priming vaccine	Boosting vaccine (TW8)	Dose boosting vaccine	Follow up (weeks)
PEACHI 03 group 1 (low dose)	5	ChAd3-hIiNSmut	5 x10^9 vp	MVA-hIiNSmut	5 x10^7 pfu	34
PEACHI 03 group 2 (standard dose)	10	ChAd3-hIiNSmut	2.5 x10^{10} vp	MVA-NSmut	2 x10^8 pfu	34

Non hIi vaccines

Study Name	Number of subjects	Priming vaccine (TW0)	Dose priming vaccine	Boosting vaccine (TW8)	Dose boosting vaccine	Follow up (weeks)
PEACHI 04 group 1	8	ChAd3-NSmut	2.5 x10^{10} vp	MVA-NSmut	2 x10^8 pfu	34
HCV003 Arm A2	11	ChAd3-NSmut	2.5 x10^{10} vp	MVA-NSmut	2 x10^8 pfu	34

Table S2. Inclusion and exclusion criteria for the recruitment of healthy volunteers in ChAd3/MVA-hIiNSmut vaccine regimen.

Inclusion Criteria	• Aged at least 18 years on the day of screening and no greater than 65 years on the day of the first vaccination • Resident in or easy access to the trial site for the duration of the study • Available for follow-up for the planned duration of the study • Able and willing (in the CI's opinion) to comply with all study requirements • Willing to allow the investigators to discuss the volunteer's medical history with their General Practitioner • For heterosexual females, willingness to practice continuous effective contraception from screening until 4 months after the last immunisation • All female volunteers must be willing to undergo urine pregnancy tests at the time points specified in the Schedule of Procedures and must have a negative pregnancy test on the day(s) of vaccination • For sexually active men, willingness to use condoms from screening until 4 months after the last vaccination • Agreement to refrain from blood donation during the course of the study • In the opinion of the Chief Investigator or designee, the volunteer has understood the information provided. Written informed consent must be given before any study-related procedures are performed • Willing to undergo HCV and HIV testing, counselling and receive test results • Specific for Groups 1 and 2: • Healthy males or females, as assessed by medical history, physical examination and laboratory tests • Specific for Group 3: • A previous diagnosis of chronic HCV infection (any HCV genotype) successfully treated with all oral DAA therapy. • Minimum duration of six months between last dose of DAA treatment and planned vaccination date • SVR 12 following last DAA treatment course • Fibroscan score of <12.5kPa within 6 months of screening
Exclusion Criteria	• Participation in another research study involving an investigational product in the 30 days preceding enrolment, or planned used during the study period • Prior receipt of a recombinant simian adenoviral vaccine • Receipt of any investigational HCV vaccine within the last 6 years • Administration of immunoglobulins and/or any blood products within the last three months preceding the planned administration of the vaccine candidate • Receipt of live attenuated vaccine within the previous 60 days or planned receipt within 60 days after vaccination with the IMP • Receipt of other vaccine, including influenza vaccine, within the previous 14 days or planned receipt within 14 days after vaccination with the IMP • Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection; asplenia; recurrent, severe infections and chronic (more than 14 days) immunosuppressive medication within the last 6 months (inhaled and topical steroids are allowed)

	• History of allergic disease or reactions likely to be exacerbated by any component of the vaccine • Any history of anaphylaxis in reaction to vaccination • Pregnancy, lactation or willingness/intention to become pregnant during the study • Personal history of autoimmune disease • History of major autoimmune disease in first degree relative, e.g. Type 1 diabetes, Graves' Disease, Systemic Lupus Erythematosus (SLE) or Spondyloarthropathy (AS). • HLA type B27 positive individuals • History of cancer (except basal cell carcinoma of the skin and cervical carcinoma in situ) • History of serious psychiatric condition • Any other serious chronic illness requiring hospital specialist supervision • Suspected or known current alcohol abuse as defined by an alcohol intake of greater than 42 units every week • Suspected or known current injecting drug use • Seropositive for hepatitis B surface antigen (HBsAg) • Any clinically significant acute or chronic medical condition that is considered unstable/progressive, or in the opinion of the Chief Investigator, may either put the volunteer at risk because of participation in the study, or may influence the result of the study, or the volunteer's ability to participate in the study • Any clinically significant abnormal finding on screening biochemistry or haematology blood test or urinalysis • Any other finding which in the opinion of the investigators would significantly increase the risk of having an adverse outcome from participating in the protocol • Vulnerable subjects (according to ICH GCP) *Specific for groups 1 and 2:* o Previous HCV infection o Reported current or previous high-risk behaviour for HCV infection (including IVDU) o Seropositive for hepatitis C virus (antibodies to HCV) at screening *Specific for group 3:* o Cirrhosis or severe fibrosis (Ishak 5 or 6) as previously defined by any of the following: o Radiological findings on CT, MR or USS o Abnormal biochemical parameters (PT, albumin and bilirubin) o clinical signs of liver decompensation (ascites, varices, encephalopathy) o Ishak score ≥5 on liver biopsy o Fibroscan at any time point in the past >12.5kPa

Table S3. Unsolicited AEs (clinical).

Volunteer	Group	Time point	Grade	Parameter	Relationship
01106	1	Day 69	1	Cough, fever, muscle ache	Possible
01107	1	Day 23	2	Flu-like syndrome	Possible
01107	1	Day 141	2	Hay fever symptoms worse than previous year	Unlikely
01111	1	Day 84	1	Runny nose, sore throat	Unlikely
01112	1	Day 234	2	Urinary tract infection	Unlikely
01117	2	Day 0	1	Tingling sensation on left arm after vaccination lasting 20 seconds, self-resolved	Definite
01117	2	Day 21	1	Feverishness, tiredness, dry cough	Possible
01119	2	Day 45	1	Acne	Unlikely
01122	2	Day 7	2	Gastrointestinal discomfort	Possible
01122	2	Day 14	1	Back pain	Unlikely
01122	2	Day 26	1	Gastrointestinal discomfort	Possible
01122	2	Day 70	1	Back pain	Unlikely

Unsolicited clinical events occurring within 30 days of receipt of ChAd-hIiNSmut or MVA-hIiNSmut vaccines. All were considered possibly related to vaccination.

Table S4. Frequency of unsolicited laboratory AEs.

Volunteer	Group	Time point	Grade	Parameter	Relationship
01106	1	Day 57	1	Low lymphocytes	Definite
01107	1	Day 28	1	Low leukocytes	Definite
01107	1	Day 28	1	Low neutrophils	Definite
01111	1	Day 57	3	Low lymphocytes	Definite
01112	1	Day 28	1	Raised bilirubin	Possible
01112	1	Day 28	1	Low leukocytes	Definite
01112	1	Day 28	1	Low neutrophils	Definite
01113	2	Day 28	1	Hypokalaemia	Possible
01113	2	Day 28	1	Low neutrophils	Definite
01113	2	Day 84	2	Hypokalaemia	Possible
01113	2	Day 84	1	Low neutrophils	Definite
01115	2	Day 57	1	Low lymphocytes	Definite
01116	2	Day 28	1	Raised bilirubin	Possible
01116	2	Day 57	2	Low lymphocytes	Definite
01117	2	Day 84	1	Hypokalaemia	Possible
01119	2	Day 57	2	Low lymphocytes	Definite
01120	2	Day 1	1	Low lymphocytes	Definite
01120	2	Day 57	2	Low lymphocytes	Definite
01120	2	Day 84	1	Low haemoglobin	Definite
01122	2	Day 57	2	Low lymphocytes	Definite
01125	2	Day 1	1	Low haemoglobin	Possible
01125	2	Day 28	1	Low haemoglobin	Possible
01125	2	Day 56	1	Low haemoglobin	Possible
01125	2	Day 57	2	Low haemoglobin	Possible
01125	2	Day 84	2	Low haemoglobin	Possible
01125	2	Day 98	2	Low haemoglobin	Possible
01125	2	Day 57	2	Low lymphocytes	Definite
01126	2	Day 57	2	Low lymphocytes	Definite

Table S5. T cell response against hIi.

Values of IFN-ɣ ELISpot response against human Ii over time.

	baseline		d14 2w post prime		d28 4w post prime		d63 4w post boost	
	DMSO (3X)	***hIi***	***DMSO (3X)***	***hIi***	***DMSO (3X)***	***hIi***	***DMSO (3X)***	***hIi***
PEA03-01117	5 (15)	1	2 (6)	1	3 (9)	1	5 (15)	2
PEA03-01113	13 (39)	5	0	1	0	2	0	8
PEA03-01114	2 (6)	1	13 (39)	1	0	7	na	na
PEA03-01115	5 (15)	4	2 (6)	1	0	2	0	5
PEA03-01118	62 (186)	75	8 (24)	33	28 (84)	65	20 (60)	22
PEA03-01119	12 (36)	11	2 (6)	6	28 (84)	10	5 (15)	3
PEA03-01116	2 (6)	13	10 (30)	5	13 (39)	12	18 (54)	17
PEA03-01120	0	3	8 (24)	8	8 (24)	3	3 (9)	8
PEA03-01122	5 (15)	4	13 (39)	10	0	13	35 (105)	0
PEA03-01125	3 (9)	0	0	0	3 (9)	8	3 (9)	4
PEA03-01126	5 (15)	2	3 (9)	4	3 (9)	1	3 (9)	6

Table S6. List of MHC class I and MHC class II multimers.

(**A-B**) List of MHC class I pentamers and MHC class II multimers encoding immunodominant epitopes on NS region restricted for specific Human leukocyte antigen (HLA) type. Assigned class I pentamer and class II tetramer number (N), sequence and HLA specificity and evidences supporting tetramer functionality.

A

N	Protein	Position	Peptide Sequence	HLA restriction	Supporting Sequence
103a	NS3	1435-1443	ATDALMTGY	A*0101	Swadling et al, 2014 Proimmune
95a	NS3	1406-1415	KLSALGINAV	A*0201	Swadling et al, 2014 Proimmune

B

N	Protein	Position	Peptide Sequence	HLA restriction	Supporting Sequence
14	NS4b	1806-1818	TLLFNILGGWVAA	DRB1*0101	Schulze zur Wiest et al, 2012 Lucas et al, 2007 Gerlach et al, 2005
15	NS3	1248-1262	GYKVLVLNPSVAATL	DRB1*0401	Schulze zur Wiesch et al, 2012 Ulsenheimer et al, 2006 Gerlach et al, 2005 Schulze zur Wiesch et al, 2005 Day 2002
17	NS3	1582-1597	NFPYLVAYQATVCARA	DRB1*1501	Schulze zur Wiesch et al, 2012 Gerlach et al, 2005 Schulze zur Wiesch et al, 2005
18	NS3	1411-1425	GINAVAYYRGLDVSV	DRB1*1501	Schulze zur Wiesch et al, 2012 Gerlach et al, 2005 Schulze zur Wiesch et al, 2005
19	NS3	1535-1551	TTVRLRAYMNTPGLPVC	DRB1*1501	Gerlach et al, 2005 Schulze zur Wiesch et al, 2005
20	NS4b	1771-1790	SGIQYLAGLSTLPGNPAIASL	DRB1*0401	Schulze zur Wiesch et al, 2005
21	NS4b	1773-1790	QYLAGLSTLPGNPAIASL	DRB1*0404	Schulze zur Wiesch et al, 2012 Gerlach et al, 2005 Schulze zur Wiesch et al, 2005
22	NS2	794-810	WPLLLLLLALPQRAYAQ	DRB1*0701	Commercially available-NIH
24	NS3	1535-1551	TTVRLRAYMNTPGLPVC	DRB1*0701	Gerlach et al, 2005 Schulze zur Wiesch et al, 2005
25	NS5b	66-81	VYYLTRDPTTPLARAA	DRB1*0301	Proimmune
29	NS5a	1957-1975	SLTITSLLRRHNWITSCS	DRB1*0701	Commercially available-NIH

Table S7. HLA typing of volunteers vaccinated with ChAd3/MVA with and without Ii and assigned class I and class II multimers.

HLA typing for volunteers vaccinated with ChAd3/MVA-hIiNSmut (**A**) and with ChAd3/MVA-NSmut (**B**).

A

N	Protein	Allele	Allele	Class I pentamer	Class II tetramer
PEA03-01113	HLA-A	26:01	02:01	95a	14
	HLA-B	38:01	15:01		
	HLA-C	12:01	01:02		
	HLA-DRB1	01:03	13:01		
	HLA-DRB3	01:01			
	HLA-DRB4	-	-		
	HLA-DRB5	-	-		
	HLA-DQB1				
		05:01	06:02		
PEA03-01115	HLA-A	24:02	11:01	-	-
	HLA-B	18:01	51:01		
	HLA-C	07:02	12:03		
	HLA-DRB1	11:01	-		
	HLA-DRB3	02:01	-		
	HLA-DRB4	-	-		
	HLA-DRB5	-	-		
	HLA-DQB1	03:01	-		
PEA03-01116	HLA-A	03:01	02:01	95a	-
	HLA-B	14:02	07:02		
	HLA-C	08:02	07:02		
	HLA-DRB1	09:01	13:01		
	HLA-DRB3	01:07	-		
	HLA-DRB4	01:01	-		
	HLA-DRB5	-	-		
	HLA-DQB1	06:02	03:03		
PEA03-01117	HLA-A	29:01	02:01	95a	14
	HLA-B	14:02	44:03		
	HLA-C	16:01	08:01		
	HLA-DRB1	01:01	03:01		
	HLA-DRB3	02:01			
	HLA-DRB4	-	-		
	HLA-DRB5	-	-		
	HLA-DQB1	02:01	05:01		
PEA03-01118	HLA-A	29:01	03:01	-	24
	HLA-B	14:02	44:03		
	HLA-C	16:01	08:02		
	HLA-DRB1	07:01	13:02		
	HLA-DRB3	02:09	-		
	HLA-DRB4	01:01	-		
	HLA-DRB5	-	-		
	HLA-DQB1	02:01	06:03		
PEA03-01119	HLA-A	02:01	24:02	95a	17,18,19
	HLA-B	07:02	-		
	HLA-C	07:02	-		
	HLA-DRB1	08:03	15:01		
	HLA-DRB3	-	-		
	HLA-DRB4	-	-		
	HLADRB5	01:01	-		
	HLA-DQB1	03:01	06:02		
PEA03-01120	HLA-A	33:03	01:01	103a	-
	HLA-B	35:01	08:01		
	HLA-C	07:01	12:03		
	HLA-DRB1	14:01	03:01		
	HLA-DRB3	02:01	01:01		
	HLA-DRB4	-	-		
	HLA-DRB5	-	-		
	HLA-DQB1	02:01	05:01		
PEA03-01122	HLA-A	01:01	26:01	103a	-14
	HLA-B	49:01	08:01		
	HLA-C	07:01	-		
	HLA-DRB1	01:01	03:01		
	HLA-DRB3	01:01	-		
	HLA-DRB4	-	-		
	HLA-DRB5	-	-		
	HLA-DQB1	02:01	05:01		
PEA03-01125	HLA-A	01:01	24:02	103a	-
	HLA-B	44:02	08:01		
	HLA-C	05:01	07:01		
	HLA-DRB1	11:01	03:01		
	HLA-DRB3	02:01+	01:01		
	HLA-DRB4	-	-		
	HLA-DRB5	-	-		
	HLA-DQB1	02:01	03:01		
PEA03-01126	HLA-A	03:01	02:01	95a	15,20,21
	HLA-B	14:02	44:02		
	HLA-C	05:01	08:02		
	HLA-DRB1	04:01	13:02		
	HLA-DRB3	03:01	-		
	HLA-DRB4	01:01	-		
	HLA-DRB5	-	-		
	HLA-DQB1	06:03	03:01		

B

N	Protein	Allele	Allele	Class I pentamer	Class II tetramer
PEA04-004	HLA-A	02:01	01:01	103a /95a	-
	HLA-B	49:01	45:01		
	HLA-C	06:02	07:01		
	HLA-DRB1	11:02	-		
	HLA-DRB3	02:01	-		
	HLA-DRB4	-	-		
	HLA-DRB5	-	-		
	HLA-DQB1	03:01	-		
PEA04-005	HLA-A	11:01	01:01	103a	
	HLA-B	45:01	57:01		
	HLA-C	03:03	06:02		
	HLA-DRB1	07:01	-		
	HLA-DRB3	-	-		
	HLA-DRB4	01:03	01:01		
	HLA-DRB5	-	-		
	HLA-DQB1	03:03	02:01		
PEA04-006	HLA-A	01:01	-	103a	-
	HLA-B	08:01	-		
	HLA-C	07:01	-		
	HLA-DRB1	11:01	03:01		
	HLA-DRB3	02:01	01:01		
	HLA-DRB4	-	-		
	HLA-DRB5	-	-		
	HLA-DQB1	02:01	03:01		
PEA04-009	HLA-A	29:01	03:01	-	14
	HLA-B	35:03	14:02		
	HLA-C	08:02	04:01		
	HLA-DRB1	01:02	12:01		
	HLA-DRB3	02:01	-		
	HLA-DRB4	-	-		
	HLA-DRB5	-	-		
	HLA-DQB1	03:01	05:01		
PEA04-011	HLA-A	23:01	25:01	-	17,18,19
	HLA-B	52:01	18:01		
	HLA-C	12:03	12:02		
	HLA-DRB1	15:01	-		
	HLA-DRB3	02:01	-		
	HLA-DRB4	-	-		
	HLA-DRB5	01:01	-		
	HLA-DQB1	06:02	06:01		
PEA04-014	HLA-A	30:01	02:01	95a	17,18,19-
	HLA-B	42:01	57:01		
	HLA-C	07:01	17:01		
	HLA-DRB1	15:01	11:02		
	HLA-DRB3	02:01	-		
	HLA-DRB4	-	-		
	HLA-DRB5	01:01	-		
	HLA-DQB1	03:01	06:02		
PEA04-028	HLA-A	32:01	03:01	-	17,18,19
	HLA-B	14:01	15:01		
	HLA-C	08:02	03:04		
	HLA-DRB1	07:01	15:01		
	HLA-DRB3	-	-		
	HLA-DRB4	01:01	-		
	HLA-DRB5	01:01	-		
	HLA-DQB1	02:01	06:02		

Table S8. List of antibodies and markers used in FACS staining panel.

ICS staining				
Antibody	**Fluorochrome**	**Origin**	**Clone**	**Brand**
Live Dead	Aqua			Invitrogen
TNFα	FITC	mouse anti human		BD Bioscience
CD4	PE/Dazzle 594	anti human	RAP-T4	Biolegend
CD8	PerCP/Cy5.5	anti human	RPA-T8	Biolegend
CD154	Pe-Cy7	anti human	24-31	Biolegend
CD107a	BV421	mouse anti human	H4A3	BD Bioscience
CD3	APC/Fire 750	anti human	SK7	Biolegend
IFN-ɣ	APC	mouse anti human		BD Bioscience
IL-2	PE	anti human	MQ1-17H12	Biolegend
MHC Class I pentamer staining				
Antibody	**Fluorochrome**	**Origin**	**Clone**	**Brand**
Live dead	NIR			Invitrogen
CD3	PO	anti human		Invitrogen
CD127	APC	anti human		Mac Myltheny Biotec
Eomes	eFluor660	anti human	WD1928	Invitrogen
CD8	PB	mouse anti human	RPA-T8	BD Bioscience
Perforin	FITC	mouse anti human		BD Bioscience
T bet	BV605	anti human	4B10	Biolegend
CD45RA	FITC	mouse anti human	HI100	BD Bioscience
HLA-DR	Alexa fluor 700	anti human	G46-6	BD Bioscience
CD38	PerCP/Cy5.5	anti human	HIT-2	Biolegend
GzA	PerCP/Cy5.5	anti human	CB9	Biolegend
CCR7 (CD197)	Pe-Cy7	rat anti human		BD Bioscience
GzB	Alexa fluor 700	mouse anti human	GB11	BD Bioscience
CD279(PD1)	Pe-Cy7	mouse anti human	EH12.1	BD Bioscience
MHC Class II tetramer staining				
Antibody	**Fluorochrome**	**Origin**	**Clone**	**Brand**
CD4	Alexa fluor 700	mouse anti human	RPA-T4	BD Bioscience
CD3	BV570	anti human	UCHT1	Biolegend
CD127 (IL-7Rα)	PB	anti human	A019D5	Biolegend
CD28	PerCP/Cy5.5	anti human	CD28.2	eBioscience
CD45RA	APC	anti human	HI100	Biolegend
CD57	FITC	anti human	HNK-1	Biolegend
CD14	APC-Cy7	anti human	HCD14	Biolegend
CD19	APC-Cy7	anti human	HIB19	Biolegend
Cell proliferation assay				
Antibody	**Fluorochrome**	**Origin**	**Clone**	**Brand**
CD3	FITC	anti human	UCHT1	Biolegend
CD8	Pe-Cy7	mouse anti human	RPA-T8	BD Bioscience
CD4	Alexa fluor 700	mouse anti human	RPA-T4	BD Bioscience
Cell trace Violet	Pacific Blue			Invitrogen
Live dead	NIR			Invitrogen