

## SUPPLEMENTARY MATERIALS

### Materials and Methods

Fig. S1. Flow chart of study participants.

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

Fig. S3. Magnitude of ChAd3/MVA-hIiNSmut low dose (group 1).

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

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

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

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

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

Fig. S9. Gating strategy for class I pentamer<sup>+</sup> CD8<sup>+</sup> T cells expressing markers.

Fig. S10. Class I phenotypic analysis of pentamer<sup>+</sup> CD8<sup>+</sup> T cells after ChAd3/MVA-NSmut vaccine regimen with or without Ii in healthy volunteers.

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

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

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

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

Table S3. Unsolicited and adverse events (clinical).

Table S4. Frequency of unsolicited laboratory adverse events.

Table S5. T cell response against Ii.

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

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

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

Data File S1. Primary data

Reference (2,8,12,15,31,32,43,45-48)