Supplementary Figure 1. Grafting of primary human T-cells with CARs. Lymphocytes T obtained from three healthy donors were retrovirally-grafted with two CARs containing scFvs derived from an anti-HCV/E2 human mAb (named e137) (e137-CAR VH+VL and e137-CAR VL+VH), or with a previously described CAR (S-CAR) used as control and recognizing the HBsAg. Flow cytometric analysis using a FITC-conjugated anti-CD4, an APC-conjugated anti-CD8 and a PE-conjugated anti-human IgG-Fc antibody, detecting the extracellular IgG1 CH2-CH3 spacer domain of the receptors, were performed to identify CARs-grafted T cells. Representative data derived from redirected T cells of a single donor are reported as dot blots. Percentages of double positive cells are given.
Supplementary Figure 2. Intracellular staining for IFN-γ, IL-2 and TNF-α of engineered T-cells redirected with e137-CARs and cultured in presence of coated antigens. Intracellular staining for human TNF-α, IFN-γ and IL-2 was performed on sorted CD4+ and CD8+ untransduced T-cells and e137-CARs-or S-CAR-transduced T cells cultured in triplicate in presence of coated HCV/sE2 or BSA (as depicted on the top of the different panels). Flow cytometric analysis using a PE-Cy7-conjugated anti-CD4, an APC-Cy7-conjugated anti-CD8 and a PE-conjugated anti-human IgG-Fc antibody were performed to identify CARs-grafted T cells. For cytokine staining FITC-conjugated anti-IFN-γ, PB-conjugated anti-TNF-α and APC-conjugated anti-IL-2 were used. EMA staining was performed in order to identify the percentage of living cells. For brevity reasons, data obtained from T cells of a triplicate of a single healthy donor and with e137-CAR VH+VL are shown. Similar results were obtained using T cells derived from other triplicates of other donors and with the e137-CAR VL+VH. Results are represented as dot plots, and percentages of positive cells are given.
**Supplementary Figure 3. Evaluation of T cell degranulation.** Staining for CD107a (LAMP-1) of untransduced, or e137-CARs (VH+VL and VL+VH)-transduced, or control S-CAR-transduced T cells after incubation with plate-coated HCV/sE2 or a control antigen (BSA) is reported.

**Supplementary Figure 4. IFN-γ, IL-2, TNF-α and granzyme B levels of CAR-engineered T-cells cultured in presence of soluble antigens.** Human IFN-γ, IL-2, TNF-α and granzyme B levels (y axis) of T cells from three different healthy donors, untransduced and transduced with e137-CARs (VH+VL and VL+VH) or S-CAR are reported. Redirected and untransduced T cells were cultured in triplicate in presence of HCVcc (JFH-1 strain, 100 FFU) viral particles, of free HCV/sE2 (H77 strain, 50 μg/mL), of medium only, of coated BSA (500 ng/well) or HCV/sE2 (500 ng/well). The mean plus standard error from the mean (error bars) are reported.
Supplementary Figure 5. HCV/E2 cell surface expression of stably transfected and infected cells. (A) Staining for HCV/E2 glycoprotein on: (A) Untransfected, HCV/E1-E2- or HCV/sE2-stably transfected HEK-293 cells; (B) Human CD81-transduced, HCV/E1-E2 or HCV/sE2-stably transfected HepG2 cells; (C) Uninfected or HCVcc-infected HuH-7.5 cells. The positive staining of HCV/sE2-secreting cells is due to HCV/E2 transient passage in the membrane before its secretion, not sufficient to significantly elicit CARs-redirected T-cells, differently than membrane-bound HCV/E1-E2.
Supplementary Figure 6. Levels of IFN-γ, IL-2, TNF-α and granzyme B of total or sorted CD4+/CD8+ T cells redirected with e137-CARs and co-cultured against untransfected and HCV/E1-E2 or HCV/sE2 stably transfected HEK-293 cells. As depicted in the graphs, CAR-transduced T cells were used at four different E:T ratios, whereas untransduced T cells were used at a single 1:4 E:T ratio. For HCV/sE2 stably transfected HEK-293 cells, levels of granzyme B and pro-inflammatory cytokines were evaluated only for total CAR-transduced and untransduced T cells. Levels of IFN-γ of total CAR-transduced and untransduced T cells are the same reported in Figure 4D-F.
Supplementary Figure 7. Cytotoxic activity of sorted CD4+ and CD8+ T-cells redirected with e137-CARs against HCV/E1-E2-stably transfected HepG2 target cells. Light microscopy (20x magnification) of HepG2 target cells transduced with human CD81 (upper panels) or stably transfected with HCV/E1-E2 glycoprotein of genotype 1a (H77 strain) (lower panels). Target cells were co-cultured for 48 hours with sorted CD4+ or CD8+ T cells (E:T 1:8) redirected with e137-CARs (VH+VL and VL+VH) or control S-CAR. Untransduced CD4+ and CD8+ T cells from the same donor were also used as control. Similar results were obtained with T cells derived from the other two donors.
Supplementary Figure 8. Levels of IFN-γ, IL-2, TNF-α and granzyme B of total or sorted CD4+/CD8+ CAR-engineered T cells co-cultured against untransfected and HCV/E1-E2- or HCV/sE2-stably transfected HepG2 cells. As depicted in the graphs, CAR-transduced T cells were used at four different E:T ratios, whereas untransduced T cells were used at a single 1:4 E:T ratio. For HCV/sE2 stably transfected HepG2 cells, levels of granzyme B and pro-inflammatory cytokines were evaluated only for total CAR-transduced and untransduced T cells. Levels of IFN-γ of total CAR-transduced and untransduced T cells are the same reported in Figure 5D-F.
Supplementary Figure 9. Levels of IFN-γ, IL-2, TNF-α and granzyme B of total or sorted CD4+/CD8+ T cells redirected with e137-CARs and co-cultured against infected and uninfected HuH-7.5. As depicted in graphs, CAR-transduced T cells were used at four different E:T ratios, whereas untransduced T cells were used at a single 1:4 E:T ratio. Levels of IFN-γ of total CAR-transduced and untransduced T cells are the same reported in Figure 6C and D.