Supplementary Data; Supplementary Methods

Glycolipid-peptide conjugate vaccines enhance $CD8^+$ T cell responses against human viral proteins

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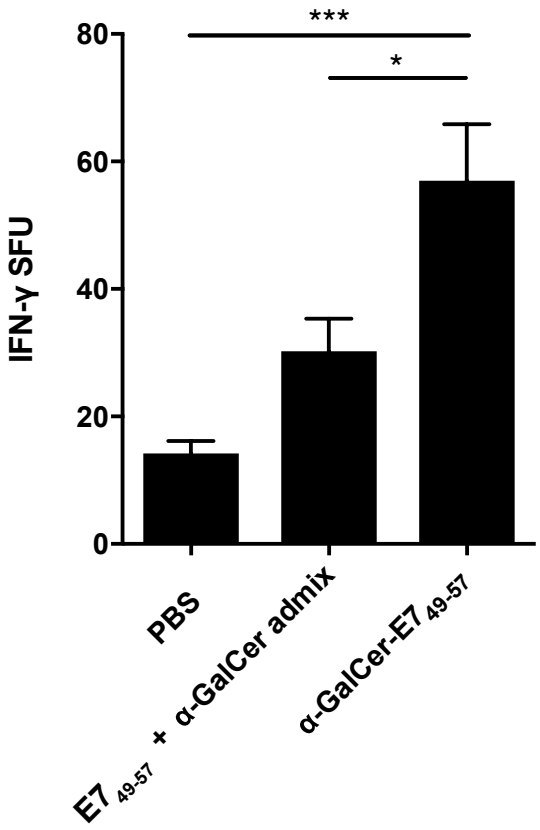
Supplementary Figure S1: C57BL/6 mice (n = 5 per group) were vaccinated intravenously with α-GalCer + $E7_{49-57}$ peptide admix, α-GalCer-$E7_{49-57}$ or PBS as a naïve control. Splenocytes from vaccinated mice were re-stimulated ex vivo with $E7_{49-57}$ peptide and production of IFN-γ was assessed by ELISpot; *** p<0.001, * p<0.05; SFU, spot-forming units.



Supplementary table S1: Expression of the 30 immune-related genes most upregulated upon culture of human PBMCs with α-GalCer-pp65$_{495-503}$, compared to pp65$_{495-503}$ peptide alone. Mean of four human donors.

Gene	Mean expression (relative to peptide alone)
CXCL10	16.88
CCL13	5.86
SPINK5	5.44
CXCL11	5.04
C8B	4.66
CCL1	3.31
CXCL9	3.20
CCL8	2.96
TNFRSF8	2.85
IFNG	2.71
COLEC12	2.66
TFEB	2.49
IDO1	2.29
IL3RA	2.24
IFIT2	2.24
TREM1	2.21
DUSP4	2.18
C9	2.14
FEZ1	2.09
MRC1	2.06
IL2RA	2.06
CTLA4	2.05
LCN2	1.96
RORC	1.95
IL1R1	1.93
TNFRSF9	1.90
GZMB	1.89
CCL24	1.82
MEFV	1.79
SERPINB2	1.78

Supplementary Methods

Ex vivo peptide-specific IFN-γ production

Conjugate vaccines or admixed controls were diluted in sterile phosphate buffered saline (PBS) solution for injection. C57BL/6 mice were injected intravenously with either 8.5 μg of α-GalCer-$E7_{49-57}$ conjugate vaccine, admixed 2.3 μg α-GalCer and 3 μg HPV16 $E7_{49-57}$ peptide (each corresponding to 2.68 nmol), or PBS alone as a naïve control. After 11 days, spleens were harvested for quantitation of *ex vivo* IFN-γ production using a mouse IFN-γ ELISpot^{PLUS} kit (Mabtech), according to manufacturer's instructions. Briefly, Millipore MultiScreen-HA 96-well filter plates (Millipore) were coated with 15 μg/mL IFN-γ mAb in PBS overnight at 4 °C. Splenocytes were seeded at 4 x 10^5 cells/well and incubated overnight with 0.1μM $E7_{49-57}$ peptide at 37°C. Following washing, plates were incubated with 1 μg/ml biotin-labeled anti-IFN-γ for 2 h at room temperature. Plates were then washed and treated with 1:1000 dilution of streptavidin-alkaline phosphatase for 1 h at room temperature. Plates were developed with nitro-blue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate substrate until all spots were clearly visible. Developed plates were dried and counted on an automated ELISpot reader (Autoimmun Diagnostika, Strassberg, Germany).