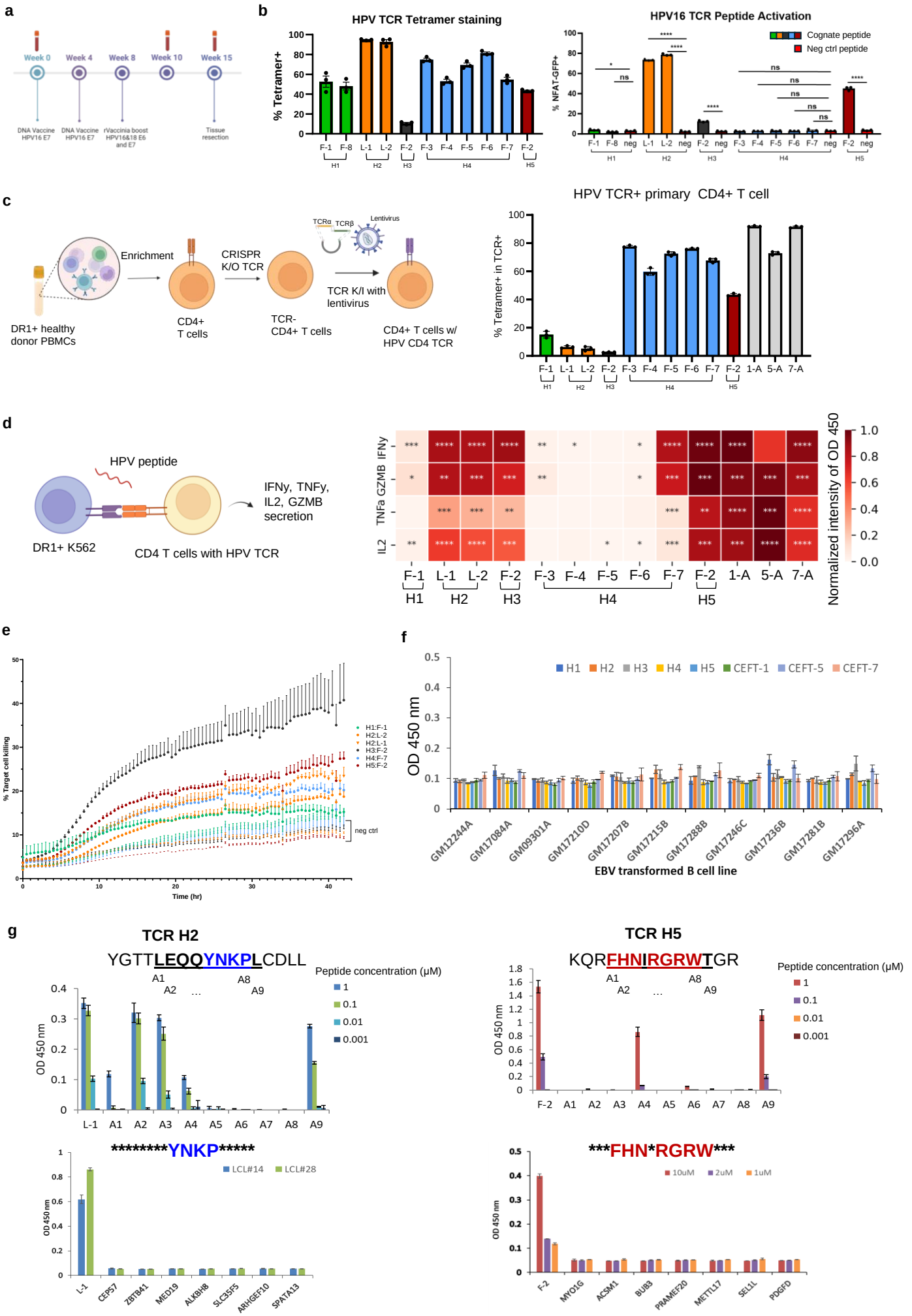
DR1-restricted HPV CD4 TCR preclinical validations



Discovery and characterization of HPV16+ specific CD4+ T cells.

a. HPV vaccine clinical trial scheme. Patients received vaccines covering E6 and E7 proteins at Week 0, 4, and 8, and PBMC samples were collected before vaccination, at Week 10, and at Week 15 after completing the entire vaccine series.

b. Validation of HPV CD4 TCRs H1-H5 against antigens 1-8 through tetramer binding and peptide-pulsed activation assays *(n=3).* ****P < 0.0001, *P ≤ 0.05, ns P > 0.05 labeled for each group relative to the negative control peptide, determined by one-tailed independent t-test assuming equal variances.

c. Engineering of HPV TCR+ primary CD4 T cells. Haplotype matched PBMC samples were enriched for CD4+ T cells and the endogenous TCRs were knocked out using CRISPR gene editing. HPV TCRs were then transduced through lentivirus. TCR expression and tetramer binding were confirmed *(n=3)*. 1-A, 5-A, and 7-A are influenza-specific CD4 TCRs used as internal positive control.

d. Functional performance of the TCRs evaluated through antigen-induced production of IFNγ, TNFα, IL2, and GZMB in an ELISA assay (*n=3*). OD 450 intensity was normalized across each cytokine. ****P < 0.0001, ***P < 0.001, **P < 0.01, *P ≤ 0.05, ns P > 0.05 labeled for each group relative to the negative control peptide, determined by one-tailed independent t-test assuming equal variances.

e. Cytotoxicity of TCR-engineered T cells toward DR1-K562 cells pulsed with cognate HPV-16 E6 antigens and tracked over 44 hours *(n=2*). Mis-matched peptides were pulsed with each TCR as the negative control.

f. Alloreactivity of HPV CD4 TCRs validated through peptide pulsed EBV-transformed cell lines (*n=2*).

g. Cross-reactivity screening. Key TCR recognition motifs were identified through alanine scanning of the 9–mer core peptides of HPV E6 antigens L-1 and F-2 at 1, 0.1, 0.01, and 0.001 uM. Human self-peptides with similar amino acid motif was identified through BLAST search. TCRs were evaluated for cross-reactivity to the self-peptides via peptide-pulsed activation and IFNγ secretion. TCR H2 was assessed for reactivity against seven self antigens at 1 uM by two EBV-derived cell lines (LCL#14 and LCL#28). TCR H5 was tested for reactivity against self-antigens at 10, 2, and 1 μM by LCL#28.