Science Advances
AAAS

Supplementary Materials for

CD8$^+$ T cell targeting of tumor antigens presented by HLA-E

Ravi F. Iyer *et al.*

Corresponding author: Klaus Früh, fruehk@ohsu.edu; Louis J. Picker, pickerl@ohsu.edu

Sci. Adv. **10**, eadm7515 (2024)
DOI: 10.1126/sciadv.adm7515

The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

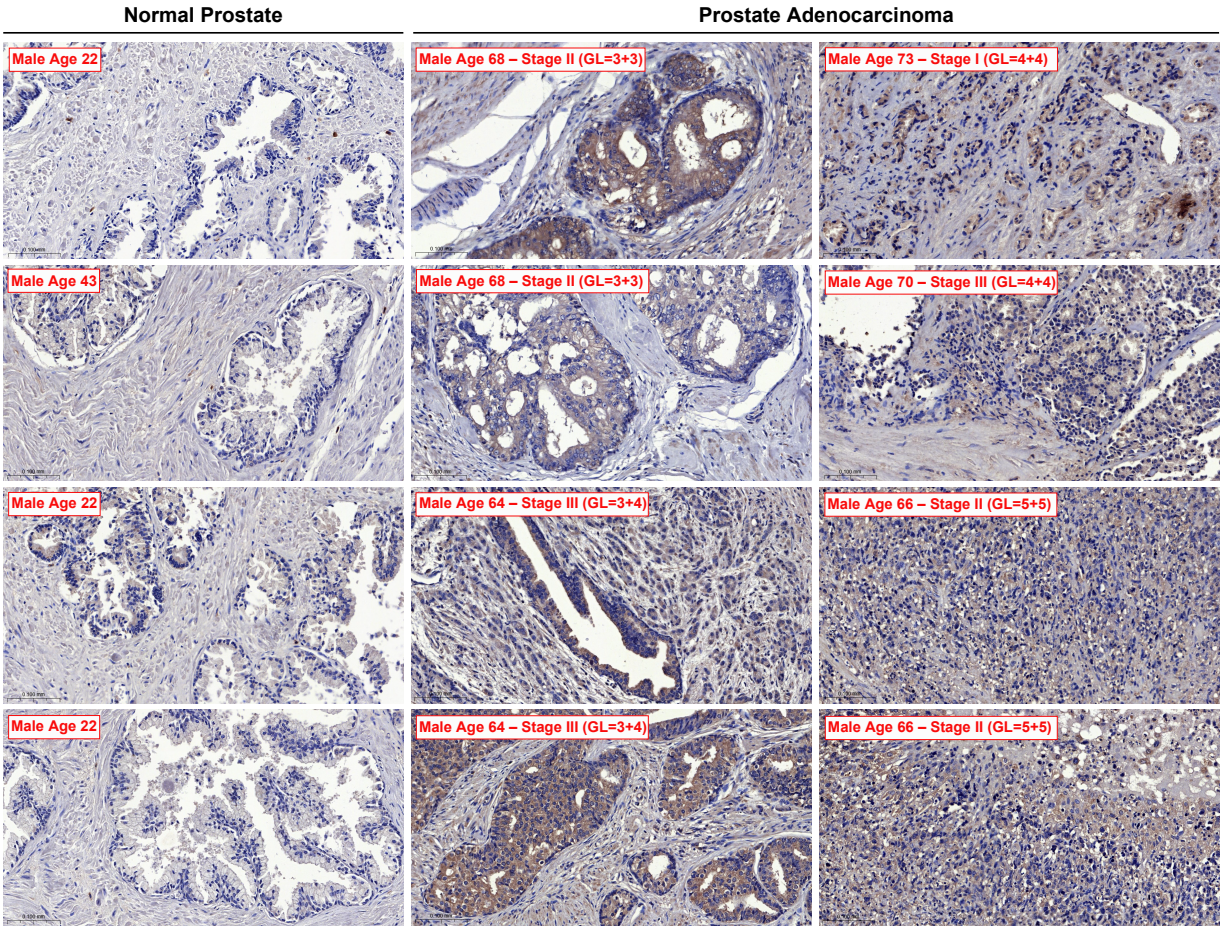


Figure S1: HLA-E expression in prostate cancer
Immuno-histochemical (IHC) analysis of prostate cancer or normal prostate tissue for expression of HLA-E. Commercial TMAs were obtained from Biomax and processed as described in the Materials and Methods section. Tissue cores were incubated with monoclonal anti-HLA-E antibody 4D12 followed by incubation with HRP-coupled secondary antibody and detection using Ventana-DAB system. Representative IHC of tumor tissue and normal prostate tissue are shown. A summary of the results in shown in data file S2.

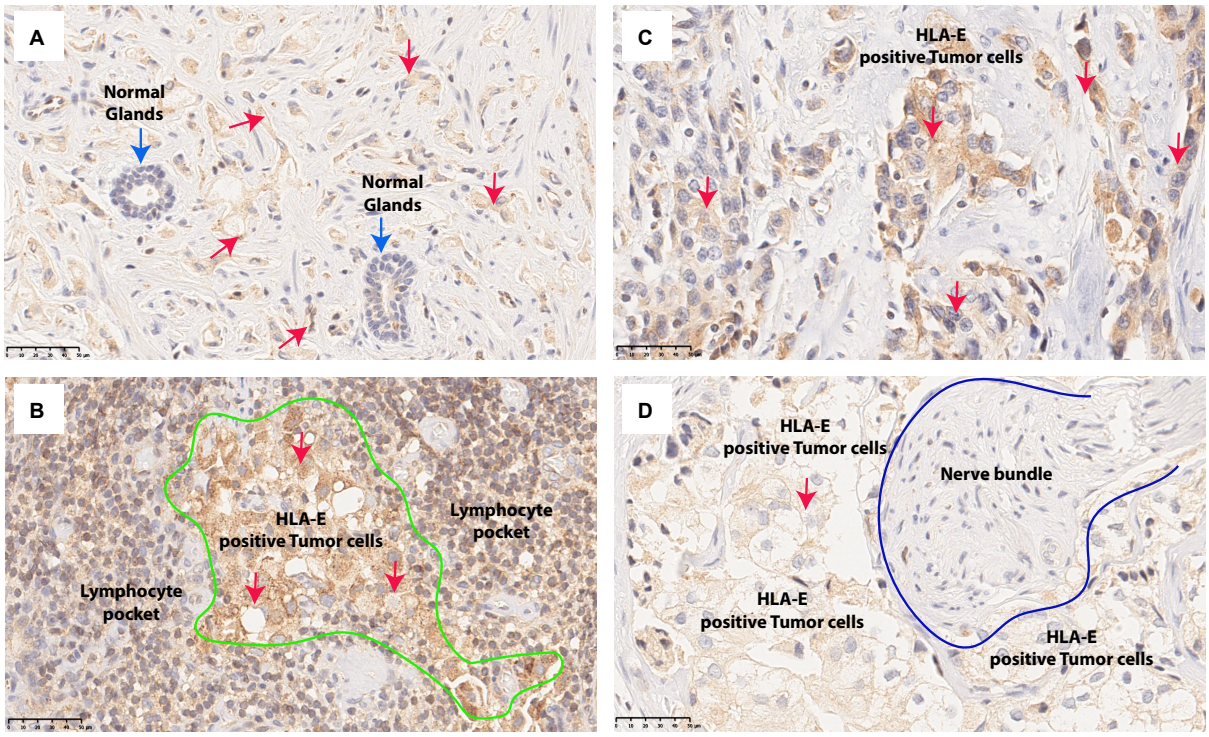


Figure S2: HLA-E expression in metastatic prostate cancer
IHC analysis of metastatic prostate cancer for expression of HLA-E. Shown are representative IHCs of tumor sections from radical prostatectomy specimens (Gleason 9) with either lymph node or perineural invasion. A) pT3bN1 Gleason 9: Prostate tissue with malignant cells. Positive tumor staining for HLA-E is indicated by red arrows. Normal glands (blue arrows) are negative for HLA-E. B) T3bN1 Gleason 9: Lymphnode tissue shows malignant cells (within green border) stained positive for HLA-E within the lymphocyte pocket. C) pT3b Gleason 9: HLA-E positive tumor cells are indicated by red arrow. D) pT3b Gleason 9: HLA-E positive tumor cells around nerve bundle (blue border) in cancer with perineural invasion.

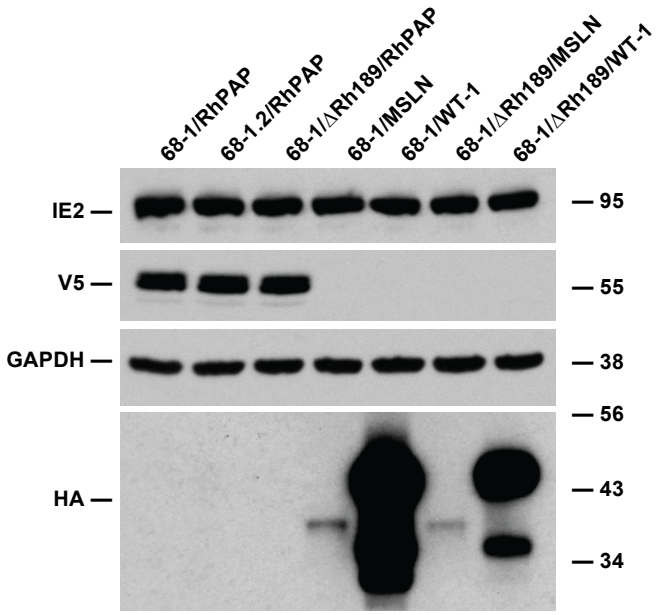
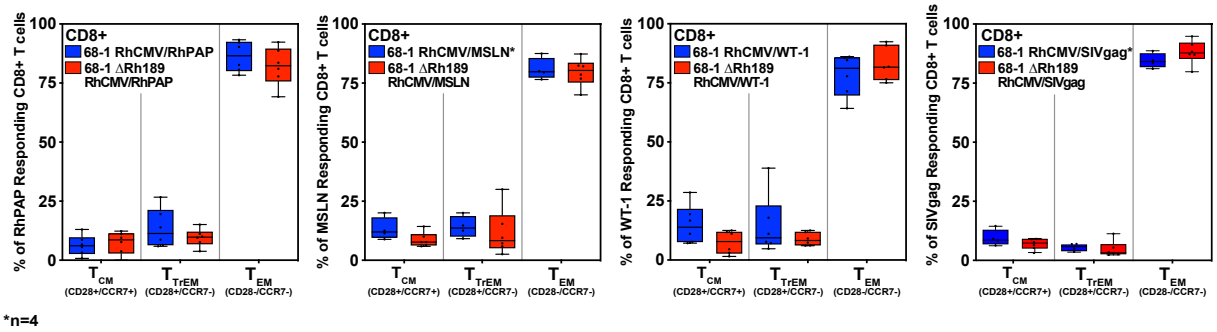


Figure S3: Immunoblots of tumor-associated antigen expression by RhCMV vectors.
Rhesus fibroblasts were infected with the indicated viral vectors at a multiplicity of infection (MOI) of 5. Cells were harvested at 24 hours post-infection. Cell lysates were electrophoretically separated by SDS-PAGE prior to immunoblotting for the RhCMV immediate early-2 (IE2) protein, the cellular protein GAPDH, RhPAP carrying a V5-epitope tag, WT-1 and MSLN which carry an HA-tag.



*n=4

Figure S4: Phenotype of TAA-specific CD8$^+$ T cell responses elicited by RhCMV vectors
Boxplots compare the memory differentiation phenotype of TAA-specific CD8$^+$ T cells in PBMC of RM co-inoculated with 68-1 RhCMV (RM 01-06) or 68-1 RhCMVΔRh189 (RM10-15) expressing RhPAP (n=6), MSLN (n=4 for 68-1, n=6 for 68-1ΔRh189), WT-1 (n=6) or SIV Gag (n=4 for 68-1, n=6 for 68-1ΔRh189). T cell responses to 15mer peptide mixes of the indicated TAA were determined by ICS for TNF-α and/or IFN-γ at >20 weeks post initial immunization. Memory differentiation state, shown as percent of each subset within the total response, was based on CD28 and CCR7 expression, delineating central memory (TCM, CD28+, CCR7+), transitional effector-memory (TrEM, CD28+, CCR7-), and effector-memory (TEM, CD28-, CCR7-) T cells.

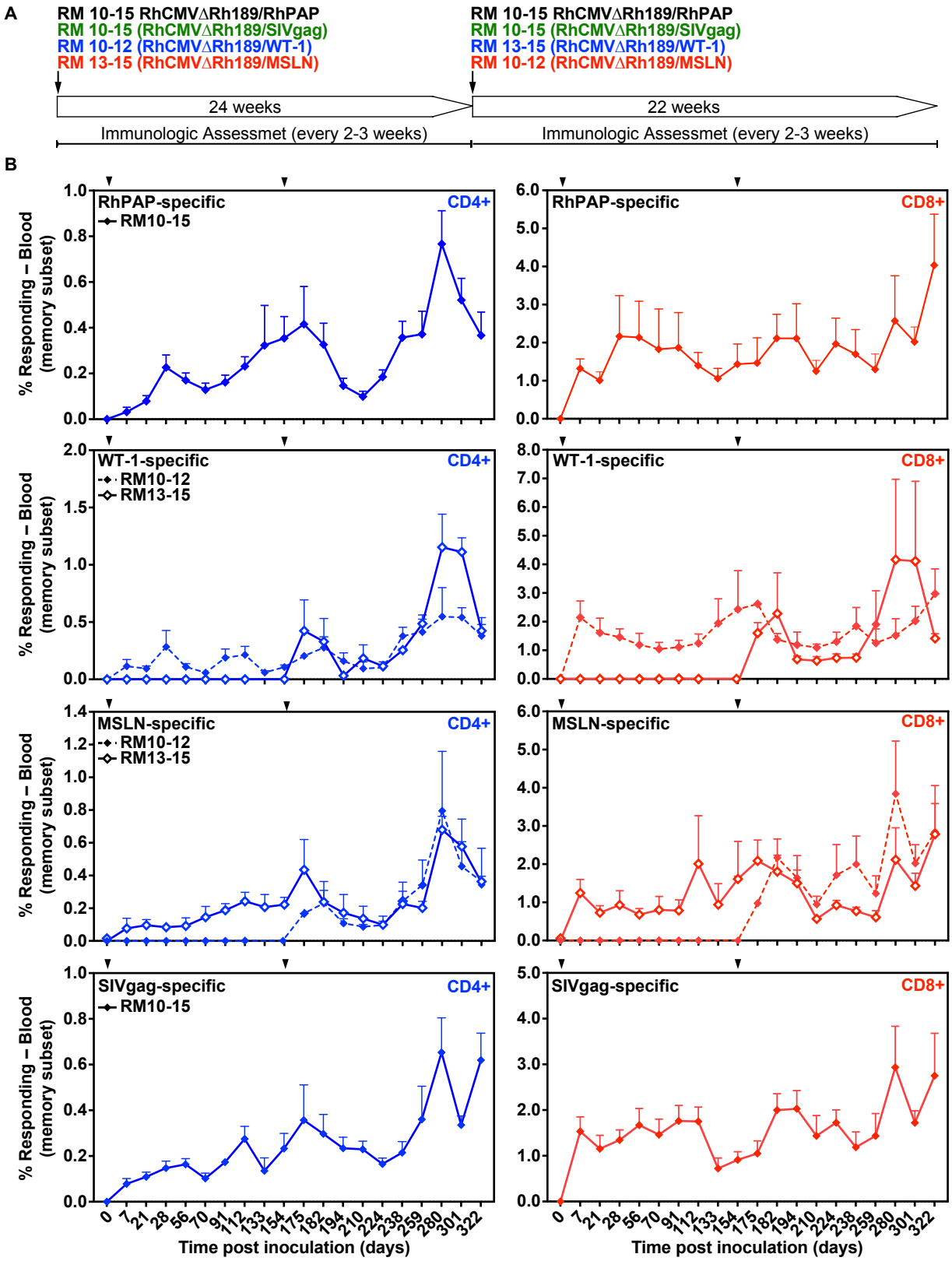

A
RM 10-15 RhCMVΔRh189/RhPAP
RM 10-15 (RhCMVΔRh189/SIVgag)
RM 10-12 (RhCMVΔRh189/WT-1)
RM 13-15 (RhCMVΔRh189/MSLN)
24 weeks
Immunologic Assessmet (every 2-3 weeks)
RM 10-15 RhCMVΔRh189/RhPAP
RM 10-15 (RhCMVΔRh189/SIVgag)
RM 13-15 (RhCMVΔRh189/WT-1)
RM 10-12 (RhCMVΔRh189/MSLN)
22 weeks
Immunologic Assessmet (every 2-3 weeks)
B
RhPAP-specific
RM10-15
CD4+
CD8+
WT-1-specific
RM10-12
RM13-15
MSLN-specific
SIVgag-specific
% Responding – Blood (memory subset)
Time post inoculation (days)
0 7 21 28 56 70 91 112 133 154 175 182 194 210 224 238 259 280 301 322

Figure S5: Longitudinal T cell analysis of RM co-inoculated inoculated with 68-1 RhCMVΔRh189 expressing RhPAP, MSLN, WT-1 or SIVgag.
A) Six male RMs were inoculated with 68-1 RhCMVΔRh189/RhPAP and with 68-1 RhCMVΔRh189/SIVgag. RMs 10-12 were additionally co-inoculated with RhCMVΔRh189/WT-1 whereas RMs 13-15 were co-inoculated with 68-1 RhCMVΔRh189/MSLN. At day 168, RMs 10-12 received 68-1 RhCMVΔRh189/MSLN whereas RMs 13-15 received 68-1 RhCMVΔRh189/WT-1. B) $CD4^+$ and $CD8^+$ T cell responses were measured in PBMC using overlapping peptide pools for each of the antigens by ICS at each of the indicated time points. The average response frequencies are shown. Individual response frequencies are shown in data file S3. Individual epitope responses and MHC-restrictions are shown for RM10-14 in Figure 4 and were measured at > 40 weeks after the initial inoculation.

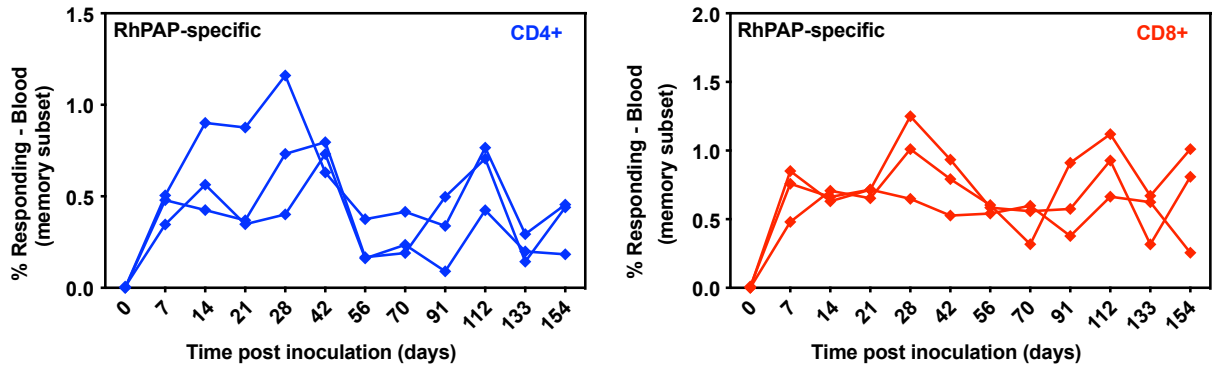


Figure S6: Longitudinal T cell analysis of 68-1 RhCMV/RhPAP-inoculated RMs.
Three male RMs (RM16-18) were inoculated with 68-1 RhCMV/RhPAP. $CD4^+$ and $CD8^+$ T cell responses were measured in PBMC using overlapping peptide pools for RhPAP by ICS at each of the indicated time points. The background-subtracted response frequencies within the $CD4^+$ and $CD8^+$ T cell memory populations are shown for each individual RM. Results for each RM are shown in data file S3.

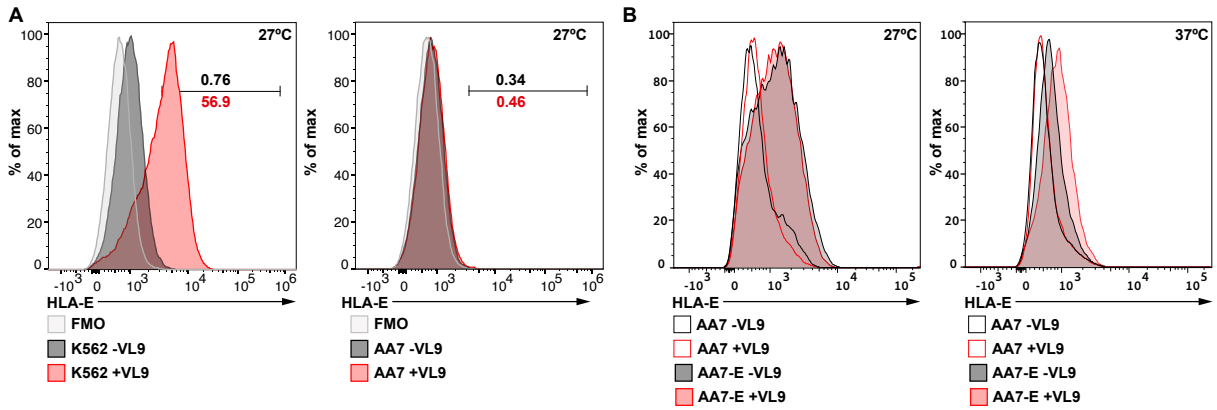


Figure S7: HLA-E surface expression in K562 cells, AA7 cells (= K562 deleted for HLA-E) and AA7-E cells (= AA7 cells transfected with HLA-E).

A) Flow cytometry of K562 cells (left) or AA7 cells (right) incubated overnight at 27°C in the presence or absence of VL9 peptide and stained with anti-HLA-E antibody 3D12. Fluorescence minus one (FMO) controls consisted of secondary antibody only. B) AA7 or AA7-E cells incubated overnight at 27°C (left) or 37°C (right). Where indicated, VL9 peptide was added for 2 hours at 37°C prior to staining with anti-HLA-E antibody 3D12.

Supplemental Data Files:

Data File S1 TMA scoring

Data File S2 HLA-E expression in prostate cancer samples

Data File S3 Individual ICS assay results for Figures 2-8 and S4-S6