Supplemental information

HLA-A*02-gated safety switch for cancer therapy
has exquisite specificity for its allelic target antigen

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Figure S1. PA2.1 LBD HLA class I binding data.
(A) scFv-Fc proteins generated from muPA2.1 IgG consisted of the PA2.1 CDRs grafted onto either a murine or humanized framework and linked to a hinge domain followed by a monomeric huFc. The muPA2.1 scFv-Fc was designed with a VL-VH orientation and a CD8-SS hinge while the huPA2.1 scFv-Fc utilized a VH-VL orientation and a LIR-1 hinge. (B) Table describing the specific HLA class I alleles arrayed in the FlowPRA™ single antigen bead kit. (C) Beads with differing relative PE fluorescence are isolated on the y-axis while binding events detected by a species-specific anti-huFc secondary antibody labelled with APC are observed on the x-axis. (D) Bar graph of the mean fluorescence intensity (MFI) of the specific HLA bead complex. Specific binding was observed on A*02 and A*69 alleles for the PA2.1 muIgG, mu- and hu-PA2.1 scFv-Fcs. The irrelevant scFv-Fc showed no detectable binding to any of the HLA alleles.
Figure S2. Paratope analysis of huPA2.1 Fab.
(A) View of the PA2.1 Fab surface at the pMHC binding interface. A surface representation of the PA2.1 Fab with the pMHC-interacting residues highlighted in red relative to the strength of the Lennard Jones potential. VH is shown in dark gray and VL in light gray. (B) The surface is removed to reveal the orientation of the CDRs. pMHC-interacting residues are highlighted as red sticks. VH and VL are shown in dark and light blue, respectively.
Figure S3. Raw vs. normalized data for Figures 5A and 5B.
(A) Unprocessed RLU data shown for huPA2.1 derived blocking receptor activity in Jurkat-NFAT luciferase cells before and (B) after data normalization. (C) Unprocessed RLU data shown for huPA2.1 derived activator function in Jurkat-NFAT luciferase cells before and (D) after data normalization.
Table S1: Crystallographic data collection and validation statistics for PA2.1 Fab-HLA-A*02:01-NY-ESO-1(V) pMHC structure.

| PDB ID | PA2.1 Fab – HLA-A*02:01 – NY-ESO-1(V) |
|--------|----------------------------------------|
|        | 8EB2                                   |

**Data collection**<sup>a,b</sup>

|                           |                                            |
|---------------------------|-------------------------------------------|
| Space group               | P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>     |
| Unit cell (Å)             | 116.8 148.8 199.6                         |
| α, β, γ (°)               | 90.0, 90.0, 90.0                          |
| Wavelength (Å)            | 0.980                                     |
| Resolution (Å)            | 49.6 -2.90 (3.01-2.90)                    |
| Unique Reflections        | 77195 (7620)                              |
| Completeness (%)          | 99.1 (95.2)                               |
| Redundancy                | 6.7 (6.9)                                 |
| CC<sub>1/2</sub> (%)      | 99.0 (38.1)                               |
| &lt;i/|f|&gt;                   | 7.19 (0.90)                               |
| R<sub>merge</sub> (%)     | 37.1 (366.5)                              |
| R<sub>pim</sub> (%)       | 15.4 (148.9)                              |
| Wilson B-factor (Å<sup>2</sup>) | 76.9                                      |

**Refinement and Validation**

|                           |                                            |
|---------------------------|-------------------------------------------|
| Resolution (Å)            | 2.90                                      |
| Number of atoms           | 19374                                     |
| Protein                   | 19374                                     |
| Ligand                    | 0                                         |
| Waters                    | 0                                         |
| R<sub>work</sub>/R<sub>free</sub> (%) | 24.0/28.5                                |
| R.m.s. deviations         |                                            |
| Bond lengths (Å)          | 0.009                                     |
| Bond angles (°)           | 1.19                                      |
| MolProbity score          | 1.37                                      |
| Clashscore (all atom)     | 8.54                                      |
| Poor rotamers (%)         | 5.70                                      |
| Ramachandran plot         |                                            |
| Favored (%)               | 94.7                                      |
| Allowed (%)               | 4.73                                      |
| Disallowed (%)            | 0.62                                      |
| Average B-factor (Å<sup>2</sup>) | 91.2                                    |

<sup>a</sup> Data collected on 12-2 beamline at the Stanford Synchrotron Radiation Lightsource (SSRL).

<sup>b</sup> Numbers in parentheses correspond to the highest resolution shell.
Table S2. Binding energy calculations for frequent HLA epitopes.
The initial PA2.1/pMHC coordinates were relaxed and used as a template for modeling pMHC
epitope variations of interest. Changes in Rosetta Energy Units (REU) were tracked and compared
to the predicted energy of the relaxed unmodified structure.

| HLA Residues in PA2.1/pMHC Interface | Clade | ΔREU (in complex with PA2.1) |
|--------------------------------------|-------|-----------------------------|
| DWRFL.EG.VE.R.E.K.Q                  | 1 (A*02:01) | 1.15                        |
| DgRLl.EG.VE.R.E.K.Q                  | 2     | 11.99                       |
| DgRFL.EG.Vd.R.E.K.Q                  | 3     | 6.06                        |
| DgRFL.dG.VE.R.E.K.Q                  | 4     | 11.31                       |
| DgRFL.EG.VE.R.E.K.Q                  | 5     | 5.26                        |
| DgRLl.EG.VE.R.k.K.Q                  | 6     | 17.02                       |
| DgRLl.EG.VE.R.E.K.e                  | 7     | 16.28                       |
| DWRFL.EG.VE.R.E.K.Q                  | (Rare)| 7.74                        |
| DWRFL.EG.Vd.R.E.K.Q                  | (Rare)| 2.59                        |