Supplementary Tables

Supplementary Table S1. Amino acid sequence of Clone45.

CDRs	1	2	3
VH	SYAMS	QIDPWGQETLYADSVKG	LTGRFDY
VL	RASQSISSYLN	SASQLQS	QQGPGTPNT
scFv	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSQIDPW GQETLYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKLTGRFDYWGQ GTLVTVSSGGGGSGGGGSGGGGSTDIQMTQSPSSLSASVGDRVTITCRASQSISSYL NWYQQKPGKAPKLLIYSASQLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ QGPGTPNTFGQGTKVEIKRA		

Supplementary Table S2. Listing of 4 most frequent sequences selected from the yeast display library.

	Heavy chain			Light chain						
Amino acid position	28	50	87	1	30	53	65	70	77	Frequency of clones
Clone45	T	Q	R	D	S	Q	S	D	S	
S3.1	T	L	G	D	S	Q	S	D	N	n=11
S3.3	T	L	R	N	S	L	N	D	S	n=14
S3.6	T	L	R	D	N	Q	S	V	S	n=1
S3.22	L	L	R	D	S	Q	S	D	S	n=3

The mutated residues are underlined. Amino acid positions are given as Kabat numbers (http://www.imgt.org).

Supplementary Table S3. Expression of HLA-A2 and immunostaining of Q2L scFv-Fc on cells.

Cell line	Tumor type	Ratio (BB7.2/isotype)*	Ratio (Q2L/isotype)*	Q2L binding**
K562	Leukemia	1.1	1.1	Negative
K562-HLA-A2	Leukemia	28.8	2.5	Weakly Positive
Molt-4	Leukemia	1.1	1.0	Negative
THP-1	Leukemia	53.9	35.6	Positive
BA25-17	Leukemia	118.5	7.2	Positive
BA25-69	Leukemia	113.6	19.1	Positive
BV-173	Leukemia	209.2	20.0	Positive
SKN-JC-1	NB	86.8	5.1	Positive
SKN-JC-2	NB	53.3	3.5	Weakly Positive
SKNJB	NB	6.4	3.2	Weakly Positive
LAN-1	NB	4.5	6.2	Positive
SKNLD	NB	0.9	1.0	Negative
SKMEL-5	Melanoma	32.1	9.0	Positive
JMN	Mesothelioma	165.3	10.2	Positive
U87	Glioblastoma	77.5	10.8	Positive
U251	glioblastoma	16.2	3.2	Weakly Positive
U2 OS	osteosarcoma	21.7	2.1	Weakly Positive
MDA-MB-231(HTB-26)	Breast	110.9	6.5	Positive
MDA-MB-361(HTB-27)	Breast	0.9	1.4	Negative
MDA-MB-468(HTB-132)	Breast	1.1	1.1	Negative
SKBR3	Breast	1.1	1.1	Negative
MCF-7	Breast	9.9	2.1	Weakly Positive
SKOV-3	Ovarian	1.0	1.2	Negative
OVCAR-3	Ovarian	7.8	1.2	Negative
OVCAR3-pp65	Ovarian	10.0	1.4	Negative
Colo 205	Colon	32.6	2.2	Weakly Positive
Caco-2 (HTB-37)	Colon	26.8	2.9	Weakly Positive
HTB37-pp65/GFP	Colon	10.9	2.3	Weakly Positive
SW480	Colon	40.5	3.2	Weakly Positive
SKHEP-1	Liver	28.3	7.5	Positive
HepG2	liver	14.4	3.4	Weakly Positive
NCI-H345	Small cell lung cancer	6.2	1.7	Negative
NCI-H522	Non-small cell lung cancer	15.8	1.5	Negative
SK-ES-1	Ewing's sarcoma	7.7	1.4	Negative
JN-DSRCT	Desmoplastic small round cell tumor	5.1	1.3	Negative
T2		178	1.7	Negative

*ratio of mean of fluorescence intensity. **define the ratio below 2 as negative, 2-4 as weakly positive, >4 as positive. BB7.2 is a monoclonal anti-HLA-A2 antibody.

Supplementary Table S4. Immunostaining of Clone 45 and Q2L on normal HLA-A2[+] and HLA-A2[-] PBMC

Cells	Ratio (BB7.2/isotype)*	Ratio (Clone45/isotype)*	Ratio (Q2L/isotype)*	Q2L binding**
HLA-A2[+] PBMC (n=10)	21.4	1.1	3.9	Weakly Positive
HLA-A2[-] PBMC (n=4)	0.8	1.1	1.2	Negative

*ratio of mean of fluorescence intensity. **define the ratio below 2 as negative, 2-4 as weakly positive, >4 as positive. BB7.2 is a monoclonal anti-HLA-A2 antibody.

Supplementary figure legends

Supplementary Figure S1. WT1 Clone 45 scFv is highly specific for the recombinant HLA-A2-RMFPNAPYL complex. (**a**) Purified WT1 Clone 45 scFv was tested for binding to recombinant, biotinylated-HLA-A2-peptide complexes by ELISA. Purified WT1 Clone 45 scFv maintained its specificity over a panel of HLA-A2-peptide complex in addition to its inability to bind to the native peptide outside of the context of MHC. The anti-HLA-A2 antibody BB7.2 was included to demonstrate that all HLA-A2-peptide complexes are adherent and presented properly on the plate. (**b**) Evidence that TAP-deficient T2 cells can be loaded with exogenous peptides. T2 cells were pulsed with (solid, unfilled lines) or without (dashed, unfilled lines) RMFPNAPYL (left panel) or LLDFVRFMGV peptides (right panel) at 40 μM in serum-free IMDM media at 37oC for 5 hours. The cells were then stained with a mouse-anti-human HLA-A2-FITC conjugated antibody (unfilled lines) or a control mouse IgG1-FITC conjugated antibody (filled lines) and analyzed on the FACS machine. The increase in florescence from the dashed line to the unfilled solid line signifies stabilization of the HLA-A2 molecule due to peptide binding and presentation. (**c**) Peptide pulsed T2 cells from A were stained with WT1 Clone 45 (unfilled lines) or a control scFv (filled lines), followed by detection using a biotinylated mouse-anti-myc-tag antibody and streptavidin-PE. Only T2 cells which had been pulsed with the RMFPNAPYL peptide (left panel), but not ones which had been pulsed with LLDFVRFMGV (right panel), could be stained by the WT1 Clone 45.

Supplementary Figure S2. Sensorgrams of binding kinetics of scFvs when measured by Biacore. Binding of scFv S3.1, S3.3, S3.6, Clone45 and Q2L were shown. Details of the experiments are described in the Materials and Methods.

Supplementary Figure S3. Epitope mapping (**a**) Model of the docked complex of Q2L with the crystal structure of HLA-A2-WT1-RMF (pdb 3HPJ). The binding epitope was predicted to involve the heavy chain CDR2 of Q2L with Tyr 8 of $WT1_{126}$. (**b**) Binding of Q2L to T2 cells pulsed with $WT1_{126}$-wildtype (RMF), Arg1Ala (A1), Phe3Ala (A3), Pro4Ala (A4), Asn5Ala (A5), Pro7Ala (A7) or Tyr8Ala (A8), measured by flow cytometry. Positions 2 and 9 were not mutated since they are anchor residues, and Position 6 is Ala in WT peptide. A 40% reduction in binding was observed when Tyr8 was mutated to Ala. All peptides were verified to bind to HLA-A2 by staining with anti-HLA-A2 clone BB7.2.

Supplementary Figure S4. (**a**) PG13 cells transfected with Q2L-CAR constructs were stained with $WT1_{126}$ tetramer. (**b**) K562 cells were transfected with Q2L-CAR and stained with $WT1_{126}$ tetramer.