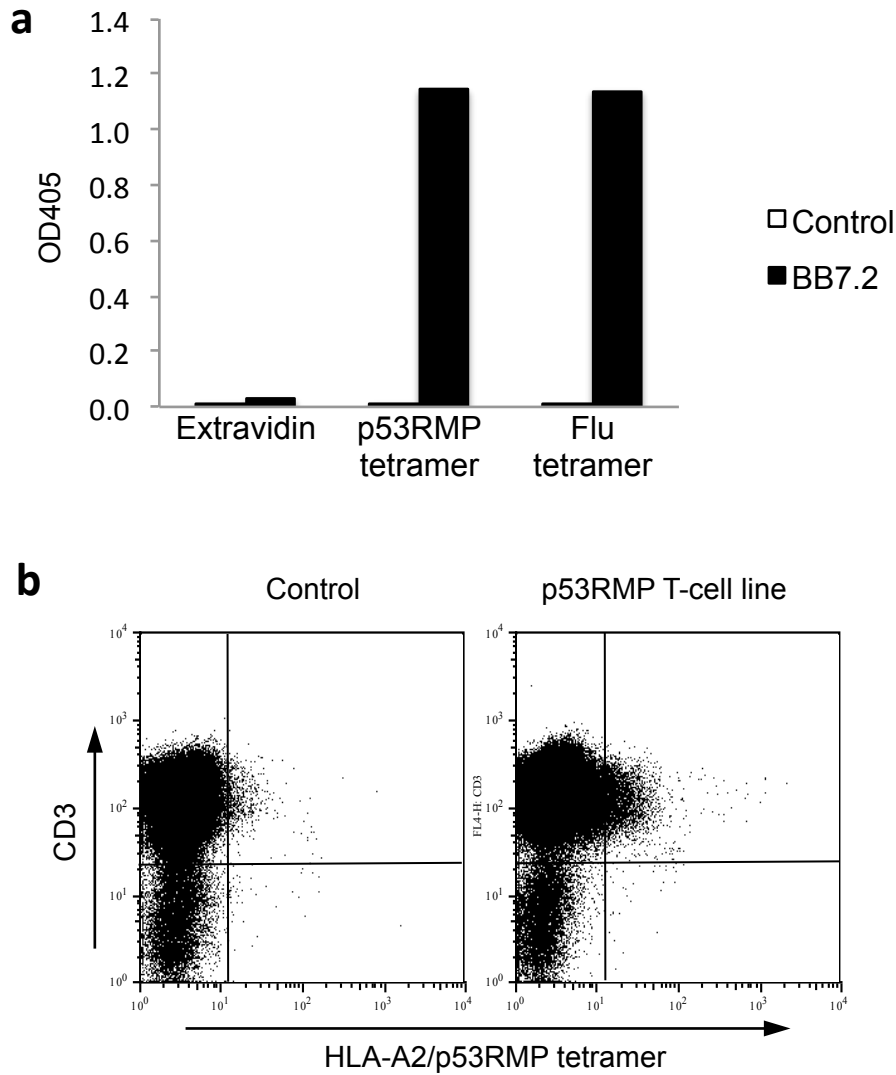
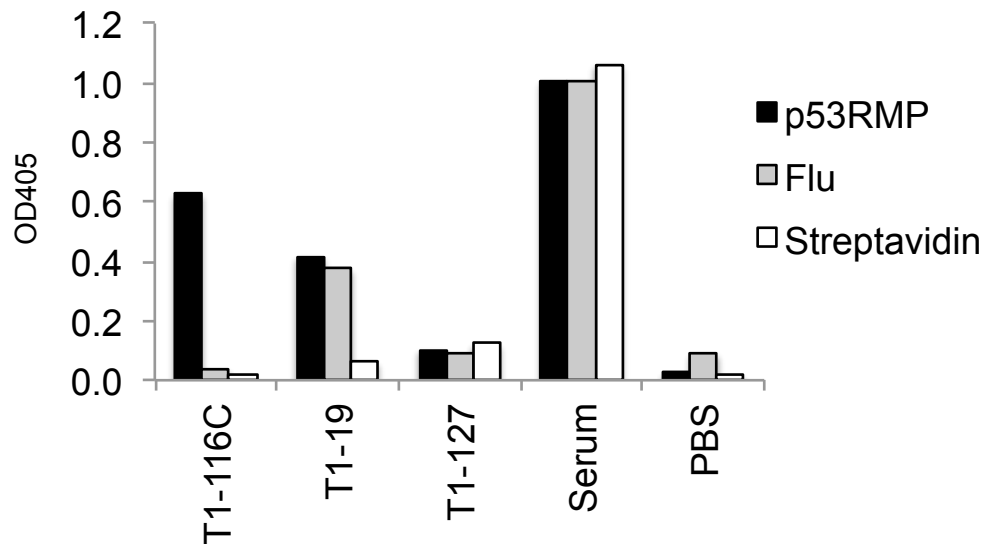


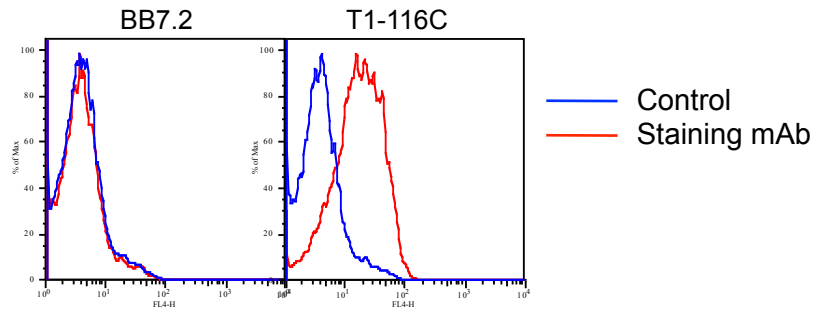
Supplementary figures



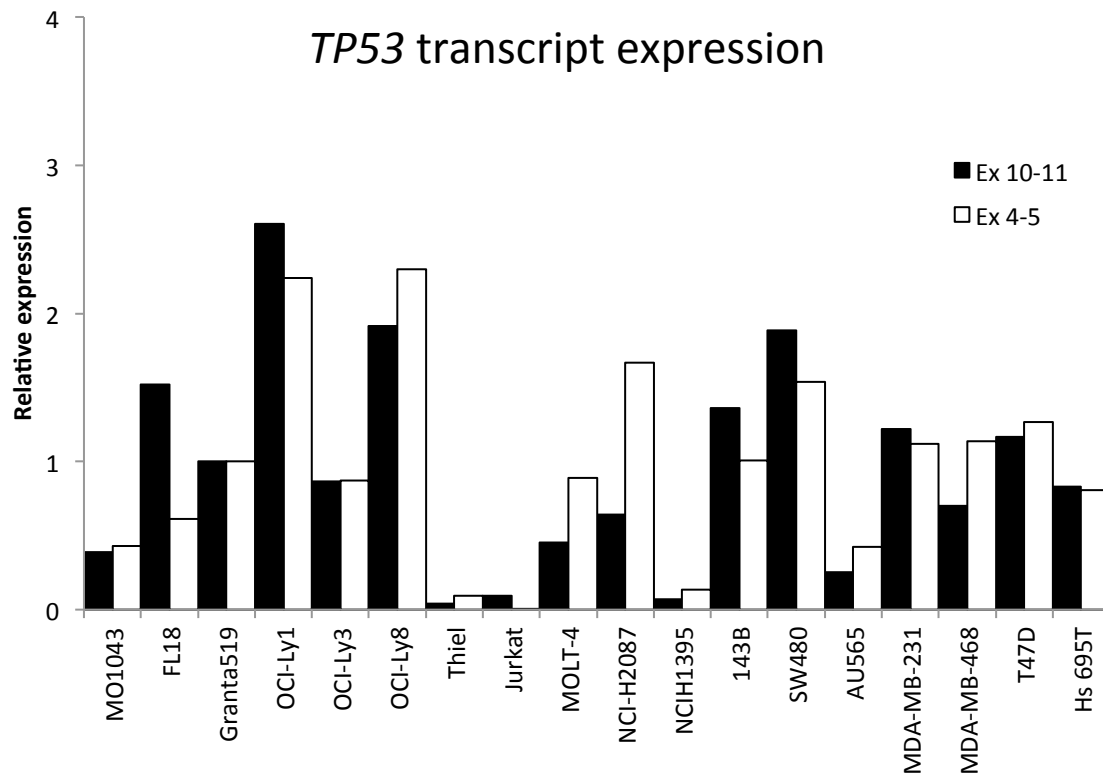
Supplementary Fig.1. Validation of the HLA-A2/p53RMP tetramer. **(a)** The HLA-A2/p53RMP tetramer and HLA-A2/Flu tetramer were shown to bind the HLA-A2 antibody BB7.2 and not an isotype control antibody by ELISA. **(b)** p53RMP-reactive T-cell lines were generated by stimulating HLA-A2 positive peripheral blood mononuclear cells (PBMCs) with autologous dendritic cells pulsed with p53RMP peptide over a 14-day period. The cell line (right) was then stained with PE-conjugated HLA-A2/p53RMP tetramer and results analysed by flow cytometry. Unstimulated cells were used as a control (left).



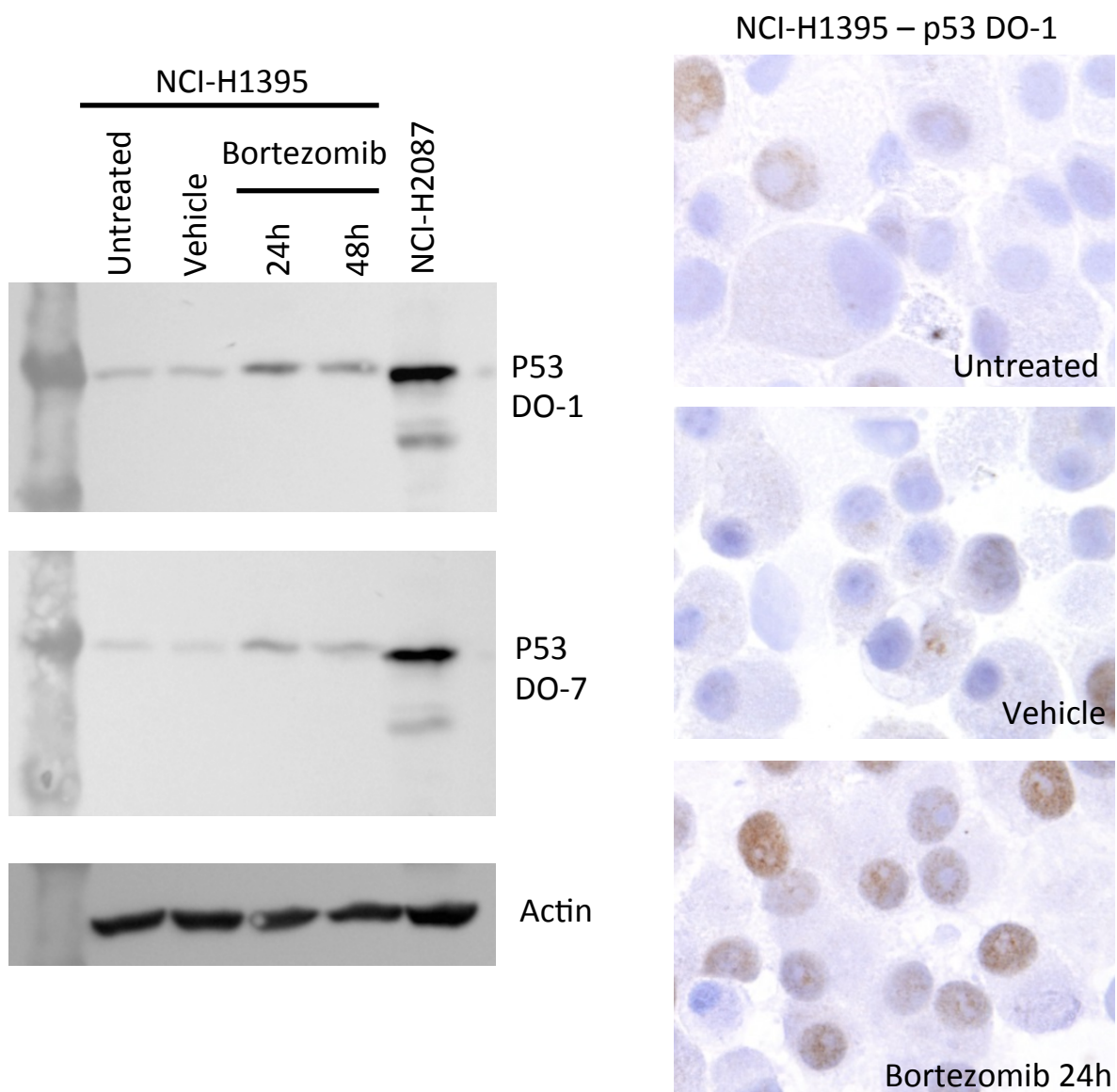
Supplementary Fig.2. ELISA screening of HLA-A2/p53RMP tetramer-reactive TCRm hybridoma supernatants. ELISA plates were coated with p53RMP tetramers, Flu tetramers or streptavidin and tested for their ability to bind antibodies in hybridoma supernatants by ELISA. The immunising serum and PBS were used as positive and negative controls. T1-116C was shown to bind p53RMP tetramers but not to Flu tetramers or streptavidin, while a representative example of a non-specific hybridoma T1-19 bound both tetramers, and a non-responding hybridoma T1-127 bound neither tetramers or streptavidin.



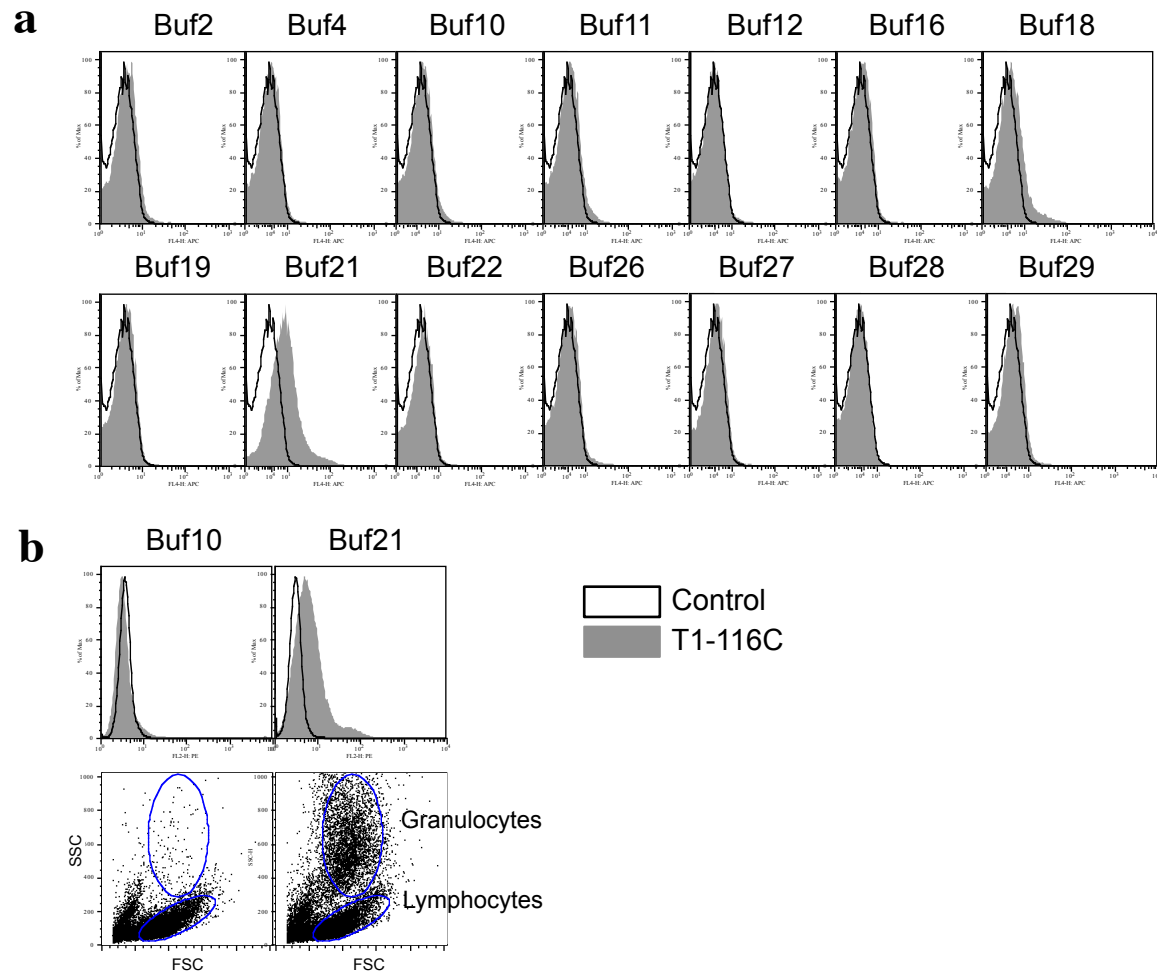
Supplementary Fig.3. T1-116C staining of HL-60 cells. The cells were stained with either the p53 TCRm antibody T1-116C or HLA-A2 antibody BB7.2 at 10 μ g/ml, followed by an APC conjugated anti-mouse secondary antibody. Isotype controls were used in control staining.



Supplementary Fig.4. *TP53* transcript levels in cancer cell lines. Total RNA was extracted from cultured cancer cells, and cDNA was synthesised using Oligo-(dT) as primer and *TP53* transcript levels were detected by quantitative real-time PCR using two pairs of human *TP53* specific intron-spanning primers Ex10-11 and Ex4-5.



Supplementary Fig.5. The proteasome actively turns over p53 in NCI-H1395 lung cancer cells. NCI-H1395 cells were grown under standard culture conditions (untreated) or with the additional of either DMSO (vehicle) or 10 μ M bortezomib in DMSO for 24 or 48 hours. Western blotting and immunocytochemistry were used to detect p53 protein expression using the anti-p53 antibodies indicated.



Supplementary Fig.6 The p53 TCRm T1-116C does not stain normal human peripheral blood mononuclear cells (PBMCs). (a) Fourteen buffy coat-derived PBMC samples were analysed for T1-116C staining by FACS. (b) Buf21 displayed granulocytosis in FACS analysis comparing with normal samples, e.g. Buf10.

Supplementary Table 1. Quantitation of T1-116C binding sites per target cell

Cell line	Peptide (T2 only)	T1-116C-PE per cell
T2	Flu-100 μ M	151
	p53RMP-100 μ M	35336
	p53RMP- 50 μ M	12207
	p53RMP- 10 μ M	1240
	p53RMP- 2 μ M	180
	p53RMP- 0.5 μ M	57
FL-18		1329
Granta 519		7491
MO1043		4145
OCI-Ly1		4016
OCI-Ly8		5819
AU565		1617
Hs695T		1101
MDA-MB-231		1956
NCI-H1395		15291
NCI-H2087		3975
SW480		551
143B		1019

Supplementary Materials and Methods

Immunocytochemistry

Acetone-fixed cytocentrifuge cell preparations of NCI-H1395 lung cancer cells were incubated in a solution of hydrogen peroxide (0.3% H₂O₂, 0.1% NaN₃ in PBS) for 10 minutes to block any endogenous peroxidase activity. Slides were washed once in PBS then once in PBS-Tween (0.05% v/v, 3 minutes each wash). Slides were then incubated with mouse anti-p53 antibody diluted in PBS (DO-1, 10ug/ml) for 30 minutes. After washing as above, the slides were incubated in secondary antibody reagent (Dako REAL™ EnVision™ Detection System, K5007) for 30 minutes. Labelling was visualized using the Liquid DAB+ Substrate Chromogen System (Dako, K3468), allowing colour to develop for 10 minutes. After washing as above, cells were counter-stained with Gill 3 Hematoxylin (Thermo Scientific, 6765009) and coverslips were mounted using Aquatex (VWR 1.08562.0050). All steps were performed at room temperature.