Legend for supplementary figures

Supplementary Figure 1. Effect of the duration of plate-bound antibody stimulation on T cell survival

CD4+CD25- T cells (1.0×10^5) from C57.BL/6 mice were stimulated by plate bound anti-CD3 and anti-CD28 antibodies as described in Figure 2A. After stimulation for 48~120 hours (indicated under the each bar), cells were gently removed from antibody-coated plates, then replaced in fresh medium without further stimulation. After 5 days from the beginning of the stimulating culture, live cell number of each group of cells was determined by trypan blue exclusion assay. The error bars represent standard deviation of triplicates.

Supplementary Figure 2. Time course analysis of changes in viability of cells stimulated with plate-bound antibody.

CD4+CD25- T cells (1.0×10^5) from C57.BL/6 mice were stimulated by plate bound anti-CD3 and anti-CD28 antibodies. After stimulation for 2,3, or 4 days (indicated above each panel), cells were gently harvested from the plates and analyzed immediately for the state of apoptosis. Each panel shows their staining profile for annexin-V and 7-AAD. The total live cell number for each condition was as follows. day 2: 1.0×10^5, day3: 1.8×10^5, and day4: 1.1×10^5.

Supplementary Figure 3. Total cell numbers of live T cells recovered after simulation with plate-bound antibodies.

Total number of live cells recovered on day 5 are shown for samples from various stimulating conditions with plate-bound anti-CD3 antibodies, anti-CD28 antibodies, and

exogenous IL-2 (as denoted by + for stimulation). Each culture was started with $1.0x10^5$ cells/plate. The error bars represent standard deviation of triplicates.

Supplementary Figure 4. Classical AICD on wildtype and p53 deficient mouse T cells

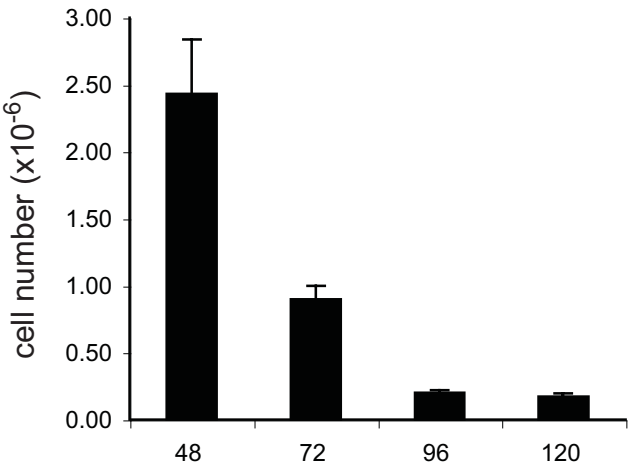
CD4 T cells from wildtype C57.BL/6 and $p53^{-/-}$ mice were activated with Con A (2μg/ml) for 2 days. Cells were washed, and cultured for 2 additional days in IL-2 (20 ng/ml) containing medium followed by stimulation with plate bound anti-CD3 antibody in the presence of IL-2 (20 ng/ml). Forty eight hours later, cells were harvested and analyzed for CD4, annexin-V and 7-AAD.

Supplementary Figure 5. Quantitative analysis of p53, MDM2 and p21 protein expression by plate-bound or beads-bound antibody stimulated $CD4^+CD25^-$ and $CD4^+CD25^+$ T cells.

Western blot shown in Fig. 7A was analyzed by NIH image and relative amount of each protein (p21, p53, and MDM2) was determined using β-actin band for normalization. 25^- res: $CD4^+CD25^-$ T cells unstimulated, 25^+ res: $CD4^+CD25^+$ T cells unstimulated, 25^- beads: $CD4^+CD25^-$ T cells stimulated with beads-bound antibody, 25^+ beads: $CD4^+CD25^+$ T cells stimulated with beads-bound antibody, 25^- plate: $CD4^+CD25^-$ T cells stimulated with plate-bound antibody, 25^+ plate: $CD4^+CD25^+$ T cells stimulated with plate-bound antibody

Supplementary Figure 6. Quantitative analysis of Bim protein expression

Relative expression level of two forms of Bim protein, BimEL and BimL, from $CD4^+CD25^-$ and $CD4^+CD25^+$ cells that were stimulated with plate bound antibody (plate) or with beads-bound antibody (beads), was determined by NIH image. The amount of HSC70 was used for normalization.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2.00
1.50
1.00
0.50
0.00
cell number (x10-6)
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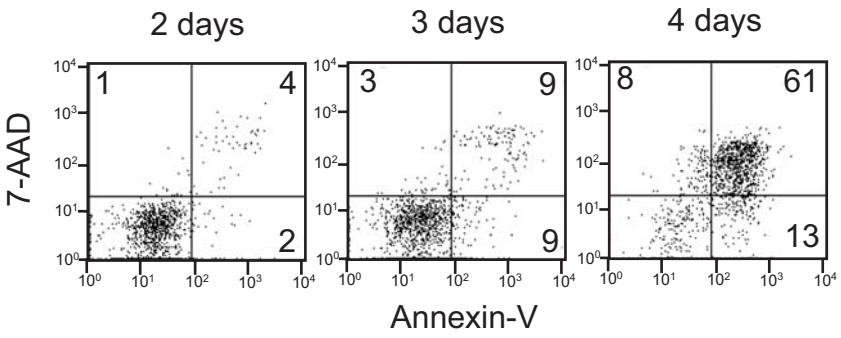
72
96
120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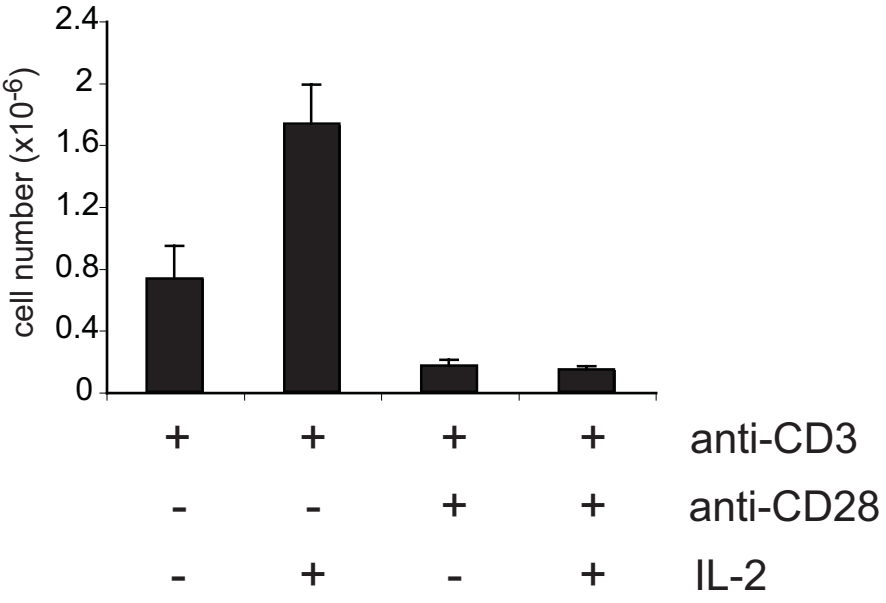
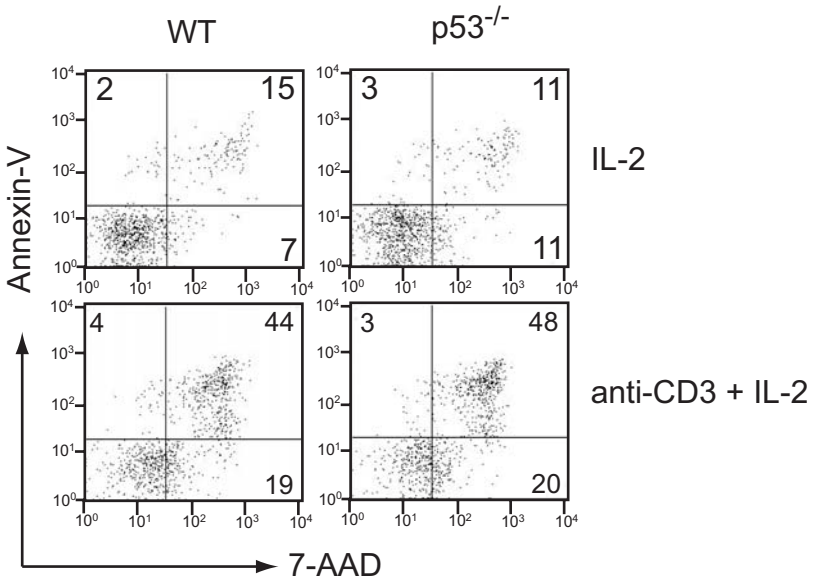
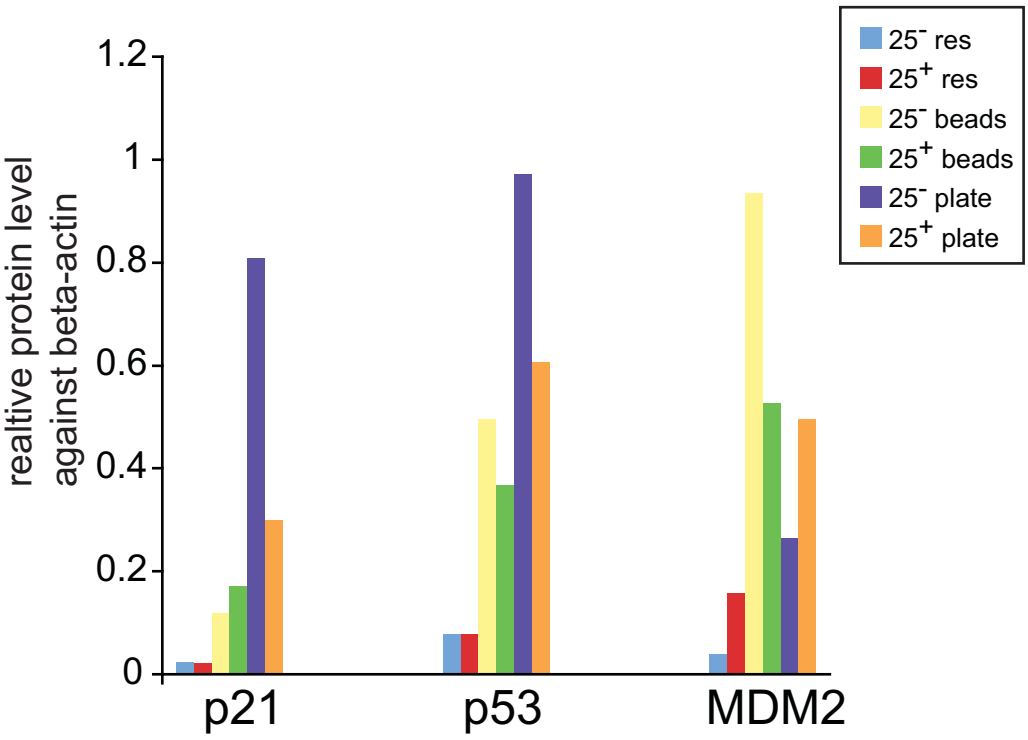
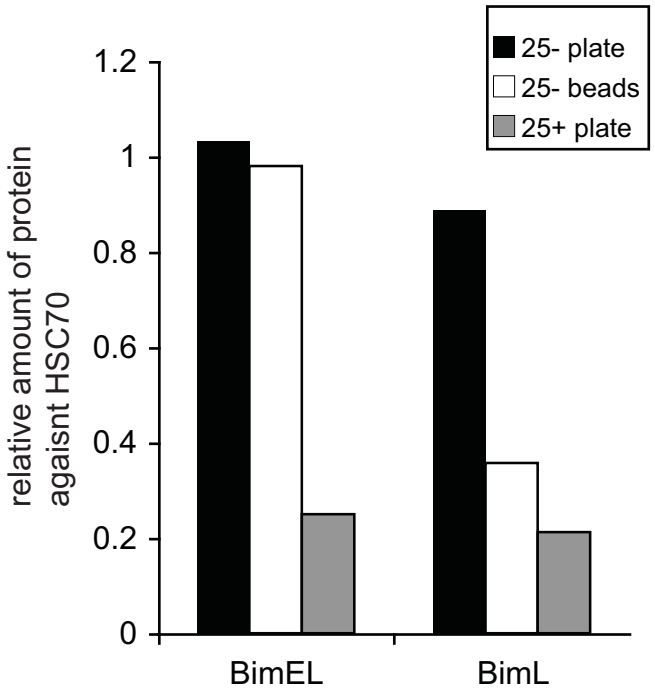
3 days
4 days
7-AAD
Annexin-V
1
4
2
3
9
9
8
61
13


2.4
2
1.6
1.2
0.8
0.4
0
cell number (x10-6)
+ + + + anti-CD3
- - + + anti-CD28
- + - + IL-2


WT
p53-/-
Annexin-V
7-AAD
IL-2
anti-CD3 + IL-2
2
15
7
3
11
11
4
44
19
3
48
20


realtive protein level
against beta-actin
1.2
1
0.8
0.6
0.4
0.2
0
p21
p53
MDM2
25- res
25+ res
25- beads
25+ beads
25- plate
25+ plate


25- plate
25- beads
25+ plate
relative amount of protein
agaisnt HSC70
1.2
1
0.8
0.6
0.4
0.2
0
BimEL
BimL