Supplementary Figure legends

Figure S1. Binding of TBP and MED23 to Ad5 CR3 E1A mutants and to CR3s from different Ad subgroups. Following GST pull-down, binding capacity was assessed by Western blotting for TBP (**A**) and MED23 (**B**) respectively. The Coomassie-stained gel showing the purity of the E1A proteins used in this instance are presented in Figure 2C of the main text. The corresponding regions of CR3 used during this study were: Ad5, residues 139-204; Ad3, residues 132-210; Ad4, residues 128-206; Ad9, residues 120-198; Ad12, residues 124-210; Ad40, residues 117-193.

Figure S2. S8 and TBP bind independently of each other to CR3. (**A**) The ability of CR3 to bind TBP (upper panel) and S8 (lower panel) was assessed by Western blotting following GST pull down using A549 cellular lysates prepared from A549 cells treated with non-silencing RNAs, or siRNAs against S8 or TBP. (**B**) The ability of Ad CR3 to bind TBP and S8 (upper panel) was also assessed by Western blotting following the immunoprecipitation of S8 and TBP from L1920A 13S E1A-A549 cells treated with non-silencing RNAs, or siRNAs against S8 or TBP (lower panel). (**C**) GST pull-down showing CR3 and TBP binding to [^{35}S]-labelled 19S ATPases. CR3 binds S8 in preference to other 19S ATPases. TBP binds all 19S ATPases, but binds S8 with highest capacity.

Figure S3. (A) L1920A 13S E1A expression does not affect 20S proteasome association with 19S proteasomal subunits. A549 and L1920A 13S E1A-A549 cellular lysates were prepared (under mild lysis conditions and in the presence of 5 mM ATP), protein concentrations determined by Bradford assay, and equivalent A549- and L1920A 13S E1A A549- lysates incubated with an antibody that

immunoprecipitates 20S proteasomal fractions. Immunoprecipitates were resolved upon SDS-PAGE and Western blotted for 19S base components, S2 (left hand panel) and S8 (right hand panel). L1920A 13S E1A does not affect 26S proteasome assembly. (**B**) L1920A 13S E1A expression does not affect S8 association with other 19S proteasomal subunits. A549 and 13S L1920A-A549 cellular lysates were prepared, protein concentrations determined by Bradford assay, and equivalent A549 and 13S L1920A A549 lysates incubated with an antibody that immunoprecipitates S8. Immunoprecipitates were resolved upon SDS-PAGE and Western blotted for 19S base components, S2 (upper left hand panel) and S7 (upper right hand panel), or S10b (middle left hand panel). Levels of S8 (middle right hand panel), 20S (lower left hand panel) and L1920A 13S (lower right hand panel) proteins were also detected by Western blotting. L1920A 13S E1A does not affect 19S proteasome assembly.

Figure S4. S8 over-expression or knock-down by RNAi does not affect CBP transactivation capacity. (**A**) Effect of S8 expression upon CBP transactivation capacity. HCT116 cells were transfected with 100 ng of pcDNA3-Gal4DBD, pcDNA3-Gal4DBD-CBP, or pcDNA3-Gal4DBD-CR3, in the presence of 2 μg of a Gal4-responsive luciferase reporter. Varying amounts of pcDNA3-N-Flag-S8 expression plasmid were included in the transfections as appropriate (see Figure). 24h post-transfection, cell lysates were prepared and luciferase activities measured. DNA levels were equalized with pcDNA3 alone. Total S8, N-FLAG S8 and β-actin were all quantified by Western blotting. Lanes 2, 3 and 4 correspond to DBD-CBP transfections possessing 0, 25, and 250 ng of Flag-S8 plasmid, respectively. Lanes 5, 6 and 7 correspond to DBD-CR3 transfections possessing 0, 25, and 250 ng of Flag-S8 plasmid, respectively. (**B**) Effect of S8 knock-down upon CBP transactivation

capacity. Following S8 knock-down, HCT116 cells were transfected with either, 100 ng pcDNA3-Gal4DBD and 2 μg of a Gal4-responsive luciferase reporter, or 100 ng pcDNA3-Gal4DBD-CBP and 2 μg of a Gal4-responsive luciferase reporter. DNA levels equalized with pcDNA3 alone. 24h post-transfection cell lysates were prepared and luciferase activities measured. S8 and β-actin levels were determined by Western blotting. Lane 1 corresponds to DBD and lane 2 to DBD-CBP under non-silencing conditions. Lane 3 corresponds to DBD and lane 4 to DBD-CBP under conditions where S8 has been silenced through RNAi.

Figure S5. (**A**) Schematic representation of the Ad genome depicting the early region transcription start sites. (**B**) Schematic representation of the Ad early region promoter regions and gene units amplified by PCR following ChIP. Grey bars indicate regions specifically amplified by oligonucleotide primer sets. The major transcription factor binding sites are also shown.

Figure S6. Role for the proteasome in 13S E1A transactivation function. (**A**) HCT116 cells were transfected with an Ad5 E4 promoter-tethered CAT reporter and pcDNA3 Ad5 13S E1A. 8h post-transfection cells were treated with proteasome inhibitors. 24h post-transfection, cell lysates were prepared and CAT activities measured (Promega). E1A levels were determined by Western blotting. Proteasome inhibitors similarly inhibited the ability of 13S E1A expressed from pLE2 to transactivate the Ad5 E4 promoter (transactivation ability correlated with E1A expression; data not shown). (**B**) HCT116 cells were transfected with a Gal4-responsive luciferase reporter and pcDNA3-Gal4DBD-CR3. 8h post-transfection cells were treated with proteasome

inhibitors. 24h post-transfection, cell lysates were prepared and CAT activities measured. Results shown are the mean +/- S.D. from three independent experiments.

Supplementary information: Figure S6.

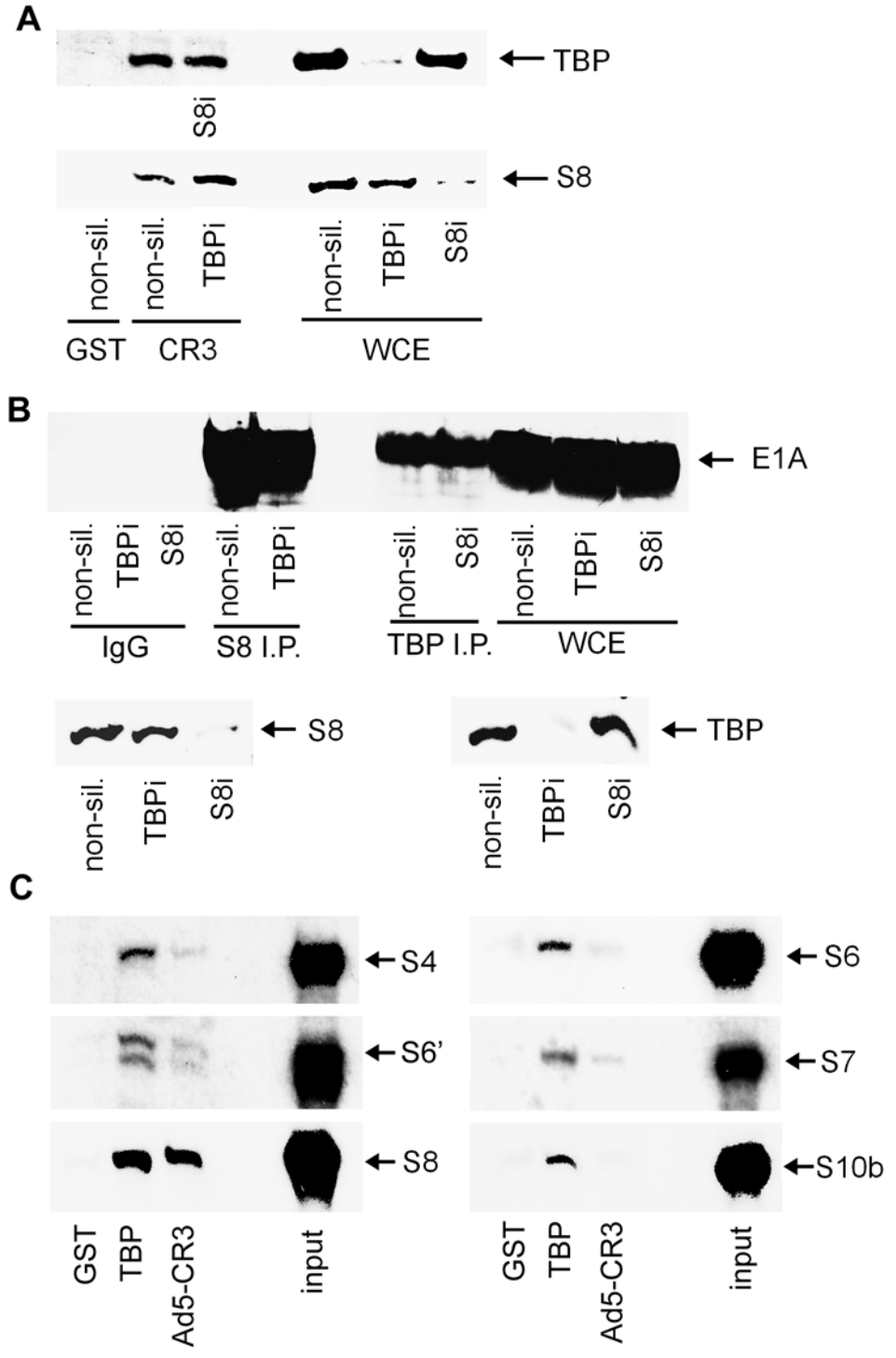
The effect of proteasome inhibition on 13S E1A transcriptional activity

Given the requirement for proteolysis in transcription we investigated whether proteasome-mediated degradation was required for 13S E1A-dependent transactivation. To examine this possibility, the transcriptional activities of 13S E1A and Gal4-CR3-E1A were assessed in HCT116 cells in the presence of specific proteasome inhibitors. Expression of 13S E1A, in the absence of inhibitors, stimulated E4 promoter activity 7.2 fold (upper panel, Figure S6), whereas proteasome inhibitors reduced 13S E1A's capacity to enhance E4 promoter activity significantly. MG132 was most potent in this regard, but calpain inhibitor II and lactacystin also reduced 13S E1A's capacity to enhance E4 promoter activity (upper panel, Figure S6). Activation of E4 promoter activity in this context reflected the protein levels of 13S E1A (middle panel, Figure S6). The apparent anomaly of proteasome inhibitor effects upon E1A levels in this regard will be considered below. Interestingly, Gal4-CR3 transactivation capacity was also reduced in cells treated with proteasome inhibitors (lower panel, Figure S6), although at the levels of plasmid used in these assays DBD-CR3 protein could not be detected (data not shown). Again, MG132 was most potent in this regard, but calpain inhibitor II and lactacystin also reduced the transcriptional activity of CR3 (lower panel, Figure S6). Collectively, these results demonstrate that proteasome-mediated degradation is required for 13S E1A-mediated transcriptional activation. We believe that the ability of proteasome inhibitors to repress E1A protein levels, rather than enhance them in this instance, reflects the fact that inhibitors were

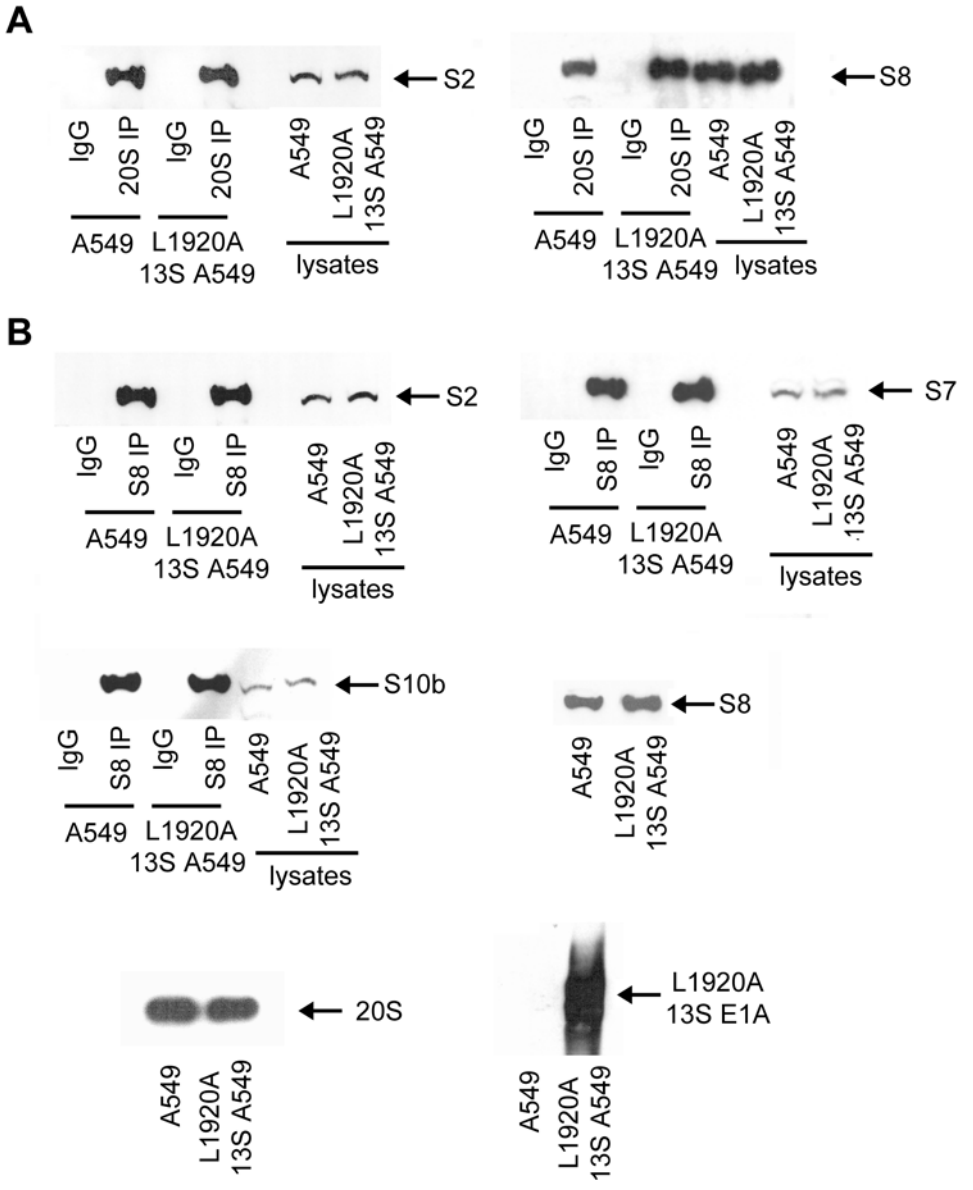
added before transiently transfected E1A protein levels were detectable. As such inhibition of 20S proteasome function at this time has a greater apparent effect on transcriptional regulation of E1A rather than apparent E1A stability; E1A protein levels are reduced through transcriptional repression. In a cell stably expressing 13S E1A, proteasome inhibition has a more obvious effect upon *w.t.* E1A protein levels (*e.g.* Figure 10B).

Rasti *et al*, Figure S1

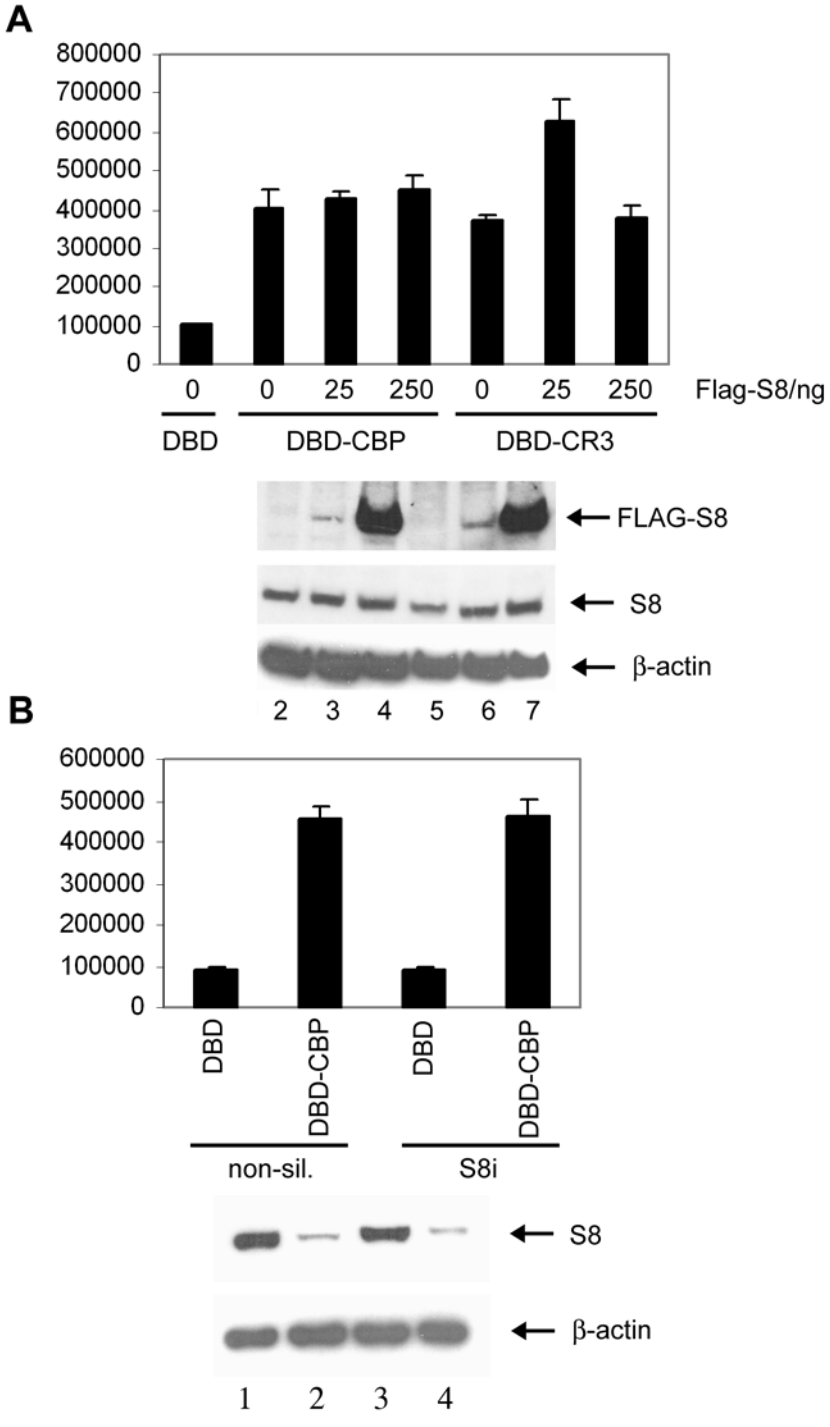
Rasti *et al*, Figure S2



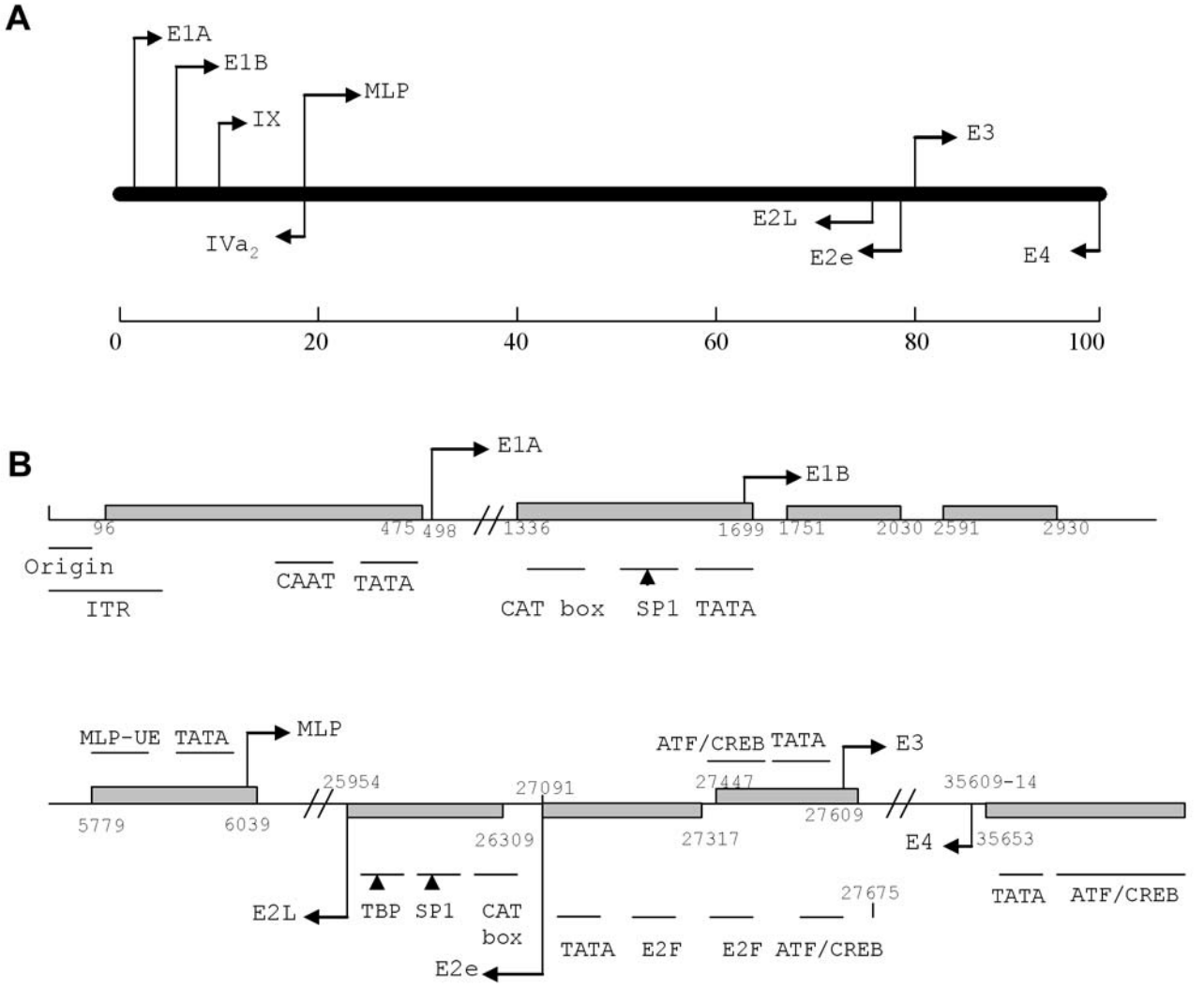
Rasti *et al*, Figure S3



Rasti *et al*, Figure S4



Rasti et al., Figure S5



Rasti *et al*, Fig S6