vp/injection on day 1, 3, and 5 with and without docetaxel at 15mg/kg administered intraperitoneally on day 2 and 8. Treatments were not significantly different ($p>0.3$). B) Representative micrographs of TRAMPC tumors stained for macrophages (CD68) and viral proteins (E1A) 15 days after intratumoral administration of one dose of the respective virus intratumorally at $1x10^{10}$vp. Magnification: 400x for CD68 and 200x for E1A. C) Survival curves for C57BL intact mice with murine colorectal CMT-93 (left panel) and lung CMT-64 (right panel) subcutaneous tumors, treated with $1x10^{10}$ vp/injection on day 1, 3, and 5 with *dl*922-947 (ΔE3B) (open diamond), AdΔCR2 (closed circle) and AdΔΔ (open circle) for CMT-93, and with *dl*922-947 and AdΔΔ for CMT-64; mock treated animals (closed square). Survival of AdΔΔ treated animals was significantly different from animals treated with *dl*922-947 (*$p<0.02$) only in the CMT-93 model, 8 animals/group.

SUPPLEMENTARY MATERIAL

Table S1. Relative sensitivity to viral mutants in NHBE and PrEC cells.

Figure S1. PCR determination of gene deletions in the novel mutants after homologous recombination and plaque expansion. Viral DNA was isolated from $CsCl_2$-purified viral preparations after amplification in HEK293 cells as described in Material and Methods. The viral DNA was amplified by qualitative PCR using specific primers followed by separation on agarose gels to determine presence or absence of the E1B19K gene and the E1ACR2 gene region. A) Verification of the 218 nucleotide E1B19K gene deletion in the AdΔ19K, AdΔΔ and *dl*337 mutants using primers amplifying a 532nt region of the wild type E1B19K gene and a 314nt region in the corresponding deletion mutants. B) Verification of the 26 nucleotide CR2 deletion in the E1A gene resulting in a 262nt band and a 236nt band in viruses with intact E1A and the ΔCR mutants respectively. The Ad5wt is wild type Ad5 previously prepared and Ad5tg is wild type virus used in this study generated from the pTG3602 plasmid as described in Material and Methods; *dl*337 is deleted in the E1B19K and E3B genes and *dl*922 is *dl*922-947 deleted in the E1ACR2 and E3B genes.

Figure S2. Sensitisation of LNCaP and 22Rv cells to Docetaxel by infection with viral mutants at low doses. Dose-response curves for docetaxel were generated after treatment of cells with serial dilutions of drug with and without addition of viral mutants at doses killing <25% cells alone. Docetaxel was added 2h after infection with 1 (not shown) or 2.5ppc in LNCaP cells (right panel) and with 2.5 and 10 ppc (not shown) in the 22Rv cells (left panel) with AdΔ19K (white bars), AdΔCR2 (grey bars) and AdΔΔ (black bars). Cells were analysed for viability 3 days after treatment initiation using the MTS-assay and EC_{50} values were determined using untreated/uninfected and untreated/infected cells as controls respectively as described in Material and Methods. Results are presented as % decrease in EC_{50} value with the combination treatments compared to that of docetaxel alone, n=3. Although differences between mutants were not significant, the AdΔΔ could greatly ($p<0.05$) decrease the EC50 value for docetaxel in the LNCaP cells with a similar trend in 22Rv cells.

Figure S3. The AdΔΔ induces activation of caspase 3 both in the presence and absence of docetaxel in the 22Rv cell line. The cells were treated with 0.1nM docetaxel 2h after infection with 10ppc of Ad5tg or AdΔΔ, cell lysates were prepared and analysed for changes in procaspase 3 and cleaved (activated) caspase 3 by immunoblotting 48h later as described in Material and Methods. Cells treated with docetaxel alone showed no or low levels of caspase activation; infection with Adtg did not induce cleavage of caspase 3 and in combination with docetaxel no or undetectable levels of cleavage-product similar to docetaxel alone; AdΔΔ potently induced caspase activation both in the presence and absence of docetaxel; staurosporin (St) treated cells were used as a positive control for cleaved activated caspase 3.

Figure S4. Efficacy *in vivo* of the newly generated adenoviral mutants AdΔ19K, AdΔCR2, AdΔΔ and the Adtg wild type virus in PC3 and DU145 tumor xenografts in athymic mice. Left panel, animals with PC3 subcutaneous tumor xenografts were treated with the viral mutants at $1x10^9$vp injected intratumorally on day 1, 3, and 5: mock treated (non-replicating *dl*312; open grey squares), Adtg (wild type virus; filled diamonds), AdΔ19K (E1B19K-deleted; filled circles), AdΔCR2 (E1ACR2-deleted; open

circles) and AdΔΔ (E1B19K and E1ACR2 deleted; filled squares). Right panel, animals with DU145 subcutaneous tumor xenografts were treated as above with the indicated viral mutants at $1x10^9$vp injected intratumorally on day 1, 3, and 5. Both models; median survival was determined by Kaplan-Meier survival analysis, 6-10 animals per group. Efficacy of viral mutants were not significantly different compared to either wild type virus or each other at this dose level, $p>0.05$. At higher viruses doses a trend (not significant) towards higher efficacy was seen for the AdΔΔ mutant in these models.