Online Supplementary Data

Adenovirus vector production and characterization: A first-generation, human Adenovirus type 5 derived replication deficient vectors (deleted for the E1 and E3 genes) were utilized in this study. Control Ad5 vectors encoding β-galactosidase (LacZ) as a transgene (Ad5-LacZ) were constructed, propagated and purified as previously described [1-3]. Viral particle (vp) and transducing unit titers (TU, bfu/ml) were determined for all Ads constructed as previously described [4,5]. Infectious titers of all Ads were determined by standard Tissue Culture Infectious Dose 50 (TCID50) method (AdEasy Adenoviral vector system manual, Qbiogene, Carlsbad, CA). Infections titer was calculated by using KARBER statistical method: TCID50/ml titer = $10 \times 10^{1 + d(S-0.5)}$, where d is the log(10) of the dilution and S is the sum of ratios from the first dilution. VP/TU/TCID results are summarized in table 1. Note, that VP/TU/TCID ratios of capsid-displaying Ads were not dramatically different from the same ratios for control Ads (Supplemental table 2). These experiments validated the viability of novel capsid-displaying Ads. Residual infectivity of Ad5-LacZ, Ad5-LacZ-IX-dCOMPinh and Ad5-LacZ-Fiber-dCOMPinh was tested after incubation with NHS (90 min, 37° C). TCID titers were dramatically reduced (approximate 10^5 times as compared to the titer noted in Supplemental Table 2, measured for intact vectors), however, there was no significant differences in the levels of reduction between three Ads tested, indicating that similar levels of Ad inactivation were observed under these conditions.

Supplementary Table 1. Novel "capsid-displaying" Ads can be propagated to high titers, similar to conventional Ad vectors. Capsid modifications do not impair infectivity and transduction efficiency of novel Ads

Virus	VP titer (vp/ml)	TU titer (BFU/ml)	TCID titer (TCID/ml)	VP/TU/TCID ratio	Silver Stain	TEM
Ad5-CMV-LacZ	$2.86x10^{12}$	$1.8x10^{11}$	$1.6x10^{10}$	179/11/1	Intact	Intact
Ad5-IX-dGFP	$3.54x10^{12}$	NA	$6.3x10^{10}$	56/NA/1	Intact	Intact
Ad-LacZ-IX-dCOMPinh	$4.75x10^{12}$	ND	$6.3x10^{10}$	75/ND/1	Intact	Intact
Ad-LacZ-Fiber-dCOMPinh	$2.06x10^{12}$	$7.0x10^{10}$	$1.0x10^{10}$	206/7/1	Intact	Intact

VP – viral particles; TU – transducing units; BFU – blue-forming units (for Ads expressing β-Gal); TCID – tissue culture infections dose (measure of Ad infectivity); TEM – transmission electron microscopy.

Supplementary Table 2. Primers used to construct and validate Ad5 vectors

Primer name	**Primer sequence (5'–3')**	**Used for**
Comp-Nhe-F	CTAGCatctgcgtgtggcaggattggggcgcccacaggtgcaccG	Cloning compinh in pIX
Comp-Nhe-R	CTAGCggtgcacctgtgggcgccccaatcctgccacacgcagatg	Cloning compinh in pIX
Comp-Xba-Fc	CTAGAatctgcgtgtggcaggattggggcgcccacaggtgcaccCG	Cloning compinh in Fiber
Comp-Not-Rc	GGCCGCggtgcacctgtgggcgccccaatcctgccacacgcagatT	Cloning compinh in Fiber
Knob-F1	AGGCAGTTTGGCTCCAATATCTG	*NotI/XbaI* insertion in Fiber
knob-Xba/Not-R1	**GCGGCCGC**ACC**TCTAGA**TGTGTCTCCTGTTTCCTGTGTA	*NotI/XbaI* insertion in Fiber
knob-Xba/Not-F2	**TCTAGA**GGT**GCGGCCGC**TCCAAGTGCATACTCTATGTCATTT	*NotI/XbaI* insertion in Fiber
knob-R2	GCTATGTGGTGGTGGGGCTATACTA	*NotI/XbaI* insertion in Fiber
CMV-F	TGGGAGTTTGTTTTGGCACC	Sequencing transgene
SV40polyA-R	TTCATTTTATGTTTCAGGTTCAGGG	Sequencing transgene
pIX-SEQ-F1	GCAAGCAGTGCAGCTTCCCG	Sequencing pIX display
pIX-SEQ-F2	GATCTGCGCCAGCAGGTTTC	Sequencing pIX display
pIX-SEQ-R	CAGGACCCTCAACGACCGAG	Sequencing pIX display
Ad5-Comp-R	CCGCCCTATCCTGATGCACG	Sequencing Fiber display
Cominh-SEQ-F	ATCTGCGTGTGGCAGGATTG	Sequencing compinh
pIX-upstream-R	CCACGCCCACACATTTCAGTACC	Sequencing of Ads to test for RCA

Silver Staining: To verify that particle number quantification was accurate across all Ads constructed, 10^{10} vps of lysed purified virions of each Ad were separated by 10% SDS-PAGE and subsequently stained with silver nitrate utilizing a Silver stain kit for proteins (Sigma, St. Louis, MO). The amount of hexon protein was quantified for each Ad vector by scanning densitometry using ImageJ software, ver. 1.29 (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). Results from this analysis indicated that the VP titers of all viruses determined by spectrophotometry fall within ~1.13 fold of each other, thus capsid-displaying Ad5 vector preparations did not contain less virions as compared to first generation Ad5 vectors based on this assay.

Western Blotting: To further verify that particle number quantification was accurate across all Ads constructed, 10^{10} of lysed purified virions of each Ad were separated by 10% SDS-PAGE and Western blotting was performed utilizing hexon specific antibodies (Abcam, Cambridge, MA). Electrophoretically separated capsid protein samples were transferred onto nitrocellulose membranes and probed with rabbit polyclonal Ad5 hexon specific antibody, followed by probing with a fluorescent secondary antibody as previously described [6]. Membranes were scanned and hexon concentrations quantified using Licor's Odyssey scanner [6]. Results from this analysis indicated that the VP titers determined by spectrophotometry fall within ~1.3 fold of each other based on this assay, thus capsid-displaying Ad5 vector preparations did not contain less virions as compared to conventional first generation Ad5 vectors based on this assay.

Electron Microscopy of purified Ad vectors: Negative staining of $CsCl_2$ purified Ad vectors was performed as follows. Ads diluted to 10^{12} vp/ml in 10 mM Tris were adsorbed to Formvar/Carbon film 300 mesh Copper grids (Electron Microscopy Sciences, Hatfield, PA) and stained with a freshly prepared, 1% solution of phosphotungstic acid (1 g PTA, 50 µl of FBS, 50 ml miliQ water,

pH 6.0, adjusted by KOH) for 30 seconds and examined by using transmission electron microscope (Philips EM410). Photographs were taken from representative areas from each sample (Supplemental Figure 2).

Capsid thermostability assay: It has been shown that an Ad capsids containing functional pIXs are more resistant to temperature inactivation as compared to Ad capsids lacking pIX or pIX functionality [7,8]. Therefore, we pre-incubated control Ads, and pIX-COMPinh displaying Ads at 45° C or 56° C, infected HEK293 cells and determined the percentage of LacZ positive HEK293 cells as previously described [4,5]. We did not detect significant differences between the ability of Ad-LacZ-IX-dCOMPinh to transduce 293 cells compared to control vectors Ad-LacZ. This confirms that COMPinh displaying vectors contain a functional protein IX.

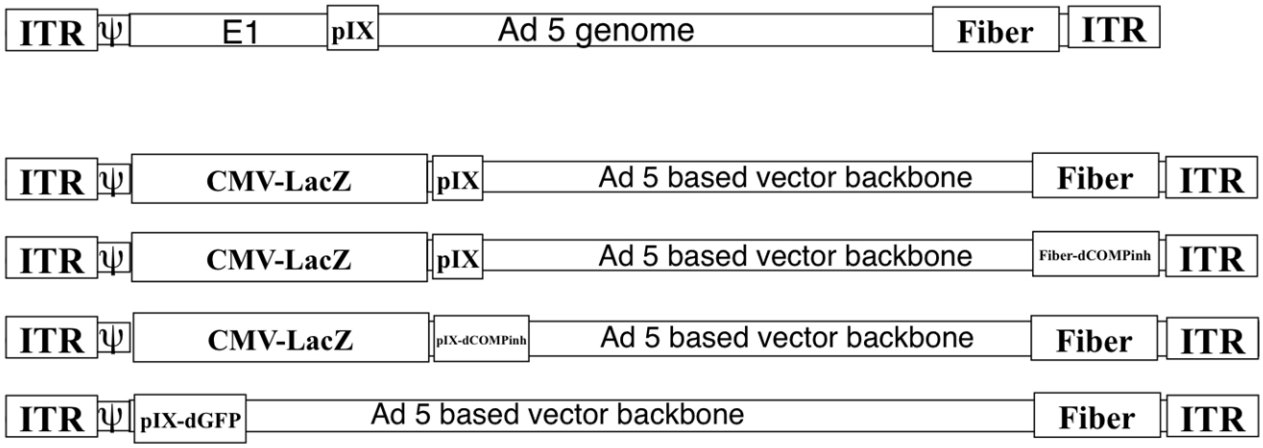
Capsid thermostability assay was performed according to previously described protocol [7-9] with modifications. 400000 HEK293 cells were plated in each well of a 24 well tissue culture plate. Ads were diluted from $CsCl_2$ purified stocks in complete media ($1.55x10^7$ vp in 500 µl of media) and were either not heat treated, or heated to 45° C or 56° C for 1 hour. Following incubation viruses were added to 293 cells. Following a 12 hour incubation, the cells were stained with X-gal [4,5] and the percentage of LacZ positive cells was determined for every sample. The results indicated that all Ads retained about 10-20% of LacZ positive cells after incubation at 45° C as compared to the non-heat treated Ads.

Complement activation AP50 serum-based assay: Alternative pathway 50 complement activation assay was performed as previously described [10] with modifications. NHS was diluted 1/5 in EGTA-$GVBS^{++}$ (8 mM EGTA) and mixed with the respective Ads ($2x10^{11}$ vp). NHS/Ad

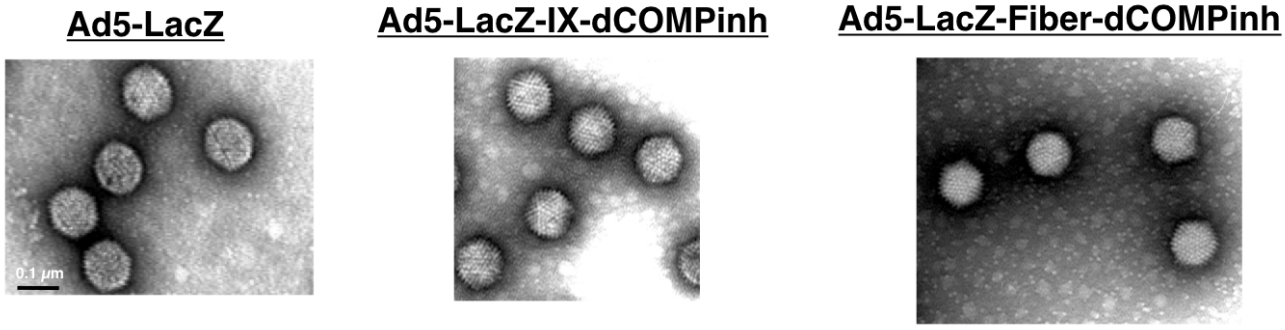
mixtures were incubated at 37° C for 30 min., and rabbit erythrocytes were added to each tube ($0.75x10^8$ in EGTA-$GVBS^{++}$)

After incubation at 37° C for 1 hour, 2 ml of EDTA-GVBS (10 mM EDTA) was added to each tube and tubes were centrifuged at 3500 rpm for 5 min. Following centrifugation absorbance of all tubes was read at 414 nm. All reagents including NHS (pooled from 30 healthy individuals) were purchased from Complement Technology Inc. (Tyler, TX). AP50 assay experiment was repeated 4 times yielding similar results. Data from one representative experiment are reported. In every experiment we have utilized N=3 technical replicates.

C3a-desArg ELISA: 100 µl of NHS was mixed with $5x10^9$ vp of each of the respective Ad vectors (final concentration of $5x10^{10}$ vp/ml of serum) and incubated at 37° C for 90 minutes. The reaction was then stopped by adding EDTA to a final concentration of 10 mM. C3a-desArg was then quantified using ELISA as per the manufacturer's instructions (Fitzgerald Industries Intl, former Research Diagnostic Inc., Concord, MA). C3a-desAgr ELISA was repeated 4 times and each experiment yielded similar results. Data from one representative experiment are reported. In every experiment we have utilized N=3 technical replicates.



Supplementary Fig.1. Schematic diagram of all Ad vectors constructed and utilized in our study. Genome maps of all Ads constructed are shown. Ad vectors were designed as described in Materials and Methods. Capsid protein IX and fiber are outlined as Ad capsid proteins utilized for fusion with COMPinh. Genome sizes are shown relative to WT Ad5 genome (top). Letter "d" prior to COMPinh or GFP defines that this peptide is "capsid-displayed". Note: genomes are not drawn to scale.



Supplementary Fig.2. Electron microscopy of purified Ad5 vectors. Cesium chloride purified Ad vectors were stained with 1% PTA and electron micrographs were taken, exactly as described in Materials and Methods. Icosahedral virion structures approximately 100 nm in diameter are clearly visible for all Ads. There is no significant amount of damaged capsids and/or free capsid proteins detected. Photographs were taken from representative areas from each sample.

Supplementary References

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