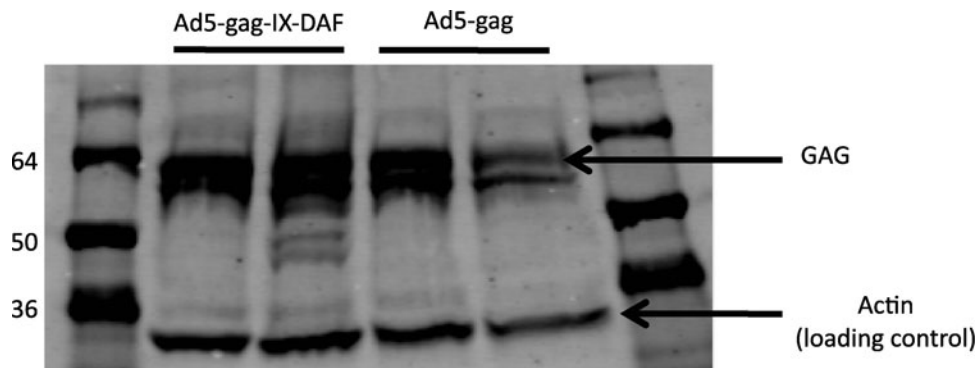


## Supplementary Data

SUPPLEMENTARY TABLE S1. Ad5 VECTORS WITH CAPSID-DISPLAYING DAF COMPLEMENT INHIBITOR EXPRESS TRANSGENE IN SPLEEN TISSUES WITH EFFICIENCY SIMILAR TO CONVENTIONAL Ad5-BASED VECTORS

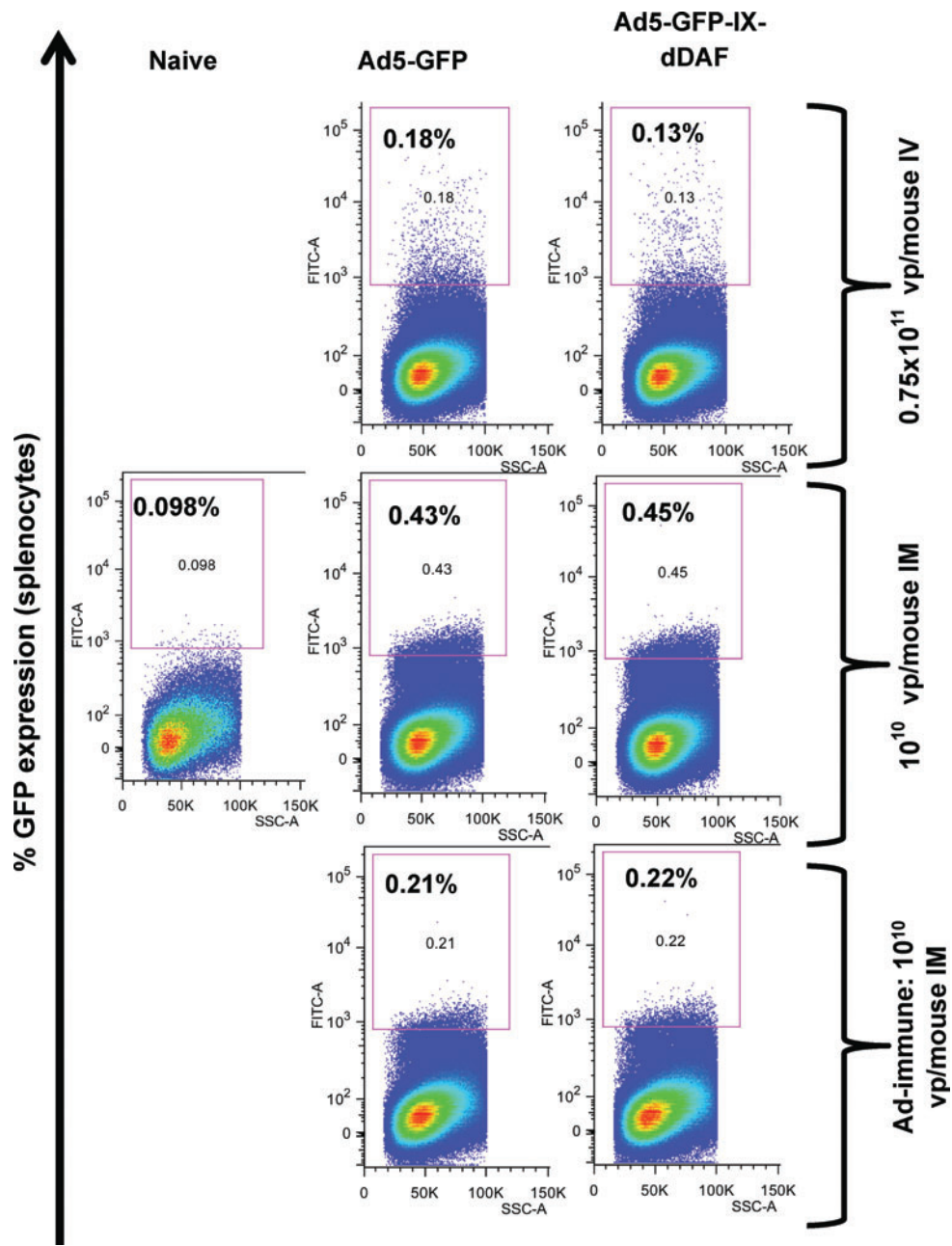
|                 | Ad5<br>preimmune<br>mice,            |                                     |                                     |
|-----------------|--------------------------------------|-------------------------------------|-------------------------------------|
|                 | IV $0.75 \times 10^{11}$<br>vp/mouse | IM $1.0 \times 10^{10}$<br>vp/mouse | IM $1.0 \times 10^{10}$<br>vp/mouse |
| Naïve           | $0.097 \pm 0.014$                    | $0.097 \pm 0.014$                   | $0.097 \pm 0.014$                   |
| Ad5-GFP         | $0.17 \pm 0.03$                      | $0.41 \pm 0.06$                     | $0.21 \pm 0.08$                     |
| Ad5-GFP-IX-Ddaf | $0.13 \pm 0.02$                      | $0.45 \pm 0.07$                     | $0.23 \pm 0.06$                     |

BALB/c mice were left untreated (naive,  $n = 3$ ) or injected IV with  $0.75 \times 10^{11}$  vp/mouse of Ad5-GFP ( $n = 3$ ) or Ad5-GFP-IX-dDAF ( $n = 3$ ). Additional mice were injected IM with  $10^{10}$  vp/mouse of the same vectors ( $n = 3$ ) comprising the Ad-naïve group, whereas a third set of mice was preimmunized with  $10^{10}$  vp/mouse of Ad5-Null (twice, 14 days apart), followed by IM injection of Ad5-GFP ( $n = 3$ ) or Ad5-GFP-IX-dDAF ( $n = 3$ ). All mice were sacrificed 24 h post injection, and splenocytes were prepared and analyzed by flow cytometry (FITC channel). The numbers represent means  $\pm$  SD. Statistical analysis was completed using two-tailed Student's *t* test to compare two groups of virus-injected animals. No significant differences were found.

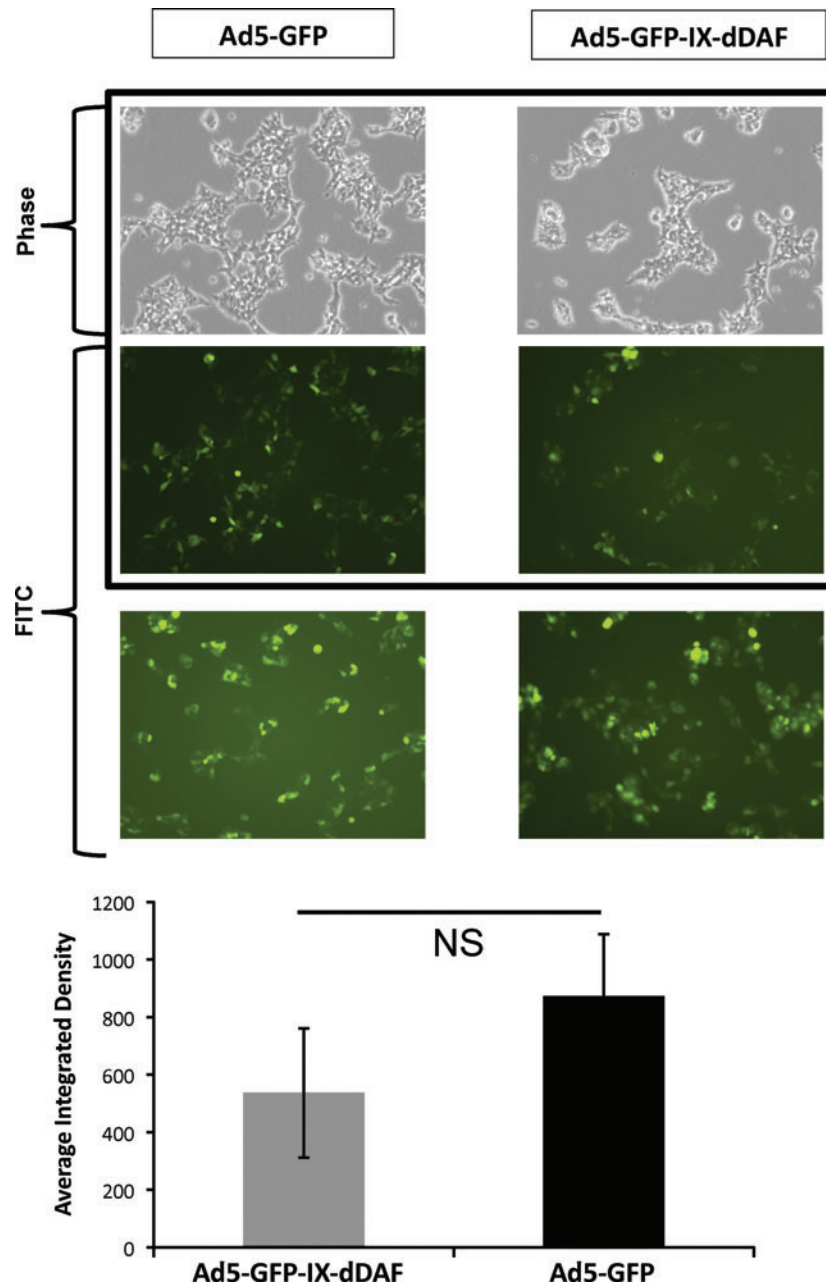


| Vector          | Fold over Ad5-gag |
|-----------------|-------------------|
| Ad5-gag         | $1.00 \pm 0.03$   |
| Ad5-gag-IX-dDAF | $1.04 \pm 0.11$   |

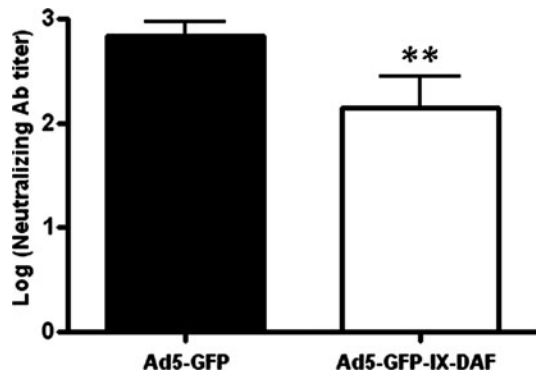
**SUPPLEMENTARY FIG. S1.** Ad vectors with capsid-displaying DAF allow for efficient transgene expression in liver tissues for at least 28 days, at levels identical to those of conventional Ad vectors. Liver tissues derived from mice, injected with medium dose of Ad vectors ( $0.75 \times 10^{11}$  vp/mouse), were snap-frozen at 28 dpi, protein samples were prepared, and western blotting with GAG- or actin-specific antibodies was performed. Representative samples are shown.



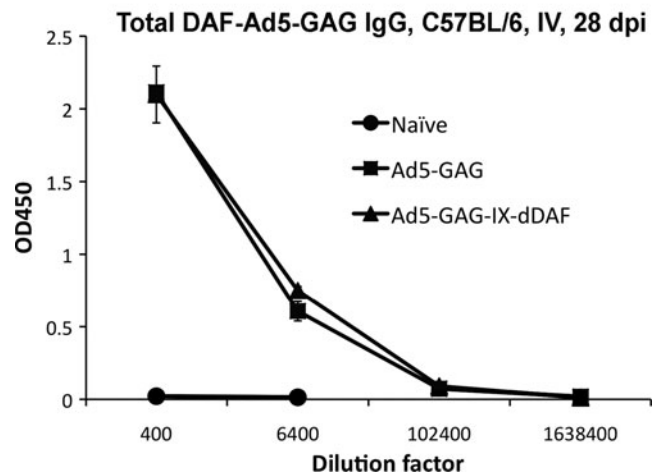
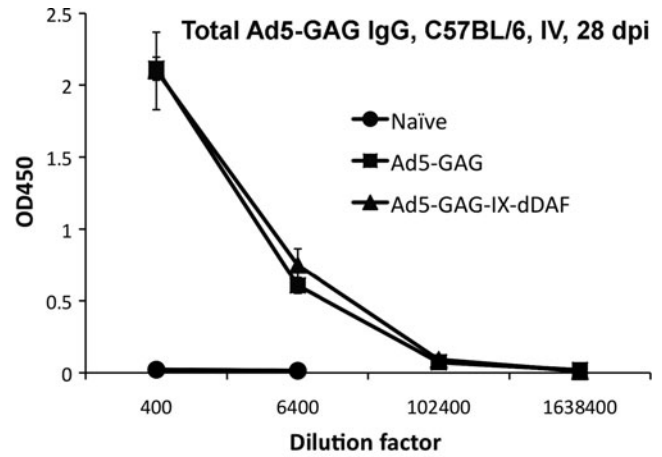
**SUPPLEMENTARY FIG. S2.** Ad5 vectors with capsid-displaying DAF complement inhibitor express transgene in spleen tissues with efficiency similar to that of conventional Ad5-based vectors. BALB/c mice were injected with Ad5-GFP or Ad5-GFP-IX-dDAF IV ( $0.75 \times 10^{11}$  vp/mouse) or IM ( $10^{10}$  vp/mouse). Preimmunized BALB/c mice were injected IM. At 24 hpi, splenocytes were isolated and analyzed by flow cytometry. Representative plots are shown.



**SUPPLEMENTARY FIG. S3.** Ad5 vectors with capsid-displaying DAF complement inhibitor express transgene with efficiency similar to that of conventional Ad5-based vectors *in vitro*. HEK293 cells were infected with 100–1,000 vp/cell of Ad5-GFP or Ad5-GFP-IX-dDAF, and GFP fluorescence was measured at 6 hpi (data for multiplicity of infection 500 are presented). Note figures in the box were taken for the same field: phase and FITC. Almost 100% cells express GFP; quantification was performed as average integrated density. The numbers represent means  $\pm$  SD. Statistical analysis was completed using two-tailed Student's *t* test to compare two groups of virus-injected animals. No significant differences were found.



**SUPPLEMENTARY FIG. S4.** Mice injected with the retro-DAF-displaying Ads generate significantly reduced titers of Ad NABs. WT C57BL/6 were injected with  $0.75 \times 10^{11}$  vp/mouse IV. At 28 dpi, plasma samples were collected from naive ( $n=2$ ) and Ad5-GFP ( $n=4$ )– and Ad5-GFP-IX-dDAF ( $n=4$ )–injected mice. Ad5-specific NABs were measured as detailed in Materials and Methods. Log of highest plasma dilution yielding >50% neutralization is graphed. Columns represent means  $\pm$  SD. Statistical analysis was completed using two-tailed Student's *t* test to compare two Ad-injected groups (\*\* $p < 0.01$ ).



**SUPPLEMENTARY FIG. S5.** Retro-DAF–displaying Ads do not generate reduced humoral responses to the Ad capsid or the transgene, when administered IV in Ad-naïve mice. Plasma from naive ( $n=2$ ), IV administered conventional Ad5-treated ( $n=4$ ), or retro-DAF-displaying Ad5-treated ( $n=4$ ) C57BL/6 mice was collected at 28 dpi, and Ad5-GFP (*top*) or Ad5-GFP-IX-dDAF (*bottom*) specific total IgG was measured by ELISA. The error bars represent  $\pm$  SD. Statistical analysis was completed using two-tailed Student's *t* test to compare two groups of virus-injected animals (no significant differences were detected). Note the identical results obtained when Ad5-GFP or Ad5-GFP-IX-dDAF was used for plate coating.