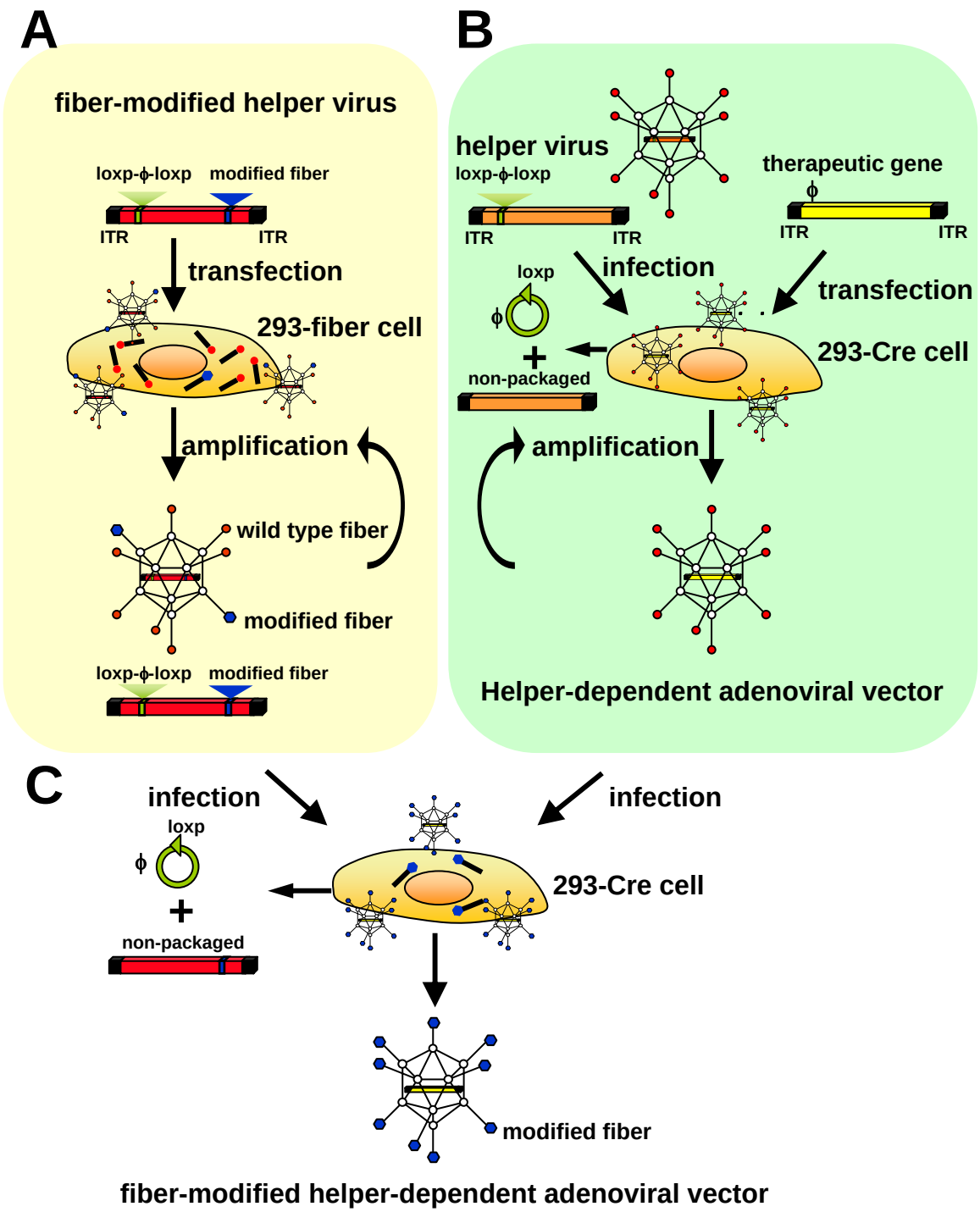
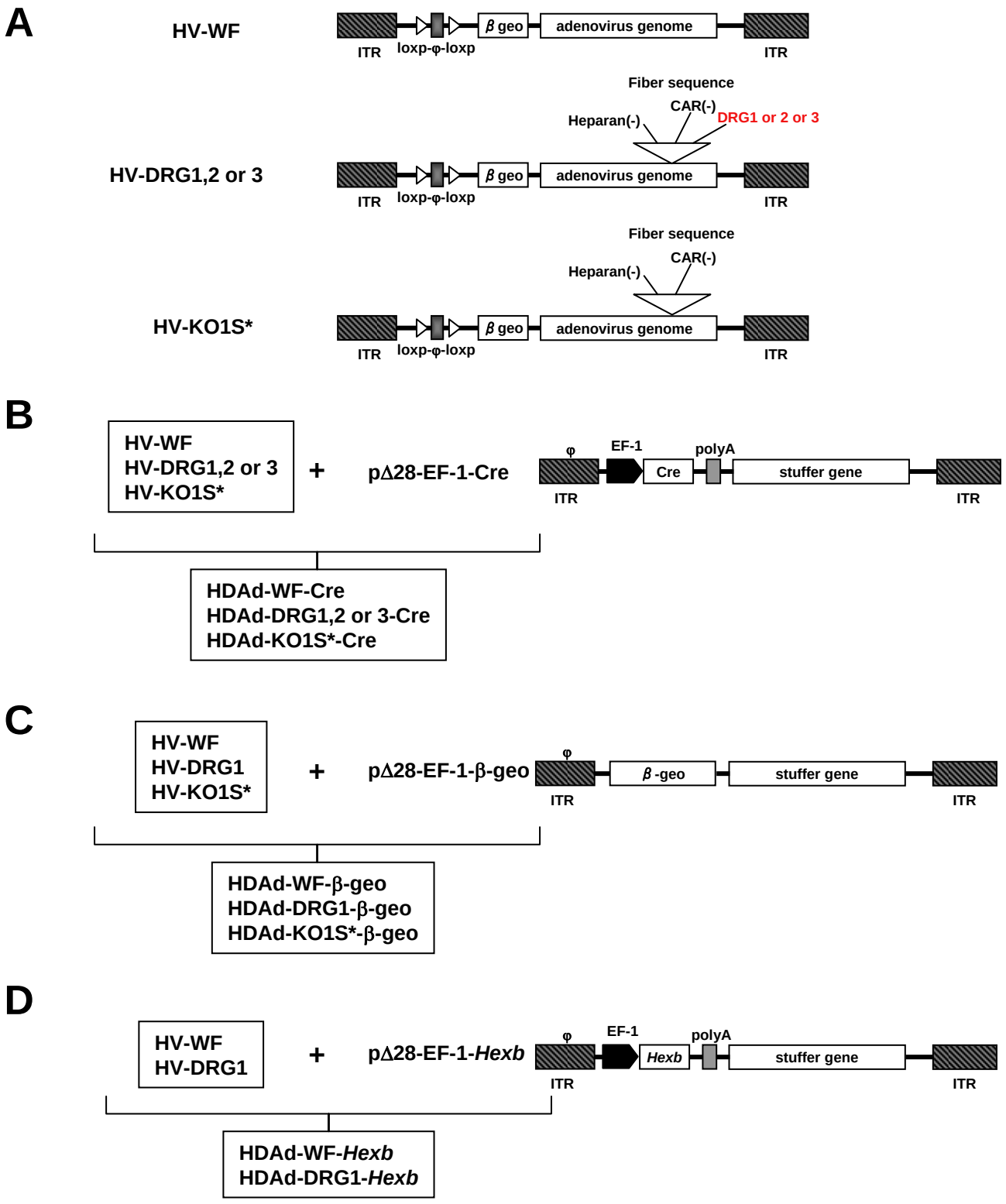
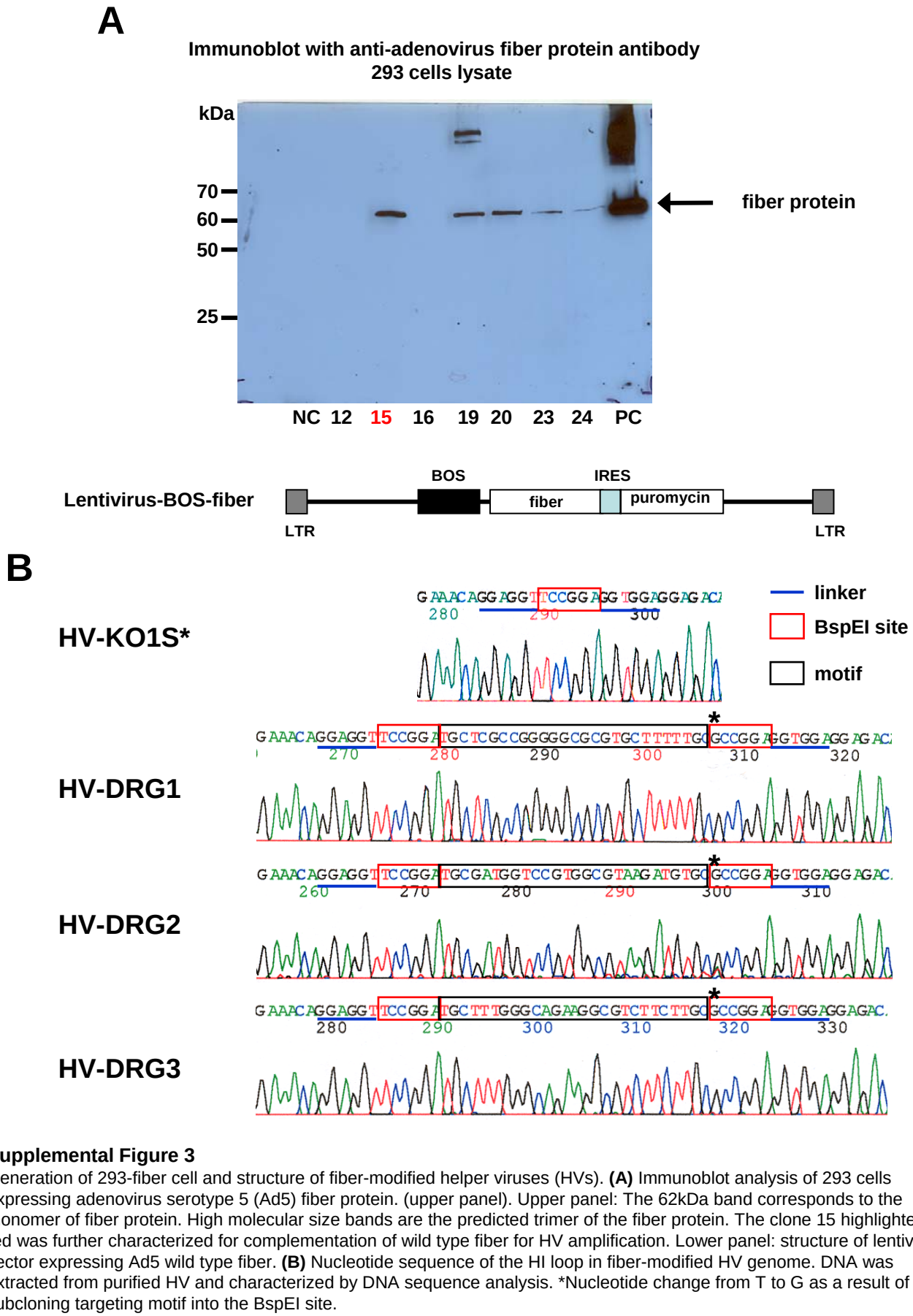


Supplemental Figure 1
Strategy for construction of fiber-modified helper-dependent adenoviral vectors (HDAd). (**A**) Construction of fiber-modified helper virus (HV). HV is a first generation E1-deleted Ad vector containing the packaging signal flanked by *loxp* sequences. This HV provides all of the components necessary for replication and packaging of HDAd genome *in trans*, but cannot be packaged by itself upon coinfection of Cre expressing packaging cells with HDAd due to the excision of the packaging signal for HV. The first step to construct targeted HDAd is to produce fiber-modified HV. In our approach, HV is detargeted by ablation of primary docking sites, CAR and HSPG, and then adding a targeting peptide ligand. HV produced by transfection contains no primary docking sites to 293 cells for infection and is unable to reinfect for amplification. To overcome this problem, we established 293 cells expressing Ad serotype 5 wild type fiber (293-fiber). HV produced on 293-fiber has both wild type and modified fiber and can infect 293 cells effectively while the modified fiber genome is maintained. But, the wild type fiber can be removed by infection of 293 cells. (**B**) Construction of HDAd. The genome of HDAd contains only essential *cis*-acting elements (inverted terminal repeats for replication and the packaging signal) and is introduced into packaging cells expressing Cre by transfection. By coinfection with HV containing wild type fiber, HDAd genome is packaged into Ad particles. HV continues to provide all of the components necessary for replication and packaging, but the HV genome cannot be packaged. (**C**) Generation of fiber-modified HDAd. Final step of production of fiber-modified HDAd is coinfection of 293Cre with HDAd and HV made on 293-fiber cells. HDAd genome is packaged into viral particles encoded by HV genome, which results in HDAd capsids containing modified fiber.

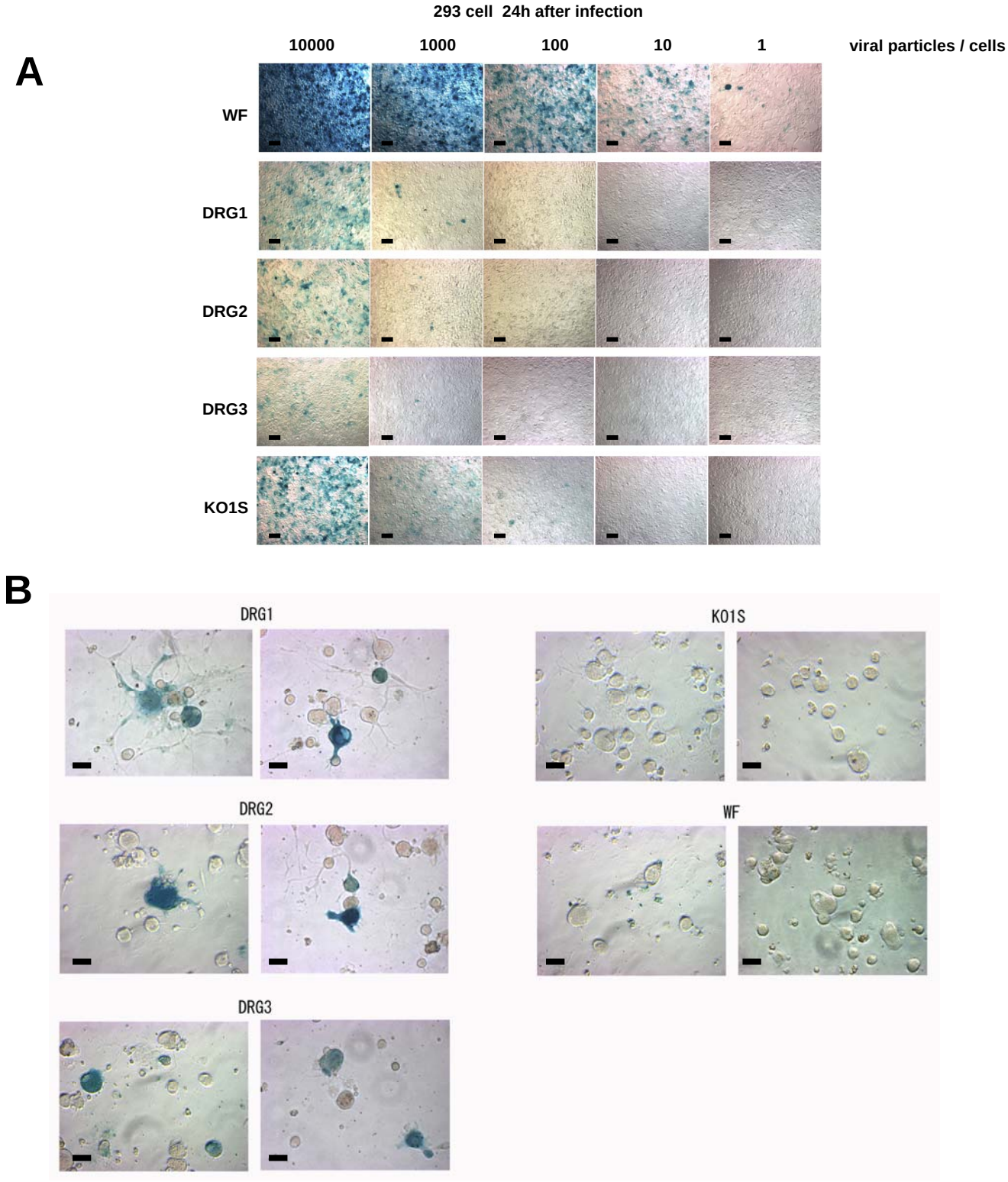


Supplemental Figure 2
Structures of fiber-modified helper virus (HV) and helper-dependent adenovirus (HDAd). (**A**) Fiber-modified HVs : HV-WF contains Ad5 wild type fiber, HV-DRG1,2 or 3 have DRG motif in KO1S* backbone fiber and HV-KO1S* has no peptide motif. (**B**) Fiber-modified HDAds expressing Cre for ROSA-GFP mice. The HDAds were produced with each type of HV. (**C**) Fiber-modified HDAds expressing β-geo for transduction and inflammatory study. The HDAds were produced with each type of HV. (**D**) Fiber-modified HDAds expressing *Hexb* for gene therapy of *Hexb* mice. The HDAds were produced with each type of HV.



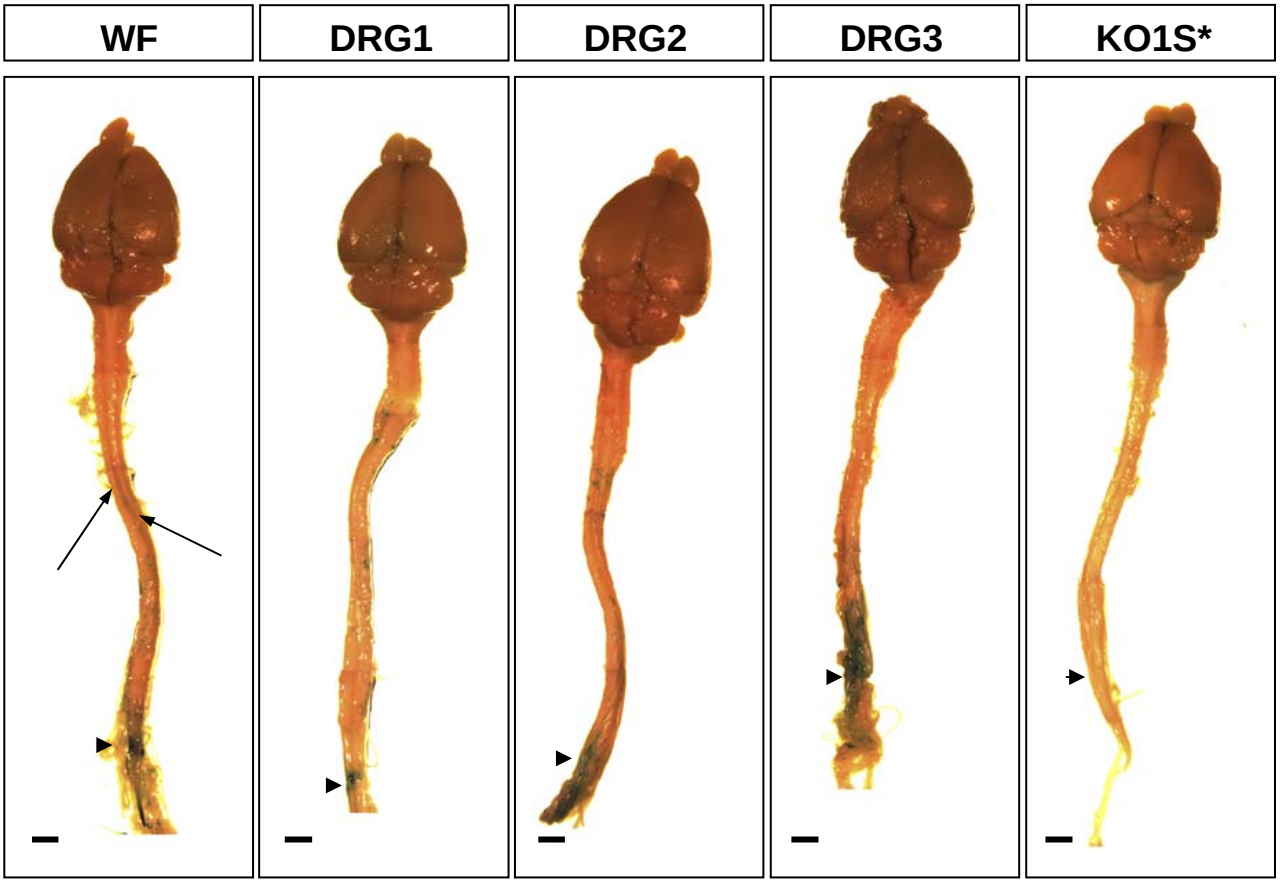
Supplemental Figure 3

Generation of 293-fiber cell and structure of fiber-modified helper viruses (HVs). **(A)** Immunoblot analysis of 293 cells expressing adenovirus serotype 5 (Ad5) fiber protein. (upper panel). Upper panel: The 62kDa band corresponds to the monomer of fiber protein. High molecular size bands are the predicted trimer of the fiber protein. The clone 15 highlighted in red was further characterized for complementation of wild type fiber for HV amplification. Lower panel: structure of lentiviral vector expressing Ad5 wild type fiber. **(B)** Nucleotide sequence of the HI loop in fiber-modified HV genome. DNA was extracted from purified HV and characterized by DNA sequence analysis. *Nucleotide change from T to G as a result of subcloning targeting motif into the BspEI site.

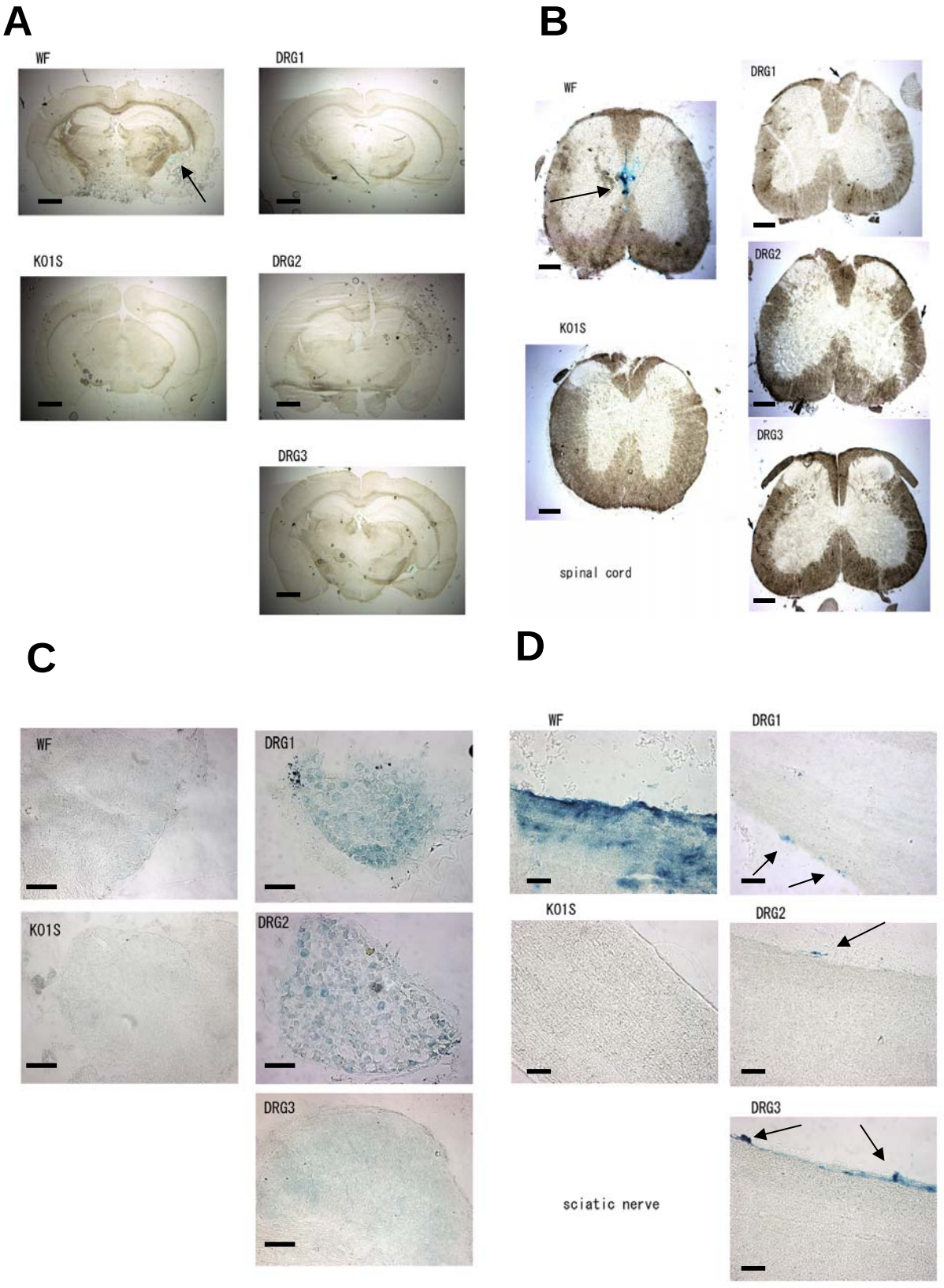


Supplemental Figure 4

LacZ expression in cells following infection with fiber-modified helper virus *in vitro*. **(A)** X-gal stain of 293 cells 24 hours after infection with fiber-modified HVs at 1- 1 x 10^4 vp/cell. Bar, 50μm. **(B)** X-gal stain of DRG neurons. Bar, 20μm.

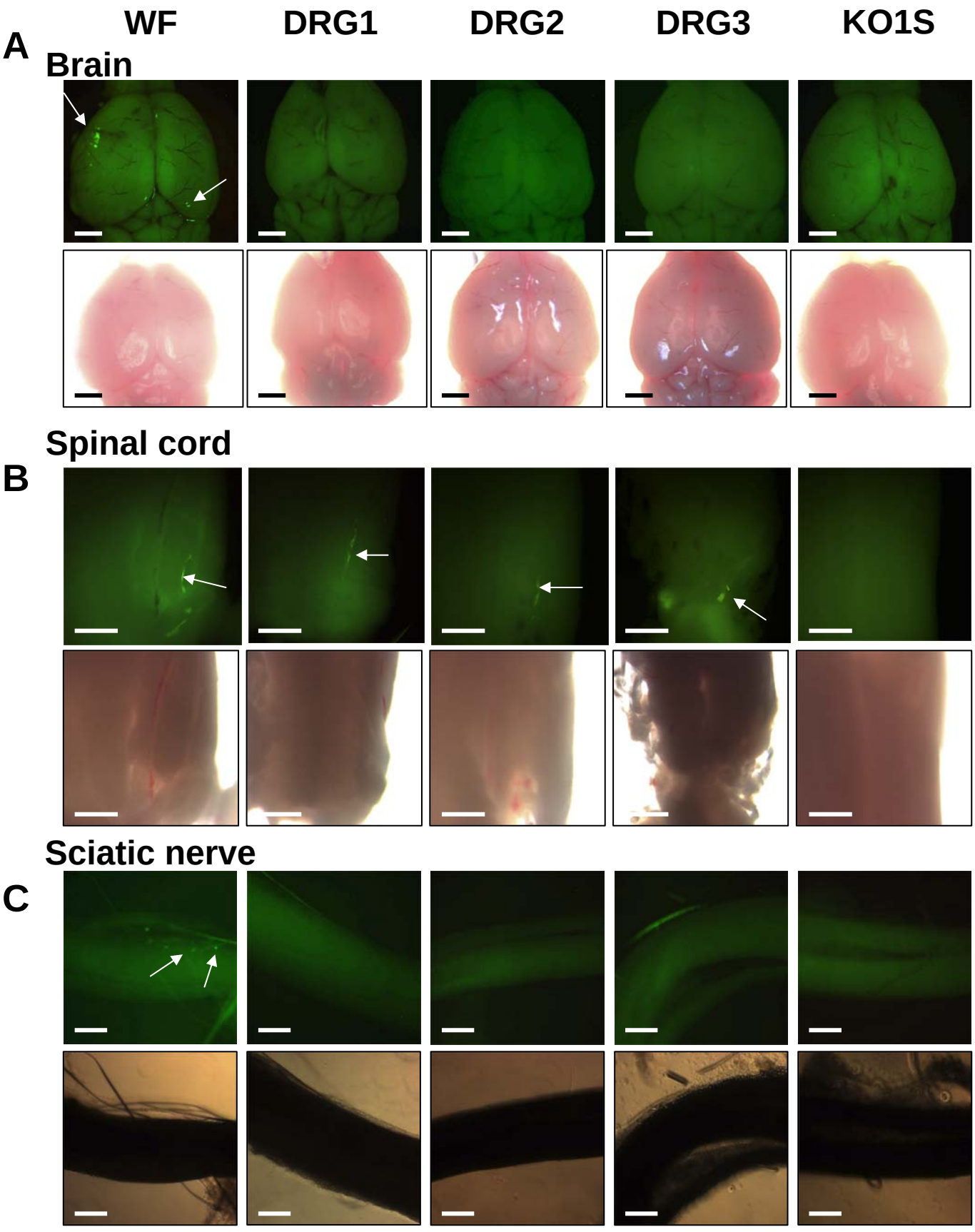


Supplemental Figure 5
LacZ expression in central nervous system. 1X10^8 vp of fiber modified HVs were injected into C57BL/6 mice through subarachnoid space at lumbar level. Mice were sacrificed 5 days after HV injection and stained for *LacZ* expression. Scale bar, 2mm. Arrows in the WF group indicate X-gal positive areas at dorsal intermediate sulcus of spinal cord. Arrowhead shows the position of vector injection.

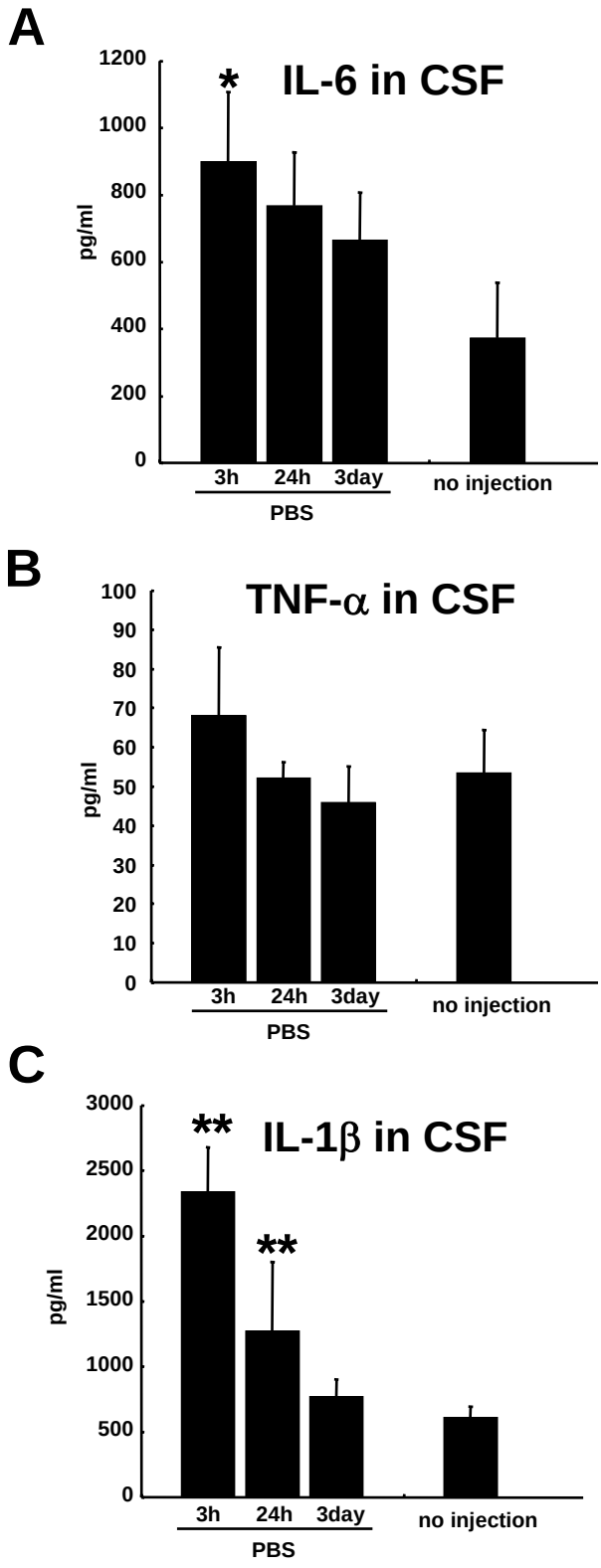


Supplemental Figure 6

X-gal stain of nervous tissues. 1 x 10^8 vp of fiber-modified HVs were injected into wild type C57/BL6 mice via subarachnoid space at the lumbar level. Mice were sacrificed for X-gal staining 5 days after HV injection. **(A)** Brain. Arrow shows X-gal stain in the lateral ventricular region. Bar, 1200μm. **(B)** Spinal cord. Arrow shows central canal of spinal cord. Bar, 250μm. **(C)** DRG. Bar, 100μm. **(D)** Sciatic nerve. Arrows indicate X-gal positive areas at the surface of nerves. Bar, 50μm.

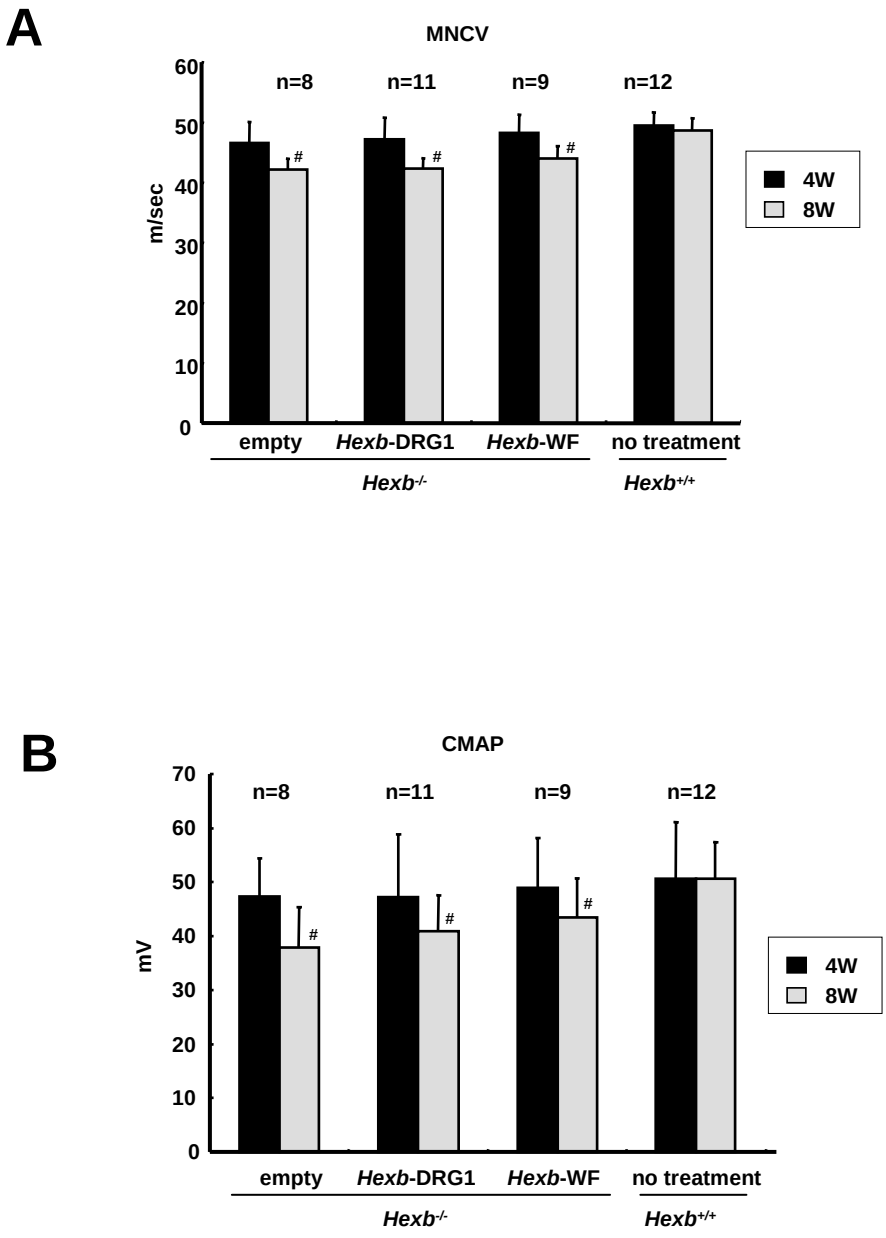


Supplemental Figure 7
Histological analysis of GFP expression in brain, spinal cord and sciatic nerve isolated from ROSA-GFP mice after administration of fiber-modified HDAd. **(A-C)** Whole tissue image. First row shows the image under GFP filter. Second row shows the same area under bright field. Bar, 2mm in **A**, 500μm in **B**, 200μm in **C**.



Supplemental Figure 8

Cytokine levels in cerebrospinal fluid (CSF). We injected PBS to C57BL/6 mice through subarachnoid space at lumbar level. CSF was collected at 3 hour, 24 hour and day3. Cytokines were measured by ELISA. **A**. IL-6, **B**. TNF-a and **C**. IL-1b. **P* <0.01 and ***P* <0.05 vs. no injection (n=3-4).



Supplemental Figure 9
Motor nerve conduction velocity (MNCV) and compound muscle action potential (CMAP) in *Hexb*–/– mice after treatment with fiber-modified HDAd expressing *Hexb*. **(A,B)** Measurement was performed at 4 and 8 weeks after injection. $^{\#}P$ <0.05 vs. *Hexb*+/+ mice (n=8-12)