

Figure W1. Bead internalization by ED1⁺ Iba1⁺ cells. Three days after treatment with Ad.TK + Ad.Flt3L, tumor-bearing rats were injected intratumorally with fluorescent microbeads. Two days later, the brains' immune cells were detected by immunofluorescence. (A) Arrows pointing cells containing beads within the brain tumor. Broken line indicates tumor border. Inset shows high-magnification microphotograph of cells containing beads within the tumor mass. (B) Confocal microphotograph showing tumor-infiltrating cells expressing the macrophage and microglia cell markers ED1 (red) and Iba1 (green) and containing beads (white) in the cytoplasm. Nuclei were stained with DAPI (blue).

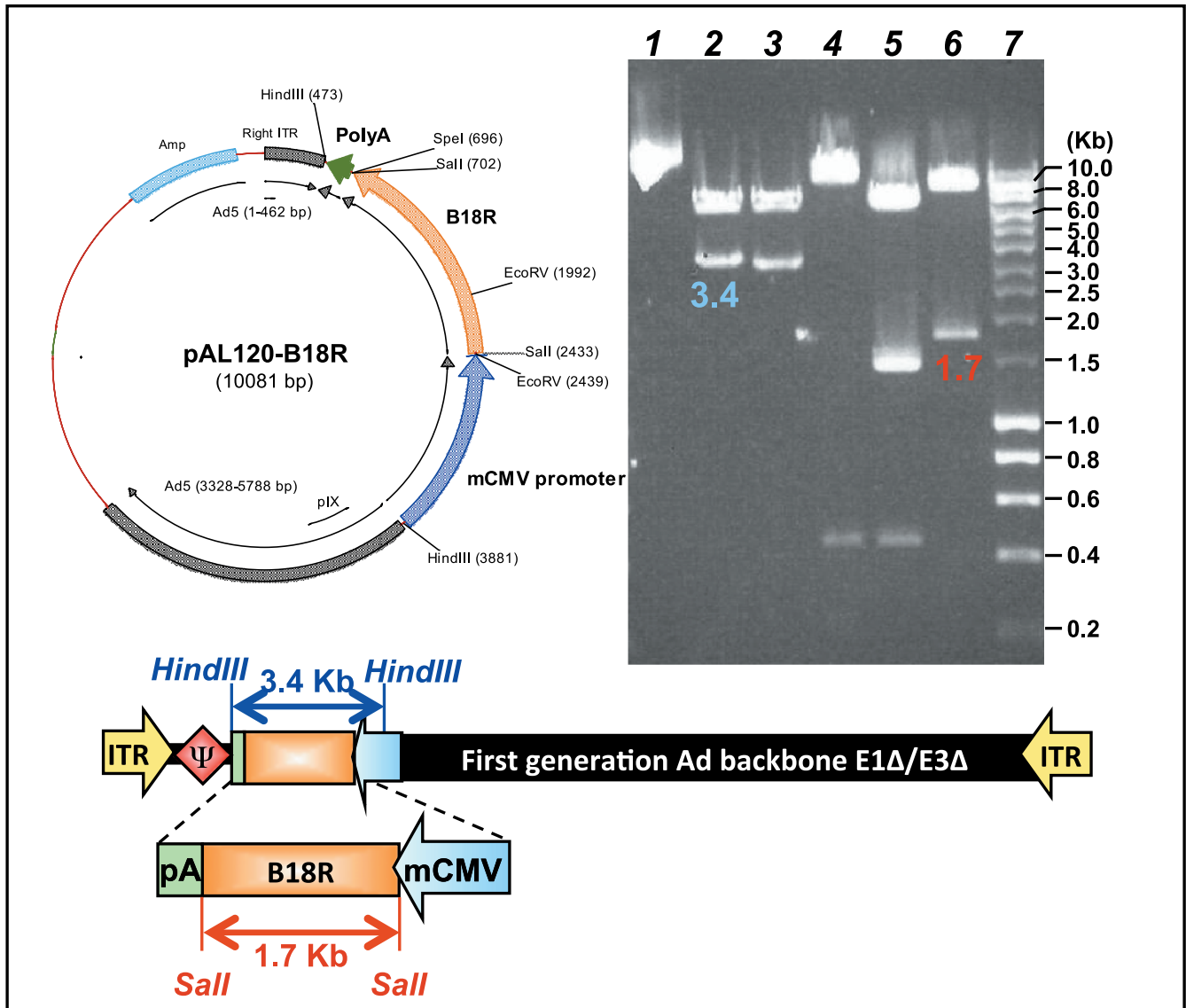


Figure W2. Construction of Ad.B18R. Adenoviral shuttle plasmid pAL120-B18R encoding the Vaccinia virus B18R cDNA (orange) that encodes a decoy receptor for IFN- α , which inhibits its function. Transgene expression is driven by the powerful murine cytomegalovirus promoter (mCMV, blue). Important restriction sites are indicated. Agarose gel electrophoresis and restriction map analysis of pAL120-B18R plasmid DNA to check for expected band sizes are shown. Lanes are as follows: lane 1, Hyladder 10 kb (Denville Scientific, Metuchen, NJ; corresponding sizes are labeled to the right of the gel); lane 2, uncut; lane 3, *HindIII*; lane 4, *HindIII* + *SpeI*; lane 5, *EcoRV*; lane 6, *EcoRV* + *HindIII*; lane 7, *SalI*; lane 8, hyperladder 1. The colored fragments correspond to the indicated fragments in the schematic of Ad-mCMV-B18R vector genome linear depiction.

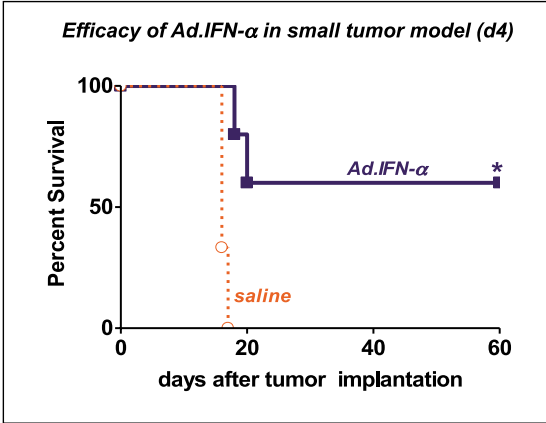


Figure W3. Efficacy of intratumoral injection of Ad.TK in combination with Ad.IFN- α . Tumor-bearing rats were treated 4 days after tumor implantation by intratumoral injection of saline or Ad.IFN- α or saline. * $P < .05$ versus saline. Mantel log-rank test.

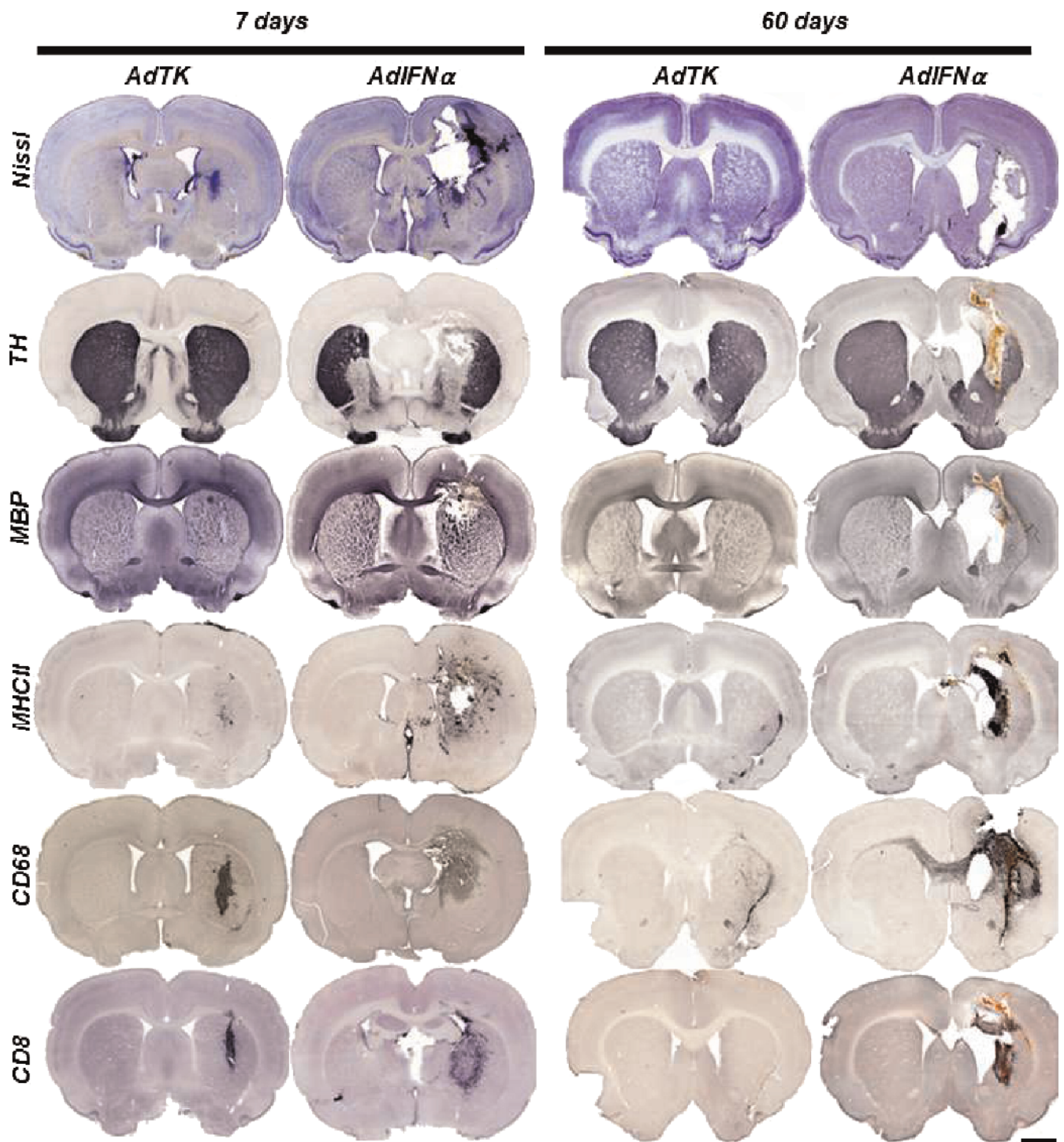


Figure W4. Acute neurotoxicity after Ad.IFN- α injection in naive rat brain. Naive Lewis rats were injected in the striatum with Ad.IFN- α or Ad.TK. Rats treated with Ad.TK received GCV treatment daily. Seven and 60 days after vector delivery, neuropathologic analysis of the brain was assessed by Nissl staining and immunocytochemistry using antibodies against TH, MBP, MHC II, CD68 (macrophages/activated microglia), and CD8 α (T cells). Scale bar, 2 mm.

Neuropathology 60 days after injection in naive brain

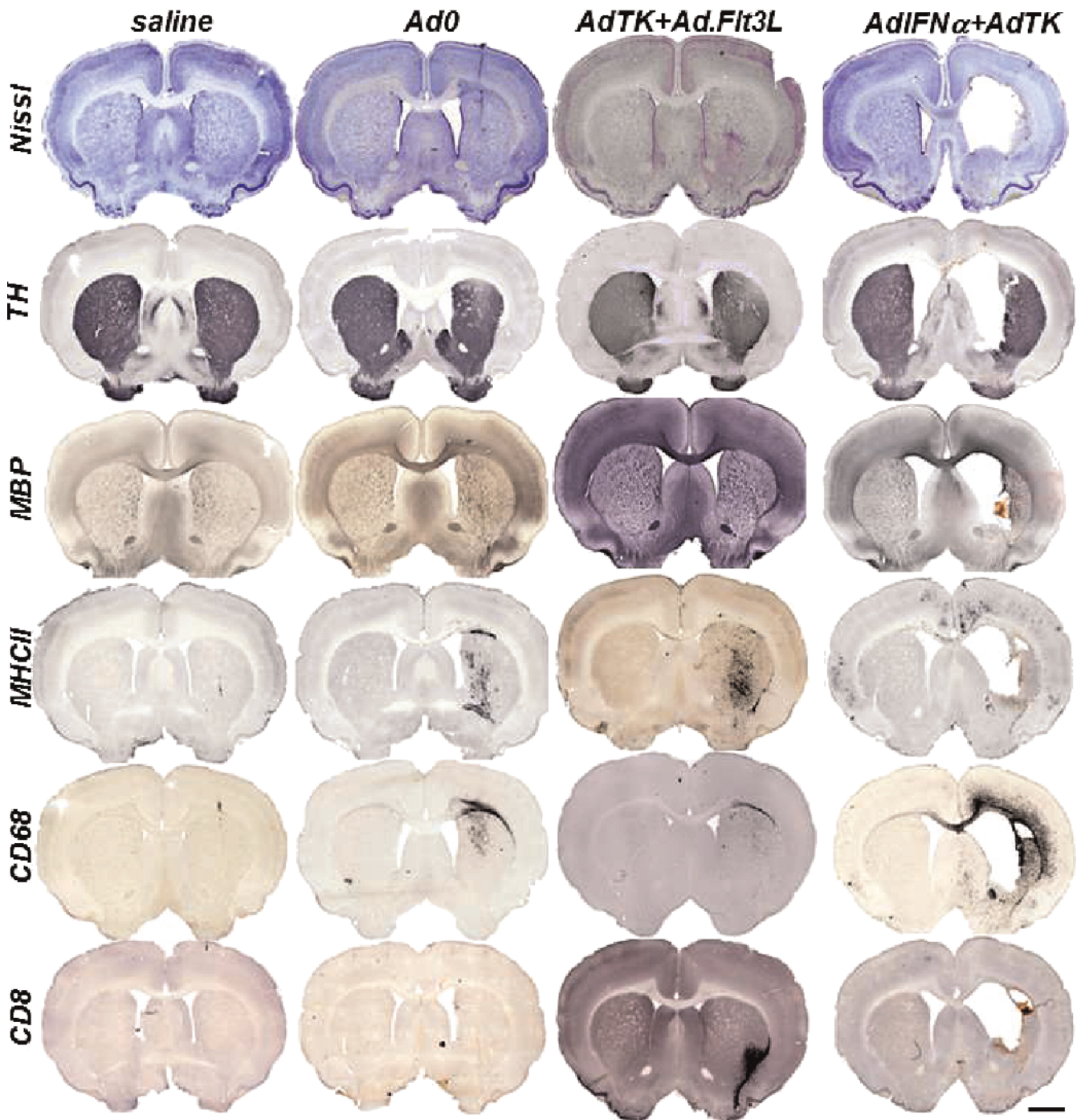


Figure W5. Chronic neuropathologic changes after Ad.IFN- α injection in naive rat brain. Naive Lewis rats were injected in the striatum with Ad.IFN- α or Ad.Flt3L and Ad-TK. As controls, rats received saline or Ad.0. Rats treated with Ad-TK received GCV treatment daily. Sixty days after vector delivery, neuropathologic analysis of the brain was assessed by Nissl staining and immunocytochemistry using antibodies against TH, MBP, MHC II, CD68 (macrophages/activated microglia) and CD8 α (T cells). Scale bar, 2 mm.