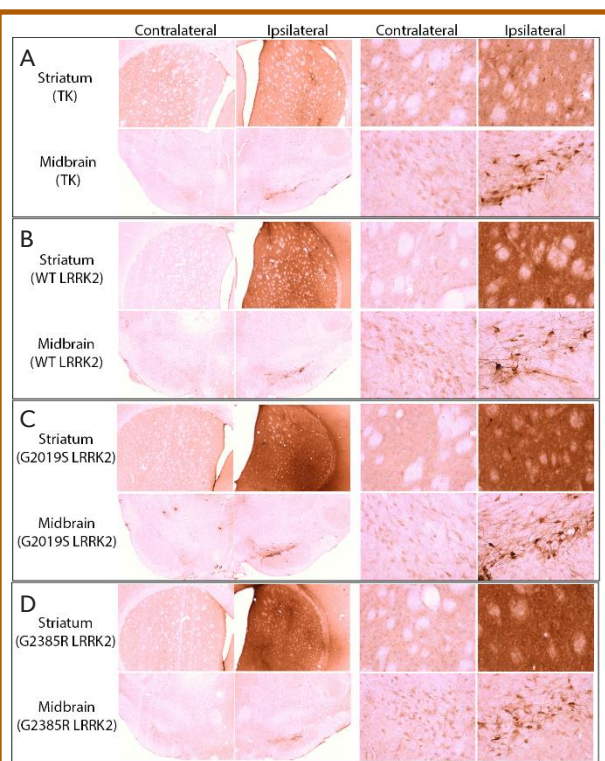


## LRRK2 OVEREXPRESSION VIRAL VECTORS FOR IN VIVO USE

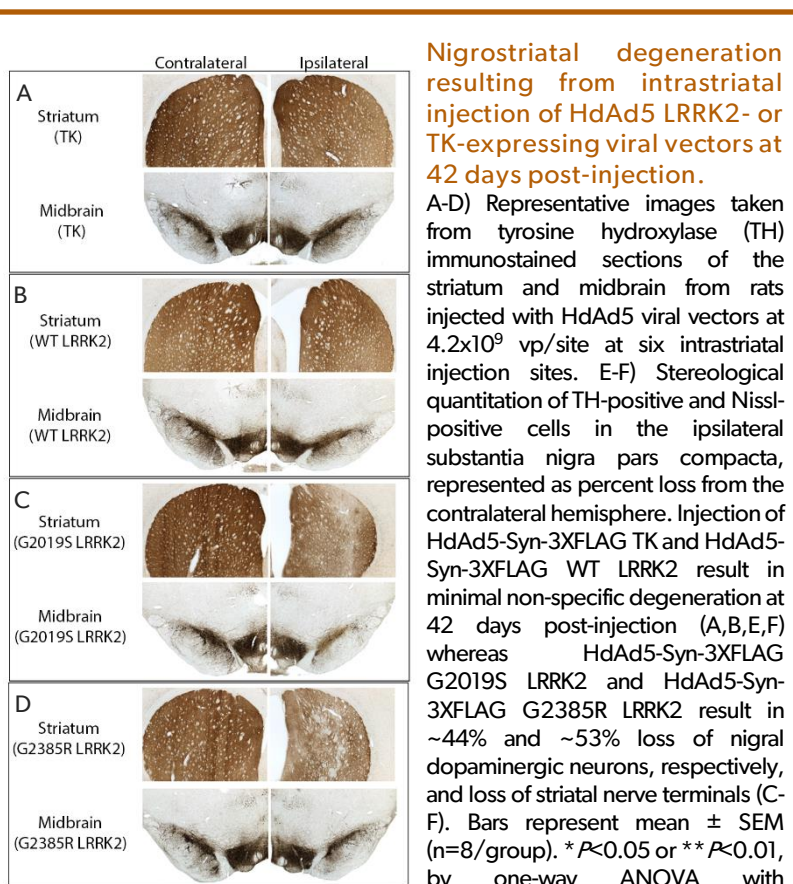
MJFF generated viral vectors expressing 3xFLAG-tagged LRRK2 (WT, G2019S, or G2385R) and 3xFLAG-tagged thymidine kinase (TK) for use in vivo. Expression is driven by the human synapsin promoter. Viral vectors were designed and generated by Dr Sam Young at the University of Iowa, characterized by Dr Darren Moore at the Van Andel Research Institute, and are available for purchase at the University of Iowa Vector Core.

	Particle Count (vp/mL)	RCA Probe -E1a (Purified Virus Copies/mL)	Viral Particle: Infectious Unit (VP:IU)	HdAd Infectivity (IU/mL)	Helper Virus Contamination (%)
HdAd5 - Syn - 3XFLAG TK	7.54E+12	0	142	5.32E+10	0.03
HdAd5 - Syn - 3XFLAG WT LRRK2	6.94E+12	0	103	6.76E+10	0.04
HdAd5 - Syn - 3XFLAG G2019S LRRK2	4.08E+12	0	76	5.38E+10	0.05
HdAd5 - Syn - 3XFLAG G2385R LRRK2	5.63E+12	0	90	6.26E+10	0.04



**Figure 1. HdAd5 transgene expression in the striatum and midbrain at 42 days post-intrastratial injection.**

Representative images from anti-FLAG immunostained sections of the striatum and ventral midbrain from adult female Wistar rats injected with HdAd5 viral vectors at  $4.2 \times 10^9$  vp/site (in 2ul) at six intrastratial injection sites in a single coronal plane. Low magnification images of the injected (ipsilateral) and uninjected (contralateral) hemispheres appear on the left. High magnification images of the ipsilateral and contralateral hemispheres appear on the right. Injection of HdAd5-Syn-3XFLAG TK (A), HdAd5-Syn-3XFLAG WT LRRK2 (B), HdAd5-Syn-3XFLAG G2019S LRRK2 (C), and HdAd5-Syn-3XFLAG G2385R LRRK2 (D) resulted in robust expression, as detected using the FLAG tag, throughout the injected striatum as well as within the dopaminergic cell bodies in the substantia nigra pars compacta in the midbrain.



**Nigrostriatal degeneration resulting from intrastratial injection of HdAd5 LRRK2- or TK-expressing viral vectors at 42 days post-injection.**

A-D) Representative images taken from tyrosine hydroxylase (TH) immunostained sections of the striatum and midbrain from rats injected with HdAd5 viral vectors at  $4.2 \times 10^9$  vp/site at six intrastratial injection sites. E-F) Stereological quantitation of TH-positive and Nissl-positive cells in the ipsilateral substantia nigra pars compacta, represented as percent loss from the contralateral hemisphere. Injection of HdAd5-Syn-3XFLAG TK and HdAd5-Syn-3XFLAG WT LRRK2 result in minimal non-specific degeneration at 42 days post-injection (A,B,E,F) whereas HdAd5-Syn-3XFLAG G2019S LRRK2 and HdAd5-Syn-3XFLAG G2385R LRRK2 result in ~44% and ~53% loss of nigral dopaminergic neurons, respectively, and loss of striatal nerve terminals (C-F). Bars represent mean  $\pm$  SEM ( $n=8$ /group). \* $P < 0.05$  or \*\* $P < 0.01$ , by one-way ANOVA with Bonferroni's post-hoc test.

