

ALPHA-SYNUCLEIN KNOCKDOWN VIRAL VECTORS

In partnership with the [Industry Research Tools Consortium](#), MJFF generated viral vectors expressing micro-RNA (miR) to knock down expression of mouse or human aSyn—including wildtype and common pathogenic mutants (A30P, E46K, A53T) of this protein. Viral vectors also express GFP as a non-toxic reporter protein to enable easy analysis of transduction efficiency. Expression is driven by the chicken beta-actin promoter hybridized with the cytomegalovirus early enhance sequence (CAG) to ensure transduction of various cell types, with enhancement by the woodchuck post-transcriptional regulatory element (WPRE) and bovine growth hormone polyadenylation sequence (BGH-polyA) to drive high expression. Viral vectors were designed, generated, and validated by GeneDetect and are available for purchase at [Vigene Biosciences](#).

aSyn Target	Viral Vector Nomenclature	<i>in vitro</i> Knockdown (Fig 1)	<i>in vivo</i> Knockdown (Fig 2)	Vigene Catalog #
miR to Human aSyn	AAV1/2-CAG-Human <i>SNCA</i> 3xmiR/GFP-WPRE-BGH-polyA	~75%	~100%	GD1009-RV-H
miR to Mouse aSyn	AAV1/2-CAG-Mouse <i>SNCA</i> 3xmiR/GFP-WPRE-BGH-polyA	~75%	~100%	GD1009-RV-M
Scrambled Control miR	AAV1/2-CAG-Scrambled Control 3xmiR/GFP-WPRE-BGH-polyA	~0%	~0%	GD1009-RV-C

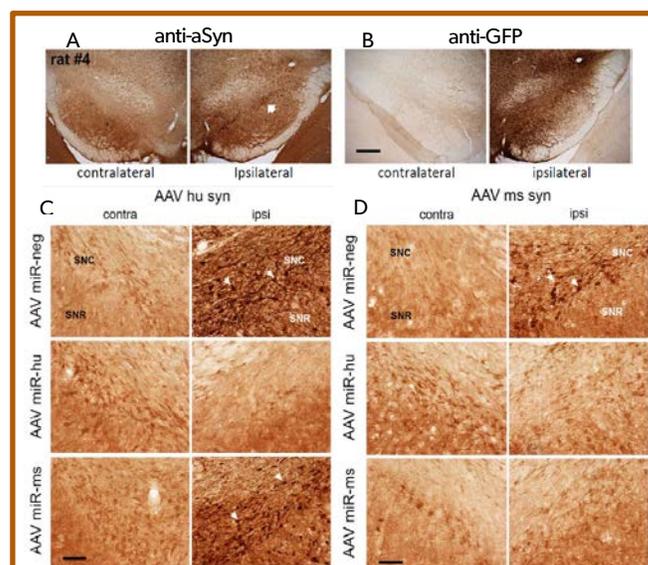
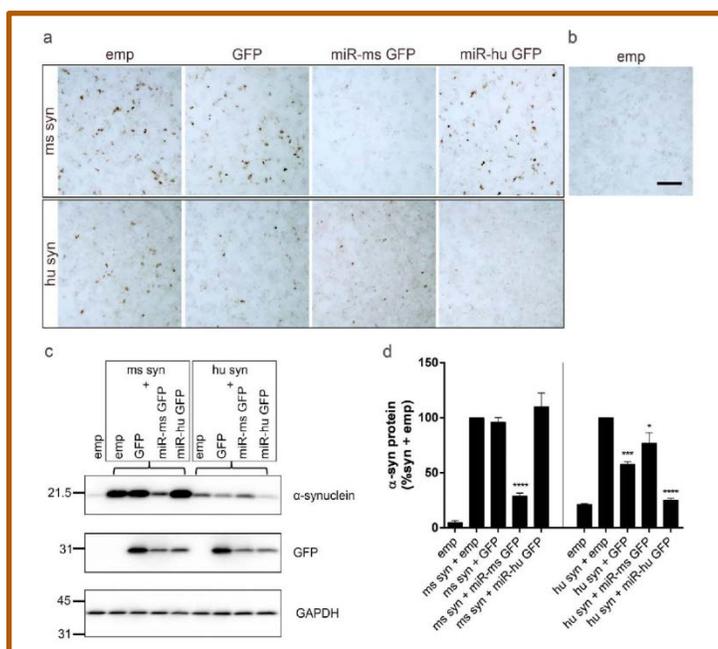


Figure 2. Transduction efficiency and aSyn knockdown after co-infusion of aSyn-overexpressing viral vectors with *SNCA* miR/GFP viral vectors in rat substantia nigra pars compacta (SNC). A-B) Validation of transduction efficiency with the human aSyn-overexpressing viral vector and the scrambled control miR viral vector at three weeks post-co-infusion. Scale bar = 500µm, arrow indicates aSyn overexpression in the SNC. A) aSyn-overexpressing viral vectors result in detectable increases in aSyn protein in the SNC three weeks post-injection. B) The scrambled control miR/GFP viral vector robustly expresses the transgene in the SNC. C-D) Co-infusion of the human or mouse aSyn-overexpressing viral vector with the various miR viral vectors. Scale bars = 100µm, arrows indicate aSyn-positive cells in the SNC adjacent to the substantia nigra pars reticulata (SNR). C) Human aSyn overexpression was abolished by the human *SNCA* miR/GFP viral vector, slightly reduced with the mouse *SNCA* miR/GFP viral vector, and unaltered with the scrambled control miR/GFP viral vector. D) Mouse aSyn overexpression was abolished by the mouse *SNCA* miR/GFP viral vector as well as the human *SNCA* miR/GFP viral vector, with no appreciable decreases in mouse aSyn overexpression observed with the scrambled control miR/GFP.

Recommended Starting Dose: For *in vitro* use, it is recommended to start with a 1:100 dilution of the stock solution supplied at 0.5×10^{12} vg/ml. Dilute further as necessary. Wait 2-3 days for knockdown in HEK293 cells and 7-9 days for knockdown in primary neurons. For *in vivo* use, it is recommended to start with a 2µl injection of the undiluted stock solution supplied at 0.5×10^{12} vg/ml. Dilute further as necessary. It is generally recommended to use a 3 week post-injection interval. Doses and recommended duration of expression will vary based on cell type, model, and desired degree of knockdown.