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Quantitative PCR of Viral Abundance

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Protocol for quantifying viral abundance by PCR used in Yoo et al 2021

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- 1 AAV-PHP.S:hSYN1-mNeonGreen was delivered systemically to wildtype mice at 6-8 weeks of age.
- 2 3-4 weeks following viral infection, 1 cm of proximal, medial, and distal small intestine (SI) and colon were harvested and flash frozen in TRIzol (ThermoFisher Scientific, Waltham, MA-Cat. No. 15596018) and RNA was extracted per manufacturer's instructions.
- 3 Viral ssDNA is present in the RNA fraction. Contaminating RNA was eliminated with RNase treatment (ThermoFisher Scientific, Waltham, MA-Cat. No. AM228) per manufacturer's instructions.
- 4 Quantitative PCR was performed on viral DNA with primers against WPRE (Forward: 5'-GGCTGTTGGGCACTGACAAT-3'; Reverse: 5'-CCGAAGGGACGTAGCAGAAG-3') and values were normalized to ubiquitous mitochondrial gene, mtRNR2 (Forward: 5'-CCGCAAGGGAAAGATGAAAA-3'; Reverse: 5'-TCGTTTGGTTTCGGGGTTTC-3').
- 5 Plotted relative abundances are mean values across three regions sampled in either SI or colon.