



Supplemental Figure S1 - AdV-Cre does not reduce Vcl or Vcl-associated proteins in wild-type NRVMs. AdV-LacZ (LacZ) and AdV-Cre (Cre) infected NRVM protein lysates were analyzed by Western blotting for expression of Vcl and Vcl-associated proteins.

Top Panel - Lysates from homozygous floxed (fl/fl) Vcl NRMVs

Lower Panel - Lysates from wild-type (wt) NRVMs (no floxed Vcl allele).

Table S1. Primer sequences and reference templates used to generate Vcl and Tjp1 (ZO-1) hybrid genes for yeast-two hybrid assays.

	Baits (in pGBKT7)
Vcl (0-3.2kb) (Construct a)	F (1-20): ATGCCGGTGTTTCACACGCGT R (3201-3182): CTA CTGGTACCAGGGAGTCT
Vcl (0-2.5kb) (Construct b)	F (1-20): ATGCCGGTGTTTCACACGCGT R (2558-2539): CGTAGCTGTTCAAGGTCTGG
Vcl (1.7-3.2kb) (Construct c)	F (1774-1794): ACTCAAGAGGTATCCGATGTT R (3201-3182): CTA CTGGTACCAGGGAGTCT
Tjp1 (0-1.5kb) (Construct 2)	F (1-20): ATGTCCGCCAGGGCCCGCGGC R (1541-1522): TCTGATTCTACAATGCGGCG
Tjp1 (1.2-2.5kb) (Construct 3)	F (1201-1220): GTCAGCCCTTCTGATGGTGC R (2568-2549): TTGATCAGTG TAGGCCCCCGC
Tjp1 (0-0.9kb) (Construct 4)	F (1-20): ATGTCCGCCAGGGCCCGCGGC R (900-881): GAATGGTCTGATGCTAGTGA
Tjp1 (0.8-1.2) (Construct 5)	F (790-809): GAGCGGGCTACCTTACTGAA R (1265-1246): ATGCTGGGCCTAAGTATCCC
Tjp1 (1.2-1.5kb) (Construct 6)	F (1201-1220): GTCAGCCCTTCTGATGGTGC R (1541-1522): TCTGATTCTACAATGCGGCG
Tjp1 (1.2-1.8kb) (Construct 7)	F (1201-1220): GTCAGCCCTTCTGATGGTGC R (1805-1786): CAGAAGTCTGCCCGATCACC

	Reporters (in pGADT7)
Tjp1 (0-2.5kb) (Construct 1)	F (1-20): ATGTCCGCCAGGGCCCGCGGC R (2568-2549): TTGATCAGTG TAGGCCCCCGC
Tjp1 (0-1.5kb) (Construct 2)	F (1-20): ATGTCCGCCAGGGCCCGCGGC R (1541-1522): TCTGATTCTACAATGCGGCG
Tjp1 (1.2-2.5kb) (Construct 3)	F (1201-1220): GTCAGCCCTTCTGATGGTGC R (2568-2549): TTGATCAGTG TAGGCCCCCGC
Vcl (0-3.2kb) (Construct a)	F (1-20): ATGCCGGTGTTTCACACGCGT R (3201-3182): CTA CTGGTACCAGGGAGTCT

Primer sequences are shown from 5'→3' and designed as follows. Forward (F) primers were designed to incorporate one random nucleotide, a linker restriction site in frame with the CDS, as well as the Kozak sequence (gccacc) and a START codon (if not present within cDNA). Reverse (R) primers were designed to incorporate one random nucleotide and a linker restriction site. Reference sequences: Mus musculus Vinculin (Vcl) CDS, NM_009502.4, Mus musculus Tight junction protein 1 (Tjp1) CDS, transcript variant 1, NM_009386.2.