Supplemental Materials

Molecular Biology of the Cell

Youker et al.

Supplementary Table I – Primers used in cloning and mutagenesis

Primers for overlap extension PCR[1,2]

p75_Nterm	sense	5-CTAGGAATTCGCCACCATGGGGGCAGGTGCCACCGG-3
p75_Cterm	antisense	5-CTAGGGGCCCGCACCGGGGATGTGGCAGTGG-3
C257A/G266I	sense	5-ATCCTGGCTGCTGTGGTTGTGATACTTGTGGCCTAC-3
C257A/G266I	antisense	5-AACCACAGCAGC**CAG**GATGGAGGCATAGACAGGGAT-3
Δ193	sense	5-GAGGCACCTCCAGAAACCACCGACAACCTCATC-3
Δ193	antisense	5-GATGAGGTTGTCGGTGGTTTCTGGAGGTGCCTC-3
Δ250	sense	5-AAGAGGTGGAACAGCCTCTACAGCAGCCTGCCC-3
Δ250	antisense	5-GGGCAGGCTGCTGTAGAGGCTGTTCCACCTCTT-3

1) Underlined sequences are mutated and the bold sequence facilitated selection of mutant but does not alter amino acid sequence.
2) Deletion mutants were created by using p75_Nterm / antisense mutant primer and p75_Cterm / sense mutant primer in first PCR reaction to generate two halves of gene. The second PCR reaction used p75_Nterm / p75_Cterm with two products of first reaction to create the full length gene with the deletion.

Primers for site-directed mutagenesis & cloning

G247C	sense	5-CGTGGTGACCCGATGCACCACCGACAAC-3
G247C	antisense	5-GTTGTCGGTGGTGCATCGGGTCACCACG-3
tandem-EGFP	sense	5-CTAGGGTACCGCCACC ATGGTGAGCAAGGGCGAGGA-3
tandem-EGFP	antisense	5-CTAGGGGCCCG CTTGTACAGCTCGTCCATGC-3

Supplementary Table II – siRNA sequences used in this study

control (firefly luciferase)	5-GAAUAUUGUUGCACGAUUUUU-3 3-AAAUCGUGCAACAAUAUUCUU-5
canine galectin-3	5-AUACCAAGCUGGAUAAUAAUU-3 3-AUAUUAUCCAGCUUGGUAUUU-5
canine galectin-4	5-GGGACAAGGUGGUGUUCAAUU-3 3-UUCCCUGUUCCACCACAAGUU-5
canine galectin-9	5-GGGUCAGAGGAGAGGAAGAUU-3 3-UCUUCCUCUCCUCUGACCCUU-5

Supplementary Figure 1. PCH analysis measurements of p75. (A) PCH analysis measures the variance of fluorescence intensity within a very small confocal volume to determine the average molecular brightness of moving particles. As an example, the variance in intensity of a tetramer of fluorescent molecules moving out of the confocal volume will be ~4x-greater than that of a four-fold higher concentration of monomer, even though the average intensity of the two samples is the same. Figure adapted from http://research.stowers-institute.org/microscopy/external/technology/PCH/index.html. **(B)** Wild type and mutant p75 were staged in the TGN of MDCK cells co-expressing the TGN marker GalT-mCherry as described in Methods. The crosshair indicates where PCH measurements were made. Cells with low levels of protein expression were chosen for analysis; therefore images shown are with a pinhole of ~3 airy units (AU) to observe signals visually. All PCH measurements were done with the pinhole set to 1 AU. Scale bar = 10 μm.

Supplemental Fig. 1
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