Supplementary material.

Figure S1. Amino acid sequence of rat p75. The extracellular domain is shown in blue, and the signal peptide is shown in yellow. The transmembrane sequence is shown in red. The linker domain of the intracellular carboxy-terminal p75 sequence is shown in green. The death domain of the intracellular sequence is shown in black, and the YXXθ sequence is shown in purple.

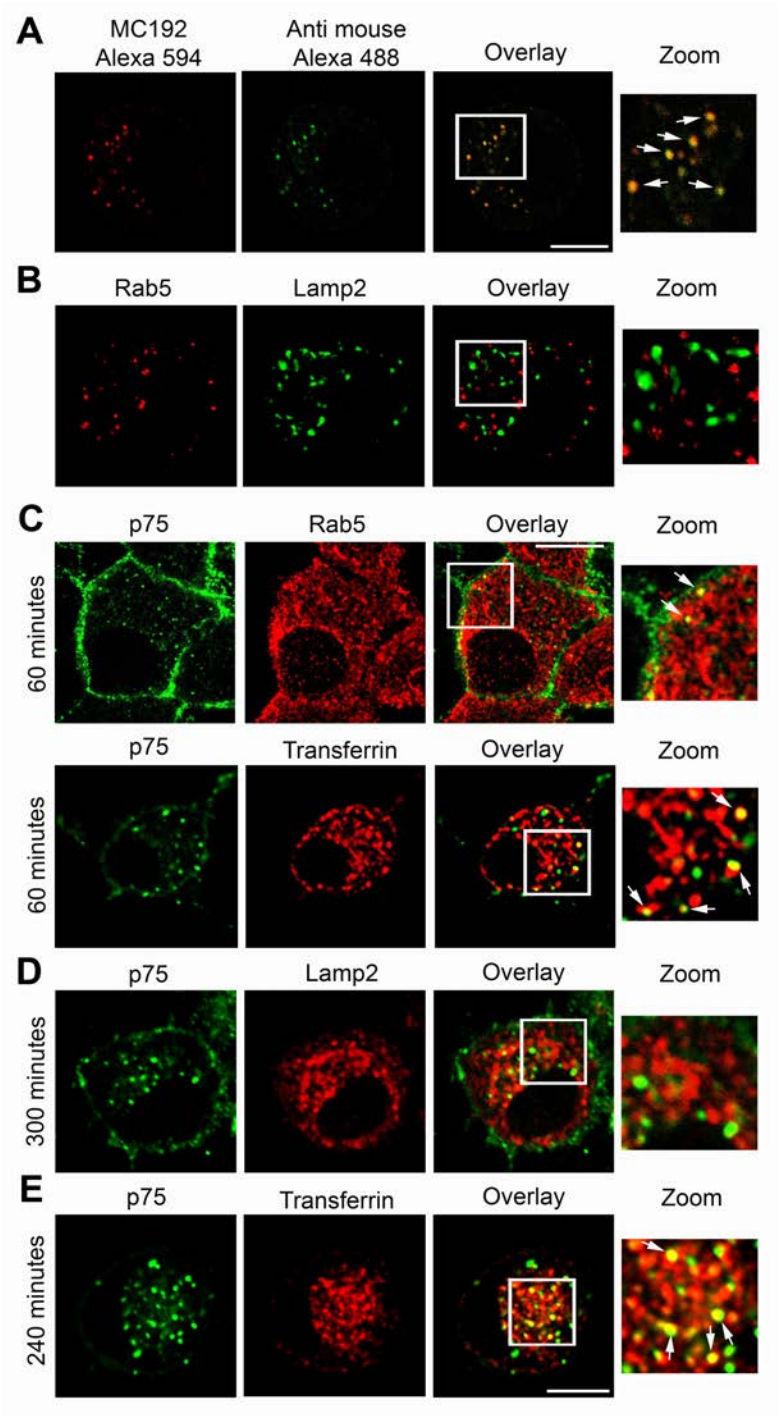
A
MC192
Alexa 594
Anti mouse
Alexa 488
Overlay
Zoom
B
Rab5
Lamp2
Overlay
Zoom
C
p75
Rab5
Overlay
Zoom
60 minutes
p75
Transferrin
Overlay
Zoom
60 minutes
D
p75
Lamp2
Overlay
Zoom
300 minutes
E
p75
Transferrin
Overlay
Zoom
240 minutes

Figure S2. Colocalisation of endocytosed p75 with different endocytic markers in sympathetic neurons. A. The maximum co-localisation that can be measured between two different fluorescent probes. Deconvoluted confocal images of p75 immunoendocytosis with anti-p75ECD-Alexa 594 (in red) in the presence of NGF (100 ng/ml) for 120 minutes in PC12 cells. The monoclonal against the extracellular domain of p75 was then observed using anti-mouse-Alexa 488 (in green). The zoom represents the white square in the overlay image. Bar, 10 μm. See table S1 in the supplementary material for the values representing the degrees of colocalisation. **B.** The minimum colocalisation that can be measured between two different fluorescent probes. Deconvoluted images of PC12 cells transfected with Rab5-GFP (in red), incubated with NGF (100 ng/ml) for 120 minutes, and then tested for the immunofluorescence of Lamp2 (in green). The zoom represents the white square in the overlay image. Bar, 10 μm. For actual values, see table S1 in the supplementary material. **C-E.** Confocal images of p75 immunoendocytosis with anti-p75ECD in the presence of BDNF (150 ng/ml) and Trf-Alexa 568 in sympathetic neurons; C is a marker for early endosomes, and E is a marker for recycled endosomes. The endogenous Rab5 (C, a marker for early endosomes) and Lamp2 (D, a marker for late endosomes) were then observed by immunofluorescence. The zoom represents the white square in the overlay images. Bar, 10 μm. The images are representative of 3 independent experiments.

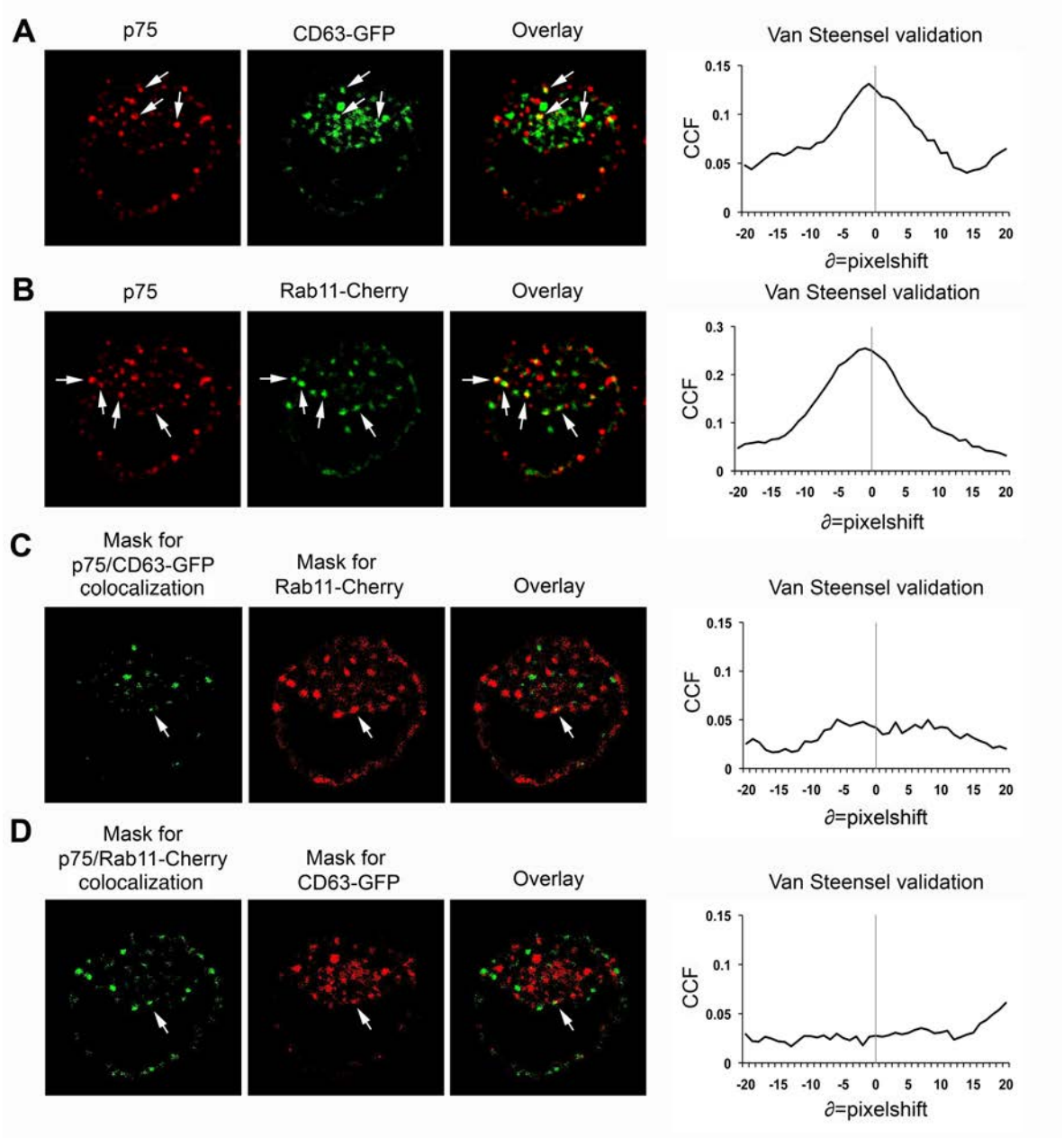
A
p75
CD63-GFP
Overlay
Van Steensel validation
CCF
∂=pixelshift
B
p75
Rab11-Cherry
Overlay
Van Steensel validation
CCF
∂=pixelshift
C
Mask for p75/CD63-GFP colocalization
Mask for Rab11-Cherry
Overlay
Van Steensel validation
CCF
∂=pixelshift
D
Mask for p75/Rab11-Cherry colocalization
Mask for CD63-GFP
Overlay
Van Steensel validation
CCF
∂=pixelshift

Figure S3. p75 accumulates in two different endosomal compartments, a CD63-positive MVB and a Rab11-positive endosome. PC12 cells transfected with Rab11-mCherry and CD63-GFP were treated with anti-p75ECD-Alexa 647 to induce p75 immunoendocytosis in the presence of NGF (100 ng/ml) for 120 minutes. The confocal images were deconvoluted and analysed to obtain the Manders coefficient. To identify true co-localisation, Van Steensel's analysis was performed, and the results were tested for Gaussian fitting (R^2). **A and B.** Left panel, p75 and CD63-GFP or Rab11-mCherry confocal images of the same cell. The arrows show the colocalisation between p75 and CD63-GFP or Rab11-mCherry. Right panel, Van Steensel's cross-correlation function (CCF) with a R^2= 0.95 and 0.99 for p75 with CD63-GFP and for p75 with Rab11-mCherry, respectively. The Van Steensel's CCF analysis indicates true colocalisation. **C and D**. Left panel, colocalisation of the masks for p75/CD63-GFP and Rab11-mCherry or the colocalisation of the masks of p75/Rab11-mCherry and CD63-GFP of the same cell shown in A and B. The arrows indicate the colocalisation between the two masks. Right panel, Van Steensel's CCF with R^2= 0.71 for the colocalisation of the p75/CD63-GFP mask and the Rab11-mCherry mask, and R^2=0.8 for the colocalisation of the p75/Rab11-mCherry mask with the CD63-GFP mask, respectively. Van Steensel's CCF indicates that these apparent colocalisations were not true because R^2 was less than 0,9.

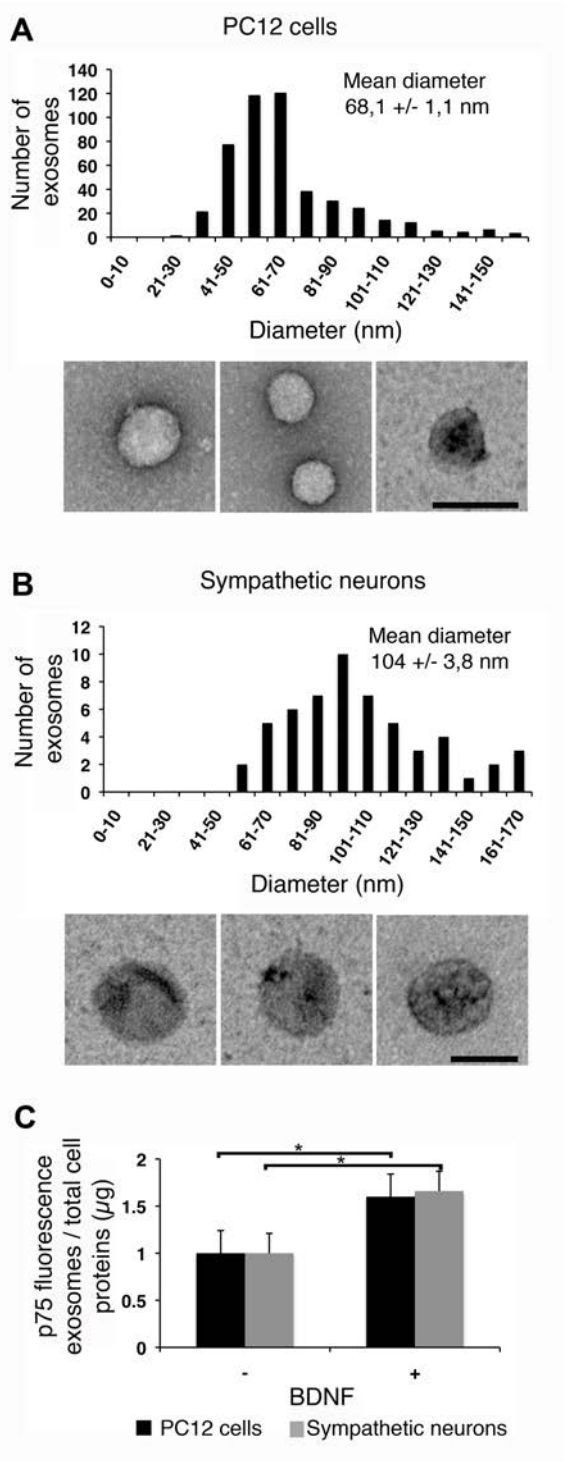
A
PC12 cells
Mean diameter
68,1 +/- 1,1 nm
Number of exosomes
140
120
100
80
60
40
20
0
0-10
21-30
41-50
61-70
81-90
101-110
121-130
141-150
Diameter (nm)
B
Sympathetic neurons
Mean diameter
104 +/- 3,8 nm
Number of exosomes
12
10
8
6
4
2
0
0-10
21-30
41-50
61-70
81-90
101-110
121-130
141-150
161-170
Diameter (nm)
C
p75 fluorescence exosomes / total cell proteins (μg)
2
1.5
1
0.5
0
*
*
-
+
BDNF
PC12 cells
Sympathetic neurons

Figure S4. Exosome size obtained by ultracentrifugation and the release of p75 in exosomes after the induction of p75 internalisation. A and B. The distribution of the diameters of the intact exosomes obtained from PC12 cells (A) and sympathetic neurons (B) was analysed using electron microscopy. PC12 cells were incubated with BDNF (150 ng/ml) for 2 hrs, and KCl (30 mM) was added to the extracellular medium for 4 hours to induce the release of exosomes. Sympathetic neurons were incubated with BDNF (150 ng/ml) for 4 hours, and KCl (30 mM) was added to the extracellular medium for 6 hours. In both cases (A and B), after the KCl treatment, the extracellular medium was ultracentrifuged to obtain an exosome-enriched sample, which was analysed by electron microscopy. The images shown are representative exosomes from three independent experiments. Bar, 100 nm. **C.** The amount of fluorescent p75 released into the extracellular medium was increased upon the addition of BDNF. PC12 cells and sympathetic neurons were incubated with anti-p75ECD-Alexa 488 in the presence of BDNF (150 ng/ml) to induce the internalisation of p75 for 2 hrs (PC12 cells) or 4 hrs (sympathetic neurons). Exosome release was then induced with KCl (30 mM) treatment for 30 minutes (PC12 cells) or 180 minutes (sympathetic neurons). The cell media were collected and centrifuged at 1,500 rpm for 5 minutes. The supernatants were subjected to sequential centrifugation consisting of a first centrifugation of 300 x *g* for 10 minutes, followed by centrifugation at 2,000 x *g* for 10 minutes and a final centrifugation for 10,000 x *g* for 30 minutes. The fluorescence of this last supernatant was measured using a Mithras LB 940 fluorimeter from Berthold Technologies. n=3. *$P < 0.05$. The control for non-specific fluorescence release by the cells was prepared by incubating the cells with anti-p75ECD-Alexa 488 for the same periods indicated above and then treating the cells with incubation medium without BDNF.

Movie 1. Time lapse of p75 labelled in red and Rab5 positive endosomes labelled in Green in PC12 cells.

Movie 2. Time lapse of p75 labelled in red and Rab11 positive endosomes labelled in Green in PC12 cells.

Movie 3. Real time microscopy of p75, a p75 positive endosome fusing with the plasma membrane

Table S1. Quantification of p75 colocalisation with endocytic markers.

*(A/B)x100	15 min	30 min	60 min	90 min	120 min
p75/Rab5	20 +/- 0.009	10 +/- 0.006	10 +/- 0.005	-	-
p75/Rab11	15 +/- 0.008	-	-	25 +/- 0.010	26 +/- 0.009
p75/Trf	28 +/- 0.017	-	-	30 +/- 0.012	33 +/- 0009
p75/Rab7	-	5 +/- 0.527	6 +/- 0.566	-	10 +/- 0.950
p75/CD63	-	9 +/- 0.720	12 +/- 1.022	-	28 +/- 1.621
Rab5/p75	7 +/- 0.010	5 +/- 0.008	5 +/- 0.008	-	-
Rab11/p75	4 +/- 0.008	-	-	8 +/- 0.011	6 +/- 0.011
Trf/Rab11	30 +/- 0.021	-	-	35 +/- 0.023	37 +/- 0.028
Rab7/p75	-	2 +/- 0.217	3 +/- 0.349	-	6 +/- 0.524
CD63/p75	-	4 +/- 0.273	5 +/- 0.337	-	11 +/- 1.323
Anti-p75ECD/ anti-mouse	-	-	-	-	86 +/- 1,6
Rab5/Lamp2	-	-	-	-	6 +/- 1,3
Lamp2/Rab5	-	-	-	-	7 +/- 1

* Percentage of co-localisation of the fluorescence intensity in A by the fluorescence intensity in B +/- SEM.

Confocal images of PC12 cells as shown in Figs. 3A and 6A, were processed using deconvolution to lower the signal-to-noise ratio, and the Manders coefficient was calculated from these images. The numbers presented in the table correspond to the proportion of p75 (A) that co-localised with different proteins (B) calculated using the Manders coefficient. The numbers were expressed as percentages. Representative images are in Fig 3A, Fig S2A and S2B and Fig.6. For quantification, 20-30 cells were analysed from 3 independent experiments. The numbers were not corrected for the maximum colocalisation that can be technically measured between two different fluorescent probes (mouse Anti-p75ECD-Alexa 594/anti-mouse Alexa 488), as shown in Fig. S2A in the supplementary material. The colocalisation number of two completely separate endosomal populations such as Rab5-positive early endosomes and the lysosomal marker LAMP2 is also shown (Fig. S2B). Less than 6-7% co-localisation can be considered no colocalisation.