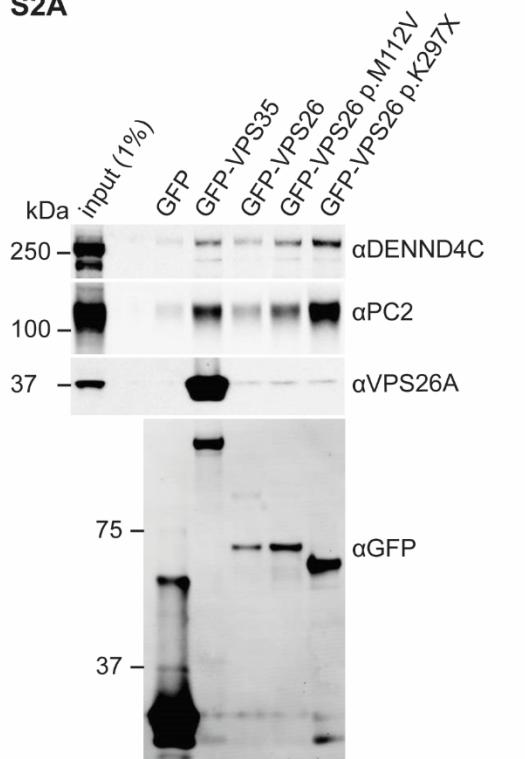


Fig. S1 The PC2 amino-terminus does not directly bind retromer subunits in isolation.

A GST-tagged retromer components VPS26A-myc, VPS29-myc and VPS35 were conjugated to beads and incubated with PC2 (1-223)-6xHis. Proteins were then eluted and binding of retromer components to the PC2 amino-terminal fragment assessed by Western analysis. **B** Purified PC2 (1-233)-6xHis was conjugated to beads and incubated with soluble retromer components VPS26A-myc, VPS29-myc and VPS35. Proteins were then eluted and binding of the PC2 amino-terminal domain to retromer assessed by Western analysis.

S2A



S2B

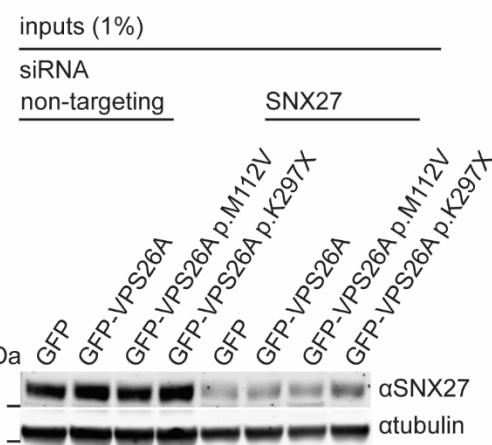
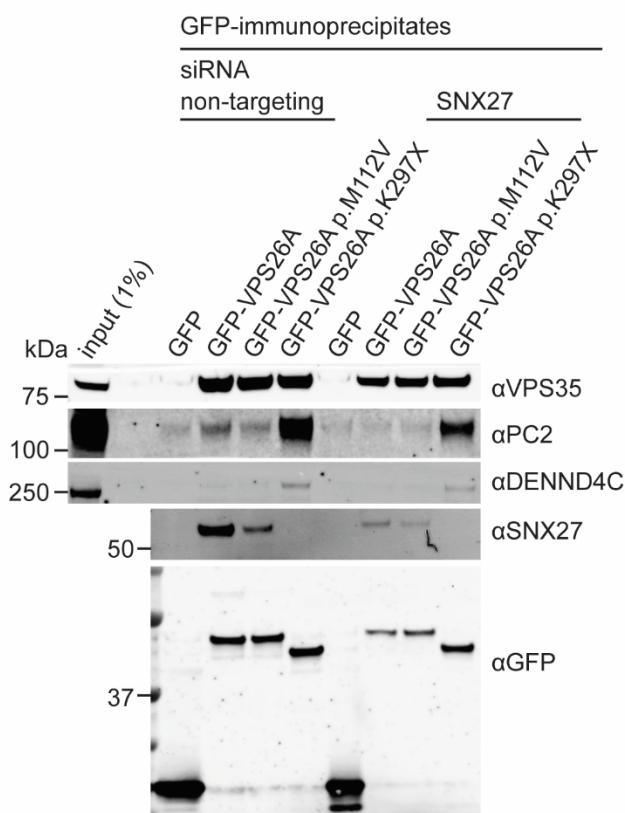


Fig. S2 Increased association of the atypical Parkinsonian-associated VPS26A variant p.K297X with PC2.

A RPE-1 cells lentivirally expressing either GFP, GFP-VPS35, GFP-VPS26A or either Parkinsonian-associated variant of VPS26A, p.M112V or p.K297X were subjected to GFP-trap. The immunoprecipitates were then resolved by SDS-PAGE and immunoblotted with the indicated antibodies. **B** SNX27 suppressed and control siRNA treated RPE-1 cells lentivirally expressing either GFP, GFP-VPS26A or the atypical Parkinsonian-associated VPS26A variants p.M112V and p.K297X were subjected to GFP-trap. The immunoprecipitates were then resolved by SDS-PAGE and immunoblotted with the indicated antibodies. Level of SNX27 suppression achieved by siRNA transfection in each condition is shown in right panel. **A** and **B** show single experiments, which are each representative of three independent biological repeats.

Table S1. Primers for subcloning.

Primer name	Sequence, 5'-3'	Restriction enzyme sites
PC2-(1-48)	Sense: ATGAATTCATGGTAACTCCAGTCGCGTG Antisense: ATGGATCCCCCTCGCAGAGGCCGCCGG	EcoRI BamHI
PC2-(1-60)	Sense: ATGAATTCATGGTAACTCCAGTCGCGTG Antisense: ATGGATCCCCCGGATGCGCTGCATCTCGAT	EcoRI BamHI
PC2-(1-70)	Sense: ATGAATTCATGGTAACTCCAGTCGCGTG Antisense: ATGGATCCCCTCCGGCCGGGGGTCCCGCGC	EcoRI BamHI
PC2-(1-84)	Sense: ATGAATTCATGGTAACTCCAGTCGCGTG Antisense: ATGGATCCCCCGGGAGCACGACGAGAGCGG	EcoRI BamHI
PC2-(1-94)	Sense: ATGAATTCATGGTAACTCCAGTCGCGTG Antisense: ATGGATCCCCGAAGCCGGGGTTATCGCG	EcoRI BamHI
PC2-(1-156)	Sense: ATGAATTCATGGTAACTCCAGTCGCGTG Antisense: ATGGATCCCCCGGCGCCGCCCTCCGCT	EcoRI BamHI
PC2-(1-223)	Sense: ACAGAATTCACATGGTAACTCCAGTCGCGTG Antisense: TGTGGATCCTTTCCCGTAAACACTTTAAGGTAT	EcoRI BamHI
PC2-(1-703)	Sense: ATCTGCAGATGGTAACTCCAGTCGCGTG Antisense: GGTACCCCAAGATCTGAGAGTTCCATTCAGCTTC	PstI KpnI
PC2-(680-968)	Sense: ACAGAATTCAATCATCAATGATACTTACTCTGA Antisense: TGTGGATCCTCATACGTGGACATTAGAACT	EcoRI BamHI

Table S2. Primers for site-directed mutagenesis reactions.

Primer name	Sequence, 5'-3'
PC2-(1-223)-GFP p.E48A	ggccccgctgcgcgcagaggccg
PC2-(1-223)-GFP p.R6G	ccaggccccgcgcctcgacaggcc
PC2-(1-223)-GFP p.V7A	cctctgcgagcaggcgggctggagatc
PC2-(1-223)-GFP p.P9A	ggccccgctgcgcgcagaggccg
PC2-(1-223)-GFP p.E48A	ggccccgctgcgcgcagaggccg
PC2-(1-223)-GFP p.Q49A	ccaggccccgcgcctcgacaggcc
PC2-(1-223)-GFP p.R50A	cctctgcgagcaggcgggctggagatc
PC2-(1-223)-GFP p.G51A	cgagcagcggccctggagatcg
PC2-(1-223)-GFP p.L52A	catctcgatctccgcgcggcgtcg
PC2-(1-223)-GFP p.E53A	ctgcatctcgatcgccaggccccgt
PC2-(1-223)-GFP p.I54A / PC2 p.I54A	gcgctgcatctcgccctccaggccccgc
PC2-(1-223)-GFP p.E55A	ggcttggagatcgcgatgcgcgttc
PC2-(1-223)-GFP p.M56A	gccggatgcgtgcgcctcgatctccaggc
PC2-(1-223)-GFP p.Q57A	cctggccggatgcgcgcattcgatctcca
PC2-(1-223)-GFP p.R58A	cgcctggccggatggccctcgatctcgatc
PC2-(1-223)-GFP p.I59A / PC2-myc p.I59A	ggccgcctgcccggcgcgctgcgtcgatctcg
PC2-(1-223)-GFP p.R60A	gcggccgcctgcgcgtgcgtcgatctcgat