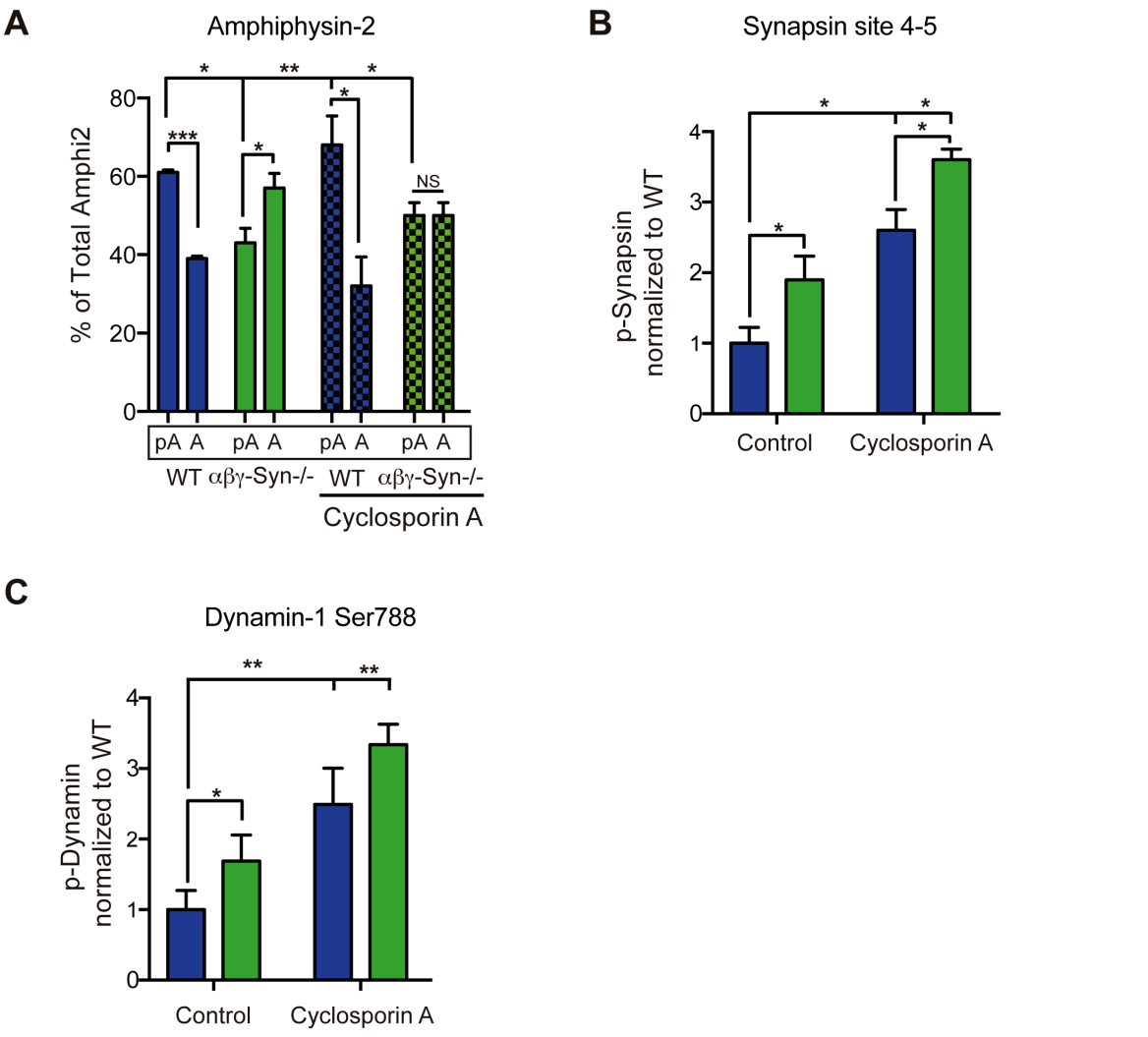
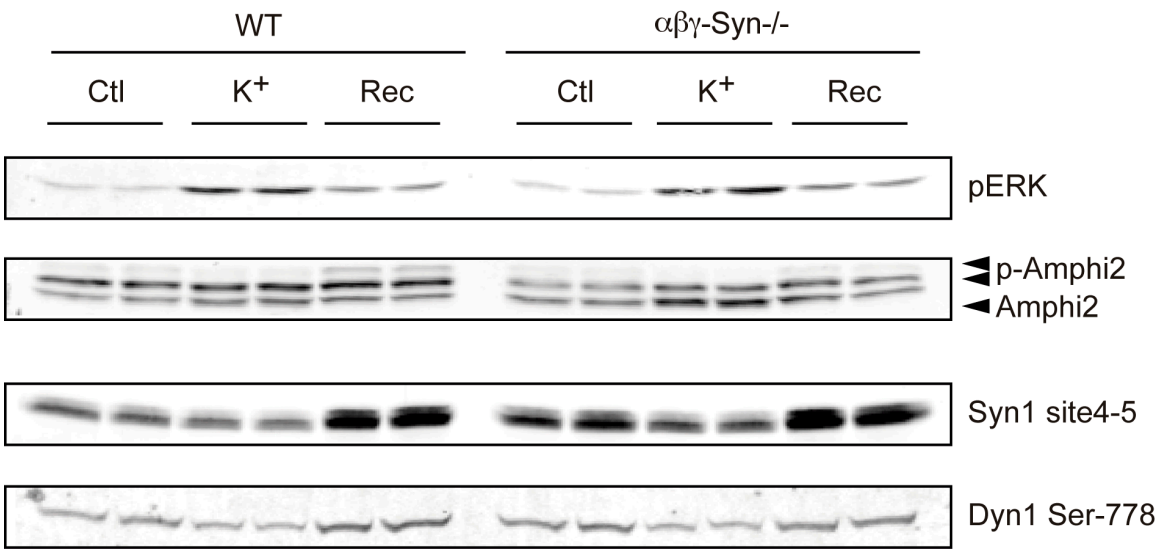
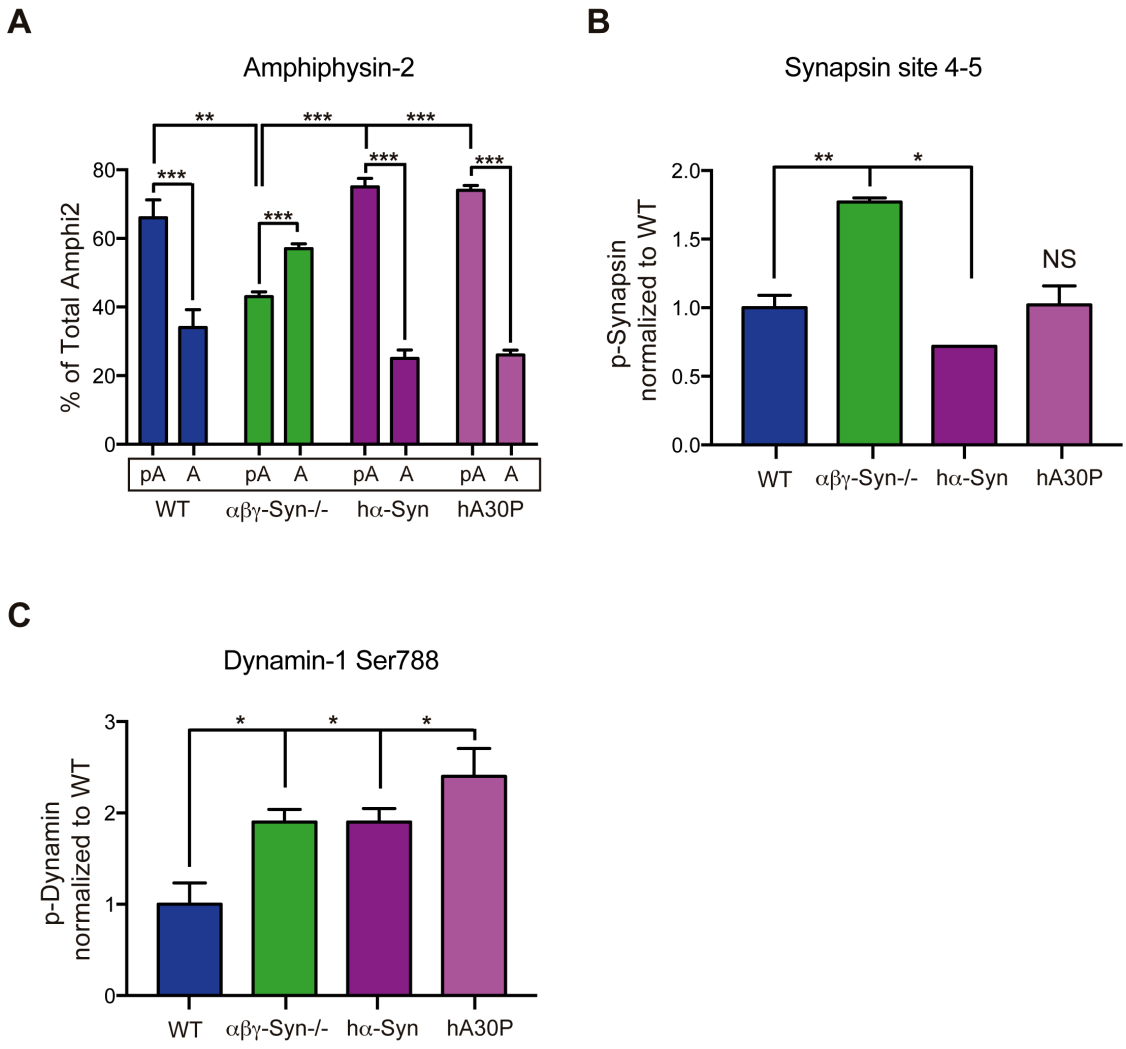


Supplementary Figure 1: Phosphorylation of endocytic proteins is regulated by Calcineurin in wild type and αβγ-Syn-/- brains, related to Figure 6. A. The decrease in amphiphysin-2 phosphorylation αβγ-Syn-/- is reverted by the use of 1 μM Cyclosporin A, a calcineurin inhibitor. While the phosphorylation in wild type is further increased upon treatment with cyclosporin A. **B-C.** The increase in phosphorylation of Synapsin site 4-5 and Dynamin-1 Ser788 is enhanced by treatment with Cyclosporin A. N = 3 independent experiments. NS, not significant, * $p<0.05$; ** $p<0.01$; *** $p<0.001$.



Supplementary Figure 2: Reversible phosphorylation of synaptic proteins, related to Figure 6. Wild type (WT) and synuclein triple knock out (αβγ-Syn-/-) synaptosomes were in control conditions (Ctl) and subsequently were subjected to KCl simulation (K+) and allowed to recover (Rec). These samples were used for western blot using specific antibodies for phosphorylated ERK (pERK), Amphiphysin-2 (Amphi-2), the phosphorylation sites 4 and 5 in synapsin and the phosphorylation in the serine 778 in Dynamin-1. N = 3 independent experiments.



Supplementary Figure 3: Phosphorylation of endocytic proteins in the different synuclein genotypes, related to Figure 6. A. Quantification of phosphor- and dephospho- amphipysin-2 in the four genotypes. The phosphorylation pattern of amphiphysin-2 in hα-Syn or hA30P is similar to wild type. **B.** The phosphorylation of Synapsin site 4-5 in αβγ-Syn-/- is increased compared to wildtype as shown in Fig. 6E. In hα-Syn mutant synapses is decreased relative to WT and the hA30P mutant is indistinguishable from WT. **C.** The phosphorylation of Dynamin-1 Ser788 is increased in all three genotypes relative to WT. N = 3 independent experiments. NS, not significant, * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Name	Aminoacid	Kinase	Phosphatase	Function	References
Syn1Site-1	Ser 9	PKACaMKI	PP2A	Resting pool maintenance	Czernik et al., 1987
Syn1Site-2	Ser 566	CaMKII	PP2A		Hutter and Greengard, 1979; Sihra et al., 1989
Syn1Site-3	Ser 603	CaMKII	PP2A		
Syn1Site-4	Ser 62	MAPK	PP2B Calcineurin		Jovanovic et al., 1996 Matsubara et al., 1996 Jovanovic et al., 2001
Syn1Site-5	Ser 67	MAPK	PP2B Calcineurin		
Syn1Site-6	Ser 549	MAPK cdk1/5	PP2B Calcineurin		
Syn1Site-7	Ser 551	cdk1/5			
Dyn1 744	Ser 774	cdk5 GSK3	Calcineurin	Synaptic vesicle scission	Tan et al., 2003 Clayton et al., 2010
Dyn1 788	Ser 9	cdk5	Calcineurin		
Amphi-1	Ser 262	cdk5	Calcineurin	Membrane curvature	Liang et al., 2007
Amphi-1	Ser 272	cdk5	Calcineurin		
Amphi-1	Ser 276	cdk5	Calcineurin		
Amphi-1	Ser 285	cdk5	Calcineurin		
Amphi-1	Thr 310		Calcineurin		
Amphi-1	Thr 350	CK2	Calcineurin	Clathrin binding inhibition	Doring et al., 2006
Amphi-1	Thr 387	CK2	Calcineurin		
Amphi-2	Serines	cdk5	Calcineurin	Membrane curvature	
Epsin	Ser 357	cdc2 kinase	Calcineurin	Clathrin binding inhibition	Chen et al., 1999
EndoA1	Ser 75	LRRK2		Membrane curvature	Ambroso et al., 2014
EndoB1		cdk5		Autophagy	Wong et al., 2011

Table I. Phosphorylation of endocytic protein related to synaptic vesicle endocytosis, related to Figure 6. Different presynaptic proteins are phosphorylated in normal conditions and dephosphorylated upon stimulation of neurons in a calcium dependent manner. Here we compile the names of the phosphorylated proteins (name), the phosphorylated amino acid residue when available (amino acid), the described kinase (kinase) and phosphatase (phosphatase) in the literature, associated and putative functions (function) and the references where these antecedents are shown (reference).

Identifier	n	Pearson's r	p-Value	Slope	Intercept
WT	26	-0.41	0.0352	-0.21	3.50
αβγ-Syn-/-	56	-0.43	0.0008	-0.27	5.09
hαsyn	27	-0.22	0.2689	-0.07	2.51
hA30P	58	-0.40	0.0016	-0.26	4.64

Table II. Correlation between the number of tethers and the vesicle distance to the AZ membrane, related to Figures 3 and 7. Pearson correlation analysis was carried on using the number of tethers and the vesicle distance to the AZ for different genotypes: Wild type (WT), synuclein triple knock out (αβγ-Syn-/-), human α-synuclein (hα-Syn) or A30P (hA30P) mutation expressed in a wild type genotype. The value 'n' is the number of data per condition, 'r' is the Pearson correlation coefficient, 'p-Value' is the statistical confidence that the result is significant, 'slope' is the slope of the correlation line and 'Intercept' is the intercept in Y axis. We see here that the only case these variables are not correlated is when human α-synuclein overexpressed.

	WT	αβγ-Syn-/-	hα-Syn	hA30P
Synapse Size	–	↓	↑	↑
Tethering	–	↑	–	–
Tether/SV	–	↑	–	↑
RRP	–	↑	–	↑
Intermediate Connectors	–	↓	–	–
Proximal Connectors	–	↓	–	–
Synapsin site 4-5	–	↑	↓	–
p-Amphiphysin	–	↓	–	–
Dyn-1 Ser788	–	↑	↑	↑

Table III. Summary of morphological and phosphorylation changes observed in synapses with different synuclein genotypes, related to all Figures. We observed different ultrastructural and phosphorylation changes when analyzing wild type (WT), αβγ-Syn-/-, hα-Syn or hA30P mutation expressed in a wild type genotype. Direction in the arrow represents and increase or decrease and a line represent no change compare to wild type.

Identifier	Animals	Synapses	SVs	Proximal SVs	Tethers	Connectors
WT	3	8	648	47	50	1103
αβγ-Syn-/-	3	6	611	67	163	636
hαsyn	2	6	535	38	53	1089
hA30P	3	9	763	81	162	1334
αβγ-Syn-/- hαsyn	2	7	618	58	-	1845

Table IV. Summary of data analyzed in CryoEM experiments, related to Figures 3, 4 and 7. Number of animals, synapses, synaptic vesicles (SVs), tethers and connectors analyzed for the denoted genotypes.