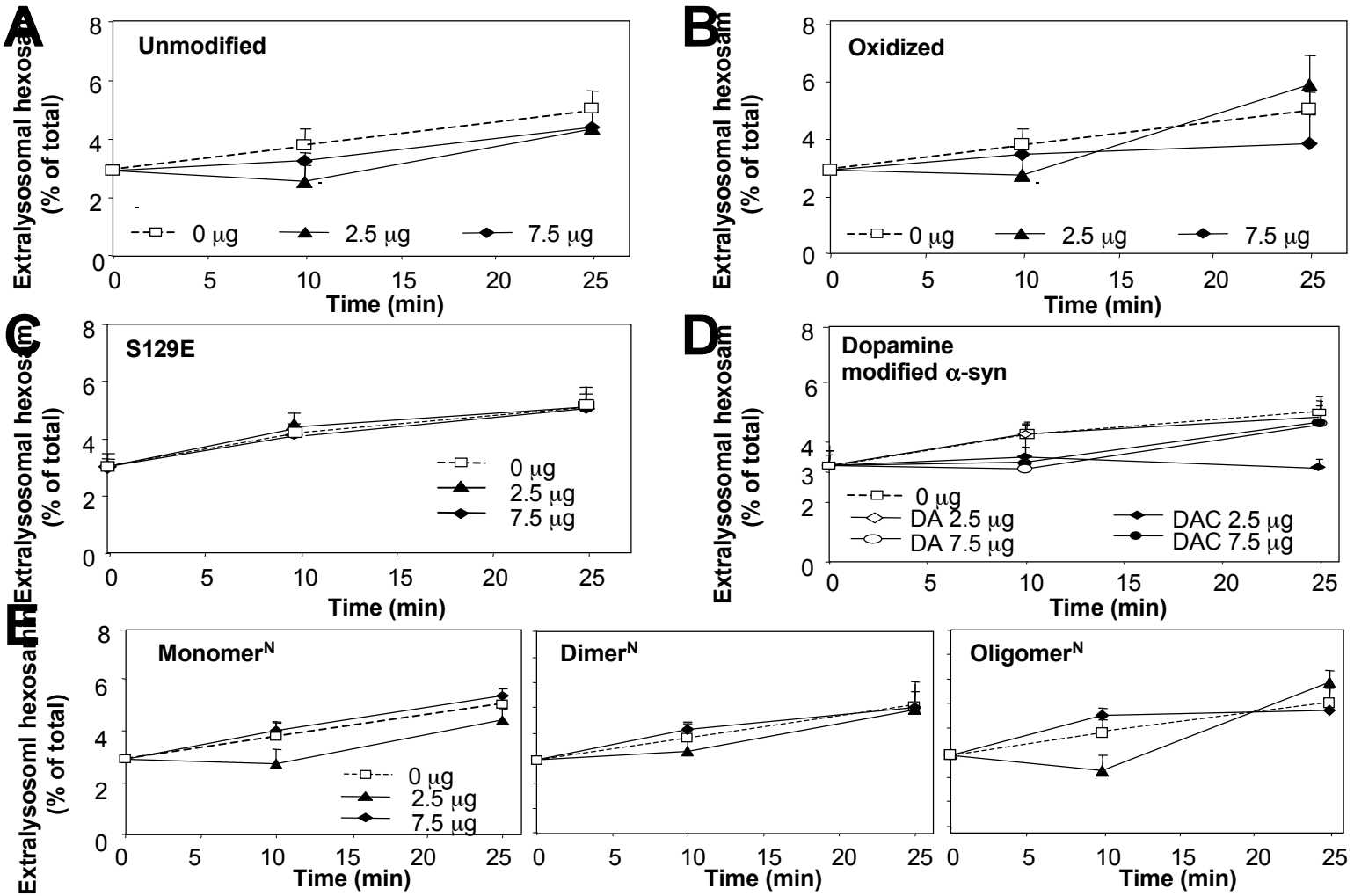
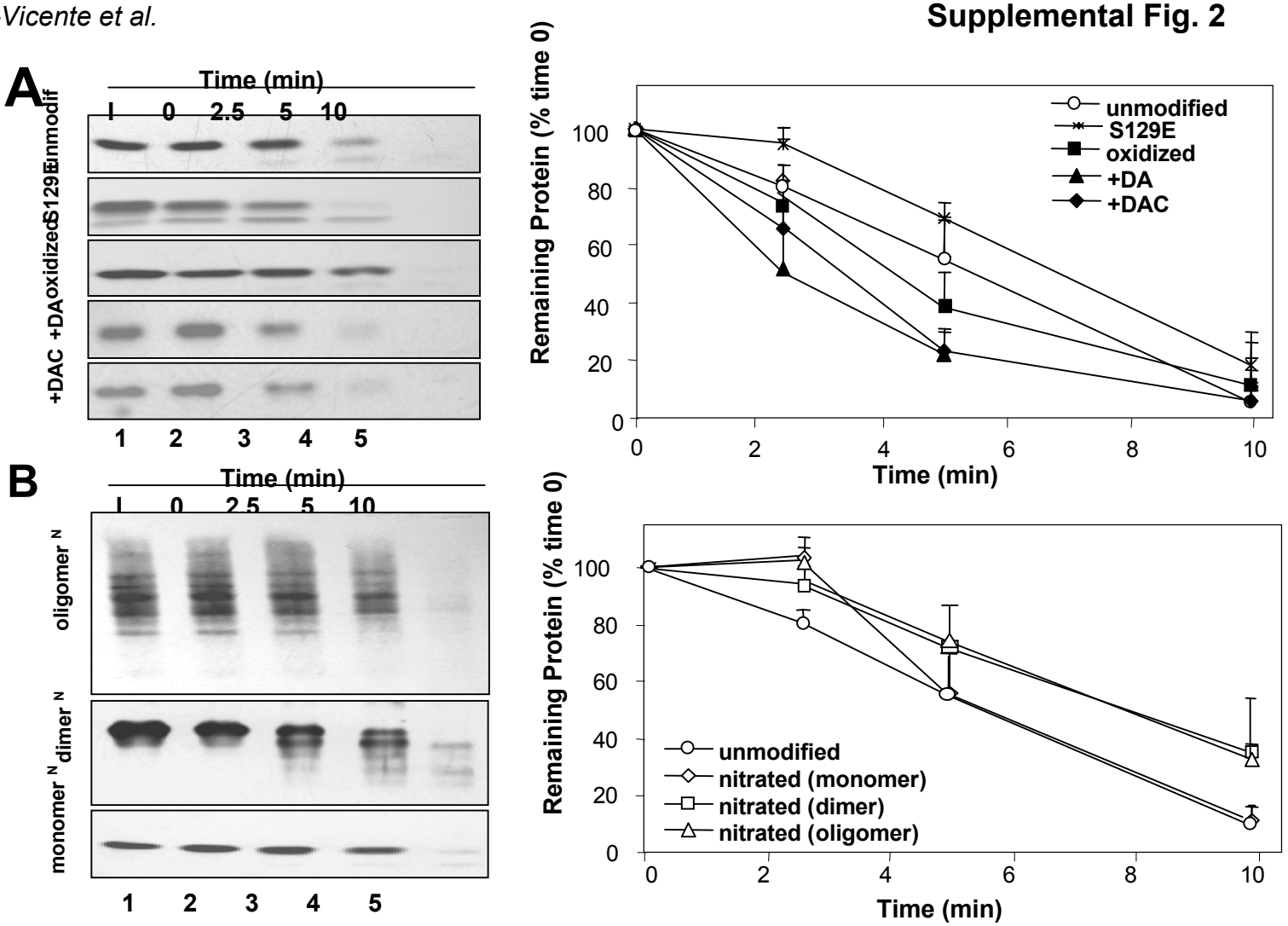


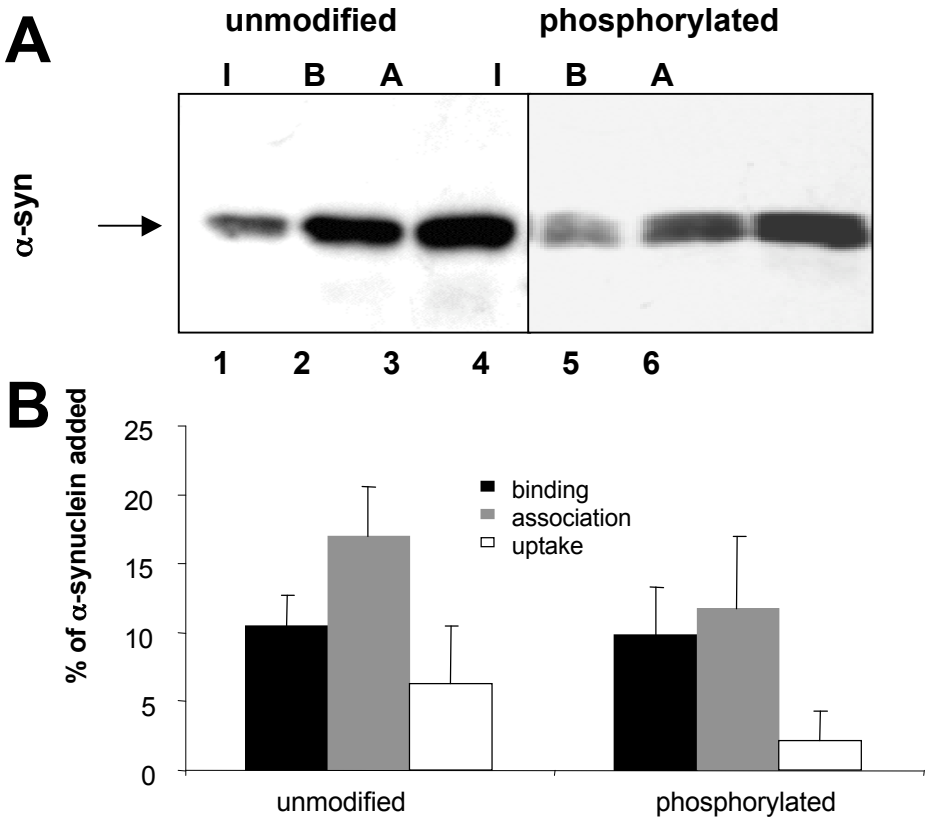
Supplementary Fig. 1. Effect of different modified forms of α-syn on lysosomal stability. The release of β-hexosaminidase from intact lysosomes incubated with the indicated concentrations of unmodified wild-type α-synuclein (**A**) or the indicated modified forms of α-synuclein (**B-E**) was measured. Values are expressed as percentage of total activity in the lysosomal fraction at time 0, and are means + S.E. of 3-6 different experiments. Differences with control values (untreated) were not significant at any time point. Release of **β**-hexosaminidase from the same batches of lysosomes incubated with 0.1% Triton X-100 was 65 $\pm$ 3 % of total (at 10 min) and 93 $\pm$ 2 % of total (at 20 min).

Supplemental Fig. 2



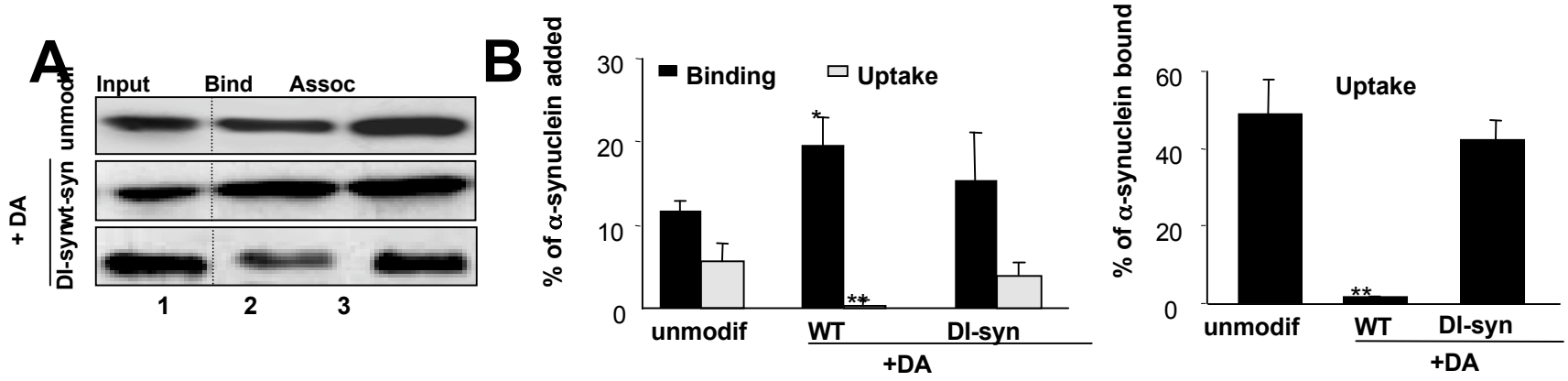
Supplementary Fig. 2. Proteolysis of different modified forms of α-syn by lysosomal proteases. A. Proteolysis of unmodified and different modified forms of α-synuclein (as labelled) α-synuclein degraded by lysosomal proteases (disrupted lysosomes) at different times was followed by immunoblot. Values in the graphic (right) are mean + S.E. of the densitometric quantification of 4-6 different experiments and are expressed as % of initial protein (time 0) present at the end of the incubation. Note that although we detected an intermediate proteolytic fragment for the three forms of the protein, both the full size and the fragment were readily degraded by lysosomal enzymes with a half life of about 4.5 min. **B.** Proteolysis of monomer, dimer and oligomers of nitrated α-syn by lysosomal proteases was analyzed as in a, but immunoblots were developed with an antibody that recognizes only the nitrated form of α-synuclein.

Supplemental Fig. 3



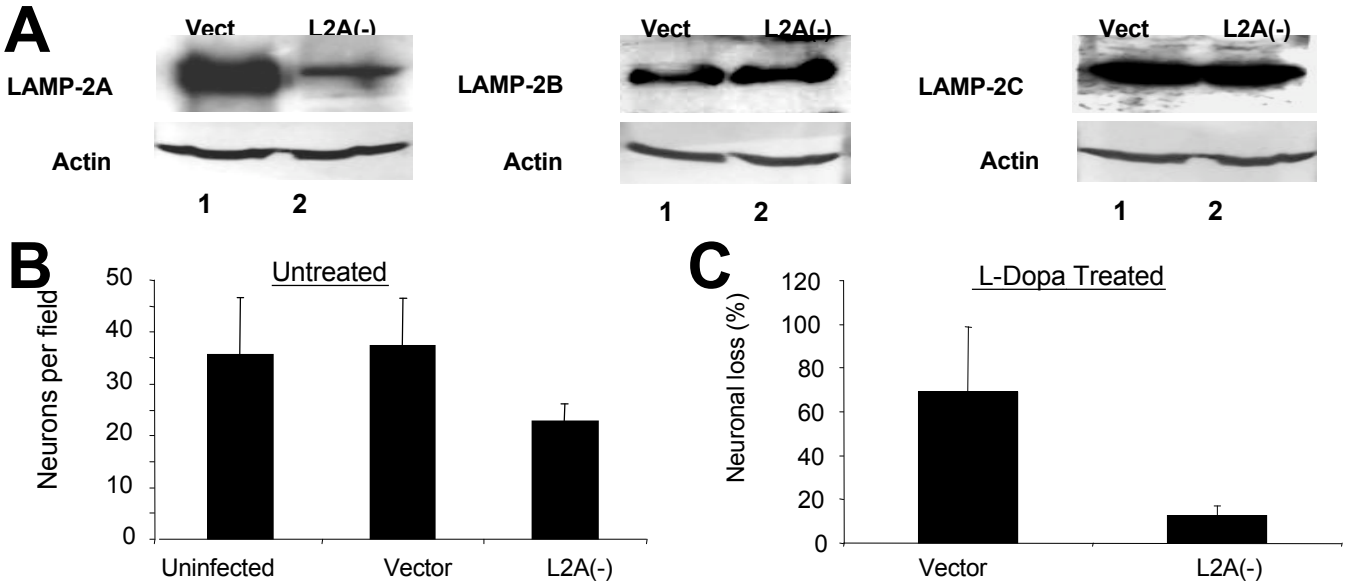
Supplementary Fig. 3. CMA of an in vitro phosphorylated form of α-syn. (**A**) Association of unmodified or *in vitro* phosphoryated α-syn with isolated lysosomes untreated (binding; Bind (B)) or previously treated with proteinase inhibitors (association: binding + uptake; Assoc (A)). Lane 1 (Input; I): 1/10 of the amount of protein added to the incubation. (**B**) The percentage of each protein bound, associated and translocated inside lysosomes and was calculated from the densitometric quantification of immunoblots (n= 4). *In almost 70% of the experiments a lower molecular weight form of the protein corresponding to dephosphorylated α-syn can be detected. Supporting our findings with the phosphorylated protein and the phosphomimetic mutant, the amount of dephopshorylated protein (lower band) associated and translocated into lysosomes was higher than of the phopshorylated from (top band).

Supplemental Fig. 4



Supplementary Fig. 4. A dopamine-insensitive mutant form of α-syn is efficiently taken up by lysosomes via CMA. (**A**) Association of dopamine-reacted wild type (WT) and dopamine-insensitive (DI) mutant of α-syn with isolated lysosomes untreated (binding; Bind) or previously treated with proteinase inhibitors (association: binding + uptake; Assoc). Lane 1 (Input; I): 1/10 of the amount of protein added to the incubation. (**B**) The percentage of each protein bound and translocated inside lysosomes (left) and the percentage of bound protein translocated into lysosomes (right) was calculated from the densitometric quantification of immunoblots (n= 4).

Supplemental Fig. 5



Supplementary Fig. 5. Neurotoxicity of L-Dopa treatment in cells with functional or impaired CMA. Ventamedial neuronal cultures plated at the same density were uninfected or infected with an empty lentivirus-based vector (Vect) or the same vector carrying a shRNAi against LAMP-2A. (**A**) Five days after infection levels of the three LAMP-2A protein variants were determined by immunoblot with specific antibodies. (**B**) Changes in neuronal number at day 5 were quantified in uninfected or the two groups of infected cells as described in Methods. Values show the remaining number of cells per field and are the mean + S.E. of 10 different fields in two independent experiments. (**C**) At day five, 100 μM L-Dopa was added to a subset of the infected cells, and 48h later the percentage of neuronal lost was calculated by similar procedures. Values are expressed as percentage of starting neurons (right before the treatment) loss after L-Dopa was added and are mean + S.E. of 10 different fields in two independent experiments.