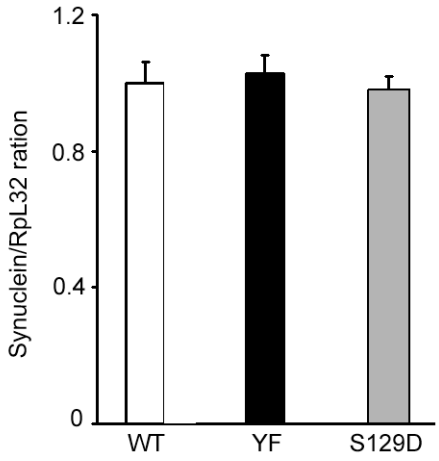
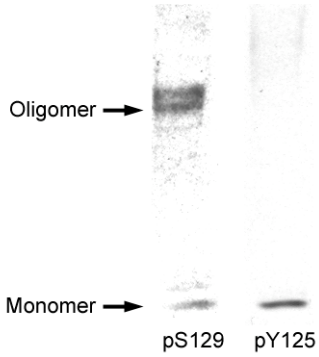
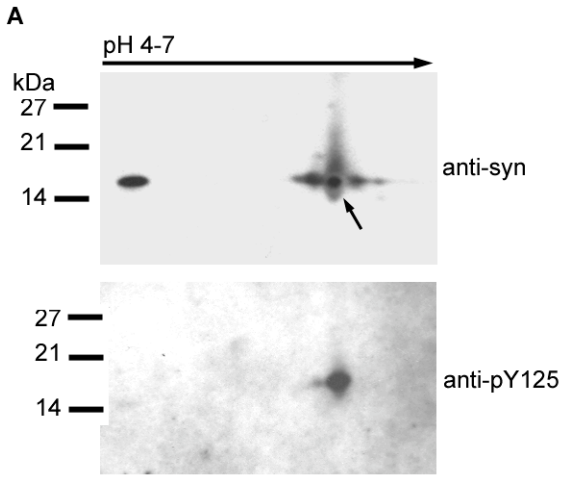
Supplemental Figures



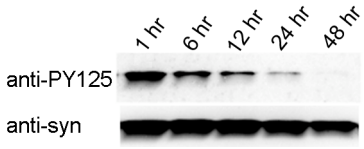
Suupplemental Figure 1. Quantitative real-time PCR comparison of α-synuclein expression levels in transgenic flies. α-synuclein expression levels in fly heads in relation to ribosomal protein RpL32. Results show similar α-synuclein expression levels in all three genotypes. Experiments were performed in triplicate and were repeated three times with no consistent differences in α-synuclein expression observed over independent experiments. Driver is *elav-GAL4.*



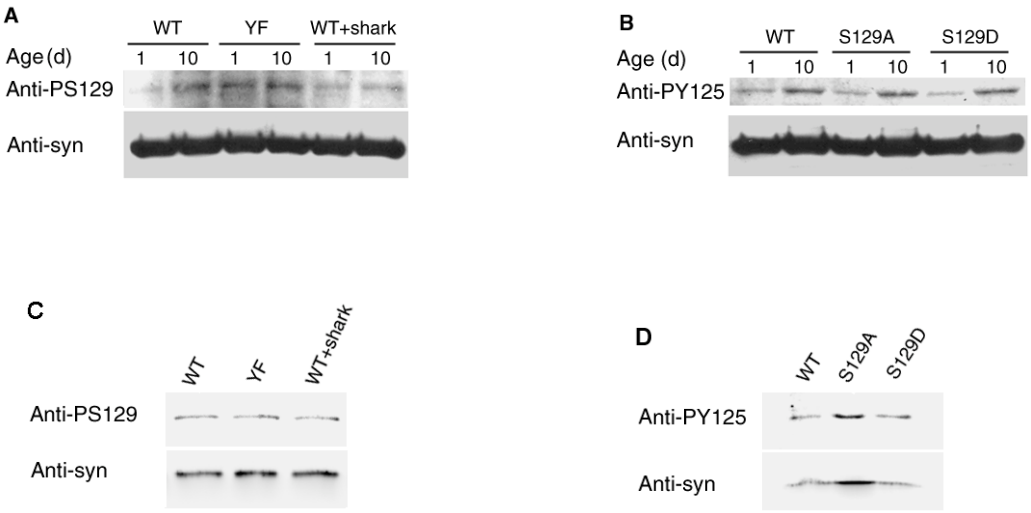
Supplemental Figure 2. Oligomerized α-synuclein is preferentially phosphorylated at Ser-129, and less phosphorylated at Tyr-125. Membranes with protein preparations from flies expressing wild-type α-synuclein were first probed with anti-PS129, followed by membrane stripping and reprobing with PY125. Exposure times were adjusted to obtain comparable hybridization signals from monomer α-synuclein. Driver is *elav-GAL4.* Flies are 20 days old.



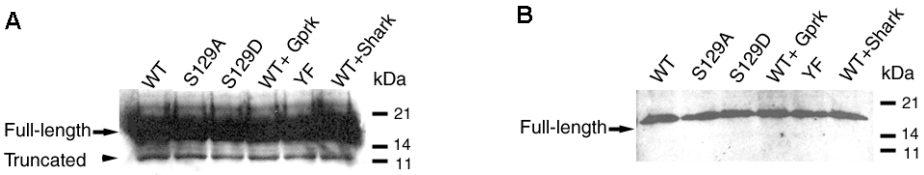
Supplemental Figure 3. Quantitative analysis of the extent of α-synuclein phosphorylation at Tyr-125. Soluble protein fractions were prepared from flies expressing wild-type α-synuclein immediately after decapitation and subjected to two-dimensional gel electrophoresis. The same membrane was probed with anti-α-synuclein, followed by stripping and reprobing with the PY125 antibody. Arrow indicates the Tyr-125 phosphorylated α-synuclein. Extent of phosphorylation was calculated as intensity of signal for phosphorylated α-synuclein as a fraction of total α-synuclein. 31.1 ± 1.4% (mean ± S.E.M. of three independent experiments) of wild type α-synuclein is phosphorylated at Tyr-125. Driver is *elav-GAL4*. Flies are 10 days old.



Supplemental Figure 4. Dephosphorylation of α-synuclein post mortem. To examine the stability of phosphorylation of Tyr-125 post mortem, heads from flies expressing wild-type α-synuclein were incubated at room temperature after decapitation for 1 hour to 48 hours prior to extraction, and soluble fractions were subjected to immunoblot analysis with PY125 (upper panel) and anti-α-synuclein (lower panel). Driver is *elav-GAL4.* Flies are 20 days old.



Supplemental Figure 5. Phosphorylation at Tyr-125 does not directly affect the phosphorylation at Ser-129 or visa versa. Western blot analysis of soluble **(A-B)** and insoluble **(C-D)** fractions of head extracts from transgenic flies expressing wild-type or mutant α-synuclein driven by *elav-GAL4*. Age refers to days after eclosion in A and B. Flies are 20 days old in C and D.



Supplemental Figure 6. Amount of naturally truncated α-synuclein is not altered by the phosphorylation status at Ser-129 or Tyr-125. (A) SDS-PAGE analysis of 20-day-old flies expressing wild-type or mutant α-synuclein as labeled above each lane under the control of the *elav-GAL4* driver. Comparable accumulation of a minor truncated form of α-synuclein migrating at 12 KDa (arrowhead), in addition to the major full-length band migrating at 17 KDa (arrow), is observed. **(B)** Immunoblot analysis reveals no clear difference in α-synuclein levels in control and experimental genotypes when using the LB509 antibody, which is directed against the C-terminal region of α-synuclein.