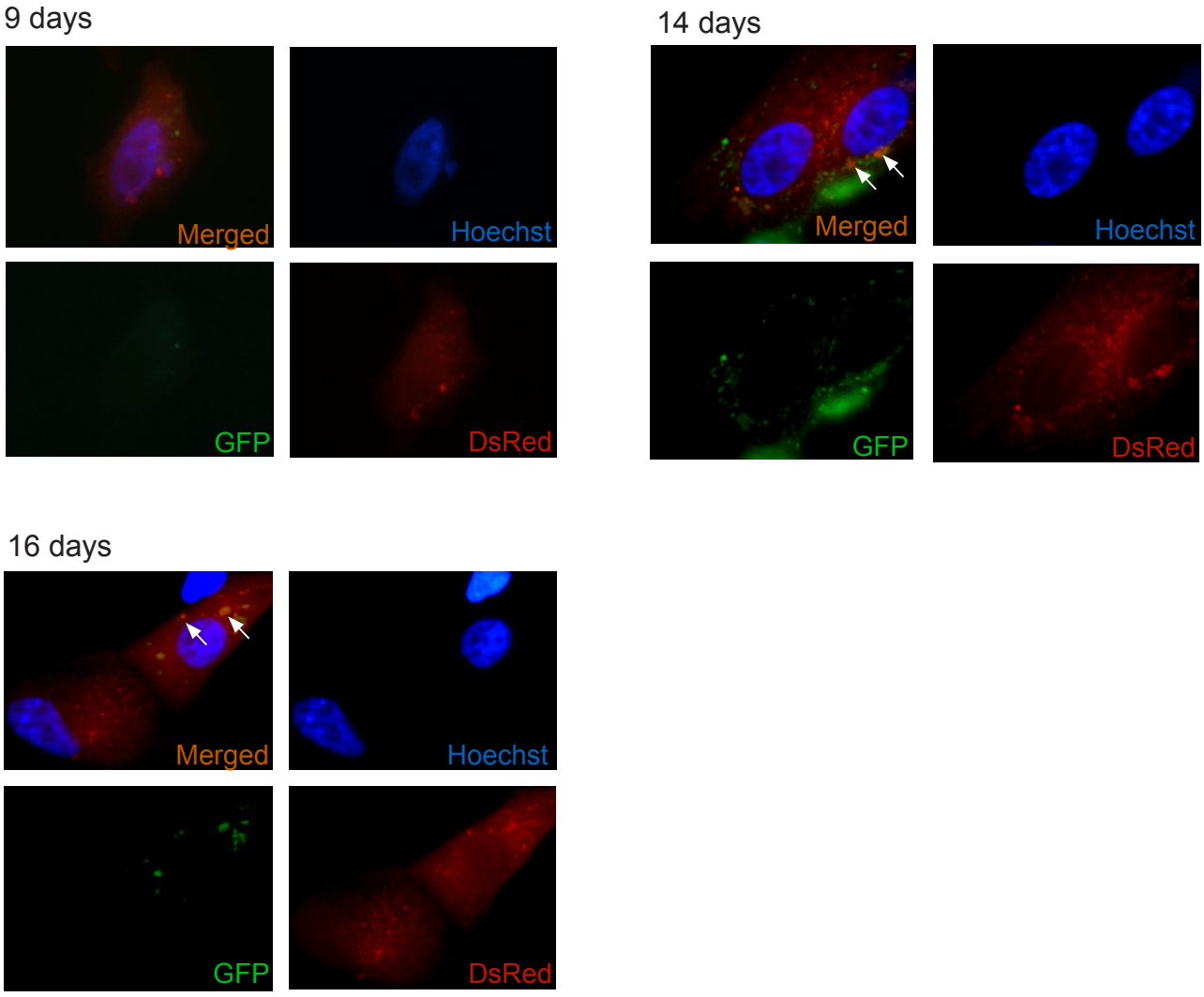

Figure S1. Intercellular propagation of α-syn in SH-SY5Y cell culture over time.
Representative pictures showing double-labeled SH-SY5Y cells after 9, 14 and 16 days of co-culture of stable SH-SY5Y cell lines expressing α-syn fused to either GFP or DsRed. The putative seeding effect, visualized by the presence of double-labeled aggregates, did not occur until day 14 (arrows).

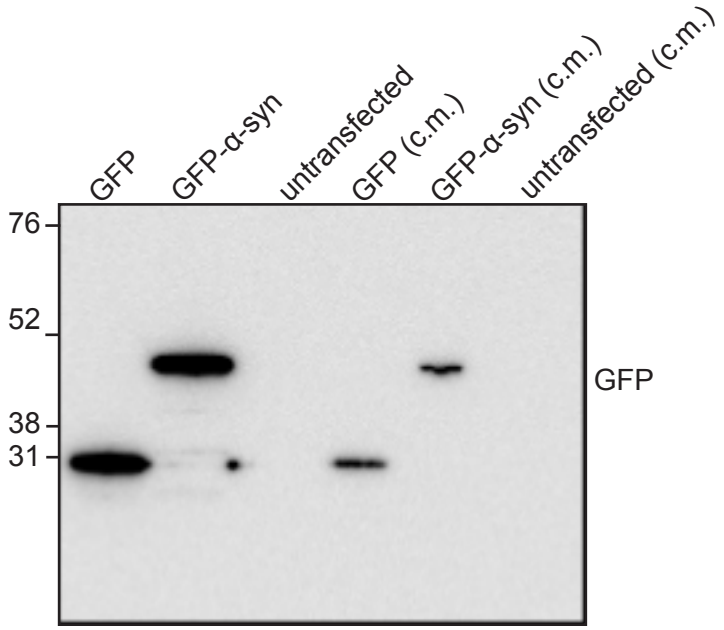

Figure S2. Comparison of GFP and GFP-α-syn secretion from HEK cells.
HEK cells were transiently transfected with either GFP or GFP-α-syn and secretion of the proteins (conditioned medium, c.m.) as well as cellular levels (three first lanes) were analyzed by Western blotting.

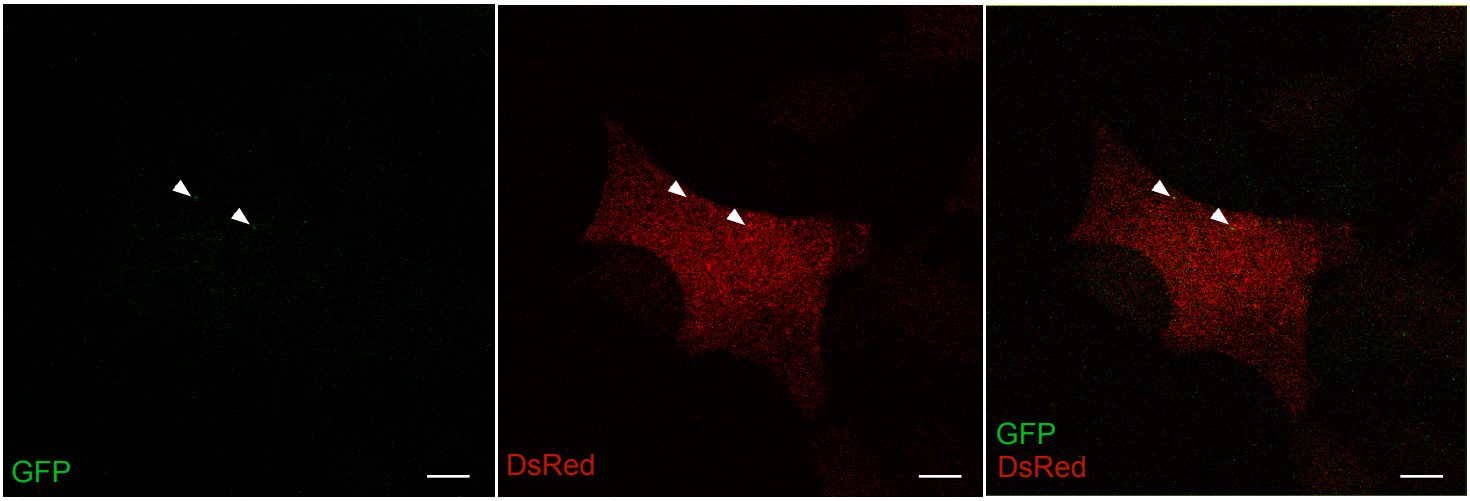

Figure S3. GFP conditioned medium does not induce seeding of intracellular α-syn.
Confocal planes of a α-syn-DsRed-expressing SH-SY5Y cell incubated with conditioned medium prepared from HEK cells transiently expressing GFP alone. This cell is one of the few cells displaying very small intracellular GFP-positive puncta (arrowheads), which are never colocalized with intracellularly expressed α-syn-DsRed aggregates. Scale bars: 10 μm.

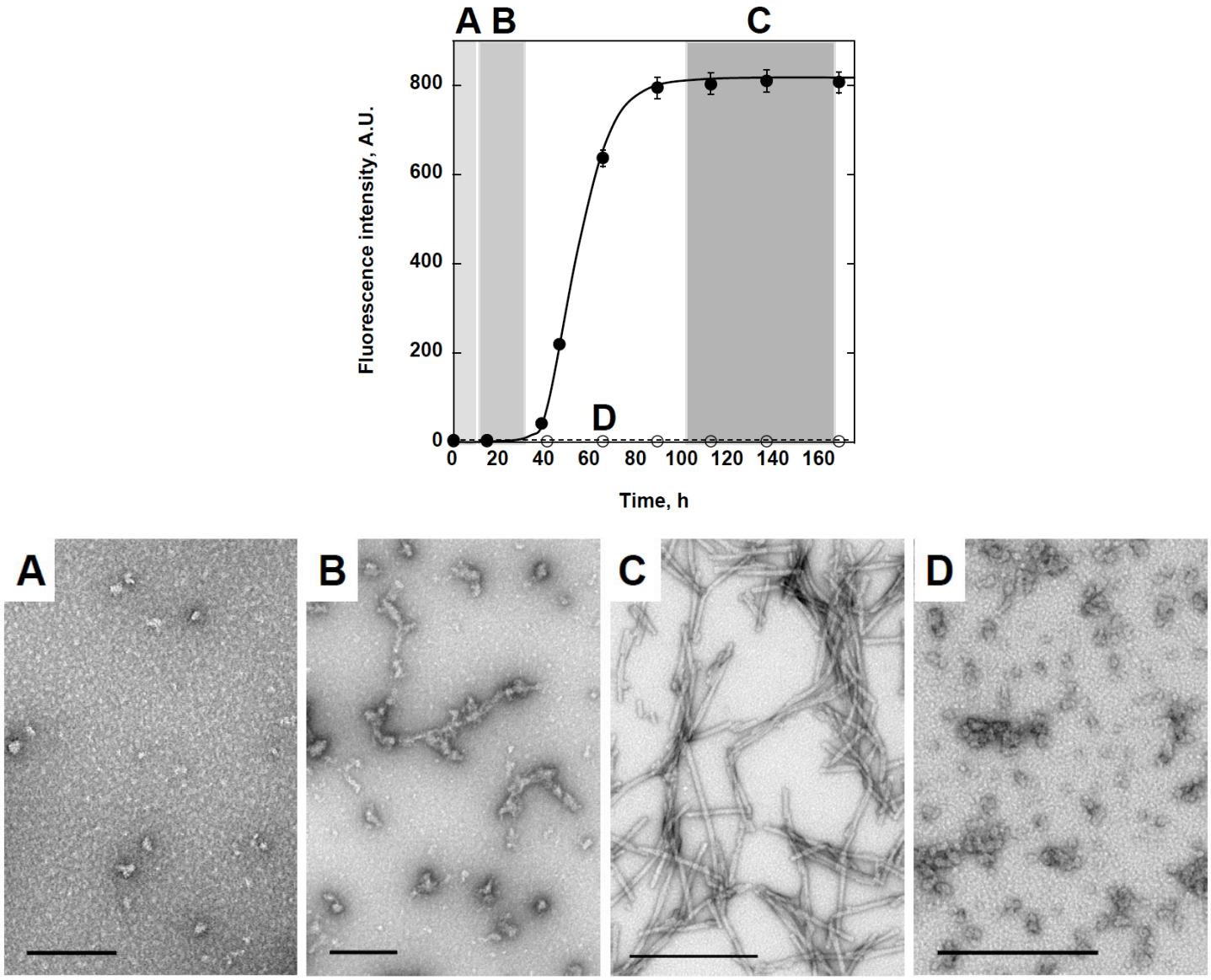

Figure S4. High molecular weight oligomeric species observed upon assembly of α-syn into fibrils.

The time course of α-syn assembly, in 20mM Tris, pH 7.5, 150mM NaCl, 1mM EGTA, 5mM ß-mercaptoethanol, at (●) 37 and (○) 4°C was monitored using Thioflavin T binding (top panel). Aliquots were removed from the solution within the time intervals indicated by the grey areas of increasing intensity, labeled A-D, and examined by transmission electron microscopy. Negatively stained electron micrographs of soluble α-syn (A), prefibrillar assemblies that form during the lag phase (B), fibrillar assemblies at steady state (C), oligomeric species that form upon incubation of α-syn under conditions where fibrils do not form (D, 4°C) and that correspond to off-fibrillar pathway assemblies. Scale bars: 0.2 μm.

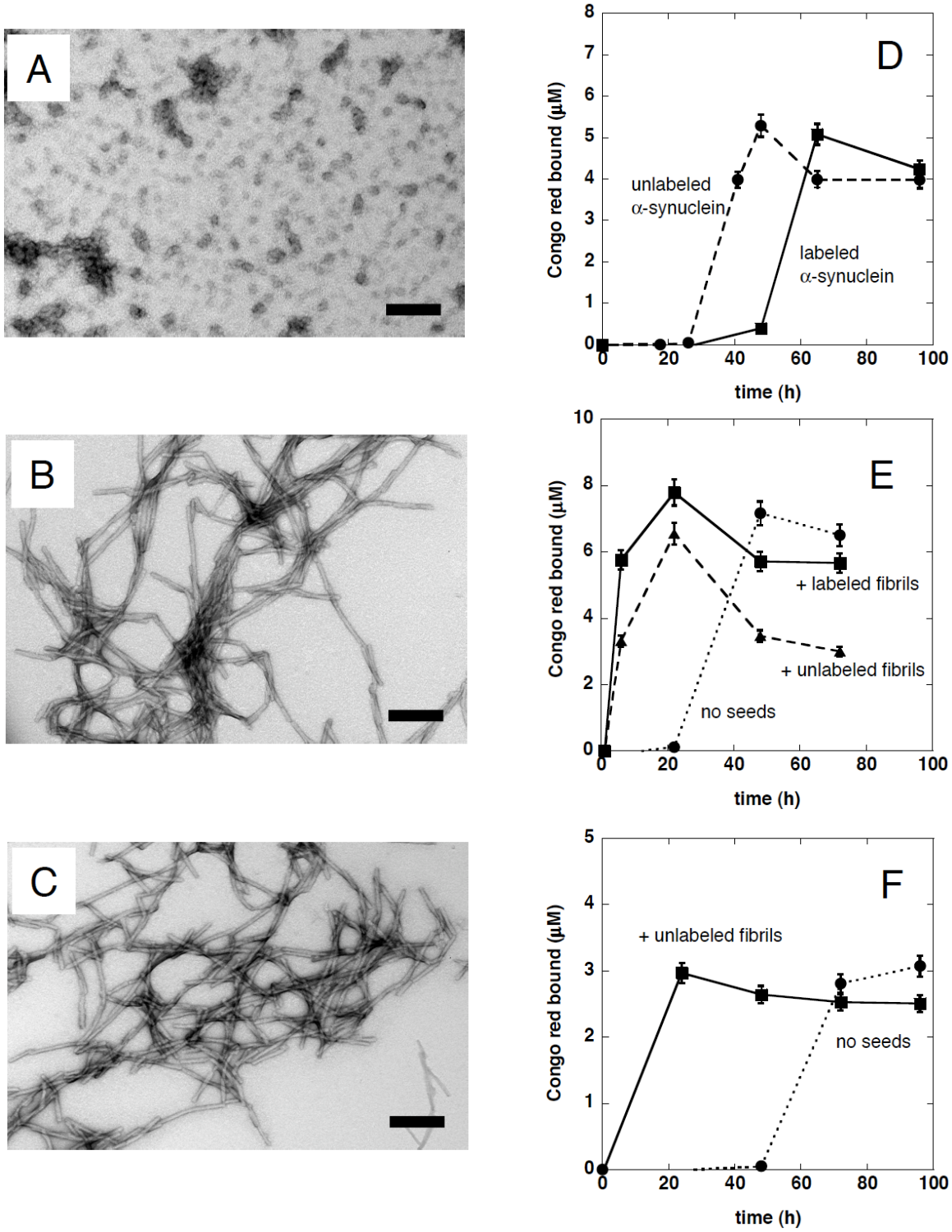

Figure S5. *In vitro* assembly and seeding properties of Alexa488-labeled α-syn.
Negatively stained electron micrographs of oligomeric species formed upon incubation of the Alexa488-labeled α-syn at 4°C (A), fibrils formed from Alexa488-labeled α-syn at steady state (B) and fibrils labeled with Alexa488 after assembly (C). (D) Assembly kinetics of soluble unlabeled (●, dashed line) and Alexa488-labeled α-syn, 120 µM (■, solid line), monitored by Congo Red binding. (E) Assembly kinetics of unlabeled α-syn, 200 µM, incubated in the absence (●, dotted line) or in the presence of 20 µM preformed unlabeled (▲, dashed line) or labeled α-syn fibrils (■, solid line), monitored by Congo Red binding. (F) Assembly kinetics of Alexa488-labeled α-syn, 100 µM, incubated in the absence (●, dotted line) or the presence of 10 µM preformed unlabeled α-syn fibrils (■, solid line), monitored by Congo Red binding. The error bars correspond to the standard deviation derived from two independent measurements. Scale bars : 0.2 µm.

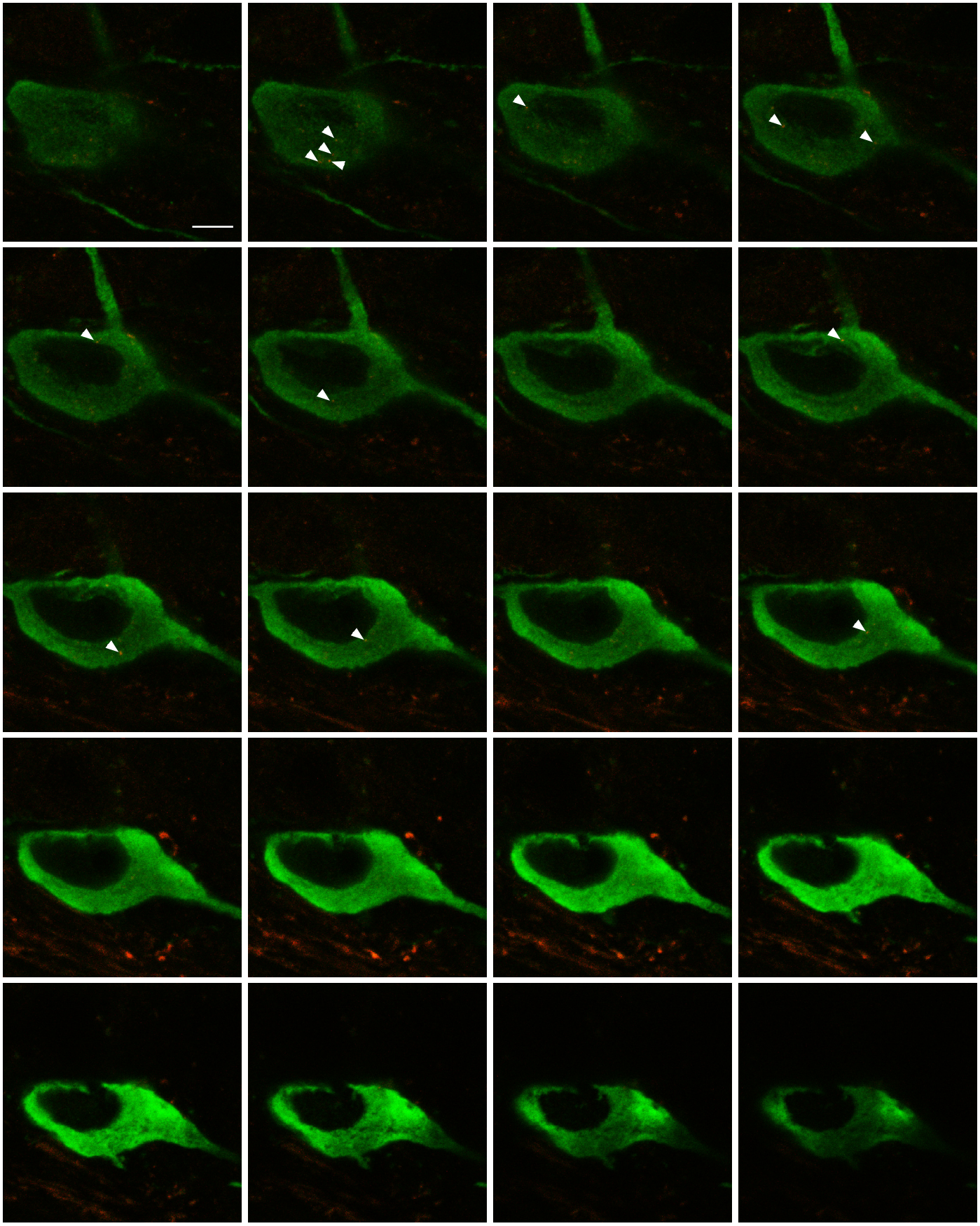

Figure S6. Multiple transferred human α-syn puncta in grafted neuron
Complete z-stack with 20 confocal planes (interval : 0.3 μm) of a grafted TH-positive neuron displaying several puncta of transferred human α-syn (arrowheads). Scale bar: 5 μm.