Cell lines:

* 1K cells (expressing E46K synuclein-Venus YFP).
* WT cells (expressing wild type synuclein-Venus YFP)

Experimental Procedure:

Cells were treated with doxycycline for 48 hours. 48 hours after the addition of doxycycline, the cells were treated with 120nM HTRA1 in DMEM before being transfected with 0.15µg (30nM) of seeds using lipofectamine 3000. Cells were imaged three days post transfection. Cells were imaged on a Zeiss LSM800 confocal with AiryScan with a 63x oil-immersion objective. Cells were excited using the 647nm laser and the 488nm laser and emission was collected from 637nm-700nm and 490-549nm, respectively.

Observations:

* Inclusions formed in all wells transfected with the seeds.
* 1K cells: The cells that were also treated with HTRA1 had a mix of inclusion presentations; some cells had lots of very small inclusions, and some had fewer small inclusions and some large inclusions. The 1K cells that received seeds alone only had lots of small inclusions but no large inclusions. FRAP analysis of the bigger inclusions in the cells that also received HTRA1 suggest that they are solid. The HTRA1 localized within the larger inclusions.
* WT cells: There was no discernable difference in appearance of the inclusions between the cells that received the seeds and the cells that received the seeds and HTRA1. Both wells showed cells with some small inclusions and few big inclusion. Again, FRAP analysis suggests that these big inclusions are solid. The cells that were treated with PFF+HTRA1 had a lower frequency of inclusions in comparison to the cells that were treated with PFF only.
* Those experiments need to be repeated 5 times in total to confirm these preliminary results.