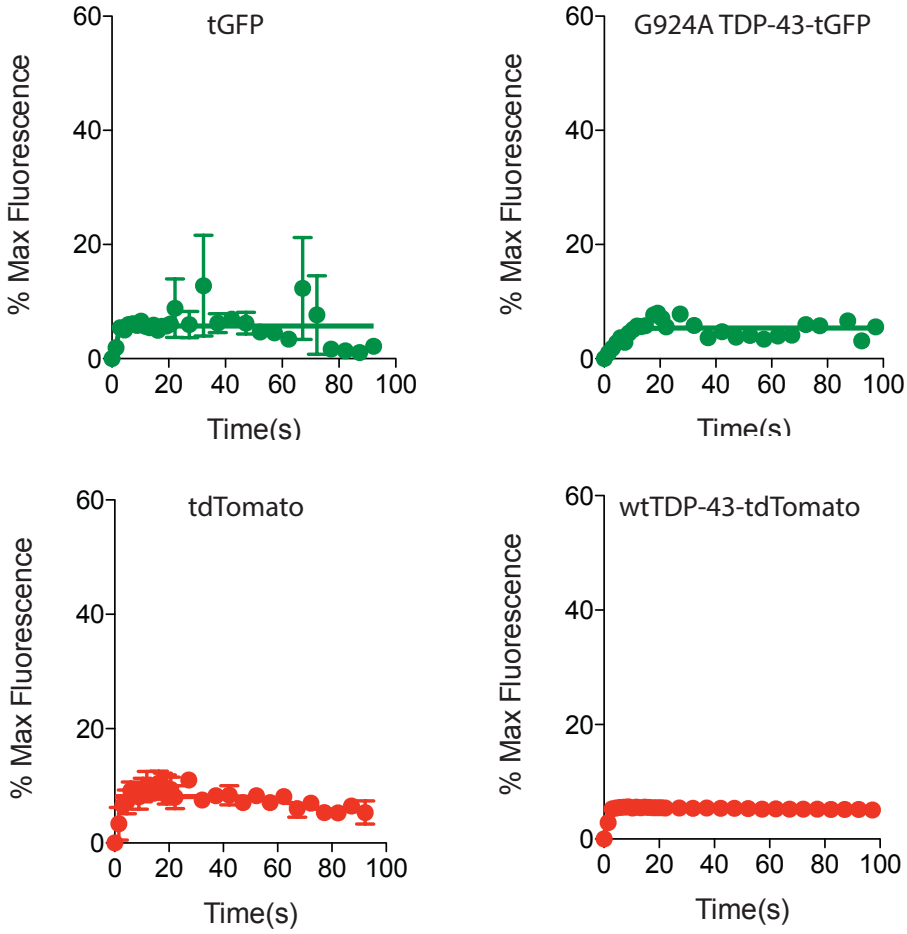

Fig. S1. FRAP analysis of inclusions containing TDP-43, tGFP or tdTomato. FRAP analysis of proteins in inclusions in NSC-34 cells expressing either tGFP, mutant G294A TDP-43-tGFP, tdTomato or wt TDP-43-tomato. Mean fluorescence intensity (from within the ROI) plotted over time. Prebleach intensity was recorded, and recovery was recorded for up to 100s. Low recovery is consistent with dense insoluble aggregates. Results are means and standard deviation from n = 3.

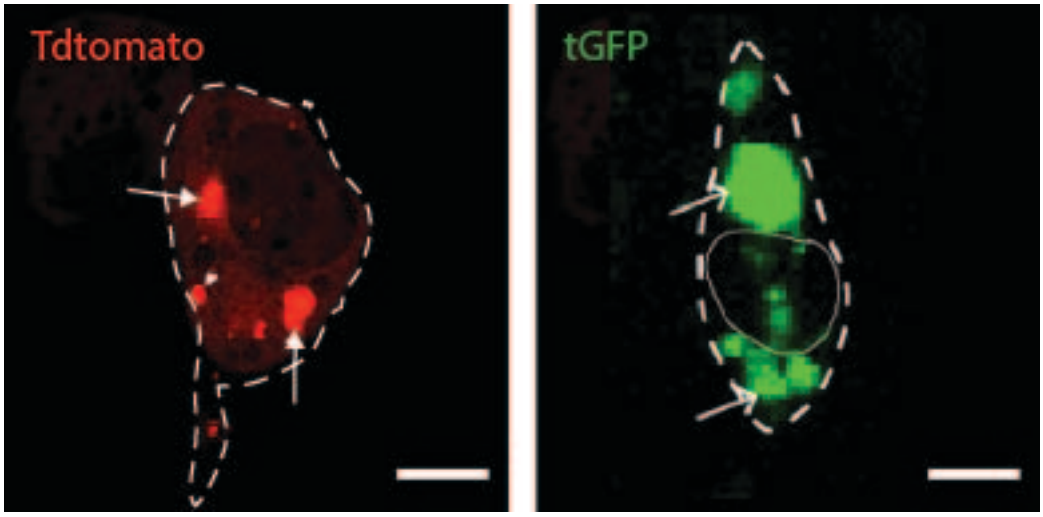

Fig. S2. Morphology of tdTomato and tGFP aggregates. Confocal microscopy identifies distinct inclusions (white arrows; Scale bar 5 μm). Dashed line represents cell border from transmission image.

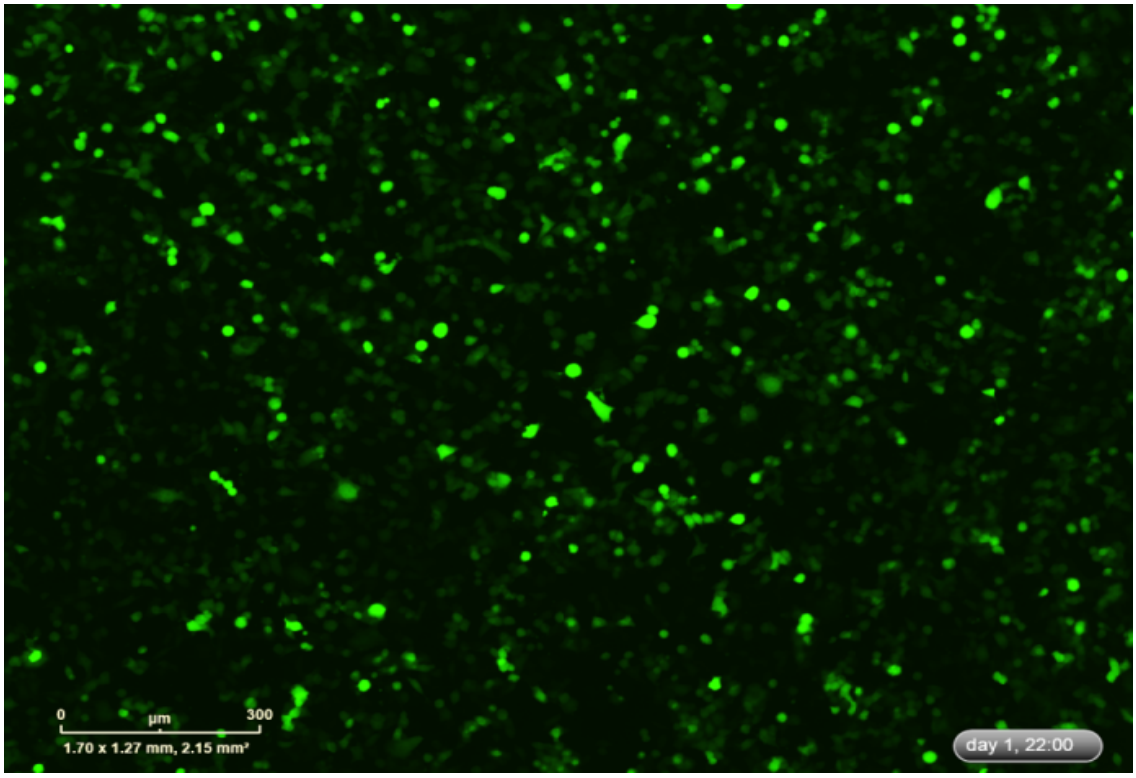

Fig. S3. Widefield view of cells expressing tGFP 48 hours after transfection. Cells were transfected and then imaged through time using an Incucyte zoom. (Scale bar 300 µm).

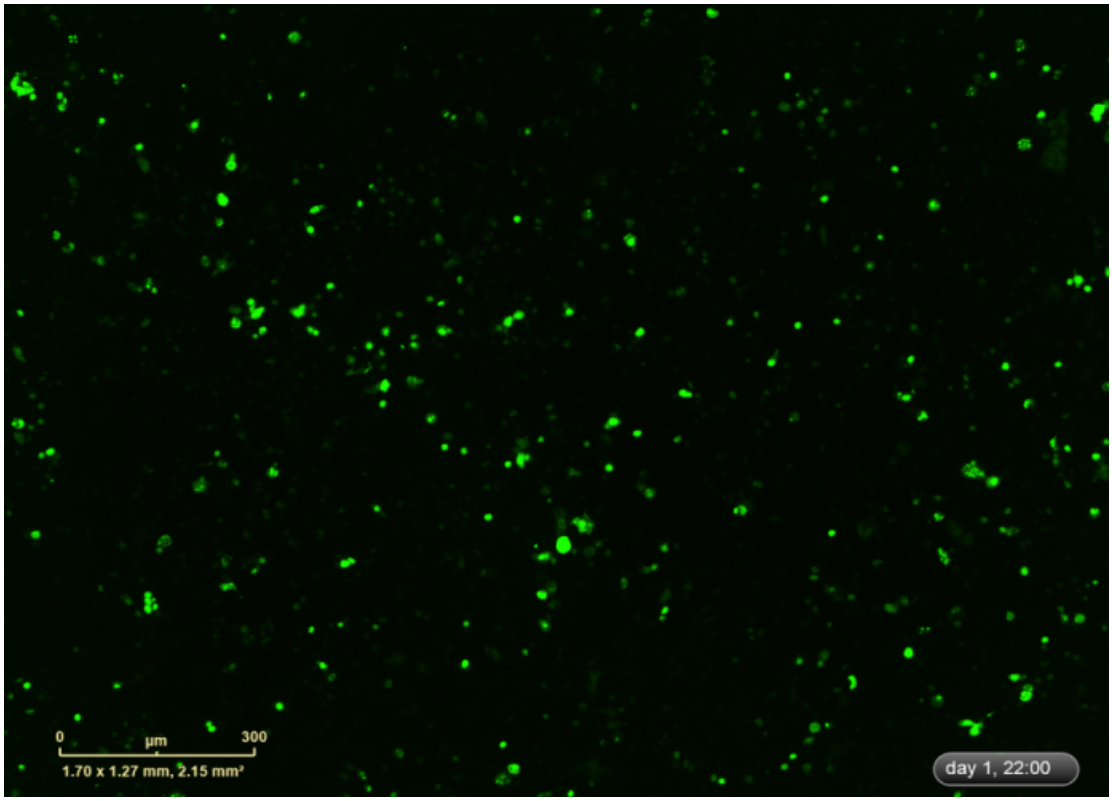

Fig. S4. Widefield view of cells expressing G294 TDP-43-tGFP 48 hours after transfection. Cells were transfected and then imaged through time using an Incucyte zoom. (Scale bar 300 µm).

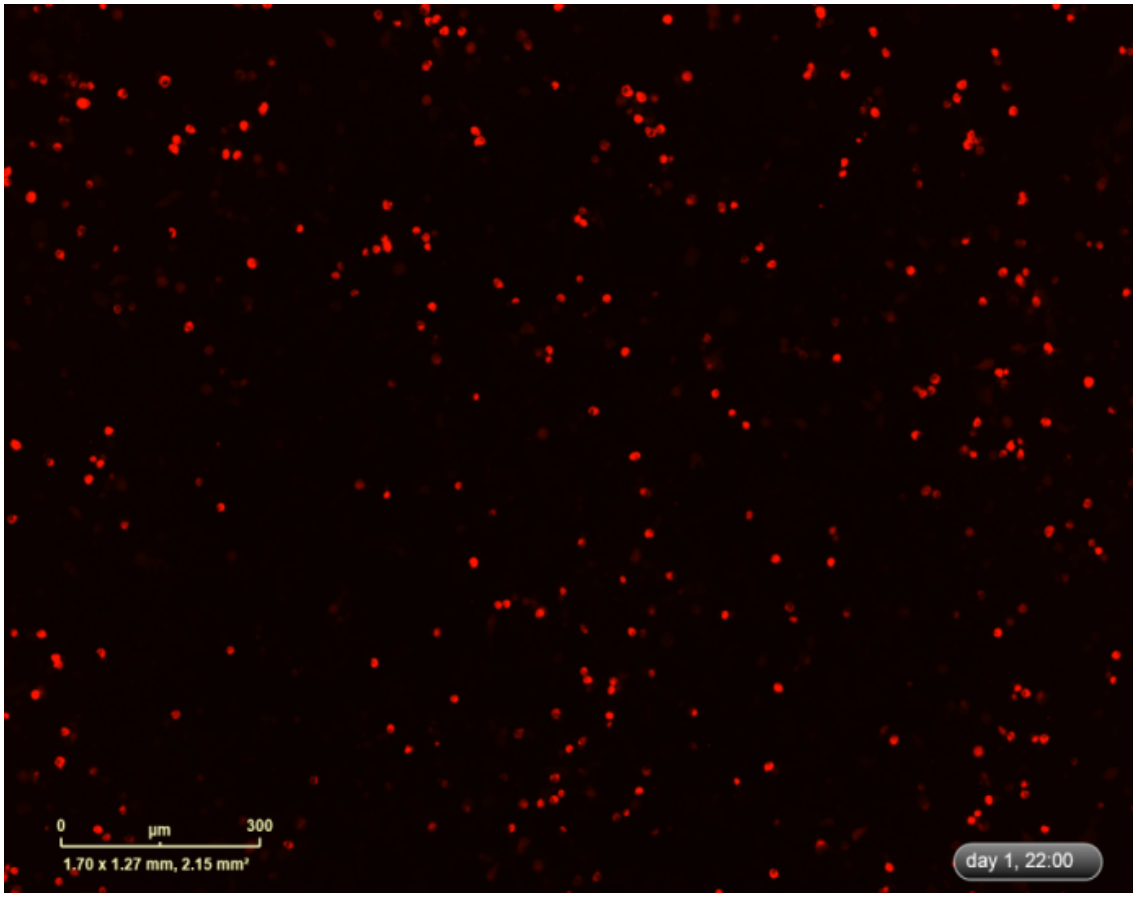

Fig. S5. Widefield view of cells expressing tdTomato 48 hours after transfection. Cells were transfected and then imaged through time using an Incucyte zoom. (Scale bar 300 µm).

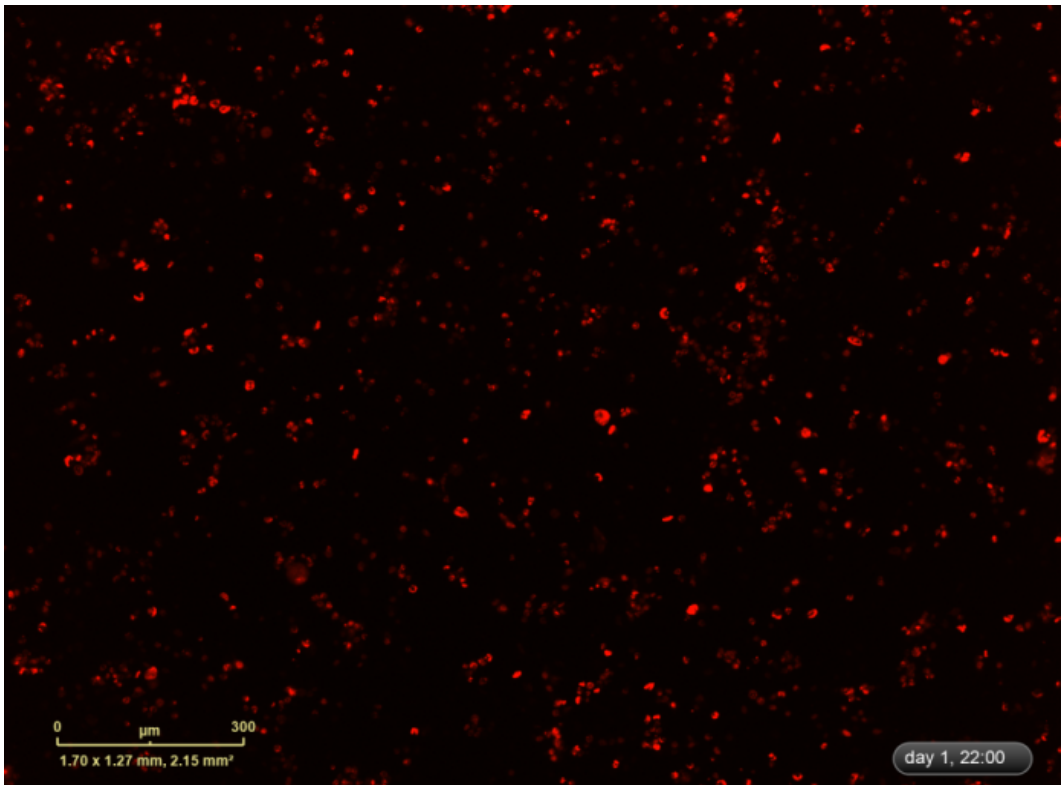

Fig. S6. Widefield view of cells expressing wt TDP-43-tdTomato 48 hours after transfection. Cells were transfected and then imaged through time using an Incucyte zoom. (Scale bar 300 µm).

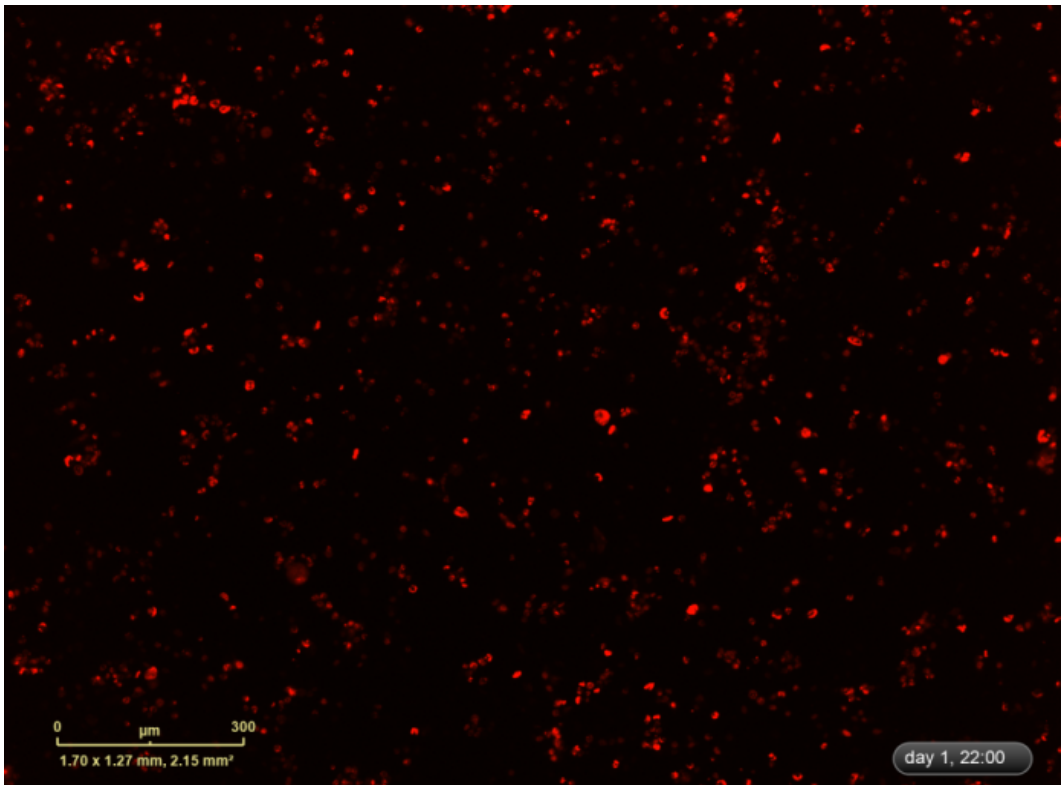

Fig. S7. Widefield view of cells expressing wt TDP-43-tdTomato 48 hours after transfection. Cells were transfected and then imaged through time using an Incucyte zoom. (Scale bar 300 µm).

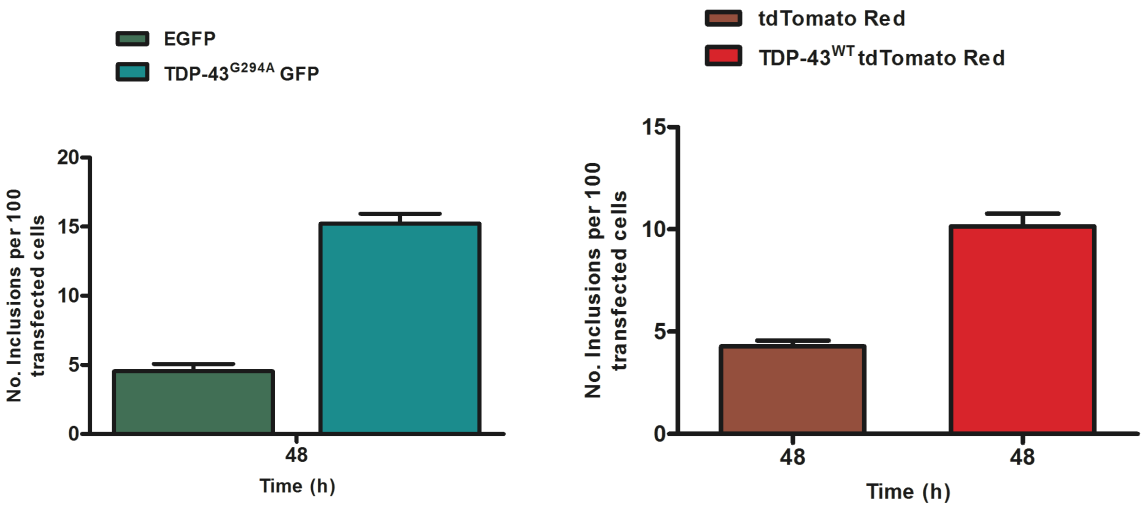

Fig. S8. Inclusions counted per transfected cell from imaging. Cells were transfected and then imaged through time using an Incucyte zoom. Inclusions were identified by eye and scored as a percentage of total cells transfected.

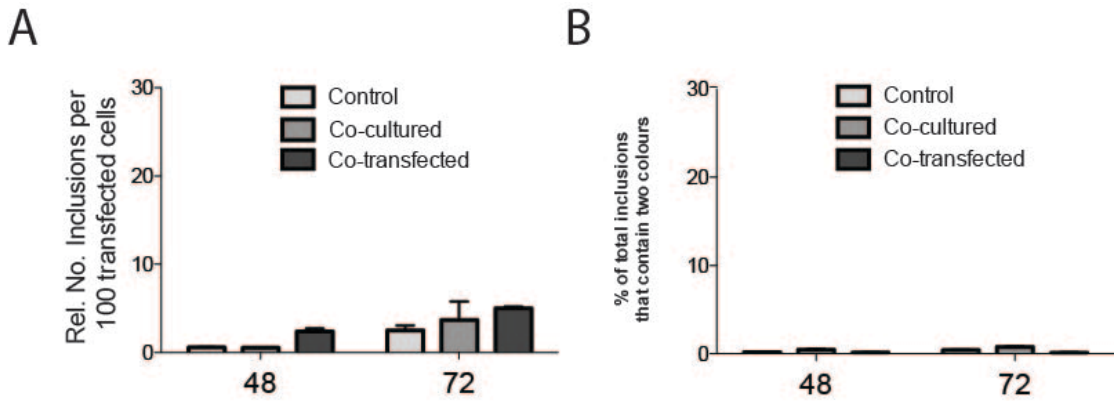

Fig. S9. FloIT detection of two-colour inclusions NSC-34 cell lysates. NSC-34 transiently transfected with tdTomato or tGFP were either mixed at the end of the incubation or immediately following transfection. Alternatively, cells were co-transfected to express both fusion proteins. After incubation for either 48 or 72 h cells were lysed and analysed by FloIT. (A) The total number of tdTomato inclusions including dual colour inclusions were enumerated by FloIT and are shown as means ± SEM (n = 3). (B) The percentage of TDP-43 inclusions formed in each treatment at time intervals that contained both tdTomato and tGFP enumerated by FloIT are shown as means ± SEM (n = 3 independent experiments). To allow comparison to the TDP-43 fusions, these results are presented on the same y-axis scale, and the inclusions counted here are relative to the level of mean fluorescence of TDP-43 fusions, as presented in Fig 3.

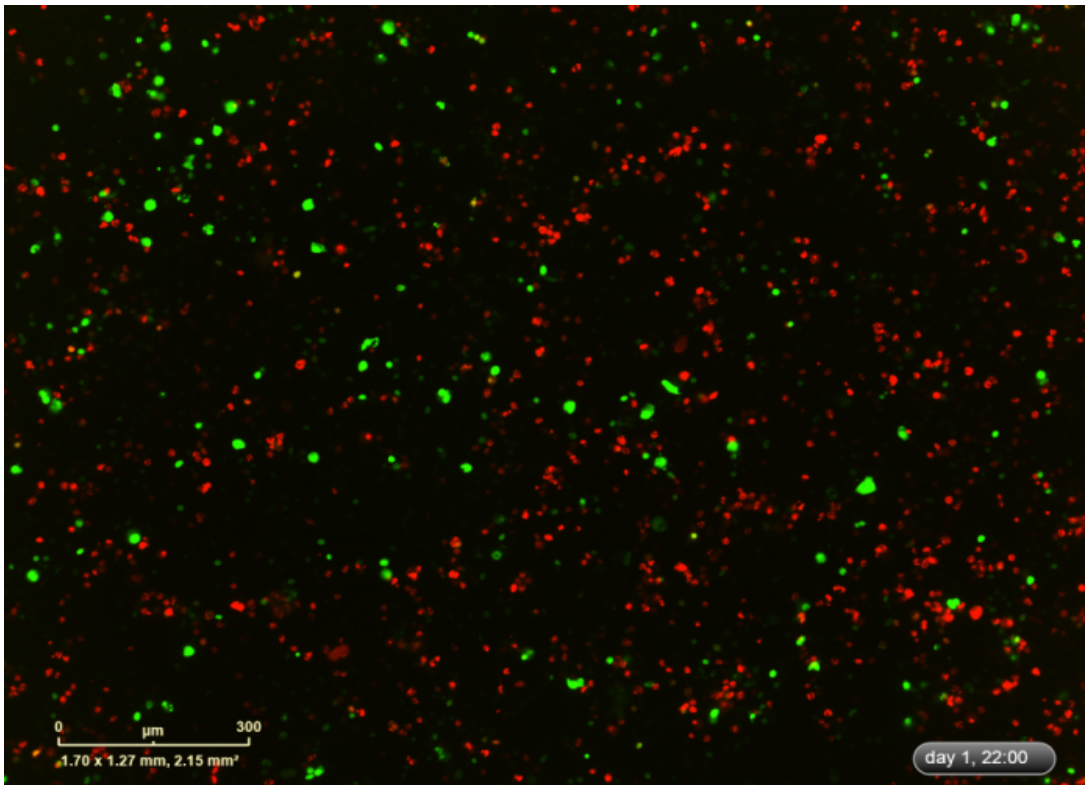

Fig. S10. Widefield view of cells expressing either G294A TDP-43-tGFPwt TDP-43-tdTomato and co-cultured for 48 hours after transfection. Cells were transfected and then imaged through time using an Incucyte zoom. (Scale bar 300 µm).

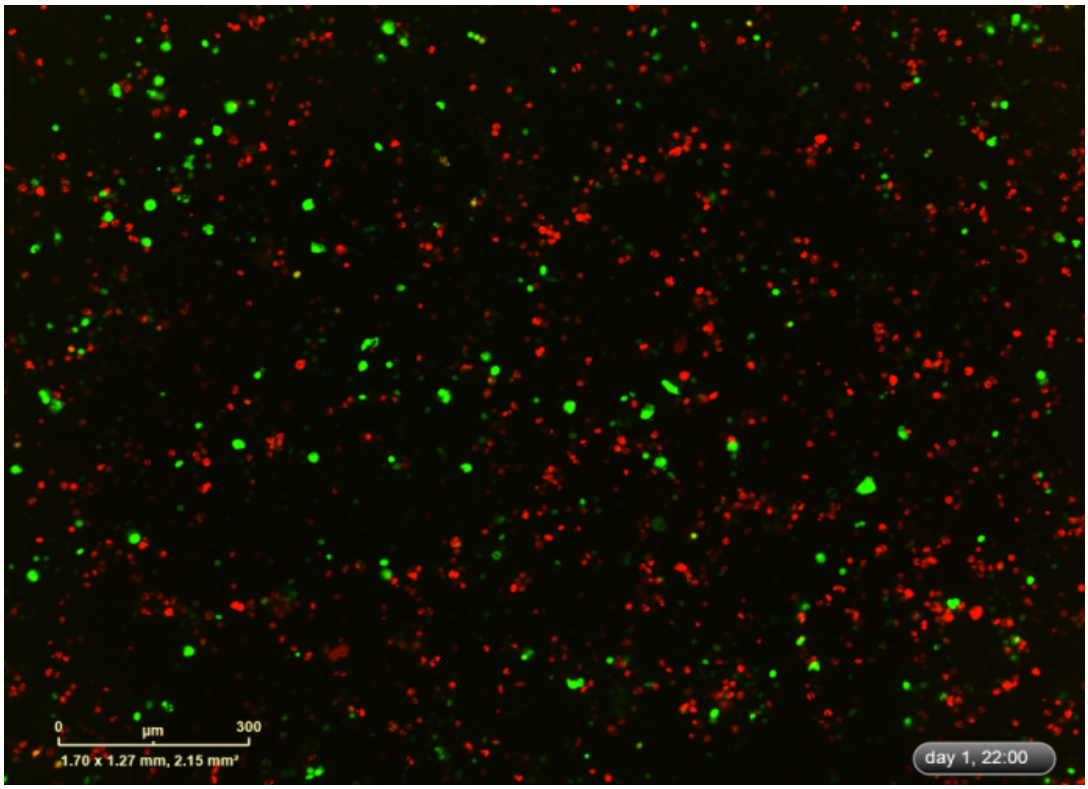

Fig. S11. Widefield view of cells expressing either G294A TDP-43-tGFPwt TDP-43-tdTomato and co-cultured for 48 hours after transfection. Cells were transfected and then imaged through time using an Incucyte zoom. (Scale bar 300 µm).

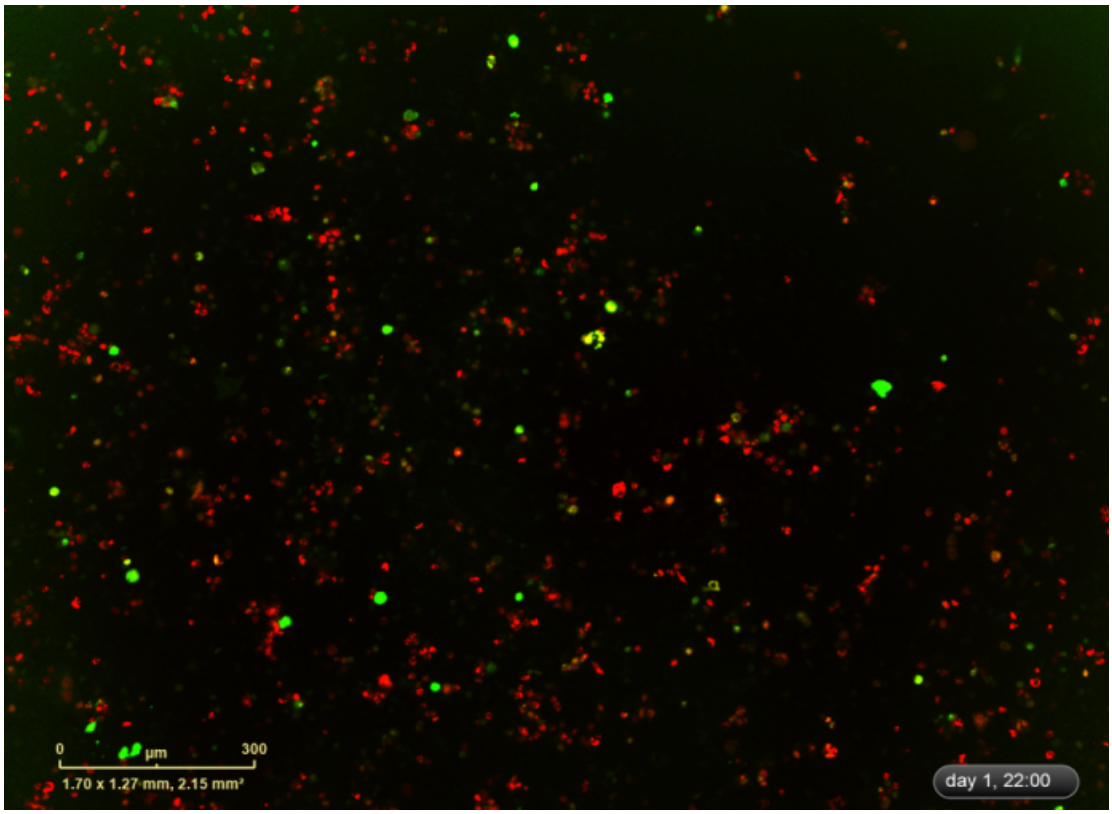

Fig. S12. Widefield view of cells co-transfected with both G294A TDP-43-tGFPwt TDP-43-tdTomato and cultured for 48 hours after transfection. Cells were transfected and then imaged through time using an Incucyte zoom. (Scale bar 300 µm).