Landes Bioscience www.landesbioscience.com

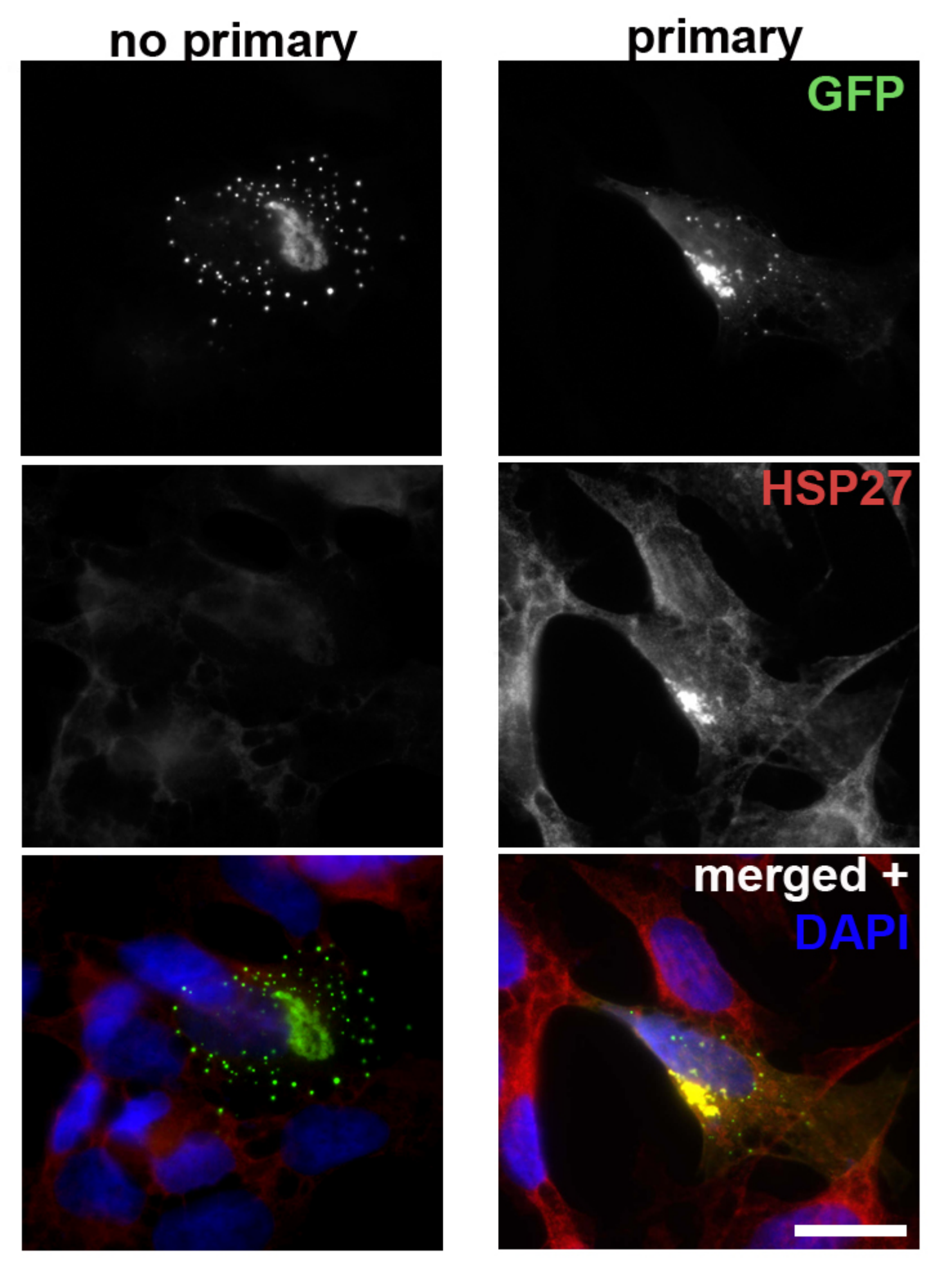
Supplemental Material to:

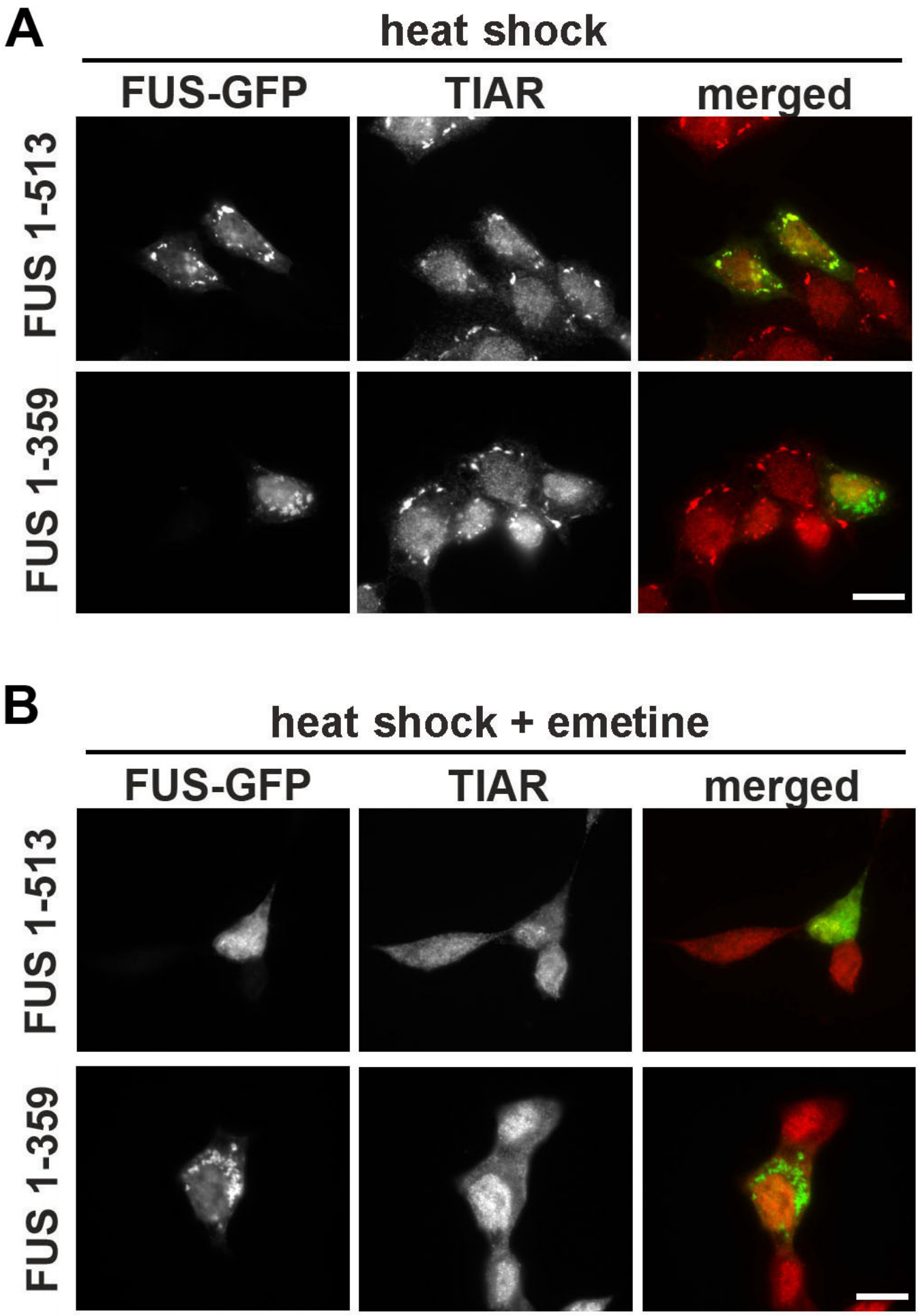
Tatyana A Shelkovnikova, Hannah Robinson, Natalie Connor-Robson, and Vladimir L Buchman

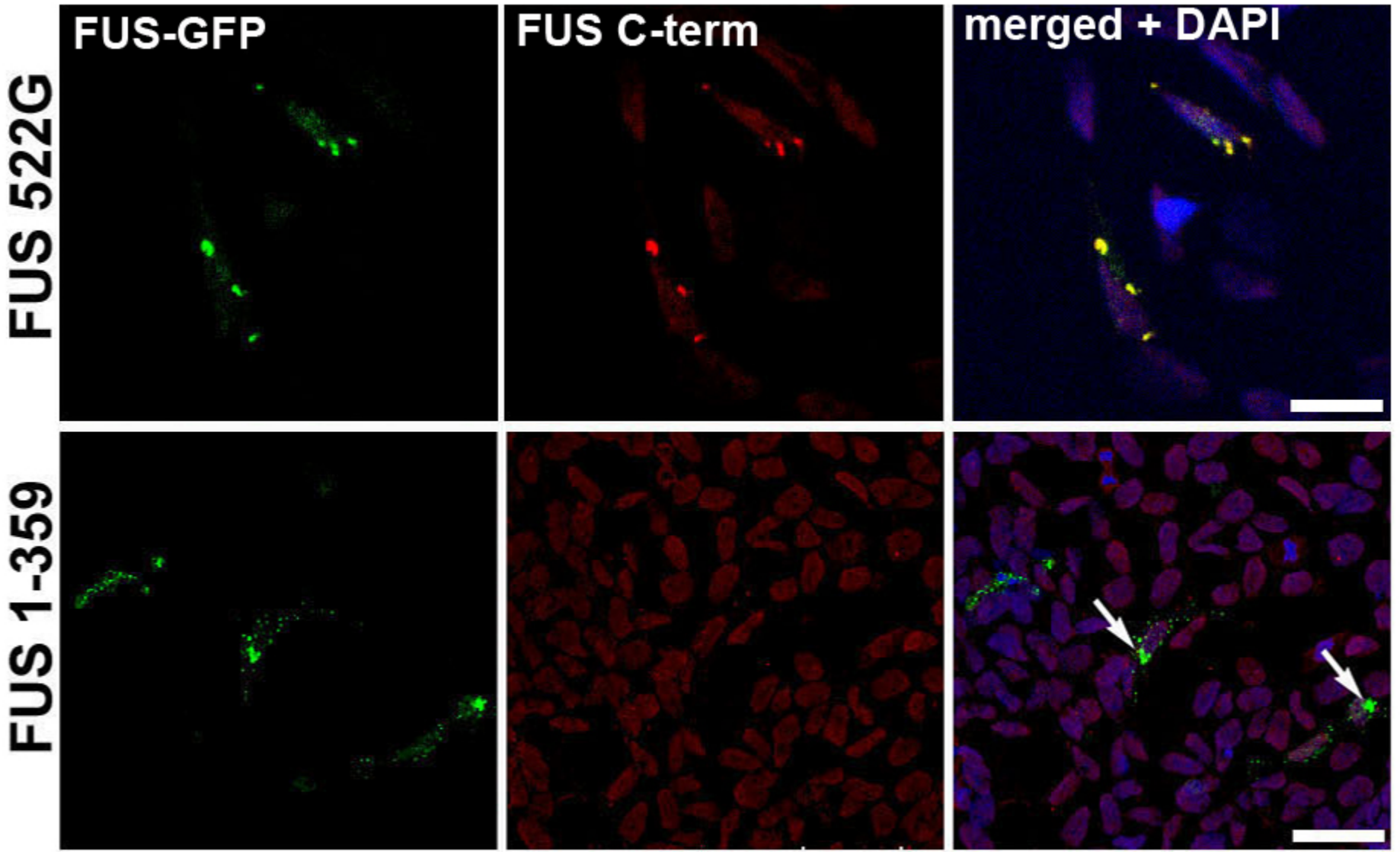
Recruitment into stress granules prevents irreversible aggregation of FUS protein mislocalized to the cytoplasm

Cell Cycle 2013; 12(19)
http://dx.doi.org/10.4161/cc.26241

http://www.landesbioscience.com/journals/cc/article/26241


no primary
primary
GFP
HSP27
merged +
DAPI


A
heat shock
FUS-GFP
TIAR
merged
FUS 1-513
FUS 1-359
B
heat shock + emetine
FUS-GFP
TIAR
merged
FUS 1-513
FUS 1-359


FUS-GFP
FUS C-term
merged + DAPI
FUS 522G
FUS 1-359

Supplementary Video and Figure Legends

Video S1. Formation of small cytoplasmic aggregates of the FUS 1-359 protein. Multiple small aggregates of GFP-FUS 1-359 protein (green) are formed throughout the cytoplasm of cells few hours after transfection with a corresponding plasmid. Time-lapse microscopy using Leica TCS SP2 MP confocal microscope (Fluotar L 63x0.70 oil objective). A sequence of images taken with 8 min intervals over a period of approximately 4 hours was further transformed into a movie using Leica Application Suite AF software.

Video S2. Fusion and translocation of small cytoplasmic aggregates of the FUS 1-359 protein. Large juxtanuclear aggresome is formed by fusion of smaller aggregates (appearing as a small round puncta) initially dispersed throughout the cytoplasm of cells expressing GFP-FUS 1-359 protein (green) after transient transfection of SH-SY5Y cells with a corresponding plasmid. Time-lapse microscopy using Leica TCS SP2 MP confocal microscope (Fluotar L 63x0.70 oil objective). A sequence of images taken with 8 min intervals over a period of approximately 4 hours was further transformed into a movie using Leica Application Suite AF software.

Fig. S1. Large perinuclear structures formed by FUS 1-359 are positive for small chaperone HSP27, a typical constituent of aggresome. Control immunostaining in the absence of primary anti-HSP27 antibody (left panels) demonstrates that the observed signal in the red cannel (right panels) is not due to the bleeding-through of green GFP fluorescence.

Fig. S2. Truncated FUS 1-359 is not recruited into stress granules upon heat shock. FUS 1-513 but not FUS 1-359 is recruited into TIAR-positive stress granules upon heat shock (43ºC for 1 hour) **(A)** and heat shock induced stress granules containing FUS 1-513 are dissipated upon concurrent emetine treatment, while FUS 1-359 aggregates remain intact **(B)**. Scale bars: 20 μm for all panels.

Fig. S3. Antibody against C-terminal epitope of FUS does not detect FUS 1-359 and therefore can be used for specific localization of full-length variants in co-transfection experiments. FUS R522G but not FUS 1-359 (arrows) expressed in SH-SY5Y neuroblastoma cells are recognized by an antibody against C-terminus of FUS. Scale bar: 35 μm the top and 45 μm for the bottom panel.