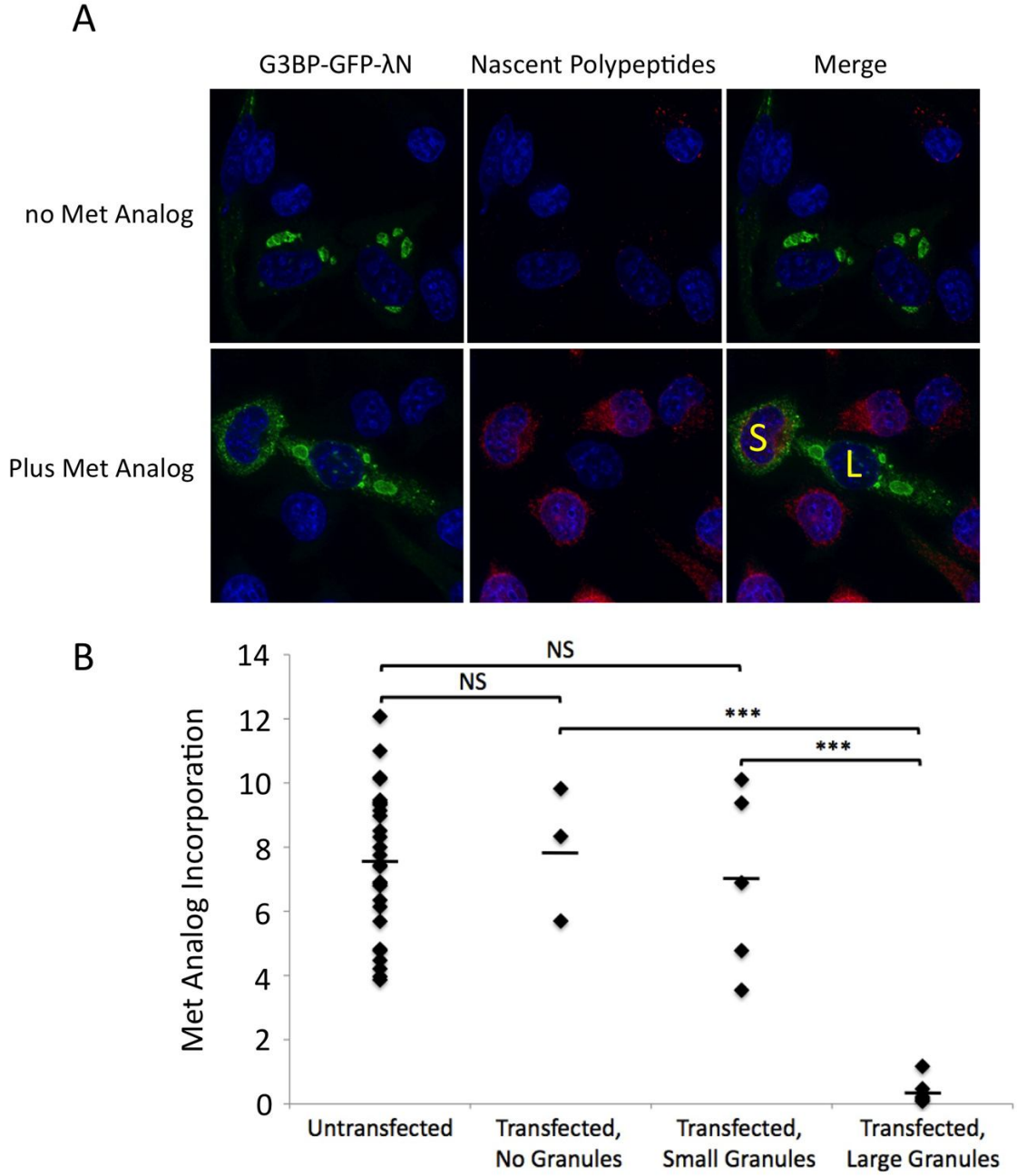
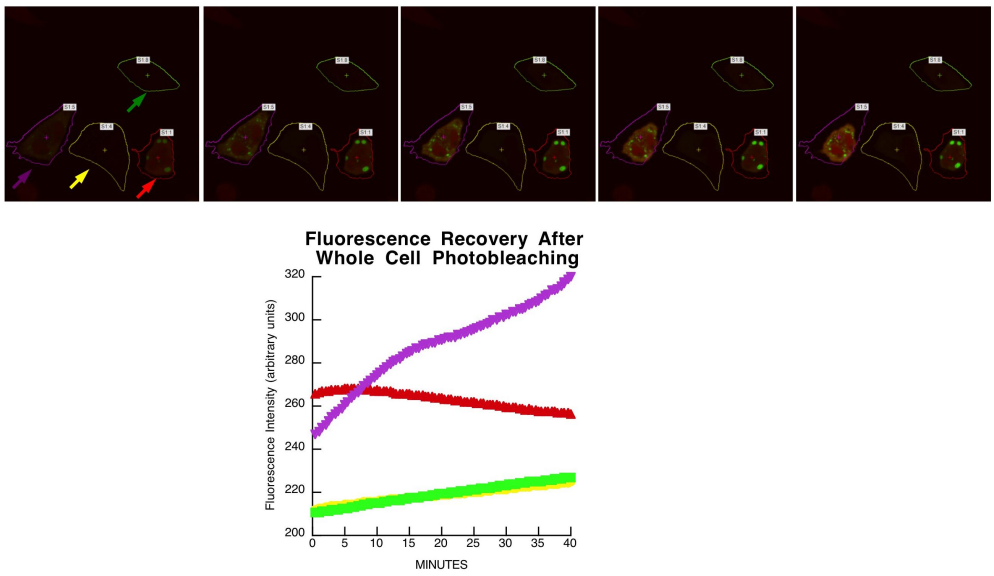
Supplementary Information:



Supplementary Figure 1: Large G3BP-induced granules exhibit translational repression measured by BONCAT. Hela cells were transfected with G3BP-GFP-λN and pulsed for 30 minutes with either methionine (no Met Analog) or L-azidohomoalanine (Plus Met Analog) prior to fixation to detect novel protein synthesis (nascent polypeptides). Cells were imaged using deconvolution microscopy (A). Quantification of BONCAT results using Image J software (B). The y-axis represents intensity of BONCAT signal/cell area*1000. Statistical analysis was done using an equivariant, two-tailed students t-test. ***, $p \leq 0.001$; NS, Not significant.

Supplementary
Figure 2



Supplementary Figure 2: Large G3BP-induced stress granules repress translation and persist during live cell imaging. Whole cell photobleaching of m-Cherry was performed and fluorescence recovery was quantified during a 45 minute live cell recovery period. Images from time points throughout the recovery period are shown, with each photobleached cell outlined in a different color and marked by colored arrow in left panel. Red, m-Cherry, intensity was quantified throughout the course of the recovery period and fluorescence intensity was plotted as a function of time. Each line color corresponds to the cell outline/label arrow displayed in the images. Cell marked with red periphery/arrow contains large stress granules and cell marked with violet is a cell with small stress granules. All cells showed increasing fluorescence intensity from translation of nascent m-cherry except the cell with large stress granules.

BONCAT Methods

Bioorthagonal noncanonical amino acid tagging (BONCAT; Dieterich et al 2007 Nat Protocols) was conducted with the cell labeling kit (Invitrogen) essentially as described previously (Qin et all 2011 J Virol). Briefly, modifications included blocking with 3% BSA/PBST and use of streptavidin 647 (Molecular Probes) for detection of the conjugated biotin tag.

Fluorescence Recovery After Photobleaching Methods

A Nikon A1 laser confocal microscope was used to photobleach m-Cherry from Hela cells transfected with both m-Cherry and G3BP-GFP-λN constructs, and expressing G3BP-induced stress granules of varying sizes. Total red fluorescence due to nascent translation was quantified over a 45 minute period following photobleaching using Nikon Elements software.