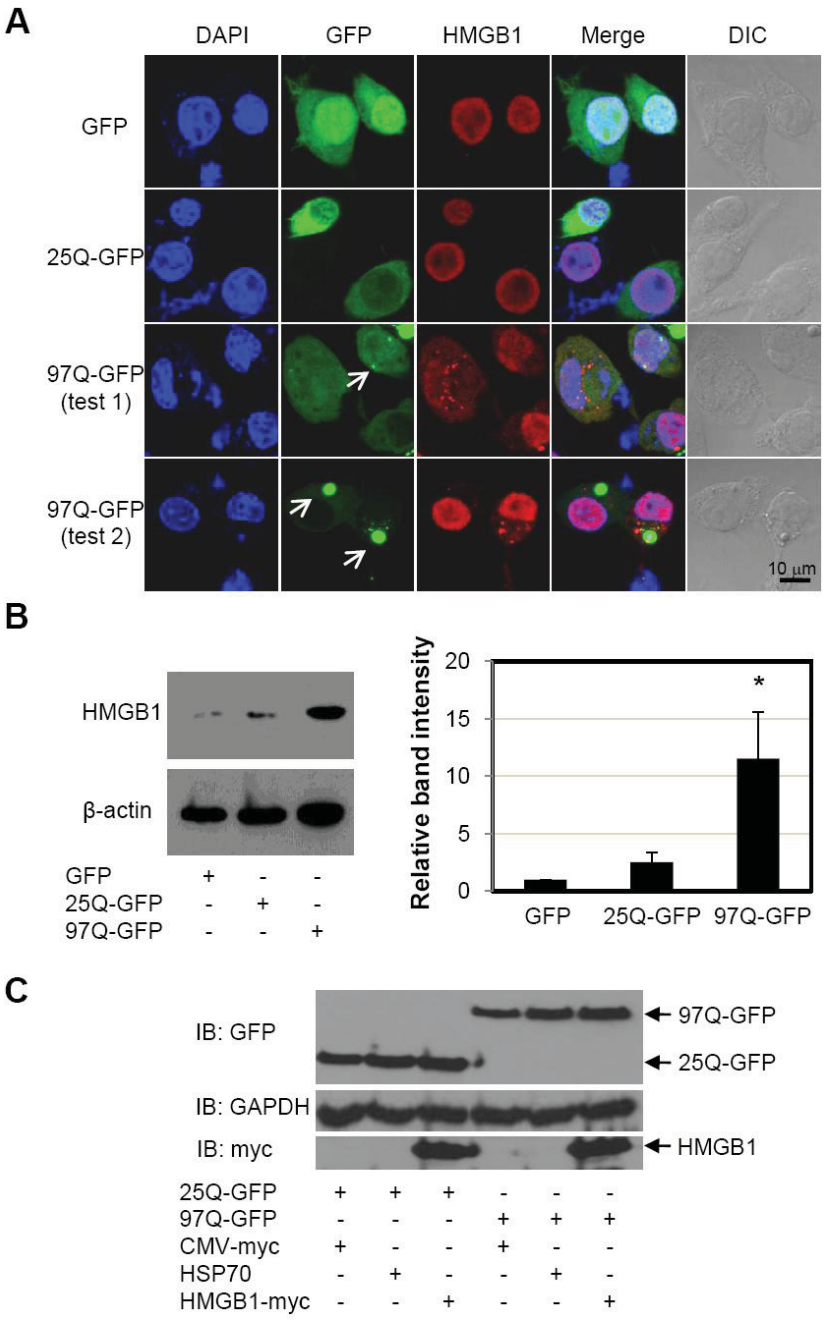

Figure S1. Overexpression of 97Q-GFP induces translocation of HMGB1. (A) SHSY5Y cells were transfected with 25Q-GFP or 97Q-GFP plasmid and incubated for 48 h. Aggregation of 97Q-GFP was observed in arrows. Endogenous HMGB1 was immunostained with anti-HMGB1 antibody and Alexa 594-conjugated secondary antibody. The intracytoplasmic translocation of HMGB1 was observed with confocal microscopy. (B)

Cytosolic fractions of SHSY5Y cells were isolated after transfection of 25Q- or 97Q-GFP plasmid and western blot analysis was performed to observe HMGB1 level in cytoplasm and the relative band intensities were measured. * p value < 0.05 (n=3). (C) SHSY5Y cells were transfected with 25Q-GFP and 97Q-GFP plasmids with or without an addition of HMGB1 or HSP70 plasmid to observe the expression of individual plasmid and the effect of HMGB1 to 25Q- or 97Q-GFP expression. Whole cell lysates were prepared and western blot analyses were performed with anti-GFP, anti-GAPDH, and anti-myc antibodies after 48 h.

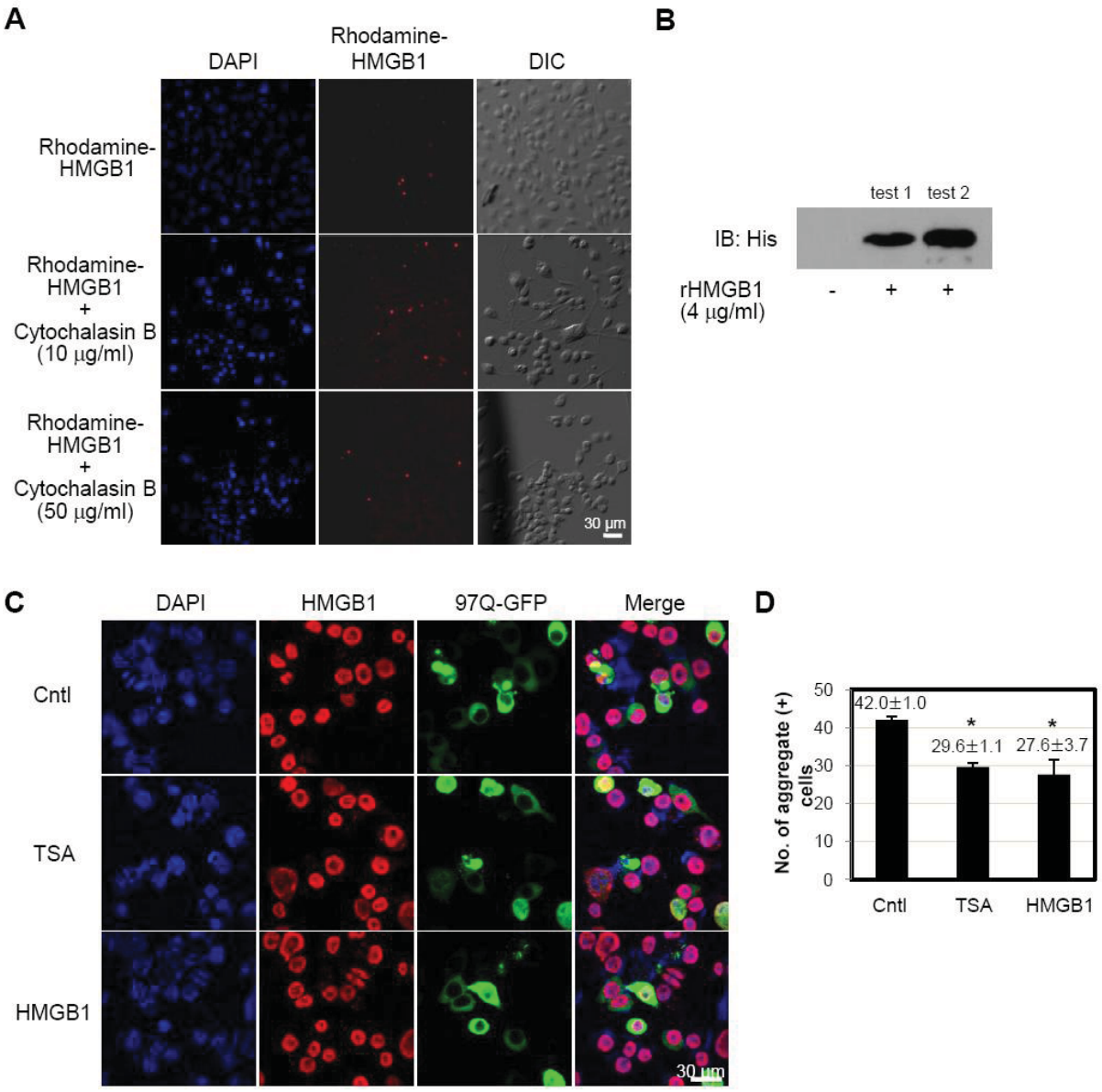

Figure S2. Exogenous rHMGB1 protein penetration and the effect of HMGB1 migration agonist (TSA) on 97Q-GFP formation. (A-B) HEK293 cells were treated with rhodamine-conjugated HMGB1 protein (4 μg/ml) for 24 h in the presence or absence 1 h pretreatment of cytochalasin B to observe the migration of exogenous HMGB1 into cells. Nuclei were stained with DAPI and observed under confocal microscopy (A). Western blot analysis was performed with the cell lysates to detect the intracellular translocation of rHMGB1 protein using anti-His antibody. Two separate tests were performed. (C-D) Effect

of HMGB1 translocation agonist on the aggregate formation of 97Q-GFP. SHSY5Y cells were transfected with 97Q-GFP plasmid and treated with 10 ng/ml of trichostatin A (TSA), which induces HMGB1 translocation into cytoplasm, for 12 h. Co-transfection of HMGB1 plasmid was used for control. After 48 h, HMGB1 translocation was observed, and 97Q-GFP aggregate (+) cells were counted among 100 GFP-positive cells (D). * p value<0.05.

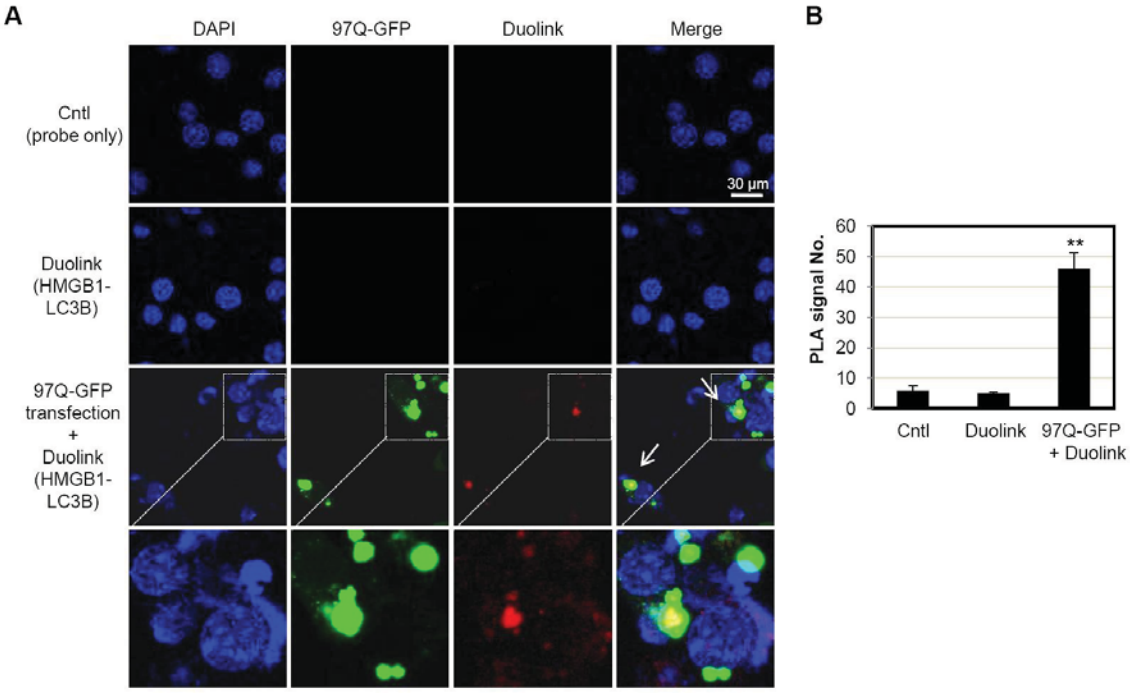

Figure S3. Autophagosome formation in 97Q-GFP and HMGB1 overexpressed cells. (A) SHSY5Y cells were co-transfected with 97Q-GFP and HMGB1 plasmids and incubated for 48 h, and proximity ligation assay (Duolink) between HMGB1 and LC3B was performed to observe the interaction of HMGB1 and LC3B. HMGB1 and LC3B binding shows red fluorescence and their bindings to 97Q-GFP shows yellow (arrow) in merged images, and PLA signal numbers were evaluated (B). ** p value<0.01 to Duolink only.