Expanded View Figures

Figure EV1. scRNA-seq analysis of the homogeneity of SCG-derived neurons.

A Classification of sympathetic neurons as either Npy^+/Dbh^- and Npy^-/Dbh^+, the latter correlating with cells that have comparatively higher proportions of sequence reads aligning to mitochondrial genes (a marker of cell stress). Note that all neurons were positive for tyrosine hydroxylase (Th^+). Markers are as follows: Npy, neuropeptide Y; Th, tyrosine hydroxylase; and Dbh, dopamine beta (β)-hydroxylase. Note that percent.mt indicates the proportion of sequence reads aligning to genes encoded by the mitochondrial genome.

B The relative proportions of four distinct cell types neurons (blue), fibroblasts (red), satellite glia (green) and Schwan cells (yellow), as detected by their transcriptomic signatures in SCG-derived cultures remained essentially constant across biological replicates and the experimental conditions used in this study, with approximately 61–85% of the population consisting of sympathetic neurons.

C WAY-150138 treatment prevents HSV-1 spreading during lytic infection. SCG neurons were infected with HSV-1 at MOI = 0.1 for productive infection for 3 days. The live-cell images were taken using an epifluorescence microscopy. Scale bar, 100 μm.

D The Us11 viral mRNA expression levels were quantified at different time-points and displayed as bar graphs with mean ± SEM. Latently infected cultures were untreated (latent) or treated with LY294002 and WAY-150138 for indicated times (n = 3 biological replicates).

Data information: P values ≤ 0.05 were considered significant, asterisks denote statistical significance (**P < 0.01; ***P < 0.001; ****P < 0.0001). P values are calculated using two-tailed unpaired Student's t test.

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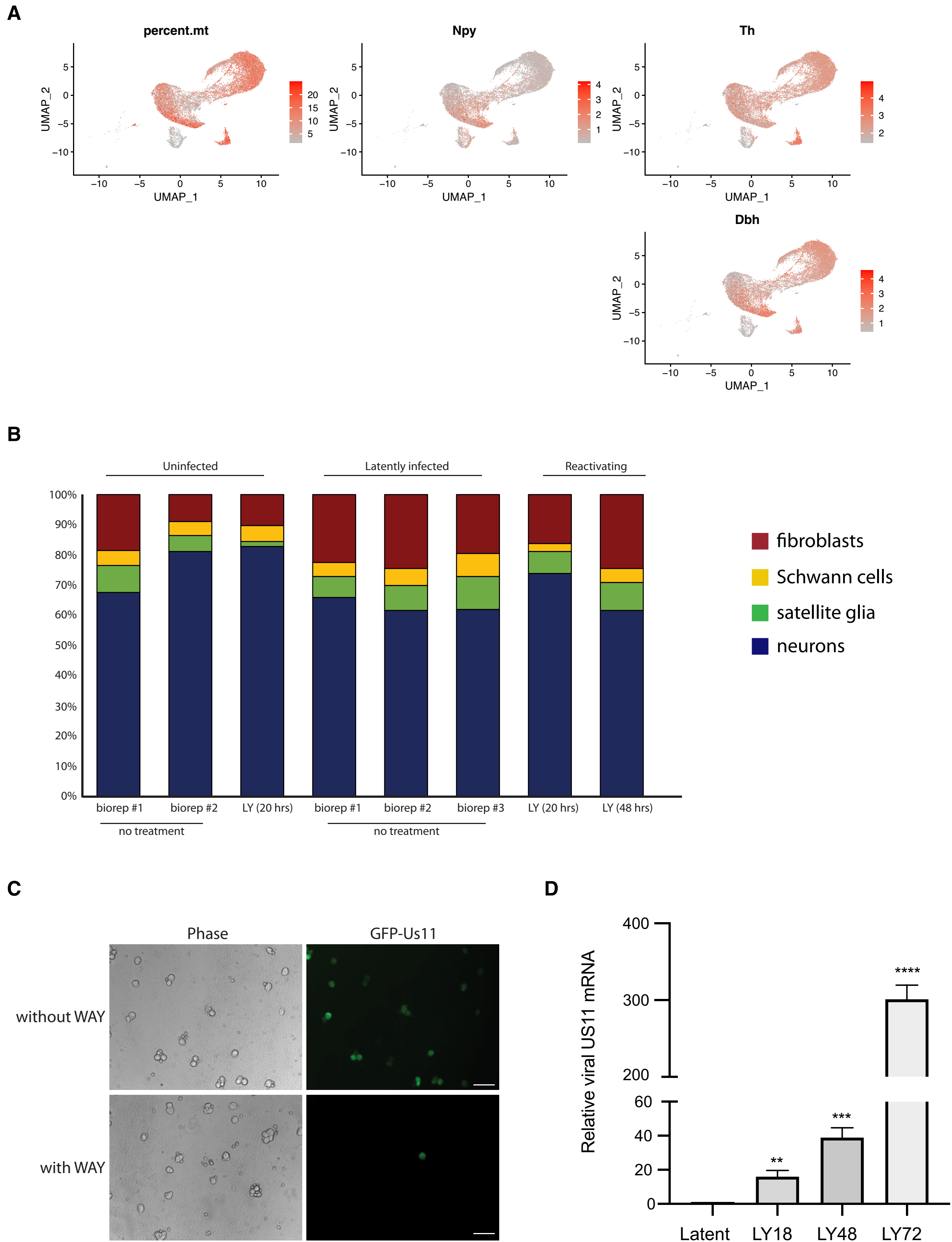

Figure EV1.

Figure EV2. scRNA-seq profiling of SCG cultures using enhanced chemistry.

A scRNA-seq profiling of SCG cultures using 10X genomics v3 chemistry identified three distinct cell populations and classified according to specified markers for neurons (Prph & Tubb3), fibroblasts (Col3a1) and Schwann cells (Sox10 and S100b). Signature transcripts correspond to: Prph, peripherin; Tubb3, beta III tubulin; Col3a1, Collagen Type III Alpha 1 Chain; Sox10, SRY-Box Transcription Factor 10; S100b, S100 calcium-binding protein B.

B smFISH images showing LY294002 treatment doesn't affect Gadd45b mRNA localization and expression levels. Cells treated LY294002 and WAY-150138 for 48 h and then smFISH was performed. Scale bar, 10 μm.

C ACV treatment by itself did not impact the subcellular localization of Gadd45b in uninfected neurons. Uninfected SCGs were treated with ACV for 7 days then treated with either DMSO or LY294002 for 72 h, and then cells were fixed and stained for Gadd45b and DAPI, and visualized by immunofluorescence. Scale bar, 10 μm.

D EdU treatment by itself did not impact Gadd45b localization or lead to EdU labeling in the uninfected, post-mitotic neurons. Uninfected SCGs were treated with ACV for 7 days, followed by treatment with DMSO or LY294002 for 72 h. Cells were then subjected to EdU pulse-labeling (6 h), followed by immunofluorescence staining of Gadd45b and EdU signal. Scale bar, 10 μm.

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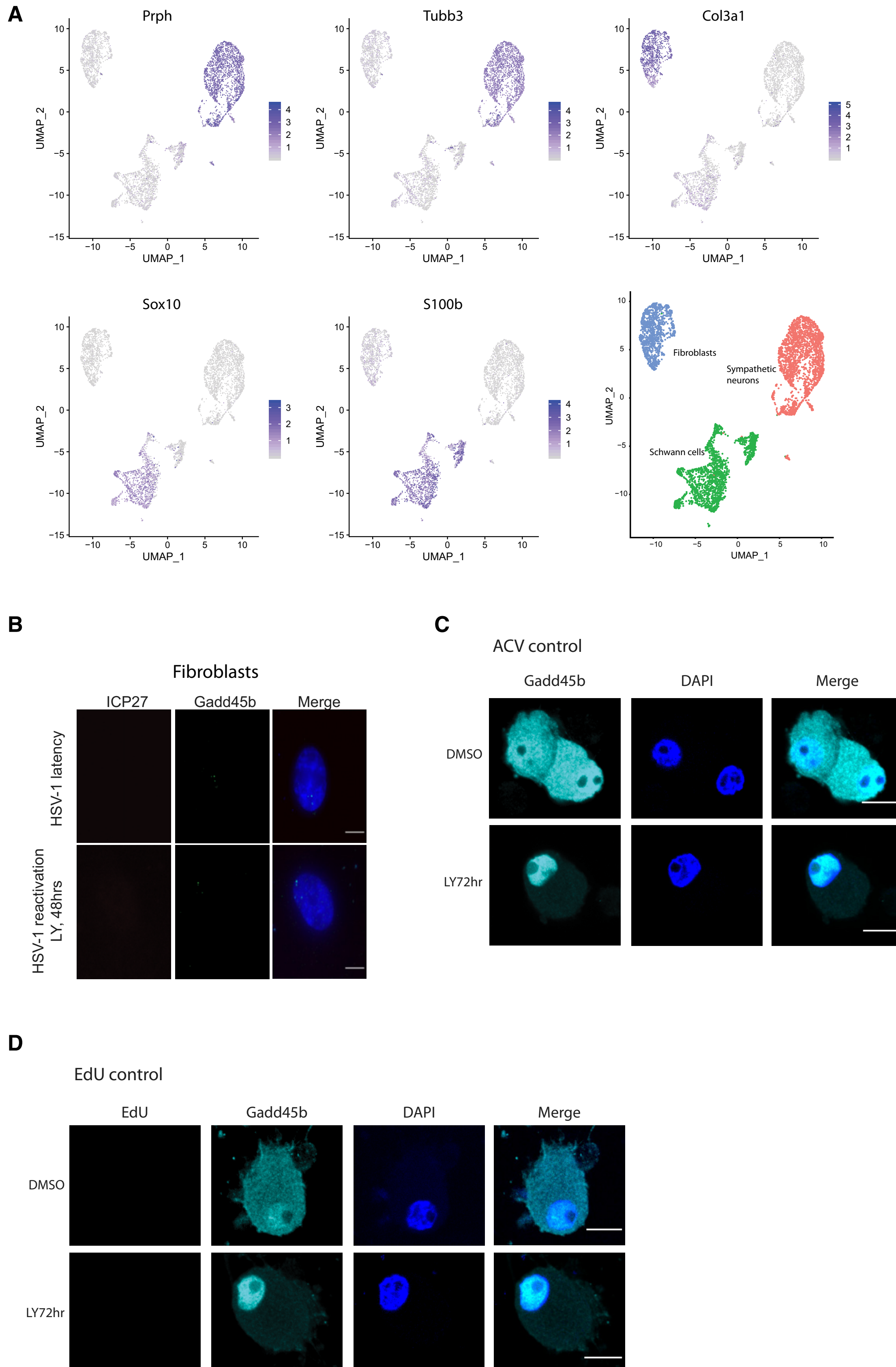

Figure EV2.

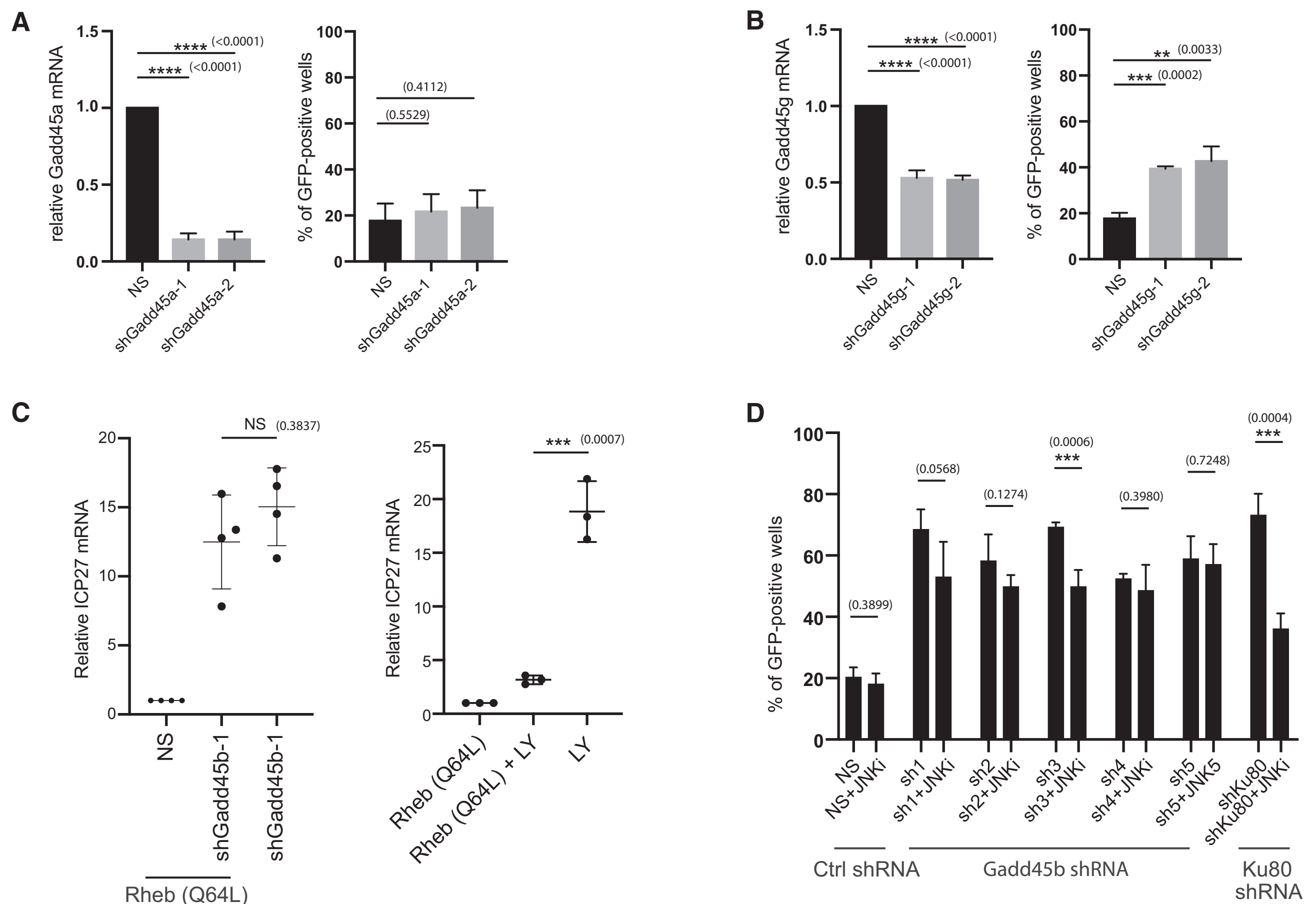

Figure EV3. Depletion of Gadd45b causes HSV-1 reactivation independent of the AKT-mTORC1-JNK signaling pathway.

A Depletion of Gadd45a expression using lentiviral-transduced shRNAs in latently infected SCG neurons does not induce HSV-1 reactivation. HSV1-GFP-Us11 latently infected cultures were infected with lentiviruses expressing either two different shRNAs against Gadd45a mRNA or a non-silencing shRNA (NS). Reactivation was quantified by scoring the percentage of wells expressing GFP after 5 days. Knockdown efficiencies for individual shRNAs in latently infected SCG neurons were confirmed by RT-qPCR using RNA collected in parallel. Reactivation rates were quantified from 30 wells for each condition; three biological replicates. RT-qPCR were quantified from three biological replicates. The bars and error bars are mean ± SD.

B Depletion of Gadd45g expression using lentiviral-transduced shRNAs in latently infected SCG neurons induces HSV-1 reactivation. Reactivation rates were quantified from 30 wells for each condition; three biological replicates. RT-qPCR were quantified from three biological replicates. The bars and error bars are mean ± SD.

C HSV-1 reactivation in response to Gadd45b depletion was independent of AKT-mTORC1 signalling. Constitutively active Flag-Rheb (Q64L) was introduced into HSV-1 latently infected SCG neurons together with either of two independent shRNAs against Gadd45b or the non-silencing shRNA (NS) and viral ICP27 mRNA levels were quantified by RT-qPCR after 48 h (left panel). Four biological replicates. The bars and error bars are mean ± SD. The ability of Rheb (Q64L) to suppress LY-induced reactivation serves as a positive control (right panle). Three biological replicates. The bars and error bars are mean ± SD.

D HSV-1 reactivation induced by Gadd45b depletion (using 5 different shRNAs) was generally not inhibited by treatment with JNK inhibitor. Reactivation induced by shRNA-mediated depletion of Ku80 served as a positive control for the JNK inhibitor. The percentage of wells expressing GFP-positive was scored as the mean ± SEM (n = 3 biological replicates). The bars and error bars are mean ± SD.

Data information: P values ≤ 0.05 were considered significant, asterisks denote statistical significance (**P < 0.01; ***P < 0.001; ****P < 0.0001). Numbers next to asterisk above the bar graphs indicate exact P values. P values are calculated using two-tailed unpaired Student's t test. P values > 0.05 were not significant (ns).

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Figure EV4. Gadd45b overexpression inhibits DNA damage response-induced HSV-1 reactivation.

A Latently infected SCG neurons were transduced with Gadd45b-Myc-Flag for 3 days and then treated with LY294002 and WAY-150138 to induce reactivation. RNA was collected at the indicated time-points and levels of viral ICP27 and UL30 mRNA quantified by RT-qPCR. three biological replicates. The bars and error bars are mean ± SD.

B Overexpression of Gadd45b-Myc-Flag suppressed viral true late Us11 gene mRNA expression. Latently infected SCG neurons were transduced with Gadd45b for 3 days and then treated with LY294002 and WAY-150138 for 72 h. mRNAs were collected and quantified by qRT-PCR for Us11 viral mRNA levels. Three biological replicates. The bars and error bars are mean ± SD.

C Ectopic expression of Gadd45b-Myc-Flag antagonizes Mirin-induced HSV-1 reactivation. Latently infected SCG neurons were either transduced with vector control (Ctrl) or Gadd45b-Myc-Flag from lentivirus for 3 days prior to addition of Mirin. Reactivation was quantified by scoring the percentage of wells expressing GFP fluorescence at the indicated days. The percentage of wells expressing GFP-positive was scored as the mean ± SEM (n = 3 biological replicates). Reactivation rates were quantified from 30 wells for each condition, three biological replicates.

D Expression of Gadd45b-Myc-Flag does not affect productive replication (lytic infection) of HSV-1 in SCG neurons, as measured by UL30 mRNA levels or visible GFP-Us11 fluorescence. SCG neurons were transduced with Gadd45b-Myc-Flag for 3 days, then secondarily infected with HSV-1 at MOI=1.5 in the absence of ACV to allow a productive infection. Levels of UL30 viral mRNA were quantified by RT-qPCR (n = 3 biological replicates). The bars and error bars are mean ± SD. Scale bar, 100 μm.

E Gadd45b overexpression does not affect HSV-1 DNA synthesis. Latent cultures were transduced with Gadd45b-Myc-Flag for 3 days and then treated with LY294002 and WAY-150138 for 72 h. Cells were pulse-labeled with EdU for 6 h prior to fixation and imaging of EdU-positive cells and anti-GFP immunofluorescence staining to detect GFP-positive cells. The percentage of EdU-positive cells and the percentage of GFP-positive cells amongst EdU-positive cells were quantified and displayed as bar graphs with mean ± SEM (n = 3 biological replicates). Scale bar, 50 μm.

Data information: P values ≤ 0.05 were considered significant, asterisks denote statistical significance (*$P < 0.05$; **$P < 0.01$; ****$P < 0.0001$). P values are calculated using two-tailed unpaired Student's t test. P values > 0.05 were not significant (ns).

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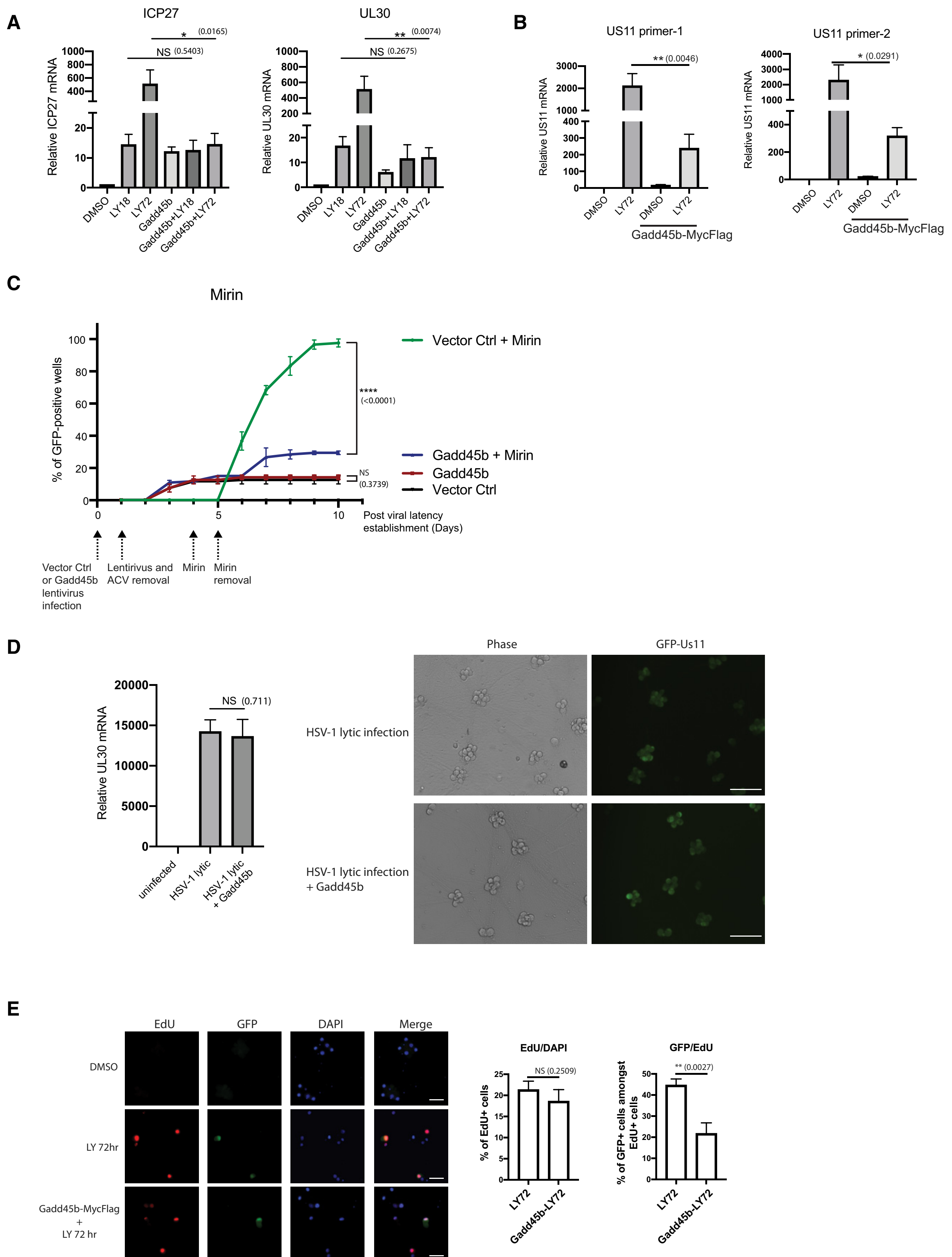

Figure EV4.

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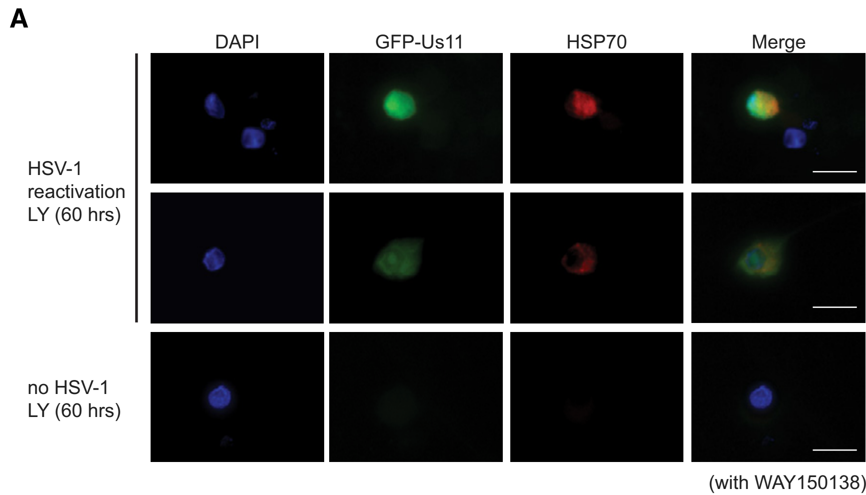

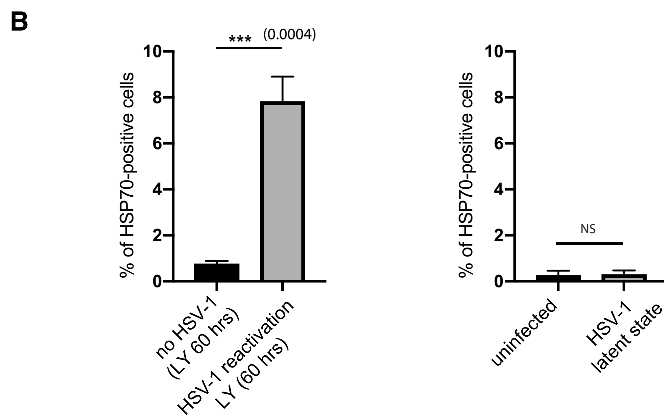

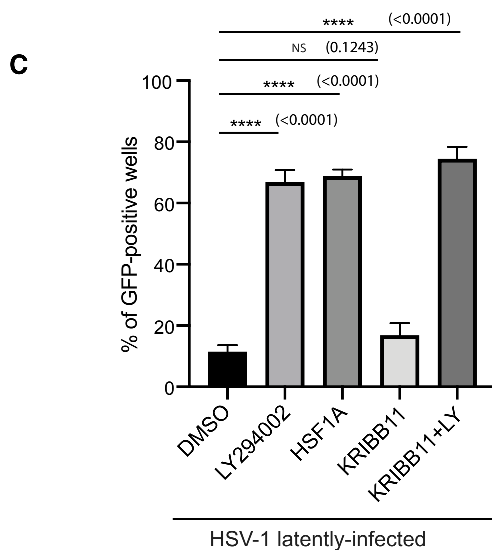

Figure EV5. Activation of the heat shock response pathway induces HSV-1 reactivation.

- A Elevated HSP70 expression in reactivated SCG neurons. Latent or uninfected neurons treated with LY294002 and WAY-150138 for 60 h and then immunofluorescence was performed probing with HSP70 and GFP. Scale bar, 20 μm.
- B The percentage of cells with HSP70 in latent or uninfected neurons were quantified from (A) and displayed as bar graphs with mean ± SEM (n = 3 biological replicates).
- C Activation of HSF1 induces HSV-1 reactivation. Reactivation assay comparing latently infected SCG neuron cultures treated with LY294002 (20 μM), HSF1A (80 μM), KRIBB11 (30 μM), or DMSO. Reactivation rates were quantified by scoring the percentage of wells with visible GFP fluorescence after 3 days (n = 3 biological replicates). The bars and error bars are mean ± SD.

Data information: P values ≤ 0.05 were considered significant, asterisks denote statistical significance (***P < 0.001; ****P < 0.0001). P values are calculated using two-tailed unpaired Student's t test. P values > 0.05 were not significant (ns).

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