Fig. S1

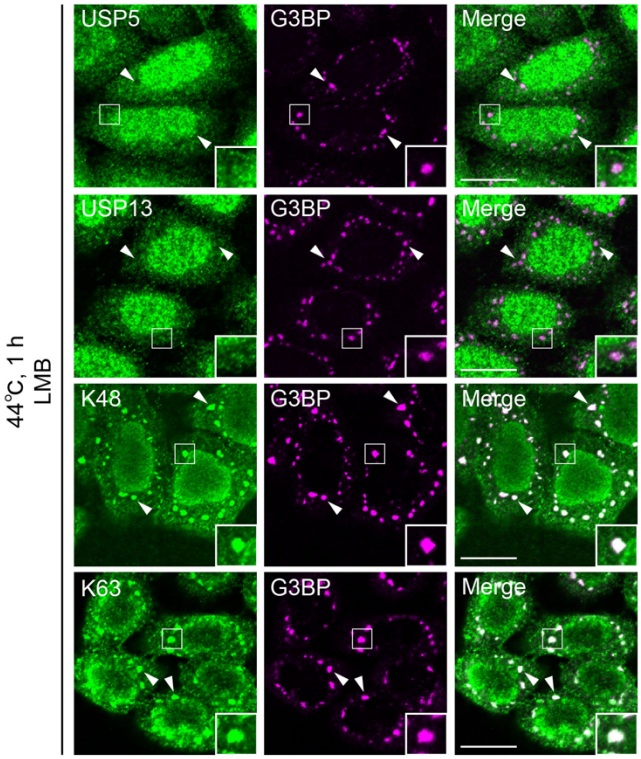

Fig. S1. A part of USP5 and USP13 in heat-induced SGs are translocated from the nucleus
Cells were cultured at 44°C for 1 h in the presence of 50 nM LMB, and stained with anti-USP5, anti-USP13, anti-K48-linked ubiquitin chain, or K63-linked ubiquitin chain antibody together with anti-G3BP antibody. Arrowheads indicate typical SGs. Insets show higher magnification images of regions indicated by squares. Bars, 20 μm. The experiments were repeated more than 3 times with similar results.

Fig. S2

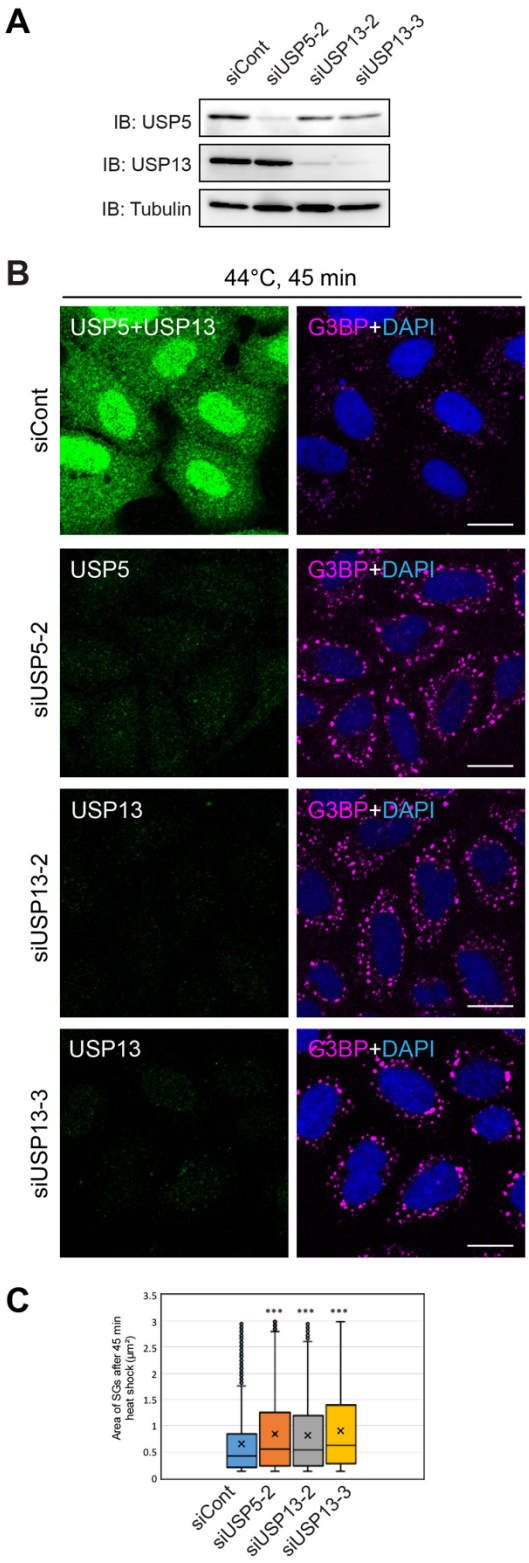

Fig. S2. Depletion of USP5 or USP13 by alternative siRNAs also accelerates assembly of heat-induced SGs (related to Fig. 6)

Cells were transfected with control siRNAs (siCont), siRNAs for USP5 (siUSP5-2) or siRNAs for USP13 (siUSP13-2 or siUSP13-3). (A) Lysates of the cells were subjected to immunoblotting with indicated antibodies. (B) The cells were subjected to heat stress (44°C for 45 min), and stained with anti-USP5 antibody and/or anti-USP13 antibody together with anti-G3BP antibody and DAPI. Bars, 20 μm. (C) The sizes of all SGs observed in 3 independent experiments were measured and shown as the box and whisker plots in the same format as Fig. 6D. ***P<0.001 versus control (two-tailed Student's t-test).

Fig. S3

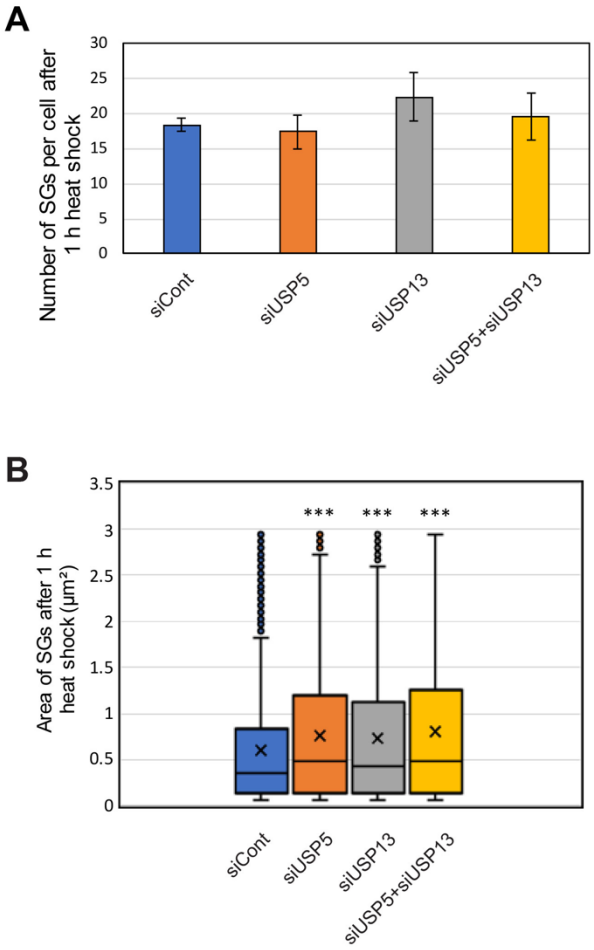

Fig. S3. The numbers and sizes of SGs in USP5/USP13-depleted cells under heat stress (44°C for 1 h) (related to Fig. 7A, B)

(A) The numbers of SGs in USP5/USP13-depleted cells under heat stress (44°C for 1 h) were shown as mean ± s.e.m. of 3 independent experiments. There was no statistically significant difference between control and knockdown samples (two-tailed Student's *t*-test). (B) The sizes of all SGs observed in 3 independent experiments were measured and shown as the box and whisker plots in the same format as Fig. 6D. ***P<0.001 versus control (two-tailed Student's *t*-test).

Fig. S4

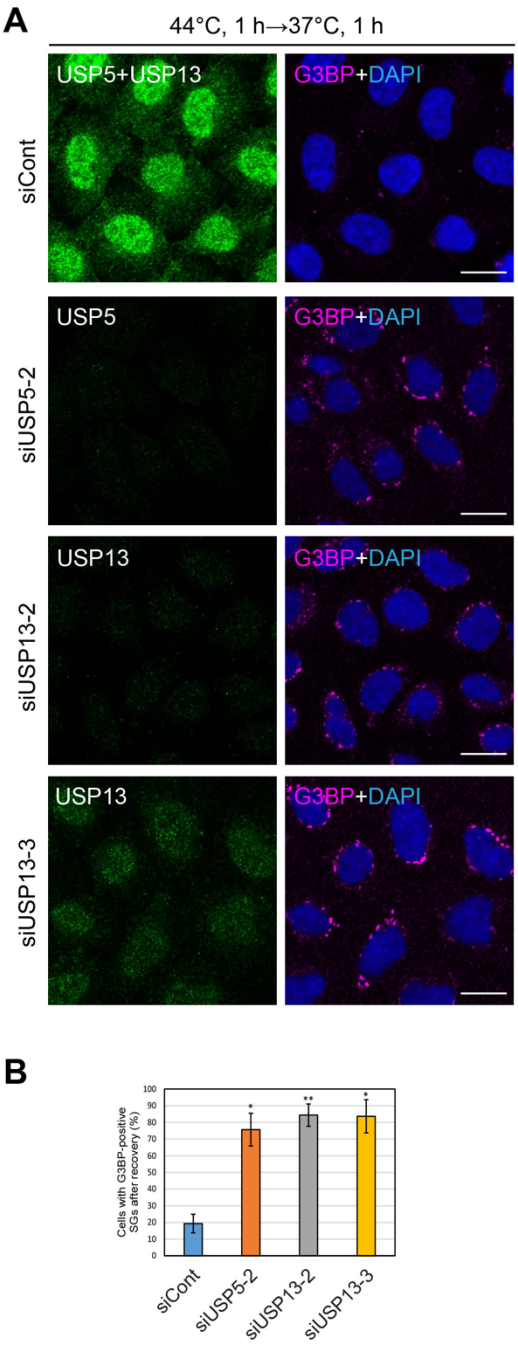

Fig. S4. Depletion of USP5 or USP13 by alternative siRNAs also represses disassembly of heat-induced SGs (related to Fig. 7C, D)

(A) Cells were transfected with control siRNAs (siCont), siRNAs for USP5 (siUSP5-2) or siRNAs for USP13 (siUSP13-2 or siUSP13-3), and subjected to heat stress (44°C for 1 h). Cells were then returned to normal conditions (37°C for 1 h), and stained with anti-USP5 antibody and/or anti-USP13 antibody together with anti-G3BP antibody and DAPI. Bars, 20 μm. (B) The numbers of SG-bearing cells in A were shown (mean ± s.e.m. of 3 independent experiments). **P*<0.05, ***P*<0.01 versus control (two-tailed Student's *t*-test).

Fig. S5

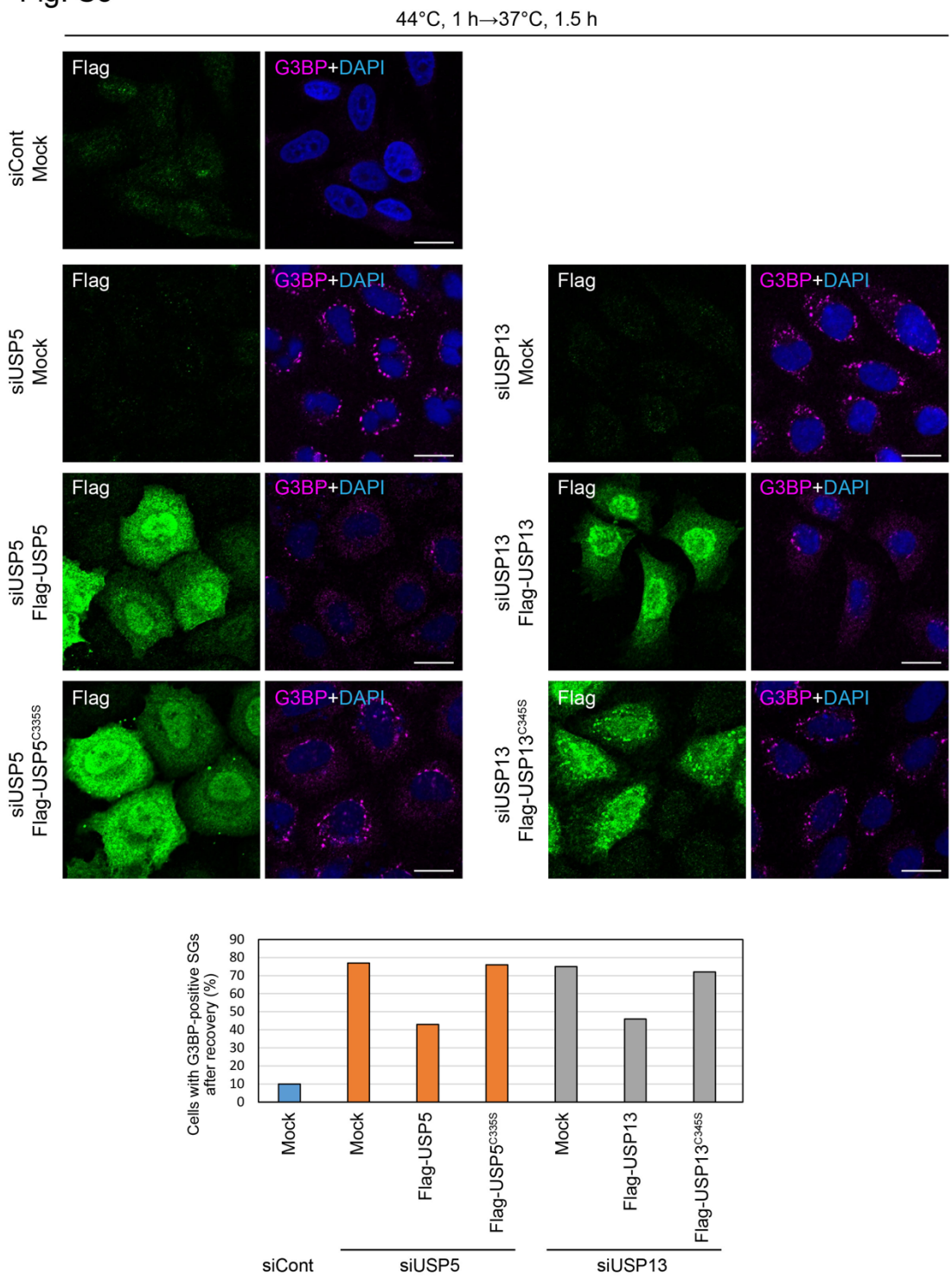

Fig. S5. Re-expression of USP5 or USP13 partially restore the repression of SG disassembly by knockdown of USP5 or USP13

Cells were transfected with control siRNAs (siCont), siRNAs for USP5 (siUSP5-1), or siRNAs for USP13 (siUSP13-2). After 24 h, cells were transfected with an empty vector or siRNA-resistant plasmids encoding Flag-tagged USP5, USP13 or their mutants in which a cysteine residue in the catalytic site was substituted [USP5, Cys335 to Ser (C335S); USP13, Cys345 to Ser (C345S)]. At 48 h after the plasmid transfection, cells were subjected to heat stress (44°C for 1 h) and then returned to normal conditions (37°C for 1.5 h). Cells were stained with anti-Flag antibody, anti-G3BP antibody and DAPI. Bars, 20 µm. In mock-transfected sample, the numbers of cells with G3BP-positive SGs in 100 cells were counted. In the other samples, the numbers of cells with G3BP-positive SGs in 100 Flag-positive cells were counted. The graph shows the representative data of 3 independent experiments.

Fig. S6

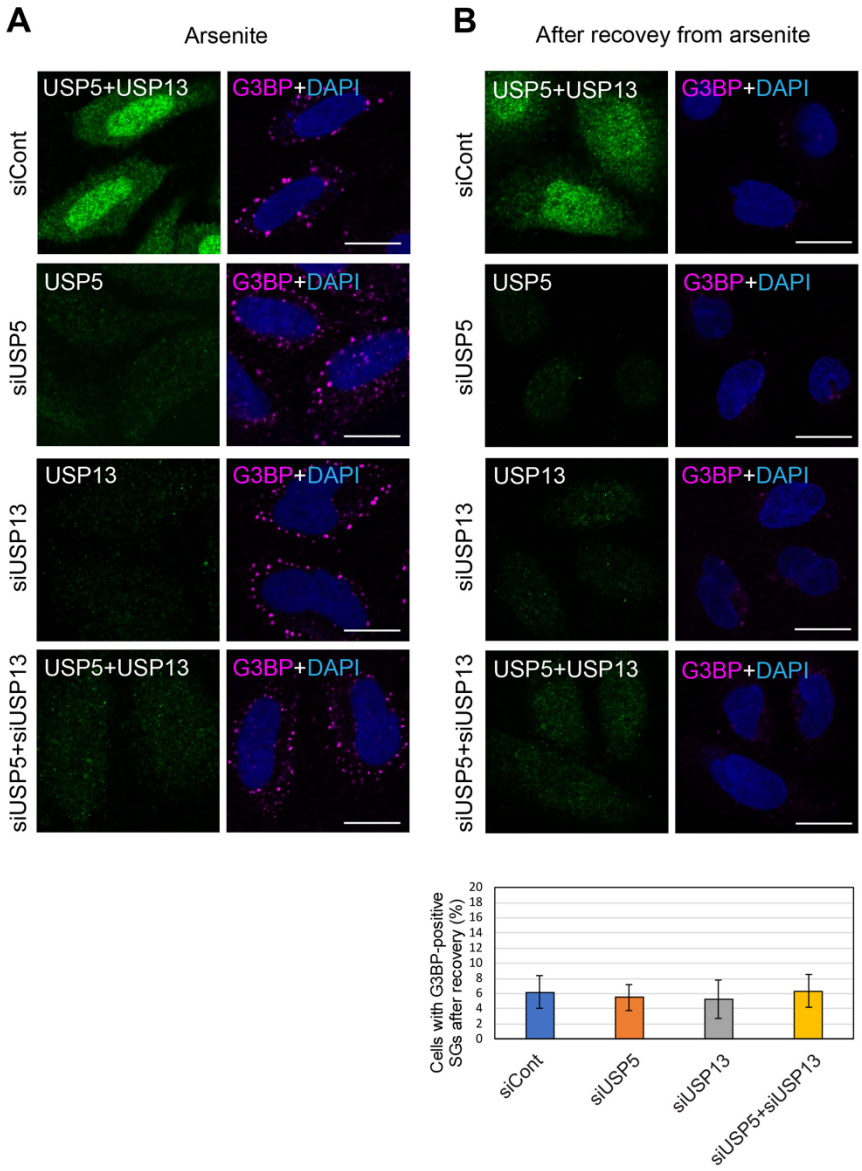

Fig. S6. Depletion of USP5 or USP13 does not affect assembly and disassembly of arsenite-induced SGs

Cells were transfected with siRNAs for USP5 (siUSP5-1) and siRNAs for USP13 (siUSP13-1) individually or in combination, subjected to treatment with arsenite (0.5 mM for 45 min) (A), and returned to normal conditions without arsenite (3 h) (B). Cells were then stained with anti-USP5 antibody and/or anti-USP13 antibody, together with anti-G3BP antibody and DAPI. Bars, 20 µm. The numbers of SG-bearing cells in B are shown as a proportion to the total number of cells (mean ± s.e.m. of 3 independent experiments) in the graph. There was no statistically significant difference between control and knockdown samples (two-tailed Student's *t*-test).