SUPPLEMENTARY FIGURES

Figure S1

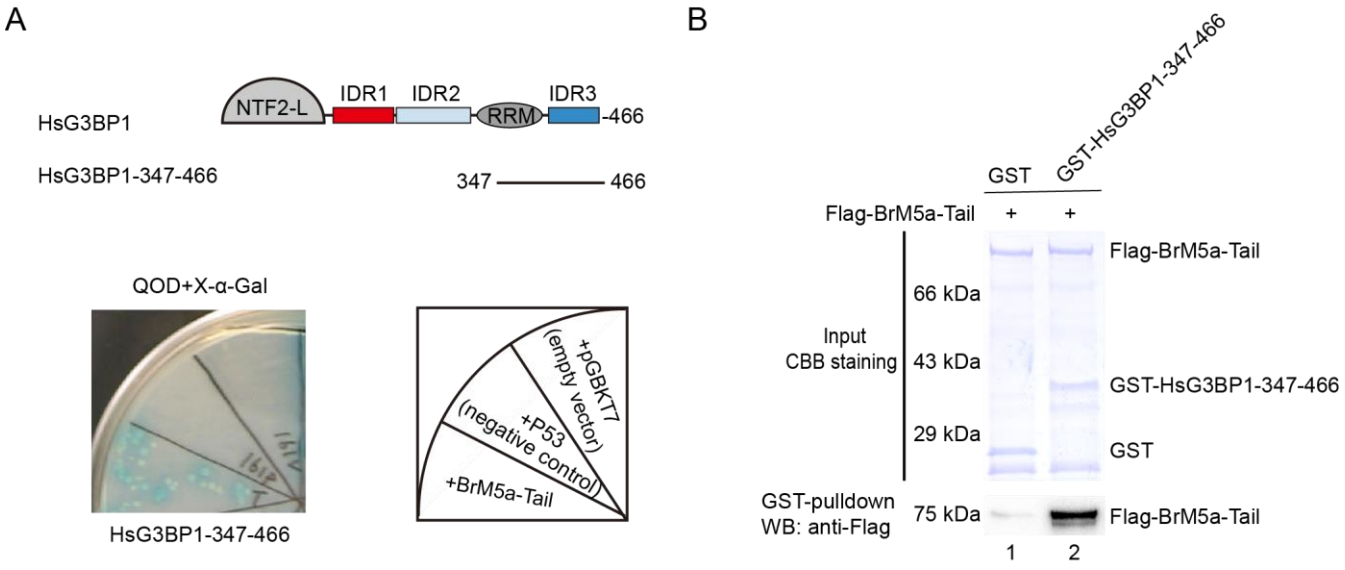

Figure S1. Identification of G3BP1 as a Myo5a-binding protein. (**A**) Yeast two-hybrid assay of HsG3BP1-347-466 (residues 347-466 of human G3BP1) and BrM5a-Tail. The interactions were assessed on QOD+X-α-Gal medium. Upper panel, diagram of HsG3BP1 domains. NTF2-L, nuclear transport factor 2-Like domain; IDR, intrinsically disordered regions; RRM, RNA recognition motif. (**B**) GST pulldown of Flag-BrM5a-Tail with GST-HsG3BP1-347-466. GST pulldown assay was performed using 2.5 μM GST-HsG3BP1-347-466 and 5 μM Flag-BrM5a-Tail. The inputs were analyzed with SDS-PAGE and visualized by Coomassie brilliant blue (CBB) staining; the GSH-Sepharose-bound proteins were eluted by GSH and analyzed by Western blot using an anti-FLAG antibody.

Figure S2

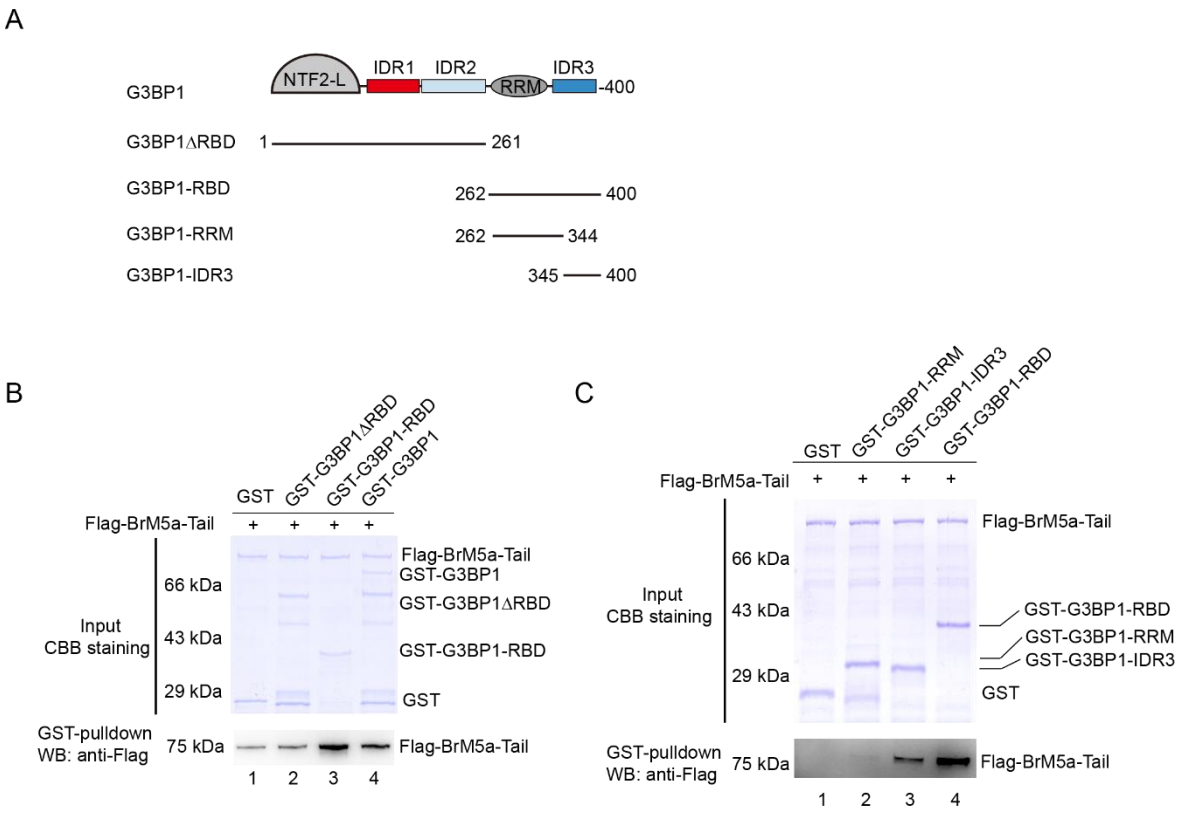

Figure S2. Identification of the Myo5a-binding region in G3BP1. (**A**) Diagram of G3BP1 domain arrangement and the truncated G3BP1 proteins used for pulldown assays. (**B**) GST pulldown of Flag-BrM5a-Tail with G3BP1 variants. (**C**) GST pulldown of Flag-BrM5a-Tail with G3BP1-RBD and the truncated variants. GST pulldown assays were performed using 2.5 μM GST-G3BP1 or its truncated variants and 5 μM Flag-BrM5a-Tail.

Figure S3

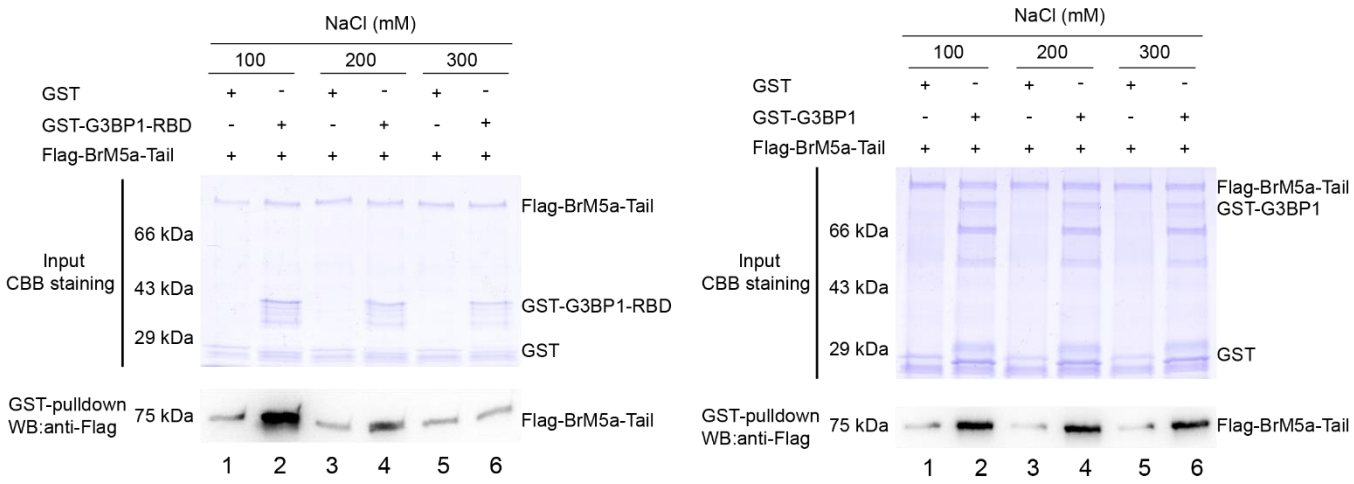

Figure S3. Ionic strength dependence of the interaction between Myo5a-tail and G3BP1 or G3BP1-RBD. GST pulldown of 2.5 μM GST-tagged G3BP1-RBD (left panel) or G3BP1 (right panel) with 5 μM Flag-BrM5a-Tail in the presence of indicated concentrations of NaCl.

Figure S4

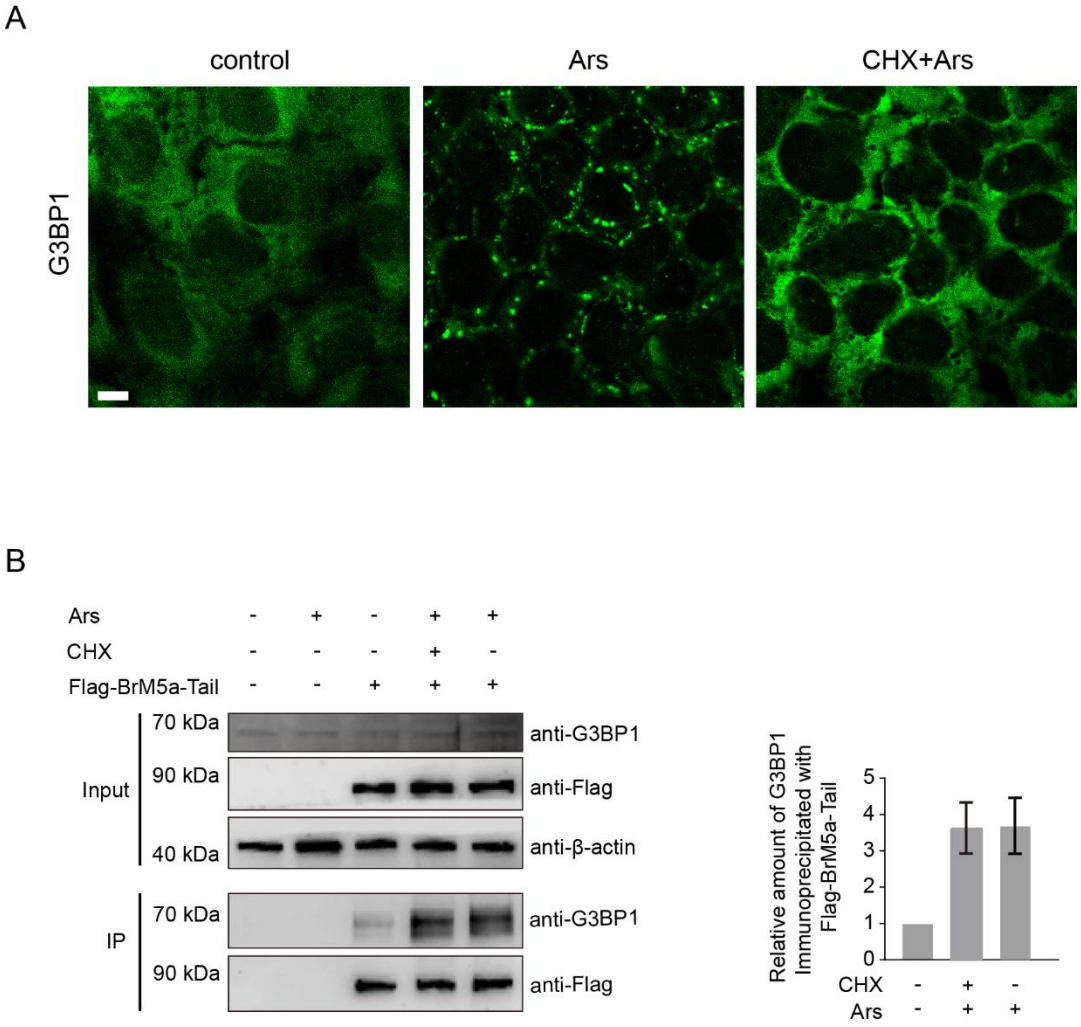

Figure S4. Effects of cycloheximide treatment on the interaction between BrM5a-Tail and G3BP1 in U2OS cells. (**A**) Cycloheximide inhibited the arsenite-induced SG formation in U2OS cells. U2OS cells were pretreated with or without 100 μg/ml cycloheximide (CHX), then exposed to 500 μM sodium arsenite (Ars) by adding 25 μl of 10 mM Ars to the culture medium. The fixed U2OS cells were stained for G3BP1 (Green) as a SG marker. Scale bars, 10 μm. (**B**) Cycloheximide treatment did not affect the interaction between BrM5a-Tail and G3BP1. U2OS cells were transfected with Flag-BrM5a-Tail and treated with or without cycloheximide and sodium arsenite as described in (**A**). Cell lysates were incubated with anti-FLAG M2 affinity agarose and the bound proteins were eluted with FLAG peptide. The inputs and the eluted proteins were detected through Western blot using the indicated antibody. Right panel shows the relative amount of G3BP1 pulled down with Flag-BrM5a-Tail (three independent assays).

Figure S5

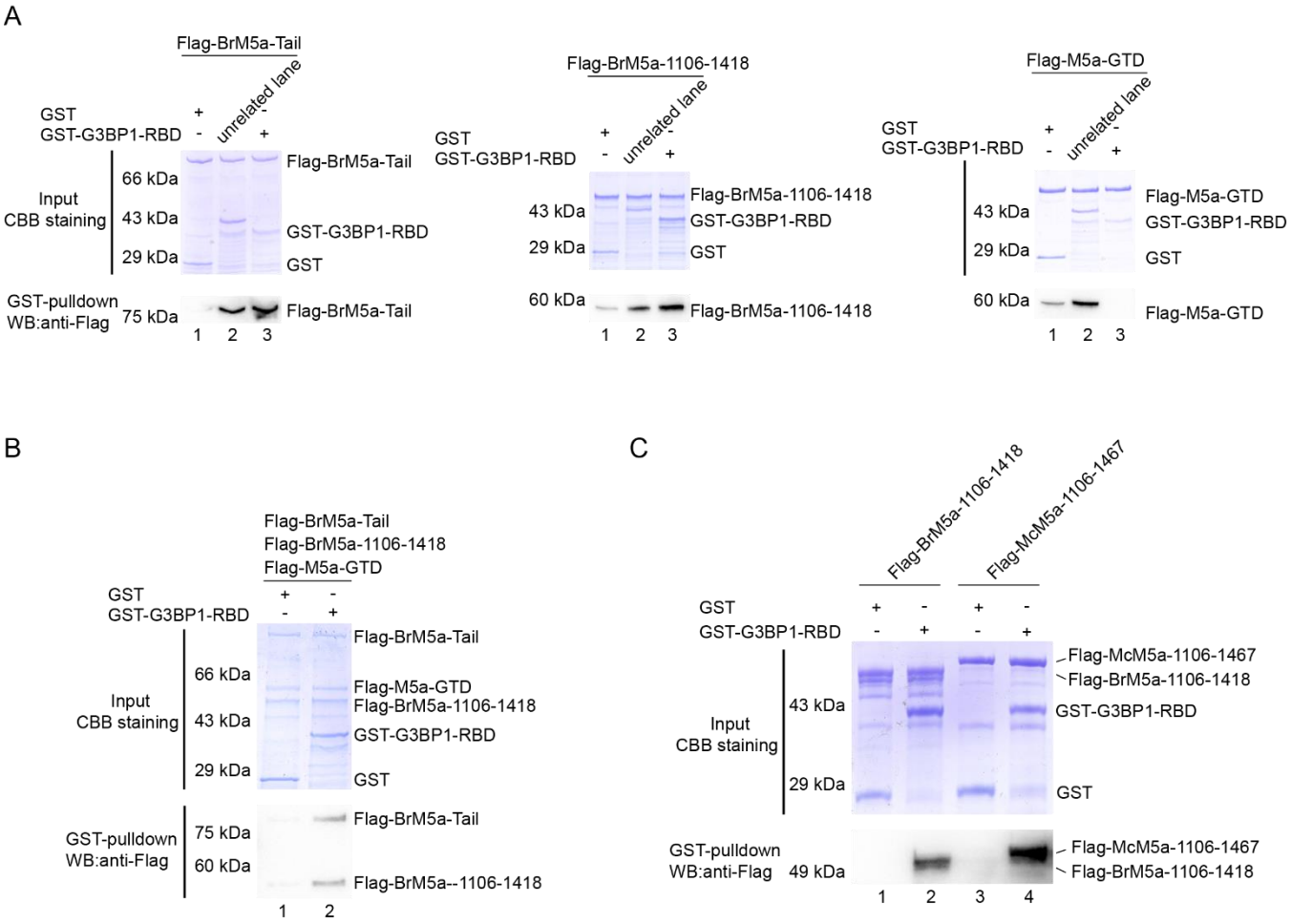

Figure S5. Identification of the G3BP1-binding region in Myo5a. (**A**) G3BP1-RBD interacts with the intermediate tail, but not the GTD, of BrM5a. GST pulldown assays were performed using 2.5 μM GST-G3BP1-RBD and 5 μM Flag-tagged BrM5a-Tail, BrM5a-1106-1418, or M5a-GTD. (**B**) G3BP1-RBD interacts with the intermediate tail of BrM5a. GST pulldown assays were performed using 4 μM GST-G3BP1-RBD and a mixture of three 1 μM Flag-tagged BrM5a-tail fragments (BrM5a-Tail, 1106-1418, and M5a-GTD). (**C**) G3BP1-RBD interacts with the intermediate tail of both BrM5a and McM5a. GST pulldown assays were performed using 2.5 μM GST-G3BP1-RBD with 2.5 μM Flag-tagged BrM5a-1106-1418 and McM5a-1106-1467.

Figure S6

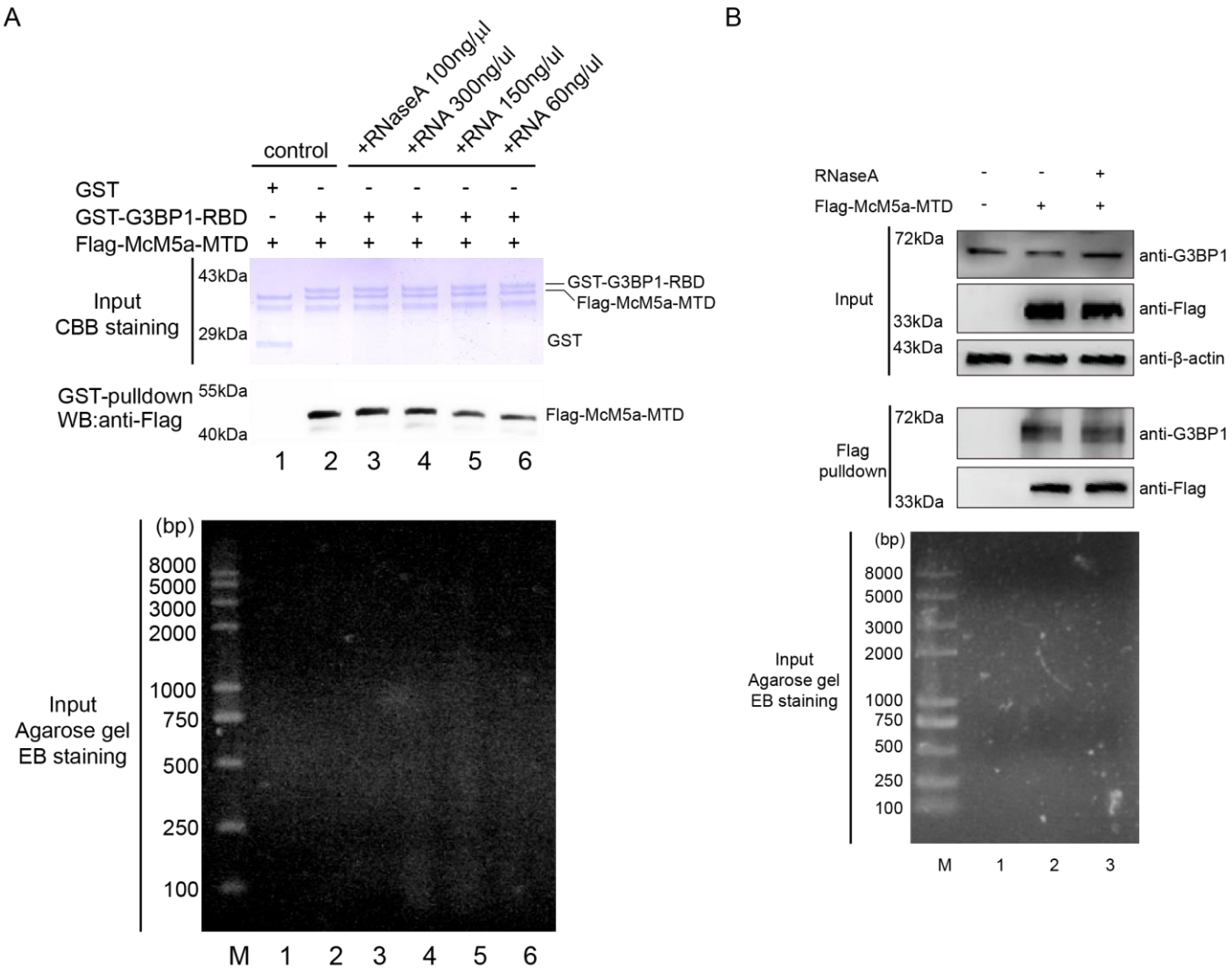

Figure S6. Effects of RNA on the interaction between G3BP1-RBD and McM5a-MTD. (**A**) GST pulldown assays were performed using 100 μl of 1.5 μM GST-G3BP1-RBD and 4.5 μM Flag-McM5a-MTD in the presence of RNase A or total RNA isolated from mouse brain. Upper panel, the proteins in the inputs (5 ul) were analyzed by SDS-PAGE and visualized by CBB staining. Middle panel, the GST-pulldown samples were analyzed by Western blot using antibody against Flag. Lower panel, the nucleotides in the inputs (5 ul) were analyzed by agarose gel and visualized by ethidium bromide staining. About 1.5 μg, 0.75 μg, and 0.3 μg RNA were loaded in lane 4, 5, and 6, respectively. (**B**) U2OS cells were transfected with Flag-McM5a-MTD, and 2 days later treated with 500 μM sodium arsenite (Ars) for 1h before lysis. Cell lysates were incubated without or with 100 ng/μl RNase A, then pulled down by anti-FLAG M2 affinity agarose, and finally the bound proteins were eluted by FLAG peptide. The inputs and the eluted proteins were detected through Western blot using the indicated antibody. Lower panel, the nucleotides in the inputs were examined by agarose gel with ethidium bromide staining.

Figure S7

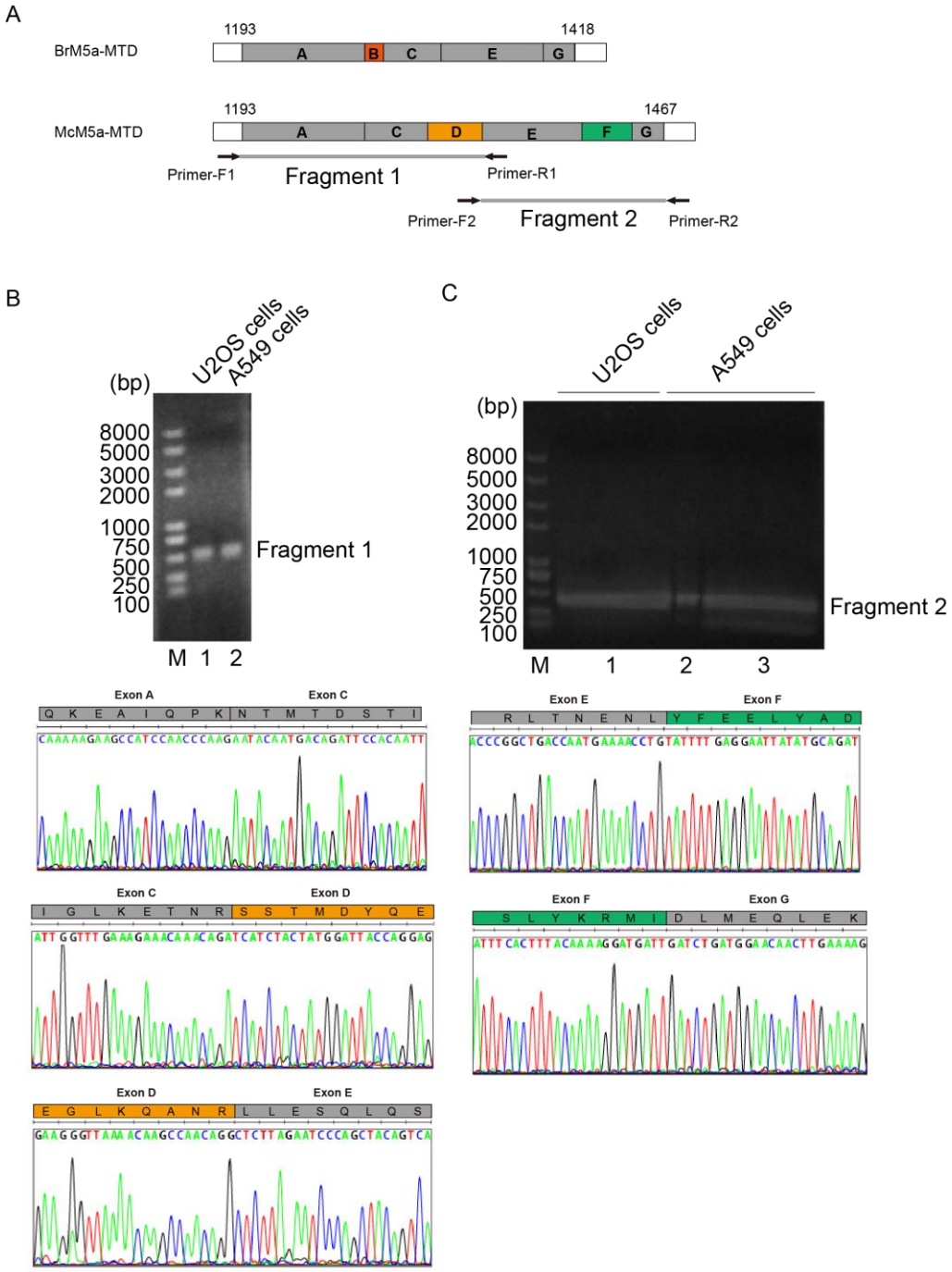

Figure S7. Identification of Myo5a isoforms expressed in U2OS cells and A549 cells. (**A**) Diagram of the tail regions of BrM5a and McM5a that are encoded by exon-A to -G of human Myo5a gene. Fragment 1 and 2 were generated by RT-PCR using the indicated primer pair. (B) Sequence analysis of Fragment 1 generated from U2OS cells. Upper panel, agarose gel of Fragment 1 generated from U2OS cells and A549 cells. (C) Sequence analysis of Fragment 2 generated from U2OS cells. Upper panel, agarose gel of Fragment 2 generated from U2OS cells and A549 cells.

Figure S8

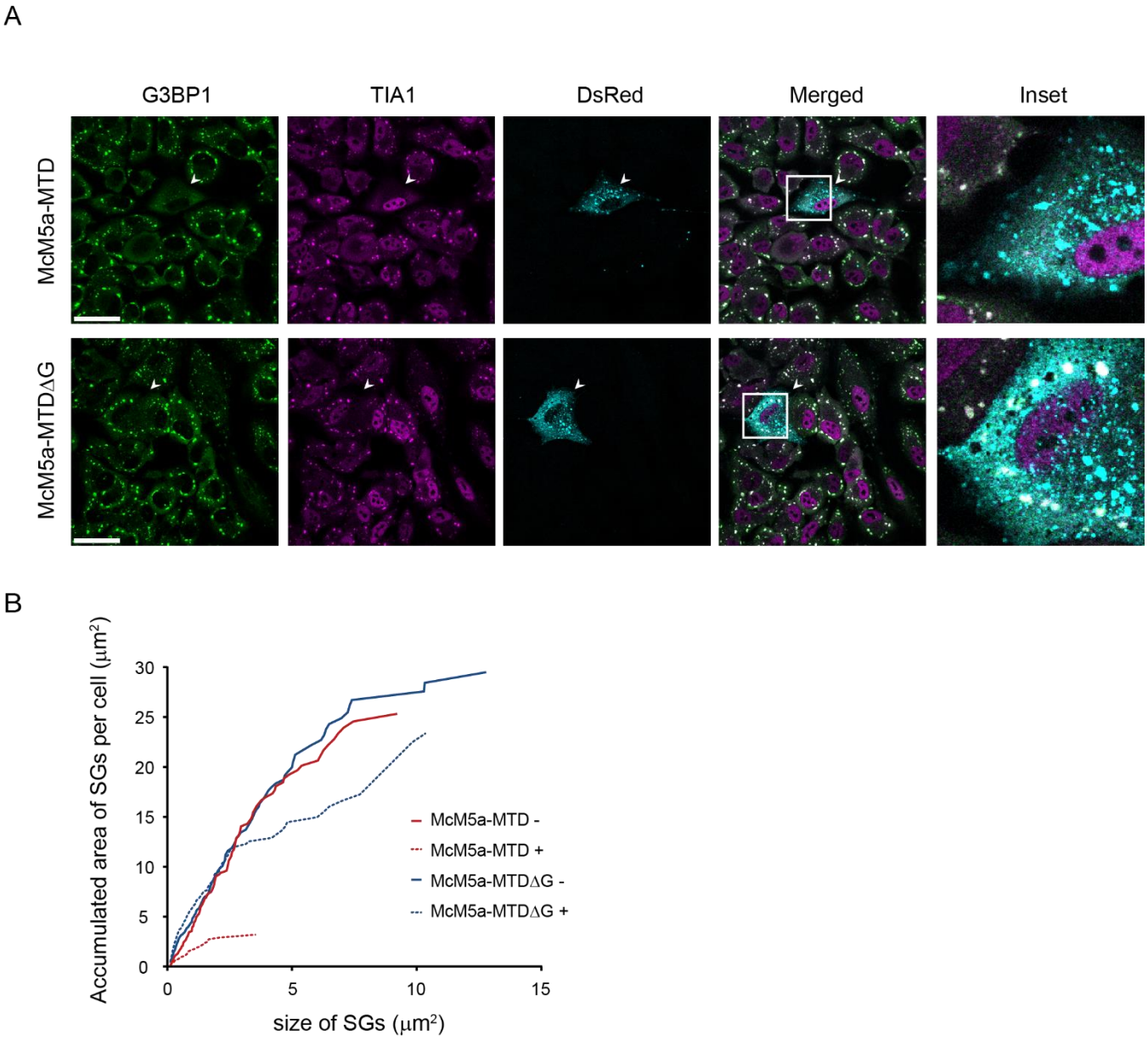

Figure S8. Overexpressing McM5a-MTD inhibits SG formation in A549 cells. (**A**) A549 cells were transfected with DsRed-McM5a-MTD or DsRed-McM5a-MTDΔG, and 2 days later treated with 500 μM sodium arsenite for 1h prior to fixation. Cells were stained with G3BP1 (Green) and TIA1 (Magenta) as SG markers. Scale bars, 40 μm. (**B**) Cumulative area of SGs per cell under arsenite-induced conditions. The area of individual SG in each cell (12 cells total for each condition) was quantified by ImageJ and a cumulative area of SGs per cell was plotted. The SG size on the X-axis represents the SGs from the 12 analyzed cells considered individually.

Figure S9

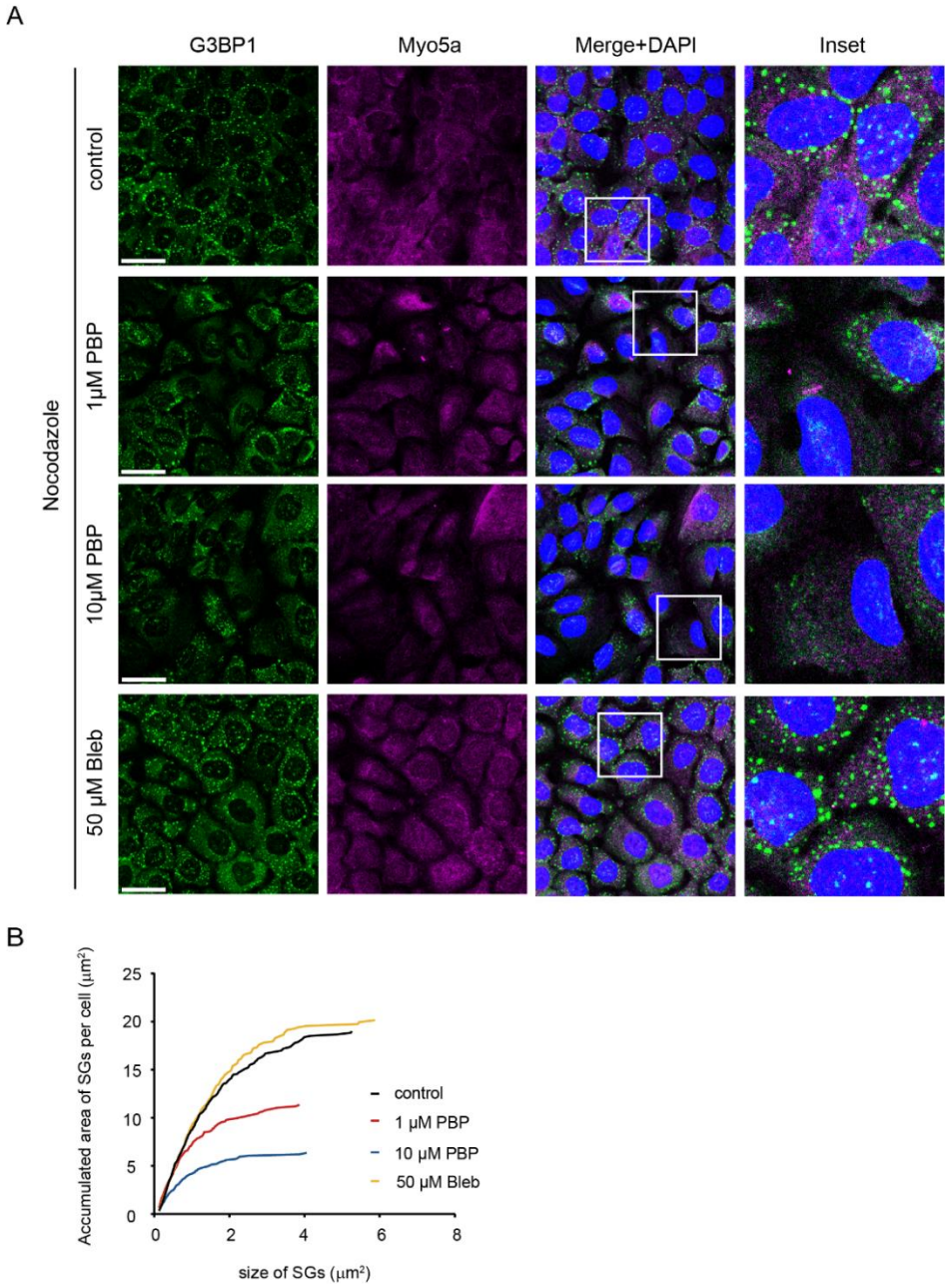

Figure S9. Effects of Nocodazole and PBP on SG formation. (**A**) U2OS cells were pretreated with 6.6 μM nocodazole for 1h, then treated with the myosin-5 inhibitor PBP or the myosin-2 inhibitor Blebbistatin (Bleb) plus nocodazole for 1h, and finally treated with 500 μM sodium arsenite for 1h. Cells were fixed and stained for G3BP1 (Green) and Myo5a (Magenta). The right lane is an enlargement of the image in the white box. Scale bar, 40 μm. (**B**) Cumulative area of SGs per cell under arsenite-induced conditions. The area of individual SG in each cell (at least 25 cells for each condition) was quantified by ImageJ and a cumulative area of SGs per cell was plotted. The SG size on the X-axis represents the SGs from all the analyzed cells considered individually.

Figure S10

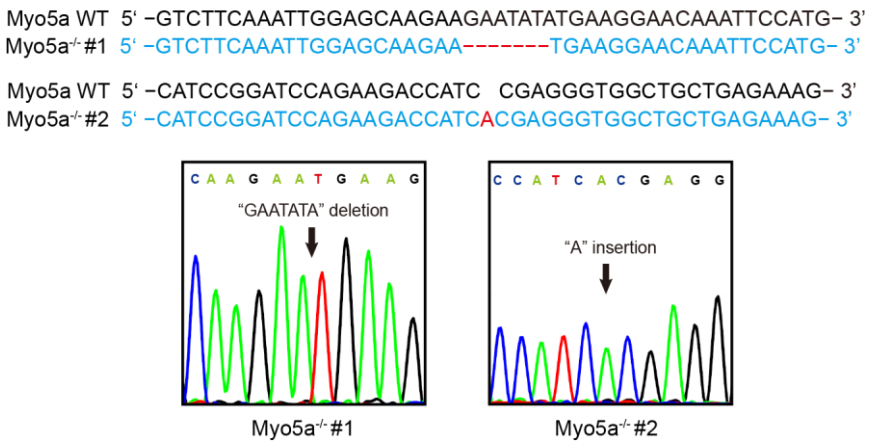

Figure S10. Knockout of Myo5a gene in U2OS cells. Sequence analysis of the Myo5a gene in genomic DNA isolated from two Myo5a-/- U2OS cell lines. Both wild-type (black) and mutant alleles (blue) are shown. The location of each mutation (deletion/insert) is marked in red.