

Supplementary Information

Cell-to-cell transmission of stress granules propagates integrated stress response

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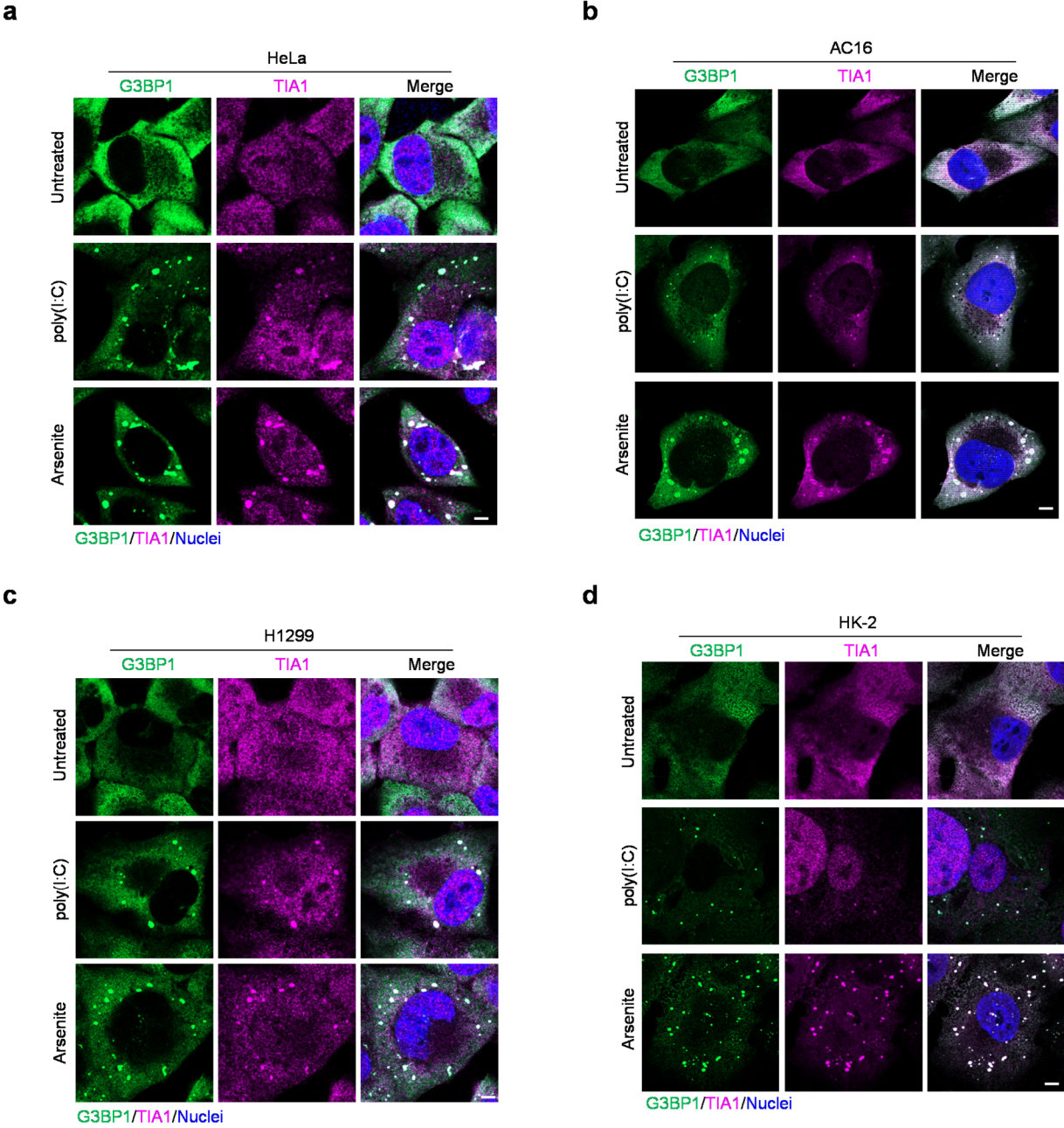
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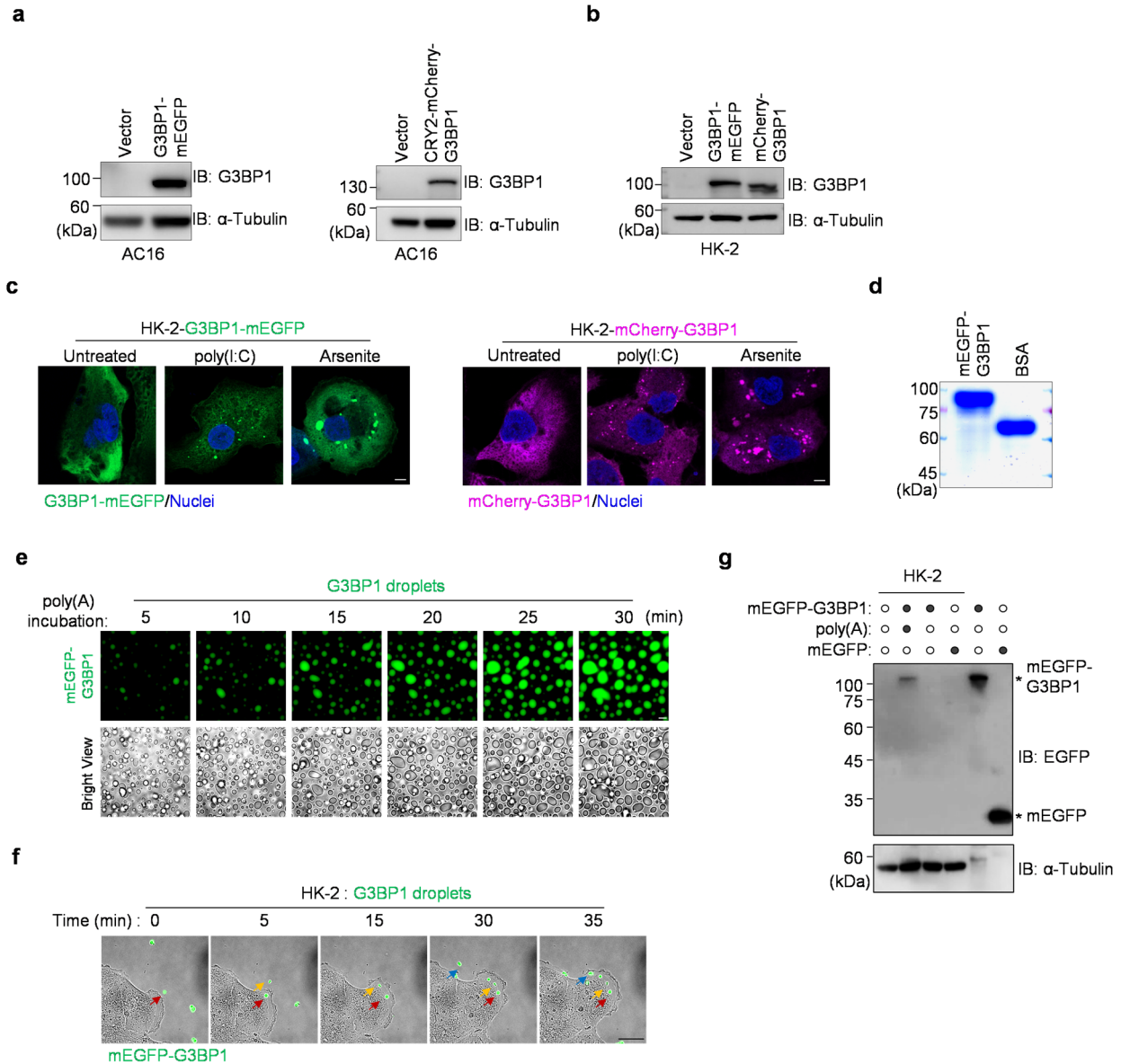
Extended Data Fig. 1



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29 **Extended Data Fig. 1 | Stress granule formation in cells.** a-d, Immunofluorescence staining of G3BP1 and
 30 TIA-1 in HeLa (a), AC16 (b), H1299(c) and HK-2 (d) cells following poly(I:C) or arsenite treatment. Scale bars,
 31 5 μm.

Extended Data Fig. 2



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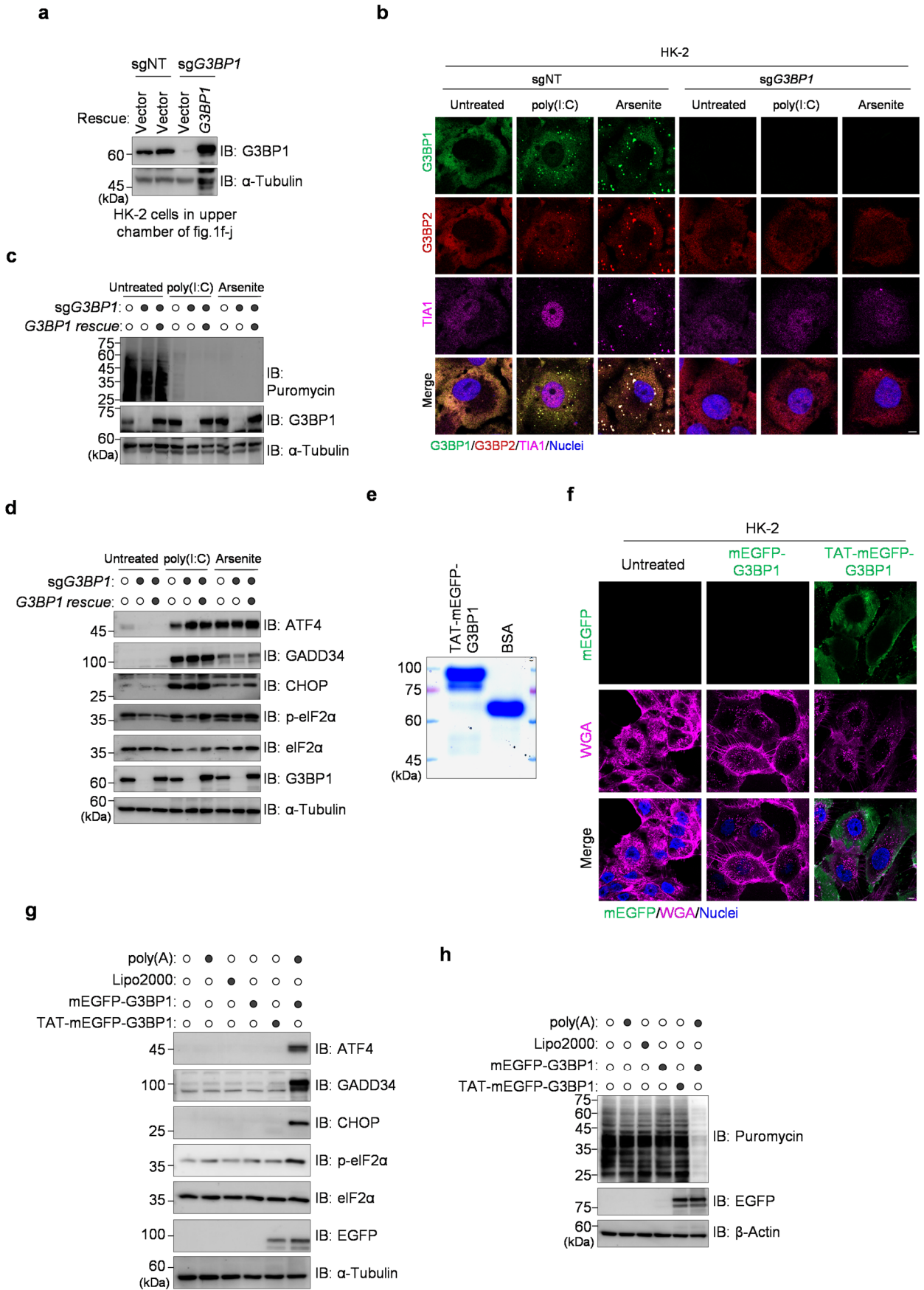
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Extended Data Fig. 2 | Stress granules undergo cell-to-cell transmission. a, Expression of G3BP1-mEGFP and Cry2-mCherry-G3BP1 in AC16 cells were assessed by immunoblotting. **b**, Expression of G3BP1-mEGFP and mCherry-G3BP1 in HK-2 cells were assessed by immunoblotting. **c**, Representative images showing SG formation in HK-2 cells expressing G3BP1-mEGFP or mCherry-G3BP1 following poly(I:C) or arsenite treatment. **d**, Coomassie blue staining of purified recombinant mEGFP-G3BP1. Bovine serum albumin (BSA), control. **e**, *In vitro* phase separation assay. Recombinant mEGFP-G3BP1 (50 μ M) was incubated with poly(A) RNA (100 ng μ L⁻¹) at room temperature, the condensate formation was monitored by time-lapse imaging. **f**, Live-cell imaging of HK-2 cells incubated with *in vitro* reconstituted mEGFP-G3BP1-poly(A) droplets. Images were acquired every 5 min. Scale bar, 25 μ m. **g**, HK-2 cells were incubated with mEGFP-G3BP1, mEGFP-G3BP1-poly(A) droplets or mEGFP for 6 h, internalized protein was detected by immunoblotting with anti-GFP. Scale bars, 5 (**c**), 10 (**e**) or 25 (**f**) μ m. Nuclei, Hoechst.

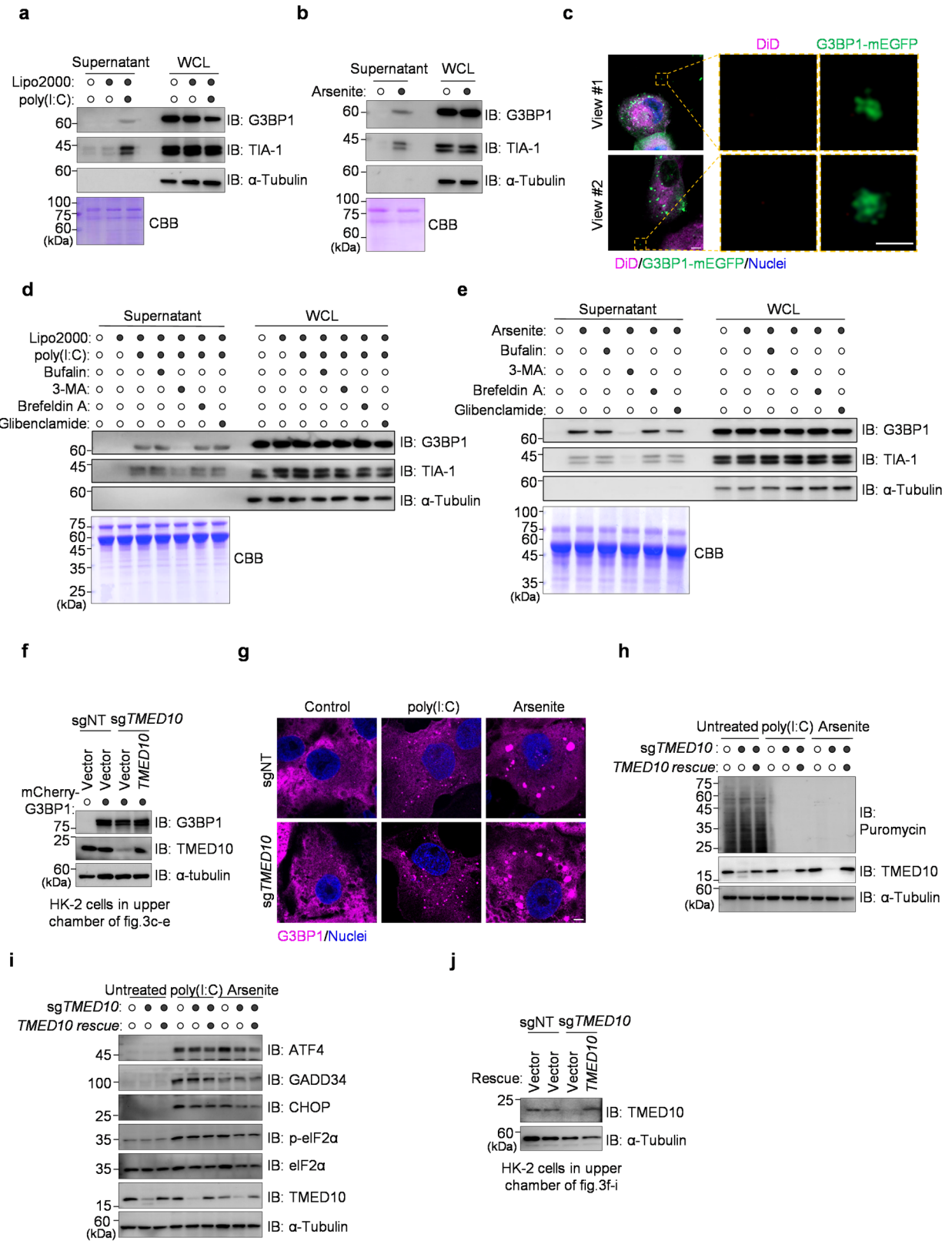
Extended Data Fig. 3



45 **Extended Data Fig. 3 | CTS mediates propagation of the ISR.** **a**, G3BP1 knockout and rescue efficiency
46 were assessed by immunoblotting. **b**, SGs formation was assessed by immunofluorescence staining in WT and
47 *G3BP1*-knockout cells. Representative images were shown. **c,d**, Genetically modified HK-2 cells (*sgNT*^{vector}
48 ^{rescue}, *sgG3BP1*^{vector rescue} or *sgG3BP1*^{*G3BP1* rescue}) were stimulated with poly(I:C) (1 $\mu\text{g mL}^{-1}$, 7 h) or arsenite (300
49 μM , 3 h). Translation (**c**) and ISR activation (**d**) was assessed. **e**, Coomassie blue staining of recombinant TAT-
50 mEGFP-G3BP1 protein. BSA, control. **f**, HK-2 cells were incubated with mEGFP-G3BP1 or TAT-mEGFP-
51 G3BP1 for 6 h. Membranes were stained with WGA (magenta). Representative images were shown. **g,h**, HK-2
52 cells were treated with poly(A), TAT-mEGFP-G3BP1 protein or mEGFP-G3BP1-poly(A) droplets. Translation
53 efficiency (**g**) and ISR activation (**h**) were assessed as above. Scale bar, 5 μm . Nuclei, Hoechst.

56 **Extended Data Fig. 4 | PERK and GCN2 are important for CTS-induced ISR activation.** **a,b**, Genetically
57 modified HK-2 cells (sgNT^{Vector rescue}, sgPERK^{Vector rescue} or sgPERK^{PERK rescue}) were stimulated with poly(I:C) (1
58 $\mu\text{g mL}^{-1}$, 7 h) or arsenite (300 μM , 3 h). Translation (**a**) and ISR activation (**b**) were assessed. **c,d**, Genetically
59 modified HK-2 recipient cells (as indicated) were incubated with G3BP1 droplets. Translation (**c**) and ISR
60 activation (**d**) were assessed. **e-i**, Transwell assay using genetically modified recipient HK-2 cells (as indicated).
61 Donor HK-2 cells were stressed with poly(I:C) (1 $\mu\text{g mL}^{-1}$, 5 h) or arsenite (300 μM , 2 h) in the upper chamber,
62 then transferred to a new lower chamber containing recipients. Schematic drawing was shown (**e**). Translation
63 (**f,h**) and ISR activation (**g,i**) were analyzed in recipient lysates. **j**, Representative images showing the
64 colocalization of internalized G3BP1 droplets with endogenous PERK after 24 h incubation, immunostaining
65 with anti-PERK. Scale bar, 5 μm . G3BP1 droplets, mEGFP-G3BP1:poly(A).

Extended Data Fig. 5



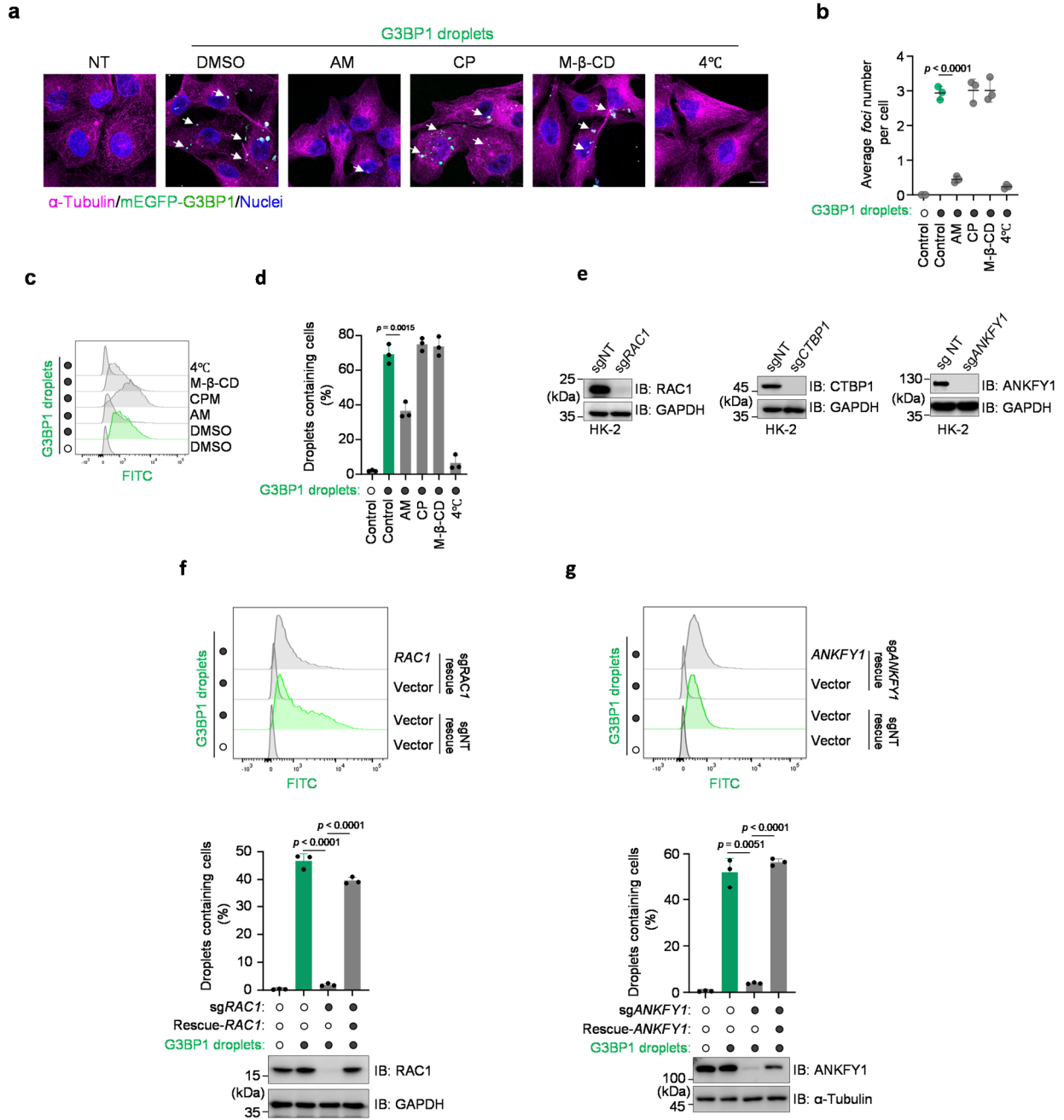
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69 **Extended Data Fig. 5 | TMED10-dependent type III UPS mediates SG release for ISR propagation. a,b,**
70 HK-2 cells were stimulated with poly(I:C) ($1 \mu\text{g mL}^{-1}$, 7 h, **a**) or arsenite ($300 \mu\text{M}$, 3 h, **b**). Supernatants were
71 collected and SGs release was assessed by immunoblotting for G3BP1 and TIA-1. Coomassie blue staining
72 (CBB) served as a loading control. **c**, HK-2-G3BP1-mEGFP cells were stimulated with arsenite. The plasma
73 membranes were labeled with DiD. **d,e**, HK-2 cells were pretreated with bufalin ($1 \mu\text{M}$), 3-MA (2.5 mM), brefeldin
74 A ($1 \mu\text{M}$) or glibenclamide ($20 \mu\text{M}$), followed by stimulation with poly(I:C) (**d**) or arsenite (**e**) as indicated. SGs
75 release into supernatants was analysed as in **a,b**. **f**, *TMED10* knockout and rescue efficiency in HK-2 cells
76 expressing mCherry-G3BP1 was assessed by immunoblotting. **g**, Representative images showing poly(I:C)-
77 and arsenite-induced SGs in wild-type and *TMED10*-knockout HK-2 cells. **h,i**, Genetically modified HK-2 cells
78 ($\text{sgNT}^{\text{Vector rescue}}$, $\text{sgTMED10}^{\text{Vector rescue}}$ or $\text{sgTMED10}^{\text{TMED10 rescue}}$) were stimulated with poly(I:C) ($1 \mu\text{g mL}^{-1}$, 7 h)
79 or arsenite ($300 \mu\text{M}$, 2 h). Translation (**h**) and ISR activation (**i**) were assessed. **j**, *TMED10* knockout and rescue
80 efficiency in HK-2 cells was verified by immunoblotting. Scale bars, $5 \mu\text{m}$. Nuclei, Hoechst.

Extended Data Fig. 6



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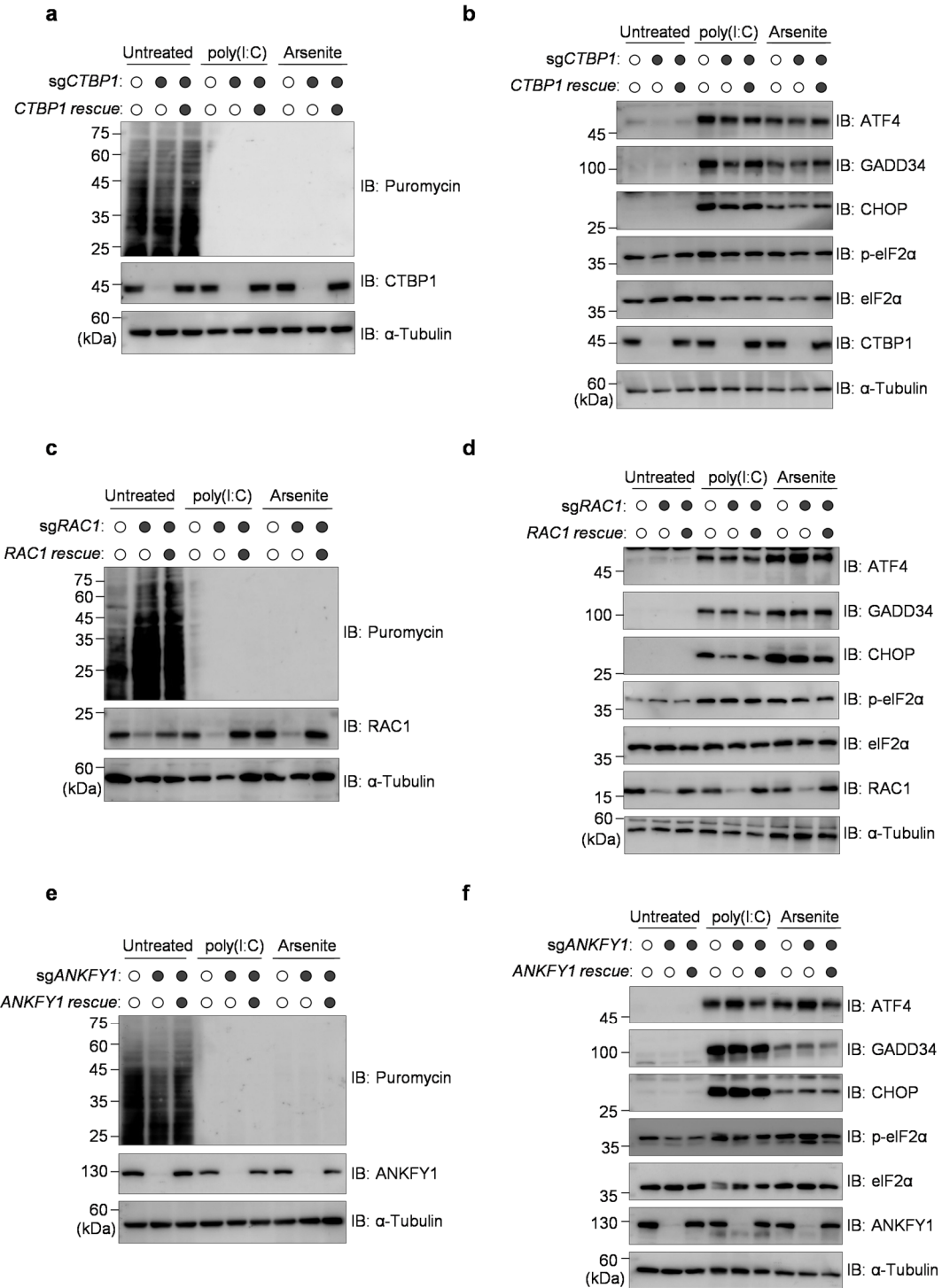
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88 **Extended Data Fig. 6 | Macropinocytosis mediates SG uptake.** **a–d**, HK-2 cells were pretreated with
89 amiloride (AM, 20 μ M), chlorpromazine (CP, 30 μ M), methyl- β -cyclodextrin (M- β -CD, 2.5 mM) or 4 $^{\circ}$ C for 1 h,
90 and then incubated with G3BP1 droplets. Uptake was quantified by immunofluorescence (**a,b**) and flow
91 cytometry (**c,d**). Representative images were shown (**a**). Average foci number per cell (b) or droplet-containing
92 cells (**d**) was analyzed. **e**, CRISPR-Cas9-mediated knockout of *CTBP1*, *RAC1* and *ANKFY1* in HK-2 cells was
93 validated by immunoblotting. **f**, Genetically modified HK-2 cells (sgNT^{vector rescue}, sg*RAC1*^{vector rescue} or
94 sg*RAC1*^{*RAC1* rescue}) were incubated with condensates for 6 h. Representative images (top) and quantification of
95 droplet-containing cells (bottom) was shown. **g**, Genetically modified HK-2 cells (sgNT^{vector rescue}, sg*ANKFY1*^{vector}
96 ^{rescue} or sg*ANKFY1*^{*ANKFY1* rescue}) were incubated with condensates for 6 h. Uptake was quantified as in **f**. G3BP1
97 droplets, mEGFP-G3BP1:poly(A). Data are presented as mean \pm s.d. Statistical significance was determined
98 by two-tailed unpaired Student's *t*-test. n = 3 biological replicates.

Extended Data Fig. 7



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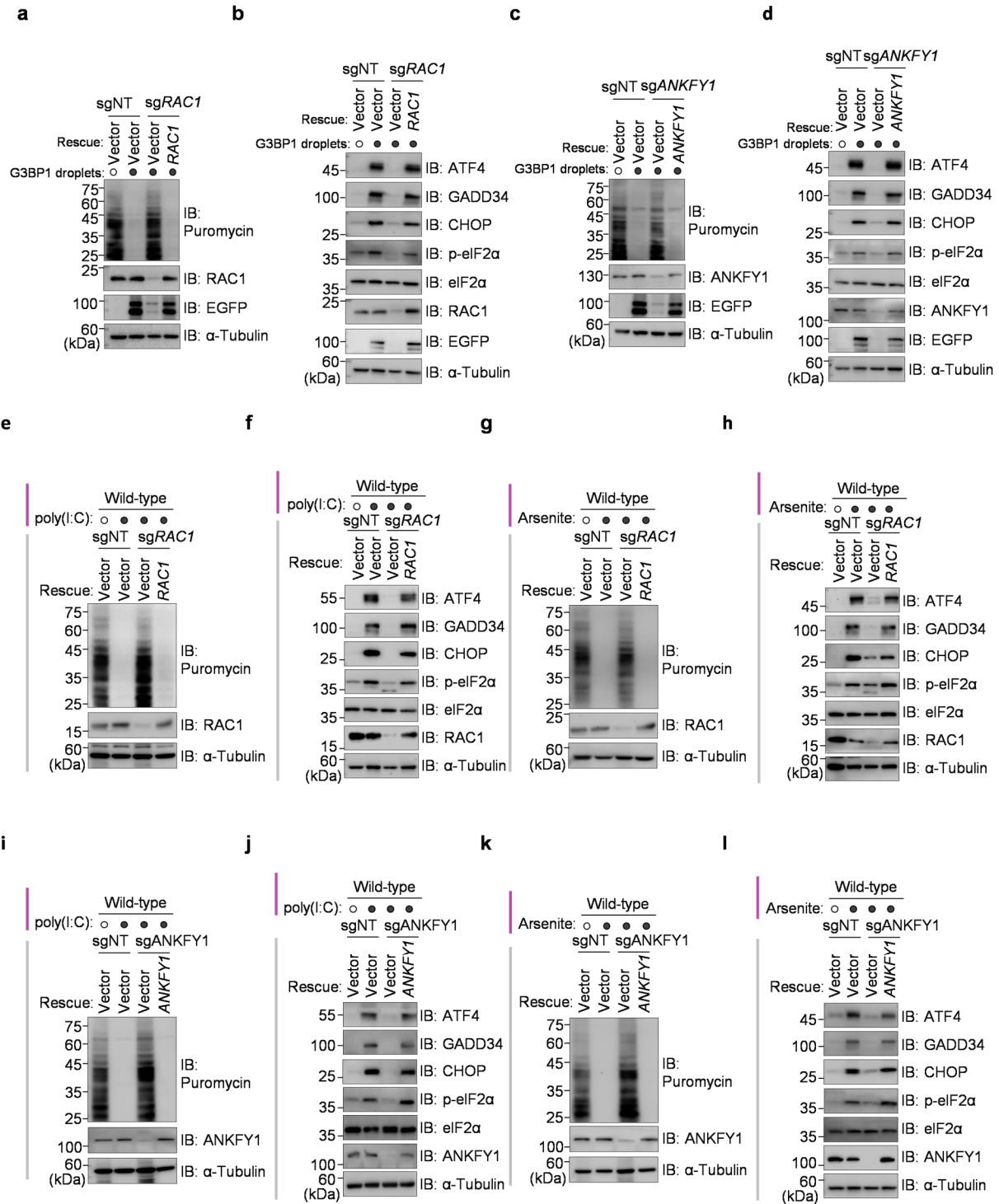
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104 **Extended Data Fig. 7 | Disruption of macropinocytosis did not affect cell-autonomous ISR activation.**
105 **a,b**, Genetically modified HK-2 cells (sgNT^{Vector rescue}, sgCTBP1^{Vector rescue} or sg CTBP1^{CTBP1 rescue}) were stimulated
106 with poly(I:C) (1 $\mu\text{g mL}^{-1}$, 7 h) or arsenite (300 μM , 2 h). Translation (**a**) and ISR activation (**b**) were assessed.
107 **c,d**, The experiment was performed as in **a,b**, using genetically modified HK-2 cells (sgNT^{Vector rescue},
108 sgRAC1^{Vector rescue} or sgRAC1^{RAC1 rescue}) stimulated with poly(I:C) or arsenite. Translation (**c**) and ISR activation
109 (**d**) were assessed. **e,f**, Genetically modified HK-2 recipient cells (sgNT^{Vector rescue}, sgANKFY1^{Vector rescue} or
110 sgANKFY1^{ANKFY1 rescue}) were stimulated with poly(I:C) or arsenite. Translation (**e**) and ISR activation (**f**) were
111 assessed.

Extended Data Fig. 8



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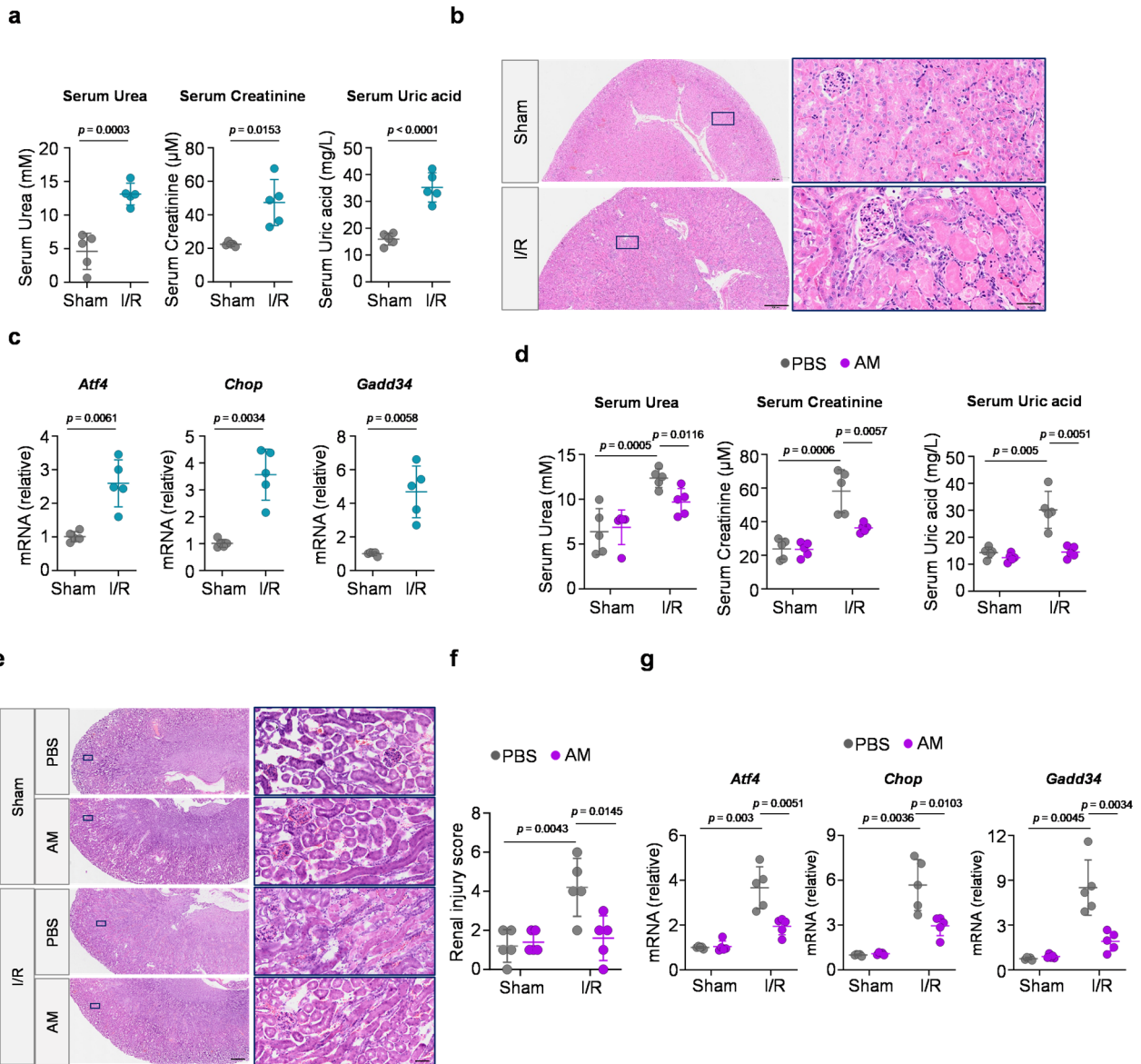
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117 **Extended Data Fig. 8 | Macropinocytosis mediates SG uptake and CTS-driven ISR activation. a,b,**
118 Genetically modified HK-2 recipient cells (sgNT^{Vector rescue}, sgRAC1^{Vector rescue} or sgRAC1^{RAC1 rescue}) were incubated
119 with G3BP1 droplets. Translation (a) and ISR activation (b) were assessed. c,d, The experiment was performed
120 as in a,b, using genetically modified HK-2 cells (sgNT^{Vector rescue}, sgANKFY1^{Vector rescue} or sgANKFY1^{ANKFY1 rescue}).
121 Translation (c) and ISR activation (d) were assessed. e-h, Transwell assay using WT HK-2 donors and
122 genetically modified recipients (sgNT^{Vector rescue}, sgRAC1^{Vector rescue} or sgRAC1^{RAC1 rescue}). Donors were stressed
123 with poly(I:C) (1 µg mL⁻¹, 5 h) or arsenite (300 µM, 2 h) in the absence of recipients, then transferred to a new
124 lower chamber containing unstimulated recipients. Translation (e,g) and ISR activation (f,h) were analyzed in
125 recipient lysates. i-l, Transwell assay performed as in e-h using genetically modified recipients (sgNT^{Vector rescue},
126 sgANKFY1^{Vector rescue} or sg ANKFY1^{ANKFY1 rescue}). Translation (i,k) and ISR activation (j,l) were analyzed in
127 recipient lysates. G3BP1 droplets, mEGFP-G3BP1:poly(A).

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Extended Data Fig. 9



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130 **Extended Data Fig. 9 | Inhibiting CTS mitigates renal ischemia-reperfusion injury (IRI) by dampening the**
 131 **ISR. a**, Serum urea, creatinine, and uric acid levels after I/R injury. **b**, Representative images of H&E-stained
 132 kidney sections from mice subjected to I/R injury. **c**, qPCR analysis of *Atf4*, *Gadd34* and *Chop* expression in the
 133 kidneys of I/R-treated mice. **d-g**, Amiloride (AM) attenuates I/R-induced kidney injury. Serum urea, creatinine
 134 and uric acid levels were measured (**d**). Representative H&E-stained kidney sections after sham or I/R as
 135 indicated (**e**). Renal injury scores were analyzed (**f**). qPCR analysis of ISR-related genes in kidney tissue (**g**)
 136 was shown. Scale bars, 500 (**b,e**, left) or 50 (**b,e**, right) μm . Data are mean \pm s.d. $n = 5$ biological replicates.
 137 Statistical significance was determined by two-tailed unpaired Student's *t*-test.