

**Title: Supplementary Movie 1.**

**Description: Time lapse movie of ABP140-3×GFP labeled actin cable remodeling response to GS.**

Time-lapse movie of ABP140-3×GFP labeled actin cable remodeling in WT, *hxx2Δ*, and *cbp2Δ* cells upon glucose starvation from 0 to 30 min. Scale bar, 2 μm.

**Title: Supplementary Movie 2.**

**Description: Time lapse movie of the ATP sensor Queen upon to GS.**

Time-lapse movie of Queen upon glucose starvation from 0 to 30 min. Scale bar, 2 μm.

**Title: Supplementary Movie 3.**

**Description: Time lapse movie of ABP140-3×GFP labeled actin cable remodeling response to GS 5 min.**

Time-lapse movie of ABP140-3×GFP labeled actin cable remodeling in WT, *spa2Δ*, and *cbp2Δ spa2Δ* cells upon glucose starvation from 0 to 5 min. Scale bar, 2 μm.

**Title: Supplementary Movie 4.**

**Description: Time lapse movie of Spa2-GFP, ABP140-Tomato response to GS 5 min.**

Time-lapse movie of Spa2-GFP, ABP140-Tomato upon glucose starvation from 0 to 5 min. Scale bar, 2 μm.

**Title: Supplementary Movie 5.**

**Description: Time lapse movie of Spa2-GFP, ABP140-Tomato response to 2-DG 5 min.**

Time-lapse movie of Spa2-GFP, ABP140-Tomato upon 2-DG from 0 to 5 min. Scale bar, 2 μm.

**Title: Supplementary Movie 6.**

**Description: Time lapse movie of Spa2-GFP response to GS.**

Time-lapse movie of Spa2-GFP in WT, *hxx2Δ*, and *cbp2Δ* cells upon glucose starvation from 0 to 30 min. Scale bar, 2 μm.

**Title: Supplementary Movie 7.**

**Description: Time lapse movie of GFP tagged Spa2 truncation variants response to GS 5min.**

Time-lapse movie of Spa2(1-535)-GFP, Spa2(281-535)-GFP, Spa2(1-281)-GFP cells upon glucose starvation from 0 to 5 min. Scale bar, 2 μm.

**Title: Supplementary Movie 8.**

**Description: Time lapse movie of GFP tagged Spa2 truncation variants response to 2-DG 5min.**

Time-lapse movie of Spa2(1-535)-GFP, Spa2(281-535)-GFP, Spa2(1-281)-GFP cells upon energy starvation from 0 to 5 min. Scale bar, 2 μm.

**Title: Supplementary Movie 9.**

**Description: Time lapse movie of ABP140-3×GFP labeled actin cable remodeling in Spa2 truncation variant response to GS 5 min.**

Time-lapse movie of ABP140-3×GFP labeled actin cable remodeling in *spa2(1-535)*, *spa2(281-535)*, *spa2(1-281)* cells upon glucose starvation from 0 to 5 min. Scale bar, 2 μm.

**Title: Supplementary Movie 10.**

**Description: Time lapse movie of ABP140-3×GFP labeled actin cable remodeling in Spa2 truncation variant response to 2-DG 5 min.**

Time-lapse movie of ABP140-3×GFP labeled actin cable remodeling in *spa2(1-535)*, *spa2(281-535)*, *spa2(1-281)* cells upon 2-DG from 0 to 5 min. Scale bar, 2 μm.

**Title: Supplementary Movie 11.**

**Description: Time lapse movie of Actin filament severing by 50nM Cof1 with or without 500 nM Spa2 variants.**

TIRF movie of actin severing in the presence of indicated Cof1 with or without Spa2 variant within a time range between 0 to 10 min. Reaction contains 0.1  $\mu\text{M}$  actin, 50 nM Cof1, 500 nM Spa2 variants. Scale bar, 2  $\mu\text{m}$ .

**Title: Supplementary Movie 12.**

**Description: Time-lapse movie of 3  $\mu\text{M}$  ADP-actin actin polymerization by 10 nM of each Spa2 variant.**

TIRF movie of Spa2 variants-mediated ADP-actin polymerization within a time range between 0 to 10 min.

Reaction contains 3  $\mu\text{M}$  ADP-actin, 10 nM Spa2 protein variants. Scale bar, 2  $\mu\text{m}$ .

**Title: Supplementary Movie 13.**

**Description: Time-lapse movie of 0.5  $\mu\text{M}$  ATP-actin polymerization by 10 nM of each Spa2 variant.**

TIRF movie of Spa2 variants-mediated ATP-actin polymerization within a time range between 0 to 10 min.

Reaction contains 0.5  $\mu\text{M}$  ATP-actin, 10 nM Spa2 protein variants. Scale bar, 2  $\mu\text{m}$ .

**Title: Supplementary Movie 14.**

**Description: Time-lapse movie of 3  $\mu\text{M}$  ADP- $\text{P}_i$ -actin polymerization by 10 nM of each Spa2 variant.**

TIRF movie of Spa2 variants-mediated ADP- $\text{P}_i$ -actin polymerization within a time range between 0 to 10 min.

Reaction contains 3  $\mu\text{M}$  ADP- $\text{P}_i$ -actin, 10 nM Spa2 protein variants. Scale bar, 2  $\mu\text{m}$ .

**Title: Supplementary Movie 15.**

**Description: Transmission light time-lapse movie of the coalescence of 5  $\mu\text{M}$  Spa2-535.**

Time-lapse movie of 5  $\mu\text{M}$  Spa2-535 phase separation droplet dynamics in 20 mM HEPES, 50 mM KCl pH 7.4 buffer. Scale bar, 5  $\mu\text{m}$ .

**Title: Supplementary Movie 16.**

**Description: Time lapse movie of 3  $\mu\text{M}$  ADP-actin polymerization and bundling in the presence of 5  $\mu\text{M}$  of Spa2 variants.**

TIRF movie of Spa2 variants-mediated ADP-actin polymerization within a time range between 3 to 61.5 min.

Reaction contains 3  $\mu\text{M}$  ADP-actin, 5  $\mu\text{M}$  Spa2 protein variants. Scale bar, 2  $\mu\text{m}$ .

**Title: Supplementary Movie 17.**

**Description: Time lapse movie of 3  $\mu\text{M}$  ADP-actin polymerization without or with 5  $\mu\text{M}$  Spa2-535 and then severing by 100nM Cof1 .**

TIRF movie of Spa2-535 mediated ADP-actin bundles severing by Cof1. Reaction contains 3  $\mu\text{M}$  ADP-actin, 5  $\mu\text{M}$  Spa2-535, 100nM Cof1. Scale bar, 2  $\mu\text{m}$ .

**Title: Supplementary Movie 18.**

**Description: Time lapse movie of 0.5  $\mu\text{M}$  ATP-actin polymerization in the presence of 5  $\mu\text{M}$  Spa2 variants.**

TIRF movie of Spa2 variants-mediated ATP-actin polymerization within a time range between 0 to 60 min.

Reaction contains 0.5  $\mu\text{M}$  ATP-actin, 5  $\mu\text{M}$  Spa2 protein variants. Scale bar, 2  $\mu\text{m}$ .

**Title: Supplementary Movie 19.**

**Description: Time lapse movie of 3  $\mu\text{M}$  ADP- $\text{P}_i$ -actin polymerization with 5  $\mu\text{M}$  Spa2-535.**

TIRF movie of Spa2-535-mediated ADP-P<sub>i</sub>-actin polymerization within a time range between 4.5 to 58.5 min. Reaction contains 3 μM ADP-P<sub>i</sub>-actin, 5 μM Spa2-535. Scale bar, 2 μm.

**Title: Supplementary Movie 20.**

**Description: Time lapse movie of 3 μM ADP-actin polymerization by engineered 10 nM Spa2-281-535-trimer.**

TIRF movie of Spa2 variants-mediated ADP-actin polymerization within a time range between 0 to 10 min. Reaction contains 3 μM ADP-actin, 10 nM Spa2 protein variants. Scale bar, 2 μm.

**Title: Supplementary Movie 21.**

**Description: Time lapse movie of 3 μM ADP-actin polymerization and bundling in the presence of 5 μM of Spa2-281-535-trimer.**

TIRF movie of Spa2-281-535-trimer-mediated ADP-actin polymerization within a time range between 0 to 60 min. Reaction contains 3 μM ADP-actin, 5 μM Spa2-281-535-trimer. Scale bar, 2 μm.

**Title: Supplementary Movie 22.**

**Description: Time lapse movie of 0.5 μM ATP-actin polymerization by engineered 10 nM Spa2-281-535-trimer.**

TIRF movie of Spa2 variants-mediated ATP-actin polymerization within a time range between 0 to 10 min. Reaction contains 0.5 μM ATP-actin, 10 nM Spa2 protein variants. Scale bar, 2 μm.

**Title: Supplementary Movie 23.**

**Description: Time lapse movie of 0.5 μM ATP-actin polymerization and bundling in the presence of 5 μM of Spa2-281-535-trimer.**

TIRF movie of Spa2-281-535-trimer-mediated ATP-actin polymerization within a time range between 0 to 60 min. Reaction contains 0.5 μM ATP-actin, 5 μM Spa2-281-535-trimer. Scale bar, 2 μm.