Description of Additional Supplementary Files

Supplementary Movie 1. Coverslip-anchored Lmod2 nucleates actin filament in presence of G-Actin monomers. No actin filaments were nucleated from streptavidin passivated coverslips lacking anchored Lmod2 (left) in the presence of a continuous flow of 1 µM G-actin (15% Alexa-488 labeled, green) in mf-TIRF buffer. In contrast, multiple nucleation events were observed on coverslips with anchored Lmod2 (right) under identical conditions (also see Supplementary Fig. 1).

Supplementary Movie 2. Lmod2 is an elongator of actin filaments. An actin filament was nucleated from coverslip-anchored Lmod2 by introducing a flow containing 1 µM G-actin (15% Alexa-568 labeled, red) in mf-TIRF buffer. The Lmod2-bound fluorescent filament was then elongated in the presence of 2 µM G-actin (15% Alexa-488 labeled, green) in mf-TIRF buffer. Filament elongation occurs at both the Lmod2-anchored PE (cyan arrowhead) and the distal free BE (yellow arrowhead; also see Fig. 1C). Pauses in the playback have been inserted to indicate the change of biochemical conditions. Additional “green” filaments appear later due to nucleation from surface-anchored Lmod2 molecules that had not initially nucleated filaments from “red” Alexa-568–labeled actin.

Supplementary Movie 3. PE Elongation by Lmod2 in the presence of capping protein (CP). An actin filament was nucleated from coverslip-anchored Lmod2 by introducing a flow containing 1 µM G-actin (15% Alexa-568 labeled, red) in mf-TIRF buffer. The Lmod2-bound fluorescent filament was then elongated in the presence of 2 µM G-actin (15% Alexa-488 labeled, green) and 25 nM CP in mf-TIRF buffer. Filament elongation occurs only at the Lmod2-anchored PE (cyan arrowhead). No elongation occurs at the distal CP-bound BE (yellow arrowhead) (also see Fig. 1D). Pauses in the playback have been inserted to indicate the change of biochemical conditions.

Supplementary Movie 4. PE by Lmod2 in the presence of capping protein (CP). Same conditions as Supplementary Movie 3, with larger field of view. Actin filaments were nucleated from coverslip-anchored Lmod2 by introducing a flow containing 1 µM G-actin (15% Alexa-568 labeled, red) in mf-TIRF buffer. The Lmod2-bound fluorescent filaments were then elongated in the presence of 2 µM G-actin (15% Alexa-488 labeled, green) and 25 nM CP in mf-TIRF buffer. Filament elongation occurs only at the Lmod2-anchored PE (cyan arrowhead) (also see Fig. 1D). Pauses in the playback have been inserted to indicate the change of biochemical conditions.

Supplementary Movie 5. Visualization of labelled CP bound to filaments elongating from coverslip-anchored Lmod2. Actin filaments were nucleated from coverslip-anchored Lmod2 by introducing a flow containing 1 µM G-actin (15% Alexa-488 labeled, green) in mf-TIRF buffer. These Lmod2-bound fluorescent filaments were then transiently exposed to fluorescently labelled 25 nM 549-CP (red) in mf-TIRF buffer. Subsequently, the filaments were elongated in the presence of unlabeled 2 µM G-actin in mf-TIRF buffer. Filament elongation occurs by insertional polymerization of unlabeled G-actin at the Lmod2-anchored PE (cyan arrowheads).

Fluorescently labelled 549-CP (red) remains associated with paused distal BEs (yellow arrowheads) (also see Fig. 1F).

Supplementary Movie 6. Coverslip-anchored biotinylated CP captures actin filament in presence of preformed actin filaments. No actin filaments were captured by streptavidin passivated coverslips lacking anchored biotinylated CP molecules (left), even after continuous flow of actin filaments (15% Alexa-488 labeled, green) in mf-TIRF buffer. In contrast, multiple filaments were captured on coverslips with anchored biotinylated CP under identical conditions.

Supplementary Movie 7. Direct visualization of fluorescently labelled Lmod2 elongating filament PEs. Streptavidin-functionalized coverslips in an mf-TIRF chamber were coated with biotinylated capping protein (CP) and subsequently exposed to a solution containing 2 µM G-actin (15% Alexa-488–labeled, green) and 10 nM 549–Lmod2 (red). The movie shows a filament, with 549–Lmod2 bound to its pointed end (PE), was captured by coverslip-bound CP. The filament was then exposed to a solution containing 2 µM G-actin (15% Alexa-488–labeled, green) and 10 nM CP. The barbed end (BE), immobilized via CP, is indicated by a yellow arrowhead, while the cyan arrowhead marks the elongating PE decorated with 549–Lmod2. The white asterisk denotes the presence of 549–Lmod2 at the PE. See also Fig. 1I

Supplementary Movie 8. Direct visualization of fluorescently labelled Lmod2 elongating filament PEs. Same experimental conditions as Supplementary Movie 7 (including solution compositions), shown over a larger field of view. Two populations of filaments captured by the CP-coated surface are observed: filaments with 549-Lmod2 bound to their pointed ends (PEs) and filaments with free PEs. Barbed ends (BEs) of filaments with Lmod2-bound PEs are indicated by upright yellow arrowheads, whereas BEs of filaments with free PEs are indicated by inverted yellow arrowheads. Cyan arrowheads mark elongating PEs decorated with 549–Lmod2, while magenta arrowheads indicate elongating free PEs. White asterisks denote the presence of 549–Lmod2 at PEs. See also Fig. 1I. The movie is looped four times for visualization.

Supplementary Movie 9. Elongation of filament PEs from profilin bound actin monomers by Lmod2 in the presence of capping protein (CP). Actin filaments were nucleated from coverslip-anchored Lmod2 by introducing a flow containing 1 µM G-actin (15% Alexa-568 labeled, red) in mf-TIRF buffer. The Lmod2-bound fluorescent filaments were then elongated in the presence of 2 µM G-actin (15% Alexa-488 labeled, green), 2 µM profilin (unlabeled) and 25 nM CP in mf-TIRF buffer. Elongation from green actin occurs only at the Lmod2-anchored PE (cyan arrowhead). No elongation occurs at the distal CP-bound BE (yellow arrowhead) (also see Fig. 2A). Pauses in the playback have been inserted to indicate the change of biochemical conditions.

Supplementary Movie 10. Depolymerization of Lmod2-bound PEs by profilin. Actin filaments (green) nucleated and elongated from coverslip-anchored Lmod2 were exposed to flow containing 50 µM of profilin (unlabeled) and 25 nM CP in mf-TIRF buffer. Depolymerization occurs at the anchored PE (left end of the filaments) after the exposure to profilin, whereas the BEs are capped by CP. Movement of the fiducial marker toward the point of attachment indicates depolymerization at the anchored PE (also see Fig. 2E,F).

Supplementary Movie 11. Deletion of WH2 domain (Lmod2ΔWH2) causes a reduction in elongation rate and processivity of Lmod2. Alexa-488 labeled (green) actin filaments nucleated from coverslip-anchored Lmod2 were exposed to a flow containing 2 µM unlabeled G-

actin and 25 nM CP. Left: Lmod2 wild-type. Right: Lmod2ΔWH2. Translocation of fluorescently labeled segment along the flow demonstrates insertional elongation of unlabeled G-actin at the location of surface-anchored Lmod2. Time-dependent detachment of filaments from coverslip-anchored Lmod2 was used to determine Lmod2ΔWH2 exhibits reduced elongation rates and faster dissociation from the PE compared with wild-type Lmod2. The movie is looped three times.

Supplementary Movie 12. **Coverslip-anchored Lmod2 molecules can renucleate new filaments after detachment of initially-nucleated filaments.** A Lmod2-bound coverslip surface was continuously perfused with a flow containing 0.5 µM G-actin (15% Alexa-488 labeled) in mf-TIRF buffer for a period of 1.5 hours. Repeated filament nucleation and elongation events were observed at the same surface locations following filament detachment (see Fig. 2M). Asterisks * mark the sites of renucleation.