-Supporting Information-

Azide-Incorporated Clickable Silk Fibroin Materials with the Ability to Photopattern

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ADDITIONAL EXPERIMENTAL PROTOCOLS

Germline Transformation. Construction of the *piggyBac* plasmid vector, pBac[3×P3-DsRed2afm]-BmPheRS_α_T407 encoding the T407A mutant of *Bombyx mori* phenylalanyl-tRNA synthetase α-subunit (BmPheRS-α), and germline transformation of *B. mori* were performed as described previously.[S1] The established transgenic line was designated as H03.

Reverse Transcription Polymerase Chain Reaction (RT-PCR). Each part of the silk glands, i.e., the anterior, middle, and posterior silk glands (respectively abbreviated as ASG, MSG, and PSG), was dissected from 5th instar larvae of silkworms (wild-type, H01, or H03 lines). The

total RNA was extracted from these materials using ISOGEN (Nippon Gene, Tokyo, Japan) according to the manufacturer's instructions followed by treatment with RQ1 RNase-free DNase (Promega, Fitchburg, WI, USA) according to the manufacturer's instructions. RT-PCR was performed to amplify cDNA fragments of the BmPheRS-α mutants using RT-PCR Quick Master Mix (TOYOBO, Osaka, Japan) according to the manufacturer's instructions with the following primer set: 5'-GATGCTGTCAGCGTTCGTTG-3' and 5'-GAATCCCACCTTTGCATTTG-3'. Control amplification was performed without RT using the same primer set. A cDNA fragment of the inherent (wild-type) BmPheRS-α was similarly amplified with the following primer set: 5'-GTGAAAGCTGGAGCTCAGTTTTG-3' and 5'-GAATCCCACCTTTGCATTTG-3'.

Urea Degumming. Amounts of fibroin in cocoons were estimated by degumming aliquots of cocoons in 8 M urea in 40 mM Tris-SO_4 (pH 7) at 80°C for 10 min as reported previously.[S1]

In-Gel Digestion and Mass Analysis. Small pieces of cocoons were dissolved in 8 M LiBr solution at 35°C at a concentration of 50 μg/μL. The dissolved solutions were used for in-gel digestion of fibroin light chain (FibL) followed by MALDI-TOF-MS analysis as previously reported.[S1]

Detection of Azides in Silk Fibroin. Azide groups in silk fibroin were detected by click reaction with biotin-PEG4-alkyne followed by Western blotting against biotin molecules attached to silk fibroin as reported previously.[S1]

REFERENCE

[S1] Teramoto, H.; Kojima, K., *Biomacromolecules* **2014**, *15*, 2682-2690.

SUPPORTING DATA

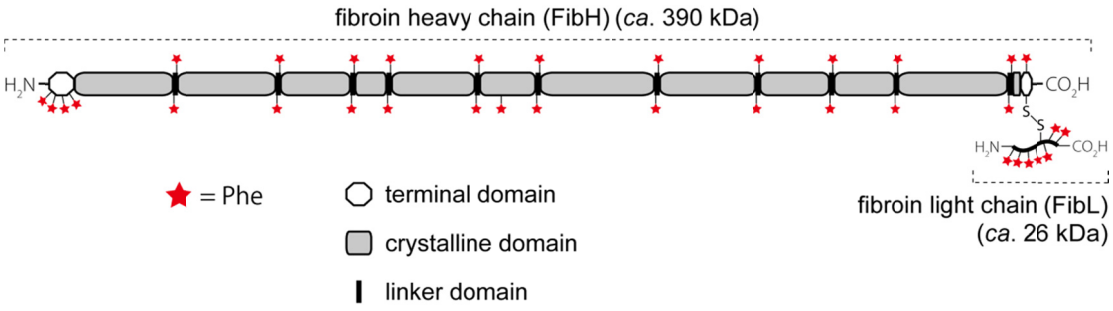

Figure S1. Schematic structure of the *B. mori* silk fibroin heterodimer. Fibroin heavy chain protein (FibH) and fibroin light chain protein (FibL) are linked by a disulfide linkage. FibH is divided into three domains: the terminal, crystalline, and linker domains. Thirty-five Phe residues in mature FibH and FibL are depicted by red stars.

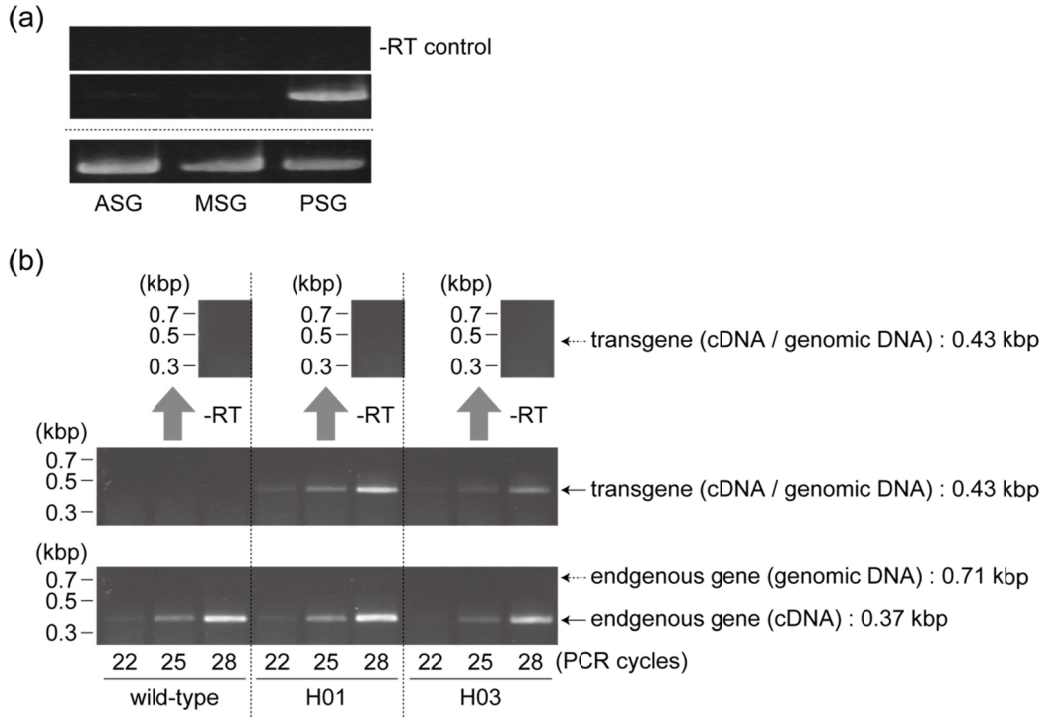

Figure S2. (a) RT-PCR amplification of the cDNA fragments of the T407A mutant (middle panel) and the wild-type (bottom panel) of BmPheRS-α from total RNAs of the anterior, middle, and posterior silk glands (respectively abbreviated as ASG, MSG, and PSG) of H03 larvae. Negative control experiments for amplification of the T407A mutant gene without RT are shown in the top panel, and verified that the DNA fragment in the middle panel was amplified from cDNA (not from genomic DNA). (b) RT-PCR amplification of the cDNA fragments of the transgenes (A450G mutant or T407A mutant of BmPheRS- α) from the total RNA extracted from PSG of 5th-instar wild-type, H01, and H03 larvae (middle panel). Control amplification without RT verified that the DNA fragments were only amplified from cDNA (not from genomic DNA) (top panel). DNA fragments of the endogenous (wild-type) BmPheRS-α gene were also amplified as control experiments (bottom panel).

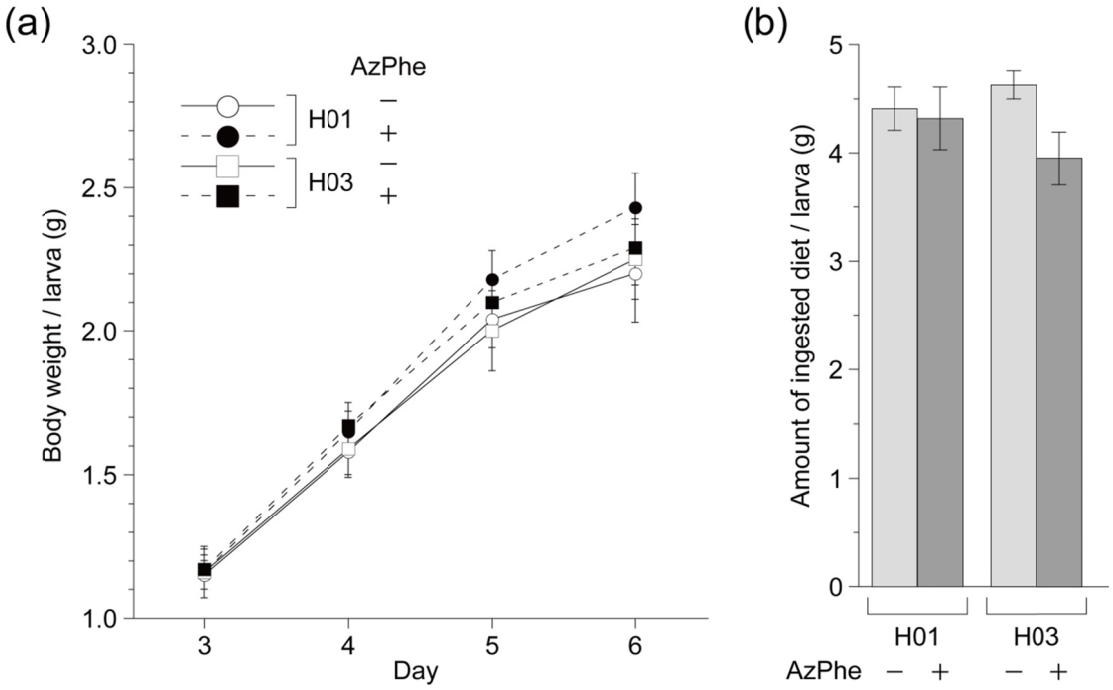

Figure S3. Growth of H01 and H03 larvae on a synthetic diet containing AzPhe (0.5 molar equiv. to Phe in the diet). (a) Average body weights of H01 and H03 larvae were plotted against the day of 5th instar. (b) Averaged total amounts of diet ingested by H01 and H03 larvae from the 3rd day of 5th instar until starting spinning. All points plotted in (a) and (b) are the average values of three independent experiments, where each experiment employed three male larvae. The error bars represent standard deviations.

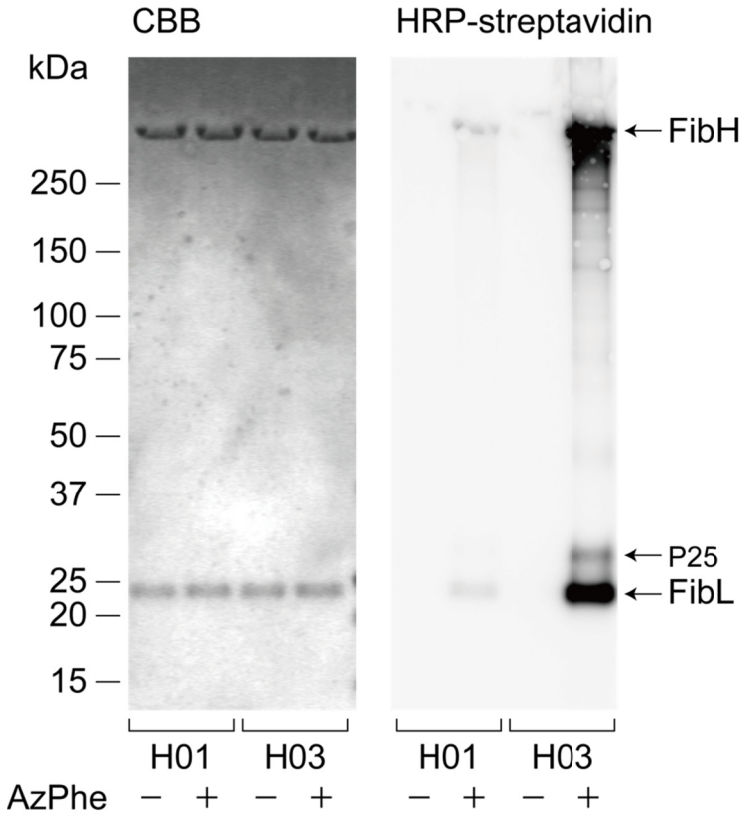

Figure S4. Raw data from the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting in Figure 2c without trimming. Silk fibroin incorporated with AzPhe was subjected to Cu-catalyzed azide alkyne cycloaddition (CuAAC) with alkyne-PEG4-biotin. After the reactions, proteins were separated by SDS-PAGE. The separated proteins were stained with Coomassie brilliant blue (CBB) (left panel) or blotted onto a polyvinylidene fluoride (PVDF) membrane followed by detection with horseradish peroxidase (HRP) conjugated-streptavidin (right panel). The signals assignable to fibroin heavy chain (FibH; ~390 kDa), fibroin light chain (FibL; ~26 kDa), and the glycoprotein P25 (~30 kDa) were observed.

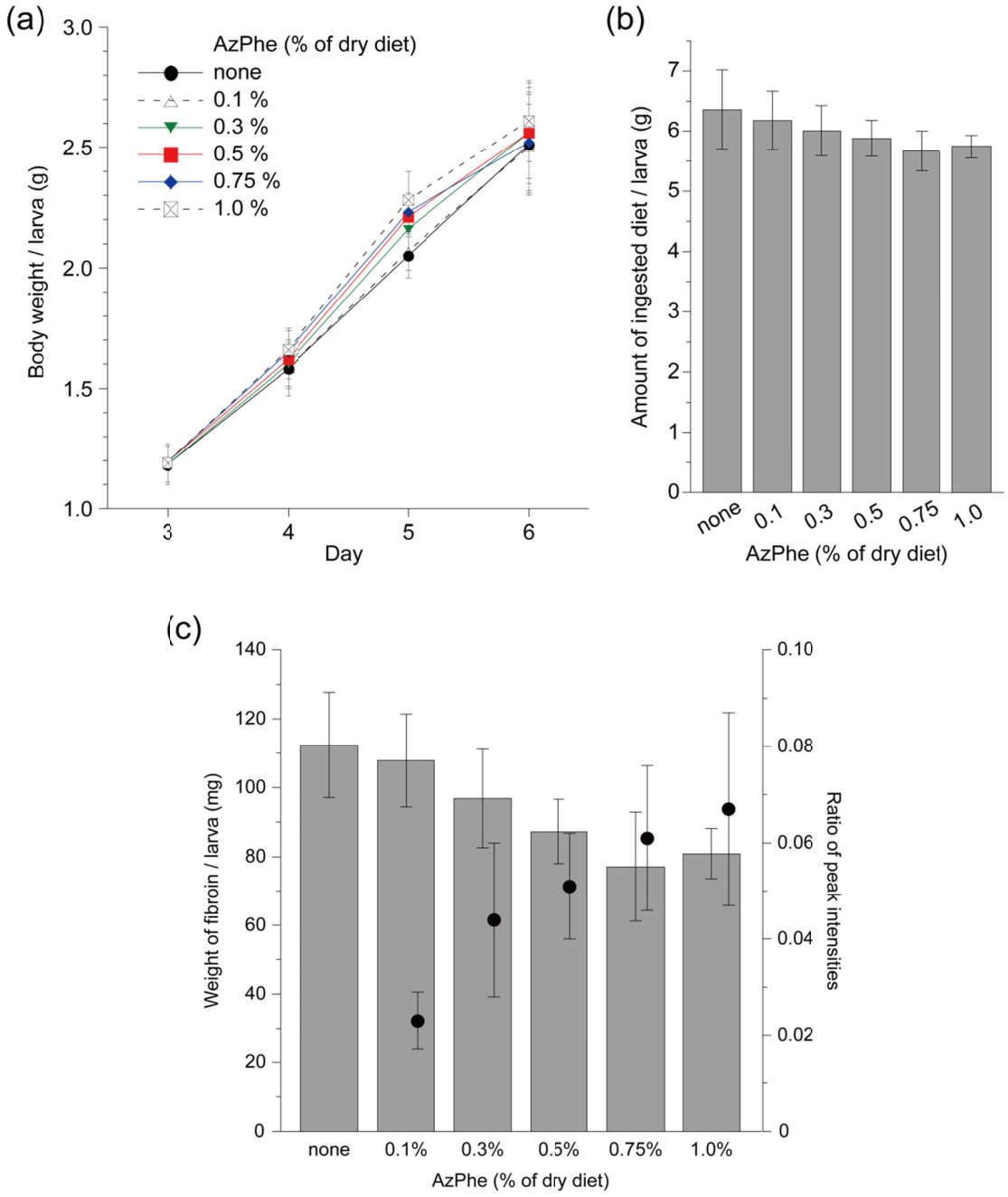

Figure S5. (a) Growth of H03 larvae on a commercially-available standard diet containing varied amounts of AzPhe. Average weights of larvae were plotted against the day of 5th instar. (b) Averaged amounts of diet ingested by larvae from the 3rd day of 5th instar until starting spinning. (c) Production of *AzidoSilk* in larvae fed a commercially-available standard diet

containing varied amounts of AzPhe. Bar plots represent the amounts of *AzidoSilk*. Closed circles represent the ratio of peak intensities (870 Da peak per parental 855 Da peak) in MALDI-TOF-MS analysis (Figure S6) as indices of AzPhe incorporation efficiency into silk fibroin. All data are the average values of three independent experiments, where each experiment employed three male larvae. The error bars represent standard deviations.

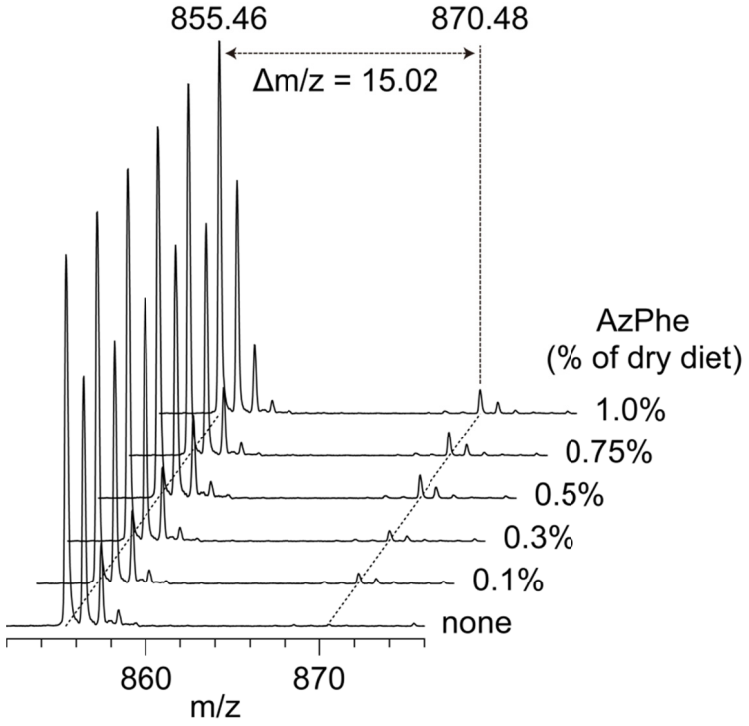

Figure S6. Detection of AzPhe incorporation in silk fibroin by MALDI-TOF-MS analysis of peptide fragments from in-gel digestion of FibL. H03 larvae were reared on a commercially-available standard diet containing varied amounts of AzPhe (0%, 0.1%, 0.3%, 0.5%, 0.75%, or 1.0% of dry diet). Enlarged views are given for the region around a peak assigned to the SGNFAGFR fragment ($[M+H]^+$ = 855.41 Da). The peak intensities at 855 Da were standardized in all spectra. The newly-emerged peaks were found at the +15 Da position (azide groups on AzPhe were reduced to amino groups during analysis).

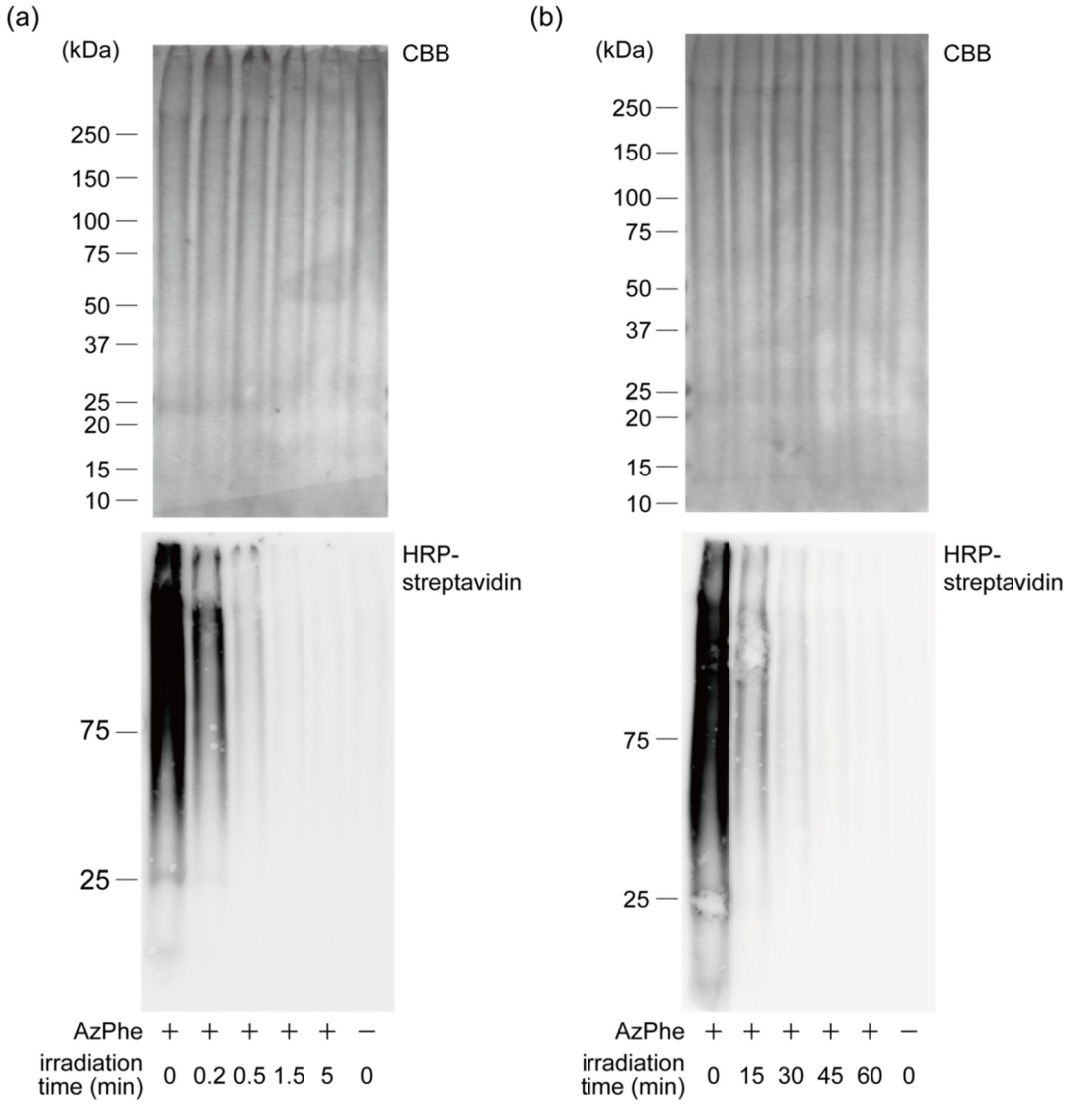

Figure S7. Photolysis of azide groups in *AzidoSilk* by UV irradiation. (a) *AzidoSilk* threads were directly irradiated with UV light (254 nm) for 0, 0.2, 0.5, 1.5, and 5 min. (b) *AzidoSilk* films were irradiated through glass substrate with UV light (352 nm) for 0, 15, 30, 45, and 60 min. The threads and films after irradiation were dissolved in LiBr solution and then reacted with biotin-PEG4-alkyne. Biotins attached to silk fibroin were detected with HRP-streptavidin by Western blotting. Normal threads and films not incorporated with AzPhe were used as negative controls.

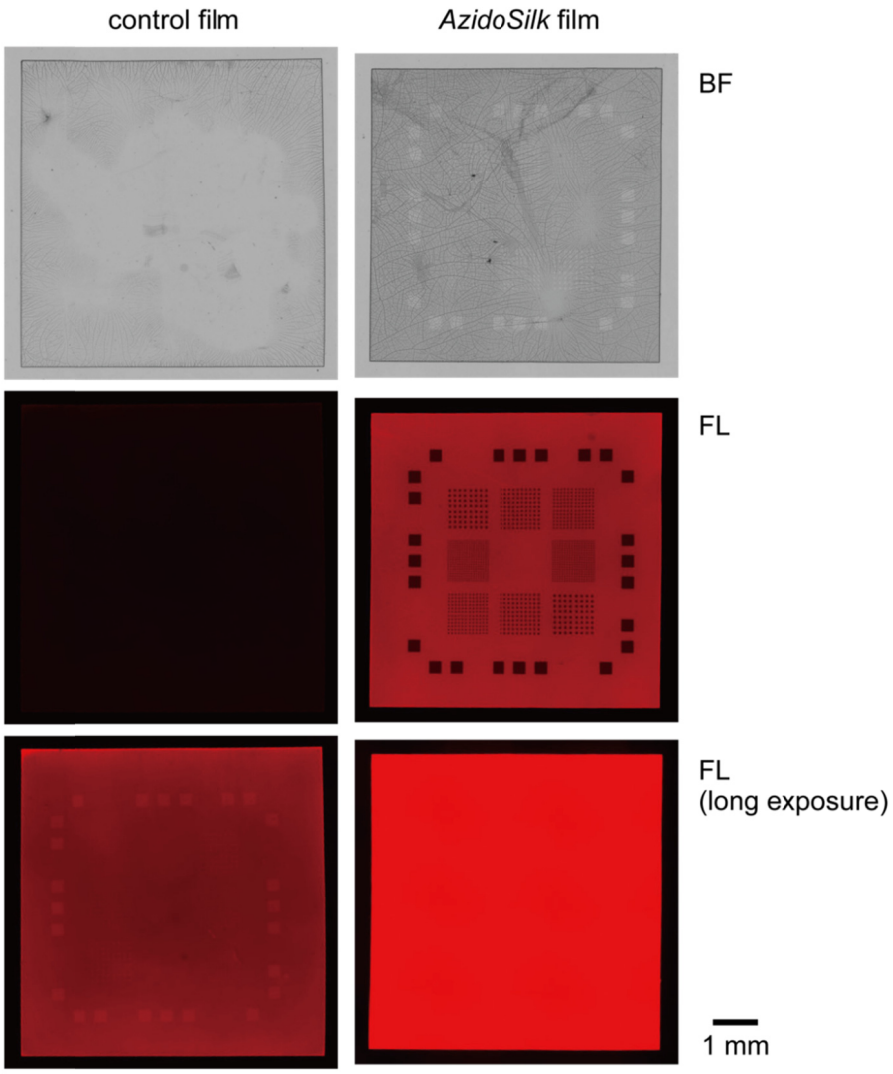

Figure S8. Photolithographic patterning of sulforhodamine B DBCO on control (left) and *AzidoSilk* (right) films. BF and FL respectively denote bright field and fluorescence images. Control and *AzidoSilk* films (7 × 7 mm^2) were irradiated with 352 nm UV light for 60 min through one of the glass photomasks shown in Figure 5. The irradiated films were clicked with sulforhodamine B DBCO followed by washing with DMSO and deionized water. A faint pattern was formed on the control film under weak background fluorescence, probably because nonspecific binding was slightly increased in the irradiated areas due to morphological or chemical changes.