Supplementary Data

Construction of a novel phagemid to produce custom DNA origami scaffolds

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Figure **Page No.**

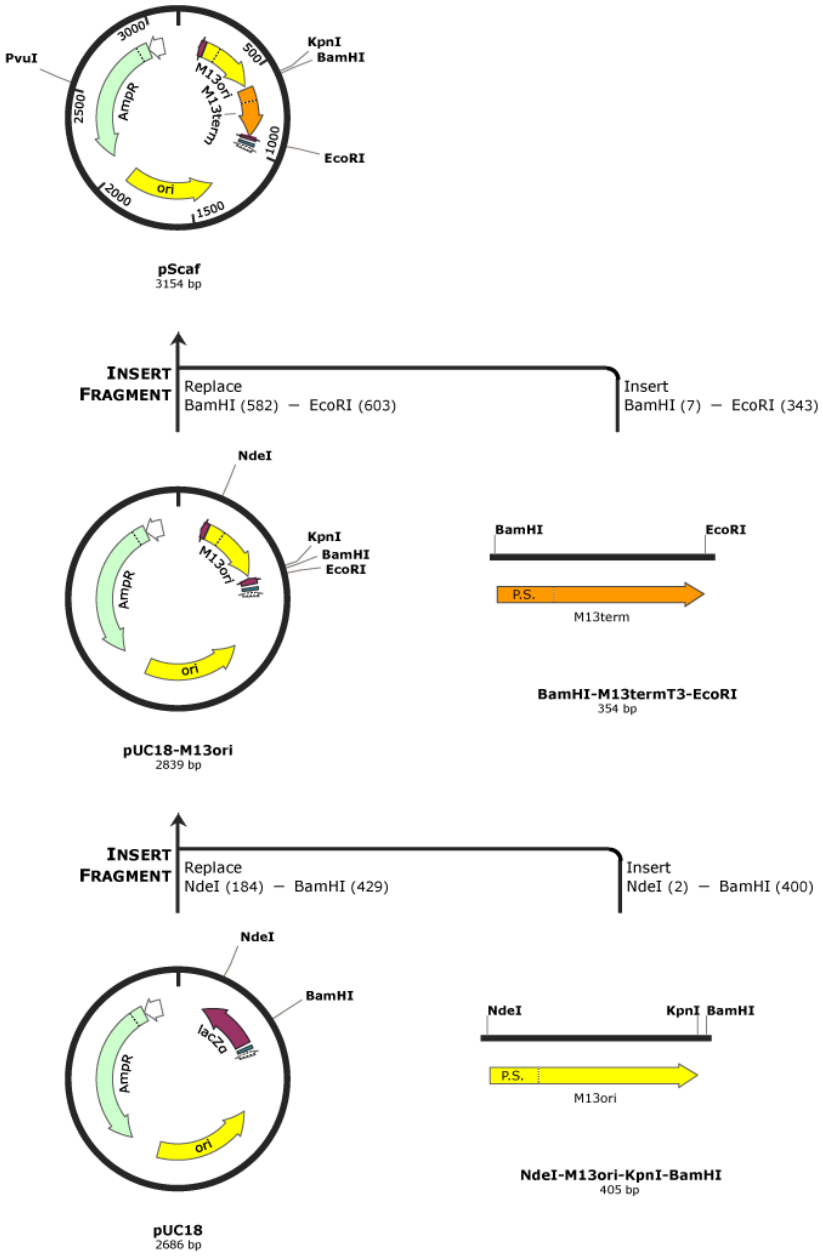

Fig. S1. pScaf construction. pUC18 was converted into pScaf by restriction cloning of a PCR-amplified *M13ori* insert flanked by 5' NdeI and 3' KpnI & BamHI, followed by restriction cloning of the packaging sequence (P.S.) and *M13 terminator,* flanked by BamHI and EcoRI. All terminator variants were similarly cloned into the BamHI-EcoRI region, and the final version named "pScaf" is the variant with three "T" substitutions in the ∂ region (See Fig. 2). Schematic generated with Snapgene.

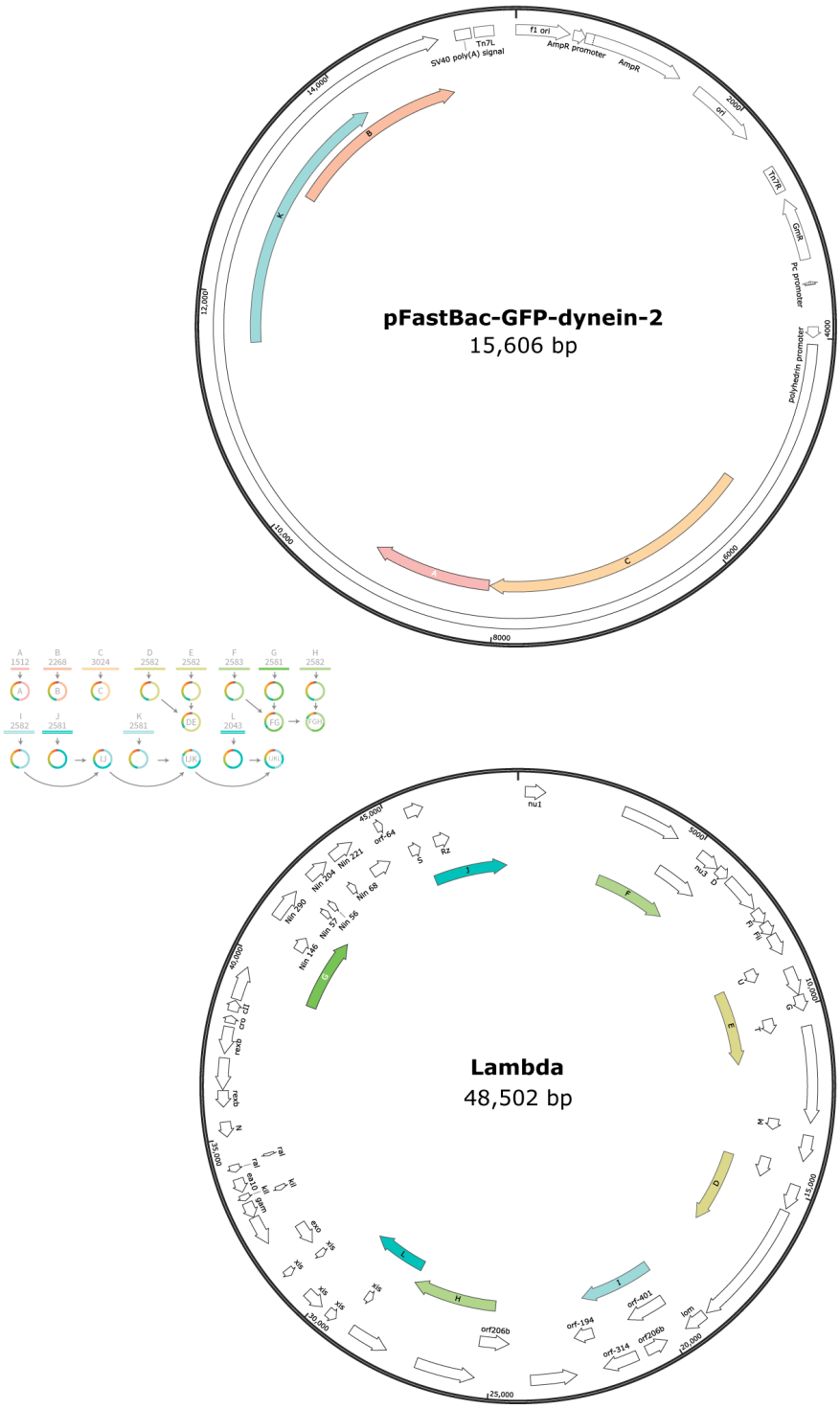

Fig. S2. Scaffold Insert Sources. Inserts A, B, C, and K were PCR-amplified from the plasmid pFastBac-GFP-dynein-2(D1091–Q4307). Inserts D, E, F, G, H, I, J, L were PCR-amplified from the Lambda phage genome. Only fragments B and K overlap by 1020 bases. Inserts highlighted in colors corresponding to Fig. 3B (inset). Plasmid feature annotations shown in white.

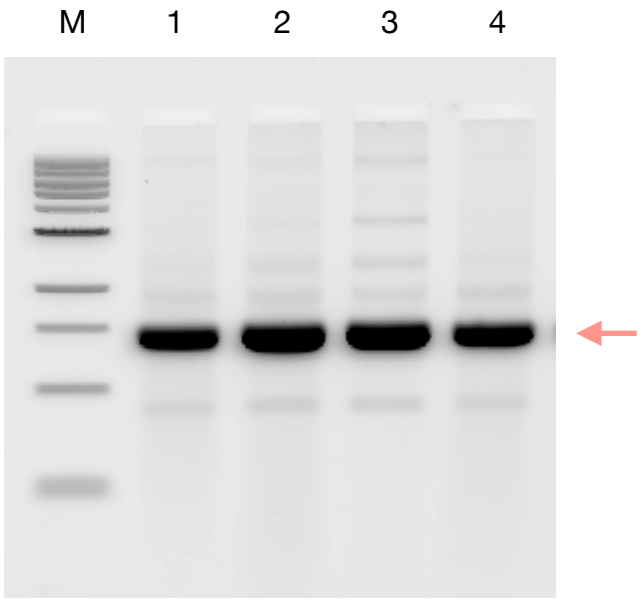

Fig. S3. Representatitive Scaffold Prep Variation. Agarose gel electrophoresis of 1kb ladder (M) and four 5544-base-long ssDNA scaffolds from separate *E.coli* transformants (lanes 1–4). Scaffolds and gel were prepared as described in Materials and Methods section. Primary ssDNA scaffold bands (red arrow) range from 70 to 75% of total integrated intensity of their respective sample lane. The preps vary in the amount of background smearing and intensity of extra (off-target) bands appear in lanes 1–4. We observed similar prep-to-prep variation for many different scaffolds.

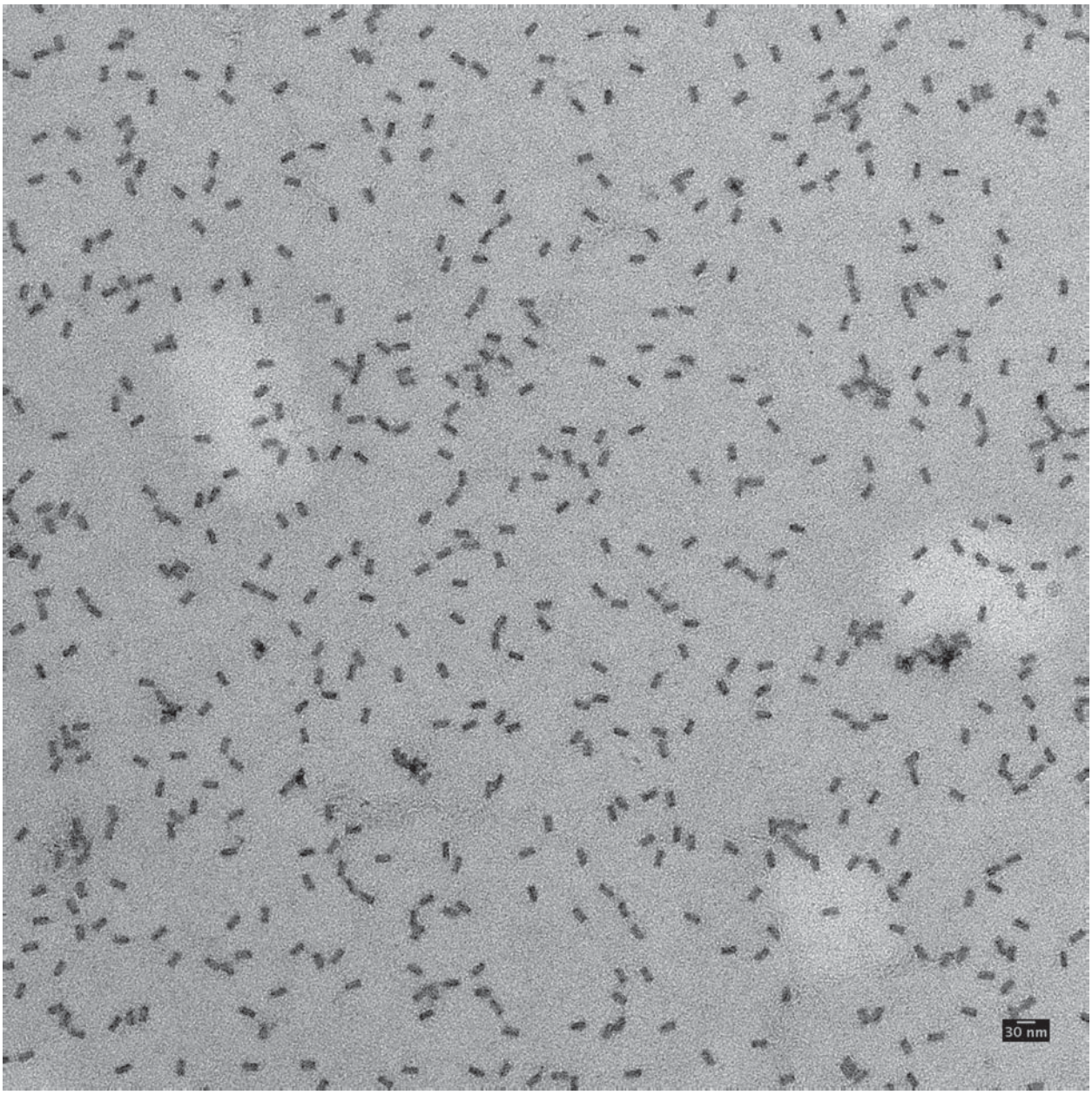

Fig. S4. A-Brick-1512. Transmission Electron Micrograph. Scale bar: 30 nm.

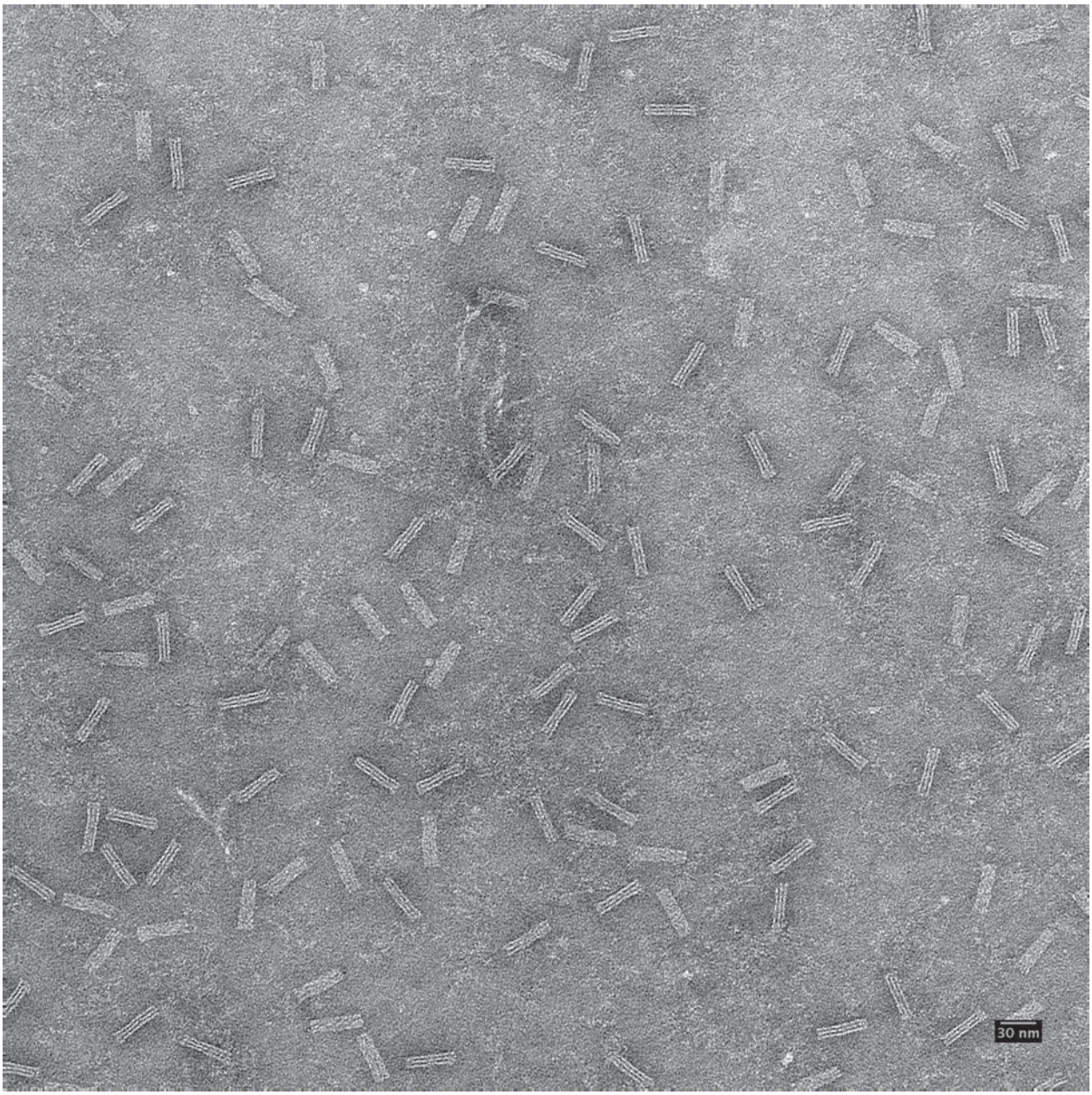

Fig. S5. B-Brick-2268. Transmission Electron Micrograph. Scale bar: 30 nm.

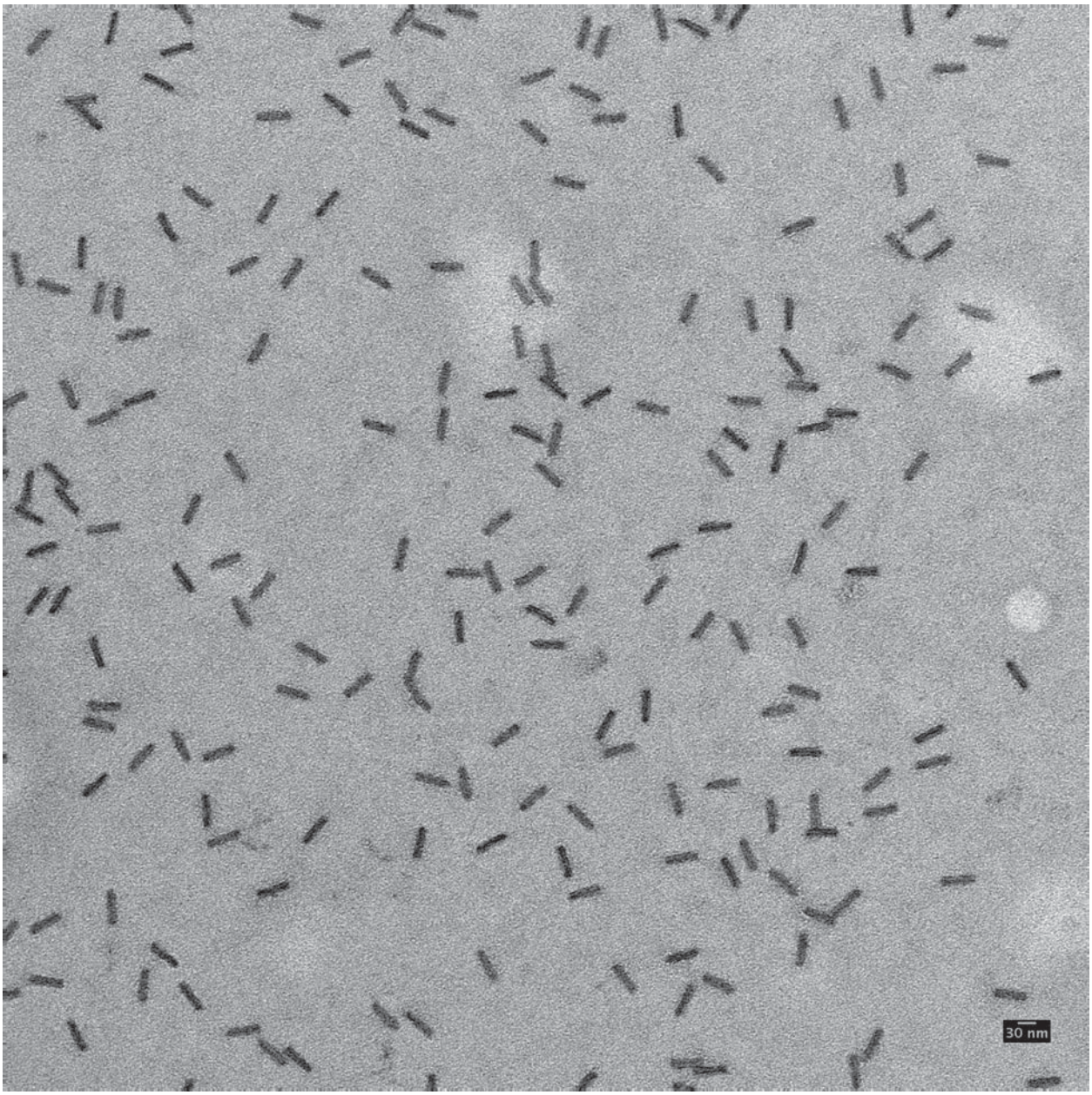

Fig. S6. C-Brick-3024. Transmission Electron Micrograph. Scale bar: 30 nm.

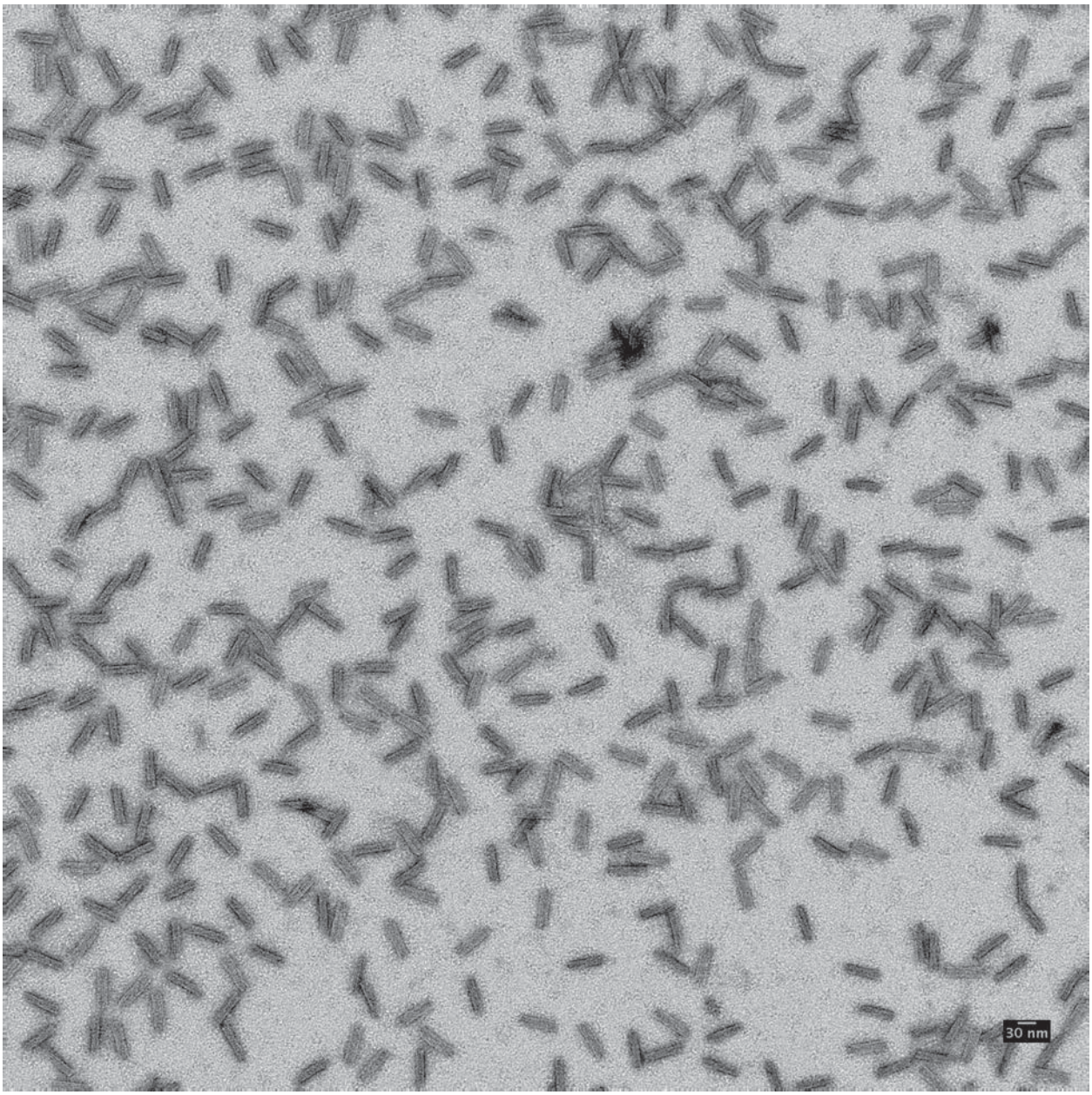

Fig. S7. DE-Brick-5544. Transmission Electron Micrograph. Scale bar: 30 nm.

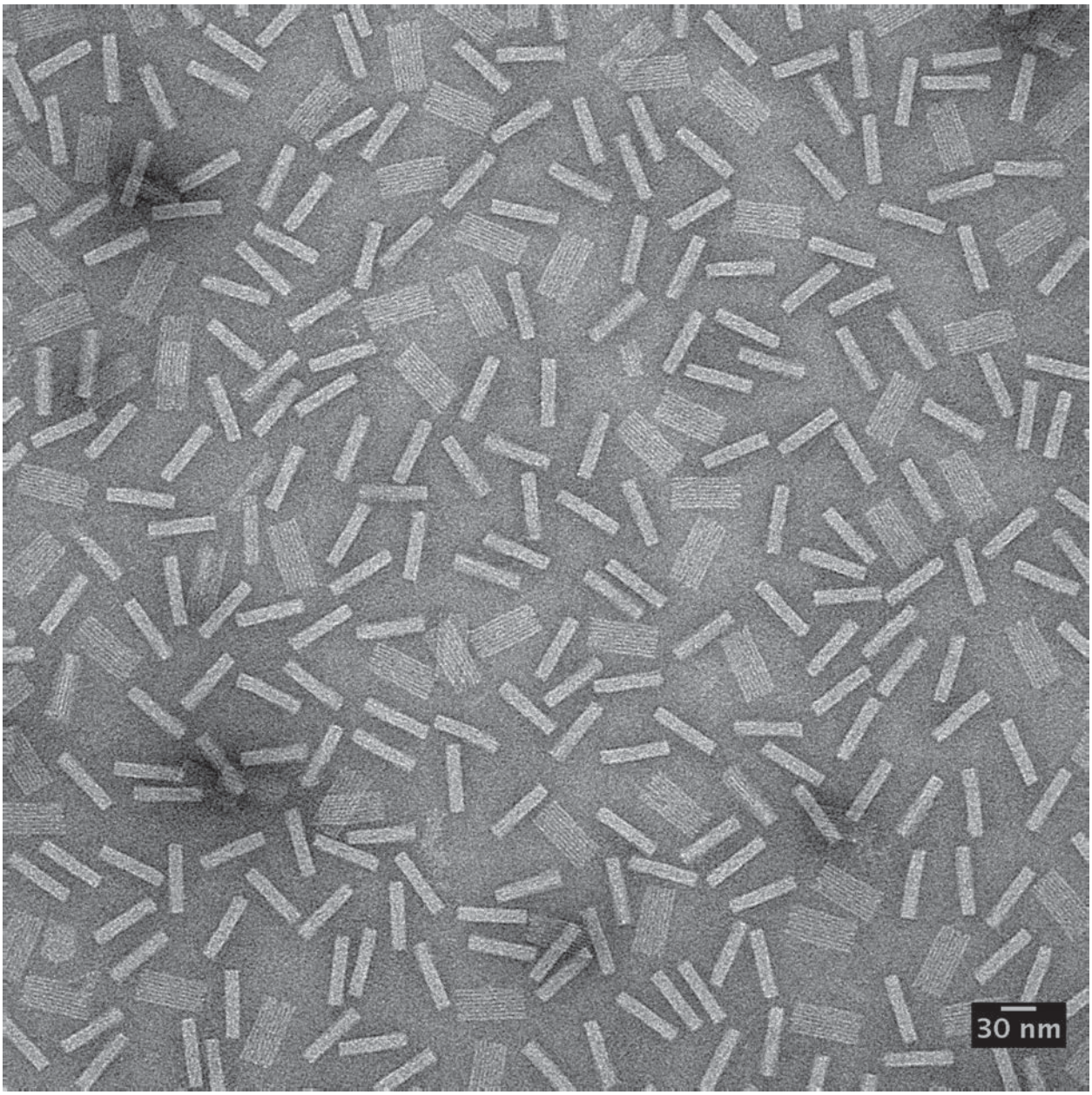

Fig. S8. FGH-Brick-8064. Transmission Electron Micrograph. Scale bar: 30 nm.

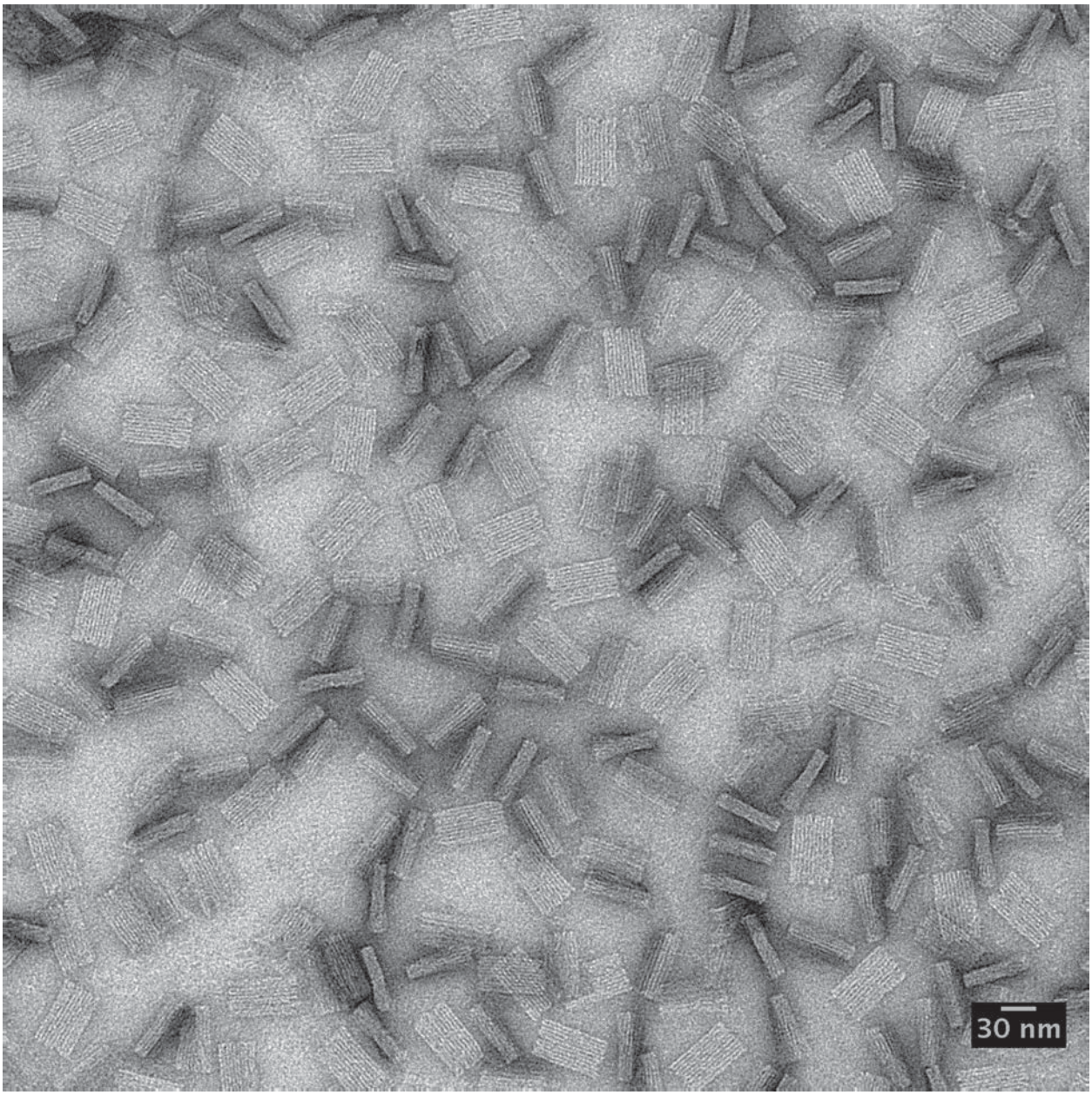

Fig. S9. IJKL-Brick-10080. Transmission Electron Micrograph. Scale bar: 30 nm.

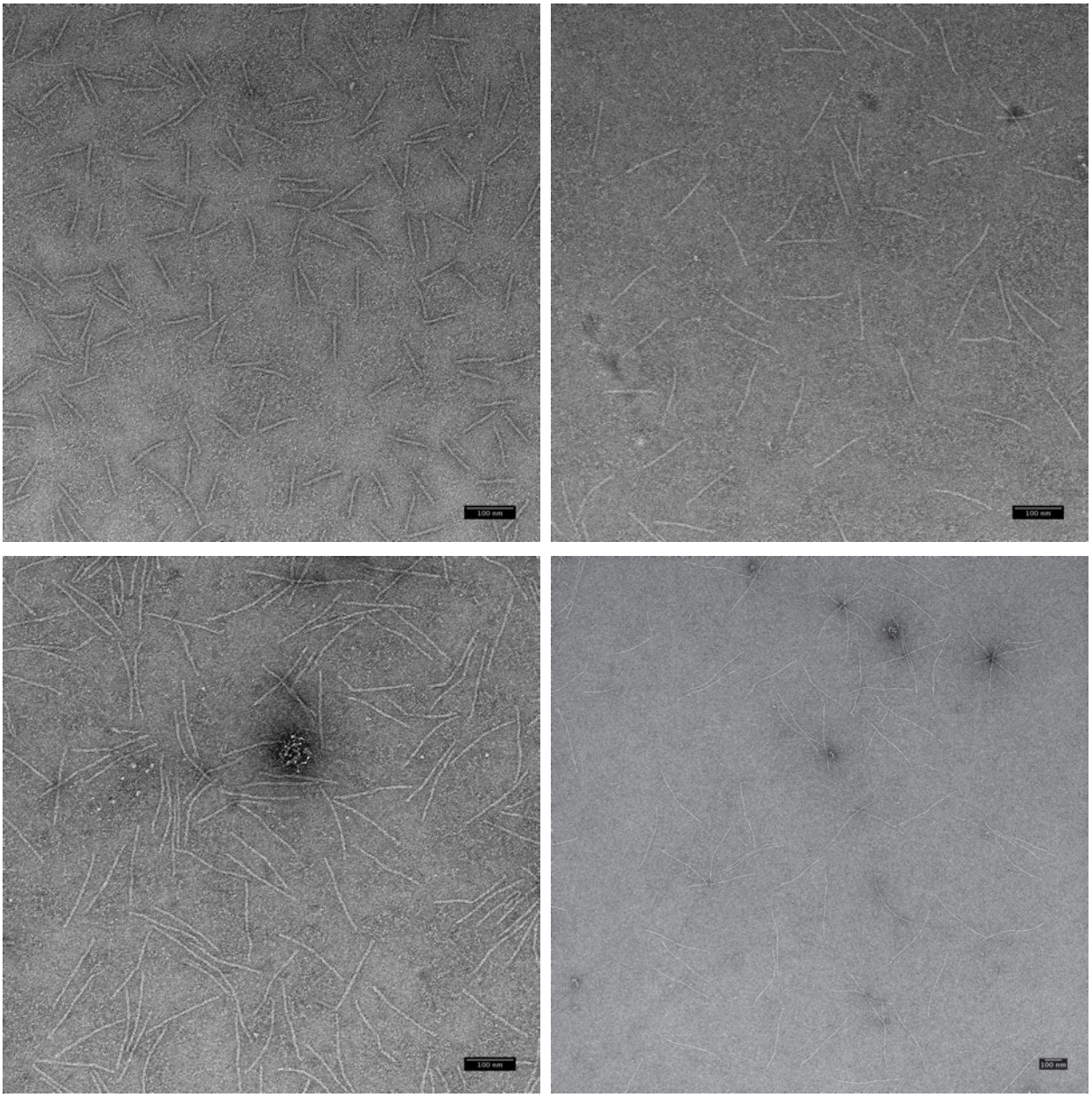

Fig. S10. 6hb monomers and trimer. Transmission Electron Micrographs.

Top left: 6hb-monomer-1512. Scale bar: 100 nm.
Top right: 6hb-monomer-2268. Scale bar: 100 nm.
Bot left: 6hb-monomer-3024. Scale bar: 100 nm.
Bot right: 6hb-trimer (1512+2268+3024). Scale bar: 100 nm.