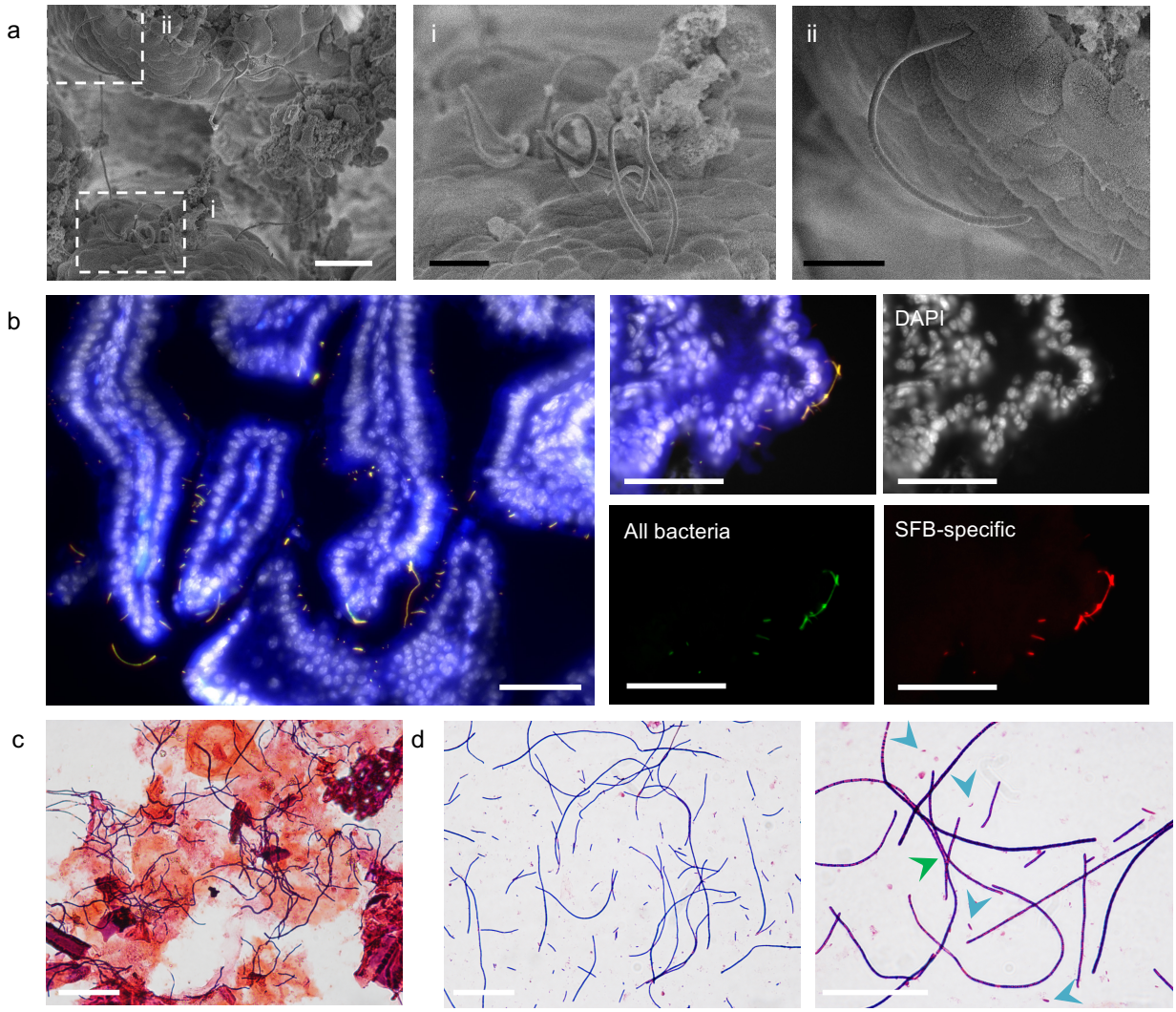
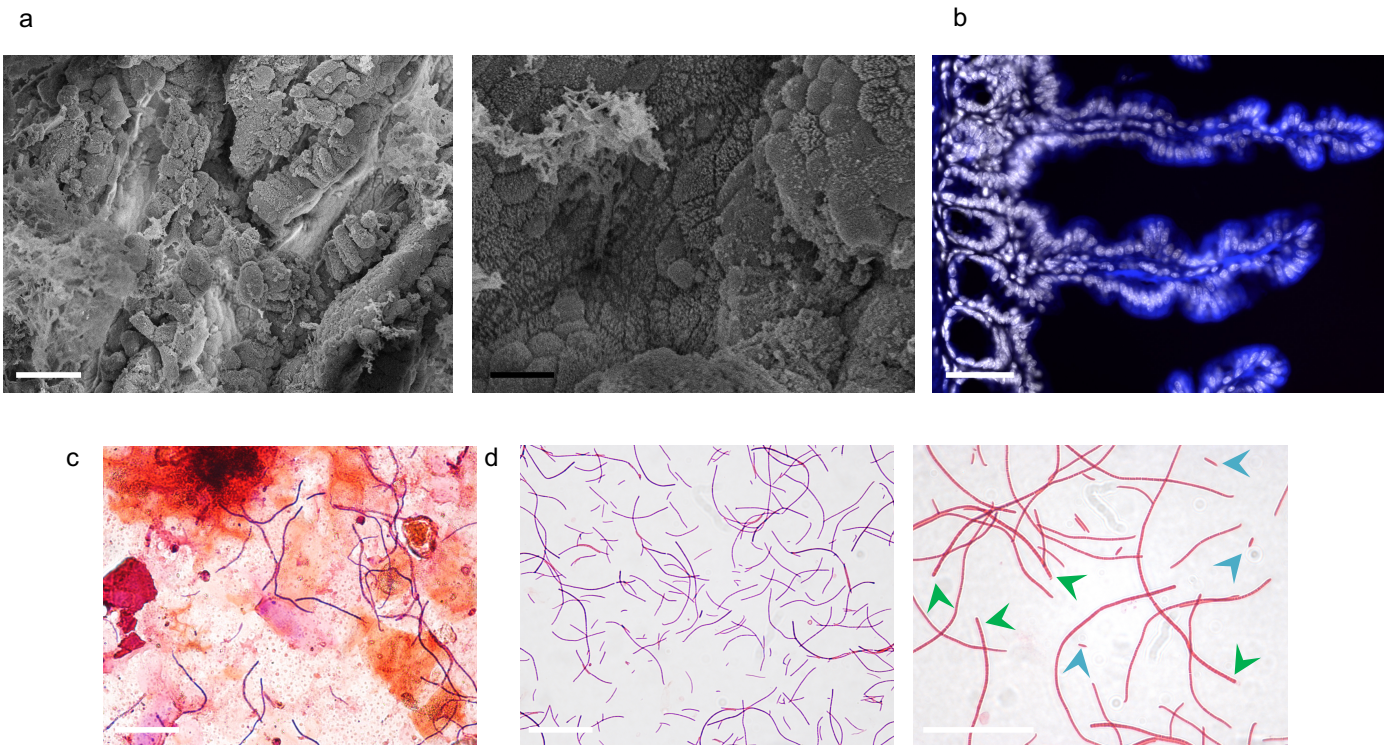
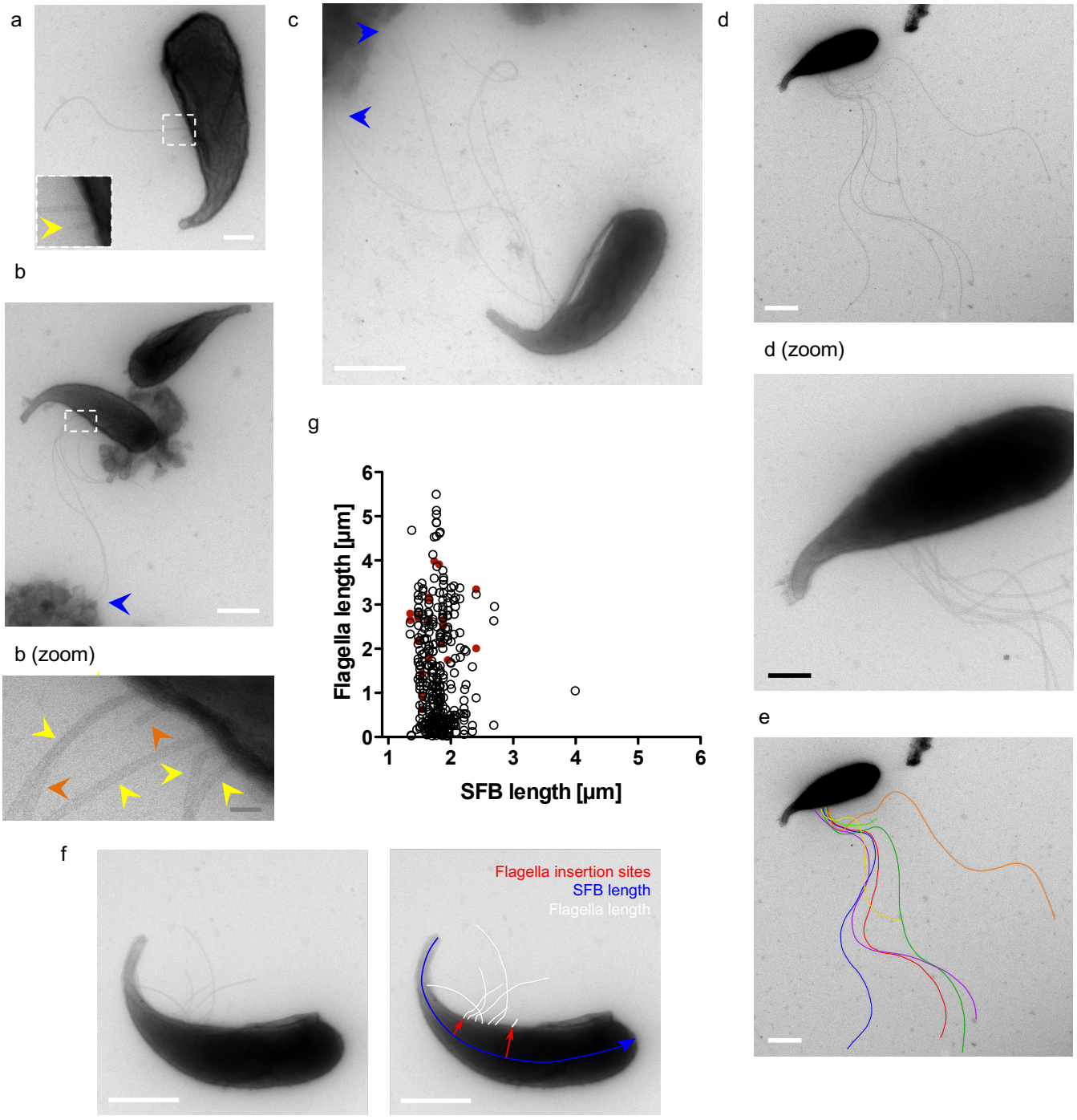
Intracellular offsprings released from SFB filaments are flagellated



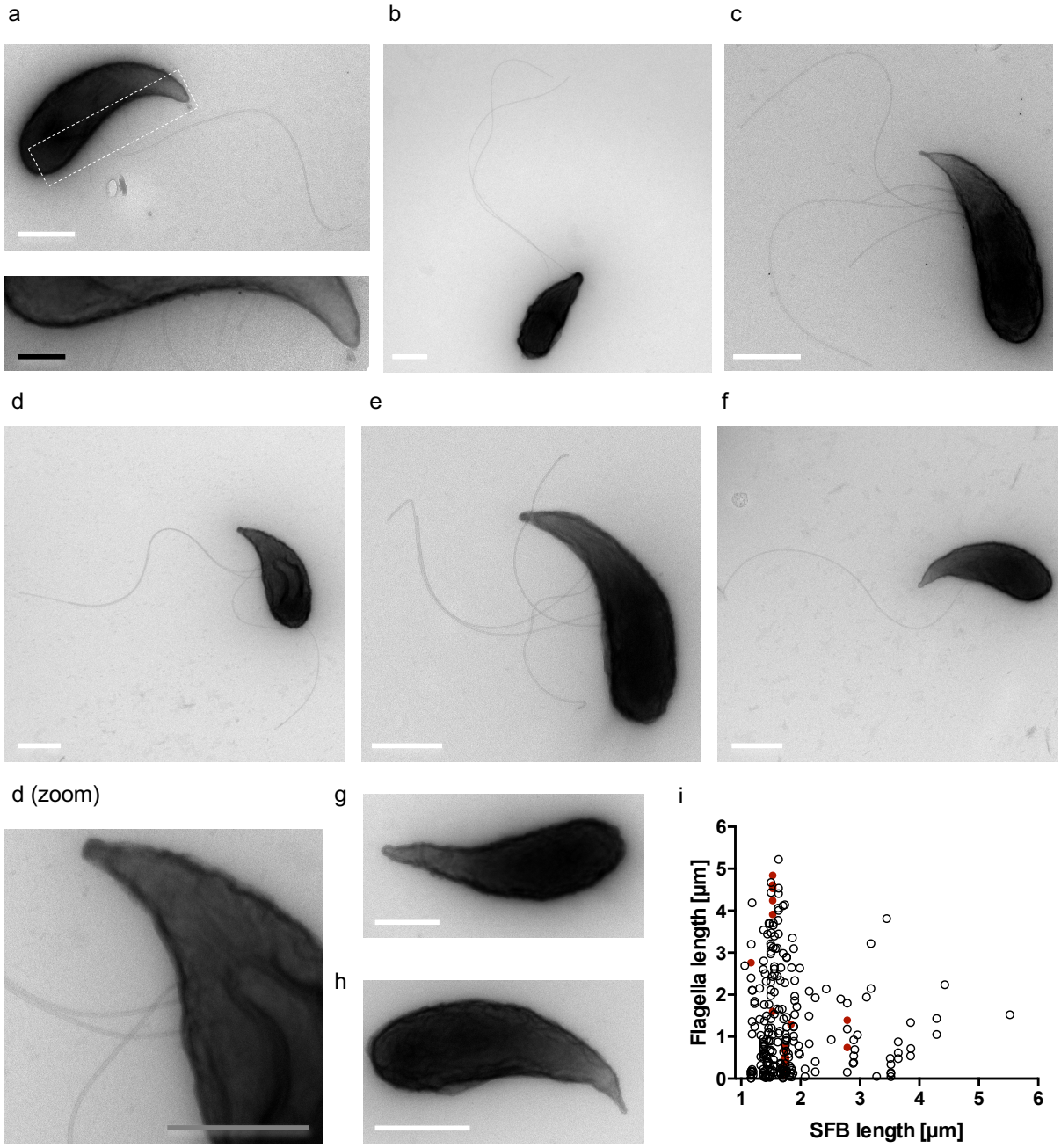
Supplementary Figure 1: Mouse SFB monocolonization, purification and analysis. a. Scanning EM and (**b**) fluorescence *in situ* hybridization, using SFB-specific and all bacterial 16S probes (autofluorescence of tissue in blue), of the terminal ileum of SFB-monocolonized mice. **c/d.** Gram stain of (c) fecal content and (d) after nycodenz purification. Green arrows highlight differentiating filaments; blue arrows highlight IOs. (a-d) Representative images from two (a), three (b) and four (c/d) independent experiments with similar results and n=14, n=11, n=8 and n=8 images taken, respectively. Scale bars: black: 10 μm; white: 25 μm.



Supplementary Figure 2: Rat SFB monocolonization, purification and analysis. a. Scanning EM and (**b**) fluorescence *in situ* hybridization, using SFB-specific and all bacterial 16S probes (autofluorescence of tissue in blue), of the terminal ileum of SFB-monocolonized mice. Scale bar: 10 μm. **c/d.** Gram stain of (c) intestinal content and (d) after nycodenz purification. Green arrows highlight differentiating filaments; blue arrows highlight IOs. (a-d) Representative images from two (a) and three (b-d) independent experiments with similar results and n=10, n=12, n=8 and n=8 images taken, respectively. Scale bars: black: 10 μm; white: 25 μm.

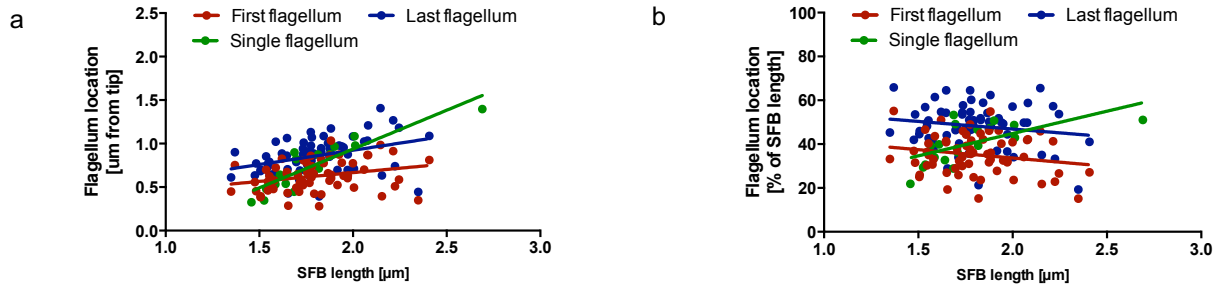


Supplementary Figure 3: TEM of mouse SFB isolated from monocolonized mice. a-e. Additional negative contrast stain TEM images of *in vivo*-grown mSFB with flagella. Yellow arrows highlight flagella; orange arrows highlight broken flagella; blue arrows highlight obstructed flagella. **e.** Image (d) with colored tracing of flagella for clarity. **f.** Schematic representation of the analysis of SFB for SFB length and flagella length and location. **g.** Analysis of flagella length compared to SFB length. Red circles denote obstructed flagella preventing full measurement. Scale bars: white: 500 nm; black 200 nm; grey: 50 nm. Representative mSFB TEM images are from four independent *in vivo* experiments with similar results (n=320, including 91 flagellated SFB).

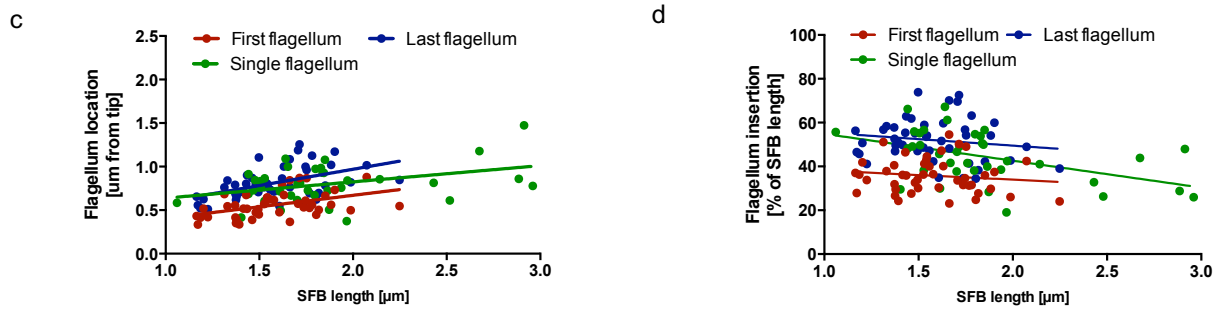


Supplementary Figure 4: TEM of rat SFB isolated from monocolonized mice. a-h. Additional TEM images of *in vivo*-grown rSFB with (a-f) and without (g/h) flagella. Representative rSFB TEM images are from three independent *in vivo* experiments with similar results (n=468, including 108 flagellated SFB). **i.** Analysis of flagella length compared to SFB length. Red circles denote obstructed flagella preventing full measurement. Scale bars: white: 500 nm; black or grey: 200 nm.

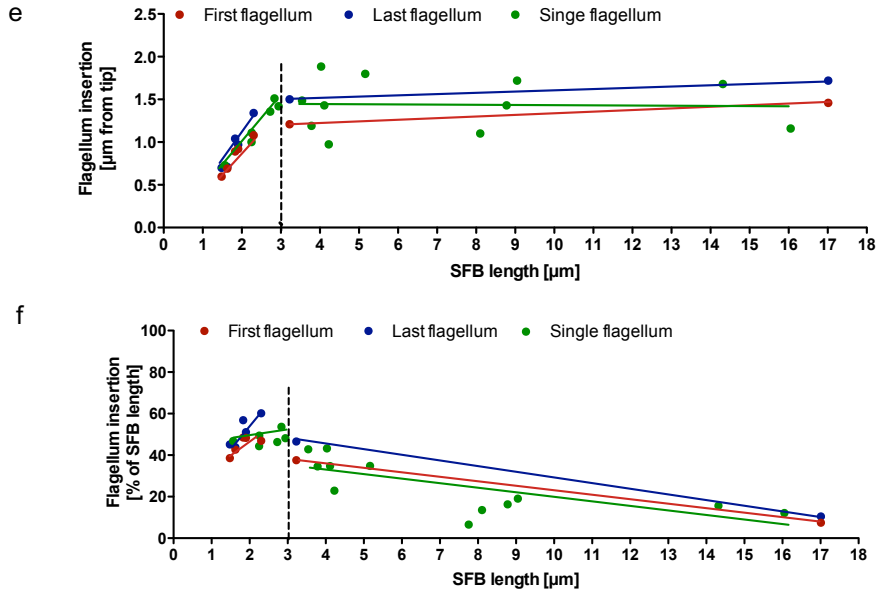
mSFB grown *in vivo*



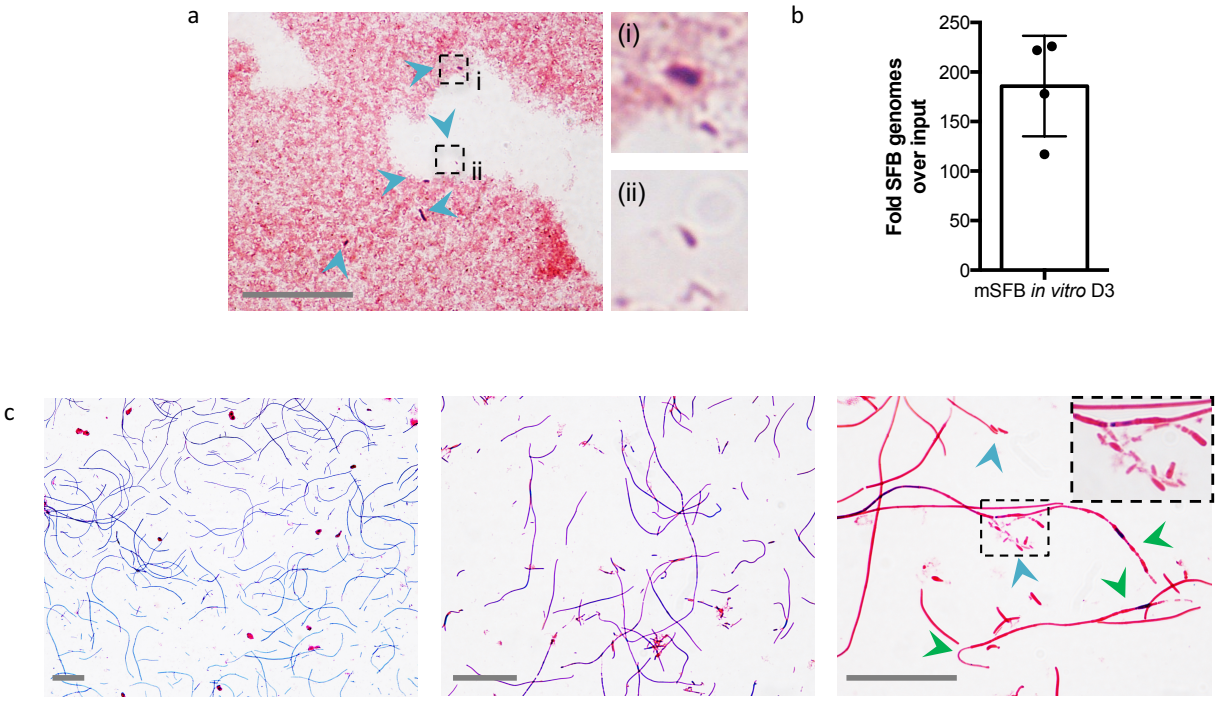
rSFB grown *in vivo*



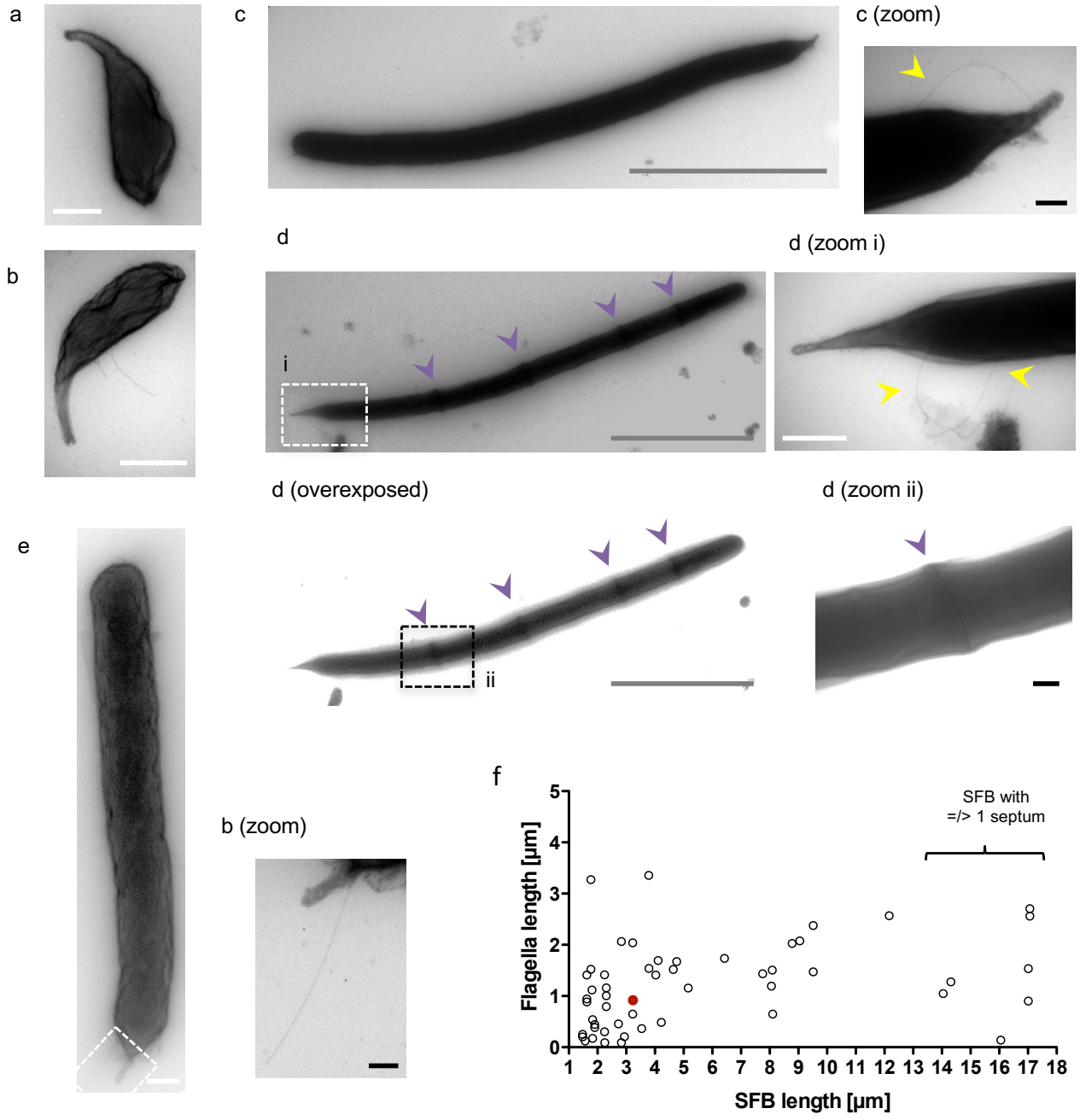
mSFB grown *in vitro*



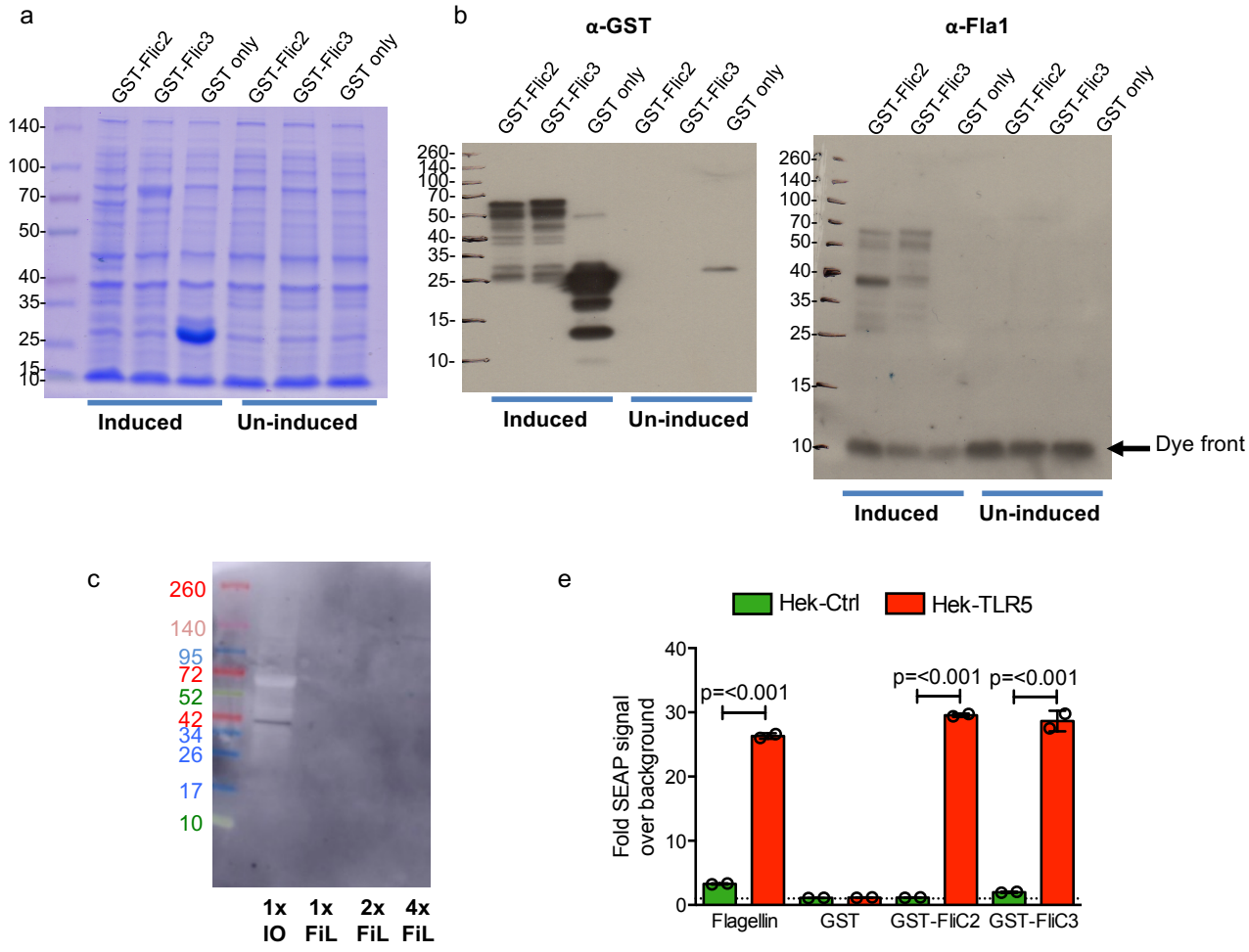
Supplementary Figure 5: Analysis of SFB flagella location. a-f. Distance of the single or first and last flagellum in terms of µm (a/c/e) and % (b/d/f) from the tip of *in vivo*-grown mSFB (a/b) or rSFB (c/d), and *in vitro*-grown mSFB (e/f). Linear regression curves are included for SFB from 1 to 3 µm (a-f) and SFB from 3 to 17 µm (e/f). Regression curves of (e) and (f) are shown as trend lines based on separate regression curve analysis for SFB up to and greater than 3 µm in length. For SFB of 1 to 3 µm, 82 bacteria were analyzed for *in vivo*-grown mSFB and rSFB and 11 bacteria were analyzed for *in vitro*-grown mSFB. An additional 14 bacteria between 3 to 17 µm in length were analyzed for *in vitro*-grown mSFB.



Supplementary Figure 6: Mouse SFB *in vitro* growth. a. Gram stain of purified IOs from monocolonized mice used as input for *in vitro* growth. **b.** qPCR analysis of the fold increase in SFB genome number per transwell over input after three days of *in vitro* growth including mean with standard deviation. Values from one of three independent experiments with similar results. **c.** Purified SFB after 3 days of *in vitro* growth. Blue arrows highlight IOs; green arrows highlight differentiating filaments. Representative images (a/c, n=8 and 10, respectively) from four independent experiments with similar results. Scale bars: (a/c) 25 μm.



Supplementary Figure 7: TEM of mouse SFB after 3 days of *in vitro* growth. a-e. Additional TEM images of *in vitro*-grown mSFB without (a) and with (b-e) flagella. Yellow arrows highlight flagella; purple arrows highlight septa. Representative mSFB TEM images are from four independent *in vitro* experiments with similar results (n=278, including 38 flagellated SFB). **f.** Analysis of flagella length compared to SFB length. Red circle denotes obstructed flagellum preventing full measurement. Scale bars: white: 500 nm; black: 200 nm; grey: 5 µm.

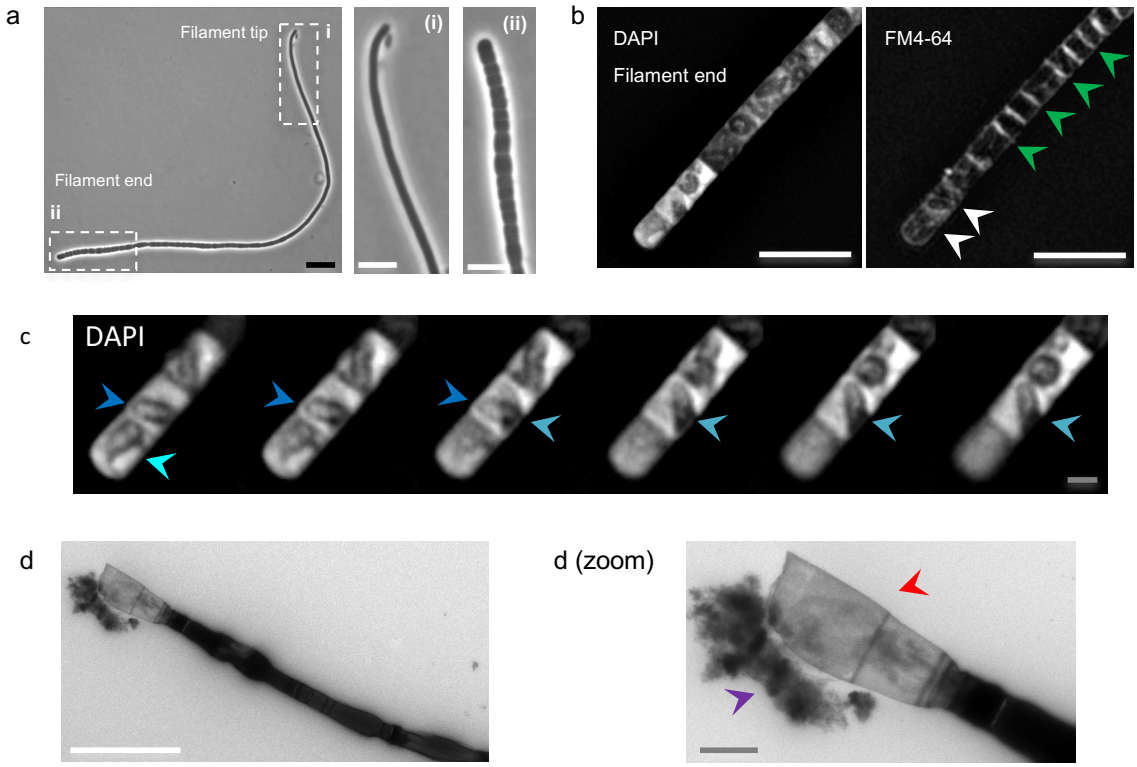


d

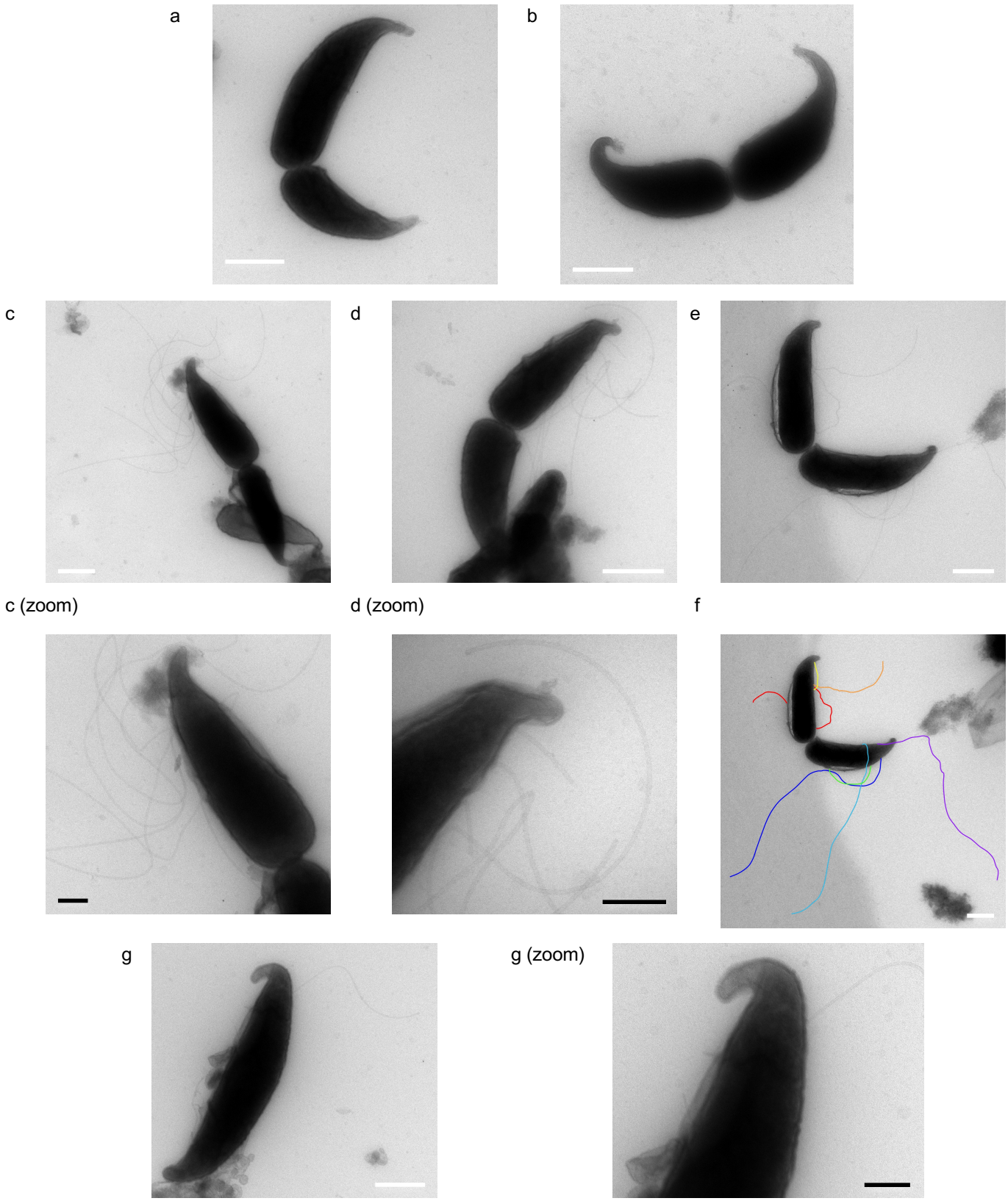
FliC3 peptides:
ELGLGGVNVSTAEDAK
ELINQEFNQLK
LNVLQQASQSMLAQANQAPQQVLSILR
NAQDGISMIZTAEGALSETZQIGQR

FliC4 peptide:
LNVLQQASQAMLAQANQAPQQVLSLLK

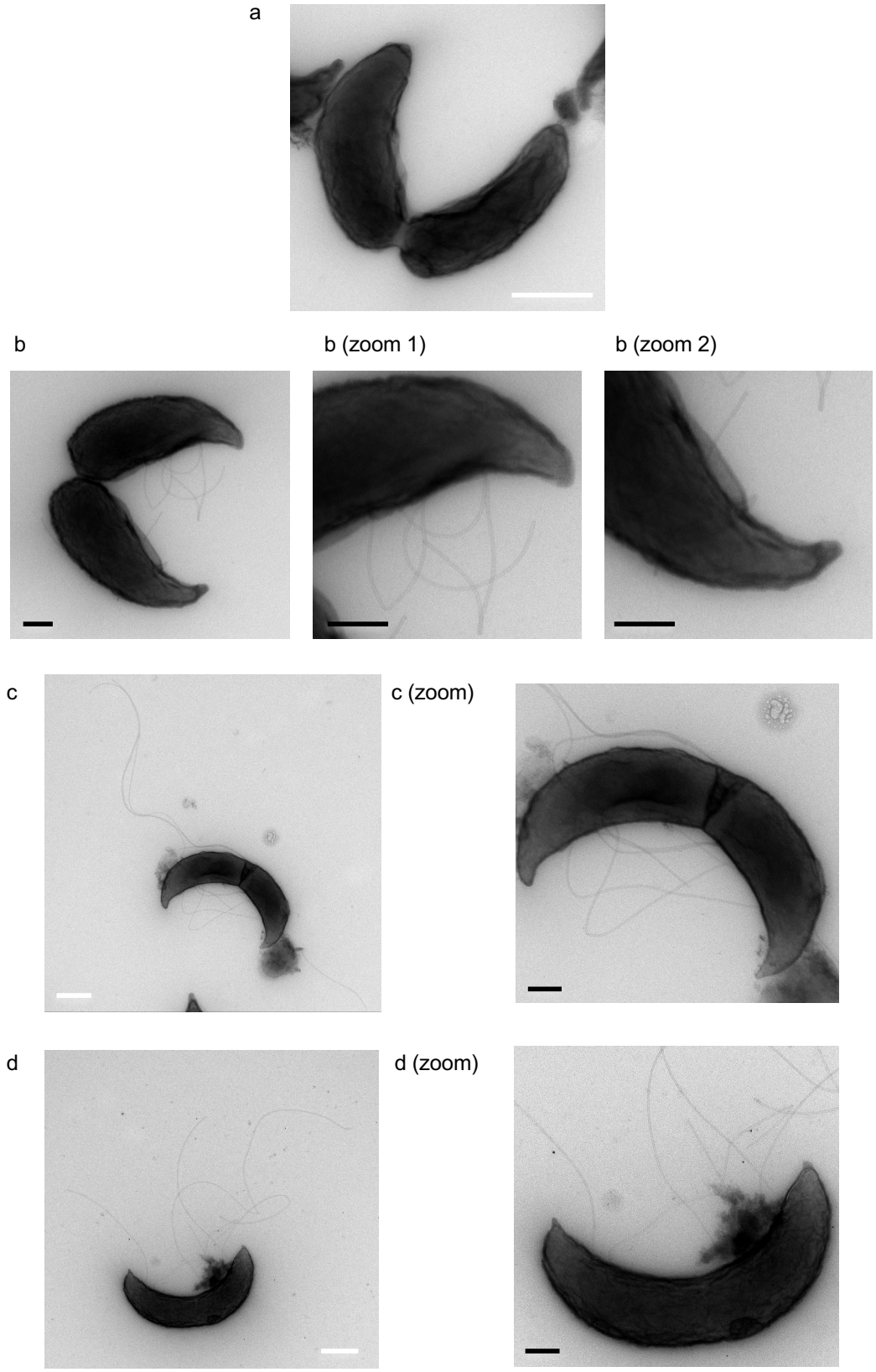
Supplementary Figure 8: SFB flagellin detection by Western blot and TLR5 reporter cell lines. a/b. Coomassie-stained SDS-page gel (a) and Western blot with anti-GST or anti-Fla1 antibody (b) of lysate from IPTG-induced and un-induced *E. coli* recombinantly expressing GST or GST translational fusions with SFB FliC2 or FliC3. **c.** Full length gel of anti-flagellin western blot of IO-only and filament-enriched (FIL) mSFB fractions from Figure 2e. "x" denotes fold increase in SFB numbers. (a-c) Representative images of two independent experiments with similar results. **d.** FliC peptides identified by mass spectrometry on the surface of mSFB from the IO-only fraction in two independent experiments with similar results. **e.** TLR5 stimulation by lysate from IPTG-induced *E. coli* recombinantly expressing GST alone or GST fused to SFB FliC2 or FliC3. Representative data of two independent experiments with similar results. Mean with standard deviation and two-sided t-test statistical analysis.



Supplementary Figure 9: Stages of the SFB developmental life-cycle. a. Bright field image of an *in vitro*-grown mSFB filament (n=5). **b.** Maximum intensity z-projection from a structured illumination image of an *in vivo*-grown mSFB filament end stained with DAPI and the membrane dye FM4-64FX (n=4). Green arrows highlight distinct septa dividing filament segments; white arrows highlight the absence of distinct septa staining. **c.** Montage of optical slices from DAPI-stained filament end of (b). Blue arrows highlight location of individual IOs within the filament. **d.** TEM image (n=3) of an *in vitro*-grown mSFB filament with an empty filament remnant (red arrow), and debris (purple arrow). Representative images from three (a) and two (b/d) independent experiments with similar results. Scale bars: black: 10 μm; white: 5 μm; grey: 1 μm.



Supplementary Figure 10: Additional TEM images of unseparated IOs from mouse SFB purified from monocolonized mice. a-g. TEM images of *in vivo*-grown mSFB without (a/b) and with (c-g) flagella during IO development. Representative images from two independent experiments with similar results (n=13 non-separated IOs, including 8 with flagellation). (f) is a zoomed-out image of (e) with colored tracing of flagella. Scale bars: white: 500 nm; black: 200 nm.



Supplementary Figure 11: TEM images of unseparated IOs from rat SFB purified from monocolonized mice. a-d. TEM images of *in vivo*-grown rSFB without (a) and with (b-d) flagella during IO development. Representative images from three independent experiments with similar results (n=8 non-separated IOs, including 4 with flagellation). Scale bars: white: 500 nm; black: 200 μm.

Supplementary Table 1: Linear regression curve analysis of SFB flagella location. Linear regression curve analysis with p-values and R-squared values for the slope's deviation from zero of the flagella insertion locations of *in vivo* and *in vitro*-grown SFB of mouse and rat origin, as shown in Supplementary Figure 5. Flagella insertion locations were calculated in terms of μm and % of the total SFB length, starting from the pointed tip of the bacterium. For *in vivo*-grown SFB, only SFB of 1-3 μm were analyzed, while for *in vitro*-grown mSFB, SFB of 1 to 3 μm and 3 to 17 μm were analyzed separately.

Distance from tip	**SFB origin, growth condition and length of SFB subset analyzed**	**p-value of regression curve** (# of images analyzed; R-squared value)		
		First flagellum	**Last flagellum**	**Single flagellum**
in μm	mSFB *in vivo* 1-3 μm	0,0286 (64; 0,075)	0,0023 (64; 0,140)	<0,0001 (18; 0,803)
	rSFB *in vivo* 1-3 μm	0,0018 (45; 0,206)	0,0006 (45; 0,242)	0,0175 (37; 0,151)
in %	mSFB *in vivo* 1-3 μm	0,1195 (64; 0,015)	0,1993 (64; 0,003)	0,0056 (18; 0,341)
	rSFB *in vivo* 1-3 μm	0,3901 (45; 0,003)	0,3268 (45; 0,019)	0,0035 (37; 0,203)
in μm	mSFB *in vitro* 1-3 μm	0,0049 (5; 0,949)	0,0060 (5; 0,942)	0,0008 (6; 0,953)
	mSFB *in vitro* 3-17 μm	N/A (2)	N/A (2)	0,8731 (14; 0,002)
in %	mSFB *in vitro* 1-3 μm	0,1707 (5; 0,518)	0,0495 (5; 0,773)	0,4274 (6; 0,163)
	mSFB *in vitro* 3-17 μm	N/A (2)	N/A (2)	0,0076 (14; 0,526)

Supplementary Data: TEM Analysis Raw Data and Summary. File 1. Summary of all TEM experiments performed and grouped per sample origin. Data analysis includes the number and percent of flagellated SFB imaged for bacteria of 1 to 2 μm and 1 to 4 μm in length. **Files 2-4.** The raw data of SFB length, flagella number and flagella length for *in vivo*-grown mSFB (File 2) and rSFB *(*File 3) and *in vitro*-grown mSFB (File 4). The *in vitro*-grown mSFB condition also includes the number of septa observed for flagellated SFB. **Files 5-7.** The subset of flagellated SFB used in the flagella insertion site analysis for *in vitro*-grown mSFB (File 5) and rSFB *(*File 6) and *in vitro*-grown mSFB (File 7), including insertion site measurements.

Supplementary Data: Raw Figure Data. Files containing the data graphed in Figures 1k, 1l, 2d, 2f, 2k and Supplementary Figures 3g, 4i, 5a-f, 6b, 7f and 8e using Prism® 5 (GraphPad) software. Files for figures with representative experiments (Figure 2k and Supplementary Figures 6b and 8e) also include data from other replicate experiments.