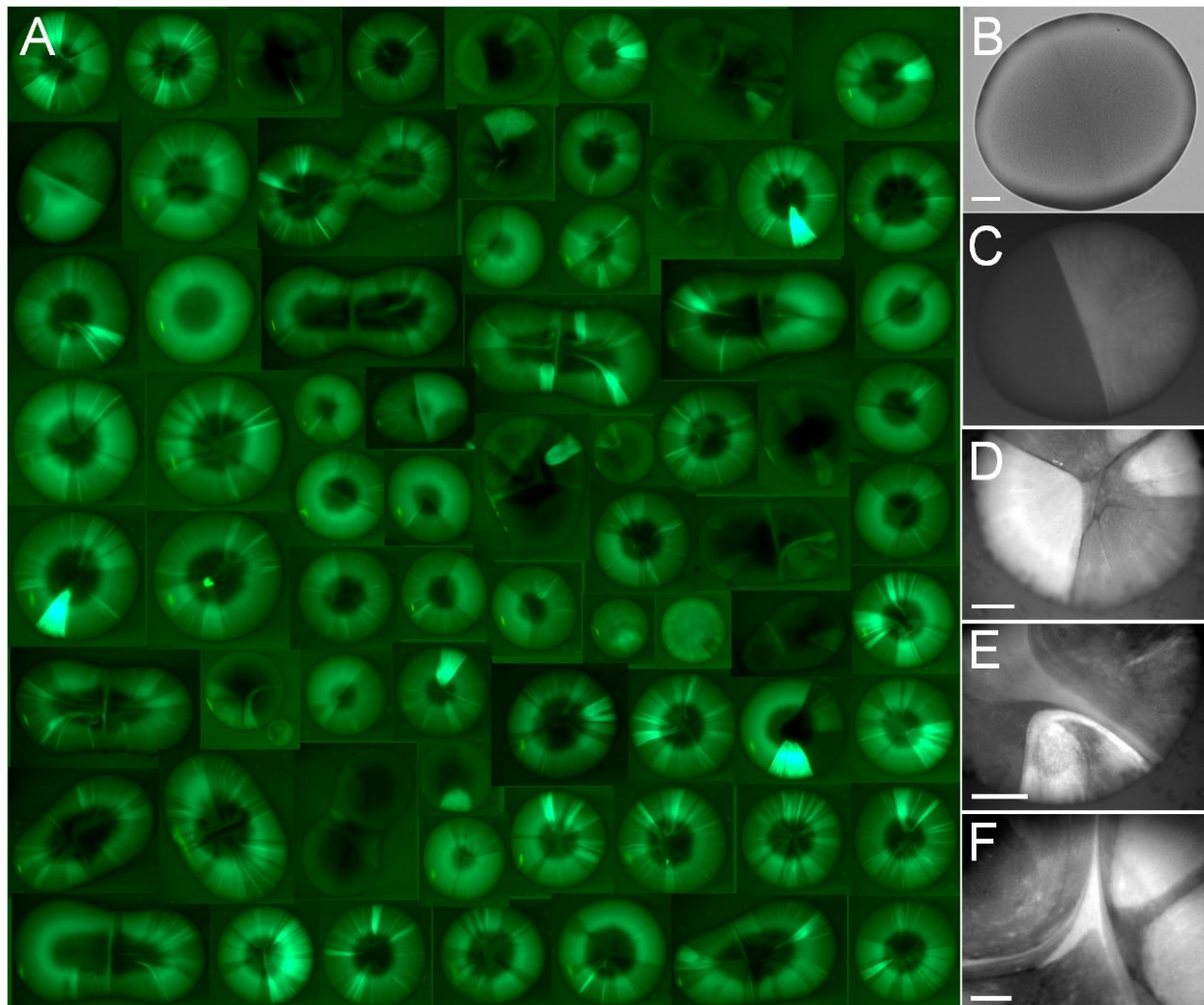


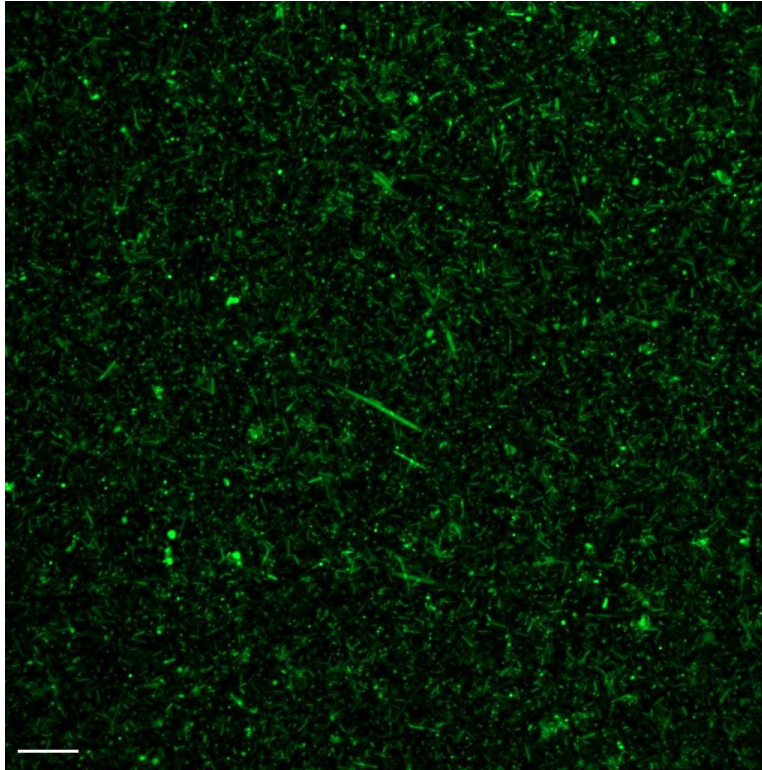
## Additional file 1: Supplemental figures and tables

Figures S1-S3

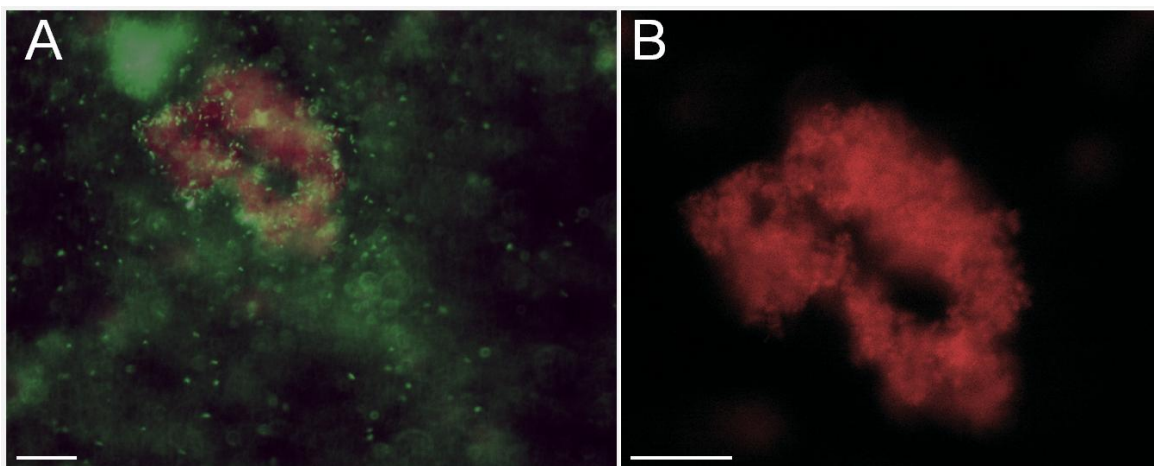
Tables S1-S3



**Figure S1 Colonies formed by *Hfx. volcanii* H1206(pSC409GFP).** Patterns shown were present after repeated subculture. (A) Randomly selected population of seventy-eight colonies on selective medium (Hv-Ca without uracil) imaged with a fluorescence dissecting microscope. (B-F) Colonies like those in (A) imaged at higher magnifications under transmitted light (B) and with blue excitation (C-F). Scale bars for (B-E) equal 100  $\mu$ m and 25  $\mu$ m for (F).



**Figure S2** Projection for a Z-stack of the first 10  $\mu\text{m}$  (or basal-layer) of a replicate 7-day *Hfx. volcanii* H53/H98 mixed SL-biofilm grown in a chamber slide and stained with SYTO 9 (imaged at the time cells were plated for recombination experiment shown in Figure 5). Scale bar equals 20  $\mu\text{m}$ .



**Figure S3** 7-day *Hfx. volcanii* H1206(pJAM1020) SL-biofilm stained with Congo red. **(A)** Overlay of images taken under blue excitation for GFP signal and green excitation for Congo red fluorescence. **(B)** Higher magnification view from **(A)** of mature cluster displaying Congo red fluorescence. Scale bars equal 20  $\mu\text{m}$ .

**Table S1 Cellular/matrix stains and fluorescent proteins used for biofilm visualization.**

Stain/fluorescent protein	Putative target/properties	Final concentration of staining solution	Excitation/emission (nm)	Method reference/source
<b>Live cells</b>				
FM 1-43	Cell membrane, lipophilic	1 µg/ml	595/615	Molecular Probes, F10317
CellMask Orange (CMO)	Cell membrane, amphipathic	2 µg/ml	554/567	Molecular Probes, C10045
red-shifted GFP	constitutive endogenous signal		490/509	[1]; see Table 1
<b>Dead Cells</b>				
Propidium Iodide (PI)	Cellular DNA	30 µM	535/617	[2]; Molecular Probes, D3566
<b>Matrix components</b>				
DAPI	eDNA	5 µg/ml	350/470	[3, 4]; Molecular Probes, D1306
Congo red (CR)	amyloid fibrils	20 µM	497/614 <sup>a</sup>	[5, 6]; Sigma, 573-58-0
Thioflavin T (ThT)	amyloid fibrils	1.5 µM	450/482 <sup>a</sup>	[7]; Acros Organics, 211760050
Concanavalin A-Texas Red (ConA)	glycoconjugates	20 µg/ml	595/615	[3]; Molecular Probes, C825
<b>SYTO 9</b>	cellular DNA and eDNA	10 µM	485/498	Molecular Probes, S34854

<sup>a</sup> When bound to amyloid fibrils

**Table S2 Strains and Plasmids.**

Strain or Plasmid	Relevant properties	Reference or source
<b>Plasmids</b>		
pTA409	Shuttle vector based on pBluescript II, with <i>pyrE2</i> and <i>hdrB</i> markers and <i>ori-pHV1/4</i> replication origin	Hölzle et al., 2008
pJAM1020	Amp <sup>R</sup> Nov <sup>R</sup> ; pHV2 replication origin; RSGFP expressed in <i>Hfx. volcanii</i> ; rRNA P2 promoter and T7 terminator	Reuter, et al., 2004
pSC409GFP	pTA409 with 933 bp KpnI/EcoRI In-Fusion fragment containing RSGFP expression construct from pJAM1020	This study
<b>Strains</b>		
<i>E. coli</i>		
HST08	Stellar competent cloning strain	Clontech, 636763
K12	<i>E. coli dam dcm</i> cloning strain	New England Biolabs, C2925I
<i>Hfx. volcanii</i>		
DS2	Wild-type	Mullakhanbhai and Larsen, 1975; Hartman et al., 2010
H26	$\Delta pyrE2$	Allers et al., 2004
H53	$\Delta pyrE2 \Delta trpA$	Allers et al., 2004
H98	$\Delta pyrE2 \Delta hdrB$	Allers et al., 2004
H1206	$\Delta pyrE2 \Delta mrr$	Allers et al., 2010
H1206(pJAM1020)	$\Delta pyrE2 \Delta mrr$ , with pJAM1020; constitutive RSGFP expression; Nov <sup>R</sup>	This study
H1206(pSC409GFP)	$\Delta pyrE2 \Delta mrr$ , with pSC409GFP; constitutive RSGFP expression	This study

**Table S3 Oligonucleotide primers.**

Primer	Sequence (5' -3') <sup>a</sup>	Properties
SC_P2promoter_F	<u>TATAGGGCGAATTGGGTACCCA</u> <u>ACCCCGATCCAAGCTTCTAGAG</u>	Forward primer for amplification of RSGFP with P2 promoter and T7 terminator
SC_T7terminator_R	<u>CGGGCTGCAGGAATTCTTCA</u> <u>GCAAAAACCCCTCAAGAC</u>	Forward primer for amplification of RSGFP with P2 promoter and T7 terminator

<sup>a</sup> In-Fusion adaptor sites are underlined.

### Supplemental references

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