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Supplemental information

In situ devices can culture the microbial dark matter of corals

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TABLE S1 - Differences in the microbial community structure from amplicon sequencing data, related to FIG 2d. Differences in the microbial community structure among the samples by performing permutational multivariate analysis of variance (PERMANOVA) on the community matrix generated by UniFrac Weighted. Groups 1 and 2 indicate the sample groups compared to each other. Symbol '*' indicates significant differences between methods/sample groups (p-value ≤ 0.05).

Sample set		PERMANOVA	PERMDISP
Group 1	Group 2	Pr(>F)	p-adj
chamber2_close_subculture	chamber1_distant_subculture	0.175	1.000
chamber2_close_subculture	chamber2_distant_subculture	0.537	1.000
chamber2_close_subculture	chamber1_close_subculture	0.216	0.997
chamber2_close_subculture	Traditional_cultivation	0.008*	1.000
chamber2_close_subculture	Mucus inoculum	0.023*	1.000
chamber1_distant_subculture	chamber2_distant_subculture	0.128	0.928
chamber1_distant_subculture	chamber1_close_subculture	0.342	0.792
chamber1_distant_subculture	Traditional_cultivation	0.006*	1.000
chamber1_distant_subculture	Mucus inoculum	0.019*	1.000
chamber2_distant_subculture	chamber1_close_subculture	0.536	1.000
chamber2_distant_subculture	Traditional_cultivation	0.01*	1.000
chamber2_distant_subculture	Mucus inoculum	0.021*	1.000
chamber1_close_subculture	Traditional_cultivation	0.015*	0.996
chamber1_close_subculture	Mucus inoculum	0.153	0.994
chamber1_distant	chamber2_distant	0.858	1.000
chamber1_distant	chamber1_close	0.082	0.457
chamber1_distant	chamber2_close	0.842	1.000
chamber1_distant	Traditional_cultivation	0.005*	0.533
chamber1_distant	Mucus inoculum	0.032*	0.552

Sample set		PERMANOVA	PERMDISP
Group 1	Group 2	Pr(>F)	p-adj
chip2_close_subculture	chip1_distant_subculture	0.095	0.907
chip2_close_subculture	chip2_distant_subculture	0.054	1.000
chip2_close_subculture	chip1_close_subculture	0.301	1.000
chip2_close_subculture	Traditional_cultivation	0.008*	0.952
chip2_close_subculture	Mucus inoculum	0.023*	0.984
chip1_distant_subculture	chip2_distant_subculture	0.452	0.999
chip1_distant_subculture	chip1_close_subculture	0.866	1.000
chip1_distant_subculture	Traditional_cultivation	0.007*	1.000
chip1_distant_subculture	Mucus inoculum	0.167	1.000
chip2_distant_subculture	chip1_close_subculture	0.62	1.000
chip2_distant_subculture	Traditional_cultivation	0.013*	1.000
chip2_distant_subculture	Mucus inoculum	0.045*	1.000
chip1_close_subculture	Traditional_cultivation	0.013*	1.000
chip1_close_subculture	Mucus inoculum	0.028*	1.000
chip1_distant	chip2_distant	0.023*	1.000
chip1_distant	chip1_close	0.099	1.000
chip1_distant	chip2_close	0.036*	1.000
chip1_distant	Traditional_cultivation	0.011*	1.000
chip1_distant	Mucus inoculum	0.026*	1.000

chamber2_distant	chamber1_close	0.119	0.145
chamber2_distant	chamber2_close	0.806	1.000
chamber2_distant	Traditional_cultivation	0.009*	0.172
chamber2_distant	Mucus inoculum	0.029*	0.195
chamber1_close	chamber2_close	0.156	0.377
chamber1_close	Traditional_cultivation	0.008*	1.000
chamber1_close	Mucus inoculum	0.023*	1.000
chamber2_close	Traditional_cultivation	0.011*	0.445
chamber2_close	Mucus inoculum	0.021*	0.467
Traditional_cultivation	Mucus inoculum	0.012*	1.000

chip2_distant	chip1_close	0.334	1.000
chip2_distant	chip2_close	0.205	1.000
chip2_distant	Traditional_cultivation	0.005*	1.000
chip2_distant	Mucus inoculum	0.039*	1.000
chip1_close	chip2_close	0.412	1.000
chip1_close	Traditional_cultivation	0.008*	1.000
chip1_close	Mucus inoculum	0.035*	1.000
chip2_close	Traditional_cultivation	0.008*	1.000
chip2_close	Mucus inoculum	0.034*	1.000

TABLE S2 - Pairwise comparison for Sørensen dissimilarity, related to FIG 2e. Symbol '*' indicates significant differences between groups (Pr[>F] < 0.05, PERMANOVA).

Sample set		PERMANOVA Pr(>F)	PERMDISP p-adj
Group1	Group2		
Chamber1_close	Chamber2_close	0.068	0.989
Chamber1_close	Traditional_cultivation	0.011*	1.000
Chamber2_close	Traditional_cultivation	0.016*	0.979
Chamber1_distant	Chamber2_distant	0.426	1.000
Chamber1_distant	Chamber1_close	0.03*	0.917
Chamber1_distant	Traditional_cultivation	0.012*	0.871
Chamber2_distant	Chamber2_close	0.225	1.000

Sample set		PERMANOVA Pr(>F)	PERMDISP p-adj
Group1	Group2		
Chip1_close	Chip2_close	0.567	1.000
Chip1_close	Traditional_cultivation	0.013*	1.000
Chip2_close	Traditional_cultivation	0.006*	1.000
Chip1_distant	Chip2_distant	0.038*	1.000
Chip1_distant	Chip1_close	0.068	1.000
Chip1_distant	Traditional_cultivation	0.009*	1.000
Chip2_distant	Chip2_close	0.422	1.000

Chamber2_distant	Traditional_cultivation	0.01*	0.691
Chamber1_close_subculture	Traditional_cultivation	0.008*	0.419
Chamber1_distant_subculture	Chamber2_distant_subculture	0.14	0.955
Chamber1_distant_subculture	Chamber1_close_subculture	0.256	0.366
Chamber1_distant_subculture	Traditional_cultivation	0.007*	1.000
Chamber2_distant_subculture	Traditional_cultivation	0.005*	0.979
Chamber2_close_subculture	Chamber2_distant_subculture	0.522	1.000
Chamber2_close_subculture	Chamber1_close_subculture	0.355	0.875
Chamber2_close_subculture	Traditional_cultivation	0.011*	1.000
Mucus inoculum	Chamber1_close	0.037*	0.278
Mucus inoculum	Chamber2_close	0.022*	0.992
Mucus inoculum	Chamber1_distant	0.026*	0.173
Mucus inoculum	Chamber2_distant	0.028*	1.000
Mucus inoculum	Chamber1_close_subculture	0.143	1.000
Mucus inoculum	Chamber1_distant_subculture	0.041*	0.173
Mucus inoculum	Chamber2_close_subculture	0.034*	0.647
Mucus inoculum	Chamber2_distant_subculture	0.028*	0.992

Chip2_distant	Traditional_cultivation	0.007*	1.000
Chip1_close_subculture	Traditional_cultivation	0.011*	0.980
Chip2_close_subculture	Chip2_distant_subculture	0.037*	1.000
Chip2_close_subculture	Chip1_close_subculture	0.036*	0.988
Chip2_close_subculture	Traditional_cultivation	0.012*	1.000
Chip1_distant_subculture	Chip2_distant_subculture	0.68	0.997
Chip1_distant_subculture	Chip1_close_subculture	0.359	1.000
Chip1_distant_subculture	Traditional_cultivation	0.011*	0.980
Chip2_distant_subculture	Traditional_cultivation	0.011*	1.000
Mucus inoculum	Chip1_close	0.031*	0.319
Mucus inoculum	Chip2_close	0.033*	0.644
Mucus inoculum	Chip1_distant	0.024*	0.757
Mucus inoculum	Chip2_distant	0.024*	0.412
Mucus inoculum	Chip1_close_subculture	0.064	0.632
Mucus inoculum	Chip2_close_subculture	0.037*	0.029*
Mucus inoculum	Chip1_distant_subculture	0.117	0.991
Mucus inoculum	Chip2_distant_subculture	0.025*	0.378

TABLE S3 - Accession IDs and NCBI's BLAST best hits, related to FIG 5. Accession IDs and NCBI's BLAST best hit of the 16S rRNA sequences of the bacterial isolates.

Isolate	Accession	Source	Best hit	Query Cover	E-value	Per. ident	Acc. Len	Best hit Accession
CL4	OQ435519	Chamber	*Marinobacter nauticus* ATCC 49840	100%	0	99.72	1520	NR_074619.1
CL5	OQ435520	Chamber	*Vibrio harveyi* strain NCIMB1280	99%	0	99.36	1436	NR_043165.1
CP1.2	OQ435521	Chamber	*Roseibium aggregatum* strain NBRC 16684	99%	0	99.7	1408	NR_113861.1
CP2	OQ435522	Chamber	*Roseivirga pacifica* strain DY53	99%	0	98.9	1442	NR_109732.1
CP3	OQ435523	Chamber	*Pseudoalteromonas flavipulchra* strain NCIMB 2033	100%	0	99.93	1483	NR_025126.1
CP4.1	OQ435524	Chamber	*Oceanicaulis stylophorae* strain GISW-4	99%	0	99.17	1401	NR_108907.1
CP4.2	OQ435525	Chamber	*Bacterioplanes sanyensis* strain GYP-2	100%	0	98.79	1412	NR_126264.1
CP5	OQ435526	Chamber	*Marinobacter* sediminum strain R65	100%	0	97.18	1512	NR_029028.1
CP6	OQ435527	Chamber	*Tritonibacter multivorans* strain MD5	100%	0	97.06	1405	NR_108509.1
CPX1.1	OQ435528	Chamber	*Alteromonas macleodii* strain NBRC 102226	99%	0	98.61	1458	NR_114053.1
CPX2	OQ435529	Chamber	*Roseibium aggregatum* strain NBRC 16684	99%	0	98.7	1408	NR_113861.1
iL2	OQ435530	Chip	*Leisingera daeponensis* DSM 23529 strain TF-218	100%	0	97.98	1419	NR_044026.1
iL6	OQ435531	Chip	*Roseibium aggregatum* strain NBRC 16684	100%	0	99.7	1408	NR_113861.1
iL8	OQ435532	Chip	*Vibrio nigripulchritudo* ATCC 27043	100%	0	98.45	1470	NR_117897.1
iL10	OQ435533	Chip	*Oceanicaulis stylophorae* strain GISW-4	97%	0	98.21	1401	NR_108907.1
iL11	OQ435534	Chip	*Roseibium aggregatum* strain NBRC 16684	100%	0	99.93	1408	NR_113861.1
iL12.1	OQ435535	Chip	*Vibrio aestivus* strain M22	99%	0	97.43	1463	NR_108873.1
iL14	OQ435536	Chip	*Roseibium aggregatum* strain NBRC 16684	100%	0	98.59	1408	NR_113861.1

iL15	OQ435537	Chip	*Leisingera aquimarina* strain R-26159	99%	0	97.52	1396	NR_042670.1
iLP	OQ435538	Chip	*Roseibium aggregatum* strain NBRC 16684	100%	0	98.21	1408	NR_113861.1
iP3	OQ435539	Chip	*Halobacillus trueperi* strain DSM 10404	99%	0	99.5	1536	NR_025459.1
iP4	OQ435540	Chip	*Robertkochia marina* strain CC-AMO-30D	97%	0	98.96	1480	NR_160524.1
iP5	OQ435541	Chip	*Microbulbifer variabilis* strain Ni-2088	98%	0	99.27	1492	NR_041021.1
iP6	OQ435542	Chip	*Roseibium aggregatum* strain NBRC 16684	98%	0	98.67	1408	NR_113861.1
iP7.1	OQ435543	Chip	*Maricaulis virginensis* strain VC 5	99%	0	99.4	1420	NR_025444.1
iP9.2	OQ435544	Chip	*Roseibium aggregatum* strain NBRC 16684	100%	0	99.85	1408	NR_113861.1
iP12	OQ435545	Chip	*Halobacillus kuroshimensis* strain IS-Hb7	100%	0	99.23	1544	NR_041262.1
iP14.1	OQ435546	Chip	*Bacterioplanes sanyensis* strain GYP-2	99%	0	98.76	1412	NR_126264.1
iP14.3	OQ435547	Chip	*Aquimarina litoralis* strain CNURIC011	100%	0	98.69	1403	NR_116703.1
iP16	OQ435548	Chip	*Roseibium aggregatum* strain NBRC 16684	100%	0	99.85	1408	NR_113861.1
iP17	OQ435549	Chip	*Muricauda aquimarina* strain SW-63	100%	0	98.67	1481	NR_042909.1
iP20.1	OQ435550	Chip	*Leisingera caerulea* DSM 24564	100%	0	97.98	1402	NR_118542.1
iP20	OQ435551	Chip	*Microbulbifer elongatus* strain DSM 6810	100%	0	98.77	1491	NR_025246.1
iP22	OQ435552	Chip	*Halobacillus kuroshimensis* strain IS-Hb7	99%	0	99.15	1544	NR_041262.1
iPX1	OQ435553	Chip	*Shewanella waksmanii* strain KMM 3823	99%	0	99.29	1540	NR_025684.1
MP1	OQ435554	Traditional	*Halobacillus trueperi* strain DSM 10404	99%	0	99.51	1536	NR_025459.1
MP3	OQ435555	Traditional	*Roseibium aggregatum* strain NBRC 16684	100%	0	98.81	1408	NR_113861.1
MP4	OQ435556	Traditional	*Pseudoalteromonas ruthenica* strain KMM300	100%	0	99.21	1489	NR_025140.1
MP5	OQ435557	Traditional	*Roseibium aggregatum* strain NBRC 16684	100%	0	99.92	1408	NR_113861.1
MP6	OQ435558	Traditional	*Ruegeria atlantica* strain NBRC 15792	98%	0	98.99	1385	NR_112615.1

MP7.2	OQ435559	Traditional	*Roseibium aggregatum* strain NBRC 16684	100%	0	99.85	1408	NR_113861.1
MP8	OQ435560	Traditional	*Roseibium album* strain 5OM6	100%	0	98.43	1477	NR_042378.1
MP9.1	OQ435561	Traditional	*Roseibium aggregatum* strain NBRC 16684	100%	0	99.93	1408	NR_113861.1
MP10	OQ435562	Traditional	*Roseibium aggregatum* strain NBRC 16684	99%	0	100	1408	NR_113861.1
MP11	OQ435563	Traditional	*Ruegeria arenilitoris* strain G-M8	99%	0	99.46	1384	NR_109635.1
MP13	OQ435564	Traditional	*Thalassotalea litorea* strain HMF4135	97%	0	98.97	1478	NR_157659.1
MP14	OQ435565	Traditional	*Ruegeria atlantica* strain NBRC 15792	100%	0	99.01	1385	NR_112615.1
MP15.1	OQ435566	Traditional	*Ruegeria conchae* strain TW15	100%	0	99.85	1384	NR_109062.1
MP15.2	OQ435567	Traditional	*Roseibium aggregatum* strain NBRC 16684	100%	0	99.78	1408	NR_113861.1
MP16	OQ435568	Traditional	*Ruegeria arenilitoris* strain G-M8	99%	0	99.38	1384	NR_109635.1
MPV	OQ435569	Traditional	*Algihabitans albus* strain HHTR 118	100%	0	99.33	1452	NR_173537.1

TABLE S4 - Sample dataset, related to STAR Methods (Method Details: Sampling, DNA extraction, and sequencing). Dataset used for compositional analysis of microbiomes from amplicon sequencing data.

	Sample type	Description	Synonyms	Replicates
1	Chip 1 close	Content retrieved from the chip interior incubated with -2 dilution, close	Chip content, device content	4
2	Chip 2 close	Content retrieved from the chip interior incubated with -3 dilution, close	Chip content, device content	4
3	Chamber 1 close	Content retrieved from the chamber interior incubated with -3 dilution, close	Chamber content, device content	4
4	Chamber 2 close	Content retrieved from the chamber interior incubated with -3 dilution, close	Chamber content, device content	4
5	Chip 1 close subculture	Colonies subcultivate in MA1:2 using the contents from chips with dilution -2, close	Chip subculture	4
6	Chip 2 close subculture	Colonies subcultivate in MA1:2 using the contents from chips with dilution -3, close	Chip subculture	4
7	Chamber 1 close subculture	Colonies subcultivate in MA1:2 using the contents from chambers with dilution -2, close	Chamber subculture	4
8	Chamber 2 close subculture	Colonies subcultivate in MA1:2 using the contents from chambers with dilution -3, close	Chamber subculture	4
9	Chip 1 distant	Content retrieved from the chip interior incubated with -2 dilution, distant	Chip content, device content	4
10	Chip 2 distant	Content retrieved from the chip interior incubated with -3 dilution, distant	Chip content, device content	4
11	Chamber 1 distant	Content retrieved from the chamber interior incubated with -3 dilution, distant	Chamber content, device content	4
12	Chamber 2 distant	Content retrieved from the chamber interior incubated with -3 dilution, distant	Chamber content, device content	4
13	Chip 1 distant subculture	Colonies subcultivate in MA1:2 using the contents from chips with dilution -2, distant	Chip subculture	4
14	Chip 2 distant subculture	Colonies subcultivate in MA1:2 using the contents from chips with dilution -3, distant	Chip subculture	4
15	Chamber 1 distant subculture	Colonies subcultivate in MA1:2 using the contents from chambers with dilution -2, distant	Chamber subculture	4
16	Chamber 2 distant subculture	Colonies subcultivate in MA1:2 using the contents from chambers with dilution -3, distant	Chamber subculture	4
17	Traditional cultivation	Conventional cultivation, using the mucus itself diluted and plated on MA1:2	Traditional cultivation	5
18	Mucus inoculum	Mucus separated from the coral by centrifugation	Mucus, Mucus inoculum	4

TABLE S5 - Statistical comparisons between groups for Shannon index, related to FIG 1a. Symbol ‘*’ indicates significant differences between groups (p-value < 0.05, ANOVA).

Sample set		
Group 1	Group 2	p-value
Chamber1_distant_subculture	Chamber1_close_subculture	0.999
Chamber2_close_subculture	Chamber1_close_subculture	0.877
Chamber2_distant_subculture	Chamber1_close_subculture	0.934
Chamber2_close_subculture	Chamber1_distant_subculture	0.999
Chamber2_distant_subculture	Chamber1_distant_subculture	0.999
Chamber2_distant_subculture	Chamber2_close_subculture	1.000
Chamber1_distant	Chamber1_close	0.124
Chamber2_close	Chamber1_close	0.997
Chamber2_distant	Chamber1_close	0.846
Chamber1_distant	Chamber1_close_subculture	< 0.001*
Chamber2_close	Chamber1_close_subculture	0.141
Chamber2_distant	Chamber1_close_subculture	0.023*
Chamber2_close	Chamber1_distant	0.867
Chamber2_distant	Chamber1_distant	0.998
Chamber2_close	Chamber1_distant_subculture	0.749
Chamber2_distant	Chamber1_distant_subculture	0.290
Chamber2_distant	Chamber2_close	0.999
Chamber2_distant	Chamber2_close_subculture	0.862
Mucus inoculum	Chamber1_close	0.072
Mucus inoculum	Chamber1_close_subculture	0.976

Sample set		
Group 1	Group 2	p-value
Chip1_distant_subculture	Chip1_close_subculture	0.999
Chip2_close_subculture	Chip1_close_subculture	0.999
Chip2_distant_subculture	Chip1_close_subculture	1.000
Chip2_close_subculture	Chip1_distant_subculture	1.000
Chip2_distant_subculture	Chip1_distant_subculture	1.000
Chip2_distant_subculture	Chip2_close_subculture	0.999
Chip1_distant	Chip1_close	0.859
Chip2_close	Chip1_close	1.000
Chip2_distant	Chip1_close	1.000
Chip1_distant	Chip1_close_subculture	< 0.001*
Chip2_close	Chip1_close_subculture	0.008*
Chip2_distant	Chip1_close_subculture	0.008*
Chip2_close	Chip1_distant	0.860
Chip2_distant	Chip1_distant	0.857
Chip2_close	Chip1_distant_subculture	< 0.001*
Chip2_distant	Chip1_distant_subculture	< 0.001*
Chip2_distant	Chip2_close	1.000
Chip2_distant	Chip2_close_subculture	< 0.001*
Traditional_cultivation	Chamber1_close	1.000
Traditional_cultivation	Chamber1_close_subculture	0.836

Mucus inoculum	Chamber1_distant	< 0.001*
Mucus inoculum	Chamber1_distant_subculture	0.439
Mucus inoculum	Chamber2_close	0.001
Mucus inoculum	Chamber2_close_subculture	0.066
Mucus inoculum	Chamber2_distant	< 0.001*
Mucus inoculum	Chamber2_distant_subculture	0.098
Mucus inoculum	Chip1_close	0.040*
Mucus inoculum	Chip1_close_subculture	1.000
Mucus inoculum	Chip1_distant	< 0.001*
Mucus inoculum	Chip1_distant_subculture	0.997
Mucus inoculum	Chip2_close	0.039*
Mucus inoculum	Chip2_close_subculture	0.959
Mucus inoculum	Chip2_distant	0.041*
Mucus inoculum	Chip2_distant_subculture	0.999

Traditional_cultivation	Chamber1_distant	0.083
Traditional_cultivation	Chamber1_distant_subculture	0.999
Traditional_cultivation	Chamber2_close	0.995
Traditional_cultivation	Chamber2_close_subculture	1.000
Traditional_cultivation	Chamber2_distant	0.797
Traditional_cultivation	Chamber2_distant_subculture	1.000
Traditional_cultivation	Chip1_close	1.000
Traditional_cultivation	Chip1_close_subculture	< 0.001*
Traditional_cultivation	Chip1_distant	0.667
Traditional_cultivation	Chip1_distant_subculture	< 0.001*
Traditional_cultivation	Chip2_close	1.000
Traditional_cultivation	Chip2_close_subculture	< 0.001*
Traditional_cultivation	Chip2_distant	1.000
Traditional_cultivation	Chip2_distant_subculture	0.003*

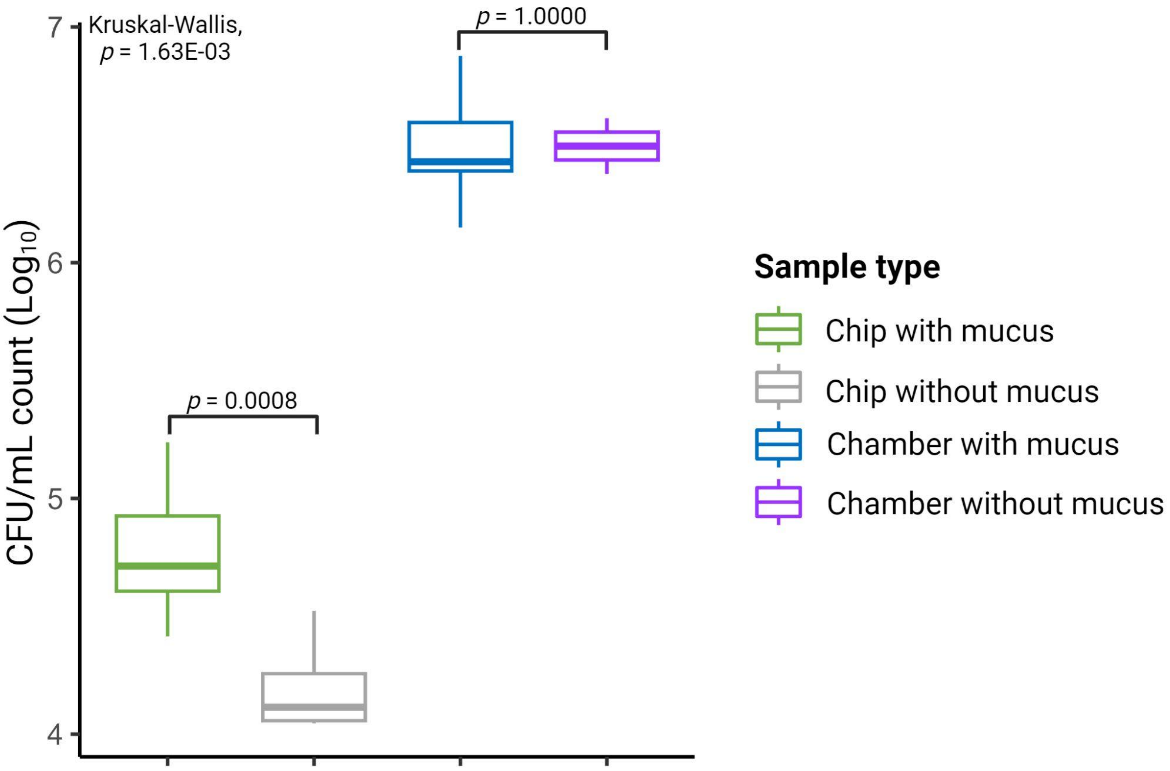

FIG S1. Colony Forming Units (CFU) counts in Log10 scale for the different culture methods in comparison to control for cell migration/leakage assay, related to STAR methods. The CFU counts were significantly lower in the chips incubated without mucus (4.199, Log10 CFU/ml, ± 0.223) in comparison to those incubated with (4.758 Log10 CFU/ml, ± 0.237) mucus (p-value = 0.0008, ANOVA), indicating that the number of CFUs was 3.75 fold higher in incubated chips. The 0.03 µm pore-size membrane used for the chips likely prevented the majority of cells in seawater from crossing into the device. There were no differences between CFU counts for chambers with (6.494 Log10 CFU/ml, ± 0.207) and without (6.494 Log10 CFU/ml, ± 0.167) inoculum (p-value = 1.000, ANOVA). This could be explained by the 0.2 µm pore-size membranes of the chambers used, allowing a greater range of microorganisms to enter. To compensate for this issue, we only considered the ASVs that were observed in the original coral mucus sample for further analysis.

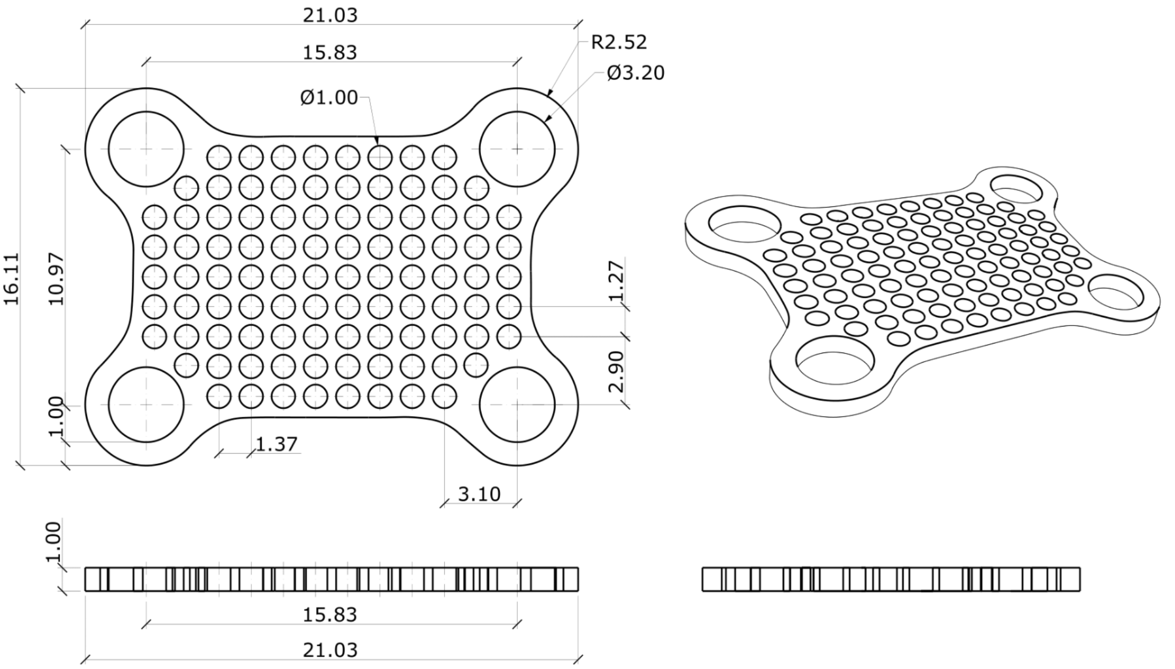

FIG S2 - Technical specifications of the chip device project, related to STAR methods. The numbers represent the dimensions of the device in millimeters.

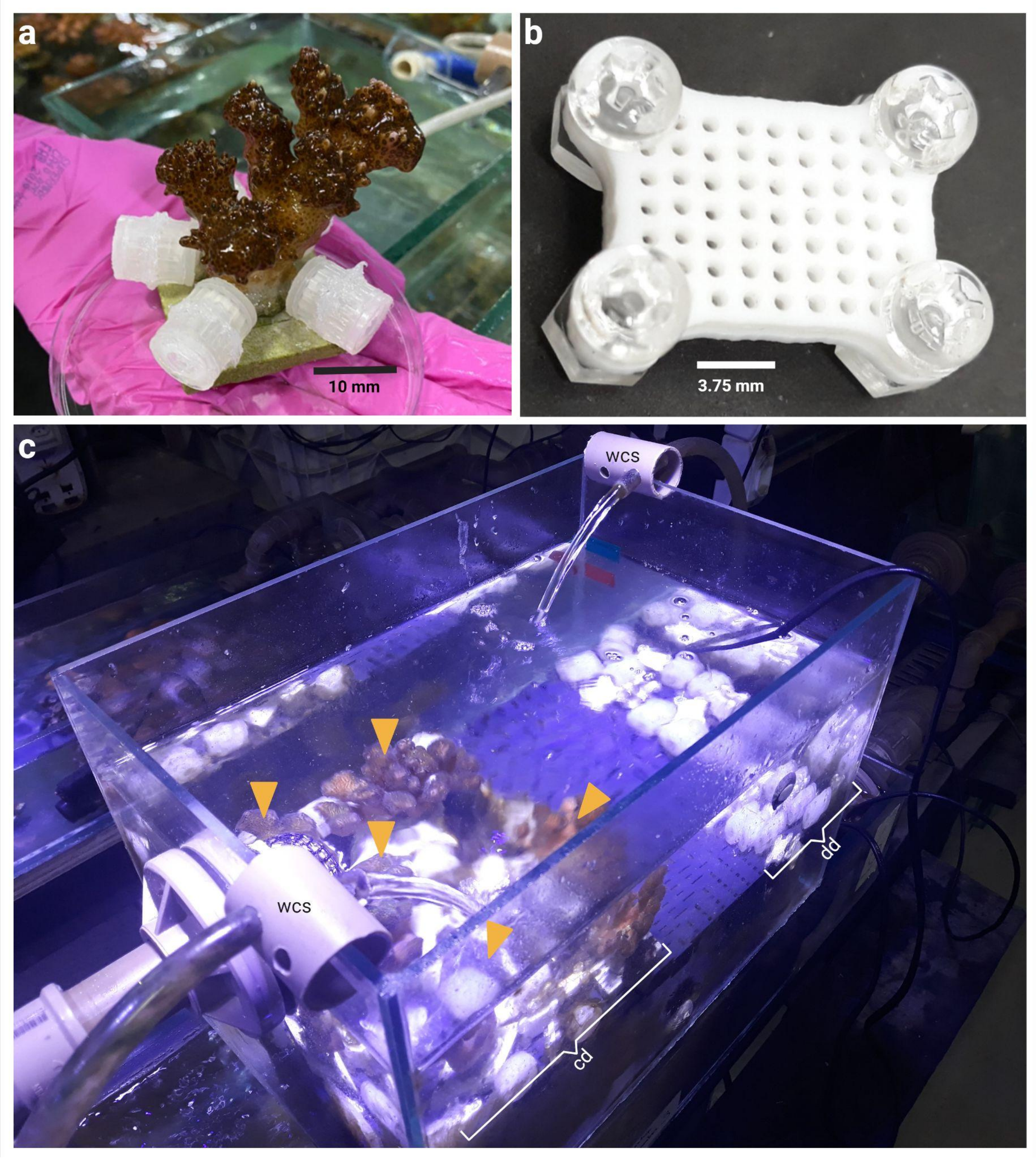

FIG S3 - Chamber, Chip and Aquarium setup, related to STAR methods. (a) the chamber devices close to the coral; (b) the chip sealed with screws and nuts; and (c) a representation of the aquarium setup with the devices incubated close (cd) to corals (yellow arrows) as well as distant devices (dd). ‘wcs’ indicates the two water circulation systems composed of two pumps (Mini A, Sarlo Better, São Caetano do Sul, Brazil) and one sump (not shown).