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Supplementary Materials for

Rapid coral bleaching recovery via symbiont transfer from healthy corals

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The PDF file includes:

Figs. S1 to S16
Tables S1 and S2
Legend for movie S1

Other Supplementary Material for this manuscript includes the following:

Movie S1

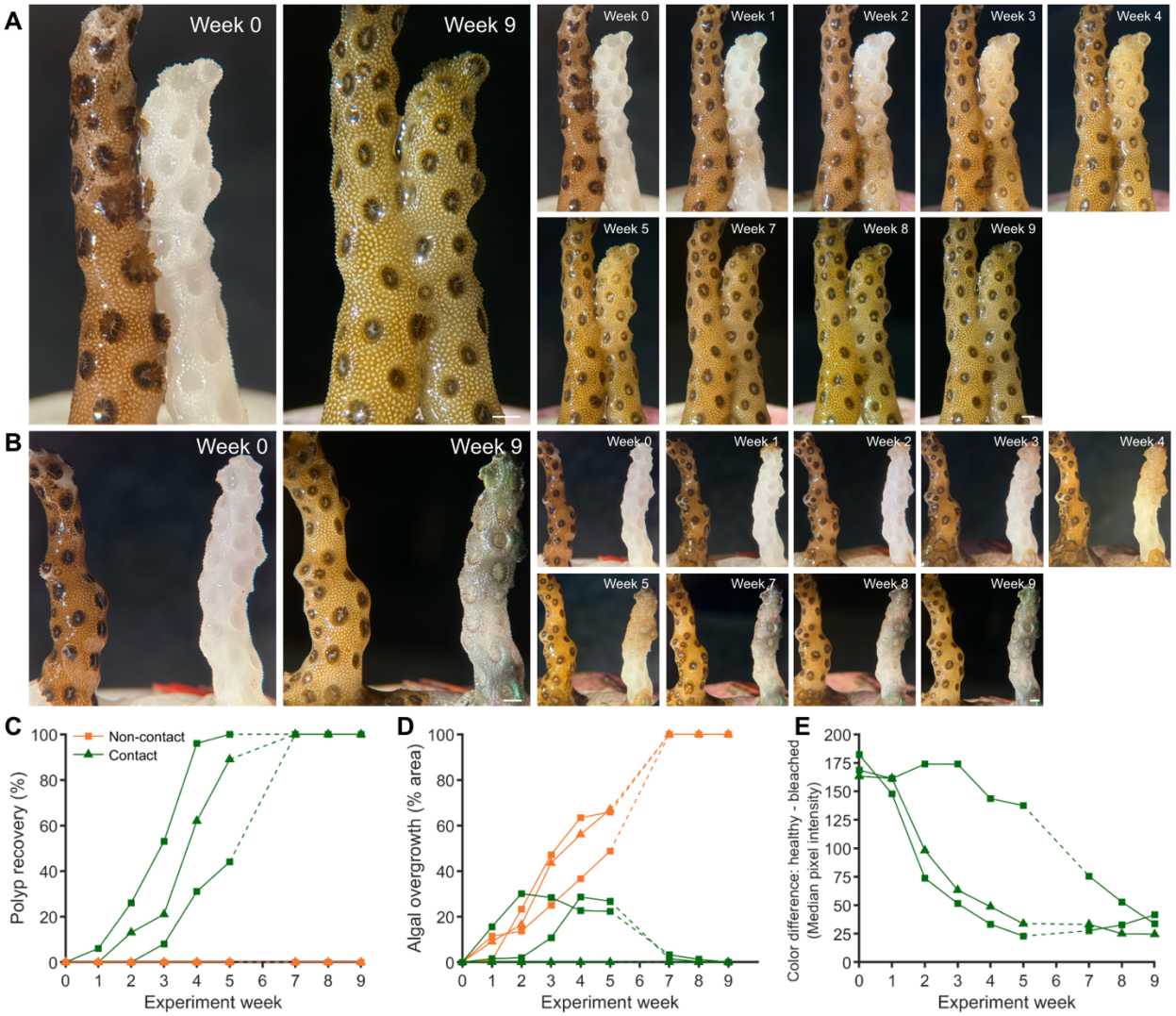


Fig. S1. Coral bleaching recovery from contact with healthy coral using larger fragments. (**A**) Example stereoscopic images of a bleached and healthy coral fragment (*P. damicornis*) in direct contact, showing weekly progression of coral bleaching recovery from week 0 to week 9. Scale bars, 1 mm. (**B**) Example stereoscopic images of a bleached and healthy coral fragment (*P. damicornis*) without contact, showing weekly progression of algal overgrowth and mortality, with minimal recovery, from week 0 to week 9. Scale bars, 1 mm. (**C**) Polyp recovery of bleached coral fragments with and without contact with healthy coral fragments from week 0 to week 9. Polyp recovery was normalized to the total number of polyps per fragment, n = 3 (contact), n = 3 (non-contact). (**D**) Area of algal overgrowth on bleached coral fragments with and without contact with healthy coral fragments from week 0 to week 9. Algal overgrowth area was normalized to fragment size, n = 3 (contact), n = 3 (non-contact). (**E**) Differences in median pixel color of bleached coral fragments in contact with healthy coral fragments from week 0 to week 9, n = 3. Coral fragments shown in panels A and B are represented by triangles. Scale bars, 1 mm. Data from week 6 was not included in analyses shown in this figure.

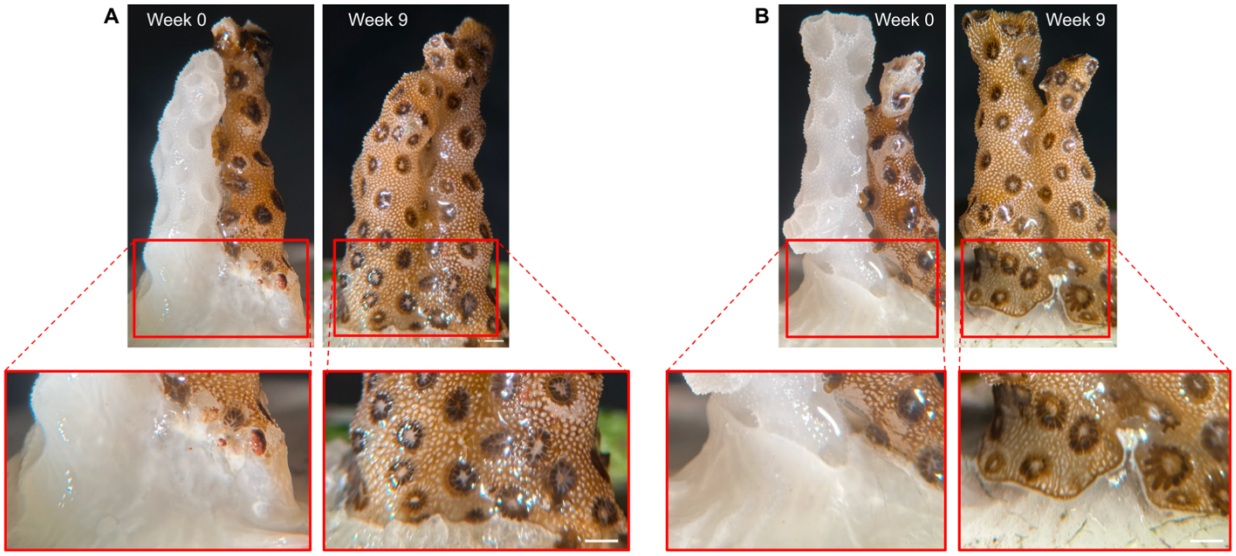


Fig. S2. Additional polyp growth on bleached coral fragments following contact. (**A, B**) Example stereoscopic image of bleached and healthy coral fragments in contact in week 0 and week 9, showing additional polyp growth on the bleached coral fragments. Scale bars, 1 mm.

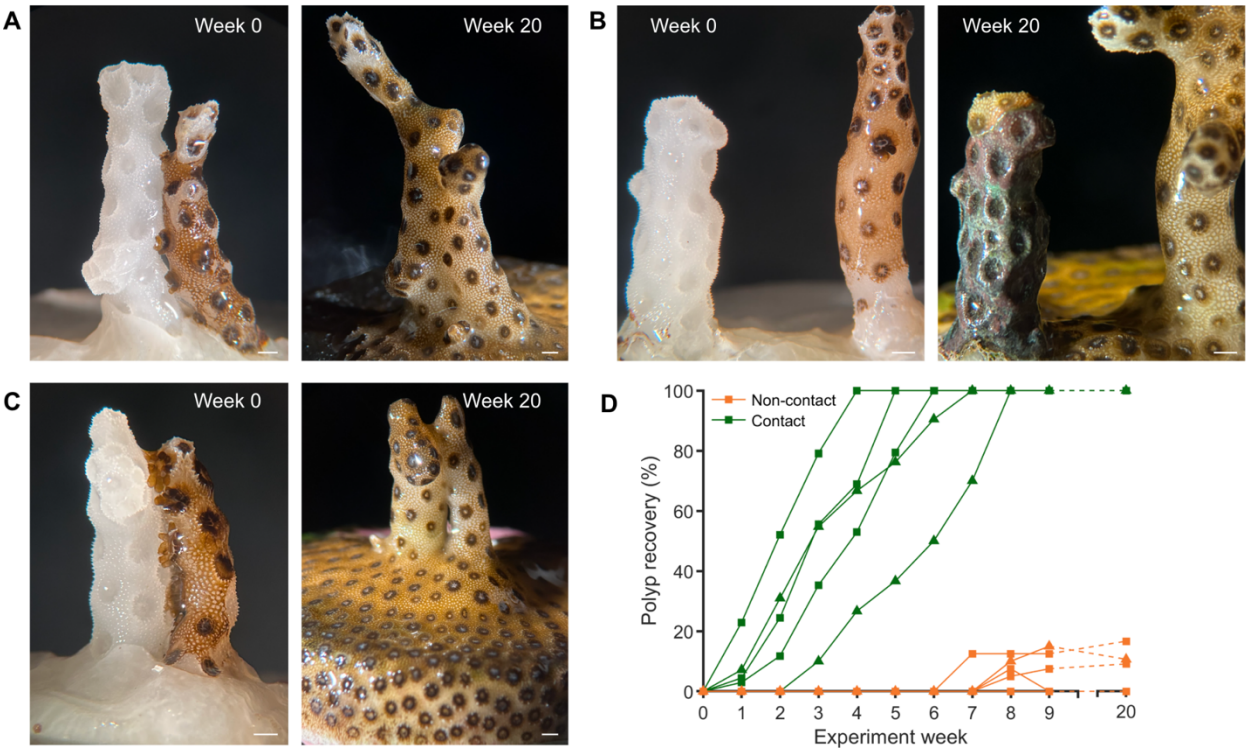


Fig. S3. Long-term polyp growth and skeletal formation of bleached corals at week 20. **(A)** Stereomicroscope images of contacting bleached and healthy coral fragments at week 0 and the same fragments at week 20, showing new skeletal formation in the previously bleached fragment. Scale bars, 1 mm. **(B)** Stereomicroscope images of non-contacting bleached and healthy coral fragments at week 0 and at week 20, showing extensive algal overgrowth and mortality of the initially bleached fragment. Scale bars, 1 mm. **(C)** Stereomicroscope images of contacting bleached and healthy coral fragments at week 0 and at week 20, showing extensive polyp growth in the previously bleached fragment. Scale bars, 1 mm. **(D)** Area of algal overgrowth on bleached coral fragments with and without contact with healthy coral fragments from week 0 to week 20. This panel extends the data shown in Fig. 1d. Algal overgrowth area was normalized to fragment size, n = 5 (contact), n = 5 (non-contact). Coral fragments shown in panels A, B, and C are represented by triangles.

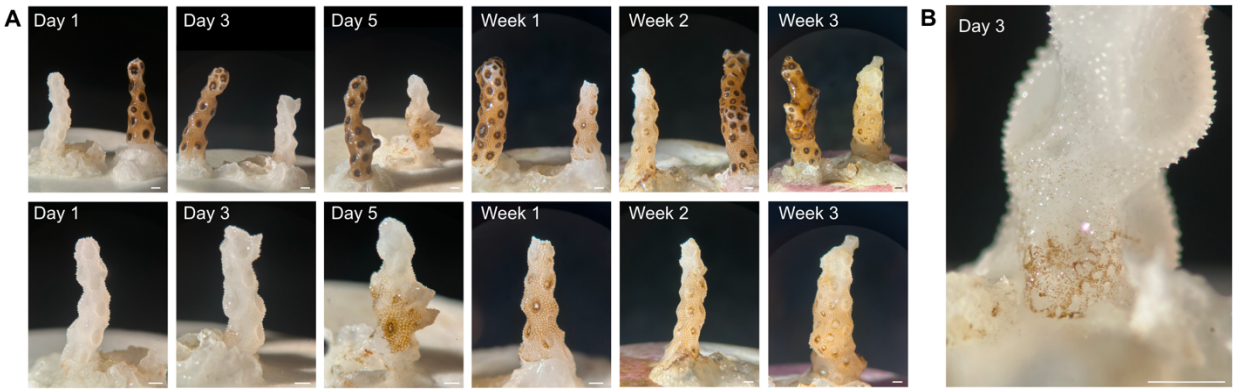


Fig. S4. Recovery stages of bleached coral following variable contact durations (1 day–3 weeks). (**A**) Stereomicroscope images of bleached and healthy coral fragments immediately after separation at multiple time points: Day 1, Day 3, Day 5, Week 1, Week 2, and Week 3. Top row: Bleached and healthy fragments; bottom row: bleached fragments only. (**B**) Close-up stereomicroscope image of bleached fragment separated at Day 3 of contact, highlighting symbiont transfer at the fragment base.

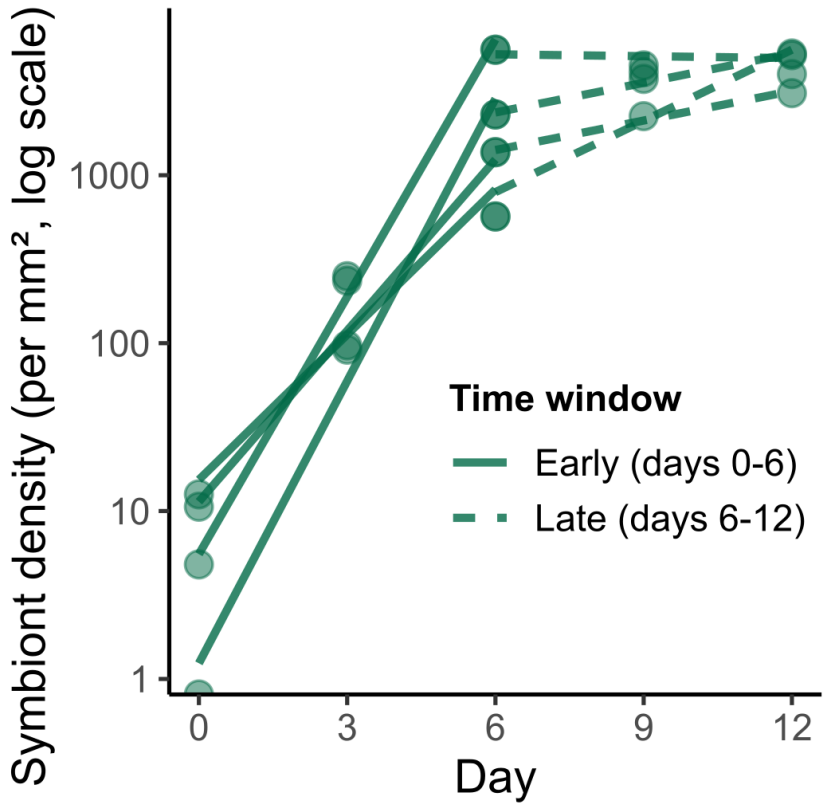


Fig. S5. Symbiont density trajectories during recovery. Symbiont density trajectories for individual coral fragments during early (0-6 days) and late (6-12 days) recovery time windows. Points represent observed mean symbiont densities per fragment. Lines represent the exponential growth models fitted separately to the early (0-6 days) and late (6-12 days) recovery windows by linear regression of log-transformed symbiont density versus time.

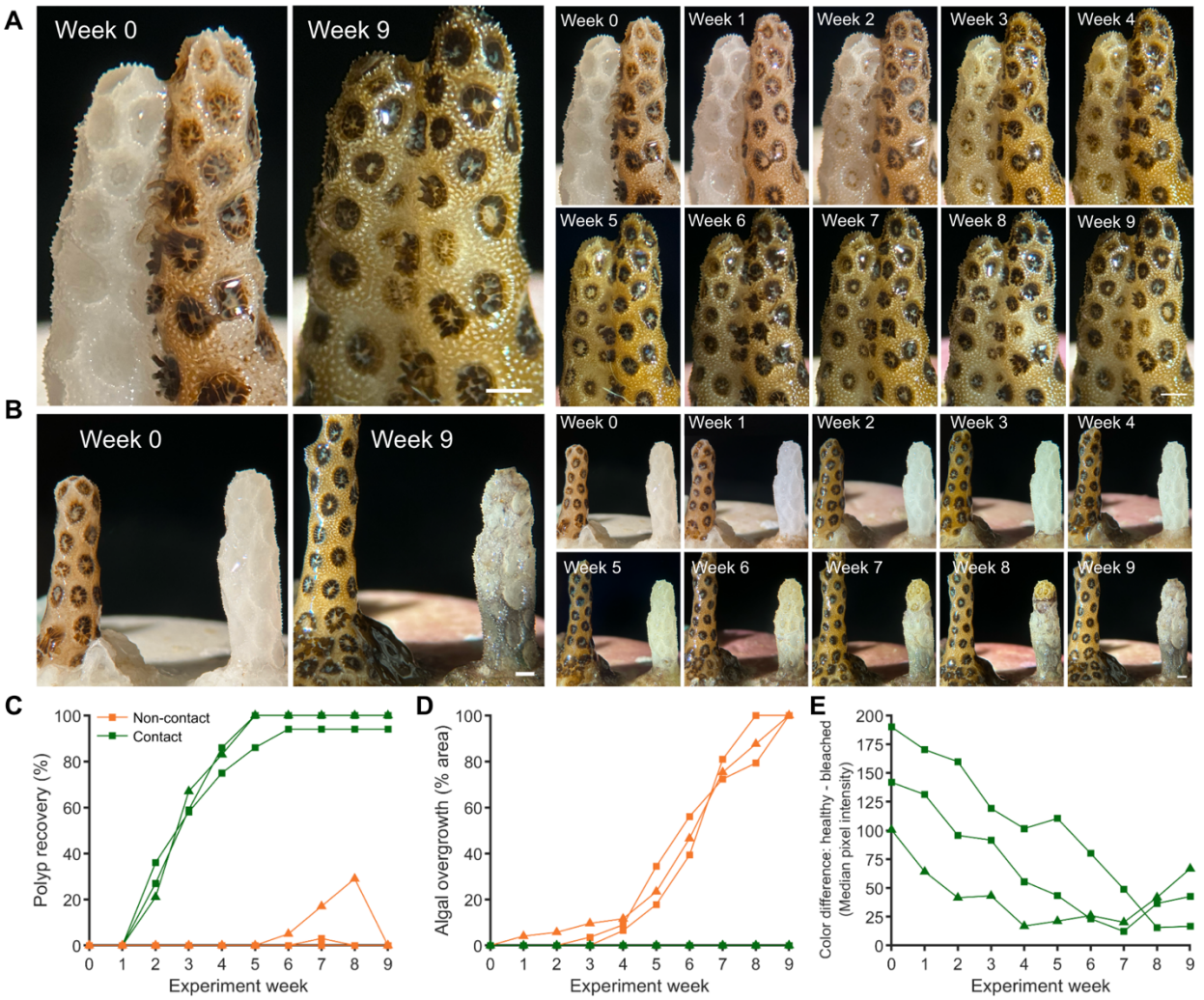


Fig. S6. Coral bleaching recovery from contact with healthy coral (genotype G). (**A**) Example stereoscopic images of a bleached and healthy coral fragment of genotype G (*P. damicornis*) in direct contact, showing weekly progression of coral bleaching recovery from week 0 to week 9. Scale bars, 1 mm. (**B**) Example stereoscopic images of a bleached and healthy coral fragment (*P. damicornis*) without contact, showing weekly progression of algal overgrowth and mortality, with minimal recovery, from week 0 to week 9. Scale bars, 1 mm. (**C**) Polyp recovery of bleached coral fragments with and without contact with healthy coral fragments from week 0 to week 9. Polyp recovery was normalized to the total number of polyps per fragment, n = 3 (contact), n = 3 (non-contact). (**D**) Area of algal overgrowth on bleached coral fragments with and without contact with healthy coral fragments from week 0 to week 9. Algal overgrowth area was normalized to fragment size, n = 3 (contact), n = 3 (non-contact). (**E**) Differences in median pixel color of bleached coral fragments in contact with healthy coral fragments from week 0 to week 9, n = 3. Coral fragments shown in panels A and B are represented by triangles. Scale bars, 1 mm.

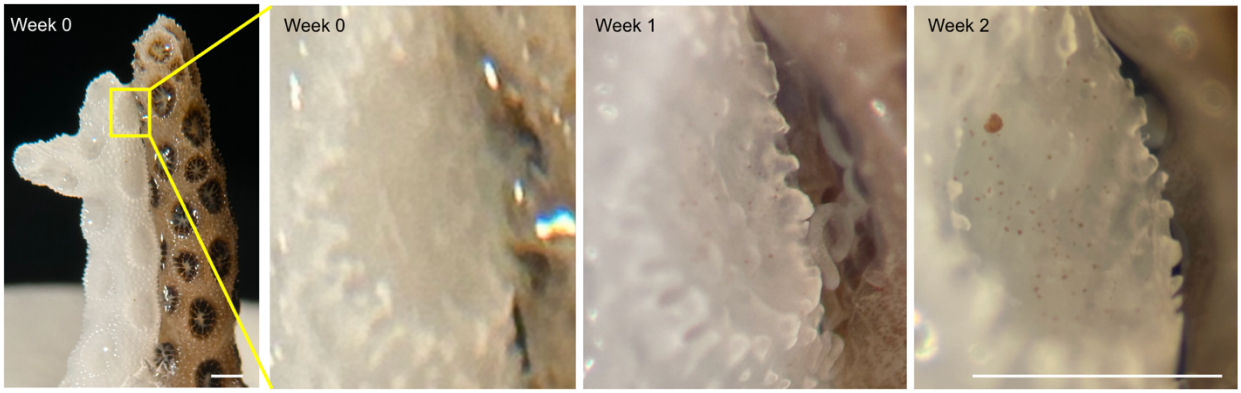


Fig. S7. Passive symbiont transfer mixed-genet corals. Example stereomicroscope images of a mixed-genet pairing (healthy green and bleached red), showing accumulation of symbionts in a bleached polyp in contact with a healthy coral, monitored weekly from week 0 to 2. Scale bars, 1 mm.



Fig. S8. Additional examples of directional symbiont spread during recovery initiating from localized points of contact. (**A**) Stereoscopic images showing symbiont transfer initiating from two tissue bridges (red circles) located at the fragment tip and midpoint, resulting in top-down and bidirectional recolonization of the fragment (blue arrows). (**B**, **C**) Example stereoscopic images showing symbiont transfer initiating from tissue bridges (red circles) located at the fragment tip (**B**) and midpoint (**C**), resulting in top-down and bidirectional recolonization of the bleached fragment, respectively (blue arrows). Images show recovery from week 0 to week 4. Scale bars, 1 mm.

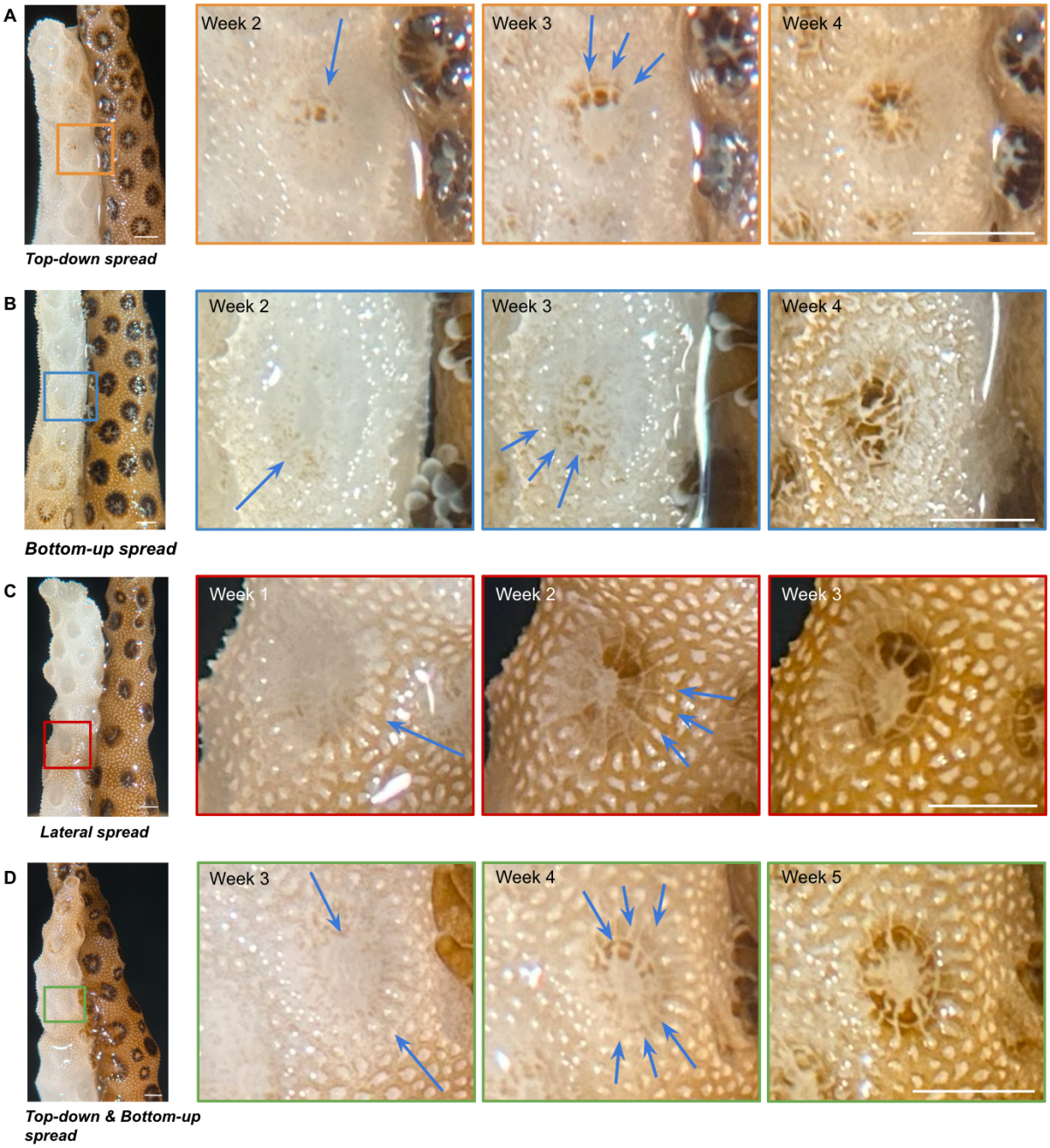


Fig. S9. Polyp-scale directional symbiont recolonization. (**A–D**) Example stereoscopic image time series of recovering bleached polyps from fragments exhibiting different recolonization patterns (**Fig. 4; fig. S8**). (**A**) Top-down recolonization (**Fig. 4**), showing symbiont appearance in upper tentacles before lower tentacles. Images span weeks 2–4 of recovery. (**B**) Bottom-up recolonization (**Fig. 4**), showing symbiont appearance in lower tentacles before upper tentacles. Images span weeks 2–4 of recovery. (**C**) Bidirectional recolonization from the fragment midpoint (**Fig. 4**), showing symbiont appearance in rightmost tentacles before leftmost tentacles. Images span weeks 1–3 of recovery. (**D**) Two sites of transfer exhibiting bidirectional and top-down recolonization (**fig. S8**), showing concurrent symbiont appearance in upper and lower tentacles. Images span weeks 3–5 of recovery. Scale bars, 1 mm.

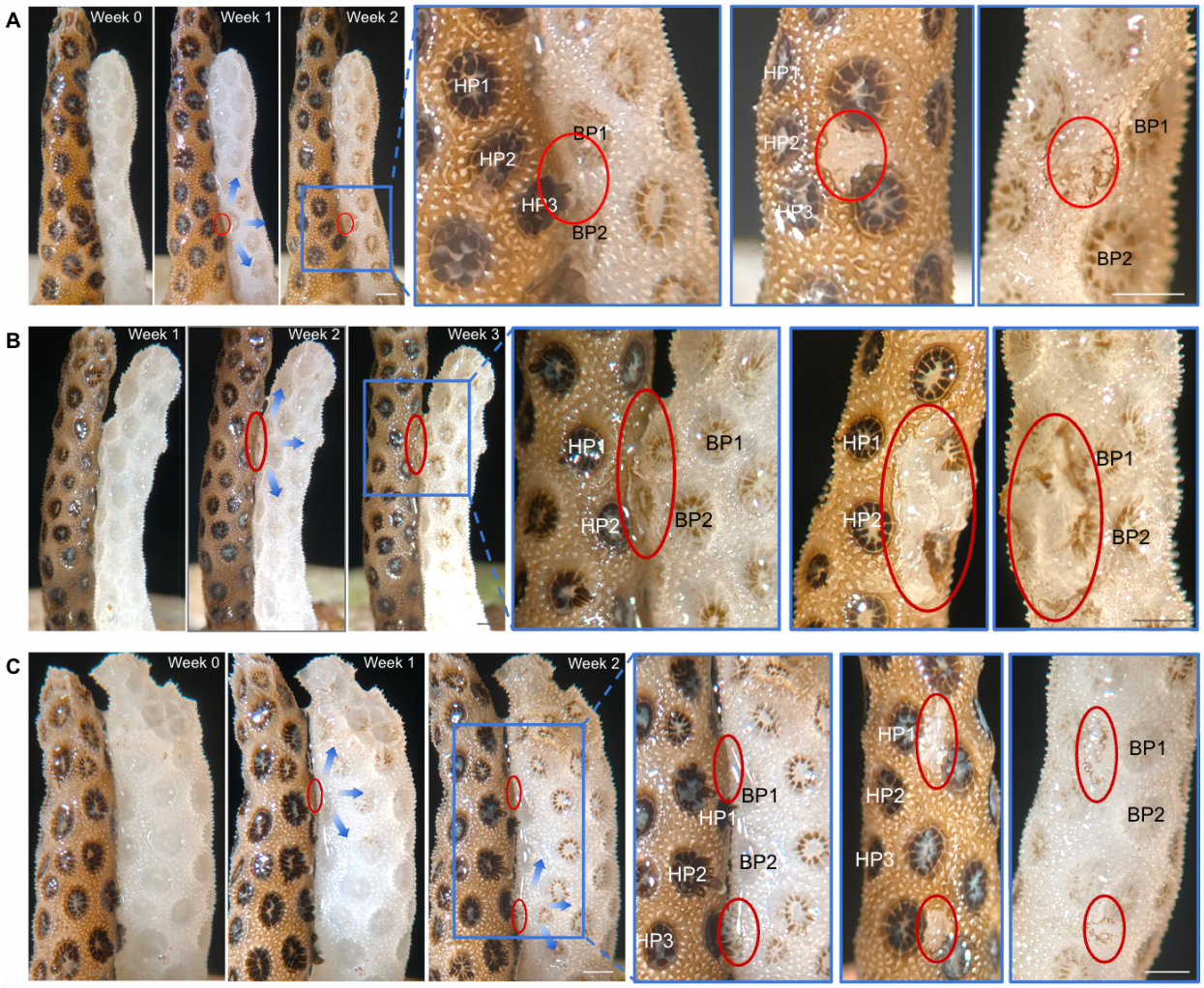


Fig. S10. Additional examples of directional symbiont spread from localized tissue fusion sites and associated tissue tearing upon separation. (**A**, **B**) Example stereoscopic images showing symbiont transfer initiating from a tissue bridge (red circle), resulting in directional recolonization (blue arrows). After two weeks of contact (**A**) and 3 weeks of contact (**B**), fragments were separated, revealing corresponding tissue rips at the sites of initial symbiont transfer on both the healthy and bleached corals. (**C**) After two weeks of contact, two tissue bridges (red circles) resulted in directional recolonization (blue arrows). Separation revealed two corresponding pairs of tissue rips at the sites of initial symbiont transfer on both fragments. The tissue rips are indicated by red circles. Labeled polyps on each coral provide reference points for comparing orientation before and after separation. Scale bars, 1 mm.

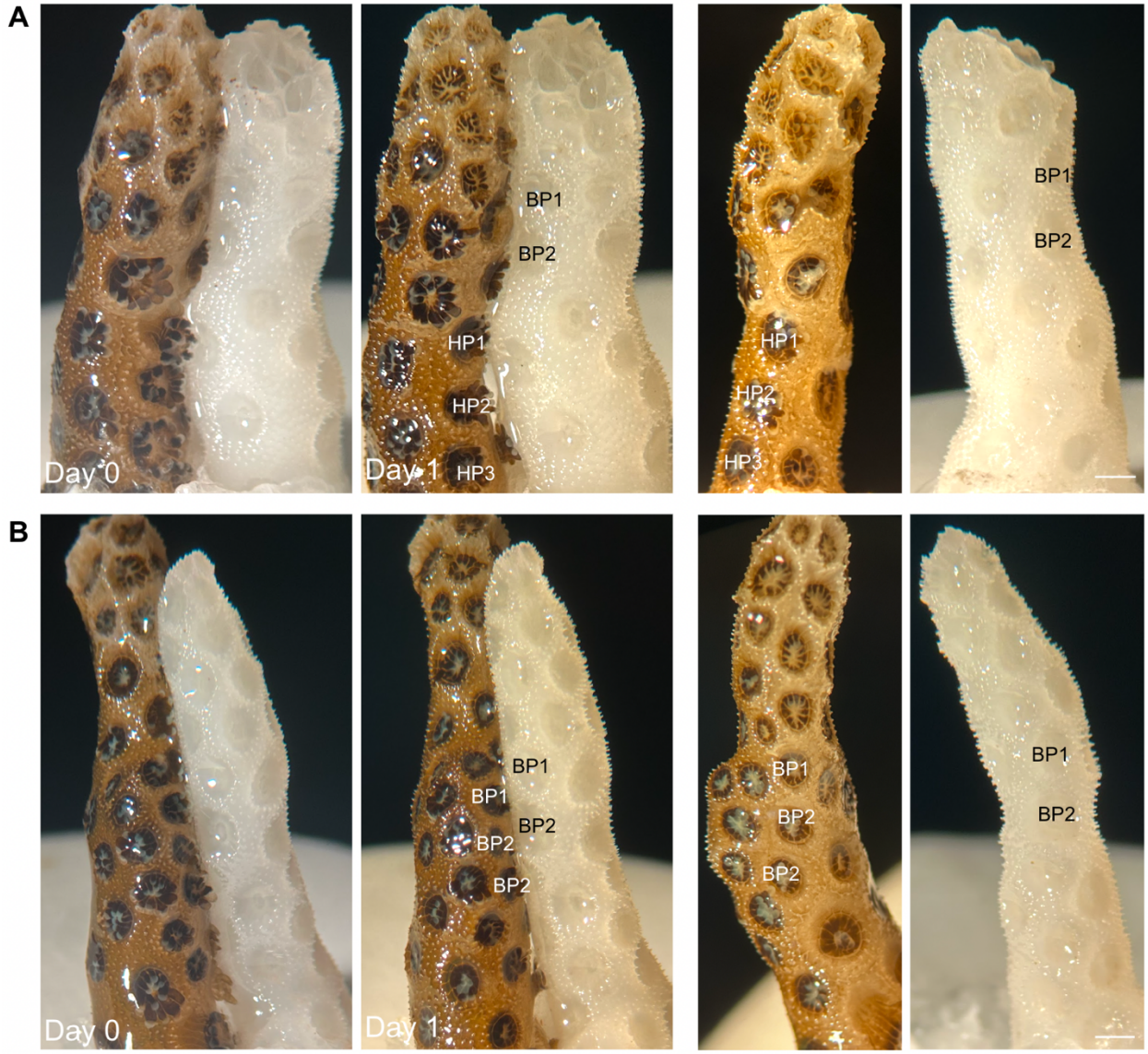


Fig. S11. Short-term contact (1 day) does not induce tissue damage upon separation. (**A**, **B**) Stereoscopic images of bleached and healthy coral fragments in contact for 1 day. Separation after 1 day of contact revealed no tissue damage or mass symbiont transfer on either fragment. Labeled polyps provide reference points for orientation before and after separation. Scale bars, 1 mm.

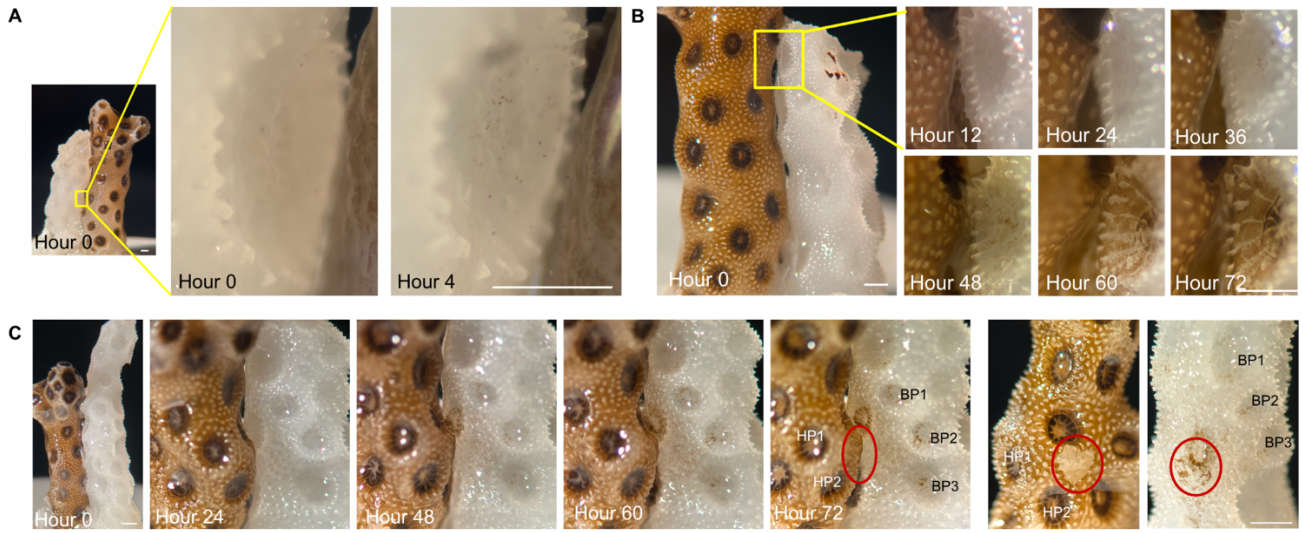


Fig. S12 Early symbiont transfer dynamics. (**A**) Example stereoscopic images of a bleached polyp in contact with a healthy coral at 0 and 4 h showing increased symbiont uptake over time. (**B**) Example stereoscopic image time series of a bleached polyp in contact with a healthy coral fragment, imaged every 12 h over 72 h, showing initial symbiont uptake from 12–36 h followed by rapid mass transfer at 48 h. (**C**) Example stereoscopic image time series of a bleached coral in contact with a healthy coral fragment, imaged every 12 h over 72 h, showing rapid mass transfer at 48 h and subsequent spread through the fragment. Separation at 72 h reveals tissue rips on both fragments at the site of initial transfer. Tissue bridges and resulting tissue tears are indicated with red circles. Labeled polyps provide reference points for orientation before and after separation. Scale bars, 1 mm.

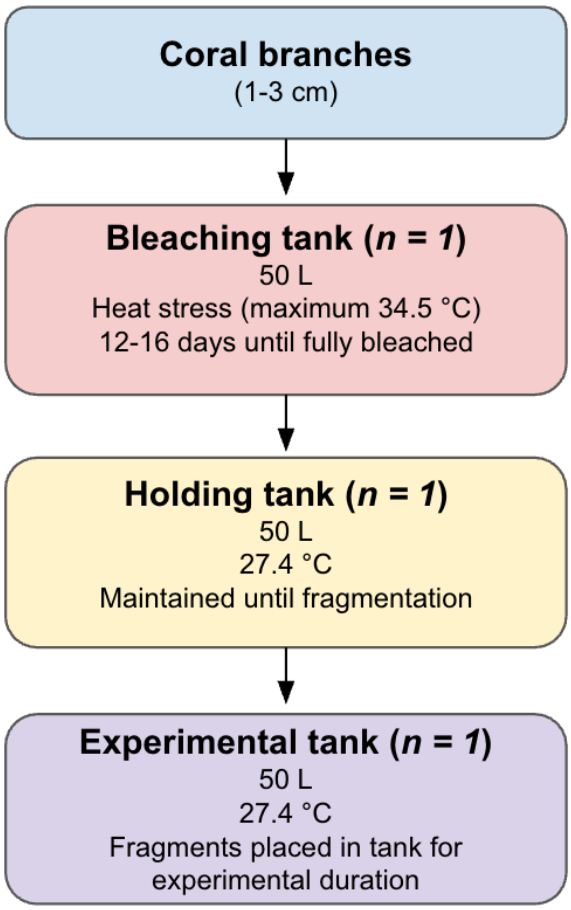


Figure S13: Overview of coral bleaching and experimental workflow. All corals were bleached simultaneously in a single bleaching tank, maintained in a holding tank, fragmented, and placed in a single experimental tank for experiments.

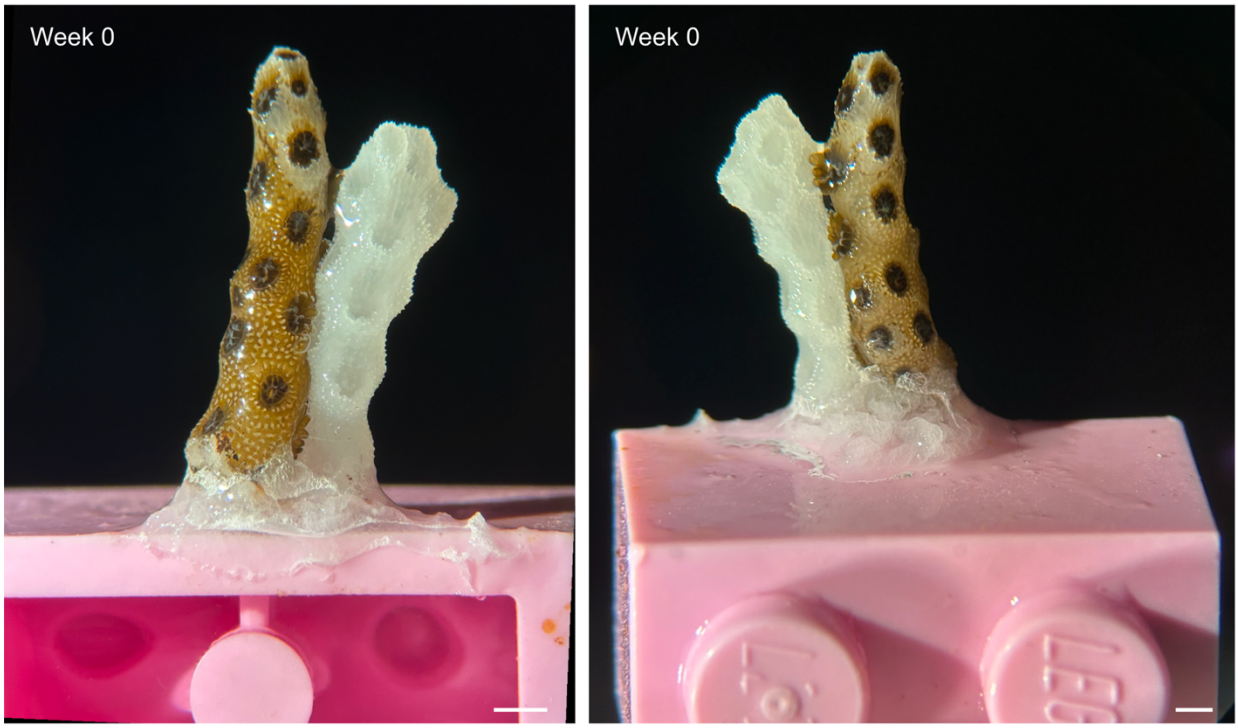


Fig. S14. Coral fragment mounting for LSCM imaging. Example stereomicroscope images of coral fragments that were affixed to 2x1 LEGO ® bricks for LSCM images depicted in **Figure 2.** Scale bars, 1 mm.

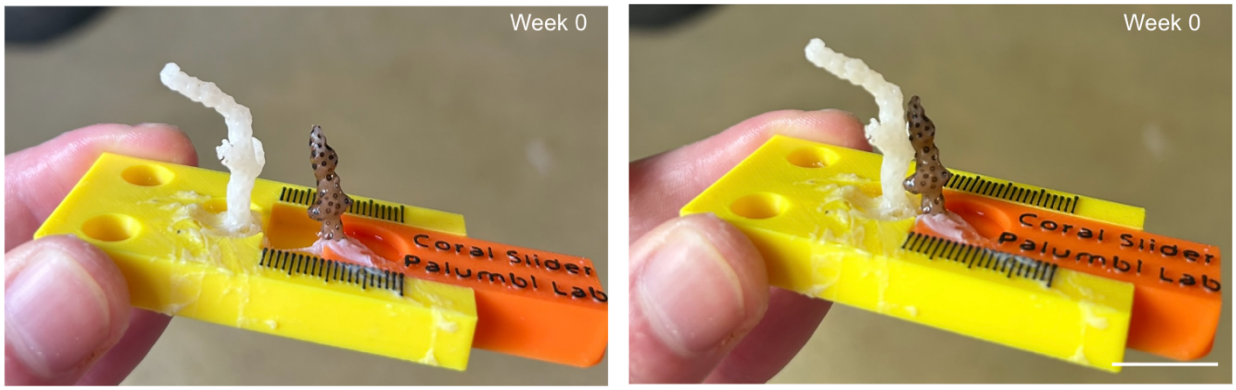


Fig. S15. Coral slider movable platform. Three-dimensional (3D)-printed coral slider showing alternating configurations of a bleached coral fragment and a healthy coral fragment separated (left) and in contact (right) at week 0. Scale bars, 2 cm.

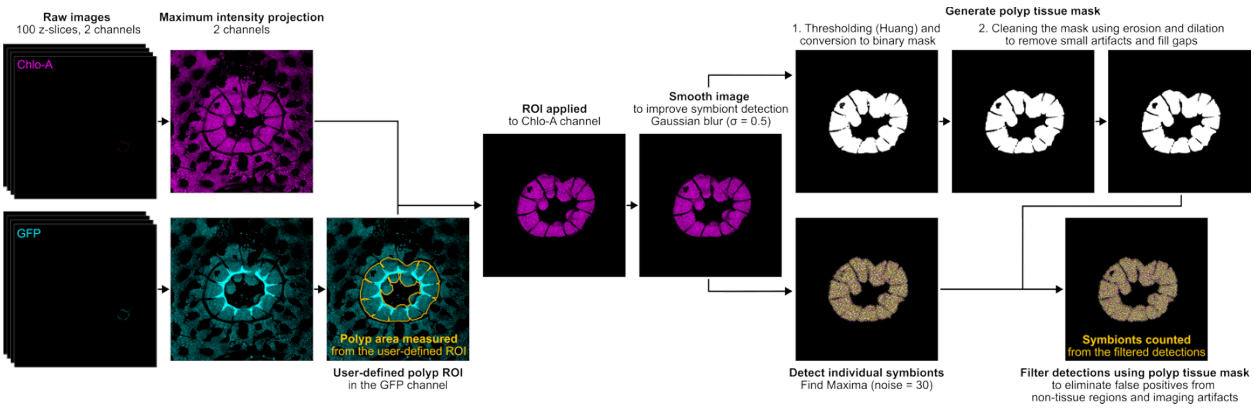


Fig. S16. Semi-automated image analysis pipeline for quantifying symbiont density. Two-channel LSCM images were separated into chlorophyll-a autofluorescence (Chlo-A; symbionts) and GFP autofluorescence (coral tissue) channels and converted to maximum intensity projections. The GFP channel was used to define a coral polyp region of interest (ROI) by subtracting the user-defined inner cavity boundary from the user-defined outer polyp boundary. The ROI area was measured and applied to the Chlo-A maximum intensity projection to restrict analysis to coral polyp tissue. The Chlo-A channel was subsequently smoothed, thresholded, and processed to generate a binary analysis mask. In parallel, symbionts were identified by local maxima detection in the smoothed Chlo-A maximum intensity projection. The binary analysis mask was then used to remove detections outside the defined coral polyp tissue region. The final symbiont count was normalized to the user-defined polyp ROI area to calculate symbiont density.

Coral fragment	Time window (days)	Exponential growth rate	Doubling time (days)	Doubling time (hours)	R^2 (coefficient of determination)
1	Early (0-6)	1.29	0.54	13.0	0.99
1	Late (6-12)	0.14	5.12	122.9	0.99
2	Early (0-6)	0.66	1.04	25.0	0.91
2	Late (6-12)	0.33	2.13	51.1	0.74
3	Early (0-6)	0.78	0.89	21.4	0.99
3	Late (6-12)	0.13	5.14	123.4	0.98
4	Early (0-6)	1.18	0.59	14.2	1.00
4	Late (6-12)	-0.01	-80.93	-1942.3	0.05
Mean ± SD	**Early (0-6)**		**0.76 ± 0.24**	**18.3 ± 5.8**	
	Late (6-12)		**4.13 ± 1.73**	**99.1 ± 41.5**	

Table S1. Symbiont doubling rates during. Exponential growth analysis of symbiont densities in bleached coral fragments across early (0–6 days) and late (6–12 days) recovery time windows. For each fragment and time window, the table shows the fitted exponential growth rate, the corresponding doubling time in days and hours, and the coefficient of determination (R^2) for the log-linear fit. These values were used to generate the predicted curves in **fig. S5**. For fragment 1, no symbionts were observed at Day 0. Therefore, a minimum value of 1 was used to allow log-transformation and exponential model fitting. Fragment 4 in the late phase (6–12 days) exhibited a negative growth rate rather than exponential growth and is therefore not considered a valid exponential fit, as reflected by its low R^2.

Experiment	Question	Sample Size	Duration	Figure
Direct contact between healthy and bleached corals	Does direct contact with healthy corals promote bleaching recovery?	n = 5 contact	9 weeks	**Fig. 1**
No contact control (5-7 mm separation)	Is contact required for recovery?	n = 5 no contact	9 weeks	**Fig. 1**
Increased fragment size	Does increased fragment size affect bleaching recovery?	n = 3 contact; n = 3 no contact	9 weeks	**Fig. S1**
Contact duration	How long of contact is required for complete bleaching recovery?	n = 12 (2 per time treatment)	1 day to 3 weeks	**Fig. 2**
LCSM-based symbiont quantification	How rapidly do the symbionts repopulate bleached polyps?	n = 4 coral pairs (3 bleached and 3 healthy polyps per pair)	12 days	**Fig. 2**
Contact recovery (green genet)	Does bleaching recovery occur in a different genet?	n = 3 contact; n = 3 no contact	9 weeks	**Fig. S6**
Mixed genet pairings	Does bleaching recovery occur cross-genet pairings?	n = 3 red-green; n = 3 green-red	9 weeks	**Fig. 3**
Symbiont redistribution during recovery	How are symbionts redistributed during recolonization of bleached tissue?	n = 6	5 weeks	**Fig. 4**
Separation of recovering coral pairs	Does separation disrupt fusion tissue bridges between corals?	n = 12	1–3 weeks	**Fig. 4**
Short-term contact and separation	Does contact and separation cause tissue damage independent of recovery?	n = 6	1 day	**Fig. S11**
Early-stage symbiont transfer dynamics	How does symbiont transfer occur during the first 3 days of contact?	n = 6	3 days	**Fig. S12**

Table S2. Experimental Design Summary.

Movie S1. Tearing of a tissue bridge between a healthy and bleached coral upon separation. A healthy and bleached coral fragment are separated after three weeks of contact, revealing corresponding tissue rips at the sites of tissue bridge formation. Rotation of both fragments shows that the tissue rips align between the two fragments.