Supporting information

Evaluating Coarse-Grained MARTINI Force-Fields for Capturing the Ripple Phase of Lipid Membranes

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Author contributions

Conceptualization: R.D.; Data curation: P.S.; Formal analysis: R.D. and P.S.; Funding acquisition: K.G.A.; Investigation: P.S. and R.D.; Methodology: R.D. and P.S.; Project administration: P.S. and R.D.; Resources: P.S., R.D. and K.G.A.; Software (simulations, analysis code): P.S. and R.D.; Supervision: K.G.A.; Validation: P.S., R.D. and K.G.A.; Visualization: P.S. and R.D.; Writing – original draft: R.D. and P.S.; Writing – review & editing: R.D., P.S. and K.G.A.

All authors have given approval to the final version of the manuscript.

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Supporting figures

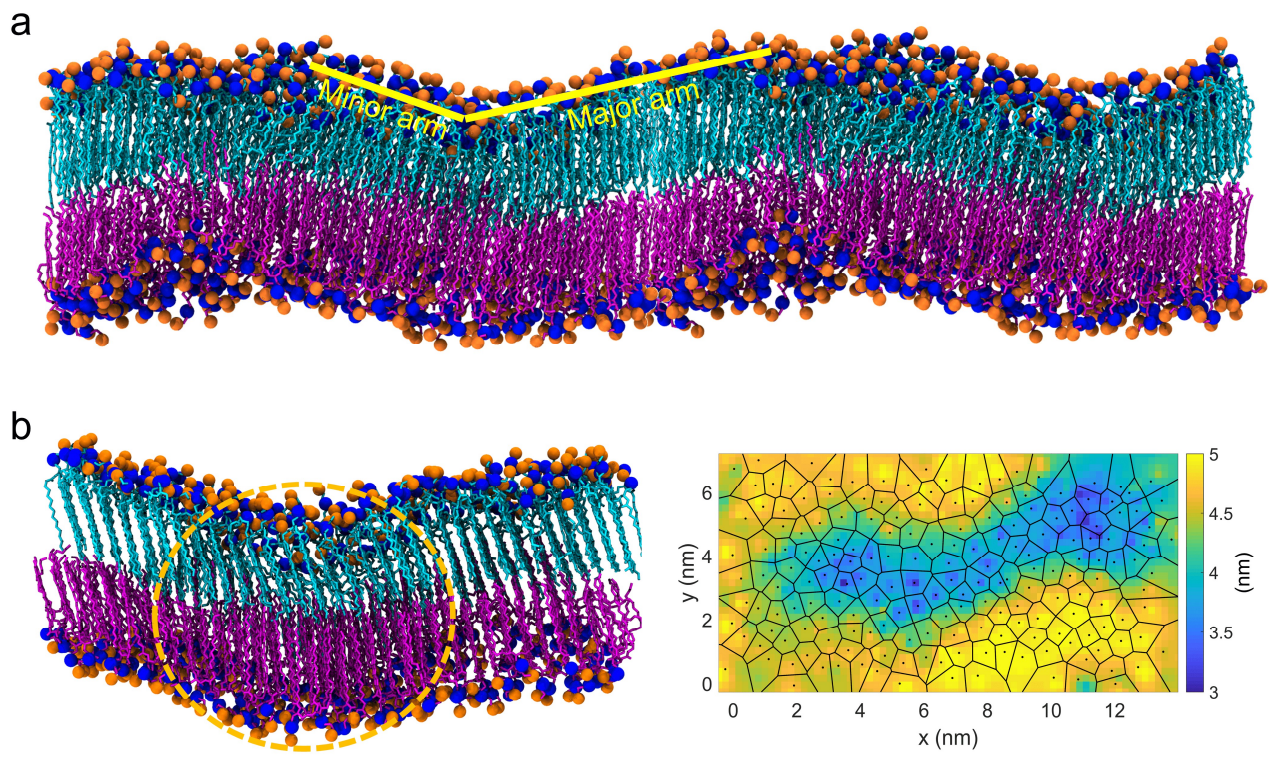

Figure S1. An independent all-atom MD replicate reproduces the ripple phase. (a) MD snapshot at time point IV from Figure 1*a* is periodically repeated along the x-axis, and the major and minor arms of the ripple in the upper leaflet are visually annotated for clarity. **(b)** Final snapshot (similar to time point IV in Figure 1) and corresponding Voronoi-thickness diagram from the independent simulated cooling replicate, using the same MD protocol as for the replicate in Figure 1, shows robust reproduction of the tilted ripple phase from all-atom simulations.

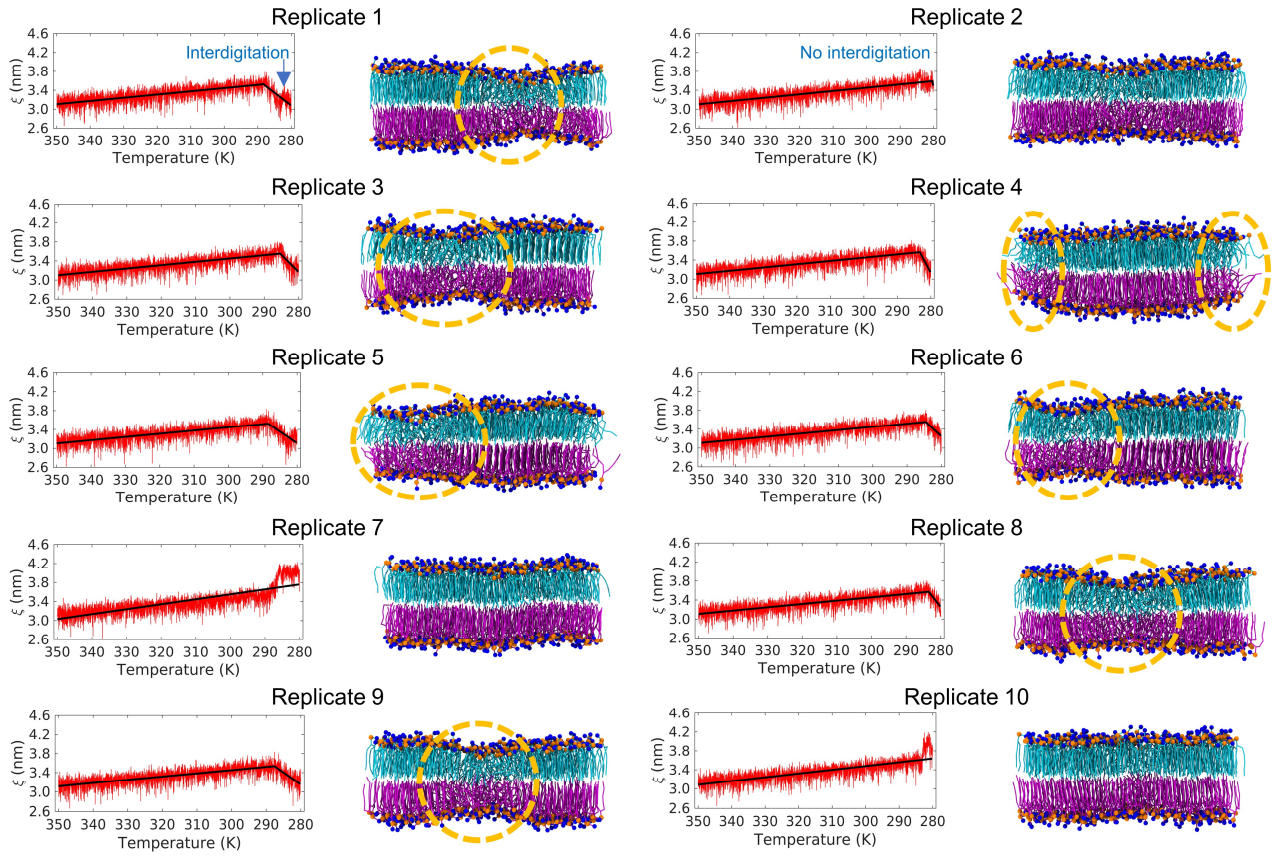

Figure S2. Summary of 10 independent cooling MD replicates with the MARTINI2n FF. Temporal evolution of the minimum inter-leaflet P-P distance (ξ), a measure of the extent of interdigitation (Figure 1*b*; $m_2 < 0$ – interdigitation, and $m_2 > 0$ – no interdigitation (Methods, Eq. 1), are highlighted in replicates 1 and 2, respectively). For example, in replicate 1, $m_1 > 0$ for T > 288.3 K, $m_2 < 0$ for T < 288.3 K and $\tau = 288.3$ K. Final snapshots corresponding to time point III at the end of cooling simulations with MARTINI2n DPPC membranes are shown for all the 10 independent MD replicates. The interdigitated domains in replicates which exhibit ripple-like structures are marked with yellow dashed circles.

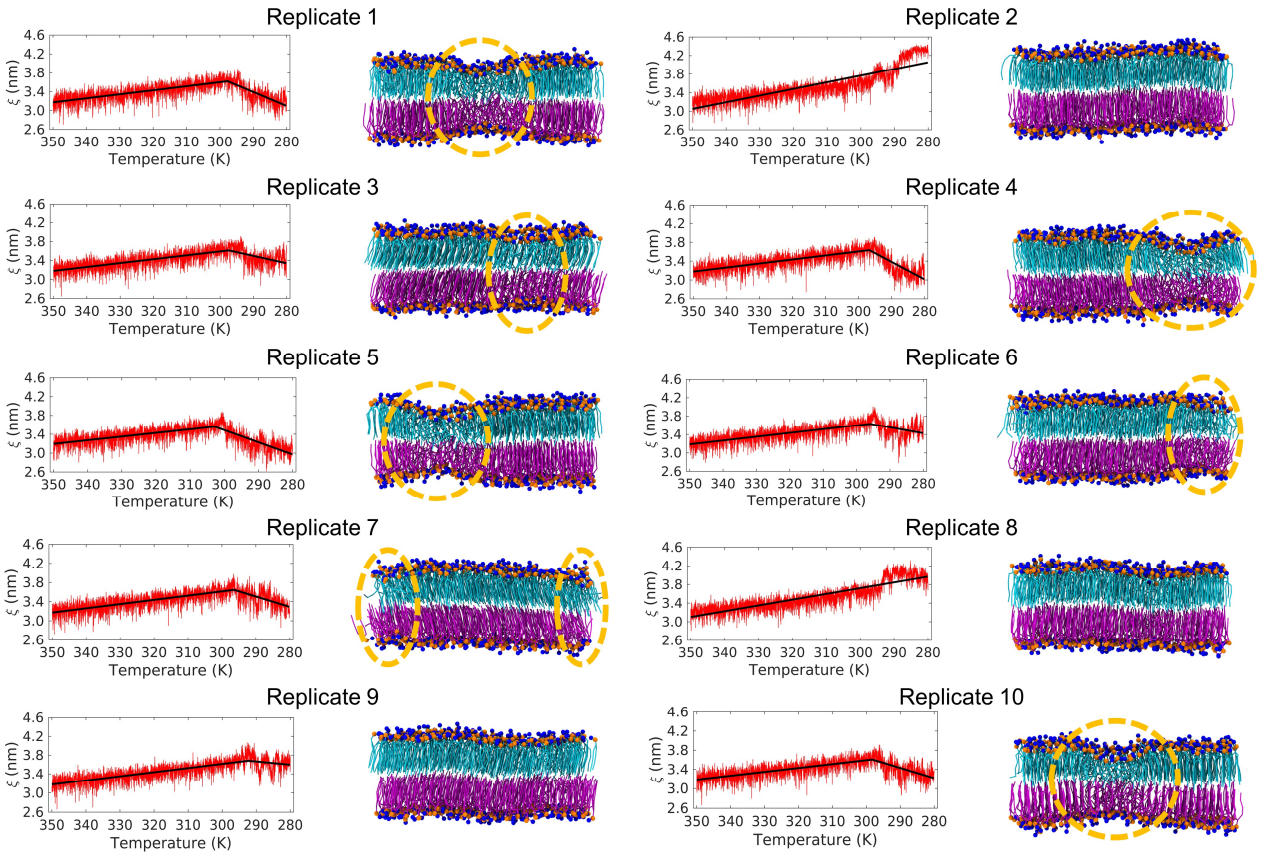

Figure S3. Summary of 10 independent cooling MD replicates with the MARTINI2On FF. Same as Figure S2, but with the MARTINI2On FF variant.

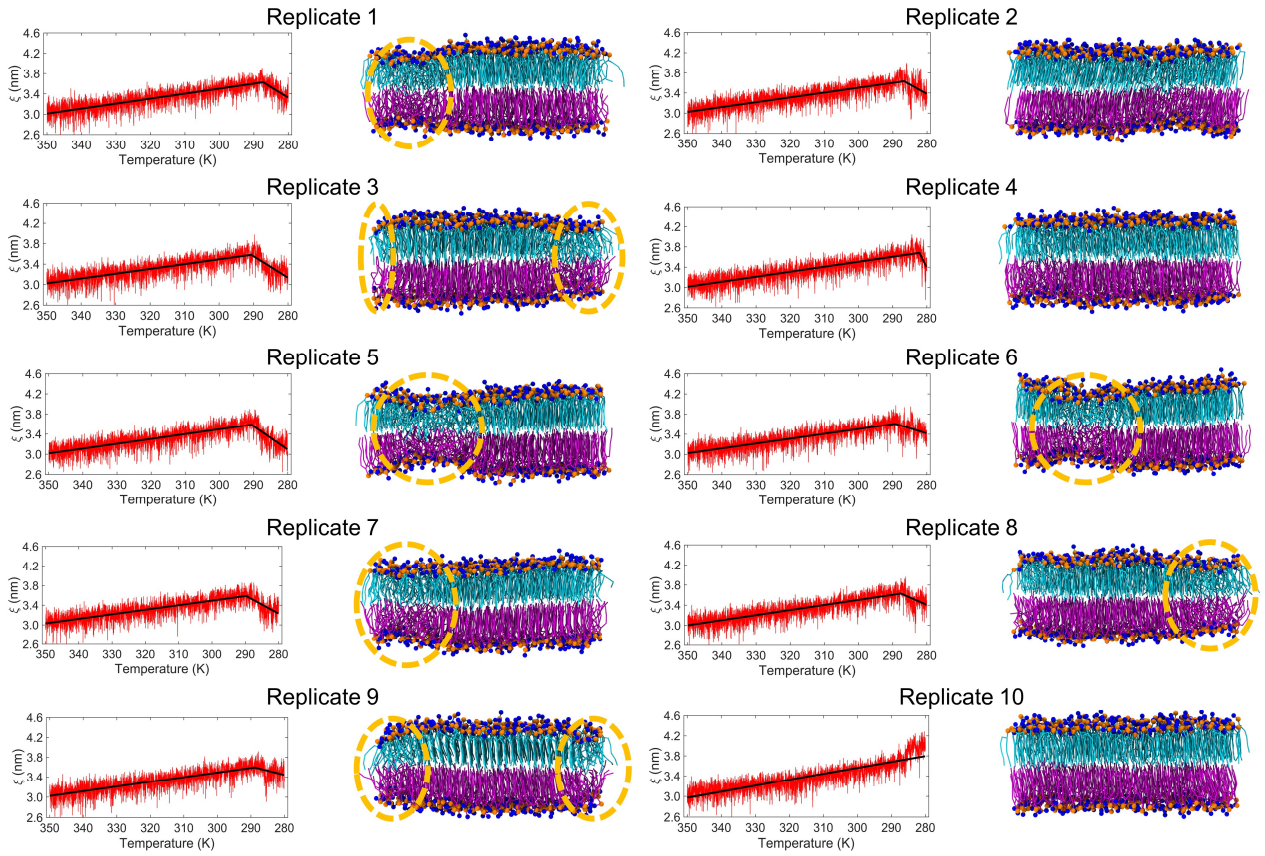

Figure S4. Summary of 10 independent cooling MD replicates with the MARTINI3a FF. Same as Figure S2, but with the MARTINI3a FF variant. Note that while the ξ trends for replicates 2 and 4 appear to indicate the onset of ripple formation, it can be inferred from visual inspection that this is due to very few interdigitating lipids and not due to the formation of a ripple-like domain. Hence, these replicates were not counted as ripple-like states.

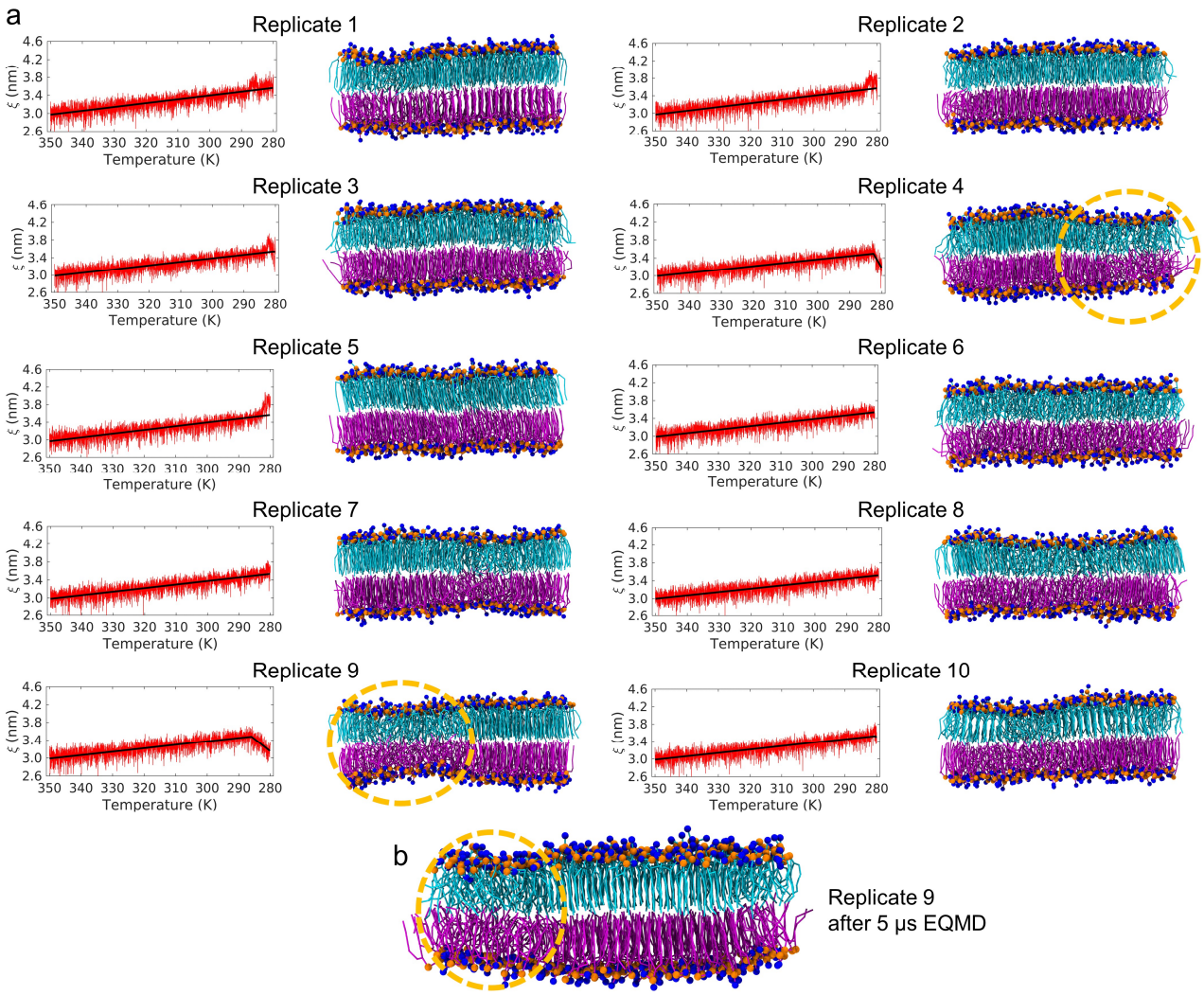

Figure S5. Summary of 10 independent cooling MD replicates with the MARTINI3b FF. (a) Same as Figure S2, but with the MARTINI3b FF variant. **(b)** Replicate 9 was further subjected to 5 μs EQMD to confirm that the ripple-like domains were stable at this timescale.

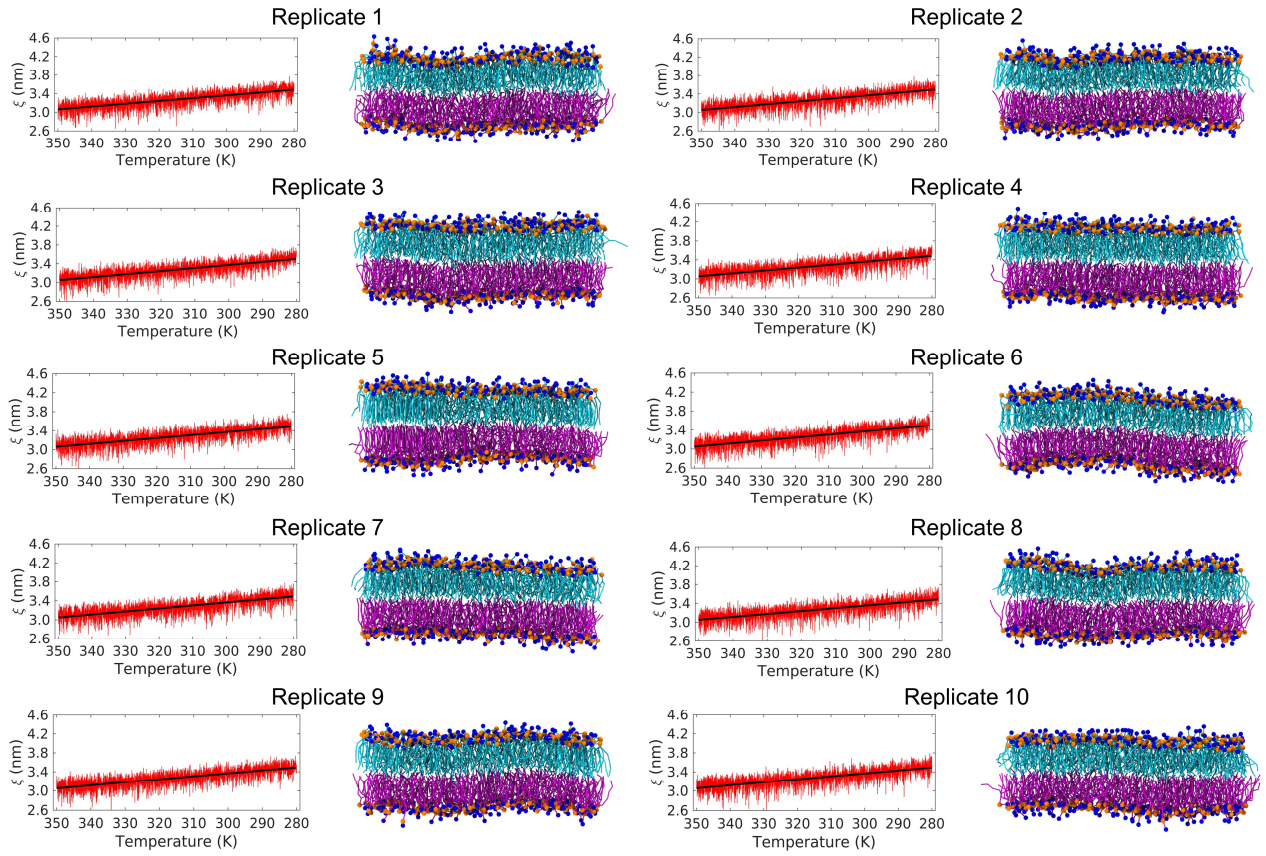

Figure S6. Summary of 10 independent cooling MD replicates with the MARTINI2p FF. Same as Figure S2, but with the MARTINI2p FF variant. Note that while none of the *ξ* plots show ripple-like domain formation during the cooling phase, replicate 5 formed shallow interdigitated ripple-like domains during the 5 μs EQMD (Figure 2*f*).

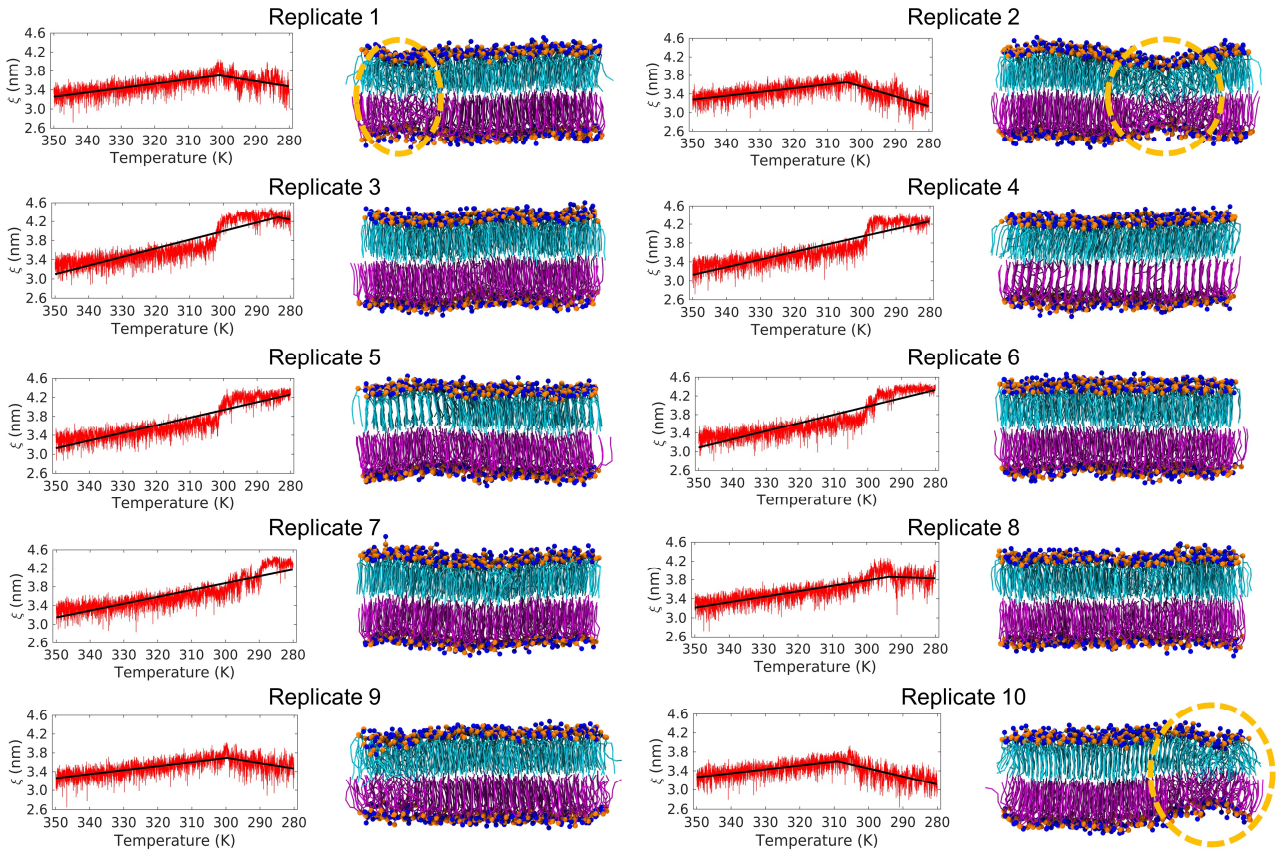

Figure S7. Summary of 10 independent cooling MD replicates with the MARTINI2Op FF. Same as Figure S2, but with the MARTINI2Op FF variant. In replicate 9, while we observed a ξ trend similar to ripple-like domain formation, visual inspection yielded no interdigitated domains, and hence this replicate was not considered as a ripple-like state.

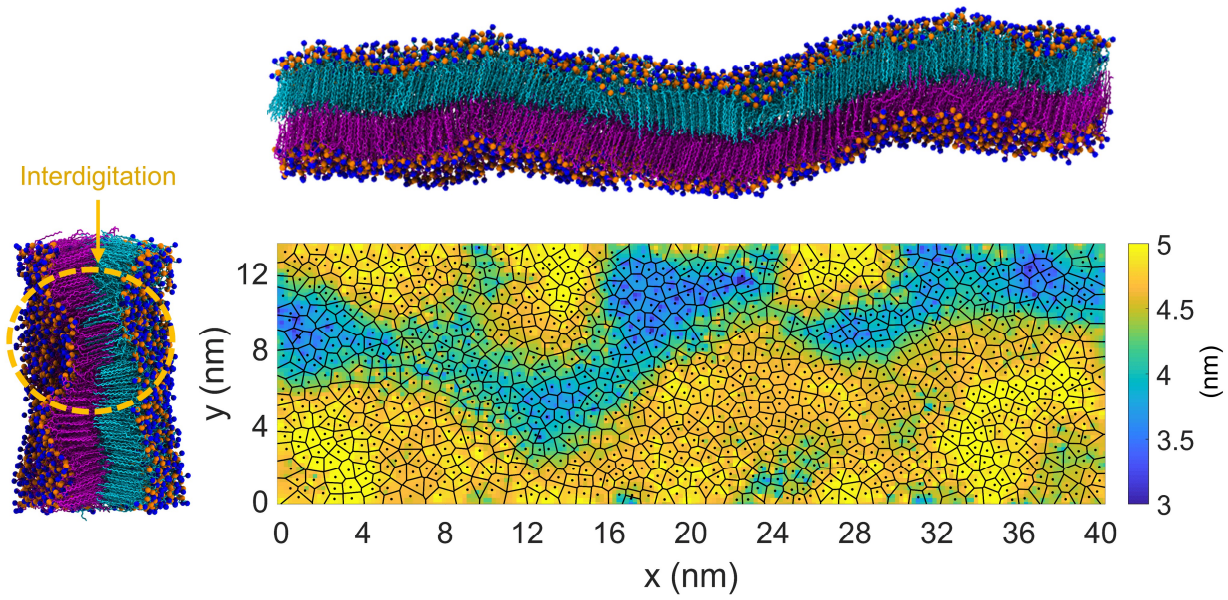

Figure S8. Independent all-atom simulation replicate also shows ripple formation in a large DPPC membrane. Similar to Figure 3, the final snapshot (front view) of a large DPPC membrane after being cooled to 290 K, and its corresponding Voronoi-thickness map, show the formation of symmetric, and asymmetric-interdigitated, ripple structures along the X-axis (longer box dimension) and Y-axis (shorter box dimension), respectively. Note that the non-interdigitated symmetric ripple along the X-axis is indiscernible from the Voronoi-thickness map.

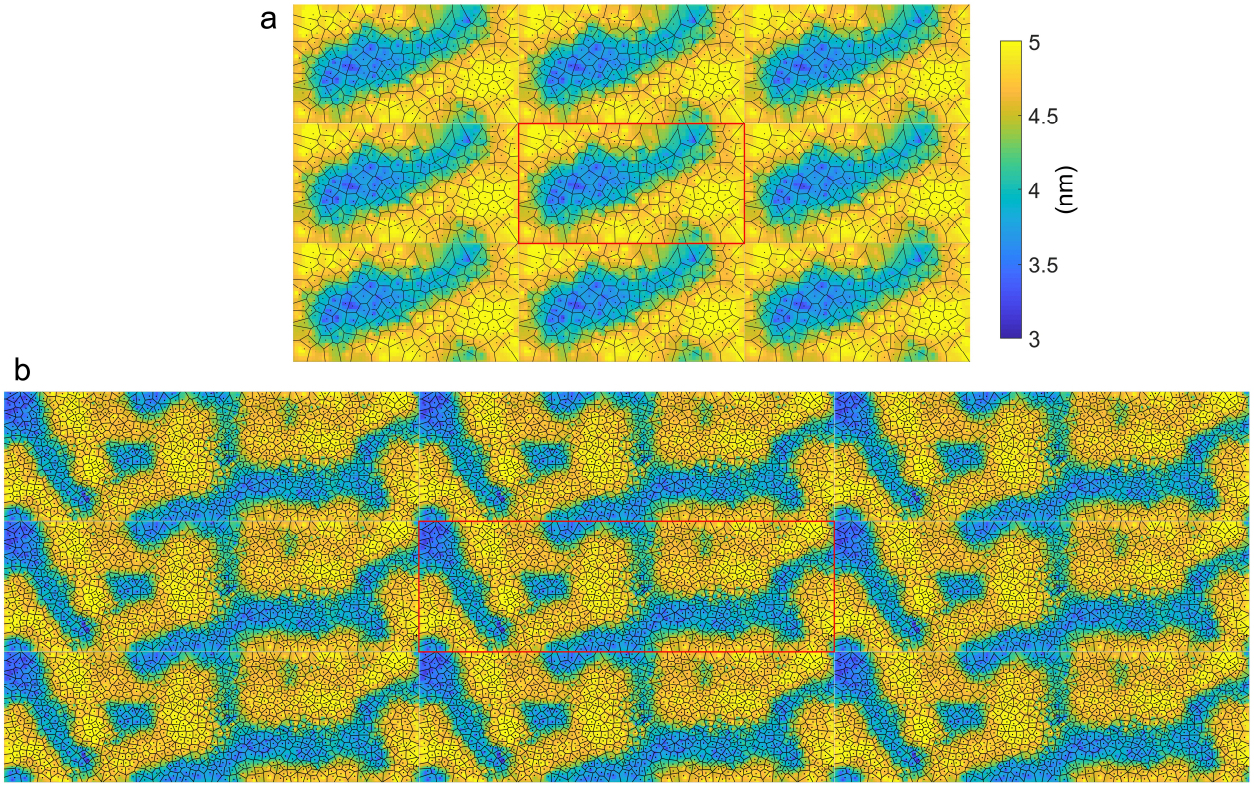

Figure S9. Periodically repeated Voronoi-thickness modulation heatmaps of atomistic DPPC membranes. Voronoi-thickness modulation heatmaps after simulated cooling of atomistic **(a)** 368-lipid (Figure 1), and **(b)** 2208-lipid (Figure 3) DPPC membranes are shown with periodic images (original simulation box in the middle shown with a red border; same thickness colorbar applies for both systems). The reticulated, interdigitated domains observed in the larger DPPC membrane are in contrast with the island-like isolated domains seen in the smaller membrane.

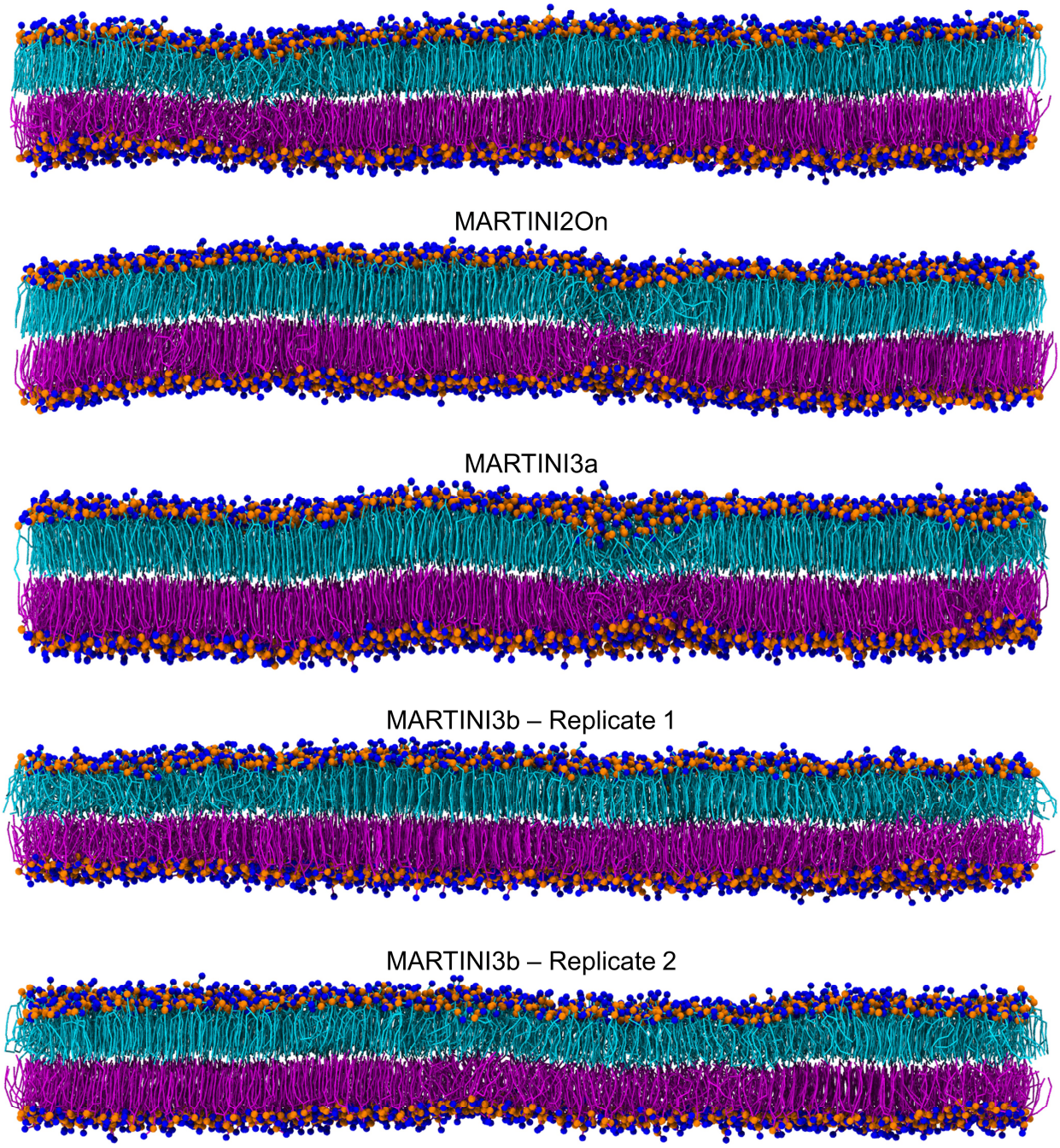

Figure S10 Independent MD replicates confirm the lack of ripple formation in large MARTINI DPPC membranes. Independent MD replicates similar to Figure 4, where large, rectangular, MARTINI2n, MARTINI2On, MARTINI3a, and MARTINI3b DPPC membranes, initially pre-equilibrated in the fluid phase at 350 K, exhibited direct transformation into the straight gel phase upon cooling to 280 K without forming either interdigitated or symmetric ripple-like structures (unlike the behaviour for smaller MARTINI membranes in Figure 2). Note: these

membranes were not subject to further EQMD after cooling, and thus a few minor domains remain in the L_α state. These are expected to disappear upon further equilibration.

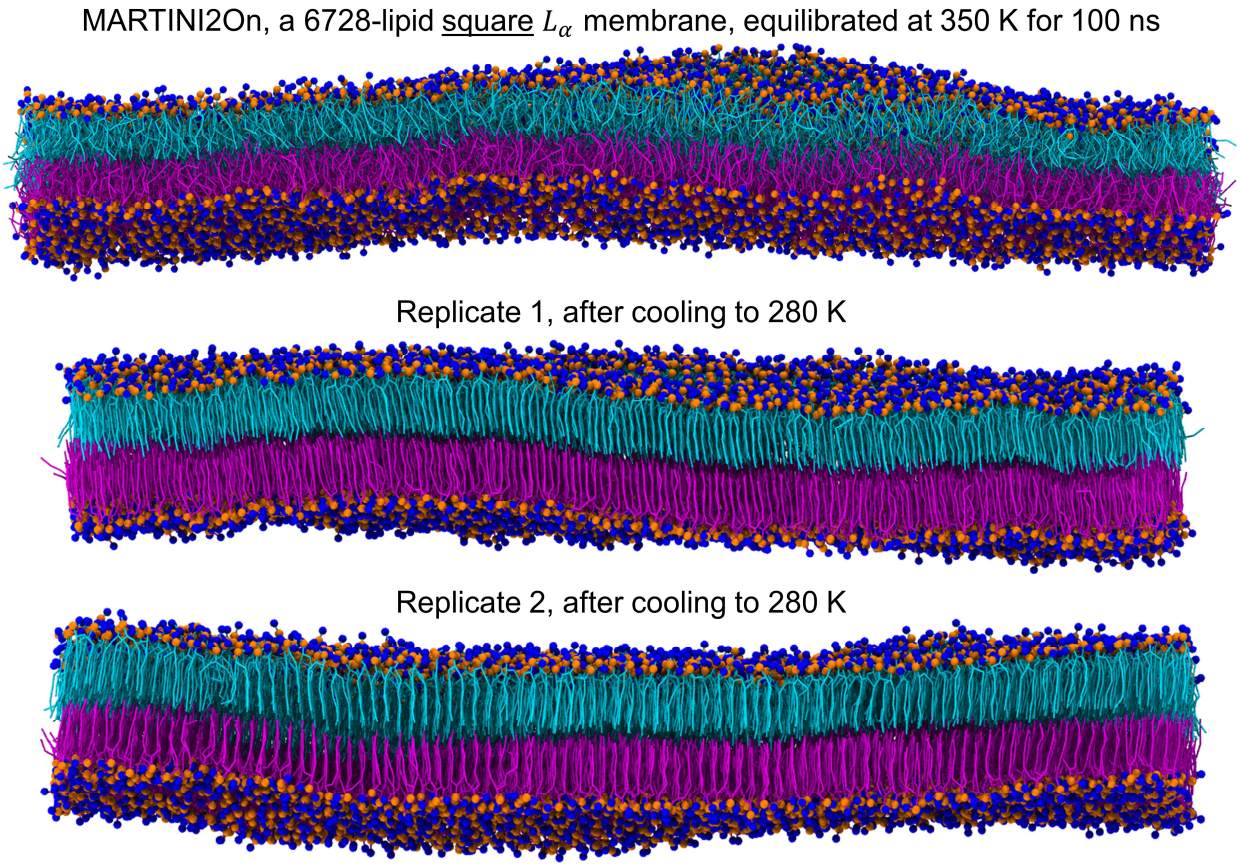

Figure S11 Large square MARTINI DPPC membranes also exhibit a lack of ripple formation. The influence of membrane geometry on ripple formation was also tested by cooling two large, square, MARTINI2On membrane replicates, from 350 K to 280 K. Similar to the rectangular membrane patches in Figure S10, both square membrane replicates directly froze into the gel phase without forming ripple membranes.

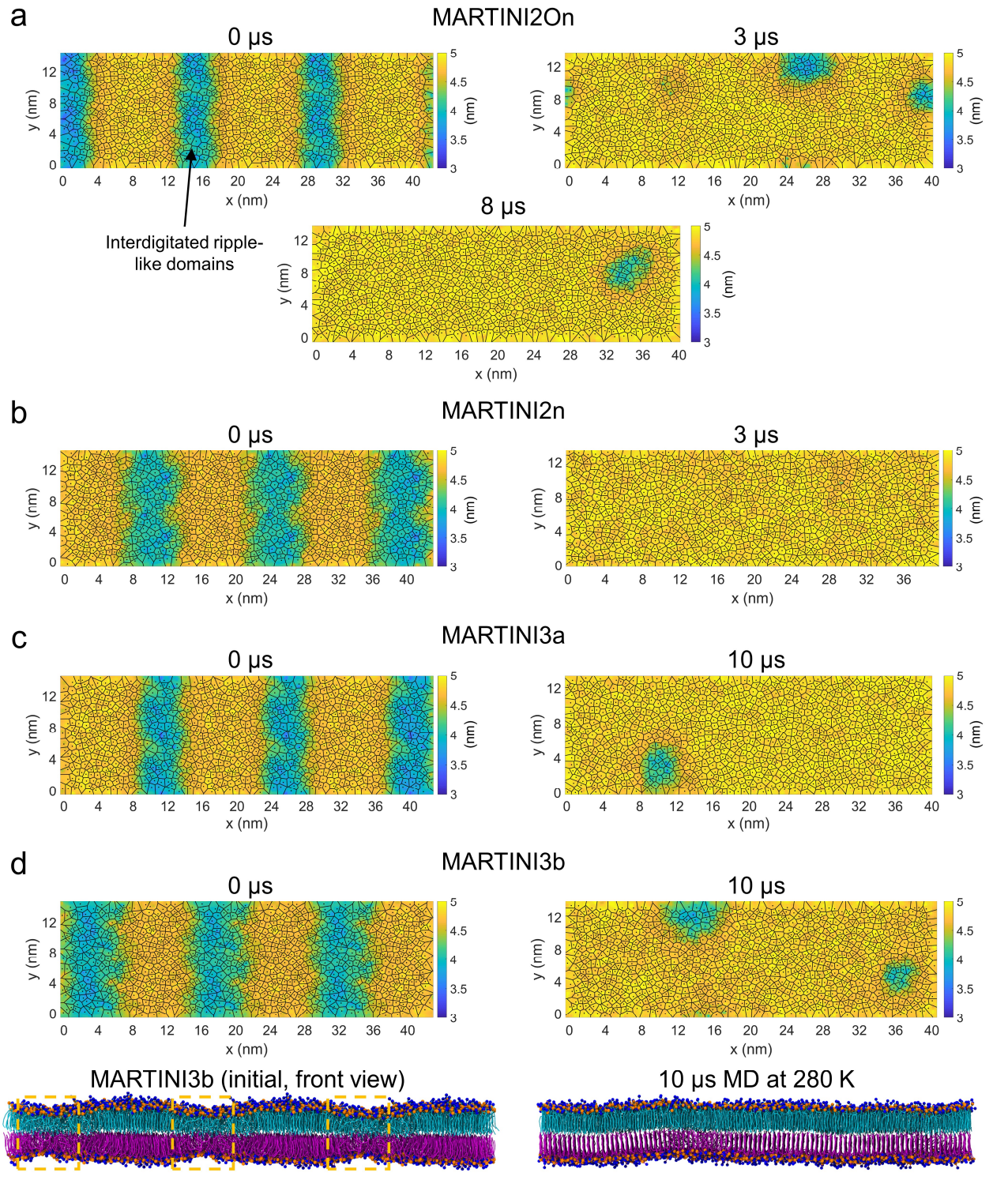

Figure S12. Voronoi-thickness maps from MD of large MARTINI membranes. Voronoi-thickness map from constant temperature MD of large **(a)** MARTINI2On, **(b)** MARTINI2n, **(c)** MARTINI3a, and **(d)** MARTINI3b membranes at 280 K, corresponding to MD snapshots in Figure 5 (snapshots for MARTINI3b shown here). Simulations starting with a periodic ripple-like configuration (0 μs), shows progressive loss of the interdigitated ripple-like domains and formation

of the gel phase by 8 μs and 10 μs for the MARTINI2On and MARTINI3a/b membranes, respectively. Notably, the MARTINI2n membrane shows complete loss of interdigitation by 3 μs. While the blue regions at the initial time points (0 μs) correspond to interdigitated domains, they could correspond to either metastable interdigitated (continuously decreasing), or non-interdigitated but partially melted domains, at the final time points. This distinction can be made by simultaneously comparing the MD snapshots (Figure 5 for MARTINI2On, MARTINI2n, MARTINI3a, and above for MARTINI3b) with the Voronoi-thickness maps above.

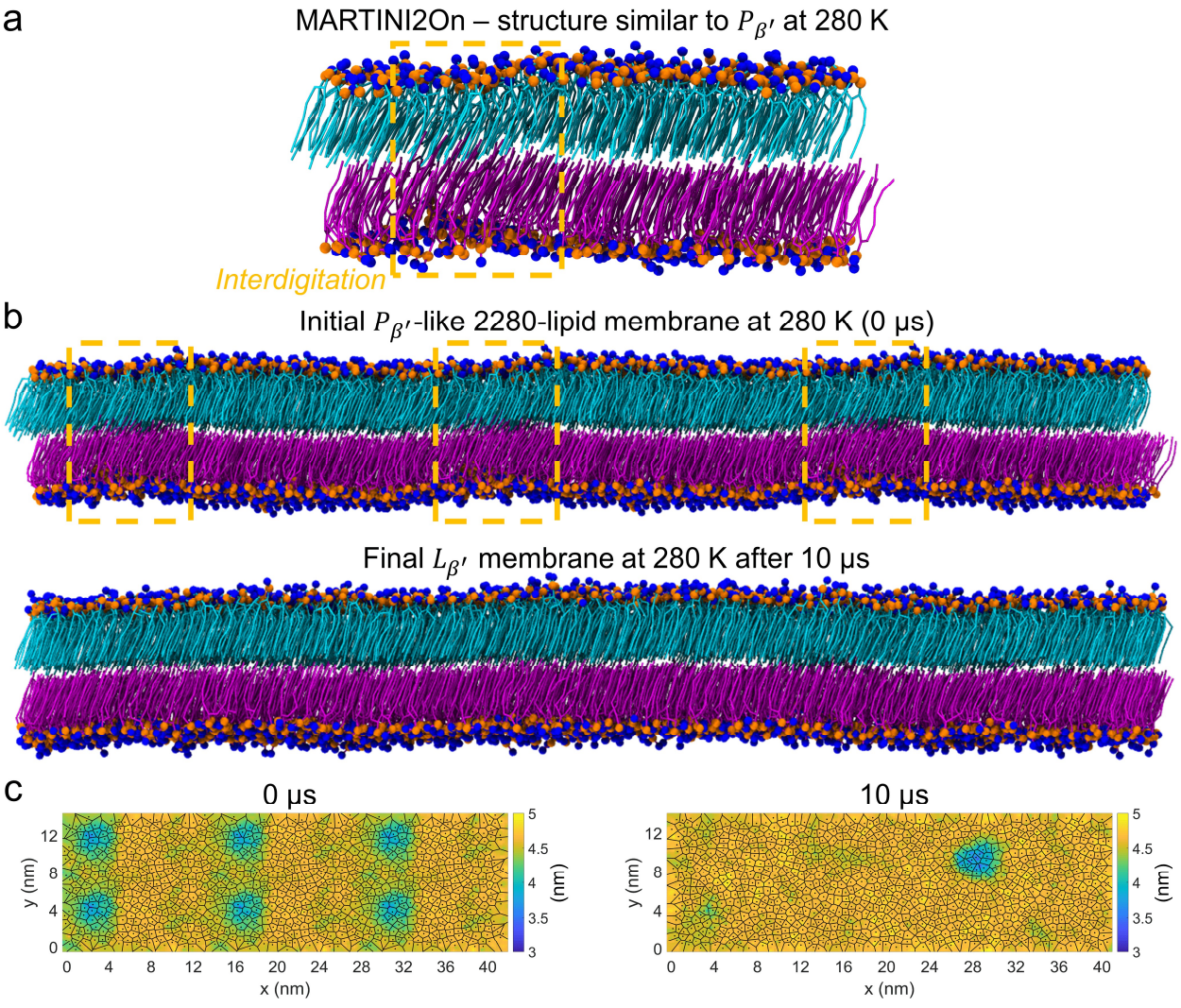

Figure S13. The tilted ripple-like state of a MARTINI2On membrane is also a finite size artefact. Simulated cooling of replicate 3 with the MARTINI2On FF (from Figure S3) showed the formation of an interdigitated domain in an otherwise $L_{\beta'}$ membrane at 280 K. The interdigitation was enhanced upon performing constant temperature MD for 5 μs: final snapshot shown in **(a)**. **(b)** To explore whether this was a finite size effect or a true ripple phase, the smaller membrane was replicated 6x across periodic boundaries to create a larger membrane with repeated ripple-like domains (snapshot at 0 μs), similar to Figure 5*a*. After 10 μs constant temperature MD, the interdigitated ripple-like regions were greatly diminished, thus confirming that the ripple-like state in (a) was indeed a finite size artefact. **(c)** Voronoi-thickness maps pertaining to MD snapshots in (b), where regions of lower thickness in blue indicate the ripple-like domains.