**Supporting Information for**

Engineering cardiolipin binding to an artificial membrane protein reveals determinants for lipid-mediated stabilization

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Tables S1 to S3

Table S1.
Cryo-EM data collection and processing statistics.

| **Data collection and processing** | **ROCKET** | **ROCKETAAXWA** |
| --- | --- | --- |
| Magnification | 165,000 | 165,000 |
| Voltage (kV) | 300 | 300 |
| Electron exposure(e–/Å2) | 60 | 60 |
| Defocus range (μm) | -0.6 to -1.8 | -0.6 to -1.8 |
| Pixel size (Å) | 0.5076 | 0.5076 |
| Number of images | 10,002 | 10,000 |
| Symmetry imposed | C4 | C4 |
| Particles refined | 98,241 | 142,434 |
| Map resolution (Å)FSC threshold | 3.770.143 | 3.890.143 |
| Map sharpening B-factor | -133 | -164 |

Table S2.

Forward and reverse primers for mutagenesis based on the ROCKET sequence.

|  |  |  |
| --- | --- | --- |
| **Mutation** | **Primer sequence** (**Forward**/Reverse) | **Tm (C°)** |
| R9A/K10A/R13A(AAXWA) | **ctgggcgACCATTATGCTGTTACTG**GTTTTCTGTGTCTTCTATCAcgccgcta | 57 |
| A61P | **GGTTGTTATTccgCTGTTGCTCAG**CGAATAGCGGAATTACGA | 60 |
| D7A/S8R | **tcgcAAGATCTGGCGTACCATTATG** CTATATGTATACAGTTTTCTGTGTCTTcgagc | 62 |

Table S3.

Protein variants; expressed sequence and theoretical molecular weight (MW).

|  |  |  |  |
| --- | --- | --- | --- |
| **Protein** | **Protein sequence (Purification tag)** | **Theoretical MW (Da)** |  |
| ROCKET | MSKDTEDSRKIWRTIMLLLVFAILLSAIIWYQITTNPDTSQIATLLSMQLLLIALMLVVIALLLSRQTEQVAESIRRDVSALAYVMLGLLLSLLNRLSLAAEAYKKAIELDPNDALAWLLLGSVLEKLKRLDEAAEAYKKAIELKPNDASAWKELGKVLEKLGRLDEAAEAYKKAIELDPEDAEAWKELGKVLEKLGRLDEAAEAYKKAIELDPNDLEHHHHHH | 25 226.44 |  |
| ROCKETAAXWA | MSKDTEDSAAIWATIMLLLVFAILLSAIIWYQITTNPDTSQIATLLSMQLLLIALMLVVIALLLSRQTEQVAESIRRDVSALAYVMLGLLLSLLNRLSLAAEAYKKAIELDPNDALAWLLLGSVLEKLKRLDEAAEAYKKAIELKPNDASAWKELGKVLEKLGRLDEAAEAYKKAIELDPEDAEAWKELGKVLEKLGRLDEAAEAYKKAIELDPNDLEHHHHHH | 24 999.13 |  |
| ROCKETA61P | MSKDTEDSRKIWRTIMLLLVFAILLSAIIWYQITTNPDTSQIATLLSMQLLLIALMLVVIPLLLSRQTEQVAESIRRDVSALAYVMLGLLLSLLNRLSLAAEAYKKAIELDPNDALAWLLLGSVLEKLKRLDEAAEAYKKAIELKPNDASAWKELGKVLEKLGRLDEAAEAYKKAIELDPEDAEAWKELGKVLEKLGRLDEAAEAYKKAIELDPNDLEHHHHHH | 25 252.48 |  |
| ROCKETD7A/S8R | MSKDTEARRKIWRTIMLLLVFAILLSAIIWYQITTNPDTSQIATLLSMQLLLIALMLVVIALLLSRQTEQVAESIRRDVSALAYVMLGLLLSLLNRLSLAAEAYKKAIELDPNDALAWLLLGSVLEKLKRLDEAAEAYKKAIELKPNDASAWKELGKVLEKLGRLDEAAEAYKKAIELDPEDAEAWKELGKVLEKLGRLDEAAEAYKKAIELDPNDLEHHHHHH | 25 251.54 |  |
| ROCKETR66A | MSKDTEDSRKIWRTIMLLLVFAILLSAIIWYQITTNPDTSQIATLLSMQLLLIALMLVVIALLLSAQTEQVAESIRRDVSALAYVMLGLLLSLLNRLSLAAEAYKKAIELDPNDALAWLLLGSVLEKLKRLDEAAEAYKKAIELKPNDASAWKELGKVLEKLGRLDEAAEAYKKAIELDPEDAEAWKELGKVLEKLGRLDEAAEAYKKAIELDPNDLEHHHHHHHHHH | 25 689.90 |  |