Supplemental Tables

Table S1. Table S1. Many of the malectin RLKs in the same subfamily as FERONIA contain the A/GXXXA/G motif, putatively for transmembrane domain association.

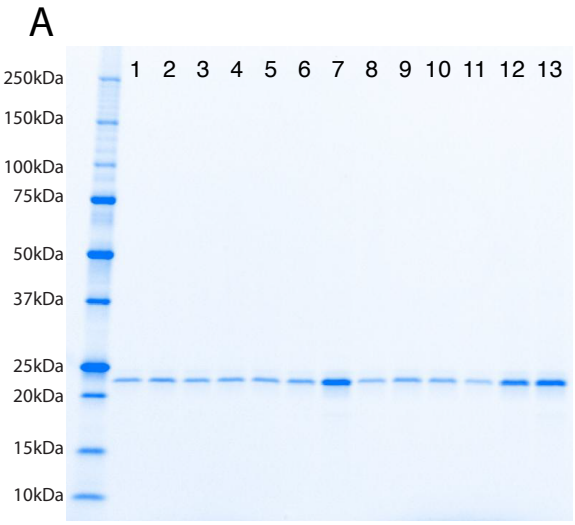
Id	Name	Uniprot	TM reg.	TM sequence	VDW	HBOND	TOTAL
AT3G04690	ANXUR1	Q9SR05	430-450	AFIIGSAGGVLAVLIGALCFT	-31.0535	-13.8915	-44.94500
AT5G28680	ANXUR2	Q3E8W4	432-452	ITAFVIGSAGGVAAVLFCALC	-39.8268	-9.1564	-48.98328
AT3G51550	FERONIA	Q9SCZ4	448-468	IIAGAASGAVVLALIIGFCVF	-26.1953	-15.5915	-41.78680
AT3G46290	HERCULES1	Q9LX66	406-426	LIVGSAIGSLLAVVFLGSCFV	-39.0302	-6.4260	-45.45628
AT5G59700		Q9FN92	407-427	IIGLTIGSLLALVVLGGFFVL	-38.9866	-4.7651	-43.75173
AT2G39360		O80623	408-428	VGLIAGLSAALCVALVFGVVV	-34.6008	-8.5028	-43.10366
AT4G39110		Q9T020	441-461	MVATAGFVMMFGAFIGLGAMV	-32.0543	-24.2691	-56.32340
AT2G21480		Q9SJT0	440-460	MVATAGFVMMFGAFVGLGAMV	-32.4248	-24.0164	-56.44120
AT5G61350	ERULUS	Q9FLJ8	426-446	IAGIGFVMALTAFLGVVVLLV	-35.0172	-5.8159	-40.83310
AT5G54380	THESEUS1	Q9LK35	416-436	AVIIGSLVGAVTLILLIAVCC	-30.6328	-9.9691	-40.60194
AT5G24010		Q9FLW0	407-427	VVWIVVGSVLGGFVFLSLFFL	-48.5259	-11.7605	-60.28640
AT1G30570	HERCULES2	Q9SA72	430-450	IIWISVGAGIAIIIFFVFLGI	-76.0560	-5.8364	-81.89244
AT2G23200		O22187	406-426	CAVAAAAASALVFSLLFMVFL	-16.5211	5.7695	-10.75156
AT5G39000		Q9FID8	446-466	LAVVGSLVVLAMFVVGVLVIM	-18.8988	0.2550	-18.64379
AT5G38990		Q9FID9	441-461	IIIAVVGSAVALAFFVLVVVL	-32.2886	-8.0407	-40.32937
AT5G39020		Q9FID6	438-458	LVIILIVVGSVIGLATFIVII	-32.7719	-8.9597	-41.73161
AT5G39030		Q9FID5	444-464	IFIAVGPGTGLATFVVVLMLW	-42.0533	-12.0869	-54.14020

Table S2. Sequences of primers used for cloning.

Primer Table			
Experiment	Specific Primers	Sequence Forward (5'->3')	Sequence Reverse, (5'->3')
Feronia into pEU cell-free vector	cHIS_Feronia_cellfree	cattctacaactacagccatgaagatcacagagggacgattcc	ctagtggtgatgatggtgatgacgtccctttggattcatgatc
Feronia Truncations using Gibson Cloning	pEU_backbone_amp	gtttaaacgaattcgagctcggtac	ggctgtagttgtagaatgtaaaa
Feronia Truncations using Gibson Cloning	KD_CT	attctacaactacagccatggatgagtcccgggtg	gctcgaattcgtttaaacctagtggtgatgatggtgatg
Feronia Truncations using Gibson Cloning	JM_KD_CT	attctacaactacagccatgggtgcttaccgcaga	gctcgaattcgtttaaacctagtggtgatgatggtg
Feronia Truncations using Gibson Cloning	TM_JM_KD_CT	attctacaactacagccatgattattgcaggcgcag	gctcgaattcgtttaaacctagtggtgatgatggtg
Feronia mutagenesis	Y495F	tgtgagtttccaaacagagatagtggaagccaccct	agggtggcttccactatctctgtttggaaactcaca
Feronia mutagenesis	K663R	catccaatagaatgtttgttgtcctcacatctctatggatgattgtg	cacaatcatccatagagatgtgaggacaacaaacattctattggatg
Feronia mutagenesis	K684R	tctagtgtaggaccagtcctcgatagaccaaaatcag	ctgattttggtctatcgaggactggtcctacactaga
Feronia mutagenesis	T696A	accacacacacgtaagcgcagttgtgaaaggaagt	acttcctttcacaactgcgcttacgtgtgtgtggt
Feronia mutagenesis	T696D	accgaaacttcctttcacaacatcgcttacgtgtgtgtggtctag	ctagaccacacacacgtaagcgatgttgtgaaaggaagtttcggt
Feronia mutagenesis	K699R	ccgaaacttcctctcacaactgtgcttacgtgtgt	acacacgtaagcacagttgtgagaggaagtttcgg
Feronia mutagenesis	S701A	ctgggtcaagataaccgaaagctcctttcacaactgtgctta	taagcacagttgtgaaaggagctttcggttatcttgacccag
Feronia mutagenesis	S701D	tctgggtcaagataaccgaaatctcctttcacaactgtgcttac	gtaagcacagttgtgaaaggagatttcggttatcttgacccaga
Feronia mutagenesis	S871A	atcttcgctggccaaagccctaccaccgatgctc	gagcatcggtggtagggctttggccagcgaagat
Feronia mutagenesis	S871D	gaatcttcgctggccaaatccctaccaccgatgctcat	atgagcatcggtggtagggatttggccagcgaagattc
Feronia mutagenesis	S877A	gagtgagtccatctgcatcttcgctggccaaact	agtttggccagcgaagatgcagatggactcactc
Feronia mutagenesis	S877D	gcacttggagtgagtccatcatcatcttcgctggccaaactcc	ggagtttggccagcgaagatgatgatggactcactccaagtgc

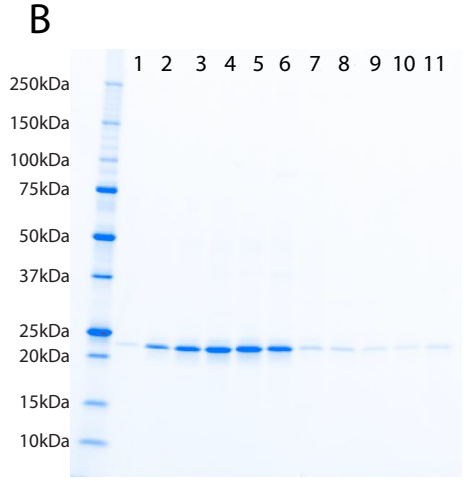
Supplemental Figures

Figure S1. A, lipid optimization, step 1. MSP1C1-C-strep(II) was synthesized with liposomes or polymersomes (1.2 mg/mL or 1.6 mM) using WEPRO2240 at ambient temperature for 17 hours. Reactions were centrifuged (14 krpm, 18000 x g, for 3 min) to separate supernatant and pellet fractions. The supernatant was used for small-scale StrepTactin purification. The eluates were fractionated into supernatant (S) and pellet (P) by centrifuging at 14 krpm (18000 x g) for 3 min. S fractions of the eluates were analyzed on SDS-PAGE. B, lipid optimization, step 2. Proteins were synthesized with cardiolipin (CL) at varying cencentrations, DDM, or phosphatidic acid (PA) using WEPRO2240 at ambient temperature for 24 hours, with the protocol as listed in (A). Post addition of CL was done after translation reaction and before centrifugation.



1 - control (no addition)
2 - Soy liposome
3 - Soy PC
4 - POPC:POPE:CL (2:2:1)
5 - POPE:POPG (3:1)
6 - POPE
7 CL (1/5; 0.32 mM)
8 - POPC:POPE (1:1)
9 - PA:PC (1:1, from egg)
10 - PA:PC (1:3, from egg)
11 - POPC:CL (4:1)
12 - POPE:CL (4:1)
13 - BdEO:CL (4:1)

Abbreviations:
PC - phosphatidylcholine
PA - phosphatidic acid
CL - Tetraoleoyl cardiolipin
POPC - 1-Palmitoyl-2-oleoylphosphatidylcholine
POPE - 1-Palmitoyl-2-oleoyl-3-phosphoatidylethanolamine
POPG - 1-Palmitoyl-2-oleoyl-3-phosphatidylglycerol
BdEO - Poly(butadiene)-block-poly(ethylene oxide)



1 - CL 0mM
2 - CL 0.16mM
3 - CL 0.32 mM
4 - CL 0.48mM
5 - CL 0.64mM
6 - CL 0.80mM
7 - DDM 0.17mM (CMC)
8 - DDM 0.085mM (1/2 CMC)
9 - PA 1.6mM
10 - PA 0.8mM
11 - Post translation addition of CL 0.32mM

Abbreviations:
PA - phosphatidic acid
CL - Tetraoleoyl cardiolipin
DDM - n-Dodecyl-β-D-maltopyranoside
CMC - Critical micelle concentration

Figure S2. Affinity-purified FERONIA translated with MSP1D1 and cardiolipin as described above was diluted 50-fold into 18MΩ H2O and spotted onto 300 mesh carbon film grids (Electron Microscopy Sciences), negatively stained with Nano-W stain (Nanoprobes), and imaged on a Philips CM120 STEM Electron Microscope operating at 80KV. Image was collected with an AMT BioSprint 12 series digital camera using AMT Image Capture Software Engine V700. Magnification is represented by scale bar shown directly on sample image.

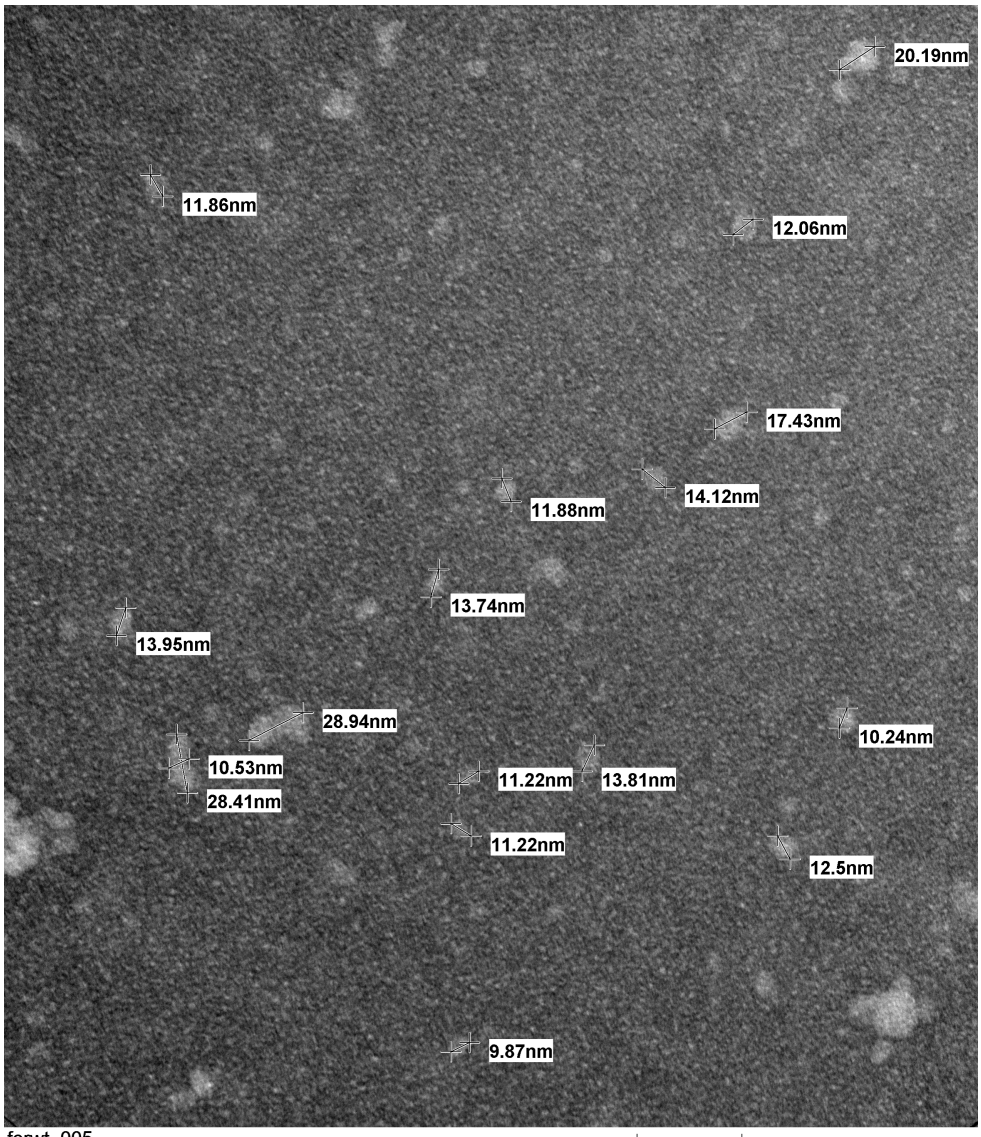


Figure S3. Batches of produced mutational variants, with respective WT control expression and molecular weight ladders. A, B, and C refer to 3 independent batches.