Supplementary Information

Dynamic pre-structuration of lipid nanodomain-segregating remorin proteins

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Supplementary Figure 1: Amino acid sequence conservation within the Remorin family¹. a Sequence motif conservation in bits based on the MEME suite². b Sequence alignment and visualizing by Blast (NCBI) and Clustal Omega, respectively.^{3,4}



Supplementary Figure 2: Ensemble of 5 monomeric structures of remorin family members predicted by Alphafold 2⁵ and colored dependent on the pLDDT score (blue: pLDDT > 90; cyan 90 > pLDDT > 70; yellow 70 > pLDDT > 50; red pLDDT < 50, see also Figure 1 in the main text).



Supplementary Figure 3: 2D ¹H-¹⁵N solution NMR spectra members recorded at 800MHz using the SOFAST-HMQC pulse sequence of 10 selected REM-CA sequences of remorin family members, as detailed in the lower right panel. The residue-specific assignments of the ¹H and ¹⁵N chemical shifts are indicated on the spectra. Residues are numbered as indicated in the lower right panel.



Supplementary Figure 4: Structure propensity of REM-CA structures simulated with atomistic molecular dynamics simulations using the AMOEBA, the AMBER ff99sb and the AMBER ff14sb forcefield over 1 μ s respectively. The color code for structural motifs is indicated on the upper left panel. Indicated below is a cartoon of the secondary structure as determined by AF2 performed on the respective REM-CA, CcpNMR analysis⁶, CNS structure calculation ⁷, the R.M.S.F of MD simulations and the R.M.S.D of the NMR structures.





Supplementary Figure 5: 2D ¹H-¹⁵N SOFAST-HMQC solution NMR spectra of 3 extended REM-CA sequences, namely StREM₁₅₀₋₁₉₈ (green), StREM₁₆₀₋₁₉₈ (pink) and StREM₁₇₁₋₁₉₈ (purple). The primary sequences of the three StREM sequences are denoted above. Residue-specific chemical shift assignment of extended StREM REM-CAs is indicated on the spectra with the color code specified in the lower right panel.



Supplementary Figure 6: Structure propensity of extended REM-CA structures of StREM₁₅₀₋₁₉₈, StREM₁₆₀₋₁₉₈ and StREM₁₇₁₋₁₉₈ simulated with atomistic molecular dynamics simulations using the AMOEBA, the AMBER ff99sb and the AMBER ff14sb forcefield over 1 μs respectively. The color code for structural motifs is indicated on the upper left panel. Indicated below is a cartoon of the secondary structure as determined by AF2 performed on the respective extended REM-CA, CcpNMR analysis⁶ and CNS structure calculation ⁷.



Supplementary Figure 7: The pearson's correlation coefficient (left panel) of full-length or truncated REM1.2 and/or REM6.1 was calculated from at least 23 cells over the course of 3 independent experiments. Significant differences were determined using a Kruskal-Wallis test followed by a Dunn's multiple comparison test. Different letters indicate significant differences (p>0.01). Scale bar = 4µm. Representative dual-color TIRF images (right panel) of the surface of epidermal cells of *Nicotiana benthamiana* transiently co-expressing full-length or truncated

AtREM1.2 and/or AtREM6.1, labeled with mRFP1.2 (red) or mVenus (cyan), with AtREM1.2 in red and AtREM6.1 in cyan when co-expressed.



Supplementary Figure 8: a C-terminal region of StREM1.3 including the coiled-coil domain. Indicated in blue on the primary sequence and on the AF2 predicted dimeric structure are the mutated L126, L137 and L155 (upper panel) with the surfaces of the interfacing residues providing the knob-into-hole arrangement (lower panel). b Ensemble (5 structures) of AF2 predictions of StREM₆₇₋₁₉₈ PPP and StREM₆₇₋₁₉₈ EEE dimers, containing the replaced residues (L126, L137 and L155 to Pro or Glu, respectively) in the AF2 structure prediction. The color code represents the pLDDT score (blue: pLDDT > 90; cyan 90 > pLDDT > 70; yellow 70 > pLDDT > 50; red pLDDT < 50, see also Figure 1 in the main text).



StREM1.3/67-198 4-Mers

Supplementary Figure 9: AF2 Multimer prediction containing a different number of the StREM_{67-198 PPP} C-terminal region for each prediction. Structures and compositions are denoted below each prediction, and for each prediction are depicted the structure in cartoon (upper left panel), colored dependent on the pLDDT score, and in surface representation colored by charge distribution (red = negative charges, blue = positive charges). The color code represents the pLDDT score (blue: pLDDT > 90; cyan 90 > pLDDT > 70; yellow 70 > pLDDT > 50; red pLDDT < 50, see also Figure 1 in the main text).



Supplementary Figure 10: Motif 1 and 2 highlighted on AF2-predicted dimeric structures for the C-terminal region of REM proteins from REM groups 1-6.

	10	20	30	40	50 . 60	. 70	80	90	100
SIREM1.3/89-188	<mark>RV</mark> SLIKAWEE	SEKSKA ENKAQK	V SA <mark>IG</mark> AWEN SK	AN LEAELKKME	É <mark>QLE</mark> KKKAE <mark>Y</mark> TEKM	KN <mark>K</mark> IALL <mark>H</mark> KEAÈI	EK <mark>R</mark> AMI EAKR	BEDLLKAEELA	AKY <mark>B</mark> AT <mark>C</mark>
SIREM1.3_R/188-89/1-100	GTA <mark>RY</mark> KAALEEAK	LLDE <mark>G</mark> RKAELMAF	KEE <mark>A</mark> EK <mark>H</mark> LLAI	NKMKET YEAKK	KK <mark>ELQ</mark> EEMKKLEAEL	NAKKSNEWA <mark>GI</mark> AS	SVKKQAKNEAI	KSKESEEWAKI	LSVB
AtREM1.1(AT3G48940.1)/66-165	<mark>Ri</mark> slikawee	A EK SK V EN KAQK <mark>K</mark>	I SS <mark>VG</mark> AWEN SK	AS <mark>VE</mark> AELKKIE	EQLNKKKAH <mark>Y</mark> TE <mark>Q</mark> M	KN <mark>K</mark> IAQI <mark>H</mark> KEAEI	EK <mark>R</mark> AMT EAKR <mark>I</mark>	GED V L K A E EMA	AK <mark>YR</mark> AT <mark>G</mark>
AtREM1.1_R/165-66/1-100	<mark>Gtarykaameeak</mark>	L VD E <mark>G</mark> RKA ET MAF	KEEAEK <mark>H</mark> IQAI	NKMQET <mark>Y</mark> HAKE	(KNLQEEIKKLEA <mark>E</mark> V	SAKKSNEWA <mark>GV</mark> S	SI <mark>K</mark> KQAKN EVI	K SK E A E EWA K I	LSIR
AtREM1.2(AT3G61260.1)/103-202	<mark>R</mark> L SFV RAWEE	SEK SKA ENKA EK	I A <mark>D V</mark> HAWEN SK	AAVEAQLKKIE	EE <mark>Q LE</mark> KKKAE <mark>Y</mark> AERM	KN <mark>K</mark> VAA I <mark>H</mark> KE <mark>AE</mark> I	ER <mark>R</mark> AMI EAKR <mark>(</mark>	EDVLKAEETA	AK <mark>YR</mark> AT <mark>G</mark>
AtREM1.2_R/202-103/1-100	GTARYKAATEEAK	LVD E <mark>G</mark> RKA E I MA F	RE EA EK <mark>H</mark> I AA V	NKMREA <mark>V</mark> EAKE	KKELQEEIKKLQAEV	AA <mark>K</mark> KSNEWAHVDA	A I <mark>K</mark> K EAKN EAH	SKESEEWARV	FSLR
AtREM1.3(AT2G45820.1)/81-180	<mark>K</mark> T SFI KAWEE	SEK SKA EN RAQK	I S <mark>DVH</mark> AWEN SK	AAVEAQLRKIE	EEKLEKKKAQ <mark>YG</mark> EKM	KN <mark>K</mark> VAA I <mark>H</mark> KLAEI	EK <mark>R</mark> AMV EAKK	GEELLKAEEM <mark>G</mark>	AKY <mark>R</mark> AT <mark>C</mark>
AtREM1.3_R/180-81/1-100	GTABYKAGMEEAK	I L E E <mark>G</mark> KKA EVMA F	KE <mark>EALKH</mark> IAAV	NKMKE <mark>GY</mark> QAKE	(KELKEEIKRLQAEV	AAKKSNEWAHVD	SI <mark>K</mark> KQARN EAH	KSKESEEWAKI	FSTK
AtREM1.4(AT5G23750.1)/93-192	<mark>R</mark> MSLIKAWEE	A EKCKVENKA EK <mark>K</mark>	L SSI <mark>G</mark> SWENNK	AAVEAELKKME	EE <mark>Q LE</mark> KKKAE <mark>Y</mark> VE <mark>Q</mark> M	KN <mark>K</mark> IAQI <mark>H</mark> KEAEI	EK <mark>R</mark> AMIEAKR <mark>I</mark>	EEILKAEELA	AKYRATG
AtREM1.4_R/192-93/1-100	Gta ry kaaleeak	L I EE <mark>G</mark> RKAELMA	KEEAEKHIQAI	NKMQEVYEAKK	(KELQEEMKKLEAEV	AA <mark>K</mark> KNNEWS <mark>GI</mark> S	SL <mark>K</mark> KEAKNEVI	CKEAEEWAKI	LSMR
AtREM1.5(AT1G63295.1)/39-138	R <mark>L</mark> ALIDAWEE	N EKAKA <mark>Q</mark> TKAYKE	ILCSIESWENNM	TALELDLKKME	EN LQVEKTEFSKKF	KK <mark>KIP</mark> EIEKIAE/	KREKTEK <mark>O</mark> KI	EQ E S I K V E K I S	EK <mark>L</mark> IAT <mark>P</mark>
AtREM1.5_R/138-39/1-100	<mark>P</mark> TAI L KESIKEVK	LI SEQEK Q KETKEF	KAEAIKEIE <mark>P</mark> I	KKEKKSEETKE	VQLNEEMKKLDLEL	AT <mark>K</mark> MNNEWSEIS(CLEKYAKTOAI	K A K E N E E WAD I	LALR
MtREM2.2/92-191	<mark>R</mark> LALIKAWEE	N EKTKV <mark>E</mark> N RA <mark>Y</mark> KM	IQ SA VD L WEDD K	ASTEAKFK <mark>G</mark> IE	EVKLDRKKSE <mark>Y</mark> VEVM	QN <mark>KIGEIH</mark> KSAEI	EKKAMIEAQK	EEILKVEETA	AK F <mark>R</mark> TR <mark>C</mark>
MtREM2.2_R/191-92/1-100	GRTRFKAATEEVK	(LIEE <mark>G</mark> KQAEIMAK	KEEA SKHIEGI	NQMVEV <mark>Y</mark> ESKK	(RDLKVEI <mark>G</mark> KEKAEI	SAKKDDEWLDVAS	SQMKYARNEVI	KTKENEEWAKI	LALR
AtREM3.1(AT1G69325.1)/14-113	MAAVVD <mark>Q</mark> WKE	ET <mark>E I SK SRKK Y</mark> EK L	SEK <mark>IV</mark> SWEDKK	RK <mark>KAK</mark> RKLHRTE	RSVEKTKLKATORF	RD EN ER I E I I V A S	SARAH <mark>A</mark> YESR	IKE <mark>E</mark> LKVK <mark>E</mark> KA	N LMRTT <mark>C</mark>
AtREM3.1_R/113-14/1-100	GTTRMLNAKEKVK	LLEEK I RSEYAHAF	ASAVIIEIRENE	ED <mark>BFR</mark> QTAKLKT	KEVSRETRHLKRKA	K R K K D EWSVIKI	ESLKE <mark>Y</mark> KKRSI	KSIETEKWODV	VAAM
AIREM3.2(AT4G00670.1)/13-112	QS <mark>KVI</mark> KAWKE	L K I T K V N N K T Q K	L L D I S <mark>G</mark> WEKK <mark>K</mark>	TT <mark>KI</mark> ESELARIO	QRK <mark>M</mark> D SKKMEK SEK L	RNE <mark>K</mark> AAV <mark>H</mark> AKAQH	KK <mark>K</mark> ADVQTRR/	AQEILDAEEAA	ABFQAAG
AIREM3.2_R/112-13/1-100	GAAQFBAAAEEAD	L I EQARRTQVDA	KKQAKAHVAAK	EN <mark>BL</mark> KESKEMKK	(SDMKRQ I RALESE I	KTTKKKEWGSIDI	LKKQTKNNVI	KTIKLEKWAKI	VKSQ
AtREM4.1(AT3G57540)/185-284	VEAKITAWQT	AKVAKINNRFKRO	DAVIN <mark>G</mark> WLNEQ\	/HRANS <mark>WMK</mark> KIE	ERK LED RRA <mark>K</mark> AMEKT	QNKVAK <mark>A</mark> QRKAEI	ERRAT <mark>A</mark> E <mark>G</mark> KR	TEVARVLEVA	N LMRAV <mark>g</mark>
AtREM4.1_R/284-185/1-100	GVARMLNAVELVF	AVET <mark>G</mark> RK <mark>G</mark> EATAF	REEAKRQAKAVI	KNQTKE <mark>MAK</mark> ARF	RDELKRE I K <mark>K</mark> MWSNA	RHVQENLW <mark>G</mark> NIVA	ADQRK FRNN I I	AVKATQWATI	Kaev
AtREM4.2(AT2G41870)/163-262	VEAKITAWQT	AKLAKINNRFKRE	DAVIN <mark>G</mark> WFNEQV	/ <mark>N</mark> K AN S <mark>WMK</mark> K I E	ERKLEERKA <mark>K</mark> AMEKT	Q <mark>N</mark> NVAKAQRKAEI	ERRAT <mark>A</mark> EAKR <mark>(</mark>	TEVAKVVEVA	N LMRAL <mark>G</mark>
AtREM4.2_R/262-163/1-100	GLARMLNAVEVVK	AVET <mark>G</mark> RKAEATAF	IREEAKRQAKAVN	NQT K EMAKAKE	REELKREIK <mark>K</mark> MWSNA	KNVQENFW <mark>G</mark> NIV/	AD ERK FRNN I H	KALKATQWATI	Kaev
AtREM5.1(AT1G45207.2)/437-536	GSKMRDHVHC	KATNHEDLTCATE	EARIISWENLQE	K <mark>a</mark> kaeaairkle	EK <mark>YEP</mark> QMKLEKKRSS	SMEKTMRKVKSAI	EKRAEEMRRS	LDN HV STASH	<mark>GK</mark> ASSFK
AtREM5.1_R/536-437/1-100	KFSSAKCHSATSV	RNDLVSRRMEEAF	KEASKVKRMIKE	Emsssrkkelkn	1Q <mark>PEY</mark> KELKRIAAEA	KAKQLNEWSIIR/	NEETACTLDE		M <mark>KSG</mark>
AtREM6.1(AT2G02170.1)/373-472	SEAR <mark>A</mark> TAWEE	A EKAKHMAR F R R E	EMKIQAWENHQ	AKSEA <mark>EMK</mark> KTE	EVK <mark>V</mark> ERIK <mark>GRAQ</mark> DRL	MK <mark>K</mark> LATIERKAEI	EKRAA <mark>AEA</mark> KKI	D <mark>H</mark> QAAKTEKQA	EQ I RRT <mark>G</mark>
AtREM6.1_R/472-373/1-100	<mark>GTRRIQEAQKETK</mark>	A A Q H D K K A E A A A F	KEEAKREITAL	KMLRD <mark>QARG</mark> KI	REVKVETK <mark>KME</mark> AES	KAK <mark>QHNEWAQIK</mark> M	MEERRFRAMHI	KAKEAEEWATA	RAES
AtREM6.2(AT1G30320)/395-494	FEKRATAWEE	A EK SKHN AR <mark>Y</mark> KRE	EIRIQAWESQE	AKLEAEMRRIE	EAKVEQMKAE <mark>AE</mark> AKI	MK <mark>K</mark> IALAKQRSEI	EKRAL <mark>AEA</mark> RK ⁻	FRDAEKA <mark>VAEA</mark>	QYIRET <mark>G</mark>
AtREM6.2_R/494-395/1-100	GTERIYQAEAVAK	EADRTKRA EA LAF		KMIKAEAEAKN	1QEVKAEIRRMEAEL	KAKEQSEWAQIRI	I EERK <mark>Y</mark> RANHI	KSKEAEEWATA	RKEF
AtREM6.3(AT1G53860.1)/323-422	ES <mark>K</mark> A <mark>P</mark> LWDDE	EDDKIKFC <mark>QRY</mark> QRE	EAKTQA <mark>WV</mark> NLEN	NAKAEAQSRKLE	EVK I QKMR SN L <mark>E</mark> EK L	MKRMDM <mark>VH</mark> RRAEL	WRATA <mark>RQ</mark> QH	VEQMQKAAET <mark>A</mark>	R <mark>K</mark> LTNRR
AtREM6.3_R/422-323/1-100	RRNTL <mark>K</mark> RATEAAK	CQMQEVHQQBATAF	WDEARRHVMDMF	RKMLKEELNSRM	IKQ I KV EL KR S <mark>Q</mark> A EA	KANELNVWAQIKA	EERQ <mark>YRQ</mark> CF	KIKDDEDDWL <mark>P</mark>	A <mark>K</mark> SE
AtREM6.4(AT4G36970.1)/300-399	SASS <mark>S</mark> SWDIS	S <mark>EP</mark> AMTL <mark>S</mark> KLQ <mark>R</mark> E	EAKIAAWENLQ	AKA EAA I RKLE	EVKLEKKKSASMDKI	LN <mark>K</mark> LQTAKTKAQI	EMR <mark>R</mark> SSV <mark>S</mark> SE	HEQQQ <mark>G</mark> NHQI <mark>S</mark>	RN SVK I T
AtREM6.4_R/399-300/1-100	TIKVSNR <mark>S</mark> IQHN <mark>O</mark>	QQQEHES <mark>S</mark> VSSRF	MEQAKIKATQL	NLIKDMSA SKR	(KELKVELKRIAAEA	KAKQLNEWAATK/	NEERQLKSLT	MA <mark>PE</mark> SSIDWS <mark>S</mark>	SSA S
AtREM6.5(AT1G67590.1)/229-328	MEARAMAWDE	A ERAK FMARYKRE	EVKIQAWENHE	RKAEM <mark>EMK</mark> KME	EVKA ERMKA <mark>R</mark> A E EK L	AN <mark>K</mark> LAATKRIAEI	ERRANAEAKLI	NE <mark>K</mark> AVKTSEKA	D <mark>Y</mark> IRRS <mark>G</mark>
AtREM6.5_R/328-229/1-100	GSRRIYDAKESTK	VAKENLKA EANAF	REEAIRKTAAL	NALKE EAR AKN	IREAKVEMK <mark>K</mark> MEMEA	KR <mark>KEH</mark> NEWAQIKI	/ EERK <mark>y</mark> ramfi	KAREAEDWAMA	RAEM
AtREM6.6(AT1G13920.1)/236-335	DD ST A <mark>D</mark> AWEK	A E L S <mark>K I K</mark> ARYE <mark>K L</mark>	NRKIDLWEAKKF	REKARRKLD I S <mark>E</mark>	QSELEQRRKR <mark>G</mark> LQR	FREDTEY <mark>I</mark> EQIAA	A <mark>GAR</mark> AQA E <mark>K</mark> D	Q SK E F K <mark>V K E</mark> K	A <mark>G</mark> VIRST
AtREM6.6_R/335-236/1-100	T SR I V <mark>G</mark> AK E K V K F	EK SQRDK EAQARA	GAAIQEIYETDI	ERFRQL <mark>G</mark> RKRR	ELESQESIDLKRRA	KERKKAEWLDIKF	RNLKEYRAK I	S L E A K E WAD A	TSDD
AtREM6.7(AT5G61280.1)/152-251	ASS <mark>K</mark> ADSWEK	[S <mark>Q</mark> K <mark>K R</mark> LR <mark>YEK</mark> M	IKA <mark>D I VG</mark> WEN ER	CLAATLLMEK RK	(S <mark>ELEK<mark>RKG</mark>INNQH<mark>Y</mark></mark>	K S <mark>K</mark> L A R I Q L I A D (GAK <mark>KQL</mark> EEKR	SKEAQV <mark>HG</mark> KV	KKMSRT <mark>G</mark>
AtREM6.7_R/251-152/1-100	GTRSMKKVKGHVC	}AEKSRRKEELQK	A <mark>GDA I</mark> LQ I RAL	SKYHQNNIGKF	K <u>ELESKR</u> KEMLLTA	A L <mark>K</mark> R EN EW <mark>G V I D</mark> /	AKMKEYRLRI	KIQSKEWSDA	KSSA

Supplementary Figure 11: Inverse alignment of the coil-coil domains centered on the symmetry axis (L137 in StREM1.3). Inverse alignment of the coiled-coil region using Clustal Omega ³ shows potential sources of different multimerization states and parallel *versus* anti-parallel coiled-coil arrangement.





AtREM5.1/451-555_Dimer_All-Atom



AtREM6.4/309-427_Dimer_All-Atom



MtREM2.2/80-208_Dimer_All-Atom



Supplementary Figure 12: Intermolecular contact map of C α of monomer 1 and all atoms of monomer 2 (range 3-10 Å) of the structures adopted throughout the 1 μ s atomistic MD simulation based on the Amber forcefield⁸. The blue scale is detailed in the right colorbars.



Supplementary Figure 13: Intermolecular contact map of C α of monomer 1 and all atoms of monomer 2 (range 3-10 Å) of the structures adopted throughout the 560 ns atomistic MD simulation based on the AMOEBA forcefield. The blue scale is detailed in the right colorbar and as in Supplementary Figure 13.



Supplementary Figure 14: Interatomic all-atom contacts detected by MAPIYA⁹, upper distance cut-off 5 Å. The blue scale is decoded in the right panel.



Supplementary Figure 15: Distance plot of the C α -C α contacts of the REM C-terminal region over 1 μ s atomistic MD simulation of the aligned residues of the three selected residues of StREM₆₇₋₁₉₈ (Leu126_{monomer_1}-Leu155_{monomer_2}, Leu137_{monomer_1}-Leu137_{monomer_2} and Leu155_{monomer_1}-Leu126_{monomer_2}), highlighted on the structure in the left panel.



Supplementary Figure 16: One representative structure of AF2 dimer predictions for the REM family members. Structures are colored as the conserved sequence motifs 1 and 2 (detailed

in Figure 1 in the main text) in the upper panel and dependent on the pLDDT score (blue: pLDDT > 90; cyan 90 > pLDDT > 70; yellow 70 > pLDDT > 50; red pLDDT < 50, see also Figure 1 in the main text) in the lower panel, for each structure.



Supplementary Figure 17: AF2 pLDDT scores of the two monomers in the predicted dimeric coiled-coil structures plotted over the primary sequence. For each REM dimer the pLDDT

scores of five dimers are shown for the full-length REM (upper panel) and C-terminal region (lower panel).



Supplementary Figure 18: Structural homology detected by Dali¹⁰ for the C-terminal region of **a** AtREM1.2, **b** AtREM6.1 and **c** StREM1.3. Three selected monomeric structures of the restricted PDB25 set are shown aligned with REM C-terminal regions. The right panel shows the ten best aligned structures of the Dali¹⁰ PDB25 set to the C-terminal regions of the respective protein.

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