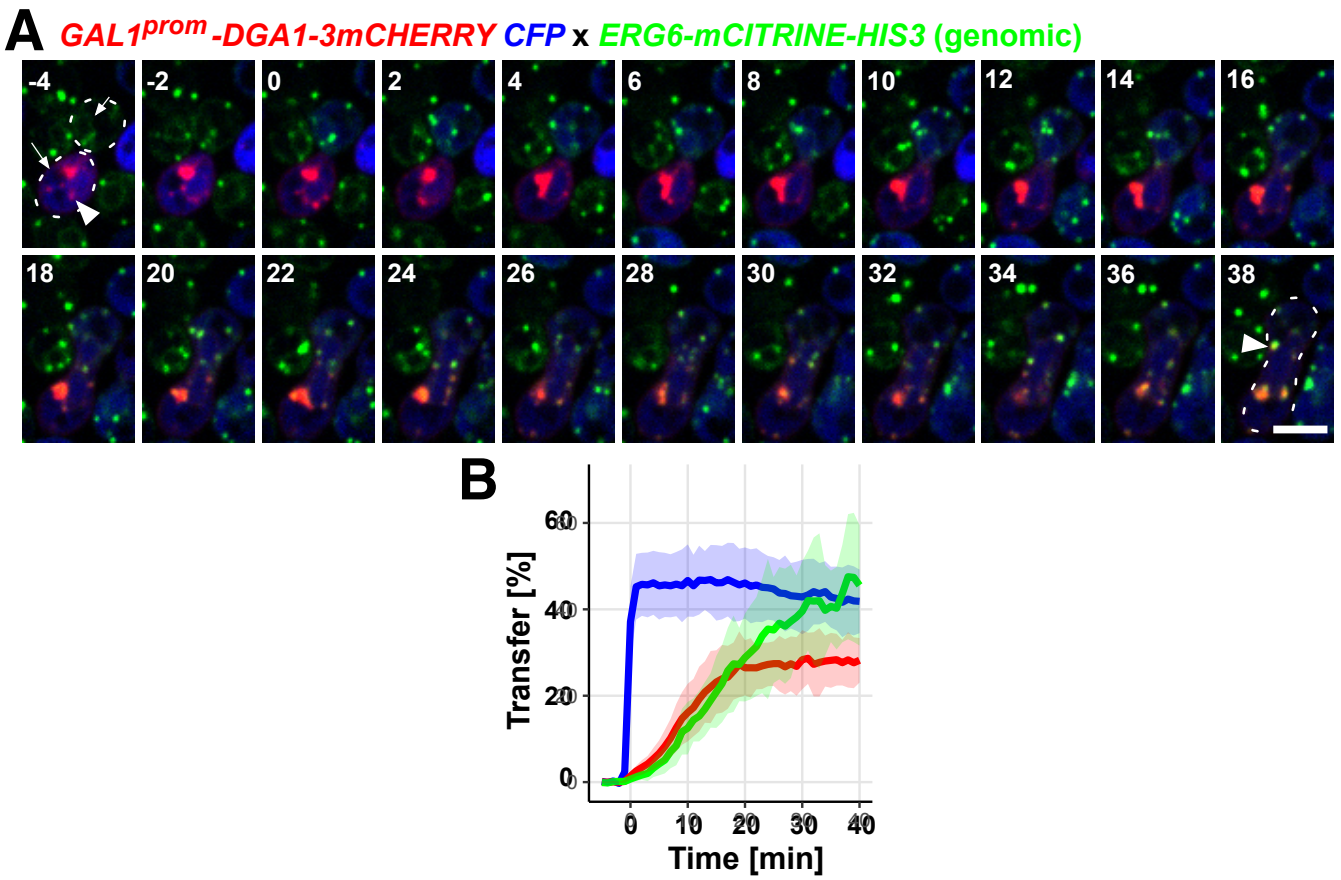


Fig. S1. Transfer of endogenous mCitrine-tagged Erg6 to LDs of the mating partner, and movement of Pet10-mScarlet labelled LDs on zygote formation.

(A) Mating reactions to monitor the exchange of endogenously tagged Erg6-mCitrine and of Dga1-3mCherry, whose expression is control by a galactose inducible promoter, in wild-type cells. Time-lapse images shown are separated by 2 min intervals over a period of 44 min, starting 2 min prior to cytoplasmic mixing (t=0 min). White arrows point to the ER membrane, and arrowheads to LDs. Scale bar, 5 µm.

(B) Rate of transfer of Erg6-mCitrine (green line), Dga1-3mCherry (red line), and the cytosolic marker CFP (blue line) between wild-type mating partners.

(C) Pet10-mScarlet labelled LDs move only slowly into the acceptor half of the zygote upon cytoplasmic mixing. Representative time-lapse images showing the transfer of Pet10-mScarlet and Erg6-mCitrine onto the LDs of mating partners. Images shown are separated by 2 min intervals, starting 4 min (t=-4) before cytoplasmic mixing (t=0), over a period of 42 min. White arrowheads point to LDs, and the red arrowhead marks an LD that has moved into the mating partner. Scale bar, 5 µm.

(D) Enlarged views of the 14 and 34 min time points shown in panel C. White arrowheads point to LDs that are still in the part of the zygote where they originate from, and the red arrowhead points to an LD that is strongly labelled with Pet10-mScarlet and that has moved into the mating partner more than 30 min after fusion had occurred. Scale bar, 5 µm.

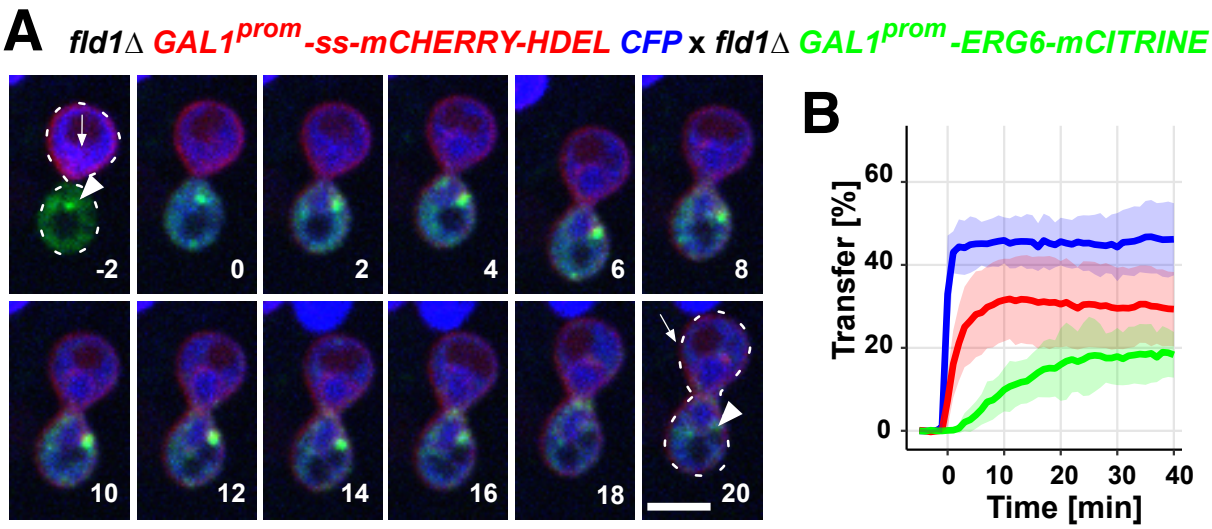


Fig. S2. Seipin affects the exchange of LD proteins but not ER fusion.

(A) Mating reactions to monitor the exchange of ss-mCherry-HDEL and Erg6-mCitrine between seipin (*fld1*Δ) mutant cells. Time-lapse images shown are separated by 2 min intervals over a period of 22 min, starting 2 min prior to cytoplasmic mixing (t=0 min). Arrows point to the ER membrane, arrowheads to LDs. Scale bar, 5 µm.

(B) Rate of transfer of the ER luminal marker ss-mCherry-HDEL and that of the LD marker Erg6-mCitrine between seipin mutant *(fld1*Δ*)* cells. The red line represents ss-mCherry-HDEL, the green line Erg6-mCitrine, and the blue line cytosolic CFP.

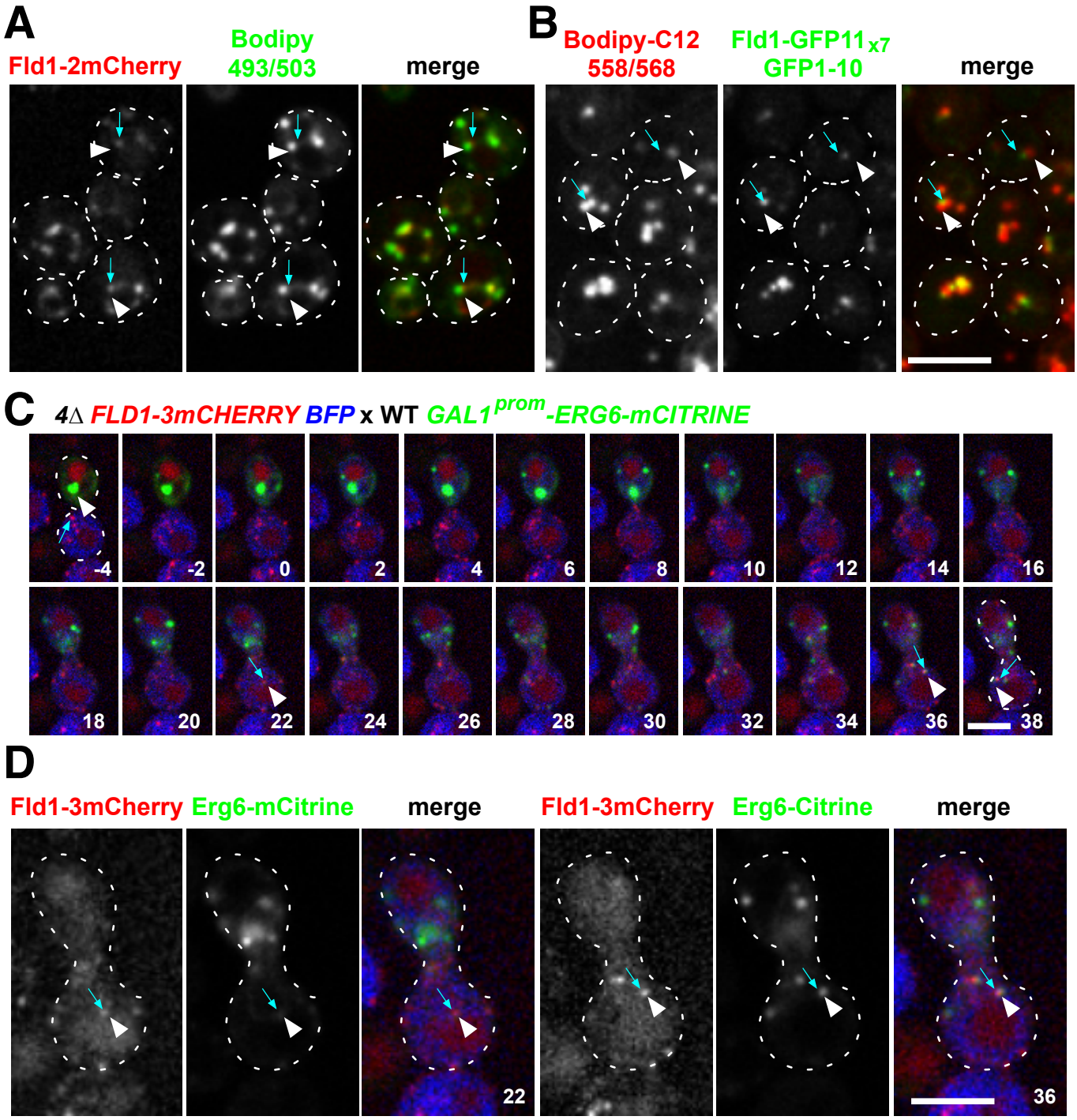


Fig. S3. mCherry- and GFP-tagged variants of seipin are functional and mark sites of LD formation.

(A, B) 2mCherry- and the split-GFP- (sfGFP11$_{x7}$+GFP1-10) tagged Fld1 localize at the base of LDs labelled with either BODIPY493/503 (panel A) or BODIPY-C12 (558/568) (panel B), respectively. Blue arrows point to Fld1 spots, arrowheads to LDs. Scale bar, 5 µm.

(C) Fld1-3mCherry marks sites of LD formation in the ER of quadruple mutant cells. Wild-type cells expressing Erg6-mCitrine were mated to quadruple mutant cells, *4*Δ (*are1*Δ *are2*Δ *dga1*Δ *lro1*Δ) expressing Fld1-3mCherry. Images shown were recorded at 2 min intervals, starting 4 min (t=-4) before cytoplasmic mixing (t=0) over a period of 42 min. Blue arrows point to Fld1 spots, arrowheads to LDs. Scale bar, 5 µm.

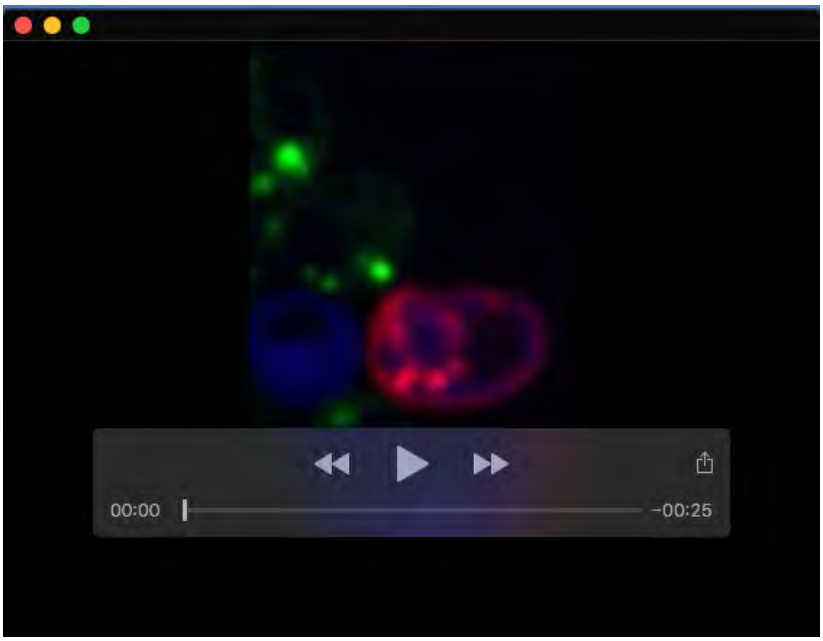
(D) Enlarged views of the 22, and 36 min time points shown in panel A. Note the accumulation of Erg6-mCitrine at ER spots marked by Fld1-3mCherry. Blue arrows point to Fld1 spots, arrowheads to LDs. Scale bar, 5 µm.

Table S1. *Saccharomyces cerevisiae* strains used in this study

Strain	Genotype	Source	Figure
BY4741	*MAT*a; *his3Δ1 leu2Δ met15Δ0 ura3Δ0*	Euroscarf	1, 2, 3, 5, 6, 7
BY4742	*MAT*α; *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	Euroscarf	1, 2, 3, 6, 7, 8
RSY5669	*MAT*α; *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 are1Δ::KanMX4 are2Δ::KanMX4 dga1Δ::KanMX4 lro1Δ::KanMX4*	This study	5, 6
RSY5795	*MAT*a; *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 are1Δ::KanMX4 are2Δ::KanMX4 dga1Δ::KanMX4 lro1Δ::KanMX4*	This study	6
MATa thr$^-$	*MAT*a; *thr*	Lab collection	
MATα thr$^-$	*MAT*α; *thr*	Lab collection	
MY14769	*MAT*a/α; *his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 met15Δ0/met15Δ0 ura3Δ0/ura3Δ0 sey1Δ::Hyg/SEY1 yop1Δ::URA3/YOP1 dsl1ΔE-NatMX/DSL1*	Mark Rose	
RSY5619	*MAT*α; *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 sey1Δ::Hyg dsl1ΔE-NatMX*	This study	4
RSY5621	*MAT*a; *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 sey1Δ::Hyg dsl1ΔE-NatMX*	This study	4
MATa fld1Δ	*MAT*a; *his3Δ1 leu2Δ met15Δ0 ura3Δ0 fld1Δ::KanMX4*	Euroscarf	7, S2
MATα fld1Δ	*MAT*α; *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 fld1Δ::KanMX4*	Euroscarf	7, S2
RSY6911	*MAT*α; *his3Δ1 leu2Δ lys2Δ0 ura3Δ0 FLD1-2mCHERRY::SpHIS5*	This study	8, S2
RSY6970	*MAT*a; *his3Δ1 leu2Δ met15Δ0 ura3Δ FLD1-GFP11x7::SpHIS5*	This study	8, S3
RSY6963	*MAT*α; *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 are1Δ::KanMX4 are2Δ::KanMX4 dga1Δ::KanMX4 lro1Δ::KanMX4 FLD1-3mCHERRY::SpHIS5*	This study	S3
RSY6756	*MAT*α; *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 ERG6-mCITRINE::SpHIS5*	This study	S1

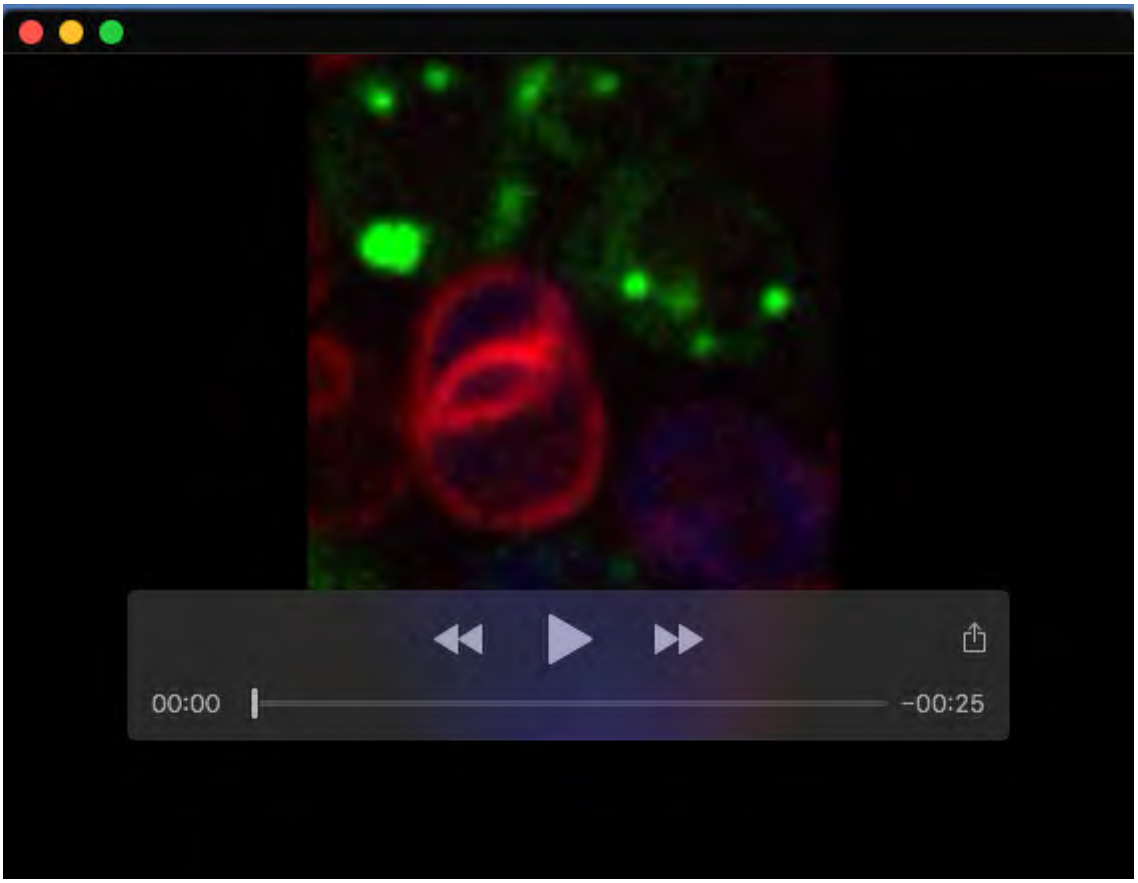
Table S2. Plasmids used in this study

Plasmids	Genotype	Source	Figure
pRS415-ADH1	*CEN/ARS, ADH1^{prom}, LEU2*	Mumberg et al., 1995	
p1174	[pRS415] *ADH1^{prom}-yECFP*	Cottier et al., 2020	1, 2, 3, 4, 5, 6, 7, 8, S1, S2
pGREG506	*CEN/ARS, GAL1^{prom}, URA3*	Jansen et al., 2005	
pKT211	[pFA6a] *link-yEmCITRINE, SpHIS5*	Sheff and Thorn, 2004	
p1307	[pGREG506] *GAL1^{prom}-ERG6-mCITRINE^{A206K}*	This study	1, 2, 3, 4, 5, 6, 7, 8, S2, S3
p30648	*POM121-3mCHERRY*	Dultz and Ellenberg, 2010	
pGREG600	*CEN/ARS, GAL1^{prom}-RecombinationSite-GFP, URA3*	Jansen et al., 2005	
p1079	[pGREG600] *GAL1^{prom}-RecombinationSite-3mCHERRY*	This study	
p1102	[p1079] *GAL1^{prom}-DGA1-3mCHERRY*	This study	1, 2, 4, 5, 7, S1
Mito-RFP	*CEN/ARS NcATP9$^{mitochondria\text{-}signal}$-RFP, LEU2*	Westermann and Neupert, 2000	
p1317	[p1079] *GAL1^{prom}- NcATP9$^{mitochondria\text{-}signal}$-3mCHERRY*	This study	3
p1312	[p1079] *GAL1^{prom}-SEC63-3mCHERRY*	This study	3
pGREG503	*CEN/ARS, GAL1^{prom}, HIS3*	Jansen et al., 2005	
MR6474	*CEN, ADH1^{prom}-PRC1^{ss}-mCHERRY-HDEL, LEU2*	Rogers et al., 2014	
p1171	[pGREG503] *GAL1^{prom}-PRC1^{ss}-mCHERRY-HDEL*	This study	4, 6, S2
pGAL-HO	*GAL^{prom}-HO-URA*	Herskowitz and Jensen, 1991	
Addgene #70224	*pHRm-NLS-dCas9-GFP11x7-NLS*	Kamiyama et al., 2016	
Addgene #129416	*pSH-EFIRES-P-GFP(1-10)opti*	Salo et al., 2019	
pRS416-ADH1	*CEN/ARS, ADH1^{prom}, LEU2*	Mumberg et al., 1995	
p2213	[pRS416] *ADH1^{prom}-sfGFP1-10*	This study	8, S3
p2216	[pRS415] *ADH1^{prom}-BFP*	This study	8, S3
p2233	[pRS415] *GAL1^{prom}-ERG6-mCITRINE*	This study	8
P1662	[pRS416] *GAL1^{prom}-PET10-mSCARLET*	This study	S1
pKT175	[pFA6a] *link-yECITRINE, CaURA3*	Sheff and Thorn, 200	



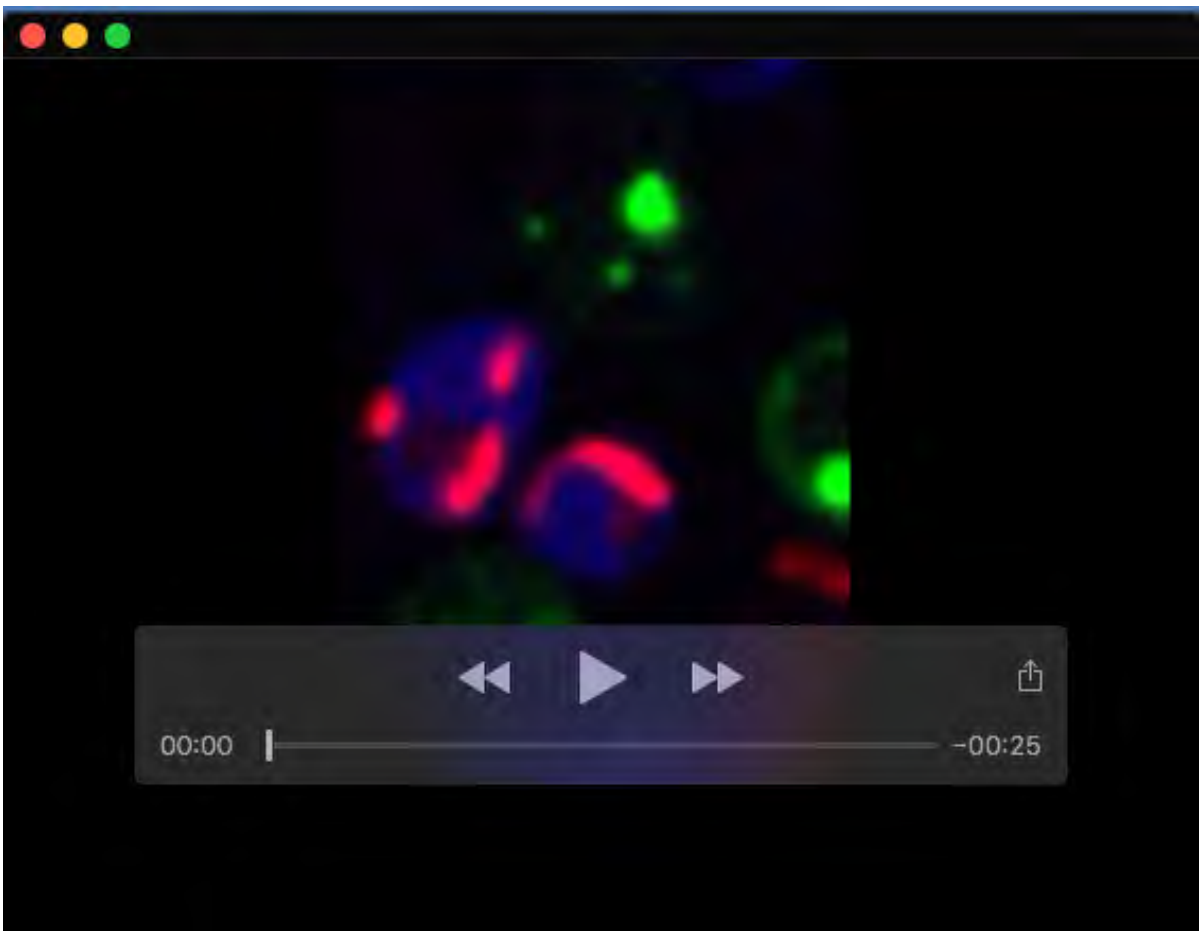
Movie 1. Redistri ution of LD markers upon zygote formation.

Time-lapse images during ygote formation between a *MAT*a cell expressing Dga1- 3mCherry and CFP and a *MAT*α cell expressing Erg6-mCitrine. Expression of the LD marker proteins under control of a galactose inducible promoter was repressed by shifting cells to glucose media 2 h before imaging. Single-plane images were recorded at 1 min intervals.



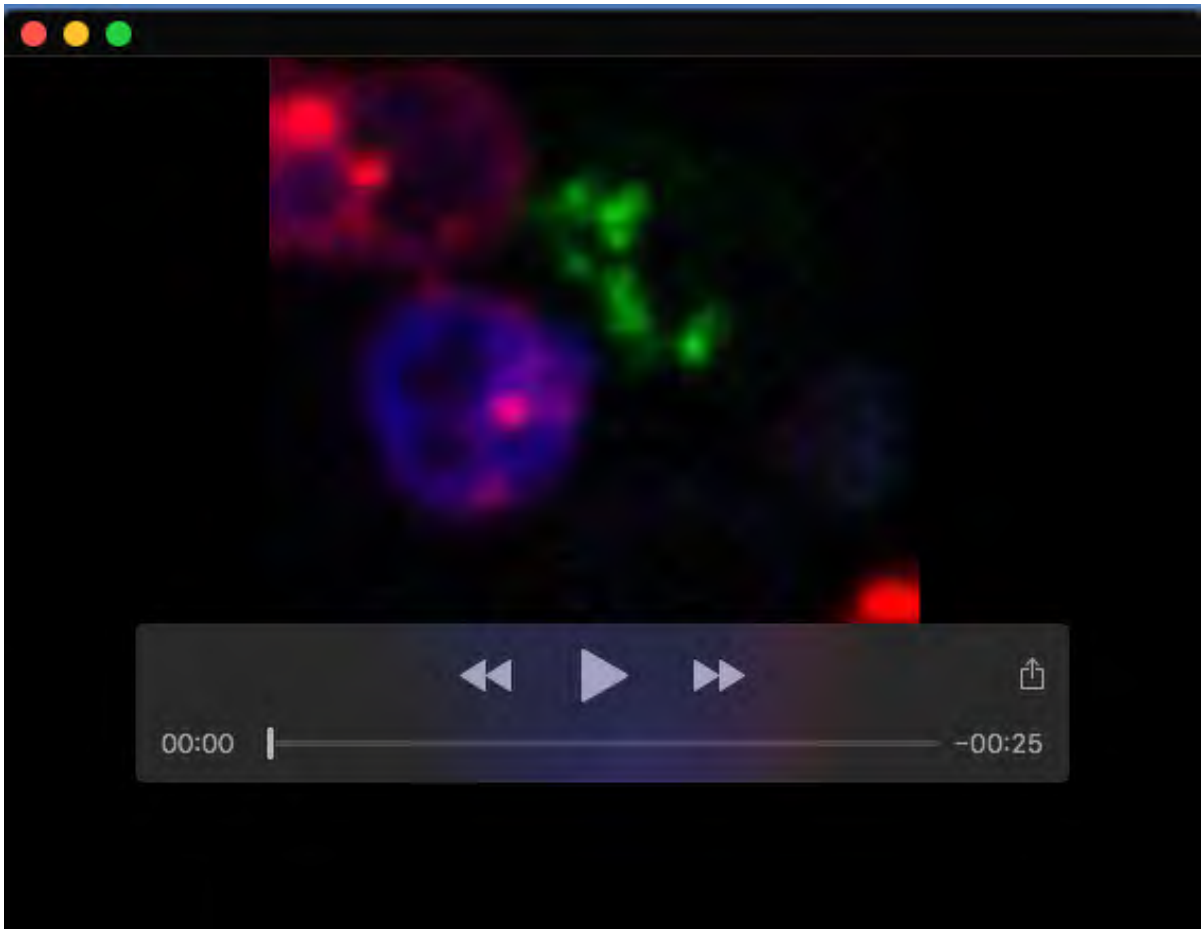
Movie 2. Redistri ution of the ER marker Sec63-mCherry and the LD marker Erg6-mCitrine upon zygote formation.

Time-lapse images of ygote formation between a *MAT*a cell expressing Sec63-mCherry and CFP and *MAT*α cell expressing Erg6-mCitrine. Expression of Sec63-mCherry and Erg6-mCitrine was switched off 2 h before imaging by shifting cells to glucose medium. Single-plane images were recorded at 1 min intervals.



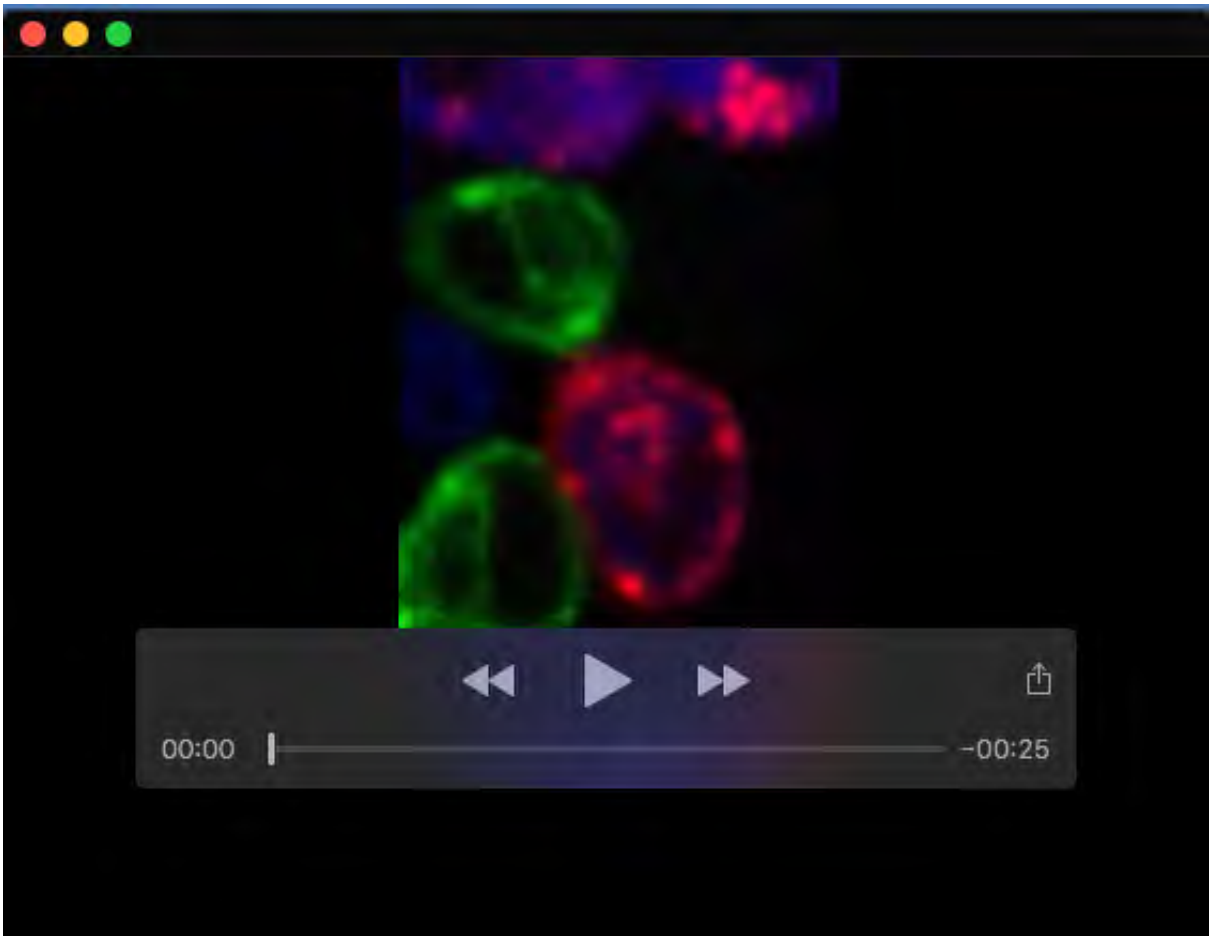
Movie 3. Dynamics of exchange of the LD marker Erg6-mCitrine and the mitochondrial matrix marker MITO-3mCherry upon zygote formation.

Time-lapse images of zygote formation between a *MAT*a cell expressing MT-3mCherry and CFP and a *MAT*α cell expressing Erg6-mCitrine. Expression of MT-3mCherry and Erg6-mCitrine was switched off 2 h before imaging by shifting cells to glucose medium. Single-plane images were recorded at 1 min intervals.



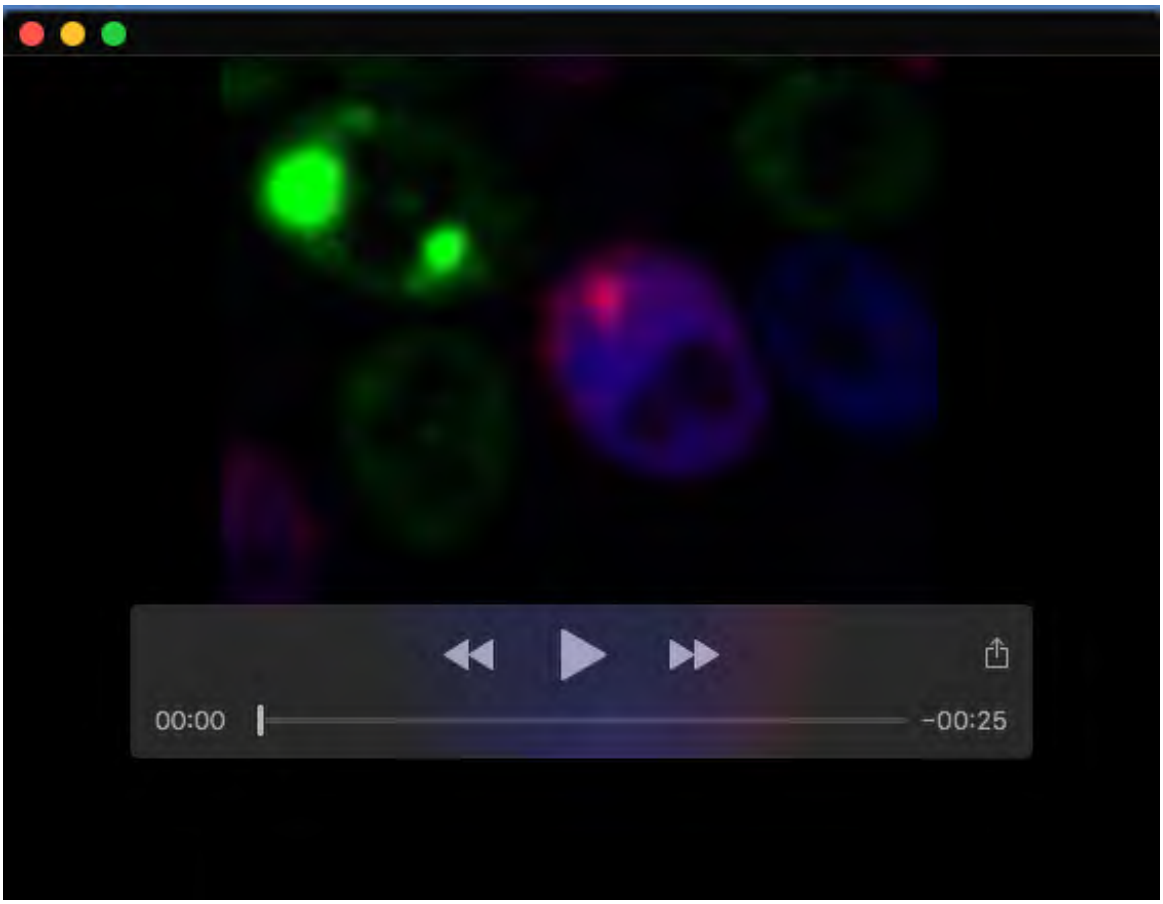
Movie 4. Exchange of Erg6-mCitrine and Dga1-3mCherry in mutants with a delay in ER fusion.

Mating between *sey1 dsl1ΔE* mutant cells expressing Erg6-mCitrine or Dga1-3mCherry and CFP. Note the accumulation of LDs at the fusion neck between the gametes. Expression of the LD marker proteins was repressed 2 h prior to imaging acquisition. Single-plane images were recorded at 1 min intervals.

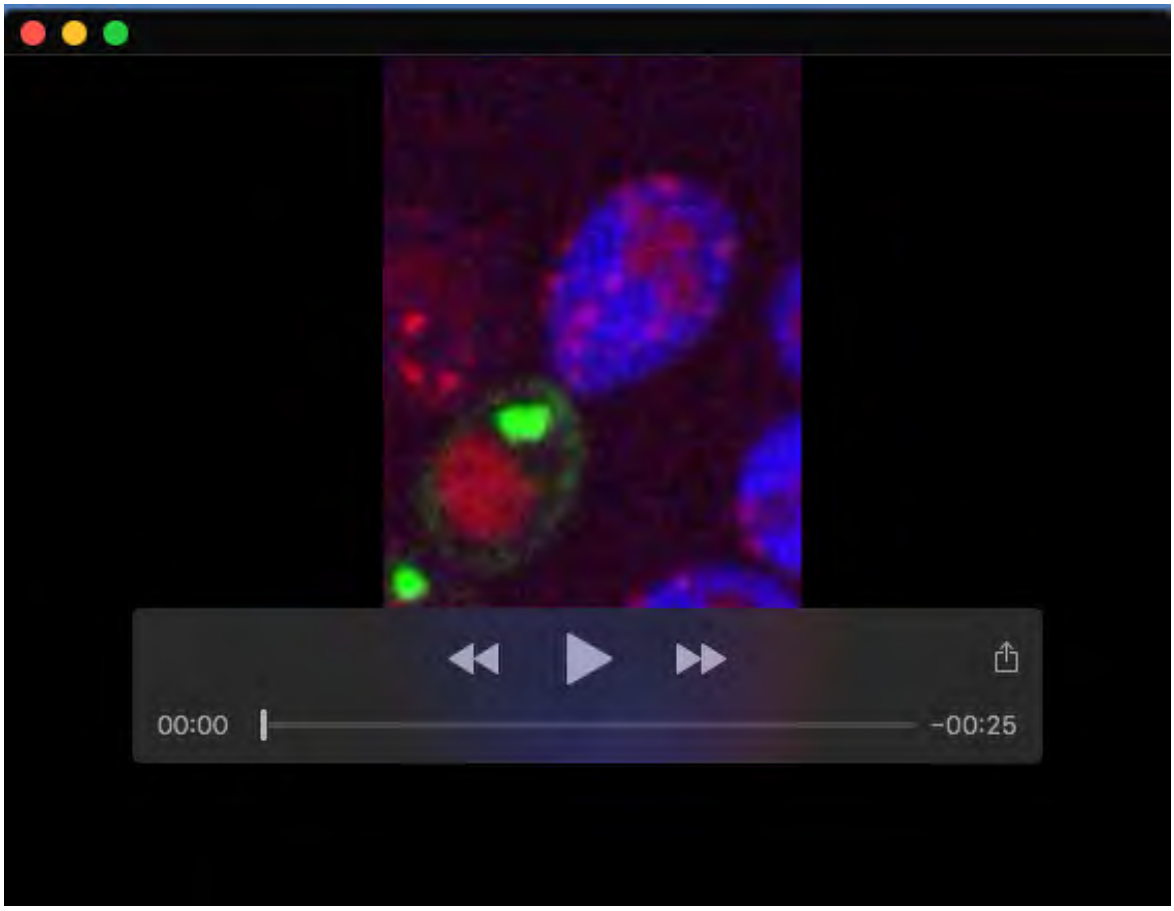


Movie 5. Erg6-mCitrine located in the ER of a quadruple mutant lacking LDs moves to LDs of the mating partner expressing Dga1-3mCherry

Time-lapse images of a *MAT*a cell expressing Dga1-3mCherry and CFP mating with a *MAT*α quadruple mutant *are1*Δ *are2*Δ *dga1*Δ *lro1*Δ) lacking LDs and expressing E -locali ed Erg6-mCitrine. Expression of the LD marker proteins was repressed 2 h prior to imaging acquisition. Single-plane images were recorded at 1 min intervals.

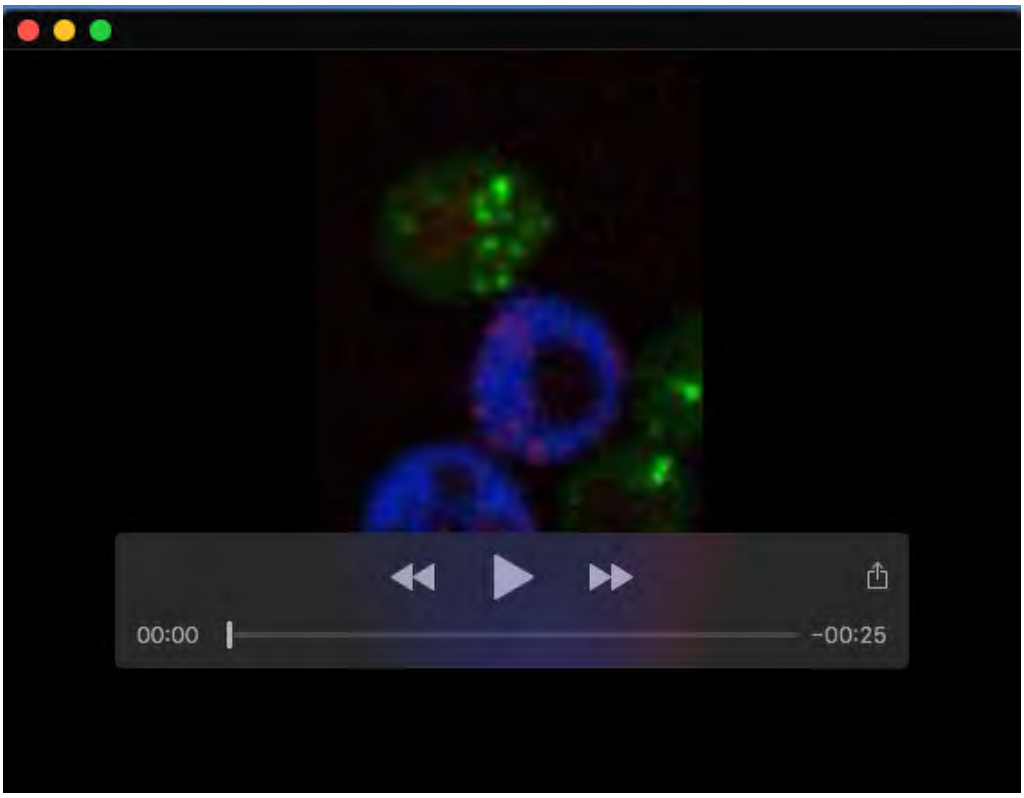


Movie 6. Seipin affects relocalization of LD markers upon zygote formation.
Zygote formation between seipin mutant cells *(fld1Δ)* expressing Dga1-3mCherry and CFP or Erg6-mCitrine. Note the large, possibly clustered LDs in both mating partners, a characteristic of seipin mutants. Expression of the LD marker proteins was repressed 2 h prior to imaging acquisition. Single-plane images were recorded at 1 min intervals.



Movie 7. ER sites marked by seipin in wild-type cells acquire the LD marker from the mating partner upon zygote formation.

Mating progression between a cell co-expressing 2mCherry-tagged seipin and CFP and a partner cell expressing Erg6-mCitrine. Erg6-mCitrine expression was repressed 2 h before imaging. Single-plane images were recorded at 2 min intervals.



Movie 8. Seipin domains in the ER are stable and do not mix upon zygote formation.

Zygote formation between mating partners expressing red- and green-fluorescently tagged seipin variants. Time-lapse images of a mating between cells of different mating types expressing either Fld1-2mCherry or a split-GFP Fld1-GFP11$_{x7}$ together with BFP and GFP1-10, respectively. Mating progression was followed by acquisition of single plan images separated by 2 min intervals.

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