Supplementary material for <A. Magenau et al.> <Discreet and distinct clustering of five model membrane proteins revealed by single molecule localization microscopy> <Molecular Membrane Biology> <2014>

Supplementary Figure S1. Localization microscopy to quantify protein clustering. (A) Localization precision of PSCFP2 and mEOS2, here fused to Lck10 was similar with an average precision of 18.6 nm ± 0.6 (PS-CFP2) and 19.1 ± 0.7 (mEOS2). (B) The number of molecules per cluster of PS-CFP2 tagged to Lck10 (orange), Src15 (pink), WT Lat34 (green), CS Lat34 (blue) and GPI-AP (red) was obtained from binary cluster maps. (A) The box extends from the 25th to the 75th percentile. The whiskers show the minimum and the maximum values, horizontal lines indicate the median and (+) indicate means. (B) Asterisks indicate *< 0.05, **$p < 0.01$, ***$p < 0.001$ and ****$p < 0.0001$. For detailed statistics please refer to Supplementary Table S1. (C) Maxima of Ripley's *K*-function curves at a radius of r = 50 nm of Lck10-PS-CFP2 fixed with 4% PFA or 4% PFA with 0.2% Glutaraldehyde. (D) Maxima of Ripley's *K*-function curves at a radius of r = 50 nm of Lck10-mEOS2 fixed with 4% PFA or 4% PFA with 0.2% Glutaraldehyde. (C–D) The box extends from the 25th to the 75th percentile. The whiskers show the minimum and the maximum values, horizontal lines indicate the median. Significances were calculated by Student's *t*-test. There were no significant differences.

Supplementary Figure S2. Expression of level of PS-CFP2 tagged protein has no effect on clustering levels. Values for max L(r)-r were plotted against the number of molecules per μm^2 for Lck10-PS-CFP2, Src15-PS-CFP2, WT Lat34-PS-CFP2, CS Lat34-PS-CFP2 and GPI-PS-CFP2. Lines indicate linear regression. Data are from five independent experiments with a total of 9–16 cells.

Supplementary Figure S3. Expression of level of mEOS2 tagged protein has no effect on clustering levels. Values for max L(r)-r were plotted against the number of molecules per μm^2 for Lck10-mEOS2, Src15-mEOS2, WT Lat34-mEOS2, CS Lat34-mEOS2 and GPI-mEOS2. Lines indicate linear regression. Data are from five independent experiments with a total of 9–17 cells.

Supplementary Figure S4. Comparison of mEOS *versus* PS-CFP2 clustering and mEOS clustering parameters for five membrane anchors. (A, B) Comparison of PS-CFP2 and mEOS2 tagged to Lck10 (orange), Src15 (pink), WT Lat34 (green), CS Lat34 (blue) and GPI-AP (red) in terms of (A) percentage of molecules in clusters and (B) number of molecules per cluster. (C–G) All membrane anchors were fused to mEOS2. (C) Maxima of Ripley's *K*-function curve at a radius of r = 50 nm. (D) Cluster radii in nm. (E) Percentage of molecules residing in clusters. (F) Number of clusters per μm^2. (G) Number of molecules per cluster. (D–G) Values were obtained from binary maps of individual image regions. (A–G) The box extends from the 25th to the 75th percentile. The whiskers show the minimum and the maximum values, horizontal lines indicate the median and (+) indicate means. *$p < 0.05$, **$p < 0.01$, $p < 0.001$ and ****$p < 0.0001$. For detailed statistics please refer to Supplementary Table S1. Data are from four independent experiments with a total of 9–17 cells.

Supplementary Figure S5. Clustering parameters of PS-CFP2-tagged and mEOS2-tagged membrane anchors with and without Latrunculin B (LatB) treatment. To depolymerize actin, cells were treated with 5 µM Latrunculin B (LatB) for 5 min. (A, D) Cluster radii in nm, (B, E) number of clusters per μm^2 and (C, F) percentage of molecules residing in clusters of PS-CFP2 (A–C) and mEOS2 (D–F) tagged to Lck10 (orange), Src15 (pink), WT Lat34 (green), CS Lat34 (blue) and GPI-AP (red). Values were obtained from binary maps of individual image regions. The box extends from the 25th to the 75th percentile. The whiskers show the minimum and the maximum values, horizontal lines indicate the median and (+) indicate means. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ and ****$p < 0.0001$ (one-way analysis of variance [ANOVA]). Data are from two independent experiments with a total of 9–17 cells.

Supplementary Figure S6. Clustering parameters of PS-CFP2-tagged and mEOS2-tagged membrane anchors with and without 7-ketocholesterol (7KC) treatment. To decrease membrane order, cells were treated with 25 µM 7-ketocholesterol (7KC) for 30 min. (A, D) Cluster radii in nm, (B, E) number of clusters per μm^2 and (C, F) percentage of molecules residing in clusters of PS-CFP2 (A–C) and mEOS2 (D–F) tagged to Lck10 (orange), Src15 (pink), WT Lat34 (green), CS Lat34 (blue) and GPI-AP (red). Values were obtained from binary maps of individual image regions. The box extends from the 25th to the 75th percentile. The whiskers show the minimum and the maximum values, horizontal lines indicate the median and (+) indicate means. *$p < 0.05$, **$p < 0.01$, $p < 0.001$ and ****$p < 0.0001$ (one-way analysis of variance [ANOVA]). Data are from two independent experiments with a total of 9–17 cells.

Figure S1

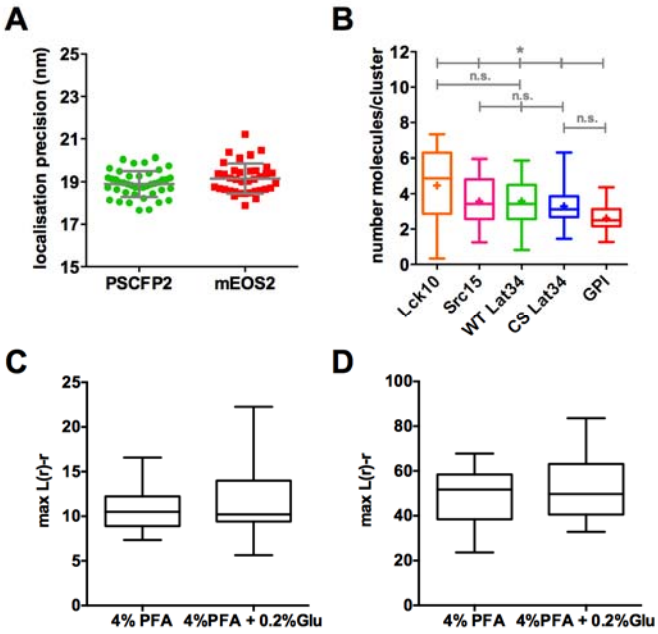

A
localisation precision (nm)
25
23
21
19
17
15
PSCFP2
mEOS2
B
number molecules/cluster
12
10
8
6
4
2
0
*
n.s.
n.s.
n.s.
Lck10
Src15
WT Lat34
CS Lat34
GPI
C
max L(r)-r
25
20
15
10
5
0
4% PFA
4%PFA + 0.2%Glu
D
max L(r)-r
100
80
60
40
20
0
4% PFA
4%PFA + 0.2%Glu

Figure S2

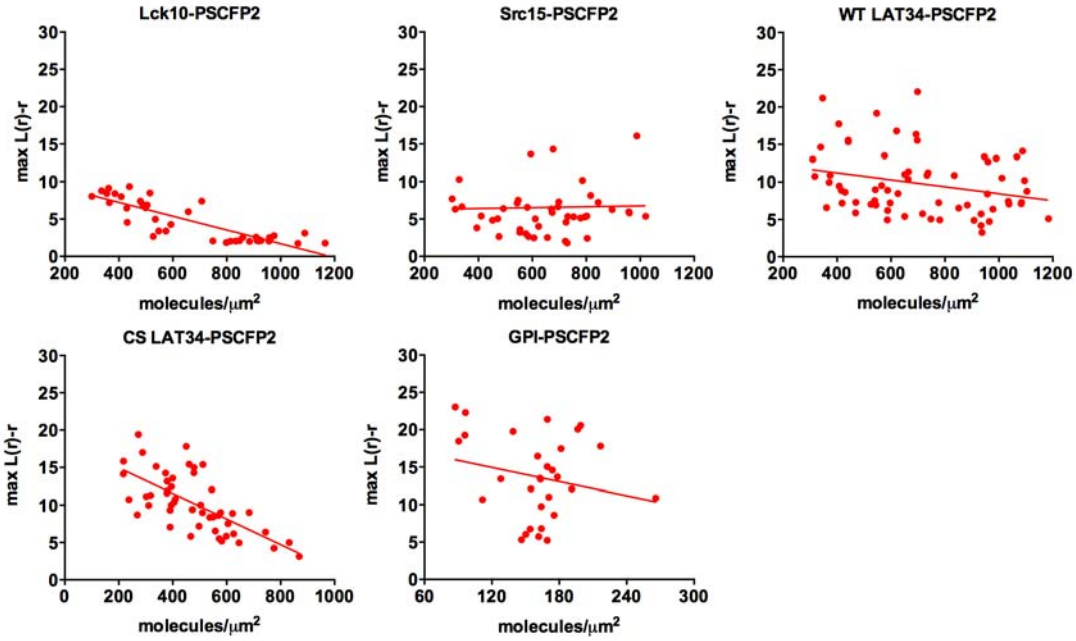

Lck10-PSCFP2
Src15-PSCFP2
WT LAT34-PSCFP2
CS LAT34-PSCFP2
GPI-PSCFP2
max L(r)-r
molecules/μm²

Figure S3

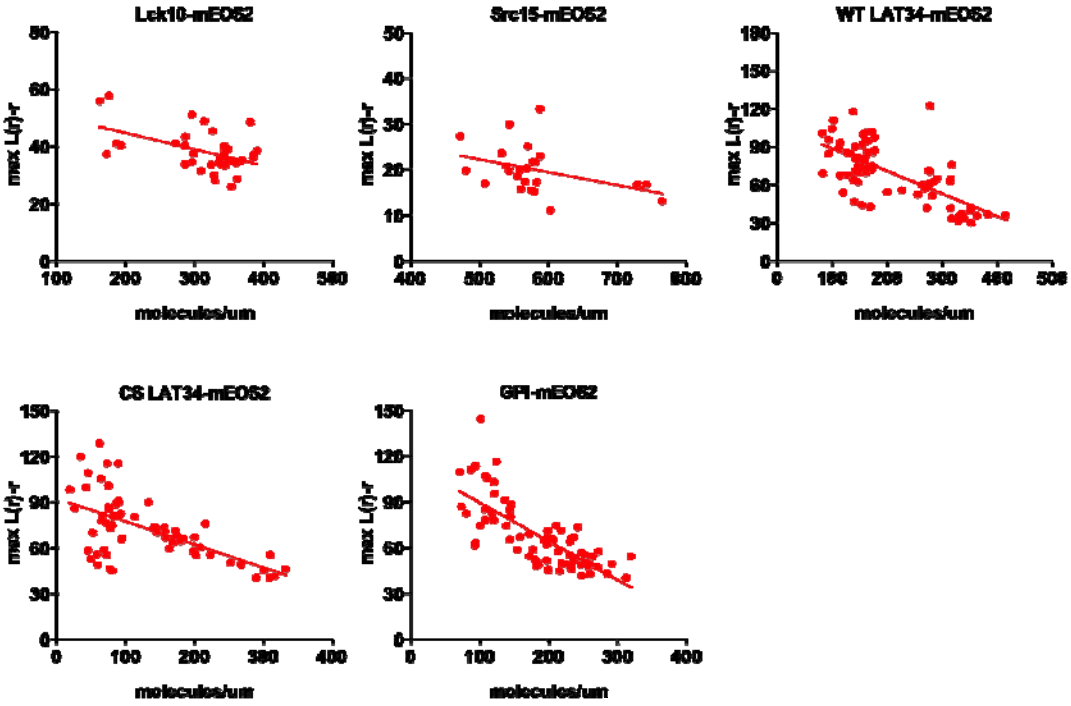

Lck10-mEOS2
max L(r)-r
molecules/um
Src15-mEOS2
max L(r)-r
molecules/um
WT LAT34-mEOS2
max L(r)-r
molecules/um
CS LAT34-mEOS2
max L(r)-r
molecules/um
GPI-mEOS2
max L(r)-r
molecules/um

Figure S4

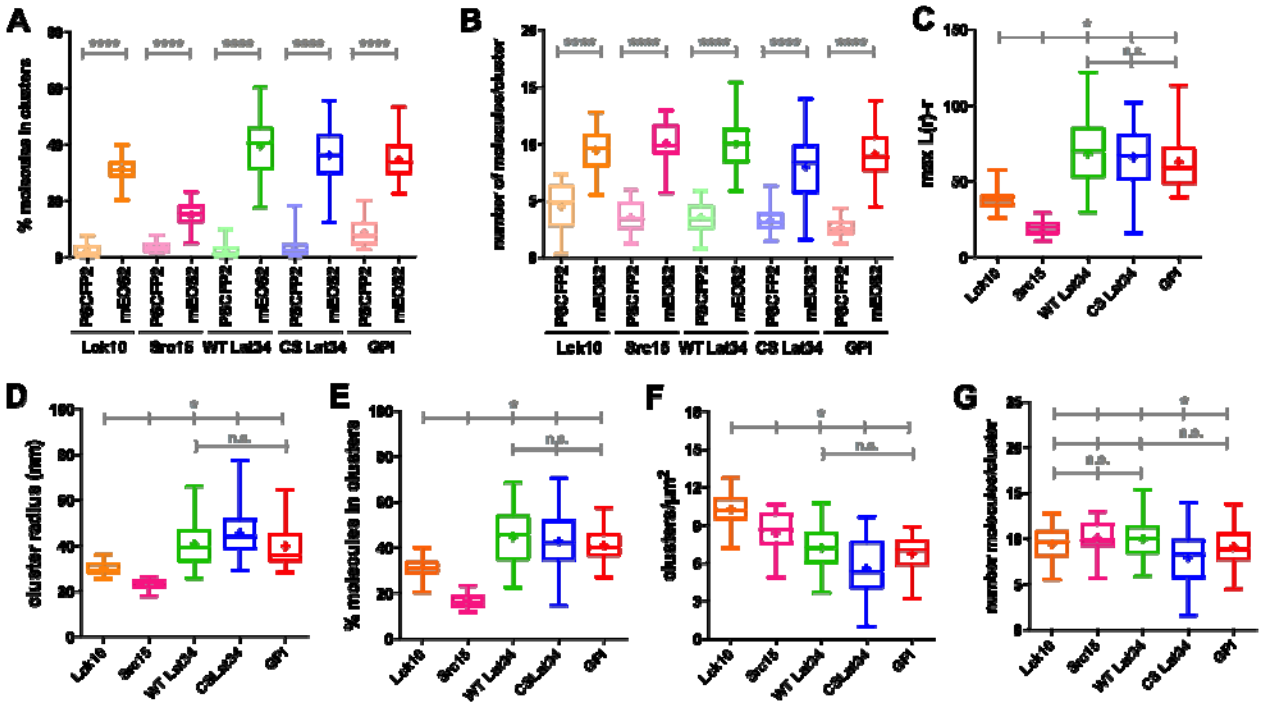

A
% molecules in clusters
PSCFP2
mEOS2
Lck10
Src15
WT Lat24
CS Lat24
GPI
B
number of molecules/cluster
C
max L(r)-r
D
cluster radius (nm)
E
% molecules in clusters
F
clusters/µm²
G
number molecules/cluster
n.s.
*
****

Figure S5

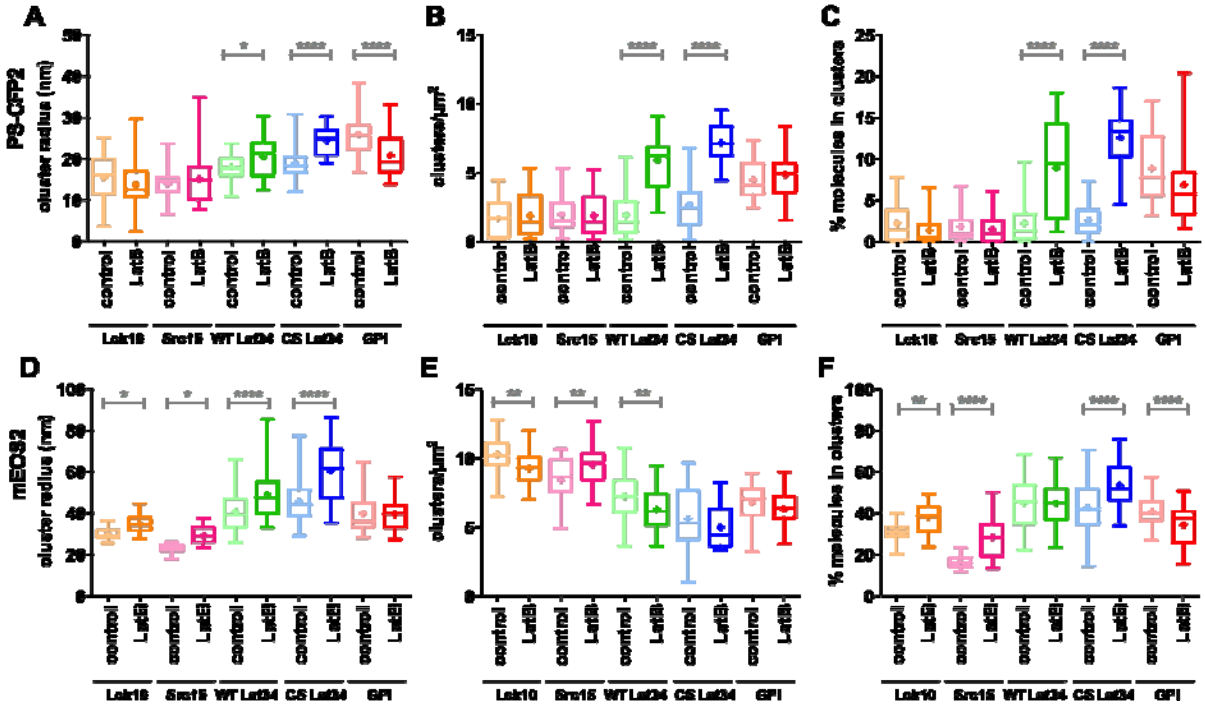
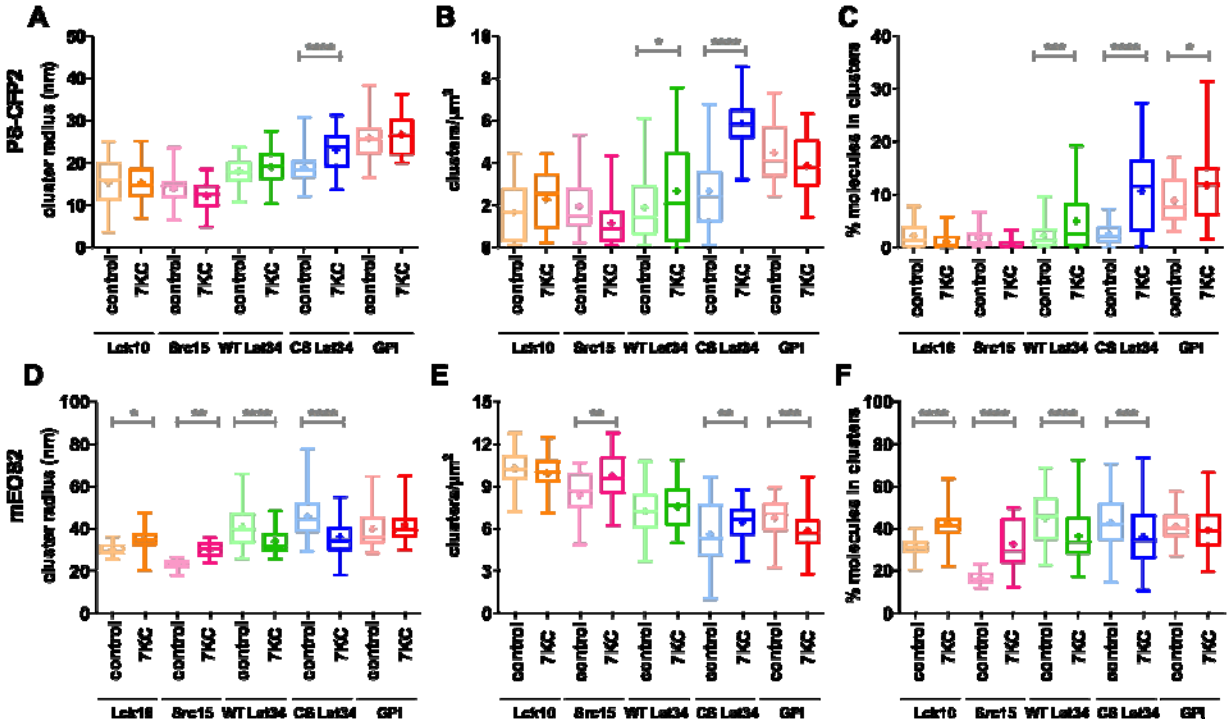

A
B
C
D
E
F
control
Latrunculin B
Lck10
Src15
WT Lat24
CS Lat24
GPI

Figure S6


A
B
C
D
E
F
PS-CFP2
cluster radius (nm)
clusters/µm²
% molecules in clusters
mEOS2
control
7KC
Lck10
Src15
WT Lat34
CS Lat34
GPI

Supplementary Table S1. Detailed statistical comparison of PS-CFP2 and mEOS2 clustering parameters in untreated control cells. The clustering parameters max L(r)-r, the percentage of molecules in clusters, number of molecules per cluster, clusters per μm^2 and the cluster radius (nm) were compared between PS-CFP2 and mEOS2-tagged membrane anchors using one-way ANOVA with Tukey's post-test. Each membrane anchor (tagged with PS-CFP2 or mEOS2 respectively) was compared to each other membrane anchor. Significant differences are indicated as follows: n.s. not significant, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ and $****p < 0.0001$.

	PS-CFP2-tagged membrane anchors					mEOS2-tagged membrane anchors					
	Lck10	Src15	WT Lat34	CS Lat 34	GPI-AP	Lck10	Src15	WT Lat34	CS Lat 34	GPI-AP	Parameter
Lck10		n.s.	**	****	****		***	****	****	****	Max L(r)-r
		n.s.	n.s.	n.s.	****		****	****	****	****	% molecules in clusters
		*	*	***	***		n.s.	n.s.	**	n.s.	Number molecules/cluster
		n.s.	n.s.	n.s.	****		****	****	****	****	Clusters/μm^2
		n.s.	n.s.	**	****		n.s.	****	****	****	Cluster radius (nm)
Src15	n.s.		n.s.	***	****	***		****	****	****	Max L(r)-r
	n.s.		n.s.	n.s.	****	****		****	****	****	% molecules in clusters
	*		n.s.	n.s.	*	n.s.		n.s.	***	n.s.	Number molecules/cluster
	n.s.		n.s.	n.s.	****	****		*	****	****	Clusters/μm^2
	n.s.		***	****	****	n.s.		****	****	****	Cluster radius (nm)
WT Lat34	**	n.s.		n.s.	****	****	****		n.s.	n.s.	Max L(r)-r
	n.s.	n.s.		n.s.	****	****	****		n.s.	n.s.	% molecules in clusters
	*	n.s.		n.s.	*	n.s.	n.s.		****	n.s.	Number molecules/cluster
	n.s.	n.s.		n.s.	****	****	*		****	n.s.	Clusters/μm^2
	n.s.	***		n.s.	****	****	****		*	n.s.	Cluster radius (nm)
CS Lat34	****	***	n.s.		***	****	****	n.s.		n.s.	Max L(r)-r
	n.s.	n.s.	n.s.		****	****	****	n.s.		n.s.	% molecules in clusters

	***	n.s.	n.s.		n.s.	**	***	****		*	Number molecules/cluster
	n.s.	n.s.	n.s.		****	****	****	****		****	Clusters/μm^2
	**	****	n.s.		****	****	****	*		**	Cluster radius (nm)
GPI-AP	****	****	****	***		****	****	n.s.	n.s.		Max L(r)-r
	****	****	****	****		****	****	n.s.	n.s.		% molecules in clusters
	***	*	*	n.s.		n.s.	n.s.	n.s.	*		Number molecules/cluster
	****	****	****	****		****	****	n.s.	****		Clusters/μm^2
	****	****	****	****		****	****	n.s.	**		Cluster radius (nm)