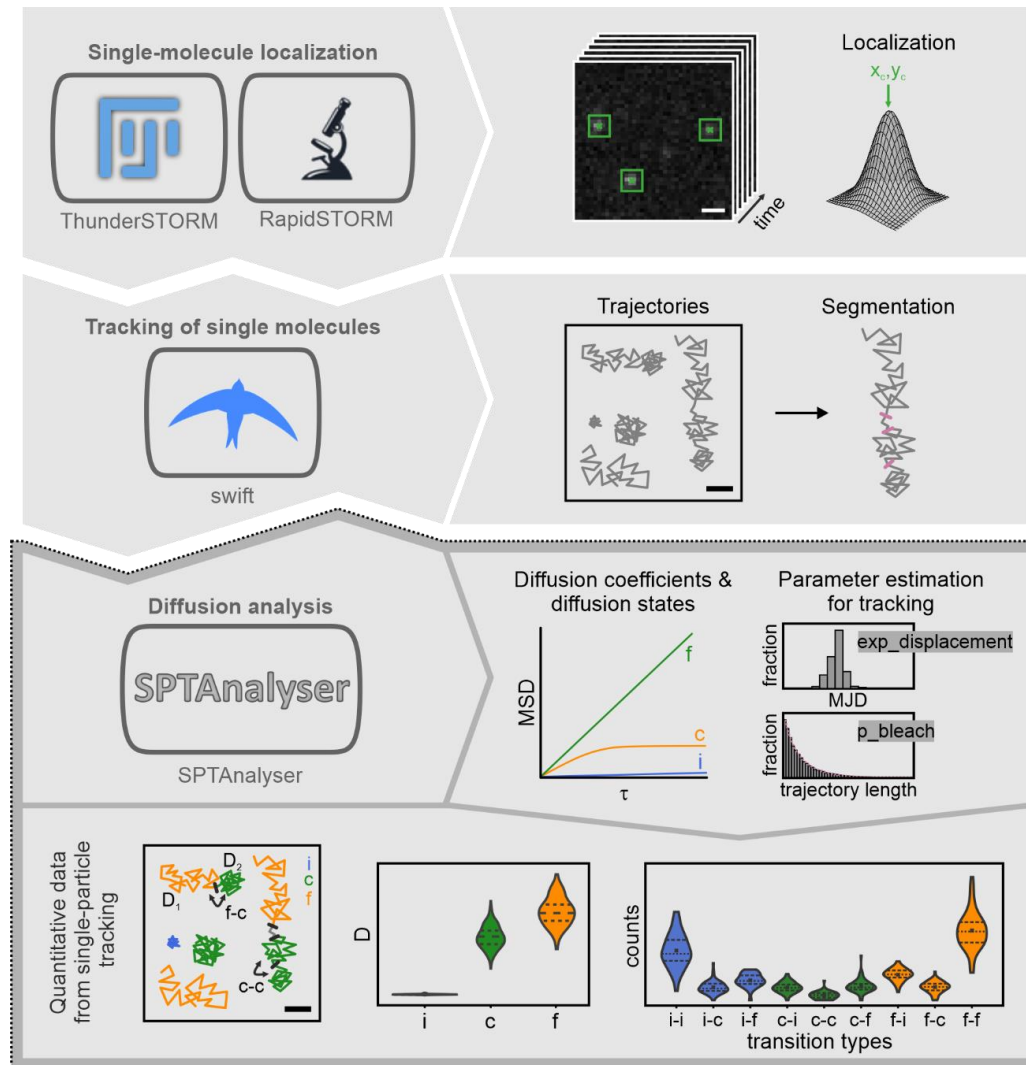
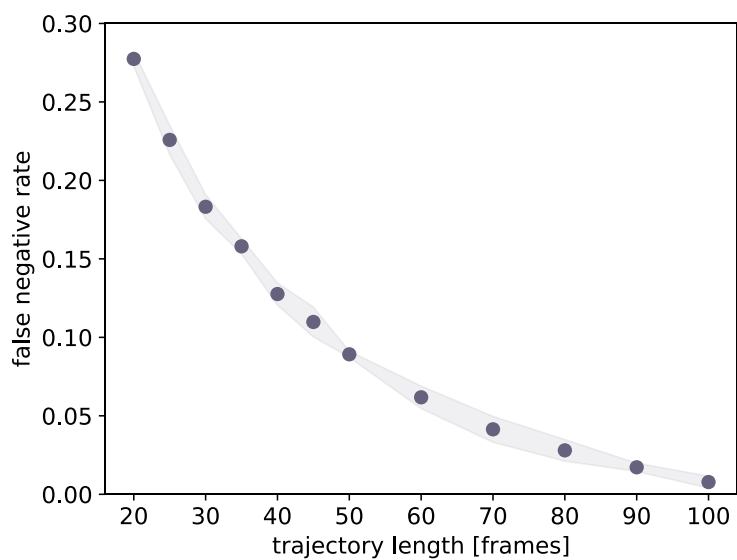


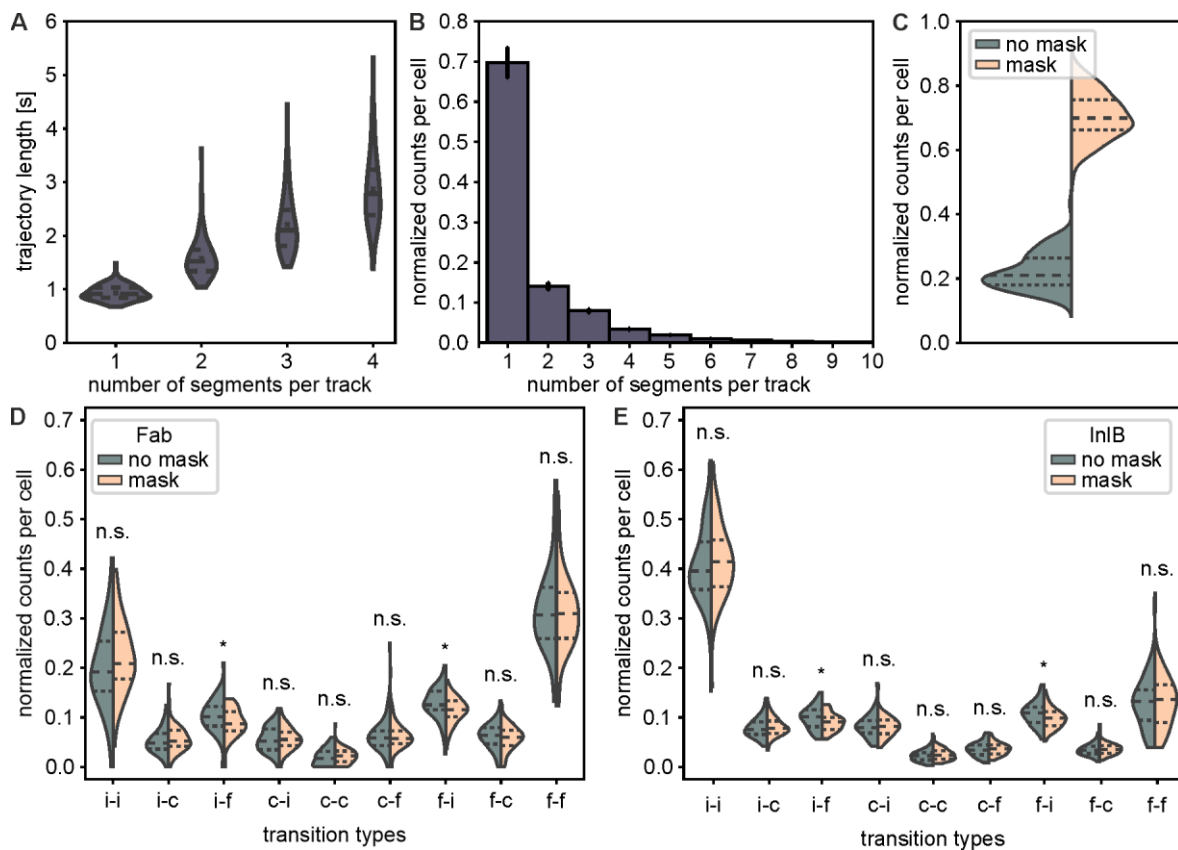
## Supplementary Material



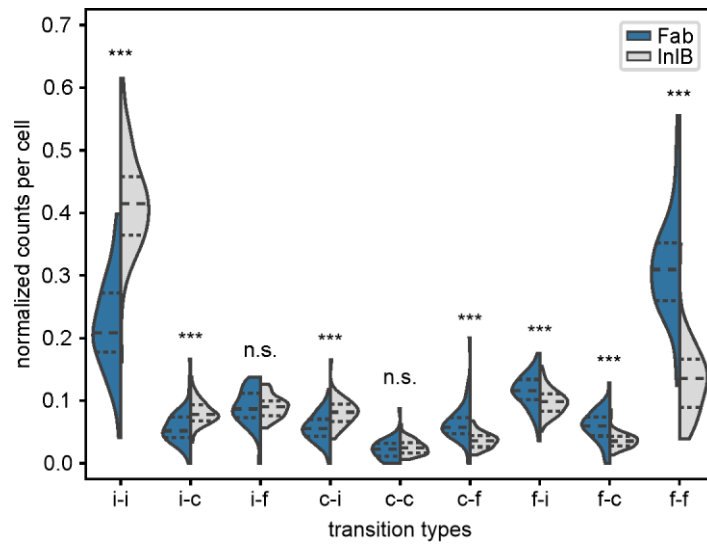
**Supplementary Figure 1:** Workflow of the analysis of single-particle tracking data with SPTAnalyser. The emission events of single molecules in the tracking movies are localized either with ThunderSTORM or RapidSTORM. The localization list is loaded into *swift*. *swift* generates trajectories and segments based on different input parameters. Several of these parameters such as the expected displacement *exp\_displacement* and the bleaching probability *p\_bleach* can be determined with a Jupyter Notebook of SPTAnalyser. SPTAnalyser also performs the analysis of the obtained segments. A MSD-based analysis extracts the diffusion coefficients as well as the diffusion states. For transition counting the diffusion state transitions between segments are counted.



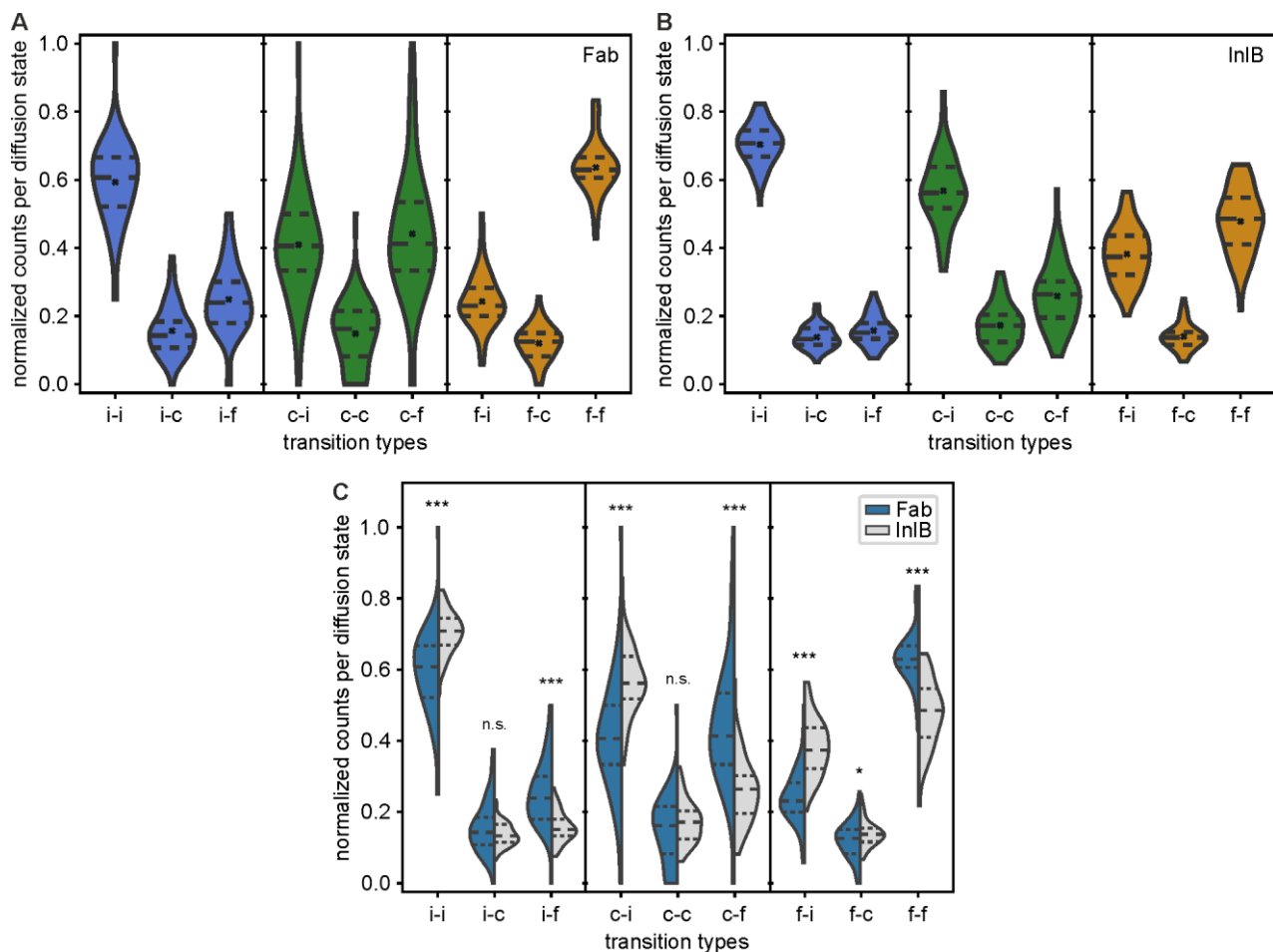
**Supplementary Figure 2:** False negative rate of trajectories of freely diffusing particles that were classified as confined. 1000 trajectories were simulated per defined trajectory length. The averages of five simulations per trajectory length are visualized as dots and the shaded areas denote the 95% confidence interval.



**Supplementary Figure 3:** Reasons for low numbers in counts and masking effects. (A) Relationship between trajectory length and the number of segments. Lengths were averaged per cell, dashed lines mark the quartiles, crosses represent the means. N per distribution = 120 cells. (B) Number of segments per trajectory. All trajectories of the 120 analyzed cells (60 Fab, 60 InlB) that contain at least one classified segment are included. Error bars mark the SEMs. (C) Violin plot of the relative occurrence of transitions between classified segments without and with masking. Masking of segments below the classification threshold of 20 frames increases the occurrence of transitions between classified segments for trajectories consisting of at least three segments. Dashed lines mark the quartiles. Violin plots of the nine different transition types between segments within trajectories in (D) Fab- and (E) InlB-treated cells (N per distribution = 60 cells, i = immobile, c = confined, f = free). Counts are normalized to one per cell. Transitions between not classified segments are neglected. Left and right sides of the violin plots compare normalized counts without and with masking (masking value = 20 frames). Significant differences of compared populations are marked in the plot (Mann-Whitney U test, significance level = 0.05,  $p > 0.05 = \text{n.s.}$ ,  $p < 0.05 = *$ ).



**Supplementary Figure 4:** Comparison of normalized transition counts per cell. Comparison of counts between Fab- and InlB-treated cells (i = immobile, c = confined, f = free). Counts are normalized to one per cell for 60 cells per condition. Dashed lines mark the quartiles. Significant differences of compared populations are marked in the plot (Mann-Whitney U test, significance level = 0.05,  $p > 0.05$  = n.s.,  $p < 0.001$  = \*\*\*).



**Supplementary Figure 5:** Comparison of normalized transition counts per diffusion state. Violin plots of the nine different transition types between segments within trajectories in (A) Fab-bound cells and (B) InlB-bound cells (i = immobile, c = confined, f = free). Counts are normalized to one per diffusion state for 60 cells per condition. Dashed lines mark the quartiles, dots the mean. (C) Comparison of counts between cells with Fab- and InlB-bound MET. Significant differences of compared populations are marked in the plot (Mann-Whitney U test, significance level = 0.05,  $p > 0.05 = \text{n.s.}$ ,  $p < 0.05 = *$ ,  $p < 0.001 = ***$ ).

**Supplementary Table 1:** Two-sample Mann-Whitney U test for segment diffusion coefficients, population of diffusion states, lengths, and confinement radii of Fab- and InlB-bound receptors (immobile = i, confined = c, free = f). Test-statistic, p-value and levels of significance (LOS) are listed. Significance level = 0.05,  $p > 0.05$  no significant difference (n.s.),  $p < 0.001$  highly significant difference (\*\*\*).

Sample 1 (Fab)	Sample 2 (InlB)	Test-statistic	p-value	LOS
diffusion coefficient i	diffusion coefficient i	4.9	3E-6	***
diffusion coefficient	diffusion coefficient	18.3	3E-36	***
diffusion coefficient f	diffusion coefficient f	15.9	3E-31	***
fraction i	fraction i	-21.1	7E-42	***
fraction c	fraction c	-6.5	2E-9	***
fraction f	fraction f	25.0	6E-49	***
segment length i	segment length i	-0.8	0.4	n.s.
segment length c	segment length c	-0.6	0.6	n.s.
segment length f	segment length f	5.7	8E-8	***
confinement radius c	confinement radius c	22.4	3E-44	***
confinement radius f	confinement radius f	17.0	2E-33	***

**Supplementary Table 2:** Wilcoxon signed-rank test for normalized transition count per cell comparisons within Fab-bound and InlB-bound receptors (immobile = i, confined = c, free = f). Test-statistic, p-value and levels of significance (LOS) are listed. Significance level = 0.05,  $p > 0.05$  no significant difference (n.s.),  $p < 0.05$  significant difference (\*),  $p < 0.01$  very significant difference (\*\*),  $p < 0.001$  highly significant difference (\*\*\*).

Sample 1 (Fab)	Sample 2 (Fab)	Test-statistic	p-value	LOS	Sample 1 (InlB)	Sample 2 (InlB)	Test-statistic	p-value	LOS
i-i	i-c	16.0	4E-23	***	i-i	i-c	32.3	4E-39	***
i-i	i-f	12.2	8E-18	***	i-i	i-f	28.1	8E-36	***
i-i	c-i	16.7	4E-24	***	i-i	c-i	33.8	3E-40	***
i-i	c-c	20.1	4E-28	***	i-i	c-c	36.4	4E-42	***
i-i	c-f	13.4	1E-19	***	i-i	c-f	34.0	2E-40	***
i-i	f-i	10.7	2E-15	***	i-i	f-i	26.6	2E-34	***
i-i	f-c	14.2	9E-21	***	i-i	f-c	33.8	3E-40	***
i-i	f-f	-4.7	2E-5	***	i-i	f-f	17.5	5E-25	***
i-c	i-f	-5.6	7E-7	***	i-c	i-f	-2.8	0.006	**
i-c	c-i	0.1	0.9	n.s.	i-c	c-i	-0.4	0.7	n.s.
i-c	c-c	9.3	4E-13	***	i-c	c-c	23.0	3E-31	***

i-c	c-f	-1.0	0.3	n.s.	i-c	c-f	13.4	1E-19	***
i-c	f-i	-10.7	2E-15	***	i-c	f-i	-4.5	3E-5	***
i-c	f-c	-0.2	0.8	n.s.	i-c	f-c	13.3	2E-19	***
i-c	f-f	-20.8	8E-29	***	i-c	f-f	-6.0	1E-7	***
i-f	c-i	7.1	2E-9	***	i-f	c-i	2.2	0.029	*
i-f	c-c	15.5	2E-22	***	i-f	c-c	24.5	1E-32	***
i-f	c-f	4.4	5E-5	***	i-f	c-f	22.1	3E-30	***
i-f	f-i	-5.5	8E-7	***	i-f	f-i	-3.1	0.003	**
i-f	f-c	6.2	6E-8	***	i-f	f-c	21.1	3E-29	***
i-f	f-f	-17.8	2E-25	***	i-f	f-f	-5.9	2E-7	***
c-i	c-c	11.0	6E-16	***	c-i	c-c	20.2	4E-28	***
c-i	c-f	-1.1	0.3	n.s.	c-i	c-f	12.1	1E-17	***
c-i	f-i	-10.8	2E-15	***	c-i	f-i	-3.9	2E-4	***
c-i	f-c	-0.4	0.7	n.s.	c-i	f-c	12.8	1E-18	***
c-i	f-f	-20.6	1E-28	***	c-i	f-f	-5.5	8E-7	***
c-c	c-f	-8.6	5E-12	***	c-c	c-f	-5.1	3E-6	***
c-c	f-i	-20.0	6E-28	***	c-c	f-i	-24.8	6E-33	***
c-c	f-c	-9.5	1E-13	***	c-c	f-c	-5.4	1E-6	***
c-c	f-f	-25.5	1E-33	***	c-c	f-f	-14.4	6E-21	***
c-f	f-i	-10.7	2E-15	***	c-f	f-i	-23.0	3E-31	***
c-f	f-c	0.9	0.4	n.s.	c-f	f-c	-0.2	0.9	n.s.
c-f	f-f	-24.3	2E-32	***	c-f	f-f	-14.9	1E-21	***
f-i	f-c	910.0	3E-14	***	f-i	f-c	23.9	5E-32	***
f-i	f-f	-16.2	2E-23	***	f-i	f-f	-5.0	6E-6	***
f-c	f-f	-23.5	1E-31	***	f-c	f-f	-14.8	2E-21	***

**Supplementary Table 3:** Two-sample Mann-Whitney U test for un-masked vs masked normalized transition counts per cell of Fab and InlB-bound receptors (immobile = i, confined = c, free = f). Test-statistic, p-value and levels of significance (LOS) are listed. Significance level = 0.05,  $p > 0.05$  no significant difference (n.s.),  $p < 0.05$  significant difference (\*).

Sample 1 (Fab no mask)	Sample 2 (Fab mask)	Test-statistic	p-value	LOS	Sample 1 (InlB no mask)	Sample 2 (InlB mask)	Test-statistic	p-value	LOS
i-i	i-i	-1.7	0.9	n.s.	i-i	i-i	-0.7	0.5	n.s.
i-c	i-c	-0.8	0.4	n.s.	i-c	i-c	-0.4	0.7	n.s.
i-f	i-f	2.3	0.026	*	i-f	i-f	2.3	0.025	*
c-i	c-i	-0.3	0.8	n.s.	c-i	c-i	0.1	1.0	n.s.
c-c	c-c	-0.7	0.5	n.s.	c-c	c-c	-1.2	0.2	n.s.
c-f	c-f	0.1	0.9	n.s.	c-f	c-f	-0.3	0.7	n.s.
f-i	f-i	2.5	0.013	*	f-i	f-i	2.4	0.016	*
f-c	f-c	0.6	0.5	n.s.	f-c	f-c	-0.7	0.5	n.s.
f-f	f-f	0.1	0.9	n.s.	f-f	f-f	-0.3	0.8	n.s.

**Supplementary Table 4:** Two-sample Mann-Whitney U test for normalized transition counts per cell of Fab and InlB bound receptors (immobile = i, confined = c, free = f). Test-statistic, p-value and levels of significance (LOS) are listed. Significance level = 0.05,  $p > 0.05$  no significant difference (n.s.),  $p < 0.001$  highly significant difference (\*\*\*).

Sample 1 (Fab)	Sample 2 (InlB)	Test-statistic	p-value	LOS
i-i	i-i	-13.7	4E-26	***
i-c	i-c	-5.4	4E-7	***
i-f	i-f	-0.3	0.8	n.s.
c-i	c-i	-6.1	2E-8	***
c-c	c-c	-1.4	0.2	n.s.
c-f	c-f	6.5	2E-9	***
f-i	f-i	3.6	4E-4	***
f-c	f-c	6.0	2E-8	***
f-f	f-f	14.0	9E-27	***



**Supplementary Table 5:** Wilcoxon signed-rank test for normalized transition count per diffusion state comparisons within Fab-bound and InlB-bound receptors (immobile = i, confined = c, free = f). Test-statistic, p-value and levels of significance (LOS) are listed. Significance level = 0.05,  $p > 0.05$  no significant difference (n.s.),  $p < 0.05$  significant difference (\*),  $p < 0.01$  very significant difference (\*\*),  $p < 0.001$  highly significant difference (\*\*\*)

Sample 1 (Fab)	Sample 2 (Fab)	Test-statistic	p-value	LOS	Sample 1 (InlB)	Sample 2 (InlB)	Test-statistic	p-value	LOS
i-i	i-c	19.2	4E-27	***	i-i	i-c	50.0	5E-50	***
i-i	i-f	12.7	2E-18	***	i-i	i-f	42.9	4E-46	***
i-i	c-i	7.0	2E-9	***	i-i	c-i	11.1	4E-16	***
i-i	c-c	22.3	2E-30	***	i-i	c-c	41.5	2E-45	***
i-i	c-f	5.2	2E-6	***	i-i	c-f	28.4	4E-36	***
i-i	f-i	20.8	7E-29	***	i-i	f-i	27.3	4E-35	***
i-i	f-c	26.1	4E-34	***	i-i	f-c	64.4	2E-56	***
i-i	f-f	-2.2	0.032	*	i-i	f-f	14.4	6E-21	***
i-c	i-f	-5.8	3E-7	***	i-c	i-f	-3.1	0.003	**
i-c	c-i	-12.7	2E-18	***	i-c	c-i	-31.5	1E-38	***
i-c	c-c	0.5	0.586	n.s.	i-c	c-c	-4.7	2E-5	***
i-c	c-f	-10.1	2E-14	***	i-c	c-f	-8.7	4E-12	***
i-c	f-i	-5.8	3E-7	***	i-c	f-i	-22.1	3E-30	***
i-c	f-c	3.3	0.002	**	i-c	f-c	-0.243	0.809	n.s.
i-c	f-f	-37.6	6E-43	***	i-c	f-f	-25.5	1E-33	***
i-f	c-i	-6.0	1E-7	***	i-f	c-i	-24.8	6E-33	***
i-f	c-c	5.4	1E-6	***	i-f	c-c	-1.7	0.091	n.s.
i-f	c-f	-7.8	1E-10	***	i-f	c-f	-9.4	3E-13	***
i-f	f-i	0.4	0.715	n.s.	i-f	f-i	-16.9	3E-24	***
i-f	f-c	9.5	2E-13	***	i-f	f-c	2.3	0.026	*
i-f	f-f	-24.2	3E-32	***	i-f	f-f	-27.5	3E-35	***
c-i	c-c	10.6	3E-15	***	c-i	c-c	22.5	1E-30	***
c-i	c-f	-0.7	0.45712	n.s.	c-i	c-f	13.0	6E-19	***
c-i	f-i	7.4	6E-10	***	c-i	f-i	16.1	3E-23	***
c-i	f-c	14.1	2E-20	***	c-i	f-c	33.4	5E-40	***
c-i	f-f	-9.3	4E-13	***	c-i	f-f	4.1	1E-4	***
c-c	c-f	-9.0	1E-12	***	c-c	c-f	-5.4	1E-6	***
c-c	f-i	-5.8	3E-7	***	c-c	f-i	-15.8	6E-23	***
c-c	f-c	2.1	0.045	*	c-c	f-c	3.6	7E-4	***
c-c	f-f	-29.3	8E-37	***	c-c	f-f	-21.0	4E-29	***

c-f	f-i	7.6	3E-10	***	c-f	f-i	-6.2	7E-8	***
c-f	f-c	12.2	9E-18	***	c-f	f-c	8.5	8E-12	***
c-f	f-f	-8.0	6E-11	***	c-f	f-f	-18.9	9E-27	***
f-i	f-c	9.1	9E-13	***	f-i	f-c	22.6	9E-31	***
f-i	f-f	-21.1	4E-29	***	f-i	f-f	-4.3	6E-5	***
f-c	f-f	-37.6	6E-43	***	f-c	f-f	-22.0	4E-30	***

**Supplementary Table 6:** Two-sample Mann-Whitney U test for normalized transition counts per diffusion state of Fab and InlB bound receptors (immobile = i, confined = c, free = f). Test-statistic, p-value and levels of significance (LOS) are listed. Significance level = 0.05,  $p > 0.05$  no significant difference (n.s.),  $p < 0.05$  significant difference (\*),  $p < 0.001$  highly significant difference (\*\*\*).

Sample 1 (Fab)	Sample 2 (InlB)	Test-statistic	p-value	LOS
i-i	i-i	-6.2883	5.6187E-9	***
i-c	i-c	1.8323	0.06942	n.s.
i-f	i-f	6.6024	1.2153E-9	***
c-i	c-i	-6.506	1.9507E-9	***
c-c	c-c	-1.6136	0.10928	n.s.
c-f	c-f	6.7898	4.802E-10	***
f-i	f-i	-9.6649	1.2491E-16	***
f-c	f-c	-2.3331	0.02134	*
f-f	f-f	10.014	1.8602E-17	***