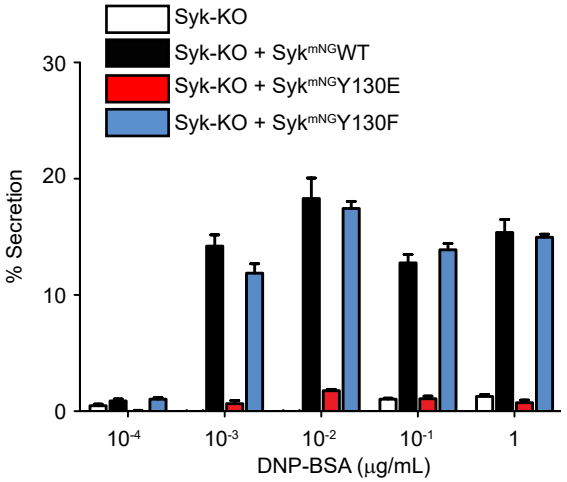
Supplemental Materials

Molecular Biology of the Cell

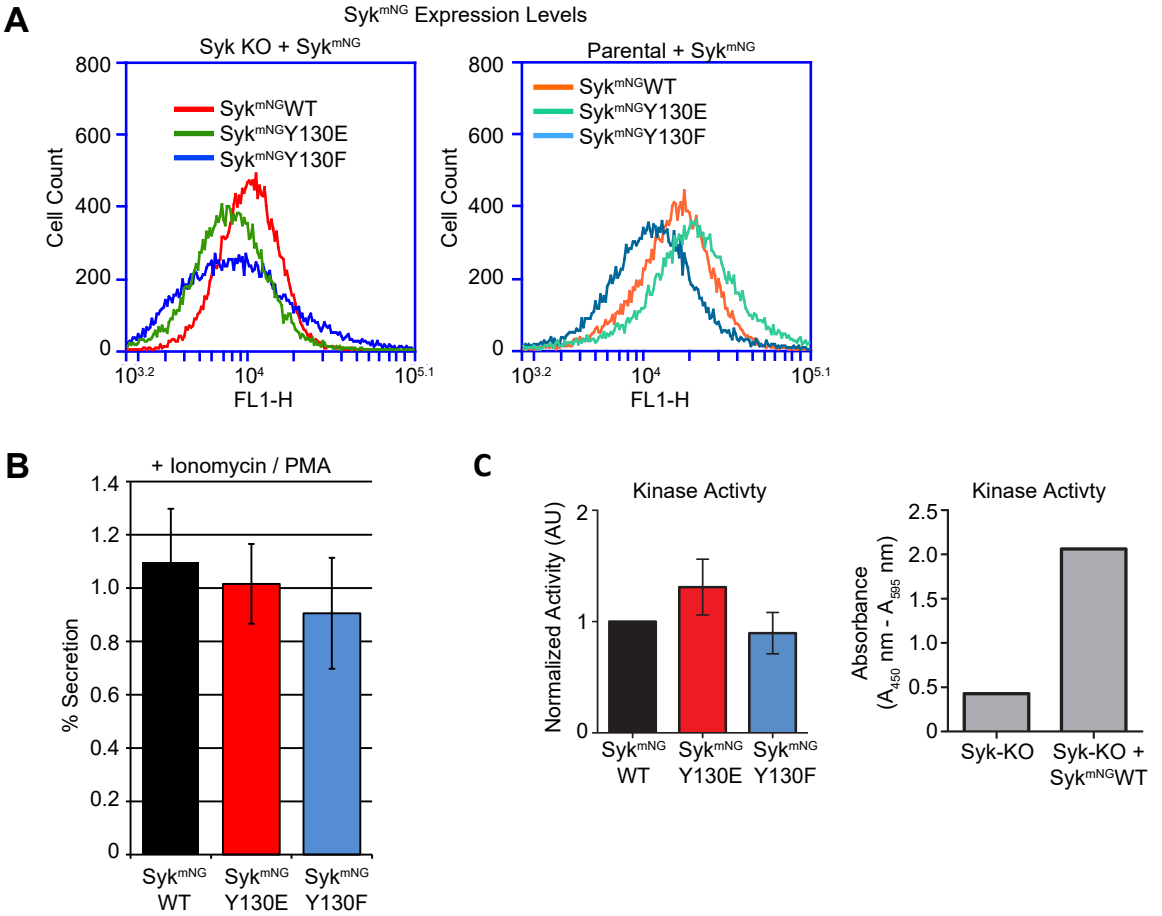
Schwartz et al.

				α_s			k_f (s^{-1})			k_s (s^{-1})			D ($\mu m^2/s$)		
Cell Type	N tracks	Drug	DNP-BSA µg/mL	Value	- std	+ std	Value	- std	+ std	Value	- std	+ std	Value	- 95% CI	+ 95% CI
WT	1434		0	0.055	0.048	0.062	2.581	2.507	2.651	0.625	0.607	0.644	0.031	0.030	0.032
WT	834		0.001	0.099	0.085	0.112							0.026	0.024	0.028
WT	337		0.01	0.114	0.094	0.137							0.024	0.022	0.027
WT	3111		0.1	0.154	0.145	0.163							0.020	0.019	0.021
WT	3105		1	0.234	0.219	0.250							0.011	0.011	0.011
WT	218	1 µM Das	0	0.012	0.004	0.020	2.581	*fixed*		0.625	*fixed*				
WT	400	1 µM Das	0.1	0.026	0.016	0.035									
Y130E	1003		0	0.041	0.034	0.048	3.313	3.204	3.422	0.871	0.831	0.907			
Y130E	702		0.001	0.079	0.066	0.094									
Y130E	1859		0.1	0.115	0.104	0.129									
Y130E	1584		1	0.232	0.209	0.253									
Y130F	872		0	0.038	0.031	0.045	2.781	2.690	2.875	0.649	0.629	0.668			
Y130F	504		0.001	0.034	0.025	0.043									
Y130F	2801		0.1	0.241	0.227	0.256									
Y130F	3476		1	0.257	0.242	0.273									

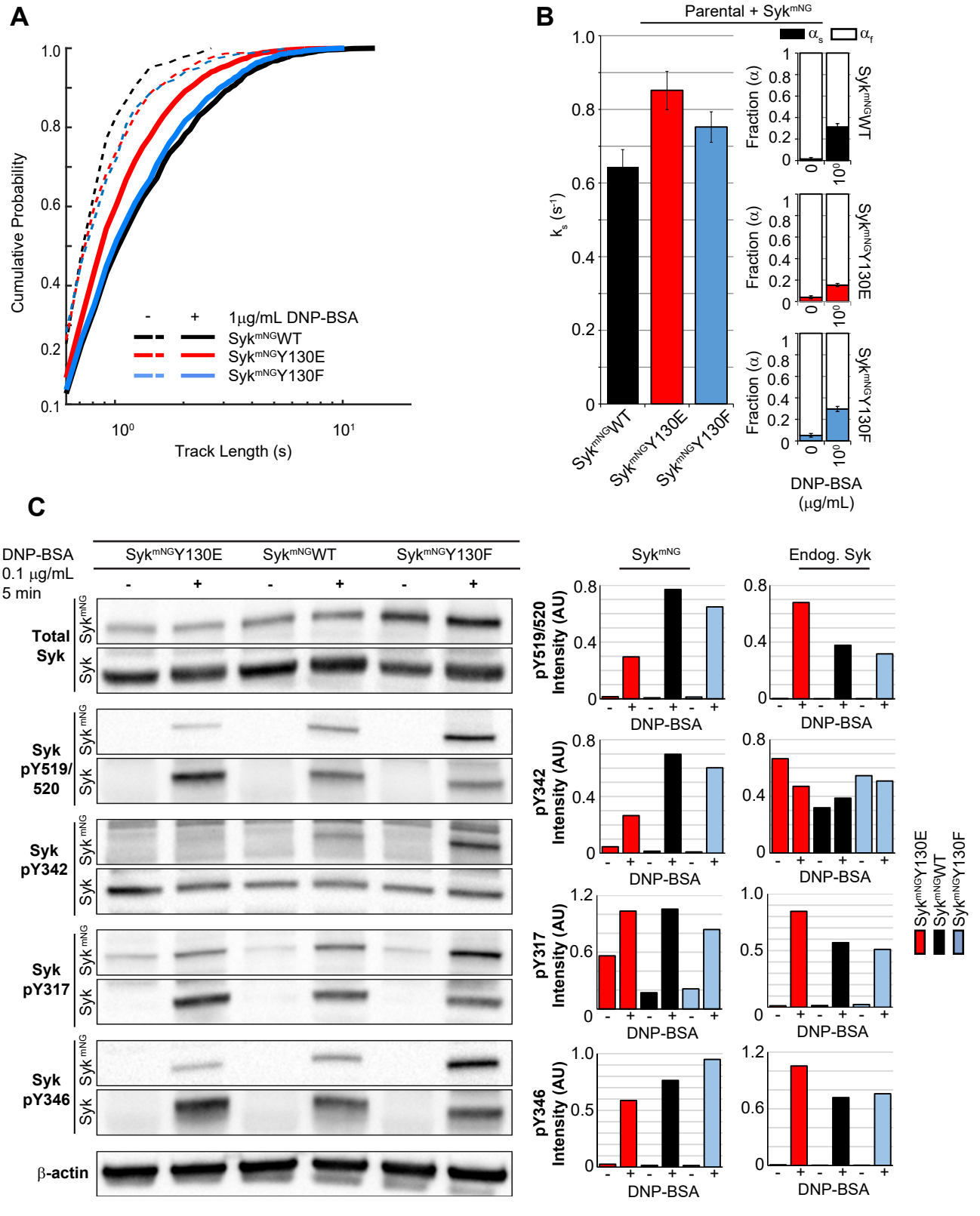
Supplemental Table S1. Measured Syk off-rates and diffusion coefficients. Off-rate parameter values found from fitting single molecule track lengths to a two-component geometric distribution mixture model, with both off-rate parameter values globally constrained across cell type in Syk-KO cells expressing Syk^{mNG}-WT, Syk^{mNG}-Y130E, or Syk^{mNG}-Y130F. Data for each condition displayed with cell type, the number of trajectories used in the analysis, if addition of 1 µM Dasatinib treatment, and dose of DNP-BSA (µg/mL). The estimated fast off-rate (k_f), the slow off-rate (k_s), and the relative fraction of slow off-rate (α_s) are reported (Value) along with a standard error below (-std) or above (+std) based on a 68% credible interval (see Methods). For the WT +1 µM Das conditions, the off-rate values were fixed to the WT untreated rate. The estimated diffusion coefficients (D) for the Syk^{mNG}-WT are reported in the right most column along with the lower (-95%CI) and upper (+95% CI) 95% confidence intervals.



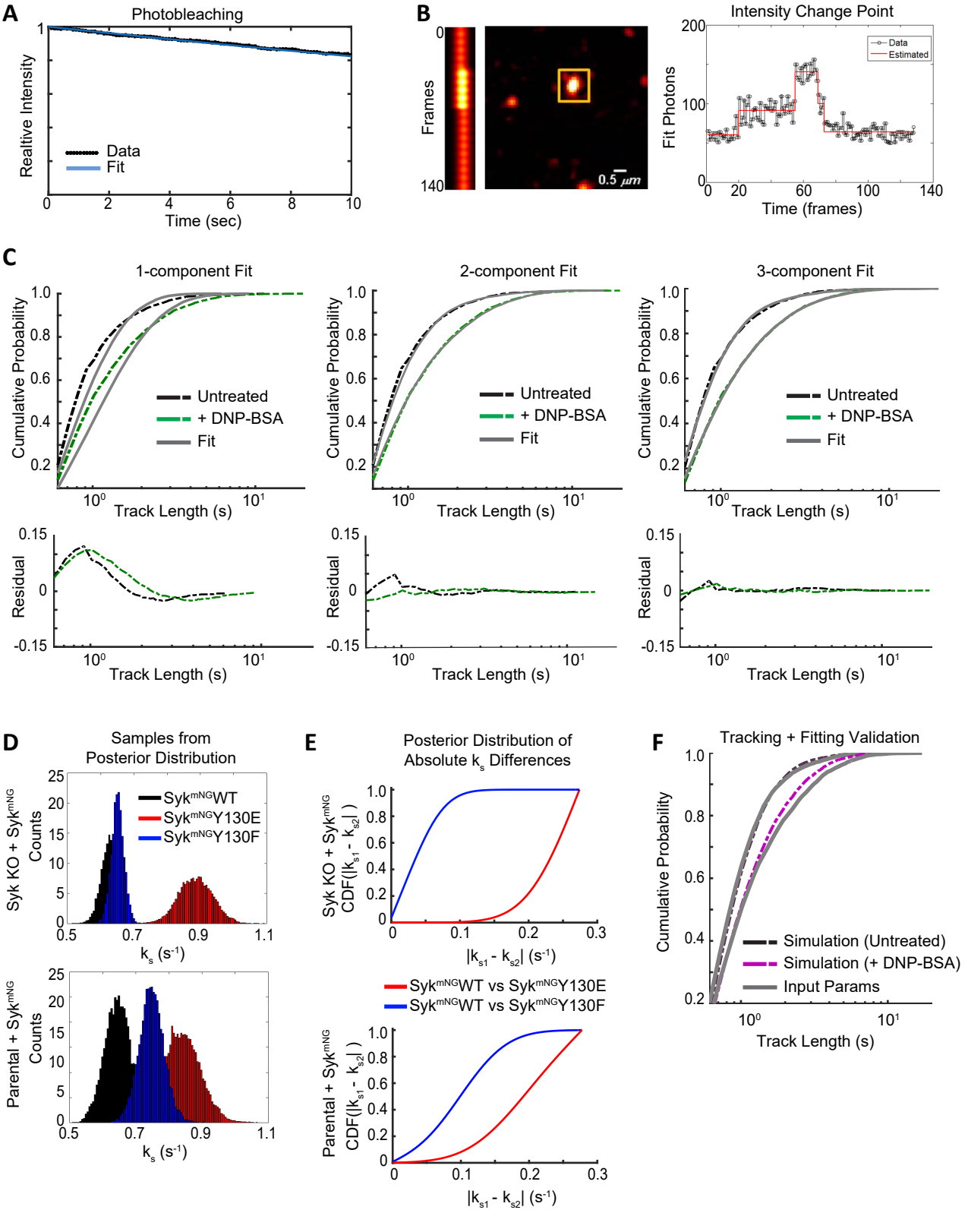
Supplemental Figure S1. Mast cell degranulation response for Syk^{mNG}-WT and mutants. Degranulation assay showing fraction of β-hexosaminidase released after 30 min of incubation over a range of DNP-BSA doses in Syk-KO cells (white bars) or in Syk-KO cells stably expressing Syk^{mNG}-WT (black bars), Syk^{mNG}-Y130E (red bars), Syk^{mNG}-Y130F (blue bars).



Supplemental Figure S2. Characterization of stable Syk^{mNG} cell lines. (A) Syk^{mNG}-WT, Syk^{mNG}-Y130E, and Syk^{mNG}-Y130F expression quantified using flow cytometry in Syk-KO or parental RBL-2H3 cell lines. (B) β-hexosaminidase release after treatment with Ionomycin and PMA in Syk-KO cell lines expressing Syk^{mNG}-WT, Syk^{mNG}-Y130E, or Syk^{mNG}-Y130F. (C) Left: Relative kinase activity for Syk-KO cell lines expressing Syk^{mNG}-WT, Syk^{mNG}-Y130E, or Syk^{mNG}-Y130F. Values normalized to Syk^{mNG}-WT for comparison. Right: Raw absorbance measurements in Syk-KO cells with (positive control) or without (negative control) expression of Syk^{mNG}-WT.


A
Cumulative Probability
Track Length (s)
1µg/mL DNP-BSA
Syk^mNGWT
Syk^mNGY130E
Syk^mNGY130F
B
Parental + Syk^mNG
k_s (s^-1)
Fraction (α)
DNP-BSA (µg/mL)
C
DNP-BSA 0.1 µg/mL 5 min
Total Syk
Syk pY519/520
Syk pY342
Syk pY317
Syk pY346
β-actin
pY519/520 Intensity (AU)
pY342 Intensity (AU)
pY317 Intensity (AU)
pY346 Intensity (AU)
Endog. Syk

Supplemental Figure S3. Syk^{mNG}-Y130E exhibits a faster off-rate and altered phosphorylation in the presence of endogenous Syk within parental RBL-2H3 cells. (A) Cumulative probability distributions for Syk^{mNG} trajectory lengths in parental RBL-2H3 cells expressing Syk^{mNG}-WT, Syk^{mNG}-Y130E, or Syk^{mNG}-Y130F both before (dashed lines) and after (solid lines) addition of 1 mg/mL DNP-BSA. (B) Slow off-rate value (k_s) found when fitting distriubtions in (A). Fraction of slow off-rate component (α_s) increases with DNP-BSA dose for Syk^{mNG}-WT and each mutant (right). Error bars are a 68% credible interval as described in Methods. (C) Western blot detection of Syk phosphorylation profile in parental RBL-2H3 cells expressing Syk^{mNG}-WT, Syk^{mNG}-Y130E, or Syk^{mNG}-Y130F in response to stimulation with 0.1 μg/mL DNP-BSA for 5 min. Blots show both endogenous Syk phosphorylation (top) and Syk^{mNG} phosphorylation (bottom) at each site for all three cell types. Quantification of Syk phosphorylation from one representative experiment; endogenous Syk phosphorylation (left) and Syk^{mNG} phosphorylation (right).


A
Photobleaching
Realtive Intensity
Data
Fit
Time (sec)
B
Frames
0.5 μm
Intensity Change Point
Fit Photons
Data
Estimated
Time (frames)
C
1-component Fit
2-component Fit
3-component Fit
Cumulative Probability
Untreated
+ DNP-BSA
Fit
Track Length (s)
Residual
D
Samples from
Posterior Distribution
Syk KO + Syk^mNG Counts
Parental + Syk^mNG Counts
Syk^mNGWT
Syk^mNGY130E
Syk^mNGY130F
k_s (s^-1)
E
Posterior Distribution of
Absolute k_s Differences
Syk KO + Syk^mNG CDF(|k_s1 - k_s2|)
Parental + Syk^mNG CDF(|k_s1 - k_s2|)
|k_s1 - k_s2| (s^-1)
Syk^mNGWT vs Syk^mNGY130E
Syk^mNGWT vs Syk^mNGY130F
F
Tracking + Fitting Validation
Cumulative Probability
Simulation (Untreated)
Simulation (+ DNP-BSA)
Input Params
Track Length (s)

Supplemental Figure S4. Overview of Syk trajectory off-rate fitting approach. (A) The photobleaching rate for mNG under the experimental imaging setup used for all single molecule data collection. The intensity of the whole cell was monitored under continuous illumination over time. A rate of 0.0193 s^{-1} with 95% confidence interval (0.01928, 0.01933) and a goodness of fit R-square value = 0.9994 was found using the Matlab Curve Fitting Tool to fit the relative intensity to a custom equation f(x) = exp(-a*x). (B) Example intensity profile and change point analysis for a single Syk^{mNG} trajectory. (Left) Kymograph of particle intensities over time. Scale bar, 0.5 μm. (Right) Intensity profile and resulting identified change points using change point analysis. (C) Comparison of fitting models for Syk^{mNG} trajectory off-rates. Cumulative probability distribution of Syk^{mNG} trajectory lengths before (black) and after (green) addition of 1 μg/mL DNP-BSA compared with fitting (grey) using a 1, 2 and 3 component geometric mixture model. Residuals of data and fit are shown below for each model. (D) The sampled posterior distribution of the k_s parameter value using Markov-Chain Monte Carlo for data from Syk^{mNG}-WT, Syk^{mNG}-Y130E, or Syk^{mNG}-Y130F trajectories in Syk-KO cells (top) or parental RBL cells (bottom). (E) Cumulative probability distributions for expected differences in k_s parameter value between Syk^{mNG}-WT and Syk^{mNG}-Y130E (red) or Syk^{mNG}-WT and Syk^{mNG}-Y130F (blue) in Syk-KO cells (top) or parental RBL cells (bottom). See Methods for more details. (F) Validation of tracking and fitting approach. Parameters similar to those found for Syk^{mNG}-WT in untreated and DNP-BSA stimulated cells were used to generate simulated image series of single molecule binding events that were then analyzed. Cumulative probability distribution of resulting trajectory lengths (untreated-black, DNP-BSA stimulated-magenta) are compared with the expected distribution given the input parameters (grey). Fit parameters found from the simulation are within one standard error of the input parameters. See Methods for more details.