

# Supporting Information

O'Neill et al. 10.1073/pnas.1205345109

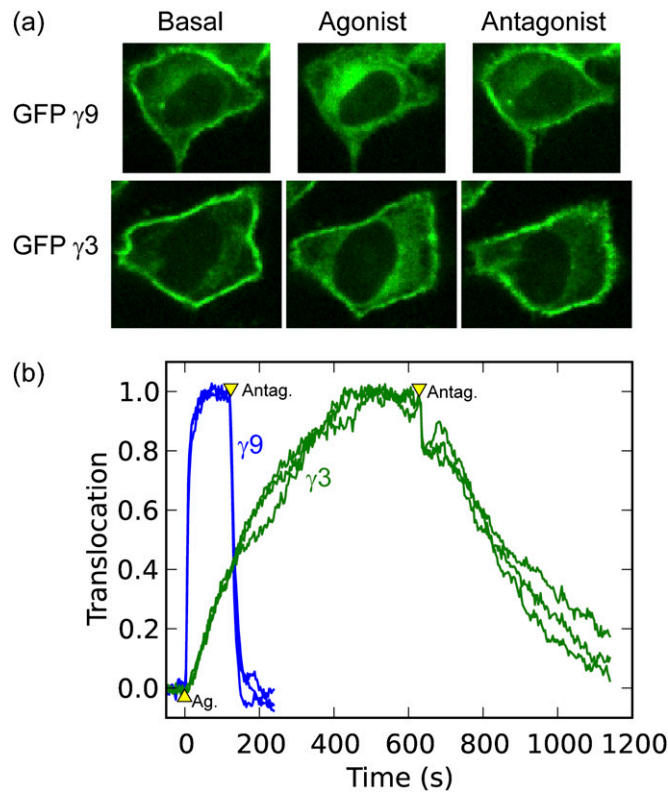
## SI Estimation of Cytosolic Diffusion Rate of GFP-Tagged $\beta\gamma$ -Subunits (Upper Bound)

To examine when cytosolic diffusion, rather than membrane dissociation, becomes rate limiting for  $\beta\gamma$ -subunit translocation, we estimated the diffusivity of a GFP-tagged  $\beta\gamma$ -subunit on the basis of its molecular weight. By measuring cytosolic diffusion of engineered GFP multimers in *Escherichia coli*, cytosolic protein diffusivities have been calibrated within the 27- to 111-kDa range (1). On the basis of those measurements, we estimated that a GFP-tagged  $\beta\gamma$ -dimer with a molecular weight of  $\sim 70$  kDa ( $\beta$ , 35 kDa;  $\gamma$ , 7–9 kDa; GFP, 27 kDa) has a diffusion coefficient of about  $7 \mu\text{m}^2/\text{s}$ . Cytosolic diffusion of the tagged  $\beta\gamma$ -complex is unlikely to be any faster than this, but it could be slower due to

hydrophobic interactions of cytosolic factors with the  $\gamma$ -subunit prenyl moiety. For 3D diffusion the mean square distance traveled  $\langle r^2 \rangle$ , is related to the diffusion time  $t$  by  $\langle r^2 \rangle = 6Dt$ , where  $D$  is the diffusion constant. Using  $D = 7 \mu\text{m}^2/\text{s}$  gives the following relationship:

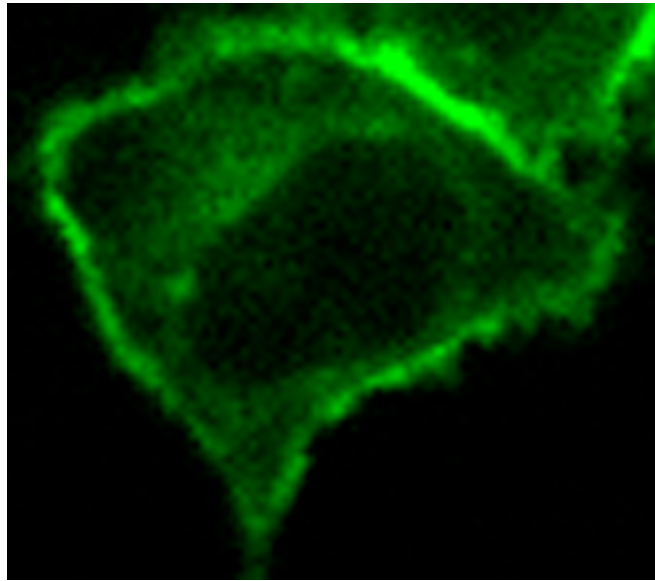
RMS displacement, $\mu\text{m}$	Time, s
1	0.02
5	0.6
10	2.3
20	9.5
30	21

1. Nenninger A, Mastroianni G, Mullineaux CW (2010) Size dependence of protein diffusion in the cytoplasm of *Escherichia coli*. *J Bacteriol* 192(18):4535–4540.



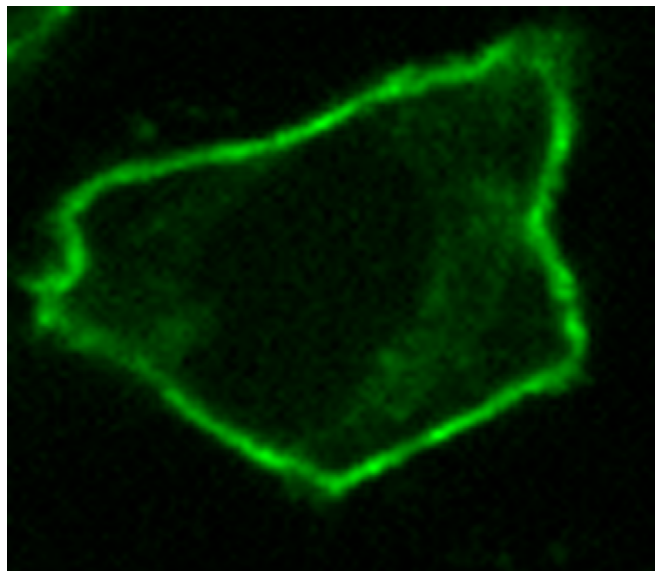
**Fig. S1.** HeLa cells were transfected with  $\alpha\text{o}$ ,  $\beta 1$ , and GFP- $\gamma 9$  or GFP- $\gamma 3$ . Endogenous CXCR4 receptors were activated with 100 ng/mL SDF-1 $\alpha$  and deactivated with 20  $\mu\text{M}$  AMD3100. (A) Confocal images before receptor activation, after receptor activation, and after receptor deactivation. (B) Forward and reverse translocation kinetics for  $\gamma 9$  and  $\gamma 3$ . Data for three different cells are shown for each subunit. Yellow markers labeled "Ag." and "Antag." indicate the addition of SDF-1 $\alpha$  and AMD3100, respectively.





**Movie S1.** Reversible  $\beta\gamma$  translocation in HeLa cells coexpressing  $\alpha$ ,  $\beta 1$ , and GFP- $\gamma 9$ . Agonist: 100 ng/mL SDF-1 $\alpha$ . Antagonist: 20  $\mu$ M AMD3100. The movie plays at 30 $\times$  real time and a running clock is displayed (h:min:s).

[Movie S1](#)



**Movie S2.** Reversible  $\beta\gamma$  translocation in HeLa cells coexpressing  $\alpha$ ,  $\beta 1$ , and GFP- $\gamma 3$ . Agonist: 100 ng/mL SDF-1 $\alpha$ . Antagonist: 20  $\mu$ M AMD3100. The movie plays at 120 $\times$  real time and a running clock is displayed (h:min:s).

[Movie S2](#)