Supplemental material

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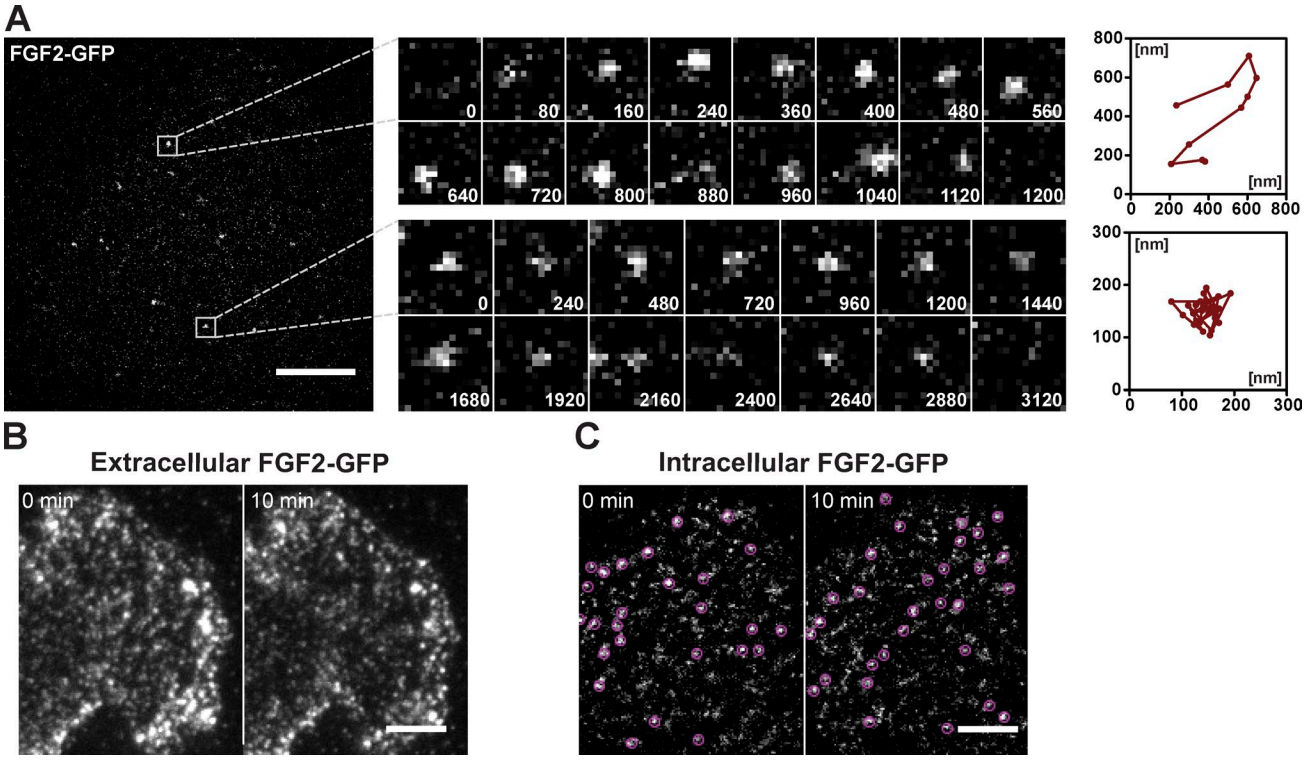


Figure S1. **Diffusion analysis of FGF2-GFP particles. (A)** Sequential frames from representative regions (white squares in left panel) tracking the diffusion of FGF2-GFP particles. In the middle panel, numbers refer to the time sequence in milliseconds. In the right panel, diffusion trajectories are determined by the series of fitted positions, connected by a straight line. Bar, 6 µm. **(B)** Cells were induced for 24 h to express FGF2-GFP and labeled with fluorescent anti-GFP nanobodies for 30 min on ice. TIRF videos were acquired with frames of 20 s. After 40 s of image acquisition, Live Cell Imaging Solution (mock) was added. Representative images from the beginning and the end of the acquired videos are shown. Bar, 6 µm. **(C)** CHO-K1 cells were cultivated under conditions of low levels of FGF2-GFP expression that allow for single particle detection based on GFP fluorescence. TIRF videos were acquired with frames of 20 s, and Live Cell Imaging Solution (mock) was added 40 s after the start of data acquisition. Representative images from the beginning and the end of the acquired videos are shown, depicting GFP particles at the inner leaflet of the plasma membrane (circles) before and after addition of the control buffer. Bar, 6 µm.

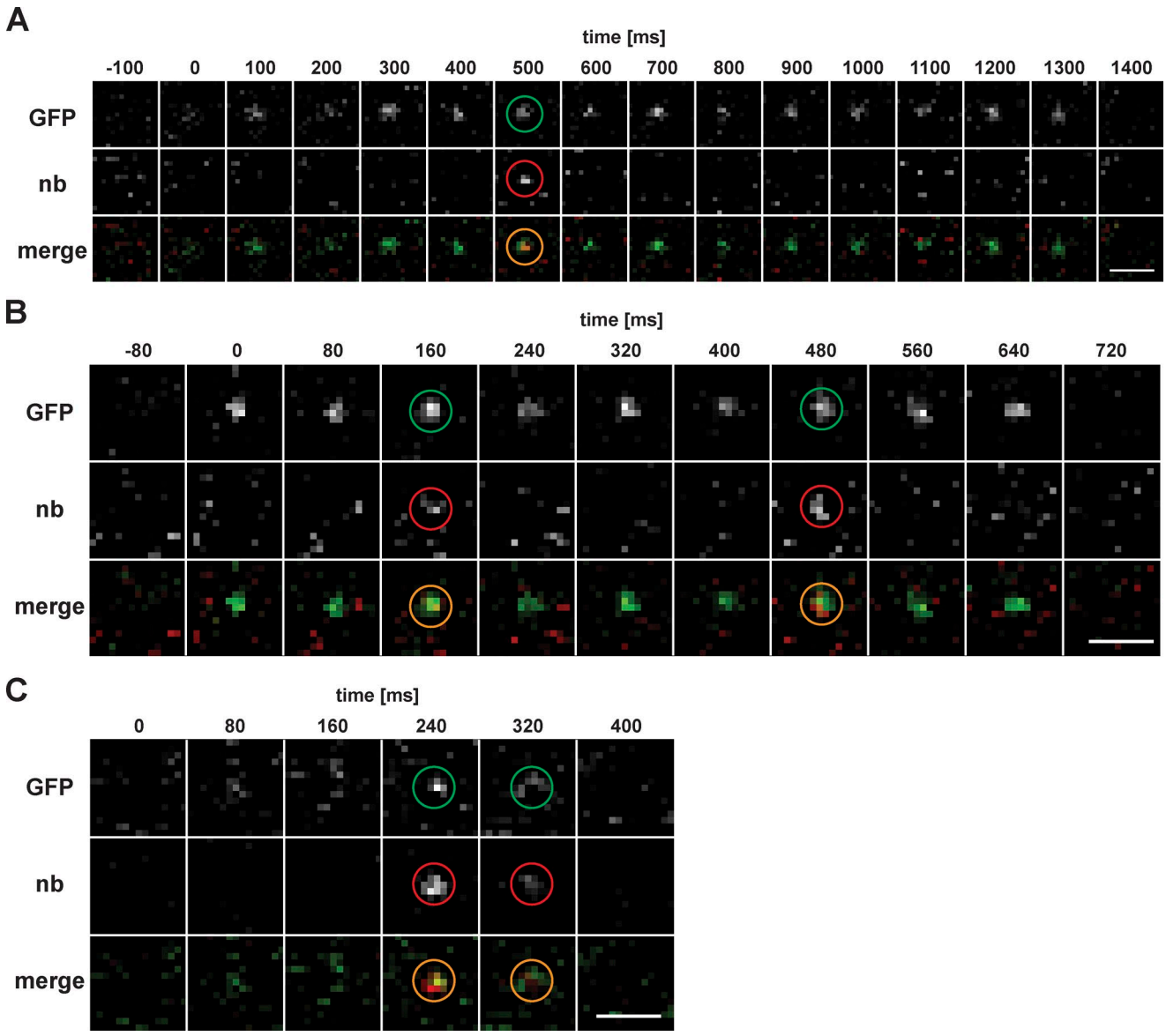
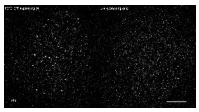
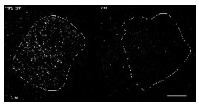


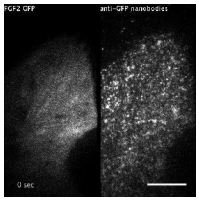
Figure S2. **Representative examples of full FGF2-GFP membrane translocation events. (A)** Representative example of an event of FGF2-GFP membrane translocation in which GFP fluorescence stably remained at the corresponding site in the plasma membrane after completion of one FGF2-GFP translocation event. Bar, 1 µm. **(B)** Representative example of an event with sequential incidents of FGF2-GFP membrane translocation accompanied by stable GFP fluorescence at the corresponding site in the plasma membrane. Bar, 1 µm. **(C)** Representative example of an event of FGF2-GFP membrane translocation in which GFP and nanobody fluorescence disappeared simultaneously at the corresponding site in the plasma membrane. Bar, 1 µm.



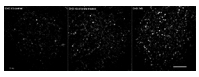
Video 1. **Live cell imaging of individual events of FGF2 membrane recruitment at the plasma membrane.** Time-lapse TIRF microscopy (80 ms per frame) was performed with CHO-K1 cells expressing either FGF2-GFP (left) or GFP (right) at concentrations that allow for single particle detection. Background signals were subtracted visualizing GFP particles in the direct vicinity of the plasma membrane. Bar, 6 µm.



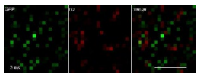
Video 2. **Detection of secreted FGF2 by fluorescently labeled anti-GFP nanobodies by live cell imaging.** CHO-K1 cells were induced with doxycycline to express either FGF2-GFP (left panel) or GFP (right panel) for 24 h. Following incubation with Alexa Fluor 647–labeled anti-GFP nanobodies (~0.5 ng/ml), the secreted population of FGF2-GFP was identified on the cell surface by single-particle live cell imaging. Bar, 10 µm.



Video 3. **Heparin treatment removes secreted FGF2-GFP from cell surfaces.** Time-lapse video (10 s/frame) of FGF2-GFP expressing cells treated with heparin (1 mg/ml). CHO-K1 cells were induced for 24 h with doxycycline followed by labeling with Alexa Fluor 647–labeled anti-GFP nanobodies. Heparin was added after 5 min following the start of image acquisition. Left: Total GFP fluorescence is recorded. Right: The secreted population of FGF2-GFP was detected by anti-GFP nanobody labeling. Bar, 10 µm.



Video 4. **Live cell imaging of FGF2 recruitment at the inner plasma membrane leaflet under conditions of impaired heparan sulfate biosynthesis.** Time-lapse TIRF microscopy (80 ms per frame) of FGF2-GFP under conditions where heparan sulfate biosynthesis is impaired as described in Fig. 3. Left: CHO WT cells. Middle: Cells that were treated with sodium chlorate to prevent sulfation of the sugar side chains of proteoglycans. Right: CHO-745 mutant cells that are incapable of synthesizing heparan sulfates as part of proteoglycans. Background signals were subtracted, visualizing GFP particles in the direct vicinity of the plasma membrane. Bar, 10 µm.



Video 5. **Representative example of a complete FGF2-GFP translocation event across the plasma membrane.** Time-lapse imaging (50 ms per frame) of a representative event of FGF2-GFP membrane recruitment and translocation to the cell surface of living cells using TIRF microscopy. Bar, 1 µm.

Provided online in Excel is Table S1, summarizing the sample size of all experiments presented in this study.