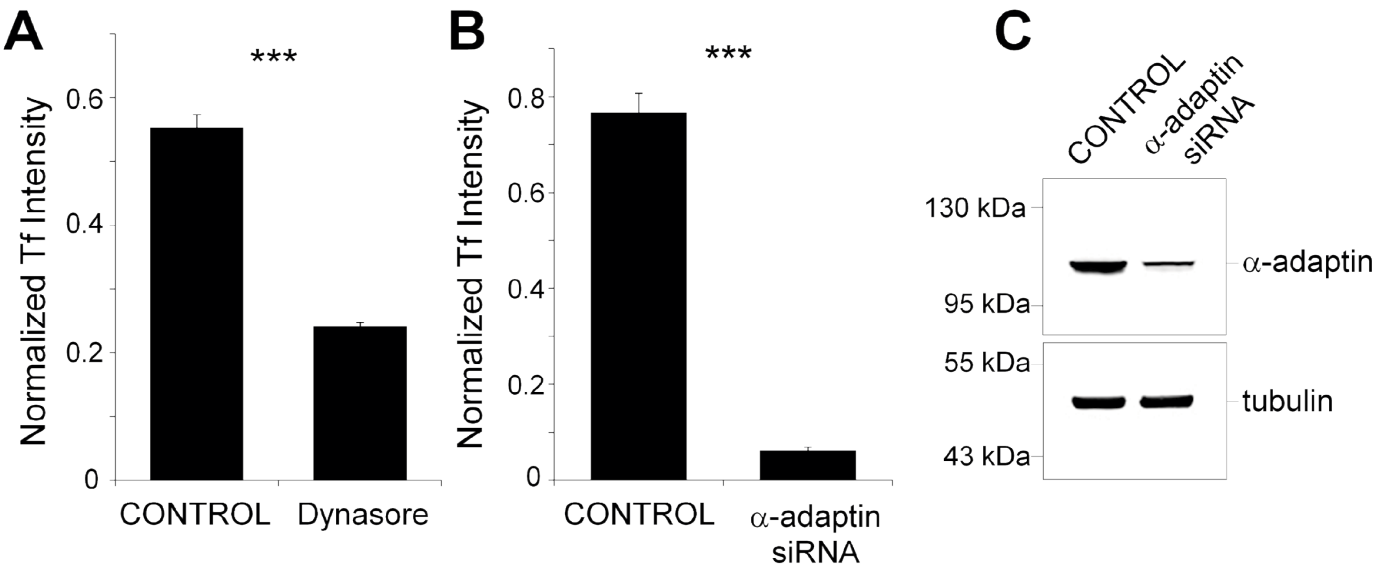

Fig S1. Silencing α-adaptin subunit of AP2 complex or inhibition of GTPase dynamin significantly reduces transferrin uptake. HeLa cells overexpressing FGFR2-GFP were either incubated for 30 minutes in the presence or absence of Dynasore inhibitor (**A**), or treated with α-adaptin siRNA (**B**). Following incubation with transferrin-Alexa-Fluor-546 (Tf) for 15 minutes at 37°C, cells were rinsed, fixed and analysed by epifluorescence microscopy. The quantification of Tf fluorescence intensity shows that both Dynasore activity and α-adaptin siRNA treatment significantly reduce transferrin entry (mean ± s.e.m., *n*=26 cells in (A), *n*=24 cells in (B) (**P*<0.05; ***P*<0.01; ****P*<0.001). (**C**) Western blot of lysates from HeLa cells transfected with α-adaptin siRNA and probed with anti-α-adaptin antibody shows potent silencing (mean ± s.e.m., *n*=3 experiments).

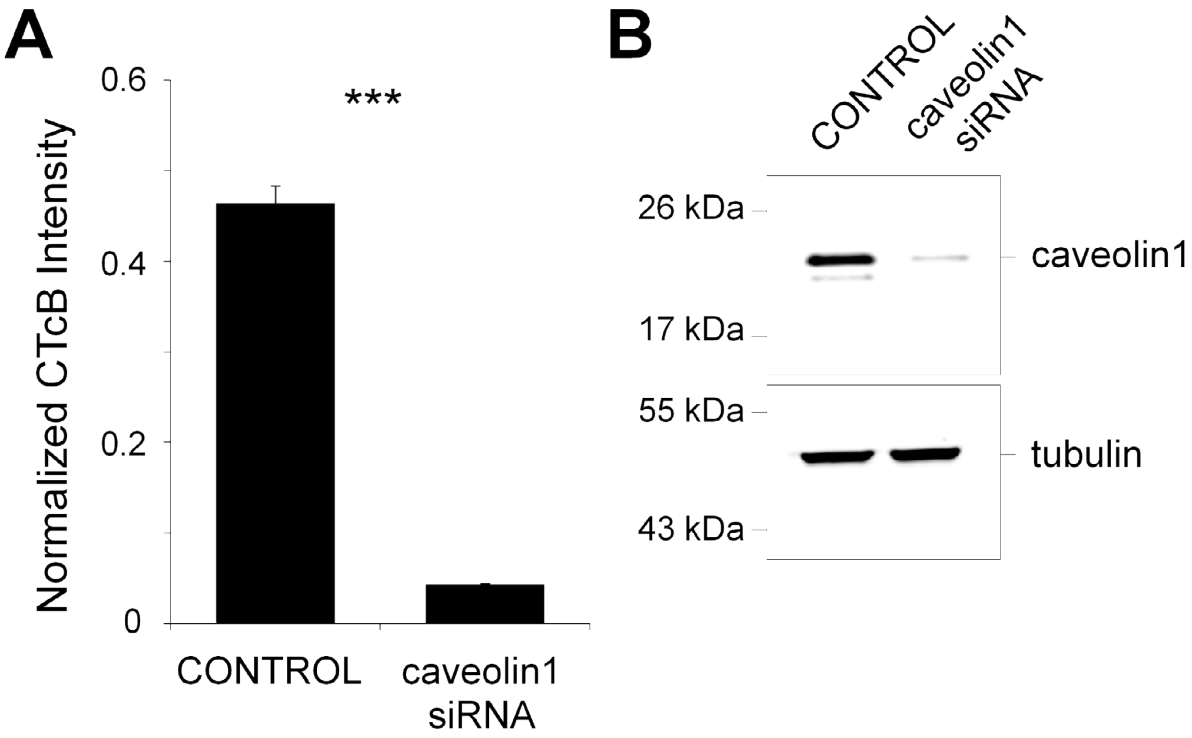

Fig S2. Silencing of caveolin1 leads to a significant reduction of choleratoxinB uptake. (**A**) HeLa cells overexpressing FGFR2-GFP were treated with caveolin1 siRNA. Following incubation with choleratoxinB-Alexa-Fluor-555 (CTcB) for 15 minutes at 37°C, cells were rinsed, fixed and analysed in epifluorescence microscopy. The quantification of CTcB fluorescence intensity shows that caveolin1 siRNA treatment leads to a significant reduction in cholera toxin uptake (mean ± s.e.m., *n*=15 cells; **P*<0.05; ***P*<0.01; ****P*<0.001). (**B**) Western blot of lysates from HeLa cells transfected with caveolin1 siRNA and probed with anti cav1 antibody shows potent silencing (mean ± s.e.m., *n*=3 experiments).

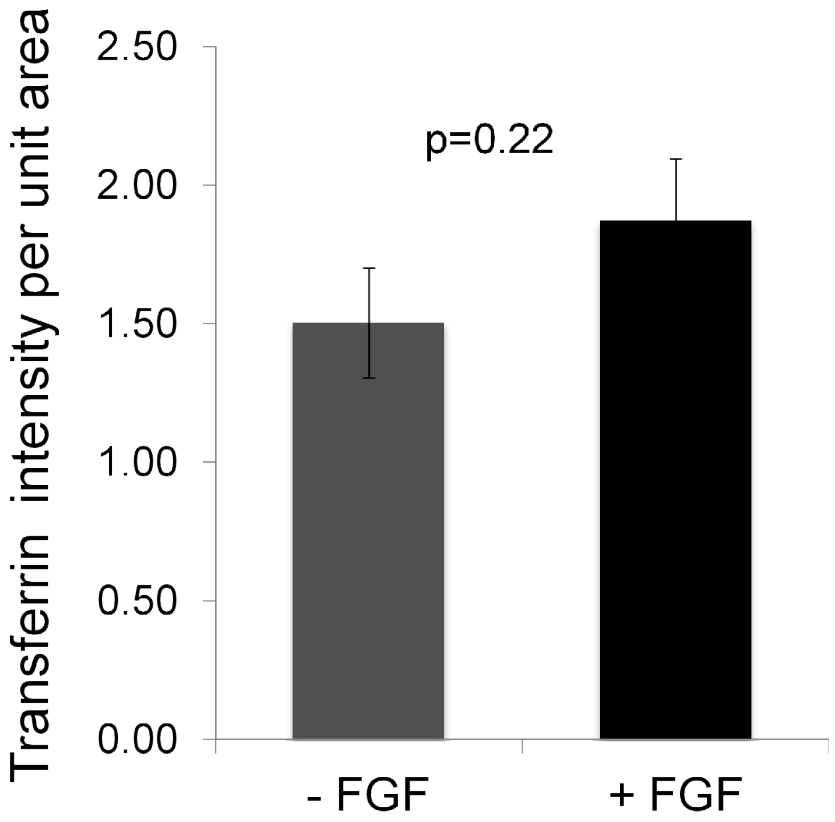

Fig S3. FGF treatment does not result in an increase in transferrin uptake. HeLa cells transiently expressing FGFR2-GFP were either stimulated with FGF2 + heparin for 15 minutes, or not stimulated, at 37°C in the presence of transferrin-Alexa-Fluor-546. Cells were then rinsed, fixed and analysed by epifluorescence microscopy. Quantification of transferrin fluorescence intensity demonstrates that FGF stimulation does not induce a significant increase in transferrin entry (mean ± s.e.m., *n*=49 cells each condition).

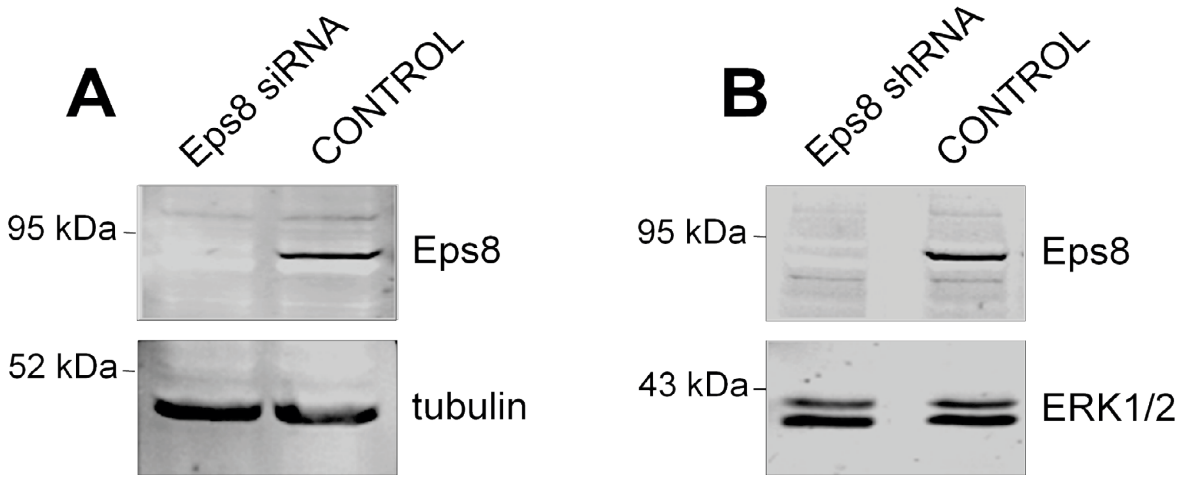

Fig S4. Eps8 expression is significantly silenced in the Eps8 knock down cell line and in Eps8 siRNA-treated HeLa cells. Cellular extracts from HeLa cells transfected with Eps8 siRNA (**A**) or from Eps8 knock down (shRNA) or control vector (control) HeLa cells (**B**) were resolved by SDS-PAGE and analysed by immunoblotting with anti-Eps8 antibody (mean ± s.e.m., *n*=3 experiments).

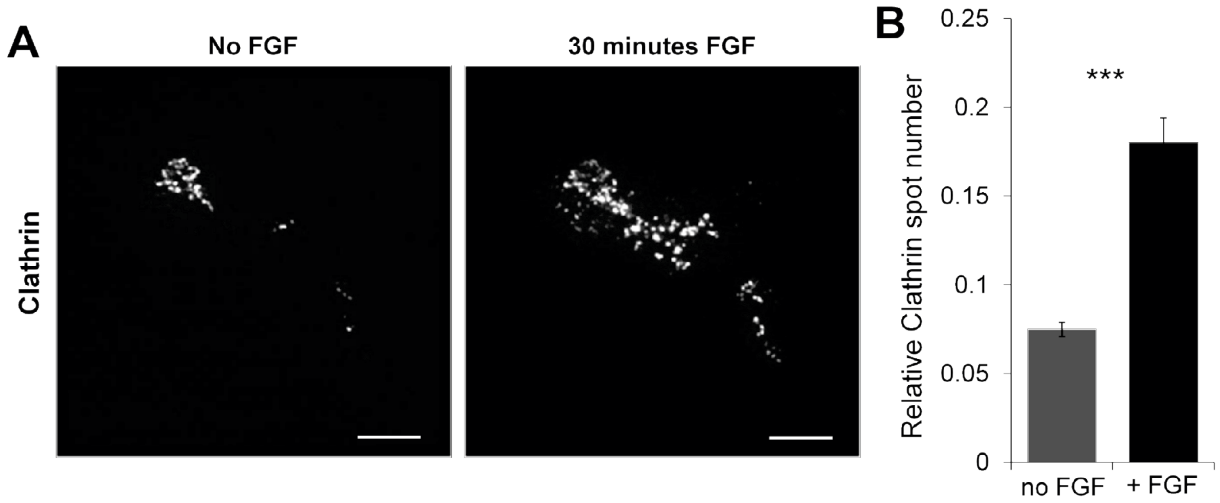

Fig S5. FGF-dependent peripheral accumulation of CCPs in FGFR-expressing LNCaP cells. LNCaP cells endogenously expressing FGFR2 and transfected with Clathrin-dsRed were analysed by TIRF microscopy before and 30 minutes after stimulation with FGF2 + heparin. (**A**) Upon FGF stimulation, cells show a significant increase in the number of clathrin spots on the plasma membrane. Scale bars: 5 μm. (**B**) Quantification analysis of clathrin spot number before and after FGF stimulation (mean ± s.e.m., *n*=45 cells; **P*<0.05; ***P*<0.01; ****P*<0.001).

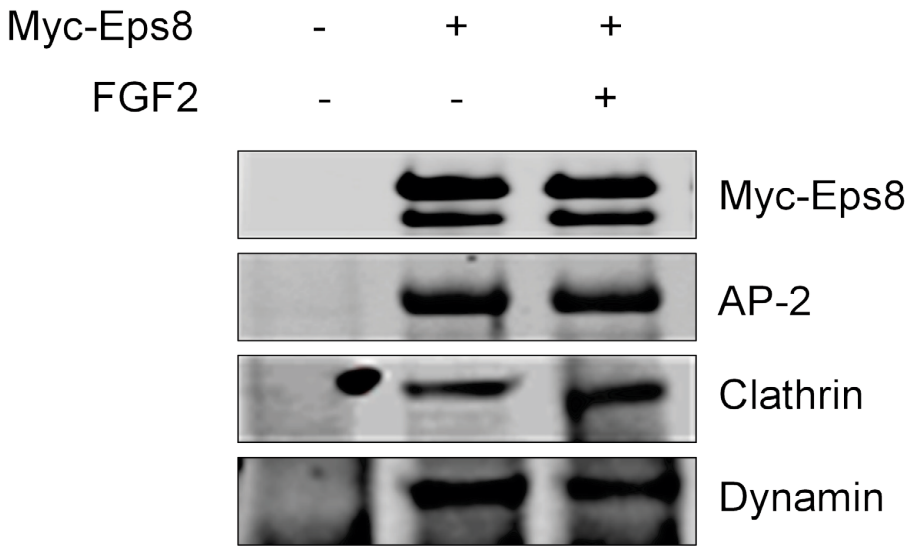

Fig S6. AP-2, Clathrin, and Dynamin interact with Eps8 in an FGF2-independent manner in HEK293T cells. Cellular extracts from HEK293T cells transfected with myc-Eps8 (where indicated) and stimulated or not with 20 ng/ml FGF + 10 μg/ml heparin for 15 min, were immunoprecipitated with anti-myc antibody, resolved by SDS-PAGE and analysed by immunoblotting for the levels of specified proteins.

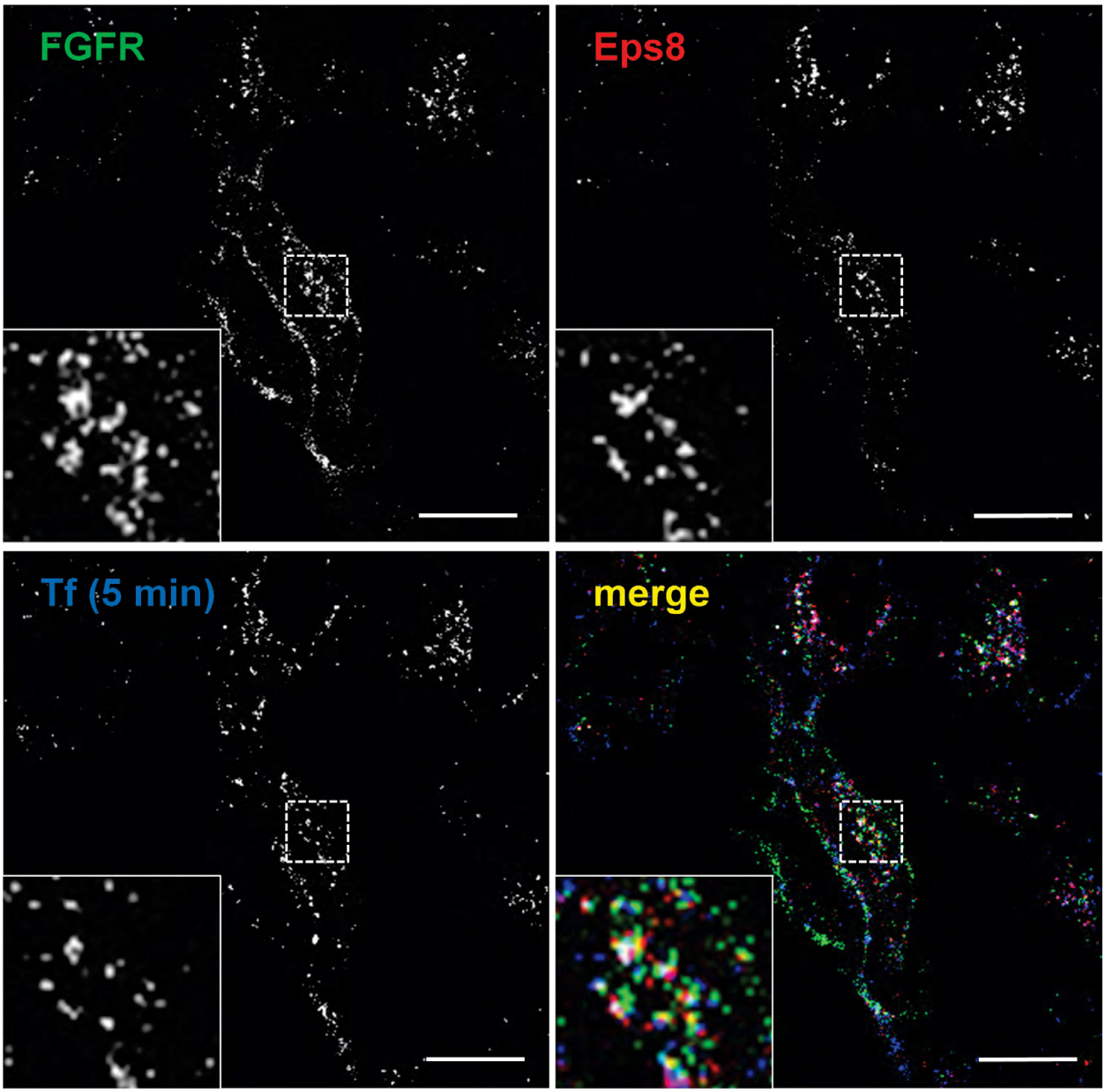

Fig S7. During early stages of trafficking FGFR colocalises with Eps8 in the early endosomal compartment. HeLa cells transiently expressing FGFR2-GFP and Eps8-mCherry were stimulated with FGF + heparin for 10 minutes. Following incubation with transferrin-Alexa-Fluor-633 (Tf) during the last 5 minutes of stimulation, cells were rinsed, fixed and analysed by confocal microscopy. Higher-magnification in merged image of selected regions from the cells show overlap of FGFR, Eps8 and 5 min internalized transferrin in white, demonstrating that during the early stages of activation (10 min FGF), FGFR colocalises with Eps8 within the early endocytic compartment. Scale bars=5 μm.

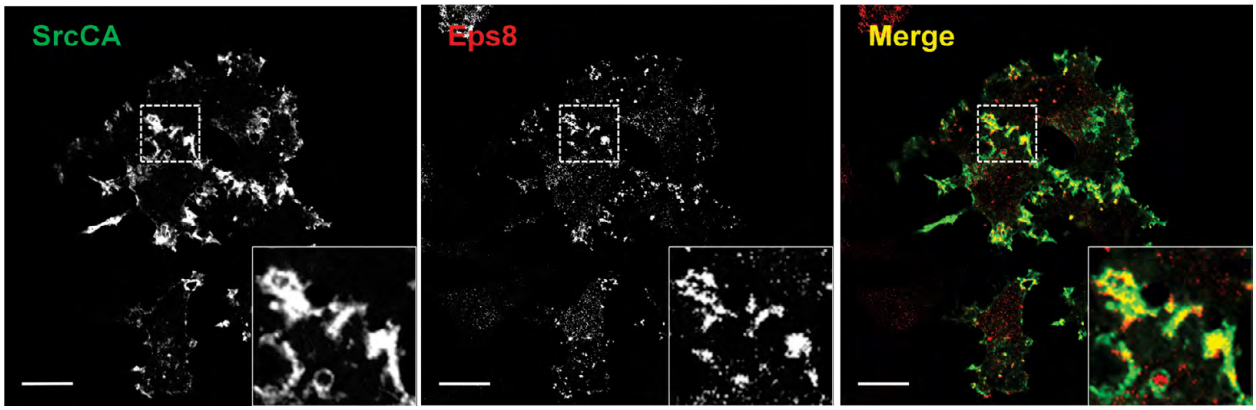

Fig S8. Colocalisation of Eps8 with active Src. HeLa cells transiently expressing constitutive active Src (SrcCA) and Eps8-mCherry were immunostained for active Src (P416Src antibody) and imaged in confocal microscopy. Higher-magnification merged image of selected regions from the cells show overlap of active Src and Eps8 in yellow. Scale bars=5 μm.

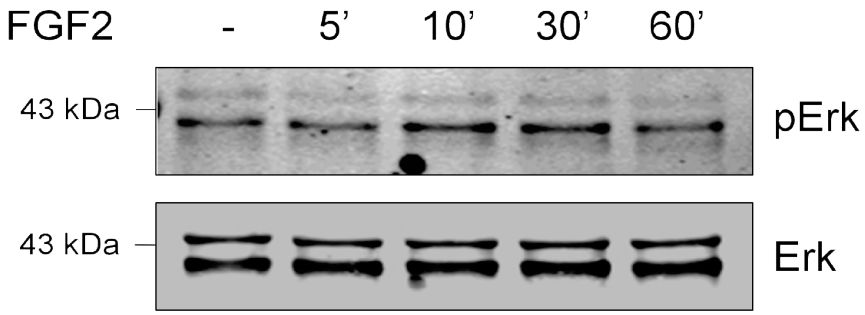

Fig S9. FGF-dependent kinetics of ERK phosphorylation in non-transfected HeLa cells. HeLa cells starved overnight in serum-free media were lysed following stimulation with FGF2 + heparin for the indicated times. Cellular extracts where resolved by SDS-PAGE and analysed by immunoblotting for the levels of specified proteins.

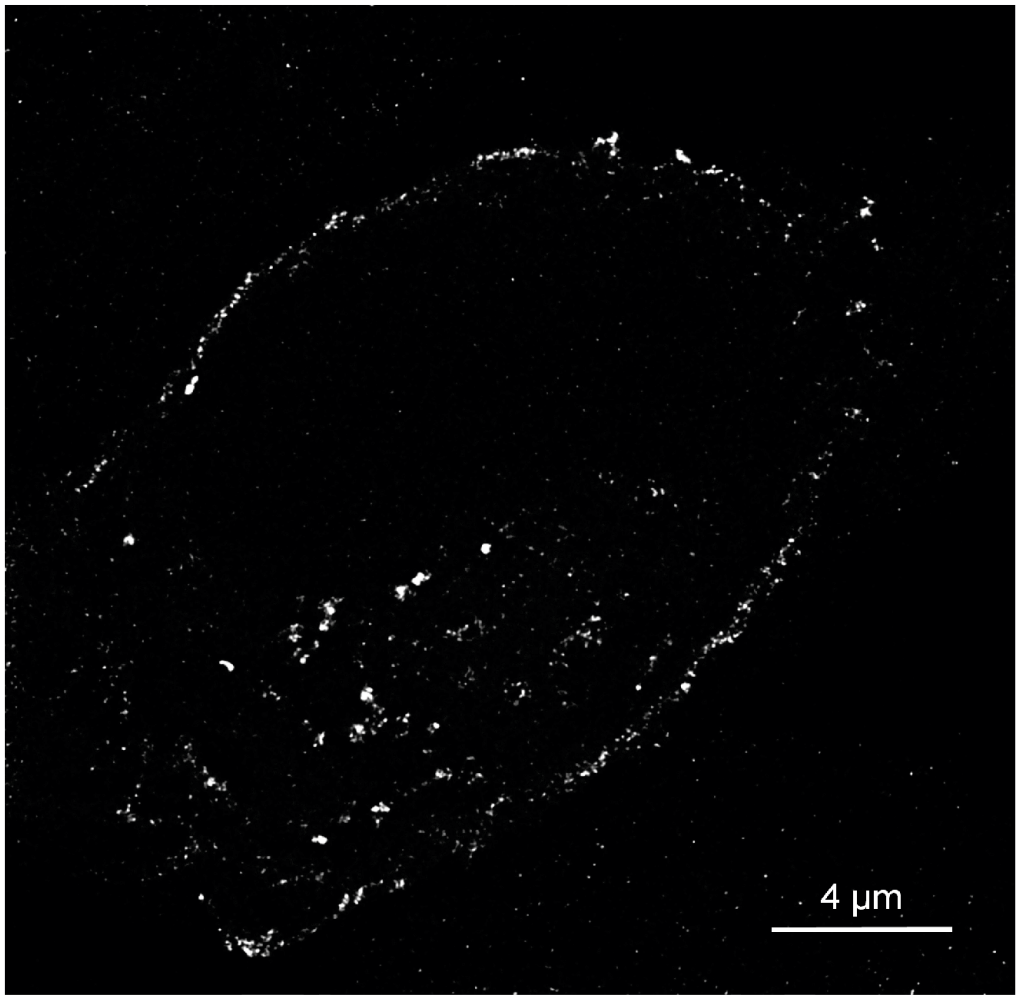

Fig S10. Super-resolution analysis of FGFR-containing vesicles in Dasatinib-treated HeLa cells. HeLa cells transiently expressing FGFR2-GFP and incubated for 30 minutes in the presence of Dasatinib inhibitor were stimulated with FGF2 + heparin for 30 minutes, fixed and analysed by super-resolution confocal microscopy on a Leica gSTED system followed by stack deconvolution. The high resolution image reveals the presence of a population of FGFR-containing vesicles accumulating at the cell periphery following Src inhibition.