

Supplemental Material

Chasing the signaling run by tri-molecular time-lapse FRET microscopy

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Keywords: Signaling; FRET microscopy; WWOX; Hyal-2; Smad4

Running title: Run, signaling run!

Supplemental Figures

Figure S1. Time-lapse FRET imaging for control vectors. COS7 cells were transiently overexpressed with ECFP and DsRed-monomer (**a**), or ECFP, EGFP and DsRed-monomer (**b**), and exposed to native hyaluronan (HAN) at 25 $\mu\text{g/ml}$ for FRET imaging at 37°C for 24 hr. The signals are at background levels.

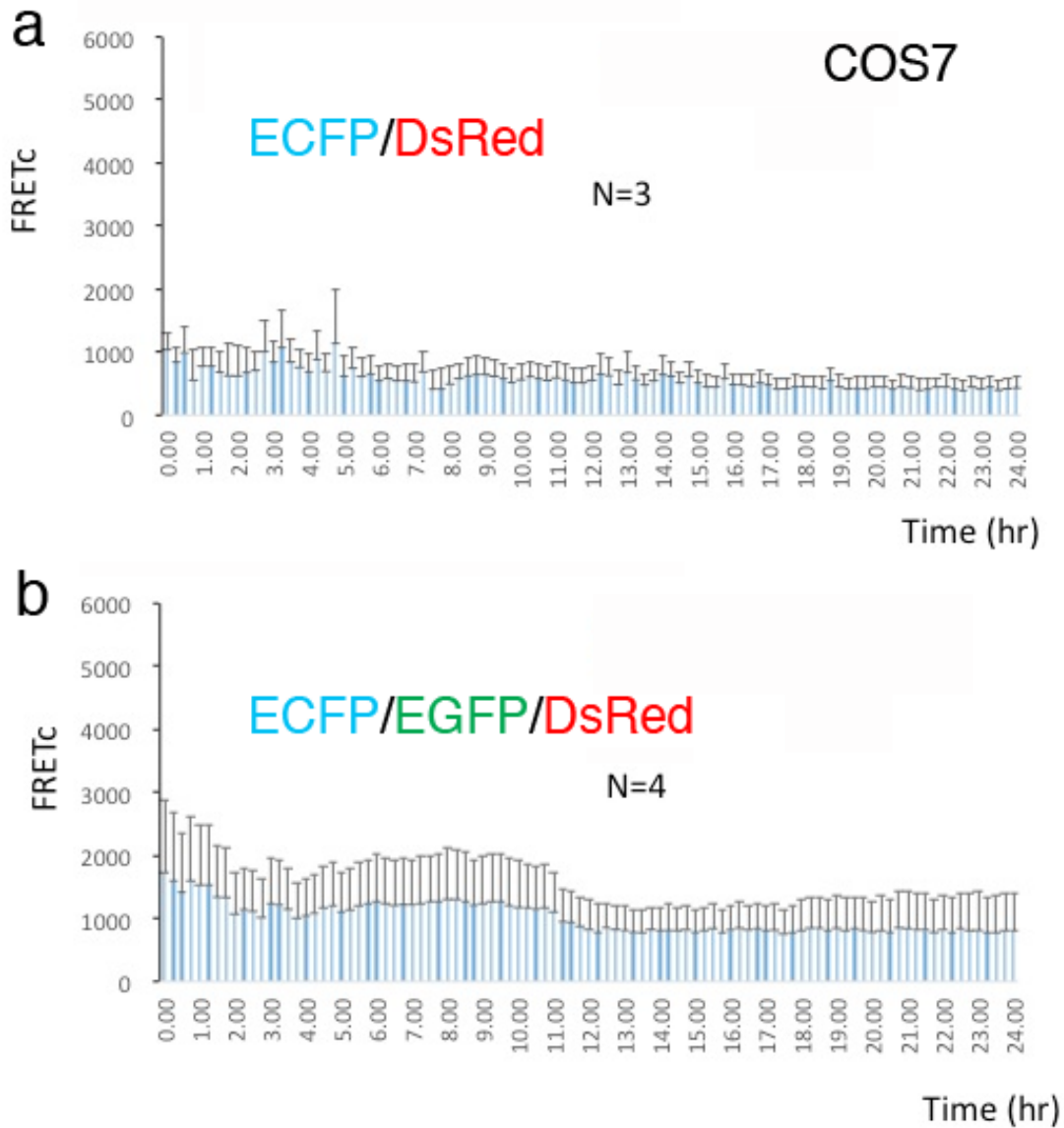
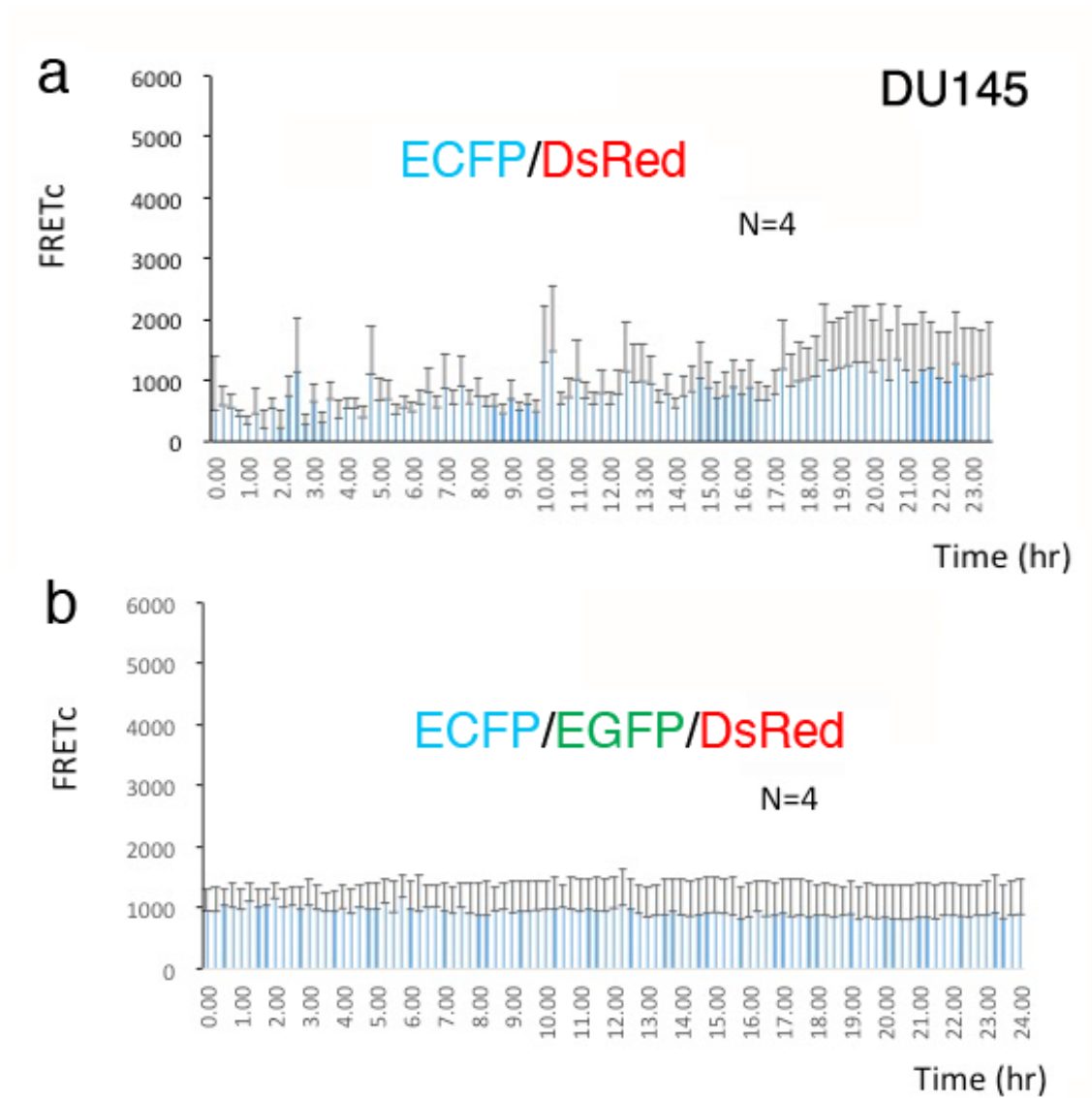


Figure S2. Time-lapse FRET imaging for control vectors. DU145 cells were transiently overexpressed with ECFP and DsRed-monomer (**a**), or ECFP, EGFP and DsRed-monomer (**b**), and exposed to native hyaluronan (HAN) at 25 $\mu\text{g/ml}$ for FRET imaging at 37°C for 24 hr. The signals are at background levels.



Supplemental Videos

Video S1. Time-lapse FRET microscopy for the binding interactions of ECFP-I κ B α , EGFP-ERK and DsRed monomer-WWOX under the influence of IoP in COS7 cells. (adapted from reference #13 with permissions)

Video S2. Time-lapse FRET analysis for the binding interactions of ECFP-I κ B α , EGFP-dnERK and DsRed monomer-WWOX under the influence of IoP in COS7 cells. (adapted from reference #13 with permissions)

Video S3. Time-lapse FRET analysis for the binding interactions of ECFP-I κ B α , EGFP-ERK and DsRed monomer-dnWWOX under the influence of IoP in COS7 cells. (adapted from reference #13 with permissions)

Video S4. Time-lapse FRET analysis for the Smad4/Hyal-2(-sp)/WWOX signaling in DU145 cells. A merged video of FRETc and bright field is shown. (adapted from reference #14 with permissions)

Video S5. Time-lapse FRET analysis for the Smad4/WWOX/p53 signaling in DU145 cells. A merged video of FRETc and bright field is shown. False positive signals are due to floating dead cells, which may be seen in other videos. (adapted from reference #14 with permissions)

Video S6. TGF- β 1-mediated TIAF1 self-aggregation and apoptosis. Lung NCI-H1299 cancer cells were transiently overexpressed with EYFP-TIAF1, followed by stimulating with TGF- β 1 (5 ng/ml). By time-lapse microscopy, aggregation of TIAF1 occurred in less than 60 min (see green punctates), followed by membrane blebbing at 180 min. (adapted from reference #31 with permissions)