General *In Vivo* Assay for the Study of Integrin Cell Membrane Receptor Microclustering

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FRET Reporters:

TCD-β mdsRED:Cherry (Bold = mCherry; underlined = transmembrane and cytoplasmic domains of βPH)

M V S K G E E D N M A I I K E F M R F K V H M E G S V N G H E F E I E G E G E G R P Y E G T Q T A K L K V T K G G P L P F A W D I L S P Q F M Y G S K A Y V K H P A D I P D Y L K L S F P E G F K W E R V M N F E D G G V V T V T Q D S S L Q D G E F I Y K V K L R G T N F P S D G P V M Q K K T M G W E A S S E R M Y P E D G A L K G E I K Q R L K L K D G G H Y D A E V K T T Y K A K K P V Q L P G A Y N V N I K L D I T S H N E D Y T I V E Q Y E R A E G R H S T G G M D E L Y K G S S S G S S S A S G Y E E Y S G P A K V F M L G I V M G V I A A I V L V G L A I L L L W K L L T T I H D R R E F A R F E K E R M N A K W D T G E N P I Y K Q A T S T F K N P M Y A G K

TCD-β mYFP:Venus(Bold = mVenus; underlined = transmembrane and cytoplasmic domains of βPH)

M V S K G E E L F T G V V P I L V E L D G D V N G H K F S V S G E G E G D A T Y G K L T L K L I C T T G K L P V P W P T L V T T L G Y G L Q C F A R Y P D H Met K Q H D F F K S A M P E G Y V Q E R T I F F K D D G N Y K T R A E V K F E G D T L V N R I E L K G I D F K E D G N I L G H K L E Y N Y N S H N V Y I T A D K Q K N G I K A N F K I R H N I E D G G V Q L A D H Y Q Q N T P I G D G P V L L P D N H Y L S Y Q S K L S K D P N E K R D H M V L L E F V T A A G I T L G M D E L Y K S S S G S S S A S G Y E E Y S G P A K V F M L G I V M G V I A A I V L V G L A I L L L W K L L T T I H D R R E F A R F E K E R M N A K W D T G E N P I Y K Q A T S T F K N P M Y A G K

FRET Controls:

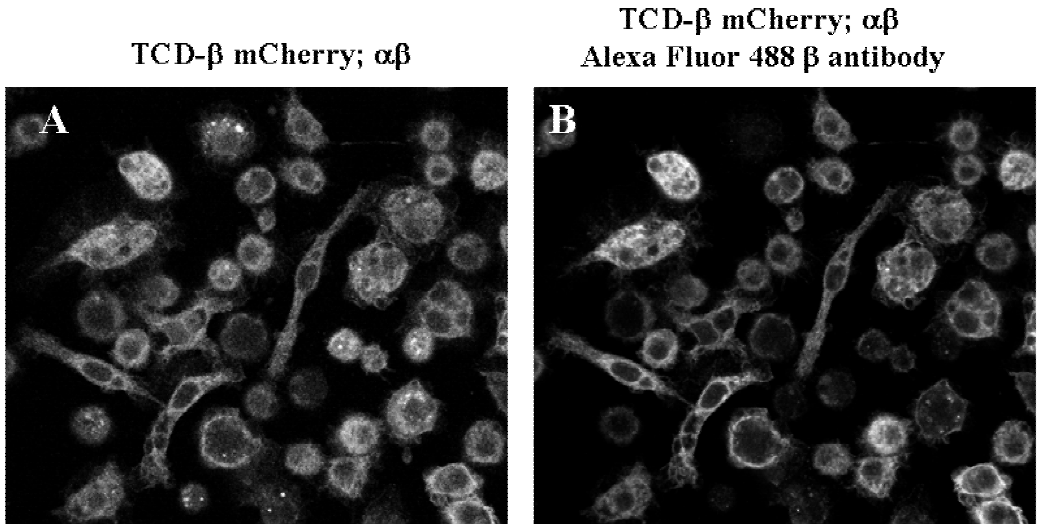
TCD-CD2 mdsRED:Cherry (Bold = mCherry; underlined = transmembrane and cytoplasmic domains of mouse CD2)

M V S K G E E D N M A I I K E F M R F K V H M E G S V N G H E F E I E G E G E G R P Y E G T Q T A K L K V T K G G P L P F A W D I L S P Q F M Y G S K A Y V K H P A D I P D Y L K L S F P E G F K W E R V M N F E D G G V V T V T Q D S S L Q D G E F I Y K V K L R G T N F P S D G P V M Q K K T M G W E A S S E R M Y P E D G A L K G E I K Q R L K L K D G G H Y D A E V K T T Y K A K K P V Q L P G A Y N V N I K L D I T S H N E D Y T I V E Q Y E R A E G R H S T G G M D E L Y K G S S S G S S S A

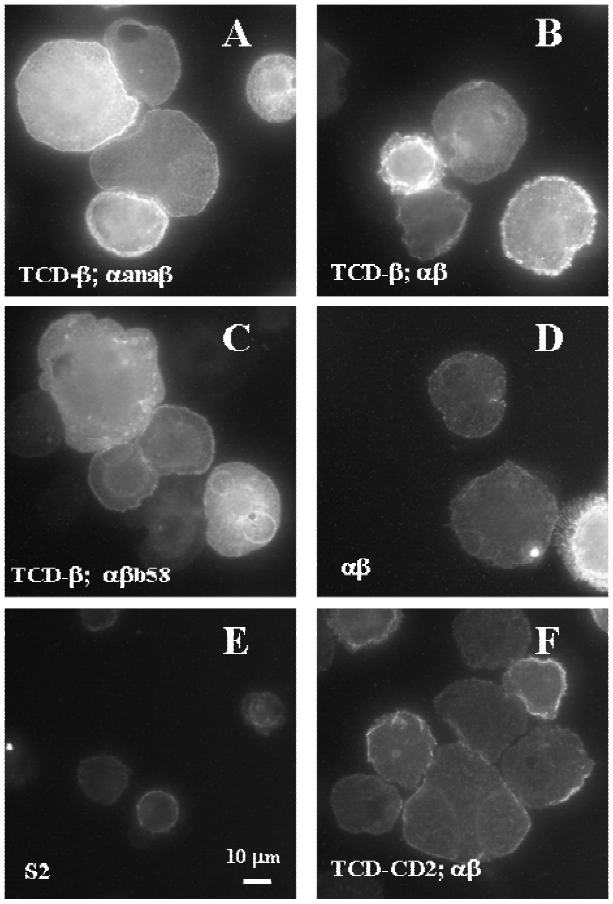
S G Y E E Y S G P A G L S F Y V T V G V G A G G L L L V L L V A L F I F C I C K R R K R N R R R K D E E L E I K A S R T S T V E R G P K P H S T P A A A A Q N S V A L Q A P P P P G H H L Q T P G H R P L P P G H R T R E H Q Q K K R P P P S G T Q I H Q Q K G P P L P R P R V Q P K P P C G S G D G V S L P P P N

TCD-CD2 mYFP:Venus (Bold = mVenus; underlined = transmembrane and cytoplasmic domains of mouse CD2)

M V S K G E E L F T G V V P I L V E L D G D V N G H K F S V S G E G E G D A T Y G K L T L K L I C T T G K L P V P W P T L V T T L G Y G L Q C F A R Y P D H M K Q H D F F K S A M P E G Y V Q E R T I F F K D D G N Y K T R A E V K F E G D T L V N R I E L K G I D F K E D G N I L G H K L E Y N Y N S H N V Y I T A D K Q K N G I K A N F K I R H N I E D G G V Q L A D H Y Q Q N T P I G D G P V L L P D N H Y L S Y Q S K L S K D P N E K R D H M V L L E F V T A A G I T L G M D E L Y K S S S G S S S A S G Y E E Y S G P A G L S F Y V T V G V G A G G L L L V L L V A L F I F C I C K R R K R N R R R K D E E L E I K A S R T S T V E R G P K P H S T P A A A A Q N S V A L Q A P P P P G H H L Q T P G H R P L P P G H R T R E H Q Q K K R P P P S G T Q I H Q Q K G P P L P R P R V Q P K P P C G S G D G V S L P P P N



Supplemental Figure 1. Confocal microscope images showing the macroscale co-localization of the FRET reporters and the full length integrin. All cells contain the fluorescent protein monomeric dsRED:Cherry cloned onto the cytoplasmic and transmembrane domains of the integrin β subunit, which is co-expressed with wild-type integrin. The cells have been allowed to spread on a tiggrin coated surface for 1 hour, and were subsequently fixed and permeabilized, which removes the bulk of the membrane associated integrin and other membrane components. This treatment is necessary to observe macroscale integrin clusters in S2 cells. The cells shown in B have been stained using an Alexa Fluor 488 labeled antibody that is specific to the βPS integrin subunit. Image A was captured using filters specific to cherry and image B was collected using filters specific for Alexa Fluor 488. There was no bleed through of cherry into the Alexa Fluor 488 image, and vise versa. In general, the cherry FRET reporter is localized with the full length integrin.



Supplemental Figure 2. Wide field microscope images showing that there is no difference in the macroscale clustering of integrins for the cell lines used in this study (A, B, C, F). The cells have been allowed to spread on a tiggrin coated surface for 1 hour, and were subsequently fixed and stained using an Alexa Fluor 488 labeled antibody that is specific to the βPS integrin subunit. (Note: these cells have not been permeabilized so they do not show the features observed in Supplemental Figure 1. These conditions are more representative of the conditions used in the FRET assay. Fixing the cells reduces the venus and cherry fluorescence). All images were obtained using filters for Alexa Fluor 488. (A) FRET reporters and αanaβ integrin (B) FRET reporters and wild-type integrin (C) FRET reporters and αβB58 integrin (D) wild-type integrin only (E) S2 cells (F) FRET controls and wild-type integrin. S2 cells contain low levels of native integrin, and do not spread well on tiggrin coated surfaces (E).