

## Binding Assay Protocols for Integrin & Talin mutants that block activation in cells

### PROTOCOL 1 - Integrin mutants that are defective in binding talin F3 and block activation.

Integrin activation is assessed using the antibody PAC1 and flow cytometry as previously described (Partridge et al., 2005). Mutants F727 and F730, which both make intimate contact with talin F3, are investigated using a mutant integrin  $\alpha$ IIb subunit, R995A. When paired with a wild-type (WT)  $\beta$ 3 subunit, the assembled integrin is in an activated state in transfected cells (Hughes et al., 1996). The activated state is dependent on endogenous talin. When  $\alpha$ IIb (R995A) is paired with  $\beta$ 3(F727A) or  $\beta$ 3(F730A),  $\beta$ 3 mutants designed to disrupt the F3-MP interaction, the activating effect of the  $\alpha$ IIb (R995A) mutation is dramatically reduced.

#### Publications cited:

- Partridge, et al. Transmembrane domain helix packing stabilizes integrin  $\alpha$ IIb $\beta$ 3 in the low affinity state J Biol Chem. 2005 Feb 25;280(8):7294-300. PubMed  
[http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list\\_uids=15591321](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15591321)
- Hughes et al., Breaking the integrin hinge. A defined structural constraint regulates integrin signaling J Biol Chem. 1996 Mar 22;271(12):6571-4. PubMed  
[http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list\\_uids=8636068](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=8636068)

### PROTOCOL 2 - Talin mutants that bind integrin but inhibit integrin activation.

$\alpha\beta$ Py cells from a chinese hamster ovary (CHO) cell line that expressed a talin-dependent, constitutively active chimeric integrin that was composed of the extracellular and transmembrane domains of  $\alpha$ IIb $\beta$ 3 as well as the cytoplasmic tails of  $\alpha$ 6 $\beta$ 1.  $\alpha\beta$ Py cells were transiently transfected with cDNAs encoding the mutant F23 constructs (L325R, S365D, S379R, or Q381V). Each of the four mutants blocked integrin activation, a result consistent with competition between endogenous full-length talin and the activation-defective F23 mutants for binding to the  $\beta$ -Membrane-Distal site. Transfection with cDNA encoding WT F23 modestly increased activation, whereas a mutant that strongly inhibits the MD-binding site, F23 (W359A), had no effect on activation, is consistent with its markedly reduced affinity for the  $\beta$  tail.