* Supplemental Data (Sato *et al*.)

FIGURE S1. **Multiple sequence alignment of the integrin-binding site of nephronectins from different vertebrate species.** Amino acid sequences in the RGD-containing linker segment of nephronectins from various vertebrate species were aligned by Clustal W (Ref. 1). The RGD and EIE motifs are highlighted in *black* and *dark gray boxes*, respectively. Two phenylalanine residues are labeled in *light gray boxes*.

1. Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) *Nucleic Acids Res* **22**, 4673-4680

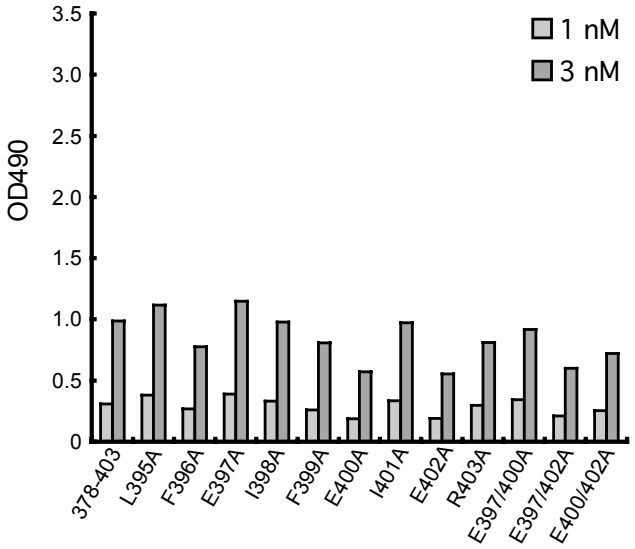

FIGURE S2. **Effect of alanine substitutions within the LFEIFEIER sequence on the αVβ3 integrin binding activity.** Microtiter plates were coated with the alanine substituted mutants and incubated with αVβ3 integrin (1 or 3 nM) in the presence of 1 mM Mn^{2+}. Bound integrins were quantified as described in the Experimental Procedures. The results represent the means of duplicate determinations.

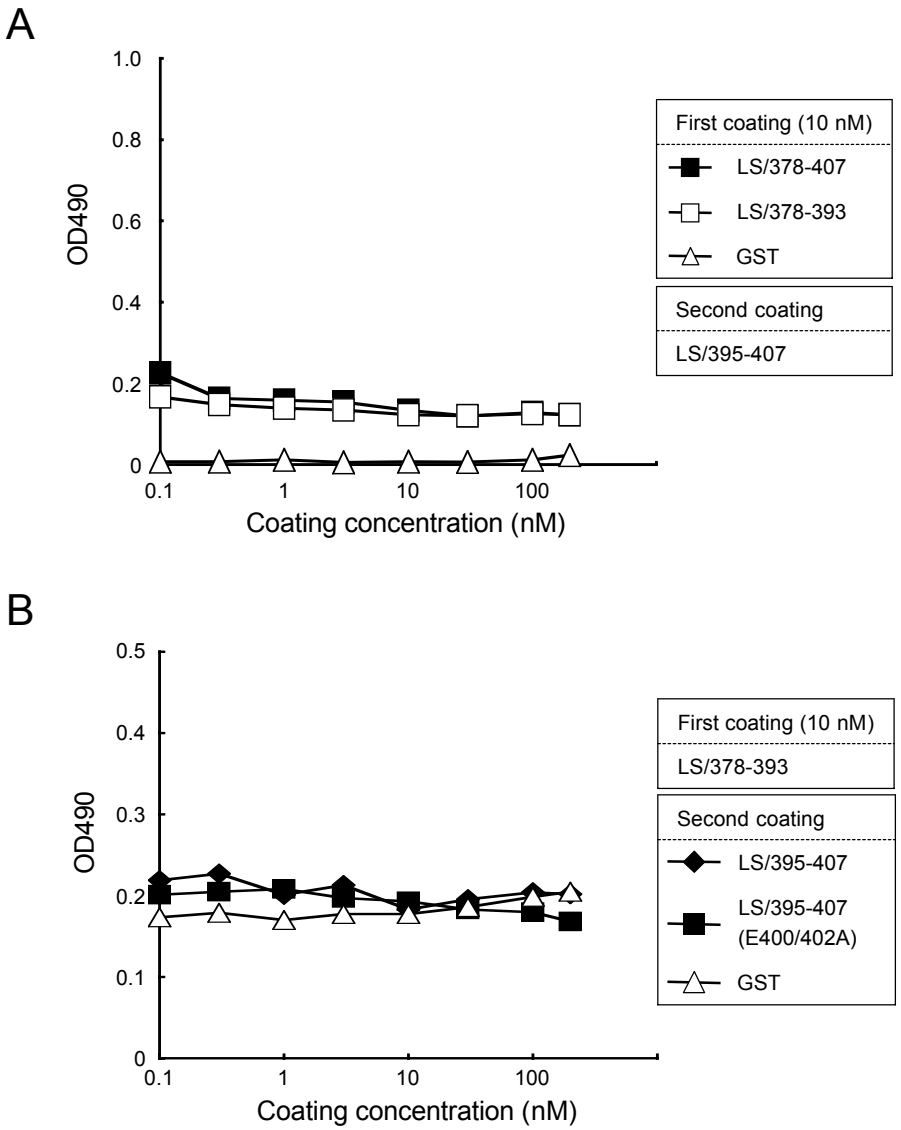

FIGURE S3. ***Trans*-complementation assays of recombinant nephronectin fragments.** *A*, 96-well microtiter plates were coated with LS/378-407 (*closed squares*), LS/378-393 (*open squares*) and GST (*open triangles*), washed with PBS, and then coated a second time with increasing concentrations of LS/395-407 lacking the RGD motif. The plates were subjected to integrin binding assays using 1 nM αVβ3 integrin as described in the Experimental Procedures. *B*, microtiter plates were coated with 10 nM LS/378-393, washed with PBS, and then coated a second time with increasing concentrations of LS/395-407 (*closed diamonds*), LS/395-407(E400/402A) (*closed squares*) and GST (*open triangles*). The plates were subjected to integrin binding assays using 1 nM αVβ3 integrin as described in the Experimental Procedures. The results represent the means of duplicate determinations.

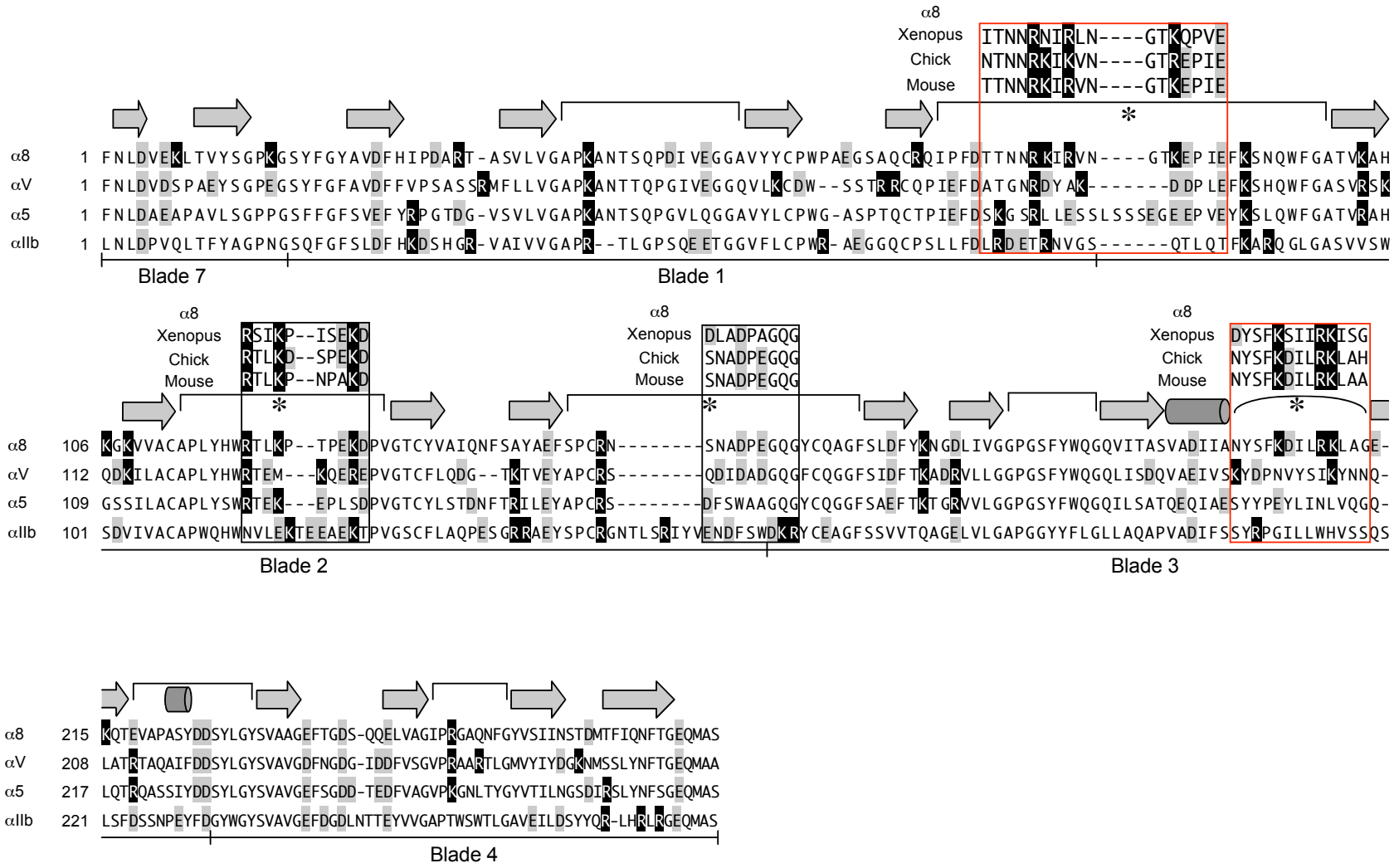

FIGURE S4. **Multiple sequence alignment of the β-propeller domain of RGD-binding integrin α subunits.** Amino acid sequences of the β-propeller domain of human integrin α8, αV, α5, and αIIb subunits were aligned by Clustal W (Ref. 1). Helices (*cylinders*) and strands (*arrows*) are predicted based on the secondary structure of αV integrin (Ref. 2). *Brackets* and an *arc* above the sequences indicate the loops located in the upper and side faces of the β-propeller, respectively. Loops that exhibit significant divergence in amino acid sequences among different α subunits are indicated by *asterisks*. Basic and acidic residues are boxed in *black* and *gray*, respectively.

1. Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) *Nucleic Acids Res* **22**, 4673-4680
2. Xiong, J. P., Stehle, T., Diefenbach, B., Zhang, R., Dunker, R., Scott, D. L., Joachimiak, A., Goodman, S. L., and Arnaout, M. A. (2001) *Science* **294**, 339-345