Supplementary Information for:

Title
Molecular basis for integrin adhesion receptor binding to p21-activated kinase 4 (PAK4)

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Supplementary Table 1. Thermodynamic properties of the interaction between PAK4cat and integrin β5 cytoplasmic tail.

| Sample Cell | Syringe       | $K_d$ (μM) | N  | $\Delta H$ (kJ/mol) | $\Delta S$ (J/molK) |
|-------------|---------------|------------|----|---------------------|---------------------|
| PAK4cat     | Integrin β5$^{743-799}$ | 5.1        | 1.4 | -7.7                | 75.7                |
| PAK4cat     | Integrin β5$^{743-799}$ | 4.8        | 0.7 | -9.2                | 70.8                |
Supplementary Table 2. RMSD difference table for between PAK4 catalytic domains.

|                  | $\beta^{743-774}$-linker-PAK4cat | $\beta^{743-774}$-linker-PAK4cat<sup>D440N,S474E</sup> | PAK4cat: $\beta^{760-770}$ | PAK4cat<sup>D440N,S474E</sup>: $\beta^{760-770}$ |
|------------------|----------------------------------|----------------------------------------------------------|-----------------------------|-----------------------------------------------|
| $\beta^{743-774}$-linker-PAK4cat | 1.1 Å (290 Ca)                    | 1.0 Å (290 Ca)                                           | 1.0 Å (290 Ca)               |                                               |
| $\beta^{743-774}$-linker-PAK4cat<sup>D440N,S474E</sup> |                                  | 0.3 Å (290 Ca)                                           |                             | 0.2 Å (290 Ca)                               |
| PAK4cat: $\beta^{760-770}$ |                                  |                                                          |                             | 0.2 Å (290 Ca) |
**Supplementary Table 3. RMSD difference table for between integrin β5 peptides.**

|                  | β5^{743-774} -linker-PAK4cat^{D440N,S474E} | PAK4cat: β5^{760-770} | PAK4cat^{D440N,S474E}: β5^{760-770} |
|------------------|---------------------------------------------|------------------------|------------------------------------|
| β5^{743-774} -linker-PAK4cat^{D440N,S474E} | 0.49 Å (6 Cα)                               | 0.14 Å (6 Cα)          |
| PAK4cat: β5^{760-770} |                                             |                        | 0.38 Å (7 Cα)                     |
Supplementary Figure 1. Electron density for integrin β5. 2Fo-Fc electron density maps contoured at 1-sigma for A) integrin β5-PAK4D440N/S474E chimera (PDB ID: 7S47). B) PAK4 co-crystallized with integrin β5 peptide -Glu^{760}-Arg-Ser-Arg-Ala-Arg-Tyr-Glu-Met-Ala-Ser^{770} (PDB ID: 7S48). C) D440N/S474E PAK4 co-crystallized with integrin β5 peptide -Glu^{760}-Arg-Ser-Arg-Ala-Arg-Tyr-Glu-Met-Ala-Ser^{770} (PDB ID: 7S46).
Supplementary Figure 2. Uncropped gels. Uncropped gels for indicated figures are shown.
Figure 4g  Cat-WT  Q358A  MW
input  β5  allb  input  β5  allb
markers

D444A
input  β5  allb

D444R
input  β5  allb

Coomassie stain of gel strip showing tail loading
input  β5  allb

Figure 4h  FL-WT  MW
input  β5  allb

Q358A

D444A  input  β5  allb

Coomassie stain of gel strip showing tail loading
input  β5  allb

M482K  input  β5  allb

ES12A
input  β5  allb

ES12R
input  β5  allb

ES12A  input  β5  allb
