Structural basis of target-binding mode of GIT1 in regulating focal adhesion dynamics

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SUPPLEMENTAL Figures

Figure S1.

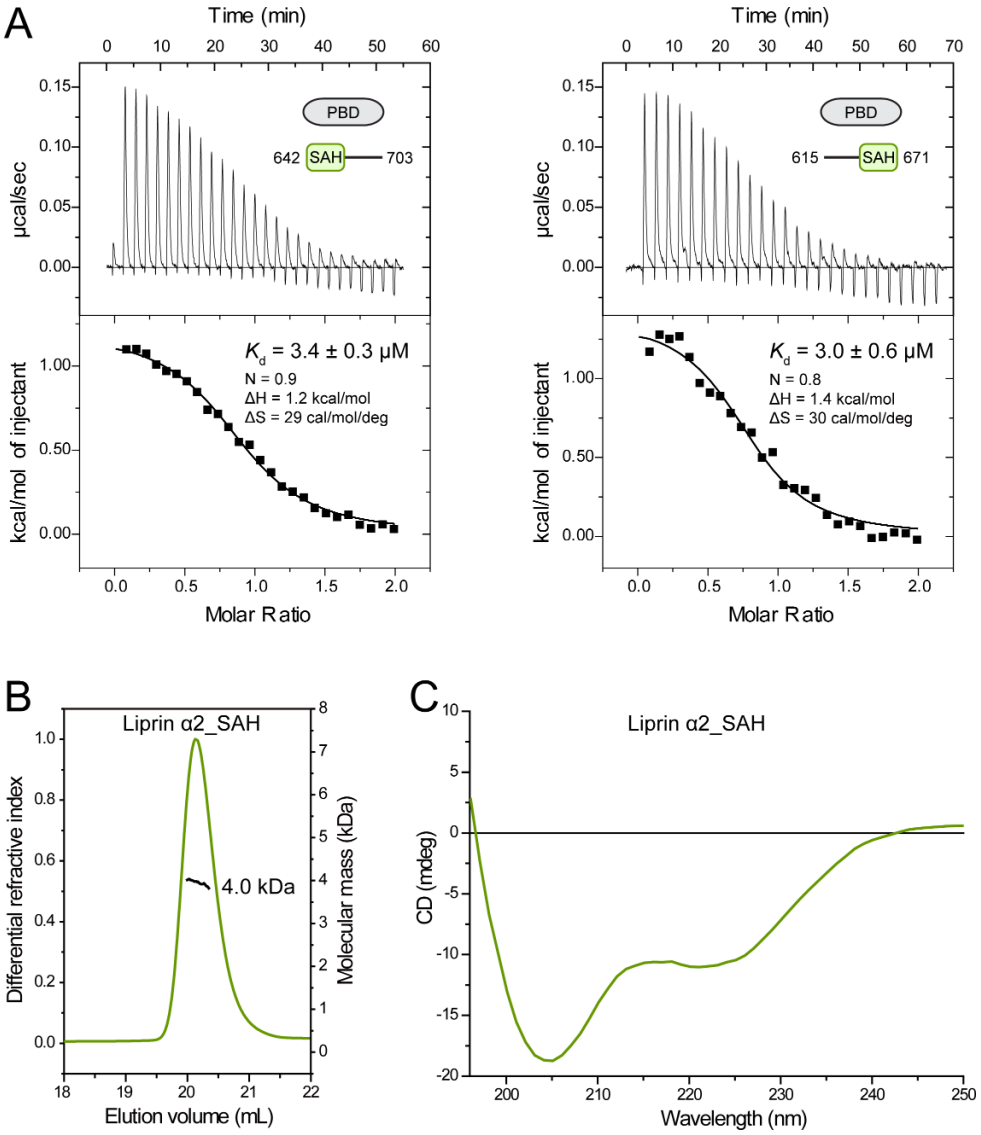

Figure S1. **Biochemical characterization of Liprin α2_SAH.** (**A**) ITC-based measurement of the binding affinities between GIT1_PBD and two Liprin α2 fragments. (**B**) Gel filtration and molecular mass analysis of Liprin-α2_SAH with Trx-tag removed. The theoretical molecular weight of monomeric Liprin-α2_SAH is 4.0 kDa. (**C**). Circular dichroism (CD) spectroscopy measurement of SAH.

Figure S2.

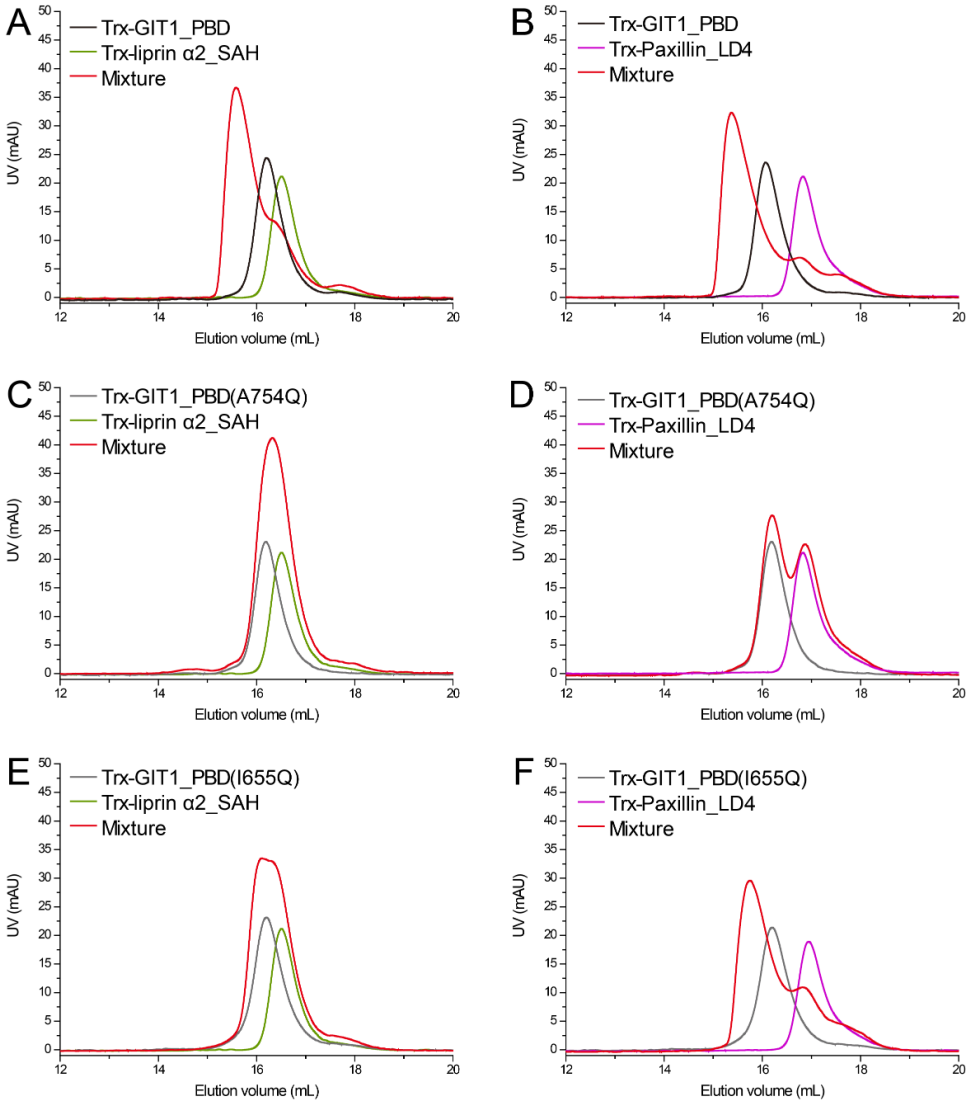

Figure S2. **Analytical gel filtration analysis of the bindings of GIT1_PBD and its mutants to either Liprin-α2_SAH or Paxillin_LD4.** (**A&B**) GIT1_PBD forms a stable complex with either Liprin-α2_SAH (**A**) or Paxillin_LD4 (**B**). (**C&D**) The A754Q mutation disrupts the binding of GIT1_PBD to both SAH (**C**) and LD4 (**D**). (**E&F**) The I655Q mutation disrupts the binding of GIT1_PBD to SAH (**E**), but not to LD4 (**F**).

Figure S3

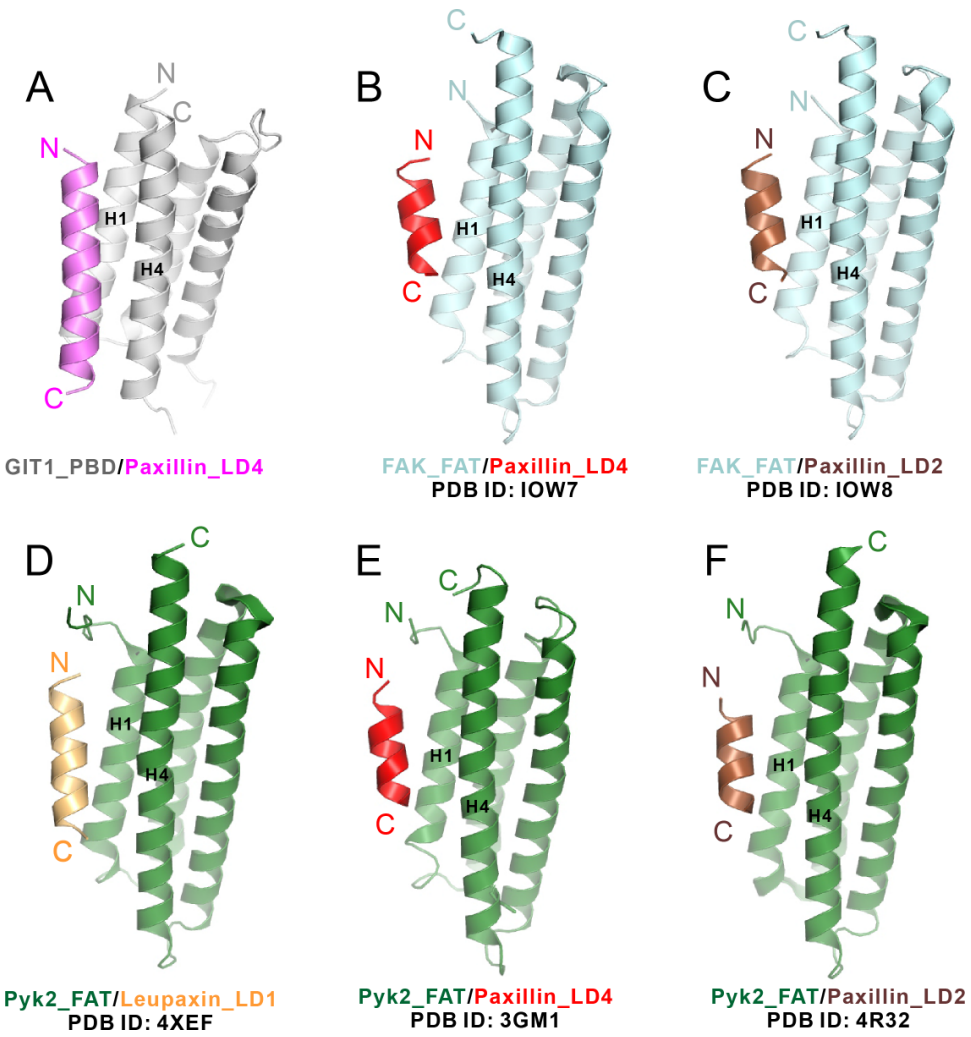

Figure S3. Structural comparison of the LD-binding modes between GIT1_PBD (A) and other FAT-like domains (B-F).

Figure S4

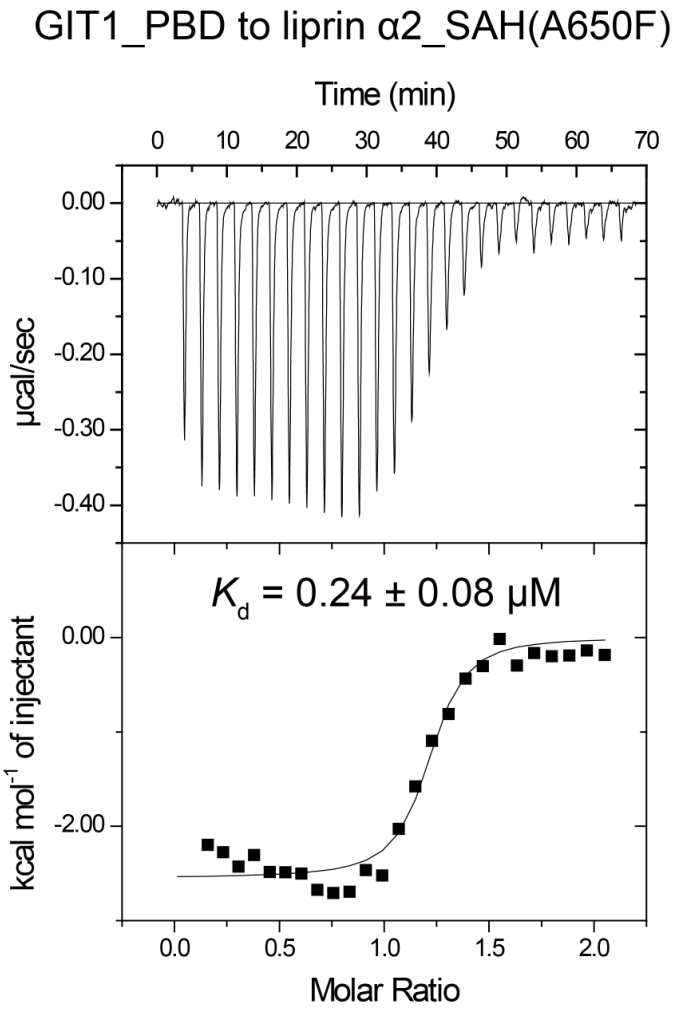

Figure S4. **ITC-based analysis of the interaction between the GIT1_PBD A650F mutant and Liprin-α2_SAH.**

Figure S5

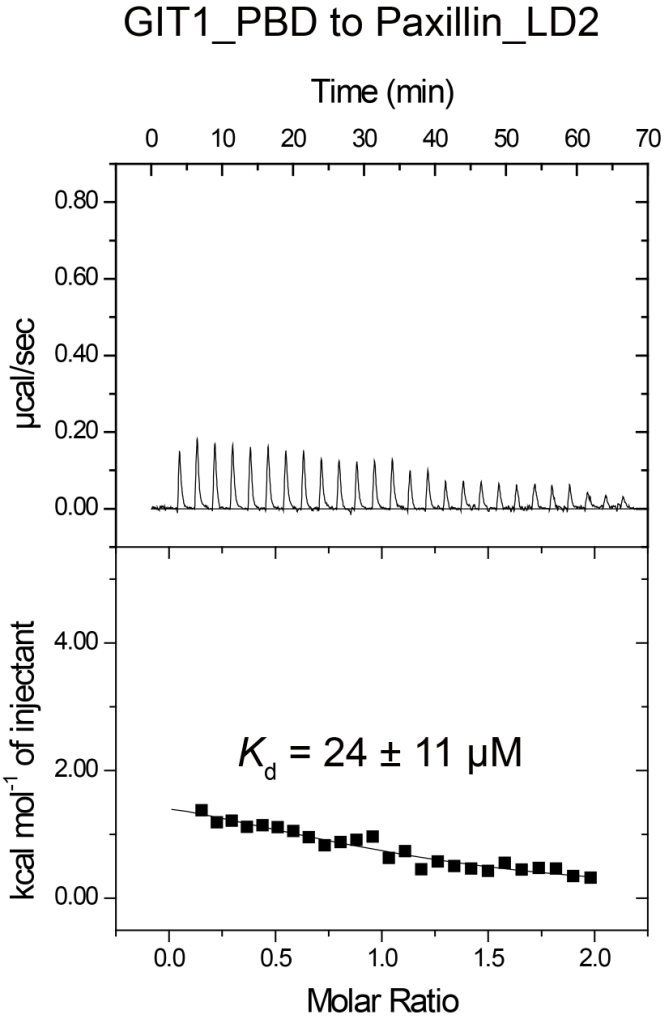

Figure S5. ITC-based analysis of the GIT1_PBD/Paxillin_LD2 interaction.

Table S1. Summary of thermodynamic parameters from ITC experiments

Interaction	K_d (μM)	N	ΔH (kcal/mol)	TΔS (kcal/mol)
GIT1_PBD/SAH	2.8 ± 0.3	0.81 ± 0.01	1.04 ± 0.02	8.61
GIT1_PBD(I665Q)/SAH	Not detectable	-	-	-
GIT1_PBD(A754Q)/SAH	Not detectable	-	-	-
GIT1_PBD(T665E)/SAH	> 200	-	-	-
GIT1_PBD/SAH(A650F)	0.24 ± 0.08	1.19 ± 0.02	-2.55 ± 0.06	6.47
GIT1_PBD/LD4	3.9 ± 0.4	1.14 ± 0.01	-6.1 ± 0.1	1.3
GIT1_PBD(I665Q)/LD4	2.4 ± 0.4	0.90 ± 0.02	-5.2 ± 0.2	2.4
GIT1_PBD(A754Q)/LD4	Not detectable	-	-	-
GIT1_PBD(T665E)/LD4	1.5 ± 0.1	0.96 ± 0.03	-6.44 ± 0.03	1.48
GIT1_PBD/LD4(Δ276-283)	Not detectable	-	-	-
GIT1_PBD/LD4(FK/VQ)	> 30	-	-	-
GIT1_PBD/LD2	24 ± 11	1.5 ± 0.1	2.4 ± 0.4	8.7
FAK_FAT/LD4	3.6 ± 0.3	0.55 ± 0.01	-8.9 ± 0.2	-1.4
FAK_FAT/LD4(Δ276-283)	8.2 ± 0.6	0.85 ± 0.02	-7.4 ± 0.2	-0.5

"-" stands for not determined.