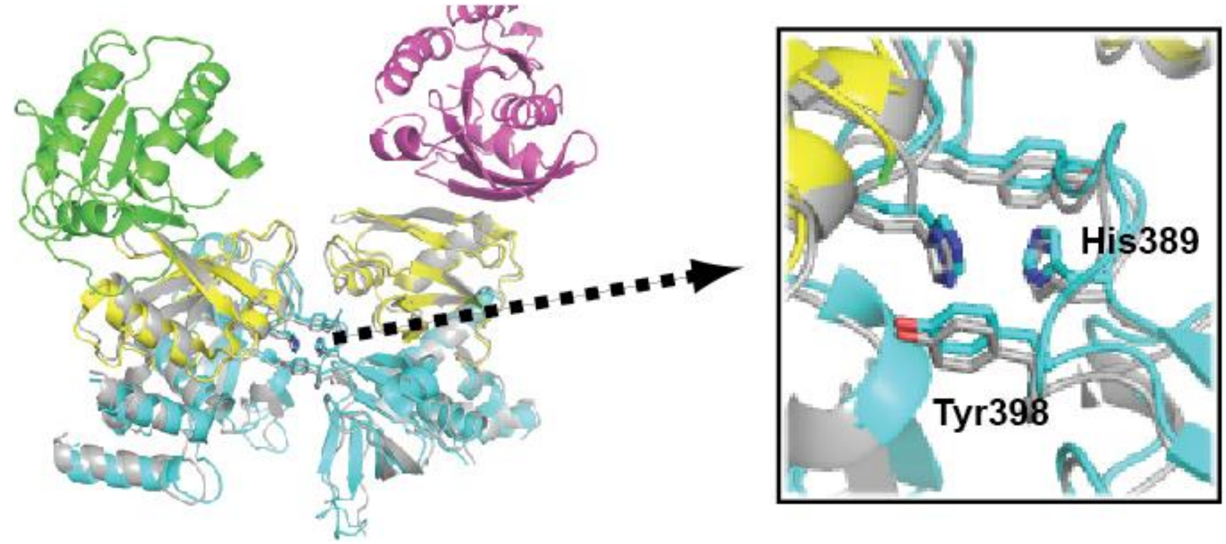

Figure S1. The “H-Y” putative interface. Related to Figure 1.

Superposition of the CC-RA-PH dimer for the original structure (3TCA) and the RA-PH: RAP1 complex dimer (4KVG) reveals a pseudo-symmetrical interface via the PH domains. The CC-RA-PH dimer is colored grey. In the RA-PH: RAP1 complex structure, the RA domain is colored yellow; the PH domain is in cyan; and RAP1 is in green or magenta. His389 and Tyr398 at the “H-Y” interface are shown in the right.

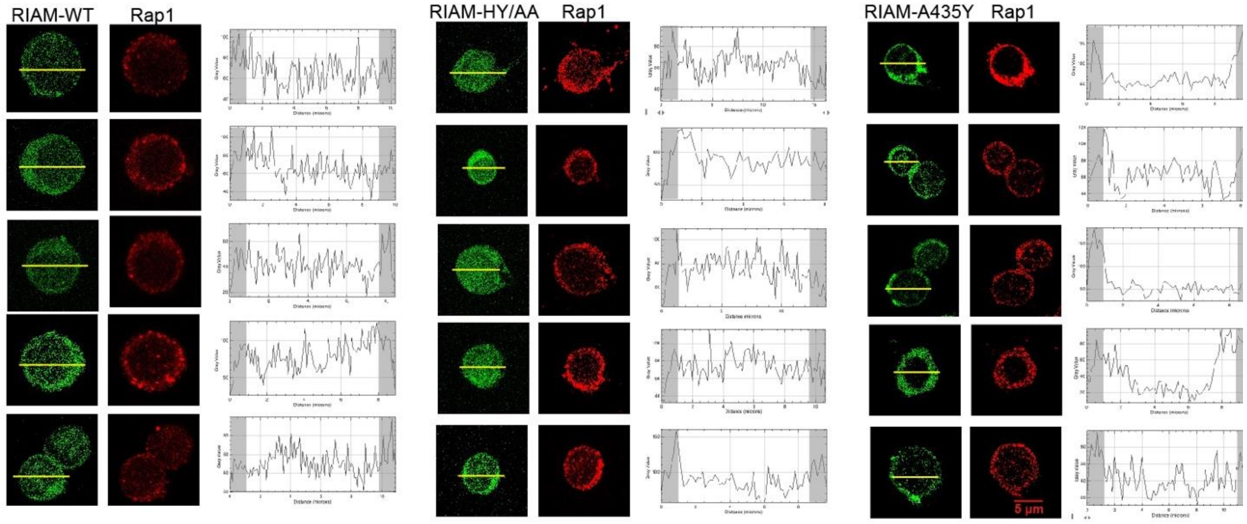

Figure S2. The PM translocation of RIAM. Related to Figure 2.

Co-expression of the HA-tagged RAP1G12V and GFP-tagged RIAM FL and mutations in Jurkat T cells. Portions defined as the PM distribution are indicated by the shadowed areas in the plots. For each sample, five cells are shown and plotted for PM co-localization of RIAM and RAP1.

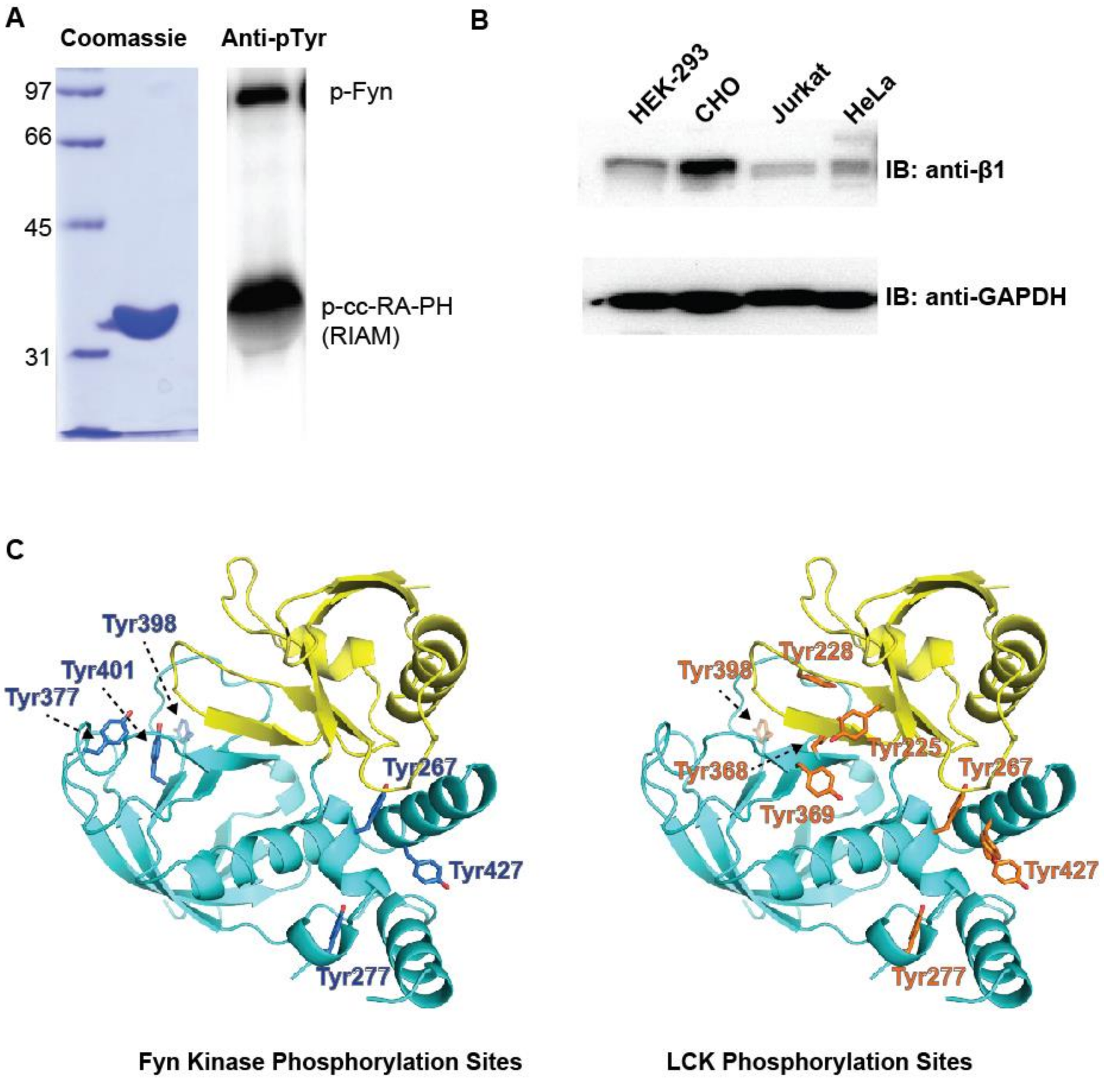

Figure S3. Tyrosine phosphorylation of RIAM by Src kinases. Related to Figures 3 and 4.

A. Tyrosine phosphorylation of RIAM cc-RA-PH by purified Fyn kinase. **B.** Expression level of endogenous β_1 integrin in HEK293, CHO, Jurkat T, and HeLa cells. **C.** *Left:* Tyrosine phosphorylation sites in RIAM RA-PH by Fyn kinase identified by mass spectrometry analysis. *Right:* Tyrosine phosphorylation sites in RIAM RA-PH by LCK identified by mass spectrometry analysis. The four common sites are shown in Figure 4A.

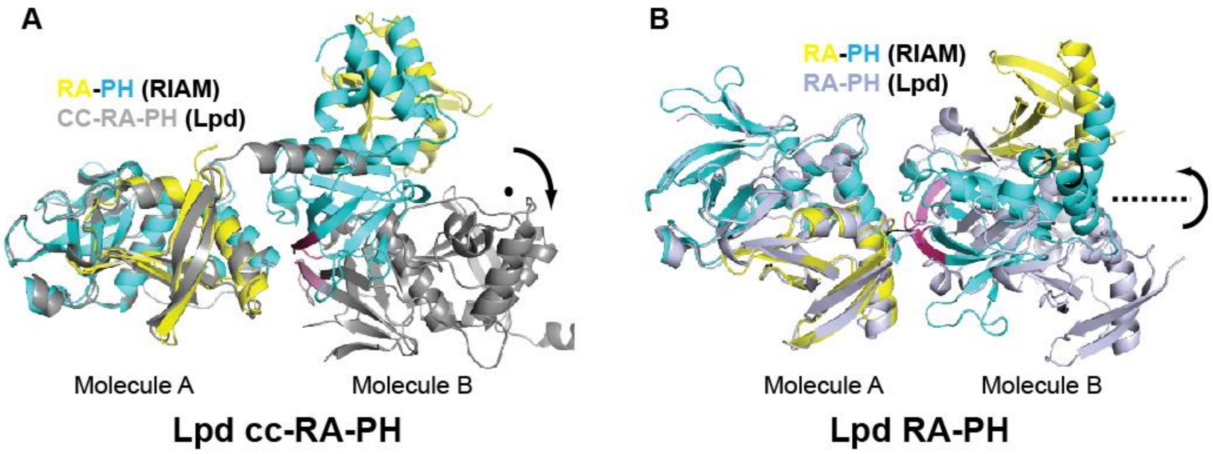

Figure S4. Similar intermolecular interface observed in Lpd structures. Related to Figures 1 and 2.

A. Superposition of the RIAM RA-PH alone structure (yellow/cyan) and the Lpd CC-RA-PH structure (gray). For each structure, two molecules (A and B) are shown, with one molecule on the left side (molecule A) superimposed. The phosphoinositide-binding site in molecule B are colored magenta (RIAM) or pink (Lpd). The relative positions of the of the two molecule B are indicated by the rotation arrow. **B.** RIAM RA-PH alone structure (yellow/cyan) and the Lpd RA-PH structure (light blue) were superimposed in a similar way as seen in panel A. The phosphoinositide-binding site in the molecule B are colored light pink (Lpd) and magenta (RIAM). The relative positions of the two molecule B are indicated by the rotation arrow.

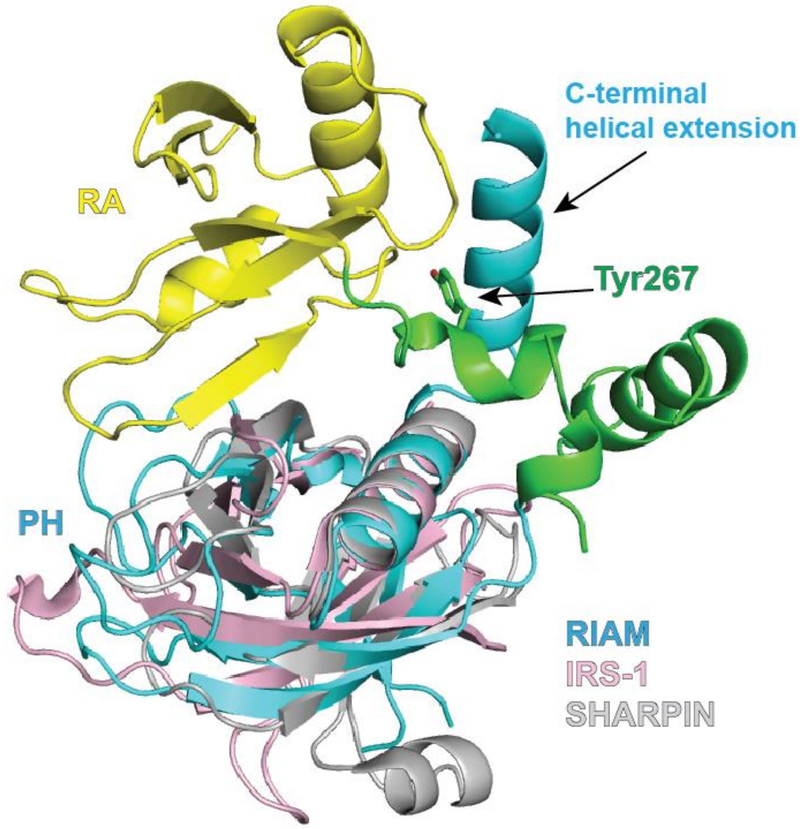

Figure S5. Comparing the PH domain of RIAM with other canonical PH domains. Related to Figure 4.

RIAM PH domain was superimposed with the PH domains of SHARPIN (gray) and IRS-1 (light pink). Connection region that links the RA (yellow) and PH (cyan) domains in RIAM is colored in green. The C-terminal helical extension is indicated. Side chain of Tyr267 is shown in stick representation.

Supplemental Table 1. Lists of data points. Related to Figures 3 and 4.

Figure 3E

	I.	II.	III.	Mean	STDEV	*t*-test (2-tailed)	Vs.
Untreated	1.00	1.00	1.00	1.00	0.000		
RK-24466	1.06	0.87	0.83	0.92	0.125	N.S.	Untreated vs. RK
anti-CD3	1.21	1.30	1.19	1.23	0.059	0.020979	Untreated vs. anti-CD3
anti-CD3+RK	0.72	0.55	0.51	0.59	0.108	0.022635	Untreated vs. anti-CD3+RK
						0.014405	anti-CD3 vs. anti-CD3+RK

Figure 3F

	I.	II.	III.	Mean	STDEV	Normalized Mean	Normalized STDEV	*t*-test (2-tailed) vs. DMSO
uncoated	0.067	0.067	0.072	0.069	0.003	0.531	0.023	
unstimulated	0.122	0.107	0.161	0.130	0.028	1.000	0.214	
PMA	0.420	0.379	0.439	0.413	0.031	3.170	0.236	
anti-CD3	0.326	0.300	0.407	0.344	0.056	2.650	0.429	
anti-CD3+DMSO	0.339	0.308	0.316	0.321	0.016	2.470	0.124	
anti-CD3+5 μM	0.186	0.185	0.215	0.195	0.017	1.500	0.131	0.014078
anti-CD3+10 μM	0.189	0.189	0.181	0.186	0.005	1.430	0.036	0.004388
anti-CD3+20 μM	0.151	0.144	0.149	0.148	0.004	1.140	0.028	0.001899

Figure 3G

	I.	II.	III.	Mean	STDEV	Normalized Mean	Normalized STDEV	*t*-test (2-tailed) vs. DMSO
Uncoated	0.105	0.099	0.105	0.103	0.004	1.000	0.034	
DMSO	1.212	1.094	1.608	1.305	0.269	12.670	2.614	
10 μM	0.710	0.665	0.641	0.672	0.035	6.520	0.340	0.064173
50 μM	0.420	0.417	0.459	0.432	0.023	4.190	0.228	0.025505
100 μM	0.233	0.212	0.207	0.217	0.014	2.110	0.134	0.020800

Figure 4E

RAP1G12V+	I.	II.	III.	Mean	STDEV	*t*-test (2-tailed)	Vs.
RIAM WT	1.00	1.00	1.00	1.00	0.000		
Y267F	0.71	0.73	0.65	0.69	0.042	0.006309	WT vs. Y267F
Y427F	0.51	0.71	0.59	0.60	0.104	0.021833	WT vs. Y427F
A435Y	1.63	1.88	1.81	1.77	0.127	0.008885	WT vs. A435Y
YY/EE	0.68	0.99	1.01	0.89	0.181	N.S.	WT vs. YY/EE

N.S.: Not Significant.