

Supplementary information

Conformation-Driven Phase Separation in the Linker Domain of Focal Adhesion Kinases

Lucy Martin^{1,2}, Daniela P. Freitas^{3,4}, Emmanuelle Boll^{1,2}, Alain Brans⁵, Justine Mortelecque^{1,2}, François-Xavier Cantrelle^{1,2}, Pierre Dourlen², Cláudio M. Gomes^{3,4}, Clément Danis^{1,2*}, Isabelle Landrieu^{1,2*}

¹CNRS EMR9002 – BSI - Integrative Structural Biology F-59000 Lille, France

²Univ. Lille, Inserm, CHU Lille, Institut Pasteur de Lille, U1167 - RID-AGE - Risk Factors and Molecular Determinants of Aging-Related Diseases, F-59000 Lille, France

³BioISI - Instituto de Biosistemas e Ciências Integrativas, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal

⁴Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal

⁵InBioS - Centre for Protein Engineering, Département des Sciences de La Vie, University of Liège, B-4000 Liège, Belgium

Fak1	691	MRMESRRQATVSWDSGGSD	<u>APPKPSRPGYPS</u>	SPRSSEGFYPS	QHMVQTNHYQVSGYP	
Pyk2	693	QER-NARYRTPKILEPTA	<u>FQEEPPPKPSRPKYR</u>	PPPQTNLLAPKLQ	-----FQVPEGL	
		*	*	*	*	*
Fak1	749	GSHGITAMAGSIYPGQASLLDQ	TDSWNHR--	PQEIAMWQPNVED	STVLDLRGIGQVLPTH	
Pyk2	744	CASSPTLTSPMEYPSVNSLHTP	PLHRHNVFKRHS	MREEDFIQF	SSREEAQQLWEAEKVK	
		*	*	*	*	
Fak1	807	LMEERLIRQQQEMEEDQ	RWLEKEERFLK	PDVRLSRGSIDREDGSLQ	GPIGNQHIIYQPVGK	
Pyk2	804	-MRQILDKQQKQMV	EDYQWLRQEEKSLD	PMVYMNDKS	-----PLTPE---	KEVGY
		*	*	*	*	*
Fak1	867	PDPAAPPKKPPRPGAPGHLGSLASL	SSPADSYNEGVKLQ	PQEISPPP	913	
Pyk2	850	<u>LEFTGPPQKPPRLGAQ</u>	-----	SIQ	868	
		*	*	*	*	

Figure S1 : Protein sequence alignment of Pyk2-KFL₆₉₃₋₈₆₈ and Fak1-KFL₆₉₁₋₉₁₃ (UniProtKB accession numbers: FAK2_HUMAN Q14289 and FAK1_HUMAN Q05397, respectively). The Fak1-KFL₇₉₁₋₈₃₄ and Pyk2-KFL₇₈₈₋₈₃₀ peptides are highlighted in bold green. Two PRRs sequences (PRR1 and PRR2) rich in “PxxP” motifs are underlined and their resonance assignment is provided in supplementary **Table S1**.

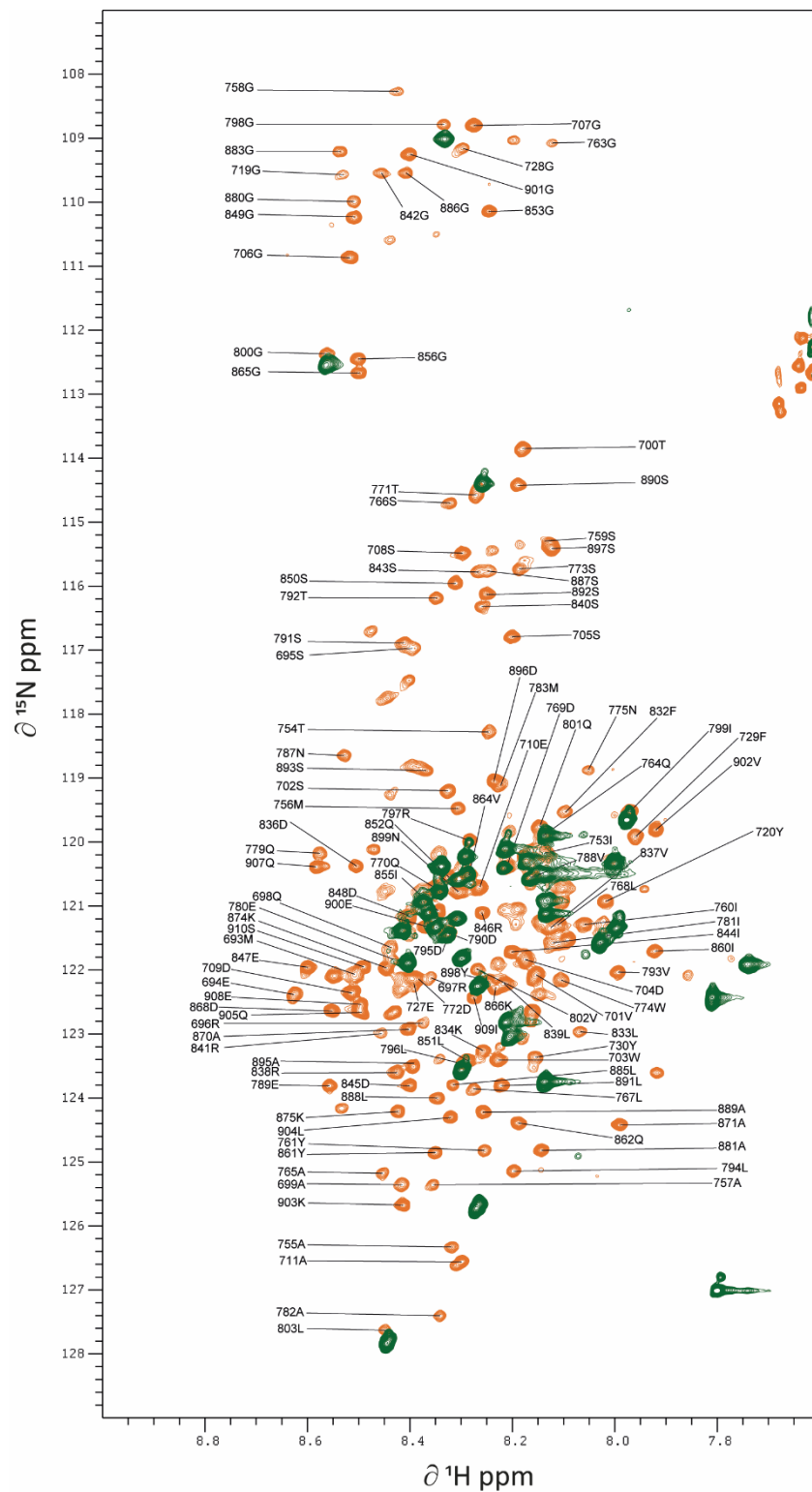


Figure S3 : Overlay of ^1H , ^{15}N HSQC two-dimensional full spectra of Fak1-KFL₆₉₁₋₉₁₃ (in orange) and of Fak1-KFL₇₉₁₋₈₃₄ peptide (superimposed in green). Resonance assignment of Fak1-KFL₆₉₁₋₉₁₃ is indicated.

Table S1

Assignment of NMR signals from peptides PRR1 and PPR2 of Pyk2-KFL

PRR1 Pyk2-KFL ₇₀₉₋₇₂₃		
<i>Residue</i>	¹⁵ <i>N</i>	¹ <i>H</i>
A709	129.863	8.238
F710	119.602	8.26
Q711	123.001	8.163
E712	124.66	8.43
K716	122.872	8.448
S718	116.905	8.488
R719	124.012	8.396
K721	121.826	8.45
Y722	121.743	8.246
R723	124.666	8.311

PRR2 Pyk2-KFL ₈₅₁₋₈₆₅		
<i>Residue</i>	¹⁵ <i>N</i>	¹ <i>H</i>
E851	126.774	8.308
F852	121.206	8.453
T853	116.31	8.146
G854	111.068	7.741
Q857	120.907	8.492
K858	124.208	8.438
R861	121.435	8.438
L862	124.251	8.407
G863	110.315	8.506
A864	124.089	8.286
Q865	119.791	8.434

Assignment of NMR signals from peptides PRR1 and PPR2 of Fak1-KFL

PRR1 Fak1-KFL ₇₀₇₋₇₂₁		
<i>Residue</i>	¹⁵ <i>N</i>	¹ <i>H</i>
G707	123.538	8.352
S708	115.654	8.404
D709	122.312	8.517
E710	120.97	8.225
A711	126.938	8.327
K714	122.89	8.449
S716	116.919	8.479
R717	114.601	8.363
G719	110.088	8.595
Y720	121.385	8.057

PRR2 Fak1-KFL ₈₆₈₋₈₈₂		
<i>Residue</i>	¹⁵ <i>N</i>	¹ <i>H</i>
D868	127.973	8.327
A870	124.257	8.416
A871	124.603	8.04
K874	122.096	8.444
K875	123.344	8.413
R878	122.239	8.422
G880	110.294	8.594
A881	124.981	8.129

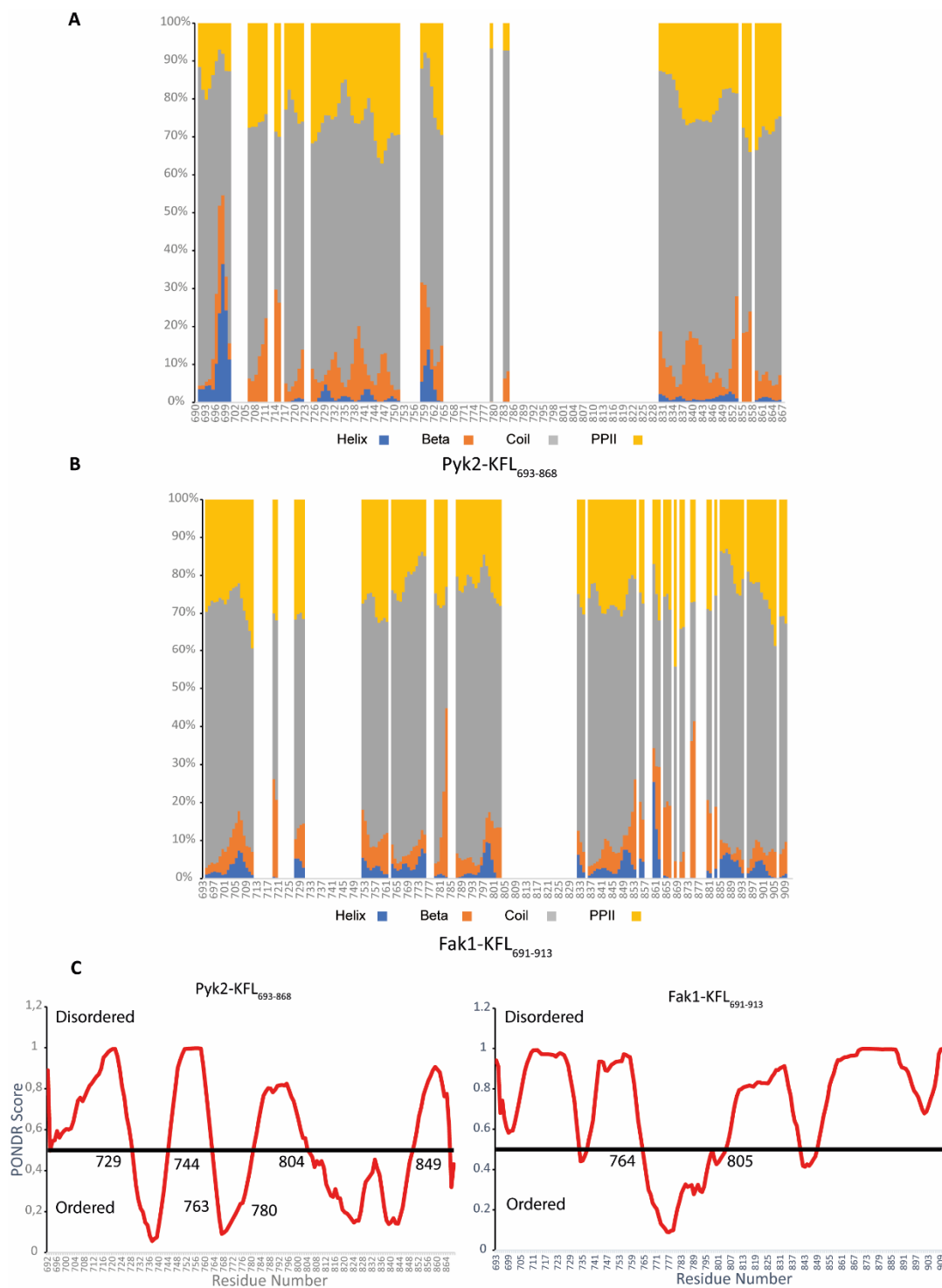


Figure S4 : A-B Delta2D analysis based on $H\alpha$, $C\alpha$, $C\beta$, CO , ^{15}N and $H^{15}N$ chemical shifts for **A.** Pyk2-KFL₆₉₃₋₈₆₈ **B.** Fak1-KFL₆₉₁₋₉₁₃ that gives the relative abundance or secondary structure propensity (SSP) for each of the secondary structures, α -helix (blue), β -strand (orange), coil (gray) and polyproline II (yellow) conformations along the peptide sequence. **C** POND sequence analysis.

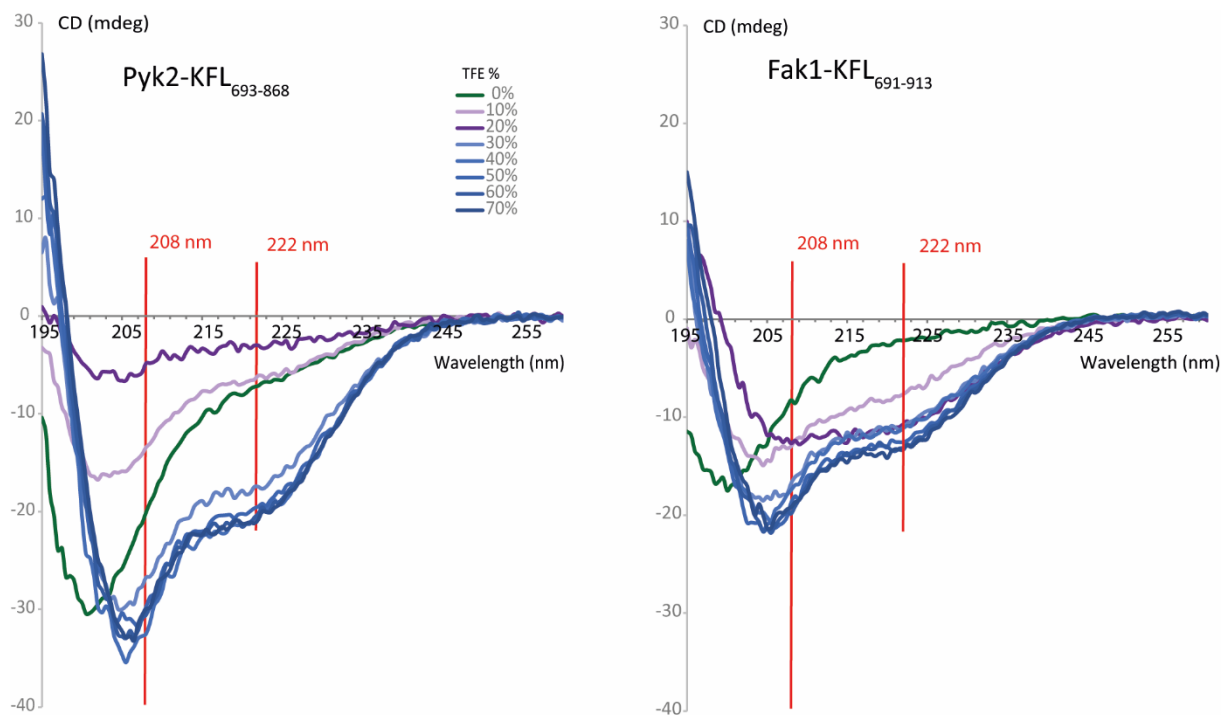


Figure S5 : Circular Dichroism (CD) spectra for FAK-KFLs, at 25°C, without and with increasing concentration of TFE. Red bars indicates the observed minimal values.

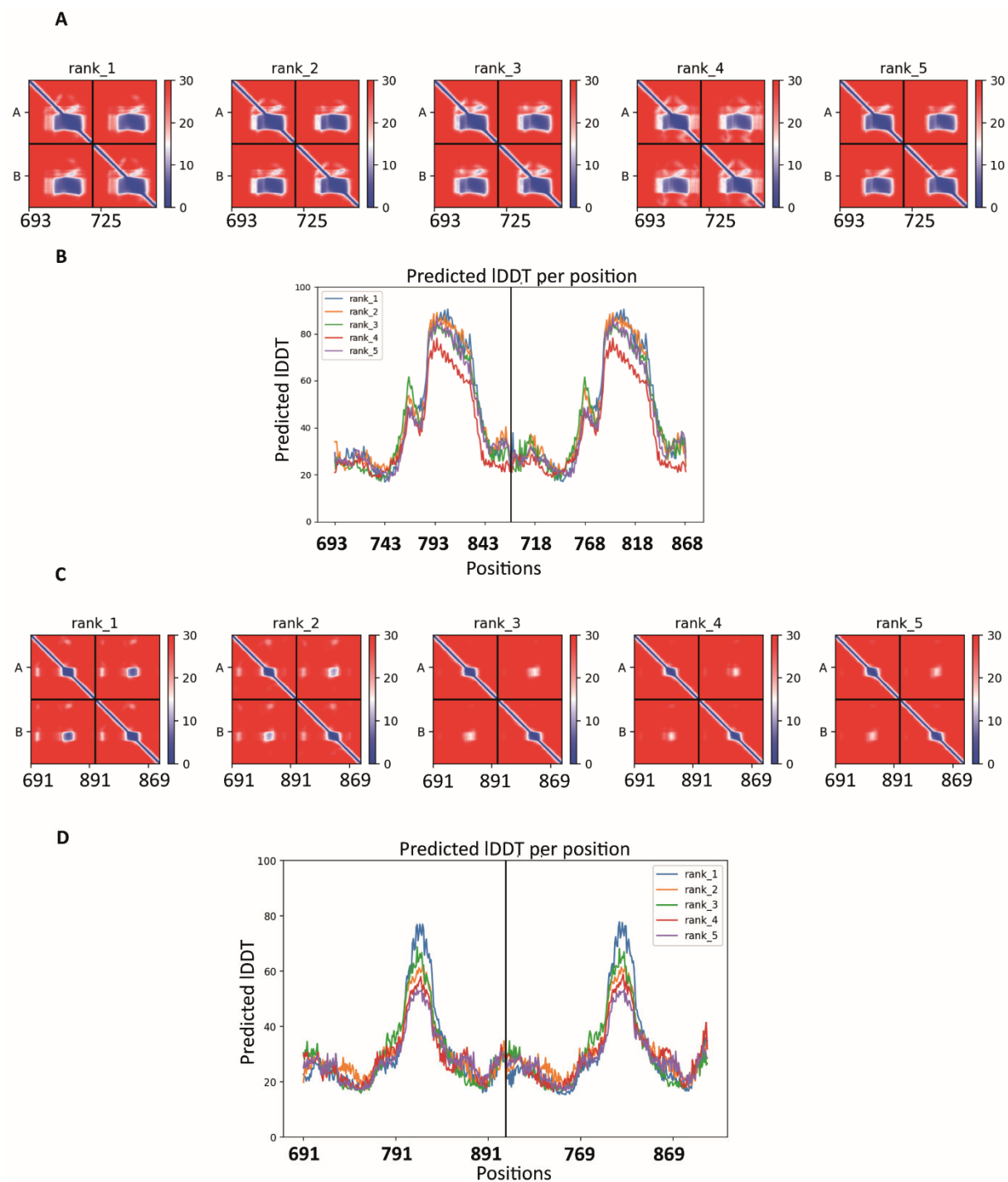


Figure S6 : AlphaFold prediction of dimer formation for **A-B** Pyk2-KFL₆₉₃₋₈₆₈ and **C-D** FAK1-KFL₆₉₁₋₉₁₃. **A, C** : color coded PAE (prediction alignment error), from blue to red, for each of the five predicted dimer molecular models. The lower the PAE values (blue) correspond to higher accuracy. **B, D** : the pLDDT (predicted local-distance difference test) score value per position of each subunit of a dimer, for five models, shows high accuracy of prediction for a dimer interface mediated by the helical peptide **B**. Pyk2-KFL₇₈₈₋₈₃₀ or **D**. Fak1-KFL₇₉₁₋₈₃₄.

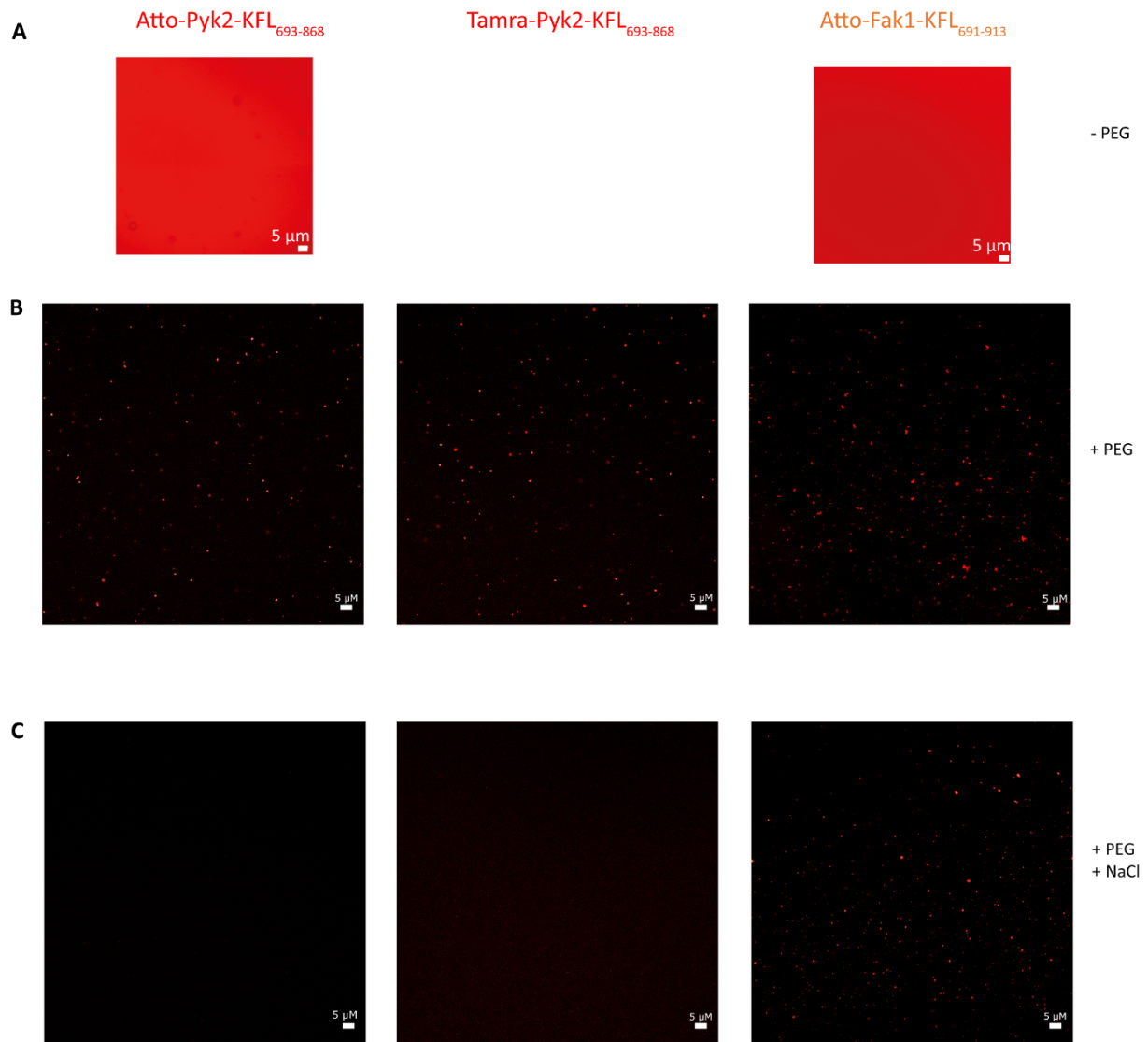


Figure S7 : **A.** Representative fluorescence microscopy images of 4 μM Atto565 (Atto) labeled-Pyk2-KFL₆₉₃₋₈₆₈ or Atto-Fak1-KFL₆₉₁₋₉₁₃ in the absence of 12 % PEG in buffer 50 mM NaPi pH 7.4, with 30 mM NaCl and **B-C.** Representative fluorescence images by confocal microscopy, of Atto-labeled FAK-KFLs and TAMRA-labeled Pyk2-KFL₆₉₃₋₈₆₈ with 12 % PEG **B.** under the same buffer conditions as A. **C.** under the same buffer conditions as A. but with the addition of 300 mM NaCl. Scale bar: 5 μm .