BC Cancer Agency

Finding new signaling complexes and understanding their function

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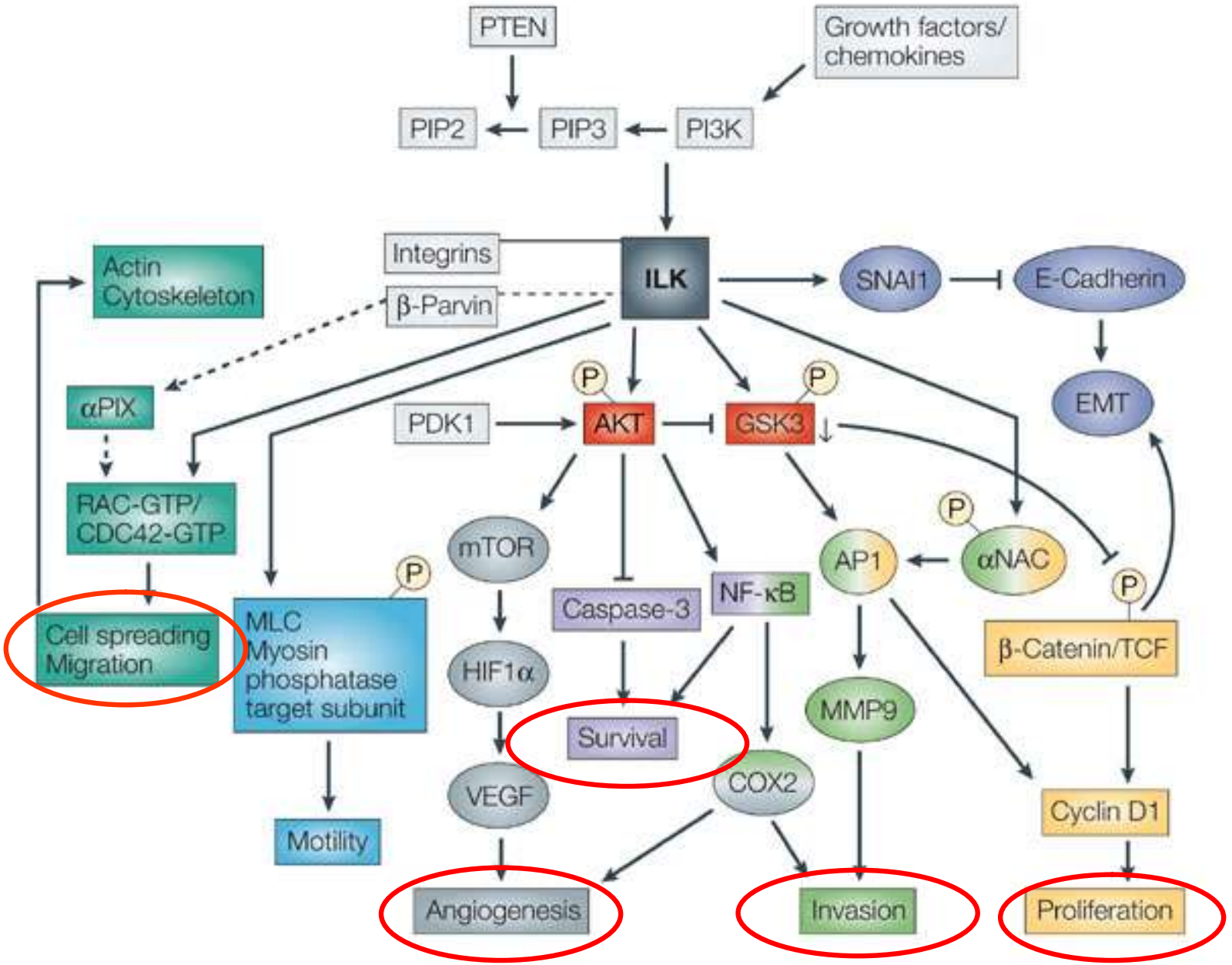
ILK-regulated signaling pathways



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On-going projects

- Characterization of ILK-protein complexes
- Functional importance of ILK for the stability of different signaling complexes
- What are the cellular targets of ILK?

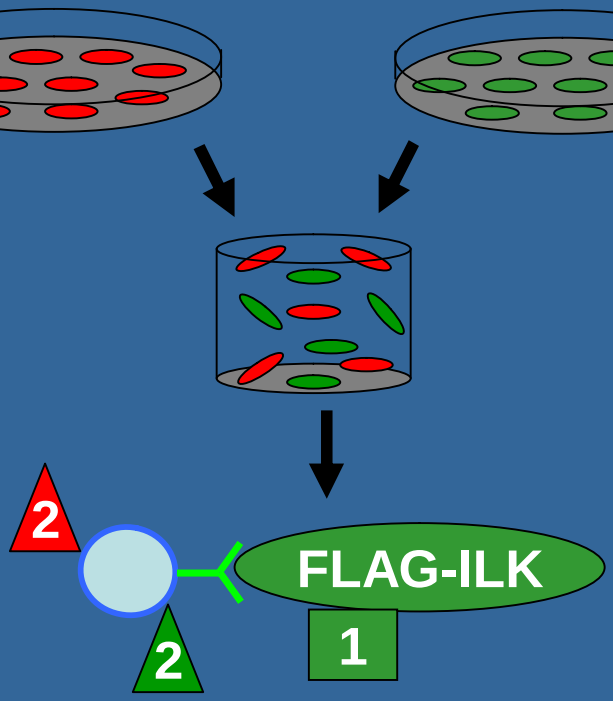
Goal 1:

Finding new ILK-binding partners

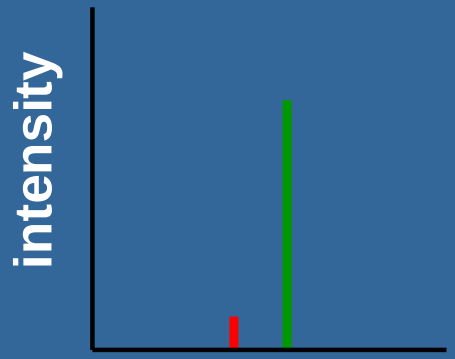
SILAC approach to identify specific ILK-binding proteins

Unlabeled HEK293 cells expressing FLAG tag

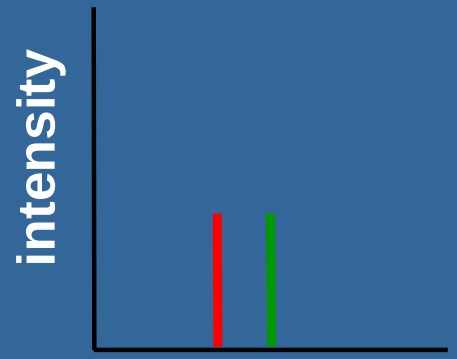
Lys-D_4 & Arg-$^{13}C_6$ labeled cells stably expressing FLAG-ILK



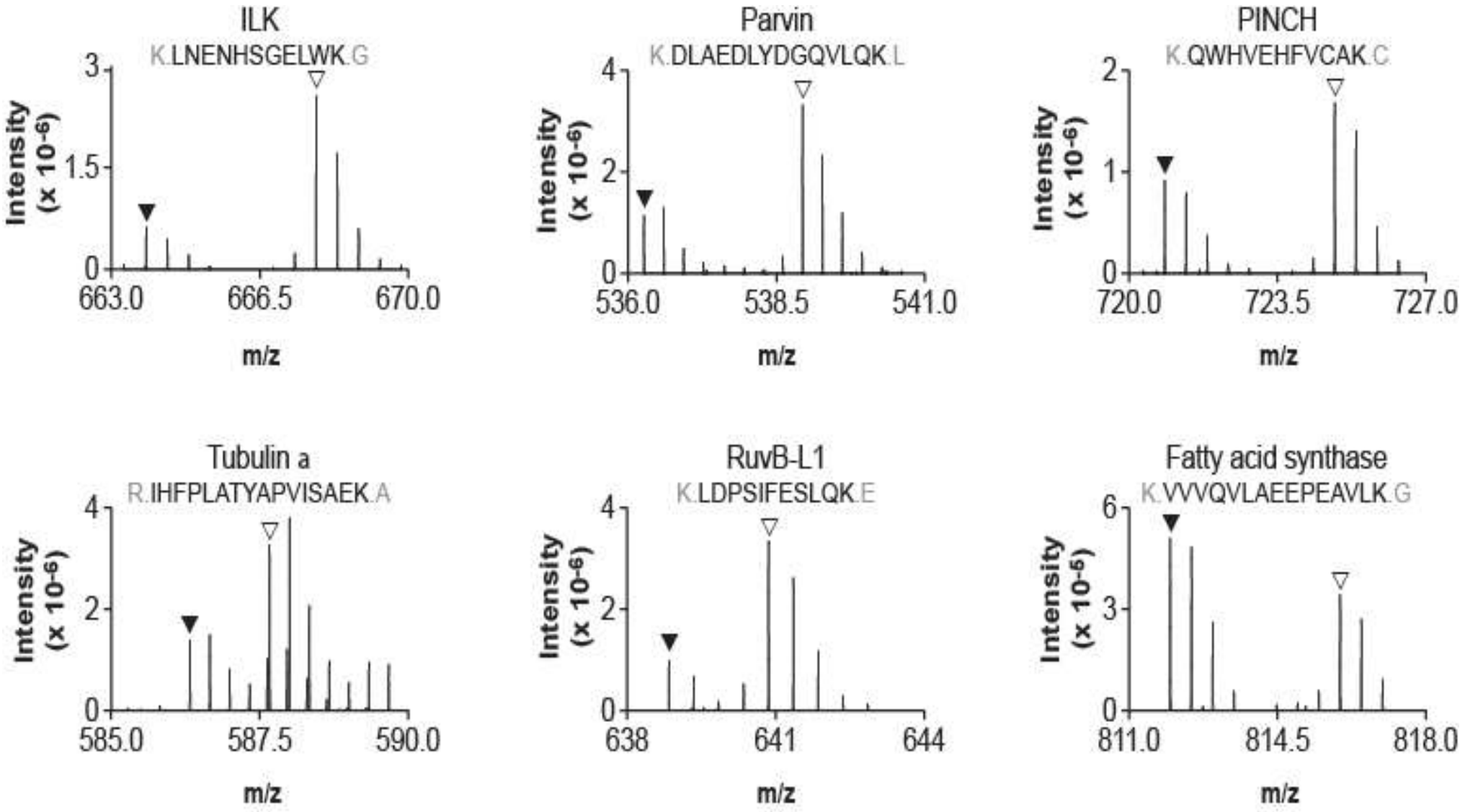
1. Specific



2. Non-specific

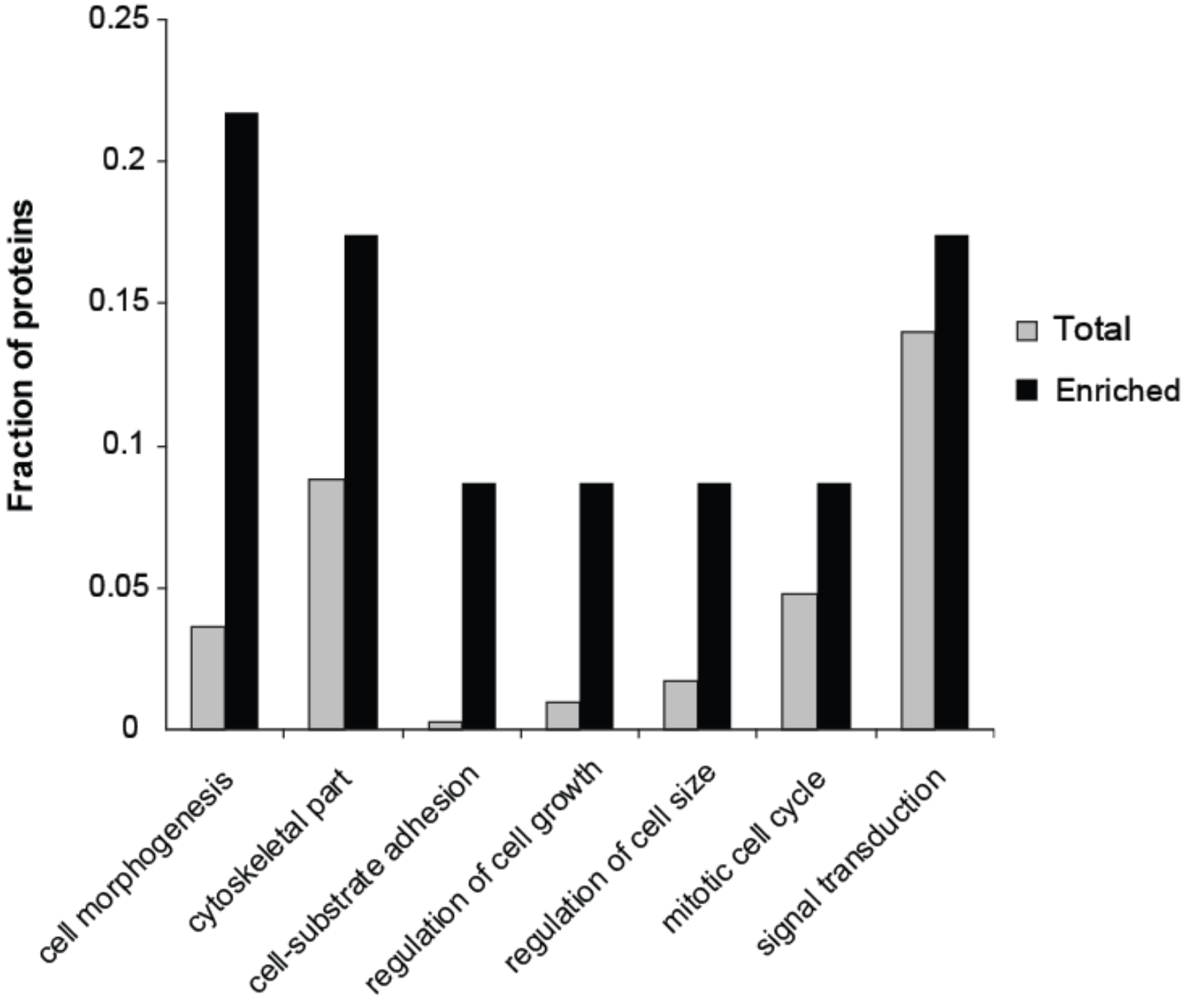


Mass spectrum of different SILAC pairs

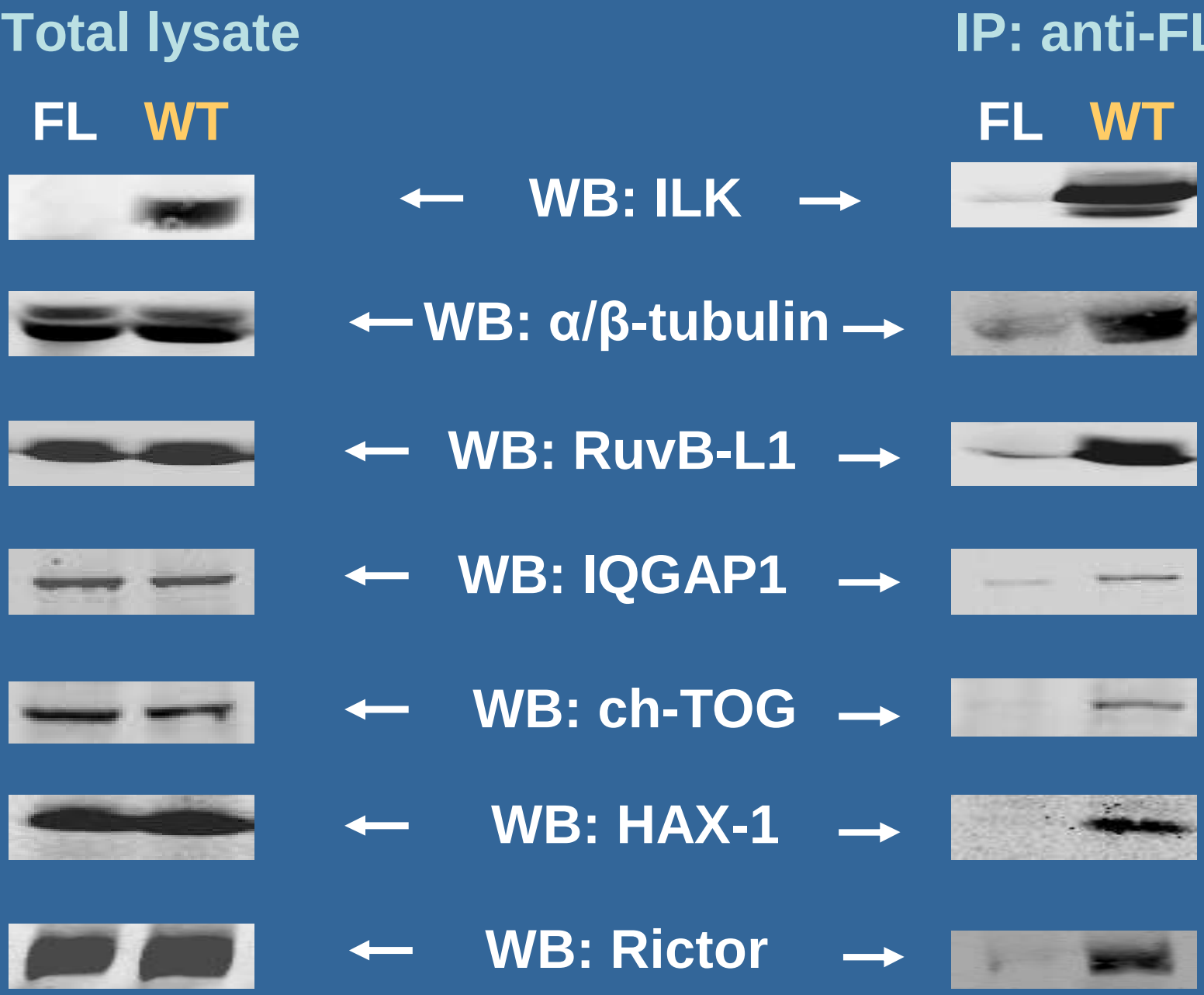


N	Gene	Description	Ave	SD	p-value
1	PARVA	Isoform 1 of Alpha-parvin	6	3	0.001
2	**ILK**	**Integrin-linked protein kinase**	**4**	**2**	**0.01**
3	RICTOR	Rapamycin-insensitive companion of mTOR (RICTOR)	3.3	0.8	6.03E-05
4	PPP6C	Serine/threonine-protein phosphatase 6	3.29	n/a	n/a
5	LGALS3BP	Galectin-3-binding protein precursor	3.16	n/a	n/a
6	DNAJA1	DnaJ homolog subfamily A member 1	2.9	0.3	n/a
7	KIAA1303	Isoform 1 of Regulatory-associated protein of mTOR	2.84	n/a	n/a
8	RUVBL2	RuvB-like 2 (Reptin 52)	2.8	0.3	0.00E+00
9	MON2	Hypothetical protein DKFZp761D20121 (Fragment)	2.75	n/a	n/a
10	SLC25A10	Isoform 1 of Mitochondrial dicarboxylate carrier	2.74	0.01	n/a
11	TUBA1B	Tubulin alpha-ubiquitous chain	2.71	n/a	n/a
12	HAX1	HS1-associating protein X-1	3	2	0.05
13	USP11	Ubiquitin specific protease 11	3	1	0.02
14	AKAP8L	A-kinase anchor protein 8-like	2.6	0.4	3.00E-07
15	CCT2	T-complex protein 1 subunit beta	3	1	0.05
16	KIAA0368	Proteasome-associated protein ECM29 homolog	2.61	n/a	n/a
17	MAGED2	Isoform 1 of Melanoma-associated antigen D2	3	2	0.09
18	TCP1	T-complex protein 1 subunit alpha	2.54	n/a	n/a
19	STT3A	Dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit STT3A	2.52	n/a	n/a
20	RUVBL1	RuvB-like 1 (Pontin 52)	3	1	0.003
21	TIMM23	Mitochondrial import inner membrane translocase subunit Tim23	2.52	n/a	n/a
22	CAD	CAD protein	2.5	0.1	0.00E+00
23	TUBB2B	Tubulin beta-2B chain	2.5	0.3	1.64E-14
24	XPOT	110 kDa protein	2.50	n/a	n/a
25	LIMS1 (also known as PINCH)	LIM and senescent cell antigen-like-containing domain protein 1	2.5	0.2	0.00E+00
140	CKAP5	Cytoskeleton-associated protein 5 (ch-TOG, XMAP215)	1.5	0.9	0.23
146	IQGAP1	Ras GTPase-activating-like protein IQGAP1	1.5	0.6	0.10

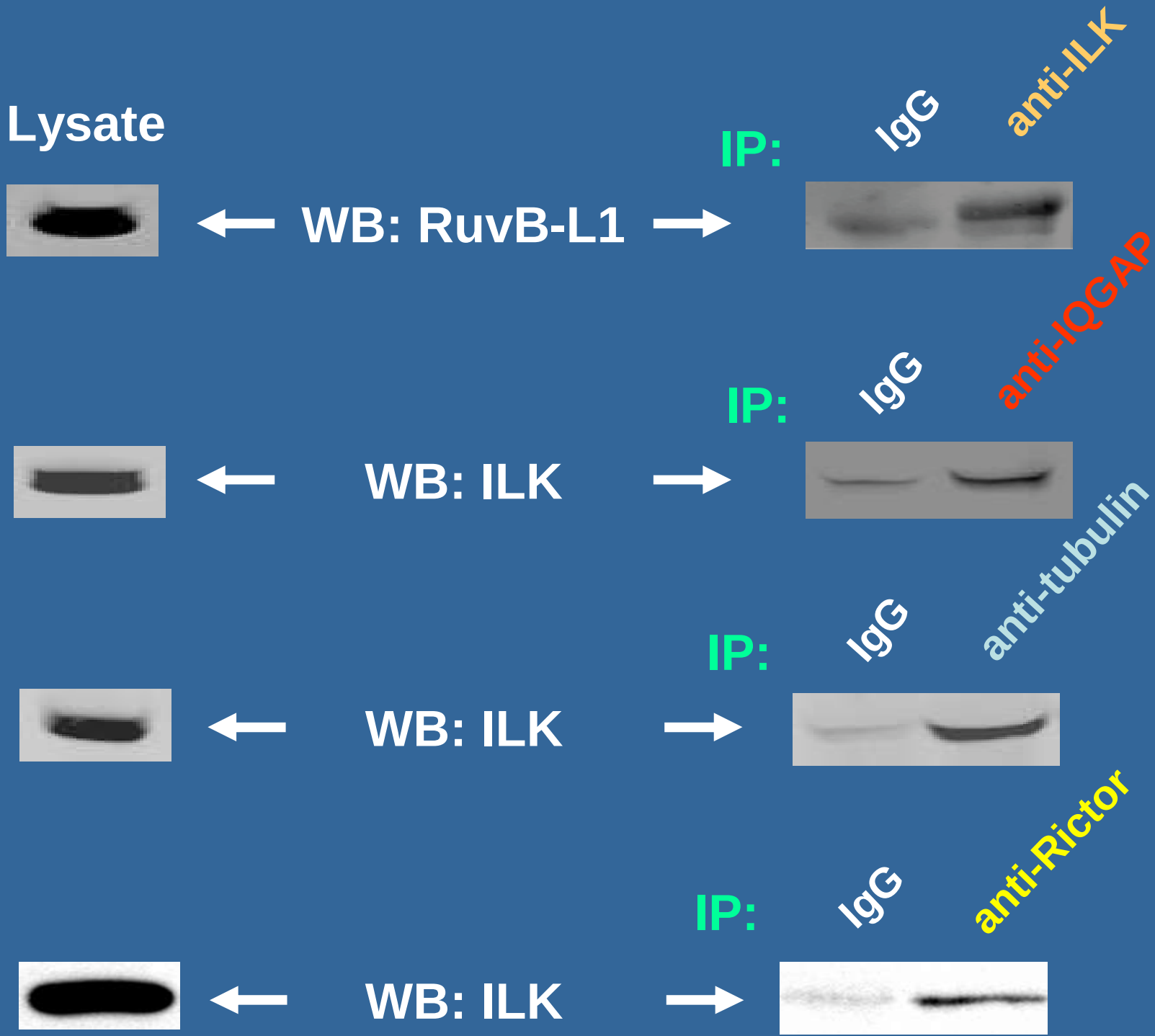
Gene ontology of ILK-binding proteins



Validation of functional interactions - exogenous co-IPs

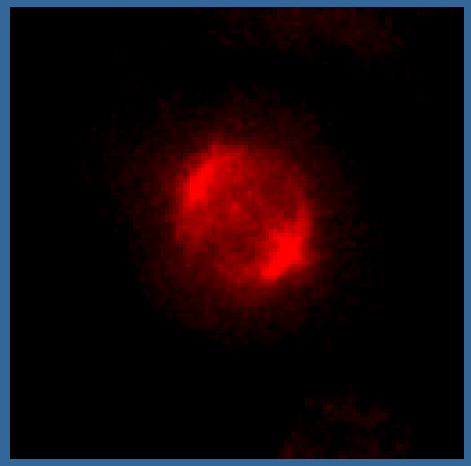


Validation of functional interactions - endogenous co-IPs

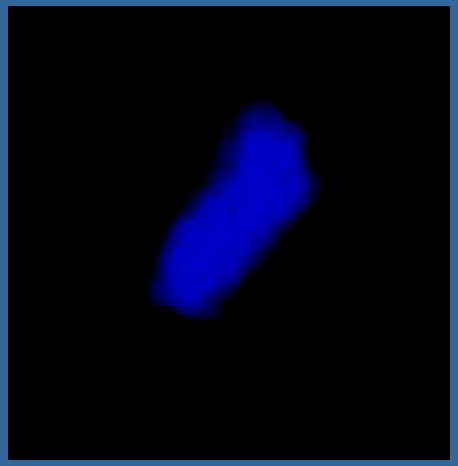


Colocalization between ILK, tubulin and RuvB-like 1

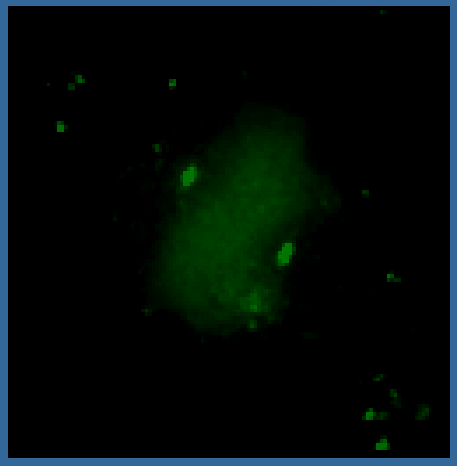
α/β Tubulin



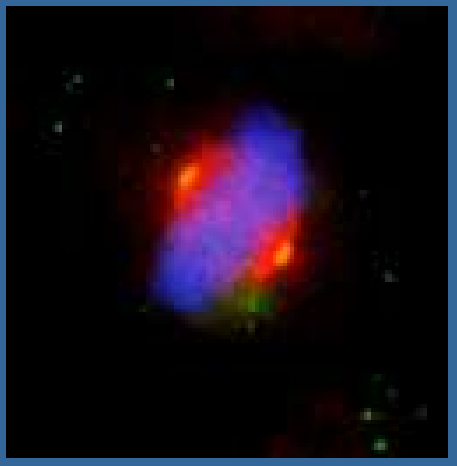
DAPI (DNA)



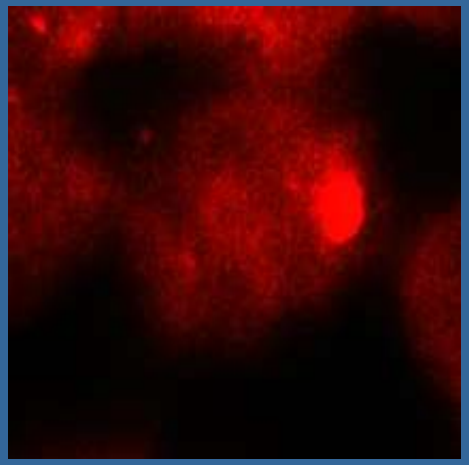
ILK



Merge



RuvB-like 1



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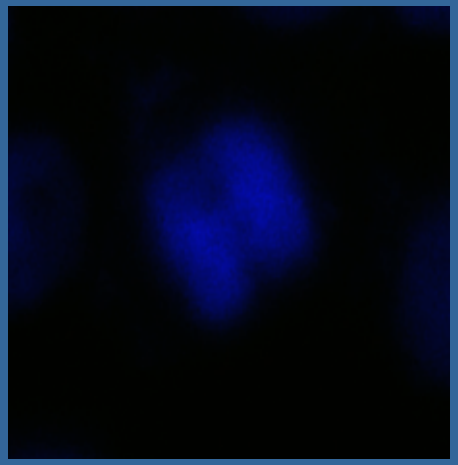
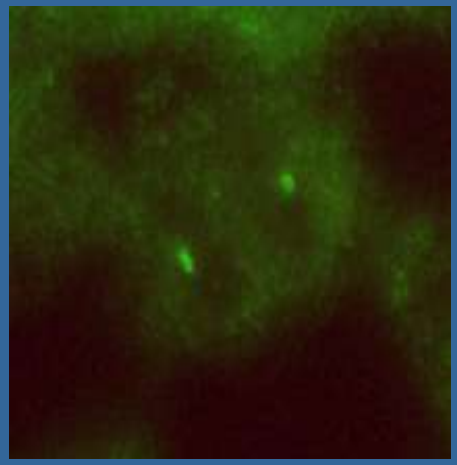


ILK



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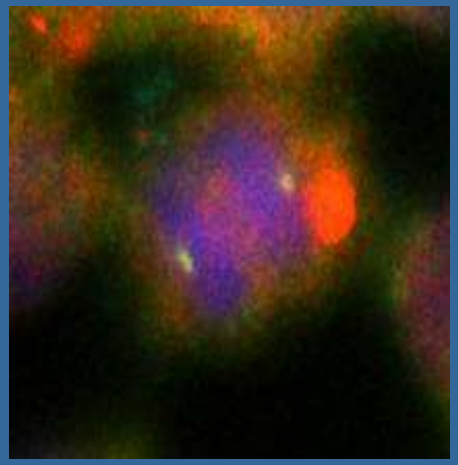


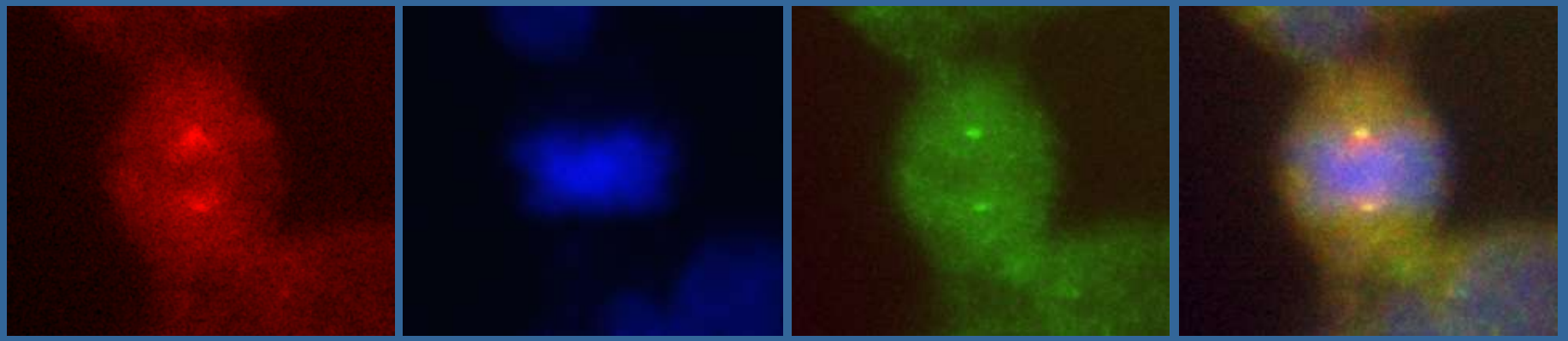
Colocalization between ILK and ch-TOG and ILK and Rictor

Ch-TOG

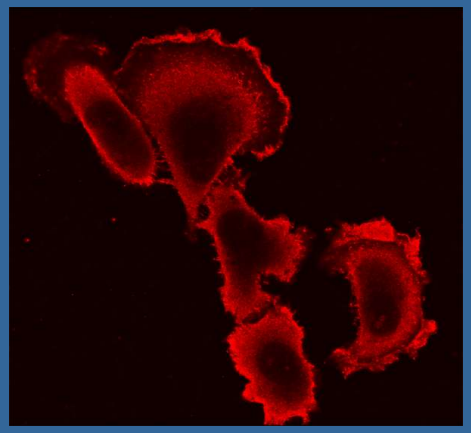
DAPI (DNA)

ILK

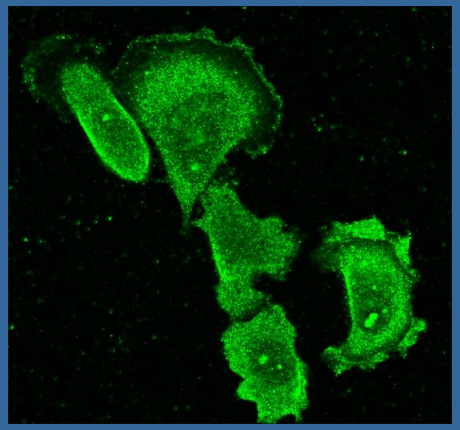
Merge



Rictor



ILK



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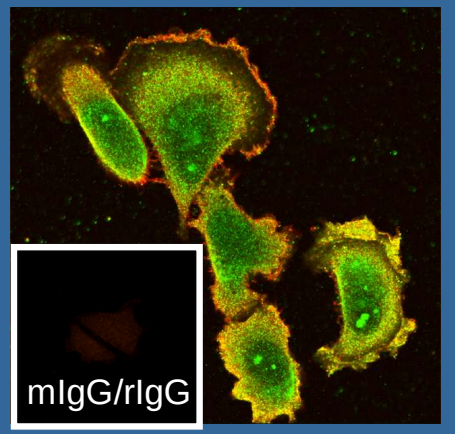


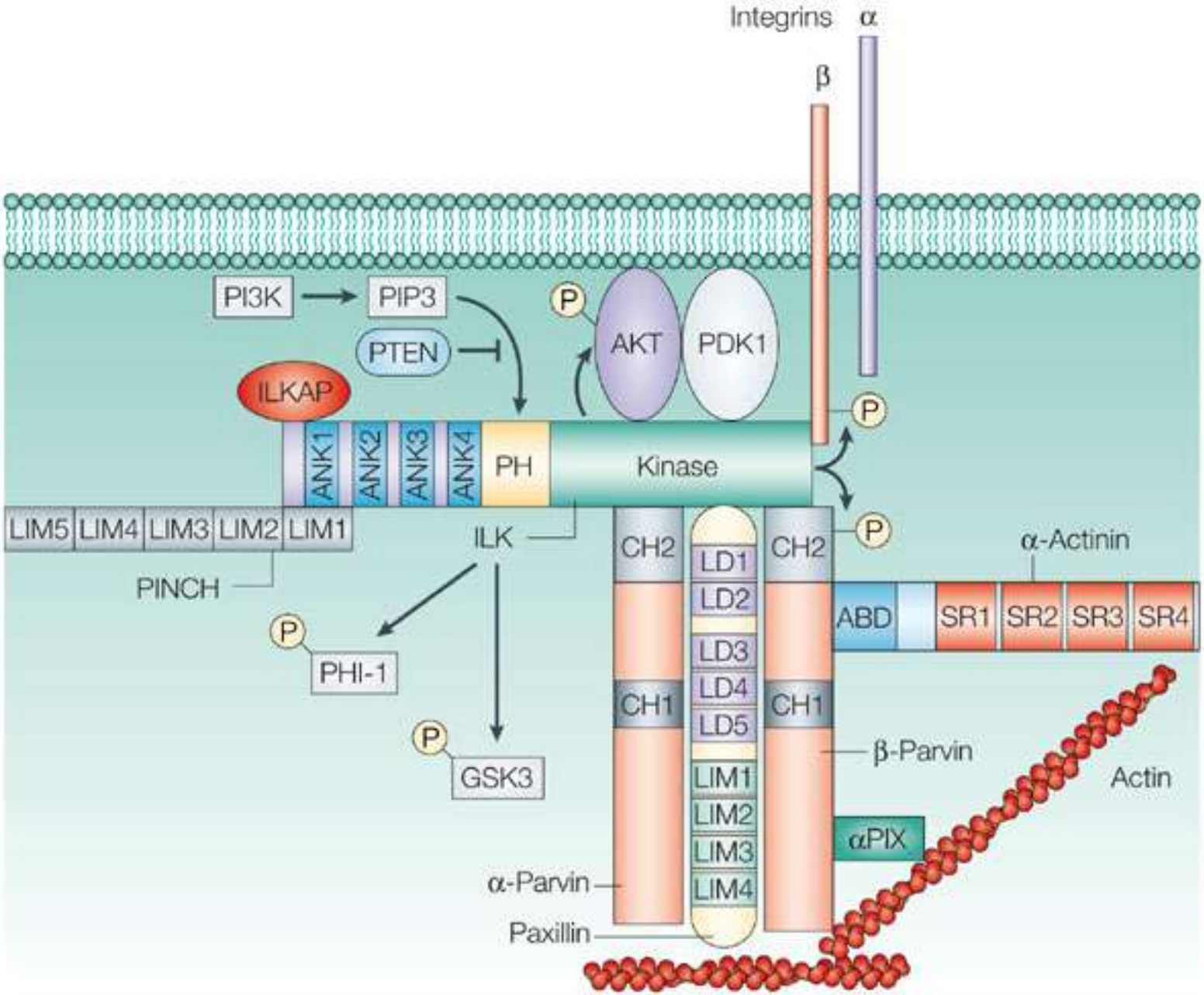
Goal 2:

Understanding the role of ILK in the formation of protein complexes

Questions:

- Do we have different ILK-containing protein complexes or just one?
- What is the role of ILK in these complexes – Does it serve as an adaptor or/and a kinase?
- How ILK could regulate the spindle integrity?

The IPP (ILK-PINCH-parvin) complex in FA



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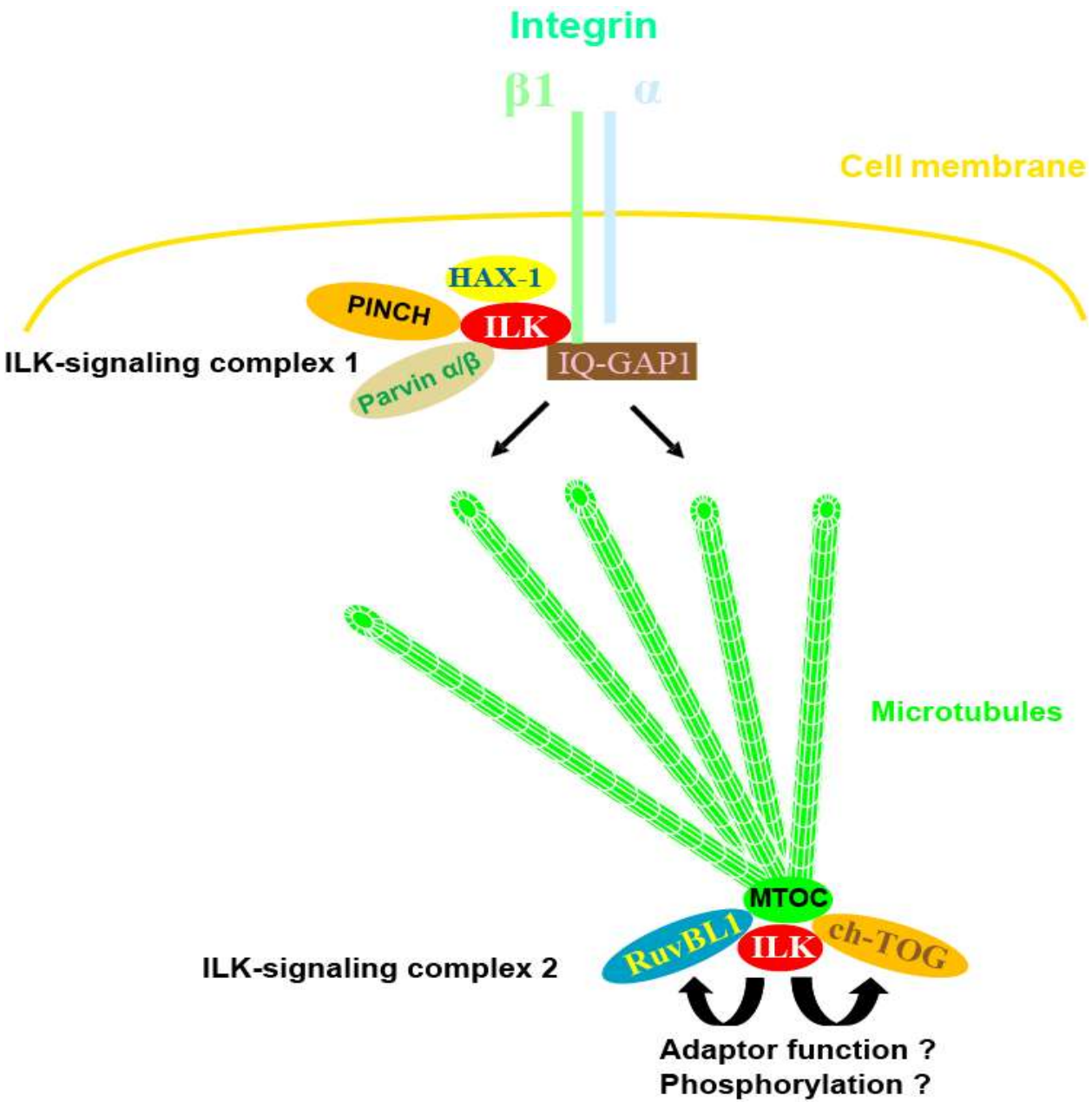
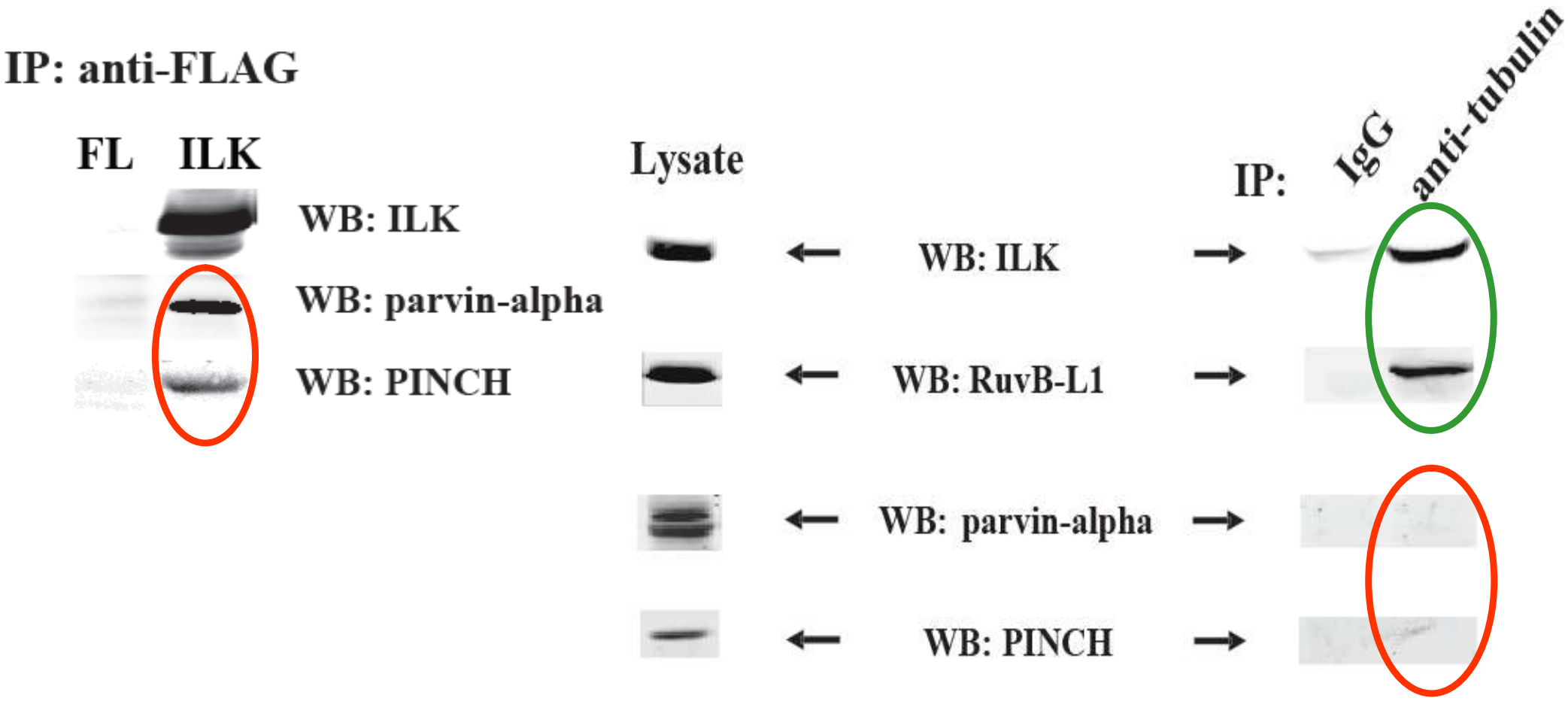
Does ILK exist in distinct protein complexes or just one big protein complex?



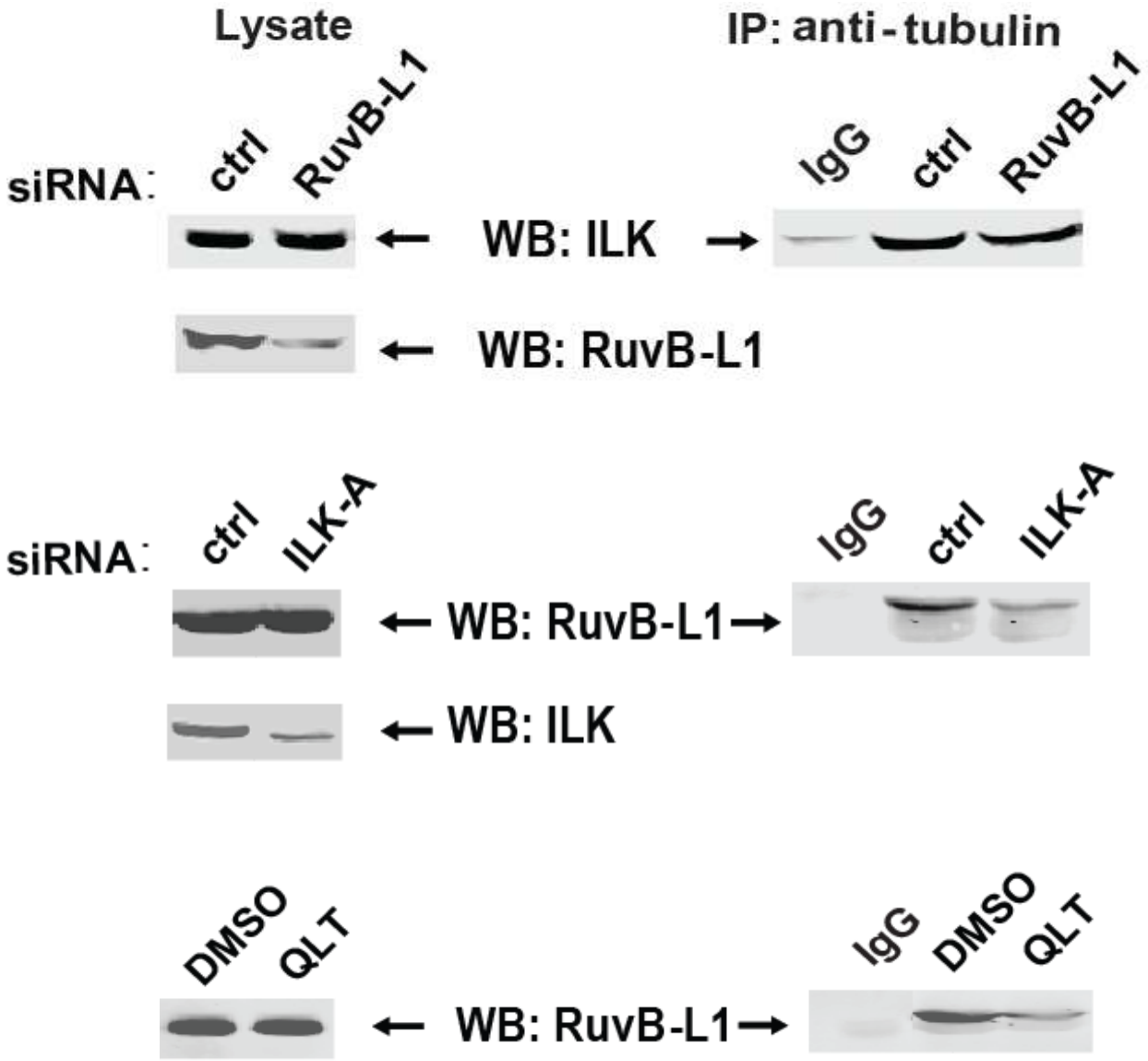
ILK forms two distinct protein complexes

ILK-signaling complex 1

ILK-signaling complex 2



ILK is important for RuvBL1 binding to tubulin



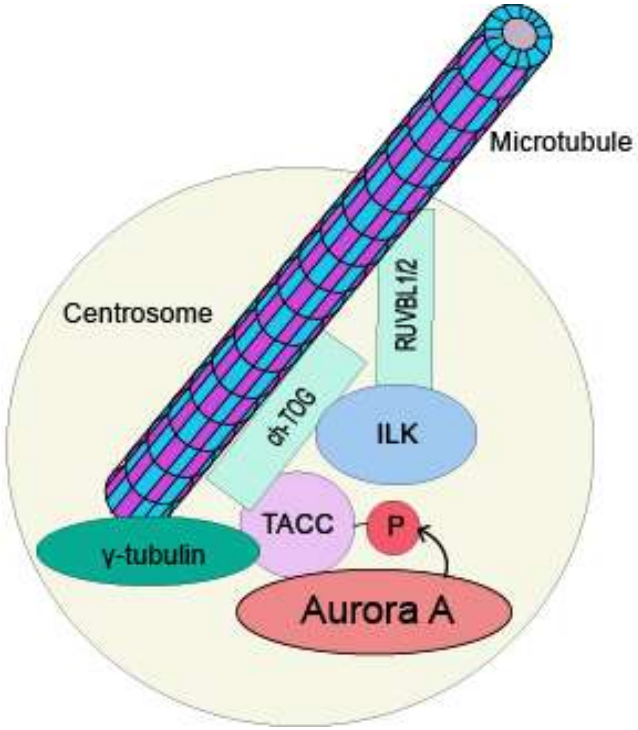
Question:

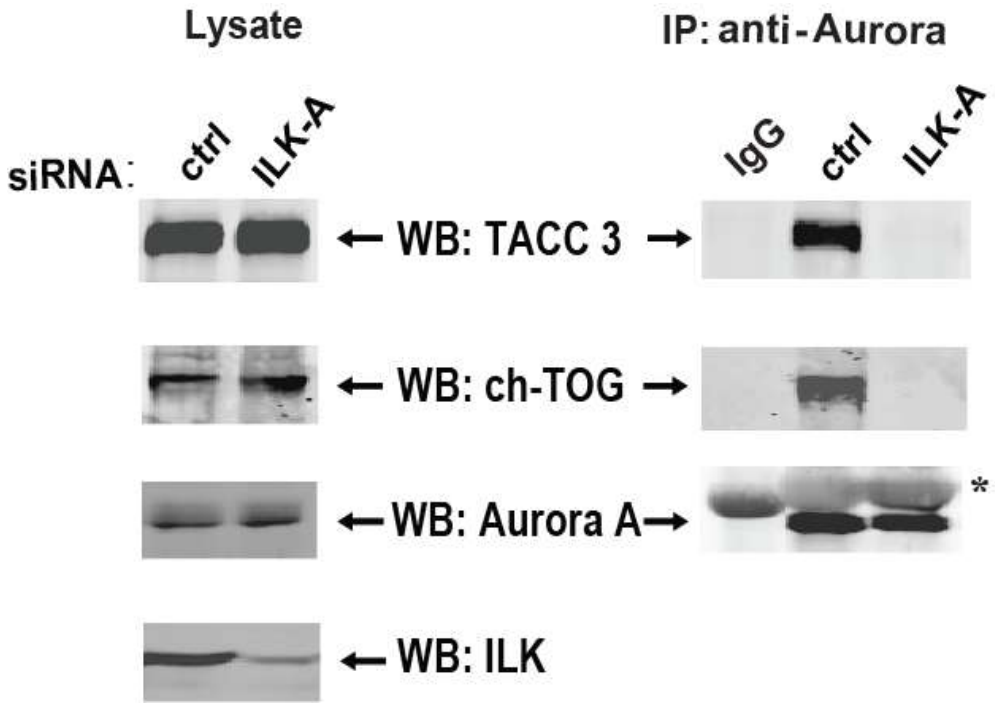
What are the targets of ILK in the mitotic spindle?

Experimental design:

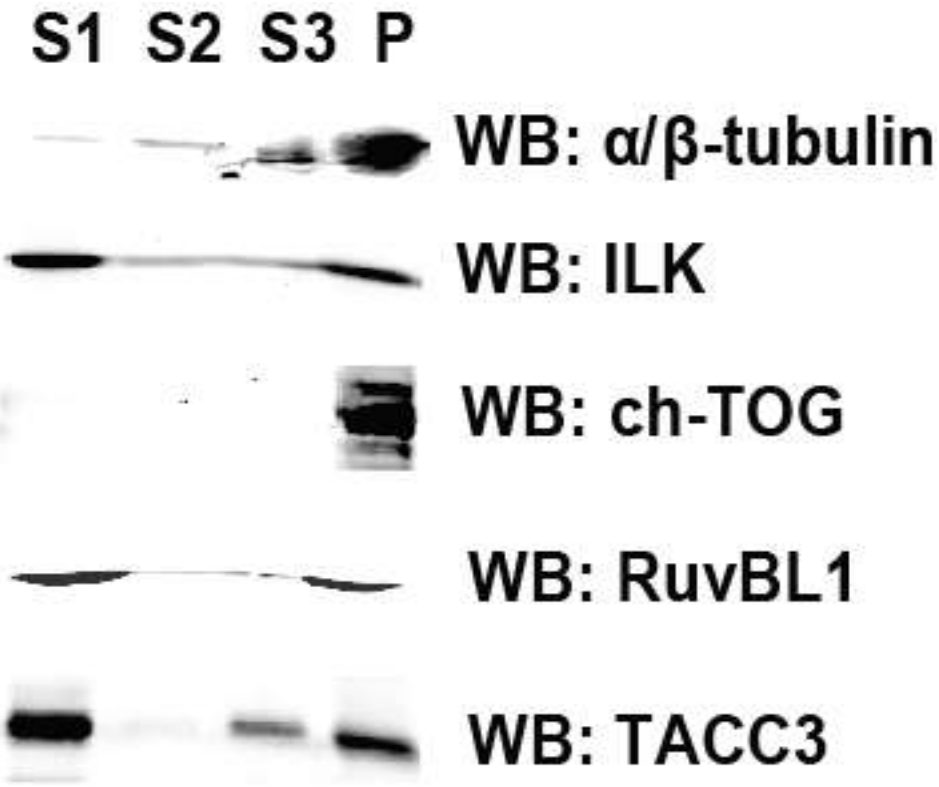
- Looking at known spindle components such as Aurora A, TACC3, ch-TOG, etc.
- Purification and MS analysis of mitotic spindles
- Phosphopeptide-enrichment of mitotic spindles
- Phosphopeptide analysis of spindles from ILK siRNA and QLT-treated cells

ILK is important for the integrity of the AuroraA-TACC3-ch-TOG complex





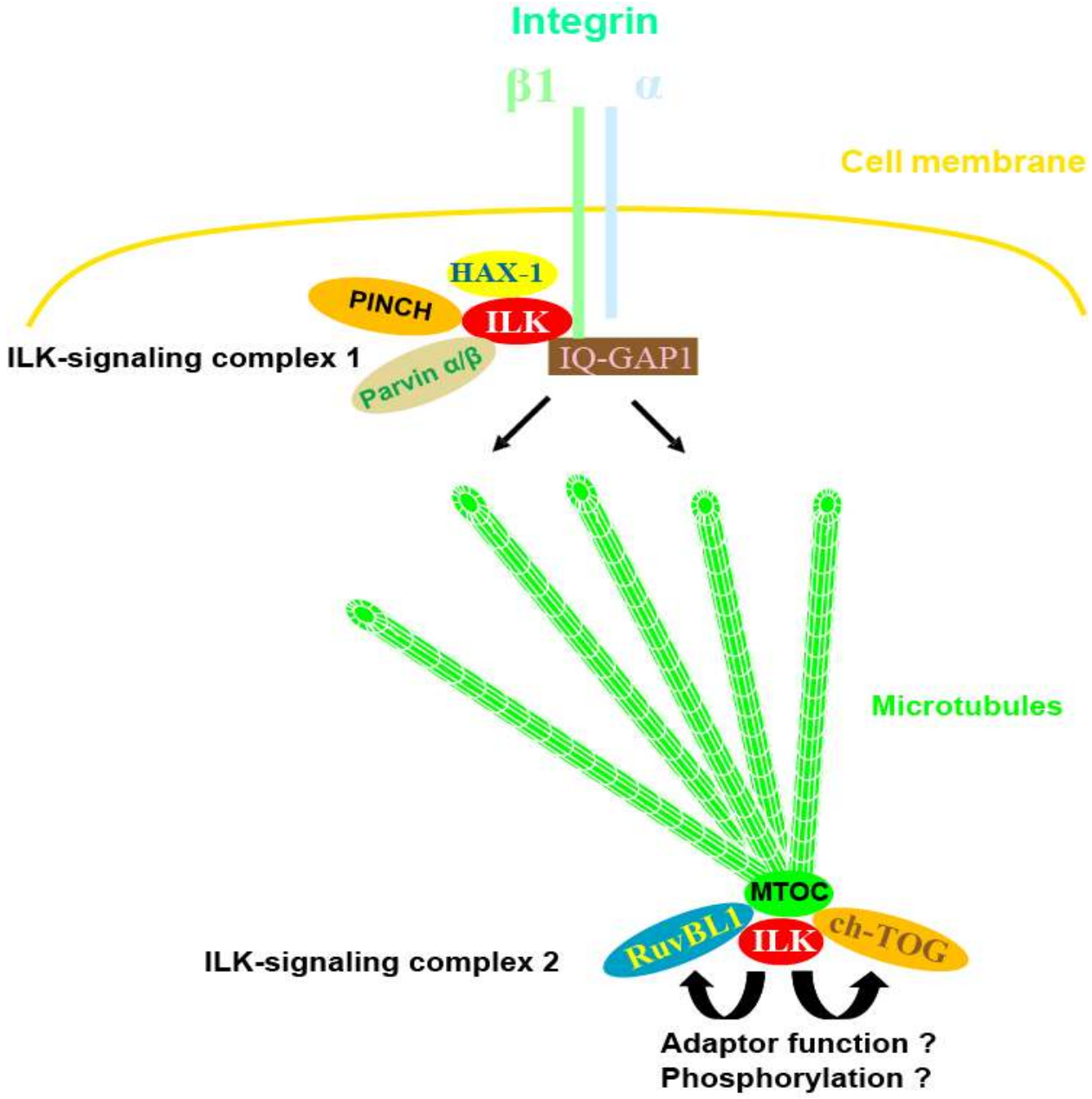
Purification and MS analysis of mitotic spindles



- The MS analysis identifies many spindle/centrosome proteins including tubulins, AuroraB, Polo-like kinase, Cdks, several coil-coil proteins, but not TACC3, ch-TOG or ILK (shed by more abundant proteins) – better separation needed

- Phosphopeptide analysis of mitotic spindles (ILK siRNA or QLT-treated cells)

Formation of distinct ILK signaling complexes



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