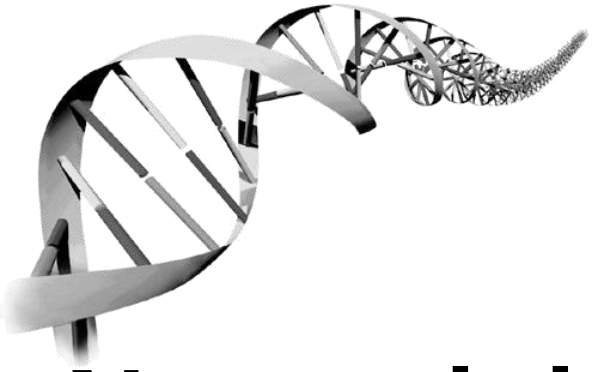


Network-based analysis of mouse testicular phosphoproteome

Yu Xue

Huazhong University of Science and Technology

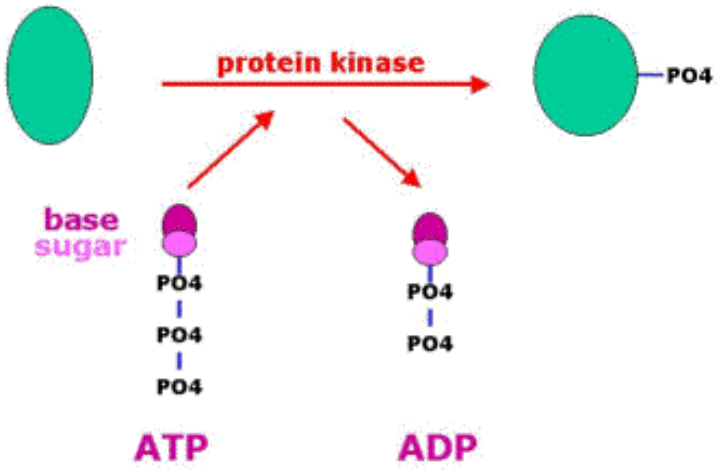
Human Proteome Project

- Little known: 2/3 of the 20,300 protein-coding human genes
- ~6000 (30%) genes lack the protein information
- HPP:
  - Mass spectrometry, antibody, and bioinformatics
  - Quantitative, sequencing, and PTMs
  - In heath and disease
- Chromosome-Centric Human Proteome Project:
  - Complete proteome
  - Genome annotation
- 2014, CNHPP: encyclopedia

Legrain et al., MCP, 2011, 10, M111.009993
Kim et al., Nature, 2014, 509:575-81

Phosphoproteomics

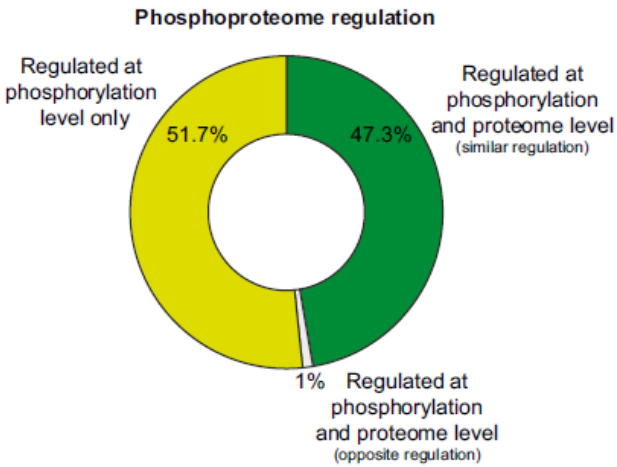
- **Large-scale identification of "*in vivo*" phosphorylation/ PTM sites**
- **Data integration & resources**
  - **dbPTM 3.0: 208,521 PTM sites**
  - **SysPTM 2.0: 471,109 PTM sites, 53,235 proteins**
- **Prediction of regulatory kinases**
  - **PKIS: composition of monomer spectrum (CMS)**
  - **PSEA: Phosphorylation Set Enrichment Analysis**



Li et al., Database, 2014, 2014:bau025
Lu et al., NAR, 2013, 41:D295-305
Zou et al., BMC Bioinformatics, 2013, 14:247
Suo et al., Sci Rep., 2014, 4:4524

What can we learn?

- Poor protein-PTM correlation
- vertebrate-specific functional modules (VFMs) are more conserved than basic functional modules (BFMs)
- Phosphorylation Sites has strong subcellular specificity
- Non-functional p-sites: 65%



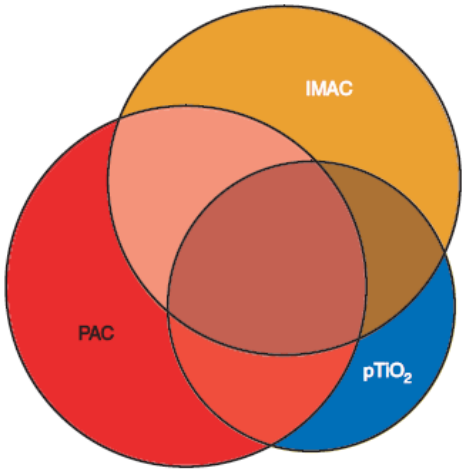
Cell Reports
Resource

In Vivo SILAC-Based Proteomics Reveals Phosphoproteome Changes during Mouse Skin Carcinogenesis

Wang et al., MBE, 2011, 28:1131-40
Chen et al. Bioinformatics. 2014, pii: btu598
Landry et al., Trends Genet., 2009, 25:193-7

Functional PTM prediction

- How many PTM events are functional?
  - Molecular mechanisms
  - Biological forecasting
  - Drug targets
- A big problem:
  - The reproducibility is low
- A network approach: reverse engineering
  - Site-specific kinase-substrate network
  - NetworKIN & iGPS: motif + PPI
  - Tissue-specific: protein expression data

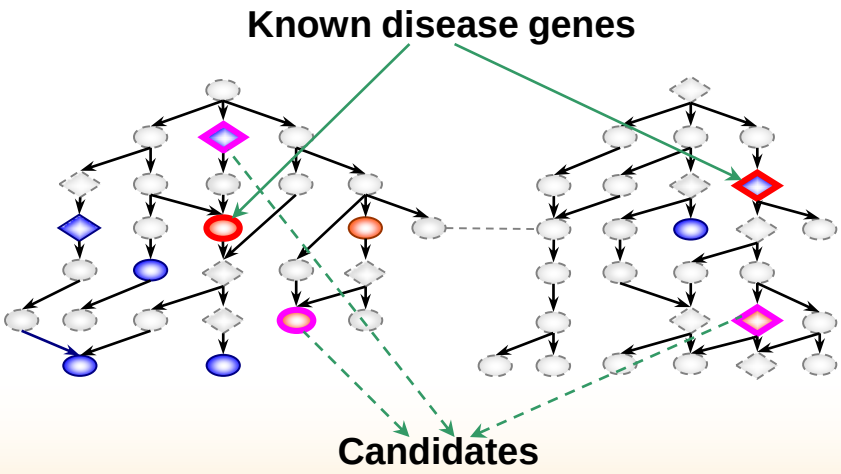


Bodenmiller et al., Nat Methods, 2007, 4:231-7
Linding et al. (2007) Cell, 129, 1415-1426
Song et al., MCP, 2012, 11: 1070-1083
Wang et al., ISB2013, 129-133

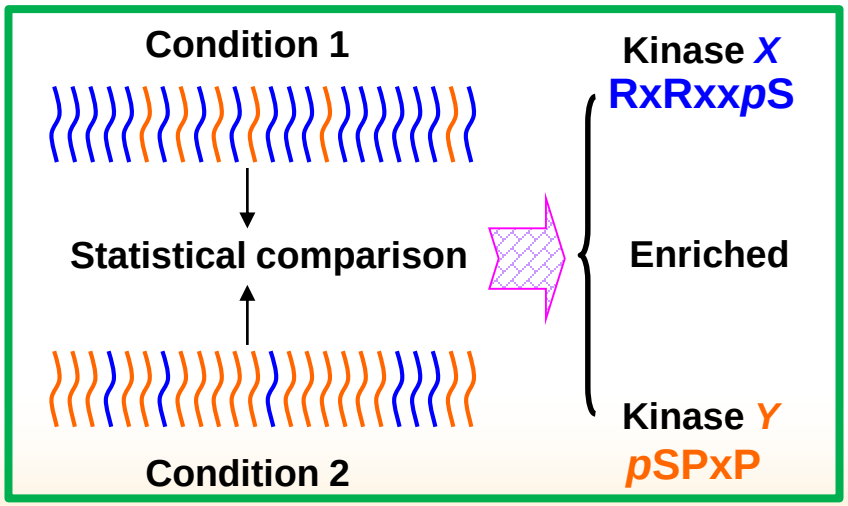
Phosphoproteomics-based network medicine

- Kinases: targets of ~75% of complex diseases
- Hypothesis: more sites, higher activity
  - **iKING**: integrative KINase Gauge
  - KSEA: Kinase-substrate enrichment analysis

Birds of a feather flock together



More sites, higher activity



Liu, et al., FEBS J, 2013, 280:5696-704
Song et al., MCP, 2012, 11: 1070-1083
Casado, et al. Sci Signal, 2013, 6, rs6

Semen quality

- Decreasing quality of semen in western countries
- Semen quality is poor in China
  - Associated: region, season & abstinence duration
  - No effect: Age, smoking, alcohol use & BMI

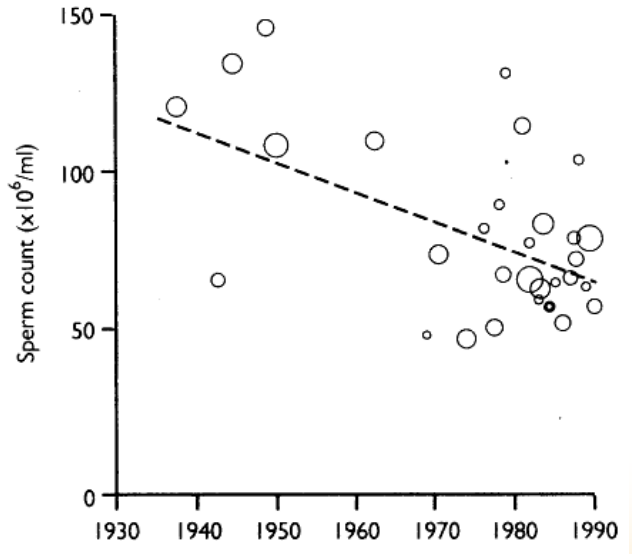


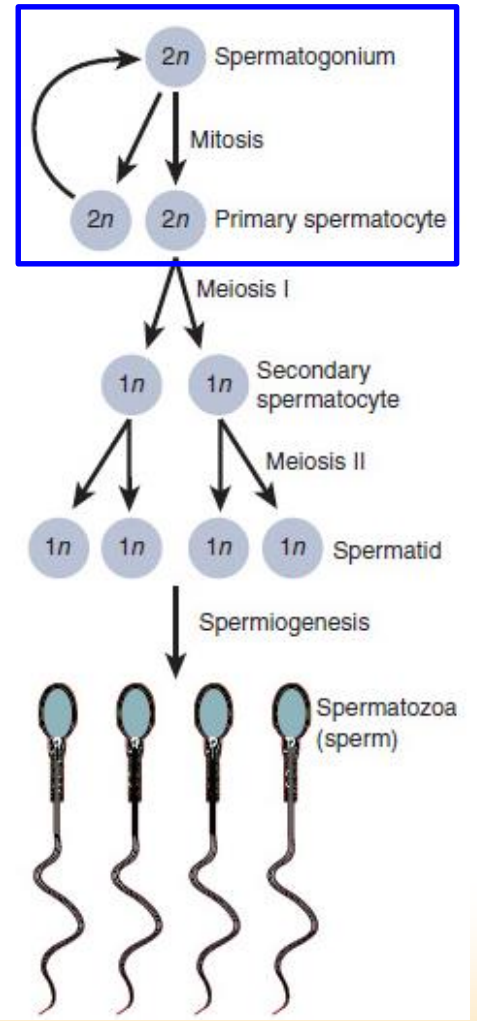
Table III. Summary of semen parameters

Semen parameters	*n*	Median	Percentage lower than WHO criteria[c]
Semen volume (ml)	1191	2.3	22.3
Sperm concentration (10^6/ml)	1191	65	4.8
Sperm count (10^6 per ejection)	1191	154	7.1
Rapid progressive motility (A%)[a]	985	19	81.9
Sperm progressive motility [(A + B)%][a]	985	46	60.7
Total motile spermatozoa [(A + B + C)%][a]	985	67	NA
Sperm viability (%)	1191	70	61.8
Normal morphology (%)[b]	1131	39	12.4

Carlsen et al., BMJ, 1992, 305:609-13
Gao et al., Hum Reprod., 2007, 22:477-84
Li et al., Hum Reprod., 2009, 24:459-69

Spermatogenesis

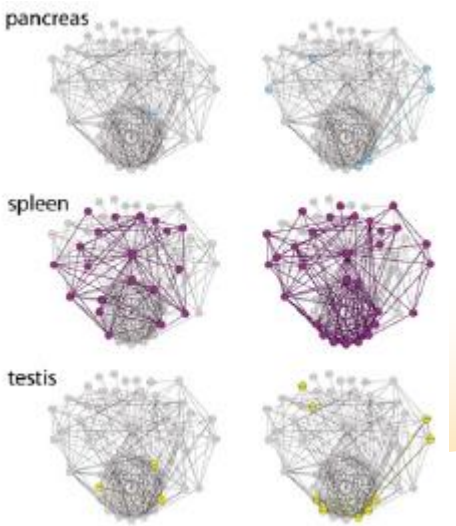
- **Sperm-generating process**
  - **Mitosis of spermatogonia (精原)**
  - **Meiosis of spermatocytes (精母)**
  - **Spermiogenesis of spermatids**
  - **~1,000 sperms per heart beat**
- **Phosphorylation regulated**
  - **MAPKs, CDC2, POLO-like kinases (PLKs)**



Li et al., Trends Mol Med., 2009, 15:159-68
Shi et al., Toxicol Lett., 2013, 221:91-101

Phosphoproteomics

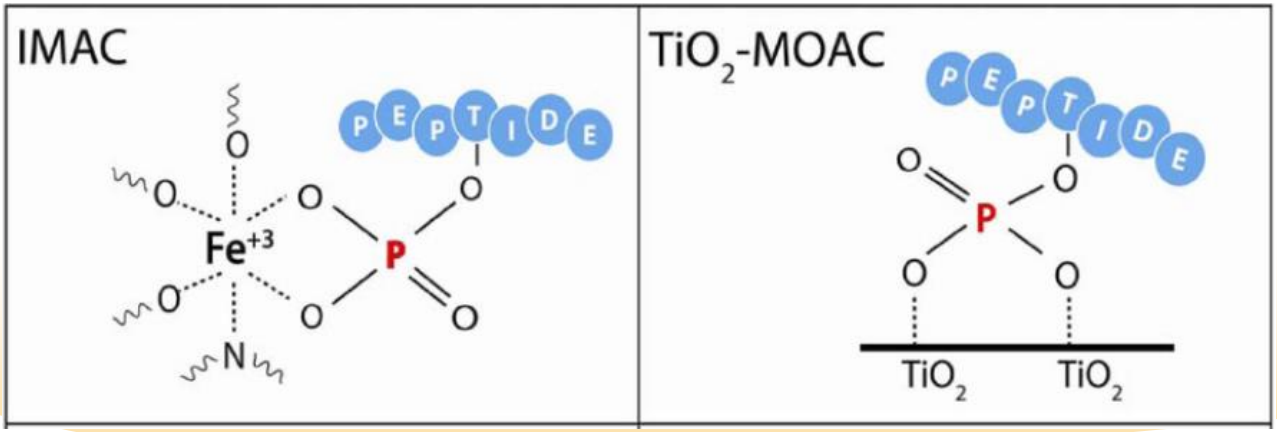
- **Swiss-Webster mice, Nine organs**
  - **brain, brown fat, heart, liver, lung, kidney, pancreas, spleen, & testis**
- **Phosphoproteomic identification**
  - **~36,000 p-sites, 6296 proteins**
  - **Different network topologies**
- **Limitations**
  - **3-week-old male**
  - **No sperms at all**



Huttlin et al., Cell, 2010, 143:1174-89

Our strategy

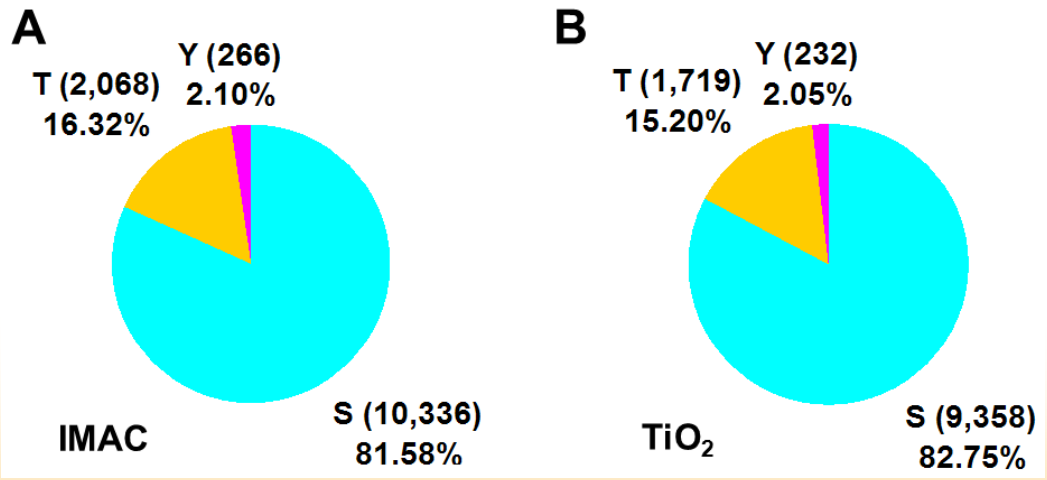
- **Adult C57BL/6 mice**
  - **8-week-old male**
  - **six testes/replicate**
- **Phosphopeptide enrichment**
  - **IMAC: doubly replicated**
  - **TiO_2: triply replicated**



Qi et al., Mol Cell Proteomics, pii: mcp.M114.039073

Testicular phosphoproteome

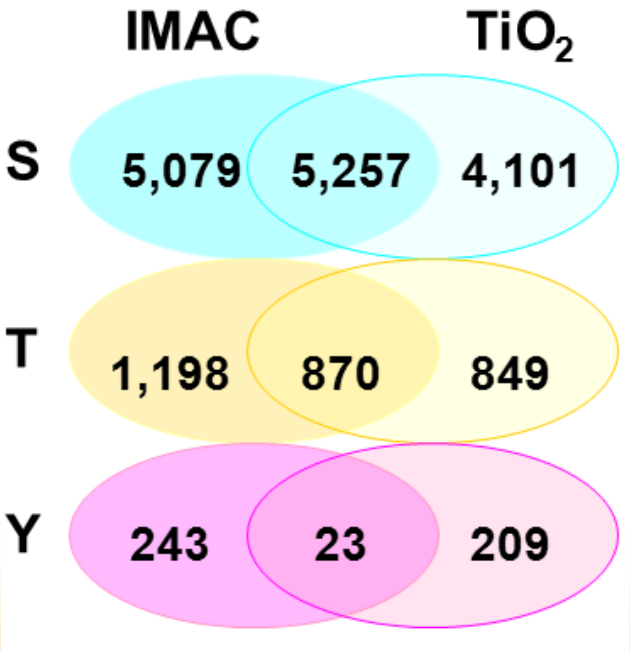
- **Total: 17,829 p-sites in 3,955 proteins**
  - **IMAC: 12,670 sites**
  - **TiO_2: 11,309 sites**
- **The residue distribution is similar**

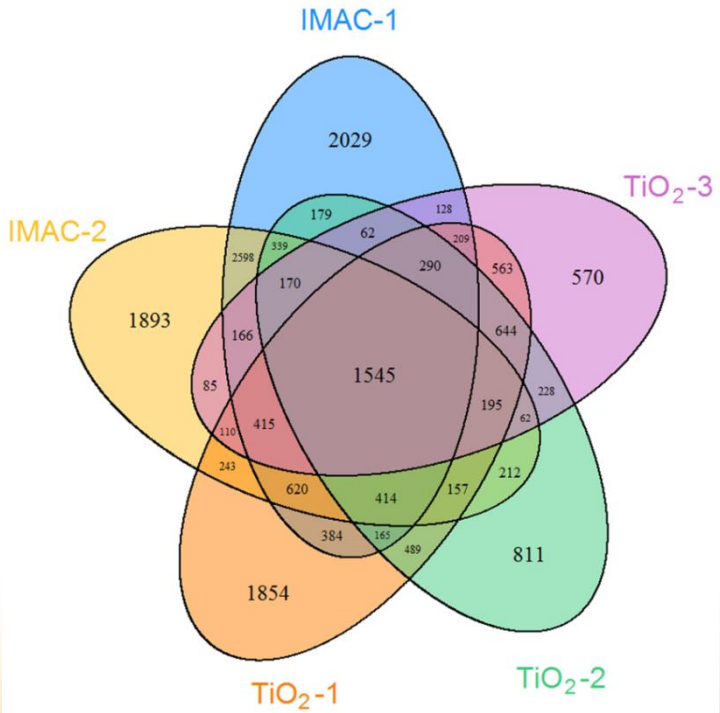


Qi et al., Mol Cell Proteomics, pii: mcp.M114.039073

IMAC & TiO_2

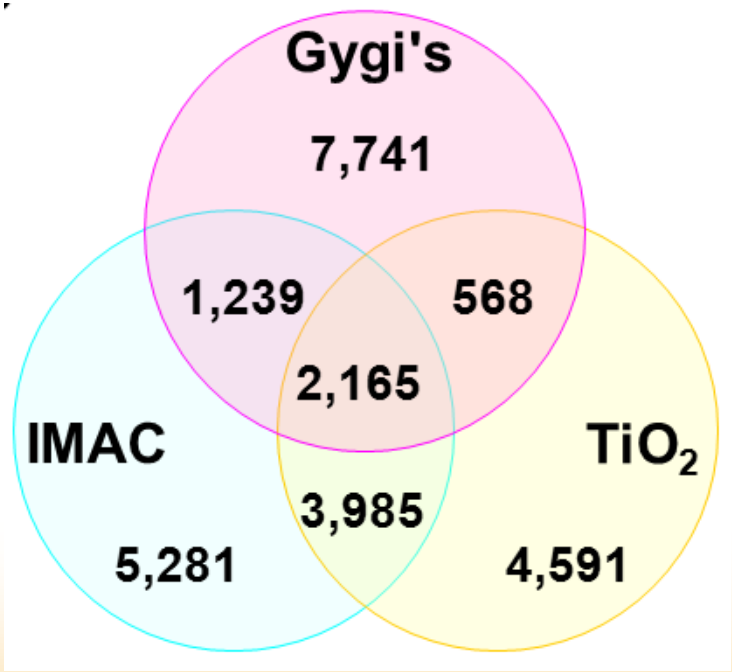
- **The data overlapping is limited**
  - **IMAC: ~48% identified by TiO_2**
  - **8.7% covered in all replicates**



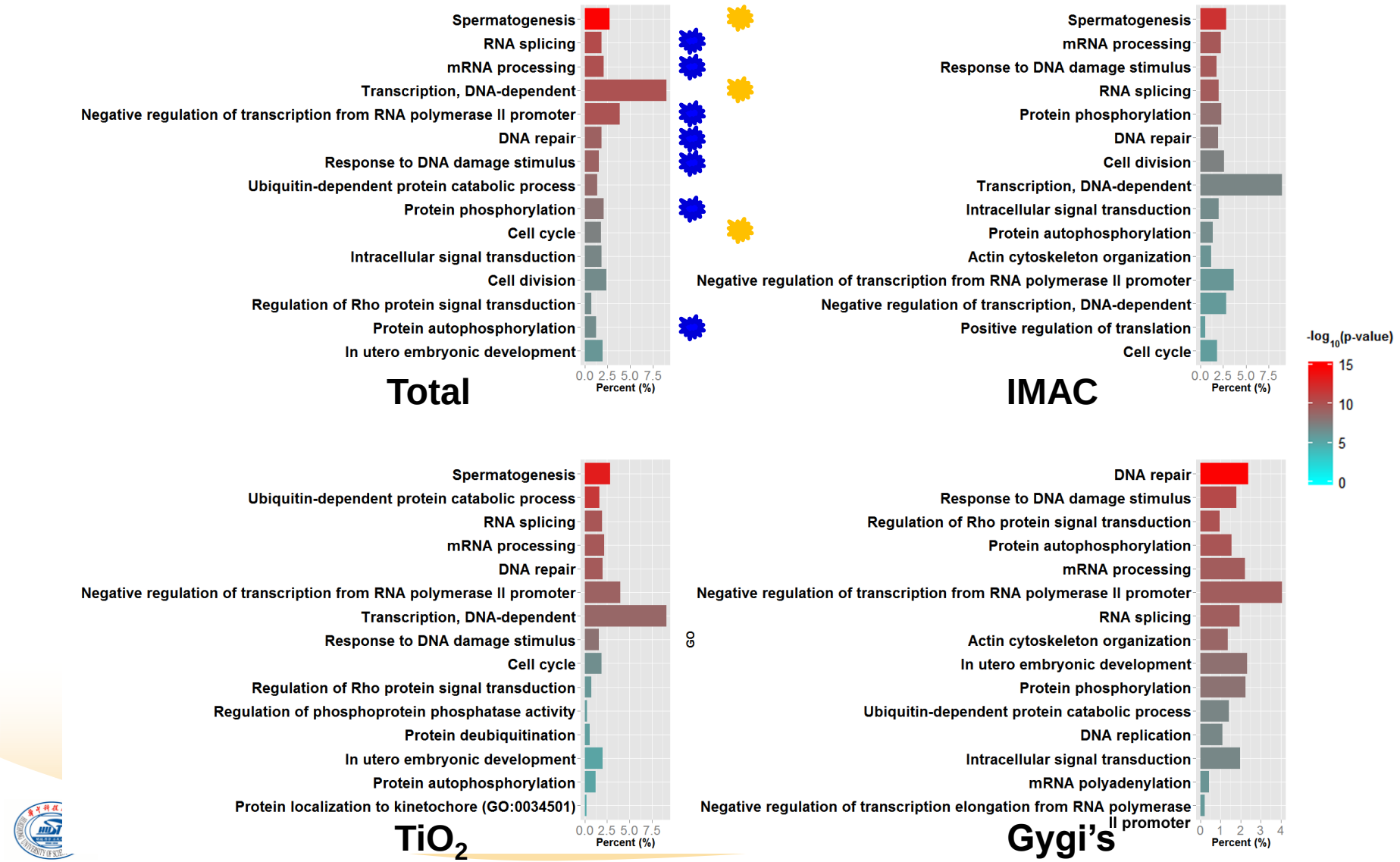


Comparison

- **Gygi's: 11,713 p-sites in 3,087 proteins**
  - **~27% covered by our data set**

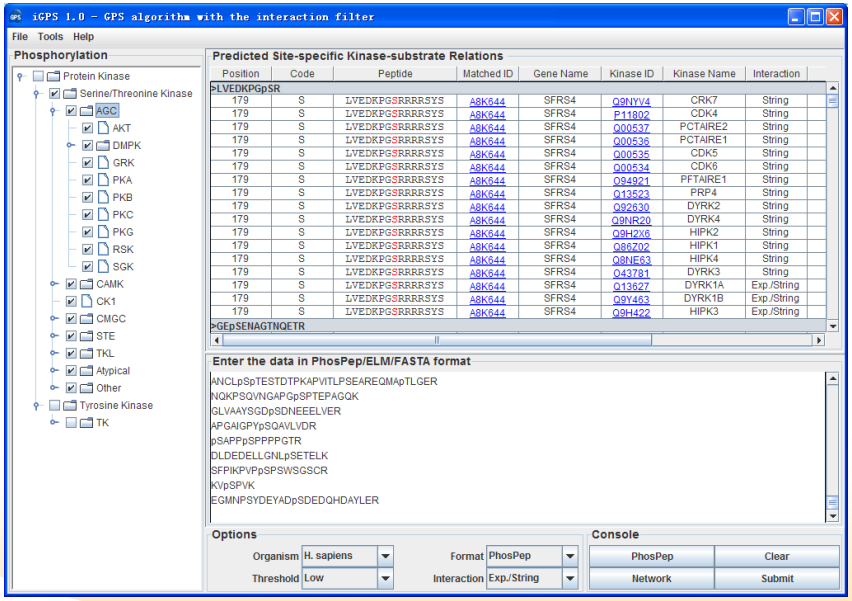


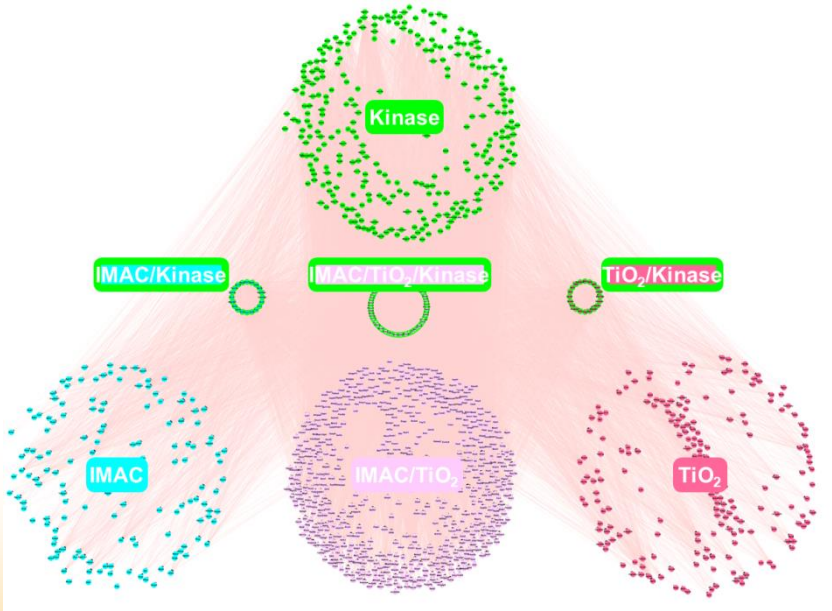
GO analysis: similar!



Network construction

- **iGPS: '*in vivo*' GPS**
  - http://igps.biocuckoo.org
  - **site-specific kinase-substrate relations (ssKSRs)**
- **kinase-substrate phosphorylation networks (KSPN)**
  - **17,065 edges, 402 kinases, 1,066 substrates**

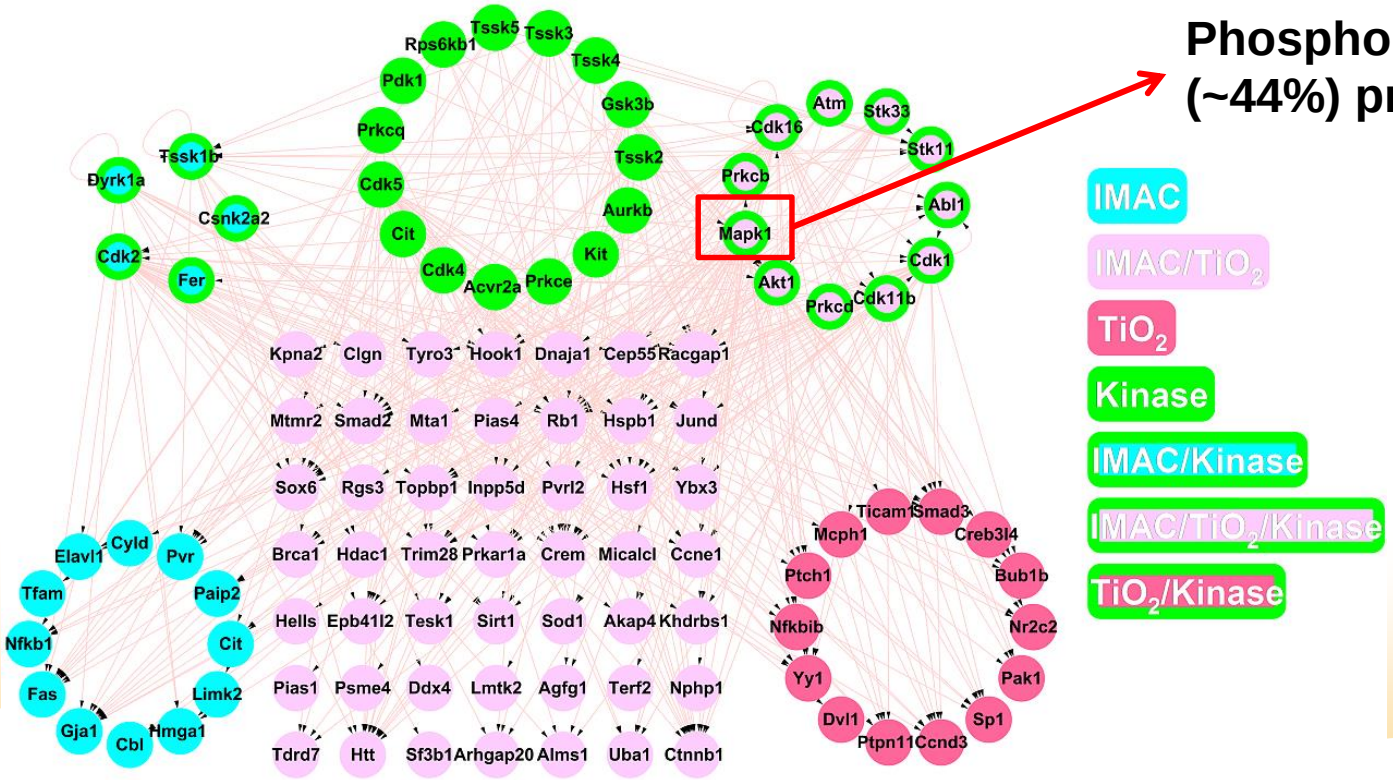




Song et al., MCP, 2012, 11, 1070-1083

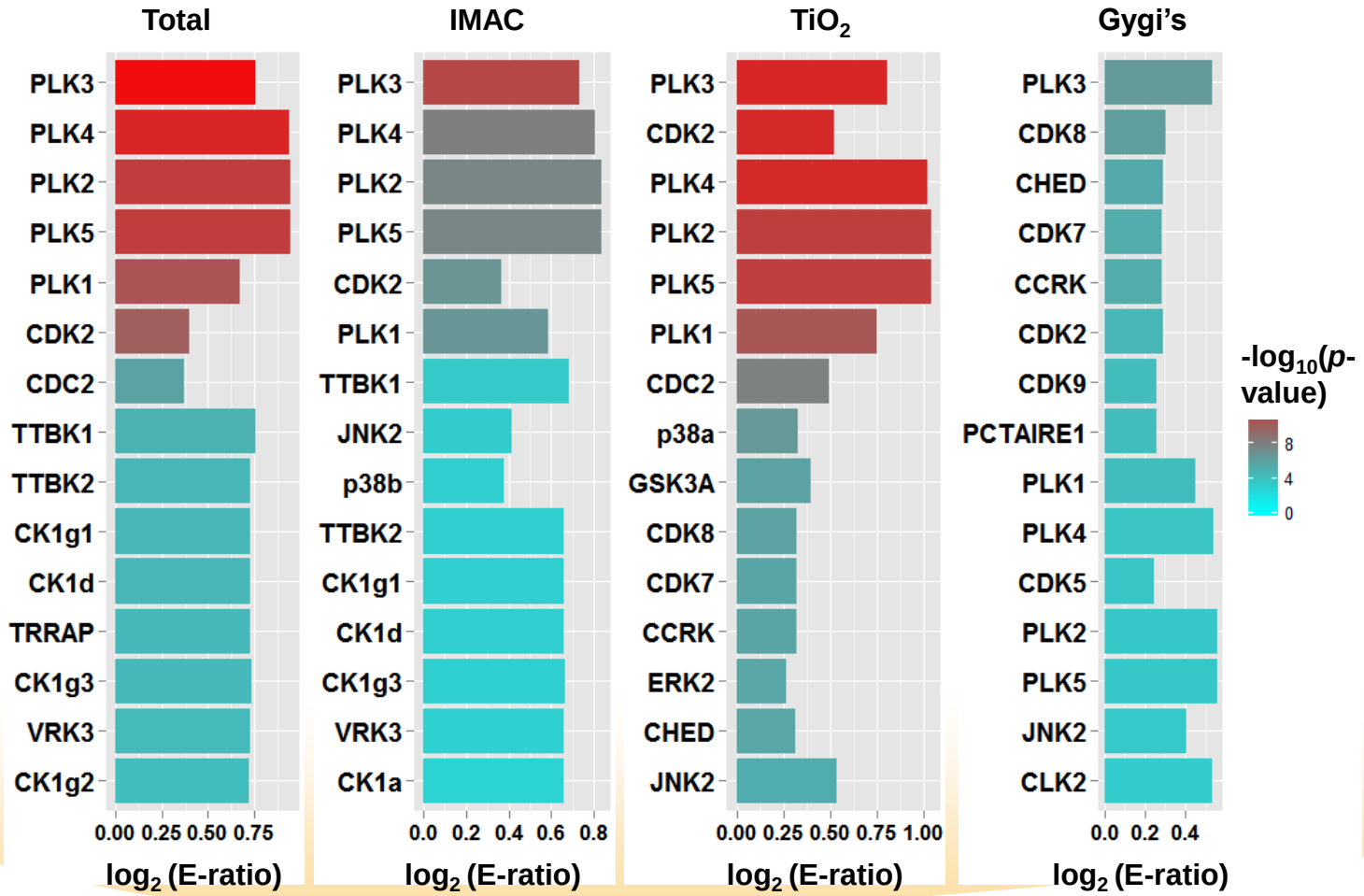
Spermatogenesis-related KSPN

- **Spermatogenesis-related proteins**
  - **QuickGO: spermatogenesis (GO:0007283)**
  - **Spermatogenesis*Online* database**
- **Sub-KSPN: 106 proteins, 371 edges**



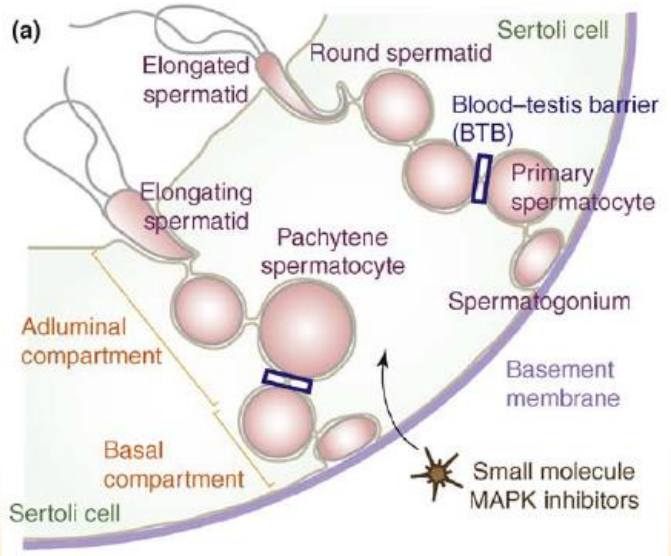
Kinases with higher activities

- Background: ~36,000 p-sites in 9 organs
- iKING: top15 kinases with highest activities



Kinase with higher activity

- **MAPKs**
  - **ectoplasmic specialization dynamics**
  - ***p*-value < 0.05: JNK2, ERK2 and p38s**
- **CDK2: chromosome pairing in meiosis**
- **CDC2: sperm activity and testicular function**

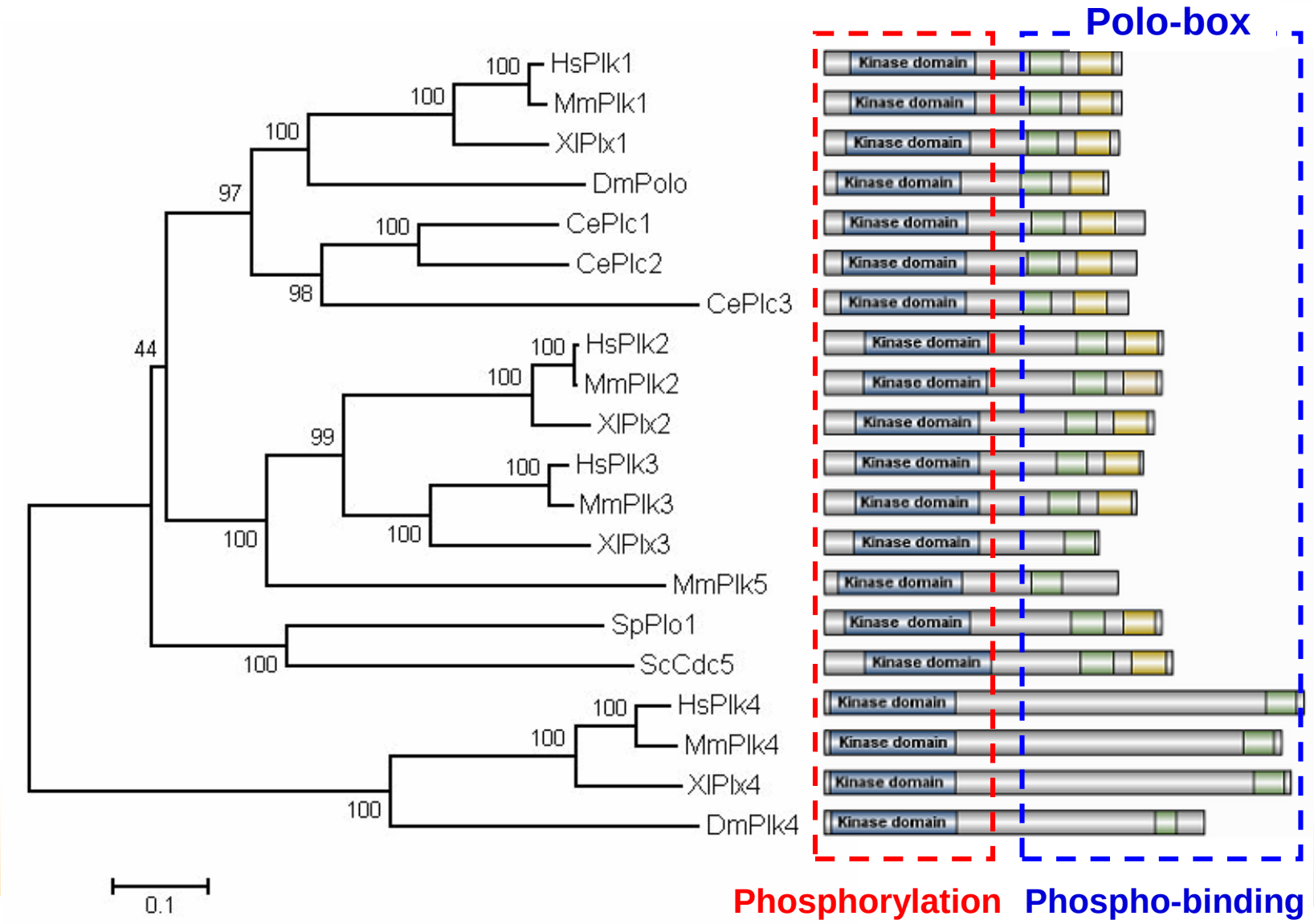


Li et al., Trends Mol Med., 2009, 15:159-68

Viera et al., J Cell Sci., 2009, 122:2149-59

Shi et al., Toxicol Lett., 2013, 221:91-101

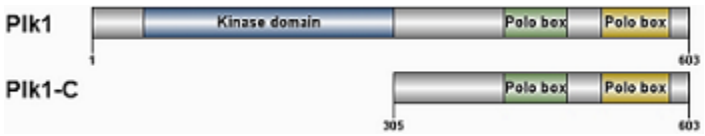
Polo-like kinases (Plks)



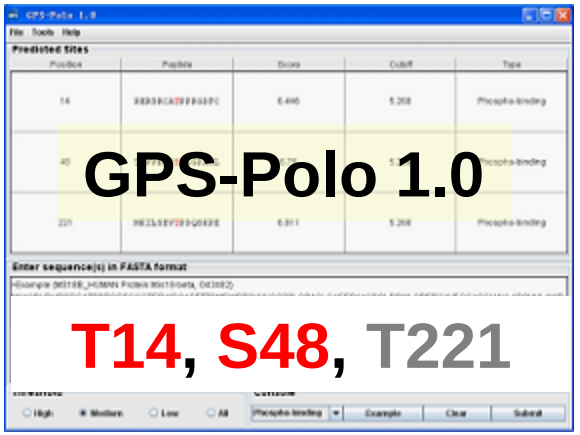
Liu, et al., Brief Bioinform, 2013, 14:344-60

Plk1-PBD interact with Phos-Mis18B

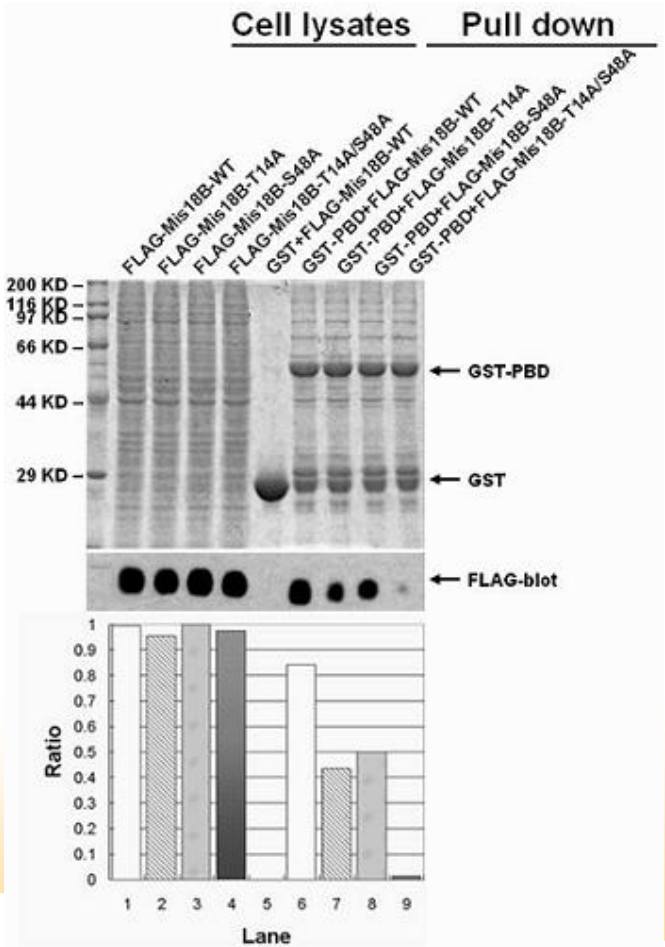
A.



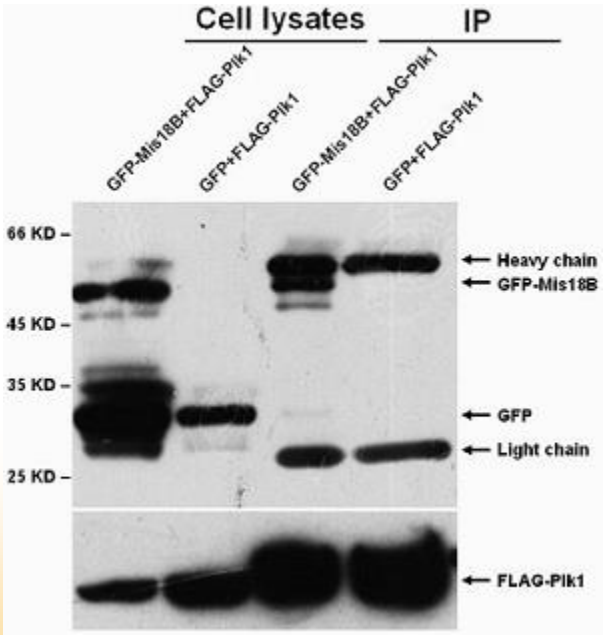
B.



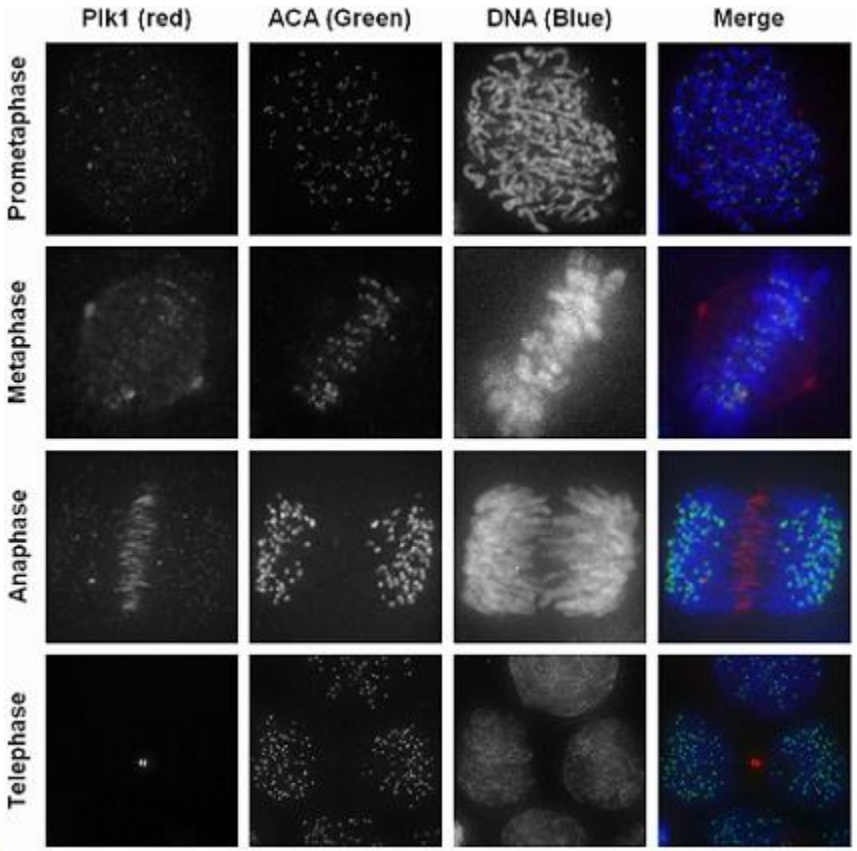
C.

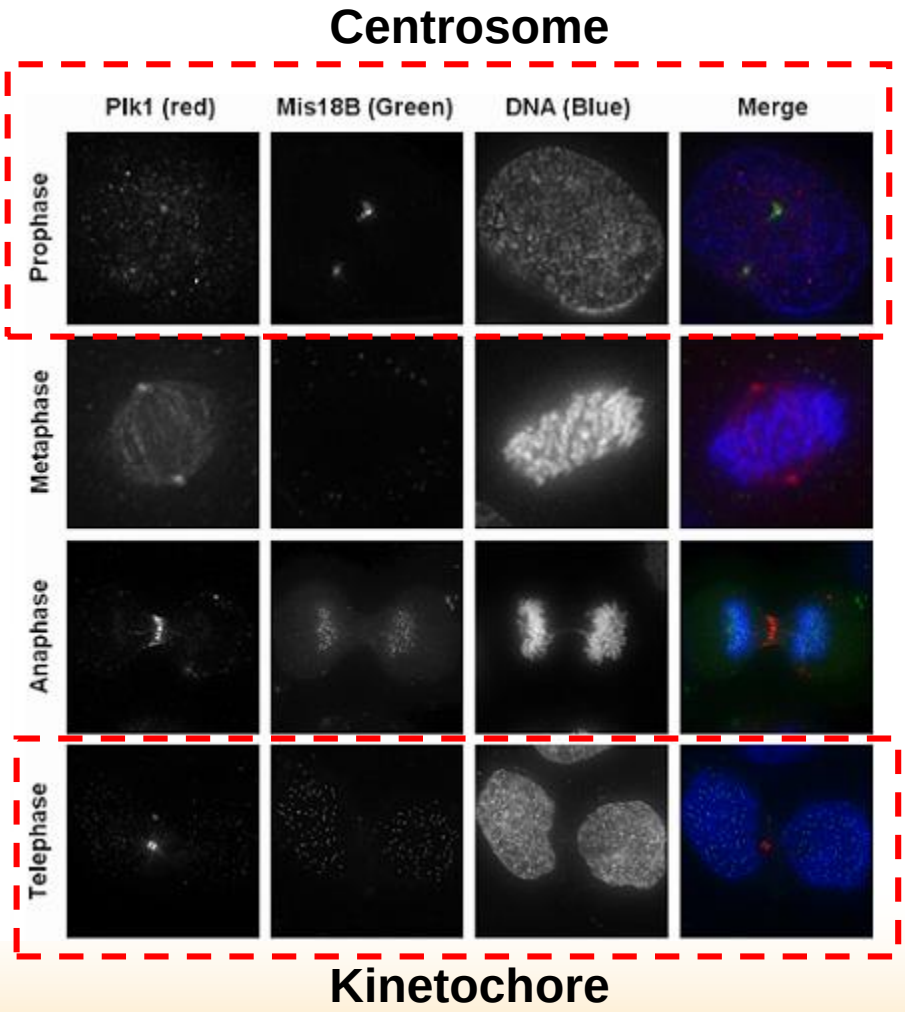


D.



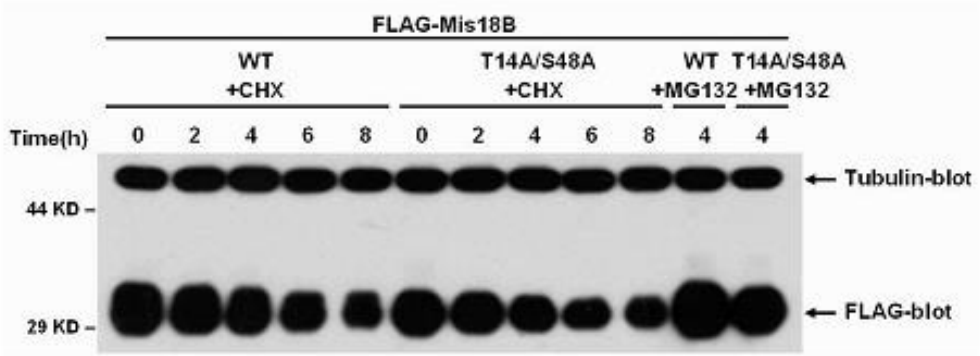
Plk1 co-localizes with Mis18B

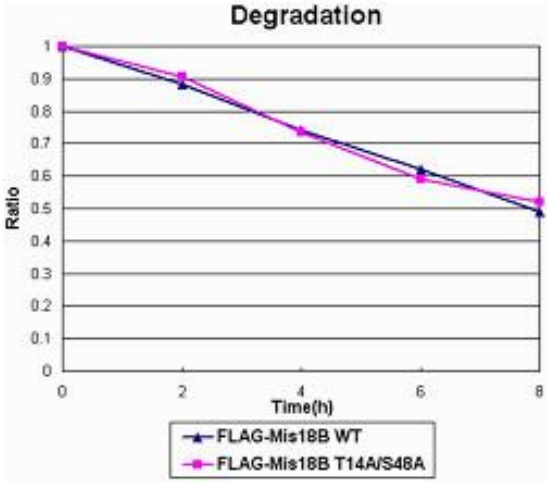




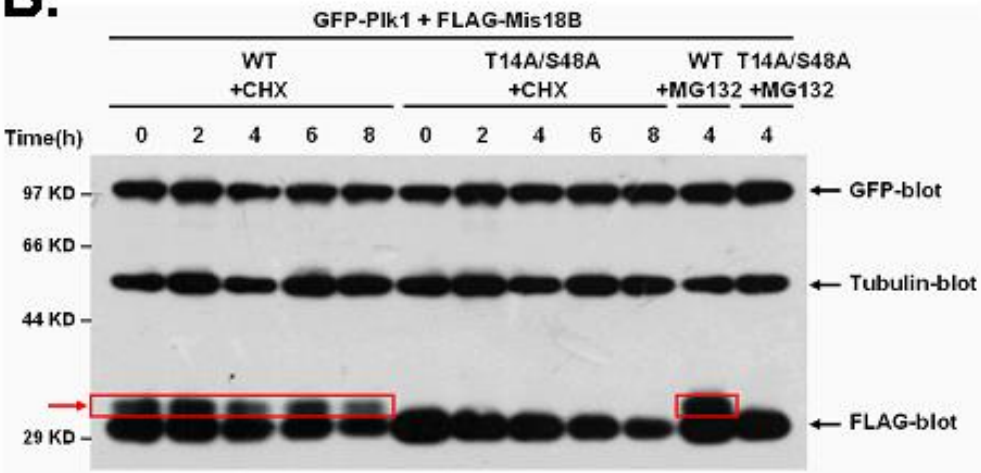
Plk1 phospho-binding regulates Mis18B stability

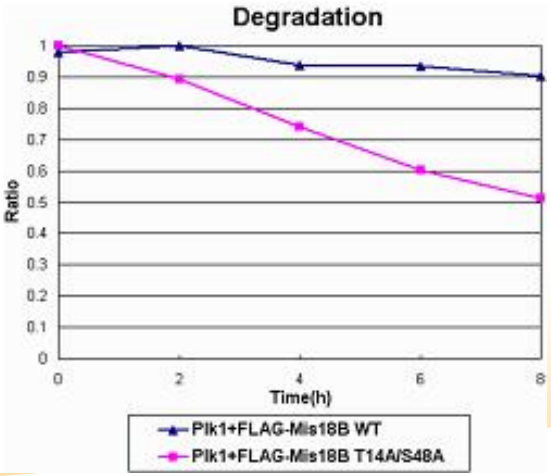
A.





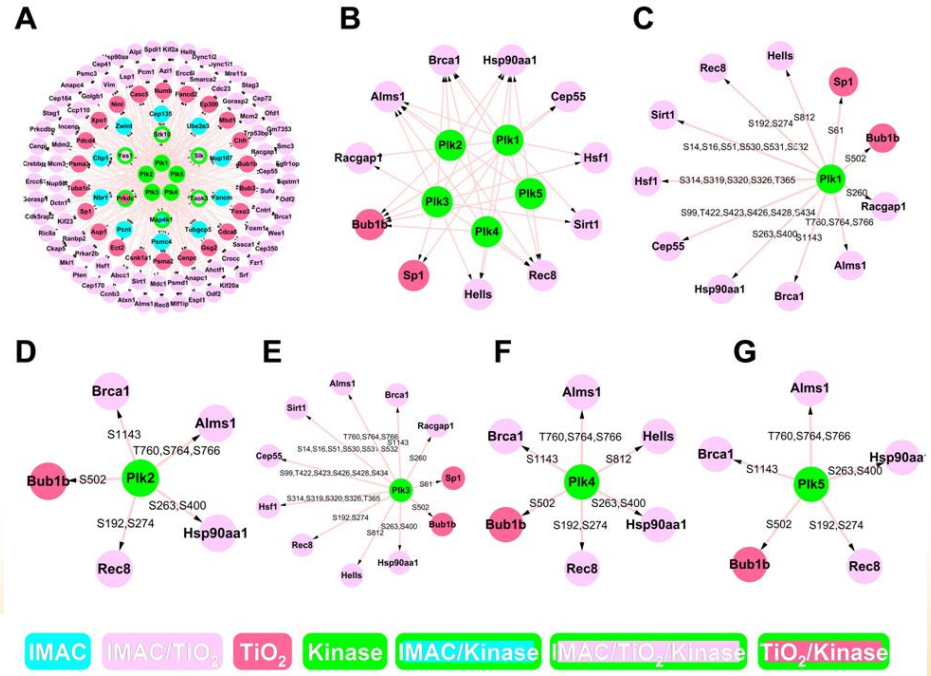
B.





A potential role in testis?

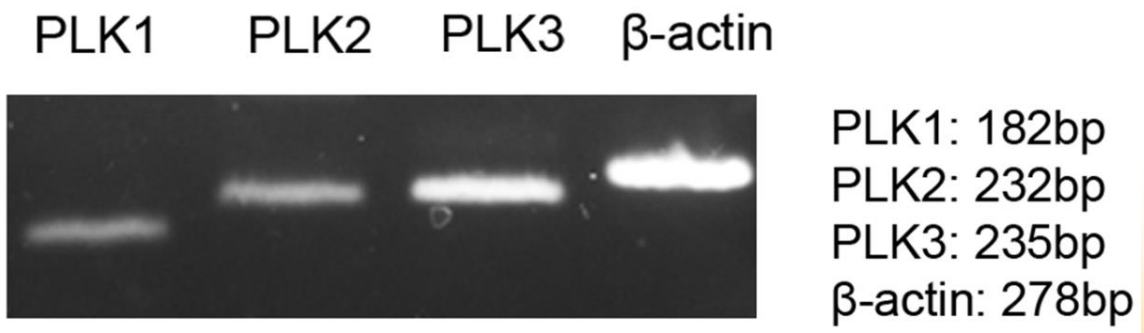
- **Plks: Not annotated in Spermatogenesis*Online***
- **Plk sub-KSPN: 122 proteins, 499 edges**
  - **Substrates: many spermatogenesis-related proteins**
  - **Phenotypes: cell morphology related**



Phenotype ID	Description	*p*-value
MP:0003111	Abnormal cell nucleus morphology	2.26E-10
MP:0003077	Abnormal cell cycle	5.71E-08
MP:0004046	Abnormal mitosis	8.76E-08
MP:0000358	Abnormal cell morphology	3.10E-07
MP:0002022	Increased lymphoma incidence	3.97E-07
MP:0002020	Increased tumor incidence	4.03E-07
MP:0010274	Increased organ/body region tumor incidence	5.83E-07
MP:0002019	Abnormal tumor incidence	9.06E-07

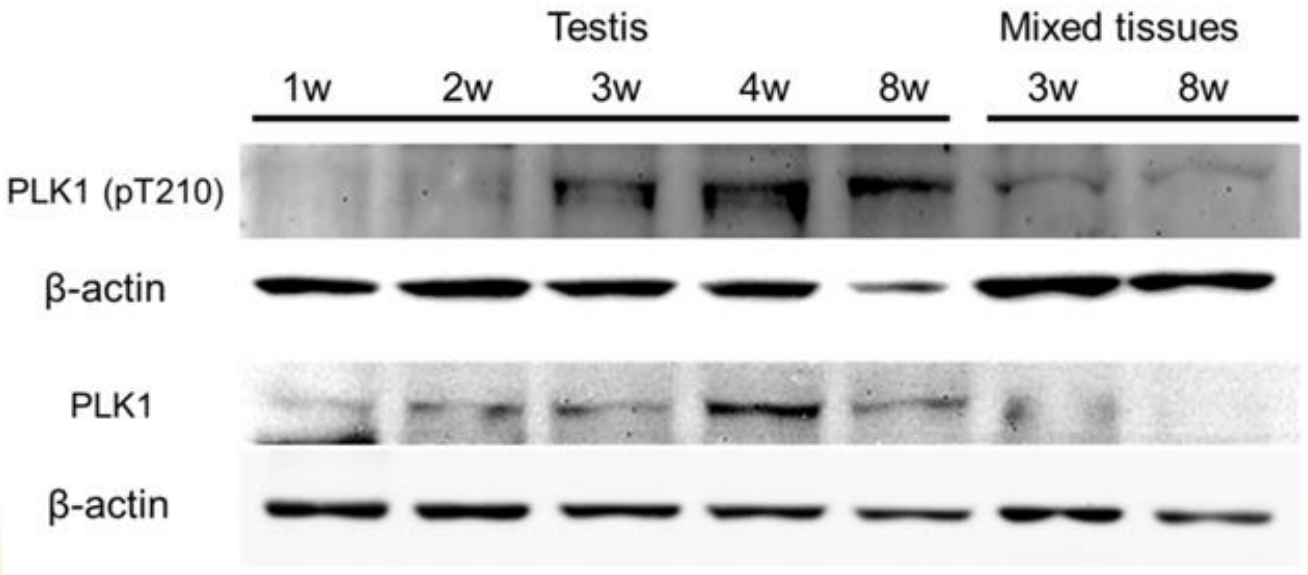
Experiments

- Spermatocyte GC2 cell line
- BI2536: Plks inhibitor
- Okadaic acid (OA)
  - Phosphatase inhibitor
  - Reverses the phosphorylation changes after inhibition of Plks
- DMSO: control
- RT-PCR: Plk1-3 existence



Validation of Plk activation

- pT210: the major p-site and correlates with Plk1 activity
- Testes: 1, 2, 3, 4, 8 weeks
- Control: 8 tissue mixtures; 3 and 8 weeks
- Plk1 activity is significantly higher

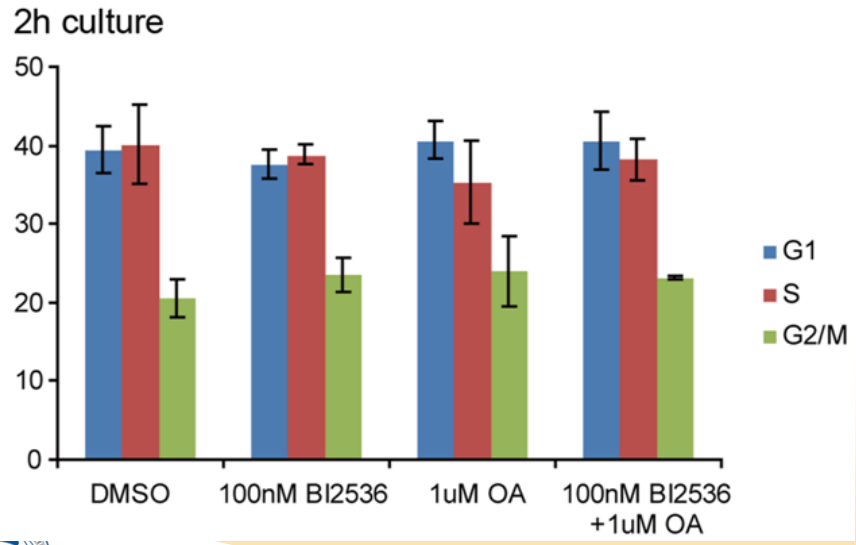


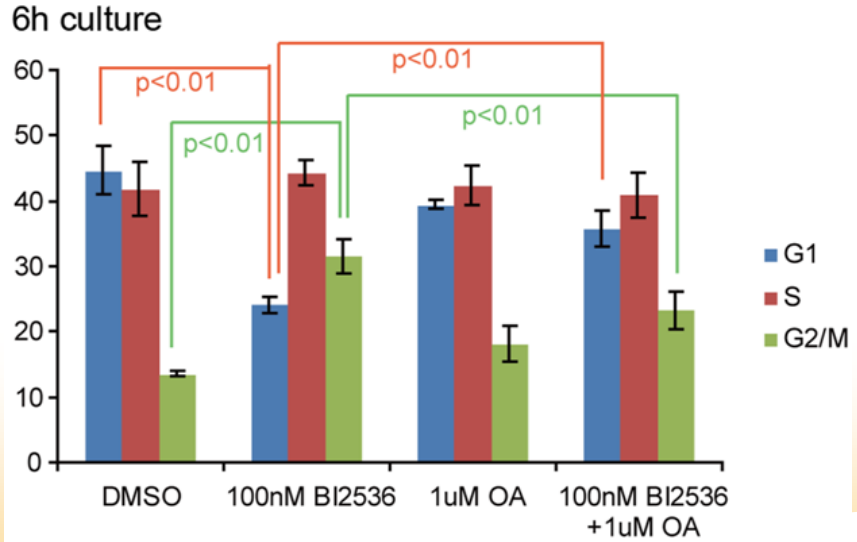
Jang et al., J Biol Chem., 2002, 277:44115-20

Kelm et al., J Biol Chem., 2002, 277:25247-56

Plk inhibition: G2/M arrest

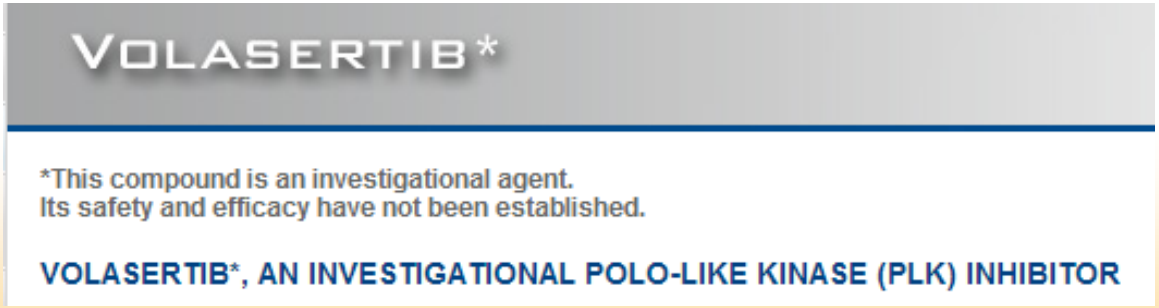
- After 6h
  - G1 cells: decreased
  - S cells: unchanged
  - G2/M cells: increased
- BI2536: induces arrest in the G2/M phase and inhibits cell proliferation





Discussion

- The differential activities of kinases can be readily and robustly predicted from poorly reproducible data
- Plk1: colon and lung cancers
- Plk inhibitors: potential anti-cancer drugs
  - BI2536: Phase I and II
  - BI6727/Volasertib: granted by the FDA in AML (with cytarabine), in older patients
  - GSK461364A: Phase I



Gjertsen et al., Leukemia. 2014, doi: 10.1038/leu.2014.222

Perspectives

- **Biological forecasting: network-based prediction**
- **Network targets in Liver cancers and neurodegenerative diseases**
- **Key regulators in Autophagy**
- **Can we learn more things from the phosphoproteomic data?**

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- Zexian Liu (HUST)
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- Ping Xu (BPRC)
- Min Li (HKBU)
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Thanks!

Any questions?