ppt结构

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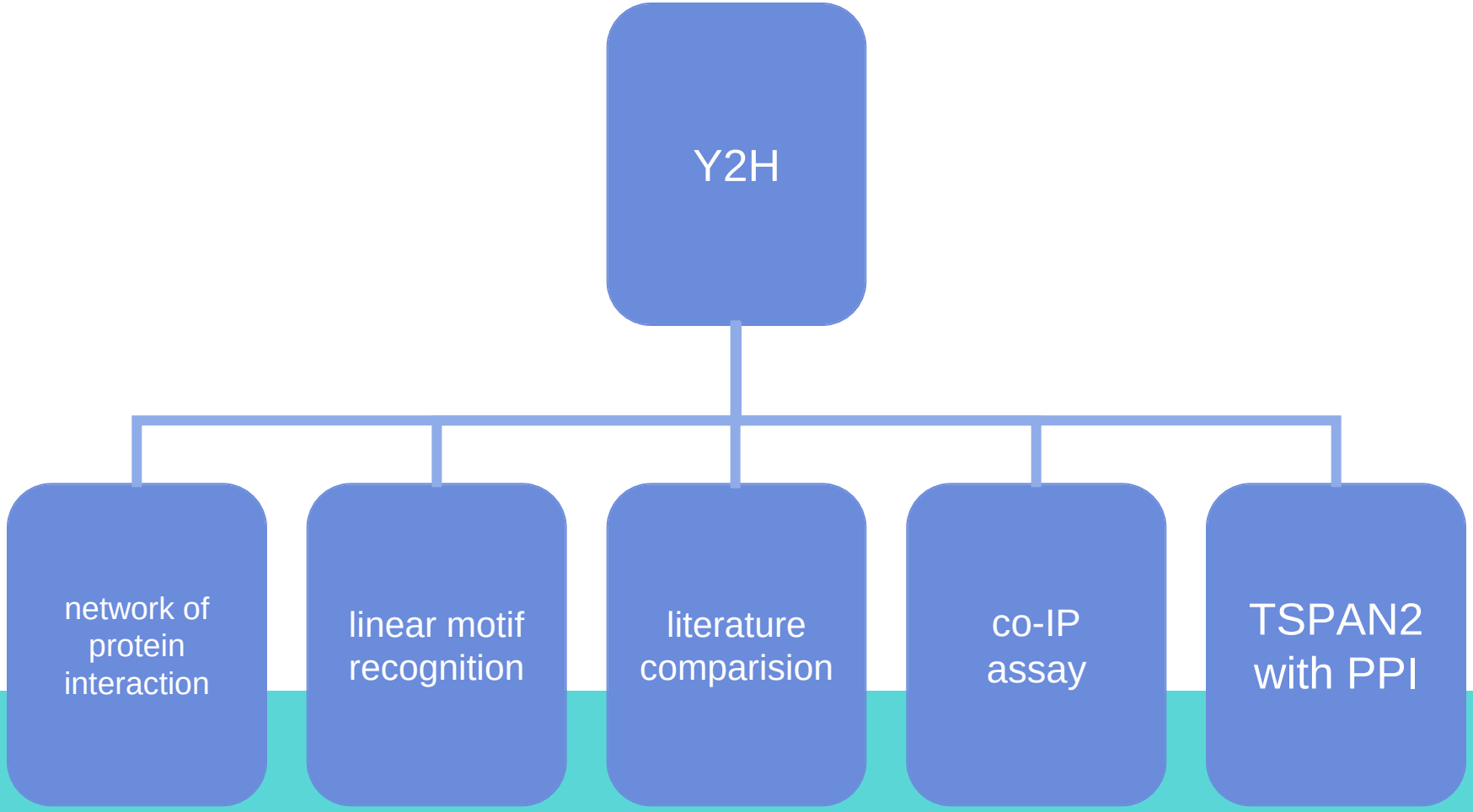
Phospho-tyrosine dependent protein–protein interaction network

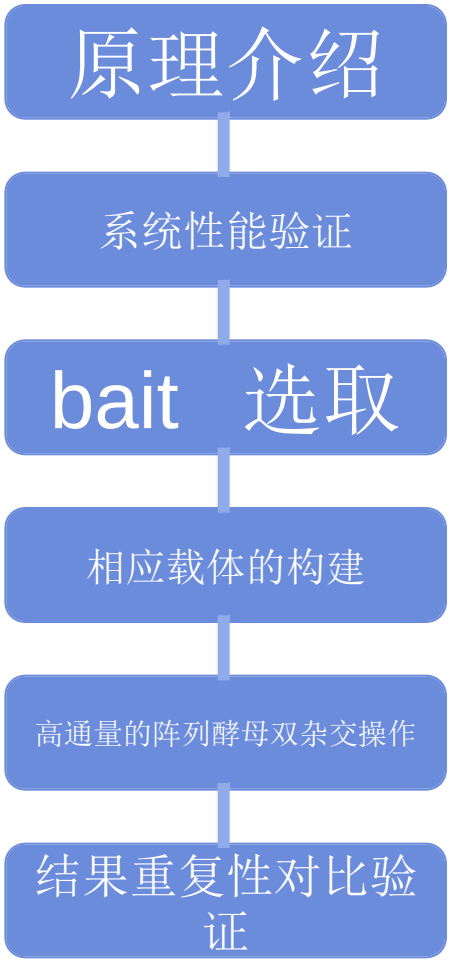
生科 生化分子 刘镠

1、依赖于酪氨酸残基磷酸化的信号转导网络，在细胞信号转导网络中占有举足轻重的地位，对其研究有着深刻的意义。

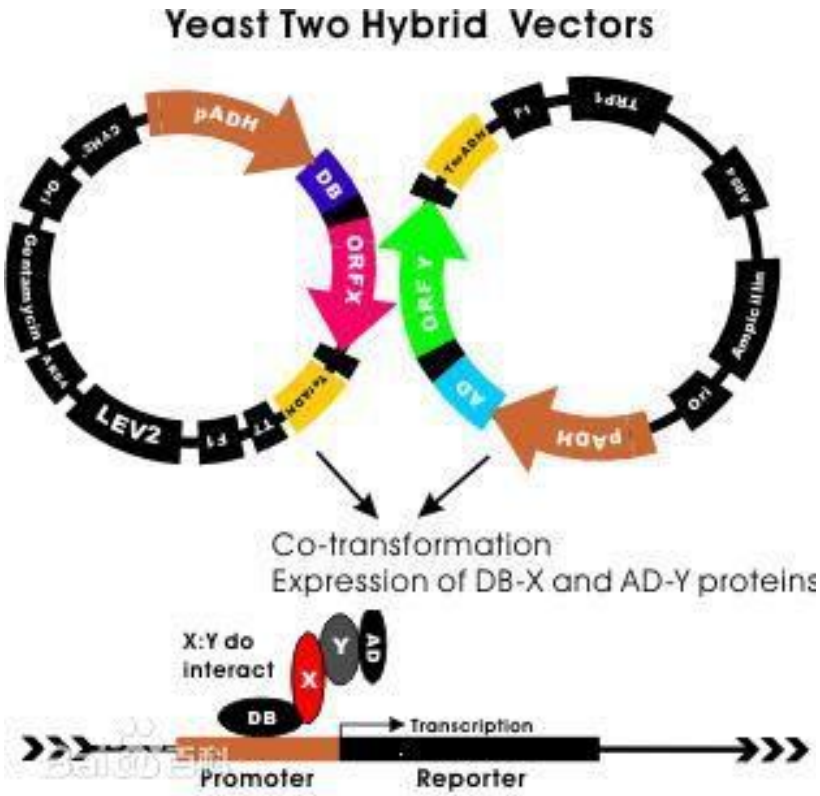
2、高通量酵母双杂交技术的出现，为实现大规模的蛋白互作验证，提供了基础。

实验结构

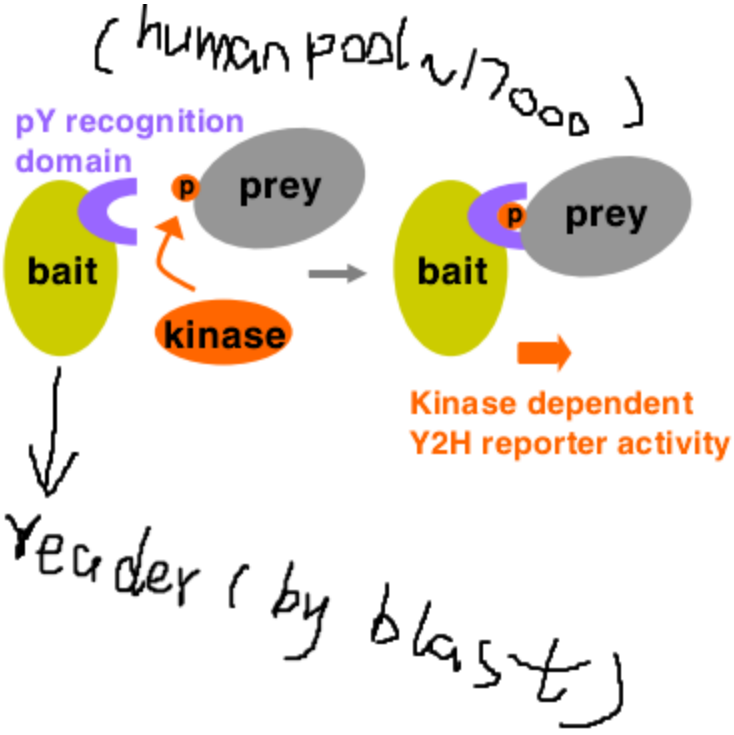

Y2H
network of protein interaction
linear motif recognition
literature comparision
co-IP assay
TSPAN2 with PPI



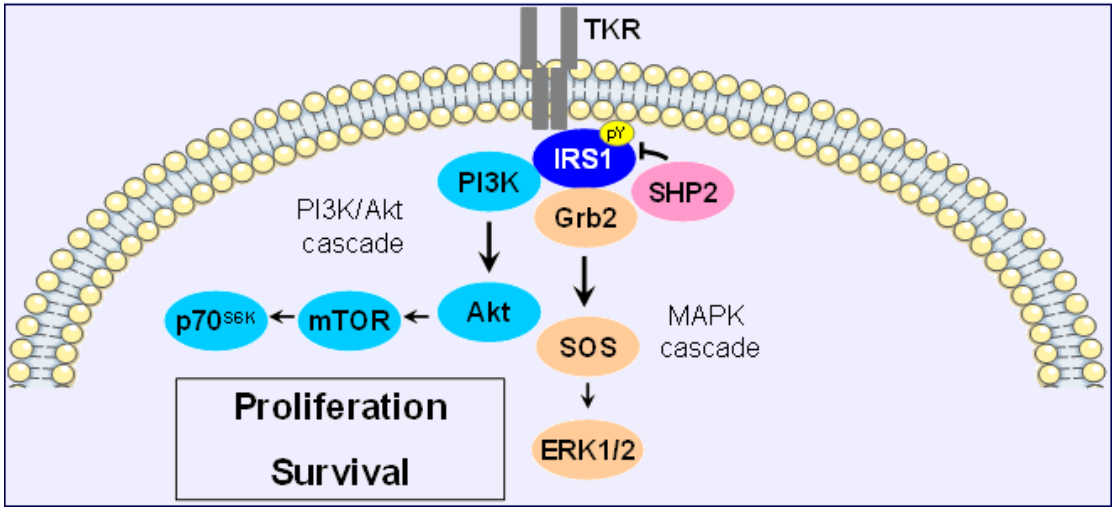
酵母双杂交操作的实现


Yeast Two Hybrid Vectors
pADH
DB
ORF X
LEV2
TRP1
ORF Y
AD
pADH
Co-transformation
Expression of DB-X and AD-Y proteins
X:Y do
interact
X
Y
AD
DB
Transcription
Promoter
Reporter

酵母双杂交技术原理


(human pool w/ 700)
pY recognition domain
P
prey
bait
kinase
P
prey
bait
Kinase dependent
Y2H reporter activity
Yeader (by blast)

本实验分子互作模型



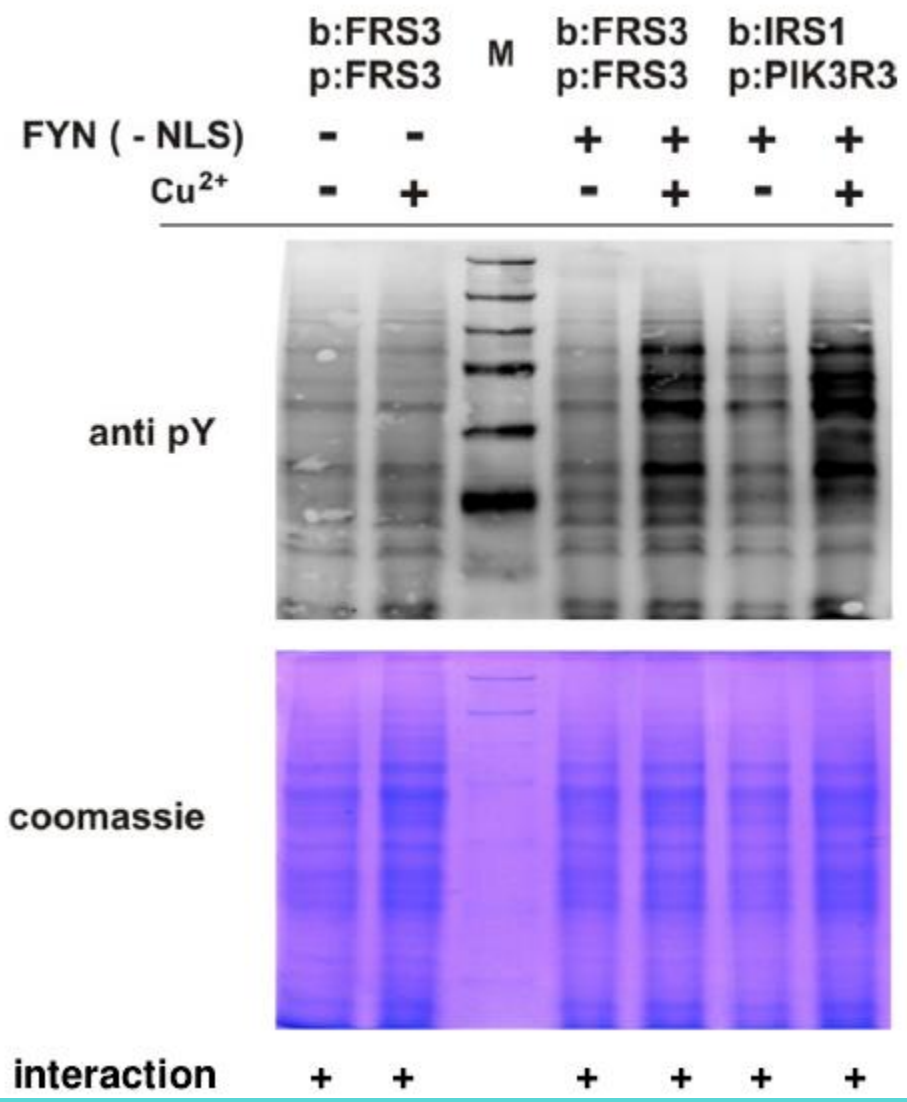
IRS1(insulin receptor substrate 1)，也称IRS-1，是胰岛素受体(insulin receptor)激酶的主要底物。IRS1含有多个酪氨酸磷酸化位点，这些位点磷酸化后可以结合含有SH2结构域的蛋白，如PI3-K和GRB2等，从而介导胰岛素诱导的代谢和促生长信号

FYN：Tyrosine-protein kinase Fyn

PIK3R3（PI3K, regulatory subunit 3）

pan-pY 4G10 antibody:检测酪氨酸残基磷酸化的抗体。可检测酪氨酸激酶表达量。

系统功能验证


b:FRS3
p:FRS3
M
b:FRS3
p:FRS3
b:IRS1
p:PIK3R3
FYN (- NLS)
- - + + + +
Cu2+
- + - + - +
anti pY
coomassie
interaction
+ + + + + +

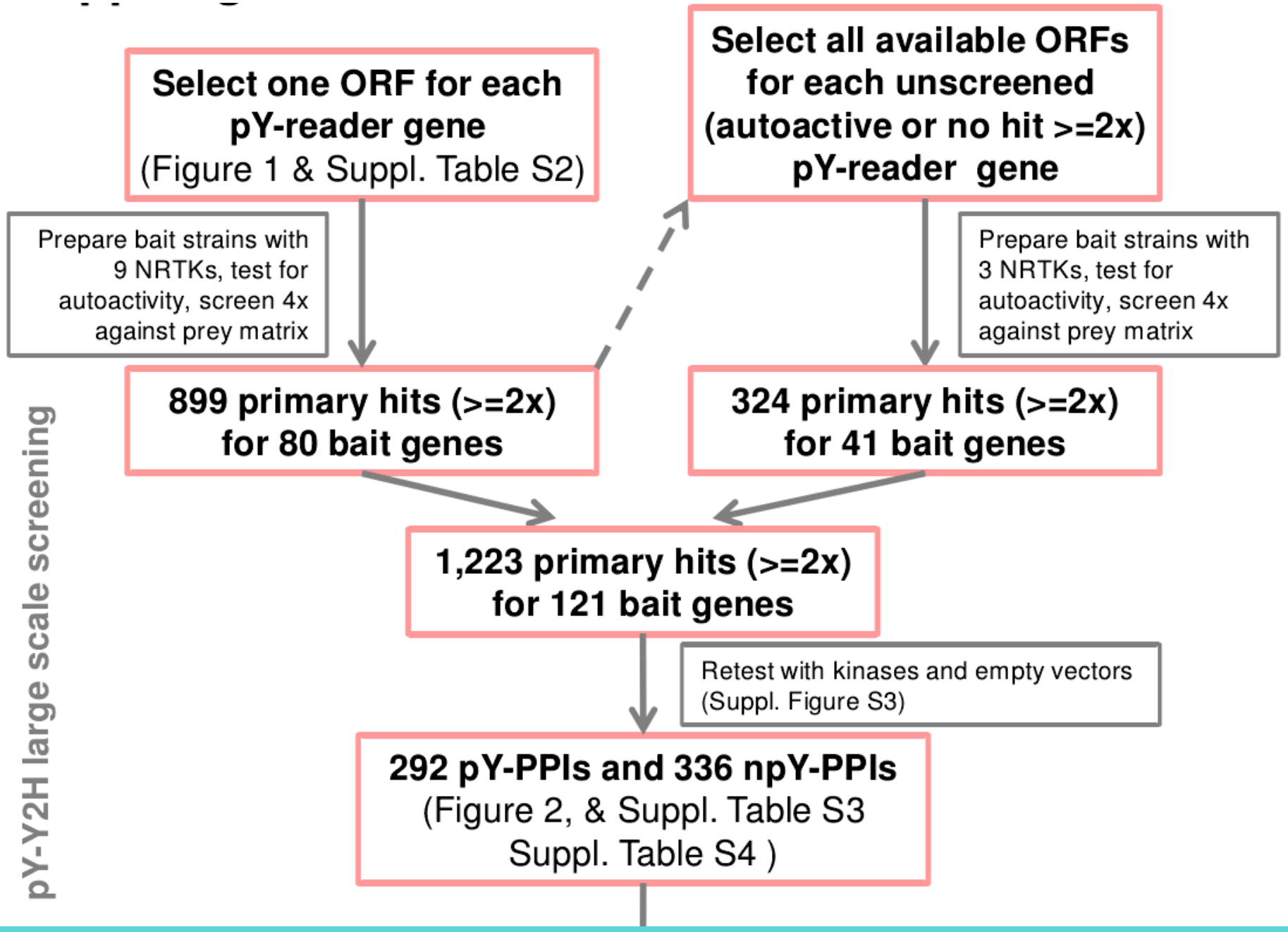
系统功能验证-激酶活性

Table S2: Bait strains

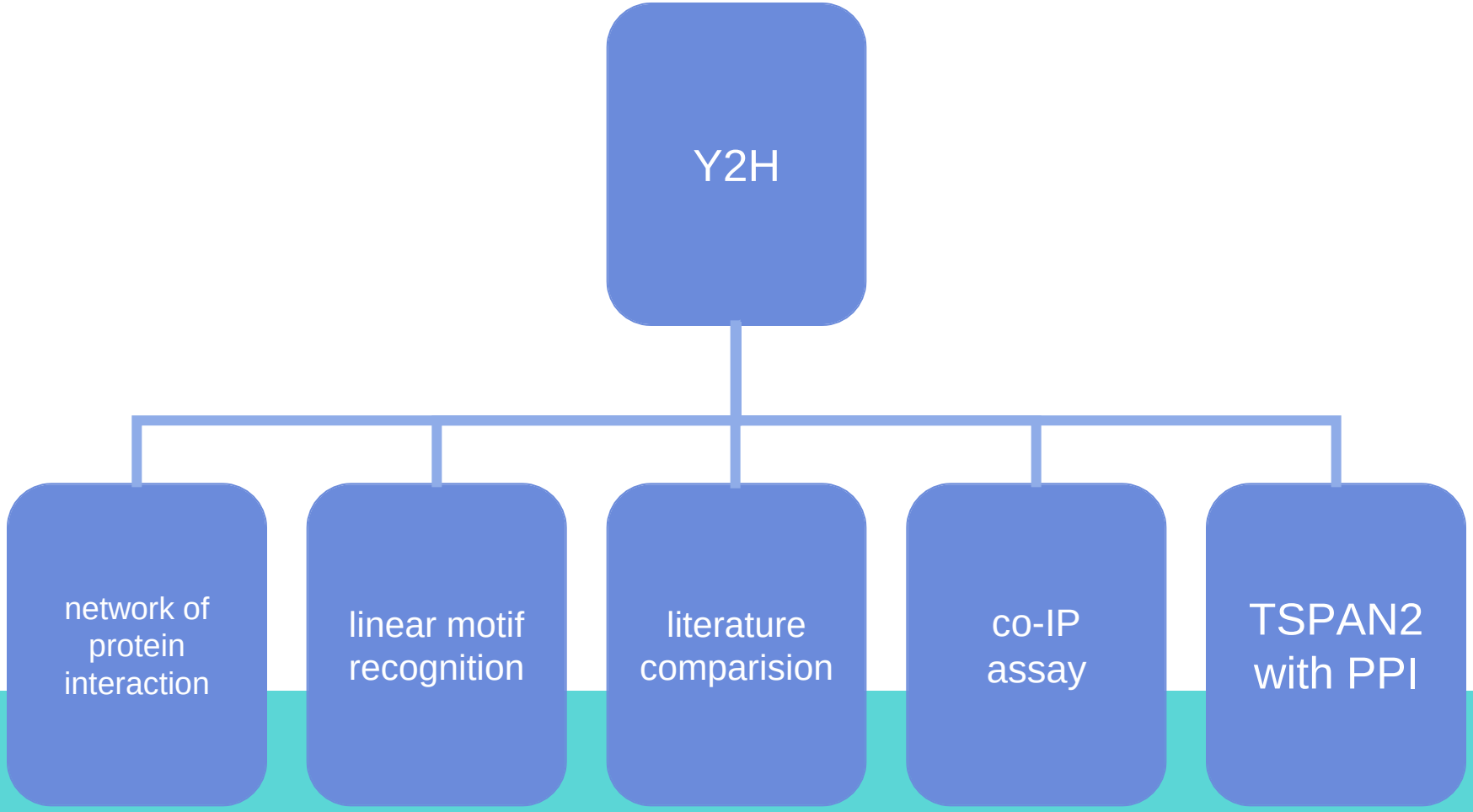
Symbol	GeneID	UniProtID	Number of Domains SH2	IRS1	PID	Number of Baits total	screenewith PPIs		Entrez Symbol	Entrez GeneID	Representative UniProtID	Number of Domains SH2	TB_IR?	TB_PI	Number of B total	screenev
ABL1	25	ABL1_HUMAN	1			0	0	0	NUMB	8650	NUMB_HUMAN			1	1	1
ABL2	27	ABL2_HUMAN	1			1	1	1	NUMBL	9253	NUMBL_HUMAN			1	0	0
ANKS1A	23294	ANKS1_HUMAN			1	1	1	1	PID1	55022	PCLI1_HUMAN			1	1	1
ANKS1B	56899	ANS1B_HUMAN			1	2	2	0	PIK3R1	5295	P85A_HUMAN	2			2	1
APBA1	320	APBA1_HUMAN			1	0	0	0	PIK3R2	5296	Q96CK7_HUMAN	2			1	1
APBA2	321	APBA2_HUMAN			1	1	1	0	PIK3R3	8503	P55G_HUMAN	2			2	2
APBA3	9546	APBA3_HUMAN			1	0	0	0	PLCG1	5335	PLCG1_HUMAN	2			1	1
APBB1	322	APBB1_HUMAN			2	2	1	0	PLCG2	5336	PLCG2_HUMAN	2			1	1
APBB2	323	APBB2_HUMAN			2	2	2	0	PTK6	5753	PTK6_HUMAN	1			1	1
APBB3	10307	APBB3_HUMAN			2	2	2	1	PTPN11	5781	PTN11_HUMAN	2			1	1
APPL1	26060	DP13A_HUMAN			1	2	2	2	PTPN6	5777	PTN6_HUMAN	2			3	3
APPL2	55198	DP13B_HUMAN			1	1	1	1	RABGAP1	23637	RBGP1_HUMAN			1	2	2
BCAR3	8412	BCAR3_HUMAN	1			1	1	0	RABGAP1L	9910	RBG1L_HUMAN			1	3	3
BLK	640	BLK_HUMAN	1			1	1	0	RASA1	5921	RASA1_HUMAN	2			2	2
BLNK	29760	BLNK_HUMAN	1			2	1	0	RGS12	6002	RGS12_HUMAN			1	0	0
BMX	660	BMX_HUMAN	1			0	0	0	RIN1	9610	RIN1_HUMAN	1			1	1
BTK	695	BTK_HUMAN	1			1	1	1	RIN2	54453	RIN2_HUMAN	1			1	1
CBL	867	A3KMP8_HUMAN	1			1	1	1	RIN3	79890	RIN3_HUMAN	1			3	3
CBLB	868	CBLB_HUMAN	1			2	1	1	SH2B1	25970	SH2B1_HUMAN	1			1	1
CBLC	23624	CBLC_HUMAN	1			2	2	0	SH2B2	10603	SH2B2_HUMAN	1			0	0
CCM2	83605	CCM2_HUMAN			1	2	2	1	SH2B3	10019	SH2B3_HUMAN	1			0	0
CHN1	1123	CHIN_HUMAN	1			2	2	0	SH2D1A	4068	SH21A_HUMAN	1			2	2
CHN2	1124	CHIO_HUMAN	1			1	1	1	SH2D1B	117157	SH21B_HUMAN	1			3	3
CISH	1154	CISH_HUMAN	1			1	1	0	SH2D2A	9047	SH22A_HUMAN	1			1	1
CRK	1398	CRK_HUMAN	1			4	3	3	SH2D3A	10045	SH23A_HUMAN	1			1	1
CRKL	1399	CRKL_HUMAN	1			2	1	1	SH2D3C	10044	SH2D3_HUMAN	1			1	1
CSK	1445	CSK_HUMAN	1			1	1	1	SH2D4A	63898	SH24A_HUMAN	1			1	1
DAB1	1600	DAB1_HUMAN			1	0	0	0	SH2D4B	387694	SH24B_HUMAN	1			0	0
DAB2	1601	DAB2_HUMAN			1	3	1	0	SH2D5	400745	SH2D5_HUMAN	1			1	1
DAPP1	27071	DAPP1_HUMAN	1			2	2	2	SH2D6	284948	SH2D6_HUMAN	1			0	0
DOK1	1796	DOK1_HUMAN		1		2	1	1	SH3BP2	6452	3BP2_HUMAN	1			1	1
DOK2	9046	DOK2_HUMAN		1		2	1	1	SHB	6461	SHB_HUMAN	1			0	0
DOK3	79930	DOK3_HUMAN		1		0	0	0	SHC1	6464	SHC1_HUMAN	1		1	0	0
DOK4	55715	DOK4_HUMAN		1		1	1	1	SHC2	25759	SHC2_HUMAN	1		1	0	0
DOK5	55816	DOK5_HUMAN		1		1	1	1	SHC3	53358	SHC3_HUMAN	1		1	1	1
DOK6	220164	DOK6_HUMAN		1		1	1	0	SHC4	399694	SHC4_HUMAN	1		1	1	1
DOK7	285489	DOK7_HUMAN		1		2	1	1	SHD	56961	SHD_HUMAN	1			2	1
EPS8	2059	EPS8_HUMAN			1	1	1	1	SHE	126669	SHE_HUMAN	1			1	1
EPS8L2	64787	ES8L2_HUMAN			1	1	1	0	SHF	90525	SHF_HUMAN	1			0	0
FER	2241	FER_HUMAN	1			1	1	1	SLA	6503	SLAP1_HUMAN	1			2	1

(The results obtained with the IRS1 and PID families of PTB domain-containing proteins are presented aside as insets.) Due to structural differences, PTB domains are divided into three groups represented by phosphotyrosine-dependent IRS-like, phosphotyrosine-dependent Shc-like, and phosphotyrosine- independent Dab-like PTBs. The last two PTBs have been named as phosphotyrosine interaction domain (PID or PI domain). PID domain has an average length of about 160 amino acids

bait 的选取-192bait


Select one ORF for each pY-reader gene (Figure 1 & Suppl. Table S2)
Select all available ORFs for each unscreened (autoactive or no hit >=2x) pY-reader gene
Prepare bait strains with 9 NRTKs, test for autoactivity, screen 4x against prey matrix
Prepare bait strains with 3 NRTKs, test for autoactivity, screen 4x against prey matrix
899 primary hits (>=2x) for 80 bait genes
324 primary hits (>=2x) for 41 bait genes
1,223 primary hits (>=2x) for 121 bait genes
Retest with kinases and empty vectors (Suppl. Figure S3)
292 pY-PPIs and 336 npY-PPIs (Figure 2, & Suppl. Table S3 Suppl. Table S4)
pY-Y2H large scale screening

实验结构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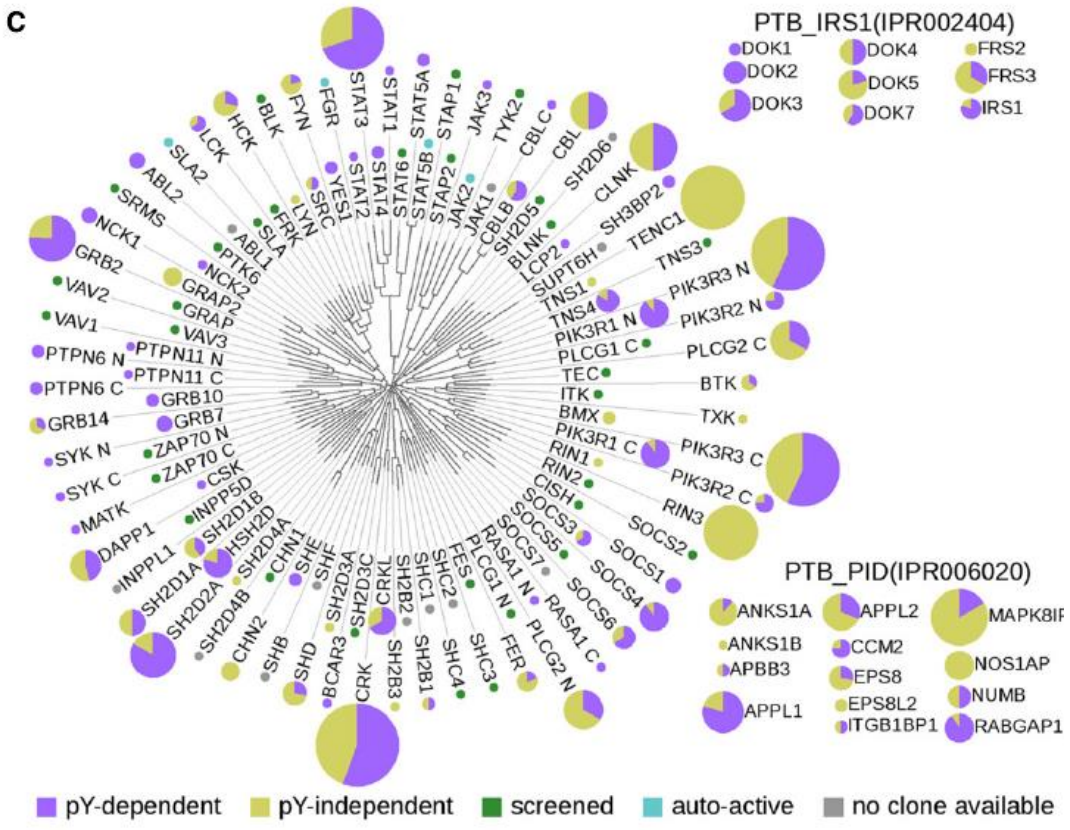
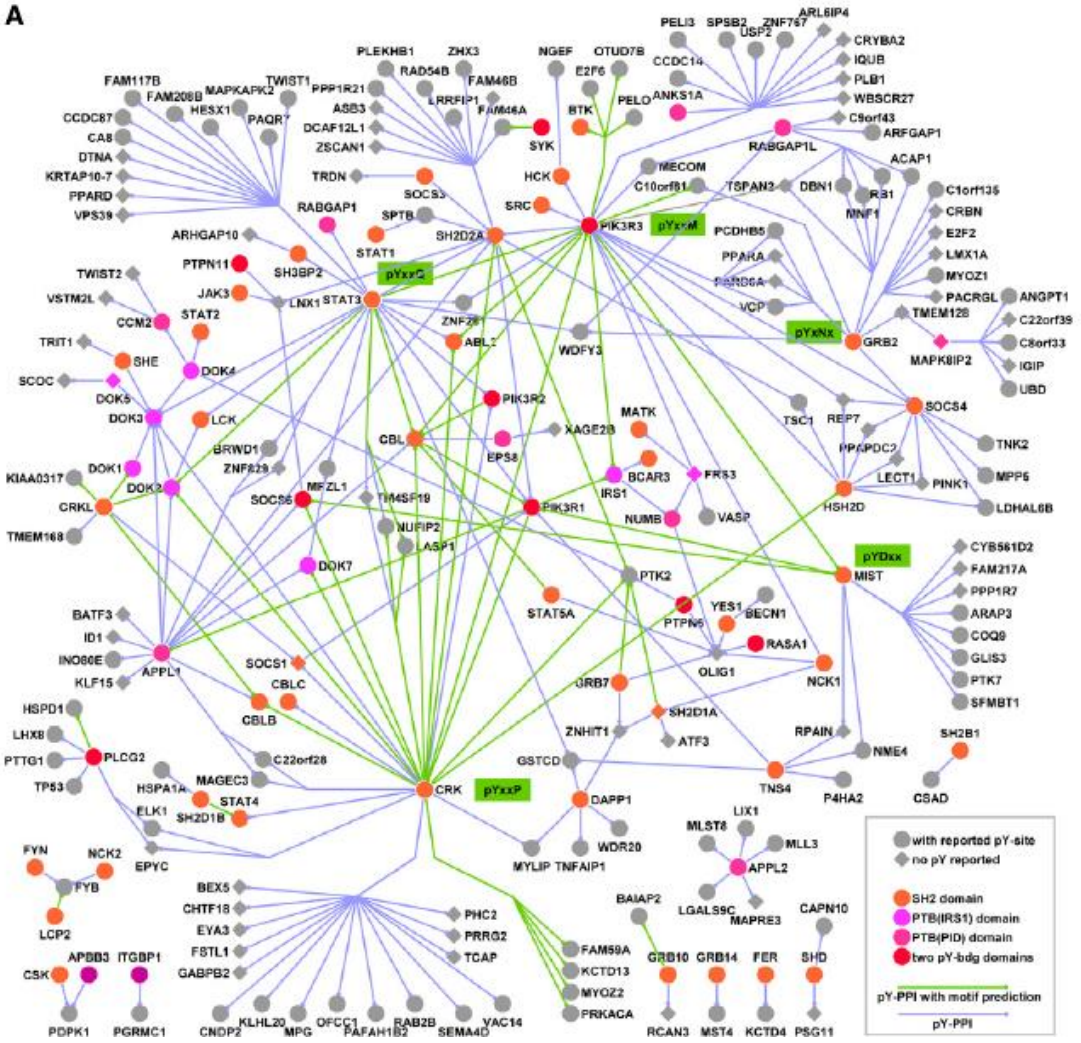


Y2H验证的bait-kinase对应关系


A
with reported pY-site
no pY reported
SH2 domain
PTB(IRS1) domain
PTB(PID) domain
two pY-bdg domains
pY-PPI with motif prediction
pY-PPI

蛋白互作网络

A

	pos	neg	nd	pos [%]
pY-dep.	57	55	22	51
independent	19	16	0	54
total	76	71	22	52

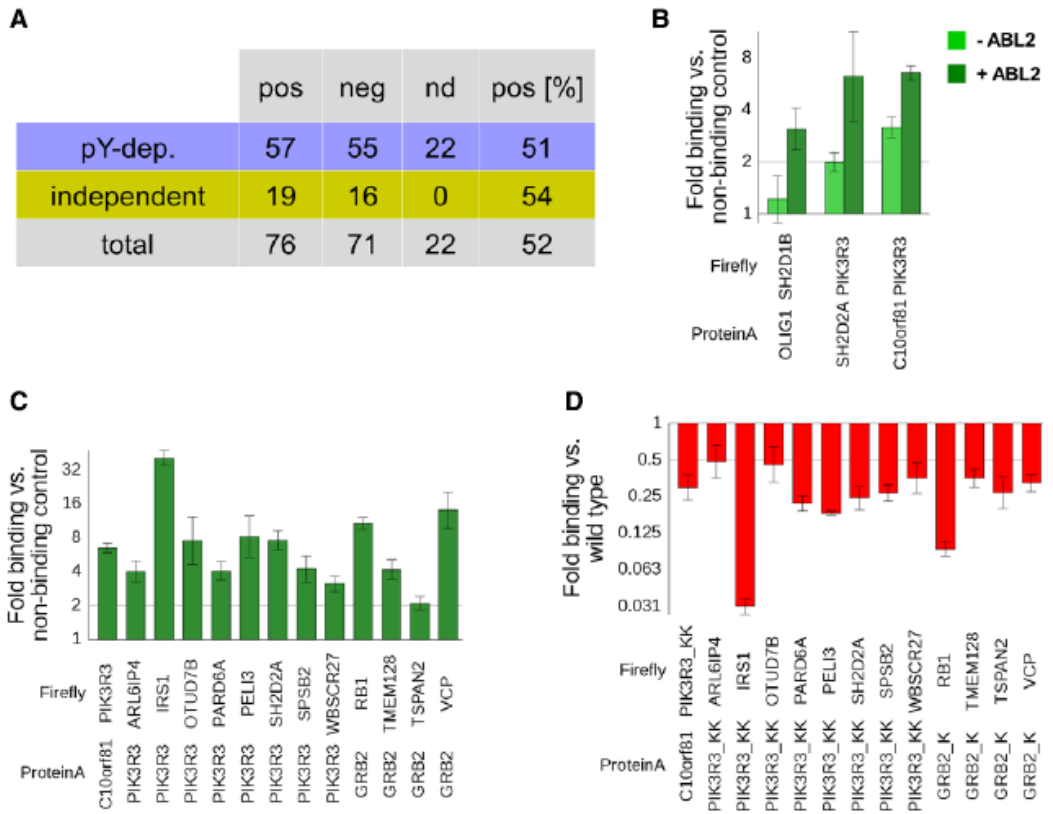


Figure 3. Validation of pY-PPIs through luciferase-based co-IP assays.

A Co-IP results: 187 PPIs were tested in co-IP assays with a success rate of ~50%. The background rate in this assay was 20–25% (Hegele *et al*, 2012; Weimann *et al*, 2013).

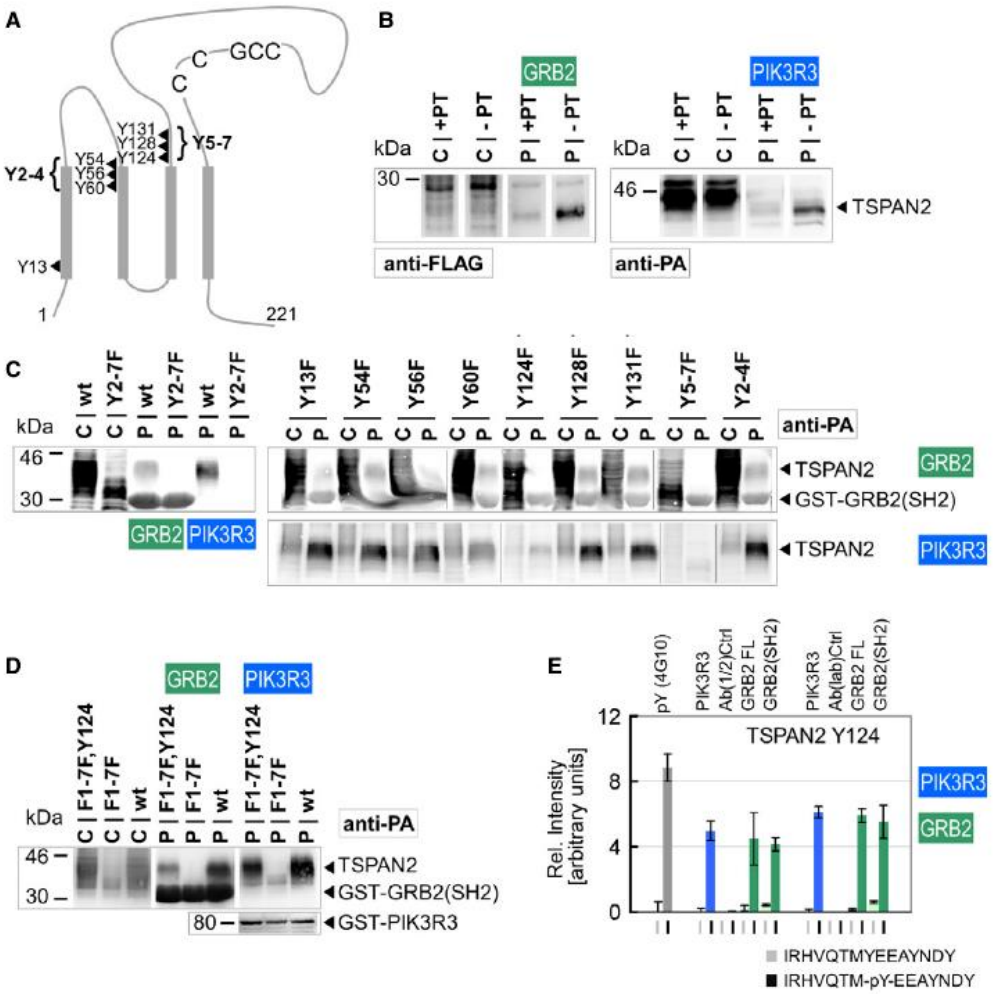
B Co-IP results for the three pY-dependent pairs that showed significantly higher binding in the co-IP assay upon induction of ABL2 (dark green) as compared to non-induced cells (light green). *y*-axis displays fold binding in comparison with a non-interacting protein tested with the same firefly-tagged protein (normalized to 1). Error bars show the standard deviation from an experiment performed with triplicate transfections.

C Selection of PIK3R3 and GRB2 co-IP results from HEK293 cells. Co-IPs were performed in HEK293 with induction of stably transfected ABL2 kinase and tyrosine phosphatase inhibition throughout the cell lysis. Error bars show the standard deviation from an experiment performed with triplicate transfections.

D The same pY-dependent protein pairs as in (C), but tested with SH2-domain mutant versions of GRB2 (R86K) and PIK3R3 (R90K+R383K). The amino acid exchange of the conserved arginine in the SH2 domain is known to reduce phosphorylation-dependent binding. Fold binding is normalized to wild-type GRB2 or PIK3R3 binding (1). Error bars show the standard deviation from an experiment performed with triplicate transfections.

重复率
50%

luciferase-免疫共沉淀实验



TSPAN2 with PI3K、GRB2

1、主要结论或创新点

本文基于高通量Y2H技术对酪氨酸激酶介导蛋白信号互作网络进行了初步的探索。创新点：高通量酵母双杂交技术对细胞信号转导网络进行分析，为构建细胞系统及各种大分子之间的作用及相互作用机制的建立提供了重要的方向和依据。

2、个人启发

一、试验设计的严密性；

二、从新技术（高通Y2H）的应用,对实现系统性的分析蛋白互作关系提供了可能可以看出：对生命科学研究的推进，技术的革新和进步是关键的手段，一个合格的科学家不仅需要具有高标准的理论知识基础，更要关注一些科学研究的新方法手段。

3、此研究的改进点

一、该实验数据的可靠性，被酵母双杂交的局限性所限制，对实现单方向的系统生物模型的三维模型（空间网络）、四维模型（引入时间坐标）的构建缺乏可靠性、可行性。

二、本实验基于网络的构建，从而给研究具体蛋白的互作机制提供了有力的参考，但并未进一步实施，从而实现科研的应用价值。可对具体的功能机制可以基于目前的分析，对感兴趣的互作蛋白信号进行深入的功能揭示，产生具体的应用价值。

Thanks for listening