染色质结合和可溶性转录因子的蛋白组学研究

Proteomic analyses reveal distinct chromatin-associated and soluble transcription factor complexes

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- Result
- Discussion

Backgrounds

信号通道是所有生物体生命生命活动的基础。

各种复杂的信号转导途径对生物体的生存和发育是必须的，一点微小的破坏都可能引起发育缺陷或一些疾病甚至是癌症。许多信号通道是通过调节某些转录因子的活性来起作用的，通常是改变它们的位置。

Transcription factors(TFs)受到与其关联的蛋白质的严格调控。

However, while the DNA-binding and the transcriptional activities of TFs on chromatin have been extensively studied, our knowledge of protein–protein interactions (PPIs) that may occur off the chromatin, which are important for the regulations and functions of these TFs, is very limited.

Knowing what proteins TFs interact with and, especially, where they interact will greatly improve our understanding of how the activities of these TFs are controlled.

- hypothesis :

TFs are engaged in different PPIs on and off chromatin, which are likely important for their regulations and diverse functions.

Forkhead box (FOX) family of TFs

FOX family TFs play important roles in regulating the expression of genes involved in a sundry of cellular processes, especially during development and tumorigenesis .

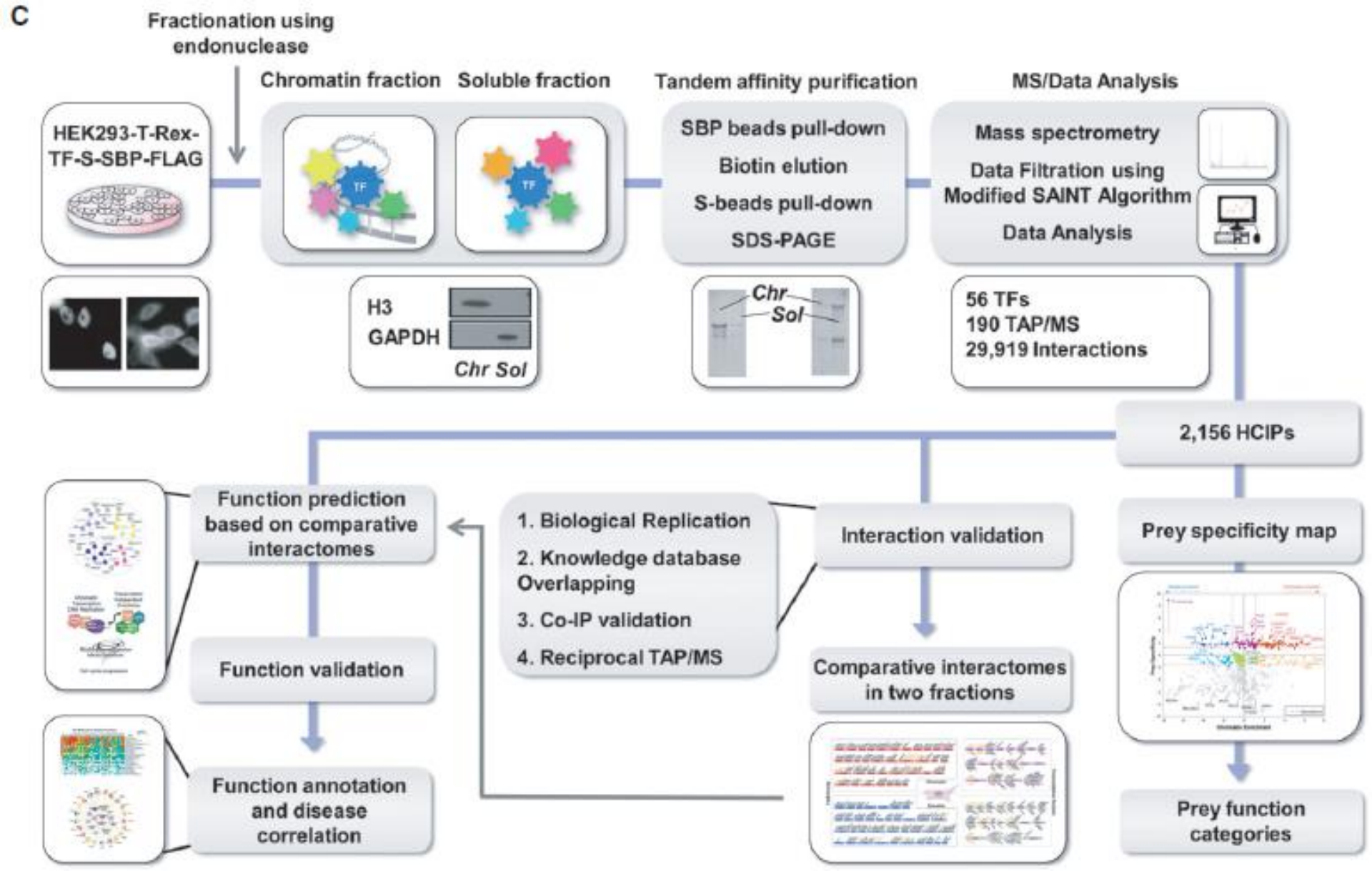
tandem affinity purification (TAP) and mass spect-

rometry (MS) analysis.

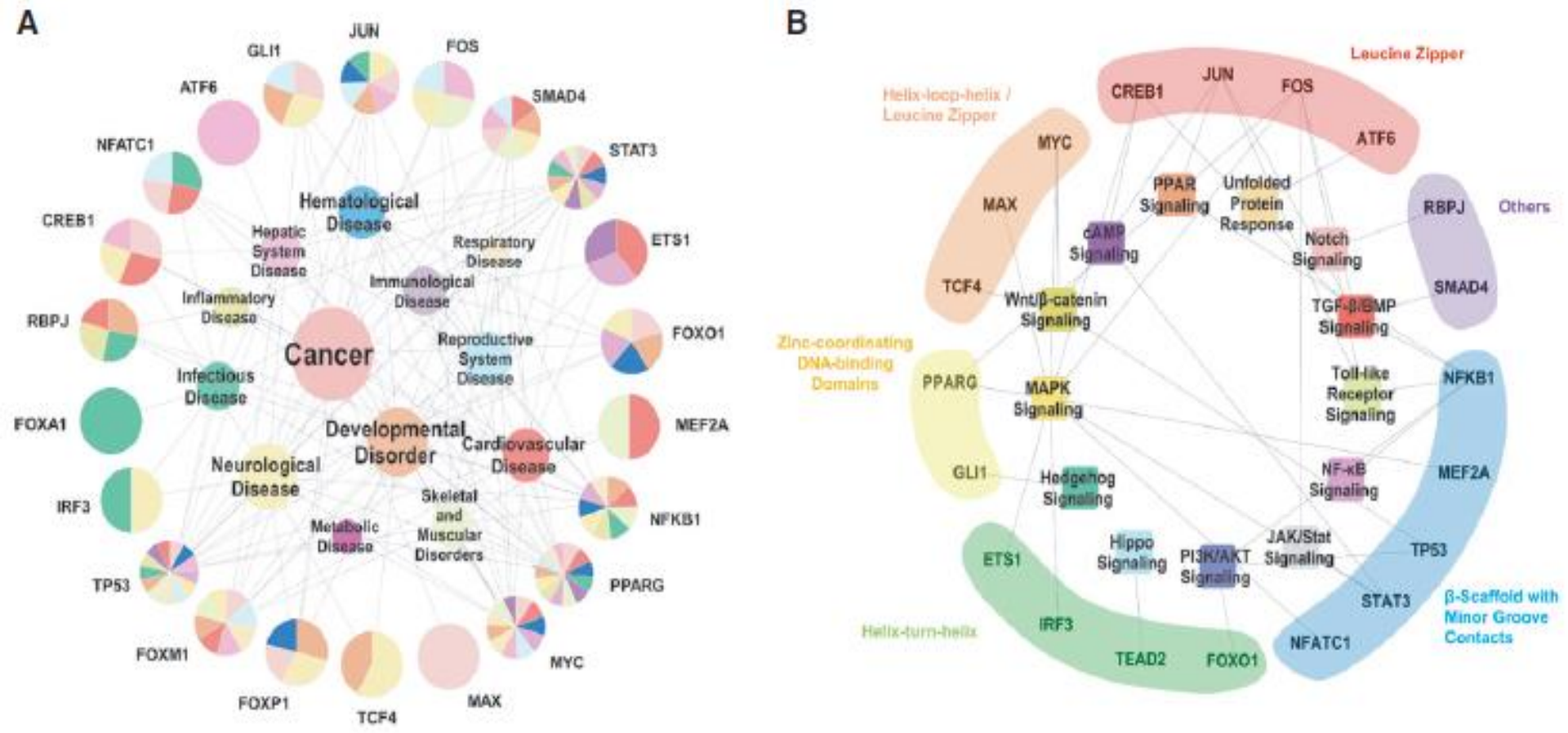
revealed that they indeed form distinct complexes on and off chromatin.

- performed TAP/MS analyses for 19 non-FOX TFs
- from five structural TF superfamilies.
- exclude the potential bias caused by the structural preference of their DNA-binding activities.
- **Hypothesis is validated.**

Methods



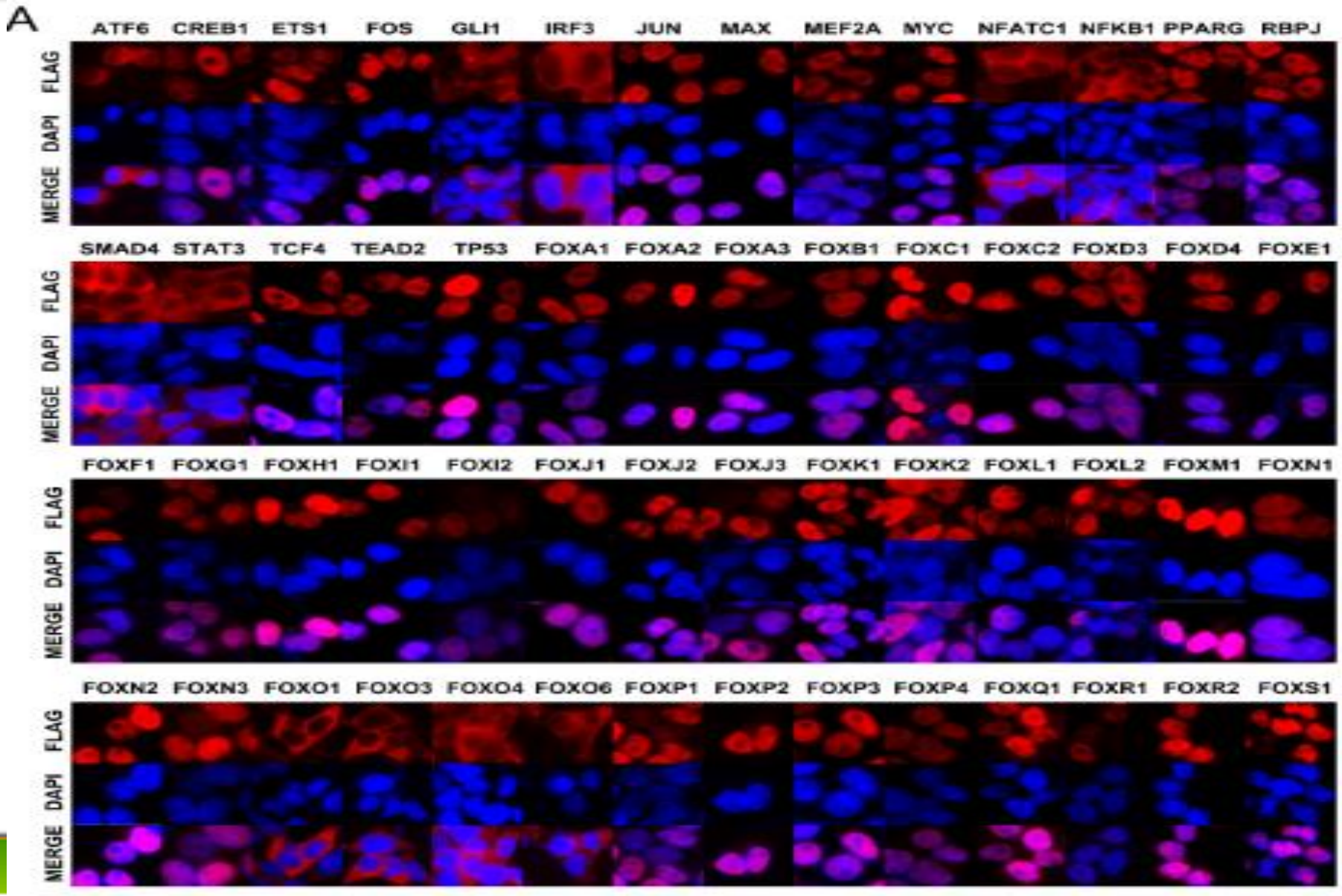
Results



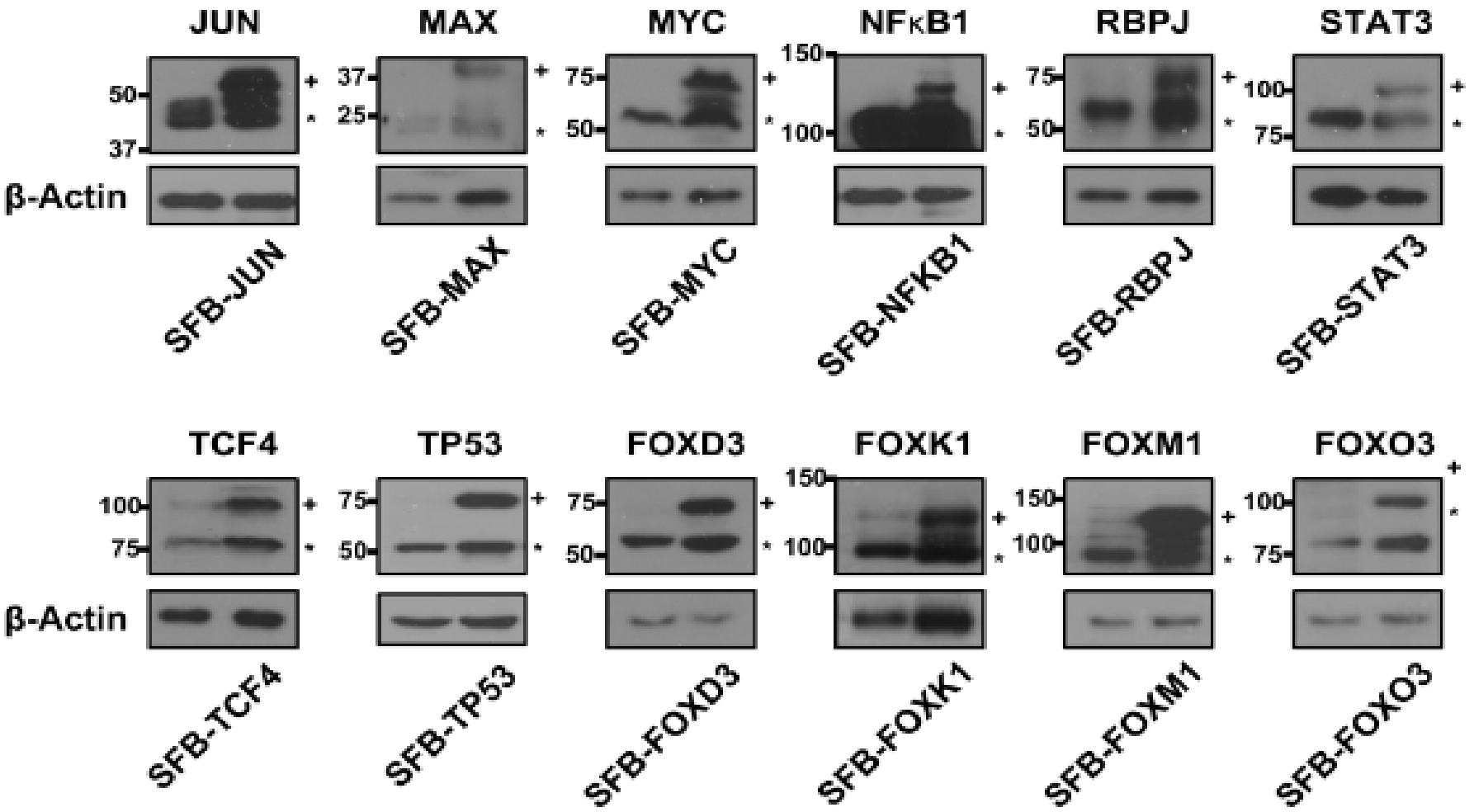
A Disease correlation of 19 TFs and 4 well-studied FOX family members, based on their GO annotations. Each colour indicates one disease. The size of each coloured pie indicates the relative ratio of –log (*P*-value) of GO annotations in the corresponding disease.

B Pathway correlation and structural superclasses of TFs. Each coloured area indicates one superfamily.

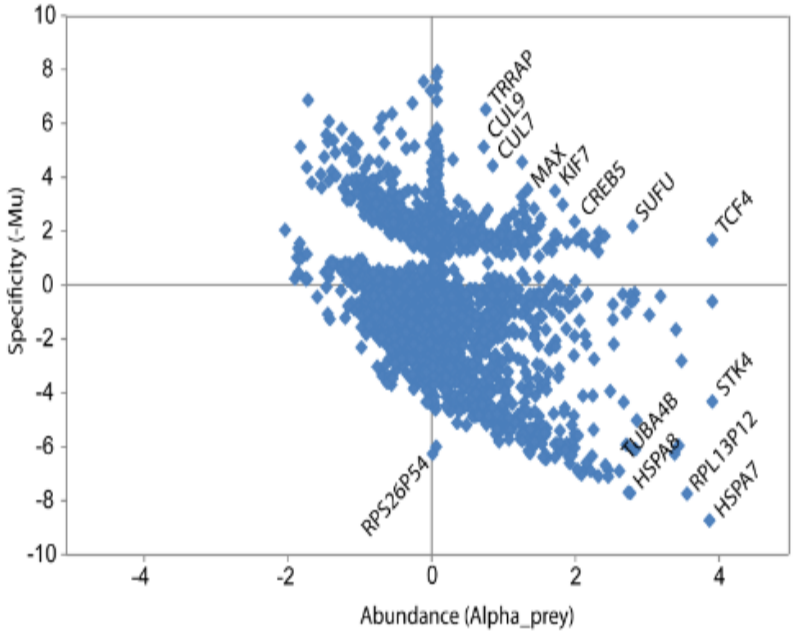
- 56 TFs, including 37 FOX family members and 19 non-FOX TFs
- TAP/MS
- We picked 12–24 single clones for each bait
- examined them by Western blotting and immunostaining
- chose the ones with the correct subcellular localizations and the lowest expression for affinity purifications.
- We compared the immunostaining results of our stable cell lines with those available in the literature.
- All of the tagged proteins were localized as previously reported.


A
ATF6 CREB1 ETS1 FOS GLI1 IRF3 JUN MAX MEF2A MYC NFATC1 NFKB1 PPARG RBPJ
FLAG
DAPI
MERGE
SMAD4 STAT3 TCF4 TEAD2 TP53 FOXA1 FOXA2 FOXA3 FOXB1 FOXC1 FOXC2 FOXD3 FOXD4 FOXE1
FLAG
DAPI
MERGE
FOXF1 FOXG1 FOXH1 FOXI1 FOXI2 FOXJ1 FOXJ2 FOXJ3 FOXK1 FOXK2 FOXL1 FOXL2 FOXM1 FOXN1
FLAG
DAPI
MERGE
FOXN2 FOXN3 FOXO1 FOXO3 FOXO4 FOXO6 FOXP1 FOXP2 FOXP3 FOXP4 FOXQ1 FOXR1 FOXR2 FOXS1
FLAG
DAPI
MERGE

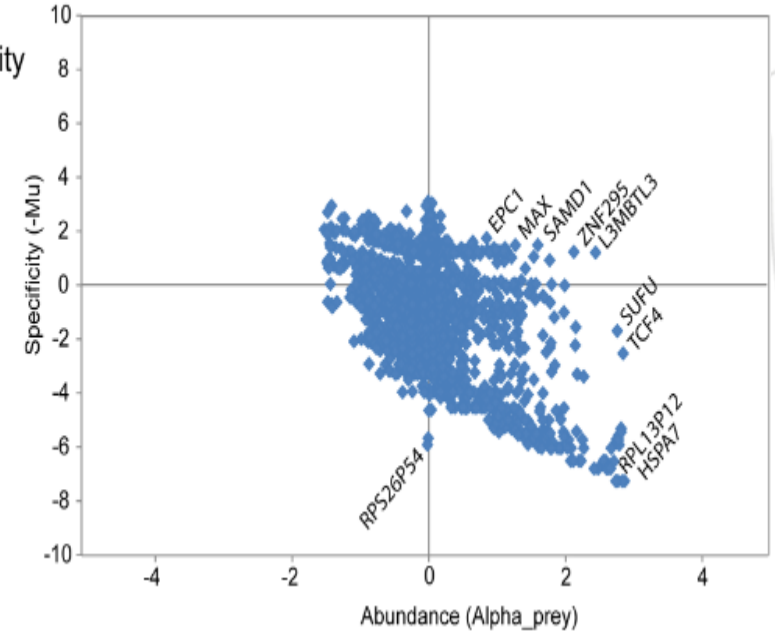
- We also compared the expression levels of 12 tagged proteins with endogenous proteins in our stable cell lines by Western blotting. Most of the tagged proteins were expressed similar to or slightly higher than that of endogenous proteins.
-

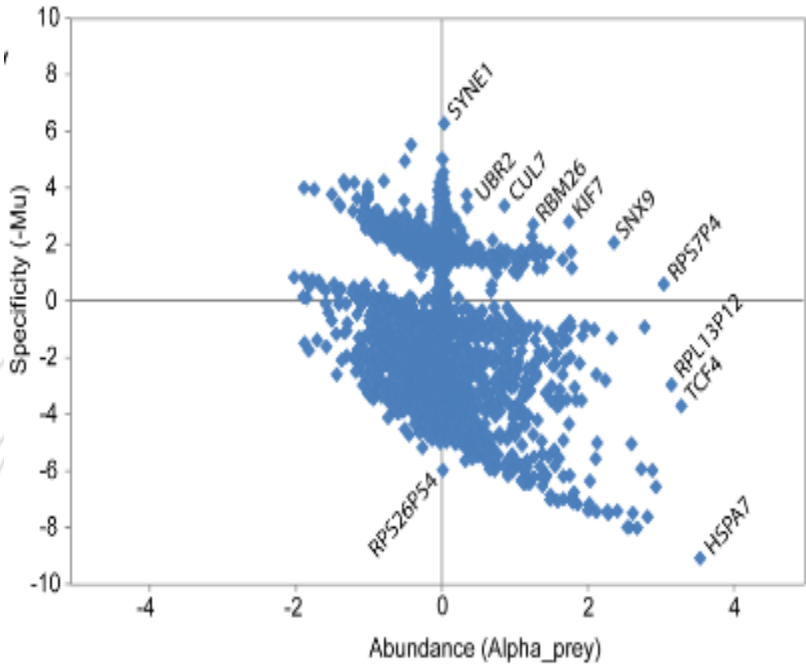

JUN
MAX
MYC
NFκB1
RBPJ
STAT3
50
37
37
25
75
50
150
100
75
50
100
75
β-Actin
SFB-JUN
SFB-MAX
SFB-MYC
SFB-NFKB1
SFB-RBPJ
SFB-STAT3
TCF4
TP53
FOXD3
FOXK1
FOXM1
FOXO3
100
75
75
50
75
50
150
100
150
100
100
75
β-Actin
SFB-TCF4
SFB-TP53
SFB-FOXD3
SFB-FOXK1
SFB-FOXM1
SFB-FOXO3

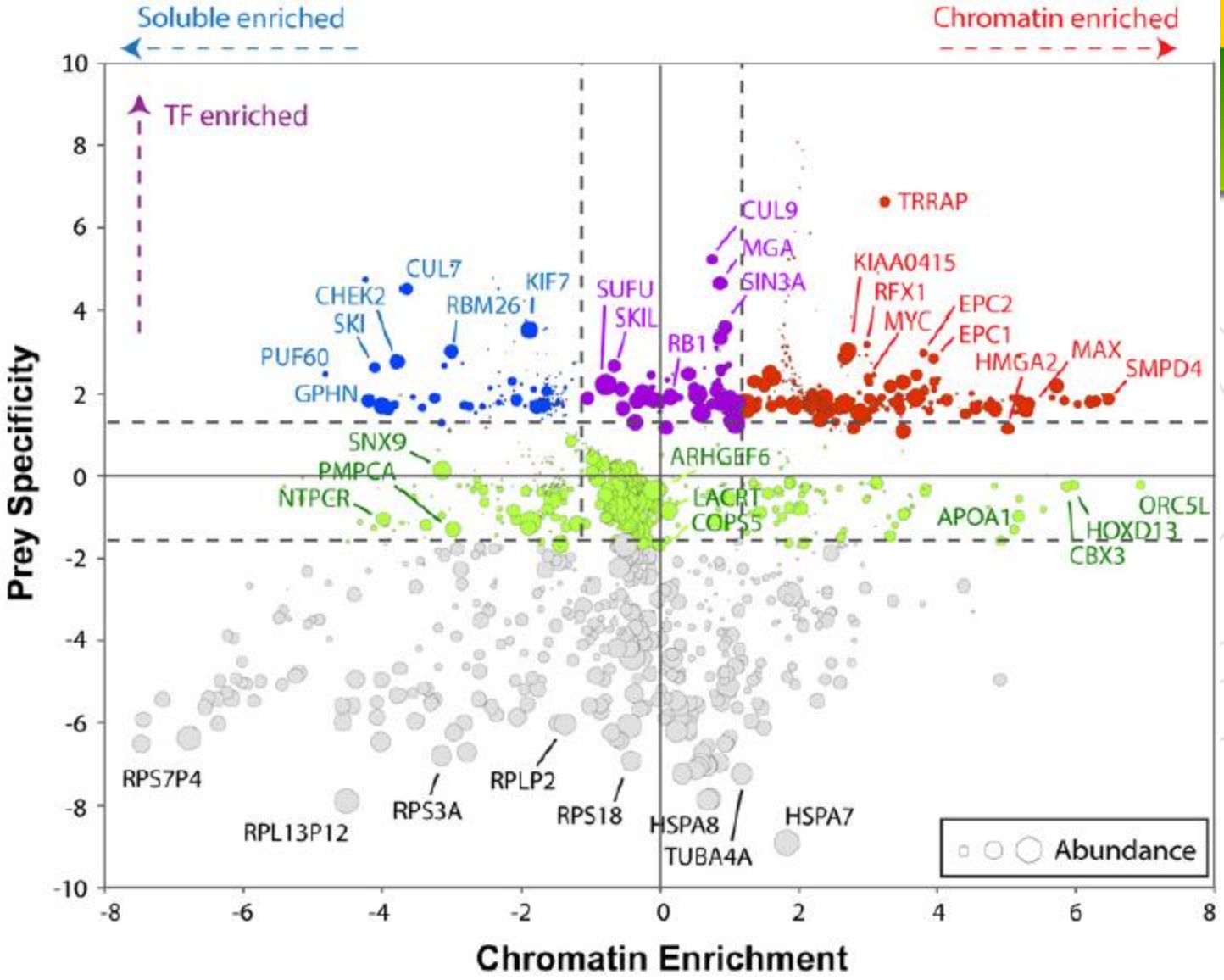
Prey Specificity



Chromatin Specificity



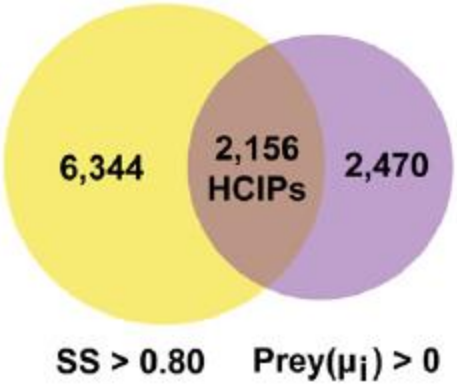




Comparative analysis of prey specificities of TFs over different fractions. The *y*-axis shows TF-binding specificities of preys: positive, specifically associate with transcription factors group; negative, no binding preference. The *x*-axis depicts fraction specificities of preys: positive and negative numbers indicate preference for their enrichment in chromatin and soluble fractions, respectively. The size of a coloured bubble indicates the log (overall abundance) of individual preys. The selected preys were categorized into four groups based on their positions highlighted with different colours: red, specific co-regulators of TFs that may be involved in transcriptional regulation; purple, regulators with no fractional preference; blue, transcription-unrelated functions or negative regulators of TFs; green, potential regulators with less specificity; plus a group of abundant proteins with no binding preference, which were shown at the bottom of the map (grey).

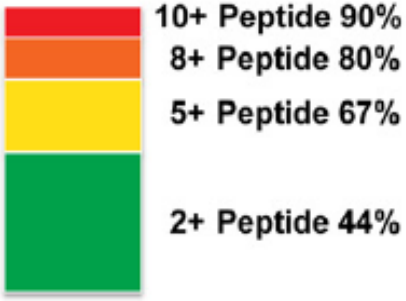
B

Data filtration and generation of HCIPs



C

Data reproducibility



The HCIP overlap ratio rises with the peptide numbers.

D

Knowledge database overlap of HCIPs

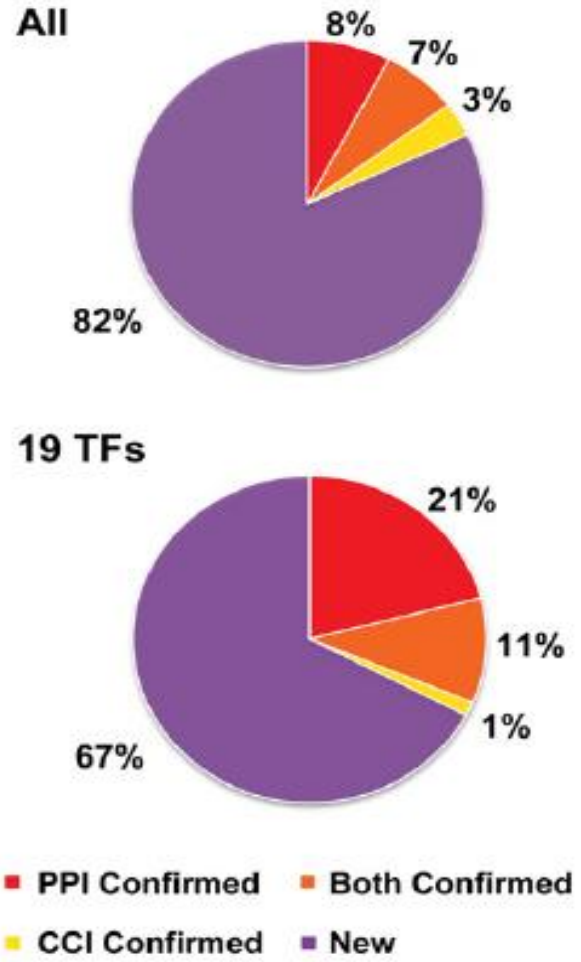
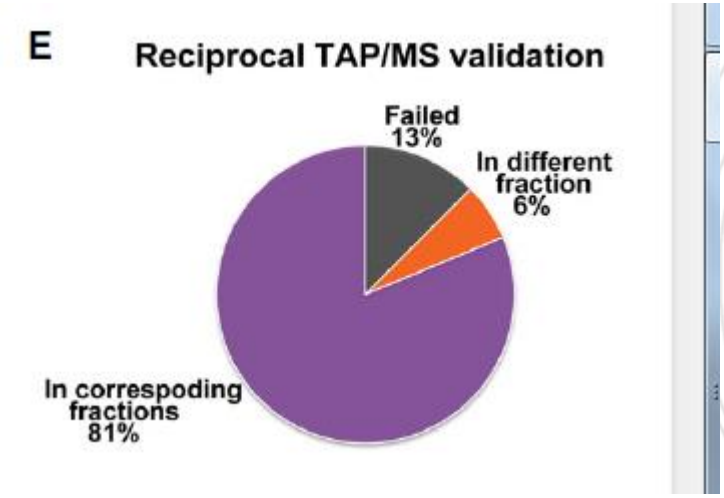


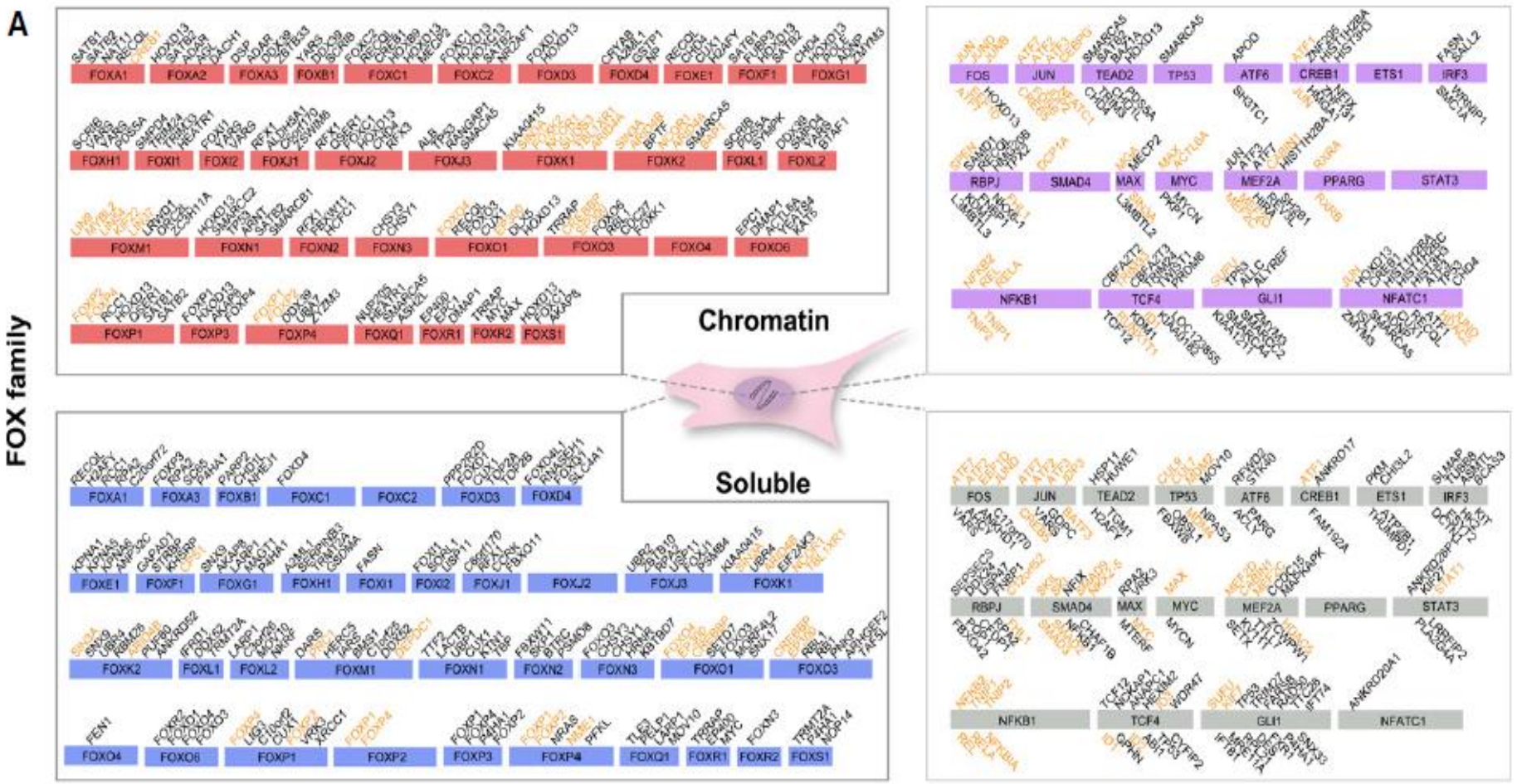
Table 2. Summary of reciprocal purifications results.

Bait	Prey	In bait fraction	In prey fraction	Bait	Prey	In bait fraction	In prey fraction
MAX	L3MBTL2	Chr	Sol	RBPJ	L3MBTL2	Chr	Sol
MAX	E2F6	Chr	Chr	RBPJ	KDM1	Chr	Chr
MAX	FOXK2	Chr	Chr	RBPJ	FBXO42	Chr/Sol	Chr/Sol
NFATC1	JUN	Chr	Chr	CREB1	ATF1	Chr/Sol	Chr/Sol
NFATC1	HOXD13	Chr	Chr	CREB1	HMGA1	Chr	N
NFATC1	CREB1	Chr	Chr	CREB1	ZNF131	Chr	N
NFATC1	ATF3	Chr	Chr	CREB1	NFIX	Chr	Chr
NFATC1	ATF1	Chr	Chr	CREB1	NFATC2	Chr	Chr

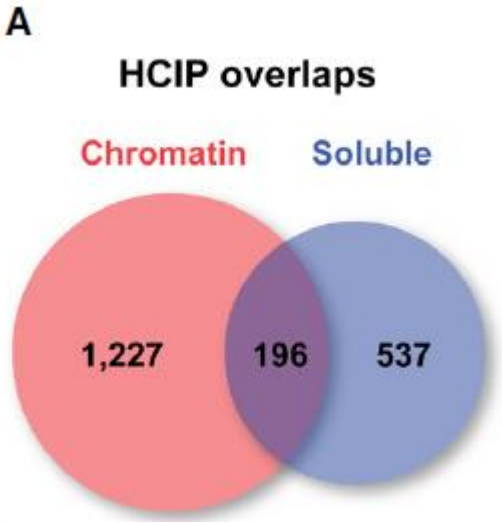
Reciprocal purifications of 16 interactions identified from MAX, NFATC1, RBPJ and CREB1 purifications were performed with the same TAP/MS protocol. Chromatin and soluble fractions were separated and whether the corresponding baits appeared in the reciprocal purification was indicated by fraction name or "N".

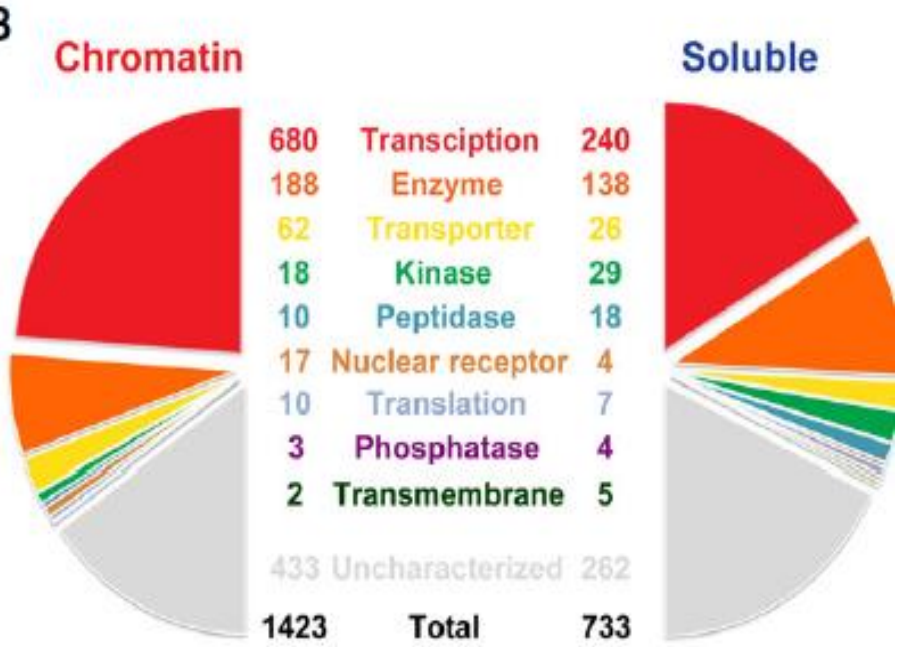


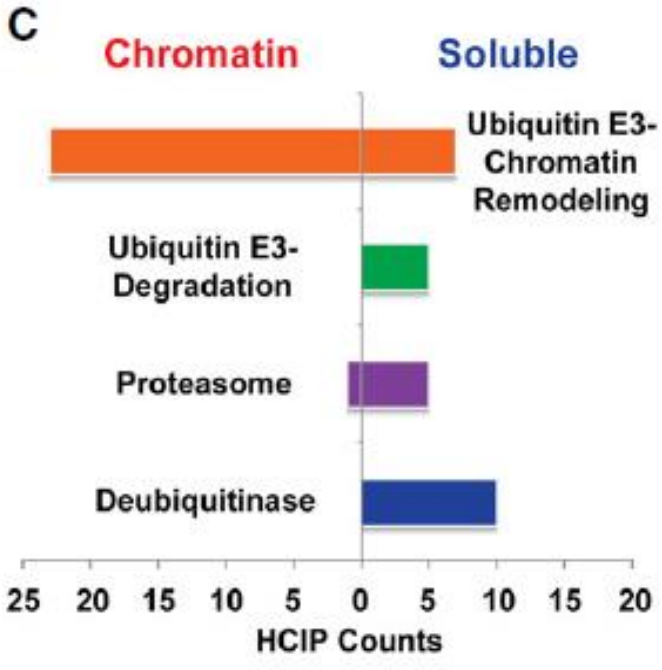
我们的数据是可靠的



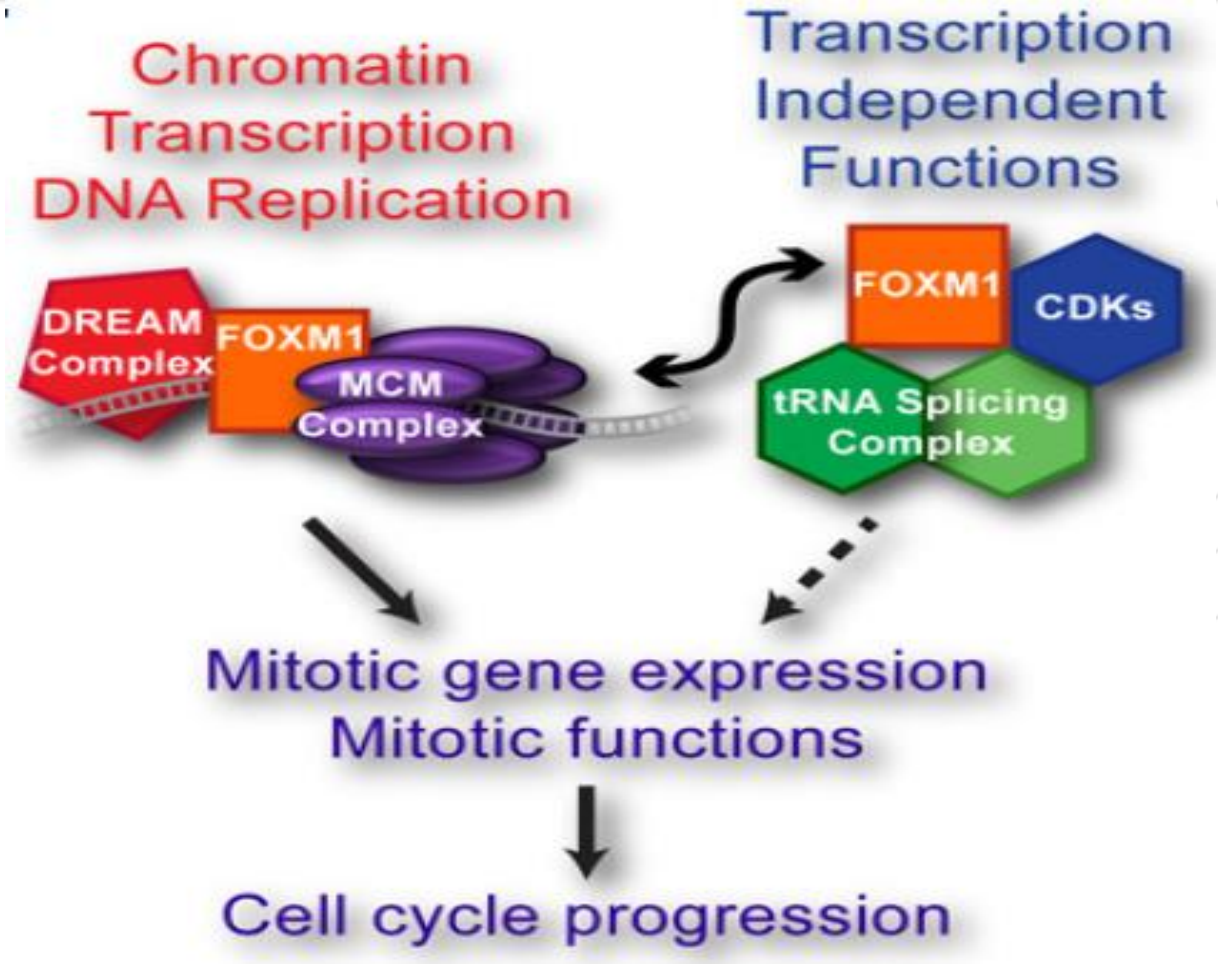
HCIPs with highest spectra counts were listed. The length of each box with the protein name on it indicates the protein size. Black fonts indicate new interactions identified by our purifications. Orange fonts indicate interactions defined by our purifications and the literature.



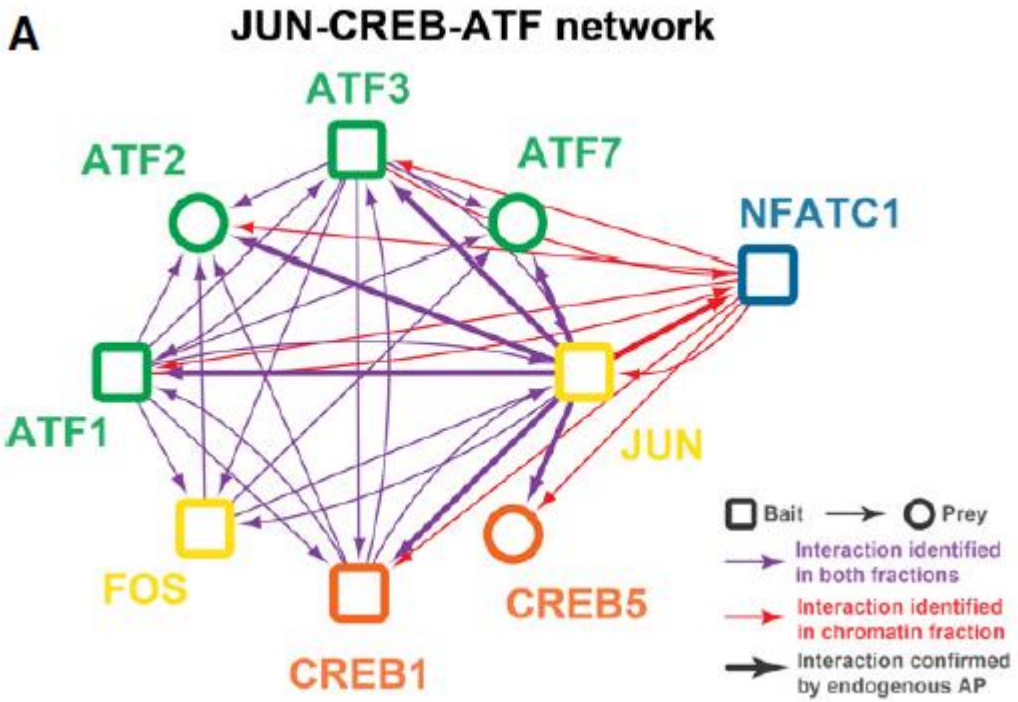




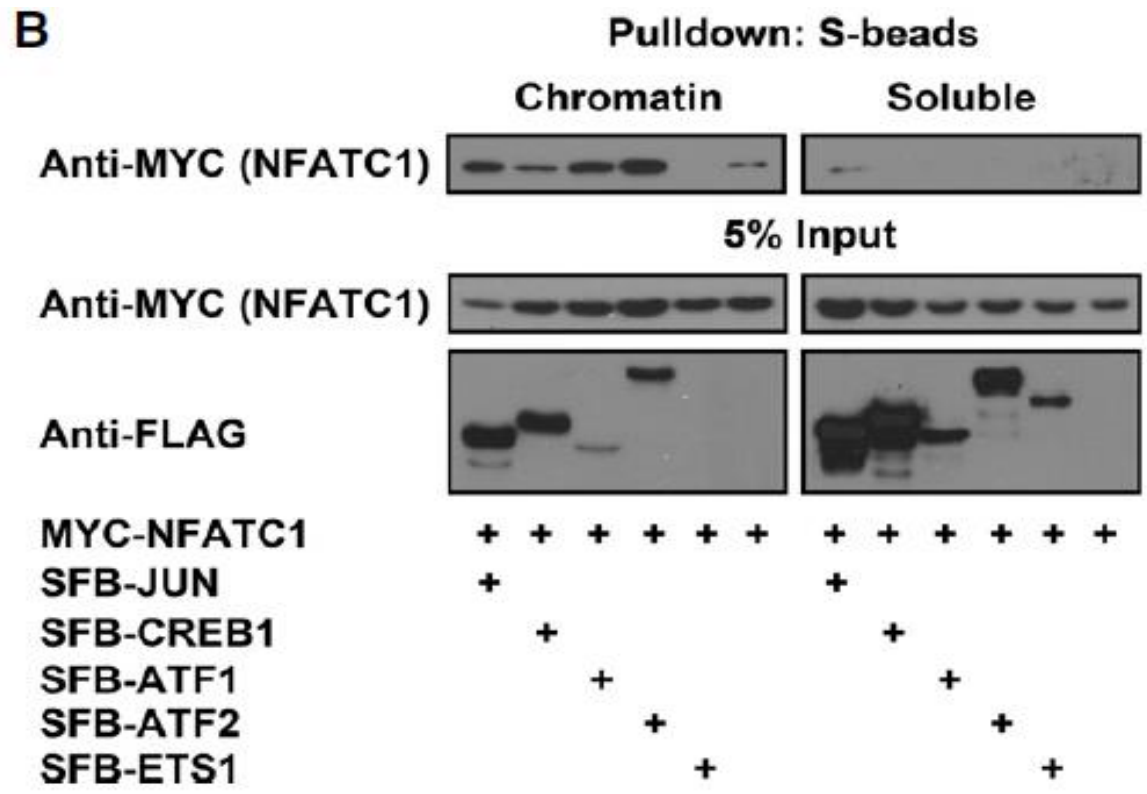
转录复合体的位点特异性是与其功能相关的



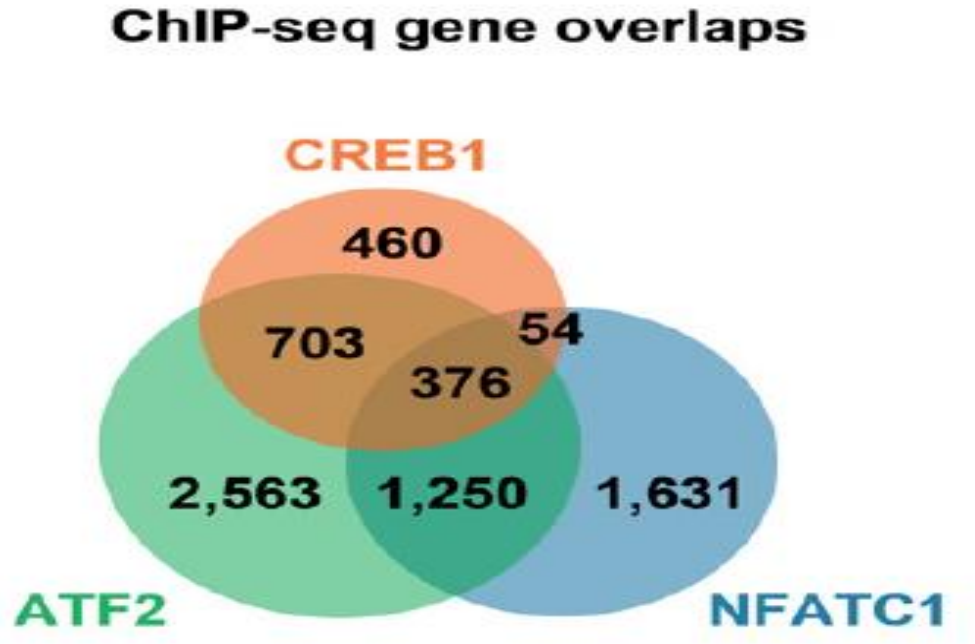
同一种转录因子可能在不同的位点与不同的蛋白质形成复合体并执行不同的功能



we built a JUN/CREB/ATF network



Western analysis confirmed that NFATC1 binds to JUN, CREB1 and ATF1/2 predominantly in the chromatin fraction



JUN/CREBs/ATFs形成稳定复合体并与NFATC一起调控基因转录。这证实了对转录复合体的位点特异性进行分析可以预测其可能的功能。

Discussion

In this study, we revealed chromatin-associated and soluble complexomes for each of the 56 TFs, which validated our hypothesis that TFs form unique protein complexes on and off chromatin. These results and other information presented in this study offer new insights into the regulation of TFs and their diverse in vivo functions.

主要结论：转录复合体具有位点特异性，不同的转录因子可能在不同的位点与不同的蛋白质形成不同的复合体并执行不同的功能。

创新点：两步筛选，多种验证方法并用，提高了数据的可信度

启发：用缜密的思维方法去做实验，并学会用系统的方法去分析实验数据

需要改进的问题：

1.有些蛋白质在排除一些通用互作蛋白如分子伴侣后无法纯化出来，因其不能正确折叠；

2.一些有瞬时相互作用的蛋白质不容易检测到，在两部亲和纯化过程中可能丢失，从而造成互作网络的小缺陷。