

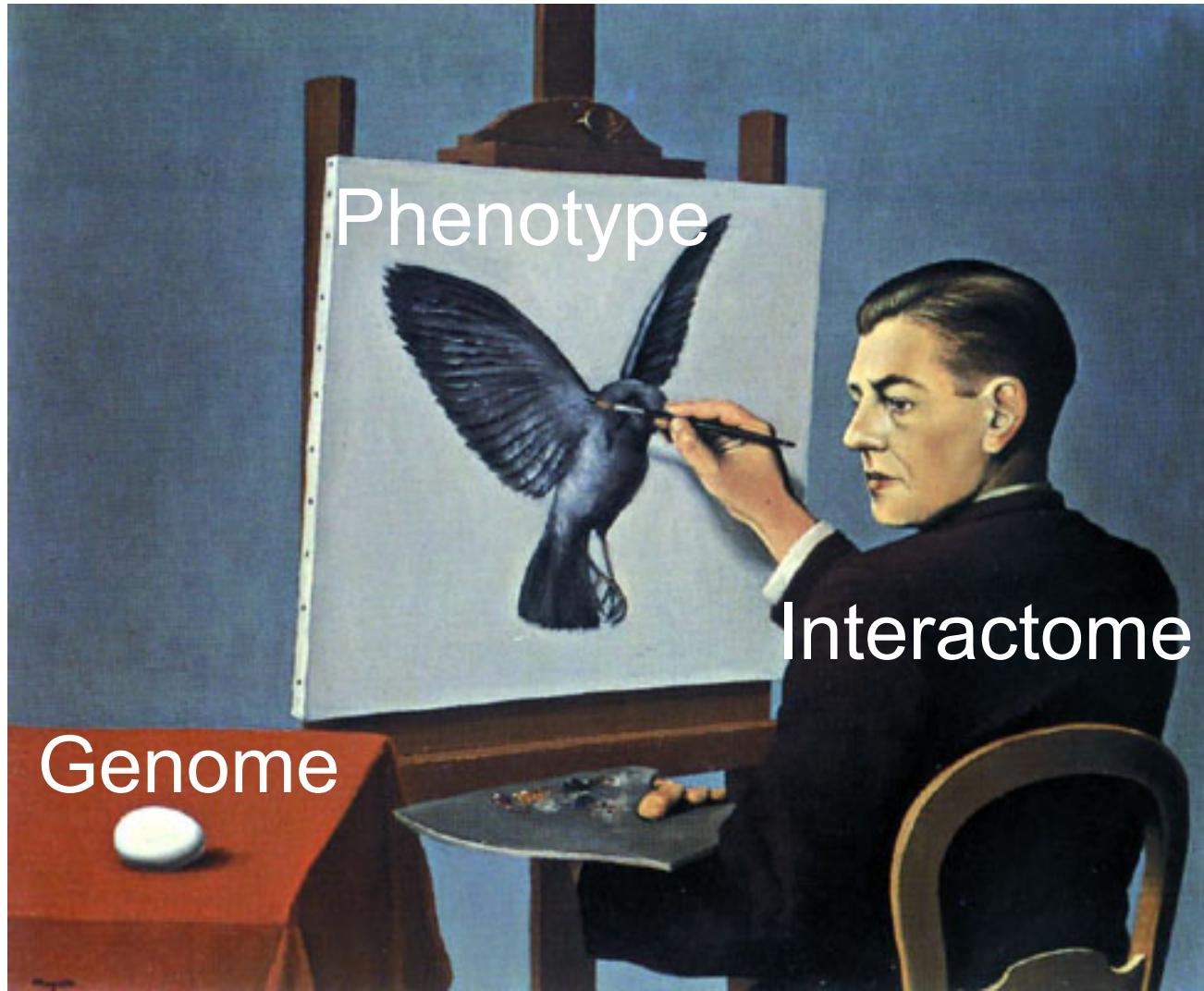


Protein – protein interactions

*Interactome networks drive
molecular organisation of the cell*

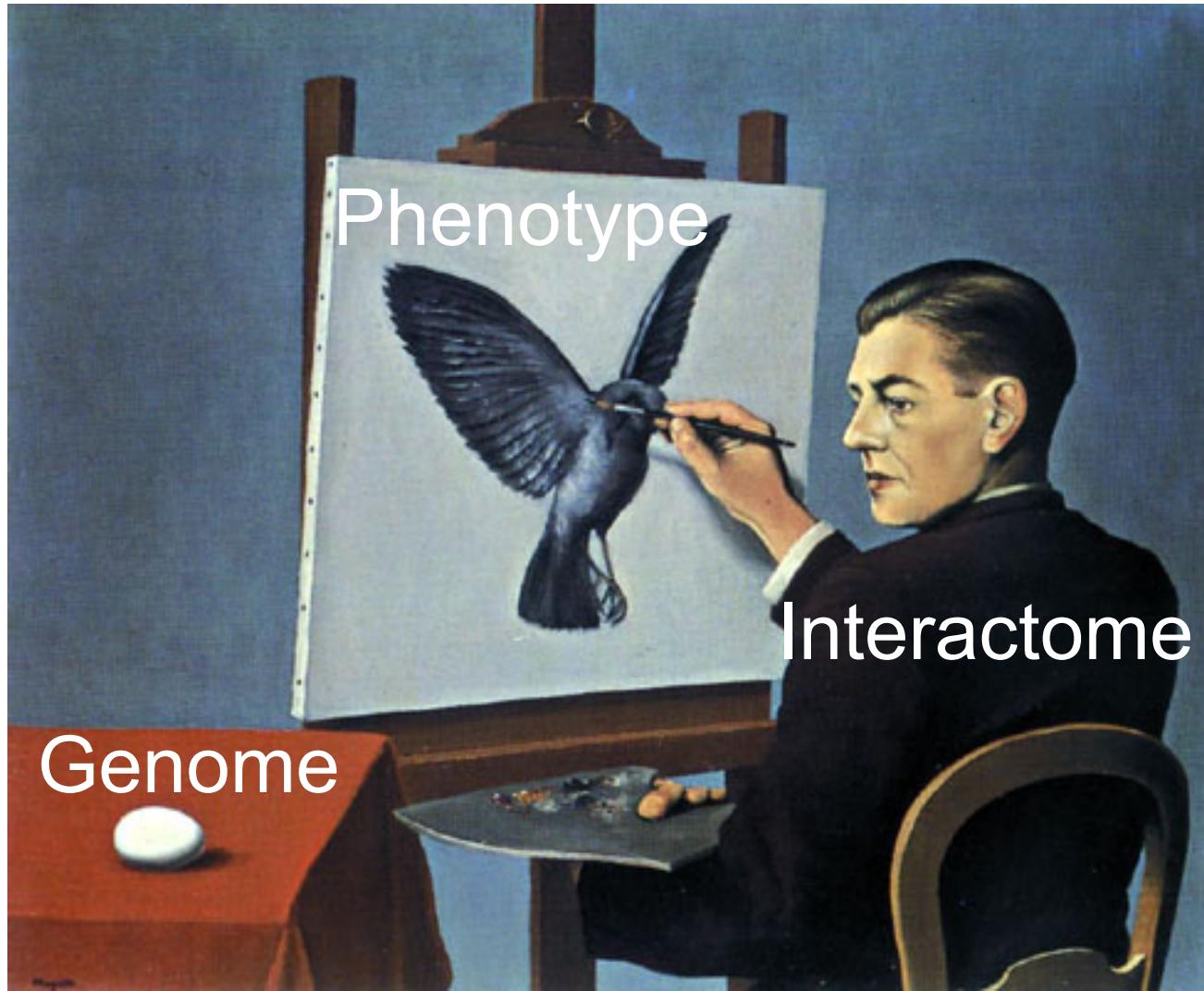
Dr. Jean-Claude Twizere

Jean-claude.twizere@ulg.ac.be

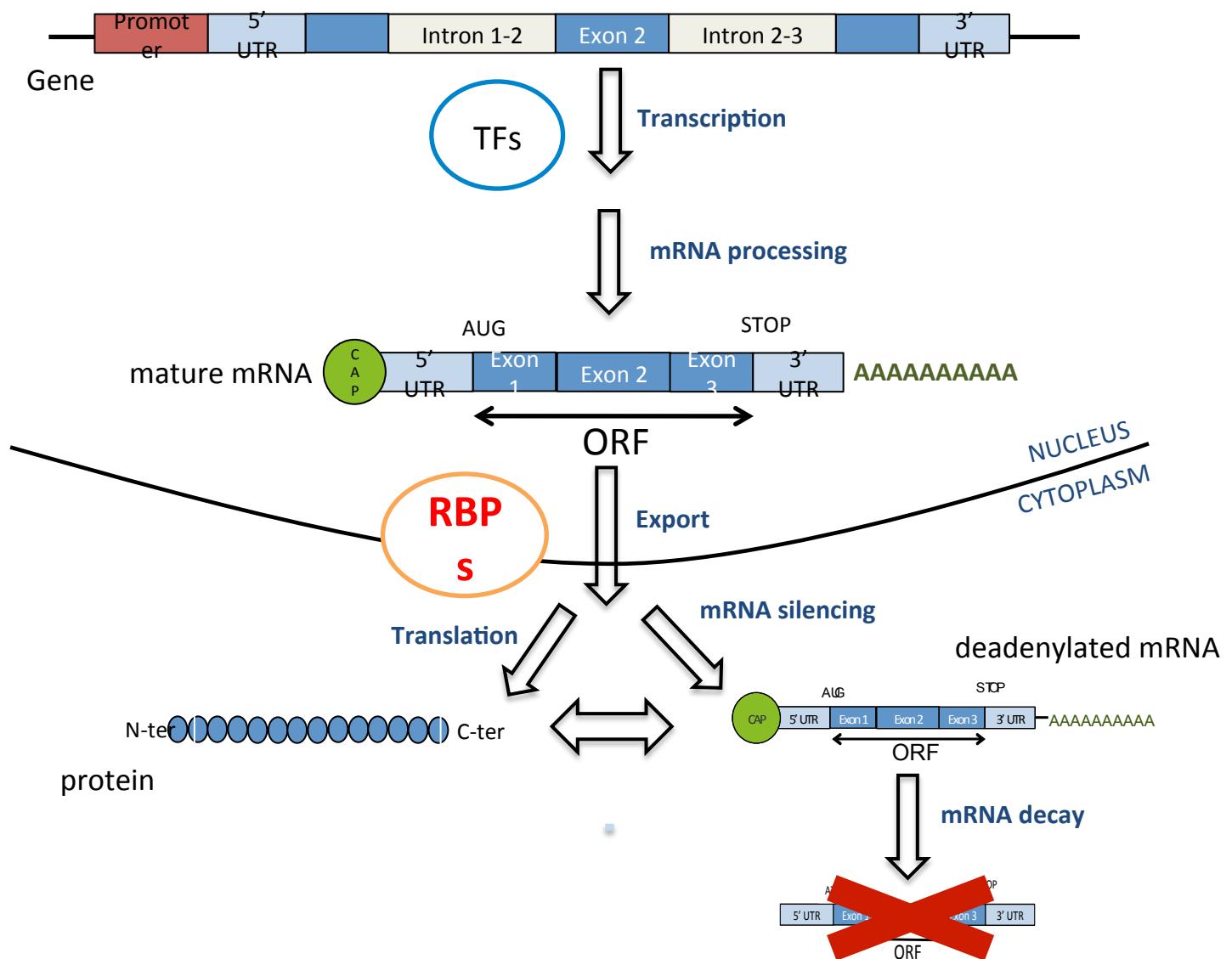


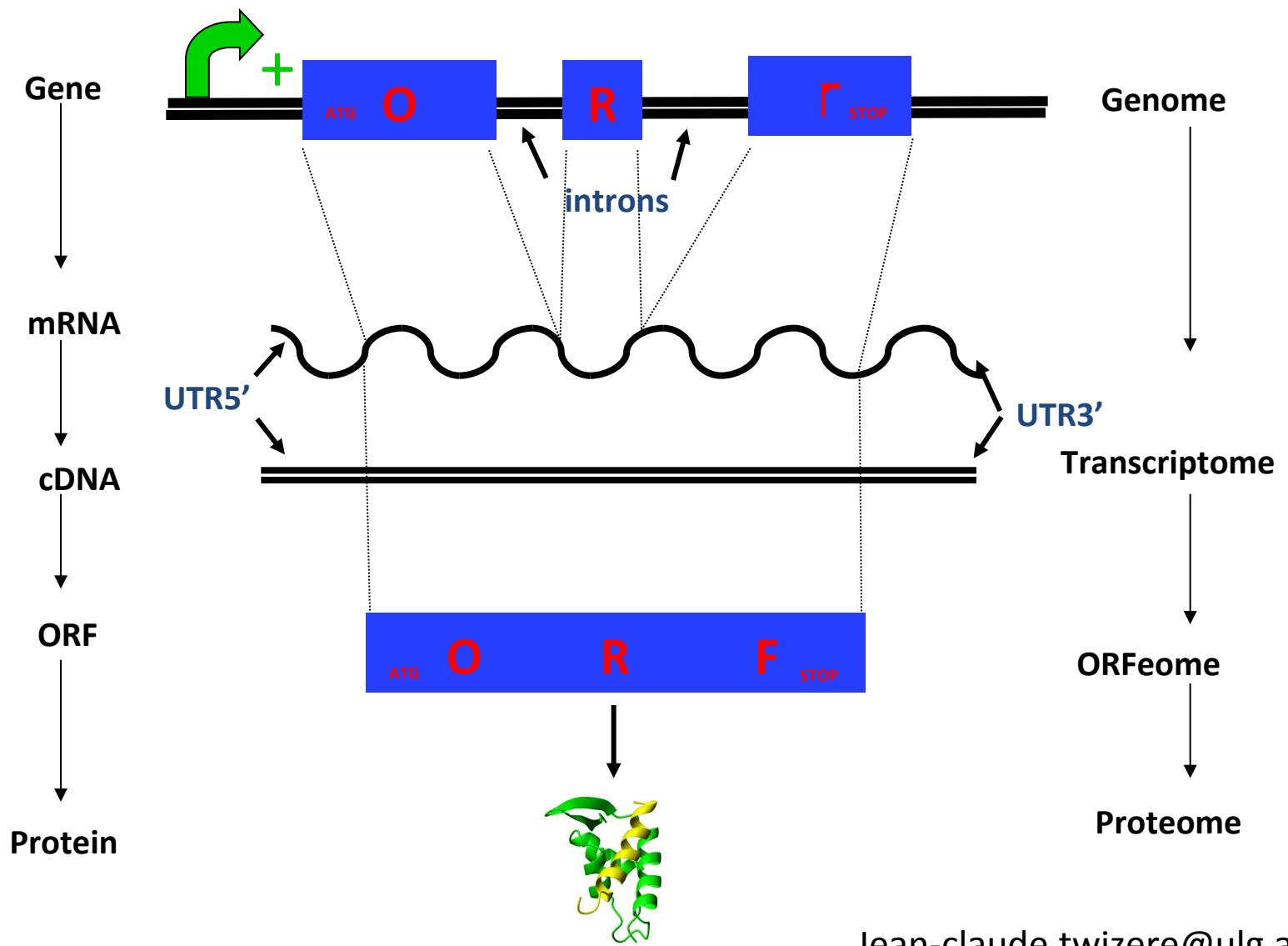
Molecular organisation of the cell

- Elucidation of Genomes, proteomes, their components and interactions
- Functional organization remains largely unknown
- Cellular function is the result of coordinated interactions
- Interaction networks essential to understand biology, disease and/or drug action

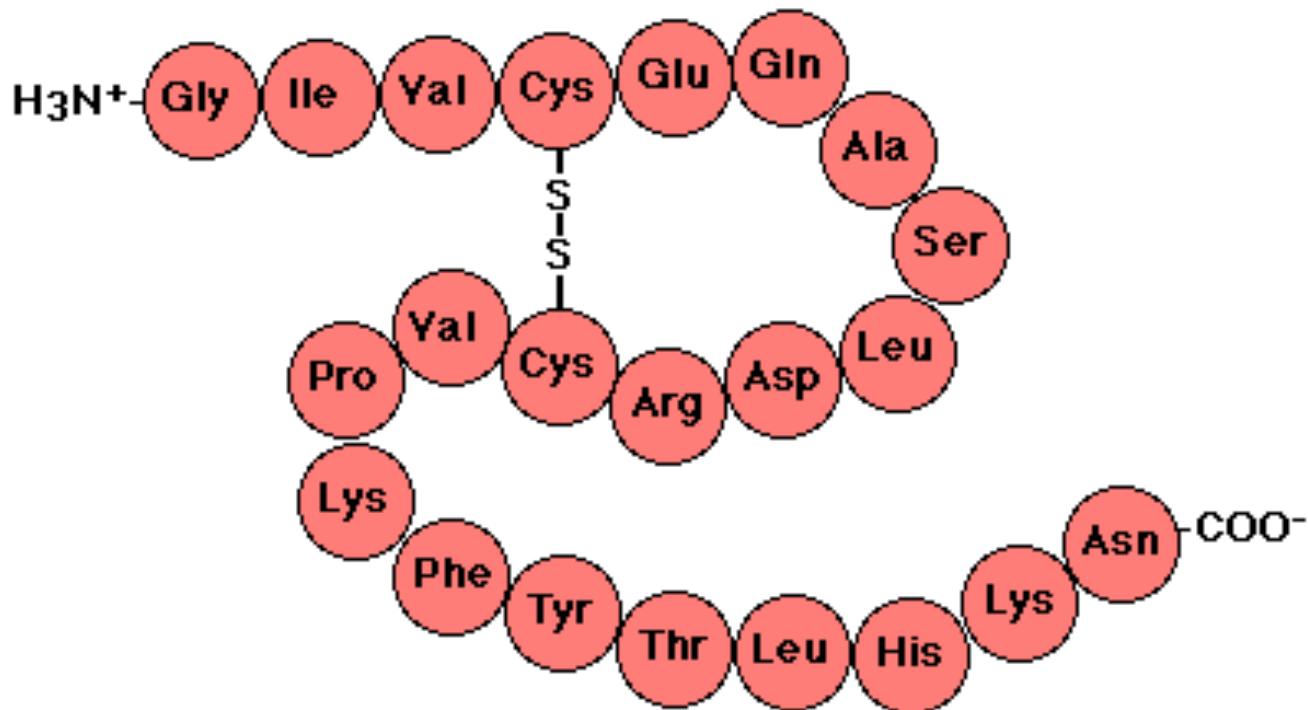


Gene expression regulation



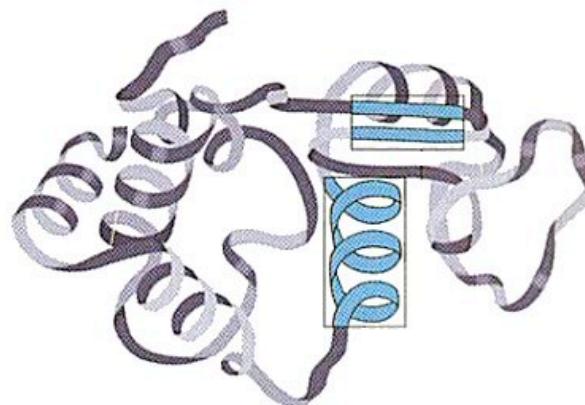


Primary structure

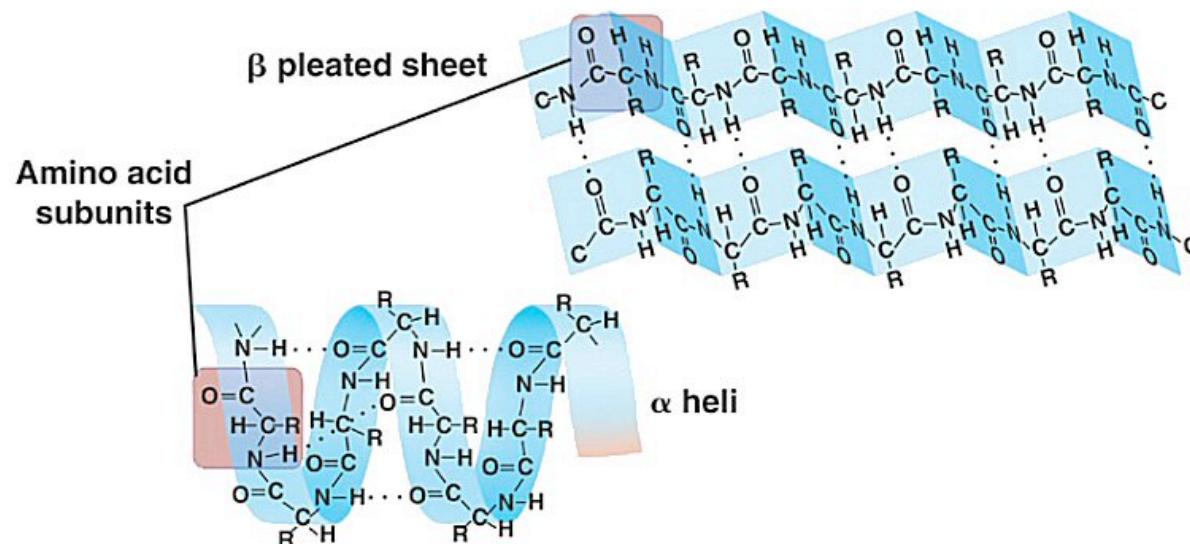


Secondary and tertiary structure

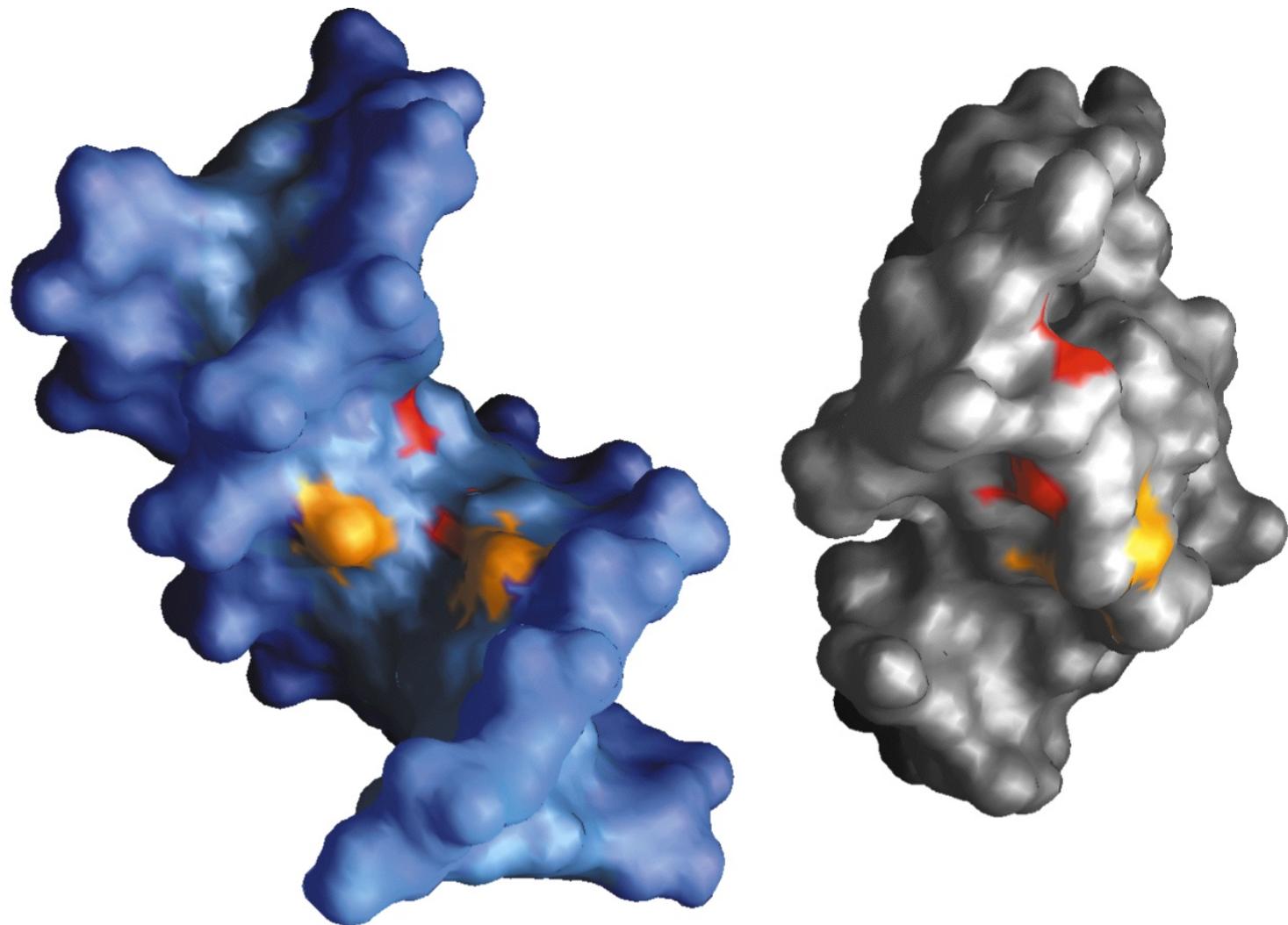
III.



II.

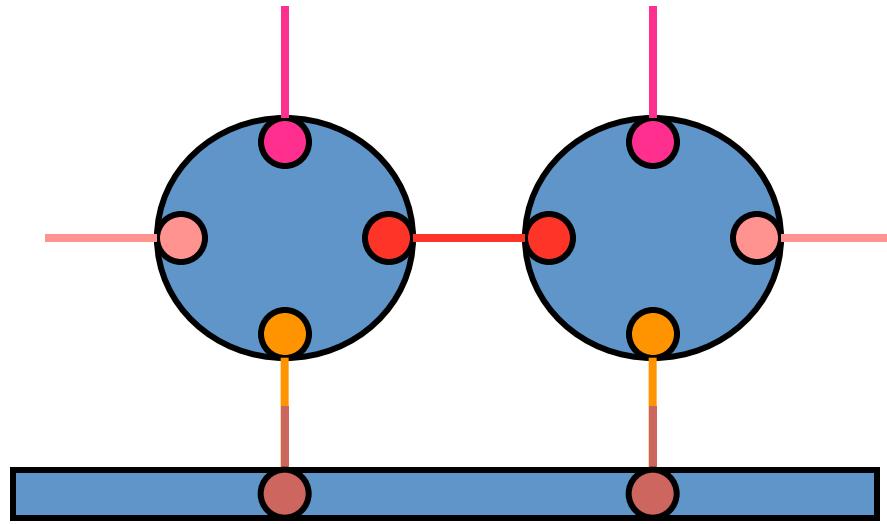


Quaternary structure



(d)

Protein Interactions

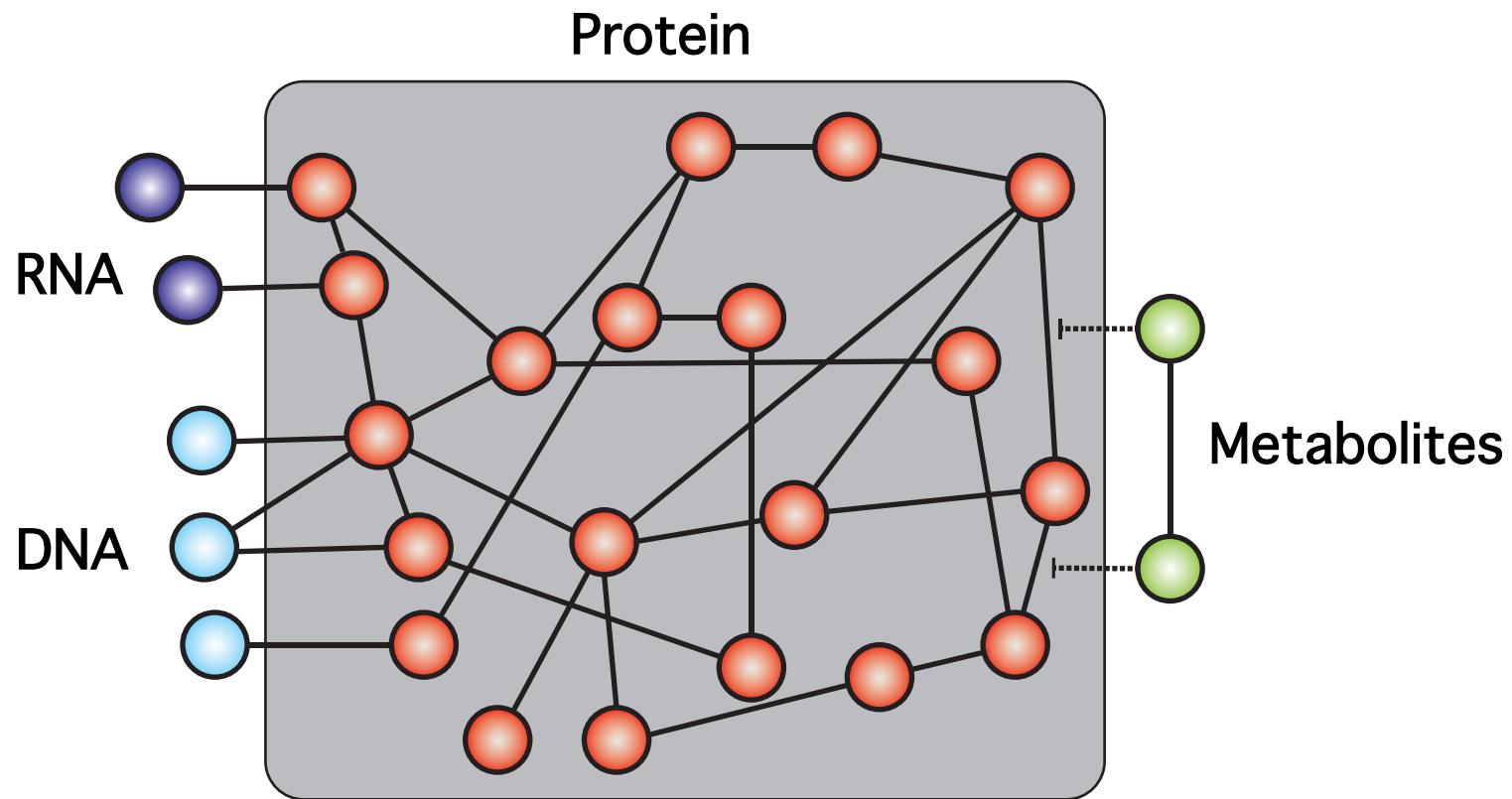


Homodimerization and DNA/protein interaction

Protein-protein interactions

- Y2H hybrid
- Affinity purification
- Energy transfer (Fluorescence = FRET)
- Co-localisation (Fluorescence based)
- Protein complementation
 - Luciferase based
 - Fluorescence based

The protein interactome network



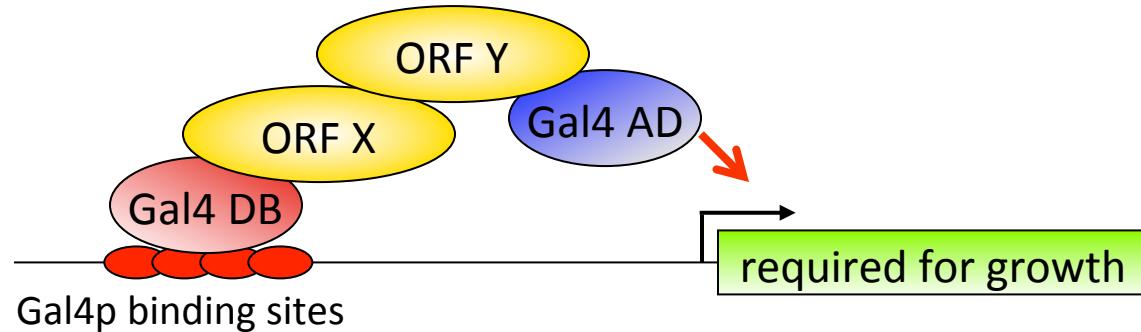
Nodes: Proteins, DNA, RNA or Metabolites

Edges: Bio-physical interactions

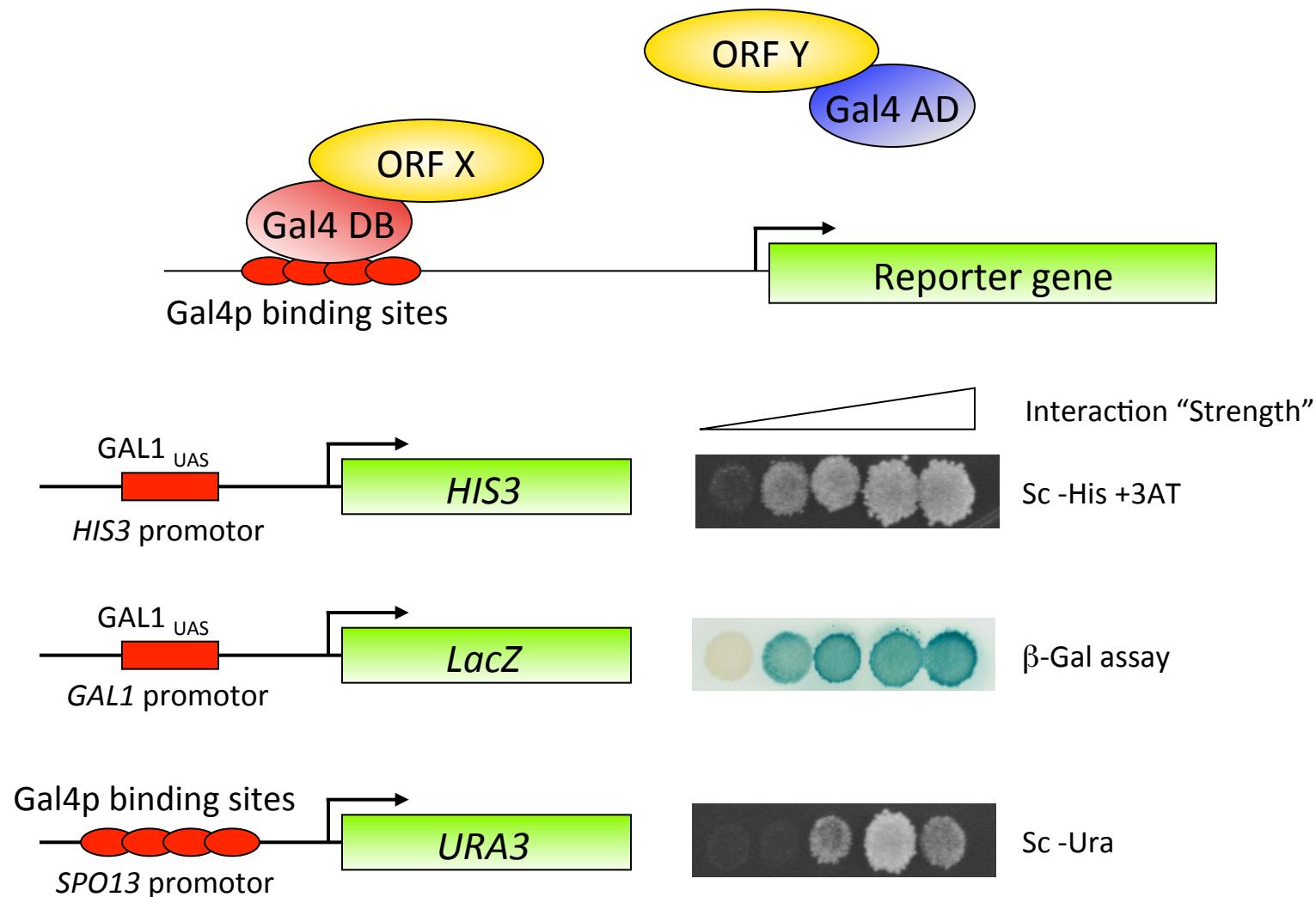
Discovering interactions: Yeast two-hybrid

Yeast two-hybrid

- Reconstitution of GAL4 transcription factor
- Fusion proteins DB-ORFX and ORFY-AD
- Reporter gene



A positive selection of the protein – protein interactions



Yeast two-hybrid

Reagents (retroviruses side)

genes + fragments



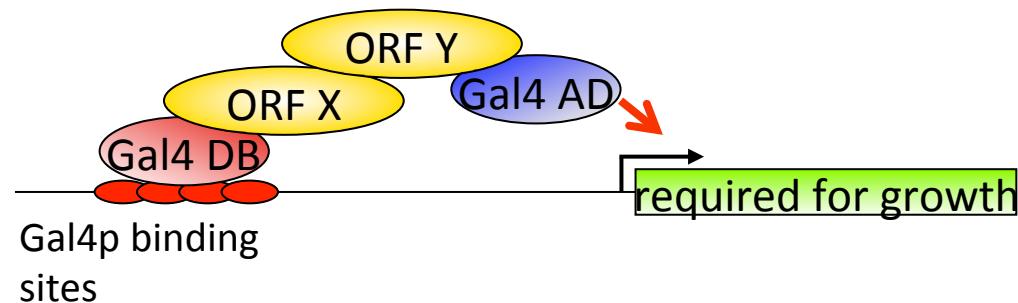
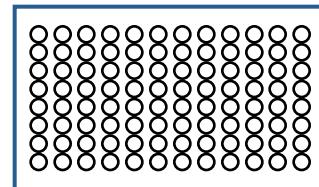
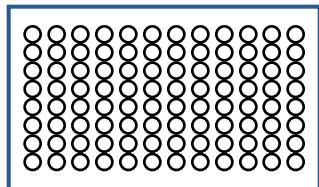
Gateway cloning

DB, AD expression vectors

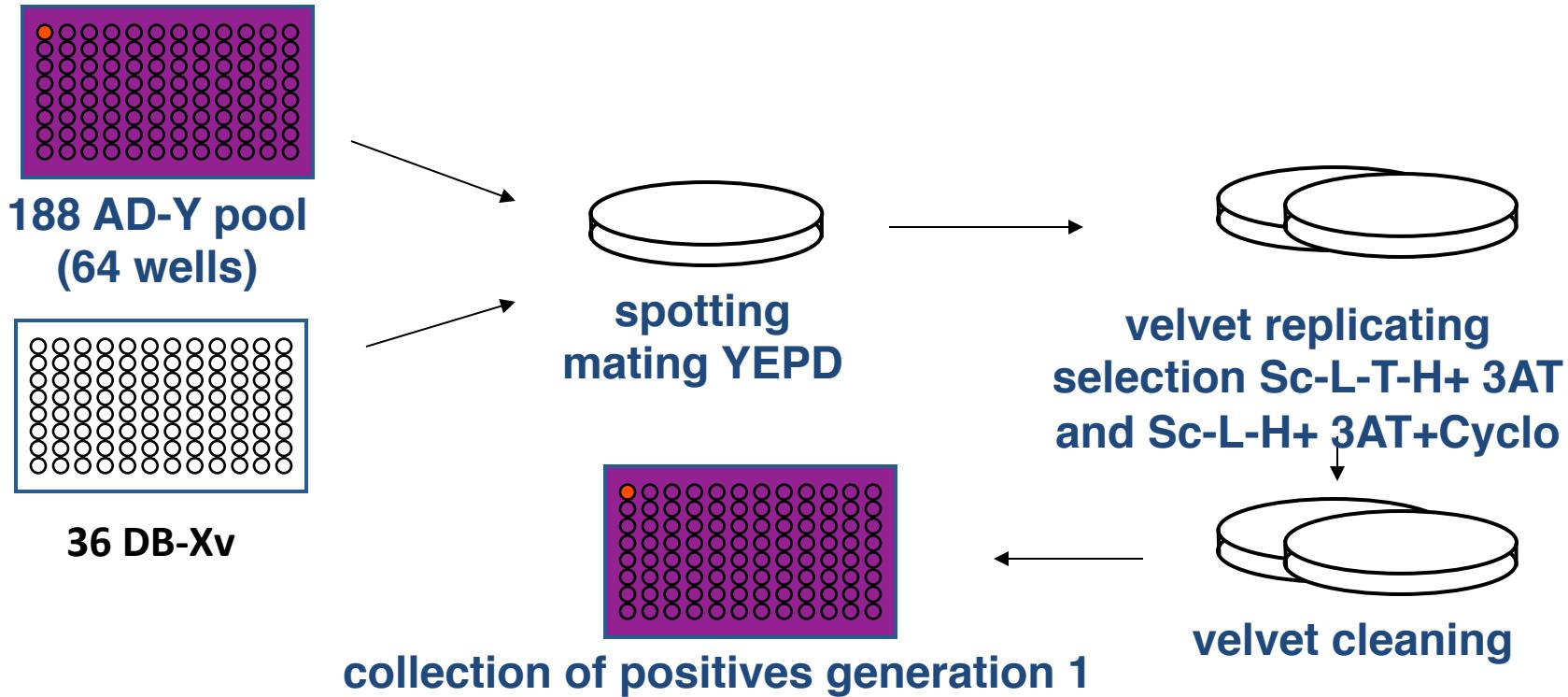
Yeast transformation

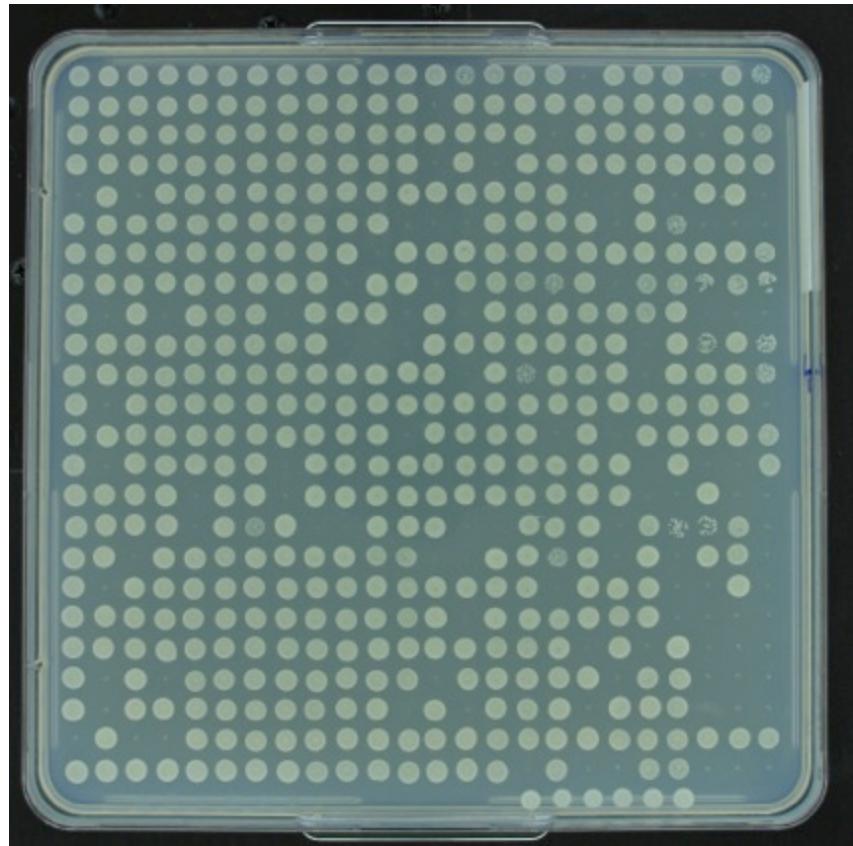
Mat a (Y8900 or Mav103)

Mat a (Y8800 or Mav203)

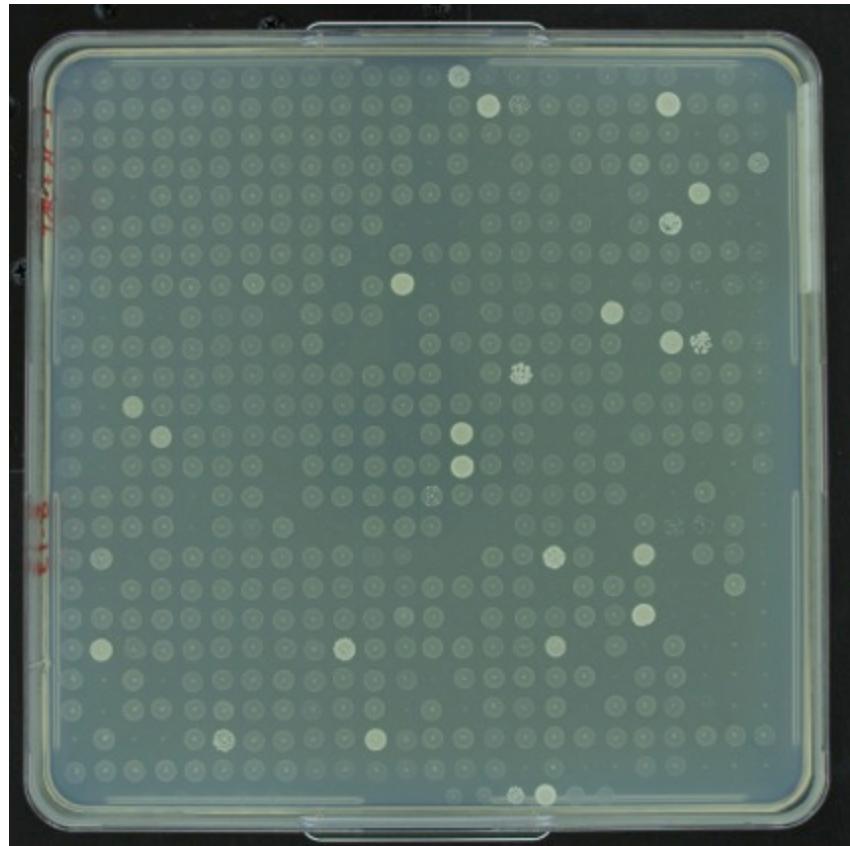


High-throughput Y2H mating



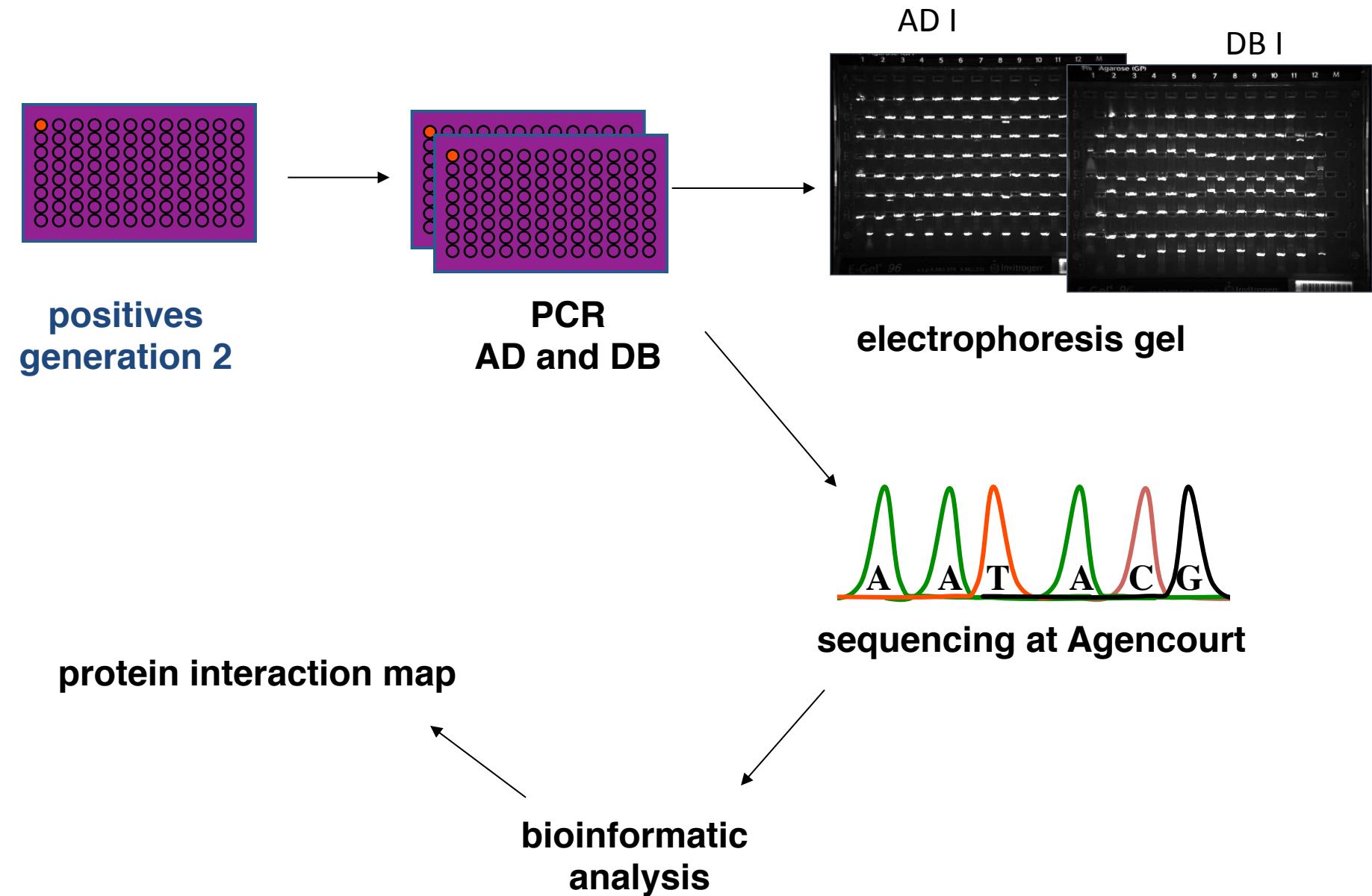


SC-LT

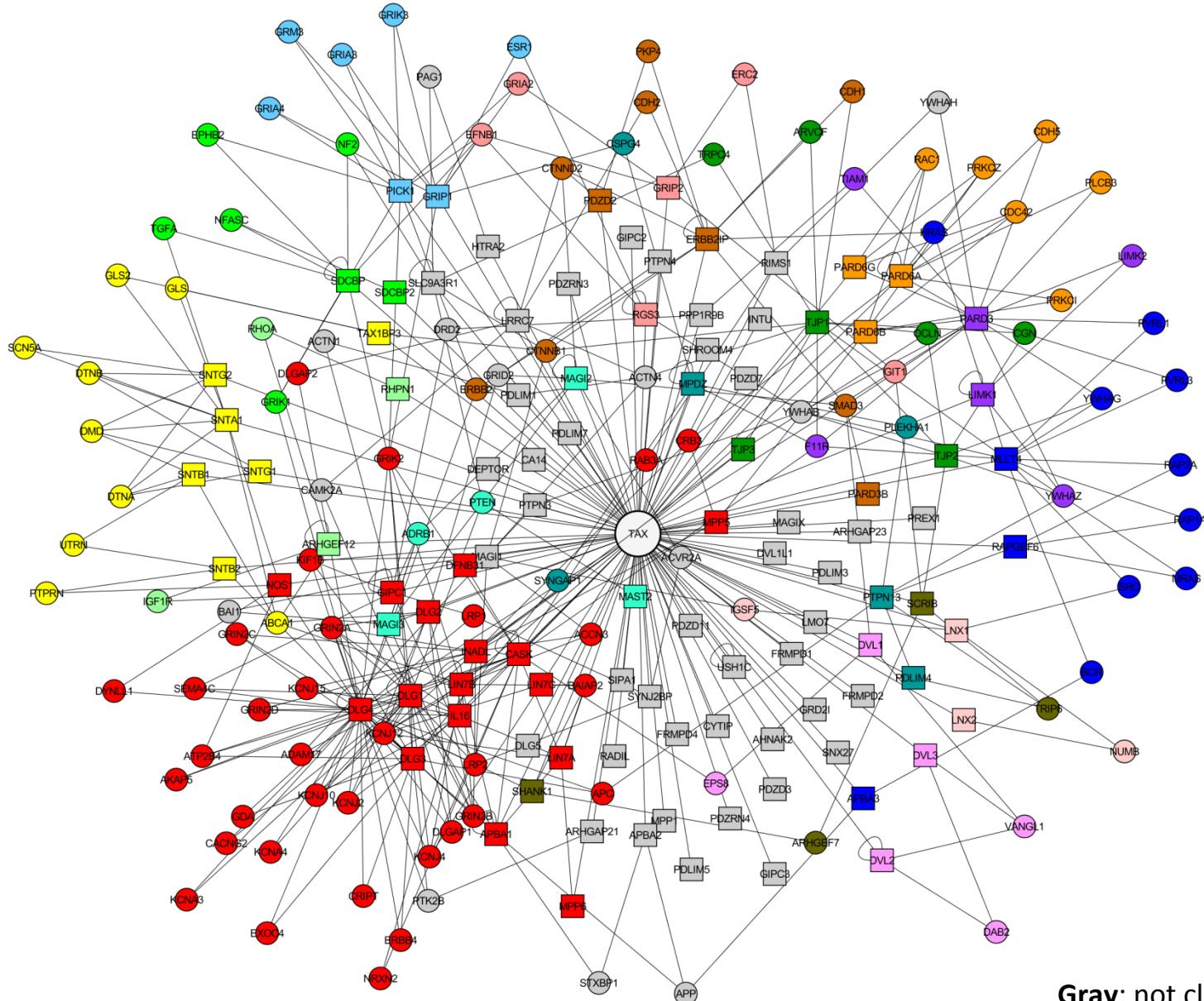


SC-LTH +1 mM 3AT

Sequencing

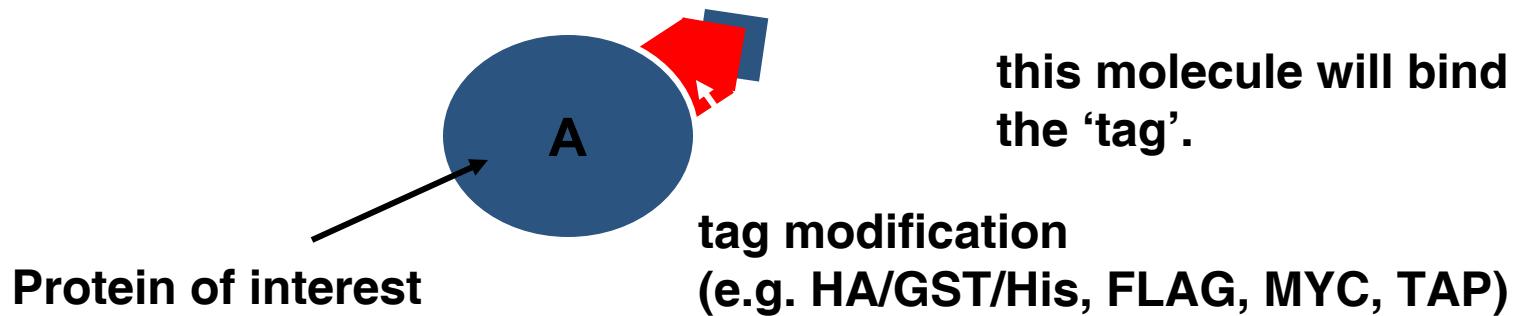


TAX / PDZ PROTEINS INTERACTOME

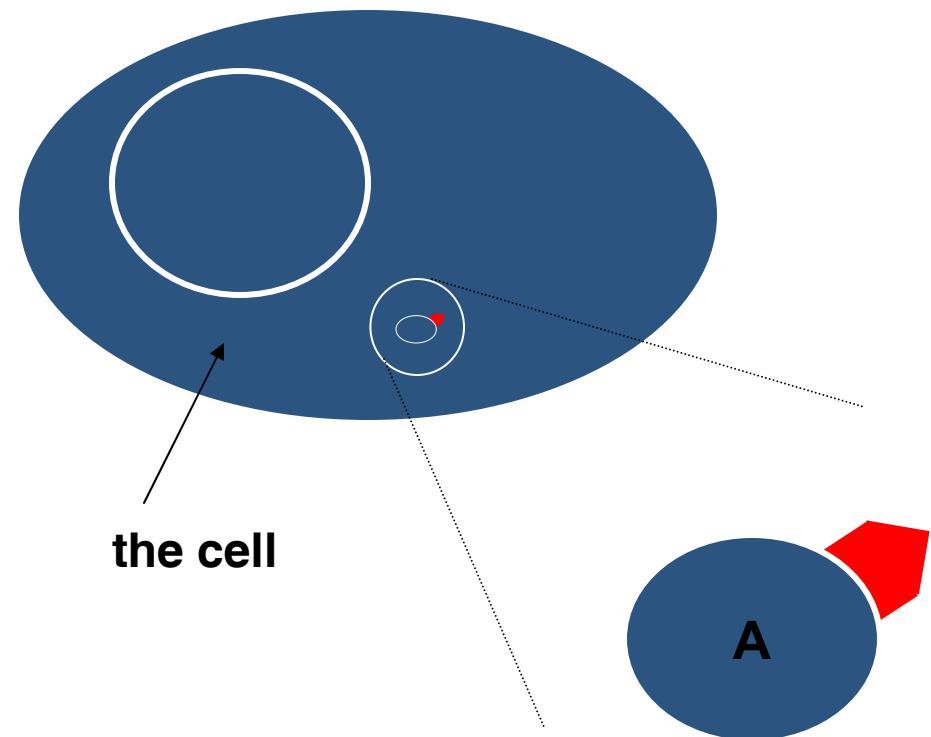


Gray: not clustered
Other color: clustered

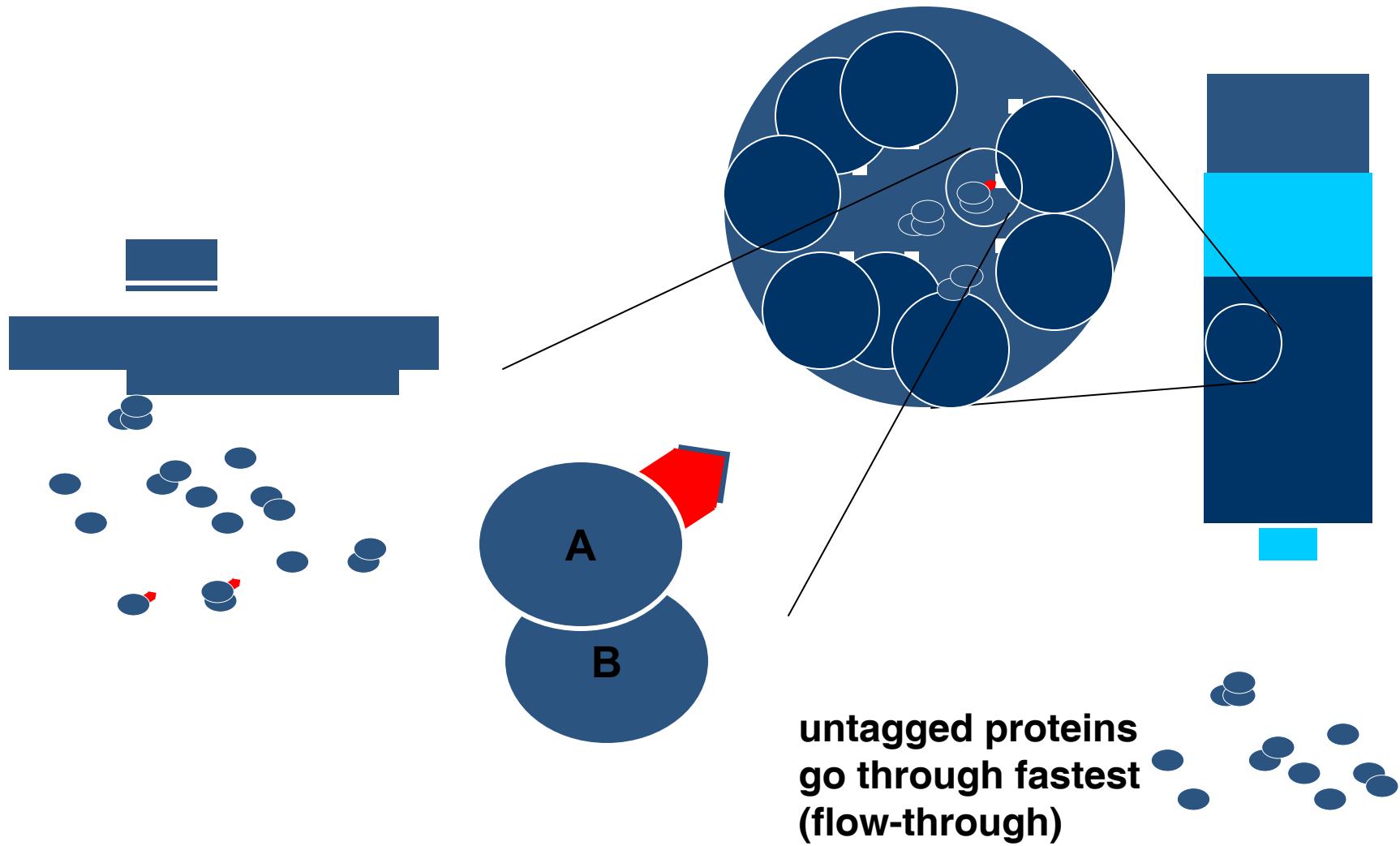
Affinity purification/mass spectrometry

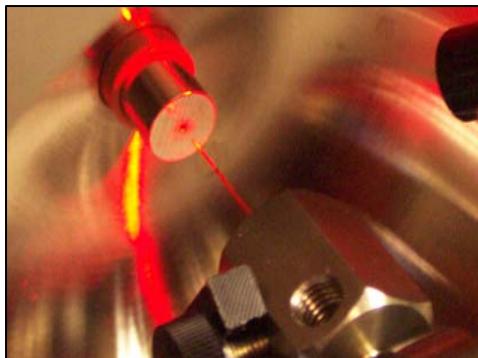
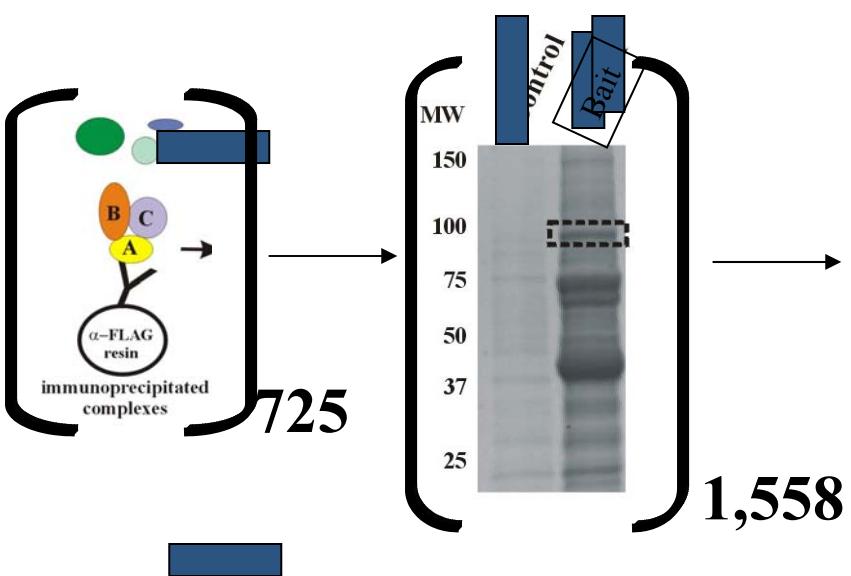


Affinity purification/mass spectrometry

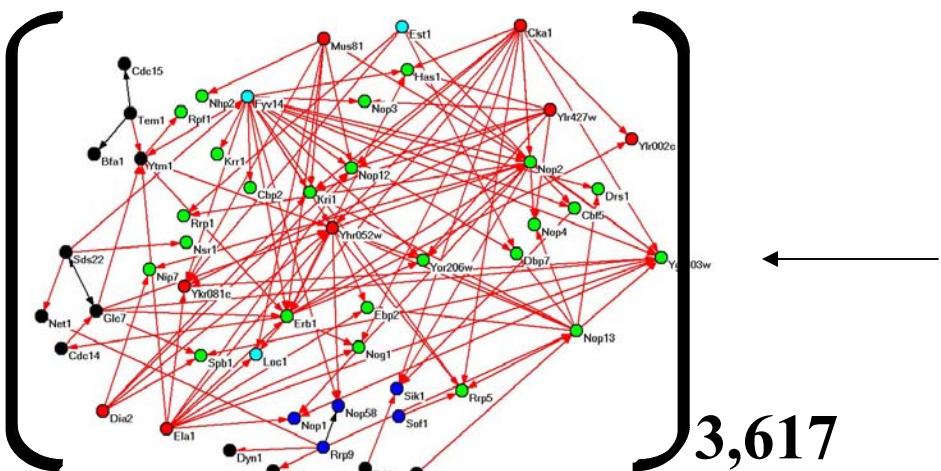
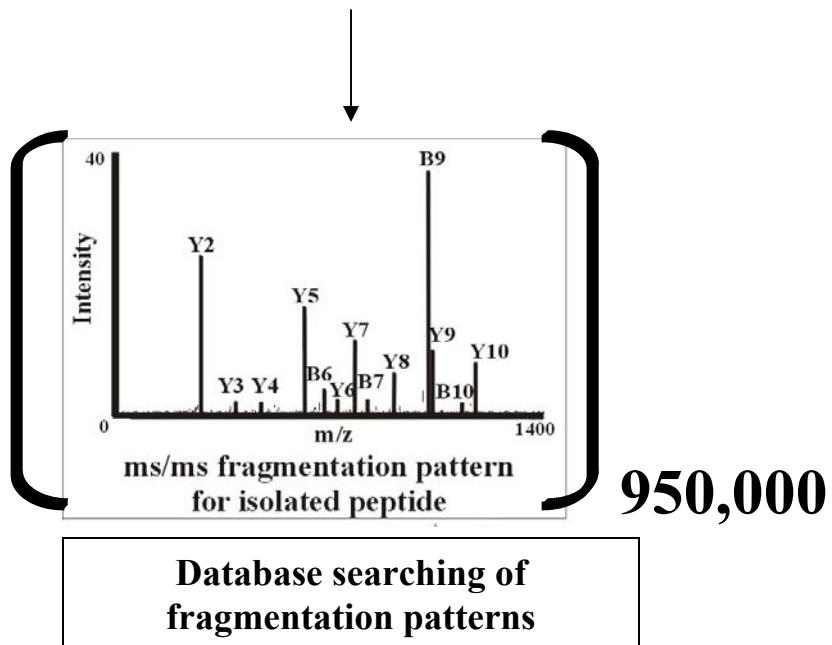


Affinity purification/mass spectrometry

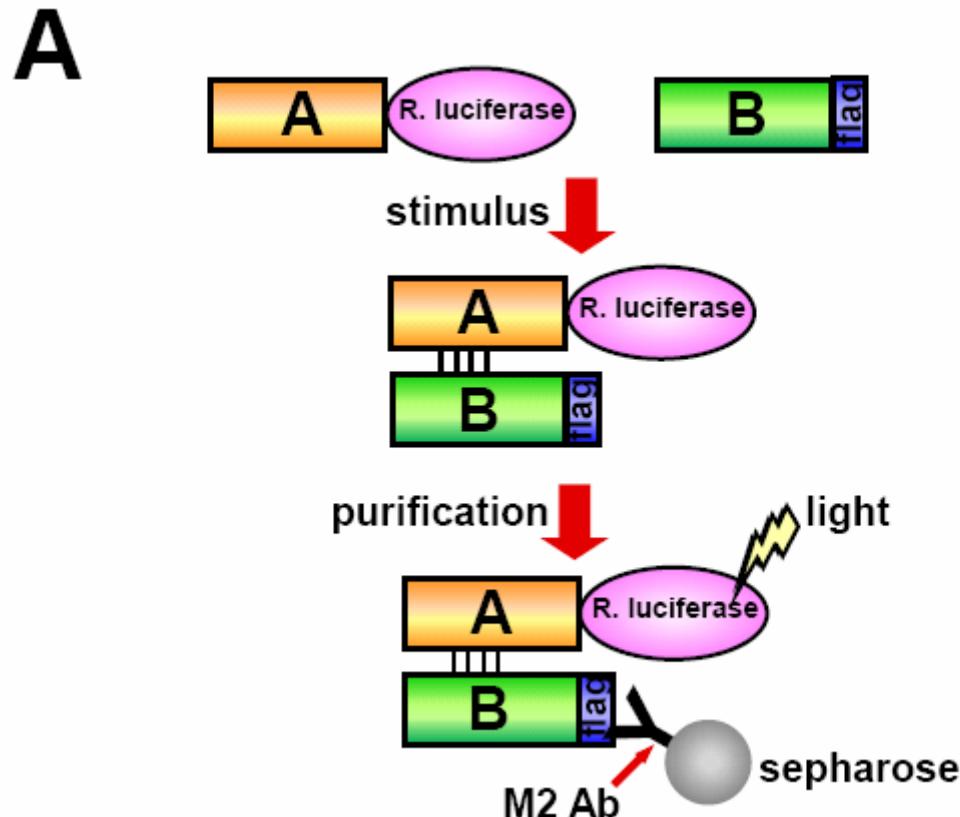




HTP-MS/MS
Network



Luminescence-based Mammalian Interactome mapping (LUMIER)

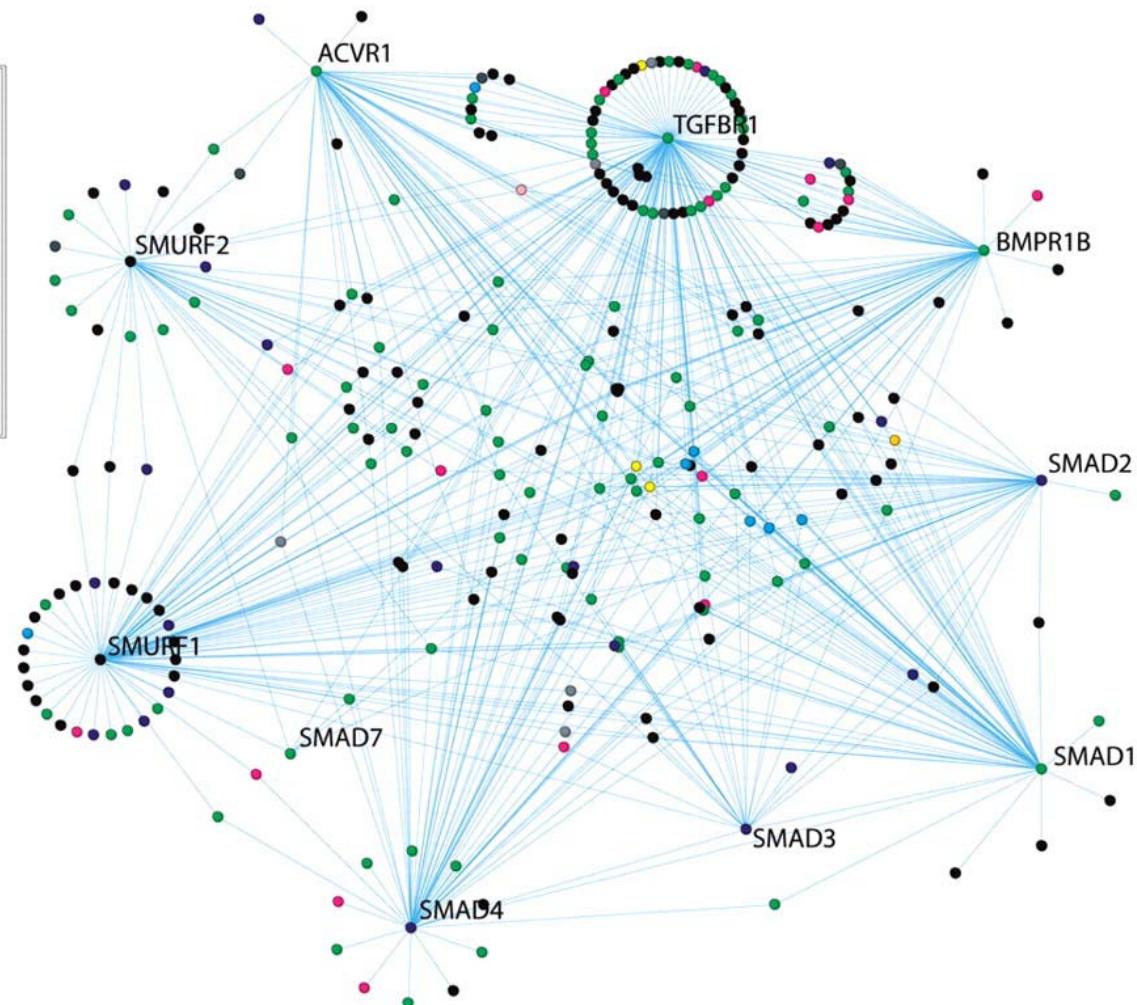
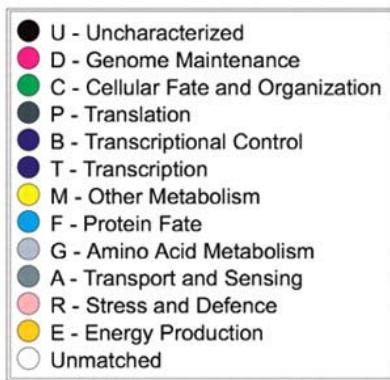


Barrios-Rodiles M, et al.

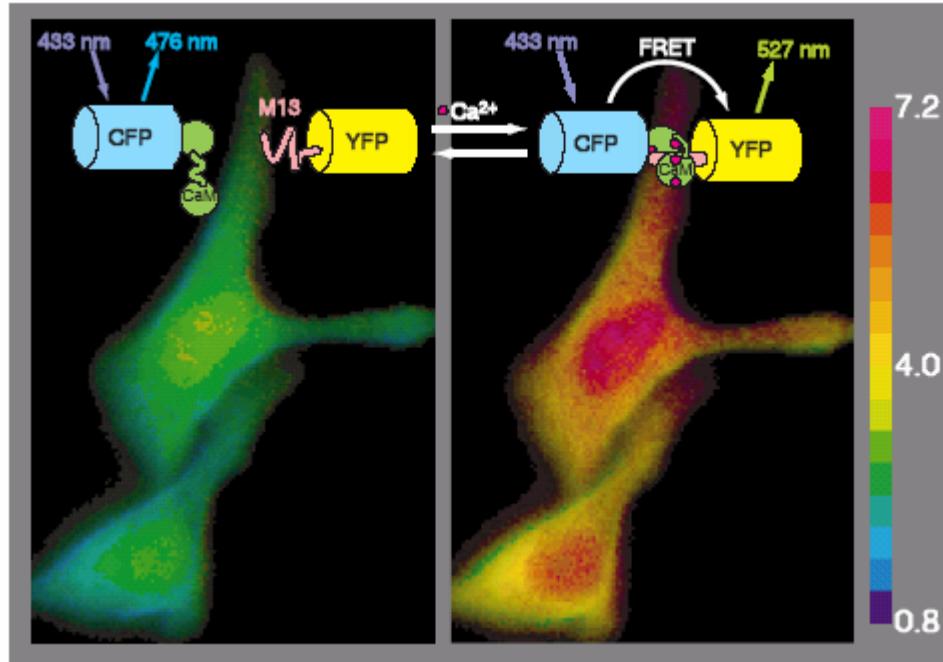
High-throughput mapping of a dynamic signaling network in mammalian cells.
Science. 2005 Mar 11;307(5715):1621-5.

High-throughput screening in 293 cells using the Lumier approach

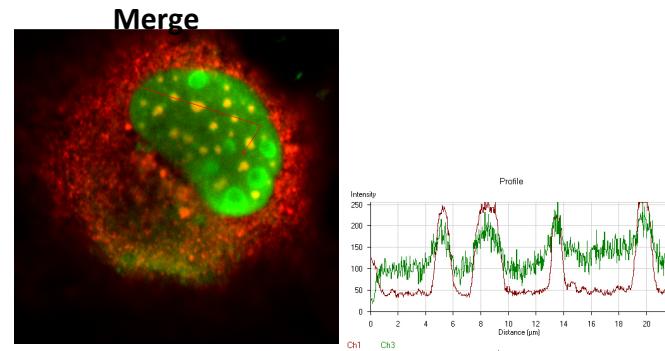
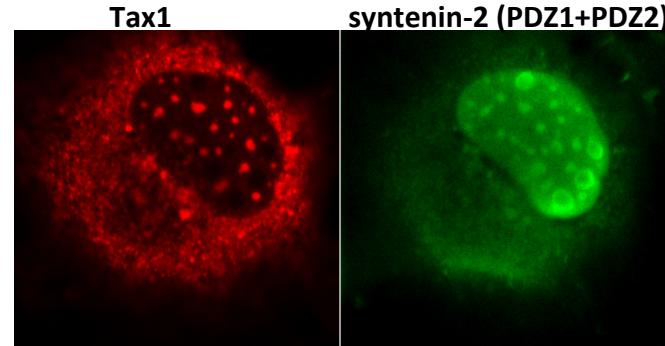
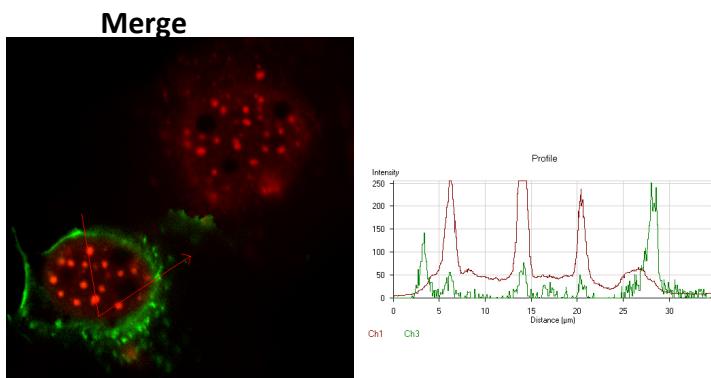
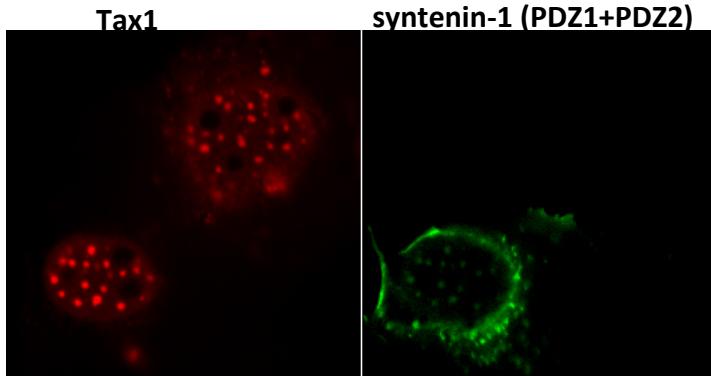
A



Monitoring assembly: FRET



Monitoring interactions: co-localization

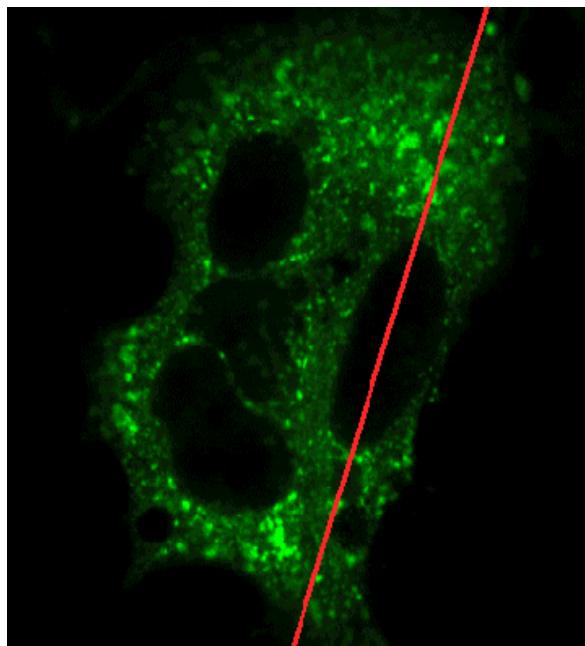


colocalization between syntenin-2 (PDZ1+PDZ2) and Tax1

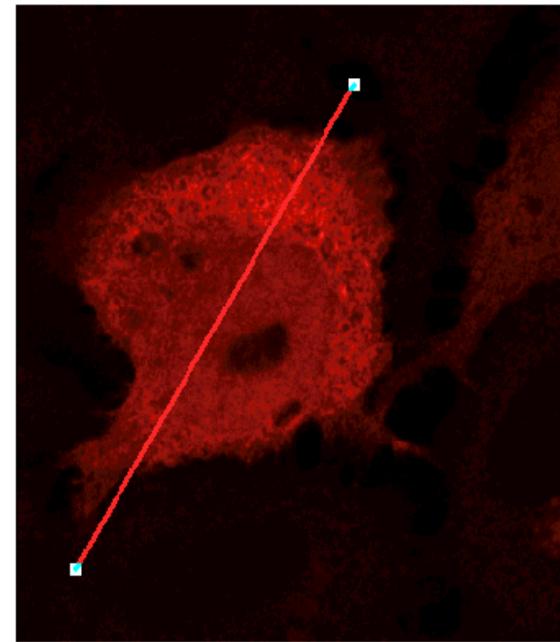
No colocalization between syntenin-1 (PDZ1+PDZ2) and Tax1

Monitoring interactions: localization change

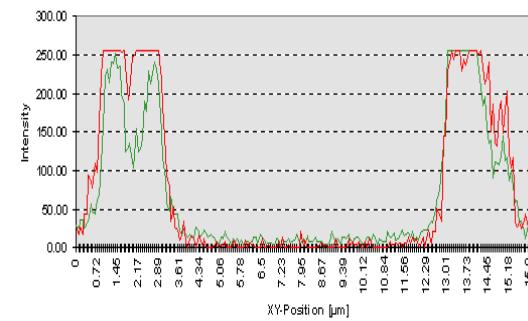
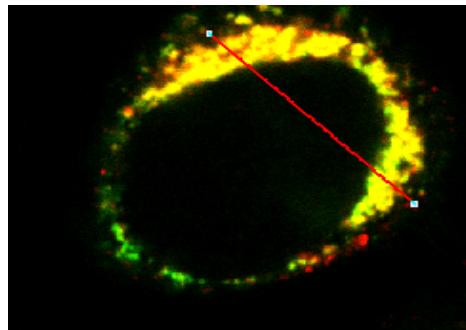
GFPTTP



Tax

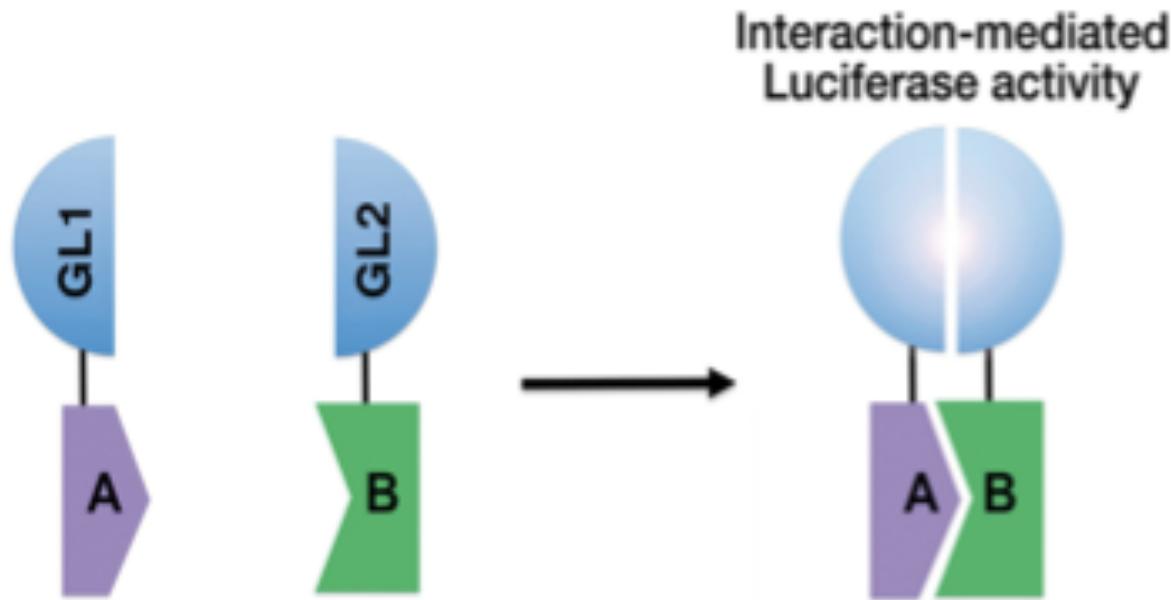


Tax1
et TTP

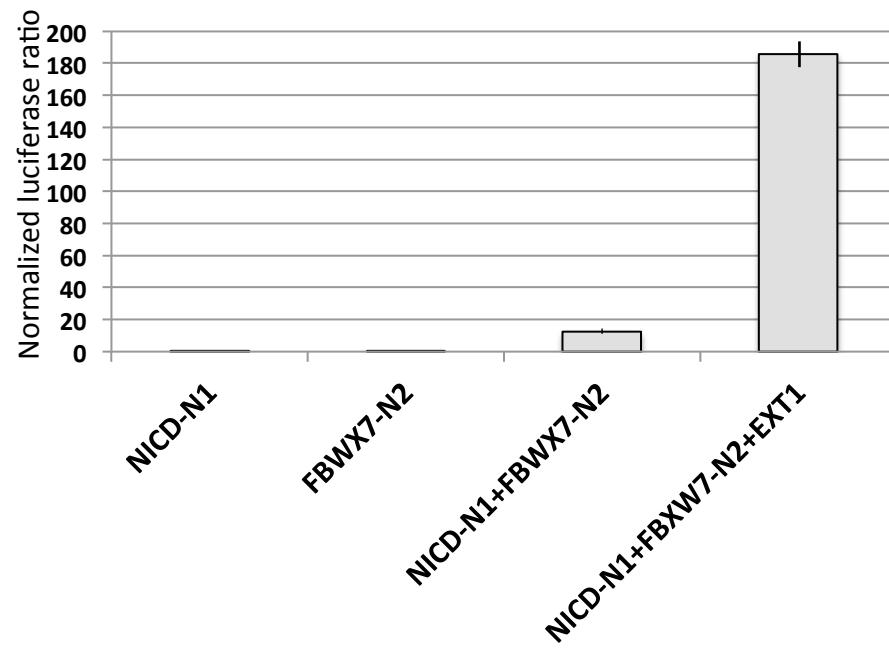


Monitoring interactions: protein complementation

Gaussia princeps luciferase (GL)-based protein complementation assay (PCA)

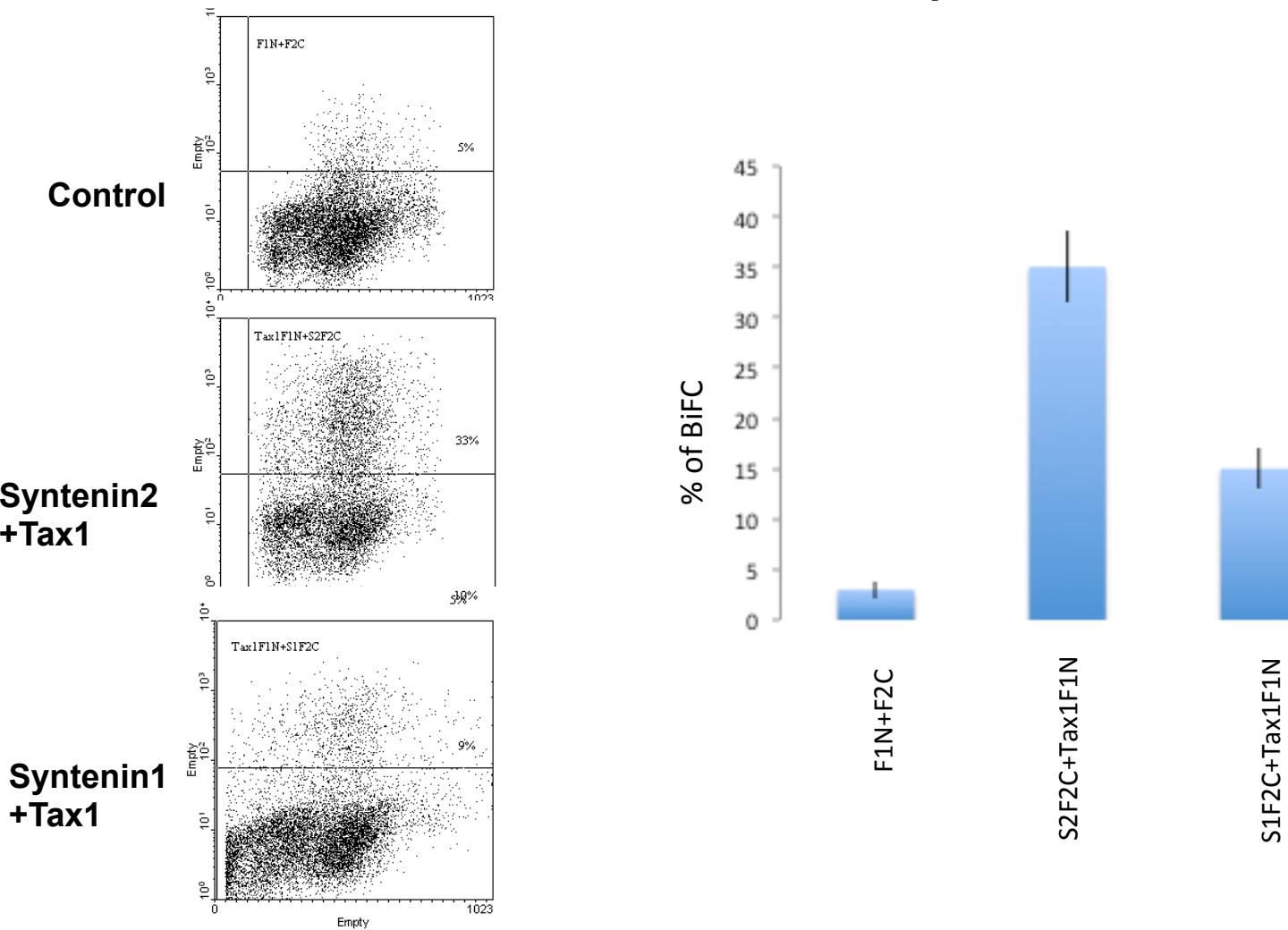


Protein complementation assay



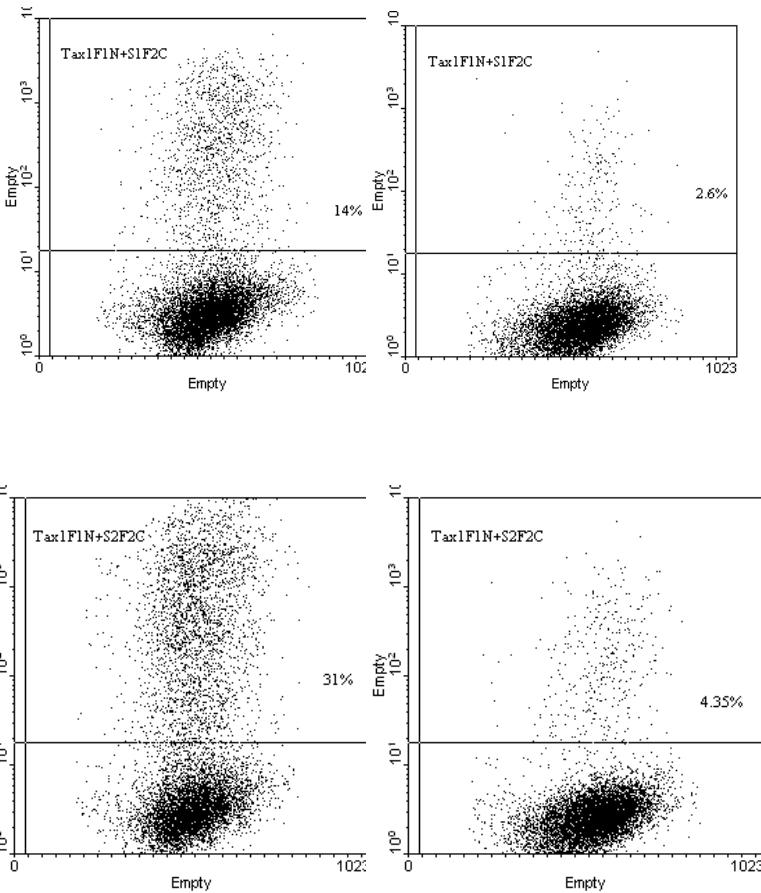
Monitoring interactions: protein complementation

Bi-molecular fluorescence complementation

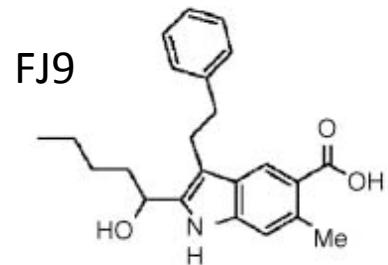
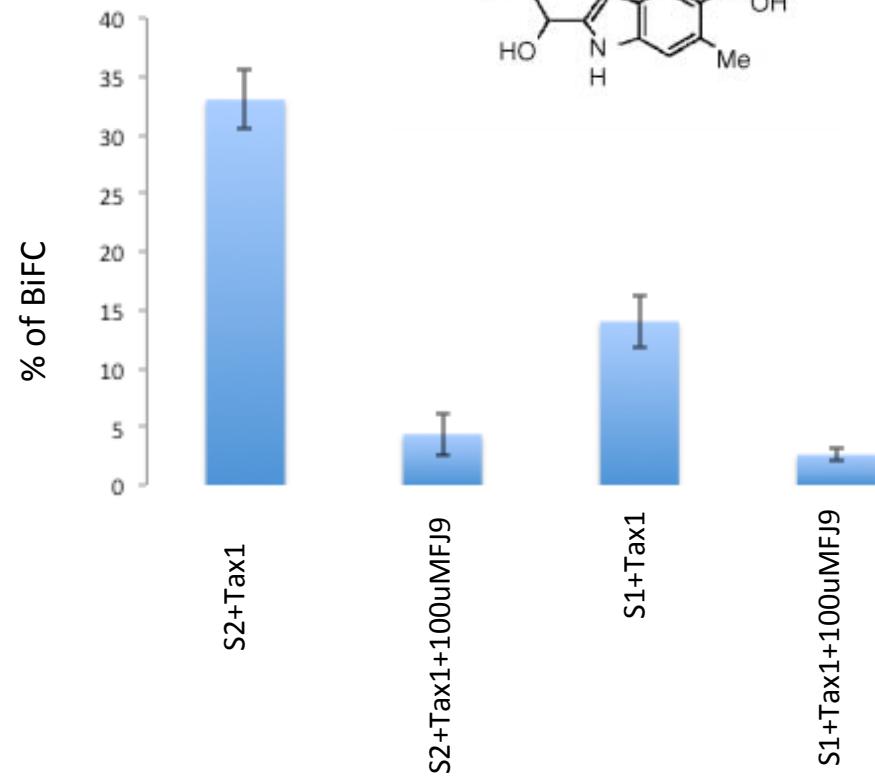


FJ9 inhibits Tax/syntenin interaction

Syntenin1+
Tax1



Syntenin2+
Tax1

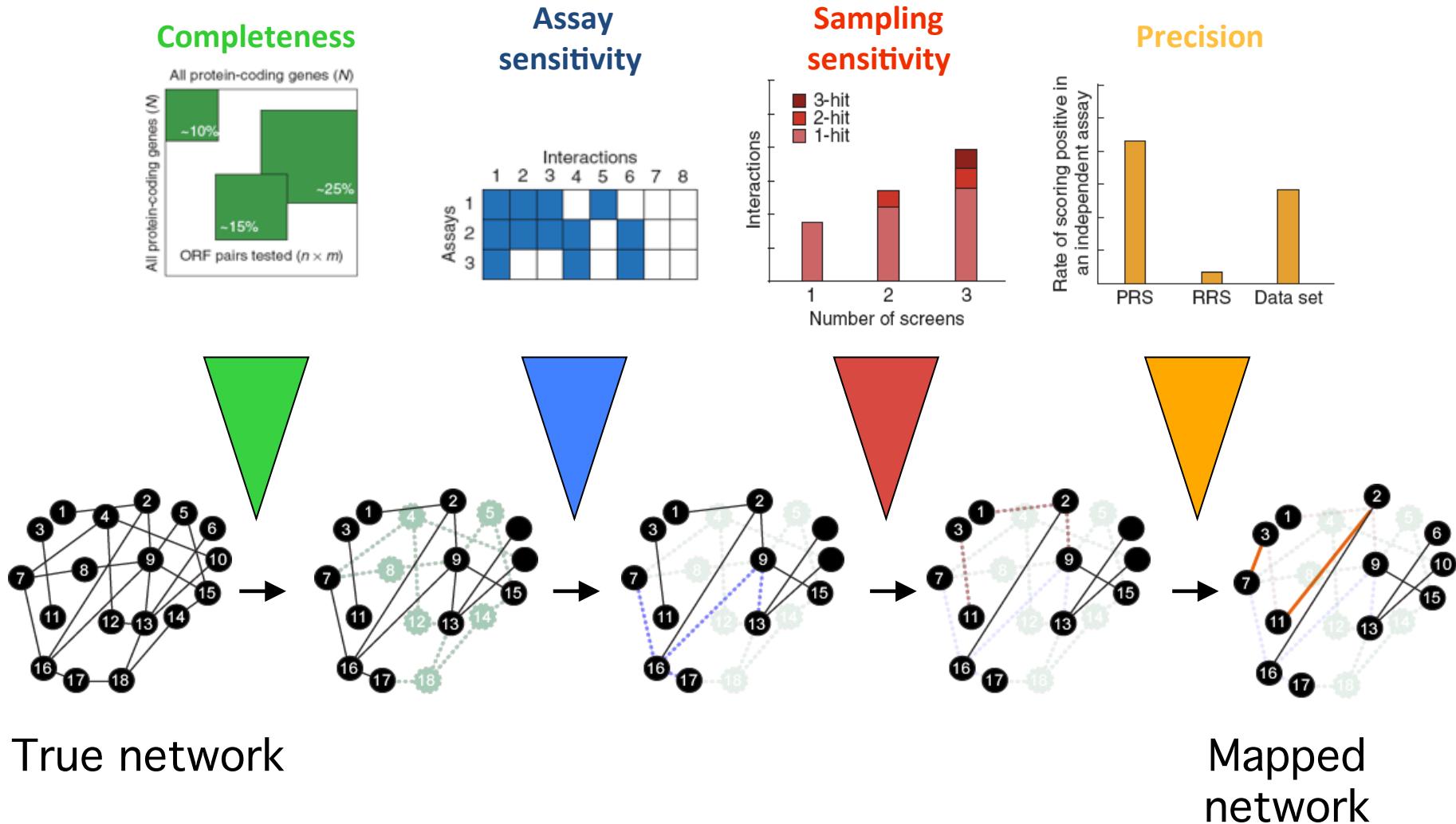


S1+Tax1+100uMFJ9

Protein-protein interactions

- Affinity purification
- Y2H hybrid
- Energy transfer (Fluorescence = FRET)
- Co-localisation (Fluorescence based)
- Protein complementation
 - Luciferase based
 - Fluorescence based

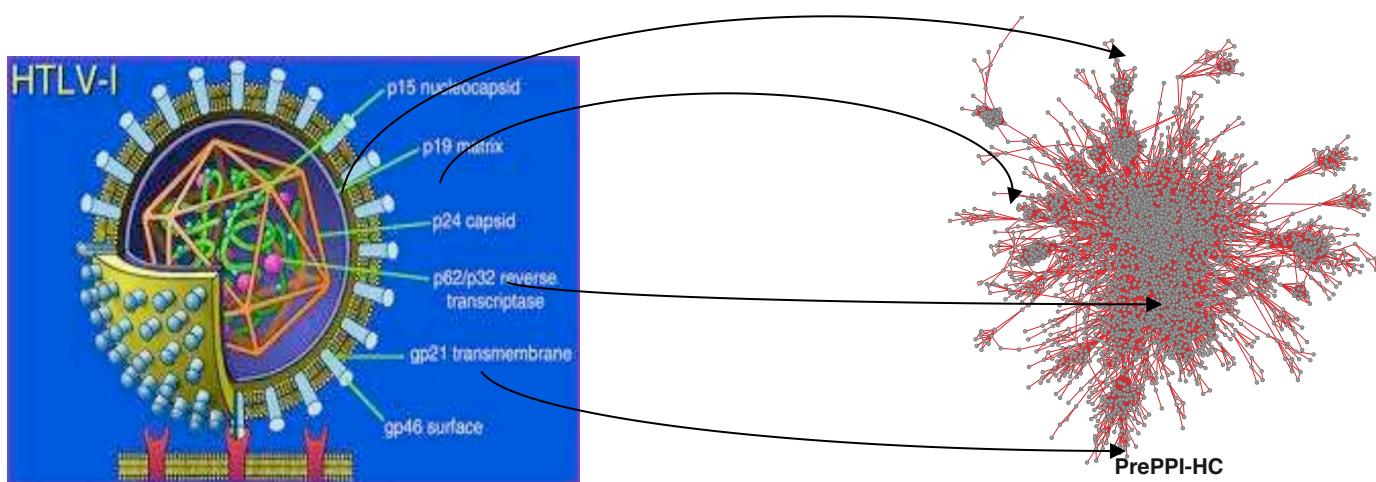
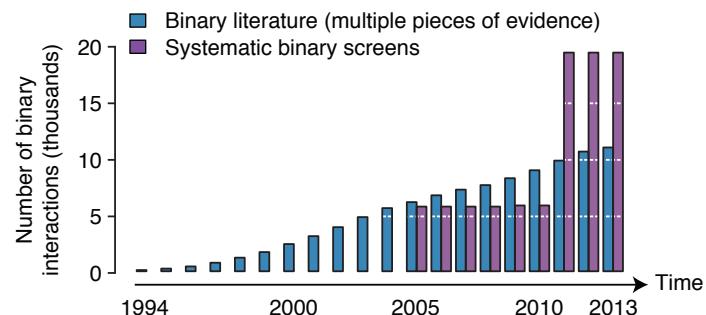
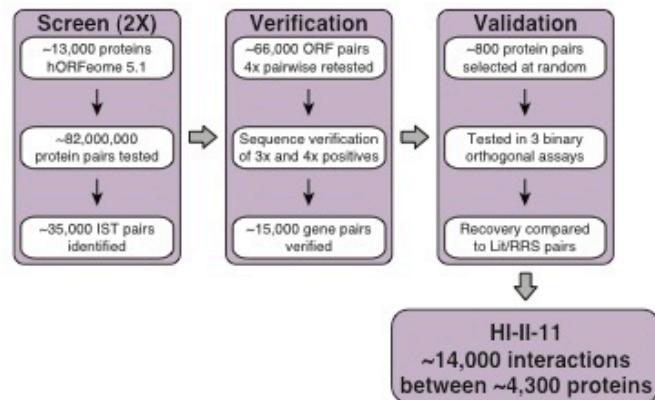
Empirical framework



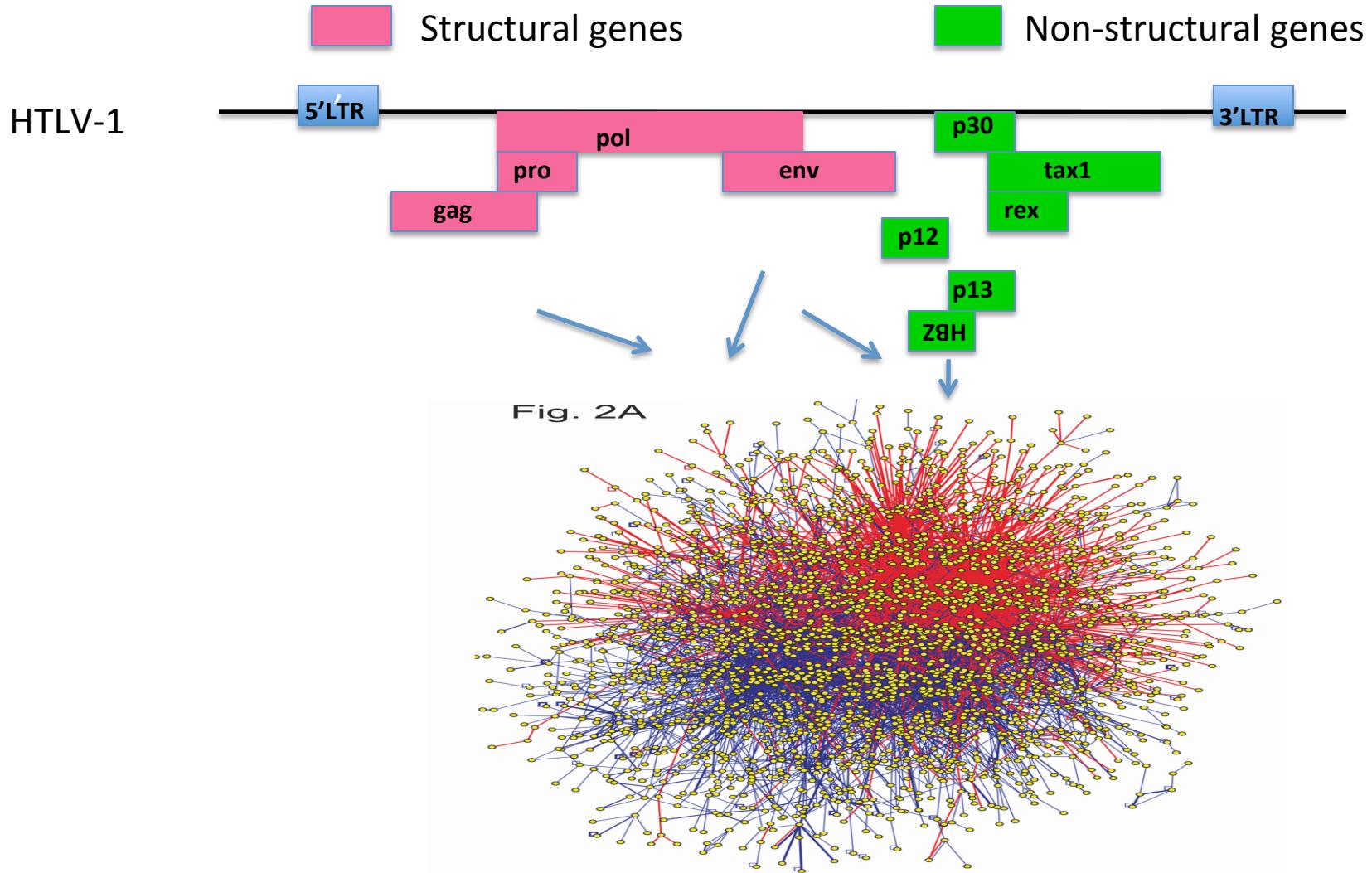
True network

Mapped network

Interactomes mapping applications

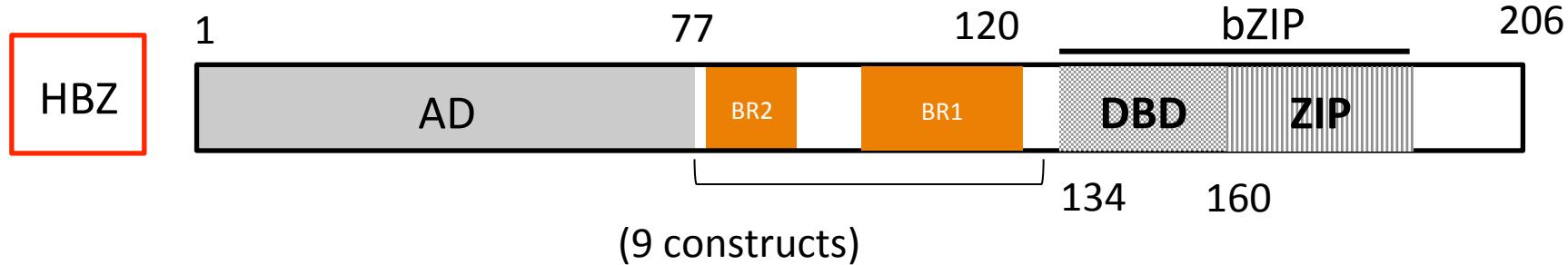
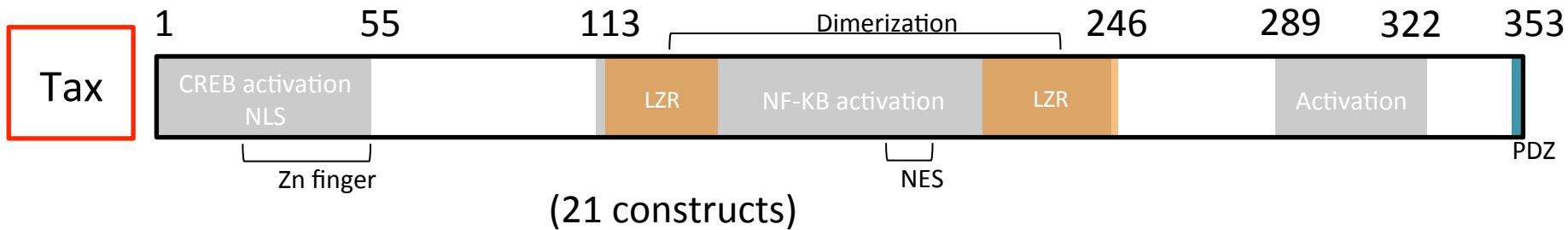


Host – Pathogens interactome

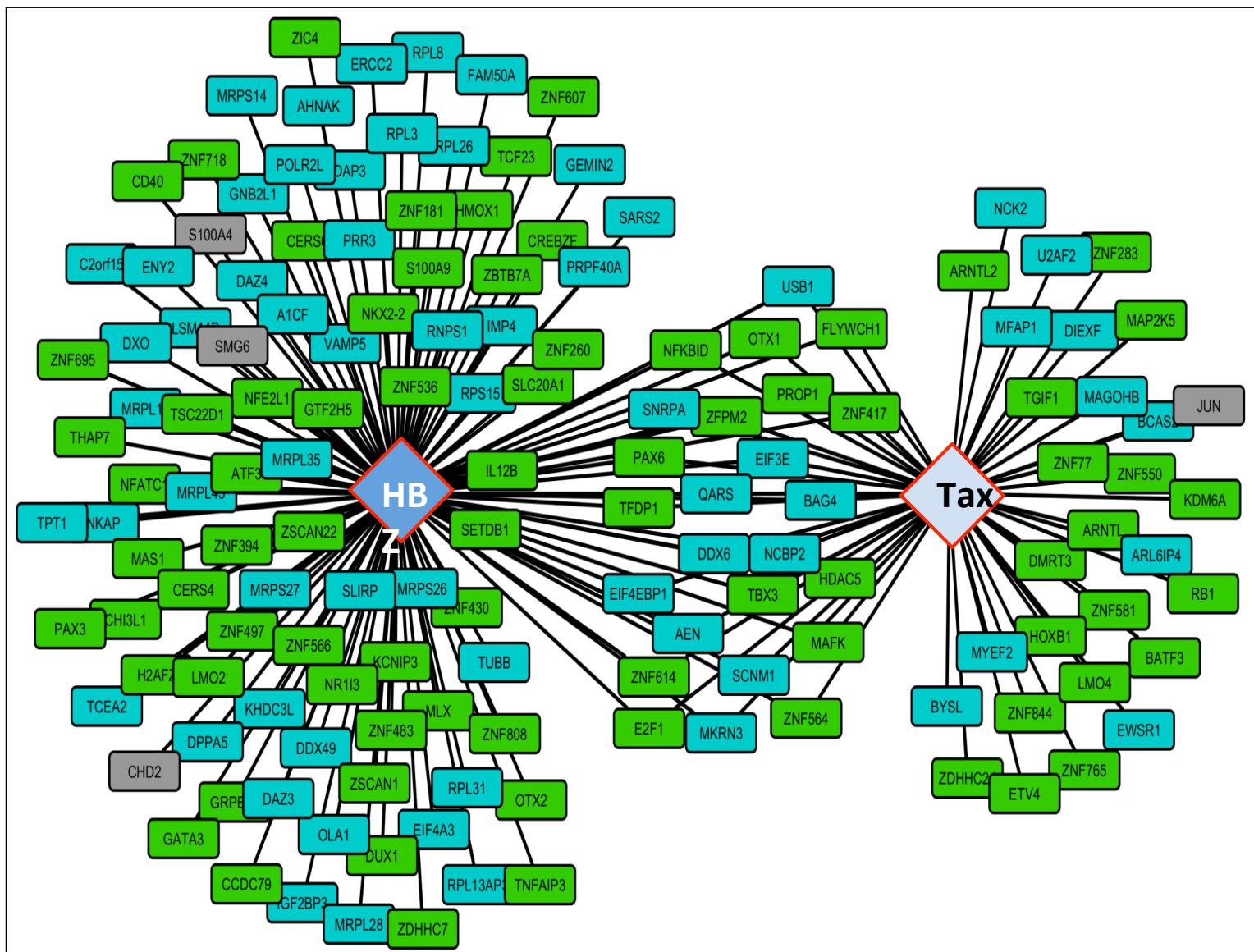


Preliminary results

Cloning of Tax and HBZ constructs



Comprehensive mapping of Tax/HBZ interactome with Transcriptional and Post-transcriptional regulators

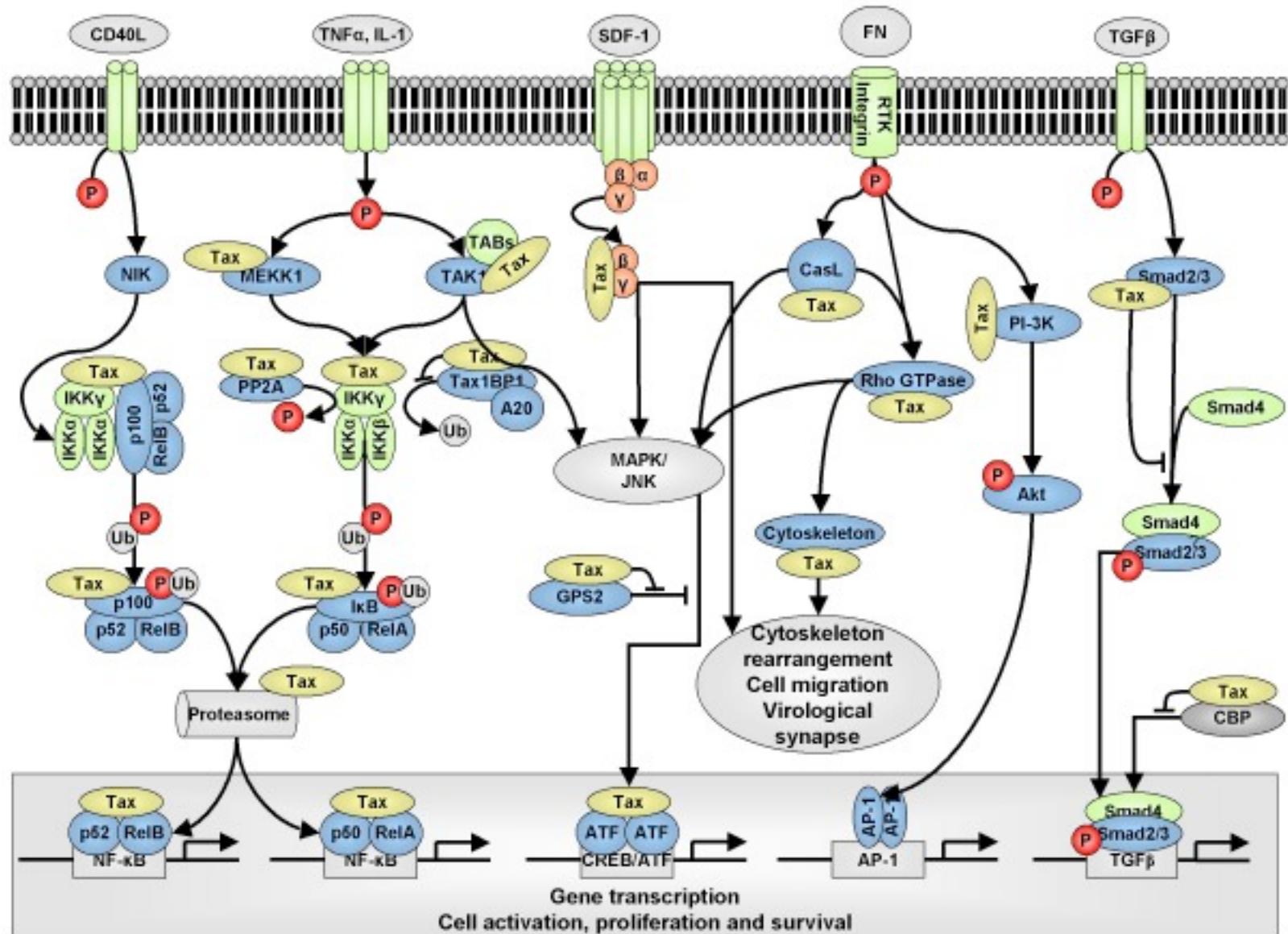


Validation:

PCA

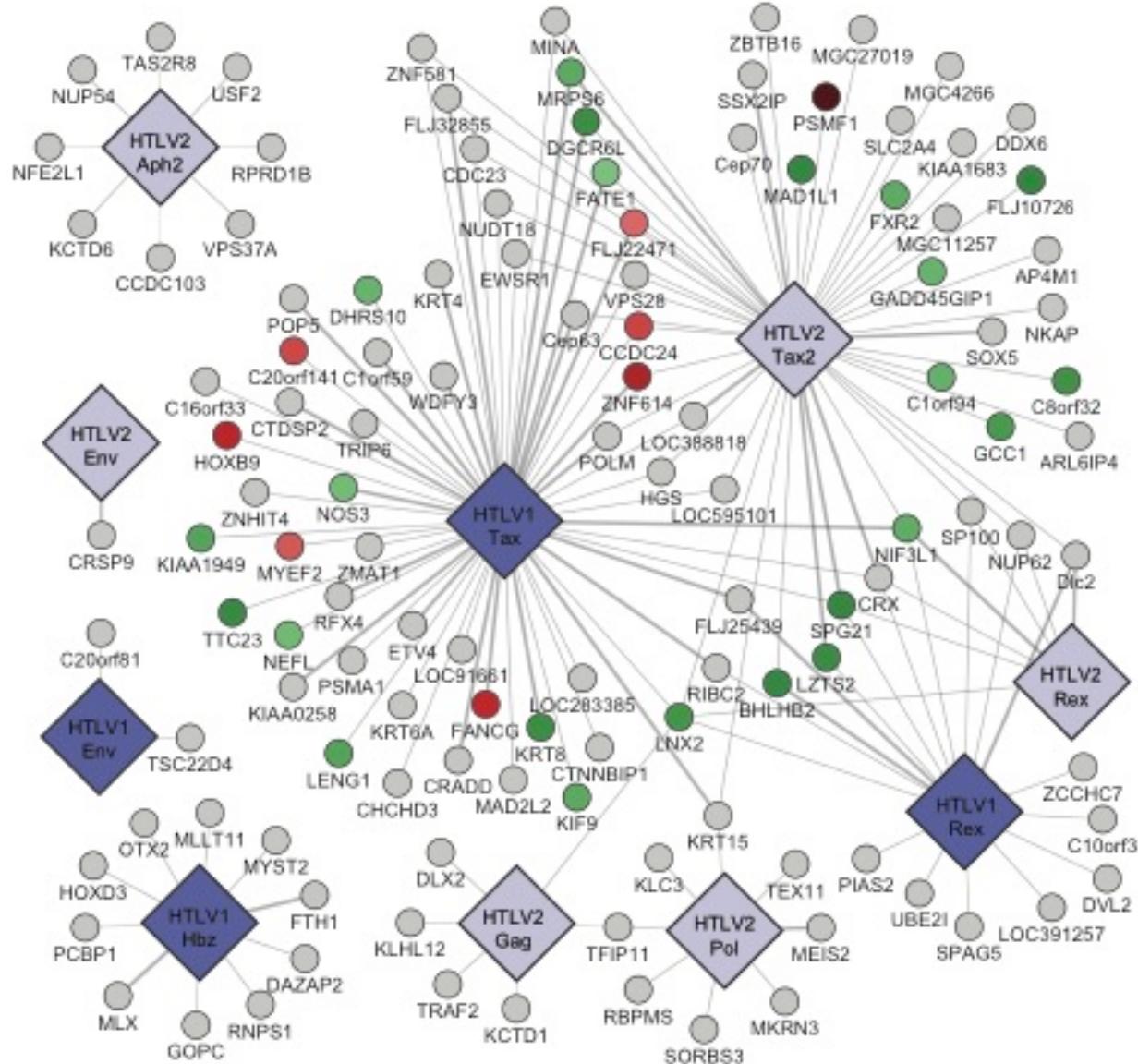
MAPPIT

History



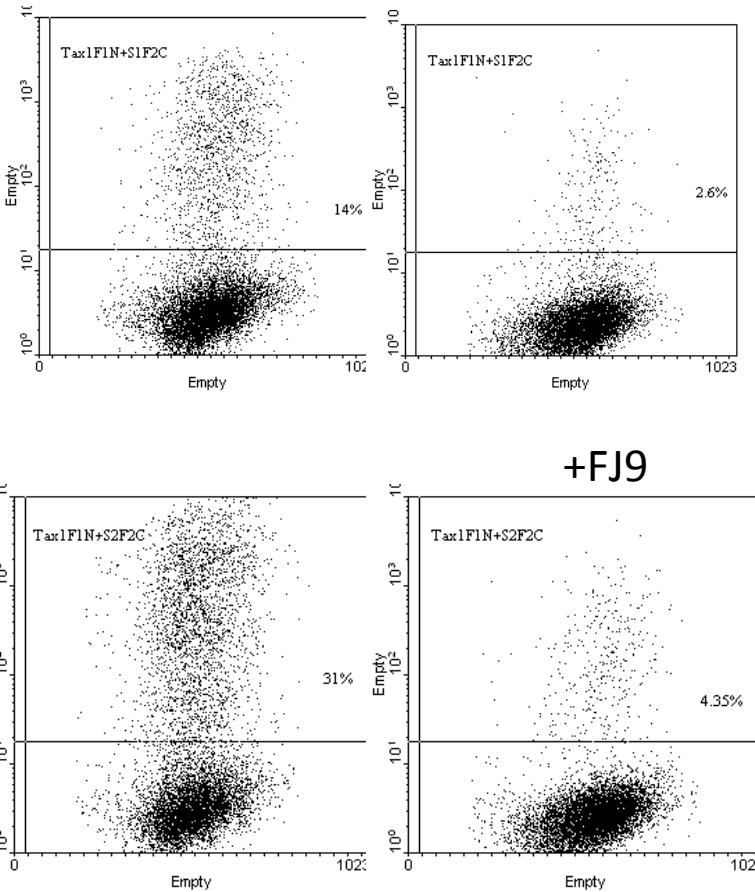
Tax1 Interactome
(Boxus et al. Retrovirology, 2008)

A host – pathogen interactome for HTLV1/2



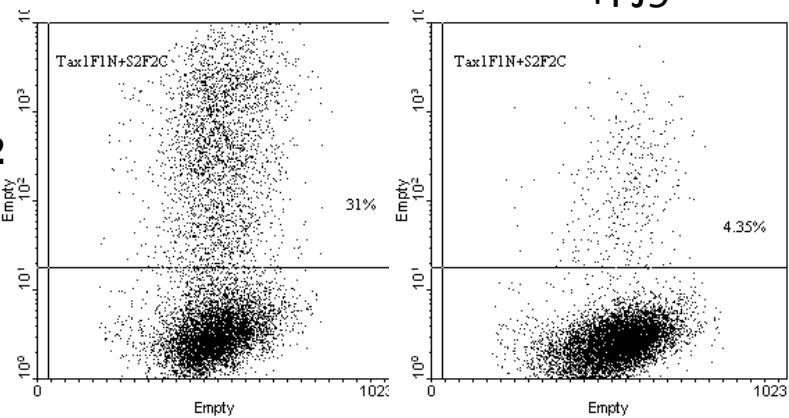
Inhibition of protein – protein interactions by small molecules

Syntenin1
+Tax1

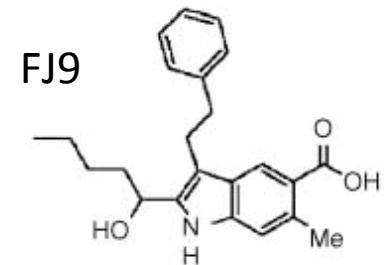
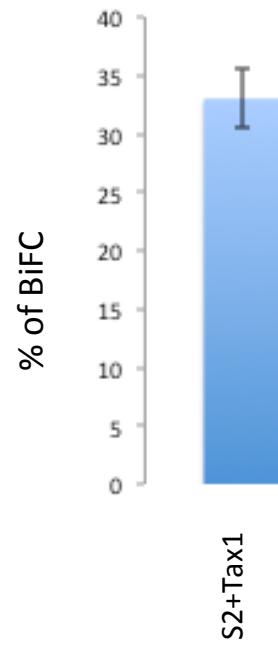


+FJ9

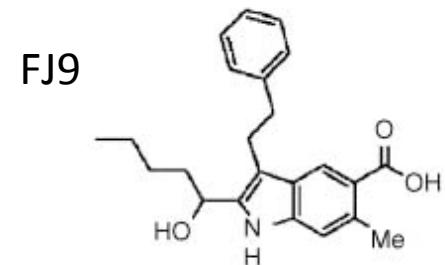
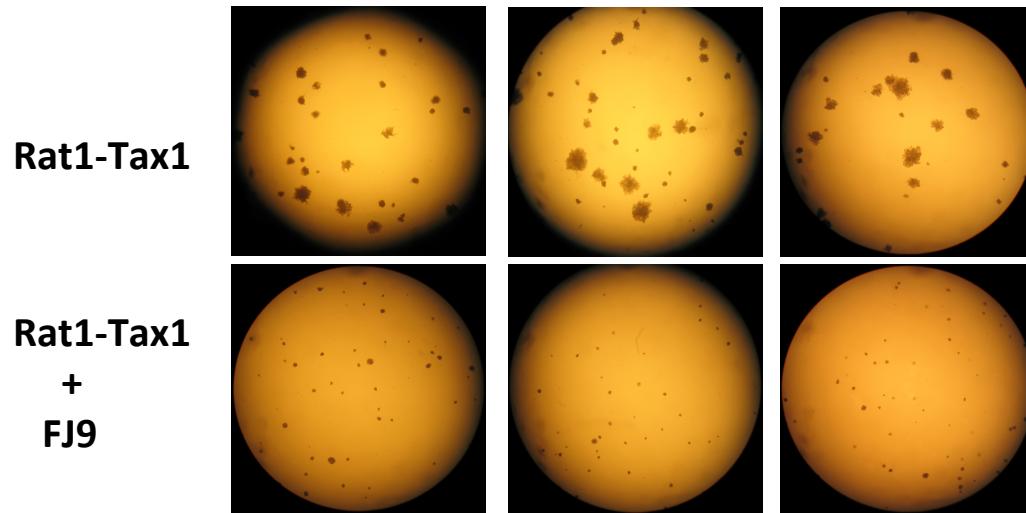
Syntenin2
+Tax1



+FJ9



Inhibition of protein – protein and cellular transformation

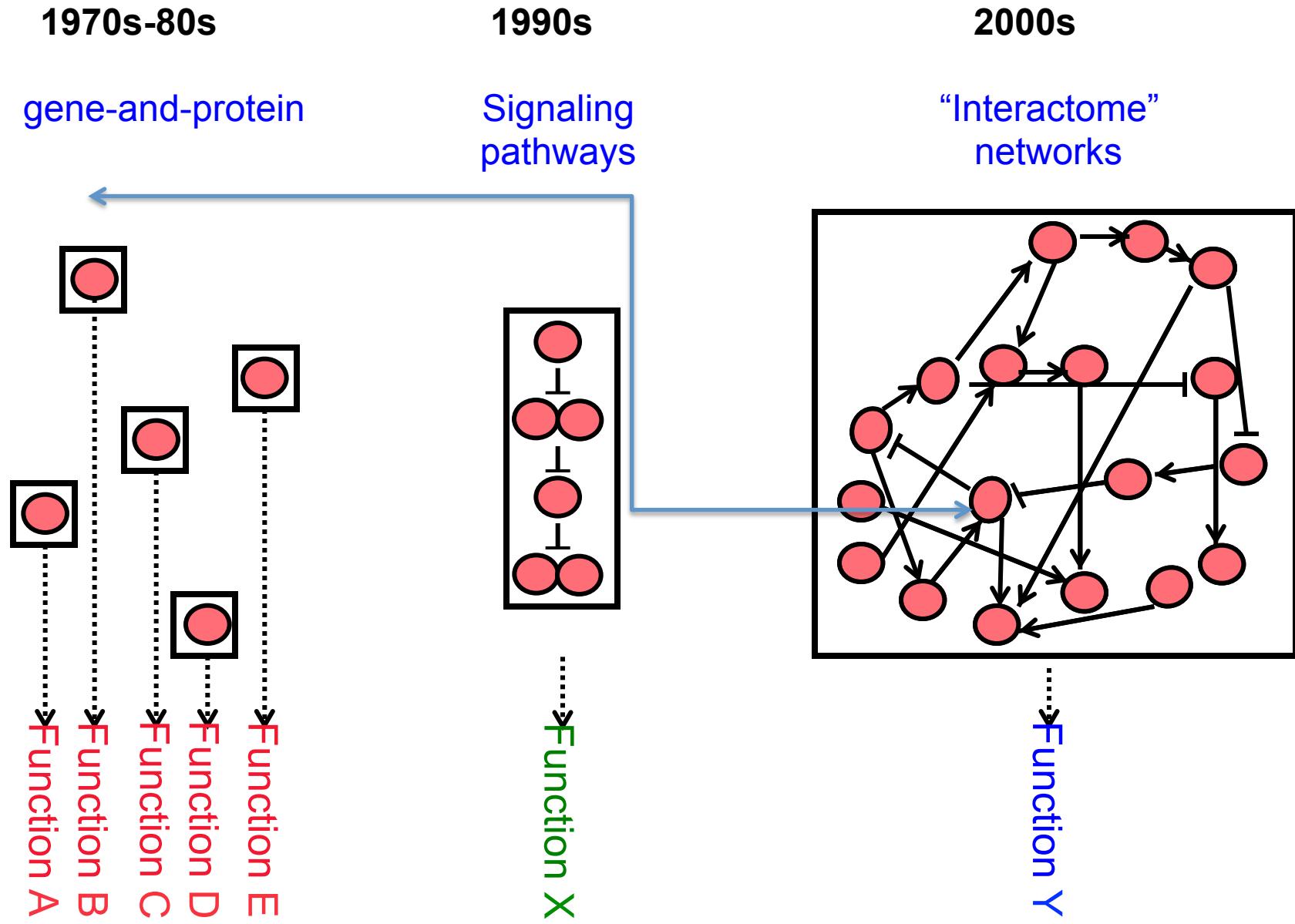


Disruption of Tax/ PDZ interaction inhibited
Tax transformation as measured by a
decrease in size and number of Tax-
induced Rat-1 foci.

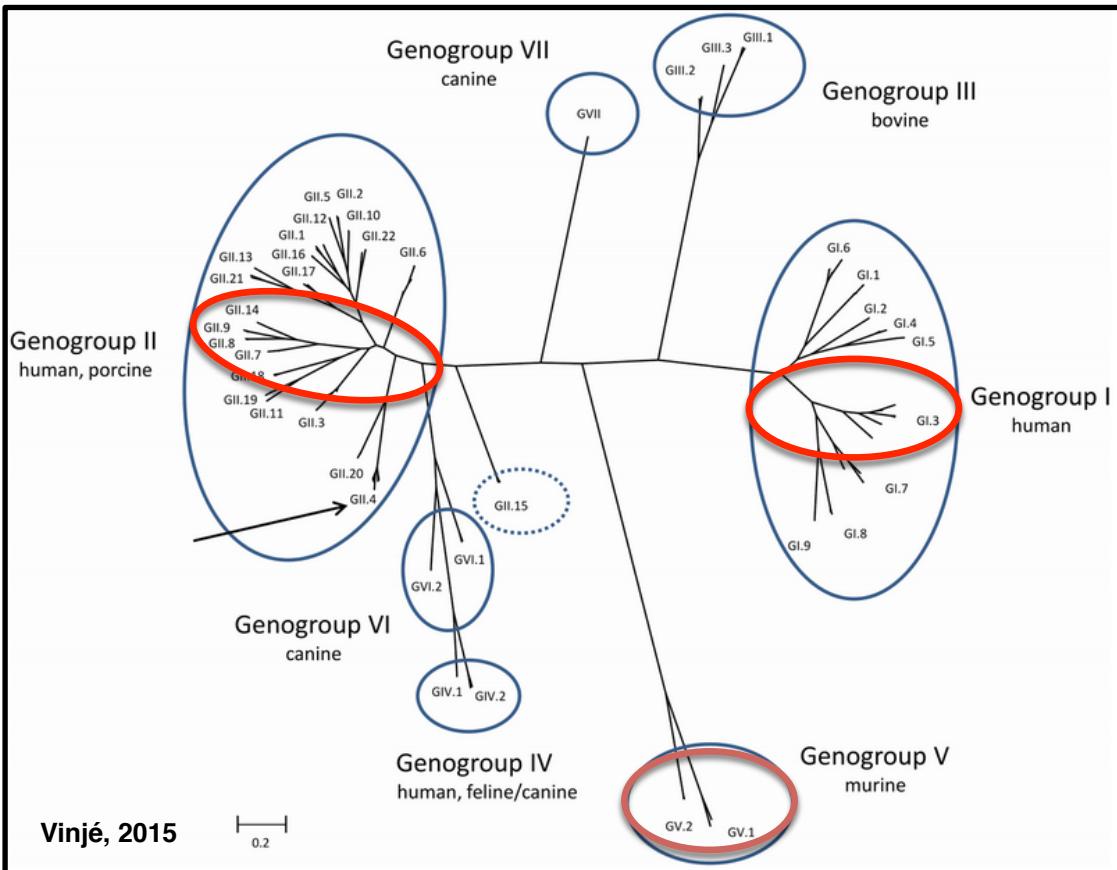


PDZ proteins involved in Tax1 transformation activity

Models for overall functional organization of the cell



Noroviruses Interactome



- GI.1 (ORF2+ORF3)** GII.1
GI.2 GII.2
GI.3 GII.3
GI.4 GII.4 GII.5
GI.5 GII.6
GI.6 GII.7
GI.7 GII.8
GI.8 GII.9
GI.9 GII.10
GII.12 GII.13
GII.14 GII.15
GII.16 GII.17
GII.18 GII.19
GII.20 GII.21
GII.22 GII.23
GII.24 GII.25
GII.26 GII.27
GII.28 GII.29
GII.30 GII.31
GII.32 GII.33
GII.34 GII.35
GII.36 GII.37
GII.38 GII.39
GII.40 GII.41
GII.42 GII.43
GII.44 GII.45
GII.46 GII.47
GII.48 GII.49
GII.50 GII.51
GII.52 GII.53
GII.54 GII.55
GII.56 GII.57
GII.58 GII.59
GII.60 GII.61
GII.62 GII.63
GII.64 GII.65
GII.66 GII.67
GII.68 GII.69
GII.70 GII.71
GII.72 GII.73
GII.74 GII.75
GII.76 GII.77
GII.78 GII.79
GII.80 GII.81
GII.82 GII.83
GII.84 GII.85
GII.86 GII.87
GII.88 GII.89
GII.90 GII.91
GII.92 GII.93
GII.94 GII.95
GII.96 GII.97
GII.98 GII.99
GII.100 GII.101
GII.102 GII.103
GII.104 GII.105
GII.106 GII.107
GII.108 GII.109
GII.110 GII.111
GII.112 GII.113
GII.114 GII.115
GII.116 GII.117
GII.118 GII.119
GII.120 GII.121
GII.122 GII.123
GII.124 GII.125
GII.126 GII.127
GII.128 GII.129
GII.130 GII.131
GII.132 GII.133
GII.134 GII.135
GII.136 GII.137
GII.138 GII.139
GII.140 GII.141
GII.142 GII.143
GII.144 GII.145
GII.146 GII.147
GII.148 GII.149
GII.150 GII.151
GII.152 GII.153
GII.154 GII.155
GII.156 GII.157
GII.158 GII.159
GII.160 GII.161
GII.162 GII.163
GII.164 GII.165
GII.166 GII.167
GII.168 GII.169
GII.170 GII.171
GII.172 GII.173
GII.174 GII.175
GII.176 GII.177
GII.178 GII.179
GII.180 GII.181
GII.182 GII.183
GII.184 GII.185
GII.186 GII.187
GII.188 GII.189
GII.190 GII.191
GII.192 GII.193
GII.194 GII.195
GII.196 GII.197
GII.198 GII.199
GII.200 GII.201
GII.202 GII.203
GII.204 GII.205
GII.206 GII.207
GII.208 GII.209
GII.210 GII.211
GII.212 GII.213
GII.214 GII.215
GII.216 GII.217
GII.218 GII.219
GII.220 GII.221
GII.222 GII.223
GII.224 GII.225
GII.226 GII.227
GII.228 GII.229
GII.230 GII.231
GII.232 GII.233
GII.234 GII.235
GII.236 GII.237
GII.238 GII.239
GII.240 GII.241
GII.242 GII.243
GII.244 GII.245
GII.246 GII.247
GII.248 GII.249
GII.250 GII.251
GII.252 GII.253
GII.254 GII.255
GII.256 GII.257
GII.258 GII.259
GII.260 GII.261
GII.262 GII.263
GII.264 GII.265
GII.266 GII.267
GII.268 GII.269
GII.270 GII.271
GII.272 GII.273
GII.274 GII.275
GII.276 GII.277
GII.278 GII.279
GII.280 GII.281
GII.282 GII.283
GII.284 GII.285
GII.286 GII.287
GII.288 GII.289
GII.290 GII.291
GII.292 GII.293
GII.294 GII.295
GII.296 GII.297
GII.298 GII.299
GII.300 GII.301
GII.302 GII.303
GII.304 GII.305
GII.306 GII.307
GII.308 GII.309
GII.310 GII.311
GII.312 GII.313
GII.314 GII.315
GII.316 GII.317
GII.318 GII.319
GII.320 GII.321
GII.322 GII.323
GII.324 GII.325
GII.326 GII.327
GII.328 GII.329
GII.330 GII.331
GII.332 GII.333
GII.334 GII.335
GII.336 GII.337
GII.338 GII.339
GII.340 GII.341
GII.342 GII.343
GII.344 GII.345
GII.346 GII.347
GII.348 GII.349
GII.350 GII.351
GII.352 GII.353
GII.354 GII.355
GII.356 GII.357
GII.358 GII.359
GII.360 GII.361
GII.362 GII.363
GII.364 GII.365
GII.366 GII.367
GII.368 GII.369
GII.370 GII.371
GII.372 GII.373
GII.374 GII.375
GII.376 GII.377
GII.378 GII.379
GII.380 GII.381
GII.382 GII.383
GII.384 GII.385
GII.386 GII.387
GII.388 GII.389
GII.390 GII.391
GII.392 GII.393
GII.394 GII.395
GII.396 GII.397
GII.398 GII.399
GII.400 GII.401
GII.402 GII.403
GII.404 GII.405
GII.406 GII.407
GII.408 GII.409
GII.410 GII.411
GII.412 GII.413
GII.414 GII.415
GII.416 GII.417
GII.418 GII.419
GII.420 GII.421
GII.422 GII.423
GII.424 GII.425
GII.426 GII.427
GII.428 GII.429
GII.430 GII.431
GII.432 GII.433
GII.434 GII.435
GII.436 GII.437
GII.438 GII.439
GII.440 GII.441
GII.442 GII.443
GII.444 GII.445
GII.446 GII.447
GII.448 GII.449
GII.450 GII.451
GII.452 GII.453
GII.454 GII.455
GII.456 GII.457
GII.458 GII.459
GII.460 GII.461
GII.462 GII.463
GII.464 GII.465
GII.466 GII.467
GII.468 GII.469
GII.470 GII.471
GII.472 GII.473
GII.474 GII.475
GII.476 GII.477
GII.478 GII.479
GII.480 GII.481
GII.482 GII.483
GII.484 GII.485
GII.486 GII.487
GII.488 GII.489
GII.490 GII.491
GII.492 GII.493
GII.494 GII.495
GII.496 GII.497
GII.498 GII.499
GII.500 GII.501
GII.502 GII.503
GII.504 GII.505
GII.506 GII.507
GII.508 GII.509
GII.510 GII.511
GII.512 GII.513
GII.514 GII.515
GII.516 GII.517
GII.518 GII.519
GII.520 GII.521
GII.522 GII.523
GII.524 GII.525
GII.526 GII.527
GII.528 GII.529
GII.530 GII.531
GII.532 GII.533
GII.534 GII.535
GII.536 GII.537
GII.538 GII.539
GII.540 GII.541
GII.542 GII.543
GII.544 GII.545
GII.546 GII.547
GII.548 GII.549
GII.550 GII.551
GII.552 GII.553
GII.554 GII.555
GII.556 GII.557
GII.558 GII.559
GII.560 GII.561
GII.562 GII.563
GII.564 GII.565
GII.566 GII.567
GII.568 GII.569
GII.570 GII.571
GII.572 GII.573
GII.574 GII.575
GII.576 GII.577
GII.578 GII.579
GII.580 GII.581
GII.582 GII.583
GII.584 GII.585
GII.586 GII.587
GII.588 GII.589
GII.590 GII.591
GII.592 GII.593
GII.594 GII.595
GII.596 GII.597
GII.598 GII.599
GII.600 GII.601
GII.602 GII.603
GII.604 GII.605
GII.606 GII.607
GII.608 GII.609
GII.610 GII.611
GII.612 GII.613
GII.614 GII.615
GII.616 GII.617
GII.618 GII.619
GII.620 GII.621
GII.622 GII.623
GII.624 GII.625
GII.626 GII.627
GII.628 GII.629
GII.630 GII.631
GII.632 GII.633
GII.634 GII.635
GII.636 GII.637
GII.638 GII.639
GII.640 GII.641
GII.642 GII.643
GII.644 GII.645
GII.646 GII.647
GII.648 GII.649
GII.650 GII.651
GII.652 GII.653
GII.654 GII.655
GII.656 GII.657
GII.658 GII.659
GII.660 GII.661
GII.662 GII.663
GII.664 GII.665
GII.666 GII.667
GII.668 GII.669
GII.670 GII.671
GII.672 GII.673
GII.674 GII.675
GII.676 GII.677
GII.678 GII.679
GII.680 GII.681
GII.682 GII.683
GII.684 GII.685
GII.686 GII.687
GII.688 GII.689
GII.690 GII.691
GII.692 GII.693
GII.694 GII.695
GII.696 GII.697
GII.698 GII.699
GII.700 GII.701
GII.702 GII.703
GII.704 GII.705
GII.706 GII.707
GII.708 GII.709
GII.710 GII.711
GII.712 GII.713
GII.714 GII.715
GII.716 GII.717
GII.718 GII.719
GII.720 GII.721
GII.722 GII.723
GII.724 GII.725
GII.726 GII.727
GII.728 GII.729
GII.730 GII.731
GII.732 GII.733
GII.734 GII.735
GII.736 GII.737
GII.738 GII.739
GII.740 GII.741
GII.742 GII.743
GII.744 GII.745
GII.746 GII.747
GII.748 GII.749
GII.750 GII.751
GII.752 GII.753
GII.754 GII.755
GII.756 GII.757
GII.758 GII.759
GII.760 GII.761
GII.762 GII.763
GII.764 GII.765
GII.766 GII.767
GII.768 GII.769
GII.770 GII.771
GII.772 GII.773
GII.774 GII.775
GII.776 GII.777
GII.778 GII.779
GII.780 GII.781
GII.782 GII.783
GII.784 GII.785
GII.786 GII.787
GII.788 GII.789
GII.790 GII.791
GII.792 GII.793
GII.794 GII.795
GII.796 GII.797
GII.798 GII.799
GII.800 GII.801
GII.802 GII.803
GII.804 GII.805
GII.806 GII.807
GII.808 GII.809
GII.810 GII.811
GII.812 GII.813
GII.814 GII.815
GII.816 GII.817
GII.818 GII.819
GII.820 GII.821
GII.822 GII.823
GII.824 GII.825
GII.826 GII.827
GII.828 GII.829
GII.830 GII.831
GII.832 GII.833
GII.834 GII.835
GII.836 GII.837
GII.838 GII.839
GII.840 GII.841
GII.842 GII.843
GII.844 GII.845
GII.846 GII.847
GII.848 GII.849
GII.850 GII.851
GII.852 GII.853
GII.854 GII.855
GII.856 GII.857
GII.858 GII.859
GII.860 GII.861
GII.862 GII.863
GII.864 GII.865
GII.866 GII.867
GII.868 GII.869
GII.870 GII.871
GII.872 GII.873
GII.874 GII.875
GII.876 GII.877
GII.878 GII.879
GII.880 GII.881
GII.882 GII.883
GII.884 GII.885
GII.886 GII.887
GII.888 GII.889
GII.890 GII.891
GII.892 GII.893
GII.894 GII.895
GII.896 GII.897
GII.898 GII.899
GII.900 GII.901
GII.902 GII.903
GII.904 GII.905
GII.906 GII.907
GII.908 GII.909
GII.910 GII.911
GII.912 GII.913
GII.914 GII.915
GII.916 GII.917
GII.918 GII.919
GII.920 GII.921
GII.922 GII.923
GII.924 GII.925
GII.926 GII.927
GII.928 GII.929
GII.930 GII.931
GII.932 GII.933
GII.934 GII.935
GII.936 GII.937
GII.938 GII.939
GII.940 GII.941
GII.942 GII.943
GII.944 GII.945
GII.946 GII.947
GII.948 GII.949
GII.950 GII.951
GII.952 GII.953
GII.954 GII.955
GII.956 GII.957
GII.958 GII.959
GII.960 GII.961
GII.962 GII.963
GII.964 GII.965
GII.966 GII.967
GII.968 GII.969
GII.970 GII.971
GII.972 GII.973
GII.974 GII.975
GII.976 GII.977
GII.978 GII.979
GII.980 GII.981
GII.982 GII.983
GII.984 GII.985
GII.986 GII.987
GII.988 GII.989
GII.990 GII.991
GII.992 GII.993
GII.994 GII.995
GII.996 GII.997
GII.998 GII.999
GII.9999 GII.9999

Mapping an interactome network

All
proteins

All proteins

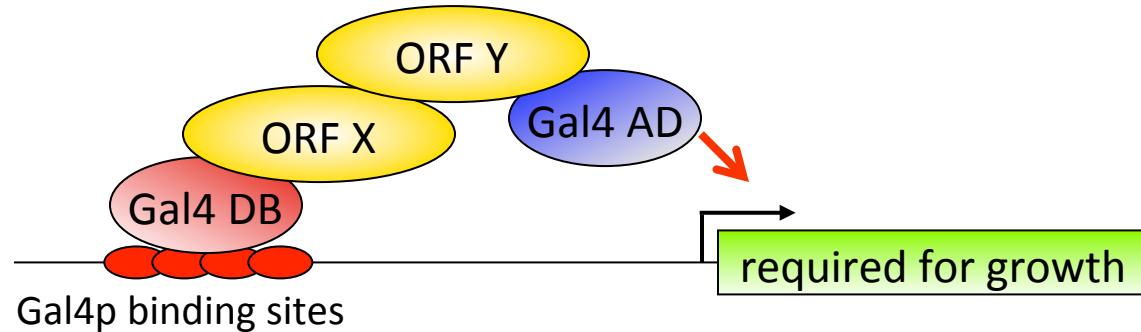
Test

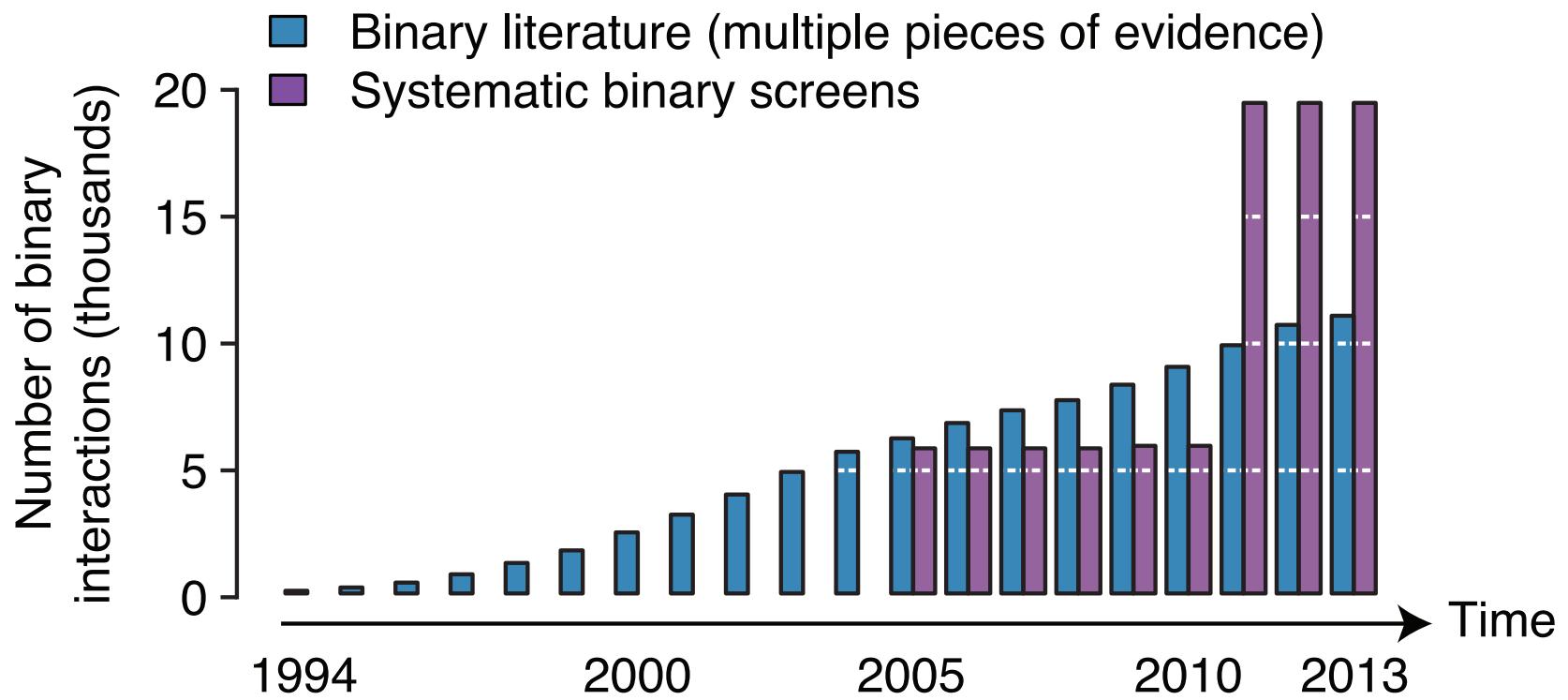
all pairwise combinations

for **possible** physical interactions

Yeast two-hybrid

- Reconstitution of GAL4 transcription factor
- Fusion proteins DB-ORFX and ORFY-AD
- Reporter gene

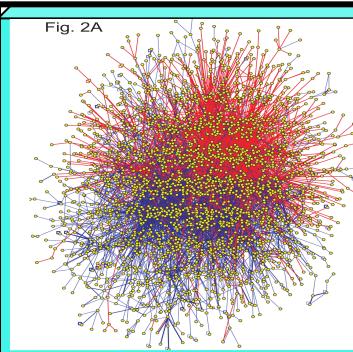




Human interactome

Rual et al.; Nature 437, 1173-1178
Stelzl et al.; Cell 122 (6), 957-68

>22,000 proteins

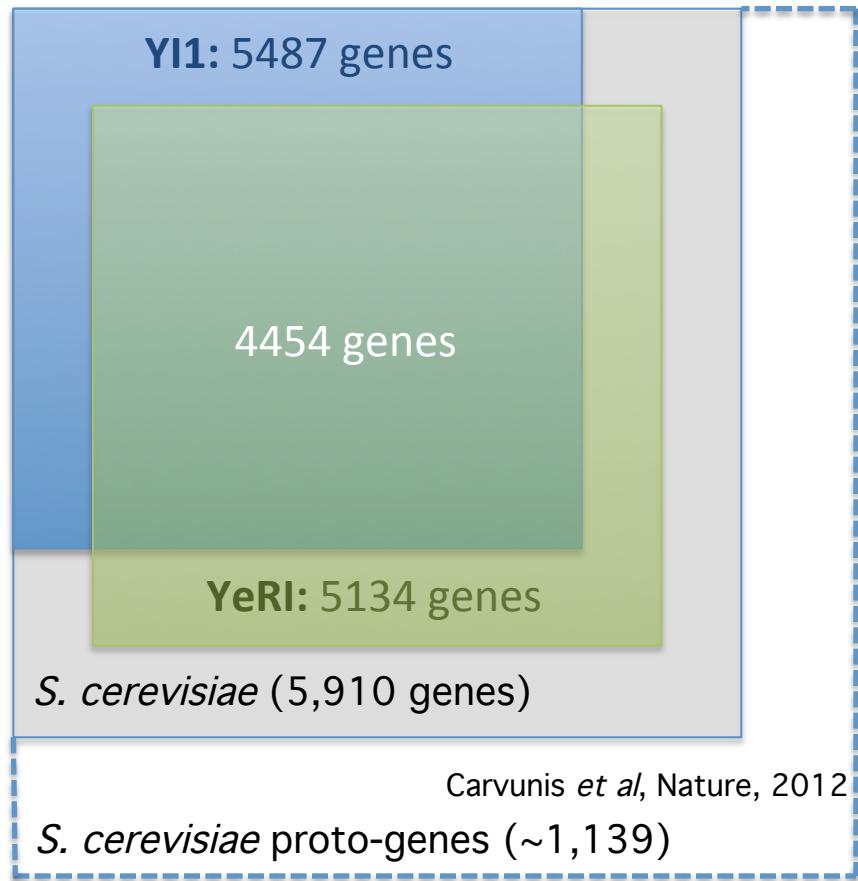


> 5 x 10⁸ pairwise combinations

various approaches

>22,000 proteins

Towards completeness of the yeast interactome

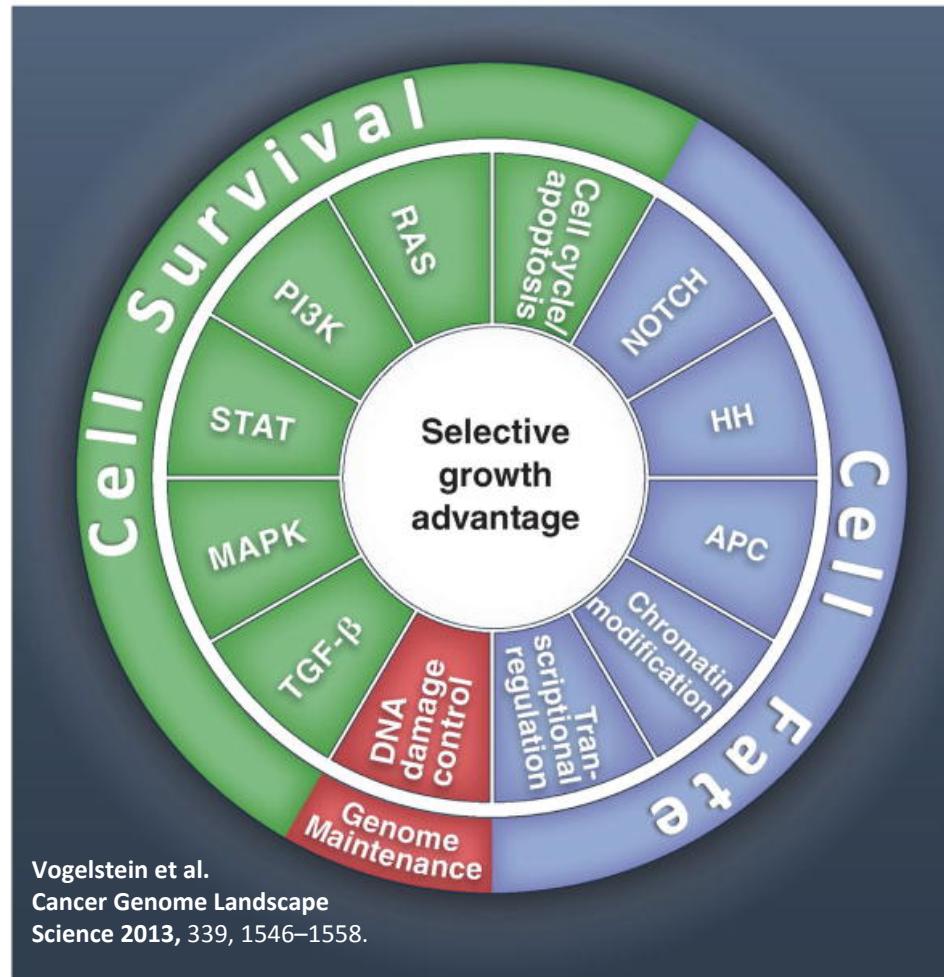


Genomic mutations landscape in cancer

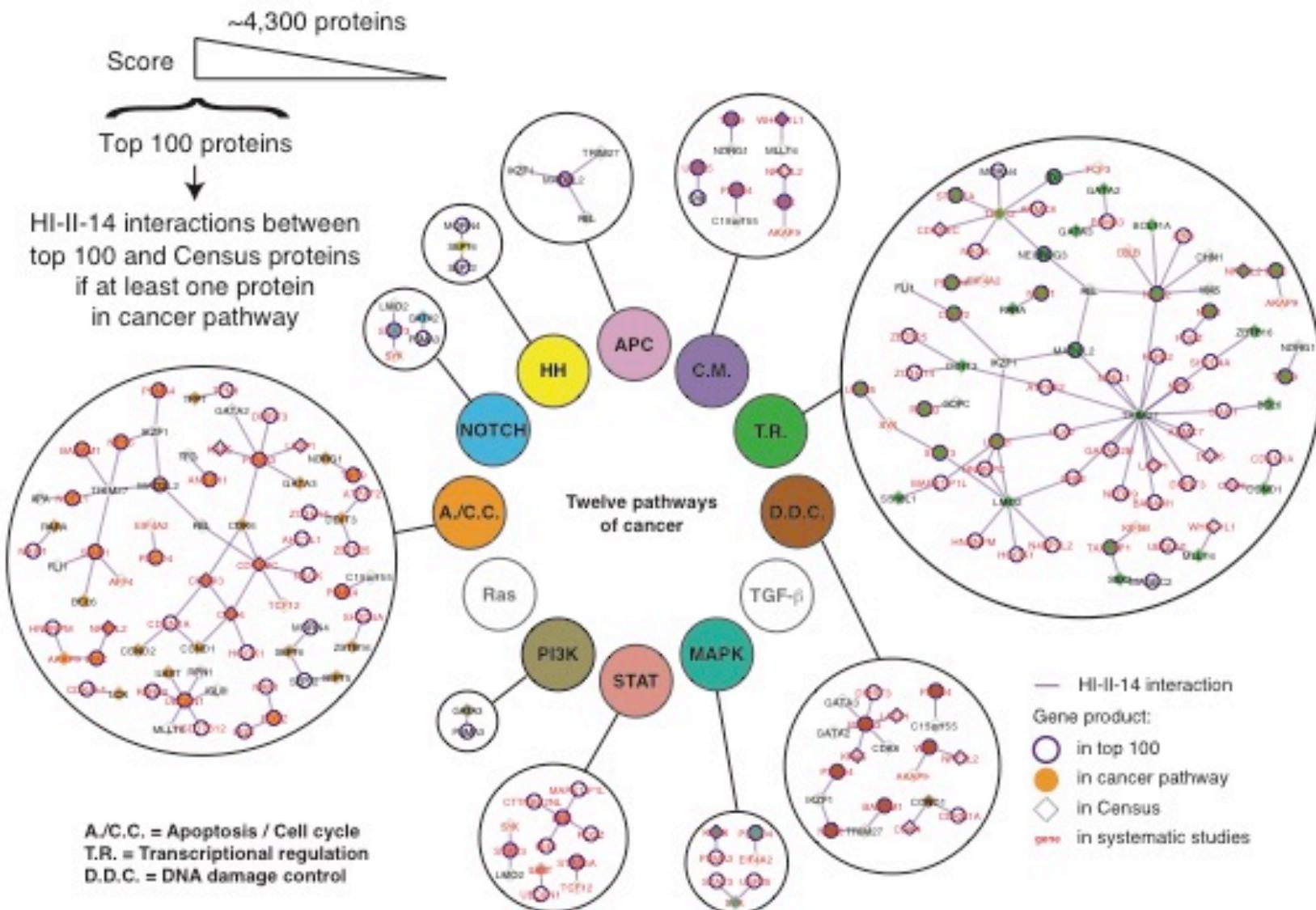
~ 500 cancer census genes

~140 cancer driver genes

Cancer Pathways



Guilt by association partners of known cancer genes

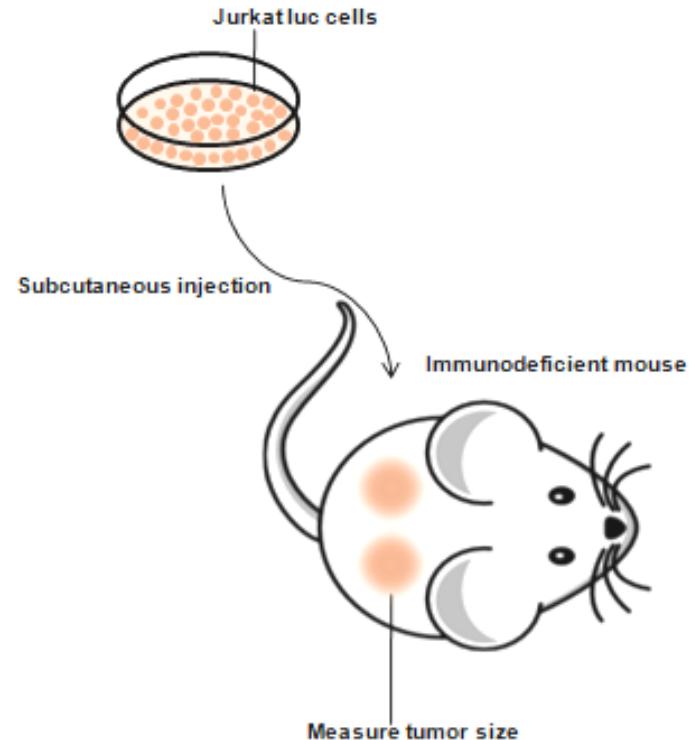
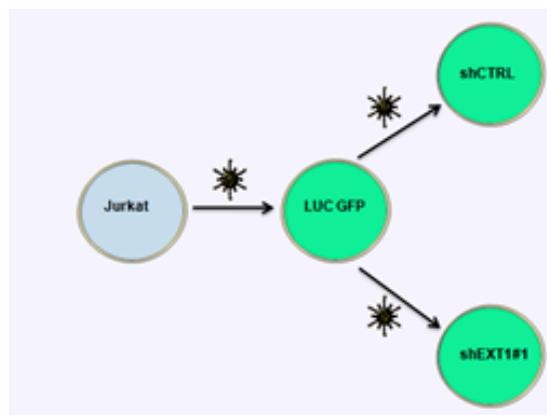
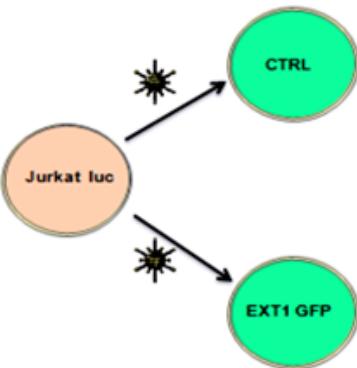


1. The role of EXT1 in T-ALL

Silencing/over-expression of EXT1 in a T-ALL *in vivo* model

Tumor xenograft experiment

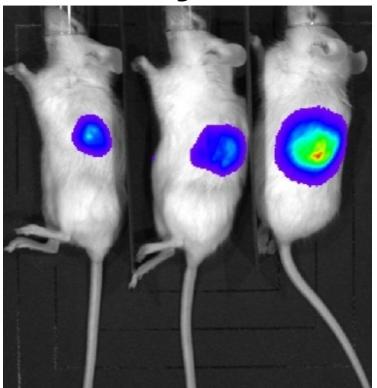
Based on bioluminescence imaging (BLI) with luciferase reporter



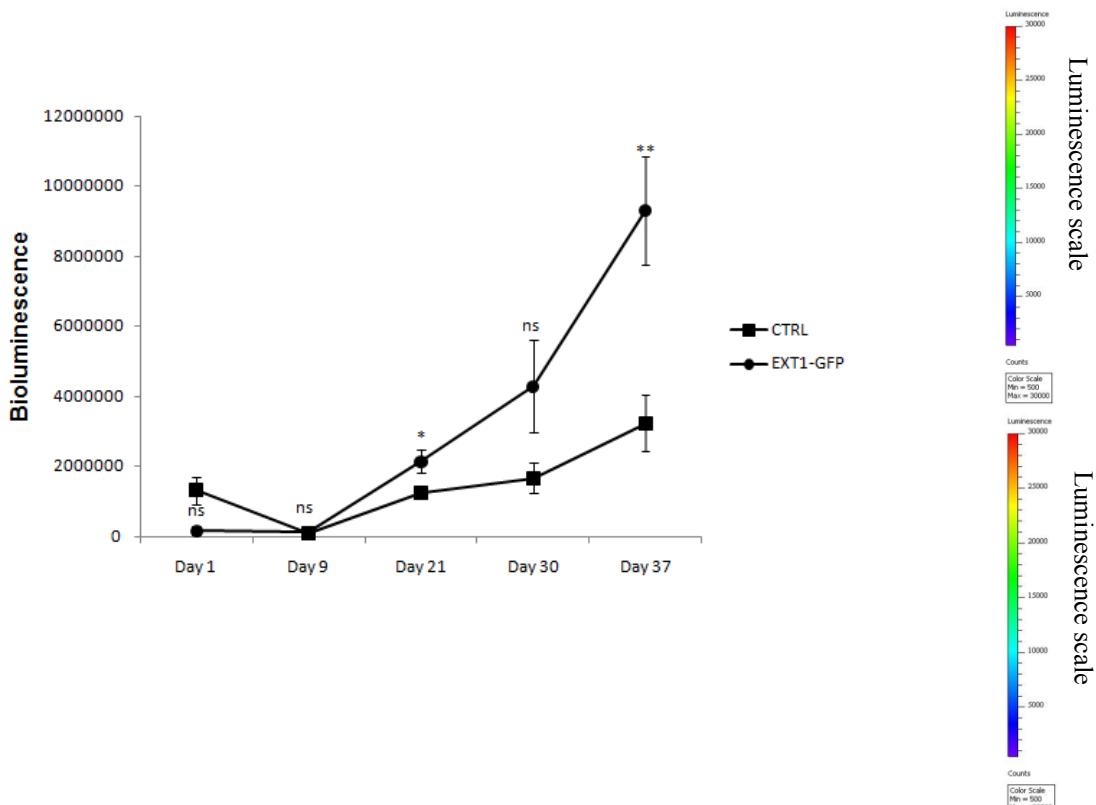
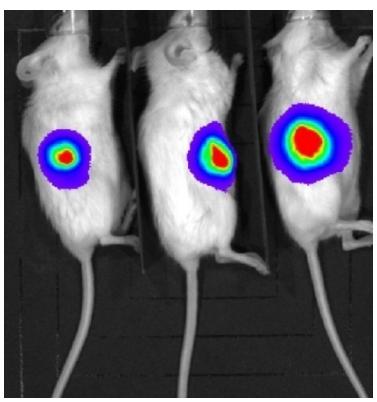
The role of EXT1 in T-ALL

Day 37

Jurkat CTRL



Jurkat EXT1



Injection of Jurkat over-expressing EXT1 in NOD-SCID mice resulted in a significant increase of the leukemic burden

Applications of interactome mapping

- Organisms Interactome mapping
- Novel disease-related genes
- Host-Pathogens interactomes