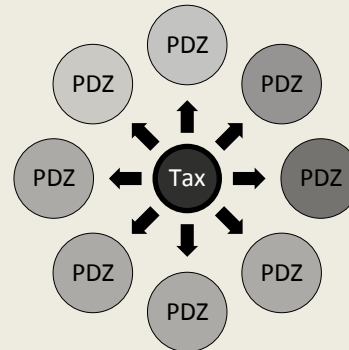


The HTLV-1 Tax Interactome: Mapping Tax interactions with PDZ-containing proteins

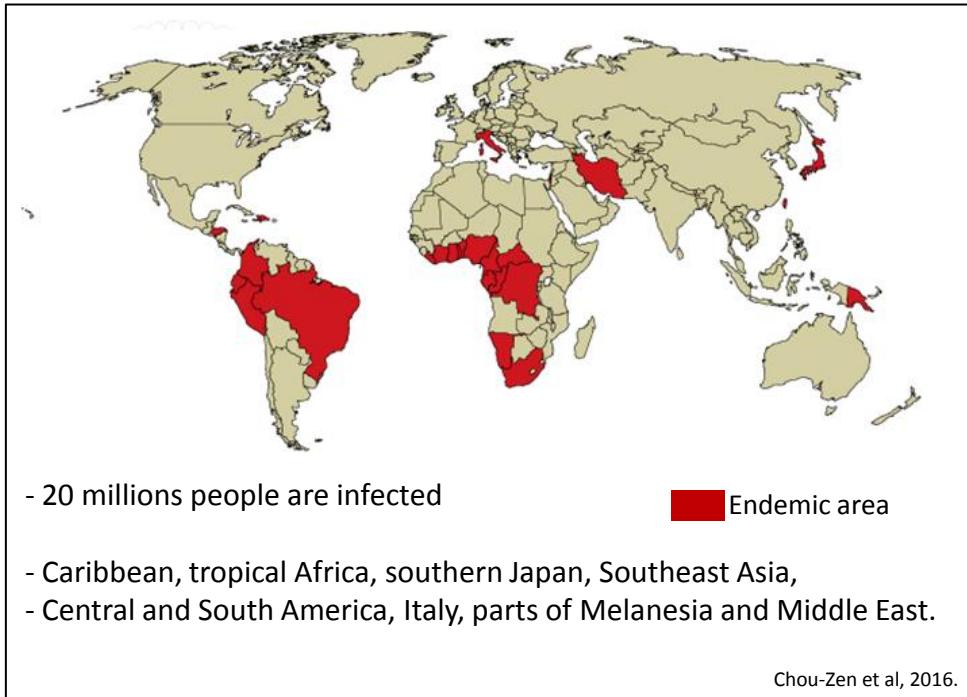


HTLV KEY FEATURES

Classification:

Deltaretrovirus

Epidemiology:



Transmission:

Contaminated blood and tissues

Breastfeeding

Sexual contact

Diseases:

Adult T Cell Leukemia

TSP/HAM

Treatment:

No efficient treatment

➔ We need a deep understanding of the molecular mechanisms

HTLV RETROVIRUSES



HTLV-1



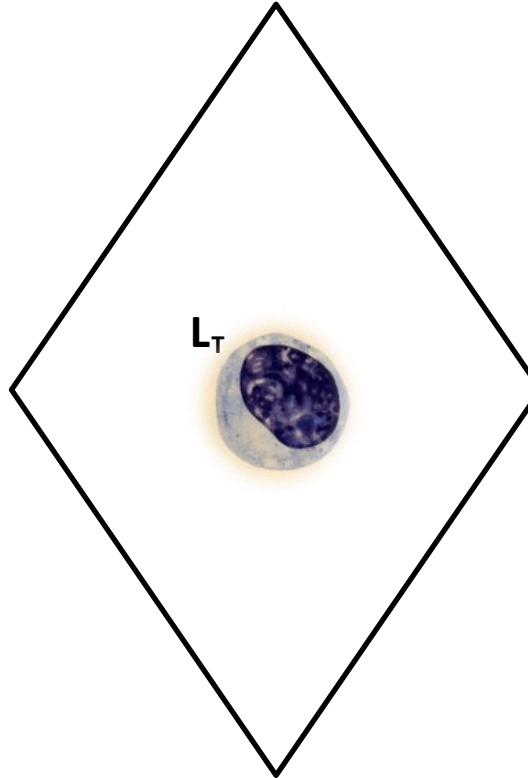
HTLV-2



HTLV-3



HTLV-4



**Two diseases:
ATLs & TSP**



Clinical complications
but **no associated
diseases.**



**No associated
diseases**



**No associated
diseases**

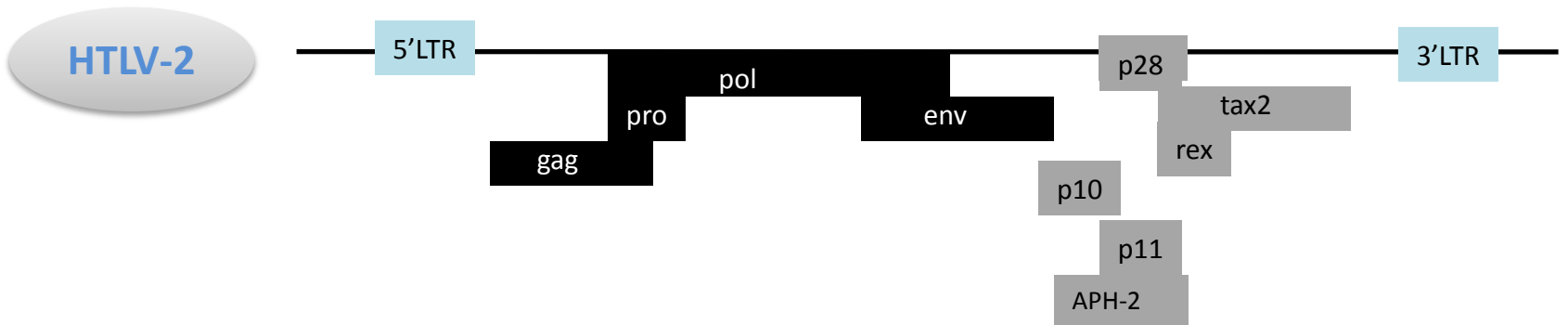
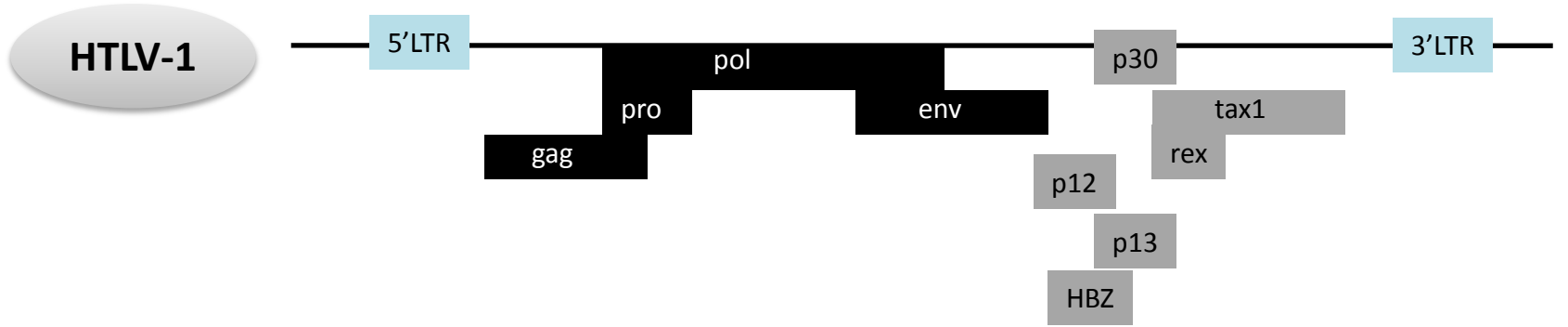
HTLV RETROVIRUSES



Structural genes



Non-structural genes



HTLV RETROVIRUSES

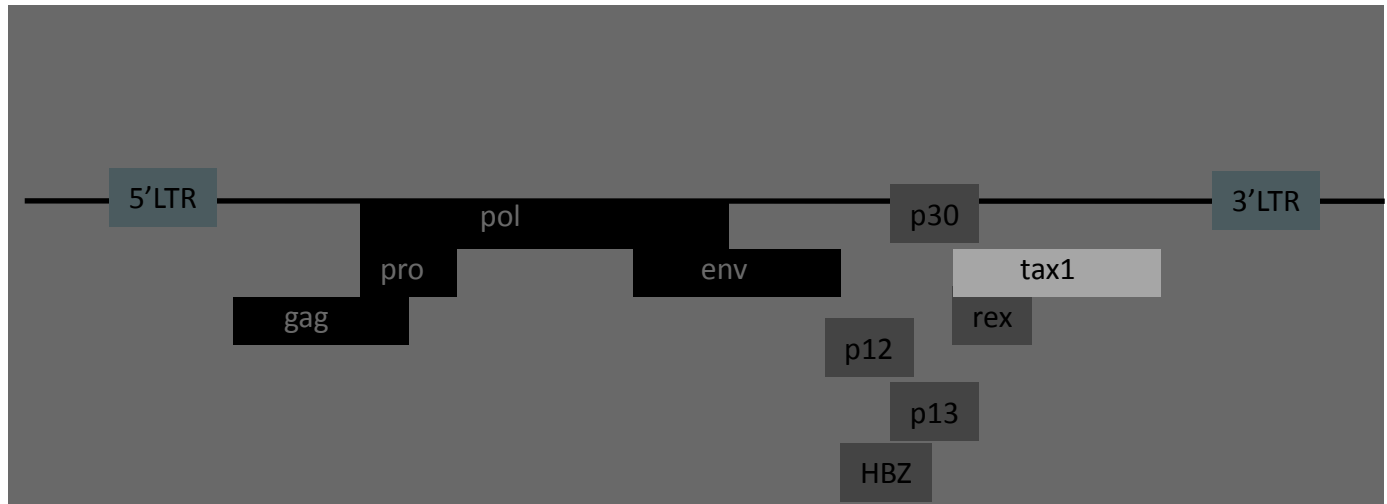


Structural genes

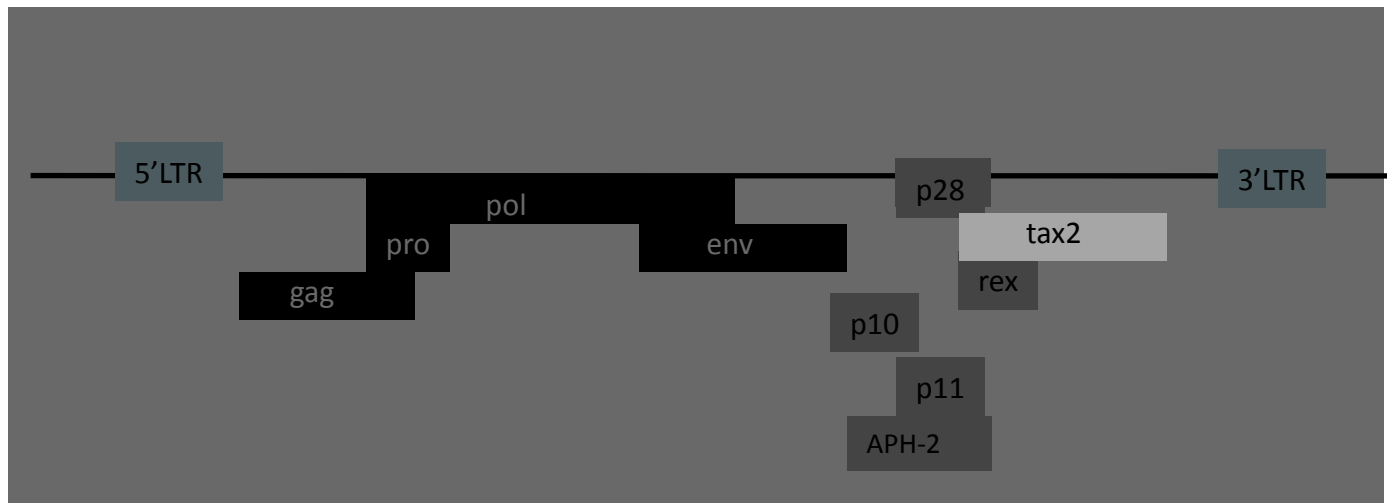


Non-structural genes

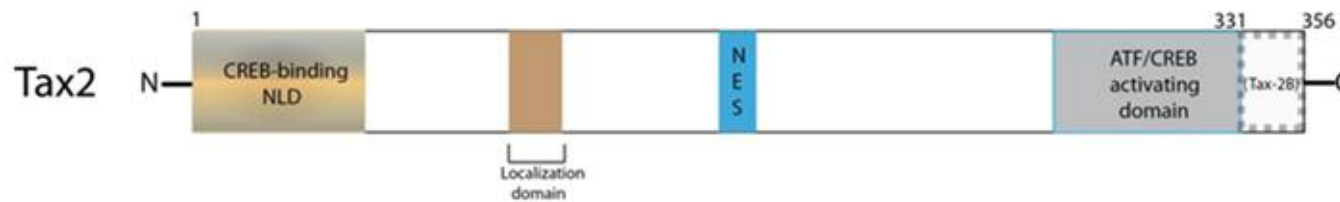
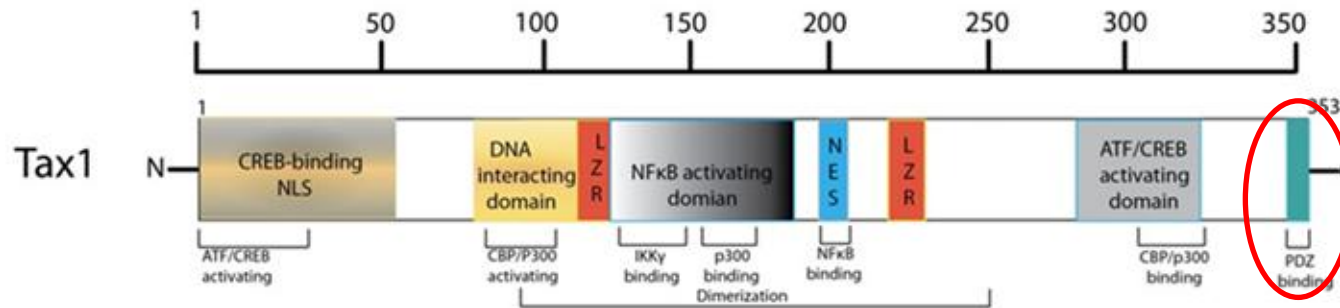
HTLV-1



HTLV-2

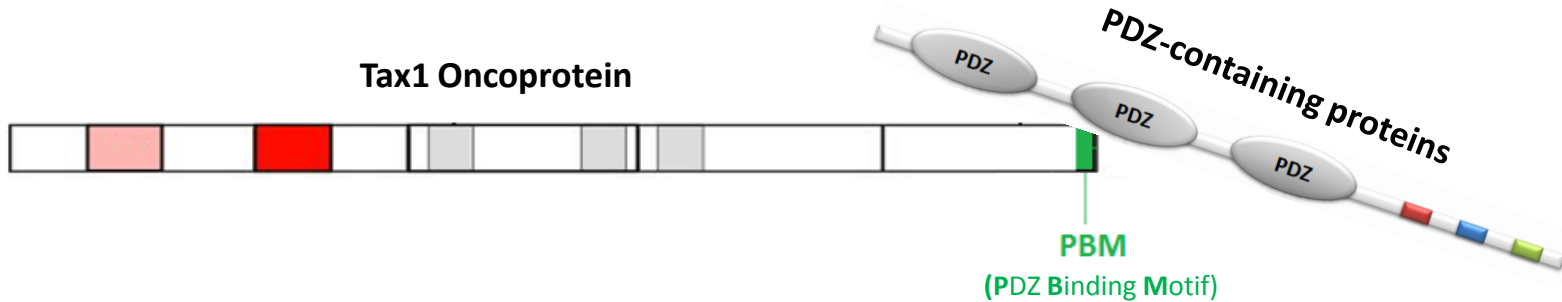


TAX1 & TAX2 DOMAINS



TAX ONCOPROTEIN AND PDZ PROTEINS

HTLV-1



PDZ-containing proteins

-Polarity

ZO

-Morphology

SCRIB

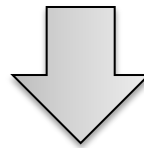
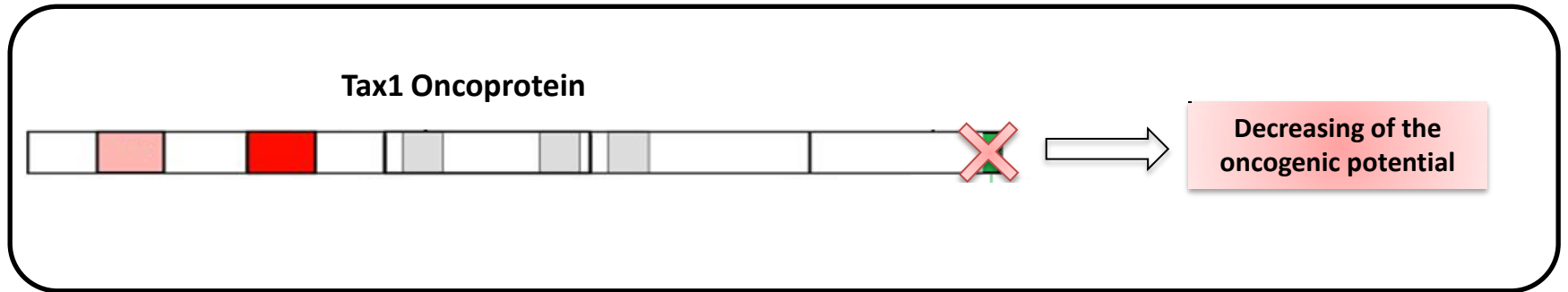
-Proliferation

DLG1

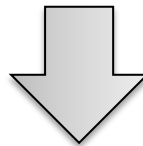
Targetted by several oncoviral proteins

E6, E7, E4-ORF1, Tax, ...

PBM



Interaction of Tax with PDZ containing proteins is one of the most important keys of HTLV-1 cell transformation.

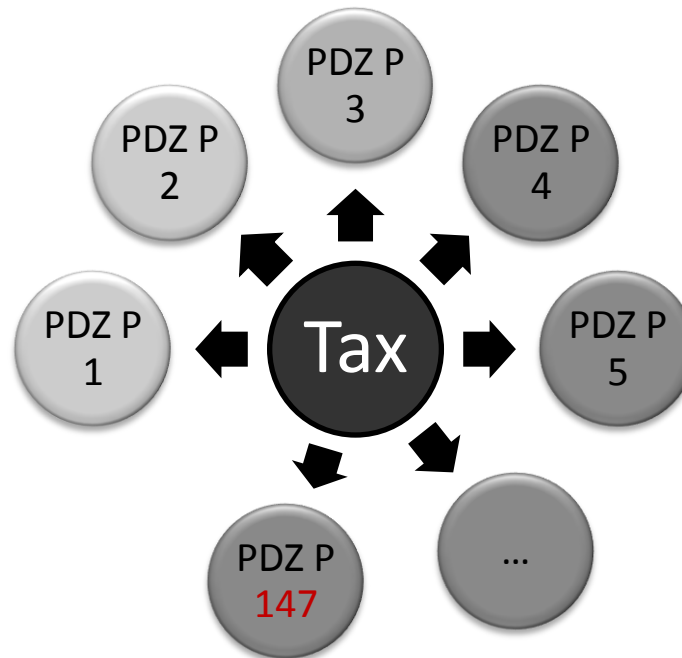


Does Tax interact with every PDZ containing protein ?

PROJECT PURPOSE

Generate a Tax/PDZ interactions map as complete as possible

147 PDZ Proteins vs Tax



Specificity ?

METHODOLOGY

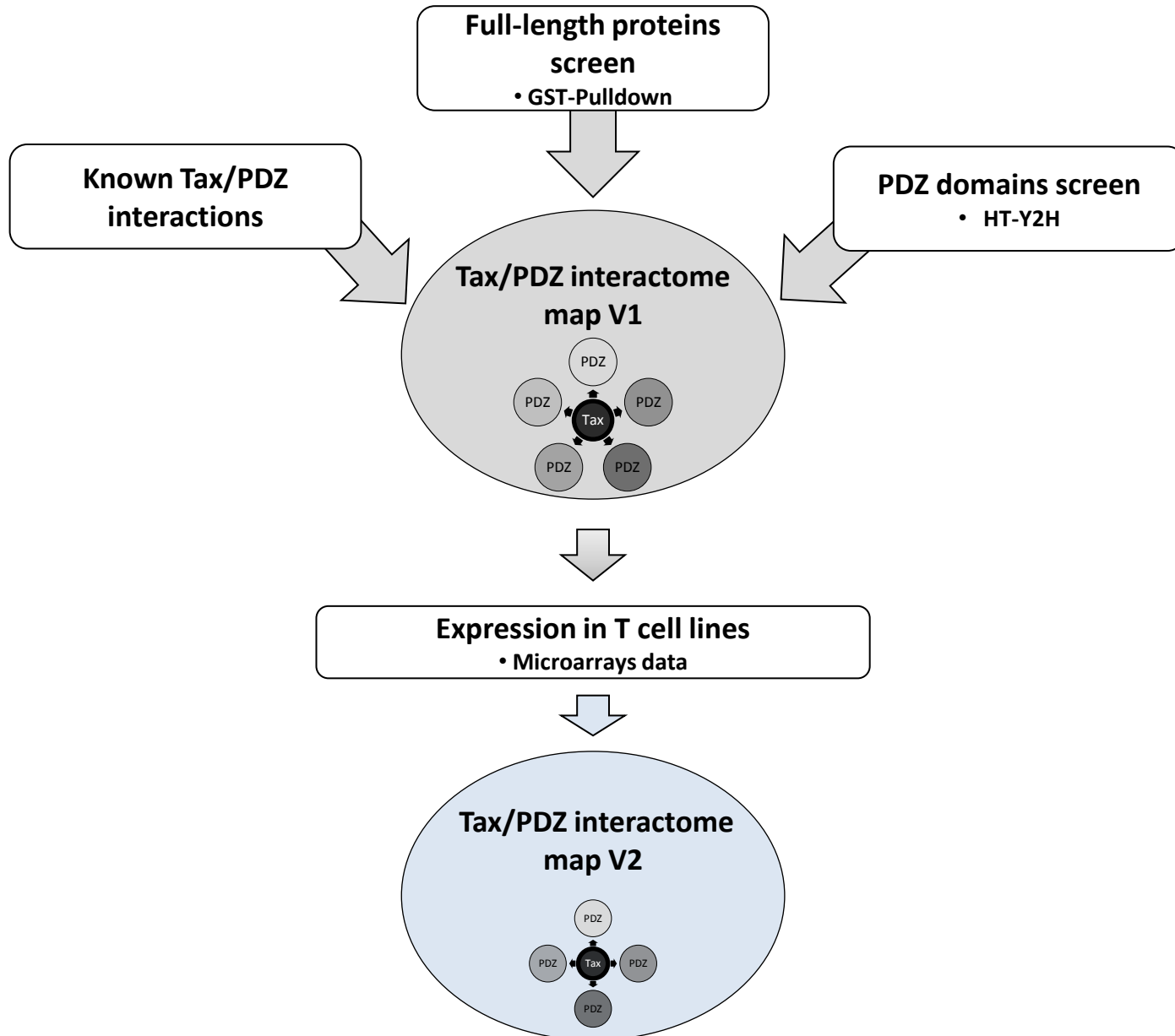


FIGURE 2

Literature screen

A.

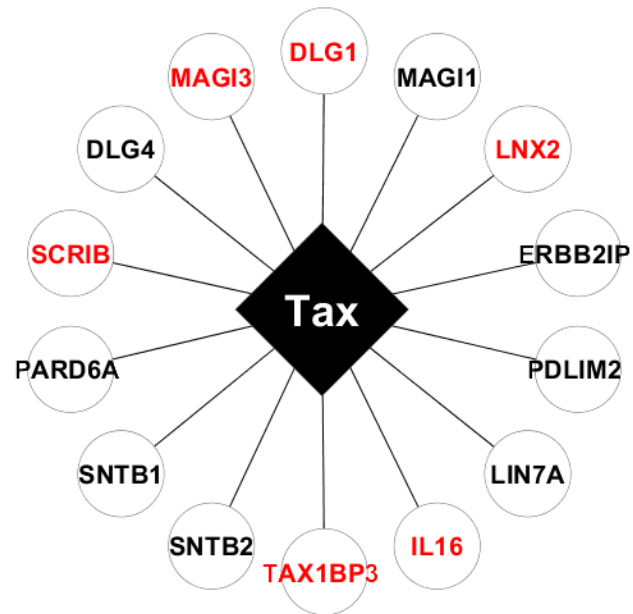


FIGURE 2

A.

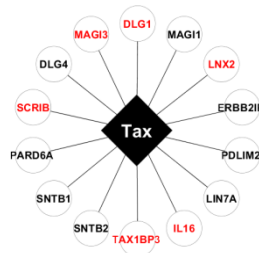


TABLE 1

Protein name	Gene ID	Uniprot Name	Uniprot ID	Pubmed article ID	HTLV-1 Biology
DLG1	1739	DLG1_HUMAN	Q12959	9192623	Cell cycle acceleration
DLG4	1742	DLG4_HUMAN	P78352	9482110	-
ERBB2IP	55914	LAP2_HUMAN	Q96RT1	17100642	-
IL16	3603	IL16_HUMAN	Q14005	12620798	Cell cycle acceleration
LIN7A	8825	LIN7A_HUMAN	O14910	9482110	-
LNX2	222484	LNX2_HUMAN	Q8N448	22458338	-
MAGI1	9223	MAGI1_HUMAN	Q96QZ7	23279616	IL-2-independent growth
MAGI3	260425	MAGI3_HUMAN	Q5TCQ9	15003862	-
PARD6A	50855	PAR6A_HUMAN	Q9NPB6	9482110	-
PDLIM2	64236	PDLI2_HUMAN	Q96JY6	19131544	Suppression of Tax-mediated tumorigenesis
SCRIB	23513	SCRIB_HUMAN	Q14160	17855372	Immune response suppression
SNTB1	6641	SNTB1_HUMAN	Q13884	9482110	-
SNTB2	6645	SNTB2_HUMAN	Q13425	9482110	-
TAX1BP3	30851	TX1B3_HUMAN	O14907	9482110	-

FIGURE 2

Full length 74 proteins screen (from ORFeome)

B.

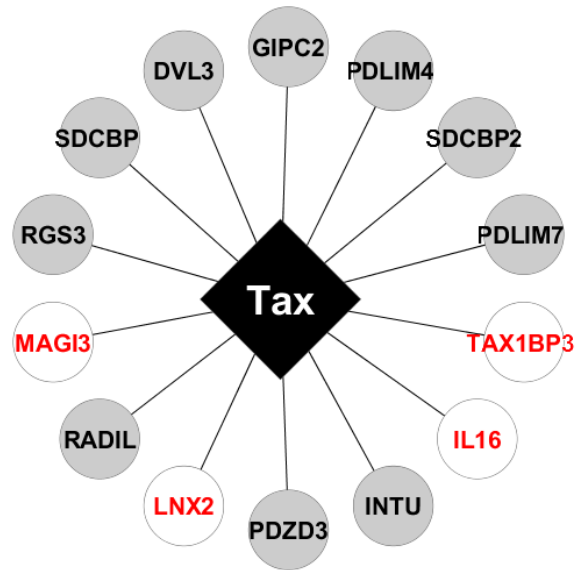


FIGURE 2

B.

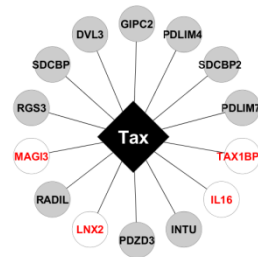


TABLE 2

Protein name	Interaction	Protein name	Interaction	Protein name	Interaction	Protein name	Interaction
AHNAK	No	INTU	Yes	PDLIM3	No	SDCBP	Yes
APBA3	No	LDB3	No	PDLIM4	Yes	SDCBP2	Yes
ARHGAP21	No	LIMK2	No	PDLIM5	No	SIPA1L2	No
CARD11	No	LIN7A	No	PDLIM7	Yes	SNTG2	No
CASK	No	LNK1	No	PDZD11	No	SNX27	No
CNKSR2	No	LNX2	Yes	PDZD3	Yes	STXBP4	No
CYTIP	No	LRRC7	No	PDZD4	No	SYNJ2BP	No
DVL1	No	MAGI3	Yes	PDZD8	No	TAX1BP3	Yes
DVL2	No	MAGIX	No	PDZK1	No	TIAF1	No
DVL3	Yes	MAST1	No	PRX	No	TIAM1	No
ERBB2IP	No	MPP1	No	PSMD9	No	TJP1	No
FRMPD1	No	MPP2	No	PTPN3	No	TJP3	No
FRMPD2	No	MPP3	No	PTPN4	No	USH1C	No
FRMPD4	No	MPP4	No	RADIL	Yes		
GIPC2	Yes	MPP5	No	RAPGEF2	No		
GIPC3	No	PARD3	No	RAPGEF6	No		
GOPC	No	PARD6A	No	RGS3	Yes		
GRIP1	No	PARD6B	No	RHPN1	No		
HTRA2	No	PDLIM1	No	RHPN2	No		
IL16	Yes	PDLIM2	No	RIMS2	No		

FIGURE 2

Separated PDZ domains screen

C.

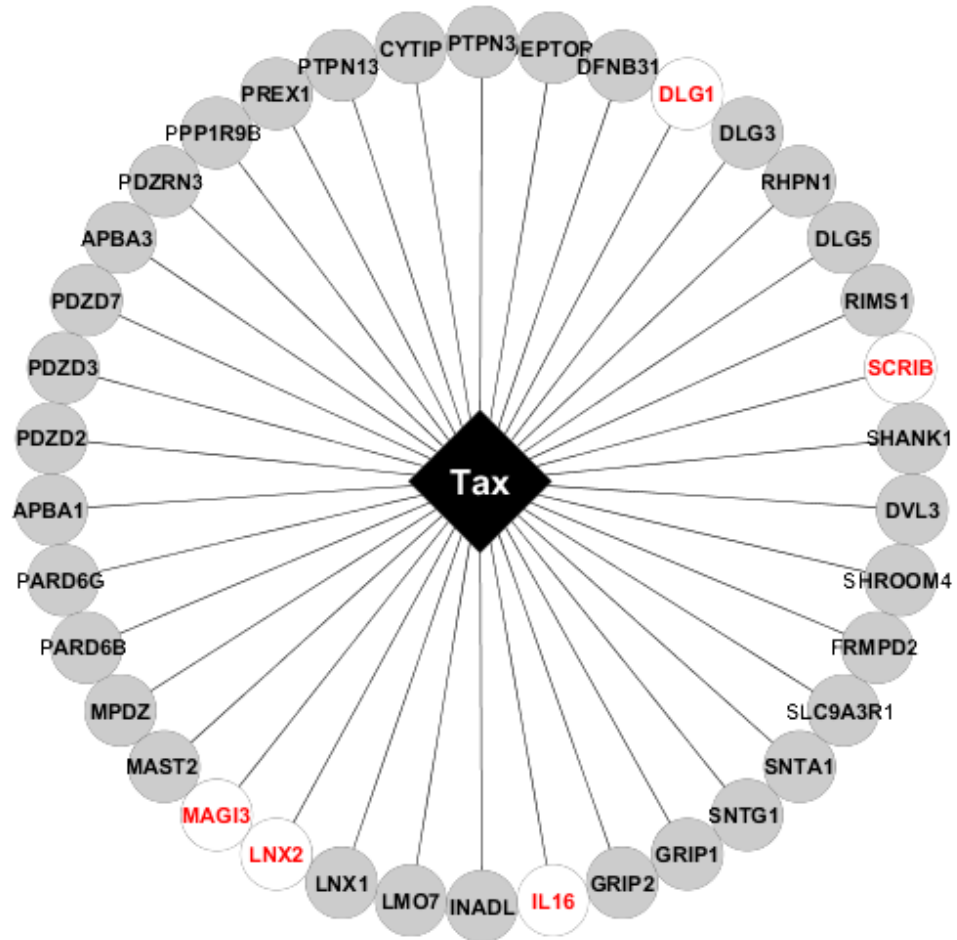


FIGURE 2

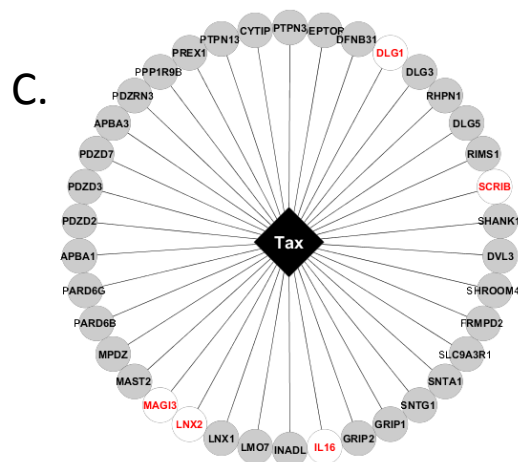


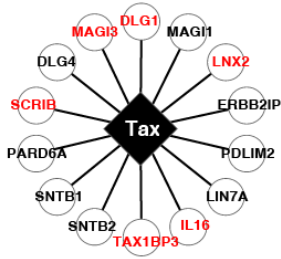
TABLE 3

APBA1_1	APBA1_2	APBA2_1	APBA2_2	APBA3_1	APBA3_2	ARHGAP21	ARHGEF11	ARHGEF12	CARD11	CARD14	CASK
DLG1_1	DLG1_2	DLG1_3	DLG2_1	DLG2_2	DLG2_3	DLG3_1	DLG3_2	DLG3_3	DLG4_1	DLG4_2	DLG4_3
DLG5_1	DLG5_2	DLG5_3	DLG5_4	HTRA2	HTRA4	INADL_1	INADL_10	INADL_2	INADL_3	INADL_4	INADL_5
INADL_6	INADL_7	INADL_8	INADL_9	LNX1_1	LNX1_3	LNX1_4	LNX2_1	LNX2_2	LNX2_3	LNX2_4	MAG1_1
MAG1_2	MAG1_3	MAG1_4	MAG1_5	MAG1_6	MAG1_6	MAGI2_1	MAGI2_2	MAGI2_3	MAGI2_4	MAGI2_5	MAGI2_6
MAGI3_2	MAGI3_3	MAGI3_4	MAGI3_5	MAGI3_6	MAGIX	MPP1	MPP2	MPP3	MPP4	MPP5	MPP6
MPP7	PARD3_1	PARD3_3	PARD3B_1	PARD3B_2	PARD3B_3	PARD6B	PARD6G	PSMD9	RAPGEF2	SCRIB_1	SCRIB_2
SCRIB_3	SHANK2	SNTB1	NEG	NEG	TJP1_2	TJP2_1	TJP2_2	TJP2_3	TJP3_1	TJP3_2	TJP3_3
AHNAK	CNKS2	CNKS3	DEPDC2_1	DEPDC2_2	DEPTOR	DFNB31_1	DFNB31_2	DFNB31_3	DVL1	DVL2	DVL3
215045	215860	FNIP1	FRMPD1	FRMPD2_1	FRMPD2_2	FRMPD2L2	FRMPD4	GIPC1	GIPC2	GIPC3	GOPC
GORASP2	GRASP	GRIP1_1	GRIP1_2	GRIP1_3	GRIP1_4	GRIP1_5	GRIP1_6	GRIP1_7	GRIP2_1	GRIP2_2	GRIP2_3
GRIP2_4	GRIP2_5	GRIP2_6	GRIP2_7	IL16_1	IL16_2	INTU	KIAA1849	LDB3	LIMK2	LMO7	NEG
MAST1	MAST2	MAST3	MAST4	MPDZ_1	MPDZ_10	MPDZ_11	MPDZ_12	MPDZ_13	MPDZ_3	MPDZ_4	MPDZ_5
MPDZ_6	MPDZ_7	MPDZ_8	MPDZ_9	MYO18A	PREX1_1	PREX1_2	PDLIM1	PDLIM2	PDLIM3	PDLIM4	PDLIM5
PDLIM7	PDZD11	PDZD2_1	PDZD2_2	PDZD2_3	PDZD2_4	PDZD2_5	PDZD2_6	PDZD3_1	PDZD3_2	PDZD3_3	PDZD3_4
PDZD4	PDZD7_1	PDZD7_2	PDZD8	PDZK1_1	PDZK1_2	PDZK1_3	PDZRN3_1	PDZRN3_2	PDZRN4	PDZRN5	PDZRN6
LRRC7	LNK1_2	SHANK1	NOS1	PARD6A	ERBB2IP	LIN7C	PICK1	HTRA3	HTRA4	TAX1BP3	LIN7B
NEG	SNTA1	SYNPO2	MLLT4	LIN7A	PARD3_2	TIAM1	SLC9A3R1_2	TJP1_3	MPDZ_2	SCRIB_4	IL16_3
PRX	CYTIP	PTPN13_1	PTPN13_2	PTPN13_3	PTPN13_4	PTPN13_5	PTPN3	PTPN4	RGS12	RGS3	RHPN1
RHPN2	RIMS1	RIMS2	SDCBP_1	SDCBP_2	SDCBP2_1	SDCBP2_2	NEG	SHROOM4	NEG	SIPA1L1	SIPA1L2
SIPA1L3	SLC9A3R1_1	SLC9A3R2_1	SLC9A3R2_2	SNTG1	SNTG2	SNX27	SYNJ2BP	TIAM2	USH1C_1	USH1C_2	USH1C_3

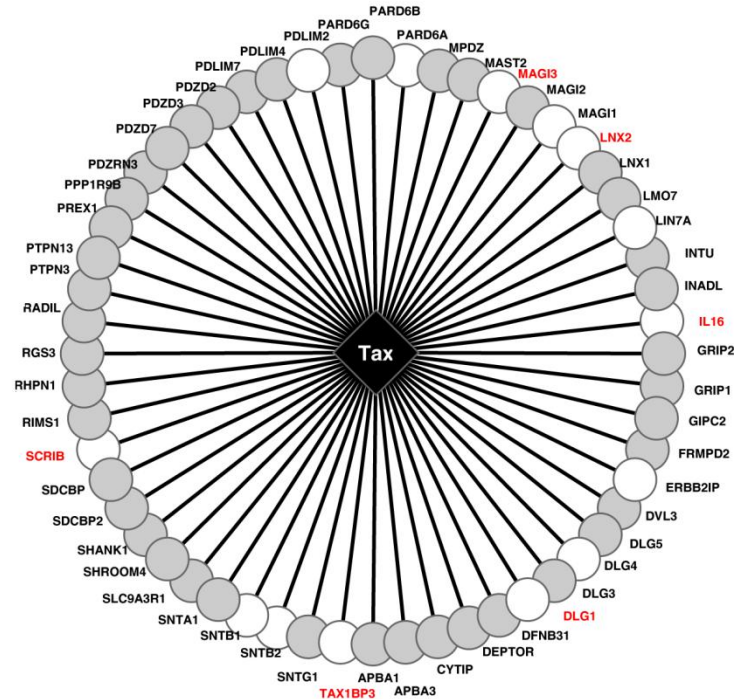
TAX/PDZ PROTEIN INTERACTOME ENRICHMENT

Literature Curated Interactions:
14 proteins

A.



D.



Tax/ PDZome network
enrichment

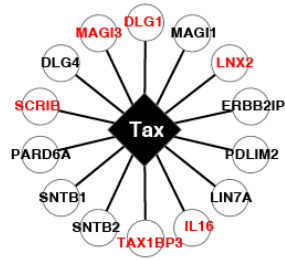
TAX/PDZ PROTEIN INTERACTOME ENRICHMENT

Literature Curated Interactions:
14 proteins

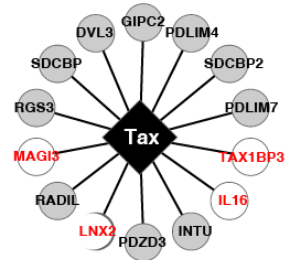
Full-length PDZ proteins:
14 proteins

PDZ domains:
38 proteins

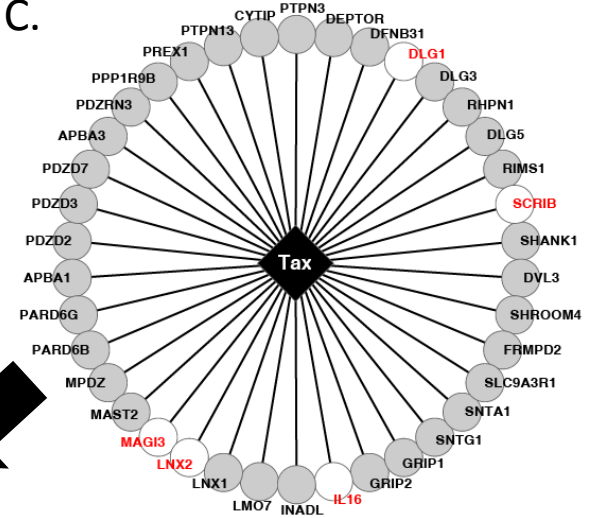
A.



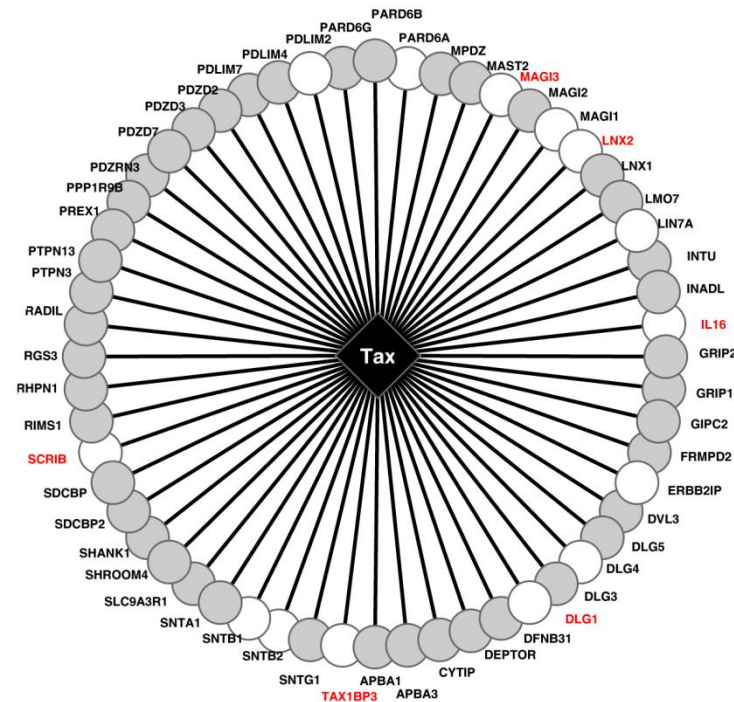
B.



C.



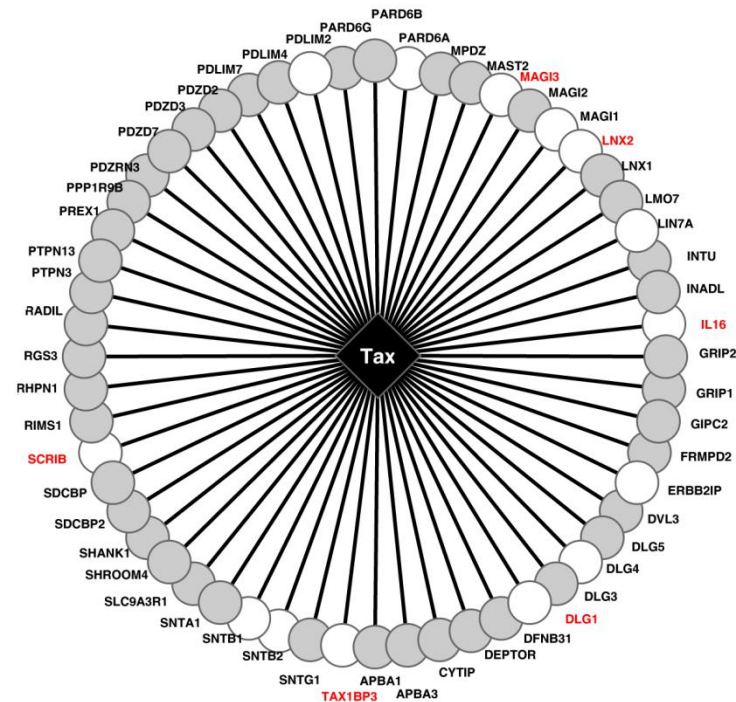
D.



Tax/ PDZome network
enrichment

TAX/PDZ PROTEIN INTERACTOME ENRICHMENT

Tax/PDZ INTERACTOME V1



PDZ PROTEINS EXPRESSION ON T-CELL LINES

24 T-cell lines:
PDZ Proteins mRNA expression levels

HTLV infected VS non-infected T cell lines:
Differential PDZ Proteins mRNA expression

T-cell lines
ALL-SIL
Be13
<u>CEM</u>
<u>CTV-1</u>
DND-41
DU.528
HPB-ALL
H-SB2
<u>JURKAT</u>
K3P
Karpas 45
KOPT-K1
LOUCY
MOLT-13
<u>MOLT-16</u>
MOLT-4
P12-ICHIKAWA
PEER
PF-382
SKW-3
SUP-T11
SUP-T13
SUP-T7

Non infected
CEM
CTV1
HUT-78
JURKAT
MOLT16

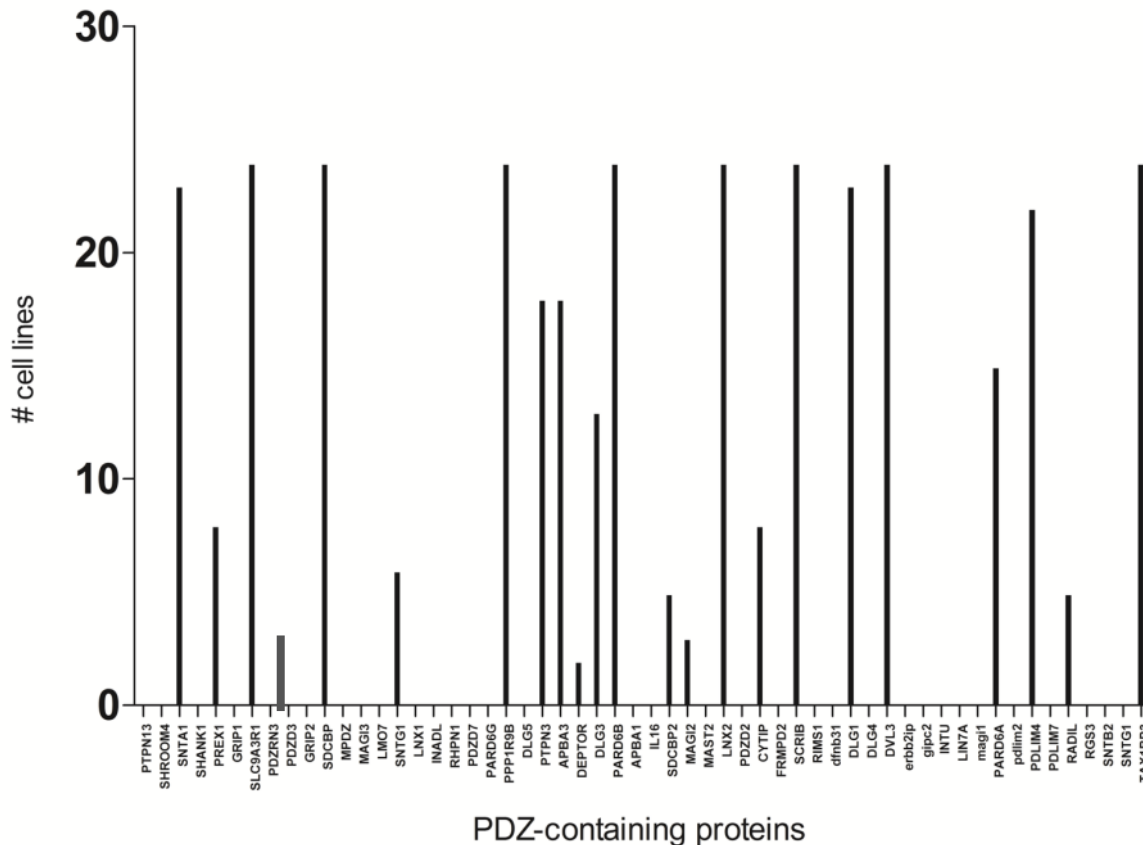
Infected
C81-45
C91PL
HUT102
MT2
MT4

PDZ PROTEINS EXPRESSION ON T-CELL LINES

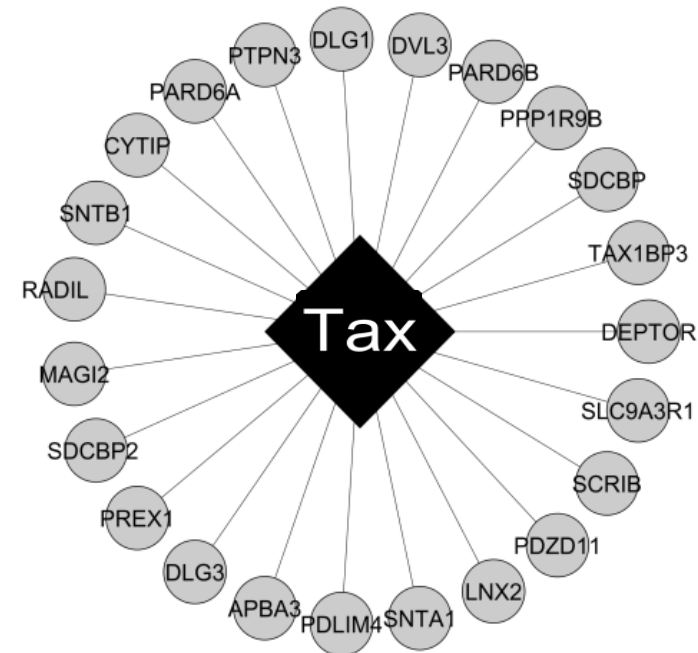
24 T-cell lines:
PDZ Proteins mRNA expression levels

HTLV infected VS non-infected T cell lines:
Differential PDZ Proteins mRNA expression

A.



B.

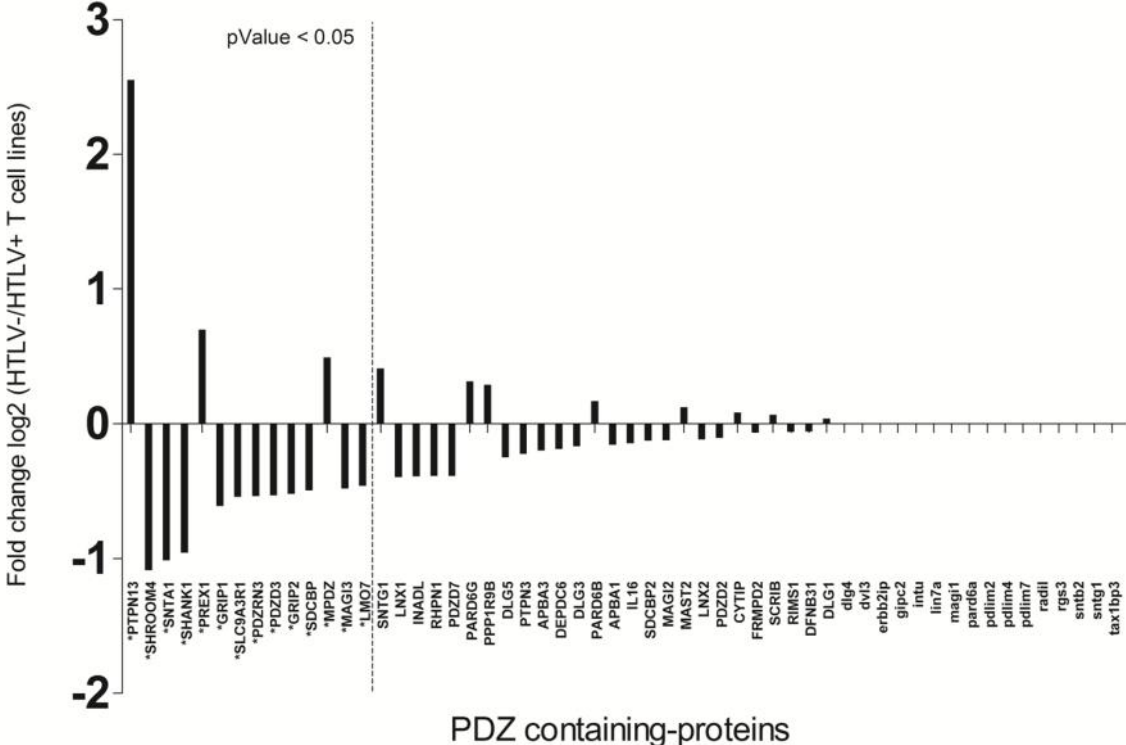


PDZ PROTEINS EXPRESSION ON T-CELL LINES

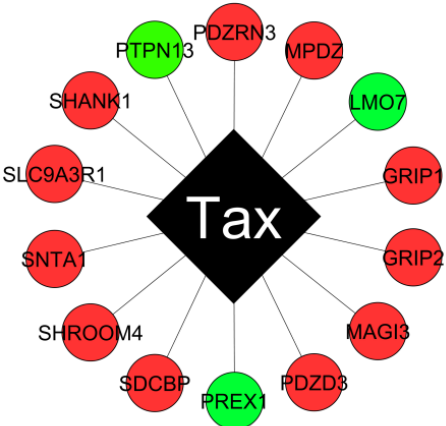
24 T-cell lines:
PDZ Proteins mRNA expression levels

HTLV infected VS non-infected T cell lines:
Differential PDZ Proteins mRNA expression

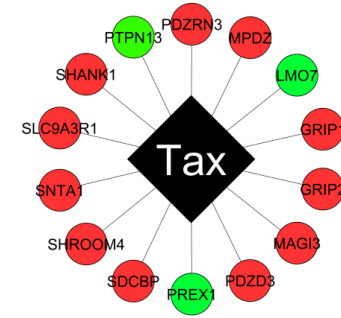
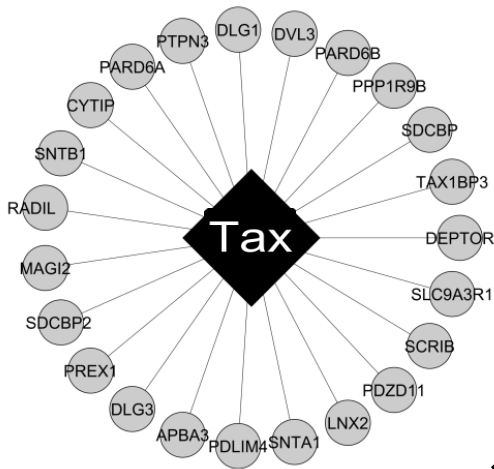
C.



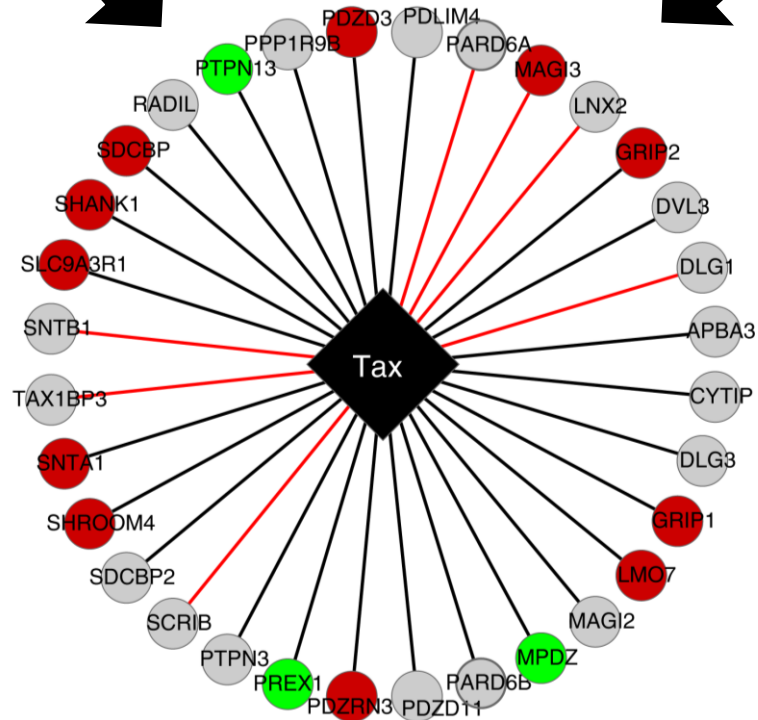
D.



Tax/PDZ INTERACTOME V2



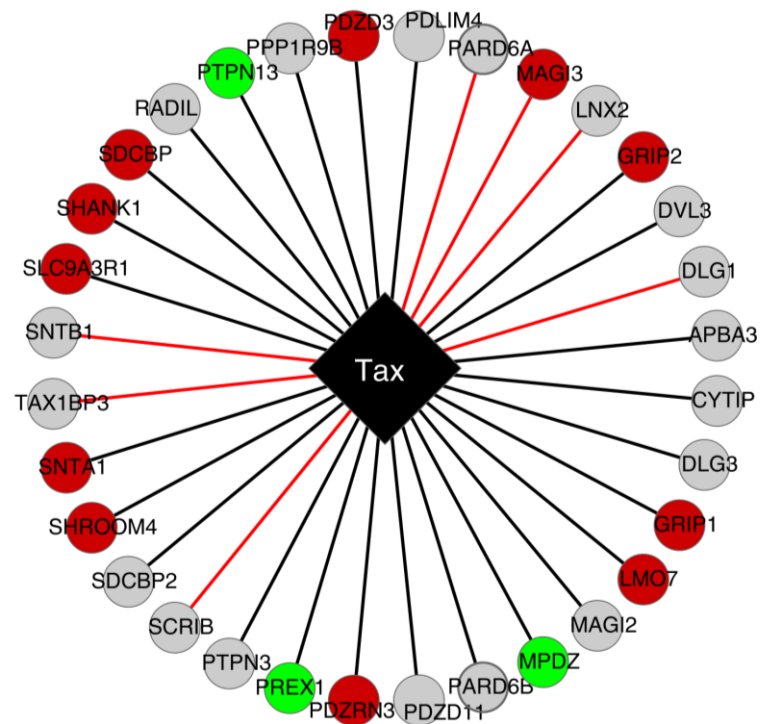
E.



Tax/PDZ INTERACTOME V2

Tax/PDZ INTERACTOME V2

E.



V2 Tax/PDZ INTERACTOME ANALYSIS

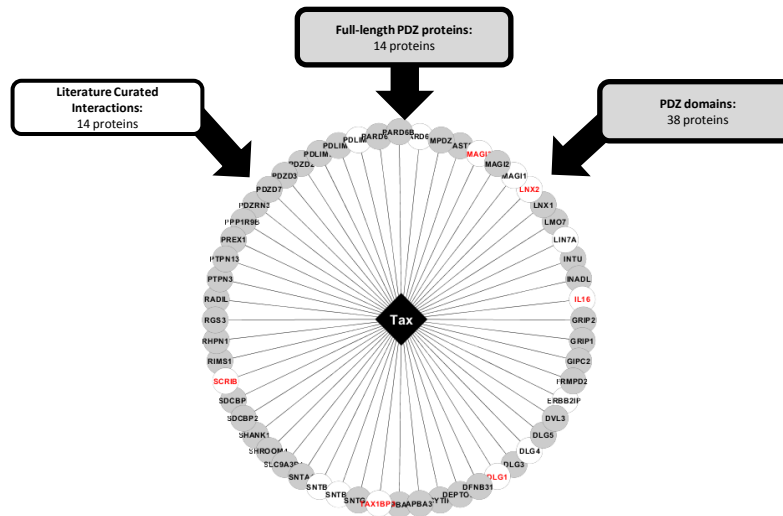
T-cells expression	Differential expression in HTLV+/- Cells	Summary
APBA3		expressed in Tcells
CYTIP		expressed in Tcells
DLG1		expressed in Tcells
DLG3		expressed in Tcells
DVL3		expressed in Tcells
	GRIP1	downregulated in HTLV+ cells
	GRIP2	downregulated in HTLV+ cells
	LMO7	upregulated in HTLV+ cells
LNX2		expressed in Tcells
MAGI2		expressed in Tcells
	MAGI3	downregulated in HTLV+ cells
	MPDZ	downregulated in HTLV+ cells
PARD6A		expressed in Tcells
PARD6B		expressed in Tcells
PDLIM4		expressed in Tcells
PDZD11		expressed in Tcells
	PDZD3	downregulated in HTLV+ cells
	PDZRN3	downregulated in HTLV+ cells
PPP1R9B		expressed in Tcells
PREX1	PREX1	expressed and upregulated in HTLV+ cells
	PTPN13	upregulated in HTLV+ cells
PTPN3		expressed in Tcells
RADIL		expressed in Tcells
SCRIB		expressed in Tcells
SDCBP	SDCBP	expressed and downregulated in HTLV+ cells
SDCBP2		expressed in Tcells
	SHANK1	downregulated in HTLV+ cells
	SHROOM4	downregulated in HTLV+ cells
SLC9A3R1	SLC9A3R1	expressed and downregulated in HTLV+ cells
SNTA1	SNTA1	expressed and downregulated in HTLV+ cells
SNTB1		expressed in Tcells
TAX1BP3		expressed in Tcells

Expressed

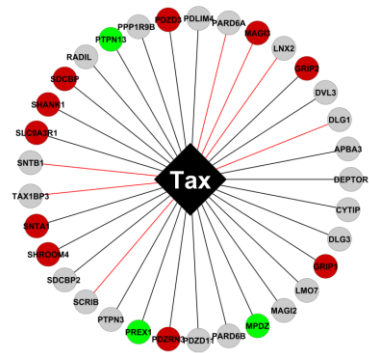
Upregulated

Downregulated

STRATEGY



Expression in T cell lines
• Microarrays data



Tax/PDZ interactome map V2

PDZ-DEPENDANT TAX PATHOGENESIS

Literature: 14 PDZ proteins

Protein name	Gene ID	Uniprot Name	Uniprot ID	Pubmed article ID	HTLV-1 Biology
DLG1	1739	DLG1_HUMAN	Q12959	9192623	Cell cycle acceleration
DLG4	1742	DLG4_HUMAN	P78352	9482110	-
ERBB2IP	55914	LAP2_HUMAN	Q96RT1	17100642	-
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MAGI1	9223	MAGI1_HUMAN	Q96QZ7	23279616	IL-2-independent growth
MAGI3	260425	MAGI3_HUMAN	Q5TCQ9	15003862	-
PAR6A	50855	PAR6A_HUMAN	Q9NPB6	9482110	-
PDLIM2	64236	PDLI2_HUMAN	Q96JY6	19131544	Suppression of Tax-mediated tumorigenesis
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SNTB1	6641	SNTB1_HUMAN	Q13884	9482110	-
SNTB2	6645	SNTB2_HUMAN	Q13425	9482110	-
TAX1BP3	30851	TX1B3_HUMAN	O14907	9482110	-

PDZ-DEPENDANT TAX PATHOGENESIS

TABLE 4

Our Study on 32 PDZ Proteins

Annotation Cluster	Enrichment Score	Gene Ontology (GO)	# genes	Name
1	0,66	cytoskeleton , cellular process organization	19	APBA3, DLG1 , LMO7, LNX2, MAGI3 , PARD6A , PARD6B, PPP1R9B, PREX1, PTPN13, PTPN3, RADIL, SCRIB , SDCBP, SDCBP2, SHANK1, SHROOM4, SLC9A3R1, SNTA1.
2	0,63	interspecies interaction between organisms, multi-organism process	3	DLG1 , MAGI3 , MPDZ
3	0,61	regulation of cell cycle, cellular developmental process	6	DLG1 , PARD6B, PPP1R9B, SCRIB , SHROOM4, SNTA1
4	0,52	negative regulation of cellular process, regulation of metabolic process, response to stimulus	11	APBA3, DEPDC6, DLG1 , DLG3, DVL3, GRIP1, PDZD3, PPP1R9B, PTPN3, SCRIB , TAX1BP3
5	0,46	regulation of cell proliferation	3	DLG3 , PPP1R9B, TAX1BP3

CYTOSKELETON CLUSTER

Figure 8

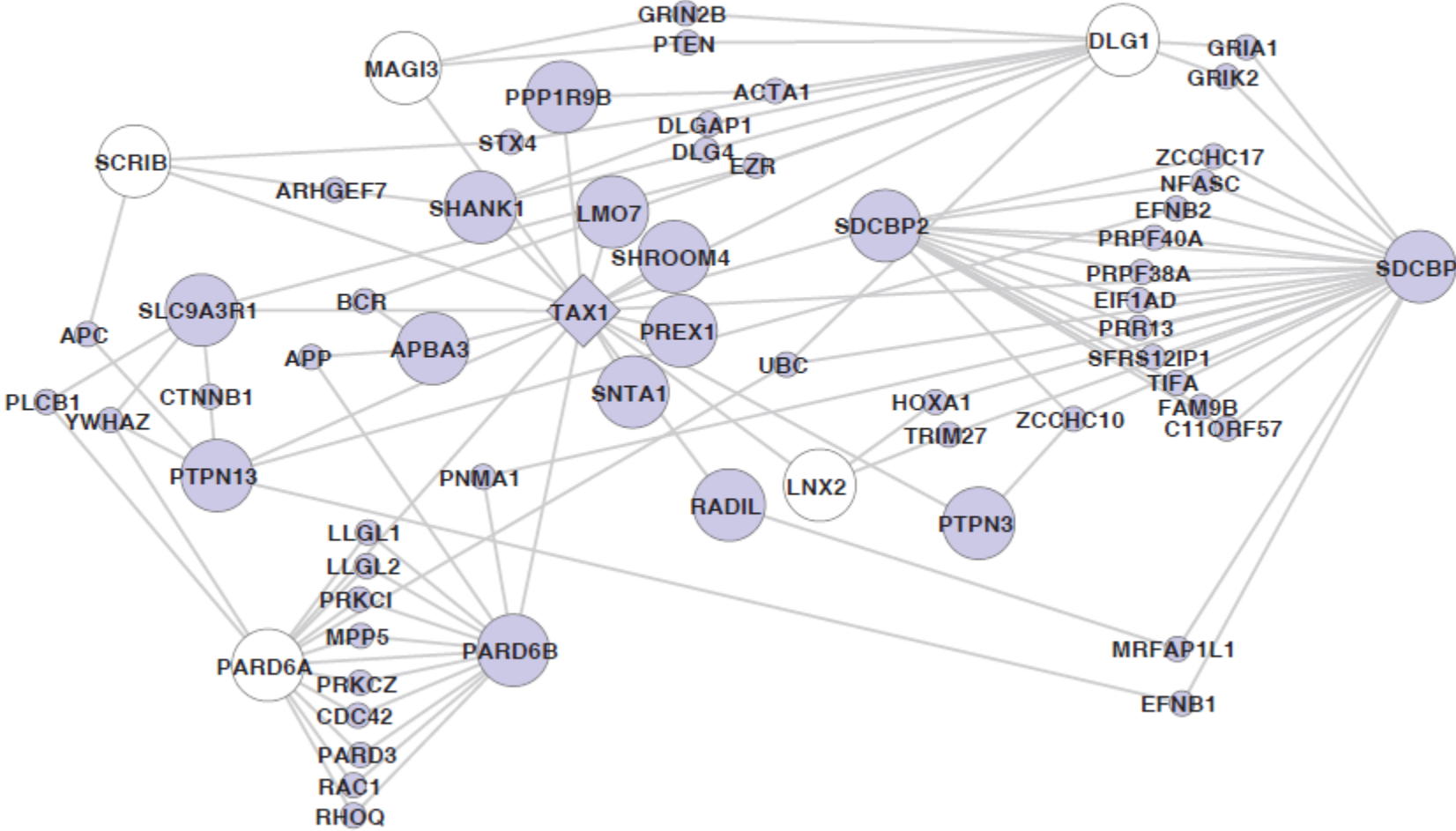
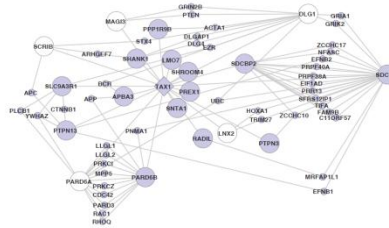


FIGURE 4

Figure 8

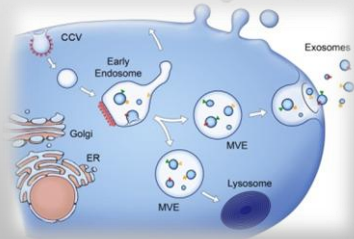


SUPPLEMENTAL TABLE 1

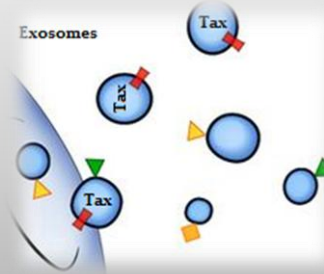
Gene ID	Interactions
SDCBP	166
SLC9A3R1	54
DLG1	47
PARD6A	35
LNX2	30
SDCBP2	27
PPP1R9B	21
PTPN13	17
PARD6B	16
SNTA1	14
SHANK1	12
MAGI3	10
SCRIB	9
RADIL	9
PTPN3	8
PREX1	5
APBA3	4
LMO7	4
SHROOM4	1

HYPOTHESIS

SDCBP: Exosomes formation



Tax: Exosomes localization



SDCBP is implicated in HTLV-1 particles transmission ?

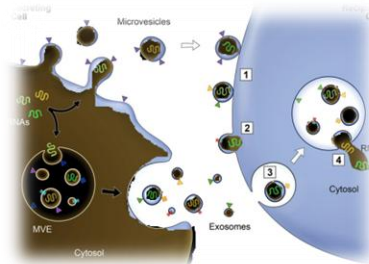
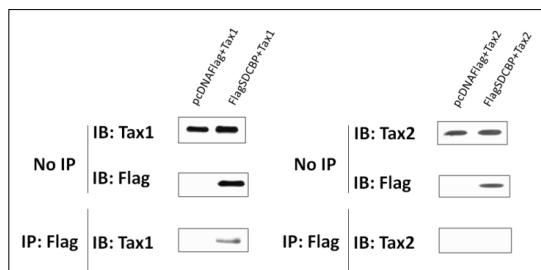
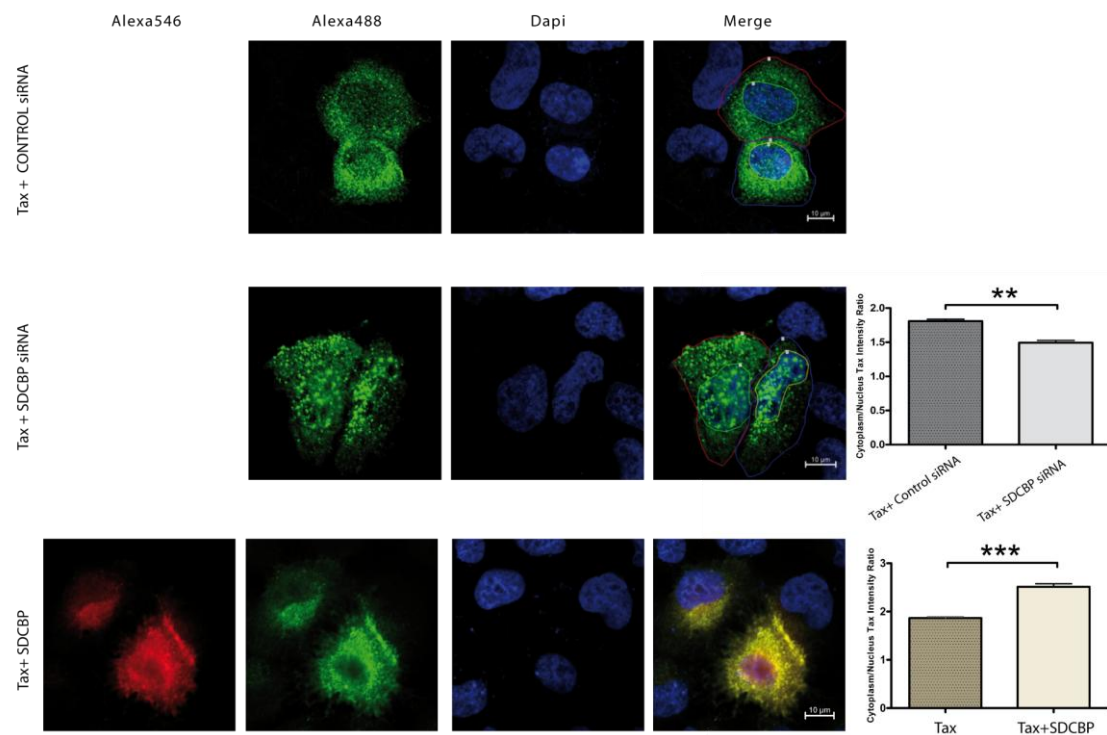


FIGURE 5

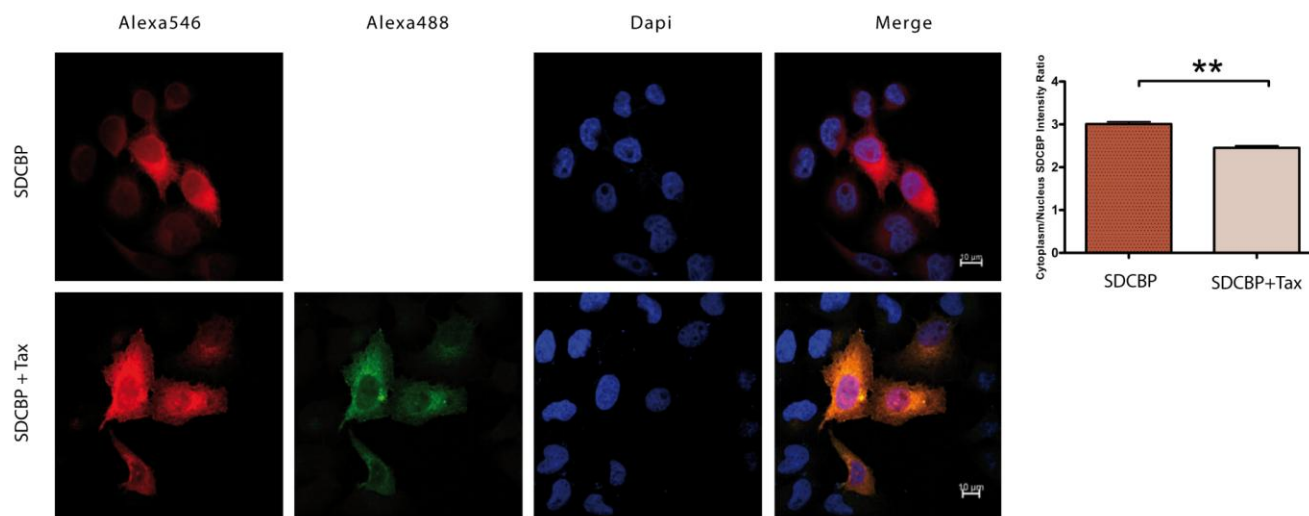
A.



B.

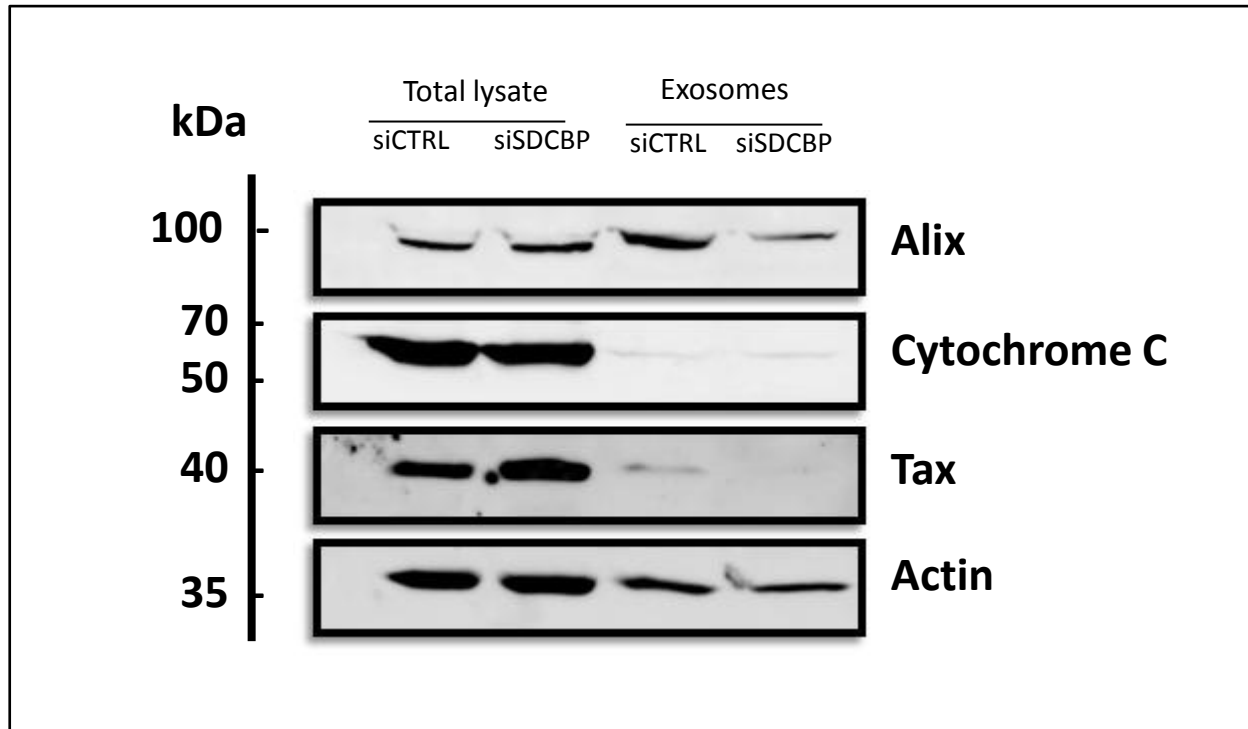


C.

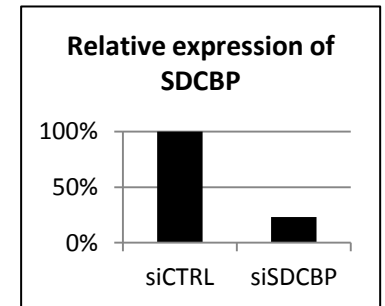


TAX EXOSOME LOCALIZATION

A.



B.



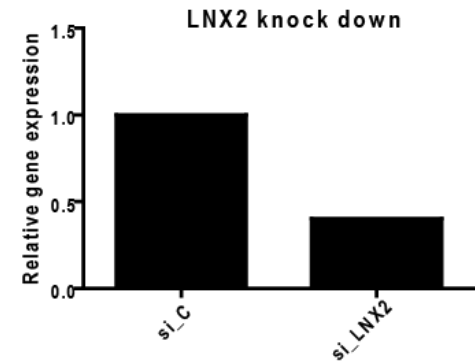
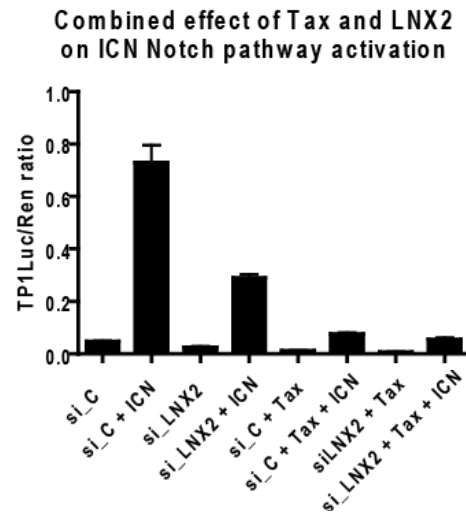
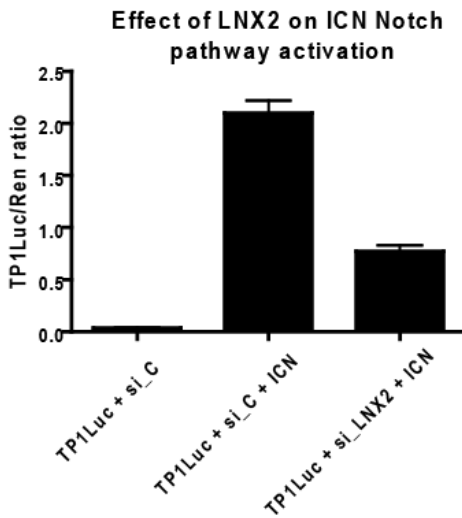
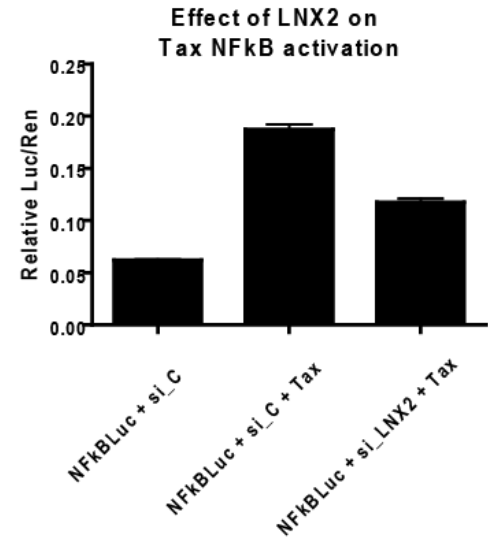
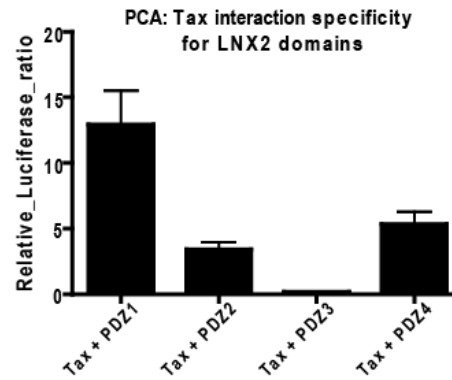
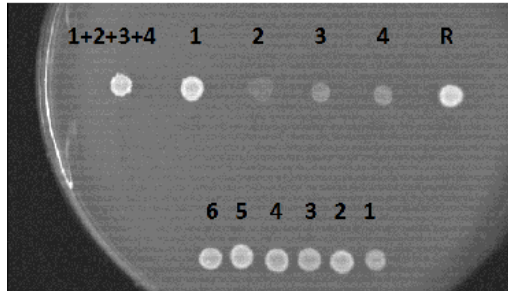
SDCBP is crucial for Tax exosome localization

CONCLUSION

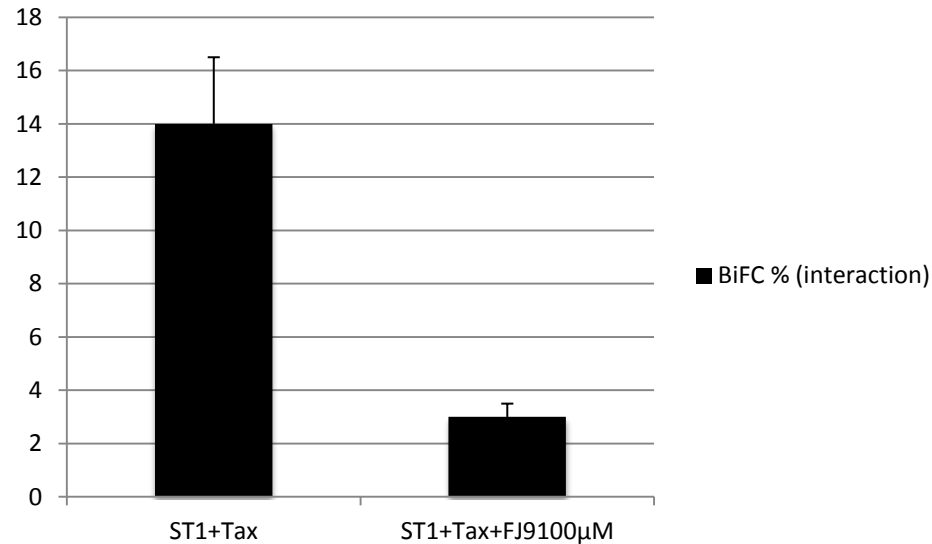
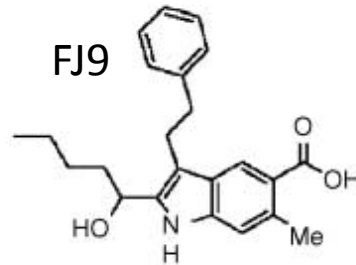
- **Tax targets 56 PDZ proteins**
- **Tax/PDZ Interactome : From 14 PDZ proteins to 56 out of 150**
- **Tax PDZ-dependant functions are related to the modification of cytoskeleton, metabolism and cell-cell contact**
- **SDCBP is an important Tax partner**
- **Tax localization is SDCBP dependant**
- **SDCBP is crucial for Tax exosomes localization**

SUPPLEMENTARY DATA

LNK2: AN INTERESTING TAX PARTNER



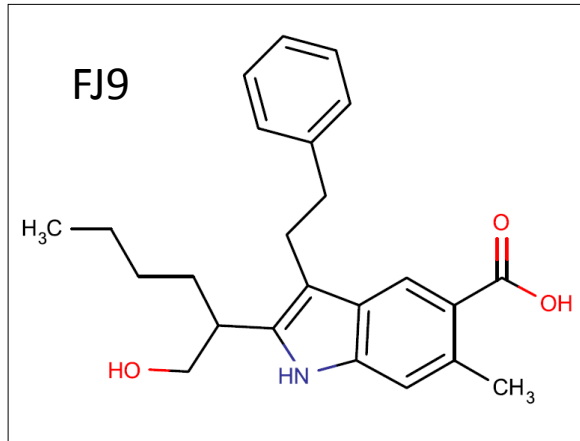
C. FJ9 CAN DISRUPT TAX-PDZ INTERACTION AND MORE



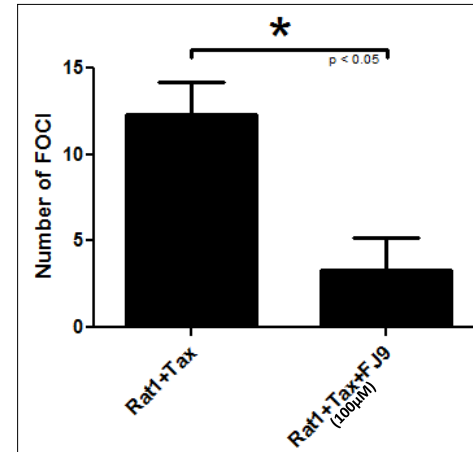
Defining a small molecule which can disrupt a Tax/PDZ interaction.

EFFECT OF FJ9 ON RAT1-TAX INDUCED TRANSFORMATION

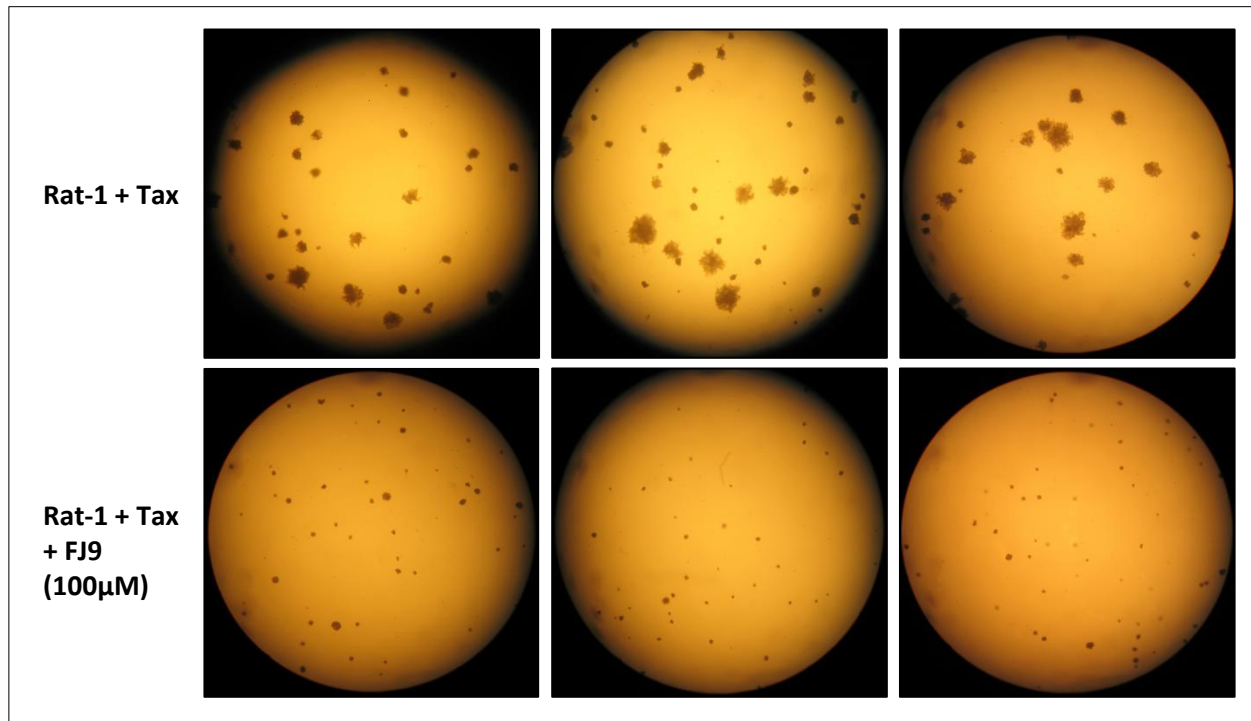
a.



b.



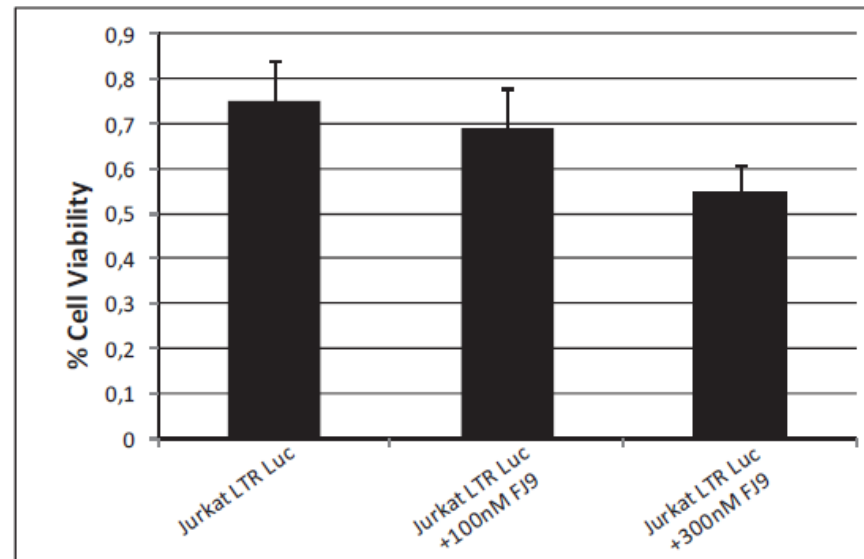
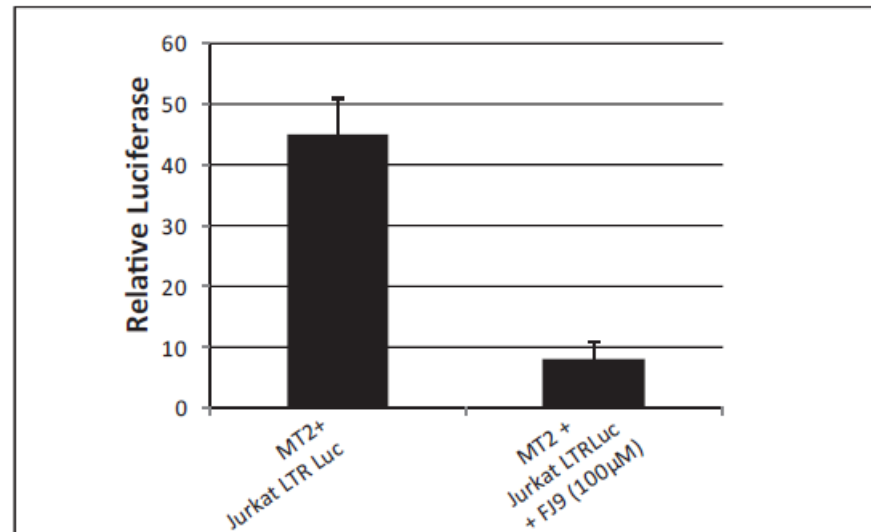
c.



Tax transformation in Rat-1 cells is inhibited in presence of FJ9 small molecule

EFFECT OF FJ9 ON TAX CELL TO CELL TRANSMISSION

Figure 14



FJ9 small molecule antagonizes the Tax cell to cell transmission



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