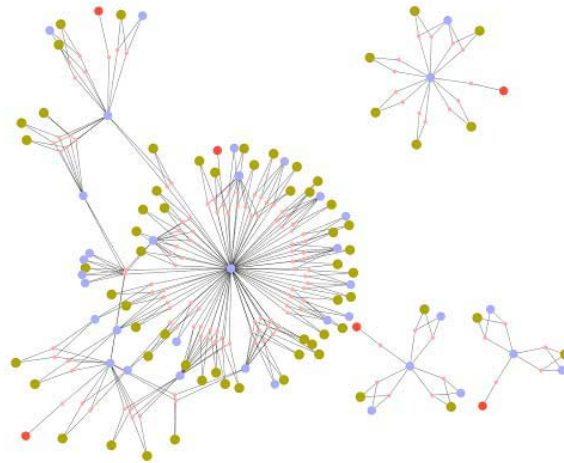
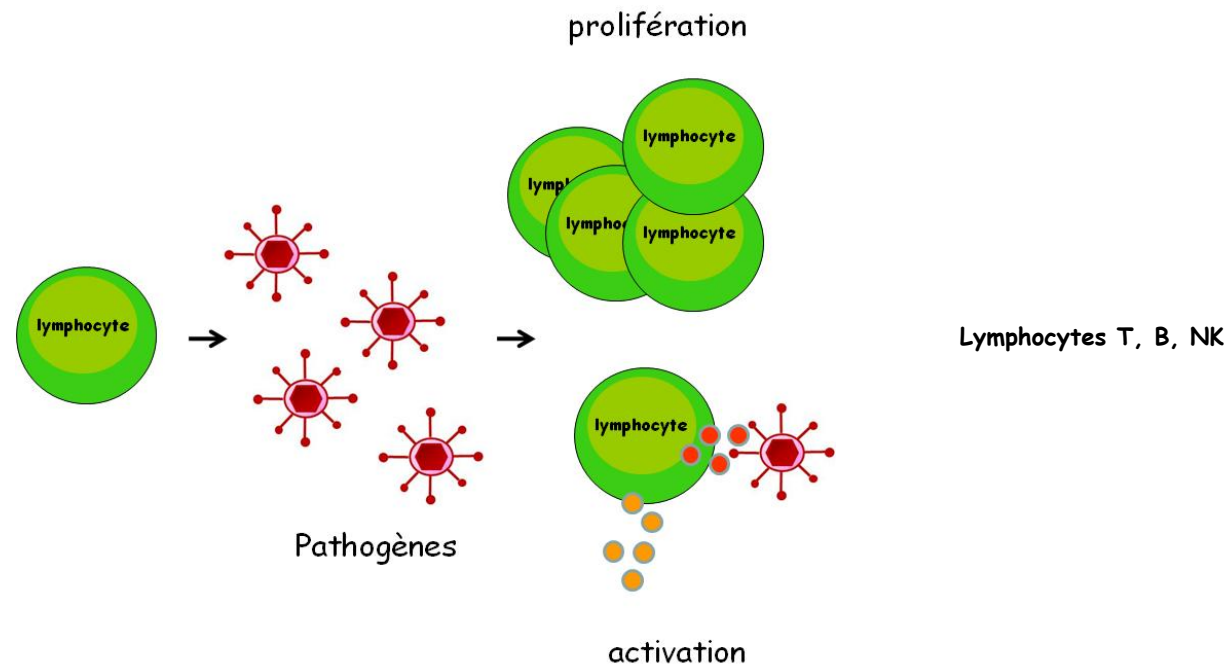


Interactome *in vivo*

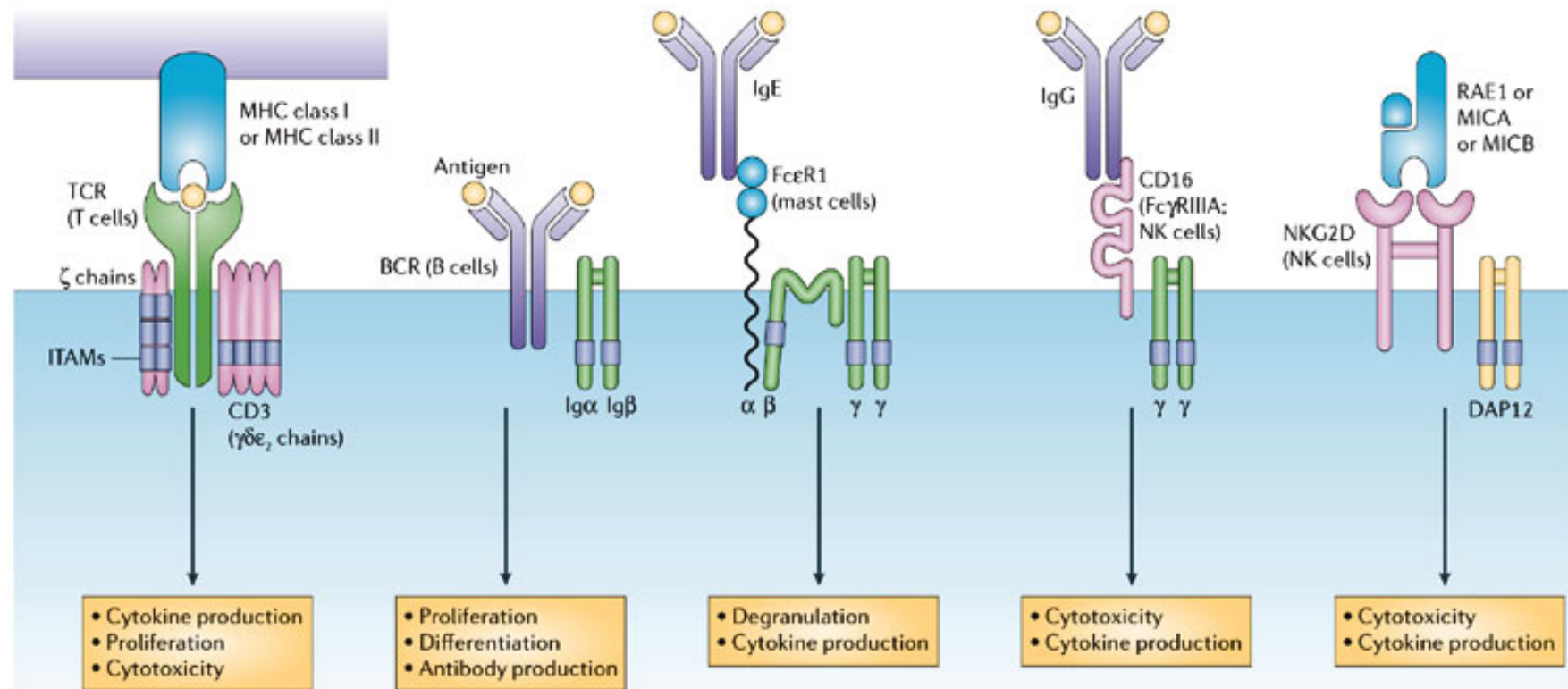


overview

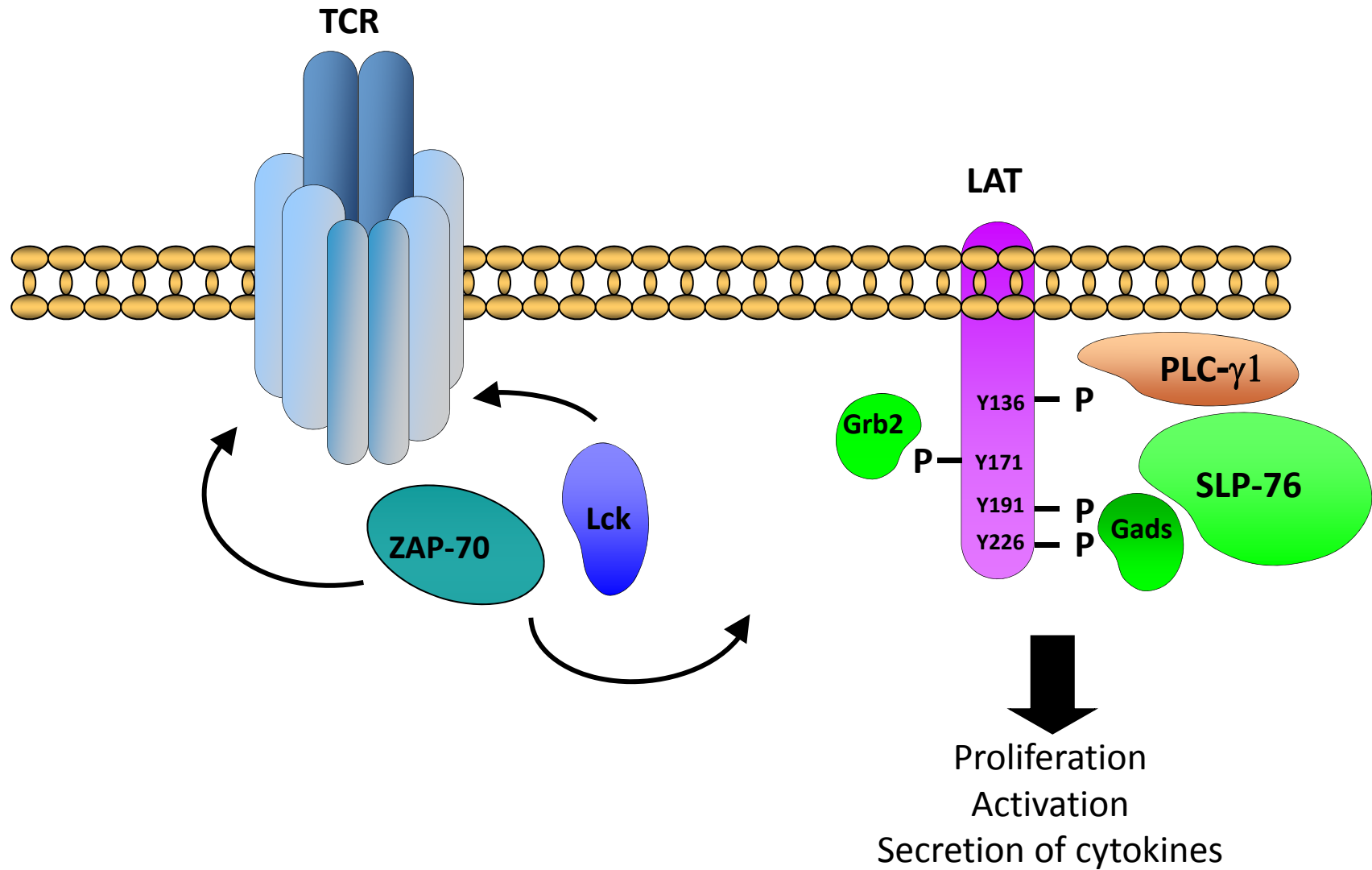
Etude de la signalisation dans les cellules immunitaires primaires



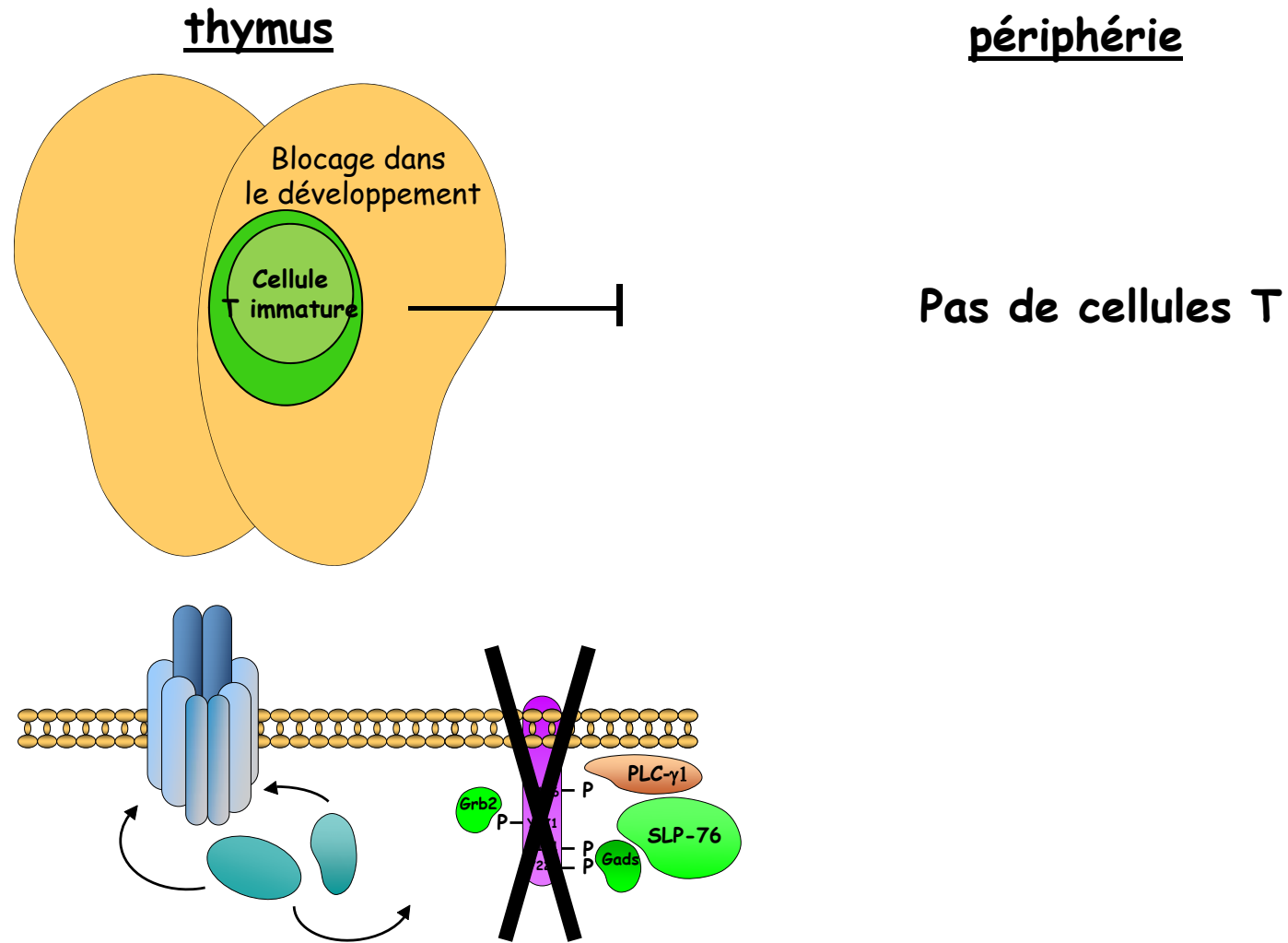
La signalisation est dependente des récepteurs antigeniques



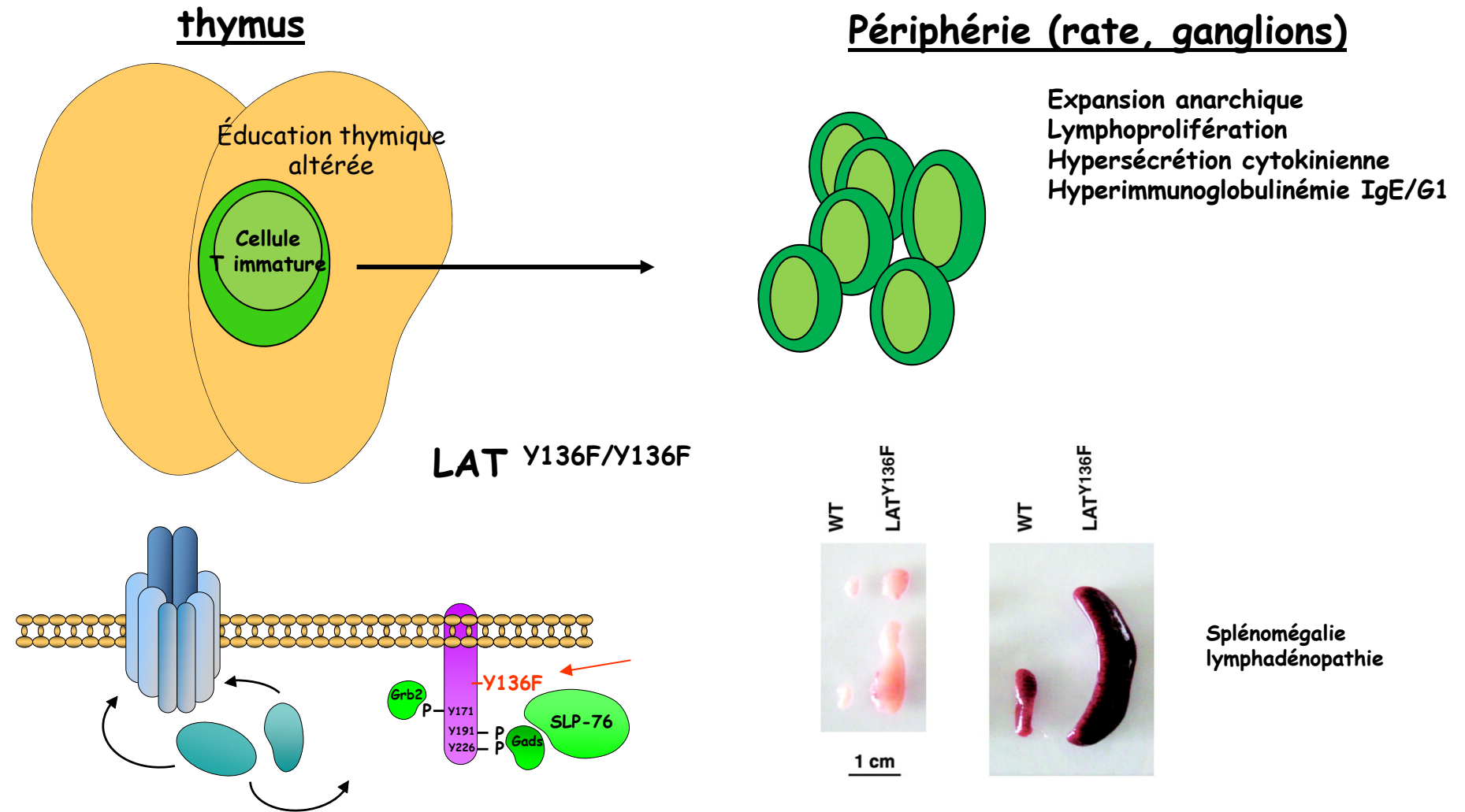
Proximal signaling in T cells



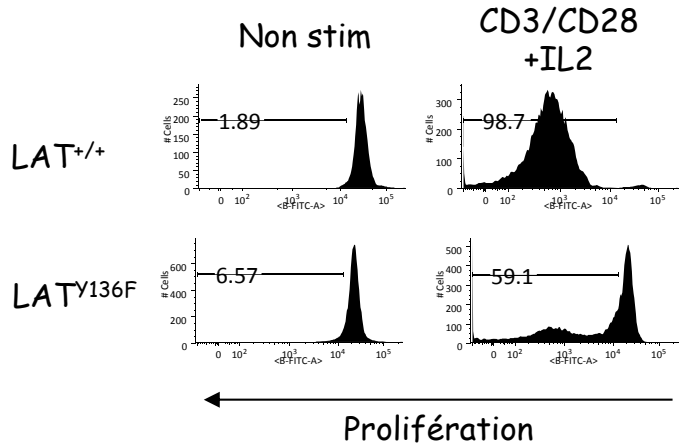
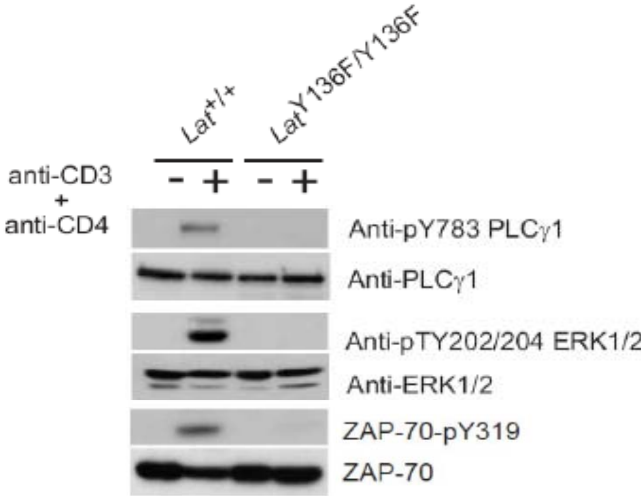
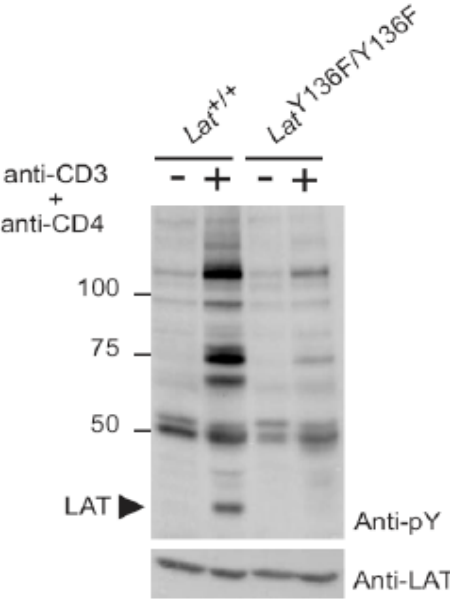
LAT est essentiel pour le développement des cellules T



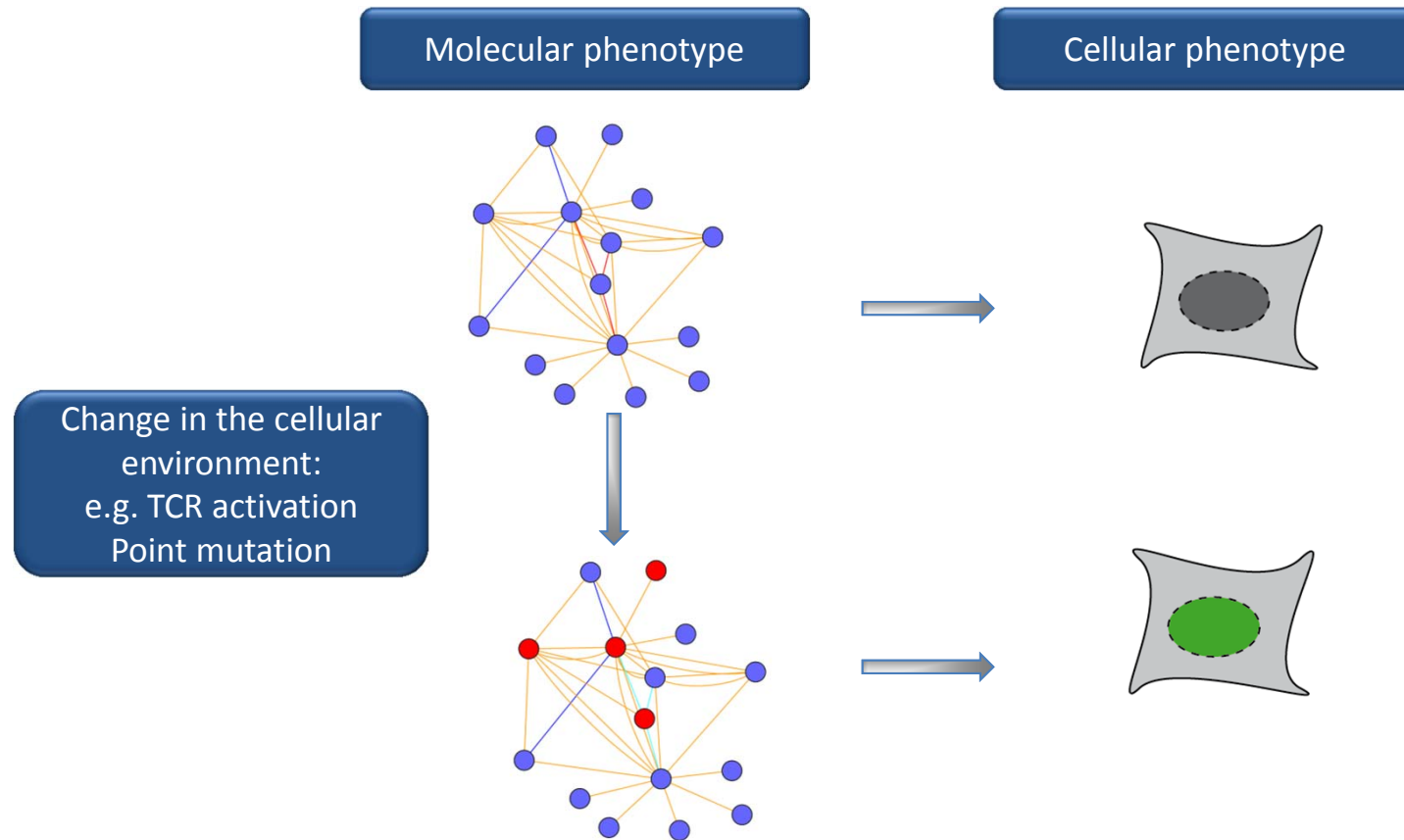
LAT est essentiel dans le maintien de l'homéostasie des cellules T



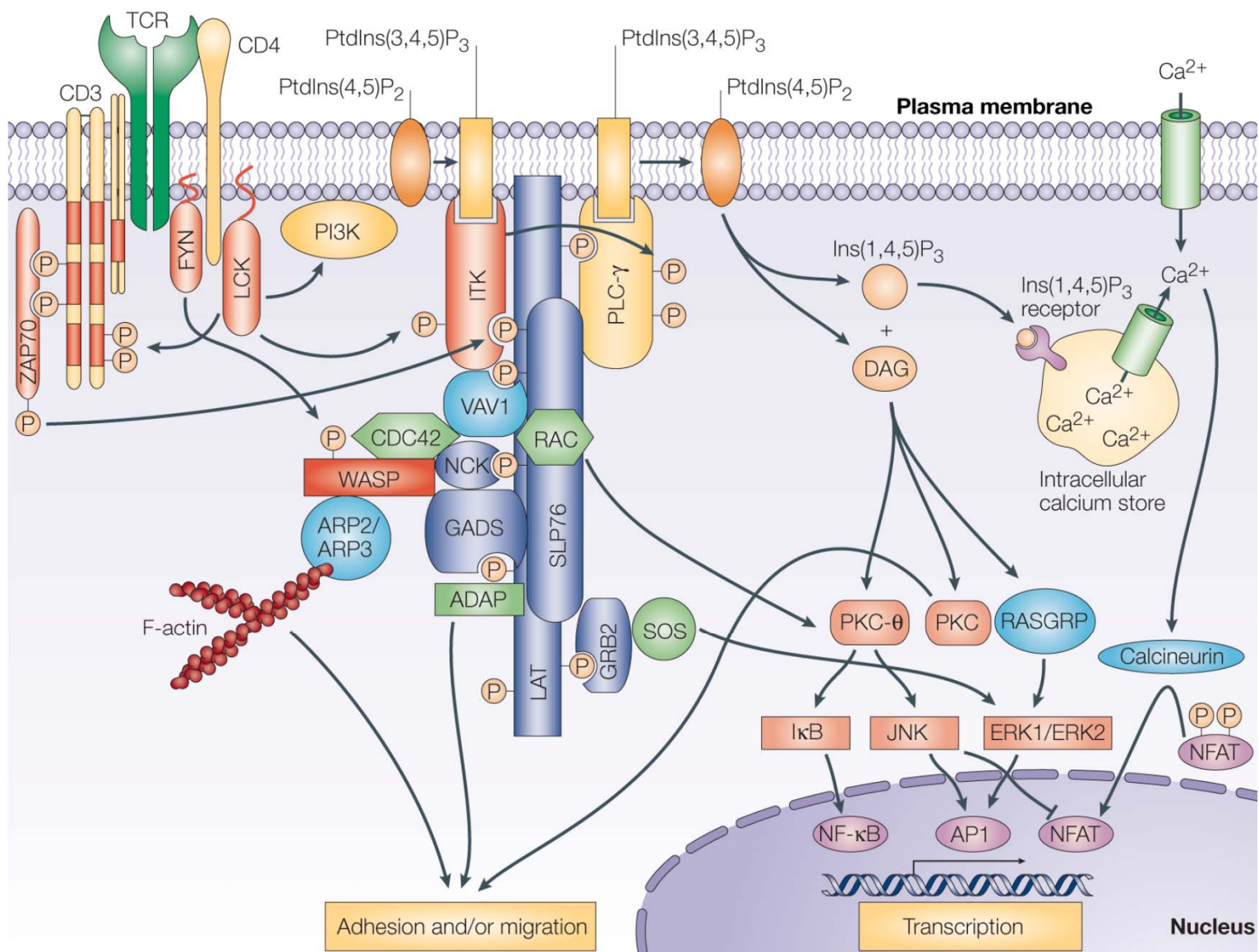
La signalisation du TCR est défectueuse dans les cellules T LATY136F



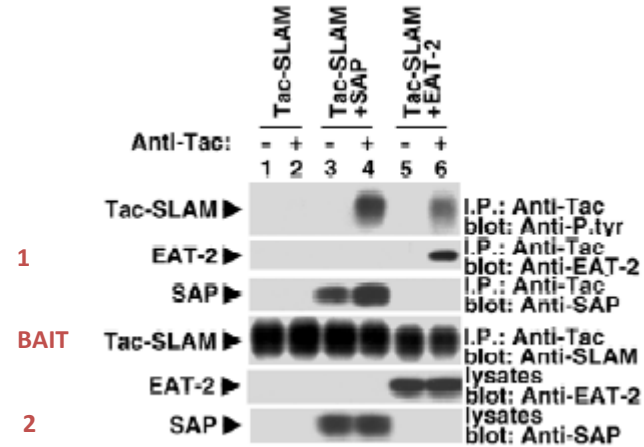
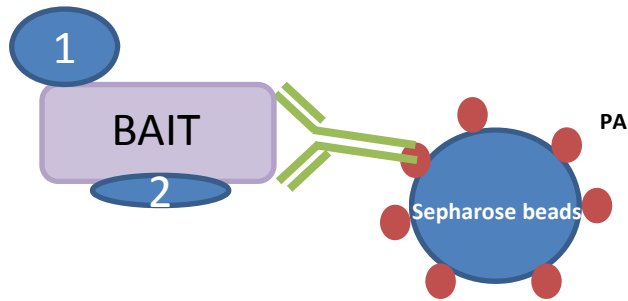
phenotypes emerge from perturbed molecular networks



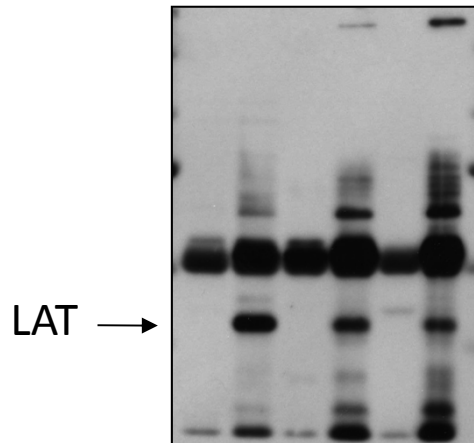
Signalisation du TCR



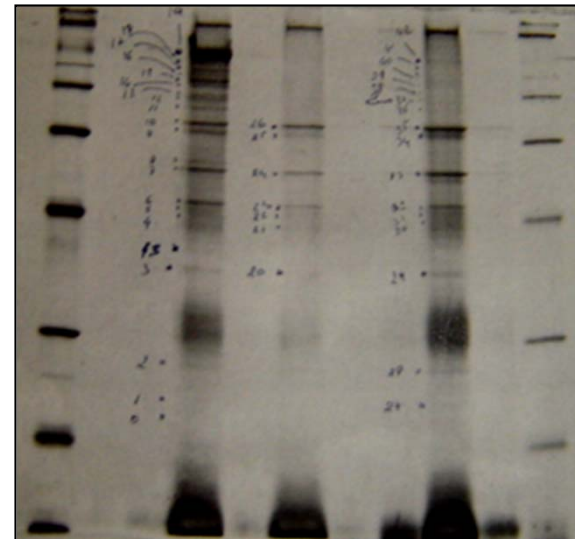
identifying the maximum of interactions, (ou pourquoi utilisé la MS/MS et ne pas trouver uniquement ce que l'on cherche)



Stimulation - + - + - +



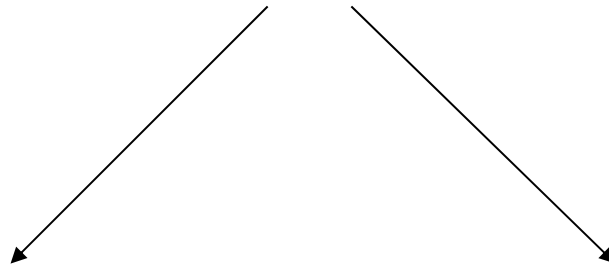
IP: LAT
IB: P-Tyr



Silver stain

**Initiate a program that will allow to integrate
all components of the TCR signaling events**

Interactome protein X



Condition A

Unstimulated

WT protein

Condition B

Stimulated

Mutant protein

Signalisation du TCR

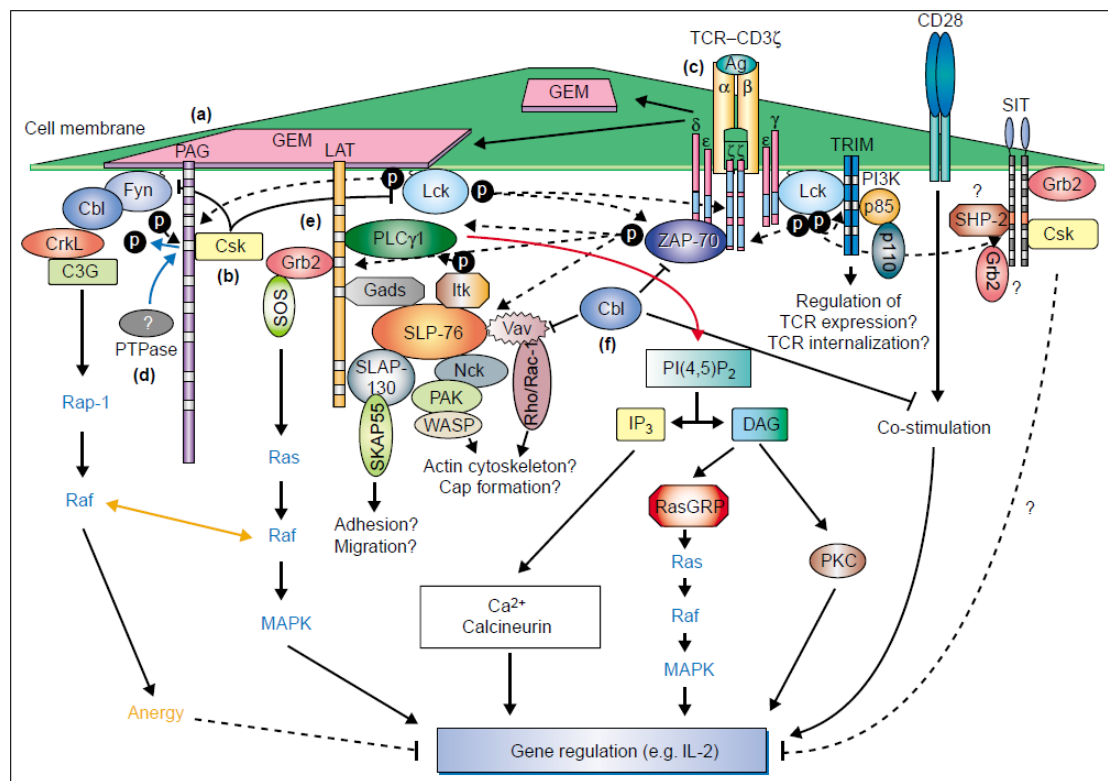
Parameters to integrate:

TCR affinity

Time of stimulation

Cell type

Costimulation



Autres parametres techniques à integrer:

Choix du Tag

Le type de stimulation (T°C, tubes, Flask...)

Le « STOP » de la réaction

Le buffer de lyse utilisé

Concentration en sels du pulldown

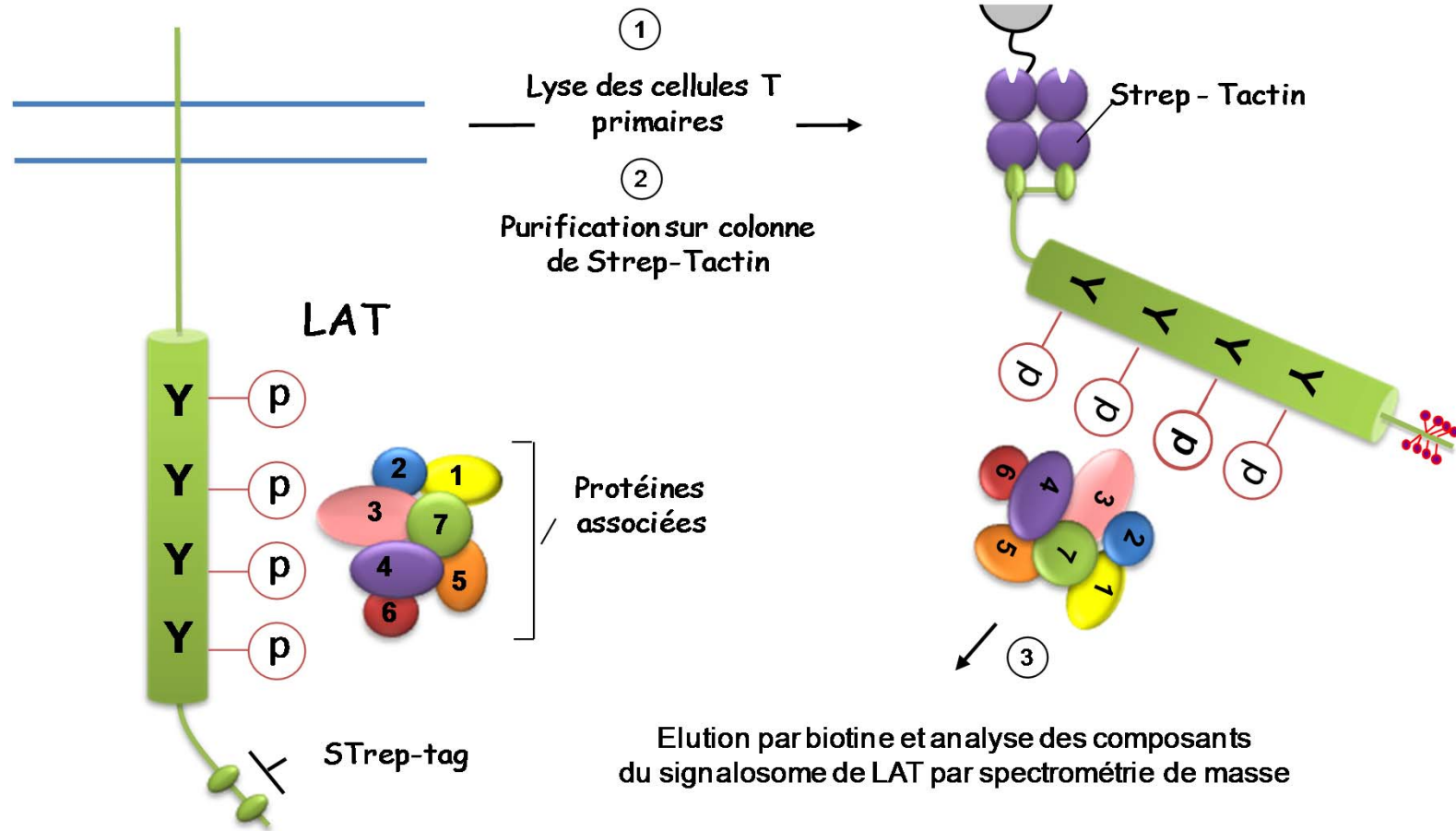
IP buffer component concentration ranges for optimization (2)	
Component	Range
Non-ionic detergents (NP-40, Triton X-100)	0.1 to 2%
Ionic detergents (SDS, sodium deoxycholate)	0.01 to 0.5%
NaCl	0 to 1M
Divalent cations	0 to 10mM
pH	6 to 9
EDTA	0 to 5mM

Le choix du TAG !!



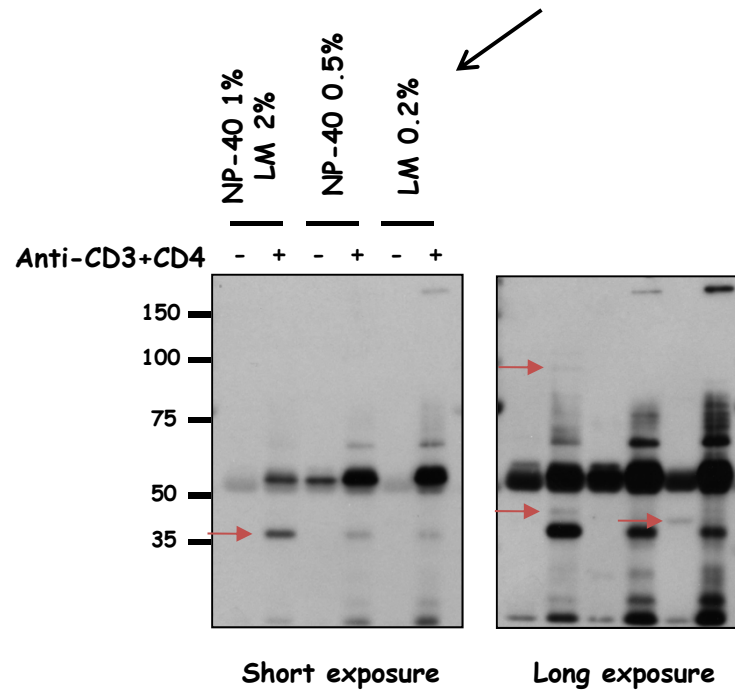
Kinase/Phosphatase
Adaptor
Transcription factor
Enzymes...

N ou C-term
Taille du Tag (encombrement sterique)
Affinité pour la purification
Spécificité pour la purification
Stringence de l'élution



Optimization of detergent conditions for affinity purification of signaling complexes in primary T cells

Currently chosen for MS experiment n-dodecyl- β -lauryl maltoside



IP: LAT
IB: P-Tyr

Properties of common detergents.

Detergent	Type	Agg. #	MW mono (micelle)	CMC mM (%w/v)	Cloud Point °C	Dialyzable
Triton X-100	Nonionic	140	647 (90K)	0.24 (0.0155)	64	No
Triton X-114	Nonionic	-	537 (-)	0.21 (0.0113)	23	No
NP-40	Nonionic	149	617 (90K)	0.29 (0.0179)	80	No
Brij-35	Nonionic	40	1225 (49K)	0.09 (0.1103)	>100	No
Brij-58	Nonionic	70	1120 (82K)	0.08 (0.0086)	>100	No
Tween 20	Nonionic	-	1228 (-)	0.06 (0.0074)	95	No
Tween 80	Nonionic	60	1310 (76K)	0.01 (0.0016)	-	No
Octyl glucoside	Nonionic	27	292 (8K)	23-24 (~0.70)	>100	Yes
Octyl thioglucoside	Nonionic	-	308 (-)	9 (0.2772)	>100	Yes
SDS	Anionic	62	288 (18K)	6-8 (0.17-0.23)	>100	No
CHAPS	Zwitterionic	10	615 (6K)	8-10 (0.5-0.6)	>100	Yes
CHAPSO	Zwitterionic	11	631 (7K)	8-10 (~0.505)	90	Yes

Agg. # = Aggregation number, which is the number of molecules per micelle

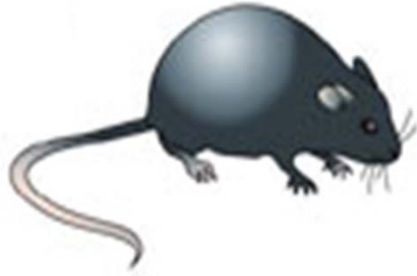
L'arrêt de la stimulation

Changement de T°

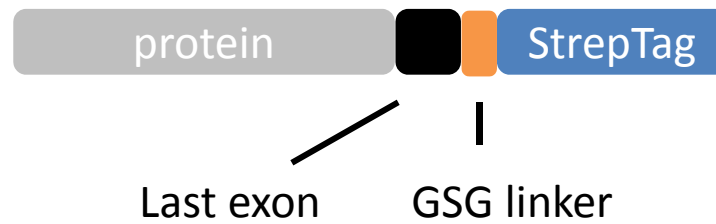
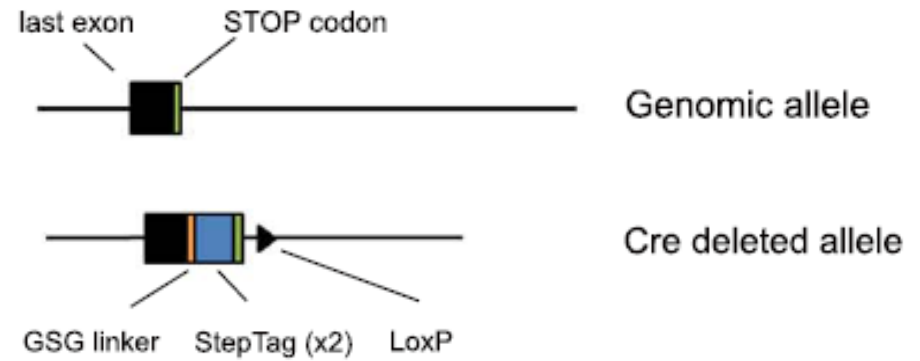
Centrifugation

Lyse des les cellules

Interactome des cellules T



Génération de souris Knock-in



Pourquoi ?

niveau d'expression physiologique de la protéine d'intérêt

étudier les interactions dans des cellules primaires

pouvoir modifier le système directement sur la souris

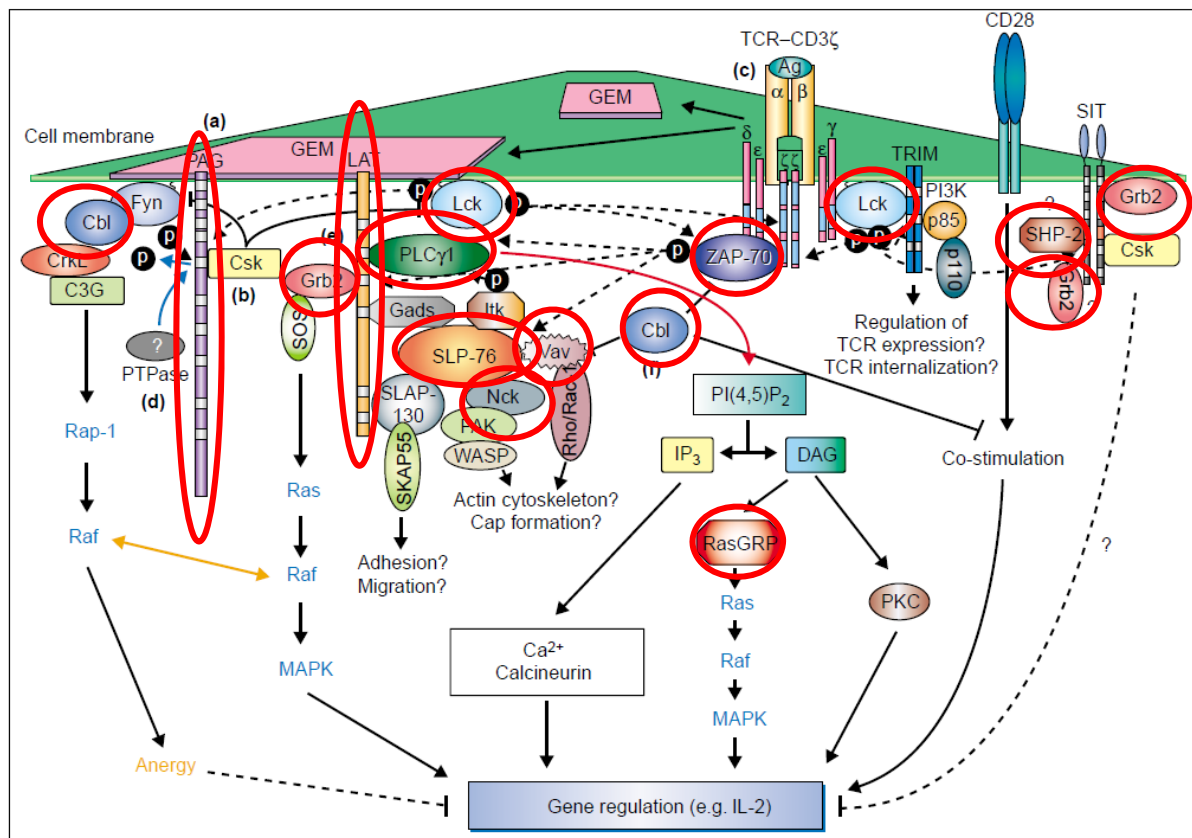


Protéines impliquées dans la signalisation du TCR

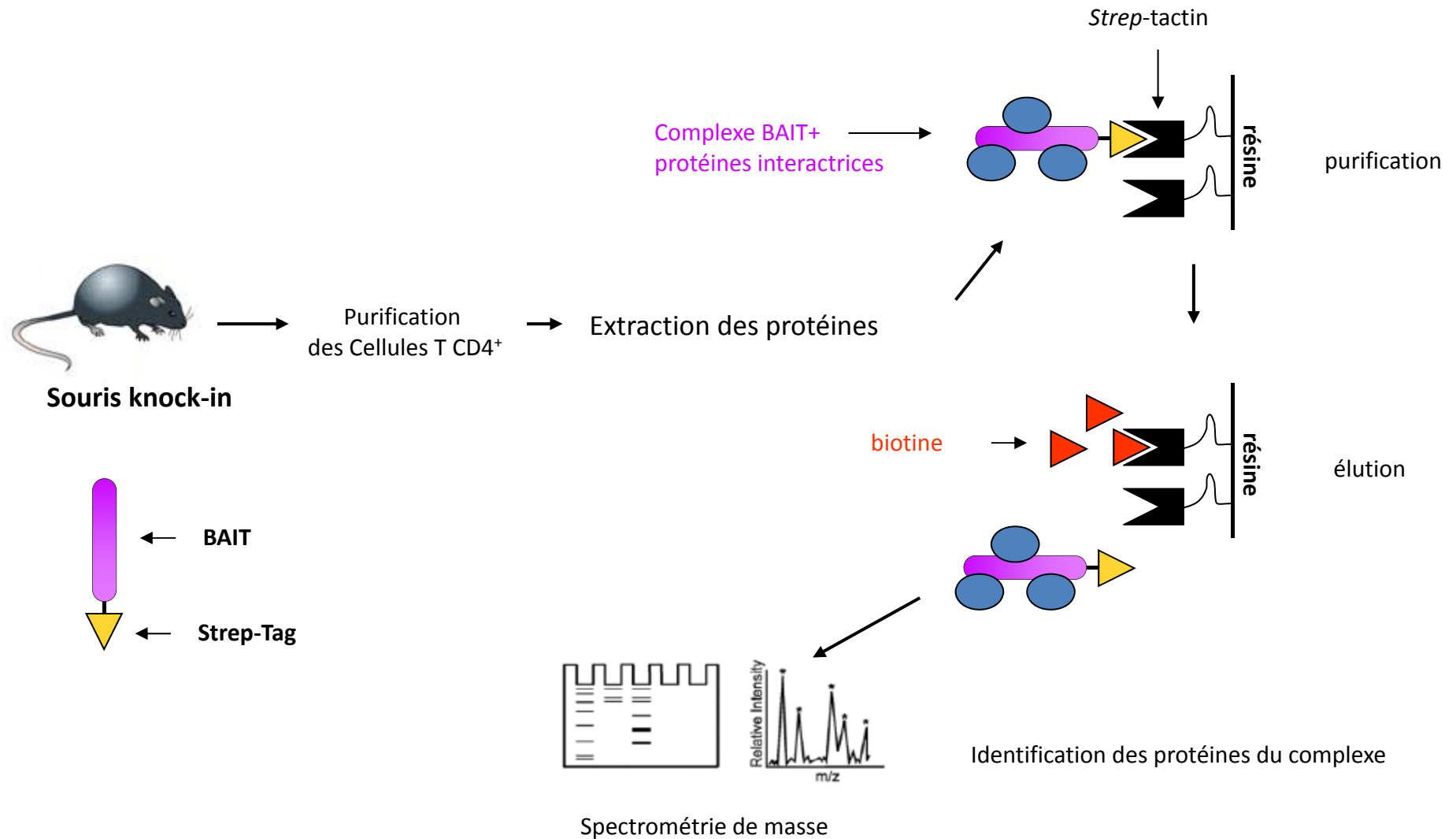
Signalisation du TCR

BAITs

CD28
Card11 (CARMA1)
ptpn6 (SHP1)
Plcg1
Rasgrp1
LAT
LATpour (LAT136)
Fyb (ADAP)
Lat2 (NTAL)
Pag1
grb2
Nck1
Inpp5d (SHIP-1)
Zap70
SLP-76
Vav-1
c-CBL
LCK



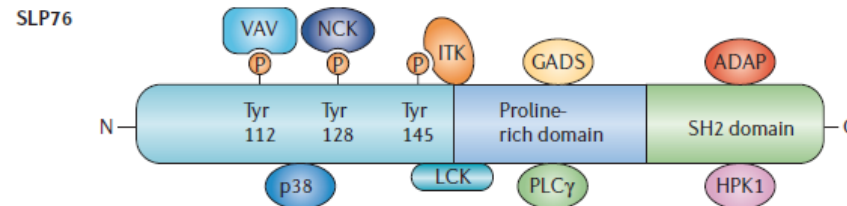
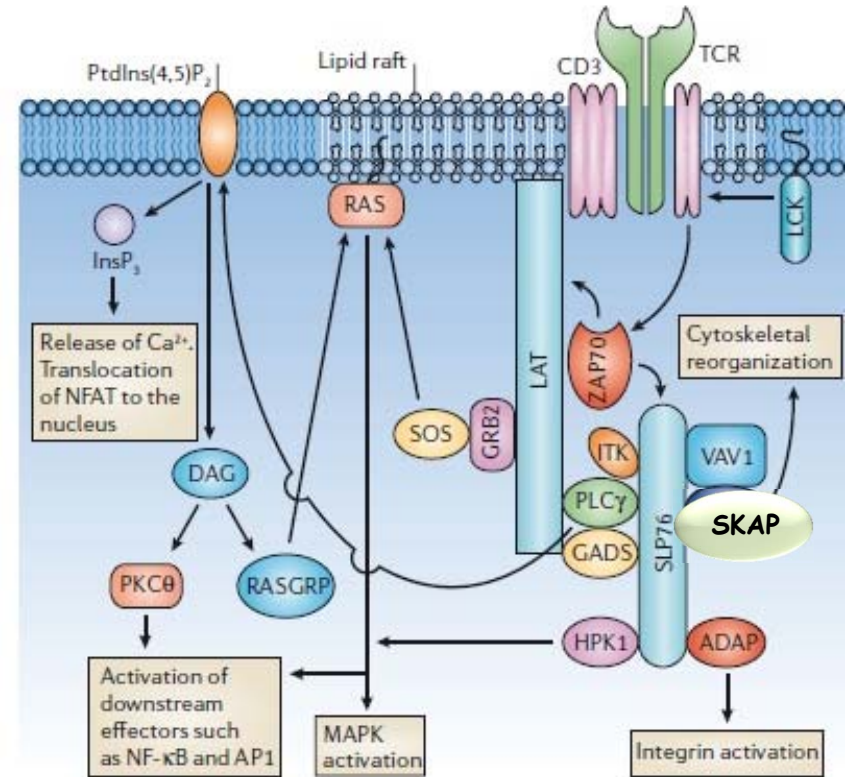
Identification de l'interactome des protéines intervenant dans la signalisation du TCR



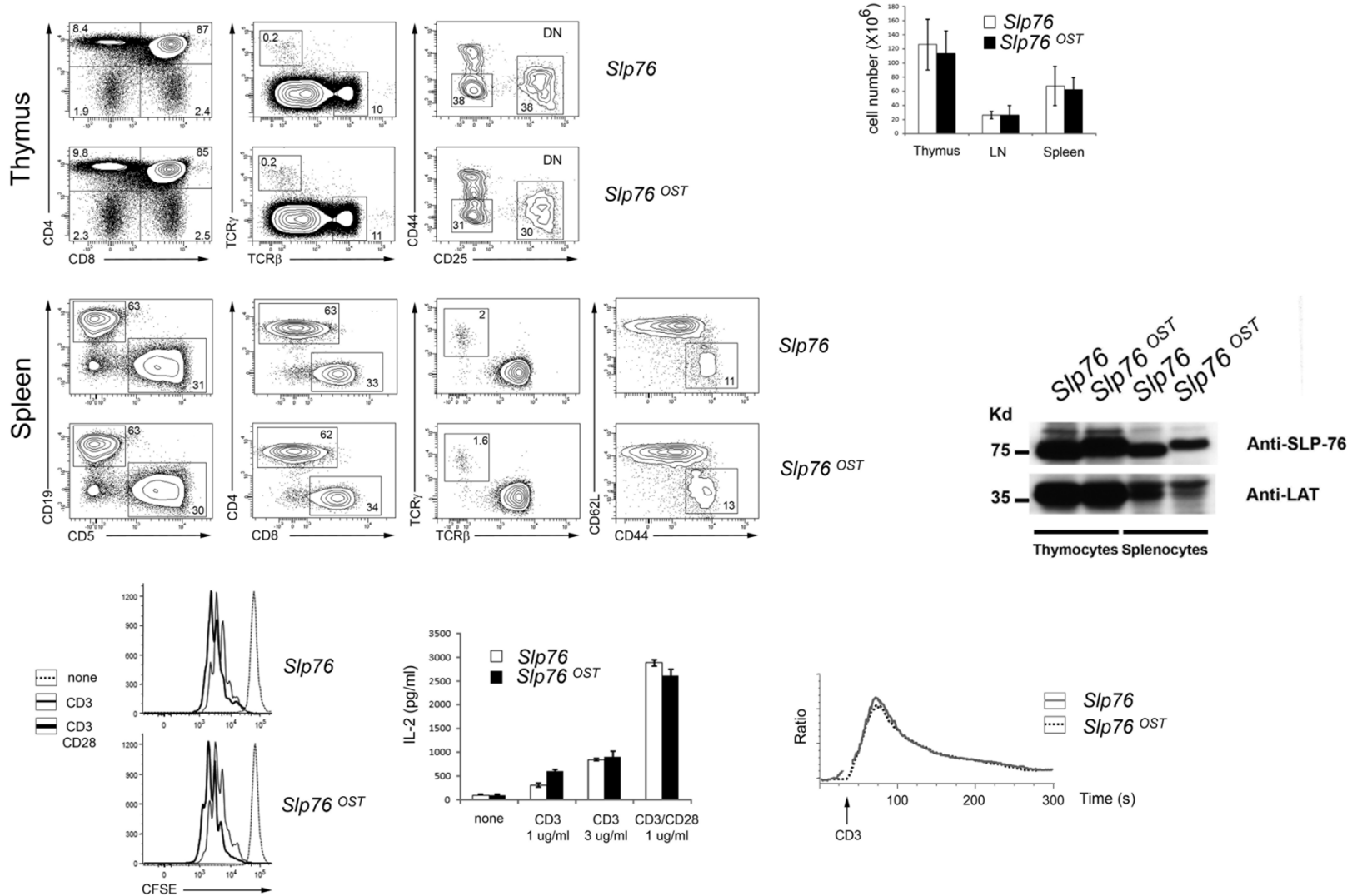
Un exemple:

SLP-76

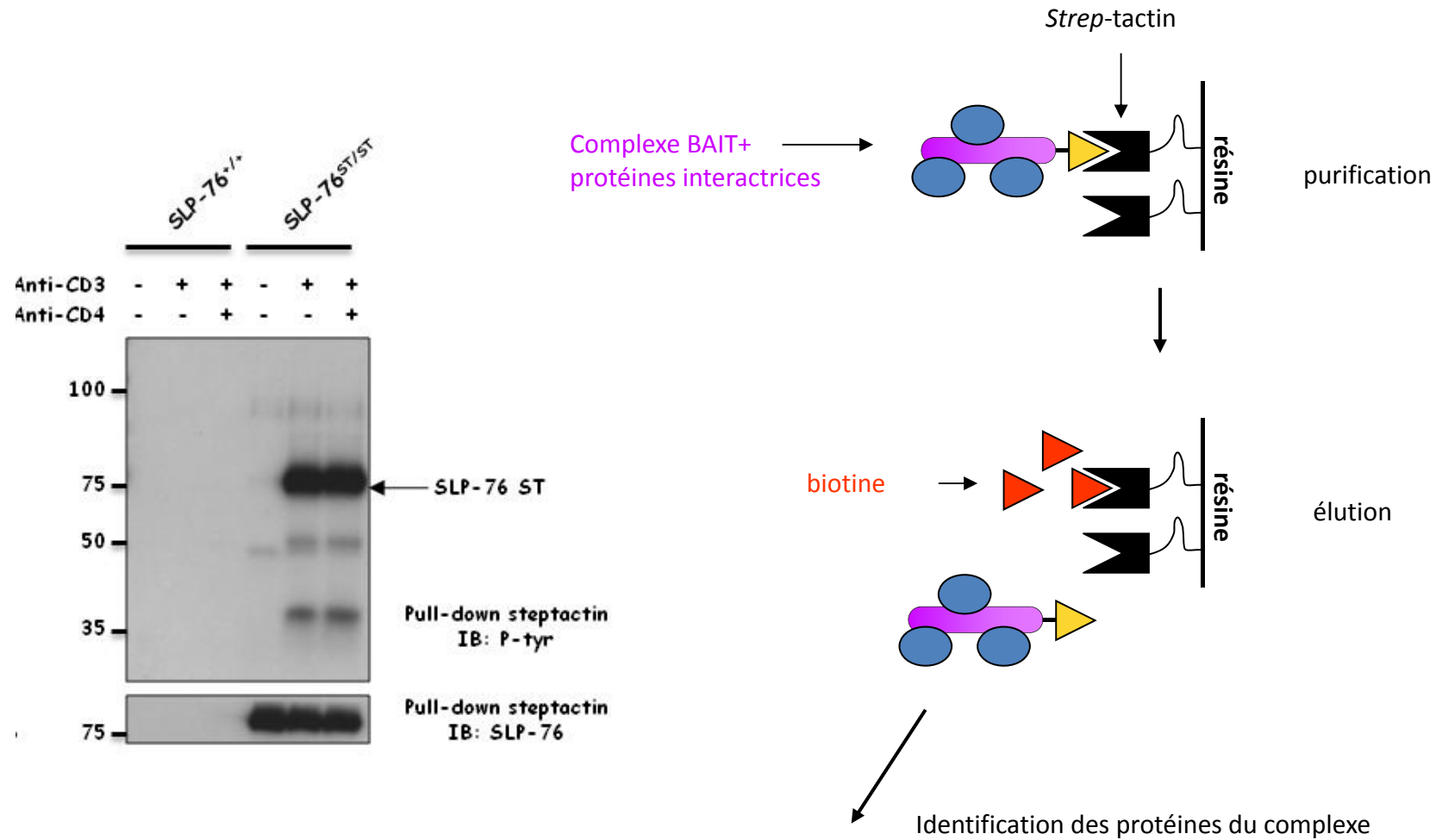
Src homology 2 (SH2) domain-
containing leukocyte protein of 76 kDa



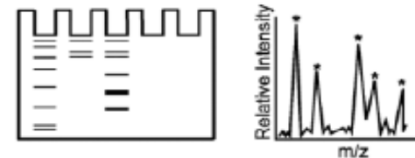
Normal development and functions of T cells from SLP-76 ST mice



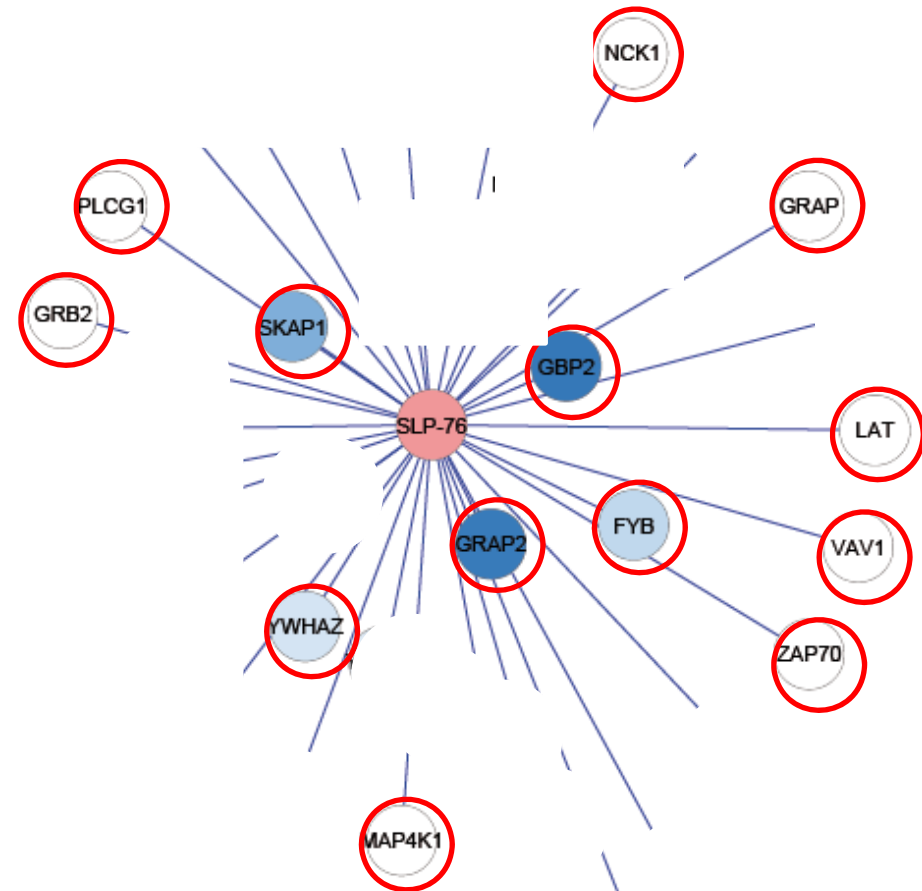
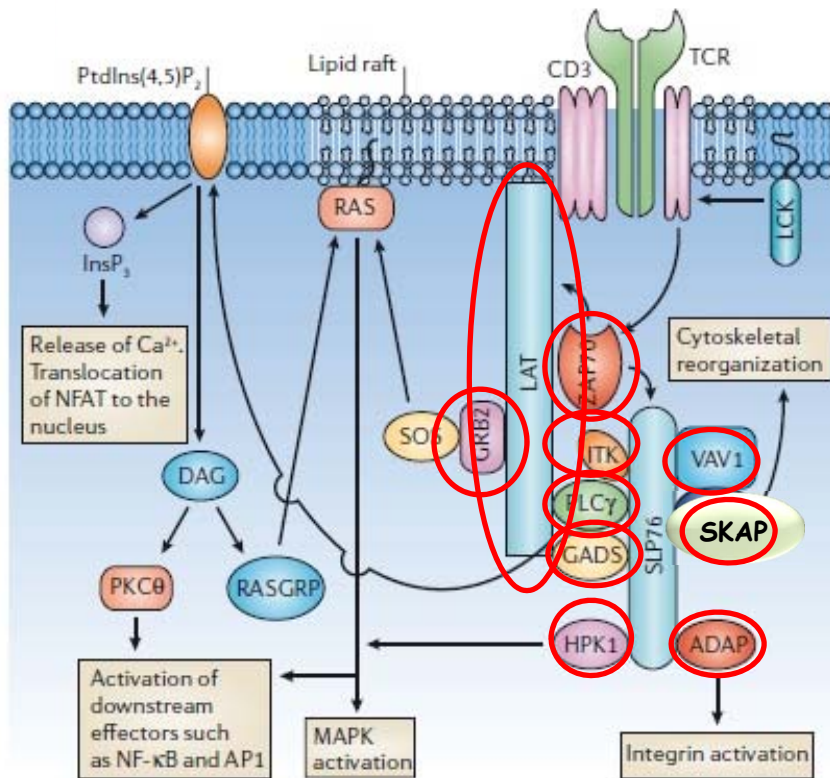
Expression and pulldown of SLP-76 ST protein



Spectrométrie de masse

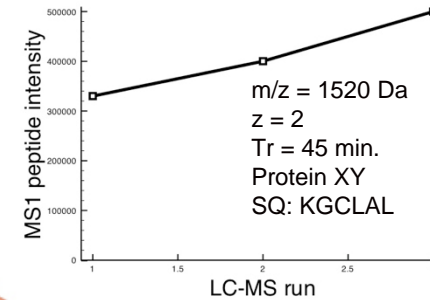


Visual Cytoscape

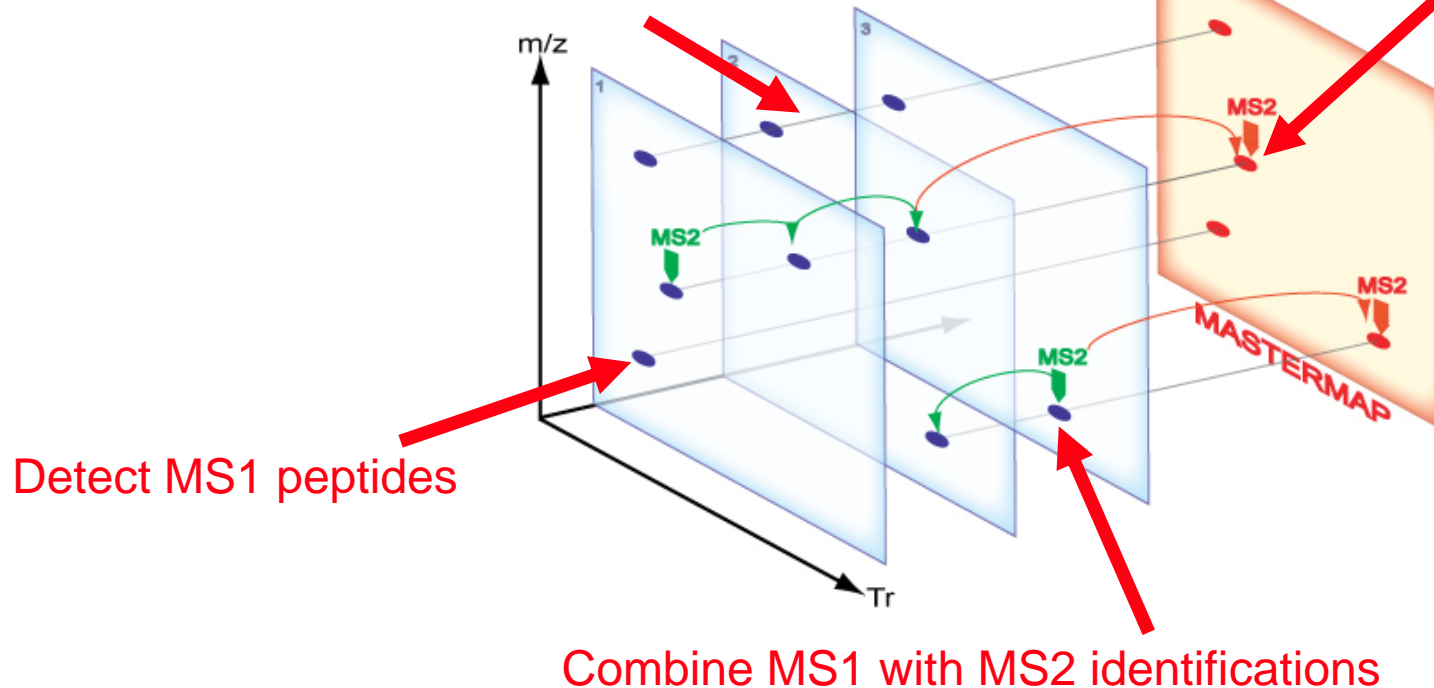


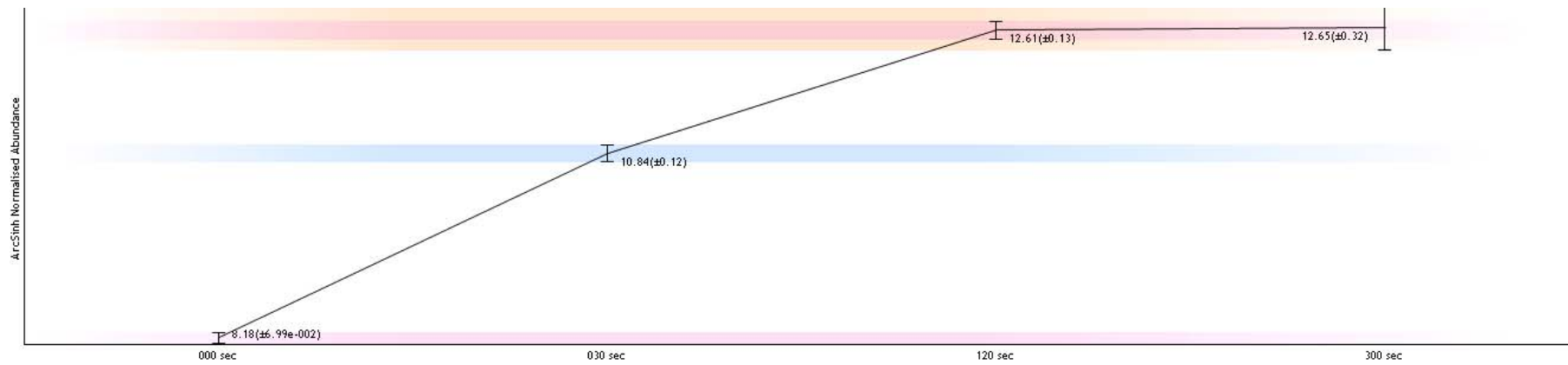
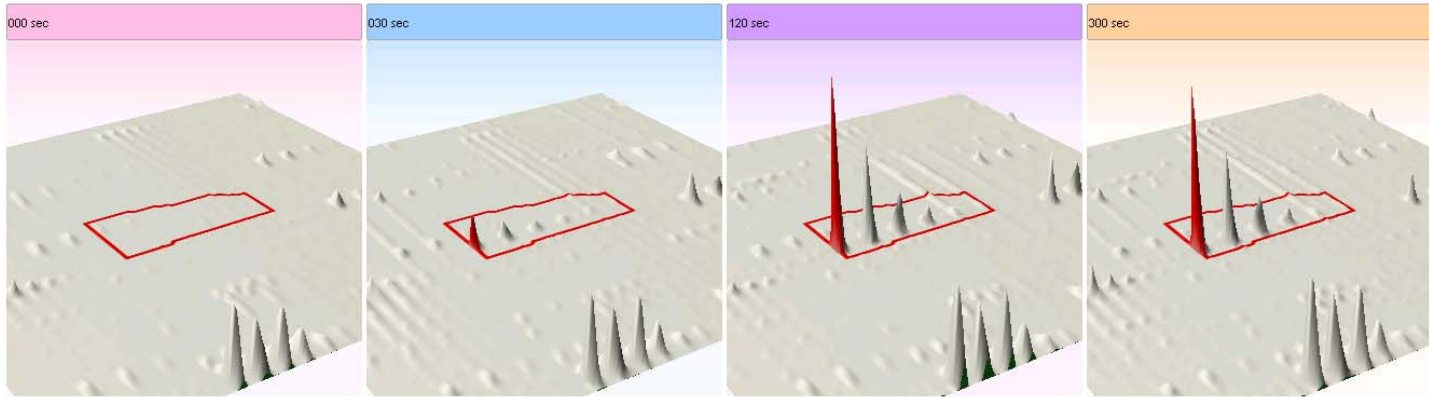
A label free approach to determine peptide abundances

Extract MS1 peptide profiles



Track MS1 peptides across LC-MS runs

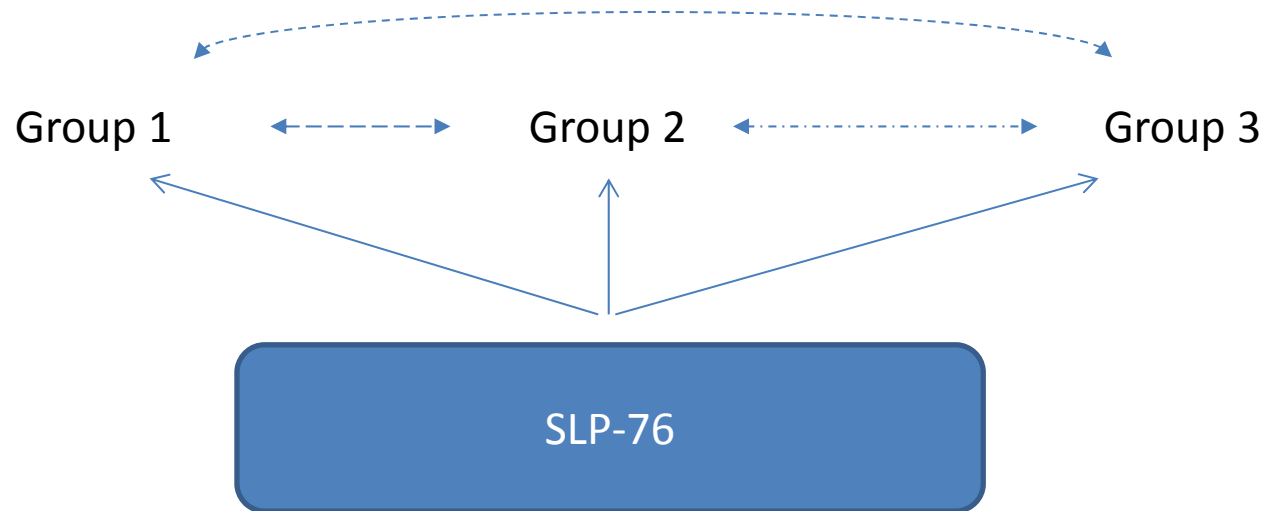
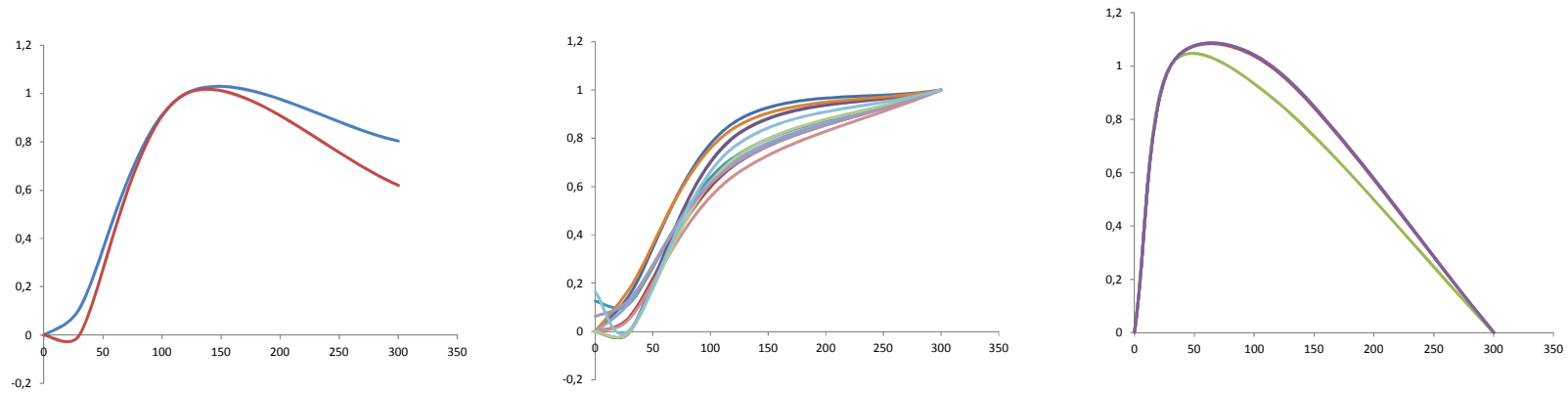




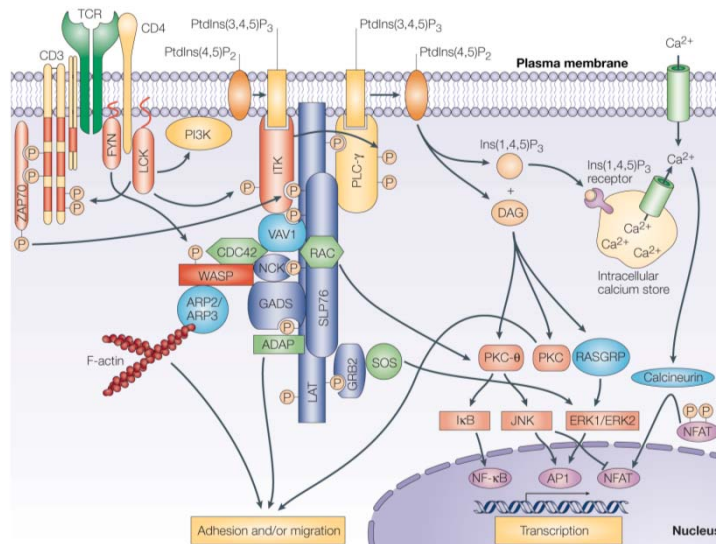
↑
Stimulation

time

Clusters based on the kinetic of interaction with SLP76



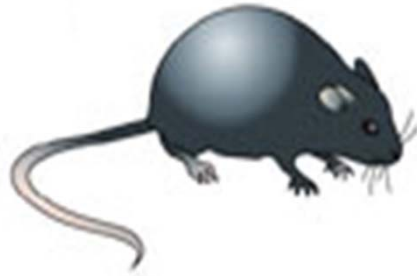
Combiner des interactomes de plusieurs BAITs, et intégrer la cinétique de stimulation



Quantifier le degres d'interaction

Quantifier de manière absolue ou relative le nombre de molecules qui interagissent entre elles

Autres intérêts du modèle *in vivo*



Comprendre les mécanismes « fins » de l'activation de cellules T



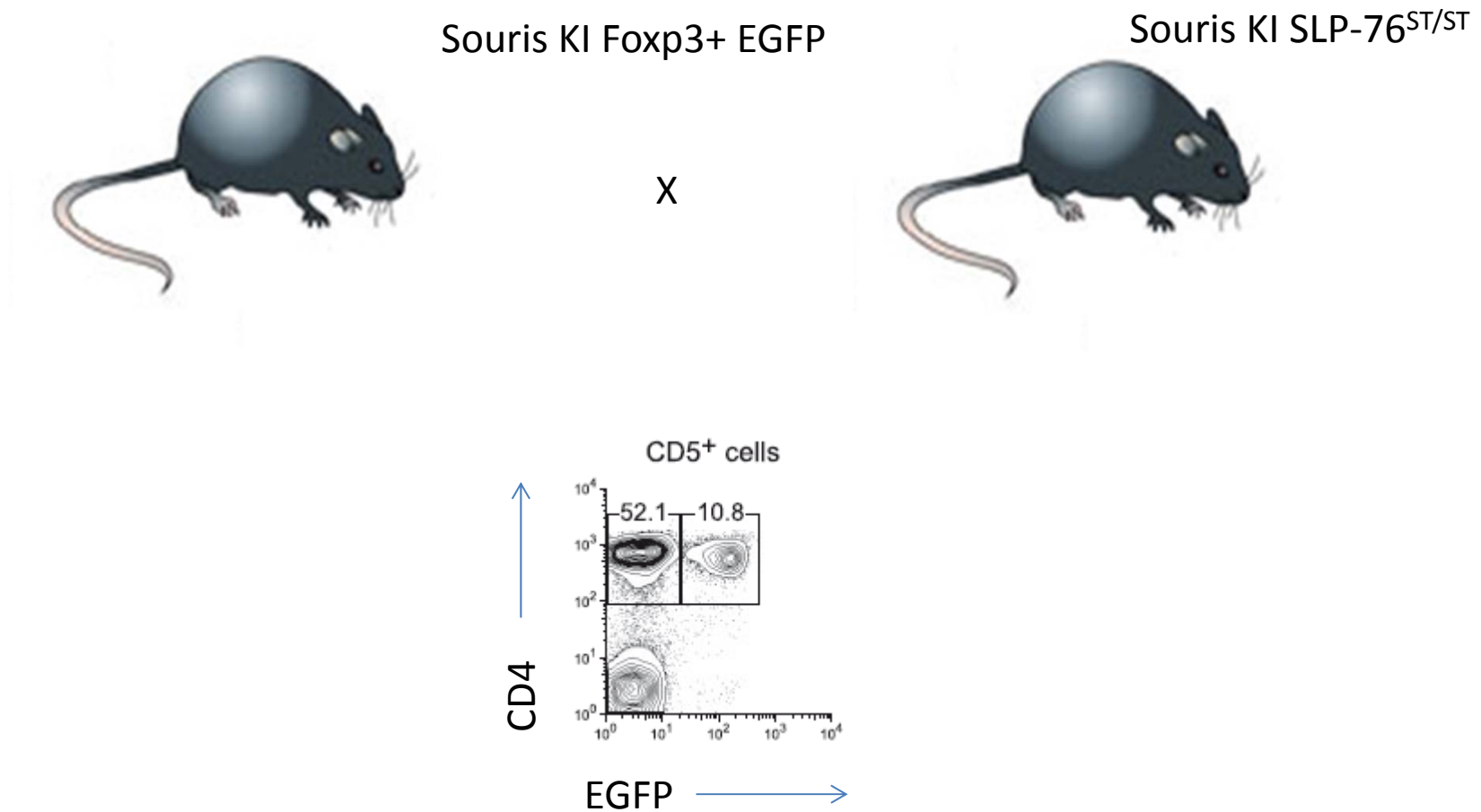
TCR transgénique

X



Souris KI SLP-76^{ST/ST}

Identifier des differences de signalisation dans des populations particulières



Definir les genes associés à un facteur de transcription *in vivo*

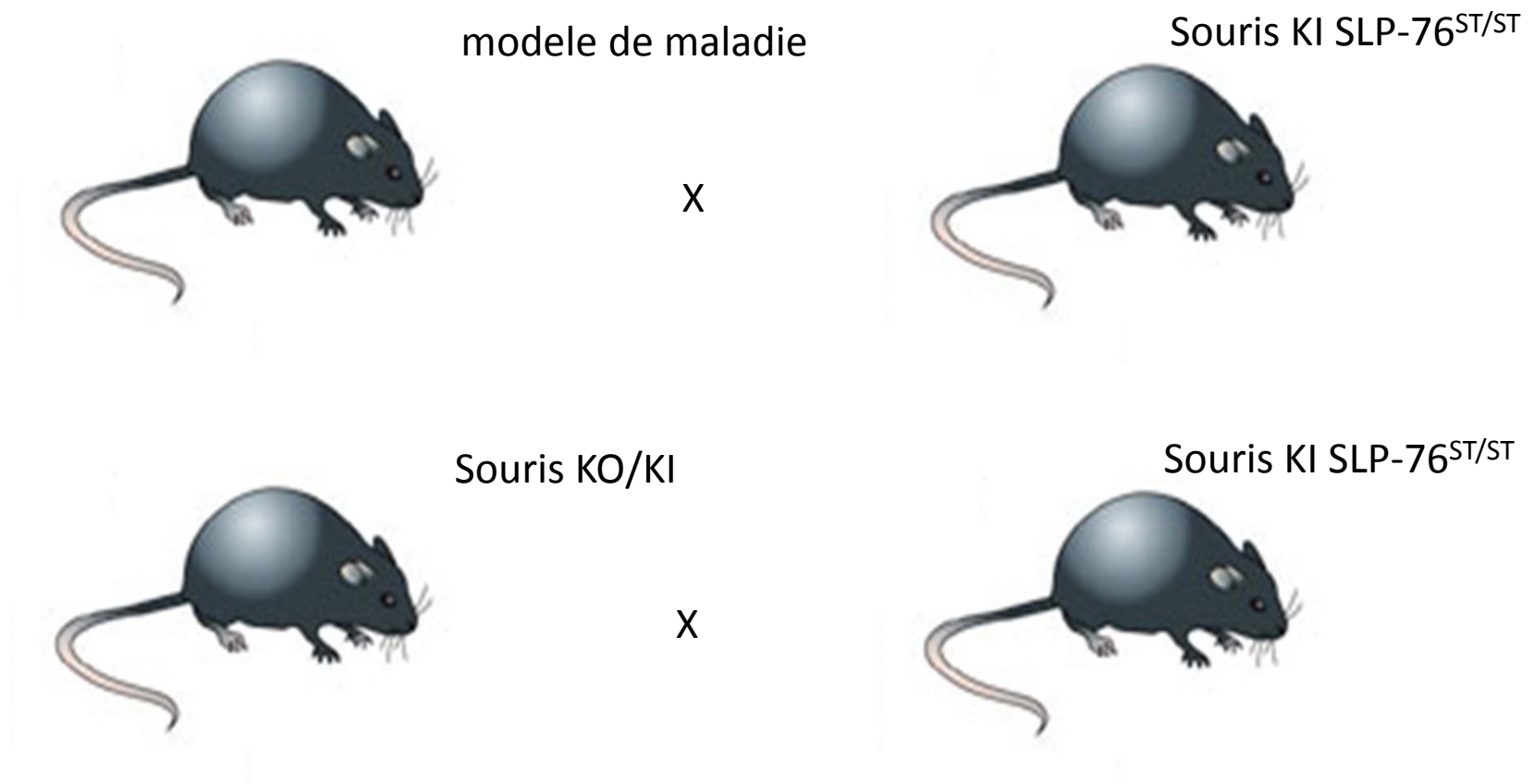
Souris KI StrepTag



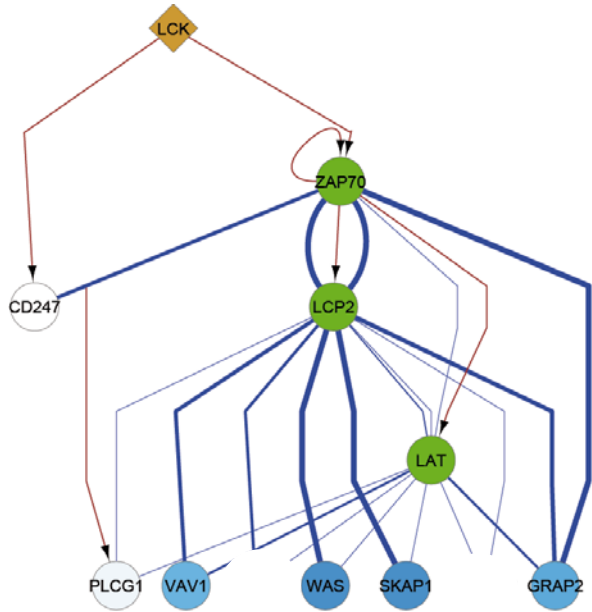
Transcriptional role of cyclin D1 in development revealed by a genetic-proteomic screen.

Nature. 2010 Jan 21;463(7279):374-8.

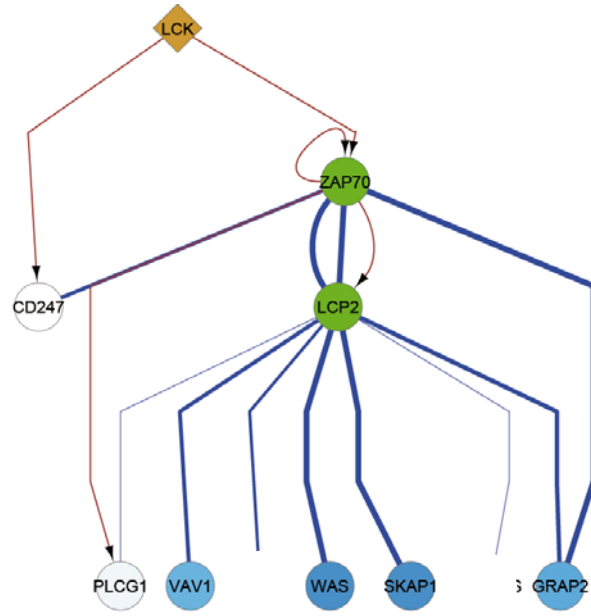
Perturber le systeme



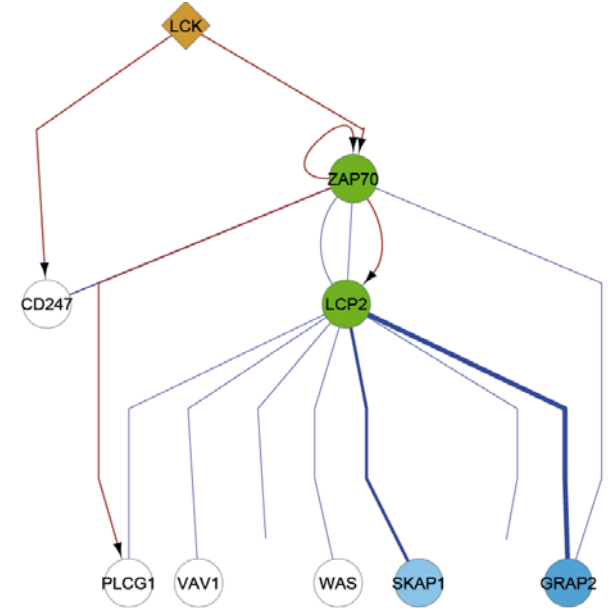
WT



LAT KO/mutant
predicted



LAT KO/mutant
Observed



Etudier et comparer les interactomes de différents types cellulaires

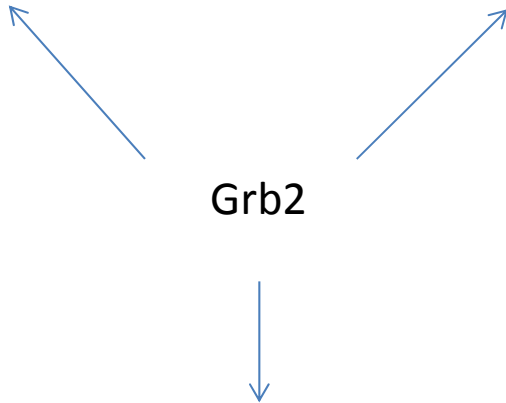
Proteine ubiquitaire

Fibroblastes

Cellules immunitaires

Grb2

Neurones



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- Claude Grégoire
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