Targeting the LRRK2 Protein Kinase for the Treatment of Parkinson's Disease

Dario Alessi

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Parkinson's Disease

- Despite intensive research, attempts to pause or even just slow the progression of Parkinson's have thus far failed

- While most cases of Parkinson's are idiopathic and with largely unknown aetiology, mutations in about 18 genes including cause rare, genetic Parkinsonism

18 Parkinson's genes

LOCUS	MODE	ONSET	GENE	FUNCTION
PARK1	AD	EARLY	α-synuclein	Protein Folding
PARK2	AR	EARLY	Parkin	Ubiquitin Biology
PARK5	AD	LATE	UCH-L1	Ubiquitin Biology
PARK6	AR	EARLY	PINK1	Phosphorylation Biology
PARK7	AR	EARLY	DJ-1	Protein Folding
PARK8	AD	LATE	LRRK2	Phosphorylation Biology
PARK9	AR	EARLY*	ATP13A2	ATPase Enzyme
PARK13	AD	LATE	HtrA2/Omi	Protease Enzyme
PARK14	AR	EARLY*	PLA2G6	Metabolic Enzyme
PARK15	AR	EARLY*	FBXO7	Ubiquitin biology
PARK17	Complex	LATE	GAK	Phosphorylation Biology
PARK18	Complex	LATE	HLA	Immune Biology
PARK19	AD	LATE	VPS35	Vesicle Trafficking
PARK20	AD	LATE	EIF4G1	Protein Translation
PARK21	X-linked	EARLY*	RAB39B	Vesicle Trafficking
PARK22	AD	LATE	CHCHD2	Transcription Factor
PARK23	AR	EARLY	VPS13C	Vesicle Trafficking
	AR	LATE	GBA	Metabolic Enzyme

* Complex syndrome

18 Parkinson's genes

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PARK8	AD	LATE	LRRK2	Phosphorylation Biology
PARK9	AR	EARLY*	ATP13A2	ATPase Enzyme
PARK13	AD	LATE	HtrA2/Omi	Protease Enzyme
PARK14	AR	EARLY*	PLA2G6	Metabolic Enzyme
PARK15	AR	EARLY*	FBXO7	Ubiquitin biology
PARK17	Complex	LATE	GAK	Phosphorylation Biology
PARK18	Complex	LATE	HLA	Immune Biology
PARK19	AD	LATE	VPS35	Vesicle Trafficking
PARK20	AD	LATE	EIF4G1	Protein Translation
PARK21	X-linked	EARLY*	RAB39B	Vesicle Trafficking
PARK22	AD	LATE	CHCHD2	Transcription Factor
PARK23	AR	EARLY	VPS13C	Vesicle Trafficking
	AR	LATE	GBA	Metabolic Enzyme

* Complex syndrome

DISCOVERY OF LRRK2 IN 2004

Neuron, Vol. 44, 601–607, November 18, 2004, Copyright ©2004 by Cell Press

Mutations in *LRRK2* Cause Autosomal-Dominant Parkinsonism with Pleomorphic Pathology

Alexander Zimprich,[1,2,11] Saskia Biskup,[3,11] Petra Leitner,[1] Peter Lichtner,[3] Matthew Farrer,[4] Sarah Lincoln,[4] Jennifer Kachergus,[4] Mary Hulihan,[4] Ryan J. Uitti,[5] Donald B. Calne,[6] A. Jon Stoessl,[6] Ronald F. Pfeiffer,[7] Nadja Patenge,[1] Iria Carballo Carbajal,[1] Peter Vieregge,[8] Friedrich Asmus,[1] Bertram Müller-Myhsok,[9] Dennis W. Dickson,[4] Thomas Meitinger,[3,10,*] Tim M. Strom,[3,10] Zbigniew K. Wszolek,[5,*] and Thomas Gasser[1,*]

Thomas Gasser (Tübingen)

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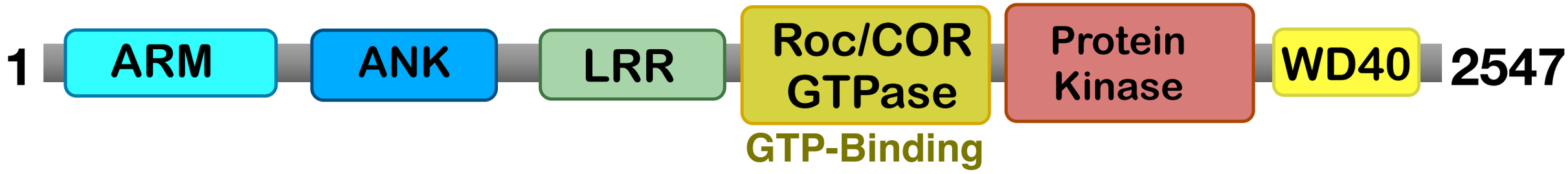
Cloning of the Gene Containing Mutations that Cause *PARK8*-Linked Parkinson's Disease

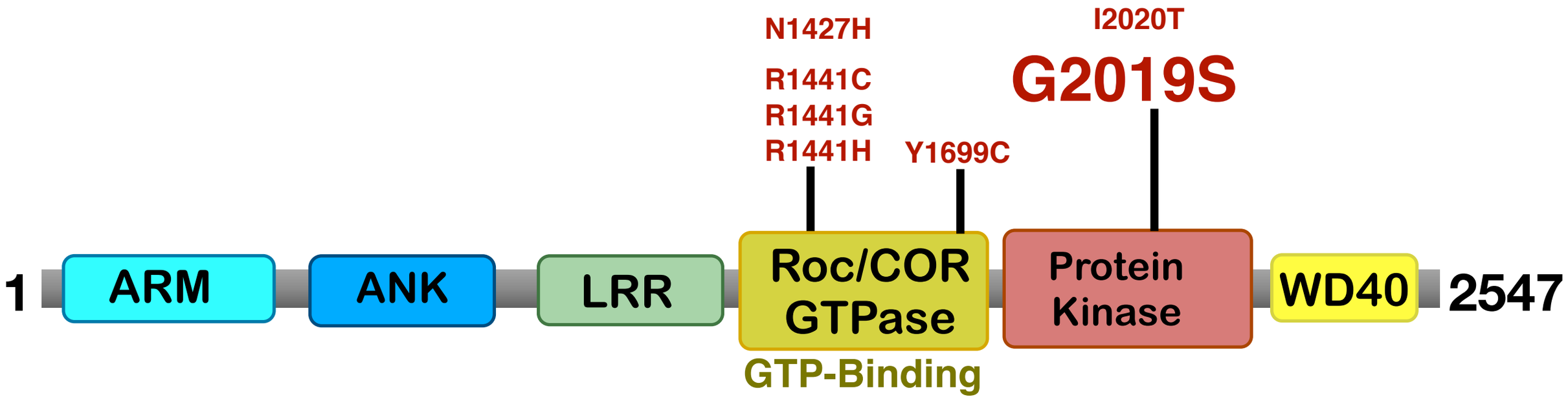
Coro Paisán-Ruíz,[1,11] Shushant Jain,[2,3,11] E. Whitney Evans,[4] William P. Gilks,[3] Javier Simón,[1] Marcel van der Brug,[5] Adolfo López de Munain,[6,7] Silvia Aparicio,[1] Angel Martínez Gil,[8] Naheed Khan,[3] Janel Johnson,[4] Javier Ruiz Martinez,[9] David Nicholl,[10] Itxaso Marti Carrera,[7] Amets Saénz Peña,[6] Rohan de Silva,[3] Andrew Lees,[3] José Félix Martí-Massó,[7] Jordi Pérez-Tur,[1,*] Nick W. Wood,[2,*] and Andrew B. Singleton[4,*]

Andrew Singleton (NIH Washington)

The LRRK2 gene encodes a large 286 kDa protein kinase



LRRK2 Domain Structure



Seven mutations result in activation of LRRK2 kinase domain and cause Parkinson's disease in an autosomal dominant fashion.

LRRK2 and Parkinson's

- Mutations in LRRK2 are one of the most common genetic causes of familial Parkinson's comprising ~5% of familial Parkinson's, and ~1% of sporadic Parkinson's patients

- LRRK2 mediated Parkinson's resemble the common sporadic form of the disease.

- G2019S is the most commonly inherited LRRK2 mutation

LRRK2 and Parkinson's

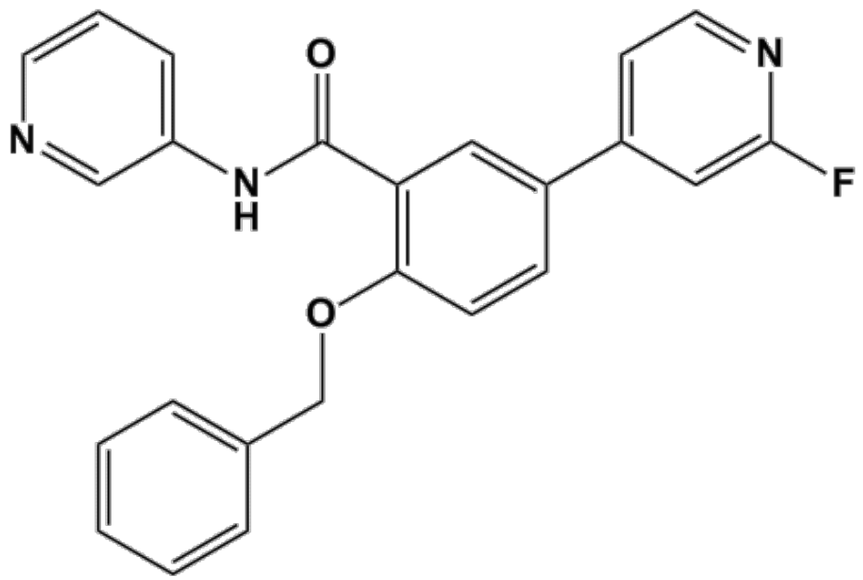
- All LRRK2 pathogenic mutations result in hyper-activation of LRRK2 protein kinase catalytic activity.

- Because of this many Pharmaceutical companies have embarked on developing drugs that target LRRK2 for the treatment of Parkinson's

- Denali Therapeutics has recently completed a Phase 1 clinical trial in healthy volunteers with a compound termed DNL201, that was claimed to achieve greater than 90% inhibition of LRRK2 kinase activity

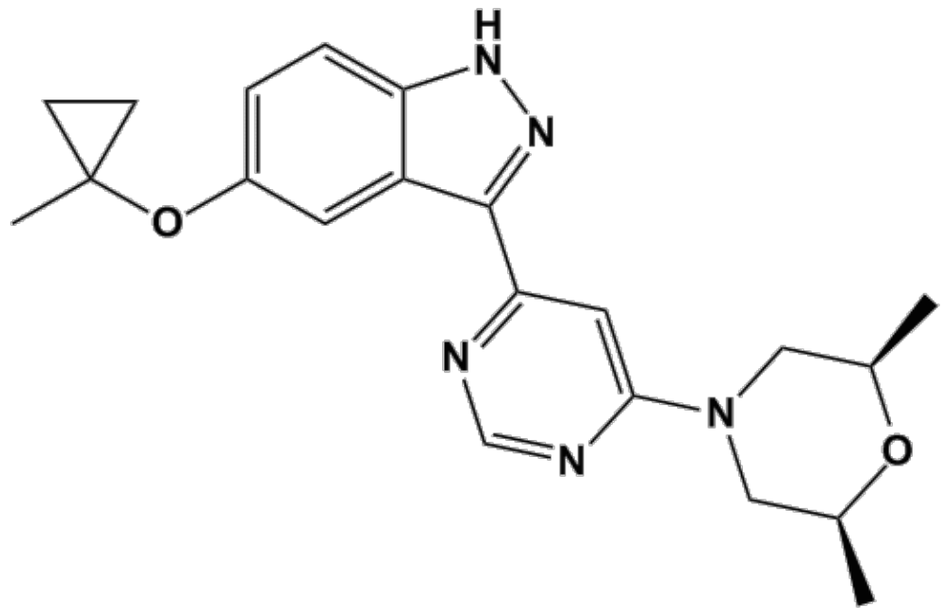
- Data is also emerging for LRRK2 involvement in idiopathic Parkinson's, suggesting that inhibitors may benefit patients beyond LRRK2 mutant carriers.

LRRK2 Inhibitors are being developed by the Pharmaceutical Industry for the treatment of Parkinson's



GSK2578215A

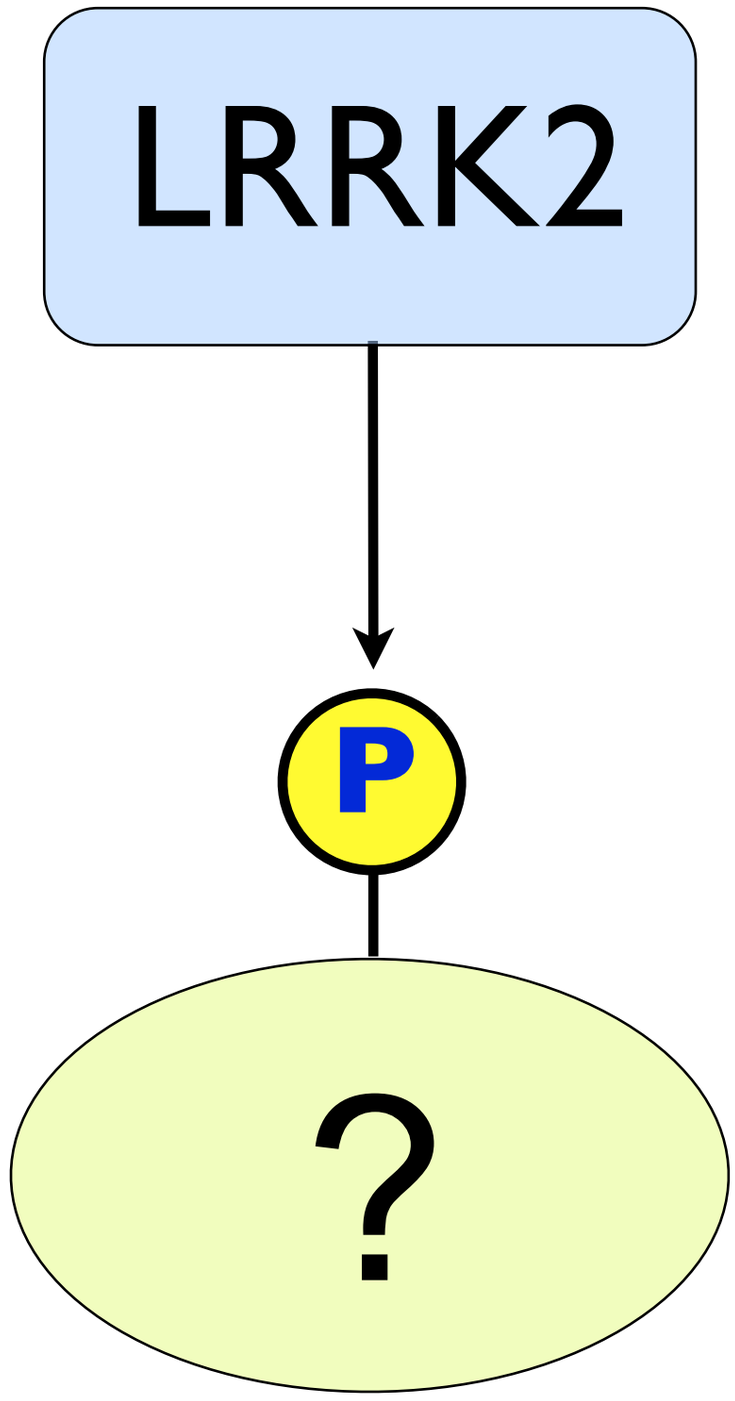
GlaxoSmithKline



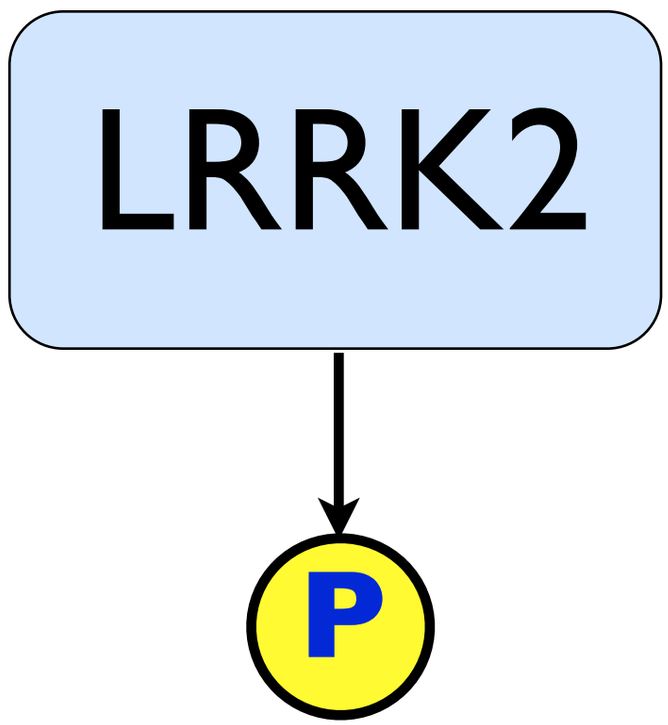
MLI-2

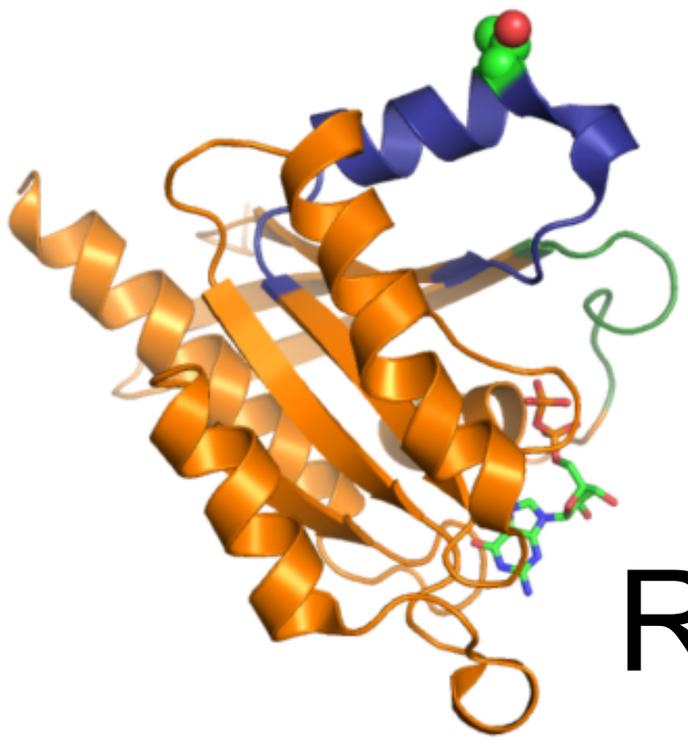
Merck

What is the cellular substrate of LRRK2?



Rab GTPases are the key physiological substrate of LRRK2





Rab10

Alessi and Mann labs, GSK (Alastair Reith) & MJFF Project

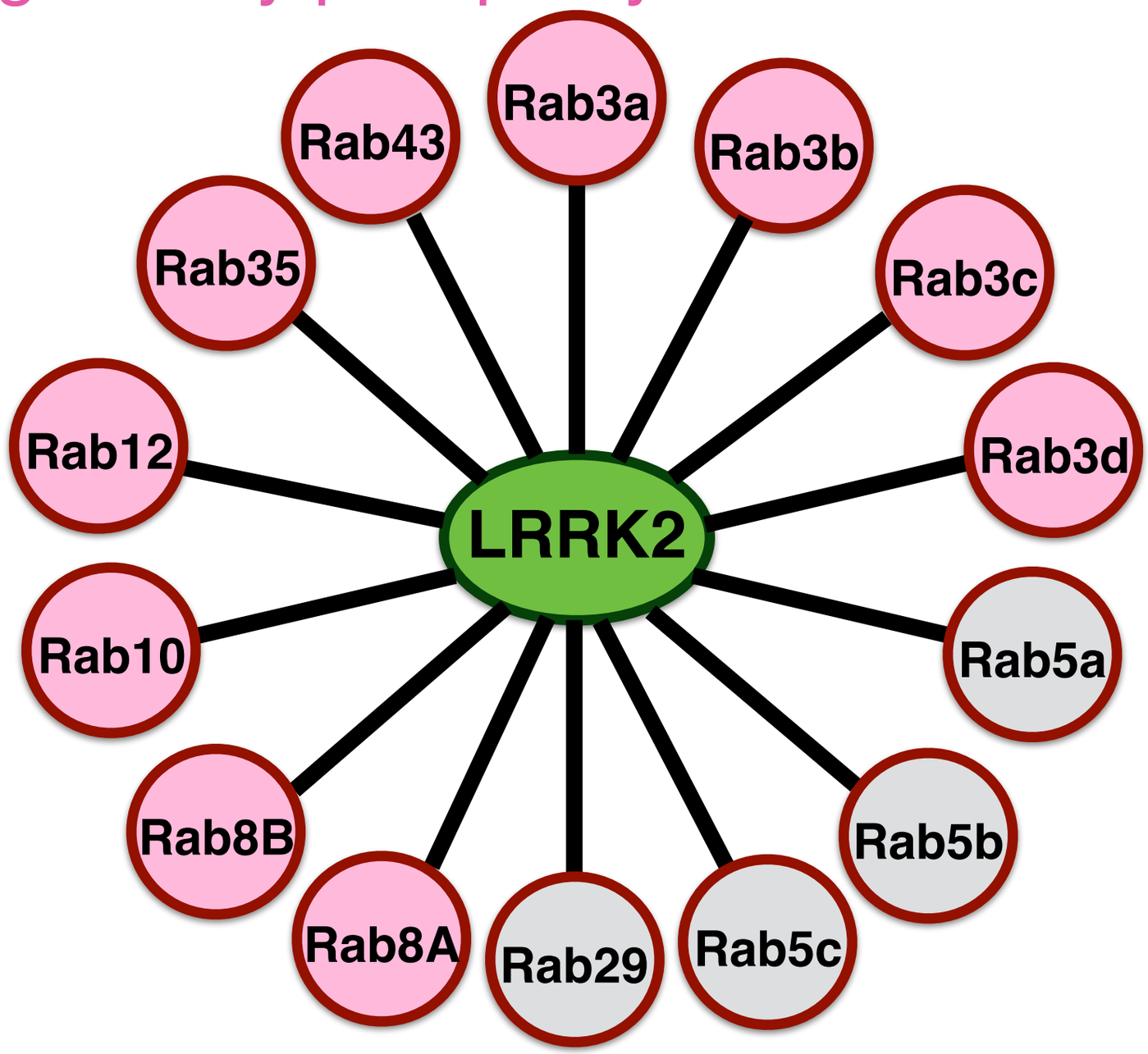
Rab GTPases Proteins

- Rab proteins are master regulators of membrane trafficking, orchestrating vesicle formation, vesicle movement along actin and tubulin networks, as well as membrane docking and fusion

- There are approximately 70 Rab proteins and their physiological roles are overall poorly understood

LRRK2 phosphorylates up to 14 Rab proteins. 10 confirmed at the endogenous level

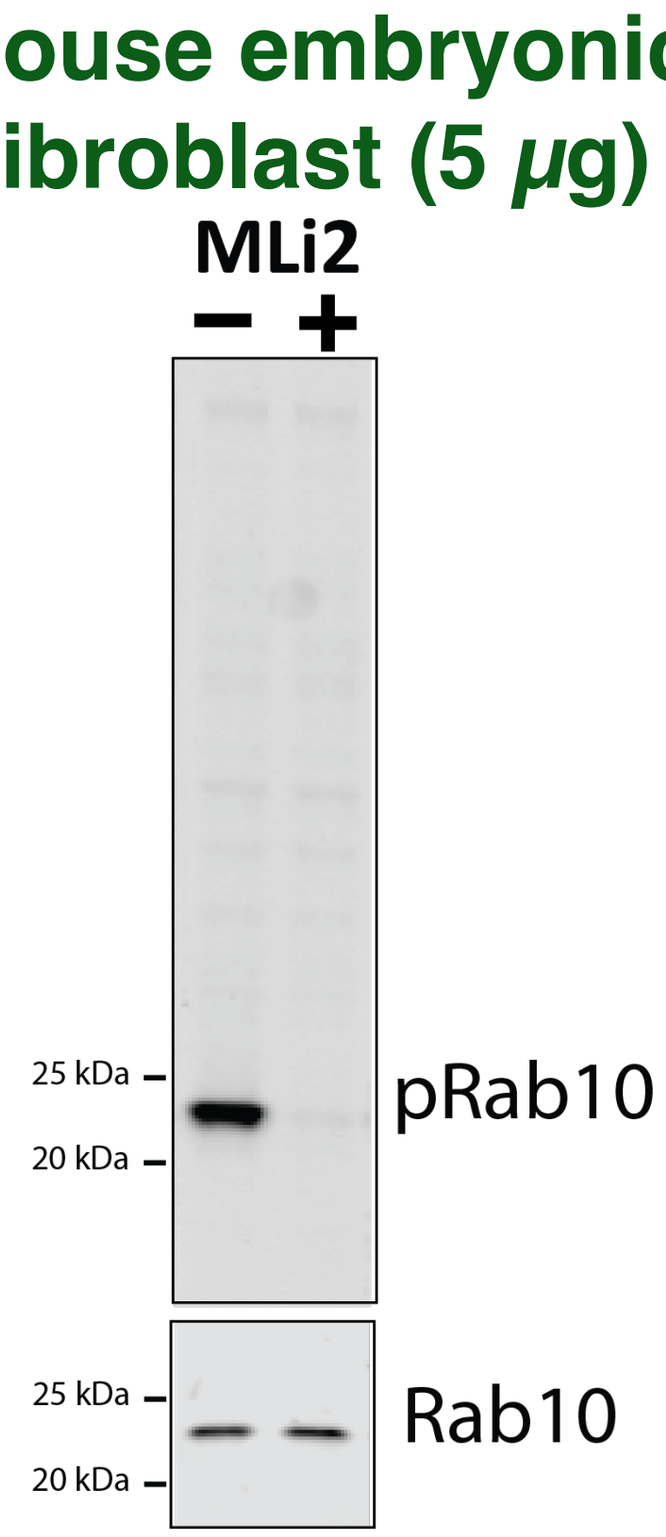
Endogenously phosphorylated



Possibly phosphorylated

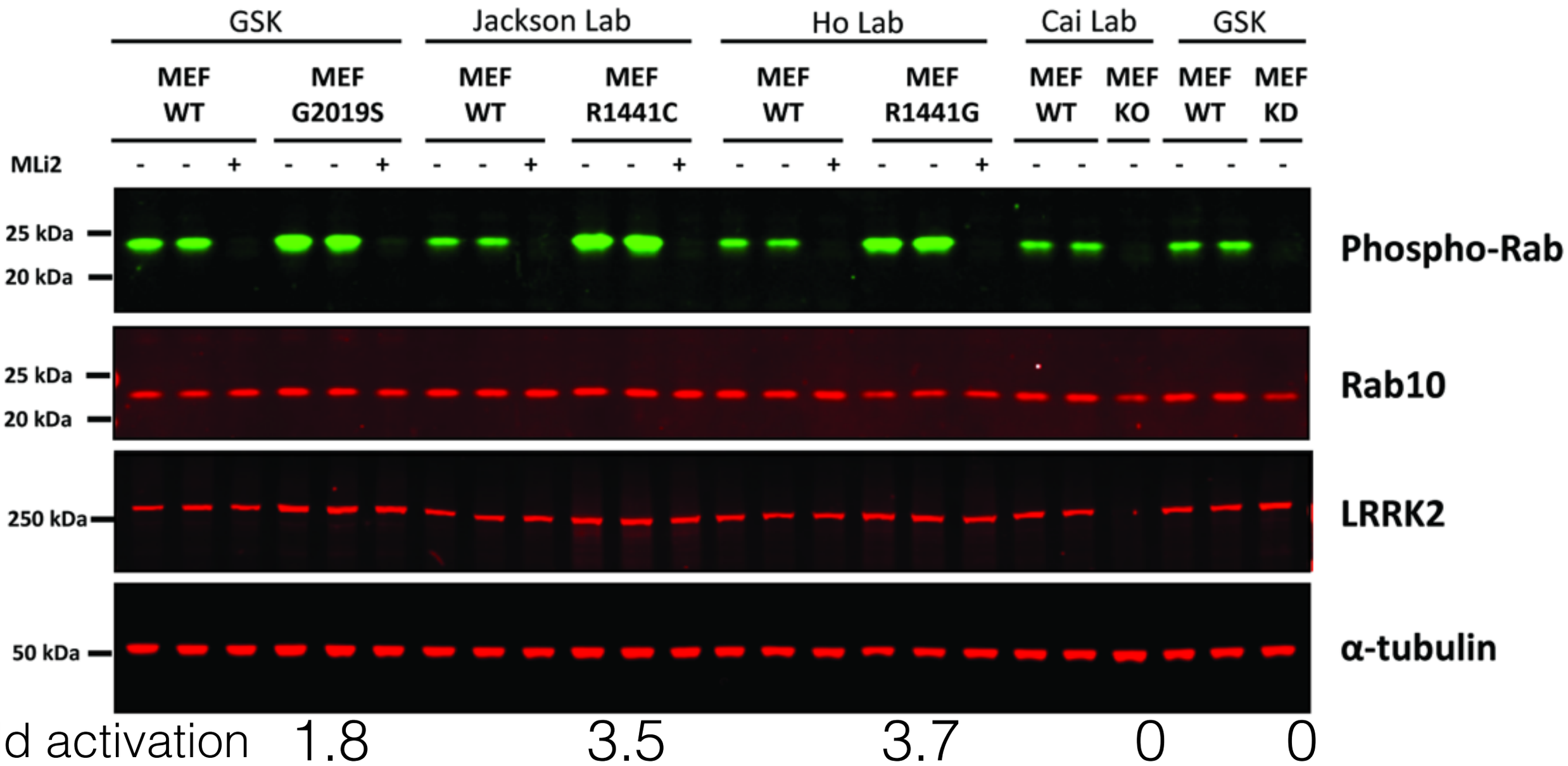
Federico Diez, Pawel Lis, Raja Nirujobi (Alessi lab)and Martin Steger (Mann lab)

Development of sensitive and selective rabbit monoclonal MJFF-pRab10 antibodies



Pawel Lis

The phospho-specific Rab10 antibodies allow facile assessment of endogenous LRRK2 activity

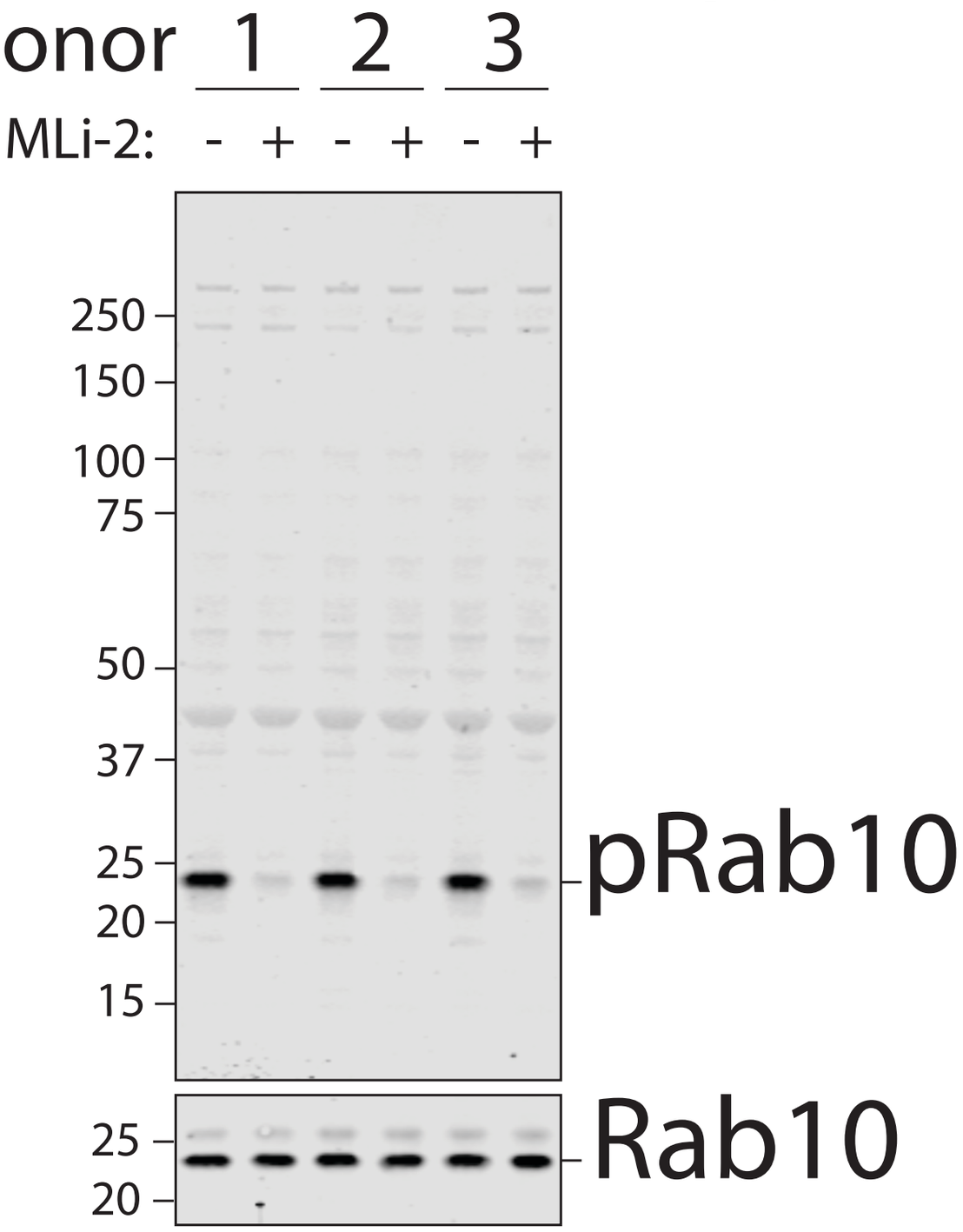


R1441G MEFs provided by Philip Wing-Lok Ho and Shu-Leong Ho (University of Hong Kong)

Pawel Lis

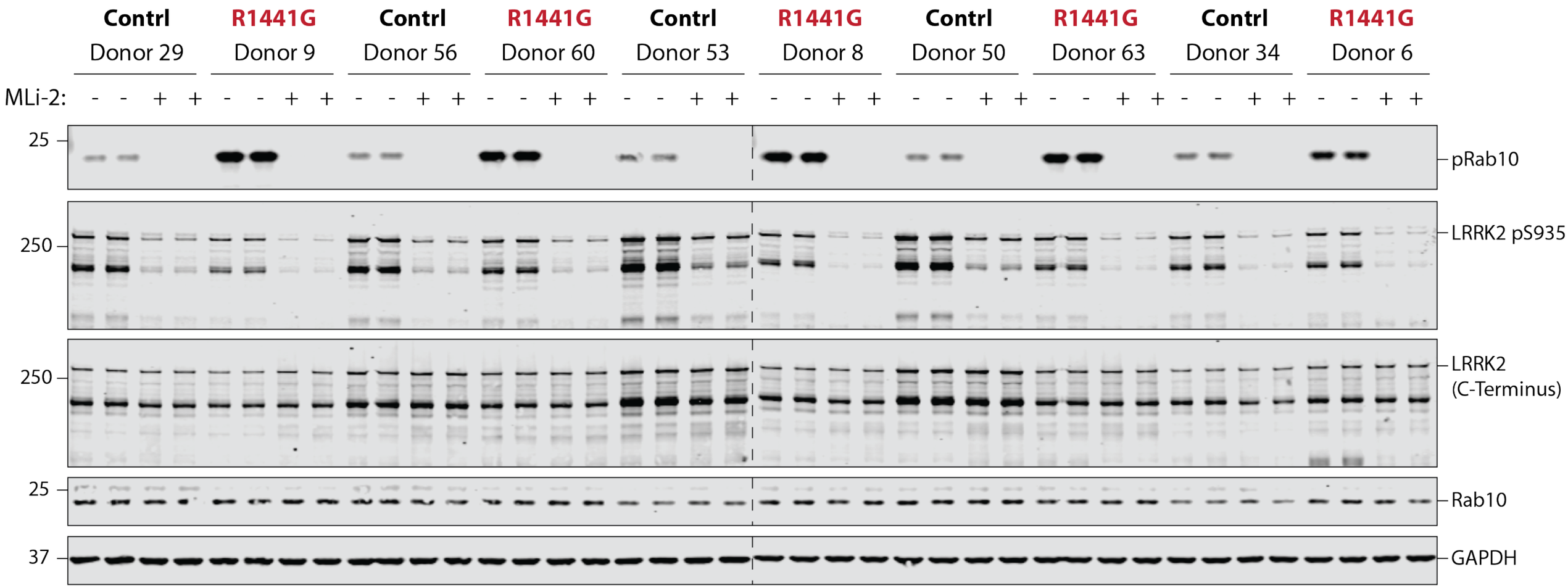
LRRK2 mediated Rab10 phosphorylation can readily be assessed in human neutrophils

Human neutrophil (10 μg)



Ying Fan, Esther Sammler & Andrew Howden

Elevated LRRK2-Dependent Rab10 Phosphorylation in R1441G Patient Neutrophils



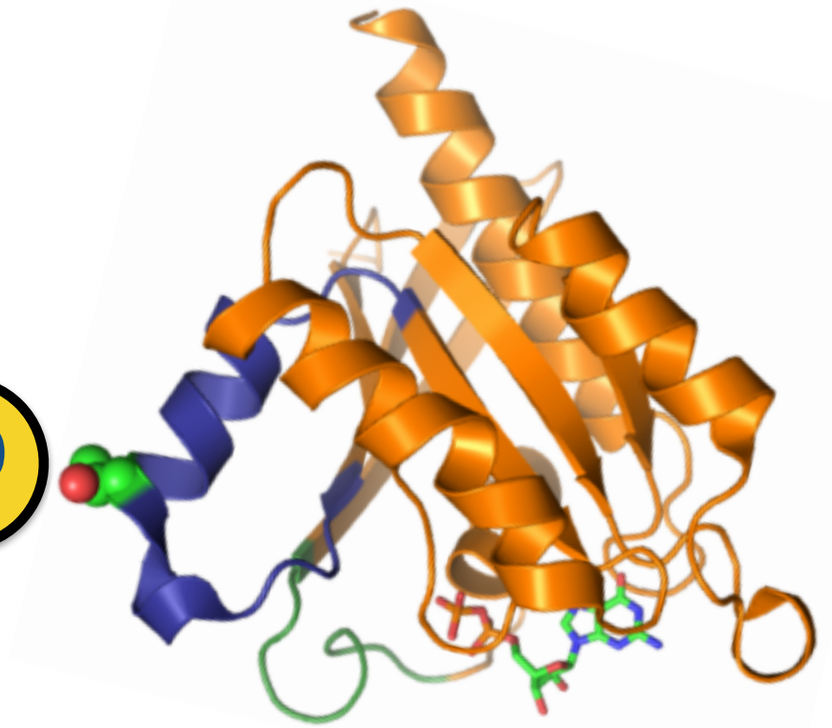
Group	Control	R1441G Carrier	Control	R1441G Carrier	Control	R1441G Carrier	Control	R1441G PD	Control	R1441G PD
Donor	29	9	56	60	53	8	50	63	34	6
Age	58	62	65	63	65	64	68	67	72	72
Gender	M	M	M	M	F	F	M	M	M	M

Collaboration with Eduardo Tools Barcelona

Ying Fan & Esther Sammler

What is downstream of LRRK2 phosphorylated Rab proteins

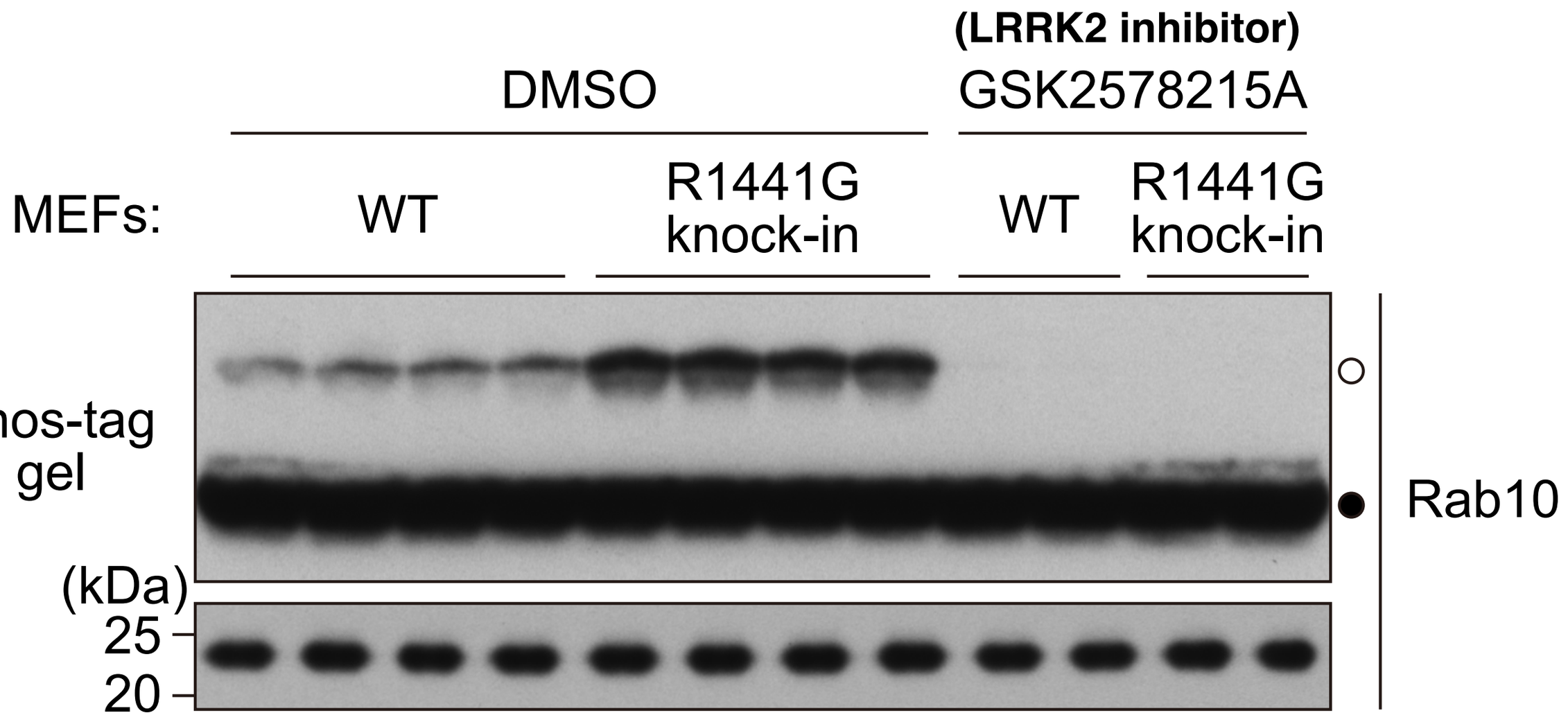
LRRK2 → **P**



Rab Protein

?

Stoichiometry of LRRK2 phosphorylation is low



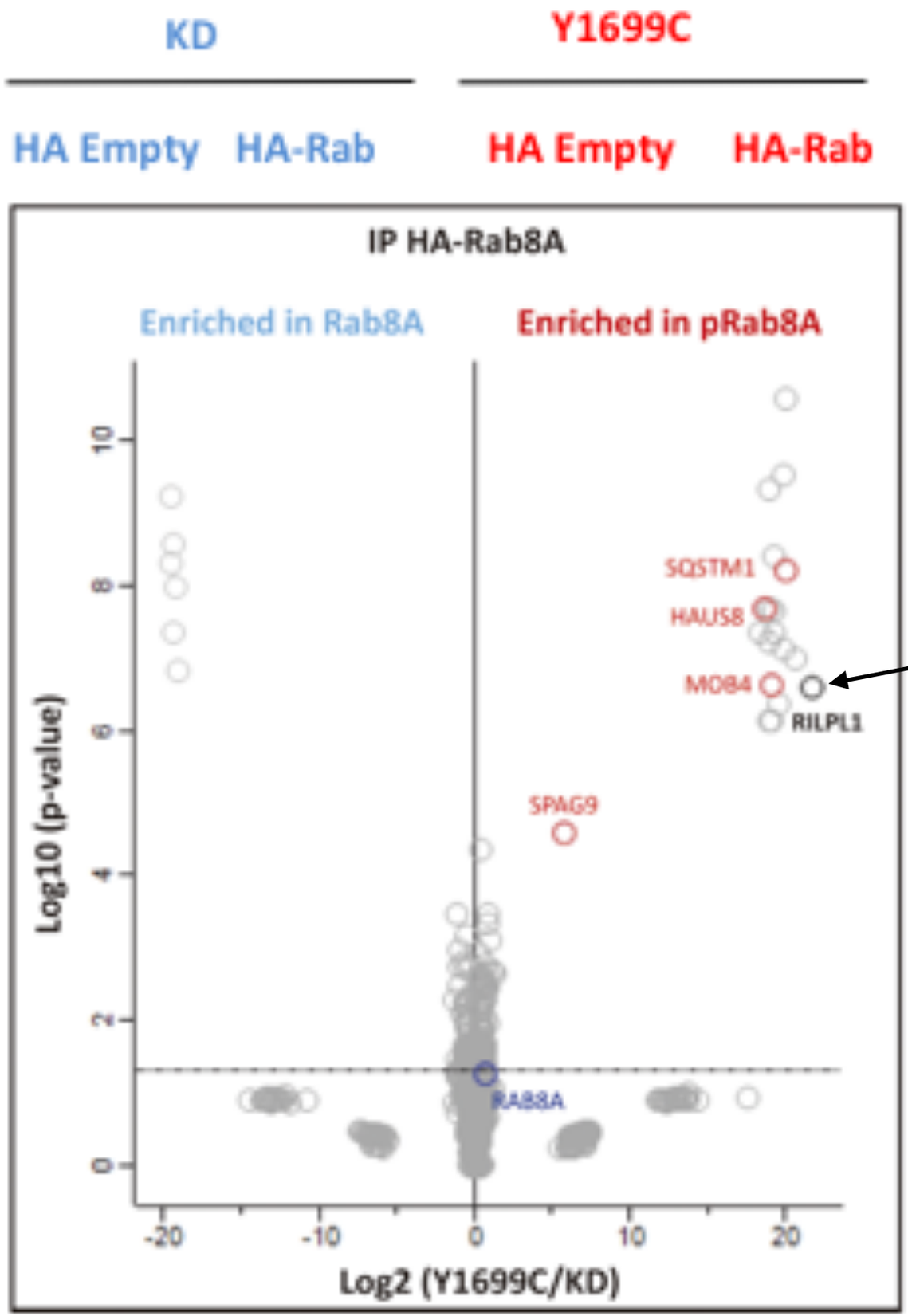
MEFs

R1441G MEFs provided by Philip Wing-Lok Ho and Shu-Leong Ho
(University of Hong Kong)

Genta Ito

Are there receptors for LRRK2 phosphorylated Rab proteins?

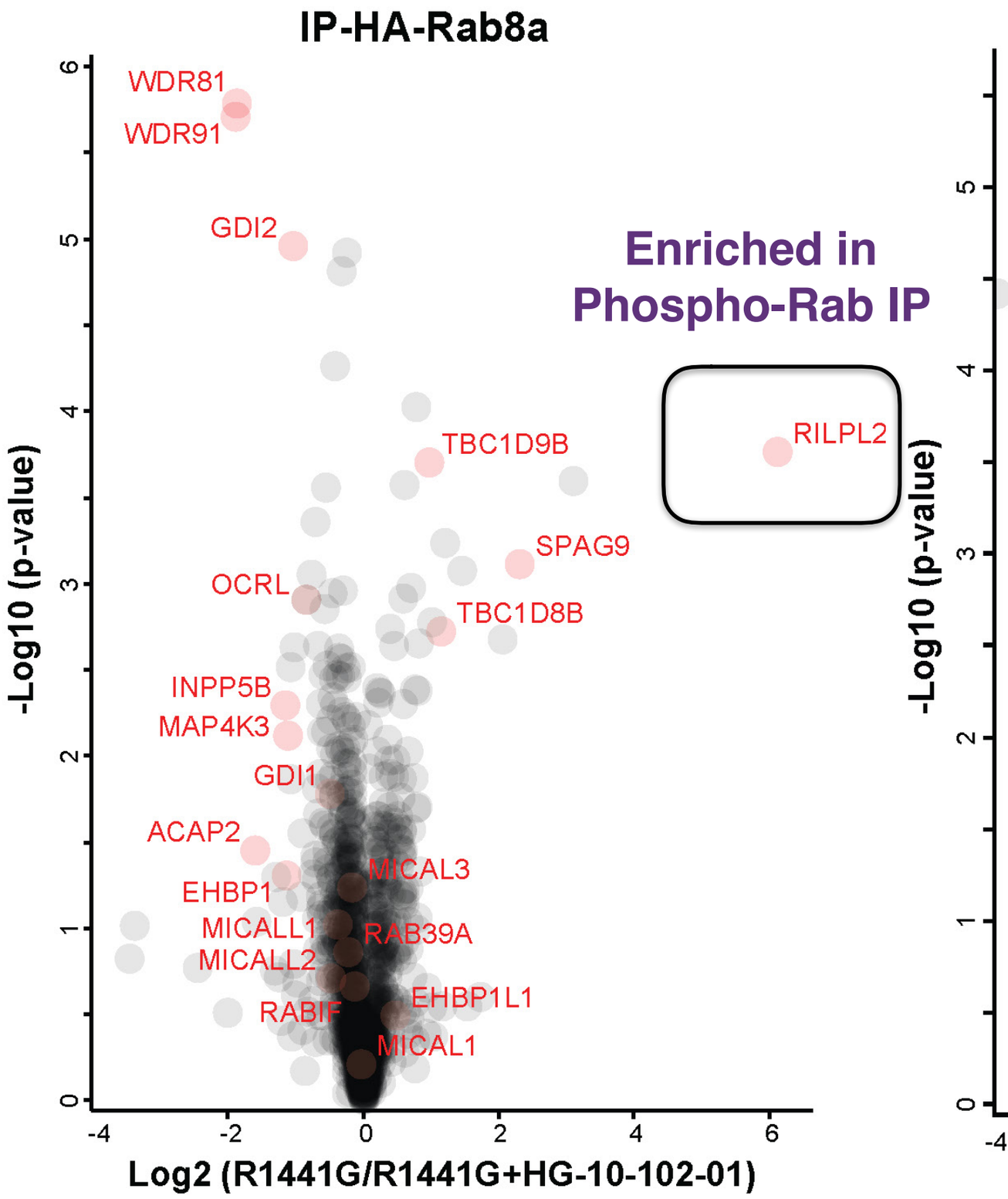
Mass Spectrometry screen to identify proteins that bind to LRRK2 phosphorylated Rab proteins

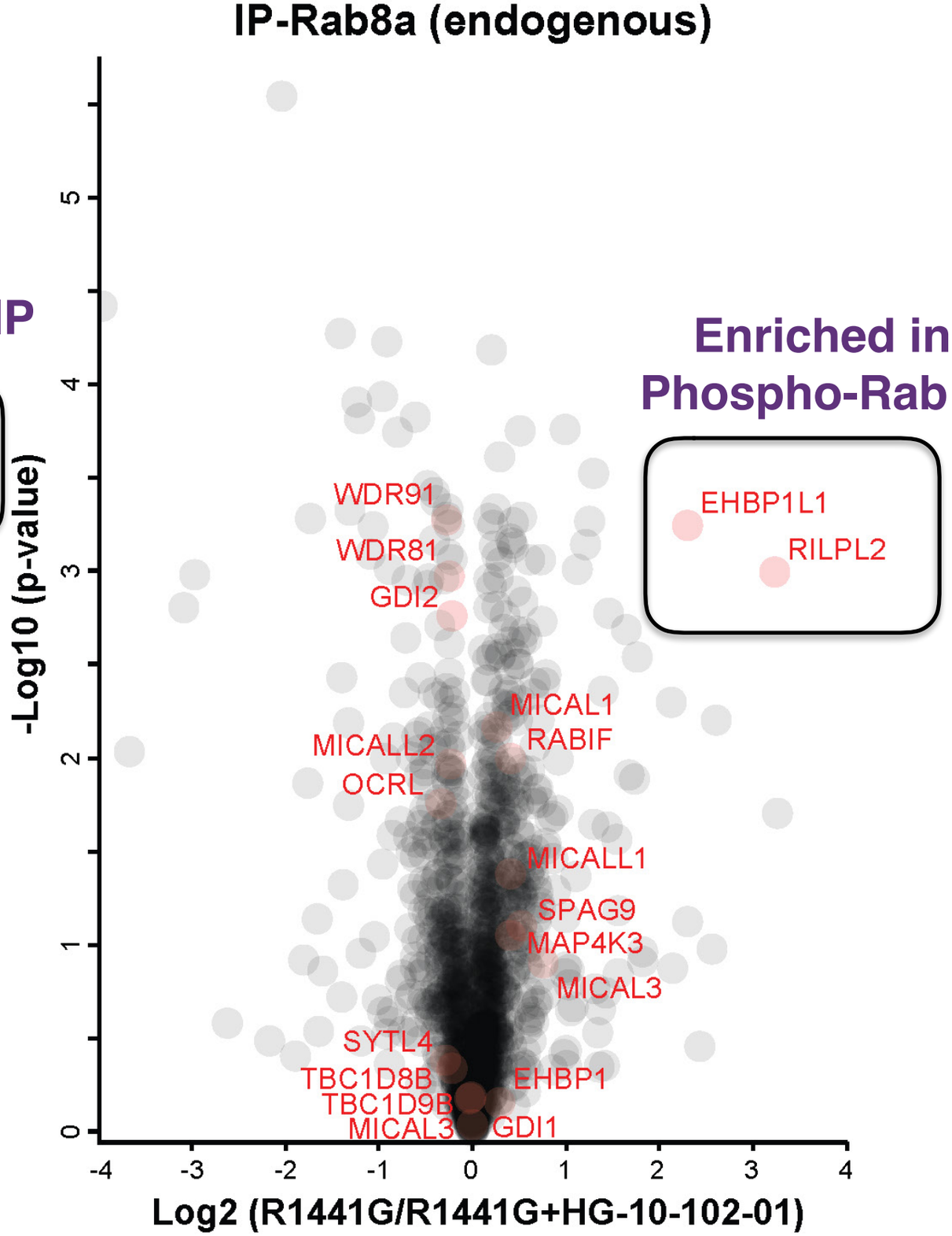


RILPL1

Federico Diez

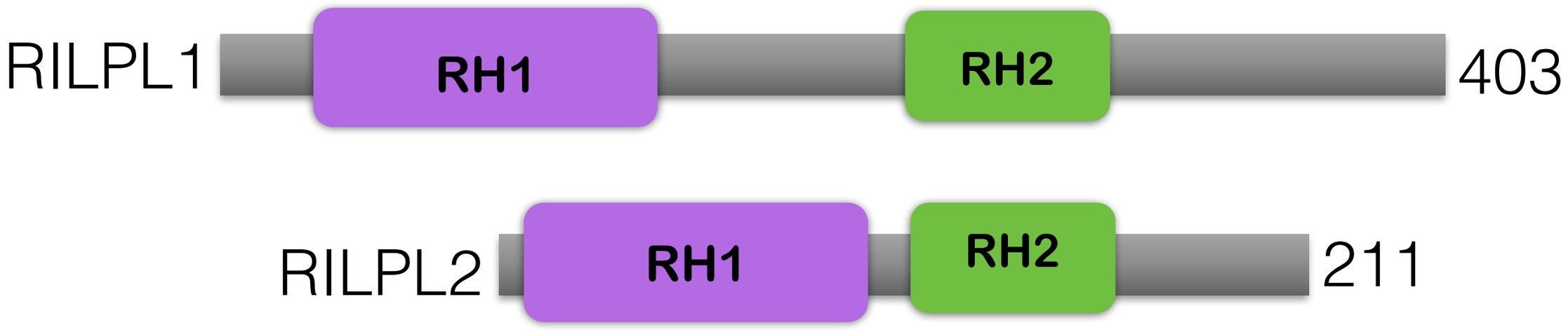
Mass Spectrometry screen to identify proteins that bind specifically to LRRK2 phosphorylated Rab proteins



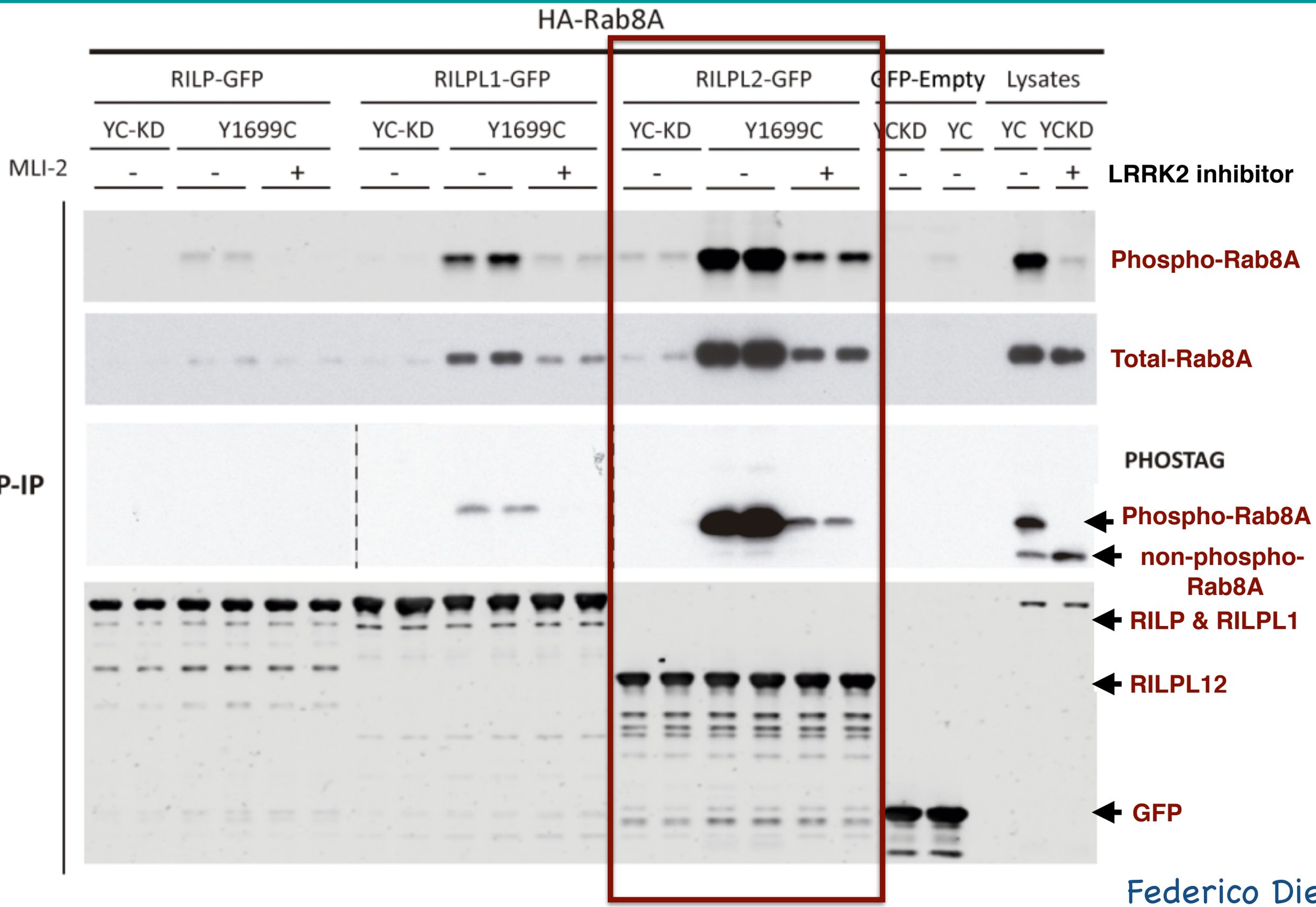


Martin Steger (Matthias Mann lab)

RILP family of protein



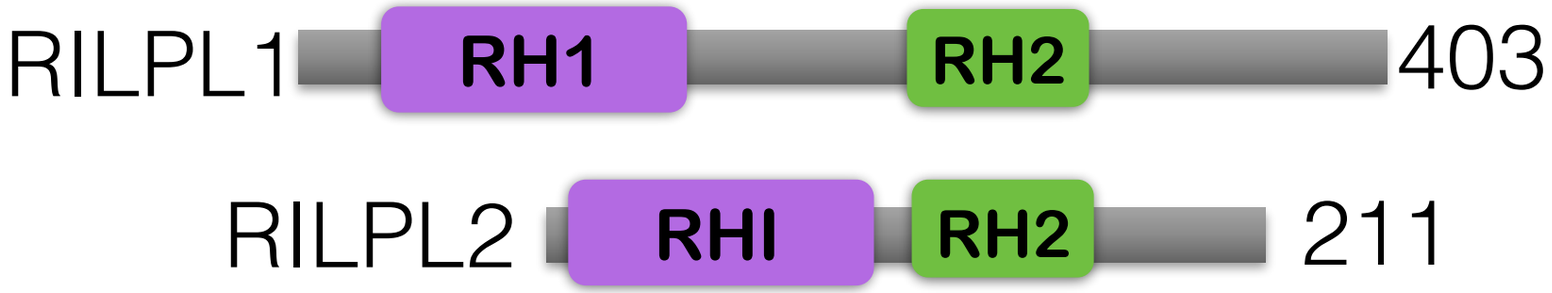
Mass Spectrometry screen to identify proteins that bind specifically to LRRK2 phosphorylated Rab proteins

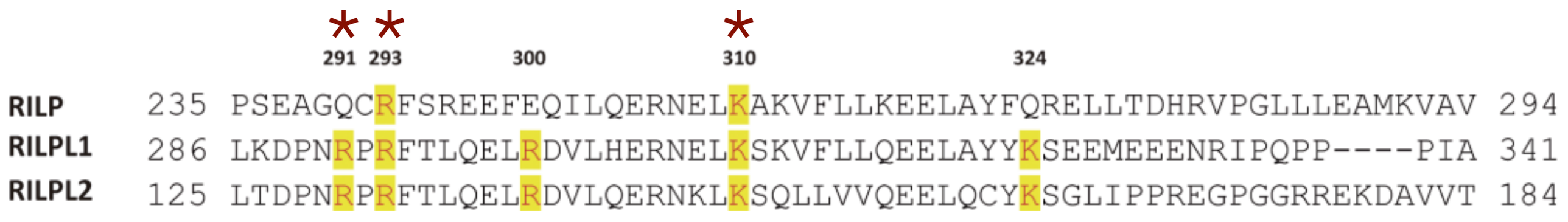


Federico Diez

RH2 domain of RILPL1 and RILPL2 mediate binding to LRRK2 phosphorylated Rab proteins

LRRK2-Phosphorylated Rab binding domain





⁎ mutation ablates binding to LRRK2 phosphorylated Rabs

Federico Diez

What is upstream of LRRK2?

?

LRRK2 → P

RabProtein

Do any Parkinson's components regulate LRRK2 mediated phosphorylation of Rab proteins?

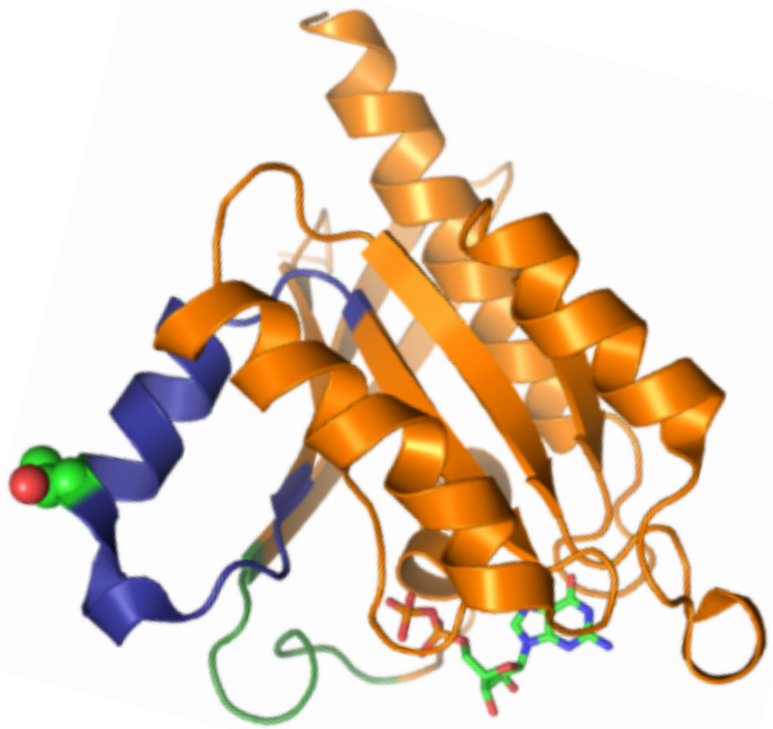
a-Syn Parkin PINK1 DJ-1 ATP13A2 PLA2G6 Fbx07

EIF4G1 DNAJC6 Rab29 VPS35 Rab39B VPS13C

GBA CHCHD2 PODXL TMEM230 GCH1

?

LRRK2 → Thr73



Rab10

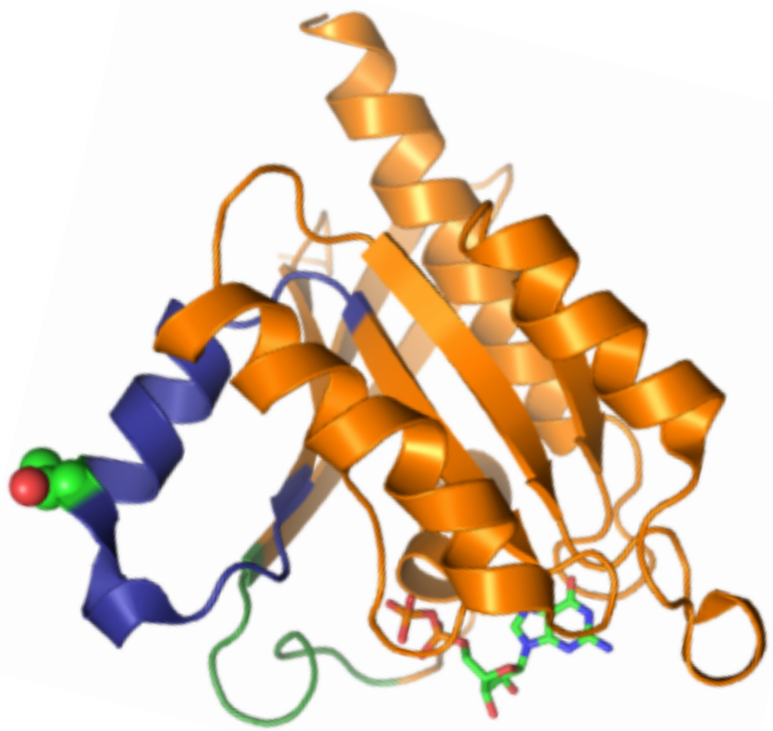
Do any Parkinson's components regulate LRRK2 mediated phosphorylation of Rab proteins?

a-Syn Parkin PINK1 DJ-1 ATP13A2 PLA2G6 Fbx07

EIF4G1 DNAJC6 **Rab29** VPS35 Rab39B VPS13C

GBA CHCHD2 PODXL TMEM230 GCH1

LRRK2 → ? → Thr73



Rab10

Connecting Rab29 and LRRK2

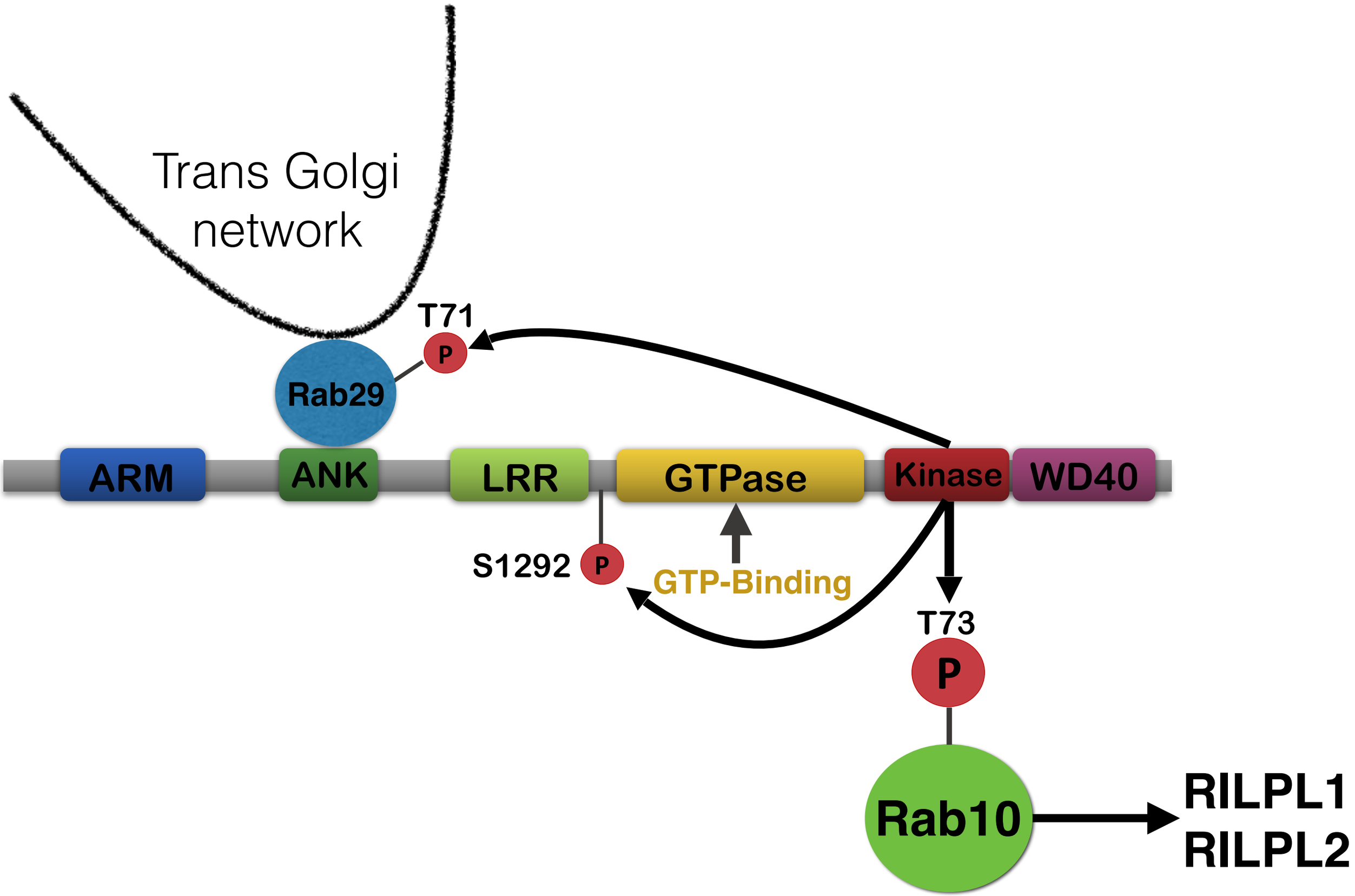
- Rab29 is one of 5 genes located within the PARK16 locus and mutations led to over expression of Rab29.

- GWAS studies indicate epistatic interactions between Rab29 and LRRK2

- Studies in C.elegans and mice indicate that LRRK2 and Rab29 lie in the same signaling network

- Rab29 is a Substrate of LRRK2 and is unique as it possesses two adjacent phosphorylation sites (Thr71 and Ser72)

Mechanism by which Rab29 activates LRRK2



Does the VPS35 Parkinson's component regulate LRRK2 mediated phosphorylation of Rab proteins?

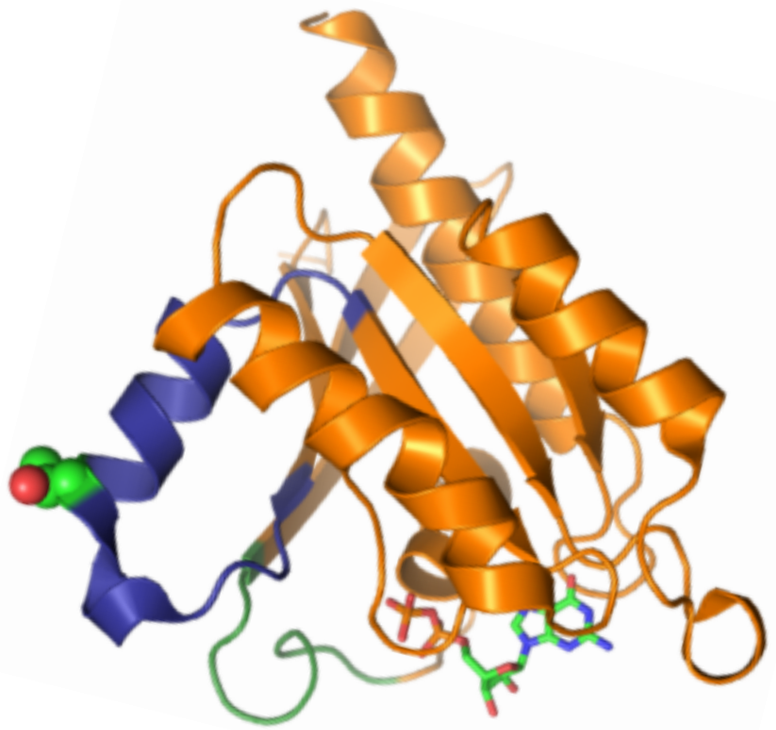
a-Syn Parkin PINK1 DJ-1 ATP13A2 PLA2G6 Fbx07

EIF4G1 DNAJC6 Rab29 **VPS35** Rab39B VPS13C

GBA CHCHD2 PODXL TMEM230 GCH1

LRRK2 → Thr73

?



Rab10

Connecting VPS35 and LRRK2

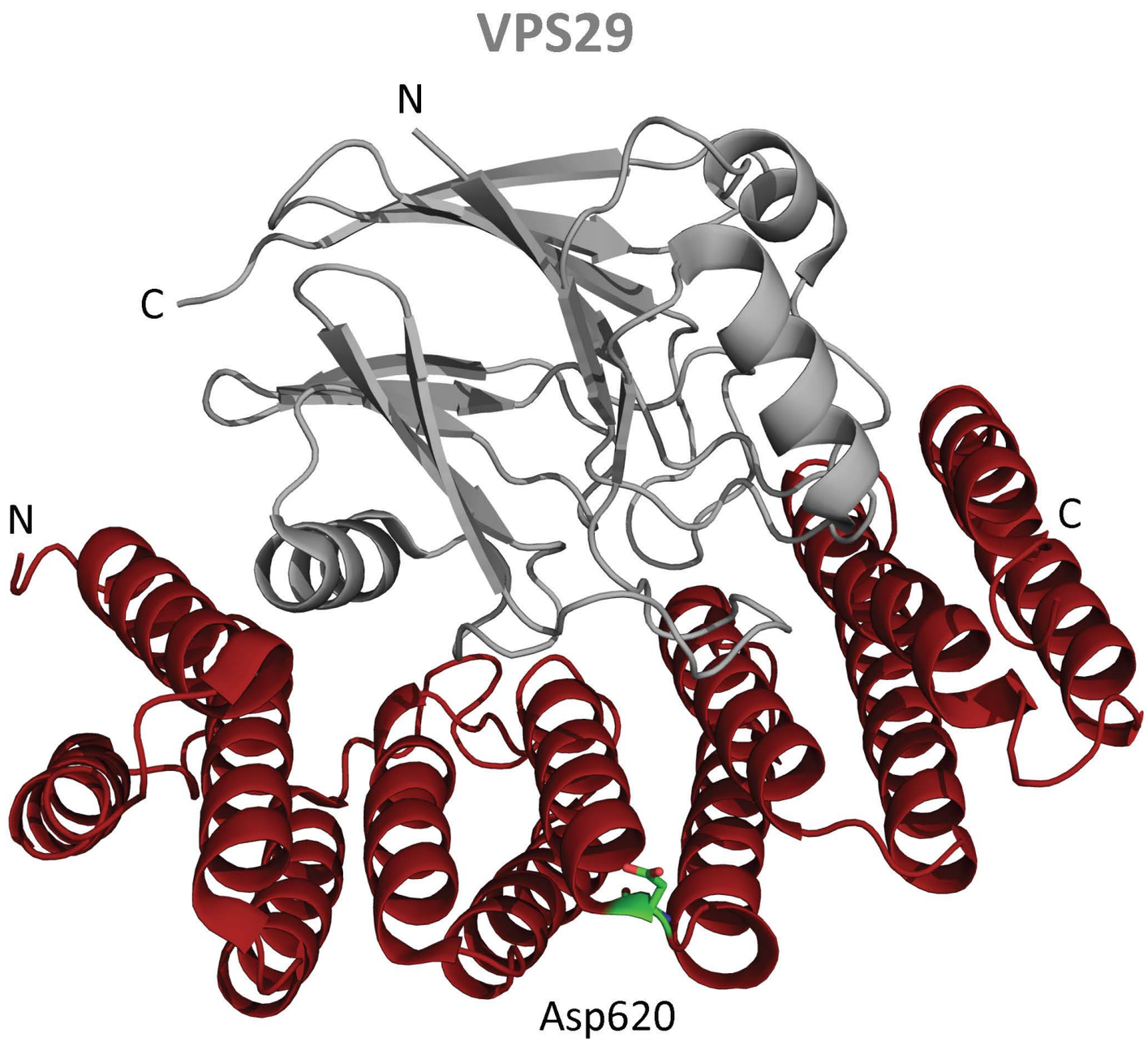
- VPS35 gene, which encodes a key component of the membrane protein–recycling retromer complex involved in retrograde transport of proteins from endosomes to the trans-Golgi network.

- Vps35 is the largest subunit of retromer complex and functions as the central platform for the assembly of Vps26 and Vps29.

- Autosomal-dominant gene mutations in VPS35 such as D620N are associated with Parkinson's disease

- Previous genetic studies hinted at VPS35 and LRRK2 operating on a same pathway

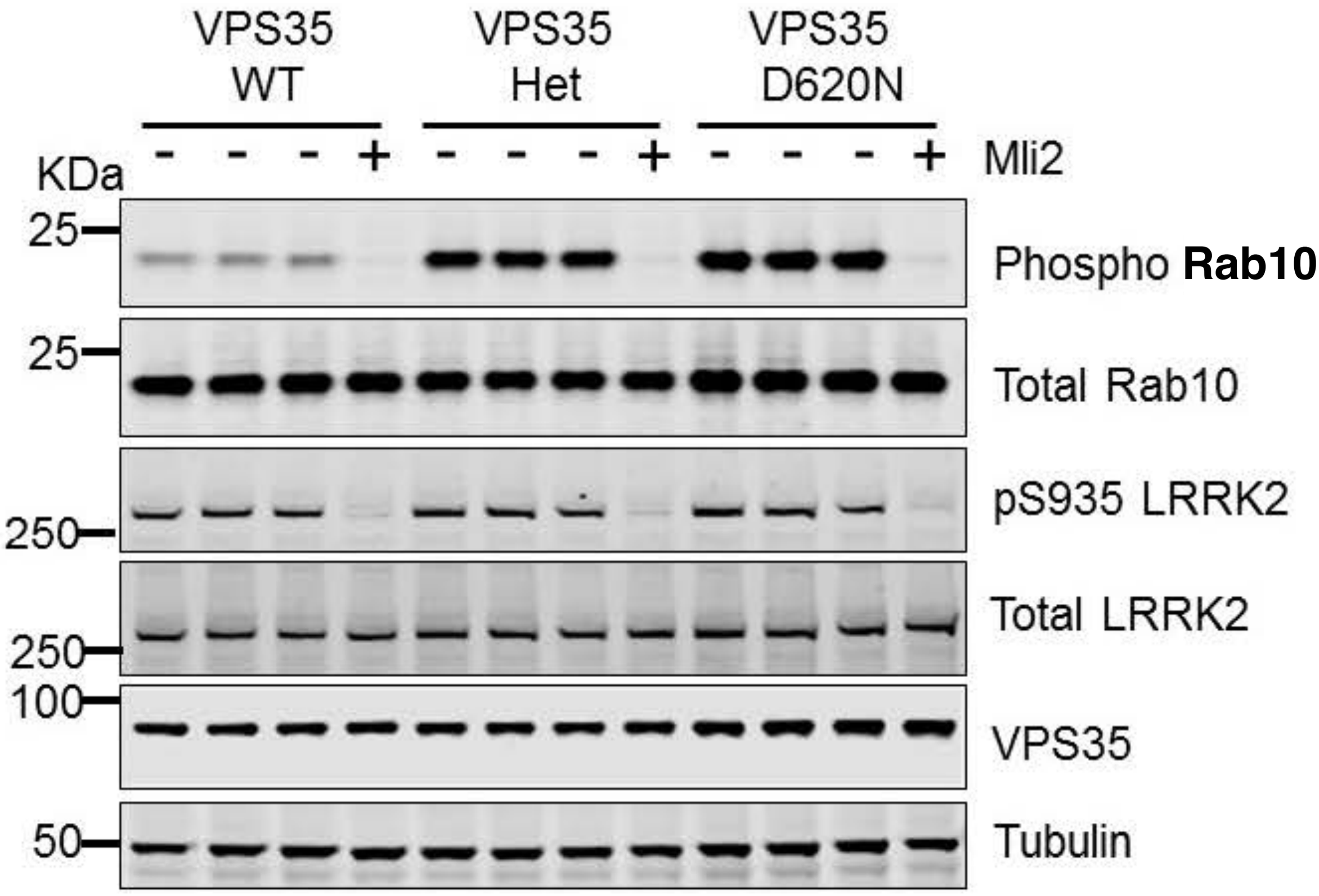
VPS35:VPS29 Structure



VPS35

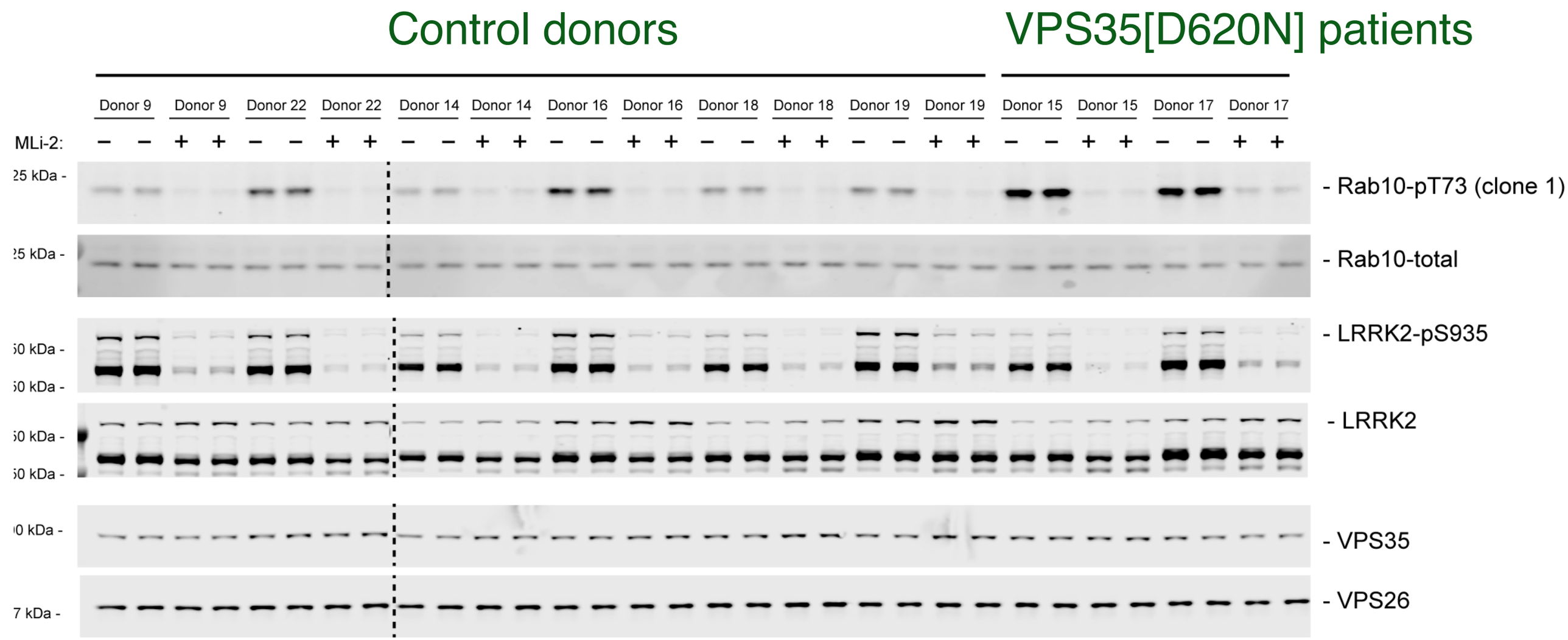
Vps35 resembles many other helical solenoid proteins including AP adaptor protein complexes that are characterized with repeated structural units in a continuous superhelix arrangement involved and function as potential cargo binding sites.

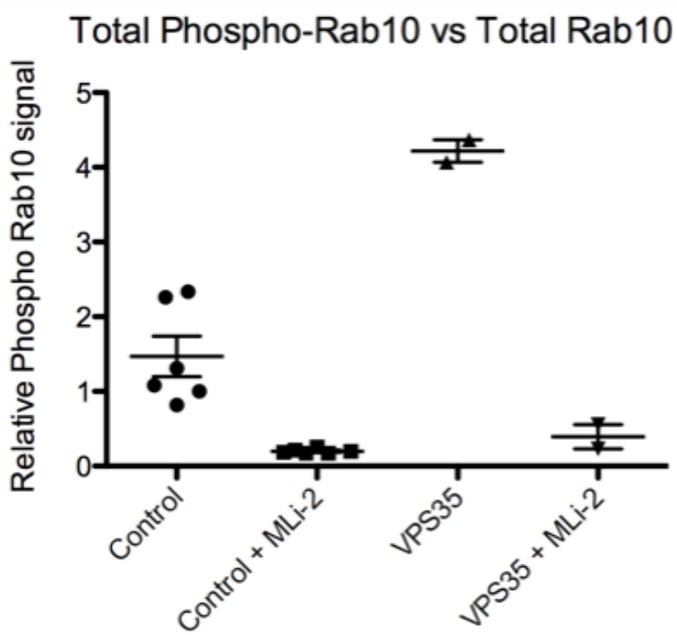
VPS35 D620N Mutation enhances LRRK2 mediated Rab phosphorylation in mouse knock-in fibroblasts



Rafeeq Mir

Initial evidence that VPS35[D620N] mutation enhances LRRK2 mediated Rab10 phosphorylation in humans

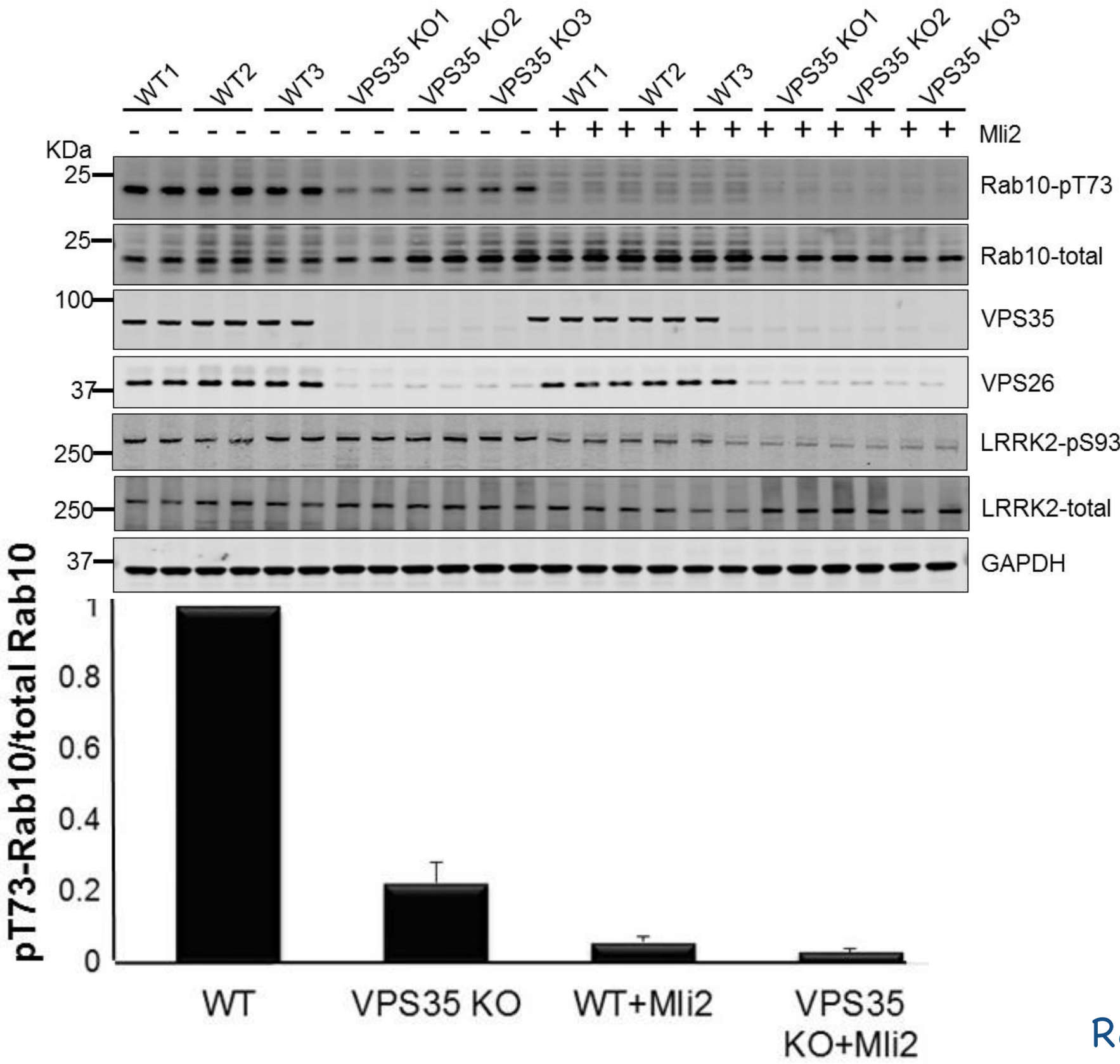




Collaboration with
Alexander Zimprich
(Vienna)

Esther Sammler
Francesca Tonelli

Knock-out of VPS35 also reduced LRRK2 activity



Rafeeq Mir

VPS35 Regulates LRRK2

- These observations provide the first evidence VPS35 controls LRRK2 activity.

- The impact of VPS35[D620N] mutation on Parkinson's could be mediated through hyper-activation of LRRK2.

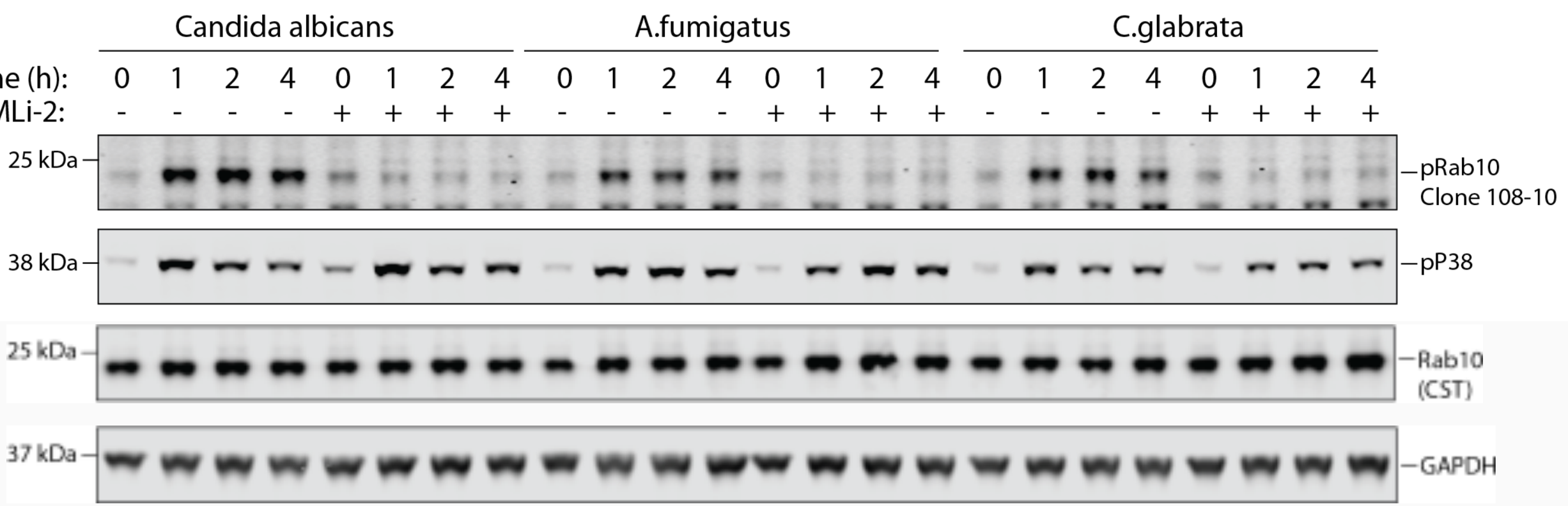
- Parkinson's patients with VPS35[D620N] mutation might benefit from future LRRK2 inhibitor therapy.

- Our findings also suggest that it may be possible to elaborate inhibitors of the retromer complex that suppress LRRK2 activity for the treatment of Parkinson's.

Is LRRK2 activated by infection?

- Inflammation plays an important role in the development of Parkinson's.
- LRRK2 is highly expressed in macrophages, monocytes and neutrophils suggesting it functions in the defence against intracellular pathogen
- In humans, single nucleotide polymorphisms within or close to the LRRK2 gene have been linked to inflammatory conditions including ulcerative colitis, Crohn's disease and also increased susceptibility to leprosy infection
- In mice, LRRK2 is required for mucosal immunity against the opportunistic pathogen, Listeria monocytogenes, and protects from S. Typhimurium infection
- The LRRK2 kinase is most closely related to the RIP kinases that are key regulators of inflammasomes

Fungal infection activates LRRK2 in mouse bone marrow derived macrophages



Ying Fan, Stephanie Laba & Simon Arthur

Parkinson's Genes, Environment, Lifestyle
Gut Microbiome, Infection

?

Trans Golgi network

Rab29

N1427H
R1441C
R1441G
R1441H

Y1699C

I2020T
G2019S

ARM | ANK | LRR | Roc/COR GTPase | Protein Kinase | WD40 | LRRK2

GTP-Binding

?

Vesicular Trafficking
Autophagy
Lysosomal dysfunction

?

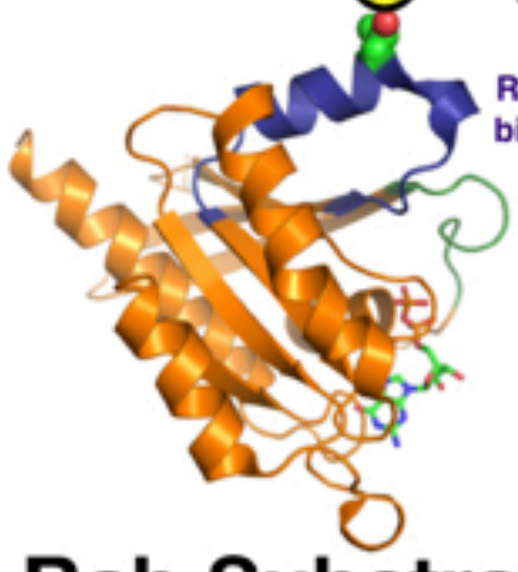
P

RILPL1
RILPL2

?

Ciliogenesis

Rab effector binding loop



Rab Substrates

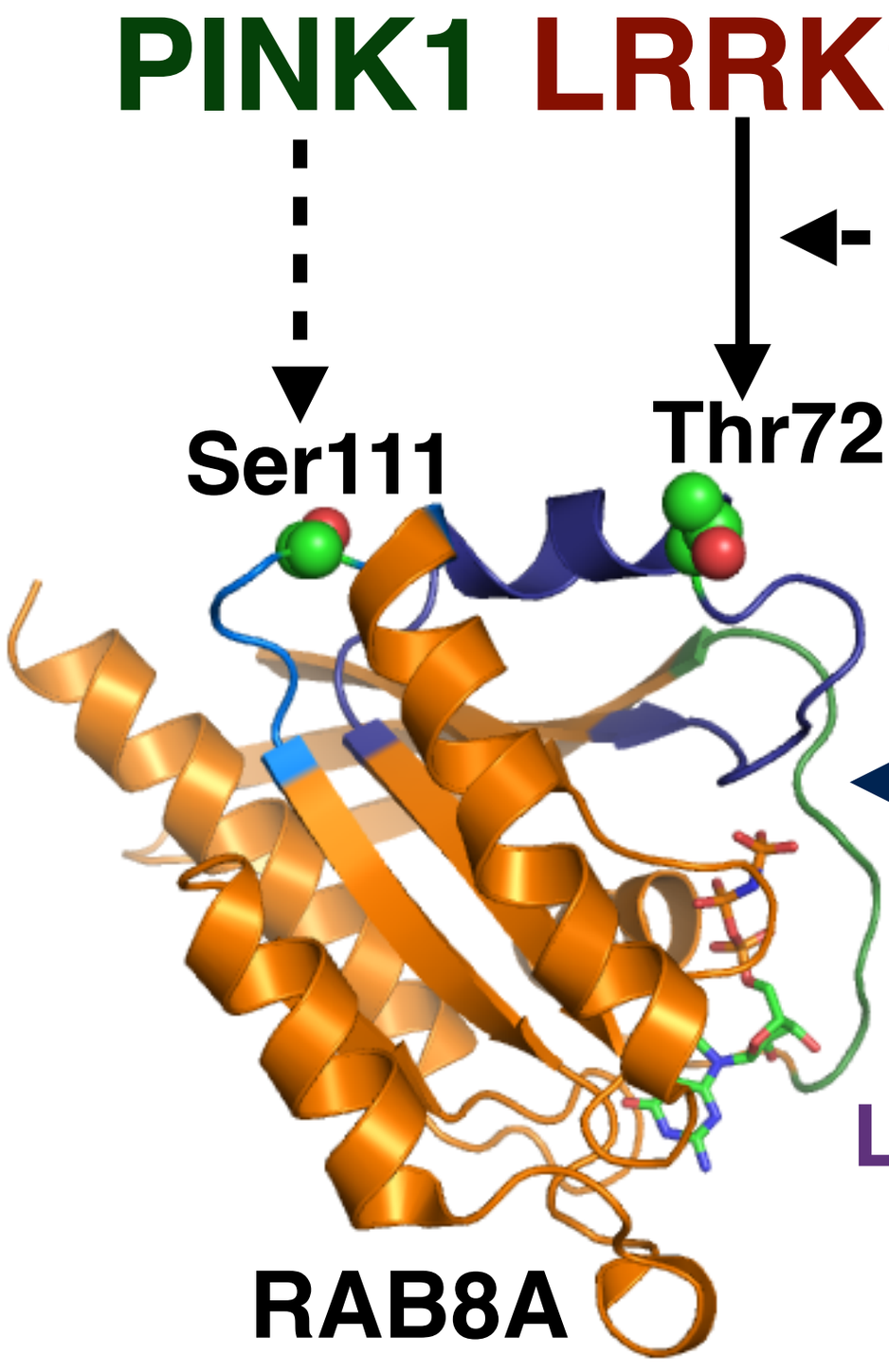
LRRK2 Activation

Pro-inflammatory responses
Protection against infection
Neuroinflammation
Parkinson's susceptibility (later life)

LRRK2 Inhibition

Increased infection risk

LRRK2, Rab29, VPS35, PINK1 and alpha-synuclein converge on Rab GTPases; Is derailment of Rab biology at the heart of understanding Parkinson's Disease?



Loss of function mutations in Rab39B also cause a Parkinson's-like disorder

ACKNOWLEDGEMENTS

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