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Role of the UPS proteolytic pathway during muscle atrophy: from animal models to human diseases

Journée Club Neuromusculaire
13 Avril 2017



D. Taillandier - Human Nutrition Unit

**UMR 1019 INRA/Université d'Auvergne
Clermont-Ferrand - France**

Background



Muscle main functions:

- Force generation (locomotion, etc.)
- Amino acids reservoir



Protein synthesis

Proteolysis

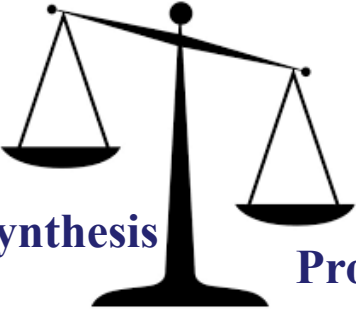
Normal situation (adult)

Chronic Renal Failure **Fasting**

Sepsis

Immobilization

Diabetes

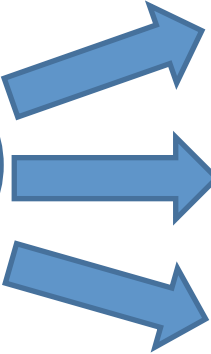


Protein synthesis

Proteolysis



Free Amino Acids



Energy

Immune response

Vital organs
Protein synthesis



Reduced efficiency of treatments
and of the immune response
General weakness



Increased
Morbidity
& Mortality



High health
care costs

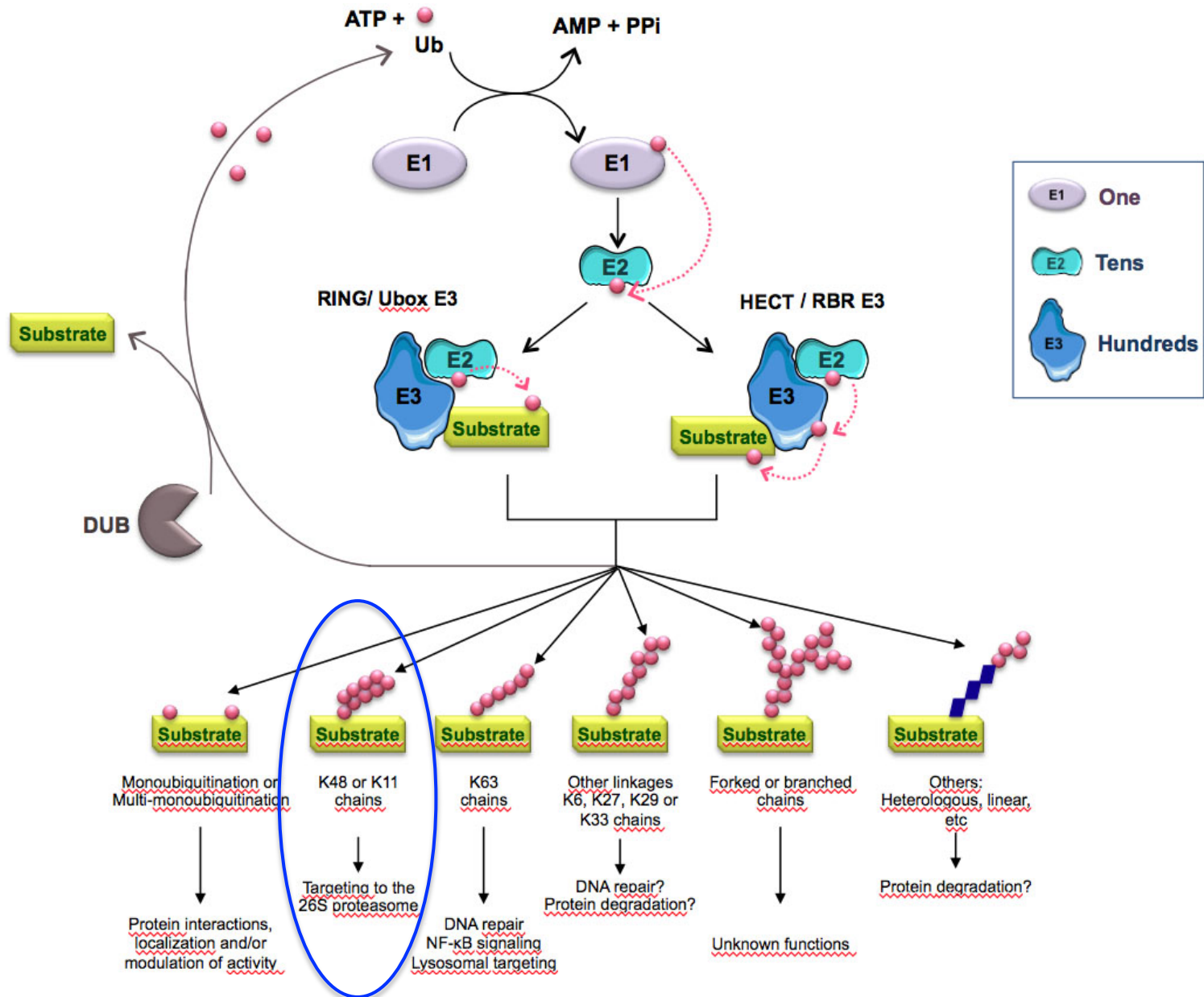
Background

Proteolysis is the main determinant

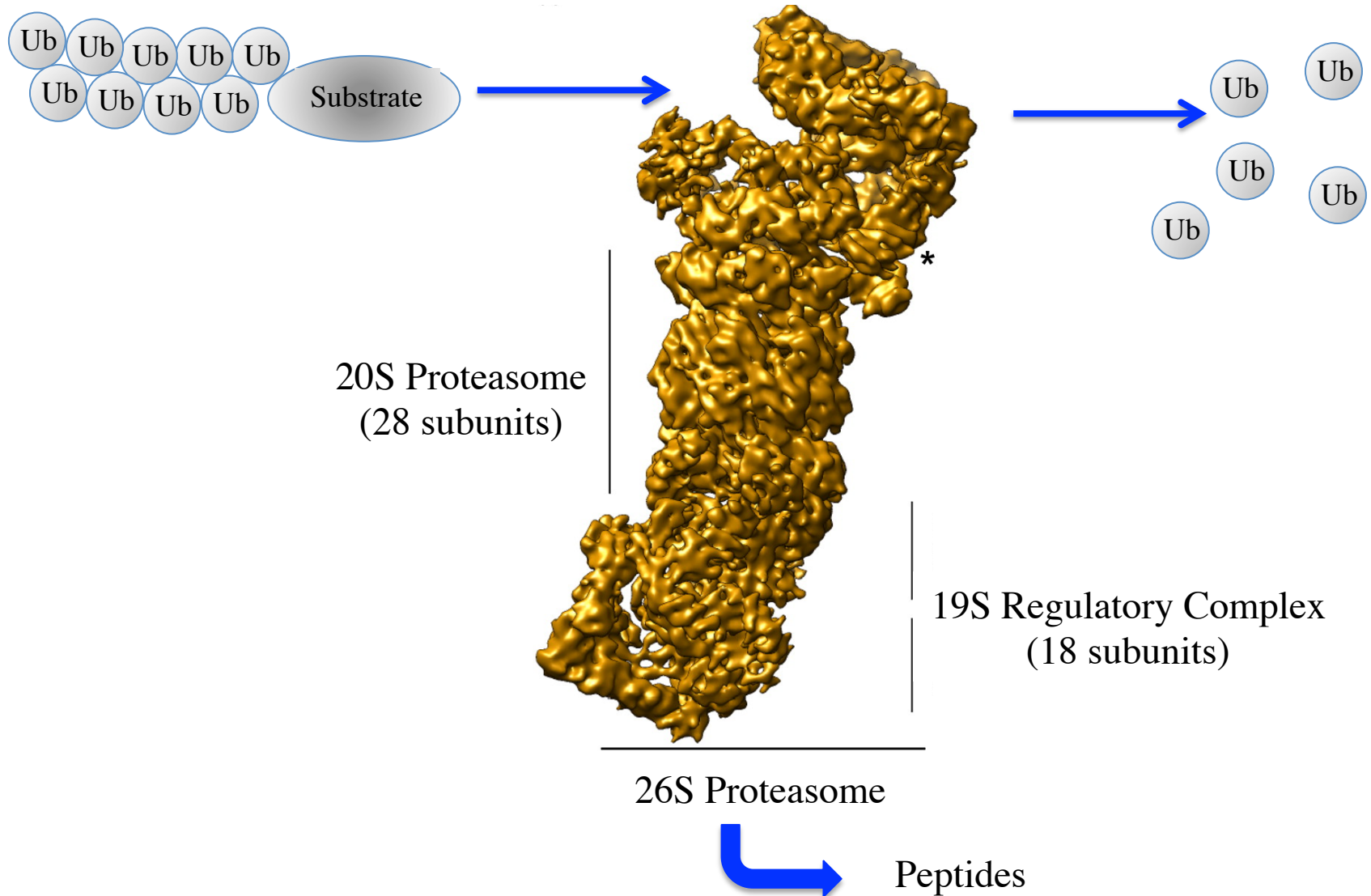
Proteolytic Systems involved

- ✓ Calpains (cytosolic, Ca^{2+} -dependent)
- ✓ Cathepsins (lysosomes, endosomes)
- ✓ Caspases (cytosolic)
- ✓ **Proteasome** (cytosolic, self-compartmentalized)
UPS: Ubiquitin Proteasome System

Ubiquitin Proteasome System (UPS)



Ubiquitin Proteasome System (UPS)



Implication of the UPS during muscle wasting

Fasting	Wing & Goldberg 1993
Denervation atrophy	Medina et al. 1995
Acidosis	Mitch et al. 1994
Burn injury	Fang et al. 1995
Diabetes	Price et al. 1996
Cancer	Temparis et al. 1994
Infection	Voisin et al. 1996
Unweighting	Taillandier et al. 1996
Etc.	

Implication of the UPS during muscle wasting

Important disease-linked muscle loss (10-60%) in animal models

Recruitment of the UPS:



ATP-dependent proteolysis using incubated muscles



mRNA levels of various components

- Ub
- Ubiquitinating Enzymes
- Deubiquitinating Enzymes
- 20S Proteasome subunits
- 19S Regulatory Complex subunits



Proteasome activity

The UPS is also implicated during the early recovery phase



mRNA levels of selected components

- Ub
- Ubiquitinating Enzymes
- Deubiquitinating Enzymes
- 20S Proteasome subunits
- 19S Regulatory Complex subunits

Implication of the UPS during muscle wasting

The atrophy-related genes “atrogenes”

Multiple types of skeletal muscle atrophy involve a common program of changes in gene expression

STEWART H. LECKER,¹ R. THOMAS JAGOE,^{*1} ALEXANDER GILBERT,
MARCELO GOMES,^{††} VICKIE BARACOS,[†] JAMES BAILEY,[‡] S. RUSS PRICE,[‡]
WILLIAM E. MITCH,[§] AND ALFRED L. GOLDBERG^{††-2}

Lecker et al. FASEB J, 2004

Am J Physiol Endocrinol Metab 287: E591–E601, 2004.
First published April 20, 2004; 10.1152/ajpendo.00073.2004.

IGF-I stimulates muscle growth by suppressing protein breakdown and expression of atrophy-related ubiquitin ligases, atrogin-1 and MuRF1

Jennifer M. Satchek,^{1*} Akira Ohtsuka,^{2*} S. Christine McLary,¹ and Alfred L. Goldberg¹

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Implication of the UPS during muscle wasting

The “Atrophy-related genes “atrogenes” in atrophying rats
(Fasting, Tumor bearing, Uremia, Diabetes)

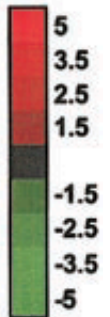
Clone	Unigene	Primary Sequence Name		F	T	U	D
3137251	Hs.183842	UBB	ubiquitin B				
2730250	Hs.183704	UBC	ubiquitin C				
2132619	Hs.3297	RPS27A	ribosomal protein S27a				
4157922	Hs.5308	UBA52	ubiquitin A-ribosomal protein fusion product				
1723142	Hs.61661	FBXO32	Atrogin-1/MAFbx				
751477	Mm.32920	Ncube1	non-canonical Ub-conjugating enzyme 1				
747318	Mm.21634	Ube4b	ubiquitination factor E4B				
2195309	Hs.82159	PSMA1	proteasome 20S subunit, alpha 1				
723267	Mm.30097	Psm1					
466041	Mm.30097	Psm1					
572285	Mm.2287	Psm5	proteasome 20S subunit, alpha 5				
1737833	Hs.82793	PSMB3	proteasome 20S subunit, beta 3				
571569	Mm.21874	Psm3					
901317	Hs.89545	PSMB4	proteasome 20S subunit, beta 4				
466254	Mm.29582	Psm4	proteasome 19S subunit, ATPase, 4				
2123183	Hs.78466	PSMD8	proteasome 19S subunit, non-ATPase, 8				
113452	Hs.90744	PSMD11					
833508	Mm.28571	Psm11	proteasome 19S subunit, non-ATPase, 11				
448976	Hs.112396	PA200	KIAA0077 protein				
1707220	Hs.75981	USP14	Ub-specific protease 14				
315082	Mm.930	Ctsl	cathepsin L				
2935790	Hs.87417	CTSL2					

Ubiquitin




Ubiquitinating enzymes

26 Proteasome

Lysosomes



Implication of the UPS during muscle wasting

	Atrogenes	Conditions
Anabolic situations		Insulin, IGF-1
Catabolic situations		Fasting Vitamin D deficiency Immobilization / unweighting Aging
Non pathological states		
Pathological and injury states		Burn injury Head trauma Hyperthermia Mechanical ventilation Glucocorticoid treatment Diabetes Cancer Sepsis Biliary cirrhosis

The E3 ligases MAFbx and MuRF1 are the more reliable atrogenes in rodents

Adapted from Polge et al. Front. Physiol., 2015

Implication of the UPS during muscle wasting

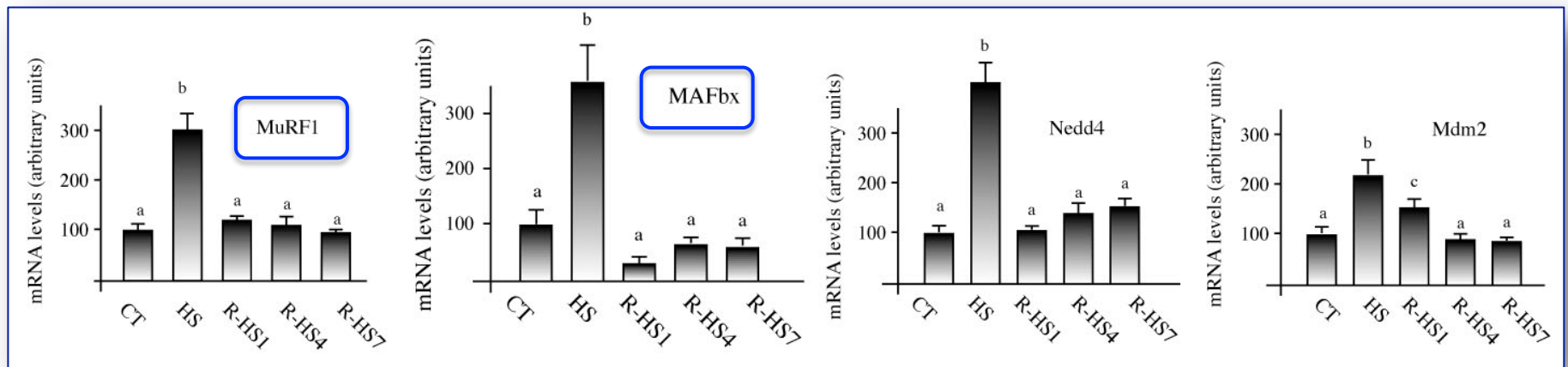
The “Atrophy-related genes “atrogenes” (Unweighting – rat soleus muscle)

Some Atrogenes are still recruited after 7 days of recovery

- Proteasome subunits
- Ub
- Specific ubiquitinating enzymes

Taillandier et al., *Int. J. Biochem. Cell Biol.* 2003 ; Polge & Taillandier, *Int. J. Biochem. Cell Biol.*, 2016

Some Atrogenes are strictly restricted to the atrophying program



Polge & Taillandier, *Int. J. Biochem. Cell Biol.*, 2016

Targets of the UPS during muscle wasting

UPS targets



Cell Cycle / Signal transduction / DNA repair / DNA replication / Apoptosis / Chromatin organization / Translation / Protein folding / Histones / Kinases / Energy metabolism / Transcription factors / UPS

Muscle UPS targets



Energy metabolism: MCK,

Transcription factors: SRF, myoD,

Translation factors: eIF3f,

Signal transduction: IRS1, NF- κ B, IKK, TRAF3,



Myofibrillar proteins: α -actin, myosin heavy and light chains, troponin I, telethonin

Heng et al. Int J Biochem Cell Biol, 2008 ; Polge et al. FASEB J, 2011

The UPS controls all the key processes for the development of the atrophying program

Implication of the UPS on muscle wasting in humans

The “Atrophy-related genes “atrogenes” MuRF1 and/or MAFbx

Catabolic situations	Atrogenes	Conditions
Non pathological states		Bed rest Immobilization / unweighting Aging
Pathological and injury states		COPD Spinal cord transection Spinal muscle atrophy Smoking Myositis Statins Cancer Pulmonary arterial hypertension Knee arthroplasty

Ubiquitination in various human diseases

E3 ligase	Targeted protein or pathway	Ubiquitin chain type	Disease
FANCL	FANCD2, FANC1	monoUb	Fanconi anemia
DDB2	Chromatin	monoUb	Xeroderma pigmentosum
Cbl family	RTKs	monoUb	Cancer
Nedd4	PTEN, α -synuclein	monoUb, Lys63, polyUb	Cowden syndrome, Parkinson
Rabex-5	Ras	monoUb	Cancer
HDM2	p53	Lys48	Cancer
APC/C10	Cyclin–CDK	Lys48	Genomic instability
SOCS1/3	IRS2	Lys48	Metabolic syndrome
MG53	IR, IRS1	Lys48	Metabolic syndrome
pVHL15	HIF	Lys48	Von Hippel Lindau
IAPs	NIK	Lys48	Multiple myeloma
Rnf168	Histones	Lys63, polyUb	Cancer, RIDDLE syndrome
TRAF6	TRAF6, NEMO, huntingtin	Lys63, polyUb	Inflammatory disease, Huntington's disease
Itch, IAPs	RIP2	Lys63, polyUb	Crohn's disease
Parkin	Mitochondrial outer membrane proteins	Lys63, polyUb	Parkinson's disease
CHIP, Parkin	Huntingtin, β -amyloid, tau	Lys63, polyUb	Huntington's disease, Alzheimer's disease
BRCA1	BRCA1	Atypical polyUb	Breast and ovarian cancer
LUBAC	NEMO	Linear polyUb	Autoinflammation, muscular amylopectinosis, bacterial infections

Implication of the UPS on muscle wasting following chronic kidney disease in humans

CHU Clermont-Fd – Nephrology Dept – Pr. A-E Heng

Several Pathologies → Kidney lesions and insufficiency

- In France :
 - Chronic Kidney Disease: **3 Millions**
 - Terminal Renal Deficiency: **30,000**
- Muscle atrophy within dialysis: **20%** incidence

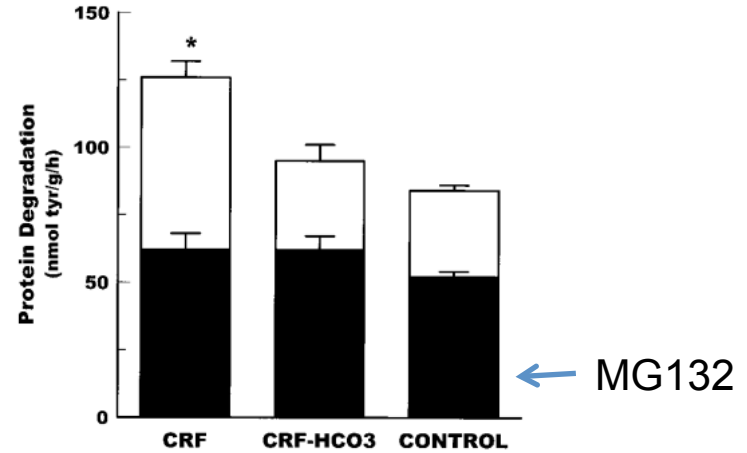
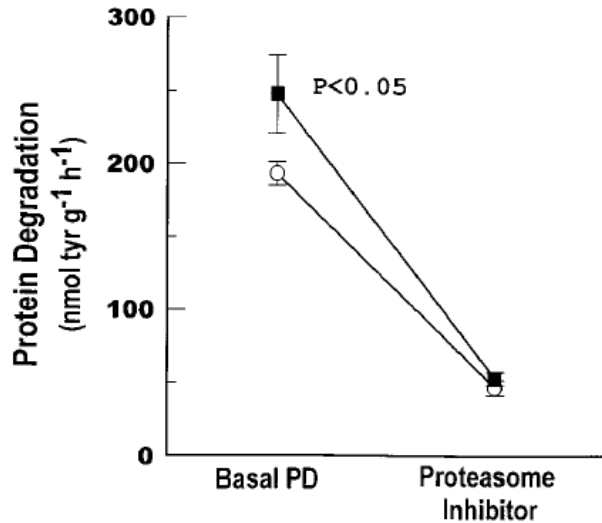
Progressive pathology



Proteolysis induction?
UPS involvement?
Long lasting induction of atrogenes?

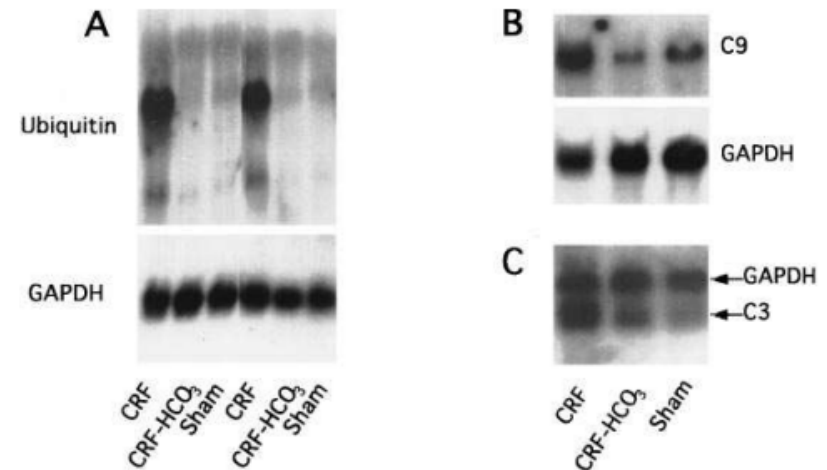
Implication of the UPS on muscle wasting upon chronic kidney disease in rats

CKD in rats



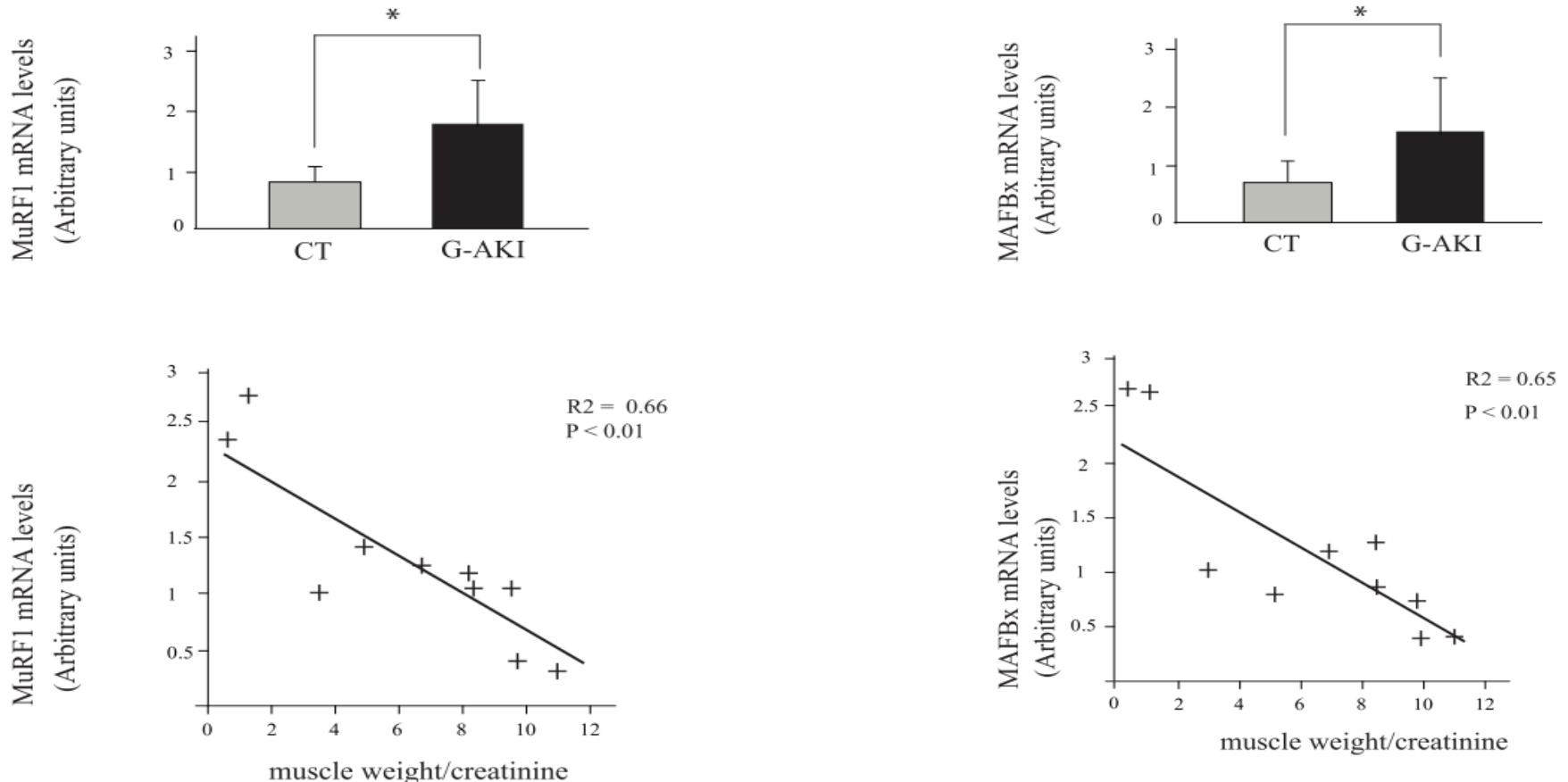
↗ Proteolysis = ↗ UPS

Acidosis is crucial for UPS-dependent protein breakdown



Implication of the UPS on muscle wasting upon acute kidney disease in rats

Acute kidney injury (gentamicin) induces a moderate atrophy (-17%) in phasic muscles



MuRF1 and MAFbx are overexpressed during Acute Kidney Injury in rats and their expression levels are correlated to the severity of kidney injury

Implication of the UPS on muscle wasting following chronic kidney disease in humans

Proteolysis induction?

 UPS involvement?

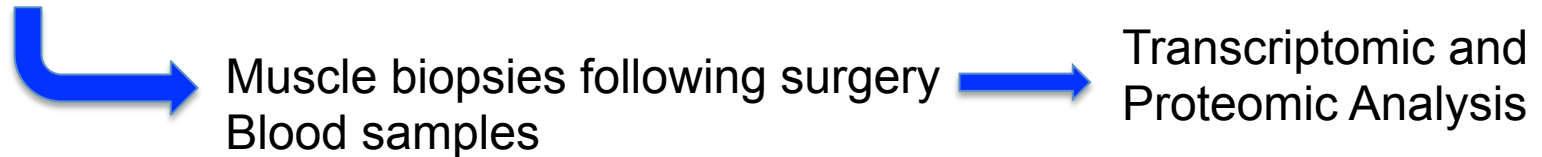
Long lasting induction of atrogenes?

 Common biomarkers with more aggressive conditions/pathologies?

Human vs. rodent biomarkers ?

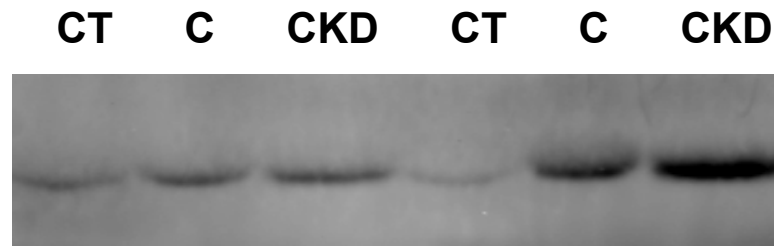
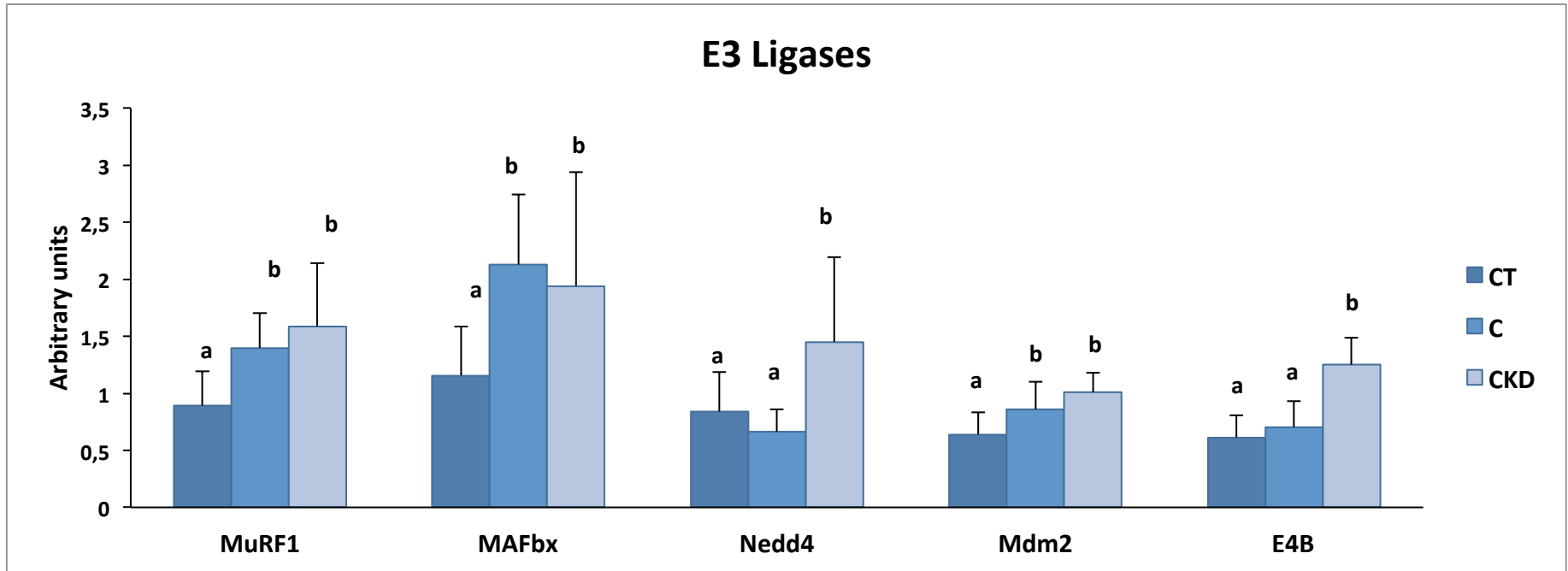
Implication of the UPS on muscle wasting following chronic kidney disease in humans: the PROMETHE cohort

- Control patients (CT)
- Hemodialysis patients (CKD)
- Cancer patients (pulmonary neoplasia, C)



	CT (n=13)	Cancer (n=14)	Hemodialysis (n=7)
Men / Women	7 / 6	9 / 5	6 / 1
Age (Years)	69 ± 11	63 ± 10	68 ± 8
Weight (kg)	77 ± 14	67 ± 13	70 ± 9
Size (cm)	168 ± 8	170 ± 7	162 ± 9
Hemoglobin (g/dL)	12 ± 8	13 ± 2	13 ± 2
Creatinine levels (µmol/L)	66 ± 14	72 ± 16	658 ± 254*
glomerular filtration rate (MDRD) (ml/min)	80 ± 18	82 ± 12	-
CRP (mg/L)	12 ± 6	61 ± 72	18 ± 14
Alkaline reserve (mmol/L)	28 ± 1	29 ± 4	27 ± 6
VIH serology	-	-	-
Hepatitis B and C serologies	-	-	-

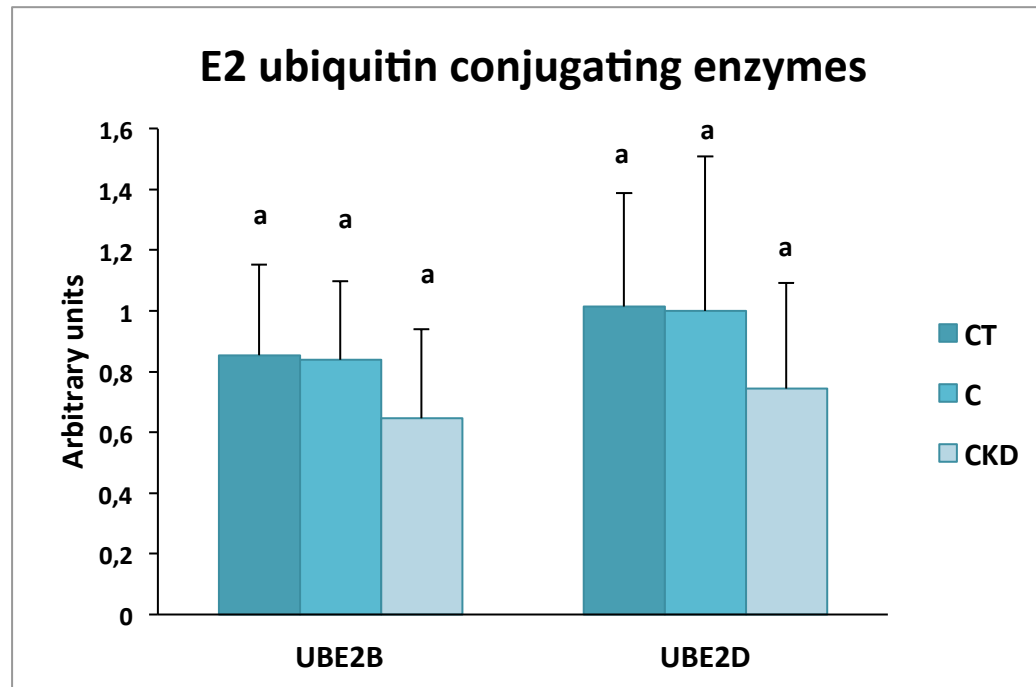
Implication of the UPS on muscle wasting in CKD and cancer patients



Immunoblot: MuRF1

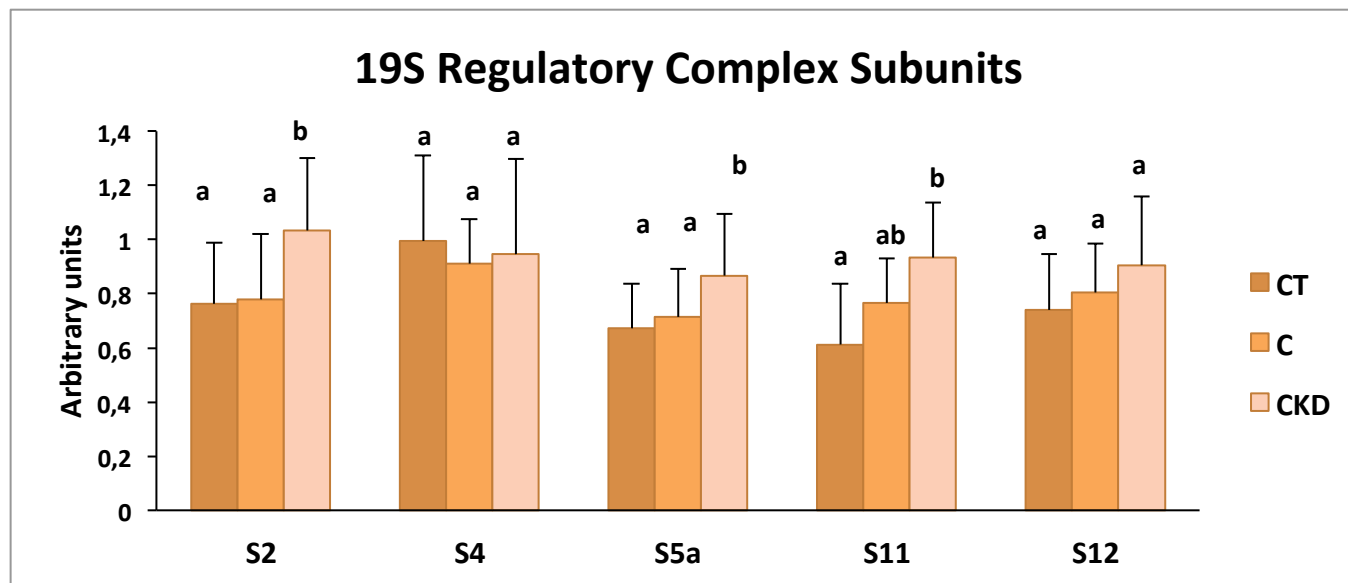
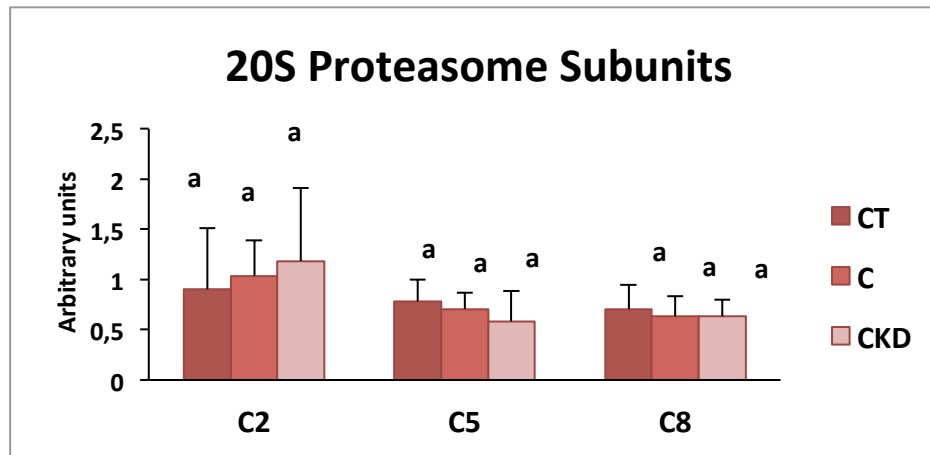
MuRF1, MAFbx and Mdm2 exhibit sustained overexpression during catabolic situations whatever the pathology

Implication of the UPS on muscle wasting in CKD and cancer patients



UBE2B, an E2 known to be part of the atrophying program, is not overexpressed in cancer and CKD patients

Implication of the UPS on muscle wasting in CKD and cancer patients



The Proteasome (20S core and 19S RC) is not a good predictor of muscle atrophy for long term UPS induction

Implication of proteolytic systems on muscle wasting in CKD and cancer patients

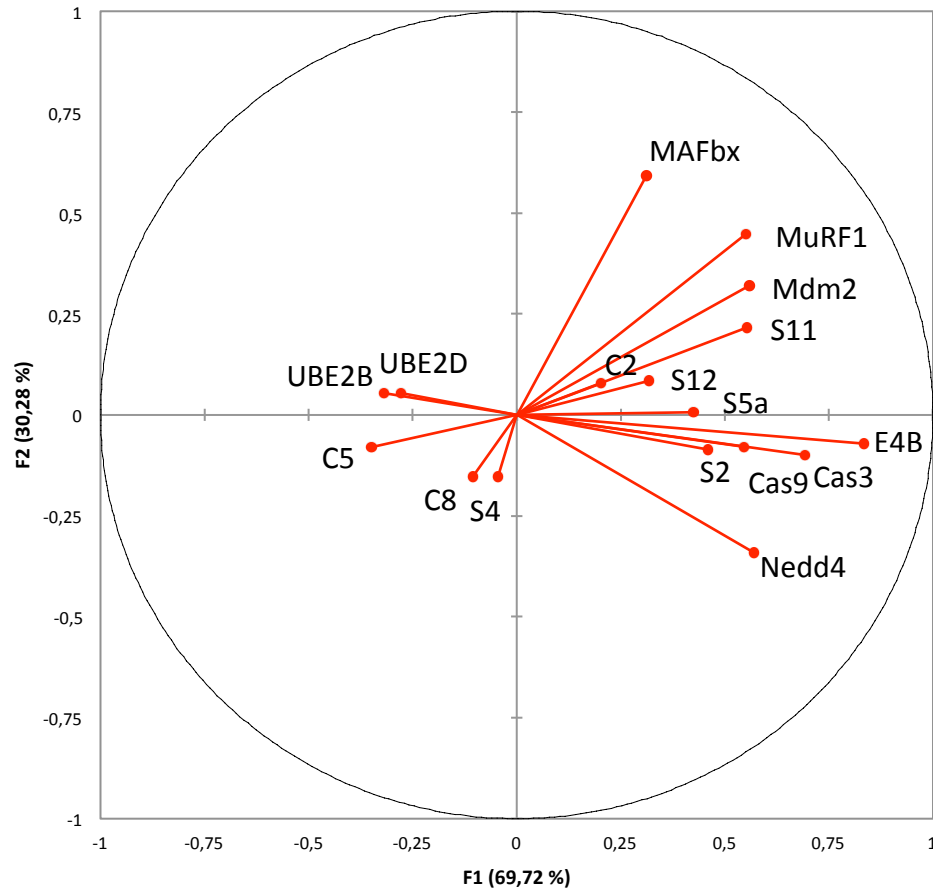
	Cancer (n=14)	CKD (n=7)
MAFbx	↗	↗
MuRF1	↗	↗
Mdm2	↗	↗
Nedd4	→	↗
E4B	→	↗
UBE2B	→	→
UBE2D2	→	→

	Cancer (n=14)	CKD (n=7)
C2, C5, C8	→	→
S2	→	↗
S4	→	→
S5a	→	↗
S11	→	↗
S12	→	→
Casp3	→	↗
Casp9	→	↗

So far, only a limited number E3 ligases are good biomarkers of muscle atrophy

Transcription analysis of muscles from CKD and cancer patients

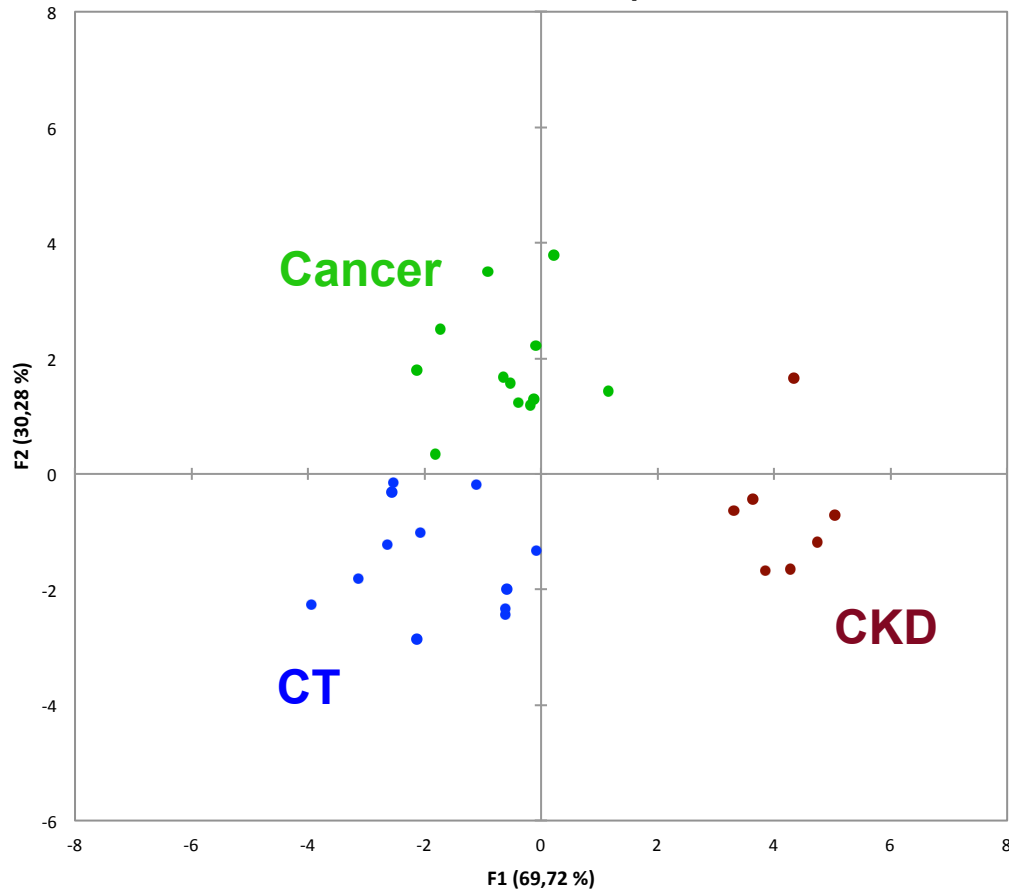
Catabolic/control expression ratio of proteolytic enzymes



Discriminant analysis of mRNA levels confirm that some E3 ligases (MuRF1, MAFbx and Mdm2) are the best predictors for muscle atrophy in CKD and cancer patients

Transcription analysis of muscles from CKD and cancer patients

Expression of proteolytic enzymes combined and used as two variables for each patient

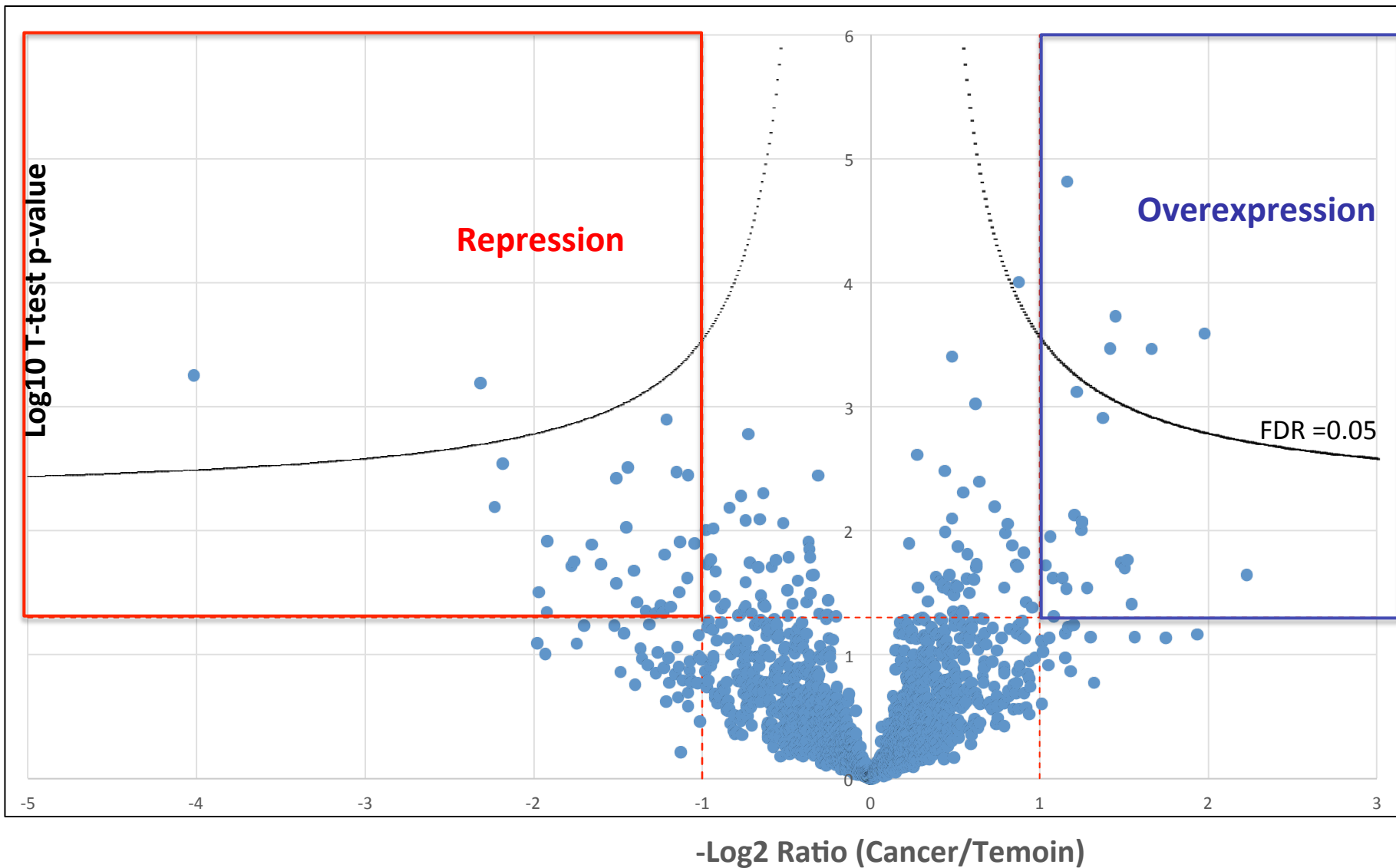


Discriminant analysis of mRNA levels from 17 actors of proteolytic systems (UPS and caspases) efficiently separates the 3 populations

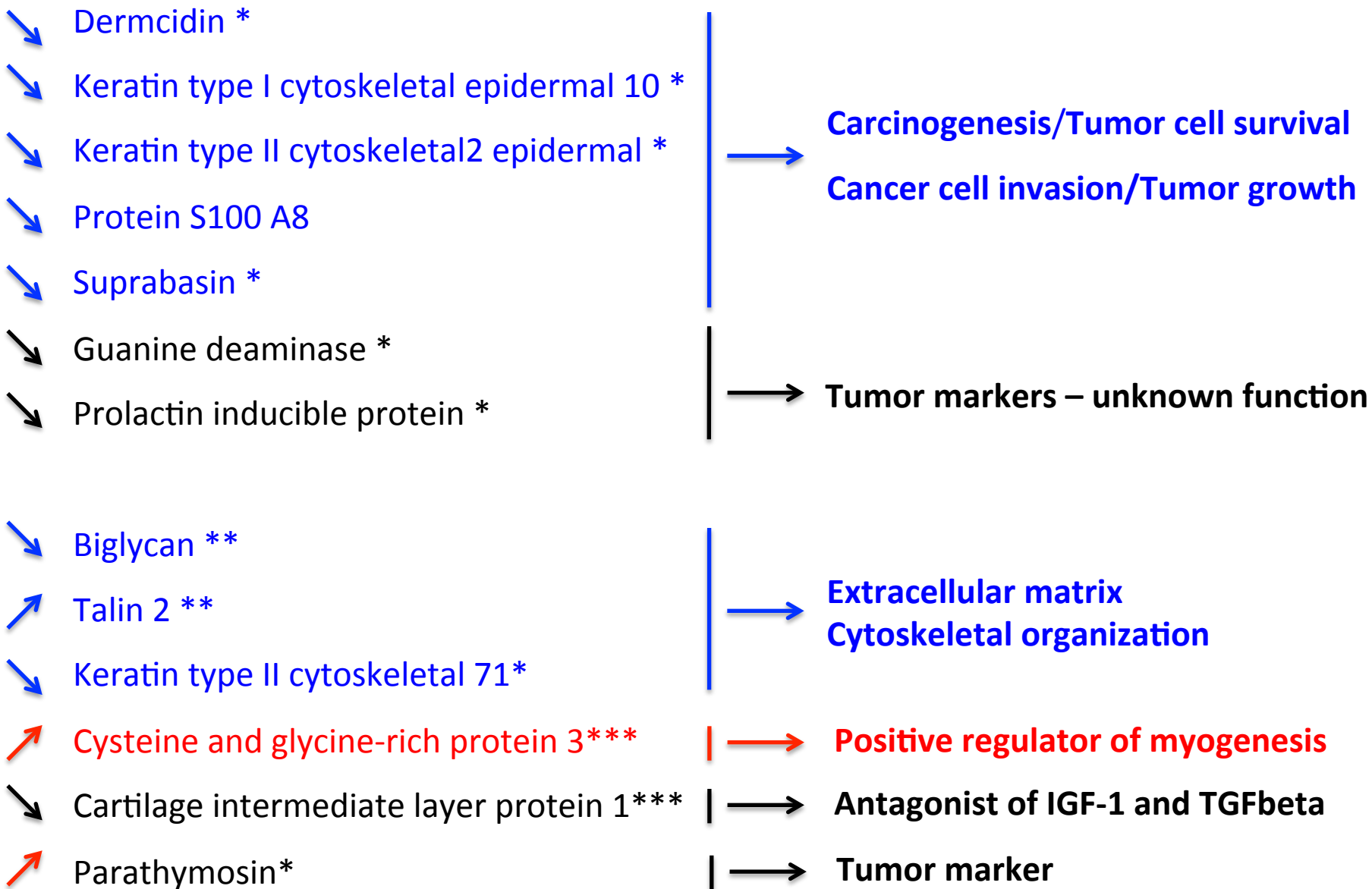
Proteomic analysis of muscles from CKD and cancer patients

- 21 patients (n = 7 per group)
- **Cytoplasmic**, myofibrillar and UPS substrates
- Shotgun analysis using nanoLC-MS/MS (Ultimate3000 system coupled to an LTQ-Orbitrap Velos mass spectrometer)
- **MaxQuant** (Max Planck Institute) and **Proline** (EDyP-Grenoble, IPBS-Toulouse, IPHC/LSMBO-Strasbourg) analysis

Mass spectrometry analysis - Volcano Plot - Cancer / CT Proline



Proteins differentially expressed in both CKD and cancer patients compared to controls



Conclusions

Common markers can be found between different pathologies in humans independently of the stage of the illness, sex, BMI and throughout a large scale of age (46-76)

- mRNA levels:

Increased expression of specific actors of proteolytic pathways

MuRF1 and MAFbx are the best (but not the only) biomarkers as in animal models

A combination of proteolysis-linked proteins discriminates healthy and pathology-developing patients but also between pathologies

- Protein levels:

Down regulation of several proteins involved in cell growth/proliferation and organization

Some actors of muscle anabolism are stimulated (but inefficient?)

Perspectives

mRNAs:

- Including other proteolytic actors: -> better isolation of specific populations?
- Using more patients/other cohorts for strengthening the conclusions
- Application to other pathologies
- Finding biomarkers in more accessible compartments (e.g. blood)

Proteins:

- Completing the proteomic analysis of soluble proteins
- Identification of UPS substrates common to the two pathologies
 - > Purification of Ub-conjugates using an in-house affinity matrix followed by mass spectrometry analysis

Acknowledgements

Lab members

Didier Attaix
Daniel Béchet
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Collaborators

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Alexandre Stella (Toulouse)



Stéphanie Cabantous (Toulouse)



Simon S. Wing (Montreal, Canada)



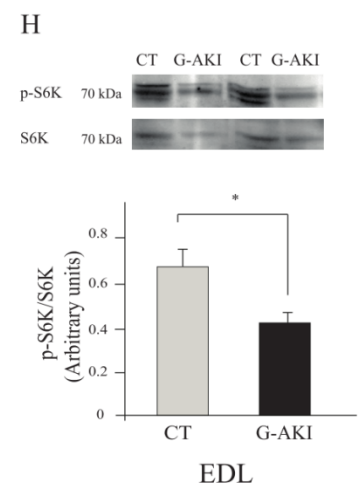
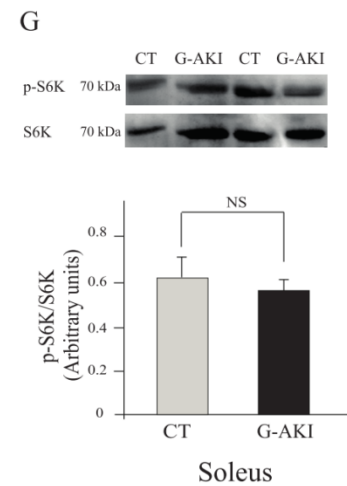
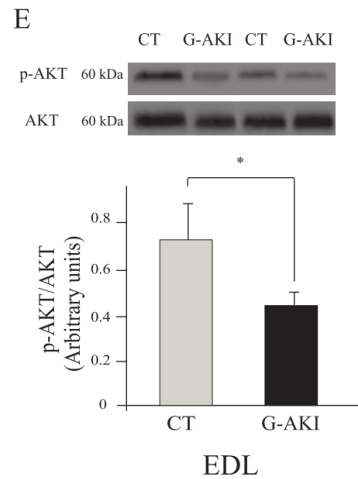
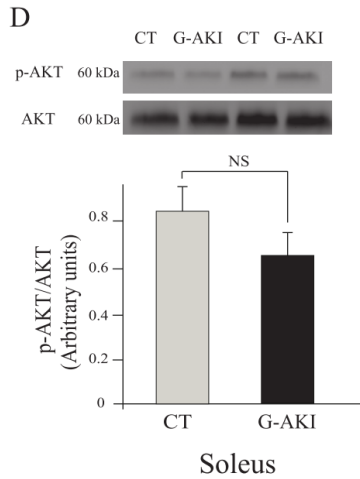
Financial support



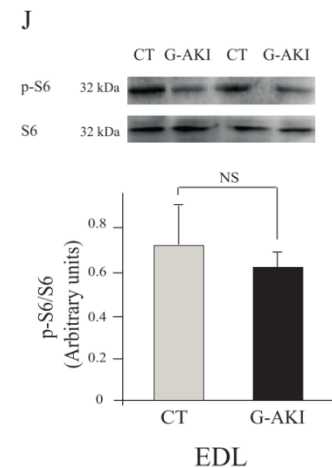
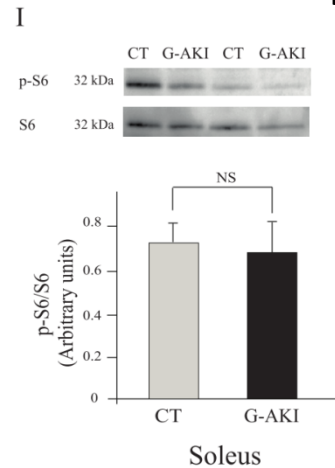
mTOR-Akt axis respond differentially in the EDL and soleus

p-AKT/AKT

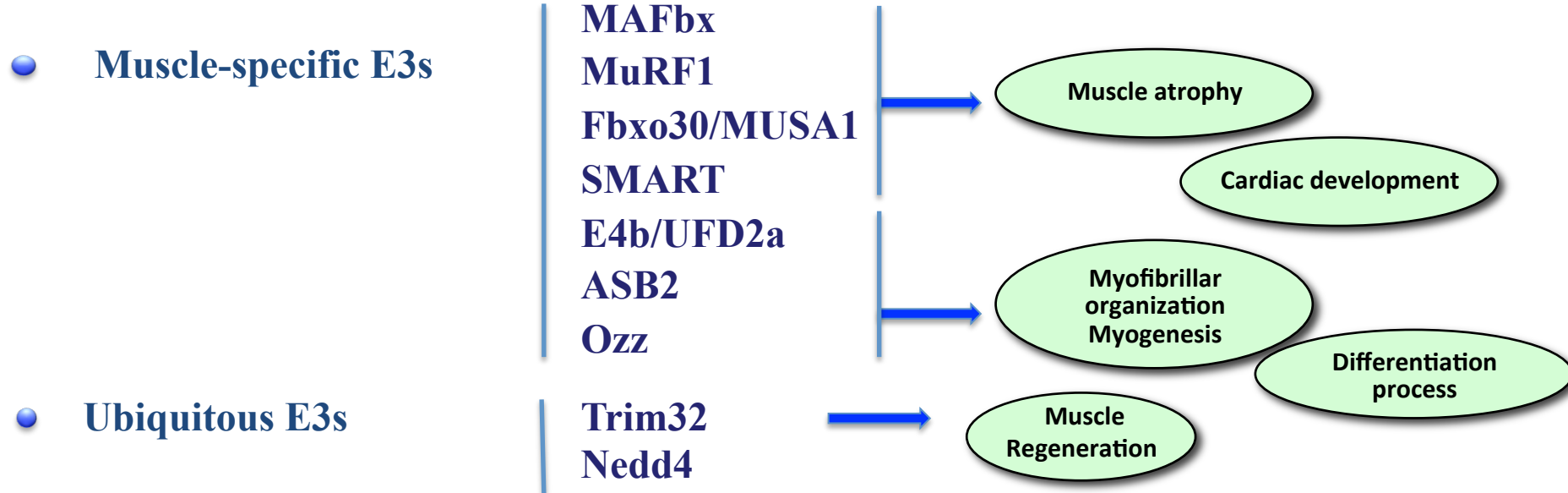
p-S6K/S6K



p-S6/S6



E3 ligases impact muscle protein homeostasis



The FASEB Journal article fj.11-180968. Published online August 2, 2011.

The FASEB Journal • Research Communication

Muscle actin is polyubiquitinated *in vitro* and *in vivo* and targeted for breakdown by the E3 ligase MuRF1

Cécile Polge,^{*,†,1} Anne-Elisabeth Heng,^{*,†,‡,1} Marianne Jarzaguet,^{*,†} Sophie Ventadour,^{†,*} Agnès Claustre,^{†,*} Lydie Combaret,^{†,*} Daniel Béchet,^{†,*} Mariette Matondo,^{S,||} Sandrine Uttenweiler-Joseph,^{S,||} Bernard Monsarrat,^{S,||} Didier Attaix,^{*,†} and Daniel Taillandier^{*,†,2}