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Daniel Taillandier

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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)

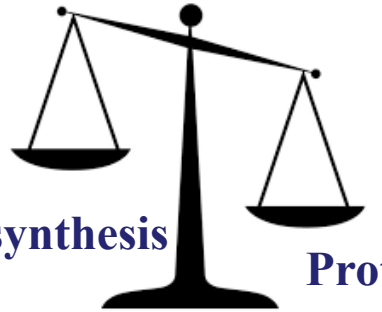
Chronic Renal Failure

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

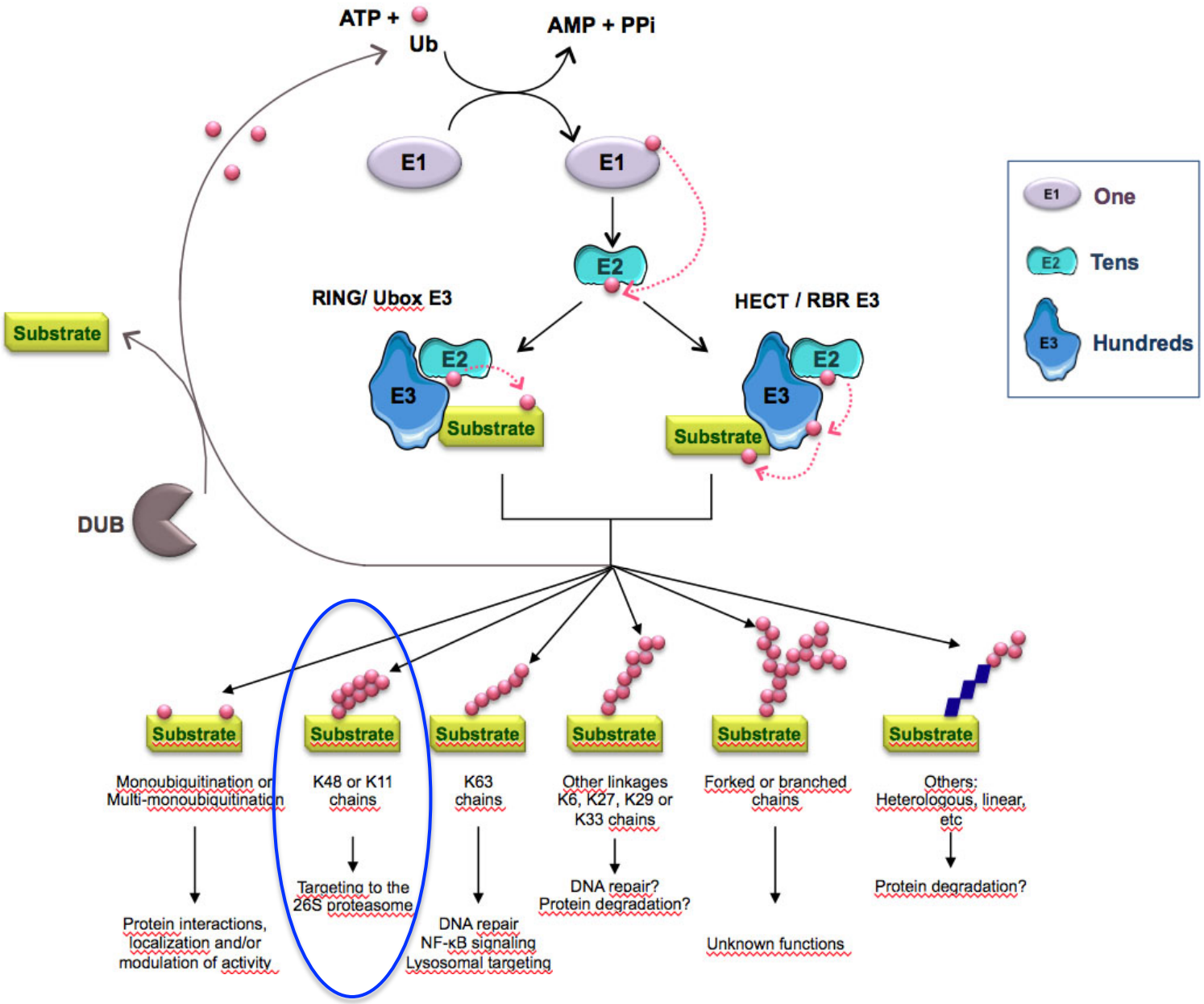
Proteolysis is the main determinant

Proteolytic Systems involved

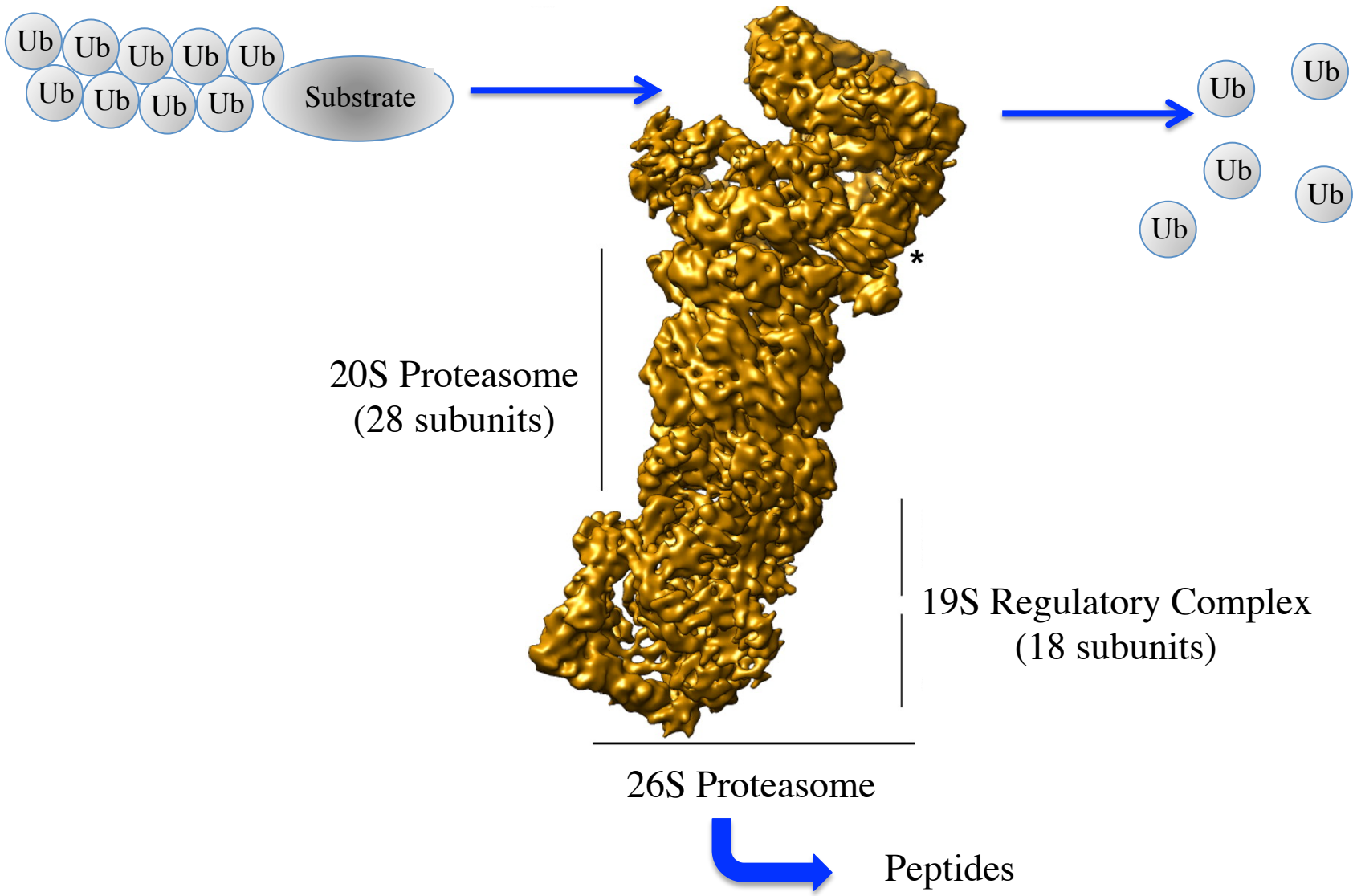
- ✓ Calpains (cytosolic, $Ca^{2+}$-dependent)
- ✓ Cathepsins (lysosomes, endosomes)
- ✓ Caspases (cytosolic)
- ✓ Proteasome (cytosolic, self-compartmentalized)

  UPS: Ubiquitin Proteasome System

# Ubiquitin Proteasome System (UPS)



# Ubiquitin Proteasome System (UPS)




*Bohn et al. PNAS 2010*

# 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.

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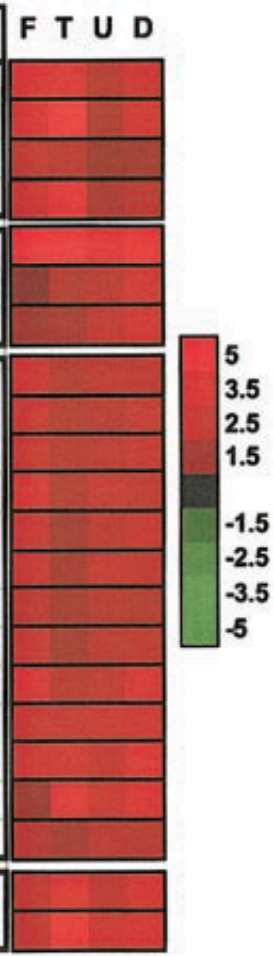
### IGF-I stimulates muscle growth by suppressing protein breakdown and expression of atrophy-related ubiquitin ligases, atrogin-1 and MuRF1

**Jennifer M. Sacheck,[1,*] Akira Ohtsuka,[2,*] S. Christine McLary,[1] and Alfred L. Goldberg[1]**
[1]*Department of Cell Biology, Harvard Medical School, Boston, Massachusetts 02115; and* [2]*Department of Biochemical Science and Technology, Faculty of Agriculture, Kagoshima University, Kagoshima 890-0065, Japan*

# 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 | |
|---|---|---|---|---|
| Ubiquitin | 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 |
| Ubiquitinating enzymes | 1723142 | Hs.61661 | FBXO32 | Atrogin-1/MAFbx |
| | 751477 | Mm.32920 | Ncube1 | non-canonical Ub-conjugating enzyme 1 |
| | 747318 | Mm.21634 | Ube4b | ubiquitination factor E4B |
| 26 Proteasome | 2195309 | Hs.82159 | PSMA1 | proteasome 20S subunit, alpha 1 |
| | 723267 | Mm.30097 | Psma1 | |
| | 466041 | Mm.30097 | | |
| | 572285 | Mm.2287 | Psma5 | proteasome 20S subunit, alpha 5 |
| | 1737833 | Hs.82793 | PSMB3 | proteasome 20S subunit, beta 3 |
| | 571569 | Mm.21874 | Psmb3 | |
| | 901317 | Hs.89545 | PSMB4 | proteasome 20S subunit, beta 4 |
| | 466254 | Mm.29582 | Psmc4 | proteasome 19S subunit, ATPase, 4 |
| | 2123183 | Hs.78466 | PSMD8 | proteasome 19S subunit, non-ATPase, 8 |
| | 113452 | Hs.90744 | PSMD11 | proteasome 19S subunit, non-ATPase, 11 |
| | 833508 | Mm.28571 | Psmd11 | |
| | 448976 | Hs.112396 | PA200 | KIAA0077 protein |
| | 1707220 | Hs.75981 | USP14 | Ub-specific protease 14 |
| Lysosomes | 315082 | Mm.930 | Ctsl | cathepsin L |
| | 2935790 | Hs.87417 | CTSL2 | |




*Lecker et al. FASEB J, 2004*

# Implication of the UPS during muscle wasting

| | Atrogenes | Conditions |
|---|---|---|
| **Anabolic situations** | ↘ | Insulin, IGF-1 |
| **Catabolic situations** | ↗ | Fasting<br>Vitamin D deficiency<br>Immobilization / unweighting<br>Aging |
| Non pathological states | | |
| Pathological and injury states | ↗ | Burn injury<br>Head trauma<br>Hyperthermia<br>Mechanical ventilation<br>Glucocorticoid treatment<br>Diabetes<br>Cancer<br>Sepsis<br>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

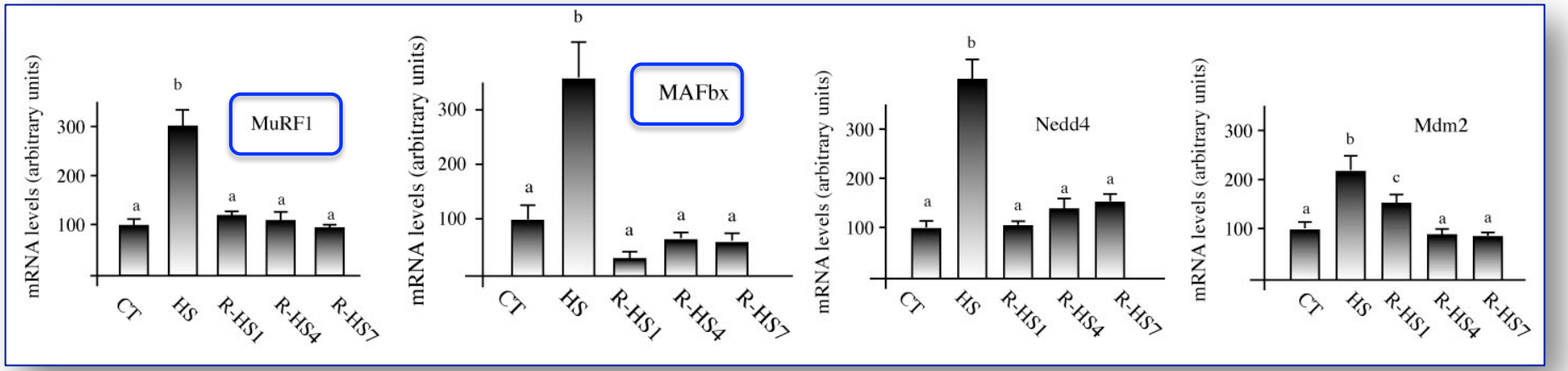
**The "Atrophy-related genes "atrogenes"**
**(Unweighting – rat soleus muscle)**

**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-kB, 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<br>Immobilization / unweighting<br>Aging |
| Pathological and injury states | ↗ | COPD<br>Spinal cord transection<br>Spinal muscle atrophy<br>Smoking<br>Myositis<br>Statins<br>Cancer<br>Pulmonary arterial hypertension<br>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

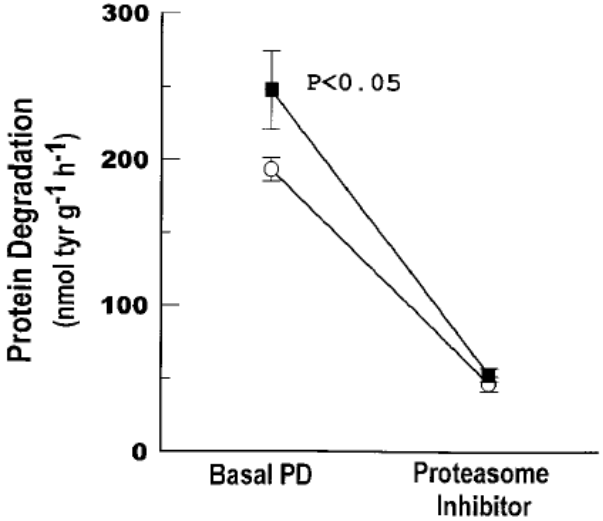
- In France :
  - Chronic Kidney Disease: **3 Millions**
  - Terminal Renal Deficiency: **30,000**
- Muscle atrophy within dialysis: **20%** incidence

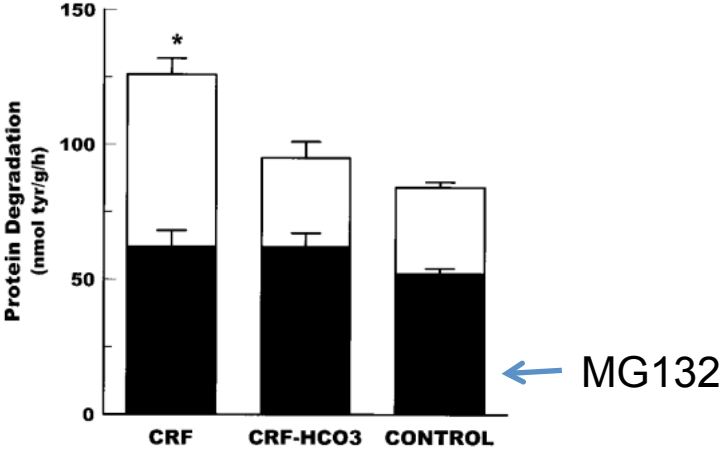
Progressive pathology

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

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

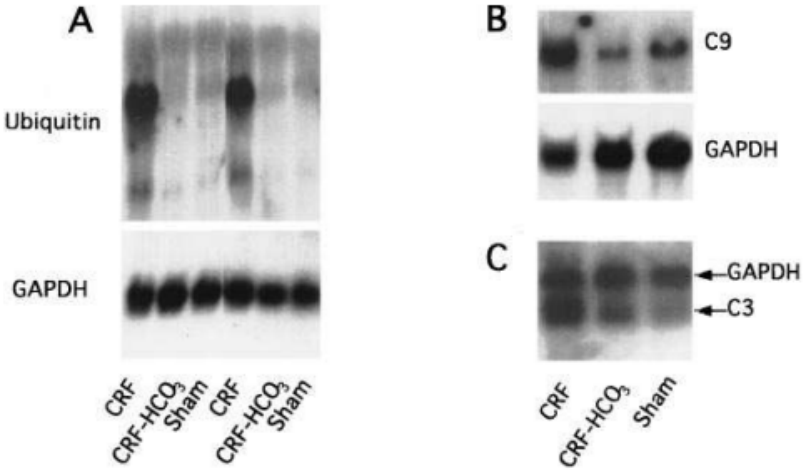
CKD in rats




↗ Proteolysis = ↗ UPS

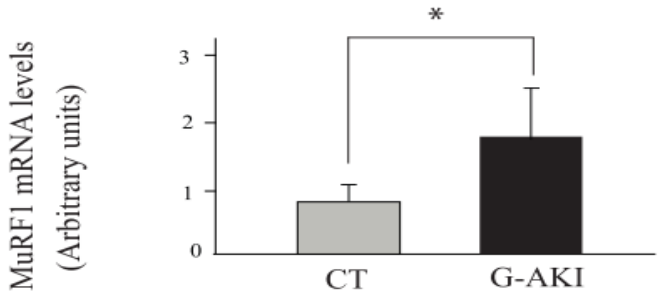
Acidosis is crucial for UPS-dependent protein breakdown

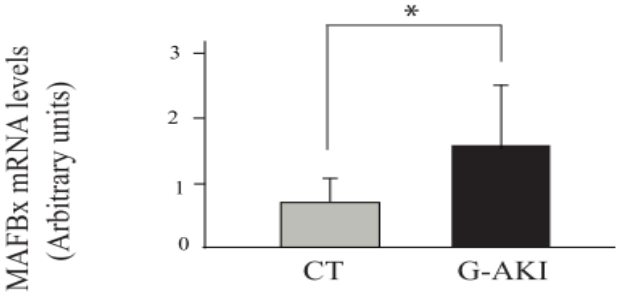


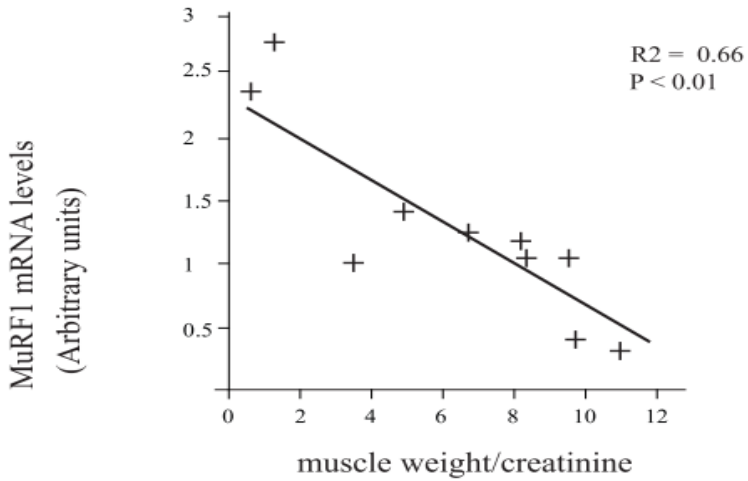

*Bailey et al, J Clin Invest 1996*

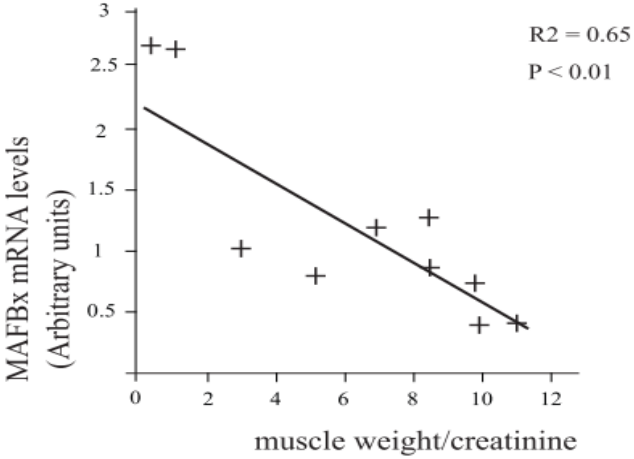
# Implication of the UPS on muscle wasting upon acute kidney disease in rats

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

*Aniort et al, Int. J. Biochem. Cell Biol. 2016*

# 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

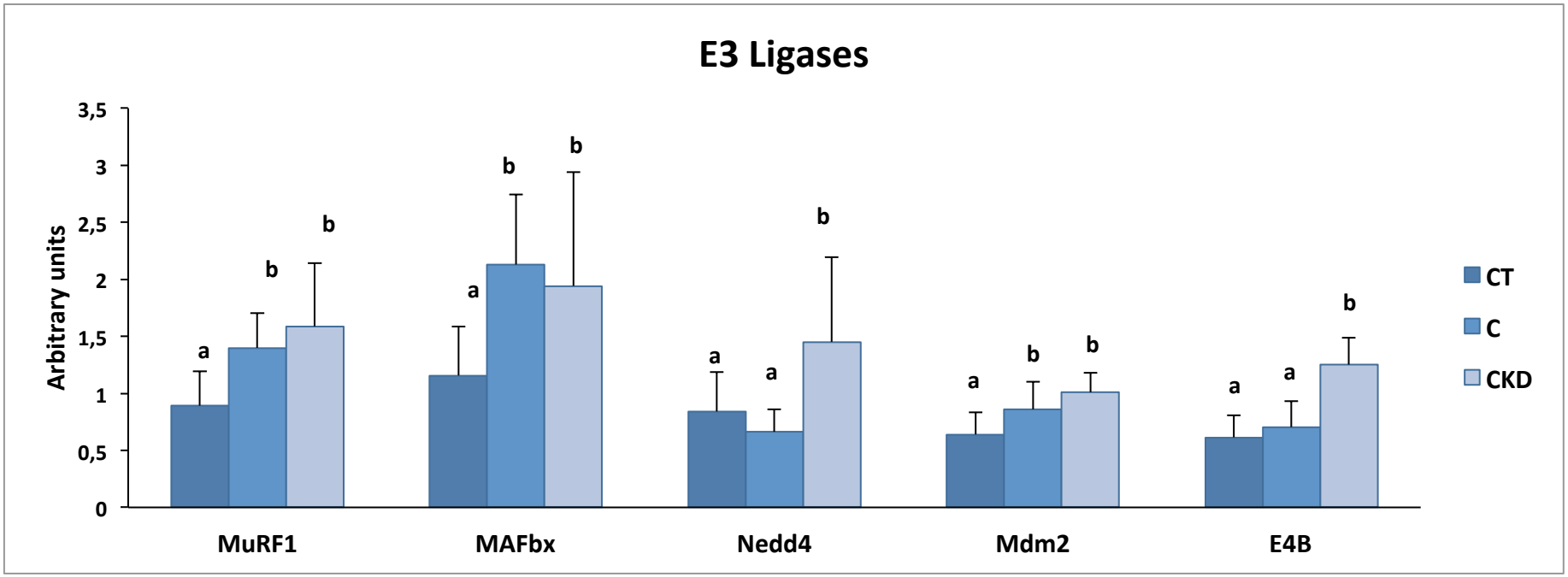
- Control patients (CT)
- Hemodialysis patients (CKD)
- Cancer patients (pulmonary neoplasia, C)

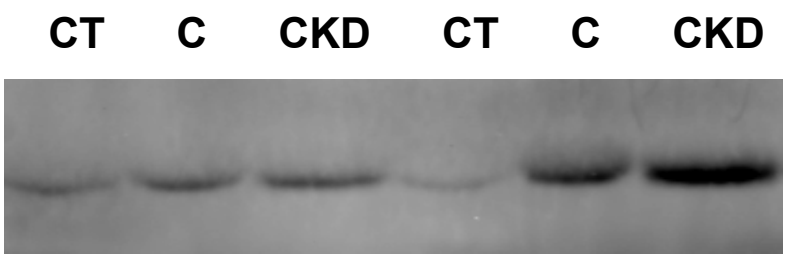
Muscle biopsies following surgery
Blood samples

Transcriptomic and Proteomic Analysis

| | 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

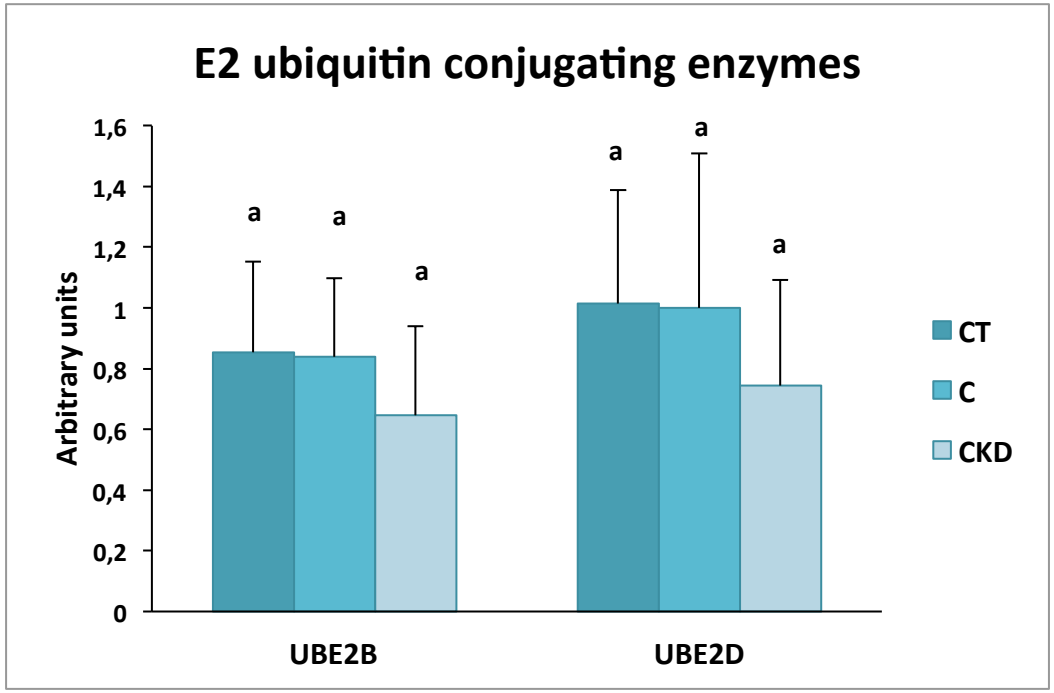



Immunoblot: MuRF1

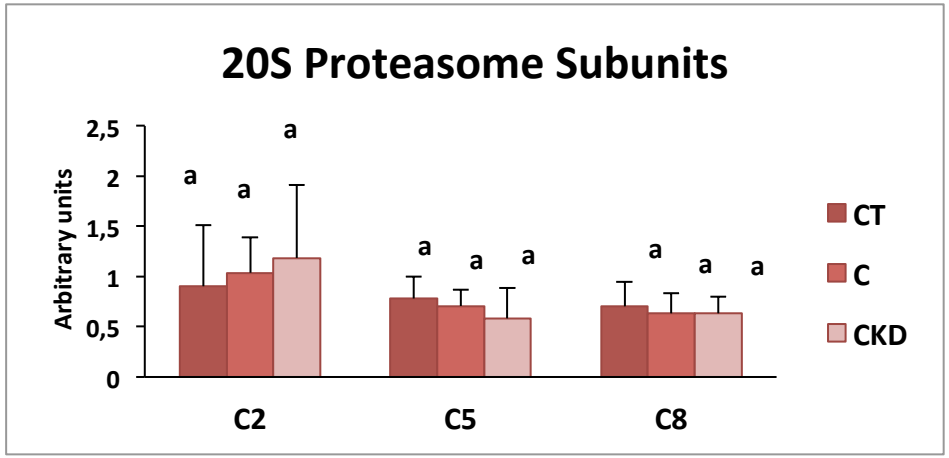
**MuRF1, MAFbx and Mdm2 exhibit sustained overexpression during catabolic situations whatever the pathology**

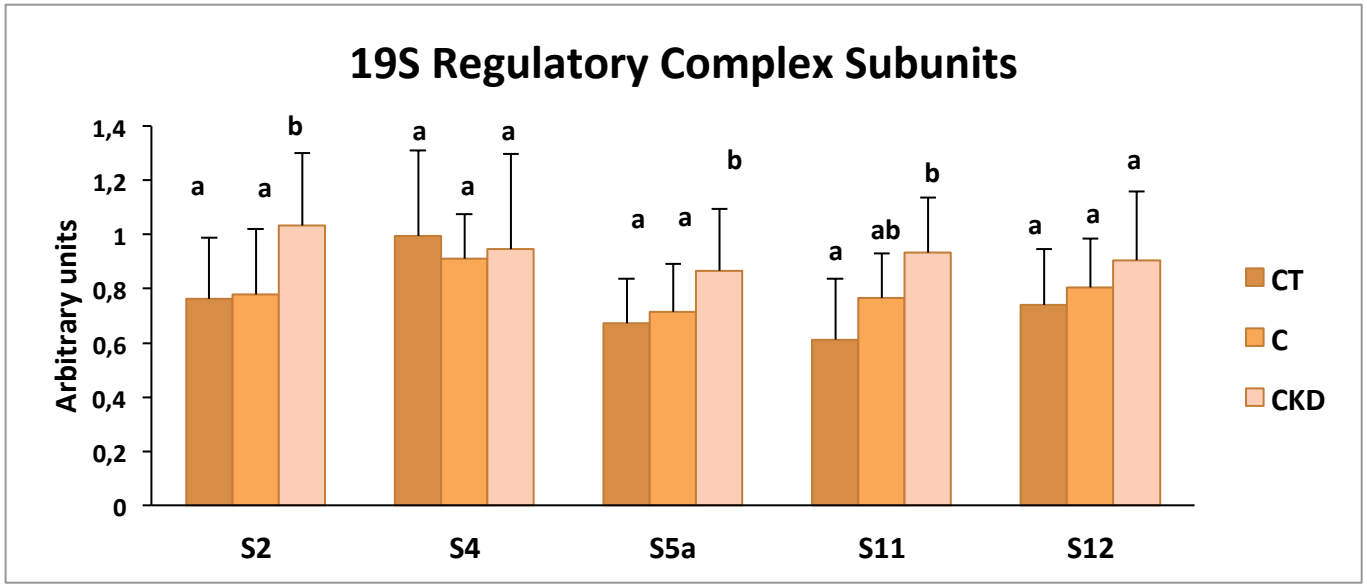
# Implication of the UPS on muscle wasting in CKD and cancer patients




**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

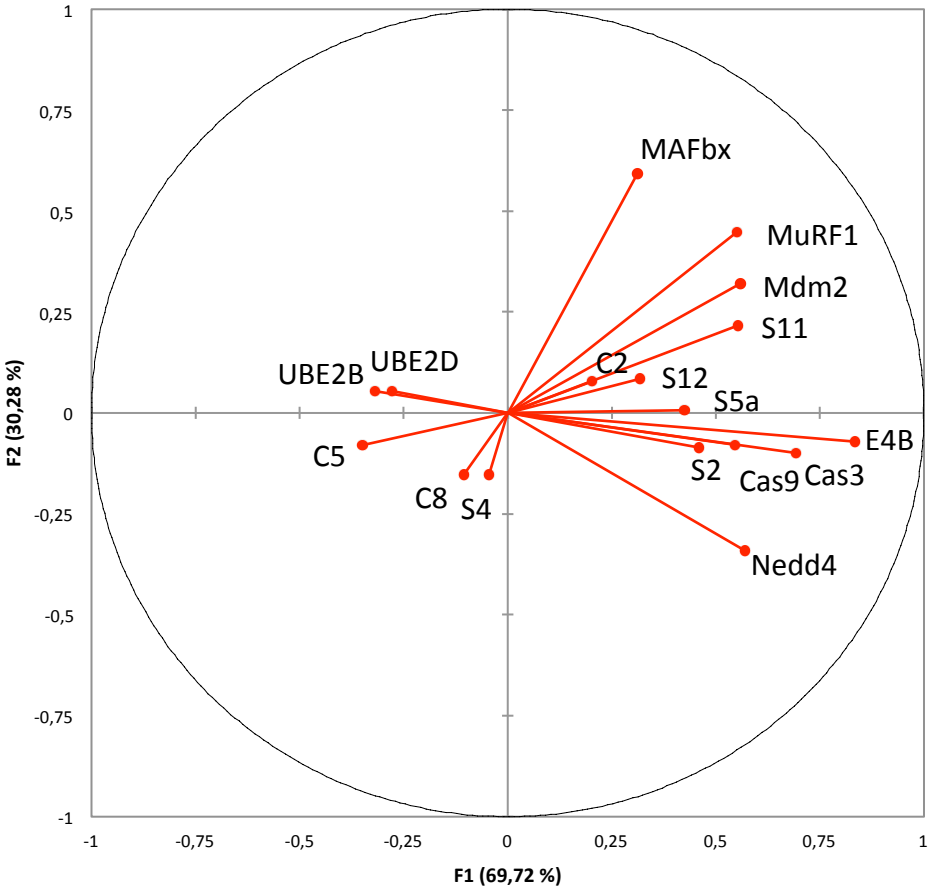
| | Cancer (n=14) | CKD (n=7) |
|---|---|---|
| MAFbx | ↗ | ↗ |
| MuRF1 | ↗ | ↗ |
| Mdm2 | ↗ | ↗ |
| Nedd4 | → | ↗ |
| E4B | → | ↗ |
| UBE2B | → | → |
| UBE2D2 | → | → |

| | 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

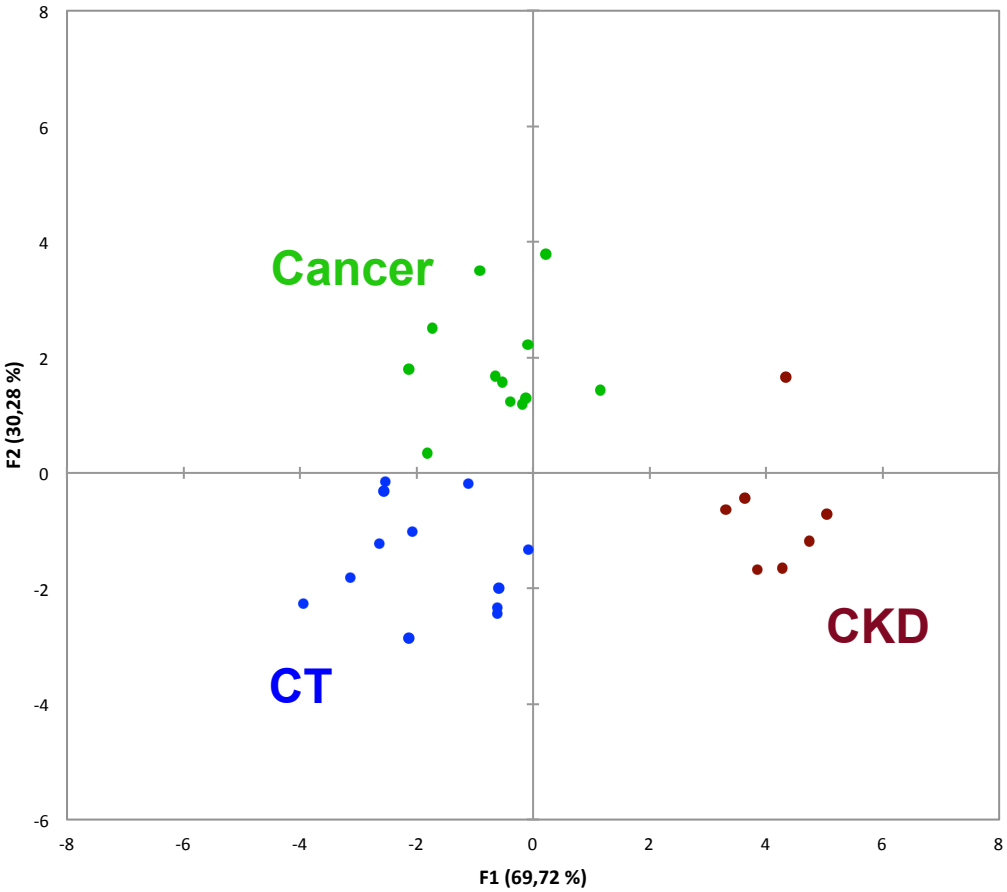
Catabolic/control expression ratio of proteolytic enzymes




**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

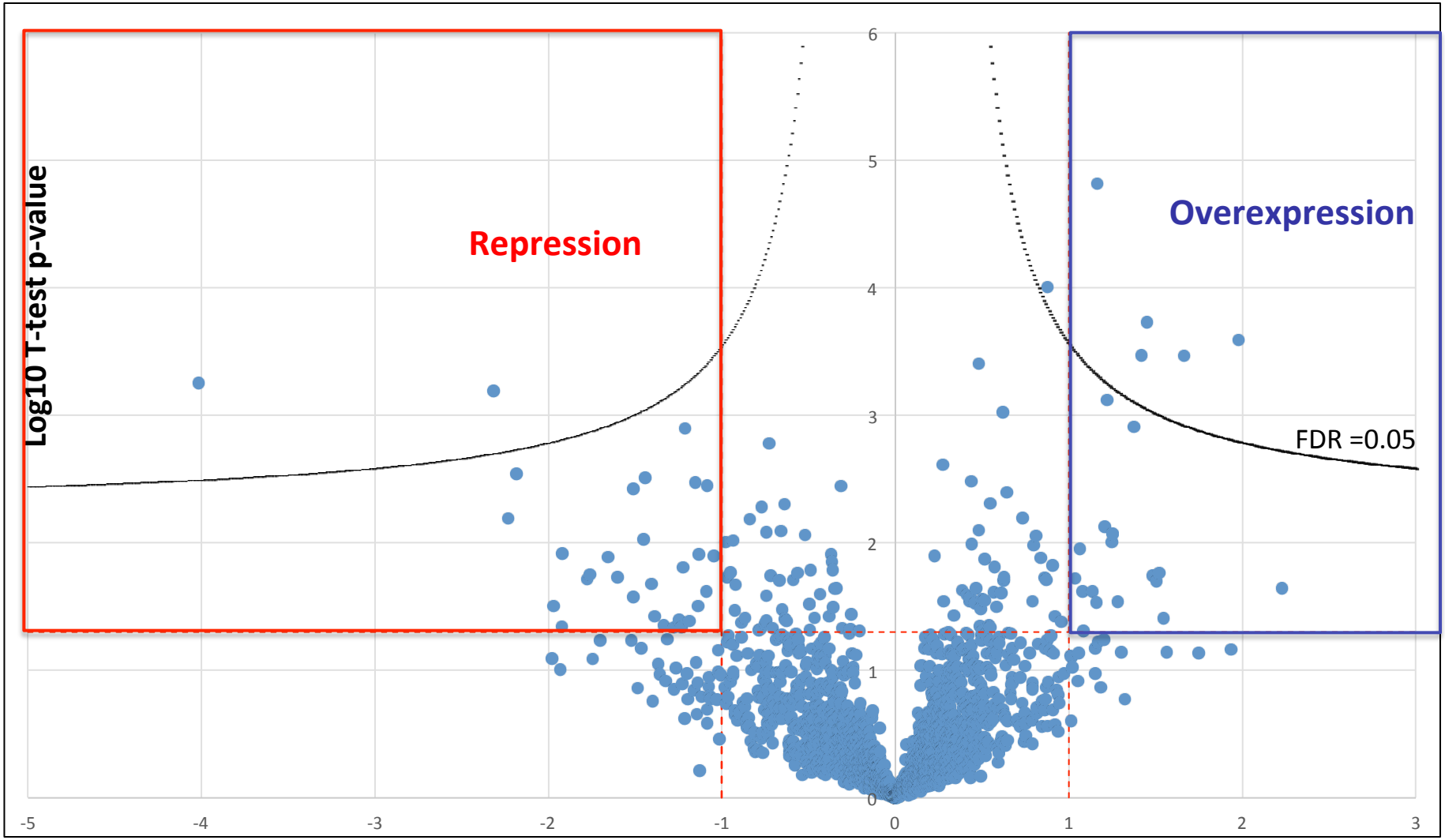



**Discriminant analysis of mRNA levels from 17 actors of proteolytic systems (UPS and caspases) efficiently separates the 3 populations**

# Proteomic analysis of muscles from CKD and cancer patients

- 21 patients (n = 7 per group)
- **Cytoplasmic**, myofibrillar and UPS substrates
- Shot gun 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

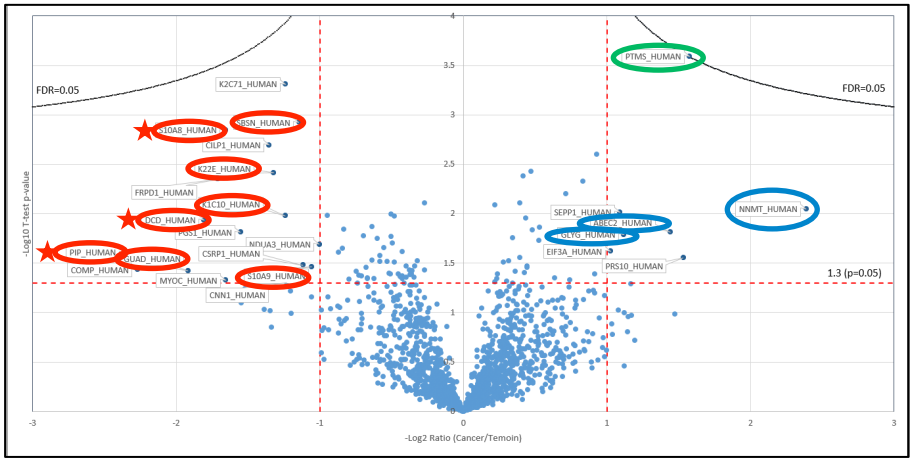
# Mass spectrometry analysis - Volcano Plot - Cancer / CT Proline

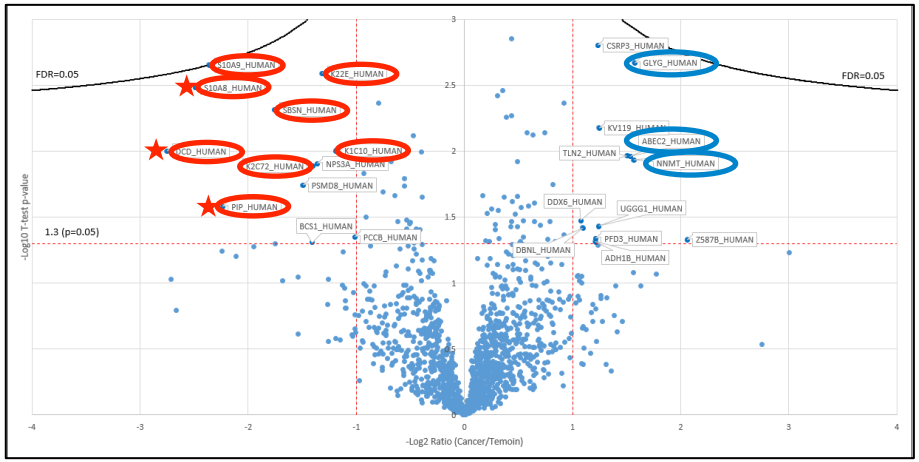


## Proline

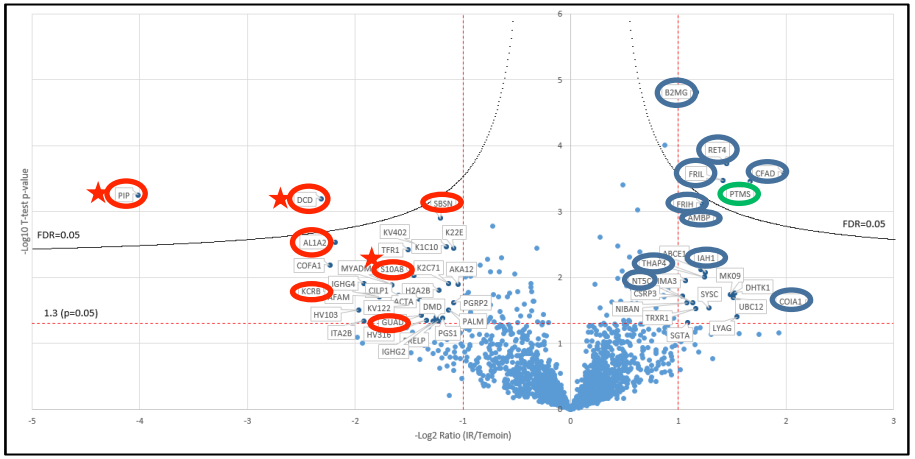
## MaxQuant

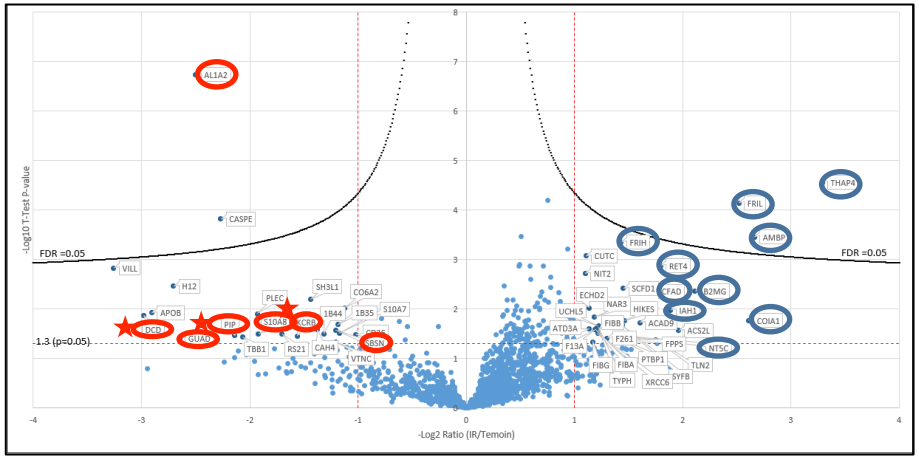
**Cancer**




**CKD**




★ Proteins differentially expressed in both cancer and CKD patients observed either with Proline and MaxQuant

**1713 proteins analyzed**
**53 differential proteins between CT and CKD patients**
**24 differential proteins between CT and C patients**
**13 proteins common to CKD and C patients**

**Threshold: 2-factor**

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

- ↘ Dermcidin *
- ↘ Keratin type I cytoskeletal epidermal 10 *
- ↘ Keratin type II cytoskeletal2 epidermal *
- ↘ Protein S100 A8
- ↘ Suprabasin *

→ **Carcinogenesis/Tumor cell survival**
**Cancer cell invasion/Tumor growth**

- ↘ Guanine deaminase *
- ↘ Prolactin inducible protein *

→ **Tumor markers – unknown function**

- ↘ Biglycan **
- ↗ Talin 2 **
- ↘ Keratin type II cytoskeletal 71*

→ **Extracellular matrix**
**Cytoskeletal organization**

- ↗ Cysteine and glycine-rich protein 3*** → **Positive regulator of myogenesis**
- ↘ Cartilage intermediate layer protein 1*** → **Antagonist of IGF-1 and TGFbeta**
- ↗ Parathymosin* → **Tumor marker**

# 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 discrimates 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

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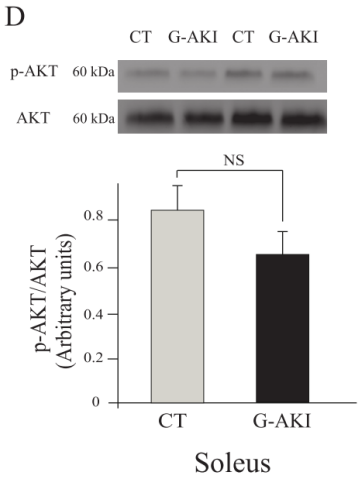
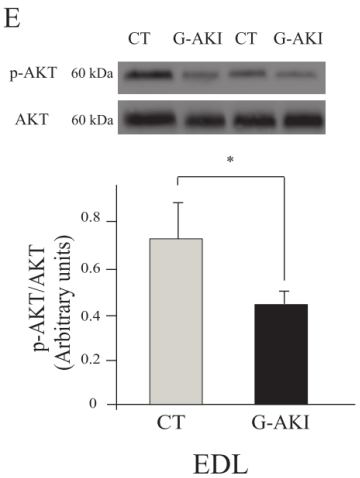
# mTOR-Akt axis respond differentially in the EDL and soleus

## p-AKT/AKT

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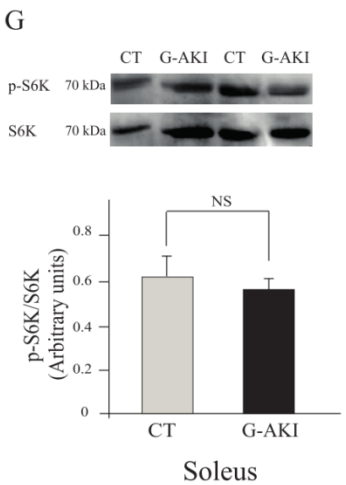
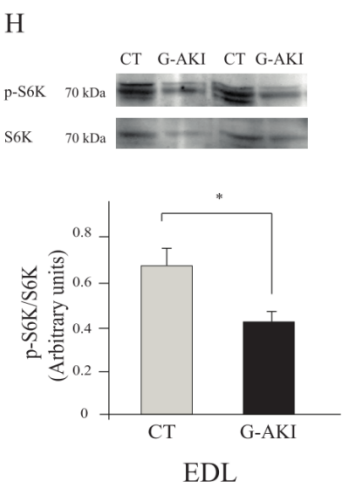
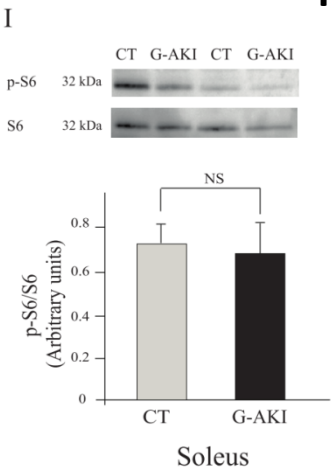
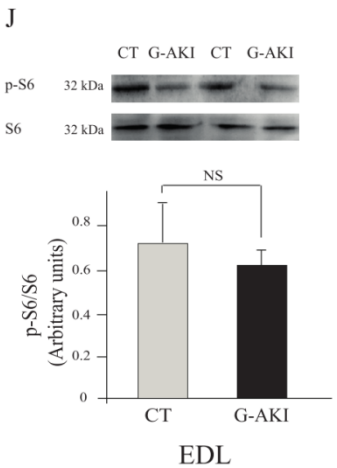


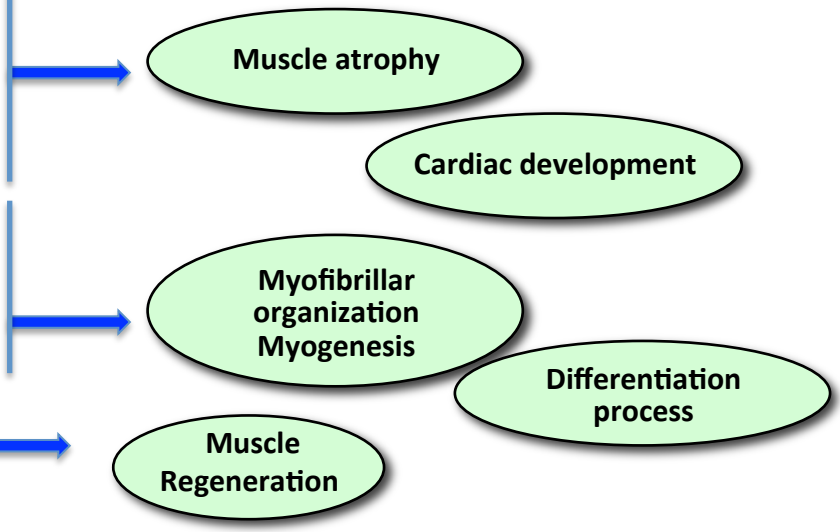

## p-S6/S6



# E3 ligases impact muscle protein homeostasis

- **Muscle-specific E3s**
  - MAFbx
  - MuRF1
  - Fbxo30/MUSA1
  - SMART
  - E4b/UFD2a
  - ASB2
  - Ozz




- **Ubiquitous E3s**
  - Trim32
  - Nedd4

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

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