Targeting the dysregulated translatome in mesothelioma

UNIVERSITY OF CAMBRIDGE

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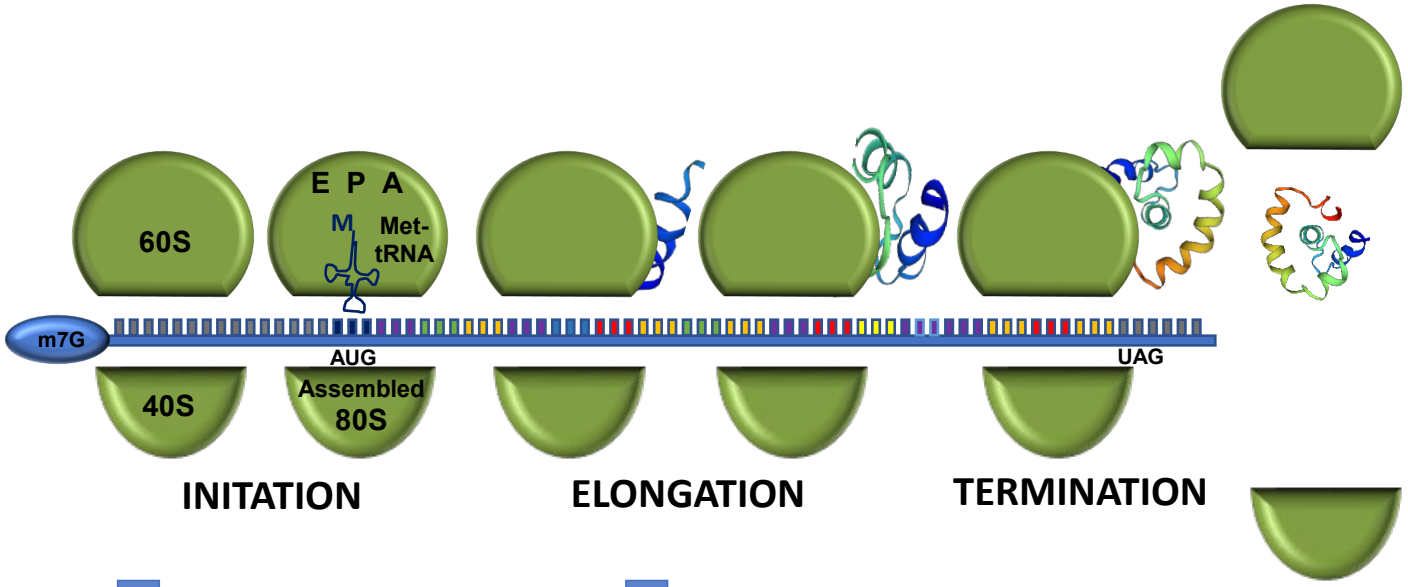
Protein synthesis



Canonical initiation factors
RNA binding proteins
Regulatory RNA motifs

Canonical elongation factors
tRNAs and coding regions

Protein synthesis and cancer

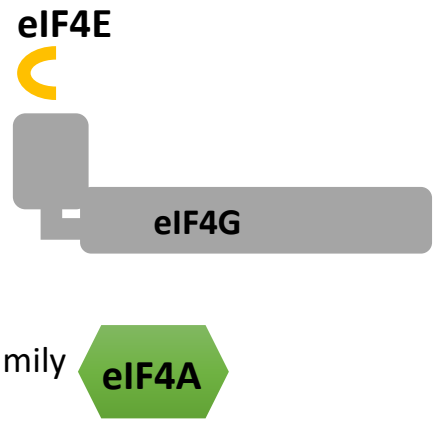
- Protein synthesis is dysregulated in the majority of cancers
- Targeting mRNA translation provides new therapeutic avenues
- Blocking mRNA translation by direct inhibition of eIFs, eEFs and additional RNA binding proteins that control this process will be less associated with development of resistance (compared to targeting the upstream signalling pathways)
- Cancer cell specific

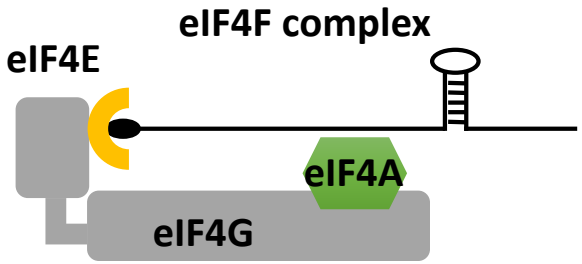
eIF4F Complex

eIF4E cap-binding protein

eIF4G large scaffold protein

eIF4A RNA helicase DEAD box family





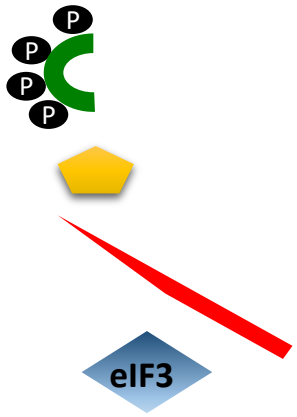
Other proteins

4E-BP1 to 3 bind eIF4E

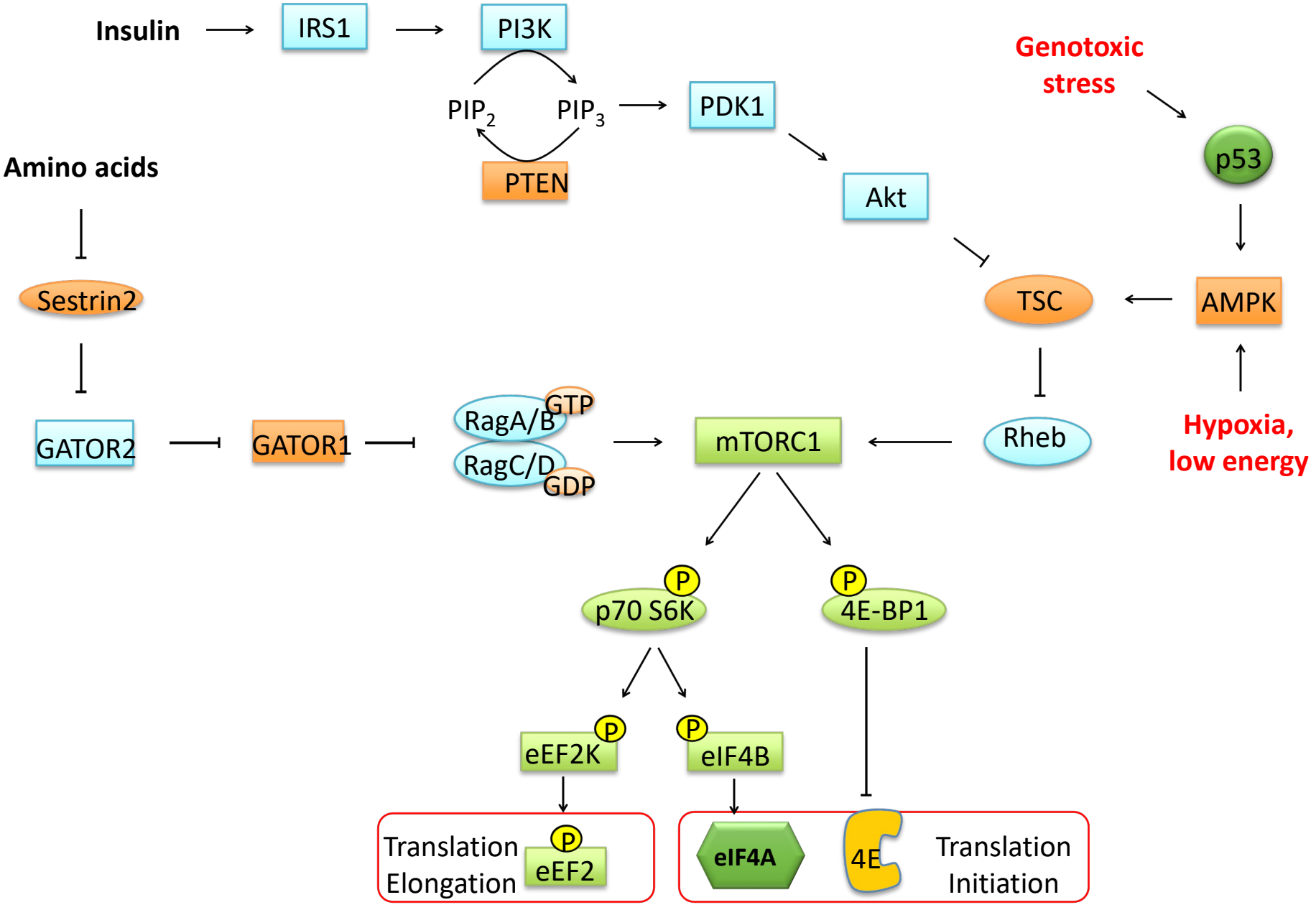
PABP binds Poly A

eIF4B (binds 4A and PABP)

eIF3 binds eIF4G and
the 40S ribosomal sub unit



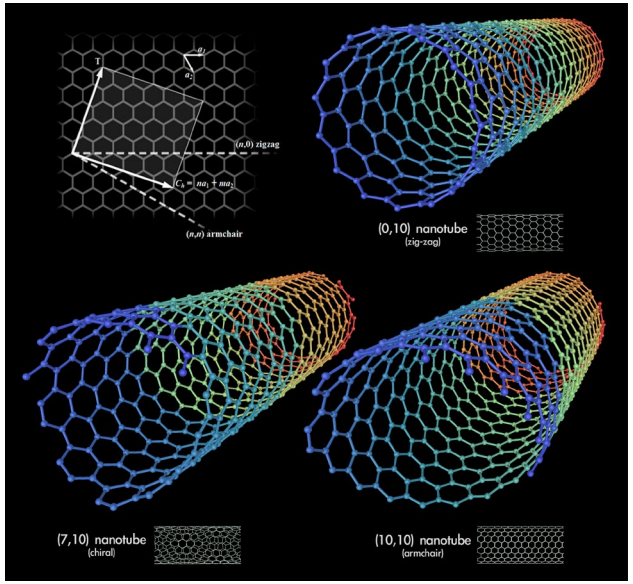
Translation and signaling through mTOR



Fibre toxicity and dysregulation of mRNA translation

Asbestos: a group of minerals that occur naturally in the environment as bundles of fibers that can be separated into thin, durable threads. These fibers are resistant to heat, fire, and chemicals and do not conduct electricity.

Carbon Nanotubes: a tube-shaped material, made of carbon, having a diameter measuring on the nanometer scale. Uses include conductive plastics, structural composite materials, flat-panel displays gas storage, antifouling paint, micro- and nano-electronics, batteries with improved lifetime, biosensors for harmful gases

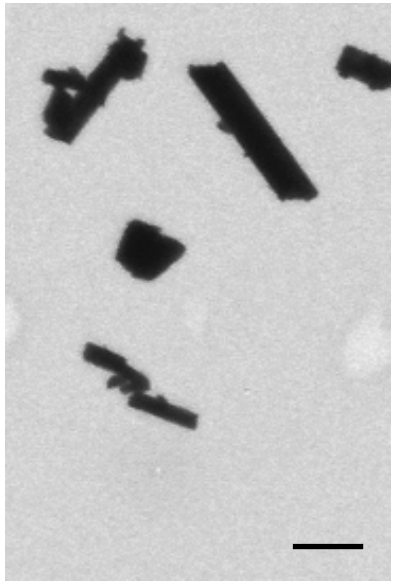


Exposure to either causes mesothelioma

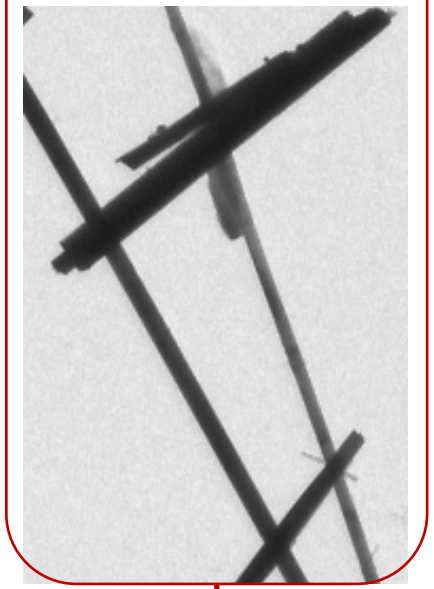
What is the link between these very different compounds?

- The **aspect ratio** of an compound describes the proportional relationship between its width and its height. Biopersistent.

Short Fibre Asbestos **(SFA)**

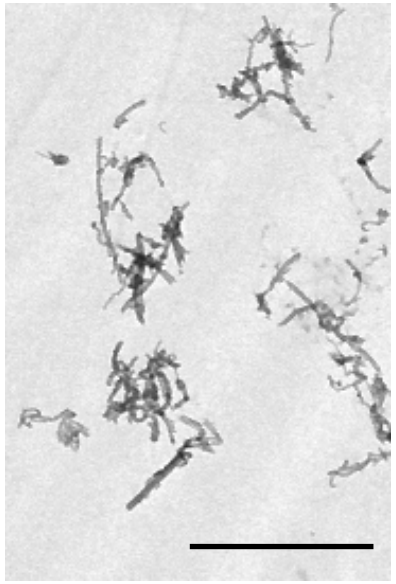


Long Fibre Asbestos **(LFA)**

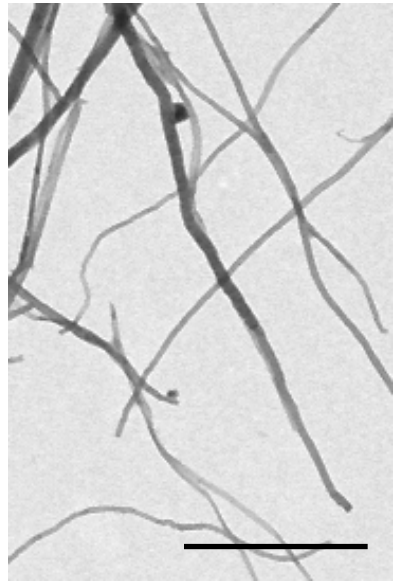


Induced lung tumours and mesothelioma *in vivo* studies

Short Carbon Nanotubes **(SNT)**



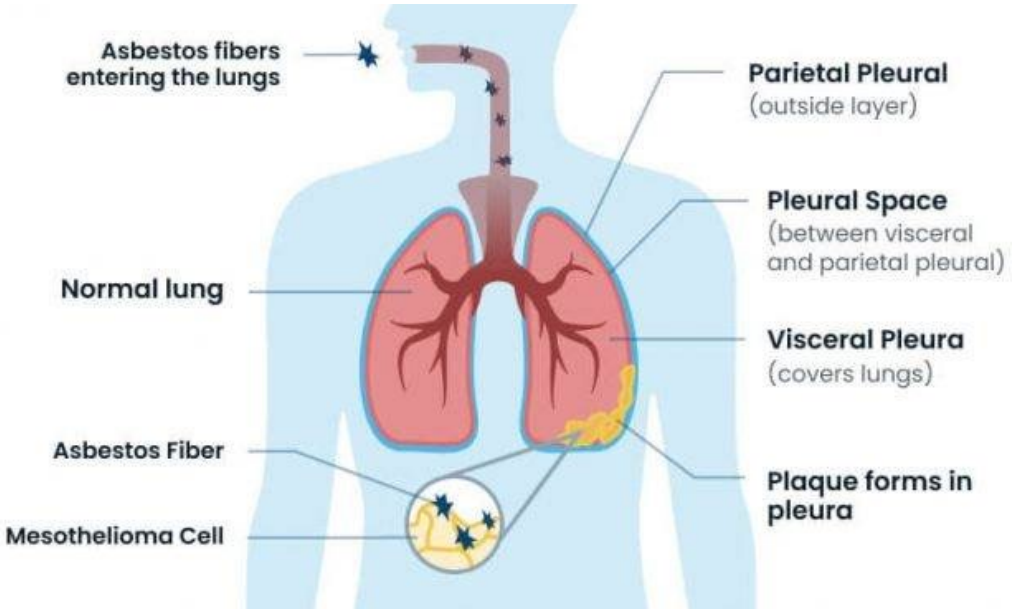
Long Carbon Nanotubes **(LNT)**



Scale bar = 1 µm

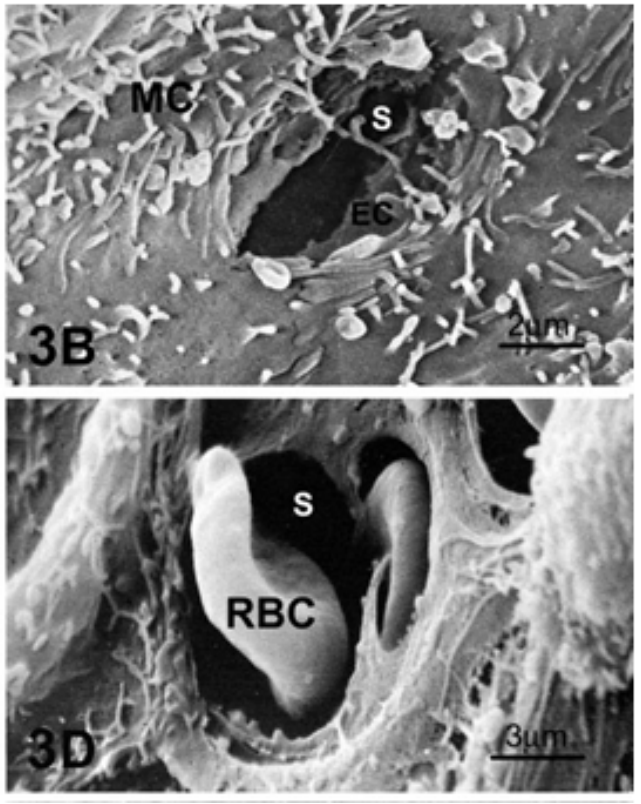
Chernova *et al* 2017 *Current Biology*

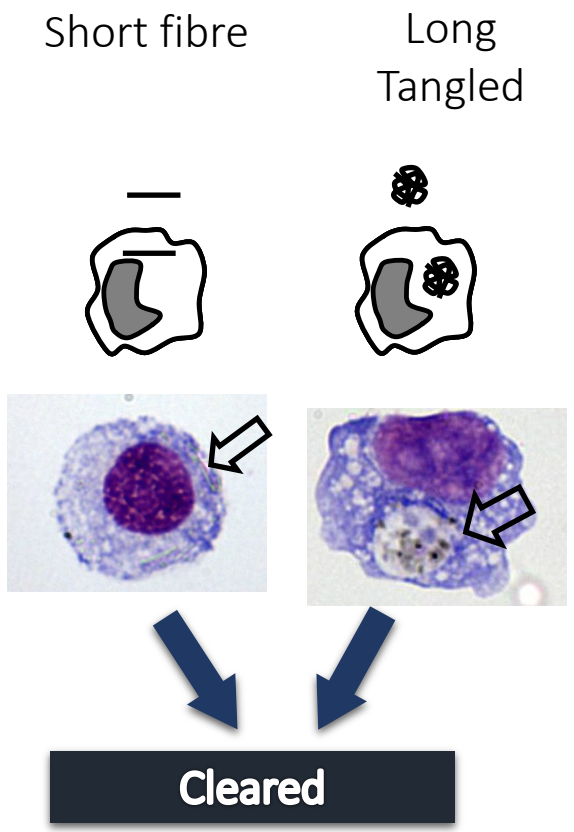
Malignant Pleural Mesothelioma

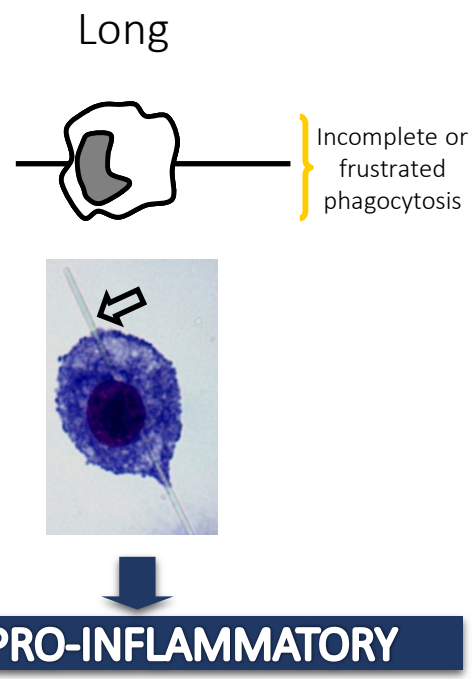


Disease of tumour suppressor loss
with no known changes in transcriptional drivers

Frustrated Phagocytosis



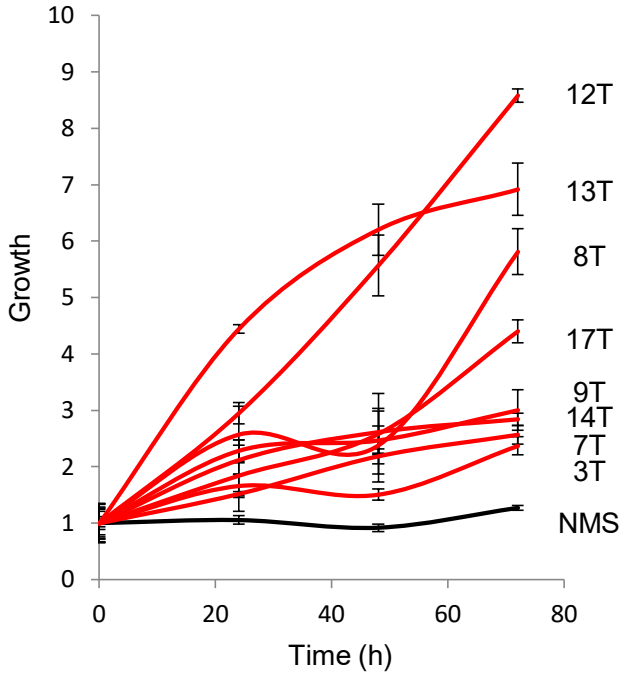




MM Primary cell lines isolated from patients

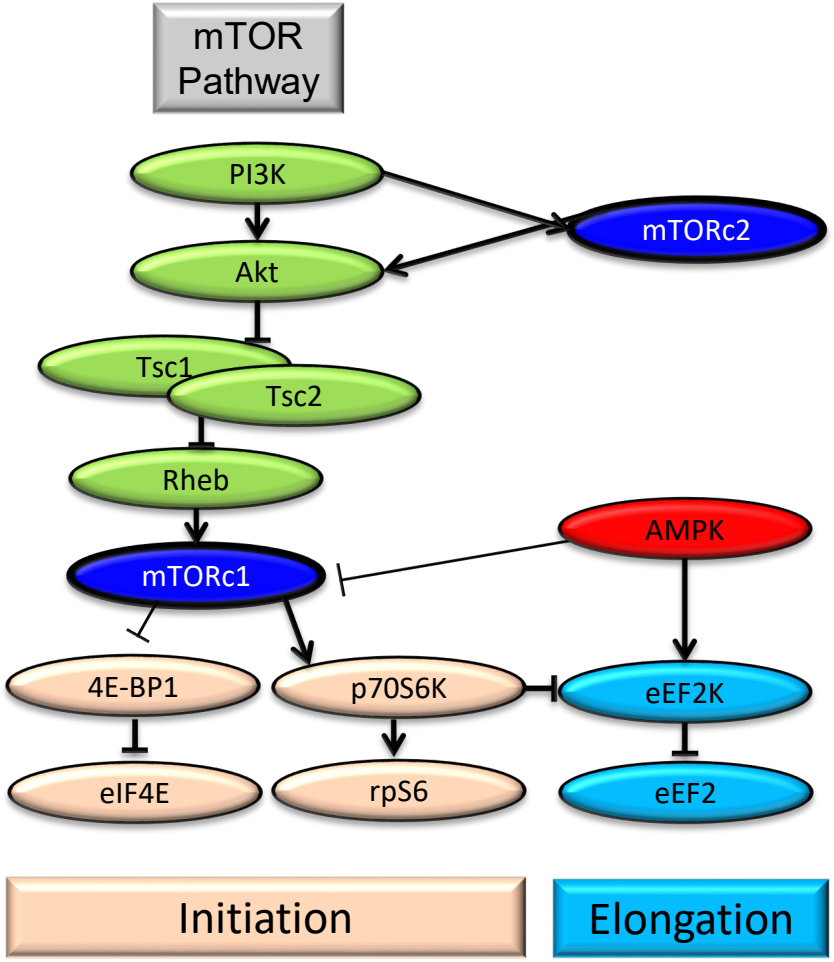
- More than 35 cell lines were established
- 8 MM Primary cell lines are used in this study
- All the Genomic and Proteomic data were generated in the first 5-10 passage

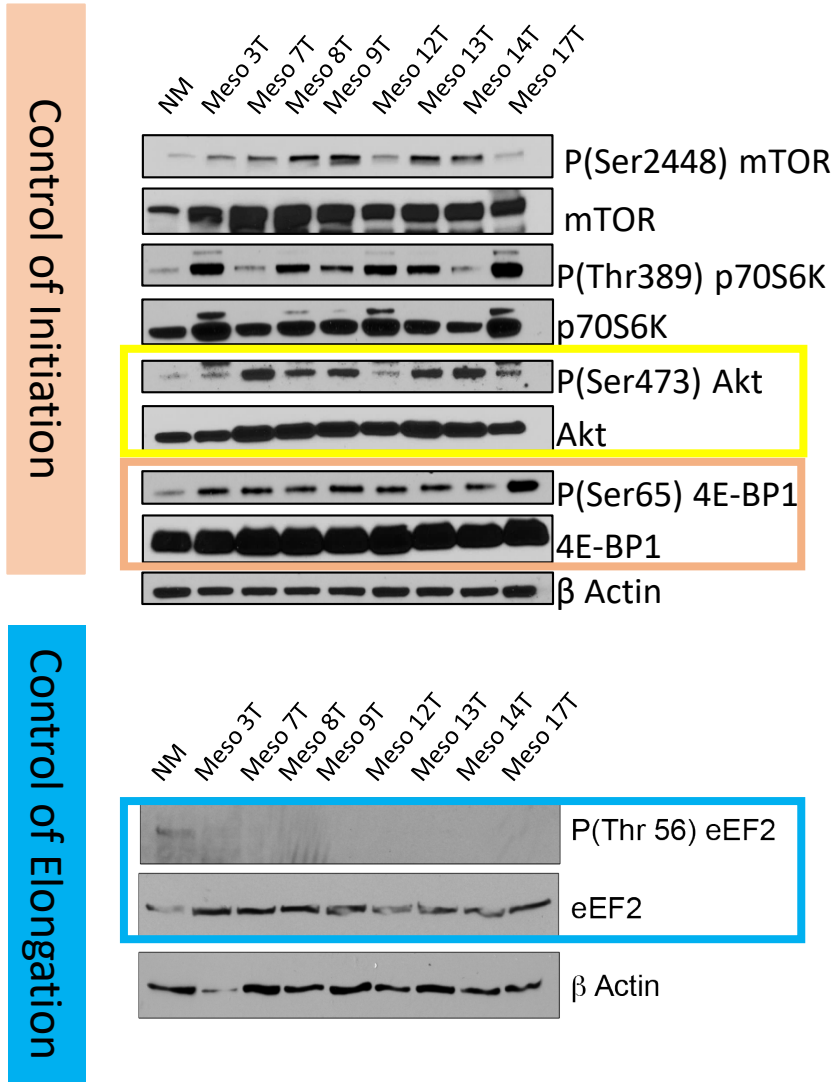
Cell line	Average Age	Gender	Histopathology	Asbestos exposure
8 Primary Malignant Mesothelioma cell lines	67.4	7 Males 1 Female	Epithelioid	Yes
2 Primary Untransformed Mesothelium	47.3	Female	Healthy	Unknown



Chernova *et al.*, 2016 *Cell Death Differ*

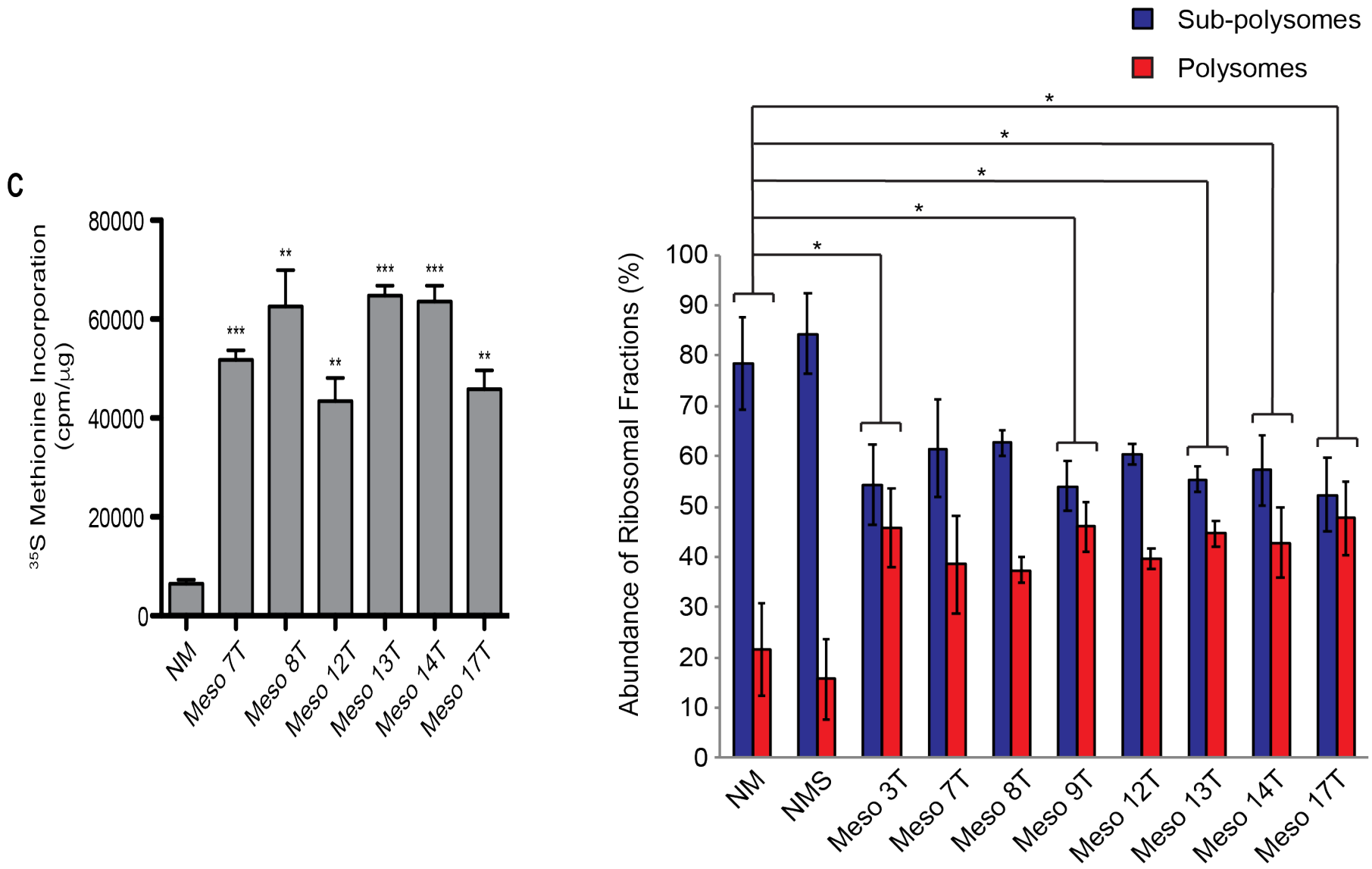
mTOR signalling pathway is highly active in primary MM cell lines



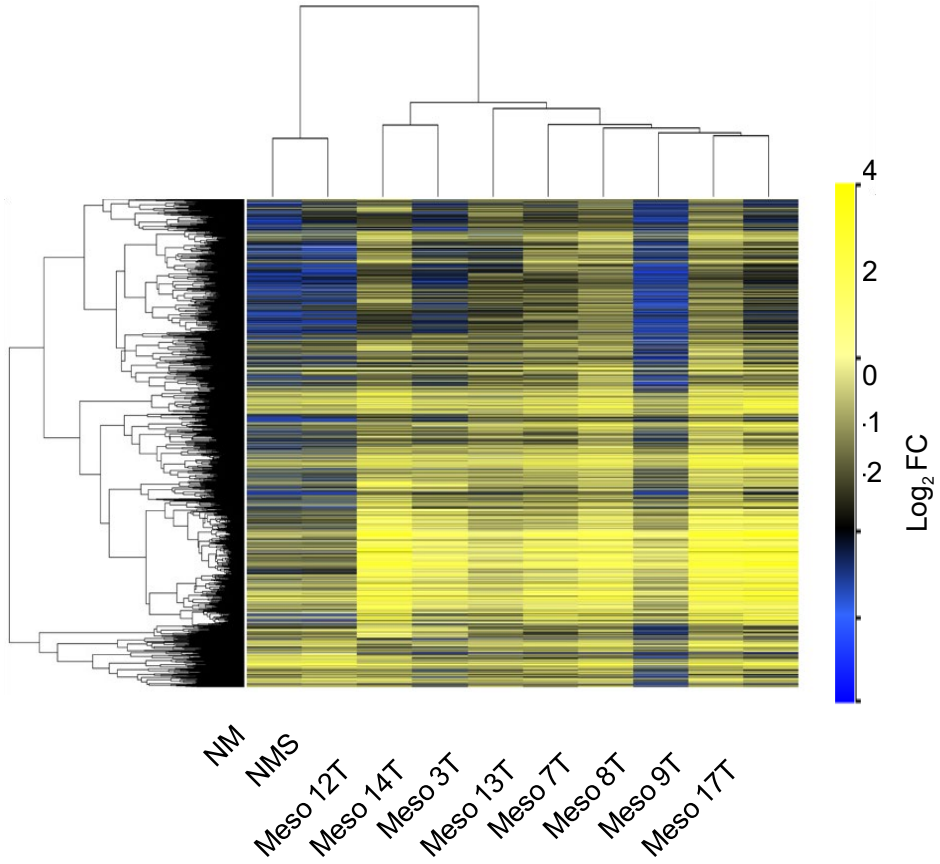


Stefano Grosso

Protein synthesis is up-regulated in primary MM cell lines



Primary MM cells express common translational drivers

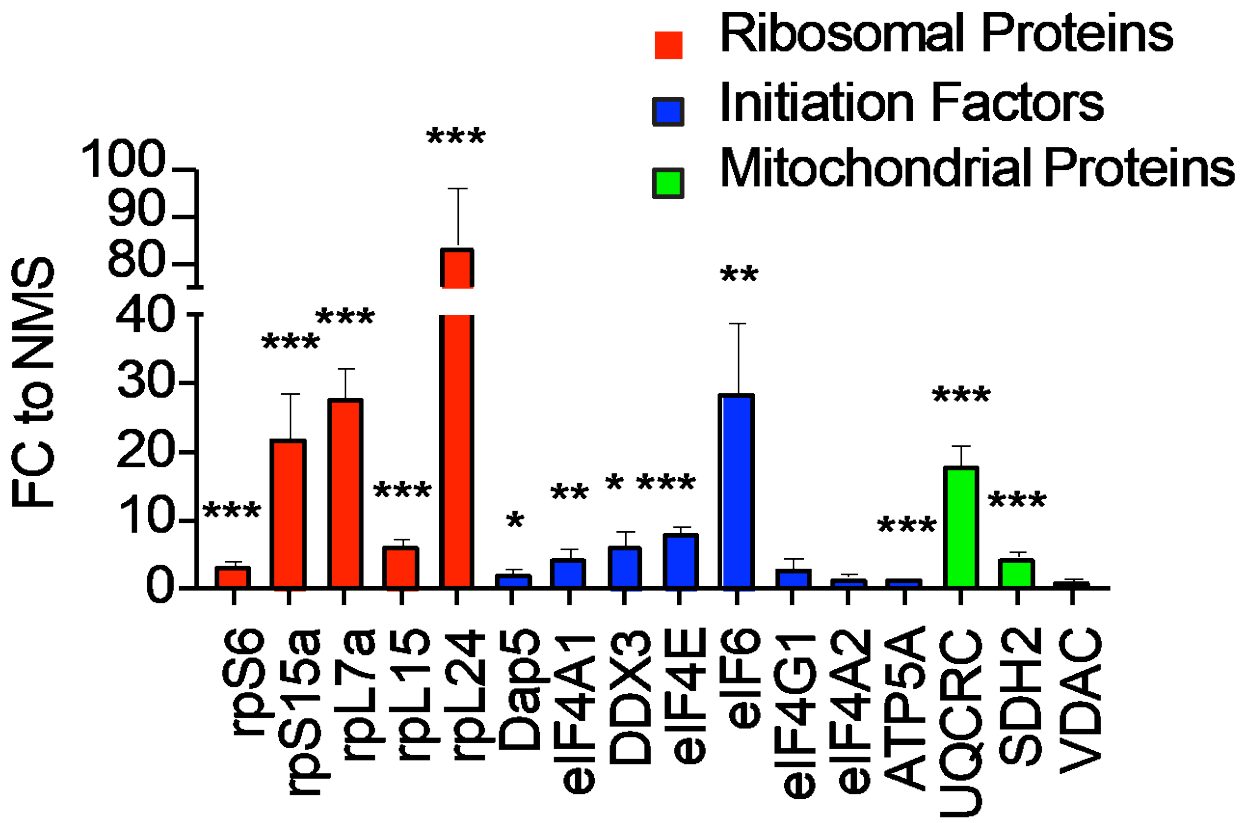


Description			p-value
shorter 5'UTR	**t test**	***	**2.805E-08**
Higher 5'UTR MFE	t test	***	1.558E-11
fewer uORF	χ^2	***	1.096E-07
TISU	χ^2	***	6.074E-06
TOP/TOP like	χ^2	n.s.	

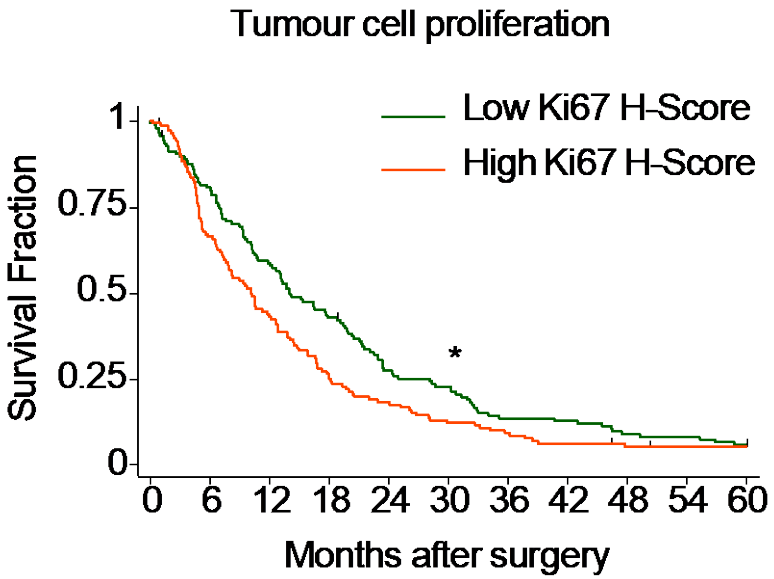
Ribosomal Machinery expression increased in Primary MM

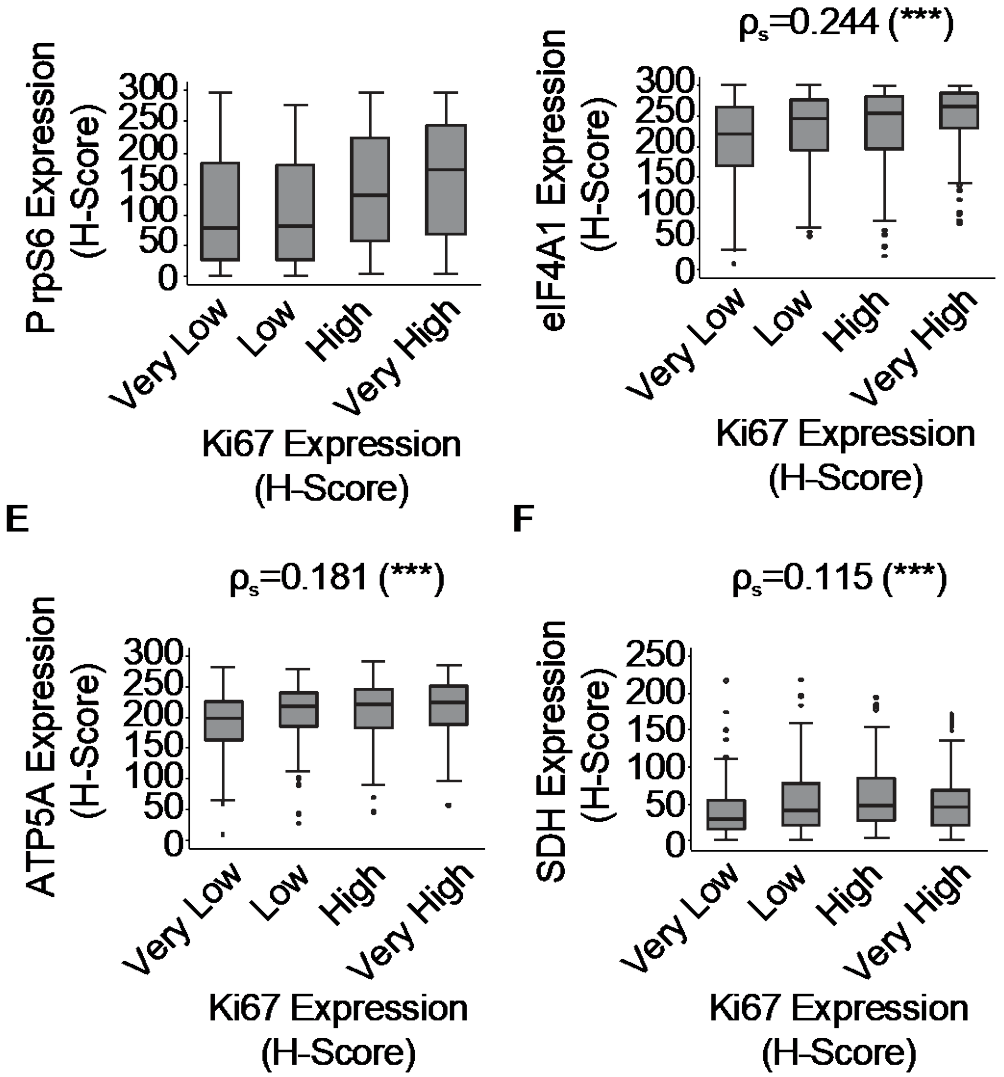
#	GO term	Description	odds ratio	p-value
1	GO:0005739	MITOCHONDRION	2,21	2.60E-13
2	GO:0022904	RESPIRATORY ELECTRON TRANSPORT CHAIN	5,73	4.68E-10
3	GO:0016070	RNA METABOLIC PROCESS	3,52	2.93E-08
4	GO:0003735	STRUCTURAL CONSTITUENT OF RIBOSOME	4,27	4.68E-07
5	GO:0006412	TRANSLATION	3,45	1.05E-06
6	GO:0010467	GENE EXPRESSION	2,36	3.56E-06
7	GO:0005743	MITOCHONDRIAL INNER MEMBRANE	2,94	5.99E-03
8	GO:0016071	mRNA METABOLIC PROCESS	3,27	5.76E-02
9	GO:0016032	VIRAL REPRODUCTION	2,77	9.52E-02
10	GO:0000216	M/G1 TRANSITION OF MITOTIC CELL CYCLE	4,87	1.77E-01

Ribosomal proteins and Initiation Factors are Translationally up-regulated



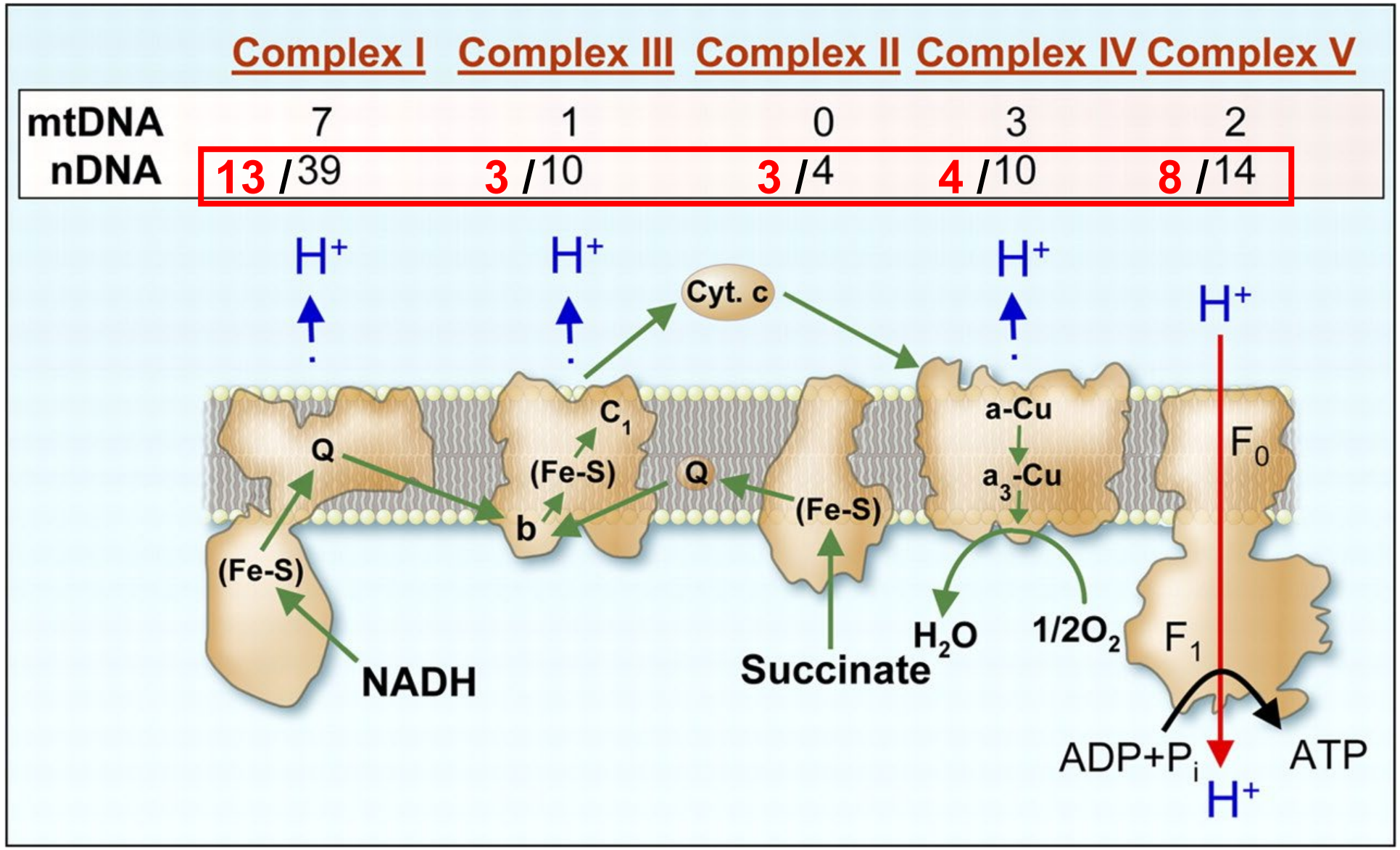
Related changes observed in patient tumours





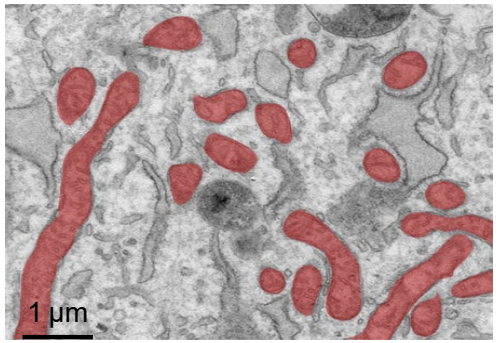
John Le Quesne MRC/CRUK
Beatson Institute

mRNAs encoding for Respiratory Electron Transport Chain are efficiently translated in MM

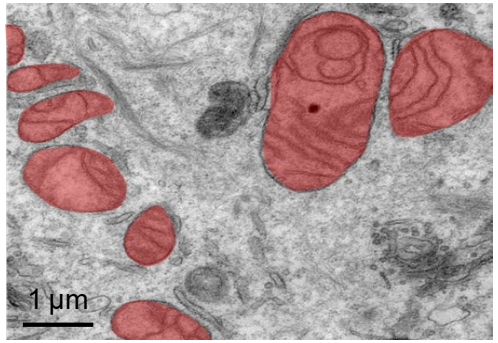


MM primary cell lines show mitochondrial fission with enlarged mitochondria

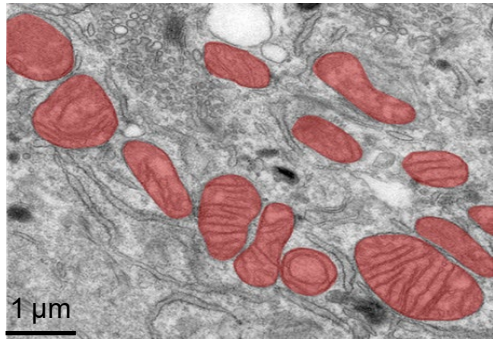
NM

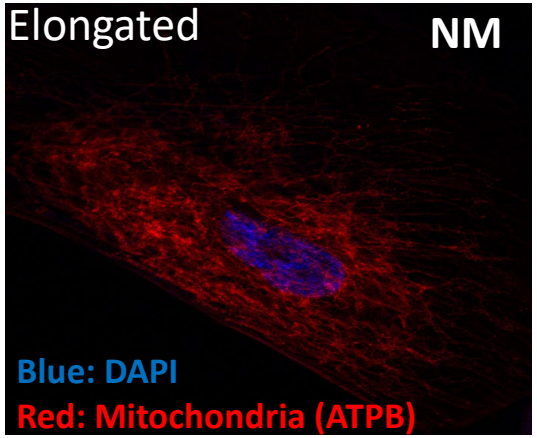


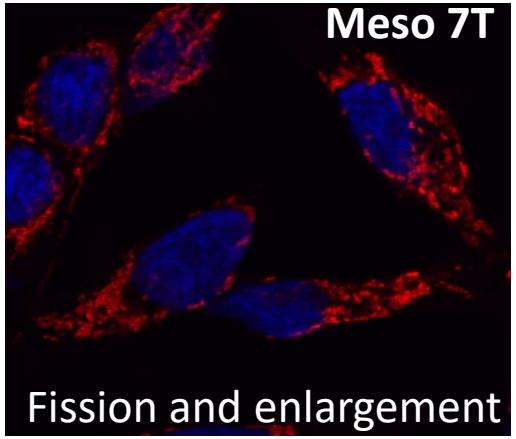
Meso 7T

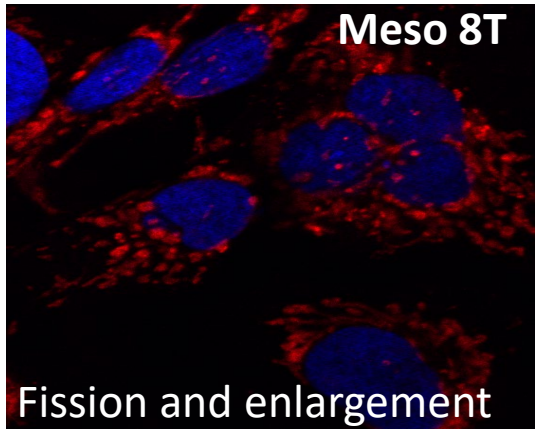


Meso 8T

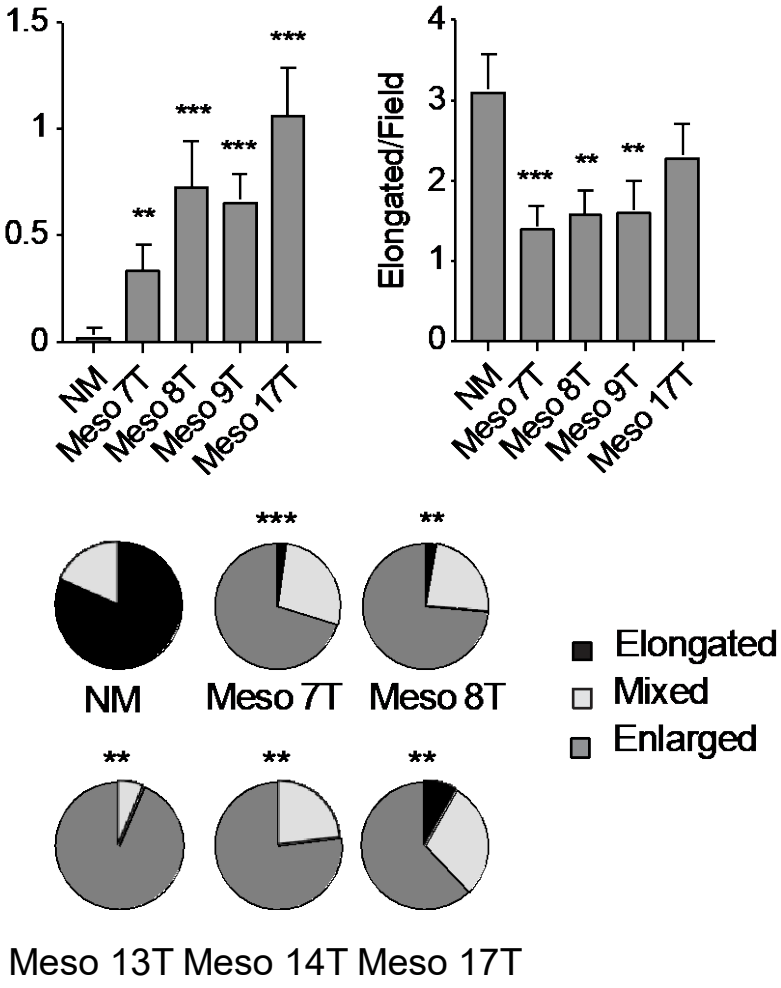


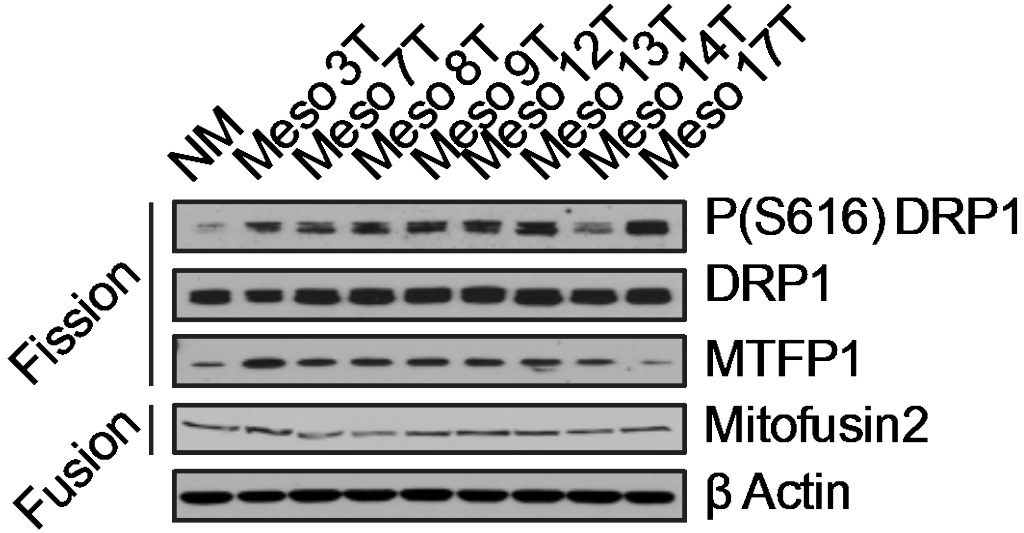




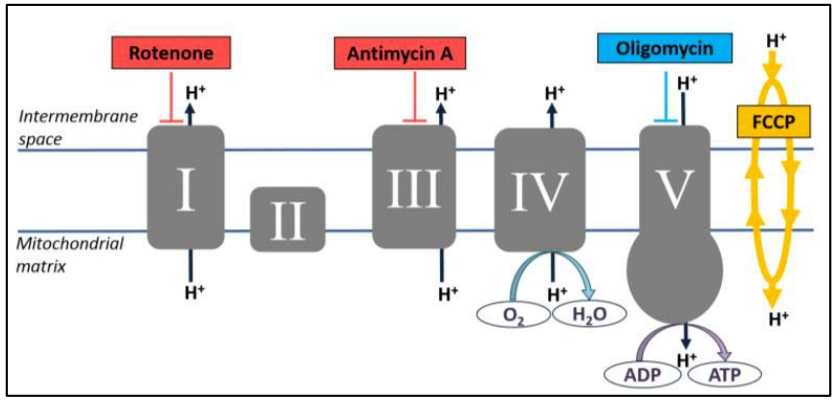


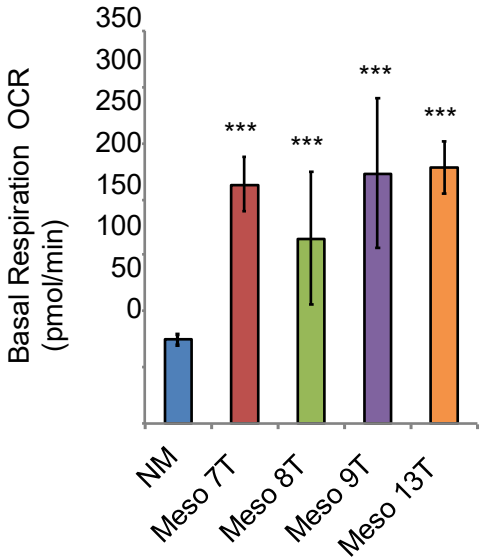
Changes in proteins that control mitochondrial fission and fusion

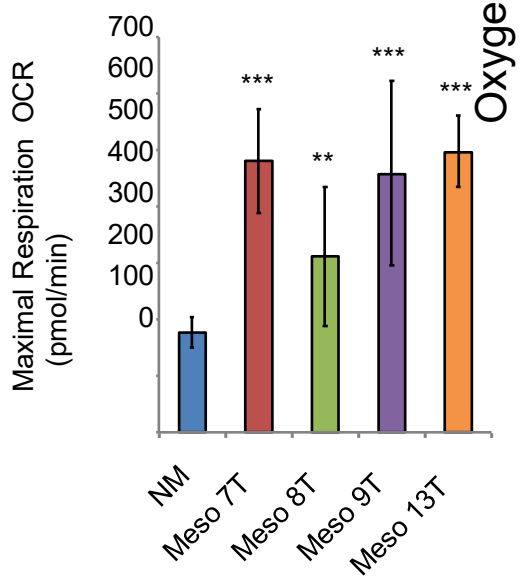


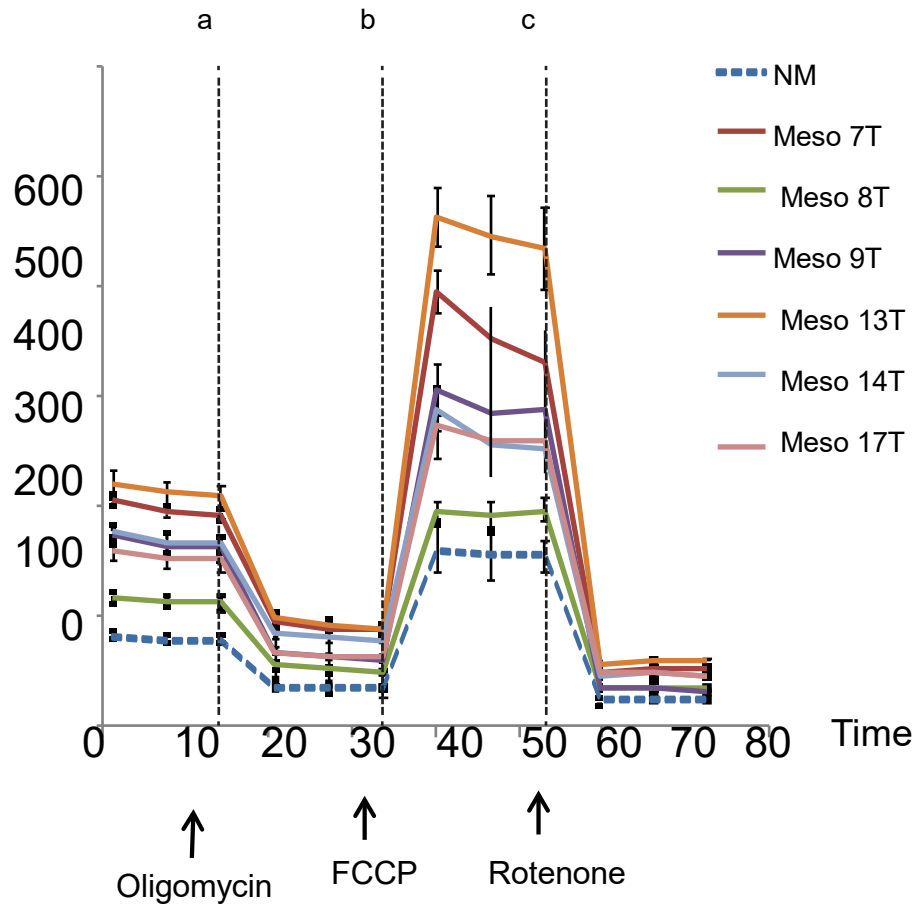


These alterations drive metabolic output with increased O_2 consumption rate

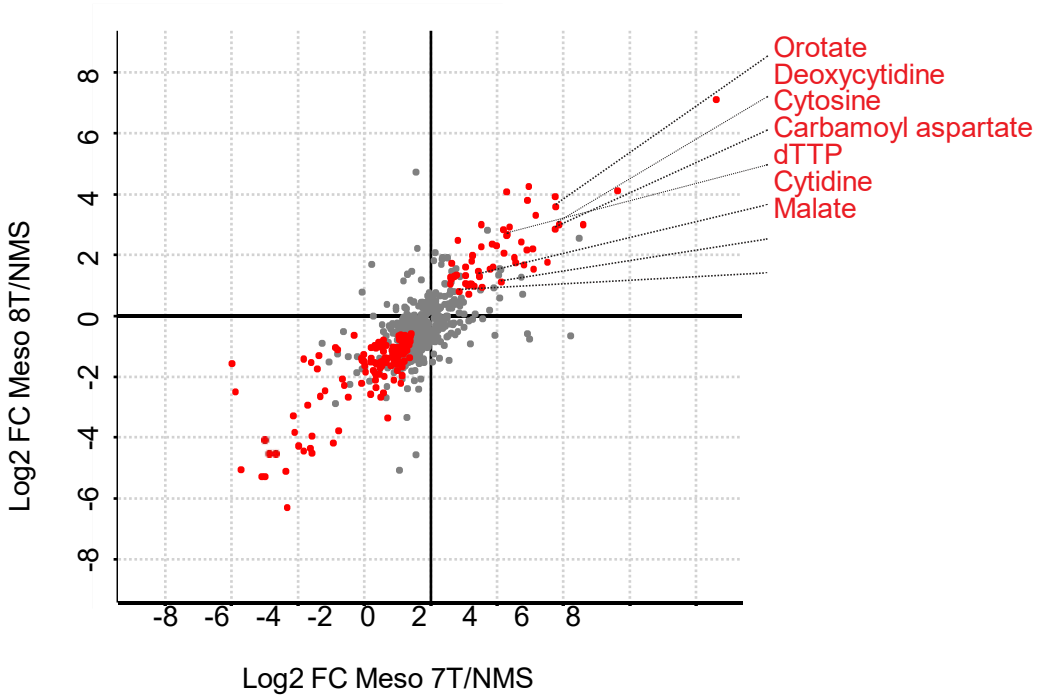


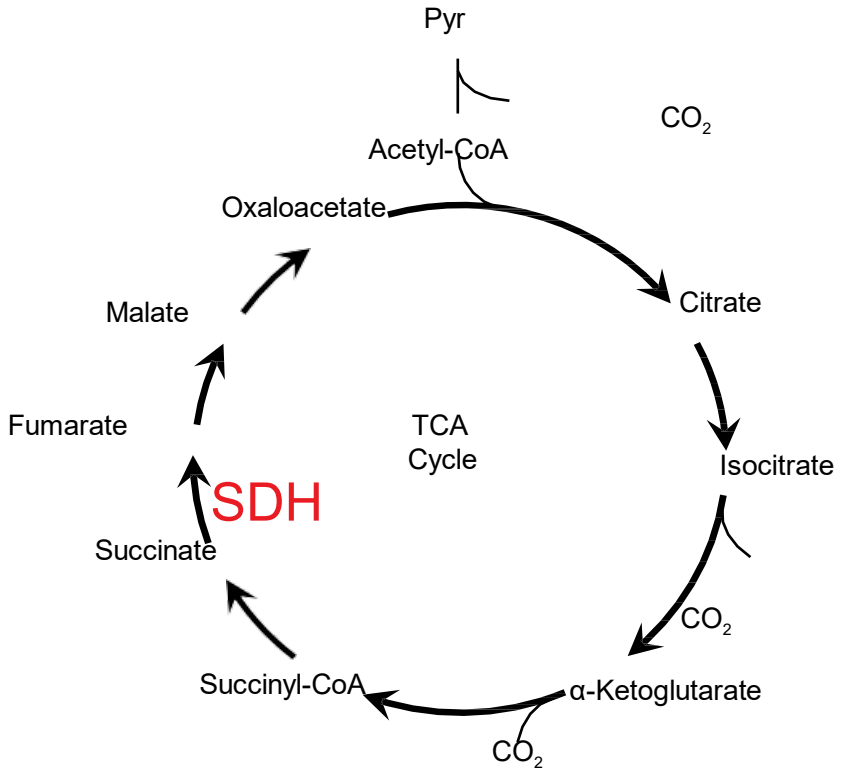


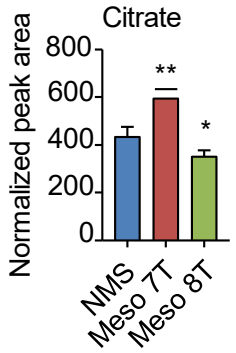


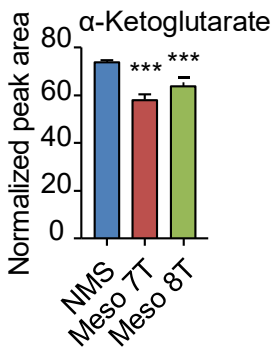


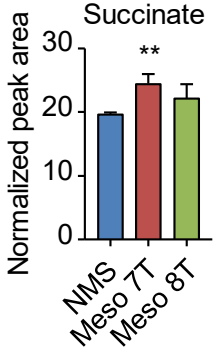
……and an altered metabolic profile

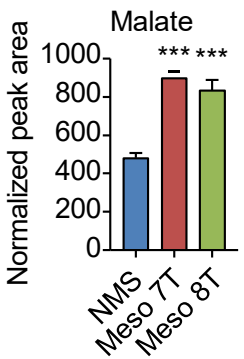








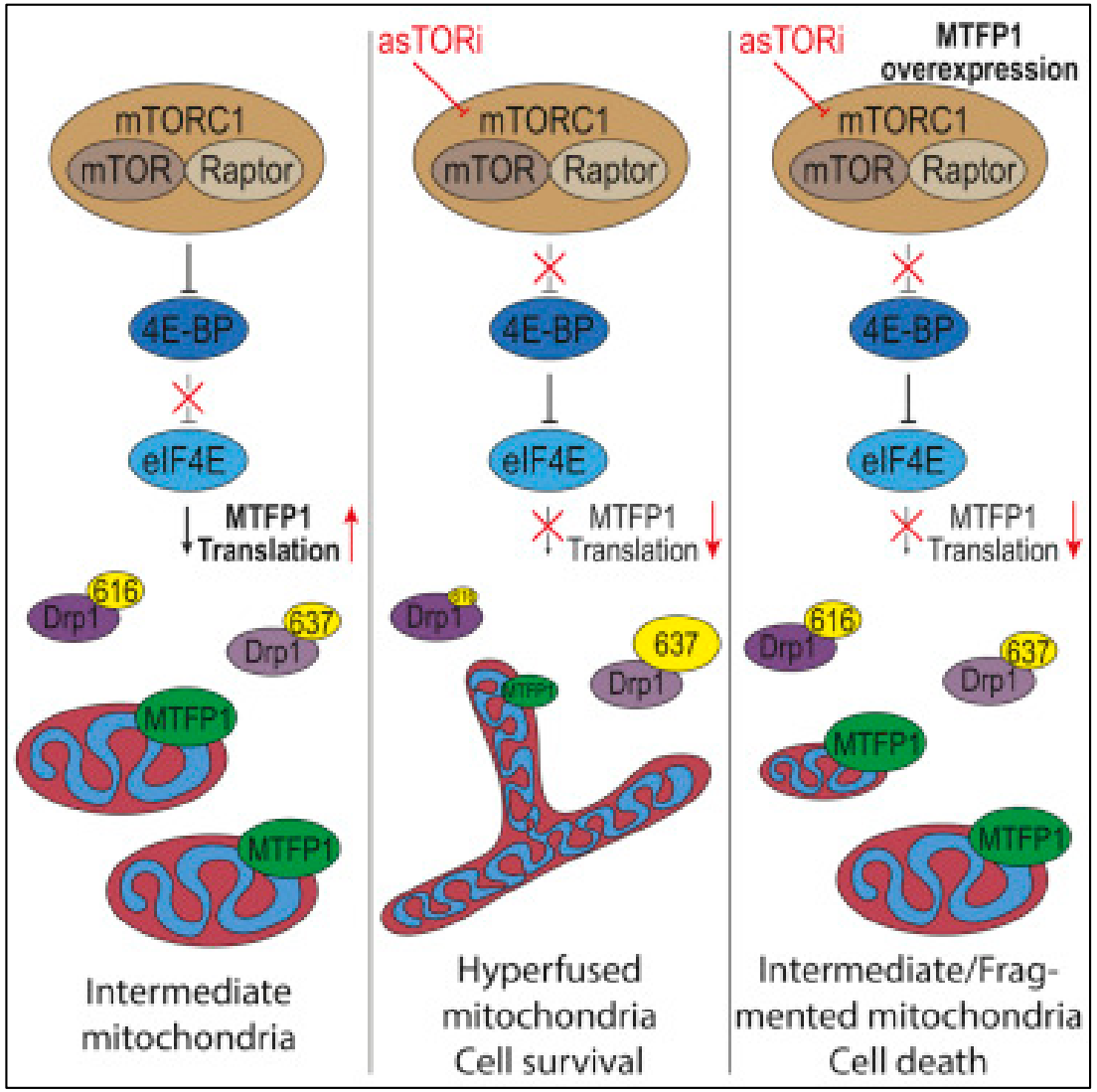




Johan Vande Voorde

Owen Sansom

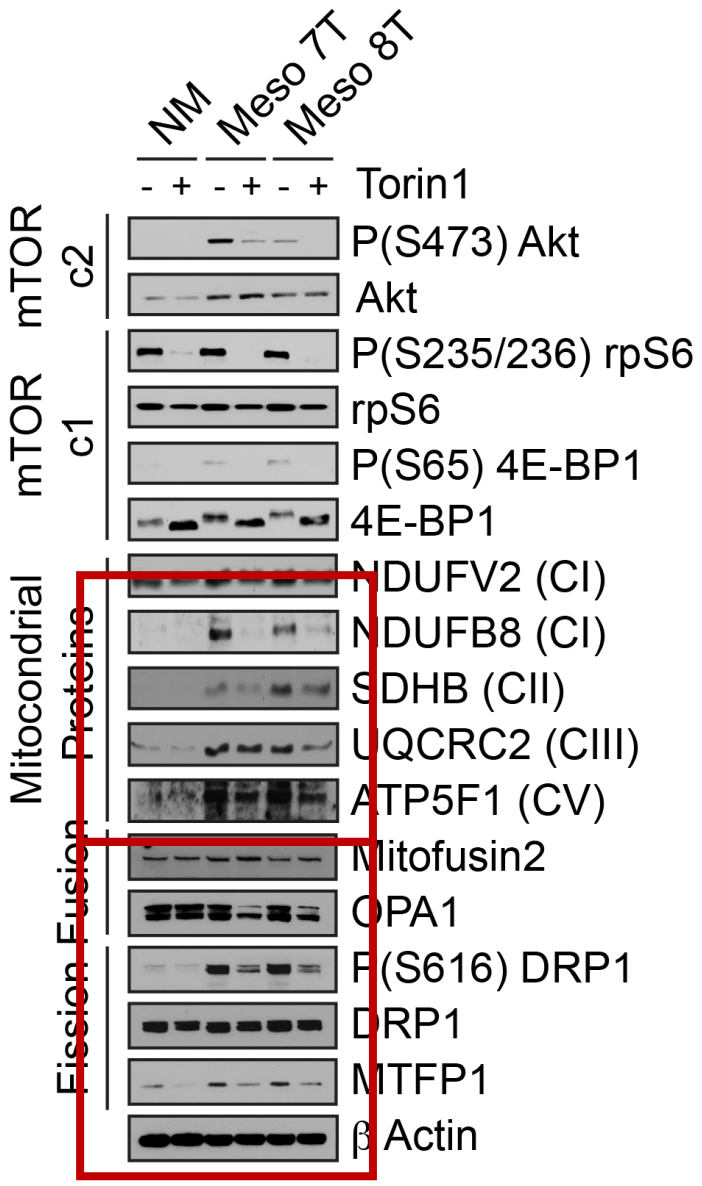
Mitochondrial dynamics and mTOR signaling pathway are linked



Morita *et al.*, *Mol Cell* 2017

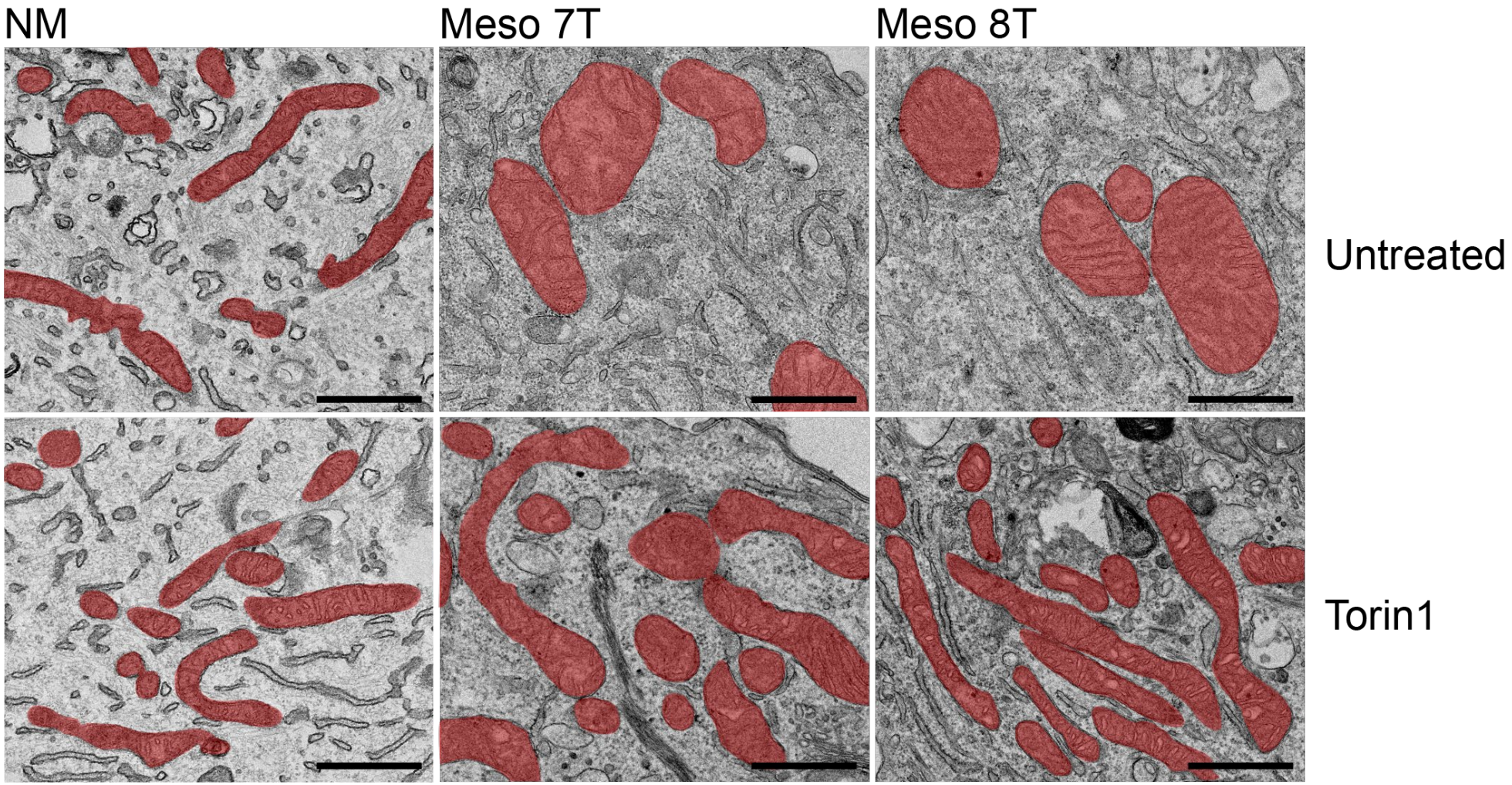
mTORC1/2 inhibition reduces expression of mitochondrial proteins and DRP1 phosphorylation

AZD2014
Torin 1

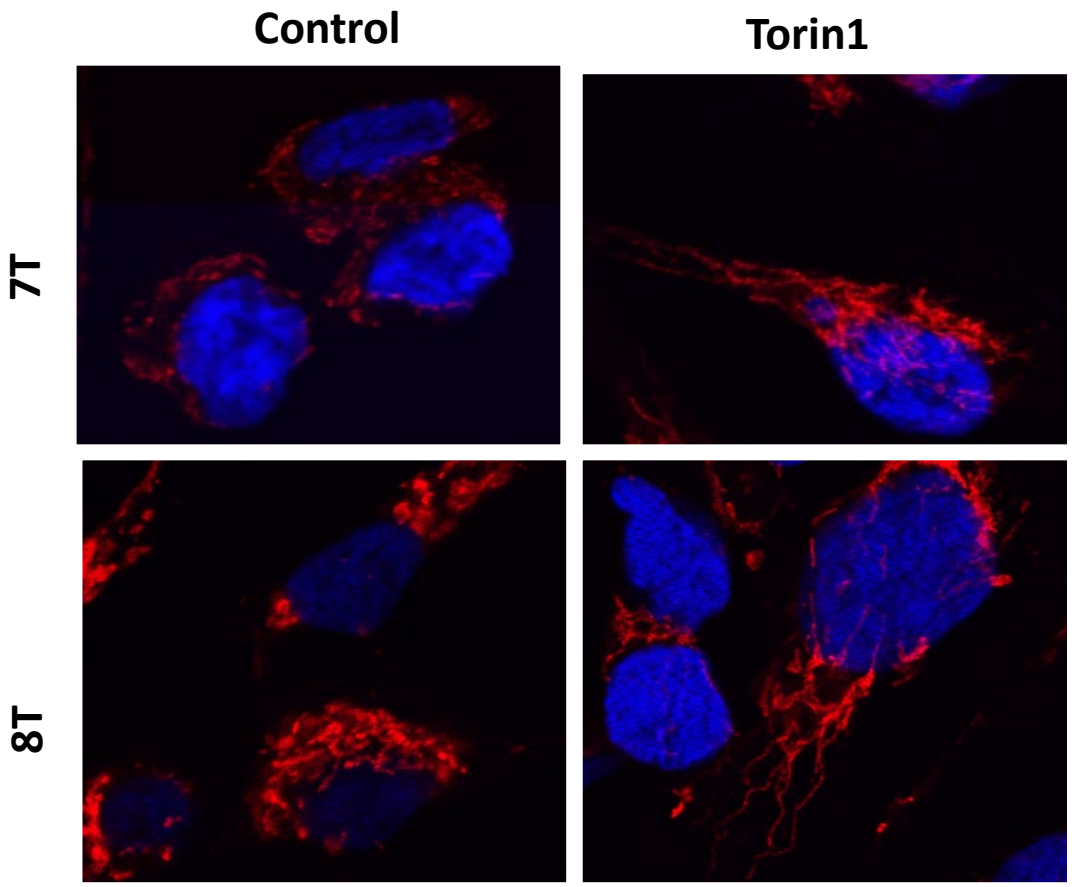


Alberto Marini

TORC1/2 inhibition induces mitochondrial elongation in MM primary cell lines



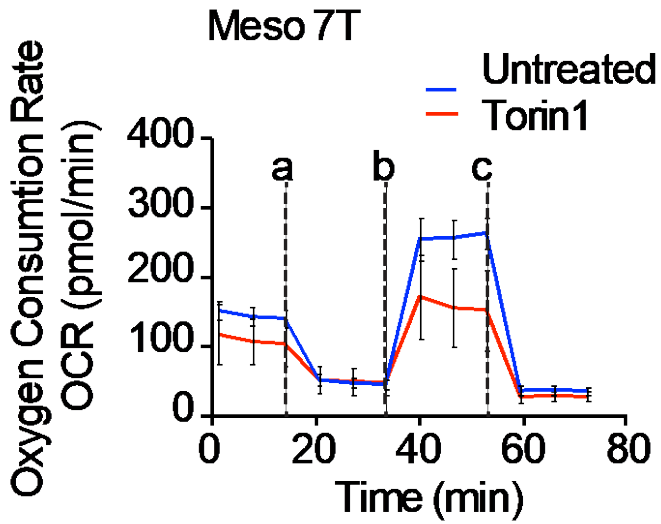
Torin1 induces mitochondrial elongation in MM primary cell lines

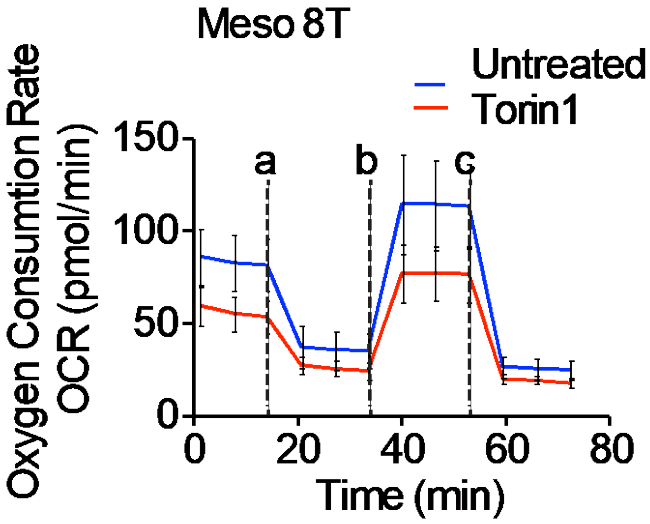


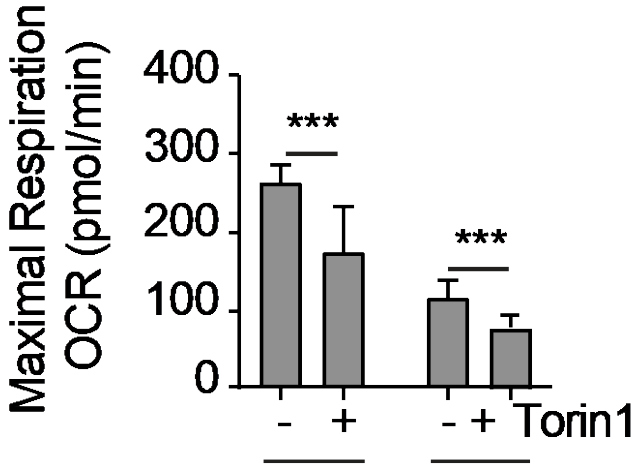
Blue: DAPI
Red: Mitochondria (ATPB)

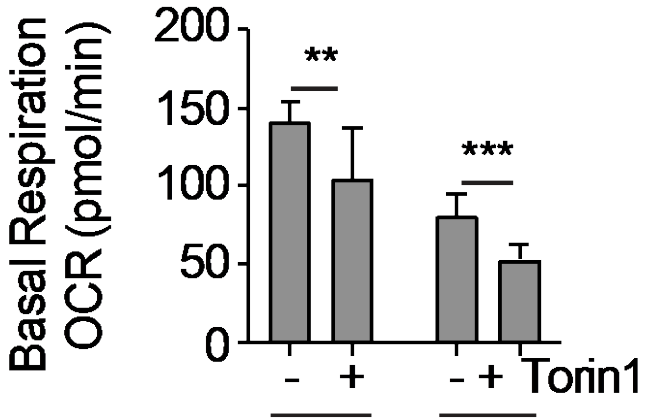
Elongated mitochondria

….reduces O_2 Consumption Rate and Respiratory Reserve Capacity

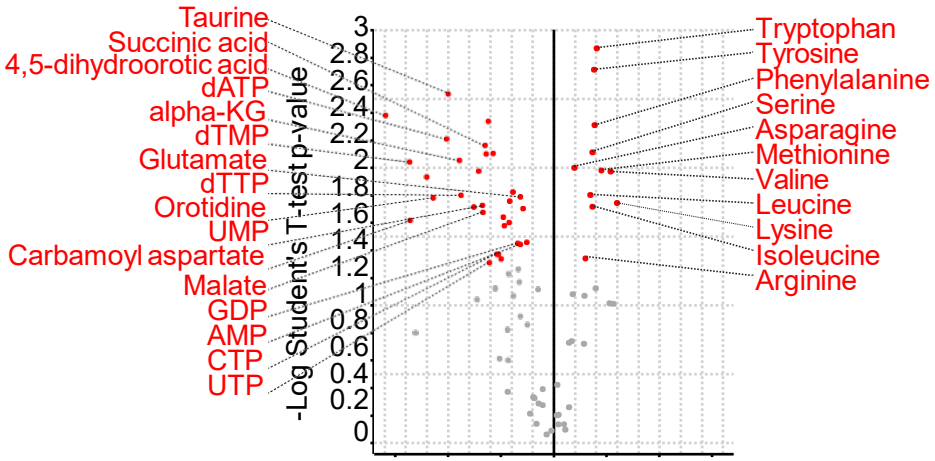


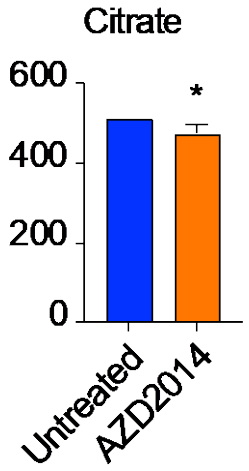


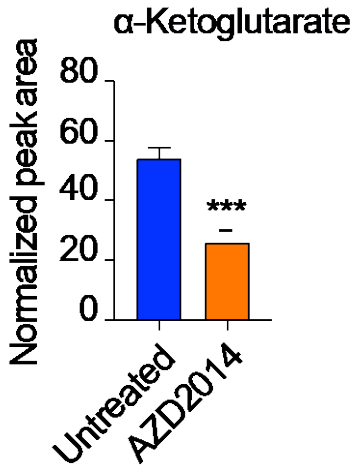


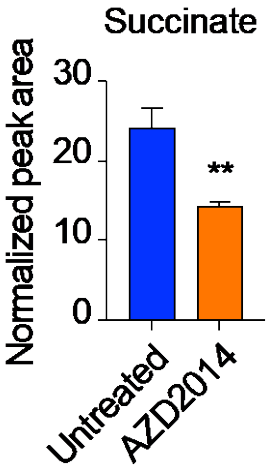


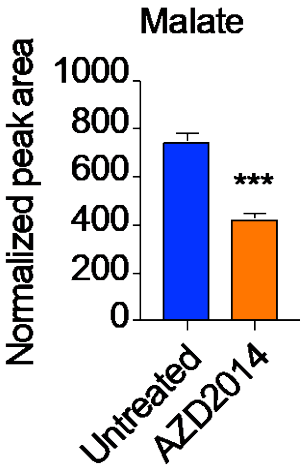
…and reprogrammes metabolic outputs





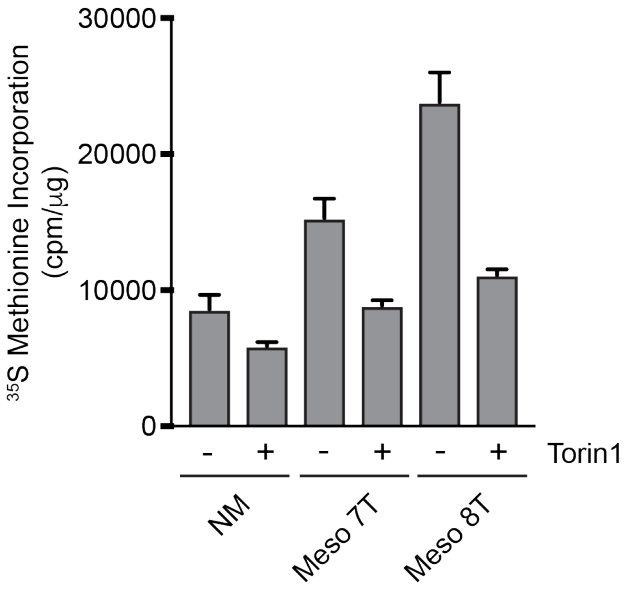


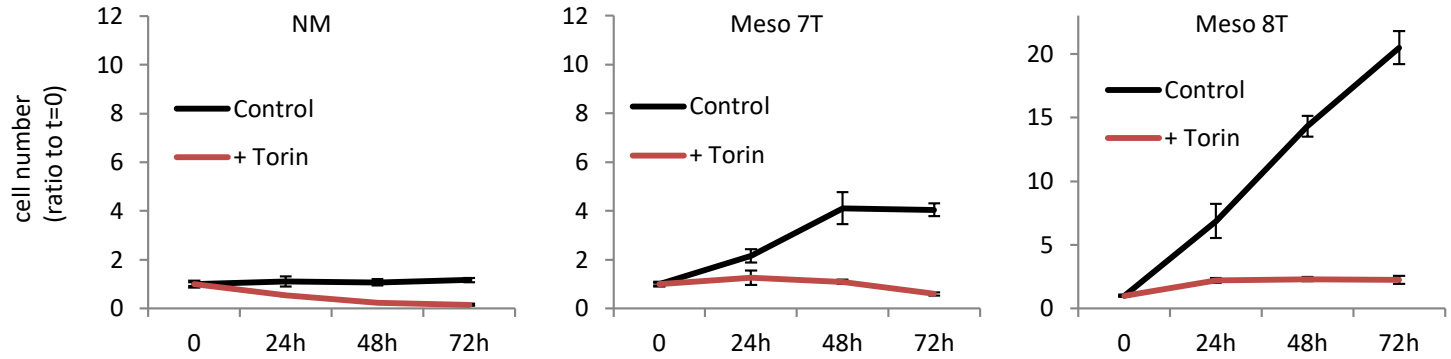




Angela Tenor

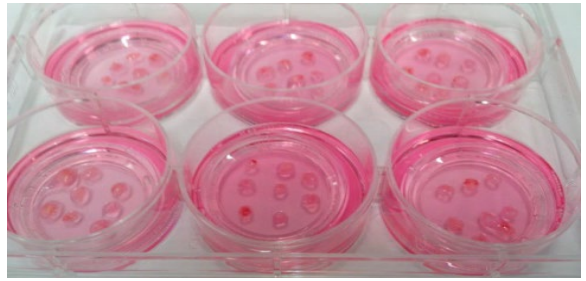
mTORC1/2 inhibition reduces mRNA translation and growth of primary MM cells





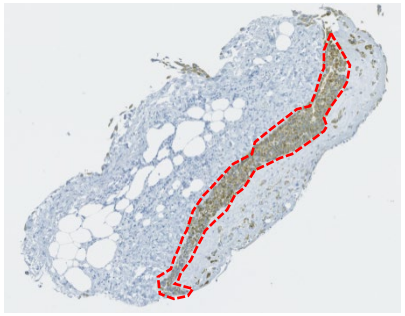
Inhibition of TORC1/2 in patient explants blocks cell proliferation

3D Tumour Explant Model

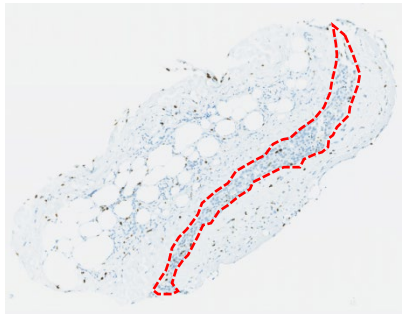


Karekla *et al,* 2016 *Cancer Res*

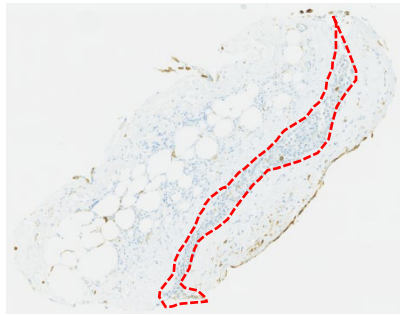
IHC staining



Cytokeratin (MNF)



Ki67

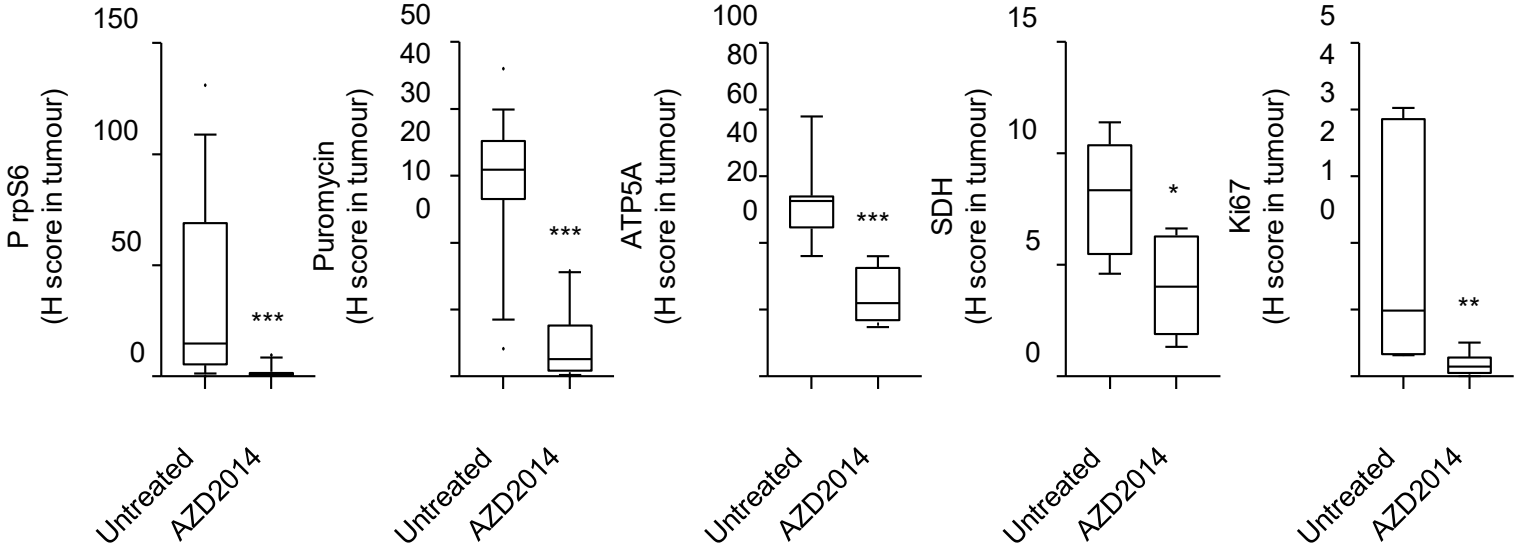


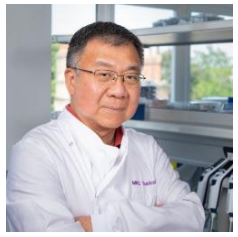
p-S6 (Ser240-244)

3 MM patients

16 different sections per sample

[Torin1]=100nM, 72h

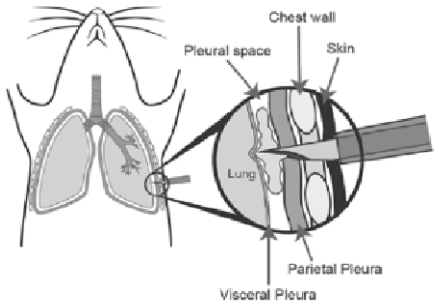




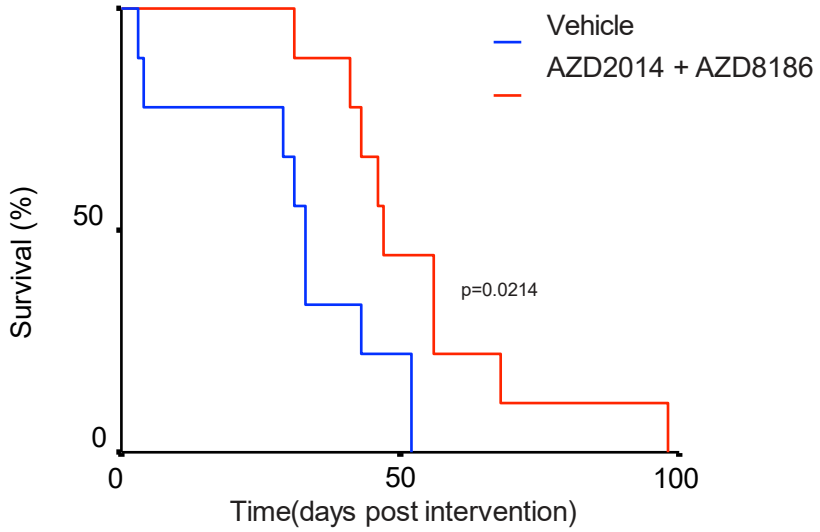
Xiao-Ming Sun

Alberto Marini

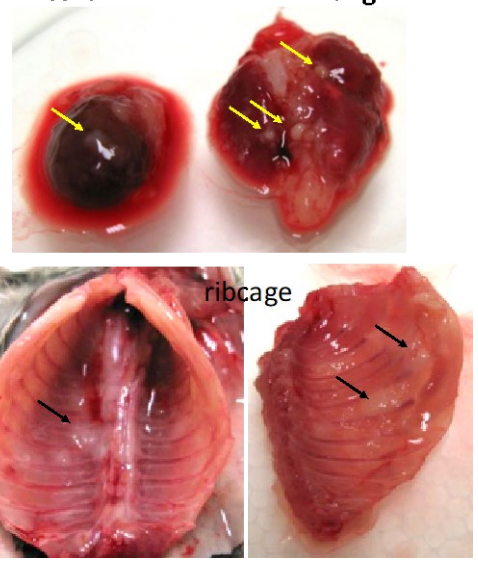
Mouse model recapitulates mesothelioma cell data

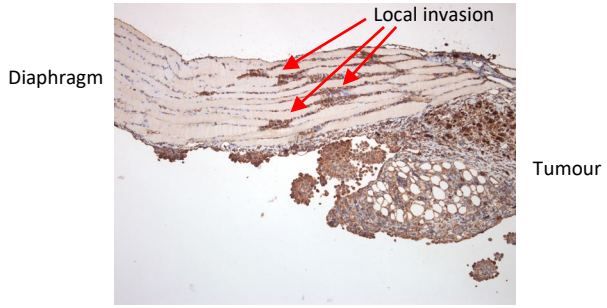


Lenti-CRE from 10^7 down → *10 days* → + asbestos intrapleural injection (25ug/mouse)



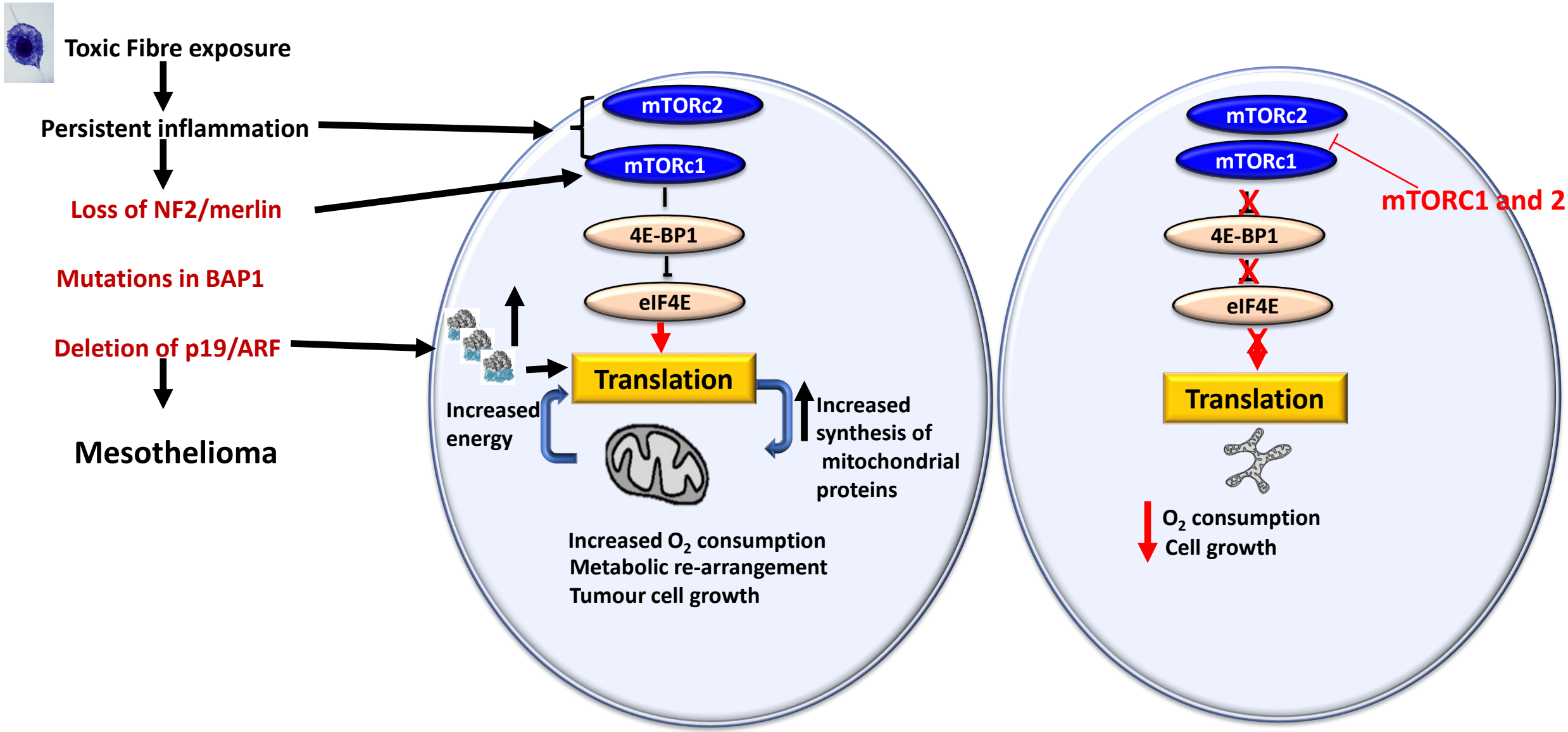
$NF2^{f/f};Cdkn2a^{-/-};p53^{f/f}$





Daniel Murphy
CRUK Beatson Institute

Loss of tumour suppressor proteins is consistent with cytoplasmic changes



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