



Actin-microtubule cytoskeletal interplay mediated by MRTF-A/SRF signaling promotes dilated cardiomyopathy caused by *LMNA* mutations

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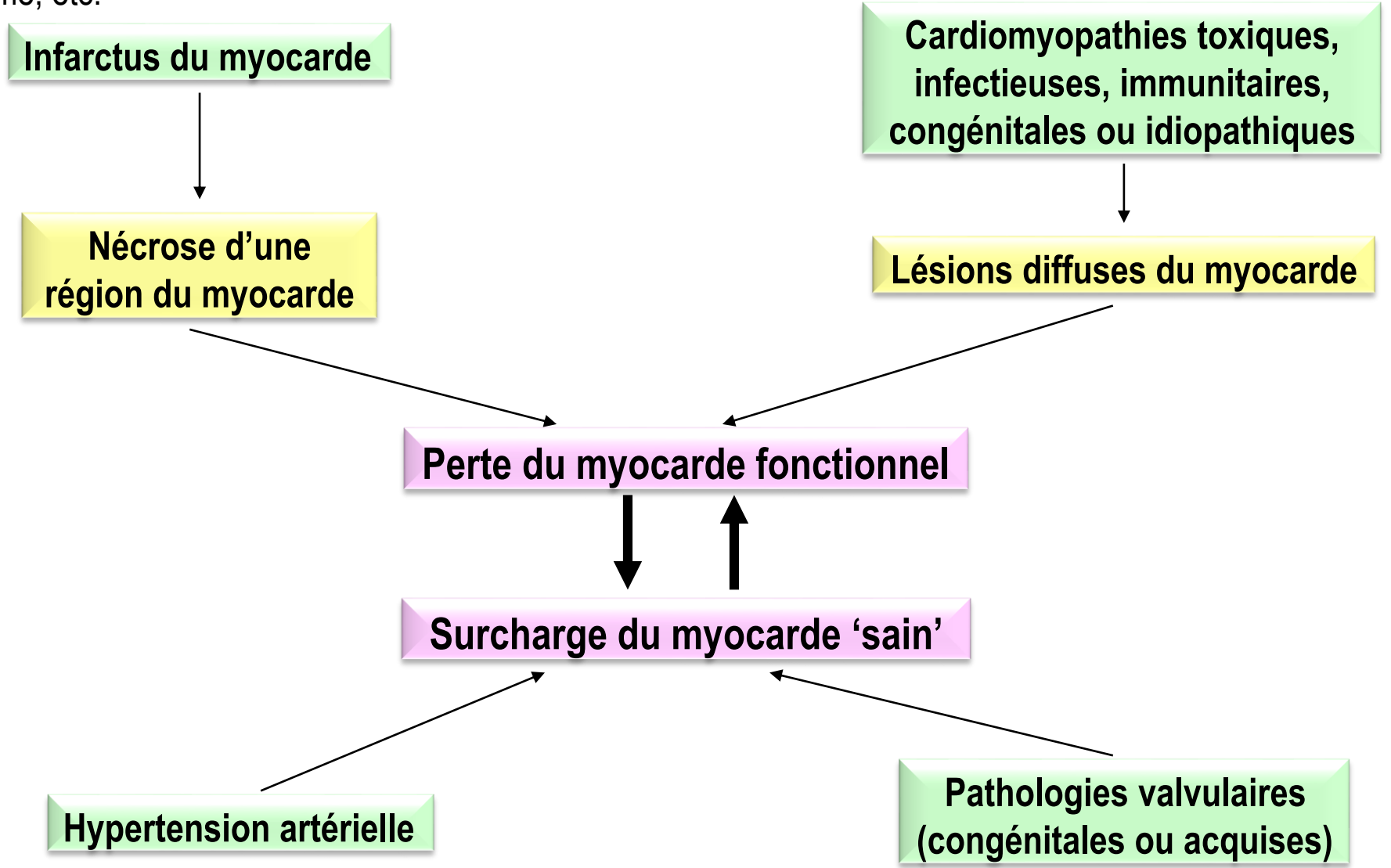
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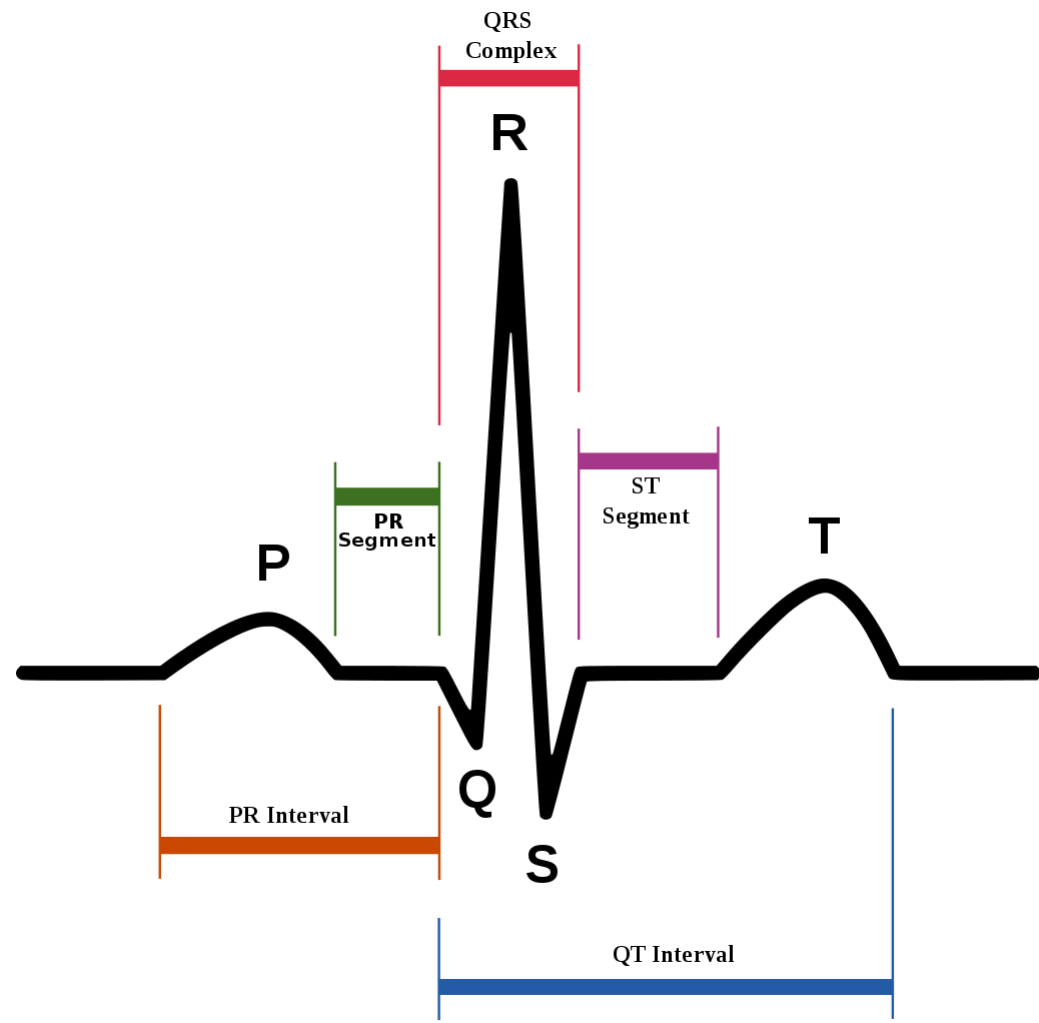
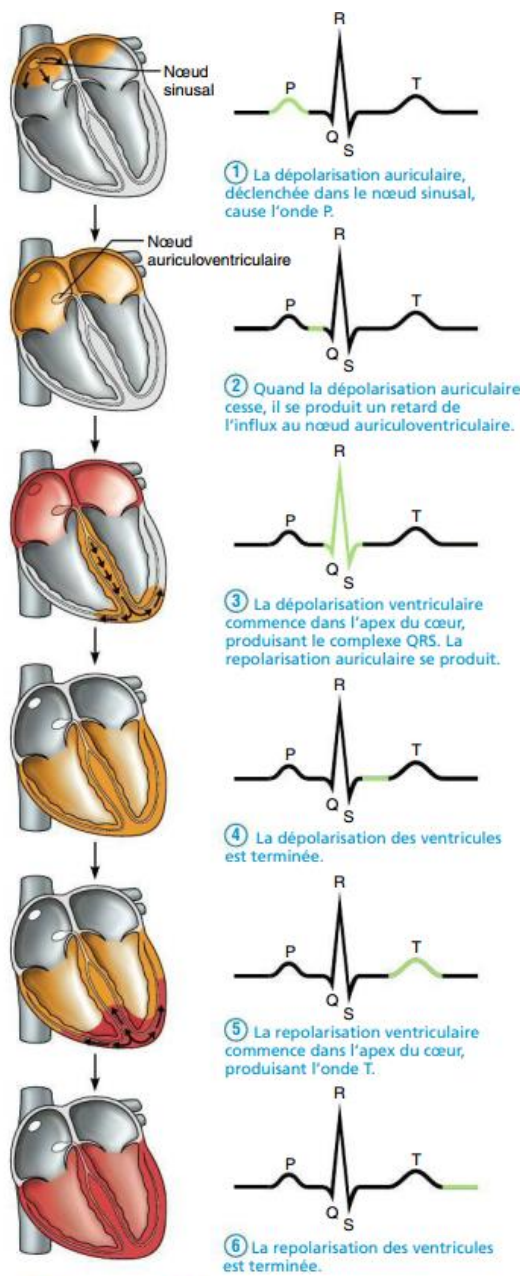
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L'IC et les étiologies

✓ L'insuffisance cardiaque est une **pathologie évolutive chronique** (elle peut être aiguë dans l'infarctus du myocarde ou la rupture de valve) qui peut être secondaire à de nombreuses étiologies : cardiopathies ischémiques, hypertension artérielle chronique, cardiomyopathies, cardiopathies valvulaires, troubles du rythme, etc.

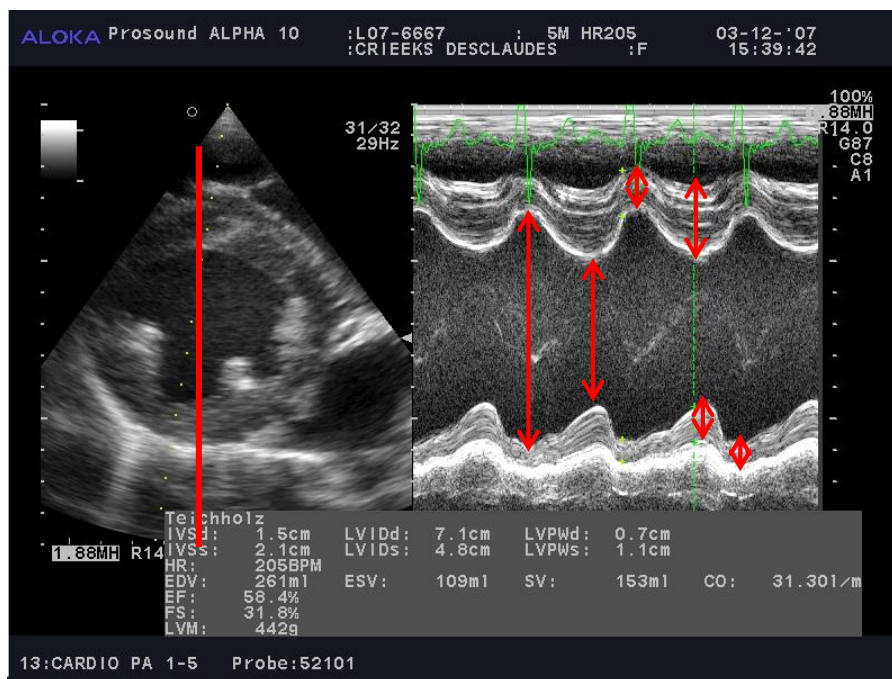


Electrocardiogramme (ECG) de repos: relation onde-propagation



- Exploration morphologique et fonctionnelle du cœur par ultrasons
- En mode bidimensionnel (BD) pour analyser en temps réel les structures cardiaques et la cinétique des parois myocardiques en 2D
- En mode temps-mouvement (TM) pour mesurer les mouvements des différentes structures cardiaques, les dimensions des parois et des cavités cardiaques et les fonctions ventriculaires en fonction du temps

Coupe parasternale petit axe



Paroi antérieure (ou du septum)
en diastole et systole (IVSd et IVSs)

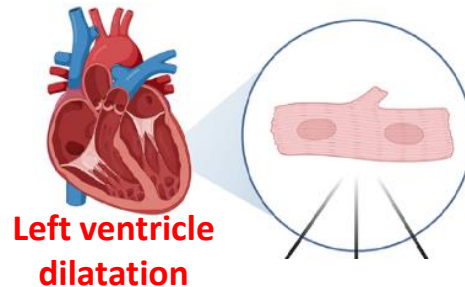
Diamètre ventriculaire en diastole et systole
(LVEDd et LVESd)

Paroi postérieure en diastole et systole
(LVPWd et LVPWs)

$FR (\%) = \frac{DTD-DTS}{DTS}$

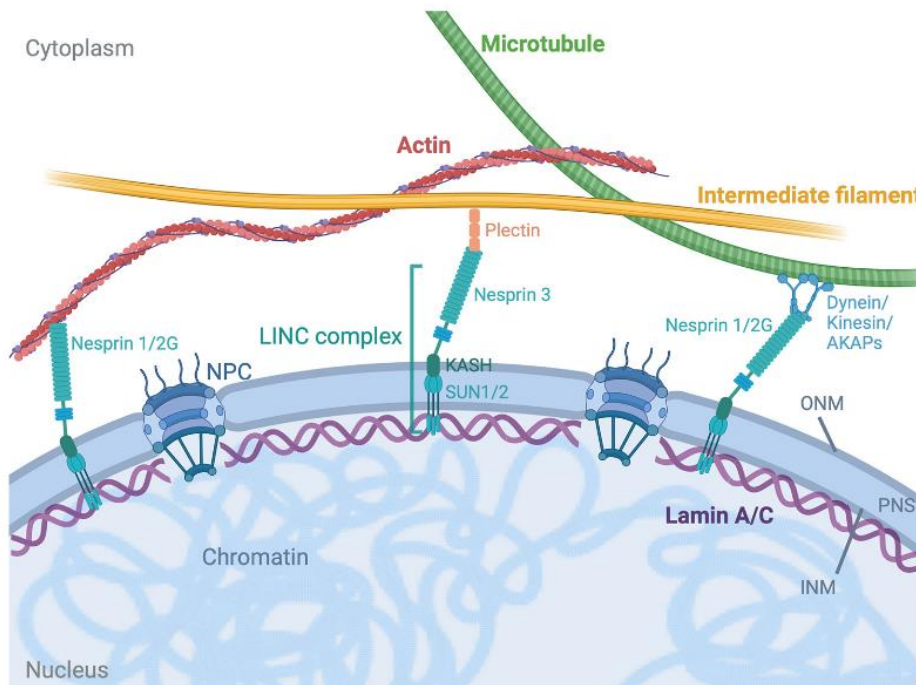
$FE (\%) = \frac{VTD-VTS}{VTS}$ (modèle de Teicholz)

Mutations in the lamin A/C gene (*LMNA*) cause dilated cardiomyopathy



Cardiomyocyte defects :

- Nuclear structure / Mechano-transduction
- Chromatin binding / Gene transcription
- Cellular signaling
- Cytoskeleton organization



CLINICAL MANIFESTATIONS

Dilated cardiomyopathy

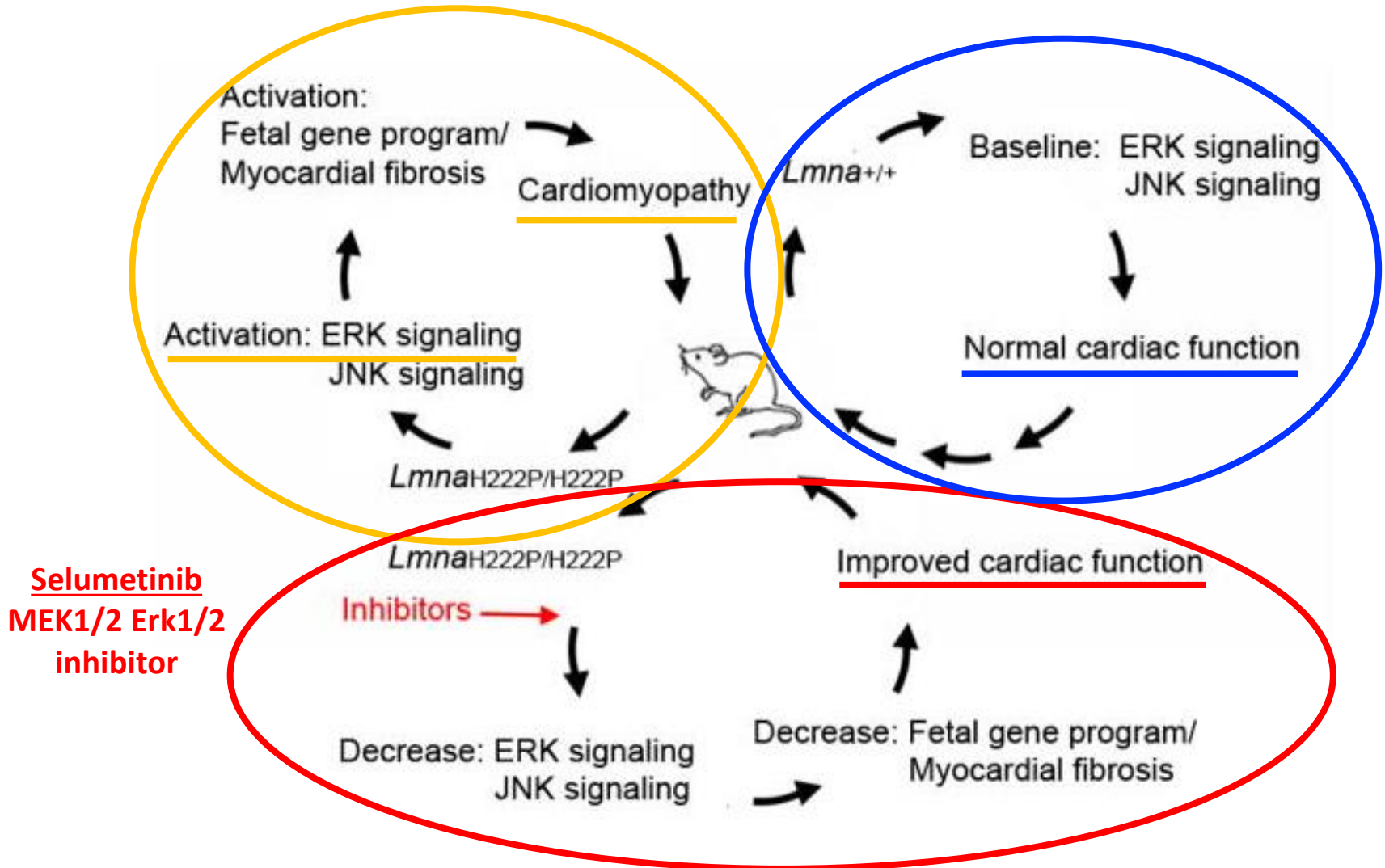
Cardiac conduction system disease

Atrial and ventricular arrhythmias

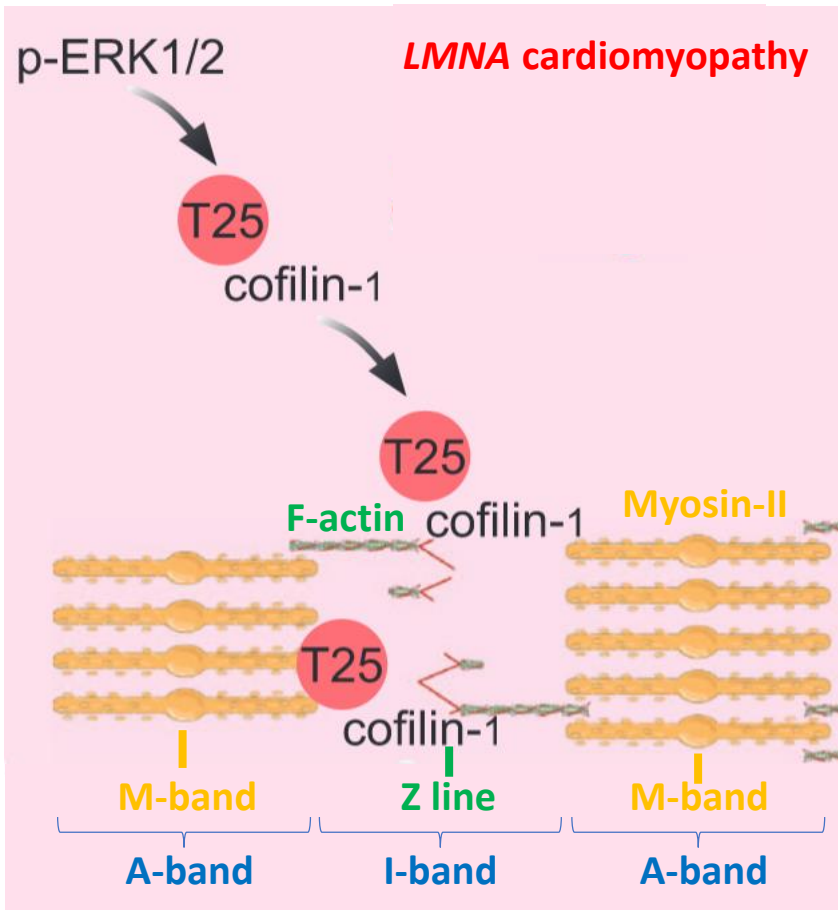
Sudden cardiac death

+/- skeletal muscle phenotypes

ERK1/2 is hyper-activated in *LMNA* cardiomyopathy



Cofilin-1 phospho-activation at Thr 25 by ERK1/2 stimulates actin disassembly in *LMNA* cardiomyopathy



Cofilin tools :

- . T25A nonphosphorylatable
- . T25D phosphomimetic
- . V20A mutated NES (nuclear export signal)

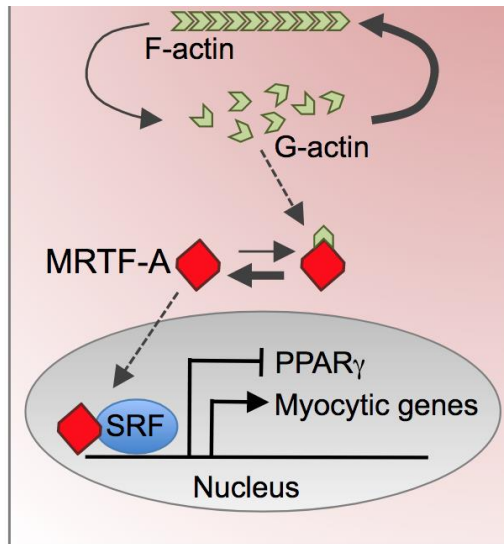
Cofilin-1 / actin binding protein / severing and depolymerizing F-actin

MRTF-A / SRF :

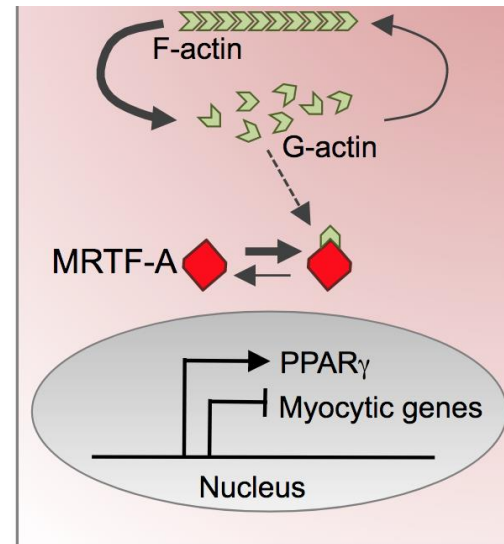
myocardin-related transcription factor A / serum response factor

SRF-mediated transcription is regulated by F/G actin

F-actin
allows transcription



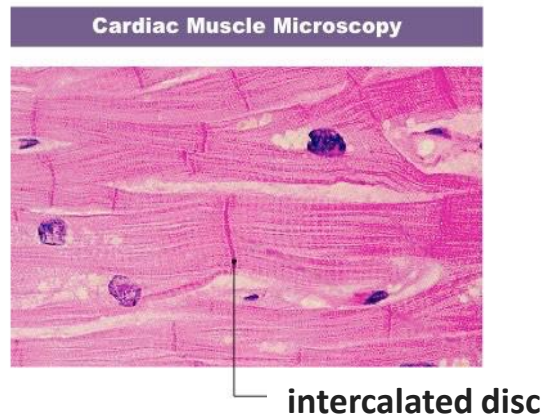
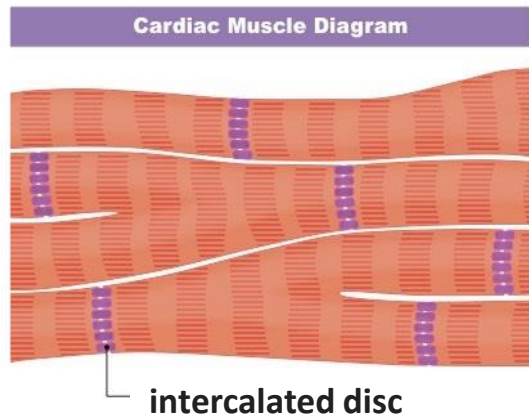
G-actin
prevents transcription



MRTF-A / SRF deregulation lead to heart failure

Disruption of SRF in adult mice heart :

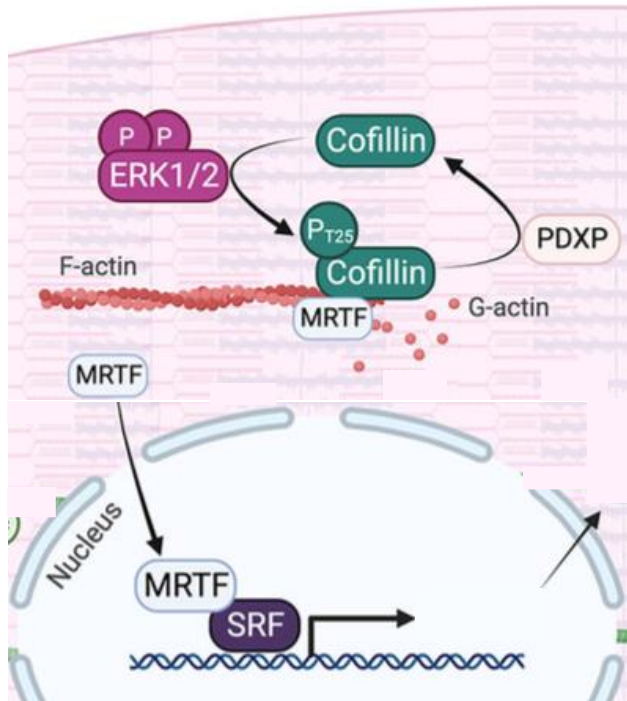
- . impaired left ventricular function : reduced contractility, progressive cardiac dilatation.
- . early decreases in the cardiac gene expression program:
cardiac alpha-actin, muscle creatine kinase, calcium-handling genes.
- . Altered cytoarchitecture of cardiomyocytes in the intercalated discs.



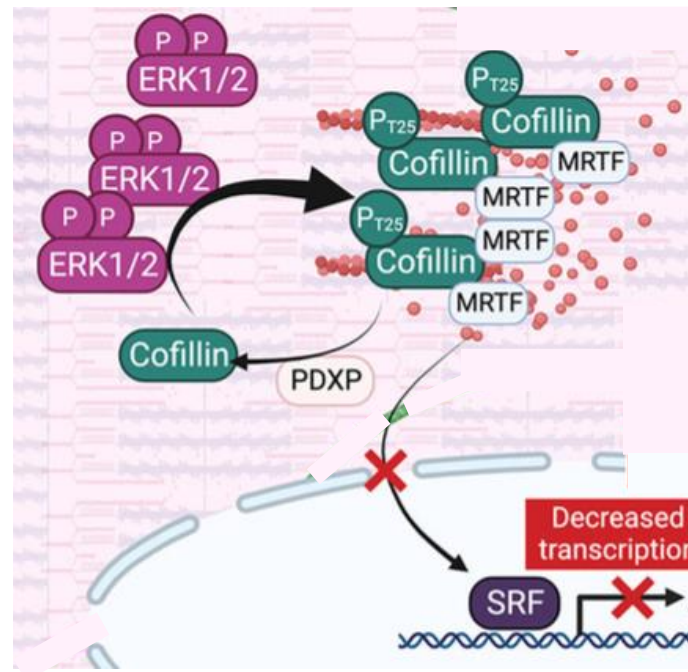
Heart failure in 10 weeks

Cofilin-1 phosphorylated on T25 binds MRTF-A and prevents its nuclear localization in *LMNA* cardiomyopathy

Healthy

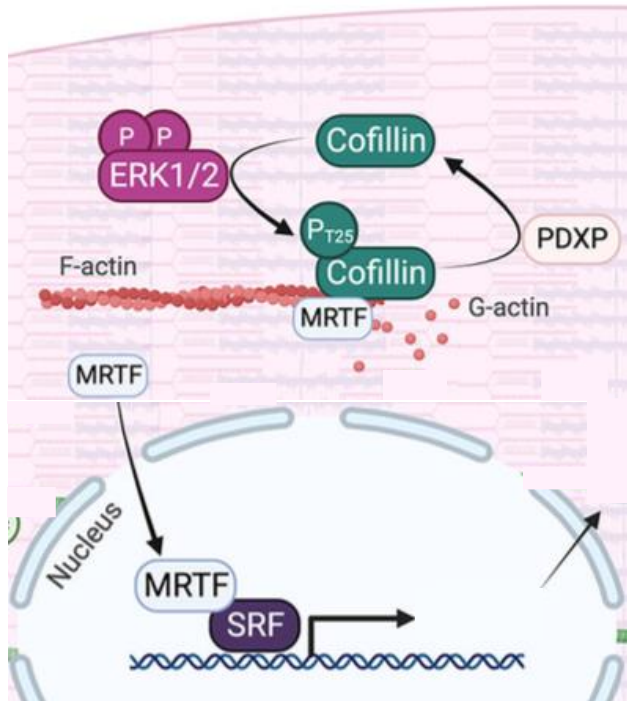


LMNA cardiomyopathy

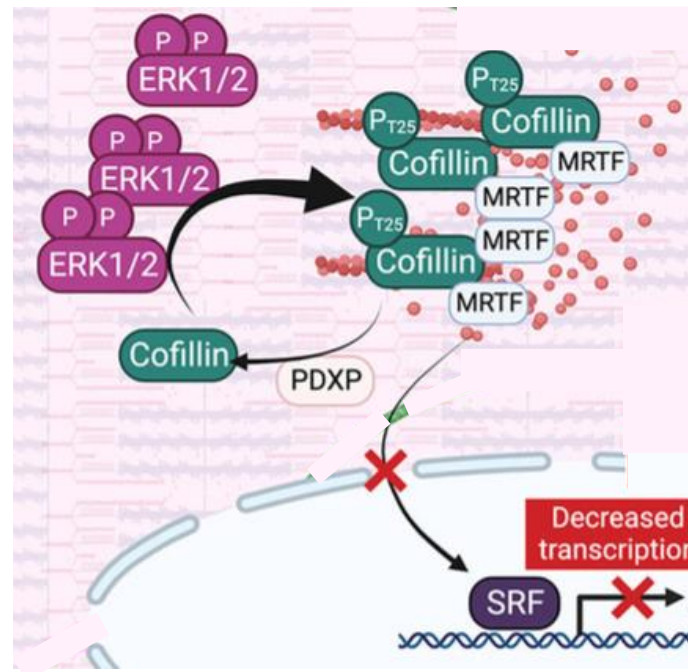


Cofilin-1 phosphorylated on T25 binds MRTF-A and prevents its nuclear localization in *LMNA* cardiomyopathy

Healthy

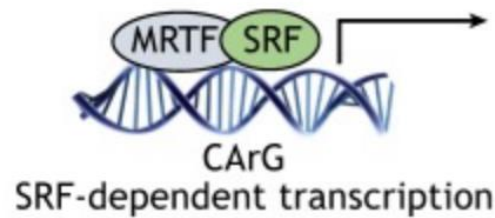


LMNA cardiomyopathy

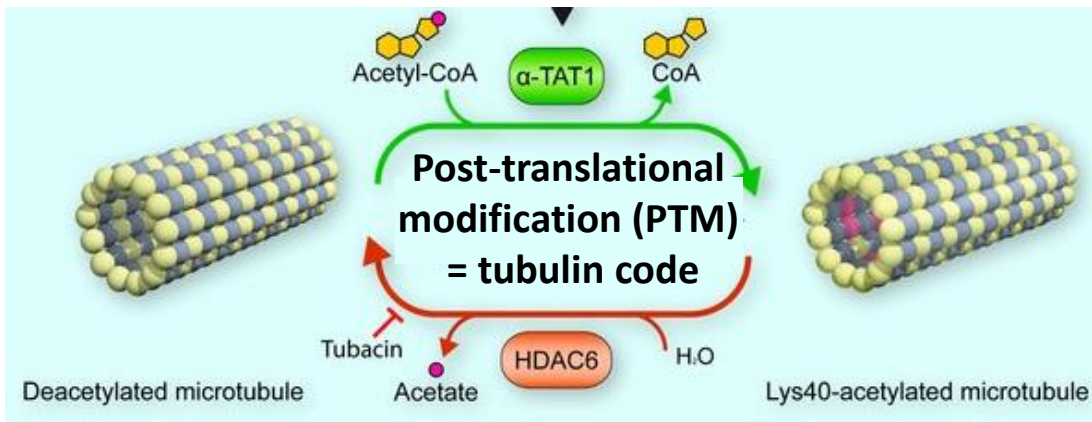


- Regulation**
- . Microtubules
 - . GAP junctions
 - . Cardiac function

α -TAT1 transcription is stimulated by SRF, leading to microtubule acetylation



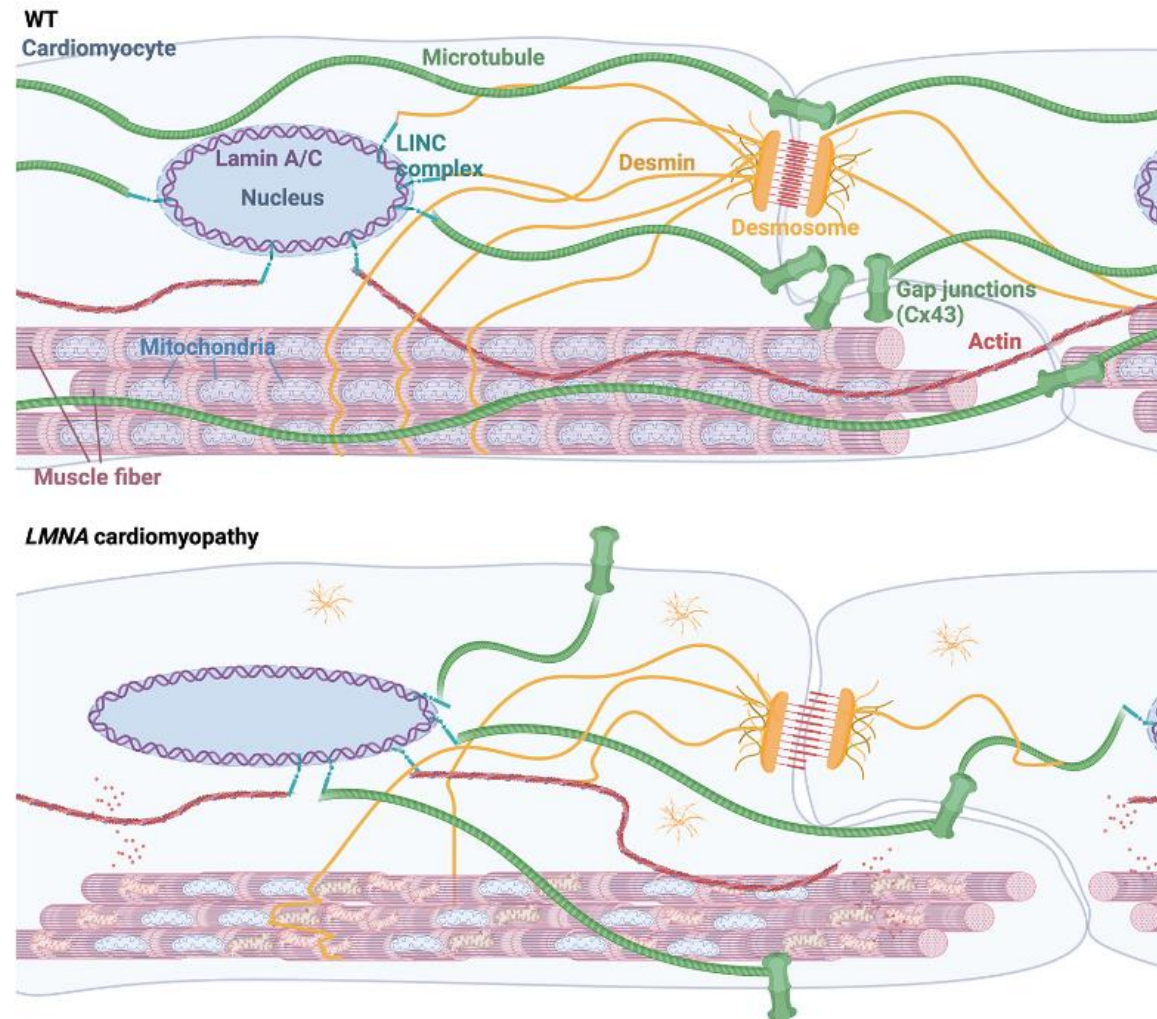
Actin, WASP Rho-GEFs Cofilin, Arp2/3 Gelsolin		Actin dynamics protrusion
LIMK Pctaire1	Cofilin phosphorylation	
Myl 9	Contractility	
Integrin, vinculin, ISG15, cadherin,CCN	Cell-cell, cell-ECM adhesion	
Ezrin, PIP5K	Membrane/cytoskeletal coupling?	
MMP	ECM degradation	
ATAT1	Microtubule control	
miRs (96,21,143/145)		



α TAT-1 : α -tubulin acetyl transferase (K40)
HDAC6 : deacetylase
(inhibited by Tubacin, Tubastatin A)

Gau & Roy, J. Cell Sci., 2018
Fernandez-Barrera et al, Aging, 2018

Gap junctions are altered in *LMNA* cardiomyopathy



Connexin 43 (Cx43) Gap junctions at the intercalated discs (microtubule / kinesin-mediated transport)

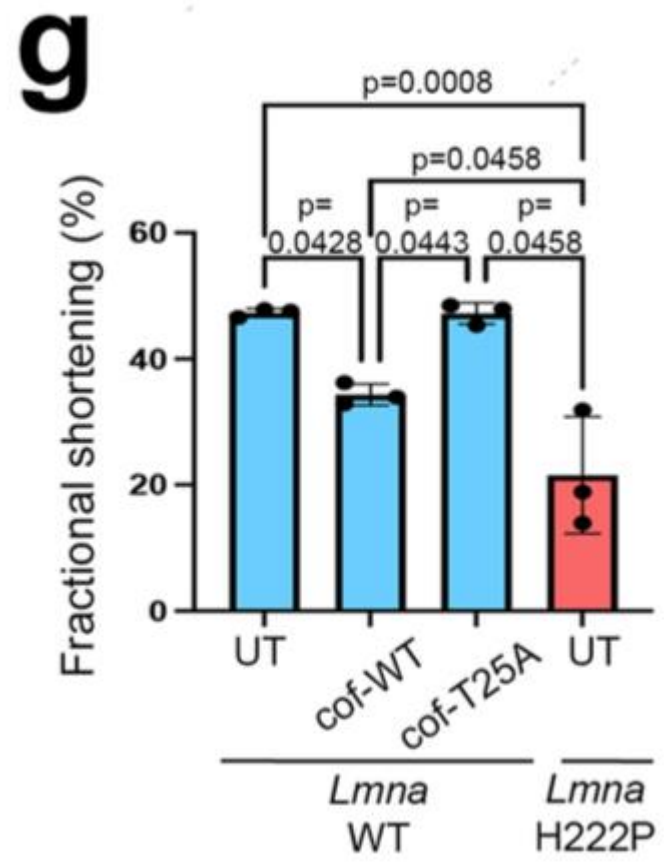
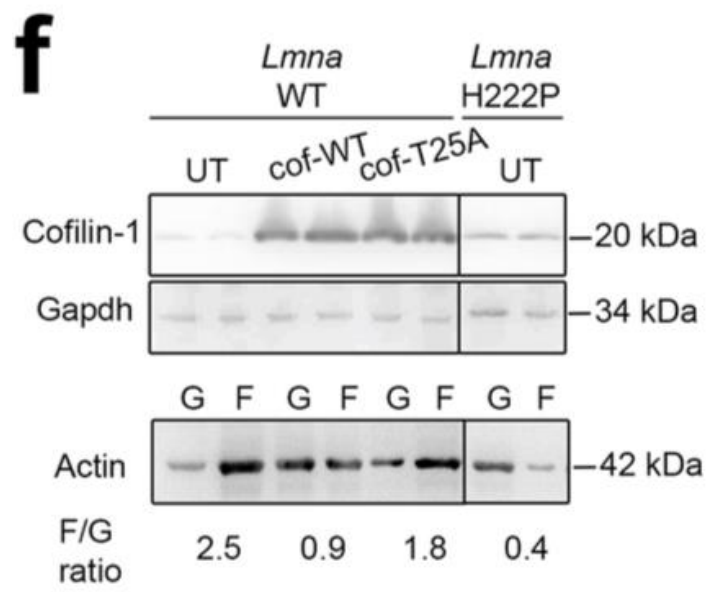
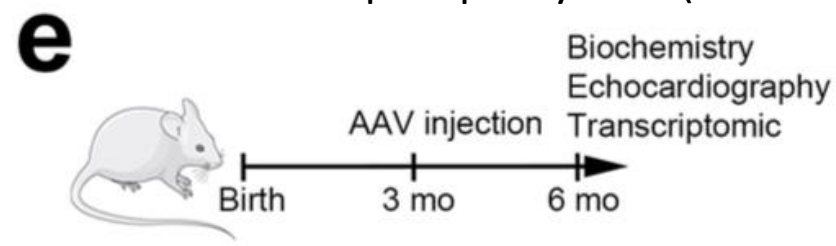
Coordinated contraction & pumping activity in heart

Lateral redistribution of Cx43

Also fibrosis with collagen fibrils (Sirius red staining in the extracellular matrix ECM)

Analysis : figure 3

Cofilin-1-WT
Cofilin-1 non phosphorylable (Cofilin-T25A)

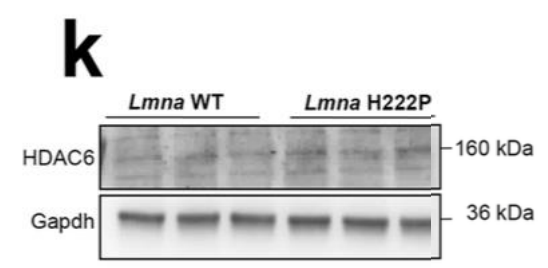
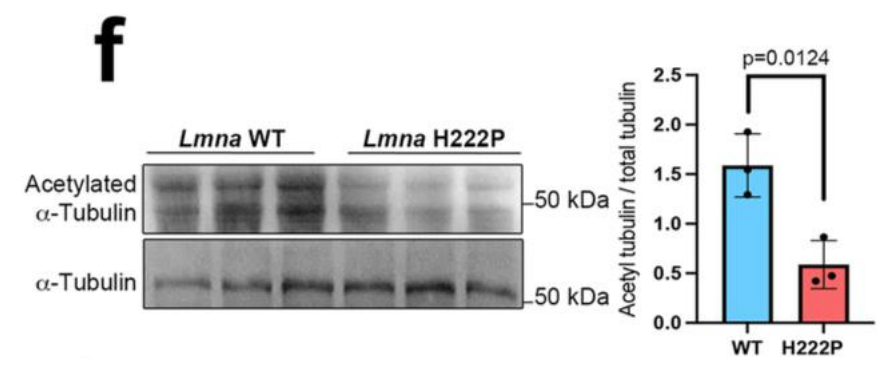
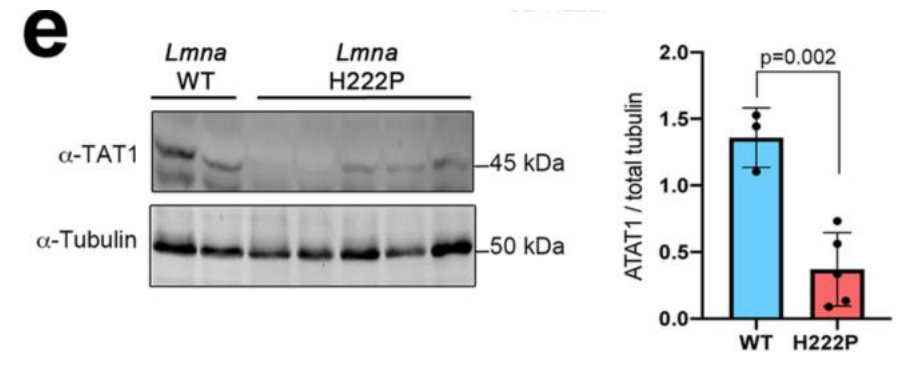
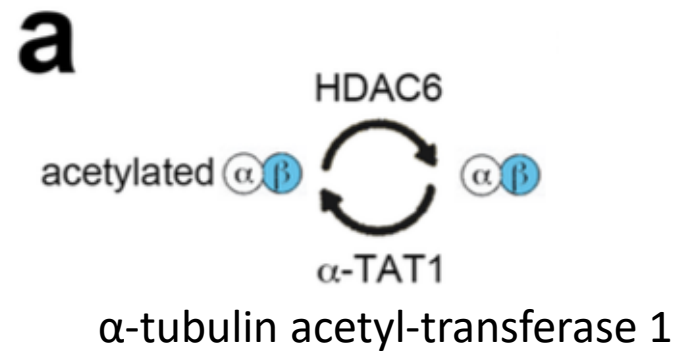


Analysis : table S1 related to figure 3

	<i>Lmna</i> WT	<i>Lmna</i> WT	<i>Lmna</i> WT	<i>Lmna</i> H222P
	NT	AAV-cofilin WT	AAV-cofilin T25A	NT
age	6 months	6 months	6 months	6 months
n	3	3	3	3
time (ms)	93,033 ± 1,86	101,82 ± 2,37 **	108,13 ± 1,66 ***	96,11 ± 2,88
heart rate (bpm)	645,07 ± 12,80	589,53 ± 13,61 **	554,9 ± 8,6 ***	624,6 ± 18,51
cardiac output (l/min)	0,06 ± 0	0,05 ± 0,01	0,06 ± 0,02	0,07 ± 0,01
IVSd (cm)	0,07 ± 0	0,06 ± 0,005	0,07 ± 0,005	0,06 ± 0
LVDd (cm)	0,35 ± 0,01	0,36 ± 0,03	0,37 ± 0,04	0,47 ± 0,07 *
LVPWd (cm)	0,07 ± 0	0,06 ± 0	0,07 ± 0,011	0,06 ± 0,005
IVSs (cm)	0,12 ± 0,01	0,09 ± 0,01 **	0,13 ± 0,005	0,09 ± 0,01 **
LVDs (cm)	0,18 ± 0,005	0,23 ± 0,025	0,19 ± 0,025	0,37 ± 0,09 **
LVPWs (cm)	0,12 ± 0,01	0,09 ± 0,005	0,11 ± 0,015	0,08 ± 0 **
LVED vol (ml)	0,113 ± 0,005	0,12 ± 0,026	0,13 ± 0,04	0,26 ± 0,11
LVES vol (ml)	0,02 ± 0	0,036 ± 0,01	0,02 ± 0,01	0,14 ± 0,10
EF (%)	84,4 ± 0,58	71,12 ± 3,67 *	84,28 ± 1,58	48,760 ± 16,50 **
FS (%)	47,32 ± 0,67	35,01 ± 2,89 *	47,22 ± 1,68	21,54 ± 9,27 ***
LV eject vol (ml)	0,09 ± 0,005	0,08 ± 0,015	0,11 ± 0,03	0,11 ± 0,02
h/r	0,39 ± 0,007	0,34 ± 0,032	0,40 ± 0,03	0,26 ± 0,03 **

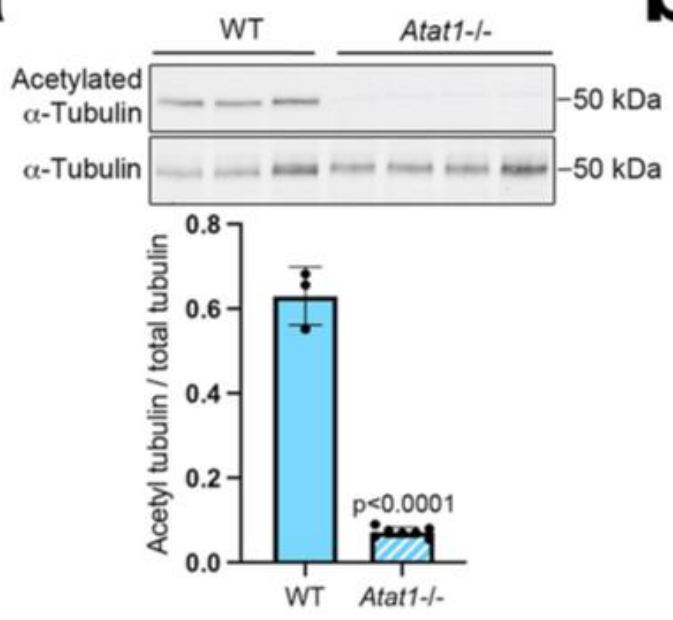
Table S1| Echocardiographic data for *Lmna*^{+/+} (WT) mice transduced or not transduced with AAV-cofilin-1 or AAV-cofilin-1-T25A at 6 months of age. IVS, inter ventricular septum; LVD, left ventricular diameter; LVPW, left ventricular posterior wall; LVED, left ventricular end diastolic; LVES, left ventricular end systolic; EF, ejection fraction; FS, fractional shortening; s, systole; d, diastole. Values are means ± SEM. *p≤0.05, **p≤0.01 and ***p≤0.001 between *Lmna*^{+/+} mice transduced and *Lmna*^{+/+} mice not transduced. Values for *Lmna*^{p.H222P/H222P} mice are shown as comparison.

Analysis : figure 4

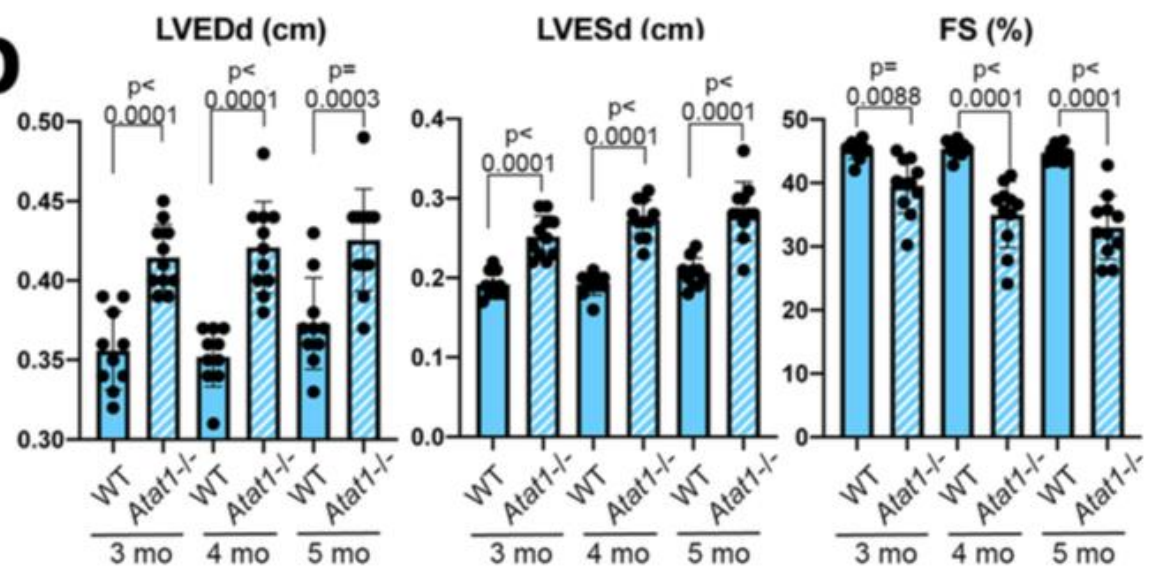


Analysis : figure 5

a

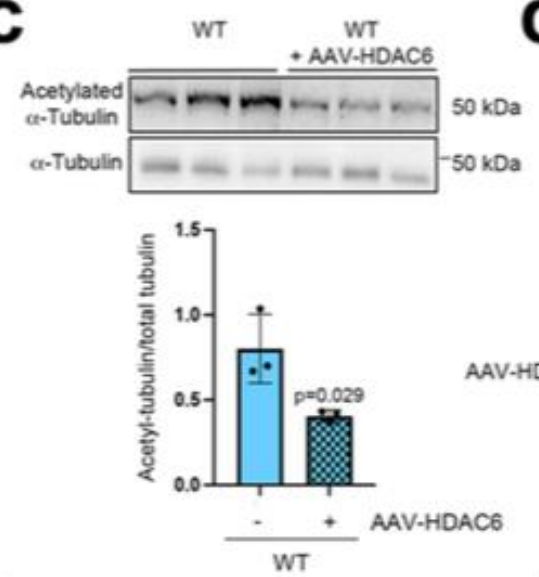


b

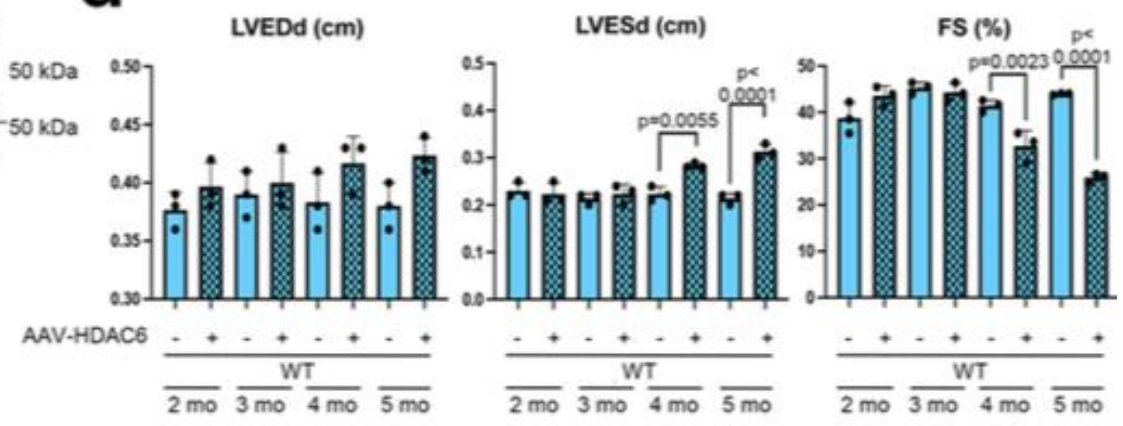


Analysis : figure 5

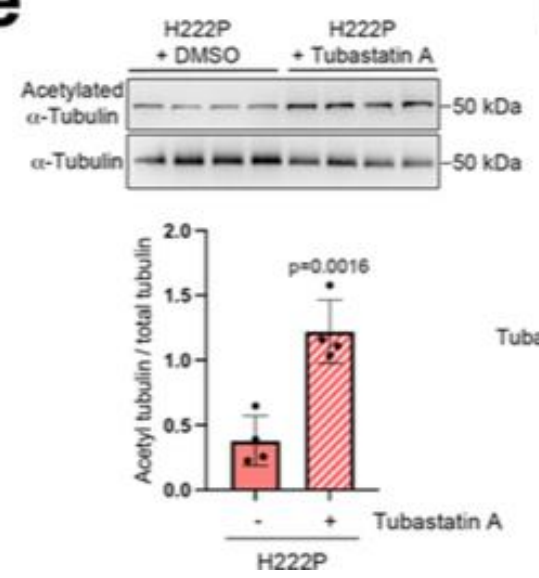
c



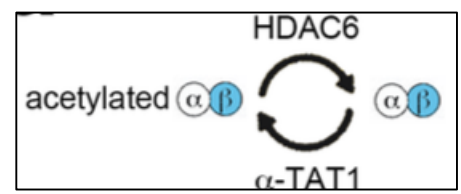
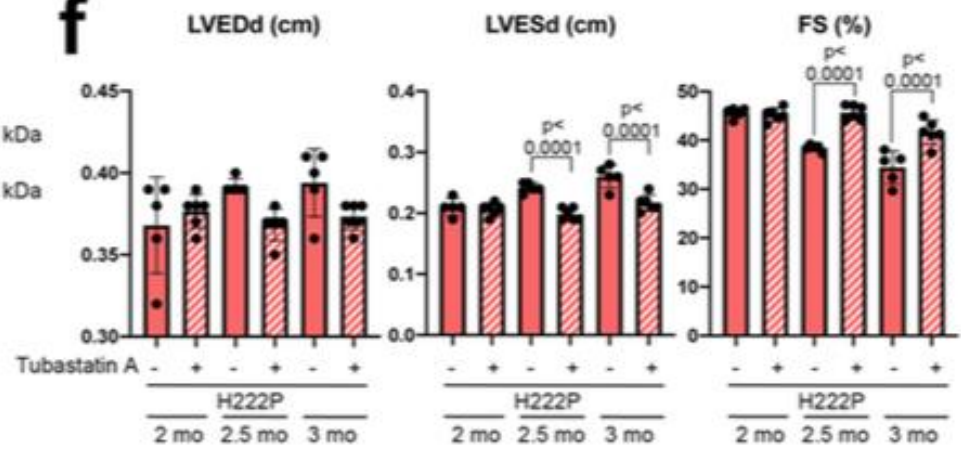
d



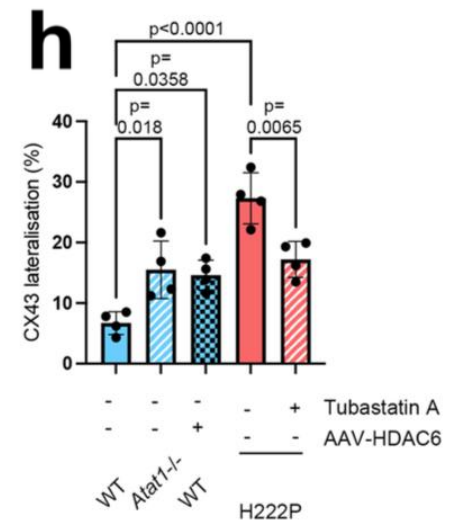
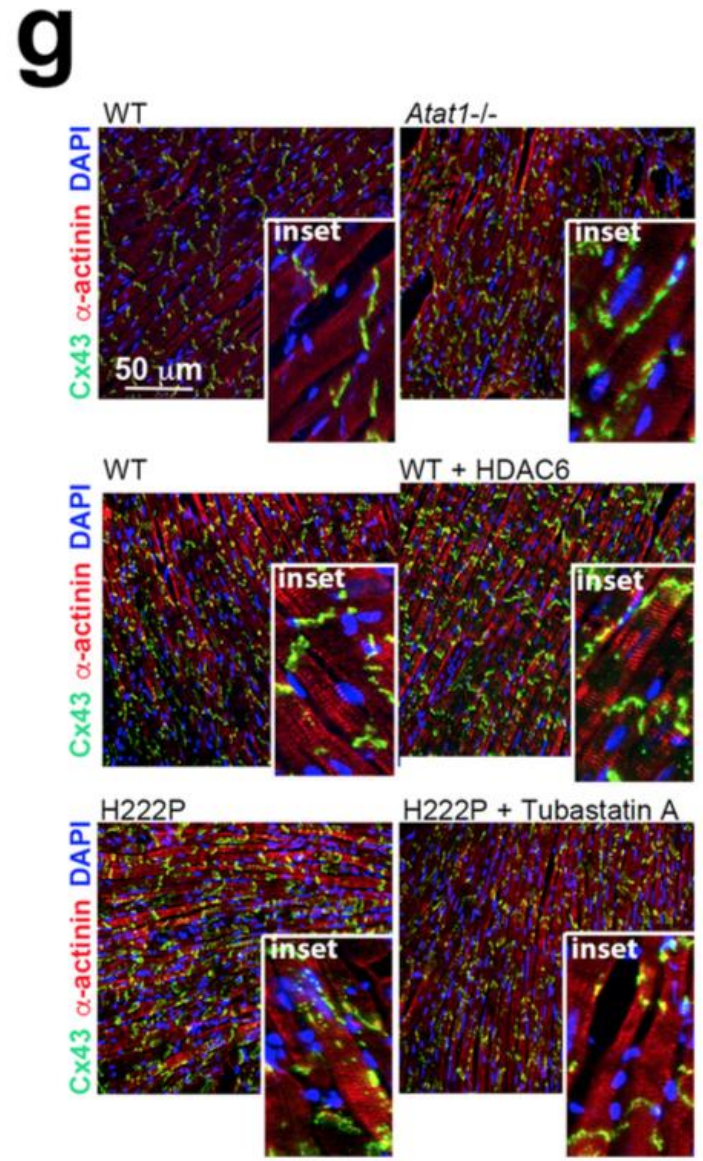
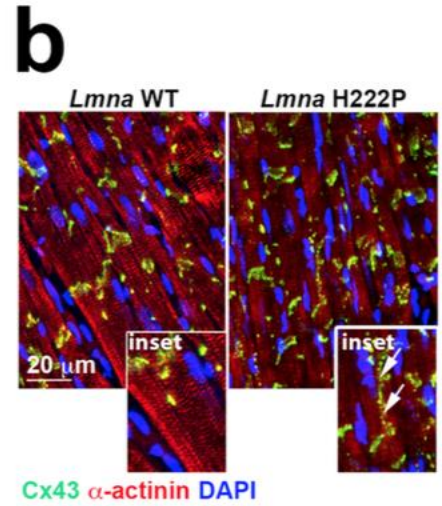
e



f



Analysis : figure 6



Analysis : table S6 related to figure 6

Table S6| ECG parameters. Baseline values of ECG parameters measured in *Lmna*^{p.H222P/H222P} mice treated with Tubastatin A as compared with DMSO-treated (top table); in *Atat1*^{-/-} (KO) mice as compared with wild-type mice (WT) (middle table) and in mice transduced with AAV overexpressing HDAC6 as compared with WT mice (bottom table).

	H222P	H222P	H222P	H222P	H222P	H222P
	DMSO	Tubastatin	DMSO	Tubastatin	DMSO	Tubastatin
age	2 months	2 months	2,5 months	2,5 months	3 months	3 months
n	4	5	4	3	4	3
RR (ms)	80.55 ± 1.9	80.84 ± 0.9	80.3 ± 1.2	78.9 ± 0.6	78.61 ± 1.1	79.3 ± 0.8
PR (ms)	34.23 ± 0.7	35.1 ± 0.6	33.7 ± 0.6	32.82 ± 0.77	34.08 ± 0.7	32.55 ± 0.38
QRS (ms)	13.64 ± 0.42	13.44 ± 0.44	13.20 ± 0.37	14.136 ± 0.39	18.38 ± 1.7	18.20 ± 1.29

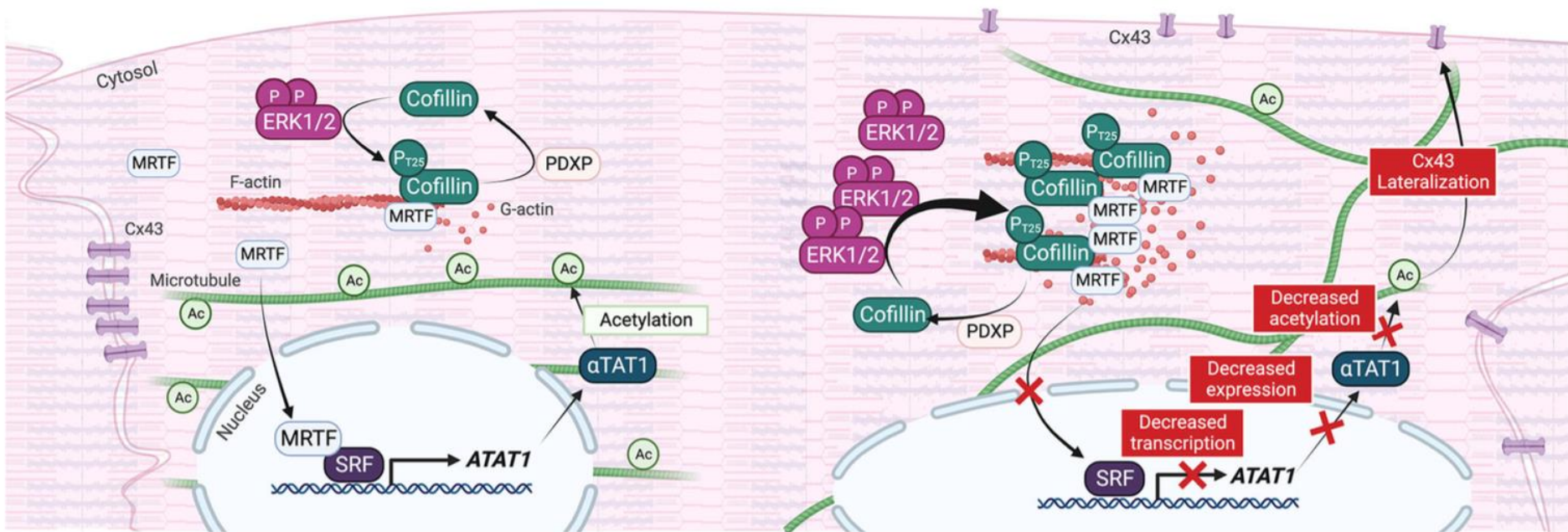
	WT	Atat1 KO	WT	Atat1 KO	WT	Atat1 KO
age	3 months	3 months	4 months	4 months	5 months	5 months
n	10	11	10	11	4	11
RR (ms)	80.55 ± 1.9	80.84 ± 0.9	80.3 ± 1.2	78.9 ± 0.6	78.61 ± 1.1	79.3 ± 0.8
PR (ms)	34.23 ± 0.7	35.1 ± 0.6	33.7 ± 0.6	32.82 ± 0.77	34.08 ± 0.7	32.55 ± 0.38
QRS (ms)	12.16 ± 0.19	11.66 ± 0.2	11.99 ± 0.19	11.46 ± 0.11	12.05 ± 0.44	11.43 ± 0.17

	WT	AAV-HDAC6	WT	AAV-HDAC6	WT	AAV-HDAC6	WT	AAV-HDAC6
age	2 months	2 months	3 months	3 months	4 months	4 months	5 months	5 months
n	3	3	3	3	3	3	3	3
RR (ms)	78.8 ± 0.32	79.72 ± 0.46	78.77 ± 0.8	81.58 ± 1.3	79.20 ± 1.6	83.11 ± 3.4	83.05 ± 1.6	86.14 ± 4.7
PR (ms)	33.2 ± 0.7	33.24 ± 0.7	32.67 ± 0.37	34.16 ± 0.9	33.15 ± 1.58	34.56 ± 1.2	36.168 ± 1.07	34.41 ± 1.14
QRS (ms)	12.27 ± 0.18	11.74 ± 0.11	12.20 ± 0.26	11.81 ± 0.18	12.11 ± 0.07	11.63 ± 0.31	11.44 ± 0.32	11.49 ± 0.23

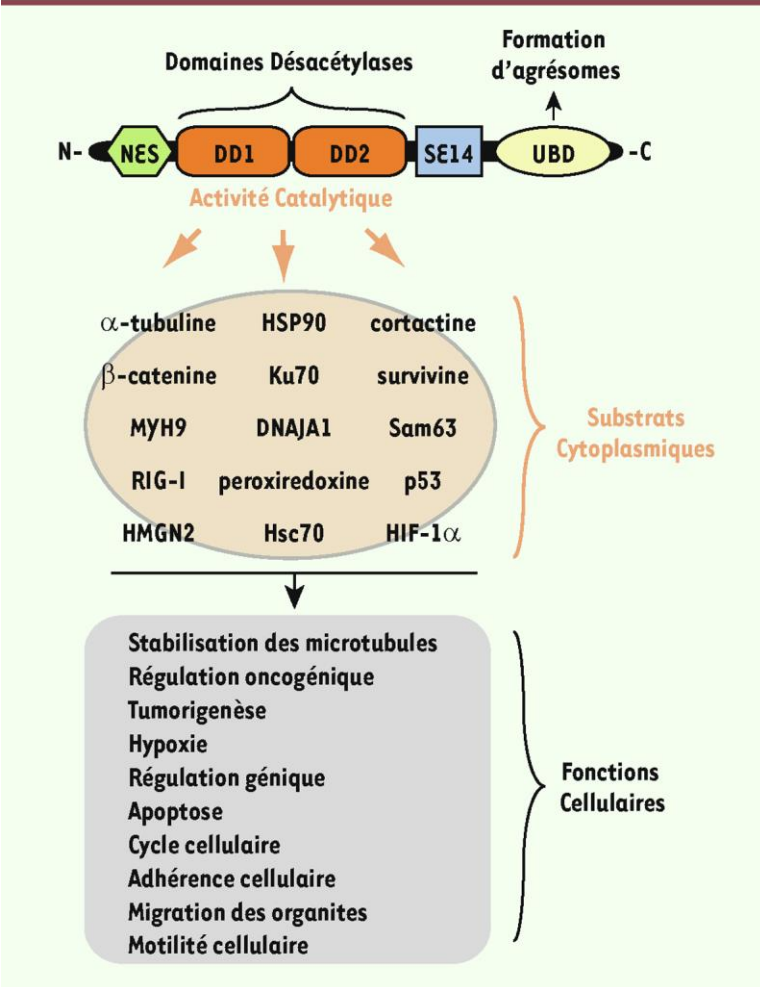
Conclusion of the study

Healthy

LMNA-cardiomyopathy



HDAC6 a new key player in the treatment of many diseases

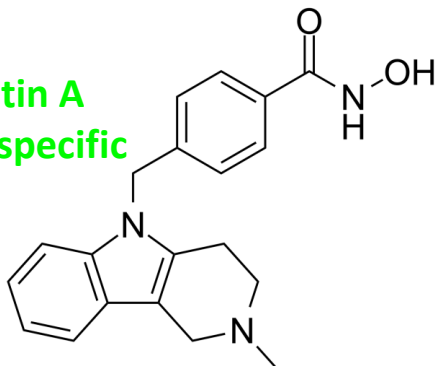


Cytoplasmic HDAC not affecting histones but with several substrates in the cytoplasm

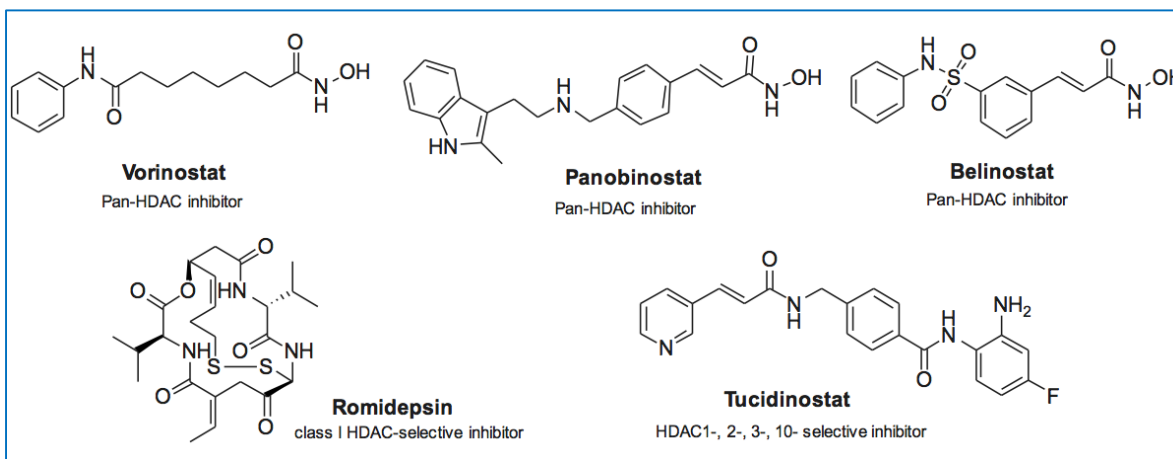
HDAC6 KO mice are fine
 HDAC6 is only a stress response factor ?

HDAC inhibitors in clinic

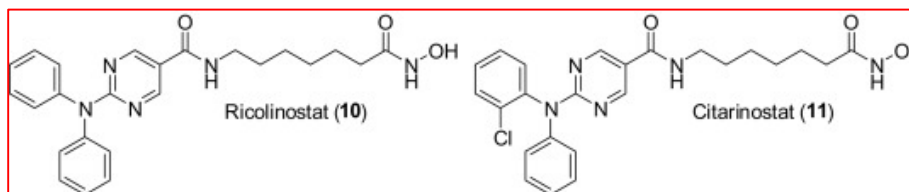
Tubastatin A
HDAC6 specific



- . displays mutagenicity/genotoxicity and poor oral delivery
- . hindering development for clinical use



Pan-HDACi (histone)
Approved for clinic

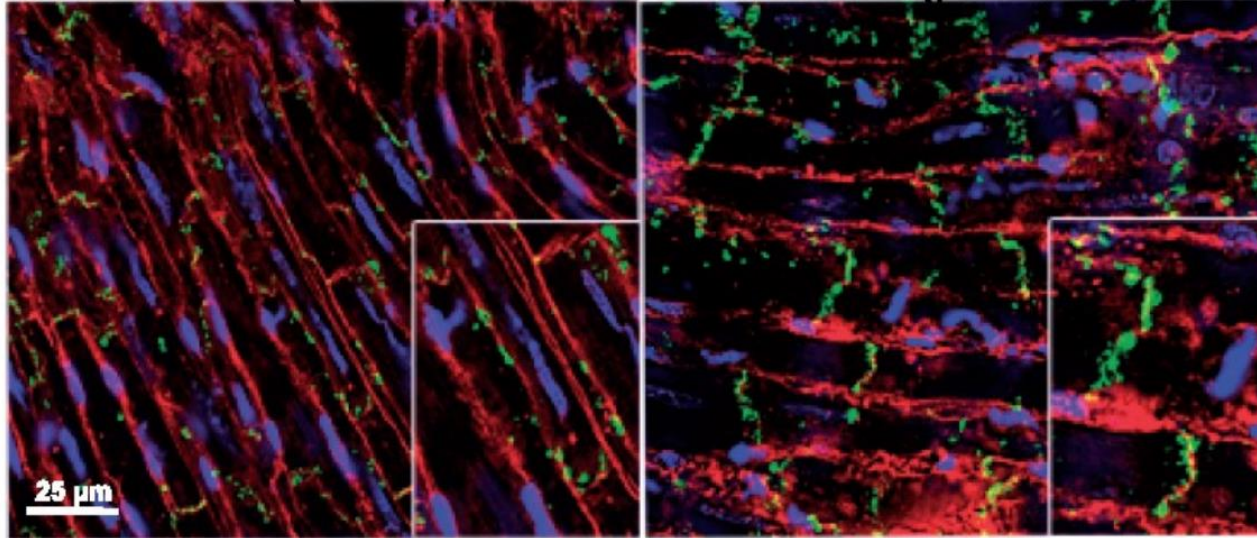


HDAC6 specific
Clinical trial

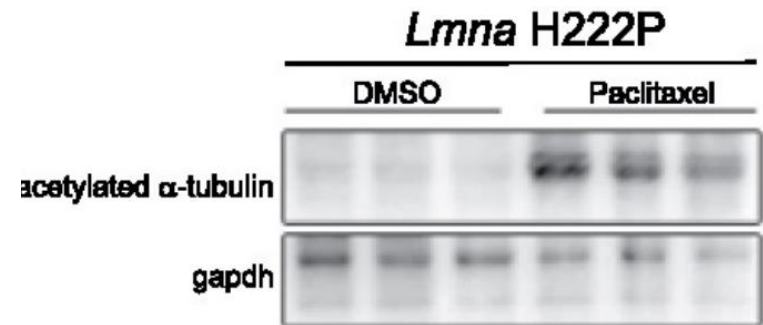
Paclitaxel / Taxol to increase α -tubulin acetylation in clinic ?

Lmna H222P (DMSO)

Lmna H222P (paclitaxel)



connexin 43
wheat germ agglutinin
dapi



Dilated cardiomyopathy

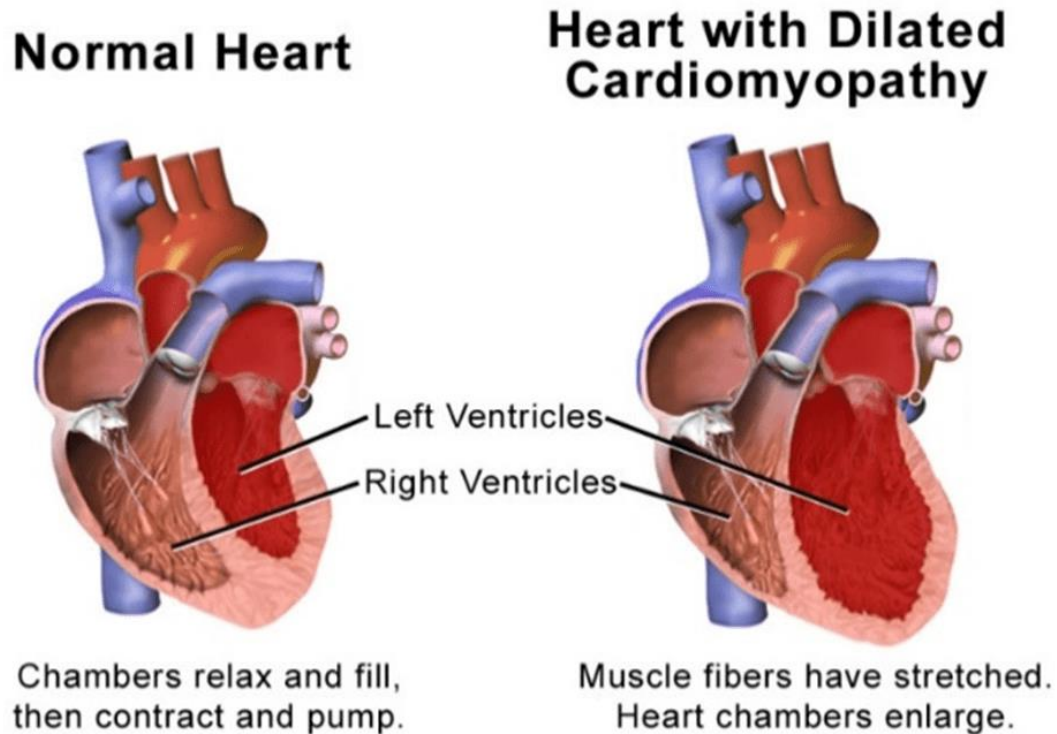


Figure 1. A comparison between a normal heart (left) and one with dilated cardiomyopathy (right), potentially caused by a mutation in LMNA.

Intercalated disc

