Supporting Information Modeling

Fusion-fission-mitophagy cycling and metabolic reprogramming coordinate Nerve Growth Factor (NGF)-dependent neuronal differentiation

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Computational Modeling: Raw data, instructions and recipes/Global quantities for the Copasi simulations

Diagram of the fusion-fission-mitophagy model

The Figure shows the diagram for the model (Figure 5, main text): Networked mitochondria are indicated by capital N and dispersed mitochondria by capital D. For each of these mitochondria there are three versions, i.e. undamaged (N, or D), somewhat damaged (Nd and Dd) and severely damaged (Ndd and Ddd). *ros* refers to ROS in the vicinity of the networked mitochondria. Nd and Ndd activate 'ros' synthesis beyond a constitutive process, whereas 'ROS' synthesis is fully dependent on Dd and Ddd. The rate constants for the three fission processes from networked to dispersed are identical and called the 'fission rate constant'. Analogously, the rate constants for the fusion of dispersed to networked mitochondria are all equal to the 'fusion rate constant'. Solid arrows refer to mass flow (mitochondria or ROS). Dashed arrows refer to influence/regulation. 'v' refers to reaction rates. It will be assumed that NGF addition increases the rate constant for fission.
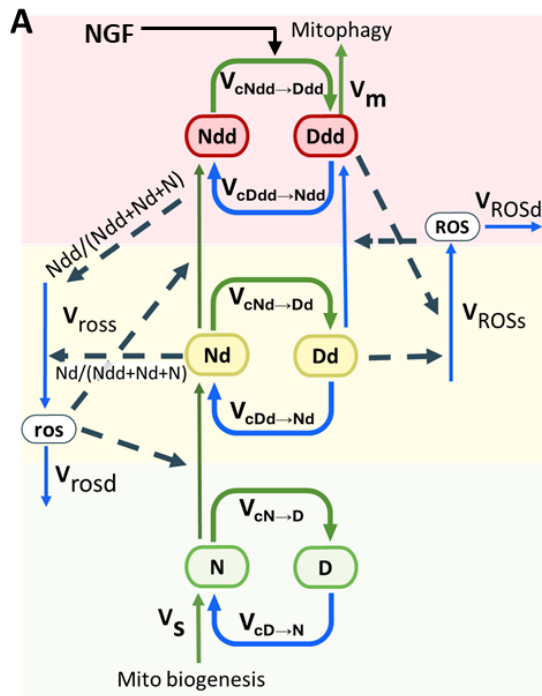
In our preliminary modeling studies, the concentration of mitochondria changed strongly by a factor of 2.5 within a few hours after NGF addition (Westerhoff, unpublished). Although this simulated the doubling of the respiratory rate observed in (Martorana et al., 2018), it was considered unrealistic as no such a strong change was observed microscopically in the mitochondrial mass. We therefore turned to a model where the total number of mitochondria did not change so fast by so much. In order to explain the 2-fold increase in respiration observed experimentally 24h after NGF addition, we instead considered the possibility that the strongly damaged mitochondria would be so damaged in their Complex I of the respiratory chain that they had strongly reduced respiration.

The preliminary model exhibited a strong decrease in mitophagy flux within a few hours (after a rapid transient 4-folds increase) (Westerhoff, unpublished). The experimental data were consistent with a substantial increase in mitophagy however (and autophagy in Martorana et al. 2018). We gathered that the strong dependence of the mitophagy flux on the Ddd concentration kept the concentration change of Ddd small.

This made us consider a variant of the model in which the rate of mitophagy was a Michaelis-Menten function ($v = \frac{Ddd \cdot \frac{V}{K_M}}{1 + \frac{Ddd}{K_M}}$) of the concentration of Ddd, where the K_M was chosen around the average Ddd concentration in the model (Westerhoff, unpublished). This was inspired by the experimental observation that the number of mitochondria around a potential mitophagy site was often much higher than the number of lysosomes, potentially making the lysosomal concentration limiting (Westerhoff and Colangelo, unpublished).

This Copasi file was optimized further. This optimization involved minor further parameter changes and a facility exhibiting the time dependence before NGF addition. This facility was added to ensure that (e.g. upon parameter changes) the model should always start from the steady state. This Copasi was used for the simulations showed in Figure 5 in the main manuscript. The corresponding files are in the directory called 'fusion fission models' including:

- The text file 'instructions'
- The model file 'fig5.cps'
- Fig5D data
- Fig 5 Global quantities of the model with values

In modeling the effect of NGF addition, the crucial parameter is the fission rate constant, which is the sole parameter changed (i.e., increased by a factor of 20) to simulate the effect of NGF addition. k_cnd0_final = 3.6 is increased from 0.18 (=k_cnd0_initial), which was the value at the initial steady state. This increase is gradual at a $t_{1/2}$ of t_half_cnd=3.33 h through the formula:

k_cycling network to disperse= {Values[k_cnd0_initial].InitialValue}+{Values[NGF added]}*({Values[k_cnd0_final].InitialValue}-{Values[k_cnd0_initial].InitialValue})*(1-EXP(-({Time}-{Values[NGF addition time]})/{Values[t_half_cnd].InitialValue})).

The model starts from the parameter setting k_cnd0_final_0 = 0.01, which is then equal to k_cnd0_initial_0 and which, if maintained at the value, corresponds to the *initial* steady condition (see

below). This may be checked by time integration for 100 h after addition of NGF (or by asking for steady state and ignoring a Copasi warning), which should show that all variables are constant in time. The *final* steady state may be obtained by setting k_cnd0_initial_0 = 0.2 (equal to k_cnd0_final_0) and then asking for steady state. These settings of k_cnd0_initial_0 and k_cnd0_final_0 lead to the settings mentioned above for the actual rate constant for fission, i.e. k_cnd0_final = 3.6 and k_cnd0_initial=3.6.

The Copasi file of the model that produces Figure 5 carries the name: Fig5.cps

The model has the following concentrations of the variables in the two steady states (initial and final steady-states):

Initial steady state	(small fission rate constant) Obtained by setting both k_cnd0_initial_0 and k_cnd0_final_0 to 0.01	
Species	Concentration [µmol/l]	Concentration/Nmitomax
D	0.000176513	0.0882565
Dd	8.20E-06	0.004097735
Ddd	0.000214598	0.107299
N	0.000151928	0.075964
Nd	9.50E-05	0.047512
Ndd	0.000306239	0.1531195
ros	0.127895	
ROS	1.0734	
final steady state	(high fission rat constant) Obtained by setting both k_cnd0_initial_0 and k_cnd0_final_0 to 0.2	
Species	Concentration [µmol/l]	Concentration/Nmitomax
D	0.000844049	0.4220245
Dd	2.30E-05	0.01151525
Ddd	0.000103507	0.0517535
N	0.000353532	0.176766
Nd	1.56E-05	0.00780955
Ndd	4.35E-05	0.02177415
ros	0.0248888	
ROS	0.518685	

The global parameters are in the model file fig5.cps and in 'Fig 5 Global quantities of the model with values.xlsx' in the folder 'fusion fission model'

The model Fig5.cps produces the simulations of Figure 5B, 5C and 5E and can be used to reproduce the simulations used for the figure 5F, 5G and 5H.

Recipe for the simulation of the figures 5B, 5C, 5E and 5E_2

Open model Fig5.cps with Copasi, press task, time course and run simulation.

Recipe for the simulation used for the figures 5F

Open model fig5.cps and set the global quantity "k_cnd0_final_0" to 0.1, 0.05 or 0.02. Then run time course.

## Recipe for the simulation used for the figures 5G and 5H

Open model fig5.cps and set the global quantity "$K_{MMitophagy}$" to $1x10^{-4}$, $1x10^{-5}$ $1x10^{-2}$. Then run time course and see the change in ROS curve. Furthermore, setting the value of $K_{MMitophagy}$ equal to $1x10^{-2}$ it is possible to reproduce the curve of active and total mitochondria shown in figure 5H.

Results of the Copasi simulations discussed in the main text and Figure 5

The Copasi in 5E_2 shows the trends of fission and fusion constants in the NGF differentiation model. After increasing the fission rate constant from 0.18 to 3.6, also fusion starts to increase to 1.8.
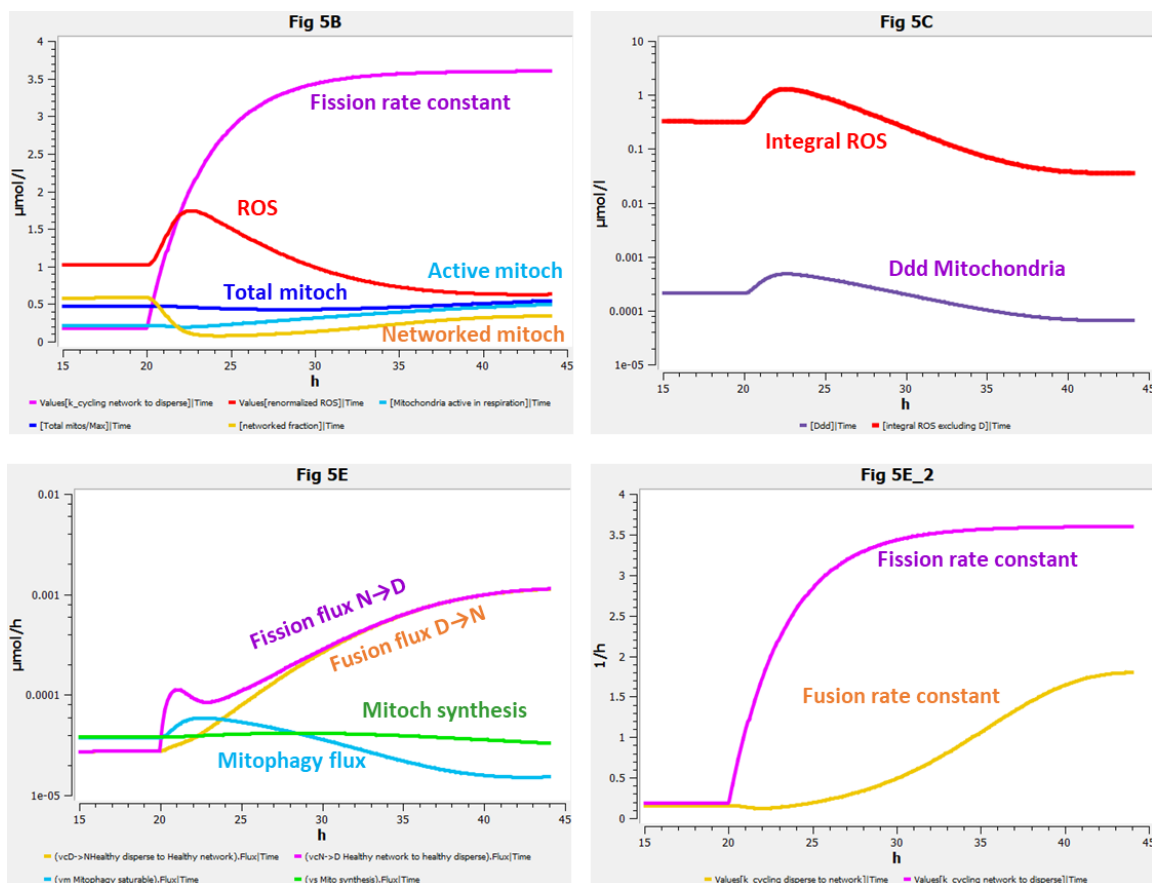
Modeling scheme representing the computational modeling procedure and its direct/indirect effect on the other variable of the network. What happens if we alter the fission rate constant? Variation in the fission rate constant directly affects the concentration of all mitochondrial populations because it acts on the fission reaction that transforms network mitochondria into dispersed mitochondria (circle 1). Indirectly, this variation alters the concentration of ROS and ros through modifications in the concentration of dispersed and networked mitochondria (circle 2). These events affect changes in the actively respiring mitochondria (including the healthy fraction plus those slightly damaged) and the ratios of dispersed and networked mitochondria over the total. Furthermore, the reactions of mitophagy ($V_{m\ mitophagy}$) and of the synthesis of new mitochondria ($V_{s\ Mito\ synthesis}$) are affected (circle 3).
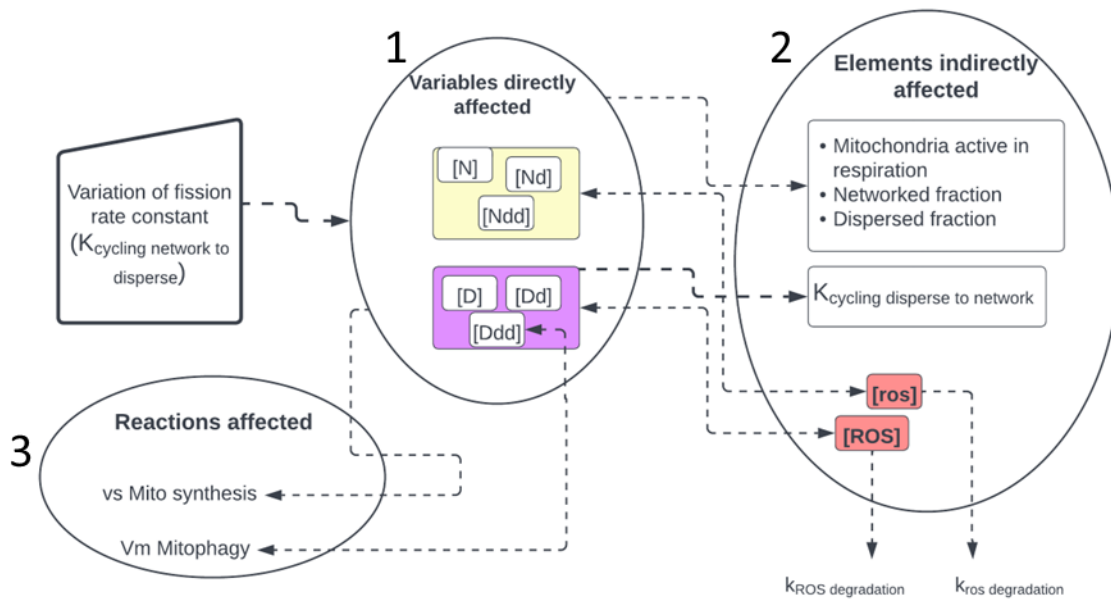
Although the mathematical model developed in this paper fits the experimental data at hand, it cannot yet be considered complete and it may not even be quite correct as it stands. For it is very difficult to measure the values of the model's kinetic parameters directly inside the living cells. In vitro experiments come with the difficulty of precisely mimicking the in vivo conditions inclusive of the spatial aspects, binding of molecules to the mitochondrial membranes, and variations due to cell type differences. Consequently, the parameter values used in the model had to be based on 'reasonable assumptions', e.g. such that the parameters do not exceed the diffusion limit and are not too disparate from parameter values used in other models for cell biology. We have also attempted to limit the number of parameters, for with an unlimited number of parameters one may fit almost every dynamic behavior. An example is our choice to keep the fission rate constant the same, independent of the state of damage of the networked mitochondria to be split. This still allows the fission rates to depend on the damage state, but

only through mass action, which is the simplest option. The assumption boils down to assuming that the damage per se does not interfere with the fission process.

An advantage of having the model at hand is that if one doubts the validity of these simplicity assumptions, one can readily modify the model so add any better information one may have available. We have simulated this for the example of the fission constant. As shown below, quite an appreciable differentiation between the three fission rate constants did affect the simulated curves, but not to the extent that the fit of the experimental data became significantly better.

Model behavior with different fission rate constants k for distinct mitochondrial states (i.e. D, Dd and Ddd)

Distinct mitochondrial populations (healthy, mildly damaged, and highly damaged mitochondria) might be differently prone to undergo fission, this implying different fission rate constants k. To examine whether this could affect our conclusions, we tested how the model would behave when applying different fission rate constants for the distinct types of mitochondria.

The following table shows the new global quantities based on three fission rate constants and three relative constants for the initial and final value, whose total sum (sum of rate constants) would be 3.6 (the value of the fission rate constant in the original model).

We arbitrarily distributed the old value of 3.6, considering a damage-dependent increase (Fig. 4B Suppl1, named after the corresponding Figure 4B in the main text).

For the reaction **N->D** the final constant is: **k_cnd0_final_D = 0.6**

For the reaction **Nd -> Dd** the final constant is **k_cnd0_final_Dd = 1**

For the reaction **Ndd -> Ddd** the final constant is **k_cnd0_final_Ddd = 2**

Name	Initial Value [Unit]	Initial Expression [Unit]	Expression [Unit] or [Unit/h]
k_cycling network to disperse_Ddd	0.18		Values[k_cnd0_initial_Ddd].InitialValue+Values[NGF added]*(Values[k_cnd0_final_Ddd].InitialValue-Values[k_cnd0_initial_Ddd].InitialValue)*(1-EXP(-(Time-Values[NGF addition time])/Values[t_half_cnd].InitialValue))
k_cycling network to disperse_D	0.18		Values[k_cnd0_initial_D].InitialValue+Values[NGF added]*(Values[k_cnd0_final_D].InitialValue-Values[k_cnd0_initial_D].InitialValue)*(1-EXP(-(Time-Values[NGF addition time])/Values[t_half_cnd].InitialValue))
k_cycling network to disperse_Dd	0.18		Values[k_cnd0_initial_Dd]+Values[NGF added]*(Values[k_cnd0_final_Dd].InitialValue-Values[k_cnd0_initial_Dd].InitialValue)*(1-EXP(-(Time-Values[NGF addition time])/Values[t_half_cnd].InitialValue))
k_cnd0_final_D	0.6	**0.6**	Values[time_factor].InitialValue*Values[k_cnd0_final_0_D].InitialValue
k_cnd0_final_Dd	1	**1**	Values[time_factor].InitialValue*Values[k_cnd0_final_0_Dd].InitialValue
k_cnd0_final_0_D	0.033333	0.033333	

k_cnd0_final_0_Dd	0.055556	0.055556	
k_cnd0_initial_D	0.18		Values[time_factor].InitialValue*Values[k_cnd0_initial_0_D].InitialValue
k_cnd0_initial_Dd	0.18		Values[time_factor].InitialValue*Values[k_cnd0_initial_0_Dd].InitialValue
k_cnd0_initial_0_D	0.01		
k_cnd0_initial_0_Dd	0.01		
k_cnd0_final_Ddd	2	**2**	Values[k_cnd0_final_0_Ddd].InitialValue*Values[time_factor].InitialValue
k_cnd0_initial_Ddd	0.18	0.18	Values[k_cnd0_initial_0_Ddd].InitialValue*Values[time_factor].InitialValue
k_cnd0_final_0_Ddd	0.111111	0.111111	0
k_cnd0_initial_0_Ddd	0.01	0.01	0

Results of Copasi simulations with 3 fission rates

The following Copasi plot shows the time dependent first-order fission rate constants *k* used for the extended model 'Fusion fission model bis' with distinct rate constants for the three types of dispersed mitochondria.
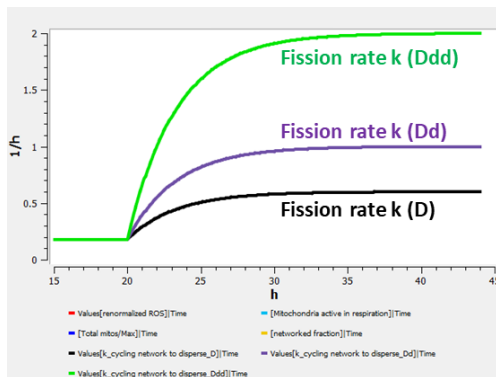
Using the above damage-dependent fission rate constant *k*, we observed that the trends of ROS, and of the distinct mitochondrial populations (total, active and network) (shown below) do not change substantially (Figure 5Bbis, 5Cbis, 5Dbis, 5Ebis), compared to the original model (Figure 5, main text). Fission, fusion and mitophagy fluxes, as well as mitochondrial synthesis behave similarly.
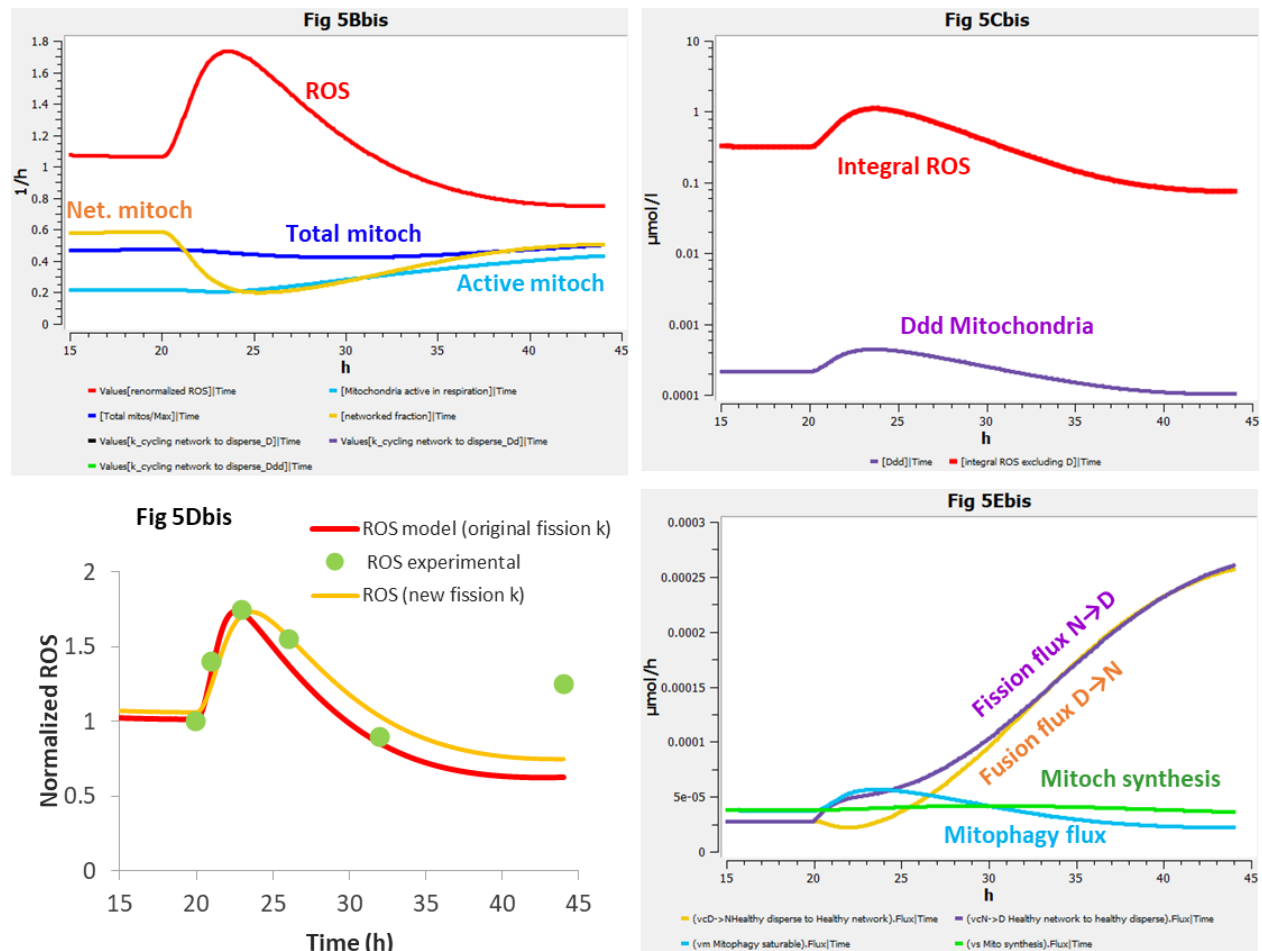
Figure 5bis. **5B-Cbis**) Modeling NGF-induced differentiation by applying different fission rate constants to distinct mitochondrial populations (for the reactions: N→D, k= 0.6; Nd→Dd, k= 1; Ndd→Ddd, k=2) and model response in terms of: ROS content, total mitochondria, active mitochondria and networked mitochondria (Bbis), Integral ROS and Ddd mitochondria (Cbis). **Dbis**) Fit of the model based on the three new fission rate k (orange line) to experimental (dots) data of ROS induced by NGF, compared to the model based on one single fission rate K (red line) (Copasi Fig5.cps). **Ebis**) Modeling the effect of increasing the fission rate constant on fission, fusion, mitophagy flux, and mitochondrial synthesis.

The Copasi figures (5Bbis, 5Cbis and 5Ebis) show that the model behavior (Ddd mitochondria, ROS, mitophagy flux, mitochondrial quality and fusion flux) does not change when applying distinct fission rate k for the distinct mitochondria populations (N->D, Nd -> Dd, and Ndd -> Ddd). Also the ROS behavior with the new fission rate constant *k* does not change significantly (Figure 5Dbis), compared to the fitting of model and experimental data observed with one single fission rate constant *k* (compared to Figure 5D, main text). However, the ROS curve with the three distinct rate constants *k* improves the fitting of the experimental point 4 (t=26 h), while slightly decreasing the fitting of the experimental point 5 (t=31.98 h).

Table S3. Description of the equations used to model and quantify the concentrations of mitochondria, ROS, mitochondrial fractions and cycling constants.

Differential equation	Variables and constants	Reactions involved
$$\frac{d\,([D] \cdot V\,compartment)}{dt}$$ $$= +V\,compartment \cdot (k\_cycling\ network\ to\ disperse \cdot [N]) - V\,compartment \cdot (k\_cycling\ disperse\ to\ network \cdot [D])$$	$[D]$= Concentration of healthy dispersed mitochondria V compartment= total volume $k_{cycling\ network\ to\ disperse}$ = first order rate constant of fission reactions $[N]$= Concentration of healthy networked mitochondria $k_{cycling\ disperse\ to\ network}$ = first order rate constant of fusion reactions	1) vcD →N: Healthy (undamage disperse to Healthy network (fu 2) vcN →D: Healthy network to healthy disperse (fission) 3) vc stands for cycle rate
$$\frac{d([N] \cdot Vcompartment)}{dt}$$ $$= -Vcompartment \cdot (k_{cyclingnetworktodisperse} \cdot [N])$$ $$- Vcompartment \cdot (k_{constitutivedamagegenerationnetworkedmitochondria} \cdot [N])$$ $$+ Vcompartment \cdot (k_{cyclingdispersetonetwork} \cdot [D])$$ $$- Vcompartment \cdot (k_{damaging} \cdot [N] \cdot [ros])$$ $$+ Vcompartment$$ $$\cdot \left(k_{mitosynthesis} \cdot \frac{N_{mitomax} - [N] - [Nd] - [Ndd] - [D] - [Dd] - [Ddd]}{N_{mitomax}}\right)$$	$k_{constitutivedamagegeneration\ networkedmitochondria}$ = first order rate constant of the reaction "N → Nd" $k_{damaging}$= second order rate constant of reaction "N → Nd; ros" and "Nd → Ndd; ros" This determines the rate of the damaging of networked mitochondria by ROS $[ros]$= Concentration of ROS produced by and affecting networked mitochondria $k_{mitosynthesis}$ = first order rate constant of the reaction "→ N; Nd Ndd D Dd Ddd" $N_{mitomax}$= maximum number of mitochondria	1) vcD →N: Healthy disperse to Healthy network (fusion) 2) vcN →D: Healthy network to healthy disperse (fission) 3) → N (synthesis of N) 4) N -> Nd (damaging of N)
Differential equation	**Variables and constants**	**Reactions involved**
$$\frac{d\,([Dd] \cdot V\,compartment)}{dt}$$ $$= +V\,compartment \cdot (k_{cycling\ network\ to\ disperse} \cdot [Nd])$$ $$- V\,compartment \cdot (k_{cycling\ disperse\ to\ network} \cdot [Dd])$$ $$- V\,compartment \cdot (k_{damaging_{dispersed}} \cdot [Dd] \cdot [ROS])$$	$[ROS]$ = Concentration of ROS produced by and affecting dispersed mitochondria	1) vcDd →Nd Damaged disperse damaged network (fusion) 2) vNd →Dd Damaged network damaged disperse (fission) 3) Dd → Ddd; Activator: ROS

Differential equation	Variables and constants	Reaction involved
$$\frac{d\,([Nd]\cdot V\,compartment)}{dt}$$ $$= -V\,compartment \cdot (\text{"}k_{cycling\ network\ to\ disperse}\text{"}$$ $$\cdot [Nd]) - V\,compartment \cdot (k_{damaging}\cdot [Nd]\cdot [ros])$$ $$+ V\,compartment$$ $$\cdot (\text{"}k_{constitutive\ damage\ generation\ networked\ mitochondria}$$ $$\cdot [N]) + V\,compartment \cdot (\text{"}k_{cycling\ disperse\ to\ network}\text{"}$$ $$\cdot [Dd]) + V\,compartment \cdot (k_{damaging}\cdot [N]\cdot [ros])$$		1) vcDd →Nd Damaged disperse... damaged network (fusion) 2) vNd →Dd Damaged network... damaged disperse (fission) 3) N → Nd; ros
$$\frac{d\,([ROS]\cdot V\,compartment)}{dt}$$ $$= +V\,compartment$$ $$\cdot \left(k_{ROS\ synthesis\ by\ damage}\cdot [S]\right.$$ $$\cdot ([Dd] + relative_{impact}\cdot [Ddd]))$$ $$- V\,compartment$$ $$\cdot (k_{ROS\ degradation}\cdot [ROS])$$	$k_{ROS\ synthesis\ by\ damage}$ = constant of ROS synthesis by damaged dispersed mitochondria (Dd and Ddd) $[S]$ = fixed concentration equal to 1. This models the substrate for the synthesis of ROS $relative\_impact$ = parameter that makes Ddd more determinant in setting ROS than Dd. This should have the effect that once there is strong damage, much more ROS is produced leading to much higher ratio of Ddd to Dd and hence to more mitophagy. $k_{ROS\ degradation}$ = first order rate constant of ROS degradation	1) S → ROS; Dd Ddd (ROS synth... by damaged dispersed mitocho... 2) ROS → (ROS degradation)

Differential equation	**Variables and constants**	**Reaction involved**
$$\frac{d\,([Ddd]\cdot V\,compartment)}{dt}$$ $$= +V\,compartment$$ $$\cdot \left(k_{\text{"}cycling\ network\ to\ disperse\text{"}}\right.$$ $$\cdot [Ndd]) - V\,compartment$$ $$\cdot (k_{\text{"}cycling\ disperse\ to\ network\text{"}}\cdot [Ddd])$$ $$- V\,compartment$$ $$\cdot \left(\frac{VbyKMmitophagy\cdot [Ddd]}{1+\frac{[Ddd]}{KMMitophagy}}\right)$$ $$+ V\,compartment$$ $$\cdot (k_{damaging\_dispersed}\cdot [Dd]\cdot [ROS])$$ $$- V\,compartment$$ $$\cdot (k_{mitophagy}\cdot [Ddd])$$	$K_{MMitophagy}$ = K_M of the Michaelis Menten reaction used to model the mitophagy reaction "Ddd →" $k_{damaging\_dispersed}$ = first order rate constant of the reaction "Dd → Ddd; ROS" It determined the damage related to ROS $k_{mitophagy}$ = first order rate constant of mitophagy from an older version of the model. When the global quantity "Mitophagy saturation switch" is equal to 1 the $k_{mitophagy}$ is equal to 0 and only v_m (i.e. the saturable mitophagy reaction) acts.	1) vcDdd →Ndd Highly damage... disperse to highly damaged net... (fusion) 2) vcNdd →Ddd Highly Damage... network to highly damaged dis... (fission) 3) "Ddd ->" vm saturable mitophagy reactio...
$$\frac{d\,([Ndd]\cdot V\,compartment)}{dt}$$ $$= -V\,compartment$$ $$\cdot (\text{"}k_{cycling\ network\ to\ disperse}\text{"}$$ $$\cdot [Ndd]) + V\,compartment$$ $$\cdot (\text{"}k_{cycling\ disperse\ to\ network}\text{"}$$ $$\cdot [Ddd]) + V\,compartment$$ $$\cdot (k_{damaging}\cdot [Nd]\cdot [ros])$$		1) "vcDdd →Ndd" Highly damag... disperse to highly damaged net... (fusion) 2) "vcNdd →Ddd" Highly Damag... network to highly damaged dis... (fission) 3) "Nd → Ndd; ros" Networked... mitochondria damaged by the R... around them (called ros)

Differential equation	Variables and constants	Reaction involved
$$\frac{d([ros] \cdot V\,compartment)}{dt}$$ $$= -V\,compartment \cdot ("k_{ros\,degradation}" \cdot [ros])$$ $$+ V\,compartment$$ $$\cdot \left(\frac{"k_{ros\,synthesis\,by\,low\,damage}" \cdot [S] \cdot [Nd]}{[Nd] + [Ndd] + [N]}\right)$$ $$+ V\,compartment \frac{\cdot ("k_{ros\,synthesis\,by\,high\,damage}" \cdot [S] \cdot [}{[Ndd] + [Nd] + [N]}$$ $$+ V\,compartment$$ $$\cdot (v("constitutive\,ros\,generation"))$$	$k_{ros\,synthesis\,by\,low\,damage}$ = constant of reaction "S$\rightarrow$ ros" (activated by Nd) $k_{ros\,synthesis\,by\,high\,damage}$ = constant of reaction "S$\rightarrow$ ros" (activated by Ndd) $k_{ros\,degradation}$ = constant of ros degradation	1) "ros $\rightarrow$" (ros degradation) 2) "$\rightarrow$ ros" (constitutive ros generation) 3) "vN $\rightarrow$ Nd" 4) "vNd $\rightarrow$ Ndd" 5) S $\rightarrow$ ros (ros synthesis **low** da activated) 6) S $\rightarrow$ ros (ros synthesis **high** damage activated)
$$[Networked\,fraction]$$ $$= \frac{[N] + [Nd] + [Ndd]}{[N] + [Nd] + [Ndd] + [D] + [Dd] + [Ddd]}$$		
$$[Dispersed\,fraction]$$ $$= \frac{[D] + [Dd] + [Ddd]}{[N] + [Nd] + [Ndd] + [D] + [Dd] + [Ddd]}$$		
$$"k\_cycling\,network\,to\,disperse"$$ $$= Values[k\_cnd0\_initial].InitialValue$$ $$+ "NGF\,added" \cdot (Values[k\_cnd0\_final].InitialValue$$ $$- Values[k\_cnd0\_initial].InitialValue) \cdot (1$$ $$- e^{\left(\frac{-(Time - "NGF\,addition\,time")}{Values[t\_half\_cnd].InitialValue}\right)}$$	$k\_cnd0\_initial$ = initial value of a rate constant that determines the starting value of k_(cycling network to disperse). k_cnd0_final = final value of a constant that determines the final value of k_(cycling network to disperse). **NGF added** = determines the beginning of the variation of the k_(cycling network to disperse). It is 0 before NGF addition (time < 20 min) and 1 after NGF addition (time>20). **t_half_cnd:** the time of change from network to dispersed as initiated by NGF addition	
Differential equation	**Variables and constants**	**Reaction involved**
$$"k\_cycling\,disperse\,to\,network" =$$ $$0.01 \cdot Values[time\_factor].InitialValue$$ $$\cdot \left(\frac{[D] + [Dd]}{[Ddd]}\right)$$	0.01 $time\_factor$ = global property used to adjust time dimension	
$$"renormalized\,ROS"$$ $$= [ROS] \cdot Values[ROSfactor].InitialValue$$ $$+ Values[ROSbackground].InitialValue$$		

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