Figure S1. Effects of Mito-Tempo (MT) and NOXs inhibitors (GKT136901,GKT) on hydrogen peroxide release rate and respiration in cells. These figures are representative graphs as these titrations were done in all cell types. A-B, Seahorse traces of different dosages of MT and GKT on respiration rates in AML12 hepatocytes. C-D, Titration of MT and GKT on basal, uncoupled, oligomycin induced, and non-mitochondrial respiration in AML12 hepatocytes. E-F, Titration of MT and GKT on hydrogen peroxide release in AML12 hepatocytes. Vertical dotted lines indicate the ranges of inhibitor concentrations that gave maximum inhibition; horizontal dotted lines indicate the rates of $H_2O_2$ release under un-inhibited and maximally-inhibited conditions. G, Rates of hydrogen peroxide release in AML12 hepatocytes. The contributions of mitochondrial matrix and NOXs were defined by the use of GKT (1 μM) and MT (1 μM) (left). The additivity of these reagents was further confirmed by incubating cells with a mix of GKT and MT (right). H-J, Rates of hydrogen peroxide release by H9c2 rat cardiomyoblasts (H), N27A dopaminergic neural cell (I) and IMR90 human lung fibroblast (J). The contributions of mitochondrial Complexes I and III, mitochondrial matrix and NOXs were defined by the use of S1QEL (1 μM), S3QEL (3 μM), MT (10 μM) and GKT (1 μM). The rate of H2O2 release from mitochondrial Complexes I and III was compared to the rate of H2O2 release from mitochondrial matrix and yield no significance. Values are means ± SEM (N = 3 independent experiments). Keys: Anti A – Antimycin A; FCCP - carbonyl cyanide p-trifluoromethoxyphenylhydrazone; Rot - rotenone

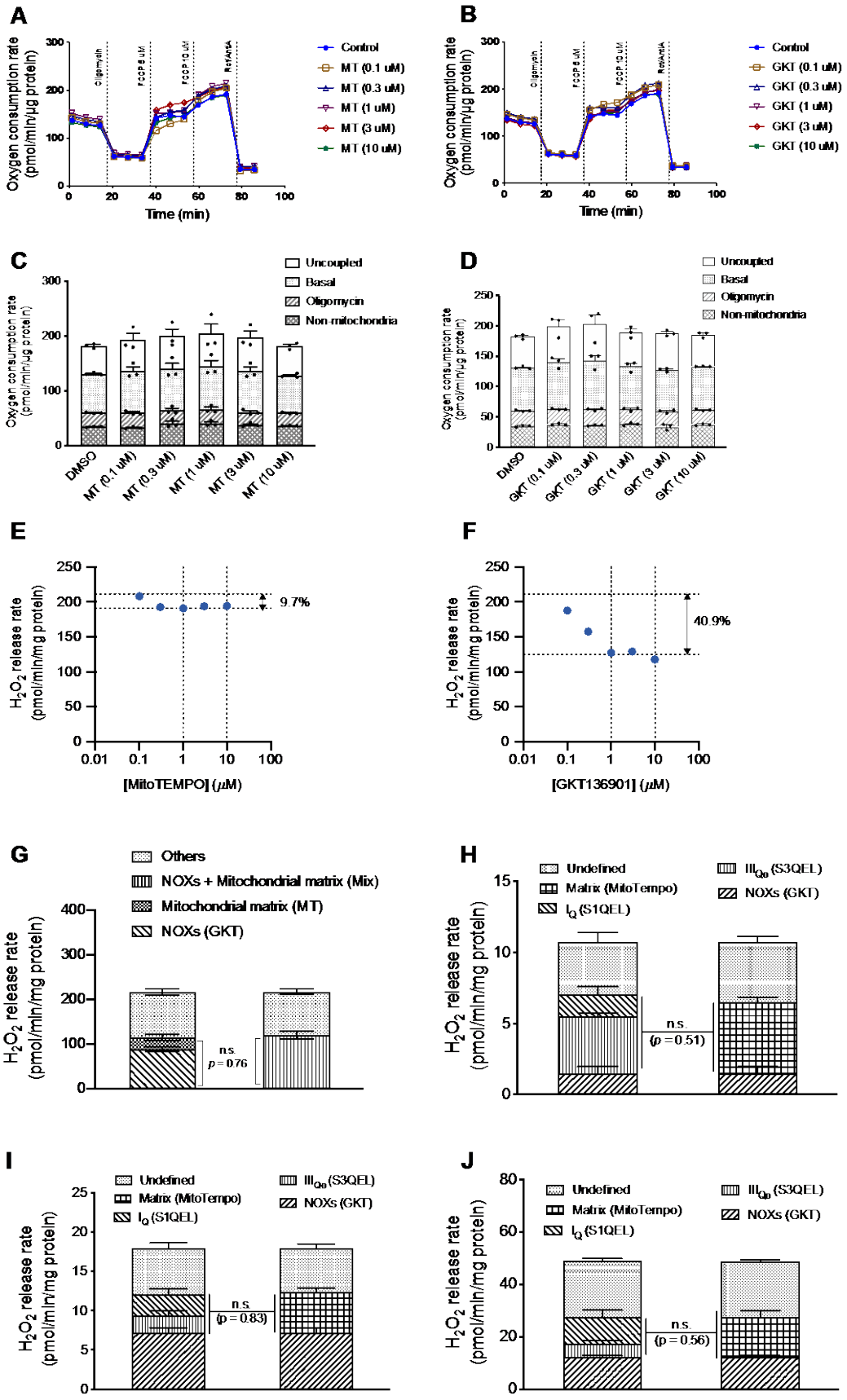
A
Oxygen consumption rate (pmol/min/µg protein)
Time (min)
Oligomycin
FCCP 1 uM
FCCP 10 uM
Rot/AntiA
Control
MT (0.1 uM)
MT (0.3 uM)
MT (1 uM)
MT (3 uM)
MT (10 uM)
B
Oxygen consumption rate (pmol/min/µg protein)
Time (min)
Control
GKT (0.1 uM)
GKT (0.3 uM)
GKT (1 uM)
GKT (3 uM)
GKT (10 uM)
C
Oxygen consumption rate (pmol/min/µg protein)
Uncoupled
Basal
Oligomycin
Non-mitochondria
DMSO
MT (0.1 uM)
MT (0.3 uM)
MT (1 uM)
MT (3 uM)
MT (10 uM)
D
Oxygen consumption rate (pmol/min/µg protein)
Uncoupled
Basal
Oligomycin
Non-mitochondria
DMSO
GKT (0.1 uM)
GKT (0.3 uM)
GKT (1 uM)
GKT (3 uM)
GKT (10 uM)
E
H2O2 release rate (pmol/min/mg protein)
9.7%
[MitoTEMPO] (µM)
F
H2O2 release rate (pmol/min/mg protein)
40.9%
[GKT136901] (µM)
G
H2O2 release rate (pmol/min/mg protein)
Others
NOXs + Mitochondrial matrix (Mix)
Mitochondrial matrix (MT)
NOXs (GKT)
n.s.
p = 0.76
H
H2O2 release rate (pmol/min/mg protein)
Undefined
Matrix (MitoTempo)
IQ (S1QEL)
IIIQo (S3QEL)
NOXs (GKT)
n.s.
(p = 0.51)
I
H2O2 release rate (pmol/min/mg protein)
Undefined
Matrix (MitoTempo)
IQ (S1QEL)
IIIQo (S3QEL)
NOXs (GKT)
n.s.
(p = 0.83)
J
H2O2 release rate (pmol/min/mg protein)
Undefined
Matrix (MitoTempo)
IQ (S1QEL)
IIIQo (S3QEL)
NOXs (GKT)
n.s.
(p = 0.56)

Table S1. Culture conditions for each cell type. H9c2, IMR90, N27A, AML12 cells were purchased from ATCC (Manassa, VA). Darmaraland mole rat lung fibroblast, Siberian hamster skin and lung fibroblast were isolated and cultured according to Seluanov et al. (2010).

| Cell type | Culture condition |
|---|---|
| H9c2 rat cardiomyoblast | Dulbecco's Modified Eagle Medium (DMEM, 25 mM glucose), 10% v/v fetal bovine serum (FBS), penicillin (100 IU/ml)-Streptomycin (100 µg/ml). Cultured in tissue culture incubator at 37°C, 5% $CO_2$, ambient $O_2$. |
| Damaraland mole rat lung fibroblast | Eagle's Minimum Essential Medium (EMEM. 5.6 mM glucose), 10% v/v FBS, penicillin (100 IU/ml)-Streptomycin (100 µg/ml). Cultured in tissue culture incubator at 37°C, 5% $CO_2$, 3% $O_2$. |
| IMR90 human lung fibroblast | DMEM (25 mM glucose, without sodium pyruvate), 10% v/v FBS, penicillin (100 IU/ml)-Streptomycin (100 µg/ml). Cultured in tissue culture incubator at 37°C, 10% $CO_2$, 3% $O_2$. |
| N27A dopaminergic neural cell | RPMI-1640 Medium (25 mM glucose), 10% v/v FBS, penicillin (100 IU/ml)-Streptomycin (100 µg/ml). Cultured in tissue culture incubator at 37°C, 5% $CO_2$, ambient $O_2$. |
| AML12 mouse hepatocytes | DMEM:F12 Medium (17.5 mM glucose), 10% v/v FBS, penicillin (100 IU/ml)-Streptomycin (100 µg/ml), insulin (10 µg/ml)-transferrin (5.5 µg/ml)-selenium (5 ng/ml), dexamethasone (40 ng/ml). Cultured in tissue culture incubator at 37°C, 5% $CO_2$, ambient $O_2$. |
| Siberian hamster dermal fibroblast | EMEM supplemented with 5.6 mM glucose, 10% v/v FBS, penicillin (100 IU/ml)-Streptomycin (100 µg/ml). Cultured in tissue culture incubator at 37°C, 5% $CO_2$, 3% $O_2$. |
| Siberian hamster lung fibroblast | |

Table S2. Hydrogen peroxide release rate (pmol/min/mg protein) from different cellular compartments in each cell types. Values are means ± STD (N = 3 independent experiments); values in brackets are percentages of total $H_2O_2$ release rate of respective cells.

| Cell types | Total | Mitochondria | NOXs | Unidentified |
|---|---|---|---|---|
| H9c2 rat cardiomyoblast | 10.7 ± 1.16 | 5.02 ± 0.08 (46.7%) | 1.48 ± 10.7 (13.8%) | 4.23 ± 0.90 (39.4%) |
| Damaraland mole rat lung fibroblast | 28.1 ± 4.85 | 9.04 ± 0.70 (32.6%) | 8.47 ± 1.31 (31.2%) | 10.59 ± 5.14 (36.2%) |
| IMR90 human lung fibroblast | 49.0 ± 0.76 | 15.2 ± 4.52 (31.0%) | 12.2 ± 1.04 (24.9%) | 21.57 ± 5.05 (44.1%) |
| N27A dopaminergic neural cell | 17.9 ± 2.11 | 5.26 ± 0.84 (29.3%) | 7.15 ± 1.19 (39.9%) | 5.52 ± 0.97 (30.8%) |
| AML12 mouse hepatocytes | 211 ± 12.6 | 20.5 ± 3.24 (9.70%) | 86.6 ± 3.1 (41.0%) | 104.1 ± 5.32 (49.3%) |
| Siberian hamster dermal fibroblast | 80.0 ± 11.1 | 7.55 ± 2.98 (9.44%) | 63.7 ± 9.40 (79.6%) | 8.76 ± 3.30 (11.0%) |
| Siberian hamster lung fibroblast | 109.1 ± 2.92 | 6.28 ± 2.52 (5.76%) | 81.4 ± 62.0 (74.6%) | 21.5 ± 13.7 (19.7%) |

## Reference

**Seluanov, A., Vaidya, A. and Gorbunova, V.** (2010). Establishing primary adult fibroblast cultures from rodents. J. Vis. Exp. e2033. doi:10.3791/2033