

1 **Ginseng extracts modulate mitochondrial bioenergetics of live**
2 **cardiomyoblasts: a functional comparison of different polar**
3 **extracts**

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13 Running title: Ginseng extracts modulate mitochondrial bioenergetics of cardiomyoblasts

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21 ginseng extracts

22 **Fig. S1.** The effects of ginseng extracts to cell viability of H9C2 cells. Cultured H9C2 cells were treated
23 with ginseng extracts (0-100 $\mu\text{g/mL}$) for 24 hours. Cell viability was determined by MTT assay. Data
24 are expressed as Mean \pm SD, $n = 5$, each with triplicate samples. *** $p < 0.001$.

25 **Fig. S2.** Schematic representation of metabolic parameters of mitochondrial respiration measured by
26 Seahorse Bioscience XFp extracellular flux analyzer. Basal respiration represents energetic demand
27 of the cell under baseline conditions. Proton leak shows the remaining basal respiration and is the
28 difference in OCR after oligomycin and rotenone/antimycin A (R&A) injection. ATP production is
29 the difference between basal respiration and proton leak and represents the portion of basal respiration
30 that is being used to drive ATP production. Maximal respiration shows the maximum rate of
31 respiration that the cell can achieve, which is calculated as the OCR after FCCP injection. Spare
32 respiratory capacity is the difference between maximal and basal OCR and can be an indicator of cell
33 fitness or flexibility. The non-mitochondrial rate was subtracted from all other rates, which is a result
34 of a subset of cellular enzymes that continue to consume oxygen after rotenone/antimycin A addition.

35 **Fig. S3.** Optimization of cell density, FCCP and tBHP dosage in XFp Mito Stress Test. **(A)** Cultured H9C2
36 cells with increasing cell density were seeded in XFp Cell Culture Miniplate and cultured for 48 hours
37 before basal OCR was measured. **(B)** H9C2 cells (5,000 cells/well) were cultured for 48 hours, then
38 treated with 1 μM oligomycin and three serial injections of FCCP at different concentrations (a high
39 concentration range of 3, 6, 12 μM and a low concentration range of 0.75, 1.5, 3 μM). The resulting
40 data set characterizes the cells' response to 6 doses of FCCP. **(C)** Cultured H9C2 cells (5,000
41 cells/well) were exposed to tBHP at various concentrations for 24 hours, and OCR was determined.
42 The above mentioned OCR values were normalized with cellular protein. Data are expressed as mean
43 \pm SD, $n = 3$, each with triplicate samples.

Table S1

Mass spectra properties of marker chemicals in ginseng extracts.

Chemical	Formula	Calculated mass [M]	Precursor ion [M-H] ¹⁾	Fragmentor energy	Collision energy	Product ion ²⁾
Ginsenoside Rb1	C ₅₄ H ₉₂ O ₂₃	1108.6	1107.6	250	41	945.5
					49	783.5
Ginsenoside Rd	C ₄₈ H ₈₂ O ₁₈	946.5	945.5	250	33	783.5
					45	621.6
Ginsenoside Re	C ₄₈ H ₈₂ O ₁₈	946.5	945.5	250	41	637.5
					53	475.5
Ginsenoside Rg1	C ₄₂ H ₇₂ O ₂₄	800.5	799.5	250	21	637.3
					37	475.4
Astragaloside IV	C ₄₁ H ₆₈ O ₁₄	784.9	829.5 ³⁾	190	5	829.5
					25	783.2

¹⁾The detected chemicals had better responses under the negative mode: the [M-H]⁻ was used as the precursor ion.

²⁾Two pairs of collision energy and product ions were used for the MRM analysis to guarantee the precision of analytes.

³⁾The precursor ion of astragaloside IV was [M + HCOOH - H]⁻ under the negative mode.

Table S2

Calibration curves, LOD, LOQ, precision, repeatability and recovery of 4 marker chemicals in ginseng extracts

Ginsenoside	Calibration curve ¹⁾	Correlation coefficient (r ²)	Range (ng)	LOD (ng) ²⁾	LOQ (ng) ³⁾	Precision (n = 6) ⁴⁾		Repeatability (n = 5)		Recovery ⁷⁾ (n = 3)	
						Intra-day ⁵⁾	Inter-day ⁶⁾	Mean (%)	RSD (%)	Mean (%)	RSD (%)
Rb1	y = 76.2x - 293.2	0.9995	2-200	0.329	1.146	1.99	1.72	98.66	1.25	98.66	1.25
Rd	y = 192.2x - 1102.8	0.9994	1-100	0.156	0.632	2.81	2.12	99.87	2.81	99.87	2.81
Re	y = 169.2x - 22.1	0.9992	1-50	0.199	0.651	2.88	2.18	98.19	2.98	98.19	2.98
Rg1	y = 401.1x - 1109.1	0.9991	1-50	0.167	0.631	1.89	1.32	99.19	2.11	99.19	2.11

¹⁾The calibration curve was derived from six data points, n = 3.

²⁾LOD refers to the limits of detection.

³⁾LOQ refers to the limits of quantification.

⁴⁾The value of RSD (%) were presented.

⁵⁾The intra-day analysis refers to the sample examined for six replicates within one day.

⁶⁾The inter-day analysis refers to the sample examined in duplicates over three consecutive days.

⁷⁾Recovery (%) = 100 × (amount found - original amount)/amount spiked. The data were presented as average of three independent determinations, and the SD was < 5% of the mean, which was not shown for clarity.

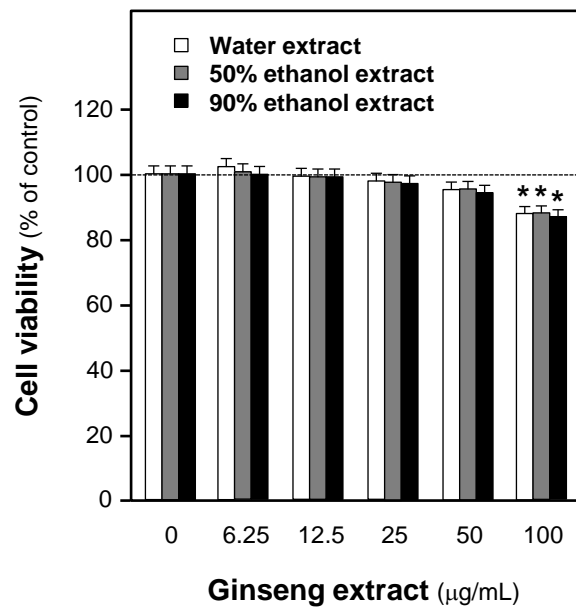


Fig. S1

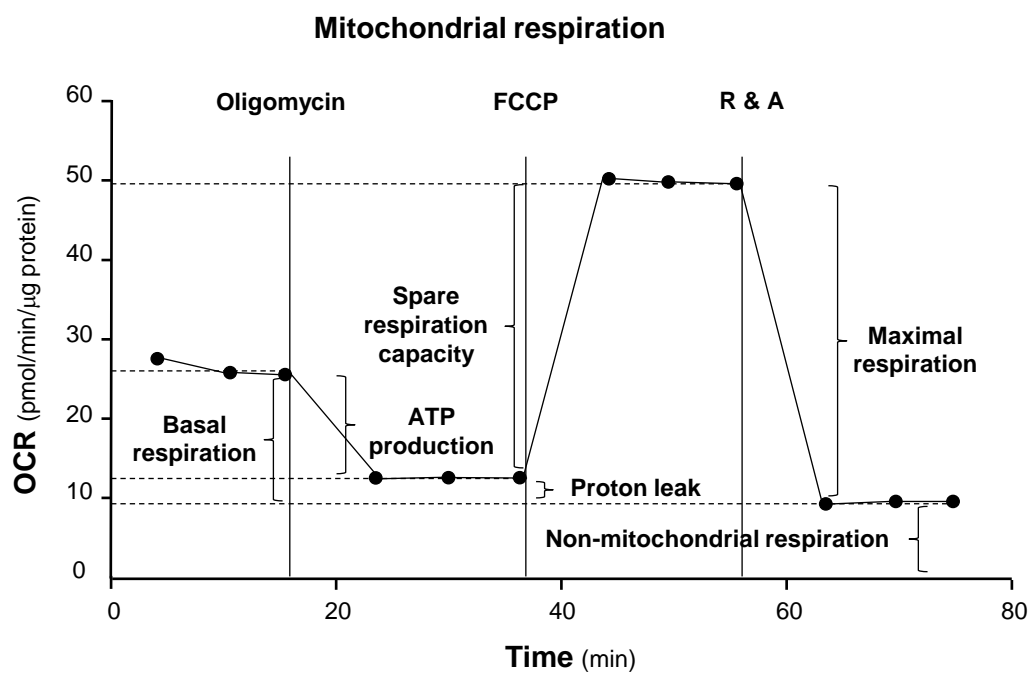


Fig. S2

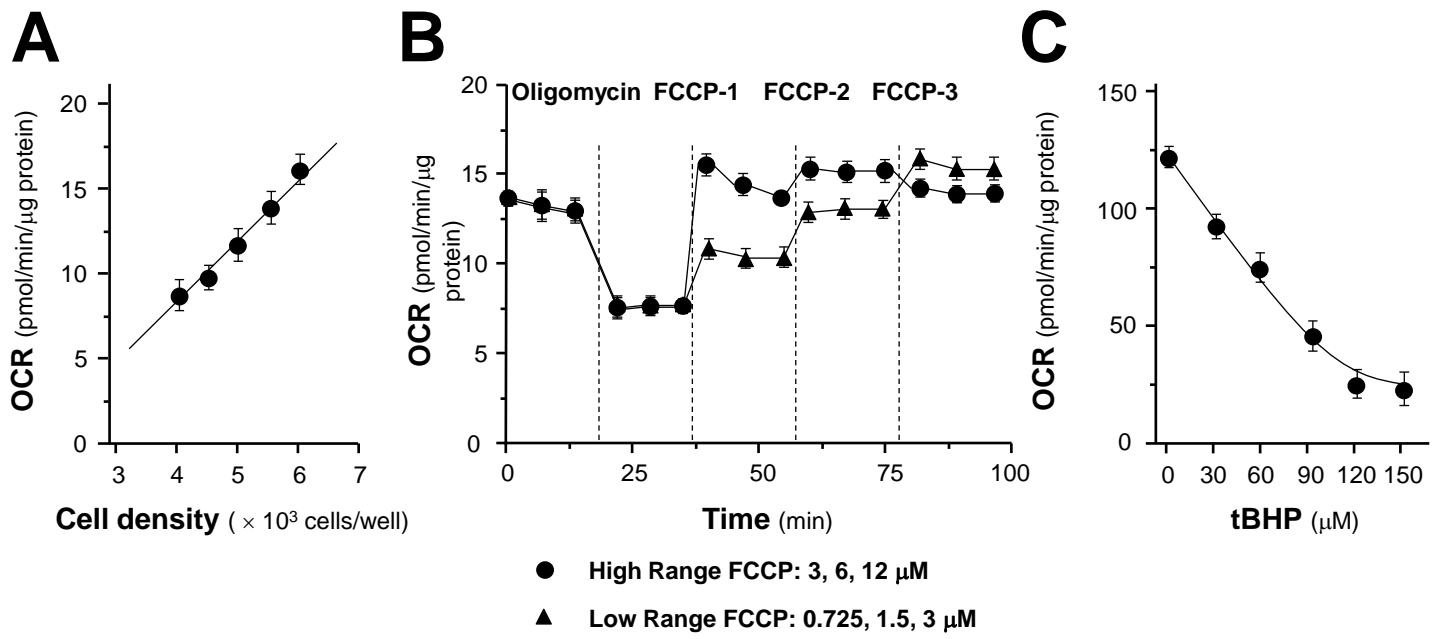


Fig. S3