SUPPLEMENTAL DATA

Table S1. Mass spectrometry sequencing of the unique band excised from SDS-PAGE of nuclear proteins bound to the S1P affinity matrix.

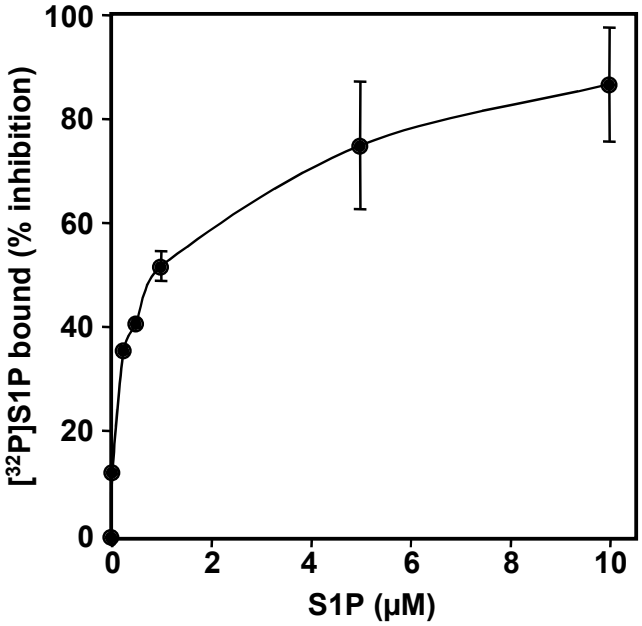
Peptide	Measured Mass	Computed Mass	Predicted Peptide Sequence
1	993.483	993.476	lgldyeer
2	1176.622	1176.625	fnasqlitqr
3	1203.620	1203.628	dlqmvnislr
4	1214.638	1214.614	ivqaegeaeaak
5	1216.512	1216.583	esvftvegghr
6	1258.736	1258.739	lllgagavaygv
7	1852.976	1852.979	iggvqqdtilaeglhfr
8	1903.993	1903.957	vlsrpnaqelpsmyqr
9	2224.107	2224.136	iyltadnlvlnlqdesftr

See Fig. 1A for more information.

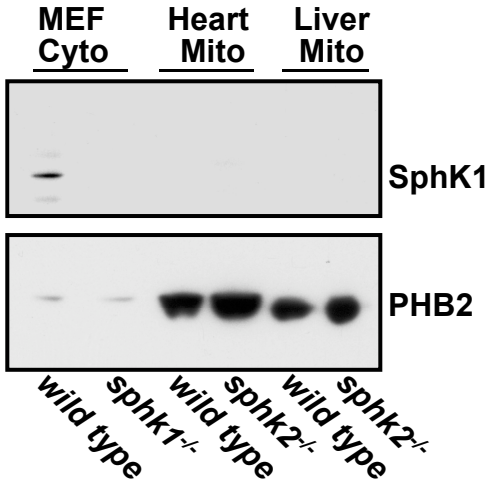
Table S2. Mass spectrometry sequencing of the additional band present in BN-PAGE of *sphk2*$^{-/-}$ mitochondria

NCBI ID	Polypeptide	Coverage (%)	Unique Peptides
NP_904331.1	cytochrome c oxidase, subunit II	24.7	12
NP_034071.1	cytochrome c oxidase, subunit IV isoform 1	48.5	10
NP_031773.2	cytochrome c oxidase, subunit Va	17.1	4
NP_034072.2	cytochrome c oxidase, subunit Vb	17.8	3
NP_079904.1	cytochrome c oxidase, subunit VIb polypeptide 1	16.3	1
NP_444301.1	cytochrome c oxidase, subunit VIc	25.0	7
NP_034074.1	cytochrome c oxidase, subunit VIIa 1	28.8	3

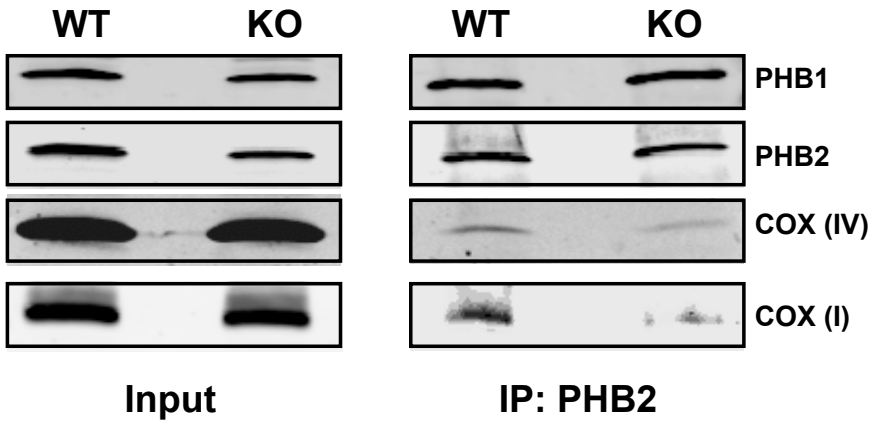
The additional band in native blue gel (BN-PAGE) of *sphk2*$^{-/-}$ mitochondria (Fig. 5A) was excised and peptide sequences of complex IV subunits identified by mass spectrometry.



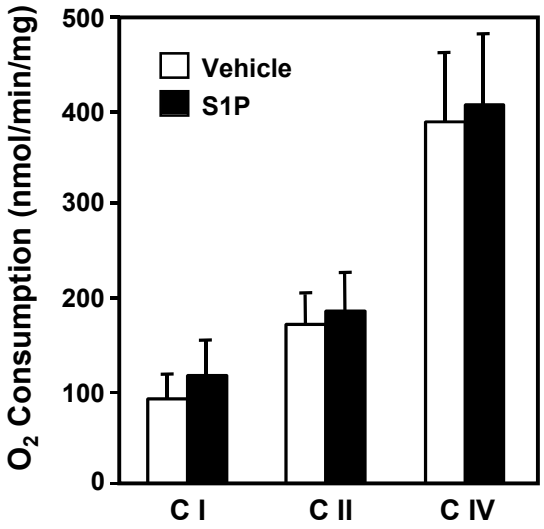
Supplemental Fig. 1. Binding affinity of S1P to PHB2. HeLa cell lysates were incubated for 30 min with [^{32}P]S1P (0.1 nM) in the absence or presence of increasing concentrations of unlabeled S1P. Cell lysates were then immunoprecipitated with antibodies against PHB2 and radioactivity in the immunocomplexes captured by Protein A/G beads was determined by scintillation counting. Data are expressed as percent inhibition and are means ± SD.



Supplemental Fig. 2. SphK1 is not expressed in mitochondria. Highly purified mitochondria (Mito) fractions were prepared from wild type and *sphk2*$^{-/-}$ hearts and livers. Equal amounts of proteins were separated by SDS-PAGE and immunoblotted with anti-SphK1 antibody. Blots were stripped and blotted with anti-PHB2 antibody to show equal loading and transfer. Cytosols (Cyto) from wild type and sphk1$^{-/-}$ MEFs were included as a positive control.



Supplemental Fig. 3. Decreased interaction of COX subunits with PHB2 in SphK2 null mitochondria. Heart mitochondria extracts from wild type and sphk2-/- mice were immunoprecipitated with anti-PHB2 antibody and immunocomplexes were analyzed by western blotting with antibodies against subunit IV of cytochrome c oxidase (COX (IV)), subunit I of cytochrome c oxidase (COX (I)), PHB1, and PHB2.



Supplemental Fig. 4. Respiratory deficits in SphK2 null cardiomyocytes are not rescued by addition of S1P. *sphk2*$^{-/-}$ cardiomyocytes treated without or with 10 μM S1P overnight. Maximum rates of respiration were measured in the presence of 2 mM ADP with respiratory complex I, II, and IV substrates. Data are expressed as nmol O_2/min/mg and are means ± SEM.