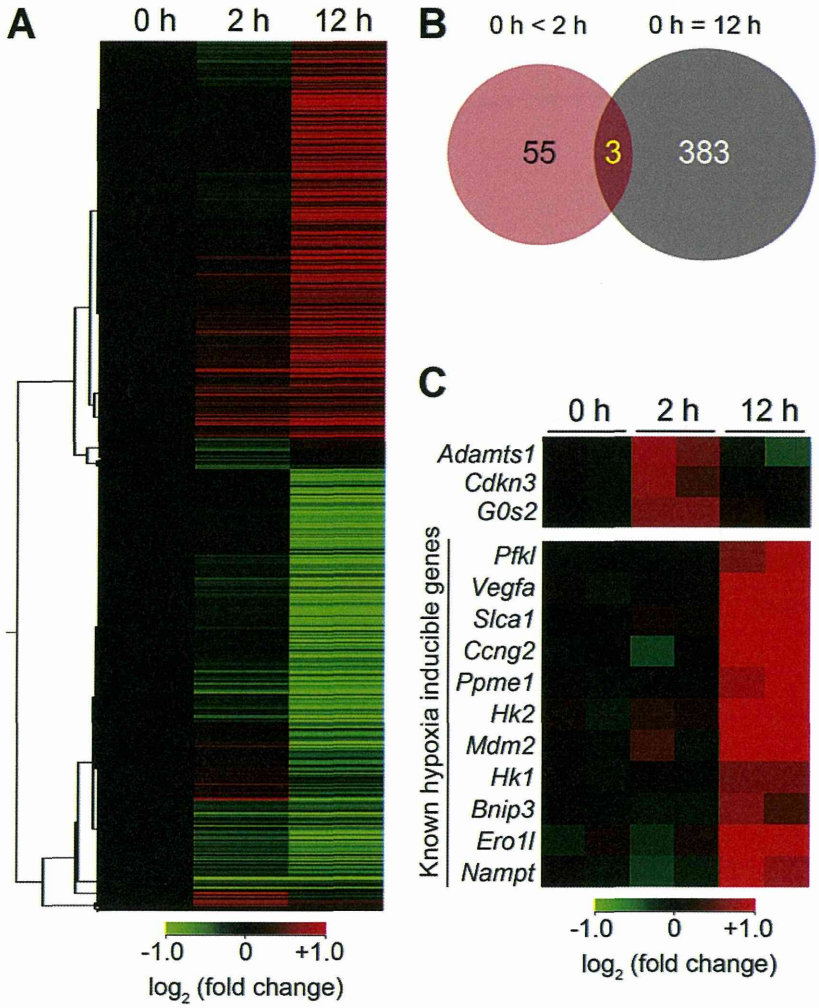


Fig. S2. Microarray analysis of hypoxia-treated cardiomyocytes and identification of *G0s2* as a rapidly inducible gene by hypoxia. (*A*) Hierarchical clustering image of 2,598 genes exhibiting significantly ($P < 0.05$; ANOVA) different expression levels at each of three time points (0, 2, and 12 h) measured in cardiomyocytes under hypoxic conditions (1% O_2). The red and green colors denote higher and lower levels of expression relative to the control sample (0 h), respectively. (*B*) Venn diagrams representing the overlap of genes that were up-regulated (>1.5-fold up-regulated) at 2 h and genes that remained unchanged (<1.2-fold change) after 12 h compared with the control at 0 h. (*C*) Heat map of the genes extracted from *A*. *Upper* shows the expression pattern of genes that were up-regulated at 2 h after the onset of hypoxia and declined to the baseline expression level at 12 h. *Lower* shows known hypoxia-inducible genes.

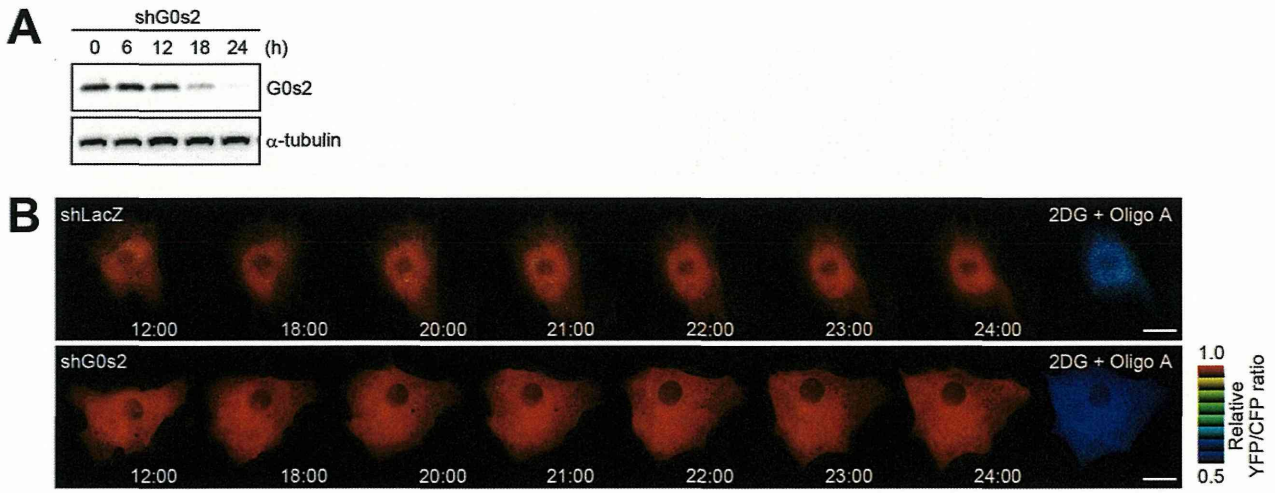


Fig. S3. G0s2 does not affect cytosolic ATP concentration. (*A*) Time course of G0s2 knockdown in cardiomyocytes. (*B*) Sequential YFP/CFP ratiometric pseudocolored images of Cyto-ATeam fluorescence in cardiomyocytes expressing shRNA for LacZ (shLacZ) or G0s2 (shG0s2; #2). Inhibitors of glycolysis [10 mM 2-deoxyglucose (2DG)] and F_oF_1-ATP synthase [1 μg/mL oligomycin A (Oligo A)] were added at the end of the time-lapse imaging to diminish the cytosolic ATP concentration. The indicated time represents the period after adenovirus infection.

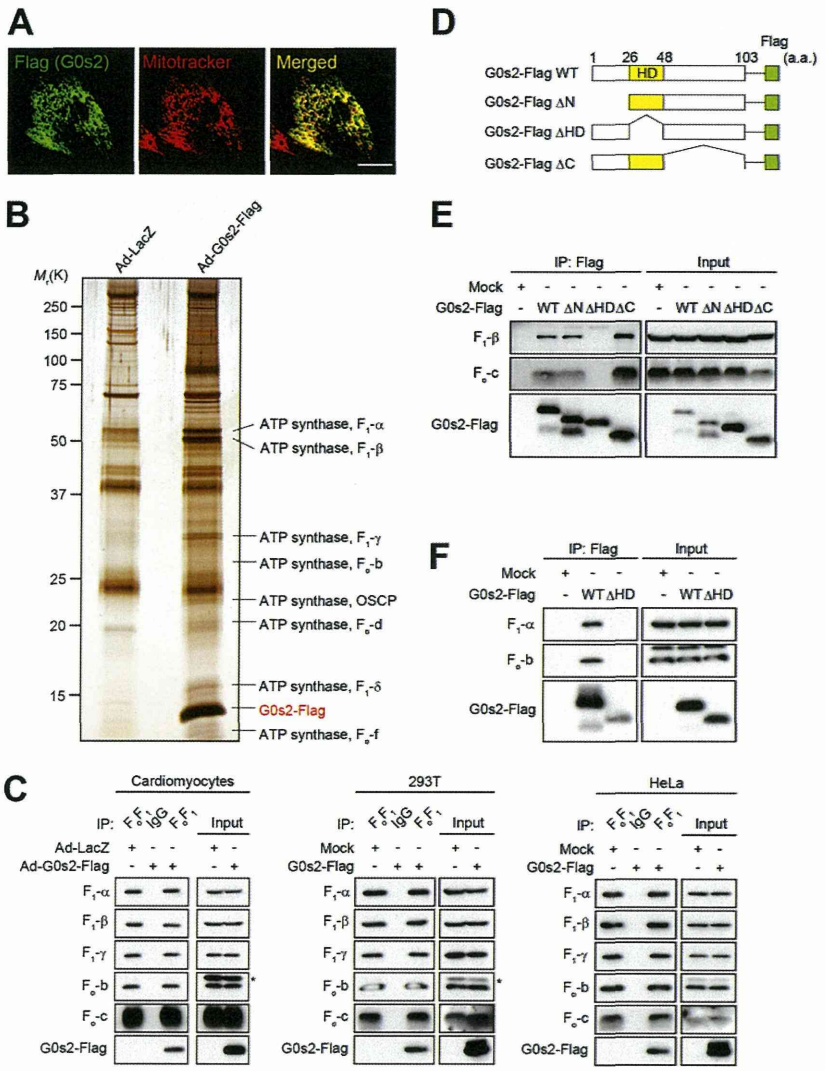


Fig. S4. Identification of F_oF_1-ATP synthase as the G0s2 binding protein. (*A*) Immunostaining of cardiomyocytes that expressed G0s2-Flag. The cells were stained with anti-Flag antibody (green) and labeled with MitoTracker Red (red). (Scale bar: 20 μm.) (*B*) A silver-stained gel of affinity-purified G0s2 binding proteins. Cell lysates from cardiomyocytes infected with adenovirus expressing G0s2-Flag or LacZ were purified with anti-Flag affinity gels. Polypeptides of the F_oF_1-ATP synthase complex identified by MS are indicated along with the G0s2-Flag protein (red). OSCP, oligomycin sensitivity conferral protein. (*C*) Immunoprecipitation (IP) of F_oF_1-ATP synthase in (*Left*) cardiomyocytes, (*Center*) 293T, and (*Right*) HeLa cells. F_oF_1, F_oF_1-ATP synthase. *Nonspecific band. (*D*) A schematic representation of the G0s2-Flag WT and deletion mutants. The hydrophobic domain (HD) of G0s2 is indicated as a yellow box. (*E* and *F*) IP of G0s2 mutants expressed in (*E*) 293T or (*F*) HeLa cells.

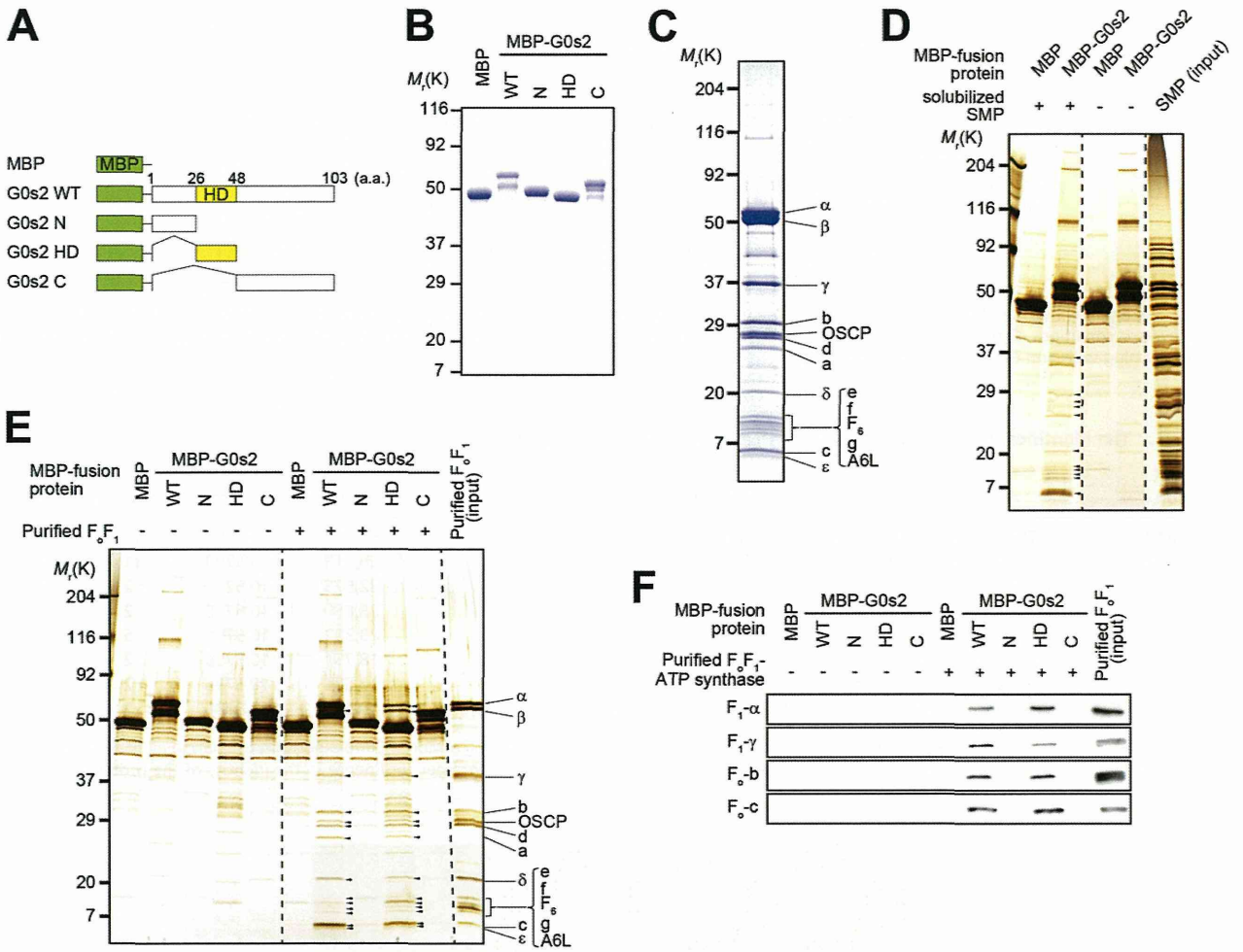


Fig. S5. G0s2 directly interacts with F_oF_1-ATP synthase. (*A*) A schematic representation of recombinant maltose-binding protein (MBP)-fusion proteins purified from *Escherichia coli*. (*B*) Coomassie Brilliant Blue-stained gel of recombinant MBP-fusion proteins purified from *E. coli*. (*C*) Coomassie Brilliant Blue-stained gel of purified F_oF_1-ATP synthase from bovine heart mitochondria. OSCP, oligomycin sensitivity conferral protein. (*D* and *E*) A silver-stained gel of an in vitro pull-down assay using (*D*) submitochondrial particles (SMPs) or (*E*) purified F_oF_1-ATP synthase from bovine heart mitochondria. Arrowheads indicate the F_oF_1-ATP synthase subunits bound to G0s2 protein. (*F*) Immunoblotting of an in vitro pull-down assay using purified F_oF_1-ATP synthase.

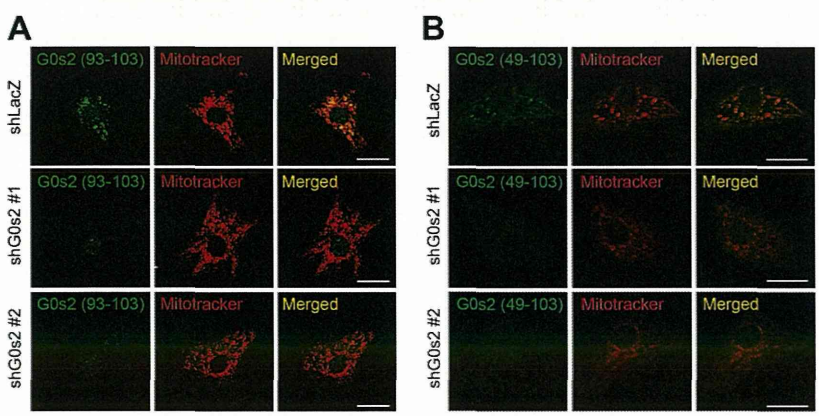


Fig. S6. G0s2 is localized to mitochondria. Immunostaining with an antibody against mouse (*A*) G0s2 (93–103 aa) or (*B*) G0s2 (49–103 aa) in cardiomyocytes expressing shLacZ, shG0s2 #1, and shG0s2 #2. (Scale bars: 20 μm.)

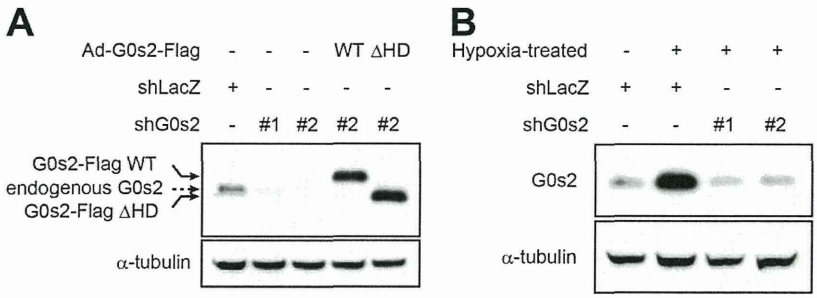
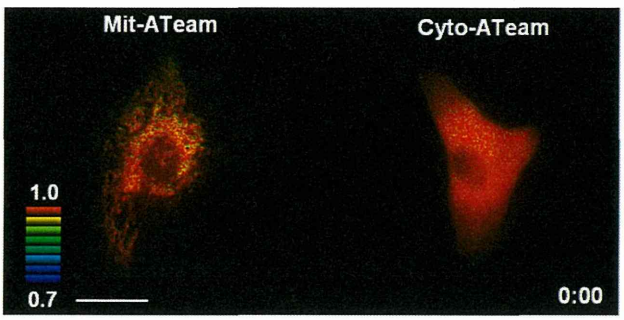


Fig. S7. (*A*) Immunoblotting of G0s2-depleted cardiomyocytes rescued by overexpression of G0s2-Flag. (*B*) Immunoblotting of G0s2-depleted cardiomyocytes under hypoxic conditions. Cardiomyocytes expressing the indicated adenovirus were exposed to hypoxia (1% O_2) for 4 h. The cell lysates were subjected to immunoblotting with anti-G0s2 or α-tubulin antibodies.

Table S1. The identification of proteins that specifically bound to G0s2 by MS

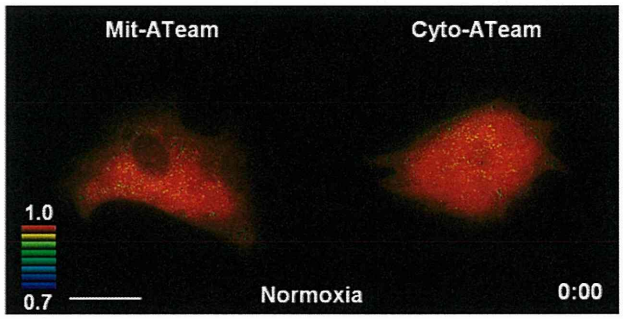
SwissProt accession	Description	Molecular mass (Da)	PLGS score	Peptides	Coverage (%)
ATPA_RAT	ATP synthase subunit-α mitochondrial	59,716	10.5798	10	18.9873
ATPB_RAT	ATP synthase subunit-β mitochondrial	56,318	10.5798	11	24.1966
ATPG_RAT	ATP synthase γ-chain	32,975	10.523	2	7.3826
ATP5F1_RAT	ATP synthase subunit b mitochondrial	28,850	10.5796	2	3.9063
ATPO_RAT	ATP synthase subunit O mitochondrial	23,382	10.5796	5	23.9437
ATP5H_RAT	ATP synthase subunit d mitochondrial	18,751	10.5796	2	13.0435
ATPD_RAT	ATP synthase subunit-δ mitochondrial	17,584	10.5796	2	13.6905
D3ZAF6_RAT	ATP synthase H transporting mitochondrial Fo complex subunit f isoform 2	10,972	10.5796	2	22.3404

A PLGS score was calculated by the Protein Lynx Global Server (PLGS; PLGS 2.4) software using a Monte Carlo algorithm to analyze all of the available MS data; this score is a statistical measure of the accuracy of assignation. A higher score implies a greater confidence in the identity of the protein.



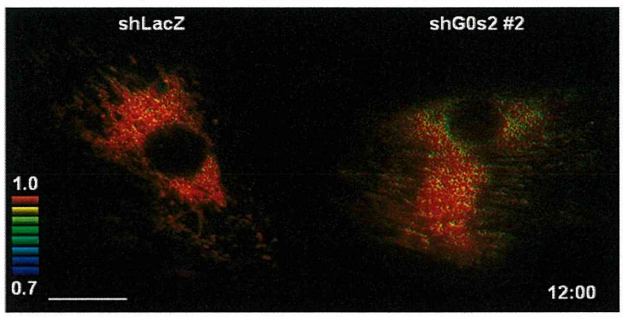
Movie S1. Time-lapse imaging of the (*Left*) Mit-ATeam and (*Right*) Cyto-ATeam fluorescence in cardiomyocytes before and after the addition of oxidative phosphorylation inhibitor. YFP/CFP ratiometric pseudocolored images were obtained every 1 min for 25 min. Oligomycin A (0.01 μg/mL), an inhibitor of F_oF_1-ATP synthase, was added at time point 5 min. (Scale bar: 20 μm.)

Movie S1



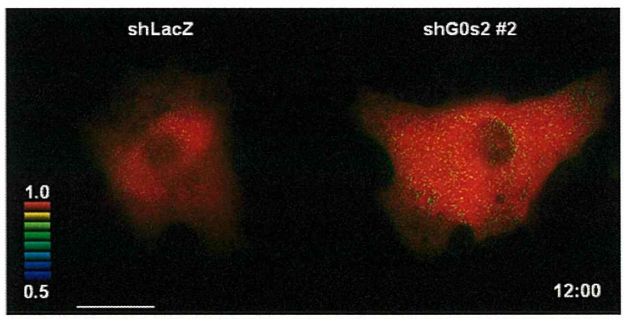
Movie S2. Time-lapse imaging of the (*Left*) Mit-ATeam and (*Right*) Cyto-ATeam fluorescence in cardiomyocytes exposed to hypoxia. YFP/CFP ratiometric pseudocolored images were obtained every 30 min for 3 h. Cells are exposed to 1% hypoxia from the time point 30 min. (Scale bar: 20 μm.)

Movie S2



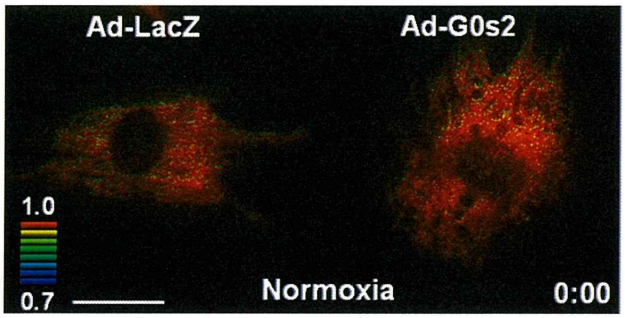
Movie S3. Time-lapse imaging of the Mit-ATeam fluorescence in cardiomyocytes that expressed (*Left*) shLacZ or (*Right*) shG0s2 #2. YFP/CFP ratiometric pseudocolored images were obtained every 1 h for 12 h. An inhibitor of F_oF_1-ATP synthase (1 μg/mL oligomycin A) was added at the end of time-lapse imaging to completely inhibit ATP synthesis. (Scale bar: 20 μm.) The indicated times designate the periods after adenovirus infection.

Movie S3



Movie S4. Time-lapse imaging of the Cyto-ATeam fluorescence in cardiomyocytes that expressed (*Left*) shLacZ or (*Right*) shG0s2 #2. YFP/CFP ratiometric pseudocolored images were obtained every 1 h for 12 h. Inhibitors of both glycolysis (10 mM 2-deoxyglucose) and F_oF_1-ATP synthase (1 μg/mL oligomycin A) were added at the end of the time-lapse imaging to decrease the cytosolic ATP concentration. (Scale bar: 20 μm.) The indicated times designate the periods after adenovirus infection.

Movie S4



Movie S5. Time-lapse imaging of the Mit-ATeam fluorescence in cardiomyocytes that expressed (*Left*) LacZ or (*Right*) G0s2 during hypoxia and reoxygenation. YFP/CFP ratiometric pseudocolored images were obtained every 30 min for 4 h. The cells were exposed and imaged under hypoxic condition from the time point 30 min to 4 h. (Scale bar: 20 μm.)

Movie S5