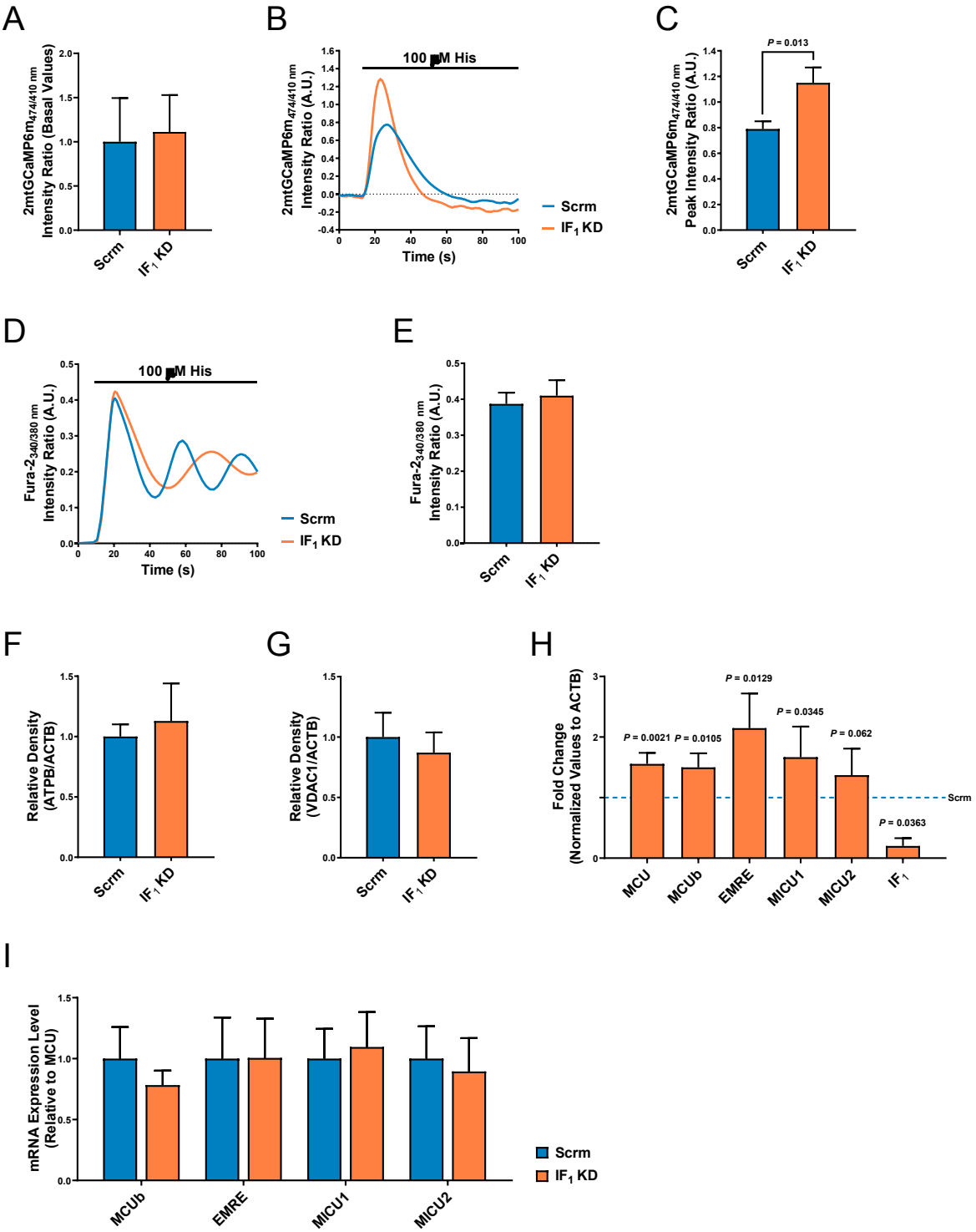
Supplementary Figure 1



Supplementary Figure 1

(A) Bar chart of average basal $[Ca^{2+}]_m$ in Scrm and IF_1 KD cells co-transfected with 2mtGCaMP6m and mtRFP (Scrm: 1.00 ± 0.49; IF_1 KD: 1.11 ± 0.42 A.U.; normalized values, n ≥ 15, N = 4).

(B) Prototypical traces depicting changes in $[Ca^{2+}]_m$ upon administration of 100 µM His in Scrm and IF_1 KD cells co-transfected with 2mtGCaMP6m and mtRFP.

(C) Quantification of average peak $[Ca^{2+}]_m$ in the two cell lines (Scrm: 0.79 ± 0.06 A.U.; IF_1 KD: 1.15 ± 0.12 A.U.; normalized values, n ≥ 15, N = 4).

(D) Representative Fura-2 AM traces of $[Ca^{2+}]_i$ dynamics in Scrm and IF_1 KD cells following administration of 100 µM His.

(E) Bar chart of average peak $[Ca^{2+}]_i$ in the two cell lines (Scrm: 0.39 ± 0.03 A.U.; IF_1 KD: 0.41 ± 0.04 A.U.; n ≥ 15, N = 4).

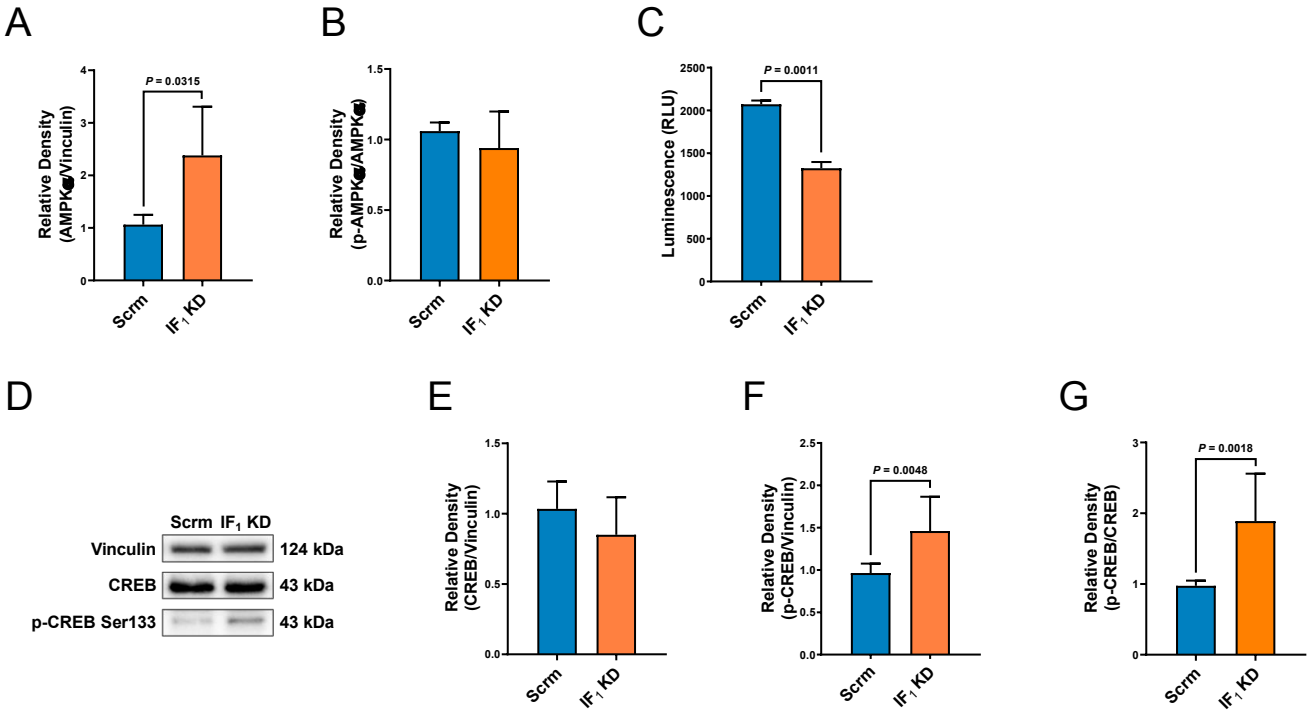
(F) Densitometry analysis of ATPB protein levels relative to GAPDH (see Figure 1G; Scrm: 1.00 ± 0.10; IF_1 KD: 1.12 ± 0.31; normalized values, N = 3)

(G) Densitometry analysis of VDAC1 protein levels relative to GAPDH (see Figure 1G; Scrm: 1.00 ± 0.20; IF_1 KD: 0.87 ± 0.17; normalized values, N = 3)

(H) Gene expression analysis of MCUC subunits in Scrm (blue line) and IF_1 KD (orange bars) cells; β-actin (ACTB) was used as internal control (normalized values, Scrm = 1; IF_1 KD: MCU = 1.56 ± 0.18; MCUb = 1.50 ± 0.23; EMRE = 2.15 ± 0.57; MICU1 = 1.67 ± 0.50; MICU2 = 1.37 ± 0.44; IF_1 = 0.20 ± 0.13; N = 5).

(I) Averaged ratios between the mRNA levels of MCU and the other subunits of the complex (normalized values, Scrm: MCUb = 1.00 ± 0.26; EMRE = 1.00 ± 0.34; MICU1 = 1.00 ± 0.24; MICU2 = 1.00 ± 0.26; IF_1 KD: MCUb = 0.78 ± 0.12; EMRE = 1.01 ± 0.32; MICU1 = 1.10 ± 0.29; MICU2 = 0.89 ± 0.27; N = 5).

Supplementary Figure 2



Supplementary Figure 2

(A) Quantification of AMPKα protein levels relative to vinculin (see Figure 1I; Scrm: 1.06 ± 0.19; IF_1 KD: 2.38 ± 0.93; N = 5)..

(B) Average ratio between phospho-AMPKα and AMPKα (see Figure 1I; Scrm: 1.06 ± 0.06; IF_1 KD: 0.94 ± 0.26; N = 5).

(C) Measurement of intracellular ATP levels in Scrm and IF_1 KD cells with an ATP-based luminescence assay (Scrm: 2071.83 ± 43.29 RLU; IF_1 KD: 1323.94 ± 73.09 RLU; N = 5).

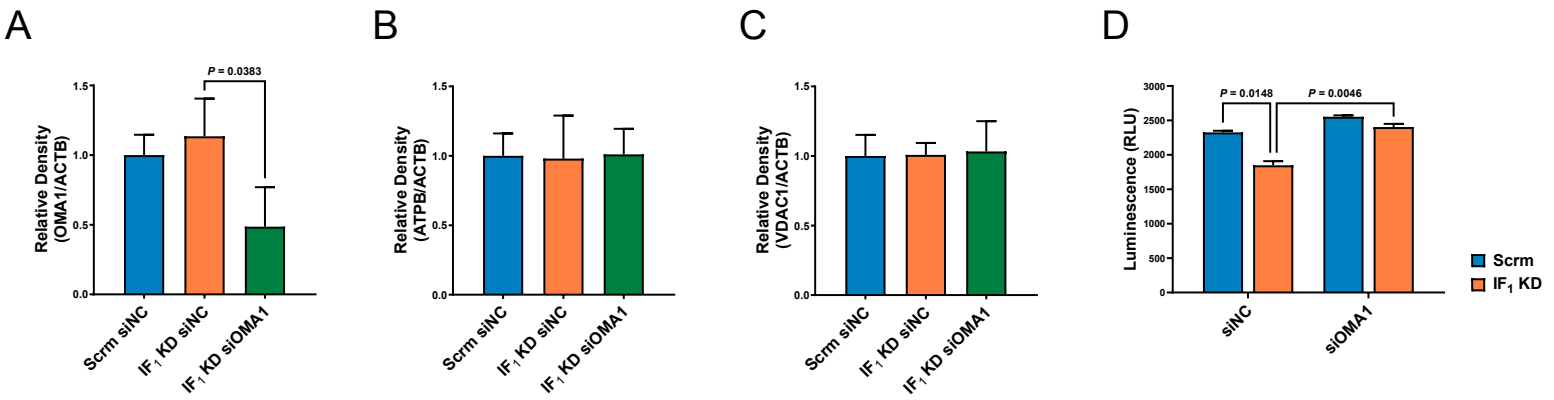
(D) Western blotting analysis of CREB and phospho-CREB (Ser133) levels in Scrm and IF_1 KD cells; vinculin was used as loading control.

(E) Densitometry analysis of CREB protein levels relative to vinculin (Scrm: 1.03 ± 0.19; IF_1 KD: 0.85 ± 0.27; N = 4).

(F) Quantification of phospho-CREB protein levels relative to vinculin (Scrm: 0.96 ± 0.11; IF_1 KD: 1.46 ± 0.41; N = 4).

(G) Average ratio between phospho-CREB and CREB (Scrm: 0.97 ± 0.07; IF_1 KD: 1.89 ± 0.67; N = 4).

Supplementary Figure 3



Supplementary Figure 3

(A) Densitometry analysis of OMA1 protein levels relative to GAPDH (see Figure 1O; normalized values, Scrm siNC = 1.00 ± 0.15; IF_1 KD siNC = 1.14 ± 0.27; IF_1 KD siOMA1 = 0.49 ± 0.28; N = 3).

(B) Quantification of ATPB protein levels relative to GAPDH (see Figure 1O; normalized values, Scrm siNC = 1.00 ± 0.16; IF_1 KD siNC = 0.98 ± 0.31; IF_1 KD siOMA1 = 1.01 ± 0.18; N = 3).

(C) Quantification of VDAC1 protein levels relative to GAPDH (see Figure 1O; normalized values, Scrm siNC = 1.00 ± 0.15; IF_1 KD siNC = 1.01 ± 0.09; IF_1 KD siOMA1 = 1.03 ± 0.22; N = 3).

(D) ATP-based luminescence analysis of total intracellular ATP levels in Scrm and IF_1 KD cells transfected with either siNC or OMA1 siRNA (Scrm siNC = 2325.72 ± 24.02 RLU; Scrm siOMA1 = 2552.33 ± 21.07 RLU; IF_1 KD siNC = 1848.33 ± 60.02 RLU; IF_1 KD siOMA1 = 2403.17 ± 46.78 RLU; N = 3).