POLG2 Disease Variants: Analyses Reveal a Dominant Negative Heterodimer, Altered Mitochondrial Localization, and Impaired Respiratory Capacity

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SUPPLEMENTARY MATERIAL

Supplementary Figure 1. Denaturing PAGE analysis of functional interaction reactions containing p55 heterodimer variants. Reactions were setup as described in Figure 2 and then loaded onto a 12% polyacrylamide gel. Primer, no protein added to the reaction; L, low salt (none added to the reaction); H, high salt (reactions supplemented with 150 mM NaCl); 140, p140 catalytic subunit only; WT, WT p55; P, WT/P205R; R, WT/R369G; G, WT/G451E; L, WT/L475DfsX2 p55 heterodimers.

Supplementary Figure 2. Standard XF24 bioenergetic assay OCR profiles. OCR profiles of the mitochondrial bioenergetics parameters of **A,** HEK293 untransfected cells and NGFP p55 transfected variant stable cell lines: **B**, P205R, **C** R369G, **D** G451E and **E** L475DfsX2. The mitochondrial stress test profile measures the four key parameters of mitochondrial function in cells grown in a microplate: basal respiration, ATP production, proton leak and maximal respiration following the addition of indicated compounds.

Supplementary Figure 3. Bioenergetic parameters for stable cell lines. A, ATP production for all cell lines as measured by the change in OCR following the addition of oligomycin. **B**, Proton leak for all cell lines as determined by the difference between oligomycin sensitive ATP coupled OCR production and non-mitochondrial O_2consumption**.**

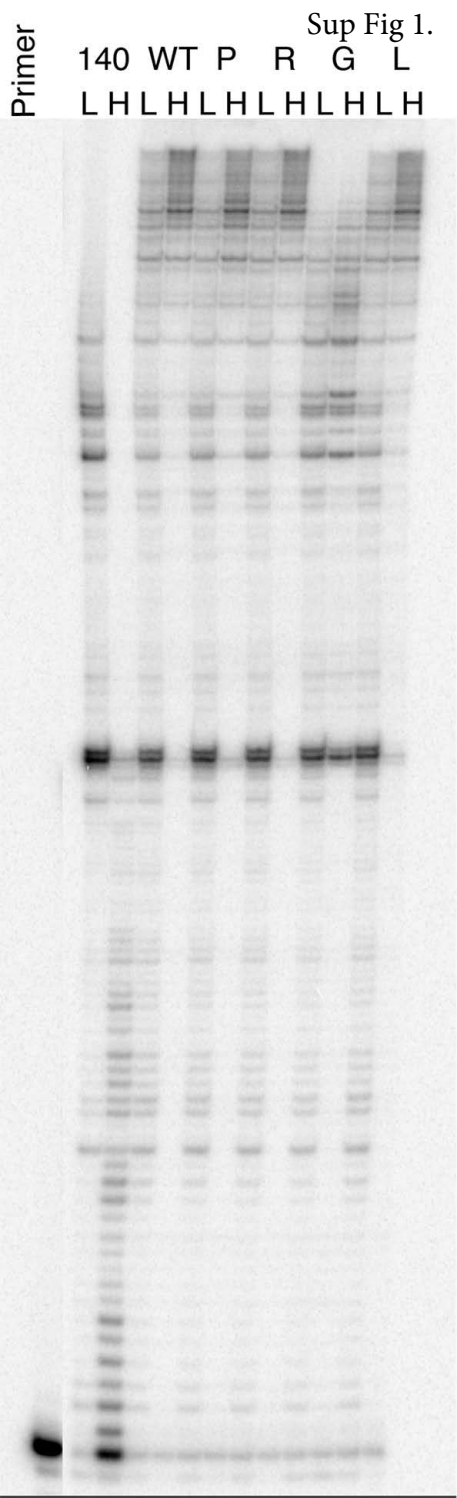
Supplementary Figure 4. Homogeneous preparations of P205R and L475DfsX2 p55 variants form aberrant reducible multimers. Partial denaturing PAGE analysis was utilized to analyze homogeneous preparations of WT and variant p55s. Samples of 0.62 mg purified WT p55 or variant were resuspended in 0.03% SDS sample buffer with or without b-mercaptoethanol and incubated with or without heating followed by visualization by Western blotting (Materials and Methods). W or WT, WT p55; P or P205R, P205R p55; L or L475D, L475DfsX2 p55. Molecular weight standards (kDa) are indicated on the left-hand-side of the gel. Note, we predict that weaker detection of the ~55 kDa band by anti-p55 in samples

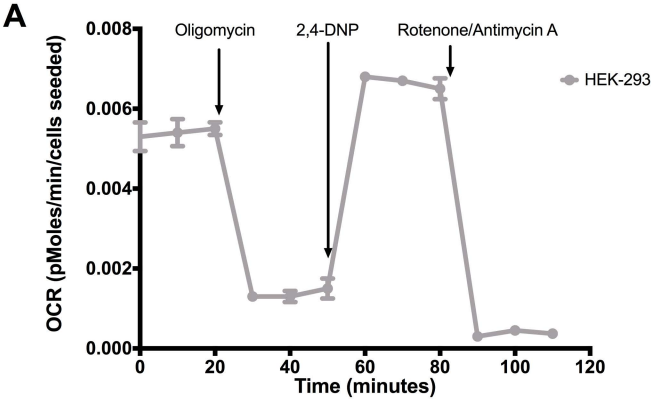
treated with heat and b-mercaptoethanol results from disruption of antibody-recognized secondary and tertiary structural elements.

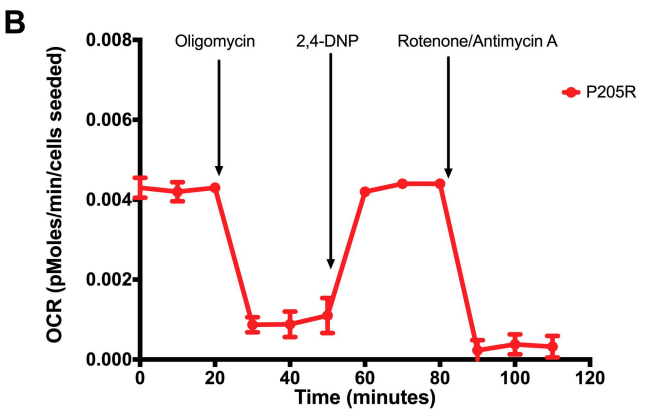
SUPPLEMENTARY METHODS

Partial denaturing PAGE – Samples of 0.62 mg purified p55 WT or variant p55 homodimers were individually prepared in 0.03% SDS sample buffer consisting of 31.25 mM Tris-HCl, pH 6.8, 7.5% glycerol, 0.005% bromophenol blue, and 0.03% sodium dodecyl sulfate (SDS). Homodimeric p55 variants were prepared as previously described (*13*). When sample buffer was supplemented with b-mercaptoethanol the final concentrations was 3%. Samples were heated at 95ºC or incubated at room temperature for 5 minutes, followed by analysis on a Criterion^{TM} XT 4-12% Bis-Tris gel in XT MES running buffer (Bio-Rad). Resolved proteins were then electrotransferred to Immobilon-P PVDF (polyvinylidene fluoride) membrane (Millipore) and bands were visualized by Western blotting and immunodetection as described above for HIS-tag protein detection with the exception that the primary antibody used for p55 detection was a 1/100 dilution of anti-p55 rabbit polyclonal antiserum (*19*) in 5 g non-fat dry milk in 100 ml TN buffer and the secondary antibody was a 1/3000 dilution of goat anti-rabbit alkaline phosphatase conjugate (Bio-Rad) in TN buffer with 1 mg/ml BSA.

Sup Fig 1.




A
Oligomycin
2,4-DNP
Rotenone/Antimycin A
HEK-293
OCR (pMoles/min/cells seeded)
0.008
0.006
0.004
0.002
0.000
0
20
40
60
80
100
120
Time (minutes)


B
Oligomycin
2,4-DNP
Rotenone/Antimycin A
P205R
OCR (pMoles/min/cells seeded)
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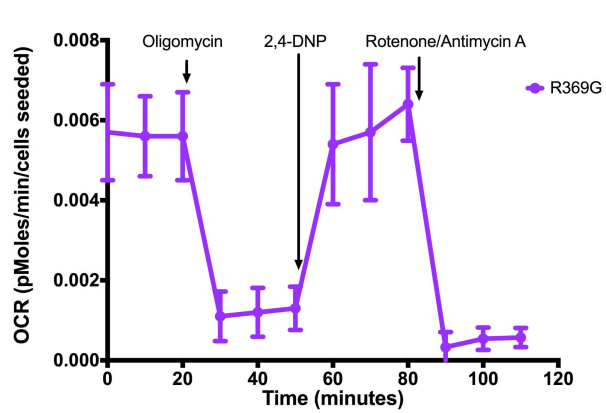
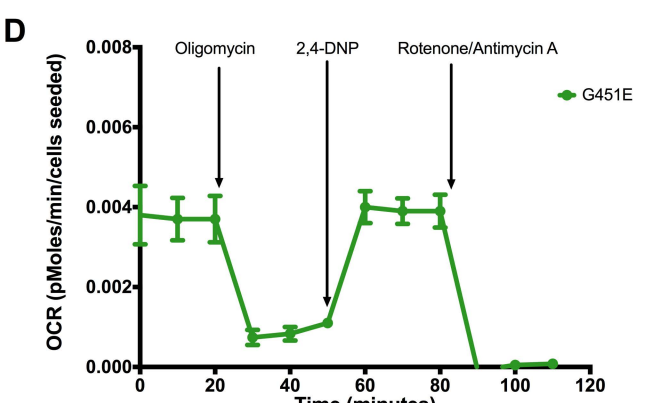
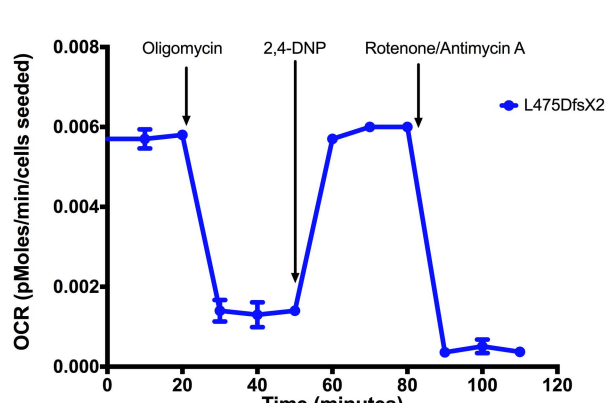
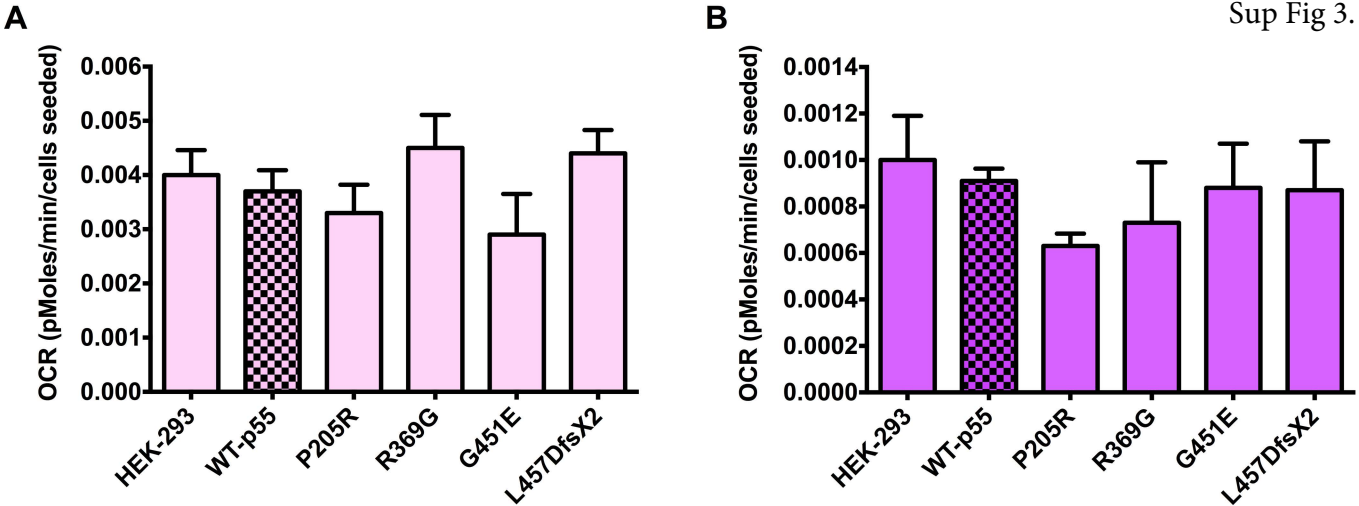
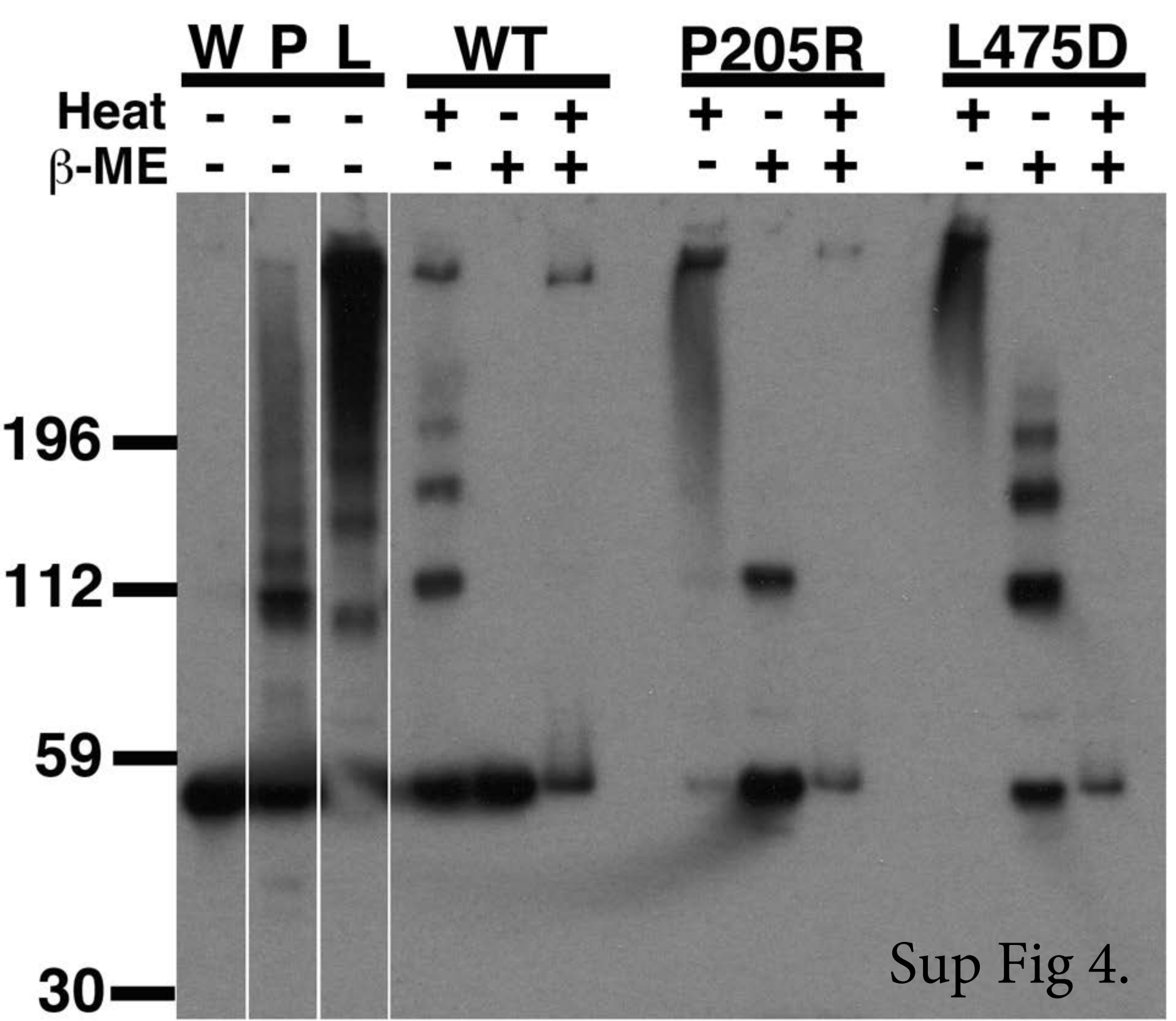
0.006
0.004
0.002
0.000
0
20
40
60
80
100
120
Time (minutes)


C
Oligomycin
2,4-DNP
Rotenone/Antimycin A
R369G
OCR (pMoles/min/cells seeded)
0.008
0.006
0.004
0.002
0.000
0
20
40
60
80
100
120
Time (minutes)


D
Oligomycin
2,4-DNP
Rotenone/Antimycin A
G451E
OCR (pMoles/min/cells seeded)
0.008
0.006
0.004
0.002
0.000
0
20
40
60
80
100
120
Time (minutes)


E
Oligomycin
2,4-DNP
Rotenone/Antimycin A
L475DfsX2
OCR (pMoles/min/cells seeded)
0.008
0.006
0.004
0.002
0.000
0
20
40
60
80
100
120
Time (minutes)


A
OCR (pMoles/min/cells seeded)
0.006
0.005
0.004
0.003
0.002
0.001
0.000
HEK-293
WT-p55
P205R
R369G
G451E
L457DfsX2
B
OCR (pMoles/min/cells seeded)
0.0014
0.0012
0.0010
0.0008
0.0006
0.0004
0.0002
0.0000
HEK-293
WT-p55
P205R
R369G
G451E
L457DfsX2


W P L
WT
P205R
L475D
Heat - - - + - + + - + + - +
β-ME - - - - + + - + + - + +
196
112
59
30
Sup Fig 4.