Appendix A. Supplementary data for

THE MMACHC PROTEOME: HALLMARKS OF FUNCTIONAL COBALAMIN DEFICIENCY IN HUMANS

Luciana Hannibal,[1,2*] Patricia M. DiBello,[1] Michelle Yu,[1] Abby Miller,[3] Sihe Wang,[3] Belinda Willard,[1] David S. Rosenblatt,[4] and Donald W. Jacobsen[1,2,5]**

[1]Department of Cell Biology, Lerner Research Institute, Cleveland Clinic

Cleveland, OH 44195

[2]School of Biomedical Sciences, Kent State University, Kent, OH 44242

[3]Department of Clinical Pathology, Cleveland Clinic, Cleveland, OH 44195

[4]Department of Human Genetics, McGill University, Montreal, QC

[5]Department of Molecular Medicine, Cleveland Clinic Lerner College of Medicine,

Case Western Reserve University, Cleveland, OH 44106

Table S1. Gel set up for 2D-DIGE experiments and fluorophore labeling scheme.

		Cy2	Cy5	Cy3
-HOCbl	Gel 1[a]	IS Pool	Control	WG1801
	Gel 2	IS Pool	Control	WG2176
	Gel 3	IS Pool	Control	WG3354
	Gel P1[b]	Unlabeled IS Pool		
+HOCbl	Gel 4	IS Pool	Control	WG1801
	Gel 5	IS Pool	Control	WG2176
	Gel 6	IS Pool	Control	WG3354
	Gel P2	Unlabeled IS Pool		

[a]Gels 1-6: analytical gels; [b]Gels P1 and P2: preparative gels. Internal standard: Pool of normal, WG1801, WG2176 and WG3354.

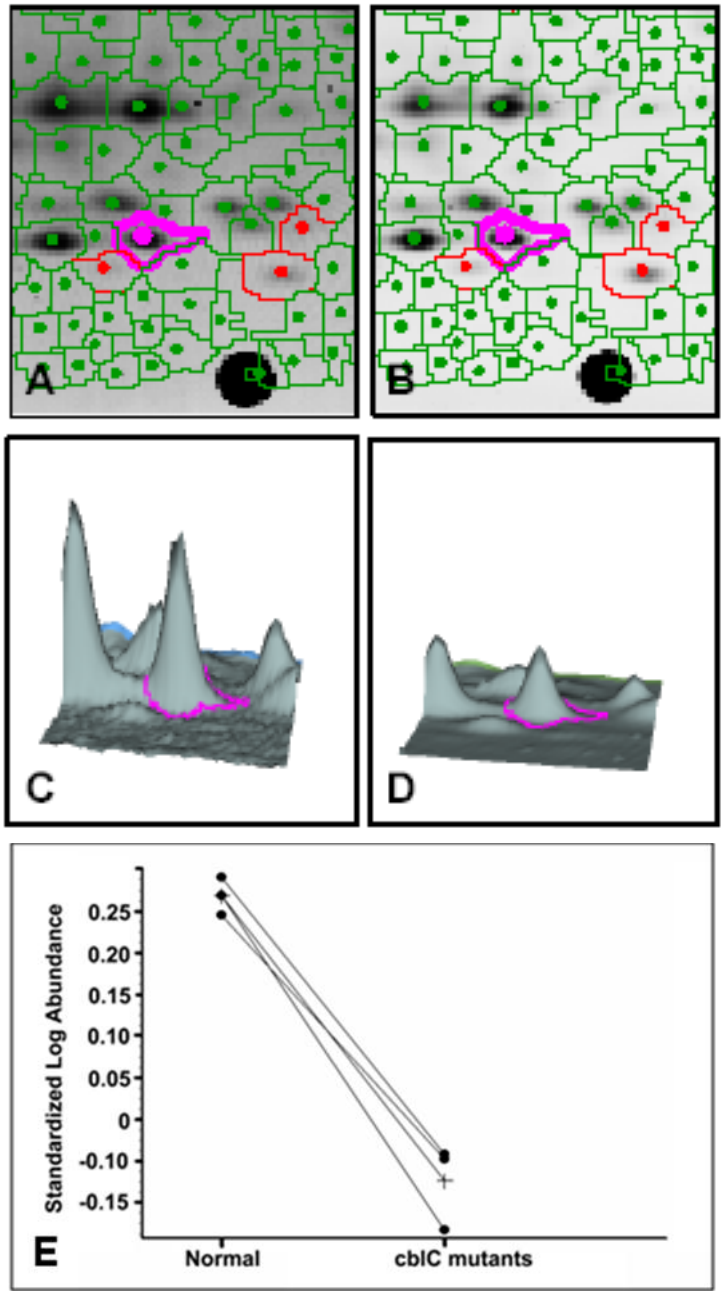
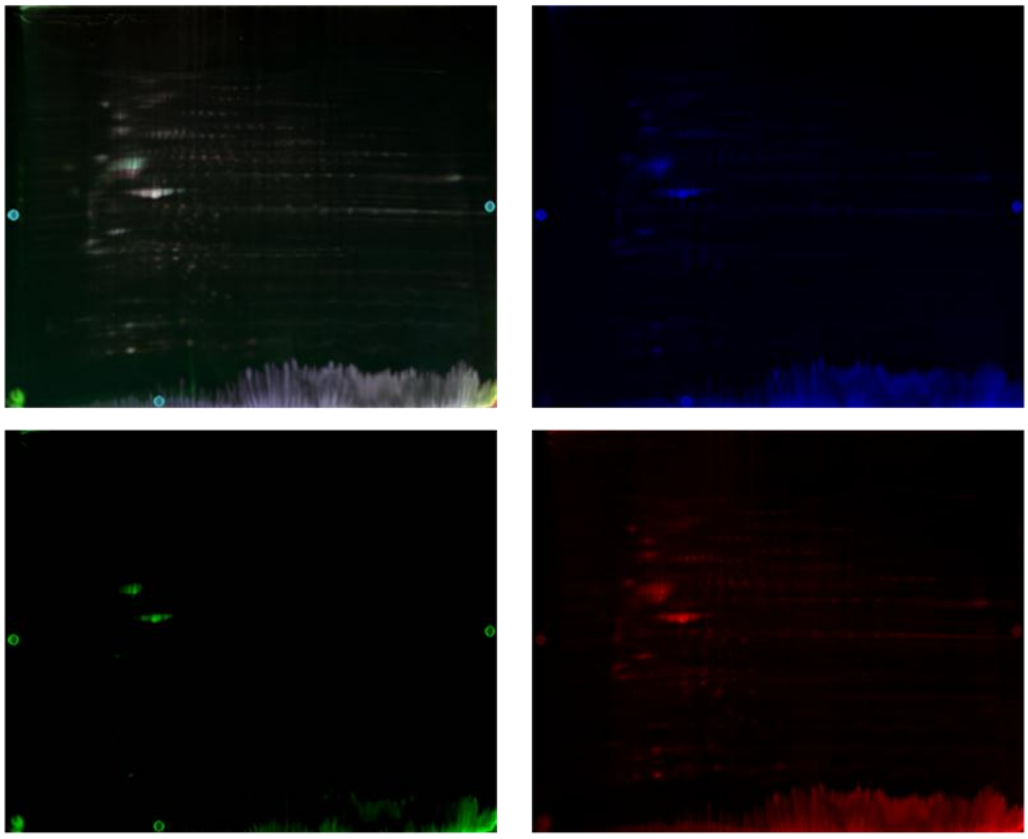


Figure S1. A representative scheme for 2D-DIGE image analysis using DeCyder® software. As an example, Spot 39 (outlined in pink) from gel P2 is shown in panels **A** (Cy5-labeled normal cells) and **B** (Cy3-labeled WG1801 mutant cells). For this particular example the cblC spot demonstrated a -2.19 fold change in volume (panels **C** and **D**, Cy5 and Cy3, respectively). A plot of log of the standardized abundance for normal and *cblC* fibroblasts is shown in panel **E** (n=3). Mass spectrometry analysis revealed that the expression change exemplified herein corresponded to protein S100A6. Fold change cut-off: ± 2.0. Statistical significance: $p < 0.05$, n=3.



1801 minus B12

Figure S2. 2D-DIGE fluorescence scans for normal (blue) and patient WG1801 (red) proteomes. The internal standard (green) and merged image are also shown.

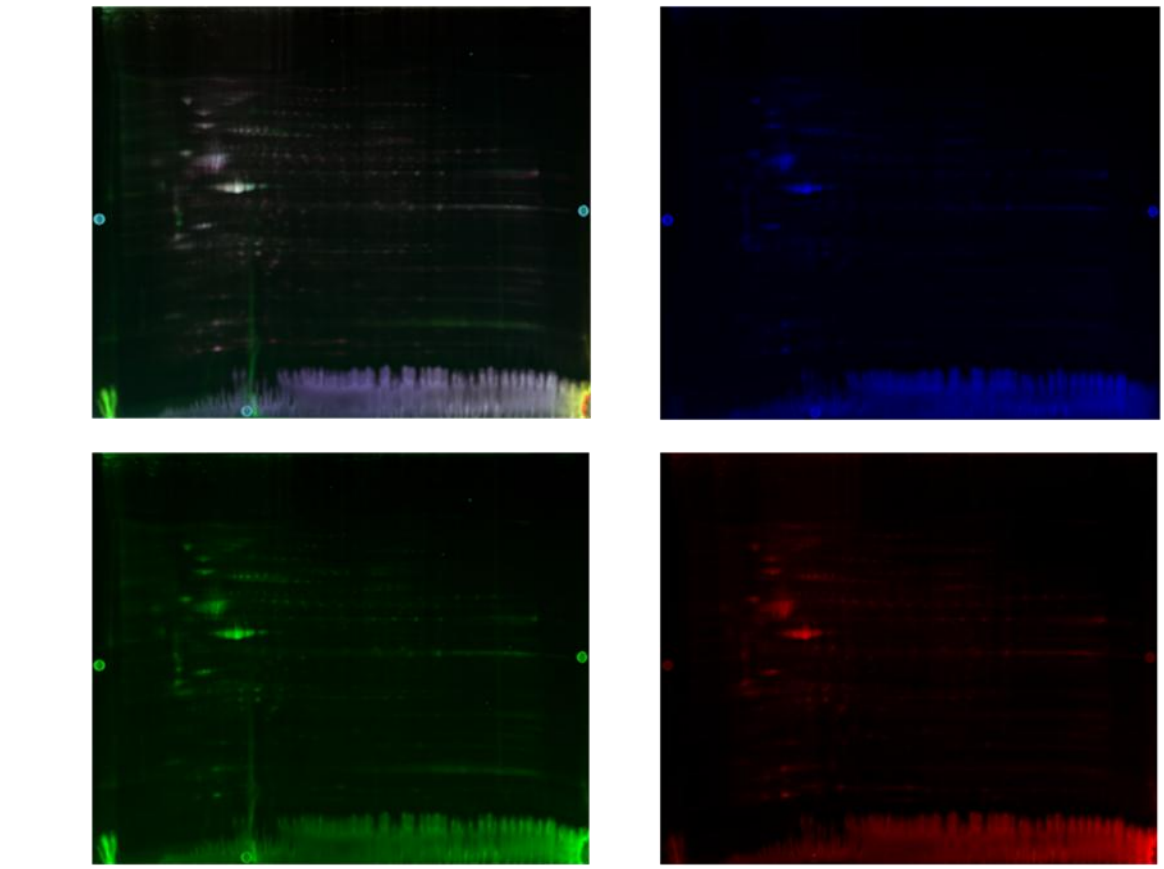


Figure S3. 2D-DIGE fluorescence scans for normal (blue) and patient WG2176 (red) proteomes. The internal standard (green) and merged image are also shown.

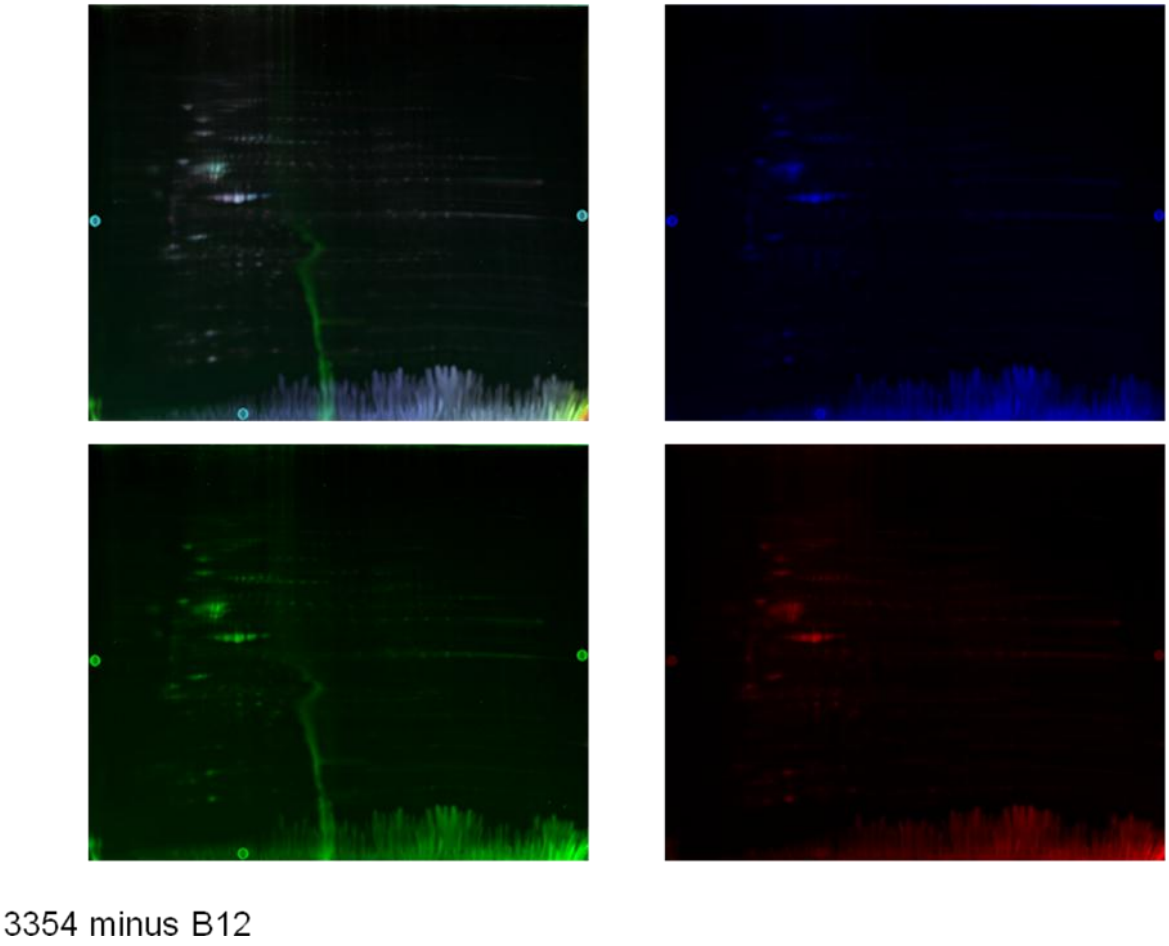
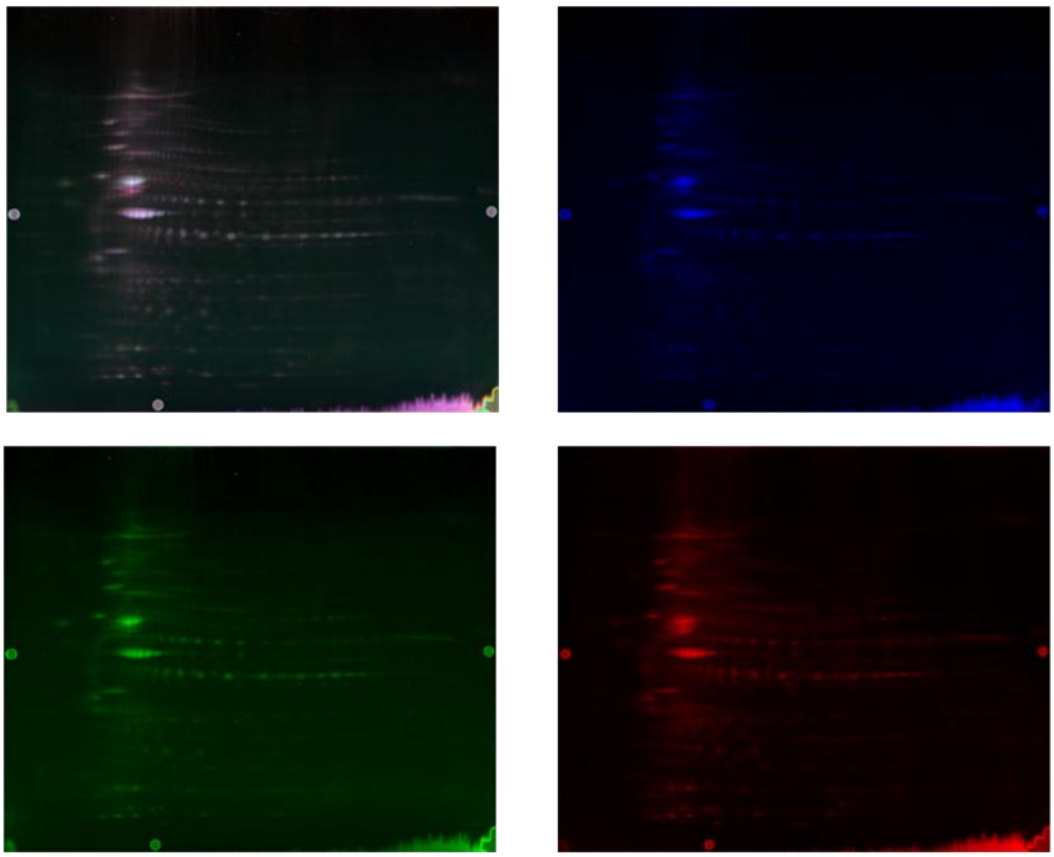


Figure S4. 2D-DIGE fluorescence scans for normal (blue) and patient WG3354 (red) proteomes. The internal standard (green) and merged image are also shown.



1801 plus B12

Figure S5. 2D-DIGE fluorescence scans for normal (blue) and patient WG1801 (red) proteomes from fibroblasts grown in the presence of 723 nM hydroxocobalamin. The internal standard (green) and merged images are also shown.

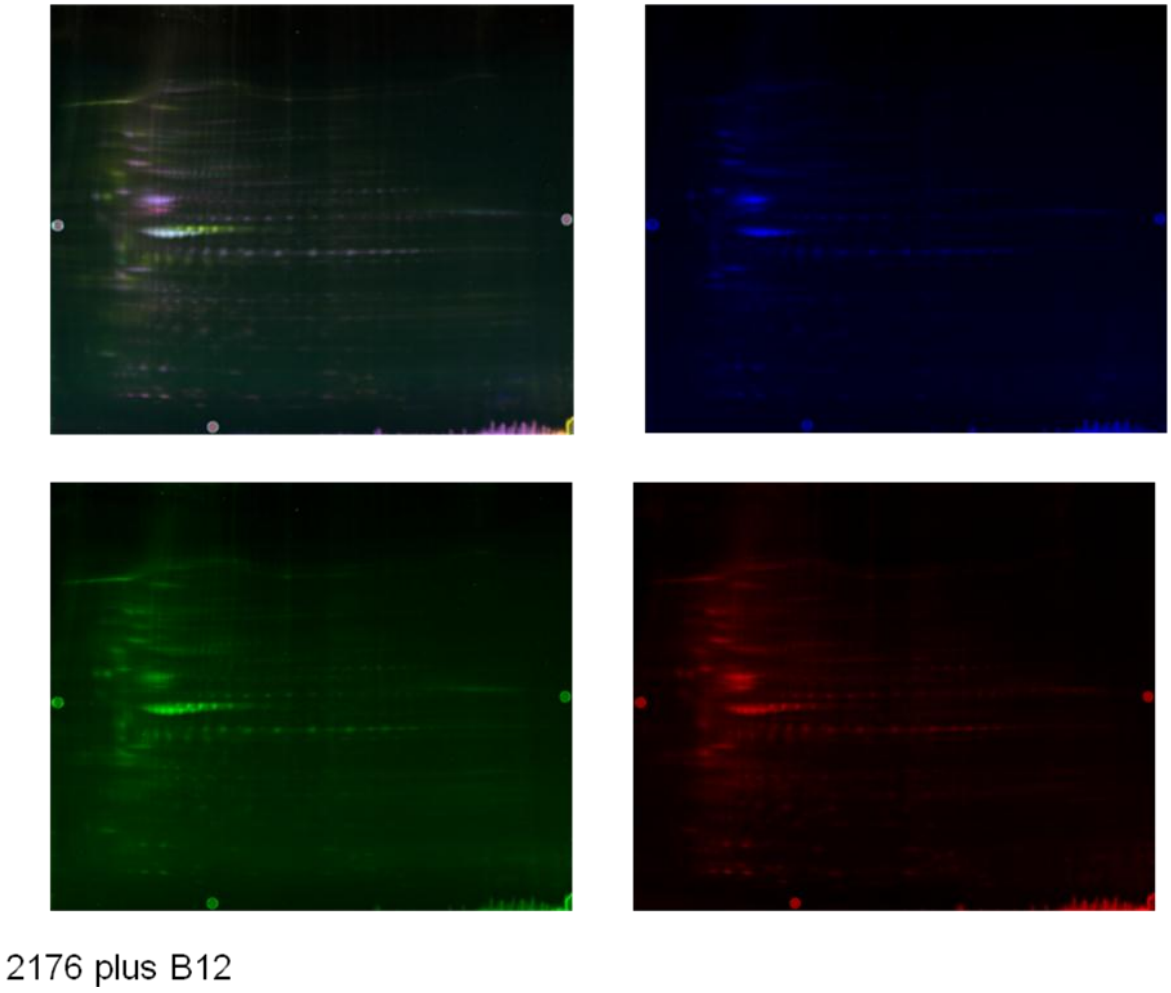


Figure S6. 2D-DIGE fluorescence scans for normal (blue) and patient WG2176 (red) proteomes from fibroblasts grown in the presence of 723 nM hydroxocobalamin. The internal standard (green) and merged images are also shown.

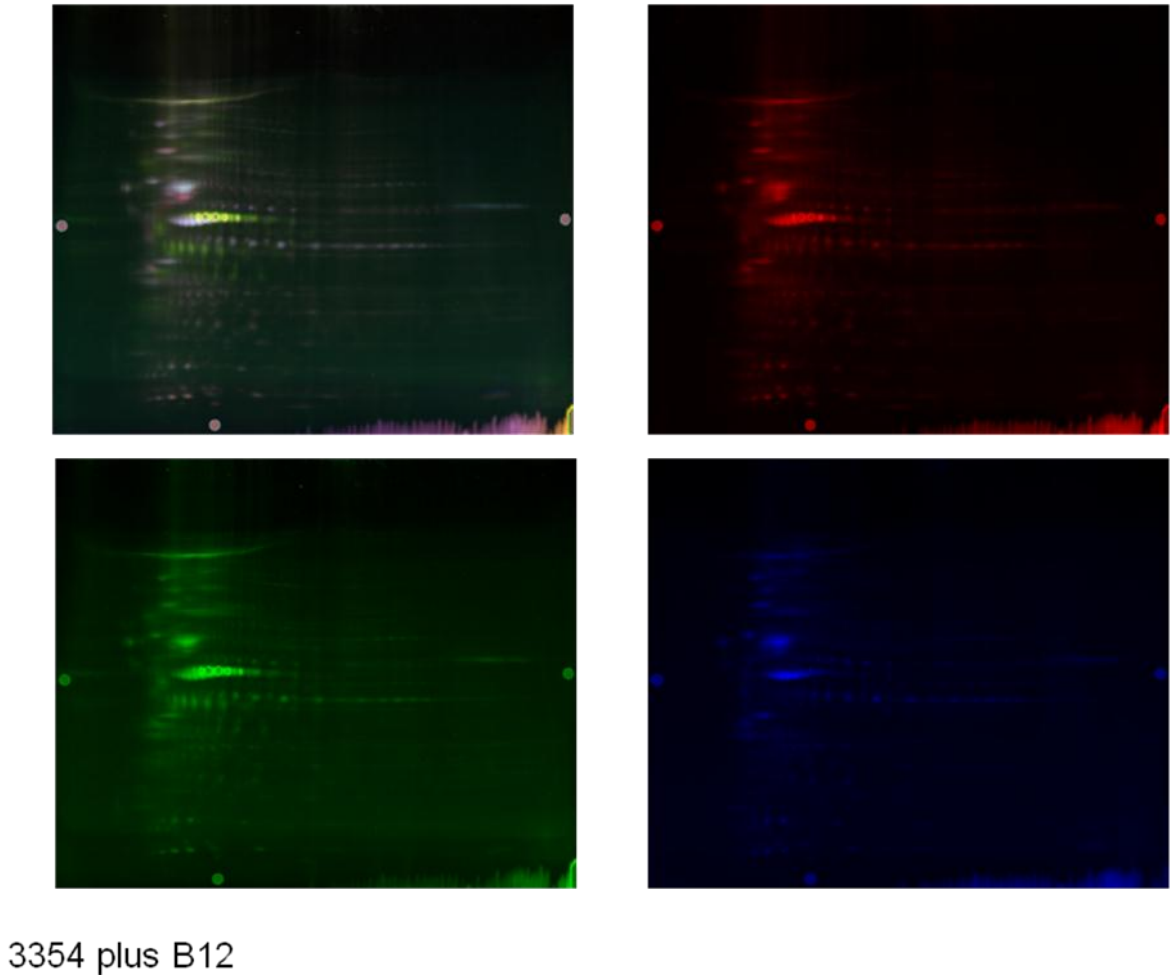


Figure S7. 2D-DIGE fluorescence scans for normal (blue) and patient WG3354 (red) proteomes from fibroblasts grown in the presence of 723 nM hydroxocobalamin. The internal standard (green) and merged images are also shown.

Assessment of cellular apoptosis (DNA laddering) and senescence (β-galactosidase activity). Cellular apoptosis (late stage) was assessed by determining the degree of genomic DNA fragmentation. Total genomic DNA was extracted (Wizard Genomic DNA purification kit, Promega, Madison, WI) from cells grown in the absence of exogenous Cbl and run on a 1% agarose gel. The gel was stained with ethidium bromide and photographed under UV light. Senescence was assessed *in situ* using a commercially available kit (Senescence Cells Histochemical Staining Kit, Sigma, St Louis, MO). Cells were seeded at ~ 30% density and grown in regular cultured medium for 24 h. The conditioned cultured medium was removed and cells were washed three times with phosphate buffered saline. The assay was conducted according to the manufacturer's instructions.

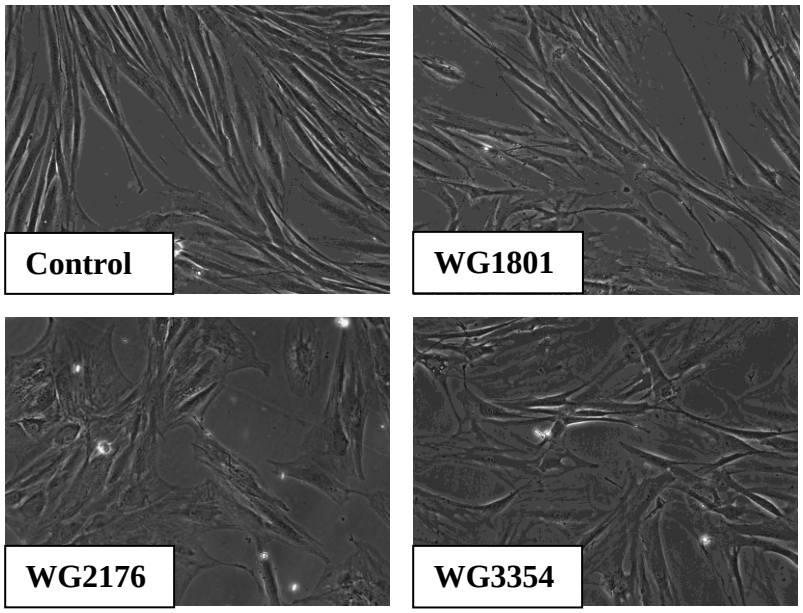


Figure S8. Morphology of control (normal fibroblasts) and *cblC* mutants by inverted phase contrast microscopy after 36 h in culture. Magnification: 100X.

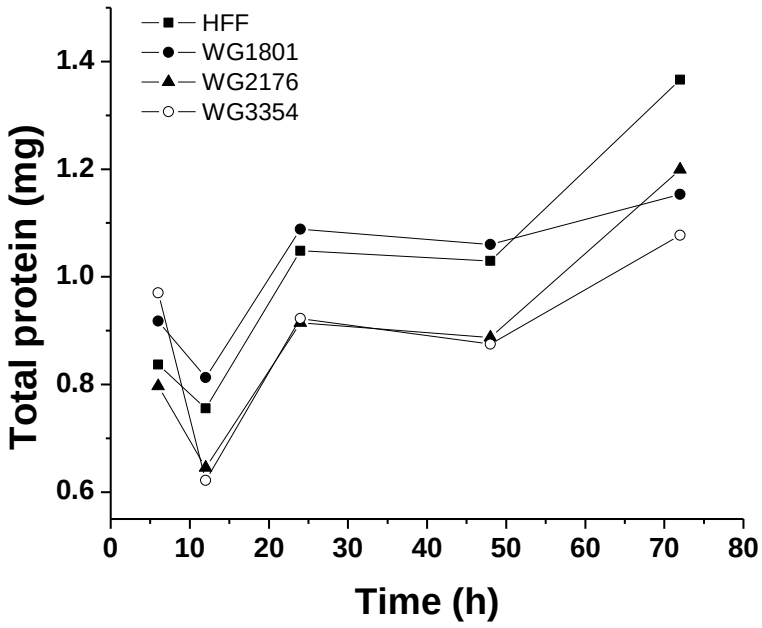


Figure S9. Growth kinetics of normal and *cblC* fibroblasts. The cultures were started with an initial density of ~30%. At each time point, the cells were washed twice with PBS, trypsinized and the total protein was measured by the BCA assay.

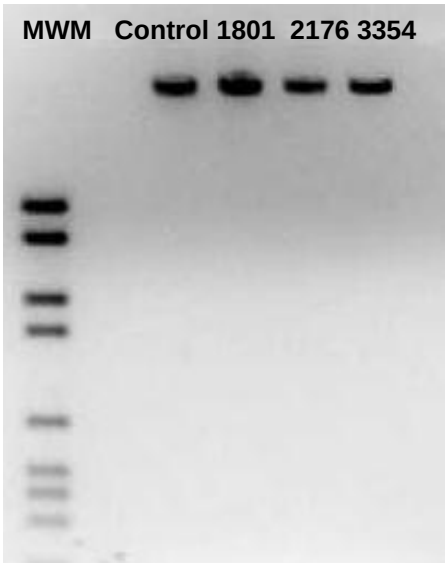


Figure S10. DNA laddering assay. MWM: molecular weight marker (1-10 Kb). ~1 μg of total DNA per lane.

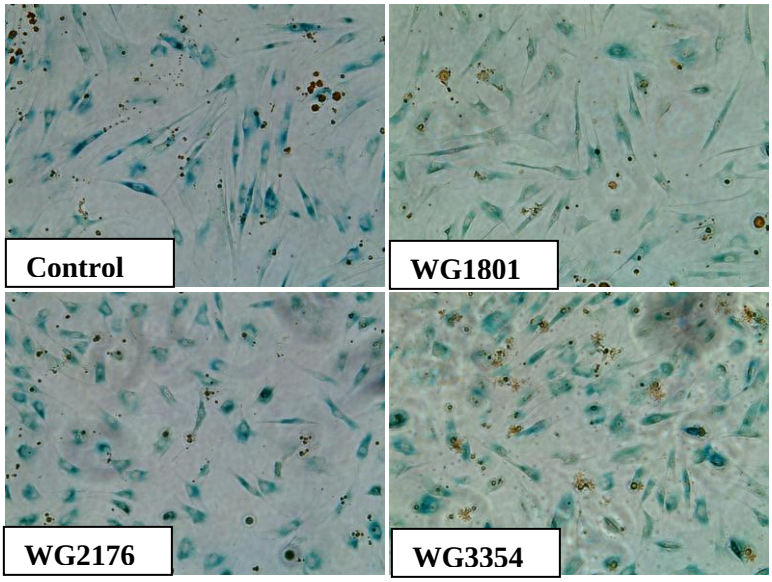


Figure S11. β-galactosidase staining of normal and *cblC* cells in culture. Brown granules are precipitates of the dye used in the assay. Senescent (viable but with an arrested cell cycle) cells express higher levels of β-galactosidase than normally dividing cells. No differences were observed between normal and *cblC* mutant cells.

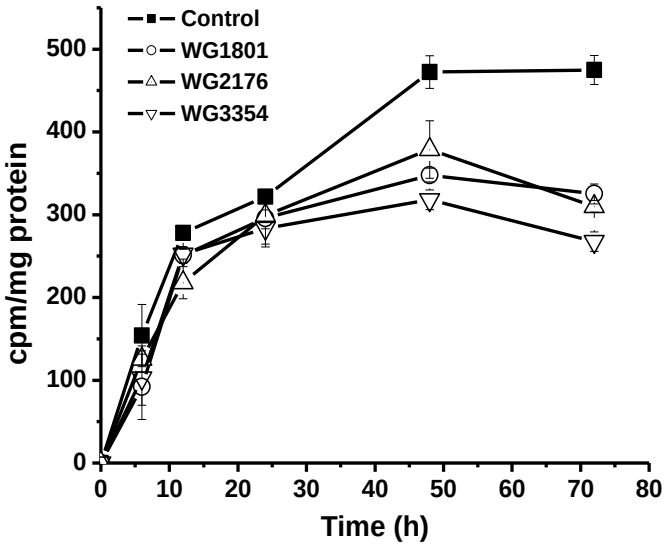


Figure S12. [^{57}Co]-HOCbl uptake by control and *cblC* fibroblasts. Cells were washed twice with PBS, lysed and the radioactivity counted on a γ-counter. Values (mean ± standard deviation, n=3) were normalized to cellular concentration of protein.