SUPPLEMENTARY INFORMATION

Detailed clinical description of patients

Patient 1 is a 2.5 year-old girl, the first child of non-consanguineous Italian parents, born at term after an uneventful pregnancy and delivery. Growth parameters were normal at birth. She presented with joint contractures and generalized joint pain from the 3rd month of life. Skin and mucosae were normal at that time. A skeletal survey did not reveal any specific abnormality. Metabolic storage disorders and rheumatic disorders were ruled out by relevant blood and urine analyses. The acute phase of pain and stiffness of all joints lasted for about 2 months. She seemed to respond initially to corticosteroid therapy, with transient improvement of joint mobility. During the first year of life gingival hypertrophy and skin nodules appeared. She was evaluated at our center at 2 years of age: she had marked growth delay (weight 7.8 kg, <P3; length 67 cm, <P3), multiple protruding gum nodules, erythematous plaques around the nose, on the neck, and on her right ear; one large subcutaneous nodule, measuring 5 cm in diameter, was present at the occipital right region of the skull; protruding perianal nodules were present, without fissures. Joint rigidity was generalized, more marked at knees (blocked in flexion), elbows and shoulders, less marked at the spine and distal joints. Cognitive development was normal for age, motor development was delayed. Failure to thrive was primary, without either diarrhea or impaired feeding, despite the oral lesions. There was no increased frequency of infections and no unexplained fever. Pain at joint mobilization during physiotherapy was partially controlled by non-steroid anti-inflammatory drugs. No continuous antalgic therapy was needed. She underwent surgery for the oral and perianal nodules at 2 years and 2 months of age. At present, she performs intensive physiotherapy and water gymnastics with some improvement in joint mobility.

Detailed medical information was not available for **Patient 2**. The patient showed signs of disease at birth and underwent long-term intensive physiotherapy as well as multiple operations for nodule removal. The patient is currently 16 and exhibits the classical signs of HFS, but was never subject to recurrent diarrhea or pulmonary infection.

Patient 3, a six week-old boy, was born five weeks preterm as the second child of consanguineous Turkish parents after an otherwise unremarkable pregnancy and delivery. Parameters for length and weight were within normal limits for gestational age and ethnicity. All other members of the family were healthy, including a three year-old sister. Initial reason for referral was a progressive painful limitation of joint mobility beginning in the first month of life. At that time, clinical examination revealed skin disease with thickening over the joints and hyperpigmentation on the areas overlying knuckles and malleoli. In the following months, pink nodules and plaques appeared on the face and extended to ears and neck; trunk, thighs and the perianal area were also affected by six months of age. Repeat examination showed generalized progressive contractures with characteristic ‘frog-leg position’, muscle wasting, and loss of subcutaneous fat. Motor milestones were delayed, but mental development was normal. Continuous morphine therapy was required for every-day handling of the child and to enable physiotherapy. Gingival hypertrophy caused difficulty during feeding. Failure to thrive was also due to recurrent episodes of diarrhea starting in the second month of life, often associated with prolonged fever and signs of bacterial infection. In spite of early and adequate therapeutic intervention, circulatory failure ensued during one infectious episode and the boy died at the age of 8 months.

Patient 4 is a 3 year-old boy, the second child of non-consanguineous Polish parents, born at term after an uneventful pregnancy. During the maternal ultrasound examination at 32 weeks of pregnancy, shorter tubular bones were suspected, but not confirmed at birth. Delivery occurred by caesarean section because of oligohydroamnios. Birth weight was 2800 g. During the first 5 weeks, the boy was treated because of talipes equinovarus. Progressive painful limitation of joint mobility began in first month of life. The baby did not tolerate physiotherapy (constant crying). At the age of 5 months, he was referred to Neurology with a tentative diagnosis of arthrogryposis. Because of a slightly coarse face, macrocephaly (head circumference at 7 months of age was 47 cm, +2,46 SD) and limited joint mobility, the patient was strongly suspected of a storage disorder, which were ruled out by repeated enzymatic examinations. At the age of 10 months, the patient was referred to the Metabolic department, were "primary connective tissue disorder" was suspected. X-ray analysis of bones suggested a diagnosis of infantile systemic hyalinosis. Because of exaggeration of protracted diarrhea, colonoscopy and gastroscopy were performed and lymphangiectasies were found in the duodenum. Alpha-1-antytripsine in stool was 6,31 mg/1 g of stool (normal values below 1,81), indicating intestinal protein loss. At that time, the first skin nodules appeared. The patient also underwent gingivectomy, which improved feeding for a short period. Nevertheless after two years, he was dependent on a nasogastric tube for feeding. Despite normal intellectual development, the patient suffered from malnutrition (weight 9,29 kg (-3,25 SD), small height 83,4 cm (-3,12 SD), smaller head circumference 49 cm (-0,94 SD)), nodules on both ears, fleshy nodules and hyperpigmentation in the perianal region, limited joint mobility disabling standing and manipulation, and recurrent diarrhea. At the age of 3 years, joints became less painful with increased mobility, but

hypertrophy of soft tissues of the mouth developed, and new skin nodules appeared.

Characterization of the 2F6 monoclonal antibody

To tested whether 2F6 indeed recognizes the CMG2 protein, we silenced CMG2 using a lentiviral vector harboring a cmg2 shRNA in three cell types: human skin fibroblasts, Human Umbilical Vein Endothelial cells (HUVEC) and human Retinal epithelial cells (RPE1). CMG2 could be detected in all three cell lines at the expected ≈50 kDa molecular weight and this band was no longer detected after cmg2 silencing (Suppl. Fig. 1A).

To further characterize the antibody, CMG2 harboring a C-terminal V5 tag was ectopically expressed in Hela cells. Total cells extract whether analyzed by Western blotting under reducing and non-reducing conditions using either an anti-V5 antibody or 2F6. As apparent from Suppl. Fig. 1B, whereas the detection of the V5 tag did not vary when reducing gels were used, detection of WT CMG2 with 2F6 was drastically decreased under reducing conditions. We also analyzed the V310F mutant. This mutation as described in the later part of the main text leads to aberrant disulfide bond formation in the CMG2 Ig-like domain. As for the WT protein, this mutant was detected beter with the 2F6 antibody under non reducing conditions. What is also apparent from Suppl. Fig. 1B, is that the V310F is not detected as efficiently as the WT protein, suggesting that 2F6 is a conformational antibody that preferentially recognize CMG2 with correctly formed disulfide bonds, i.e. properly folded protein. That 2F6 poorly recognizes the V310F mutant was further confirmed by immunofluorescence. While the V310F mutant could be readily detected with the anti-V5 antibody, no staining was observed with 2F6.

LEGENDS FOR SUPPLEMENTARY FIGURES

Supplementary Figure 1: Characterization of the anti-hCMG2 2F6 monoclonal antibody.

A. AFF11 fibroblasts, Rpe1 or HUVEC cells were transfected with a inducible-lentivirus expressing a shRNA against CMG2. AFF11 and Rpe1 cells were grown in DMEM medium supplemented with fetal calf serum and penicillin/streptomycin; HUVEC cells in medium 199 supplemented with L-glutamine, penicillin/streptomycin and fetal calf serum. Cells were incubated for 3 or 4 days with doxycycline (1 µg/mL). 40 µg of cell lysates were analyzed by SDS-PAGE in reducing or non-reducing conditions, and Western blotting with the anti-hCMG2 2F6 monoclonal antibody or an anti-Actin antibody. **B.** HeLa cells were transfected for 48 h with WT or V310F CMG2-V5 constructs. 40 µg of cell extracts were analyzed by SDS-PAGE in non-reducing or reducing conditions and Western blotting with an anti-V5, the anti-hCMG2 2F6 monoclonal, or an anti-Actin antibodies. **C.** 20 µg of cells extracts from patients-derived fibroblasts were analyzed by SDS-PAGE in reducing or non-reducing conditions, and Western blotting with the anti-hCMG2 2F6 monoclonal antibody or an anti-Actin antibody.

Note: We have observed using transfection of mouse CMG2 that the 2F6 antibody fails to recognize mouse-CMG2 both by western blotting and immunofluorescence. Thus 2F6 appears to be a species specific antibody.

Supplementary Figure 2: Detection of CMG2 protein in HFS patient fibroblasts

CMG2 was analyzed in 20 µg of cell lysates from patient-derived fibroblasts by SDS-PAGE under non-reducing conditions. Western blotting was performed using the 2F6 anti-hCMG2 antibody.

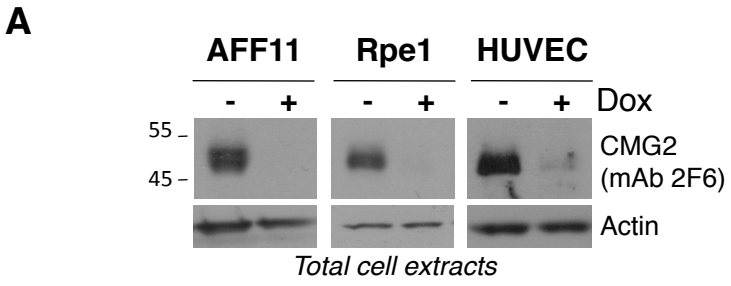
Supplementary Figure 3: Structural alignment of representative Ig-like folds.

A. Multiple sequence alignment of the Ig-like domain of different CMG2 and TEM8 orthologues. Sequences from *Homo sapiens* (human), *Mus musculus* (mouse), *Rattus norvegicus* (rat), *Bos taurus* (bovine) and *Danio rerio* (zebrafish) were aligned using the computer program MUSCLE. Residue conservation is denoted by a blue gradient, from dark blue for highly conserved to light blue for low residue conservation. **B.** Alignment of Human CMG2 (swissprot entry P58335) onto a representative set of 17 Ig-like folds. The name of each sequence/structure is indicated in the first column. The second column indicates the percent identity between CMG2 and each structure, whereas the second column indicates the percentage of cysteines conserved between CMG2 and each structure. Residues are colored by a color gradient indicating their backbone Root Mean Square Deviation (RMSd) to 2cxk. Dark blue indicates a perfect superposition: RMSd < 0.5A; light blue <1.0A; green < 2.5A; yellow <3.5A; orange <4.5A; red > 4.5A or not present in the reference structure.

Supplementary Figure 4:

A. CHO cells were transfected for 48 h with WT or mutants CMG2-V5 constructs. Cell extracts were analyzed by SDS-PAGE in reducing conditions and Western blotting with an anti-V5 antibody. **B.** CHO cells were transfected for 48 h with WT or mutants CMG2-V5 constructs. Cell extracts were analyzed by SDS-PAGE in non-reducing conditions and Western blotting with an anti-V5 antibody. **C.** Fibroblasts

derived from Patient 5 were incubated with 10 μM of MG132 or not, for 16h. Cell lysates (300 μg of total proteins) were immunoprecipitated with an anti-hCMG2 antibody. Samples were analyzed by SDS-PAGE under non-reducing conditions and Western blotting with an anti-hCMG2 antibody. **D.** Control and Patient 1-derived fibroblasts were incubated with 10 μM MG132 or not, for 16h in complete cell medium and subsequently incubated with 0.2 mg/ml NHS-SS-biotin. Cell lysates (300 μg of total proteins) were submitted to immunoprecipitation with streptavidin-agarose beads. Samples and total extracts (20 μg of proteins) were analyzed by SDS-PAGE and Western blotting with the mouse monoclonal anti-hCMG2 antibody 1H8. **E**. Fibroblasts derived from Patients 1, 2 and 5 and control cells were incubated with 10 μM BZ or not, for 16h and subsequently incubated with 0.2 mg/ml NHS-SS-biotin. Cell lysates (20 μg of total proteins) were analyzed by non-reducing SDS-PAGE and Western blotting and probed with anti-hCMG2 and anti-calnexin. **F.** Fibroblasts derived from Patient 1 and control cells were incubated with anthrax PA at a 500 ng/mL final concentration. After different time points, cell lysates were immunoprecipitated with an anti-hCMG2 antibody. Samples were analyzed by SDS-PAGE under reducing or non-reducing conditions and Western blotting with an anti-hCMG2 and anti-ubiquitin antibodies.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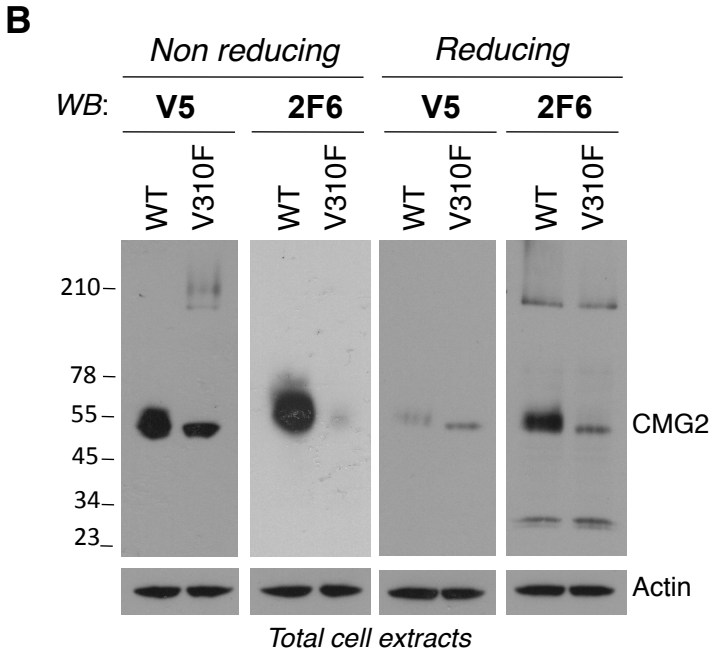
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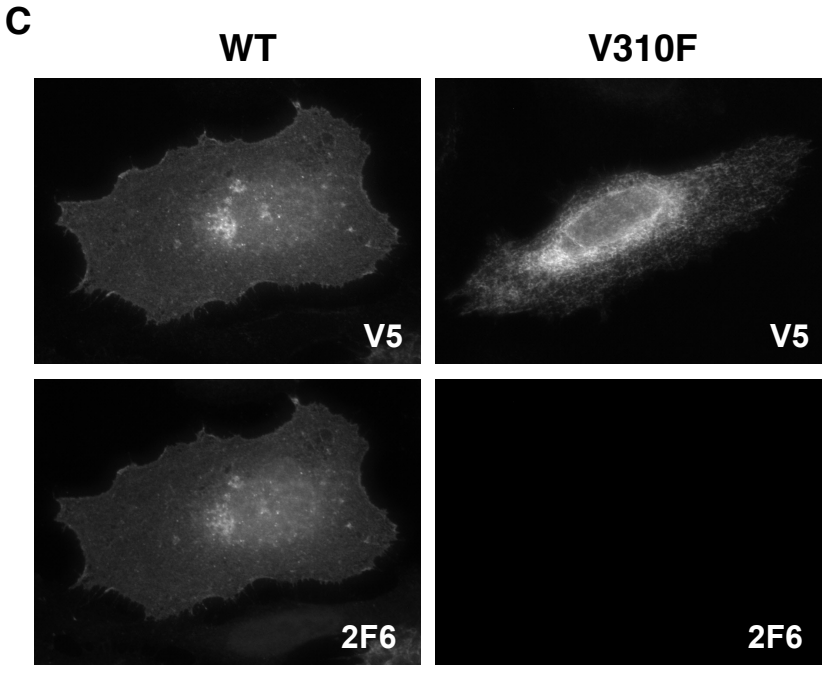
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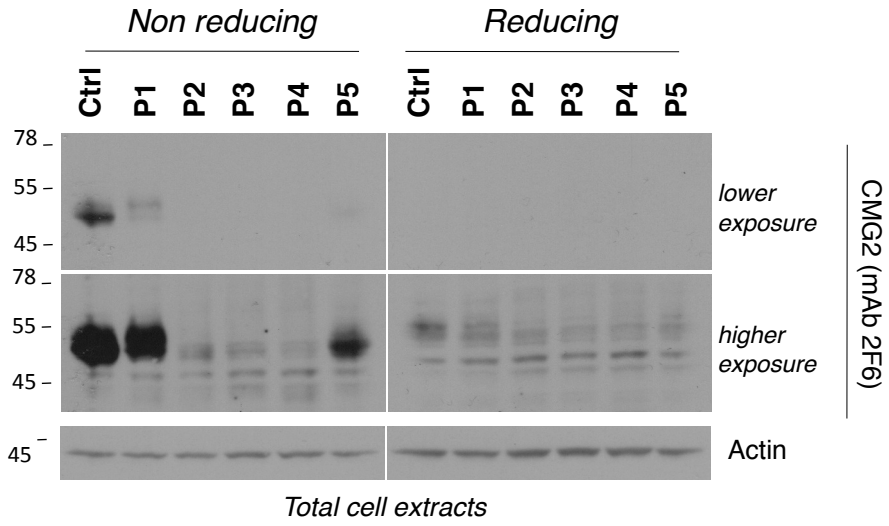
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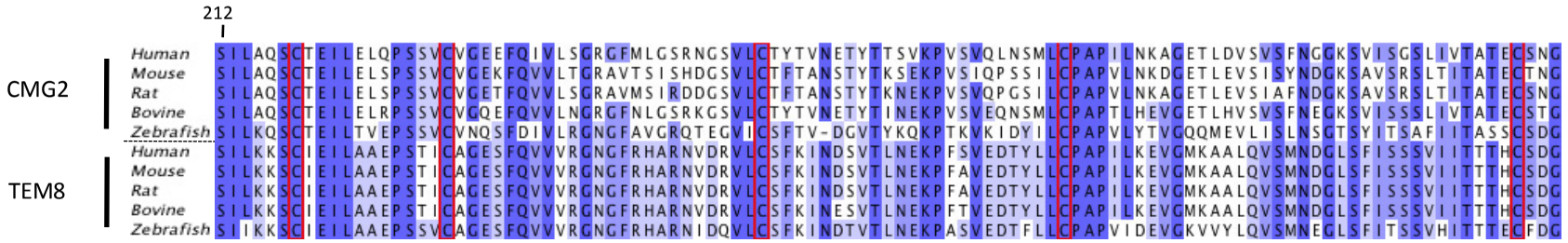

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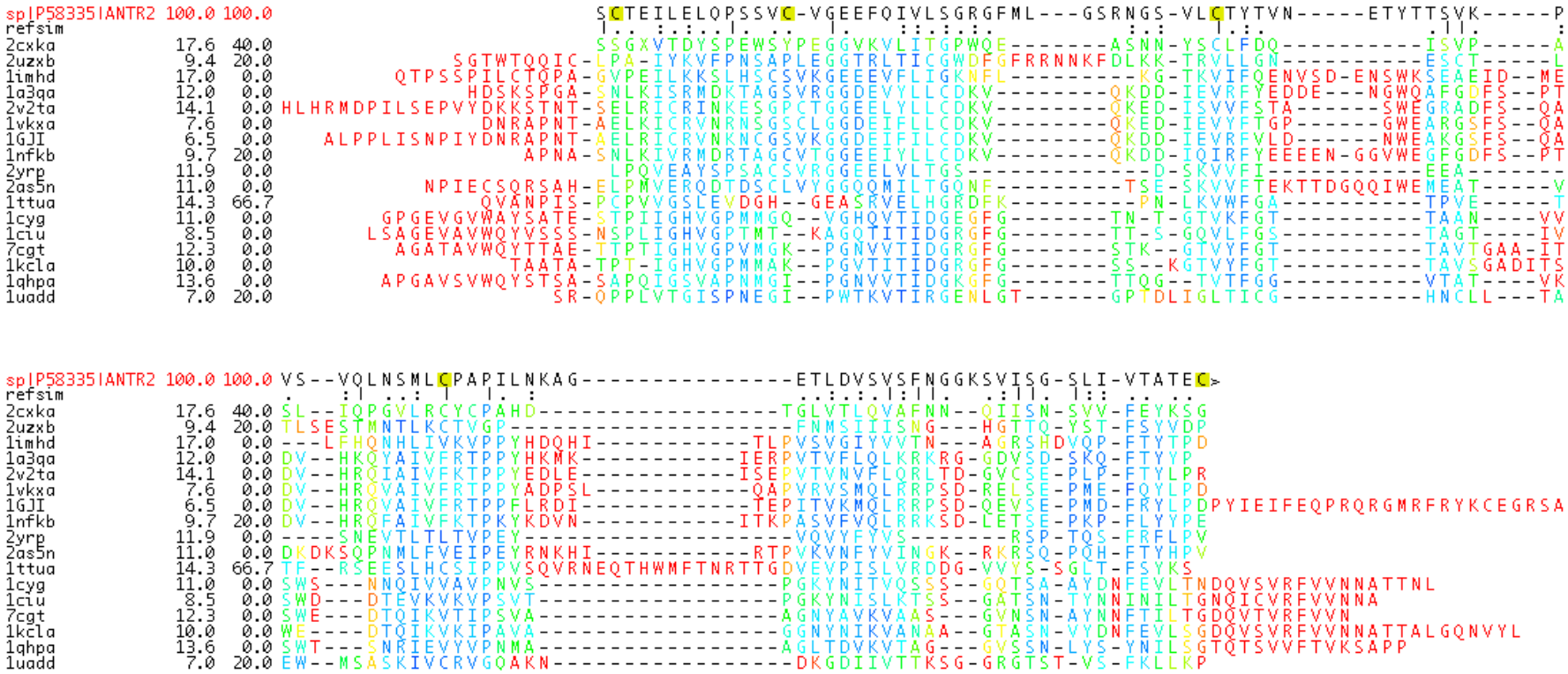

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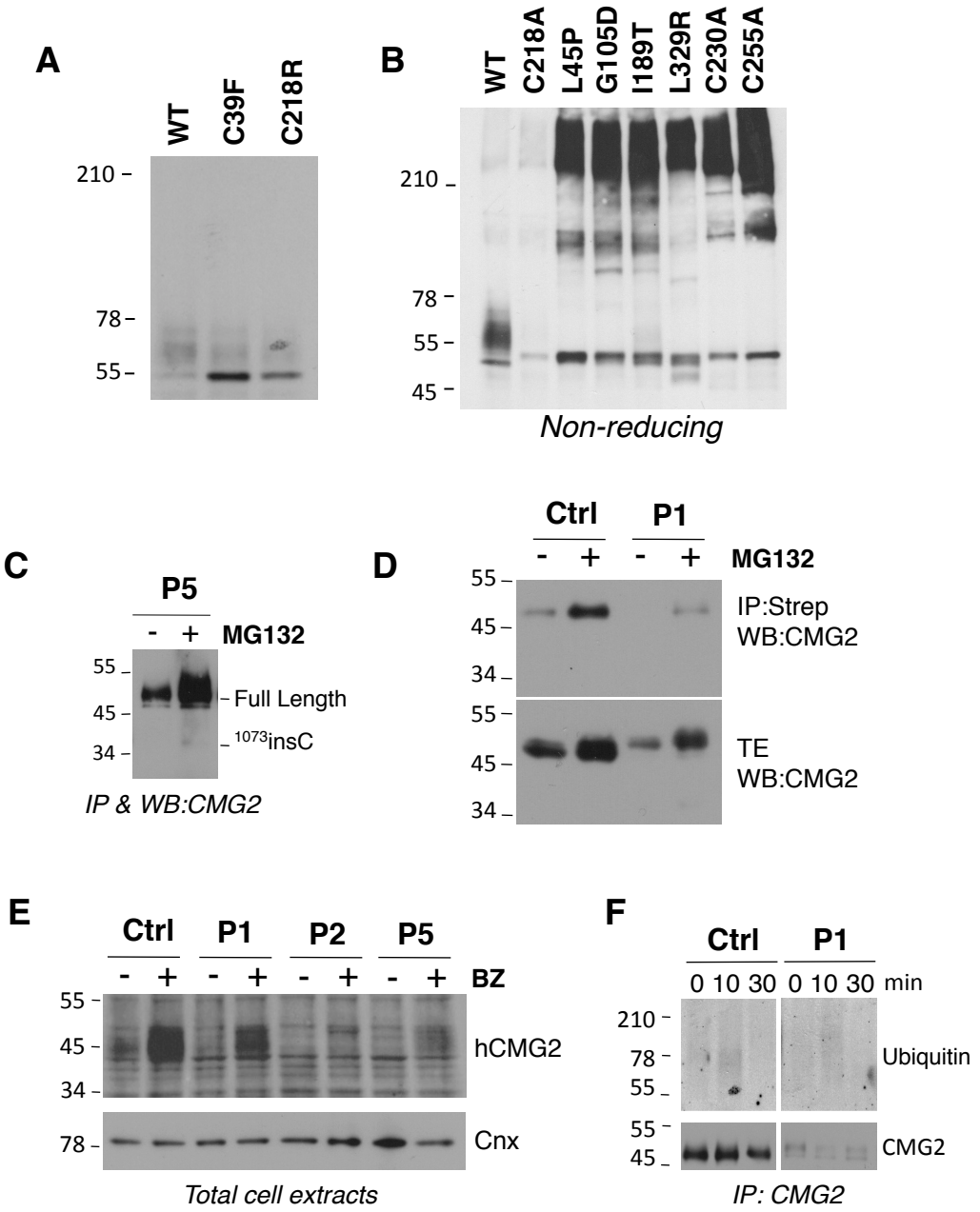
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