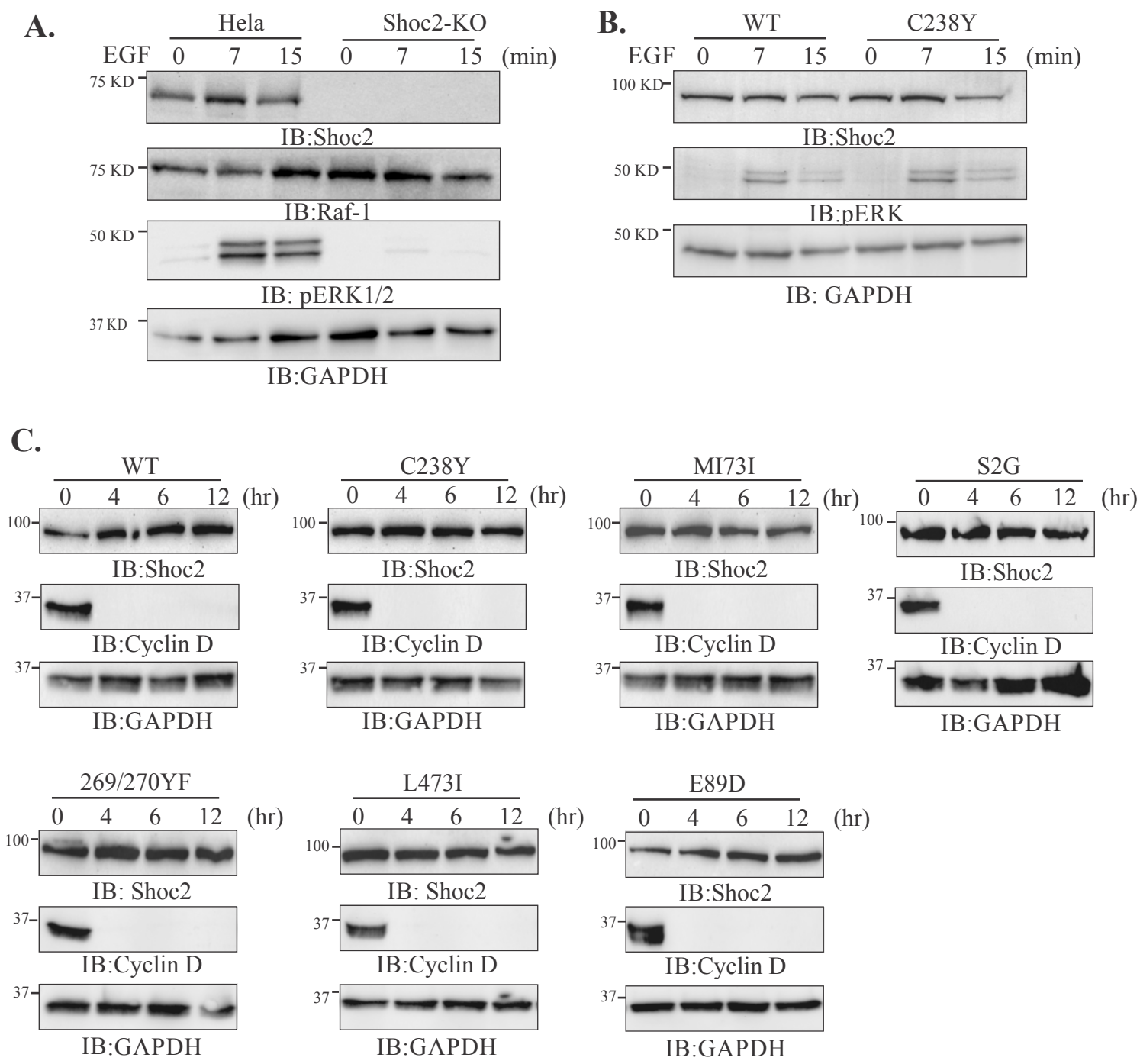



**Fig. S1. Full-length Shoc2-tRFP and Shoc2-tRFP NSLAH-associated mutants have similar protein half-life.**

**A. Parental and** CRISPR/Cas9 Shoc2 KO HeLa cells were serum-starved for 16 hr and then stimulated with EGF (0.2 ng/ml) for 7 and 15 min. Immunoblots were analyzed with anti-Shoc2, -RAF-1, -pERK1/2, and -GAPDH antibodies. The results in each panel are representative of those from three independent experiments.

**B**. HeLa Shoc2 CRISPR KO cells transiently transfected with the Shoc2 C238Y mutant were serum-starved for 16 hr and then stimulated with EGF (0.2 ng/ml) for 7 and 15min. Cell lysates were analyzed using anti-pERK1/2, -GAPDH and -Shoc2 antibodies.

**C.** CRISPR/Cas9 Shoc2 KO HeLa cells were transiently transfected with full-length Shoc2-tRFP or Shoc2-tRFP mutants. Thirty-six hours post-transfection cells were treated with 30 μM Cycloheximide for indicated times at 37°C. The lysates were probed by immuno-blotting (IB) for Shoc2, Cyclin D (half-life 30 min, experimental control) and GAPDH (loading control).

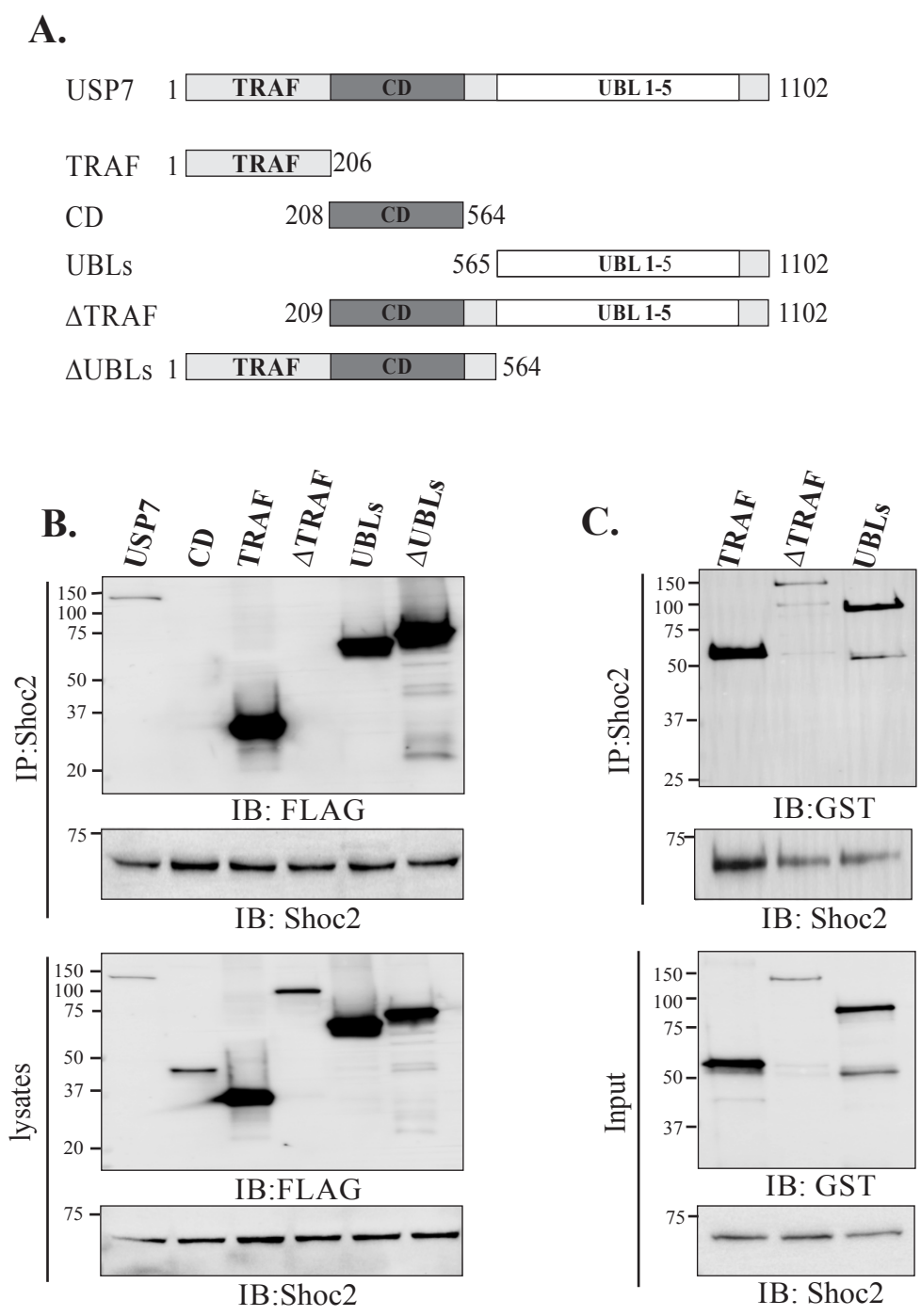



**Fig. S2. Mapping the architecture of the Shoc2-USP7 complex.**

**A.** Schematic representation of the full-length and truncated FLAG-USP7 constructs.

**B**. Co-immunoprecipitation studies reveal the importance of TRAF (aa1-206) and UBL1-5 (aa564-1102) domains for Shoc2 interaction in cells. The indicated constructs were expressed in 293FT cells for 48 hrs before cell lysates were immunoprecipitated with anti-Shoc2 and immunoblotted with anti-FLAG (USP7). Cell lysates were immunoblotted with anti-FLAG antibody to monitor expression of USP7 and corresponding truncated mutants used in IP panel or Shoc2 Abs to monitor expression of Shoc2.

**C.** Purified indicated recombinant fragments of USP7 were used in *in vitro* Shoc2-pulldown assays to determine the regions on USP7 that Shoc2 binds. GST or GST-USP7 fragments were incubated with recombinant His-Shoc2 bound to Sepharose A beads. Bound proteins were detected by anti-GST and Shoc2 immunoblotting.

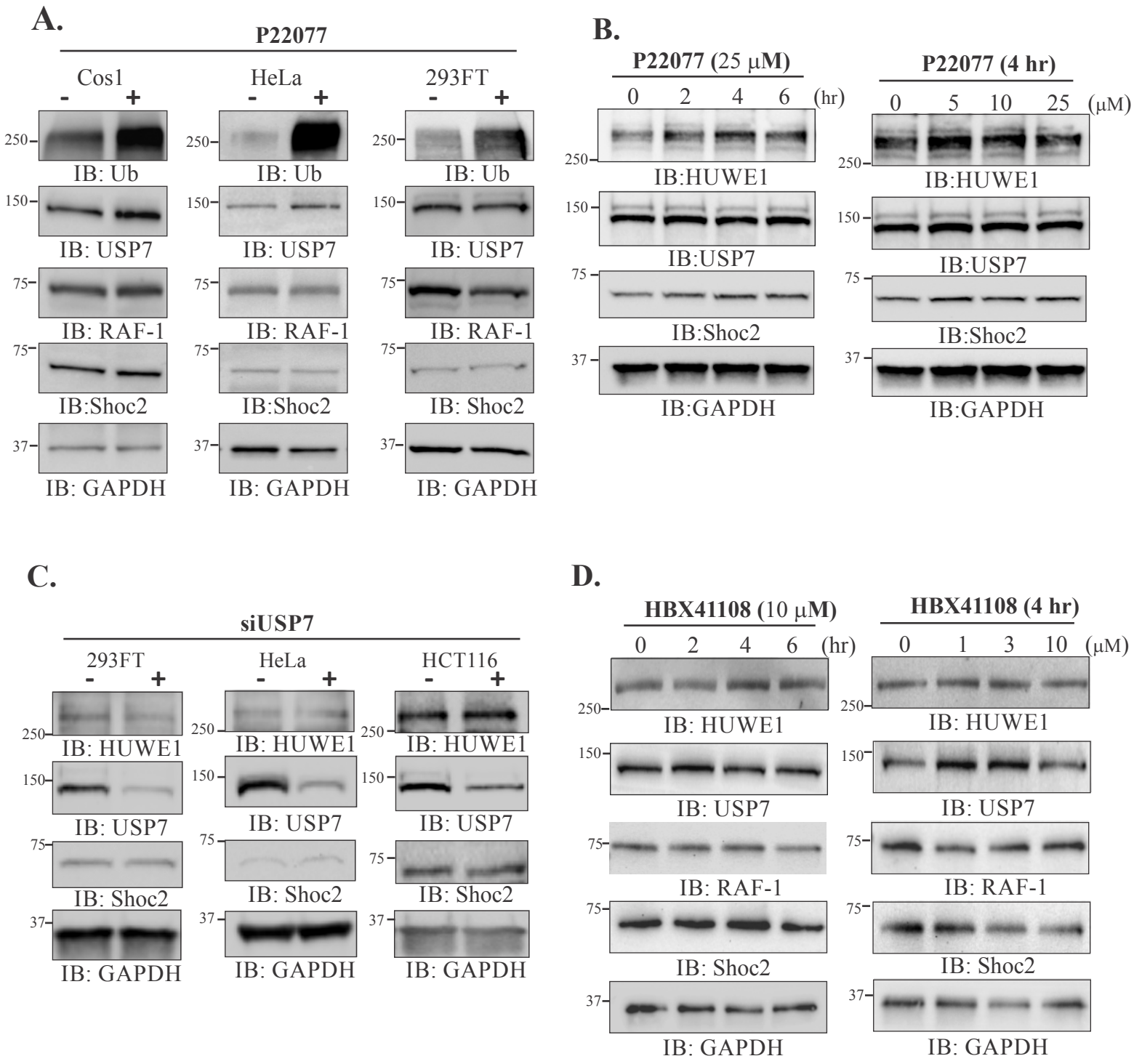
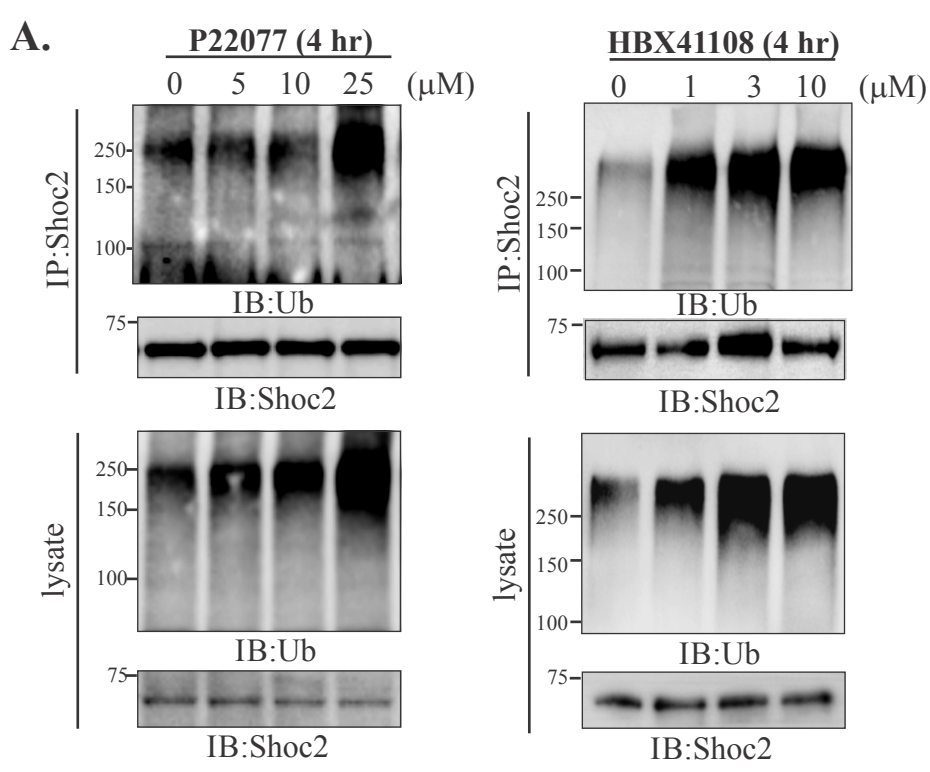


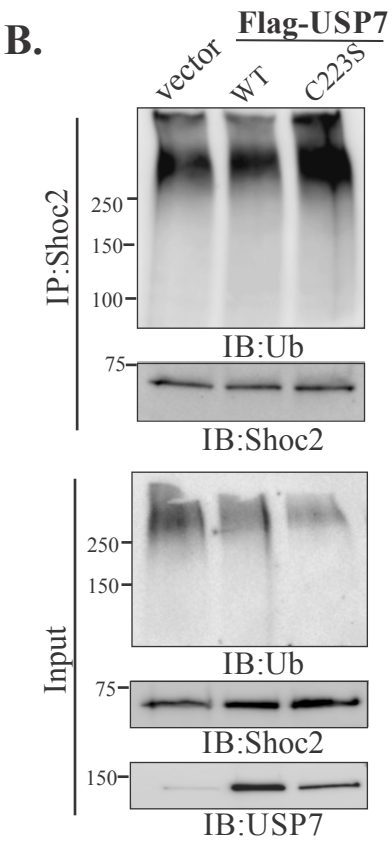

**Fig. S3. USP7 does not modify levels of the proteins in the Shoc2 complex.**

**A.** Cos-1, HeLa or 293FT cells were treated with the vehicle (DMSO) or 25 of P22077 for 4 hr. Cell lysates were analyzed using anti-Ub, -USP7, -RAF-1, -Shoc2 and -GAPDH antibodies. **B.** 293FT cells were treated with the vehicle (DMSO) or 10 μM of P22077 at the time period indicated or indicated doses of P22077. Cell lysates were analyzed using anti-HUWE1, -USP7, -Shoc2, and -GAPDH antibodies.

**C.** HeLa, HCT116 and 293FT cells were transiently transfected with non-targeting siRNA (siNT) or USP7 siRNA (siUSP7). Cell lysates were analyzed using anti-HUWE1, -USP7, -Shoc2 and -GAPDH antibodies.

**D.** 293FT cells were treated with the vehicle (DMSO) or 10 μM of HBX41108 at the time period indicated or indicated doses of HBX41108. Cell lysates were analyzed using anti-HUWE1, -USP7, -RAF-1, -Shoc2, and -GAPDH antibodies.



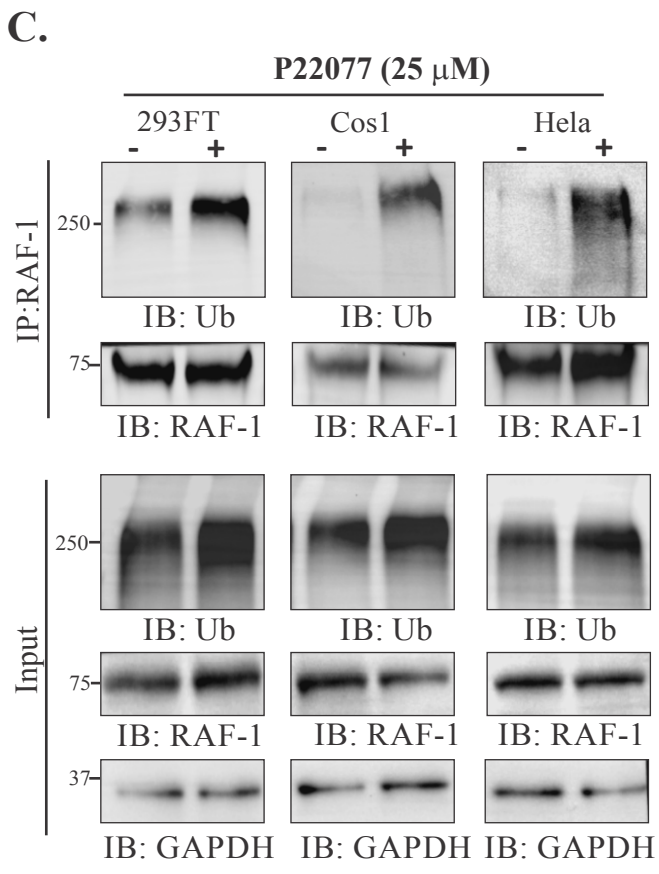



**Fig. S4. USP7 does not modify levels of the proteins in the Shoc2 complex. A.** Endogenous Shoc2 was immunoprecipitated from 293FT cells treated with USP7 inhibitor P22077 or HBX41108. Shoc2 ubiquitination was detected by immunoblotting using anti-ubiquitin (Ub) antibody. The immunoprecipitates and cell lysates were analyzed by immunoblotting with using anti-Ub, and -Shoc2 antibodies. The results in each panel are representative of those from three independent experiments.

**B.** 293FT cells were transfected with WT FLAG-USP7 or FLAG-USP7 with the C223S substitution. Shoc2 was immunoprecipitated using anti-Shoc2, and Shoc2 ubiquitination was detected with anti-Ub antibodies. Cell lysates were analyzed using anti-Ub, -USP7 and -Shoc2 antibodies.

**C.** Endogenous RAF-1 was immunoprecipitated from 293FT, Cos1 or HeLa cells treated with USP7 inhibitor P22077. RAF-1 ubiquitination was detected by immunoblotting using anti-ubiquitin (Ub) antibody. The immunoprecipitates and cell lysates were analyzed by immunoblotting with using anti-Ub, -RAF-1 and GAPDH antibodies. The results in each panel are representative of those from three independent experiments.

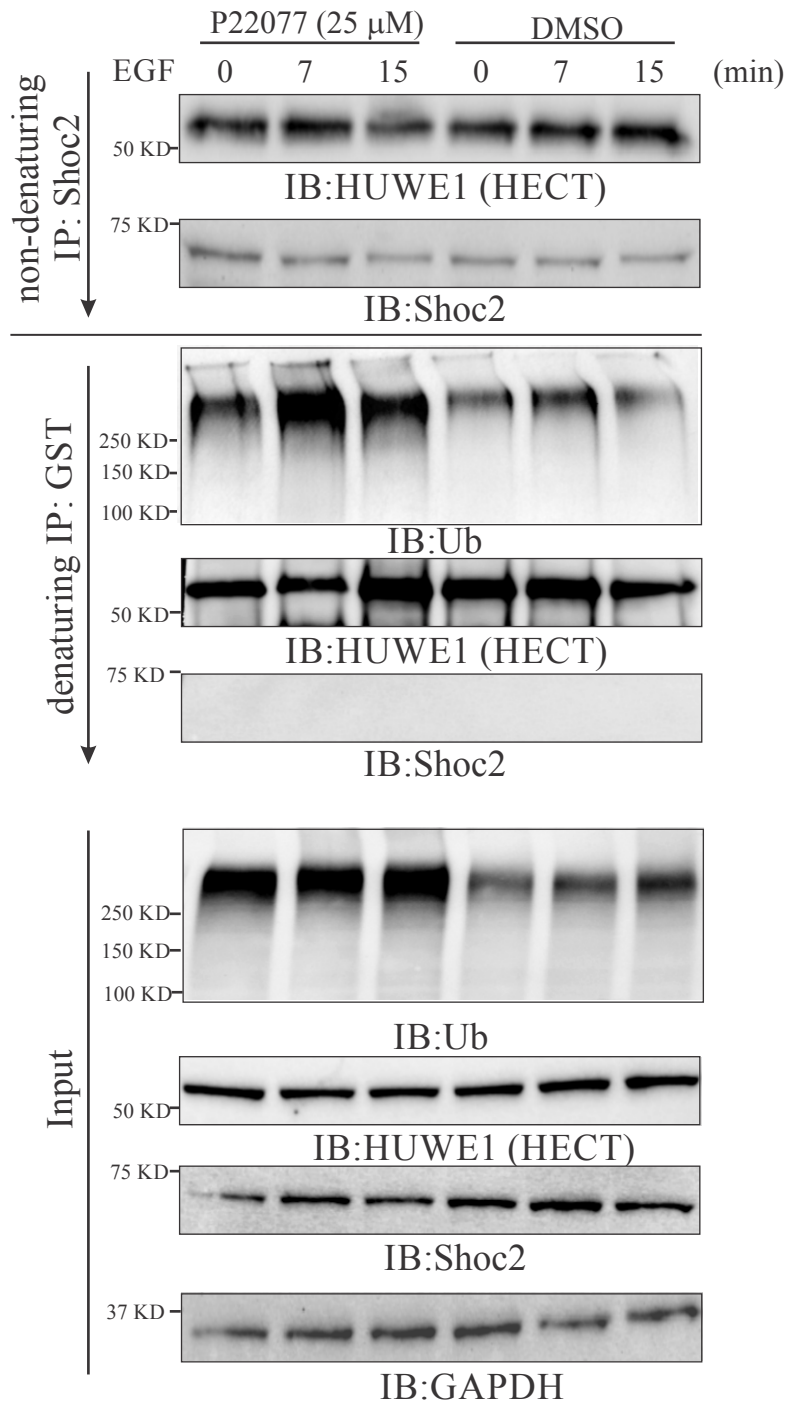



**Fig. S5. USP7 regulates ubiquitination of Shoc2- bound HECT domain of HUWE1.**

293FT cells were transiently transfected with GST-tagged HECT domain of HUWE1. 48 hours after transfections cells were serum-starved for 16 hr, treated with 25 μM of P22077 for 4 hr and then stimulated with EGF (0.2 ng/ml) for 7 and 15min. Endogenous Shoc2 was precipitated under non-denaturing conditions. Shoc2 immuno-precipitates were then denatured and subjected for immunoprecipitation using anti-HUWE1 antibody. HUWE1 ubiquitination was detected with anti-ubiquitin (Ub) antibody. Immunoblots were analyzed with anti-Shoc2, - HUWE1, -Ub, and GAPDH antibodies. The results are representative of at least three independent experiments.

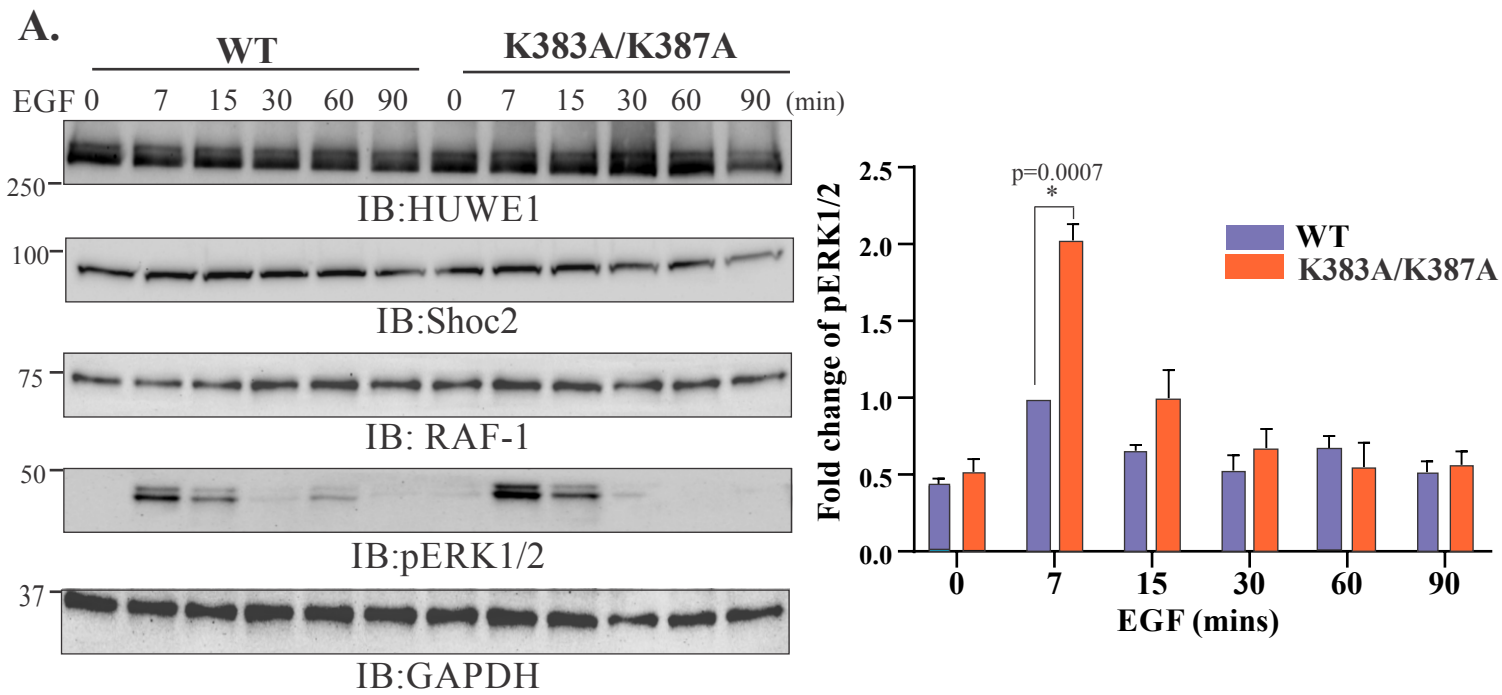



**Fig. S6. ERK1/2 pathway activation in cells expressing USP7-binding deficient mutants of Shoc2.**

HeLa cells were transfected with WT Shoc2-tRFP, Shoc2 (K383/387A) (**A**) or Shoc2 (K22/26A) (**B**) mutants respectively. Cells were serum-starved for 16 hr and then stimulated with EGF (0.2 ng/ml). Immunoblots were analyzed with anti -HUWE1, -Shoc2, -RAF-1, -pERK1/2 and -GAPDH antibodies. Bars represent the mean amount of pERK1/2 normalized to the total amount of GAPDH in arbitrary units ± S.E. ($n$=3) ($p$<0.0007 (*7 min K383/387A*), by Student's *t*-test and $p$<0.0488 (*7 min K22/26A*), by Student's *t*-test). The results in each panel are representative of those from three independent experiments.

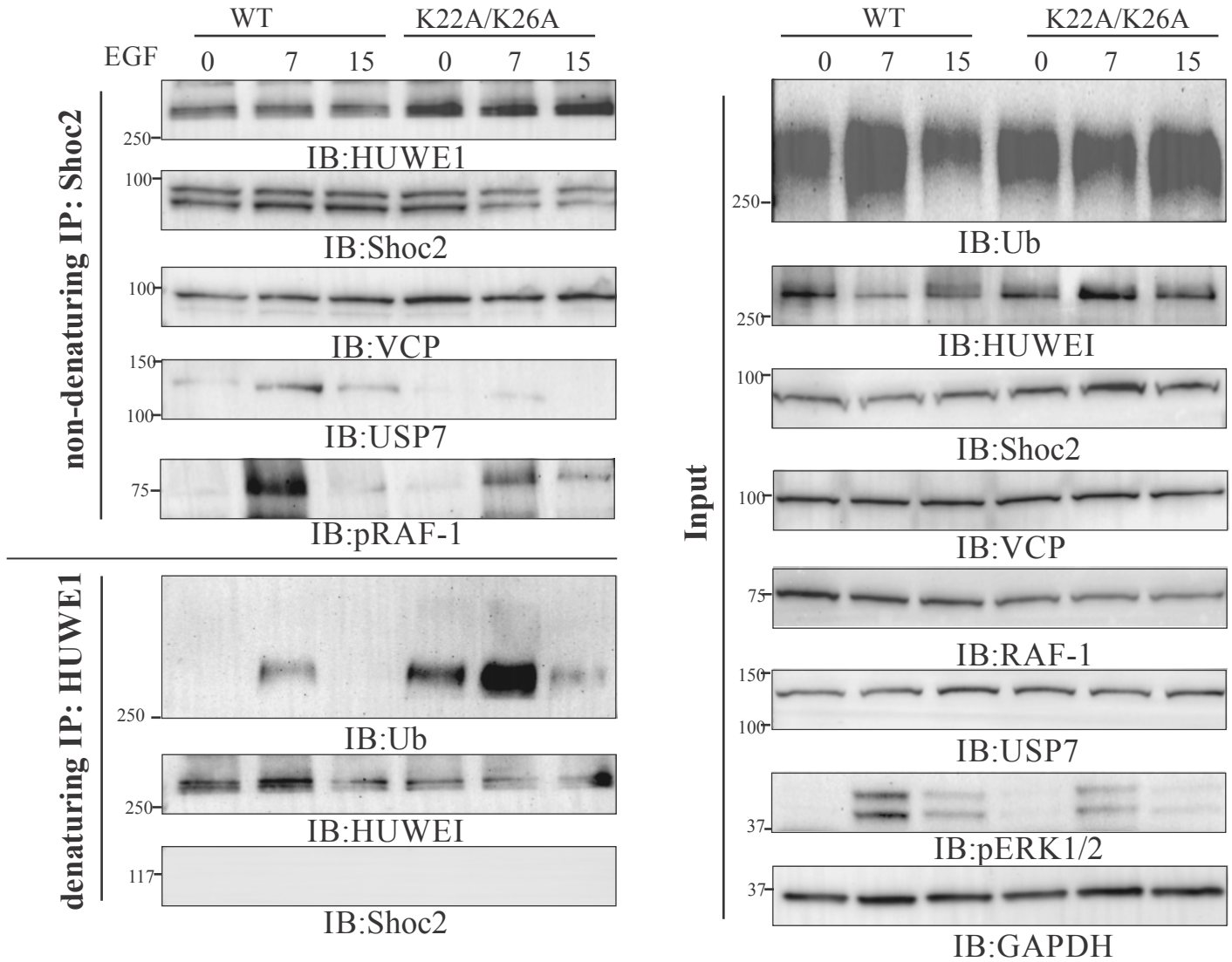



**Fig. S7. USP7 regulates ubiquitination of Shoc2- bound HUWE1.**

HeLa Shoc2 CRISPR KO cells expressing WT Shoc2-tRFP or the Shoc2 mutant K22/26A were serum-starved for 16 hr and then stimulated with EGF (0.2 ng/ml) for 7 and 15min. Endogenous Shoc2 was precipitated under non-denaturing conditions. 50% of Shoc2 immunoprecipitates were analyzed with anti-HUWE1, -VCP, -Shoc2, -USP7 and RAF-1 antibody. The rest of Shoc2 immunoprecipitates were then denatured and subjected for immunoprecipitation using anti-HUWE1 antibody. Ubiquitination was detected with anti-ubiquitin (Ub) antibody. Immunoblots were analyzed with anti-Shoc2, -Ub and -HUWE1 antibodies. Cell lysates were probed with anti-Ub, -HUWE1, -VCP, -Shoc2, -USP7, pERK1/2, GAPDH and RAF-1 antibody. The results in each panel are representative of those of at least three independent experiments.

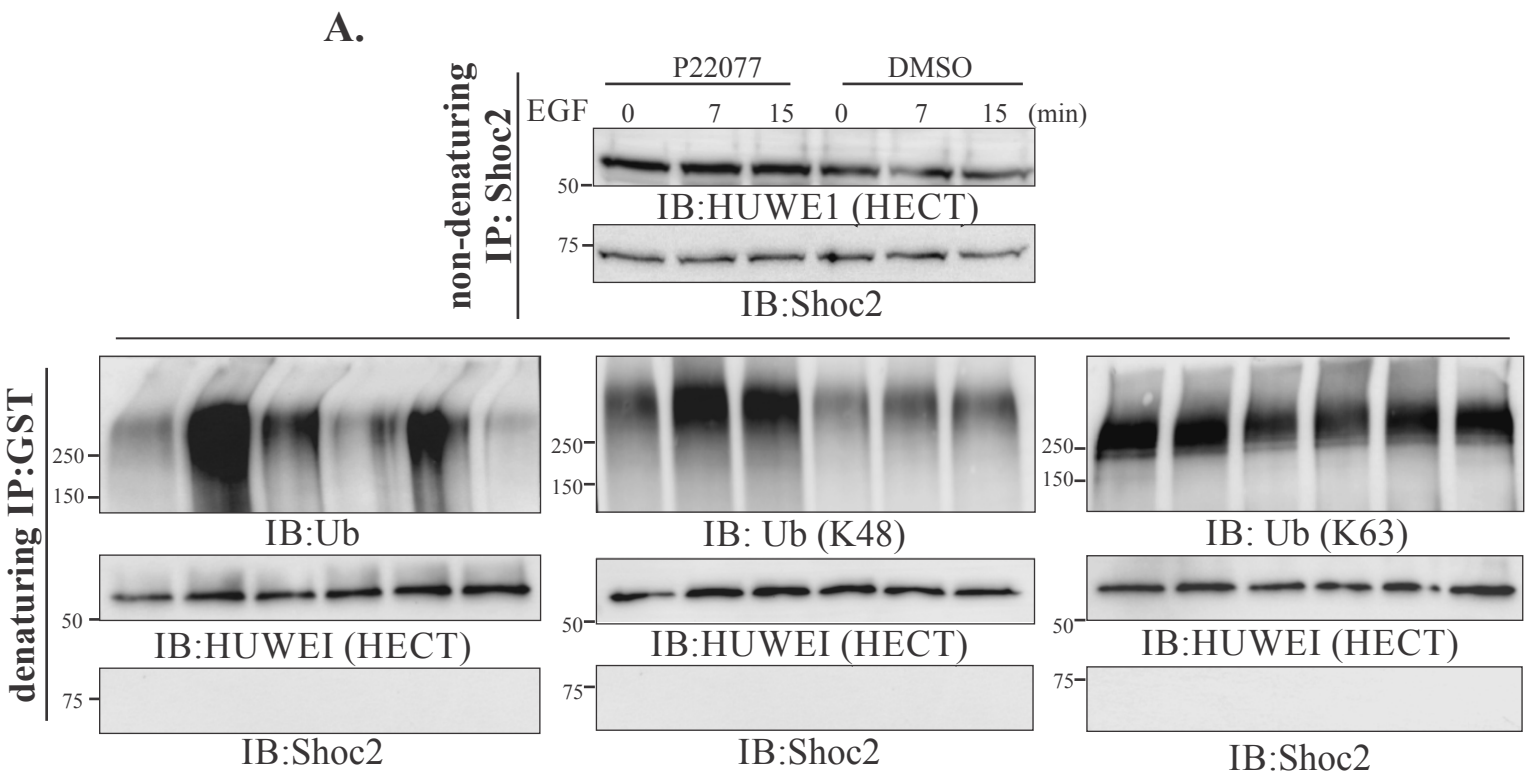



**Fig. S8. USP7 modulated K48 linkages on Shoc2-bound HUWE1 in cells stimulated with EGF.**

**A.** 293FT cells were transfected with GST-tagged HECT domain of HUWE1. Endogenous Shoc2 was precipitated under non-denaturing conditions from cells treated with P22077 (25μM) for 4 hours. Shoc2 immuno-precipitates were analyzed with anti-HUWE1 and Shoc2 antibody. Shoc2 precipitates were then denatured and subjected for immunoprecipitation using anti-HUWE1 antibody. Ubiquitination was detected with anti-K48 ubiquitin (K48), -K63 ubiquitin (K63) or -Ub antibody. Cell lysates were probed with anti-HUWE1, -Shoc2, and -GAPDH antibody. The results in each panel are representative of those of at least three independent experiments.

**B.** The mean amount of Ub normalized to the total amount of HECT ubiquitination at 0 min ± SE from three experiments is presented on the graph. The results in each panel are representative of those from three independent experiments.

| Table 1 | Mutation Summary and Clinical Phenotypes of Seven Individuals with Shoc2 mutations | | | | | | |
|---|---|---|---|---|---|---|---|
| | Subject Number | | | | | | |
| DECIPHER ID | 1 (254516) | 2 (259095) | 3 (3284096) | 4 (287439) | 5 (287504) | 6 (296587) | 7 (318097) |
| Characteristic | | | | | | | |
| Sex | M | M | M | M | M | F | M |
| Age | >1 | 12 | 3 | 3 | 3 | 18 | ? |
| Mutation type | Del | missense c.A4>G p.S2G | missense c.G713>A p.C238Y | missense c.G267>C p.E89D | missense c.T1417>A p.L473I | missense c.A4>G p.S2G | missense c.A4>G p.S2G |
| Inheritance | de novo | de novo | UN | UN | maternally inherited | de novo | UN |
| Genomic size | 2.19 Mb | 1b | 1b | 1b | 1b | 1b | 1b |
| Genes affected (n) | 10 | 1 | 4 | 2 | 4 | 2 | 1 |
| Symptoms | | | | | | | |
| Heart defects | V | | | V | V | V | |
| Hair | | | V | | V | V | |
| Short stature | | V | | | | V | |
| Intellectual disability | | V | V | N/A | V | V | V |
| Skin tags | V | | V | | | | |

**b,** bases; **Del**, deletion; **F,** female; **M**, male; **Mb**, megabases; **N/A**, not applicable; **UN,** unknown; **v,** present.

| Table 2: Supplemental Clinical Notes |
|---|
| **DECIPHER ID 254516 – (deletion 2.19 Mb)** |
| Abnormal heart morphology, Abnormality of the kidney, Coarctation of aorta, Preauricular skin tag, Sacral dimple, Single transverse palmar crease, Skin tags, Tricuspid regurgitation |
| **DECIPHER ID 259095 - (SNV A>G)** |
| Abnormal fundus morphology, Abnormality of dental morphology, Bilateral ptosis, Epistaxis, Highly arched eyebrow, Long palpebral fissure, Moderately short stature, Specific learning disability. |
| **DECIPHER ID 284096 - (SNV G>A)** |
| Absent septum pellucidum, Clinodactyly of the 5th finger, Numerous nevi, Optic atrophy, Septo-optic dysplasia, Short distal phalanx of finger, Skin tags<br>At age 8 years old: Septo-optic dysplasia with right optic nerve hypoplasia, absent septum pellucidum, normal pituitary and corpus callosum, visual impairment of unknown cause. Previous suboptimal Synacthen test, now normal pituitary function and Synacthen Hypermobility. Behavioral problems and mild learning difficulty very white/blond hair and a small black hair patch a number of cutaneous pink/brown nevi including on his scalp and which appear to be growing both in number and in dimensions. |
| **DECIPHER ID 287439 - (SNV G>C)** |
| Penile hypospadias, Talipes, Tetralogy of Fallot |
| **DECIPHER ID 287504 - (SNV T>A)** |
| Bilateral conductive hearing impairment, Brachycephaly, Bruxism, Cleft palate, Clinodactyly of the 5th finger, Developmental regression, Gastroesophageal reflux, Gastrostomy tube feeding in infancy, Global developmental delay, Micropenis, Mitral regurgitation, Recurrent lower respiratory tract infections, Seizures, Talipes equinovarus, Upslanted palpebral fissure. |
| **DECIPHER ID 296587 - (SNV A>G)** |
| Atrial septal defect, Bilateral ptosis, Broad neck, Chronic otitis media, Delayed puberty, Downslanted palpebral fissures, Dry skin, Feeding difficulties in infancy, Fine hair, Gray matter heterotopias, Intellectual disability, moderate, Pulmonic stenosis, Severe short stature.<br>At age 23 patient had pulmonary stenosis and an ASD. She has longstanding short stature adult height around 135cm. Very significant learning problems – very limited reading and writing and needs supervision in daily activities. |
| **DECIPHER ID 318097 - (SNV A>G)** |
| Intellectual disability. |