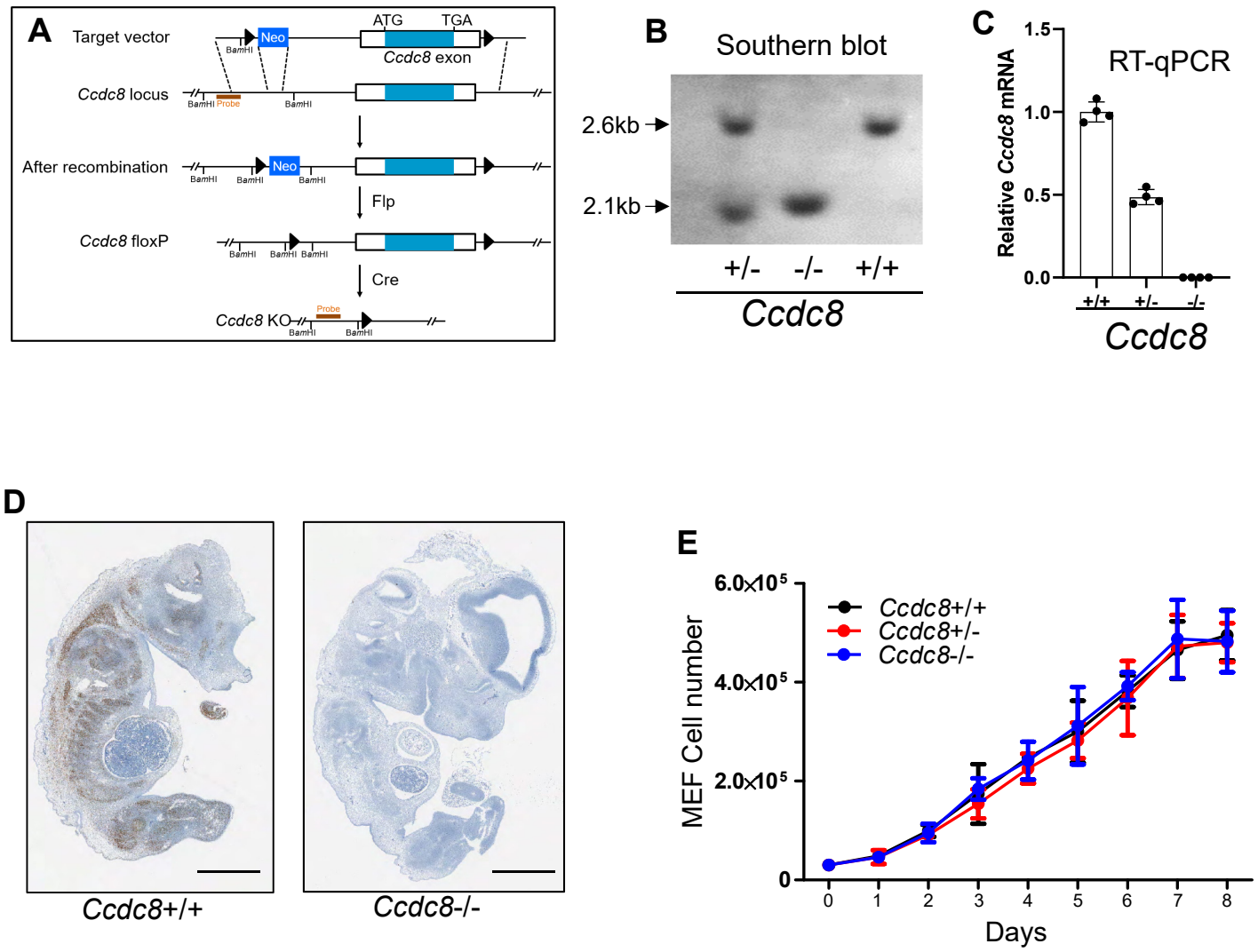
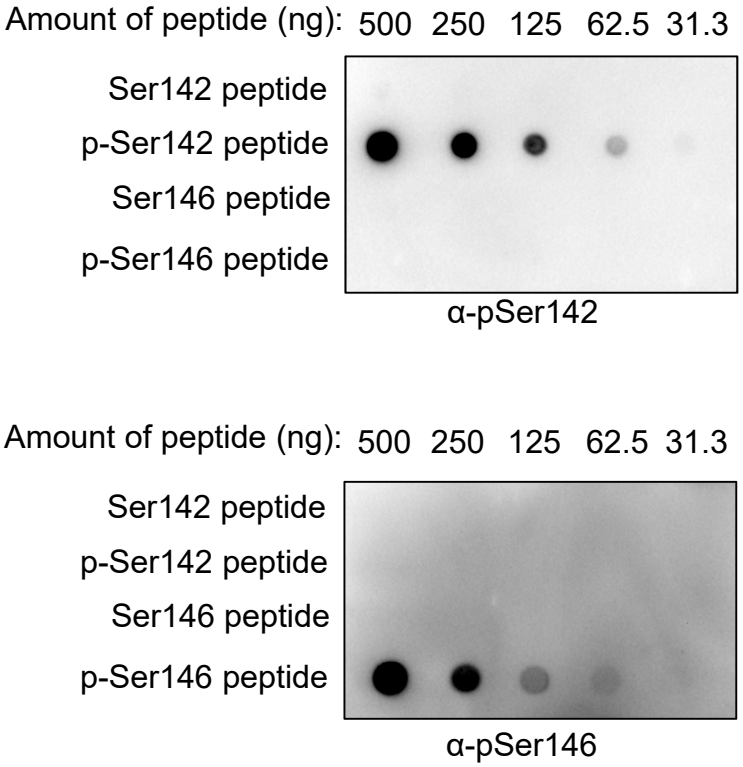
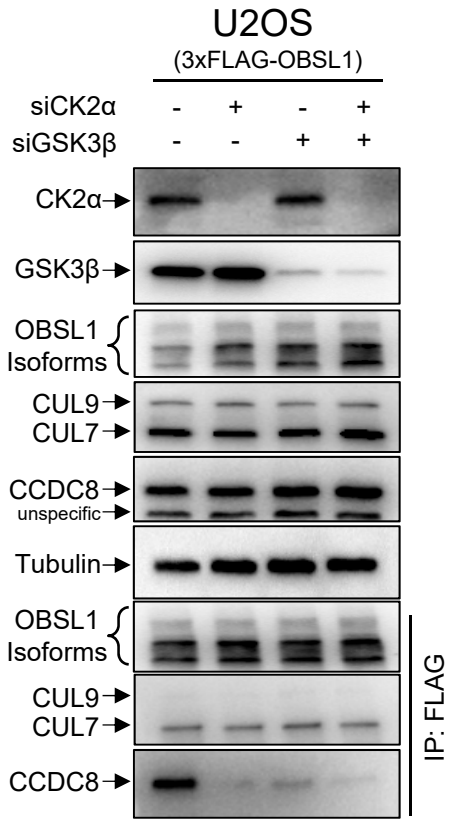


Supplemental Figure 1. Generation and validation of *Ccdc8* knocking out mice. (**A**) Schematic representation of target vector and mouse *Ccdc8* locus before and after homologue recombination. (**B**) Southern blot of *Ccdc8*+/+, *Ccdc8*+/- and *Ccdc8*-/- genomic DNA prepared from E13.5 mouse embryo fibroblast (MEF). Probes and B*am*HI restriction site used for southern blot are shown in Supplemental Figure 1A. (**C**) RT-qPCR of *Ccdc8*+/+, *Ccdc8*+/- and *Ccdc8*-/- cDNA prepared from MEF. Data are represented as mean ± SEM from four technical replicates. (**D**) RNA in situ hybridization of *Ccdc8*+/+ and *Ccdc8*-/- of sections from E12.5 embryo. Scale bar = 1 mm. (**E**) Growth curve of *Ccdc8*+/+, *Ccdc8*+/- and *Ccdc8*-/- MEF cells. Data are represented as mean ± SEM from four replicates.



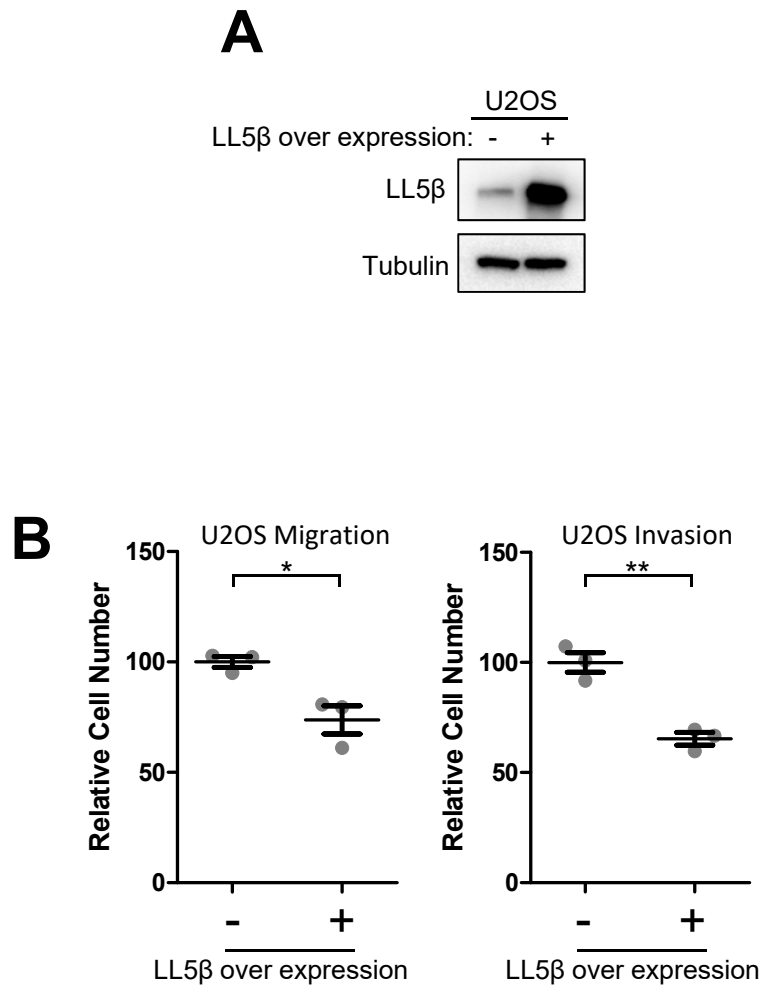
Supplemental Figure 2. Validation of CCDC8 pSer142 and pSer146 antibodies.

Validation of antibodies recognizing phosphorylated serine 142 (pSer142) or phosphorylated serine 146 (pSer146) CCDC8 peptides. Peptide antigen containing phosphorylated or non-phosphorylated serine was blotted on nitrocellulose membrane at the indicated amount and subjected to immunoblotting with purified pSer142 or pSer146 antibodies.



Supplemental Figure 3. GSK3 and CK2 are required for 3-M complex assembly.

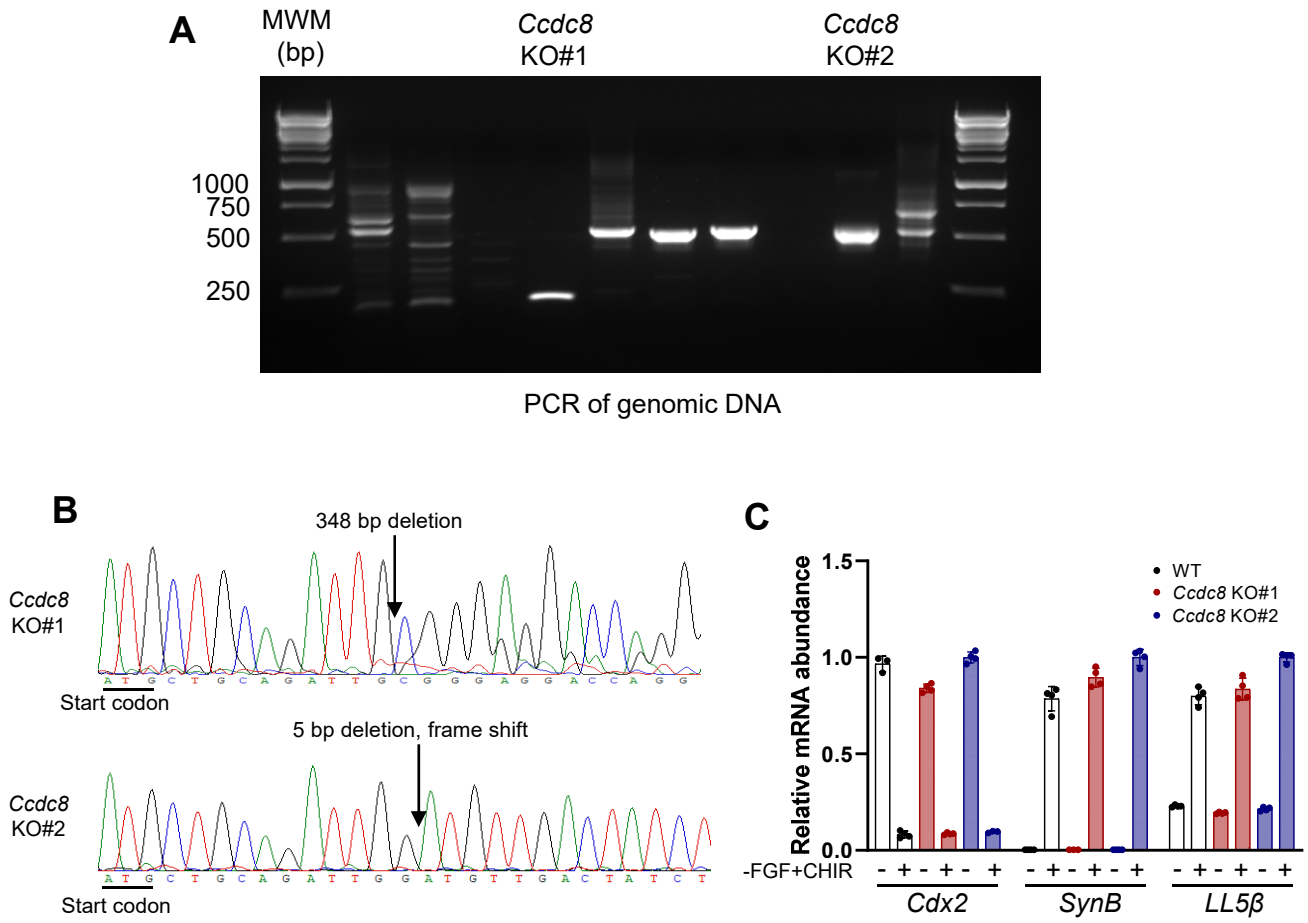
U2OS with endogenous 3XFLAG-OBSL1 tagged by CRISPR were transfected with pooled siRNA targeting CK2α or GSK3β. The binding between OBSL1 with CUL7 and CCDC8 was determined by IP-western analyses.



Supplemental Figure 4. Over expression of LL5β reduces U2OS cell migration and invasion.

(**A**) U2OS cells transduced with empty lentivirus vector or lentivirus vector encoding for LL5β were analyzed by Western Bot.

(**B**) U2OS cells with or without LL5β over expression were subjected to transwell cell migration or invasion assay. Data are represented as mean ± SEM from three replicates. Significance is determined by Student's t-test. * indicates $p < 0.05$; ** indicates $p < 0.01$

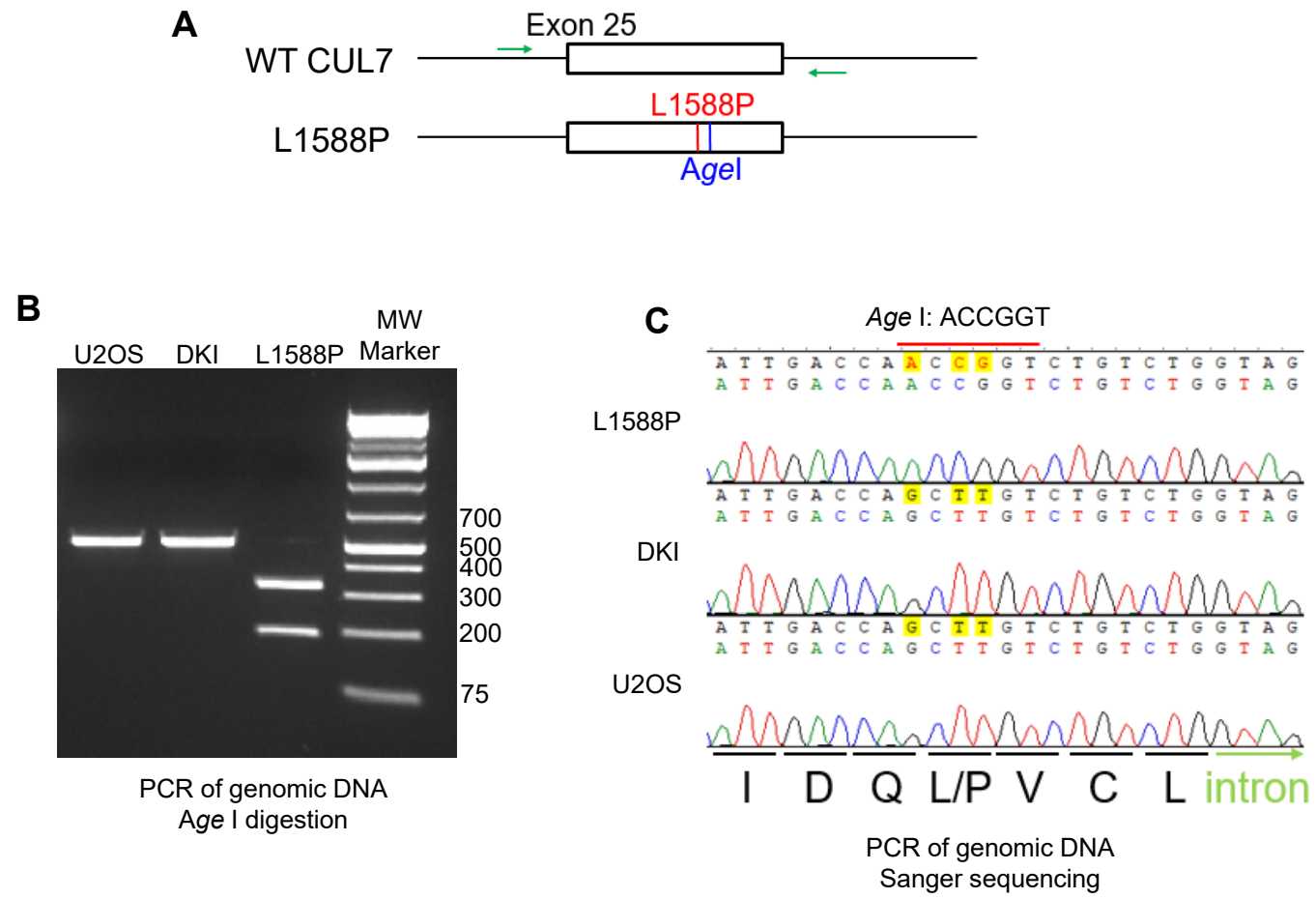


Supplemental Figure 5. Validation of Ccdc8 knock out in trophoblast stem cells.

(**A**) Validation of *Ccdc8* knock out in trophoblast stem cells (TSC). Mouse TSC were transfected with plasmids encoding for Cas9 and sgRNA targeting *Ccdc8*. Genomic DNA from individual TSC clones were prepared and subjected to PCR with primers specifically amplifying sequences around sgRNA cleavage site. EtBr-agarose gel of PCR product was shown. The predicted size of amplification from wild type genome was 550 base pairs. *Ccdc8* KO#1 and KO#2 clones were chosen for studies shown in Figure 6F and Figure 6G. MWM, molecular weight marker.

(**B**) Sanger sequencing of *Ccdc8* KO#1 and KO#2 PCR products.

(**C**) RT-qPCR showed *Ccdc8* and *LL5β* mRNA expression in mouse trophoblast stem cell (TSC) and in vitro differentiated trophoblast giant cells (TGC) or syncytiotrophoblast layer-II (SynT-II) cells.



Supplemental Figure 6. Generation of cell line harboring 3-M syndrome patients-derived mutation

(A) Schematic representation shows L1588P knock in strategy and PCR primers (green) used for validation. A new and diagnostic restriction site AgeI was introduced into the genome without changing amino acid sequence.

(B) PCR validation of homozygous knocking in. PCR product amplified from genomic DNA from U2OS, DKI or L1588P cells were digested with AgeI enzyme, separated by Agarose gel and stained with EtBr.

(C) Sanger sequencing of PCR product amplified from genomic DNA from parental, DKI and DKI/L1588P U2OS cells.