Figure S1

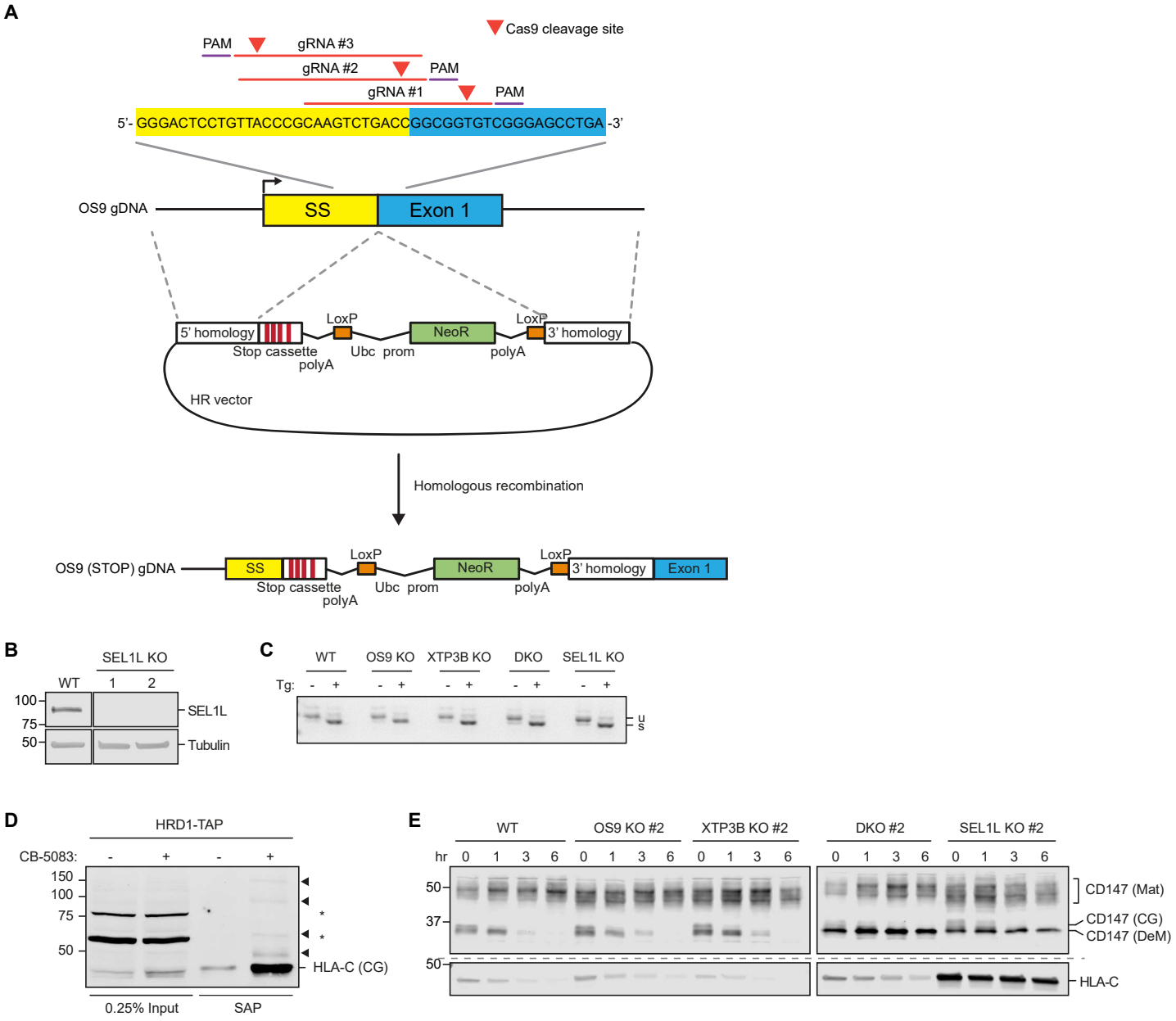

Figure S1. Related to Figure 1. Characterization of knockout cell lines generated by CRISPR/Cas9-mediated genome editing. (A) Schematic outline of the genome-editing strategy to disrupt endogenous OS9. Detailed information about guide RNA sequences, homologous recombination vectors, and selection procedures used to obtain clonal cell lines deficient for OS and XTP3B, individually or together, as well as cell lines deficient for SEL1L can be found in the Supplementary Tables S1-3. (B) Steady-state levels of SEL1L in two independent clonal SEL1L knockout cell lines. Immunoblot analysis of cell lysates. Same immunoblot as Figure 1A. For simplicity, only SEL1L KO cells are shown. (C) *Xbp1* mRNA splicing in knock-out cell lines that were mock treated or treated with 300 nM thapsigargin for 2 hr. (D) HRD1 complexes were captured by tandem-affinity purification from HEK293 cells expressing endogenously TAP-tagged HRD1. Cells were mock treated or treated with the VCP/p97 inhibitor CB-5083 at a final concentration of 5 μM for 6 hr. Input and S affinity-purified (SAP) HRD1 protein complexes were analyzed by immunoblotting. Arrow heads indicate likely ubiquitinated HLA-C species and asterisks indicate background bands. (E) Cells were treated with the protein synthesis inhibitor cycloheximide (PSI-chase) for the indicated times and CD147 or HLA-C turnover was determined by immunoblotting with an anti-CD147 or an anti-HLA-C antibody. These experiments were performed once in independent clonal cell lines that were generated with different guide RNA sequences.

Figure S2

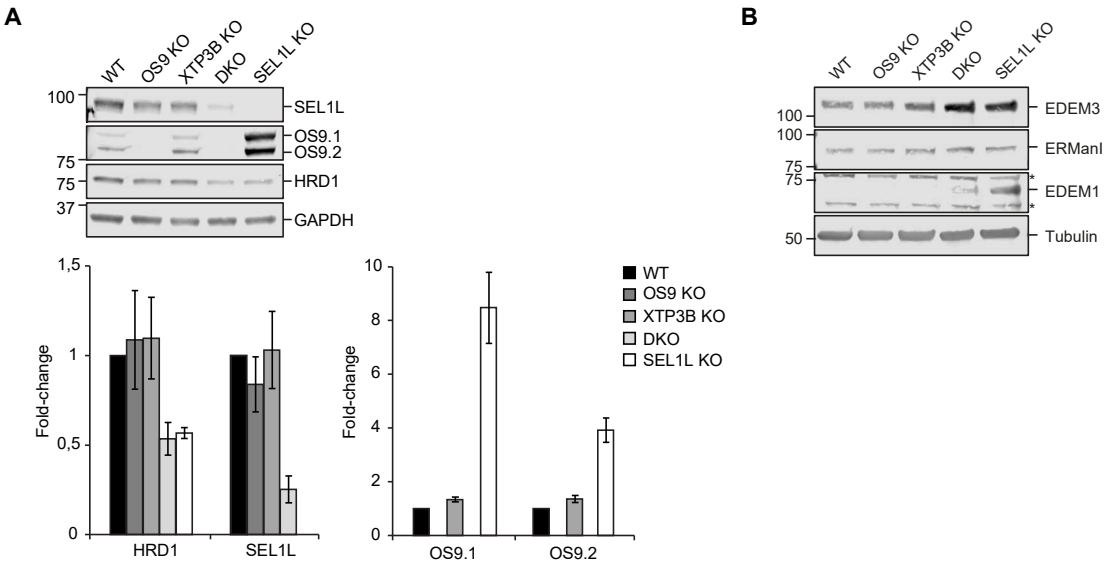

Figure S2. Related to Figure 2. Destabilization of SEL1L/HRD1 complex upon loss of OS9 and XTP3B. (A) Immunoblot analysis of cell lysates from wild type and the indicated knockout cell lines. Fold-change relative to wild type cells was calculated after normalization to GAPDH. Same blot as shown in Figure 2A but including SEL1L KO. Quantification represents mean±SEM (*n*=3). (B) Steady-state levels of EDEM1, EDEM3, and ERManI in the indicated knockout cell lines. Immunoblot analysis of cell lysates from wild type and the indicated knockout cell lines. Asterisks indicate background bands.

Figure S3

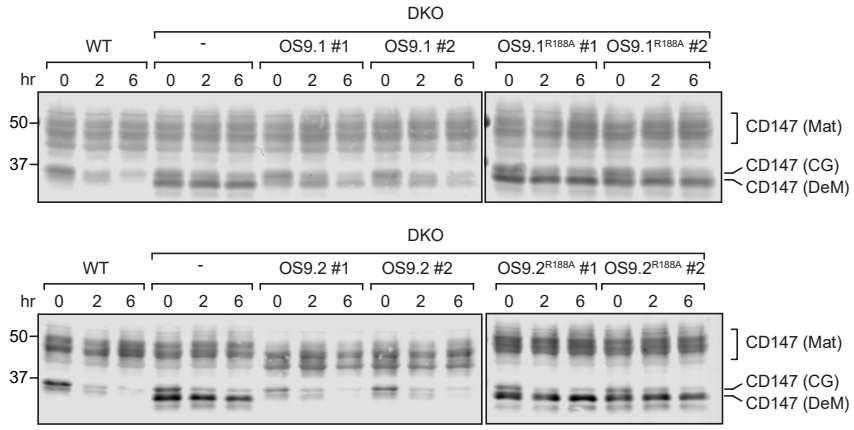

Figure S3. Related to Figure 3. OS9 isoform-specificity in targeting CD147 for ERAD. PSI-chase of CD147 in WT, DKO, and DKO cells stably expressing the indicated OS9 constructs in independently isolated clonal cell lines. This experiment was done once in independent clonal cell lines stably expressing the indicated OS9 variants. Data were used for the quantification in Fig. 3A. Mat=mature; CG=core-glycosylated; DeM=demannosylated.

Figure S4

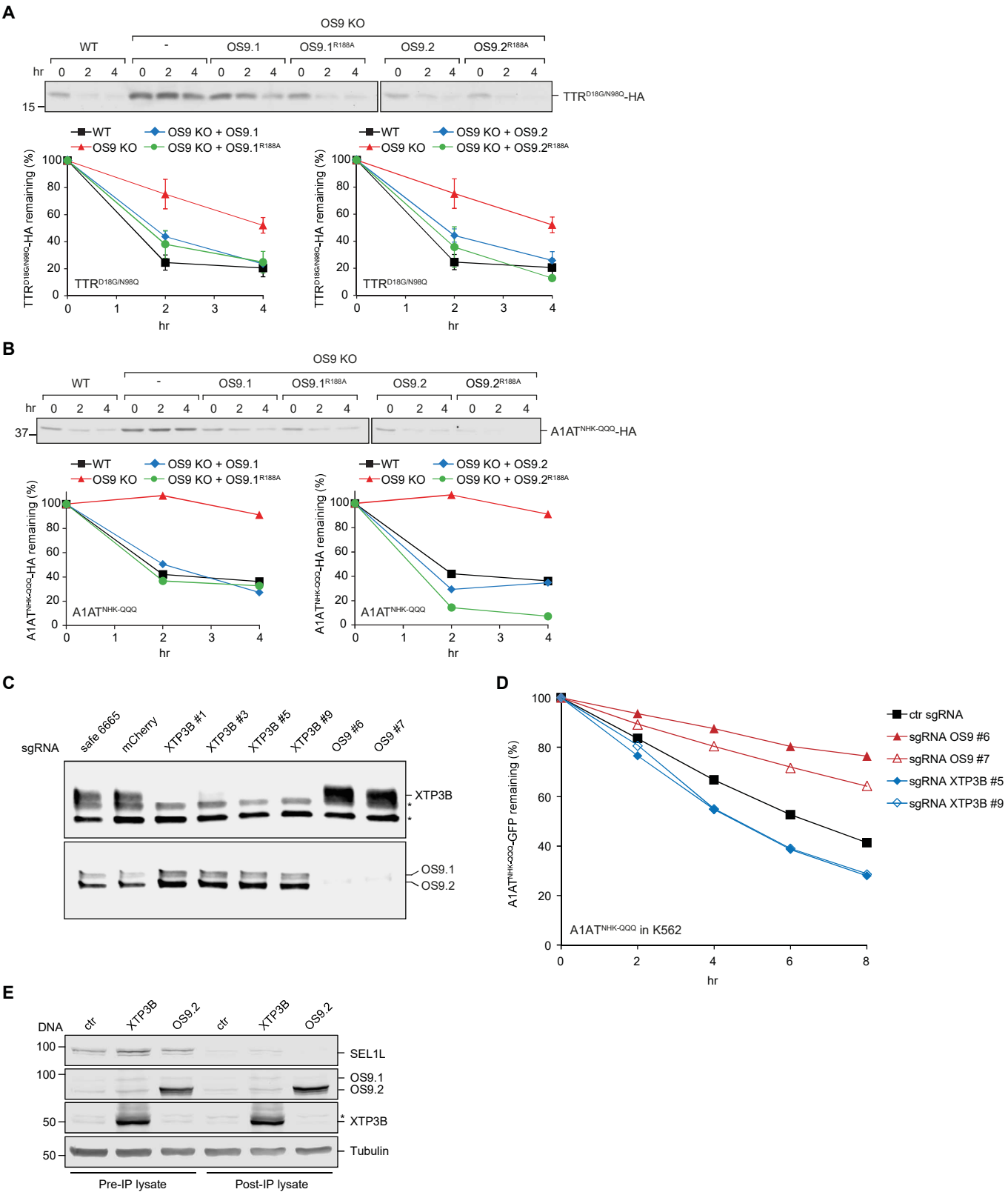

Figure S4. Related to Figure 5. Inhibition of non-glycosylated protein ERAD by XTP3B is antagonized by OS9. (A and B) PSI-chase analysis ~72hrs after transfection with $TTR^{D18G/N98Q}$ (A; *n*=3) or $A1AT^{NHK-QQQ}$-HA (B; *n*=1) in WT, OS9 KO, and OS9 KO cells stably expressing the indicated OS9 constructs. Error bars indicate SEM. (C) Immunoblot analysis of steady-state levels of XTP3B and OS9 in K562 cells stably expressing the indicated sgRNA constructs. (D) K562 cells stably expressing dox-inducible $A1AT^{NHK-QQQ}$-GFP and the indicated sgRNA were treated with 0.1 μg/mL doxycycline (dox) for 16 hours. Cells were subsequently treated with emetine and GFP median fluorescence intensity was measured by flow cytometry analysis at the indicated times. Quantification is based on one experiment. Data for ctr sgRNA, sgRNA OS9 #6 and sgRNA XTP3B #5 are as in Figure 5D. (E) Immunoblot analysis of pre- and post-IP cell extracts of SEL1L IP samples shown in Figure 5F. One representative blot is shown out of three independent experiments. Asterisks indicate background bands.

Figure S5

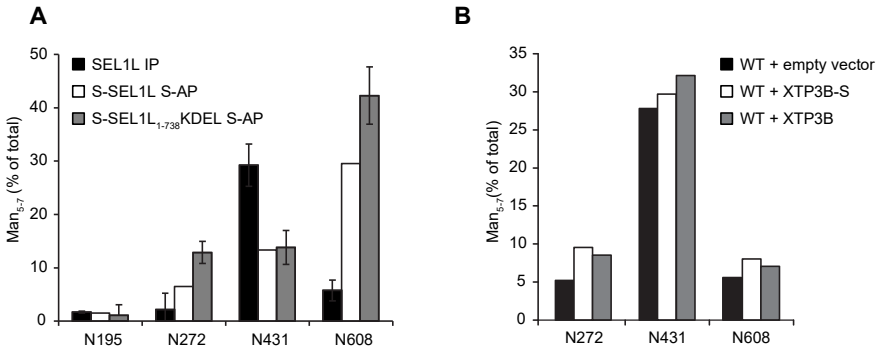

Figure S5. Related to Figure 6. Overexpressed SEL1L is demannosylated at Asn608. (A) Quantification of tryptic glycopeptides from endogenous SEL1L immunoprecipitated (*n*=2) or S-affinity purified from cells expressing S-SEL1L (*n*=1), or S-SEL1L$_{1-738}$KDEL (*n*=3). Data for endogenous SEL1L and S-SEL1L as in Fig. 6B. (B) Quantification of tryptic glycopeptides from endogenous SEL1L immunoprecipitated from cells expressing empty vector (*n*=1), XTP3B-S (*n*=1), or XTP3B (*n*=1). Quantification represents the mean±STDEV.

Table S1. Homology-directed repair vectors generated and used in this study. Related to Methods.

			5’ homology arm		3’ homology arm	
Gene	Insertion cassette	Selection marker	Fw primer	Rv primer	Fw primer	Rv primer
OS9	TAP tag	Puromycin	GACTAGATC TGGAGGTCA TTTTACTGGA GG	CTAGCGATC GGGTCAGAC TTGCGGGTA ACA	GAATTTAATT AAGGCGGTG TCGGGAGCC TGAA	CTAGGGTAC CGCTGGCAG GCGACACTC ATAG
OS9	STOP	Geneticin	GACTAGATC TGGAGGTCA TTTTACTGGA GG	CTAGCGATC GGGTCAGAC TTGCGGGTA ACA	GAATTTAATT AAGGCGGTG TCGGGAGCC TGAA	CTAGGGTAC CGCTGGCAG GCGACACTC ATAG
XTP3B	TAP tag	Puromycin	GCCTAGATC TGCCCTGCG TTAGTCAGA AGCTGTTAA G	CTATCGATC GGGCTCGGC CGCCGCCGG ACG	GAATTTAATT AACTTCCTCA ACTCAGCGA TGA	CTAGGGTAC CCCGCGAGG TTACAAATGT TC
SEL1L	TAP tag	Puromycin	GACTAGATC TCACCCTGGT TTCCGTACTT C	CTAGCGATC GCGCCGAGG CCAAGCTCA GCA	GAATTTAATT AATCCTCGG GTCAGTATC CGCC	CTAGGGTAC CCCCAAAAC GTCAAGCCA CAG

Table S2. CRISPR constructs generated in this study. Related to Methods.

Cell type	Gene	sgRNA	Sequence
HEK293	OS9	2	TTACCCGCAAGTCTGACCGG
HEK293	OS9	3	CGGTCAGACTTGCGGGTAAC
HEK293	XTP3B	1	CCGCAGAGGACCAGTAACAC
HEK293	XTP3B	2	CCCGGGCGGGCCGGTGTTAC
HEK293	SEL1L	1	GAGCTTGGCCTCGGCGTCCT
HEK293	SEL1L	2	GCAGCAGCGTCAGCCCTATC
K562	control 6665		GCGAAGAGATGGTCCTT
K562	XTP3B	5	GACACCATACTATCCTGT
K562	XTP3B	9	GTACTACCTTGGGAATATGT
K562	OS9	6	AGTGTCGCCTGCCAGC
K562	OS9	7	AGGAAACACCTGCTTACCA

Table 3. Knockout cell lines generated in this study. Related to Methods.

Cell line	**sgRNA**	**Clone #**	**Resistance**	**Remarks**
OS9 KO #1	2	B8	Puromycin	
OS9 KO #2	3	A3	G418	
XTP3B KO #1	2	2A3	Puromycin	
XTP3B KO #2	1	1B5	Puromycin	
OS9 KO; XTP3B KO (DKO) #1	3	C5	Puromycin; G418	Generated from XTP3B KO #1
OS9 KO; XTP3B KO (DKO) #2	2	2A2	Puromycin; G418	Generated from XTP3B KO #1
SEL1L KO #1	1	3B2	Puromycin	
SEL1L KO #2	2	3A5	Puromycin	

Table 4. Stable rescue cell lines generated in this study. Related to Methods.

Cell line	**Clone #**	**Resistance**
DKO + OS9.1 #1	A3	Puromycin, G418, Zeocin
DKO + OS9.1 #2	B8	Puromycin, G418, Zeocin
DKO + OS9.1^{R188A} #1	A5	Puromycin, G418, Zeocin
DKO + OS9.1^{R188A} #2	B4	Puromycin, G418, Zeocin
DKO + OS9.2 #1	D2	Puromycin, G418, Zeocin
DKO + OS9.2 #2	E4	Puromycin, G418, Zeocin
DKO + OS9.2^{R188A} #1	F6	Puromycin, G418, Zeocin
DKO + OS9.2^{R188A} #2	E5	Puromycin, G418, Zeocin
DKO + XTP3B	A8	Puromycin, G418, Zeocin
DKO + XTP3B^{R207A} #1	A1	Puromycin, G418, Zeocin
DKO + XTP3B^{R428A} #1	D3	Puromycin, G418, Zeocin
DKO + XTP3B$^{R207A/R428A}$ #1	C7	Puromycin, G418, Zeocin
OS9 KO + OS9.1 #1	A2	G418/Zeocin
OS9 KO + OS9.1 #2	B7	G418/Zeocin
OS9 KO + OS9.1^{R188A} #1	D2	G418/Zeocin
OS9 KO + OS9.1^{R188A} #2	F1	G418/Zeocin
OS9 KO + OS9.2 #1	C3	G418/Zeocin
OS9 KO + OS9.2 #2	C5	G418/Zeocin
OS9 KO + OS9.2^{R188A} #1	E1	G418/Zeocin
OS9 KO + OS9.2^{R188A} #2	F4	G418/Zeocin