

## SUPPLEMENTARY TABLES

**Table S1: List of enriched proteins in CWC22 IPs**

List of protein significantly enriched in CWC22 immuno-precipitation compared to control experiments and grouped by function. Proteins were classified according to their function.

Protein	Name	Function
NSRP1;CCDC55	Nuclear speckle splicing regulatory protein 1	Splicing
THOC1	THO complex subunit 1	Splicing/mRNA transport
THOC3	THO complex subunit 3	Splicing/mRNA transport
DDX24	ATP-dependent RNA helicase DDX24	Splicing
PPIL4	Peptidyl-prolyl cis-trans isomerase-like 4	Splicing
EXOSC6	Exosome complex component MTR3	RNA degradation
MRTO4	mRNA turnover protein 4 homolog	RNA degradation
WDR36	WD repeat-containing protein 36	Ribosome/translation
PES1	Pescadillo homolog	Ribosome/translation
UTP20	Small subunit processome component 20 homolog	Ribosome/translation
RSL1D1	Ribosomal L1 domain-containing protein 1	Ribosome/translation
RPLP0;RPLP0P6	60S acidic ribosomal protein P0:60S acidic ribosomal protein P0-like	Ribosome/translation
CARS	Cysteine-tRNA ligase, cytoplasmic	Ribosome/translation
EIF6	Eukaryotic translation initiation factor 6	Ribosome/translation
LACTB	Serine beta-lactamase-like protein LACTB, mitochondrial	Ribosome/translation
TCOF1	Treacle protein	Ribosome/translation
EIF3A	Eukaryotic translation initiation factor 3 subunit A	Ribosome/translation
WDR43	WD repeat-containing protein 43	Ribosome/translation
GNL3	Guanine nucleotide-binding protein-like 3	Ribosome/translation
GTPBP4	Nucleolar GTP-binding protein 1	Ribosome/translation
NAT10	N-acetyltransferase 10	Ribosome/translation
RNMTL1	rRNA methyltransferase 3, mitochondrial	Ribosome/translation
PCF11	Pre-mRNA cleavage complex 2 protein Pcf11	Polyadenylation
CLUH	Clustered mitochondria protein homolog	mRNP
KPNA3	Importin subunit alpha-4	mRNA transport
THOC6	THO complex subunit 6 homolog	mRNA transport
ERCC3	TFIIH basal transcription factor complex helicase XPB subunit	Chromatin/transcription
GTF2H3	General transcription factor IIH subunit 3	chromatin/transcription
ZMYND8	Protein kinase C-binding protein 1	Chromatin/transcription
GTF2H1	General transcription factor IIH subunit 1	Chromatin/transcription
TRIM32	E3 ubiquitin-protein ligase TRIM32	Chromatin/transcription
GTF2H2;GTF2H2 C	General transcription factor IIH subunit 2;General transcription factor IIH subunit 2-like protein	Chromatin/transcription
MDC1	Mediator of DNA damage checkpoint protein 1	Chromatin/transcription
ZNF638	Zinc finger protein 638	Chromatin/transcription
MINA	Bifunctional lysine-specific demethylase and histidyl-hydroxylase MINA	Chromatin/transcription
MIER1	Mesoderm induction early response protein 1	Chromatin/transcription
DOT1L	Histone-lysine N-methyltransferase, H3 lysine-79 specific	Chromatin/transcription
PHF3	PHD finger protein 3	Chromatin/transcription
GTF2H4	General transcription factor IIH subunit 4	Chromatin/transcription
ZNF622	Zinc finger protein 622	Chromatin/transcription
DDRKG1	DDRKG domain-containing protein 1	Chromatin/transcription
SPEN	Msx2-interacting protein	Chromatin/transcription
PDS5B	Sister chromatid cohesion protein PDS5 homolog B	Chromatin/transcription
TCF20	Transcription factor 20	Chromatin/transcription
AFF4	AF4/FMR2 family member 4	Chromatin/transcription
ZC3H4	Zinc finger CCCH domain-containing protein 4	Chromatin/transcription
C14orf166	UPF0568 protein C14orf166	Chromatin/transcription
CDYL	Chromodomain Y-like protein	Chromatin/transcription
MKI67	Antigen KI-67	Cell division
KIF18A	Kinesin-like protein KIF18A	Cell division
CDK5RAP3	CDK5 regulatory subunit-associated protein 3	Cell division
BOK	Bcl-2-related ovarian killer protein	Apoptosis
RAI14	Ankycorbin	Actin regulation
RBBP6	E3 ubiquitin-protein ligase RBBP6	Ubiquitin ligase
DPYSL2	Dihydropyrimidinase-related protein 2	Signaling
ATXN2	Ataxin-2	Signaling
CRMP1	Dihydropyrimidinase-related protein 1	Signaling
ITPR3	Inositol 1,4,5-trisphosphate receptor type 3	Signaling
ITPR1	Inositol 1,4,5-trisphosphate receptor type 1	Signaling
ARHGAP17	Rho GTPase-activating protein 17	Signaling
DUSP12	Dual specificity protein phosphatase 12	Signaling
YLPM1	YLP motif-containing protein 1	Protein phosphatase
FAM98A	Protein FAM98A	Protein methylation

UFL1	E3 UFM1-protein ligase 1	Protein ligase
DUT	Deoxyuridine 5-triphosphate nucleotidohydrolase. mitochondrial	Nucleotide metabolism
C2CD5	C2 domain-containing protein 5	Metabolism
OSBPL10	Oxysterol-binding protein-related protein 10	Metabolism

**Table S2: List of enriched proteins in e14FA3 IPs**

List of protein significantly enriched in e14FA3 immuno-precipitation compared to control experiments and grouped by function. Proteins were classified according to their function.

Protein	Name	Function
CASC3	Protein CASC3	EJC
ACIN1	Acinus	EJC
PNN	Pinin	EJC
MAGOHB	Protein mago nashi homolog 2	EJC
RBM8A	RNA-binding protein 8A	EJC
EIF4A3	Eukaryotic initiation factor 4A-III;Eukaryotic initiation factor 4A-III. N-terminally processed	EJC
RNPS1	RNA-binding protein with serine-rich domain 1	EJC
SAP18	Histone deacetylase complex subunit SAP18	EJC
UPF2	Regulator of nonsense transcripts 2	EJC
SMG6	Telomerase-binding protein EST1A	EJC
UPF3B	Regulator of nonsense transcripts 3B	EJC
LUC7L3	Luc7-like protein 3	Splicing
PPIE	Peptidyl-prolyl cis-trans isomerase E;Peptidyl-prolyl cis-trans isomerase	Splicing
PRPF40A	Pre-mRNA-processing factor 40 homolog A	Splicing
ISY1	Pre-mRNA-splicing factor ISY1 homolog	Splicing
SON	Protein SON	Splicing
PRPF6	Pre-mRNA-processing factor 6	Splicing
PRPF4B	Serine/threonine-protein kinase PRP4 homolog	Splicing
PRPF38A	Pre-mRNA-splicing factor 38A	Splicing
PPIG	Peptidyl-prolyl cis-trans isomerase G;Peptidyl-prolyl cis-trans isomerase	Splicing
CRNKL1	Crooked neck-like protein 1	Splicing
SNRNP40	U5 small nuclear ribonucleoprotein 40 kDa protein	Splicing
DDX46	Probable ATP-dependent RNA helicase DDX46	Splicing
DDX41	Probable ATP-dependent RNA helicase DDX41	Splicing
BCAS2	Pre-mRNA-splicing factor SPF27	Splicing
TFIP11	Tuftelin-interacting protein 11	Splicing
RBM22	Pre-mRNA-splicing factor RBM22	Splicing
BUD31	Protein BUD31 homolog	Splicing
PPIL3	Peptidyl-prolyl cis-trans isomerase-like 3;Peptidyl-prolyl cis-trans isomerase	Splicing
MFAP1	Microfibrillar-associated protein 1	Splicing
RBM15	Putative RNA-binding protein 15	Splicing
THRAP3	Thyroid hormone receptor-associated protein 3	Splicing
LUC7L2	Putative RNA-binding protein Luc7-like 2	Splicing
DHX8	ATP-dependent RNA helicase DHX8	Splicing
IK	Protein Red	Splicing
PRPF31	U4/U6 small nuclear ribonucleoprotein Prp31	Splicing
SYF2	Pre-mRNA-splicing factor SYF2	Splicing
PPIL1	Peptidyl-prolyl cis-trans isomerase-like 1	Splicing
WTAP	Pre-mRNA-splicing regulator WTAP	Splicing
SNRNP70	U1 small nuclear ribonucleoprotein 70 kDa	Splicing
DGCR14	Protein DGCR14	Splicing
FAM50A	Protein FAM50A	Splicing
PAXBP1	PAX3- and PAX7-binding protein 1	Splicing
DHX38	Pre-mRNA-splicing factor ATP-dependent RNA helicase PRP16	Splicing
GPKOW	G patch domain and KOW motifs-containing protein	Splicing
PRPF4	U4/U6 small nuclear ribonucleoprotein Prp4	Splicing
EFTUD2	116 kDa U5 small nuclear ribonucleoprotein component	Splicing
SF3B1	Splicing factor 3B subunit 1	Splicing
SNRPA1	U2 small nuclear ribonucleoprotein A	Splicing
CCDC12	Coiled-coil domain-containing protein 12	Splicing
SART1	U4/U6.U5 tri-snRNP-associated protein 1	Splicing
USP39	U4/U6.U5 tri-snRNP-associated protein 2	Splicing
DDX23	Probable ATP-dependent RNA helicase DDX23	Splicing
ZC3H18	Zinc finger CCCH domain-containing protein 18	Cap binding protein
NCBP2	Nuclear cap-binding protein subunit 2	Cap binding protein
NUP98	Nuclear pore complex protein Nup98-Nup96;Nuclear pore complex protein Nup98;Nuclear pore complex protein Nup96	RNA transport
RAE1	mRNA export factor	RNA transport
MRPL1	39S ribosomal protein L1. mitochondrial	Ribosome/Translation
ICT1	Peptidyl-tRNA hydrolase ICT1. mitochondrial	Ribosome/Translation
CARS2	Probable cysteine--tRNA ligase. mitochondrial	Ribosome/Translation
RBM45	RNA-binding protein 45	RBP
PDCD4	Programmed cell death protein 4	RBP
CPSF2	Cleavage and polyadenylation specificity factor subunit 2	Polyadenylation
FIP1L1	Pre-mRNA 3-end-processing factor FIP1	Polyadenylation
WDR33	pre-mRNA 3 end processing protein WDR33	Polyadenylation
PABPN1	Polyadenylate-binding protein 2	Polyadenylation
DPF2	Zinc finger protein ubi-d4	Chromatin/transcription
SMARCC1	SWI/SNF complex subunit SMARCC1	Chromatin/transcription

SNW1	SNW domain-containing protein 1	Chromatin/transcription
BCLAF1	Bcl-2-associated transcription factor 1	Chromatin/transcription
ARID1A	AT-rich interactive domain-containing protein 1A	Chromatin/transcription
SMARCA4	Transcription activator BRG1	Chromatin/transcription
SMARCE1	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily E member 1	Chromatin/transcription
INTS3	Integrator complex subunit 3	Chromatin/transcription
SMARCC2	SWI/SNF complex subunit SMARCC2	Chromatin/transcription
ARID1B	AT-rich interactive domain-containing protein 1B	Chromatin/transcription
SMARCB1	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1	Chromatin/transcription
TOP3B	DNA topoisomerase 3-beta-1;DNA topoisomerase	Chromatin/transcription
SMARCD2	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D member 2	Chromatin/transcription
PTCD3	Pentatricopeptide repeat domain-containing protein 3, mitochondrial	Chromatin/transcription
SMARCD1	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D member 1	Chromatin/transcription
CAMK2D	Calcium/calmodulin-dependent protein kinase type II subunit delta	Chromatin/transcription
TDRD3	Tudor domain-containing protein 3	Chromatin/transcription
INTS6	Integrator complex subunit 6	Chromatin/transcription
C9orf78	Uncharacterized protein C9orf78	Chromatin/transcription
CCDC9	Coiled-coil domain-containing protein 9	Cell division
KIF20A	Kinesin-like protein KIF20A	Cell division
CDK11B;CDK11A	Cyclin-dependent kinase 11B;Cyclin-dependent kinase 11A	Cell division
KIF1C	Kinesin-like protein KIF1C	Cell division
DIABLO	Diablo homolog, mitochondrial	Apoptosis
XIAP	E3 ubiquitin-protein ligase XIAP	Apoptosis
CXorf56	UPF0428 protein CXorf56	?
CHMP4B	Charged multivesicular body protein 4b	Endocytosis
NOSIP	Nitric oxide synthase-interacting protein	Ubiquitin ligase
SEN1	Sentrin-specific protease 1	SUMO protease
CAMK2G	Calcium/calmodulin-dependent protein kinase type II subunit gamma	Signaling

**Table S3: List of shared enriched proteins in CWC22 and eIF4A3 IPs**

List of protein significantly enriched in both CWC22 and eIF4A3 immuno-precipitation compared to control experiments and their function. Splicing factors were indicated in blue while mRNA processing factors in orange. For each protein, enrichment ratio is shown in a log<sub>2</sub> scale for CWC22 and eIF4A3 immuno-precipitations respectively.

Protein	Name	Function	Log <sub>2</sub> fold enrichment eIF4A3/Control	Log <sub>2</sub> fold enrichment CWC22/Control
AQR	Intron-binding protein aquarius	Splicing (IBC complex)	8.4	5.3
SLU7	Pre-mRNA-splicing factor SLU7	Splicing (step 2)	5.2	3.1
RBM25	RNA-binding protein 25	Splicing associated factor	6.1	3.4
PRPF38B	Pre-mRNA-splicing factor 38B	Splicing associated factor	6.4	3.4
CWC27	Peptidyl-prolyl cis-trans isomerase CWC27 homolog	Splicing (Bact)	7.7	7.4
CDC5L	Cell division cycle 5-like protein	Splicing (PRP19-complex)	6.2	3.6
XAB2	Pre-mRNA-splicing factor SYF1	Splicing (IBC complex)	8.0	5.2
CWC15	Spliceosome-associated protein CWC15 homolog	Splicing (PRP19-complex)	6.5	3.9
SRRM2	Serine/arginine repetitive matrix protein 2	Splicing (Bact)	5.8	4.5
CWC22	Pre-mRNA-splicing factor CWC22 homolog	Splicing (Bact-P)	8.1	5.5
YTHDC1	YTH domain-containing protein 1	mRNA processing factor (m6A reader)	4.2	4.3
NCBP1	Nuclear cap-binding protein subunit 1	mRNA processing factor (Cap binding Complex)	6.2	5.3
KIAA1429	Protein virilizer homolog	mRNA processing factor (m6A writer)	4.1	3.7
ZC3H14	Zinc finger CCCH domain-containing protein 14	mRNA processing factor (polyadenylation, etc...)	6.2	3.7
THOC2	THO complex subunit 2	mRNA processing factor (Export)	3.9	6.5
SRRT	Serrate RNA effector molecule homolog	mRNA processing factor (Cap associated protein)	5.1	4.6

**Table S4: Data collection, phasing and refinement statistics**

<b>CWC27/CWC22/eIF4A3</b>	
<b>Data collection</b>	
Space group	<i>P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub></i>
Cell dimensions	
<i>a, b, c</i> (Å)	151.255 163.627 181.131
$\alpha, \beta, \gamma$ (°)	90 90 90
Wavelength	0.99989
Resolution (Å)	94.69 -3.192 (3.306 -3.192)
<i>R</i> <sub>sym</sub> or <i>R</i> <sub>merge</sub>	0.1287 (2.598)
<i>I</i> / $\sigma I$	16.57 (0.95)
Completeness (%)	99.46 (95.11)
Redundancy	9.3 (9.1)
CC(1/2)	0.999 (0.48)
<b>Refinement</b>	
Resolution (Å)	94.69 -3.192 (3.306 -3.192)
No. reflections	74871 (7048)
<i>R</i> <sub>work</sub> / <i>R</i> <sub>free</sub>	0.2324/0.2617
No. atoms	14536
Protein	14536
<i>B</i> -factors	118.9
Protein	118.9
R.m.s deviations	
Bond lengths (Å)	0.005
Bond angles (°)	1.07

\*Values in parentheses are for highest-resolution shell.

**Table S5: GO analysis of genes upregulated in CWC27 KD**

GO analysis with GOrilla (Eden et al., 2009) and REVIGO (Supek et al., 2011) of all genes significantly up-regulated (p-value <0.05) in CWC27 KD.

term_ID	description	frequencyInDb	log10(pvalue)	uniqueness	dispensability	representative
GO:0002376	<b>immune system process</b>	0.600%	-115 287	0.983	0.000	immune system process
GO:0003014	renal system process	0.017%	-30 137	0.968	0.000	renal system process
GO:0006952	defense response	0.568%	-131 688	0.708	0.000	defense response
GO:1901700	response to oxygen-containing compound	0.503%	-41 343	0.648	0.550	defense response
GO:0043207	response to external biotic stimulus	0.300%	-68 268	0.622	0.336	defense response
GO:0043434	response to peptide hormone	0.056%	-39 957	0.671	0.645	defense response
GO:0048583	regulation of response to stimulus	1120%	-44 547	0.608	0.487	defense response
GO:0035556	intracellular signal transduction	4000%	-31 232	0.519	0.641	defense response
GO:0034097	<b>response to cytokine</b>	0.136%	-40 595	0.654	0.688	defense response
GO:0019722	calcium-mediated signaling	0.040%	-46 073	0.642	0.413	defense response
GO:0032103	positive regulation of response to external stimulus	0.055%	-37 423	0.525	0.635	defense response
GO:0019221	<b>cytokine-mediated signaling pathway</b>	0.093%	-126 990	0.519	0.304	defense response
GO:0071456	cellular response to hypoxia	0.022%	-39 872	0.647	0.592	defense response
GO:0019932	second-messenger-mediated signaling	0.079%	-48 570	0.633	0.359	defense response
GO:0009607	response to biotic stimulus	0.342%	-65 884	0.734	0.340	defense response
GO:0009605	response to external stimulus	1370%	-56 234	0.705	0.501	defense response
GO:0006950	response to stress	4575%	-110 706	0.673	0.447	defense response
GO:0006954	<b>inflammatory response</b>	0.110%	-119 586	0.720	0.491	defense response
GO:0007166	cell surface receptor signaling pathway	0.920%	-74 828	0.570	0.475	defense response
GO:0042221	response to chemical	3071%	-42 573	0.684	0.562	defense response
GO:0010033	response to organic substance	0.900%	-38 601	0.633	0.675	defense response
GO:0007186	G-protein coupled receptor signaling pathway	0.882%	-30 026	0.572	0.538	defense response
GO:0009987	cellular process	63780%	-33 788	0.994	0.000	cellular process
GO:0022610	biological adhesion	0.550%	-31 726	0.983	0.000	biological adhesion
GO:0040011	locomotion	0.997%	-34 881	0.983	0.000	locomotion
GO:0043900	regulation of multi-organism process	0.079%	-48 125	0.809	0.000	regulation of multi-organism process
GO:0001934	positive regulation of protein phosphorylation	0.227%	-40 757	0.703	0.247	regulation of multi-organism process
GO:0043903	regulation of symbiosis, encompassing mutualism through parasitism	0.039%	-33 893	0.804	0.648	regulation of multi-organism process
GO:0045124	regulation of bone resorption	0.006%	-30 857	0.754	0.472	regulation of multi-organism process
GO:0048584	positive regulation of response to stimulus	0.461%	-40 283	0.543	0.694	regulation of multi-organism process
GO:0042592	homeostatic process	1661%	-43 546	0.727	0.224	regulation of multi-organism process
GO:0001817	<b>regulation of cytokine production</b>	0.108%	-30 246	0.793	0.608	regulation of multi-organism process
GO:0050896	response to stimulus	12210%	-73 536	0.985	0.000	response to stimulus
GO:0050900	<b>leukocyte migration</b>	0.060%	-82 351	0.591	0.000	leukocyte migration
GO:0032787	monocarboxylic acid metabolic process	2485%	-36 498	0.843	0.339	leukocyte migration
GO:0022614	membrane to membrane docking	0.001%	-45 186	0.907	0.101	leukocyte migration
GO:0032879	regulation of localization	0.726%	-31 864	0.753	0.281	leukocyte migration
GO:0006636	unsaturated fatty acid biosynthetic process	0.018%	-31 261	0.877	0.476	leukocyte migration
GO:0042119	<b>neutrophil activation</b>	0.009%	-30 438	0.684	0.608	leukocyte migration
GO:0001775	cell activation	0.171%	-65 436	0.881	0.136	leukocyte migration
GO:0046456	icosanoid biosynthetic process	0.009%	-39 666	0.872	0.111	leukocyte migration
GO:0032940	secretion by cell	0.763%	-32 823	0.837	0.224	leukocyte migration
GO:0046903	secretion	0.810%	-34 157	0.868	0.557	leukocyte migration
GO:0015939	pantothenate metabolic process	0.144%	-34 881	0.867	0.432	leukocyte migration
GO:0051704	multi-organism process	0.751%	-53 270	0.983	0.000	multi-organism process
GO:0007159	<b>leukocyte cell-cell adhesion</b>	0.090%	-38 041	0.898	0.057	leukocyte cell-cell adhesion
GO:0090673	endothelial cell-matrix adhesion	0.000%	-32 048	0.952	0.519	leukocyte cell-cell adhesion

**Table S6: GO analysis of genes upregulated in CWC22 KD**

GO analysis with GOrilla (Eden et al., 2009) and REVIGO (Supek et al., 2011) of all genes significantly up-regulated (p-value <0.05) in CWC22 KD.

term_ID	description	frequencyIn Db	log10 (pvalue)	uniqueness	dispensability	representative
GO:0002376	<b>immune system process</b>	0.600%	-54 976	0.982	0.000	immune system process
GO:0009308	amine metabolic process	0.521%	-32 765	0.976	0.000	amine metabolism
GO:0040011	locomotion	0.997%	-38 570	0.982	0.000	locomotion
GO:0050896	response to stimulus	12210%	-65 171	0.984	0.000	response to stimulus
GO:0051704	multi-organism process	0.751%	-42 916	0.982	0.000	multi-organism process
GO:0065007	biological regulation	20498%	-34 547	0.986	0.000	biological regulation
GO:0070098	<b>chemokine-mediated signaling pathway</b>	0.018%	-115 391	0.488	0.000	chemokine-mediated signaling pathway
GO:0043903	regulation of symbiosis, encompassing mutualism through parasitism	0.039%	-32 457	0.794	0.648	chemokine-mediated signaling pathway
GO:1901700	response to oxygen-containing compound	0.503%	-47 878	0.587	0.675	chemokine-mediated signaling pathway
GO:0043207	response to external biotic stimulus	0.300%	-70 114	0.573	0.415	chemokine-mediated signaling pathway
GO:0043900	regulation of multi-organism process	0.079%	-32 472	0.801	0.205	chemokine-mediated signaling pathway
GO:0048583	regulation of response to stimulus	1120%	-35 969	0.580	0.487	chemokine-mediated signaling pathway
GO:1902882	regulation of response to oxidative stress	0.020%	-31 765	0.627	0.537	chemokine-mediated signaling pathway
GO:0042592	homeostatic process	1661%	-36 421	0.746	0.337	chemokine-mediated signaling pathway
GO:0032101	regulation of response to external stimulus	0.160%	-41 129	0.508	0.691	chemokine-mediated signaling pathway
GO:0032103	positive regulation of response to external stimulus	0.055%	-38 416	0.489	0.691	chemokine-mediated signaling pathway
GO:0048871	multicellular organismal homeostasis	0.067%	-37 825	0.757	0.573	chemokine-mediated signaling pathway
GO:0050794	regulation of cellular process	18840%	-36 364	0.726	0.499	chemokine-mediated signaling pathway
GO:0042127	regulation of cell proliferation	0.313%	-55 670	0.777	0.189	chemokine-mediated signaling pathway
GO:0033993	response to lipid	0.226%	-54 101	0.567	0.636	chemokine-mediated signaling pathway
GO:0019932	second-messenger-mediated signaling	0.079%	-36 055	0.600	0.408	chemokine-mediated signaling pathway
GO:0009607	response to biotic stimulus	0.342%	-67 747	0.699	0.421	chemokine-mediated signaling pathway
GO:0009605	response to external stimulus	1370%	-42 518	0.667	0.501	chemokine-mediated signaling pathway
GO:0051928	positive regulation of calcium ion transport	0.017%	-37 799	0.700	0.478	chemokine-mediated signaling pathway
GO:0006952	<b>defense response</b>	0.568%	-80 773	0.678	0.447	chemokine-mediated signaling pathway
GO:0006950	response to stress	4575%	-76 289	0.633	0.316	chemokine-mediated signaling pathway
GO:0006954	<b>inflammatory response</b>	0.110%	-83 716	0.703	0.491	chemokine-mediated signaling pathway
GO:0035584	calcium-mediated signaling using intracellular calcium source	0.003%	-43 188	0.659	0.336	chemokine-mediated signaling pathway
GO:0007166	cell surface receptor signaling pathway	0.920%	-50 835	0.537	0.538	chemokine-mediated signaling pathway
GO:0007165	signal transduction	6621%	-54 841	0.461	0.679	chemokine-mediated signaling pathway
GO:0042221	response to chemical	3071%	-51 226	0.645	0.562	chemokine-mediated signaling pathway
GO:0065008	regulation of biological quality	3395%	-38 477	0.789	0.275	chemokine-mediated signaling pathway
GO:0010033	response to organic substance	0.900%	-48 210	0.570	0.507	chemokine-mediated signaling pathway
GO:0007186	G-protein coupled receptor signaling pathway	0.882%	-72 832	0.538	0.472	chemokine-mediated signaling pathway
GO:0070374	positive regulation of ERK1 and ERK2 cascade	0.034%	-51 096	0.510	0.333	chemokine-mediated signaling pathway
GO:0060384	innervation	0.005%	-31 367	0.927	0.044	innervation
GO:0007568	aging	0.088%	-31 002	0.926	0.417	innervation
GO:0050900	<b>leukocyte migration</b>	0.060%	-85 575	0.563	0.051	leukocyte migration
GO:0022614	membrane to membrane docking	0.001%	-37 077	0.905	0.101	leukocyte migration
GO:0001775	cell activation	0.171%	-36 737	0.880	0.136	leukocyte migration
GO:0006959	humoral immune response	0.035%	-30 462	0.530	0.662	leukocyte migration
GO:0006928	movement of cell or subcellular component	0.973%	-36 696	0.866	0.170	leukocyte migration
GO:0007159	<b>leukocyte cell-cell adhesion</b>	0.090%	-35 817	0.932	0.057	leukocyte cell-cell adhesion



**Table S7: List of upregulated genes in CWC27 KD**

Genes significantly upregulated in CWC27 KD compared to control untreated RPE-1 cells (p-value < 0.05). Genes are classified according to their Fold Change (FC) in CWC27 KD compared to control ( $\log_2(\text{FC CWC27}/\text{Control})$ ).  $\log_2(\text{FC CWC22}/\text{Control})$  is indicated for the same genes.

Symbol	Base mean	$\log_2(\text{FC CWC22}/\text{Control})$	$\log_2(\text{FC CWC27}/\text{Control})$	Gene name	Inflammatory Category
CCR1	5.7	3.58	8.19	C-C chemokine receptor type 1	Cytokine Receptor
ACOD1	3.3	4.32	7.10	aconitate decarboxylase 1	Inflammatory pathway modulator
KYNU	15.3	7.27	6.66	L-kynureninase	Inflammatory pathway modulator
MEFV	1.7	4.84	6.60	Pyrrin	Inflammasome subunit
CCL20	5.3	7.49	6.05	C-C motif chemokine 20	Cytokine
LYPD3	8.3	4.30	5.08	LY6/PLAUR domain containing 3	
EHF	1.0	6.54	5.07	ETS homologous factor	
VCAM1	7.7	6.93	5.05	Vascular cell adhesion protein 1	Leukocyte Adhesion molecule
CCL5	169.7	6.07	5.03	C-C motif chemokine 5	Cytokine
ICAM1	260.0	6.07	4.99	Intercellular adhesion molecule 1	Monocyte Adhesion molecule
OTOF	0.0	0.99	4.98	Otoferlin	
H1F0	159.3	3.88	4.93	Histone H1 F0	
TNFRSF9	14.7	5.05	4.90	Tumor necrosis factor receptor superfamily member 9	Cytokine receptor
BCL2A1	0.7	6.31	4.89	Bcl-2-related protein A1	
PTGES	8.3	7.68	4.89	prostaglandin E synthase-1	Inflammatory pathway modulator
AQP3	2.0	6.21	4.87	Aquaporin 3	
NR5A2	0.0	5.12	4.71	Nuclear receptor subfamily 5 group A member 2	
PRR20G	0.3	1.05	4.69	Proline-rich protein 20G	
CXCL1	47.0	5.24	4.65	Growth-regulated alpha protein	Cytokine
HLA-DRA	5.7	2.76	4.62	HLA class II histocompatibility antigen. DR alpha chain	Antigen presentation
GOLGA7B	2.7	2.46	4.62	Golgin subfamily A member 7B	
L1CAM	0.3	4.30	4.39	Neural cell adhesion molecule L1	Adhesion molecule
CD74	34.0	3.40	4.38	H-2 class II histocompatibility antigen gamma chain	Antigen presentation
C15orf48	0.7	4.10	4.38	Normal mucosa of esophagus-specific gene 1 protein	
NOTCH3	11.0	3.31	4.35	Notch3	
GDF15	4.7	3.36	4.35	Growth/differentiation factor 15	Cytokine receptor
UNC13A	3.7	5.14	4.31	Protein unc-13 homolog A	
POU2F2	10.7	2.18	4.30	POU domain, class 2, transcription factor 2	
CXCL11	116.3	5.48	4.29	C-X-C motif chemokine 11	Cytokine
ARHGAP30	3.3	1.61	4.28	Rho GTPase-activating protein 30	
VNN3	0.7	4.72	4.27	Vascular non-inflammatory molecule 3	
DES	1.3	3.37	4.26	Desmin	
TCIM	0.3	3.38	4.25	Transcriptional and immune response regulator	Inflammatory pathway modulator
HR	23.0	3.55	4.24	Lysine-specific demethylase hairless	
TNFRSF8	2.0	4.32	4.21	Tumor necrosis factor receptor superfamily member 8	Cytokine receptor
HSD11B1	13.7	3.08	4.20	Corticosteroid 11-beta-dehydrogenase isozyme 1	
PTPRN	4.3	3.35	4.20	protein tyrosine phosphatase receptor type N	
LINC01537	8.3	1.36	4.13	lncRNA	
PTGIR	7.7	3.50	4.11	Prostacyclin receptor	Inflammatory pathway modulator
ALDOC	6.3	2.99	4.06	Fructose-bisphosphate aldolase C	
SLCO4A1	52.3	2.76	4.06	Solute carrier organic anion transporter family member 4A1	
PECAM1	22.7	2.00	4.04	Platelet endothelial cell adhesion molecule	Leukocyte Adhesion molecule
ADAMTS9	2.0	1.97	4.03	Disintegrin and metalloproteinase with thrombospondin motifs 9	
ADAM8	16.3	1.90	4.03	Disintegrin and metalloproteinase domain-containing protein 8	Leukocyte migration in inflammation
PLCG2	16.0	1.98	4.03	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase gamma-2	Inflammatory pathway modulator
SERPING1	339.7	3.01	3.97	Plasma Protease C1 inhibitor	Activation of complement complex C1
CXCL6	2.7	4.55	3.97	C-X-C motif chemokine ligand 6	Cytokine
PTPRH	8.7	3.30	3.96	protein tyrosine phosphatase receptor type H	
STMN3	21.3	2.03	3.90	Stathmin 3	
SERPINA1	1.7	3.81	3.89	Alpha anti-trypsin	Protection against leukocyte elastase
CCRL2	9.0	3.61	3.87	C-C chemokine receptor-like 2	Cytokine receptor
ACVRL1	1.0	3.36	3.87	Serine/threonine-protein kinase receptor R3	
NECTIN4	3.3	5.17	3.87	Nectin-4	Adhesion molecule
TLR7	0.7	0.83	3.86	Toll-like receptor 7	Inflammasome subunit
SYP	9.0	3.04	3.86	Synaptophysin	
ADCY8	0.3	0.32	3.86	Adenylate cyclase type 8	
ALK	12.7	1.85	3.85	ALK tyrosine kinase receptor	Receptor
CYP4F11	0.7	2.70	3.82	Phylloquinone omega-hydroxylase	
CXCL8	712.3	4.93	3.79	Interleukine 8	Cytokine

CARD16	63.7	3.46	3.78	Caspase recruitment domain-containing protein 16	Inflammasome subunit
KLHDC7B	10.7	1.14	3.77	Kelch domain-containing protein 7B	
NCALD	3.0	0.70	3.77	Neurocalcin-delta	
AIM2	41.7	2.08	3.76	Interferon-inducible protein AIM2	Inflammasome subunit
ELOVL3	2.7	2.28	3.76	Elongation of very long chain fatty acids protein 3	
MRGPRX3	0.3	4.17	3.76	Mas-related G-protein coupled receptor member X3	
TNFAIP6	143.0	5.54	3.76	Tumor necrosis factor, alpha-induced protein 6	Inflammatory pathway modulator
PTGS1	0.7	1.52	3.75	Prostaglandin-Endoperoxide Synthase 1	Inflammatory pathway modulator
SIRLNT	0.3	5.18	3.75	SIRT1 regulating lncRNA tumor promoter	
MEP1B	8.7	2.30	3.73	Meprin A subunit beta	Cytokine processing
SERPINB2	198.3	5.00	3.72	Plasminogen activator inhibitor 2	
LTF	0.7	2.66	3.72	Lactotransferrin	Inflammatory pathway modulator
KLK10	0.3	2.56	3.72	Kallikrein 10	
CLCA3P	9.7	2.34	3.71	Calcium-activated chloride channel regulator family member 3	
IFI27	687.7	3.32	3.71	Interferon alpha-inducible protein 27, mitochondrial	Inflammatory pathway modulator
LINC01285	1.7	2.59	3.70	lncRNA	
GPSM3	5.7	0.75	3.69	G-protein-signaling modulator 3	Inflammatory pathway modulator
ADGRB3	2.3	1.09	3.68	Adhesion G protein-coupled receptor B3	
MTSS1	10.0	4.05	3.66	metastasis suppressor 1	
CX3CL1	35.3	3.67	3.64	Fractalkine	Cytokine
COL5A3	0.0	2.79	3.63	Collagen alpha-3(V) chain	
IL24	0.0	1.90	3.61	Interleukine 24	Cytokine
GRIP2	91.3	2.28	3.60	Glutamate receptor-interacting protein 2	
SIRPB1	12.7	0.89	3.59	Signal-regulatory protein beta-1	Receptor
RRAD	23.0	4.24	3.58	GTP-binding protein RAD	
IL20RB	18.0	1.70	3.56	Interleukin-20 receptor subunit beta	Cytokine receptor
VNN2	4.0	2.35	3.56	Vascular non-inflammatory molecule 2	
TYMP	18.7	1.57	3.55	Thymidine phosphorylase	Inflammatory pathway modulator
CH25H	45.3	0.95	3.54	Cholesterol 25-hydroxylase	
GUCY1A2	0.7	4.05	3.54	Guanylate cyclase soluble subunit alpha-2	
C3	726.7	3.61	3.54	Complement C3	Inflammatory pathway modulator
COL1A2	3.3	0.81	3.52	Collagen alpha-2(I) chain	
GBP5	244.3	3.10	3.51	guanylate binding protein 5	Inflammasome subunit
C11orf96	2.0	3.05	3.51	Uncharacterized	
MAPK8IP2	22.0	2.91	3.50	C-Jun-amino-terminal kinase-interacting protein 2	
IFNL1	44.0	2.81	3.48	Interferon lambda 1	Cytokine
EDNRB	4.0	3.15	3.48	Endothelin Receptor	
VNN1	0.0	3.04	3.47	Vanin-1	
CADM3	0.0	0.63	3.46	Cell adhesion molecule 3	Adhesion molecule
MCAM	21.3	4.06	3.46	Cell surface glycoprotein MUC18	Adhesion molecule
IL11	201.7	2.35	3.46	Interleukine 11	Cytokine
EPHA7	11.7	0.43	3.45	Ephrin type-A receptor 7	
CD40	0.7	1.16	3.45	Tumor necrosis factor receptor superfamily member 5	Cytokine
AQP1	5.3	0.77	3.45	Aquaporin 1	
CCL4	11.0	4.44	3.44	C-C motif chemokine 4	Cytokine
CSAG3	10.0	2.78	3.44	Chondrosarcoma-associated gene 2/3 protein	
CA9	332.0	2.79	3.43	Carbonic anhydrase 9	
ISM2	19.0	2.81	3.43	Isthmin-2	
MUC16	1.7	2.78	3.40	mucin 16	
SLC8A3	0.0	1.62	3.40	Sodium/calcium exchanger 3	
IL32	56.7	3.80	3.39	Interleukine 32	Cytokine
TNFAIP2	625.3	3.72	3.39	TNFA induced protein	Inflammatory pathway modulator
SPHKAP	2.0	1.04	3.38	A-kinase anchor protein SPHKAP	
HLA-DRB1	4.3	1.64	3.38	HLA class II histocompatibility antigen, DRB1-14 beta chain	Antigen presentation
NOTUM	2.7	0.51	3.37	Palmitoleoyl-protein carboxylesterase	
TNFSF13B	108.3	1.89	3.37	Tumor necrosis factor ligand superfamily member 13B	Cytokine
IGSF10	2.7	4.32	3.35	Immunoglobulin superfamily member 10	
CCL4L2	0.3	3.52	3.35	CCL4-like	Cytokine
PURPL	0.3	3.35	3.35	p53 upregulated regulator of p53 levels	
NRN1	3.7	1.15	3.34	Neuritin	
CXCL10	201.7	3.53	3.33	C-X-C motif chemokine 10	Cytokine
RSAD2	2246.0	3.04	3.32	Radical S-adenosyl methionine domain-containing protein 2, viperin	Inflammatory pathway modulator
APOBEC3G	57.7	2.15	3.31	DNA dC->dU-editing enzyme APOBEC-3G	
DHRS2	13.7	3.40	3.30	Dehydrogenase/reductase SDR family member 2, mitochondrial	
PLA2G4C	85.3	1.60	3.29	Cytosolic phospholipase A2 gamma	Inflammatory pathway modulator
NIM1K	41.3	2.52	3.28	Serine/threonine-protein kinase NIM1	
BEX5	2.0	1.31	3.27	Nerve growth factor receptor-associated protein 2	
CCR3	0.3	0.81	3.27	C-C chemokine receptor type 3	Cytokine receptor
IL1A	114.0	3.05	3.27	IL1 alpha	Cytokine
CSAG1	23.7	2.37	3.27	Putative chondrosarcoma-associated gene 1 protein	
IFNL2	8.0	2.64	3.26	Interferon lambda	Cytokine

**Table S8: List of down-regulated genes in CWC27 KD**

Genes significantly downregulated in CWC27 KD compared to control untreated RPE-1 cells (p-value < 0.05). Genes are classified according to their Fold Change (FC) in CWC27 KD compared to control ( $\log_2(\text{FC CWC27}/\text{Control})$ ).  $\log_2(\text{FC CWC22}/\text{Control})$  is indicated for the same genes.

Symbol	Base mean	$\log_2(\text{FC CWC22}/\text{Control})$	$\log_2(\text{FC CWC27}/\text{Control})$	Name	Category
CLSTN2	12690.7	-3.90	-4.38	Calsyntenin	Cadherin
TAGLN	14976.3	-4.48	-4.00	Transgelin	TGF pathway. Actin cytoskeleton
TGFB2-AS1	640.3	-3.16	-3.93	TGF $\beta$ 2 antisense	
CWC27	1131.0	0.17	-3.92		
TGFB2	8419.0	-2.79	-3.72	TGF $\beta$ 2	TGF pathway
GDF6	5621.0	-4.04	-3.67	Growth differentiation factor 6	TGF pathway
LBH	11556.7	-2.06	-3.56	limb bud and heart	
SLC26A2	2150.7	-3.17	-3.48	solute carrier family 26	
ST6GAL2	1765.7	-4.20	-3.36	beta-galactoside alpha-2.6-sialyltransferase	
DCDC2	258.0	-2.74	-3.30	doublecortin domain containing 2	
ANKRD1	21385.3	-2.04	-3.26	Ankyrin Repeat Domain-Containing 1	TGF pathway
ADAMTS14	399.7	-2.72	-3.25	A disintegrin and metalloproteinase with thrombospondin motifs 14	TGF pathway
CDH6	2399.7	-0.83	-3.20	Cadherin-6	Adhesion molecule. TGF pathway
IDH1	3376.0	-0.17	-3.16	Isocitrate dehydrogenase	
NRXN3	369.3	-1.42	-3.15	Neurexin-3-beta	Adhesion molecule
CTGF	23161.3	-2.02	-3.11	Connective Tissue Growth Factor	TGF pathway
PLCE1-AS1	17.7	-2.82	-3.06	antisense -1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase epsilon-1	
HHIP	3437.7	-2.07	-3.01	Hedge-hog interacting protein	TGF pathway
RAB11FIP4	29.7	-1.73	-3.00	Rab11 family-interacting protein 4	
OXTR	3169.0	-1.49	-2.90	Oxytocin receptor	
CHAC1	207.3	-3.33	-2.88	glutathione specific gamma-glutamylcyclotransferase	
ACTG2	59.0	-2.07	-2.88	$\gamma$ Actin	TGF pathway. Actin cytoskeleton
KCNH1	48.7	-2.12	-2.87	Potassium voltage-gated channel subfamily H member 1	
ALDH1B1	1251.3	-2.18	-2.82	Aldehyde dehydrogenase X, mitochondrial	
EPYC	14.3	-2.03	-2.82	epiphycan	
ALCAM	12898.3	-0.49	-2.77	activated leukocyte cell adhesion molecule	Adhesion molecule. TGF pathway
MYL9	11838.7	-2.33	-2.74	Myosin Light Chain	Actin cytoskeleton
TPM1	50346.3	-2.36	-2.74	Tropomyosin	TGF pathway. Actin cytoskeleton
PRICKLE1	82.7	-2.06	-2.69	Prickle-like protein 1	
SAMD12	522.0	-0.08	-2.67	Sterile alpha motif domain-containing protein 12	
MT-ATP8	1801.7	-1.92	-2.63	ATP synthase protein 8	Mitochondrial gene
MTATP6P1	3509.3	-2.01	-2.62	<i>MT-ATP6 pseudogene</i>	
MT-ATP6	19764.0	-2.00	-2.59	ATP synthase subunit a	Mitochondrial gene
NPM3	354.7	-0.92	-2.59	nucleoplasmin	
HSD17B14	163.7	-3.66	-2.59	17 $\beta$ -hydroxysteroid dehydrogenase 14	
SERTAD4-AS1	69.7	-2.70	-2.59	SERTAD4 antisense RNA 1	
MT-CO3	37238.0	-2.02	-2.58	Cytochrome c oxidase subunit 3	Mitochondrial gene
CEMIP2	1628.0	-1.57	-2.58	Cell surface hyaluronidase	
FHDC1	194.7	-1.15	-2.56	FH2 domain-containing protein 1	
B3GALT2	35.7	-1.49	-2.56	Beta-1.3-galactosyltransferase 2	
LMOD1	1237.7	-1.83	-2.54	Leiomodin 1	Actin cytoskeleton
MT-ND3	12014.0	-2.19	-2.54	NADH-ubiquinone oxidoreductase chain 3	Mitochondrial gene
SLC26A4	241.7	-3.04	-2.54	Pendrin	
GSKIP	779.3	-0.45	-2.52	GSK3B-interacting protein	
ROR1	843.3	-0.96	-2.50	Inactive tyrosine-protein kinase transmembrane receptor ROR1	
C1orf198	2997.7	-1.86	-2.49	Uncharacterized protein C1orf198	
FAM84B	4169.7	-2.16	-2.48	Protein LRATD2	
SETD7	4056.3	-0.77	-2.48	Histone-lysine N-methyltransferase SETD7	
OR2T8	213.0	-2.38	-2.46	Olfactory receptor 2T8	
KLF2	333.3	-1.02	-2.46	Krueppel-like factor 2	TGF pathway
MT-CYB	29097.3	-1.80	-2.45	Cytochrome b	Mitochondrial gene
EPPK1	10.3	-2.15	-2.44	Epiplakin	
SELENOI	1879.0	-1.75	-2.43	Pyroline-5-carboxylate reductase 1, mitochondrial	
PYCR1	527.0	-2.20	-2.43	Ethanolaminephosphotransferase 1	
RIMS1	392.3	-1.70	-2.42	Regulating synaptic membrane exocytosis protein 1	
WNT5B	5281.3	-1.33	-2.42	Wnt-5b	TGF pathway
MATN3	125.0	-0.32	-2.41	Matrilin-3	
NXPH4	84.0	-1.72	-2.41	Neurexophilin-4	

JPH2	368.7	-1.33	-2.41	Junctophilin-2	
EEF1B2	8790.0	-2.05	-2.40	Elongation factor 1-beta	Protein synthesis
CCDC144NL-AS1	29.7	-2.62	-2.38	Putative coiled-coil domain-containing protein 144 N-terminal-like anti sense	
PDGFB	96.7	-3.76	-2.38	Platelet-derived growth factor subunit B	
CDH4	203.7	-1.50	-2.37	Cadherin-4	Adhesion molecule
GALNT10	26751.0	-2.41	-2.37	Polypeptide N-acetylgalactosaminyltransferase 10	
EEF1B2P3	143.0	-2.04	-2.37	<i>EEF1B2 Pseudo gene</i>	<i>pseudogene</i>
DDAH1	9944.3	-2.04	-2.35	N(G),N(G)-dimethylarginine dimethylaminohydrolase 1	
SPP1	5603.0	-1.51	-2.35	Sphingosine-1-phosphate phosphatase 1	
COL4A4	3776.0	-2.86	-2.35	Collagen alpha-4(IV) chain	
POLR3B	417.0	0.20	-2.33	DNA-directed RNA polymerase III subunit RPC2	
ARHGEF16	43.0	-3.69	-2.32	Rho guanine nucleotide exchange factor 16	
B4GALT4	671.3	0.23	-2.31	Beta-1.4-galactosyltransferase 4	
LIMS2	124.3	-1.74	-2.30	LIM and senescent cell antigen-like-containing domain protein 2	Actin cytoskeleton
FAT1	15170.3	-1.87	-2.29	Protocadherin Fat 1	TGF pathway. Actin cytoskeleton
ACTG1	96886.7	-1.76	-2.29	$\gamma$ Actin	Actin cytoskeleton
MEGF6	2575.0	-2.17	-2.29	Multiple epidermal growth factor-like domains protein 6	
ALPK2	74.7	-2.59	-2.29	Alpha-protein kinase 2	

## SUPPLEMENTARY FIGURES LEGENDS

### Supplementary Figure 1: CRISPR/Cas9 mediated gene tagging

(a) Strategy used for genome editing of HeLa cells in order to fuse FLAG tags in N-terminal extremity of CWC27. A transgene containing the puromycin resistance gene (flanked by 2 LoxP sites) followed by a P2A site and 3xFLAG tag was inserted into the 5'-UTR of CWC27 gene. The sequence between LoxP sites was eliminated with Cre recombinase. (b) Western blots of CWC27 and GAPDH on protein extracts from selected HeLa cell clones expressing homogenous FLAG-CWC27 proteins after Cre recombinase treatment (lanes 1-9), before treatment (lane 9) or from WT HeLa cells (lane 10).

### Supplementary Figure 2: Study of CWC27 mutants

(a) Western blots of input and proteins coimmunoprecipitated with FLAG-CWC27 truncations or FLAG only (-) transiently expressed in HeLa cells. Detected proteins are indicated on the left. Unspecific contaminating protein detected by anti-FLAG antibodies are marked (\*). (b) HeLa cells transfected with GFP-CWC27 (1-472, 1-306, 1-388 or 170-472) were fixed and stained using anti-FLAG (green) and counterstained with DAPI (blue). Merged images are shown on the right panels.

### Supplementary Figure 3: CWC22 and CWC27 form a complex with eIF4A3

(a) Interaction of eIF4A3 with CWC22-S and with CWC22-S/CWC27 heterodimer. Mixed recombinant proteins (input: 35% of total) and proteins retained with CBP-CWC22-S (lane 2), with preformed heterodimer CBP-CWC22-S/CWC27 (lane 3) or not (lane 1) on calmodulin beads (precipitate) were analyzed by SDS-PAGE. (b) CWC27/CWC22/eIF4A3 complex formation using a Superdex 200 column. The complex CWC27 and CWC22 was mixed with a small excess of eIF4A3 and subjected to the SEC column. The main peak corresponds to the trimeric complex. The SDS PAGE below shows a stoichiometric complex.

### Supplementary Figure 4: Sequence alignment of CWC27 c-terminal region

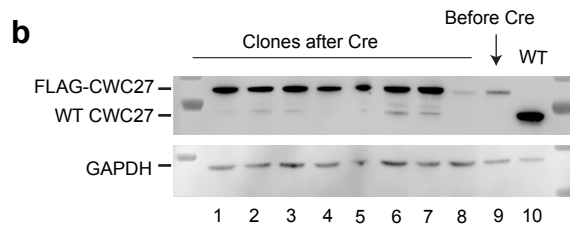
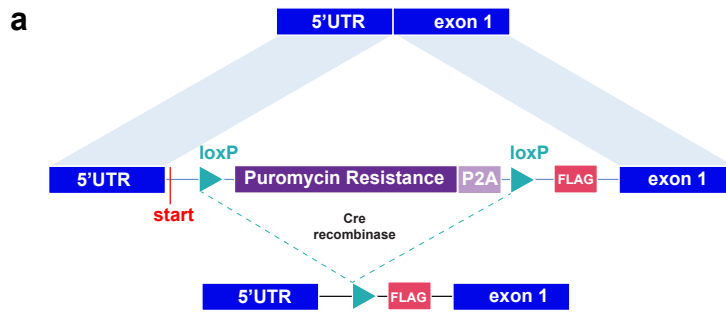
(a) Alignment of protein sequences of CWC27 from *Homo sapiens* (H.s., Q6UX04), *Mus musculus* (M.m., Q3TKY6), *Gallus gallus* (G.g., E1C0I5), *Xenopus laevis* (X.l., Q6GLX7), *Danio rerio* (D.r., Q7ZW86), *Drosophila melanogaster* (D.m., Q9VTN7), *Caenorhabditis elegans* (C.e., Q9XXI7), *Schizosaccharomyces pombe* (S.p., O42941) and *Saccharomyces cerevisiae* (S.c., Q02770). The number of residues of each protein is indicated. Residues

showing some conservation are framed in blue boxes. Residues conserved between all the species are shaded in red while residues in only some species or similar are in red. The position of the helix and the loop are indicated. Dashed lines indicate sequences not-seen on the CWC27/CWC22/eIF4A3 3D structure. Yellow stars indicate residues in direct contact with CWC22. **(b)** Conservation of CWC27 residues (378-426) interacting with the CWC22 MIF4G domain (green).

### **Supplementary Figure 5: Impacts of CWC27 and CWC22 KD on gene expression in RPE-1 cells**

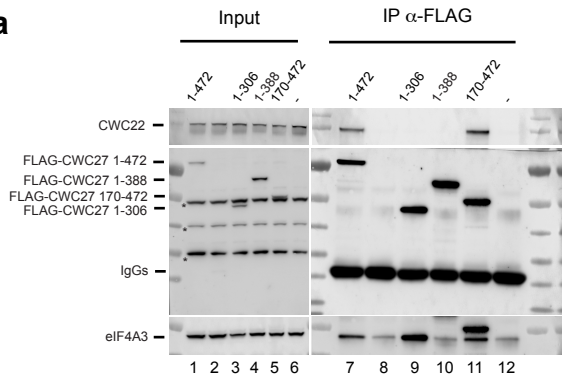
**(a)** Western blots of proteins coimmunoprecipitated with anti-Rab5 (as control), anti-eIF4A3, anti-Y14, anti-CWC22 and anti-CWC27 from hTERT RPE-1 cell extracts. Detected proteins are indicated on the left. **(b)** Expression level of CWC27 (blue) and CWC22 (green) mRNAs in RPE-1 cells measured by RT-qPCR. Cells were treated with CWC27, CWC22 or control DsiRNAs. Expression levels in control cells serve as reference. **(c)** Principal component analysis of RNA-seq samples using gene counts as inputs. The first two components are shown. **(d-g)** RNA-seq read counts from all biological replicates exemplifying alternative splicing events differentially affected in RPE-1 cells treated by DsiRNAs control (black), CWC22 (green) or CWC27 (blue). Selected splice sites of NEK1, ABI1, ADD3 and UPF3 mRNAs are shown.

# Supplementary Figure 1: CRISPR/Cas9 mediated gene tagging

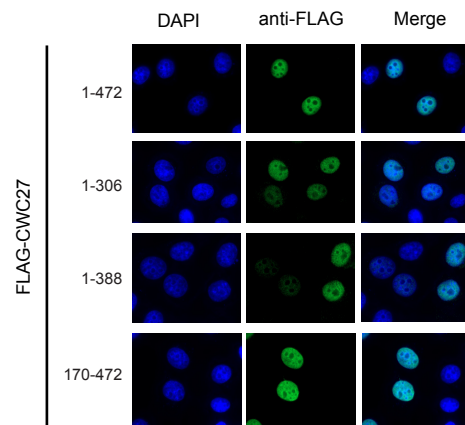


## Supplementary Figure 2: Study of CWC27 mutants

**a**

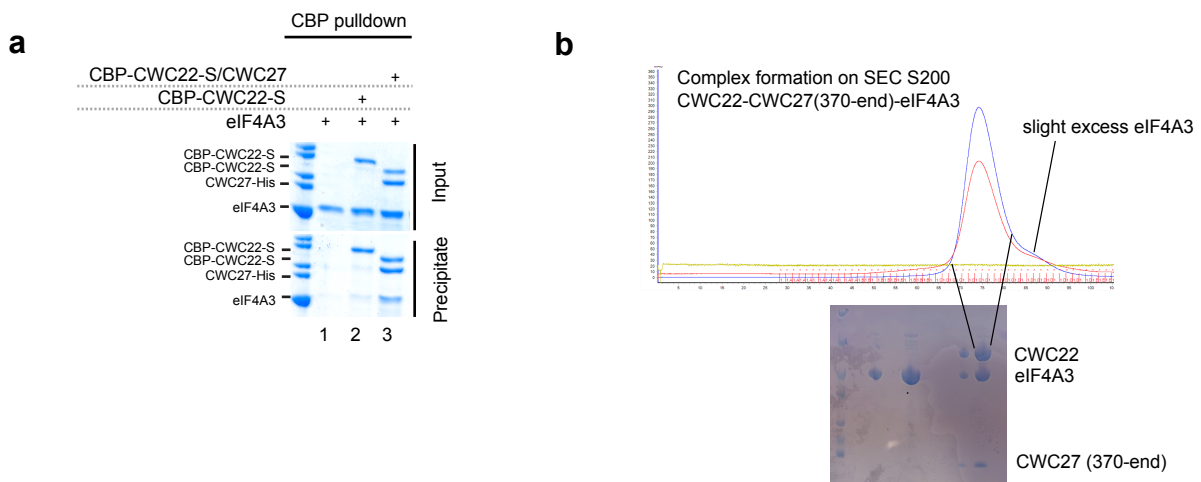


**b**





### Supplementary Figure 3: CWC27 and CWC22 form a complex with eIF4A3



# Supplementary Figure 4: Sequence alignment of CWC27 C-terminal region

**a**

	370	380	390	400
CWC27_H.s./1-472	KQKY	EALR	KQQS.K	KGT
CWC27_M.m./1-469	KQKY	EALR	KQQP.K	KGT
CWC27_G.g./1-477	KRKY	EDKR	KQQP.K	KGV
CWC27_X.l./1-477	KEKY	EEVR	KKNT.N	KGV
CWC27_D.r./1-470	RKKY	DDMR	KQKL.K	KGS
CWC27_D.m./1-502	KEKY	TAKV	KLQP.	KGS
CWC27_C.e./1-483	KMRF	KSKT	KDVK	KQ
CWC27_S.p./1-463	KK.	VVLG	KRNLE	ND
CWC27_S.c./1-301	TESQ	ENIK	EE.	PMD

	370	380	390	400
CWC27_H.s./1-472	OTL	ADL	LDL	NSQ
CWC27_M.m./1-469	OTL	ADL	LDL	ISQ
CWC27_G.g./1-477	OTL	ADL	LDL	DR
CWC27_X.l./1-477	OTL	ADL	LDL	DR
CWC27_D.r./1-470	OTL	ADL	LDL	ES
CWC27_D.m./1-502	OTL	ADL	LDL	ES
CWC27_C.e./1-483	OTL	ADL	LDL	ES
CWC27_S.p./1-463	OTL	ADL	LDL	ES
CWC27_S.c./1-301	OTL	ADL	LDL	ES

\*
\*
\*
\*
\*

Helix
Loop

	410	420	430	440
CWC27_H.s./1-472	D	T	P	.
CWC27_M.m./1-469	S	V	P	.
CWC27_G.g./1-477	E	V	S	.
CWC27_X.l./1-477	E	A	S	.
CWC27_D.r./1-470	D	V	E	.
CWC27_D.m./1-502	D	I	.	.
CWC27_C.e./1-483	E	G	E	E
CWC27_S.p./1-463	N	V	P	.
CWC27_S.c./1-301	.	.	.	.

	410	420	430	440
CWC27_H.s./1-472	E	T	E	.
CWC27_M.m./1-469	A	E	.	.
CWC27_G.g./1-477	E	S	E	.
CWC27_X.l./1-477	D	V	E	.
CWC27_D.r./1-470	E	A	E	.
CWC27_D.m./1-502	D	I	.	.
CWC27_C.e./1-483	E	G	E	E
CWC27_S.p./1-463	N	V	P	.
CWC27_S.c./1-301	.	.	.	.

	410	420	430	440
CWC27_H.s./1-472	D	D	D	D
CWC27_M.m./1-469	S	D	D	D
CWC27_G.g./1-477	E	T	E	.
CWC27_X.l./1-477	E	A	.	.
CWC27_D.r./1-470	D	V	E	.
CWC27_D.m./1-502	D	I	.	.
CWC27_C.e./1-483	E	G	E	E
CWC27_S.p./1-463	N	V	P	.
CWC27_S.c./1-301	.	.	.	.

	410	420	430	440
CWC27_H.s./1-472	S	R	.	.
CWC27_M.m./1-469	T	R	.	.
CWC27_G.g./1-477	S	R	.	.
CWC27_X.l./1-477	S	G	.	.
CWC27_D.r./1-470	S	.	.	.
CWC27_D.m./1-502	T	P	.	.
CWC27_C.e./1-483	S	S	.	.
CWC27_S.p./1-463	T	.	.	.
CWC27_S.c./1-301	.	.	.	.

	410	420	430	440
CWC27_H.s./1-472	R	K	V	K
CWC27_M.m./1-469	D	A	S	M
CWC27_G.g./1-477	Q	D	.	.
CWC27_X.l./1-477	D	.	.	.
CWC27_D.r./1-470	S	.	.	.
CWC27_D.m./1-502	S	.	.	.
CWC27_C.e./1-483	S	.	.	.
CWC27_S.p./1-463	R	.	.	.
CWC27_S.c./1-301	.	.	.	.

	450	460	470
CWC27_H.s./1-472	E	I	Y
CWC27_M.m./1-469	E	I	Y
CWC27_G.g./1-477	E	I	Y
CWC27_X.l./1-477	E	I	Y
CWC27_D.r./1-470	E	I	Y
CWC27_D.m./1-502	D	A	Y
CWC27_C.e./1-483	H	I	T
CWC27_S.p./1-463	K	D	V
CWC27_S.c./1-301	.	.	.

	450	460	470
CWC27_H.s./1-472	P	R	N
CWC27_M.m./1-469	P	R	N
CWC27_G.g./1-477	P	R	N
CWC27_X.l./1-477	P	R	N
CWC27_D.r./1-470	P	R	N
CWC27_D.m./1-502	P	R	N
CWC27_C.e./1-483	P	R	N
CWC27_S.p./1-463	P	R	N
CWC27_S.c./1-301	.	.	.

	450	460	470
CWC27_H.s./1-472	V	N	K
CWC27_M.m./1-469	V	N	K
CWC27_G.g./1-477	V	N	K
CWC27_X.l./1-477	V	N	K
CWC27_D.r./1-470	V	N	K
CWC27_D.m./1-502	V	N	K
CWC27_C.e./1-483	V	N	K
CWC27_S.p./1-463	V	N	K
CWC27_S.c./1-301	.	.	.

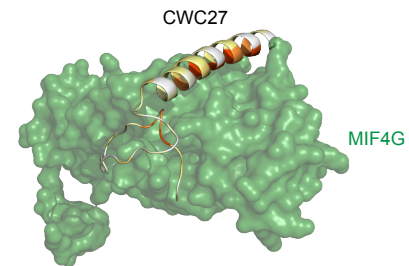
  

	450	460	470
CWC27_H.s./1-472	S	K	K
CWC27_M.m./1-469	S	K	L
CWC27_G.g./1-477	S	K	I
CWC27_X.l./1-477	S	K	I
CWC27_D.r./1-470	S	K	L
CWC27_D.m./1-502	T	G	S
CWC27_C.e./1-483	E	.	.
CWC27_S.p./1-463	.	.	.
CWC27_S.c./1-301	.	.	.

	450	460	470
CWC27_H.s./1-472	L	M	.
CWC27_M.m./1-469	L	.	.
CWC27_G.g./1-477	L	.	.
CWC27_X.l./1-477	L	.	.
CWC27_D.r./1-470	L	.	.
CWC27_D.m./1-502	L	.	.
CWC27_C.e./1-483	L	.	.
CWC27_S.p./1-463	L	.	.
CWC27_S.c./1-301	.	.	.

**b**



more conserved  less conserved

## Supplementary Figure 5: Impacts of CWC27 and CWC22 KD on gene expression in RPE-1 cells

