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Supplemental information

Unannotated microprotein EMBOW regulates the interactome and chromatin and mitotic functions of WDR5

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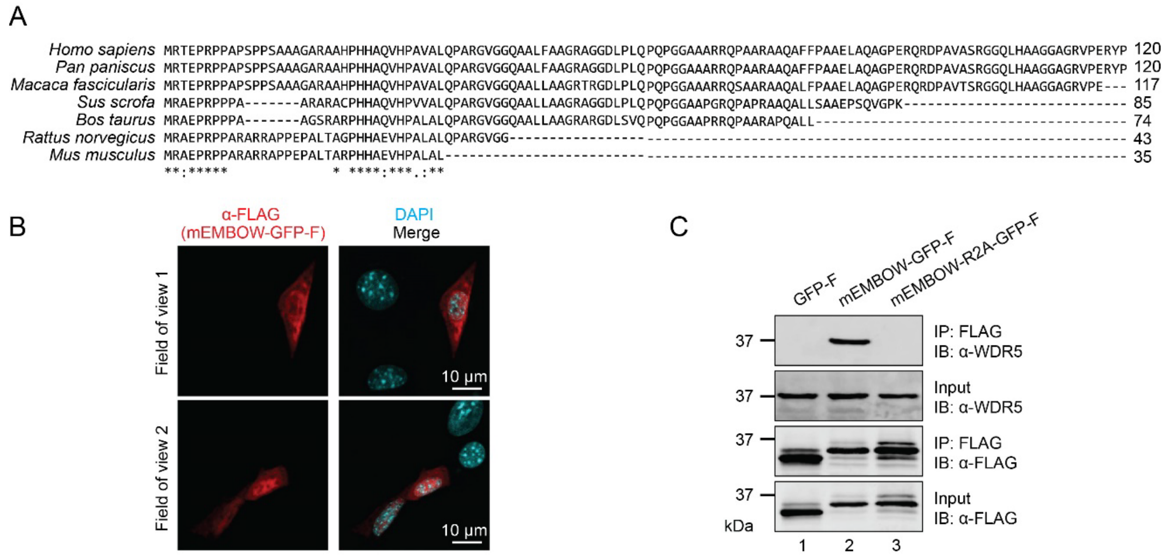

Figure S1. EMBOW is conserved in mice. Related to Figure 1 and 2.

(A) *SCRIB* mRNAs from *Homo sapiens* (NM_182706.5), *Pan paniscus* (XM_034966823.1), *Macaca fascicularis* (XM_005564272.2), *Sus scrofa* (XM_021090546.1), *Bos taurus* (XM_025001931.1), *Rattus norvegicus* (NM_001191879) and *Mus musculus* (NM_134089.2) were obtained from the NCBI nucleotide database and translated in the +1, +2 and +3 frames using the Expasy translate tool to identify upstream ORFs. The full-length sequences of hypothetical EMBOW homologs were predicted in each transcript and aligned with ClustalW2.

(B) 3T3 cells transfected with a C-terminal GFP-FLAG dual tagged mouse EMBOW (mEMBOW-GFP-F) expression construct were immunostained with anti-FLAG (red) and DAPI (cyan); scale bar, 10 μm. Data are representative of three biological replicates.

(C) HEK 293T cells were transfected with GFP-FLAG (GFP-F, lane 1), wild-type (mEMBOW-GFP-F, lane 2) or R2A mutated (mEMBOW-R2A-GFP-F, lane 3) mEMBOW-GFP-FLAG, and FLAG IP was performed, followed by IB. Data are representative of three biological replicates.

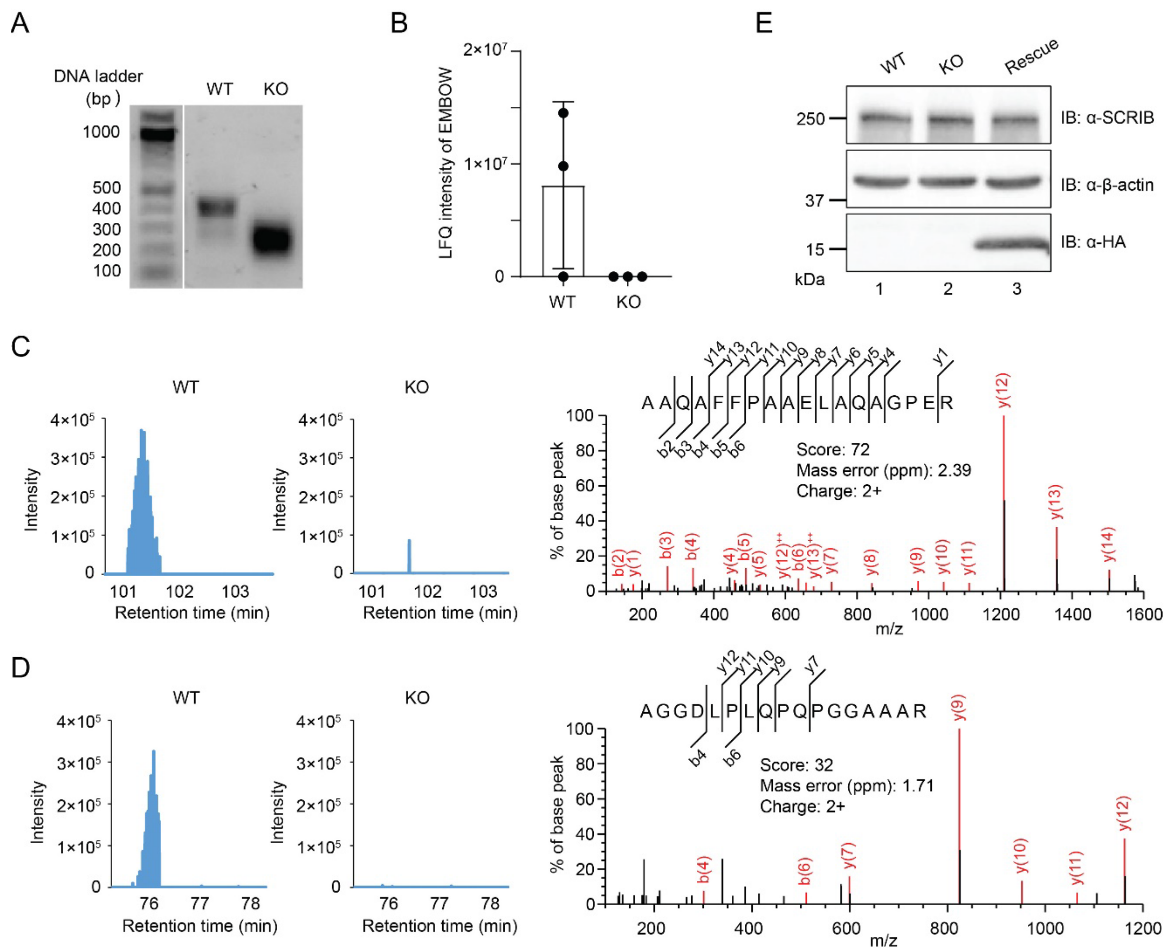

Figure S2. Validation of the EMBOW knock-out HEK 293T cell line. Related to Figure 3.

(A) Genomic DNA PCR and agarose gel electrophoresis of wild-type (WT) or EMBOW KO (KO) HEK 293T cells.

(B) Label-free quantitation (LFQ, *N* = 3 biologically independent experiments) of endogenous EMBOW co-purifying with WDR5-FLAG in an anti-FLAG co-IP from wild-type (WT) or EMBOW KO (KO) HEK 293T cells stably expressing WDR5-FLAG. Data represent mean values ± s.e.m..

(C-D) Shown are MS1 extracted ion chromatograms (EICs, left) corresponding to MS/MS spectra (right) of two EMBOW tryptic peptides detected in WT HEK 293T cells. EIC analysis for each EMBOW peptide was performed in both WT and EMBOW KO HEK 293T cells.

(E) Western blot analysis of SCRIB expression in wild-type (WT), EMBOW knock-out (KO) and rescue (KO stably expressing EMBOW-FLAG-HA) HEK 293T cells with the indicated antibodies. Data are representative of three biological replicates.

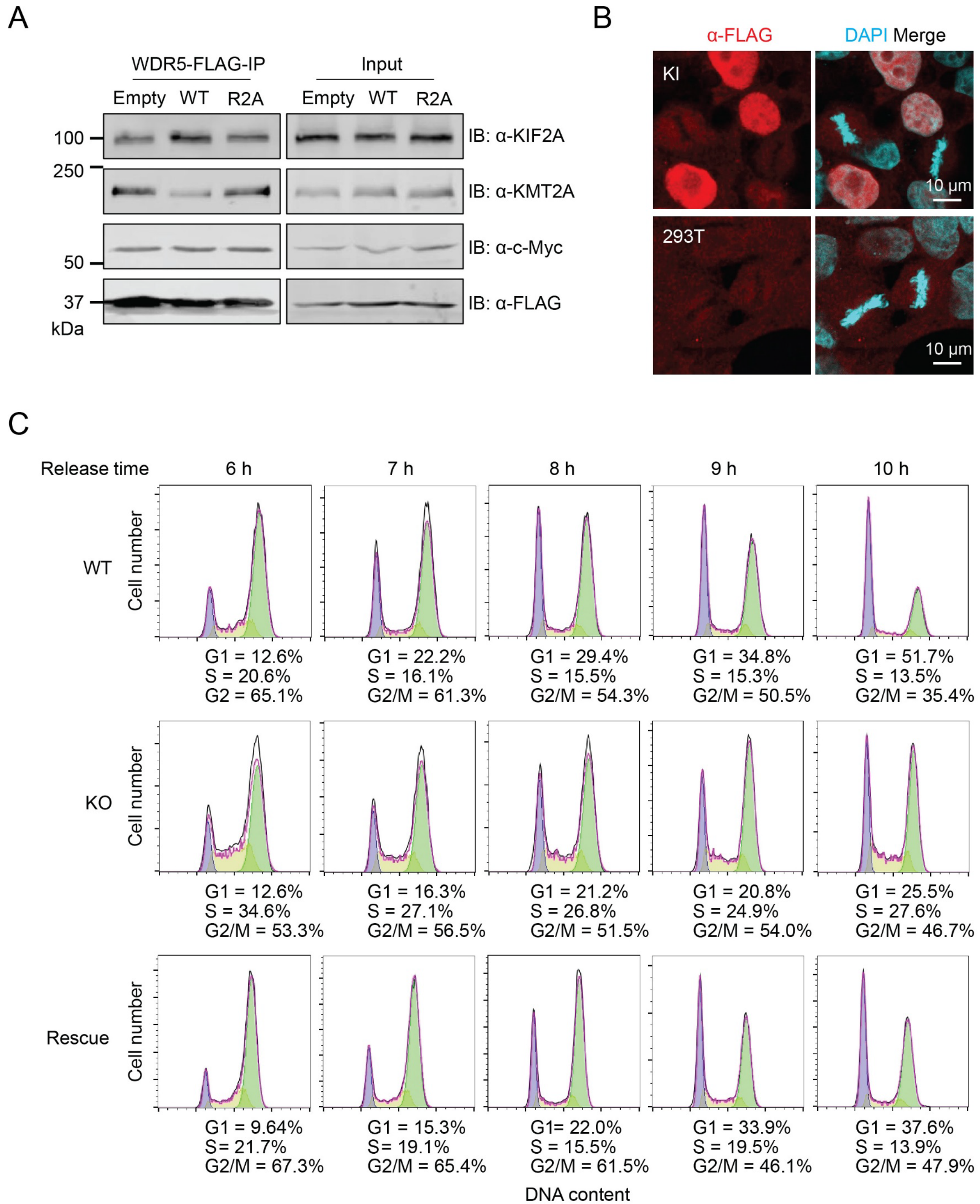

Figure S3. EMBOW does not regulate the interaction between WDR5 and MYC, and loss of EMBOW prolongs G2/M phase. Related to Figures 3 and 4.

(A) HEK 293T cells stably expressing WDR5-FLAG were transfected with pcDNA 3.1 empty vector (Empty), wild-type (WT) or R2A mutated (R2A) EMBOW-myc, followed with WDR5-FLAG IP and immunoblotting with antibodies indicated on the right. Cell

lysates (4%) before IP (input) were used as loading controls. Data are representative of two biological replicates.

(B) Immunostaining of EMBOW-FLAG-HA KI cells (KI) and HEK 293T cells (293T) as a control with anti-FLAG (red) and DAPI (cyan). Scale bar, 10 μm. Data are representative of three biological replicates.

(C) Flow cytometry analysis of synchronized wild-type (WT, top panel), EMBOW knock-out (KO, middle panel) and rescue (KO stably expressing EMBOW-FLAG-HA, bottom panel) HEK 293T cells released from the G1/S boundary. Shown are cells released from 6-hour to 10-hour time point. The time points after release are indicated at the top, and the cell cycle distributions are quantified below. Data are representative of three biological replicates.

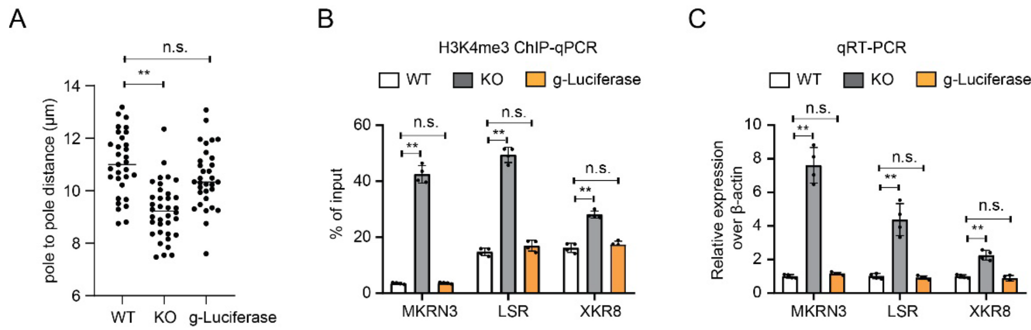

Figure S4. Loss of EMBOW regulates spindle formation and off-target genes independent of Cas9 expression. Related to Figures 4 and 6.

(A) Quantitation of the pole-to-pole distances of parental HEK 293T (WT), EMBOW KO (KO), or HEK 293T transient transfected with gRNA plasmid targeting luciferase (g-luciferase) via KIF2A immunostaining, totaling > 30 cells for each measurement. Data represent mean values ± s.e.m., and significance was evaluated by one-way ANOVA (Dunnett's test). ** $p < 0.01$, n.s., not significant.

(B-C) H3K4me3 ChIP-qPCR (B) or quantitative RT-PCR (qRT-PCR, C) results of three *de novo* target genes in the three cell lines described above. Data represent mean values ± s.e.m.; $N = 4$ biologically independent samples. Significance was evaluated with one-way ANOVA (Dunnett's test). ** $p < 0.01$, n.s., not significant.

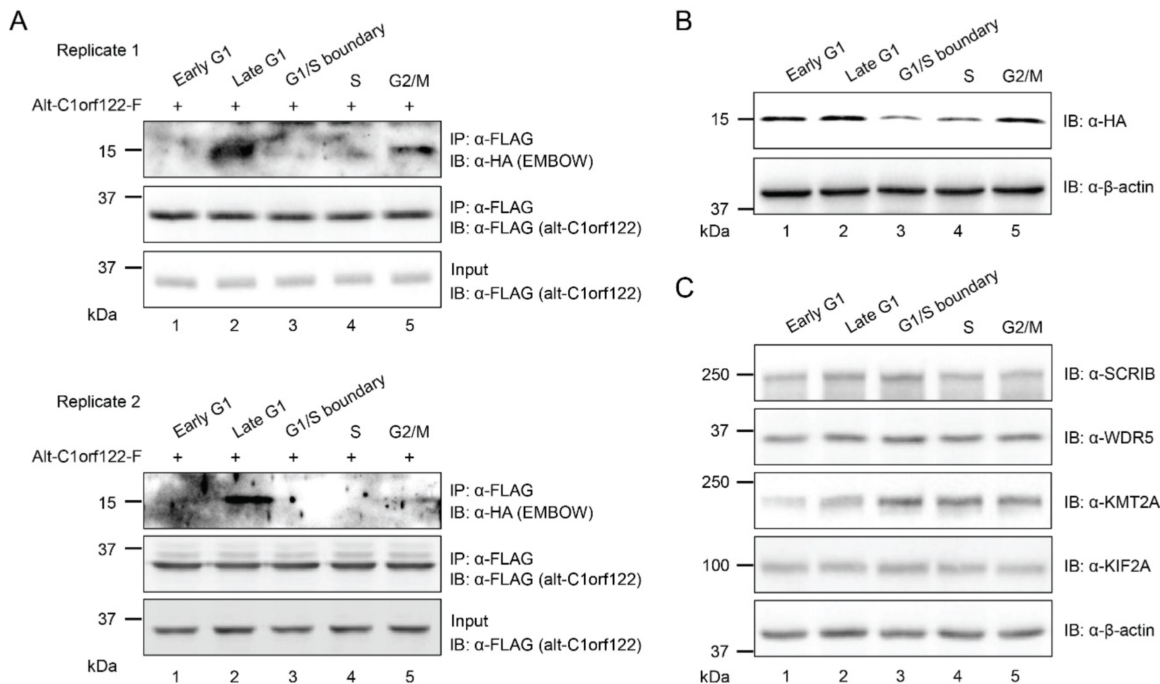

Figure S5. EMBOW is cell-cycle regulated. Related to Figures 4-6.

(A) EMBOW-FLAG-HA knock-in (KI) HEK 293T cells were transiently transfected with a plasmid encoding alt-C1orf122-FLAG, serving as a FLAG immunoprecipitation (IP) positive control, and cells were synchronized and released from G2/M phase (lanes 1 and 2, release time point 3 h and 8 h, respectively), or G1/S boundary (lanes 3-5, release time point 0 h, 3 h and 7 h, respectively), followed by FLAG IP and immunoblotting (IB). Cell lysates (4%) before IP (input) were used as the loading controls. Shown are two biological replicates.

(B) Synchronized HEK 293T cells stably expressing EMBOW-FLAG-HA were released from G2/M phase (lanes 1 and 2, release time point 3 h and 8 h, respectively), or G1/S boundary (lanes 3-5, release time point 0 h, 3 h and 7 h, respectively), followed by immunoblotting with antibodies indicated on the right. Data are representative of three biological replicates.

(C) HEK 293T cells were released from G2/M phase (lanes 1 and 2, release time point 3 h and 8 h, respectively), or G1/S boundary (lanes 3-5, release time point 0 h, 3 h and 7 h, respectively), followed by immunoblotting with antibodies indicated on the right. Data are representative of three biological replicates.

Rank	Log_2 (LFQ intensity EMBOW-FH/293T)	- Log_{10} (*p*-value)	Gene name	Description
1	31.61	6.40	EMBOW	Unannotated microprotein EMBOW
2	27.06	6.20	WDR5	WD repeat-containing protein 5
3	24.48	6.09	SNRPD3	Small nuclear ribonucleoprotein Sm D3
4	23.99	5.87	RPL13	60S ribosomal protein L13
5	23.82	5.83	BOLA2	BolA-like protein 2. Acts as a cytosolic iron-sulfur (Fe-S) cluster assembly factor.
6	23.80	6.02	RBM8A	RNA-binding protein 8A
7	23.23	5.88	RPS23	40S ribosomal protein S23
8	22.88	5.89	LIMA1	LIM domain and actin-binding protein 1
9	22.81	5.94	JUP	Junction plakoglobin. Common junctional plaque protein.
10	22.76	5.94	DSC1	Desmocollin-1, component of intercellular desmosome junctions.
11	22.24	5.94	CAMSAP3	Calmodulin-regulated spectrin-associated protein 3, key microtubule-organizing protein.

Table S1 | List of proteins enriched by EMBOW co-immunoprecipitation. Related to Figure 2.

Shown are top 10 proteins enriched by EMBOW identified by quantitative LC-MS/MS analysis with $-Log_{10}$ (*p*-value) > 1.0 (two sample *t*-test evaluated with Perseus).

Gene name	Log_2 (LFQ intensity OE/Empty)	- Log_{10} (*p*-value)
CXXC1	-0.2676	1.45264
KMT2A	-0.2118	1.0525
RBBP5	-0.1885	0.88717
KMT2B	-0.1755	0.571822
KMT2C	-0.0728	0.361724
KMT2D	-0.03627	0.130469
DPY30	0.195867	0.318485

Table S2 | Enriched fold-changes and significance of other KMT2 or WRAD complex members upon EMBOW overexpression. Related to Figure 3. Significance was evaluated with Perseus (two sample *t*-test). Statistically significant changes were defined as $-\log_{10}$ (p value) ≥ 1.

qPCR primers (5' - 3')			
Target genes	Use	Forward primer	Reverse primer
RPL5	ChIP-qPCR	CCTTTTCCCACCCCCTAGC	CCATGCATCTAAAGCCCCGA
RPL35	ChIP-qPCR	CTCGCCATCGACTACGAGTG	CTGGGTTACTCTGCCCAAGG
RPS24	ChIP-qPCR	TGAGCTATAGGCACGCGAAG	CCCTCGACAACCATCCTCTG
MKRN3	ChIP-qPCR	GCGCTTCTAACACAGTGGGA	TCACCAGCAAGGCATTAGCA
LSR	ChIP-qPCR	CGTCTTCGTGTGGCTTCTGC	ACTCCATCCATCCCCCTCTAC
XKR8	ChIP-qPCR	AATGTGCCTTTGCGTTTCGT	TTTTACCTTTGAGGCGCCCG
RPL5	qRT-PCR	GCCTTGCCAGAACTACCACT	TTCTGGCCCATGATGTGCTT
RPL35	qRT-PCR	CGAGTCGTCCGGAAATCCAT	CAGGTTCTCCTCGTGCTTGT
RPS24	qRT-PCR	ATGAGGGAATTGGGGCTTGG	TTCTACCTGCCACACAACCC
MKRN3	qRT-PCR	CAGCGTGGTATGGACAAGGT	TCCACCTGCGGATACACCTA
LSR	qRT-PCR	TCACCGGAAATGCTGACCTG	GAAGAGCCAGTCAAGGACGAT
XKR8	qRT-PCR	AGGAGGAGCCCTCTGAGTTT	GATGCCAACCCACTGGTAGT
SCRIB	qRT-PCR	TGCCCTTGTCTGGAAAGAAGT	TCAGCAAAGTCCGGTGACTG
β-actin	qRT-PCR	AGGCACCAGGGCGTGAT	GCCCACATAGGAATCCTTCTGAC

Table S3 | qPCR primers (5' - 3'). Related to Figures 5 and 6.