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2 **Figure S1. Validation of EMC2 Gene Manipulation**

3 **A-B.** Quantitative RT-PCR validation of EMC2 mRNA expression following plasmid-mediated

4 knockdown or overexpression.

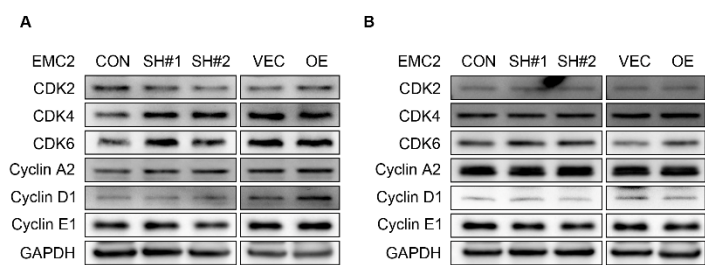
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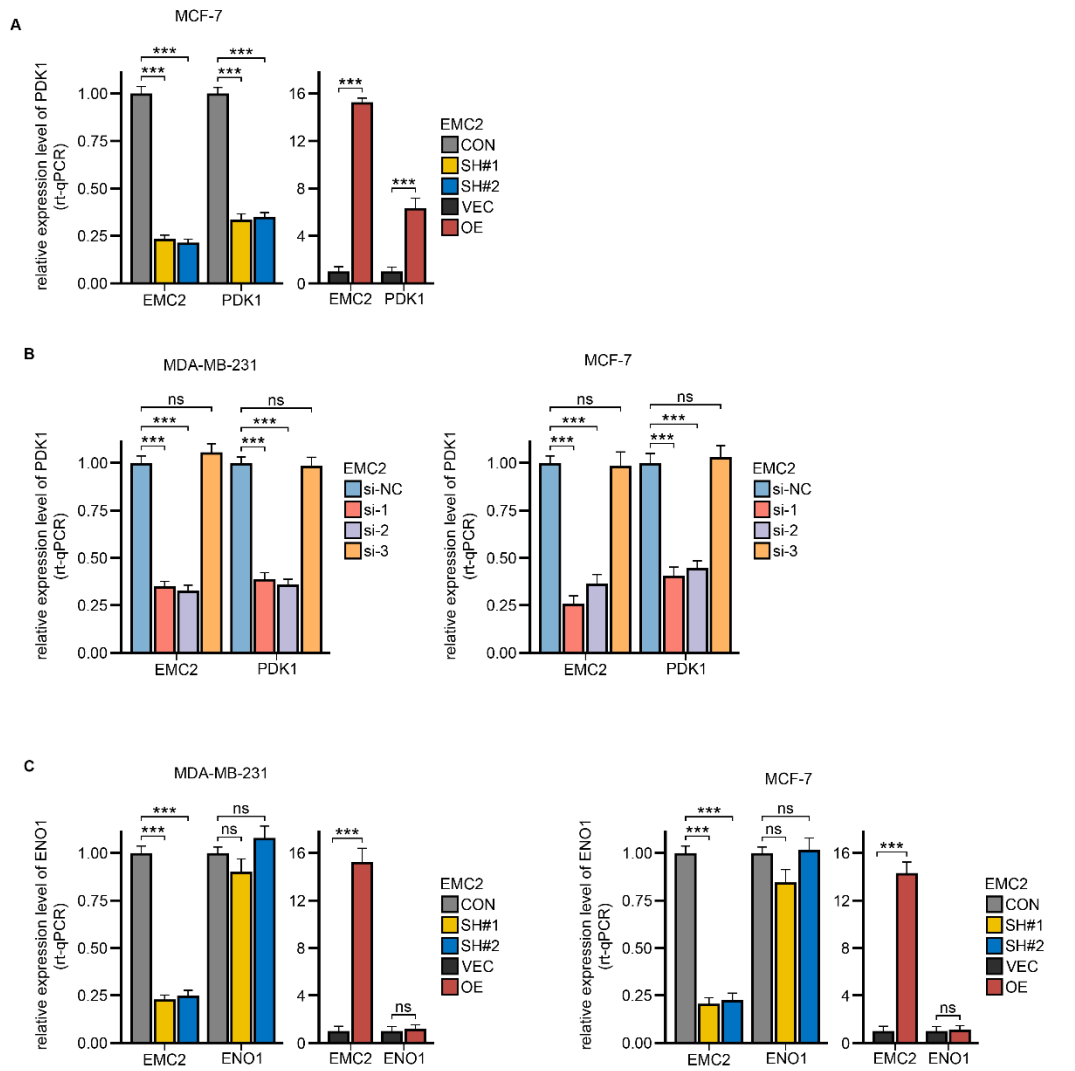
11 **Figure S2. Additional Cell Cycle Analysis**

12 A-B. Western blot analysis of cell cycle checkpoint proteins.

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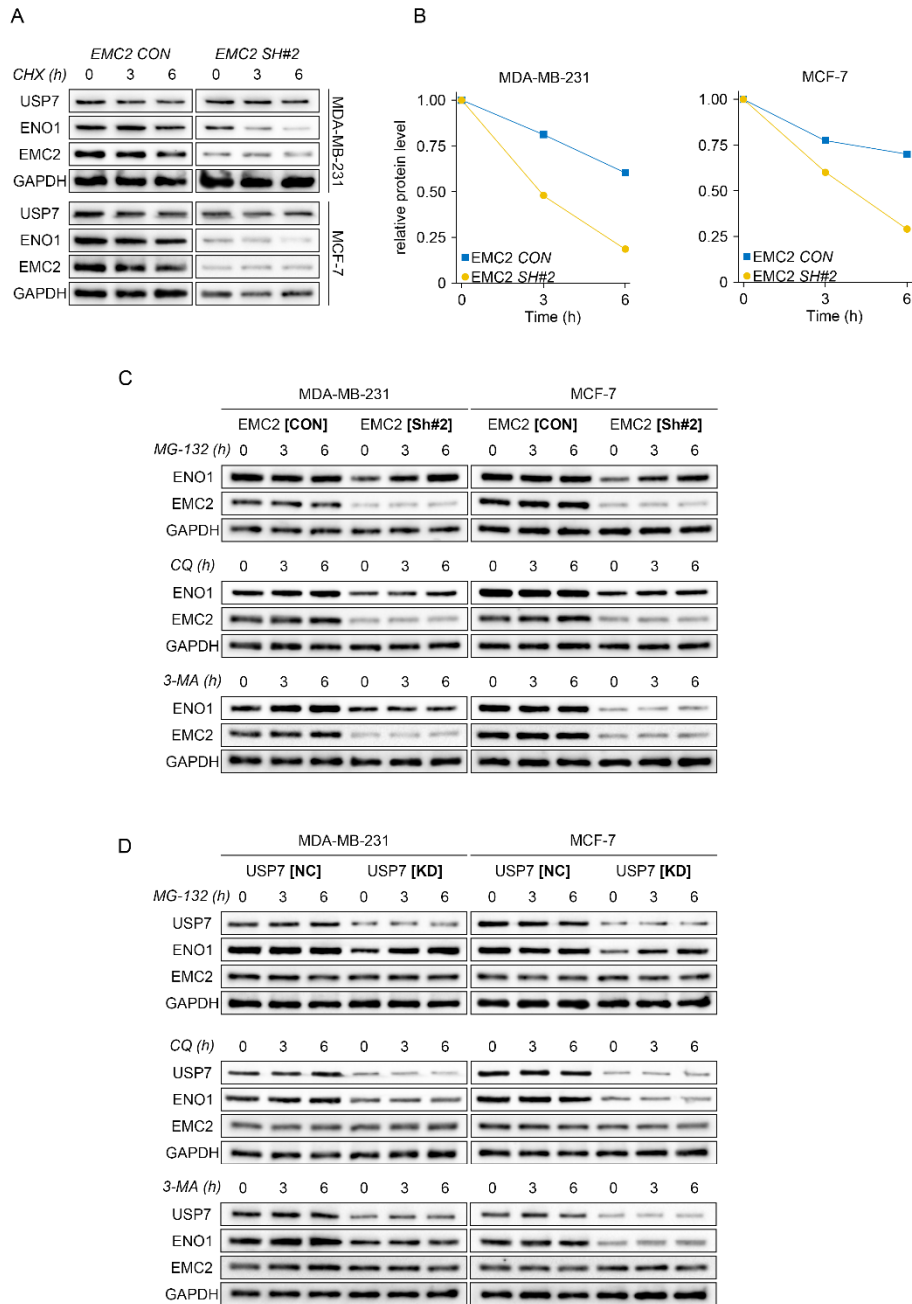
17 **Figure S3. Additional Expression Analysis**

18 **A.** qRT-PCR analysis of PDK1 expression following EMC2 knockdown or overexpression.

19 **B.** qRT-PCR analysis of PDK1 expression following transient EMC2 knockdown using siRNA.

20 **C.** qRT-PCR analysis of ENO1 expression following EMC2 knockdown or overexpression.

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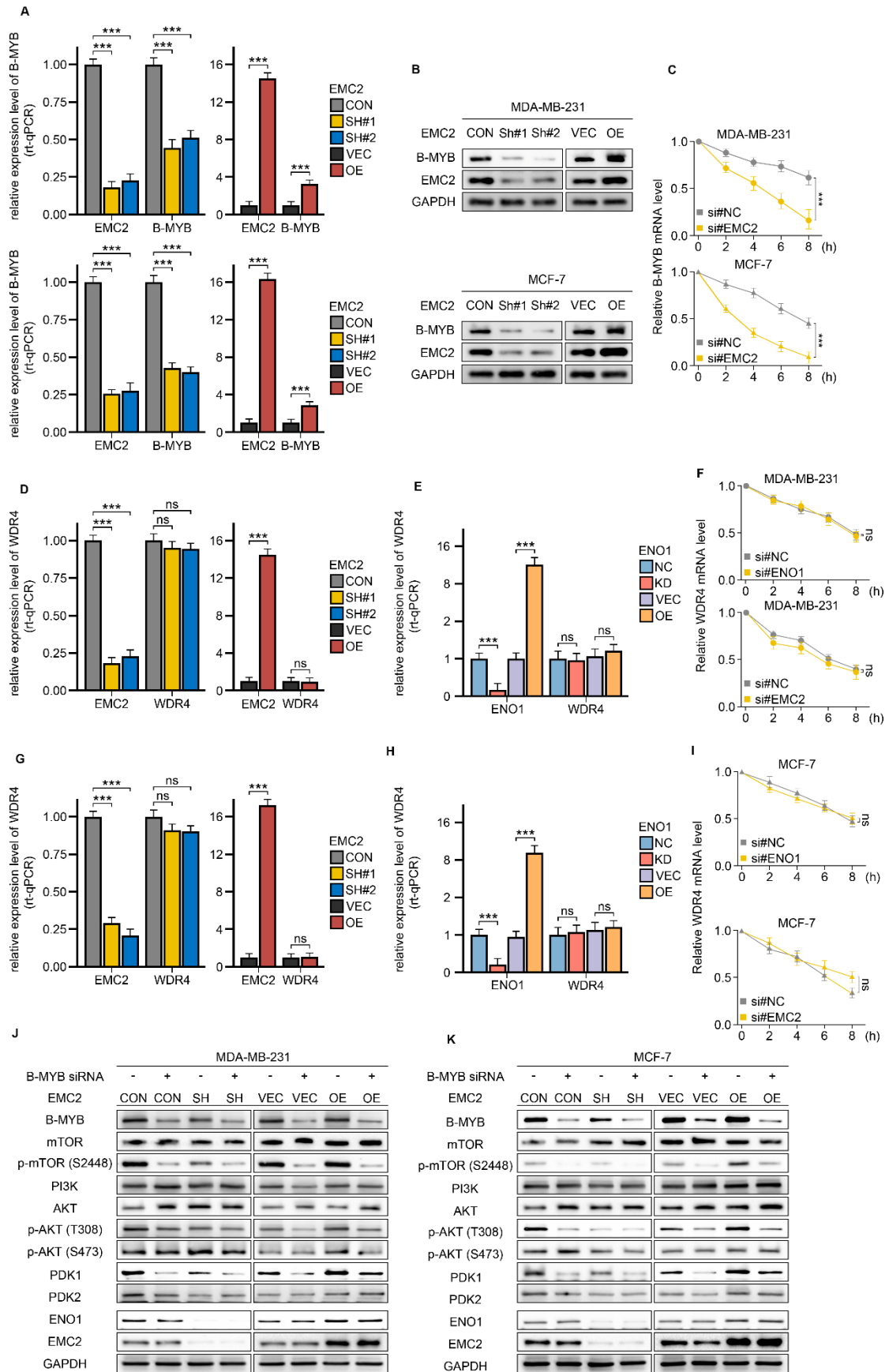
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23 **Figure S4. ENO1 protein Half-life assay and Degradation inhibit assay**

24 **A-B.** Protein stability analysis using cycloheximide chase assay (CHX, 50 $\mu\text{g/mL}$) at 0, 3, and 6
 25 hours.

26 **C-D.** ENO1 protein levels following treatment with protein degradation inhibitors:

- 27 • MG-132 (proteasome inhibitor, 10 μ M)
- 28 • Chloroquine (CQ, lysosomal inhibitor, 50 μ M)
- 29 • 3-Methyladenine (3-MA, autophagy inhibitor, 10 mM)



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32 **Figure S5. ENO1-Mediated Regulation of B-MYB and PDK1**

33 **A.** B-MYB expression following EMC2 knockdown or overexpression (qRT-PCR).

34 **B.** B-MYB expression following EMC2 knockdown or overexpression (Western blot).

35 **C.** B-MYB mRNA stability analysis after treating with Actinomycin D (ActD, 5 μ M) at 0, 2, 4, 6,
36 and 8 hours.

37 **D.** WDR4 expression following EMC2 knockdown or overexpression (qRT-PCR; MDA-MB-231).

38 **E.** WDR4 expression following ENO1 knockdown or overexpression (qRT-PCR; MDA-MB-231).

39 **F.** WDR4 mRNA stability analysis after treating with Actinomycin D (ActD, 5 μ M) at 0, 2, 4, 6, and
40 8 hours (MDA-MB-231).

41 **G.** WDR4 expression following EMC2 knockdown or overexpression (qRT-PCR; MCF-7).

42 **H.** WDR4 expression following ENO1 knockdown or overexpression (qRT-PCR; MCF-7).

43 **I.** WDR4 mRNA stability analysis after treating with Actinomycin D (ActD, 5 μ M) at 0, 2, 4, 6, and
44 8 hours (MCF-7).

45 **J.** PDK1 and ENO1 expression following B-MYB knockdown (Western blot; MDA-MB-231).

46 **K.** PDK1 and ENO1 expression following B-MYB knockdown (Western blot; MCF-7).

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