

Supplementary Materials: Phosphorylation of OGFOD1 by Cell Cycle-Dependent Kinase 7 Enhances the Transcriptional Activity of RNA Polymerase II in Breast Cancer Cells

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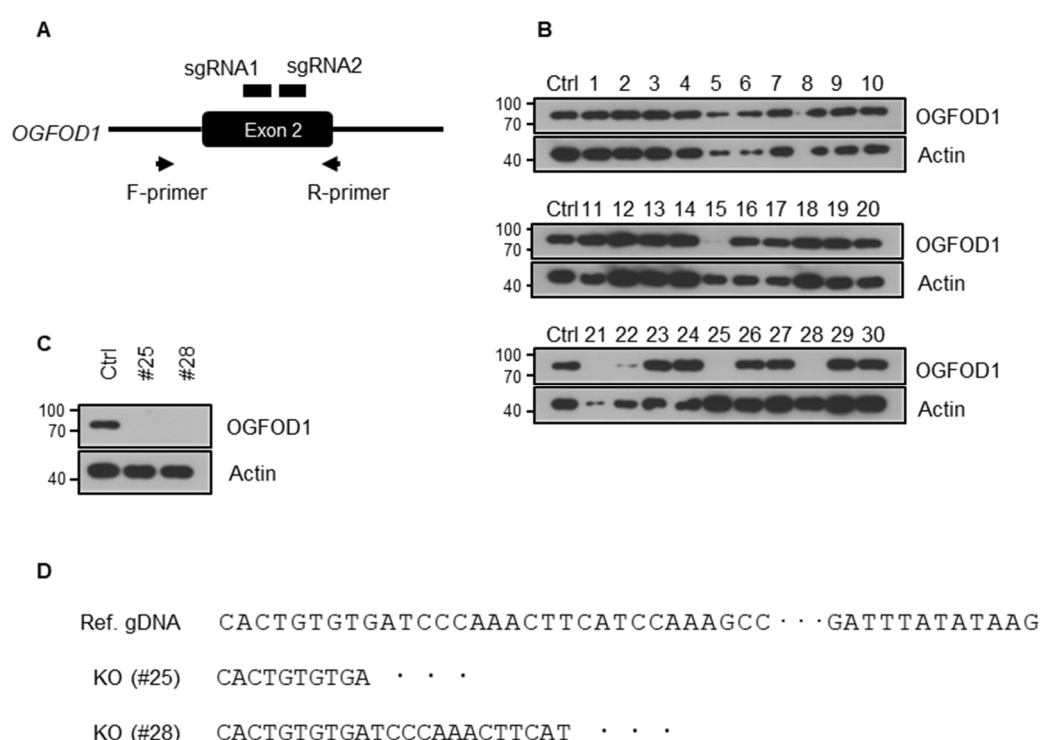


Figure S1. Knockout of OGFOD1 in MDA-MB-231 by CRISPR/Cas9 technology. **(A)** a model of OGFOD1 knockout strategy. sgRNAs show target sites of guide RNA. Each of sites was digested using nickase Cas9 (D10A) enzyme. F-primer and R-primer are primer sets for DNA amplification to analysis the deletion sequence. **(B,C)** The screening results of OGFOD1 knockout. Protein levels were confirmed by western blot assay. Actin were used as control. **(D)** Sanger sequencing results in knockout #25 and #28 cell lines.

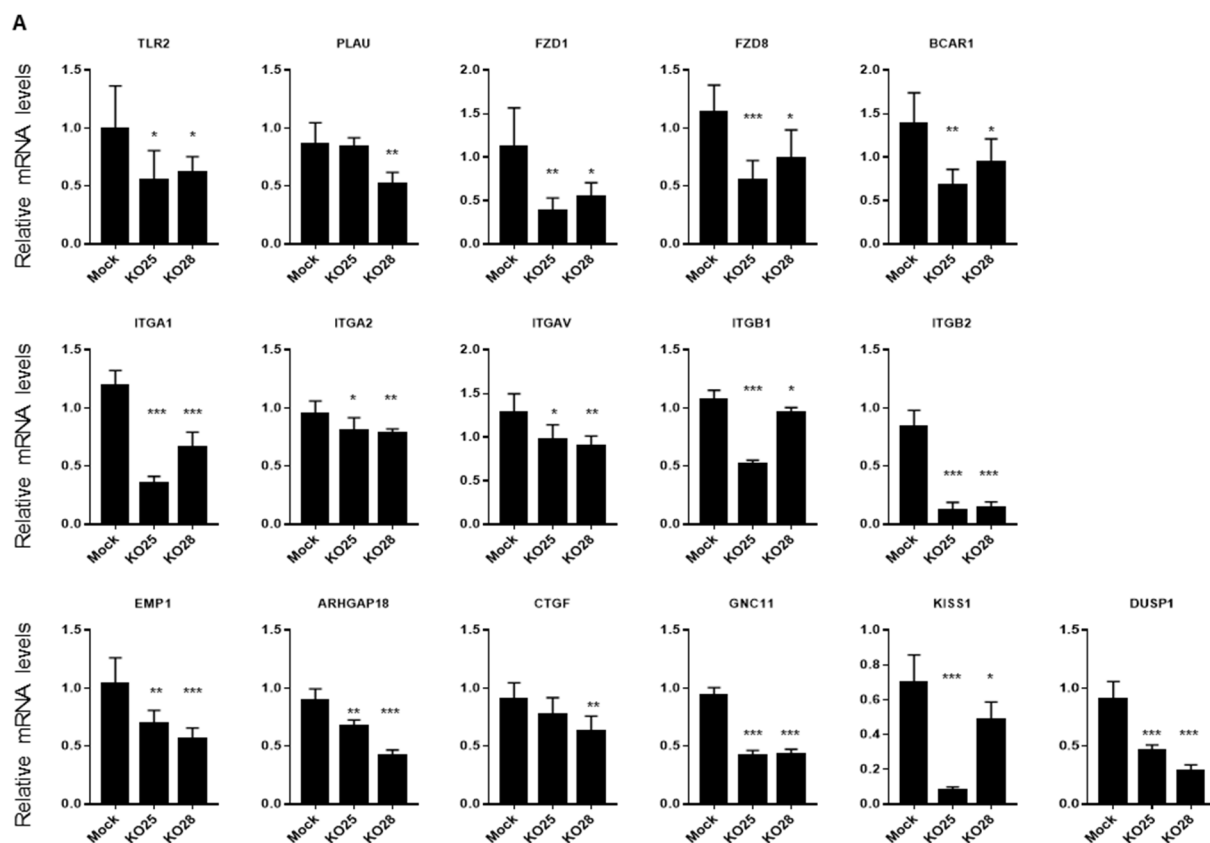


Figure S2. Altered genes by OGFOD1 KO in MDA-MB-231. (A) mRNA expression of target genes. Relative mRNA levels were analyzed by qRT-PCR. In comparison with Mock cells, OGFOD1 KO cells exhibits decreased mRNA levels. Statistical data were presented as mean \pm SD. Two-tailed unpaired student t-test was performed for statistical analysis. ($n = 6$), (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

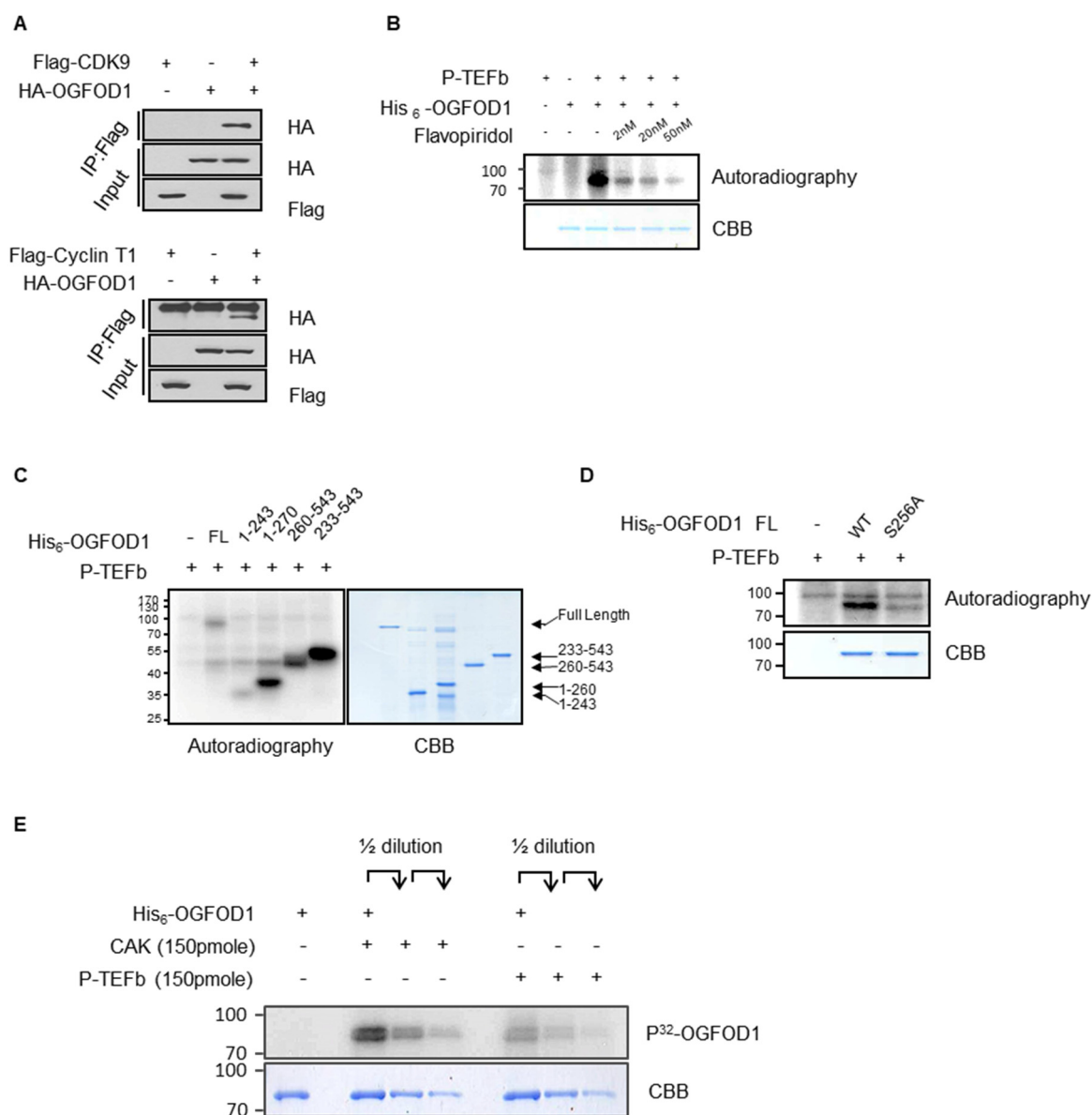


Figure S3. P-TEFb phosphorylates serine 256 residue of OGFOD1 in vitro. **(A)** P-TEFb complex (CDK9, Cyclin T1) was transiently transfected in HEK293T with or without OGFOD1. Immunoprecipitation was performed by anti-Flag. **(B)** *In vitro* P-TEFb phosphorylation assay. Recombinant P-TEFb and OGFOD1 proteins were used. Flavopiridol was used as an inhibitor of kinase assay. OGFOD1 protein amounts were confirmed by coomassie brilliant blue (CBB). **(C)** *In vitro* P-TEFb phosphorylation assay with truncated OGFOD1. 100ng of P-TEFb was used for *in vitro* phosphorylation assay. **(D)** Radioactive kinase assay of wild type or S256A OGFOD1. Kinase assay was done with same amount of wild type or mutant OGFOD1. Input proteins were verified with coomassie brilliant blue staining. **(E)** *In vitro* phosphorylation assay of CDK7 and CDK9 with serial dilution of OGFOD1 proteins.

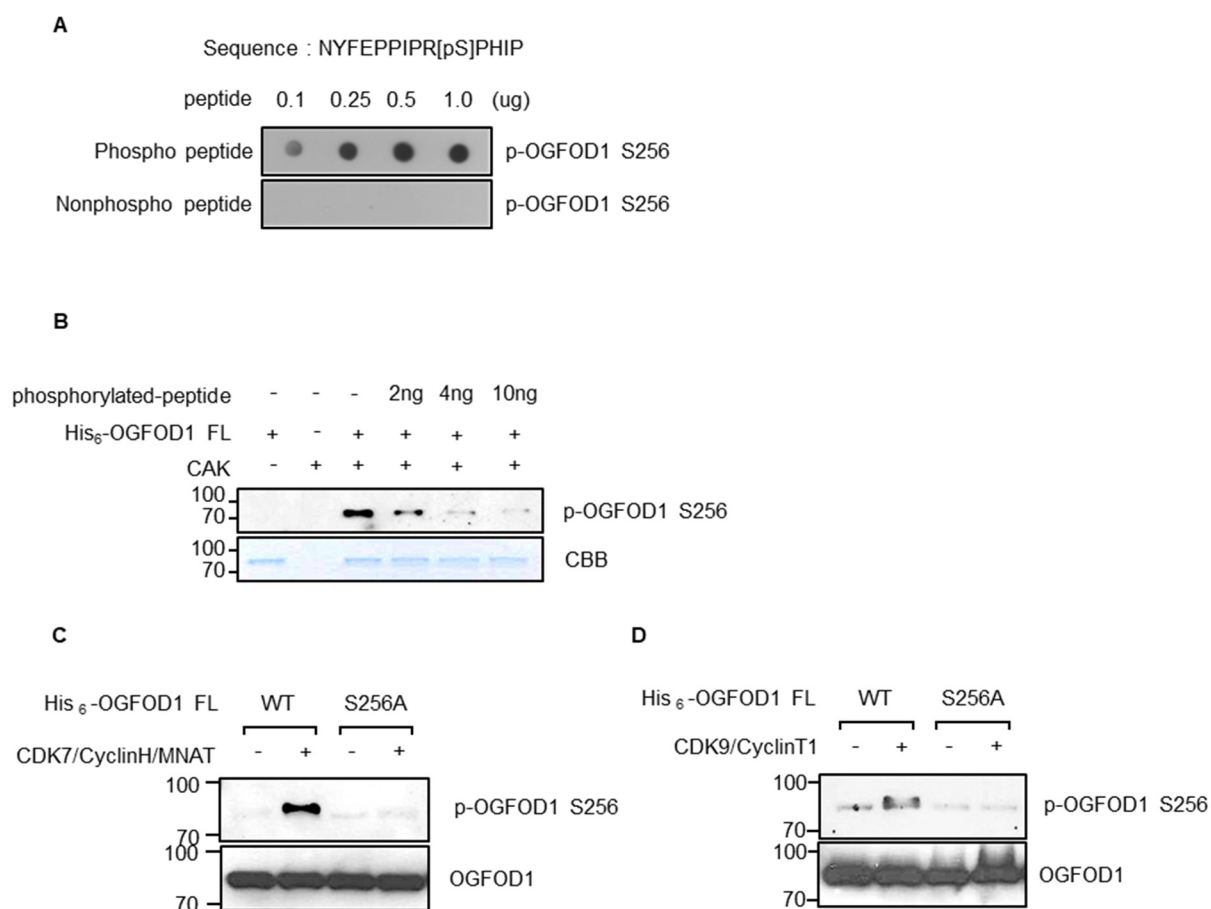


Figure S4. The generation of customized anti-phosphor serine 256 OGFOD1 antibody. **(A)** Dot blot assay. Anti-phosphor serine 256 OGFOD1 antibody was generated against NYFEPPIR[pS]PHIP. Phosphor or non-phosphor peptide was blotted on nitrocellulose membrane with indicated amounts. Dot blot assay was conducted to confirm its specificity. **(B)** Peptide competition assay. Recombinant OGFOD1 was phosphorylated by CAK and separated by SDS-PAGE. Phosphor peptide was treated with primary antibody. **(C)** *In vitro* CAK phosphorylation. Recombinant OGFOD1 was phosphorylated by CAK complex. Immunoblotting was performed using p-OGFOD1 S256 antibody. **(D)** *In vitro* P-TEFb phosphorylation. Recombinant OGFOD1 was phosphorylated by CAK complex. Immunoblotting was performed using p-OGFOD1 S256 antibody.

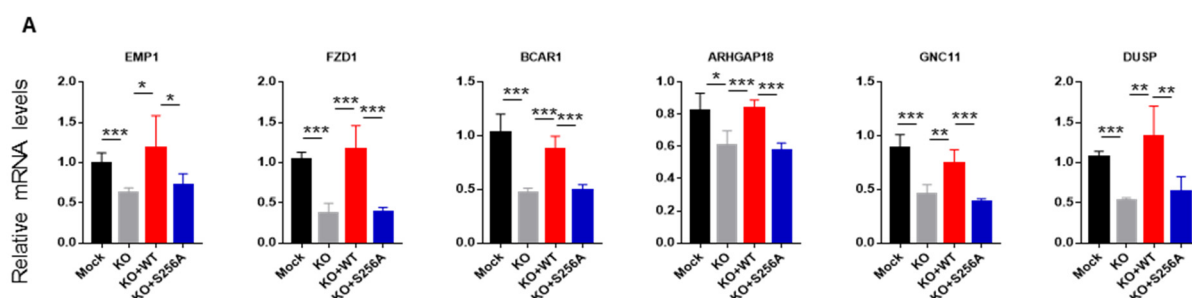


Figure S5. Altered gene expression levels in Mock, OGFOD1 KO, KO+WT, KO+S256A. **(A)** mRNA expression of target genes in OGFOD1 rescued cells. Statistical data were presented as mean \pm SD. Two-tailed unpaired student t-test was performed for statistical analysis. ($n = 5$), (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

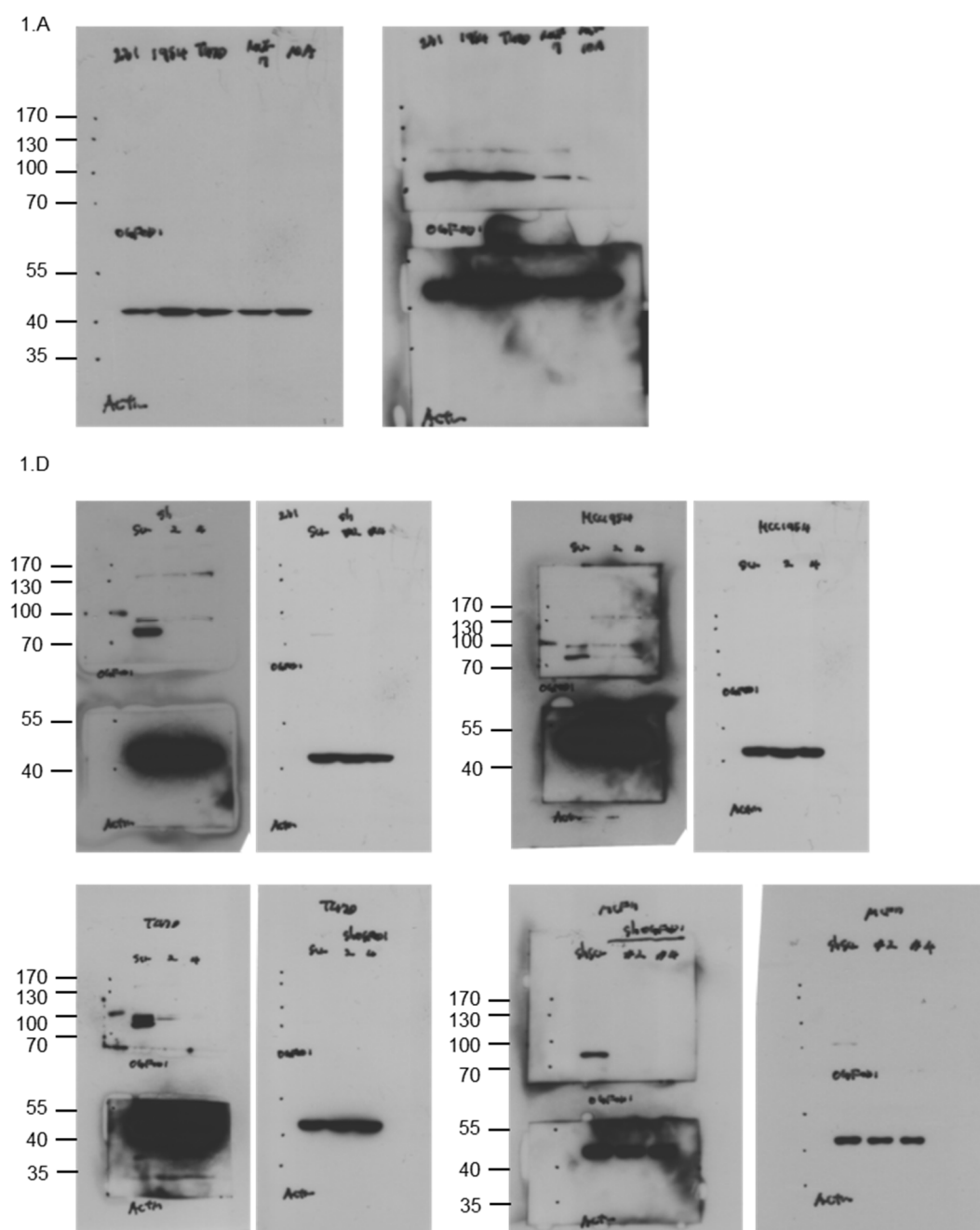
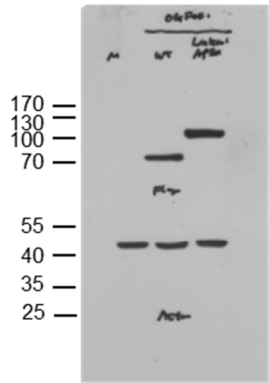
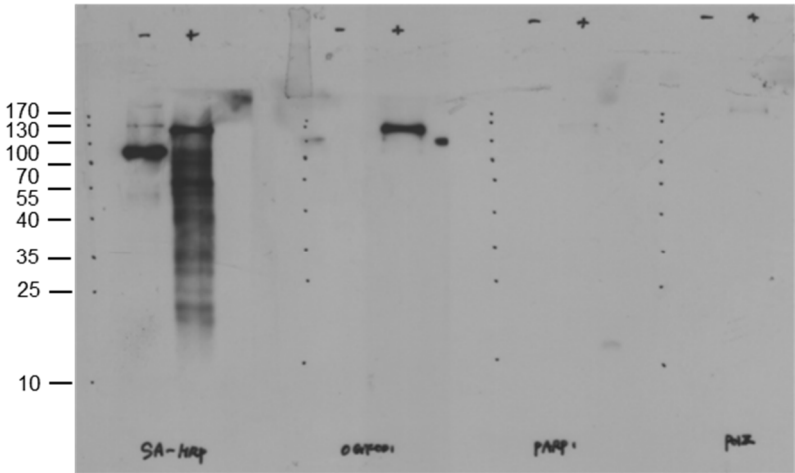


Figure S6. Full western blot of Figure 1.

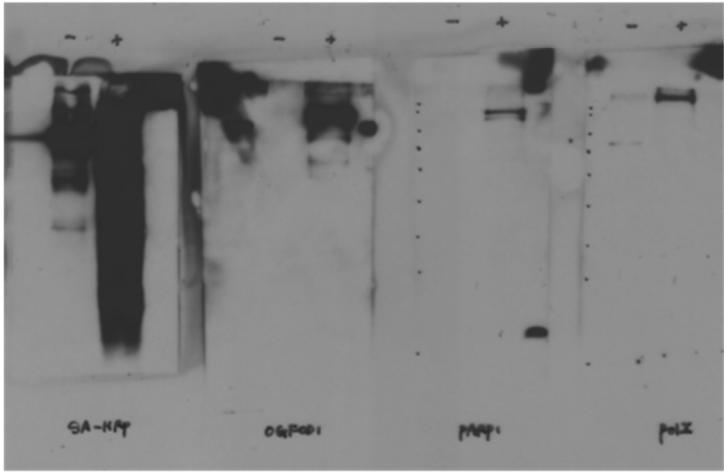
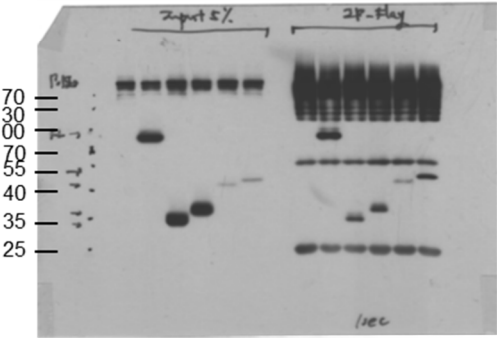
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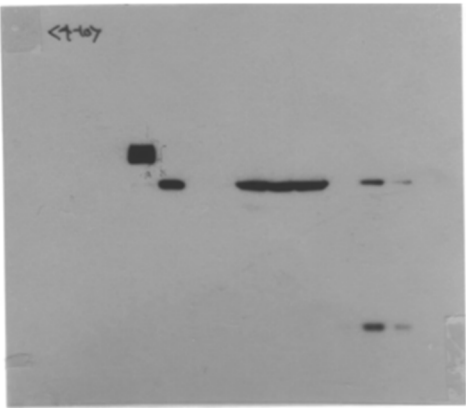
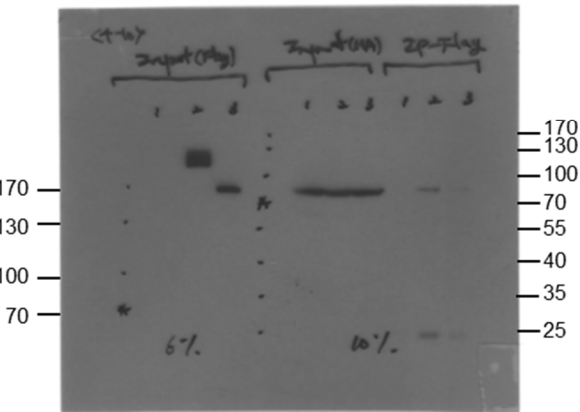
2.D



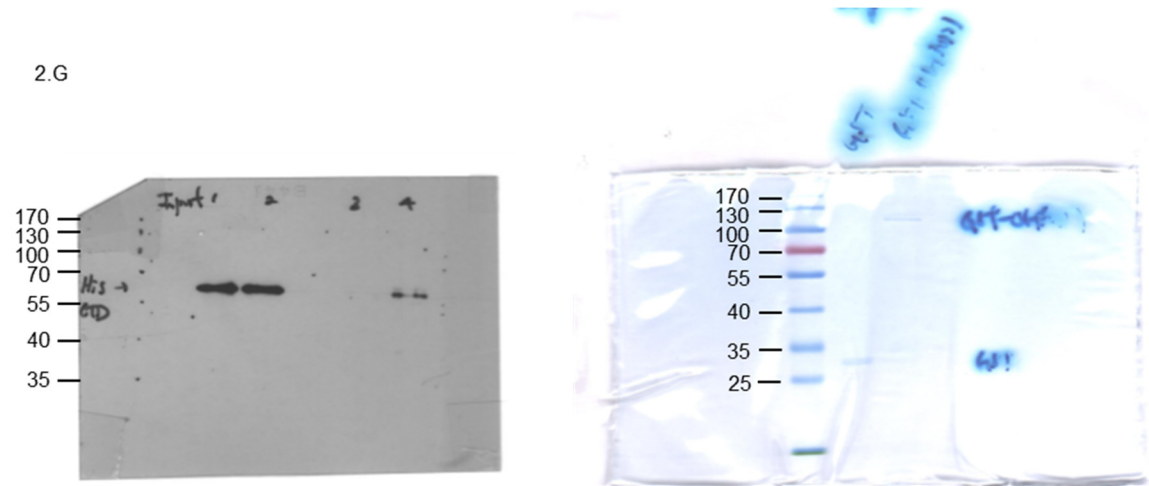
2.E



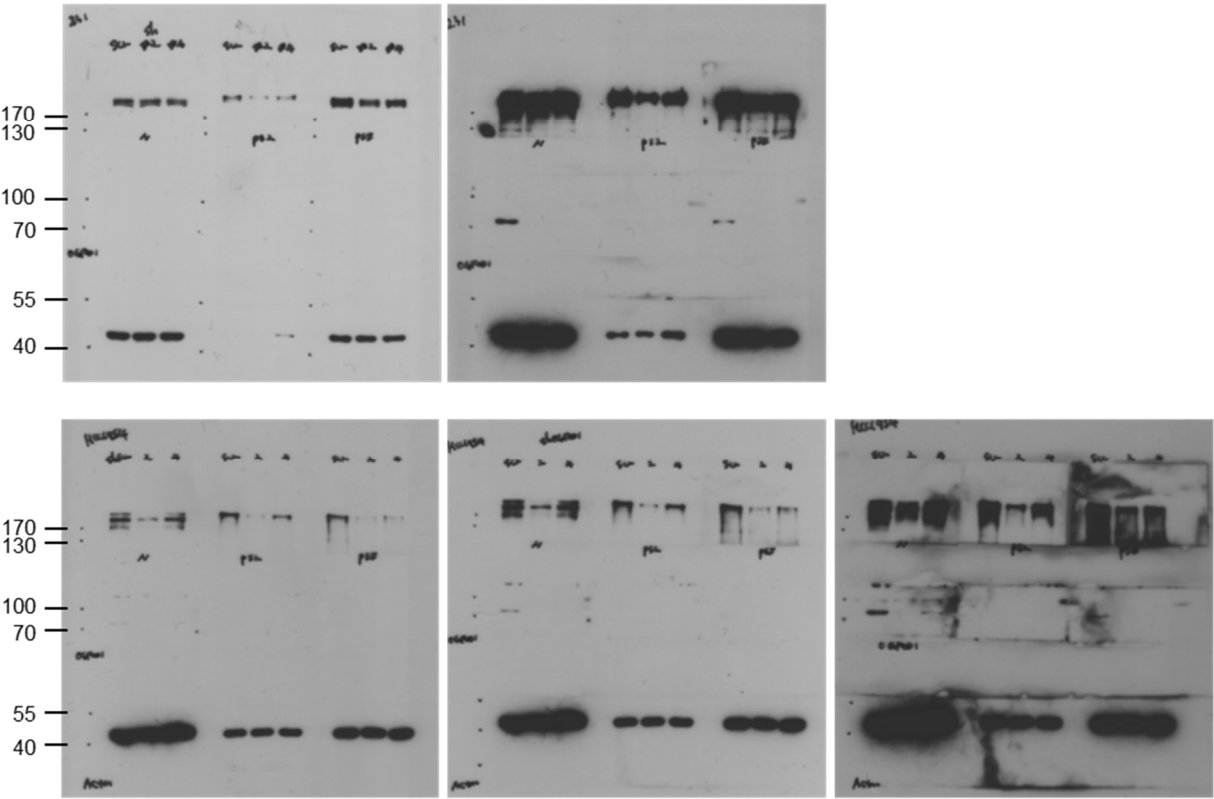
2.F



2.G



2.H



2.H

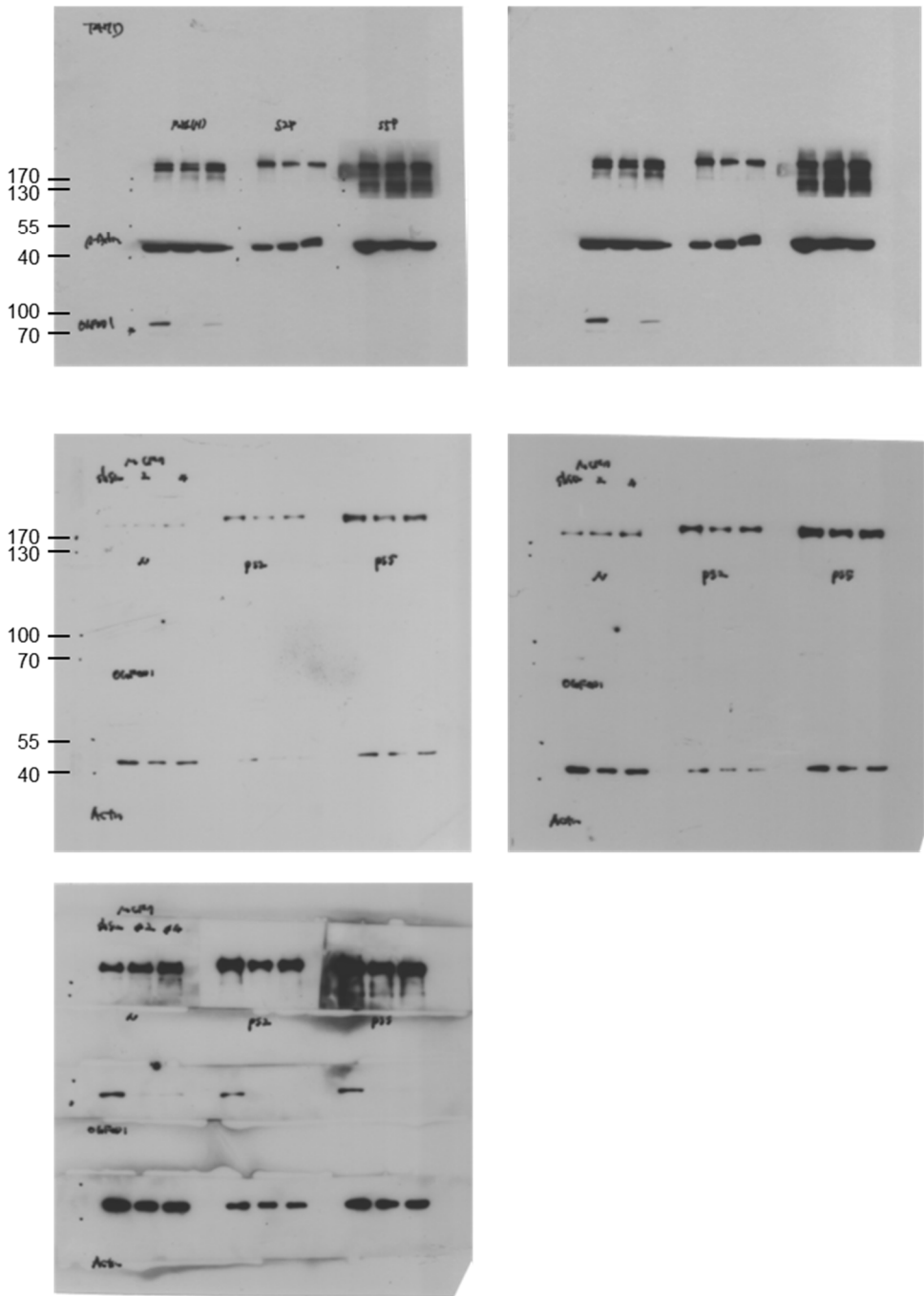
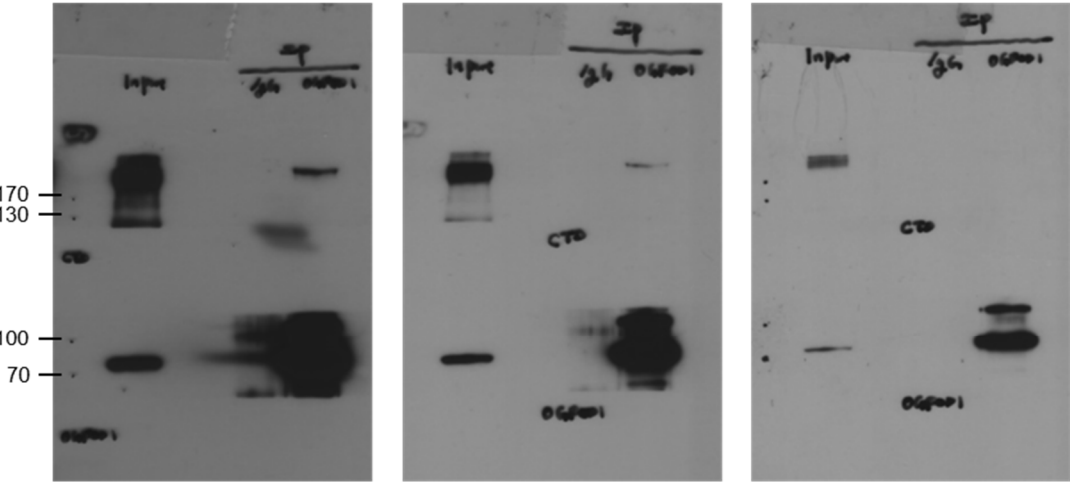
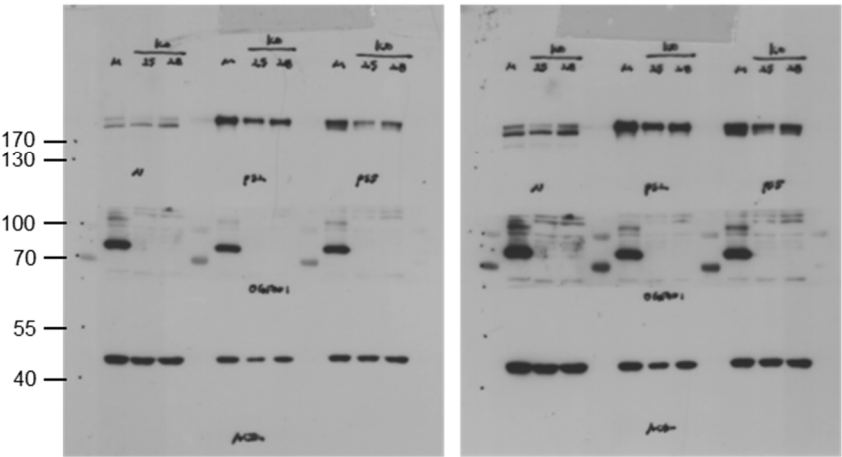


Figure S7. Full western blot of Figure 2.

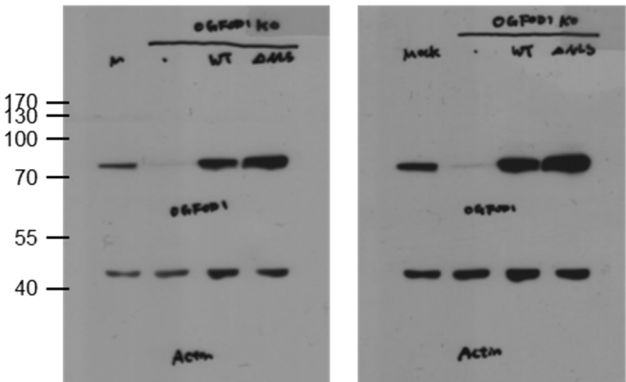
3.A



3.B



3.D



3.F

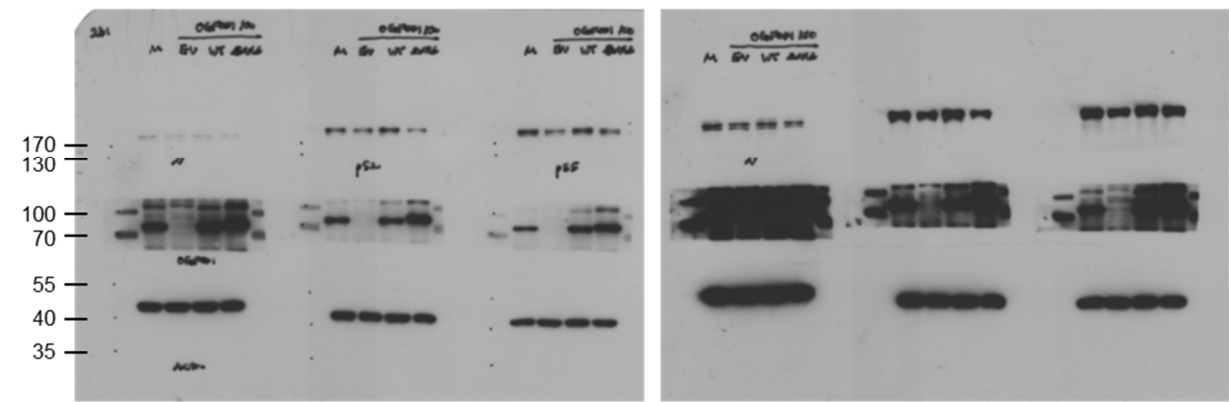
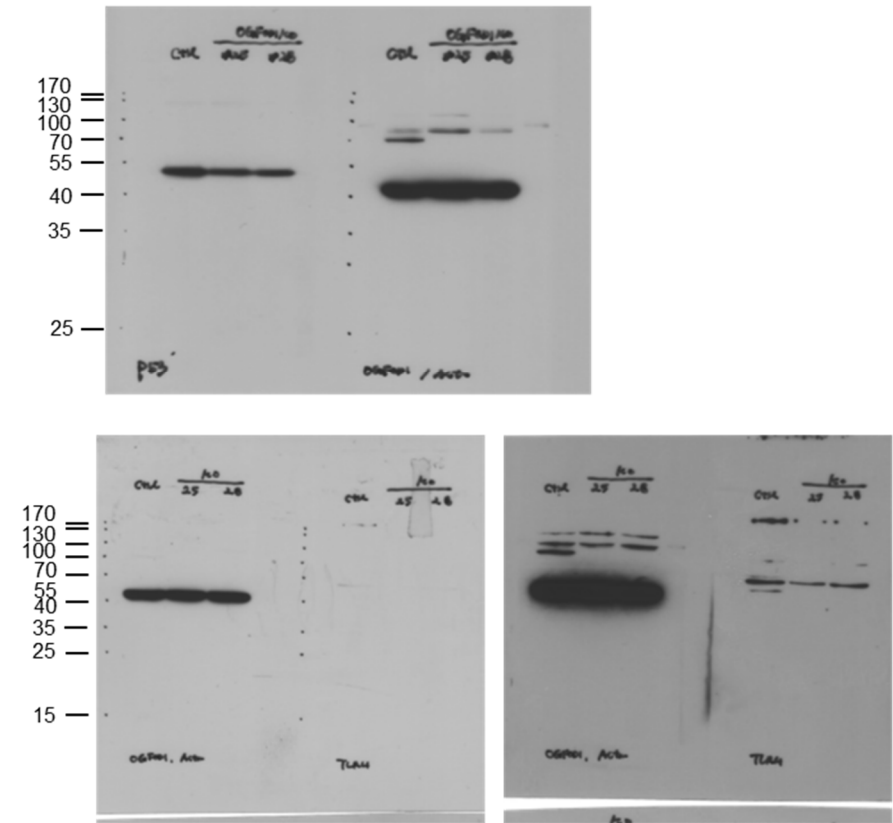


Figure S8. Full western blot of Figure 3.

4.D



4.D

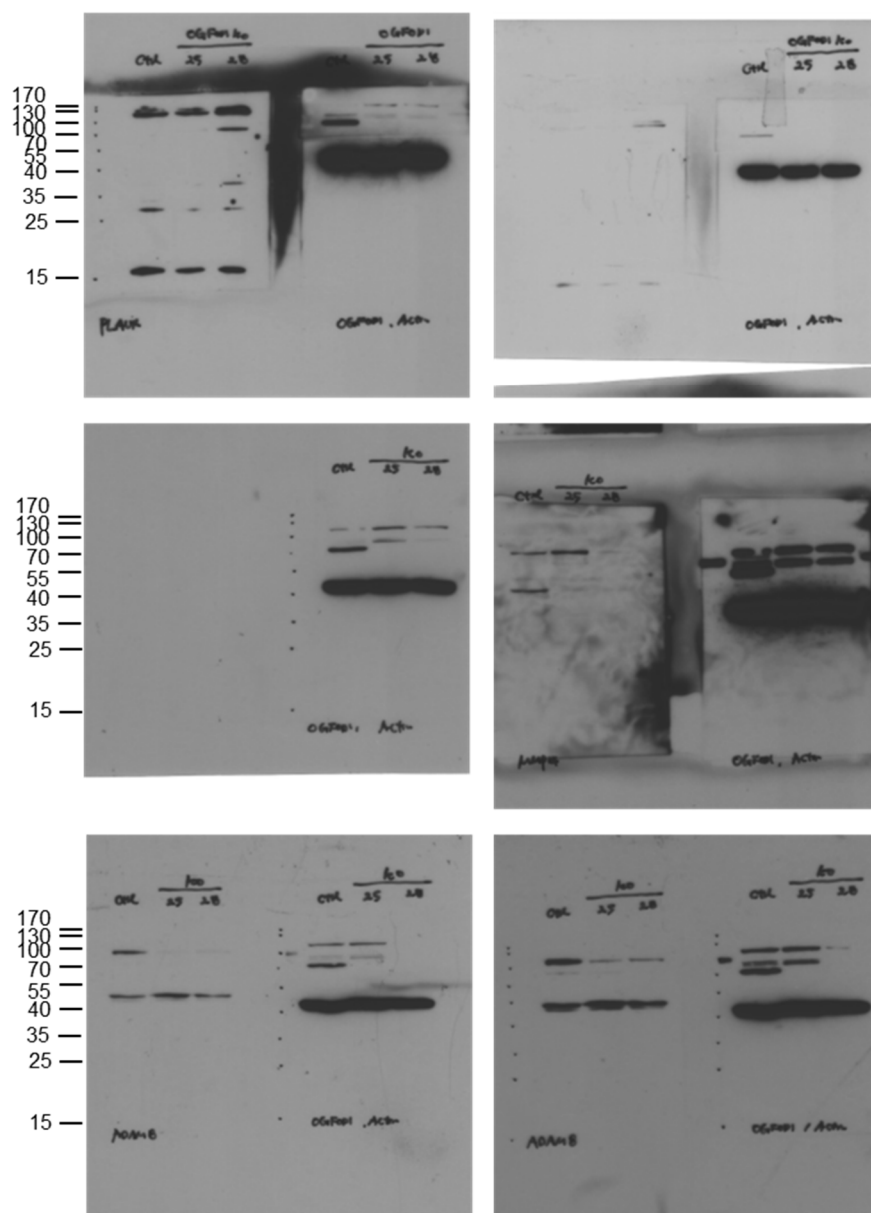
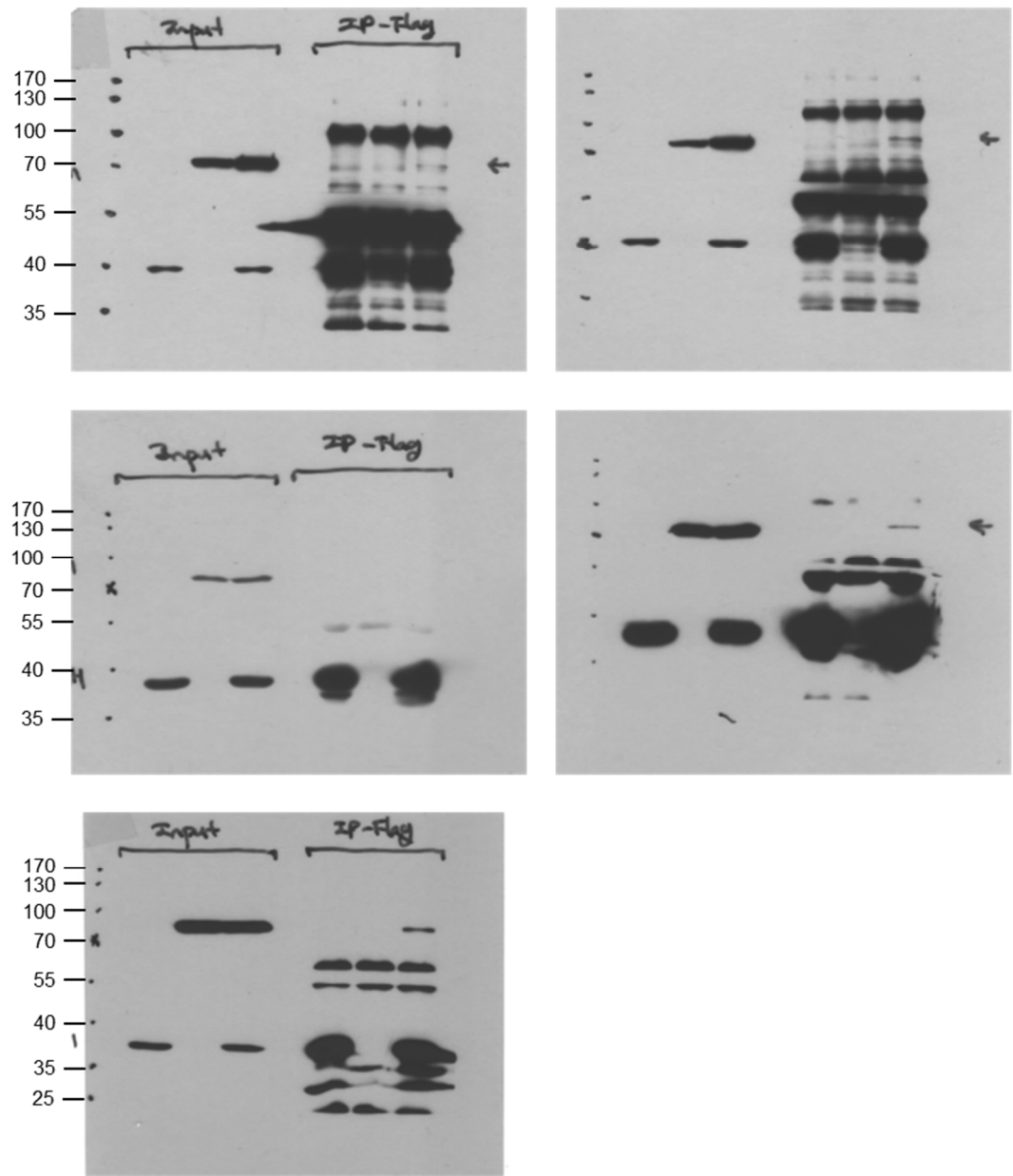
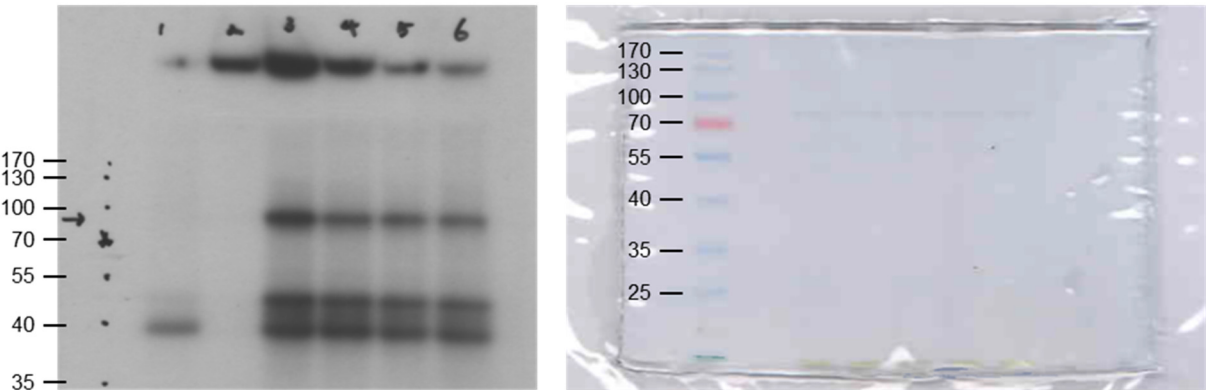


Figure S9. Full western blot of Figure 4.

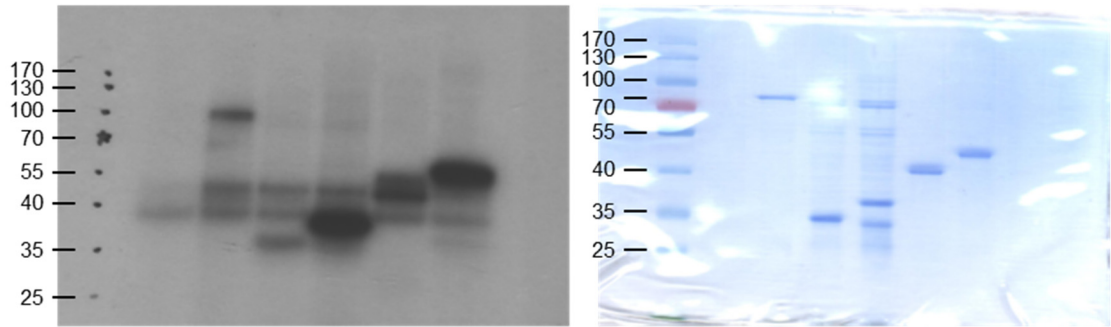
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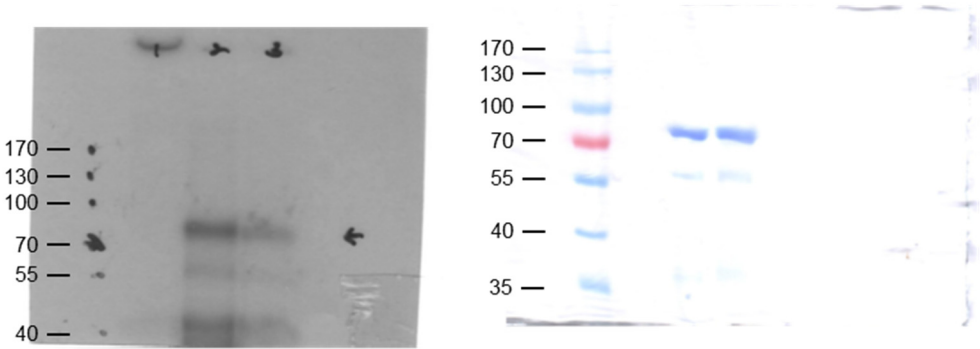
5.B



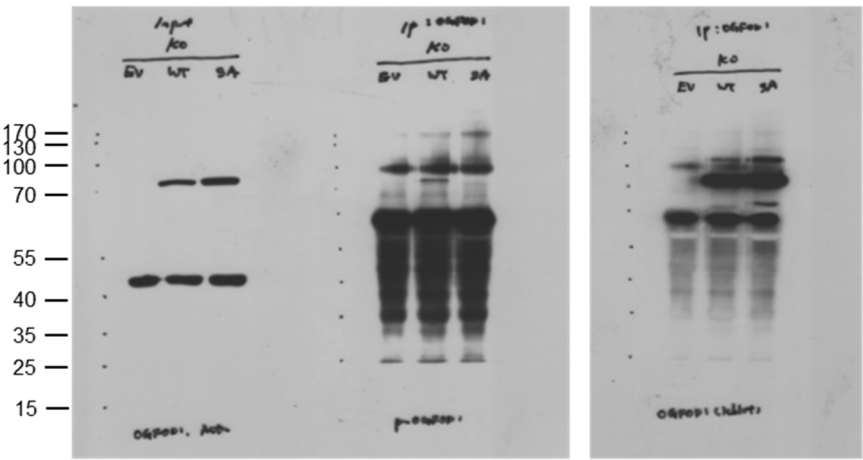
5.D



5.F



5.G



5.H

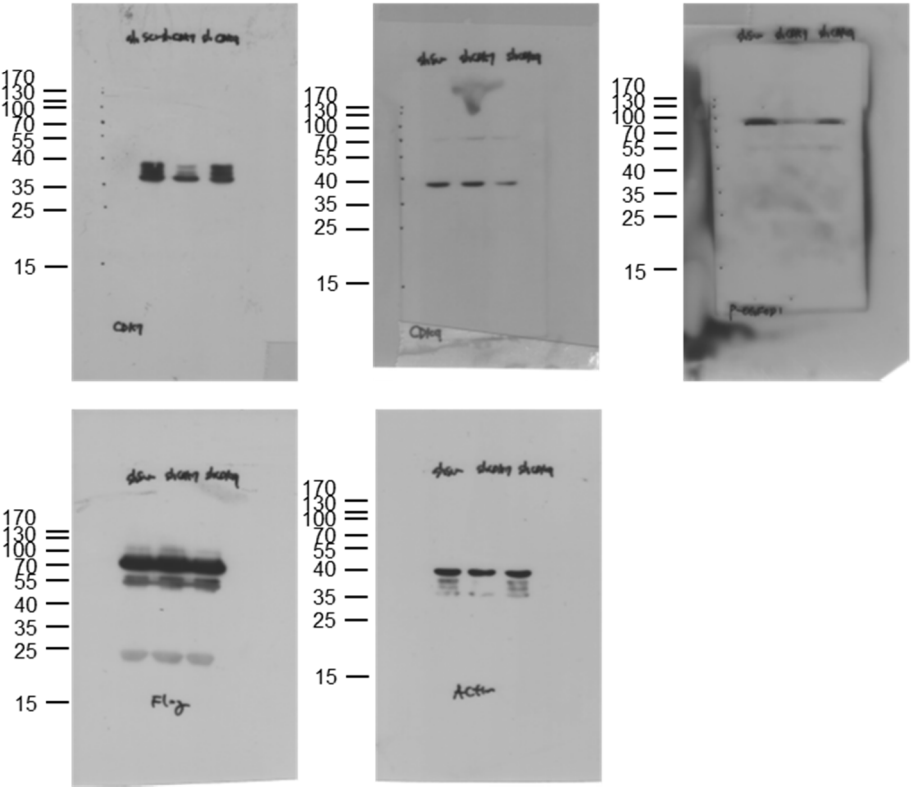
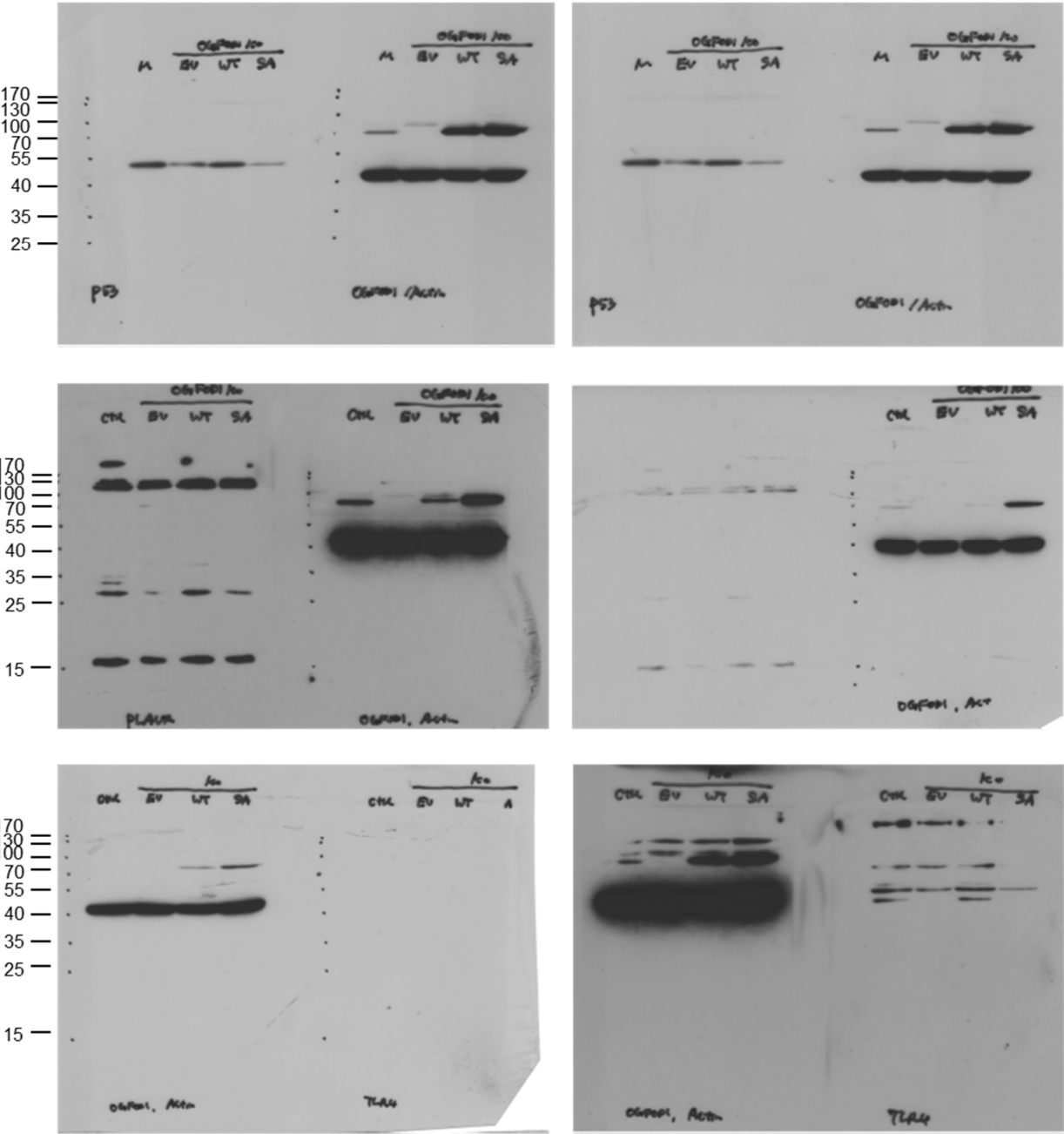


Figure S10. Full western blot of Figure 5.

6.B



6.B

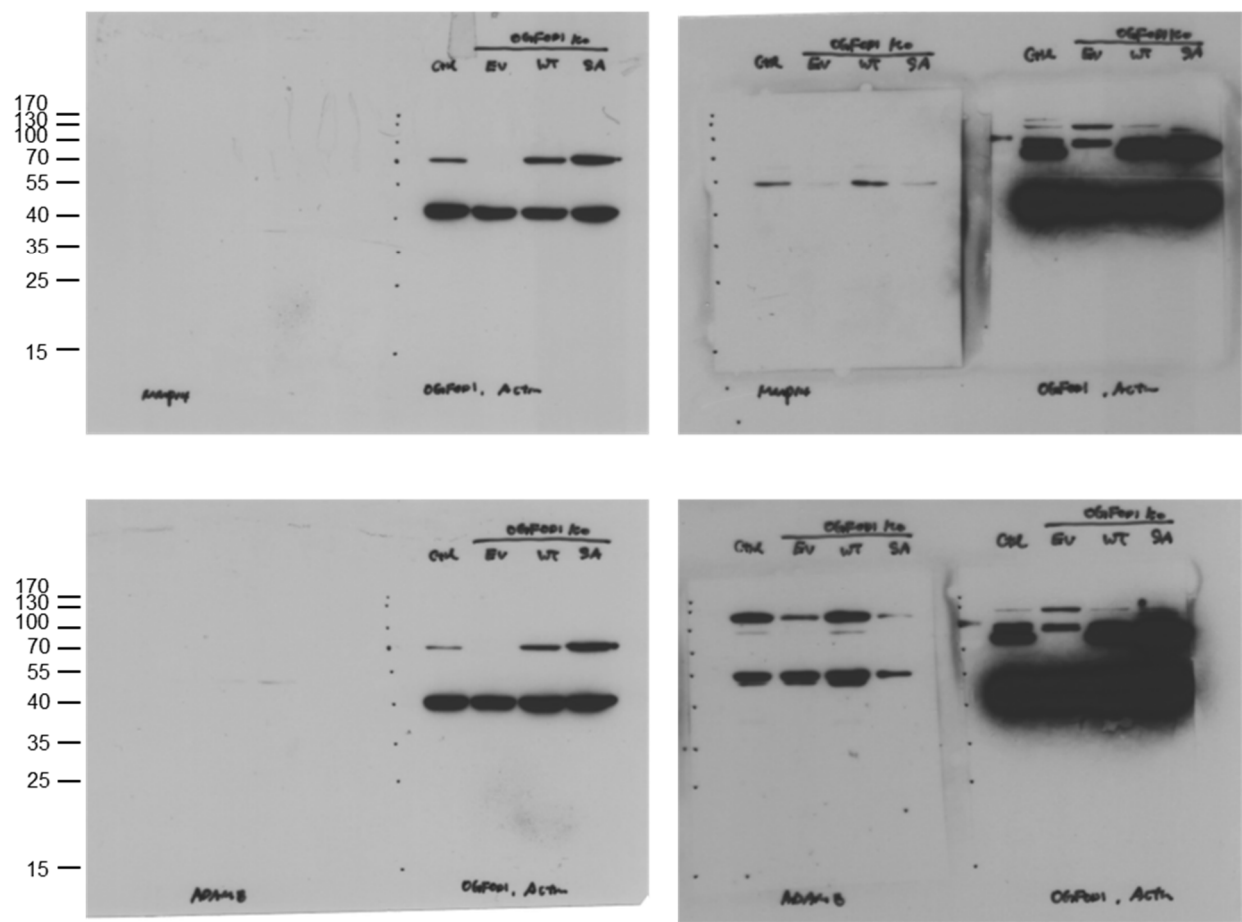
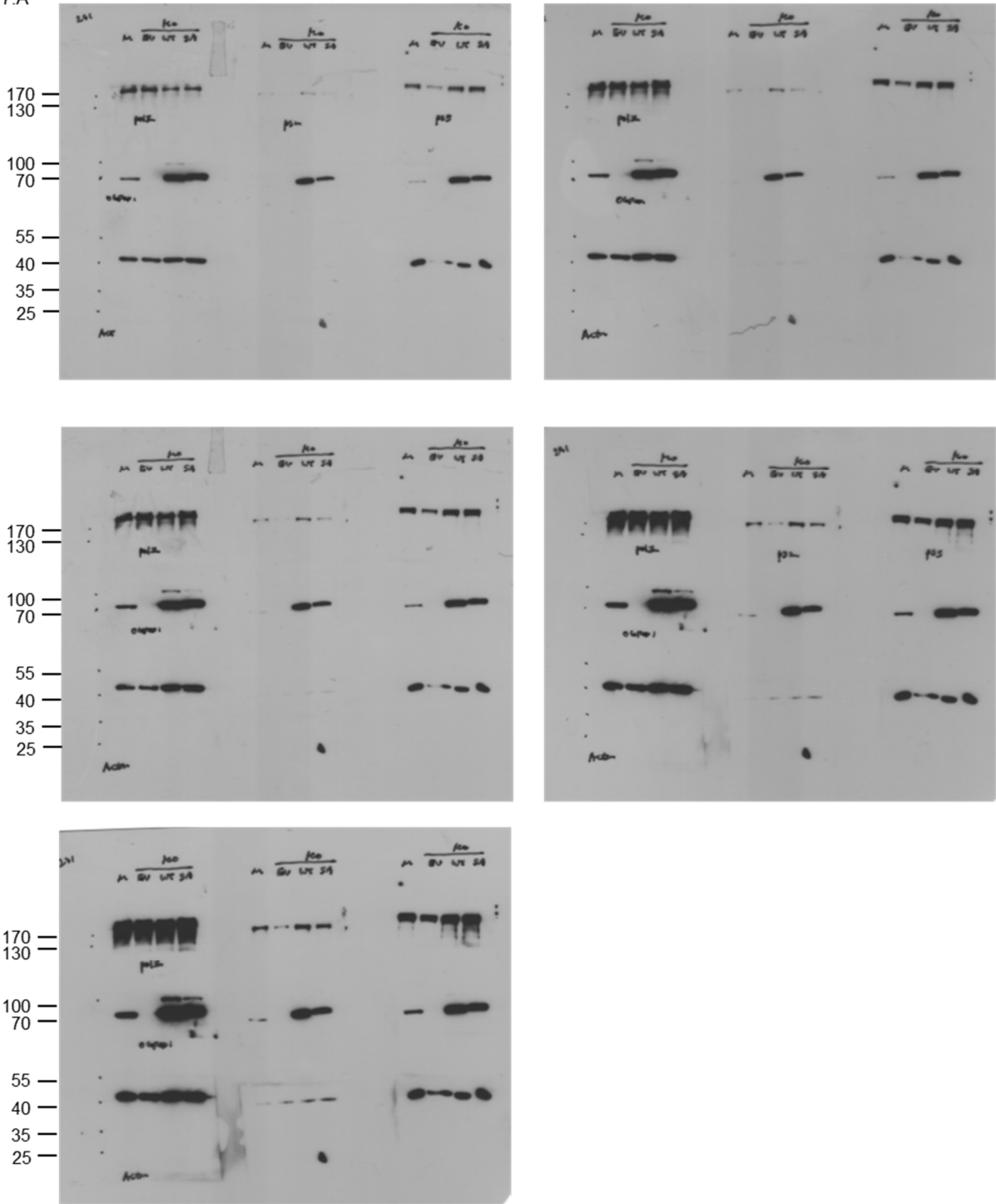


Figure S11. Full western blot of Figure 6.

7.A



7.J

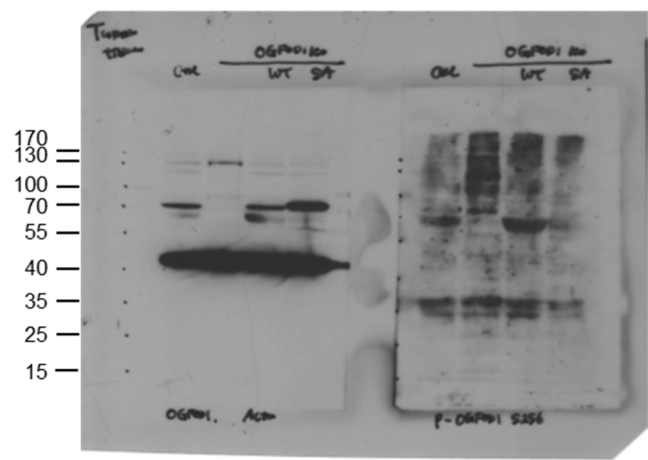
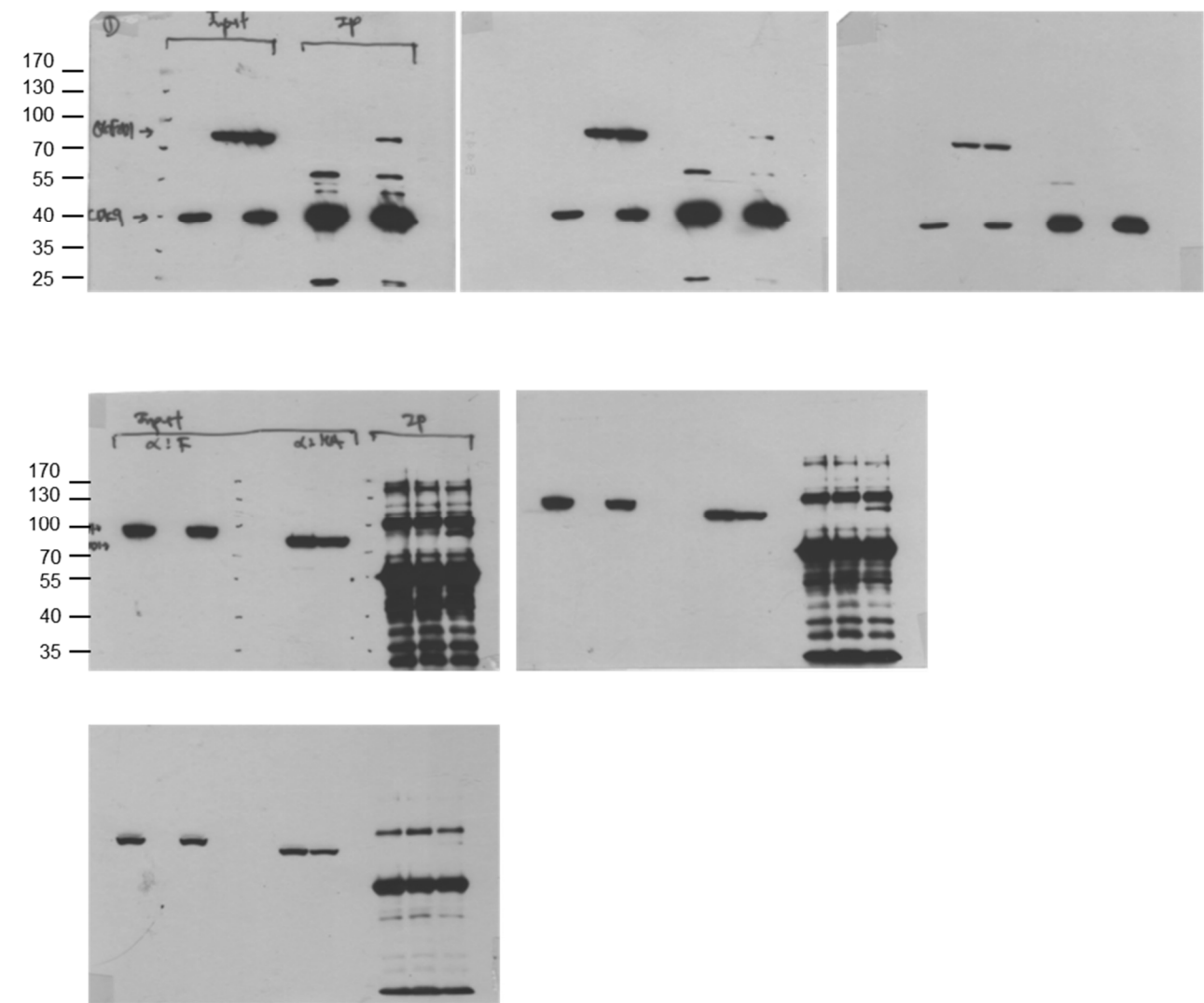


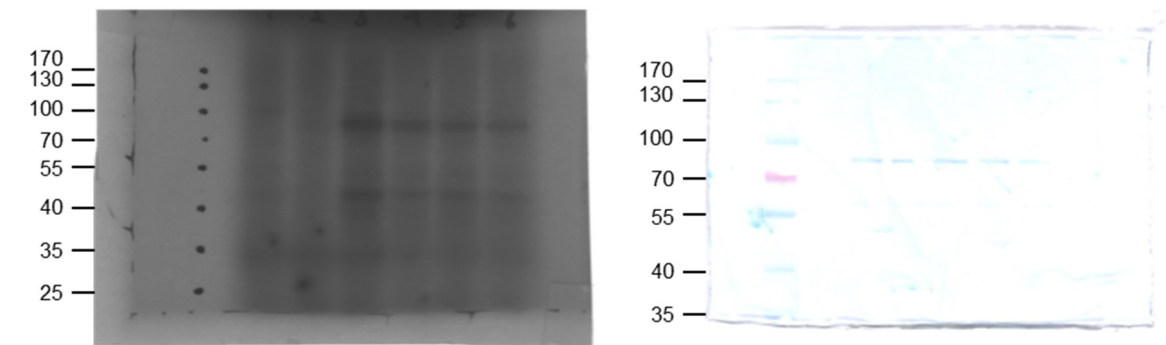
Figure S12. Full western blot of Figure 7.



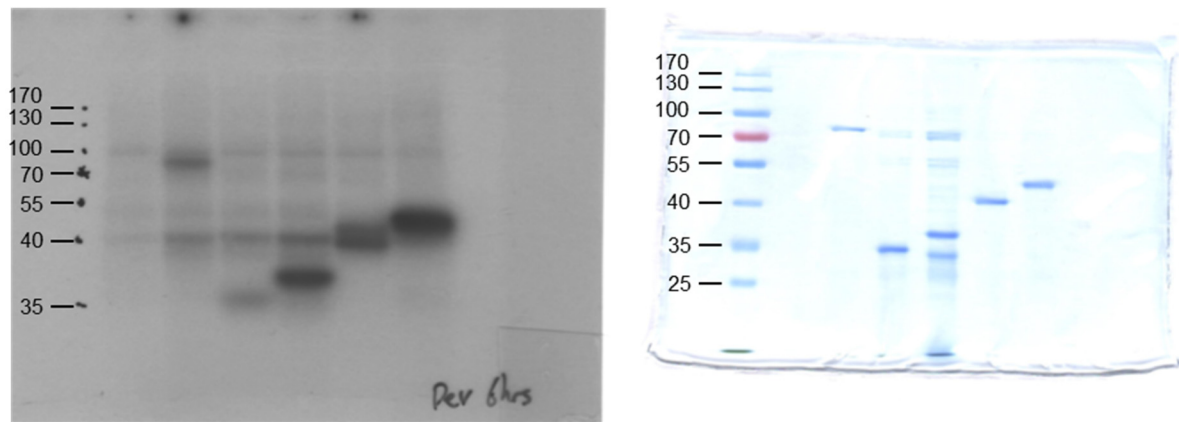
3.A



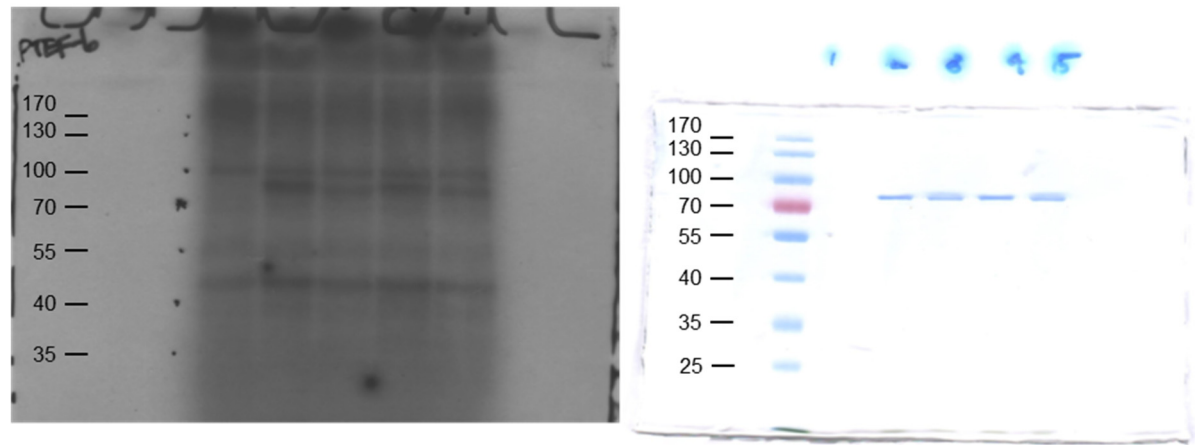
3.B



3.C



3.D



3.E

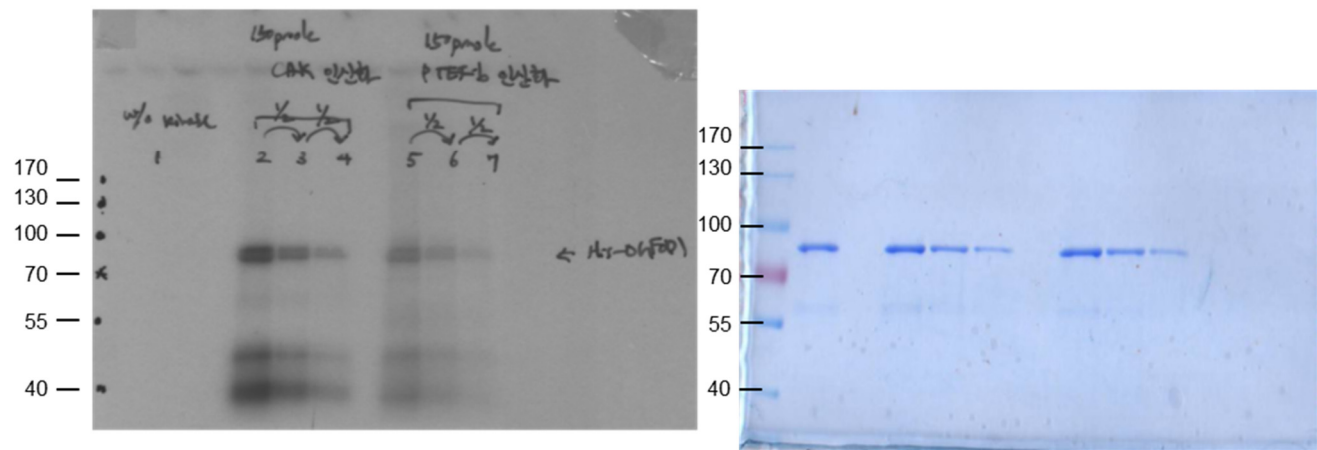
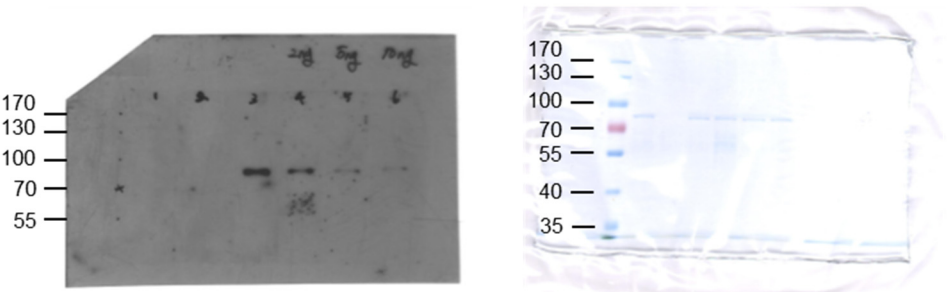


Figure S14. Full western blot of Figure S3.

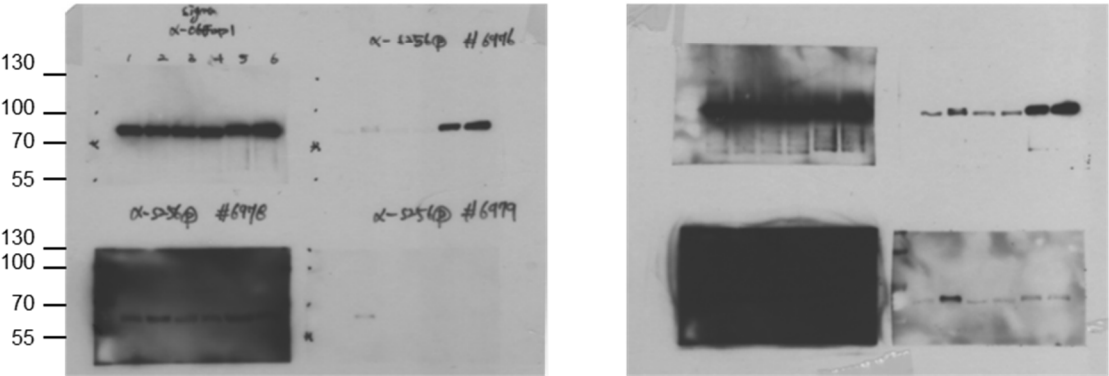
4.A



4.B



4.C



4.D

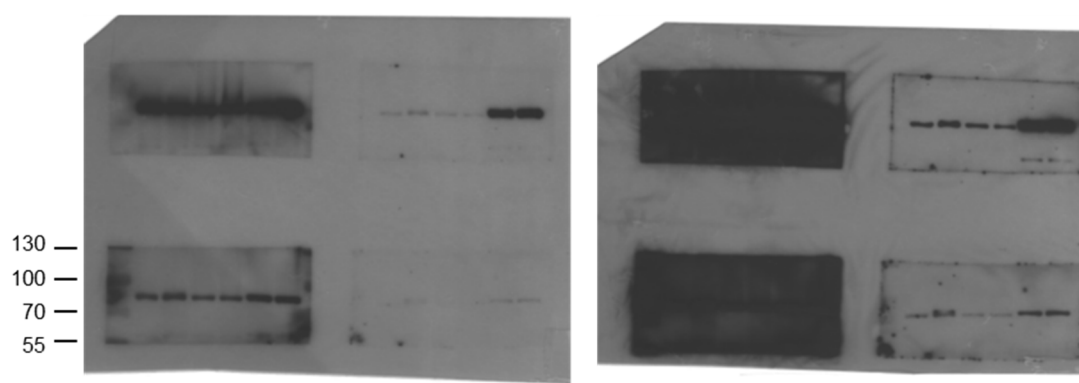


Figure S15. Full western blot of Figure S4.

Table S1. Primer list for knockout, RT-qPCR and ChIP-qPCR.

Name	Sequence
sgRNA oligonucleotides for OGFOD1 knock-out 1-1	CACCGTCAGAAGGAACTGATGAACT
sgRNA oligonucleotides for OGFOD1 knock-out 1-2	AAACAGTTCATCAGTTCCTTCTGAC
sgRNA oligonucleotides for OGFOD1 knock-out 2-1	CACCGCTTCTAAGAAGTCTTGGCTT
sgRNA oligonucleotides for OGFOD1 knock-out 2-2	AAACAAGCCAAGACTTCTTAGAAGC
Oligonucleotides for OGFOD1 exon 2_F	CCCAAATTCATGACCATCC
Oligonucleotides for OGFOD1 exon 2_R	GGGAATAAATACCTGCTGGA
hOGFOD1 S256A mutagenesis_F	CCCCCATACCTCGGGCCCCCTCACATCCCACA
hOGFOD1 S256A mutagenesis_R	TGTGGGATGTGAGGGGCCCCGAGGTATGGGGGG
qRT-PCR primer for TP53_F	TCAACAAGATGTTTTGCCAACTG
qRT-PCR primer for TP53_R	ATGTGCTGTGACTGCTTGTAGATG
qRT-PCR primer for PLAUR_F	TGAAGAAGGGCGTCCAAAG
qRT-PCR primer for PLAUR_R	GTGGTGTTCAGCATTTTCAG
qRT-PCR primer for TLR4_F	GGACCTTCCAGCAACAAGA
qRT-PCR primer for TLR4_R	CAGGGACAGGTCTAAAGAGAGA
qRT-PCR primer for MMP14_F	CGGCCTTCTGTTCTTGATAAA
qRT-PCR primer for MMP14_R	AGCGCTCCTTGAAGACAAA
qRT-PCR primer for ADAM8_F	GGGCTGGAGATTGGAATAG
qRT-PCR primer for ADAM8_R	CGTGATGAGCTGTACGTTGT
qRT-PCR primer for TLR2_F	CCTACTGGGTGGAGAACCTTAT
qRT-PCR primer for TLR2_R	CAGGAATGAAGTCCCCGCTTATG
qRT-PCR primer for PLAU_F	CGCATGACTTTGACTGGAATTG
qRT-PCR primer for PLAU_R	TCCAGGGTAAGAAGTGTGAGA
qRT-PCR primer for FZD1_F	AAGACCGAGTGGTGTGTAATG
qRT-PCR primer for FZD1_R	TGGCCATGCTGAAGAAGTAG
qRT-PCR primer for FZD8_F	CATCGGCTACAACCTACACCTA
qRT-PCR primer for FZD8_R	CTGCACAGGAAGAACTTGAGA
qRT-PCR primer for BCAR1_F	ACTATGACTACGTCCACCTACA

qRT-PCR primer for BCAR1_R	CTCCTGTTCAGTCGTTCAAA
qRT-PCR primer for ITGA1_F	GGAAAGAGTATGCACAACGTATTC
qRT-PCR primer for ITGA1_R	CTGTCAGACCGTCACCATTTA
qRT-PCR primer for ITGA2_F	CTGTTCAAGGAGGAGACAACCTT
qRT-PCR primer for ITGA2_R	CTGCACCCAGCATCAGAATA
qRT-PCR primer for ITGAV_F	ACTCTTAGCTGGTCTTCGTTTC
qRT-PCR primer for ITGAV_R	TGTGAGATACAACCTGGGCTTAC
qRT-PCR primer for ITGAB1_F	AATTAGGCCTCTGGGCTTTAC
qRT-PCR primer for ITGAB1_R	CCTTCATGACACTTGGGACTT
qRT-PCR primer for ITGB2_F	GTGACCAGTAGGATGGTGAAG
qRT-PCR primer for ITGB2_R	GACCCTGGAGGAGAGTTTATTG
qRT-PCR primer for EMP1_F	GCCAATGTCTGGTTGGTTTC
qRT-PCR primer for EMP1_R	CACTGTCTTGAGGGCATCTT
qRT-PCR primer for ARHGAP18_F	GCTCAGTGTGGAGTATCTCAAAG
qRT-PCR primer for ARHGAP18_R	TCAGGTAGGAGGATGACAAGAA
qRT-PCR primer for CTGF_F	GCTGACCTGGAAGAGAACATTA
qRT-PCR primer for CTGF_R	CTCCACAGAATTTAGCTCGGTAT
qRT-PCR primer for GNC11_F	TGCAGAGACAACAAGTGTCTAA
qRT-PCR primer for GNC11_R	TGTCTTCTGGAATTCCTTTACT
qRT-PCR primer for KISS1_F	AGAAGGACCTGCCGAACATA
qRT-PCR primer for KISS1_R	CTTCTGCCGTGGTTCCC
qRT-PCR primer for DUSP1_F	TTTGAGAACGCAGGAGAGTTTA
qRT-PCR primer for DUSP1_R	TGTACCAAGACACCACAGTTC
ChIP primer for TP53_1_F	CTCTAGCCAAGCTTCCATCC
ChIP primer for TP53_1_R	CTGAACCTGATGAGTCCTCTCTG
ChIP primer for TP53_2_F	CTCCCGAATAGCTGGGATTAC
ChIP primer for TP53_2_R	GGGCGGATTACTTGAGGATAG
ChIP primer for TP53_3_F	TGTGACTGCTTGATAGATGGC
ChIP primer for TP53_3_R	TCTGTCTCCTTCCTCTTCCTAC
ChIP primer for TP53_4_F	CCTGGTTAGTACGGTGAAGTG
ChIP primer for TP53_4_R	AGGGATGTTTGGGAGATGTAAG
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