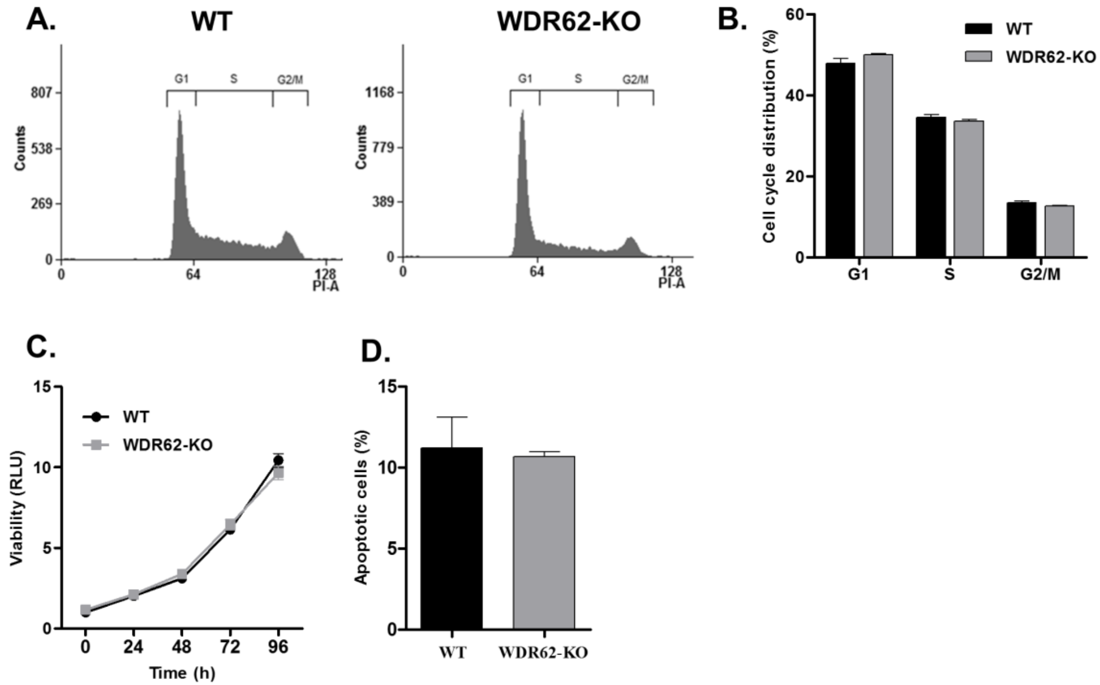
Supplemental Materials

Molecular Biology of the Cell

Prinz et al.



Supplemental Figure 1: Cell cycle and proliferation analysis of WDR62-KO cells. (A-B) Cell cycle analysis. WT and WDR62-KO cells were harvested, fixed with 70% ethanol and stained with propidium iodide, then analyzed by flow cytometry. Results are expressed as the mean ± SEM of three replicates. C. Proliferation assay. 3,000 cells were plated in 96-well plate in triplicates. Viability was assayed with CellTiter-Glo reagent on the indicated time points. The viability level obtained from WT cells on day zero was determined as one while all other viability measurements were determined relatively. Results are shown as relative light units (RLU) and expressed as the mean ± SEM of four replicates. D. Cell death analysis. WT and WDR62 cells were harvested and stained with annexin V-FITC and 7-aad, then analyzed by flow cytometry. Cells that were positive to annexin V were considered as apoptotic cells. Results are expressed as the mean ± SEM of three replicates.

A.

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     WT GTGTCACACTCGAGAAGGTGCTTGGCATCACAGCCCAGAACAGCAGTGGCCTAACCTGTGACCCCGGCACAGGCCATGTGGCCTACCTGGCAGG
KO #1a  GTGTCACACTCGAGAAGGTGCTTGGCATCACAGCCCAGAACAGCAGTGGCCTAACCTGTGACCCCGGCACAGGCCATGTGGCCTACCTGGCAGG 212 ins
KO #1b  GTGTCACACTCGAGAAGGTGCTTGGCATCACAGCCCAGAACAGCAGTGGCCTAACCTGTGACCCCGGCACAGGCCATGTGGCCTACCTGGCAGG 320 ins
KO #2a  GTGTCACACTCGAGAAGGTGCTTGGCATCACAGCCCAGAACAGCAGTGGCCTAACCTGTGACCCCG-CACAGGCCATGTGGCCTACCTGGCAGG   1 del
KO #2b  GTGTCACACTCGAGAAGCCGCTTGGCATCACAGCCCAGAACAGCAGTGGCCTAACCTGTGACCCCGGCACAGGCCATGTGGCCTACCTGGCAGG 231 ins
KO #3a  GTGTCACACTCGAGAAGCCGCTTGGCATCACAGCCCAGAACAGCAGTGGCCTAACCTGTG--------------------GCCTACCTGGCAGG  20 del
KO #3b  GTGTCACACTCGAGAAGCCG--------------------------------------------------------------------------  74 del
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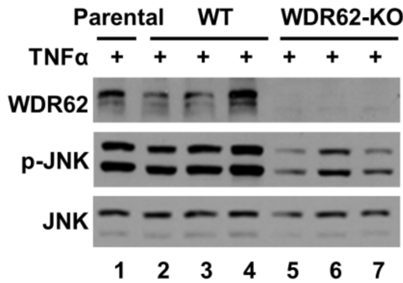
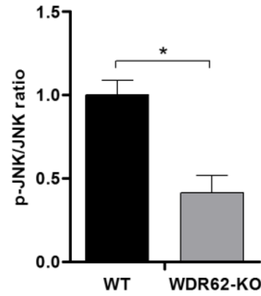
B.



C.



Supplemental Figure 2: WDR62 mediates TNFα-induced JNK activation in HCT116 cells. (A) Sequence of exon 2 of WT WDR62 and three WDR62-KO clones of HCT116 cells. The sequences a and b in each clone represents the two mutated alleles. The gRNA sequence that was used is shown in red and the protospacer adjacent motif (PAM) is shown in green. Hyphens indicate deleted nucleotides, arrowheads indicate site of insertion. All insertions included a stop codon within the open reading frame. (B) Parental HCT116 cells, three WT clones and three WDR62-KO clones were treated with TNFα (50 ng/ml) for 15 min. Following stimulation, cells were harvested and subjected to Western blot analysis with WDR62, p-JNK and JNK antibodies. (C) The ratio of p-JNK/JNK obtained with TNFα-treated WT cells was determined as 1 while the ratio obtained with TNFα-treated WDR62-KO cells was determined relatively to their appropriate WT control. Results are expressed as the mean ratio ± SEM of the three independent clones. *$p \leq 0.05$ compared to WT cells.

A.

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WT     GTGTCACACTCGAGAAGGTGCTTGGCATCACAGCCCAGAACAGCAGTGGCCTAACCTGTGACCCCGGCACAGGCCATGTGGCCTACCTGGCAGG
KO #1a GTGTCACACTCGAGAAGGTGCTTGGCATCACAGCCCAGAACAGCAGTGGCCTAACCTGTG-------CACAGGCCATGTGGCCTACCTGGCAGG   7 del
KO #1b GTGTCACACTCGAGAAGGTGCTTGGCATCACAGCCCAGAACAGCAGTGGCCTAACCTGTGACCCCGGCACAGGCCATGTGGCCTACCTGGCAGG   1 ins
KO #2a GTGTCACACTCGAGAAGGTGCTTGGCATCACAGCCCAGAACAGCAGTGGCCTAACCTGTGACC--------------------TACCTGGCAGG  20 del
KO #2b GTGTCACACTCGAGAAGCCGCTTGGCATCACAGCCCAGAACAGCAGTGGCCTAACCTGTGACCCCGGCACAGGCCATGTGGCCTACCTGGCAGG   2 ins
KO #3a GTGTCACACTCGAGAAGCCGCTTGGCATCACAGCCCAGAACAGCAGTGGCCTAACCTG--------------------TGGCCTACCTGGCAGG  20 del
KO #3b GTGTCACACTCGAGAAGCCGCTTGGCATCACAGCCCAGAACAGCAGTGGCCTAACCTG-------------------------------GCAGG  31 del
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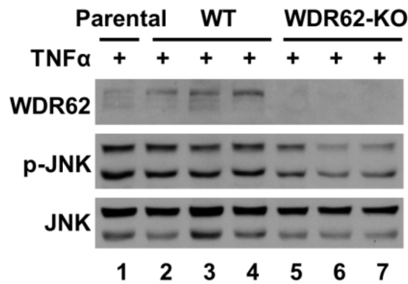
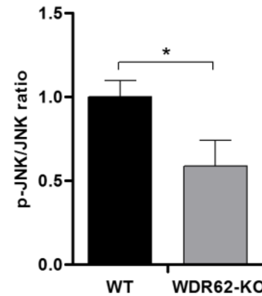
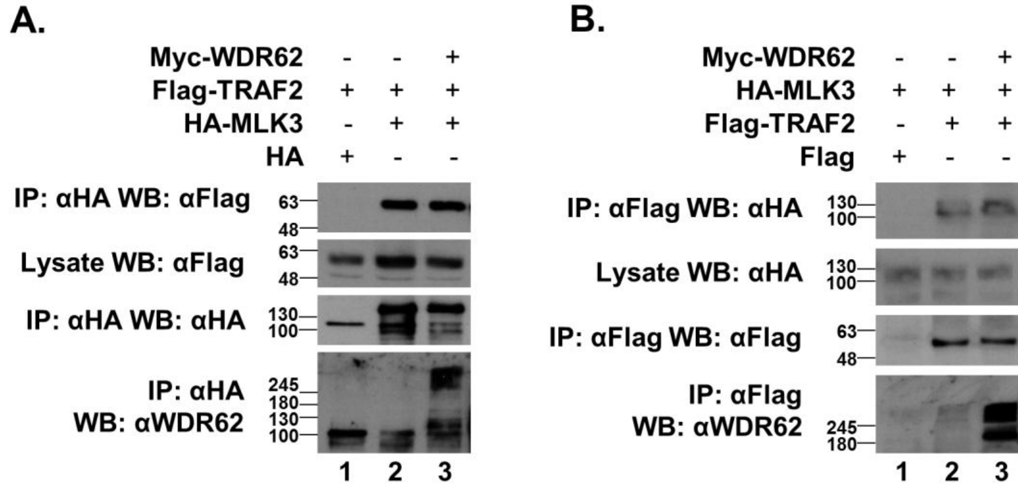
B.



C.



Supplemental Figure 3: WDR62 mediates TNFα-induced JNK activation in HeLa cells. (A) Sequence of exon 2 of WT WDR62 and three clones of HeLa cells with WDR62-KO. The sequences a and b in each clone represents the two mutated alleles. The gRNA sequence that was used is shown in red and the protospacer adjacent motif (PAM) is shown in green. Hyphens indicate deleted nucleotides, arrowheads indicate site of insertion. (B) Parental HeLa cells, three WT clones and three WDR62-KO clones were treated with TNFα (50 ng/ml) for 15 min. Following stimulation, cells were harvested and subjected to Western blot analysis with WDR62, p-JNK and JNK antibodies. (C) The ratio of p-JNK/JNK obtained with TNFα-treated WT cells was determined as 1 while the ratio obtained with TNFα-treated WDR62-KO cells was determined relatively to their appropriate WT control. Results are expressed as the mean ratio ± SEM of the three independent clones. *$p \leq 0.05$ compared to WT cells.



Supplemental Figure 4: HEK-293T cells were co-transfected with expression plasmids as indicated. Cell lysates were subjected to immuno-precipitation (IP) with the indicated antibodies. Eluted proteins were separated by SDS-PAGE, followed by Western blot with the appropriate antibodies as indicated. The expression level of transfected plasmids was determined by blotting the total cell lysate with the appropriate antibodies.