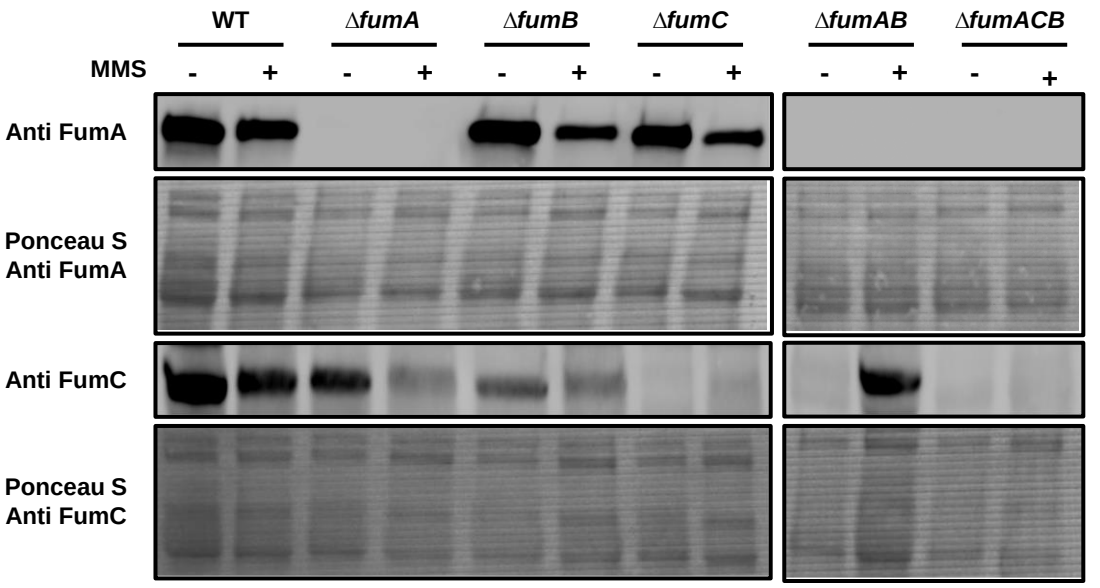
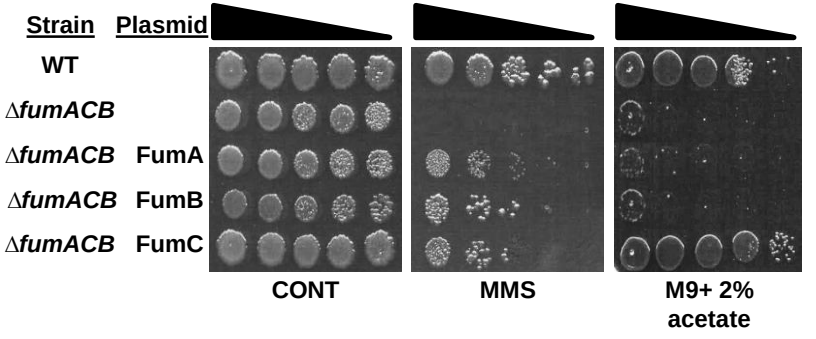
# Supplemental Information

**S1A**




**S1B**



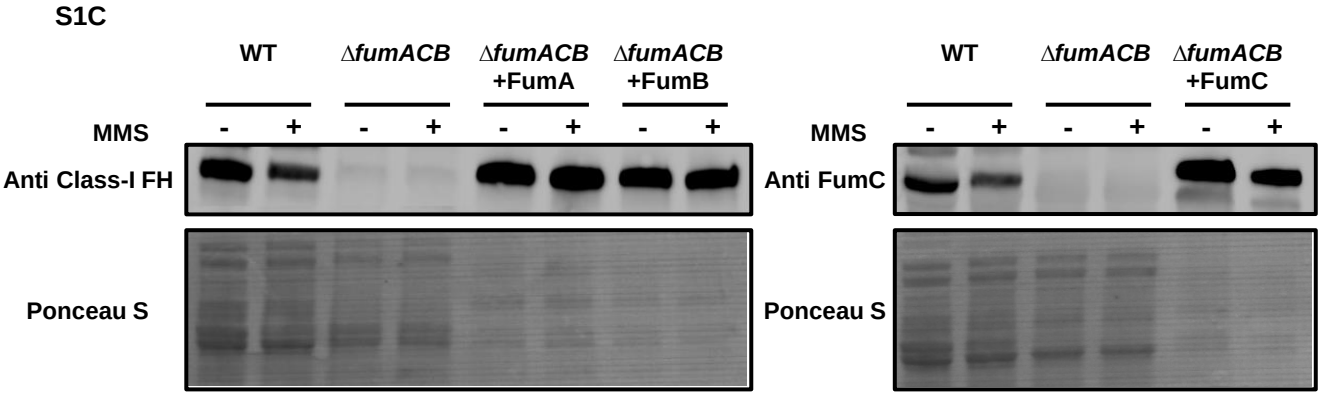
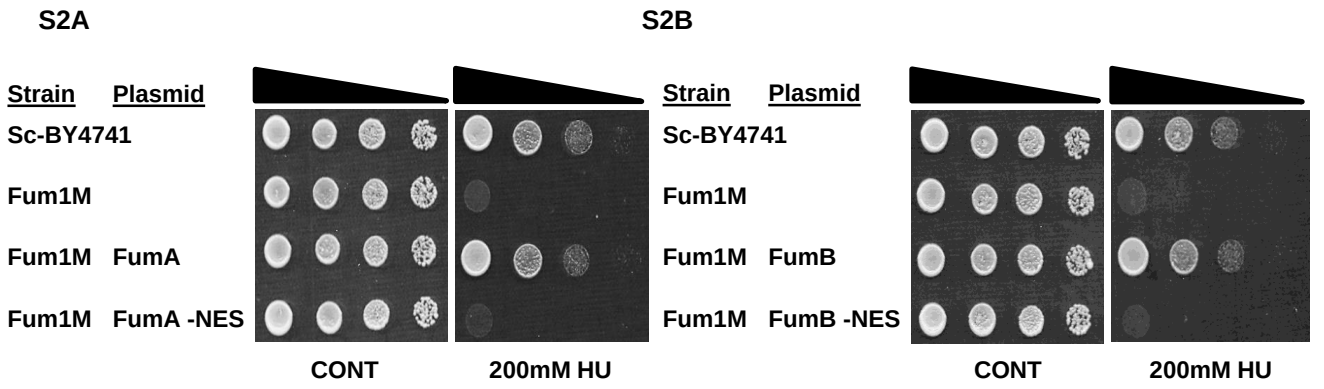



**Figure S1- In the absence of FumA and FumB, FumC participates in the DDR in *E. coli*.** (**A**) *E. coli* wild type (WT), *ΔfumA*, *ΔfumB*, *ΔfumC*, *ΔfumAB, ΔfumACB* strains were grown to mid-exponential phase and treated with MMS (0.35% (v/v) for 45 minutes at 37ºC). Total cell extracts were prepared from the indicated samples and subjected to Western blotting, using the indicated antibodies followed by Ponceau S staining. Each result is representative of three independent experiments. (**B**) *E. coli* wild type (WT), *ΔfumACB* and *ΔfumACB* harboring plasmids encoding the indicated *E. coli fum* genes grown to early-exponential phase (OD600nm=0.3), and treated with MMS (0.35% [v/v] for 45 minutes at 37ºC). The cells were then washed and serially diluted (spot test) onto LB plates. (**C**) *E. coli* wild type (WT), *ΔfumACB* and *ΔfumACB* harboring plasmids encoding the indicated *E. coli fum* genes were grown as in S1A. Total cell extracts were prepared from the indicated samples and subjected to Western blotting, using the indicated antibodies followed by Ponceau S staining. Each result is representative of three independent experiments.

**Host- *S. cerevisiae***



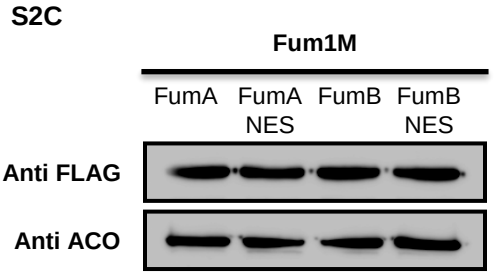
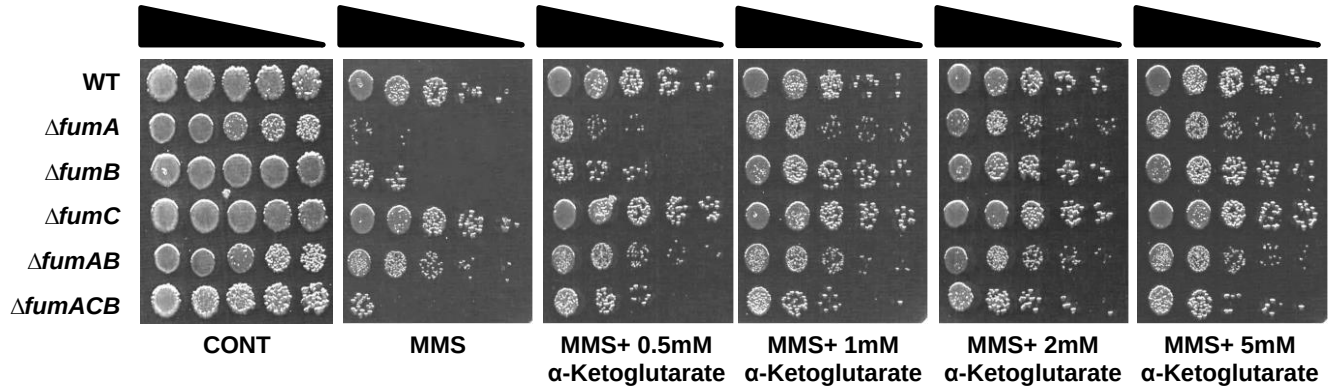
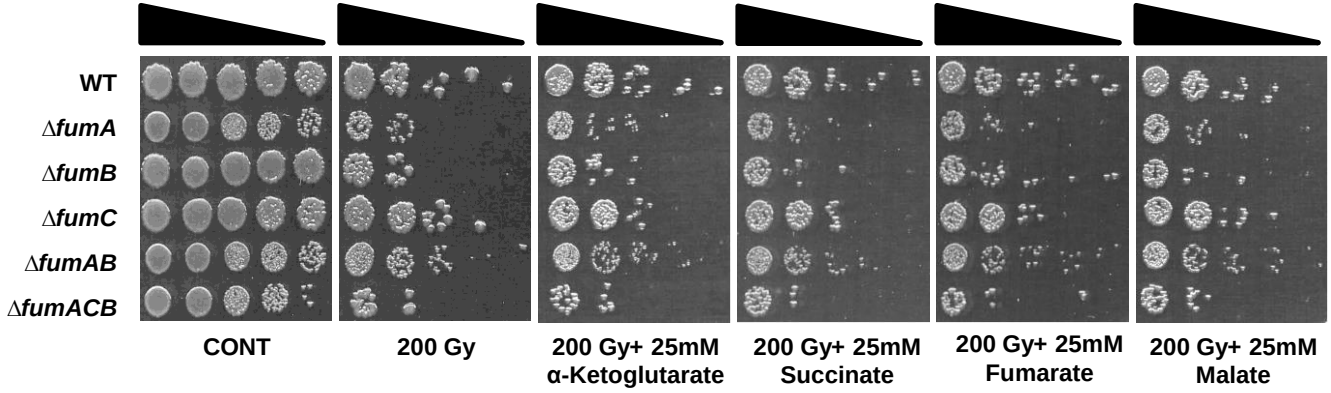




**Figure S2- FumA-NES and FumB-NES do not complement DNA damage sensitivity in *S. cerevisiae*.** (**A, B**) *S. cerevisiae* wild type Sc-BY4741, *Fum1M* and *Fum1M* harboring plasmids encoding the indicated *E. coli fum* genes, were grown to exponential phase in SC-Gal medium and serially diluted onto SC-Dex or SC-Dex+ 200 mM HU. (**C**) These strains were also lysed and centrifuged to obtain the supernatant and extracts were subjected to Western blotting, using the indicated antibodies (anti Aco1 as the loading control). Each result is representative of at least three independent experiments.

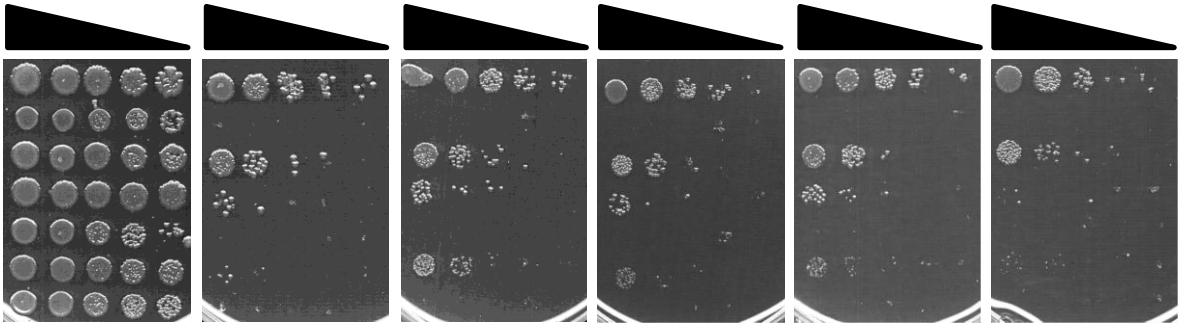
**S3A**


WT
ΔfumA
ΔfumB
ΔfumC
ΔfumAB
ΔfumACB
CONT
MMS
MMS+ 0.5mM α-Ketoglutarate
MMS+ 1mM α-Ketoglutarate
MMS+ 2mM α-Ketoglutarate
MMS+ 5mM α-Ketoglutarate

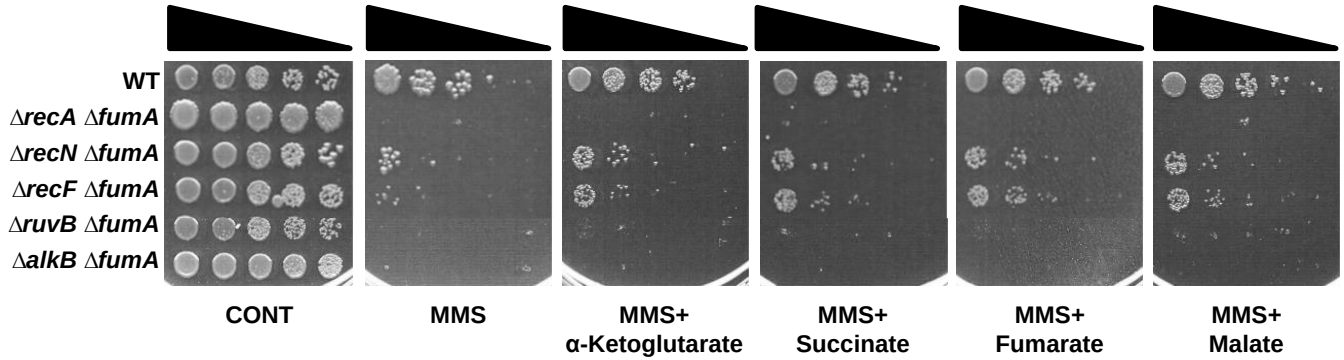
**S3B**


WT
ΔfumA
ΔfumB
ΔfumC
ΔfumAB
ΔfumACB
CONT
200 Gy
200 Gy+ 25mM α-Ketoglutarate
200 Gy+ 25mM Succinate
200 Gy+ 25mM Fumarate
200 Gy+ 25mM Malate

**S3C**


WT
ΔrecA
ΔrecN
ΔrecB
ΔrecF
ΔruvB
ΔrecC
CONT
MMS
MMS+ α-Ketoglutarate
MMS+ Succinate
MMS+ Fumarate
MMS+ Malate

**S3D**


WT
ΔrecA ΔfumA
ΔrecN ΔfumA
ΔrecF ΔfumA
ΔruvB ΔfumA
ΔalkB ΔfumA
CONT
MMS
MMS+ α-Ketoglutarate
MMS+ Succinate
MMS+ Fumarate
MMS+ Malate

**S3E**

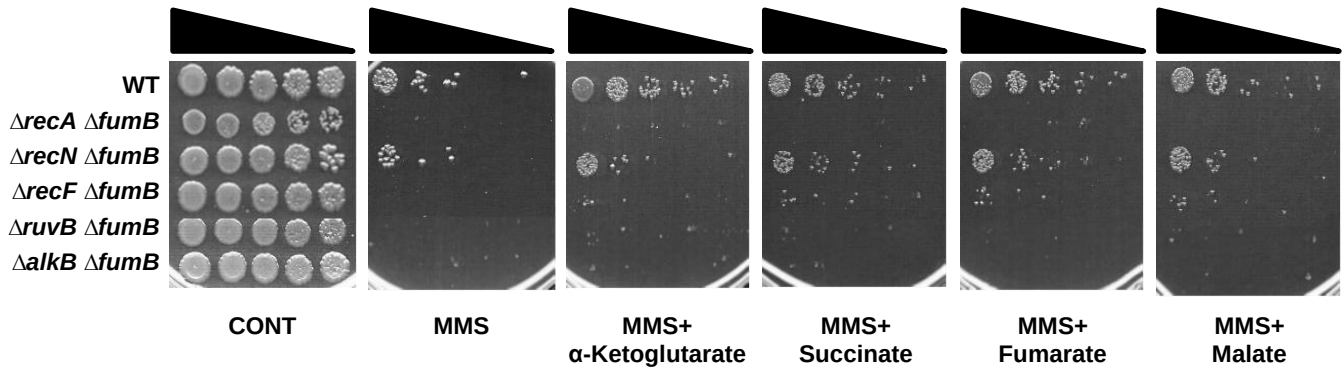
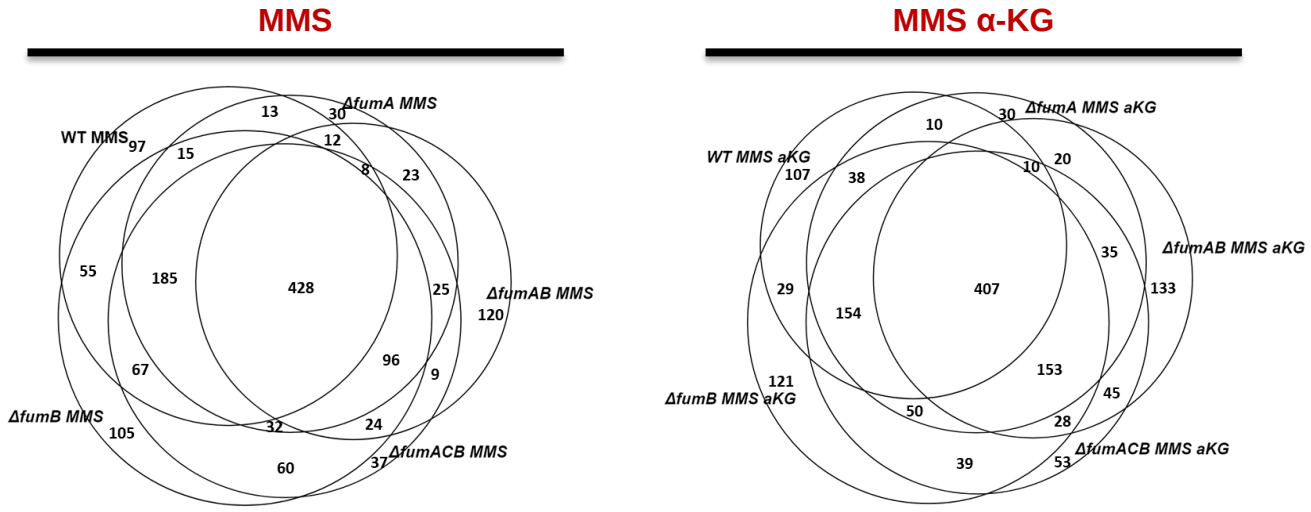

WT
ΔrecA ΔfumB
ΔrecN ΔfumB
ΔrecF ΔfumB
ΔruvB ΔfumB
ΔalkB ΔfumB
CONT
MMS
MMS+ α-Ketoglutarate
MMS+ Succinate
MMS+ Fumarate
MMS+ Malate

**Figure S3- α-KG complementation of DNA damage sensitivity is specific to *fum* null mutants following MMS treatment.** *E. coli* wild type (WT), *ΔfumA, ΔfumB, ΔfumC*, *ΔfumAB* and *ΔfumACB* strains were grown to mid-exponential phase (OD600nm=0.3) and (**A**) treated with MMS (0.35% MMS (v/v) for 45 min) or (**B**) the cells were irradiated (200Gy). The cells were then washed and serially diluted (spot test) onto LB plates containing or lacking the indicated organic acids. (**C**) *E. coli* DNA damage repair genes null mutants, and (**D**) composite null mutants, *ΔfumA*+ DNA damage repair genes, or (**E**) *ΔfumB*+ DNA damage repair genes mutants, were grown to early-exponential phase (OD600nm=0.3), and treated with MMS (0.35% (v/v) for 45 minutes at 37$^{o}$C). The cells were then washed and serially diluted (spot test) onto LB plates and LB containing the indicated organic acid plates. Each result is representative of three independent experiments.

Differentially expressed genes (DEGs) log2FC ≥2.0

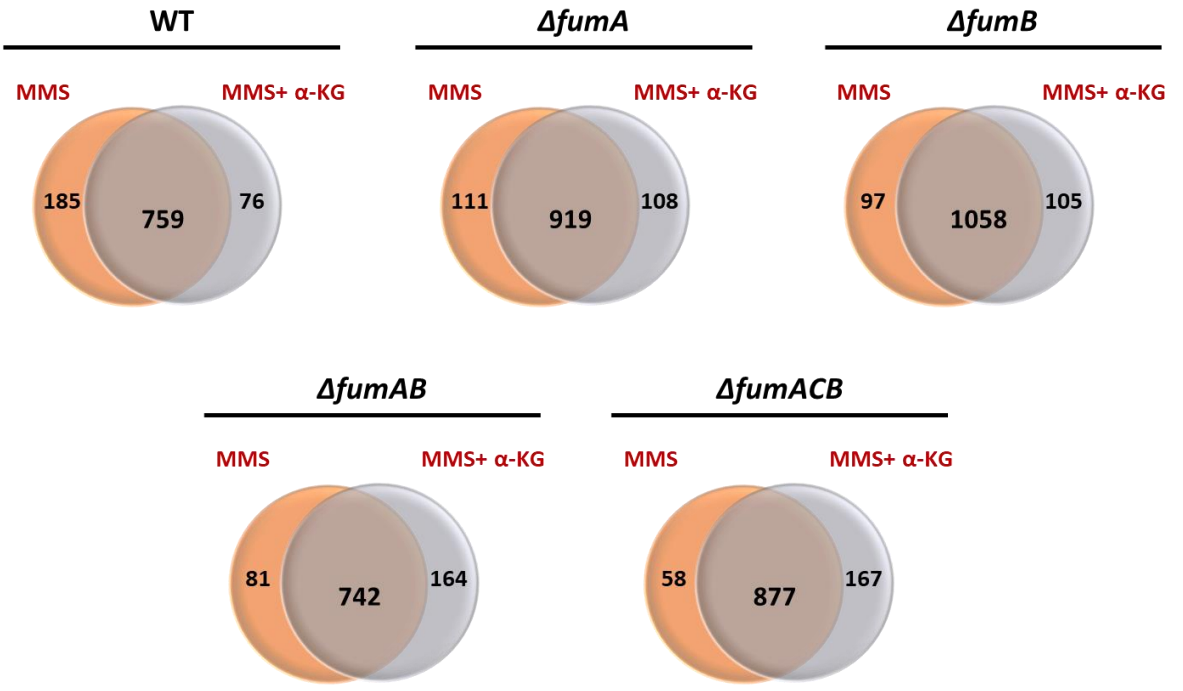
**Altered expression in WT vs *fum* null mutants**

**S4A**




**S4B**

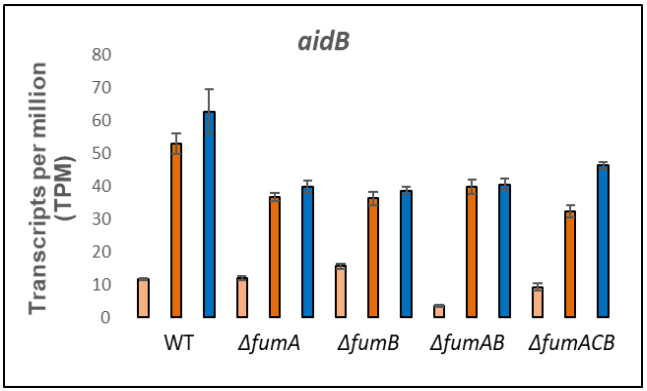
## α-KG effect on DEGs



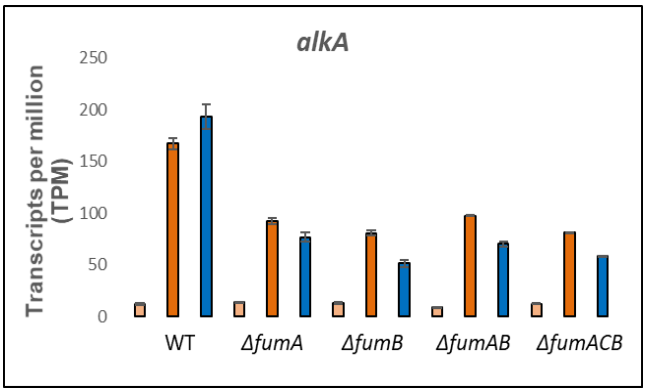
## mRNA expression (RNA-seq)

Control MMS MMS+α-KG

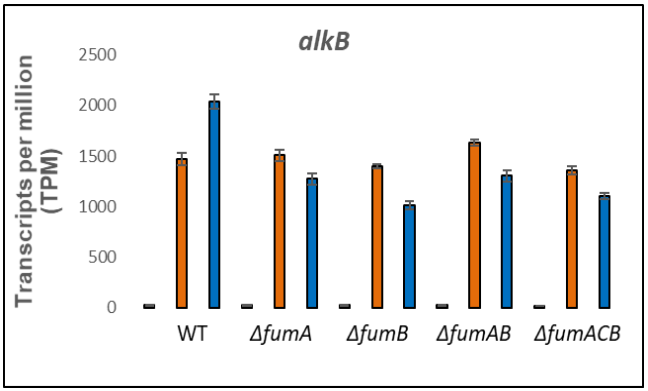
S4C



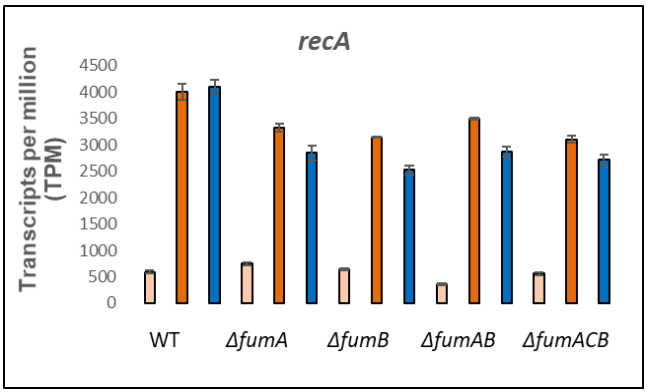

S4D



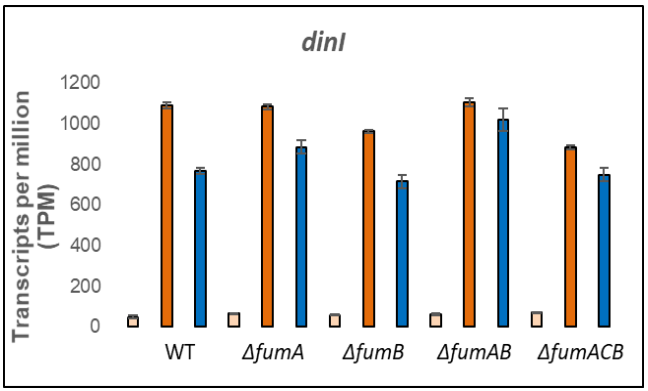

S4E



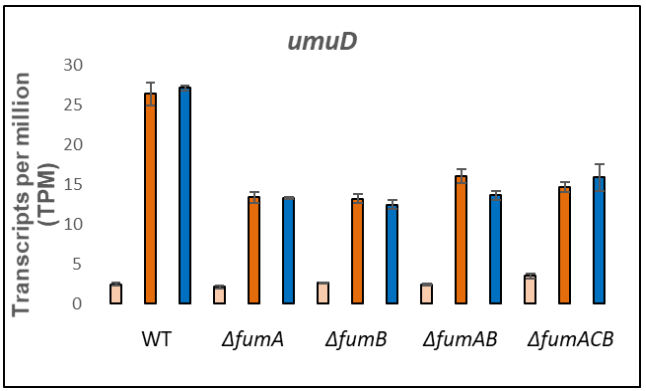

S4F



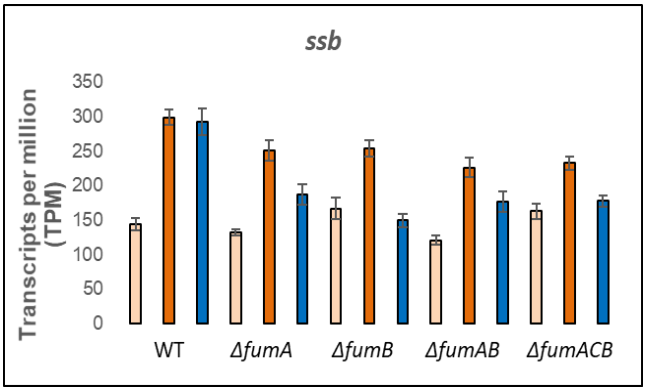

S4G




S4H



**S4I**

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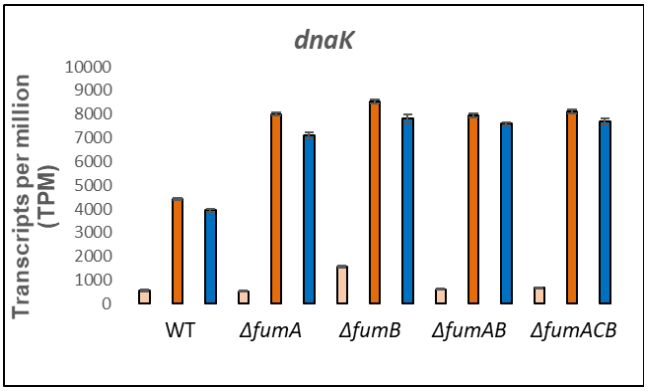
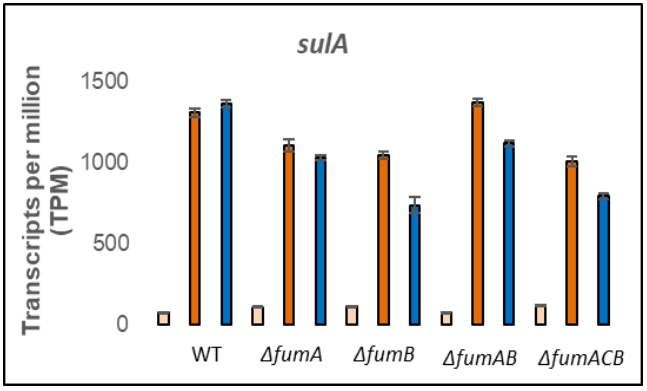
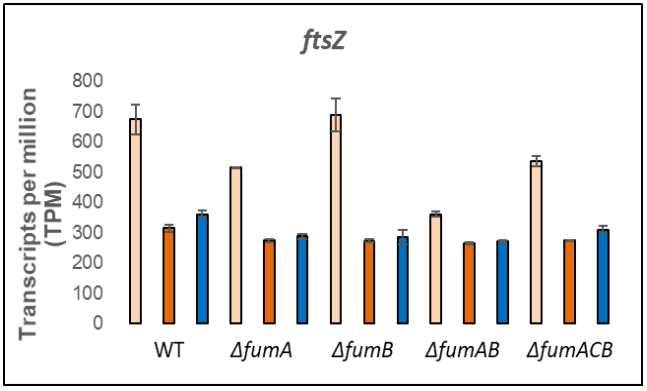


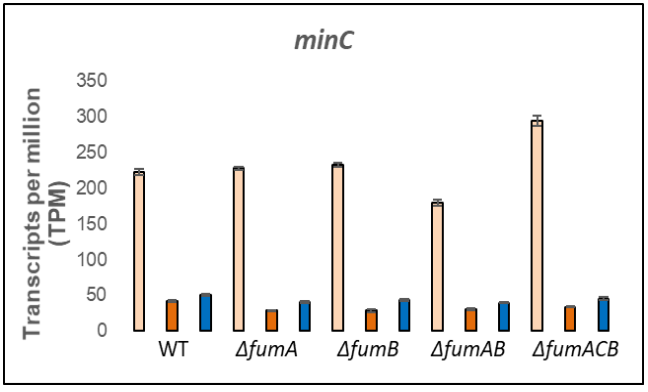

**S4K**




**S4L**




**S4M**




**S4N**

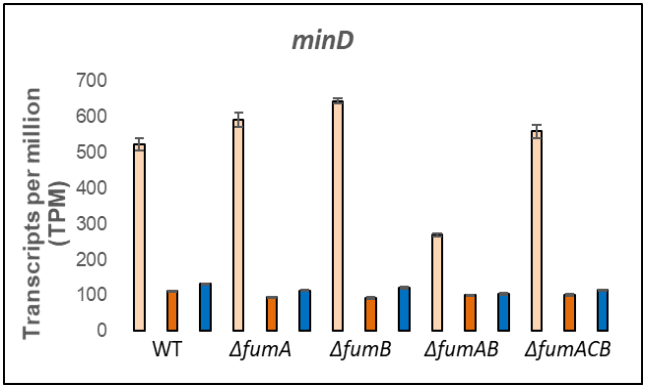


**Figure S4- RNA-seq analysis of differential expressed genes.**

RNA-seq analysis; *E. coli* wild type (WT), *ΔfumA*, *ΔfumB*, *ΔfumAB* and *ΔfumACB* strains were grown to early-exponential phase (OD600nm=0.3), and treated with MMS (0.35% MMS (v/v) for 30 min). The cells were collected and total RNA was extracted and subjected to RNA-seq analysis (see materials and methods). (**A**) Venn diagrams showing the common and differential genes (transcripts) between *E. coli* wild type (WT), *ΔfumA*, *ΔfumB, ΔfumAB* and *ΔfumACB* strains following treatment with MMS or MMS+25mM α-KG. (**B**) comparison of shared transcripts in the presence or absence of α-KG *in E. coli* wild type (WT), *ΔfumA, ΔfumB, ΔfumAB* and *ΔfumACB* strains. (**C-N**) Gene expression levels revealed by RNA-seq for (**C**) *aidB*, Putative acyl-CoA dehydrogenase AidB (**D**) *alkA*, DNA-3-methyladenine glycosylase 2 (**E**) *alkB*, Alpha-ketoglutarate-dependent dioxygenase AlkB (**F**) *recA*, Protein RecA (**G**) *dinI*, DNA damage-inducible protein I (**H**) *umuD*, Protein UmuD (**I**) *ssb*, single-stranded DNA-binding protein (**J**) *dnaK*, Chaperone protein DnaK (**K**) *sulA*, Cell division inhibitor SulA (**L**) *ftsZ*, Cell division protein FtsZ (**M**) *minC*, Septum site-determining protein MinC (**N**) *minD*, Septum site-determining protein *MinD*. TPM (transcripts per kilobase million) shown as Normalized gene expression from RNA-seq are mean ± standard error (n=3).

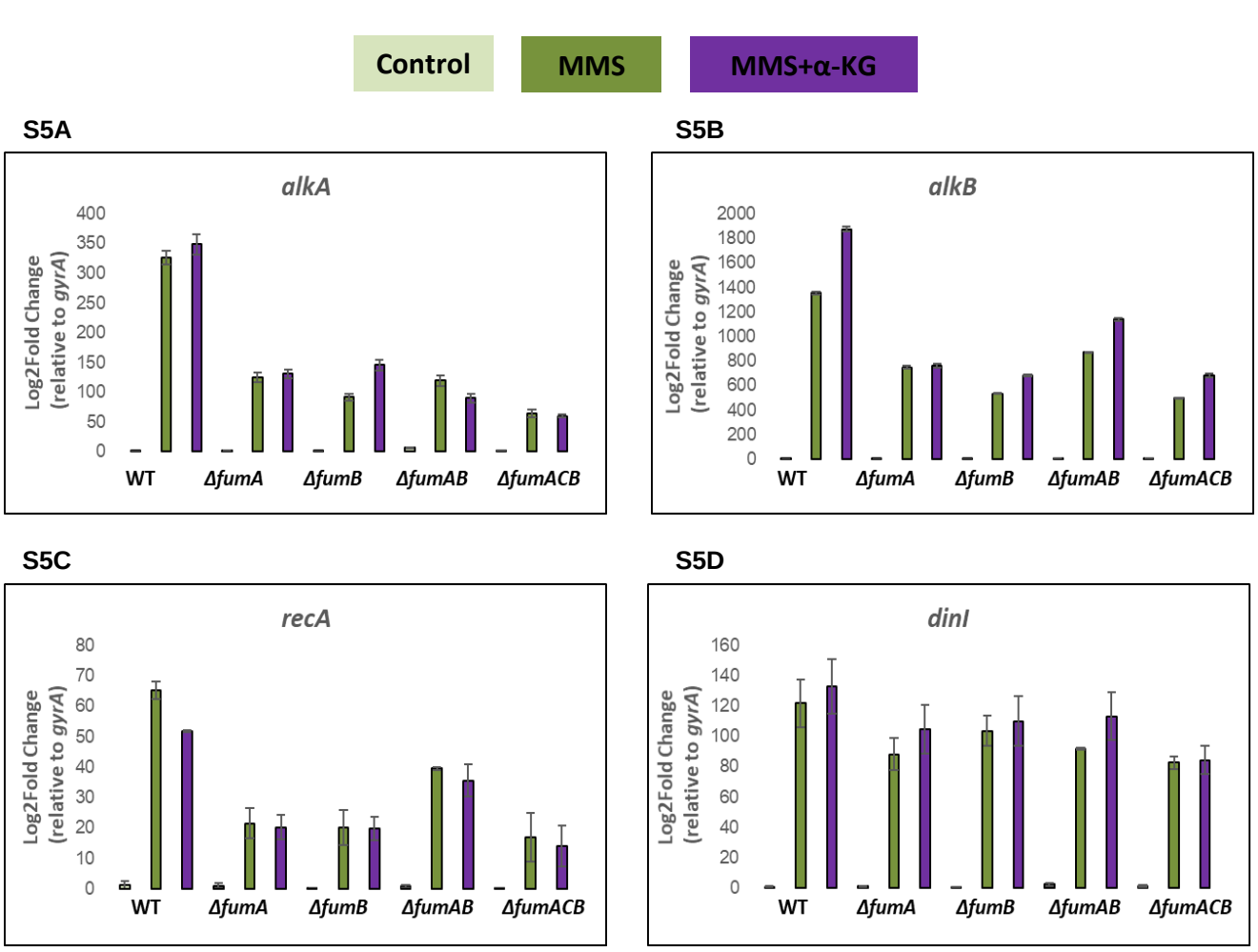
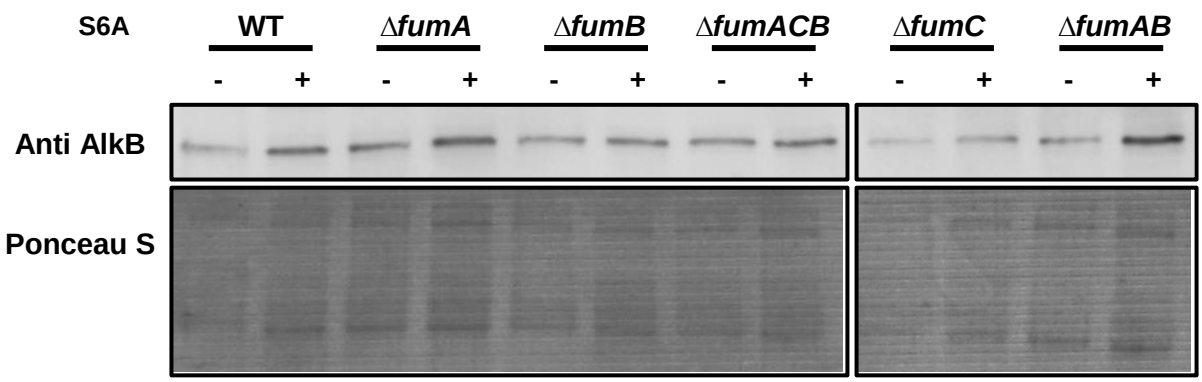



**Figure S5- Validation of RNA-seq data.** (**A-D**) *E. coli* wild type (WT), *ΔfumA, ΔfumB, ΔfumAB* and *ΔfumACB* strains were grown in LB and LB+25mM α-KG to early-exponential (OD600nm=0.3) phase, (OD 600nm=0.3), and treated with 0.35% [v/v] MMS for 35 minutes at 37°C. Cells were collected and total RNA was extracted. Graphs show the transcript

levels of (**A**) *alkA* (**B**) *alkB* (**C**) *recA* and (**D**) *dinI* normalized against *gyrA* as an internal control and calculated using the ΔΔCT method. Data is represented as mean ± standard error (*n=3).*


S6A
WT
ΔfumA
ΔfumB
ΔfumACB
ΔfumC
ΔfumAB
- + - + - + - + - + - +
Anti AlkB
Ponceau S

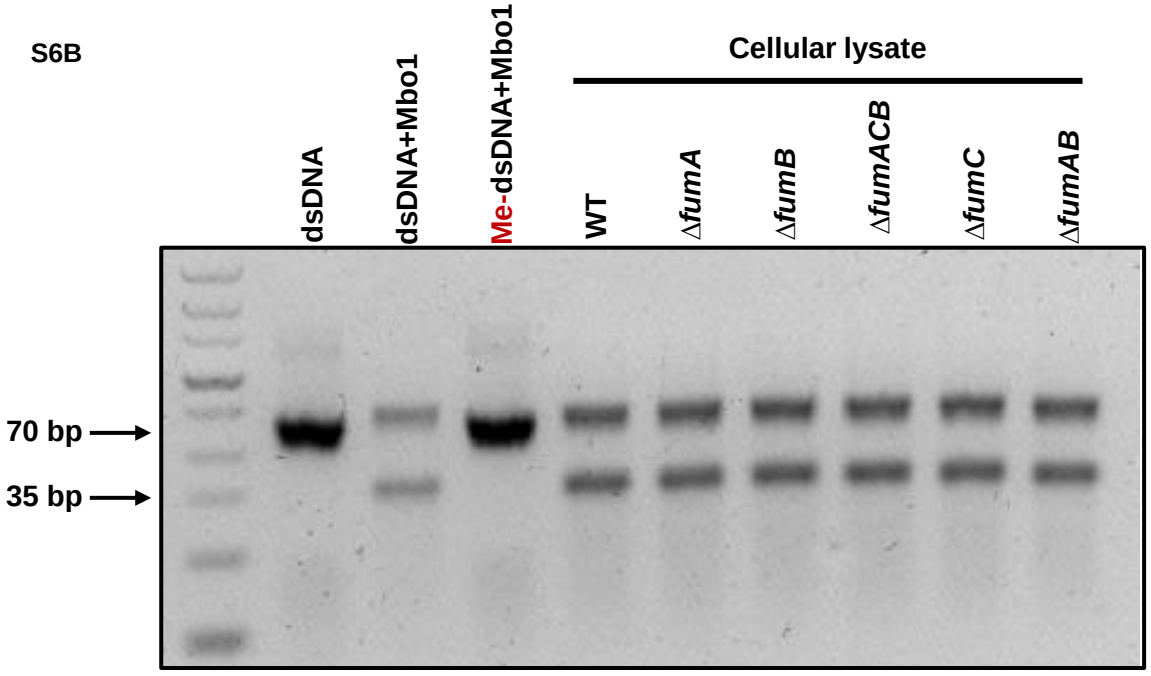

S6B
dsDNA
dsDNA+Mbo1
Me-dsDNA+Mbo1
Cellular lysate
WT
ΔfumA
ΔfumB
ΔfumACB
ΔfumC
ΔfumAB
70 bp
35 bp

**Figure S6- AlkB activity in *fum* null mutant strains. (A)** *E. coli* wild type (WT), *ΔfumA, ΔfumB, ΔfumC*, *ΔfumAB* and *ΔfumACB* strains were grown to mid-exponential phase (OD600nm=0.3) and treated with MMS (0.35% MMS (v/v) for 45 min). The cells were lysed and centrifuged to obtain a supernatant which was subjected AlkB in vitro repair assay, Western blotting (using the indicated antibodies) and Ponceau S staining. **(B)** Repair of methylated oligonucleotides for 4 hours at 37$^{o}$C by 40µM lysate from the indicated strains. Following in vitro repair, the reaction was mixed with equi-molar concentration of complementary ssDNA, the resultant dsDNA was digested with MboI and subjected to agarose electrophoresis and staining with safeU. Following successful demethylation, digestion with MboI produces a 35bp product.