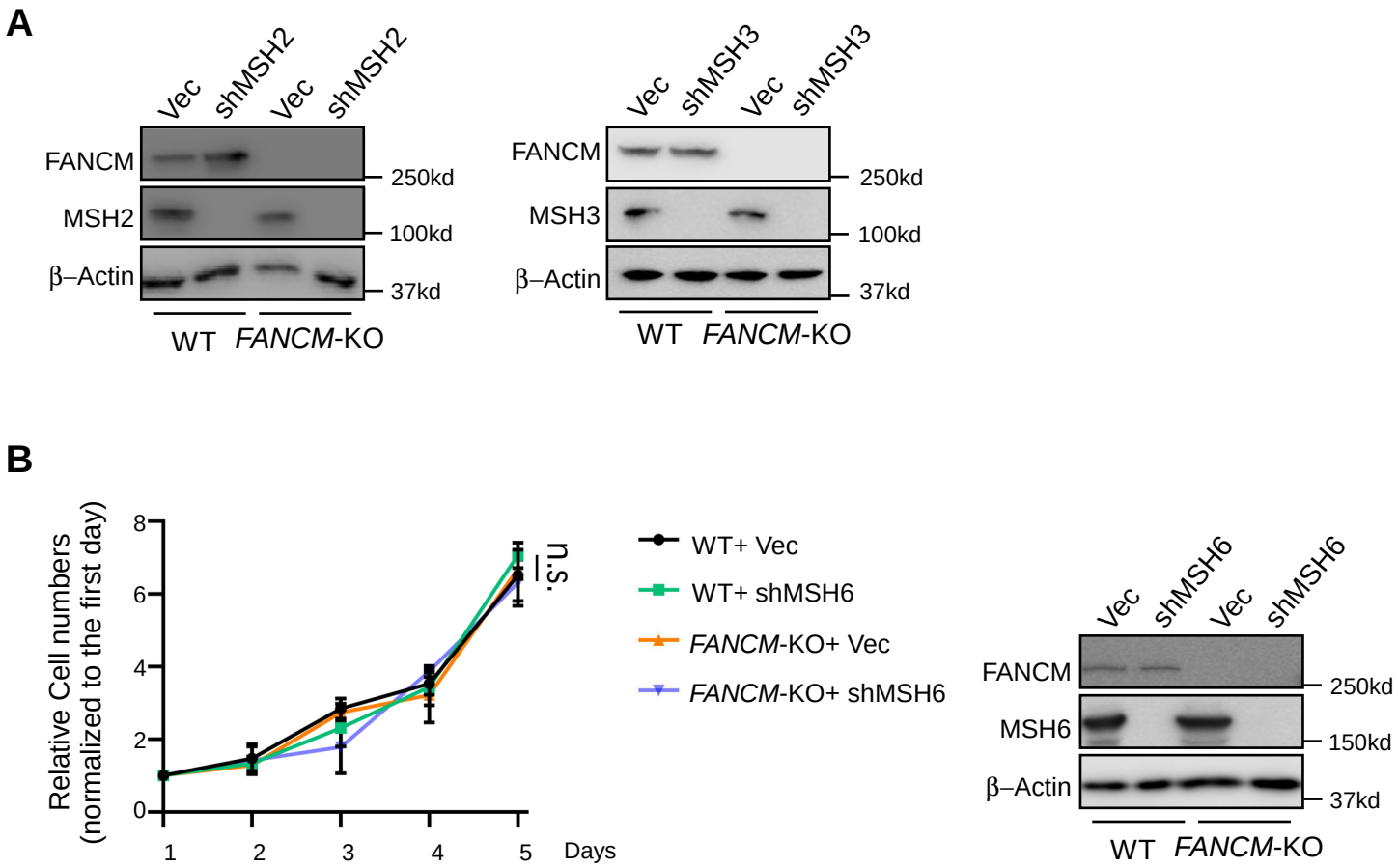
A
Vec
shMSH2
Vec
shMSH2
FANCM
MSH2
β–Actin
250kd
100kd
37kd
WT
FANCM-KO
Vec
shMSH3
Vec
shMSH3
FANCM
MSH3
β–Actin
250kd
100kd
37kd
WT
FANCM-KO
B
Relative Cell numbers
(normalized to the first day)
8
6
4
2
0
1
2
3
4
5
Days
n.s.
WT+ Vec
WT+ shMSH6
FANCM-KO+ Vec
FANCM-KO+ shMSH6
Vec
shMSH6
Vec
shMSH6
FANCM
MSH6
β–Actin
250kd
150kd
37kd
WT
FANCM-KO
C
Vec
shMSH2
MSH2
β–Actin
100kd
37kd
Vec
shMSH3
MSH3
β–Actin
100kd
37kd

Figure S1

Figure S1: MSH2/MSH3 is synthetically lethal with FANCM.
(A). Depletion of MSH2 or MSH3 in WT and *FANCM*-KO HCT116 cells was confirmed by Western blot analysis. These cell lines were used in the experiments shown in Figure 1A.
(B). Growth curves (left panel) of HCT116 WT and *FANCM*-KO cells were plotted after infection with lentiviruses expressing MSH6 shRNA or a vector control (Vec). Error bars represent the standard deviation of three independent experiments. Depletion of MSH6 and *FANCM* KO in HCT116 cells were confirmed by Western blot analysis (right panel).
(C). Depletion of MSH2 or MSH3 in HCT116 cells was confirmed by Western blot analysis. These cell lines were used in the experiments shown in Figure 1C.

Figure S1

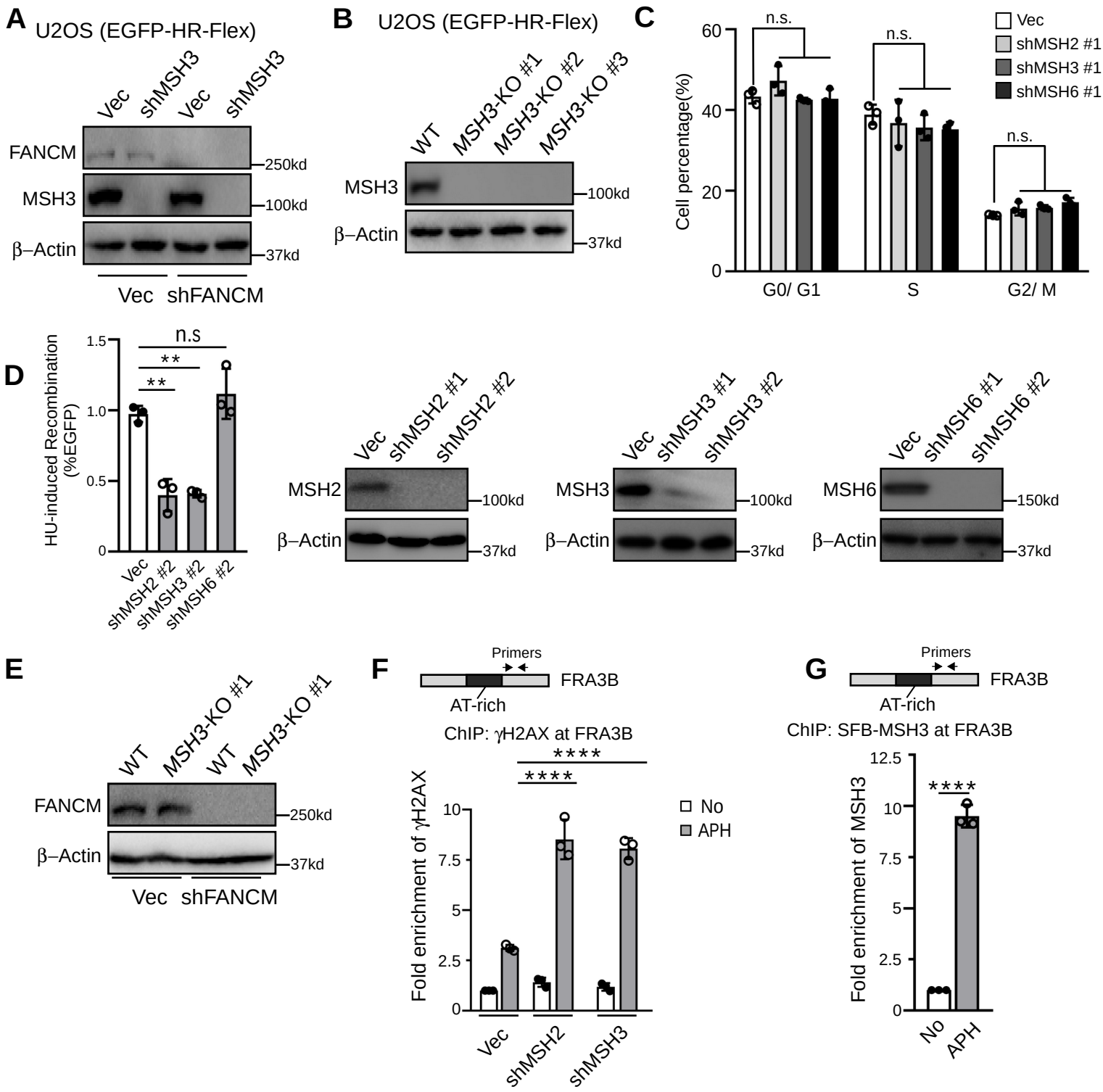
A
U2OS (EGFP-HR-Flex)
Vec
shMSH3
Vec
shMSH3
FANCM
MSH3
β–Actin
250kd
100kd
37kd
Vec
shFANCM
B
U2OS (EGFP-HR-Flex)
WT
MSH3-KO #1
MSH3-KO #2
MSH3-KO #3
MSH3
β–Actin
100kd
37kd
C
Cell percentage(%)
60
40
20
0
n.s.
n.s.
n.s.
Vec
shMSH2 #1
shMSH3 #1
shMSH6 #1
G0/ G1
S
G2/ M
D
HU-induced Recombination (%EGFP)
1.5
1.0
0.5
0
n.s
**
**
Vec
shMSH2 #2
shMSH3 #2
shMSH6 #2
Vec
shMSH2 #1
shMSH2 #2
MSH2
β–Actin
100kd
37kd
Vec
shMSH3 #1
shMSH3 #2
MSH3
β–Actin
100kd
37kd
Vec
shMSH6 #1
shMSH6 #2
MSH6
β–Actin
150kd
37kd
E
WT
MSH3-KO #1
WT
MSH3-KO #1
FANCM
β–Actin
250kd
37kd
Vec
shFANCM
F
Primers
FRA3B
AT-rich
ChIP: γH2AX at FRA3B
****
****
Fold enrichment of γH2AX
10
7.5
5
2.5
0
No
APH
Vec
shMSH2
shMSH3
G
Primers
FRA3B
AT-rich
ChIP: SFB-MSH3 at FRA3B
Fold enrichment of MSH3
12.5
10
7.5
5
2.5
0
****
No
APH
H
Vec
shMUS81
MUS81
β–Actin
55kd
37kd
I
Vec
shPCNA
PCNA
β–Actin
25kd
37kd
J
PCNA binding region
MSH3-WT
NLS
SFB
WT: 1-1137 aa
MSH3-ND
NLS
SFB
ND: 201-1137 aa
SFB-WT
SFB-ND
Flag
β–Actin
100kd
37kd

Figure S2

Figure S2: MutSβ is required for mitotic recombination at Flex1.
(A). Western blot analysis was performed to confirm MSH3 and FANCM depletion with β-Actin as a loading control. These cell lines were used in the experiments shown in Figure 2B.
(B). MSH3 was knocked out in U2OS (EGFP-HR-Flex) cells and three KO clones were subjected to MSH3 Western blot analysis. These cell lines were used in the experiments shown in Figure 2C.
(C). Cell cycle analysis was performed in U2OS (EGFP-HR-Flex) cells after infection with lentiviruses expressing MSH2, MSH3, MSH6 shRNAs or a vector control (Vec). The proportion of cells in G0/G1 phase, S phase, G2/M phase as a percentage of the total number of cells is shown.
(D). Western blot analysis was performed to confirm MSH2, MSH3, and MSH6 depletion with β-Actin as a loading control. These cell lines were used in the experiments shown in Figure 2D.
(E). Western blot analysis was performed to confirm FANCM depletion in U2OS (EGFP-HR-Flex) WT and *MSH3*-KO cells with β-Actin as a loading control. These cell lines were used in the experiments shown in Figure 2E, right.
(F). Anti-γH2AX ChIP analysis at FRA3B around AT-rich sequences was performed in HCT116 cells after depleting MSH2 or MSH3 with corresponding shRNA#1 or shRNA vector (Vec) and using indicated primer sets before and after APH treatment (0.8 μM, 16 h). The ChIP value in HCT116 cells with no treatment in the Vec control sample was set as 1 for normalization.
(G). Anti-streptavidin ChIP analysis at FRA3B around AT-rich sequences was performed in HCT116 cells expressing SFB-MSH3 before and after APH (0.8 μM, 16 h) treatment. The ChIP value in the HCT116 cells with no treatment was set as 1 for normalization.
(H, I). Western blot analysis was performed to confirm depletion of MUS81 (H) and PCNA (I) with β-Actin as a loading control. These cell lines were used in the experiments shown in Figure 2H and 2I.
(J). A schematic drawing depicts SFB-MSH3 and SFB-MSH3-DN (Δ1-200aa) (left). The expression of SFB-MSH3-WT and SFB-MSH3-DN is shown by anti-Flag Western with β-Actin as a loading control (right). SFB: S protein-2xFlag-Streptavidin-Binding peptide tag, NLS: nuclear localization signal. ND: N-terminal deletion. These cell lines were used in the experiments shown in Figure 2J.

Figure S2

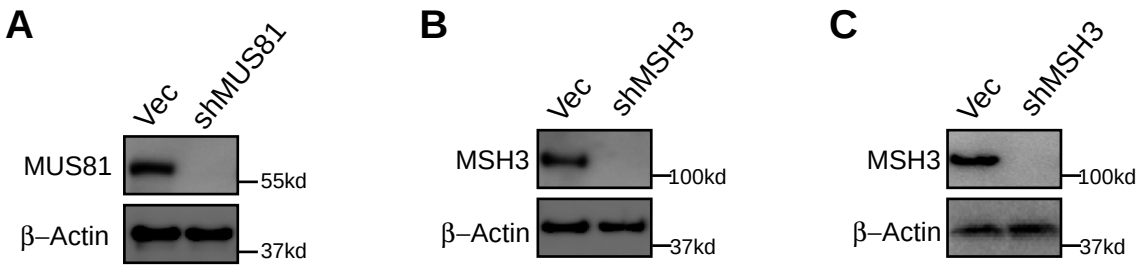
A
Vec
shMUS81
MUS81
55kd
β–Actin
37kd
B
Vec
shMSH3
MSH3
100kd
β–Actin
37kd
C
Vec
shMSH3
MSH3
100kd
β–Actin
37kd

Figure S3

Figure S3: MutSβ recruits RAD52 to Flex1 after DSB formation.
Western blot analysis was performed to show MUS81 depletion in U2OS (EGFP-HR-Flex) cells expressing SFB-RAD52 (A), MSH3 depletion in U2OS cells (B) and MSH3 depletion in U2OS (EGFP-HR-Flex) cells expressing SFB-RAD52 (C) with β-Actin as a loading control. These cell lines were used in the experiments shown in Figure 3A-C.

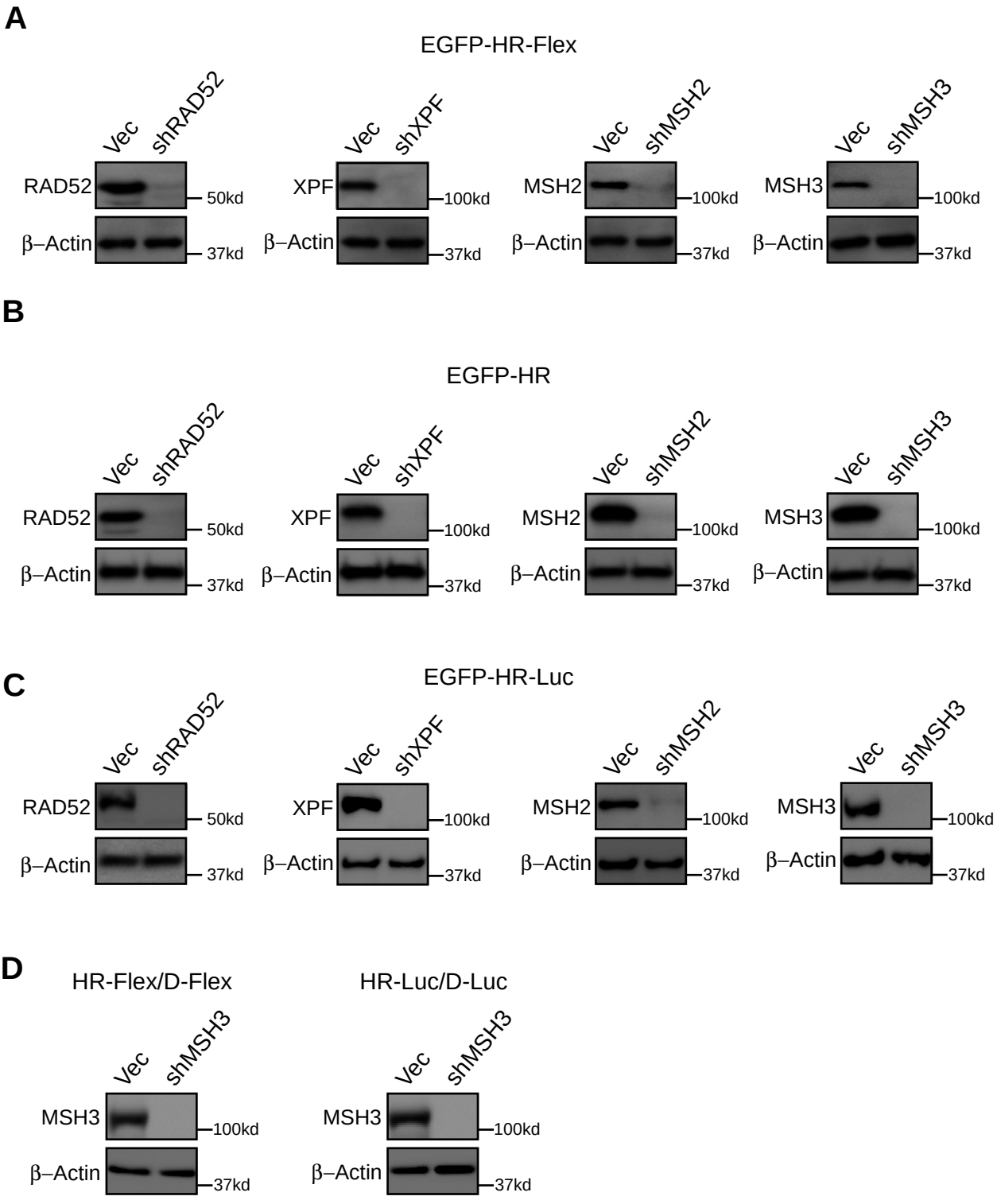
A
EGFP-HR-Flex
Vec
shRAD52
RAD52
50kd
β–Actin
37kd
Vec
shXPF
XPF
100kd
β–Actin
37kd
Vec
shMSH2
MSH2
100kd
β–Actin
37kd
Vec
shMSH3
MSH3
100kd
β–Actin
37kd
B
EGFP-HR
Vec
shRAD52
RAD52
50kd
β–Actin
37kd
Vec
shXPF
XPF
100kd
β–Actin
37kd
Vec
shMSH2
MSH2
100kd
β–Actin
37kd
Vec
shMSH3
MSH3
100kd
β–Actin
37kd
C
EGFP-HR-Luc
Vec
shRAD52
RAD52
50kd
β–Actin
37kd
Vec
shXPF
XPF
100kd
β–Actin
37kd
Vec
shMSH2
MSH2
100kd
β–Actin
37kd
Vec
shMSH3
MSH3
100kd
β–Actin
37kd
D
HR-Flex/D-Flex
Vec
shMSH3
MSH3
100kd
β–Actin
37kd
HR-Luc/D-Luc
Vec
shMSH3
MSH3
100kd
β–Actin
37kd

Figure S4

Figure S4: MutSβ is important for removing Flex1 at DSBs to complete HR.
(A-C). Western blot analysis was performed to confirm RAD52, XPF, MSH2 and MSH3 depletion in U2OS (EGFP-HR-Flex) cells (A), U2OS (EGFP-HR) cells (B) and U2OS (EGFP-HR-Luc) cells (C) with β-Actin as a loading control. These cell lines were used in the experiments shown in Figure 4A and 4B.
(D). Western blot analysis was performed to confirm MSH3 depletion in U2OS (HR-Flex/D-Flex) and U2OS (HR-Luc/D-Luc) with β-Actin as a loading control. These cell lines were used in the experiments shown in Figure 4C.

Figure S4

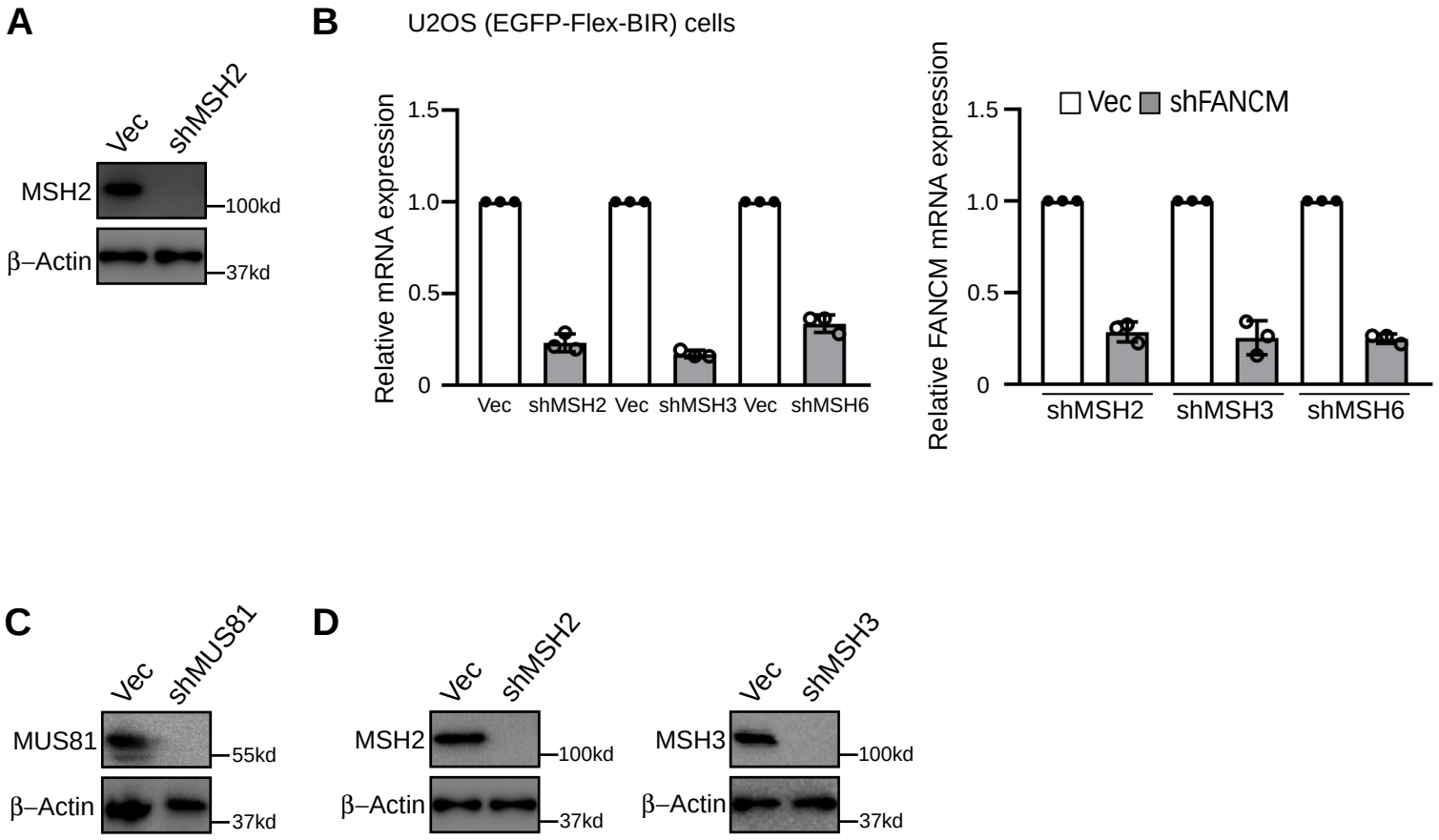
A
Vec
shMSH2
MSH2
100kd
β–Actin
37kd
B
U2OS (EGFP-Flex-BIR) cells
Relative mRNA expression
1.5
1.0
0.5
0
Vec
shMSH2
Vec
shMSH3
Vec
shMSH6
Vec
shFANCM
Relative FANCM mRNA expression
shMSH2
shMSH3
shMSH6
C
Vec
shMUS81
MUS81
55kd
β–Actin
37kd
D
Vec
shMSH2
MSH2
100kd
β–Actin
37kd
Vec
shMSH3
MSH3
100kd
β–Actin
37kd

Figure S5

Figure S5: MutSβ is important for MiDAS and preserving CFS stability.
(A). Western blot analysis was perform to confirm MSH2 depletion in U2OS cells with β-Actin as a loading control. These cell lines were used in the experiments shown in Figure 6A.
(B). qPCR was performed to show depletion of MSH2, MSH3 and MSH6 by shRNAs followed by FANCM depletion in U2OS (EGFP-Flex-BIR) cells. These cell lines were used in the experiments shown in Figure 6B.
(C). Western blot analysis was perform to confirm MUS81 depletion in U2OS cells with β-Actin as a loading control. These cell lines were used in the experiments shown in Figure 6E.
(D). Western blot analysis was perform to confirm MSH2 and MSH3 depletion in HCT116 cells with β-Actin as a loading control.

Figure S5