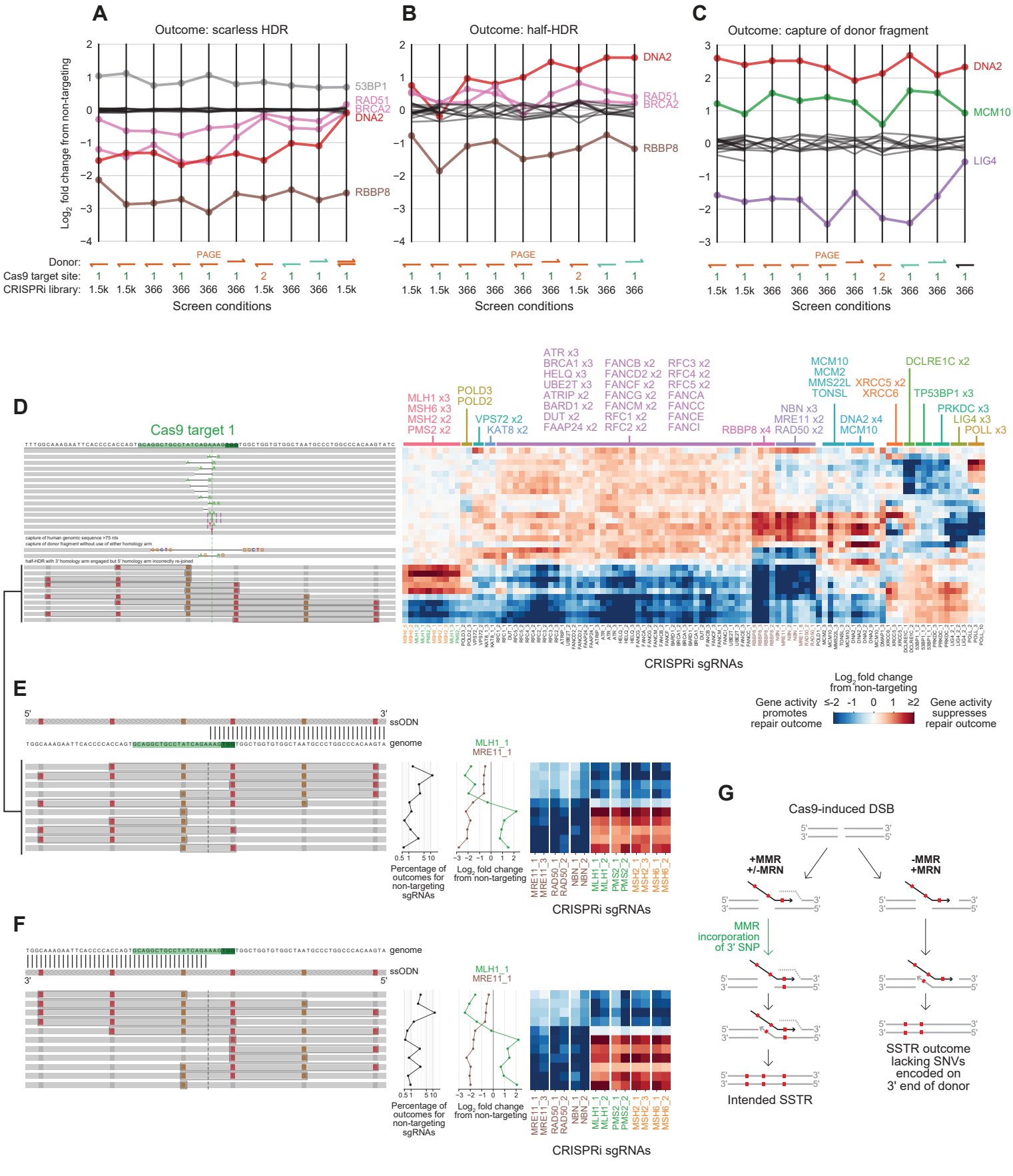
A
Outcome: scarless HDR
B
Outcome: half-HDR
C
Outcome: capture of donor fragment
Log2 fold change from non-targeting
53BP1
RAD51
BRCA2
DNA2
RBBP8
MCM10
LIG4
PAGE
Donor:
Cas9 target site:
CRISPRi library:
Screen conditions
D
Cas9 target 1
capture of human genomic sequence >75 nts
capture of donor fragment without use of either homology arm
half-HDR with 3' homology arm engaged but 5' homology arm incorrectly re-joined
MLH1 x3
MSH6 x3
MSH2 x2
PMS2 x2
POLD3
POLD2
VPS72 x2
KAT8 x2
ATR x3
BRCA1 x3
HELQ x3
UBE2T x3
ATRIP x2
BARD1 x2
DUT x2
FAAP24 x2
FANCB x2
FANCD2 x2
FANCF x2
FANCG x2
FANCM x2
RFC1 x2
RFC2 x2
RFC3 x2
RFC4 x2
RFC5 x2
FANCA
FANCC
FANCE
FANCI
RBBP8 x4
NBN x3
MRE11 x2
RAD50 x2
DNA2 x4
MCM10
MCM10
MCM2
MMS22L
TONSL
XRCC5 x2
XRCC6
DCLRE1C x2
TP53BP1 x3
PRKDC x3
LIG4 x3
POLL x3
CRISPRi sgRNAs
Log2 fold change from non-targeting
Gene activity promotes repair outcome
≤-2 -1 0 1 ≥2
Gene activity suppresses repair outcome
E
5'
3'
ssODN
genome
TGGCAAAGAATTCACCCCACCAGTGCAGGCTGCCTATCAGAAAGGTGGCTGGTGTGGCTAATGCCCTGGCCCACAAGTA
MLH1_1
MRE11_1
Percentage of outcomes for non-targeting sgRNAs
Log2 fold change from non-targeting
MRE11_1
MRE11_3
RAD50_1
RAD50_2
NBN_1
NBN_2
MLH1_1
MLH1_2
PMS2_1
PMS2_2
MSH2_1
MSH2_3
MSH6_1
MSH6_2
CRISPRi sgRNAs
F
genome
ssODN
3'
5'
MLH1_1
MRE11_1
Percentage of outcomes for non-targeting sgRNAs
Log2 fold change from non-targeting
CRISPRi sgRNAs
G
Cas9-induced DSB
+MMR
+/-MRN
-MMR
+MRN
MMR incorporation of 3' SNP
Intended SSTR
SSTR outcome lacking SNVs encoded on 3' end of donor