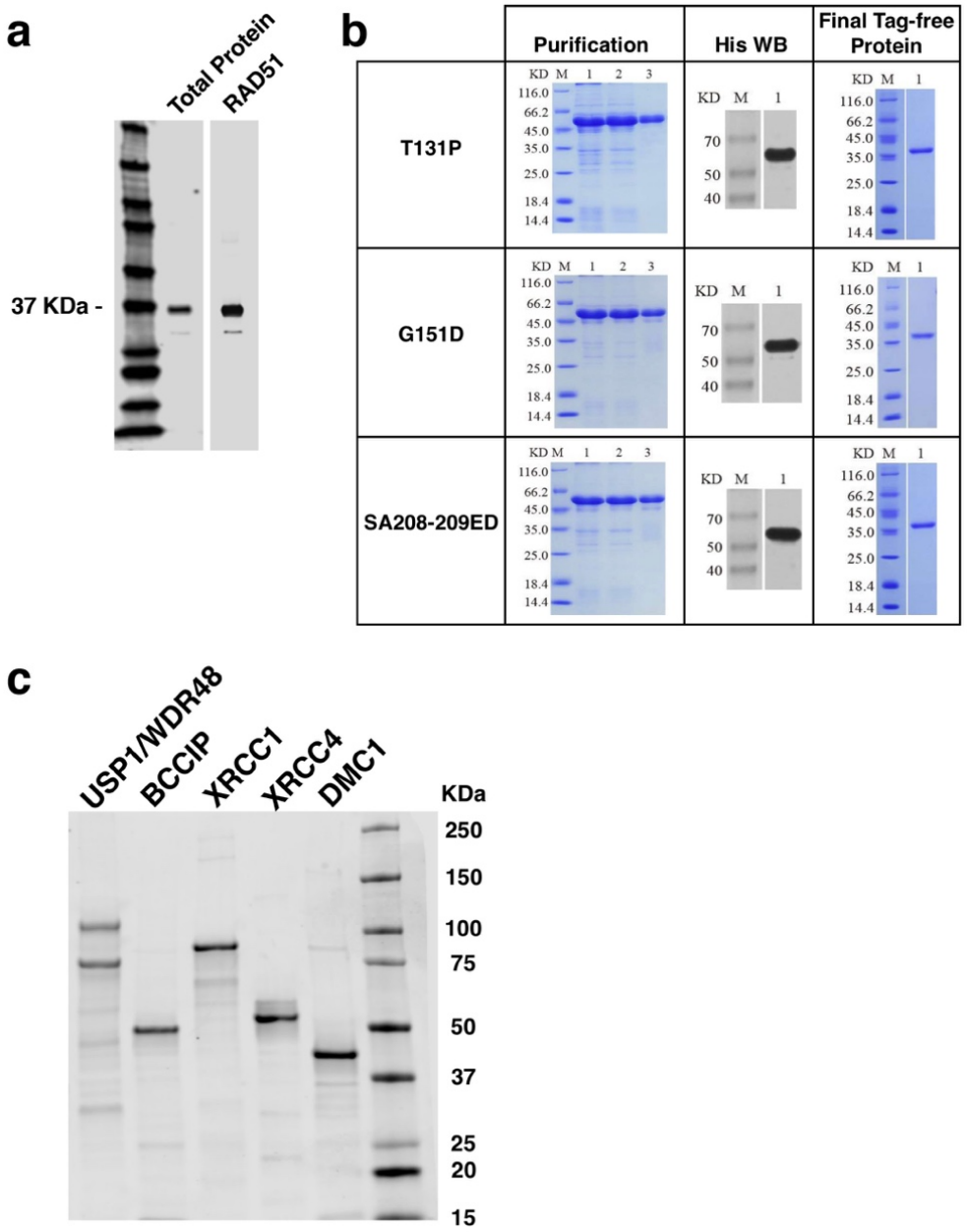

Supplementary Figure 1 | Verification of Recombinant Protein Identity and Purity. **a,** Total protein staining and Western blotting of recombinant RAD51. Membrane was stained for total protein, imaged, then stripped and probed with an anti-RAD51 antibody. **b,** Coomassie staining (left, prior to His-tag removal; right, after tag removal) and His-tag Western blotting of recombinant RAD51 mutants produced for injections described in **Figure 4**. **c,** Total protein staining of recombinant DSB repair- and HR-associated proteins used in **Figure 4c** (protein marker on far-right).

Component	Sequence
tracrRNA	AAACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGU UAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU
Chd2^{R1684H} crRNA	GCGGUAGCUCCCAGAACGGUGUUUUAGAGCUAUGC UGUUUUG
Chd2^{R1684H} ssODN	TCAGTATGAGCAGCATTGGTATAAGGACCACCACTA TGGTGACCGGAGGCATATGGATGCTCACCATTCTGG GAGCTACCGCCCTAACAACATGTCCAGAAAGAGGCC GTATGAGCAGTACAACAG
Tyr^{C89S} crRNA	GAAGUUGCCUGAGCACUGGCGUUUUAGAGCUAUGC UGUUUUG
Tyr^{C89S} ssODN	GTTCCCCTTCAAAGGGGTGGATGACCGTGAGTCCTGG CCCTCTGTGTTTTATAATAGGACAAGCCAGTGCTCAG GCAACTTCATGGGTTTCAACTGCGGAAACTGTAAGTT TGGATTTGGGG

Supplementary Table 1 | Sequences of tracrRNA, crRNAs, and HR donors. Guide sequences are highlighted in red.

RAD51 Mutant	DNA Sequence
T131P	atggcaatgcagatgcagcttgaagcaaatgcagatacttcagtggaagaagaaagctttggcccacaacccatttcacggttag agcagtgtggcataaatgccaacgatgtgaagaaattggaagaagctggattccatactgtggaggctgttgcctatgcgccaaag aaggagctaataaatattaagggaattagtgaagccaaagctgataaaattctgacggagtctcgctctgttgccaggctggagtg caatagcgtgatcttggtctactgcaccctccgcctctcaggttcaagtgattctcctgcctcagcctcccgagtagttgggactacag gtggaattgagactggatctatcacagaaatgtttggagaattccga**c**ctgggaagacccagatctgtcatacgctagctgtcacct gccagcttcccattgaccggggtggaggtgaaggaaaggccatgtacattgacactgagggtacctttaggccagaacggctgct ggcagtggctgagaggtatggtctctctggcagtgatgtcctggataatgtagcatatgctcgagcgttcaacacagaccaccagac ccagctcctttatcaagcatcagccatgatggtagaatctaggtatgcactgcttattgtagacagtgccaccgcccttacagaaca gactactcgggtcgaggtgagctttcagccaggcagatgcacttggccaggtttctgcggatgcttctgcgactcgctgatgagtttgg tgtagcagtggtaatcactaatcaggtggtagctcaagtggatggagcagcgatgtttgctgctgatcccaaaaaacctattggagg aaatatcatcgcccatgcatcaacaaccagattgtatctgaggaaaggaagaggggaaaccagaatctgcaaaatctacgactc tccctgtcttcctgaagctgaagctatgttcgccattaatgcagatggagtgggagatgccaaagactga
G151D	atggcaatgcagatgcagcttgaagcaaatgcagatacttcagtggaagaagaaagctttggcccacaacccatttcacggttag agcagtgtggcataaatgccaacgatgtgaagaaattggaagaagctggattccatactgtggaggctgttgcctatgcgccaaag aaggagctaataaatattaagggaattagtgaagccaaagctgataaaattctgacggagtctcgctctgttgccaggctggagtg caatagcgtgatcttggtctactgcaccctccgcctctcaggttcaagtgattctcctgcctcagcctcccgagtagttgggactacag gtggaattgagactggatctatcacagaaatgtttggagaattccgaactgggaagacccagatctgtcatacgctagctgtcacct gccagcttcccattgaccggg**a**tggaggtgaaggaaaggccatgtacattgacactgagggtacctttaggccagaacggctgct ggcagtggctgagaggtatggtctctctggcagtgatgtcctggataatgtagcatatgctcgagcgttcaacacagaccaccagac ccagctcctttatcaagcatcagccatgatggtagaatctaggtatgcactgcttattgtagacagtgccaccgcccttacagaaca gactactcgggtcgaggtgagctttcagccaggcagatgcacttggccaggtttctgcggatgcttctgcgactcgctgatgagtttgg tgtagcagtggtaatcactaatcaggtggtagctcaagtggatggagcagcgatgtttgctgctgatcccaaaaaacctattggagg aaatatcatcgcccatgcatcaacaaccagattgtatctgaggaaaggaagaggggaaaccagaatctgcaaaatctacgactc tccctgtcttcctgaagctgaagctatgttcgccattaatgcagatggagtgggagatgccaaagactga
SA208-209ED	atggcaatgcagatgcagcttgaagcaaatgcagatacttcagtggaagaagaaagctttggcccacaacccatttcacggttag agcagtgtggcataaatgccaacgatgtgaagaaattggaagaagctggattccatactgtggaggctgttgcctatgcgccaaag aaggagctaataaatattaagggaattagtgaagccaaagctgataaaattctgacggagtctcgctctgttgccaggctggagtg caatagcgtgatcttggtctactgcaccctccgcctctcaggttcaagtgattctcctgcctcagcctcccgagtagttgggactacag gtggaattgagactggatctatcacagaaatgtttggagaattccgaactgggaagacccagatctgtcatacgctagctgtcacct gccagcttcccattgaccggggtggaggtgaaggaaaggccatgtacattgacactgagggtacctttaggccagaacggctgct ggcagtggctgagaggtatggtctctctggcagtgatgtcctggataatgtagcatatgctcgagcgttcaacacagaccaccagac ccagctcctttatcaagca**ga**ag**a**catgatggtagaatctaggtatgcactgcttattgtagacagtgccaccgcccttacagaaca gactactcgggtcgaggtgagctttcagccaggcagatgcacttggccaggtttctgcggatgcttctgcgactcgctgatgagtttgg tgtagcagtggtaatcactaatcaggtggtagctcaagtggatggagcagcgatgtttgctgctgatcccaaaaaacctattggagg aaatatcatcgcccatgcatcaacaaccagattgtatctgaggaaaggaagaggggaaaccagaatctgcaaaatctacgactc tccctgtcttcctgaagctgaagctatgttcgccattaatgcagatggagtgggagatgccaaagactga

Supplementary Table 2 | DNA Sequences for the Cloning and Production of Recombinant RAD51 Mutant Proteins. Mutated nucleotides are **bold, underlined, and highlighted in red.**

Forward Primer	Sequence	Reverse Primer	Sequence	Product Size (bp)
C2RH-Long_F1	ACTGACACATGGGAGAAGCC	C2RH-Long_R1	TCTCCTTATAACAGGCCAACC	429
C2RH-short_F1	AGTGCCTCACCTCTCACACC	C2RH-short_R1	ACTGGCAGAGGGAAAGAAAG	266
C2RH-Long_F2	TCACTGGGTCACAAGCAAAG	C2RH-Long_R2	GAAGCAGATGCCCATCTCAG	1279
C2RH-Short_F2	ACTGACACATGGGAGAAGCC	C2RH-Short_R2	GATCGAGGAGACTGGCAGAG	358
Tyr-Long_F	TCAATTTAGTTACCTCACTATGGGC	Tyr-Long_R	CAAGTACTCATCTGTGCAAATGTC	992
Tyr-Short_F	TTTGGCCATAGGTGCCTG	Tyr-Short _R	GAGCCTGTGCCTCCTCTAAG	411
Shank3-Long_F	AGGAAAGCAAGGTTGAGCTG	Shank3-Long_R	CTCTGAGGCTTGCAGACGG	584
Shank3-Short_F	GAGCTCTACTCCCTTAGGACTT	Shank3-Short_R	TCCCCCTTTCACTGGACACCC	316
Gapdh-Long_F	TACGGGTGCACGTAGCTCAG	Gapdh-Long_R	CGAAGGACACCAGGCAGTC	396
Gapdh-Short_F	TCCCTAGACCCGTACAGTGC	Gapdh -Short_R	CTCTGCTCCTCCCTGTTCC	133

Supplementary Table 3 | Sequences of PCR primers and expected product sizes. C2RH-Long/short_F1/R1 primers were used for experiments performed in **Figures 1-3**, as well as **Extended Data Figures 1 and 2**. In an attempt to further rule out large deletions as a source of apparent homozygosity, we designed a new genotyping strategy with an initial ~1.3kb amplimer and used primer sets C2RH-Long/Short_F2/R2 for all other experiments.