

Table S1 REC3 domain in conformation-4OO8 has more residues that within 5Å of the hybrid than other conformations.

PDB ID	Hybrid (PAM proximal)	REC3 (497-712) residues within 5Å of the hybrid
6O0Z	RNA:1-18 DNA:1-18	497N, 510K, 515Y, 659W, 661R, (absent: V530-P537, C574-N588, D672-G676, D686-A689)
6O0Y	RNA:1-20 DNA:1-3, 4-20	497N, 510K, 515Y, 518F, 519T, 526K, 582G, 583V, 584E, 588N, 589A, 627E, 657T, 658G, 659W, 660G, 661R, 662L, 692N, 693F, 694M, 695Q, 698H,
5F9R	RNA:1-20 DNA:1-20	497N, 515Y, 518F, 519T, 522N, 526K, 581S, 582G, 583V, 584E, 588N, 589A, 624T, 657T, 658G, 659W, 660G, 661R, 662L, 692N, 693F, 694M, 695Q, 698H
4OO8	RNA:1-20 DNA:1-20	497N, 500K, 507V, 510K, 515Y, 518F, 519T, 522N, 523E, 526K, 528K, 539F, 581S, 582G, 583V, 584E, 588N, 589A, 624T, 627E, 657T, 658G, 659W, 660G, 661R, 662L, 692N, 693F, 694M, 695Q, 698H
4UN3	RNA:1-20 DNA:1-20	497N, 500K, 510K, 515Y, 518F, 519T, 522N, 526K, 581S, 582G, 583V, 584E, 588N, 589A, 624T, 627E, 657T, 658G, 659W, 660G, 661R, 662L, 692N, 693F, 694M, 695Q, 698H, (absent: 711A-712Q)

Table S2.1 On and off-target sequences.

Definition	NCBI Reference Sequence	Region	Gene site	Spacer (5' - 3') /Non-target strand	PAM (5' - 3')	sgRNA (5' - 3') guide sequence
Homo sapiens chromosome 2, GRCh38.p13 Primary Assembly	NC_000002.12	72933853 to 72933875	EMX1-1	GAGTCCGAGCAGAAGAAGAA	GGG	GAGUCCGAGCAGAAGAAGAA
Homo sapiens chromosome 5, GRCh38.p13 Primary Assembly	NC_000005.10	45358981 to 45358959	EMX1-1-OT1	GAGTTAGAGCAGAAGAAGAA	AGG	
Homo sapiens chromosome 15, GRCh38.p13 Primary Assembly	NC_000015.10	43817549 to 43817571	EMX1-1-OT2	GAGTCTAAGCAGAAGAAGAA	GAG	
Homo sapiens chromosome 2, GRCh38.p13 Primary Assembly	NC_000002.12	72933937 to 72933959	EMX1-2	GTCACCTCCAATGACTAGGG	TGG	GUCACCUCCAAUGACUAGGG
Homo sapiens chromosome 1, GRCh38.p13 Primary Assembly	NC_000001.11	187475834 to 187475856	EMX1-2-OT2	GCTACCTCCAGTGACTAGGG	AAG	
Homo sapiens chromosome 18 genomic patch of type FIX, GRCh38.p13 PATCHES HG2213_PATCH	NW_013171814.1	201394 to 201416	EMX1-2-OT3	ACAACCCCAATGACCAGGG	AGG	
Homo sapiens chromosome 17, GRCh38.p13 Primary Assembly	NC_000017.11	73140959 to 73140981	EMX1-2-OT4	GTCACCTGTAATGACTAGGG	AGA	
Homo sapiens chromosome 11, GRCh38.p13 Primary Assembly	NC_000011.10	22625792 to 22625814	FANCF-1	GCTGCAGAAGGGATTCCATG	AGG	GCUGCAGAAGGGAUCCAUG
Homo sapiens chromosome 22, GRCh38.p13 Primary Assembly	NC_000022.11	36556970 to 36556948	FANCF-1-OT1	GCTGCAGAAGGGATTCCAAG	GGG	
Homo sapiens chromosome 6, GRCh38.p13 Primary Assembly	NC_000006.12	27764865 to 27764843	FANCF-1-OT2	GACGCAGAAGGGACTCCATG	GGG	
Homo sapiens chromosome 17, GRCh38.p13 Primary Assembly	NC_000017.11	59421407 to 59421385	FANCF-1-OT3	GGTACAGAAGGGCTTCCATG	AGG	
Homo sapiens chromosome 11, GRCh38.p13 Primary Assembly	NC_000011.10	22625610 to 22625632	FANCF-3	GGCGGCTGCACAACCACTGG	AGG	GGCGGCUGCACAACCAGUGG
Homo sapiens chromosome 19, GRCh38.p13 Primary Assembly	NC_000019.10	3511570 to 3511592	FANCF-3-OT2	ATAGGCTGCACCCCACTGG	GGG	
Homo sapiens chromosome 20, GRCh38.p13 Primary Assembly	NC_000020.11	41510398 to 41510420	FANCF-3-OT4	GCAAGGCTACACAACCACTGA	GGG	
Homo sapiens chromosome 6, GRCh38.p13 Primary Assembly	NC_000006.12	43769554 to 43769576	VEGFA-5	GGGTGGGGGGAGTTTGCTCC	TGG	GGGUGGGGGGAGUUUGCUC
Homo sapiens chromosome 5, GRCh38.p13 Primary Assembly	NC_000005.10	32945152 to 32945174	VEGFA-5-OT1	GCGTGGGGGGTGTGTGCTCC	CGG	
Homo sapiens chromosome 17, GRCh38.p13 Primary Assembly	NC_000017.11	41640070 to 41640092	VEGFA-5-OT2	TAGTGGAGGGAGCTTGCTCC	TGG	
Homo sapiens chromosome 6, GRCh38.p13 Primary Assembly	NC_000006.12	43770819 to 43770841	VEGFA-1	GACCCCTCCACCCCGCCTC	CGG	GACCCCUCCACCCCGCCUC
Homo sapiens chromosome 6, GRCh38.p13 Primary Assembly	NC_000006.12	43769717 to 43769739	VEGFA-2	GGTGAGTGAGTGTGTGCGTG	TGG	GGUGAGUGAGUGUGUGCGUG

Table S2.2 Primers and probes of ddPCR.

Gene site	Forward primer	Reference probe	Drop-off probe	Reverse primer	Annealing temperature (°C)	Wild-type amplicon size (bp)
EMX1-1	cggaggacaaagtacaaacgg	ccattggcctgcttcgtggcaatgcg (5' 6-FAM, 3' BHQ1)	cgagcagaagaagaag (5' VIC, 3' MGB)	gtcattggagggtgacatcgatg	60	144
EMX1-1-OT1	gtacacctgtacatctgcacaag	ctgtcttggccatgccataagcccctatt (5' 6-FAM, 3' BHQ1)	atgcctttctcttc (5' VIC, 3' MGB)	aagaaatgcccaatcattgatgc	60	148
EMX1-1-OT2	agcctcttttcaatgtgcttc	ccatcacggccttggcaaatagagccct (5' 6-FAM, 3' BHQ1)	ctaagcagaagaagaag (5' VIC, 3' MGB)	agagtagatgggtggtagtgg	60	117
EMX1-2	ccacgaagcaggccaatg	cagcaagcagcactctgccctcg (5' VIC, 3' BHQ1)	acctccaatgactaggg (5' 6-FAM, 3' MGB)	tggccaggagtgccaga	60	145
EMX1-2-OT2	gatctctttctcctactgcacaca	accctgaggattgctagactgagatgc (5' VIC, 3' BHQ1)	tccagtactagggaa (5' 6-FAM, 3' MGB)	gggtgttatttggtcattgatagg	60	132
EMX1-2-OT3	agcacgcatgagggacaaac	ctcttgagctcacaagtgaagagt (5' VIC, 3' BHQ1)	ccaatgaccaggag (5' 6-FAM, 3' MGB)	ggctgtgtgtttgagacatgg	60	108
EMX1-2-OT4	ccttccccatgttccaat	tgttctgggtccaagcagggtcagg (5' VIC, 3' BHQ1)	ctgtaatgactagggaga (5' 6-FAM, 3' MGB)	ctgcatttgcattggaacagga	60	119
FANCF-1	tcggaagcgatccaggtg	agtacgcagagagtcgccctcca (5' VIC, 3' BHQ1)	agaagggttccatg (5' 6-FAM, 3' MGB)	cgtatcatttcgggtgttcc	60	123
FANCF-1-OT1	agcctctgtctcacctcatc	cctactgactgacagaggtgcctgg (5' VIC, 3' BHQ1)	agaagggttccaag (5' 6-FAM, 3' MGB)	tgcctcagagatcccactctg	60	146
FANCF-1-OT2	ctccaatgccaggagtggg	acaagggtgagaagagggtggggaga (5' VIC, 3' BHQ1)	agaagggttccatg (5' 6-FAM, 3' MGB)	agcatgggggcaagagc	60	126
FANCF-1-OT3	tggcaccatgaaaacacagg	caaaggcctggctgacctgtttct (5' VIC, 3' BHQ1)	agaagggttccatg (5' 6-FAM, 3' MGB)	ttggccttctgatagcagg	60	140
FANCF-3	cacatccatggcgctttg	cggcatggccccattgcacg (5' VIC, 3' BHQ1)	ttgcctccactggt (5' 6-FAM, 3' MGB)	agtcgctaatacccgaactg	60	121
FANCF-3-OT2	gaccctgacccccatctcta	ctgacccaggcgccgcaaacg (5' VIC, 3' BHQ1)	ctgcacaccagtg (5' 6-FAM, 3' MGB)	atgtgccatgtgggttctagg	60	126
FANCF-3-OT4	cattcagagaagcgacctgtg	ctggcctccgaccaaggcctg (5' VIC, 3' BHQ1)	tacacaaccagtggg (5' 6-FAM, 3' MGB)	aaaatcactcggttaagccgatct	60	115
VEGFA-5	ctaaagagggaatgggctttgg	gccctgggctctctgtacatgaagca (5' VIC, 3' BHQ1)	agtttgcctctggac (5' 6-FAM, 3' MGB)	gtcagagggacacactgtgg	60	156
VEGFA-5-OT1	gcaaagctaagcagagatgcc	agaccaagactgagtttt (5' VIC, 3' BHQ1)	attccccgggagca (5' 6-FAM, 3' MGB)	tgagatctgggaaagaagcc	60	137
VEGFA-5-OT2	accacagtgtgcaggagt	caggcctgcttctccatccaggaag (5' VIC, 3' BHQ1)	agcttgcctctggc (5' 6-FAM, 3' MGB)	atggagtgttctgcctgagc	60	123

Table S2.3 Primers of T7EI assay.

Gene site	Spacer (5' - 3') /Non-target strand	PAM (5' - 3') /Non-target strand	Forward primer	Reverse primer	Annealing temperature (°C)	Wild-type amplicon size (bp)
EMX1-1	GAGTCCGAGCAGAAGAAGAA	GGG	cttcagagcctgcactct	aggctctccgaggagaaggc	71	720
EMX1-1-OT1	GAGTTAGAGCAGAAGAAGAA	AGG				
EMX1-1-OT2	GAGTCTAAGCAGAAGAAGAA	GAG				
EMX1-2	GTCACCTCCAATGACTAGGG	TGG	ctgccatccccttctgtgaa	aatctaccacccaggtct	69	797
EMX1-2-OT2	GCTACCTCCAATGACTAGGG	AAG				
EMX1-2-OT3	ACAACCCCAATGACCAGGG	AGG				
EMX1-2-OT4	GTCACCTGTAATGACTAGGG	AGA				
FANCF-1	GCTGCAGAAGGGATTCCATG	AGG	aaagagtgctgcaccaggt	agagcggttcctcacgtcac	68	770
FANCF-1-OT1	GCTGCAGAAGGGATTCCAAG	GGG				
FANCF-1-OT2	GACGCAGAAGGGACTCCATG	GGG				
FANCF-1-OT3	GGTACAGAAGGGCTTCCATG	AGG				
FANCF-3	GGCGGCTGCACAACCAGTGG	AGG	acacggataaagacgctggg	ctcttctggggccgacga	69	807
FANCF-3-OT2	ATAGGCTGCACACCCAGTGG	GGG				
FANCF-3-OT4	GCAGGCTACACAACCAGTGA	GGG				
VEGFA-5	GGGTGGGGGGAGTTTGCTCC	TGG	tgagagccgttccctctttg	agacgttccttagtgctggc	68	836
VEGFA-5-OT1	GCGTGGGGGGTGTTTGCTCC	CGG				
VEGFA-5-OT2	TAGTGGAGGGAGCTTGCTCC	TGG				
VEGFA-1	GACCCCTCCACCCCGCCTC	CGG	gctcggtgctggaatttgat	tcggcgagctactcttct	67	835
VEGFA-2	GGTGAGTGAGTGTGTGCGTG	TGG	tgccgctcactttgatgtct	ccgctaccagccgacttta	68	807