

Supplementary Table 2: Oligo nucleotides used to clone the CRISPR/Cas9 constructs and luciferase reporter plasmids

Oligo sequence (5' to 3')	Oligo name
CACCGACTCAGCTGTACACGGACTGCA	sgR124C22ntTOP
AAACTGCAGTCCGTGTACAGCTGAGTC	sgR124C22ntBOTTOM
CACCGCTCAGCTGTACACGGACTGCA	sgR124C21ntTOP
AAACTGCAGTCCGTGTACAGCTGAGC	sgR124C21ntBOTTOM
CACCGTCAGCTGTACACGGACTGCA	sgR124C20ntTOP
AAACTGCAGTCCGTGTACAGCTGAC	sgR124C20ntBOTTOM
CACCGCAGCTGTACACGGACTGCA	sgR124C19ntTOP
AAACTGCAGTCCGTGTACAGCTGC	sgR124C19ntBOTTOM
CACCGAGCTGTACACGGACTGCA	sgR124C18ntTOP
AAACTGCAGTCCGTGTACAGCTC	sgR124C18ntBOTTOM
CACCGGCTGTACACGGACTGCA	sgR124C17ntTOP
AAACTGCAGTCCGTGTACAGCC	sgR124C17ntBOTTOM
CACCGCTGTACACGGACTGCA	sgR124C16ntTOP
AAACTGCAGTCCGTGTACAGC	sgR124C16ntBOTTOM
CACCG ACCACTCAGCTGTACACGGACCACA	sgR124H22ntTOP
AAACTGTGGTCCGTGTACAGCTGAGTGTC	sgR124H22ntBOTTOM
CACCG CCACTCAGCTGTACACGGACCACA	sgR124H21ntTOP
AAACTGTGGTCCGTGTACAGCTGAGTGCC	sgR124H21ntBOTTOM
CACCG CACTCAGCTGTACACGGACCACA	sgR124H20ntTOP
AAACTGTGGTCCGTGTACAGCTGAGTGC	sgR124H20ntBOTTOM
CACCG ACTCAGCTGTACACGGACCACA	sgR124H19ntTOP
AAACTGTGGTCCGTGTACAGCTGAGTC	sgR124H19ntBOTTOM
CACCG CTCAGCTGTACACGGACCACA	sgR124H18ntTOP
AAACTGTGGTCCGTGTACAGCTGAGC	sgR124H18ntBOTTOM
CACCG TCAGCTGTACACGGACCACA	sgR124H17ntTOP
AAACTGTGGTCCGTGTACAGCTGAC	sgR124H17ntBOTTOM
CACCG CAGCTGTACACGGACCACA	sgR124H16ntTOP
AAACTGTGGTCCGTGTACAGCTGC	sgR124H16ntBOTTOM
CACCG ACTCAGCTGTACACGGACCTCA	sgR124L22ntTOP
AAACTGAGGTCCGTGTACAGCTGAGTC	sgR124L22ntBOTTOM
CACCG CTCAGCTGTACACGGACCTCA	sgR124L21ntTOP
AAACTGAGGTCCGTGTACAGCTGAGC	sgR124L21ntBOTTOM
CACCG TCAGCTGTACACGGACCTCA	sgR124L20ntTOP
AAACTGAGGTCCGTGTACAGCTGAC	sgR124L20ntBOTTOM
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AAACTGAGGTCCGTGTACAGCTGC	sgR124L19ntBOTTOM

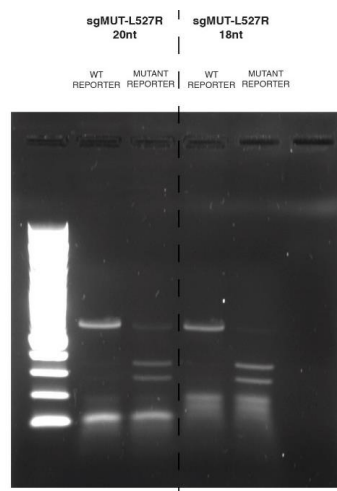
CACCG AGCTGTACACGGACCTCA	sgR124L18ntTOP
AAACTGAGGTCCGTGTACAGCTC	sgR124L18ntBOTTOM
CACCG GCTGTACACGGACCTCA	sgR124L17ntTOP
AAACTGAGGTCCGTGTACAGCC	sgR124L17ntBOTTOM
CACCG CTGTACACGGACCTCA	sgR124L16ntTOP
AAACTGAGGTCCGTGTACAGC	sgR124L16ntBOTTOM
CACCG CCAAGAGTCTGCTCCATTCTCT	sgR555W22ntTOP
AAACAGAGAATGGAGCAGACTCTTGGC	sgR555W22ntBOTTOM
CACCG CAAGAGTCTGCTCCATTCTCT	sgR555W21ntTOP
AAACAGAGAATGGAGCAGACTCTTGC	sgR555W21ntBOTTOM
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AAACAGAGAATGGAGCAGACTCTTC	sgR555W20ntBOTTOM
CACCG AGAGTCTGCTCCATTCTCT	sgR555W19ntTOP
AAACAGAGAATGGAGCAGACTCTC	sgR555W19ntBOTTOM
CACCG GAGTCTGCTCCATTCTCT	sgR555W18ntTOP
AAACAGAGAATGGAGCAGACTCC	sgR555W18ntBOTTOM
CACCG AGTCTGCTCCATTCTCT	sgR555W17ntTOP
AAACAGAGAATGGAGCAGACTC	sgR555W17ntBOTTOM
CACCG GTCTGCTCCATTCTCT	sgR555W16ntTOP
AAACAGAGAATGGAGCAGACC	sgR555W16ntBOTTOM
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CACCG AGTCTGCTCTGTTCTCT	sgR555Q17ntTOP
AAACAGAGAACAGAGCAGACTC	sgR555Q17ntBOTTOM
CACCG GTCTGCTCTGTTCTCT	sgR555Q16ntTOP
AAACAGAGAACAGAGCAGACC	sgR555Q16ntBOTTOM
AGATGCGGAGAAGCTGAGGCCTGAG	sgR124L AsCpf1 TOP
AAAACCTCAGGCCTCAGCTTCTCCGC	sgR124L AsCpf1 BOTTOM
CACCGCCTCCGAGCCCTGCCACCAA	sgR555W sg21 S.aureus TOP
AAACTTGGTGGCAGGGCTCGGAAGGC	sgR555W sg21 S.aureus BOTTOM
CACCGCCTCCGAGCCCTGCCACCAA	sgR555W sg22 S.aureus TOP
AAACTTGGTGGCAGGGCTCGGAAGGCC	sgR555W sg22 S.aureus BOTTOM
ACCACCACTCAGCTGTACACGGACCGCACGGAGAAGCTGAGGCCTGAGATG	R124 Rep WT Top
CTAGCATCTCAGGCCTCAGCTTCTCCGTGCGGTCCGTGTACAGCTGAGTGGTGGTGTAC	R124 Rep WT Bottom
ACCACCACTCAGCTGTACACGGACTGCACGGAGAAGCTGAGGCCTGAGATG	R124C Rep Mutant Top
CTAGCATCTCAGGCCTCAGCTTCTCCGTGAGGTCCGTGTACAGCTGAGTGGTGGTGTAC	R124C Rep Mutant Bottom
ACCACCACTCAGCTGTACACGGACCACCGAGAAGCTGAGGCCTGAGATG	R124H Rep Mutant Top

CTAGCATCTCAGGCCTCAGCTTCTCCGTGGTCCGTGTACAGCTGAGTGGTGGTGTAC	R124H Rep Mutant Bottom
ACCACCACTCAGCTGTACACGGACCTCACGGAGAAGCTGAGGCCTGAGATG	R124L Rep Mutant Top
CTAGCATCTCAGGCCTCAGCTTCTCCGTGAGGTCGTGTACAGCTGAGTGGTGGTGTAC	R124L Rep Mutant Bottom
TTCCGAGCCCTGCCACCAAGAGAACGGAGCAGACTCTGGGAGATGCCAAG	R555 Rep WT Top
CTAGCTTGGCATCTCCAAGAGTCTGCTCCGTTCTTGGTGGCAGGGCTCGGAAGTAC	R555 Rep WT Bottom
TTCCGAGCCCTGCCACCAAGAGAACAGAGCAGACTCTGGGAGATGCCAAG	R555Q Rep Mutant Top
CTAGCTTGGCATCTCCAAGAGTCTGCTCTGTTCTTGGTGGCAGGGCTCGGAAGTAC	R555Q Rep Mutant Bottom
CTTCCGAGCCCTGCCACCAAGAGAATGGAGCAGACTCTGGGAGATGCCAA	R555W MUT REP TOP
CTAGTTGGCATCTCCAAGAGTCTGCTCCATTCTTGGTGGCAGGGCTCGGAAGGTAC	R555W MUT REP BOTTOM

Supplementary Table 3: Off-target scores for each guide RNA utilised

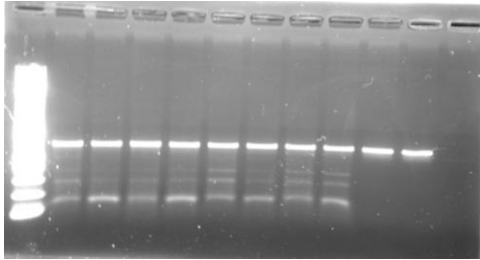
		Benchling		MIT Optimised CRISPR Design Tool
		Off-target score	On-target score	Off-target score
<i>S. pyogenes</i> Cas9 NGG PAM	R124WT	95	83.7	94.4
	R124C	62	65.4	80.1
	R124H	70	81.4	79.5
	R124L	71	47.9	82
	R555WT	65.6	48.1	83
	R555Q	46.6	0.9	31
	R555W	56.7	3.7	50
<i>S. aureus</i> Cas9 NNGRRT PAM	R555W	30.4	79.1	Program does not look for <i>S. aureus</i> Cas9 PAM
Mutant AsCpf1	R124L	Program does not look for mutant AsCpf1 PAM		

Supplementary Figure 1: Allele-specific cleavage of L527R TGFBI mutation utilising a PAM-specific approach

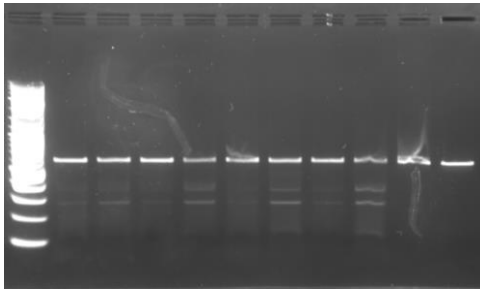


Supplementary Figure 2: Confirmation of the specificity achieved using a guide-specific system targeted to prevalent TGFBI mutations

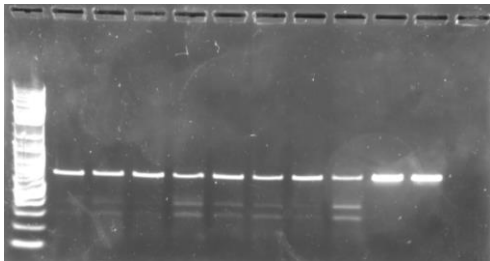
R124C:



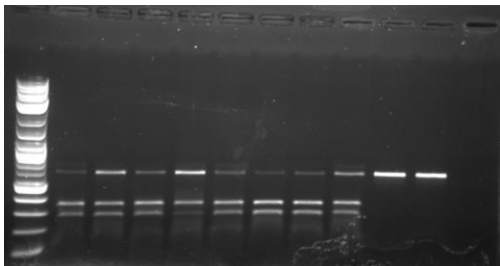
R124H:



R124L:



R555Q:



R555W:

