

Insertion site (amino acid)	Epitope tag			
	myc	bio	mEOS2	GFP
158		1		
171			1	
246				5
257		1		
263		1		
275		1		
277	1			
278		1		
279		1		
289		1		
294		1		
338		1		
351		1		2
352		1		
370		4		
380	1	1		
393	3			
395	15			
396		1		
399		1		
405		1		
408				1
413	3			
414		2		
458	5			
475				1
507		2		
536	1			2
543			1	
544	1			
546		1		
548		1		
550			1	
551			1	
552			2	
553				1
573	2			1
574	1			
575				1
590			2	
629				1
645		1		
667		1	1	
668		1		
669			1	
712		1	1	
713			1	
716	2	1	1	
717		1	1	
721	1			1
749			1	
767				1

Table S7. Insertion sites of select in-frame complementing tagged Sir3-alleles

Complementing clones from the mutagenized Sir3 pools were analyzed with colony PCR to qualitatively determine the location of the epitope tag within the *SIR3* ORF. PCR products from a subset of these complementing clones were sequenced and the precise location of the tag insertion was identified. Each of the 107 unique locations within Sir3 where a tag was found in a functional allele is listed in the “insertion site” column, based on the amino acid of insertion. The final two insertion sites listed are downstream of the Sir3 STOP codon. The remaining columns list the number of complementing clones from each mutagenized pool found at a given insertion location. In total, the epitope tag was located in frame in 65 Sir3-myc, 52 Sir3-bio, 46 Sir3-mEOS2, and 47 Sir3-GFP complementing clones.

Insertion Site (Amino acid)	Epitope tag			
	myc	bio	mEOS2	GFP
769		1	4	
771				1
772			1	
773			1	
775				1
777		2		
793				1
795	2			
796		3	2	4
797				1
800		1		
804			1	
807	7	3	6	6
808				1
809	2			
826	27			
829			1	
857			1	1
858			2	
859			1	
860			1	
863	1		1	
870			1	
877				1
878			1	
887				1
889		1		
894			2	1
895	2		1	1
896				1
918			1	
922				1
931				1
972				1
975	7		1	1
976		1		
977				4
981	3			
984		1		
997		1	1	
998		1		
1002	1			
1012		1		
1013		1		
1015		1		
1016				1
1017		1		
1019		1		
1081			1	
1084		1		
+6 bp	1			
+17 bp	1			

Table S7 (continued). Insertion sites of select in-frame complementing tagged Sir3-alleles

Complementing clones from the mutagenized Sir3 pools were analyzed with colony PCR to qualitatively determine the location of the epitope tag within the *SIR3* ORF. PCR products from a subset of these complementing clones were sequenced and the precise location of the tag insertion was identified. Each of the 107 unique locations within Sir3 where a tag was found in a functional allele is listed in the “insertion site” column, based on the amino acid of insertion. The final two insertion sites listed are downstream of the Sir3 STOP codon. The remaining columns list the number of complementing clones from each mutagenized pool found at a given insertion location. In total, the epitope tag was located in frame in 95 Sir3-myc, 52 Sir3-bio, 46 Sir3-mEOS2, and 47 Sir3-GFP complementing clones.