

**S1 Table. Summary of CRISPR-Cas9 targeting efficiency for *Ss-unc-22*.** Results for combined nicotine assay data presented in Fig 3B. The estimated number of  $F_1$  iL3s collected from each injection experiment was based on the average number of iL3s per injected adult calculated in S14 Table.

target	delivery	injection experiment	# free-living adults injected ( $P_0$ )	estimated # $F_1$ iL3s collected	# iL3s screened	# twitching (%)
wild type	—	—	—	—	1,314	2 (0.15%)
1	DNA	a	29	~957	254	59 (23%)
		b	31	~1,023	192	19 (10%)
			60	~1,980	446	78 (17%)
1	RNP	a	27	~891	473	63 (13%)
		b	22	~726	435	82 (19%)
			49	~1,617	908	145 (16%)
2	DNA	a	31	~1,023	222	56 (25%)
		b	30	~990	123	20 (16%)
		c	20	~660	332	70 (21%)
			81	~2,673	677	146 (22%)
2	RNP	a	27	~891	668	201 (30%)
		b	22	~726	362	153 (42%)
		c	25	~825	254	116 (46%)
			74	~2,442	1,284	470 (37%)
3	DNA	a	24	~792	368	88 (24%)
		b	20	~660	235	80 (34%)
			44	~1,452	603	168 (28%)
3	RNP	a	33	~1,089	191	70 (37%)
		b	28	~924	428	118 (28%)
			61	~2,013	619	188 (30%)