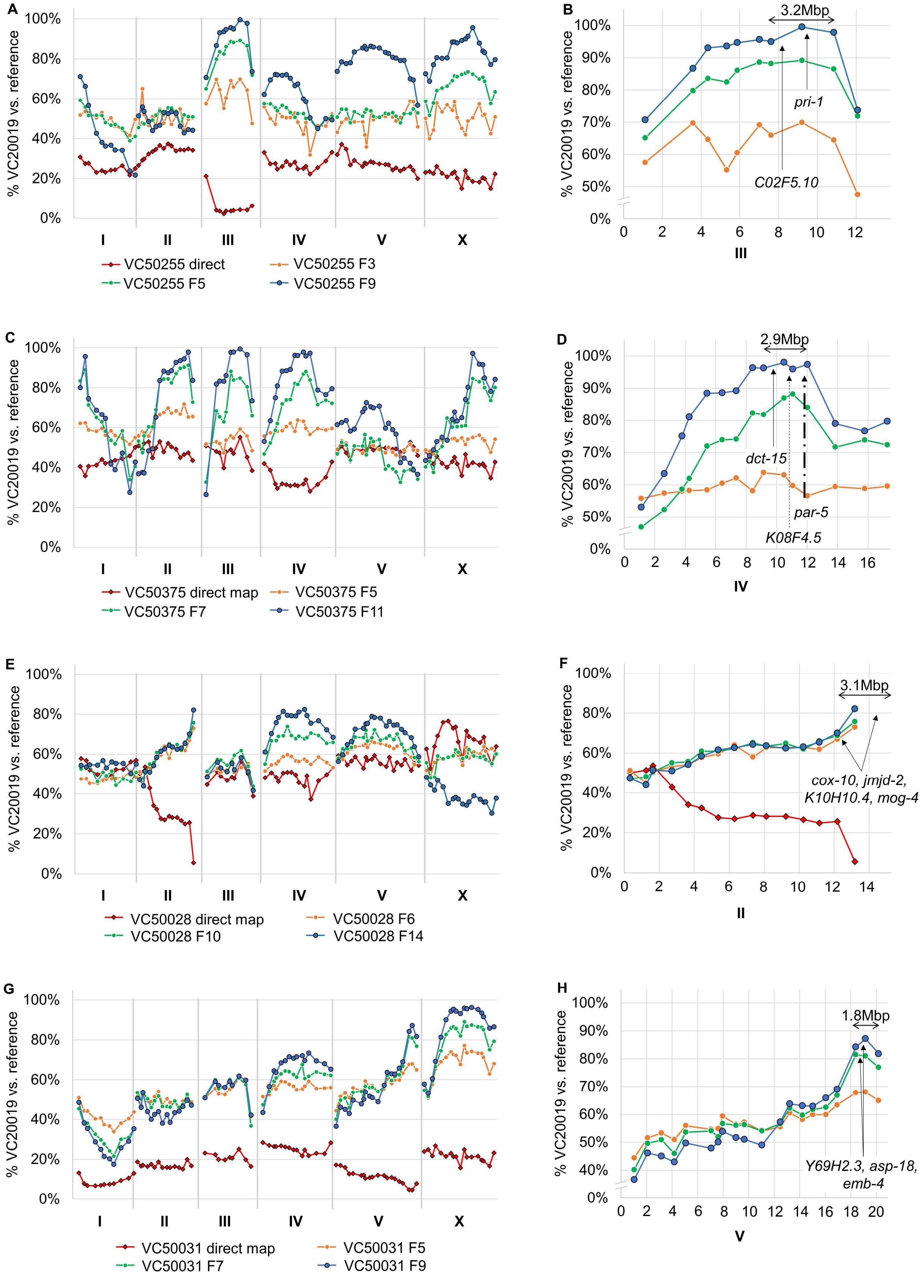
A
% VC20019 vs. reference
100%
80%
60%
40%
20%
0%
I
II
III
IV
V
X
VC50255 direct
VC50255 F3
VC50255 F5
VC50255 F9
B
3.2Mbp
pri-1
C02F5.10
III
C
VC50375 direct map
VC50375 F5
VC50375 F7
VC50375 F11
D
2.9Mbp
dct-15
par-5
K08F4.5
IV
E
VC50028 direct map
VC50028 F6
VC50028 F10
VC50028 F14
F
3.1Mbp
cox-10, jmjd-2,
K10H10.4, mog-4
II
G
VC50031 direct map
VC50031 F5
VC50031 F7
VC50031 F9
H
1.8Mbp
Y69H2.3, asp-18,
emb-4
V

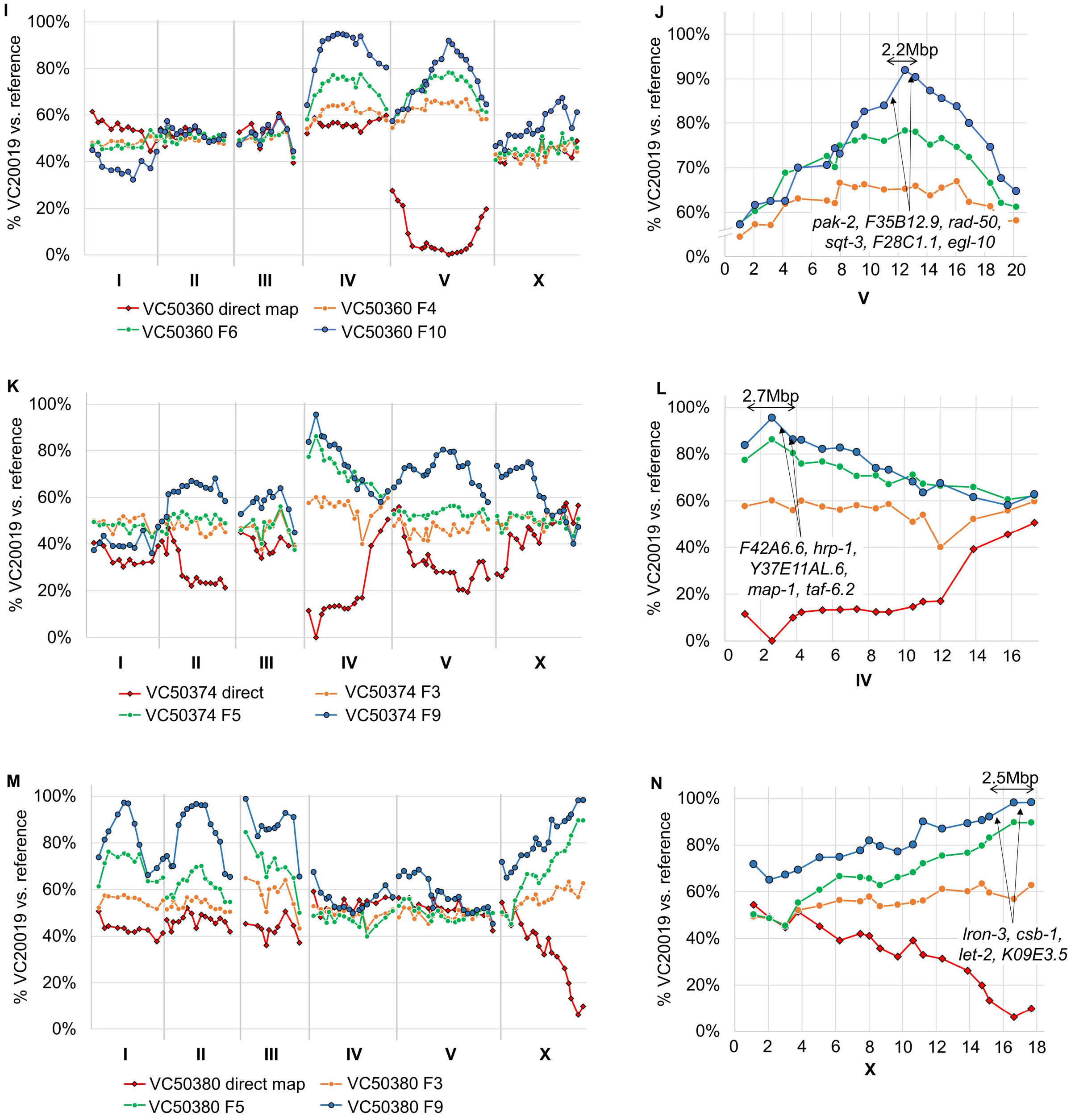
I
% VC20019 vs. reference
100%
80%
60%
40%
20%
0%
I
II
III
IV
V
X
VC50360 direct map
VC50360 F4
VC50360 F6
VC50360 F10
J
% VC20019 vs. reference
100%
90%
80%
70%
60%
0%
2.2Mbp
pak-2, F35B12.9, rad-50,
sqt-3, F28C1.1, egl-10
0
2
4
6
8
10
12
14
16
18
20
V
K
% VC20019 vs. reference
100%
80%
60%
40%
20%
0%
I
II
III
IV
V
X
VC50374 direct
VC50374 F3
VC50374 F5
VC50374 F9
L
% VC20019 vs. reference
100%
80%
60%
40%
20%
0%
2.7Mbp
F42A6.6, hrp-1,
Y37E11AL.6,
map-1, taf-6.2
0
2
4
6
8
10
12
14
16
IV
M
% VC20019 vs. reference
100%
80%
60%
40%
20%
0%
I
II
III
IV
V
X
VC50380 direct map
VC50380 F3
VC50380 F5
VC50380 F9
N
% VC20019 vs. reference
100%
80%
60%
40%
20%
0%
2.5Mbp
lron-3, csb-1,
let-2, K09E3.5
0
2
4
6
8
10
12
14
16
18
X

Figure S9. TS strains can carry multiple strong fitness-defective loci. In three cases, the competitive fitness mapping protocol identified multiple loci within these strains that conferred an overall reduced fitness to the population at a fixation rate that made it difficult to identify the correct TS-specific locus. Additional mapping with the liquid-format bulk segregant assay elucidated the correct linkage group for the TS phenotype - albeit with lower resolution. The TS-associated locus in VC50255 was identified at LGIII (A) with the prime candidate as *pri-1* based on phenotype and Wormbase reports (B). A 2.9 Mbp TS interval was identified in VC50375 on LGIV (C) containing 3 candidates (D) with the prime candidate as *par-5* based on a shared cell-cycle delay phenotype in the strain. In VC50028 a 3.1 Mbp interval on LGII was identified (E). The primary candidate based on shared phenotype data between the strain and Wormbase sources is *mog-4* (F). The strain VC50031 had multiple loci with a TS-associated hit on a 1.8 Mbp interval on LGV and a likely hit for *lin-2* on LGX (G). The primary candidate in this interval is *emb-4* (H) based on VC50031's sterility phenotype and a reported high early embryonic expression of this gene. The strain VC50360 produced a mapping profile (I) with two very strong candidate loci and resolved to a 2.2M bp interval on LGV (J) with a primary candidate of *sqt-3* as the strain's phenotype overlapped with reported RNAi and allele phenotypes. VC50374 exhibited a number of peaks that were mirrored in liquid-format segregant mapping with the TS-locus resolved at LGIV (K). The 2.7 Mbp interval (L) contained many candidates with the primary candidate as *taf-6.2*, a gene reported to have embryonic lethal phenotypes and TS lethal alleles. One such allele is located in the same exon as the VC50374 SNV. The TS strain VC50380 (M) identified 4 strong low-fitness loci with liquid-format bulk segregant mapping resolving the TS locus to LGX. Within this interval (N) the primary candidate is *let-2* based on reported phenotypes compared to this strain. Single arrows indicate positions of genes while double-arrows identify the interval spanned by a list of genes.