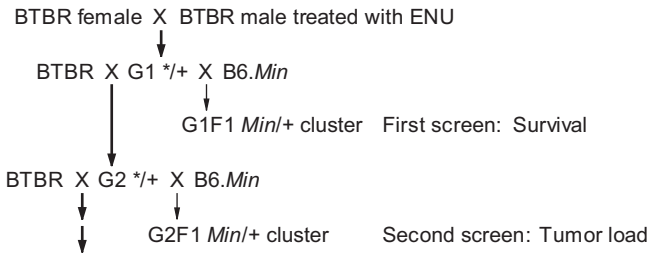
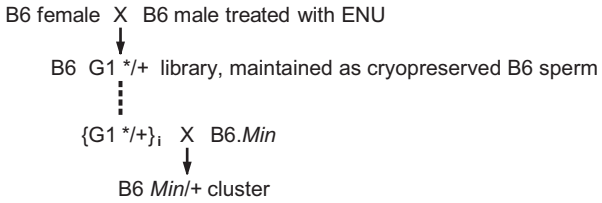
Figure 1

A. Outcross design:



B. Isogenic design:



C. Isogenic mapping partner:

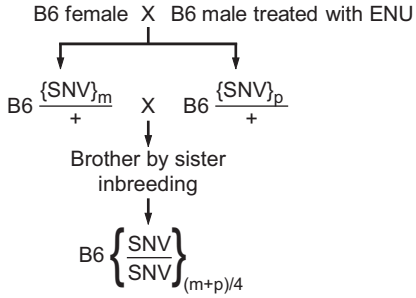


Figure 2

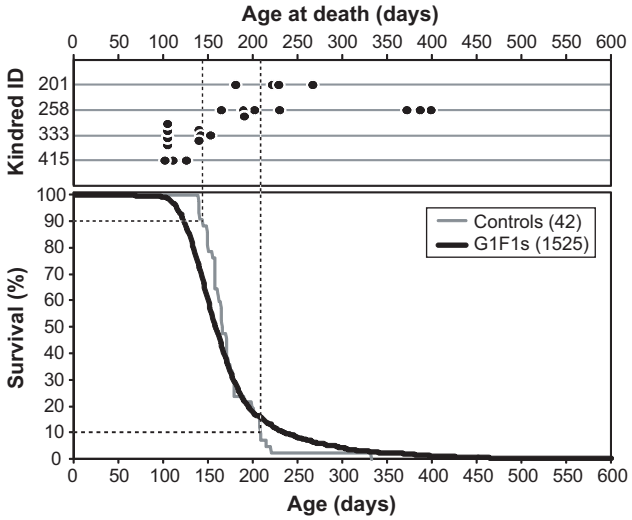

Age at death (days)
0 50 100 150 200 250 300 350 400 450 500 550 600
Kindred ID
201
258
333
415
100
90
80
70
60
50
40
30
20
10
0
Survival (%)
Controls (42)
G1F1s (1525)
0 50 100 150 200 250 300 350 400 450 500 550 600
Age (days)

Figure 3

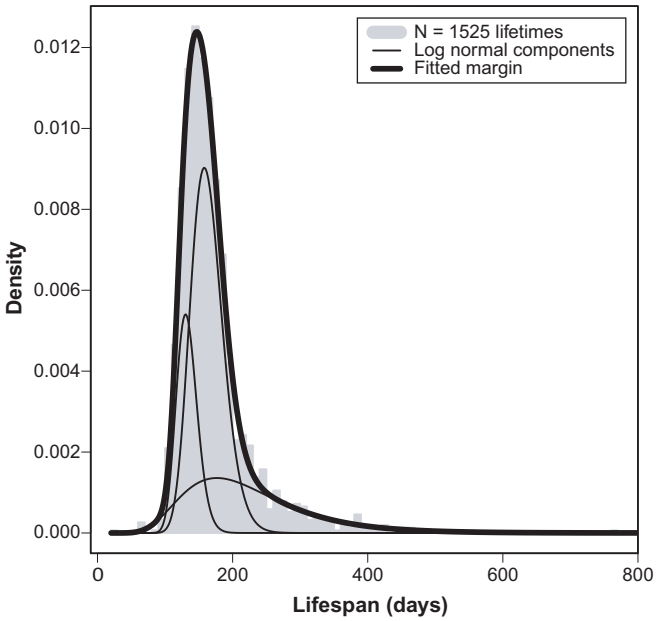

N = 1525 lifetimes
Log normal components
Fitted margin
0.012
0.010
0.008
0.006
0.004
0.002
0.000
Density
0
200
400
600
800
Lifespan (days)

Figure 4

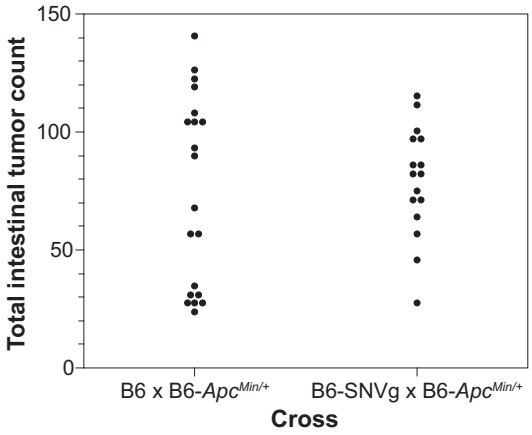

150
100
50
0
Total intestinal tumor count
B6 x B6-*Apc*$^{Min/+}$
B6-SNVg x B6-*Apc*$^{Min/+}$
Cross

Figure 5

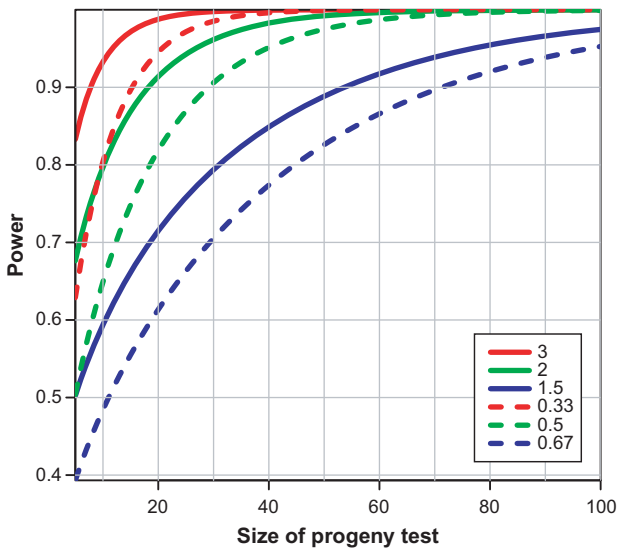

Power
0.9
0.8
0.7
0.6
0.5
0.4
20
40
60
80
100
Size of progeny test
3
2
1.5
0.33
0.5
0.67

Figure 6

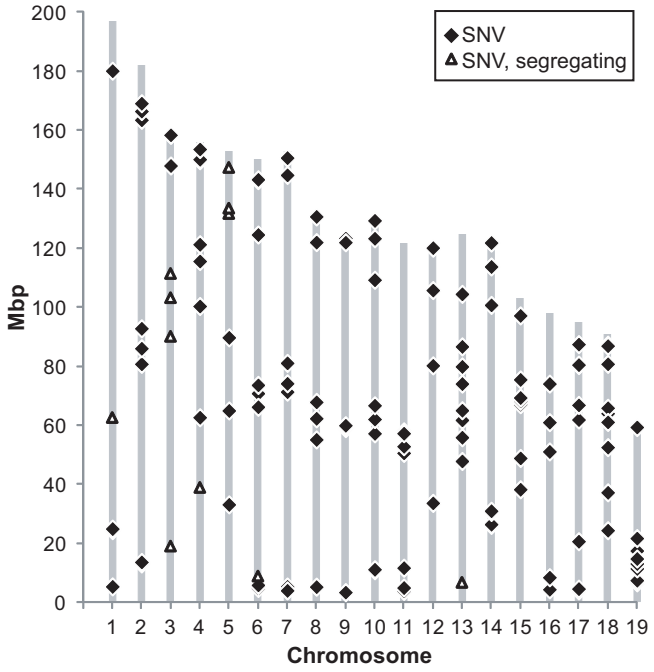

SNV
SNV, segregating
Mbp
200
180
160
140
120
100
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Chromosome

Table S1 Sequencing of the B6-SNV lines.

Line	Raw (Gbp)	Mapped (Gbp)	Coverage (times)	N of all candidate SNVs*	N of line-specific candidate SNVs**
SNVb	27.40	23.83	7.94	9864	3246
SNVc	20.61	17.21	5.74	9028	2932
SNVe	14.64	12.24	4.08	7094	2051
SNVf	6.05	5.14	1.71	2925	1198
SNVg	28.38	24.25	8.08	6324	1225
SNVh	23.85	20.03	6.68	9559	2520

*There were 22911 sites overall: 13172 appeared in only 1 line, 3590 in 2, 2187 in 3, 2212 in 4, 1467 in 5, and 283 in all 6 lines.

**In addition, lines B6.SNVg and B6.SNVh, which are known to be related, share 792 candidate variants that are found in none of the other lines.

Table S2 The spectrum of distances between 13172 adjacent line-specific candidate variants.
Note that 13172 SNVs on 120 chromosomes yield 13052 distances.

Distance between adjacent SNVs (bp)	N of SNVs	Cumulative total	Cumulative %
1	58	58	0.4
2	62	120	0.9
3	50	170	1.3
4	66	236	1.8
5	66	302	2.3
6	38	340	2.6
7	29	369	2.8
8	33	402	3.1
9	27	429	3.3
10	28	457	3.5
11	25	482	3.7
12	28	510	3.9
13	15	525	4.0
14	17	542	4.2
15	20	562	4.3
16	11	573	4.4
17	10	583	4.5
18	16	599	4.6
19	8	607	4.7
20	18	625	4.8
21	15	640	4.9
22	10	650	5.0
23	5	655	5.0
24	9	664	5.1
25	10	674	5.2
26-1000	683	1357	10.4
1001-10,000	970	2327	17.8
10001-100,000	1696	4023	30.8
100,001-1,000,000	5448	9471	72.6
1,000,001-45,000,000	3581	13052	100.0

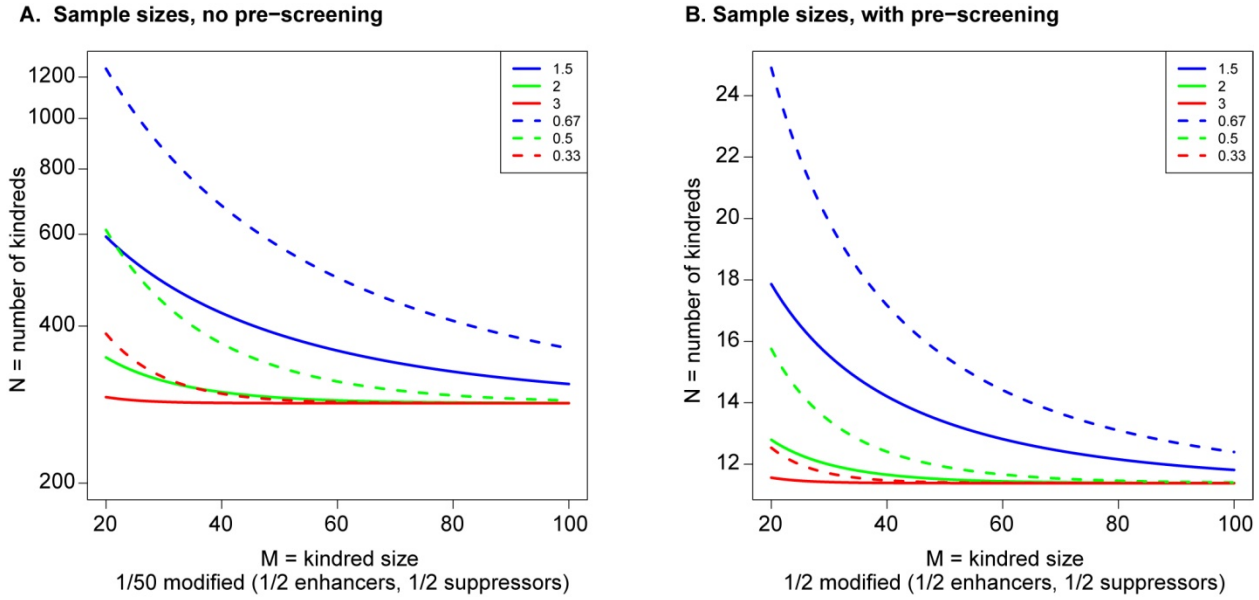


Figure S1. Sample size requirements in a progeny test for various fold effects modifying the expected tumor count, both (A) without prescreening, assuming 1/100 gametes have a modifier with directional effect shown, and (B) with survival-based pre-screening such that ¼ of gametes have directional effect shown. Sample sizes are calculated such that a 5% FDR-controlled list of modified kindreds is non-empty with 95% probability. In all cases the non-modified tumor-count distribution is Negative Binomial, with mean 99.8 tumors and shape parameter 9.8, as estimated from control data. Modifiers are assumed to affect the mean (and thus the variance), but not the shape parameter. (Recall that a Negative Binomial distribution has mean mu and variance mu*(1+mu/shape)). Calculations allow segregation of each mutant modifier within a carrier kindred and use a normal approximation for the distribution of average tumor count. We reckoned that a one-hit mutagenesis library will produce 1/50 gametes carrying some modifier, and that ½ of these may be in a specific direction, and this determined the rates used above. Without pre-screening, the burden of a tumor-count-based progeny test is especially high in terms of the number of kindreds required to be tested.

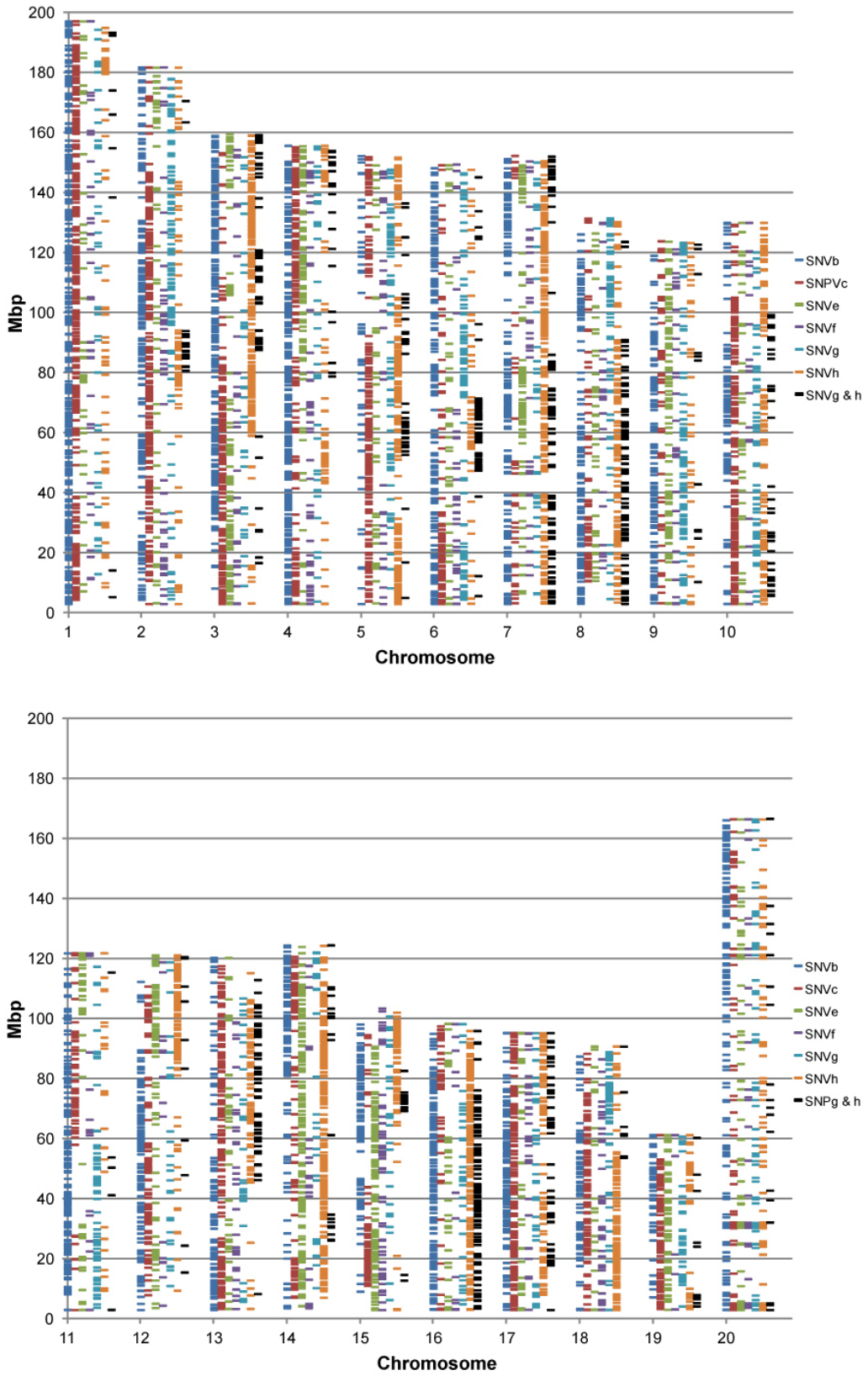
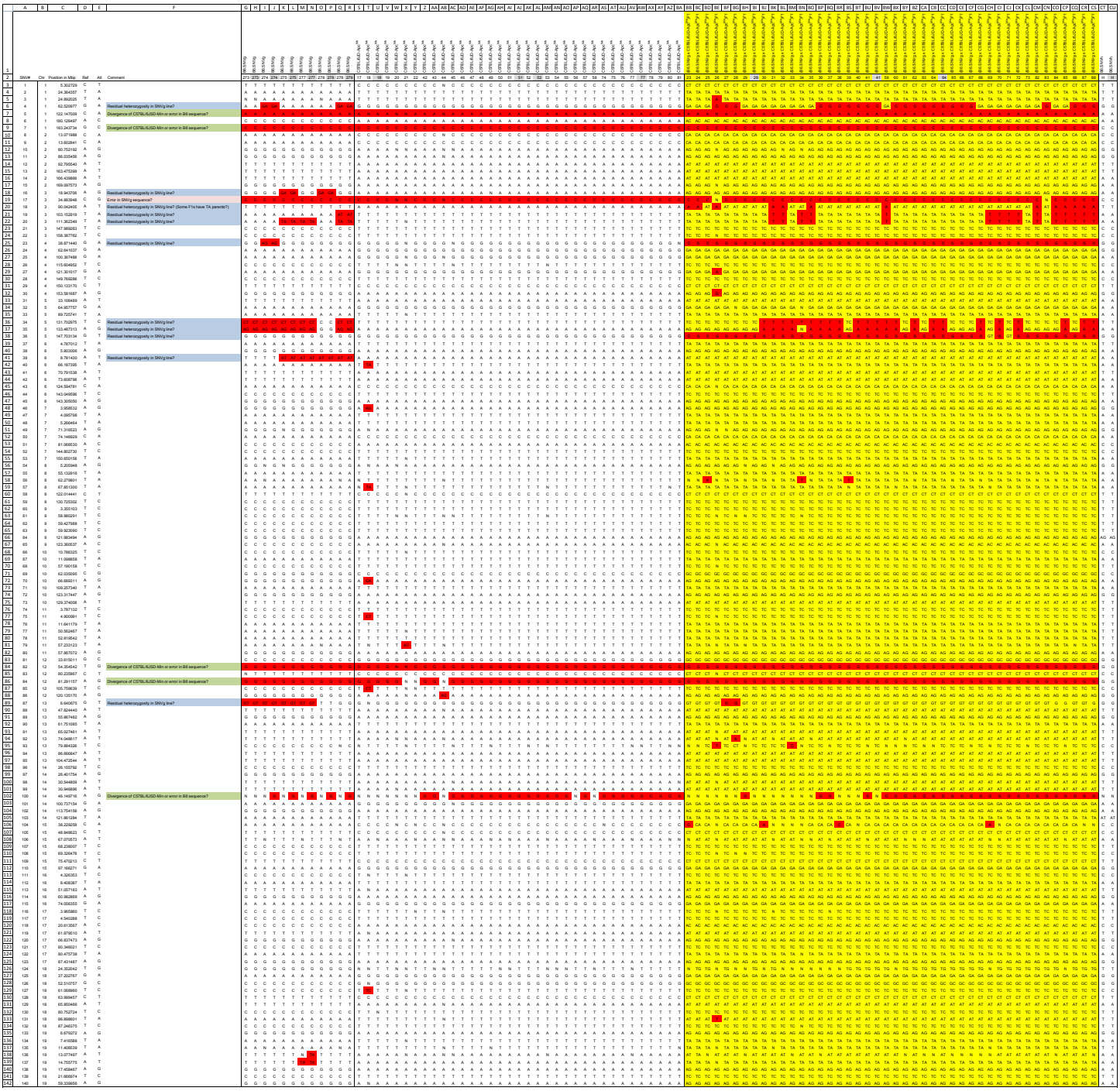


Figure S2 The candidate SNV sites that are line-specific or specific to only lines B6.SNVg and B6.SNVh.



Supporting Data File S1

Results of Sequenom validation tests of DNA from 31 C57BL6/6JD-*Apc*^{Min}, 6 B6.SNVg, 1 B6.SNVh, and 41 (C57BL/6JD-*Apc*^{Min} x B6.SNVg)F1 mice. Note that 14 of the samples were run twice (gray-coded animal ID cells). Data from B6 and B6.SNVg sequences are shown in columns D and E, respectively. Unexpected Sequenom results are highlighted in red. For 11 of the positions, the unexpected results in the B6.SNVg mice and/or the F1s are consistent with residual heterozygosity in the B6.SNVg line (see comment column). The unexpected results at 5 positions are consistent with either an error in the canonical B6 sequence or genetic drift in C57BL/6JD-*Apc*^{Min}.