Supplemental Methods

Calling LM-SNVs

We attempted calling of LM-SNVs in twelve hiPSC lines from four children (i.e., for 03-03, 03-04, 07-03, and S1123-03) by comparing their genomes with the genomes of both their corresponding parents. As reasoned above, such a strategy offers the advantage of filtering out germline SNPs while not missing high frequency mosaic SNVs in children's fibroblasts (**Supplemental Fig. S1**, step 1). However, the large number of germline SNPs, together with the less than ideal sensitivity (i.e. below 100%), can impair the detection of several parental germline SNPs. The subset of such (undetected) SNPs inherited by the child, can potentially be introduced as false positives in the candidate LM-SNV sets. Therefore we used the catalogue of known germline variants from the 1000 Genomes Project to additionally filter out germline SNPs to arrive to the final sets of LM-SNVs calls (**Supplemental Fig. S1**, step 2). Data for one cell line did not match the genome of the corresponding child and was not included in the analysis. One more line had insufficient coverage to call SNVs and was also excluded.

To call LM-SNVs in each hiPSC line from children with data for both parents we did the following: i) call SNVs using GATK; ii) remove SNVs that had at least one supporting read in ether of the parents (in data from either fibroblasts or derived hiPSC lines), as such SNVs are likely inherited germline SNPs; iii) remove SNVs where we saw some support for a third allele rather than for the reference and alternative ones, as such sites are likely problematic for read mapping or prone to sequencing error; iv) remove SNVs that had less than 70% of average coverage in the sample; v) remove SNVs that had less than 35% or higher than 65% TAF for the alternative allele. SNVs mosaic in fibroblasts and transmitted to hiPSCs should be present in the hiPSC lines at about 50% TAF. Hence, the value of TAF is an important criterion for considering an LM-SNV putatively mosaic, as opposed to a culture artifact or sequencing error. The occurrence of SNVs during culture can happen at any time in the hiPSC colony, but only SNVs created at hiPSC's first division will have a high TAF of around 25%. The choice of 35% TAF cut off is aimed at differentiating between true mosaic variants and such culture artifacts.

We attempted LM-SNVs calling in nineteen hiPSC lines from six parents. Lines from individual 07-02 had insufficient coverage to call SNVs and were excluded from the analysis. To call LM-SNVs we did the following: i) call SNVs using GATK (see above); ii) remove SNVs that had at least one supporting read in child/children (in data from either fibroblasts or derived hiPSC lines), as such SNVs are likely transmitted germline SNPs; ii) remove SNVs that had at least one supporting read in matching fibroblasts, as such SNVs are likely germline variants (**Supplemental Fig. S2**); iv) remove SNVs where we saw some support for a third allele rather than for the reference and alternative ones, as such sites are likely problematic for read mapping or prone to sequencing error; v) remove SNVs that had less than 70% of average coverage in the sample; vi) remove SNVs that had less than 35% or higher than 65% TAF for the alternative allele. The same criteria were utilized to call LM-SNVs in children with one parent, where in item (ii) we compared hiPSC genome against parental genome.

Note, calling LM-SNVs in parents can miss high TAF SNVs as those may have supporting evidence in fibroblasts. Validation results demonstrated that true high TAF SNVs are discovered even with such an approach. The generated calls comprised candidate LM-SNV sets (**Supplemental Table S1**). Guided by validation we filtered out SNVs found in the catalogue of known germline variants from the 1000 Genomes Project to arrive to the final LM-SNV sets for both children and parent hiPSC lines.

LM-SNV validation in hiPSC of children

LM-SNVs were validated with target site amplification and re-sequencing (amplicon-seq) and with PCR and Sanger band sequencing in hiPSC lines where they were originally discovered. For the amplicon-seq experiment, we randomly selected 69 LM-SNVs found in hiPSC lines #1 and #6 from 03-04. For each site we designed primers with MacVector such that primers flanked the sites and amplified a region up to 450 bp around it. After amplification around the sites, pooled DNA for all sites was sequenced with Illumina MiSeq using 2x300 bp reads. An LM-SNV was considered validated if it was covered by more than 100 reads and its TAF was above 35% (**Supplemental Fig. S1**, step 4). This experiment revealed that in each hiPSC line ~11% sites were false positives, due to sequencing errors (**Supplemental Table S1**). Presence of an SNV in hiPSC at around 50% TAF does not guarantee that it is mosaic, as it could still be a germline SNV. Thus, we performed (see below) further confirmatory experiments in fibroblasts, which showed that 4 LM-SNVs (~7% of 57 validated) were actually present in the corresponding fibroblasts at 50% AF and, thus, are likely germline SNPs.

Furthermore, some of the LM-SNVs in our callset could be created *de novo* in the children germline. To verify this, we randomly selected 10 *de novo* candidate SNPs from the hiPSC of 03-03 and tested their presence in the corresponding and parents fibroblasts with PCR and Sanger sequencing. PCR reactions were performed using

Thermo Scientific Phusion High-Fidelity DNA Polymerase and 50 ng of genomic DNA amplified for 30 cycles. Products were purified using the Qiagen PCR purification kit. Fluorescent sequencing was carried out on Applied Biosystems 3730 capillary instrument. Three SNVs were confirmed as *de novo* SNPs, 3 more were likely *de novo* by could also be mosaic (peak heights in Sanger sequencing were different for the reference and alternative alleles), for 1 SNVs we could not get clear results in one of parents, 2 sites were validated LM-SNVs (present only in one hiPSC line), and one SNVs was an inherited SNPs (**Supplemental Table S1**). This validation demonstrated that some germline SNPs within LM-SNV sets can be created *de novo* in the child.

We did additional PCR validations in fibroblasts for hiPSC #9 from 07-03 as it was having a large number of LM-SNV calls. Ten LM-SNVs were randomly selected and analyzed with PCR and Sanger sequencing. Only one SNV was validated indicating that data for this line lead to high false positive rate. But note, 47 of the LM-SNV in the set were confirmed as mosaic in fibroblasts using the targeted capture sequencing validation approach (**Table 1**).

Confirmation with targeted capture and re-sequencing in children fibroblasts

For 6,280 candidate LM-SNVs sites (which included 5,203 of the final set) discovered in the nine hiPSC lines: S1123-03#1, S1123-03#8, 03-03#2, 03-03#3, 03-03#4, 03-04#1, 03-04#6, 07-03#7, 07-03#9, we designed an Agilent SureSelectXT Custom 0.5-2.9Mb library with standard array software provided by the company. For each of four fibroblast samples (S1123-03, 03-03, 03-04, and 07-03) we performed capture and library construction four times, then barcoded the libraries with a unique barcode for each person, pooled the DNA and sequenced it with HiSeq. All the samples were process in one batch, thus minimizing systematic inter-sample experimental variability. Reads for each sample were differentiated by barcode, and aligned to the reference genome with BWA.

For 670 sites (10% of total; 6% to 25% per hiPSC line) the sequencing coverage was less than 50 reads in each sample, and these sites were excluded (**Fig. 2A**, gray bars). For the remaining LM-SNV we counted number of reads with bases, having base quality score of at least 20, supporting reference and alternative alleles in each sample. For each LM-SNV we also assigned a fibroblast sample, where we expected to find it as a mosaic SNV, as the sample that was used to derive the hiPSC line where the LM-SNVs was discovered. 225 sites (4%) were deemed germline based on TAF above 35%. Counts of such sites per hiPSC line ranged from 17 to 36 (average of 25), or 56 to 97 sites (average of 72), when adjusted for discovery sensitivity (**Fig. 2A**, red bars). These sites are likely *de novo* SNVs present in fibroblasts and hence in each hiPSC line but absent from parental genomes. These numbers are consistent with other measurements of *de novo* SNVs from sequencing (Besenbacher et al. 2015; Kloosterman et al. 2015; Conrad et al. 2011) and provide a positive control for our analyses.

To confirm that the remaining LM-SNVs are mosaic, one needs to establish that the support for SNVs (i.e., for the non-reference allele) is above background sequencing noise. For data from each fibroblast we used data from other three as control to evaluate background. The rationale is that the exact same mosaic SNVs, i.e., occurring at the same position in the genome and having the same mutation are not likely to occur in different people. In support of this, when comparing LM-SNV lists (i.e., putative mosaic SNVs) from unrelated individuals we found not a single one that is shared. Such case control comparison allowed us to decide when base mismatches in reads covering a site represent mosaic SNV or noise (see **Methods**). We then applied the Fischer's exact test to get a p-value (for each remaining LM-SNVs) that the proportion of reads supporting the alternative allele in the assigned sample is higher than in the other tree samples combined. Given that we were testing many sites and conducted multiple hypothesis testing, we considered a LM-SNVs confirmed as mosaic if this p-value was lower than 0.001.

Most of the LM-SNVs could not be confirmed by the capture method (76% of total; 48% to 85% per hiPSC line; **Fig. 2A** and **C**, blue bars). These could be real mosaic SNVs that have such low TAF that the capture experiment was not sensitive enough to reveal them. Indeed, the TAF for most of the sites in the inconclusive category was below 0.5%. This is consistent with the notion that almost none of the captured sites had coverage over 2,000X, for which a TAF lower than 0.05% is not observable, while detectable TAF with statistical significance, i.e., TAF significantly above background of sequencing error, is higher than this lower limit.

Finally, for 588 putative LM-SNVs (9.4%) across the 9 hiPSC lines, we observed a sufficient number of reads above background supporting the alternative allele and, hence, confirmed them as mosaic in fibroblasts at a *p*-value cut off of 0.001. At such stringency, only 6 sites (out of the 6,280 tested) could be erroneously confirmed as mosaic, which is only 1% of the 588 confirmed SNVs.

We additionally cross-validated mosaic SNVs with high TAF in fibroblasts in individual 03-03. For this we randomly selected 7 mosaic SNVs with roughly 25% TAF and conducted local PCR amplification with Sanger sequencing. For all of the sites we could clearly see the presence of the SNVs in the fibroblasts with frequencies

lower that those in the derived hiPSC lines, consistent with SNVs being mosaic rather than germline (**Supplemental Fig. S5**).

Confirmation with target site amplification and re-sequencing in children fibroblasts

The same 57 sites that were validated with amplicon-seq in hiPSC lines #1 and #6 from 03-04, were amplified using the same primers in the fibroblasts of 03-04. Amplified DNA was pooled and sequenced with MiSeq using 2x300 bp reads. As PCR primers were designed such that amplicons are never longer than 450 bps, paired reads coming from them will overlap in their 3'-end, allowing constructing one single genomic fragment, where sequencing errors in the overlapping parts can be corrected. 75% of the target sites fell into such areas of overlap.

To find the best overlap between paired reads we slide them against each other increasing overlap by one base at a time. At each step we count matching and mismatching bases in the overlap. Given the count we calculate p-value using binomial test and assuming 0.25 as random chance to have matched bases. The best overlap is chosen by the lowest p-value and by requiring to have at least 50 overlapping bases and at least 75% of matching bases in the overlap. Once the best overlap is found, the bases and corresponding base qualities from the two reads in the overlapping part are unified. If bases match, the resulting bases are the same with the resulting quality equal to the sum of qualities in each read. If bases mismatch, the resulting bases are those having higher quality with the resulting quality being the absolute difference between the qualities in each read. Only constructed fragments (or "long reads") from fusing paired reads were used for further analysis.

"Long reads" were aligned with BWA. To estimate base errors, which could be sequencing error or polymerase error during DNA amplification, we tabulated mismatches in aligned reads in genomic positions between PCR primers but not coinciding with positions of LM-SNVs (**Supplemental Fig. S6B**). Note, there are 384 times more of such positions than of tested LM-SNVs. We assumed that all mismatches represent base error, while some may reflect mosaic SNVs. Tabulation was done for each mismatch type (e.g., C to A), rather than for all mismatches. For analysis, we considered bases with at least 20 quality score and at least 100X coverage. Then for each LM-SNV (e.g., chr2:40177413, C to T), we calculated its TAF as number read supporting expected alternative allele (i.e, non-reference) and the proportion of tabulated positions having the same mismatch type (i.e., C to T) and a mismatch frequency higher than the TAF. This was the empirical p-value that a LM-SNV is a mosaic variant. We used p-value cut off of 0.05. LM-SNVs with TAF higher than 35% were considered as germline SNPs. Described data processing steps are implemented in Leucippus software (https://github.com/abyzovlab/Leucippus).

Confirmation with ddPCR

All ddPCR assays were prepared following the protocol as described in (Hindson et al. 2013); 20ng of gDNA was used and RPP30 was used as the reference allele. All primer and probe concentrations were kept at 0.5μM. To calibrate experiments we used few loci for which copies of synthesized dsDNA up to 500bp (so called, gBlocks ordered from IDT) containing the designed amplicon sequence and SNV of interest at 0.3-3% allele frequency with respect to RPP30 were mixed with 20ng NA12878 gDNA to serve as positive control. NA12878 gDNA only was used as negative control for all assays (**Supplemental Fig. S10**). For all SNV loci tested, negative controls were assayed in an annealing temperature gradient 52.5-62.5 °C with increments of 2.5 °C and 40 cycles of amplification. The optimal annealing temperature was discerned and used to assay the sample of interest. SNV frequencies were calculated according to (Dube et al. 2008).

Digital droplet PCR primers (15bp-28bp) were designed to amplify a 60-200bp sized amplicon containing the SNV site. GC content of primers were limited between 50-60%, and T_m was kept between 50-65 °C with ≤ 5 °C difference between both primers. Stretches of four or more C's or G's were avoided in the primer sequence, and all primers were tested for the absence of non-specific priming using in silico PCR (UCSC Genome Browser). TaqMan probes (15-25bp) with FAM fluorophore and ZEN and Black hole quenchers were designed and ordered from Integrated DNA Technologies (IDT). The probe T_m was kept at least 5-10 °C higher than its corresponding primers. Probe sequences starting with a G at the 5'-end were avoided.

Comparing counts of mosaic SNVs in children and parents

When comparing against one relative, rare germline SNPs could be missed and included in the LM-SNV sets, which is reflected in inflated counts for parents relative to children. Thus, while in estimating the maximum number of mosaic SNVs for parents we accounted for possible germline variants contaminating the LM-SNV sets, the estimates of LM-CNVs for children and parents (**Table 1**) are not directly comparable, as input values for the estimates were calculated with different approaches. For example, the comparison against fibroblasts can miss

high TAF mosaic SNVs, thus the actual sensitivity of discovering mosaic SNVs in hiPSC from parents could be lower than estimated.

To understand how counts for children and parent hiPSCs compare, we conducted an LM-SNV calling exercise in the hiPSCs from the four children with two parents by mimicking the strategy we used for the parents, i.e., by comparing hiPSC genomes to the genome of only one parent and against the matching fibroblasts. This exercise revealed that the comparison against one parent gives an estimate of maximum count of mosaic SNVs that is, on average, 20% higher than the one previously obtained when comparing children genomes against two parents (**Supplemental Table S3**). A similar increase is observed for child S1120-03, which had data for only one parent (**Table 1**).

To provide a corroboration strategy, for a set of 147 unique random LM-SNVs (non-unique LM-SNVs, i.e., those present in two or more lines, could be germline SNPs) from the hiPSC lines of individual S1123-02 we conducted an amplicon-seq experiment in fibroblasts. Among other things (see below), this experiment measured the fraction of germline SNPs by estimating their precise TAF. By subtracting this fraction from the total LM-SNVs set we estimated counts of non-germline LM-SNVs for each hiPSC line from individual S1123-02. We compared these estimates with those derived by excluding non-unique LM-SNVs in different hiPSC from the same individual (**Supplemental Table S4**). The two estimates are consistent within 30% precision.

From these two comparisons, we expect that on average the maximum counts of mosaic SNVs for hiPSCs from parents can be overestimated by up to 30%.

Calculating correlations with epigenome marks

Somatic SNVs were downloaded from ICGC release 20 (https://dcc.icgc.org/api/v1/download?fn=/release_20/Projects/LINC-JP/simple_somatic_mutation.open.LINC-JP.tsv.gz). Broad peaks of histone marks were downloaded from RoadMap Epigenomics Project (Roadmap Epigenomics Consortium et al. 2015) (http://egg2.wustl.edu/roadmap/data/byFileType/peaks/consolidated/broadPeak) which also included uniformly processed data from ENCODE project (ENCODE Project Consortium et al. 2012). Only data that were deemed high quality (https://docs.google.com/spreadsheets/d/1yikGx4MsO9Ei36b64yOy9Vb6oPC5IBGlFbYEt-N6gOM/edit?pref=2&pli=1#gid=15) were used in the subsequent analysis. We divided the reference genome into 10 Mbp bins and utilized only those bases that are unlikely to have artifacts in next generation sequencing data, i.e., using only "P" (passed) bases from strict mask by the 1000 Genomes Project (ftp://ftp-trace.ncbi.nih.gov/1000genomes/ftp/technical/working/20120417_phase1_masks). Next, we counted density of somatic/mosaic SNVs and bases in broad histone peaks for "passed" bases. Calculated per bin densities of SNVs and histone marks were correlated. As in previous analysis (Polak et al. 2015) we used Spearman's rank correlation for quantification.

Supplemental Tables

Supplemental Table S1. LM-SNV calls, validation results and confirmation results for hiPSC lines for children with both parents.
See additional .xlsx file.

Supplemental Table S2. LM-SNV calls and confirmation results for hiPSC lines from parents and child S1120-03.
See additional .xlsx file.

Supplemental Table S3. Estimated maximum count of somatic mosaic SNVs

Individual	hiPSC	Estimated counts of SNV		
		vs both parents	vs father	vs mother
07-03	#3	1,200	1,563	1,659
	#7	1,065	1,311	1,318
03-04	#1	725	678	695
	#6	712	566	584
03-03	#2	1,288	1,802	1,777
	#3	1,189	1,757	1,563
	#4	1,036	1,320	1,357
S1123-03	#1	1,087	1,189	1,168
	#8	1,013	1,055	1,040

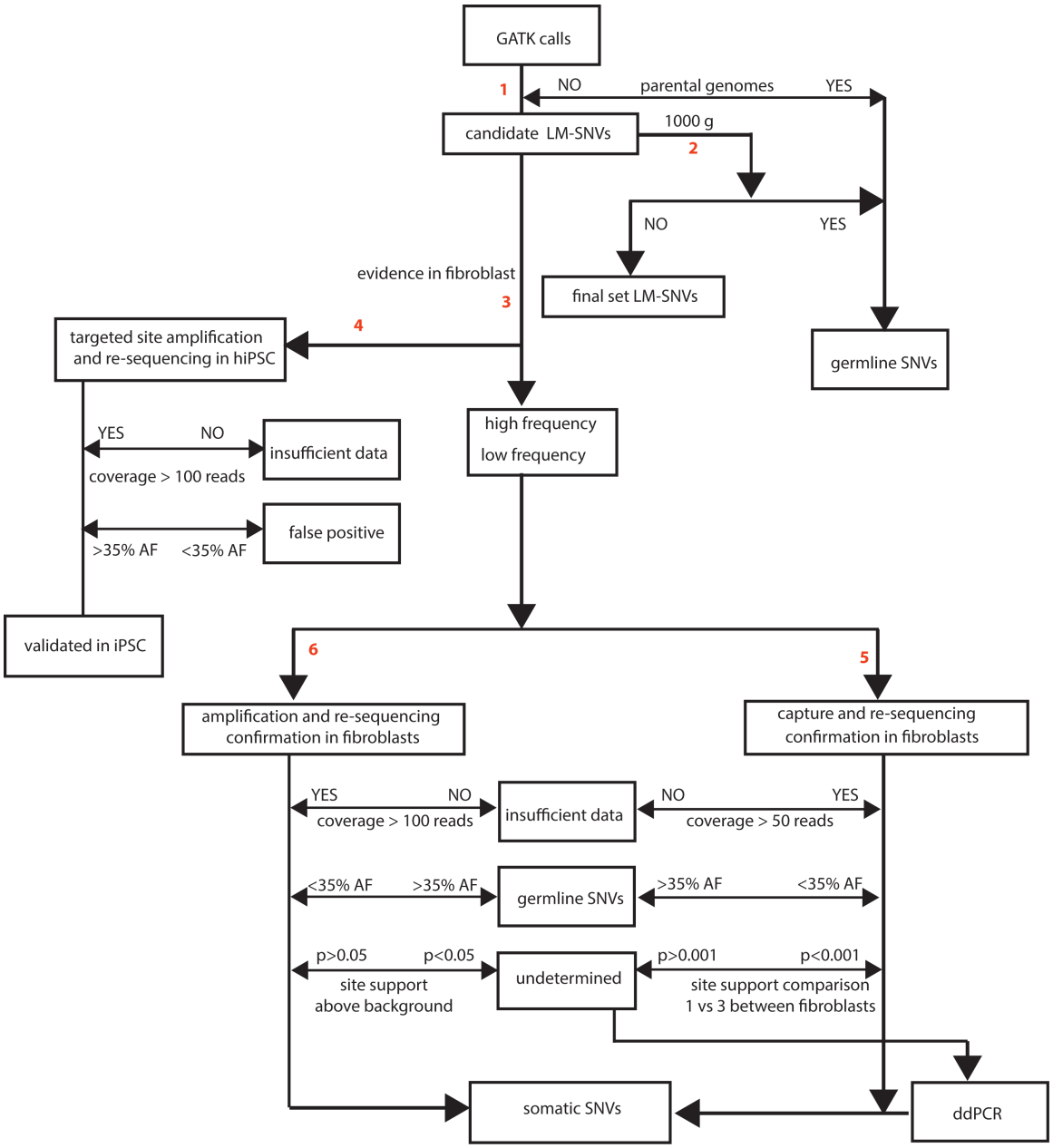
Supplemental Table S4. Estimates counts of non-germline LM-SNVs calls for hiPSC lines from S1123-02.

hiPSC	Sensitivity	Counts of LM-SNVs				
		called	non overlapping	overlapping	not germline (by overlap)	not germline (by amplicon-seq)
#2	48%	2,830	1,602	1457	968	703
#11	36%	2,198	1,366		801	1,048
#17	41%	2,360	1,214		759	714

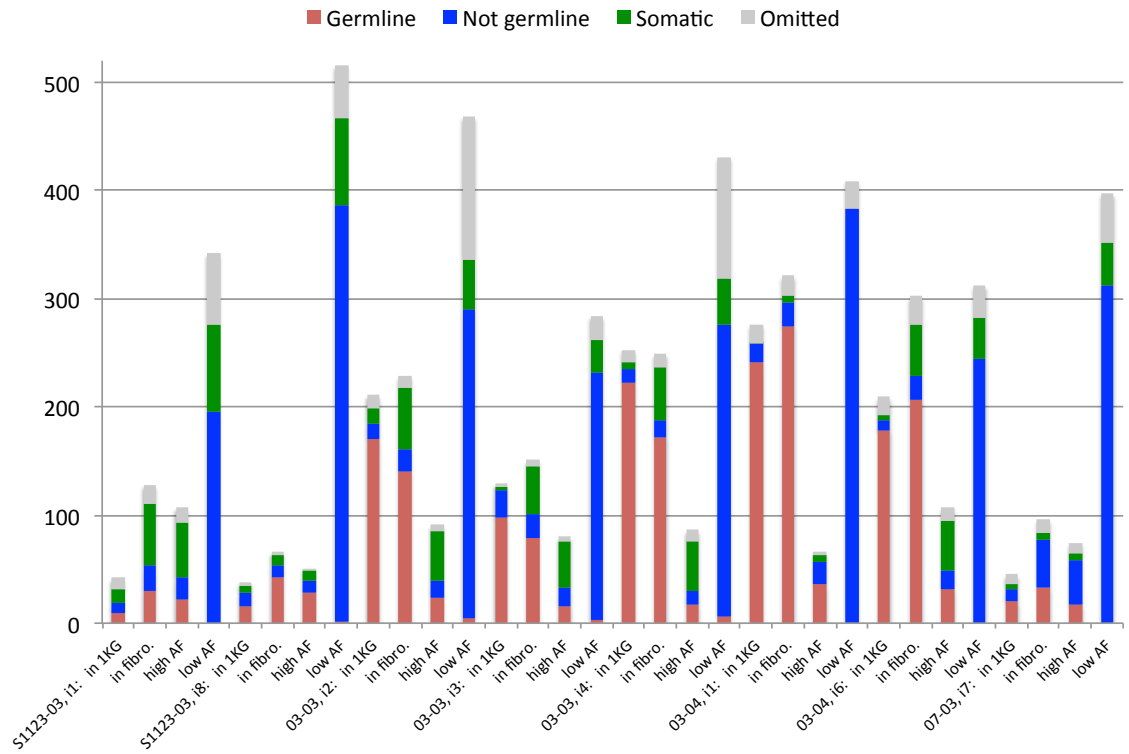
Supplemental Table S5. List of LM-SNVs predicted by VEP and FunSeq2 as having functional effect.
See additional .xlsx file.

Supplemental Table S6. Primer and probe sequences for ddPCR experiments.

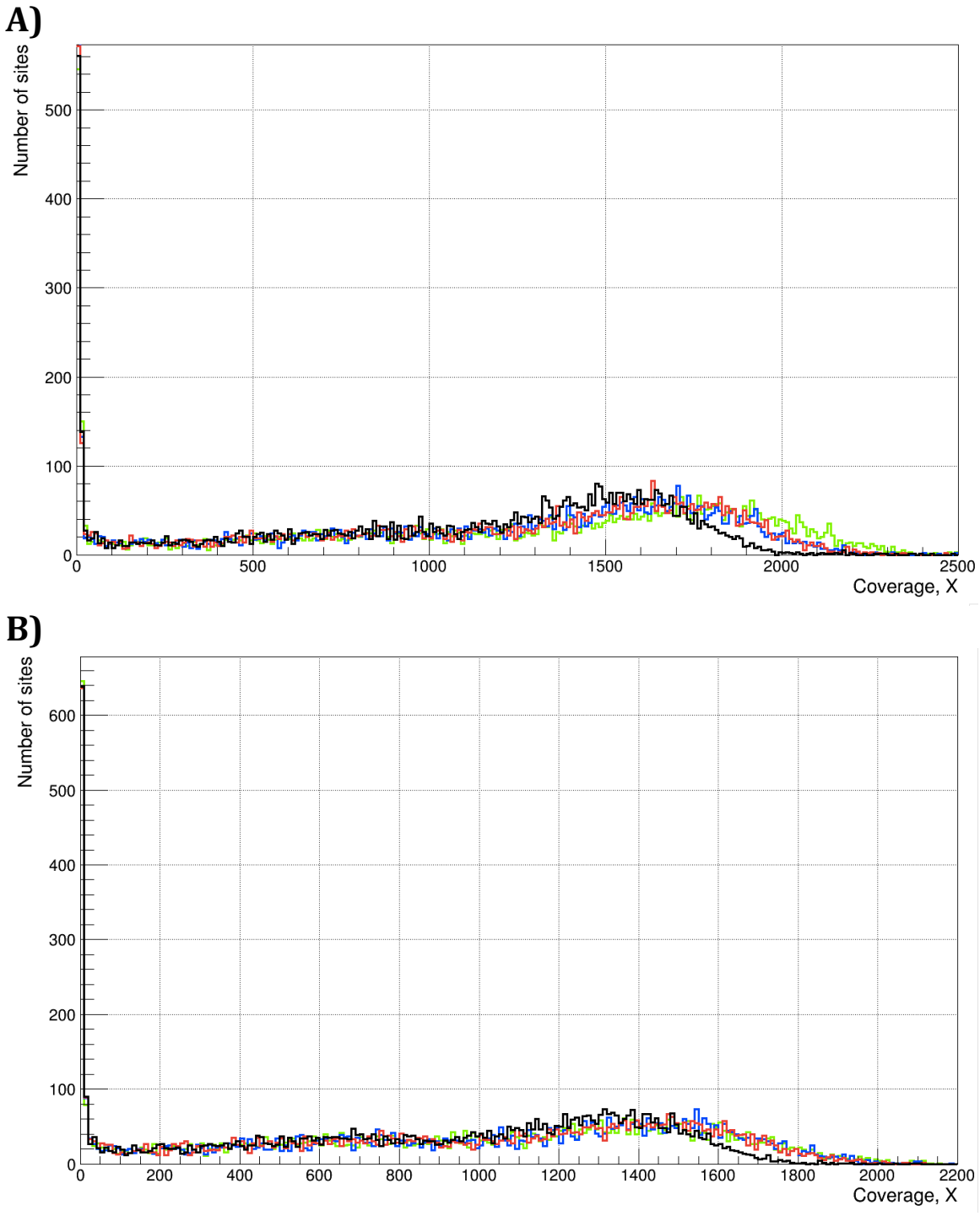
Supplemental Figures



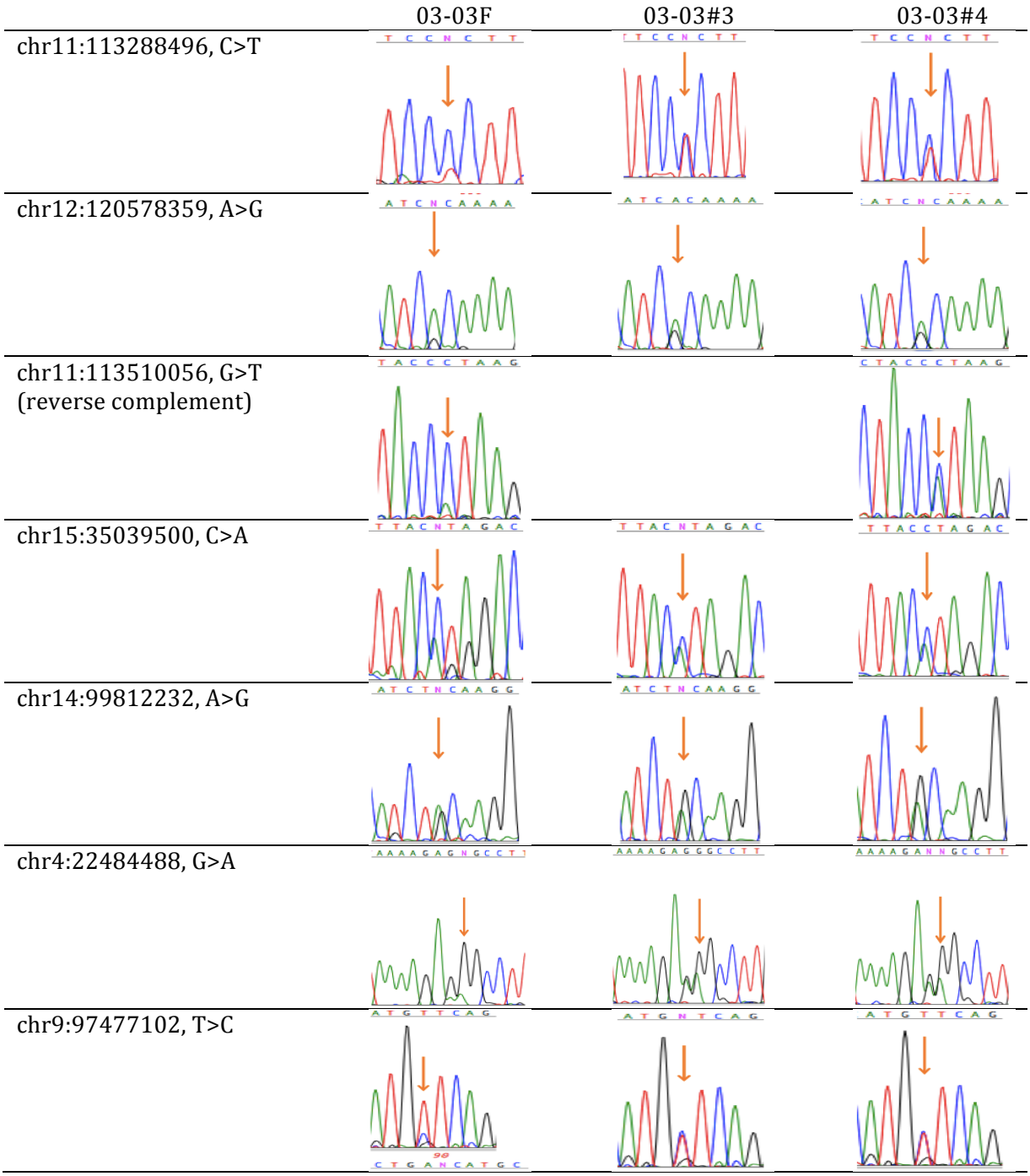
Supplemental Figure S1. Outline of SNV discovery pipeline, of validation and confirmation experiments, and of criteria used for SNV classification. Step numbers are given in red.



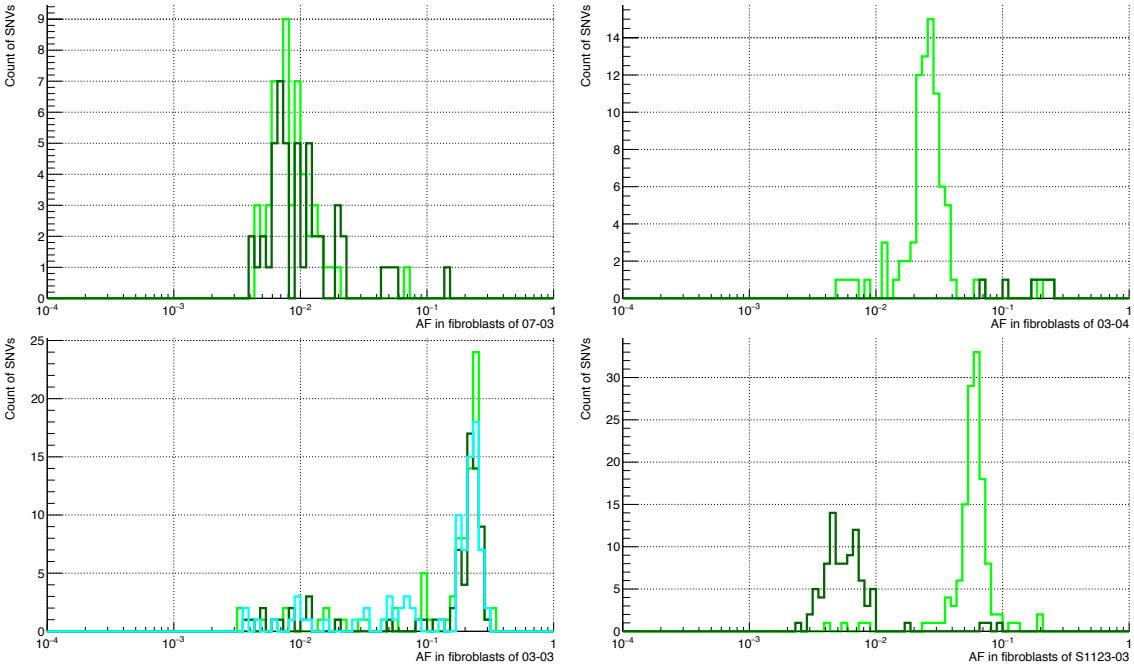
Supplemental Figure S2. Justification for LN-SNV discovery and filtering strategy in children. Summary of targeted DNA capture and re-analysis results (STAGE 2 in Fig. 1, step 5 in Fig. S1) of candidate LM-SNVs discovered in children. For the purpose of demonstrating the validity of our confirmation strategy, the candidate LM-SNVs sets that were subjected to confirmation re-analyses are broken into subsets: (1) SNVs found in the catalogue of commons SNPs by the 1000 Genomes Project (category "in 1KG"); (2) SNVs with support in fibroblast samples (category "in fibro."). Subsets (1) and (2) overlap. The remaining "final set" of LM-SNVs after removing 1000 genome SNPs was subdivided into (3) putative high and (4) low TAF LM-SNVs according to whether some support was found in the original fibroblast sequence data (see text). Deep sequencing of LM-SNVs by the capture approach allows determining the actual TAF of the LM-SNVs candidates in fibroblast tissue. TAF is then used to define SNVs as germline (red, denoting SNVs with TAF consistent with 50%) and somatic mosaic (green, denoting SNVs with TAF less than 50% but above background created by sequencing errors). The data show that filtering of LM-SNV candidates by intersection with SNPs called in corresponding fibroblasts (category "in fibro") would remove both germline and somatic variants, the last overlapping with high TAF somatic variants, while filtering the original LM-SNV set by intersection with SNPs by the 1000 Genomes Project effectively removes almost exclusively germline variants. The final LM-SNV subsets consisting of putative high and low TAF SNVs in corresponding fibroblasts contain almost no germline variants. We, therefore, only applied the latter filter, which is sufficient to remove germline variants. Gray bars represent counts of LM-SNVs for which no sufficient data were collected in the capture-seq experiments. Blue bars represent count of LM-SNVs, which were not germline (TAF was much less than 50%) and their support was consistent with sequencing noise (i.e., we cannot confirm them as somatic).



Supplemental Figure S3. Depth of sequencing from capture experiment. **A)** shows the site coverage when applying no base quality cut off. **B)** shows the site coverage by bases with the quality of at least 30. Each color curve represents a child: 03-03 (green), 03-04 (blue), 07-03 (red), and S1123-03 (black).

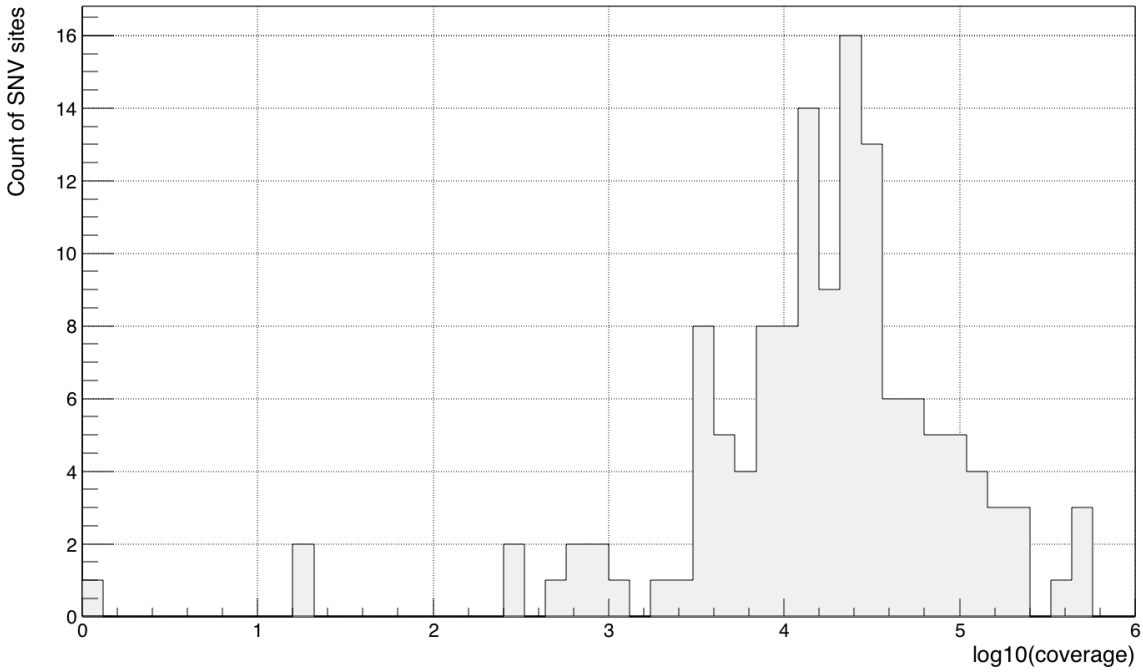


Supplemental Figure S4. Cross-validation of somatic SNVs with ~25% TAF in 03-03 fibroblasts with PCR and Sanger sequencing. 7 randomly selected SNVs could be clearly seen in the fibroblasts with the frequencies lower than those in derived hiPSC lines, consistent with SNVs being somatic rather than germline.

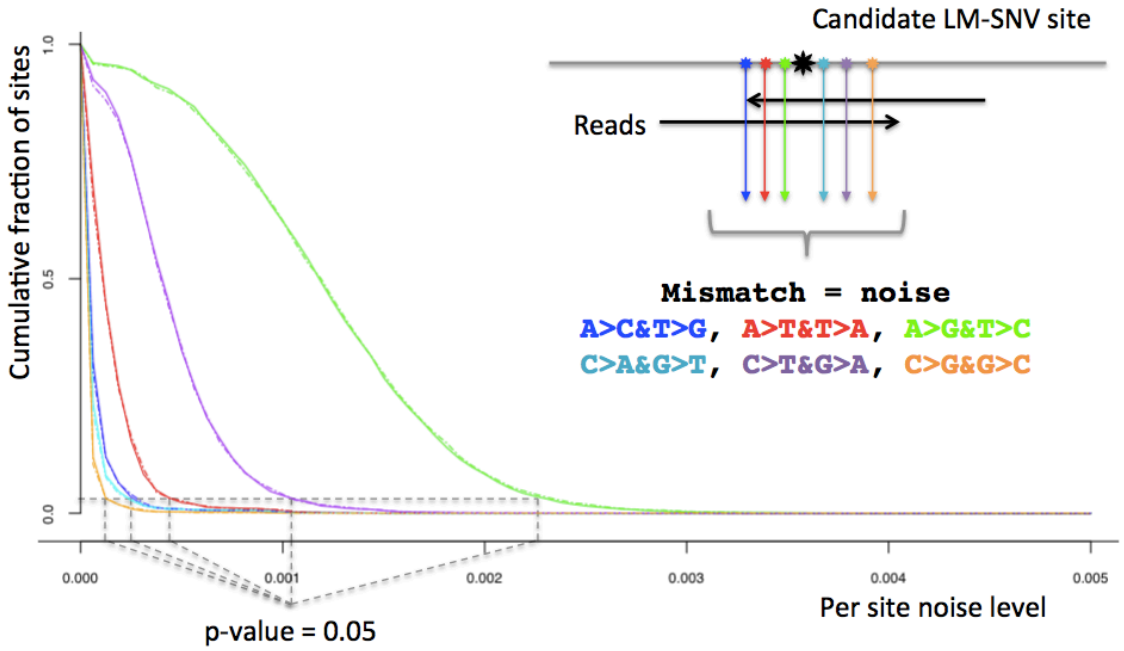


Supplemental Figure S5. Capture-seq experiment in fibroblasts revealed that mosaic SNVs were present in the fibroblast tissue with TAF ranging from 0.25% to 35%. Distributions of TAF have clear peaks. The peaks are likely to reflect clonal expansion of fibroblast cells, resulting in TAF of SNVs present in an expanded cell rising to high values. Strongly supporting this suggestion, each of the two peaks in the TAF spectrum of S1123-03 child represent SNVs found in different hiPSC colonies, i.e., different fibroblast cell (for each person SNVs found in different hiPSC colony are colored by different color: green, dark green and cyan). Based on TAF spectrum analysis we conclude that two cells giving rise to iPSC lines in 07-03 are likely clonally related, two cells giving rise to iPSC lines in 03-04 in are likely not clonally related, three cell giving rise to iPSC lines in 03-03 are likely clonally related and two clones giving rise to two iPSC lines in S1123-03 are likely not clonally related.

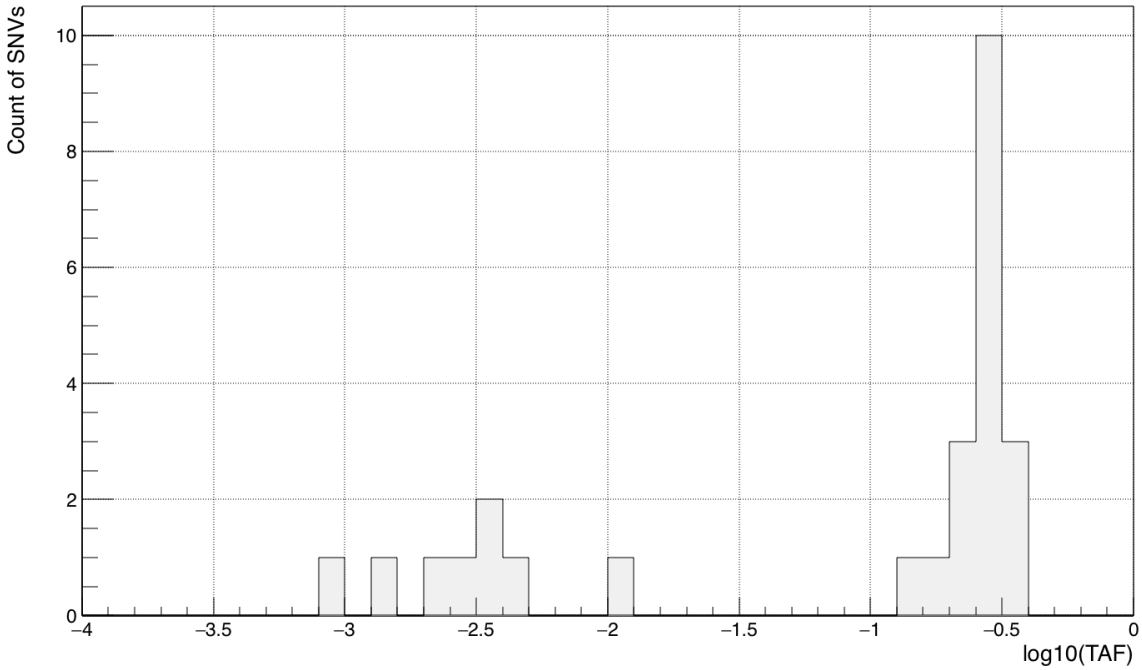
A)



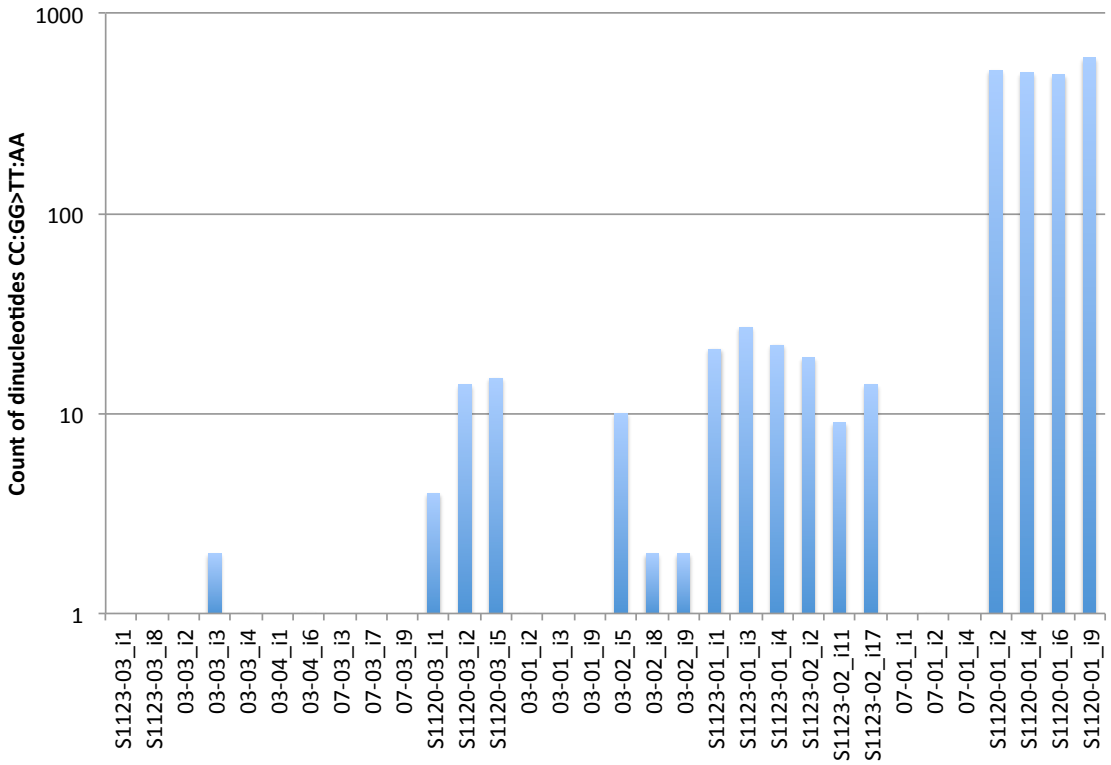
B)



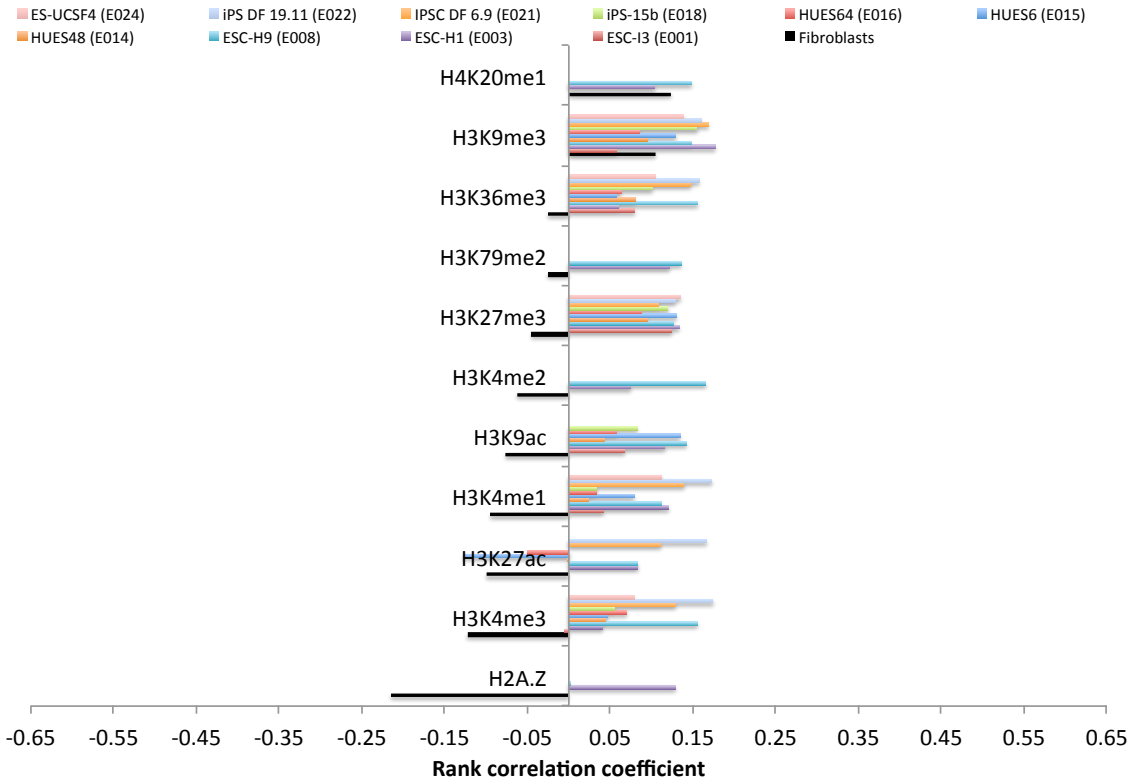
Supplemental Figure S6. A) Sequencing coverage of targeted sites in the amplification and re-sequencing experiment in fibroblasts of 03-04. B) Empirical estimation of amplification and sequencing noise in data for fibroblasts of 03-04.



Supplemental Figure S7. TAF spectrum for somatic SNVs determined from amplicon-seq experiment in fibroblasts of S1123-02. The spectrum has a distinct peak between 20-30% TAF. Existence of such peaks was also observed in fibroblast of four children (**Fig. 2**).

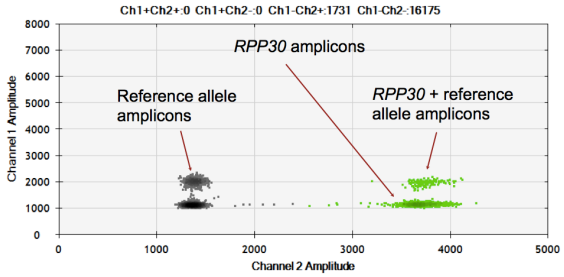


Supplemental Figure S8. Counts of dinucleotide CC:GG>TT:AA variants found in each hiPSC sample. Fathers are denominated as -01, mothers as -02 and children as -03 and -04. Parents consistently show higher fraction of such dinucleotide substitutions. Large numbers of dinucleotide substitutions partially explains larger LM-SNV call set for these lines. As the majority of these dinucleotide sites were shared between at least two hiPSC lines, the estimated maximum count of mosaic SNV is likely underestimated, due to considering them as germline variants in the estimation.



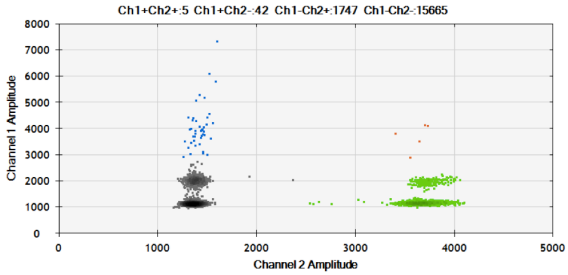
Supplemental Figure S9. Correlation coefficients of density of somatic SNVs with histone marks in primary fibroblasts cells, ESC and iPSC. Correlation are almost always (except for H3K27ac) consistent across various stem cells and different from correlation in fibroblasts.

Negative Control



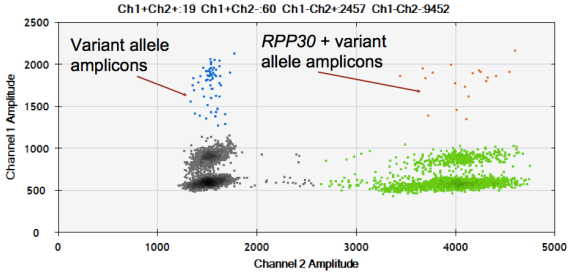
template: 20ng of 12878 genomic DNA

Positive Control



template: 20ng of 12878 genomic DNA + synthesized DNA containing variant allele at 3% reference allele frequency

03-04F

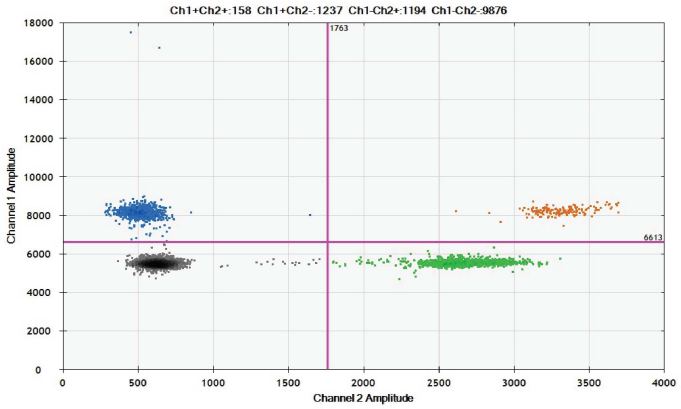


template: 20ng of F0304 genomic DNA

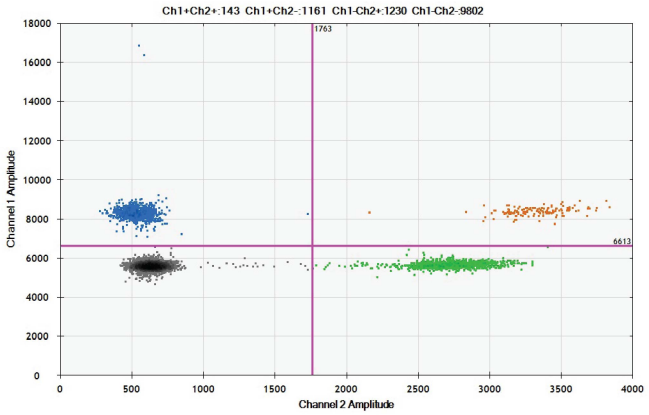
Supplemental Figure S10. Example of calibrating and running ddPCR. SNV chr2:234244563, G>T found in hiPSC line of 03-04 is used in this example.

Chr4:176850524, G>C

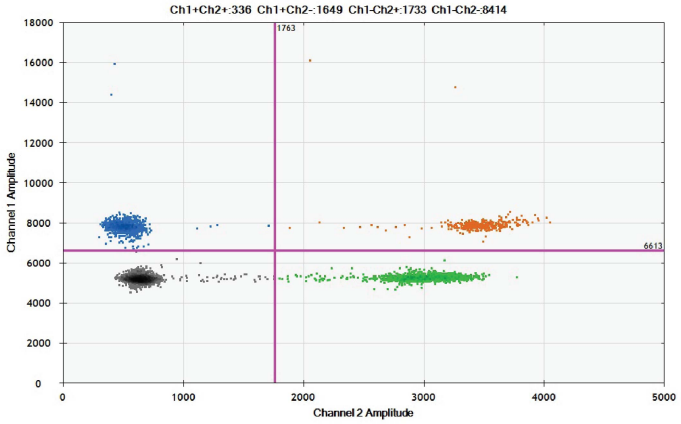
F1123-03 Replicate #1



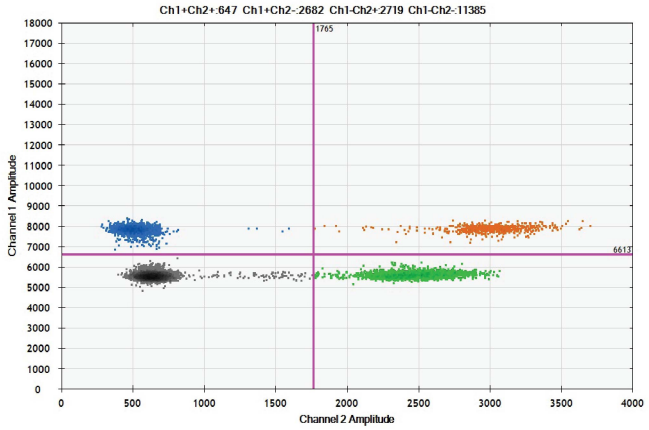
F1123-03 Replicate #2



0.3% gBlock spike into WT background



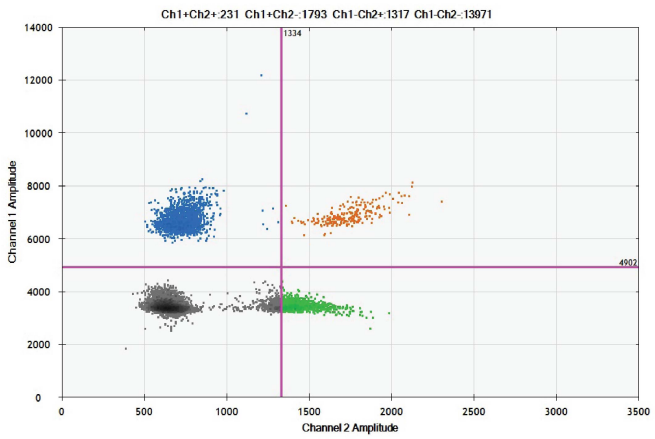
WT control



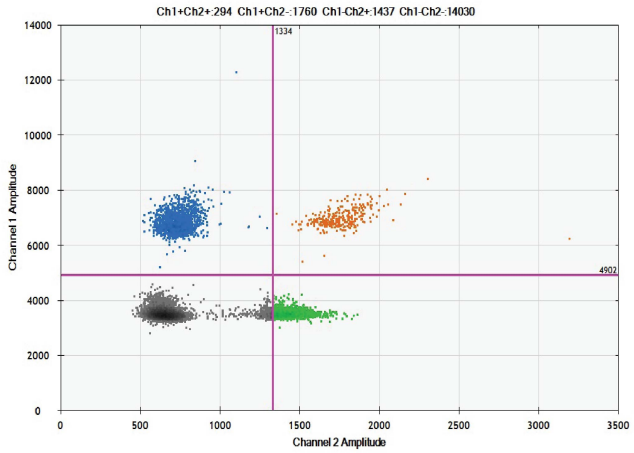
Supplemental Figure S11. ddPCR for SNV chr4:176850524, G>C in S1123-03F and positive and negative controls.

chr4:173292503, G>A

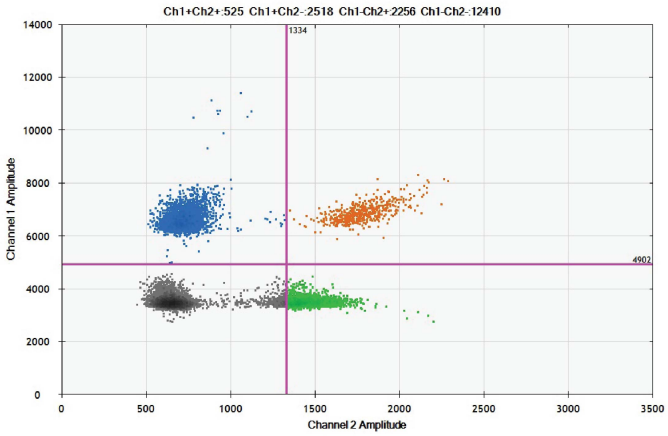
F1123-03 Replicate #1



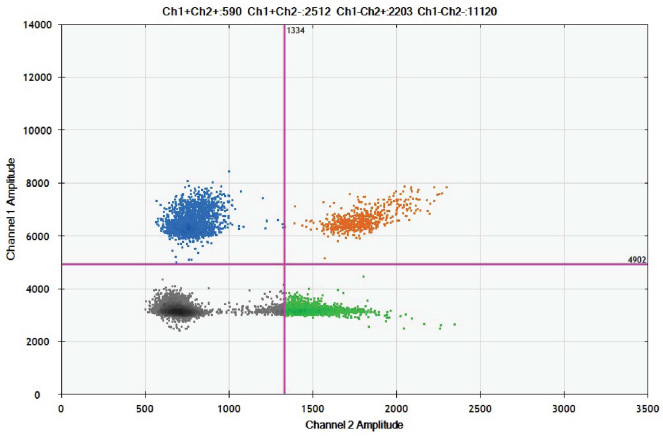
F1123-03 Replicate #2



0.3% gBlock spike into WT background



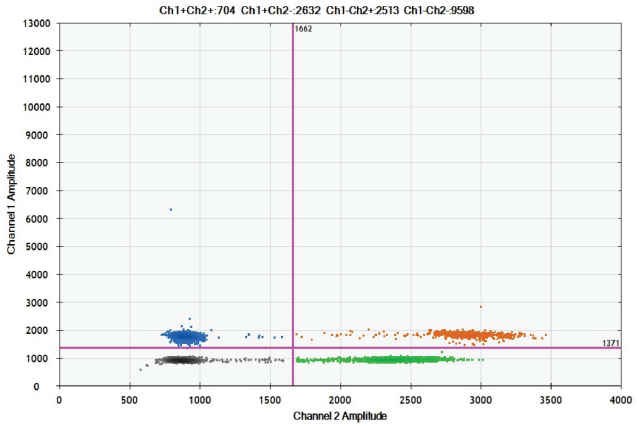
WT control



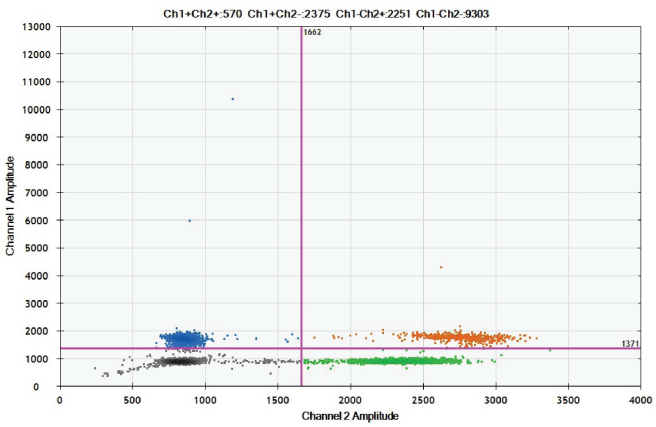
Supplemental Figure S12. ddPCR for SNV chr4:173292503, G>A S1123-03F and positive and negative controls.

chr11:78386317, T>C

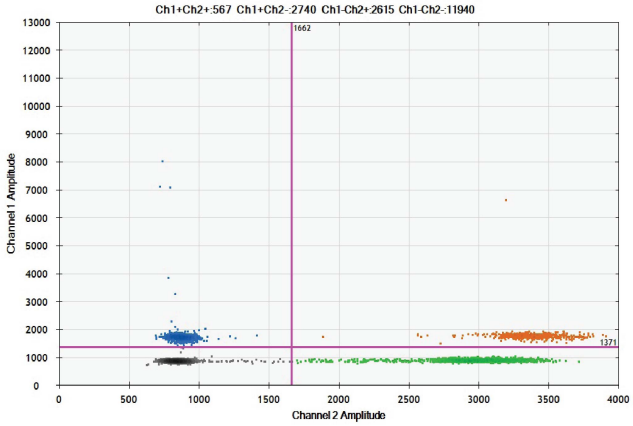
F03-04 Replicate #1



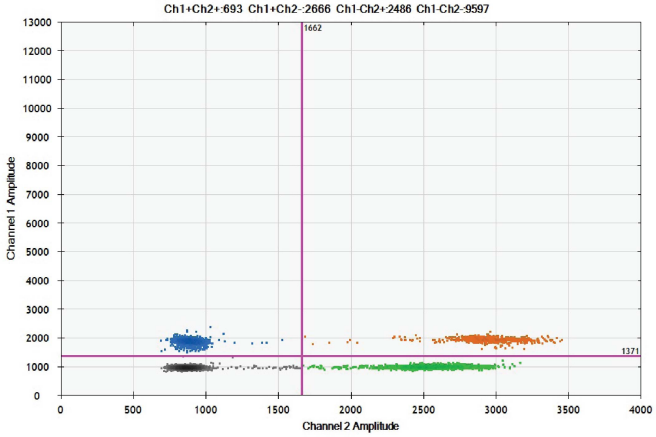
F03-04 Replicate #2



0.3% gBlock spike into WT background



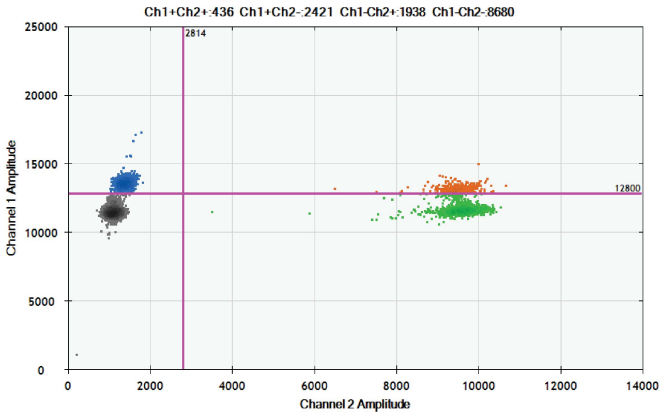
WT control



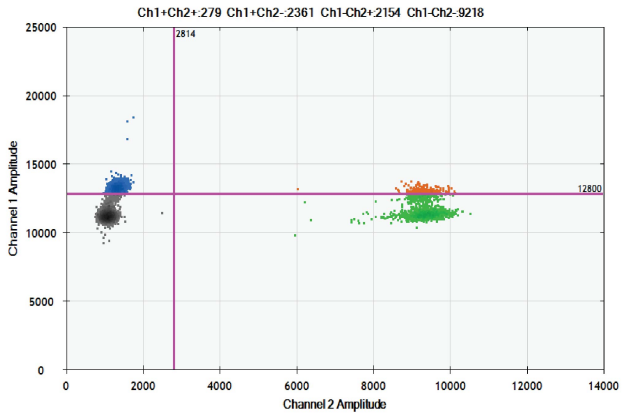
Supplemental Figure S13. ddPCR for SNV chr11:78386317, T>C in 03-04F and positive and negative controls.

chr3:44770233, G>A

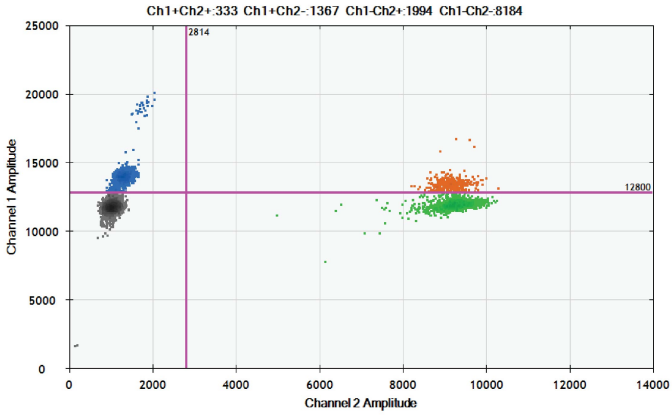
F03-04 Replicate #1



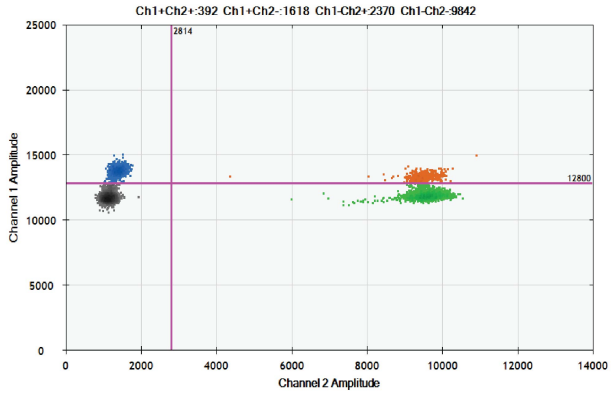
F03-04 Replicate #2



3% gBlock spike into WT background



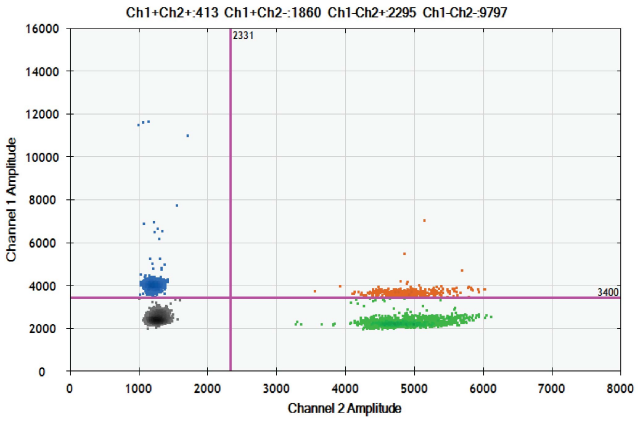
WT control



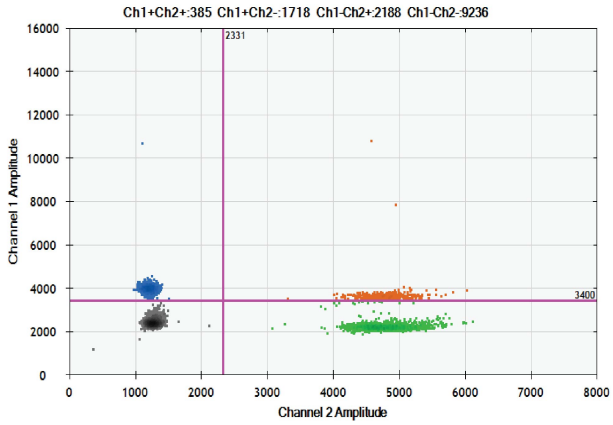
Supplemental Figure S14. ddPCR for SNV chr3:44770233, G>A in 03-04F and positive and negative controls.

chr9:103881356, C>G

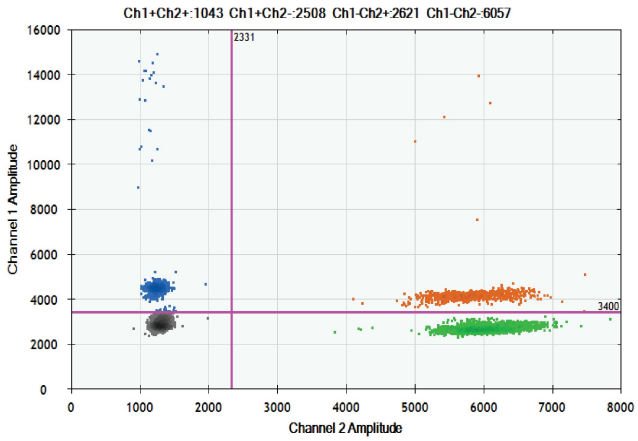
F1123-03 Replicate #1



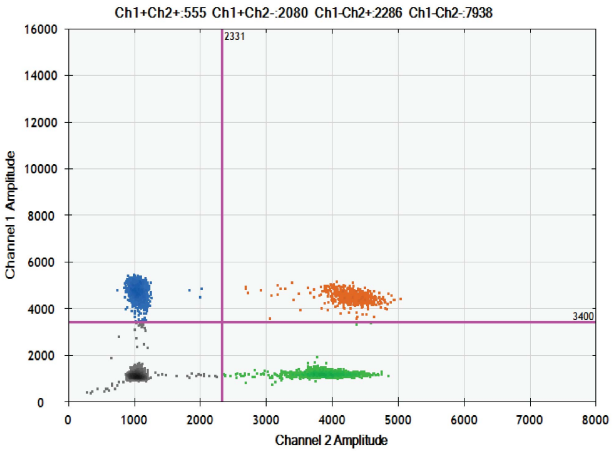
F1123-03 Replicate #2



0.3% gBlock spike into WT background



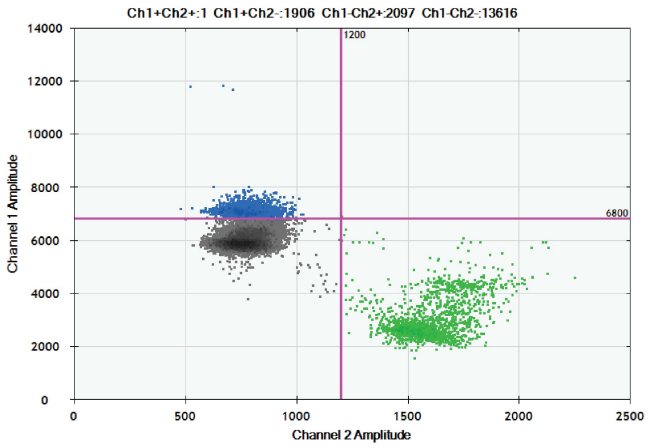
WT control



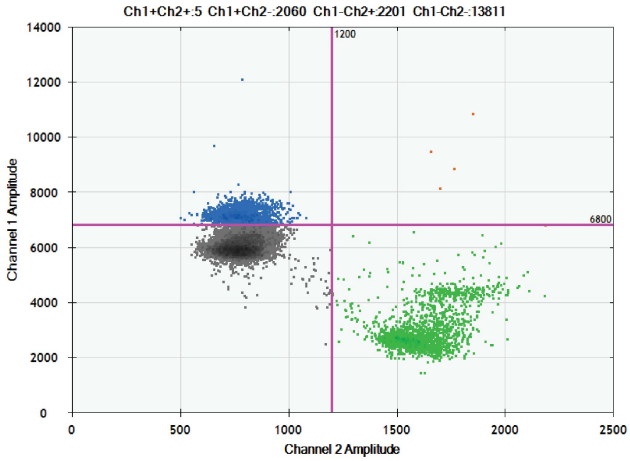
Supplemental Figure S15. ddPCR for SNV chr9:103881356, C>G in S1123-03F and positive and negative controls.

chr6:4785972, T>C

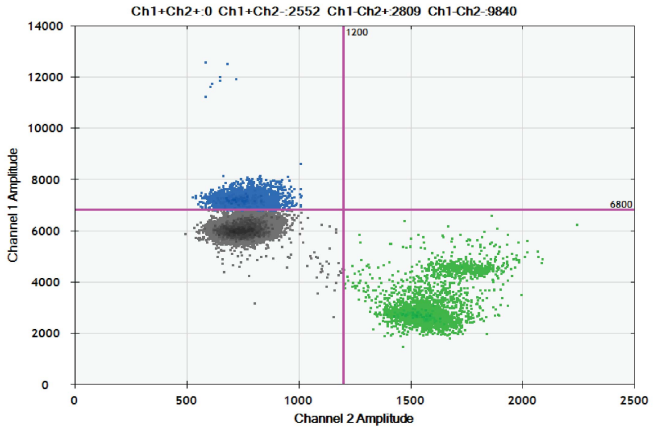
F1123-03 Replicate #1



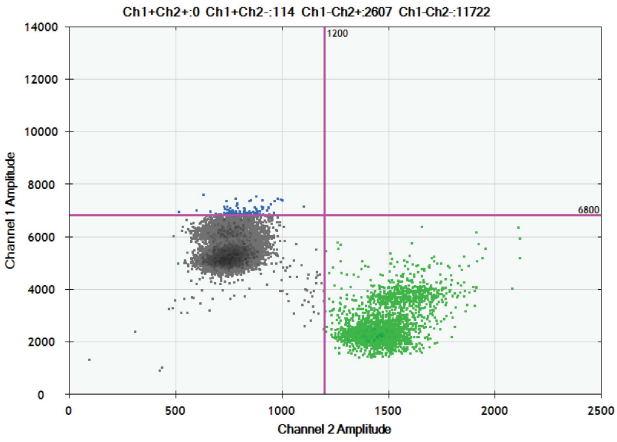
F1123-03 Replicate #2



0.3% gBlock spike into WT background



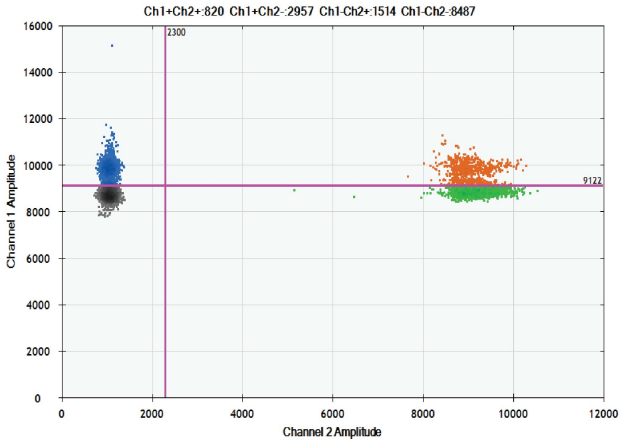
WT control



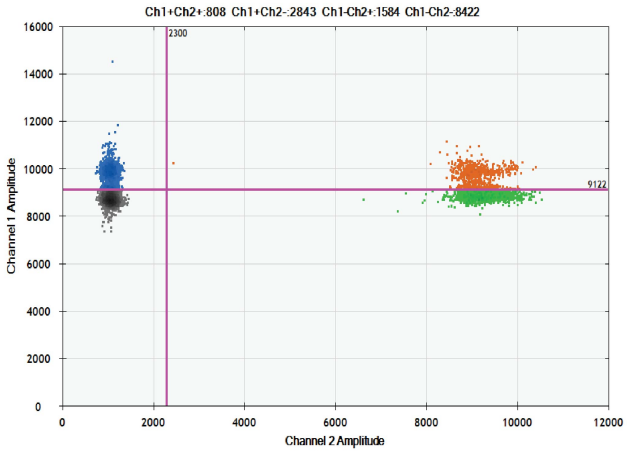
Supplemental Figure S16. ddPCR for SNV chr6:4785972, T>C in S1123-03F and positive and negative controls.

chr18:6836418, G>A

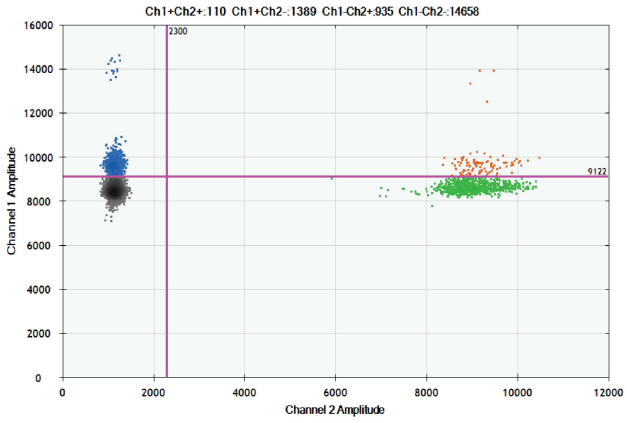
F03-04 Replicate #1



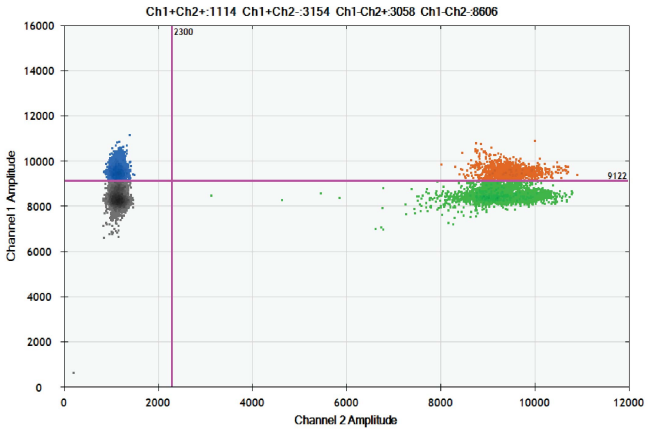
F03-04 Replicate #2



0.3% gBlock spike into WT background



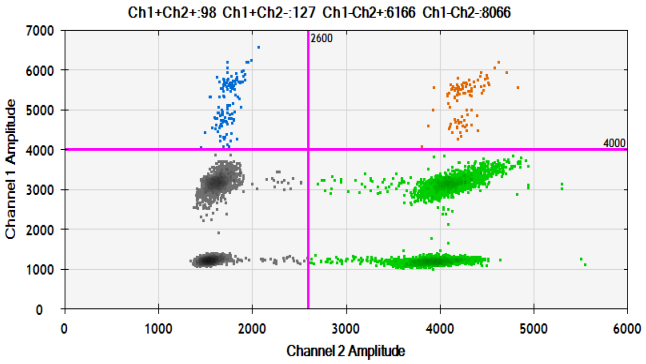
WT control



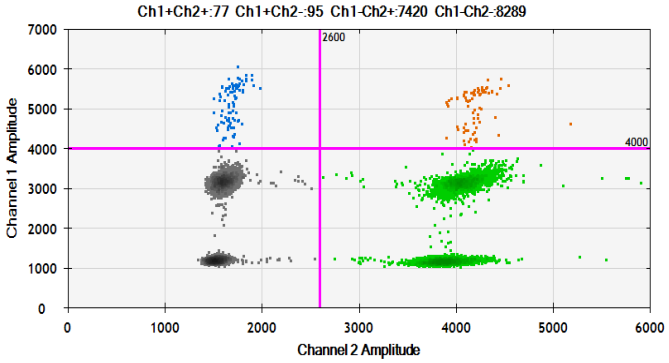
Supplemental Figure S17. ddPCR for SNV chr18:6836418, G>A in 03-04F and positive and negative controls.

chr5:176266361, G>T

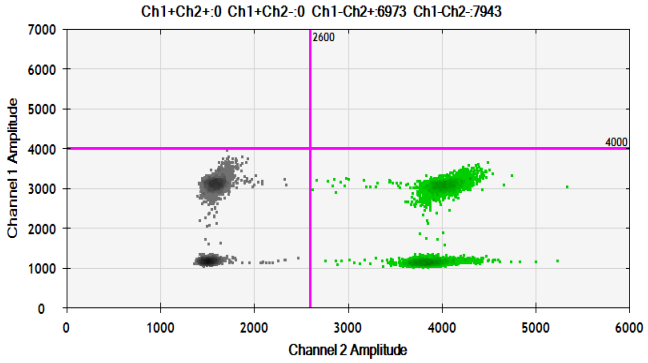
03-04F Replicate #1



gBlock spike into WT background



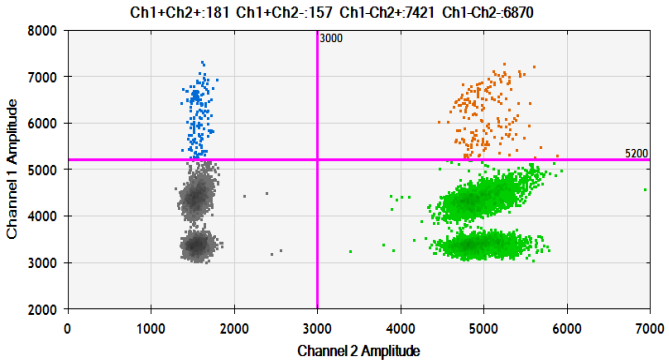
WT background



Supplemental Figure S18. ddPCR for SNV chr5:176266361, G>T in 03-04F and positive and negative controls

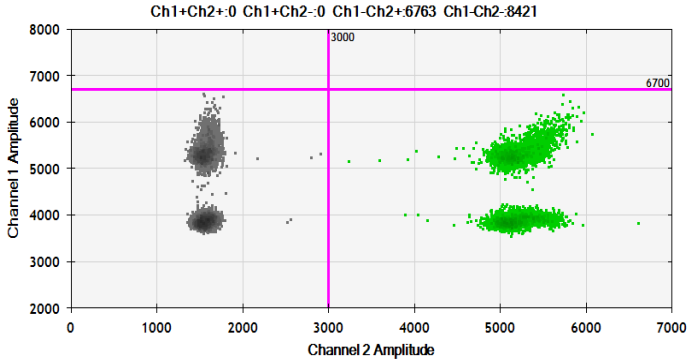
chr2:40177413, C>T

03-04F Replicate #1



gBlock spike into WT background

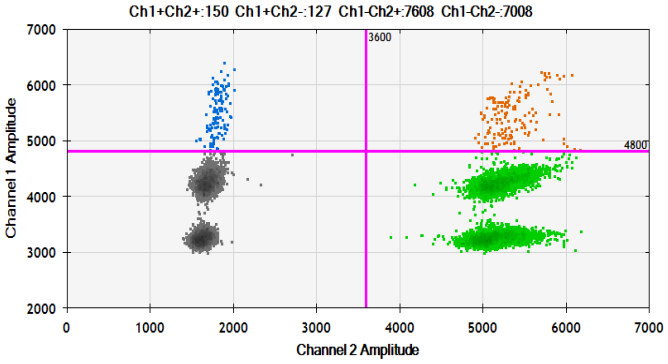
WT background



Supplemental Figure S19. ddPCR for SNV chr2:40177413, C>T in 03-04F and negative control

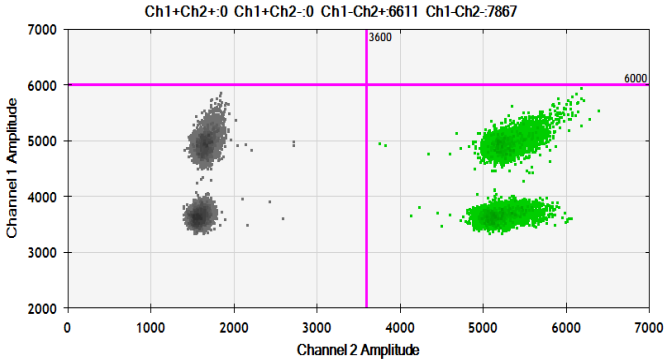
chr5:160336307, T>C

03-04F Replicate #1



gBlock spike into WT background

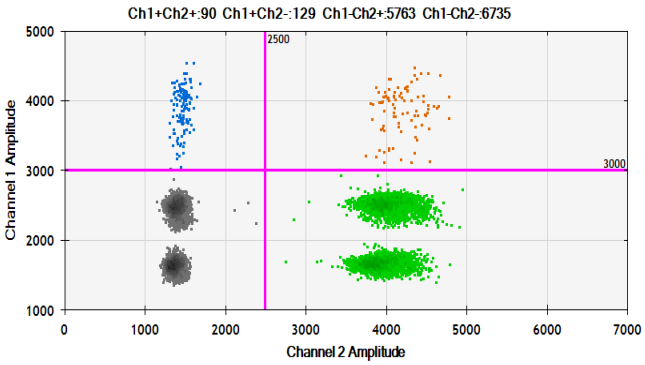
WT background



Supplemental Figure S20. ddPCR for SNV chr5:160336307, T>C in 03-04F and negative control

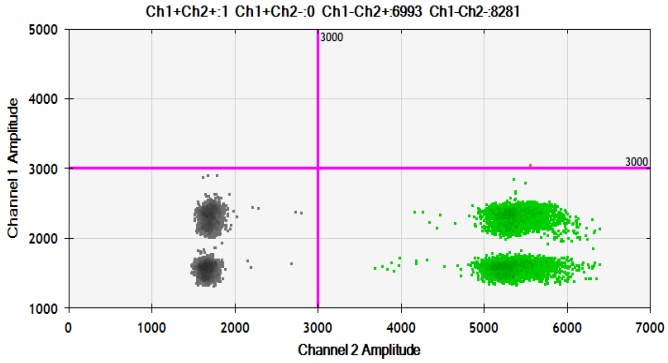
chr18:22640972, A>G

03-04F Replicate #1



gBlock spike into WT background

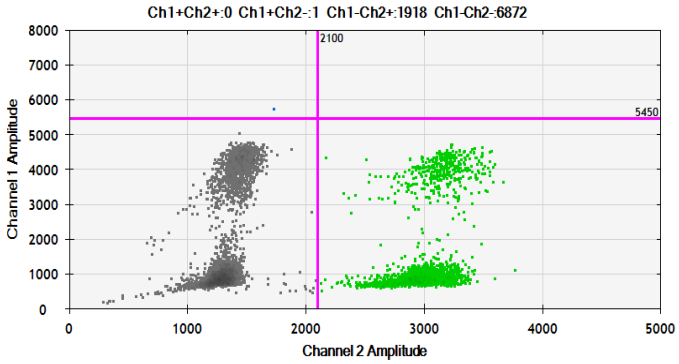
WT background



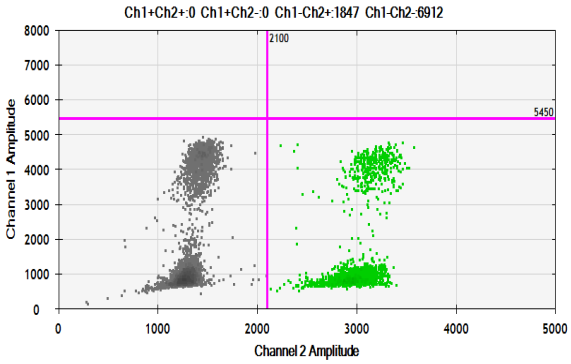
Supplemental Figure S21. ddPCR for SNV chr18:22640972, A>G in 03-04F and negative control

chr2:39382871, A>G

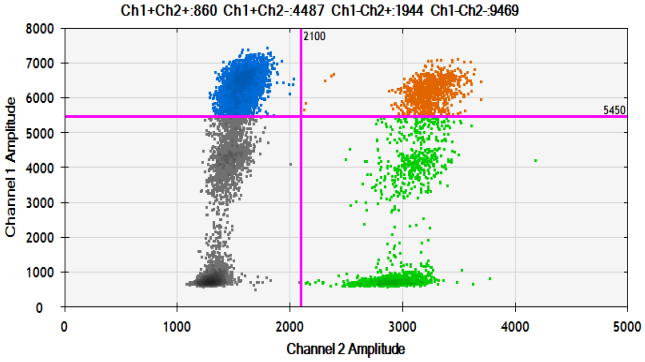
03-03F Replicate #1



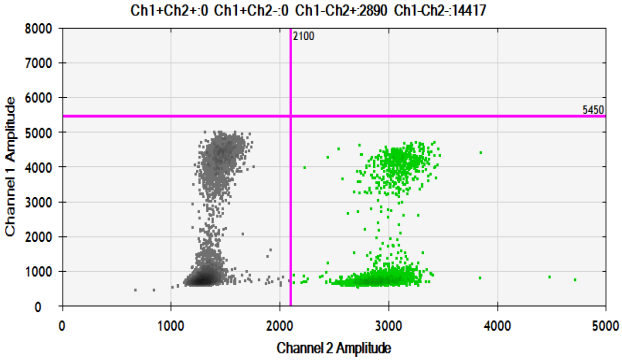
03-03F Replicate #2



gBlock spike into WT background



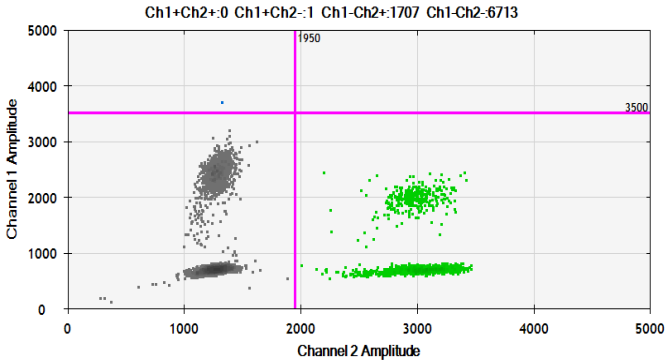
WT background



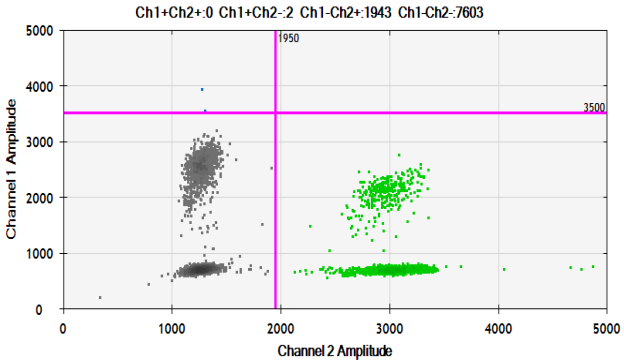
Supplemental Figure S22. ddPCR for SNV chr2:39382871, A>G in 03-03F and positive and negative controls

chr3:11437689, G>A

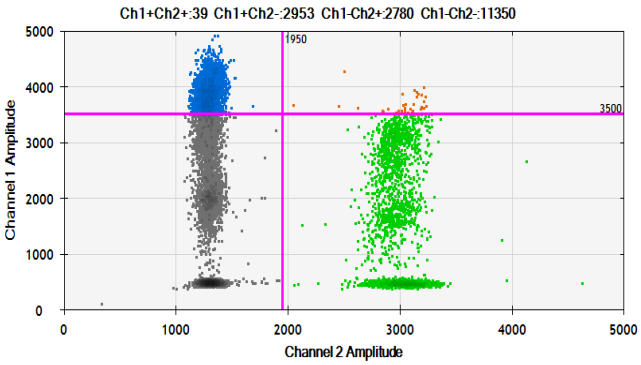
03-03F Replicate #1



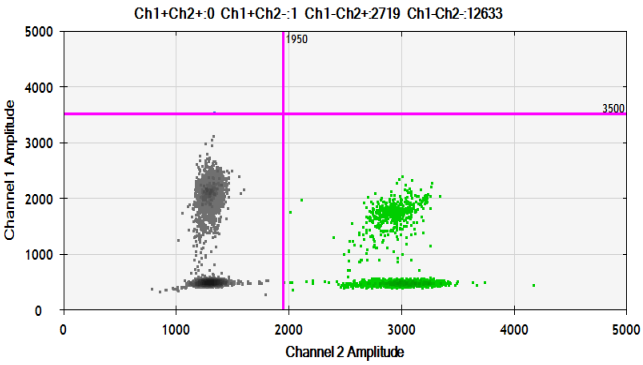
03-03F Replicate #2



gBlock spike into WT background

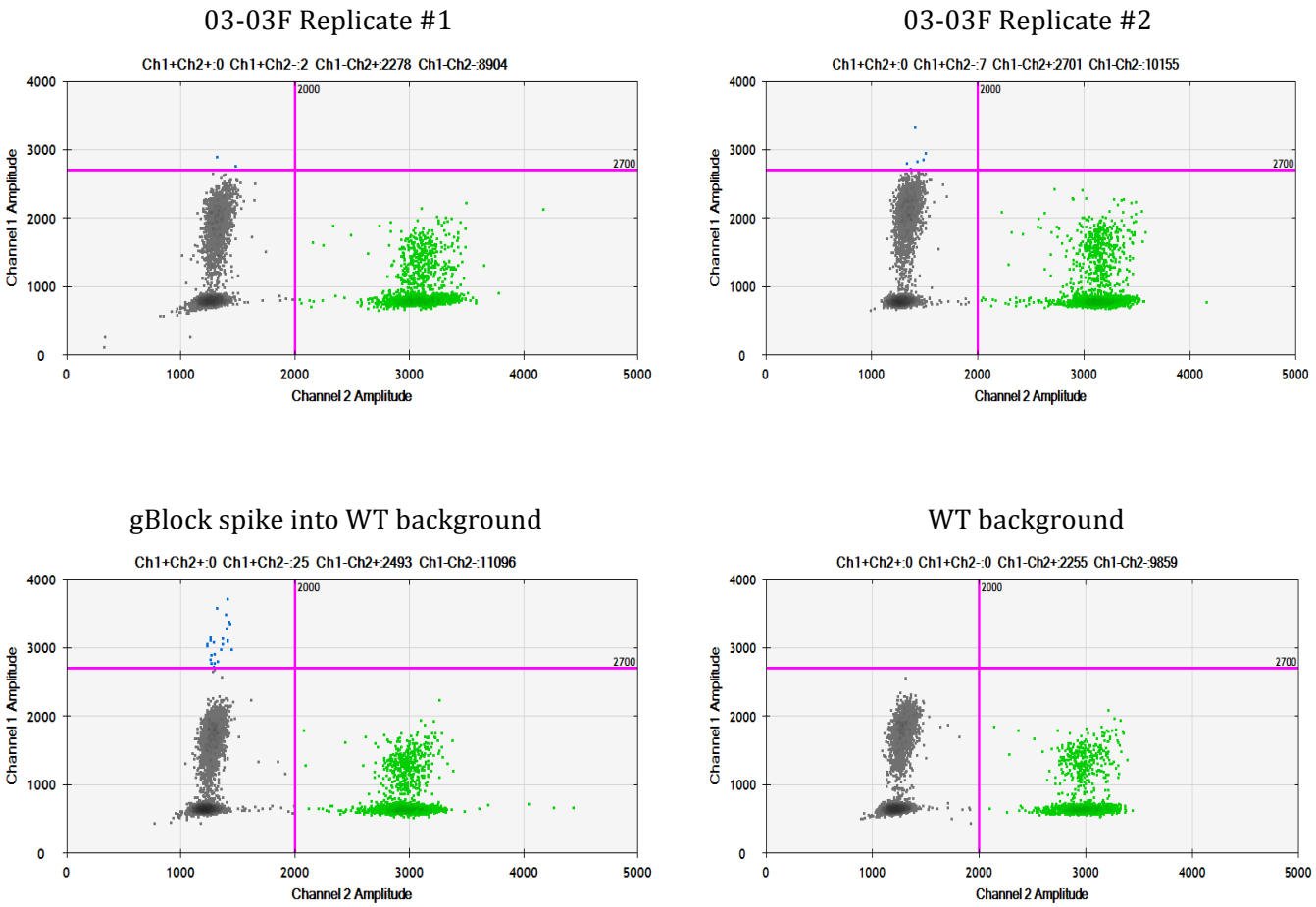


WT background



Supplemental Figure S23. ddPCR for SNV chr3:11437689, G>A in 03-03F and positive and negative controls

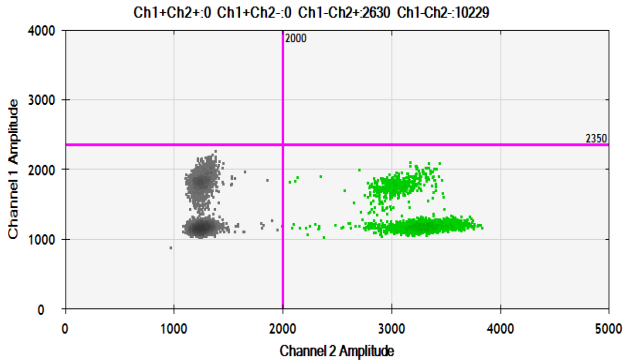
chr17:60090380, T>C



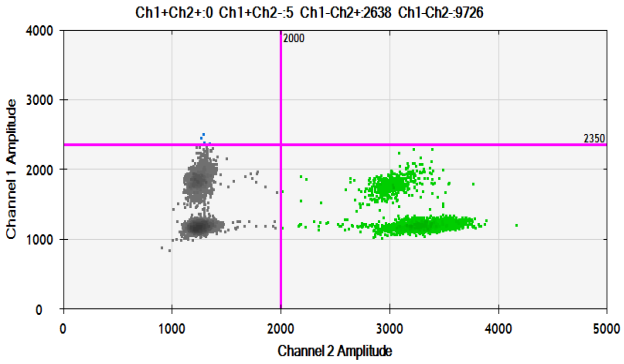
Supplemental Figure S24. ddPCR for SNV chr17:60090380, T>C in 03-03F and positive and negative controls

chr3:85271680, T>C

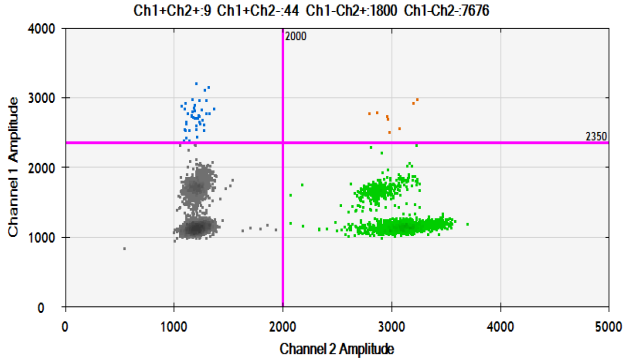
03-03F Replicate #1



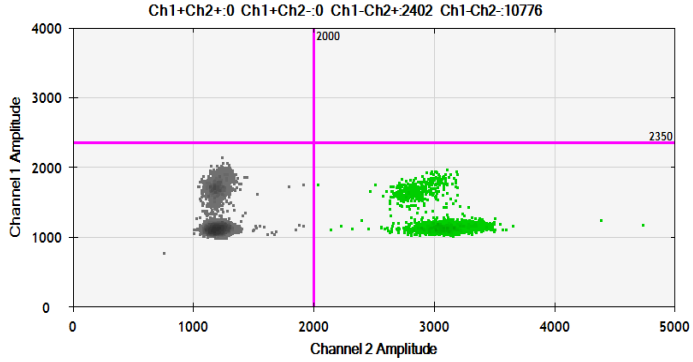
03-03F Replicate #2



gBlock spike into WT background



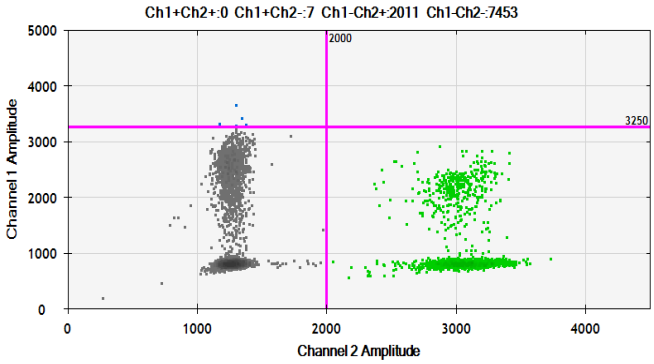
WT background



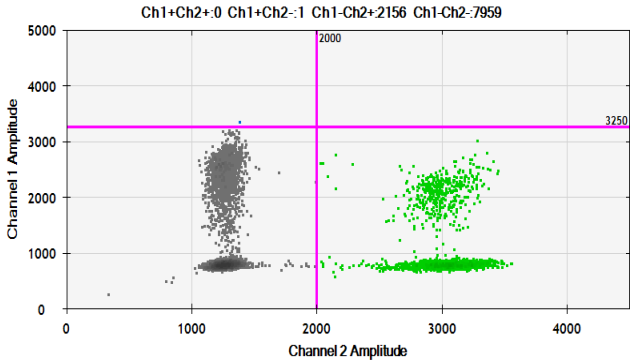
Supplemental Figure S25. ddPCR for SNV chr3:85271680, T>C in 03-03F and positive and negative controls

chr3:47837575, C>T

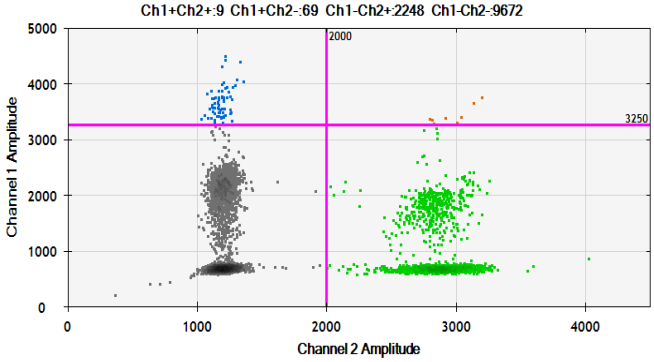
03-03F Replicate #1



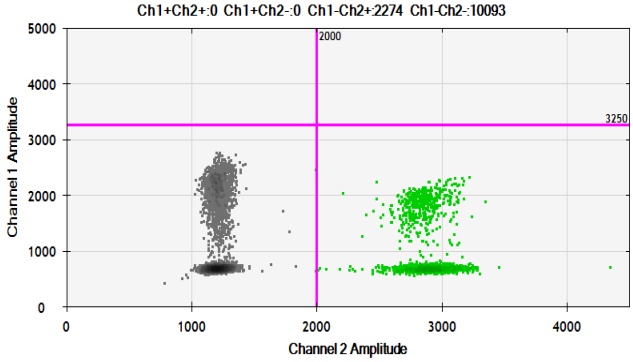
03-03F Replicate #2



gBlock spike into WT background



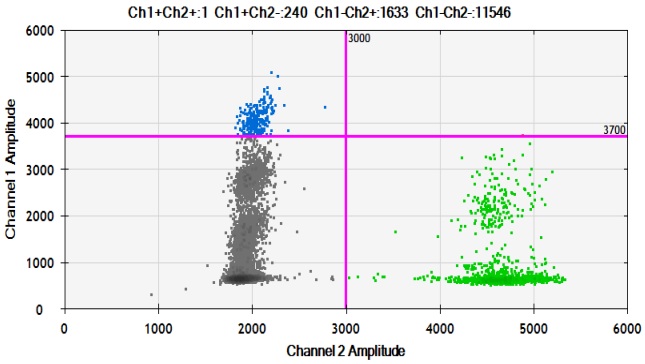
WT background



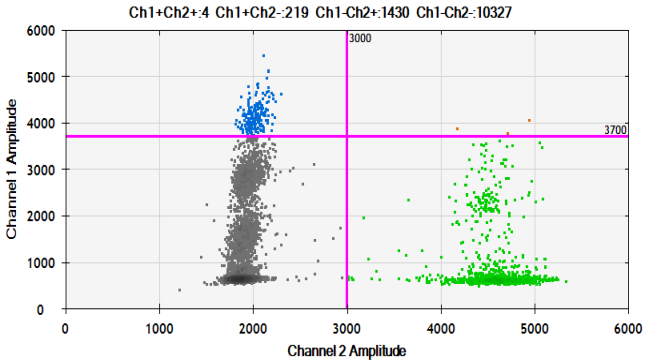
Supplemental Figure S26. ddPCR for SNV chr3:47837575, C>T in 03-03F and positive and negative controls

chr4:72999731, C>T

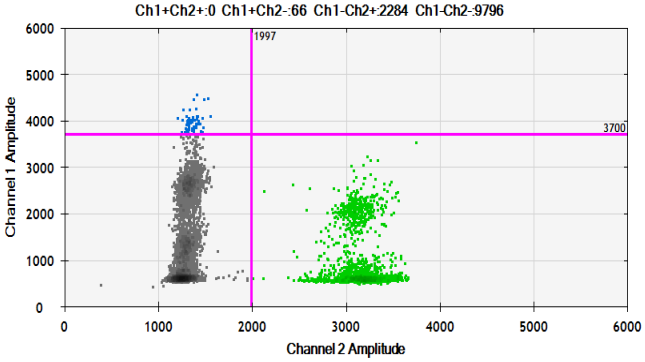
03-03F Replicate #1



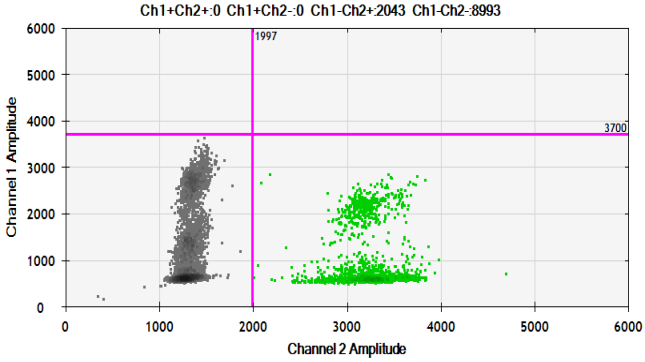
03-03F Replicate #2



gBlock spike into WT background



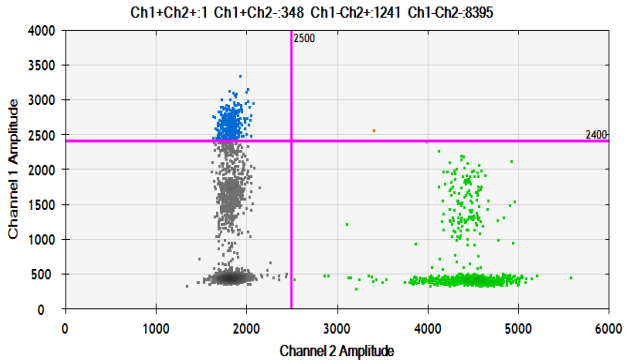
WT background



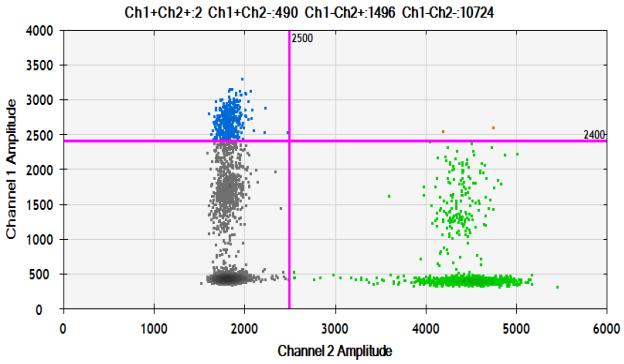
Supplemental Figure S27. ddPCR for SNV chr4:72999731, C>T in 03-03F and positive and negative controls

chr7:107614314, G>A

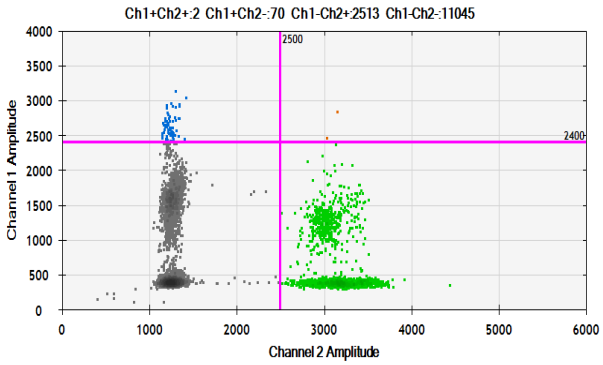
03-03F Replicate #1



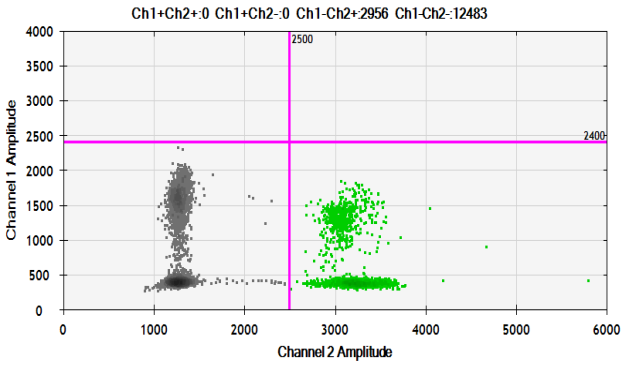
03-03F Replicate #2



gBlock spike into WT background



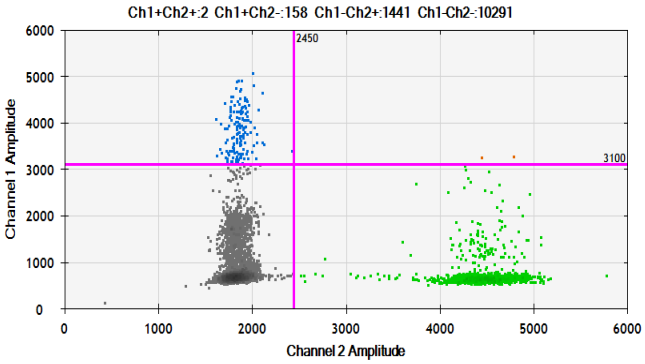
WT background



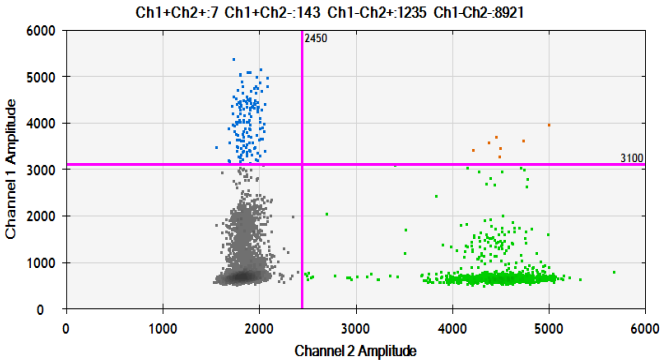
Supplemental Figure S28. ddPCR for SNV chr7:107614314, G>A in 03-03F and positive and negative controls

chr10:94330569, A>T

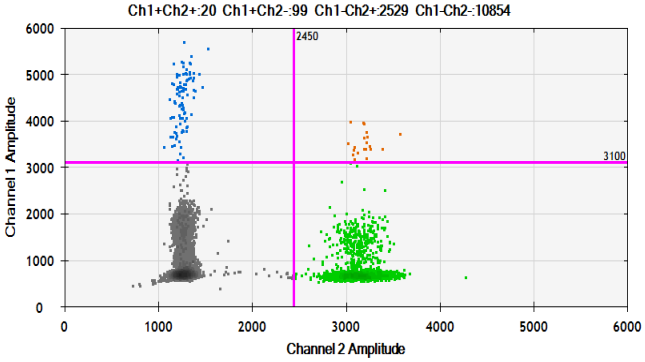
03-03F Replicate #1



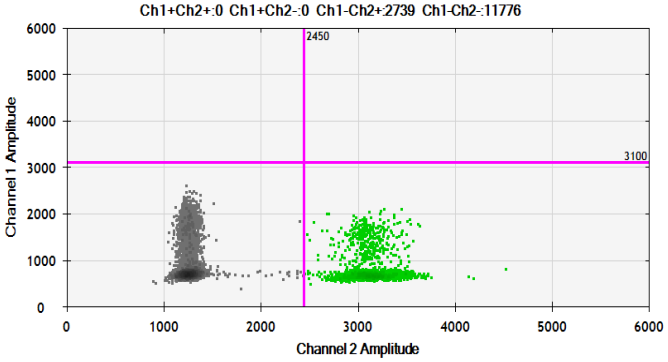
03-03F Replicate #2



gBlock spike into WT background



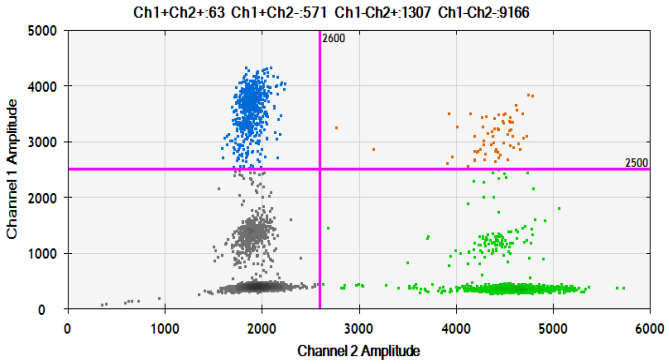
WT background



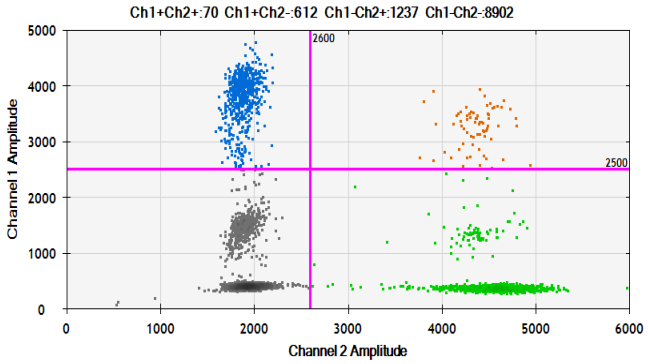
Supplemental Figure S29. ddPCR for SNV chr10:94330569, A>T in 03-03F and positive and negative controls

chr19:46874059, G>A

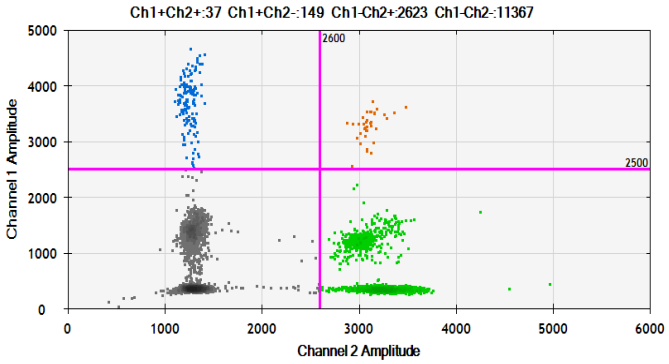
03-03F Replicate #1



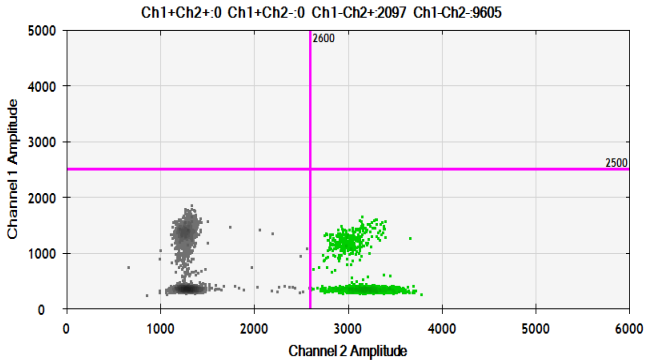
03-03F Replicate #2



gBlock spike into WT background



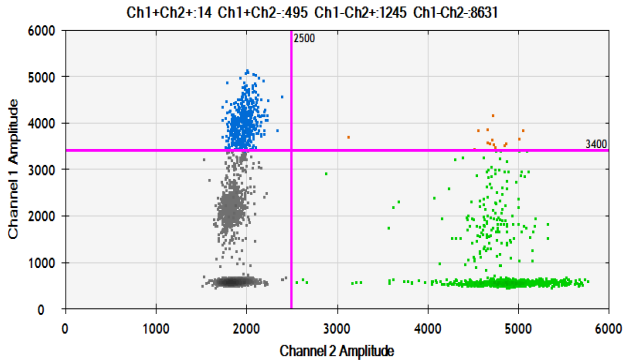
WT background



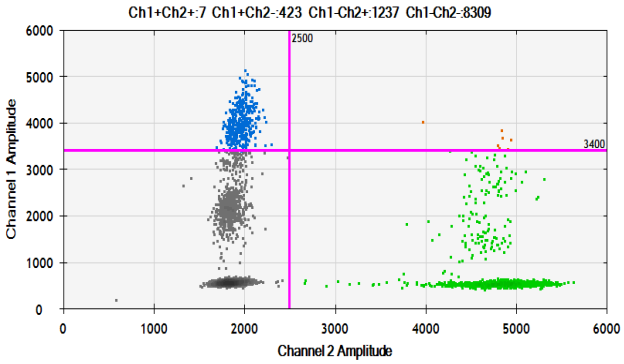
Supplemental Figure S30. ddPCR for SNV chr19:46874059, G>A in 03-03F and positive and negative controls

chr4:129432139, A>G

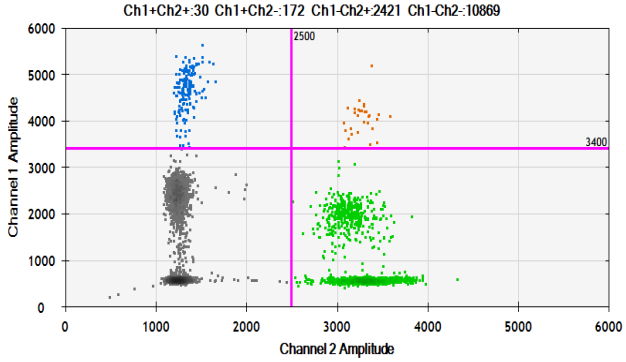
03-03F Replicate #1



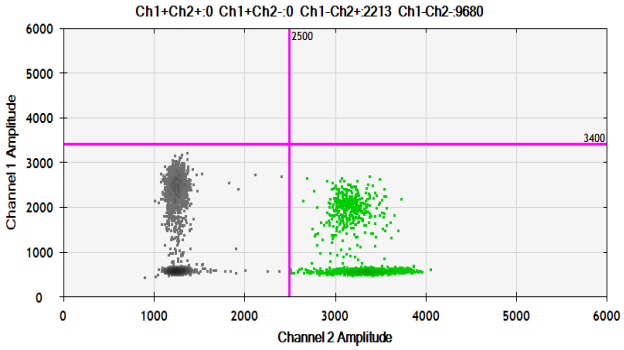
03-03F Replicate #2



gBlock spike into WT background



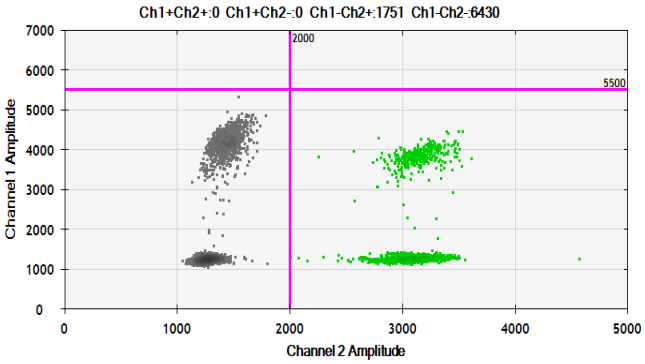
WT background



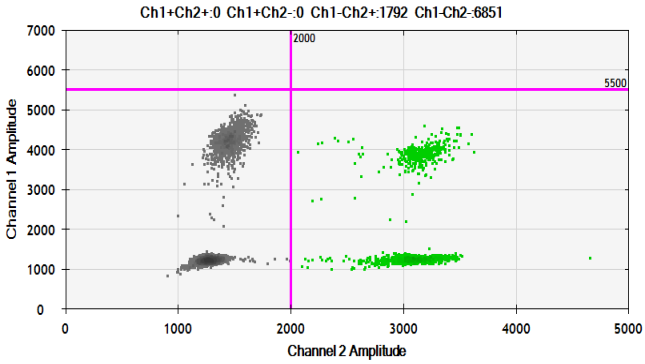
Supplemental Figure S31. ddPCR for SNV chr4:129432139, A>G in 03-03F and positive and negative controls

chr2:109542477, T>C

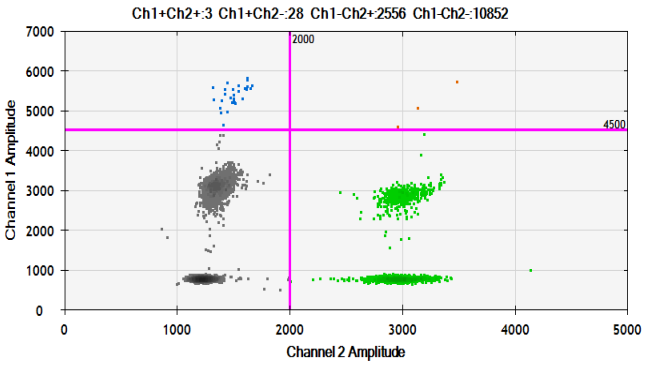
03-03F Replicate #1



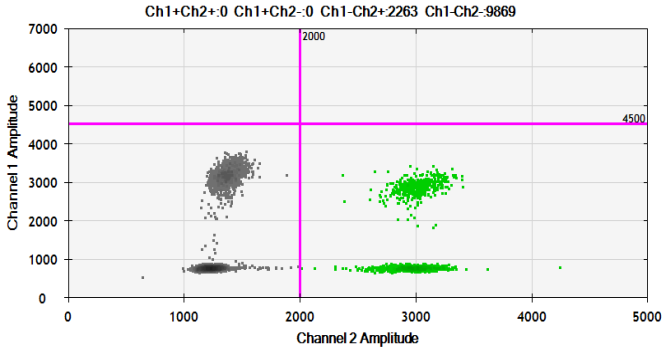
03-03F Replicate #2



gBlock spike into WT background



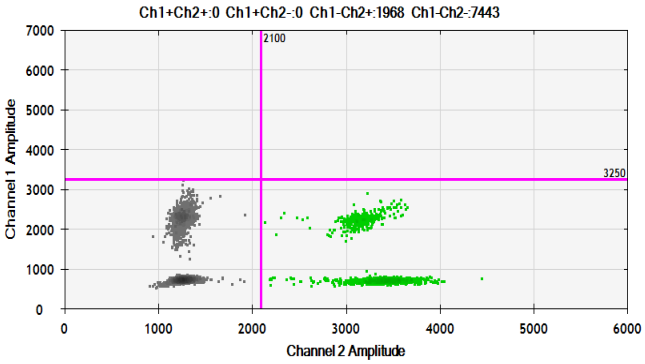
WT background



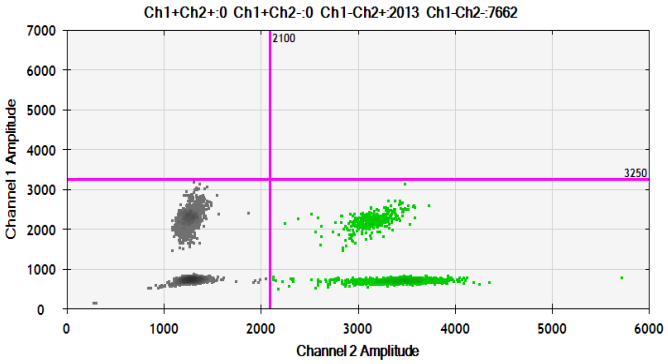
Supplemental Figure S32. ddPCR for SNV chr2:109542477, T>C in 03-03F and positive and negative controls

chr10:24138267, G>C

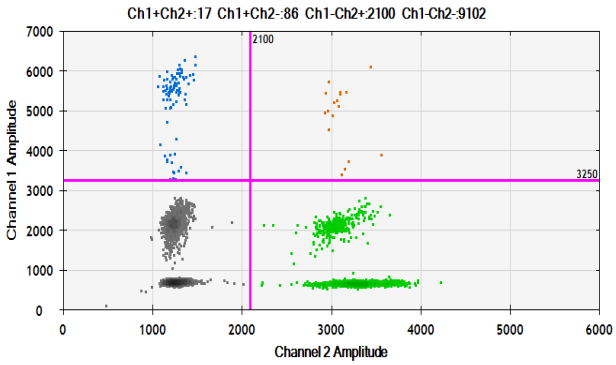
03-03F Replicate #1



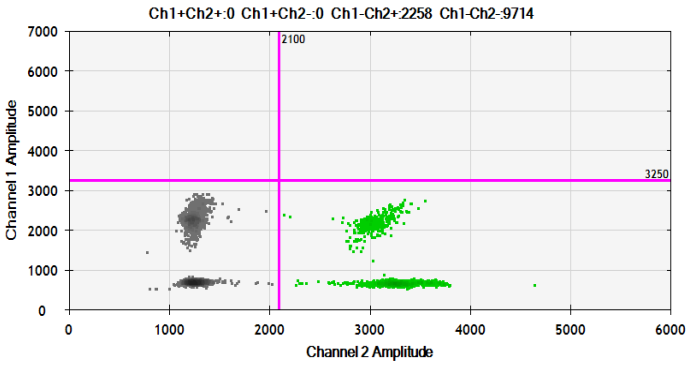
03-03F Replicate #2



gBlock spike into WT background



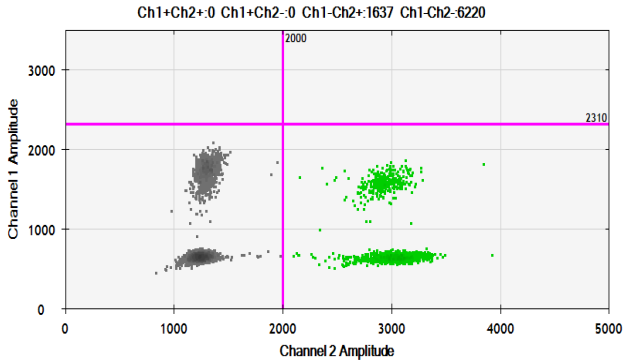
WT background



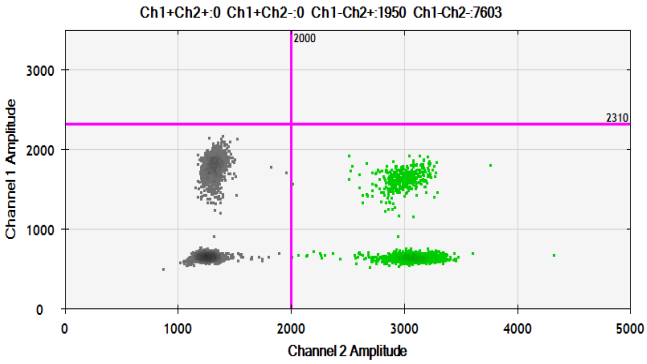
Supplemental Figure S33. ddPCR for SNV chr10:24138267, G>C in 03-03F and positive and negative controls

chr2:83643848, C>G

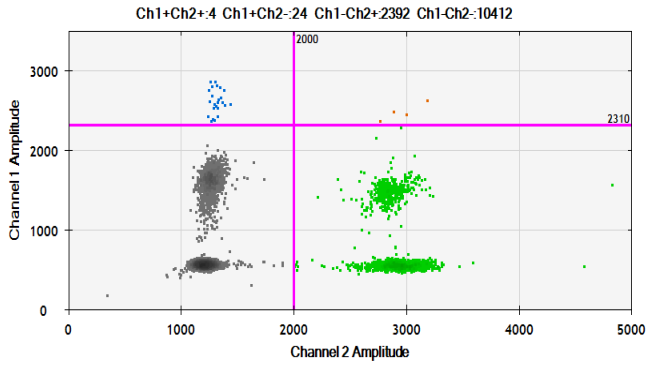
03-03F Replicate #1



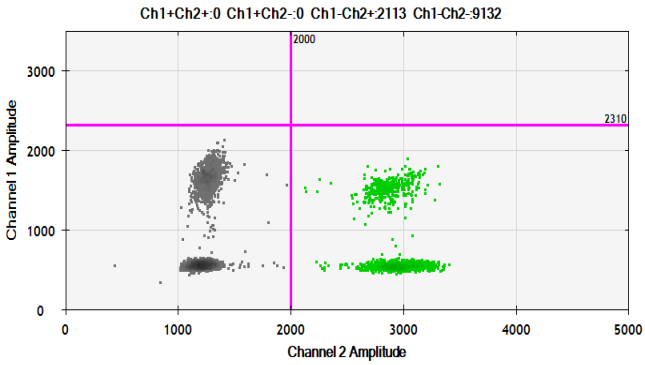
03-03F Replicate #2



gBlock spike into WT background



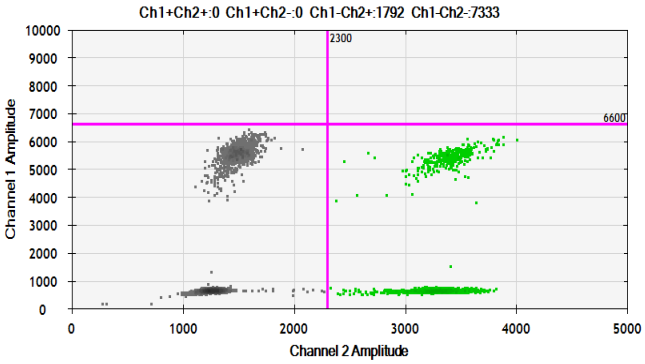
WT background



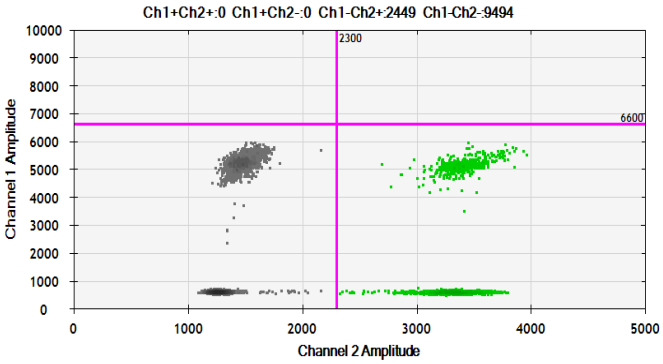
Supplemental Figure S34. ddPCR for SNV chr2:83643848, C>G in 03-03F and positive and negative controls

chr7:26034784, C>G

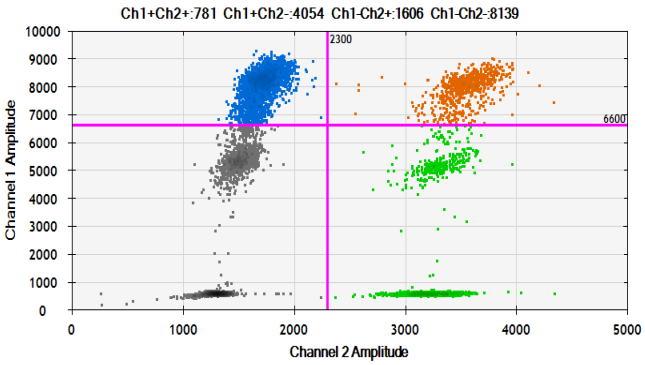
03-03F Replicate #1



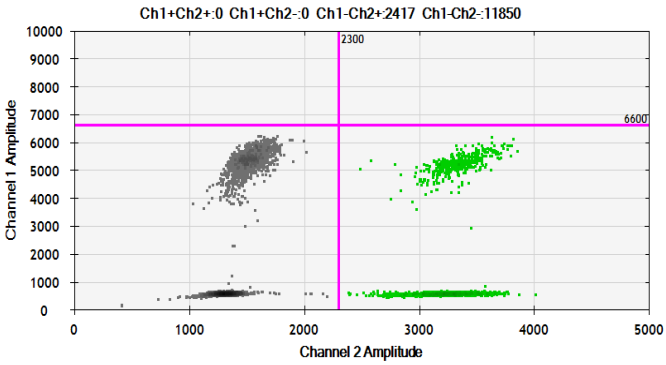
03-03F Replicate #2



gBlock spike into WT background



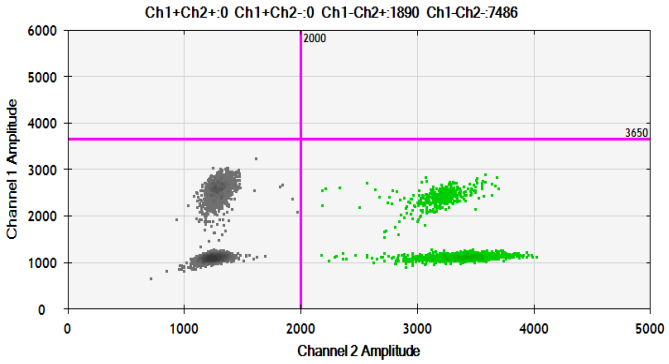
WT background



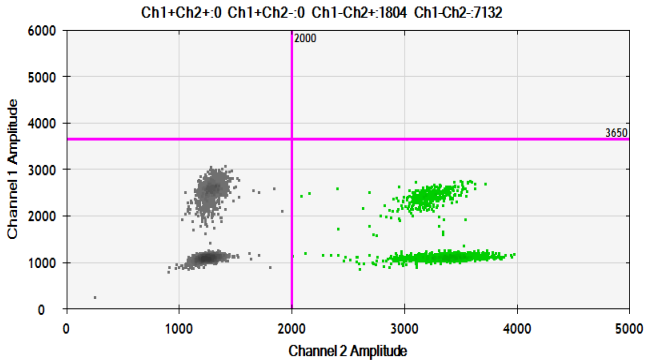
Supplemental Figure S35. ddPCR for SNV chr7:26034784, C>G in 03-03F and positive and negative controls

chr2:6695877, T>C

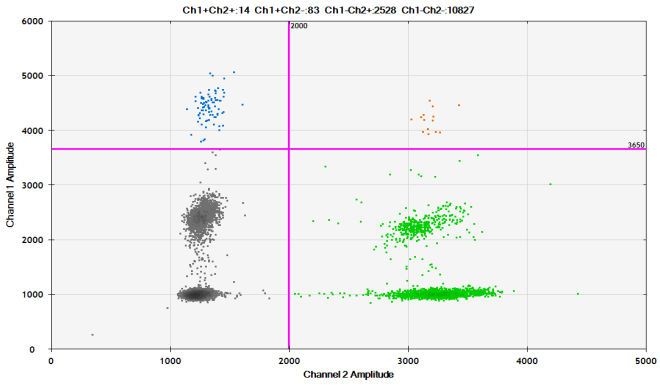
03-03F Replicate #1



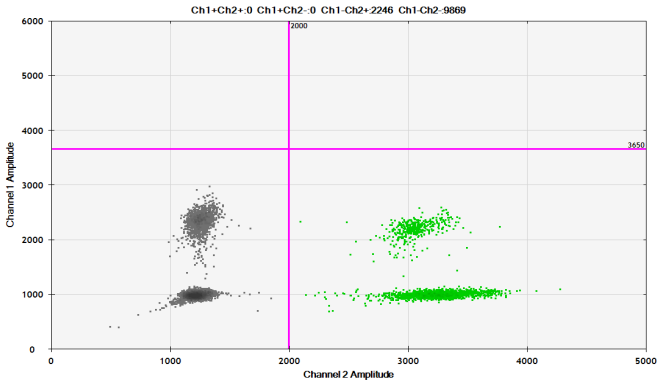
03-03F Replicate #2



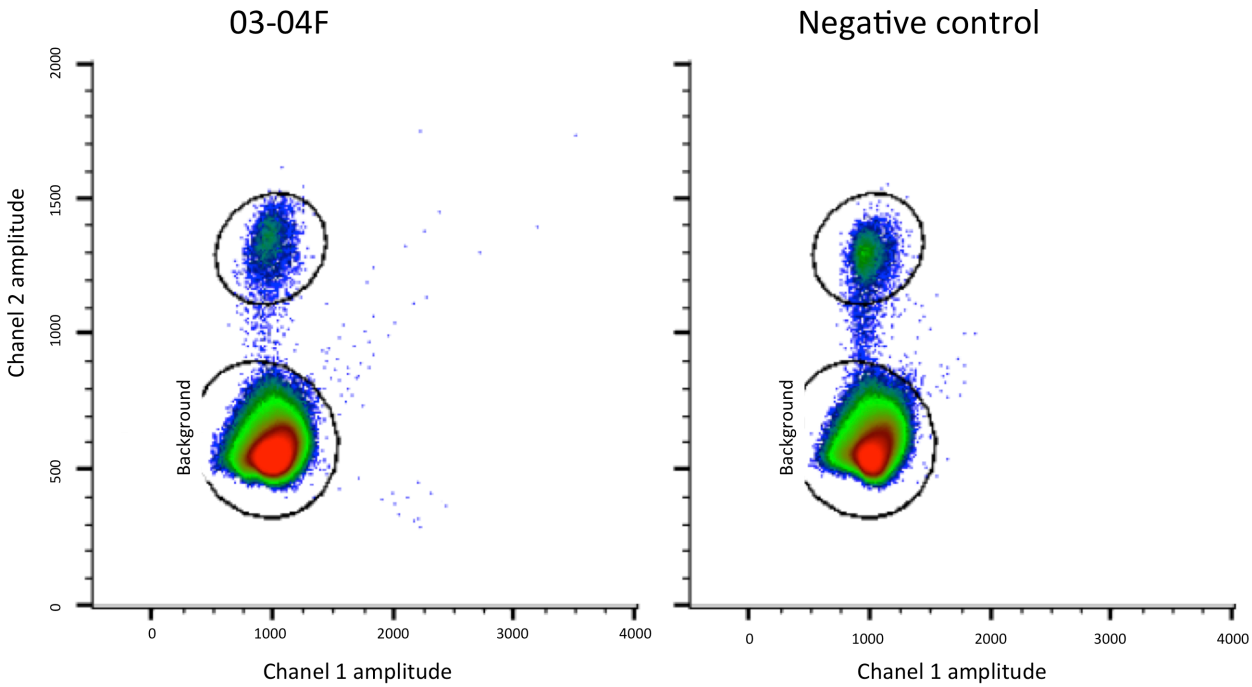
gBlock spike into WT background



WT background



Supplemental Figure S36. ddPCR for SNV chr2:6695877, T>C in 03-03F and positive and negative controls



Supplemental Figure S37. ddPCR for SNV chr5:160440768, T>C in 03-04F and negative control.