Supplemental Methods and Data

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Supplemental Methods

Standardized skin biopsy processing and culture protocol

Between the time of initial skin biopsy and culture set-up, skin biopsy samples were kept in sterile RPMI at room temperature (18-25 $^{\circ}$ *Celsius).* Using sterile technique, samples were measured, trimmed of hair, adipose, and connective tissue, minced, and incubated in collagenase solution at 37°C (with 5% CO_2) for 75 minutes, then aspirated and expelled with a 5 mL, 20-gauge syringe three times, and finally re-incubated overnight or until the end of the work day. Samples were then aspirated three more times with the syringe and spun down at 1000 rpm for 10 minutes before collagenase was removed and the tissue was resuspended in 0.6 - 1.2 mL AmnioMax Media (Invitrogen, # 12556-015 or # 17001-082) for plating in a T12 vented flask. The flask was then placed back into the incubator. Samples were gently flooded with 2 mL additional media 24 hours after initiation and were then allowed to grow to 75-80% confluence before being split. When ready to split, media was aspirated and samples were washed in HBSS and then left in 0.8 mL of TrypLE Express (Invitrogen, # 12605-010) for 5 minutes before transfer to a T75 flask. After the flask was filled with fresh media, samples were returned to the incubator for further growth. When the samples reached 80-100% confluency in T75 flasks, they were again trypsinized before being removed from the flask and spun down. After media supernatant was removed, 3-5 mL of PBS was added, and the cells were spun down again to prepare a pellet for DNA extraction. Time from culture initiation to DNA extraction was recorded in the laboratory culture log. Samples failing to culture were noted in the laboratory culture log, and the ordering physician was notified.

Skin biopsy culture trouble shooting

For samples slow to grow in the initial T12 flask compared to other samples, the sample can be tyrpsinized per above but instead of transferring to a T75, 2 ml of fresh AminoMax media can be added, the sample gently agitated to allow the cells to spread out, and returned to the incubator for further growth. Culture failure is more likely at the initial T12 stage (never plating down > never reaching 75% confluency). Failing to recover after splitting the sample from the T12 into a T75 is rare with this protocol.

Bioinformatics and Variant calling

FASTQ files were mapped, indels realigned, duplicates marked, and variants called and annotated as previously described[1] and outlined in Supplemental Figure S2. Annotated variants were then filtered according to set filters detailed in Supplemental Figure S2.

VNTR Analysis

Multiple loci VNTR analysis (MLVA) was performed on cultured skin fibroblast samples from 23 subjects who had undergone allogeneic stem cell transplant prior to biopsy. Results from each subject's cultured skin fibroblast sample was compared to his/her stem cell donor and his/her own pre-transplant blood or buccal swab based MLVA results at 16 specific loci. Loci were mapped and visualized using GeneMapper 5.0 (Applied Biosystems).

References

1. Guidugli L, Johnson AK, Alkorta-Aranburu G, Nelakuditi V, Arndt K, Churpek JE, Godley LA, Townsley D, Young NS, Fitzpatrick C, Del Gaudio D, Das S, Li Z. Clinical utility of gene panel-based testing for hereditary myelodysplastic syndrome/acute leukemia predisposition syndromes. Leukemia 2017, 31:1226-1229

Supplemental Figures

FIGURE S1 Study consort diagram

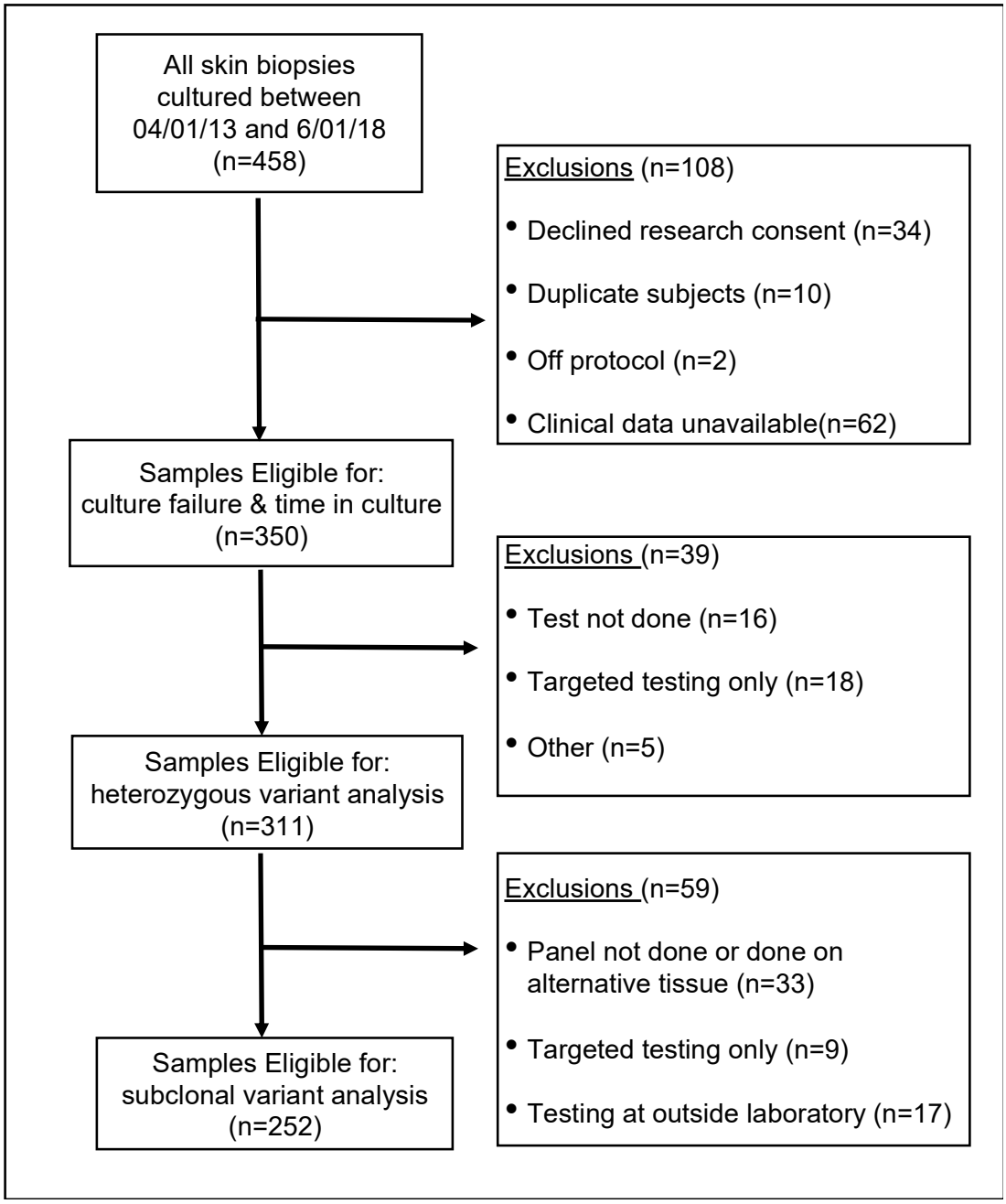

FIGURE S2 Variant filtering schema

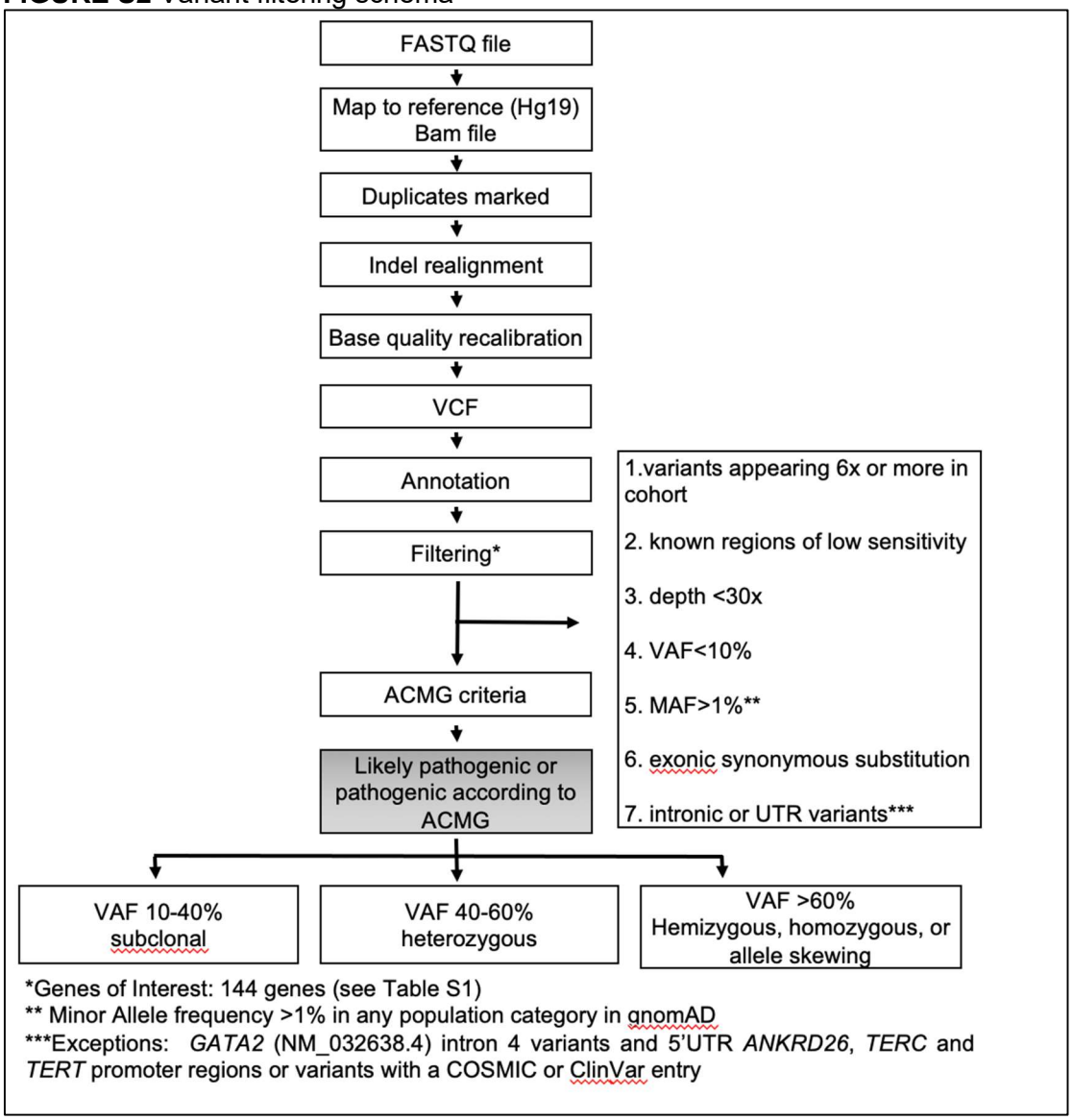

FIGURE S3 Time from culture initiation to confluency by (a) total population and (b) by heterozygous pathogenic/likely pathogenic variant status

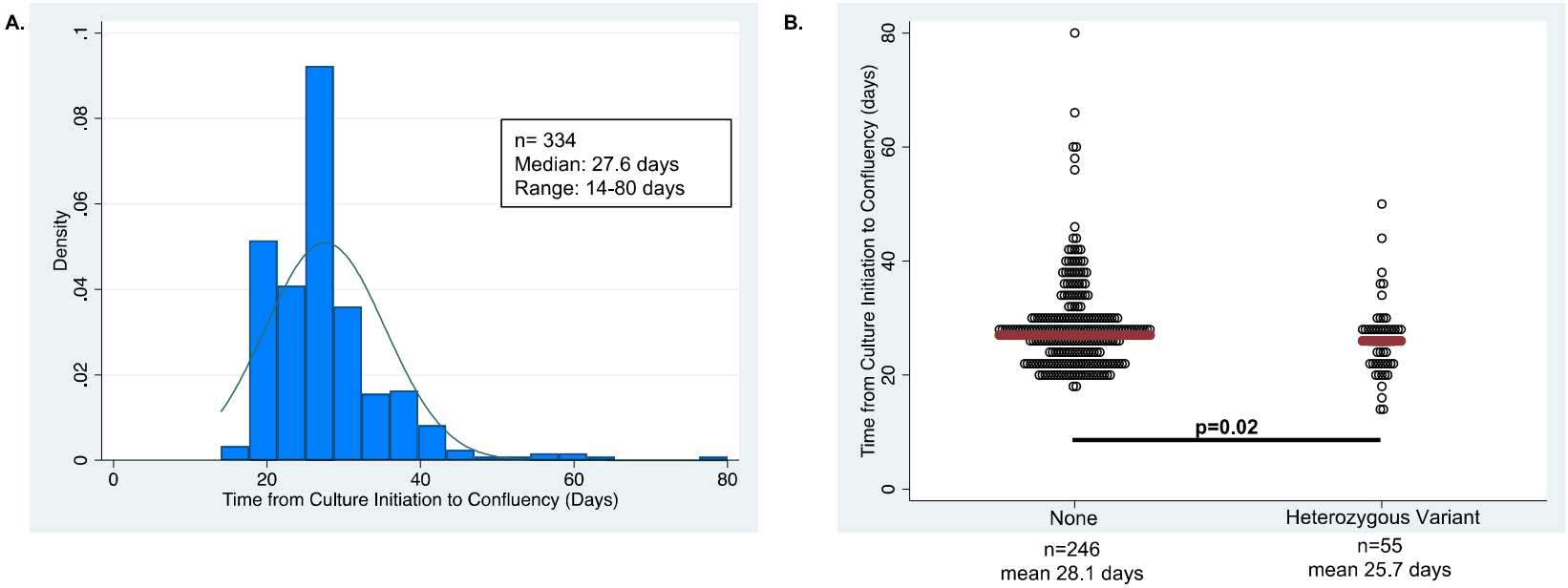

FIGURE S4 Chromosome 3 heterozygous deletion including *GATA2* and *TERC* (a) confirmed on microarray and qPCR of initial cultured skin fibroblast sample, but (b) absent on second independent cultured skin fibroblast sample

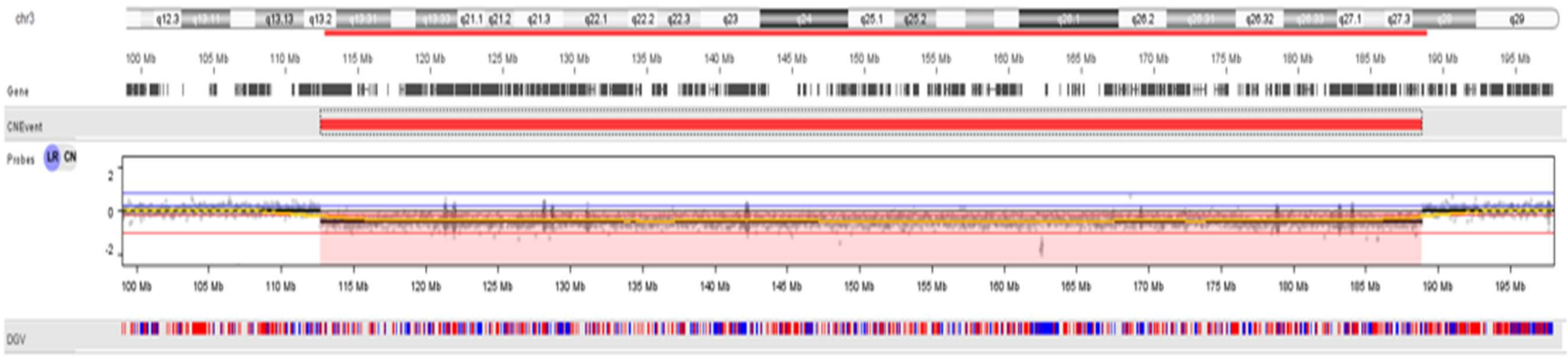

Figure S5 Subclonal variant confirmatory testing. Sanger sequencing electropherograms from nascent DNA from a control sample (top) and each subject's initial skin fibroblast culture (bottom) are shown below for all variants with minor allele frequencies 10-40%.

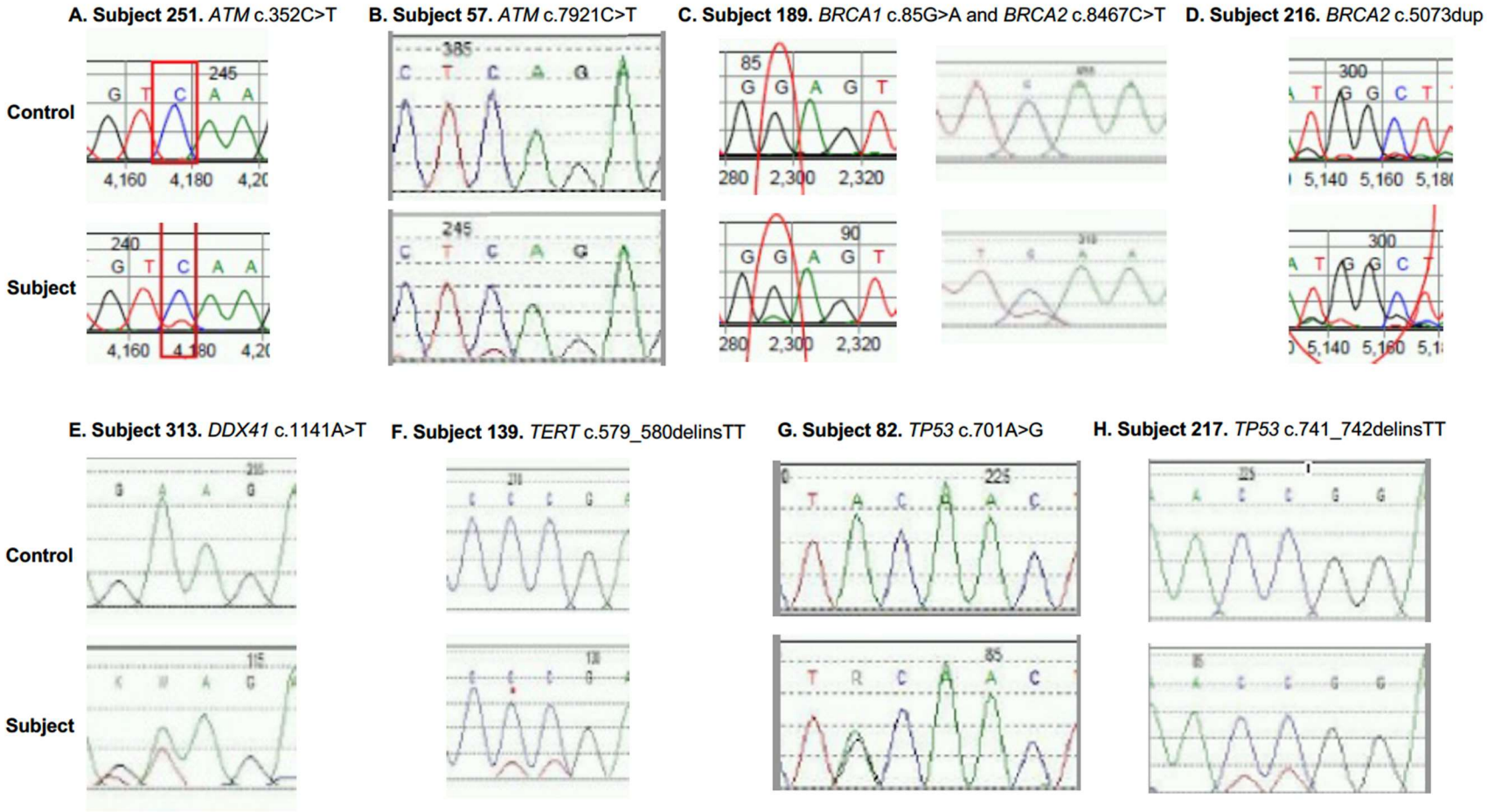

FIGURE S5 Continued

I. Subject 110. *CTC1* c.131_134del

J. Subject 247. *SBDS* c.183_184delinsCT