

SUPPLEMENTARY MATERIAL

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SUPPLEMENTARY MATERIALS AND METHODS

Cell line culture: OVCAR8 (RRID: CVCL_1629) cells were grown in GIBCO's RPMI 1640 (Catalog number 11875-093) supplemented with 10% heat inactivated FBS and 1% Penicillin streptomycin. The line is tested in the lab every six months for mycoplasma using Lonza's MycoAlert Mycoplasma detection kit (Catalog Number LT07-418), and experiments were performed on passages 1-15 of the cells after thawing and testing.

siRNAs and transfection: siRNAs included Qiagen's AllStars negative control siRNA (Catalog number 1027280) and a *BRCA1* siRNA targeting exon 13 of the coding sequence (GGGAUACCAUGCAACAUAA). OVCAR8 cells were plated on day 0, transfected with 20pmol of the respective siRNA on days 1 and 2, and then plated for appropriate experiments or harvested for western blotting on day 3.

Cell line sensitivity assays: OVCAR8 cells were transfected as described above. On day three, cells were seeded into 96 well plates at a density of 1000 cells per well. The cells were allowed to settle for 4 hours, and then media was changed to media containing the following drugs for 7 days. Each dose was performed in triplicate, and three biologic replicates of each experiment

were performed. Carboplatin (Hospira NDC 61703-339-50), 0uM (contained ddH₂O at a volume equal to the volume used in the highest carboplatin dose), 0.05 uM, 0.5 uM, 5 uM, 10 uM, 25 uM, 50 uM, and 75 uM. Olaparib (Selleck Catalog number S1060), 0uM (contained DMSO at a volume equal to the volume used in the highest olaparib dose), 0.0005uM, 0.005uM, 0.05uM, 0.5uM, 1uM, 10uM, 25uM, and 50uM. VE-822 (Selleck Catalog number S7102) 0uM (contained DMSO at a volume equal to the volume used in the highest VE-822 dose), 0.005uM, 0.05uM, 0.1uM, 0.5uM, 1uM, and 5uM. Prexasertib (Eli Lilly) 0uM (contained DMSO at a volume equal to the volume used in the highest prexasertib dose), 0.00001uM, 0.00005uM, 0.0001uM, 0.0005uM, 0.05uM, and 1uM. Doxorubicin (Selleck Catalog Number S1208) 0uM (contained DMSO at a volume equal to the volume used in the highest doxorubicin dose), 0.00005uM, 0.00005uM, 0.0005uM, 0.005uM, 0.05uM, and 0.5uM. Gemcitabine (Selleck Catalog Number S1714) 0uM (contained DMSO at a volume equal to the volume used in the highest gemcitabine dose), 0.00005uM, 0.00005uM, 0.0005uM, 0.005uM, 0.05uM, and 0.5uM.. Paclitaxel (Fresenius Kabi Catalog Number 760350) 0uM (contained EtOH at a volume equal to the volume used in the highest paclitaxel dose), 0.00005uM, 0.00005uM, 0.0005uM, 0.005uM, 0.05uM, and 0.5uM. After 7 days, an equal volume of Cell Titer Glo (Promega Catalog number G7572) was added to each well, and the luminescence was read on a ClarioStar plate reader.

IC₅₀s were calculated using GraphPad Prism software.

Organoid sensitivity assays: Organoids were either plated on day 0 or split in a final concentration of 75% Matrigel with approximately 10,000 or more cells/cell groups per 10uL droplet. 3uL droplets were plated into each well of a 96 well plate. The growth factor rich culture medium described in the main text methods containing the below drug doses was added

to the plates. Media containing drug was left on for 5-7 days. Carboplatin (Hospira NDC 61703-339-50), 0uM (contained ddH₂O at a volume equal to the volume used in the highest carboplatin dose), 5 uM, 10 uM, 25 uM, 50 uM, and 75 uM. Olaparib (Selleck Catalog number S1060), 0uM (contained DMSO at a volume equal to the volume used in the highest olaparib dose), 0.05uM, 0.5uM, 1uM, 10uM, 25uM, and 50uM. VE-822 (Selleck Catalog number S7102) 0uM (contained DMSO at a volume equal to the volume used in the highest VE-822 dose), 0.005uM, 0.05uM, 0.1uM, 0.5uM, 1uM, and 5uM. Prexasertib (Eli Lilly) 0uM (contained DMSO at a volume equal to the volume used in the highest prexasertib dose), 0.0005uM, 0.001uM, 0.005uM, 0.05uM, 0.5uM, and 1uM. Doxorubicin (Selleck Catalog Number S1208) 0uM (contained DMSO at a volume equal to the volume used in the highest doxorubicin dose), 0.0005uM, 0.001uM, 0.005uM, 0.05uM, 0.5uM, and 1uM. Gemcitabine (Selleck Catalog Number S1714) 0uM (contained DMSO at a volume equal to the volume used in the highest gemcitabine dose), 0.0005uM, 0.001uM, 0.005uM, 0.05uM, 0.5uM, and 1uM. Paclitaxel (Fresenius Kabi Catalog Number 760350) 0uM (contained EtOH at a volume equal to the volume used in the highest paclitaxel dose), 0.0005uM, 0.005uM, 0.05uM, 0.1uM, 0.5uM, and 1uM. After 5-7 days, an equal volume of Cell Titer Glo (Promega Catalog number G7572) was added to each well, and the luminescence was read on a ClarioStar plate reader. IC₅₀s were calculated using GraphPad Prism software.

Splitting organoids: Once the organoids become confluent, they can be split either mechanically or through digestion. In both methods, the organoids and Matrigel were scraped out of the well along with the media, and pipetted into a 50mL conical. For mechanical disruption, the suspensions were vigorously pipetted until organoid clumps were not visible.

Alternatively, the organoids can be added to 1mL of TRYPLE (Life Technologies # 12605010), incubated at 37°C for 3 minutes, and then vigorously disrupted with pipetting until clumps are not visible anymore. In either case, the suspensions were spun for 3 minutes at 3000RPM, the media aspirated, and the pellets placed on ice. Ideally, the organoids should be split 1:2-1:3. Matrigel was added into the cell pellet to a final concentration of 75% Matrigel with approximately 10,000 or more cells/cell groups per 10uL droplet. The cells were plated to be carried or to be tested for drug sensitivity in the appropriate plates (48-well to carry, 96-well for sensitivity testing), the Matrigel allowed to briefly solidify, and growth factor rich culture media (described in the main text methods) was added.

Fiber Assay: For OVCAR8 cells, the cells were transfected as described above. On day 2, after the second transfection was complete, the cells were plated into a 6-well plate at a density of 500,000 cells per well. On the following day, the medium was replaced with culture medium containing 0.2mM CldU (Sigma Aldrich Catalog number C6891) and incubated at 37°C for 30 minutes. The media was removed, the cells washed three times with PBS, and new pre-warmed media containing 0.25mM IdU (Sigma Aldrich Catalog number I7125) was added and the cells incubated at 37°C for 30 minutes. The cells were washed three times with PBS. Pre-warmed media containing one of the following combinations was then added for a 3 hour incubation at 37°C: 0.04uM Prexasertib alone, 0.05uM VE-822 alone, 25uM Carboplatin alone, 1uM Gemcitabine alone, 2mM Hydroxyurea (Sigma Aldrich H8627) alone, 0.04uM Prexasertib+25uM Carboplatin, 0.05uM VE-822+25uM Carboplatin, 0.04uM Prexasertib+0.05uM VE-822, 0.04uM Prexasertib+1uM Gemcitabine, or control media with a volume of ddH₂O equal to the volume of carboplatin used, or a volume of DMSO equal to the

volume of VE-822, Gemcitabine, or Prexasertib used. Please note, there was no HU in the media during any step for treatment with control media, prexasertib, carboplatin, VE-822, prexasertib+carboplatin, gemcitabine, prexasertib+VE-822, or gemcitabine+prexasertib, so any fork destabilization seen with these drugs or combinations was due solely to these individual drugs or combinations. After three hours, the cells were trypsinized, pelleted, washed once with PBS and resuspended in an equal volume of 1X PBS with 0.05% trypsin and low melt agarose. This mixture was placed in a plug mold (BioRad catalog number 1703713) and allowed to set at 4°C for one hour. The plug was then pushed out of the mold into a buffer containing 0.5M EDTA pH8, 1% Sarkosyl, and proteinase K (Fisher Scientific Catalog number EO0491). The agarose plugs were incubated in this buffer at 50°C overnight. Agarose plugs were washed (1M Tris, 0.1M EDTA pH8) then digested with beta-agarase overnight in 0.5M MES pH5.5. Samples were then poured into FiberComb wells and combed onto silanized coverslips (Genomic Vision COV-002) using the Molecular Combing System from Genomic Vision. Coverslips were probed with rat anti-BrdU antibody (clone BU1/75 (ICR1) specific to CldU, Life Technologies MA182088) and mouse anti-BrdU antibody (specific to IdU, BD Biosciences 347580) and visualized by fluorescence microscopy. Pictures were taken of 100 fibers per condition. Fibers were measured with ImageJ. The ratio of IdU:CldU tracks in each fiber was calculated and graphed using GraphPad Prism.

For organoid fiber assays, the organoids were scraped from the wells 3-10 days after initial plating or 2-3 days after passage. While still in Matrigel, the organoid cultures were pelleted, the media aspirated, and fresh pre-warmed media containing 0.2mM CldU was added and organoids were incubated at 37°C for 30 minutes. The organoids were pelleted, the media was removed, the cells washed two times with PBS with re-pelleting between each wash, and

new pre-warmed media containing 0.25mM IdU was added and the organoids incubated at 37°C for 30 minutes. The organoids were pelleted, the media was removed, the cells washed two times with PBS with re-pelleting between each wash, and new pre-warmed media containing one of the following drugs or drug combinations was added and the organoids incubated at 37°C for 3 hours: 2mM Hydroxyurea, 0.04uM Prexasertib alone, 25uM Carboplatin alone, 1uM Gemcitabine alone, 0.04uM Prexasertib+25uM Carboplatin, 0.04uM Prexasertib+1uM Gemcitabine, or control media with a volume of ddH₂O equal to the volume of carboplatin used, or a volume of DMSO equal to the volume of Gemcitabine or Prexasertib used. Please note, there was no HU in the media during any step for treatment with prexasertib, carboplatin, prexasertib+carboplatin, gemcitabine, or gemcitabine+prexasertib, so any fork destabilization seen with these drugs or combinations was due solely to these individual drugs or combinations. After three hours, the organoids were pelleted and the media removed. The organoids were washed once with PBS, and then ice-cold Corning cell recovery solution (Catalog number 354253) was added and the organoids rotated end over end at 4°C for 20 minutes. The organoids were pelleted, washed once with PBS, and then resuspended in an equal volume of 1X PBS with 0.05% trypsin and low melt agarose. This mixture was placed in a plug mold as described above, and the remainder of the assay was carried out exactly as above. Two to three biologic replicates were performed for each organoid and cell line with each drug treatment.

Histology and Immunohistochemistry: All IHC was performed on the Leica Biosystems Bond III automated staining platform. Antibody p53 from Immunotech catalogue # 1767, clone DO-1, was run at 1:500 dilution using the Leica Biosystems Refine Detection Kit with citrate antigen retrieval. Antibody PAX8 from Proteintech, catalogue # 10336-1-AP, Polyclonal, was run at

1:500 dilution using the Leica Biosystems Refine Detection Kit with citrate antigen retrieval. For the Rad51 assay, staining was performed as described previously (1), and Geminin staining was performed using the NCL-L-Geminin antibody, Leica Biosystems. Cells with 1-3 RAD51-foci were called positive. Percentage of geminin positive cells indicates proliferation rate. A sample was designated HR-proficient when Rad51-foci positive cells are present in a minimum of two 40x fields. A sample was designated HR-deficient when >3% of the cells were Geminin positive and RAD51-foci positive cells were absent. The HR status of a sample is indeterminate when RAD51-foci positive cells are absent and <3% of the cells are Geminin positive.

For human tissue, tissue was fixed in 10% formalin overnight, processed and paraffin embedded in the Brigham and Women's Hospital pathology department, cut to 5um sections on positively charged slides, and then stained with Hematoxylin & Eosin or stained with p53 or PAX8 antibodies as described above. For organoids, organoids were harvested five to seven days after initial plating or passage, treated with 0 or 10Gy X-Ray using a RadSource RS-2000 Biological System X-Ray source, and then fixed in 10% formalin for one hour four hours later. The organoids were spun down to a pellet and resuspended in warmed HistoGel (Thermo Fisher Catalog number 22110678). The mixture was solidified at 4°C for approximately one hour, and then processed and paraffin embedded in the Brigham and Women's Hospital pathology department, cut to 5um sections on positively charged slides, and then stained with Hematoxylin & Eosin or stained with p53, PAX8, or Rad51 (1) antibodies as described above.

Western blots: For post-treatment westerns, all cell lines or organoids were treated with either 0.04 uM Prexasertib or media containing an equivalent volume of DMSO for 14-16 hours and compared by western. For select organoids, the organoids were treated with a) media containing

an equivalent volume of DMSO as the highest drug treatment, 0.04 uM Prexasertib, 25uM Carboplatin, or 0.04uM Prexasertib+25uM Carboplatin for 3 hours and 14 hours and compared by western or with b) media containing an equivalent volume of DMSO as the highest drug treatment, 0.04 uM Prexasertib, 1uM Gemcitabine, or 0.04uM Prexasertib+1uM Gemcitabine for 3 hours and 14 hours and compared by western. For siRNA experiments, cell lines were transfected as described above. After the relevant treatment, for the organoids, the Matrigel was removed as described previously, and then the pelleted cells or organoids were lysed in NETN300 (1% NP-40, 300mM NaCl, 0.1mM EDTA, 50mM Tris-HCL pH7.5) with protease inhibitors (Roche Catalog number 1836153). Loading buffer (NuPAGE LDS Sample Buffer (4x) (Life Technologies Catalog number NP0007) with NuPAGE Sample Reducing Agent (10x) (Life Technologies Catalog number NP0009)) was added, and equal amounts of each sample were loaded into 4-12% Bis-Tris Gels (Invitrogen Catalog number NP0336BOX). Proteins were transferred to 0.45um nitrocellulose membranes, and the membranes were blotted with the following antibodies: KAP1 (phosphoS824) (Abcam catalog number ab84077), Chk1 (G-4) (Santa Cruz Biotechnology catalog number sc-8408), Phospho-Chk1 (Ser345) (Cell Signaling Technology Catalog number 2348S), Anti- γ H2A.X (phosphor S139) (Abcam catalog number ab2893), anti-RPA2 (p Ser33) (Novus catalog number NB100-544), alpha-tubulin (Cell Signaling Technology catalog number 2144S), and Beta-actin (Cell Signaling Technology catalog number 49675).

Whole Exome Sequencing: Fresh tissue, organoid cell pellets, whole blood samples, and paraffin embedded normal tissue controls were sent to the Broad Institute for DNA extraction and whole exome sequencing.

Per the Broad Institute sequencing protocol:

“the Illumina exome specifically targets approximately 37.7Mb of mainly exonic territory made up of all targets from our Agilent exome design (Agilent SureSelect All Exon V2), all coding regions of Gencode V11 genes, and all coding regions of RefSeq gene and KnownGene tracks from the UCSC genome browser (<http://genome.ucsc.edu>). The Illumina exome uses in-solution DNA probe-based hybrid selection to generate Illumina exome sequencing libraries. Pooled libraries were normalized to 2nM and denatured using 0.2 N NaOH prior to sequencing. Flowcell cluster amplification and sequencing were performed according to the manufacturer’s protocols using either the HiSeq 2000 v3 or HiSeq 2500. Each run was a 76bp paired-end with a dual eight-base index barcode read. Data was analyzed using the Broad Picard Pipeline which includes de-multiplexing and data aggregation.”

Somatic indels in tumors and organoids were called using Oncotate_Capture_Indel_Workflow of the general Production Analysis (Capture) Workflow pipeline for somatic short variant discovery in Firehose (Broad Institute). Somatic single nucleotide variants were called using Mutect2 (2) in the Firecloud environment (Broad Institute). Candidate somatic variants were filtered using quality, depth, position, and population frequency thresholds.

The genomic copy number alterations from the tumors and organoids were analyzed using two methods. For general comparison of copy number and allelic fraction across the whole genome, the copy number variants were discovered using GATK4 CNV pipeline following GATK Best Practices for somatic CNV discovery. The clean BAMs of each pair of tumor/organoid and matched-normal samples were analyzed using the WDL pipeline `gatk/CNV_Somatic_Pair_Workflow` (snapshot 7) with GATK4 docker image version 4.0.4.0 in

FireCloud (Broad Institute; <https://portal.firecloud.org>). In addition, for analysis of specific genes, copy number alterations of specific alterations were discovered using GISTIC2.0 analysis (99% confidence interval using default settings) (3) of segmented files generated using the ReCapSeg workflow in Firehose (Broad Institute).

Mutations were visualized with the cBioPortal OncoPrinter script (4,5).

Supplementary References

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2. Cibulskis K, Lawrence MS, Carter SL, Sivachenko A, Jaffe D, Sougnez C, *et al.* Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. *Nat Biotechnol* **2013**;31(3):213-9 doi 10.1038/nbt.2514.
3. Mermel CH, Schumacher SE, Hill B, Meyerson ML, Beroukhi R, Getz G. GISTIC2.0 facilitates sensitive and confident localization of the targets of focal somatic copy-number alteration in human cancers. *Genome Biol* **2011**;12(4):R41 doi 10.1186/gb-2011-12-4-r41.
4. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, *et al.* Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal* **2013**;6(269):p11 doi 10.1126/scisignal.2004088.

5. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, *et al.* The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov* **2012**;2(5):401-4 doi 10.1158/2159-8290.CD-12-0095.

SUPPLEMENTARY TABLE LEGENDS

Supplementary Table S1. WES metrics: The table shows the number of pass-filter reads, the sample coverage, mean target coverage, and contamination for each organoid and tumor sequenced.

Supplementary Table S2. Somatic mutation concordance between patient tumor material and organoids.

Supplementary Table S3. Filtered somatic mutations. Mutect2 SNVs were filtered to remove variants with: dbSNP_Val_Status,=Any value Variant_Classification=IGR, chr=M, variants not present on bidirectional reads, $t_alt_f < 0.05$, $Pair_Alt_Cov < 20$. IndelLocator indels were filtered to remove variants with: dbSNP_Val_Status,=Any value Variant_Classification=IGR, chr=M, $t_alt_f < 0.05$, $Pair_Alt_Cov < 20$.

Supplementary Table S4. Clinical outcome data. The patients are listed in three groups according to their treatment status at the time of tissue procurement for organoid generation. The patient groups include 1) untreated, 2) post-neoadjuvant, and 3) other for those on clinical trials after multiple modalities of therapy. For the untreated patients, we provide outcome after adjuvant therapy up to July 24, 2018, indicating whether or not the patient was deemed platinum

sensitive during this period. For the neoadjuvant patients, we judge neoadjuvant response by imaging and CA125 at the time of the surgery where tissue was obtained, and we also provide outcome and clinical follow up until July 24, 2018. For the third group on clinical trials, we provide the response of the tumor sites we tested to the relevant clinical trial drug. We also provide general outcome information for the patient up until July 24, 2018. We also provide genetic status and histologic tumor type for each patient. Next to all outcomes, we provide the organoid drug sensitivity data, RAD51 foci data, and fiber data to allow for an easy side-by-side comparison. S stands for sensitive, R stands for resistant, U stands for unstable, and St stands for stable.

Supplementary Table S5. Number and characteristics of SNVs and indels found in each tumor-organoid pair.

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1. Generation of Low-Grade Serous, Borderline Mucinous, and Fallopian tube organoids. A) Comparison of a low grade serous parent tumor H&E and p53 stain (top panel) to the paired organoid (bottom panel). B) H and E stains of a parent fallopian tube and its paired organoid. The arrows point to ciliated cells. C) Comparison of H&Es of a parent gastrointestinal type borderline mucinous tumor (left) to its paired organoid (middle) and a mucicarmine stain (right) of the organoid. Arrows point to goblet cells in the parent tumor and organoid.

Supplementary Figure S2. Assessment of somatic mutation similarity of organoids to parent tumors. 33 of the 34 organoid and parent tumor lines presented underwent successful WES analysis. For each sample, the scatter plot compares the somatic mutant SNV allele fraction in the parent tumor and matched organoid. Most mutations have an organoid mutant allele fraction that is similar to the parent tumor, supporting the conclusion that the organoid derivation process does not select a single clone or induce large-scale genomic alterations. Each page represents a single parent tumor/organoid line comparison with the same layout for supplementary figures S2A-S2FF.

Supplementary Figure S3. Assessment of copy number and allelic imbalance similarity of organoids to parent tumors. For each organoid and parent tumor, relative copy number and allelic imbalance analysis was performed. On the top for each panel is a plot of the copy number variation and allelic imbalance across each chromosome for the parent tumor, and on the bottom of each panel is a plot of the copy number variation and allelic imbalance across each chromosome for the matched organoid. The X chromosome was not assessed by copy number analysis. Each page represents a single parent tumor/organoid line comparison with the same layout for supplementary figures S3A-S3HH.

Supplementary Figure S4. BRCA1 depletion leads to increased therapeutic sensitivities and decreased replication fork stability. A) OVCAR8 cells were transfected with either a control siRNA or a *BRCA1* specific siRNA and then treated with varying doses of carboplatin, olaparib, prexasertib, VE-822, doxorubicin, paclitaxel, and gemcitabine. In the top panel dose curves are shown for the cells with various treatments, and in the bottom panel, the average IC50 over three

replicates is shown for the cells with the same treatments. Error bars represent the standard deviation between three biologic replicates. B) OVCAR8 cells were transfected with either a control siRNA or a *BRCA1* specific siRNA, treated in the top panels with control media with DMSO or H₂O added at a volume equal to the highest drug dose, HU alone, Carboplatin (carbo) alone, Prexasertib (Prex) alone, VE822 alone, Carbo+Prex, Carbo+VE-822, or Prex+VE-822 or in the bottom panels with control media with DMSO added at a volume equal to the highest drug dose, Prexasertib (Prex) alone, Gemcitabine (Gem) alone, or Prex+Gem. For the VE-822, prex, carbo, prex+VE-822, carbo+prex, carbo+VE-822, prex, gem, and prex+gem experiments, there is no HU in the media at any step, meaning that any fork instability observed is the result of the single drug or drug combination. The IdU to CldU ratio is shown for 100 fibers for each treatment for each cell line. The experiment was repeated three times with similar results. In each graph, a black line marks the average stable ratio, and a grey line marks the average of the prexasertib+carboplatin or prexasertib+gemcitabine biologic replicates. C) Western blot analysis of OVCAR8 cells transfected with the siRNAs used for experiments in panels A and B. The BRCA1 western is on the left and the tubulin control western is on the right. An arrow indicates the position of the p220 and Δ 11b isoforms.

Supplementary Figure S5. IC₅₀s of the organoid panel for carboplatin and olaparib. A) Bar graph plotting the IC₅₀s for tested organoid lines with Carboplatin. Each bar represents the IC₅₀ from 1-3 experiments, and the error bars represent the standard deviation between replicates. The black line at 20 μ M represents our cut off for sensitivity. B) Bar graph plotting the IC₅₀s for tested organoid lines with Olaparib. Each bar represents the IC₅₀ from 1-3 experiments, and the error bars represent the standard deviation between replicates. The black

line at 5uM represents our cut off for sensitivity. For each graph, the Y-axis represents the IC50 and the X-axis reveals the patient number and tumor site.

Supplementary Figure S6. IC50s of the organoid panel for prexasertib and VE-822. A) Bar graph plotting the IC50s for tested organoid lines with prexasertib. Each bar represents the IC50 from 1-3 experiments, and the error bars represent the standard deviation between replicates. The black line at 0.05uM represents our cut off for sensitivity. B) Bar graph plotting the IC50s for tested organoid lines with VE-822. Each bar represents the IC50 from 1-3 experiments, and the error bars represent the standard deviation between replicates. The black line at 0.4uM represents our cut off for sensitivity. For each graph, the Y-axis represents the IC50 and the X-axis reveals the patient number and tumor site.

Supplementary Figure S7. IC50s of the organoid panel for paclitaxel and doxorubicin. A) Bar graph plotting the IC50s for tested organoid lines with paclitaxel. Each bar represents the IC50 from 1-3 experiments, and the error bars represent the standard deviation between replicates. The black line at 0.01 uM represents our cut off for sensitivity. B) Bar graph plotting the IC50s for tested organoid lines with doxorubicin. Each bar represents the IC50 from 1-3 experiments, and the error bars represent the standard deviation between replicates. The black line at 0.5 uM represents our cut off for sensitivity. For each graph, the Y-axis represents the IC50 and the X-axis reveals the patient number and tumor site.

Supplementary Figure S8. IC50s of the organoid panel for gemcitabine. A) Bar graph plotting the IC50s for tested organoid lines with gemcitabine. Each bar represents the IC50 from

1-3 experiments, and the error bars represent the standard deviation between replicates. The black line at 0.02 uM represents our cut off for sensitivity.

Supplementary Figure S9. Chromosome-level copy ratio and allelic imbalance for subjects

with germline risk alleles. Allelic imbalance of heterozygous variants (Blue dots, normal is less than approximately 0.15) and Log2 copy ratio (orange dots) were plotted in 5-point sliding windows for each chromosome harboring a germline variant. Parent tumors and organoid lines were then assessed for copy number status at the known germline mutation locus to predict the functional status of the gene, given the observed combination of germline and somatic changes. A) In patient DF-17-126, examination of the *RAD51C/FANCO* locus reveals a copy number of two and increased allelic imbalance in favor of the mutant allele, consistent with copy neutral LOH of the wild type allele. B) In patient DF-18-30, analysis of the *BRCA2* locus revealed that there was somatic single copy loss, high allelic imbalance, and increased risk allele fraction, consistent with LOH and retention of the risk allele. C) In patient DF-17-107 left ovary mass, analysis of the *BRCA1* locus is impaired by low tumor purity, but shows mildly increased allelic imbalance across the locus and a copy number of two, consistent with copy neutral LOH causing clonal or subclonal loss of the wild type allele. The other tumor sites do not have evidence of copy number changes at the *BRCA1* locus. D) In patient DF-17-39, analysis of the *BRCA1* locus reveals a somatic single copy loss, locus-wide increased allelic imbalance, and elevated risk allele fraction, consistent with LOH of the wild type allele. E) In patient DF-17-115, analysis of the *BRCA2* locus reveals that there was somatic single copy loss, high allelic imbalance, and increased risk allele fraction, consistent with LOH and retention of the risk allele. F) In patient DF-18-23, analysis of the *BRCA2* locus reveals that there was somatic single copy loss, high

allelic imbalance, and increased risk allele fraction, consistent with LOH and retention of the risk allele. G) In patient DF-18-8, a plot of allelic imbalance and estimated copy number at the *BRCA1* locus, reveals a copy number of two and increased allelic imbalance in favor of the mutant allele, consistent with copy neutral LOH of the wildtype allele. For all locus-specific interpretations, copy number and allelic balance values were compared to genome-wide baselines, found in Figure S3 generated by GATK CNV analysis.

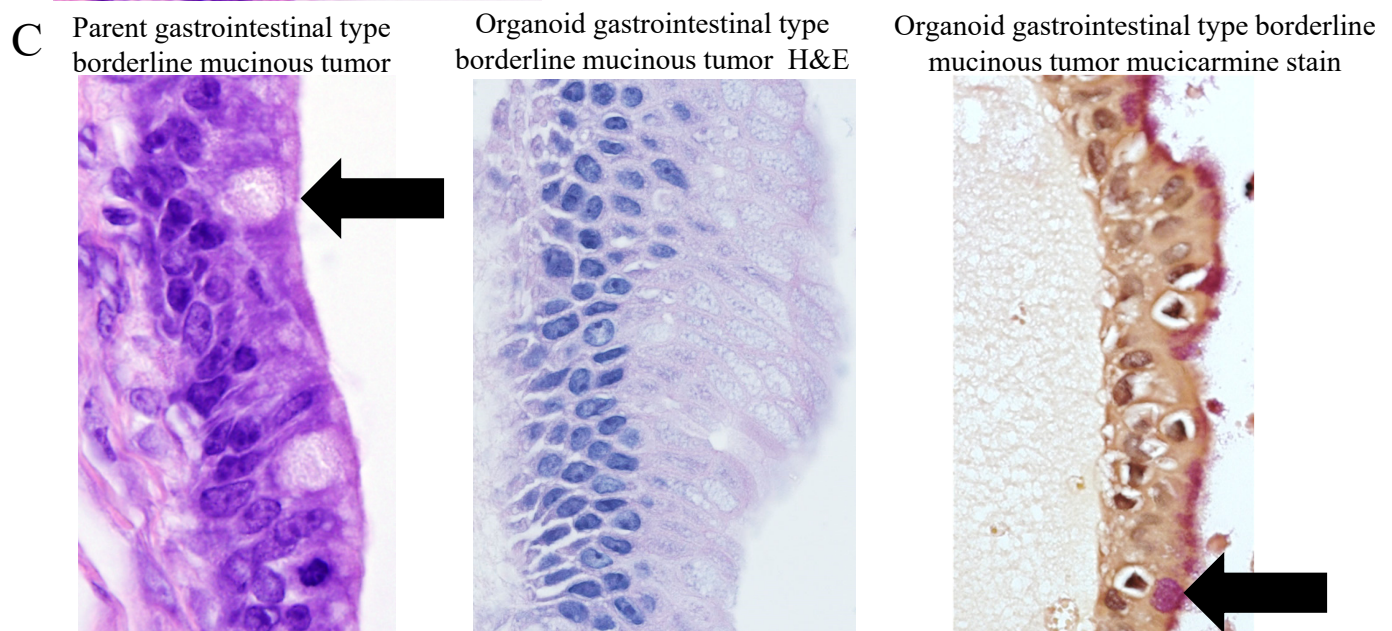
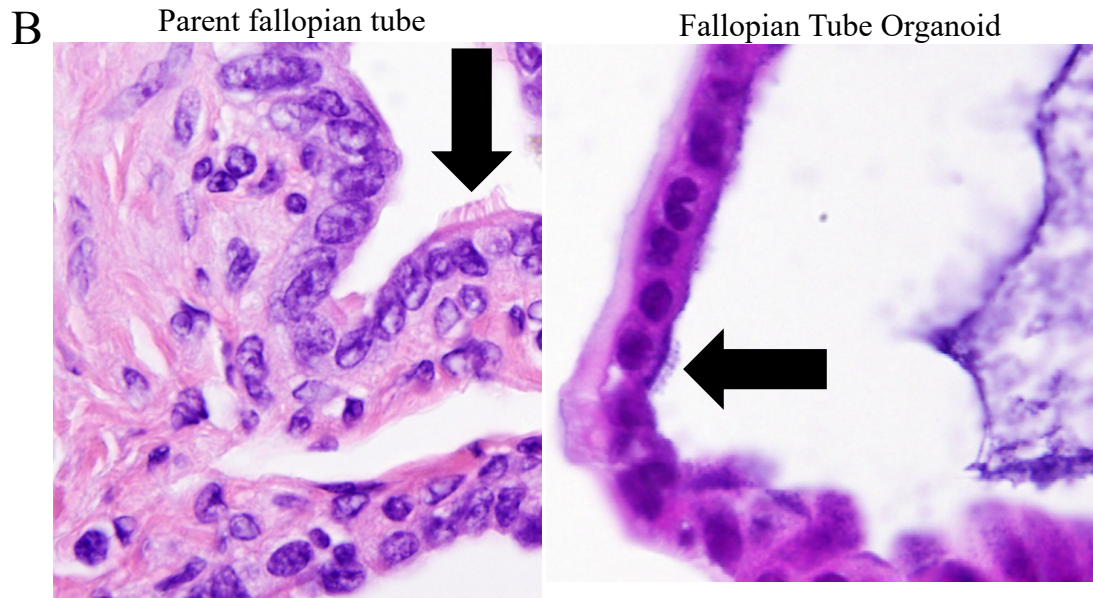
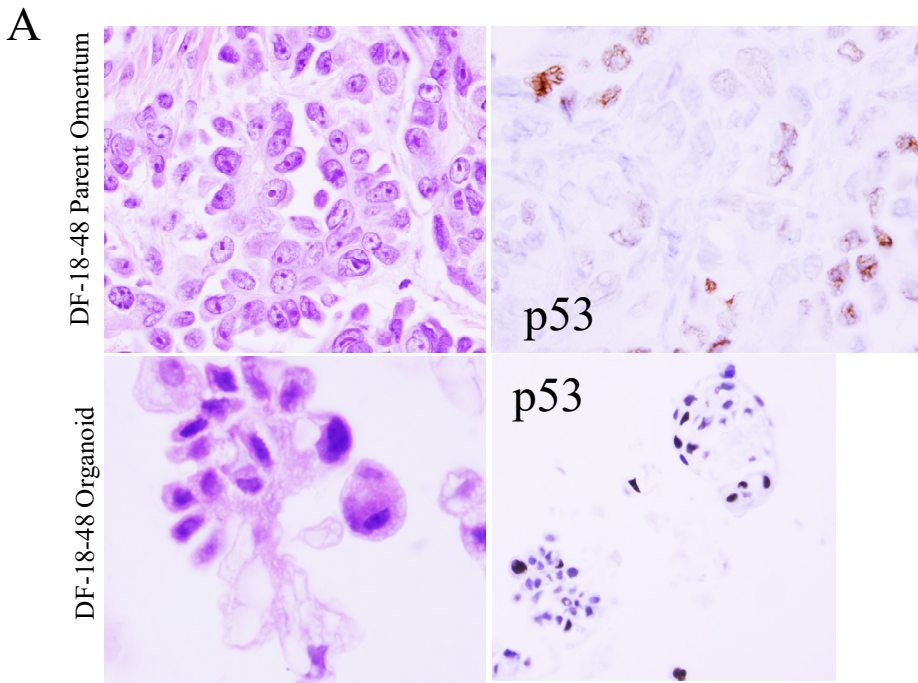


Figure S2 A

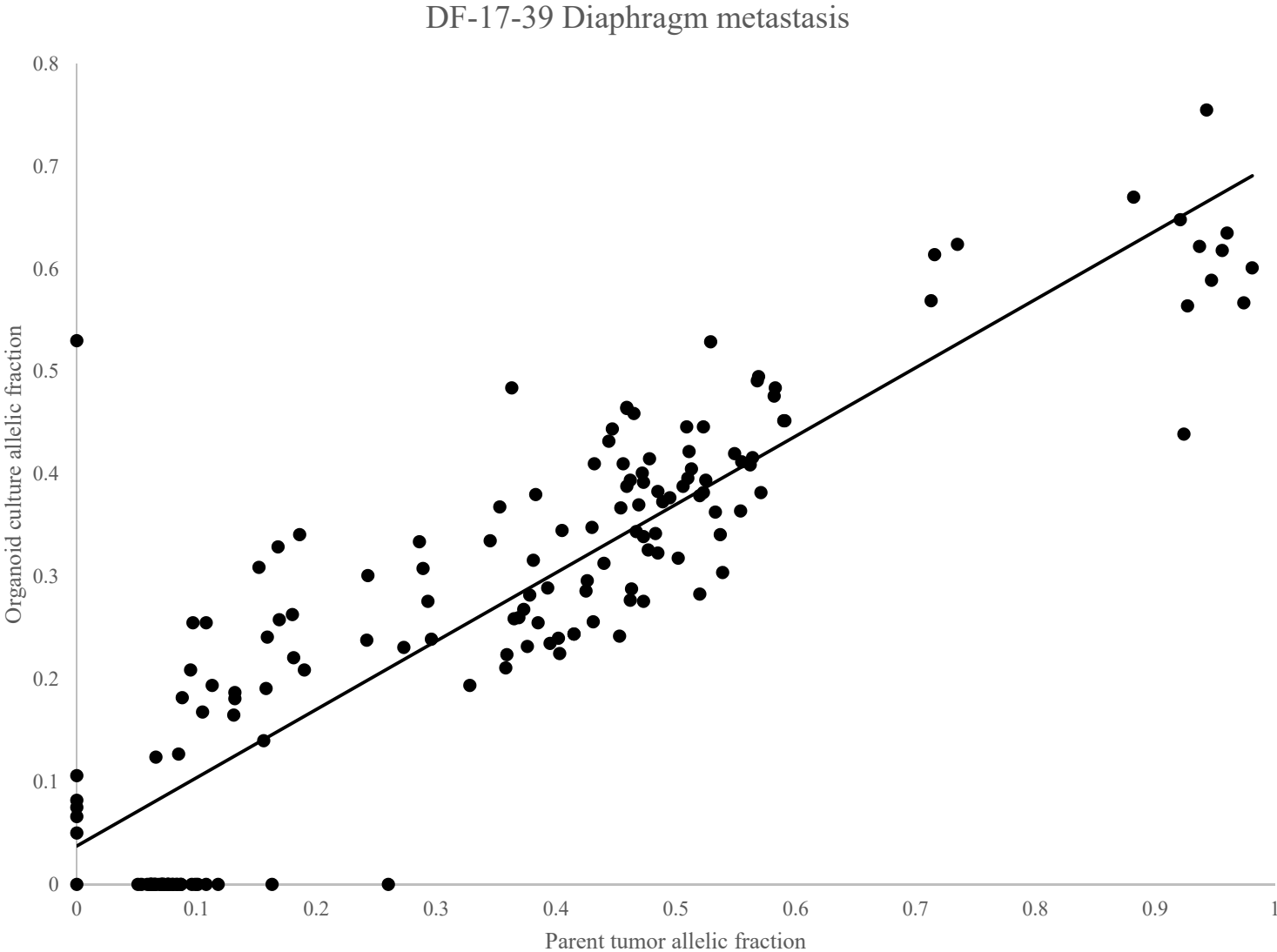


Figure S2 B

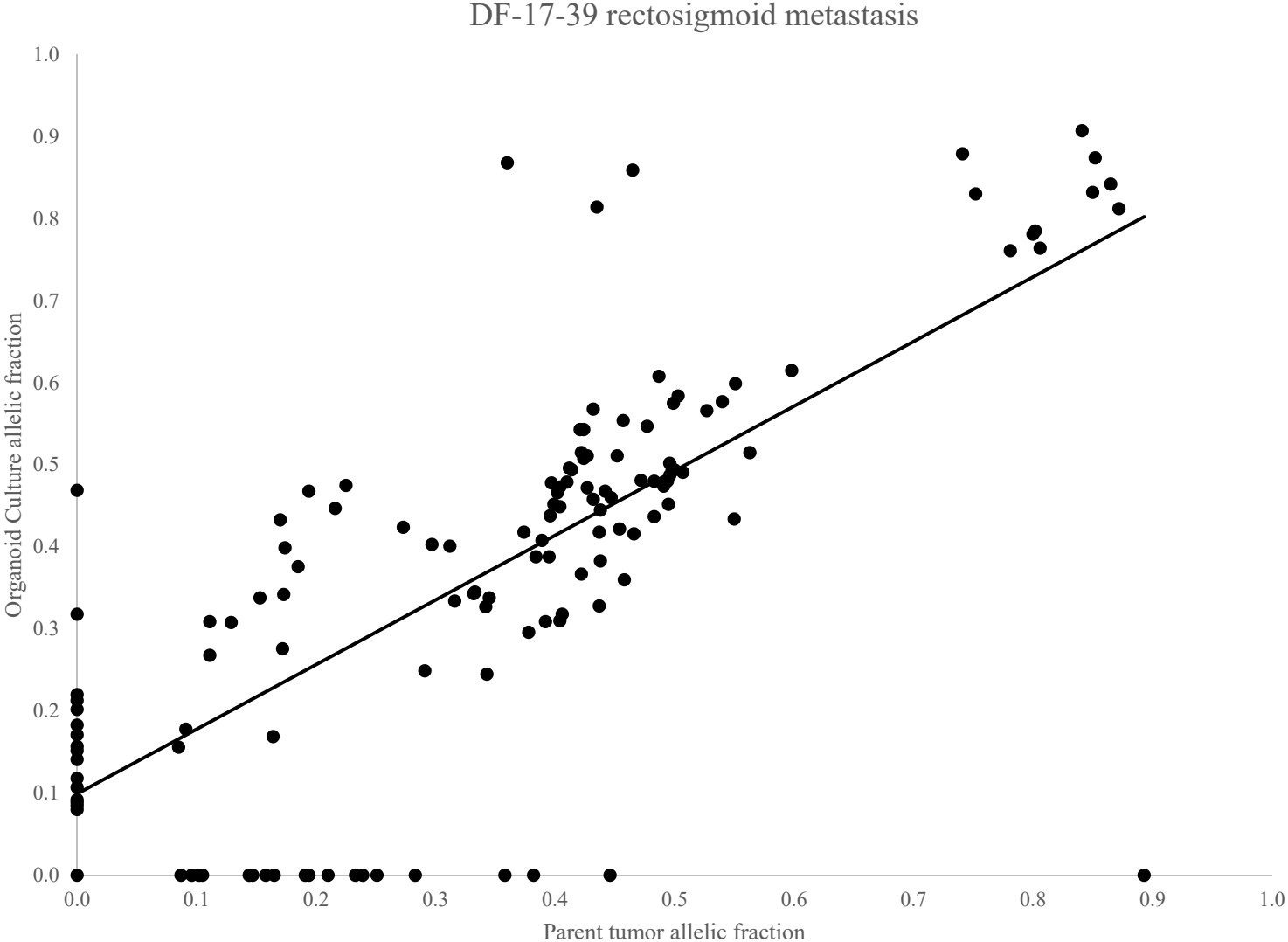


Figure S2 C

DF-17-39 Supracolic Omentum Metastasis

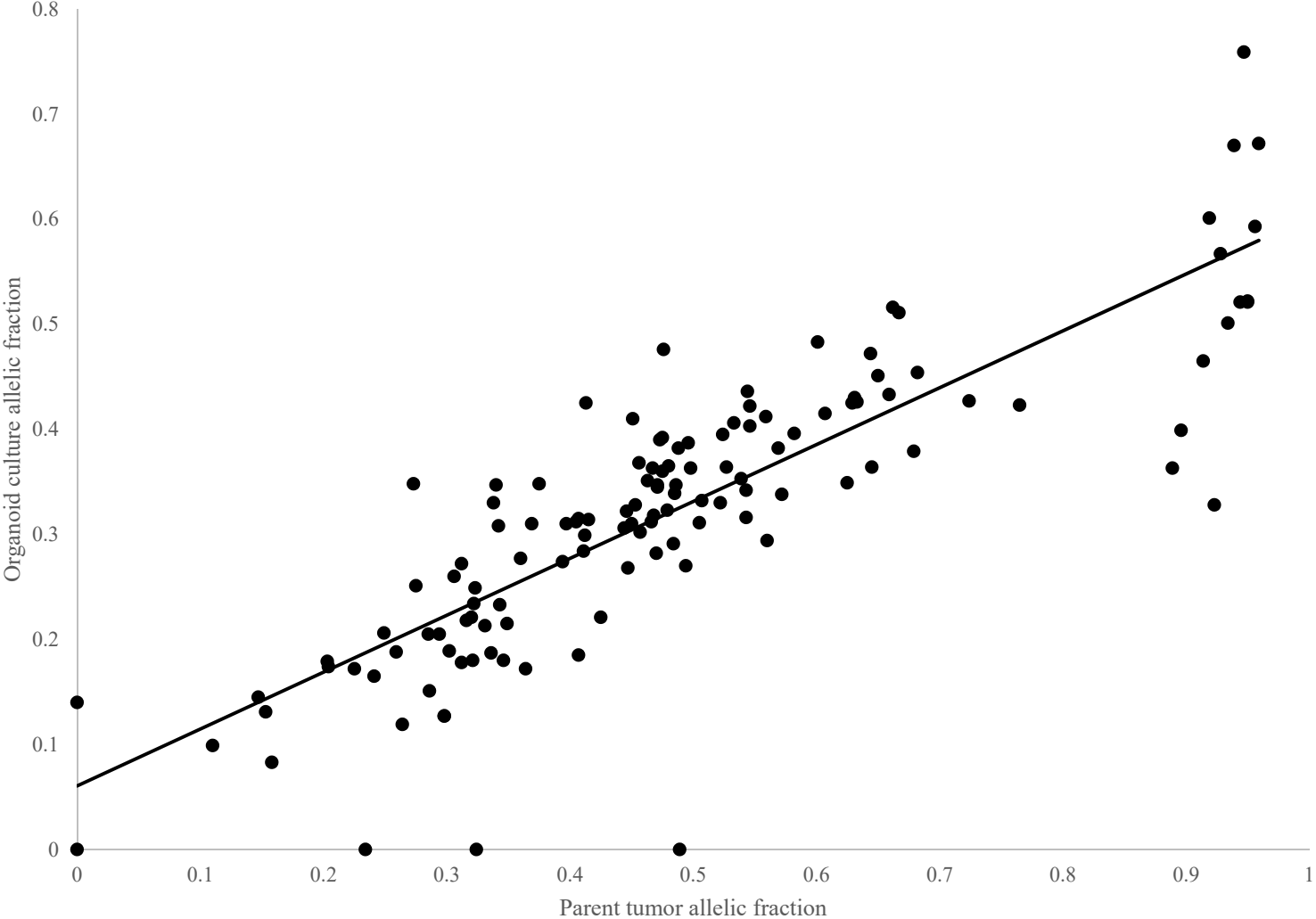


Figure S2 D

DF-17-39 Transverse colon mesentery metastasis

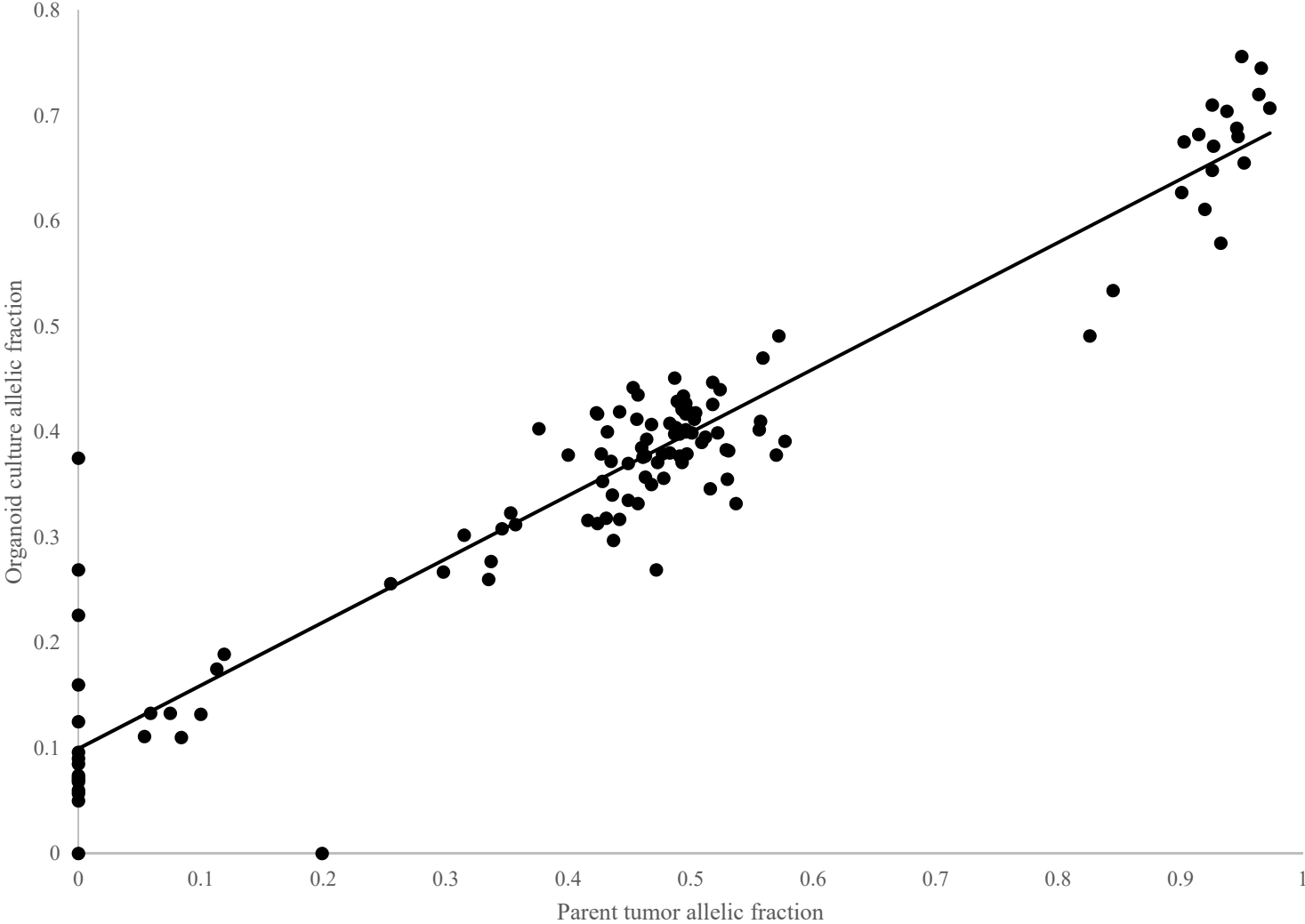


Figure S2 E

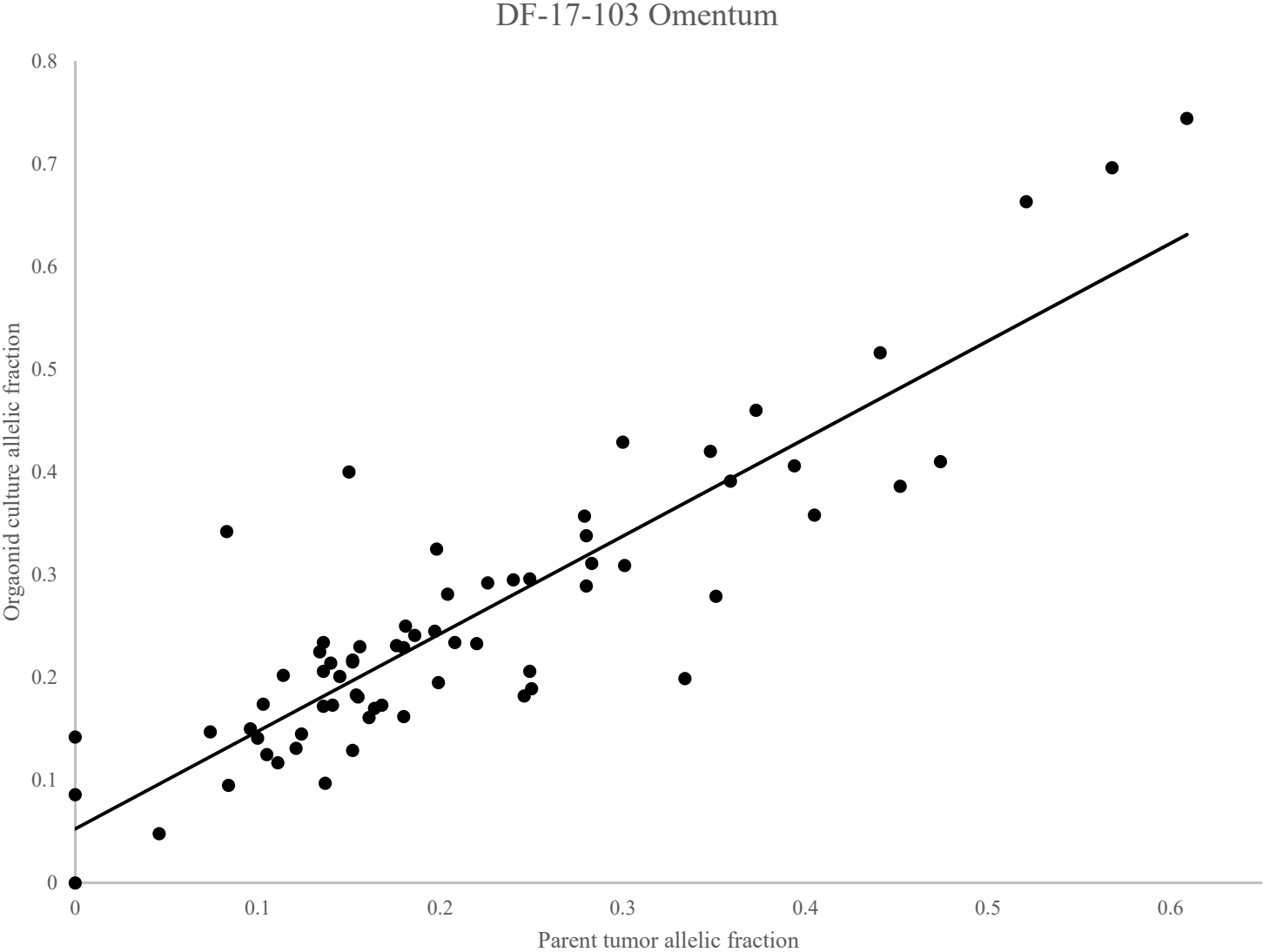


Figure S2 F

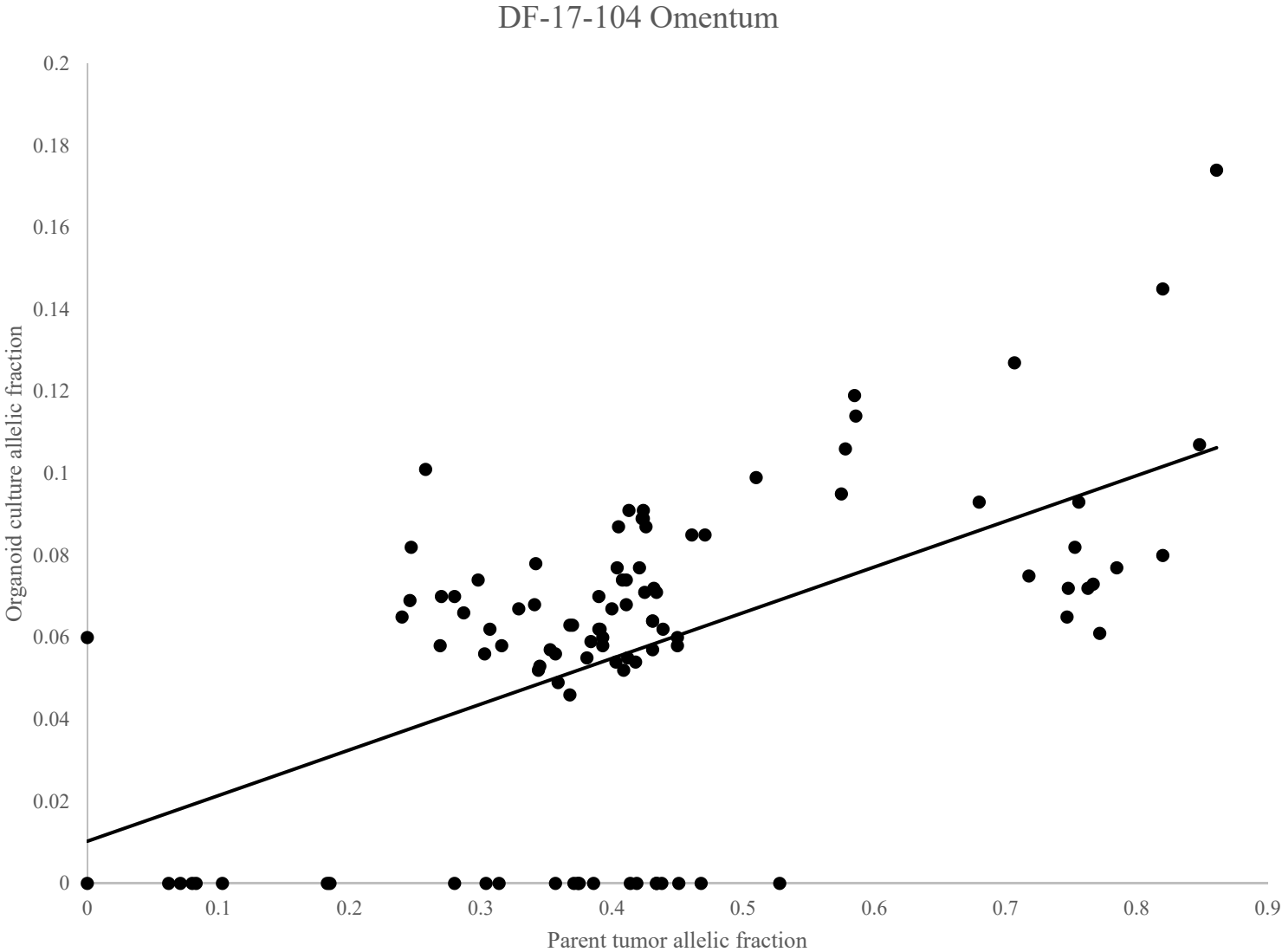


Figure S2 G

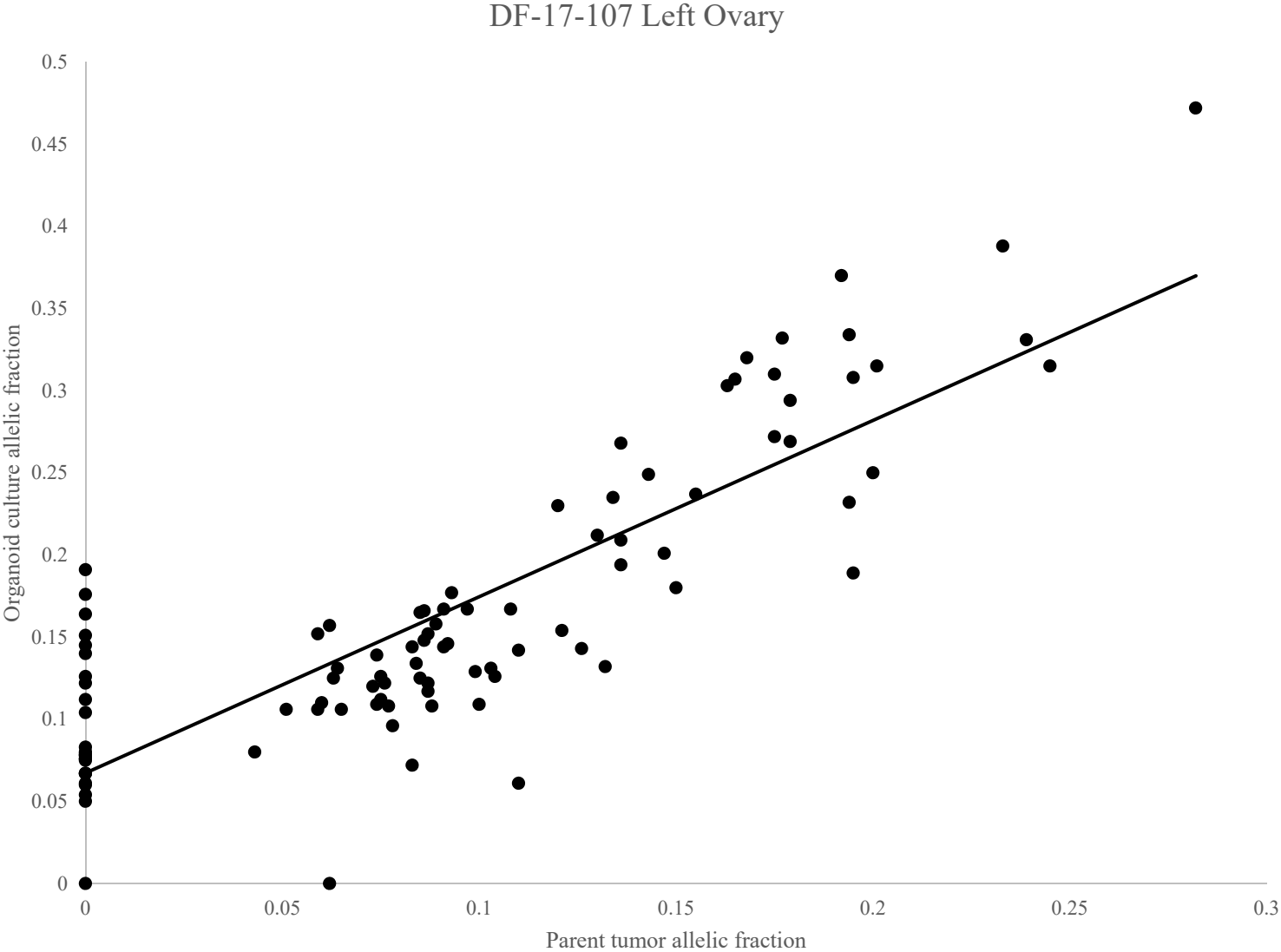


Figure S2 H

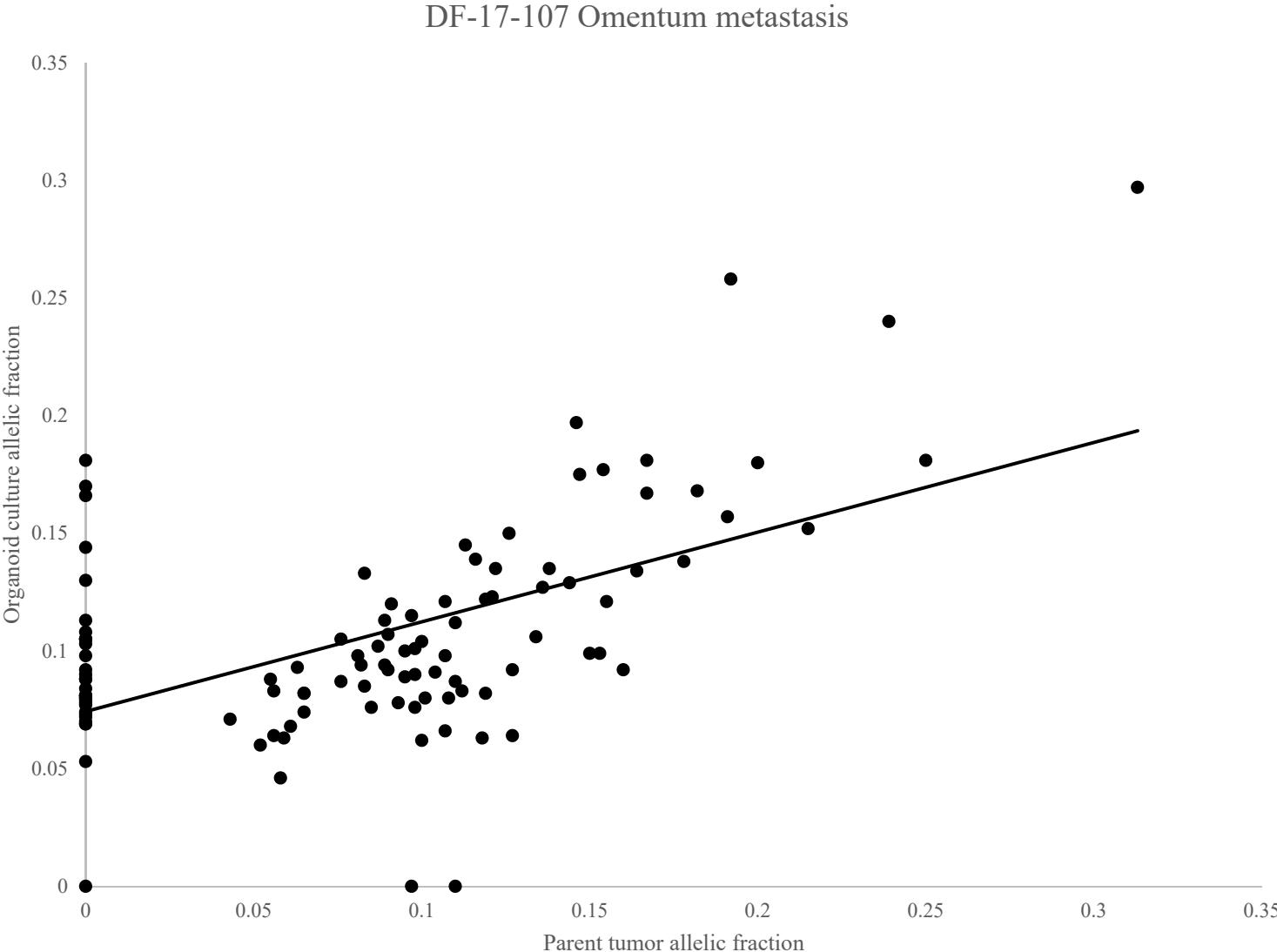


Figure S2 I

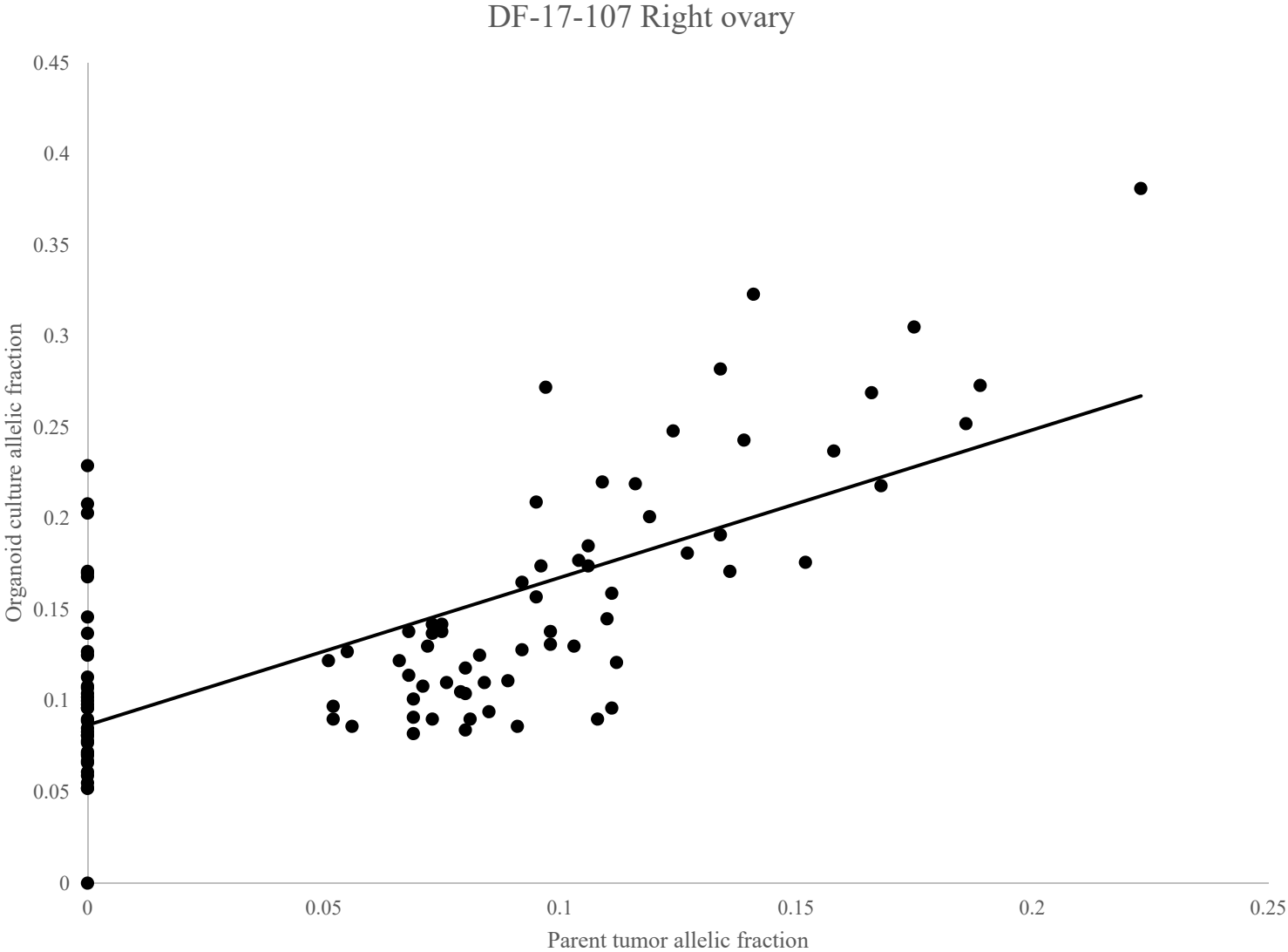


Figure S2 J

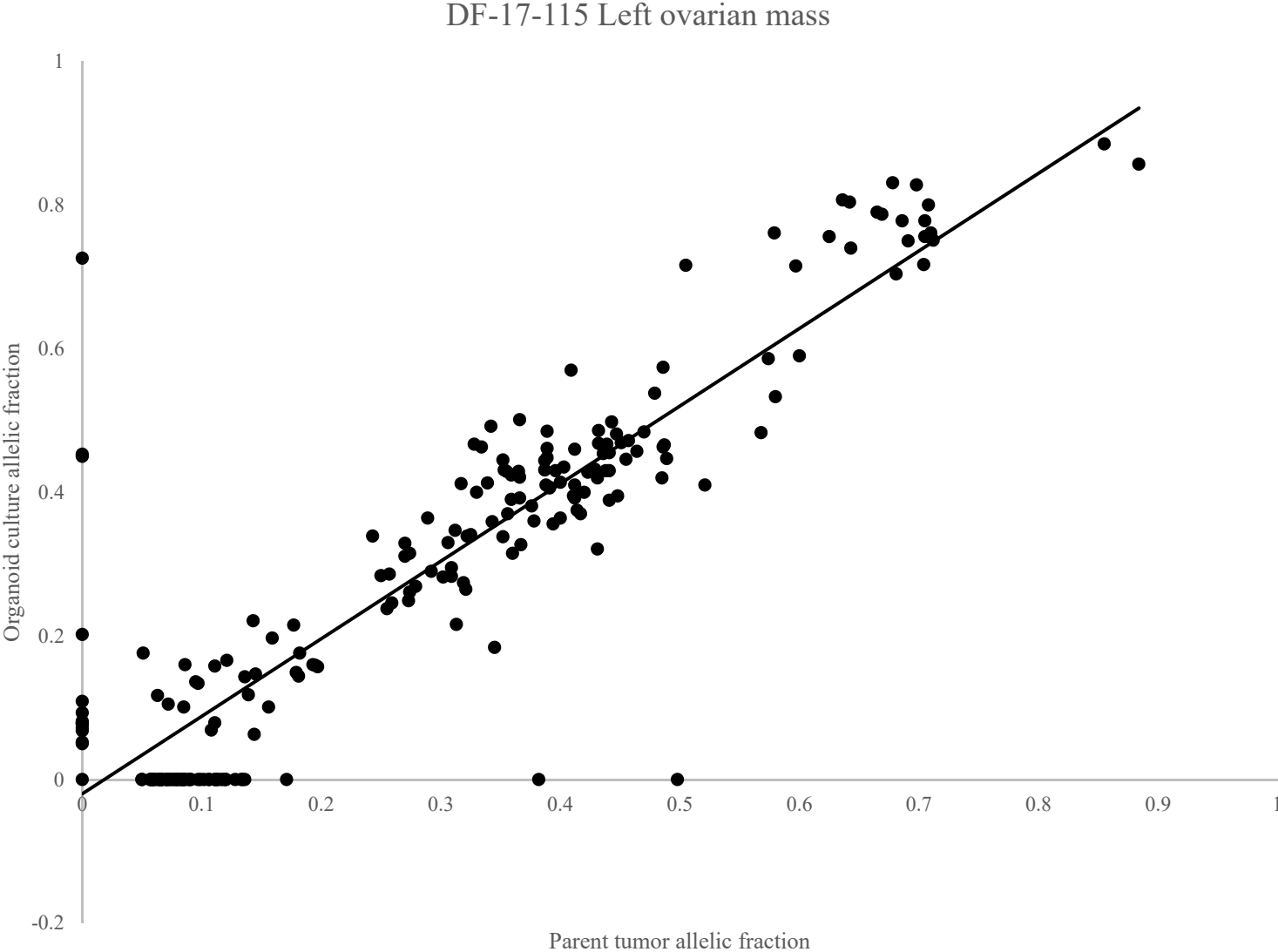


Figure S2 K

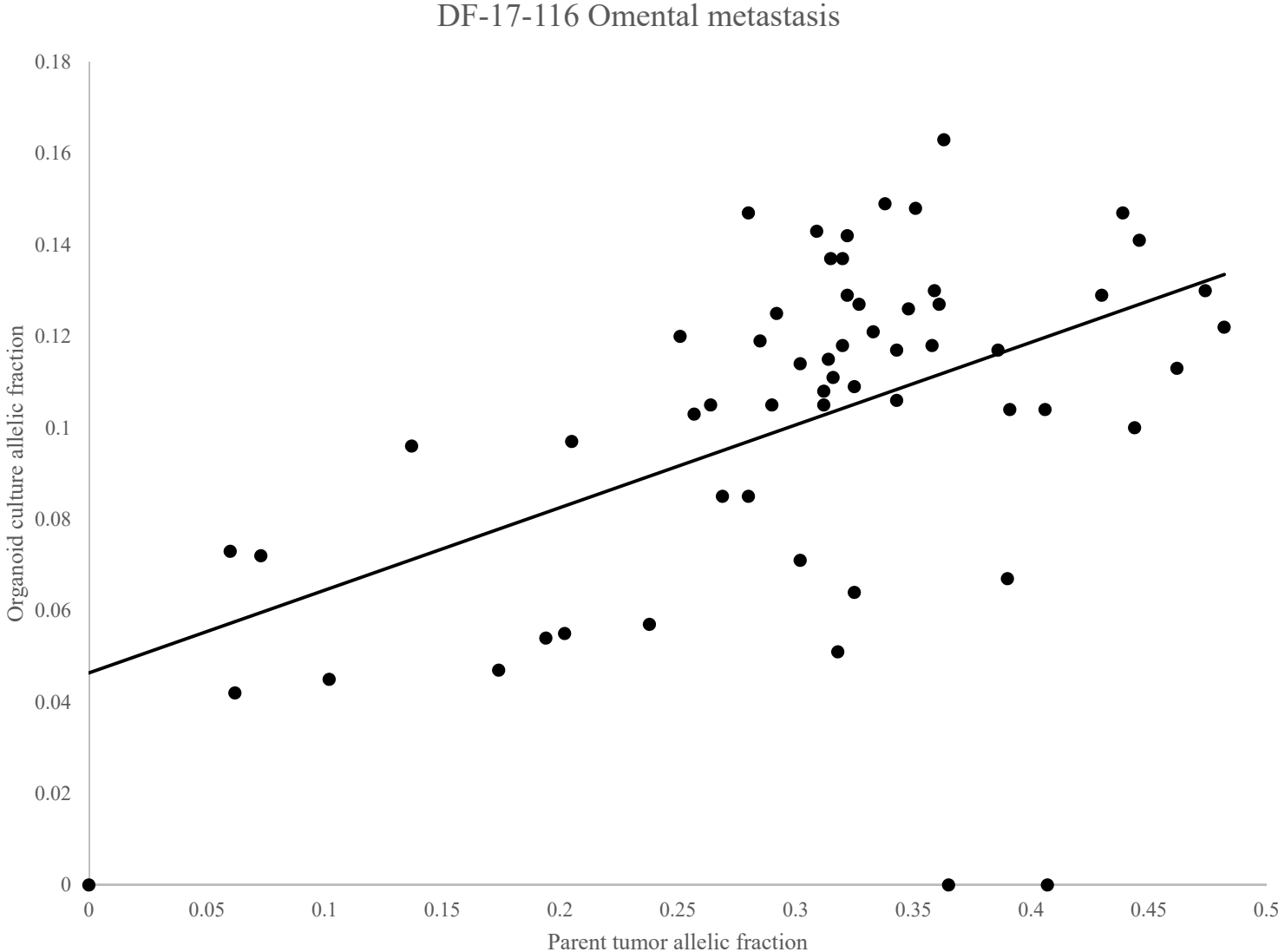


Figure S2 L

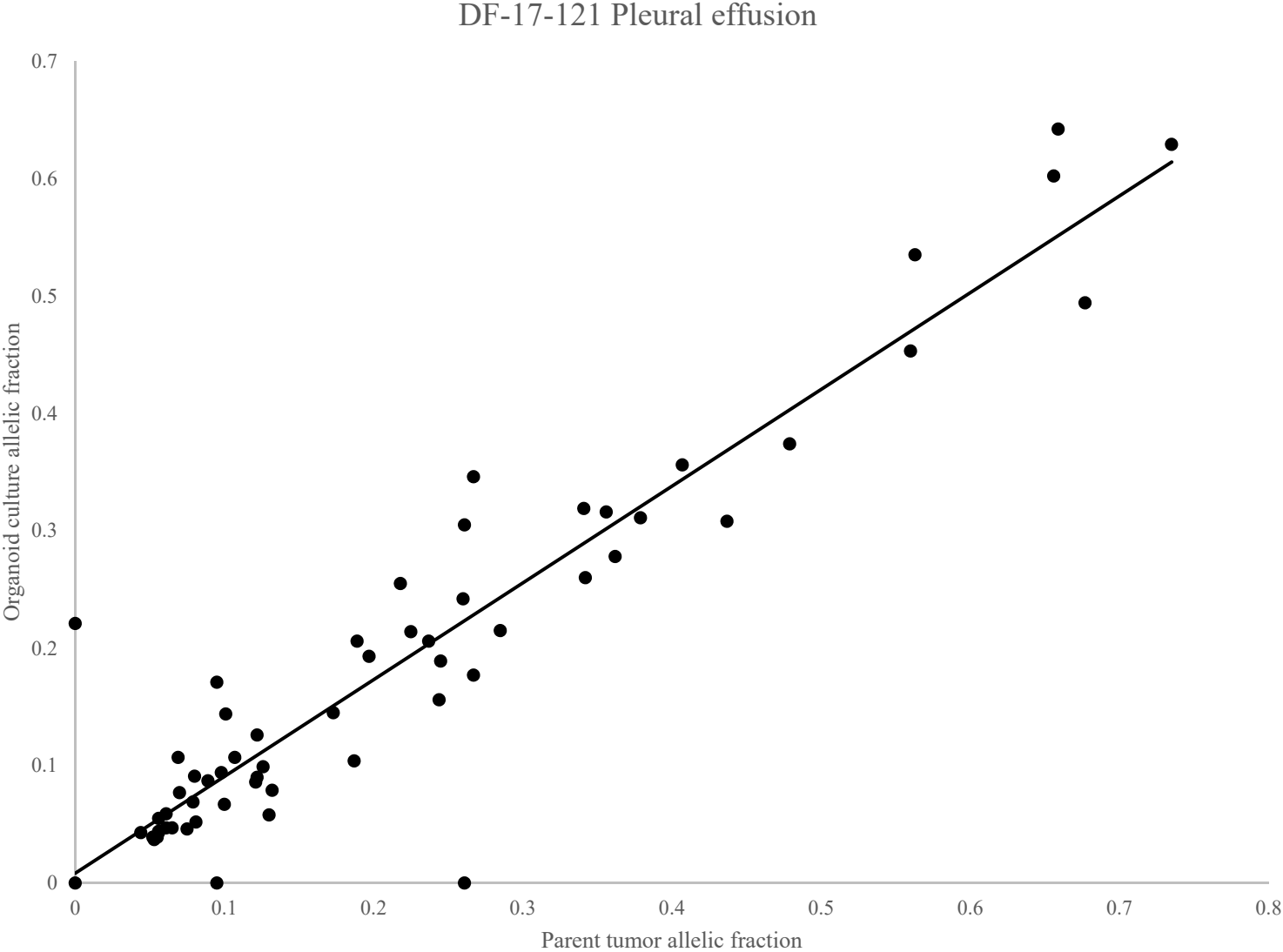


Figure S2 M

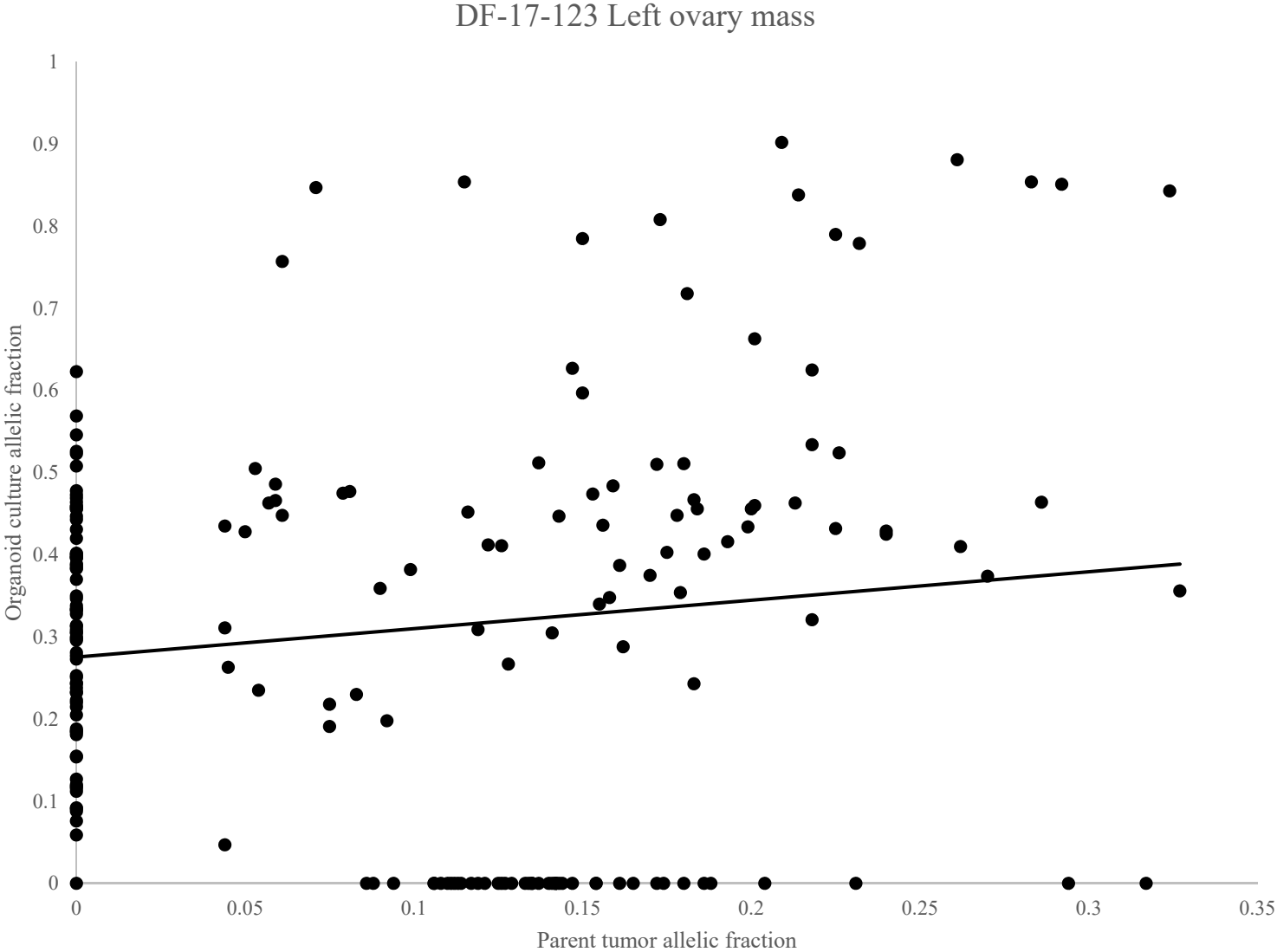


Figure S2 N

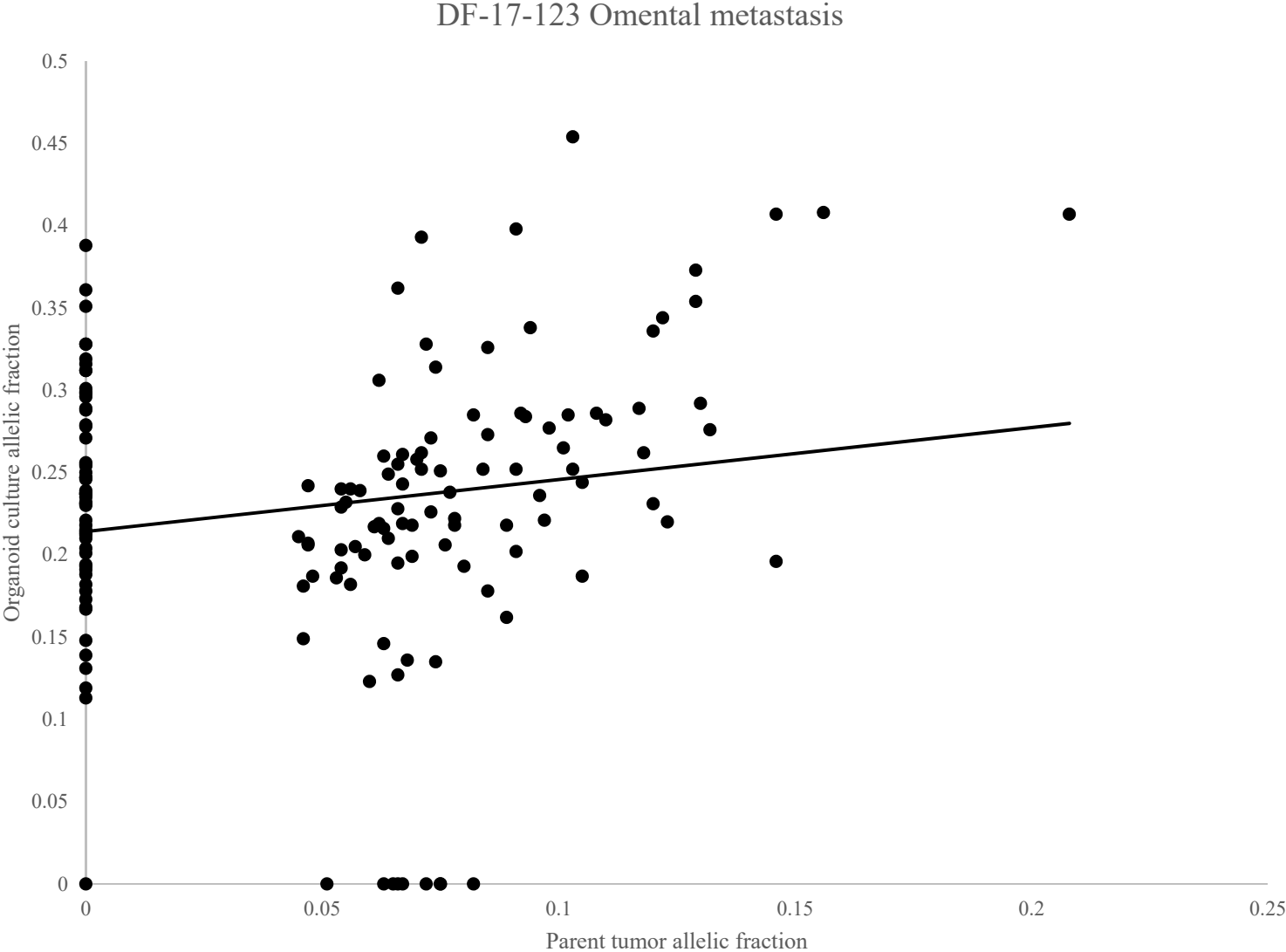


Figure S2 O

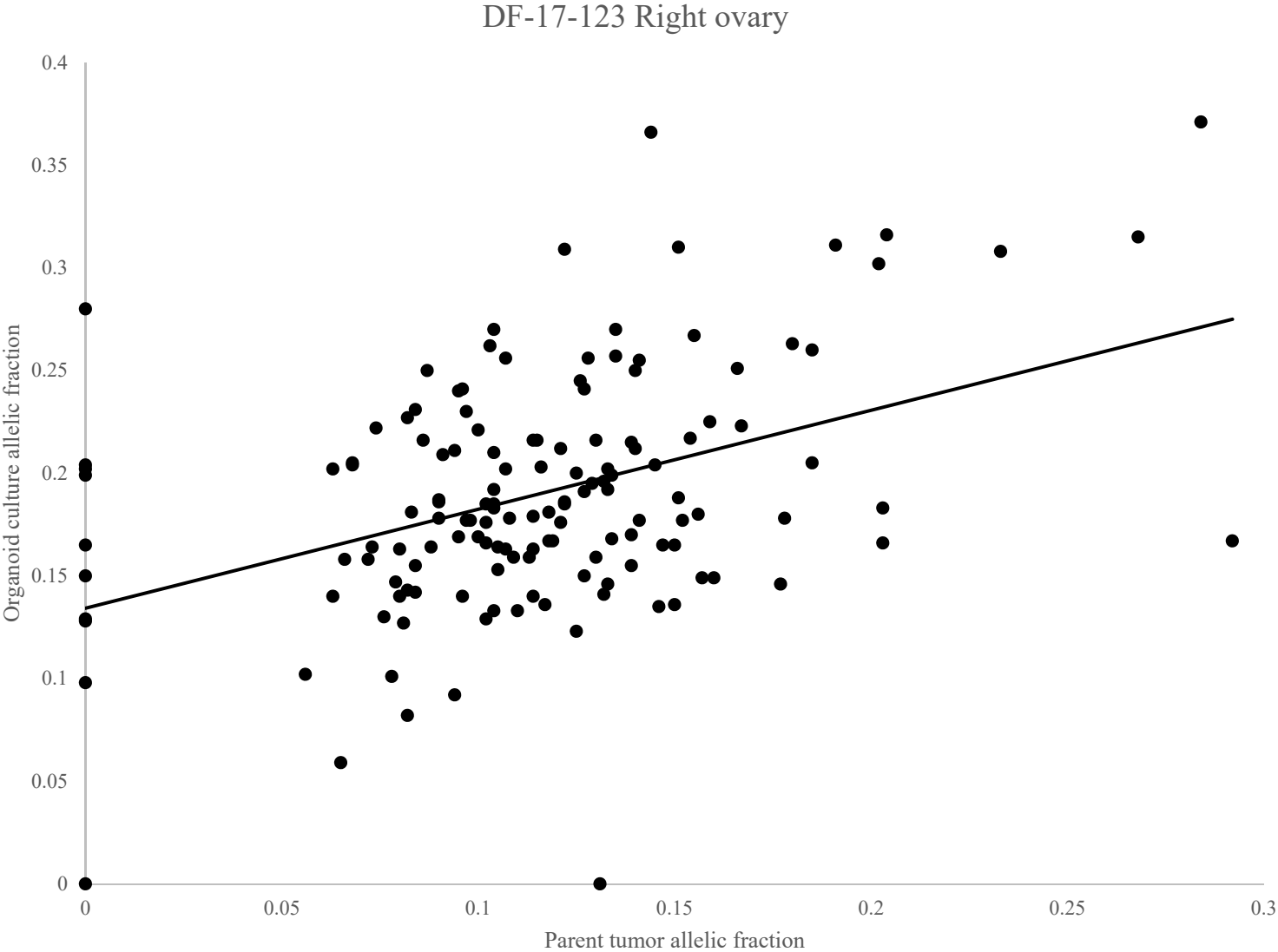


Figure S2 P

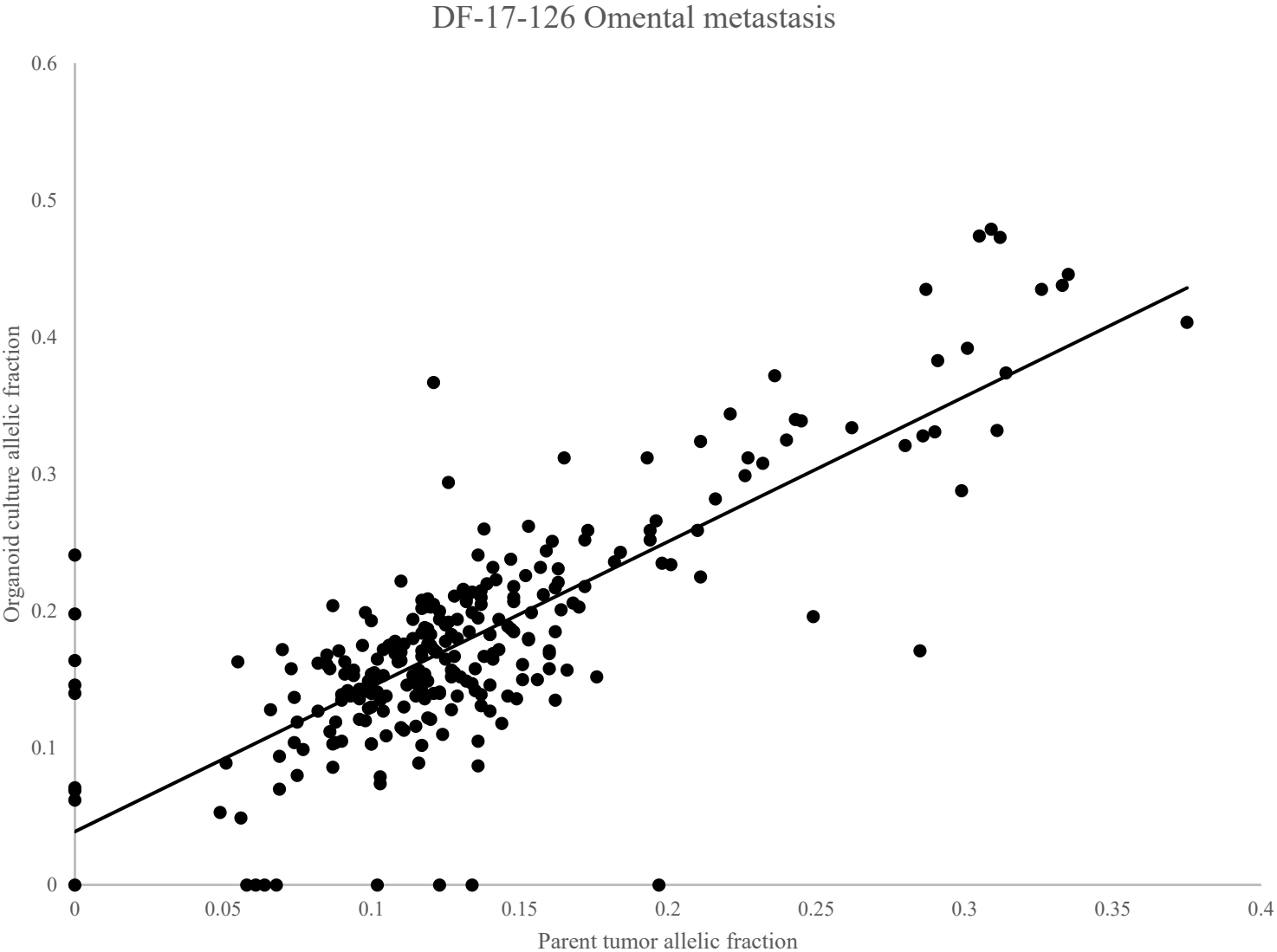


Figure S2 Q

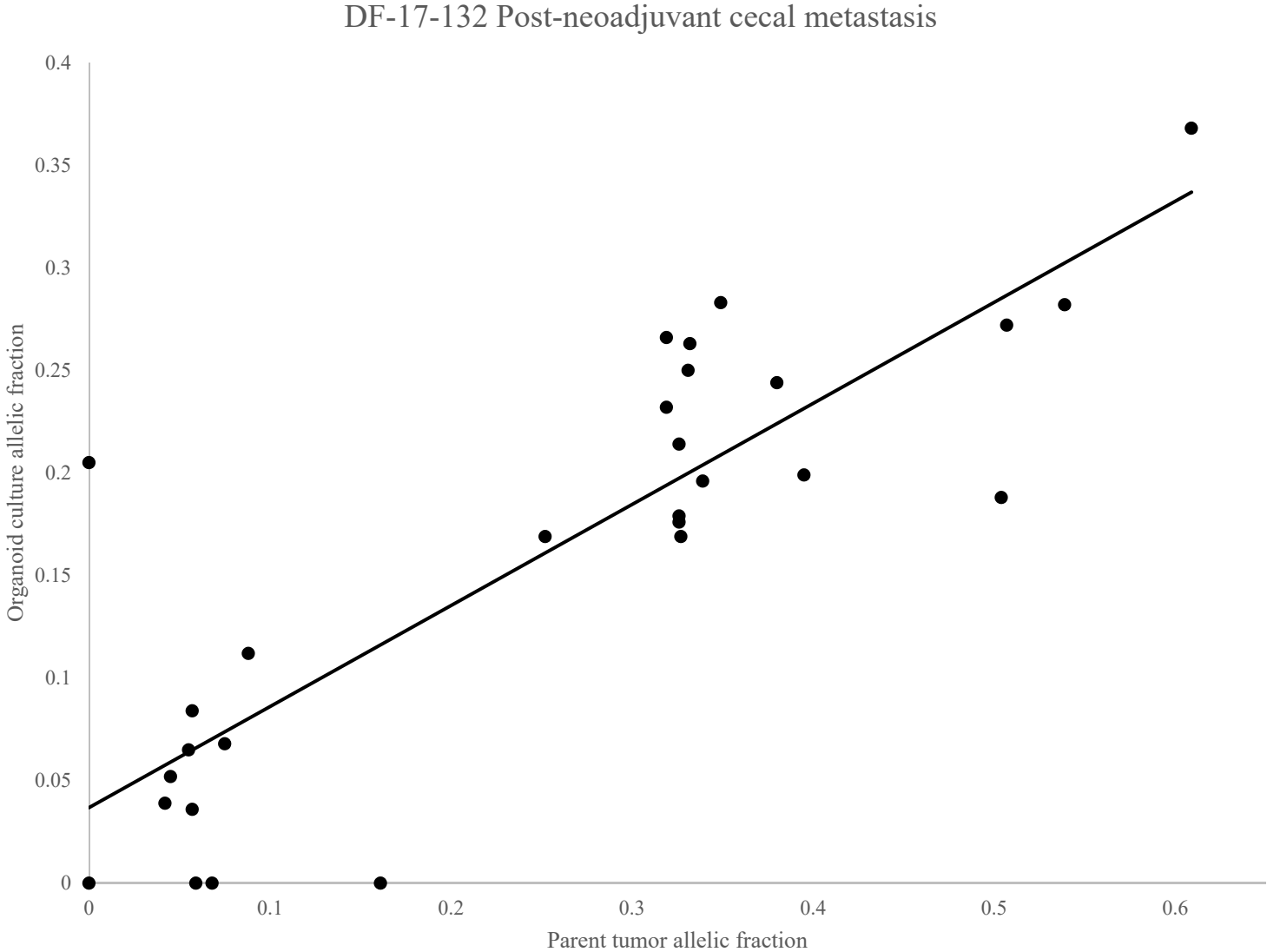


Figure S2 R

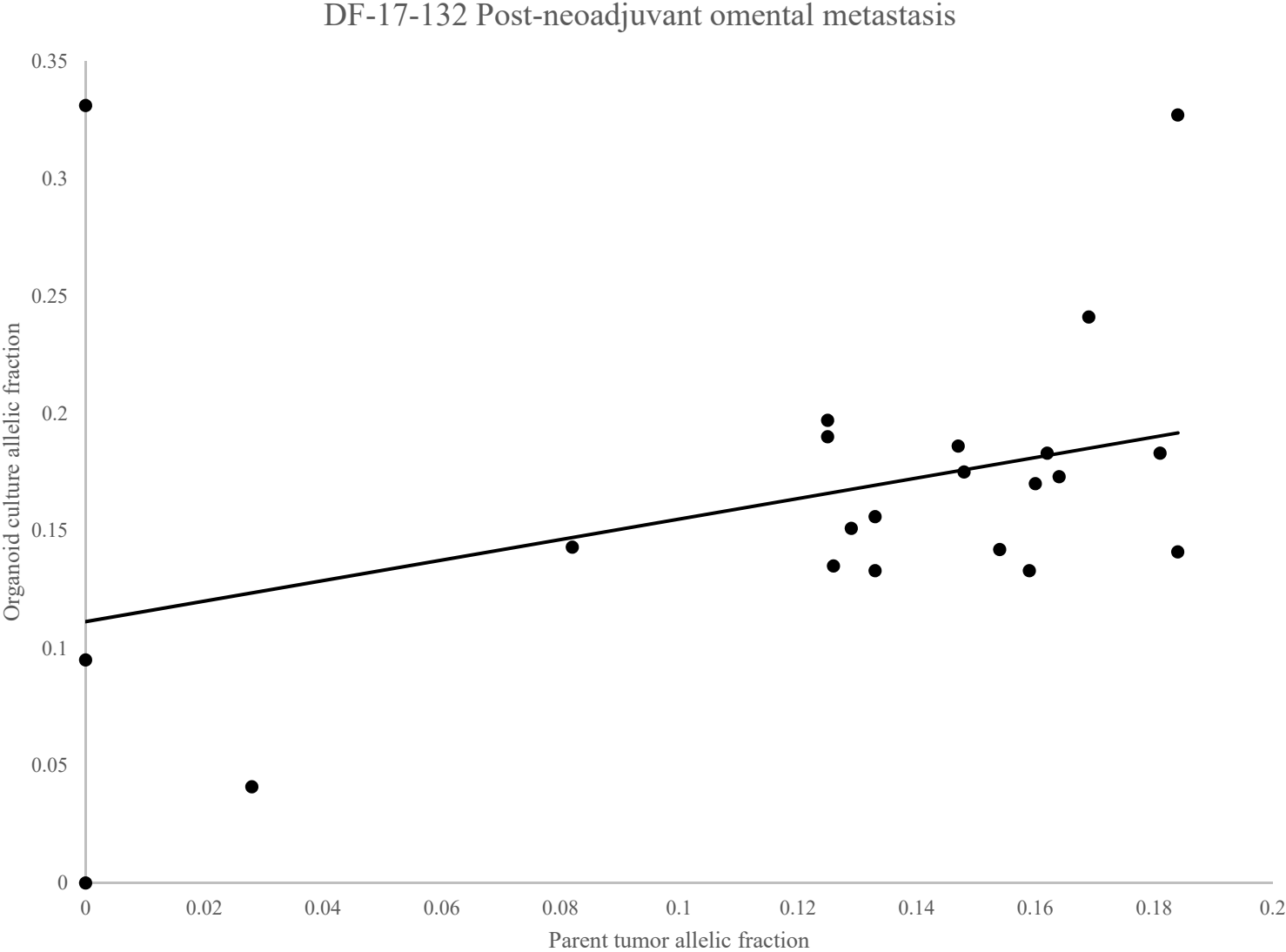


Figure S2 S

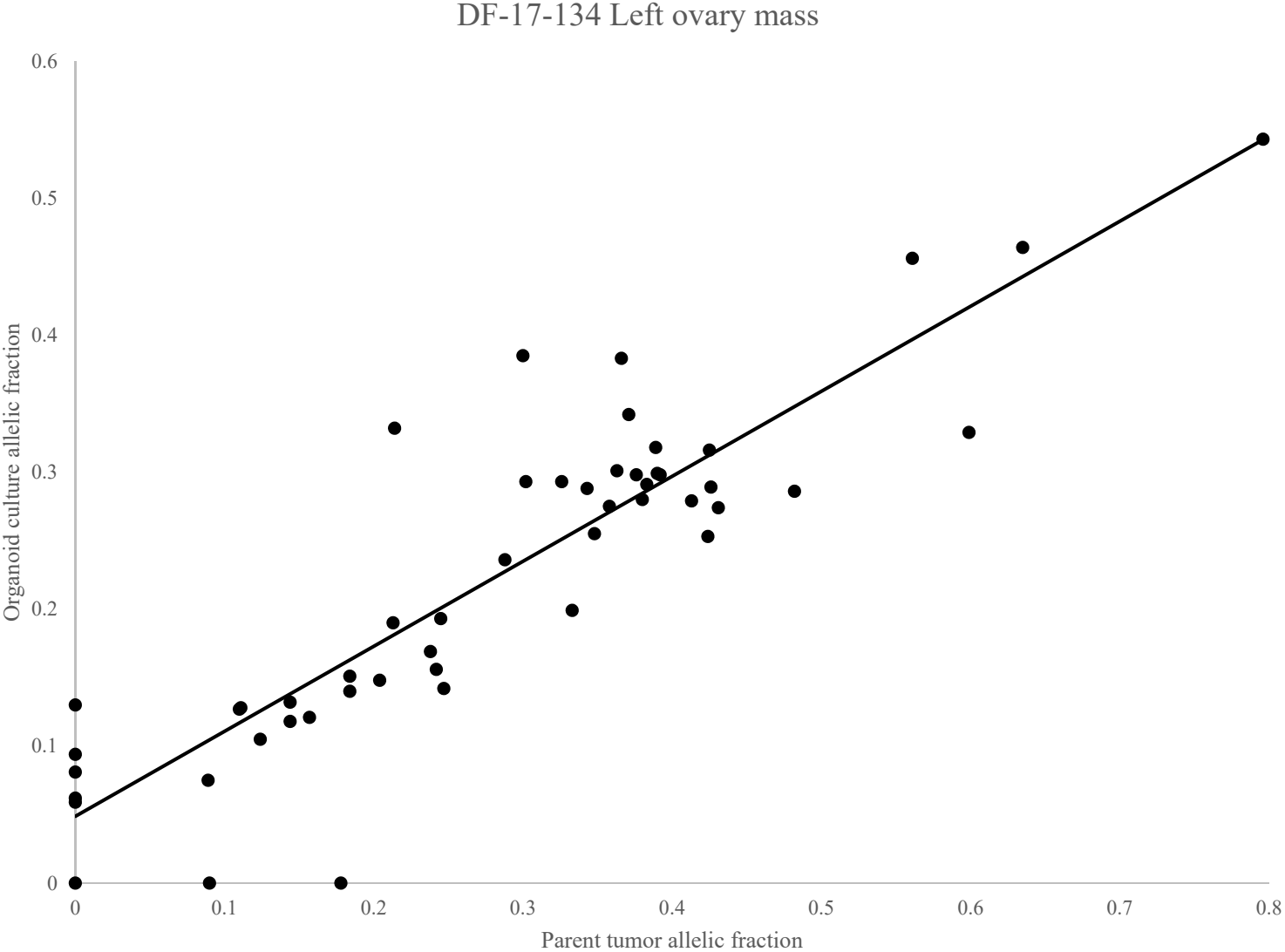


Figure S2 T

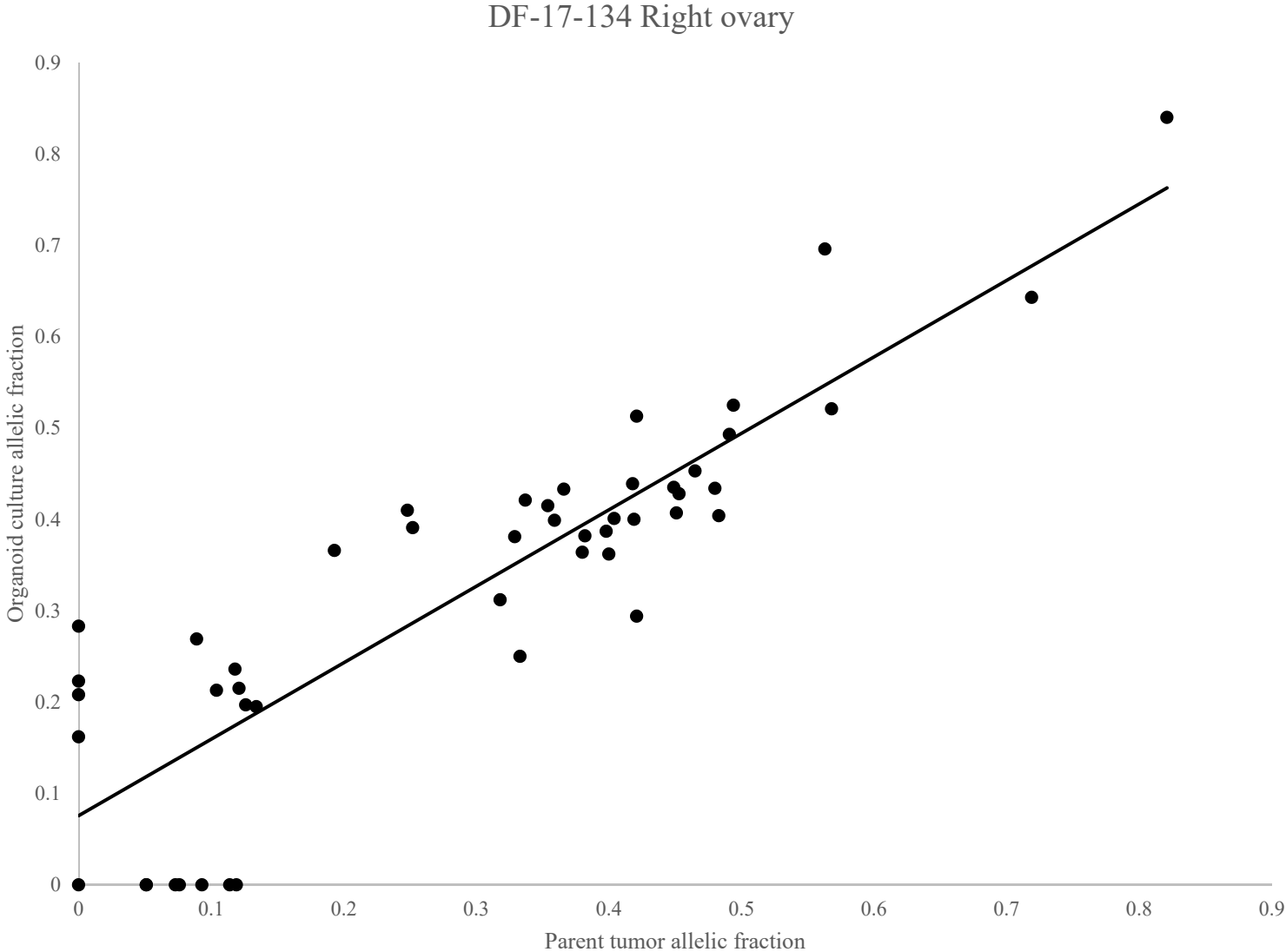


Figure S2 U

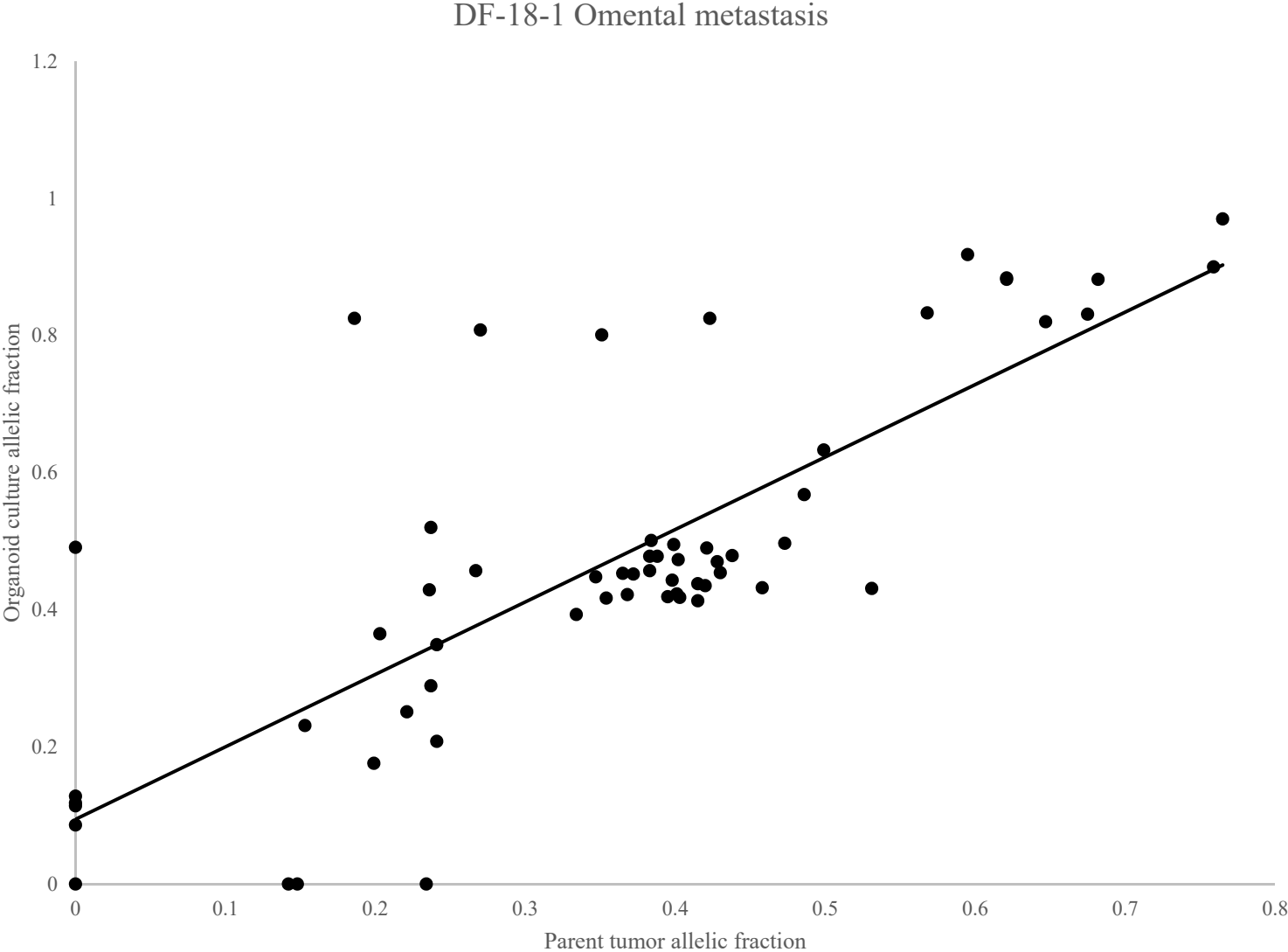


Figure S2 V

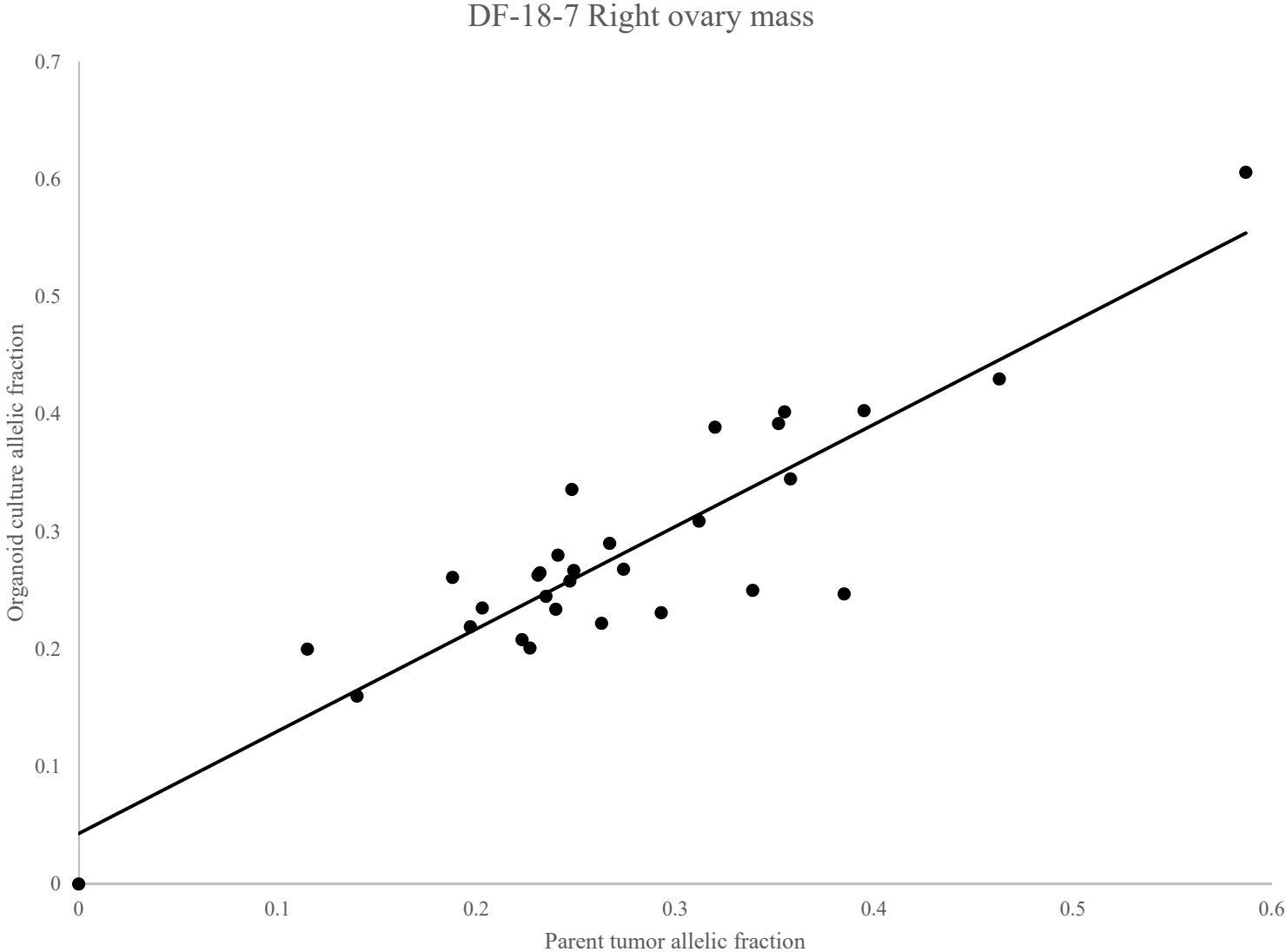


Figure S2 W

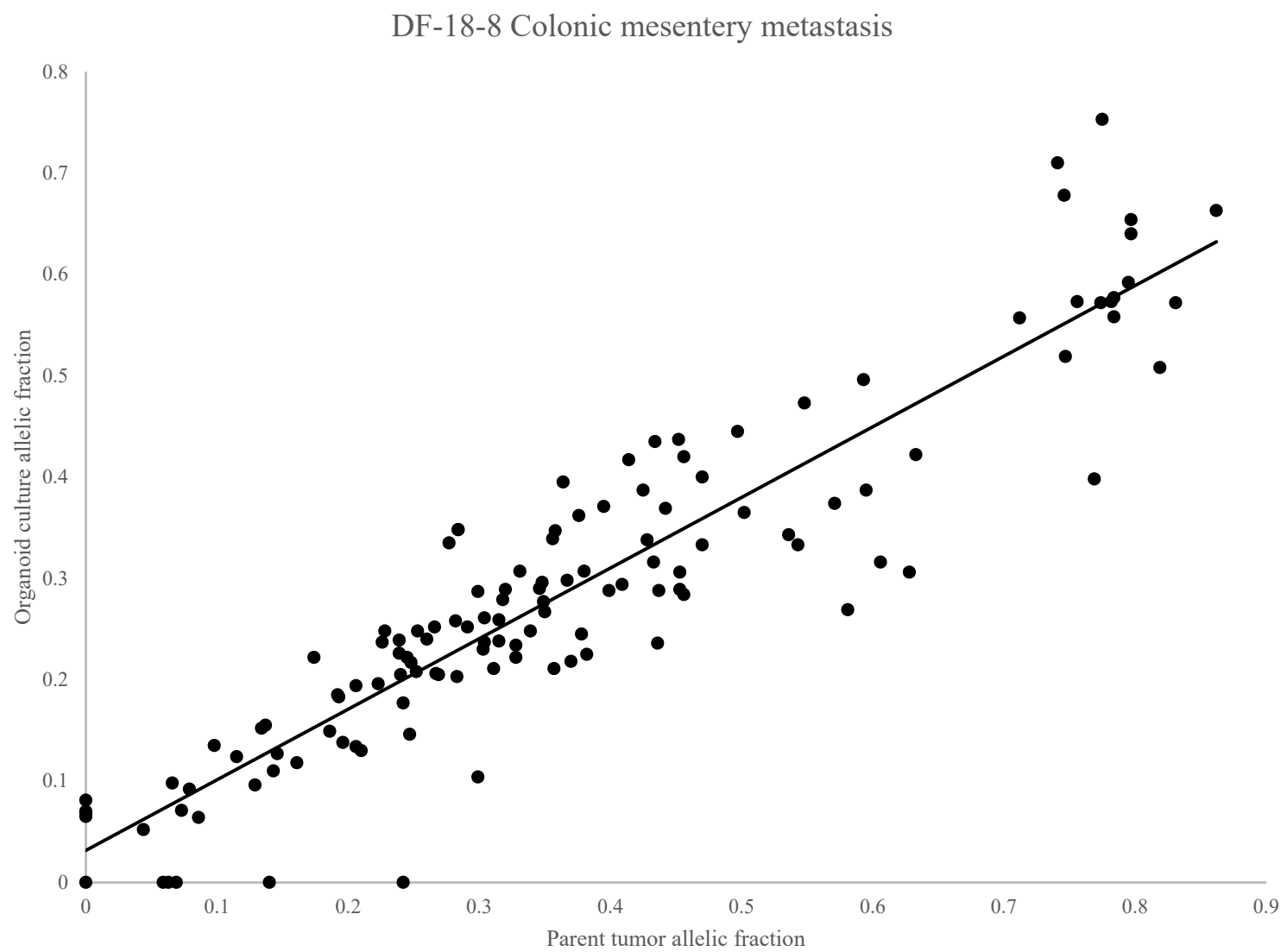


Figure S2 X

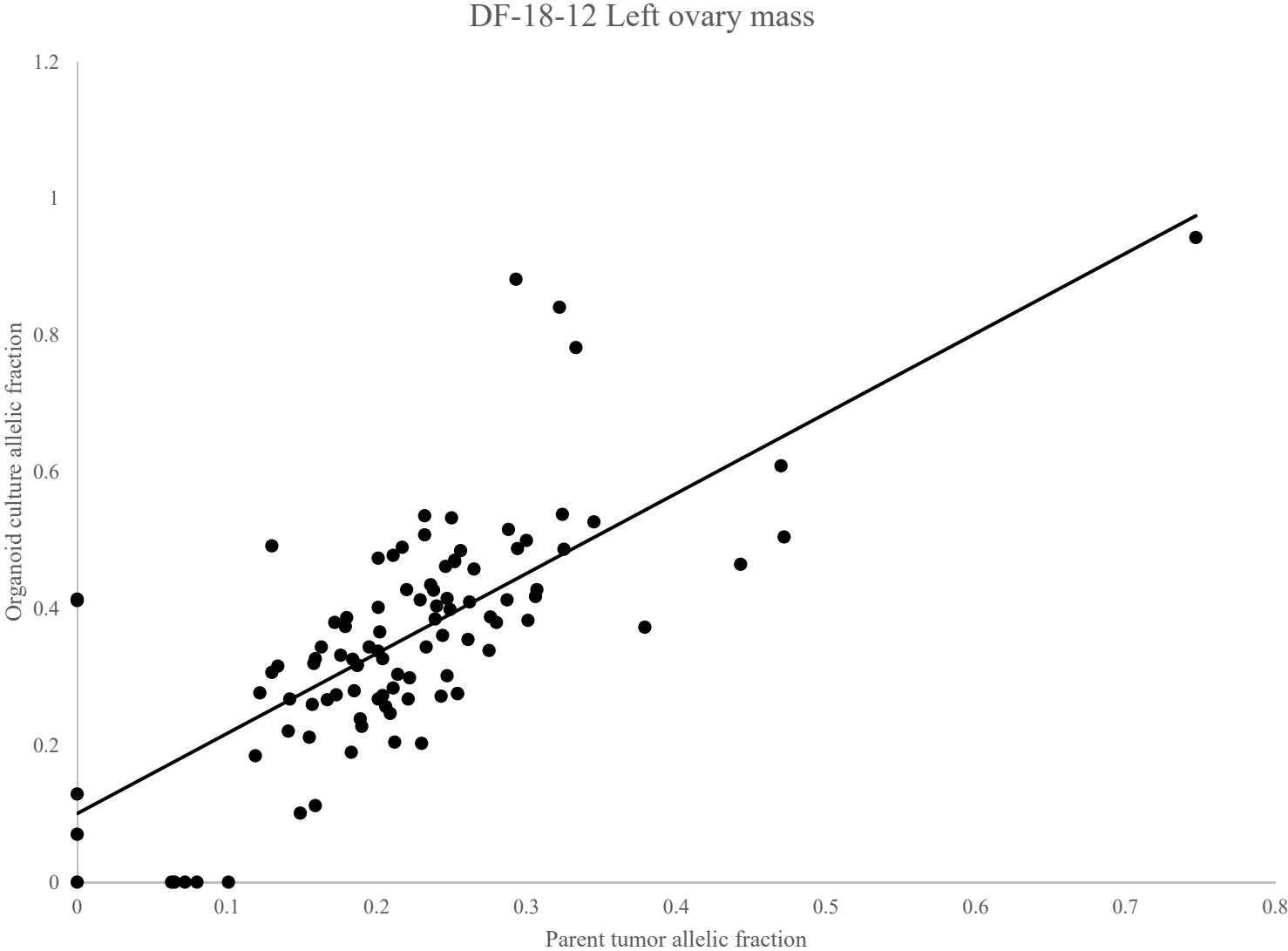


Figure S2 Y

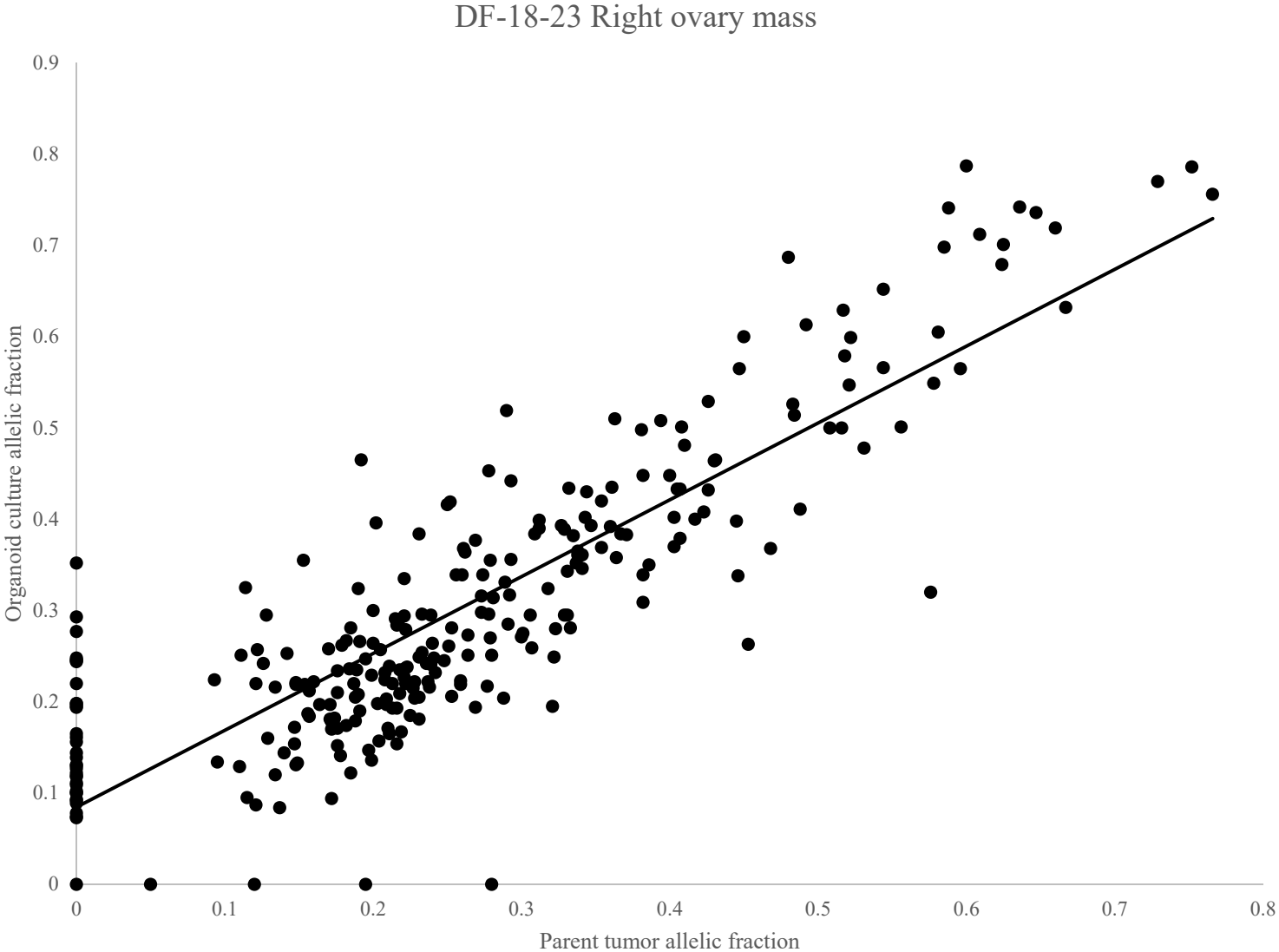


Figure S2 Z

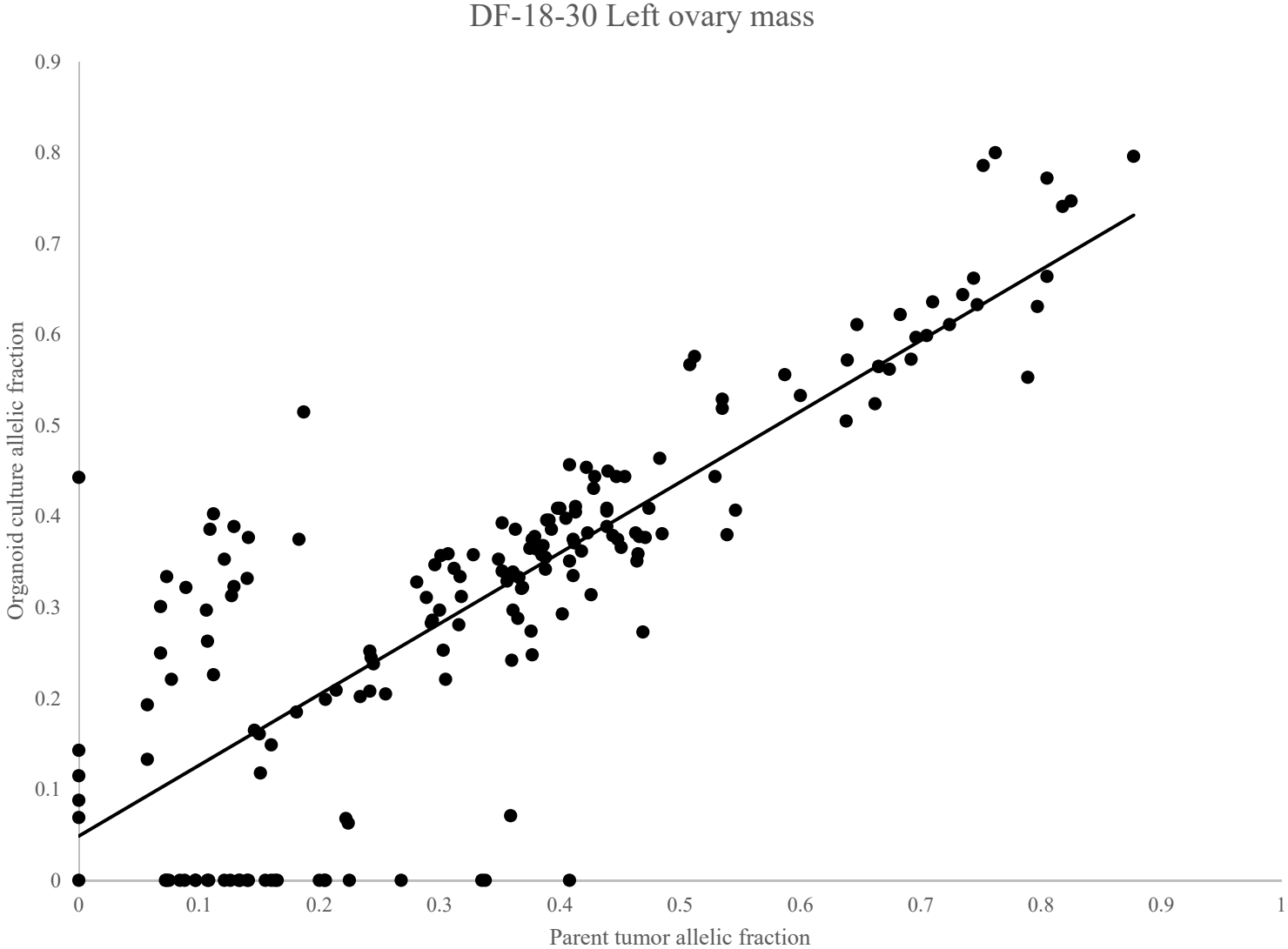


Figure S2 AA

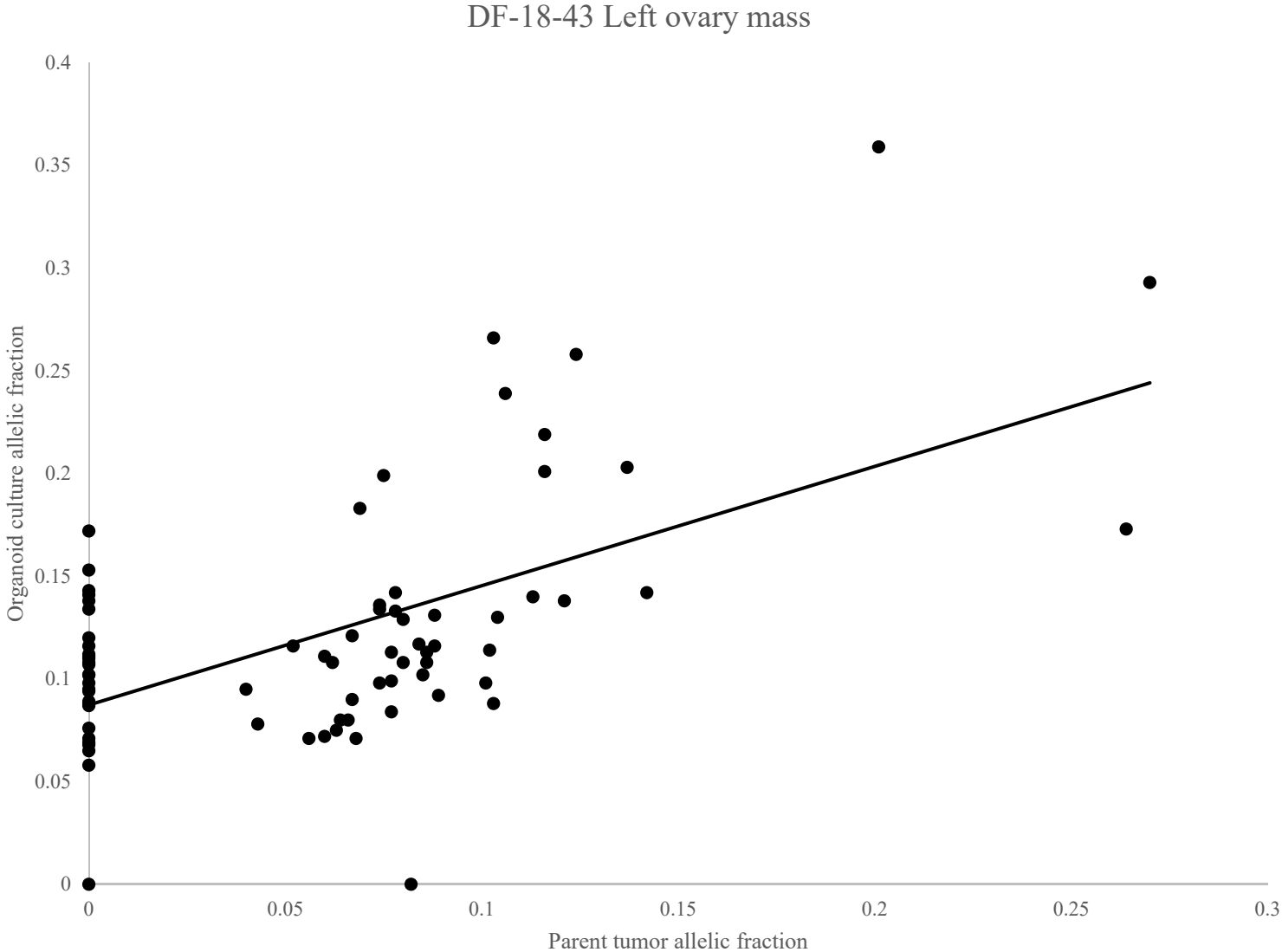


Figure S2 BB

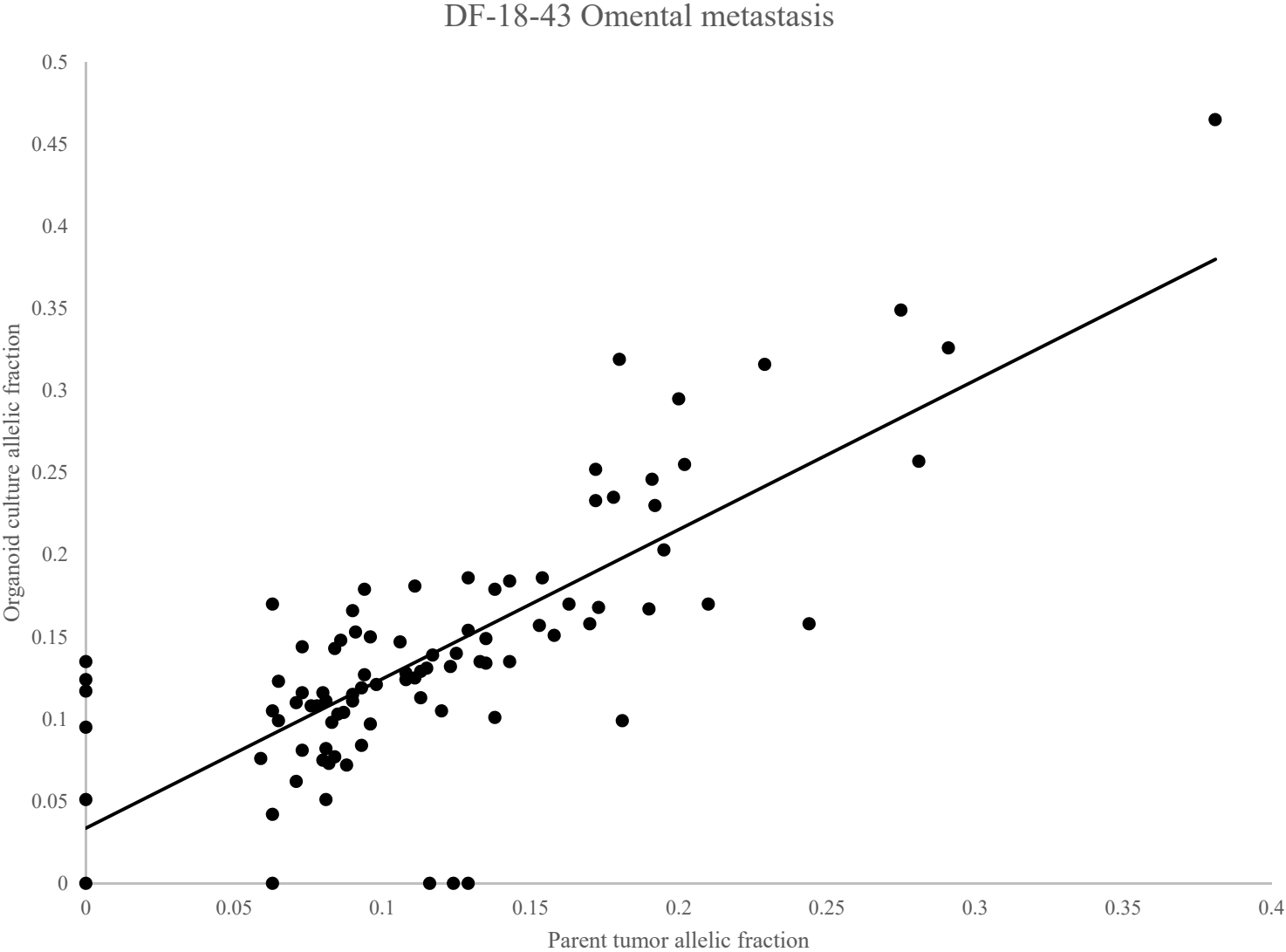


Figure S2 CC

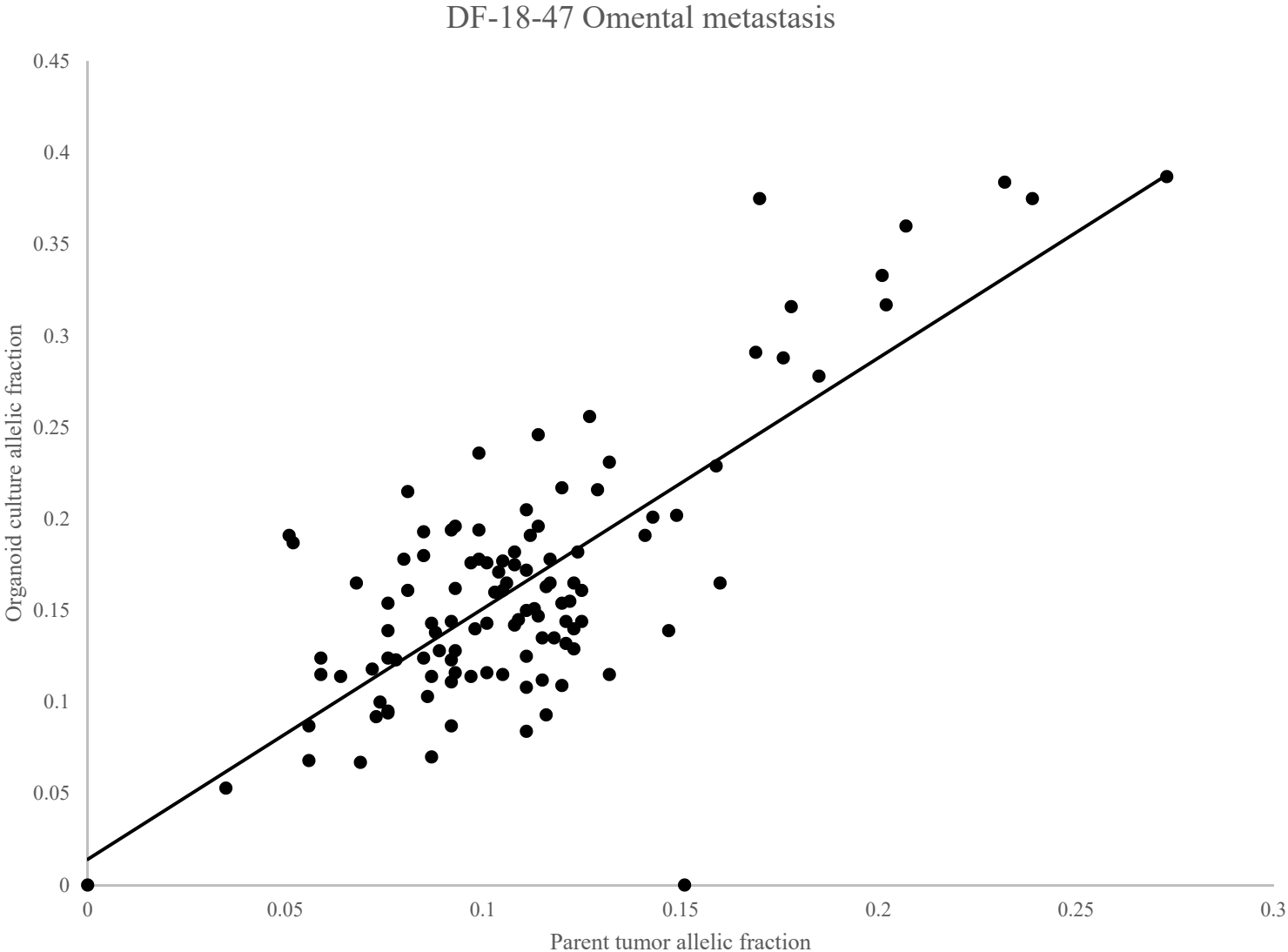


Figure S2 DD

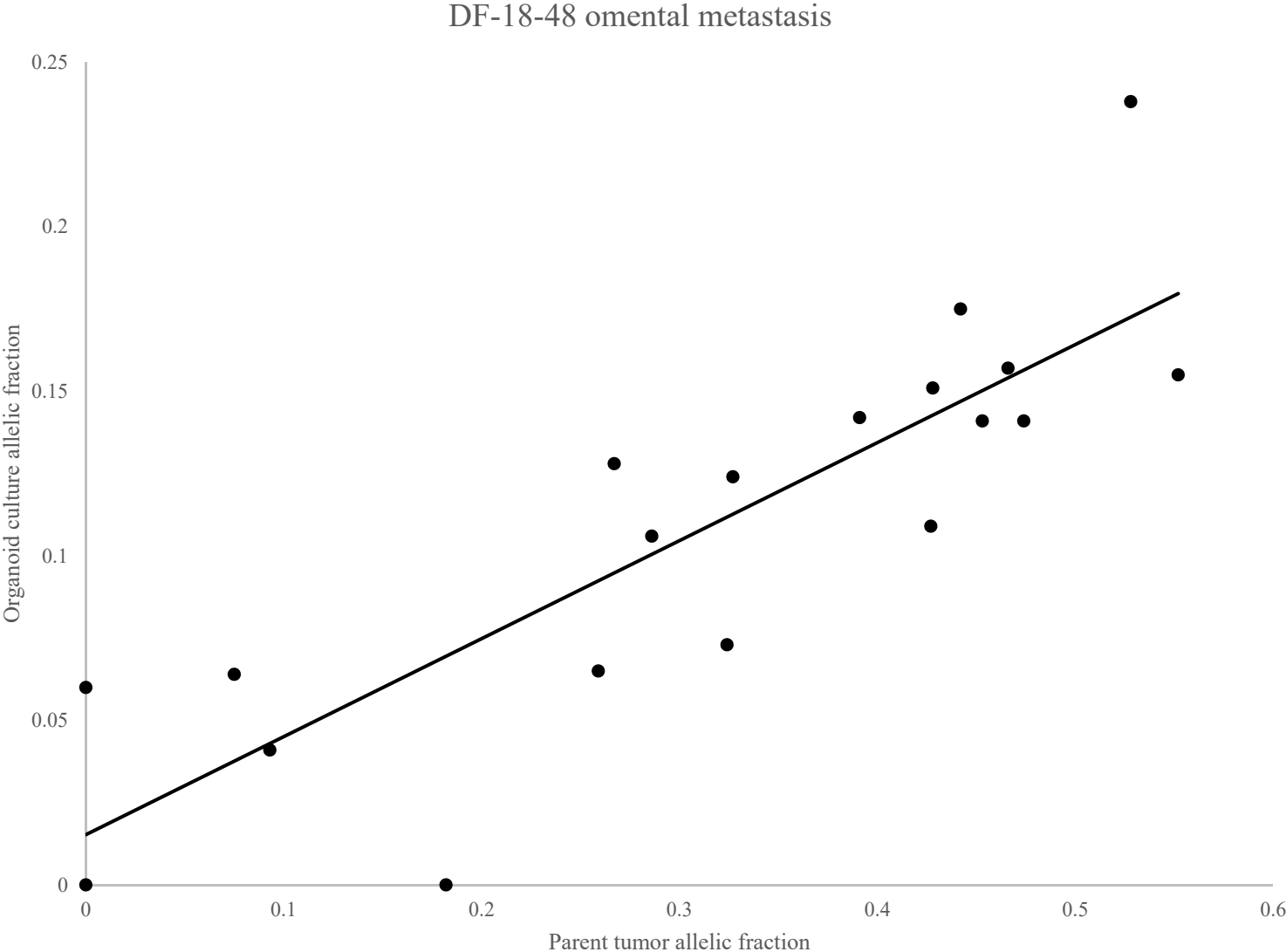


Figure S2 EE

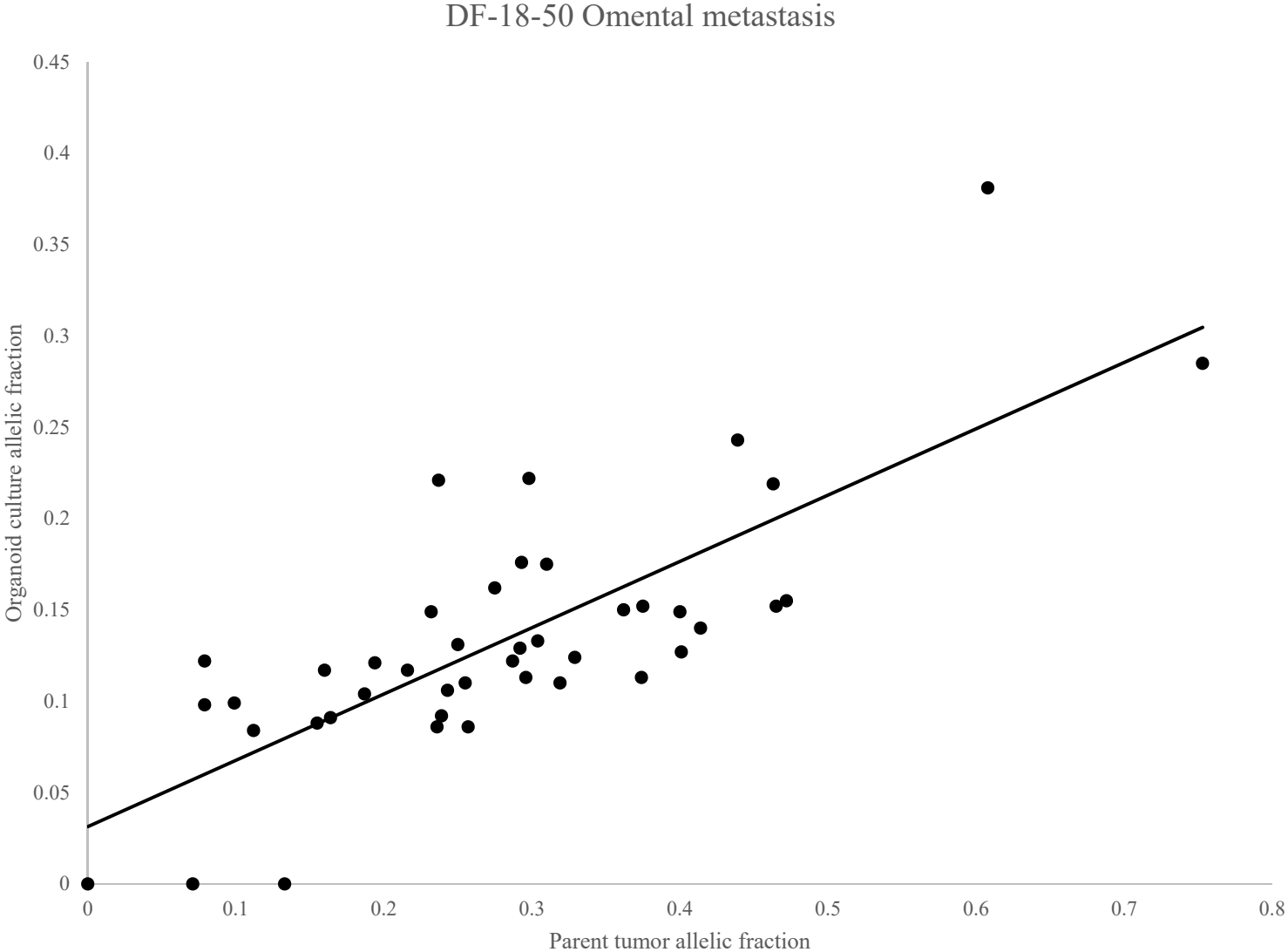


Figure S2FF

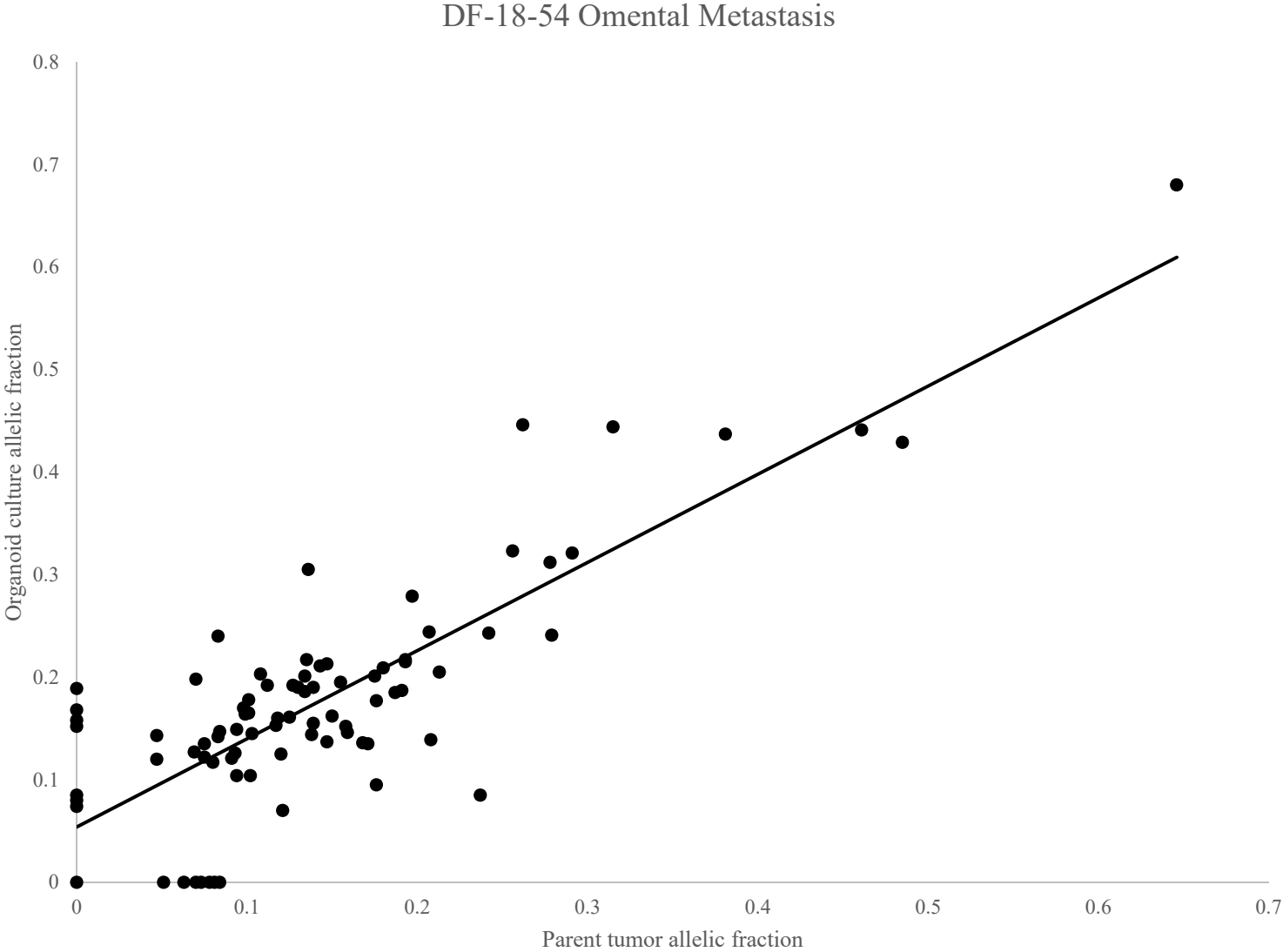


Figure S3 A DF-17-39 Rectosigmoid Mesentery Metastasis

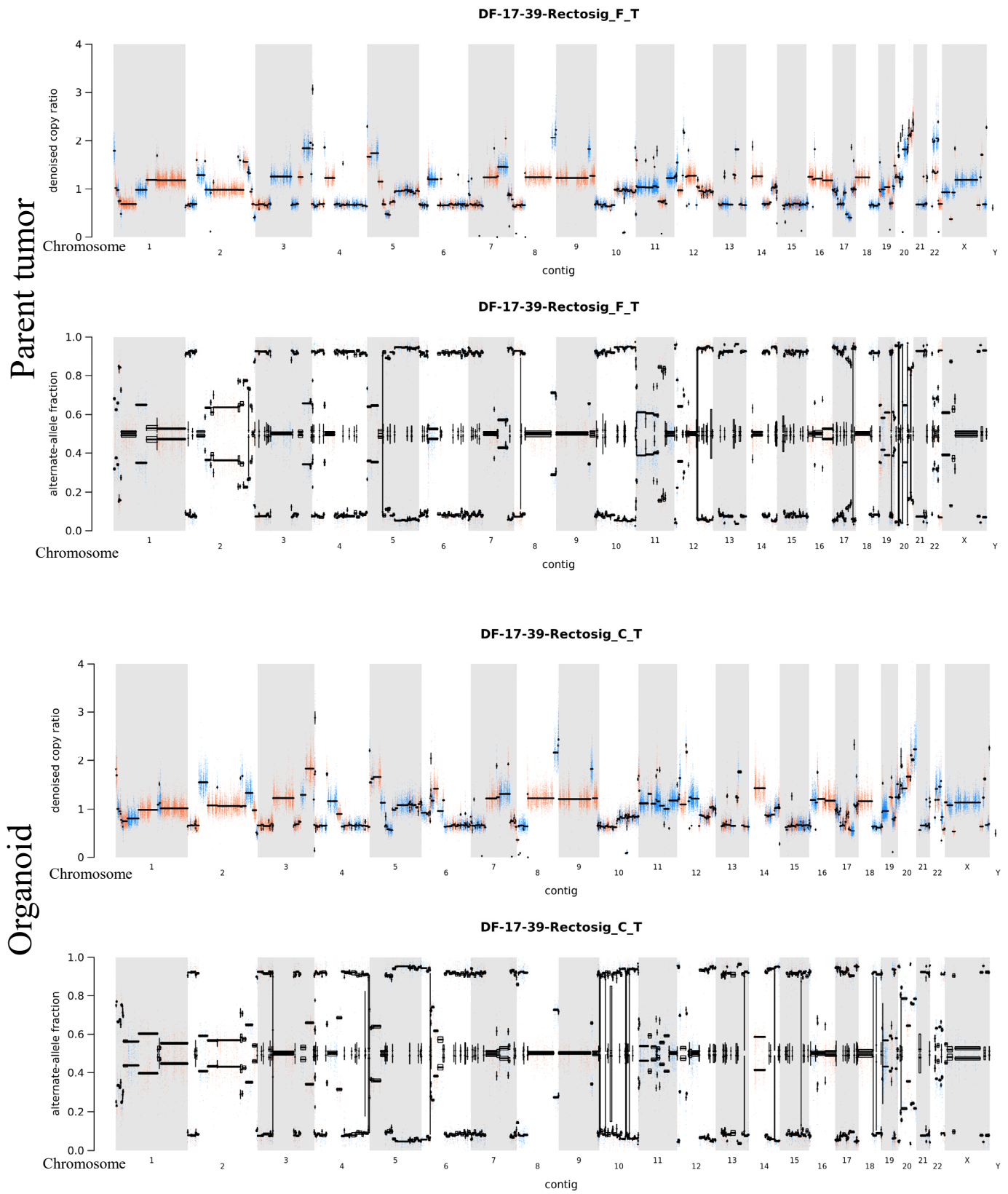


Figure S3 B DF-17-39 Transverse Colon Mesentery Metastasis

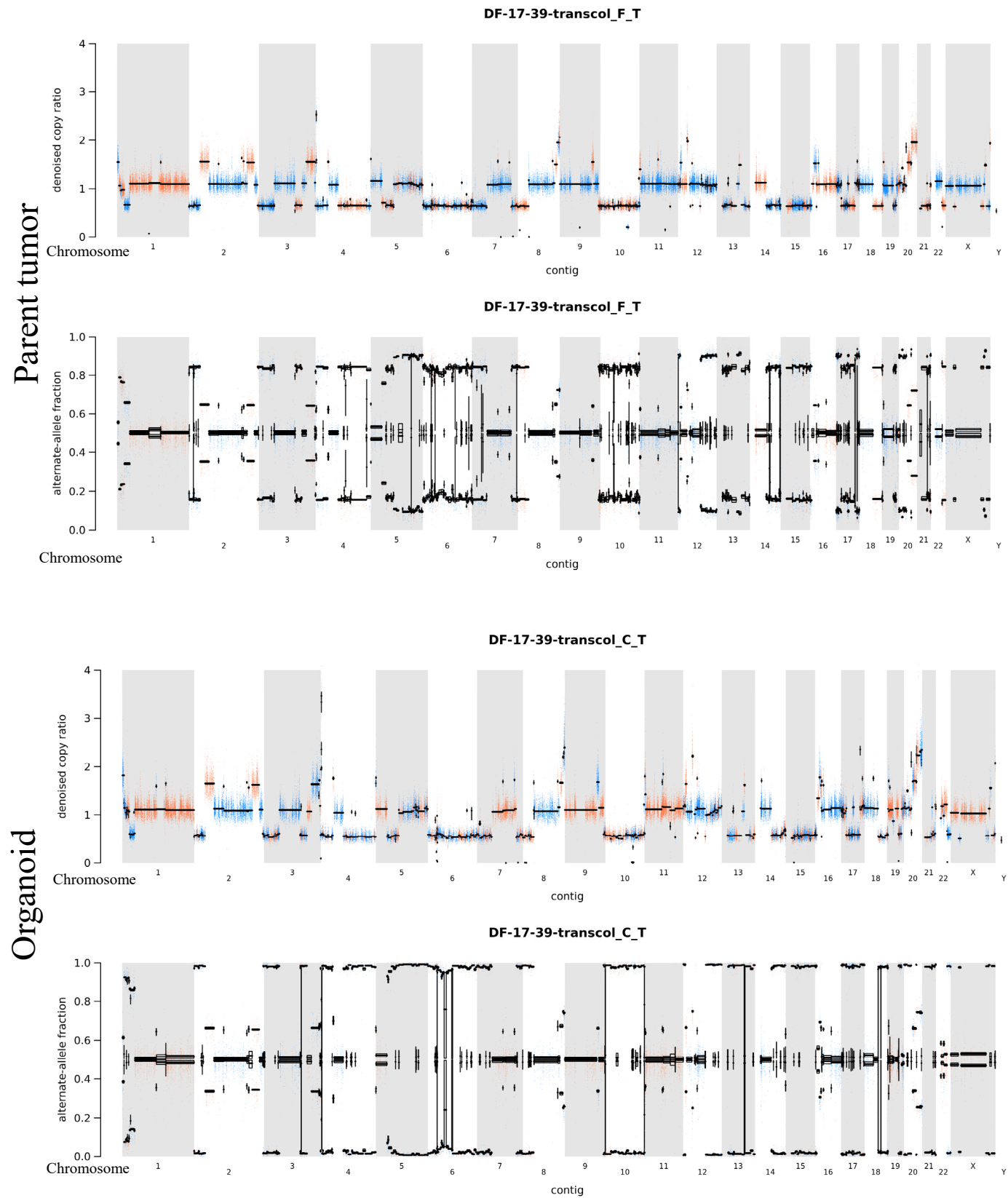


Figure S3 C DF-17-39 Supracolic Omentum Metastasis

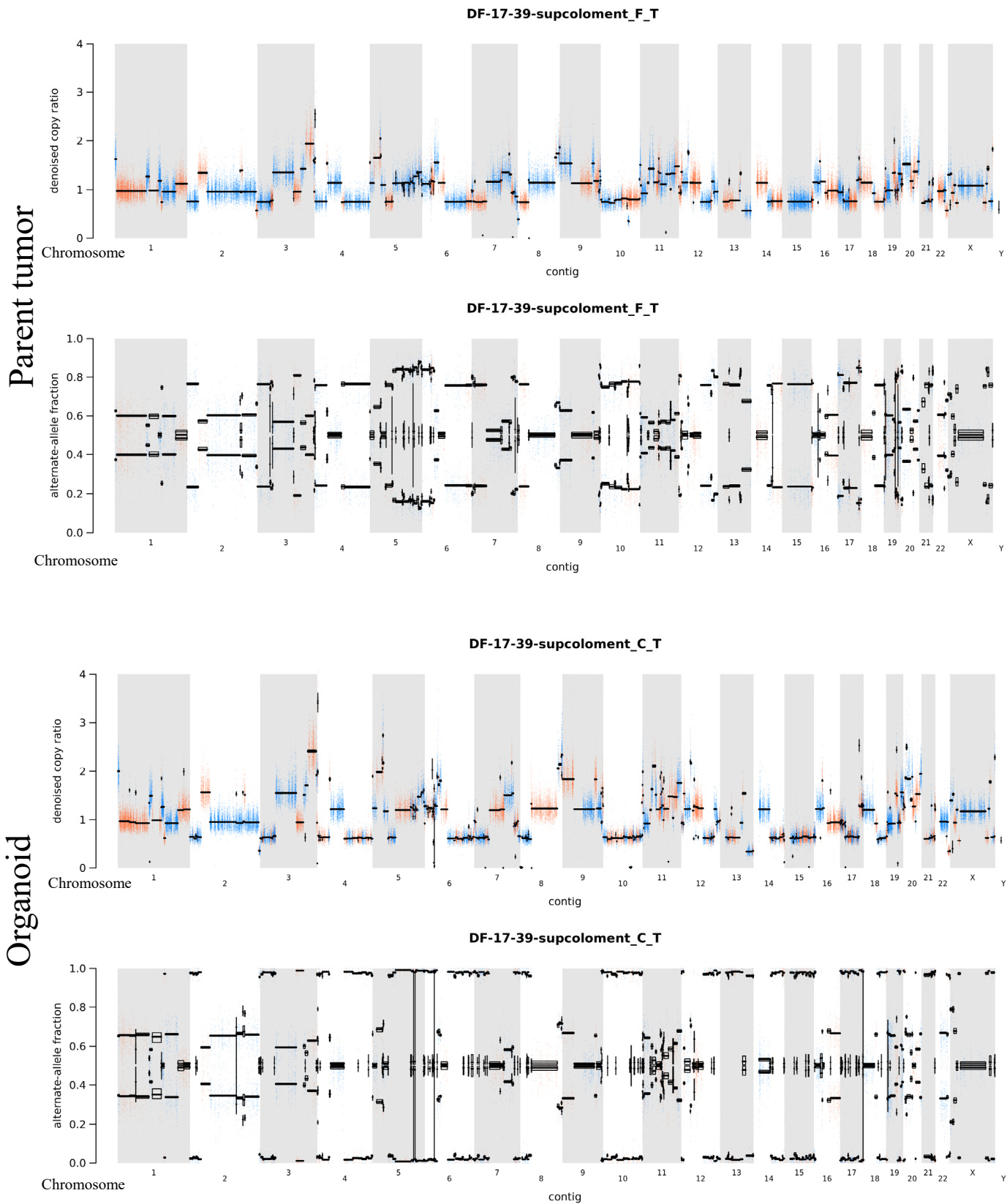


Figure S3 D

DF-17-39 Diaphragm Metastasis

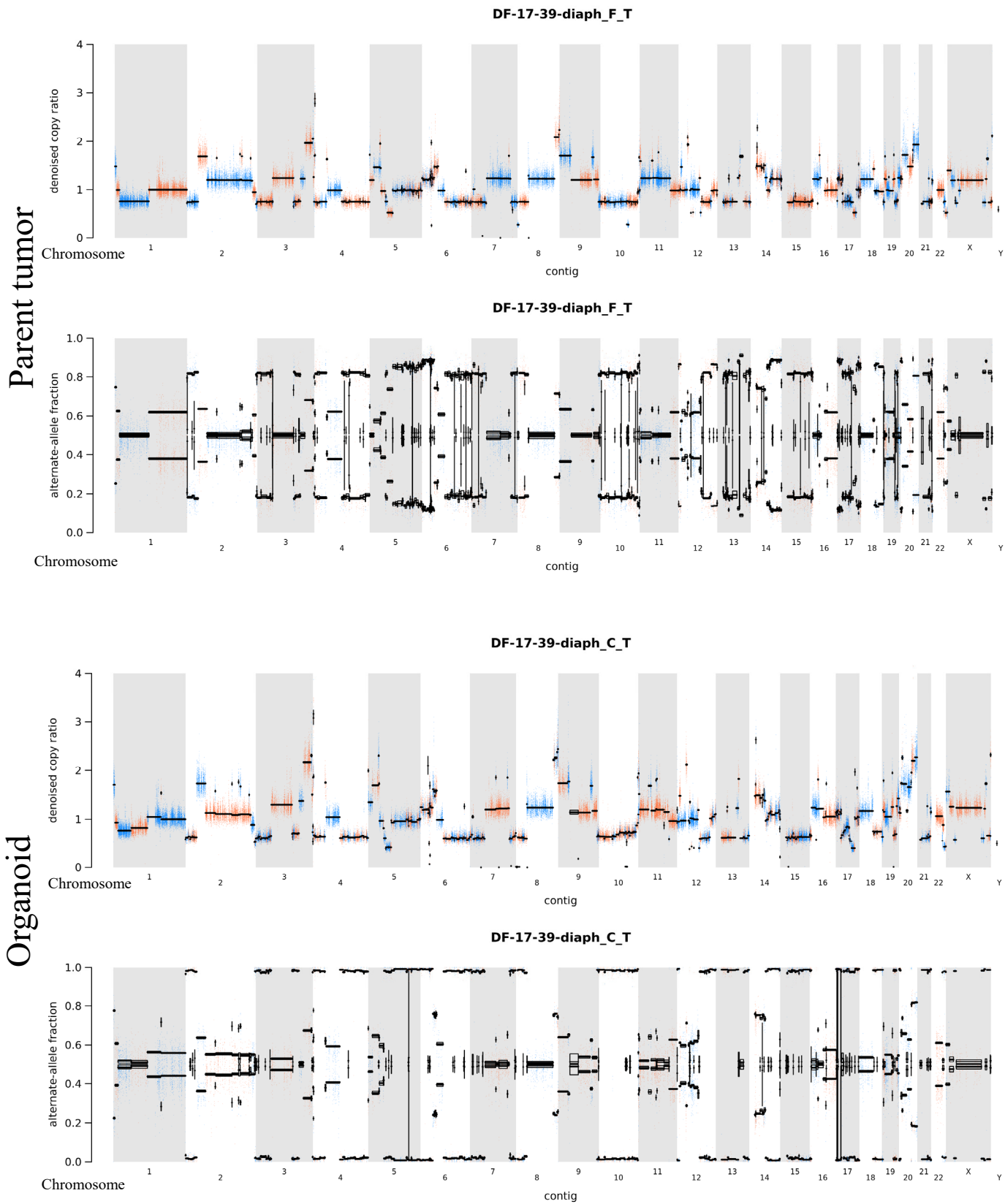


Figure S3 E

DF-17-103 Omental Metastasis

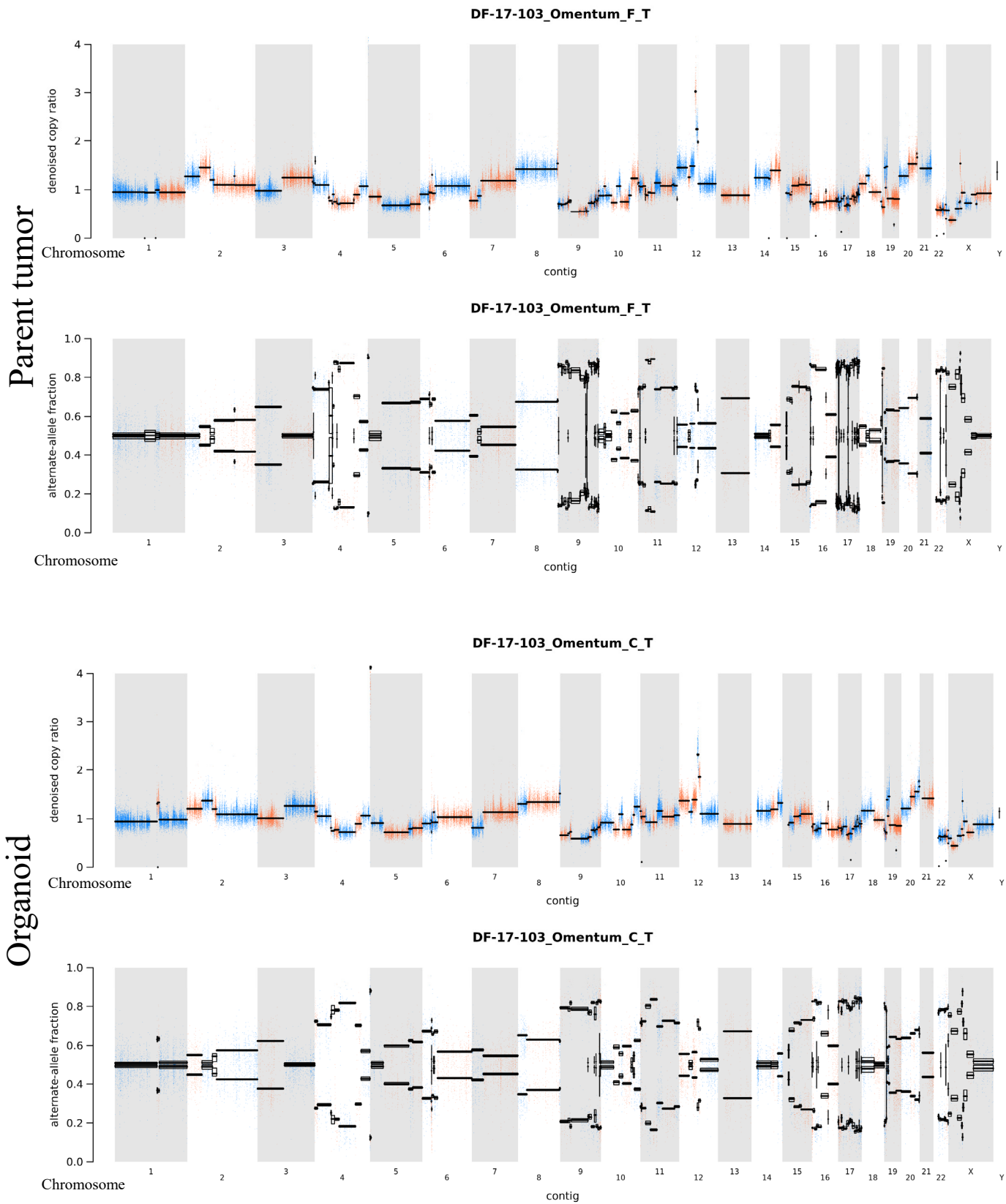


Figure S3 F

DF-17-104 Omental Metastasis

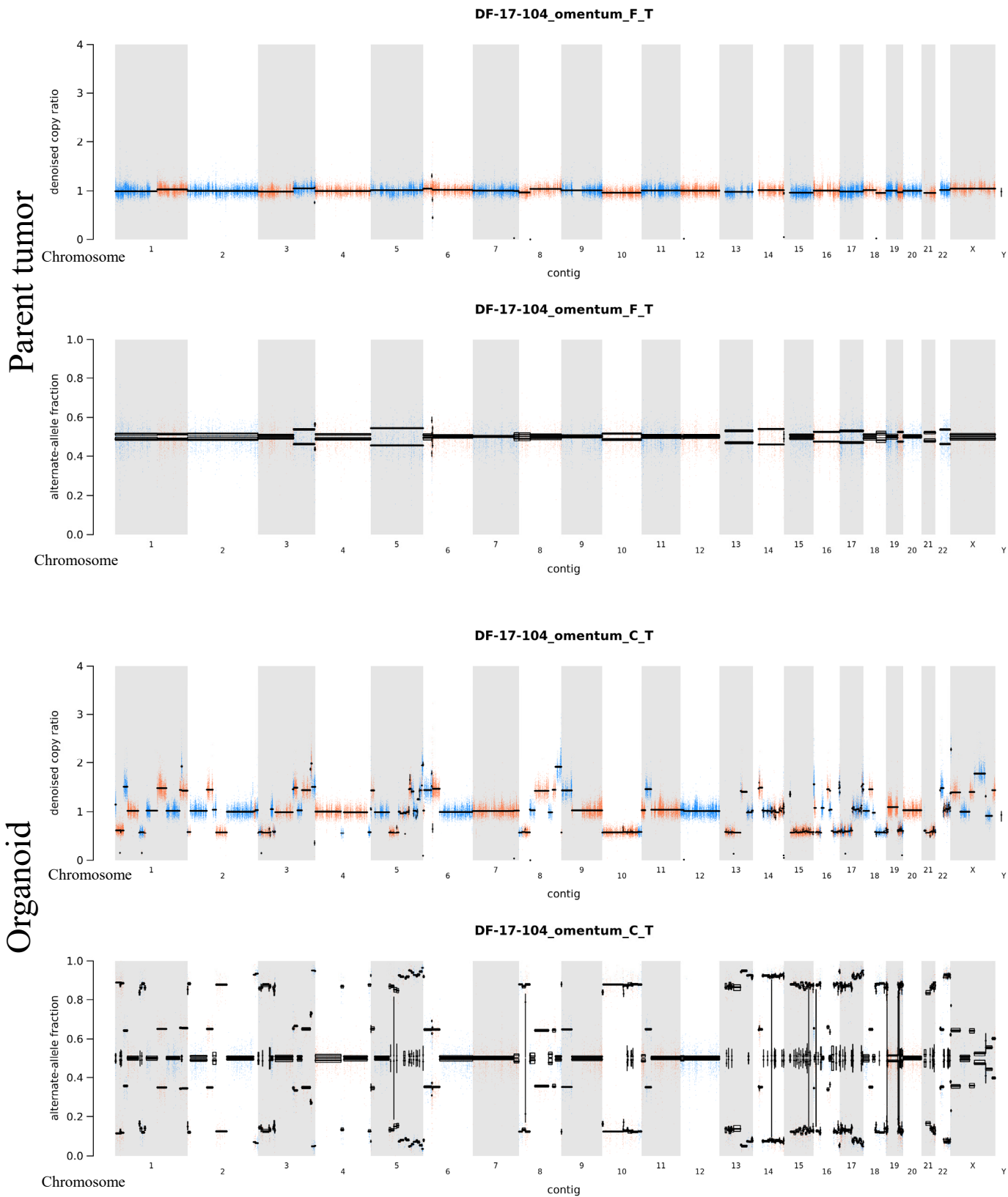


Figure S3 G

DF-17-107 Left Ovary Mass

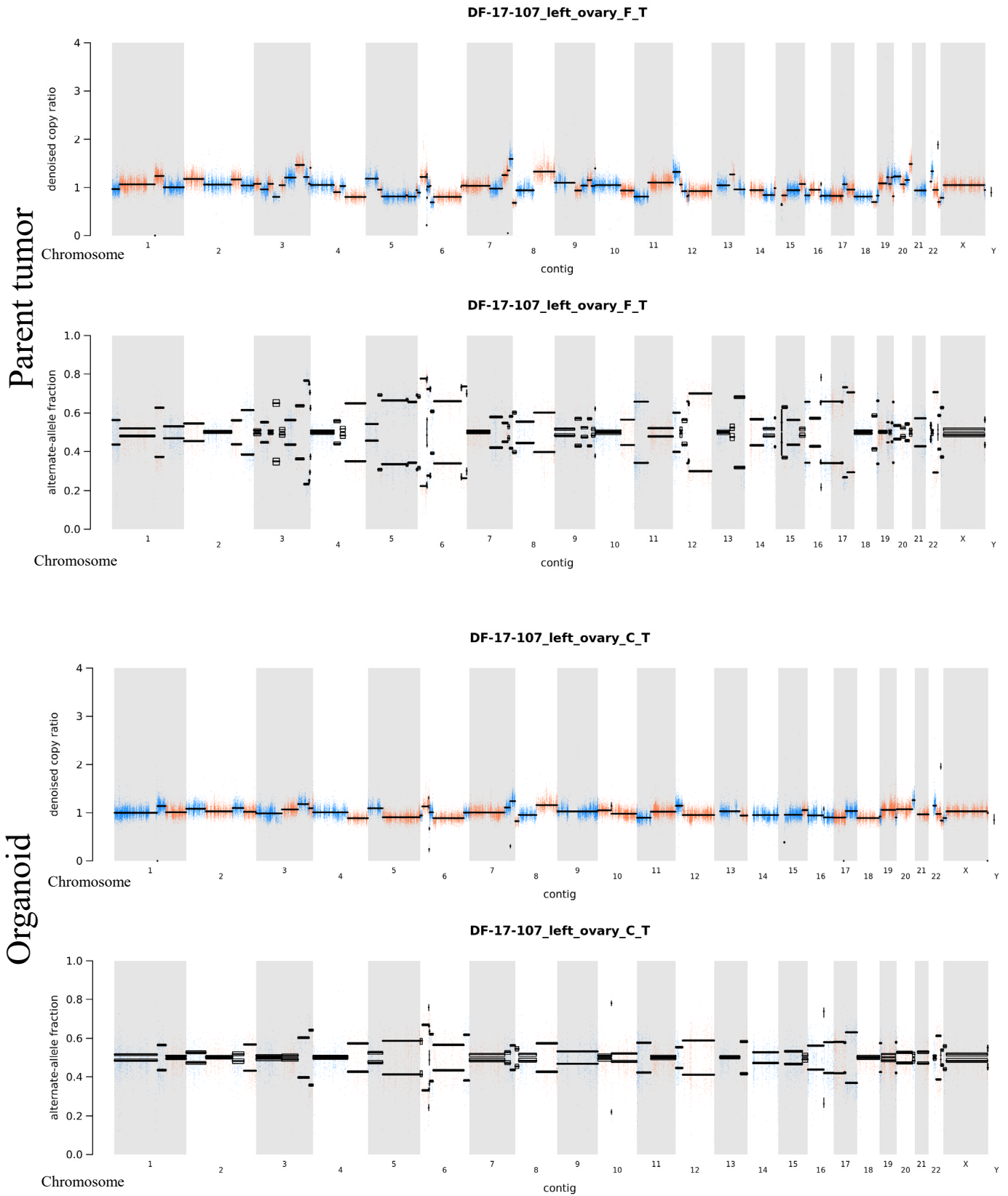


Figure S3 H

DF-17-107 Right Ovary Mass

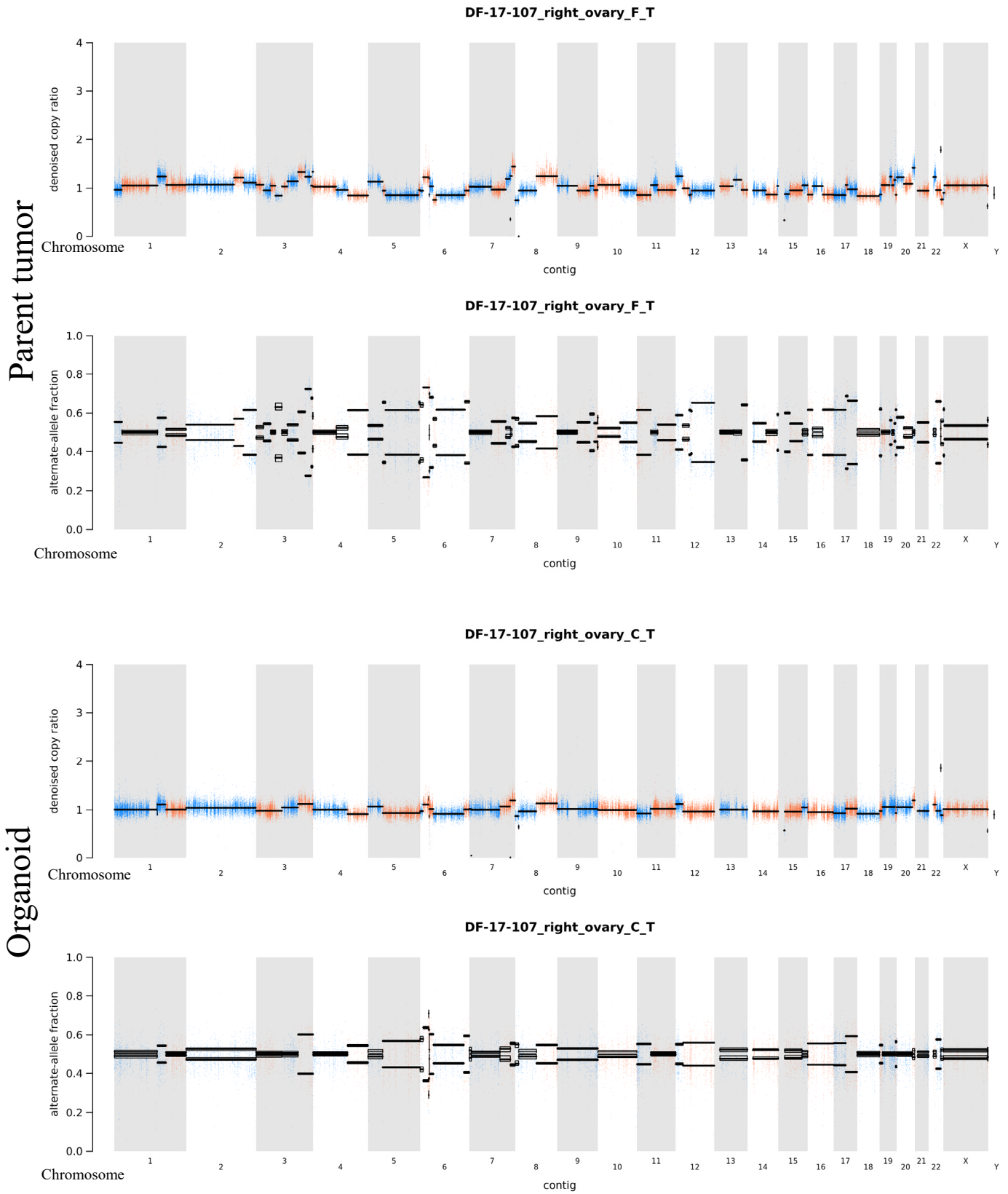


Figure S3 I

DF-17-107 Omental Metastasis

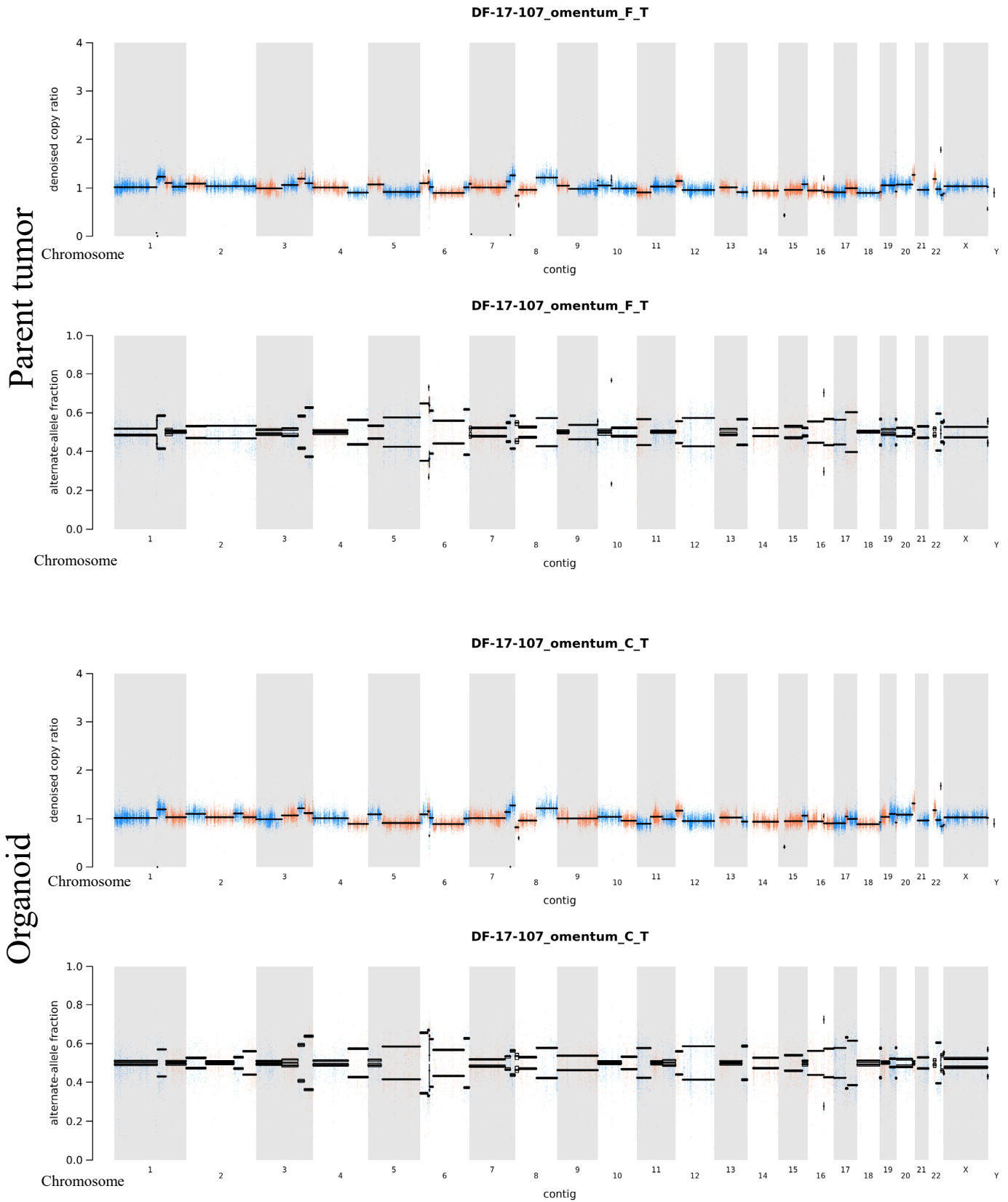


Figure S3 J

DF-17-115 Left Ovary Mass

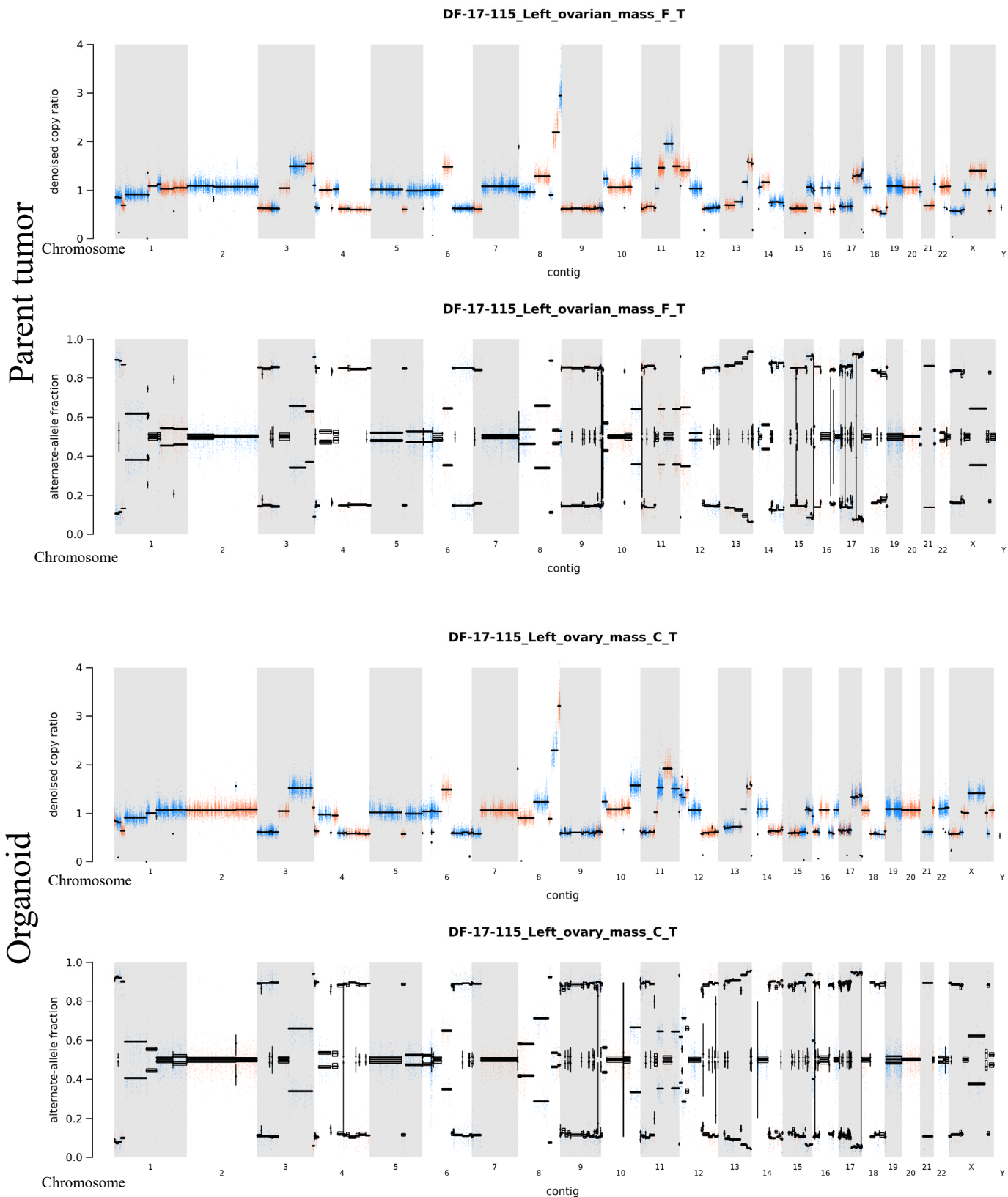


Figure S3 K

DF-17-116 Omental Metastasis

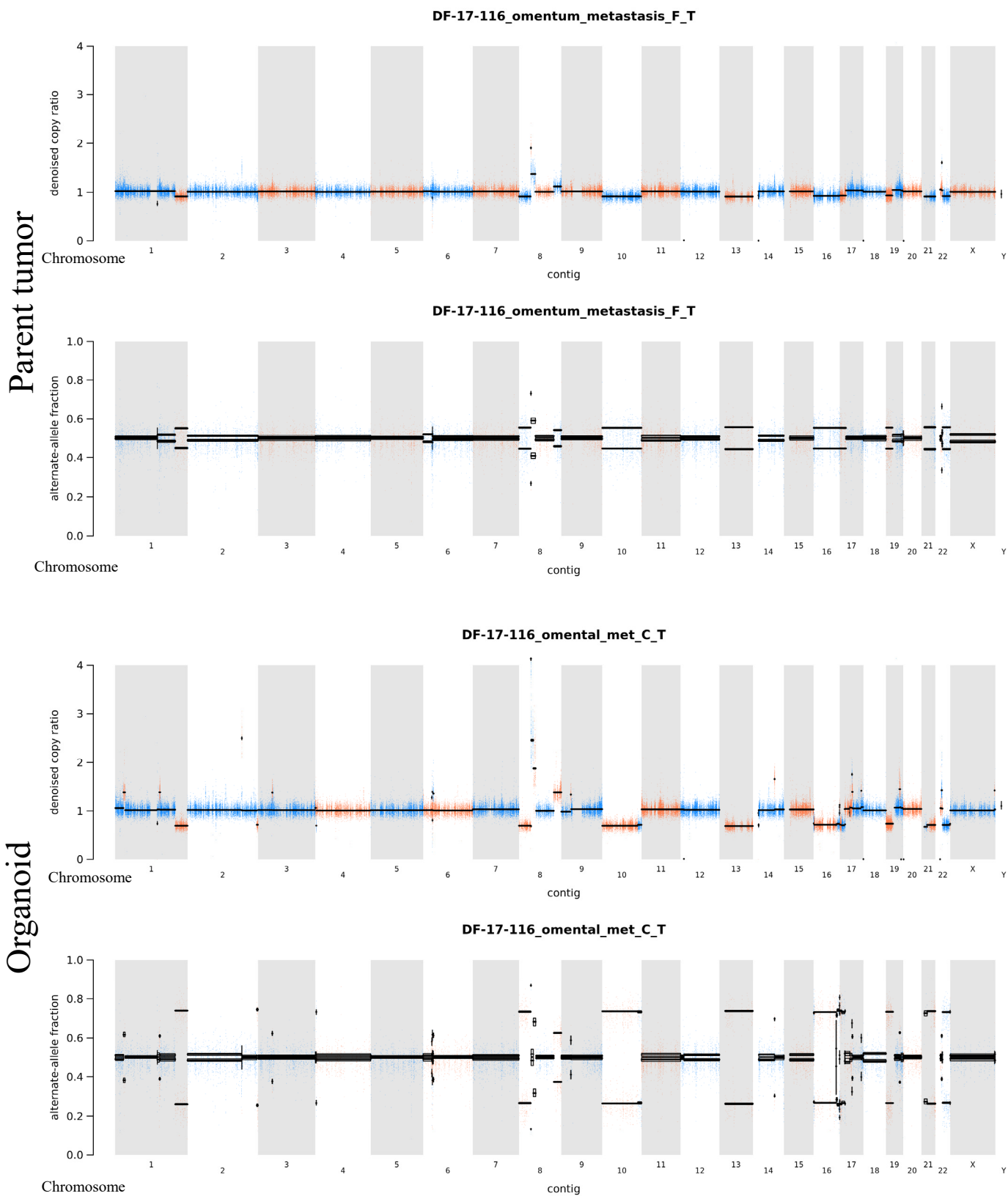


Figure S3 L

DF-17-121 Pleural Effusion

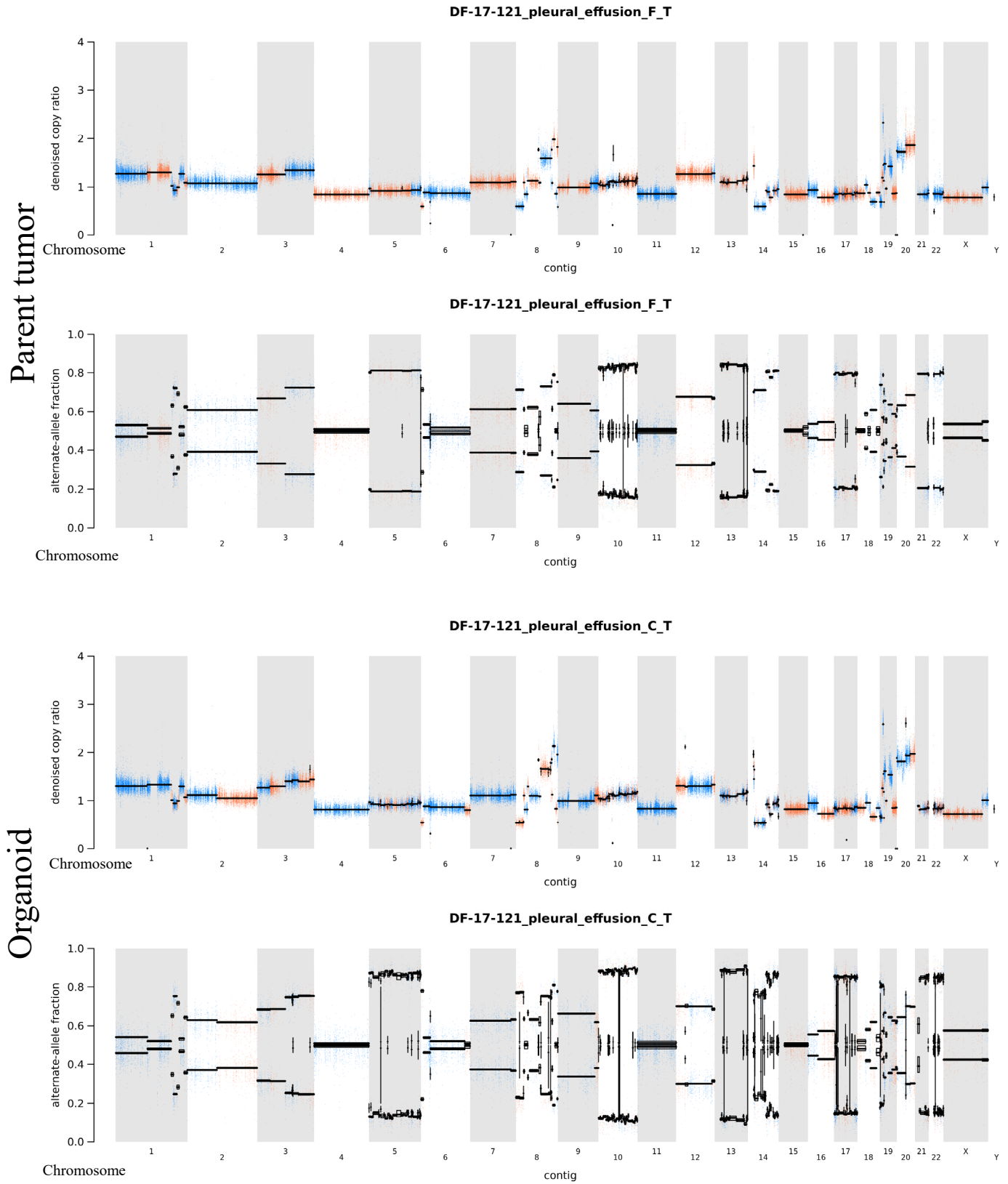


Figure S3 M

DF-17-123 Right Ovary Mass

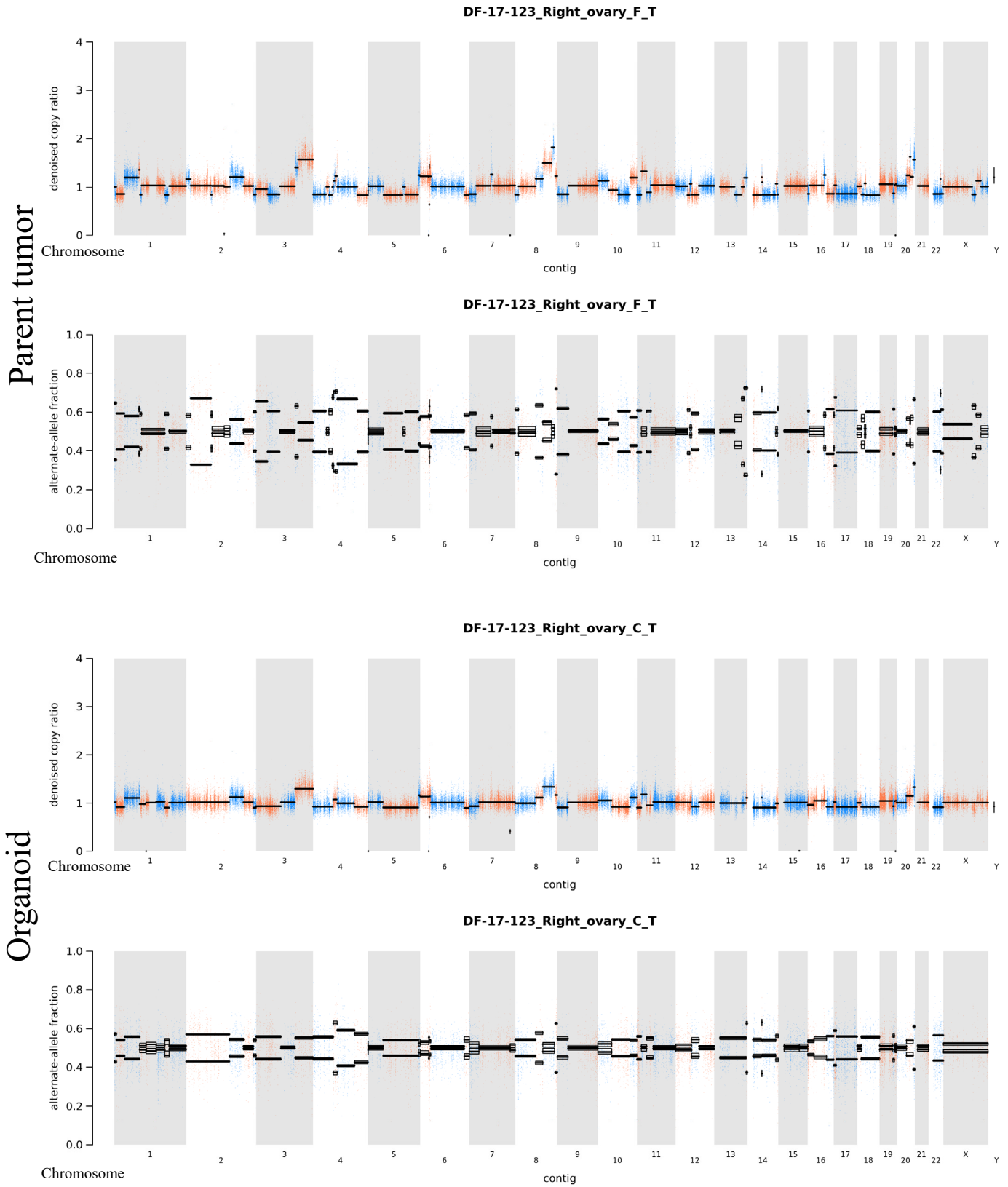


Figure S3 N

DF-17-123 Left Ovary Mass

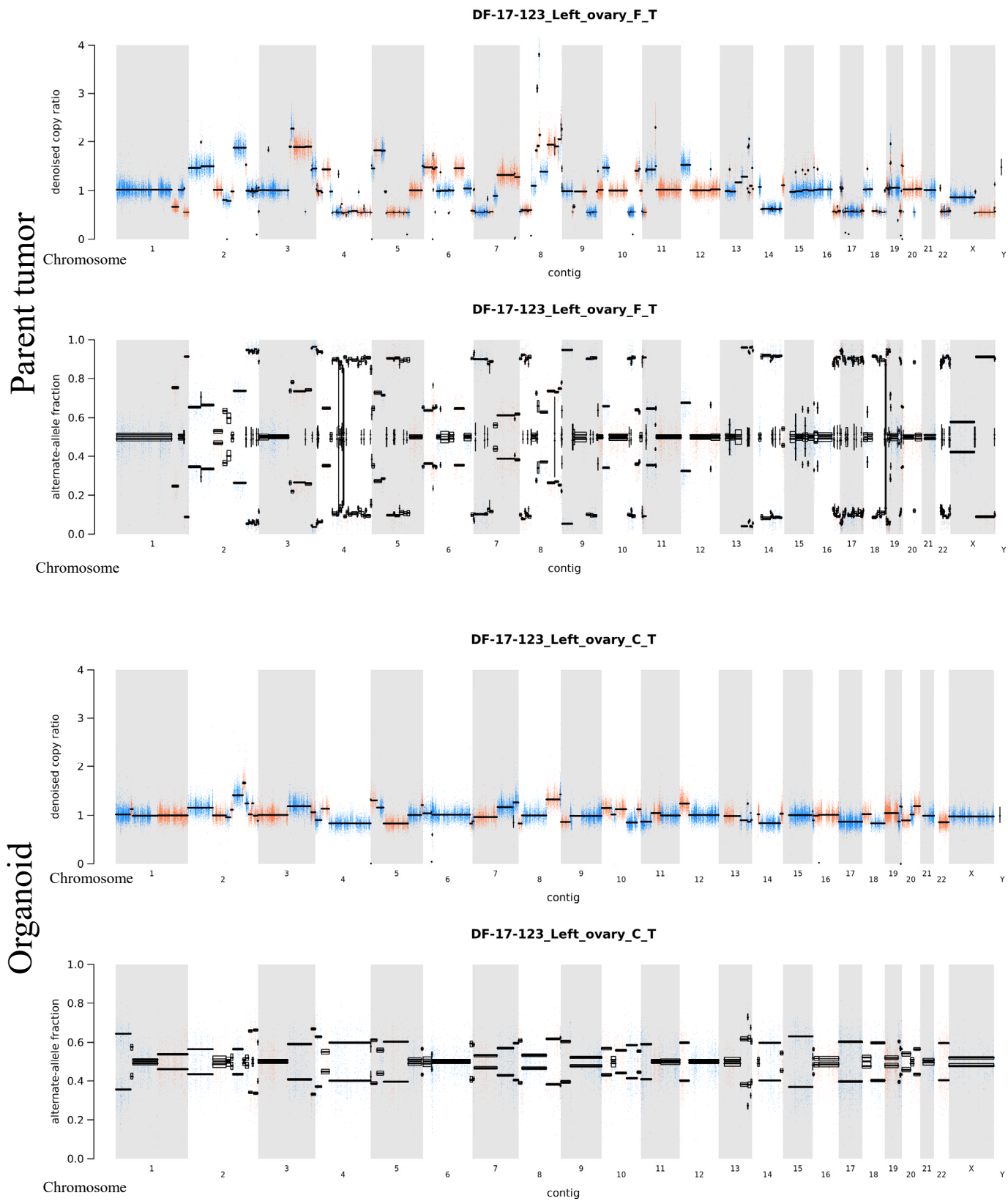


Figure S3 O

DF-17-123 Omental Metastasis

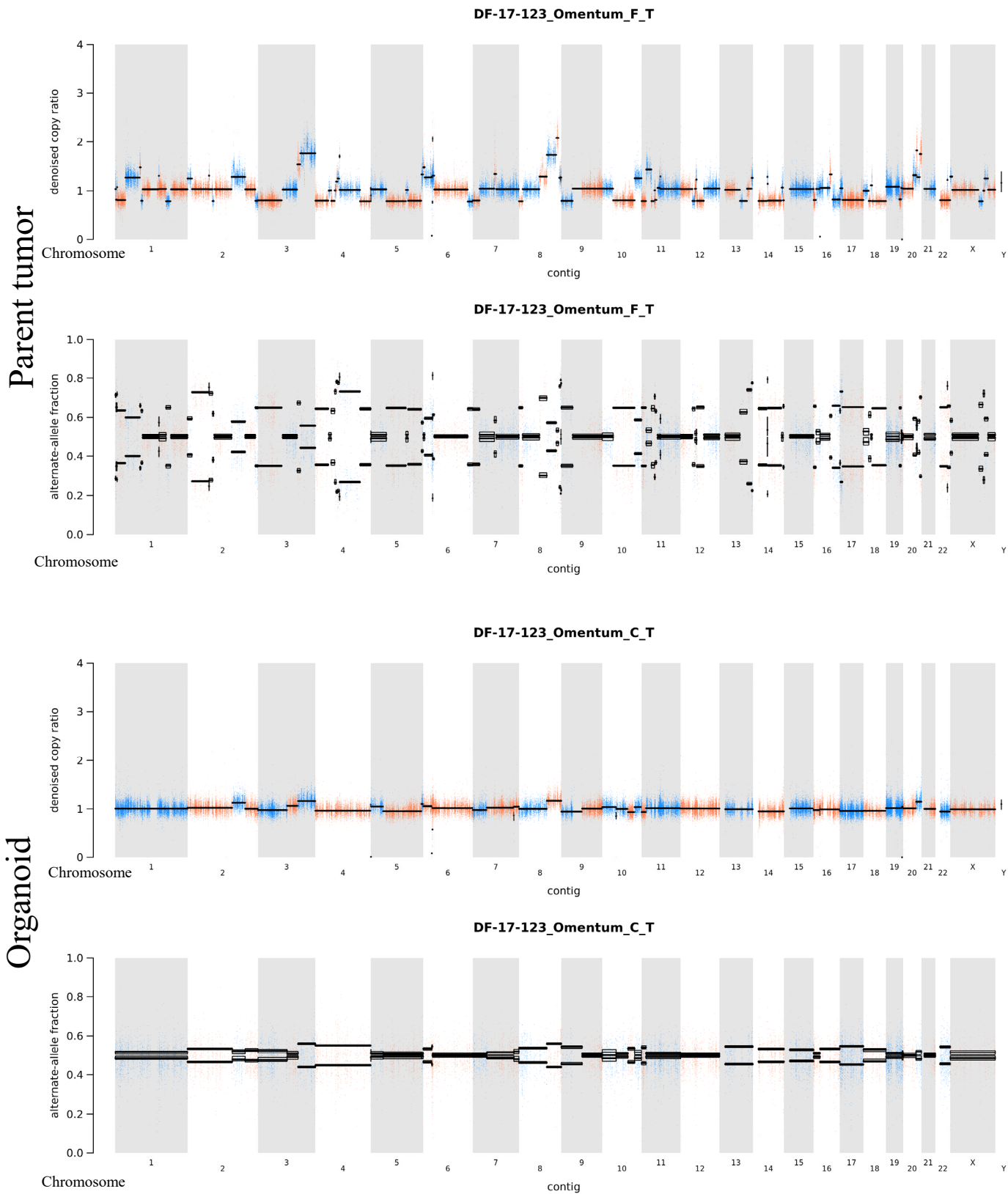


Figure S3 P

DF-17-126 Omental Metastasis

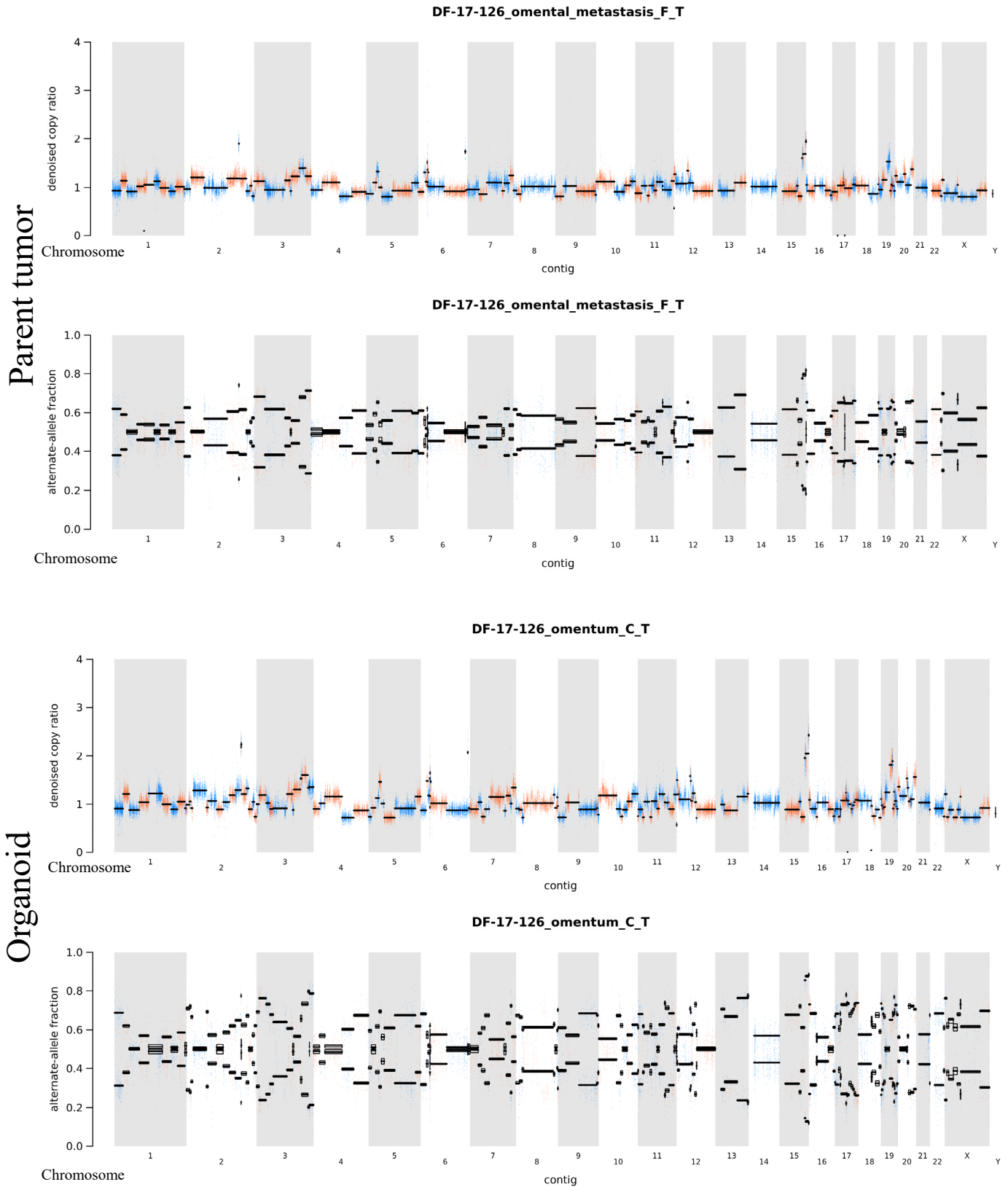


Figure S3 Q DF-17-132 Omental Metastasis Pre-Treatment

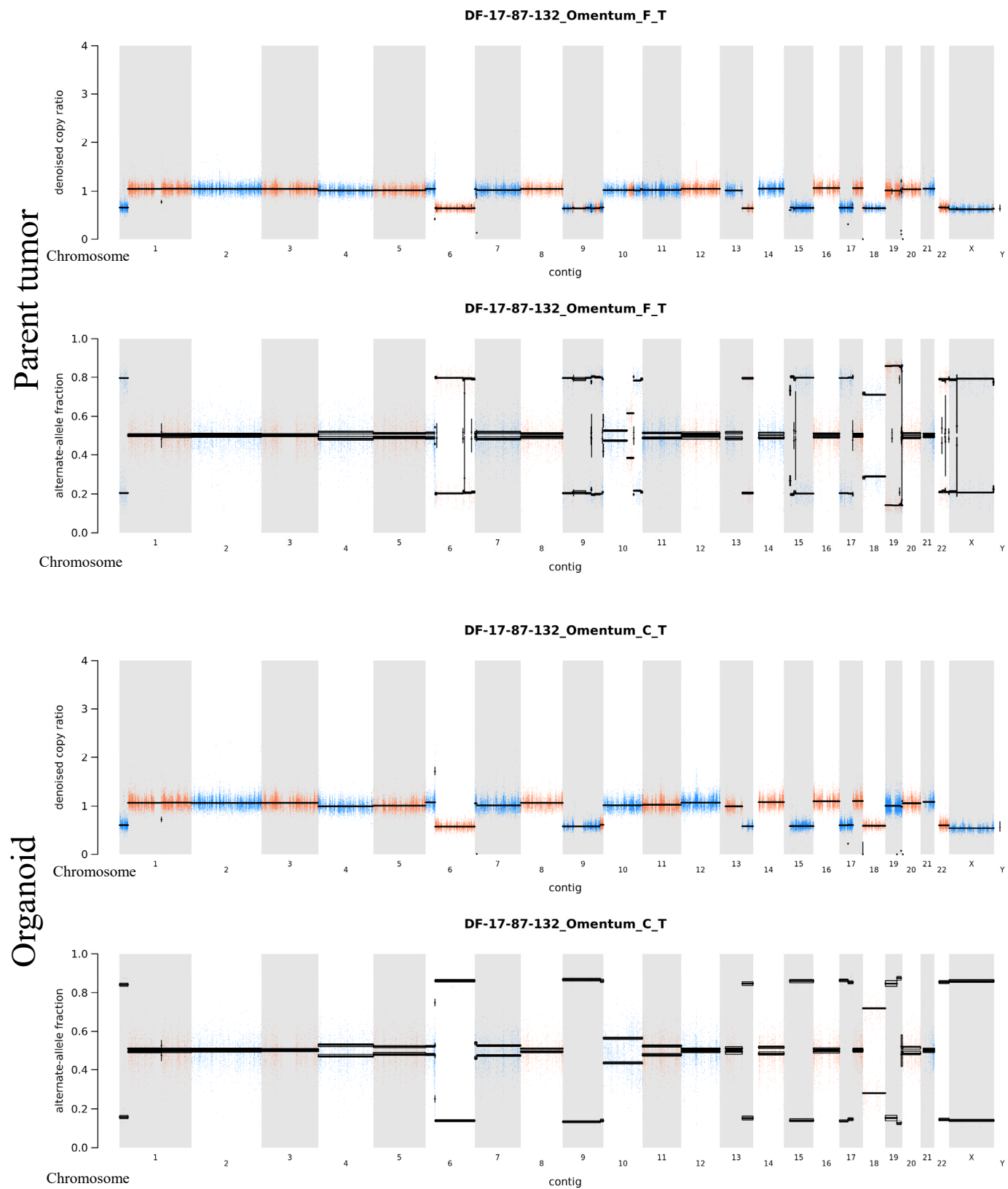
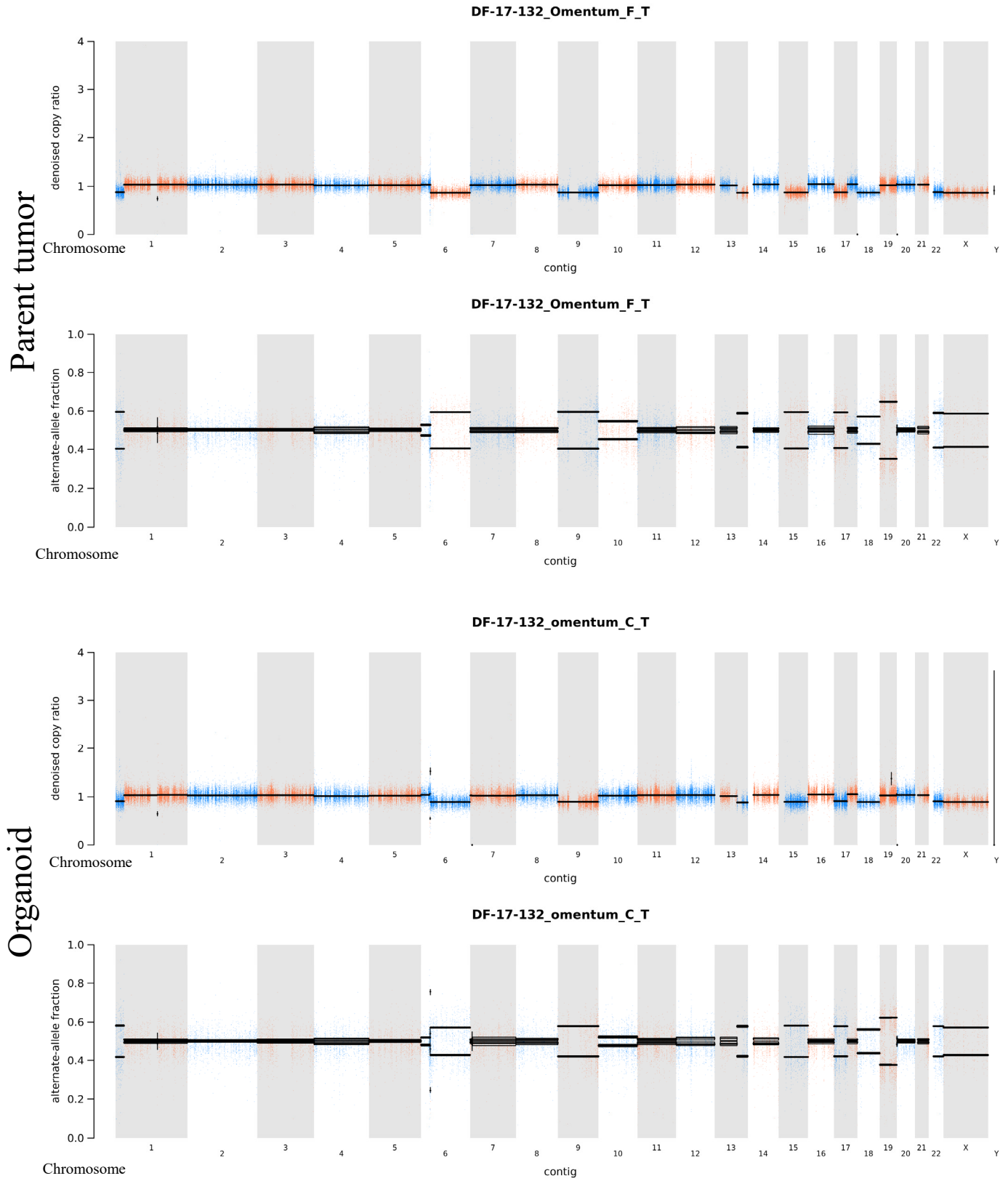


Figure S3 R DF-17-132 Omental Metastasis Post-Neoadjuvant Therapy



DF-17-132 Cecal Mesentery Metastasis Post-Neoadjuvant Therapy

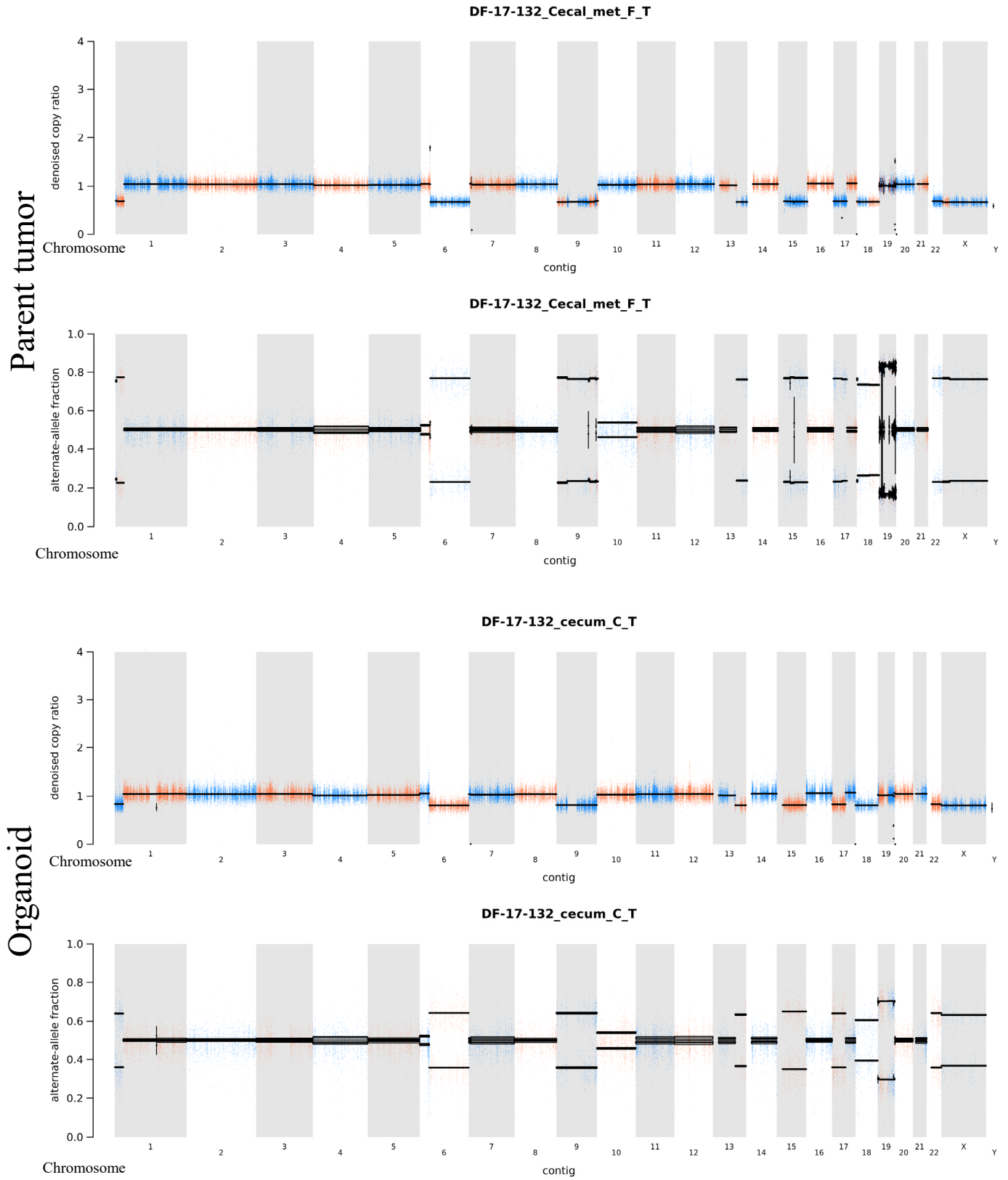


Figure S3 S

Figure S3 T

DF-17-134 Left Ovary Mass

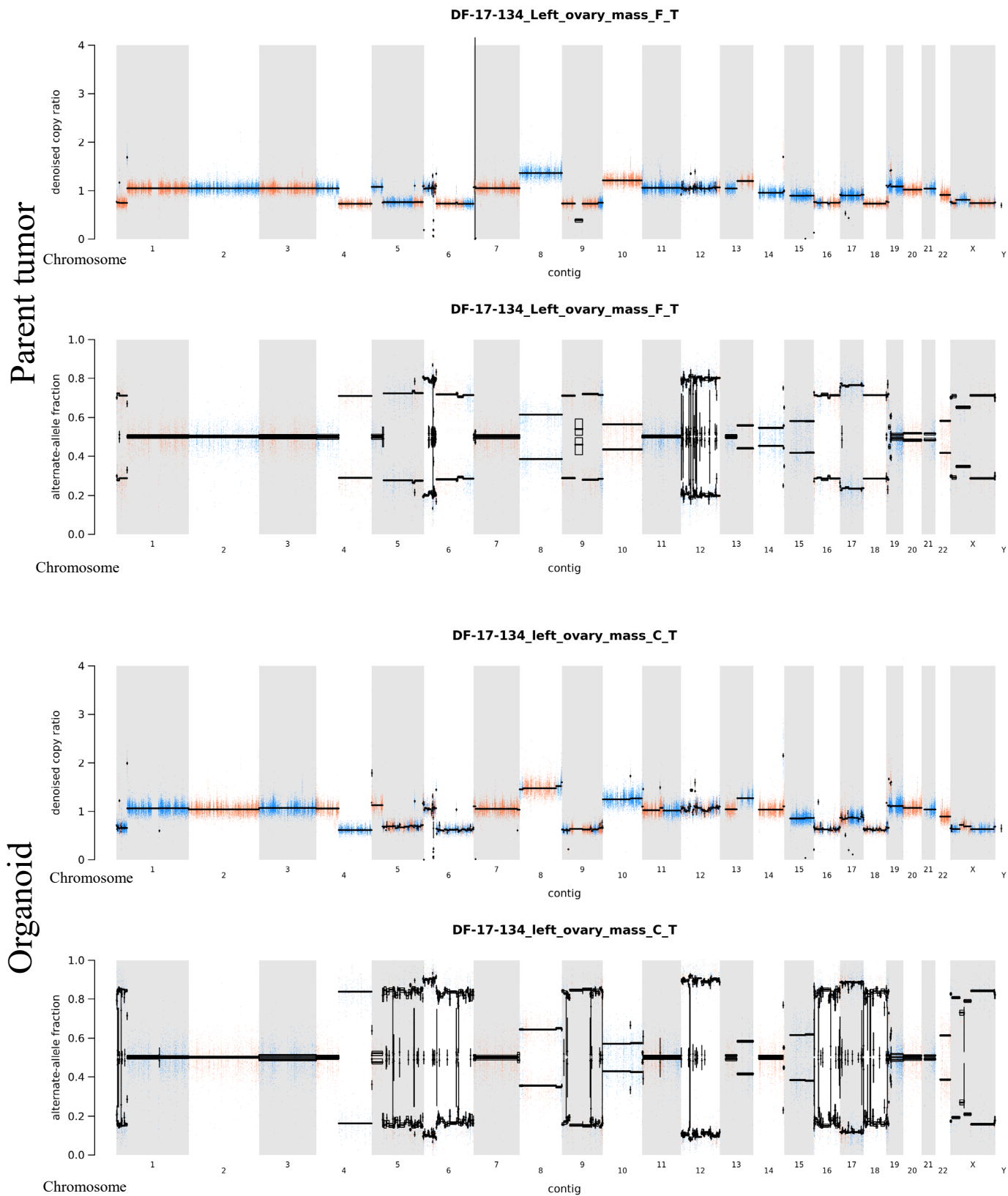


Figure S3 U

DF-17-134 Right Ovary Mass

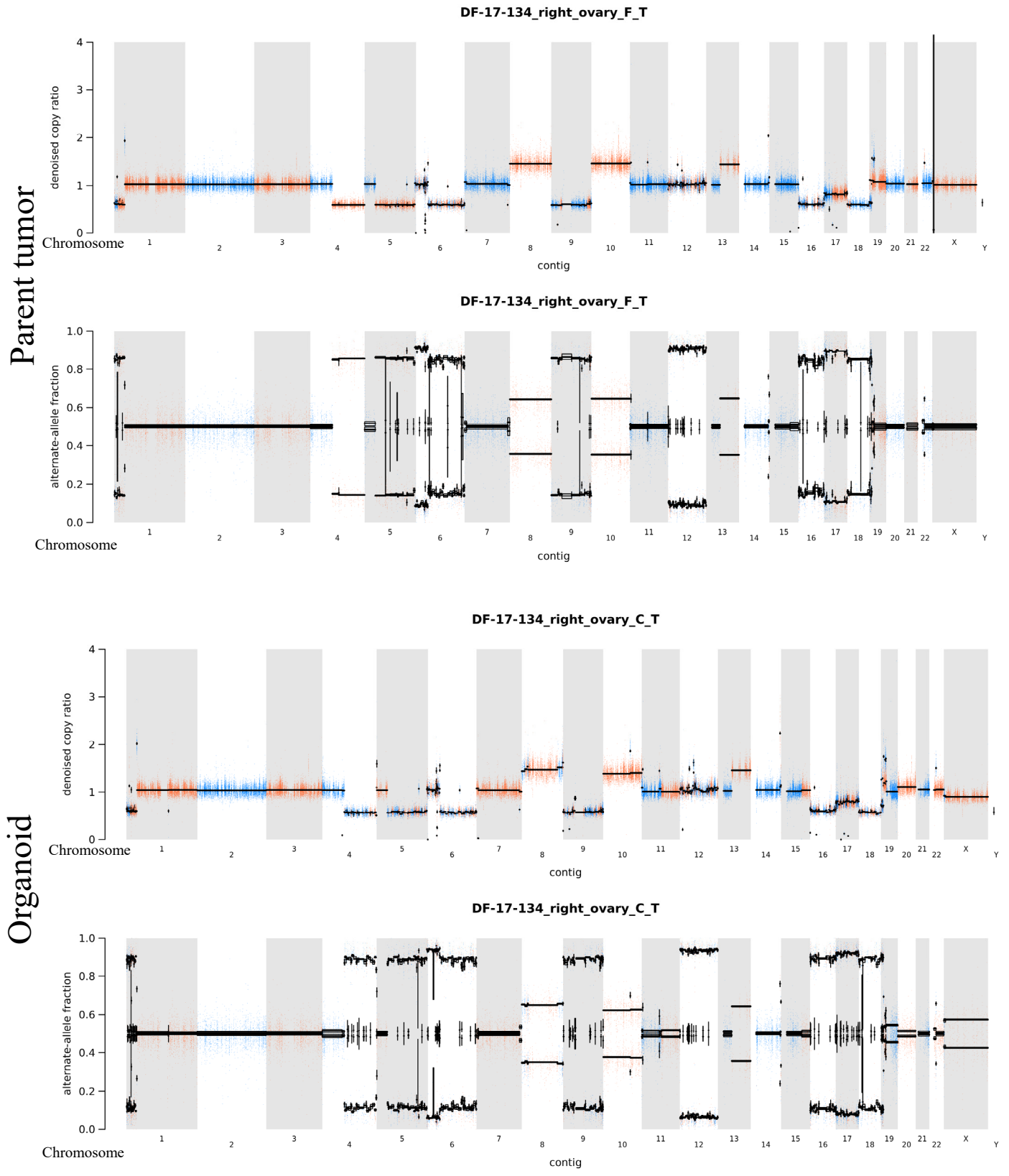


Figure S3 V

DF-18-1 Omental metastasis

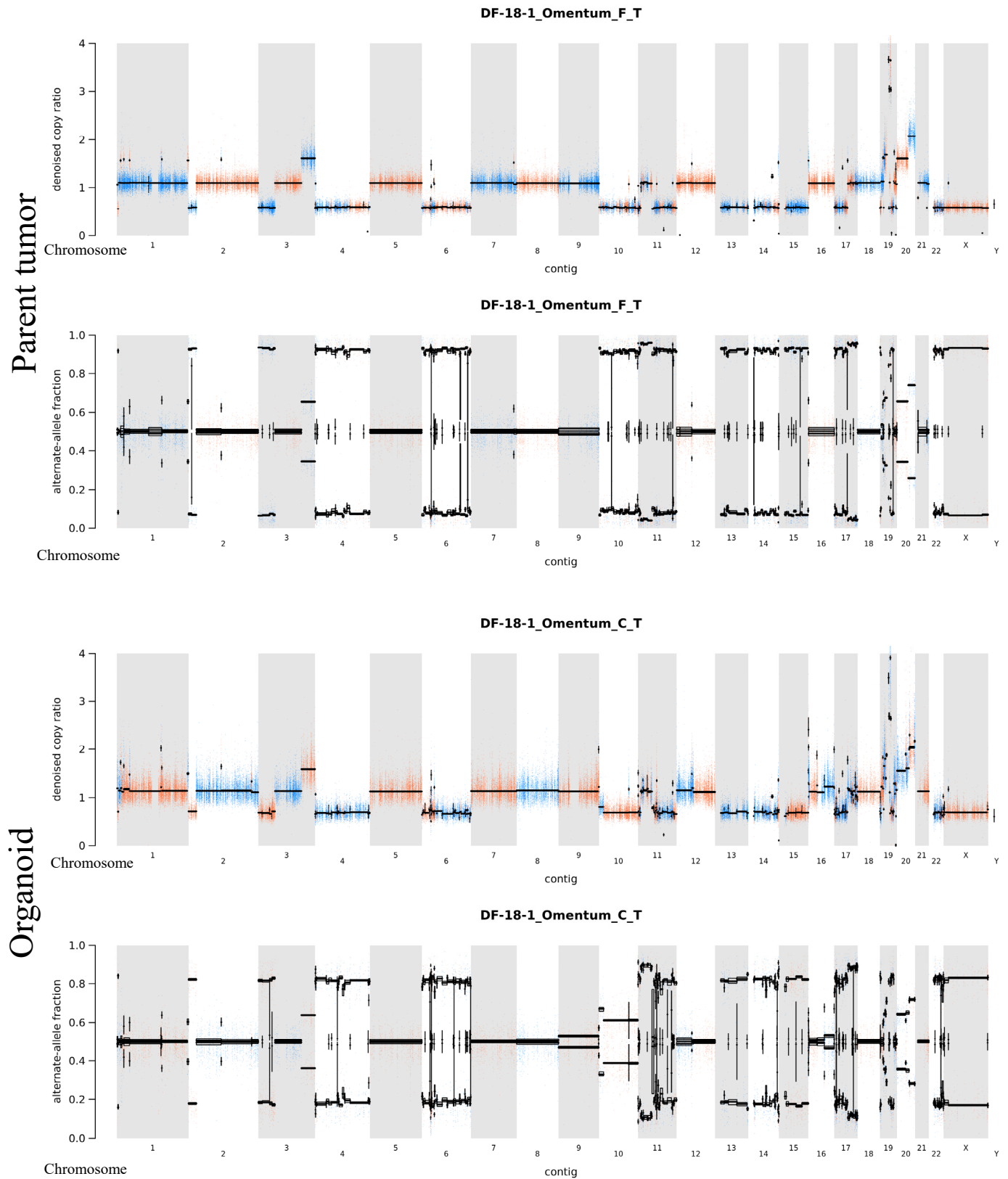


Figure S3 W

DF-18-7 Right ovary mass

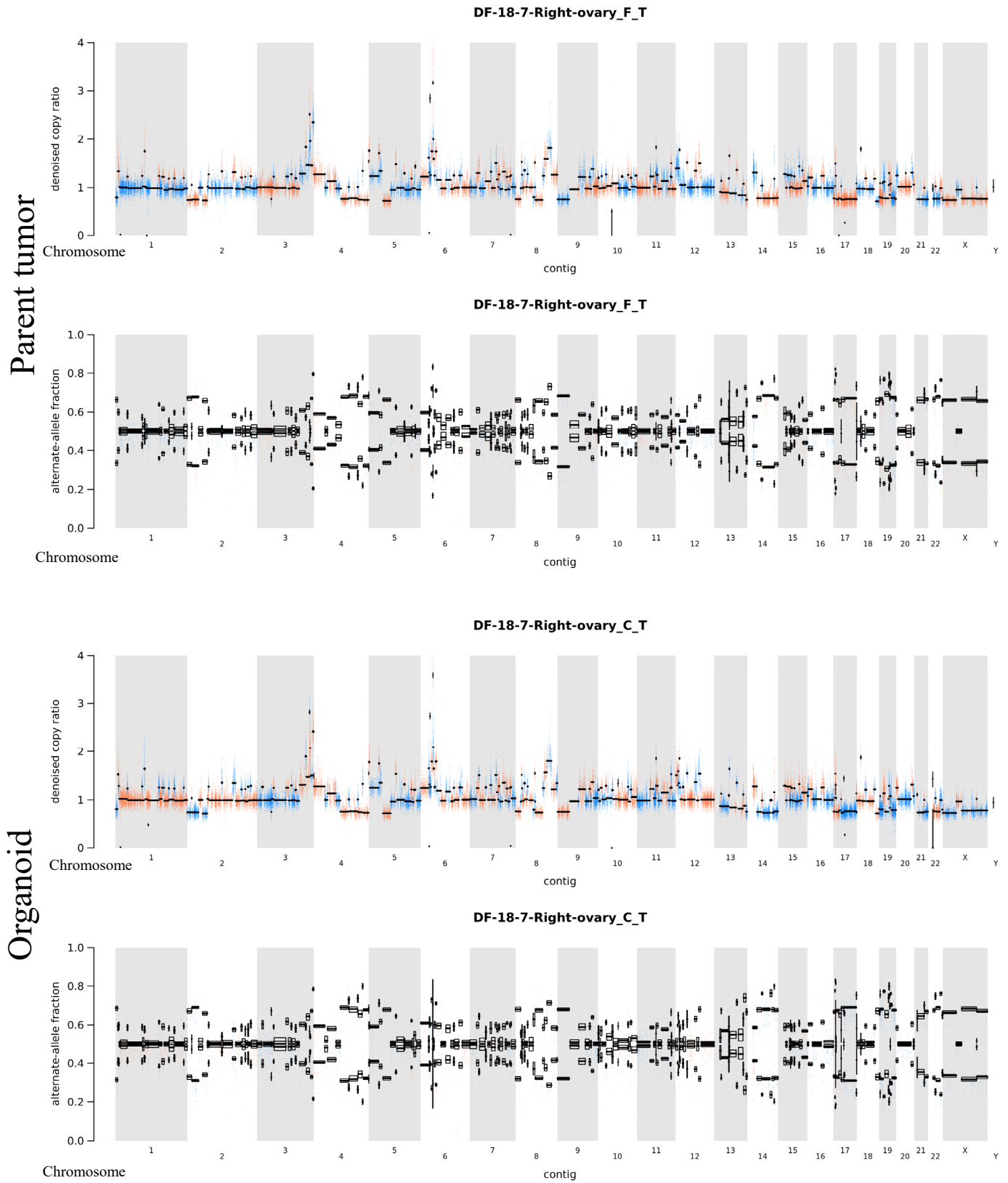


Figure S3 X

DF-18-8 Colonic mesentery metastasis

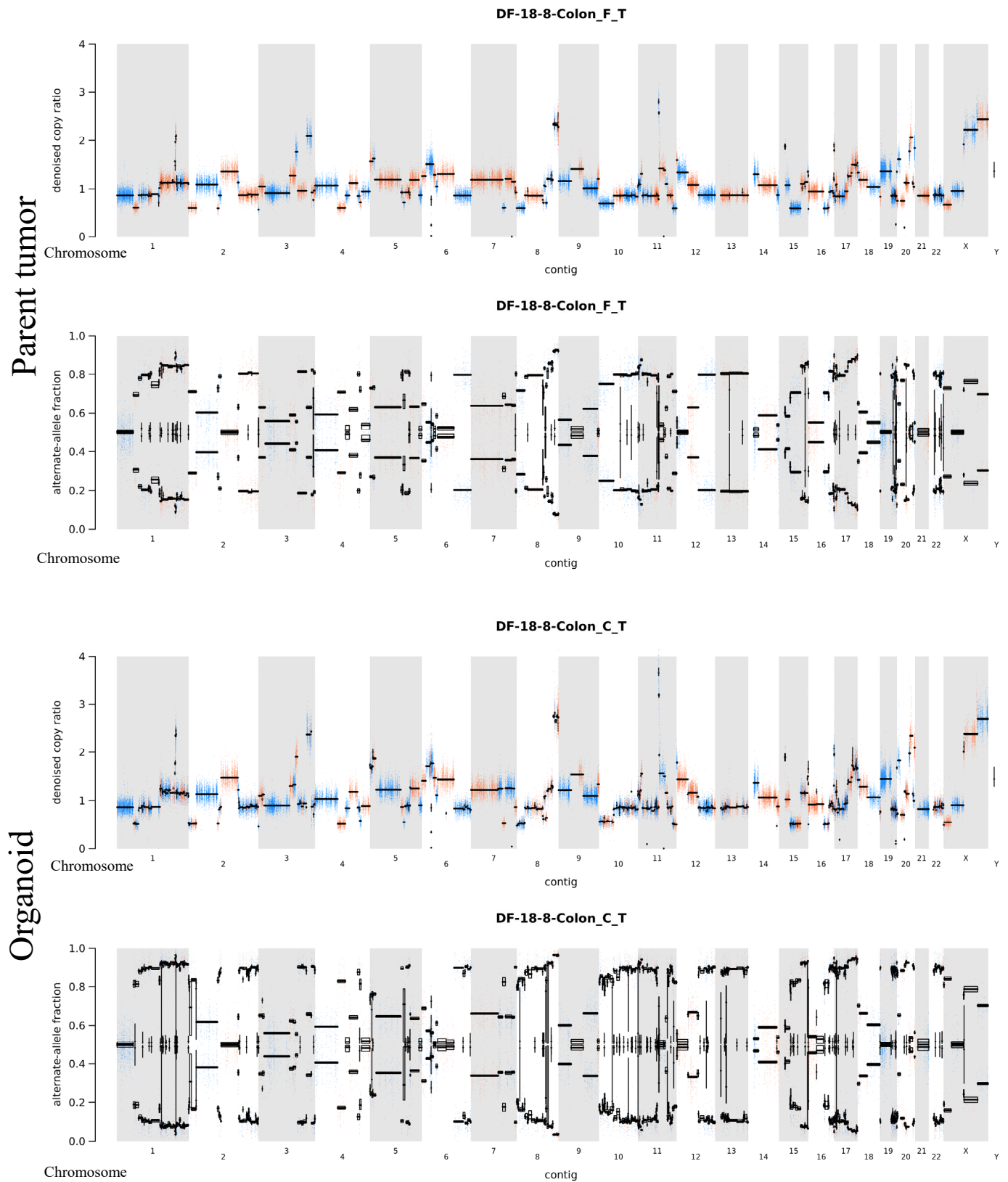


Figure S3 Y

DF-18-12 Left ovary mass

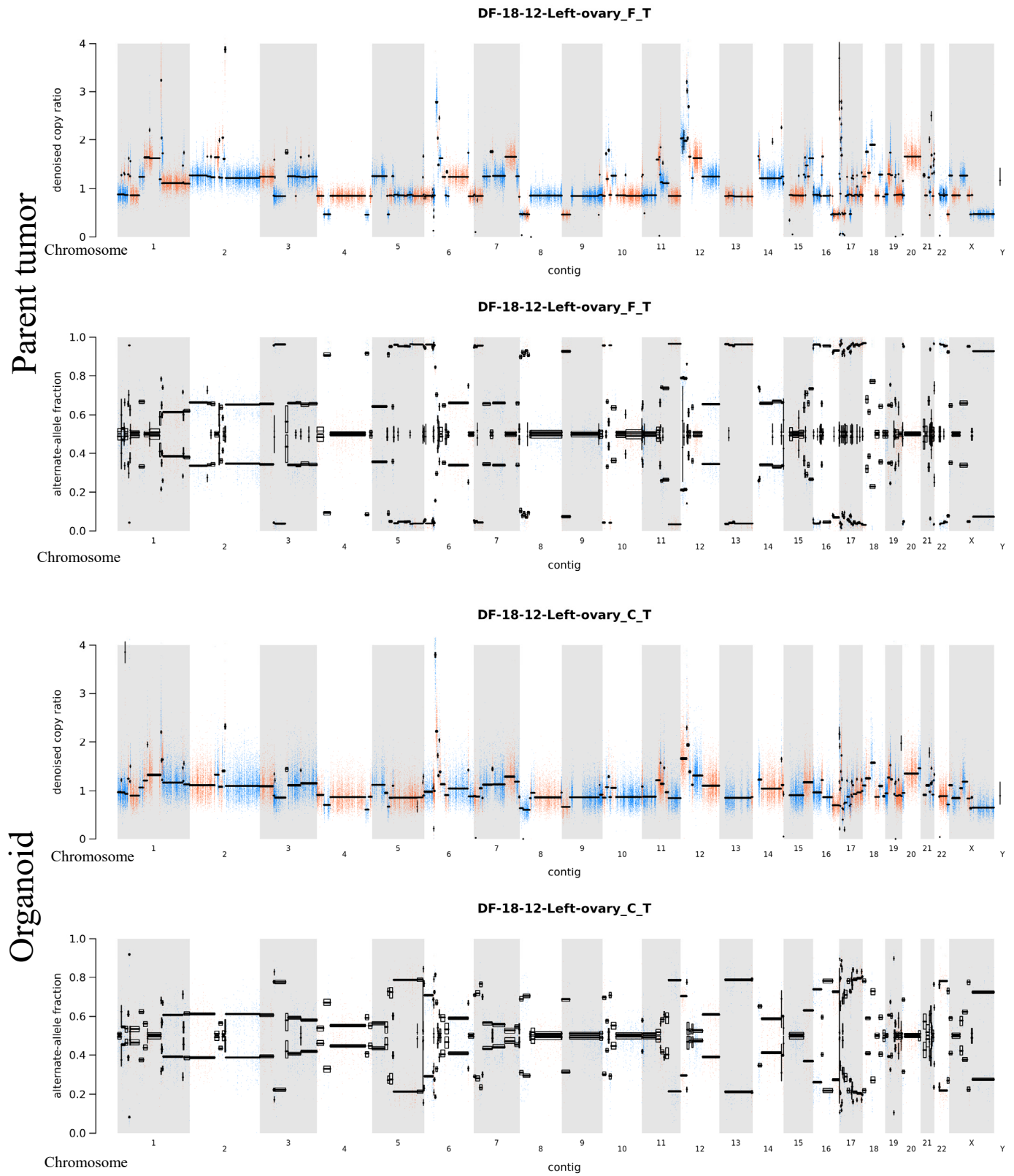


Figure S3 Z

DF-18-17 Omental metastasis

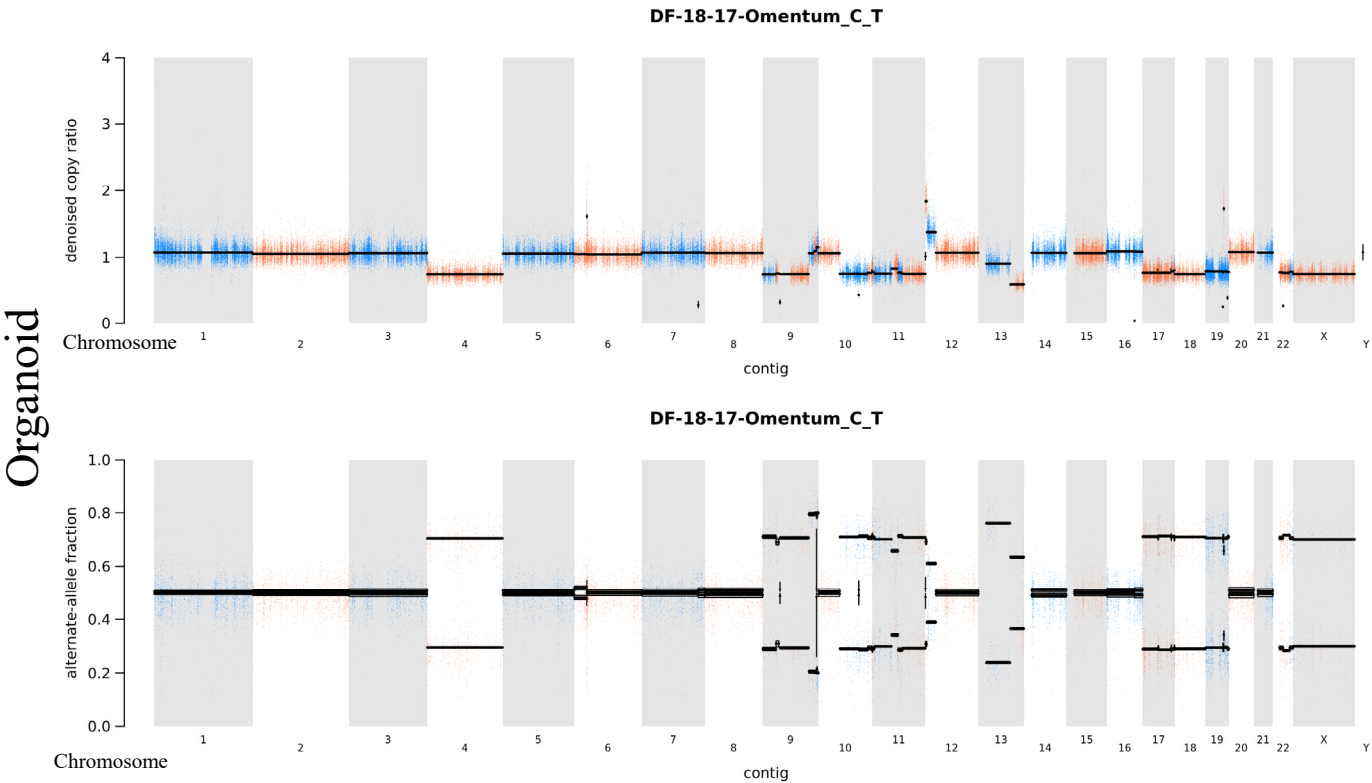


Figure S3 AA

DF-18-23 Right ovary mass

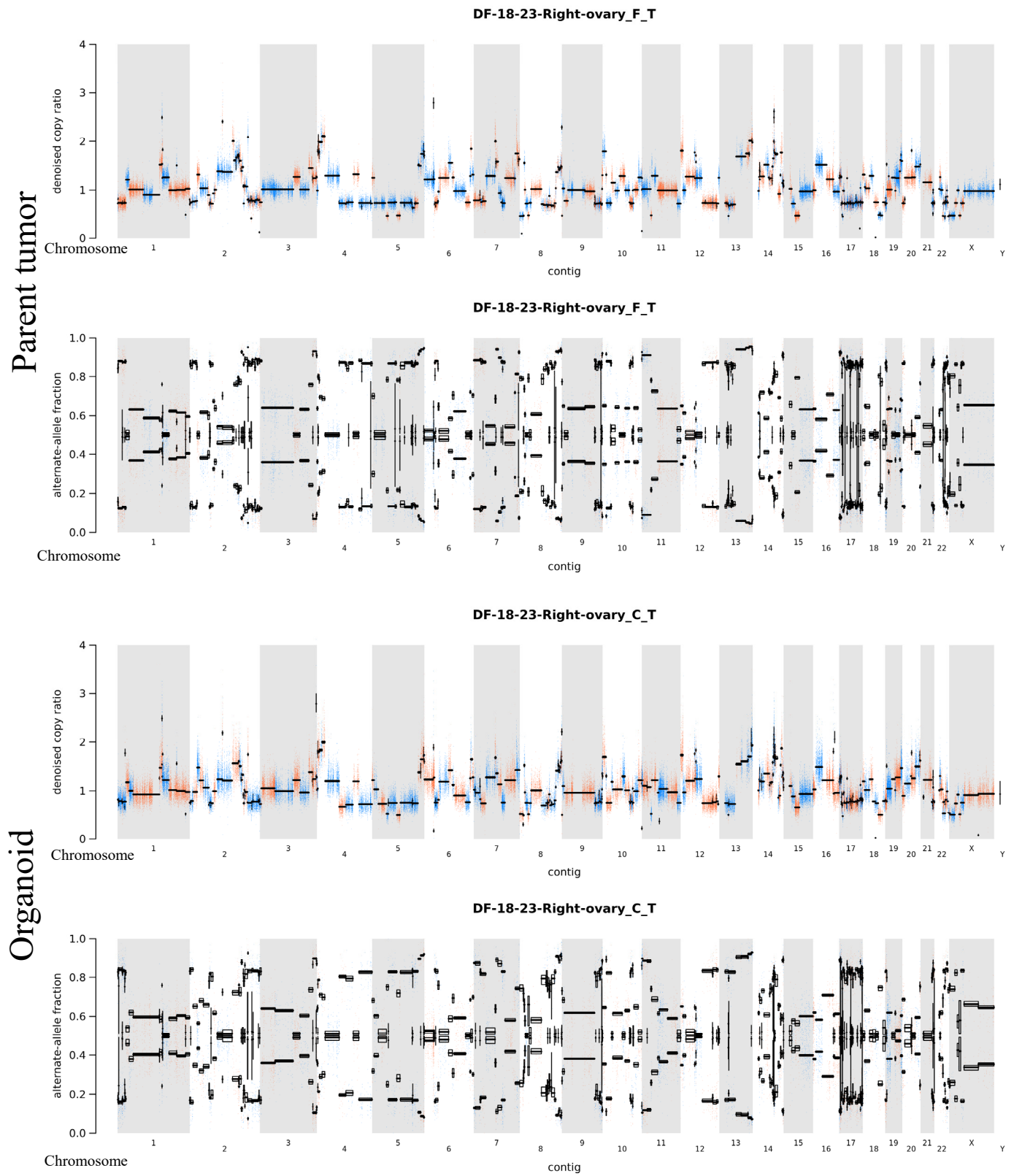


Figure S3 BB

DF-18-30 Left ovary mass

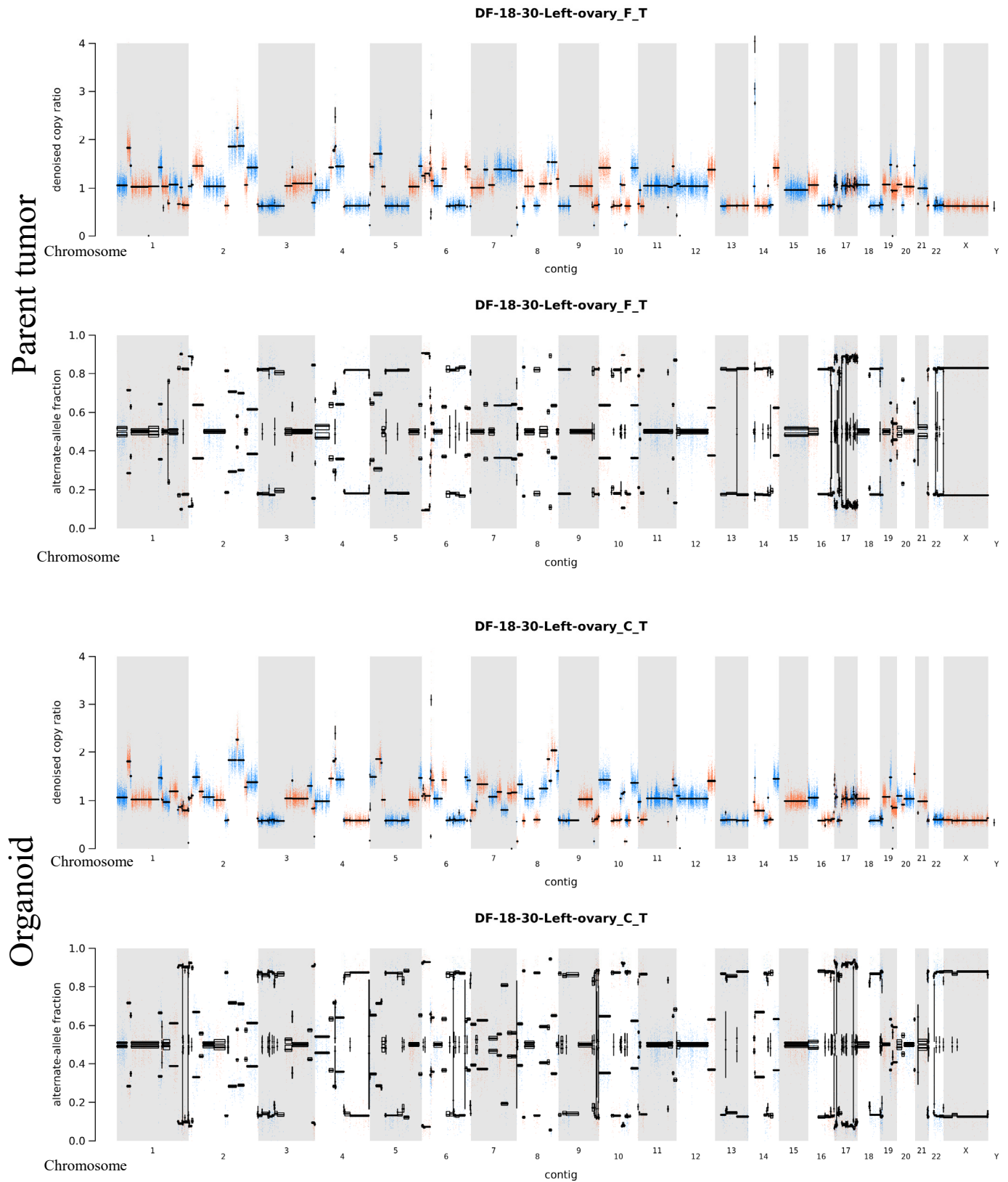


Figure S3 CC

DF-18-43 Left Ovary Mass

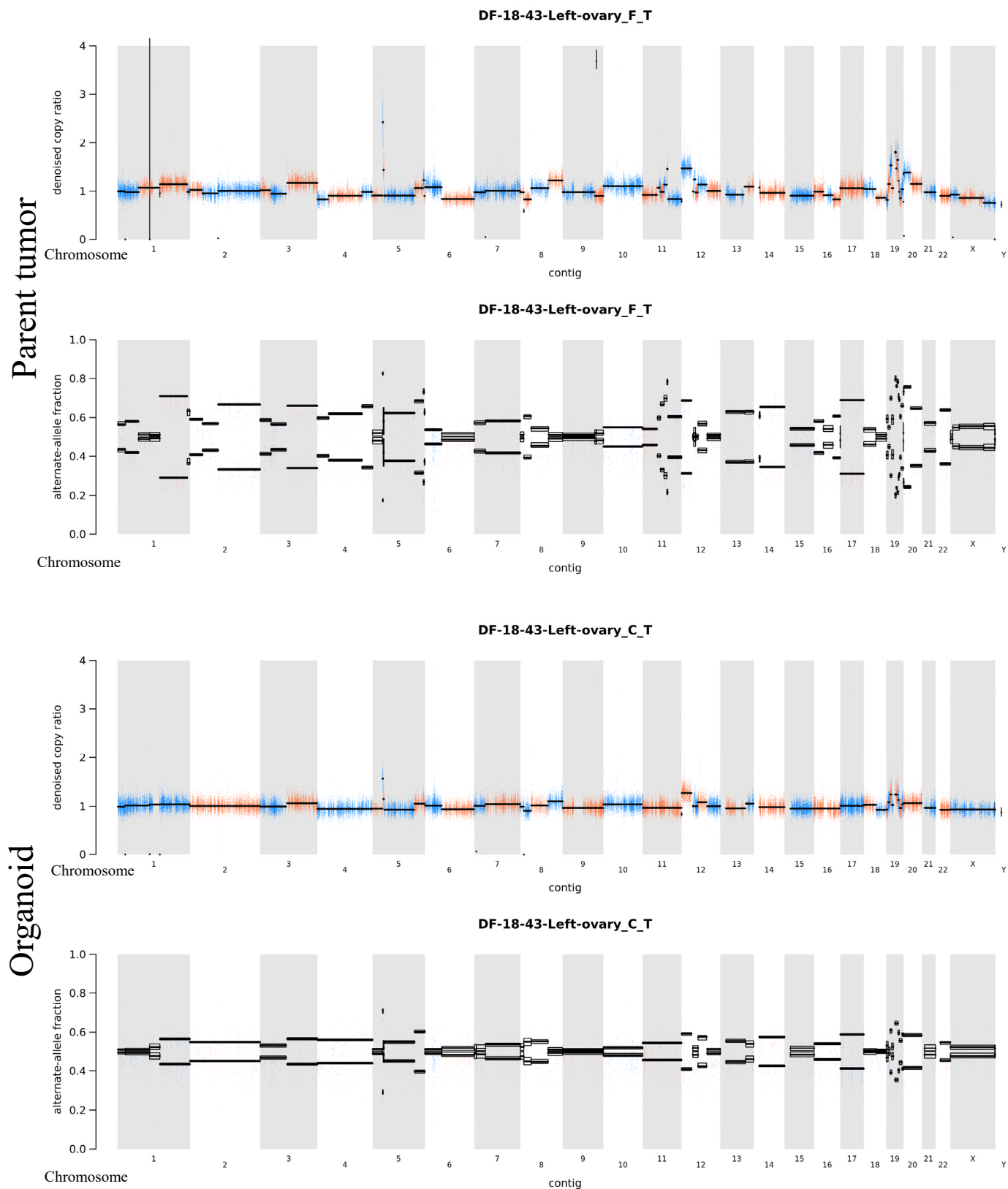


Figure S3 DD

DF-18-43 Omental Metastasis

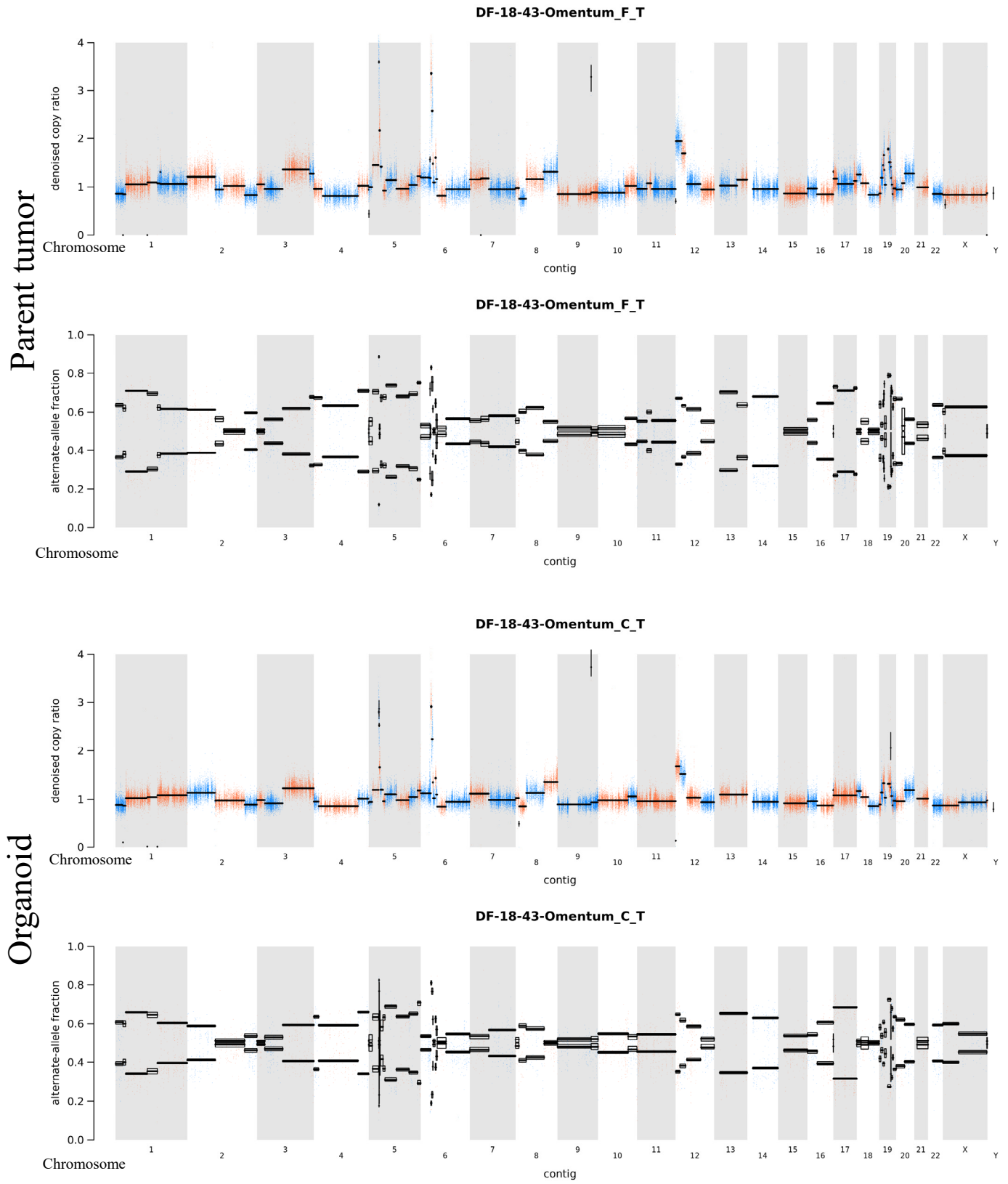


Figure S3 EE

DF-18-47 Omental metastasis

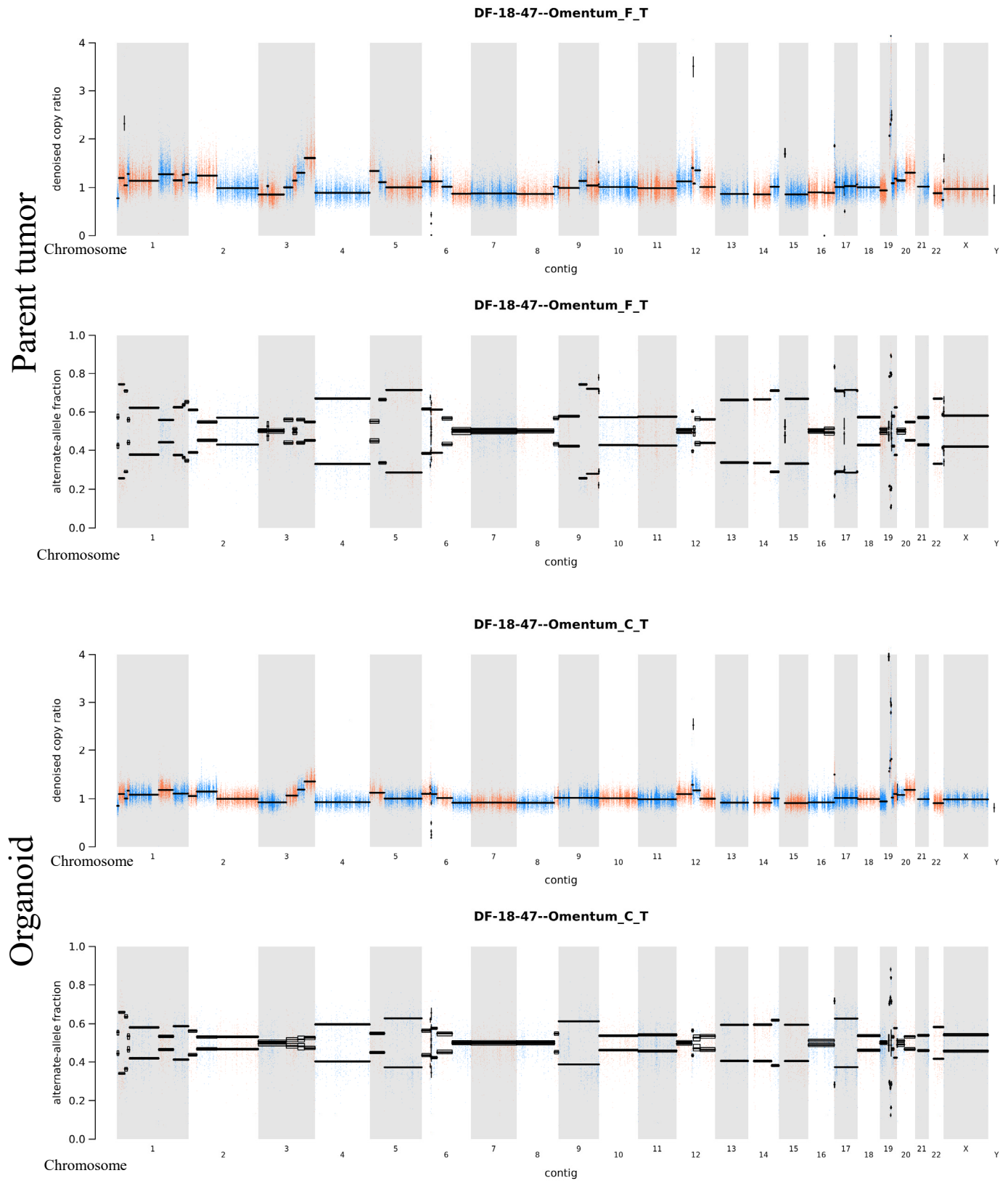


Figure S3 FF

DF-18-48 Omental metastasis

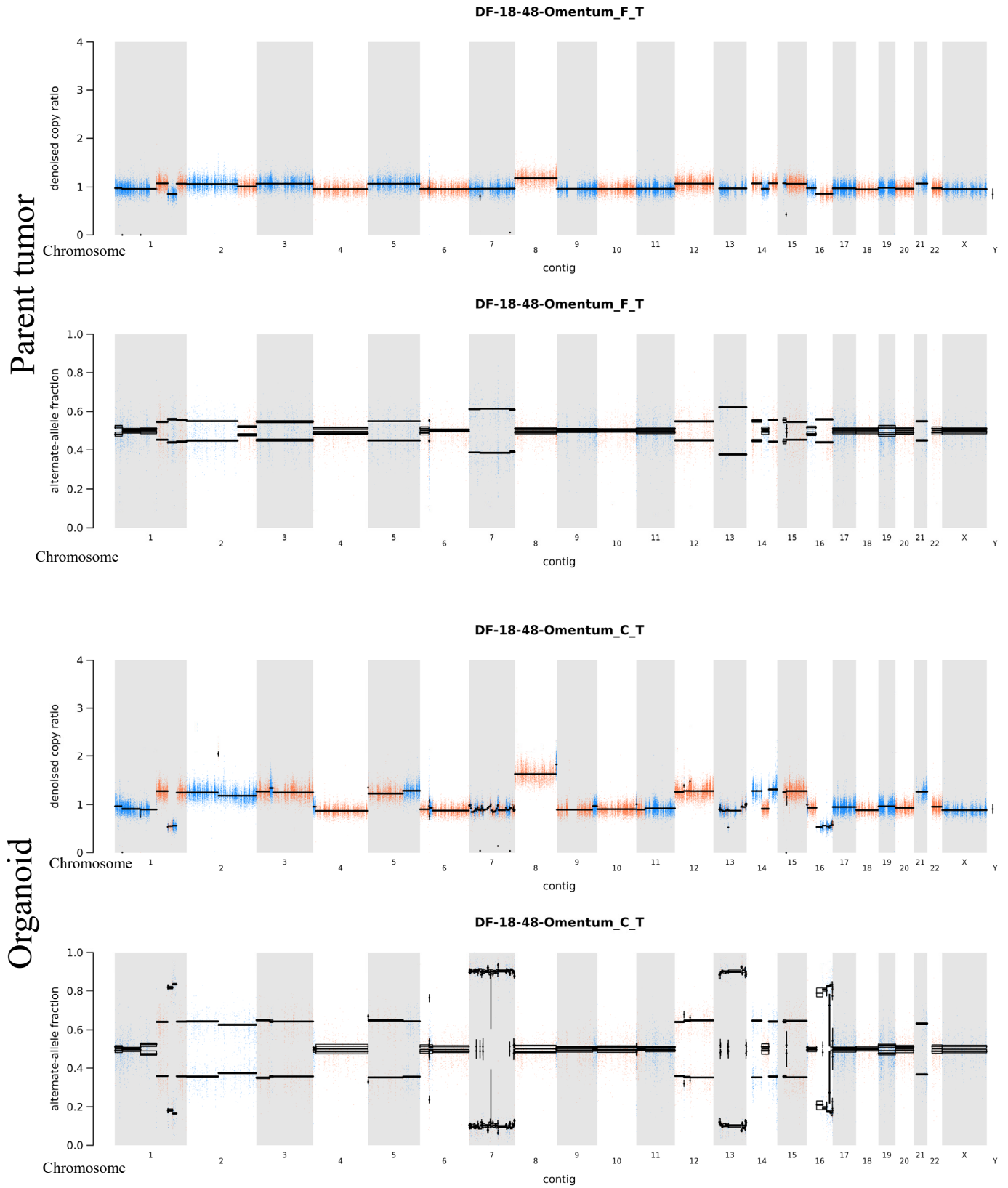


Figure S3 GG

DF-18-50

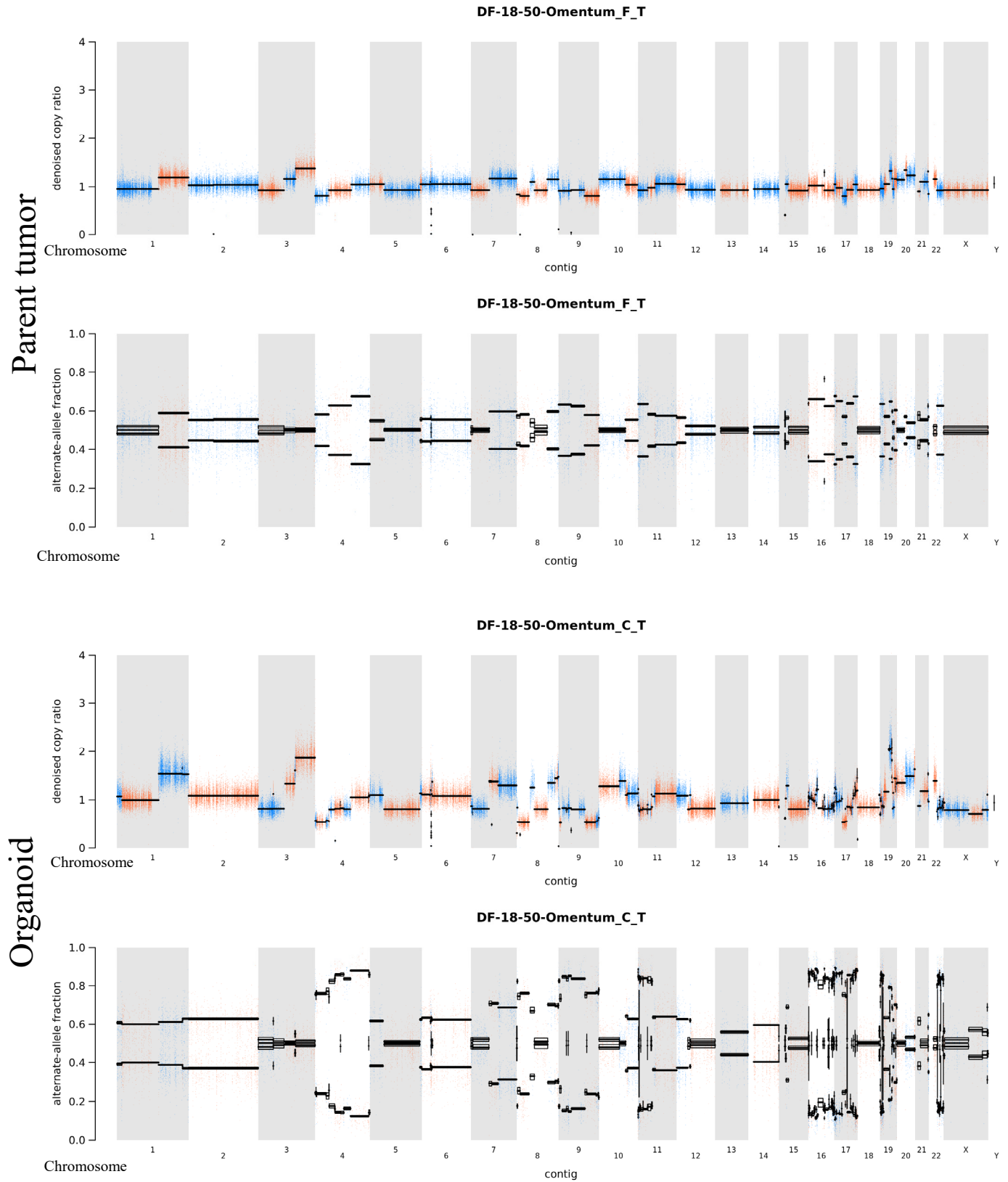


Figure S3 HH

DF-18-54 Omental metastasis

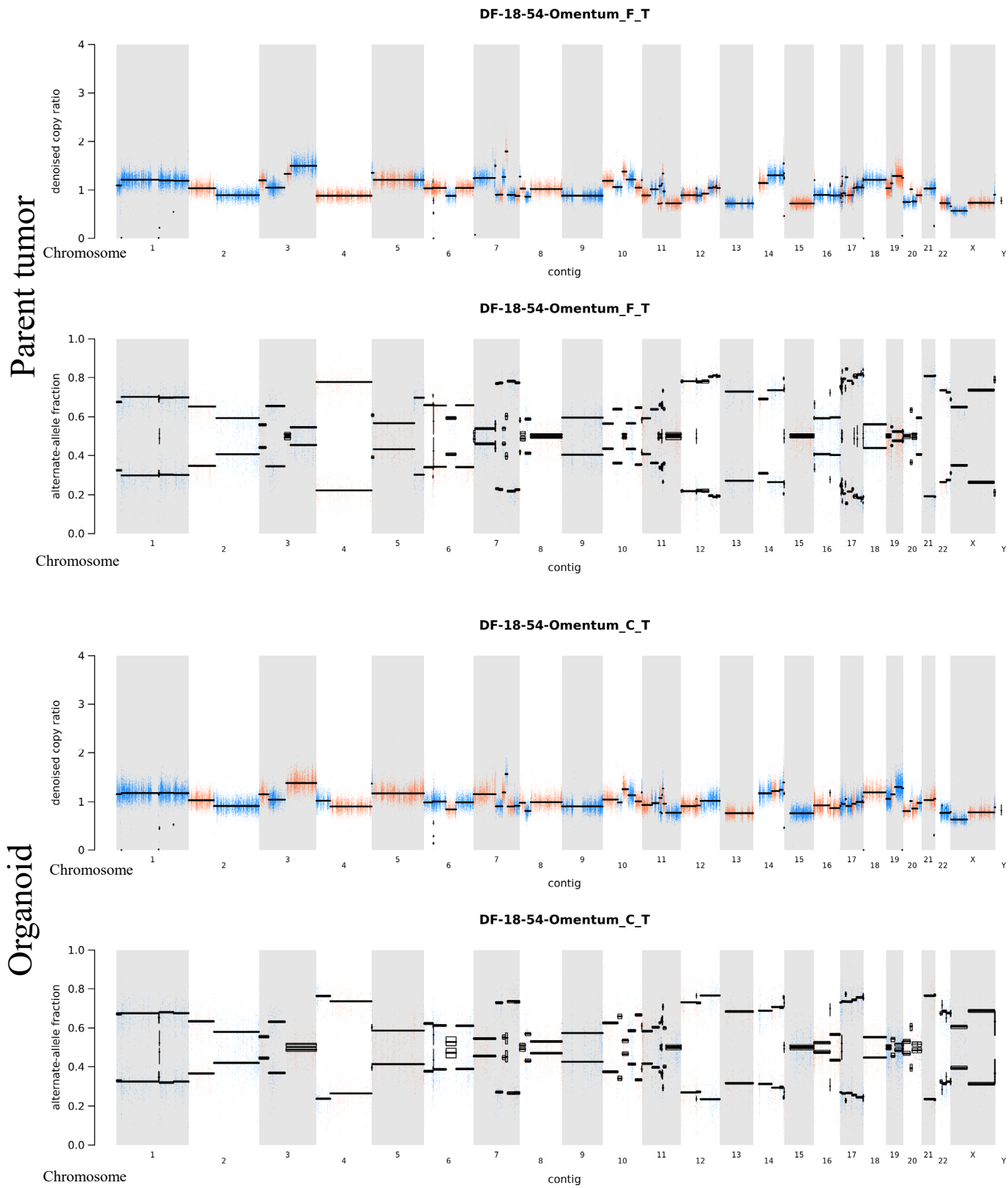


Figure S4

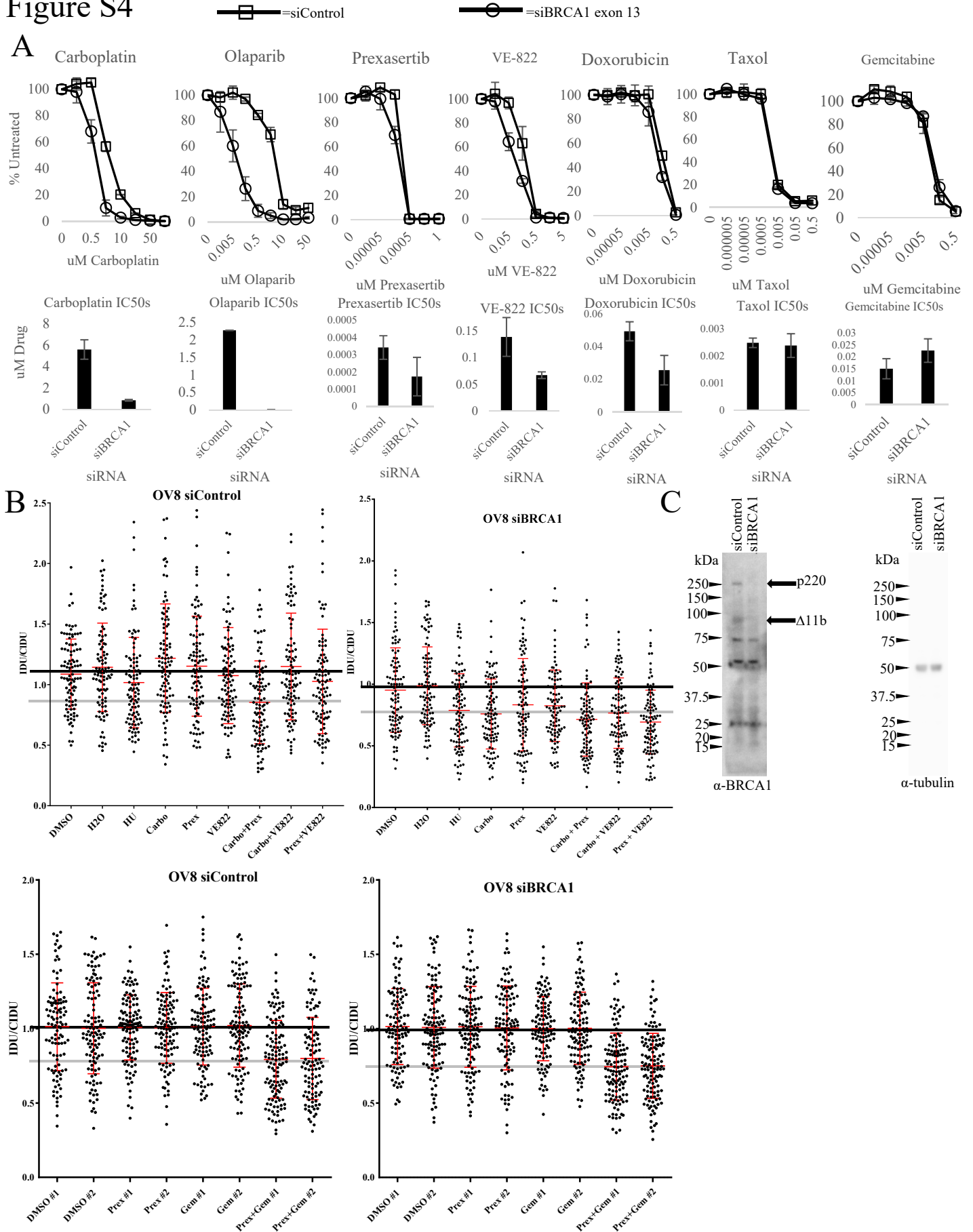


Figure S5

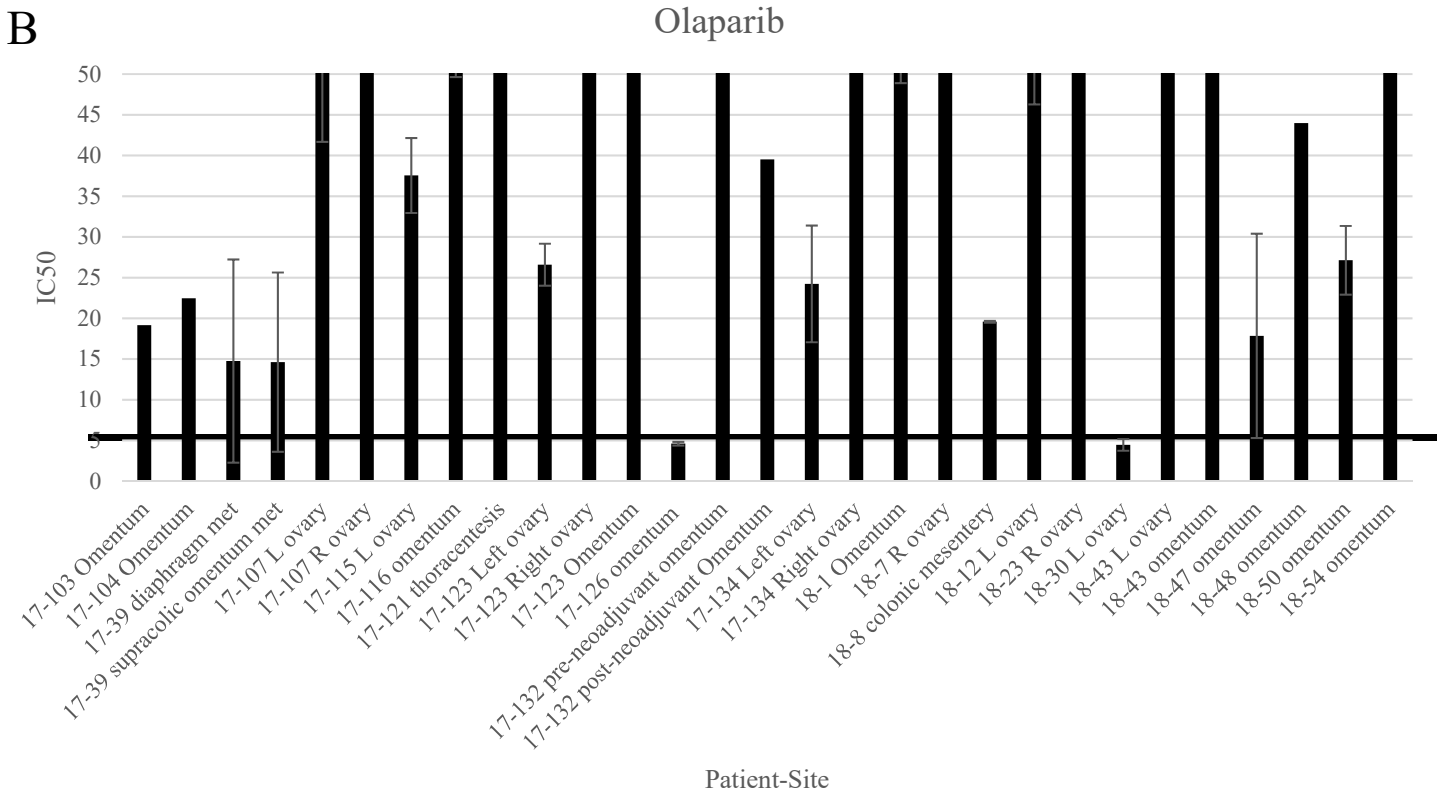
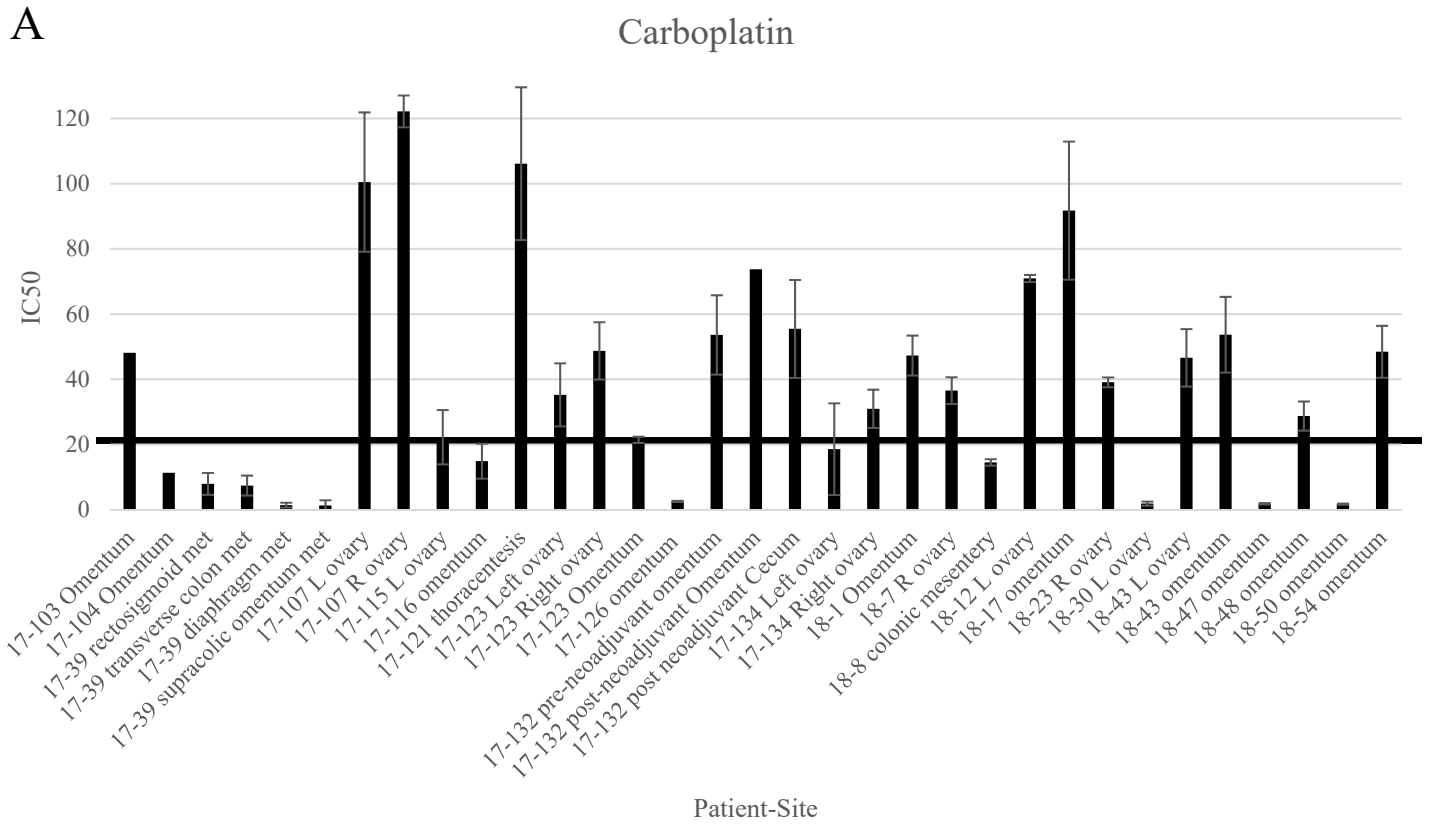


Figure S6

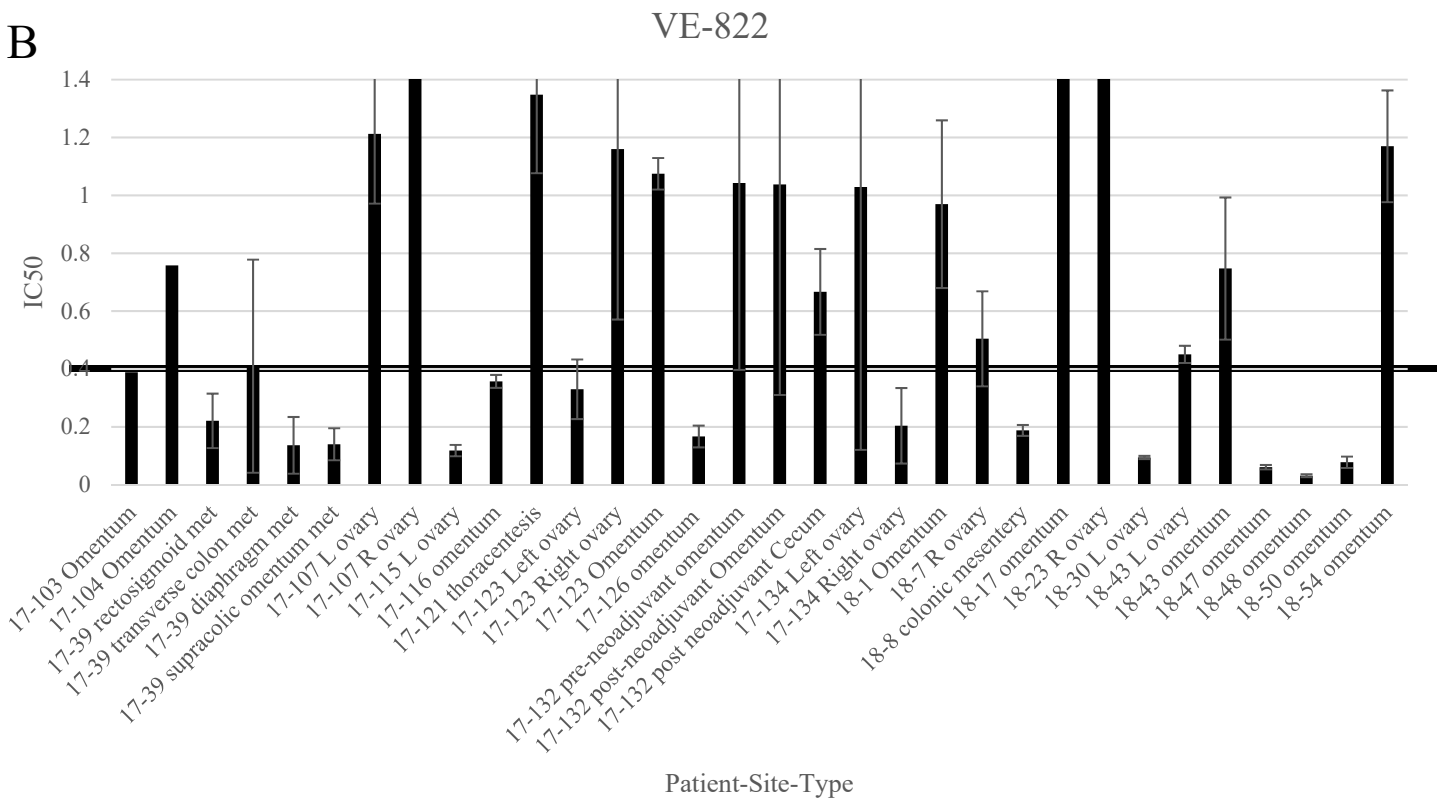
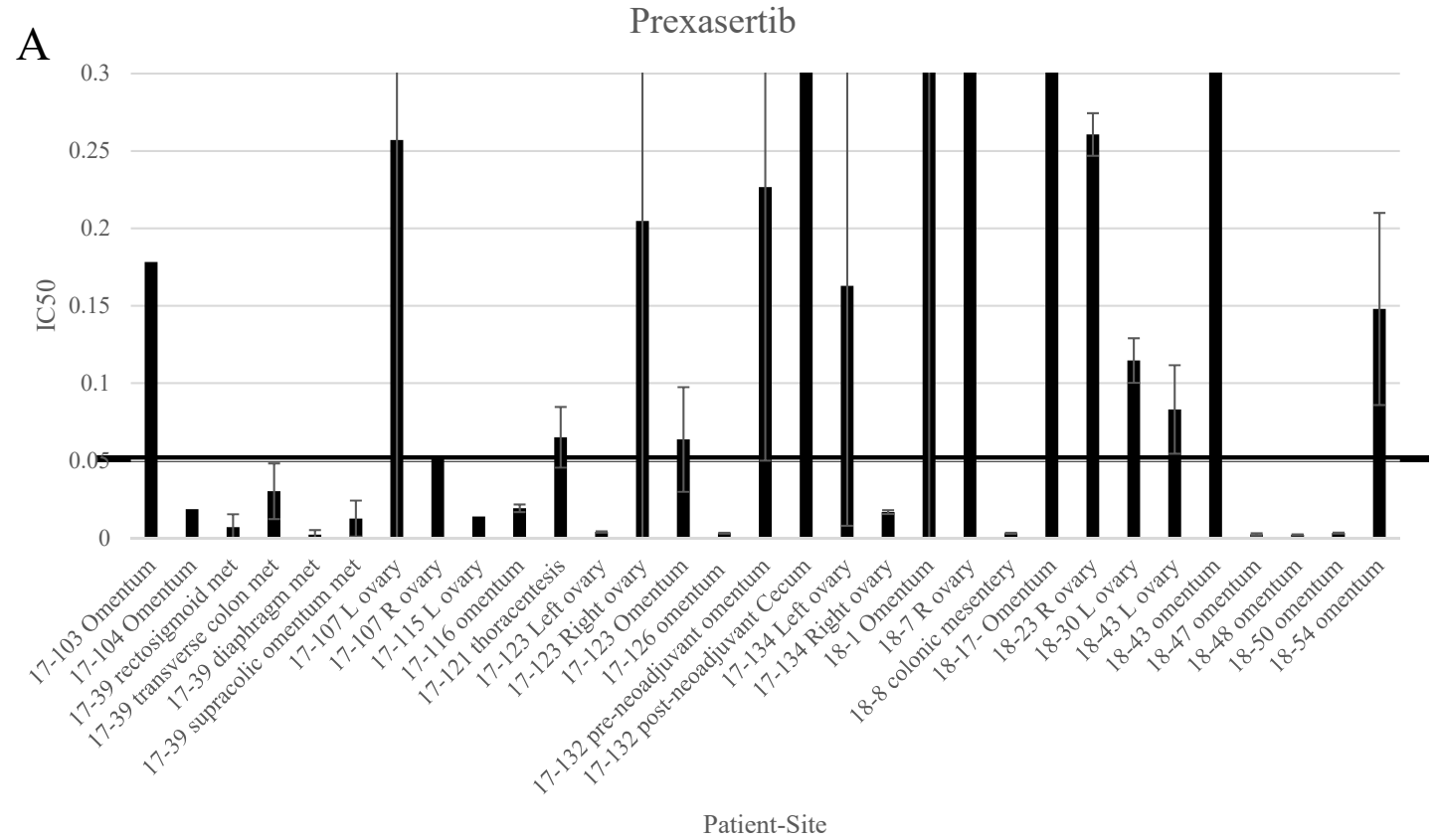


Figure S7

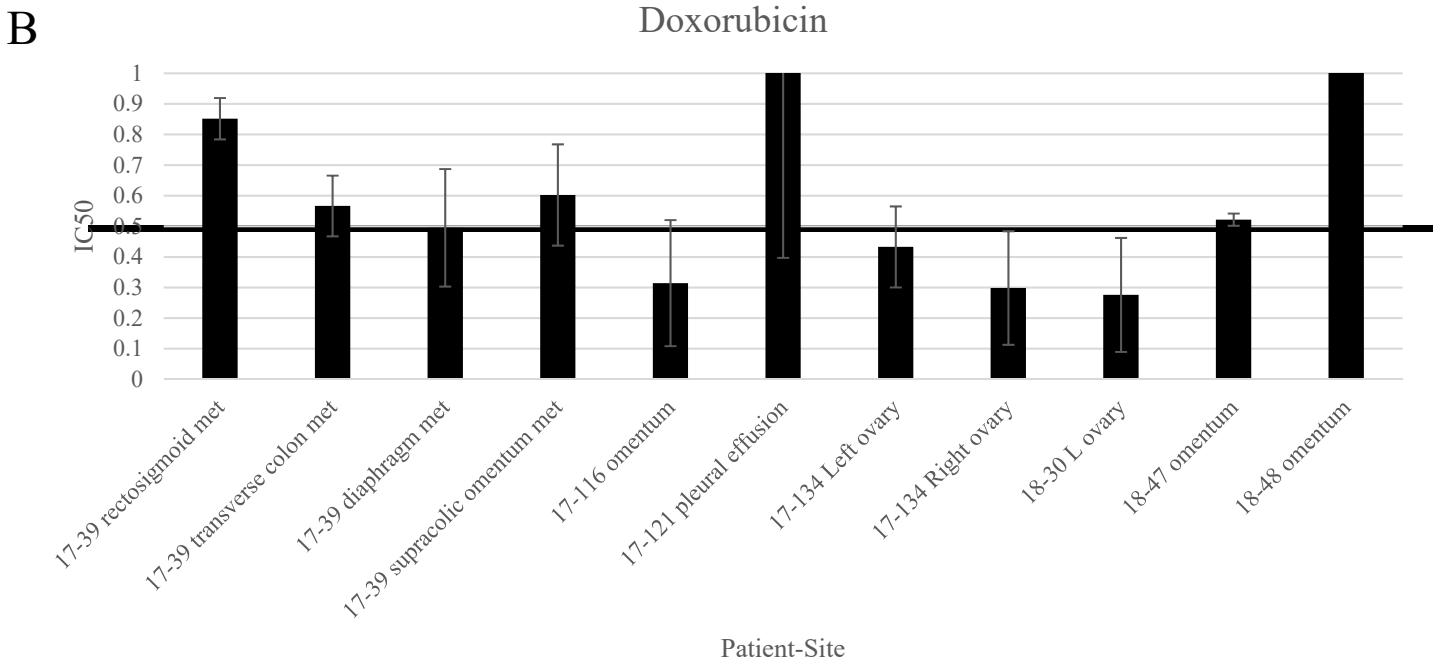
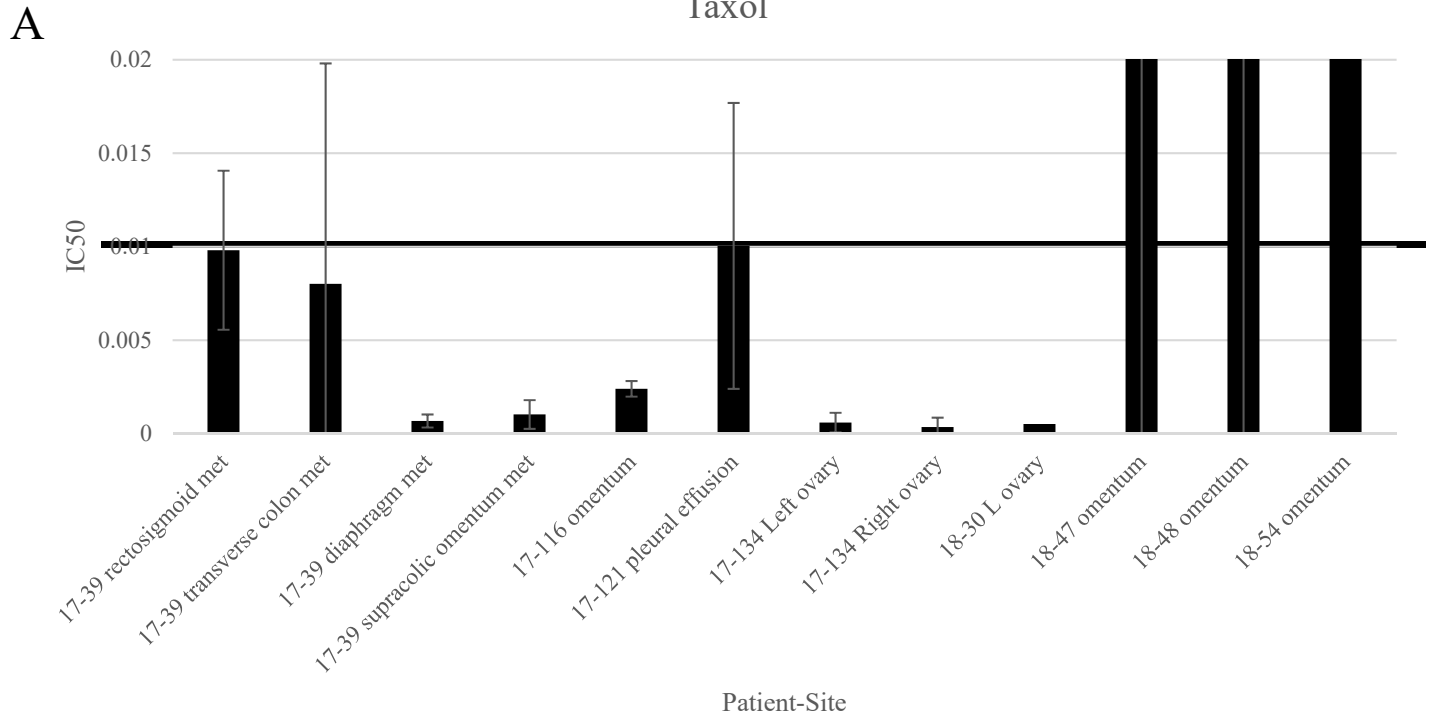


Figure S8

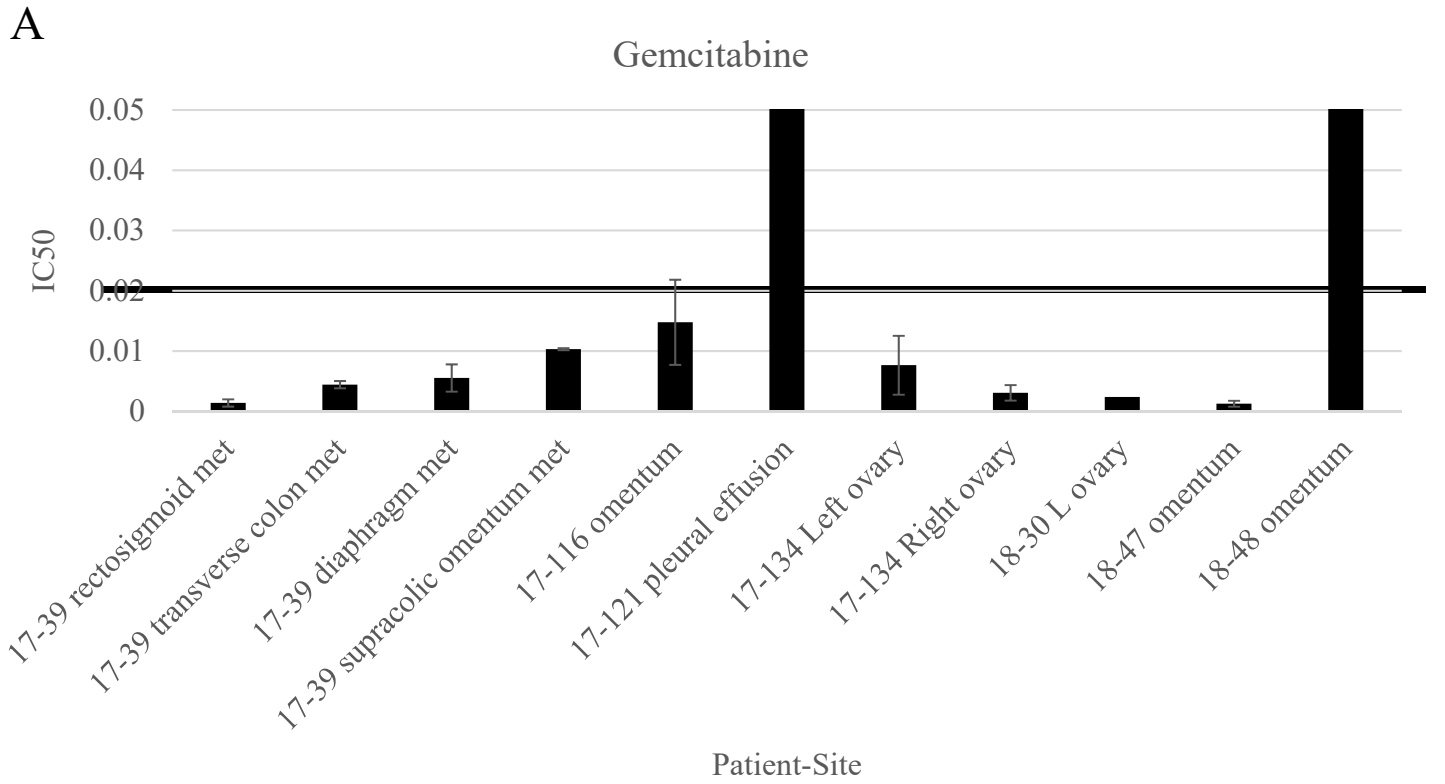


Figure S9A

DF-17-126 Omental metastasis

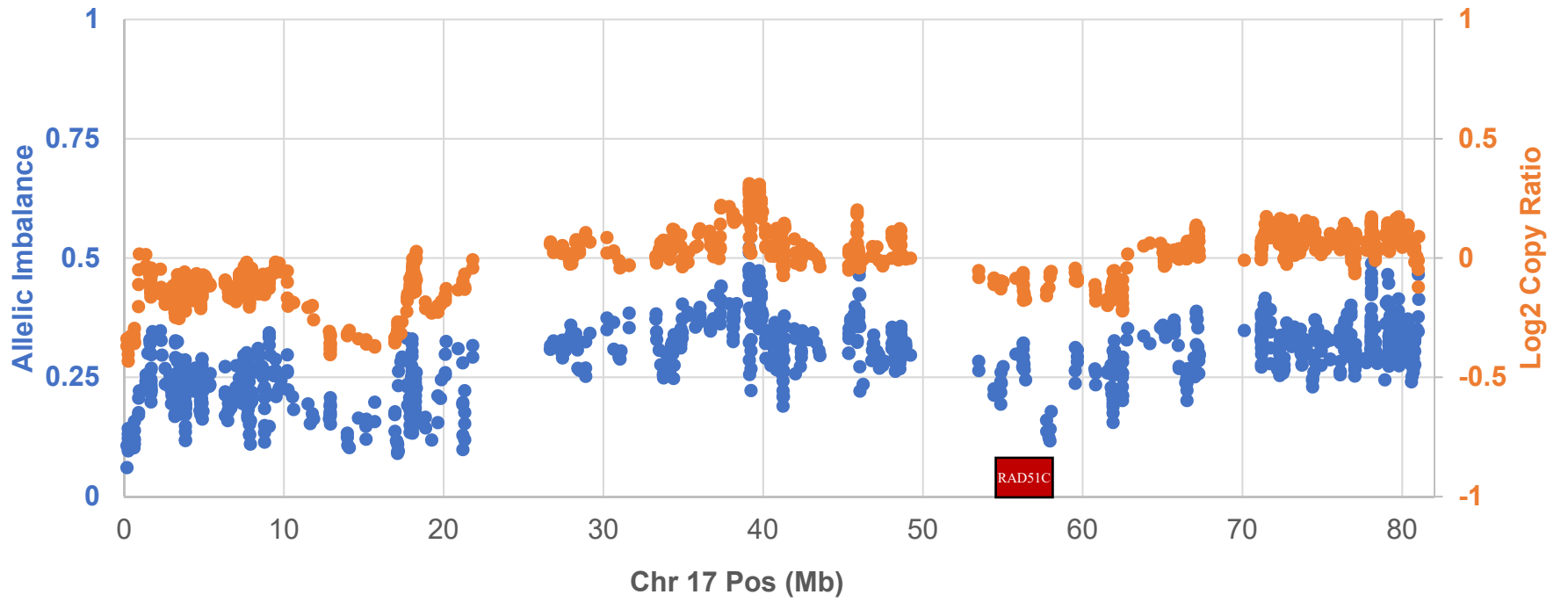


Figure S9B

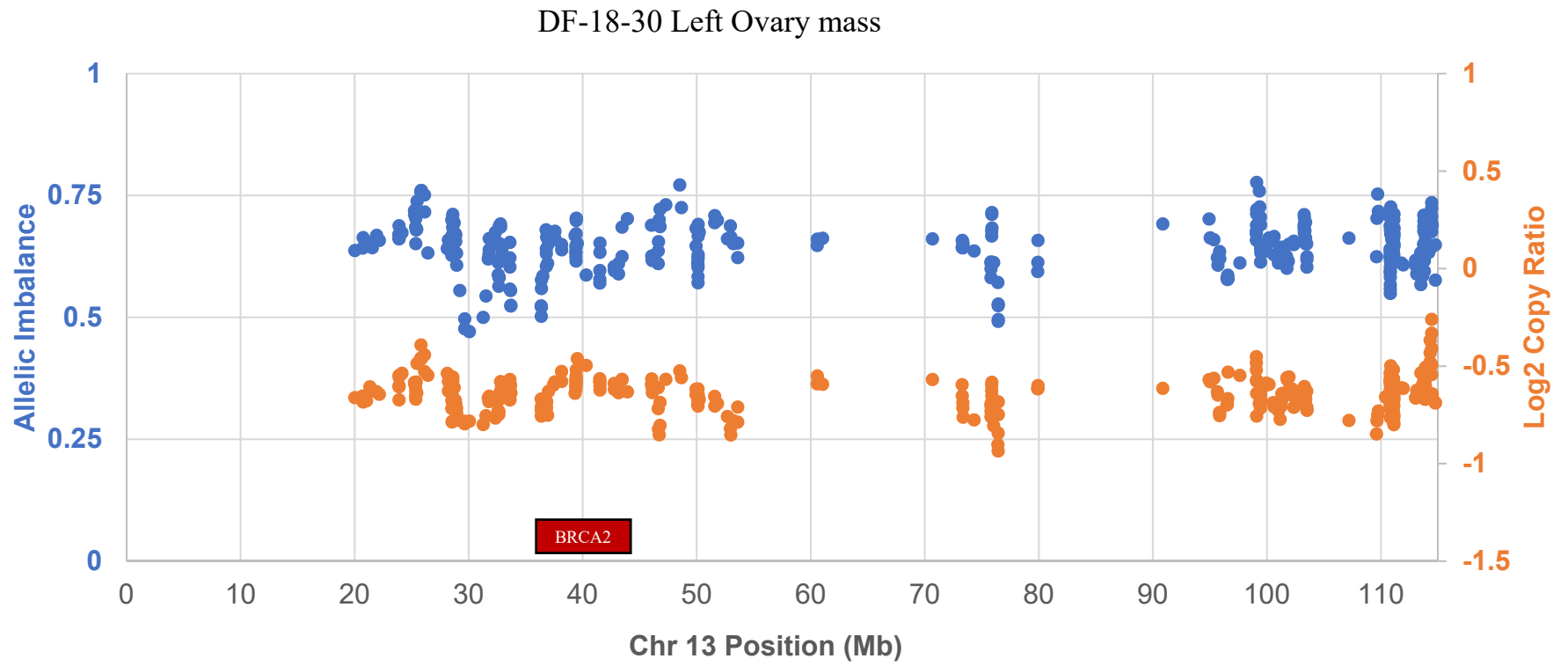


Figure S9C

DF-17-107 Left Ovary Mass

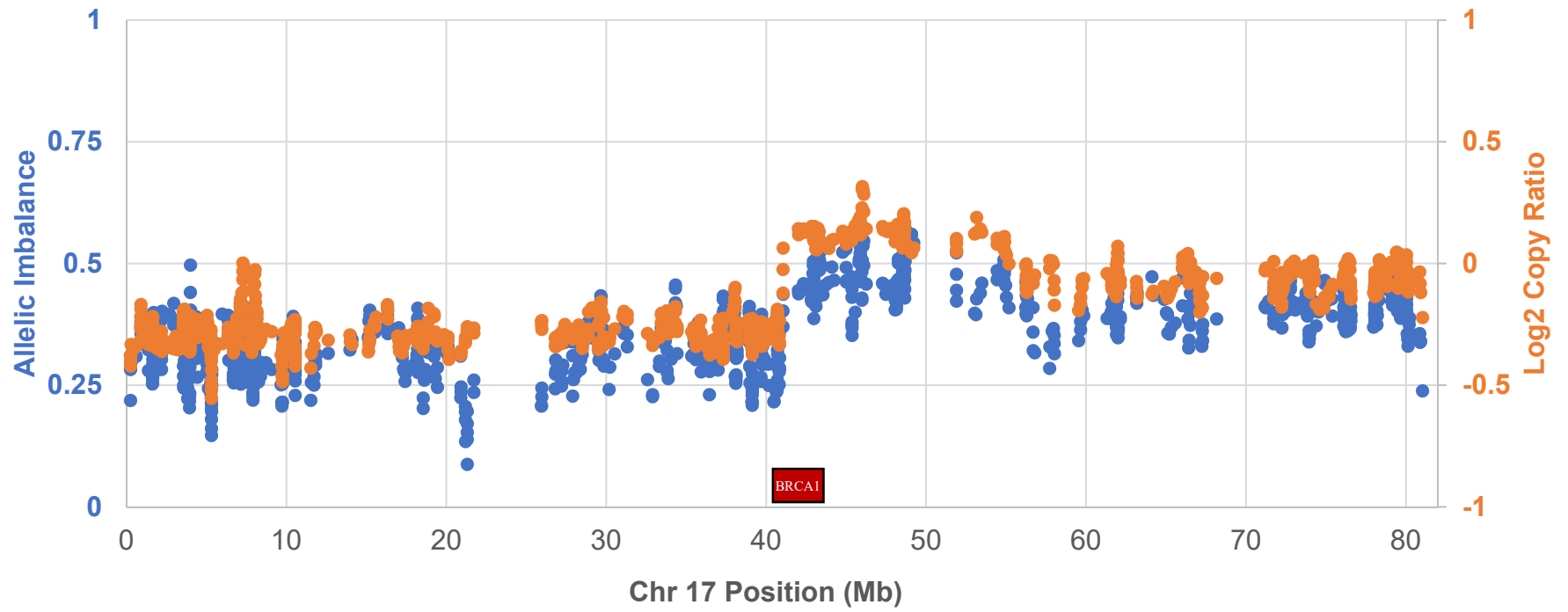


Figure S9D

DF-17-39 Transverse colon mass

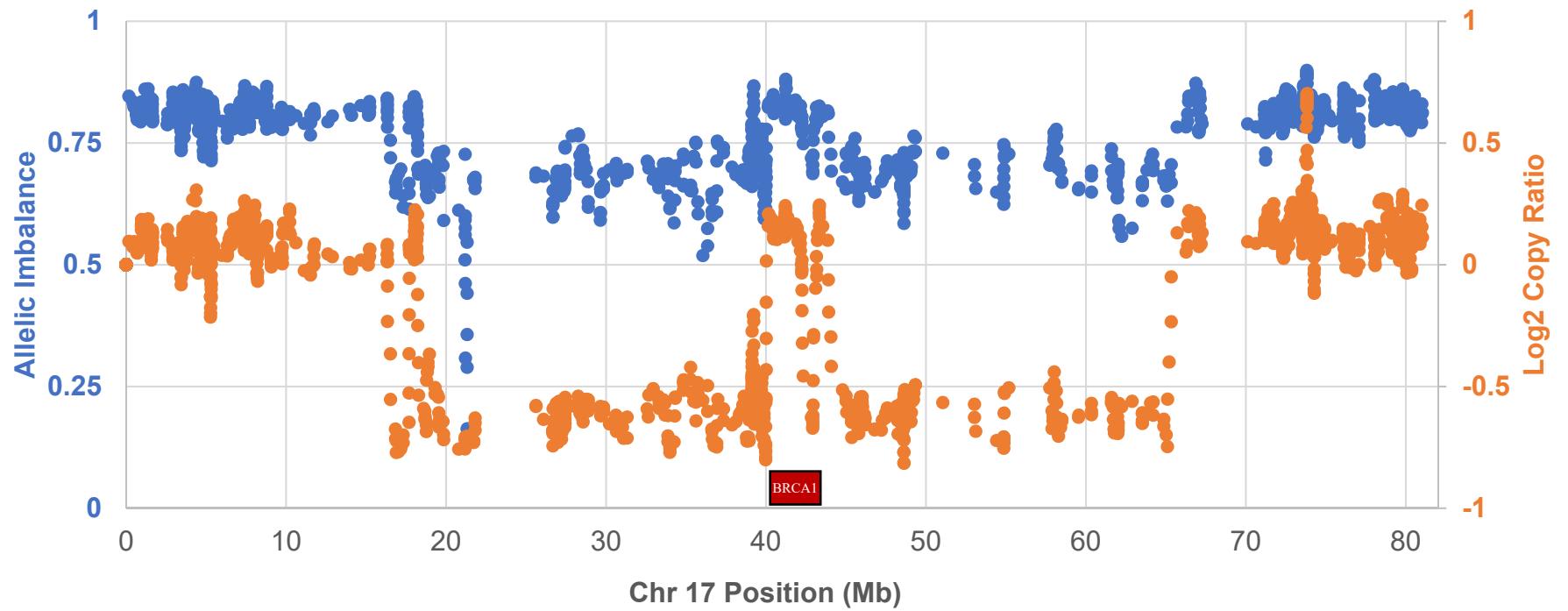


Figure S9E

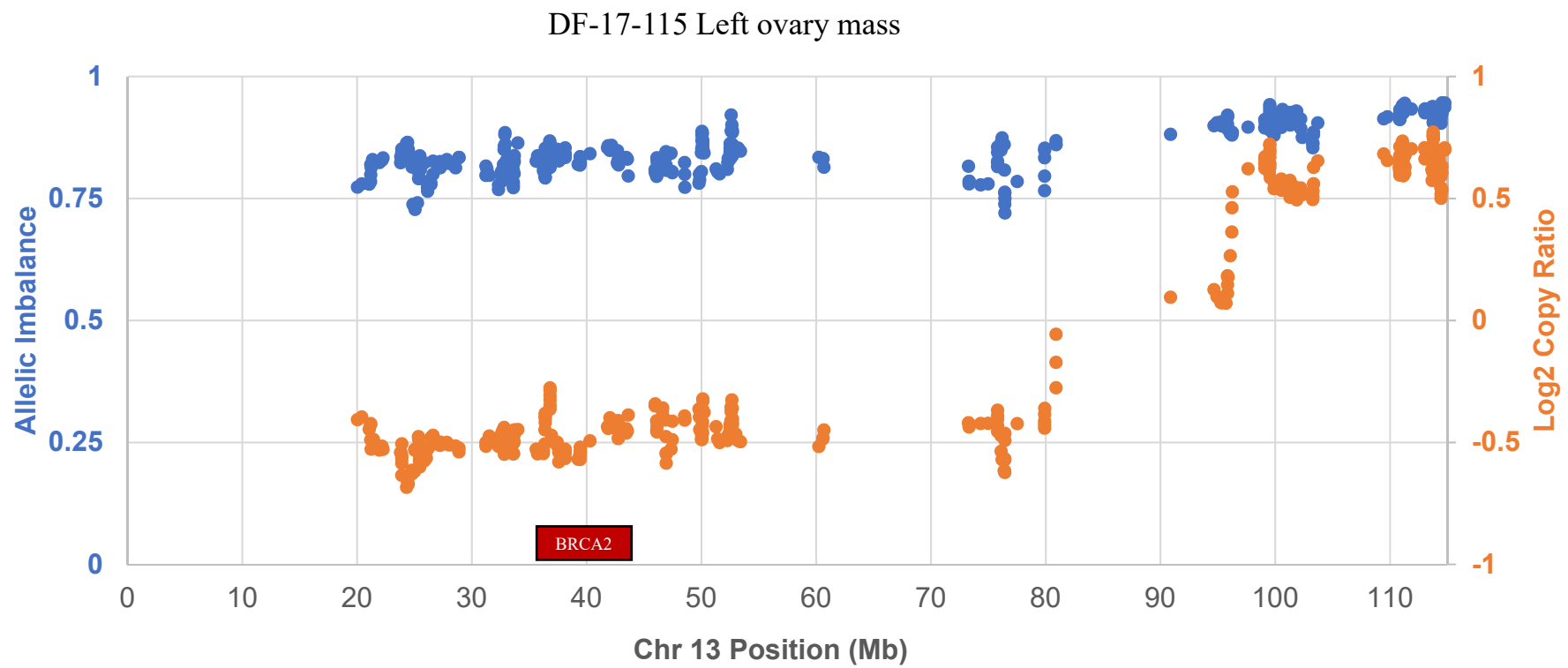


Figure S9F

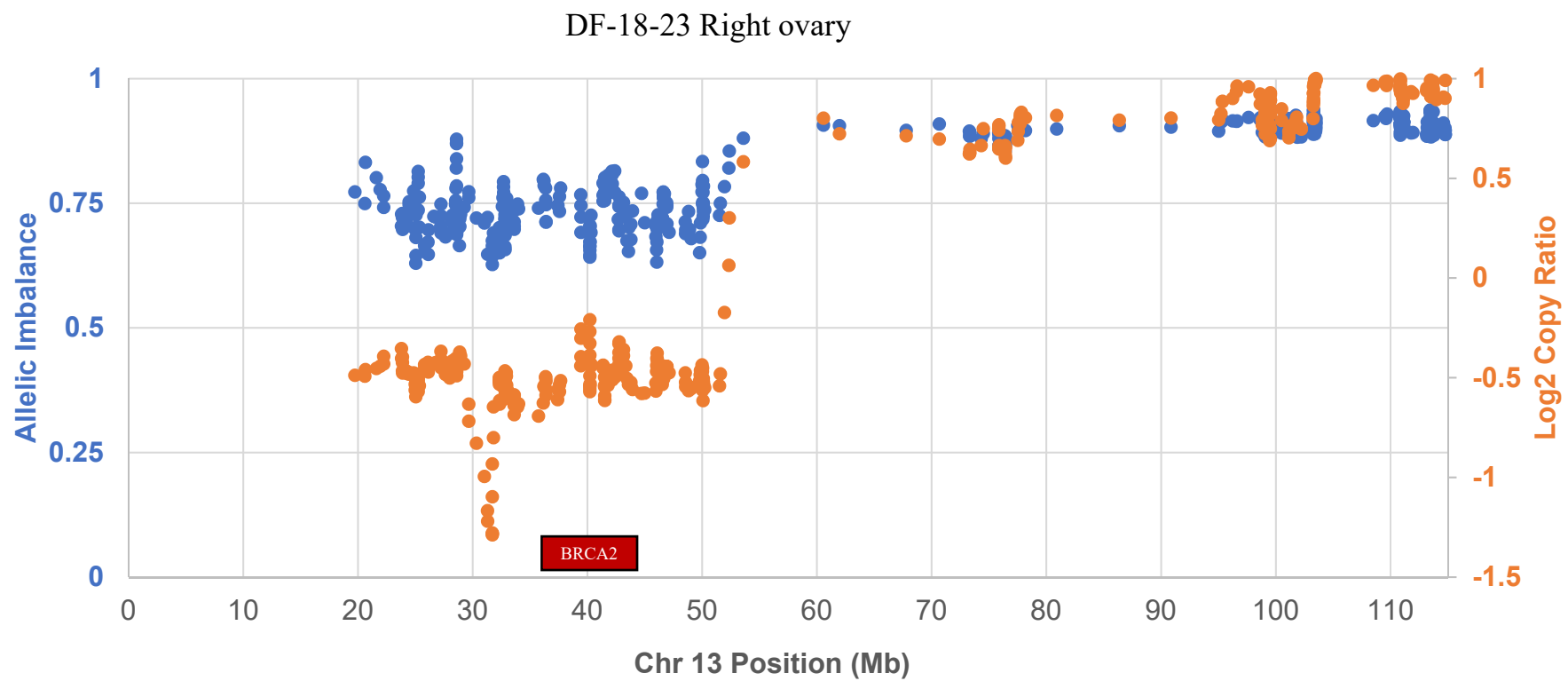


Figure S9G

DF-18-8 Colonic mesentery mass

