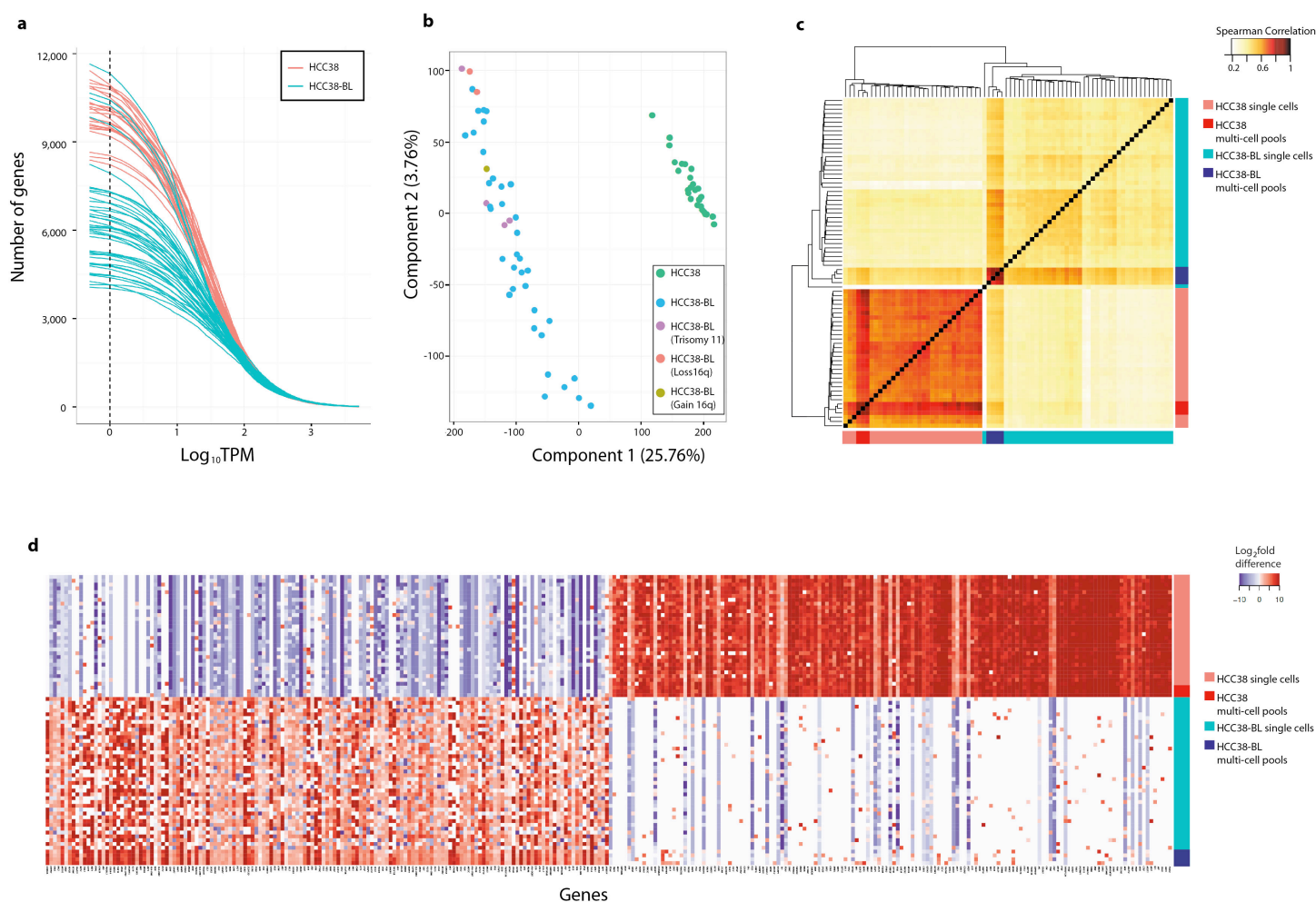


**Supplementary Figure 1**

Performance of G&T-seq whole-genome amplification in HCC38 and HCC38-BL cells.

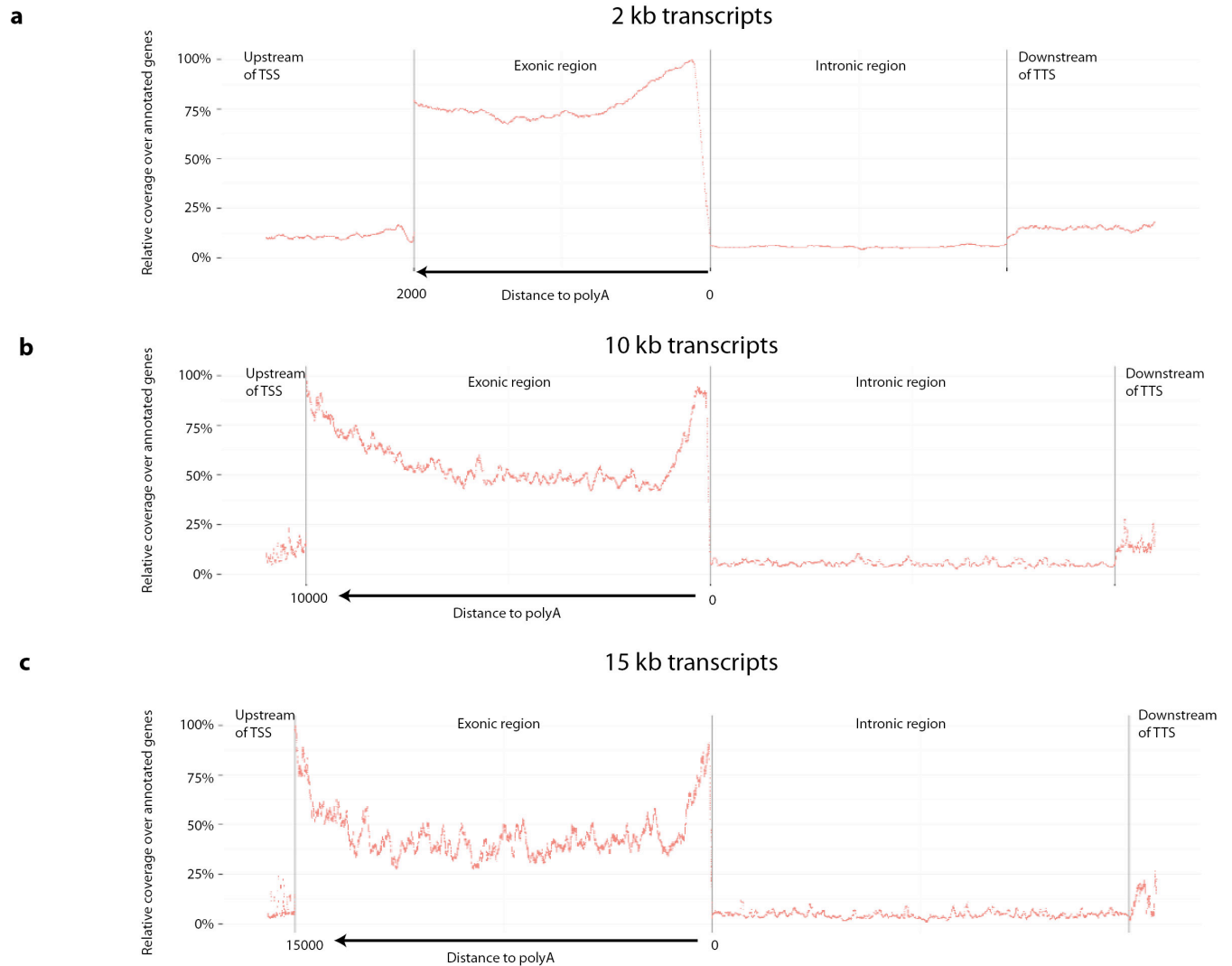
**(a)** Copy-number concordance between bulk DNA sequencing of HCC38(-BL) cells and single-cell or multicell G&T-seq following MDA or PicoPlex WGA. For reference, single-cell DNA copy-number concordances obtained with conventional MDA and PicoPlex are shown. **(b)** Heat map of the genome-wide DNA copy number (LogR) in single cells and in multicell controls isolated from HCC38 and HCC38-BL cells and amplified using MDA. For reference, the copy-number profile derived from bulk HCC38 DNA (not subjected to WGA) is shown on the left. **(c)** Lorenz curve illustrating the relationship between the cumulative fraction of the genome covered (x-axis) and the cumulative fraction of mapped bases (y-axis). **(d)** Normalized read count as a function of %GC content. The distributions are shown for all HCC38 G&T-seq samples amplified with MDA (purple) and PicoPlex (green). For comparison, the distributions for bulk (no WGA, blue), conventional single-cell MDA (black) and conventional single-cell PicoPlex (orange) are shown.



## Supplementary Figure 2

Performance of G&T-seq whole-transcriptome amplification in HCC38 and HCC38-BL cells.

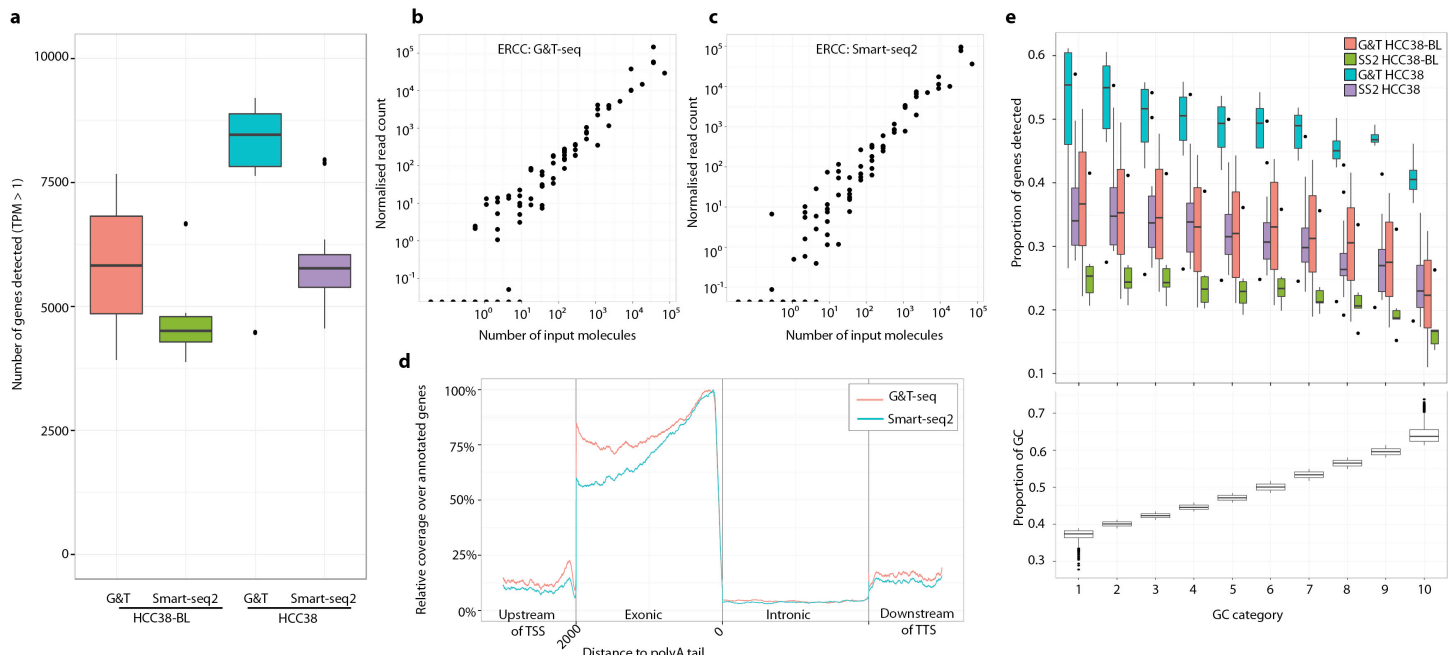
**(a)** Transcript detection following G&T-seq of HCC38 and HCC38-BL single cells. The number of expressed genes (y-axis) in HCC38 single cells (red lines) and HCC38-BL single cells (blue lines) versus TPM (x-axis). At TPM > 1 (dashed line), between 4,000 and 11,000 transcripts were detected per cell, with substantially more transcripts detected in HCC38 cells. **(b)** Principal-component analysis of HCC38 and HCC38BL single-cell transcriptomes. Cells in which genomic aneuploidies were detected are highlighted. **(c)** Heat map displaying Spearman correlation of 8,237 protein-coding genes expressed in at least 32 samples with TPM > 1. **(d)** Expanded heat map showing the top 200 differentially expressed genes between HCC38 and HCC38-BL cells. The TPM of each gene is 'normalized' by the median of the TPM of this gene across all samples and is presented as the log<sub>2</sub>-fold difference from this median.



### Supplementary Figure 3

Sequence coverage over transcript length and intronic and gene flanking regions in single-cell G&T-seq transcriptome data.

Read coverage in (a) 2 kb, (b) 10 kb and (c) 15 kb transcripts is shown. Numbers indicate the distance from the poly(A) tail in the exonic region only. Regions upstream of the transcription start site (TSS) and transcription termination site (TTS), as well as intronic regions, are also shown.

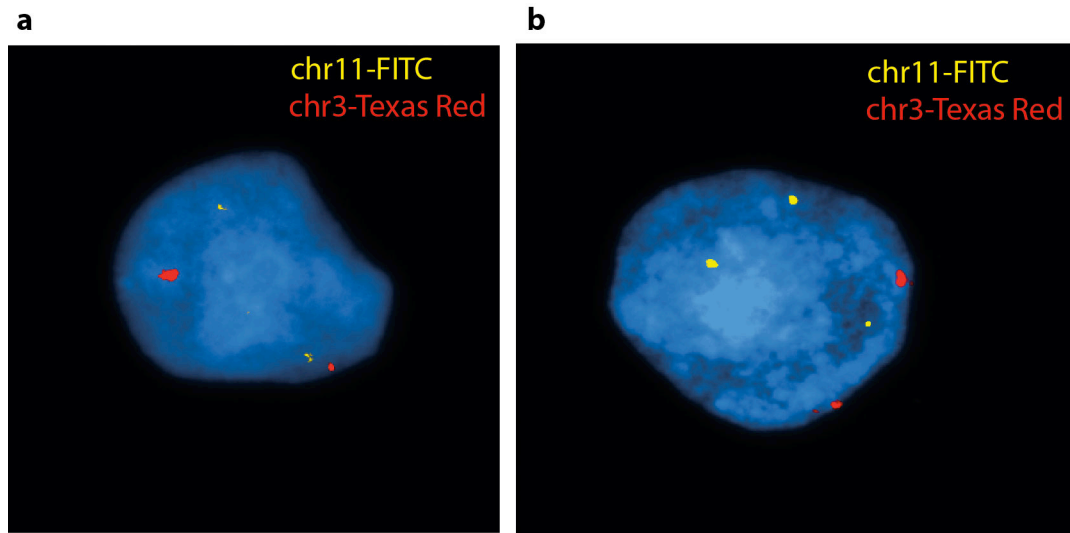


## Supplementary Figure 4

Comparison of RNA-seq data generated with the G&T-seq and conventional Smart-seq2 protocols.

In this comparison, 28 single cells (8 HCC38 and 20 HCC38-BL single cells) were used for G&T-seq, and 20 single cells (14 HCC38 and 6 HCC38-BL single cells) were applied for conventional Smart-seq2. Importantly, these cells came from the same cultures, were isolated at the same time, were processed (when possible) with the same batches of reagents, and were eventually sequenced together. **(a)** Transcript detection following G&T-seq or conventional Smart-seq2 amplification of HCC38 and HCC38-BL single cells. The number of transcripts detected at TPM > 1 is displayed. **(b)** Detection of ERCC transcripts relative to ERCC input amount; the plot shows the averaged normalized read count across all single-cell samples in a G&T-seq experiment versus the number of molecules of each ERCC sequence that was spiked in. **(c)** Detection of ERCC transcripts relative to ERCC input amount in a parallel Smart-seq2 experiment. **(d)** Sequence coverage over transcript length and intronic and gene flanking regions in single-cell G&T-seq and Smart-seq2 transcriptome data. Read coverage in 2 kb transcripts is shown. Numbers indicate the distance from the poly(A) tail in the exonic region only. Regions upstream of the transcription start site (TSS) and transcription termination site (TTS), as well as intronic regions, are also shown. **(e)** Transcript detection in bins of transcript GC content for HCC38 and HCC38-BL single-cell transcriptomes generated by G&T-seq and Smart-seq2 (SS2). The upper panel shows the proportion of genes detected in each bin, and the lower panel displays the proportion of GC content in each bin.

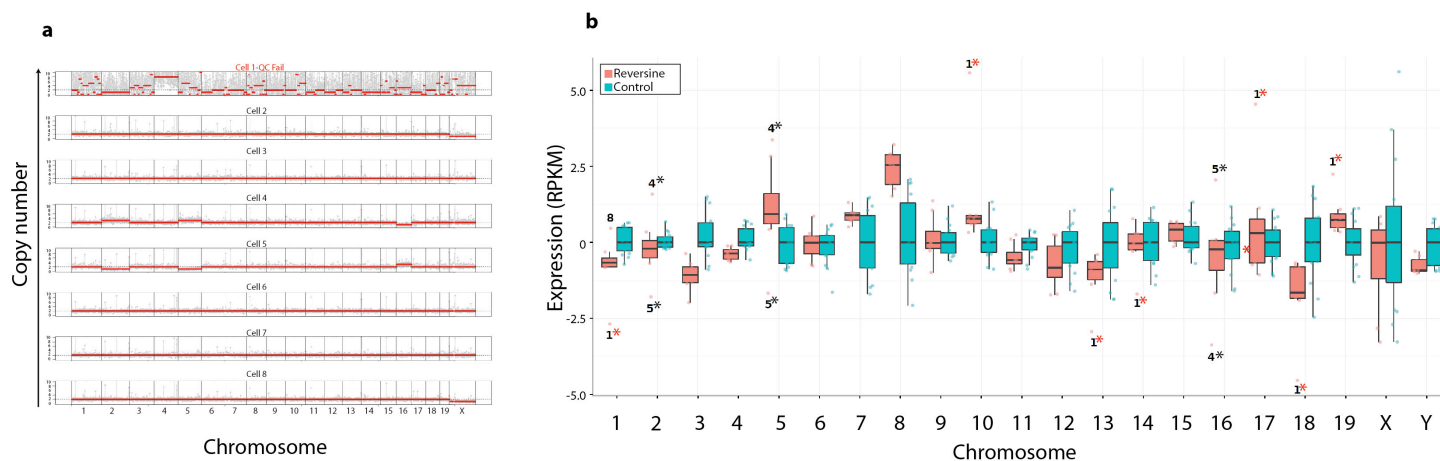




### Supplementary Figure 5

Interphase FISH to detect trisomy 11 in a subset of HCC38-BL cells.

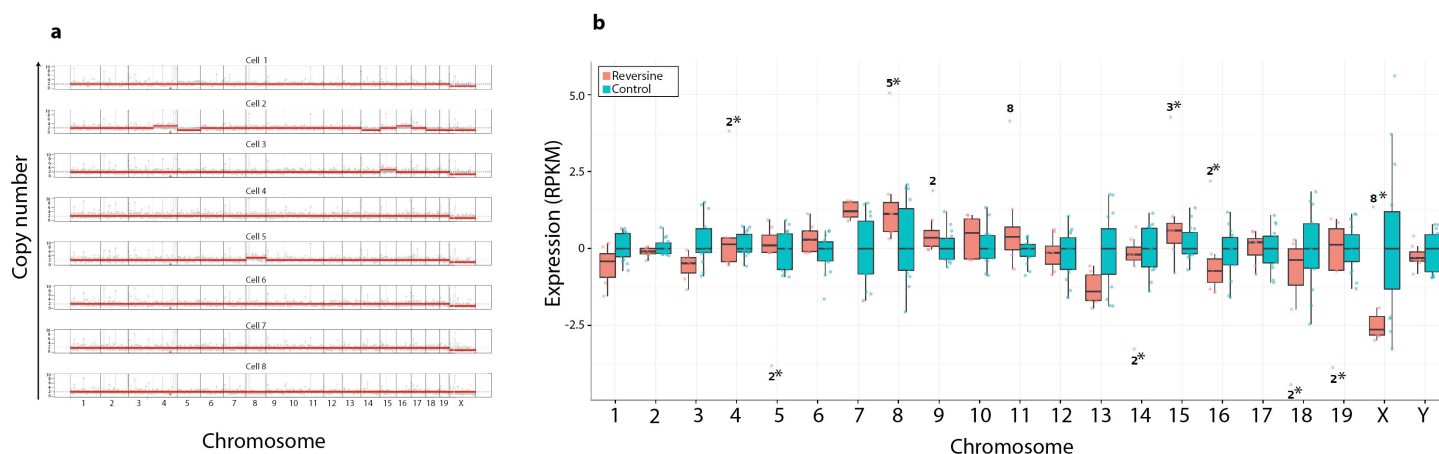
Chromosomes 11 and 3 were hybridized with a centromeric probe (labeled with FITC and Texas Red, respectively). The majority of HCC38-BL cells had disomy 11 (**a**), whereas trisomy 11 was observed in 2 out of 100 HCC38-BL cells analyzed (**b**).



## Supplementary Figure 6

Relationship between chromosomal copy number and chromosome-wide expression in a mouse embryo at the eight-cell stage.

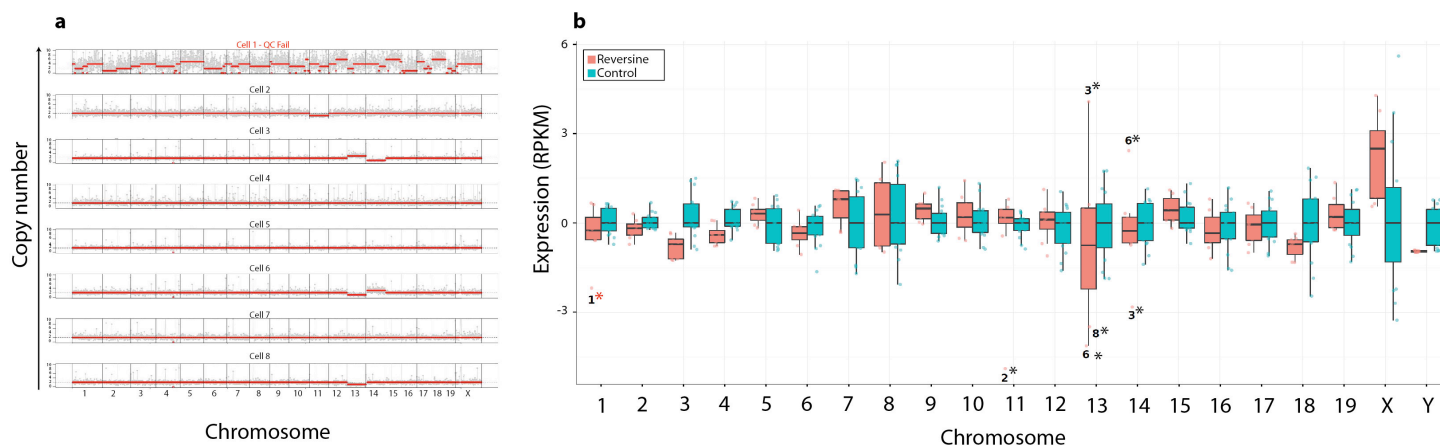
Reversine-treated mouse embryo at the eight-cell stage (embryo A) containing sister cells with reciprocal aneuploidies. **(a)** The genome-wide copy-number profile is shown for all eight cells in the embryo (numbered 1–8). Cell 1 failed QC at the genome level. Reciprocal aneuploidies were observed for cells 4 and 5 at chromosomes 2, 5 and 16. **(b)** Genome-wide expression binned per chromosome in the control ( $n = 16$  cells, untreated and shown in blue) and reversine-treated ( $n = 8$  cells, shown in red) embryos (RPKM of the latter are relative to the median-centered control RPKMs). The expected expression dosage resulting from the aneuploidies for chromosomes 2, 5 and 16 in the blastomeres (cells 4 and 5) was detected in the correct cell's transcriptome. Cells displaying concordantly higher and lower overall expression per chromosome are highlighted with a black asterisk. Cell 1, which also failed DNA-seq QC, is highlighted with a red asterisk. For all box plots, the lower and upper boundaries of the box represent, respectively, the 25th and 75th percentiles, with the bar being equal to the median. The whiskers represent the 5th and 95th percentiles.



### Supplementary Figure 7

Relationship between chromosomal copy number and chromosome-wide expression in a mouse embryo at the eight-cell stage.

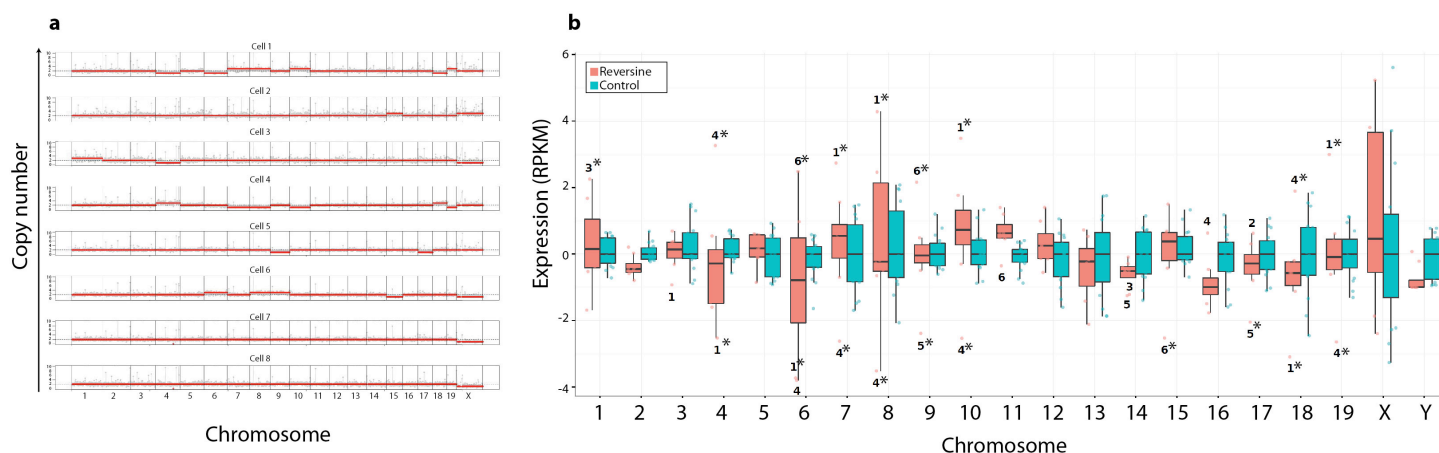
Reversine-treated mouse embryo at the eight-cell stage (embryo B) containing sister cells with reciprocal and nonreciprocal aneuploidies. **(a)** The genome-wide copy-number profile is shown for all eight cells in the embryo (numbered 1–8). A complex pattern of aneuploidy was observed in cell 2 (gain of chromosomes 4 and 16 and loss of chromosomes 5, 14, 18 and 19). Cell 3 had a gain in chromosome 15, and cell 5 had a gain in chromosome 8, while cell 8 gained an X-chromosome. **(b)** Genome-wide expression binned per chromosome comparing the cells from embryo B (reversine-treated, shown in red,  $n = 8$ ) with those from control embryos ( $n = 16$  cells, untreated and shown in blue). Cells displaying concordantly higher and lower overall expression per chromosome are highlighted with an asterisk. For all box plots, the lower and upper boundaries of the box represent, respectively, the 25th and 75th percentiles, with the bar being equal to the median. The whiskers represent the 5th and 95th percentiles.



### Supplementary Figure 8

Relationship between chromosomal copy number and chromosome-wide expression in a mouse embryo at the eight-cell stage.

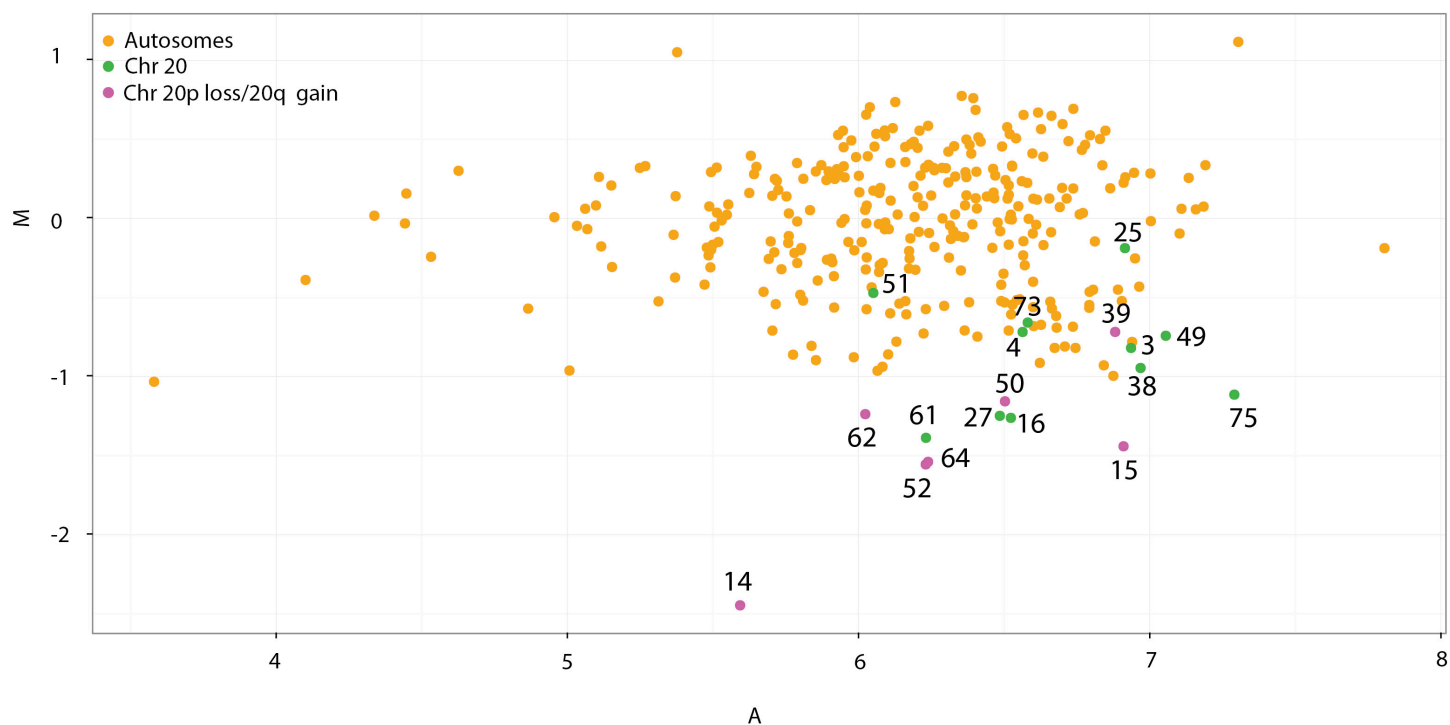
Reversine-treated mouse embryo at the eight-cell stage (embryo C) containing sister cells with reciprocal and nonreciprocal aneuploidies. **(a)** The genome-wide copy-number profile is shown for all eight cells in the embryo (numbered 1–8). Cell 1 failed QC following DNA-seq. Cell 2 had a loss of chromosome 11, whereas cells 3 and 6 showed reciprocal gains and losses at chromosomes 13 and 14. Cell 8 had lost a copy of chromosome 13. **(b)** Genome-wide expression binned per chromosome comparing the cells from embryo C (reversine-treated, shown in red,  $n = 8$ ) with those from control embryos ( $n = 16$  cells, untreated and shown in blue). Cells displaying concordantly higher and lower overall expression per chromosome are highlighted with a black asterisk. Cell 1, which failed DNA-seq QC, is highlighted with a red asterisk. For all box plots, the lower and upper boundaries of the box represent, respectively, the 25th and 75th percentiles, with the bar being equal to the median. The whiskers represent the 5th and 95th percentiles.



### Supplementary Figure 9

Relationship between chromosomal copy number and chromosome-wide expression in a mouse embryo at the eight-cell stage.

Reversine-treated mouse embryo at the eight-cell stage (embryo E) containing sister cells with reciprocal and nonreciprocal aneuploidies. **(a)** The genome-wide copy-number profile is shown for all eight cells in the embryo (numbered 1–8). Cells 1 and 4 had reciprocal aneuploidies for chromosomes 4, 7, 8, 10, 18 and 19, with cell 1 having an additional nonreciprocal loss of chromosome 6. Cell 2 had a gain at chromosomes 15 and X. Cell 3 had a gain of chromosome 1 and losses of chromosomes 4 and X. Cell 5 had a loss of chromosome 9 and 17. Cell 6 had a gain of chromosomes 6, 8 and 9 and losses of chromosomes 15 and X. Cells 7 and 8 had a loss of chromosome X. **(b)** Genome-wide expression binned per chromosome comparing the cells from embryo E (reversine-treated, shown in red) with those from control embryos ( $n = 16$  cells, untreated and shown in blue). Cells displaying concordantly higher and lower overall expression per chromosome are highlighted with an asterisk. For all box plots, the lower and upper boundaries of the box represent, respectively, the 25th and 75th percentiles, with the bar being equal to the median. The whiskers represent the 5th and 95th percentiles.



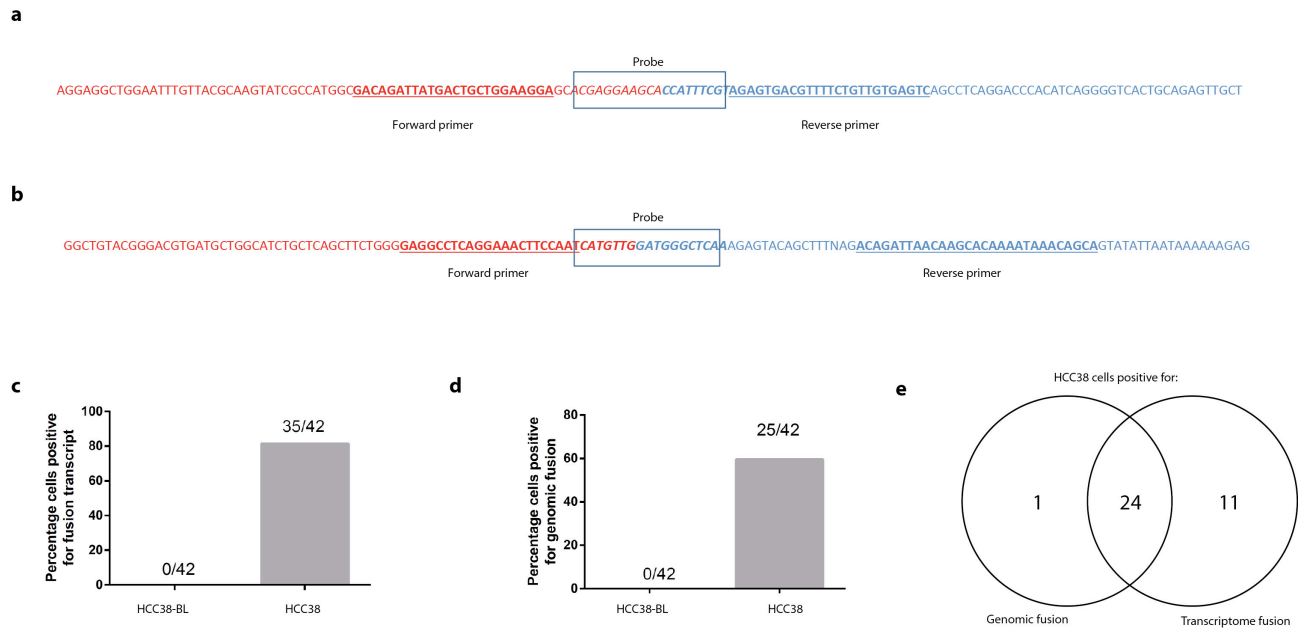
### Supplementary Figure 10

Relationship between chromosomal-arm copy number and chromosome-arm-wide expression in iPSC-derived neurons.

MA plot comparing the log<sub>2</sub> ratio in mRNA expression levels between p and q chromosomal arms (M) to the average expression across the chromosome arms (A) for all cells containing trisomy 21. The acrocentric chromosomes 13, 14, 15, 21 and 22 and chromosome Y have been excluded. The values for chromosome 20 are shown in green for cells without evidence of gain or loss of the chromosomal arms, and cells with genomic evidence for loss of 20p and gain of 20q are shown in purple. Numbers indicate cell identifiers.







## Supplementary Figure 12

Confirmation of *MTAP-PCDH7* expression and detection of the associated genomic fusion by qPCR.

Taqman primer and probe sets were designed to detect (a) the *MTAP-PCDH7* fusion transcript and (b) the genomic breakpoint that fuses chromosomes 4 and 9. Examples of consensus reads mapping across both breakpoints are shown, with the *MTAP* side colored red and the *PCDH7* side colored blue. Primer/probe sets were specifically designed to span the breakpoints in both cases. (c) Detection of the *MTAP-PCDH7* fusion transcript in cDNA from G&T-seq of HCC38 and HCC38-BL cells. (d) Detection of the *MTAP-PCDH7* genomic fusion in MDA-amplified DNA from G&T-seq of HCC38 and HCC38-BL cells. (e) Venn diagram showing the overlap of detection of the fusion transcript and associated genomic rearrangement in parallel from the same single cells.

Sample	WGA method	Total Single cells	Total cells failing QC (% of all cells)	Total cells failed on Genome QC (<2% mapped reads)	Total cells failed on Genome QC (MAPD)	Total cells failed on Transcriptome QC (<3500 detected genes)	Cells failing on both Genome and Transcriptome QC (% of all QC fails)
HCC38(BL) MDA	MDA	86	18 (20.9%)	9	9	10	10 (55.5%)
HCC38(BL) PicoPlex	PicoPlex	86	24 (27.9%)	18	9	18	16 (66.6%)
Total HCC38(BL)		172	42 (24.4%)	27	18	28	26 (61.9%)
Mouse Induced Aneuploidy	PicoPlex	56	2 (3.5%)	0	2	0	0 (0%)
Trisomy 21 iPSC	PicoPlex	56	15 (26.8%)	0	10	11	7 (46.6%)
All cells		284	59 (20.7%)	27	30	39	33 (55.9%)

**Supplementary table 1: Overview of QC statistics for all single cells sequenced using the G&T-seq approach.** In total, 284 single cells were amplified, of which 225 (79%) passed QC. Many of those cells which failed QC in FACS based HCC38 and HCC38BL experiments failed both genome and transcriptome QC (61.9%), indicating that a cell may not have been sorted into the particular well.

Sample	Sequenced Reads (million)	Mapped Reads (million)	Mapped %	Duplicates (million)	Duplicate %	Depth of Coverage	Breadth of genome %
HCC38_21	874.5	852.4	97.5	74.3	8.7	33.6	73.4
HCC38_23	853.1	836.1	98.0	63.9	7.6	33.9	78.3
HCC38_24	882.9	855.0	96.8	78.8	9.2	32.4	64.8
HCC38_29	857.0	838.7	97.9	70.8	8.4	34.5	71.2
HCC38-BL_3	867.9	848.4	97.8	73.3	8.6	34	57.5
HCC38-BL_7	874.3	842.5	96.4	80.9	9.6	31.9	58.8
HCC38-BL_38	870.1	844.0	97.0	83.0	9.8	33.2	59.1
HCC38-BL_43	815.1	798.8	98.0	64.6	8.1	33.1	74.1

**Supplementary Table 2: Summary statistics of deep sequencing of 8 single cell genomes generated by G&T-seq.**