The needle in the haystack - searching for genetic and epigenetic differences in monozygotic twins discordant for Tetralogy of Fallot

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Supplementary Materials
Figure S1. Statistics of reads obtained from whole genome sequencing. (A) Ranges of GC content, duplication level and read quality scores of total reads over all samples (n=16, i.e., values for each lane of four samples) as well as mean read depth per base after mapping (n=4). (B) Average sequencing read depth across the human reference genome (hg38).
Figure S2. Statistics of input reads obtained from whole genome bisulfite sequencing. Values for each lane of four samples (i.e., n=24).
Figure S3. Bisulfite conversion efficiency. (A) Base compositions on forward and reverse strand over all samples. (B) Bisulfite conversion rate over all samples. Each sample was sequenced on six sequencing lane (n=6).
Figure S4. Quality measurements of deduplicated mapped reads obtained from whole genome bisulfite sequencing. (A) Ranges of GC content, read quality scores and duplication level of deduplicated mapped reads over all samples as well as mean read depth per base. (B) Average sequencing read depth across the human reference genome (hg38).
Figure S5. Methylation bias and rates. (A) CpG methylation bias (M-bias) over forward and reverse reads over all bases. (B) Methylation rates over CpGs, CHGs, CHHs and Cs. Note that ‘H’ denotes for A, T or C.
Figure S6. Filtering of possible disease-relevant local variations identified in affected TOF twins based on zygosity differences between healthy and affected sibling. CADD, combined annotation dependent depletion; H, healthy; INDEL, insertion and deletion; M, million; MAF, minor allele frequency; SNV, single nucleotide variation; TOF, Tetralogy of Fallot.
**Figure S7.** Filtering of possible disease-relevant structural variations identified in affected TOF twins using whole genome sequencing. H, healthy; TOF, Tetralogy of Fallot; SV, structural variation; VEP, Ensembl variant effect predictor.
Figure S8. Global DNA methylation levels of twins. Line plots show the DNA methylation along the chromosomal length (GRCh38.p13/hg38). (A) Methylation levels in Twin1_H and Twin1_TOF. (B) Methylation levels in Twin2_H and Twin2_TOF. H, healthy; TOF, Tetralogy of Fallot.
Figure S9. DNA methylation rate and coverage of CpGs. (A) Methylation and coverage in Twin1_H and Twin1_TOF. (B) Methylation and coverage in Twin2_H and Twin2_TOF. H, healthy; TOF, Tetralogy of Fallot.
| Sample                        | Dups (%) | GC (%) | Total reads (million) | Mean base quality (Pred) |
|-------------------------------|----------|--------|-----------------------|--------------------------|
| Twin1_H_TD180611543_HNCVWCCXY_L4_1 | 11.70%  | 40%    | 78.5                  | 38.6                     |
| Twin1_H_TD180611543_HNCVWCCXY_L4_2 | 10.50%  | 41%    | 78.5                  | 37.2                     |
| Twin1_H_TD180611543_HNCVWCCXY_L5_1 | 11.30%  | 40%    | 77.5                  | 38.6                     |
| Twin1_H_TD180611543_HNCVWCCXY_L5_2 | 10.70%  | 41%    | 77.5                  | 37.2                     |
| Twin1_H_TD180611543_HNCVWCCXY_L6_1 | 10.30%  | 40%    | 78.3                  | 38.7                     |
| Twin1_H_TD180611543_HNCVWCCXY_L6_2 | 10.30%  | 41%    | 78.3                  | 37.3                     |
| Twin1_H_TD180611543_HNCVWCCXY_L7_1 | 10.90%  | 40%    | 78.0                  | 38.6                     |
| Twin1_H_TD180611543_HNCVWCCXY_L7_2 | 10.30%  | 41%    | 78.0                  | 37.2                     |
| Twin1_TOF_TD180611542_HNCVWCCXY_L4_1 | 10.70%  | 40%    | 79.6                  | 38.6                     |
| Twin1_TOF_TD180611542_HNCVWCCXY_L4_2 | 9.60%   | 41%    | 79.6                  | 37.0                     |
| Twin1_TOF_TD180611542_HNCVWCCXY_L5_1 | 10.10%  | 40%    | 79.0                  | 38.6                     |
| Twin1_TOF_TD180611542_HNCVWCCXY_L5_2 | 9.60%   | 41%    | 79.0                  | 37.0                     |
| Twin1_TOF_TD180611542_HNCVWCCXY_L6_1 | 9.30%   | 40%    | 79.8                  | 38.6                     |
| Twin1_TOF_TD180611542_HNCVWCCXY_L6_2 | 9.10%   | 41%    | 79.8                  | 37.1                     |
| Twin1_TOF_TD180611542_HNCVWCCXY_L7_1 | 9.90%   | 40%    | 79.4                  | 38.6                     |
| Twin1_TOF_TD180611542_HNCVWCCXY_L7_2 | 9.20%   | 41%    | 79.4                  | 37.0                     |
| Twin2_H_TD180611545_HNCVWCCXY_L4_1 | 11.30%  | 40%    | 85.3                  | 38.6                     |
| Twin2_H_TD180611545_HNCVWCCXY_L4_2 | 10.20%  | 41%    | 85.3                  | 37.2                     |
| Twin2_H_TD180611545_HNCVWCCXY_L5_1 | 10.80%  | 40%    | 84.8                  | 38.6                     |
| Twin2_H_TD180611545_HNCVWCCXY_L5_2 | 10.20%  | 41%    | 84.8                  | 37.2                     |
| Twin2_H_TD180611545_HNCVWCCXY_L6_1 | 10.00%  | 40%    | 85.6                  | 38.6                     |
| Twin2_H_TD180611545_HNCVWCCXY_L6_2 | 9.90%   | 41%    | 85.6                  | 37.3                     |
| Twin2_H_TD180611545_HNCVWCCXY_L7_1 | 10.70%  | 40%    | 85.3                  | 38.6                     |
| Twin2_H_TD180611545_HNCVWCCXY_L7_2 | 10.00%  | 41%    | 85.3                  | 37.2                     |
| Twin2_TOF_TD180611544_HNCVWCCXY_L4_1 | 11.70%  | 40%    | 89.7                  | 38.7                     |
| Twin2_TOF_TD180611544_HNCVWCCXY_L4_2 | 10.40%  | 40%    | 89.7                  | 37.3                     |
| Twin2_TOF_TD180611544_HNCVWCCXY_L5_1 | 11.40%  | 40%    | 89.1                  | 38.6                     |
| Twin2_TOF_TD180611544_HNCVWCCXY_L5_2 | 10.70%  | 40%    | 89.1                  | 37.3                     |
| Twin2_TOF_TD180611544_HNCVWCCXY_L6_1 | 10.70%  | 40%    | 90.0                  | 38.7                     |
| Twin2_TOF_TD180611544_HNCVWCCXY_L6_2 | 10.40%  | 40%    | 90.0                  | 37.4                     |
| Twin2_TOF_TD180611544_HNCVWCCXY_L7_1 | 11.20%  | 40%    | 89.7                  | 38.6                     |
| Twin2_TOF_TD180611544_HNCVWCCXY_L7_2 | 10.40%  | 40%    | 89.7                  | 37.3                     |
| **average**                  | **10.42%** | **40.4%** | **83.1**          | **37.91**                  |

**Table S1.** Overview of sequencing reads obtained from whole genome sequencing. Paired-end Illumina sequencing (2x150 bp) was performed. For each sample, ‘L’ denotes the sequencing lane and ‘*_1’/’*_2’ represents the forward and reverse reads, respectively.
Table S2. Statistics of read mapping, coverage and read depth in each sample obtained from whole genome sequencing. PE, paired-end; SE, single-end.
| Sample         | Dups (%) | GC (%) | Total reads (million) | Mean base quality (Pred) |
|---------------|----------|--------|-----------------------|-------------------------|
| Sp333_L1_1    | 10.7%    | 21%    | 60.7                  | 38.61                   |
| Sp333_L1_2    | 7.2%     | 21%    | 60.7                  | 35.96                   |
| Sp333_L2_1    | 10.8%    | 21%    | 60.9                  | 38.59                   |
| Sp333_L2_2    | 8.8%     | 21%    | 60.9                  | 35.42                   |
| Sp333_L3_1    | 11.1%    | 21%    | 61.5                  | 38.62                   |
| Sp333_L3_2    | 10.9%    | 21%    | 61.5                  | 35.93                   |
| Sp333_L4_1    | 11.4%    | 21%    | 60.7                  | 38.59                   |
| Sp333_L4_2    | 10.8%    | 21%    | 60.7                  | 35.84                   |
| Sp333_L5_1    | 11.4%    | 21%    | 60.8                  | 38.57                   |
| Sp333_L5_2    | 10.7%    | 21%    | 60.8                  | 35.88                   |
| Sp333_L6_1    | 11.6%    | 21%    | 60.8                  | 38.59                   |
| Sp333_L6_2    | 10.6%    | 21%    | 60.8                  | 35.79                   |
| Sp334_L1_1    | 9.5%     | 21%    | 52.8                  | 38.61                   |
| Sp334_L1_2    | 6.9%     | 21%    | 52.8                  | 35.28                   |
| Sp334_L2_1    | 9.6%     | 21%    | 52.9                  | 38.60                   |
| Sp334_L2_2    | 7.9%     | 21%    | 52.9                  | 34.74                   |
| Sp334_L3_1    | 9.9%     | 21%    | 53.1                  | 38.62                   |
| Sp334_L3_2    | 9.3%     | 21%    | 53.1                  | 35.21                   |
| Sp334_L4_1    | 10.1%    | 21%    | 52.9                  | 38.60                   |
| Sp334_L4_2    | 9.4%     | 21%    | 52.9                  | 35.11                   |
| Sp334_L5_1    | 10.2%    | 21%    | 53.0                  | 38.58                   |
| Sp334_L5_2    | 9.4%     | 21%    | 53.0                  | 35.16                   |
| Sp334_L6_1    | 10.2%    | 21%    | 52.9                  | 38.59                   |
| Sp334_L6_2    | 9.2%     | 21%    | 52.9                  | 35.06                   |
| Sp335_L1_1    | 9.9%     | 21%    | 58.1                  | 38.57                   |
| Sp335_L1_2    | 6.7%     | 21%    | 58.1                  | 35.33                   |
| Sp335_L2_1    | 10.3%    | 21%    | 58.4                  | 38.56                   |
| Sp335_L2_2    | 8.4%     | 21%    | 58.4                  | 34.87                   |
| Sp335_L3_1    | 10.5%    | 21%    | 58.5                  | 38.58                   |
| Sp335_L3_2    | 10.1%    | 21%    | 58.5                  | 35.36                   |
| Sp335_L4_1    | 10.8%    | 21%    | 58.1                  | 38.56                   |
| Sp335_L4_2    | 10.2%    | 21%    | 58.1                  | 35.26                   |
| Sp335_L5_1    | 10.8%    | 21%    | 58.2                  | 38.53                   |
| Sp335_L5_2    | 10.1%    | 21%    | 58.2                  | 35.30                   |
| Sp335_L6_1    | 10.9%    | 21%    | 58.2                  | 38.55                   |
| Sp335_L6_2    | 10.0%    | 21%    | 58.2                  | 35.21                   |
| Sp336_L1_1    | 10.6%    | 21%    | 57.4                  | 38.53                   |
| Sp336_L1_2    | 7.3%     | 22%    | 57.4                  | 35.32                   |
| Sp336_L2_1    | 10.7%    | 21%    | 57.5                  | 38.52                   |
| Sp336_L2_2    | 8.7%     | 22%    | 57.5                  | 34.81                   |
| Sp336_L3_1    | 10.8%    | 21%    | 57.7                  | 38.54                   |
| Sp336_L3_2    | 10.3%    | 22%    | 57.7                  | 35.30                   |
| Sp336_L4_1    | 11.0%    | 21%    | 57.4                  | 38.52                   |
| Sp336_L4_2    | 10.3%    | 22%    | 57.4                  | 35.20                   |
| Sp336_L5_1    | 11.2%    | 21%    | 57.5                  | 38.50                   |
| Sp336_L5_2    | 10.3%    | 22%    | 57.5                  | 35.25                   |
| Sp336_L6_1    | 11.3%    | 21%    | 57.4                  | 38.51                   |
| Sp336_L6_2    | 10.2%    | 22%    | 57.4                  | 35.15                   |
| **average**   | 10.0%    | 21.1%  | 57.4                  | 36.95                   |

Table S3. Overview of sequencing reads obtained from whole genome bisulfite sequencing. Paired-end Illumina sequencing (2x150 bp) was performed. For each sample, 'L' denotes the sequencing lane and "*_1"/"*_2" represents the forward and reverse reads, respectively.
| Sample        | Total reads  | Aligned reads | Unaligned reads | Ambiguously aligned reads | Duplicates (removed) | Unique reads (remaining) |
|--------------|--------------|---------------|-----------------|---------------------------|---------------------|--------------------------|
| Sp333_RDM00799_L1   | 60713900     | 47385992      | 11020584        | 2307323                   | 5189315             | 42196677                 |
| Sp333_RDM00799_L2   | 60893917     | 46199044      | 12445190        | 2249682                   | 5134995             | 41064049                 |
| Sp333_RDM00799_L3   | 61492618     | 47941470      | 11220346        | 2330802                   | 5487141             | 42453429                 |
| Sp333_RDM00799_L4   | 60709131     | 47151752      | 11261855        | 2295523                   | 5388325             | 41763427                 |
| Sp333_RDM00799_L5   | 60734267     | 47279615      | 11151329        | 2303322                   | 5500015             | 41779600                 |
| Sp333_RDM00799_L6   | 60772843     | 47201470      | 11270561        | 2300811                   | 5579960             | 41621510                 |
| Sp334_RDM00800_L1   | 52881578     | 42765055      | 8112712         | 1933811                   | 3788216             | 38976839                 |
| Sp334_RDM00800_L2   | 52869973     | 41547389      | 9448694         | 1873888                   | 3745131             | 37802258                 |
| Sp334_RDM00800_L3   | 53073278     | 42862058      | 8277457         | 1933762                   | 3975946             | 38861112                 |
| Sp334_RDM00800_L4   | 52907527     | 42575157      | 8410260         | 1922110                   | 3951599             | 38623558                 |
| Sp334_RDM00800_L5   | 52970756     | 42740812      | 8298848         | 1931094                   | 4042902             | 38697910                 |
| Sp334_RDM00800_L6   | 52880739     | 42553677      | 8406457         | 1920605                   | 4086371             | 38467306                 |
| Sp335_RDM00801_L1   | 58057558     | 43962495      | 11948026        | 2147033                   | 4600494             | 39362001                 |
| Sp335_RDM00801_L2   | 58331772     | 42931982      | 13304995        | 2094791                   | 4569984             | 38361998                 |
| Sp335_RDM00801_L3   | 58434542     | 44208796      | 12070948        | 2154794                   | 4838393             | 39370403                 |
| Sp335_RDM00801_L4   | 58102451     | 43793880      | 12175556        | 2133013                   | 4789273             | 39004607                 |
| Sp335_RDM00801_L5   | 58145454     | 43929444      | 12074280        | 2141726                   | 4889574             | 39039870                 |
| Sp335_RDM00801_L6   | 58218810     | 43881767      | 12194631        | 2142107                   | 4963750             | 39818017                 |
| Sp336_RDM00802_L1   | 57337526     | 38671981      | 16656058        | 2009485                   | 3765512             | 34906469                 |
| Sp336_RDM00802_L2   | 57461325     | 37640382      | 17867931        | 1953011                   | 3723665             | 33916717                 |
| Sp336_RDM00802_L3   | 57656206     | 38835755      | 16807423        | 2013024                   | 3947861             | 34887894                 |
| Sp336_RDM00802_L4   | 57403215     | 38509393      | 16895107        | 1998711                   | 3912006             | 34597387                 |
| Sp336_RDM00802_L5   | 57474641     | 38653792      | 16814326        | 2006521                   | 4003857             | 34649935                 |
| Sp336_RDM00802_L6   | 57411843     | 38510060      | 16898712        | 2003071                   | 4044545             | 34465515                 |

Table S4. Mapping result of reads obtained from whole genome bisulfite sequencing. For each sample, 'L' denotes the sequencing lane.
|                      | Twin1_H | Twin1_TOF | Twin2_H | Twin2_TOF |
|----------------------|---------|-----------|---------|-----------|
| Mean depth           | 24.0    | 22.1      | 22.4    | 19.8      |
| SD depth             | 37.31   | 37.57     | 36.59   | 43.77     |
| Mean mapping quality (Pred) | 36.01   | 35.83     | 35.62   | 35.43     |
| Mean insert size (bp) | 256.24  | 255.93    | 251.37  | 255.33    |
| SD insert size (bp)  | 54.78   | 56.80     | 55.08   | 56.77     |
| GC (%)               | 20.9    | 20.6      | 21.5    | 22.2      |
| Methylated CpG (%)   | 83.5    | 84.6      | 80.9    | 81.1      |
| Methylated CHG (%)   | 0.5     | 0.6       | 0.5     | 0.5       |
| Methylated CHH (%)   | 0.4     | 0.5       | 0.5     | 0.5       |
| Cs (million)         | 10504   | 9347      | 9875    | 8860      |
| Dups (%)             | 12.1    | 10.0      | 11.5    | 11.1      |
| Deduplicated reads (million) | 249.1 | 229.5      | 232.4   | 205.2     |
| Duplicated reads (million) | 34.1   | 25.5      | 30.3    | 25.6      |

Table S5. Read statistics after mapping and deduplication as well as methylation rates over CpGs, CHGs and CHHs in each sample obtained from whole genome bisulfite sequencing.
| Chr | Start   | End       | Copy number | Type | Associated gene(s) |
|-----|---------|-----------|-------------|------|--------------------|
| 4   | 68550000| 68600000  | 4           | gain | UGT2B17 |
| 5   | 46450000| 46500000  | 3           | gain | - |
| 7   | 38250000| 38350000  | 3           | gain | TRGJ2, TRGJP2, TRGC1, TRGJ1, TRGJP, TARP |
| 9   | 60700000| 60750000  | 3           | gain | - |
| 10  | 39650000| 39700000  | 3           | gain | - |
| 13  | 86200000| 86250000  | 3           | gain | - |
| 18  | 20850000| 20900000  | 1           | loss | - |
| 19  | 54850000| 54900000  | 3           | gain | KIR3DL2, FCAR |
| 21  | 54500000| 55000000  | 3           | gain | - |
| 21  | 58000000| 58500000  | 3           | gain | - |
| 21  | 71500000| 72000000  | 1           | loss | - |
| 1   | 228550000| 228600000 | 3           | gain | RNA5SP19 |
| 5   | 46450000| 46500000  | 3           | gain | - |
| 14  | 41150000| 41200000  | 1           | loss | - |
| 18  | 20850000| 20900000  | 1           | loss | - |

**Table S6.** Copy number variations identified in Twin1_TOF and Twin2_TOF based on whole genome sequencing data. Positions based human reference genome (GRCh38.p13/hg38).
| Chr | Pos | ID       | REF | ALT | Associated gene | GT:FT:GQ:PL:PR(:SR) |
|-----|-----|----------|-----|-----|-----------------|---------------------|
| 21  | 3868664 | MantaINS:117:0:0:0:2:0 | A | T | MantaDEL:82892:0:0:0:0:0 | GGGGAGGACGACAGGGGAGG |
| 21  | 4567462 | MantaINS:523:0:0:0:0:0 | CCGGGCGGGGGGACGAGCGCCGGGTTCCGTCCAAGCCCT | GRM4(chr1:234776356[A]GTT:FT:GQ:PL:PR(:SR)
| 21  | 7373258 | MantaINS:45134:0:0:0:0:0 | AGCATTGCATAGGCATGGCATCACATACACGAGCATTGCATAGGCACGG | DNAH17(chr1:205209646[GTGAATATCTGTGGCTCAGAGCTGAGCCCTGCCGTCCCCTGCCAGGAGAGGAGGCTGCTGGGCGGCGCAGGCAGTGTGGCTCAGAGCTGAGCCGAGATCGCGCCACTGCACTCCAGCCTGGGCGACAGAGCGGCAGGAGAATGGCGTGAACCCGGGAGGCGGAGCTTGAGAGAAATCAAGCTGTGTTGCAGGCCTGATAAATTCTCAGTCCCACGGTGCTTCAATACCTGTGAAAAGAAGGGAGTGAAAGCTGGATTGGGCAAAGA |
| 21  | 82409 | MantaINS:82409:0:0:0:0:0 | GA | TA | MantaINS:13464:0:0:0:0:1 | ACCACACCAGGCCACACCACACCACACCACACCACGCCACACCACACAGCCACTGGGACTACAGGCGCCCGCCACTACGGGGATCTCGGCTCACTGCAAGCTCCGCCTCCCGGGTTCACGCCATTCTC |
| 21  | 90162 | MantaINS:90162:0:0:0:0:0 | TGTGTTGTTCCTCTCATGTGTCCATGTGTTTTCATTGTTCAGCTCCCACTTA | DSTYK(chr1:205209646[GTGAATATCTGTGGCTCAGAGCTGAGCCCTGCCGTCCCCTGCCAGGAGAGGAGGCTGCTGGGCGGCGCAGGCAGTGTGGCTCAGAGCTGAGCCGAGATCGCGCCACTGCACTCCAGCCTGGGCGACAGAGCGGCAGGAGAATGGCGTGAACCCGGGAGGCGGAGCTTGAGAGAAATCAAGCTGTGTTGCAGGCCTGATAAATTCTCAGTCCCACGGTGCTTCAATACCTGTGAAAAGAAGGGAGTGAAAGCTGGATTGGGCAAAGA |
| 21  | 91423 | MantaINS:91423:0:0:0:0:0 | CCGGGCGGGGGGACGAGCGCCGGGTTCCGTCCAAGCCCT | GRM4(chr1:234776356[A]GTT:FT:GQ:PL:PR(:SR)
| 21  | 93747 | MantaINS:93747:0:0:0:0:0 | CCGGGCGGGGGGACGAGCGCCGGGTTCCGTCCAAGCCCT | GRM4(chr1:234776356[A]GTT:FT:GQ:PL:PR(:SR)
| 21  | 702739 | MantaINS:702739:0:0:0:0:0 | GTGAGAATATC | CT | MantaBND:68499:0:1:0:0:0 | A
| 21  | 397401 | MantaBND:616:0:1:0:0:0 | 0/1:PASS:348:576,0,345:10,6:25,12 | RETREG1(chr1:236374908[CTCTGCCGCAGGTGGATGTGCGTGGGGGACACTGTGATGACCTGTGTCCTCCCTGCCGTCCCCTGCCAGGAGAGGAGGCTGCTGGGCGGCGCAGGCAGTGTGGCTCAGAGCTGAGCCGAGATCGCGCCACTGCACTCCAGCCTGGGCGACAGAGCGGCAGGAGAATGGCGTGAACCCGGGAGGCGGAGCTTGAGAGAAATCAAGCTGTGTTGCAGGCCTGATAAATTCTCAGTCCCACGGTGCTTCAATACCTGTGAAAAGAAGGGAGTGAAAGCTGGATTGGGCAAAGA |
| 21  | 39991 | MantaBND:7212:0:1:0:0:0 | 1/1:PASS:17:219,20,0:0,0:0,9 | 
| 21  | 41174 | MantaBND:45134:0:1:0:0:1 | 1/1:PASS:203:339,0,200:9,2:18,7 | AL355377.3(chr22:17720154[T]AA
| 21  | 45675 | MantaBND:93747:0:0:0:0:0 | 0/1:PASS:137:187,0,999:23,1:68,10 | L3MBTL4(chr1:205209478[ATTAGTG]

**Table S7.** Structural variations uniquely identified in Twin1_TOF based on whole genome sequencing data. Positions based human reference genome (GRCh38.p13/hg38).
| Chr | Pos  | ID           | REF | ALT | Assoicated gene | GT:FT:GQ:PL:PR (:SR)                  |
|-----|------|--------------|-----|-----|----------------|-------------------------------------|
| chr1| 3868664 | MantaINS:126:0:0:0:0:0 | A   | ACCACCCCAGGCCACGCCACACCAAGCCACACCACACCAGGCCACACCACACCATACCACACCACACCAGGCCACACCAC | DFFB 1/1:PASS:25:387,28,0:0,16 |
| chr1 | 16725255 | MantaBND:687:0:1:0:0:0:0 | C   | C[chr1:234776370]C | 0/1:PASS:297:347,0,488:36,29      |
| chr1 | 206923954 | MantaDUP:TANDEM:6517:0:1:0:0:0:0 | T   | T<DUP:TANDEM>PIGR | 0/1:PASS:30:80,0,593:19,4:34,5    |
| chr11| 63931436 | MantaDEL:64286:0:1:0:0:0:0 | A   | <DEL>NAA40   | 0/1:PASS:431:481,0,568:25,10:35,8 |
| chr11 | 122084984 | MantaINS:64833:0:0:0:0:0:0 | T   | TCTCCCCACCCTCCACACCCCACCCCTCATTCTTCCCCACCCCCACTCCTCCTCCACCCACCCTTC | TENM4 1/1:PASS:33:557,36,0:0,0:0,12 |
| chr15 | 41559198 | MantaBND:72779:0:2:0:0:0:0 | C   | [chr13:43495691]GC | 0/1:PASS:195:245,0,488:10,7:25,5  |
| chr16 | 56518771 | MantaINS:82820:0:0:0:0:0:0 | A   | AGCATTTTGGGCATGCCTCCGTCAAATACACAGGGCTCTGATTTGCCTTG | BBS2 0/1:PASS:174:224,0,305:1,0:20,7 |
| Gene        | Overlap | Tissue (RV) | iPSC-derived CMs (day 15) | iPSC-derived CMs (day 60) |
|-------------|---------|-------------|----------------------------|---------------------------|
|             |         | NH (n=4)    | TOF (n=18)                | Healthy (n=2; 2-3 clones each) | Healthy (n=2; 3 clones each) | TOF (n=1, 3 clones) |
| Twin1_TOF & Twin2_TOF |         |             |                            |                           |                          |                     |
| AL355377.3  |         | 0.0         | 0.0                        | 0.0                       | 0.0                      | 0.0                 |
| DFFB        | 1,4     | 2.6         | 3.9                        | 7.3                       | 8.7                      | 4.4                 |
| FTH1P20     |         | 0.0         | 0.0                        | 4.4                       | 3.7                      | 6.4                 |
| NAA40       | 3       | 6.0         | 9.2                        | 14.2                      | 20.4                     | 14.5                |
| RIPOR2      |         | 0.0         | 0.0                        | 0.0                       | 0.0                      | 0.0                 |
| Twin1_TOF   |         |             |                            |                           |                          |                     |
| ANTXR1      | 2,3     | 4.5         | 9.9                        | 24.7                      | 29.5                     | 64.7                |
| AVL9        |         | 2.1         | 1.9                        | 6.2                       | 6.1                      | 4.3                 |
| CCNA1       |         | 0.0         | 0.0                        | 0.6                       | 0.1                      | 0.4                 |
| DNAH17      |         | 0.2         | 0.2                        | 0.5                       | 0.4                      | 0.2                 |
| DPP6        |         | 0.1         | 0.1                        | 0.4                       | 0.4                      | 0.1                 |
| DSTYK       |         | 2.0         | 2.5                        | 4.4                       | 3.7                      | 4.8                 |
| GRM4        | 3       | 0.1         | 0.2                        | 0.4                       | 0.1                      | 0.0                 |
| KAZN        | 3       | 0.7         | 0.5                        | 6.4                       | 6.1                      | 4.1                 |
| L3MBTL4     | 2,3,4,6 | 0.2         | 1.2                        | 4.5                       | 2.6                      | 4.5                 |
| LINC01348   |         | 0.0         | 0.0                        | 0.0                       | 0.0                      | 0.0                 |
| NDUFA13     | 1       | 1836.7      | 1749.8                     | 114.7                     | 163.8                    | 123.0               |
| PRDM15      |         | 1.3         | 1.0                        | 2.5                       | 3.1                      | 2.1                 |
| RETREG1     |         | 0.0         | 0.0                        | 0.0                       | 0.0                      | 0.0                 |
| SAMD10      | 4       | 0.5         | 1.9                        | 2.0                       | 1.6                      | 1.2                 |
| SIMC1       | 4       | 0.5         | 0.7                        | 12.1                      | 10.2                     | 6.6                 |
| Twin2_TOF   |         |             |                            |                           |                          |                     |
| BBS2        | 1,3     | 7.1         | 8.6                        | 54.6                      | 45.0                     | 34.2                |
| EIF2S3      | 1       | 13.0        | 18.3                       | 184.1                     | 110.4                    | 121.5               |
| ERG         |         | 3.4         | 3.8                        | 0.6                       | 0.3                      | 3.0                 |
| GAK         | 4       | 7.0         | 7.5                        | 17.1                      | 20.1                     | 12.3                |
| MIR100HG    |         | 3.7         | 7.0                        | 7.0                       | 1.6                      | 2.5                 |
| PIGR        |         | 0.7         | 0.8                        | 0.1                       | 0.0                      | 0.0                 |
| TENM4       | 3       | 0.1         | 0.1                        | 8.8                       | 7.2                      | 3.5                 |
| TYRO3       |         | 1.1         | 1.1                        | 15.3                      | 26.0                     | 8.1                 |

Table S9. Candidate genes with structural variations in Twin1_TOF and/or Twin2_TOF. Expression is given in RPKM (tissue; Grunert et al. 2014) and TPM (CMs; Grunert et al. 2020) values, respectively. Overlap with list of cardiovascular-associated genes is indicated by ‘1’ and overlaps with CHD-related datasets are indicated by ‘2’ (expression), ‘3’ (methylation), ‘4’ (CNV), ‘5’ (CHD gene), and ‘6’ (miRtarget). CHD, congenital heart disease; CNV, copy number variation; CMs, cardiomyocytes; H, healthy; iPSC, induced pluripotent stem cell; NH, normal heart; RPKM, reads per kilo base per million mapped reads; RV, right ventricle; TOF, Tetralogy of Fallot; TPM, transcript per million.