**Supplementary information for:**

# CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes

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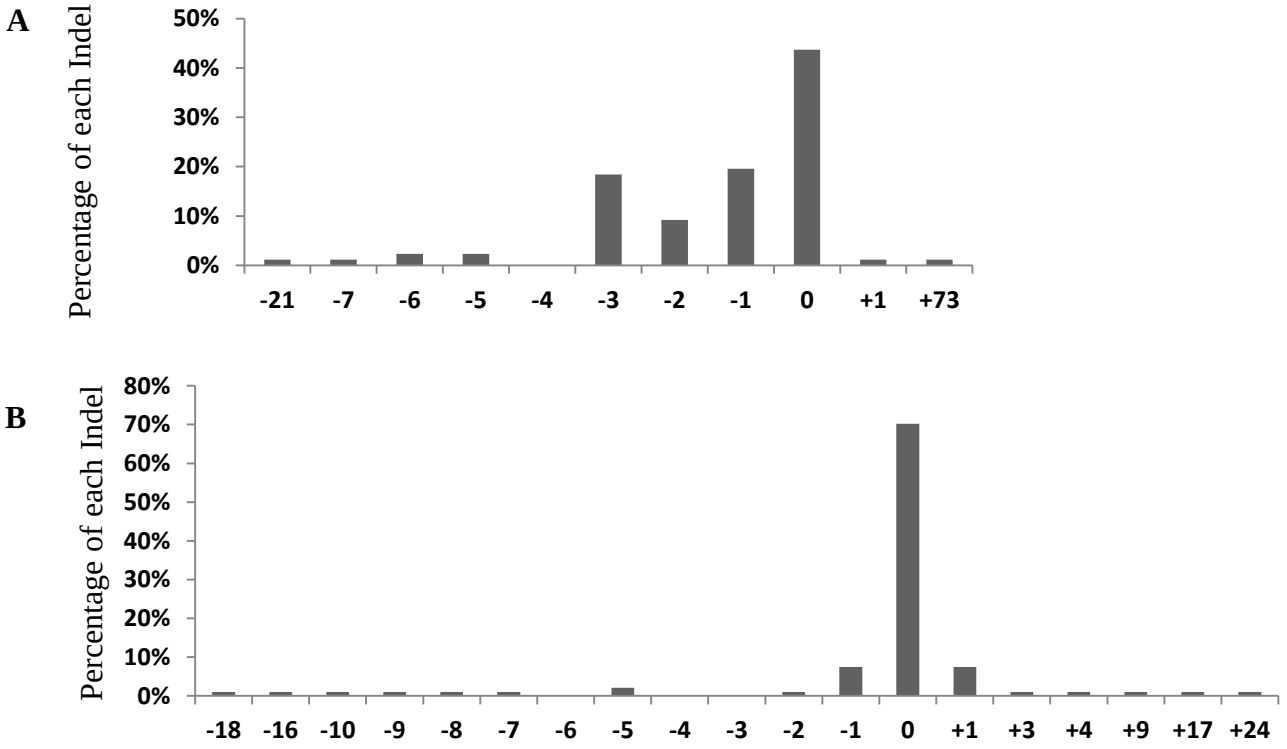
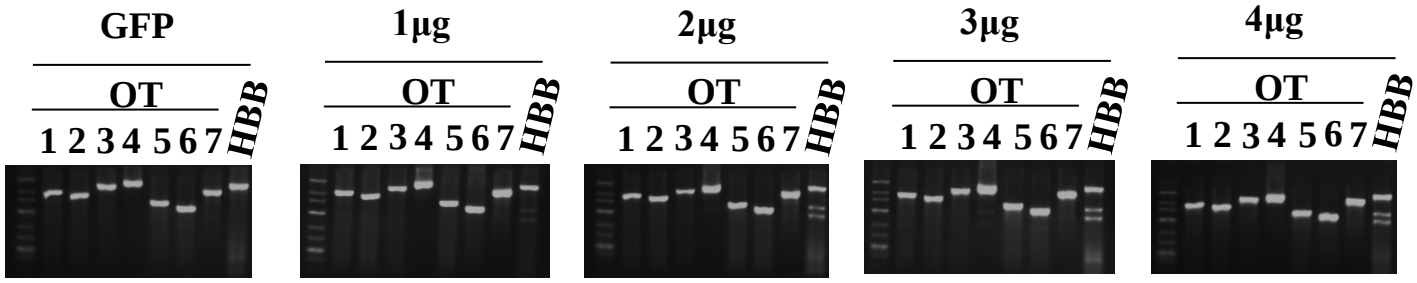
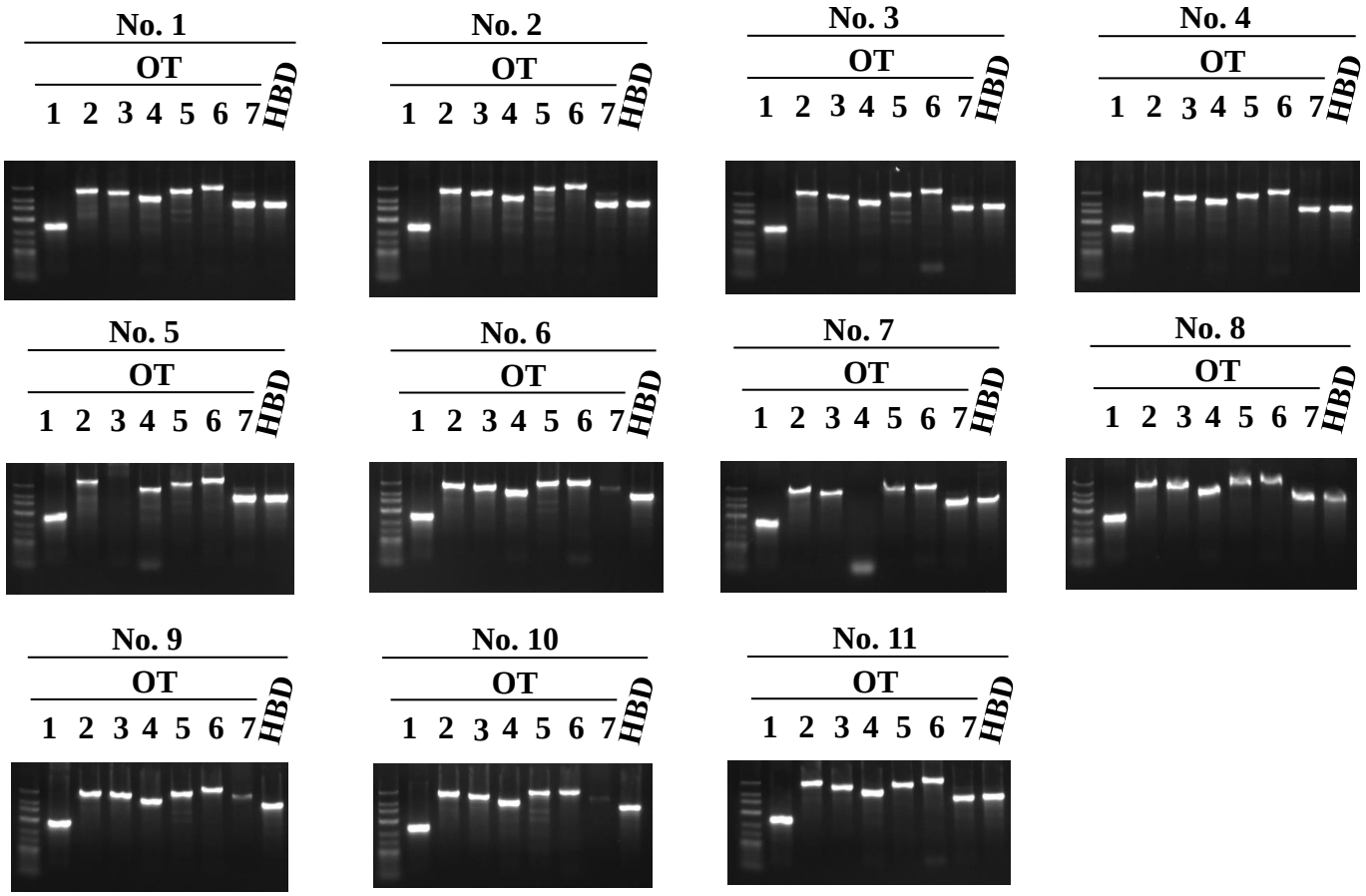
## Supplementary figures



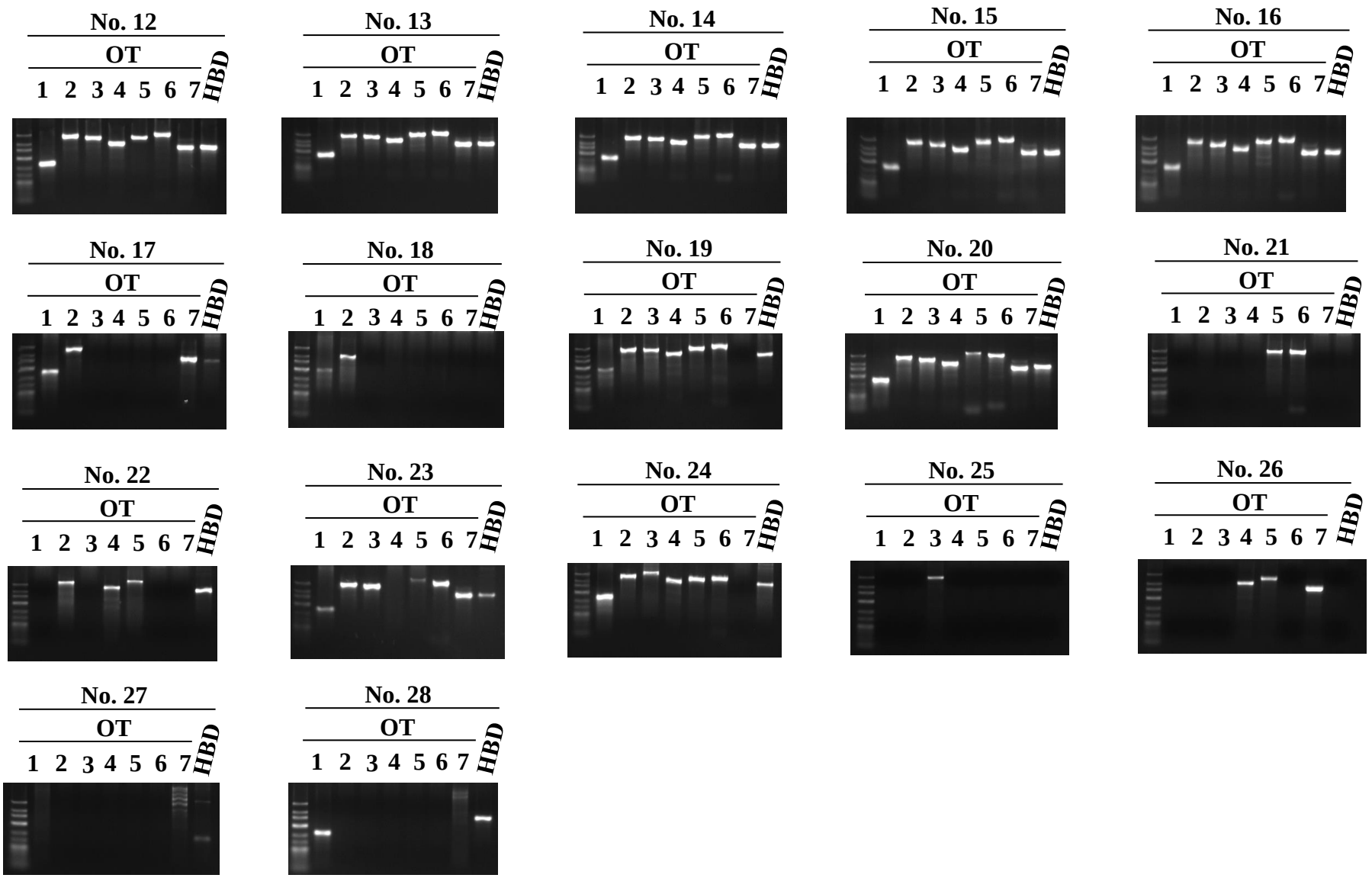

**Supplementary Fig 1. Indel spectra of G1 and G2 gRNAs.** 293T cells were transfected with 2μg of pX330-G1 **(A)** or pX330-G2 (**B**). The region spanning each target site was PCR amplified. And the PCR products were subcloned into TA vectors and sequenced. About 50 clones were sequenced, and the results are summarized here. The size of each indel was calculated. The y-axis shows the percentage of each indel.



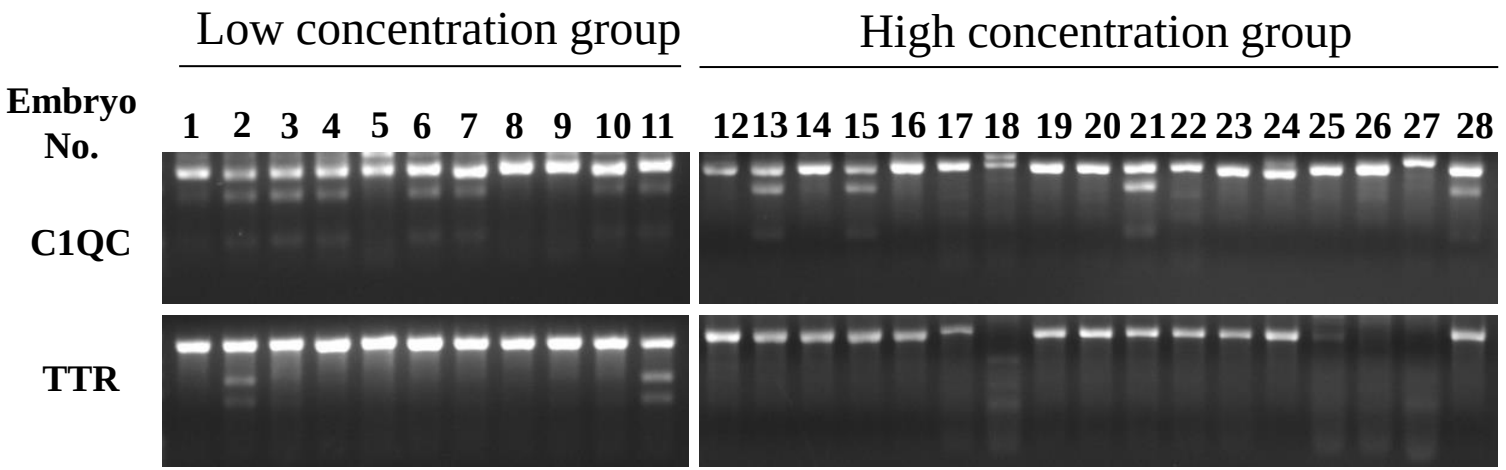

**Supplementary Fig 2. G2 gRNA showed detectable off-target cleavage at OT-4 site.** 293T cells were transfected with increasing concentrations (1µg, 2µg, 3µg, 4µg) of the G2 gRNA-Cas9 vector. A GFP expression vector was used as transfection control. Regions spanning the top 7 predicted off-target sites for each gRNA were PCR amplified for the T7E1 assay. OT, off-target. HBB, on-target editing in the HBB gene locus.



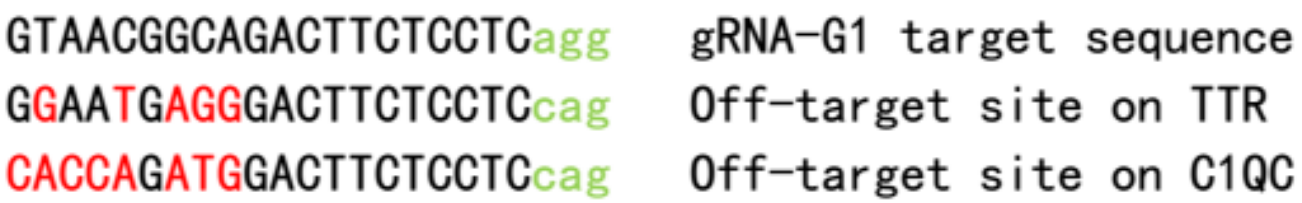

**Supplementary Fig 3. Assessment of off-target cleavage in human 3PN embryos in the low concentration (100/20) group by the T7E1 assay.** Seven predicted potential off-target (OT) sites and the site in *HBD* locus were amplified by PCR for the T7E1 assay to detect CRISPR/Cas9–mediated cleavage. The smaller bands in each lane indicate off-target cleavage.




**Supplementary Fig 4. Assessment of off-target cleavage in human 3PN embryos in the high concentration (200/40) group by the T7E1 assay**. Eight potential off-target sites were amplified by PCR for the T7E1 assay. The smaller bands in each lane indicate off-target cleavage. Some of the target sites failed to be amplified in some samples.




**Supplementary Fig 5**. **Confirmation of off-target cleavage in the C1QC and TTR loci in human 3PN embryos by the T7E1 assay**. Two candidate off-target sites, detected by exome sequencing, were amplified by PCR for the T7E1 assay to detect CRISPR/Cas9–mediated cleavage. The smaller bands in each lane indicate off-target cleavage. Some of the target sites failed to be amplified in some samples.




**Supplementary Fig 6. The off-target sites in the *TTR* gene and *C1QC* gene**.
PAM, green. Mismatched nucleotides, red.

**Supplementary Table 1. List of PCR primers to amplify genomic regions, assess editing status, and examine off-target sites for G1 and G2 gRNAs.**

| Primers | Locus | Direction | Sequence (5' to 3') |
|---|---|---|---|
| HBB-T7E1 | chr11:5248231 | F | AGTCCAACTCCTAAGCCAGTG |
| | | R | GAGGTTGTCCAGGTGAGCC |
| G1-OT1 | chr3:181783903 | F | TGTCAAGGTTTATGAGAGGTCTG |
| | | R | CATGTGTTCTGTGTATGTGTGTGTA |
| G1-OT2 | chr1:227894389 | F | AGAGGGGGCTGACACGTTA |
| | | R | TTTGTGTTCTCATGATGCAGCG |
| G1-OT3 | chrX:149810034 | F | GGTTCCGTATCGTGCCTCA |
| | | R | GAACTAGGTGCAGTGATACCGT |
| G1-OT4 | chr11:132762118 | F | CCCTATACCTGGGCTCCGTT |
| | | R | GAAAGGGCCTCTCTCTTTGTAATG |
| G1-OT5 | chr6:158896257 | F | AAGCTCTACAAGGGCAGAGAATG |
| | | R | TCAAAGCTCCCAGATTCACGTT |
| G1-OT6 | chr1:204671648 | F | GGCTCTAGGTGAGCTTGTGG |
| | | R | CCCACCACACTGTCAGTACC |
| G1-OT7 | chr20:30590029 | F | CTGAGACCTGGGGCTGGG |
| | | R | TGGGGGGATTTGGGTGAG |
| HBD- T7E1 | chr11: 5234396 | F | AGAACAGCCAATCTCAGGG |
| | | R | CCAAGGGTAGACCACCAGTA |
| G2-OT1 | chr9:104595866 | F | CGAAATGATTGGAACCATGGGA |
| | | R | CCTCCAGTTTCTAAGAGCGGTG |
| G2-OT2 | chr6:157157457 | F | AGGTAACAGTCGACGTCAGTA |
| | | R | CTTTAACAGGCAAGGACTCAACC |
| G2-OT3 | chr2:179603682 | F | ACCATGCTGAATGGGAACACT |
| | | R | TGAGGCCTTGAATGACAGCG |
| G2-OT4 | chr8:568439 | F | TGCCCTATGCGTGCTCACT |
| | | R | GTAATGTTGCCCAAGGTCTCTG |
| G2-OT5 | chr4:18681607 | F | CAGGAGCTTCCCTTCACAGA |
| | | R | TGGGCAGCAGGAATGAATGA |
| G2-OT6 | chr4:88057690 | F | ATTGCCTAGAGCGCTGCAC |
| | | R | TGGCTGGACAACATGAGTTACC |
| G2-OT7 | chr19:1379107 | F | CTGGGGAGGCTTAGATGGGA |
| | | R | AAAGCGTGCAGGCTTCTGAG |

**Supplementary Table 2. Summary of Indels and Single Nucleotide Variants (SNVs) Detected by Exome Sequencing**

| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| Raw indel | 32,546 | 39,506 | 37,707 | 40,919 | 36,355 | 41279 |
| Indel in exon | 1,182 | 1,267 | 1,180 | 1,327 | 1,232 | 1275 |
| Reads number >2 indel | 1,091 | 1,131 | 1,085 | 1,199 | 1,133 | 1153 |
| Post Low-complexity filter | 698 | 767 | 712 | 795 | 739 | 740 |
| Post Homopolymeric filter | 487 | 521 | 489 | 517 | 487 | 516 |
| F1anking region with potential off-target sites indel | 2 | 1 | 2 | 1 | 2 | 4 |
| On-target indels | 1 | 1 | 1 | 1 | 2 | 4 |
| Candidate off-target indels | 1 | - | 1 | - | - | 0 |
| sample-specific indels | 1 | - | 1 | - | - | 0 |
| Raw SNV | 319,448 | 379,535 | 353,982 | 389,704 | 356,904 | 395349 |
| SNV in exon | 30,987 | 30,444 | 30,168 | 32,701 | 32,126 | 31652 |
| Reads number >2 SNV | 29,178 | 28,531 | 28,512 | 30,838 | 30,202 | 29699 |
| Post Low-complexity filter | 25,979 | 25,146 | 25,291 | 27,373 | 26,854 | 26256 |
| Post Homopolymeric filter | 24,319 | 23,513 | 23,668 | 25,628 | 25,158 | 24572 |
| F1anking region with potential off-target sites SNV | 19 | 15 | 24 | 19 | 25 | 23 |
| sample-specific SNVs | 3 | 1 | 9 | 2 | 9 | 7 |