

## **Supplementary Material**

### **Hi-C analysis of genomic contacts revealed karyotype abnormalities in chicken HD3 cell line**

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**Additional Table 1. List of FISH probes used for verification of translocations identified by Hi-C in HD3 cell line.**

| Probe ID | Chromosomal localization                                                                                                  | Probe origin                                                    | References                                                                                          |
|----------|---------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|
| 54J7     | GGA1: 185647688-185877592                                                                                                 | BAC-clone                                                       | <a href="https://bacpacresources.org/chicken261.htm">https://bacpacresources.org/chicken261.htm</a> |
| 48K18    | GGA2qter*                                                                                                                 | BAC-clone                                                       |                                                                                                     |
| 20I12    | GGA2qter*                                                                                                                 | BAC-clone                                                       |                                                                                                     |
| 177B7    | GGA4: 5725062-5925483                                                                                                     | BAC-clone                                                       |                                                                                                     |
| 33C6     | GGA4: 6419141-6610711                                                                                                     | BAC-clone                                                       |                                                                                                     |
| 48P9     | GGA4: 7108351-7324973                                                                                                     | BAC-clone                                                       |                                                                                                     |
| 47F11    | GGA4: 41547668-41747405                                                                                                   | BAC-clone                                                       |                                                                                                     |
| 4E13     | GGA4: 52490280-52709710                                                                                                   | BAC-clone                                                       |                                                                                                     |
| 161B22   | GGA5: 57365803-57524533                                                                                                   | BAC-clone                                                       |                                                                                                     |
| 94G14    | GGA6: 811765-995465                                                                                                       | BAC-clone                                                       |                                                                                                     |
| 179F2    | GGA6: 35037508-35206756                                                                                                   | BAC-clone                                                       |                                                                                                     |
| 112D24   | GGA7: 121180-292753                                                                                                       | BAC-clone                                                       |                                                                                                     |
| 126G14   | GGA7: 13125892-13385143                                                                                                   | BAC-clone                                                       |                                                                                                     |
| 38E18    | GGA7: 36600879-36791726                                                                                                   | BAC-clone                                                       |                                                                                                     |
| 106I17   | GGA21: 135847-355071                                                                                                      | BAC-clone                                                       |                                                                                                     |
| Cen4     | Centromere region of GGA4                                                                                                 | PCR with specific primers from Cen4 reference sequence AB556725 | Shang et al., 2010; Krasikova et al., 2012                                                          |
| 4p       | GGA4p                                                                                                                     | Needle-based microdissection of GGA4p                           | Maslova et al., 2015                                                                                |
| 4q       | GGA4q                                                                                                                     | Needle-based microdissection of GGA4q                           | Maslova et al., 2015                                                                                |
| Wpaint   | GGAW                                                                                                                      | Needle-based microdissection of GGAW                            |                                                                                                     |
| Zqter    | q-terminal heterochromatic region of GGAZ                                                                                 | Needle-based microdissection of GGAZqter                        |                                                                                                     |
| CNM      | Centromere and pericentromere regions of microchromosomes; centromere regions of GGA6, GGA9, two clusters on GGA3q, GGAWp | Oligonucleotide                                                 | Matzke et al., 1990; Krasikova et al., 2006                                                         |
| PO41     | Subtelomere and pericentromere regions of microchromosomes; subtelomere regions of GGA1p, GGA1q, GGA2q, GGAWp, and GGAZp  | Oligonucleotide                                                 | Wicker et al., 2005; Deryusheva et al., 2007                                                        |
| Tel      | Terminal regions of all chromosomes; interstitial sites on GGA1, GGA2q, GGA3q, and GGAW                                   | Oligonucleotide                                                 | Meyne et al., 1989; Nanda and Schmid, 1994                                                          |

\* - the position of these CHORI-261 BAC clones was specified by FISH on metaphase chromosomes of chicken embryonic fibroblasts (CEF). In GalGal5 chicken genome assembly 48K18 and 20I12 are erroneously placed on GGA14: 12868028-13032026 and GGA14: 13386106-13599690 correspondingly.

## References

1. Shang W-H, Hori T, Toyoda A, Kato J, Popendorf K, Sakakibara Y, et al. Chickens possess centromeres with both extended tandem repeats and short non-tandem-repetitive sequences. *Genome Res.* 2010;20:1219–28.
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4. Matzke MA, Varga F, Berger H, Schernthaner J, Schweizer D, Mayr B, et al. A 41–42 bp tandemly repeated sequence isolated from nuclear envelopes of chicken erythrocytes is located predominantly on microchromosomes. *Chromosoma.* 1990;99:131–7.
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6. Wicker T, Robertson JS, Schulze SR, Feltus FA, Magrini V, Morrison JA, et al. The repetitive landscape of the chicken genome. *Genome Res.* 2005;15(1):126-36.
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8. Meyne J, Ratliff RL, MoYzIs RK. Conservation of the human telomere sequence (TTAGGG) among vertebrates. *Proc Natl Acad Sci USA.* 1989;86(18):7049-53.
9. Nanda I, Schmid M. Localization of the telomeric (TTAGGG)<sub>n</sub> sequence in chicken (*Gallus domesticus*) chromosomes. *Cytogenet Genome Res.* 1994;65:190–3.

**Additional Table 2. Small interchromosomal translocations identified in HD3 cell line based on the analysis of Hi-C heatmaps.**

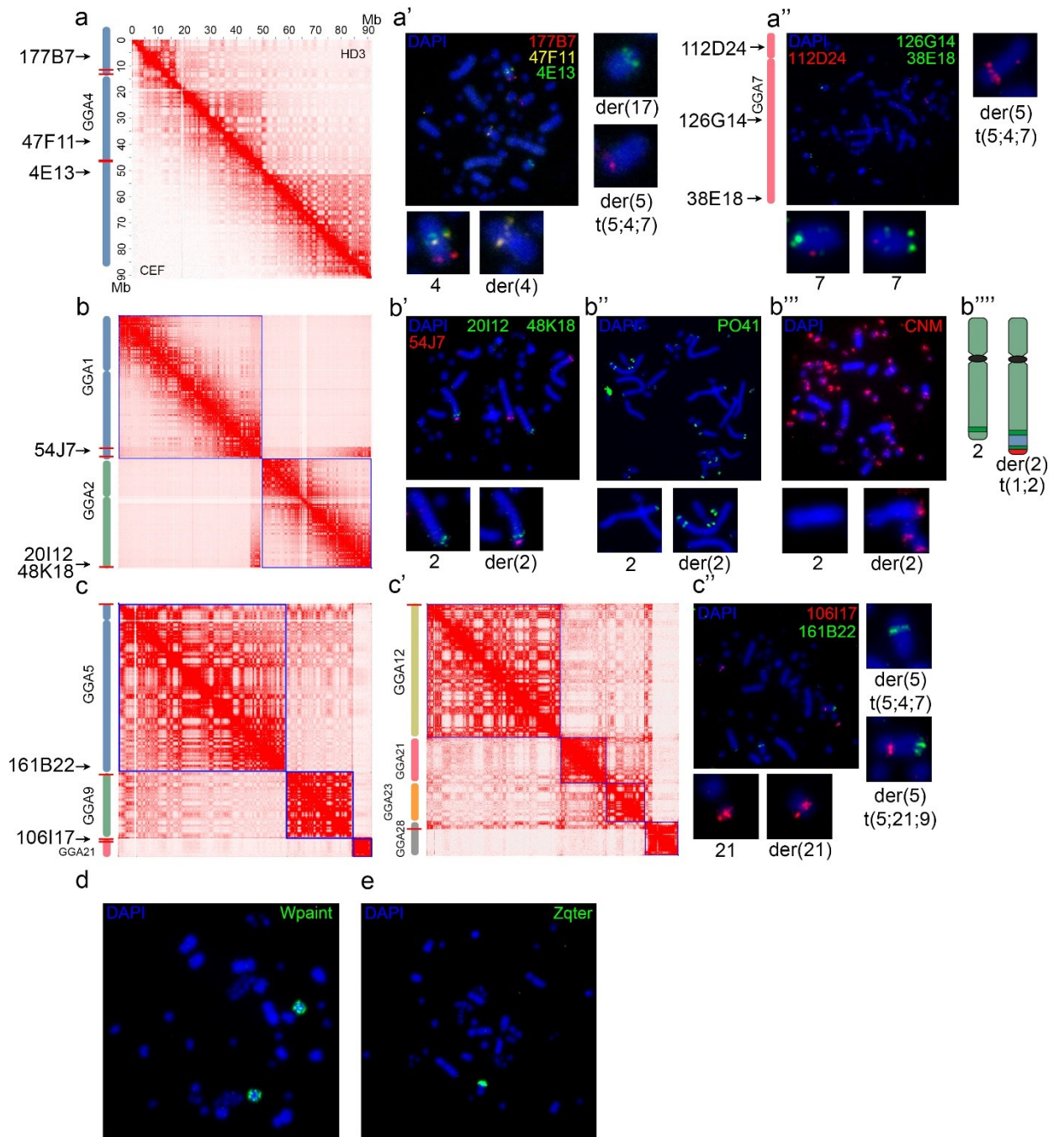
| <b>Interacting chr1</b> | <b>start, ± 5000 bp</b> | <b>end, ± 5000 bp</b> | <b>Interacting chr2</b> | <b>Insertion site / approximate breakpoint position, ± 5000 bp</b> |
|-------------------------|-------------------------|-----------------------|-------------------------|--------------------------------------------------------------------|
| <b>1</b>                | 196140000               | 196200000             | <b>2</b>                | q: ~149460000                                                      |
| <b>1</b>                | 6690000                 | 6710000               | <b>3</b>                | q: ~ 81065000                                                      |
| <b>3</b>                | 3290000                 | 3370000               | <b>1</b>                | q: ~76 815 000                                                     |
| <b>2</b>                | 74715000*               |                       | <b>Z</b>                | 56065000                                                           |
| <b>4</b>                | 45000                   | 110000                | <b>24</b>               | pter: 0 - 20000                                                    |
| <b>4</b>                | 19905000                | 19910000              | <b>Z</b>                | p: ~ 8450000                                                       |
| <b>5</b>                | 21170000*               |                       | <b>20</b>               | ~ 2075000                                                          |
| <b>13</b>               | 15325000*               |                       | <b>27</b>               | ~ 65000                                                            |
| <b>Z</b>                | 38685000*               |                       | <b>14</b>               | ~14315000                                                          |
| <b>Z</b>                | 81855000                | 82005000              | <b>3</b>                | p: ~2200000                                                        |
| <b>Z</b>                | 81855000                | 82005000              | <b>24</b>               | qter: ~6270000                                                     |

\* - small ambiguous translocation of ~5 Kbp regions.

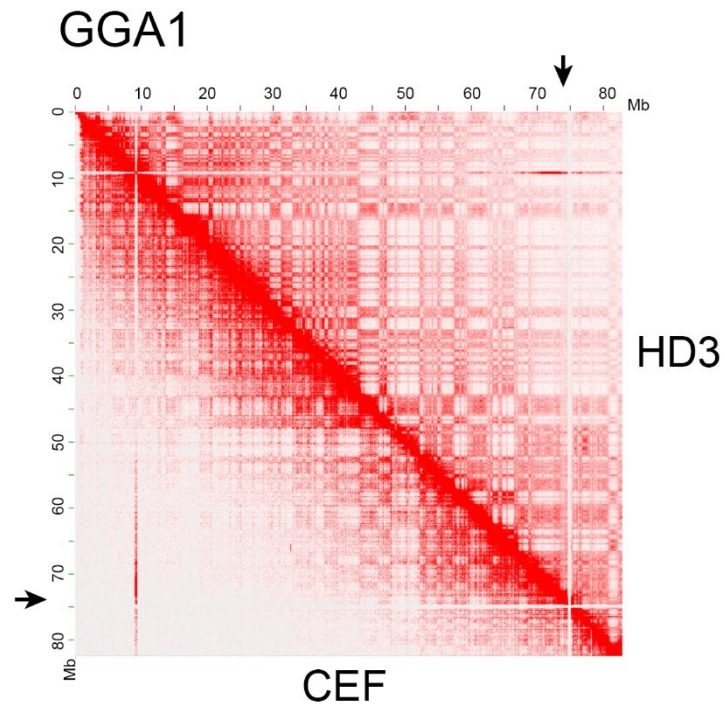
**Additional Table 3. Intrachromosomal translocations identified in HD3 cell line based on the analysis of Hi-C heatmaps.**

| <b>Chromosome #</b> | <b>start, ± 5000 bp</b> | <b>end, ± 5000 bp</b> | <b>SV type, comment</b>                                           |
|---------------------|-------------------------|-----------------------|-------------------------------------------------------------------|
| <b>1</b>            | 180 580 000             | 194 260 000           | duplication, associated with t(1;2)                               |
| <b>1</b>            | 194 265 000             | 196 140 000           | partial deletion                                                  |
| <b>2</b>            | 25 780 000              | 25 875 000            | deletion                                                          |
| <b>2</b>            | 54 325 000              | 61 685 000            | partial deletion                                                  |
| <b>3</b>            | 0                       | 2 195 000             | partial deletion, associated with t(3;24)                         |
| <b>4</b>            | 16 840 000              | 18 830 000            | partial deletion, associated with t(4;5), t(4;6), t(4;7), t(4;19) |
| <b>5</b>            | 605 000                 | 795 000               | partial deletion                                                  |
| <b>7</b>            | 0                       | 4 105 000             | partial deletion, associated with t(7;33)                         |
| <b>7</b>            | 9255000-9255000         | 10745000              | small translocation                                               |
| <b>9</b>            | 6070000                 | 6990000               | duplication                                                       |
| <b>11</b>           | 19 170 000              | 20 175 000            | amplification                                                     |
| <b>17</b>           | 4 780 000               | 5 025 000             | partial deletion                                                  |
| <b>21</b>           | 0                       | 325 000               | duplication                                                       |
| <b>22</b>           | 45 000                  | 760 000               | partial deletion                                                  |
| <b>24</b>           | 0-6275000*              |                       | duplication/amplification                                         |
| <b>27</b>           | 0-5035000               |                       | duplication/amplification                                         |
| <b>27</b>           | 5 040 000               | 5 645 000             | partial deletion                                                  |
| <b>W</b>            | 0-5155000*              |                       | duplication                                                       |

\* - copy number variation involving the whole chromosome.



**Additional Figure 1. Interchromosomal rearrangements in HD3 cell line involving macro- and microchromosomes.** **a, b, c, c'** – assemblies of HD3 Hi-C heatmaps, indicating intrachromosomal contacts of GGA4 in HD3 cells versus CEF (**a**), translocation between GGA1 and GGA2 (**b**), translocations between macro- and microchromosomes (**c, c'**). Blue squares on the heatmaps represent intrachromosomal contacts (chromosome territories). Relative positions of breakpoints are shown as red lines on the schematic depictions of chromosomes. Arrows point to the approximate positions of BAC-probes. **a'-a'** – 3-color FISH verification of breakage of one of the homologs of GGA4 on metaphase spreads of HD3. **a''** – FISH verification of translocation of a part of GGA7p and a deletion of this fragment from both homologs of GGA7 on metaphase spreads of HD3 using BAC-based probes. **b'-b'''** – FISH verification of translocation between GGA1 and GGA2 on metaphase spreads of HD3 using BAC-based probes (**b'**) and detection of new clusters of PO41 repeats (**b''**) and CNM repeats (**b'''**) on the derivative homolog of GGA2 with repeat-specific probes. **b''''** – schematic depiction of normal and derivative homologs of GGA2. **c''** – FISH verification of translocation between GGA5 and GGA21 on metaphase spreads of HD3. Note the trisomy of the translocated region in the short arm of GGA21 and different size of chromosomes containing material of GGA21 (enlarged images). **d, e** – disomy of the W sex chromosome (**d**) in the presence of the Z chromosome (**e**) in the HD3 karyotype confirmed by FISH with W chromosome paint and microdissected q-terminal heterochromatic region of GGAZ as probes. Chromosomes are counterstained with DAPI.



**Additional Figure 2. Abnormal intrachromosomal contacts caused by genome assembly errors in GalGal5.** View of Hi-C heatmap of genomic contacts in HD3 (upper right) versus CEF (lower left) with a normal karyotype. Arrow points to the increased number of genomic contacts near the SEMA3A and LOC107053979 loci observed in both HD3 and CEF.