## Supplemental Tables

### Table S1. Cell preparations for Hi-C.

| Sample | Days post-RA injection | Pre-sort purity (SYCP3-positive cells) | Post-sort purity | Total sorted cells |
|---|---|---|---|---|
| **Zygonema #1** | 25 (third wave)[a] | 33% zygonema / 67% diplonema | 94% zygonema / 1% round spermatids / 5% DAPI[b] | 600,000 |
| **Zygonema #2** | 33 (fourth wave) | 0.6% leptonema / 42% zygonema / 58% diplonema | 5% leptonema / 91% zygonema / 1% diplonema, 3% DAPI | 743,750 |
| **Pachynema #1** | 30 (third wave) | 99% pachynema / 1% diplonema | 77% pachynema / 23% diplonema[c] / 1% round spermatids | 807,250 |
| **Pachynema #2** | 47 (fifth wave) | Not available | 85% pachynema / 14% diplonema / 1% round spermatids | 600,000 |
| **Pachynema #3** | 21 (second wave) | 100% pachynema | 79% pachynema / 21% diplonema | 620,000 |

[a] ”Second wave, ”third wave,” “fourth wave,” and “fifth wave” refer to synchronized waves of spermatogenesis.

[b] DAPI refers to DAPI-positive, SYCP3-negative cells that are likely somatic cells from surrounding tissue.

[c] Cells were scored as diplonema if even a single chromosome pair showed evidence of desynapsis.

**Table S2. Hi-C read counts.**

| | Zygonema #1 | Zygonema #2 |
|---|---|---|
| Total mapped read pairs | 197,614,046 | 153,901,397 |
| B6 (% of mapped)[a] | 74,259,687 (37.6%) | 57,821,970 (37.6%) |
| CAST (%)[b] | 54,628,271 (27.6%) | 42,564,406 (27.7%) |
| B6-CAST (%)[c] | 6,537,482 (3.3%) | 5,124,838 (3.3%) |
| Ambiguous (%)[d] | 61,065,373 (30.9%) | 47,493,793 (30.9%) |
| Unassignable (%)[e] | 1,123,233 (0.6%) | 896,390 (0.6%) |
| GEO accession number[f] | GSM3476500 | GSM3476501 |

| | Pachynema #1 | Pachynema #2 | Pachynema #3 | E14 ES Cells |
|---|---|---|---|---|
| Total mapped read pairs | 176,921,227 | 155,119,468 | 155,072,169 | 522,314,158 |
| B6 (% of mapped)[a] | 65,861,659 (37.3%) | 56,354,326 (36.3%) | 56,304,306 (36.3%) | N/A |
| CAST (%)[b] | 48,728,160 (27.5%) | 43,068,165 (27.8%) | 43,130,548 (27.8%) | N/A |
| B6-CAST (%)[c] | 6,164,297 (3.5%) | 5,882,454 (3.8%) | 5,645,524 (3.6%) | N/A |
| Ambiguous (%)[d] | 55,104,704 (31.2%) | 49,049,415 (31.6%) | 49,251,537 (31.8%) | N/A |
| Unassignable (%)[e] | 1,062,407 (0.6%) | 765,108 (0.5%) | 740,254 (0.5%) | N/A |
| GEO accession number[f] | GSM3476502 | GSM3476503 | GSM3476504 | GSM1908921 |

[a] Read pairs were assigned as B6 if both reads were assigned to B6, or if one read was assigned to B6 and the other was ambiguous (i.e. contained no SNPs).

[b] Read pairs were assigned as CAST if both reads were assigned to CAST, or if one read was assigned to CAST and the other was ambiguous (i.e. contained no SNPs).

[c] Read pairs were assigned as B6-CAST if one read was assigned to B6, and the other to CAST.

[d] Read pairs were assigned as ambiguous if neither read contained SNPs. These read pairs were added to the B6 and CAST read pairs for total Hi-C maps and contact probability graphs.

[e] Read pairs were assigned as unassignable if either read contained SNPs mapping to both B6 and CAST. These could represent reads with internal inter-homolog ligation junctions, sequencing errors, or meiotic gene conversion tracts. These read pairs were excluded from downstream analyses.

[f] Data for meiotic prophase is publicly available at the NIH Gene Expression Omnibus under series GSE122622 and the listed individual dataset accession codes. E14 ES cell data is available under series GSE74055 and the listed individual dataset accession code.