**Supplementary File 1**

**Supplementary file 1a. Whole Exome Sequencing and mapping data for the POI patients and the unaffected sister.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Read Pairs (millions) | Mean Depth | Coverage  > 25 X | Mismatch Read 1 | Mismatch Read 2 |
| POI III-2 | 56.2 | 79.05 X | 80.1 % | 0.13 % | 0.23 % |
| POI III-3 | 36.5 | 26.81 X | 89.86 % | 0.15 % | 0.29 % |
| NA III-10 | 31.1 | 38.39X | 92.1 % | 0.16 % | 0.25 % |

**Supplementary file 1b. Filtering of the variants identified by Whole Exome Sequencing.**

|  |  |  |  |
| --- | --- | --- | --- |
| Variants called in | POI III-2 | POI III-3 | NA III-10 |
| Total | **118545** | **105899** | **107840** |
| SNPs | 108106 | 96854 | 98621 |
| Indels | 10439 | 9045 | 9219 |
| Homozygous variants | 42695 | 39741 | 39640 |
| Heterozygous variants | 75850 | 66158 | 68200 |
| Variant filters | | | | | **# of variants** |
| Coverage > 9 reads in the 3 exomes | | | | | 130021 |
| Homozygous in patients, Homozygous Ref or Heterozygous in non-affected sister | | | | | 8897 |
| In coding regions | | | | | 4553 |
| With protein impact (i.e not synonymous) | | | | | 2295 |
| not Homozygous in unrelated fertile controls | | | | | 66 |
| MAF < 1% in GnomAD | | | | | 5 |
| with coherent functional information | | | | | 1 |

**Supplementary file 1c: Pathogenicity predictions for the S167L variant in *HSF2BP.***

|  |  |  |  |
| --- | --- | --- | --- |
| Software | Score for the variant | Pathogenicity threshold | Pathogenicity  prediction |
| SIFT | 0.114 | < 0.05 | Tolerated |
| SIFT4G | 0.004 | < 0.05 | Damaging |
| PolyPhen 2 | 0.135 | > 0.8 | Benign (indicated as  probably\_damaging in GnomAD |
| M-CAP | 0.0232 | > 0.025 | Tolerated (borderline) |
| FATHMM-MKL | 0.8975 | > 0.5 | Damaging |
| LRT | 0.000293 | Score is a p-value | Deleterious |
| MutationTaster | 0.994943 | 0.5 | Disease-causing |
| MutationAssessor | 1.87 | > 0.65 | Low |
| FATHMM | -0.06 | < -1.5 | Tolerated |
| FATHMM-MKL coding | 0.89755 | 0.5 (default) 0.80 (stringent) | Deleterious |
| PROVEAN | -2.88 | -2.28 | Damaging |
| MetaSVM | -0.8926 | 0 | Tolerated |
| MetaLR | 0.1613 | 0.5 | Tolerated |
| REVEL | 0.068 | 0.5 (default) 0.75 (stringent) | Benign |
| DANN | 0.996469 | 0.96 | Damaging |
| CADD | 5.470242 | 1.75 | Damaging |
| GERP++ RS | 4.36 | > 4.4 | Conserved |
| phyloP100way\_vertebrate | 5.335 | > 1.6 | Highly conserved |

**Supplementary file 1d. Quantification of HSF2BP colocalization with RPA1, DMC1 and BRME1.** The quantification was carried out from three cells corresponding to each stage. Non-specific colocalization (random colocalization) was quantified in the same cells but after rotating one of the images 90 degrees.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| STAGE | HSF2BP foci stained positive for BRME1 | | BRME1 foci stained positive for HSF2BP | |
| **Specific**  **(w/o rotation)** | **Non-specific**  **(with rotation)** | **Specific**  **(w/o rotation)** | **Specific**  **(w/o rotation)** |
| **Early zygotene** | 91,5% ± 2,0 | 4,1% ± 1,3 | 68,7% ± 1,0 | 3,1% ± 1,0 |
| **Late zygotene** | 94,1% ± 1,2 | 3,1% ± 0,7 | 88,0% ± 3,0 | 2,9% ± 0,7 |
| **Early pachytene** | 92,6% ± 2,6 | 2,8% ± 1,7 | 89,6% ± 2,8 | 2,7% ± 1,7 |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| STAGE | HSF2BP foci stained positive for DMC1 | | DMC1 foci stained positive for HSF2BP | |
| **Specific**  **(w/o rotation)** | **Non-specific**  **(with rotation)** | **Specific**  **(w/o rotation)** | **Non-specific**  **(with rotation)** |
| **Early zygotene** | 74,0% ± 11,1 | 1,0% ± 0,5 | 87,3% ± 8,1 | 1,2% ± 0,7 |
| **Late zygotene** | 55,3% ± 13,4 | 1,9% ± 1,1 | 89,1% ± 6,1 | 3,3% ± 2,5 |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| STAGE | HSF2BP foci stained positive for RPA1 | | RPA1 foci stained positive for HSF2BP | |
| **Specific**  **(w/o rotation)** | **Non-specific**  **(with rotation)** | **Specific**  **(w/o rotation)** | **Specific**  **(w/o rotation)** |
| **Early zygotene** | 76,3% ± 2,0 | 5,5% ± 4,4 | 69,2% ± 0,8 | 5,0% ± 4,1 |
| **Late zygotene** | 81,8% ± 3,1 | 3,0% ± 0,9 | 82,4% ± 3,4 | 3,0% ± 1,0 |
| **Early pachytene** | 79,7% ± 3,5 | 2,8% ± 1,7 | 88,6% ± 3,5 | 3,1% ± 2,0 |

**Supplementary file 1e. Quantification of BRME1 colocalization with RPA2 and DMC1.** The quantification was carried out from three cells corresponding to each stage. Non-specific colocalization (random colocalization) was quantified in the same cells but after rotating one of the images 90 degrees.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| STAGE | BRME1 foci stained positive for RPA2 | | RPA2 foci stained positive for BRME1 | |
| **Specific**  **(w/o rotation)** | **Non-specific**  **(with rotation)** | **Specific**  **(w/o rotation)** | **Non-specific**  **(with rotation)** |
| **Early zygotene** | 90,5% ± 2,3 | 2,2% ± 0,9 | 84,6 ± 3,9 | 2,1% ± 0,8 |
| **Late zygotene** | 90,2% ± 1,5 | 2,0% ± 0,4 | 95,2% ± 0,6 | 2,1% ± 0,4 |
| **Early pachytene** | 87,7% ± 5,0 | 1,8% ± 0,2 | 92,4% ± 4,8 | 1,9% ± 0,3 |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| STAGE | BRME1 foci stained positive for DMC1 | | DMC1 foci stained positive for BRME1 | |
| **Specific**  **(w/o rotation)** | **Non-specific**  **(with rotation)** | **Specific**  **(w/o rotation)** | **Non-specific**  **(with rotation)** |
| **Early zygotene** | 58,3% ± 4,6 | 5,1% ± 2,4 | 55,5% ± 5,0 | 4,9% ± 2,5 |
| **Late zygotene** | 30,8% ± 2,6 | 2,7% ± 1,0 | 78,8% ± 13,7 | 7,0% ± 1,7 |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Supplementary file 1f. Summary table comparing the different alterations in the meiotic recombination pathway on each genotype (both sexes) for the different meiotic players analyzed (gH2AX, RPA1, SPATA22, DMC1, RAD51 and MLH1).** |  |  | Lepto/Early zygo | Zygo/Zygo-like | Pachy |
| gH2AX | *Hsf2bp*S167L/S167L | males |  |  | **>>** |
| females |  |  | **>** |
| *Hsf2bp-/-* | males |  | ∞ | N/A |
| females |  |  | **>>>** |
| *Brme1-/-* | males |  | ∞ | N/A |
| females |  |  | **>>** |
| RPA1 | *Hsf2bp*S167L/S167L | males | **=** | **=** | **>>** |
| females |  | **=** | **=** |
| *Hsf2bp-/-* | males | **=** | **>** | N/A |
| females |  | **=** | **=** |
| *Brme1-/-* | males | **=** | **>** | N/A |
| females |  | **=** | **=** |
| SPATA22 | *Hsf2bp*S167L/S167L | males |  | **>** | **>** |
| females |  | **>? ns** | **>? ns** |
| *Hsf2bp-/-* | males |  | **>>** | N/A |
| females |  | **>>** | **>>** |
| *Brme1-/-* | males |  | >>> | N/A |
| females |  | **>>** | **>>>** |
| DMC1 | *Hsf2bp*S167L/S167L | males | **<** | **-?ns** | **<** |
| females |  | **<<** | **<<** |
| *Hsf2bp-/-* | males | **<<<** | **<<<** | N/A |
| females |  | **<<<** | **<<<** |
| *Brme1-/-* | males | **<<<** | **<<<** | N/A |
| females |  | **<<<** | **<<<** |
|  | | | | | |
|  | | | | | |
|  |  |  | **Lepto/Early zygo** | **Zygo/Zygo-like** | **Pachy** |
| RAD51 | *Hsf2bp*S167L/S167L | males | **<<** | **<<** | **<** |
| females |  | **<<** | **<<<** |
| *Hsf2bp-/-* | males | **<<<** | <<< | N/A |
| females |  | **<<<** | **<<<** |
| *Brme1-/-* | males | **<<** | **<<<** | N/A |
| females |  | **<<** | **<<<** |
|  | | | **Total foci** | **% cells w/o foci in XY** | **% cells w/o foci in autosomes** |
| MLH1 | Wild type | males | 25 | 27% | 3% |
| females | 24 | N/A | 21% |
| *Hsf2bp*S167L/S167L | males | 21 | 55% | 20% |
| females | 22 | N/A | 48% |
| *Hsf2bp-/-* | males | 0 | N/A | N/A |
| females | 19 | N/A | 75% |
| *Brme1-/-* | males | 0 | N/A | N/A |
| females | 19 | N/A | 79% |

**The wild type mean has been considered as the reference value:**

**>** :Higher than wild type

**<** : Lower than wild type

= : Similar to wild type

∞: The signal is similar in zygotene-like arrested cells and in wild type zygotene cells, but in the arrested cells it remains permanently.

ns: non-significant differences (Two-tailed Welch´s t-test analysis).

N/A: Not Applicable due to a previous meiotic arrest in *Hsf2bp-/-* and *Brme1-/-* malesor due to the absence of XY bivalent in females (MLH1 data).

Empty cells: not tested.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Gene names | Protein IDs | R1-Num of unique peptides | R2- Num of unique peptides | Control-Num of unique peptides | R1 iBAQ | R2 iBAQ | Control iBAQ | Group |
| Hsf2bp | Q9D4G2 | 20 | 16 | 0 | 168840000 | 37607000 | 0 | R1 and R2 only |
| C19orf57  (Brme1) | Q6DIA7 | 16 | 13 | 1 | 77473000 | 22181000 | 59511 | R1 and R2 only |
| Brca2 | P97929 | 78 | 77 | 0 | 5296400 | 3125000 | 0 | R1 and R2 only |
| Palb2 | Q3U0P1 | 7 | 11 | 0 | 1102300 | 896460 | 0 | R1 and R2 only |
| Rad51 | Q08297 | 2 | 4 | 0 | 509370 | 367390 | 0 | R1 and R2 only |
| Rpa2 | Q62193 | 3 | 0 | 0 | 235990 | 0 | 0 | R1 only |

**Supplementary file 1g. Meiotic recombination players identified as putative BRME1 interactors by immunoprecipitation coupled to mass spectrometry of whole testis extracts with two antibodies (R1 and R2) against BRME1.** See extended data on supplementary file 1h.

**Supplementary file 1h. Extended data of BRME1 putative interactors identified by immunoprecipitation coupled to mass spectrometry of whole testis extracts with two different antibodies (R1 and R2) against BRME1 (see Methods).**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Gene names | Protein IDs | R1-Num of unique peptides | R2-Num of unique peptides | Control-Num of unique peptides | R1 iBAQ | R2 iBAQ | Control iBAQ | Group |
| Hsf2bp | Q9D4G2 | 20 | 16 | 0 | 168840000 | 37607000 | 0 | R1 and R2 only |
| Edc4 | Q3UJB9 | 3 | 46 | 1 | 193300 | 24305000 | 59266 | R1 and R2 only |
| C19orf57  (Brme1) | Q6DIA7 | 16 | 13 | 1 | 77473000 | 22181000 | 59511 | R1 and R2 only |
| Dhrs11 | Q3U0B3 | 3 | 11 | 0 | 424760 | 10761000 | 0 | R1 and R2 only |
| Rpl27 | P61358 | 4 | 3 | 1 | 14163000 | 6680600 | 189540 | R1 and R2 only |
| Ccar2 | Q8VDP4 | 24 | 22 | 0 | 10790000 | 5672600 | 0 | R1 and R2 only |
| Sec13 | Q9D1M0 | 10 | 9 | 0 | 4987100 | 5509600 | 0 | R1 and R2 only |
| Dynll2 | Q9D0M5 | 3 | 3 | 1 | 18915000 | 4495000 | 449620 | R1 and R2 only |
| Rpl18 | P35980 | 3 | 4 | 0 | 6704400 | 4425100 | 0 | R1 and R2 only |
| Usp4 | P35123;Q99K46 | 37 | 24 | 0 | 40187000 | 3667700 | 0 | R1 and R2 only |
| Brca2 | P97929 | 78 | 77 | 0 | 5296400 | 3125000 | 0 | R1 and R2 only |
| Hspd1 | P63038 | 8 | 11 | 1 | 1885200 | 2681000 | 43682 | R1 and R2 only |
| Rpl10a | P53026 | 6 | 3 | 0 | 27684000 | 2361300 | 0 | R1 and R2 only |
| Chtf8 | P0CG14 | 17 | 16 | 0 | 4464800 | 1811600 | 0 | R1 and R2 only |
| Rpl13a | P19253 | 3 | 3 | 0 | 7370600 | 1474600 | 0 | R1 and R2 only |
| Rpl7 | P14148 | 10 | 4 | 1 | 8627200 | 1416200 | 188040 | R1 and R2 only |
| Dnajb11 | Q99KV1 | 3 | 6 | 1 | 1009800 | 1414900 | 137790 | R1 and R2 only |
| Rpl6 | P47911 | 7 | 5 | 1 | 4884800 | 1182700 | 41048 | R1 and R2 only |
| Rpl30 | P62889 | 4 | 4 | 0 | 3876500 | 1174300 | 0 | R1 and R2 only |
| Arhgef2 | Q60875 | 0 | 12 | 0 | 0 | 942440 | 0 | R2 only |
| Palb2 | Q3U0P1 | 7 | 11 | 0 | 1102300 | 896460 | 0 | R1 and R2 only |
| Wnk1 | P83741;Q80UE6 | 16 | 9 | 0 | 2979600 | 835530 | 0 | R1 and R2 only |
| Rpl27a | P14115 | 2 | 1 | 0 | 4934400 | 768520 | 0 | R1 only |
| Rpl8 | P62918 | 3 | 3 | 1 | 4026000 | 754840 | 53677 | R1 and R2 only |
| Exosc5 | Q9CRA8 | 2 | 3 | 0 | 492230 | 714900 | 0 | R1 and R2 only |
| Tex33 | Q9D9J2 | 4 | 4 | 1 | 2079400 | 686300 | 249520 | R1 and R2 only |
| Bag5 | Q8CI32 | 3 | 10 | 0 | 98819 | 677230 | 0 | R1 and R2 only |
| Rpap3 | Q9D706 | 0 | 8 | 0 | 0 | 624360 | 0 | R2 only |
| Ybx1 | P62960 | 4 | 3 | 1 | 1559000 | 610700 | 36539 | R1 and R2 only |
| Ilf2 | Q9CXY6 | 7 | 4 | 1 | 1504400 | 592600 | 11488 | R1 and R2 only |
| Tdrd1 | Q99MV1 | 2 | 10 | 0 | 86505 | 561490 | 0 | R1 and R2 only |
| Ctnnb1 | Q02248 | 0 | 6 | 0 | 0 | 533930 | 0 | R2 only |
| Arhgdia | Q99PT1 | 2 | 3 | 1 | 673000 | 506600 | 145900 | R1 and R2 only |
| Rpl32 | P62911 | 3 | 3 | 0 | 1224000 | 479360 | 0 | R1 and R2 only |
| Ybx2 | Q9Z2C8 | 7 | 4 | 1 | 4297400 | 477700 | 156530 | R1 and R2 only |
| Dnaja4 | Q9JMC3 | 4 | 7 | 1 | 729390 | 461060 | 54926 | R1 and R2 only |
| Rpl24 | Q8BP67 | 3 | 2 | 0 | 2133500 | 458720 | 0 | R1 and R2 only |
| Hspa1l | P16627 | 2 | 2 | 0 | 684830 | 438960 | 0 | R1 and R2 only |
| Cenpj | Q569L8 | 11 | 11 | 0 | 544000 | 423970 | 0 | R1 and R2 only |
| Rpl4 | Q9D8E6 | 6 | 3 | 0 | 1783600 | 410860 | 0 | R1 and R2 only |
| Ewsr1 | Q61545 | 4 | 3 | 1 | 3332800 | 408390 | 74676 | R1 and R2 only |
| Rpl28 | P41105 | 2 | 2 | 0 | 1146400 | 385370 | 0 | R1 and R2 only |
| Rbm14 | Q8C2Q3 | 6 | 5 | 0 | 1316300 | 378870 | 0 | R1 and R2 only |
| Eif4e | P63073 | 3 | 2 | 1 | 1875700 | 375610 | 71180 | R1 and R2 only |
| Aifm1 | Q9Z0X1 | 1 | 6 | 0 | 99312 | 374800 | 0 | R2 only |
| Rps8 | P62242 | 5 | 2 | 0 | 2807000 | 369660 | 0 | R1 and R2 only |
| Rad51 | Q08297 | 2 | 4 | 0 | 509370 | 367390 | 0 | R1 and R2 only |
| Xlr3c;Xlr3b;Xlr3a | Q61806;Q6P205;Q60595 | 1 | 3 | 0 | 58041 | 367380 | 0 | R2 only |
| Ranbp1 | P34022 | 0 | 2 | 0 | 0 | 365060 | 0 | R2 only |
| Vapa | Q9WV55 | 5 | 2 | 0 | 2393500 | 359790 | 0 | R1 and R2 only |
| Amy1;Amy2 | P00687;P00688 | 2 | 2 | 0 | 4730900 | 355380 | 0 | R1 and R2 only |
| Echs1 | Q8BH95 | 3 | 3 | 1 | 1090100 | 347120 | 180080 | R1 and R2 only |
| Dnaja2 | Q9QYJ0 | 5 | 4 | 0 | 508220 | 329450 | 0 | R1 and R2 only |
| Rpl19 | P84099 | 2 | 2 | 0 | 2200900 | 329190 | 0 | R1 and R2 only |
| Fahd2 | Q3TC72 | 3 | 2 | 1 | 659830 | 326080 | 90862 | R1 and R2 only |
| Exosc4 | Q921I9 | 3 | 2 | 0 | 159550 | 320140 | 0 | R1 and R2 only |
| Glt8d1 | Q6NSU3 | 8 | 2 | 1 | 3636300 | 315810 | 8110,6 | R1 and R2 only |
| Eif2b4 | Q61749 | 0 | 3 | 0 | 0 | 308760 | 0 | R2 only |
| Exosc2 | Q8VBV3 | 3 | 4 | 0 | 225990 | 294010 | 0 | R1 and R2 only |
| Bag2 | Q91YN9 | 1 | 3 | 0 | 301170 | 291950 | 0 | R2 only |
| Hnrnpm | Q9D0E1 | 4 | 6 | 0 | 476840 | 286690 | 0 | R1 and R2 only |
| Anxa1 | P10107 | 4 | 4 | 1 | 599940 | 274100 | 16392 | R1 and R2 only |
| Kif21a | Q9QXL2;Q9QXL1;B7ZNG0 | 0 | 8 | 0 | 0 | 263910 | 0 | R2 only |
| Sorbs3 | Q9R1Z8 | 0 | 3 | 0 | 0 | 260670 | 0 | R2 only |
| Rpgr | Q9R0X5 | 0 | 4 | 0 | 0 | 258990 | 0 | R2 only |
| Psmc4 | P54775 | 5 | 4 | 1 | 450370 | 255350 | 17166 | R1 and R2 only |
| Caprin1 | Q60865 | 6 | 2 | 0 | 1890700 | 254830 | 0 | R1 and R2 only |
| Pcbp2 | Q61990 | 4 | 2 | 1 | 1067500 | 252810 | 148480 | R1 and R2 only |
| Pgrmc1 | O55022 | 1 | 2 | 0 | 181560 | 241760 | 0 | R2 only |
| Eif6 | O55135 | 5 | 2 | 0 | 2835600 | 237780 | 0 | R1 and R2 only |
| Mamld1 | P0C6A2 | 1 | 4 | 0 | 75258 | 236850 | 0 | R2 only |
| Rpl21 | O09167 | 1 | 2 | 0 | 648660 | 225840 | 0 | R2 only |
| Rps24 | P62849 | 1 | 2 | 0 | 591820 | 223810 | 0 | R2 only |
| Exosc6 | Q8BTW3 | 1 | 2 | 0 | 186290 | 213080 | 0 | R2 only |
| Wdr34 | Q5U4F6 | 1 | 3 | 0 | 186700 | 211000 | 0 | R2 only |
| Rpgrip1 | Q9EPQ2 | 0 | 4 | 0 | 0 | 196320 | 0 | R2 only |
| Hagh | Q99KB8 | 6 | 4 | 0 | 1000700 | 193140 | 0 | R1 and R2 only |
| Gapvd1 | Q6PAR5 | 0 | 6 | 0 | 0 | 182050 | 0 | R2 only |
| Slc2a3 | P32037 | 5 | 2 | 1 | 4020900 | 180730 | 282890 | R1 and R2 only |
| Pfkl | P12382 | 1 | 4 | 0 | 57749 | 176310 | 0 | R2 only |
| Rbm17 | Q8JZX4 | 1 | 4 | 0 | 26197 | 173970 | 0 | R2 only |
| Eif2b5 | Q8CHW4 | 0 | 2 | 0 | 0 | 169930 | 0 | R2 only |
| Dnajb6 | O54946;Q9QYI8 | 1 | 2 | 0 | 195800 | 163940 | 0 | R2 only |
| Tprn | A2AI08 | 0 | 2 | 0 | 0 | 162190 | 0 | R2 only |
| Ropn1l | Q9EQ00 | 2 | 1 | 0 | 697100 | 156610 | 0 | R1 only |
| Npm1 | Q61937 | 2 | 2 | 1 | 296120 | 154640 | 38063 | R1 and R2 only |
| Ddx5;Ddx17 | Q61656;Q501J6 | 2 | 2 | 1 | 704920 | 150620 | 70359 | R1 and R2 only |
| Lyar | Q08288 | 2 | 2 | 1 | 154100 | 148270 | 29145 | R1 and R2 only |
| Tbl1xr1 | Q8BHJ5 | 0 | 3 | 0 | 0 | 140050 | 0 | R2 only |
| Pfkp | Q9WUA3 | 5 | 3 | 0 | 382140 | 139640 | 0 | R1 and R2 only |
| Clcc1 | Q99LI2 | 0 | 4 | 0 | 0 | 132410 | 0 | R2 only |
| Ubap2l | Q80X50 | 1 | 3 | 0 | 66946 | 130510 | 0 | R2 only |
| Vapb | Q9QY76 | 2 | 2 | 0 | 461320 | 125430 | 0 | R1 and R2 only |
| Dlst | Q9D2G2 | 2 | 2 | 1 | 235820 | 121910 | 112620 | R1 and R2 only |
| Ndel1 | Q9ERR1;Q9CZA6 | 0 | 3 | 0 | 0 | 120480 | 0 | R2 only |
| Htra2 | Q9JIY5 | 0 | 2 | 0 | 0 | 119120 | 0 | R2 only |
| Polr2e | Q80UW8 | 0 | 2 | 0 | 0 | 115740 | 0 | R2 only |
| Rbm7 | Q9CQT2 | 2 | 2 | 0 | 176710 | 115380 | 0 | R1 and R2 only |
| Psma8;Psma7 | Q9CWH6;Q9Z2U0 | 5 | 2 | 1 | 1189900 | 111700 | 113360 | R1 and R2 only |
| Stk39 | Q9Z1W9 | 4 | 3 | 0 | 564060 | 110480 | 0 | R1 and R2 only |
| Pld3 | O35405 | 2 | 1 | 0 | 546090 | 107090 | 0 | R1 only |
| Eif2b2 | Q99LD9 | 0 | 3 | 0 | 0 | 106890 | 0 | R2 only |
| Tdrd6 | P61407 | 11 | 9 | 0 | 461070 | 106510 | 0 | R1 and R2 only |
| Shcbp1l | Q3TTP0 | 3 | 2 | 1 | 179970 | 106190 | 14360 | R1 and R2 only |
| Tarbp2 | P97473 | 0 | 2 | 0 | 0 | 105740 | 0 | R2 only |
| Spata20 | Q80YT5 | 4 | 3 | 0 | 410910 | 104640 | 0 | R1 and R2 only |
| Trafd1 | Q3UDK1 | 1 | 2 | 0 | 0 | 104460 | 0 | R2 only |
| Serpina1b | P22599 | 2 | 0 | 0 | 2856200 | 102550 | 678480 | R1 only |
| Hprt1 | P00493 | 3 | 2 | 1 | 354350 | 102310 | 79077 | R1 and R2 only |
| Hnrnpk | P61979 | 5 | 1 | 0 | 512010 | 100340 | 0 | R1 only |
| Strap | Q9Z1Z2 | 9 | 3 | 0 | 874350 | 98155 | 0 | R1 and R2 only |
| C1sa;C1sb | Q8CG14;Q8CFG8 | 4 | 2 | 0 | 312700 | 95781 | 0 | R1 and R2 only |
| Akap12 | Q9WTQ5 | 5 | 4 | 0 | 241290 | 95498 | 0 | R1 and R2 only |
| Kif27 | Q7M6Z4 | 0 | 6 | 0 | 0 | 95153 | 0 | R2 only |
| Stk31 | Q99MW1 | 4 | 4 | 1 | 133550 | 93796 | 5679,5 | R1 and R2 only |
| Sec23a;Sec23b | Q01405;Q9D662 | 2 | 4 | 0 | 108710 | 92427 | 0 | R1 and R2 only |
| Lrrc34 | Q9DAM1 | 4 | 4 | 0 | 68517 | 89375 | 0 | R1 and R2 only |
| Hip1 | Q8VD75 | 0 | 3 | 0 | 0 | 87633 | 0 | R2 only |
| Exosc7 | Q9D0M0 | 0 | 2 | 0 | 0 | 87082 | 0 | R2 only |
| Stk36 | Q69ZM6 | 0 | 3 | 0 | 0 | 86558 | 0 | R2 only |
| Dkkl1 | Q9QZL9 | 1 | 2 | 0 | 84507 | 86106 | 0 | R2 only |
| Sdhb | Q9CQA3 | 5 | 2 | 0 | 821740 | 85886 | 0 | R1 and R2 only |
| Mael | Q8BVN9 | 2 | 3 | 1 | 333530 | 80932 | 40646 | R1 and R2 only |
| Hspa4 | Q61316 | 3 | 3 | 0 | 260230 | 80515 | 0 | R1 and R2 only |
| Fam184b | Q0KK56 | 0 | 4 | 0 | 0 | 79391 | 0 | R2 only |
| Atxn2l | Q7TQH0 | 7 | 2 | 0 | 960960 | 78810 | 0 | R1 and R2 only |
| Atp5b | P56480 | 4 | 3 | 1 | 248730 | 75502 | 17119 | R1 and R2 only |
| Als2 | Q920R0 | 0 | 4 | 0 | 0 | 73670 | 0 | R2 only |
| Fn1 | P11276 | 0 | 6 | 0 | 0 | 70072 | 0 | R2 only |
| Cope | O89079 | 1 | 2 | 0 | 70934 | 68740 | 0 | R2 only |
| Nup133 | Q8R0G9 | 0 | 3 | 0 | 0 | 68014 | 0 | R2 only |
| Ruvbl2 | Q9WTM5 | 0 | 2 | 0 | 0 | 67203 | 0 | R2 only |
| Tekt4 | Q149S1 | 1 | 2 | 0 | 54604 | 65884 | 0 | R2 only |
| Rtcb | Q99LF4 | 3 | 2 | 1 | 415760 | 65057 | 66810 | R1 and R2 only |
| Lancl2 | Q9JJK2 | 4 | 2 | 0 | 229810 | 63780 | 0 | R1 and R2 only |
| Pygm;Pygb | Q9WUB3;Q8CI94;Q9ET01 | 2 | 2 | 1 | 53539 | 61107 | 13916 | R1 and R2 only |
| Eif3k | Q9DBZ5 | 2 | 1 | 0 | 303390 | 60567 | 0 | R1 only |
| Hspbp1 | Q99P31 | 0 | 2 | 0 | 0 | 59812 | 0 | R2 only |
| Dpysl3;Crmp1 | Q62188;P97427 | 2 | 1 | 0 | 0 | 58906 | 0 | R1 only |
| Snrpb;Snrpn | P27048;P63163 | 4 | 1 | 0 | 665630 | 58830 | 0 | R1 only |
| Atp2a2 | O55143;Q64518;Q8R429 | 4 | 3 | 0 | 338550 | 58794 | 0 | R1 and R2 only |
| Acly | Q91V92 | 1 | 4 | 0 | 28857 | 56271 | 0 | R2 only |
| Sox30 | Q8CGW4 | 0 | 2 | 0 | 0 | 55897 | 0 | R2 only |
| Kif5b | Q61768;P28738;P33175 | 11 | 3 | 1 | 595910 | 54255 | 20536 | R1 and R2 only |
| Hnrnpa3 | Q8BG05 | 3 | 2 | 0 | 442780 | 53115 | 0 | R1 and R2 only |
| Clgn | P52194 | 3 | 2 | 1 | 344620 | 49523 | 62274 | R1 and R2 only |
| Skt | A2AQ25 | 0 | 5 | 0 | 0 | 48382 | 0 | R2 only |
| Rqcd1 | Q9JKY0 | 2 | 1 | 0 | 91131 | 47676 | 0 | R1 only |
| Gmps | Q3THK7 | 2 | 2 | 0 | 131990 | 46712 | 0 | R1 and R2 only |
| D1Pas1;Ddx3x;Ddx3y | P16381;Q62167;Q62095 | 2 | 2 | 0 | 149500 | 46676 | 0 | R1 and R2 only |
| Odf1 | Q61999 | 2 | 1 | 0 | 332760 | 44049 | 0 | R1 only |
| Ruvbl1 | P60122 | 0 | 2 | 0 | 0 | 43574 | 0 | R2 only |
| Kifap3 | P70188 | 2 | 1 | 0 | 231490 | 41384 | 0 | R1 only |
| Actl7a | Q9QY84 | 0 | 2 | 0 | 0 | 40644 | 0 | R2 only |
| Ubr5 | Q80TP3 | 0 | 6 | 0 | 0 | 38908 | 0 | R2 only |
| Rock1 | P70335;P70336 | 0 | 3 | 0 | 0 | 38476 | 0 | R2 only |
| Rps2 | P25444 | 5 | 1 | 0 | 1473300 | 38357 | 0 | R1 only |
| RtcA | Q9D7H3 | 2 | 1 | 0 | 114410 | 37842 | 0 | R1 only |
| Kiaa1324 | A2AFS3 | 10 | 1 | 0 | 5900000 | 37639 | 0 | R1 only |
| Apoa1bp | Q8K4Z3 | 2 | 1 | 0 | 246830 | 36405 | 0 | R1 only |
| Txnrd3 | Q99MD6 | 7 | 1 | 0 | 1342700 | 36342 | 0 | R1 only |
| Tbc1d1 | Q60949 | 0 | 3 | 0 | 0 | 36202 | 0 | R2 only |
| Timm50 | Q9D880 | 2 | 1 | 0 | 173140 | 35819 | 0 | R1 only |
| Blvra | Q9CY64 | 3 | 2 | 1 | 287230 | 35317 | 26522 | R1 and R2 only |
| Cpsf1 | Q9EPU4 | 7 | 3 | 0 | 407640 | 34499 | 0 | R1 and R2 only |
| Flna | Q8BTM8 | 1 | 5 | 0 | 9298,2 | 34102 | 0 | R2 only |
| Ccdc13 | D3YV10 | 2 | 2 | 0 | 65841 | 33416 | 0 | R1 and R2 only |
| Fyttd1 | Q91Z49 | 3 | 1 | 0 | 412970 | 33406 | 0 | R1 only |
| Poldip3 | Q8BG81 | 2 | 2 | 0 | 45321 | 31020 | 0 | R1 and R2 only |
| Piwil1 | Q9JMB7 | 10 | 3 | 0 | 767380 | 30549 | 0 | R1 and R2 only |
| Tpp2 | Q64514 | 6 | 2 | 0 | 219590 | 28832 | 0 | R1 and R2 only |
| Kif3a | P28741;P33174 | 3 | 1 | 0 | 160620 | 28635 | 0 | R1 only |
| Uso1 | Q9Z1Z0 | 0 | 2 | 0 | 0 | 28261 | 0 | R2 only |
| Vim | P20152;P31001 | 2 | 2 | 0 | 53445 | 26731 | 0 | R1 and R2 only |
| Tln1 | P26039;Q71LX4 | 5 | 5 | 0 | 68758 | 26539 | 0 | R1 and R2 only |
| Pc | Q05920 | 2 | 1 | 0 | 98436 | 26509 | 0 | R1 only |
| Hsp90R1 | P11499 | 5 | 1 | 0 | 1037800 | 26372 | 0 | R1 only |
| Map4 | P27546 | 1 | 2 | 0 | 67052 | 25354 | 0 | R2 only |
| Upf1 | Q9EPU0 | 18 | 2 | 0 | 2328000 | 25293 | 0 | R1 and R2 only |
| Golga1 | Q9CW79 | 0 | 2 | 0 | 0 | 22905 | 0 | R2 only |
| Myh8;Myh4;Myh1 | P13542;Q5SX39;Q5SX40 | 2 | 2 | 0 | 44360 | 22133 | 0 | R1 and R2 only |
| Hnrnpu | Q8VEK3 | 3 | 1 | 0 | 396380 | 22088 | 0 | R1 only |
| Ahsa1 | Q8BK64 | 2 | 1 | 0 | 171790 | 21464 | 0 | R1 only |
| Uhrf1bp1l | A2RSJ4 | 7 | 1 | 0 | 442990 | 21354 | 0 | R1 only |
| Pgam5 | Q8BX10 | 3 | 1 | 0 | 504060 | 21146 | 0 | R1 only |
| Man2c1 | Q91W89 | 2 | 2 | 1 | 63138 | 20969 | 12134 | R1 and R2 only |
| Tjp1 | P39447 | 10 | 4 | 0 | 426880 | 20721 | 0 | R1 and R2 only |
| Eif4a3 | Q91VC3 | 2 | 1 | 0 | 52120 | 20420 | 0 | R1 only |
| Eci1 | P42125 | 2 | 1 | 0 | 107850 | 19520 | 0 | R1 only |
| Stat4 | P42228 | 1 | 2 | 0 | 18273 | 18517 | 0 | R2 only |
| Il4i1 | O09046 | 2 | 1 | 0 | 144390 | 16047 | 0 | R1 only |
| Prps1;Prps2 | Q9D7G0;Q9CS42 | 6 | 1 | 0 | 523570 | 14765 | 0 | R1 only |
| Xpnpep1 | Q6P1B1 | 2 | 1 | 0 | 63335 | 14765 | 0 | R1 only |
| Huwe1 | Q7TMY8 | 2 | 3 | 0 | 19703 | 13376 | 0 | R1 and R2 only |
| Ndufa10 | Q99LC3 | 2 | 1 | 0 | 41505 | 12590 | 0 | R1 only |
| Eprs | Q8CGC7 | 1 | 2 | 0 | 30547 | 12325 | 0 | R2 only |
| Kif3b;Kif3c | Q61771;O35066 | 6 | 2 | 0 | 329860 | 12146 | 0 | R1 and R2 only |
| Nup160 | Q9Z0W3 | 0 | 2 | 0 | 0 | 10456 | 0 | R2 only |
| Drg1 | P32233 | 3 | 1 | 0 | 128230 | 8231,4 | 0 | R1 only |
| Usp15 | Q8R5H1 | 3 | 1 | 0 | 101310 | 7650 | 0 | R1 only |
| Sf3a1 | Q8K4Z5 | 2 | 1 | 0 | 86356 | 5839,5 | 0 | R1 only |
| Vcl | Q64727 | 2 | 1 | 0 | 46706 | 5739,2 | 0 | R1 only |
| Cnot1 | Q6ZQ08 | 0 | 3 | 0 | 0 | 5397 | 0 | R2 only |
| Odf2 | A3KGV1 | 2 | 1 | 0 | 21144 | 2948,3 | 0 | R1 only |
| Dync1h1 | Q9JHU4 | 2 | 2 | 1 | 12517 | 843,22 | 5755,4 | R1 and R2 only |
| Tcea2 | Q9QVN7;P10711 | 15 | 0 | 0 | 77583000 | 0 | 0 | R1 only |
| Slc4a1 | P04919 | 17 | 0 | 0 | 35820000 | 0 | 0 | R1 only |
| Kif17 | Q99PW8;O35231 | 33 | 1 | 0 | 21062000 | 0 | 0 | R1 only |
| Cap2 | Q9CYT6 | 14 | 0 | 0 | 16491000 | 0 | 0 | R1 only |
| Kank3 | Q9Z1P7 | 15 | 0 | 0 | 8436100 | 0 | 0 | R1 only |
| Rpl9 | P51410 | 5 | 0 | 0 | 7949900 | 0 | 0 | R1 only |
| Dynll1 | P63168 | 3 | 0 | 0 | 6071100 | 0 | 0 | R1 only |
| Kctd9 | Q80UN1 | 9 | 0 | 0 | 3984300 | 0 | 0 | R1 only |
| Gtsf1 | Q9DAN6 | 3 | 0 | 0 | 2817800 | 0 | 0 | R1 only |
| Bcap29 | Q61334 | 3 | 0 | 0 | 2109700 | 0 | 0 | R1 only |
| Dnali1 | Q8BVN8 | 5 | 0 | 0 | 1832800 | 0 | 0 | R1 only |
| Ctnnd1 | P30999 | 12 | 0 | 0 | 1456600 | 0 | 0 | R1 only |
| Tmem239 | Q9DA47 | 2 | 0 | 0 | 1390700 | 0 | 0 | R1 only |
| Sh3d21 | Q7TSG5 | 6 | 0 | 0 | 1203800 | 0 | 0 | R1 only |
| Gnptg | Q6S5C2 | 4 | 0 | 0 | 1177600 | 0 | 0 | R1 only |
| Golga3 | P55937 | 20 | 0 | 0 | 1123200 | 0 | 0 | R1 only |
| Golga5 | Q9QYE6 | 10 | 0 | 0 | 988130 | 0 | 0 | R1 only |
| Pspc1 | Q8R326 | 2 | 0 | 0 | 960890 | 0 | 0 | R1 only |
| Rps13 | P62301 | 3 | 0 | 0 | 814080 | 0 | 0 | R1 only |
| Osbp | Q3B7Z2 | 4 | 0 | 0 | 805840 | 0 | 0 | R1 only |
| Cbr4 | Q91VT4 | 3 | 0 | 0 | 759080 | 0 | 0 | R1 only |
| Lrrc46 | Q9DAP0 | 5 | 0 | 0 | 736280 | 0 | 0 | R1 only |
| Rel | P15307 | 5 | 0 | 0 | 658640 | 0 | 0 | R1 only |
| Ccdc136 | Q3TVA9 | 8 | 0 | 0 | 566240 | 0 | 0 | R1 only |
| Psma2 | P49722 | 4 | 0 | 0 | 506470 | 0 | 0 | R1 only |
| Zmym4 | A2A791 | 7 | 0 | 0 | 479830 | 0 | 0 | R1 only |
| Dynlt1 | P51807 | 2 | 0 | 0 | 479170 | 0 | 0 | R1 only |
| Snap29 | Q9ERB0 | 2 | 0 | 0 | 478880 | 0 | 0 | R1 only |
| Ssr1 | Q9CY50 | 2 | 0 | 0 | 466850 | 0 | 0 | R1 only |
| Rpn1 | Q91YQ5 | 5 | 0 | 0 | 457730 | 0 | 0 | R1 only |
| Rsph9 | Q9D9V4 | 2 | 0 | 0 | 384920 | 0 | 0 | R1 only |
| Srsf1 | Q6PDM2 | 2 | 0 | 0 | 379540 | 0 | 0 | R1 only |
| Usp28 | Q5I043 | 5 | 0 | 0 | 340430 | 0 | 0 | R1 only |
| Hnrnpa2b1 | O88569 | 2 | 0 | 0 | 334490 | 0 | 0 | R1 only |
| Crybg3 | Q80W49 | 5 | 0 | 0 | 314850 | 0 | 0 | R1 only |
| Pdzd8 | B9EJ80 | 5 | 0 | 0 | 314600 | 0 | 0 | R1 only |
| Ubxn1 | Q922Y1 | 2 | 0 | 0 | 307630 | 0 | 0 | R1 only |
| Rnf220 | Q6PDX6 | 2 | 0 | 0 | 295420 | 0 | 0 | R1 only |
| Klc2 | O88448;O88447 | 4 | 0 | 0 | 294240 | 0 | 0 | R1 only |
| Otud4 | B2RRE7 | 4 | 0 | 0 | 291460 | 0 | 0 | R1 only |
| Wdr61 | Q9ERF3 | 3 | 0 | 0 | 290590 | 0 | 0 | R1 only |
| Mapkap1 | Q8BKH7 | 2 | 0 | 0 | 276910 | 0 | 0 | R1 only |
| Isyna1 | Q9JHU9 | 2 | 0 | 0 | 270340 | 0 | 0 | R1 only |
| Cttnbp2 | B9EJA2 | 6 | 0 | 0 | 252000 | 0 | 0 | R1 only |
| Dld | O08749 | 2 | 0 | 0 | 247930 | 0 | 0 | R1 only |
| Bag6 | Q9Z1R2 | 4 | 0 | 0 | 246860 | 0 | 0 | R1 only |
| Sh3gl2 | Q62420 | 3 | 0 | 0 | 242500 | 0 | 0 | R1 only |
| Hspb1 | P14602 | 2 | 0 | 0 | 241770 | 0 | 0 | R1 only |
| Otub2 | Q9CQX0 | 2 | 0 | 0 | 237760 | 0 | 0 | R1 only |
| Arhgap17 | Q3UIA2 | 3 | 0 | 0 | 237560 | 0 | 0 | R1 only |
| Rpa2 | Q62193 | 3 | 0 | 0 | 235990 | 0 | 0 | R1 only |
| Skiv2l2 | Q9CZU3 | 5 | 0 | 0 | 232690 | 0 | 0 | R1 only |
| Otub1 | Q7TQI3 | 2 | 0 | 0 | 220290 | 0 | 0 | R1 only |
| Rictor | Q6QI06 | 7 | 0 | 0 | 220020 | 0 | 0 | R1 only |
| Atp5f1 | Q9CQQ7 | 2 | 0 | 0 | 212740 | 0 | 0 | R1 only |
| Acot7 | Q91V12 | 2 | 0 | 0 | 190940 | 0 | 0 | R1 only |
| Rdh11 | Q9QYF1 | 2 | 0 | 0 | 183580 | 0 | 0 | R1 only |
| Polr2c | P97760 | 2 | 0 | 0 | 182800 | 0 | 0 | R1 only |
| Rps18 | P62270 | 2 | 1 | 0 | 177970 | 0 | 0 | R1 only |
| Phb2 | O35129 | 4 | 0 | 0 | 150050 | 0 | 0 | R1 only |
| Ccnb1ip1 | D3Z3K2 | 2 | 0 | 0 | 148000 | 0 | 0 | R1 only |
| Cryz | P47199 | 2 | 0 | 0 | 146680 | 0 | 0 | R1 only |
| Aimp2 | Q8R010 | 2 | 0 | 0 | 139050 | 0 | 0 | R1 only |
| Adsl | P54822 | 2 | 0 | 0 | 137730 | 0 | 0 | R1 only |
| Ift122 | Q6NWV3 | 3 | 0 | 0 | 136580 | 0 | 0 | R1 only |
| Fam213a | Q9CYH2 | 2 | 0 | 0 | 128440 | 0 | 0 | R1 only |
| Sugt1 | Q9CX34 | 2 | 0 | 0 | 127610 | 0 | 0 | R1 only |
| Ndufv2 | Q9D6J6 | 3 | 0 | 0 | 127350 | 0 | 0 | R1 only |
| Stx12 | Q9ER00 | 2 | 0 | 0 | 121450 | 0 | 0 | R1 only |
| Wtap | Q9ER69 | 2 | 0 | 0 | 119330 | 0 | 0 | R1 only |
| Fxr1;Fmr1;Fxr2 | Q61584;P35922;Q9WVR4 | 2 | 0 | 0 | 111570 | 0 | 0 | R1 only |
| Pa2g4 | P50580 | 2 | 0 | 0 | 111040 | 0 | 0 | R1 only |
| Cacybp | Q9CXW3 | 2 | 0 | 0 | 110630 | 0 | 0 | R1 only |
| Lrrc59 | Q922Q8 | 2 | 0 | 0 | 110050 | 0 | 0 | R1 only |
| Adad1 | Q5SUE7 | 2 | 0 | 0 | 97389 | 0 | 0 | R1 only |
| Pacs1 | Q8K212 | 2 | 0 | 0 | 96109 | 0 | 0 | R1 only |
| Farsb | Q9WUA2 | 2 | 0 | 0 | 95382 | 0 | 0 | R1 only |
| Ddost | O54734 | 2 | 0 | 0 | 94283 | 0 | 0 | R1 only |
| Ppp1r7 | Q3UM45 | 2 | 0 | 0 | 92202 | 0 | 0 | R1 only |
| Tnrc6b | Q8BKI2 | 3 | 0 | 0 | 91699 | 0 | 0 | R1 only |
| Cul9 | Q80TT8 | 5 | 0 | 0 | 90586 | 0 | 0 | R1 only |
| Nt5c1b | Q91YE9 | 2 | 0 | 0 | 90406 | 0 | 0 | R1 only |
| Nudt16l1 | Q8VHN8 | 2 | 0 | 0 | 86393 | 0 | 0 | R1 only |
| Gart | Q64737 | 2 | 0 | 0 | 84251 | 0 | 0 | R1 only |
| Actl6a;Actl6b | Q9Z2N8;Q99MR0 | 2 | 0 | 0 | 82171 | 0 | 0 | R1 only |
| Prkaca;Prkacb | P05132;P68181 | 2 | 0 | 0 | 82106 | 0 | 0 | R1 only |
| Psmc3 | O88685 | 3 | 0 | 0 | 81001 | 0 | 0 | R1 only |
| Mrps22 | Q9CXW2 | 2 | 0 | 0 | 76739 | 0 | 0 | R1 only |
| Ipo4 | Q8VI75 | 2 | 0 | 0 | 73788 | 0 | 0 | R1 only |
| Psat1 | Q99K85 | 2 | 0 | 0 | 70751 | 0 | 0 | R1 only |
| Get4 | Q9D1H7 | 2 | 0 | 0 | 70049 | 0 | 0 | R1 only |
| Hk1 | P17710;O08528 | 3 | 0 | 0 | 67956 | 0 | 0 | R1 only |
| Cbr1;Cbr3 | P48758;Q8K354 | 2 | 0 | 0 | 67005 | 0 | 0 | R1 only |
| Cab39 | Q06138 | 2 | 0 | 0 | 58078 | 0 | 0 | R1 only |
| Prr5 | Q812A5 | 2 | 0 | 0 | 54652 | 0 | 0 | R1 only |
| Dnajc7 | Q9QYI3 | 2 | 0 | 0 | 48839 | 0 | 0 | R1 only |
| Dhx30 | Q99PU8 | 2 | 0 | 0 | 46771 | 0 | 0 | R1 only |
| Matr3 | Q8K310 | 2 | 0 | 0 | 41923 | 0 | 0 | R1 only |
| Usp9x | P70398 | 2 | 0 | 0 | 24195 | 0 | 0 | R1 only |
| Ctsd | P18242 | 2 | 0 | 0 | 20903 | 0 | 0 | R1 only |
| Myh9;Myh10 | Q8VDD5;Q61879 | 2 | 0 | 0 | 14942 | 0 | 0 | R1 only |

**Supplementary file 1i. crRNAs employed for the generation of the different mouse models.**

|  |  |  |
| --- | --- | --- |
| Mouse model | crRNA ID | Sequence |
| *Hsf2bp*-/- | sgRNA1 | 5’-TCACAAAACTCTCCATCGTC-3’ |
| sgRNA2 | 5’-ATTGGATGGGGATGTCAAGG-3’ |
| *Hsf2bp*S167L/S167L | sgRNA1 | 5’-TCACAAAACTCTCCATCGTC-3’ |
| sgRNA2 | 5’-ATTGGATGGGGATGTCAAGG-3’ |
| *Brme1* Δ142-472 /Δ142-472 | sgRNA3 | 5’-AACCTCAGGGACTCTCTCTG-3’ |
| sgRNA4 | 5’-GAAGTCTAGTTCCATTGCTG-3’ |
| *Brme1*-/- | sgRNA3 | 5’-AACCTCAGGGACTCTCTCTG-3’ |
| *Spo11*-/- | sgRNA5 | 5’-TATGTCTCTATGCAGATGCA-3’ |
| sgRNA6 | 5’- ACACTGACAGCCAGCTCTTT-3’ |
| *Rnf212*-/- | sgRNA7 | 5’- ACCCACGTGAGACTCGCGCG-3’ |
| sgRNA8 | 5’- CCTCAAAGGTCCGCGTATTC-3’ |
| *Hei10*-/- | sgRNA9 | 5’- GAAAGGGTACTGTTGCAAGC-3’ |

**Supplementary file 1j. ssODN employed for the different mouse models generation.**

|  |  |
| --- | --- |
| Mouse model | ssODN sequence |
| *Hsf2bp*S167L/S167L | 5’CTTTGGAAAGATGTGACAGTTCTATCTTTTTTATCTTTCAGGACAAAGCATTGAAGTTTTTCAACATAACTGG**A**CAGACGATGGAGAGTTTTGTGAAGT**T**ATTGGATGGGGATGTCAAGGA**A**GTTGATTCTGATGAAAATCAATTTGTCTTTGCACTGGCTGGAATTGTAACAAGTAGGTAACTTTTCAGATACAGCGCT3’ |
| *Brme1* Δ142-472 /Δ142-472 | 5’CTTCAGAGTGCTTGCTTATTGAAGGCCAGGACTGAATCTTCTTTTTCCACAGGAAACAAGGCCAGAGCTGGGAGCCCTCAAAGCAGCCAGCCAGCCACAGGCAATGGAACTAGACTTCCTGCCTGACAGCCAGATACAGGATGCCCTGGATGCCACTAACATGGAGCAGGTAAGAGCTTTCTGTACTCAAATGTACACCC3’ |
| *Spo11-/-* | 5’GTTTCCTGCGGTATGTGTTCTCTGCCGTGGTCTGTGTTTGTCACCGTCCAGGAGCAATGCTCATTCTGTGTTGA**G**CTTGCATCTGCATAGAGACATAT**T**CT**T**CACTGACAGCCAGCTCTTTGG**C**AACCAGGCTGCGGTGGACAGCGCCATCGATGACATTTCCTGTATGCTGAAAGTGCCCAGGAGGAGTCTGCACGTGG3’ |

**Supplementary file 1k. Primers and expected product size for genotyping each mutant mouse model.**

|  |  |  |  |
| --- | --- | --- | --- |
| Mouse model | Genotyping Primer | Sequence | Size (bp) |
| *Hsf2bp*-/-  *Hsf2bp*S167L/S167L | F1 | 5’-TTCTTTGGAAAGATGTGACAGTTC-3’ | WT and KI alelle: 383  KO alelle: 355 |
| R1 | 5’-ACCTGGGTTTCCTTTAGATCAGTTA-3’ |
| *Brme1 Δ142-472/Δ142-472* | F2 | 5’-GAAAGTTCTTCAGAGTGCTTGCT-3’ | WT alelle: 1228  Δ alelle: 235 |
| R2 | 5’-AGCCCTATCTTGTCACCTAAAG-3’ |
| F3 | 5’-CCCAGCAGATGCCTCTCTTAT-3’ | WT alelle: 150  Δ alelle: No PCR product |
| R3 | 5’-CTCAGCAGAGTTCCAATGCAG-3’ |
| *Brme1* -/- | F2 | 5’-GAAAGTTCTTCAGAGTGCTTGCT-3’ | WT alelle: 646  KO alelle: 606 |
| R3 | 5’-CTCAGCAGAGTTCCAATGCAG-3’ |
| *Spo11*-/- | F4 | 5’- AGAGCCCCCAGTGCTCTTAAC-3’ | WT alelle: 416  KO alelle: 417 |
| R4 | 5’- GGCAGACCCCTCTACCTCTGT-3’ |
| *Rnf212*-/- | F5 | 5’- TTTCTTTGCCTCCGTACTTTTGG-3’ | WT alelle: 557  KO alelle: 415 |
| R5 | 5’- CCCAGGCTTTACTTCAACAACAA -3’ |
| *Hei10*-/- | F6 | 5’- CTGCCTGTTCTCACATCTTC-3’ | WT alelle: 100  KO alelle: 96 |
| R6 | 5’- AGCTTTCCAGAAAGGGTACTG -3’ |

|  |  |  |  |
| --- | --- | --- | --- |
| **Supplementary file 1l. Summary table of all the main and supplementary figures/tables and the associations between them.** |  | | Associated  Suppl. Figures |
| Figure 1 | **Pedigree of the consanguineous family with the variant HSF2BP-S167L** | |  |
| Figure 2 | **Mice carrying the HSF2BP S167L variant show a partial reduction of fertility** | | Fig2\_S1 |
| a | Fertility assessment *Hsf2bp*S167L/S167L females and males |  |
| b | Ovaries histology *Hsf2bp*S167L/S167L and *Hsf2bp-/-* |  |
| c | Follicles quantification *Hsf2bp*S167L/S167L and *Hsf2bp-/-* | Fig2\_S2a |
| d | Testes images *Hsf2bp*S167L/S167L and *Hsf2bp-/-* | Fig2\_S2b |
| e | Testes histology *Hsf2bp*S167L/S167L and *Hsf2bp-/-* |  |
| f | TUNEL of testis *Hsf2bp*S167L/S167L |  |
| g | Epididymis sperm counts *Hsf2bp*S167L/S167L |  |
| Figure 3 | **Meiocytes from *Hsf2bp*S167L/S167L mice show a decrease in the expression of HSF2BP** | |  |
| a | HSF2BP staining in *Hsf2bp*S167L/S167L males. Immunofluorescence |  |
| b | HSF2BP staining in *Hsf2bp*S167L/S167L females. Immunofluorescence |  |
| c | Western blot aHSF2BP in *Hsf2bp*S167L/S167L and *Hsf2bp-/-*13 dpp males |  |
| Figure 4 | **DNA repair in *Hsf2bp*****S167L/S167L** **mice** | |  |
| a | gH2AX staining in *Hsf2bp*S167L/S167L and *Hsf2bp-/-* males. Immunofluorescence |  |
| b | RPA1 staining in *Hsf2bp*S167L/S167L and *Hsf2bp-/-* males. Immunofluorescence | Fig4\_S1a |
| c | gH2AX staining in *Hsf2bp*S167L/S167L and *Hsf2bp-/-* females. Immunofluorescence |  |
| d | RPA1 staining in *Hsf2bp*S167L/S167L and *Hsf2bp-/-* females. Immunofluorescence | Fig4\_S1b |
| Figure 5 | **The loading of recombinases is compromised in *Hsf2bp*****S167L/S167L** **mice** | |  |
| a | DMC1 staining in *Hsf2bp*S167L/S167L and *Hsf2bp-/-* males. Immunofluorescence | Fig5\_S1a |
| b | RAD51 staining in *Hsf2bp*S167L/S167L and *Hsf2bp-/-* males. Immunofluorescence | Fig5\_S1b |
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