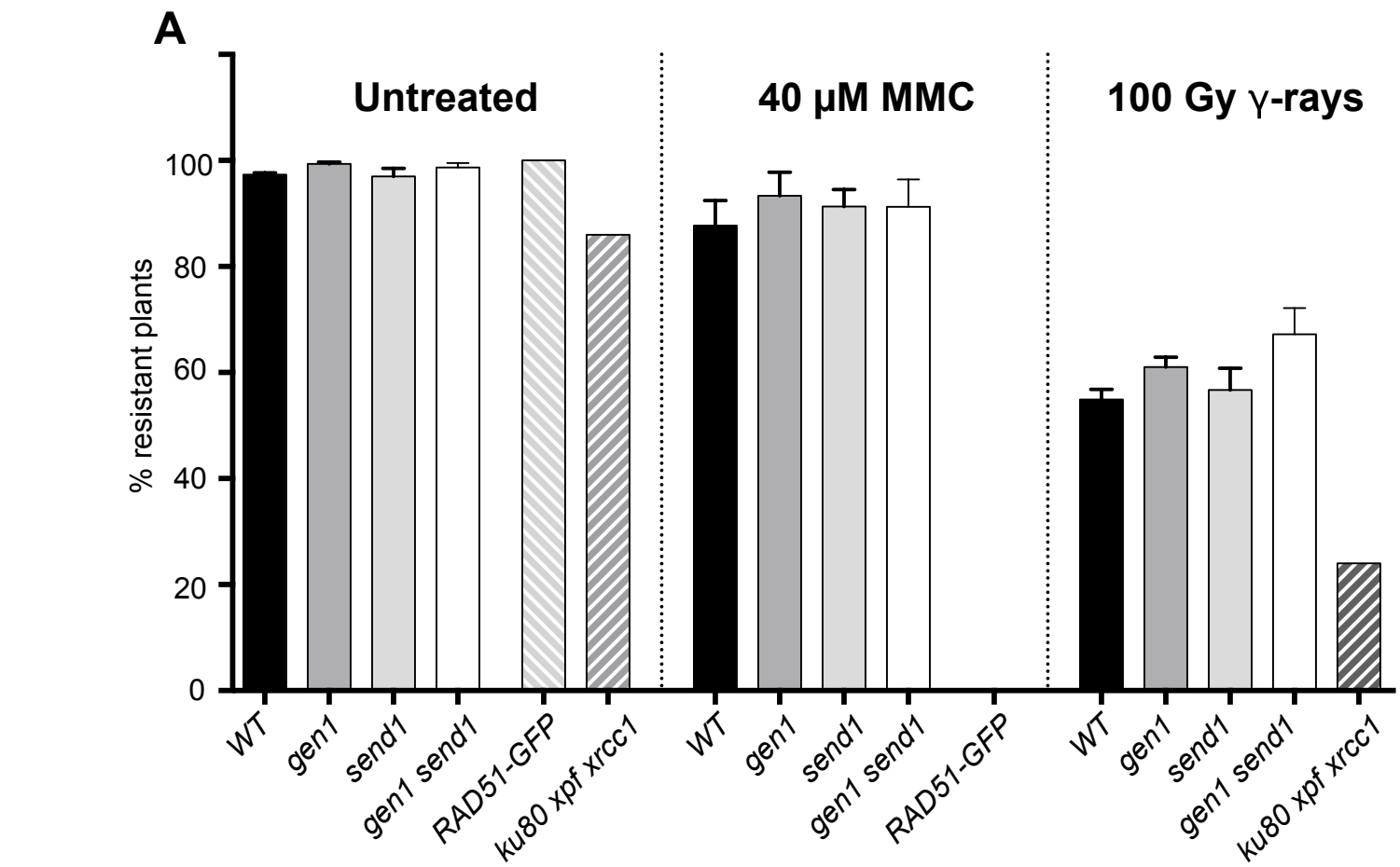
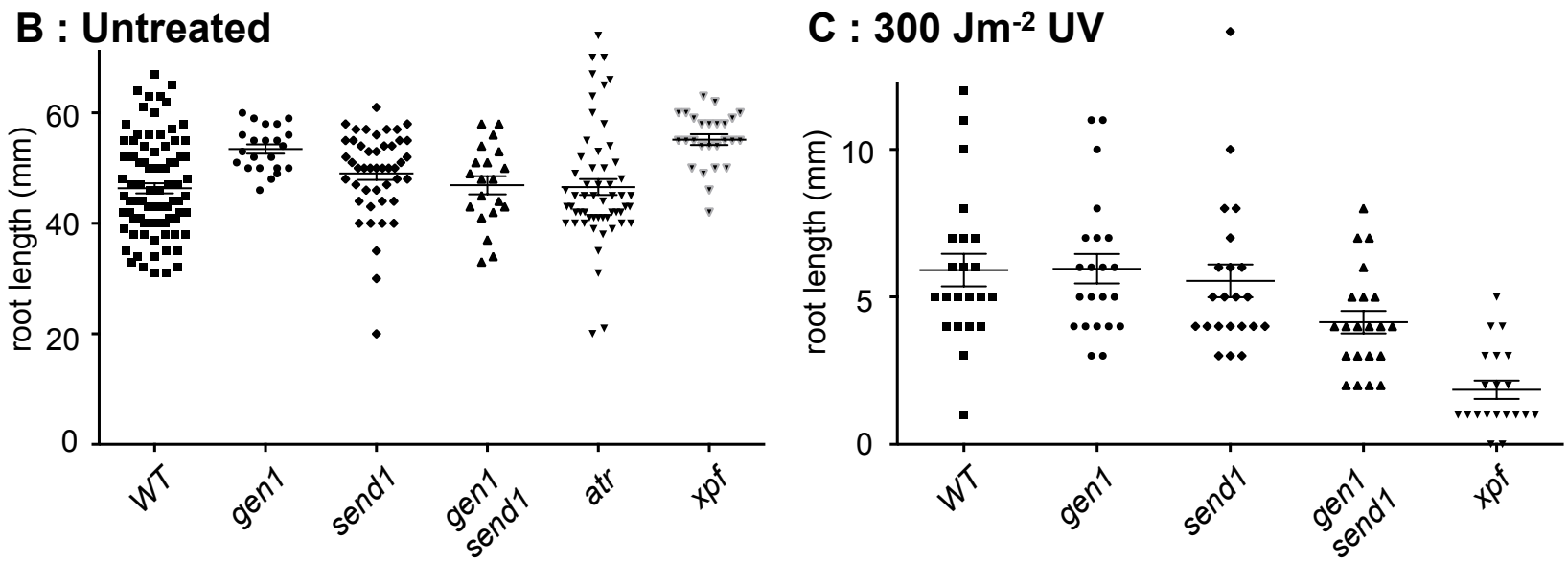
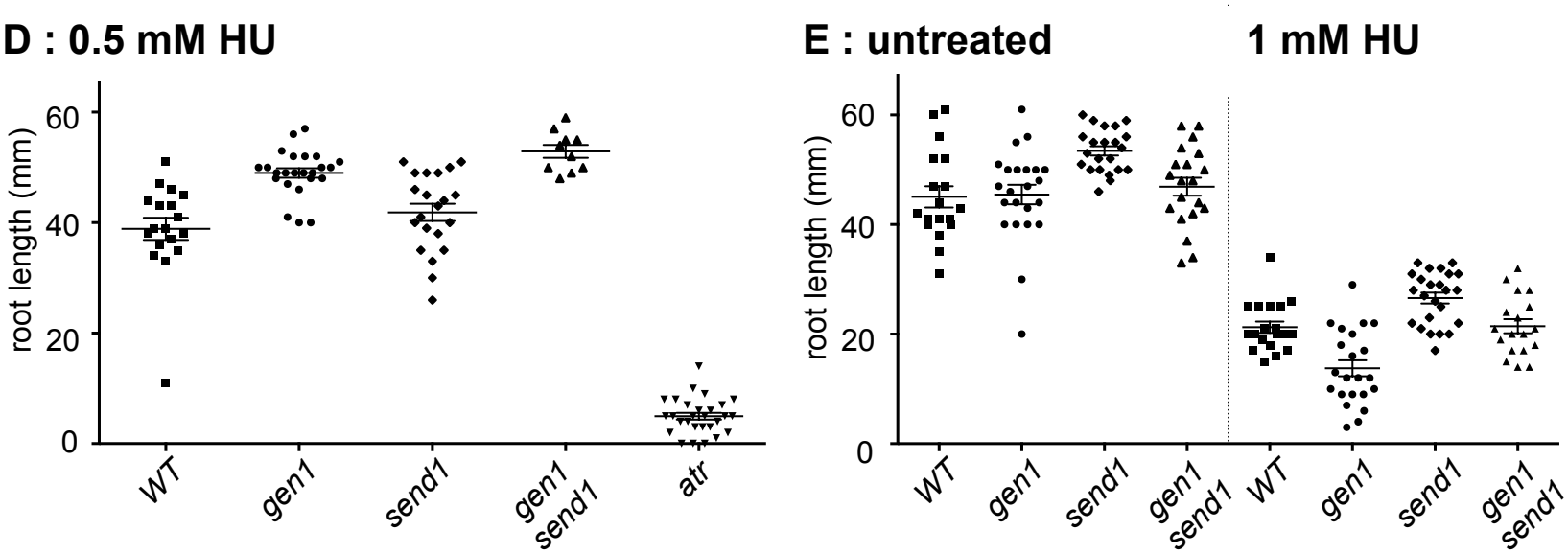
```
Hs-GEN1      MGV-NDLWQILEPVKQHIPL-----------RNLGGKTIAVDLSLWVCEAQTVKKMMG--          46
Sc-YEN1      MGV-SQIWEFLKPYLQDSRIPLRKFVIDFNKSQKRAPRIAIDAYGWLFECGFIQNIDISA          59
At-GEN1      MGVGGNFWDLLRPYAQQQGF-----------DFLRNKRVAVDLSFWIVQHET--AVKG--          45
At-SEND1     MGV-KYLWDVLEPCKKTFPL-----------DHLQNKRVCVDLSCWMVELHKVNKSYC--          46
             ***   :*:.*.*  :   :                 :.:*   *: :

Hs-GEN1      ----------------------------SVMKPHLRNLFFRI-SYLTQMDVKLVFVMEG          76
Sc-YEN1      RSRSRSRSPTRSPRDSDIDSSQEYYGSRSYTTTGKAVINFISRLKELLSLNVEFLLVFDG         119
At-GEN1      ----------------------------FVLKPHLRLTFFRTINLFSKFGAYPVFVVDG          76
At-SEND1     ----------------------------ATKEKVYLRGFFHRLRALIALNCSIILVSDG          77
                                        .       *:     :  :    ::* :*

Hs-GEN1      EPPKLKADVISKRNQSRYGSSGK--------SWSQ---------------KTGRSHFKSV         113
Sc-YEN1      VMKPS----FKRKFNHEQNATTCDDEKEYYSSWEQHVKNHEVYGNCKGLLAPSDPEFIS-         174
At-GEN1      TPSPLKSQARISRFFRSSGIDTCNLPV--IKDGVS---------------VERNKLFSEW         119
At-SEND1     AIPGIKVPTYKRRLKARFEIADDGVEPS-KETSLK---------------RNMGSEFSCI         121
                         :                    .                    *

Hs-GEN1      LRECLHMLECLGIPWVQAAGEAEAMCAYLNAGGHVDGCLTNDGDTFLYGAQTVYRNFTMN         173
Sc-YEN1      --LVRKLLDLMNISYVIACGEGEAQCVWLQVSGAVDFILSNDSDTLVFGGEKILKNYSKF         232
At-GEN1      VRECVELLELLGIPVLKANGEAEALCAQLNSQGFVDACITPDSDAFLFGAMCVIKDIKPN         179
At-SEND1     IKEAKVIASTLGILCLDGIEEAEAQCALLNSESLCDACFSFDSDIFLFGAKTVYREICLG         181
                   : . : *  : .  *.** *. *:  .  *  :: *.* :::*.  : ::

Hs-GEN1      TKD-------------------PHVDCYTMSSIK--SKLGLDRDALVGLAILLGCDYLPK         212
Sc-YEN1      YDDFGPSSITSHSPSRHHDSKESFVTVIDLPKINKVAGKKFDRLSLLFFSVLLGADYN-R         291
At-GEN1      SRE--------------------P-FECYHMSHIE--SGLGLKRKHLIAISLLVGNDYDSG         217
At-SEND1     EGG--------------------Y-VVCYEMDDIK--KKLGLGRNSLIALALLLGSDYS-Q         218
                                  .    :  *:      : *  *: :::*:* **

Hs-GEN1      GVPGVGKEQALKLIQILKGQSLLQRFNRWNETSCNSS------------PQLLVTKKLAH         260
Sc-YEN1      GVKGLGKNKSLQLAQCEDPNFSMEFYDIFKDFNLEDLTSE---SLRKSRYRLFQKRLY--         346
At-GEN1      GVLGIGVDKALRIVREFSEDQVLERLQDIGNGLQPAVPGGIKSGDDGEEFRSEMKKRSPH         277
At-SEND1     GVRGLRQEKACELVRSIGDNVILEKVASEGLSFAEKP-------------R--KSKKQVR         263
             ** *:  ::: .: :    :  ::                          :   .:

Hs-GEN1      CSVCSHPGSPKDHERNGCRLCKSDKYCEPHDYEYCCPCEWHRTEHDRQLSEVENNIKKKA         320
Sc-YEN1      ------------------------LYCKDHSVELFG-----RNYPVLLNQGSFEGWPSTV         377
At-GEN1      CSRCGHLGSKRTHFKSSCEHCGCDSGCIKKPLGFRCECSFCSKDRDLREQKKTNDWWIKV         337
At-SEND1     PSVCSKKGTLPLVVINGNN-------------------------RDP---ERLEE-----         290
                                                           :

Hs-GEN1      C----CCEGFPFHEVIQEFLLNKDK--L---------------VKVIRYQRPDLLLFQRFT         360
Sc-YEN1      A------IMHYFHPIVQPYFDEEVLSDKYINMAGNGHYRNLNFNELKYFLQSLN-LPQ-I         429
At-GEN1      CDKIALAPEFPNRKIIELYLSDGLMTGD--------------GSSMSWGTPDTGMLVDLM         383
At-SEND1     -----------IKQVIDAFMNPKCHQADSNTVS-------RALAEFSFQRT---KLQEIC         329
                      : ::: ::                          : :       :

Hs-GEN1      LEKMEWPNHYACEKLLVLLTHYDMIERKLGSR-------N--------SNQLQPIRIVKT         405
Sc-YEN1      SSFDKWFHDSMHEMFLL-------REFLSIDESDNIGKGNMRITEE--------KIMNID         474
At-GEN1      VFKLHWDPSYVRKMLLPMLSTIYLREKARNNT-------GY----ALLCDQYEFHSIKCI         432
At-SEND1     HQFFEWPPEKTDEYILPKVAERNLRRFANLQSRSTEVEVNLPLHKPQMPEKCPVSEIIKT         389
                 .*      : :*         .    .                      :

Hs-GEN1      RIRNGVHCFEIEWEKPEHYAMED-----KQHGEF--ALLTIEE-----------------         441
Sc-YEN1      GGKFQIPCFKIRYTTFLPNIPISSQSPLKRSNSPSRSKSPTRR------QMDIMEHP---         525
At-GEN1      KTRYGHQSFVIRWRKPKSTSGYS-----HSHSEPEESIVVLEEEEESVDPLDGLNEPQVQ         487
At-GEN2      RKVQGRECFEVSWNDLEGLESS---------------IVP--------------------         414
                    .* : :

Hs-GEN1      -------------ESLFEAAYPEIVAVYQKQKLEIKGKKQKRIKPKENNLPEPDEVM-SF         487
Sc-YEN1      ---NSLW-LP---KYLIPQSHPLVIQYYETQQLIQKEKEKKGKKSNKSRLPQKNNLDEFL         578
At-GEN1      NDNGDCFLLTDECIGLVQSAFPDETEHFLHEKK-LRESKKKNVSEEETATPRA-------         539
At-SEND1     -------------ADLVERACPEKIIEFKEKMAAKKKKPKPKQKQKETSSPTKSSSLVEL         461
                        *.  : *     :  :    : . :   . ::.  *

Hs-GEN1      Q---SHMTL---------------------------------------KPTCEIFHKQNSK         506
Sc-YEN1      RK--HTSPIKSIGKVGESRKEILEPVRKRLFVDTDEDTSLEEIPAPTRLTTVDEHSDNDD         636
At-GEN1      ----TTMGV---------------------------------------QRSITDFYRSAKK         557
At-SEND1     SLELQHLDLNSTSLVSRSTLE--EAEQENEQQNSKKHDYLRLIDSPDRENCNNAWSNRDR         519
                    :                                        : .     . .

Hs-GEN1      L--------NSGISPDPTLPQESISASLNSLLLPKNTPCLNAQEQFMSSLRPLAIQQIKA         558
Sc-YEN1      DSLIFVDEITNSQSVLDSSPGKRIRDLT-------------QDEQVDVWKDVIEISPIK-         682
At-GEN1      A--------AAGQ-------------------------------------------SIET         566
At-SEND1     L--------GVGMSSFPLYPETEVID--------LISPCPEARSRSV-------------         550
                        .

Hs-GEN1      VSKSLISES---SQPNTS-SHNIS--VIADLHLSTIDWEGTSFSNSPAIQRNTFSHDLKS         612
Sc-YEN1      --KSRTTNAEKN-PPESGLKSRSSITINARL-------QGTKML-PPNLTAPRLEREHSS         731
At-GEN1      GGSSKASAEKKRQAT-STS-SSNLTKSVRRRLL----------------------FG-----         599
At-SEND1     -SRSY---------QEQK-SH---------------------------------DHQLET         566
                *           .   .

Hs-GEN1      EVESELSAIPDGFENIPEQLSCESERYTANIKKVLDEDSDGISPEEHLLSGITDLCLQDL         672
Sc-YEN1      VLDQLVTD---AQDTVDRFVACDSDSSST---------------IE*-------------         759
At-GEN1      ------------------------------------------------------------         599
At-SEND1     VI--ELSDS-----ETDDEEHCKKAREL--------------------------------         587

Hs-GEN1      PLKERIFTKLSYPQDNLQPDVNLKTLSILSVKESCIANSGSDCTSHLSKDLPGIPLQNES         732
Sc-YEN1      ------------------------------------------------------------         759
At-GEN1      ------------------------------------------------------------         599
At-SEND1     ----RIFL------QNIRKDIIL---------------------------------------         600

Hs-GEN1      RDSKILKGDQLLQEDYKVNTSVPYSVSNTVVKTCNVRPPNTALDHSRKVDMQTTRKILMK         792

Hs-GEN1      KSVCLDRHSSDEQSAPVFGKAKYTTQRMKHSSQKHNSSHFKESGHNKLSSPKIHIKETEQ         852

Hs-GEN1      CVRSYETAENEESCFPDSTKSSLSSLQCHKKENNSGTCLDSPLPLRQRLKLRFQST             908
```

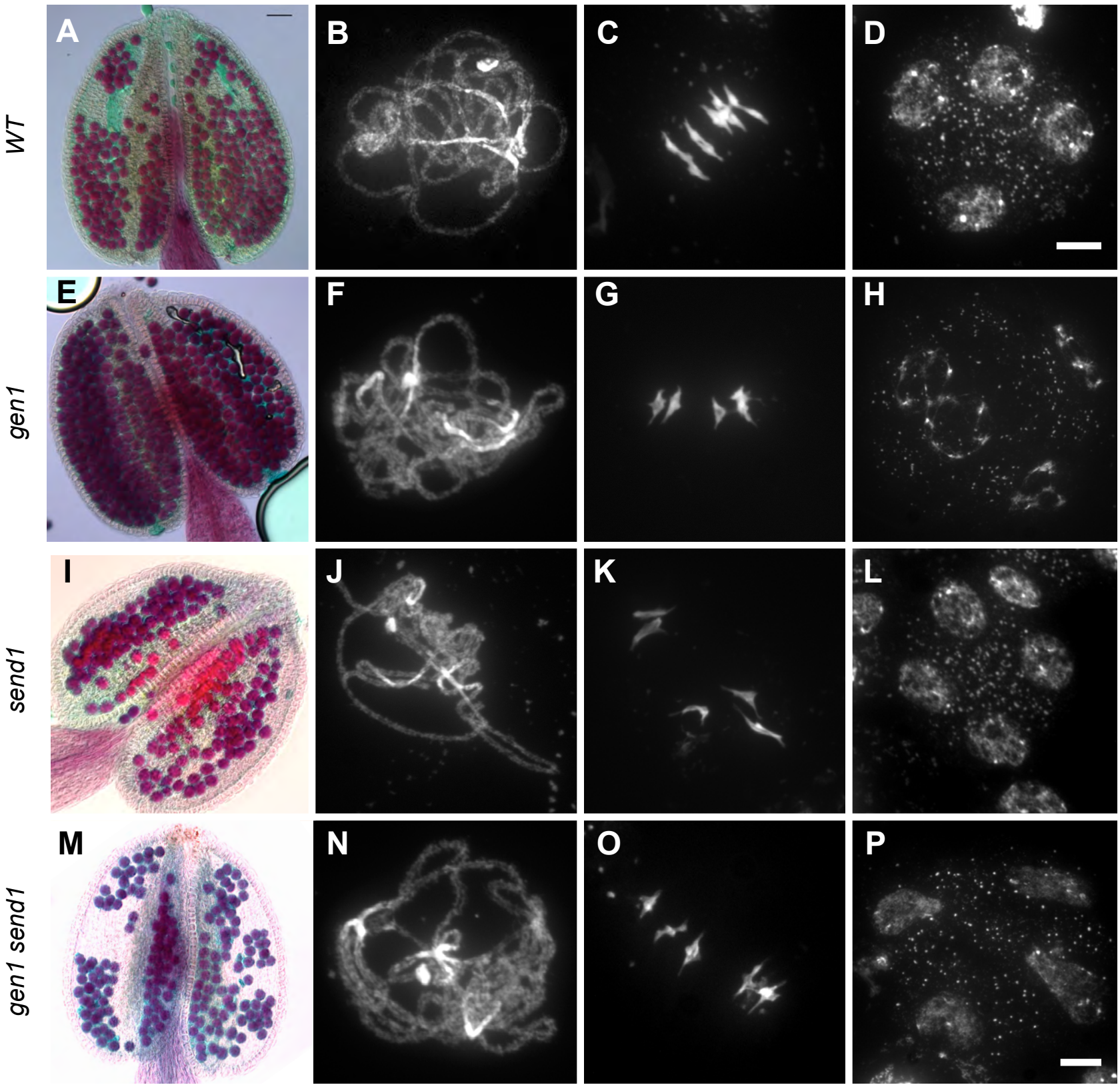
Supplemental Figure 1. Sequence alignment of GEN1/Yen1 proteins. Alignment of *Homo sapiens* Hs-GEN1, *Saccharomyces cerevisiae* Sc-YEN1 and *Arabidopsis thaliana* At-GEN1 and At-SEND1 with ClustalW. Numbers indicate amino acid positions. Magenta letters indicate the amino acids that have been shown to be essential for nuclease activity. The blue, magenta and green lines show (respectively) the positions of the XPG N-terminal (XPG-N) and internal (XPG-I) nuclease domains and helix-hairpin-helix domain. Asterisks, colons and dots indicate identical, conserved and semi-conserved residues, respectively.





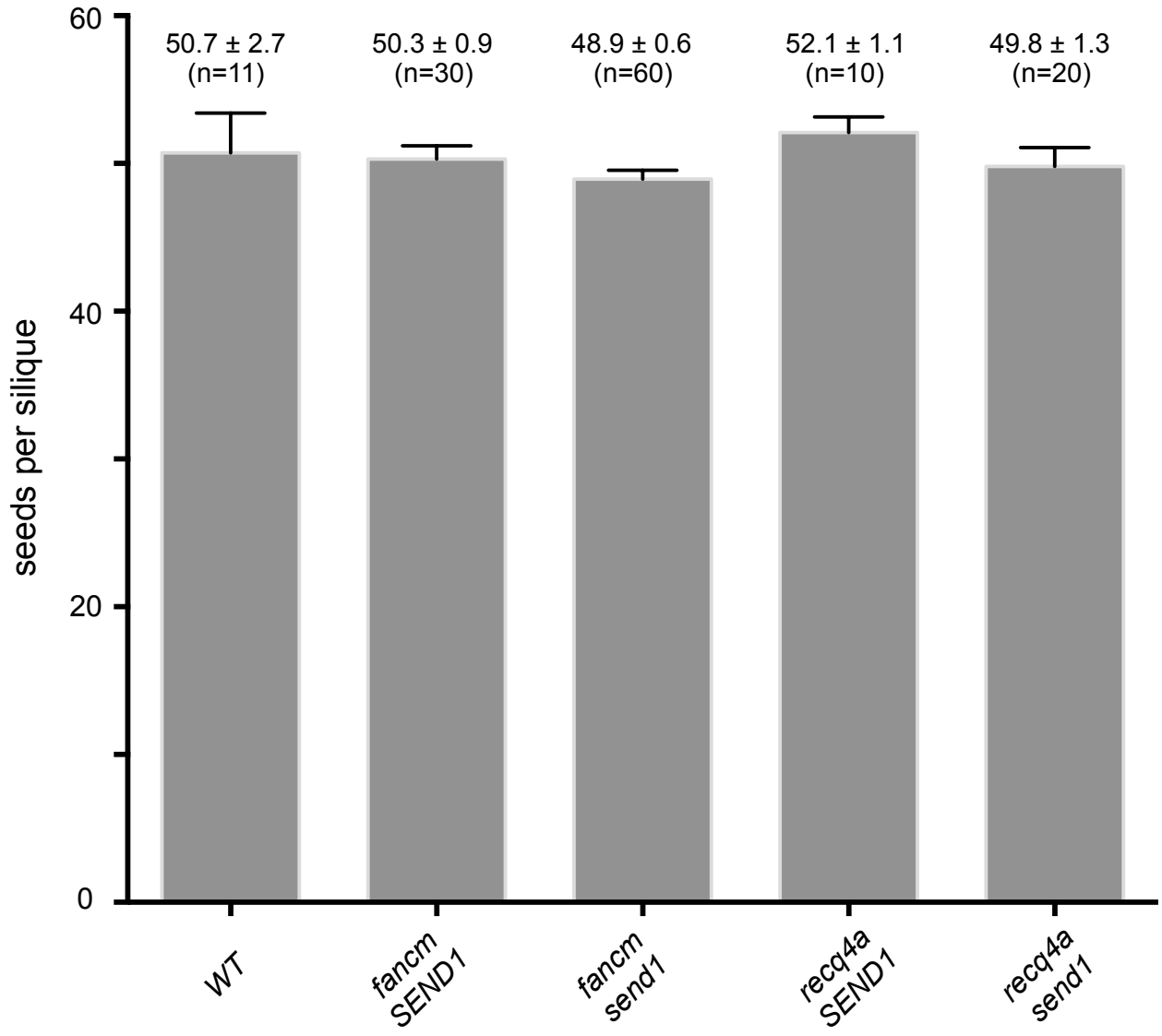


Supplemental Figure 2. Sensitivity of *gen1, send1 and gen1 send1* to DNA damage.
(A) Sensitivities to MMC and gamma-rays. Positive controls are the dominant-negative RAD51-GFP and *ku80 xpf xrcc1* knockout plants. Sensitivities were analysed by counting leaves of two-week old seedlings and scoring those with >3 true leaves as resistant. Data is from scoring 100 plants in each case with 3 replicates of each except for WT+MMC and *send1* +10 Gy gamma-rays (2 replicates each). *RAD51-GFP* and *ku80 xpf xrcc1* controls are from single experiments. HU-treated and UV-treated. Root growth of untreated **(B)**, UV-irradiated **(C)** and HU treated **(D, E)** plants. *xpf* and *atr* mutant plants were included as positive controls for sensitivity to UV and HU respectively **(B, C, D)**. Individual plant measurements showing means +SEM from >18 plants in each case (points on graphs are individual plant measurements).



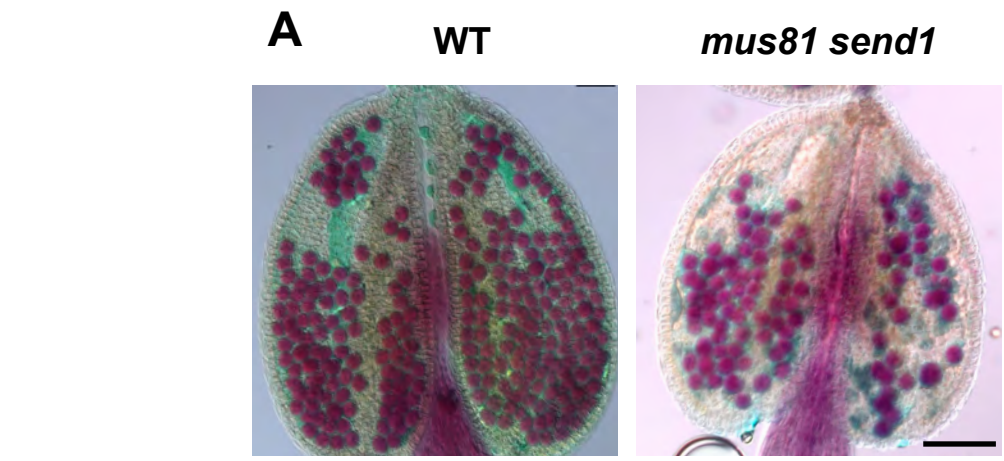
Supplemental Figure 3. Pollen viability and Meiosis in WT and mutant plants. Pollen viability and Meiotic progression in WT (**A to D)**, *gen1* **(E to H)**, *send1* **(I to L)** and *gen1 send1* **(M to P)**. **A, E, I, M**: Alexander staining showing viable pollen (purple-red). DAPI staining of pollen mother cell nuclei showing full synapsis at pachytene (B, F, J, N), 5 bivalents at metaphase I **(C, G, K, O)** and tetrads showing four meiotic products **(D, H, L, P)**. (N = 28 (*gen1*), 36 (*send1*) and 30 (*gen1 send1*)). (Scale Bar: 10 µm).

Supplemental Figure 4. Developmental defects in double and triple mutant plants.
The triple *mus81 send1 gen1* mutant shows similar growth defects to *mus81 send1* plants. 3-week-old plants. A 2cm scale bar is shown at the bottom right.



Supplemental Figure 5. Fertility of *fancm send1 and recq4a send1* mutants. Mean (±SEM) numbers of seeds per silique in wild-type, *fancm*, *fancm send1*, *recq4A*, and *recq4A send1* mutants.

Supplemental Figure 6. Growth of *trd1* and *trd1 send1* mutants.
The single *trd1* and double *trd1 send1* mutants grow normally. The severe growth phenotype of the *mus81 send1 gen1* mutant is shown for comparison. Two-week old plants. A 1cm scale bar is shown at the bottom right.



Supplemental Figure 7. Pollen viability and tetrad analysis in *mus81 send1* mutants
(A) Viable (purple) and inviable (green) Alexander staining of pollen from WT and *mus81 send1* anthers. **(B)** and **(C)** Tetrads resulting from male meiosis in wild-type and *mus81 send1* plants. Meiotic products were stained with toluidine blue and numbers of normal tetrads and aberrant dyads, triads and pentads monitored. A 100µm scale bar is included at bottom right.

Supplemental Table 1.

PCR primers used for characterising *gen1* and *send1* T-DNA mutants

GEN1	
Primer Name	**Sequence**
a	TTAAGGGTTTCGTCCTTAAAC
b	TTCAGAAAACTCACGAACAAT
c	ATGCTTGCATTACTCCTGATA
d	TCGTATTGATCACACAACAAA
TAG6 (T-DNA Left Border)	CACTCAGTCTTTCATCTACGGCA
SEND1	
Primer Name	**Sequence**
e	AGCCTTGTAAGAAGACTTTCC
f	CGTAGCAAACAACATAACCTC
g	CTCAAAAGAAATATGGGGTCT
h	ACTCAAAGAACTGATGGCATA
i (*send1-1* reverse)	CACCTGCTTGATCTCTTCCAG
j	CAAGATCAACTGAGGTTGAAG
k	TCATAGGATAATGTCTTTCCTGA
l (*send1-1* forward)	TATGCCTTGGTCAGTGGAAAG
Lba1 (SALK T-DNA Left Border)	TGGTTCACGTAGTGGGCCATCG