

1 Avid lysosomal acidification in fibroblasts of the Mediterranean mouse *Mus*
2 *spretus*
3

4 Melissa Sui¹, Joanne Teh¹, Kayleigh Fort¹, Daniel Shaw², Peter Sudmant³, Tsuyoshi Koide⁴,
5 Jeffrey M. Good², Juan M. Vazquez^{*3}, Rachel B. Brem^{*1}

6 Departments of ¹Plant and Microbial Biology and ³Integrative Biology, University of California, Berkeley,
7 Berkeley, CA 94720, USA

8 ²Division of Biological Sciences, University of Montana, Missoula, MT 59812, USA

9 ⁴National Institute of Genetics, Mishima, Shizuoka 411-8540, Japan

10 *To whom correspondence should be addressed: juan@vazquez.bio, rbrem@berkeley.edu

11

12 Abstract

13

14 Failures of the lysosome-autophagy system are a hallmark of aging and many disease states.
15 As a consequence, interventions that enhance lysosome function are of keen interest in the
16 context of drug development. Throughout the biomedical literature, evolutionary biologists have
17 discovered that challenges faced by humans in clinical settings have been resolved by non-
18 model organisms adapting to wild environments. Here, we used a primary cell culture approach
19 to survey lysosomal characteristics in selected species of the genus *Mus*. We found that cells
20 from *M. musculus*, mice adapted to human environments, had weak lysosomal acidification and
21 high expression and activity of the lysosomal enzyme β -galactosidase, a classic marker of
22 cellular senescence. Cells of wild relatives, especially the Mediterranean mouse *M. spretus*, had
23 more robustly performing lysosomes and dampened β -galactosidase levels. We propose that
24 classic laboratory models of lysosome function and senescence may reflect characters that
25 diverge from the phenotypes of wild mice. The *M. spretus* phenotype may ultimately provide a
26 blueprint for interventions that ameliorate lysosome breakdown in stress and disease.

27

28 Introduction

29

30 Many aspects of metazoan health hinge on the ability of the lysosome-autophagy pathway to
31 recycle damaged macromolecules and to direct cell growth decisions (Shin & Zoncu 2020).
32 Indeed, interventions that act broadly to promote health and longevity often require the
33 lysosome-autophagy system (Aman *et al.* 2021; Hansen *et al.* 2018; Bareja *et al.* 2019). More
34 specific mechanisms to boost autophagy are also of potential clinical interest, particularly for
35 treatment of proteinopathies and aging etiologies (Bonam *et al.* 2019; Hansen *et al.* 2018). In
36 practice, whether and how to stimulate proteostasis machinery to advance organismal health
37 remains an open question, and the literature describing such manipulations is in its infancy
38 (Simonsen *et al.* 2008; Pyo *et al.* 2013; Pickford *et al.* 2008; Leiva-Rodríguez *et al.* 2018; Shin
39 *et al.* 2013; Liu *et al.* 2023; Bhuiyan *et al.* 2013).

40

41 Cellular senescence is a tumor-suppressive program characterized by cell cycle arrest,
42 resistance to apoptosis, and activation of immune signaling in response to stress (Campisi &
43 d'Adda di Fagagna 2007; Campisi 2005; Hornsby 2002). Though senescence mitigates cellular
44 stress and safeguard health, the accumulation of senescent cells in aged tissues leads to
45 dysfunctional tissue remodeling and chronic inflammation (Krtolica *et al.* 2001; Parrinello *et al.*
46 2005; Davalos *et al.* 2010; Olivieri *et al.* 2018; Wan *et al.* 2021). In senescent cells, lysosomes
47 increase in size, and lysosomal β -galactosidase becomes active at sub-optimal pH (Robbins *et*
48 *al.* 1970, Magalhães & Passos 2018, Dodig *et al.* 2019, Curnock *et al.* 2023). Though the exact
49 role of lysosomes in senescence has not been fully elucidated, enhancements to their function
50 have been associated with reduced cellular degeneration and diminished inflammation (Green
51 *et al.* 2011, Carmona-Gutierrez *et al.* 2016, Rovira *et al.* 2022).

52

53 Against a backdrop of decades of work in laboratory systems, ecologists have cataloged stress-
54 and disease-resistance traits in wild genotypes from unusual niches, perhaps most famously in

55 long-lived animal species (Oka *et al.* 2023; Chusyd *et al.* 2021; Finch 2009). Cell-based surveys
56 represent a powerful approach to find and dissect these natural resilience programs. One
57 particularly fruitful discipline has profiled the variation in chemical stress resistance across
58 primary cells from panels of non-model animal species, including, in landmark cases, discovery
59 of the underlying mechanisms (Tian *et al.* 2019; Harper *et al.* 2007; Attaallah *et al.* 2020; Sulak
60 *et al.* 2016). Cellular senescence has also been shown to vary across animal species in *in vitro*
61 models (Attaallah *et al.* 2020; Kang *et al.* 2023; Zhao *et al.* 2018; Gomez *et al.* 2012).

62
63 In this study, we aimed to leverage species-specific variation in lysosomal markers of cellular
64 senescence within the *Mus* genus to investigate how evolutionary processes have shaped
65 lysosomal function. Mouse species in this genus shared a common ancestor 7-8 million years
66 ago, and as they radiated across Eurasia, *M. musculus* subspecies came to occupy human-
67 associated niches, whereas other taxa are still found exclusively in the wild (Pagès *et al.* 2015,
68 Smissen & Rowe 2018). Our previous case study (Kang *et al.* 2023) found differences in
69 senescence behaviors, including lysosome markers, across fibroblasts from *M. musculus*
70 subspecies and *M. spretus*, a wild relative that diverged 1-3 million years ago (Morgan *et al.*
71 2022; Dejager *et al.* 2009). Here, we aimed to build on these observations to gain a deeper
72 understanding of lysosomal programs in non-model mice, focusing on the contrasts between the
73 genotypes of human commensal and laboratory mice and those of their wild relatives.
74

75 Results

76 ***M. spretus* fibroblasts exhibit reduced lysosomal β-galactosidase activity**

77 To extend our initial observation of divergent cellular senescence behaviors across *Mus* (Kang
78 *et al.* 2023), we established a panel of primary tail skin fibroblasts from four wild-derived strains
79 of *M. m. musculus* (PWK/PhJ, BLG2/Ms, CHD/Ms, MSM/Ms), two wild-derived strains of *M. m.*
80 *domesticus* (ManB/NachJ, TUCA/NachJ), an admixed classic laboratory strain of mostly *M. m.*
81 *domesticus* origin (C57BL/6J), a wild-derived strain of the steppe mouse *M. spicilegus* (ZRU),
82 and two wild-derived strains of the Mediterranean mouse *M. spretus* (STF/Pas, SFM/Pas). We
83 subjected each culture to 15 Gy of infrared irradiation and incubated for 10 days, which is
84 sufficient to induce DNA damage response, arrested cell growth, and senescence expression
85 programs in fibroblasts from across the genus (Kang *et al.* 2023). We first assayed each culture
86 for lysosomal β-galactosidase, as a standard marker of senescence that reports exhaustion of
87 protein quality control in the lysosome (Campisi & d'Adda di Fagagna 2007; Kang *et al.* 2023;
88 Casella *et al.* 2019; Guerrero-Navarro *et al.* 2022; Carmona-Gutierrez *et al.* 2016). Fibroblasts
89 of each genotype displayed the anticipated increase in β-galactosidase activity following
90 irradiation, compared to their respective control counterparts (Figure 1B). Whereas cells from
91 strains of each species behaved similarly, we observed significant differences between species:
92 irradiated *M. spretus* fibroblasts exhibited β-galactosidase signal ~2-fold lower than did cells
93 from *M. m. musculus* and *M. m. domesticus*, and staining of *M. spicilegus* cells was between the
94 two extremes (Figure 1B). To follow up on this difference, we used an alternative approach for
95 senescence induction with the radiomimetic drug neocarzinostatin, which induces senescence
96 in mouse cells at 3.6 µM (Ito *et al.* 2018, Correia Melo *et al.* 2016). Fibroblasts treated with
97

99 neocarzinostatin exhibited a pattern mimicking that we had seen under radiation, with the
100 treatment inducing elevated β -galactosidase activity in all cultures, and cells from *M. spretus*
101 staining lower than those of sister taxa (Figure 1C). Furthermore, the same trend was
102 detectable in cells cultured in the absence of DNA damage: fibroblasts from *M. musculus*
103 subspecies exhibited higher β -galactosidase staining than those of *M. spicilegus*, which stained
104 higher than *M. spretus*. The latter species differences from untreated cells were of smaller
105 magnitude than those manifesting after stress, and this dependence on treatment was
106 statistically significant (Figures 1 and S1). Thus, *M. spretus* fibroblasts exhibited damped β -
107 galactosidase activity relative to other genotypes, with amplification of the effect under stress.
108 Controls ruled out culture passage number as a driver of the variation (Figure S2).

109

110 In principle, differences in the senescence-apoptosis fate choice after DNA damage (Childs *et*
111 *al.* 2014; Zhao *et al.* 2018; Attaallah *et al.* 2020) could contribute to the divergence between
112 mouse genotypes that we had seen in fibroblast β -galactosidase staining. To explore this, we
113 measured Caspase 3/5 activity in fibroblasts in response to irradiation (Figure S3A) and
114 neocarzinostatin treatment (Figure S3B). The results showed no consistent patterns of
115 apoptosis either within or between species' genotypes, arguing against a role for apoptosis
116 activation in species differences in fibroblast β -galactosidase activity.

117

118 To establish further the robustness of *Mus* species differences in fibroblast β -galactosidase
119 activity, we considered the dose-response relationship with stress. We compared primary
120 fibroblasts from *M. m. musculus* PWK and *M. spretus* STF as representatives of their respective
121 species, in each case assaying β -galactosidase upon irradiation at increasing doses. The
122 results revealed a consistent ~2-fold difference between the genotypes at each dose (Figure
123 S4), a magnitude slightly exceeding the species divergence in the untreated control, consistent
124 with our survey across genotypes at fixed stress doses (Figure 1). These data ruled out a switch
125 by *M. spretus* fibroblasts into a high-amplitude, *M. musculus*-like program above a certain
126 threshold of stress exposure. We conclude instead that *M. spretus* cells are hard-wired for lower
127 β -galactosidase activity in all tested conditions—including the resting state.

128

129 **No consistent divergence in senescence signaling across *Mus* species fibroblasts**

130

131 We reasoned that the differences in β -galactosidase activity in fibroblasts among the *Mus*
132 species we tested were likely mediated by mechanisms unrelated to senescence itself. As a first
133 investigation of this idea, we explored senescence signaling, using as a readout P21, a
134 regulator of immune recruitment and cell cycle arrest in senescence (Gu *et al.* 2013; Yew *et al.*
135 2011). We quantified levels of P21 protein in response to either irradiation or neocarzinostatin,
136 with fibroblasts from *M. m. musculus* PWK and *M. spretus* STF as a testbed. Results showed
137 the expected induction of P21 in fibroblasts from both species in response to irradiation or
138 neocarzinostatin (Figure S5). In investigating quantitative patterns among these P21 induction
139 behaviors, we noted some species-specific differences, though they were not of consistent
140 direction: *M. spretus* cells induced P21 protein abundance more than did *M. musculus* under
141 neocarzinostatin treatment but less following irradiation and in untreated controls (Figure S5).
142 These changes could reflect the differential activity of alternate senescence signaling pathways

143 (Saul *et al.* 2023) between species in some treatments. However, given our focus on lysosomal
144 β-galactosidase differences between *M. musculus* and *M. spretus* across all tested conditions
145 (Figure 1), we concluded that P21 regulation was not a consistent correlate of this phenotype
146 and not a compelling candidate for its underlying mechanism. Indeed, in analysis of mRNA
147 expression profiles (Kang *et al.* 2023), we detected no differences in induction of the P21 gene
148 (*CDKN1A*) between *M. musculus* and *M. spretus* fibroblasts in response to stress (Figure S6).

149

150 ***M. spretus* fibroblasts exhibit strongly acidified lysosomes**

151

152 To advance our search to understand *Mus* species differences in β-galactosidase activity in the
153 fibroblast model, we next focused on regulation of the enzyme itself. Transcriptional profiling
154 data from fibroblasts revealed 1.5 to 2-fold higher expression of the lysosomal β-galactosidase
155 *GLB1* in *M. musculus* fibroblasts than in those of *M. spretus*, regardless of treatment (Figure
156 S7A). Allele-specific expression measurements in stressed and control fibroblasts from an
157 interspecies F1 hybrid made clear that this change was regulated in *cis*: that is, the *M. musculus*
158 allele of *GLB1* drove higher expression of its own encoding locus than the *M. spretus* allele,
159 when both were in the same nucleus (Figure S7B). Thus, the species changes in β-
160 galactosidase activity we had noted in terms of cell biology in fibroblasts (Figure 1) were
161 mirrored by regulation of gene expression, including robust differences between genotypes in
162 unstressed control conditions.

163

164 We hypothesized that the higher β-galactosidase expression and activity we had seen in *M. musculus*
165 fibroblasts could be a consequence of heightened need owing to failures elsewhere in
166 the proteostasis system, relative to cells of *M. spretus*. To explore this, we assayed lysosomal
167 acidity with the Lysotracker stain, again making use of *M. m. musculus* PWK and *M. spretus*
168 STF as a test system. Conforming to our prediction, *M. spretus* cells exhibited 3-fold stronger
169 Lysotracker signal relative to cells of *M. musculus*, in untreated cultures (Figure 2, left). The
170 species difference persisted in cultures induced to senesce with irradiation (Figure 2, middle)
171 and neocarzinostatin (Figure 2, right). Taken together, our results establish a trait syndrome of
172 elevated lysosomal acidification and low β-galactosidase expression and activity in *M. spretus*
173 fibroblasts relative to those of other mice, whether the cells are senescent or not.

174

175 **Discussion**

176

177 For decades, biomedical researchers have relied on strains from the *M. musculus* clade as the
178 standard for studying lysosomal and senescence biology. In this work, we have demonstrated
179 that lysosomal phenotypes vary quantitatively across *Mus* in fibroblast culture models, and that
180 in contrast to *M. musculus*, cells from the non-commensal mouse *M. spretus* exhibit a lysosomal
181 acidity and β-galactosidase program of the kind that, in the experimental literature, has been
182 associated with healthy aging and disease resistance.

183

184 When experimentally induced in laboratory cell models, a backup in the lysosomal-autophagy
185 system triggers compensatory increases in lysosomal mass and number, for which high β-
186 galactosidase activity is a robust marker (Curnock *et al.* 2023; Delfarah *et al.* 2021; Lee *et al.*

187 2006, Dimri *et al.* 1995, Rovira *et al.* 2022). We propose a similar causal relationship between
188 the two phenotypes we have observed as they vary across *Mus* fibroblasts. Under this model,
189 the elevated β -galactosidase expression and activity in *M. musculus* cells would represent
190 compensation for their weak lysosomal acidification in comparison to *M. spretus* genotypes,
191 even in the absence of experimental stress. The *M. musculus* cis-regulatory allele upregulating
192 β -galactosidase that we have noted in fibroblasts could well represent a genetically encoded
193 component of such a program, a constitutive boost in protein degradation capacity by a
194 regulatory mechanism, in the face of lysosomal acidification defects.
195

196 Our work leaves open the question of whether weak lysosomal acidity and high β -galactosidase
197 in fibroblasts represent a state ancestral to *Mus* that was resolved in the *M. spicilegus*-*M.*
198 *spretus* lineage, or an evolutionary novelty that arose with commensalism in *M. musculus*. In
199 either case, the evidence that we have seen for conservation across *M. musculus* strains and
200 subspecies strongly suggests a history of constraint in this lineage. If the lysosomal behaviors
201 we have studied here prove to have evolved under selection, they could conceivably relate to
202 body size, response to immune challenges, or association with human ecology as they differ
203 between *M. musculus* and *M. spretus* (Dejager *et al.* 2009, Mahieu *et al.* 2006, Kawakami &
204 Yamamura 2008, Staelens *et al.* 2002, Blanchet *et al.* 2010, Pérez del Villar *et al.* 2013, Harr *et*
205 *al.* 2016).

206 Ultimately, as its mechanism is revealed, the high-acidification phenotype of *M. spretus*
207 fibroblasts may prove to be well suited for the development of mimetics that would boost
208 lysosomal function in a human clinical setting. More broadly, our results provide a way forward
209 for the use of wild mouse species as models for lysosomal function and senescence, without the
210 peculiarities of commensal lineages.
211

212 Methods

213

214 Primary tail fibroblasts were extracted as described by Khan and Gasser (2016). Subsequent
215 culture and experiments used complete medium (DMEM, 10% FBS, 1% pen-strep) in T25 flasks
216 at 37°C, 3% O₂, and 10% CO₂. Cells were passaged based on confluence using trypsin.
217 Experimental treatments included 15 GY of ionizing irradiation or 3.6 μ M neocarzinostatin.
218 Analysis kits used were the Abcam Ltd. Senescence Detection Kit (Cat. #ab65351), ApoTox-
219 Glo™ Triplex Assay (Promega Cat. #G6321), and LysoTracker™ Green DND-26 (Thermo
220 Fisher Cat. #L7526). Primary and secondary antibodies used in western blots were the rabbit
221 monoclonal Anti-p21 antibody (ab188224, Abcam, 1:1000), mouse monoclonal anti- β -tubulin
222 (T9026, Sigma-Aldrich, 1:1000), Goat Anti-Mouse IgG(H+L) Human ads-HRP (Cat#1031-05,
223 Southern Biotech, 1:5000), and Goat Anti-Rabbit IgG(H+L) Human ads-HRP (Cat#4050-05,
224 Southern Biotech, 1:5000). Additional details of methods available in Supplementary Materials.
225

226

227 Acknowledgements

228

229 The authors thank Vera Gorbunova, Emilie Tu, Helen Bateup, Samantha Jackson, Linda
230 Wilbrecht, Polly Campbell, and Michael Nachman for animal material; Diana Bautista, Britt
231 Glaunsinger, José Pablo Vázquez-Medina, and Mary West for their generosity with space and
232 resources; Juliana Valencia Lesmes, Mara Baylis, Sam Rider and Harriet Song for technical
233 contributions; and members of the Brem lab for helpful discussions. This work was supported by
234 National Institutes of Health R01NS116992 to RBB and R01GM120430 to RBB and JMG. JMV
235 was supported by the National Science Foundation Postdoctoral Research Fellowship in
236 Biology (#2109915) and by the National Institute for Health T32AG000266 and 1K99AG088361.
237

238

239

240

241

242

243

244

245

246

247

248

249

250

251

252

253

254

255

256

257

258

259

260

261

262

263

264

265

266 Figure captions

267

268 **Figure 1. Low β -galactosidase activity in *M. spretus* fibroblasts regardless of treatment.**

269 In a given panel, each bar length displays the mean percentage of primary fibroblasts of the
270 indicated strain and species that stained positive after administration of the colorimetric β -
271 galactosidase substrate X-Gal. From top to bottom, complete names of the strains are as
272 follows: *M. m. musculus* (PWK/PhJ, BLG2/Ms, CHD/Ms, MSM/Ms), laboratory strain of mostly
273 *M. m. domesticus* origin (C57BL/6J), *M. m. domesticus* (TUCA/NachJ, ManB/NachJ), *M.*
274 *spicilegus*, and *M. spretus* (SFM, STF/Pas). Panels report results from (A) untreated cells, (B)
275 cells treated with infrared irradiation followed by a 10-day incubation, or (C) cells after 1 hour of
276 neocarzinostatin treatment followed by 24 hours of incubation. Points report biological and
277 technical replicates collected over at least two separate days. Error bars report one standard
278 error above and below the mean. ***, two-tailed Wilcoxon $p < 10^{-7}$ comparing *M. spretus* with all
279 other genotypes; *M. spretus* and *M. spicilegus* also differed in each panel (Wilcoxon $p < 0.05$).
280 For (B) and (C), in a comparison between the respective treatment and the untreated control in
281 (A), a two-factor ANOVA with treatment and genotype as factors yielded $p < 2e^{-16}$ for the
282 interaction between the two factors.

283

284 **Figure 2. High lysosomal acidity in *M. spretus* cells across all treatments.**

285 Shown are results from assays of the lysosomal acidity reporter Lysotracker on primary
286 fibroblasts of the indicated genotype (*M. spretus*, strain STF; *M. m. musculus*, strain PWK). The
287 y-axis reports mean fluorescence of the indicated culture normalized to the control *M. musculus*
288 sample for each experiment. Pairs of bars report results from untreated cells (left), or cells
289 treated with irradiation followed by a 10-day incubation (middle) or after 1 hour of
290 neocarzinostatin treatment followed by 24 hours of incubation (right). Data points correspond to
291 biological and technical replicates collected over at least two different days, and the bar height
292 reports their mean. Error bars report one standard error above and below the mean. *, Wilcoxon
293 $p < 0.05$, **, Wilcoxon $p < 0.01$.

294

295

296

297

298

299

300

301

302

303

304

305

306

307 **Supplementary figure captions**

308

309 **Figure S1. Low normalized β-galactosidase activity in *M. spretus* fibroblasts.**

310 Data are as in Figure 1 of the main text, except that measurements from the culture of a given
311 genotype and DNA damage treatment were normalized to the average of all measurements
312 from untreated controls of that genotype. ***, two-tailed Wilcoxon p < .001 comparing *M. spretus*
313 with all other genotypes for both treatments.

314

315 **Figure S2. Fibroblast passage number has no detectable effect on X-Gal staining.**

316 In a given panel, each row reports results from assays of the β-galactosidase substrate X-Gal
317 on fibroblasts of the indicated genotype shown in Figure 1 of the main text, and each point
318 reports one replicate culture. The x-axis reports the passage number of the respective culture
319 and the y-axis reports X-Gal staining. (A) Cells induced to senesce with irradiation (right) and
320 their paired controls (left). (B) Cells induced to senesce with neocarzinostatin (right) and their
321 paired controls (left).

322

323 **Figure S3. No consistent variation between *Mus* species fibroblasts in apoptosis activity.**

324 Data are as in Figure 1 of the main text, except that for a given genotype and treatment, the x-
325 axis reports caspase activity of the culture measured with the Apotox-Glo assay, normalized to
326 the average from untreated cultures of the respective genotype for each experiment. (A)
327 Outliers trimmed for visualization. (B) Full data set view.

328

329 **Figure S4. Dose dependence of β-galactosidase staining in *Mus* fibroblasts.**

330 Each trace shows results from assays of the β-galactosidase substrate X-Gal on primary
331 fibroblasts of the indicated genotype (*M. spretus*, strain STF; *M. m. musculus*, strain PWK). For
332 a given trace, the x-axis reports irradiation dosage (in centigray, cGy) at 0 (Control), 1000, 1500,
333 2000, and 2500 cGy, and the y-axis reports the percentage of X-Gal positive cells in untreated
334 controls or in irradiated cells after 10 days of incubation. Smaller points represent biological and
335 technical replicates, and larger points indicate their mean values. ***, two-factor ANOVA with
336 dosage and species as factors yielded p < 2e⁻¹⁶ for each factor.

337

338 **Figure S5. No consistent variation between *Mus* fibroblasts in P21 abundance.**

339 Shown are results of Western blot assays of abundance of the senescence regulator P21 in
340 primary fibroblasts of the indicated genotype (*M. spretus*, strain STF; *M. m. musculus*, strain
341 PWK). (A) Top, representative blot showing tubulin (55 kDa) and P21 (21 kDa) levels in 25 µg of
342 whole cell lysate (WCL) from untreated control cells or those irradiated (Xray) and incubated for
343 10 days to establish senescence. Bottom, blot as at top except that cells were treated with 1
344 hour of neocarzinostatin (NCS) and incubated for 24 hours to establish senescence. (B) Each
345 column reports quantification of P21 protein abundance normalized to tubulin for the indicated
346 genotype and treatment. Data points correspond to biological and technical replicates collected
347 over at least two different days, and the bar height reports their mean. Error bars report one
348 standard error above and below the mean. No comparisons were significant in pairwise
349 Wilcoxon tests. *, two-factor ANOVA with condition and species as factors yielded p < 0.05 for
350 species.

351 **Figure S6. No significant variation between *Mus* fibroblasts in *Cdkn1a* mRNA expression.**

352 (A) Shown are measurements of mRNA expression of the β -galactosidase gene *Cdkn1a*, from
353 profiling of fibroblasts of the indicated genotype (*M. spretus*, strain STF; *M. m. musculus*, strain
354 PWK), untreated or treated with irradiation followed by a 10-day incubation (Kang *et al.* 2023).
355 Data points within each column represent biological and technical replicates and the bar height
356 reports their mean. Error bars report one standard error above and below the mean. Only
357 condition had a significant impact on expression in a two-factor ANOVA with treatment and
358 genotype as factors ($p < 0.005$, **).

359

360 **Figure S7. Variation between *Mus* fibroblasts in *Glb1* mRNA expression.**

361 (A) Shown are measurements of mRNA expression of the β -galactosidase gene from profiling of
362 fibroblasts of the indicated genotype (*M. spretus*, strain STF; *M. m. musculus*, strain PWK),
363 untreated or treated with irradiation followed by a 10-day incubation (Kang *et al.* 2023). Data
364 points within each column represent biological and technical replicates and the bar height
365 reports their mean. Only the effect of genotype was significant in a two-factor ANOVA with
366 condition and species as factors (*, $p < 0.05$). (B) Data are as in (A), except that allele-specific
367 mRNA expression of the β -galactosidase gene was measured from profiling of fibroblasts of an
368 F1 hybrid between STF and PWK. (*M. spretus*, strain STF; *M. m. musculus*, strain PWK)(Kang
369 *et al.* 2023). In a two-factor ANOVA with condition and genotype as factors, both had significant
370 effects (species $p < 0.0005$, ***; condition $p < 0.05$, *).

371

372

373

374

375

376

377

378

379

380

381

382

383

384

385

386

387

388

389

390

391

392

393

394

395 **References**

- 396
- 397 Admasu TD, Rae MJ & Stolzing A (2021) Dissecting primary and secondary senescence to
398 enable new senotherapeutic strategies. *Ageing Research Reviews* 70, 101412.
- 399 Aman Y, Schmauck-Medina T, Hansen M, Morimoto RI, Simon AK, Bjedov I, Palikaras K,
400 Simonsen A, Johansen T, Tavernarakis N, Rubinsztein DC, Partridge L, Kroemer G,
401 Labbadia J & Fang EF (2021) Autophagy in healthy aging and disease. *Nat Aging* 1,
402 634–650.
- 403 Attaallah A, Lenzi M, Marchionni S, Bincoletto G, Cocchi V, Croco E, Hrelia P, Hrelia S, Sell
404 C & Lorenzini A (2020) A pro longevity role for cellular senescence. *Geroscience* 42,
405 867–879.
- 406 Bareja A, Lee DE & White JP (2019) Maximizing Longevity and Healthspan: Multiple
407 Approaches All Converging on Autophagy. *Front Cell Dev Biol* 7, 183.
- 408 Bhuiyan MS, Pattison JS, Osinska H, James J, Gulick J, McLendon PM, Hill JA, Sadoshima
409 J & Robbins J (2013) Enhanced autophagy ameliorates cardiac proteinopathy. *J Clin
410 Invest* 123, 5284–5297.
- 411 Blanchet C, Jaubert J, Carniel E, Fayolle C, Milon G, Szatanik M, Panthier J-J & X
412 Montagutelli (2011) Mus spretus SEG/Pas mice resist virulent *Yersinia pestis*, under
413 multigenic control. *Genes Immun* 12, 23–30.
- 414 Bonam SR, Wang F & Muller S (2019) Lysosomes as a therapeutic target. *Nat Rev Drug
415 Discov* 18, 923–948.
- 416 Campisi J (2005) Senescent cells, tumor suppression, and organismal aging: good citizens,
417 bad neighbors. *Cell* 120, 513–522.
- 418 Campisi J & d'Adda di Fagagna F (2007) Cellular senescence: when bad things happen to
419 good cells. *Nat Rev Mol Cell Biol* 8, 729–740.
- 420 Carmona-Gutierrez D, Hughes AL, Madeo F & Ruckenstuhl C (2016) The crucial impact of
421 lysosomes in aging and longevity. *Ageing Res Rev* 32, 2–12.
- 422 Casella G, Munk R, Kim KM, Piao Y, De S, Abdelmohsen K & Gorospe M (2019)
423 Transcriptome signature of cellular senescence. *Nucleic Acids Res* 47, 7294–7305.
- 424 Childs BG, Baker DJ, Kirkland JL, Campisi J & van Deursen JM (2014) Senescence and
425 apoptosis: dueling or complementary cell fates? *EMBO Rep* 15, 1139–1153.

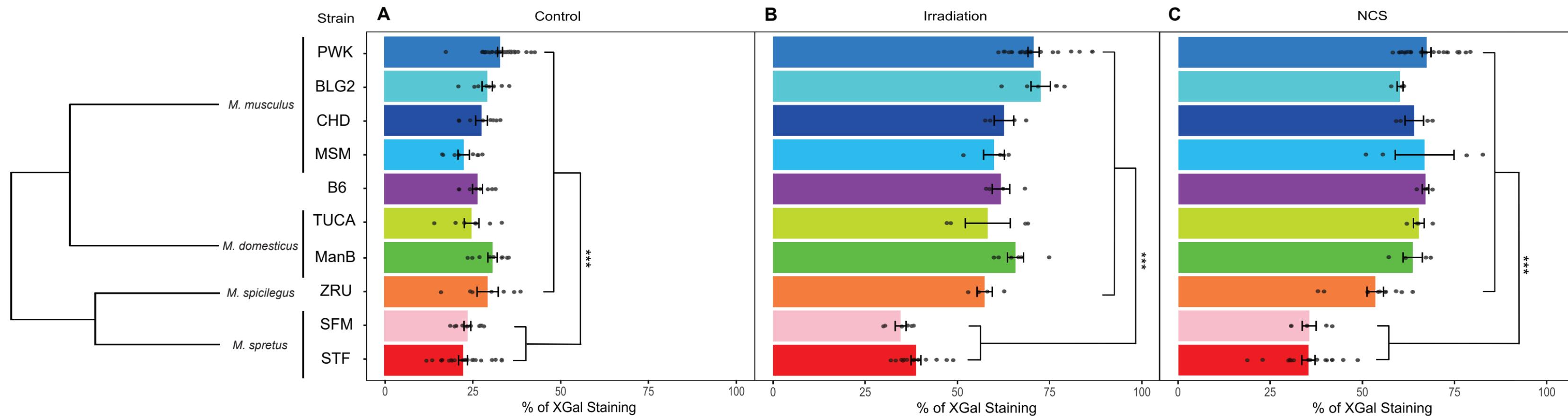
- 426 Chusyd DE, Ackermans NL, Austad SN, Hof PR, Mielke MM, Sherwood CC & Allison DB
427 (2021) Aging: What We Can Learn From Elephants. *Front Aging* 2, 726714.
- 428 Correia□Melo C, Marques FD, Anderson R, Hewitt G, Hewitt R, Cole J, Carroll BM, Miwa
429 S, Birch J, Merz A, Rushton MD, Charles M, Jurk D, Tait SW, Czapiewski R, Greaves
430 L, Nelson G, Bohlooly-Y M, Rodriguez-Cuenca S, Vidal-Puig A, Mann D, Saretzki G,
431 Quarato G, Green DR, Adams PD, Von Zglinicki T, Korolchuk VI & Passos JF (2016)
432 Mitochondria are required for pro-ageing features of the senescent phenotype. *The*
433 *EMBO Journal* 35, 724–742.
- 434 Curnock R, Yalci K, Palmfeldt J, Jäättelä M, Liu B & Carroll B (2023) TFEB□dependent
435 lysosome biogenesis is required for senescence. *EMBO J* 42, e111241.
- 436 De Magalhães JP & Passos JF (2018) Stress, cell senescence and organismal ageing.
437 *Mechanisms of Ageing and Development* 170, 2–9.
- 438 Del Villar LP, Vicente B, Galindo-Villardón P, Castellanos A, Pérez-Losada J & Muro A
439 (2013) Schistosoma mansoni experimental infection in Mus spretus (SPRET/EiJ strain)
440 mice. *Parasite*, 20, 27.
- 441 Dejager L, Libert C & Montagutelli X (2009) Thirty years of Mus spretus: a promising future.
442 *Trends Genet* 25, 234–241.
- 443 Delfarah A, Hartel NG, Zheng D, Yang J & Graham NA (2021) Identification of a Proteomic
444 Signature of Senescence in Primary Human Mammary Epithelial Cells. *J Proteome*
445 *Res* 20, 5169–5179.
- 446 Dodig S, Čepelak I & Pavić I (2019) Hallmarks of senescence and aging. *Biochem Med*
447 (*Zagreb*) 29, 030501.
- 448 Finch CE (2009) Update on slow aging and negligible senescence--a mini-review.
449 *Gerontology* 55, 307–313.
- 450 Gomes NMV, Ryder OA, Houck ML, Charter SJ, Walker W, Forsyth NR, Austad SN,
451 Venditti C, Pagel M, Shay JW & Wright WE (2011) Comparative biology of mammalian
452 telomeres: hypotheses on ancestral states and the roles of telomeres in longevity
453 determination. *Aging Cell* 10, 761–768.
- 454 Green DR, Galluzzi L & Kroemer G (2011) Mitochondria and the autophagy-inflammation-
455 cell death axis in organismal aging. *Science* 333, 1109–1112.

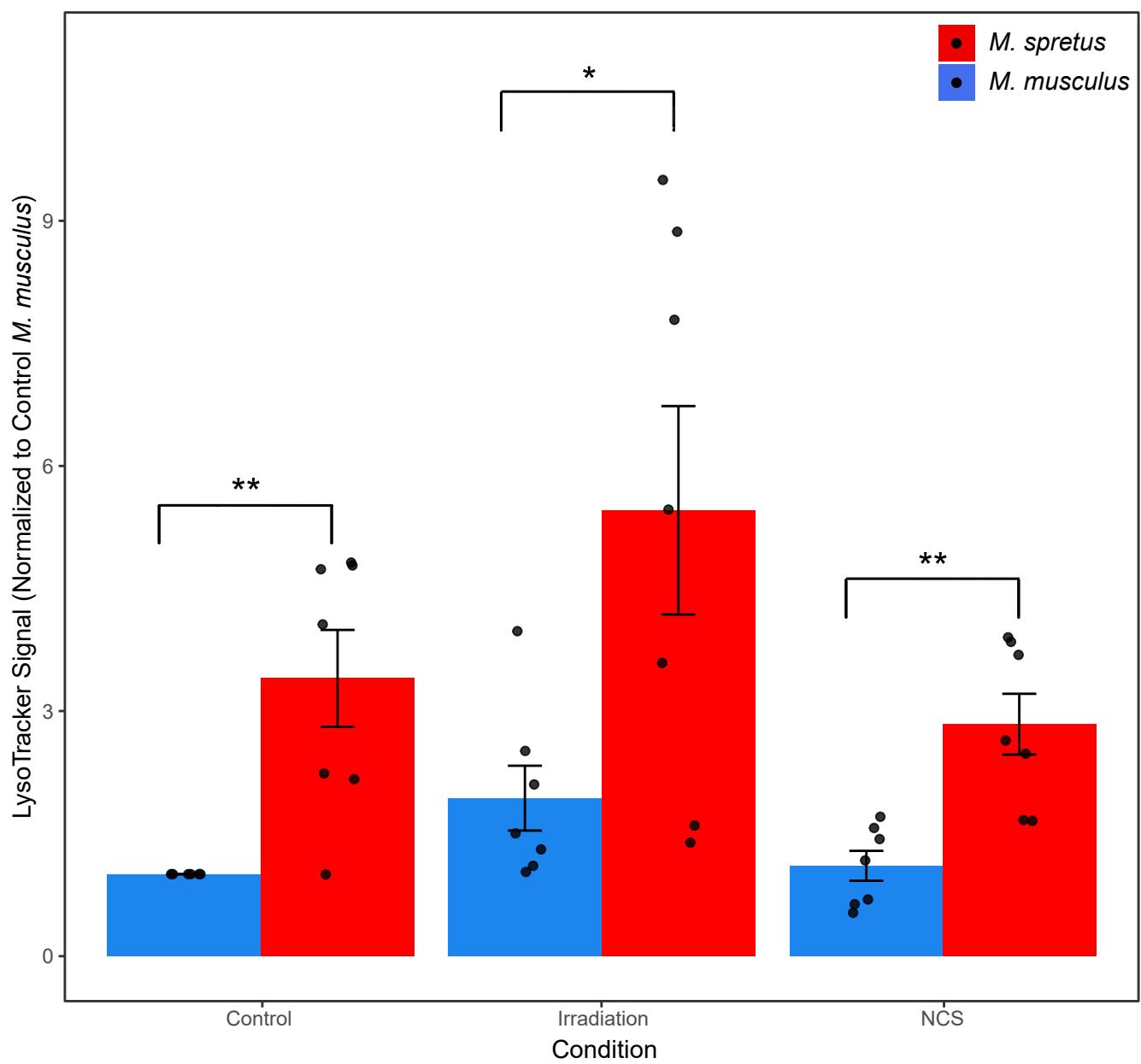
- 456 Gu Z, Jiang J, Tan W, Xia Y, Cao H, Meng Y, Da Z, Liu H & Cheng C (2013) p53/p21
457 Pathway involved in mediating cellular senescence of bone marrow-derived
458 mesenchymal stem cells from systemic lupus erythematosus patients. *Clin Dev*
459 *Immunol* 2013, 134243.
- 460 Guerrero-Navarro L, Jansen-Dürr P & Cavinato M (2022) Age-Related Lysosomal
461 Dysfunctions. *Cells* 11, 1977.
- 462 Hansen M, Rubinsztein DC & Walker DW (2018) Autophagy as a promoter of longevity:
463 insights from model organisms. *Nat Rev Mol Cell Biol* 19, 579–593.
- 464 Harper JM, Salmon AB, Leiser SF, Galecki AT & Miller RA (2007) Skin-derived fibroblasts
465 from long-lived species are resistant to some, but not all, lethal stresses and to the
466 mitochondrial inhibitor rotenone. *Aging Cell* 6, 1–13.
- 467 Harr B, Karakoc E, Neme R, Teschke M, Pfeifle C, Pezer Ž, Babiker H, Linnenbrink M,
468 Montero I, Scavetta R, Abai MR, Molins MP, Schlegel M, Ulrich RG, Altmüller J,
469 Franitzka M, Büntge A, Künzel S & Tautz D (2016) Genomic resources for wild
470 populations of the house mouse, *Mus musculus* and its close relative *Mus spretus*. *Sci*
471 *Data* 3, 160075.
- 472 Hernandez-Segura A, Brandenburg S & Demaria M (2018) Induction and Validation of
473 Cellular Senescence in Primary Human Cells. *J Vis Exp*, 57782.
- 474 Hooper JM, Rainer E & Banks RA (1996) Haemodialysis patients' knowledge and beliefs
475 about medication. *EDTNA ERCA J* 22, 38–40.
- 476 Hornsby PJ (2002) Cellular senescence and tissue aging in vivo. *J Gerontol A Biol Sci Med*
477 *Sci* 57, B251-256.
- 478 Imai Y, Tanave A, Matsuyama M & Koide T (2022) Efficient genome editing in wild strains
479 of mice using the i-GONAD method. *Sci Rep* 12, 13821.
- 480 Ito T, Teo YV, Evans SA, Neretti N & Sedivy JM (2018) Regulation of Cellular Senescence
481 by Polycomb Chromatin Modifiers through Distinct DNA Damage-and Histone
482 Methylation-Dependent Pathways. *Cell Rep* 22, 3480–3492.
- 483 Kang T, Moore EC, Kopania EEK, King CD, Schilling B, Campisi J, Good JM & Brem RB
484 (2023) A natural variation-based screen in mouse cells reveals USF2 as a regulator of
485 the DNA damage response and cellular senescence. *G3 (Bethesda)* 13, jkad091.

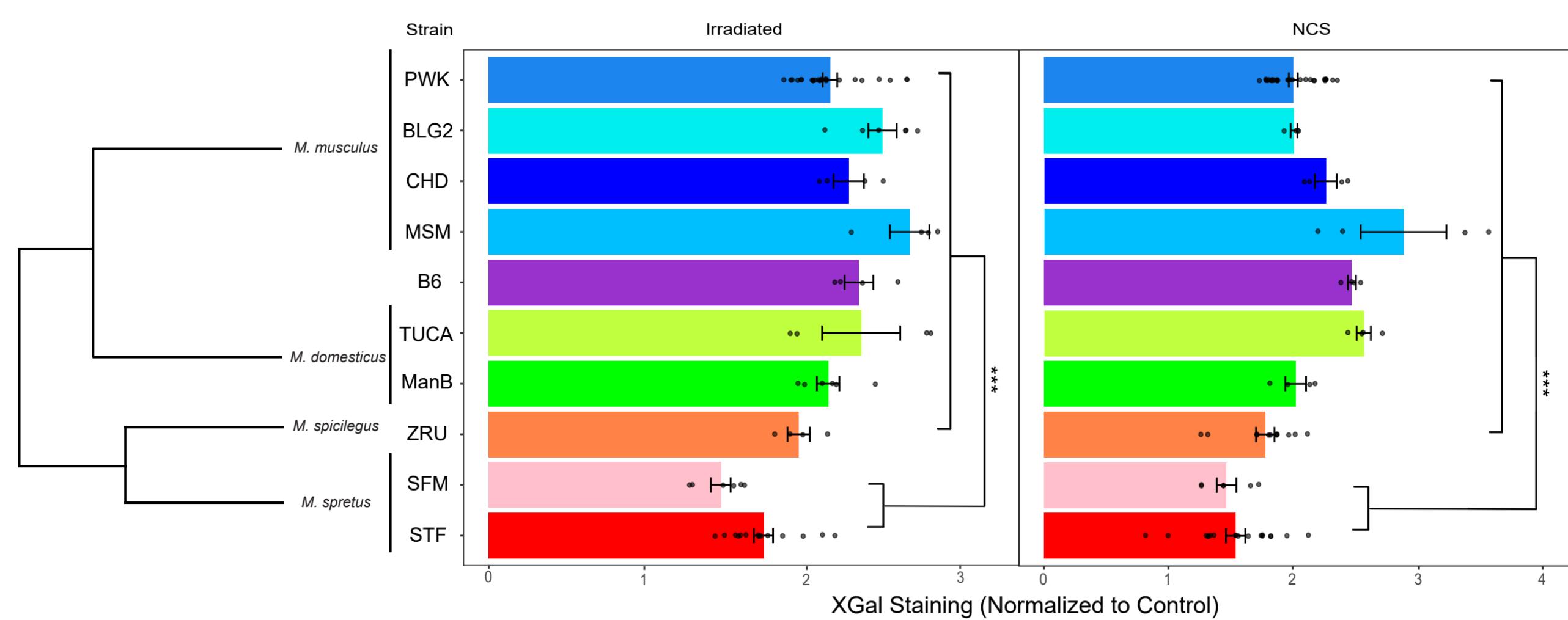
- 486 Kawakami M & Yamamura K-I (2008) Cranial bone morphometric study among mouse
487 strains. *BMC Evolutionary Biology*, 8 (1), 73.
- 488 Kirschner K, Rattanavirotkul N, Quince MF & Chandra T (2020) Functional heterogeneity in
489 senescence. *Biochemical Society Transactions* 48, 765–773.
- 490 Kreis N-N, Friemel A, Zimmer B, Roth S, Rieger MA, Rolle U, Louwen F & Yuan J (2016)
491 Mitotic p21Cip1/CDKN1A is regulated by cyclin-dependent kinase 1 phosphorylation.
492 *Oncotarget* 7, 50215–50228.
- 493 Lee BY, Han JA, Im JS, Morrone A, Johung K, Goodwin EC, Kleijer WJ, DiMaio D & Hwang
494 ES (2006) Senescence-associated beta-galactosidase is lysosomal beta-
495 galactosidase. *Aging Cell* 5, 187–195.
- 496 Leiva-Rodríguez T, Romeo-Guitart D, Marmolejo-Martínez-Artesero S, Herrando-Grabulosa
497 M, Bosch A, Forés J & Casas C (2018) ATG5 overexpression is neuroprotective and
498 attenuates cytoskeletal and vesicle-trafficking alterations in axotomized motoneurons.
499 *Cell Death Dis* 9, 626.
- 500 Liu X, Ye Q, Huang Z, Li X, Zhang L, Liu X, Wu Y-C, Brockmeier U, Hermann DM, Wang Y-
501 C & Ren L (2023) BAG3 Overexpression Attenuates Ischemic Stroke Injury by
502 Activating Autophagy and Inhibiting Apoptosis. *Stroke* 54, 2114–2125.
- 503 Mahieu T, Park JM, Revets H, Pasche B, Lengeling A, Staelens J, Wullaert A, Vanlaere I,
504 Hochedepied T, Van Roy F, Karin M, Libert C (2006) The wild-derived inbred mouse
505 strain SPRET/Ei is resistant to LPS and defective in IFN- β production. *Proceedings of
506 the National Academy of Sciences* 103 (7), 2292–2297.
- 507 Morgan AP, Hughes JJ, Didion JP, Jolley WJ, Campbell KJ, Threadgill DW, Bonhomme F,
508 Searle JB & de Villena FP-M (2022) Population structure and inbreeding in wild house
509 mice (*Mus musculus*) at different geographic scales. *Heredity (Edinb)* 129, 183–194.
- 510 Mylonas A & O'Loghlen A (2022) Cellular Senescence and Ageing: Mechanisms and
511 Interventions. *Front Aging* 3, 866718.
- 512 Oka K, Yamakawa M, Kawamura Y, Kutsukake N & Miura K (2023) The Naked Mole-Rat
513 as a Model for Healthy Aging. *Annu Rev Anim Biosci* 11, 207–226.
- 514 Pagès M, Fabre P, Chaval Y, Mortelliti A, Nicolas V, Wells K, Michaux JR & Lazzari V
515 (2015) Molecular phylogeny of South-East Asian arboreal murine rodents. *Zoologica
516 Scripta* 45 (4), 349–364.

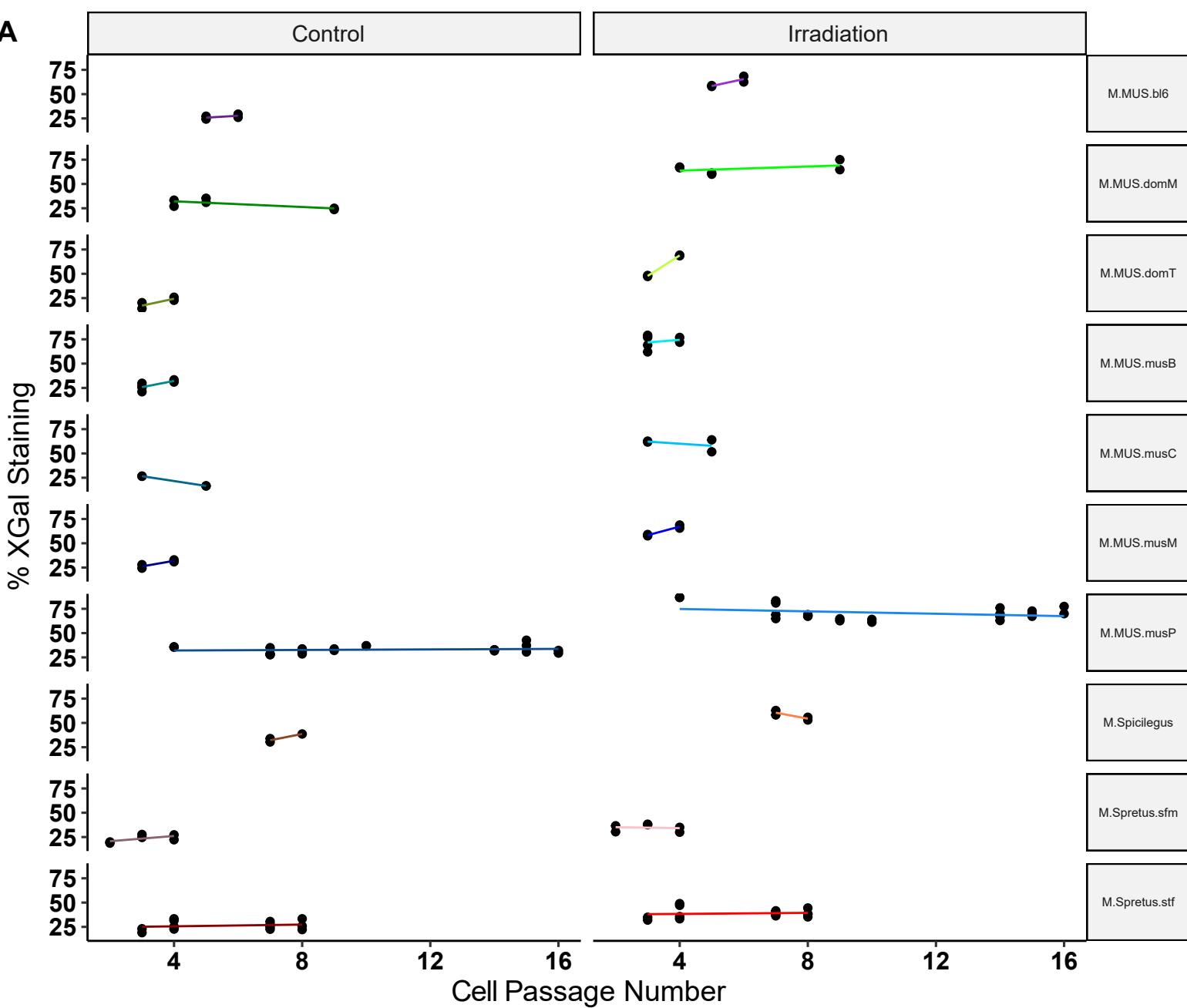
- 517 Pickford F, Masliah E, Britschgi M, Lucin K, Narasimhan R, Jaeger PA, Small S, Spencer B,
518 Rockenstein E, Levine B & Wyss-Coray T (2008) The autophagy-related protein beclin
519 1 shows reduced expression in early Alzheimer disease and regulates amyloid beta
520 accumulation in mice. *J Clin Invest* 118, 2190–2199.
- 521 Pyo J-O, Yoo S-M, Ahn H-H, Nah J, Hong S-H, Kam T-I, Jung S & Jung Y-K (2013)
522 Overexpression of Atg5 in mice activates autophagy and extends lifespan. *Nat
523 Commun* 4, 2300.
- 524 Romanov VS & Rudolph KL (2016) p21 shapes cancer evolution. *Nat Cell Biol* 18, 722–
525 724.
- 526 Rovira M, Sereda R, Pladevall-Morera D, Ramponi V, Marin I, Maus M, Madrigal-Matute J,
527 Díaz A, García F, Muñoz J, Cuervo AM & Serrano M (2022) The lysosomal proteome
528 of senescent cells contributes to the senescence secretome. *Aging Cell* 21, e13707.
- 529 Shin HR & Zoncu R (2020) The Lysosome at the Intersection of Cellular Growth and
530 Destruction. *Dev Cell* 54, 226–238.
- 531 Shin JY, Hong S-H, Kang B, Minai-Tehrani A & Cho M-H (2013) Overexpression of beclin1
532 induced autophagy and apoptosis in lungs of K-rasLA1 mice. *Lung Cancer* 81, 362–
533 370.
- 534 Simonsen A, Cumming RC, Brech A, Isakson P, Schubert DR & Finley KD (2008)
535 Promoting basal levels of autophagy in the nervous system enhances longevity and
536 oxidant resistance in adult Drosophila. *Autophagy* 4, 176–184.
- 537 Smissen PJ & Rowe KC (2018) Repeated biome transitions in the evolution of Australian
538 rodents. *Molecular Phylogenetics and Evolution* 128, 182–191.
- 539 Staelens J, Wielockx B, Puimège L, Van Roy F, Guénet J-L & Libert C (2002)
540 Hyporesponsiveness of SPRET/Ei mice to lethal shock induced by tumor necrosis
541 factor and implications for a TNF-based antitumor therapy. *Proceedings of the National
542 Academy of Sciences*, 99 (14), 9340–9345.
- 543 Sulak M, Fong L, Mika K, Chigurupati S, Yon L, Mongan NP, Emes RD & Lynch VJ (2016)
544 TP53 copy number expansion is associated with the evolution of increased body size
545 and an enhanced DNA damage response in elephants. *eLife* 5, e11994.
- 546 Takasugi M, Yoshida Y, Hara E & Ohtani N (2023) The role of cellular senescence and
547 SASP in tumour microenvironment. *The FEBS Journal* 290, 1348–1361.

- 548 Tian X, Firsanov D, Zhang Z, Cheng Y, Luo L, Tombline G, Tan R, Simon M, Henderson S,
549 Steffan J, Goldfarb A, Tam J, Zheng K, Cornwell A, Johnson A, Yang J-N, Mao Z,
550 Manta B, Dang W, Zhang Z, Vijg J, Wolfe A, Moody K, Kennedy BK, Bohmann D,
551 Gladyshev VN, Seluanov A & Gorbunova V (2019) SIRT6 Is Responsible for More
552 Efficient DNA Double-Strand Break Repair in Long-Lived Species. *Cell* 177, 622-
553 638.e22.
- 554 Yan J, Chen S, Yi Z, Zhao R, Zhu J, Ding S & Wu J (2024) The role of p21 in cellular
555 senescence and aging-related diseases. *Molecules and Cells* 47, 100113.
- 556 Yew T-L, Chiu F-Y, Tsai C-C, Chen H-L, Lee W-P, Chen Y-J, Chang M-C & Hung S-C
557 (2011) Knockdown of p21(Cip1/Waf1) enhances proliferation, the expression of
558 stemness markers, and osteogenic potential in human mesenchymal stem cells. *Aging*
559 *Cell* 10, 349–361.
- 560 Zhao Y, Tyshkovskiy A, Muñoz-Espín D, Tian X, Serrano M, de Magalhaes JP, Nevo E,
561 Gladyshev VN, Seluanov A & Gorbunova V (2018) Naked mole rats can undergo
562 developmental, oncogene-induced and DNA damage-induced cellular senescence.
563 *Proc Natl Acad Sci U S A* 115, 1801–1806.

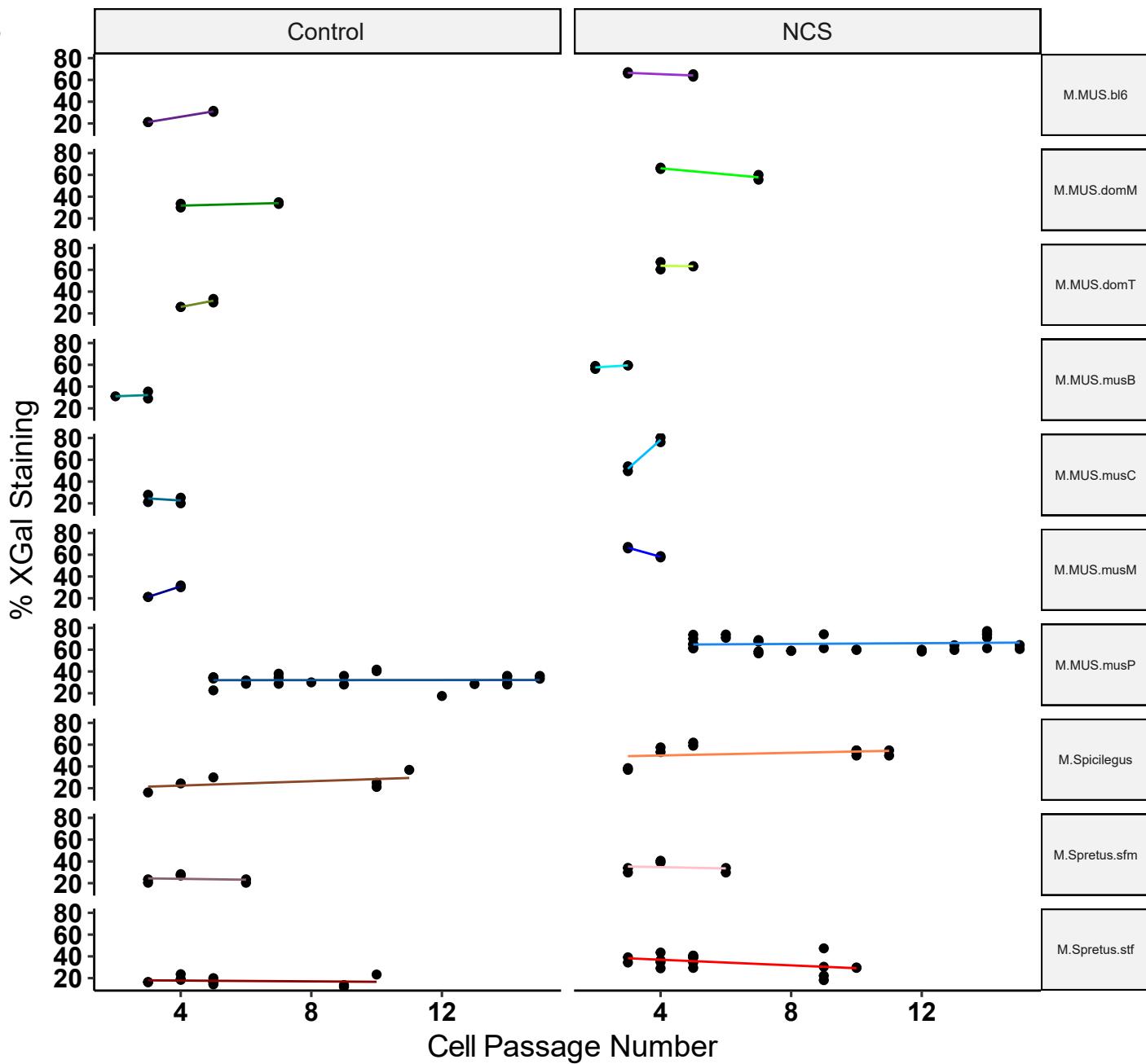


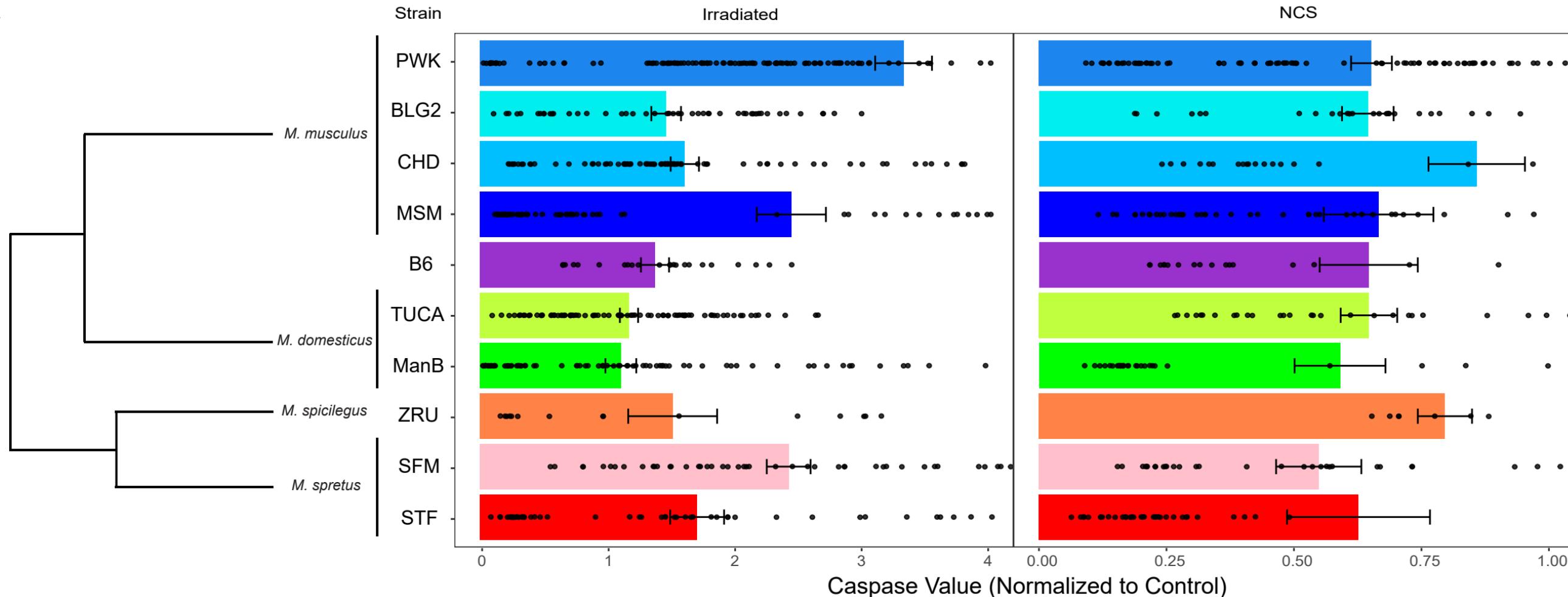


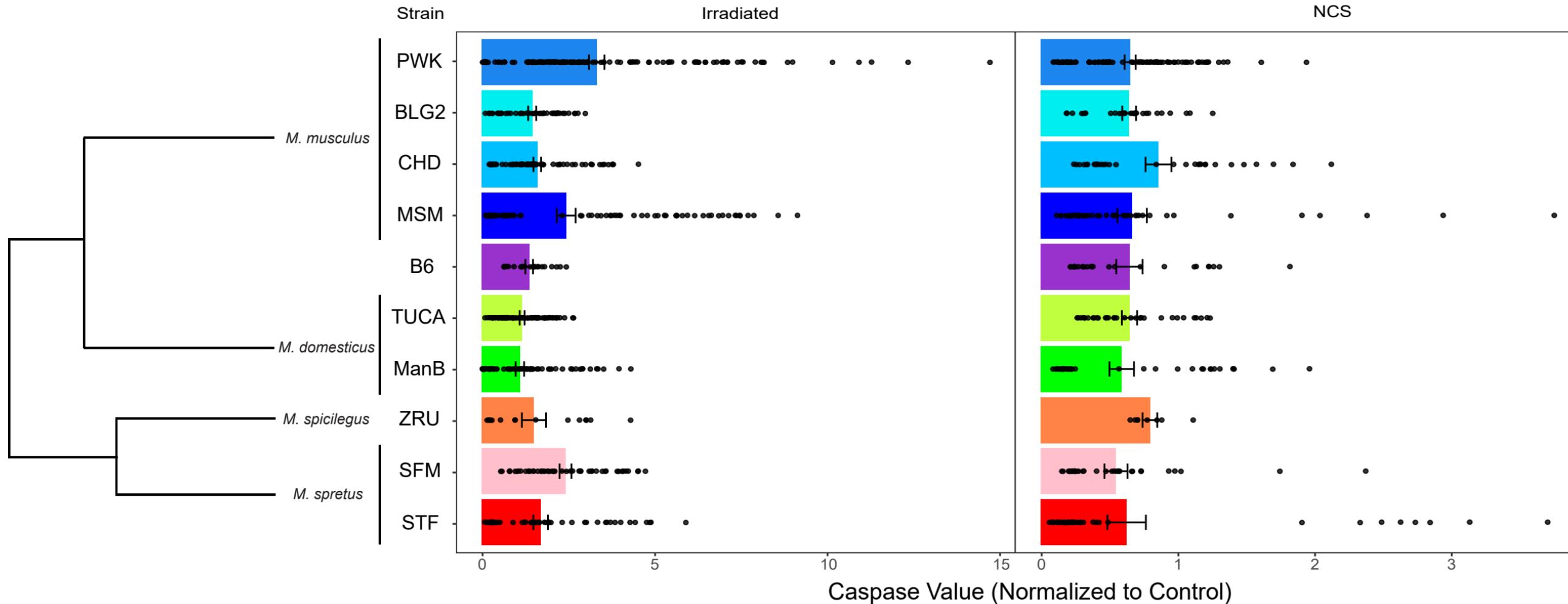


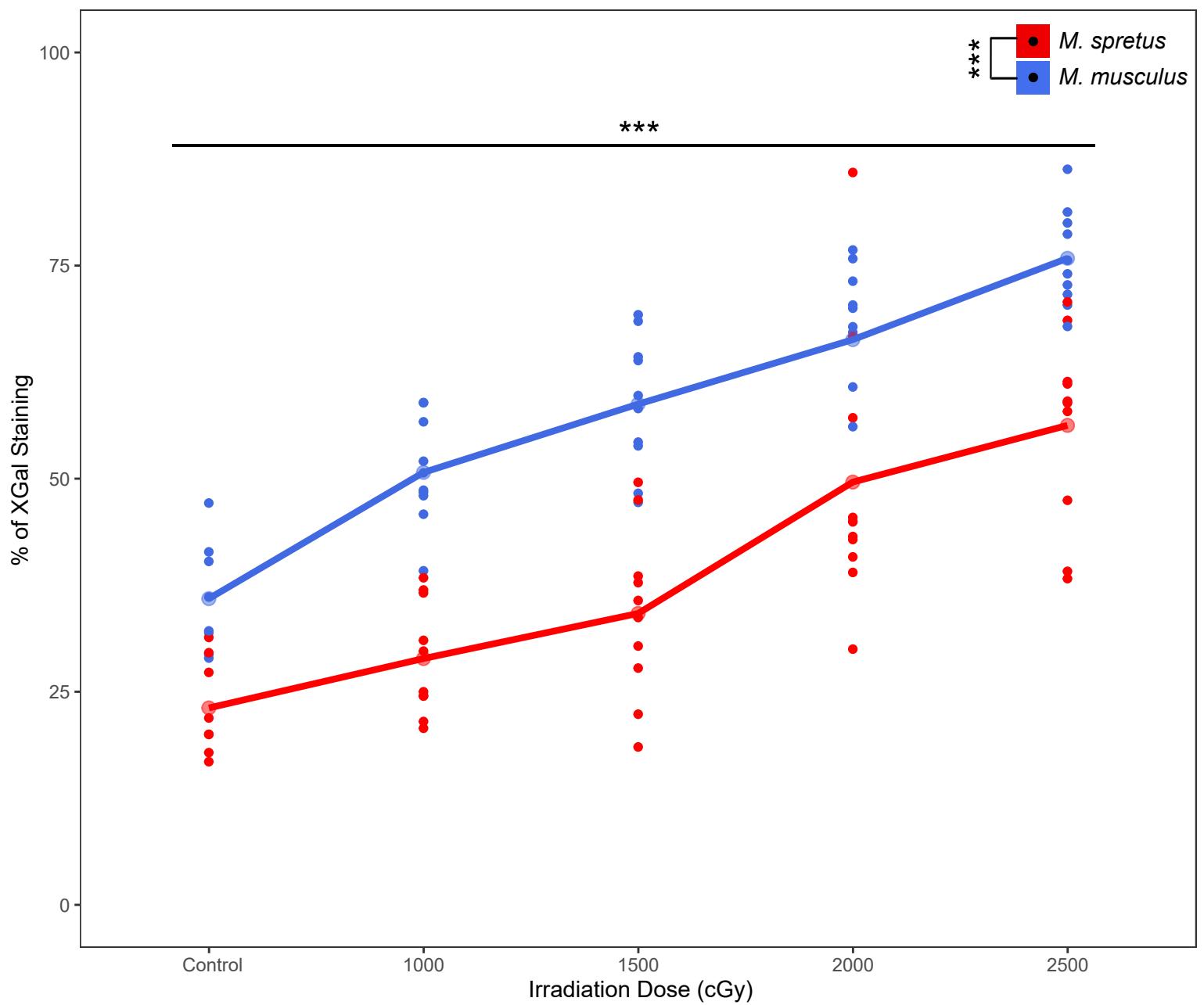
A

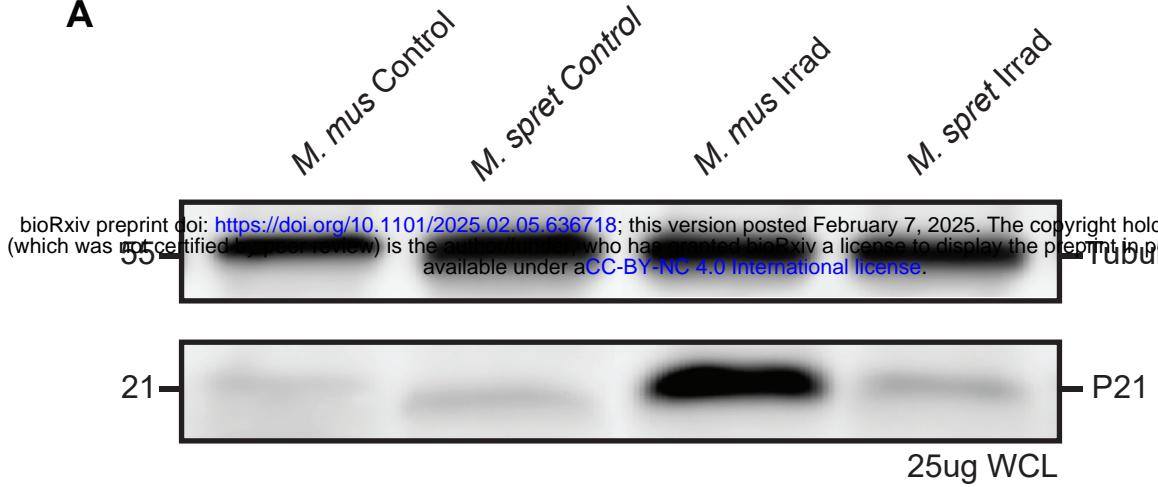
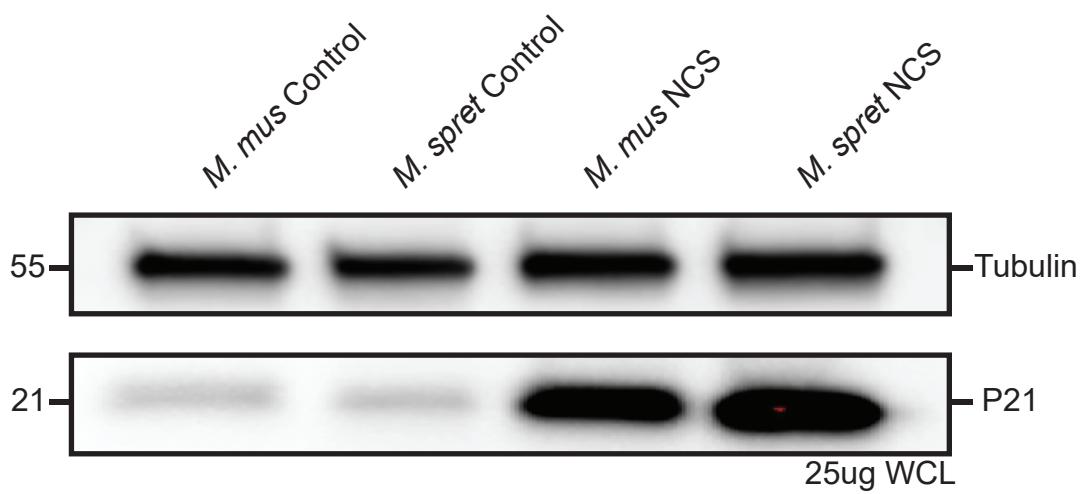
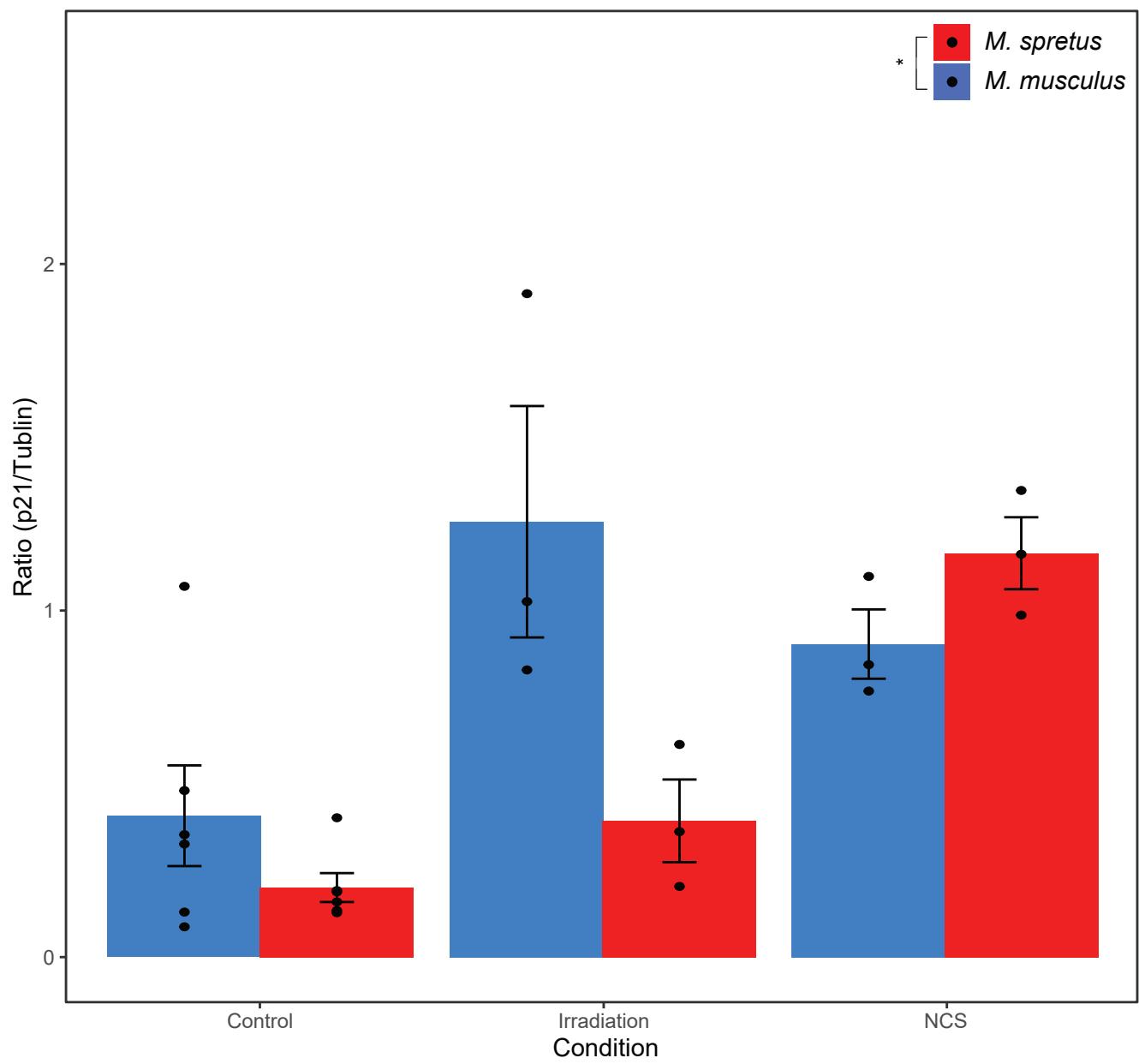
B

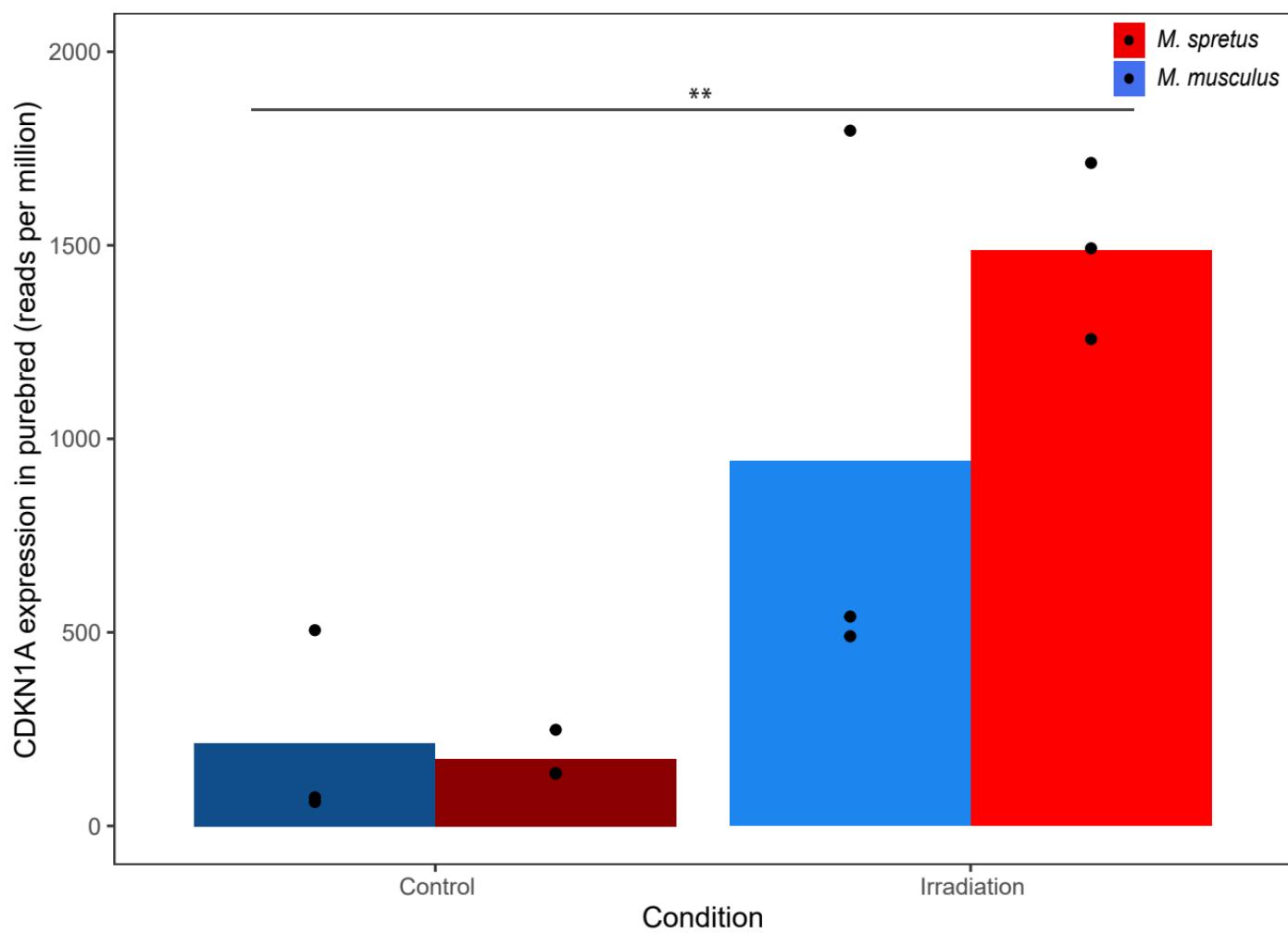


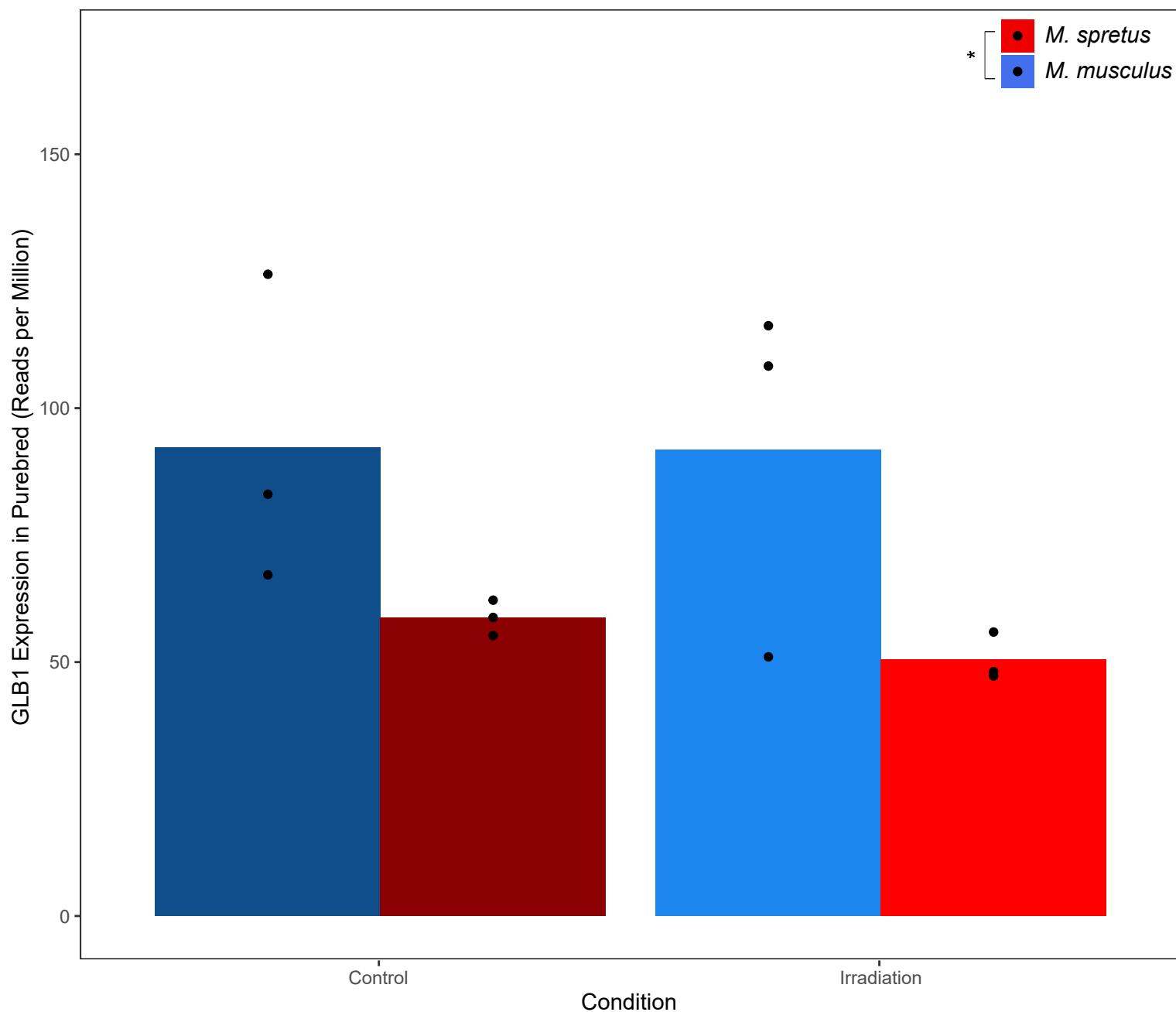
A

B



A**B****C**



A

B