

1 ***TP53* mutations and drug sensitivity in acute myeloid**

2 **leukaemia cells with acquired MDM2 inhibitor resistance**

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28

29 **Abstract**

30 Background: MDM2 inhibitors are under investigation for the treatment of acute
31 myeloid leukaemia (AML) patients in phase III clinical trials. To study resistance
32 formation to MDM2 inhibitors in AML cells, we here established 45 sub-lines of the
33 AML *TP53* wild-type cell lines MV4-11 (15 sub-lines), OCI-AML-2 (10 sub-lines), OCI-
34 AML-3 (12 sub-lines), and SIG-M5 (8 sub-lines) with resistance to the MDM2 inhibitor
35 nutlin-3.

36 Methods: Nutlin-3-resistant sub-lines were established by continuous exposure to
37 stepwise increasing drug concentrations. The *TP53* status was determined by next
38 generation sequencing, cell viability was measured by MTT assay, and p53 was
39 depleted using lentiviral vectors encoding shRNA.

40 Results: All MV4-11 sub-lines harboured the same R248W mutation and all OCI-AML-
41 2 sub-lines the same Y220C mutation, indicating the selection of pre-existing *TP53*-
42 mutant subpopulations. In concordance, rare alleles harbouring the respective
43 mutations could be detected in the parental MV4-11 and OCI-AML-2 cell lines. The
44 OCI-AML-3 and SIG-M5 sub-lines were characterised by varying *TP53* mutations or
45 wild type *TP53*, indicating the induction of *de novo TP53* mutations. Doxorubicin,
46 etoposide, gemcitabine, cytarabine, and fludarabine resistance profiles revealed a
47 noticeable heterogeneity among the sub-lines even of the same parental cell lines.
48 Loss-of-p53 function was not generally associated with decreased sensitivity to
49 cytotoxic drugs.

50 Conclusion: We introduce a substantial set of models of acquired MDM2 inhibitor
51 resistance in AML. MDM2 inhibitors select, in dependence on the nature of a given
52 AML cell population, pre-existing *TP53*-mutant subpopulations or induce *de novo TP53*
53 mutations. Although loss-of-p53 function has been associated with chemoresistance

54 in AML, nutlin-3-adapted sub-lines displayed in the majority of experiments similar or
55 increased drug sensitivity compared to the respective parental cells. Hence,
56 chemotherapy may remain an option for AML patients after MDM2 inhibitor therapy
57 failure. Even sub-lines of the same parental cancer cell line displayed considerable
58 heterogeneity in their response to other anti-cancer drugs, indicating the need for the
59 detailed understanding and monitoring of the evolutionary processes in cancer cell
60 populations in response to therapy as part of future individualised treatment protocols.

61

62 **Key words:** acquired resistance, MDM2, TP53, acute myeloid leukaemia, nutlin-3,
63 cross-resistance, heterogeneity

64

65

66 **Background**

67 MDM2 inhibitors are under development as novel class of anti-cancer drugs for
68 the treatment *TP53* wild-type cancer cells from different cancer entities including acute
69 myeloid leukaemia (AML) [1]. *TP53* encodes p53, a major tumour suppressor protein.
70 *MDM2* is a p53 target gene that encodes for MDM2, a major endogenous inhibitor of
71 p53. MDM2 physically interacts with p53 and mediates its ubiquitination and
72 proteasomal degradation. MDM2 inhibitors activate p53 signalling by interference with
73 the MDM2/ p53 interaction [1-3].

74 Various MDM2 inhibitors have been shown to exert anti-cancer effects in pre-
75 clinical models of AML, alone or in combination with other drugs [4-20]. Moreover,
76 different MDM2 inhibitors are under investigation in clinical studies for their effects on
77 AML [18,21-23], with idasanutlin currently being tested in phase II and III trials for the
78 treatment of AML (NCT02670044, NCT02545283).

79 Drug-adapted cancer cell lines have been used to identify and investigate
80 clinical resistance mechanisms [24-33]. The adaptation of cancer cell lines to MDM2
81 inhibitors indicated that the treatment of *TP53* wild-type cancer cells may be associated
82 with the formation of *TP53* mutations as resistance mechanisms [3,34-39]. In
83 concordance, treatment of liposarcoma patients harbouring *TP53* wild type cancer
84 cells with the MDM2 inhibitor SAR405838 resulted in the emergence of *TP53*
85 mutations [40].

86 The origin of MDM2 inhibitor-induced *TP53* mutations in *TP53* wild-type cell
87 lines is not entirely clear. In dependence of the cell line model, MDM2 inhibitors may
88 induce a range of different *de novo* *TP53* mutations in a given model or select small,
89 pre-existing cell fractions that harbour *TP53* mutations [35,36,39,41].

90 To study acquired resistance formation to MDM2 inhibitors in AML cells, we here
91 established and analysed a panel of sub-lines of the *TP53* wild-type AML cell lines
92 MV4-11, OCI-AML-2, OCI-AML-3, and SIG-M5, with acquired resistance to the MDM2
93 inhibitor nutlin-3 [3,42]. In total, this included 45 nutlin-3-adapted sub-lines (15 MV4-
94 11 sub-lines, 10 OCI-AML-2 sub-lines, 12 OCI-AML-3 sub-lines, 8 SIG-M5 sub-lines).
95

96 **Methods**

97 **Cells**

98 The AML cell lines MV4-11, OCI-AML-2, OCI-AML-3, and SIG-M5 were
99 obtained from DSMZ (Braunschweig, Germany). The nutlin-3-resistant sub-lines were
100 established by adaption to growth in the presence of increasing drug concentrations
101 as previously described [35,36] and derived from the resistant cancer cell line (RCCL)
102 collection [43].

103 All cells were propagated in IMDM supplemented with 10 % FBS, 100 IU/mL
104 penicillin and 100 µg/mL streptomycin at 37°C. Cells were routinely tested for
105 mycoplasma contamination and authenticated by short tandem repeat profiling.

106 p53-depleted SIG-M5 cells were established as described previously [44] using
107 the Lentiviral Gene Ontology (LeGO) vector technology [45,46].

108 **Viability assay**

109 Cell viability was tested by the 3-(4,5-dimethylthiazol-2-yl)-2,5-
110 diphenyltetrazolium bromide (MTT) dye reduction assay after 120 h incubation
111 modified as described previously [35,36]. 2×10^4 cells suspended in 100 µL cell culture
112 medium were plated per well in 96-well plates and incubated in the presence of various
113 drug concentrations for 120 h. Then, 25µL of MTT solution (2 mg/mL (w/v) in PBS)
114 were added per well, and the plates were incubated at 37°C for an additional 4h. After
115 this, the cells were lysed using 100µL of a buffer containing 20% (w/v) sodium
116 dodecylsulfate and 50% (v/v) N,N-dimethylformamide with the pH adjusted to 4.7 at
117 37°C for 4h. Absorbance was determined at 560 nm to 620 nm for each well using a
118 96-well multiscanner. After subtracting of the background absorption, the results are
119 expressed as percentage viability relative to control cultures which received no drug.

120 Drug concentrations that inhibited cell viability by 50% (IC₅₀) were determined using
121 CalcuSyn (Biosoft, Cambridge, UK).

122 ***TP53* next generation sequencing**

123 The *TP53* status was determined by next generation sequencing as previously
124 described [47]. All coding exonic and flanking intronic regions of the human *TP53* gene
125 were amplified from genomic DNA with Platinum™ Taq DNA polymerase (Life
126 Technologies) by multiplex PCR using two primer pools with 12 non-overlapping primer
127 pairs each, yielding approximately 180 bp amplicons. Each sample was tagged with a
128 unique 8-nucleotide barcode combination using twelve differently barcoded forward
129 and eight differently barcoded reverse primer pools. Barcoded PCR products from up
130 to 96 samples were pooled, purified and an indexed sequencing library was prepared
131 using the NEBNext® ChIP-Seq Library Prep Master Mix Set for Illumina in combination
132 with NEBNext® Multiplex Oligos for Illumina (New England Biolabs). The quality of
133 sequencing libraries was verified on a Bioanalyzer DNA High Sensitivity chip (Agilent)
134 and quantified by digital PCR. 2 x 250 bp paired-end sequencing was carried out on
135 an Illumina MiSeq (Illumina) according to the manufacturer's recommendations at a
136 mean coverage of 300x.

137 Read pairs were demultiplexed according to the forward and reverse primers
138 and subsequently aligned using the Burrows-Wheeler Aligner against the Homo
139 sapiens Ensembl reference (rev. 79). Overlapping mate pairs were combined and
140 trimmed to the amplified region. Coverage for each amplicon was calculated via
141 SAMtools (v1.1) [48]. To identify putative mutations, variant calling was performed
142 using SAMtools in combination with VarScan2 (v2.3.9) [49]. Initially, SAMtools was
143 used to create pileups with a base quality filter of 15. Duplicates, orphan reads,
144 unmapped and secondary reads were excluded. Subsequently, Varscan2 was applied

145 to screen for SNPs and InDels separately, using a low-stringency setting with minimal
146 variant frequency of 0.1, a minimum coverage of 20 and a minimum of 10 supporting
147 reads per variant to account for cellular and clonal heterogeneity. Minimum average
148 quality was set to 20 and a strand filter was applied to minimize miscalls due to poor
149 sequencing quality or amplification bias. The resulting list of putative variants was
150 compared against the IARC TP53 (R17) database to check for known p53 cancer
151 mutations.

152 **Statistics**

153 Results are expressed as mean \pm S.D. of at least three experiments.
154 Comparisons between two groups were performed using Student's t-test. Three and
155 more groups were compared by ANOVA followed by the Student-Newman-Keuls test.
156 P values lower than 0.05 were considered to be significant.

157

158 **Results**

159 **Nutlin-3 sensitivity/ resistance status of the nutlin-3-adapted AML sub-lines**

160 To study acquired resistance formation to MDM2 inhibitors in AML cells, we
161 established and analysed a panel of sub-lines of the *TP53* wild-type AML cell lines
162 MV4-11, OCI-AML-2, OCI-AML-3, and SIG-M5, with acquired resistance to the MDM2
163 inhibitor nutlin-3. The parental cell lines MV4-11, OCI-AML-2, OCI-AML-3, and SIG-
164 M5 displayed sensitivity to nutlin-3 in a range of 0.90 to 2.33 μ M (Suppl. Table 1). The
165 nutlin-3 IC₅₀ values in the nutlin-3-adapted sub-lines of MV4-11 (nutlin-3 IC₅₀:
166 2.33 μ M) ranged from 13.3 to 22.6 μ M resulting in resistance factors (fold change nutlin-
167 3 IC₅₀ in nutlin-3-adapted MV4-11 sub-lines/ nutlin-3 IC₅₀ in MV4-11) ranging
168 between 5.7 (MV4-11^rNutlin^{20 μ M}XII) and 9.7 (MV4-11^rNutlin^{20 μ M}II) (Figure 1, Suppl.
169 Table 1).

170 In the nutlin-3 adapted sub-lines of OCI-AML-2 (nutlin-3 IC₅₀: 0.90 μ M), the
171 nutlin-3 IC₅₀s ranged from 14.8 μ M (OCI-AML-2^rNutlin^{20 μ M}XI, resistance factor: 16.4)
172 to 19.9 μ M (OCI-AML-2^rNutlin^{20 μ M}II, resistance factor: 22.1) (Figure 2, Suppl. Table 1).
173 In the OCI-AML-3 (nutlin-3 IC₅₀: 1.75 μ M) sub-lines, the nutlin-3 IC₅₀s ranged from
174 11.3 μ M (OCI-AML-3^rNutlin^{20 μ M}XII, resistance factor: 6.5) to 20.62 μ M (OCI-AML-
175 3^rNutlin^{20 μ M}XI, resistance factor 11.8) (Figure 3, Suppl. Table 1) and in the SIG-M5
176 (Nutlin-3 IC₅₀: 1.27 μ M) sub-lines from 3.64 μ M (SIG-M5^rNutlin^{20 μ M}XV, resistance
177 factor: 2.9) to 23.5 μ M (SIG-M5^rNutlin^{20 μ M}XI, resistance factor: 18.5) (Figure 4, Suppl.
178 Table 1).

179

180 ***TP53* status of nutlin-3-adapted AML cell lines and nutlin-3 resistance**

181 The determination of the *TP53* status in the nutlin-3-adapted AML sub-lines
182 revealed that all MV4-11 sub-lines harboured the same heterozygous R248W mutation
183 and that all OCI-AML-2 sub-lines harboured the same heterozygous Y220C mutation
184 (Table 1). In contrast, the OCI-AML-3 and SIG-M5 sub-lines harboured a range of
185 different *TP53* mutations and included sub-lines that had retained wild-type *TP53*.
186 (Table 1). In concordance, 219 out of 12418 reads of the appropriate *TP53* region in
187 the parental MV4-11 cell line indicated the presence of alleles with an R248W mutation
188 and 98 out of 907 reads indicated the presence of alleles with a Y220C mutation in the
189 parental OCI-AML-2 cell line. In contrast, the mutations detected in the nutlin-3-
190 adapted OCI-AML-3- and SIG-M5-sub-lines could not be detected in the respective
191 parental cell lines. Also, MV4-11 and OCI-AML-2 could be adapted to nutlin-3 in 12-15
192 passages, whereas the nutlin-3 adaptation of OCI-AML-3 and SIG-M5 required 30-35
193 passages. This indicates that MV4-11 and OCI-AML-2 contain pre-existing *TP53*-
194 mutant subpopulation that are selected by nutlin-3 treatment, while nutlin-3 treatment
195 resulted in *de novo* *TP53* mutations in OCI-AML-3 and SIG-M5. These results are
196 consistent with those obtained from other cancer entities [35,36,39,41].

197 Most of the *TP53* mutations are in the DNA binding domain (aa 102-292). The
198 R248W mutation in the nutlin-3-adapted MV4-11 sub-lines and the Y220C mutation in
199 the nutlin-3-adapted OCI-AML-2 sub-lines belong to the ten most commonly mutated
200 *TP53* positions. 12 of the further 13 mutations are also located in the DNA binding
201 domain and are known or expected to affect p53 function. Codon 27 is located in the
202 transactivation domain, which is relevant for the MDM2-p53 interaction. The P27S
203 mutation is known to increase the binding affinity of p53 to MDM2 [50-53].

204 There was no obvious relationship between the nutlin-3 IC₅₀ in the parental cell
205 lines in which nutlin-3 selected pre-existing *TP53*-mutant subpopulations (MV4-11:
206 2.33 μ M, OCI-AML-2: 0.90 μ M) and those parental cell lines in which nutlin-3 induced
207 *de novo TP53*-mutations (OCI-AML-3: 1.75 μ M, SIG-M5: 1.27 μ M). The nutlin-3-
208 adapted sub-lines displayed similar nutlin-3 IC₅₀s independently of the mechanism of
209 resistance formation or nutlin-3 sensitivity of the respective parental cell line (Figure
210 5). The fold changes (nutlin-3 IC₅₀ resistant sub-line/ nutlin-3 IC₅₀ respective parental
211 cell line) were typically higher in parental cell lines that displayed lower nutlin-3 IC₅₀
212 values (Figure 5). In the OCI-AML-3- and SIG-M5- sub-lines, there was no significant
213 difference between the nutlin-3 IC₅₀s in the *TP53*-mutant and *TP53* wild-type cell lines
214 (Figure 5).

215 **Cross-resistance profiles in the nutlin-3-adapted AML sub-lines**

216 Next, we determined sensitivity profiles of the nutlin-3-adapted AML sub-lines
217 to doxorubicin, etoposide, gemcitabine, cytarabine, and fludarabine (Figure 1-4, Suppl.
218 Table 1). According to the relative sensitivity of the nutlin-3-adapted sub-lines relative
219 to the respective parental cell lines, sub-lines were categorised as more sensitive (IC₅₀
220 nutlin-3-adapted sub-line/ IC₅₀ respective parental cell line <0.5), less sensitive (IC₅₀
221 nutlin-3-adapted sub-line/ IC₅₀ respective parental cell line >2), or similarly sensitive
222 (IC₅₀ nutlin-3-adapted sub-line/ IC₅₀ respective parental cell line >0.5 and <2) (Figure
223 6).

224 The sensitivity profiles indicated drug- and cell line-specific differences. Nutlin-
225 3-resistance was not generally associated with increased resistance to other drugs
226 (Figure 6). There was a noticeable heterogeneity in the drug response within the nutlin-
227 3-resistant sub-lines of each parental cell line (Figure 1-4, 7). This included the MV4-
228 11 and OCI-AML-2 sub-lines, although nutlin-3 had selected pre-existing *TP53*-mutant

229 subpopulations in them. The maximum fold difference between nutlin-3-adapted sub-
230 lines of the same parental cell line was 11.4 with MV4-11rNutlin^{20μM}XII having a
231 doxorubicin IC50 of 2.28ng/mL and MV4-11rNutlin^{20μM}VII having a doxorubicin IC50 of
232 26.0ng/mL (Figure 7).

233 Finally, the drug response patterns were more similar between doxorubicin and
234 etoposide than between these two drugs and the other agents (Figure 1-4, 6).

235

236 Discussion

237 MDM2 inhibitors are currently being investigated in phase II and III clinical trials
238 for AML (NCT02670044, NCT02545283). In various cell types, resistance formation to
239 MDM2 inhibitors has previously been shown to be associated with the selection of pre-
240 existing *TP53*-mutant cancer cell populations or the induction of *de novo TP53*
241 mutations [3,35,36,39,41]. A clinical trial in liposarcoma patients confirmed that MDM2
242 inhibitor therapy is also associated with the emergence of *TP53* mutations in the clinic
243 [40]. Here, we present a new set of models of acquired MDM2 inhibitor resistance in
244 AML, in total 45 nutlin-3-adapted sub-lines of the AML cell lines MV4-11 (15 sub-lines),
245 OCI-AML-2 (10 sub-lines), OCI-AML-3 (12 sub-lines), and SIG-M5 (8 sub-lines). Our
246 results indicate that both mechanisms, selection of pre-existing *TP53*-mutant cancer
247 cells and induction of *de novo TP53* mutations, are relevant in AML. Nutlin-3
248 consistently selected pre-existing *TP53*-mutant subpopulations in MV4-11 (R248W)
249 and OCI-AML-2 (Y220C) cells. Interestingly, two other studies had also reported the
250 emergence of R248W mutations in MV4-11 sub-lines. One study reported on an MDM2
251 inhibitor (SAR405838)-adapted MV4-11 sub-line with an R248W mutation [38].
252 Another one presented an R248W-mutant MV4-11 sub-line that had emerged during
253 prolonged cell line cultivation [9]. This suggests the consistent presence of an MV4-11
254 subpopulation that harbours an R248W *TP53* mutation.

255 In contrast, the 12 nutlin-3-adapted OCI-AML-3 sub-lines included 9 *TP53*-
256 mutant sub-lines, which all harboured different mutations, and 3 sub-lines that had
257 retained wild-type *TP53*. Similarly, the 8 SIG-M5 sub-lines consisted of 4 *TP53*-mutant
258 sub-lines, again each harbouring a different mutation, and 4 *TP53* wild-type sub-lines.

259 Notably, loss-of-p53-function has been associated with aggressive disease,
260 chemoresistance, and dismal outcome in AML [54]. In patients with therapy-related

261 AML, cytotoxic chemotherapy selected pre-existing *TP53*-mutant clones that were
262 highly resistant to therapy [55,56]. However, resistance formation to nutlin-3 was not
263 generally associated with cross-resistance to other anti-cancer drugs in AML cells.
264 Hence, loss-of-p53-function does not always seem to mediate resistance to cytotoxic
265 therapies directly. Indeed, RNAi-mediated depletion of p53 in SIG-M5 cells resulted in
266 increased resistance to nutlin-3 but not to doxorubicin (Suppl. Figure 1). Notably, loss-
267 of-p53 function may also indirectly increase the adaptability of AML cells to cytotoxic
268 anti-cancer therapies, for example due to increased genomic instability [54].

269 In addition, the nutlin-3-adapted AML sub-lines displayed a noticeable
270 heterogeneity in their responses to the anti-cancer drugs doxorubicin, etoposide,
271 gemcitabine, cytarabine, and fludarabine. This also included the MV4-11 and OCI-
272 AML-2 sub-lines, in which pre-existing *TP53*-mutant subpopulations had been selected
273 by nutlin-3 treatment. Indeed, the highest fold change in the IC₅₀ between the most
274 sensitive and the most resistant nutlin-3-adapted sub-line of a given parental cell line
275 was observed in MV4-11. The most doxorubicin-resistant MV4-11 sub-line (MV4-
276 11^rNutlin^{20μM}VII) displayed a doxorubicin IC₅₀ of 26.0ng/mL, while the most
277 doxorubicin-sensitive sub-line (MV4-11^rNutlin^{20μM}XII) displayed a doxorubicin IC₅₀ of
278 2.28ng/mL, resulting in an 11.4-fold difference. This indicates that the drug sensitivity
279 profile of a nutlin-3-adapted AML subline cannot be predicted even if a defined pre-
280 existing subpopulation of *TP53* mutant cells has been selected.

281 The doxorubicin and etoposide response profiles were more similar across the
282 nutlin-3-adapted AML sub-lines than the sensitivity profiles of the other drugs. This
283 may reflect a higher level of similarity between the mechanisms of action of doxorubicin
284 and etoposide, which are both topoisomerase II inhibitors [57], compared to the other
285 agents that are nucleoside analogues [58,59].

286 In conclusion, the investigation of 45 nutlin-3-adapted sub-lines of the AML cell
287 lines MV4-11, OCI-AML-2, OCI-AML-3, and SIG-M5 showed that MDM2 inhibitors
288 select, in dependence on the nature of a given AML cell population, pre-existing *TP53*-
289 mutant subpopulations or induce *de novo TP53* mutations. Since MDM2 inhibitors are
290 currently undergoing phase III clinical trials for the treatment of AML, patients should
291 be monitored for the emergence of *TP53*-mutant leukaemia cells. The nutlin-3-adapted
292 AML sub-lines showed a noticeable heterogeneity in their response to the cytotoxic
293 anti-cancer drugs doxorubicin, etoposide, gemcitabine, cytarabine, and fludarabine.
294 This indicates that even if a given cancer cell population is repeatedly adapted to the
295 same drug in independent experiments, each adaptation follows an individual process
296 resulting in a subpopulation with unique features. A substantial heterogeneity in the
297 drug response was even observed in the MV4-11 and OCI-AML-2 sub-lines, in which
298 nutlin-3 had selected pre-existing *TP53*-mutant subpopulations. Hence, future
299 individualised treatment protocols will depend on the detailed monitoring of the
300 evolutionary processes in cancer cell populations in response to therapy and an in-
301 depth understanding of the therapeutic implications of the observed changes.

302 **Abbreviation list**

303 AML, acute myeloid leukaemia; IC50, concentration that inhibits cell viability by 50%;

304 MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

305 **Ethics approval and consent to participate**

306 Not applicable

307

308 **Consent for publication**

309 Not applicable

310

311 **Availability of data and materials**

312 All data generated or analysed during this study are included in this published article

313 and its supplementary information files.

314

315 **Competing interests**

316 The authors declare that they have no competing interests.

317

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322 the study, the collection, analysis, and interpretation of data, and in writing the

323 manuscript.

324

325 **Authors' contributions**

326 All authors analysed data and read and approved the final manuscript. MMi and JCjr
327 directed the study and wrote the manuscript. CS, FR, TR, and JCjr were involved in
328 the generation of the nutlin-3-resistant cell lines and sensitivity testing. MMe, AN, and
329 TS were involved in the *TP53* sequencing and analysed the resulting data together
330 with MM, DS, and JCjr.

331

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334

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- 556

557 **Table 1.** *TP53* mutation status of AML cell lines and their nutlin-3-adapted sub-lines.
558

Cell Line	<i>TP53</i> mutation status
MV4-11	wild type
MV4-11 ^r Nutlin ^{20μM} I-XV	R248W (het) ^{1,2}
OCI-AML-2	wild type
OCI-AML-2 ^r Nutlin ^{20μM} I-V, VII, VIII, X, XI, XV	Y220C (het) ²
OCI-AML-3	wild type
OCI-AML-3 ^r Nutlin ^{20μM} I	R196* ³ (hom)
OCI-AML-3 ^r Nutlin ^{20μM} IV	R273S (het)
OCI-AML-3 ^r Nutlin ^{20μM} V	S215G (het)
OCI-AML-3 ^r Nutlin ^{20μM} VI	C176F (het)
OCI-AML-3 ^r Nutlin ^{20μM} VII	G244S (het)
OCI-AML-3 ^r Nutlin ^{20μM} VIII	wild-type
OCI-AML-3 ^r Nutlin ^{20μM} IX	c.485 del 6bp (TCTACA) het, IYK->K (p.162..p.164)
OCI-AML-3 ^r Nutlin ^{20μM} XI	G266V (het)
OCI-AML-3 ^r Nutlin ^{20μM} XII	wild type
OCI-AML-3 ^r Nutlin ^{20μM} XIII	wild type
OCI-AML-3 ^r Nutlin ^{20μM} XIV	S215G (het)
OCI-AML-3 ^r Nutlin ^{20μM} XV	R248Q (het)
SIG-M5	wild type
SIG-M5 ^r Nutlin ^{20μM} III	wild type
SIG-M5 ^r Nutlin ^{20μM} IV	K132E (hom)
SIG-M5 ^r Nutlin ^{20μM} VI	R282W (het)
SIG-M5 ^r Nutlin ^{20μM} VIII	P27S (het)
SIG-M5 ^r Nutlin ^{20μM} IX	wild type
SIG-M5 ^r Nutlin ^{20μM} XI	c.196 del A (->Stop in Codon), V173L (het)
SIG-M5 ^r Nutlin ^{20μM} XV	wild type
SIG-M5 ^r Nutlin ^{20μM} XX	wild type

559
560 ¹ het, heterozygous; hom, homozygous
561 ² All sub-lines share the identical mutation
562 ³ stop codon

563 **Figure legends**

564 **Figure 1.** Drug sensitivity profiles of the AML cell line MV4-11 and its sub-lines adapted
565 to nutlin-3 (20 μ M). Concentrations that inhibit cell viability by 50% (IC₅₀) as determined
566 by MTT assay after 120h incubation and relative sensitivity expressed as fold change
567 (IC₅₀ nutlin-3-resistant MV4-11 sub-line/ IC₅₀ MV4-11). Numerical data are presented
568 in Suppl. Table 1.

569

570 **Figure 2.** Drug sensitivity profiles of the AML cell line OCI-AML-2 and its sub-lines
571 adapted to nutlin-3 (20 μ M). Concentrations that inhibit cell viability by 50% (IC₅₀) as
572 determined by MTT assay after 120h incubation and relative sensitivity expressed as
573 fold change (IC₅₀ nutlin-3-resistant OCI-AML-2 sub-line/ IC₅₀ OCI-AML-2). Numerical
574 data are presented in Suppl. Table 1.

575

576 **Figure 3.** Drug sensitivity profiles of the AML cell line OCI-AML-3 and its sub-lines
577 adapted to nutlin-3 (20 μ M). Concentrations that inhibit cell viability by 50% (IC₅₀) as
578 determined by MTT assay after 120h incubation and relative sensitivity expressed as
579 fold change (IC₅₀ nutlin-3-resistant OCI-AML-3 sub-line/ IC₅₀ OCI-AML-3). Numerical
580 data are presented in Suppl. Table 1.

581

582 **Figure 4.** Drug sensitivity profiles of the AML cell line SIG-M5 and its sub-lines adapted
583 to nutlin-3 (20 μ M). Concentrations that inhibit cell viability by 50% (IC₅₀) as determined
584 by MTT assay after 120h incubation and relative sensitivity expressed as fold change
585 (IC₅₀ nutlin-3-resistant SIG-M5 sub-line/ IC₅₀ SIG-M5). Numerical data are presented
586 in Suppl. Table 1.

587

588 **Figure 5.** Distribution of the nutlin-3 IC50 values in the nutlin-3-adapted AML sub-lines.
589 The IC50 values are presented as they are and as fold changes (nutlin-3 IC50 nutlin-
590 3-adapted sub-line/ nutlin-3 IC50 respective parental cell line). In addition, the
591 distribution of the nutlin-3 IC50 values is presented in the nutlin-3-adapted OCI-AML-
592 3- and SIG-M5-sub-lines in dependence of their *TP53* mutation status. Numerical data
593 are presented in Suppl. Table 1.

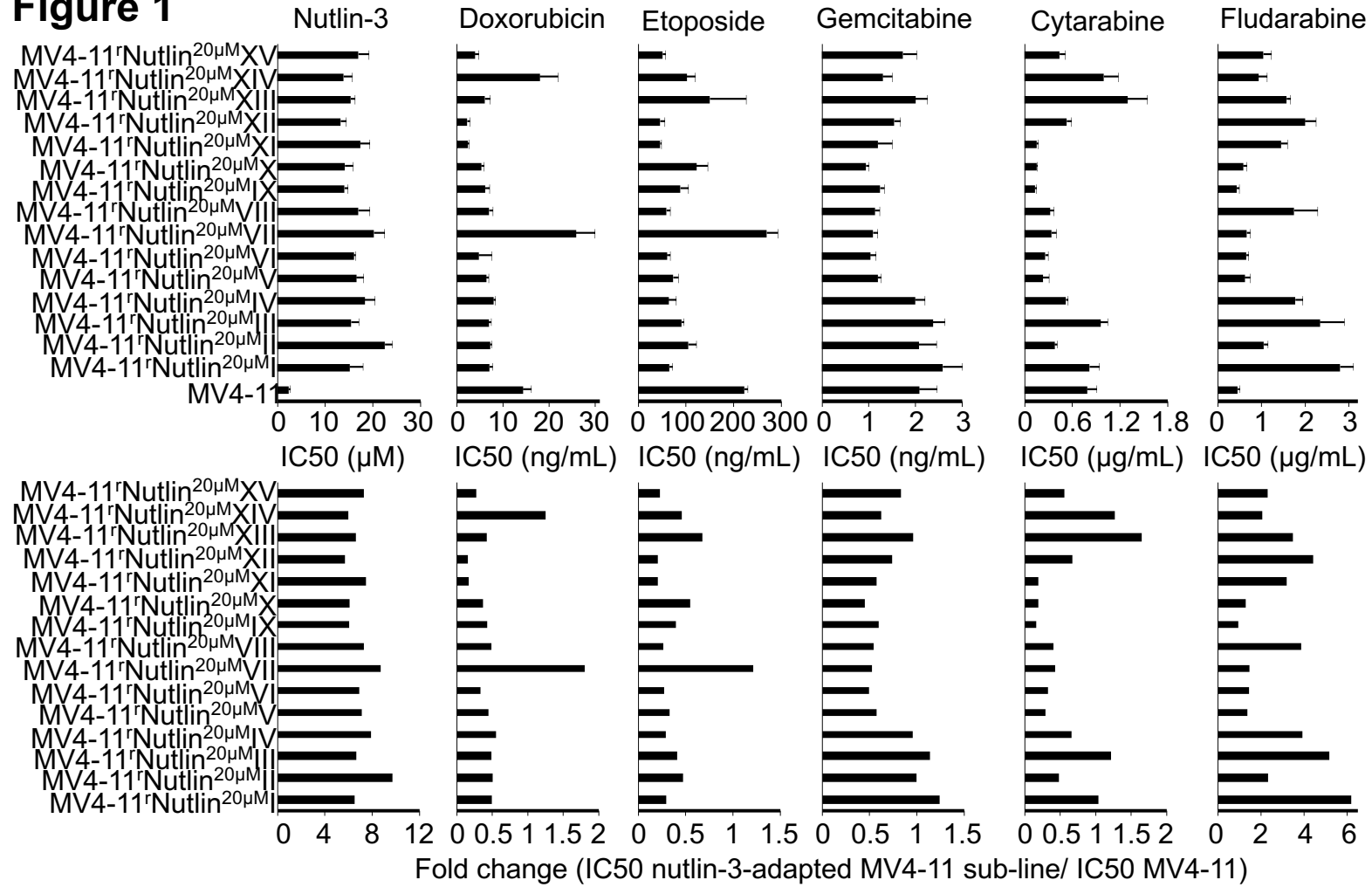
594

595 **Figure 6.** Nutlin-3-adapted AML sub-lines that display decreased, similar, or increased
596 sensitivity to doxorubicin, etoposide, gemcitabine, cytarabine, or fludarabine relative to
597 the respective parental cell lines. The nutlin-3-adapted AML sub-lines were
598 categorised as cell lines that display a higher drug sensitivity than the respective
599 parental cell line (IC50 nutlin-3-adapted sub-line/ IC50 respective parental cell line
600 <0.5, blue bars), a similar drug sensitivity as the respective parental cell line (IC50
601 nutlin-3-adapted sub-line/ IC50 respective parental cell line >0.5 and <2, yellow bars),
602 or a lower drug sensitivity than the respective parental cell line (IC50 nutlin-3-adapted
603 sub-line/ IC50 respective parental cell line >2, purple bars). Numerical data are
604 presented in Suppl. Table 1.

605

606 **Figure 7.** Comparison of the response of individual nutlin-3-adapted AML sub-lines to
607 doxorubicin, etoposide, gemcitabine, cytarabine, or fludarabine. The fold change IC50
608 sub-line with the highest IC50/ IC50 sub-line with the lowest IC50 are presented for
609 each drug in the nutlin-3-adapted sub-lines of MV4-11, OCI-AML-2, OCI-AML-3, and
610 SIG-M5. In addition, the distribution of the IC50s of the individual cell lines are shown.

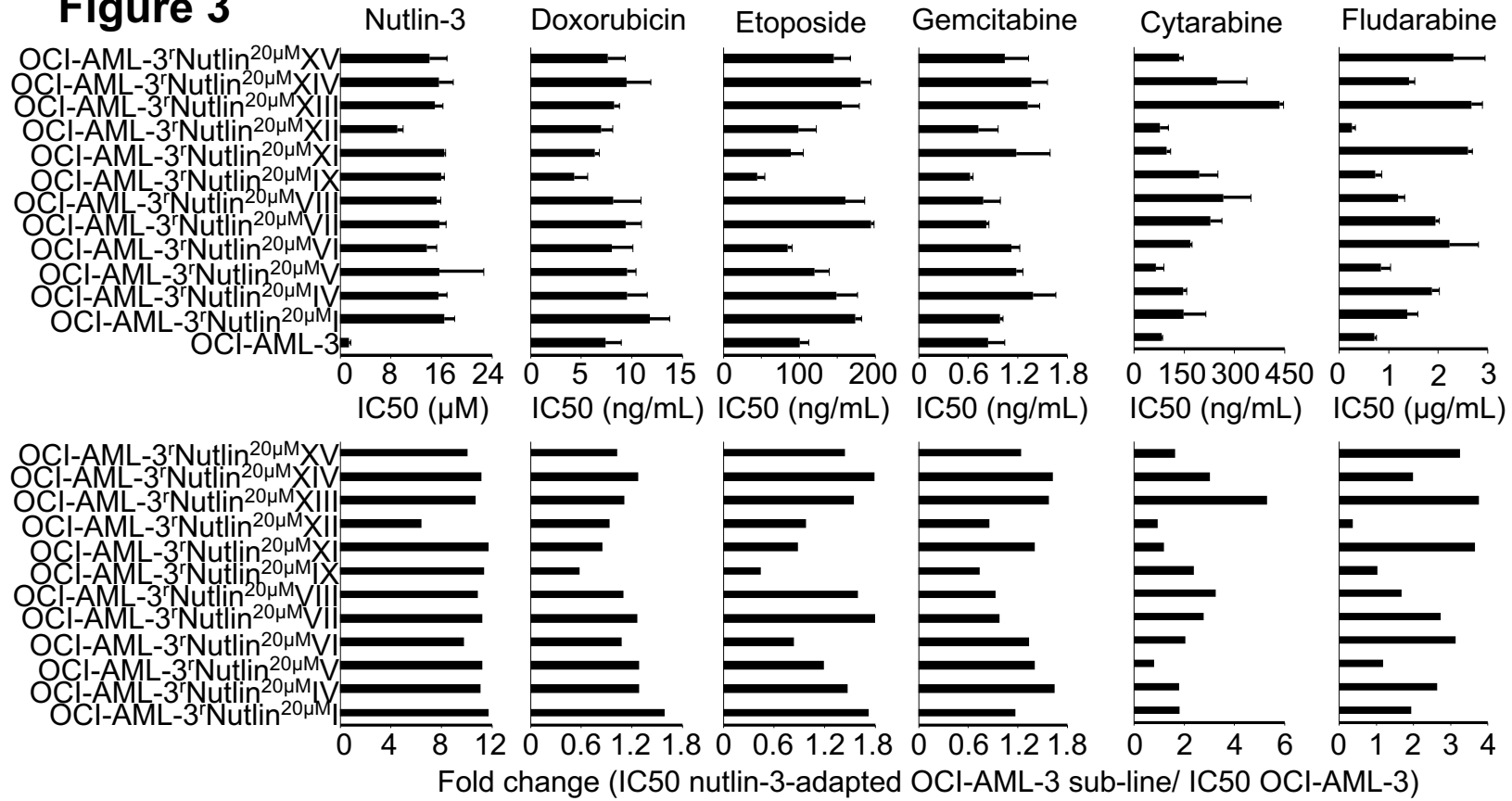
Figure 1



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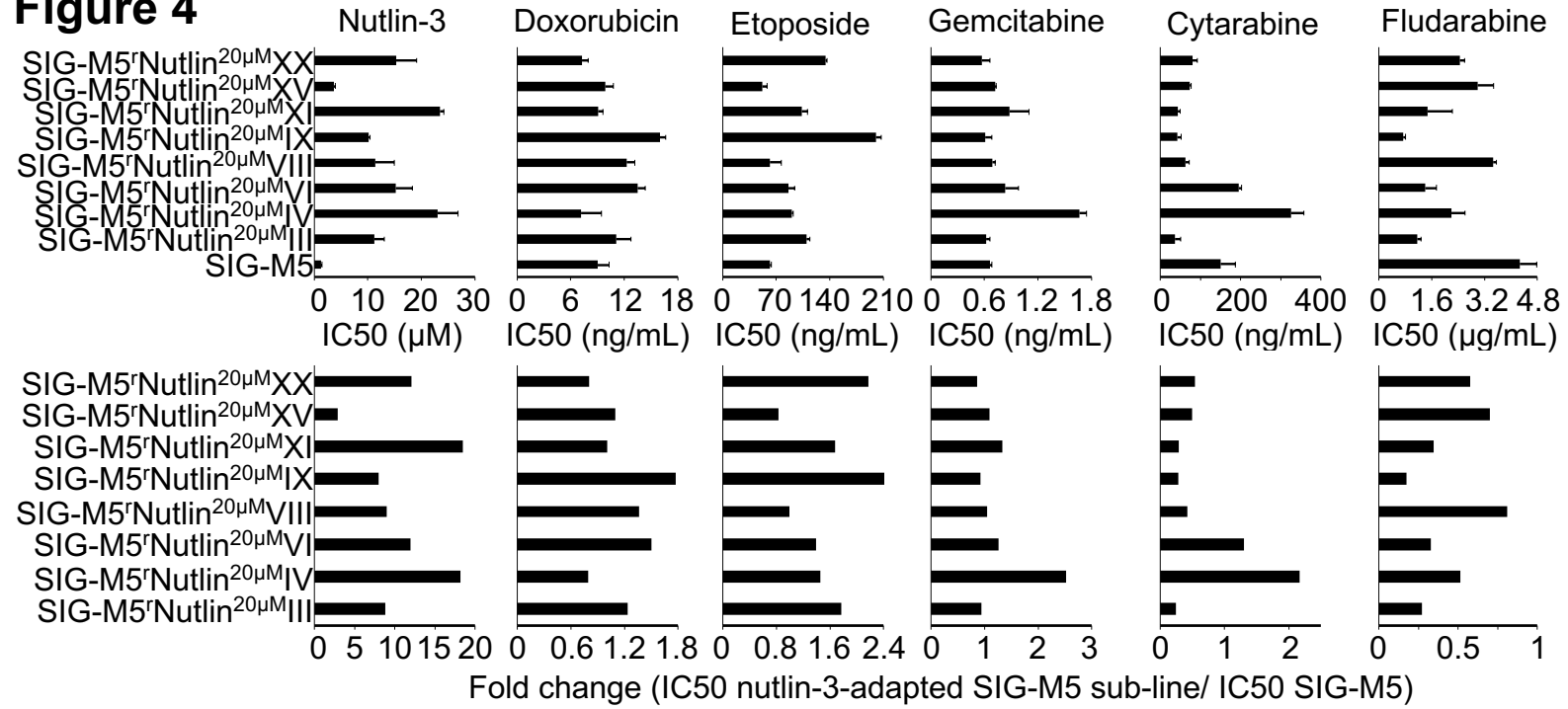
Figure 3



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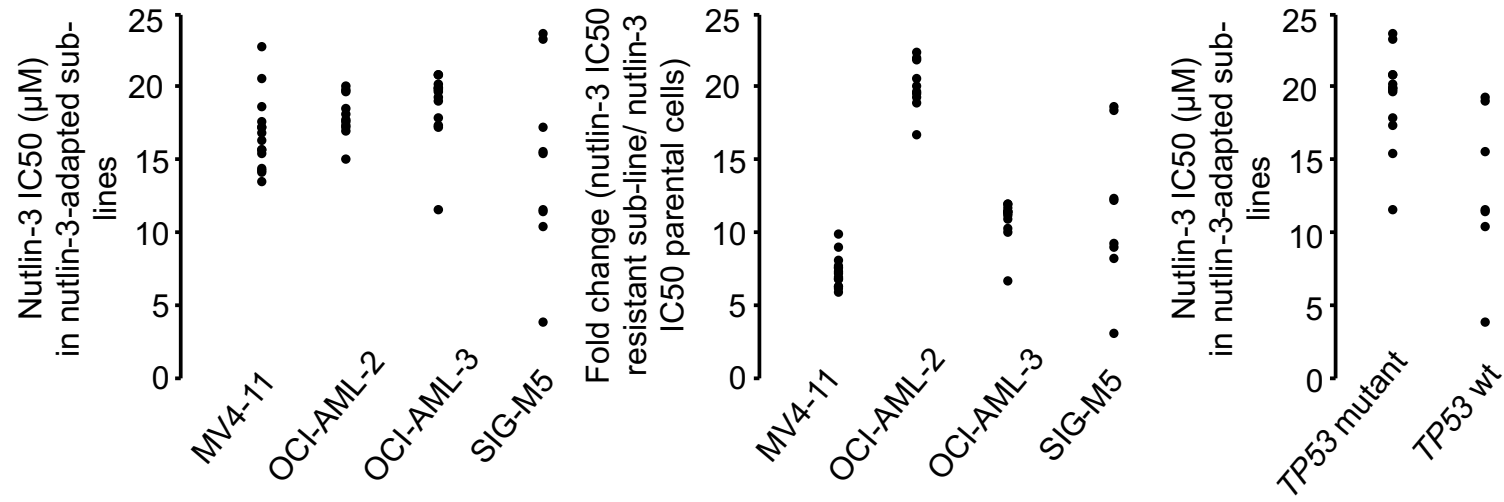
Figure 4



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Figure 5



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Figure 6

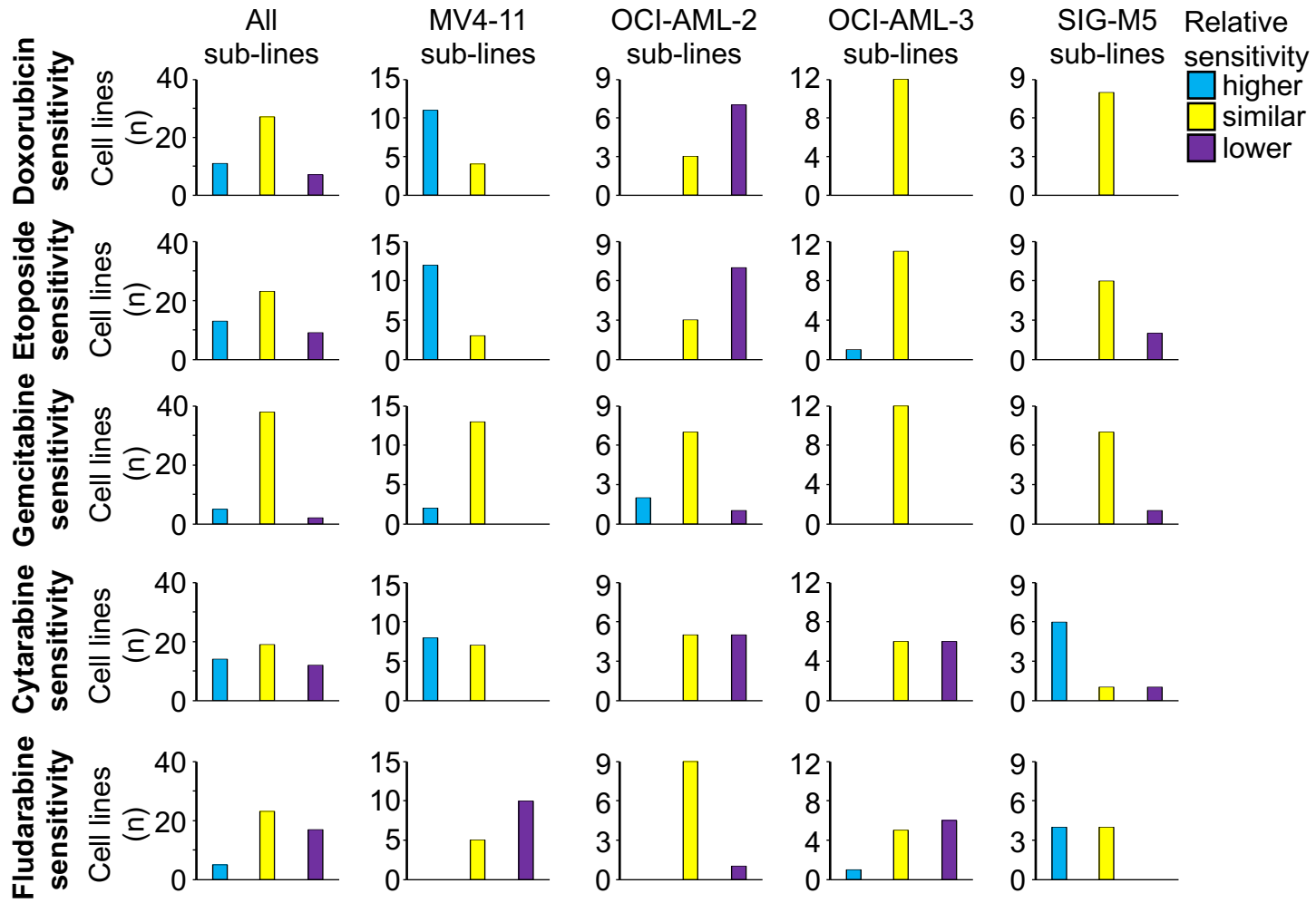
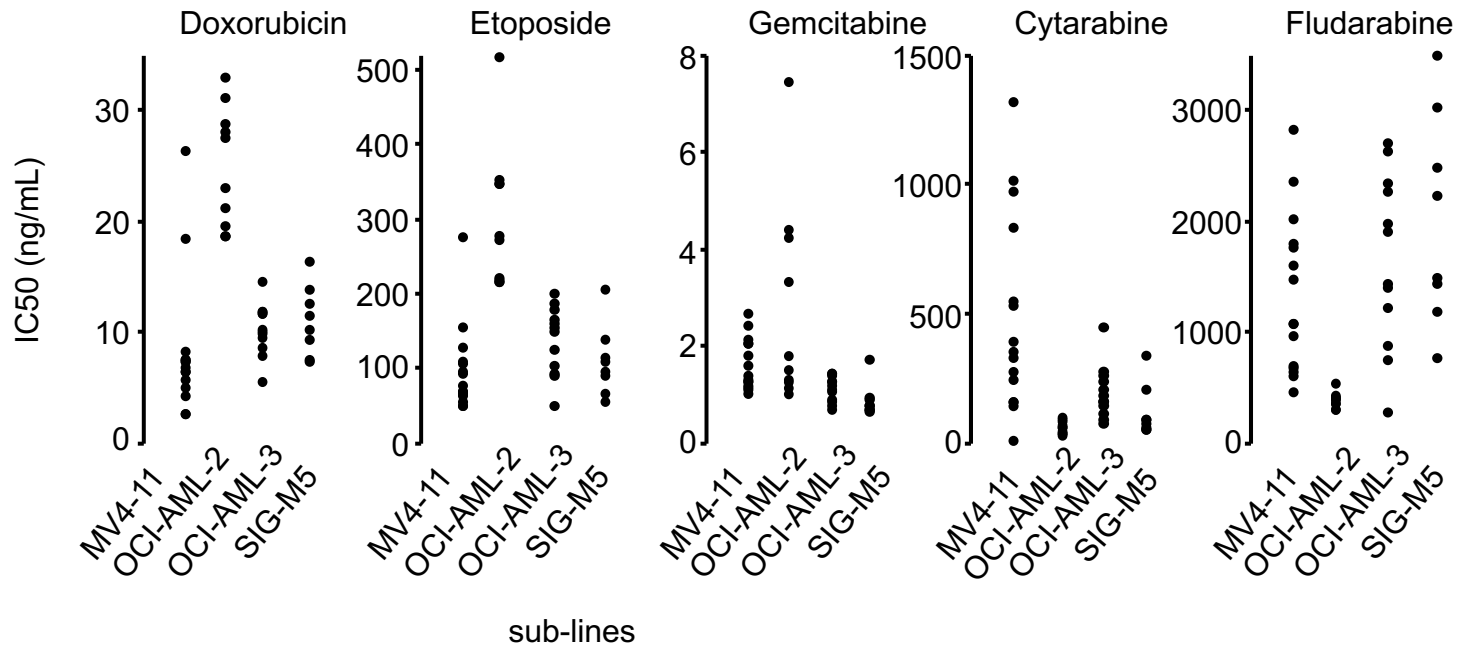
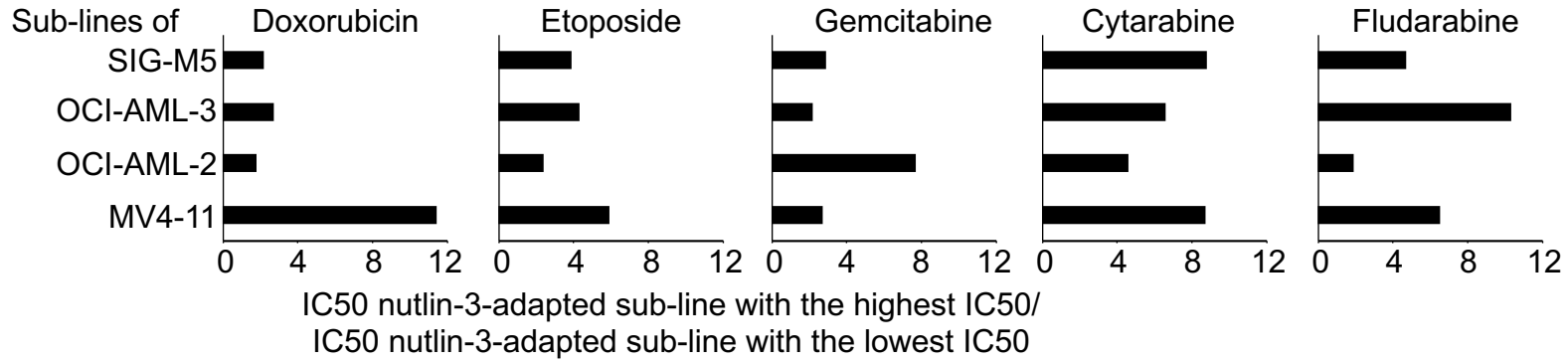
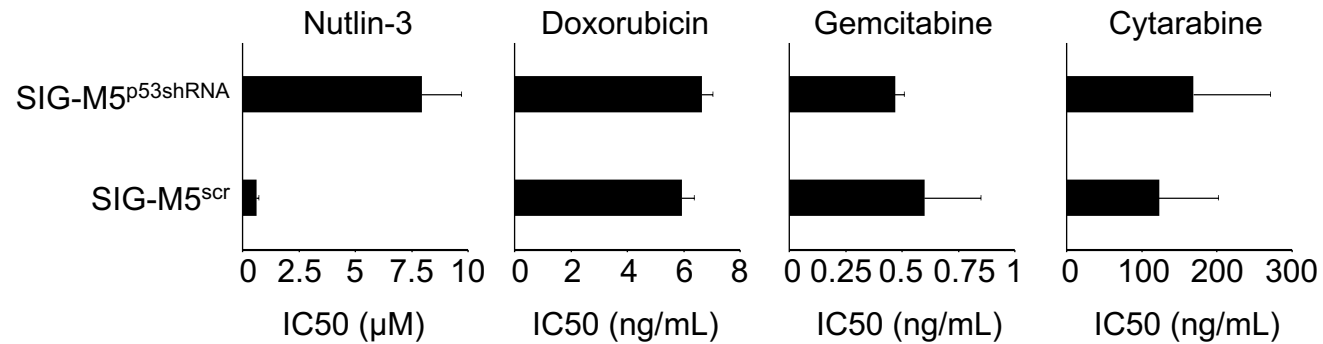


Figure 7



Suppl. Figure 1



Suppl. Figure 1. Drug sensitivity in SIG-M5 cells transduced with a lentiviral control vector encoding non-targeting ('scrambled') shRNA (SIG-M5^{scr}) and SIG-M5 cells transduced with a lentiviral vector encoding shRNA targeting p53 (SIG-M5^{p53shRNA}). Concentrations that reduce cell viability by 50% (IC50) were determined by MTT assay after 120h of incubation.

623

624

625

Suppl. Table 1. TP53 status and drug sensitivity profiles in AML cell lines and their sub-lines adapted to nutlin-3 (20µM).

Cell line	TP53 status	Drug concentration that reduces cell viability by 50% (IC50) ¹					
		nutlin-3 (µM)	doxorubicin (ng/mL)	etoposide (ng/mL)	gemcitabine (ng/mL)	cytarabine (µg/mL)	fludarabine (µg/mL)
MV4-11	wild type	2.33 ± 0.35	14.4 ± 1.8	223 ± 7	2.08 ± 0.38	0.79 ± 0.12	0.45 ± 0.05
MV4-11 ^r Nutlin ^{20µM} I	R248W (het) ²	15.2 ± 2.8 (6.52) ³	7.15 ± 0.68 (0.49)	65.3 ± 6.9 (0.29)	2.58 ± 0.42 (1.24)	0.82 ± 0.12 (1.04)	2.79 ± 0.31 (6.20)
MV4-11 ^r Nutlin ^{20µM} II	R248W (het)	22.6 ± 1.5 (9.70)	7.23 ± 0.36 (0.50)	106 ± 17 (0.47)	2.07 ± 0.38 (1.00)	0.38 ± 0.03 (0.48)	1.05 ± 0.10 (2.33)
MV4-11 ^r Nutlin ^{20µM} III	R248W (het)	15.5 ± 1.6 (6.65)	6.99 ± 0.56 (0.48)	91.2 ± 4.9 (0.41)	2.37 ± 0.26 (1.14)	0.96 ± 0.09 (1.22)	2.33 ± 0.56 (5.18)
MV4-11 ^r Nutlin ^{20µM} IV	R248W (het)	18.4 ± 2.1 (7.90)	8.00 ± 0.42 (0.55)	64.3 ± 15.5 (0.29)	1.99 ± 0.21 (0.96)	0.52 ± 0.03 (0.66)	1.77 ± 0.17 (3.93)
MV4-11 ^r Nutlin ^{20µM} V	R248W (het)	16.6 ± 1.5 (7.12)	6.46 ± 0.52 (0.45)	73.5 ± 10.9 (0.33)	1.19 ± 0.07 (0.57)	0.23 ± 0.08 (0.29)	0.62 ± 0.12 (1.38)
MV4-11 ^r Nutlin ^{20µM} VI	R248W (het)	16.1 ± 0.3 (6.91)	4.77 ± 2.85 (0.33)	60.5 ± 7.3 (0.27)	1.03 ± 0.12 (0.49)	0.26 ± 0.04 (0.33)	0.65 ± 0.05 (1.44)
MV4-11 ^r Nutlin ^{20µM} VII	R248W (het)	20.3 ± 2.2 (8.71)	26.0 ± 4.1 (1.80)	271 ± 23 (1.21)	1.09 ± 0.10 (0.52)	0.34 ± 0.06 (0.43)	0.66 ± 0.08 (1.47)
MV4-11 ^r Nutlin ^{20µM} VIII	R248W (het)	17.0 ± 2.4 (7.30)	7.05 ± 0.84 (0.49)	59.3 ± 7.8 (0.27)	1.13 ± 0.10 (0.54)	0.32 ± 0.05 (0.41)	1.74 ± 0.54 (3.87)
MV4-11 ^r Nutlin ^{20µM} IX	R248W (het)	14.1 ± 0.7 (6.05)	6.18 ± 0.96 (0.43)	88.7 ± 17.1 (0.40)	1.24 ± 0.09 (0.60)	0.13 ± 0.02 (0.16)	0.43 ± 0.06 (0.96)
MV4-11 ^r Nutlin ^{20µM} X	R248W (het)	14.2 ± 1.7 (6.09)	5.33 ± 0.65 (0.37)	123 ± 24 (0.55)	0.94 ± 0.06 (0.45)	0.15 ± 0.01 (0.19)	0.58 ± 0.08 (1.29)
MV4-11 ^r Nutlin ^{20µM} XI	R248W (het)	17.4 ± 2.0 (7.47)	2.42 ± 0.27 (0.17)	46.1 ± 3.1 (0.21)	1.19 ± 0.31 (0.57)	0.15 ± 0.02 (0.19)	1.44 ± 0.15 (3.20)
MV4-11 ^r Nutlin ^{20µM} XII	R248W (het)	13.3 ± 1.2 (5.71)	2.28 ± 0.56 (0.16)	46.1 ± 9.7 (0.21)	1.54 ± 0.14 (0.74)	0.53 ± 0.06 (0.67)	1.99 ± 0.25 (4.42)

MV4-11 ^r Nutlin ²⁰ μ MXIII	R248W (het)	15.4 \pm 0.9 (6.61)	6.13 \pm 0.48 (0.42)	151 \pm 76 0.68	2.00 \pm 0.25 (0.96)	1.30 \pm 0.25 (1.65)	1.57 \pm 0.09 (3.49)
MV4-11 ^r Nutlin ²⁰ μ MXIV	R248W (het)	13.9 \pm 1.8 (5.97)	18.1 \pm 3.9 (1.25)	102 \pm 17 (0.46)	1.30 \pm 0.20 (0.63)	1.00 \pm 0.18 (1.27)	0.93 \pm 0.20 (2.07)
MV4-11 ^r Nutlin ²⁰ μ MXV	R248W (het)	17.0 \pm 2.3 (7.30)	3.95 \pm 0.80 (0.27)	51.3 \pm 6.2 (0.23)	1.73 \pm 0.29 (0.83)	0.44 \pm 0.07 (0.56)	1.04 \pm 0.18 (2.31)

Cell line	TP53 status	Drug concentration that reduces cell viability by 50% (IC ₅₀) ¹					
		nutlin-3 (μ M)	doxorubicin (ng/mL)	etoposide (ng/mL)	gemcitabine (ng/mL)	cytarabine (ng/mL)	fludarabine (μ g/mL)
OCI-AML-2	<i>wild type</i>	0.90 \pm 0.22	9.80 \pm 2.61	107 \pm 24	2.59 \pm 1.48	23.9 \pm 13.9	0.22 \pm 0.03
OCI-AML-2 ^r Nutlin ²⁰ μ M I	Y220C (het)	19.5 \pm 1.6 (21.7)	27.3 \pm 5.7 (2.79)	348 \pm 41 (3.24)	7.40 \pm 4.16 (2.86)	81.5 \pm 55.1 (3.41)	0.36 \pm 0.17 (1.64)
OCI-AML-2 ^r Nutlin ²⁰ μ M II	Y220C (het)	19.9 \pm 2.1 (22.1)	27.7 \pm 12.6 (2.83)	273 \pm 117 (2.54)	1.23 \pm 0.29 (0.47)	17.8 \pm 2.4 (0.74)	0.32 \pm 0.09 (1.45)
OCI-AML-2 ^r Nutlin ²⁰ μ M III	Y220C (het)	16.8 \pm 3.4 (18.7)	30.8 \pm 9.5 (3.14)	342 \pm 83 (3.18)	4.34 \pm 2.88 (1.68)	80.6 \pm 44.2 (3.37)	0.37 \pm 0.18 (1.68)
OCI-AML-2 ^r Nutlin ²⁰ μ M IV	Y220C (het)	17.4 \pm 3.6 (19.3)	28.5 \pm 5.2 (2.91)	342 \pm 104 (3.18)	4.18 \pm 2.01 (1.61)	71.2 \pm 40.0 (2.98)	0.39 \pm 0.17 (1.77)
OCI-AML-2 ^r Nutlin ²⁰ μ M V	Y220C (het)	19.6 \pm 2.9 (21.8)	20.9 \pm 2.9 (2.13)	216 \pm 61 (2.01)	3.27 \pm 1.51 (1.26)	48.7 \pm 18.0 (2.04)	0.33 \pm 0.19 (1.50)
OCI-AML-2 ^r Nutlin ²⁰ μ M VII	Y220C (het)	17.5 \pm 4.2 (19.4)	19.3 \pm 2.4 (1.97)	212 \pm 121 (1.97)	1.21 \pm 0.49 (0.47)	27.4 \pm 7.5 (1.15)	0.36 \pm 0.21 (1.64)
OCI-AML-2 ^r Nutlin ²⁰ μ M VIII	Y220C (het)	18.3 \pm 3.1 (20.3)	22.8 \pm 10.7 (2.33)	214 \pm 90 (1.99)	0.96 \pm 0.14 (0.37)	25.8 \pm 9.1 (1.08)	0.27 \pm 0.10 (1.23)
OCI-AML-2 ^r Nutlin ²⁰ μ M X	Y220C (het)	17.2 \pm 4.1 (19.1)	18.4 \pm 6.1 (1.88)	212 \pm 80 (1.97)	1.08 \pm 0.20 (0.42)	26.6 \pm 10.5 (1.11)	0.27 \pm 0.06 (1.23)
OCI-AML-2 ^r Nutlin ²⁰ μ M XI	Y220C (het)	14.8 \pm 4.2 (16.4)	32.7 \pm 6.1 (3.34)	511 \pm 47 (4.76)	1.75 \pm 0.63 (0.68)	46.5 \pm 16.2 (1.95)	0.37 \pm 0.19 (1.68)

OCI-AML-2 ^r Nutlin ^{20μM} XV	Y220C (het)	17.9 ± 3.7 (19.9)	18.4 ± 3.66 (1.88)	268 ± 101 (2.50)	1.43 ± 0.33 (0.55)	55.5 ± 18.7 (2.32)	0.51 ± 0.10 (2.32)
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Cell line	TP53 status	Drug concentration that reduces cell viability by 50% (IC50) ¹					
		nutlin-3 (μM)	doxorubicin (ng/mL)	etoposide (ng/mL)	gemcitabine (ng/mL)	cytarabine (ng/mL)	fludarabine (μg/mL)
OCI-AML-3	wild type	1.75 ± 0.30	8.90 ± 1.89	101 ± 12	0.84 ± 0.20	82.1 ± 3.4	0.71 ± 0.04
OCI-AML-3 ^r Nutlin ^{20μM} I	R196*4 (hom)	20.6 ± 2.1 (11.7)	14.2 ± 2.4 (1.60)	174 ± 9 (1.73)	0.98 ± 0.04 (1.17)	148 ± 66 (1.80)	1.38 ± 0.21 (1.94)
OCI-AML-3 ^r Nutlin ^{20μM} IV	R273S (het)	19.5 ± 1.6 (11.1)	11.5 ± 2.4 (1.29)	149 ± 28 (1.48)	1.38 ± 0.28 (1.64)	147 ± 10 (1.49)	1.87 ± 0.15 (2.63)
OCI-AML-3 ^r Nutlin ^{20μM} V	S215G (het)	19.7 ± 8.7 (11.3)	11.5 ± 1.0 (1.29)	121 ± 19 (1.20)	1.18 ± 0.08 (1.40)	65.3 ± 23.4 (0.80)	0.84 ± 0.20 (1.18)
OCI-AML-3 ^r Nutlin ^{20μM} VI	C176F (het)	17.2 ± 1.9 (9.83)	9.65 ± 2.51 (1.08)	84.8 ± 5.8 (0.84)	1.12 ± 0.10 (1.33)	168 ± 5 (2.05)	2.23 ± 0.58 (3.14)
OCI-AML-3 ^r Nutlin ^{20μM} VII	G244S (het)	19.7 ± 1.3 (11.3)	11.3 ± 1.9 (1.27)	195 ± 4 (1.94)	0.82 ± 0.03 (0.98)	228 ± 34 (2.78)	1.94 ± 0.08 (2.73)
OCI-AML-3 ^r Nutlin ^{20μM} VIII	wild-type	19.1 ± 0.9 (10.9)	9.83 ± 3.29 (1.10)	161 ± 26 (1.60)	0.78 ± 0.21 (0.93)	266 ± 83 (3.24)	1.19 ± 0.13 (1.68)
OCI-AML-3 ^r Nutlin ^{20μM} IX	c.485 del 6bp (TCTACA) het, IYK->K (p.162..p.164)	20.0 ± 0.6 (11.4)	5.19 ± 1.61 (0.58)	44.9 ± 9.6 (0.45)	0.62 ± 0.04 (0.74)	195 ± 55 (2.38)	0.73 ± 0.13 (1.03)
OCI-AML-3 ^r Nutlin ^{20μM} XI	G266V (het)	20.6 ± 0.3 (11.8)	7.63 ± 0.55 (0.86)	89.3 ± 16.3 (0.89)	1.18 ± 0.40 (1.40)	97.0 ± 11.8 (1.18)	2.60 ± 0.09 (3.66)
OCI-AML-3 ^r Nutlin ^{20μM} XII	wild type	11.3 ± 1.2 (6.46)	8.37 ± 1.42 (0.94)	99 ± 24 (0.98)	0.72 ± 0.24 (0.86)	77.8 ± 25.2 (0.95)	0.26 ± 0.07 (0.37)
OCI-AML-3 ^r Nutlin ^{20μM} XIII	wild type	18.8 ± 1.6 (10.7)	9.91 ± 0.67 (1.11)	157 ± 23 (1.55)	1.32 ± 0.14 (1.57)	434 ± 12 (5.29)	2.67 ± 0.22 (3.76)

OCI-AML-3 ^r Nutlin ²⁰ μ MXIV	S215G (het)	19.6 \pm 2.8 (11.2)	11.4 \pm 2.9 (1.28)	181 \pm 14 (1.80)	1.36 \pm 0.20 (1.62)	248 \pm 89 (3.02)	1.41 \pm 0.12 (1.99)
OCI-AML-3 ^r Nutlin ²⁰ μ MXV	R248Q (het)	17.7 \pm 3.5 (10.1)	9.18 \pm 2.10 (1.03)	146 \pm 22 (1.45)	1.04 \pm 0.29 (1.24)	134 \pm 13 (1.63)	2.31 \pm 0.64 (3.25)

630

Cell line	TP53 status	Drug concentration that reduces cell viability by 50% (IC ₅₀) ¹					
		nutlin-3 (μ M)	doxorubicin (ng/mL)	etoposide (ng/mL)	gemcitabine (ng/mL)	cytarabine (ng/mL)	fludarabine (μ g/mL)
SIG-M5	wild type	1.27 \pm 0.16	9.01 \pm 1.26	61.9 \pm 1.6	0.66 \pm 0.02	150 \pm 37	4.27 \pm 0.52
SIG-M5 ^r Nutlin ²⁰ μ MIII	wild type	11.2 \pm 1.9 (8.80)	11 \pm 1.6 (1.23)	109 \pm 4 (1.77)	0.62 \pm 0.04 (0.94)	36.9 \pm 13.7 (0.25)	1.16 \pm 0.11 (0.27)
SIG-M5 ^r Nutlin ²⁰ μ MIV	K132E (hom)	23.0 \pm 3.8 (18.1)	7.12 \pm 2.31 (0.79)	90.0 \pm 2.3 (1.45)	1.66 \pm 0.08 (2.52)	325 \pm 32 (2.17)	2.20 \pm 0.41 (0.52)
SIG-M5 ^r Nutlin ²⁰ μ MVI	R282W (het)	15.2 \pm 3.2 (11.9)	13.5 \pm 0.85 (1.50)	86.2 \pm 7.6 (1.39)	0.83 \pm 0.15 (1.26)	195 \pm 7 (1.30)	1.40 \pm 0.34 (0.33)
SIG-M5 ^r Nutlin ²⁰ μ MVIII	P27S (het)	11.4 \pm 3.5 (8.98)	12.3 \pm 0.9 (1.36)	61.6 \pm 15.1 (1.00)	0.69 \pm 0.03 (1.05)	63.0 \pm 8.5 (0.42)	3.46 \pm 0.10 (0.81)
SIG-M5 ^r Nutlin ²⁰ μ MIX	wild type	10.1 \pm 0.3 (7.97)	16.0 \pm 0.7 (1.77)	200 \pm 7 (3.23)	0.61 \pm 0.07 (0.92)	42.5 \pm 9.3 (0.28)	0.74 \pm 0.07 (0.17)
SIG-M5 ^r Nutlin ²⁰ μ MXI	c.196 del A (->Stop in Codon), V173L (het)	23.5 \pm 0.7 (18.5)	9.07 \pm 0.54 (1.01)	103 \pm 7 (1.67)	0.88 \pm 0.22 (1.33)	43.1 \pm 6.6 (0.29)	1.47 \pm 0.75 (0.34)
SIG-M5 ^r Nutlin ²⁰ μ MXV	wild type	3.64 \pm 0.29 (2.87)	9.87 \pm 0.90 (1.10)	51.5 \pm 6.4 (0.83)	0.72 \pm 0.01 (1.09)	73.7 \pm 3.4 (0.49)	2.99 \pm 0.48 (0.70)
SIG-M5 ^r Nutlin ²⁰ μ MXX	wild type	15.3 \pm 3.8 (12.1)	7.23 \pm 0.71 (0.80)	134 \pm 2 (2.17)	0.57 \pm 0.09 (0.86)	80.7 \pm 11.1 (0.54)	2.46 \pm 0.13 (0.58)

631

632 ¹ Determined by MTT after a 120h incubation period633 ² het, heterozygous; hom, homozygous

634 ³ Fold change (IC50 nutlin-3-adapted sub-line/ IC50 respective parental cell line)

635 ⁴ Stop codon

636