

Chromosome 5q deletion drives evolution of aneuploidy in myeloid neoplasms with complex karyotype

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Abstract:

Clonal acquisition of multiple chromosomal abnormalities in hematopoietic stem and progenitor cells (HSPCs) is a hallmark of high-risk acute myeloid leukemias with complex karyotype (AML-CK). AML-CK is associated with TP53 mutations and chromosome 5q deletions (del5q); however, the drivers and clonal trajectories of aneuploid evolution in HSPCs remain unknown. We have developed a patient-derived induced pluripotent stem cell (iPSC) model in which preleukemic HSPCs clonally evolve to distinct, highly aneuploid states following transient mitotic inhibition. By tracking chromosome evolution at single cell resolution, we show that TP53-mutant HSPCs with del5q, but not TP53 mutation alone, evolved complex chromosomal changes. Clonal evolution was marked by stepwise acquisition of numerical and structural chromosome changes seen in AML-CK patients, with individual abnormalities conferring fitness advantage. iPSC-derived aneuploid HSPCs and primary AML-CK patient samples exhibited a conserved gene expression signature marked by upregulation of PTEN, cohesins, and anti-apoptotic factor BCL2, indicative of a shared aneuploid cell state in HSPCs. Clinical BCL2 inhibitor venetoclax eradicated BCL2-dependent aneuploid clones, with resistant clones undergoing a lineage switch to upregulate alternative BCL2 factors. In summary, we demonstrate that mutant TP53 and del5q drive chromosome evolution marked by stepwise acquisition of individual abnormalities. Moreover, aneuploid HSPCs exhibit a shared gene expression state which confers unique targetable therapeutic vulnerabilities in AML-CK.

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1 **Chromosome 5q deletion drives evolution of aneuploidy in myeloid neoplasms**
2 **with complex karyotype**

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51 **Key Points**

- 52 • Chromosome 5q deletion drives progressive chromosome evolution in *TP53*-mutant preleukemic
53 HSPCs.
- 54 • Aneuploid HSPCs exhibit a shared gene expression signature which confers unique therapeutic
55 vulnerabilities in AML-CK.
- 56
- 57

58 **Abstract**

59 Clonal acquisition of multiple chromosomal abnormalities in hematopoietic stem and progenitor cells
60 (HSPCs) is a hallmark of high-risk acute myeloid leukemias with complex karyotype (AML-CK). AML-CK
61 is associated with *TP53* mutations and chromosome 5q deletions (del5q); however, the drivers and clonal
62 trajectories of aneuploid evolution in HSPCs remain unknown. We have developed a patient-derived
63 induced pluripotent stem cell (iPSC) model in which preleukemic HSPCs clonally evolve to distinct, highly
64 aneuploid states following transient mitotic inhibition. By tracking chromosome evolution at single cell
65 resolution, we show that *TP53*-mutant HSPCs with del5q, but not *TP53* mutation alone, evolved complex
66 chromosomal changes. Clonal evolution was marked by stepwise acquisition of numerical and structural
67 chromosome changes seen in AML-CK patients, with individual abnormalities conferring fitness
68 advantage. iPSC-derived aneuploid HSPCs and primary AML-CK patient samples exhibited a conserved
69 gene expression signature marked by upregulation of *PTEN*, cohesins, and anti-apoptotic factor *BCL2*,
70 indicative of a shared aneuploid cell state in HSPCs. Clinical *BCL2* inhibitor venetoclax eradicated *BCL2*-
71 dependent aneuploid clones, with resistant clones undergoing a lineage switch to upregulate alternative
72 *BCL2* factors. In summary, we demonstrate that mutant *TP53* and del5q drive chromosome evolution
73 marked by stepwise acquisition of individual abnormalities. Moreover, aneuploid HSPCs exhibit a shared
74 gene expression state which confers unique targetable therapeutic vulnerabilities in AML-CK.

75 Introduction

76

77 Aneuploidy involves changes in chromosome number or structure¹⁻³ and is pervasive in cancers⁴.
78 Aneuploidy is a hallmark of high-risk acute myeloid leukemias (AML) with complex karyotype (AML-CK),
79 defined as three or more chromosomal abnormalities⁵. AML-CK is almost universally associated with
80 mutations in *TP53*, very poor outcomes, and lower response to current antileukemic therapies⁶⁻⁸. Most
81 AML-CK patients present with widespread aneuploidy at diagnosis, making it challenging to reconstruct
82 clonal trajectories of early disease. It remains unknown how hematopoietic stem and progenitor cells
83 (HSPCs) with aneuploidy first arise and evolve, ultimately giving rise to these aggressive leukemias.

84

85 While relatively infrequent in *de novo* AML, complex karyotype with *TP53* mutations is enriched in
86 therapy-related myeloid neoplasms (t-MN), implicating cytotoxic therapy in inducing chromosome
87 instability or creating selective pressure for the mutant clones⁹. *TP53* mutant clones are detectable at low
88 frequency years prior to t-MN diagnosis, and in many cases are antecedent to chemotherapy^{10,11}. *TP53*
89 mutations are found in age-related clonal hematopoiesis, which is associated with an increased risk of
90 myeloid malignancy^{12,13}. These observations broadly suggest that *TP53* mutations are early events in
91 AML-CK pathogenesis, and that clonal progression to AML-CK requires the occurrence of additional
92 genetic events often in the context of cytotoxic therapy. Despite the advent of improved single cell copy
93 number detection methods, detection of early clones in this disease remains rare¹⁴. Consequently, little
94 is known about the genetic drivers and trajectories of AML-CK evolution from preleukemic HSPCs.

95

96 Specific chromosomal abnormalities are recurrently observed in certain cancers, suggesting that they
97 impact genes that contribute to cancer fitness and progression^{15,16}. Most patients with AML-CK harbor
98 recurrent deletions on chromosomes 5q (del5q), 7q, and/or 17p (encoding *TP53*), with del(5q) being the
99 most common change found in over 80% of AML-CK cases, suggesting that this region encodes critical
100 genes for disease progression^{7,17-19}. Classical studies have established the contribution of individual 5q
101 genes to leukemogenesis¹⁹⁻²³. However, it remains unknown if 5q deletion acts as a driver of AML-CK
102 progression, owing to lack of experimental model systems in which normal HSPCs evolve to complex
103 karyotypes. Understanding the role of del5q as a driver in AML-CK could nominate mechanisms and
104 molecular dependencies due to haploinsufficiency of 5q-encoded genes. Aneuploid states more broadly
105 may confer specific molecular dependencies due to cellular stresses on proteostasis, replication, and
106 mitosis²⁴, which could be therapeutically harnessed for incurable *TP53*-mutated leukemias²⁵.

107

108 Here we report an induced pluripotent stem cell (iPSC) model in which patient-derived *TP53*-mutant
109 preleukemic HSPCs evolve to distinct highly aneuploid states following mitotic mis-segregation. We show
110 that HSPCs with *TP53* mutation and del5q undergo progressive aneuploid evolution resembling AML-CK

111 patients. By tracking the trajectories of chromosome evolution at the single cell level, we show that
112 aneuploid evolution is characterized by stepwise acquisition of abnormalities which converged on shared
113 gene expression signature with AML-CK patients, revealing unique molecular dependencies associated
114 with acquisition of aneuploidy in HSCs which can be therapeutically targeted in *TP53*-mutated leukemias.

116 **Materials and Methods**

117
118 **MDS/AML iPSC-derived HSPC culture.** *TP53^{mut}* and *TP53^{mut}* del5q iPSC lines were previously derived
119 from bone marrow cells of a female MDS/AML-CK patient^{20,26}. Primary AML samples were obtained from
120 the Fred Hutchinson Cancer Center Co-Operative Center for Excellence in Hematology core or the Mayo
121 Clinic Biorepository for Acute Leukemia Research after Institutional Review Board approval. Written
122 informed consent was obtained from all participants. iPSC lines were differentiated into HSPCs, and
123 established as expandable iPSC-HSPCs by the introduction of five transcription factors^{20,26}. iPSC-HSPCs
124 were cultured in StemSpan SFEM II (StemCell Technologies) plus 50 ng/mL SCF, 50 ng/ml FLT3,
125 50 ng/ml TPO, 50 ng/ml IL-6, 10 ng/ml IL-3 (PeproTech), and doxycycline (2 µg/ml) (Sigma)²⁷. Cells were
126 maintained at 0.5-1x10⁶ cells/ml and media changed every 3 to 4 days. For evolution to aneuploidy, iPSC-
127 HSPC cell lines were treated with 1 µM of NMS-P715 MPS1 inhibitor (Sigma) in DMSO for 24 hours,
128 followed by a complete media change. Cultures were maintained at 0.5-1x10⁶ cells/ml and cultured for
129 60-70 days or as specified. Complete details are provided in Supplementary Methods.

130
131 **Single cell RNA sequencing and CNV analysis.** Patient samples were thawed by dropwise addition of
132 IMDM + 1% BSA, washed, and then purified using the CD34+ MACS (Miltenyi) selection column, followed
133 by the Dead Cell Removal Kit (Miltenyi). iPSC-HSPC cell lines were thawed and dead cells removed by
134 the Dead Cell Removal Kit. Single cell sequencing was performed using the 10X Chromium Next GEM
135 Single Cell 3' Reagents kit v.3.1 on a Chromium Controller. Libraries were sequenced using Illumina NGS
136 NextSeq/NovaSeq 150/100 PE cycle kits. After alignment to hg38 using the 10X genomics Cell Ranger
137 count tool, the Seurat R Package²⁸ v4.1.3 was used to perform integration where multiple runs were
138 analyzed together, with mitochondrial gene regression (SCTransform function), dimensional reduction,
139 UMAP generation, and clustering. Copy number inference was performed using the Numbat package²⁹.
140 Complete details are provided in Supplementary Methods.

142 **Data Availability**

143 Raw and processed single cell RNA sequencing data and CNV analysis have been deposited to GEO
144 under accession #GSE282370.

146 Results

147 148 Experimental model of aneuploid evolution in preleukemic HSPCs

149 We have previously reprogrammed bone marrow cells from a Li-Fraumeni patient who developed AML-
 150 CK and isolated two isogenic preleukemic iPSC lines: an ancestral line with a heterozygous *TP53* R209fs
 151 mutation (herein *TP53^{mut}*), and a derived line with *TP53* R209fs and an isolated del(5)(q22q31) (herein
 152 *TP53^{mut}del5q*)^{20,26}. Reprogramming of these clones suggested that *TP53*-mutant preleukemic clones first
 153 acquired isolated del(5q), followed by other complex abnormalities (which were not compatible with
 154 reprogramming). We differentiated *TP53^{mut}* and *TP53^{mut}del5q* iPSCs to CD34⁺ HSPCs and established
 155 conditionally immortalized HSPCs, which can be continuously expanded for ~5 months while maintaining
 156 a developmental hierarchy with multipotent CD34⁺ HSPCs and more differentiated myeloid cell types^{20,26}.
 157 *TP53^{mut}* and *TP53^{mut}del5q* HSPCs expanded with similar cell division characteristics and maintained a
 158 stable karyotype over extended culture (**Figure 1A; Supplementary Figure 1A-B**).

159
 160 Errors in chromosome segregation due to defective mitotic checkpoint is a major mechanism of
 161 chromosome instability in cancers^{3,30}. MPS1 kinase inhibitors (MPSi) robustly induce chromosome mis-
 162 segregation giving rise to aneuploid clones³¹⁻³³. We transiently treated *TP53^{mut}* and *TP53^{mut}del5q* HSPCs
 163 with the MPSi NMS-P715 for 24 hours and monitored by conventional karyotyping over extended 5 to
 164 10-week culture (**Figure 1A**). Strikingly, we observed robust evolution to distinct highly aneuploid states
 165 following MPSi treatment across multiple experiments. Even more surprisingly, *TP53^{mut}del5q* HSPCs
 166 almost invariably evolved to aneuploidy (mean ~95.1%), whereas parental *TP53^{mut}* HSPCs infrequently
 167 gave rise to aneuploid clones (mean = 5.8%) ($p < 0.0001$) (**Figure 1B**). *TP53^{mut}del5q* cultures harbored a
 168 greater number of abnormalities per clone ($p = 0.0002$) and distinct clones ($p < 0.0001$) compared to
 169 *TP53^{mut}* HSPCs (**Figure 1C,D**). The clones derived from *TP53^{mut}del5q* HSPCs typically gained ~2
 170 additional abnormalities, with several subclones observable by conventional karyotyping (at least 2 out
 171 of 20 cells counted) after ~5 weeks of culture (**Table 1**). The abnormalities observed in the *TP53^{mut}del5q*
 172 MPSi culture were reflective of patients with AML-CK, including trisomy 8 (+8), trisomy 10 (+10), and
 173 unbalanced translocations or deletions of chromosome 21 (chr. 21) (**Figure 1E; Table 1; Supplementary**
 174 **Table 1**). These observations contrasted with *TP53^{mut}* HSPCs, which never reached >20% of total culture
 175 (**Figure 1B**). Taken together our findings show that iPSC-derived HSPCs undergo robust chromosome
 176 evolution upon mitotic checkpoint inhibition and that 5q loss is a cooperating abnormality required for
 177 complex karyotype evolution.

178 179 Evolution of aneuploidy is a stepwise process shaped by clonal competition

180 The clonal trajectories by which preleukemic *TP53*-mutant HSPCs evolve to aneuploidy in AML-CK are
 181 unknown. To examine these evolutionary dynamics, we followed paired *TP53^{mut}del5q* “evolving” and

182 *TP53^{mut}* “non-evolving” HSPCs in long-term culture following segregation error (**Figure 2A**). In a
183 representative experiment, 3 independent *TP53^{mut}del5q* subclones emerged ~1 month after MPSi
184 treatment with additional material on chr. 21 [del(5q),add(21p)], monosomy 21 [del(5q),-21], and trisomy
185 10 [del(5q),+10] (**Figure 2B**, top; **Supplementary Figure 2A**, left). After another month in culture, the
186 add(21p) emerged as the dominant clone reaching fixation (~100%), with the other clones no longer
187 detectable by conventional karyotyping (**Figure 2B**, top). Interestingly, the add(21p) clone further
188 diversified by acquiring add(11p), not detected at the previous time point (**Figure 2B**; **Supplementary**
189 **Figure 2A**, middle). The paired *TP53^{mut}* culture exhibited limited karyotype changes, with the presence
190 of a minor +8 clone at ~10% (**Supplementary Figure 2B**). Whole genome sequencing showed that a
191 limited number of single nucleotide variants were acquired during aneuploid evolution that were private
192 to individual lines and did not map to known cancer driver genes (**Supplementary Table 2**). These data
193 suggest that chromosome evolution is driven by clonal competition, with a single clone emerging as the
194 “winner” and further gradually diversifying.

195

196 The initial mis-segregation event is expected to produce small numbers of abnormal clones which may
197 be lost by genetic drift. To test this possibility, we set up a parallel culture from a cryopreserved sample
198 immediately after MPSi treatment (**Figure 2A**). By repeating the evolution experiment, we observed a
199 completely different winning clone with deletion 1p [del(5q),del(1p),dup(2),-21] gaining dominance over
200 the add(21p) clone, the latter failing to further diversify (**Figure 2B**; bottom; **Supplementary Figure 2A**,
201 right). One possible explanation is the bottleneck effect, wherein the rare del(1p) clone was randomly
202 split into the cryopreserved sample. To determine if individual abnormalities act as drivers, we estimated
203 the relative fitness of each clone using a Bayesian probabilistic framework designed to fit existing clone
204 frequency measurements over time³⁴. Notably, add(21p) clone had a relative fitness advantage (mean
205 $1+s \sim 1.014$) in the original culture in which the del(1p) clone was not detected (**Figure 2C**, top). By
206 contrast, its fitness advantage was lost in the repeat culture (mean $1+s \sim 0.971$) (**Figure 2C**, bottom).
207 These data suggest that certain chromosome abnormalities act as drivers to confer highest relative
208 fitness, with the repertoire of competing clones shaped by genetic drift and clonal selection.

209

210 To better examine evolutionary dynamics in early culture, we modeled a long-term evolution
211 experiment^{35,36} by splitting an initial culture into four separate sub-cultures (A-D) after the 24-hour MPSi
212 treatment (**Figure 2D**). We observed multiple competing subclones in the four cultures, notably distinct
213 from the ones seen in the previous experiment, followed by emergence of the +8 founder as the “winning”
214 clone in all cultures (**Figure 2E**). Interestingly, the +8 clone further diversified by acquiring unique
215 abnormalities in each culture, including add(11), +19, or +10 (**Figure 2E**). We observed some
216 diversification of the founding clone in *TP53^{mut}* cultures, but with limited selection (**Supplementary**
217 **Figure 2C**). Mixing of the cultures after ~2 weeks resulted in a similar pattern of clonal expansion of the

218 +8 founder (**Supplementary Figure 2D**). Estimates of relative fitness (1+s) varied for each sub-culture,
219 but were consistent with +8 acting as the driver event (**Figure 2F**). Taken together, these data suggest
220 that AML-CK evolution is shaped by genetic drift limiting the initial repertoire of aberrant clones, with
221 clonal competition selecting a winning founder clone with the highest relative fitness, with subsequent
222 evolution of progressively fitter clones. These findings help explain the highly variable karyotypes of
223 individual AML-CK patients, which often contain derivatives of a single dominant clone (e.g., stemline).

224

225 **Single cell analysis identifies gene expression signature of aneuploidy**

226 Conventional cytogenetic analysis is a gold standard in clinical practice but limited to manual examination
227 of a small number of metaphases. To gain deeper insight into the clonal dynamics and concurrent cell
228 state changes, we utilized single cell RNAseq to quantify changes in gene expression in aneuploid clones
229 identified by copy number inference (CNI)^{29,37}. We profiled sequential samples in the representative
230 experiment in Figure 2A by single cell transcriptomics, followed by haplotype-aware copy number calling
231 (**Figure 3A; Supplementary Figure 3A**). As validation, unbiased calling of del(5q) in a combined *TP53^{mut}*
232 and *TP53^{mut}del5q* dataset revealed >95% confidence in correct calling (**Supplementary Figure 3B**).
233 Consistent with conventional karyotyping, *TP53^{mut}* culture was comprised of several clones at <10%
234 frequency, whereas *TP53^{mut}del5q* culture reached ~60% abnormal cells (**Figure 3B; Supplementary**
235 **Figure 3C**). Unbiased lineage reconstruction further identified distinct normal and del(5q) clonal branches
236 (**Figure 3C**). Analysis of *TP53^{mut}del5q* culture by conventional karyotyping showed the winning add(21p)
237 clone (**Figure 2B**), but surprisingly, no chr. 21 abnormalities were called by CNI. Instead, gain of chr. 10q
238 material (10q+) was identified (**Figure 3C**), suggesting that the additional material on chr. 21p was
239 derived from 10q. To confirm this, whole genome sequencing identified a gain of 10q22qter including the
240 *PTEN* gene, which was localized to chr. 21p by metaphase FISH (**Supplementary Figure 3D,E**). These
241 data show that single cell CNI enables robust identification of aneuploid clones, with improved ability to
242 define the origin of rearranged material.

243

244 We next sought to define the gene expression changes associated with acquisition of aneuploidy in
245 HSPCs. To this end, we compared gene expression between the ancestral del(5q) and the derived
246 10q+/add(21p) clone identified by CNI. Both cell types shared a similar distribution of myeloid, lymphoid,
247 and progenitor cell populations (**Supplementary Figure 3F; Supplementary Table 3**). We identified 272
248 differentially expressed genes, including 116 upregulated genes, of which 37 were encoded within the
249 10q+ interval (**Supplementary Table 4**)³⁸. Upregulated genes included the 10q *PTEN* tumor suppressor,
250 which has an essential role in genome integrity^{39,40}, anti-apoptotic factor *BCL2*, and *CD34* (**Figure 3D**).
251 Upregulated genes were enriched for gene ontology terms related to mitosis and cohesin loading, TGF β
252 pathway, p53 pathway, apoptotic signaling, and cell cycle (**Figure 3E**). Key mitotic factors included
253 cohesin subunits *STAG1*, *STAG2*, *WAPL*, and *SMC3*, regulators of sister chromatid segregation *CENPF*,

254 *KIF11*, *KIF20B*, *TOP2A*, and genome integrity factors *SHLD2*, *HELLS*, and *PTEN* (**Figure 3D**;
255 **Supplementary Table 4**), many of which are encoded in the 10q+ interval. These alterations suggest
256 that aneuploid HSPCs upregulate chromosome segregation factors which may facilitate the maintenance
257 of aneuploid cell state.

258

259 **Aneuploidy signature is shared with AML-CK patients**

260 To determine if these gene expression changes are broadly associated with aneuploidy in hematopoietic
261 cells, we next performed single cell transcriptomics on two primary AML-CK patient samples with *TP53*
262 mutation and del(5q) (**Figure 4A**). CNI analysis of patient RO20518 identified del(5q) [add(5)(q11.2)], +8,
263 chr. 15 abnormalities [t(2;15)], and -16, found in the clinical karyotype (**Figure 4B**; **Supplementary Table**
264 **5**). CNI analysis of patient R332 identified -5, -7, +8, +19, +21, and +22 identified in karyotype analysis
265 (**Supplementary Figure 4A**; **Supplementary Table 5**), in addition to copy number changes that were
266 not resolved by karyotype. The R332 sample was comprised of four highly complex CK subclones (CK1-
267 4) (**Supplementary Figure 4A**), while in the RO20518 sample we also detected ancestral clones with
268 isolated loss of 5q and 15p. To determine if the aneuploidy signature in our iPSC model was shared with
269 AML-CK patients, we generated a module score based on 116 genes upregulated in the +10q/add(21p)
270 clone as the iPSC-Complex Karyotype signature (i-CK) (**Supplementary Figure 4B**). The i-CK signature
271 was upregulated in all R332 CK1-4 clones and some of the RO20518 clones over normal copy number
272 cells identified by CNI in each patient (**Figure 4C**). No chr. 10q gain was detected by CNI or conventional
273 karyotyping in either patient, suggesting that i-CK is a generalizable signature induced in aneuploid
274 HSPCs in AML-CK irrespective of specific abnormalities. *PTEN* and cohesin component *WAPL* were
275 consistently upregulated in aneuploid clones in AML-CK patients (**Figure 4D-E**). *BCL2* was upregulated
276 in R332, but not RO20518 which expressed *CD14* (**Figure 4D-E**), consistent with low expression of *BCL2*
277 in myelo-monocytic AML⁴¹. To characterize the aneuploidy signature across larger patient cohorts, we
278 analyzed single cell RNAseq data from 5 AML-CK patients reported by Wang et al⁴², 8 AML-CK patients
279 reported by Leppä et al¹⁴, and 1 AML-CK patient from Petti et al^{43,44}. The samples included those with
280 *TP53* mutation, *TP53*^{mut} with 17p/-17, and *TP53* WT cases, with multiple cytogenetic abnormalities
281 detected by single cell CNI (**Supplementary Figure 4C**; **Supplementary Table 5**). All 5 patients from
282 Wang et al⁴² showed significant enrichment of the i-CK signature, including *PTEN* and cohesins, in
283 complex subclones compared to euploid cells from the same patient (**Figure 4F,G**). Of the 8 patients
284 from Leppä et al¹⁴, 7 showed significant i-CK signature enrichment compared to normal immune cells
285 from the same patient (**Supplementary Figure 4D**). Similarly, i-CK signature was upregulated in the
286 AML-CK patient from Petti et al^{43,44} (**Supplementary Figure 5A-C**), but not in patients with del(5q) MDS
287 without complex karyotype (**Supplementary Figure 5D-F**). Taken together, the i-CK signature was
288 broadly upregulated in 14 of 16 AML-CK patients with diverse mutational status and cytogenetic
289 abnormalities. The i-CK signature was also largely distinct from the leukemic stem cell (LSC-17)

290 signature, which was not positively enriched in aneuploid clones (**Supplementary Figure 5G**). We
291 propose that a generalized gene expression state is associated with aneuploidy in AML-CK, despite
292 different acquired chromosomal abnormalities.

293 294 **BCL2 is a targetable molecular dependency of aneuploid clones**

295 The anti-apoptotic factor *BCL2*, upregulated in aneuploid HSPCs (**Figure 3D**), is the target of venetoclax,
296 a *BCL2* inhibitor clinically used to treat high-risk AML⁴⁵⁻⁴⁷. We sought to determine whether aneuploid
297 clones are sensitized to venetoclax and how evolution to aneuploidy is impacted by venetoclax treatment.
298 Venetoclax response can be modulated by the levels of *BCL2*, other anti-apoptotic *BCL2* family proteins,
299 and pro-apoptotic factors (such as *BAX*)^{47,48}. *TP53^{mut}del5q* and the aneuploid +10q/add(21p) HSPCs
300 selectively upregulated *BCL2* (**Figure 3D**), but not other anti-apoptotic paralogs *MCL1*, *BCL2L1*, or
301 *BCL2A1* (**Figure 5A**). Consistent with this, parental *TP53^{mut}del5q* HSPCs were highly sensitive to
302 venetoclax compared to *TP53^{mut}* HSPCs (IC₅₀ ~0.250 μM vs. ~40 μM) (**Figure 5B**). To test how treatment
303 with venetoclax impacts evolution to aneuploidy, we evolved cryopreserved *TP53^{mut}del5q* HSPCs (**Figure**
304 **2A**) with continuous treatment with vehicle control or venetoclax at the 250 nM IC₅₀ dose (**Figure 5C**).
305 In the vehicle-treated culture, the same add(21p) [del(5q),add(21p),add(11p)] clone gained relative
306 dominance as in the original experiment, though interestingly it underwent whole genome duplication
307 event to give rise to a 4N subclone (**Figure 5D**, left). In marked contrast, the *BCL2*-high add(21p) clone
308 was eradicated in the venetoclax-treated culture, and instead the del(1p) [del(1p),dup2,del(5q),-21]
309 minor clone became dominant (**Figure 5D**, right). The del(1p) founder clone massively diversified under
310 venetoclax selection into several complex subclones, which gained dic(11;19) and -16 (**Figure 5D**, right).
311 Venetoclax-treated culture displayed a marked resistance to venetoclax (IC₅₀ ~ 70 μM) compared to the
312 vehicle-treated culture with dominant add(21p) clone, which had even greater sensitivity to venetoclax
313 (IC₅₀ ~ 0.02 μM) than the parental *TP53^{mut}del5q* HSPCs (IC₅₀ = 0.25 μM) (**Figure 5E**). These data show
314 that some aneuploid clones are sensitized to venetoclax, with therapy resistance driven by subclonal
315 diversity.

316 317 **Venetoclax-resistant aneuploid HSPCs undergo a lineage switch**

318 To understand the mechanisms driving venetoclax resistance in AML-CK, we next performed single cell
319 karyotyping. This analysis readily detected the add(21p)/10q+ and the del(1p) clone harboring dup2 and
320 -21 (**Figure 5F**). Venetoclax almost entirely eradicated add(21p)/10q+ and parental *TP53^{mut}del5q* clones,
321 with the culture largely comprised of the del(1p) clone (**Figure 5G; Supplementary Figure 6A,B**).
322 Interestingly, the venetoclax-resistant culture markedly downregulated *BCL2* and upregulated *BCL2A1*
323 expression by qPCR (**Figure 6A**). Single cell analysis showed that *BCL2* expression was reduced in the
324 del(1p) clone in both vehicle- and venetoclax-treated cultures (**Figure 6B**), suggesting that this shift is an
325 intrinsic property of this aneuploid genotype rather than a response to venetoclax. Since HSPC

326 populations express different levels of *BCL2* factors, we next investigated the HSPC composition. The
327 add(21p) clone was largely similar to immature lymphoid-primed multipotent progenitors (LMPP-like). By
328 contrast, the del(1p) clone showed a significant contribution from more mature erythroid-megakaryocyte
329 progenitors (MEP-like) (**Figure 6C, Supplementary Table 6**). Megakaryocyte and erythroid genes were
330 induced in the GO annotation of del(1p)-upregulated genes (**Figure 6D**), including *FLI1*, *TAL1*, *ITGA2B*
331 (CD41) (**Supplementary Figure 6C**), and interestingly JAK/STAT genes *STAT1*, *STAT3*, and *STAT5B*
332 (**Figure 6E, Supplementary Table 7**). Importantly, the gene signature from the MEP-like del(1p) clone
333 was enriched in a subset of AML-CK patients, for example RO20518 that expressed low *BCL2* and was
334 not highly enriched for the i-CK signature (**Supplementary Figure 6D,E**). The patient cohort from Leppä
335 et al¹⁴ included an AML-CK patient (P9) treated with venetoclax. The diagnostic sample was comprised
336 of a dominant CK3 clone which expressed high i-CK signature/*BCL2* and immature markers (CD34,
337 CD117), and a minor clone CK1 which expressed low i-CK signature/*BCL2* and MEP markers (CD71,
338 CD41) (**Supplementary Figure 6F-I**). Consistent with our model, venetoclax treatment depleted the
339 immature CK3 clone leading to clonal dominance of the MEP-like CK1 clone (**Supplementary Figure**
340 **6H**). These data suggest that venetoclax-resistant aneuploid clones can undergo a lineage switch from
341 a more primitive to an MEP-like progenitor cell identity that intrinsically express lower levels of *BCL2*.

342

343 Since aneuploid MEP-like HSPCs may be sensitized to distinct drug classes, we screened the parental,
344 vehicle- and venetoclax-treated lines with a panel of FDA-approved drugs for hematological malignancies
345 (**Supplementary Figure 7A, Supplementary Table 8**). Interestingly, venetoclax-resistant HSPCs with
346 dominant del(1p) clone were sensitized to multiple JAK2 inhibitors clinically used to treat
347 myeloproliferative neoplasms, including ruxolitinib, momelotinib, fedratinib, and pacritinib
348 (**Supplementary Figure 7B**). We further validated that venetoclax-resistant HSPCs were sensitized to
349 ruxolitinib compared to vehicle-treated cells (**Figure 6F**). To test the combination of venetoclax and JAK2
350 inhibitor, we treated aneuploid HSPCs with venetoclax as before (Figure 5C), allowing outgrowth of
351 resistant clones, followed by treatment with ruxolitinib. Ruxolitinib at the IC₅₀ dose of 50 nM suppressed
352 the outgrowth of the venetoclax-resistant MEP-like del(1p) clone by ~2.5 fold (**Figure 6G-I**). Lastly, we
353 tested venetoclax and JAK2 inhibitors in three primary AML-CK samples. Two of these samples had poor
354 response to venetoclax alone (IC₅₀ >2 μM; **Supplementary Figure 7C**), but were resensitized in
355 combination with JAK2 inhibitor, especially pacritinib (**Supplementary Figure 7D**). Taken together, we
356 show that venetoclax monotherapy resistance can arise from aneuploid HSPCs with MEP-like lineage
357 identity that are sensitized to distinct drug classes.

358

359 Discussion

360

361 The clonal origins of myeloid neoplasms with complex karyotype remain largely unknown. Here, we
362 develop an iPSC model system in which preleukemic HSPCs evolve to complex and diverse aneuploid
363 states following transient mitotic inhibition. Using this model, we demonstrate that iPSC-derived AML-CK
364 preleukemic HSPCs with *TP53* mutation and del(5q), but not *TP53* mutation alone, undergo complex
365 karyotype evolution marked by sequential acquisition of chromosomal abnormalities. By tracking this
366 process at single cell resolution, we identify a gene expression signature shared with AML-CK patients.
367 We propose that despite distinct collections of chromosome abnormalities, aneuploidy in AML-CK is
368 associated with generalized gene expression states, reflecting the clonal origin and molecular
369 dependencies of aneuploidy, such as anti-apoptotic *BCL2* factors. Aneuploid clones were sensitized to
370 standard of care venetoclax therapy, with resistance driven by lineage switching to HSPC states with low
371 *BCL2* expression. These findings show the existence of molecular dependences in aneuploid HSPCs
372 which can be therapeutically targeted in *TP53*-mutant leukemias.

373
374 AML-CK presents with widespread aneuploidy at diagnosis making it difficult to reconstruct clonal origins.
375 Near-complete penetrance of *TP53* mutations and their detection in clonal hematopoiesis and in
376 preleukemic clones from AML-CK patients^{10,11} suggests that these are initiating mutations. Most patients
377 with AML-CK harbor recurrent deletions on chromosome 5q, suggesting that this region encodes critical
378 genes that cooperate with mutant *TP53* to drive AML-CK progression^{7,17-19}. Here, we provide the first
379 experimental evidence that 5q loss is a driver of AML-CK progression. Loss of 5q is also an early event
380 in myeloid oncogenesis, and isolated del(5q) defines a subtype of MDS with favorable prognosis and
381 macrocytic anemia¹⁹, which can progress to high-risk MDS/AML via secondary mutations, including in
382 *TP53*⁴⁹. This suggests that del(5q) may be the initiating lesion in some patients followed by secondary
383 *TP53* mutations. The commonly deleted 5q region in AML-CK spans 5q22-q31 which encodes multiple
384 genes implicated in disease pathogenesis, including *EGR1*, *APC*, *CDC25C*, *CTNNA1*, and *LMNB1*²⁰⁻²³.
385 The identification of 5q tumor suppressor genes with roles in regulating genome stability remains an
386 important goal. We propose a common origin of AML-CK that requires cooperative genetic loss of *TP53*
387 and del(5q) and inhibition of mitotic checkpoint integrity via genotoxic or cytotoxic therapy.

388
389 Clonal trajectories of chromosomal evolution in the human hematopoietic system have not been studied
390 experimentally. Chromosomal shattering, termed chromothripsis, is observed in genetically unstable
391 tumors suggestive of a rapid, catastrophic evolution^{3,50}. Evolution in our model is consistent with a more
392 gradualist model of neoplastic progression, in which rare aneuploid cells arising after mitotic checkpoint
393 inhibition diversify in a sequential fashion. Other studies of *TP53*-mutated secondary AML are also
394 suggestive of a sequential model of aneuploidy⁵¹. Recent single cell analysis in AML-CK patients has
395 uncovered evidence for both sequential and punctuated evolution¹⁴, although detection of early clones
396 remains rare.

397

398 By using single cell CNI to track this evolutionary process, we identify an i-CK gene expression signature
399 shared with AML-CK patients. This signature is marked by upregulation of HSC genes and genome
400 integrity factors including *PTEN*⁴⁰ and cohesin subunits, including *SMC3* and *WAPL*. Aneuploidy imposes
401 unique cellular stresses on proteostasis, replication, and mitosis²⁴. Inappropriate chromatid pairing during
402 mitosis induces high levels of genome instability that can be incompatible with cell survival⁵². Interestingly,
403 *PTEN* has a non-canonical role in promoting genome integrity in mitosis³⁹. Cohesins mediate
404 chromosome pairing in early mitosis and cohesin cleavage is required for anaphase progression.
405 Critically, while cohesin mutations in *STAG2* portend a poor prognosis in myeloid neoplasms, they are
406 mutually exclusive with *TP53* mutations and are almost never observed in AML-CK⁵³. We hypothesize
407 that upregulation of *PTEN* and cohesins enables aneuploid cells to stabilize chromosome pairing in
408 mitosis, explaining why diverse CK genotypes converge on this i-CK signature. Our model of AML-CK
409 will enable further dissection of the mechanisms required for the maintenance of an aneuploid cell state
410 in HSPCs.

411

412 *TP53* mutation and complex karyotype defines a high-risk subgroup of myeloid neoplasms with poor
413 response to antileukemic therapies^{17,25}, including *BCL2* inhibitor venetoclax^{45,54-56}. We find that *BCL2* is
414 often upregulated as part of the i-CK signature and may promote the survival of aneuploid clones.
415 Treatment with venetoclax eradicates clones expressing high *BCL2* levels, however, resistance is driven
416 by aneuploid clones with reduced expression of *BCL2* and induction of alternative *BCL2* family factors,
417 such as *BCL2L1* and *BCL2A1*. The switch to alternative *BCL2* factors characterizes normal
418 hematopoietic differentiation; *BCL2* expression declines during myeloid differentiation with dependence
419 on *MCL1*⁴¹, whereas erythroid and megakaryocyte lineages are dependent on *BCL2L1*⁵⁷. Interestingly,
420 AMLs in which LSCs display expression of mature monocytic or erythroid/megakaryocyte programs
421 display poor responses to venetoclax^{41,48,57}. Consistent with this, our data suggests that venetoclax-
422 resistant aneuploid clones undergo an erythroid/megakaryocyte lineage switch which sensitizes them to
423 *JAK2* inhibition. We propose that similarly to non-aneuploid leukemias, LSCs in AML-CK likely exist in a
424 spectrum of cell states from more primitive (HSC-like) to more mature (MEP-like) among diverse clones,
425 with distinct molecular vulnerabilities⁵⁸. More broadly, we propose that aneuploid cell states confer unique
426 molecular vulnerabilities which can be therapeutically targeted in high-risk *TP53*-mutant leukemias.

427

428

429

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443

444 **Author Contributions**

445 J.P.C., and S.D. conceived the project and designed the experiments; J.P.C., performed the experiments
446 with help from S.R., S.S., V.J., and J.W.; J.P.C., S.R., S.G., F.Y.H., AM.L., V.J., P.S.B, A.T., M.F., E.M.S.,
447 and S.D., analyzed and interpreted the data; J.L.A., J.S.A., S.H.K., P.S.B, A.T., V.J., M.F., and E.M.S.,
448 provided input on experimental design and interpretation. B.W., and H.A.A. provided patient sample data.
449 A.N.S., S.H.K., J.S.A., J.L.A., and E.M.S., provided patient samples and clinical interpretation; J.P.C.,
450 and S.D. prepared the manuscript.

451

452 **Competing Interests**

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585

Table 1

Exp	Gen	Sample	Treat	Days	% Aneupl.	Karyotype
1	Pooled	1A	NMS	38	57	46,XX,del(5)(q22q31)[16]/46,idem,add(2)(p13)[16]/48,idem,+8,+20[5]/46,XX[3] (6/16 del5q non-clonal)
2	TP53	P53-2A	NMS	35	0	46,XX[30] (3 non-clonal)
2	TP53	P53-2A	NMS	76	10	47,XX,+8[2]/46,XX[18] (3/18 non-clonal)
2	del5q	del5q-2A	NMS	35	33	46,XX,del(5)(q22q31)[20]/47,sl,+10[4]/45,sl,-21[3]/46,sl,add(21)(p11.2)[3]
2	del5q	del5q-2A	NMS	76	100	46,XX,del(5)(q22q31),add(21)(p12)[12]/46,idem,add(11)(p15)[8]
2	TP53	P53-2A (rethaw)	NMS	57	0	46,XX[22] (4/22 non-clonal)
2	del5q	del5q-2A (rethaw)	NMS	57	100	45,XX,del(5)(q15q31),del(1)(p36.1p?32),dup(2)(q21q31),-21[18]/46,XX,del(5)(q15q31),add(21)(p11.2)[2]
3	TP53	P53-3control	DMSO	60	15	46,XX,dup(1)(q21q32)[3]/46,XX[17] (2 non-clonal)*
3	TP53	P53-3A	NMS	38	10	46,XX,dup(1)(q21q32)[2]/48,XX,+10,+19[2]/46,XX[16] (2 non-clonal)*
3	TP53	P53-3B	NMS	38	0	46,XX,dup(1)(q21q32)[2]/46,XX[18] (2 non-clonal +3/+6)*
3	TP53	P53-3C	NMS	38	0	46,XX,dup(1)(q21q32)[3]/46,XX[17] (2 non-clonal -15/-17)*
3	TP53	P53-3D	NMS	38	0	46,XX,dup(1)(q21q32)[6]/46,XX[14] (3/14 non-clonal -8-13-5-14-15/+1+7)*
3	TP53	P53-3A	NMS	72	10	46,XX,dup(1)(q21q32)[2]/46,XX,+del(1)(p13),-17[2]/46,XX[16]*
3	TP53	P53-3B	NMS	72	0	46,XX,dup(1)(q21q32)[3]/46,XX[17]*
3	TP53	P53-3C	NMS	72	15	46,XX,dup(1)(q21q32)[2]/46,XX,+1,add(1)(p12),del(1)(q12),-17[3]/46,XX[15]*
3	TP53	P53-3D	NMS	72	0	46,XX,dup(1)(q21q32)[4]/46,XX[16]*
3	del5q	del5q-3control	DMSO	60	0	46,XX,del(5)(q22q33)[20]
3	del5q	del5q-3A	NMS	38	10	46,XX,del(5)(q22q31)[18]/45,XX,sl,-15[2] (4 non-clonal -19/-20/-11-18/+8-19)
3	del5q	del5q-3B	NMS	38	30	46,XX,del(5)(q22q31)[14]/45,sl,dic(11;19)(p15;q13.3)[3]/47,sl,+10[2]/47,sl,+8[1]
3	del5q	del5q-3C	NMS	38	40	46,XX,del(5)(q22q31)[12]/47,idem,+8[8] (3 non-clonal -10/-18/-21)
3	del5q	del5q-3D	NMS	38	45	46,XX,del(5)(q22q31)[11]/47,idem,+8[8]/47,idem,+10[1] (3 non-clonal -11/-13/-16)
3	del5q	del5q-3A+B+C+D	NMS	57	90	46,XX,del(5)(q22q33)[2]/47,XX,del(5),+8[15]/48,XX,del(5),+8,+10[4] (7 non-clonal, in +8 samples?)
3	del5q	del5q-3A	NMS	72	90	46,XX,del(5)(q22q33)[2]/47,XX,del(5)(q22q33),+8[12]/47,XX,del(5)(q22q33),+10[1]/47,XX,del(5)(q22q33),+8,add(11)(p15)[4]/47,XX,del(5),+10,add(11)(p15),add(19)(q13.4)[1]
3	del5q	del5q-3B	NMS	72	95	46,XX,del(5)(q22q33)[1]/47,XX,del(5)(q22q33),+8[6]/48,XX,del(5)(q22q33),+8,+10[2]/48,XX,del(5)(q22q33),+8,+19[6]/47,XX,del(5)(q22q33),+8,add(11)(p15)[2]/47,XX,del(5)(q22q33),+10,add(11)(p15)[1]/47,XX,del(5)(q22q33),+8,add(19)(q13.4)[2]/45,XX,del(5),+8,add(11)(p15),-19[1]
3	del5q	del5q-3C	NMS	72	90	46,XX,del(5)(q22q33)[2]/47,XX,del(5)(q22q33),+8[13]/47,XX,del(5)(q22q33),+10[1]/48,XX,del(5)(q22q33),+8,+10[3]/47,XX,del(5)(q22q33),+8,add(11)(p15),add(19)(q13.4)[1]
3	del5q	del5q-3D	NMS	72	100	47,XX,del(5)(q22q33),+8[11]/47,XX,del(5)(q22q33),+10[4]/48,XX,del(5)(q22q33),+8,+10[3]/48,XX,del(5)(q22q33),+8,add(11)(p15)[2]
4	TP53	P53-4control	DMSO	38	0	46,XX[20]
4	TP53	P53-4A	NMS	38	0	46,XX[20]
4	del5q	del5q-4control	DMSO	38	15	46,XX,del(5)(q22q31)[17]/46,XX,del(5)(q22q31),dic(11;19)(p15;q13.3)[3]
4	del5q	del5q-4A	NMS	38	45	46,XX,del(5)(q22q31)[11]/47,XX,del(5)(q22q31),+8[7]/47,XX,del(5)(q22q31),+10[2]

587 **Table 1. Karyotypes of HSPC evolution to aneuploidy experiments.** Complete karyotype reports of
588 *TP53^{mut}* and *TP53^{mut}del5q* HSPC experiments after treatment with NMS-P715 or DMSO vehicle control
589 shown in Figure 1B and Figure 1E. Columns are organized as follows: experiment (Exp) 1-4; genotype
590 (Gen): *TP53* = *TP53^{mut}*, *del5q* = *TP53^{mut}del5q*; sample name (A,B,C, etc.); treatment (NMS or DMSO
591 control); days: culture period after NMS treatment when karyotype was determined; % Aneupl.:
592 percentage of cells with altered karyotype from the parental line. New abnormalities detected in *TP53^{mut}*
593 HSPCs are shown in blue font. New abnormalities detected in *TP53^{mut}del5q* HPSCs are shown in red
594 font. Experiment #3 corresponds to the parallel evolution experiment in Figure 2D in which sub-cultures
595 A,B,C,D were split from the same primary culture. In experiment #3, a spontaneous *dup(1)(q21q32)*
596 abnormality was observed in *TP53^{mut}* DMSO control cultures, and clones with this abnormality (marked
597 with *) were not included in the calculated percentage of aneuploid cells for NMS-treated cultures from
598 the same original culture.

599 **Figure Legends**

600

601 **Figure 1. Experimental model of aneuploid evolution in *TP53*-mutant preleukemic HSPCs. (A)**

602 Schematic of reprogramming of *TP53*^{mut} and *TP53*^{mut}del5q iPSCs from a patient with MDS/AML-CK and
603 differentiation into iPSC-HSPCs. Cells were exposed to 1 μM MPS1 inhibitor NMS-P715 for 24 hours,
604 washed, and cultured continuously for 5-10 weeks. Cytogenetic analysis was performed using
605 conventional karyotyping to analyze clonal dynamics of aneuploid cells. **(B)** Percentage of aneuploid cells
606 over time post NMS-P715 treatment as reported by karyotyping (left), and a representative aneuploid
607 clone with trisomy 8 and 20 evolved from *TP53*^{mut}del5q HSPCs. Connected line indicates measurements
608 over time in the same culture (n = 7 independent cultures derived from n = 3 NMS-P715 treated samples).
609 **(C)** Number of chromosome abnormalities per clone compared to parental lines (karyotype complexity);
610 n=8 independent cultures derived from n = 4 NMS-P715 treated samples, two tailed Mann Whitney Test
611 (**p=0.002). **(D)** Number of distinct aneuploid clones detected by karyotyping in each culture; n = 8
612 independent experiments, two-tailed Mann Whitney Test (****p<0.0001). **(E)** Summary of chromosome
613 abnormalities identified by karyotyping in aneuploid clones derived from *TP53*^{mut}del5q HSPCs. Each row
614 is a separate clone with experiments grouped by color (n = 18 clones derived from n = 4 NMS-P715
615 treated samples), individual chromosomes are in columns, and chromosome rearrangements are shown
616 in color: red = amplification, blue = hemizygous deletion, purple = unknown material/rearrangement.
617 Detailed information is included in Table 1.

618

619 **Figure 2. Evolution of aneuploidy is a stepwise process shaped by clonal competition. (A)**

620 Experimental scheme of HSPC evolution to aneuploidy following 24-hour NMS-P715 MPSi treatment. A
621 portion of the culture was cryopreserved, thawed, and compared to the primary culture. **(B)** Clonal
622 dynamics fish plot of a *TP53*^{mut}del5q evolution experiment, with primary experiment (top plot) and repeat
623 experiment from cryopreserved sample (bottom plot). The proportion of each clone over time is shown
624 as determined by manual karyotyping at time points indicated by hashed lines. Individual clones are
625 shown in colors with inferred lineage indicated. **(C)** Mean relative fitness (s + 1) of each clone in the
626 primary vs. cryopreserved cultures in (B); mean and 1-99th percentile of 9000 replicates. Dotted line
627 indicates neutral fitness. **(D)** Experimental scheme of the parallel culture experiment, in which *TP53*^{mut} or
628 *TP53*^{mut}del5q HSPCs were split into 4 separate cultures (A,B,C,D) immediately after 24-hour NMS-P715
629 MPSi treatment, cultured in parallel, and analyzed by karyotyping at time points indicated by hashed
630 lines. **(E)** Clonal dynamics fish plots of the parallel A,B,C,D cultures showing the proportion of each clone
631 determined by manual karyotyping. Clones are shown in colors with inferred lineage indicated. **(F)** Mean
632 relative fitness (s + 1) for each clone in parallel cultures in (D). Neutral fitness (1+s = 1) is shown with
633 dotted line. Each point is a mean of 9000 replicates for each culture, bars indicate mean of means ± S.D.

634

635 **Figure 3. Single cell analysis identifies gene expression signature of aneuploidy. (A)** Experimental
 636 scheme of time course experiment. *TP53^{mut}* and *TP53^{mut}del5q* HSPCs were evolved and sequentially
 637 sampled by single cell RNAseq at time points indicated, and clonal makeup defined by CNI. **(B)** Clonal
 638 dynamic fish plot of the *TP53^{mut}del5q* culture corresponding to Figure 2B determined by single cell CNI
 639 using the Numbat package at time points indicated by hashed lines. **(C)** Copy number abnormalities in
 640 single cells identified using Numbat; data combined from all timepoints in *TP53^{mut}* and *TP53^{mut}del5q* lines.
 641 CNAs are shown in color: red = amplification, blue = deletion, green = copy number-neutral loss of
 642 heterozygosity. Clonal identity based on probability that the cell belongs to each inferred clone is shown
 643 as color bar on the left: gray = *TP53^{mut}* only, green = *TP53^{mut}del5q* , blue = *TP53^{mut}del5q+10q*. **(D)**
 644 Normalized gene expression level in single cell clones identified by Numbat; log scale, black dot indicates
 645 mean. **(E)** Top gene ontology categories in Metascape for genes upregulated in aneuploid
 646 *TP53^{mut}del5q+10q* compared to parental *TP53^{mut}del5q* HSPCs. Upregulated genes were defined by
 647 Wilcoxon Rank Sum Test, $q < 0.05$. Log (q-value) of each term is displayed ($q < 0.05$).

648

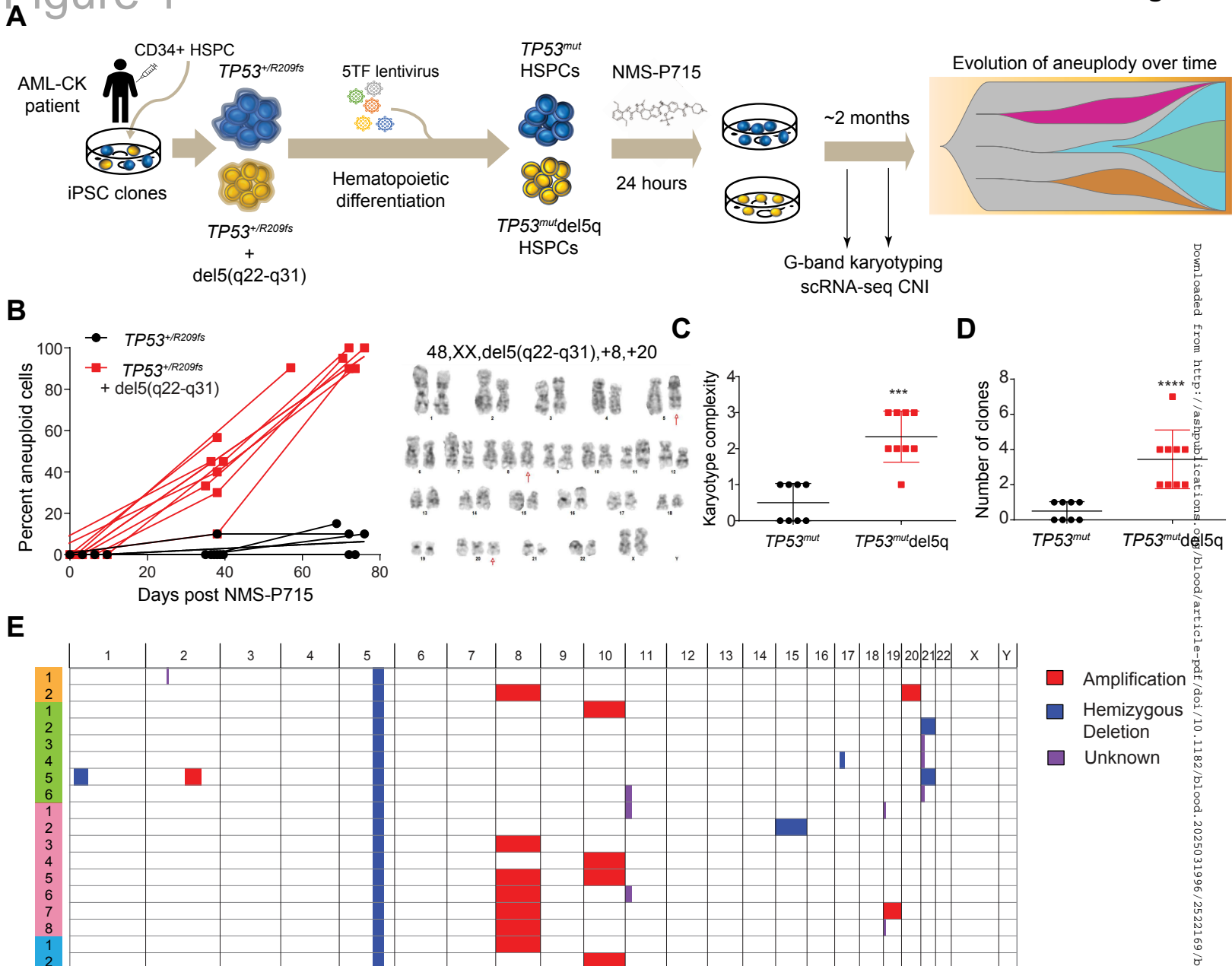
649 **Figure 4. Aneuploidy signature is shared with AML-CK patients. (A)** Experimental scheme of single
 650 cell transcriptomic analysis comparing *TP53^{mut}del5q* iPSC-HSPCs and patients with AML-CK sequenced
 651 in the study, as well as Leppä et al¹⁴, Wang et al⁴², and Petti et al^{43,44} published datasets. **(B)** Copy number
 652 abnormalities identified in single cells of AML-CK patient RO20518. Red = amplification, blue = deletion,
 653 green = copy number-neutral loss of heterozygosity. Clonal identity based on probability that the cell
 654 belongs to each inferred clone is shown as color bar on the left: grey = WT, orange = del(5q)+del(15p),
 655 green/blue = CK clones #1-2. **(C)** Normalized expression of the i-CK signature genes in single cell clones
 656 of AML-CK patients RO20518 and R332; log scale, black dot indicates median; statistical testing: R332
 657 all CK clones enriched for i-CK ($p < 0.0001$), RO20518 del5q ($p < 0.0001$) (Holm Adjusted Wilcox Rank
 658 Sum test). **(D)** Normalized gene expression level in single cell clones (WT, del5q, CK1-2) in AML-CK
 659 patient RO20518; log scale, black dot indicates median. **(E)** Normalized gene expression level in single
 660 cell clones (WT, CK1-4) in AML-CK patient R332; log scale, black dot indicates mean. **(F)** Normalized
 661 expression of the i-CK signature genes in single cell aneuploid (CK) or euploid (WT) clones of AML-CK
 662 patients from Wang et al⁴²; black dot indicates mean; statistical testing: PT6A, PT10A, PT16A, PT27A,
 663 PT29A all CK clones enriched for i-CK, $p < 0.0001$, Holm Adjusted Wilcox Rank Sum test. **(G)** Normalized
 664 gene expression level of *PTEN* and *SMC3* in single cell euploid (WT) or aneuploid (CK) clones
 665 in AML-CK patients from Wang et al; log scale.

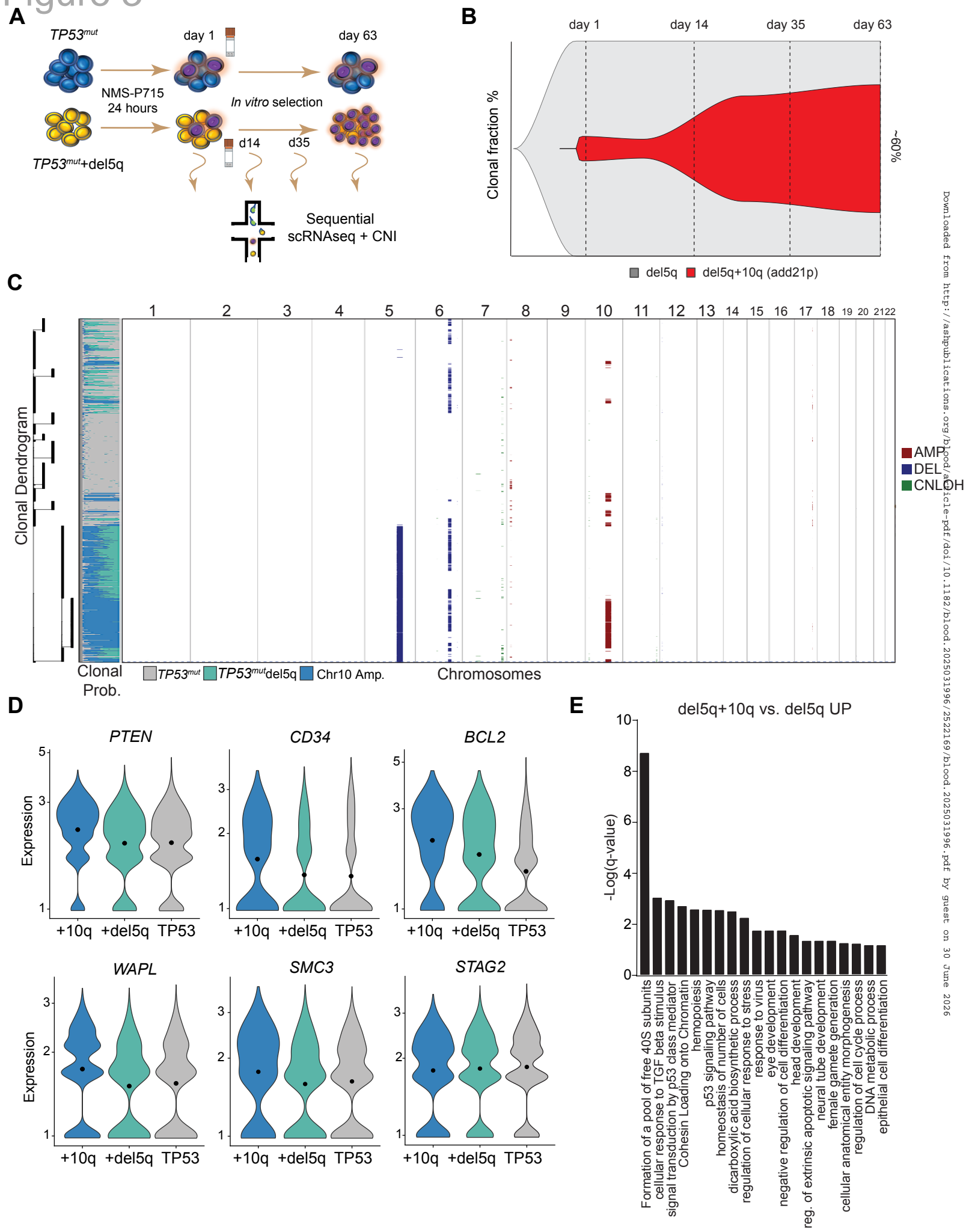
666

667 **Figure 5. BCL2 is a targetable molecular dependency of aneuploid clones. (A)** Normalized gene
 668 expression level in single cell clones identified by Numbat from iPSC HSPCs; log scale, black dot
 669 indicates mean. **(B)** Relative cell viability of parental *TP53^{mut}* and *TP53^{mut}del5q* HSPCs treated with
 670 venetoclax in dose response format, for 7 days. Concentration plotted as log₁₀ nM. **(C)** Experimental

671 scheme of venetoclax (VEN) treatment, in which HSPCs harboring the *BCL2*-high del5q+10q subclone
672 (Figure 2B) are evolved with 250 nM VEN or DMSO vehicle for ~48 days and sampled periodically for
673 karyotype. **(D)** Clonal dynamics fish plots of the *TP53^{mut}*del5q cultures evolved in the presence of DMSO
674 vehicle (left) or VEN (right) (day 14-62) showing the proportion of each clone determined by manual
675 karyotyping. Clones are shown in colors with inferred lineage indicated. **(E)** Relative cell viability of
676 *TP53^{mut}*del5q-derived cultures evolved with VEN or DMSO and parental lines treated with VEN in dose
677 response format, for 7 days. Concentration plotted as log₁₀ nM. **(F)** Copy number abnormalities identified
678 by Numbat in single cells of pooled vehicle and venetoclax-treated samples. Red = amplification, blue =
679 deletion, green = copy number-neutral loss of heterozygosity. Clonal identity based on probability that
680 the cell belongs to each inferred clone is shown as color bar on the left: grey/orange = del5q parental,
681 green = add21p/+10q, and blue = del(1p). **(G)** Percent clonal contribution for each culture as determined
682 by Numbat single cell CNI; add21p = [del(5q);add(21p)/+10q], del1p = [del(5q);del(1p);dup(2);-21].
683

684 **Figure 6. Venetoclax-resistant aneuploid HSPCs undergo a lineage switch.** **(A)** Expression of *BCL2*
685 family genes in bulk *TP53^{mut}*del5q HSPCs evolved in the presence of vehicle or venetoclax compared to
686 parental lines. Expression measured relative to *GAPDH* by qPCR and normalized to *TP53^{mut}* HSPCs. **(B)**
687 Violin plots of expression of *BCL2* family genes in single cells with clonal identity determined by CNI.
688 Color indicates treatment condition (vehicle or venetoclax), log scale, black dot indicates mean. **(C)**
689 Lineage composition identified by SingleR for add(21p) and del(1p) clones determined by CNI. Cell types
690 with < 5 cells in both conditions were excluded. **(D)** Top gene ontology categories in Metascape analysis
691 on 284 upregulated (LFC > 0.5, Wilcoxon Rank Sum Test q value > 0.05) genes in the del(1p) clone vs
692 the parental *TP53^{mut}*del5q clone. Log (q-value) of each term is displayed (q < 0.05). **(E)** Violin plots of
693 expression of *STAT* family genes differentially expressed between del(1p) and *TP53^{mut}*del5q parental
694 HSPCs, colored by clone, log scale, black dot indicates mean. **(F)** Normalized relative mean cell viability
695 of aneuploid cultures treated with the JAK2 inhibitor ruxolitinib. *TP53^{mut}*del5q HSPCs evolved in the
696 presence of vehicle or venetoclax and parental lines were treated with ruxolitinib in dose response
697 format for 5 days. n=2, concentration plotted as log₁₀ nM. **(G)** Cell growth of HSPCs from the add(21p)/del(1p)
698 culture (Figure 5C) treated sequentially with venetoclax and JAK2 inhibitor ruxolitinib. HSPCs were
699 treated with 250 nM venetoclax (VEN) or DMSO vehicle (Veh) between day 14 to 35, and subsequently
700 treated with 50 nM ruxolitinib (RUX) or vehicle between day 35 to 49. Left: cell growth of vehicle- or VEN-
701 treated cultures as fold expansion relative to day 14. Right: cell growth of vehicle- or RUX-treated cultures
702 as fold expansion relative to day 35. **(H)** Clonal expansion of venetoclax-untreated cultures treated with
703 vehicle or RUX. Clonal composition was determined by karyotyping, with fold expansion relative to day
704 35. **(I)** Clonal expansion of venetoclax-treated cultures subsequently treated with vehicle or RUX. Clonal
705 composition was determined by karyotyping, with fold expansion relative to day 35.





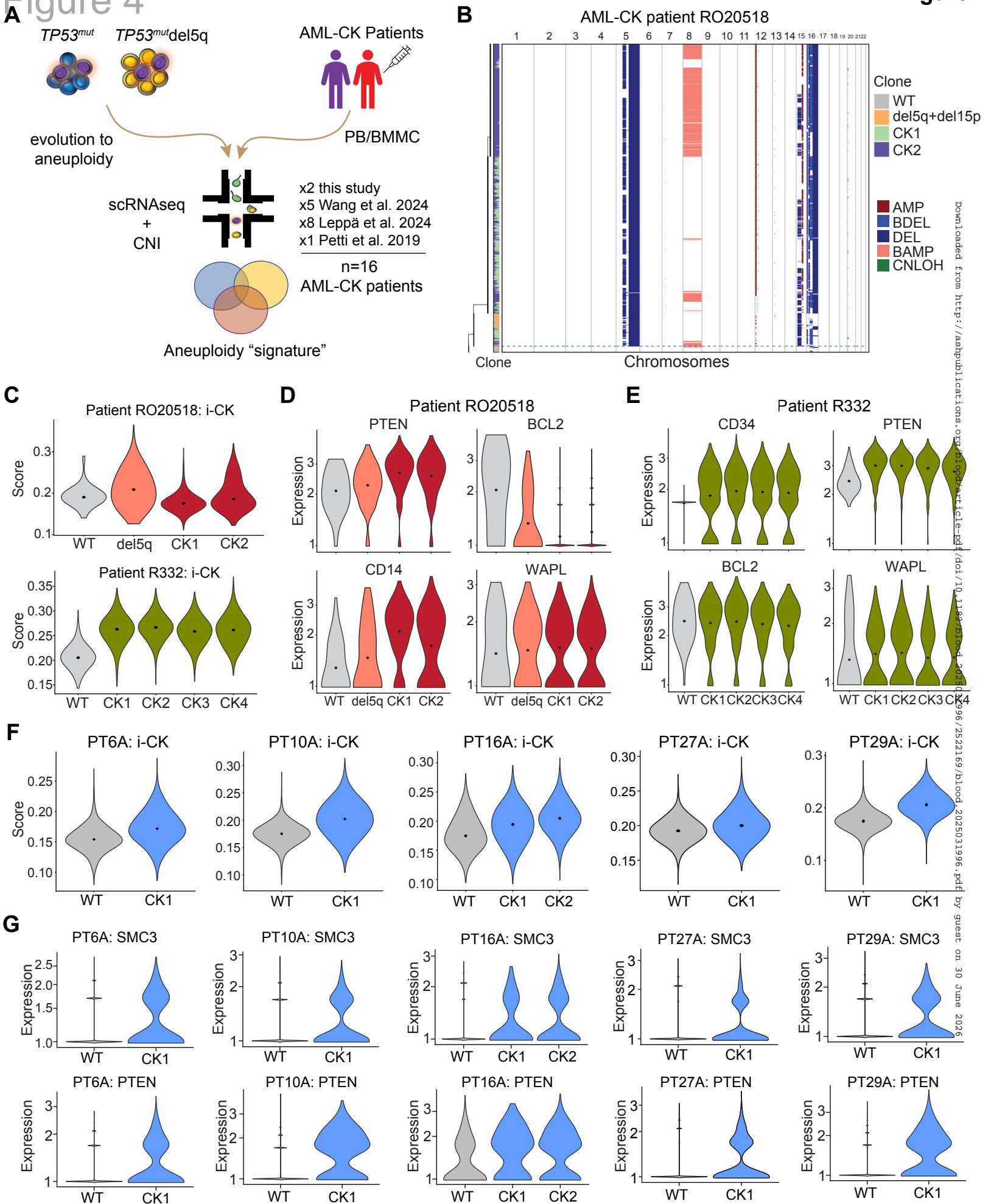


Figure 5

Figure 5

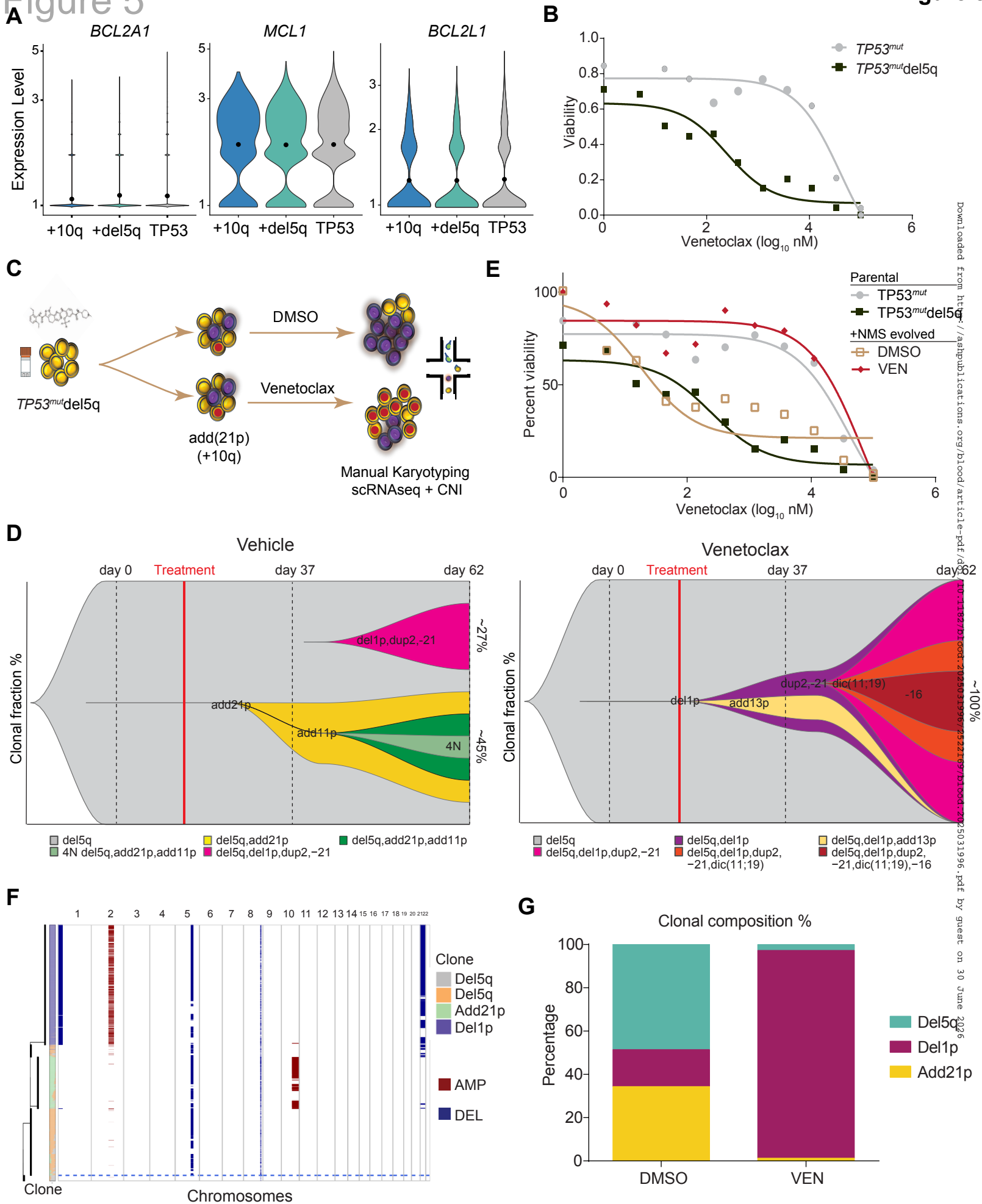
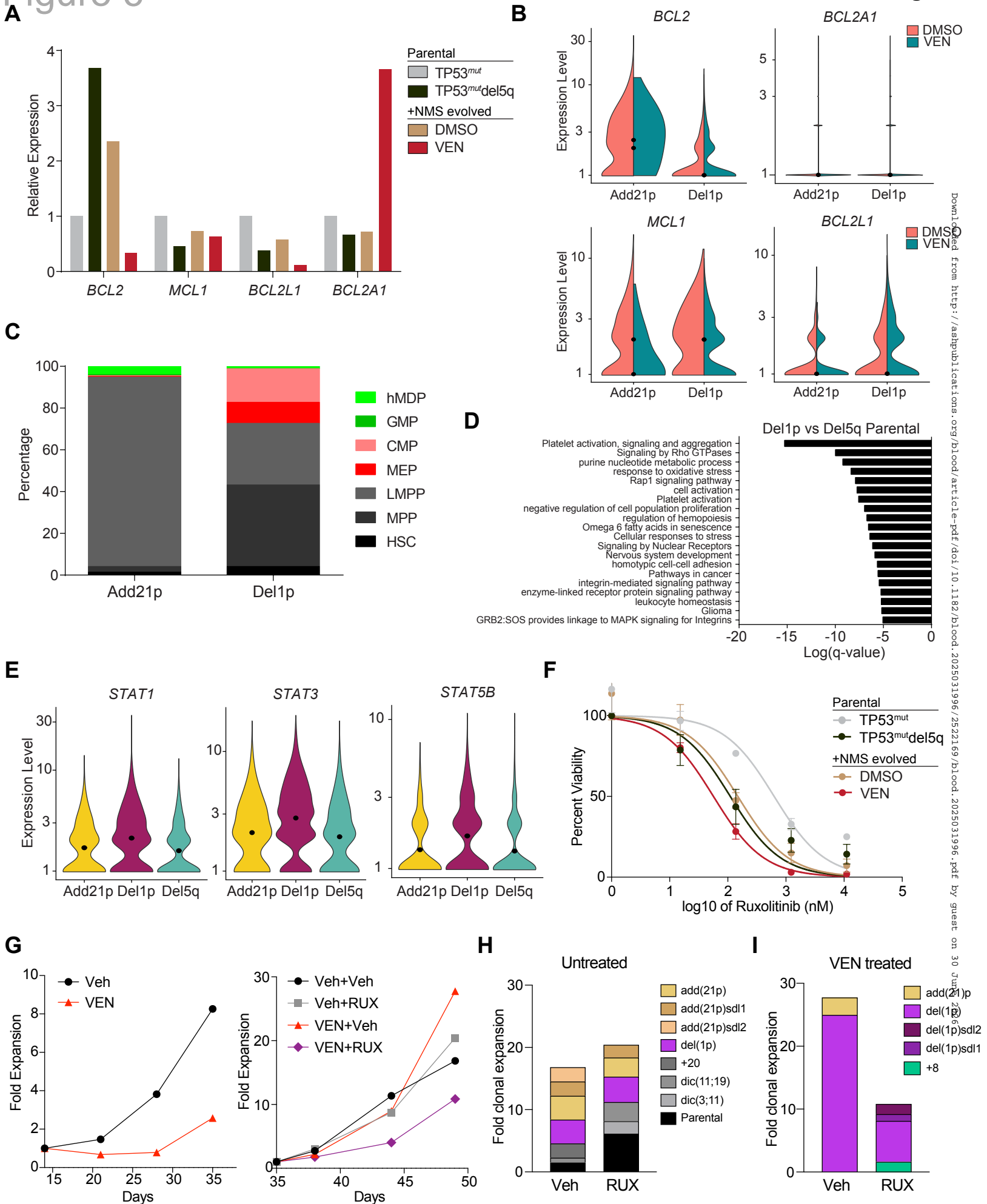


Figure 6

Figure 6



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