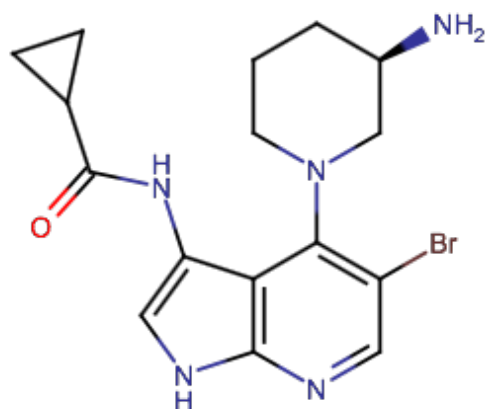


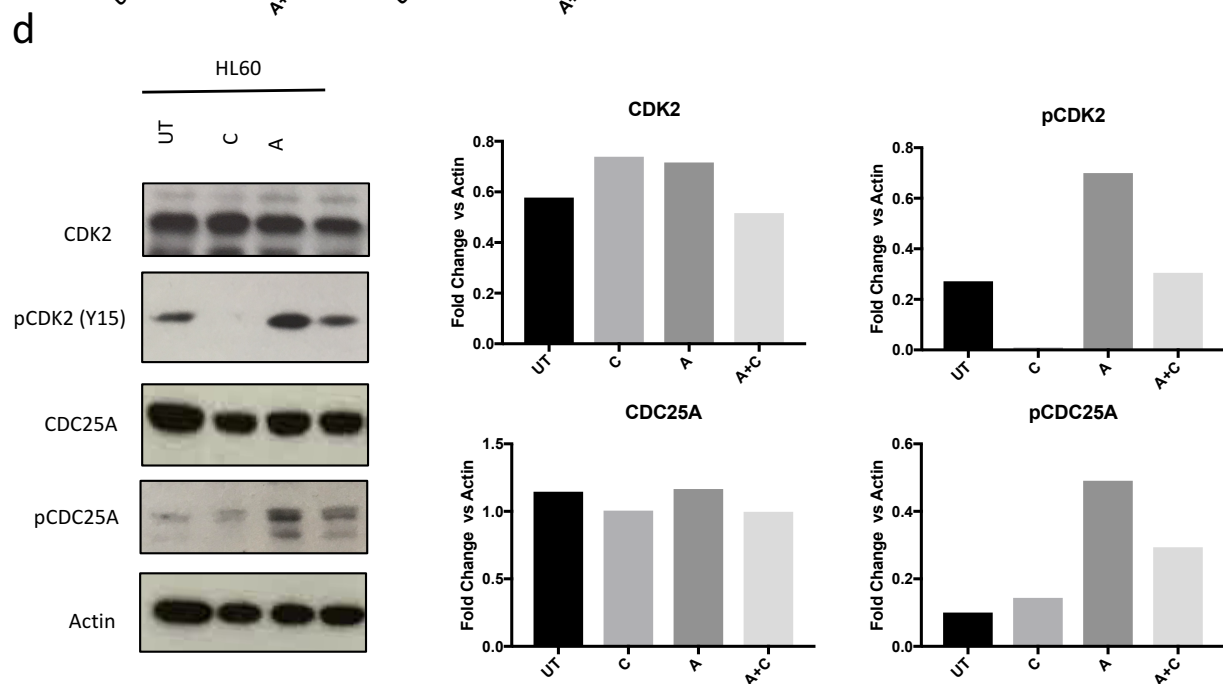
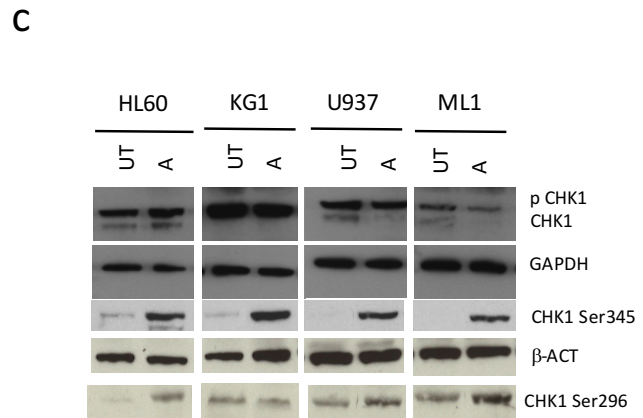
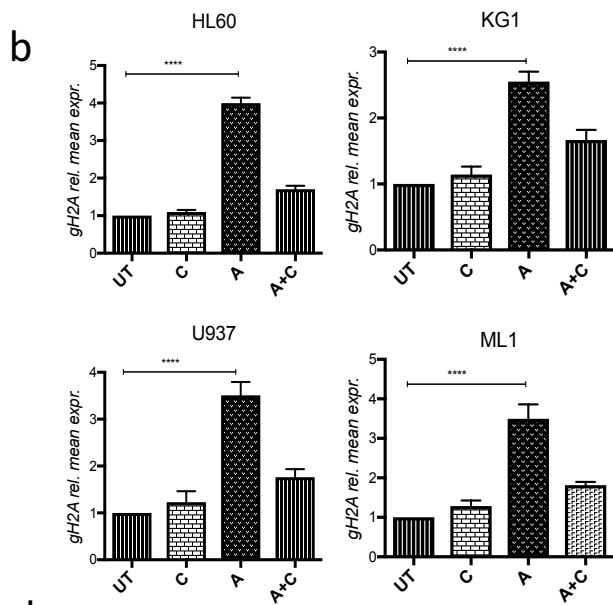
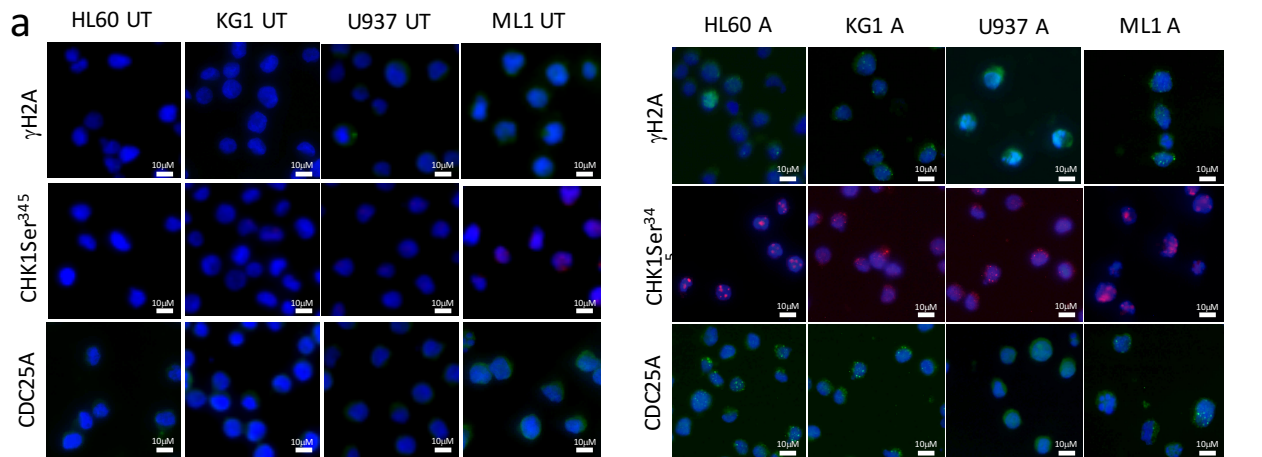


**Supplementary Figure 1. Chemical structure of GDC-0575**

Formula: C<sub>16</sub>H<sub>20</sub>BrN<sub>5</sub>O

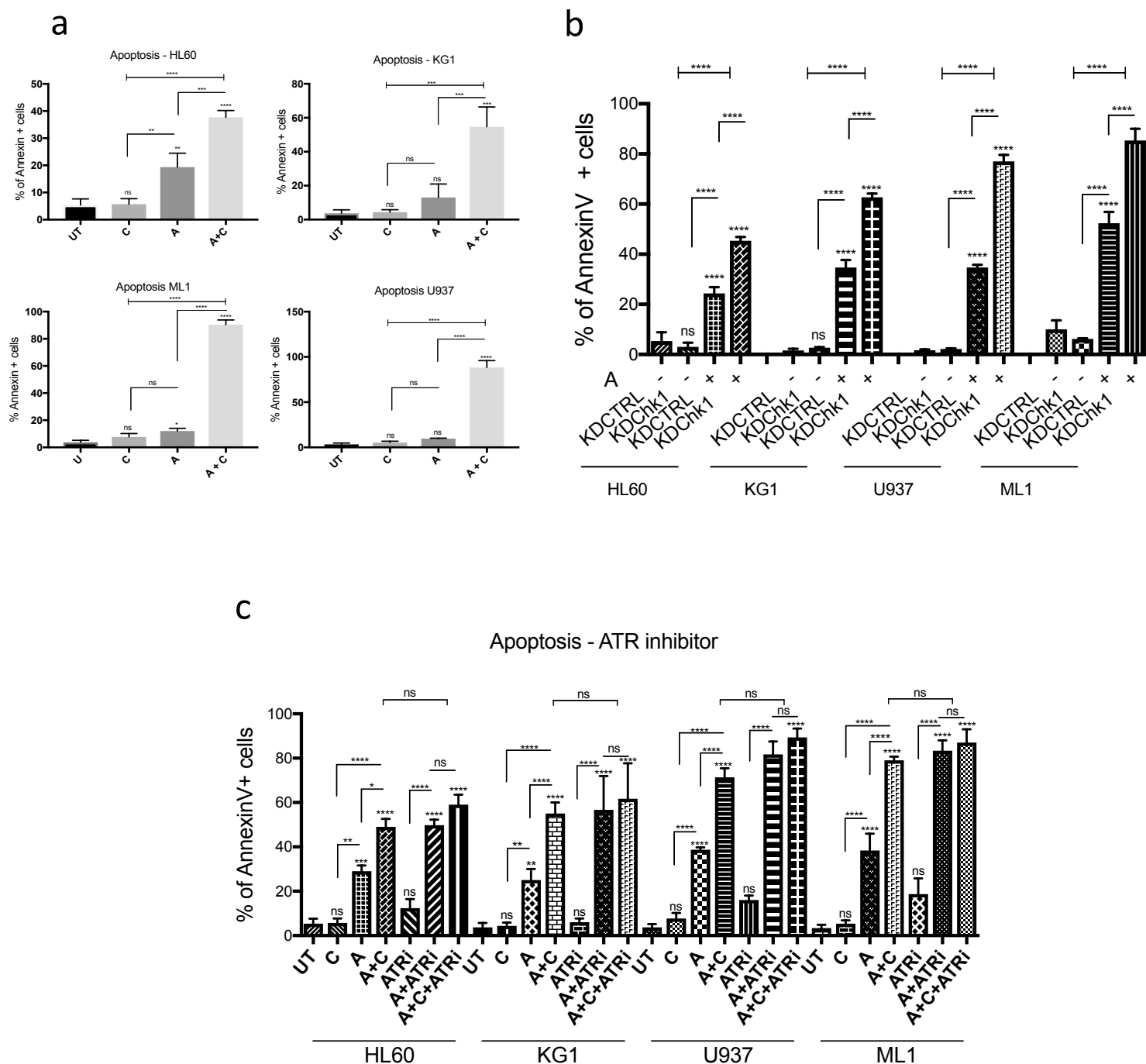
Molecular Weight: 378.27

(*R*)-*N*-(4-(3-aminopiperidin-1-yl)-5-bromo-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)  
cyclopropanecarboxamide

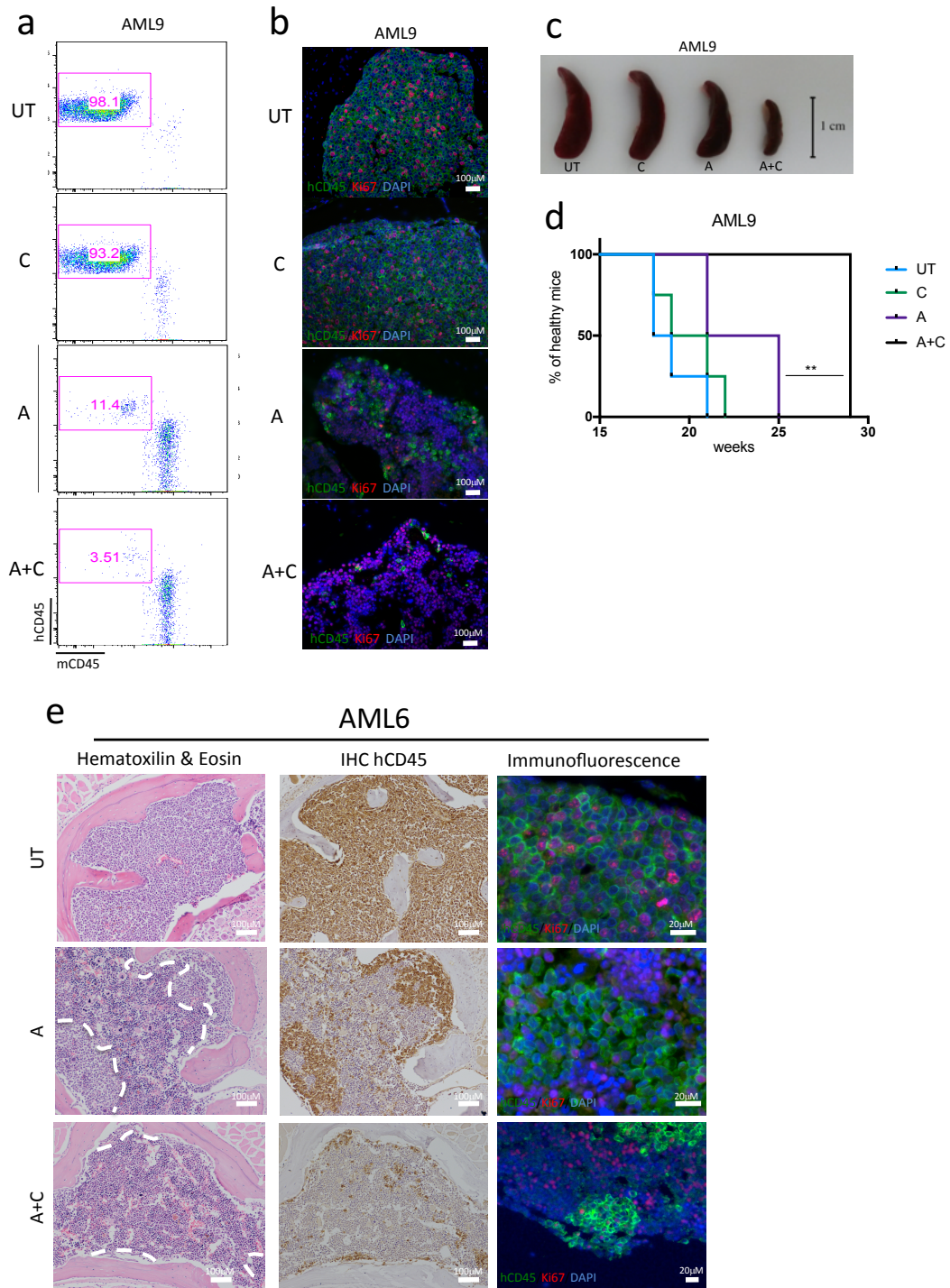




**Supplementary Figure 2. Ara.C induced DNA damag and activation of Chk.1.** **a)** Immunofluorescence on AML cell lines untreated (left panel) or treated for 24h with AraC (right panel) showing activation of DNA damage-associated markers. **b)** Facs analysis showing the percentage of  $\gamma$ H2A on the same AML cell lines as in a and under the described treatment conditions. **c)** Western blot showing both the global phosphorylation of Chk1 and its specific phosphorylation on the Ser345 and auto-phosphorylation Ser296 in AML cell lines upon 1d AraC treatment.



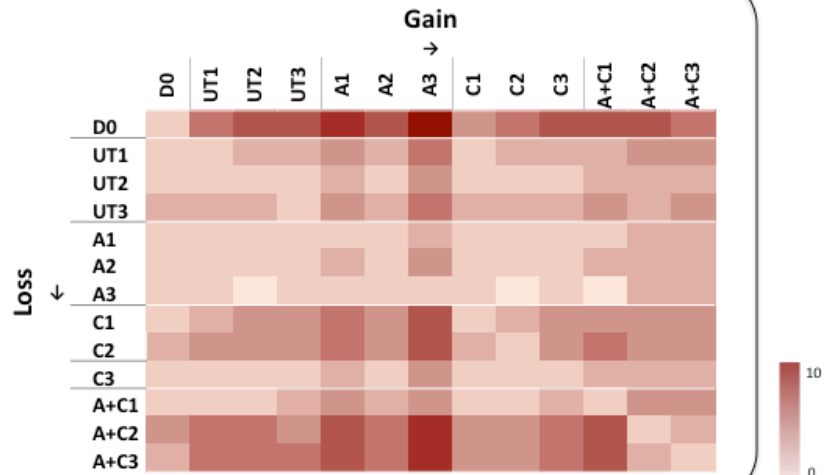
**Supplementary Figure 3. CHK1inhibition via Chk.1 inh or KD of chk.1 or use of ATR inh enhance the cytotoxicity of AraC in different AML cell lines *in vitro*** **a)** Percentage of apoptosis of four AML cell lines after 24hr of low dose Ara.C (100nM)  $\pm$  Chk.1 inh; untreated (UT). UT n=3; Chk.1 (C) n=3, Ara.C (A) n=3 and A+C n=3. **b)** Percentage of apoptosis in four AML cell lines transfected with KDCTRL and KDChk1 after one-day treatment with AraC (A). n=3 for all samples. **c)** Percentage of apoptosis in four AML cell lines after 2 day treatment with A, AraC+CHK1i (A+C), ATR inhibitor (ATRi), A+ATRi, A+C+ATRi. n=3 for all samples.



**Supplemental Figure 4. CHK1i enhances the cytotoxicity of AraC in different primary AML *in vivo*.** **a)** Percentage of human cells in the bone marrow of AML9-transplanted mice one week post-treatment. **b)** Immunofluorescence for Ki67 proliferation on mice's spine for the same patient and treatment conditions as in a. Scale bar is 100mM. **c)** Comparison of mouse spleens size for the same patient and treatment conditions as in a. **d)** Sickness curves monitoring AML9-injected mouse under different treatment conditions at various time points. (UT n=4; C n=4; A n=4; A+C n=4. Stat using Gahan-Breslow-Wilcoxon) **e)** Remaining cells in the bone marrow of AML6-transplanted mice one week post-treatment shown by H&E (left panel), CD45 IHC (middle panel) and IF (right panel; green cells are human CD45<sup>+</sup>, red cells are Ki67<sup>+</sup> and blue cells are the nuclei). Scale bar is indicated in the picture.

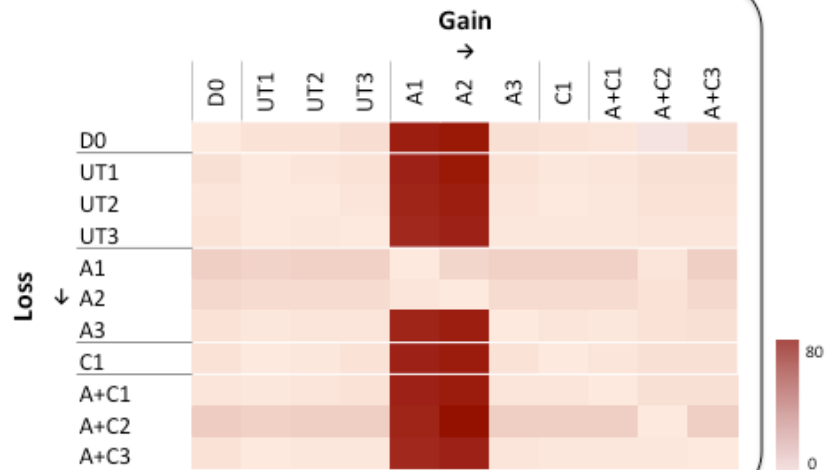
AML 5

|      | Total SNVs |
|------|------------|
| D0   | 26         |
| UT1  | 29         |
| UT2  | 30         |
| UT3  | 29         |
| A1   | 31         |
| A2   | 30         |
| A3   | 32         |
| C1   | 38         |
| C2   | 38         |
| C3   | 30         |
| A+C1 | 30         |
| A+C2 | 28         |
| A+C3 | 28         |



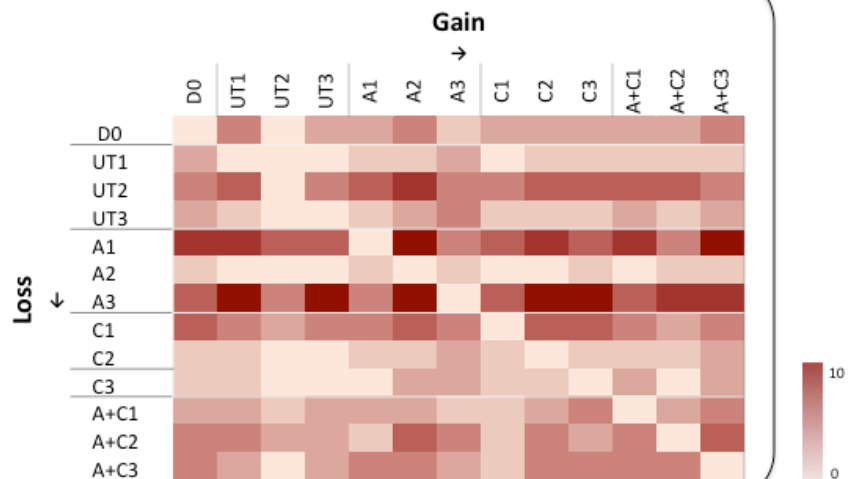
AML 6

|      | Total SNVs |
|------|------------|
| D0   | 40         |
| UT1  | 39         |
| UT2  | 41         |
| UT3  | 42         |
| A1   | 99         |
| A2   | 105        |
| A3   | 41         |
| C1   | 40         |
| A+C1 | 40         |
| A+C2 | 33         |
| A+C3 | 43         |

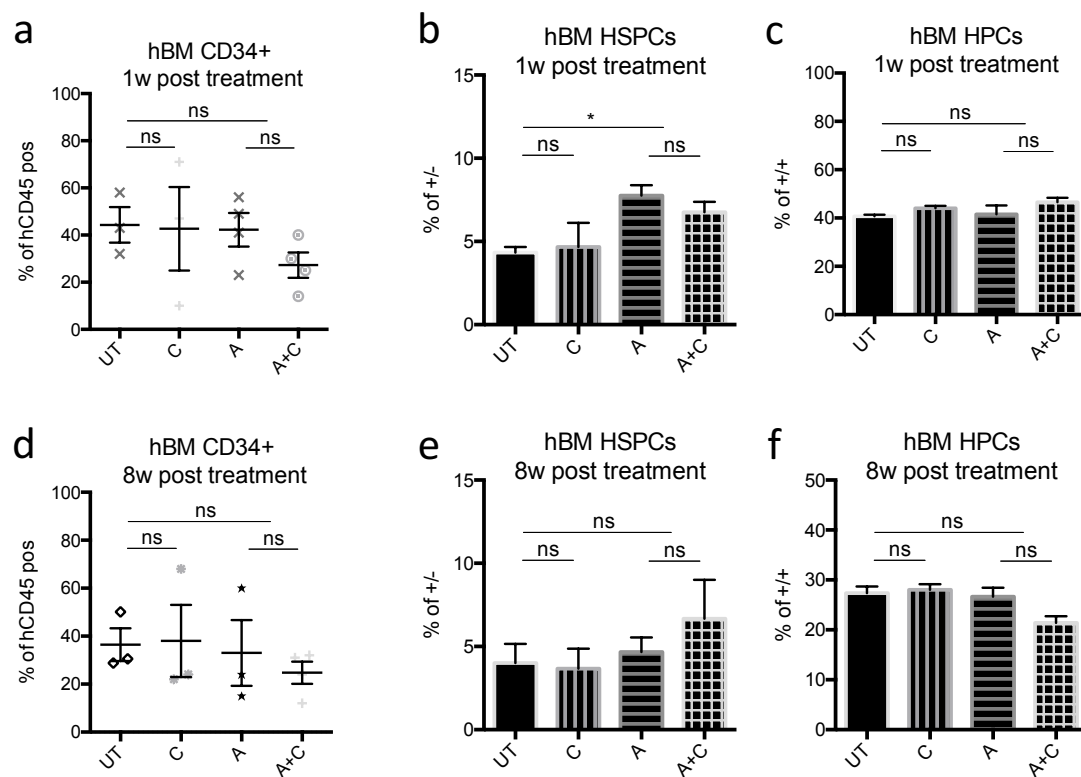


AML 7

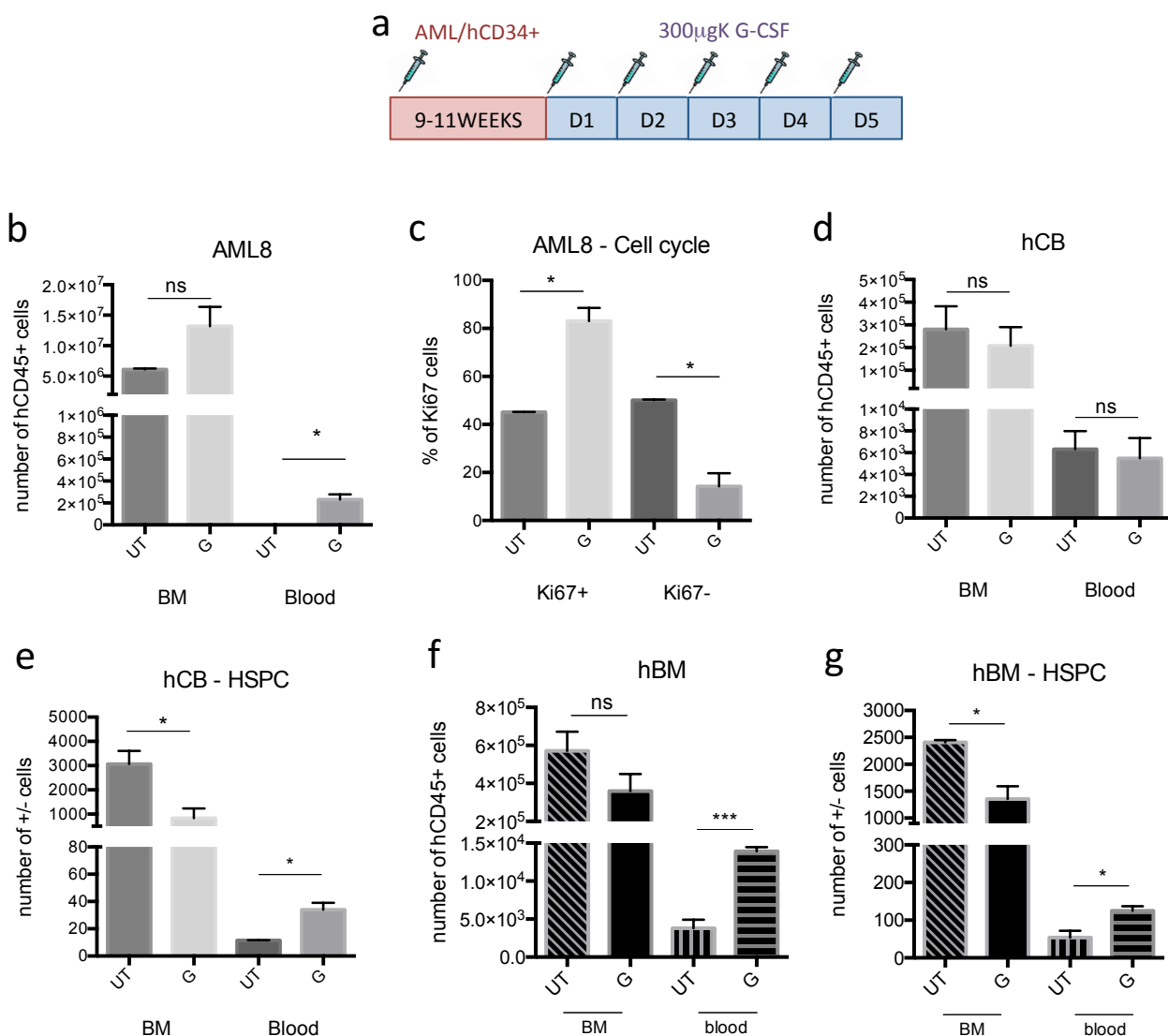
|      | Total SNVs |
|------|------------|
| D0   | 32         |
| UT1  | 33         |
| UT2  | 29         |
| UT3  | 32         |
| A1   | 29         |
| A2   | 34         |
| A3   | 29         |
| C1   | 30         |
| C2   | 33         |
| C3   | 33         |
| A+C1 | 32         |
| A+C2 | 31         |
| A+C3 | 32         |



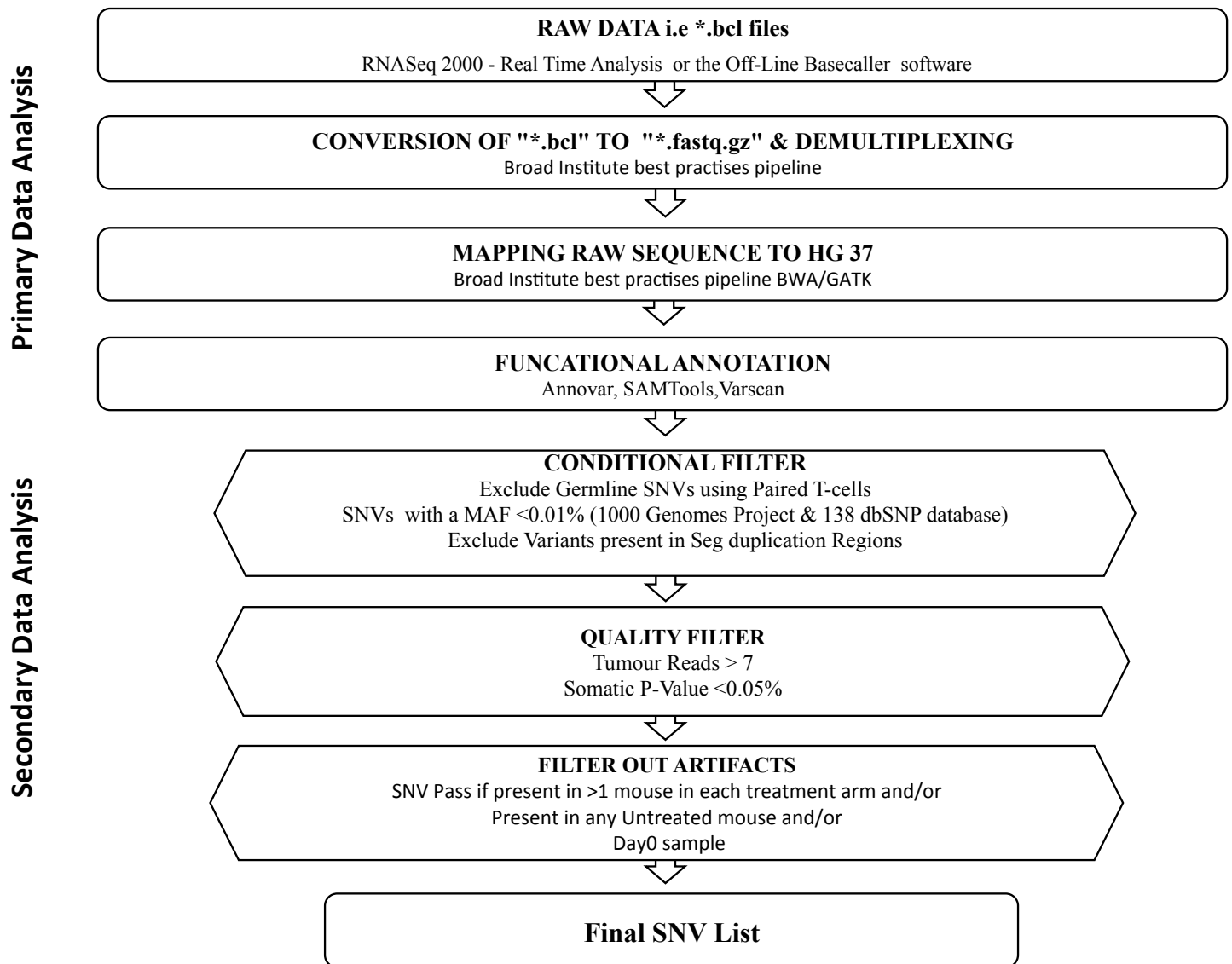
**Supplemental Figure 5. CHK1i plus AraC treatment does not generate new mutations.** Table (left) for each patient shows the total number of mutations (SNVs) detected in each condition. Heatmap (right) for each patient shows the changes in the mutation spectrum observed in each condition. Mutations present at D0 sample were compared to UT, A, C and A+C-mice.



**Supplementary Figure 6. CHK1i plus AraC treatment does not affect human hematopoiesis derived from bone marrow *in vivo*.** **a)** Percentage of human cells in the BM aspirates of hBM-transplanted mice one week post-treatment. Each dot represents an individual mouse. UT n=3; C n=3; A n=4; A+C n=4. ns= not significant. Total number of mice= 14. **b)** Percentage of HSPCs (Lin<sup>-</sup>CD34<sup>+</sup>CD38<sup>-</sup>) in the BM aspirates of hNM-transplanted mice one week post-treatment. The analysis was performed on the same mice as in a. **c)** Same as in b, but percentage of HPCs (Lin<sup>-</sup>CD34<sup>+</sup>CD38<sup>+</sup>). **d)** Same as in a but 8 weeks post-treatment when mice were sacrificed for the analysis. **e)** Same as in B but 8 weeks post-treatment. **f)** Same as in c but 8 weeks post-treatment.



**Supplementary Figure 7. G-CSF mobilizes both AML and normal hematopoietic cells *in vivo*.** **a)** Treatment regimen for G-CSF with AML or hCD34<sup>+</sup> cells, *in vivo*. **b)** Absolute number of hCD45<sup>+</sup> cells in AML8-transplanted mice after the treatment in BM and blood. UT n=3; G n=3. ns= not significant. **c)** Percentage of Ki67 positive and negative cells in the BM of AML8-transplanted mice after the treatment. UT n=3; G n=3. ns= not significant. **d)** Same as in b but mice were transplanted with hCB CD34<sup>+</sup> cells. UT n=4; G n=4. ns= not significant. **e)** Same as in d but looking at HSPC cells. **f)** Same as in b but mice were transplanted with hBM CD34<sup>+</sup> cells. UT n=4; G n=4. ns= not significant. **g)** Same as in f but looking at HSPC cells.



**Supplementary Figure 8.** Sequencing data analysis pipeline used for whole-exome sequencing data analysis. SNV-Single Nucleotide Variants

**Supplementary Table 1.** GDC-0575 Kinase Selectivity Profile (KinomeScan® at 100 nM)

| % Residual Activity | n=460   | Kinases Inhibited                                            |
|---------------------|---------|--------------------------------------------------------------|
| < 1 %               | n = 1   | ChK1                                                         |
| 5 - 20 %            | n = 3   | SLK, YSK4, MYLK                                              |
| 20 - 50%            | n = 9   | FLT3, GSG2, DAPK3, DMPK, MELK, CSNK2A2, JAK2, RPS6KA4, MARK3 |
| 50 -100%            | n = 447 | All others, including ChK2                                   |

ChK1 enzyme IC<sub>50</sub> = 1.2 nM

\*Additional IC<sub>50</sub> determinations confirmed greater than 30-fold selectivity in potency for ChK1 inhibition over the off-target kinases (See Table Supplementary S2).



**Supplementary Table 2.** Description of AML patient samples features.

| AML ID | FEATURE       | CYTOGENETICS | CYTO RISK    | NPM    | FLT3 | EXPERIMENT              |
|--------|---------------|--------------|--------------|--------|------|-------------------------|
| AML1   | Non Engrafter | Inv(16)      | good         | n.a.   | n.a. | <i>in vitro</i>         |
| AML2   | Non Engrafter | t(9,6)       | intermediate | n.a.   | n.a. | <i>in vitro</i>         |
| AML3   | Engrafter     | normal       | intermediate | wt     | ITD  | <i>in vitro</i>         |
| AML4   | Engrafter     | t(9,11)      | intermediate | n.a.   | n.a. | <i>in vitro</i>         |
| AML5   | Engrafter     | complex      | poor         | wt     | n.a. | <i>in vitro/in vivo</i> |
| AML6   | Engrafter     | normal       | intermediate | mutant | ITD  | <i>in vitro/in vivo</i> |
| AML7   | Engrafter     | normal       | intermediate | mutant | ITD  | <i>in vivo</i>          |
| AML8   | Engrafter     | Del 7q+8+1   | poor         | wt     | wt   | <i>in vivo</i>          |
| AML9   | Engrafter     | normal       | intermediate | mutant | ITD  | <i>in vivo</i>          |

| Expt no | Gene      | Ensembl_ID      | Chr_position    | Ref>Var | Amino Acid Change | Read 1 | Read 2 | Tumour Variant Frequency | Somatic P Value | cosmic70       | SNP138_1000G       |
|---------|-----------|-----------------|-----------------|---------|-------------------|--------|--------|--------------------------|-----------------|----------------|--------------------|
| AML5    | LDLRAD3   | ENSG00000179241 | chr11_36248720  | C>T     | S131S             | 62     | 33     | 34.74%                   | 1.27E-14        | ID=COSM237653  | rs142823958_0.0004 |
| AML5    | STMN2     | ENSG00000104435 | chr8_80577757   | G>A     | UTR3              | 135    | 104    | 43.51%                   | 6.79E-35        |                |                    |
| AML5    | FUZ       | ENSG0000010361  | chr19_50314725  | C>T     | splicing          | 58     | 73     | 55.73%                   | 2.73E-22        |                |                    |
| AML5    | STK32B    | ENSG00000152953 | chr4_5128847    | A>G     | intronic          | 206    | 127    | 38.14%                   | 1.77E-43        |                |                    |
| AML5    | WDR17     | ENSG00000150627 | chr4_177067106  | A>G     | intronic          | 28     | 26     | 48.15%                   | 5.37E-07        |                |                    |
| AML5    | COL27A1   | ENSG00000196739 | chr9_116945255  | C>T     | intronic          | 20     | 8      | 28.57%                   | 5.41E-04        |                |                    |
| AML5    | DSG2      | ENSG00000046604 | chr18_29099714  | T>C     | intronic          | 9      | 21     | 70%                      | 1.37E-09        |                |                    |
| AML5    | SOGA1     | ENSG00000149639 | chr20_35425414  | G>C     | intronic          | 96     | 70     | 42.17%                   | 4.33E-26        |                |                    |
| AML5    | RIMKLA    | ENSG00000177181 | chr1_42880383   | A>T     | N305I             | 236    | 60     | 20.27%                   | 6.68E-15        |                |                    |
| AML5    | TTL7      | ENSG00000137941 | chr1_84376926   | A>C     | Y570D             | 31     | 44     | 58.67%                   | 3.88E-16        |                |                    |
| AML5    | NMI       | ENSG00000123609 | chr2_152132156  | A>G     | I159T             | 41     | 31     | 43.06%                   | 9.55E-11        |                |                    |
| AML5    | FSIP2     | ENSG00000188738 | chr2_186654687  | C>T     | L1031F            | 73     | 38     | 34.23%                   | 1.35E-17        |                |                    |
| AML5    | KPNA4     | ENSG00000186432 | chr3_160283015  | C>G     | G19A              | 104    | 50     | 32.47%                   | 1.64E-16        |                |                    |
| AML5    | VCAN      | ENSG00000038427 | chr5_82785951   | C>T     | S35S              | 27     | 63     | 70%                      | 1.07E-41        |                |                    |
| AML5    | STK31     | ENSG00000196335 | chr7_23825098   | A>G     | N694S             | 168    | 108    | 39.13%                   | 6.41E-42        |                |                    |
| AML5    | TRPA1     | ENSG00000104321 | chr8_72958796   | T>A     | K671N             | 18     | 12     | 40%                      | 1.09E-04        |                |                    |
| AML5    | NAA40     | ENSG00000110583 | chr11_63713403  | A>G     | N12S              | 43     | 38     | 46.91%                   | 3.03E-13        |                |                    |
| AML5    | HYKK      | ENSG00000188266 | chr15_78825996  | C>G     | S369C             | 71     | 55     | 43.65%                   | 8.81E-24        |                |                    |
| AML5    | XPO6      | ENSG00000169180 | chr16_28167651  | C>G     | G281R             | 142    | 91     | 39.06%                   | 2.22E-35        |                |                    |
| AML5    | CNTNAP4   | ENSG00000152910 | chr16_76486419  | T>G     | F289L             | 77     | 62     | 44.60%                   | 9.68E-26        |                |                    |
| AML5    | PLIN5     | ENSG00000214456 | chr19_4525739   | C>T     | R209H             | 135    | 98     | 42.06%                   | 1.66E-40        |                |                    |
| AML5    | ZNFT71    | ENSG00000197951 | chr19_57133272  | G>A     | R206H             | 237    | 95     | 28.61%                   | 6.65E-26        |                |                    |
| AML5    | FBLN1     | ENSG00000077942 | chr22_45944540  | C>T     | R497C             | 35     | 36     | 50.70%                   | 1.24E-16        |                |                    |
| AML5    | COL20A1   | ENSG00000101203 | chr20_61952223  | C>T     | intronic          | 7      | 5      | 41.67%                   | 0.00555766      |                |                    |
| AML5    | ITIH3     | ENSG00000162267 | chr3_52833569   | T>A     | intronic          | 11     | 9      | 45%                      | 7.93E-04        |                |                    |
| AML5    | TP53      | ENSG00000141510 | chr17_7577538   | C>T     | R116Q             | 20     | 70     | 77.78%                   | 2.70E-33        | ID=COSM99021   | rs11540652         |
| AML7    | QSOX1     | ENSG00000116260 | Chr1_180165613  | C>T     | P562L             | 145    | 142    | 49.48%                   | 5.24E-13        |                |                    |
| AML7    | NLRP3     | ENSG00000162711 | Chr_1247587549  | A>G     | T268T             | 201    | 215    | 51.68%                   | 4.43E-13        |                |                    |
| AML7    | APOB      | ENSG00000084674 | Chr2_21231407   | C>T     | G2778E            | 225    | 213    | 48.63%                   | 1.28E-46        |                |                    |
| AML7    | GALNT5    | ENSG00000136542 | Chr2_158115938  | C>G     | P448P             | 81     | 81     | 50%                      | 2.52E-10        |                |                    |
| AML7    | PLXNB1    | ENSG00000164050 | Chr3_48463201   | G>A     | S512F             | 71     | 61     | 46.21%                   | 2.26E-05        |                |                    |
| AML7    | TRMT44    | ENSG00000155275 | Chr4_8477547    | G>A     | W698X             | 190    | 186    | 49.47%                   | 0.00240843      |                |                    |
| AML7    | KLHL8     | ENSG00000145332 | Chr4_88104494   | T>G     | A88A              | 110    | 100    | 47.62%                   | 1.44E-08        |                |                    |
| AML7    | SPEF2     | ENSG00000152582 | Chr5_35704757   | C>G     | Q834E             | 73     | 59     | 44.70%                   | 2.21E-05        |                |                    |
| AML7    | CREB5     | ENSG00000146592 | Chr7_28610101   | C>T     | S104L             | 266    | 218    | 45.04%                   | 6.03E-20        |                |                    |
| AML7    | KMT2E     | ENSG00000005483 | Chr7_104731706  | G>A     | S556N             | 64     | 45     | 41.28%                   | 6.28E-06        |                |                    |
| AML7    | KIAA1549L | ENSG00000110427 | Chr11_33566484  | C>A     | A685D             | 163    | 149    | 47.76%                   | 1.93E-12        |                | 0.0002             |
| AML7    | ARN12     | ENSG00000172379 | Chr15_80800561  | C>A     | M229I             | 94     | 74     | 44.05%                   | 6.25E-11        |                |                    |
| AML7    | MYADM     | ENSG00000179820 | Chr19_54377434  | G>A     | A217A             | 287    | 300    | 51.11%                   | 3.35E-26        |                |                    |
| AML7    | LAIR1     | ENSG00000167613 | Chr19_54868189  | T>C     | Y147C             | 70     | 75     | 51.72%                   | 4.01E-05        |                |                    |
| AML7    | ZBBX      | ENSG00000169064 | Chr3_166966513  | A>G     | intronic          | 78     | 59     | 43.07%                   | 6.38E-04        |                |                    |
| AML7    | ITGA1     | ENSG00000213949 | Chr5_52204889   | T>C     | intronic          | 80     | 77     | 49.04%                   | 7.93E-05        |                |                    |
| AML7    | GLYT1L3   | ENSG00000203972 | Chr6_49485109   | G>A     | intronic          | 104    | 120    | 53.57%                   | 5.95E-21        |                |                    |
| AML7    | AIMP2     | ENSG00000106305 | Chr7_6054619    | G>A     | intronic          | 12     | 8      | 40%                      | 0.04052952      |                | 0.0004             |
| AML7    | ZC3HC1    | ENSG00000091732 | Chr7_129685254  | T>C     | intronic          | 94     | 100    | 51.55%                   | 2.23E-07        |                |                    |
| AML7    | CNGB3     | ENSG00000170289 | Chr8_87066558   | C>T     | intronic          | 20     | 16     | 44.44%                   | 0.00486144      |                |                    |
| AML7    | VPS13B    | ENSG00000132549 | Chr8_100847343  | A>G     | intronic          | 39     | 36     | 48%                      | 0.00455566      |                |                    |
| AML7    | BTA1F1    | ENSG00000095564 | Chr10_93702171  | T>G     | intronic          | 67     | 18     | 21.18%                   | 0.01817019      |                |                    |
| AML7    | NUP160    | ENSG00000030066 | Chr11_47834373  | C>A     | intronic          | 62     | 50     | 44.64%                   | 1.84E-04        |                |                    |
| AML7    | BRMS1     | ENSG00000174744 | Chr11_66109761  | G>C     | intronic          | 46     | 73     | 61.34%                   | 1.94E-10        |                |                    |
| AML7    | TMEM135   | ENSG00000166575 | Chr11_86782682  | C>T     | intronic          | 32     | 18     | 36%                      | 5.81E-04        |                |                    |
| AML7    | SLC2A14   | ENSG00000173262 | Chr12_7970731   | T>C     | intronic          | 6      | 6      | 50%                      | 0.00686499      |                | 0.001              |
| AML7    | NPAS3     | ENSG00000151322 | Chr14_34249952  | C>A     | intronic          | 133    | 75     | 36.06%                   | 8.36E-10        |                | 0.0002             |
| AML7    | SPAG7     | ENSG00000091640 | Chr17_4863006   | G>T     | intronic          | 101    | 104    | 50.73%                   | 9.70E-17        |                |                    |
| AML7    | CCDC40    | ENSG00000141519 | Chr17_78032910  | A>G     | intronic          | 22     | 20     | 47.62%                   | 0.00662656      |                |                    |
| AML7    | IL1RAPL1  | ENSG00000169306 | ChrX_29935507   | G>A     | intronic          | 25     | 14     | 35.90%                   | 0.04415273      |                |                    |
| AML7    | MAOB      | ENSG00000069535 | ChrX_43697074   | G>A     | intronic          | 20     | 17     | 45.95%                   | 0.00161519      |                | 0.0013             |
| AML7    | NCOR1     | ENSG00000141027 | Chr17_16062188  | C>T     | splicing          | 32     | 39     | 54.93%                   | 0.00151075      |                |                    |
| AML6    | EBF2      | ENSG00000221818 | chr8_25702020   | G>A     | UTR3              | 24     | 28     | 53.85%                   | 8.56E-14        |                |                    |
| AML6    | SLAMF7    | ENSG00000026751 | chr1_160721107  | C>T     | intronic          | 54     | 48     | 47.06%                   | 4.22E-19        |                |                    |
| AML6    | TBC1D1    | ENSG00000065882 | chr4_38037425   | A>G     | intronic          | 16     | 20     | 55.56%                   | 1.87E-07        |                |                    |
| AML6    | SPEF2     | ENSG00000152582 | chr5_35790110   | C>T     | intronic          | 151    | 77     | 33.77%                   | 1.29E-32        |                |                    |
| AML6    | KIF13B    | ENSG00000197892 | chr8_28967641   | C>T     | intronic          | 35     | 34     | 49.28%                   | 7.62E-15        |                |                    |
| AML6    | HSPA12A   | ENSG00000165868 | chr10_118440546 | G>A     | intronic          | 35     | 20     | 36.36%                   | 4.09E-06        |                | 0.0002             |
| AML6    | NOX4      | ENSG00000086991 | chr11_89224224  | G>A     | intronic          | 37     | 33     | 47.14%                   | 3.67E-13        |                |                    |
| AML6    | FREM2     | ENSG00000150893 | chr13_39451239  | T>C     | intronic          | 76     | 97     | 56.07%                   | 3.67E-44        |                |                    |
| AML6    | LDLRAD4   | ENSG00000168675 | chr18_13611030  | G>A     | intronic          | 21     | 20     | 48.78%                   | 5.02E-10        |                |                    |
| AML6    | SYMPK     | ENSG00000125755 | chr19_46366108  | G>A     | intronic          | 27     | 29     | 51.79%                   | 1.30E-12        |                |                    |
| AML6    | AP2A1     | ENSG00000196961 | chr19_50306467  | G>A     | intronic          | 73     | 55     | 42.97%                   | 2.85E-20        |                |                    |
| AML6    | ATP6V1E1  | ENSG00000131100 | chr22_18082755  | G>A     | intronic          | 20     | 42     | 67.74%                   | 1.26E-15        |                |                    |
| AML6    | SYN1      | ENSG00000080806 | chrX_47464548   | G>A     | intronic          | 34     | 16     | 32%                      | 3.50E-05        |                |                    |
| AML6    | LRR14M4   | ENSG00000176204 | chr2_77746270   | C>T     | R243H             | 100    | 104    | 50.98%                   | 5.56E-42        | ID=COSM266868  |                    |
| AML6    | TET2      | ENSG00000168769 | chr4_106156729  | C>T     | R544X             | 117    | 114    | 49.35%                   | 7.55E-44        | ID=COSM41850   |                    |
| AML6    | CMYA5     | ENSG00000164309 | chr5_79034662   | C>T     | H3358H            | 89     | 75     | 45.73%                   | 4.11E-25        |                |                    |
| AML6    | DNAH11    | ENSG00000105877 | chr7_21730446   | C>T     | N1996N            | 84     | 83     | 49.70%                   | 2.12E-33        |                |                    |
| AML6    | SVEP1     | ENSG00000165124 | chr9_113242031  | G>A     | R791C             | 111    | 85     | 43.37%                   | 1.68E-33        |                |                    |
| AML6    | AHNAK     | ENSG00000124942 | chr11_62294702  | T>C     | K2396R            | 68     | 37     | 35.24%                   | 3.87E-17        |                |                    |
| AML6    | RYR3      | ENSG00000198838 | chr15_33842399  | G>A     | R285Q             | 60     | 28     | 31.82%                   | 6.02E-09        |                |                    |
| AML6    | DCC       | ENSG00000187323 | chr18_50976873  | C>T     | P1078L            | 16     | 7      | 30.43%                   | 1.50E-04        | ID=COSM226365  |                    |
| AML6    | KIF16B    | ENSG00000089177 | chr20_16360726  | G>A     | R641C             | 60     | 69     | 53.49%                   | 2.42E-25        | ID=COSM1681548 |                    |
| AML6    | KLHL13    | ENSG00000030906 | chrX_117044028  | C>T     | R195Q             | 60     | 67     | 52.76%                   | 2.68E-23        |                |                    |
| AML6    | PRKCB     | ENSG00000166501 | chr16_24043511  | T>C     | C115R             | 67     | 58     | 46.40%                   | 4.57E-23        |                |                    |
| AML6    | CSE1L     | ENSG00000124207 | chr20_47675011  | G>A     | S4N               | 16     | 15     | 48.39%                   | 1.03E-06        |                |                    |
| AML6    | CENPJ     | ENSG00000151849 | chr13_25485908  | G>A     | intronic          | 13     | 15     | 53.57%                   | 7.04E-07        |                |                    |
| AML6    | ACTN2     | ENSG00000077522 | chr1_236914648  | G>A     | intronic          | 11     | 9      | 45%                      | 6.15E-04        |                |                    |
| AML6    | UNC79     | ENSG00000133958 | chr14_94041513  | C>A     | S550N             | 73     | 72     | 49.66%                   | 1.17E-30        |                |                    |
| AML6    | CELSR1    | ENSG00000075275 | chr22_46787632  | C>T     | D2016N            | 23     | 19     | 45.24%                   | 1.76E-07        |                | 0.0004             |
| AML6    | AP2M1     | ENSG00000161203 | chr3_183895917  | G>A     | intronic          | 67     | 43     | 39.09%                   | 5.72E-14        |                |                    |
| AML6    | HBS1L     | ENSG00000112339 | chr6_135317932  | A>G     | T274T             | 97     | 75     | 43.60%                   | 1.40E-33        |                |                    |
| AML6    | GFR2      | ENSG00000168546 | chr8_21608385   | A>G     | L37P              | 84     | 60     | 41.67%                   | 1.61E-25        |                |                    |
| AML6    | SBF2      | ENSG00000133812 | chr11_10051353  | T>G     | N158H             | 30     | 34     | 53.12%                   | 7.47E-18        |                |                    |
| AML6    | CYTH1     | ENSG00000108669 | chr17_76694387  | A>C     | F200V             | 38     | 21     | 35.59%                   | 4.96E-10        |                |                    |
| AML6    | SHANK1    | ENSG00000161681 | chr19_51217442  | C>T     | G213R             | 23     | 22     | 48.89%                   | 2.49E-08        |                |                    |
| AML6    | GPR101    | ENSG00000165370 | chrX_136113330  | G>A     | Y168Y             | 58     | 58     | 50%                      | 4.96E-22        | ID=COSM3231809 |                    |
| AML6    | YTHDF3    | ENSG00000185728 | chr8_64122434   | C>A     | UTR3              | 10     | 7      | 41.18%                   | 0.01070425      |                |                    |
| AML6    | D2HGDH    | ENSG00000180902 | chr2_242694669  | A>G     | intronic          | 32     | 21     | 39.62%                   | 5.53E-06        |                |                    |
| AML6    | PPP5D1    | ENSG00000230510 | chr19_46984436  | A>G     | UTR3              | 2      | 6      | 75%                      | 0.0015083       |                |                    |
| AML6    | KCMN3     | ENSG00000143603 | chr1_154841416  | C>A     | intronic          | 10     | 9      | 47.37%                   | 0.00130833      |                |                    |

**Supplementary Table 3. Table showing the whole-exome Sequencing data for 3 Patient samples.** Primary CD33+ cells from each patient were used for whole-exome sequencing. High-confidence mutations were selected following the primary data and secondary data analysis. WGA-Whole Genome Amplification.

|            |                      | 10 Reads | 20 Reads | 30 Reads | 40 Reads | 50 Reads | 60 Reads | 70 Reads | 80 Reads | 90 Reads | 100 Reads |
|------------|----------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|-----------|
| AML6 D0    | Depth Coverage Level | 98%      | 96%      | 94%      | 91%      | 87%      | 84%      | 80%      | 76%      | 72%      | 68%       |
| AML6 T     |                      | 98%      | 97%      | 95%      | 93%      | 91%      | 88%      | 86%      | 83%      | 80%      | 77%       |
| AML6 UT1   |                      | 98%      | 95%      | 92%      | 88%      | 83%      | 79%      | 74%      | 69%      | 65%      | 60%       |
| AML6 UT2   |                      | 98%      | 97%      | 95%      | 93%      | 90%      | 87%      | 84%      | 81%      | 78%      | 74%       |
| AML6 UT3   |                      | 98%      | 96%      | 94%      | 91%      | 87%      | 84%      | 80%      | 76%      | 73%      | 69%       |
| AML6 A1    |                      | 98%      | 97%      | 94%      | 92%      | 89%      | 85%      | 82%      | 78%      | 75%      | 71%       |
| AML6 A2    |                      | 98%      | 96%      | 94%      | 90%      | 87%      | 83%      | 79%      | 75%      | 71%      | 67%       |
| AML6 A3    |                      | 98%      | 97%      | 95%      | 93%      | 91%      | 88%      | 85%      | 82%      | 79%      | 75%       |
| AML6 C1    |                      | 98%      | 95%      | 92%      | 88%      | 83%      | 79%      | 74%      | 69%      | 65%      | 60%       |
| AML6 A+C1  |                      | 98%      | 97%      | 94%      | 91%      | 88%      | 85%      | 81%      | 77%      | 73%      | 69%       |
| AML6 A+C2  |                      | 98%      | 94%      | 91%      | 86%      | 81%      | 76%      | 70%      | 65%      | 60%      | 55%       |
| AML6 A+C3  |                      | 98%      | 95%      | 91%      | 86%      | 82%      | 76%      | 71%      | 66%      | 61%      | 56%       |
| AML5 D0    |                      | 98%      | 96%      | 92%      | 89%      | 84%      | 80%      | 75%      | 71%      | 66%      | 62%       |
| AML5 T     |                      | 98%      | 96%      | 94%      | 92%      | 89%      | 85%      | 82%      | 79%      | 75%      | 71%       |
| AML5 UT1   |                      | 98%      | 96%      | 92%      | 89%      | 85%      | 81%      | 76%      | 72%      | 68%      | 63%       |
| AML5 UT2   |                      | 99%      | 97%      | 96%      | 94%      | 91%      | 89%      | 86%      | 83%      | 80%      | 77%       |
| AML5 UT3   |                      | 98%      | 97%      | 94%      | 92%      | 89%      | 85%      | 82%      | 78%      | 75%      | 71%       |
| AML5 A1    |                      | 98%      | 96%      | 94%      | 91%      | 88%      | 84%      | 80%      | 77%      | 73%      | 69%       |
| AML5 A2    |                      | 98%      | 96%      | 94%      | 91%      | 88%      | 84%      | 80%      | 77%      | 73%      | 69%       |
| AML5 A3    |                      | 99%      | 97%      | 95%      | 93%      | 91%      | 88%      | 86%      | 83%      | 80%      | 77%       |
| AML5 C1    |                      | 98%      | 96%      | 93%      | 90%      | 87%      | 83%      | 79%      | 75%      | 71%      | 67%       |
| AML5 C2    |                      | 98%      | 96%      | 93%      | 90%      | 86%      | 82%      | 78%      | 74%      | 70%      | 66%       |
| AML5 C3    |                      | 98%      | 96%      | 94%      | 91%      | 88%      | 84%      | 80%      | 77%      | 73%      | 69%       |
| AML5 A+C1  |                      | 98%      | 95%      | 91%      | 87%      | 82%      | 77%      | 72%      | 67%      | 62%      | 57%       |
| AML5 A+C2  |                      | 98%      | 95%      | 90%      | 86%      | 81%      | 75%      | 70%      | 65%      | 60%      | 55%       |
| AML5 A+C3  |                      | 98%      | 94%      | 90%      | 86%      | 81%      | 76%      | 71%      | 66%      | 61%      | 57%       |
| AML7 D0    |                      | 99%      | 98%      | 96%      | 95%      | 93%      | 92%      | 90%      | 88%      | 86%      | 84%       |
| AML7 T_wga |                      | 53%      | 39%      | 31%      | 25%      | 21%      | 18%      | 15%      | 13%      | 12%      | 10%       |
| AML7 UT1   |                      | 99%      | 98%      | 96%      | 95%      | 93%      | 91%      | 89%      | 87%      | 84%      | 82%       |
| AML7 UT2   |                      | 97%      | 92%      | 87%      | 80%      | 74%      | 67%      | 60%      | 54%      | 48%      | 42%       |
| AML7 UT3   |                      | 99%      | 98%      | 96%      | 94%      | 92%      | 90%      | 87%      | 84%      | 81%      | 78%       |
| AML7 A1    |                      | 96%      | 92%      | 86%      | 79%      | 72%      | 65%      | 58%      | 51%      | 45%      | 39%       |
| AML7 A2    |                      | 98%      | 97%      | 95%      | 92%      | 89%      | 87%      | 83%      | 80%      | 77%      | 73%       |
| AML7 A3    |                      | 97%      | 93%      | 87%      | 81%      | 75%      | 68%      | 62%      | 55%      | 49%      | 44%       |
| AML7 C1    |                      | 98%      | 95%      | 91%      | 87%      | 82%      | 77%      | 72%      | 67%      | 62%      | 57%       |
| AML7 C2    |                      | 97%      | 94%      | 90%      | 85%      | 80%      | 74%      | 68%      | 63%      | 57%      | 52%       |
| AML7 C3    |                      | 99%      | 97%      | 96%      | 94%      | 92%      | 90%      | 87%      | 84%      | 82%      | 79%       |
| AML7 A+C1  |                      | 97%      | 93%      | 88%      | 83%      | 76%      | 70%      | 63%      | 57%      | 51%      | 45%       |
| AML7 A+C2  |                      | 97%      | 94%      | 89%      | 84%      | 78%      | 72%      | 66%      | 61%      | 55%      | 50%       |
| AML7 A+C3  |                      | 98%      | 95%      | 89%      | 80%      | 71%      | 61%      | 52%      | 44%      | 36%      | 29%       |

**Supplementary Table 4. Whole-exome Sequencing coverage data.** Sequencing coverage across the exonic regions for primary patient CD33+ D0 cells, post-transplanted xenografted cells for all patients samples used in this study.

| GROUP | SECONDARY ENGRAFTMENT OF hBM CELLS<br>> 0.05% |
|-------|-----------------------------------------------|
| UT    | 2/4                                           |
| C     | 1/4                                           |
| A     | 2/2                                           |
| A+C   | 1/2                                           |
| A+C+G | 1/2                                           |

**Supplementary Table 5.** Secondary transplantation of hCD45<sup>+</sup> cells, pooled from hBM-transduced mice one week post-treatment. Mice were sacrificed at 16w. Mice engraftment with >0.05% human cells were considered positive. UT n=4; C n=4; A n=2; A+C n=2; A+C+G n=2.