ACCSCIENCE PUBLISHING

International Journal of Bioprinting

ACCEPTED PAPER · SUPPLEMENTARY FILE

Engineering a biocompatible 3D model of the bone marrow niche for drug screening against KMT2A-rearranged acute myeloid leukaemia cell line MOLM-13

Article ID: IJB026190172

Citation: Hope L, Sanchez-Rubio A, Berry C, Salmeron-Sanchez M, Copland M, Wheadon H. Engineering a biocompatible 3D model of the bone marrow niche for drug screening against KMT2A-rearranged acute myeloid leukaemia cell line MOLM-13. *Int J Bioprint*. 2026. doi: 10.36922/IJB026190172

AccScience Publishing
RESEARCH ARTICLE
Volume X Issue X (2026)
doi: 10.36922/IJB026190172

Engineering a biocompatible 3D model of the bone marrow niche for drug screening against *KMT2A*-rearranged acute myeloid leukaemia cell line MOLM-13

Running title: Bioprinted leukaemia model for drug screening

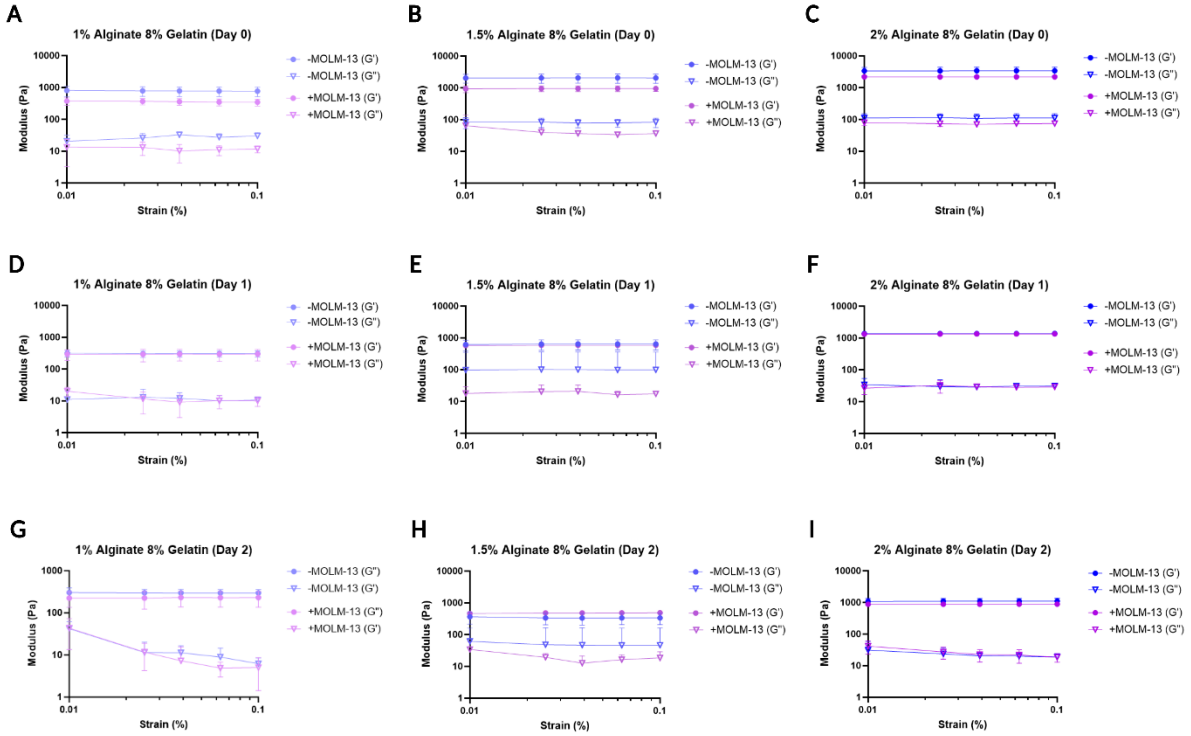
Supplementary File

(A) Supplementary methods

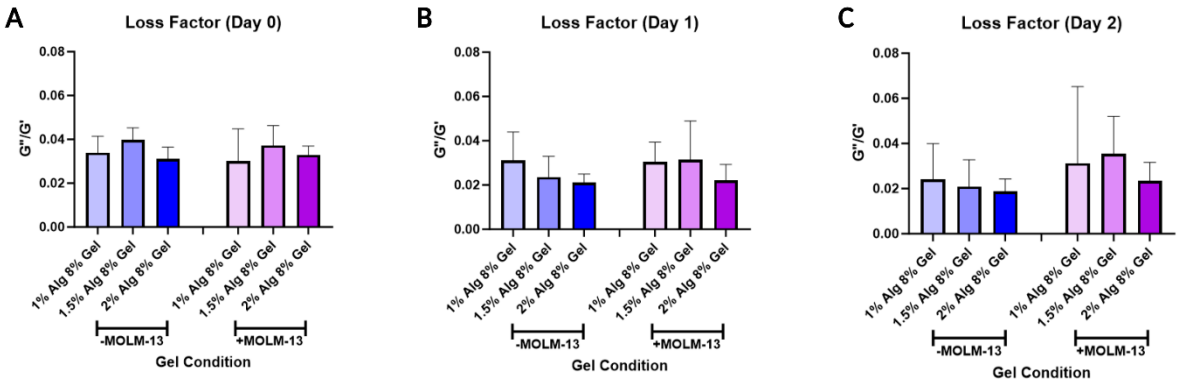
Actin/DAPI staining

2%Alg 8%Gel hand-cast and 3D bioprinted hydrogels containing MOLM-13 cells or MOLM-13 HS5 spheroid co-culture were washed in PBS supplemented with calcium and magnesium (PBS+; Gibco), then fixed in 4% paraformaldehyde in PBS+ at 37 °C for 15 minutes. Hydrogels were washed with PBS+ then permeabilised (10.3 g sucrose, 0.292 g sodium chloride, 0.06 g magnesium chloride (hexahydrate), 0.476 g HEPES in 100 mL PBS+, 0.5 mL Triton X) for five minutes at 4°C. Gels were washed with PBS+ then blocked in 1% bovine serum albumin (BSA) for five minutes at 37°C. Gels were submerged in 1% BSA containing Alexa Fluor™ 594 Phalloidin stain (Invitrogen) and incubated for 1 hour at 37°C. After incubating, the gels were washed three times with 0.5% PBS+-Tween20 under gentle agitation. Gels were stained with VECTASHIELD Antifade mounting media containing DAPI (Vector Laboratories, USA) prior to imaging. Images were captured at 10X and 20X magnification using the Zeiss Axiovert (hand-cast) and EVOS M7000 Imager (3D bioprinted) (Ex/Em: Alexa Fluor™ 594 Phalloidin: 581/609 nm; DAPI: 360/460 nm).

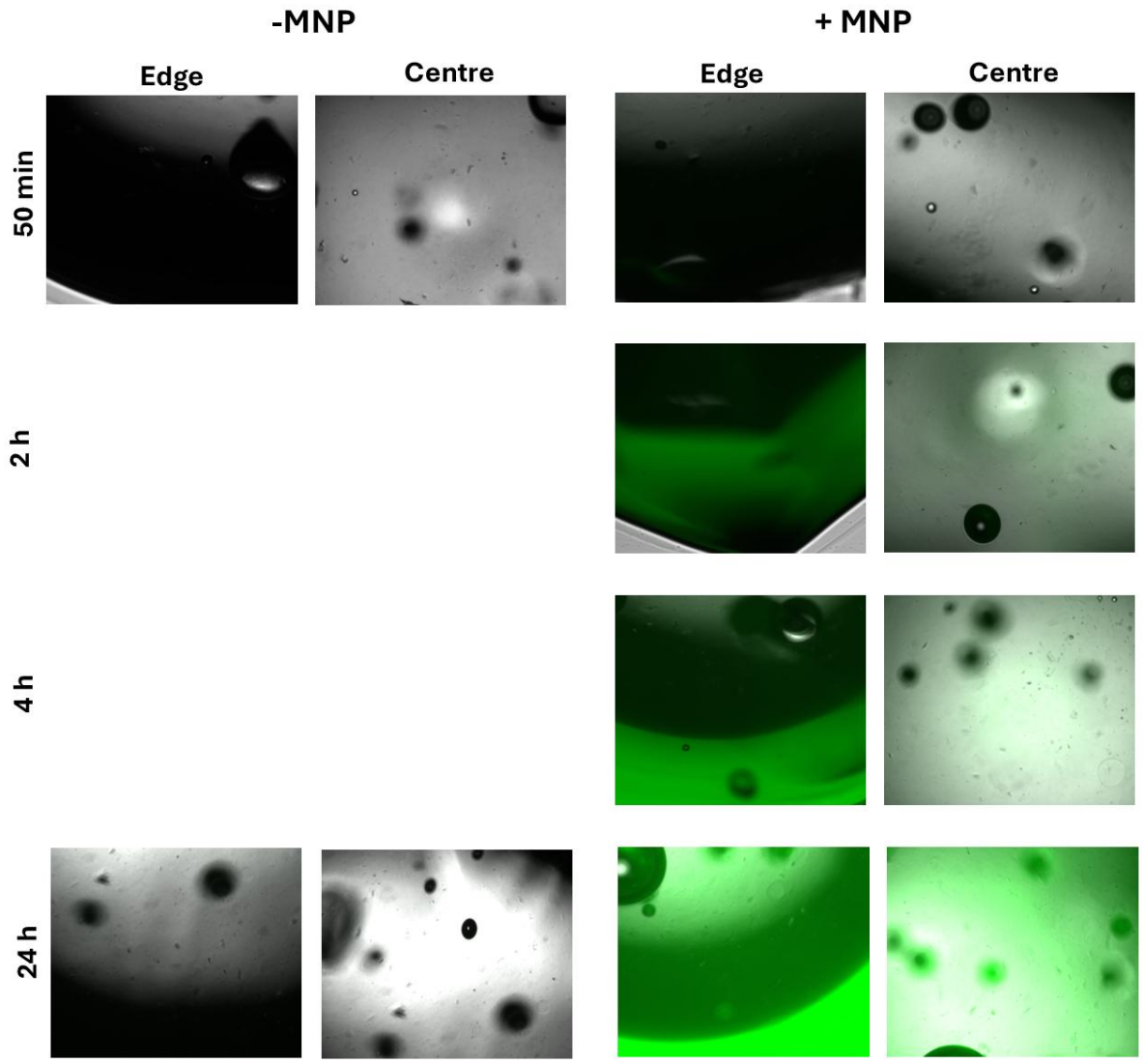
(B) Supplementary figures and tables



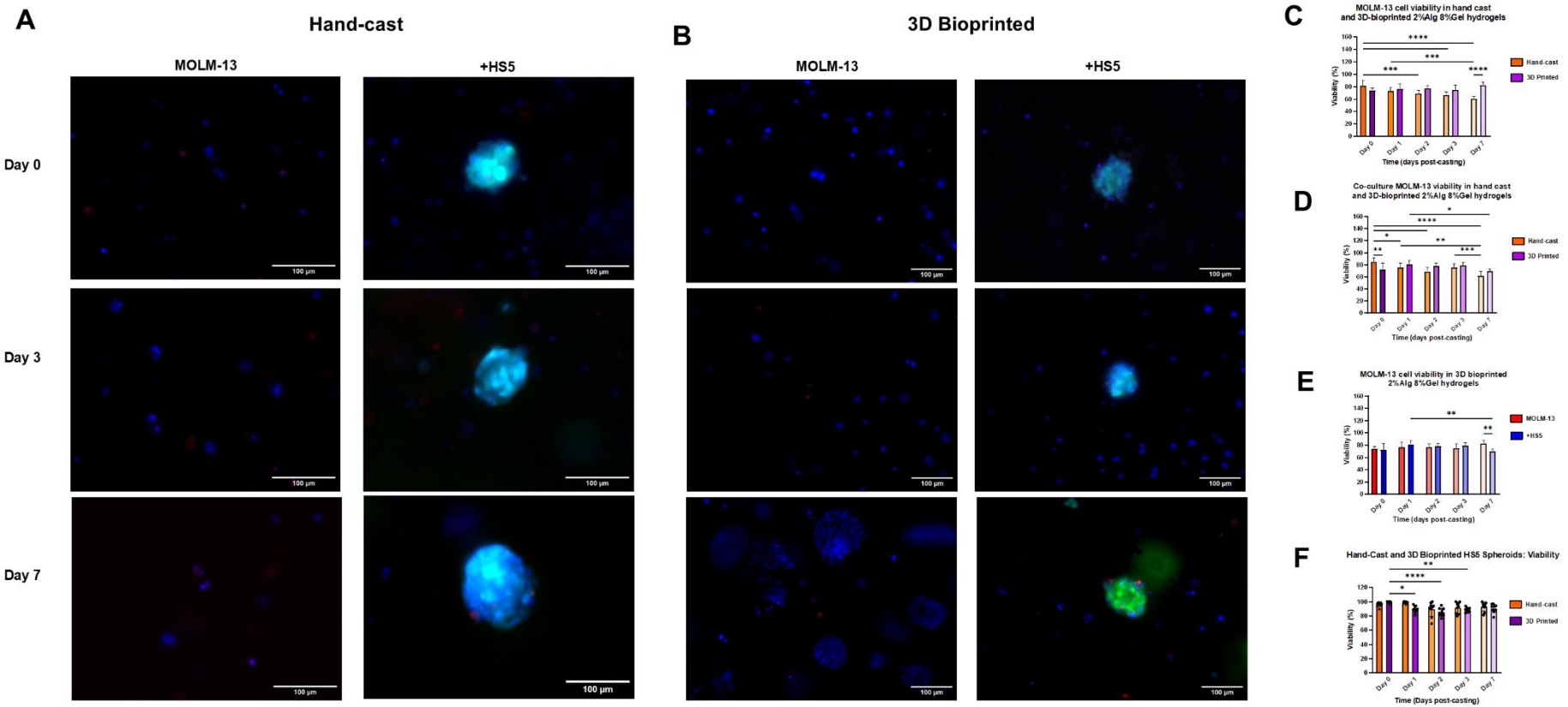
Supplementary Figure 1: Strain sweeps of 1%, 1.5% and 2% Alg 8% Gel hydrogels at days 0, 1 and 2 after crosslinking. A, D and E depict 1%Alg 8%Gel at days 0, 1, 2 respectively; B, E and H depict 1.5% Alg 8%Gel hydrogels; and C, F, I show 2%Alg 8%Gel. Closed circles show the storage modulus (G') and open triangles show the loss modulus (G"). Strain range was 0.01-0.1% strain and angular frequency was 10 rad/s. Error bars depict standard deviation.



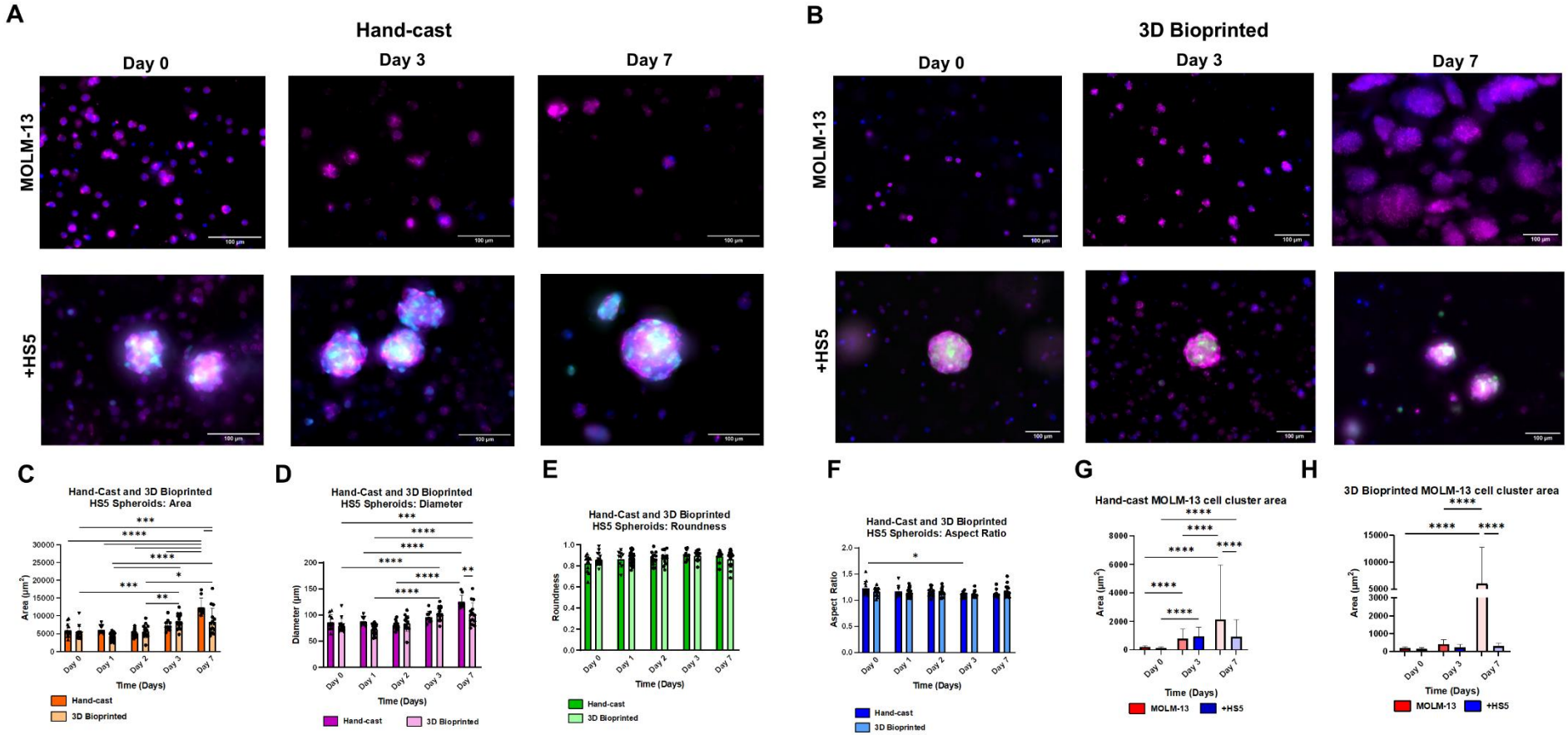
Supplementary Figure 2: Loss factor of 1%, 1.5% and 2% Alginate/8% gelatin hydrogels with and without MOLM-13 cells over two days. Loss factor was calculated as outlined in equation 2. Error bars depict standard deviation.



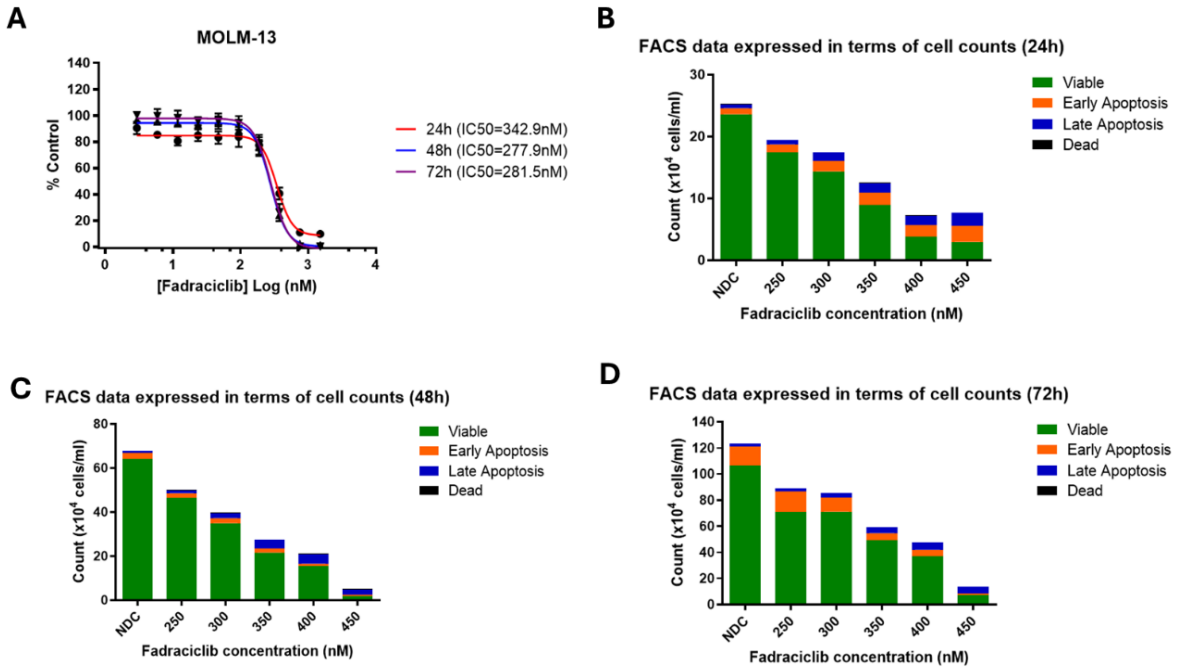
Supplementary Figure 3: Diffusion of fluorescent nanoparticles through 2%Alg 8%Gel hand-cast hydrogels to simulate diffusion of chemotherapeutic drugs. Hydrogels were submerged in DMEM containing FluorMag nanoparticles conjugated to FITC at 200 µg/mL and imaged at regular intervals over 24 hours. Here, it shows that small particles can diffuse through the hydrogels after at least two hours. Images were taken using a Zeiss Axiovert microscope at 5X, with the FITC channel exposure set at 800 ms across all timepoints.


A
Hand-cast
MOLM-13
+HS5
Day 0
Day 3
Day 7
100 µm
B
3D Bioprinted
MOLM-13
+HS5
100 µm
C
MOLM-13 cell viability in hand cast and 3D-bioprinted 2%Alg 8%Gel hydrogels
Viability (%)
Time (days post-casting)
Hand-cast
3D Printed
D
Co-culture MOLM-13 viability in hand cast and 3D-bioprinted 2%Alg 8%Gel hydrogels
E
MOLM-13 cell viability in 3D bioprinted 2%Alg 8%Gel hydrogels
MOLM-13
+HS5
F
Hand-Cast and 3D Bioprinted HS5 Spheroids: Viability
Time (Days post-casting)

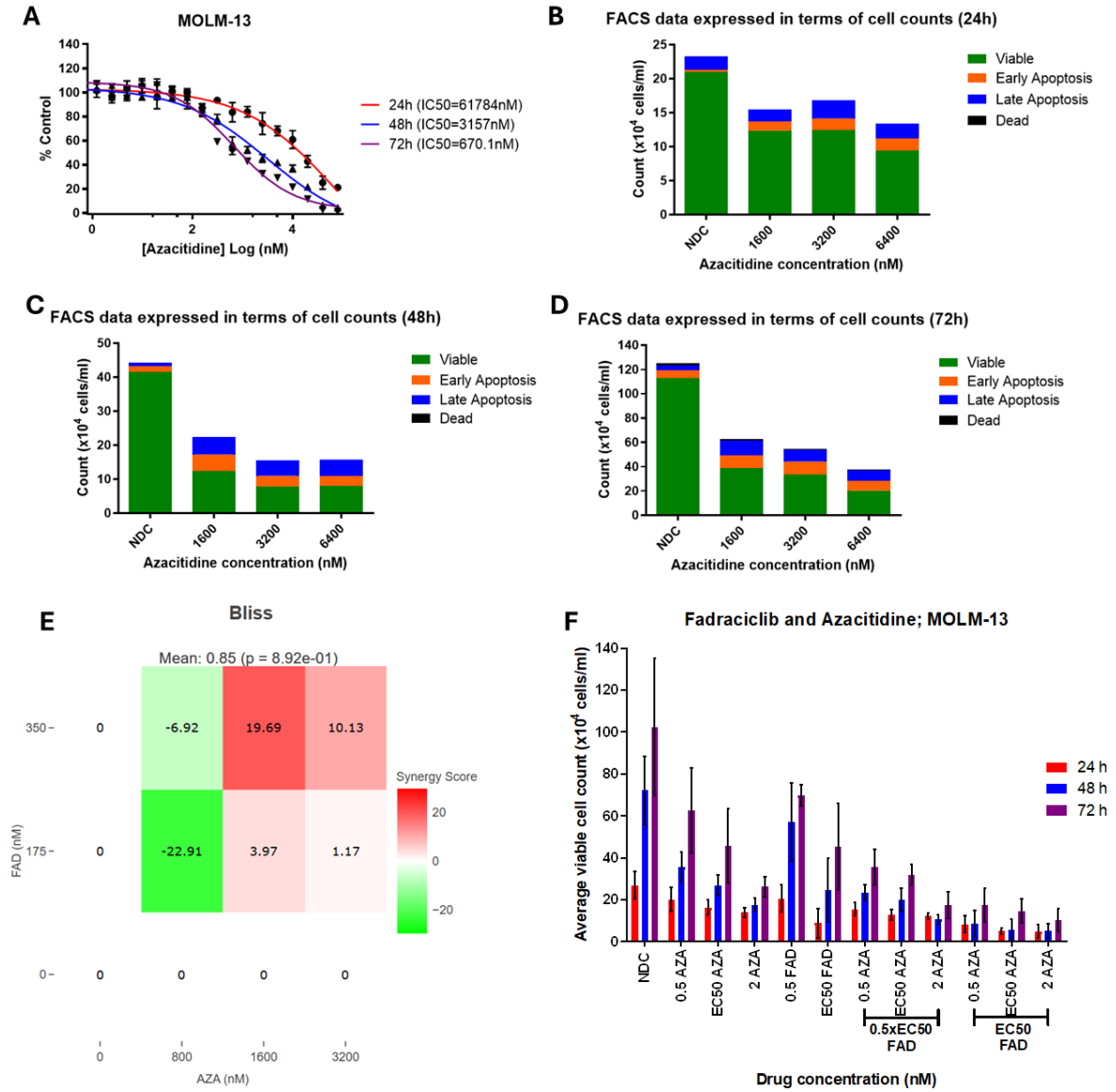
Supplementary Figure 4: Viability MOLM-13 cells and HS5 spheroids within hand-cast and 3D bioprinted 2%Alg 8%Gel hydrogels. A: MOLM-13 cells in monoculture and co-cultured with GFP-HS5 spheroids within hand-cast 2%Alg 8%Gel hydrogels stained with live/dead stain; B: 3D bioprinted alginate/gelatin hydrogels containing MOLM-13 cells in monoculture and co-cultured with GFP-HS5 spheroids stained with live/dead stain (20X magnification; scale bar: 100 μm); C: Comparison of monoculture MOLM-13 viability in hand-cast and 3D bioprinted alginate/gelatin gels; D: Comparison of co-cultured MOLM-13 cell viability in hand-cast and 3D bioprinted alginate/gelatin gels; E: Comparison of MOLM-13 monoculture and HS5 co-culture viability. Green: HS5 cells (GFP-HS5 cells); blue: live cells; red: dead cells; F: Comparison of HS5 spheroid viability in hand-cast and 3D bioprinted hydrogels for up to seven days. HS5 spheroid viability was calculated using the JACOP plugin on Fiji, whereby the Mandler's coefficient was used to determine the degree of overlap of GFP (green channel) with either live (blue channel) or dead (red channel). Hand-cast viability images were taken using Zeiss Axiovert microscope, and 3D bioprinted viability images were taken using the EVOS M7000 Imager. Single plane images were taken at 10X for analysis and 20X for representative images. One-way ANOVAs with multiple comparisons were used in C-F. Significance is shown using asterisks, and an absence of this indicates a lack of significance. Significance: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$ **** $P \leq 0.0001$. Bars represent the average of nine technical replicates (three images per hydrogel, total of nine datapoints) with standard deviation shown as error bars.


A
Hand-cast
Day 0
Day 3
Day 7
MOLM-13
+HS5
B
3D Bioprinted
Day 0
Day 3
Day 7
MOLM-13
+HS5
C
Hand-Cast and 3D Bioprinted HS5 Spheroids: Area
Area (μm²)
Time (Days)
Hand-cast
3D Bioprinted
D
Hand-Cast and 3D Bioprinted HS5 Spheroids: Diameter
Diameter (μm)
Time (Days)
Hand-cast
3D Bioprinted
E
Hand-Cast and 3D Bioprinted HS5 Spheroids: Roundness
Roundness
Time (Days)
Hand-cast
3D Bioprinted
F
Hand-Cast and 3D Bioprinted HS5 Spheroids: Aspect Ratio
Aspect Ratio
Time (Days)
Hand-cast
3D Bioprinted
G
Hand-cast MOLM-13 cell cluster area
Area (μm²)
Time (Days)
MOLM-13
+HS5
H
3D Bioprinted MOLM-13 cell cluster area
Area (μm²)
Time (Days)
MOLM-13
+HS5

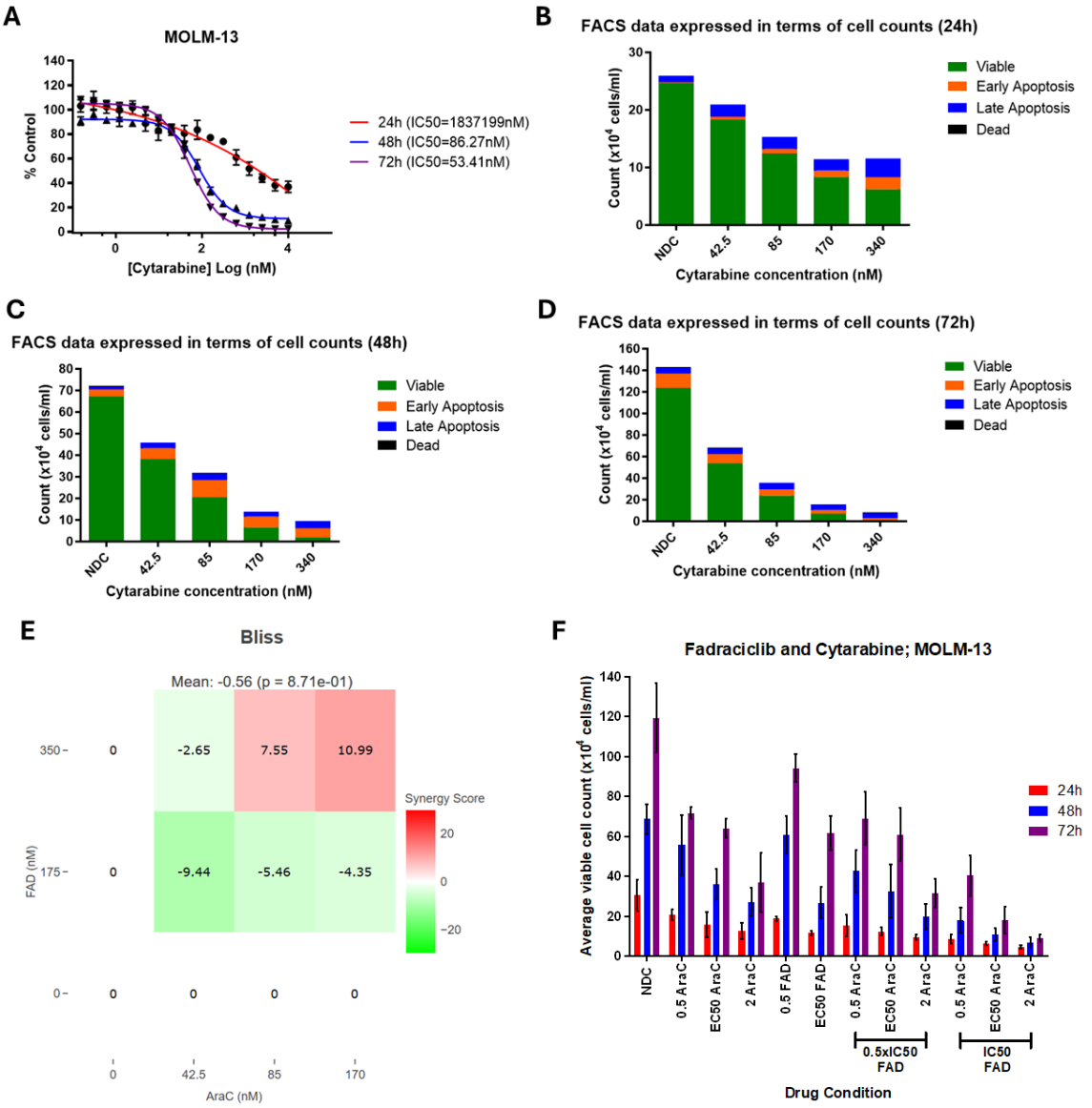
Supplementary Figure 5: Morphology of MOLM-13 cells within hand-cast and 3D bioprinted 2%Alg 8%Gel hydrogels. A: Representative images of MOLM-13 cells in monoculture and co-cultured with GFP-HS5 spheroids within hand-cast 2%Alg 8%Gel hydrogels stained for F-Actin and DAPI nuclear stain (20X magnification; scale bar: 100 µm); B: MOLM-13 cells in monoculture and co-cultured with GFP-HS5 spheroids within 3D bioprinted 2%Alg 8%Gel hydrogels stained for F-Actin and DAPI nuclear stain (20X magnification; scale bar: 100 µm). Green: HS5 cells; blue: DAPI (Nuclei); magenta: F-Actin. Spheroids were quantified for hand-cast and 3D bioprinted spheroids and area (C), diameter (D), roundness (E), and aspect ratio (F) are depicted as bar charts. The cluster size of MOLM-13 cells in monoculture and HS5 co-culture in hand-cast (G) and 3D bioprinted (H) hydrogels were quantified using Fiji and shown as bar charts. Analyses were completed using images taken at 10X magnification. Representative z-stack images were taken at 20X magnification, and the Maximal Intensity Projection was selected to produce the final images. Hand-cast hydrogel images were taken using Zeiss Axiovert microscope, and 3D bioprinted images were taken using the EVOS M7000 Imager. One-way ANOVAs with multiple comparisons were used in C-H to detect significant differences between conditions. Significance is shown using asterisks, and an absence of this indicates a lack of significance. Significance: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$ **** $P \leq 0.0001$. Bars represent the average of nine technical replicates (three images per hydrogel, total of nine datapoints) with standard deviation shown as error bars.



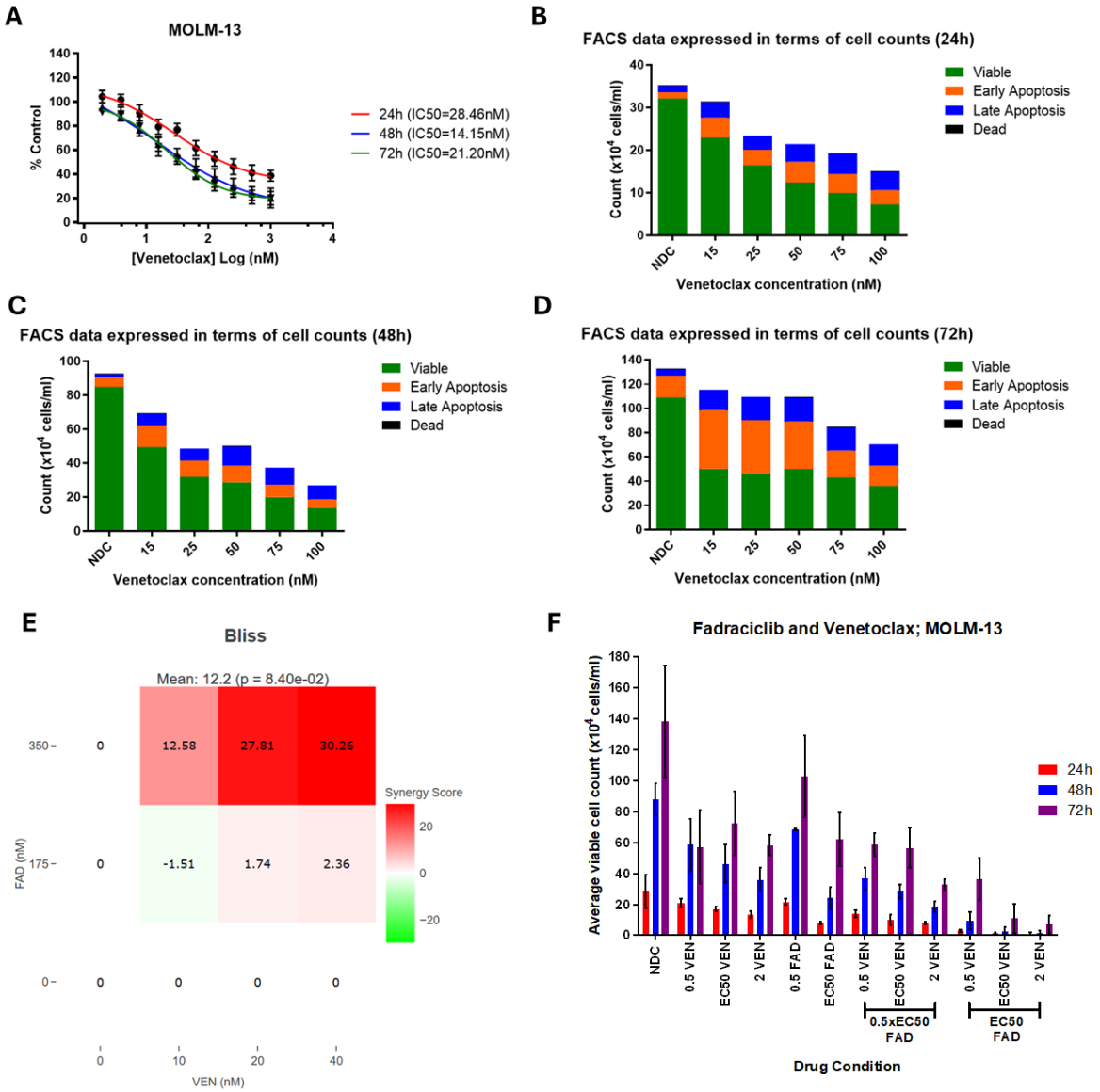
Supplementary Figure 6: Determining the EC50 of fadraciclib (FAD) on MOLM-13 cells. The cells were treated with fadraciclib in a range of 250-450 nM and analysed after 24, 48 and 72 hours. A: Logarithmic graph depicting the results of the resazurin reduction assays (n=3) at 24h (red), 48h (blue), and 72h (purple). N=3. Error bars depict standard deviation. B-D: Annexin V/DAPI data expressed in terms of absolute cell counts per condition at 24, 48 and 72 hours (respectively). Viable (green – Annexin V negative, DAPI negative), Early Apoptosis (orange – Annexin V positive, DAPI negative), Late Apoptosis (orange – Annexin V positive, DAPI positive), Dead (orange – Annexin V negative, DAPI positive). N=1.



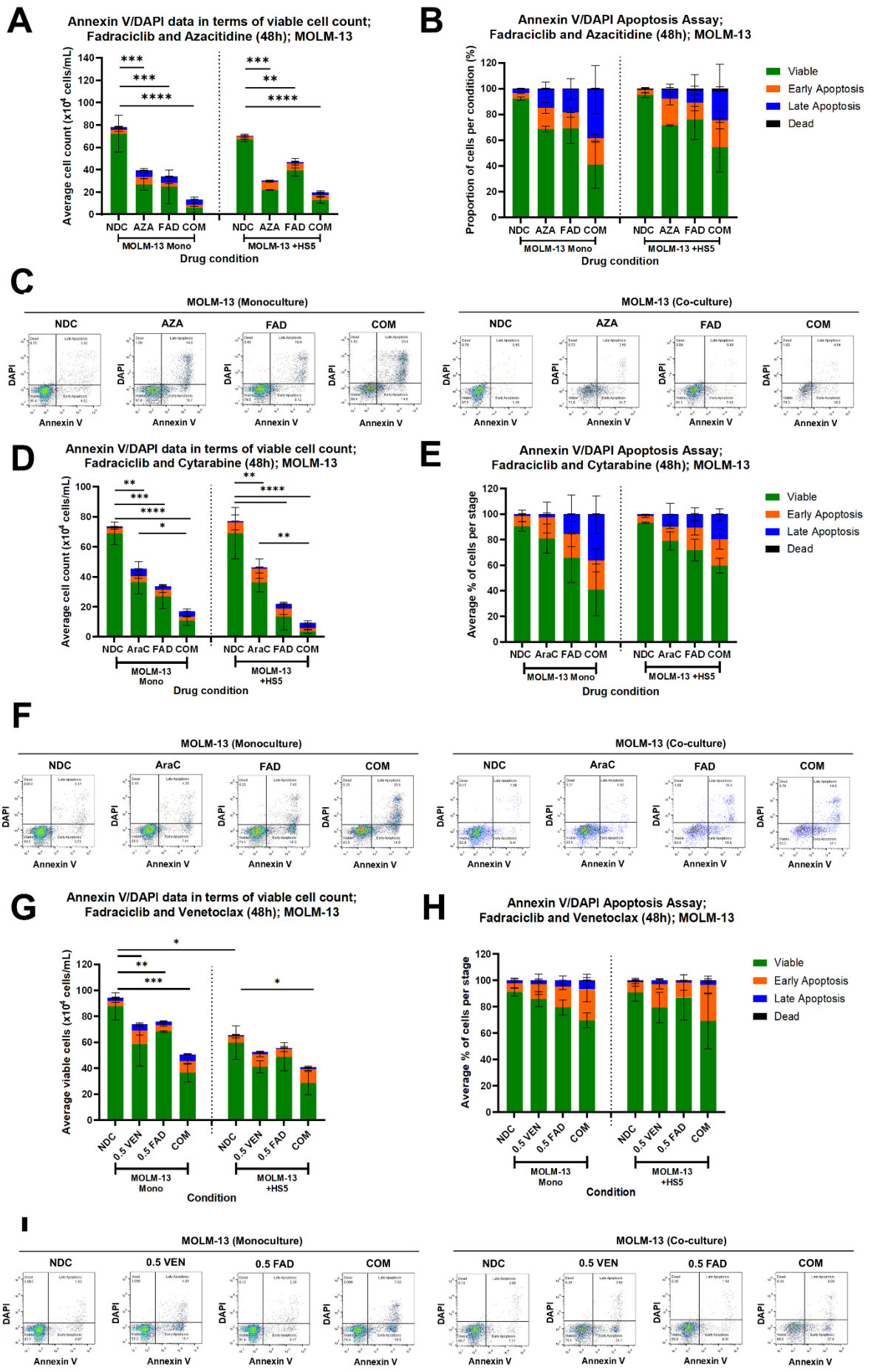
Supplementary Figure 7: Determining the EC50 of azacitidine (AZA) on MOLM-13 cells and optimal combination with fadraciclib. The concentrations used were 1600 nM, 3200 nM and 6400 nM (0.5x EC50, EC50 and 2xEC50 from 48 hours resazurin assay). A: Logarithmic graph showing the results of the resazurin assays (n=3) at 24h (red), 48h (blue), and 72h (purple). B-D: Annexin V/DAPI data expressed in terms of absolute cell counts per condition at 24, 48 and 72 hours (respectively). E: Heatmap displaying the synergy calculated for each dosage combination using the Bliss Independence model at 48h. Heatmap produced using Synergy Finder+ software [1]. F: A bar chart depicting all fadraciclib and azacitidine combinations assessed on MOLM-13 cells. The cell numbers were calculated using trypan blue staining and the proportion of viable cells were taken from Annexin V/DAPI staining. FAD EC50: 350 nM, AZA EC50: 1600 nM. 24h: Red, 48h: blue, 72h: purple. Bars represent the average of three biological replicates with standard deviation depicted as error bars.



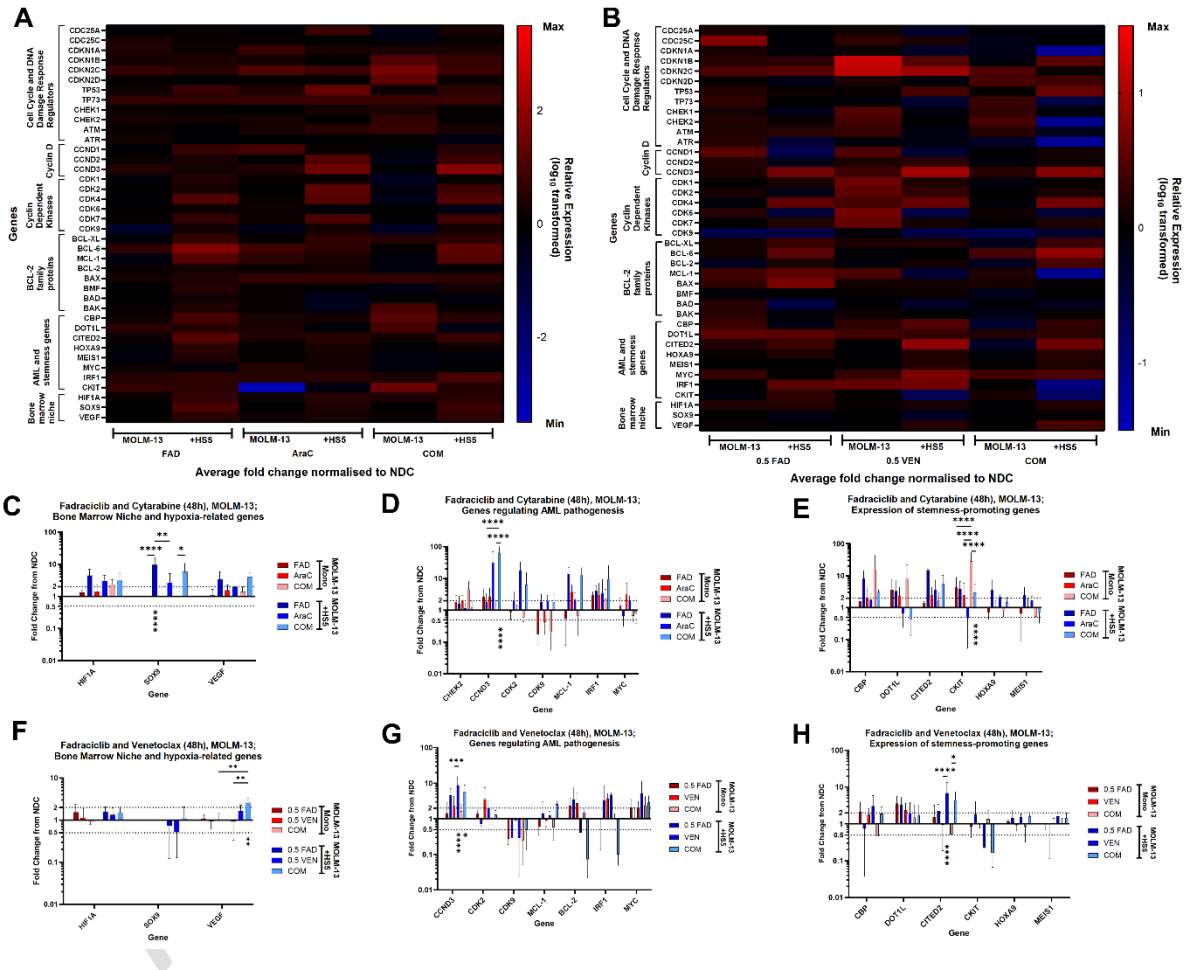
Supplementary Figure 8: Determining the EC50 of cytarabine (AraC) on MOLM-13 cells and dose optimisation with fadraciclib. A: Logarithmic graph displaying the results of the resazurin assays (n=3) at 24h (red), 48h (blue), and 72h (purple). B-D: Annexin V/DAPI data expressed in terms of absolute cell counts per condition at 24, 48 and 72 hours (respectively). The cells were treated with 42.5 nM, 85 nM, 170 nM and 340 nM (0.5x EC50, EC50, 2xEC50 and 4xEC50 from 48h resazurin). E: Heatmap displaying the synergy calculated for each dosage combination using the Bliss Independence model at 48h. Heatmap produced using Synergy Finder+ software [1]. F: A bar chart depicting all fadraciclib and cytarabine combinations assessed on MOLM-13 cells. The cell numbers were calculated using trypan blue staining and the proportion of viable cells were taken from Annexin V/DAPI staining. FAD EC50: 350 nM, AraC EC50: 42.5 nM. 24h: Red, 48h: blue, 72h: purple. Bars represent the average of three biological replicates with standard deviation depicted as error bars.



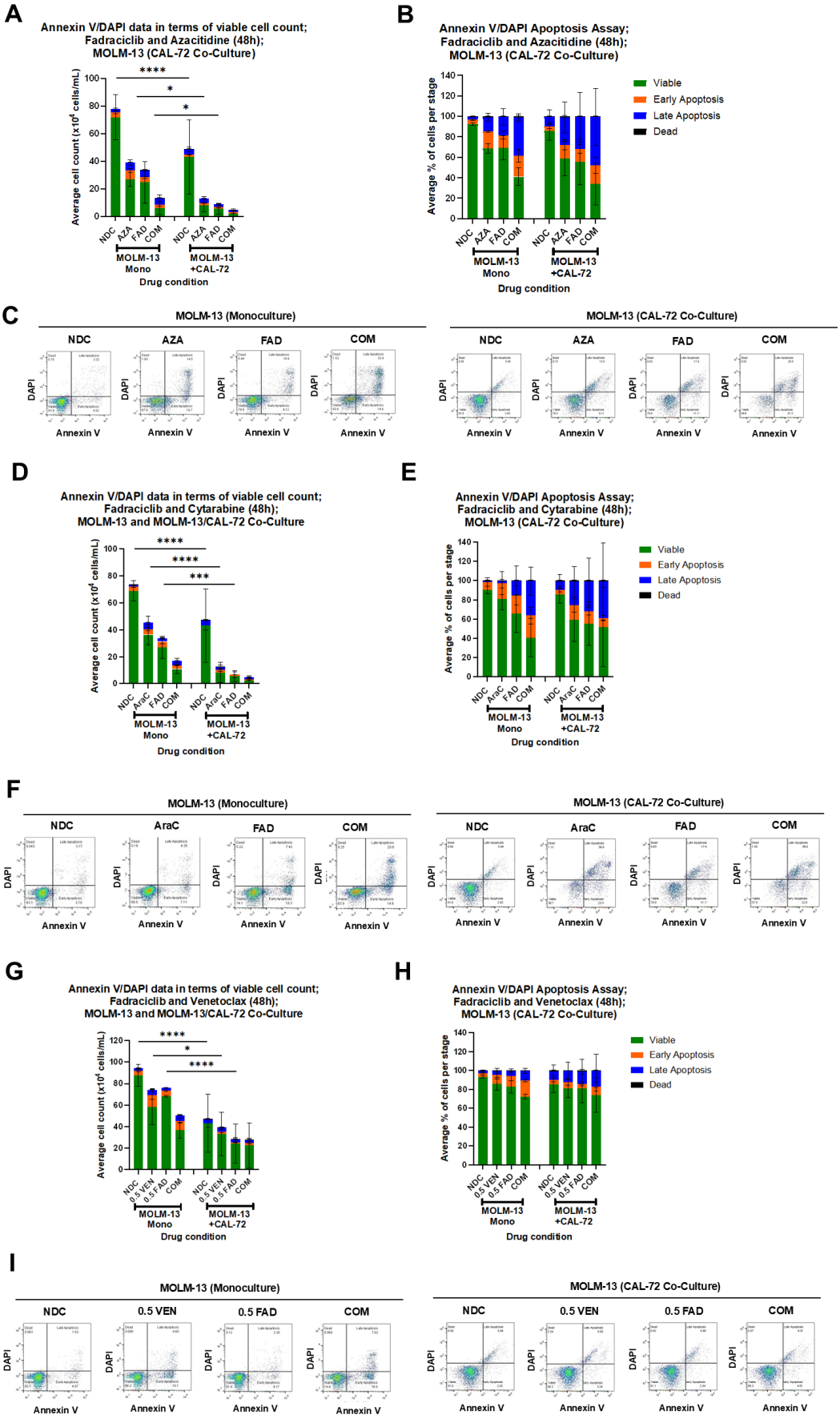
Supplementary Figure 9: Determining the EC50 of venetoclax (VEN) and the optimal combination of venetoclax and fadraciclib on MOLM-13 cells. A: Logarithmic graph displaying the results of the resazurin reduction assays (n=3) at 24h (red), 48h (blue), and 72h (purple). B-D: Annexin V/DAPI data expressed as absolute cell counts per condition at 24, 48 and 72 hours (respectively). E: Heatmap displaying the synergy calculated for each dosage combination using the Bliss Independence model at 48h. Heatmap produced using Synergy Finder+ software [1]. F: A bar chart depicting all fadraciclib and venetoclax combinations assessed on MOLM-13 cells. The cell numbers were calculated using trypan blue staining and the proportion of viable cells were taken from Annexin V/DAPI staining. Due to the potency of these combinations, the 0.5xIC50 values of fadraciclib and venetoclax were selected (FAD 0.5xEC50: 175 nM, VEN 0.5xEC50: 10 nM). 24h: Red, 48h: blue, 72h: purple. Bars represent the average of three biological replicates with standard deviation depicted as error bars.


A
Annexin V/DAPI data in terms of viable cell count;
Fadraciclib and Azacitidine (48h); MOLM-13
Average cell count (x10⁴ cells/mL)
NDC AZA FAD COM
MOLM-13 Mono
MOLM-13 +HS5
Drug condition
B
Annexin V/DAPI Apoptosis Assay;
Fadraciclib and Azacitidine (48h); MOLM-13
Proportion of cells per condition (%)
Viable
Early Apoptosis
Late Apoptosis
Dead
C
MOLM-13 (Monoculture)
MOLM-13 (Co-culture)
NDC
AZA
FAD
COM
DAPI
Annexin V
D
Annexin V/DAPI data in terms of viable cell count;
Fadraciclib and Cytarabine (48h); MOLM-13
Average cell count (x10⁴ cells/mL)
NDC AraC FAD COM
MOLM-13 Mono
MOLM-13 +HS5
Drug condition
E
Annexin V/DAPI Apoptosis Assay;
Fadraciclib and Cytarabine (48h); MOLM-13
Average % of cells per stage
F
MOLM-13 (Monoculture)
MOLM-13 (Co-culture)
NDC
AraC
FAD
COM
G
Annexin V/DAPI data in terms of viable cell count;
Fadraciclib and Venetoclax (48h); MOLM-13
Average viable cells (x10⁴ cells/mL)
NDC 0.5 VEN 0.5 FAD COM
MOLM-13 Mono
MOLM-13 +HS5
Condition
H
Annexin V/DAPI Apoptosis Assay;
Fadraciclib and Venetoclax (48h); MOLM-13
Average % of cells per stage
I
MOLM-13 (Monoculture)
MOLM-13 (Co-culture)
NDC
0.5 VEN
0.5 FAD
COM

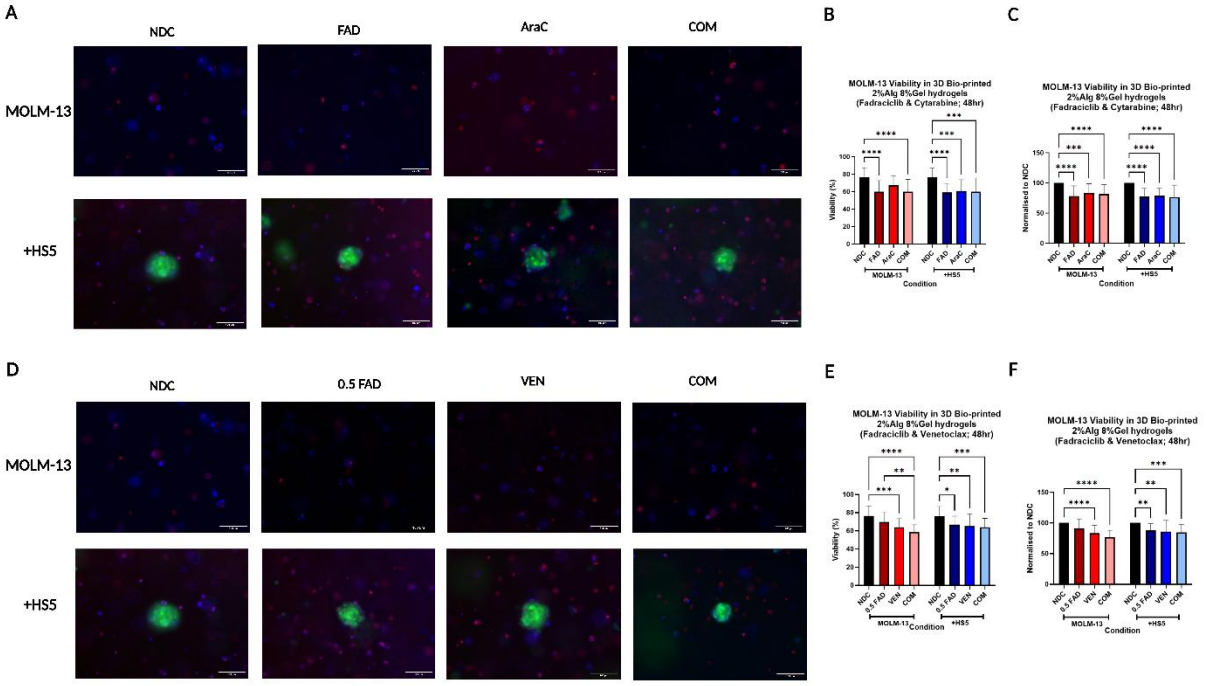
Supplementary Figure 10: MOLM-13 cells in 2D monoculture and HS5 co-culture treated with fadraciclib and current AML therapies. A, D, G: Annexin V/DAPI data shown in terms of cell counts for MOLM-13 cells treated with fadraciclib (FAD) alone, in combination with azacitidine (AZA; A), cytarabine (AraC; D) or venetoclax (VEN; G) and combination (COM) therapy in monoculture and co-culture. B, E, H: Bar chart showing the proportion of cells in each apoptotic stage through Annexin V/DAPI apoptosis staining for FAD alone and in combinaton with AZA (B), AraC (E), or VEN (H). A one-way ANOVA was used to detect significant differences between conditions in B, E, H. C, F, I: Representative Annexin V/DAPI scatter plots showing the proportions of NDC and drug treated MOLM-13 cells in monoculture (left) and co-culture (right) in each stage of apoptosis 48 hours post treatment. Significance: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$ **** $P \leq 0.0001$. Bars represent the mean of three biological replicates, and standard deviation depicted as error bars.



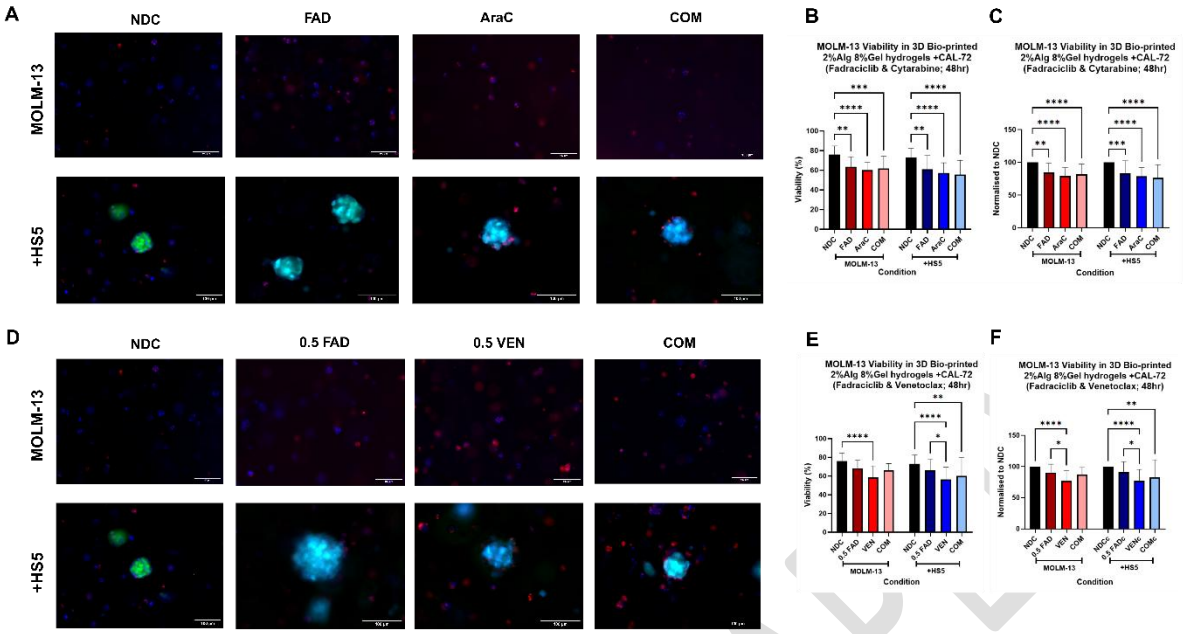
Supplementary Figure 11: The effect of fadraciclib alone and combination with cytarabine and venetoclax on MOLM-13 gene expression. A, B: Heat maps displaying differences in gene expression for MOLM-13 cells in monoculture and HS5 co-culture following treatment with fadraciclib (FAD) alone and in combination with cytarabine (AraC, A) or venetoclax (VEN, B), normalised to NDC. Blue: downregulated compared to NDC; red: upregulated. C, F: bone marrow niche and hypoxia-related gene expression after treatment with cytarabine (C) and venetoclax (F); D, G: expression of genes regulating AML pathogenesis after cytarabine (D) and venetoclax (G) treatments; E, H: stemness-promoting gene expression after cytarabine (E) and venetoclax (H) single and combination treatments. Two-way ANOVAs with multiple comparisons were used to detect significant differences between conditions in B, C, E, F. Significance: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$ **** $P \leq 0.0001$. Bars represent the mean of three biological replicates, and standard deviation depicted as error bars.


A
Annexin V/DAPI data in terms of viable cell count;
Fadraciclib and Azacitidine (48h);
MOLM-13 (CAL-72 Co-Culture)
Average cell count (x10⁴ cells/mL)
NDC
AZA
FAD
COM
MOLM-13 Mono
MOLM-13 +CAL-72
Drug condition
B
Annexin V/DAPI Apoptosis Assay;
Fadraciclib and Azacitidine (48h);
MOLM-13 (CAL-72 Co-Culture)
Average % of cells per stage
Viable
Early Apoptosis
Late Apoptosis
Dead
C
MOLM-13 (Monoculture)
MOLM-13 (CAL-72 Co-Culture)
DAPI
Annexin V
D
Annexin V/DAPI data in terms of viable cell count;
Fadraciclib and Cytarabine (48h);
MOLM-13 and MOLM-13/CAL-72 Co-Culture
AraC
E
Annexin V/DAPI Apoptosis Assay;
Fadraciclib and Cytarabine (48h);
MOLM-13 (CAL-72 Co-Culture)
F
G
Annexin V/DAPI data in terms of viable cell count;
Fadraciclib and Venetoclax (48h);
MOLM-13 and MOLM-13/CAL-72 Co-Culture
0.5 VEN
0.5 FAD
H
Annexin V/DAPI Apoptosis Assay;
Fadraciclib and Venetoclax (48h);
MOLM-13 (CAL-72 Co-Culture)
I

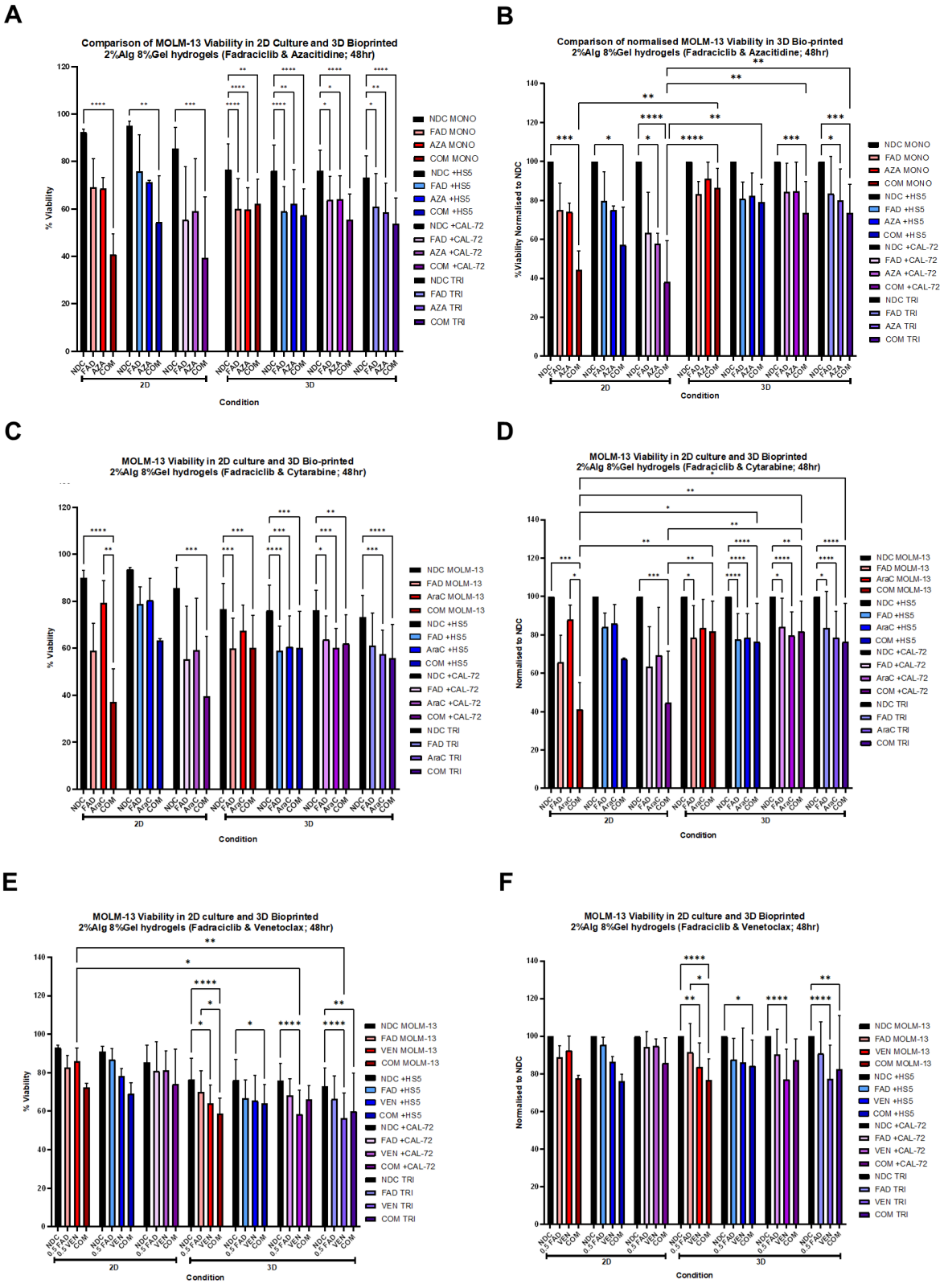
Supplementary Figure 12: MOLM-13 cells in 2D monoculture and CAL-72 co-culture treated with fadraciclib and selected AML therapies. A, D, G: Annexin V/DAPI data shown in terms of cell counts for MOLM-13 cells treated with fadraciclib (FAD), azacitidine (AZA; A), cytarabine (AraC; D) or venetoclax (VEN; G) and combination (COM) therapies of fadraciclib with these drugs in monoculture and CAL-72 co-culture. B, E, H: Bar chart showing the proportion of cells in each apoptotic stage through Annexin V/DAPI apoptosis staining for FAD alone and in combinaton with AZA (B), AraC (E), or VEN (H) in monoculture and CAL-72 co-culture. A one-way ANOVA was used to detect significant differences between conditions in B, E, H. C, F, I: Representative Annexin V/DAPI scatter plots showing the proportions of NDC and drug treated MOLM-13 cells in monoculture (left) and CAL-72 co-culture (right) in each stage of apoptosis. Significance: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$ **** $P \leq 0.0001$. Bars represent the mean of three biological replicates, and standard deviation depicted as error bars.



Supplementary Figure 13: MOLM-13 cells in 3D monoculture and HS5 spheroid co-culture treated with fadraciclib with cytarabine or venetoclax. A, D: Representative images of 3D bioprinted MOLM-13 cells in monoculture and HS5 spheroid co-culture 48 hours after treatment with fadraciclib (FAD), cytarabine (AraC), venetoclax (VEN) or a combination of FAD with AraC or VEN (COM). Images taken at 20X using the M7000 Imager (scale bar 100 µm). Green: HS5 cells (GFP-HS5 cells); blue: live cells; red: dead cells. B, E: MOLM-13 cell viability within 3D bioprinted 2%Alg 8%Gel hydrogels following drug treatment. C, F: MOLM-13 cell viability normalised to NDC. One-way ANOVAs with multiple comparisons were used to find significant differences between conditions in B, C, E, F. Significance: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$ **** $P \leq 0.0001$. Bars represent the mean of three biological replicates, and standard deviation depicted as error bars.

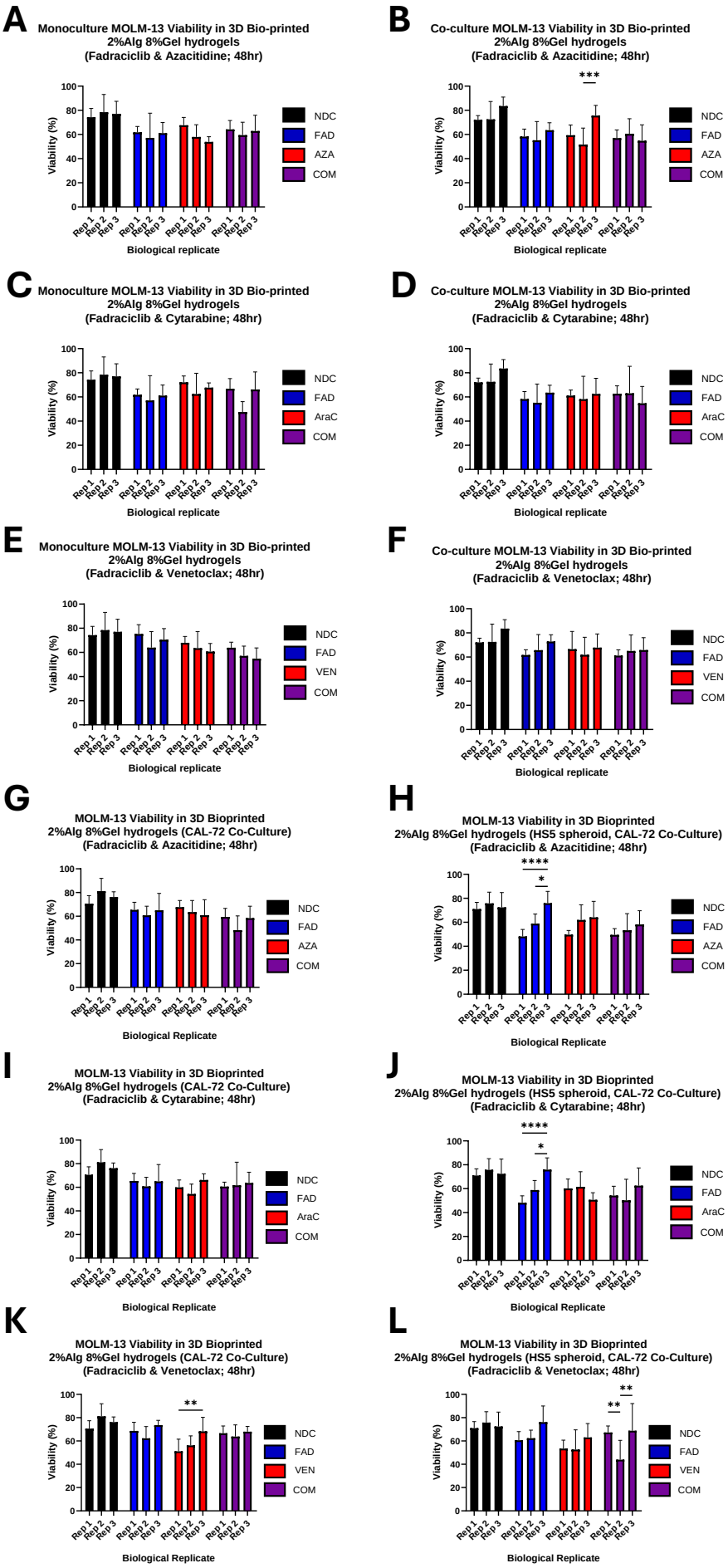


Supplementary Figure 14: Assessment of fadraciclib with cytarabine or venetoclax within the final 3D bioprinted model. A, D: Representative images of the final 3D bioprinted AML model, comprising MOLM-13 cells in monoculture and HS5 spheroid co-culture cultured with a CAL-72 monolayer. Images taken 48 hours after treatment with fadraciclib (FAD), cytarabine (AraC), venetoclax (VEN) or a combination of FAD with AraC or VEN (COM). Images taken at 20X using the M7000 Imager (scale bar 100 μm). Green: HS5 cells (GFP-HS5 cells); blue: live cells; red: dead cells. B, E: MOLM-13 cell viability within 3D bioprinted 2%Alg 8%Gel hydrogels following drug treatment. C, F: MOLM-13 cell viability normalised to NDC. One-way ANOVAs with multiple comparisons were used to find significant differences between conditions in B, C, E, F. Significance: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$ **** $P \leq 0.0001$. Bars represent the mean of three biological replicates, and standard deviation depicted as error bars.



Supplementary Figure 15: Bar charts comparing all conditions assessed within this study for fadraciclib in combination with azacitidine, cytarabine and venetoclax. A, C, E: MOLM-13 viability after treatment with fadraciclib (FAD), azacitidine (AZA; A), cytarabine (AraC, C) and venetoclax (VEN, E) in all 2D and 3D conditions. B, D, F: MOLM-13 cell viability normalised to NDC following treatment with FAD and AZA (B), AraC (D) and VEN (F). One-way ANOVAs with multiple comparisons were used to detect significant differences

between conditions. Significance: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$ **** $P \leq 0.0001$. Bars represent the average of three biological replicates with standard deviation shown as error bars.


A
Monoculture MOLM-13 Viability in 3D Bio-printed
2%Alg 8%Gel hydrogels
(Fadraciclib & Azacitidine; 48hr)
B
Co-culture MOLM-13 Viability in 3D Bio-printed
2%Alg 8%Gel hydrogels
(Fadraciclib & Azacitidine; 48hr)
***
C
Monoculture MOLM-13 Viability in 3D Bio-printed
2%Alg 8%Gel hydrogels
(Fadraciclib & Cytarabine; 48hr)
D
Co-culture MOLM-13 Viability in 3D Bio-printed
2%Alg 8%Gel hydrogels
(Fadraciclib & Cytarabine; 48hr)
E
Monoculture MOLM-13 Viability in 3D Bio-printed
2%Alg 8%Gel hydrogels
(Fadraciclib & Venetoclax; 48hr)
F
Co-culture MOLM-13 Viability in 3D Bio-printed
2%Alg 8%Gel hydrogels
(Fadraciclib & Venetoclax; 48hr)
G
MOLM-13 Viability in 3D Bioprinted
2%Alg 8%Gel hydrogels (CAL-72 Co-Culture)
(Fadraciclib & Azacitidine; 48hr)
H
MOLM-13 Viability in 3D Bioprinted
2%Alg 8%Gel hydrogels (HS5 spheroid, CAL-72 Co-Culture)
(Fadraciclib & Azacitidine; 48hr)
****
*
I
MOLM-13 Viability in 3D Bioprinted
2%Alg 8%Gel hydrogels (CAL-72 Co-Culture)
(Fadraciclib & Cytarabine; 48hr)
J
MOLM-13 Viability in 3D Bioprinted
2%Alg 8%Gel hydrogels (HS5 spheroid, CAL-72 Co-Culture)
(Fadraciclib & Cytarabine; 48hr)
****
*
K
MOLM-13 Viability in 3D Bioprinted
2%Alg 8%Gel hydrogels (CAL-72 Co-Culture)
(Fadraciclib & Venetoclax; 48hr)
**
L
MOLM-13 Viability in 3D Bioprinted
2%Alg 8%Gel hydrogels (HS5 spheroid, CAL-72 Co-Culture)
(Fadraciclib & Venetoclax; 48hr)
**
**
Viability (%)
Biological replicate
Biological Replicate
Rep 1
Rep 2
Rep 3
NDC
FAD
AZA
AraC
VEN
COM

Supplementary figure 16: Assessing inter-replicate variability in 3D bioprinted MOLM-13 cells treated with selected chemotherapies. Each biological replicate was compared to the other biological replicates (n=9 technical replicates each) to assess whether the results obtained from the intermediate 3D model (A-F, no CAL-72 monolayer) and the final 3D model (G-L, with CAL-72 monolayer) were reproducible across experiments. One Way ANOVAs with multiple comparisons were completed to detect significant differences. Overall, few significant differences were found between replicates, highlighting that this model is highly reproducible. Significance: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$ **** $P \leq 0.0001$. Bars represent the mean of three biological replicates, and standard deviation depicted as error bars. Rep: replicate. FAD: fadraciclib. AZA: azacitidine. AraC: cytarabine. VEN: venetoclax. COM: combination therapy of fadraciclib plus the additional selected chemotherapy in each graph.

Supplementary Table 1: **A table summarising the drug concentrations which induce 50% apoptosis in MOLM-13 cells.**

Drug (nM)	**24h**	**48h**	**72h**
Fadraciclib	350	350	350
Azacitidine	6400	1600	1600
Cytarabine	85	85	42.5
Venetoclax	25	20	15

Notes: These concentration ranges were estimated using resazurin assays and determined using Annexin V/DAPI staining and trypan blue exclusion staining. Concentrations measured in nM.

Supplementary Table 2: Primers selected for multiplex qRT-PCR.

Primer	Gene	Forward (5'to3')	Reverse (5'to3')
ATM	Ataxia-Telangiectasia Mutated	CGGAGCTGATTGTAGCAACATACTA	CAGATAGAGCCTGAAGTACACAGAG
ATP5B	ATP synthase F1 subunit beta	TCCATCCTGTCAGGGACTATG	ATCAAACTGGACGTCCACCAC
ATR	Ataxia Telangiectasia and Rad3-related	CAGCTCTCTATGAAGGCCATTCAA	GTTCTACTGTTTCACTGTCTGTTGC
B2M	beta-2 microglobulin	GTCTTTCAGCAAGCACTGGTCT	CTTAGATGTCTCGATCCCACTT
BAD	BCL2 Associated Agonist Of Cell Death	CTTTAAGAAGGGACTTCCTCGCC	CAAGTTCCGATCCCACCAGG
BAK	BCL2 Antagonist/Killer 1	TCATCGGGGACGACATCAAC	CAAACAGGCTGGTGGCAATC
BAX	BCL2-associated X protein	GACATTGGACTTCCTCCGGG	ACAGGGACATCAGTCGCTTC
BCL2	B-cell CLL/lymphoma 2	CCCTGTGGATGACTGAGTACC	GTTCCACAAAGGCATCCCAGC
BCL6	B-cell lymphoma 6	GCACTTGGGACCACAGAGAAA	ATGCCTTGCTTCACAGTCCA
BCLXL	B-cell lymphoma extra large	AGCTGGTTTTTTTGCCAGCC	TCTCTTCTAAGATCCAAAGCC
BMF	Bcl-2-modifying factor	GAACCCCAGCGACTCTTTTA	TTTCGGGCAATCTGTACCTC
CBP	CREB-binding protein	TTCTTCCCGGAGACTGAGGT	GACAGGGAGGATGGAGGAGT
CCND1	Cyclin D1	GATCAAGTGTGACCCGGACTG	CCTTGGGGTCCATGTTCTGC
CCND2	Cyclin D2	CACCAACACAGACGTGGATTG	CTCCGACTTGGATCCGTCAC
CCND3	Cyclin D3	CAGAGCCTGCTCCGCC	CTGCTCCTCACATACCTCCA
CDC25A	Cell Division Cycle 25A	GTCTAGATTCTCCTGGGCCATTG	CAGAATGGCTCCTCTTCAGAGC
CDC25B	Cell Division Cycle 25B	GTTTGTTGGCTGCCCTGC	CGACAGGGAGGATTCGGATG
CDC25C	Cell Division Cycle 25C	AATGTAGCCCAGCACAGCTT	GTAAGGCAGCCACCTTGGAA
CDK1	Cyclin Dependent Kinase 1	ATGAAGTGTGGCCAGAAGTG	CAGAAATTCGTTTGGCTGGATCA

CDK2	Cyclin Dependent Kinase 2	GCTTGTTATCGCAAATGCTGC	GATGGGGTACTGGCTTGGTC
CDK4	Cyclin Dependent Kinase 4	GAAACTCTGAAGCCGACCAG	AGGCAGAGATTCGCTTGTGT
CDK6	Cyclin Dependent Kinase 6	CCTGCAGGGAAAGAAAAGTGC	CCTCCTCTTCCCTCCTCGAA
CDK7	Cyclin Dependent Kinase 7	GTGGCCGGACATGTGTAGTC	GCCGTAATTCGAGCACATGG
CDK9	Cyclin Dependent Kinase 9	ATGGAAAACGAGAAGGAGGGG	TAGGGGGAAGCTTTGGTTCG
CDKN1A	Cyclin Dependent Kinase Inhibitor 1A	TCTTGTACCCTTGTGCCTCG	CGGCGTTTGGAGTGGTAGAA
CDKN1B	Cyclin Dependent Kinase Inhibitor 1B	GGCTAACTCTGAGGACACGC	TGAGTAGAAGAATCGTCGGTTGC
CDKN2C	Cyclin Dependent Kinase Inhibitor 2C	AGAAACTGTCACTGGGAGCG	AGGCTCGGCCATTCTTTAGG
CDKN2D	Cyclin Dependent Kinase Inhibitor 2D	GGCAGTTCAAGAGGGTCACA	GTCCCTGCGATGGAGATCAG
CHEK1	Checkpoint Kinase 1	GGTCACAGGAGAGAAGGCAATA	GGAAGAATCTCTGAGCATCTGG
CHEK2	Checkpoint Kinase 2	AGTGGATCCAAAGGCACGTT	CCTGGGGTAGAGCTGTGGAT
CITED2	Cbp/P300-Interacting Transactivator With Glu/Asp Rich Carboxy-Terminal Domain 2	GCGAGCACATACACTACGGC	CCATGAACTGGGAGTTGTTAAACC
CKIT	KIT proto-oncogene	GCATTCAAGCACAATGGCAC	CCAATCAGCAAAGGAGTGAA
DOT1L	Disruptor of Telomeric Silencing 1-Like	CAGTGAGAAGGGCCTGAGAG	TGGAGTTGAGGTTGAGGGGA
ENOX2	Ecto-NOX disulfide-thiol exchanger 2	CACTGGCACTACCAAACTGCA	GAGCTGGAGGGAACCTGATTT

GUSB	Glucuronidase beta	GAGCAAGACAGTGGGCTGG	CCATTCGCCACGACTTTGTT
HIF1A	Hypoxia Inducible Factor 1 Subunit Alpha	TACTCATCCATGTGACCATGAGG	TAGTTCTTCCTCGGCTAGTTAGG
HOXA9	Homeobox A9	CCCCATCGATCCCAATAACCC	TCCCTGGTGAGGTACATGTTG
IRF1	Interferon Regulatory Factor 1	ACCTCTGCCTTCTTCCCTCT	CATCCGAGTGATGGGCATGT
JUN	Jun proto-oncogene, AP-1 transcription factor subunit	GGGGTTGACTGGTAGCAGAT	CTGTGTCTGTCTGTCTGCCT
MCL1	Myeloid Cell Leukaemia 1	GCCTTCCAAGGATGGGTTTG	TATGCCAAACCAGCTCCTACTC
MEIS1	Meis Homeobox 1	ATGGCGCAAAGGTACGACG	TACTGATGCGAGTGCAGAGG
MYC	MYC proto-oncogene, bHLH transcription factor	GACTCTGAGGAGGAACAAGA	TCGTCGCAGTAGAAATACGG
TP53	Tumour Protein p53	TGTGACTTGCACGTACTCCC	TCCGTCATGTGCTGTGACTG
SOX9	SRY-box transcription factor 9	ATCTCCCCCAACGCCATCTT	CTGGGATTGCCCCGAGTG
TBP	TATA-box binding protein	ATCTTTGCAGTGACCCAGCAT	CTTGCGCTGGAACTCGTCT
TP73	Tumour Protein p53	ACTTTGAGATCCTGATGAAGC	GAGGACCGGCCCGTAGGA
TYW1	TRNA-YW Synthesizing Protein 1 Homolog	CTCCTGATAGCACACAGAAAGTTTA	TGCGCTGAACGTTTTTGATCC
VEGF	Vascular Endothelial Growth Factor	AGAAGGAGGAGGGCAGAATCA	AGGGTACTCCTGGAAGATGTCC