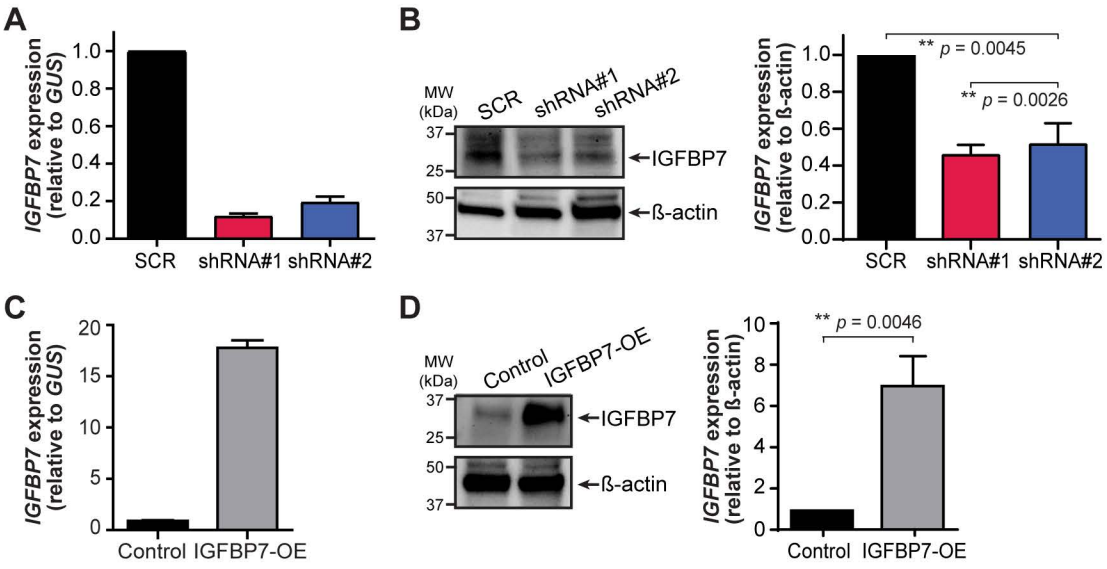
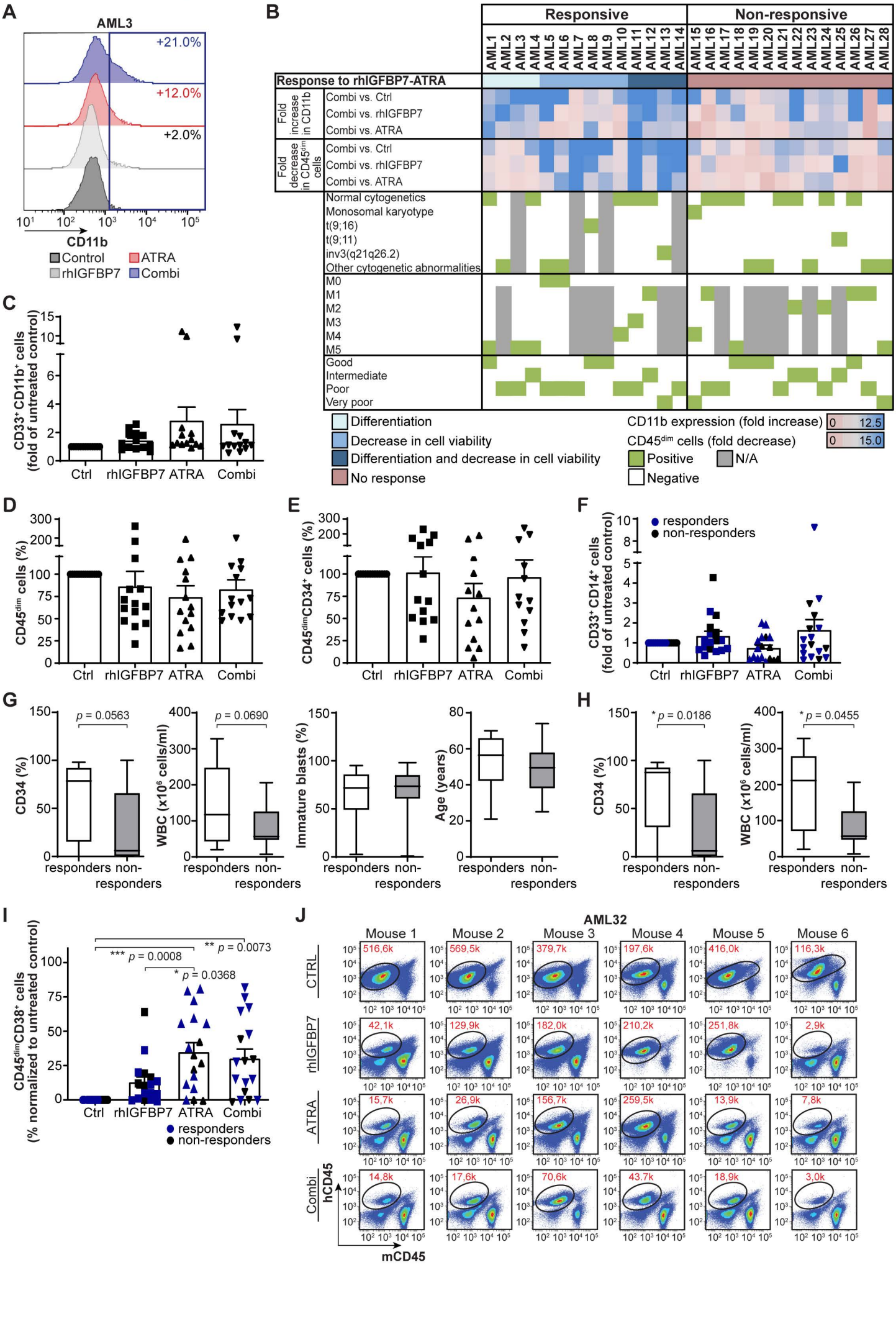
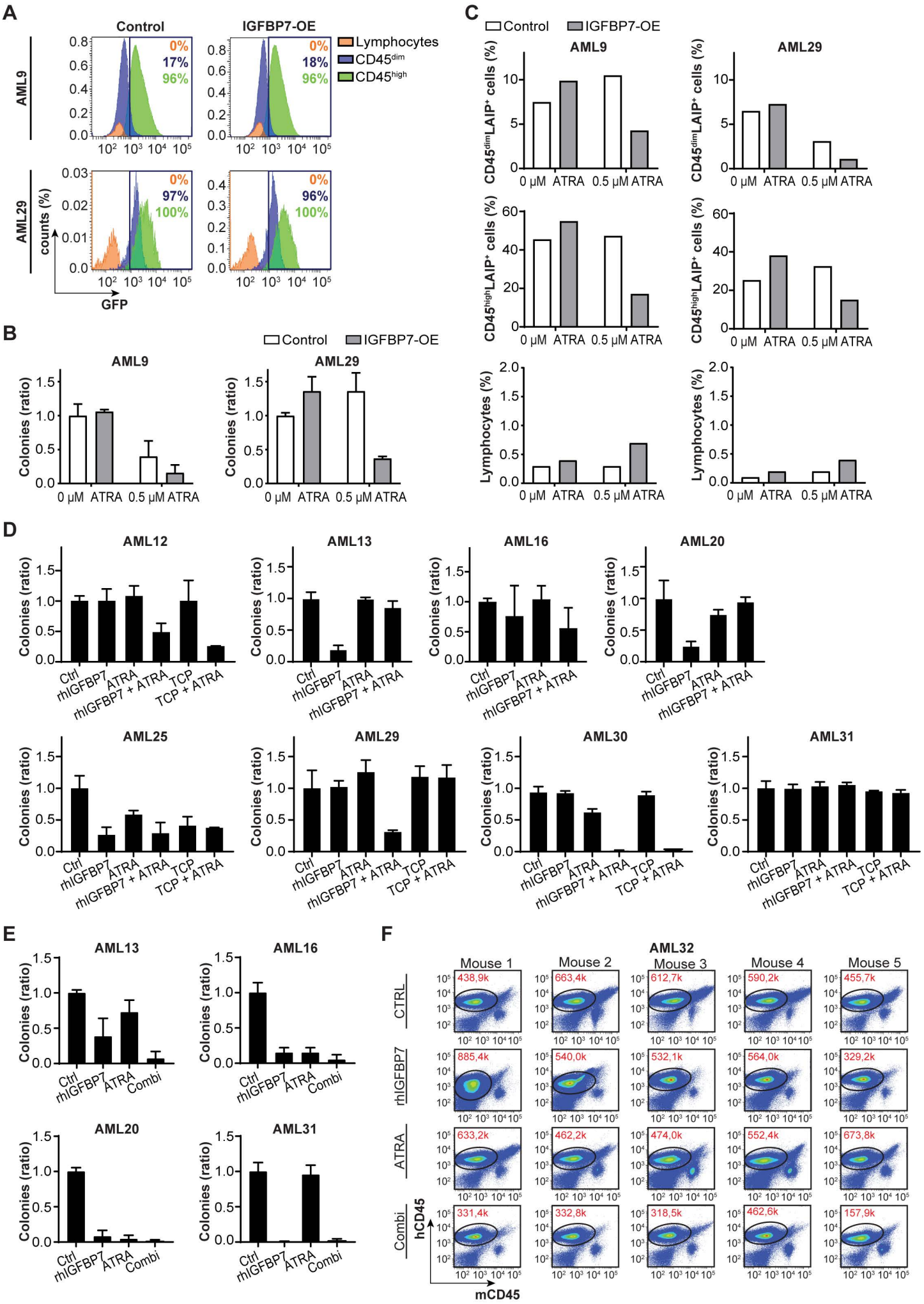
Supplemental Figure 1



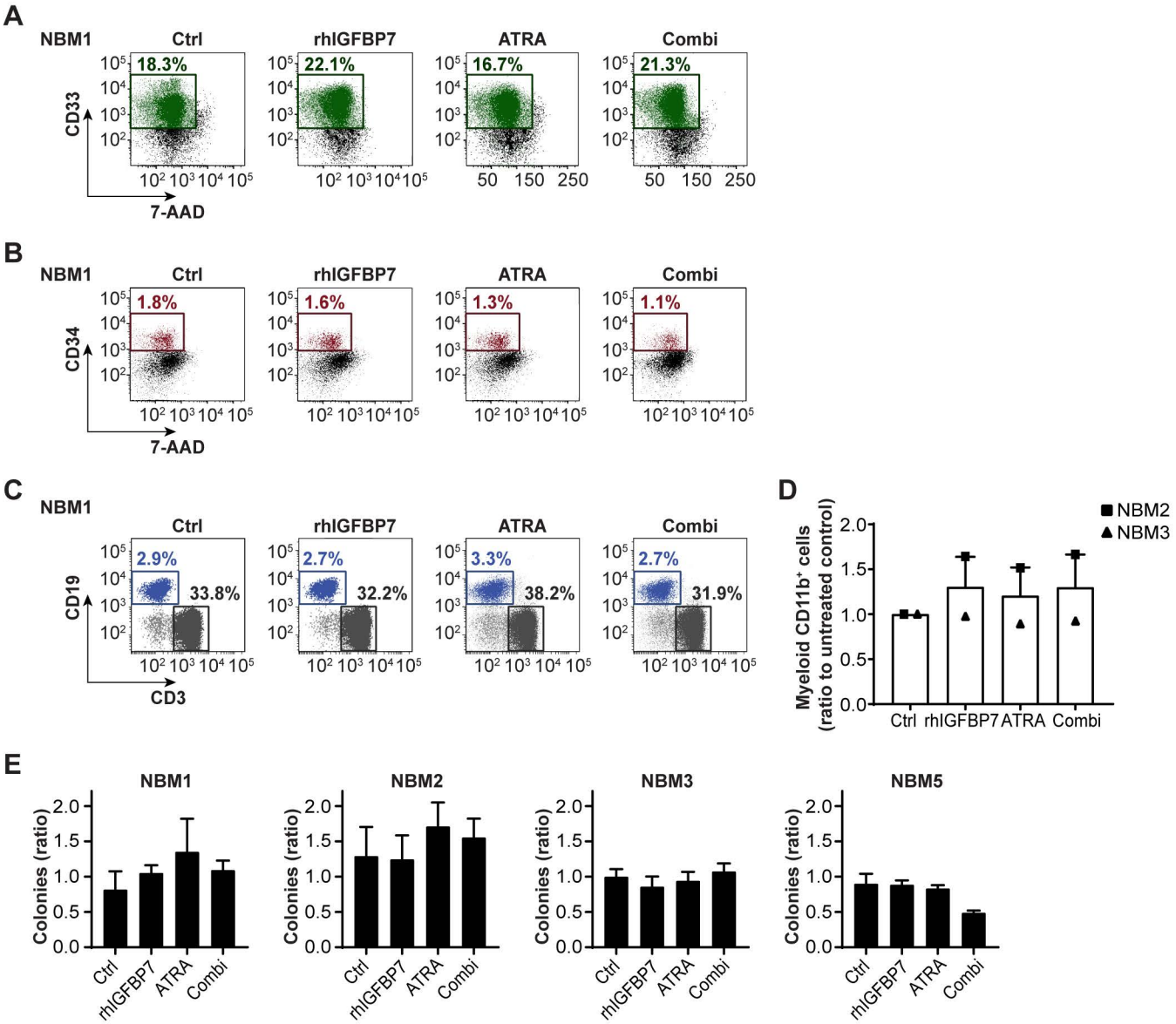
Supplemental Figure 2



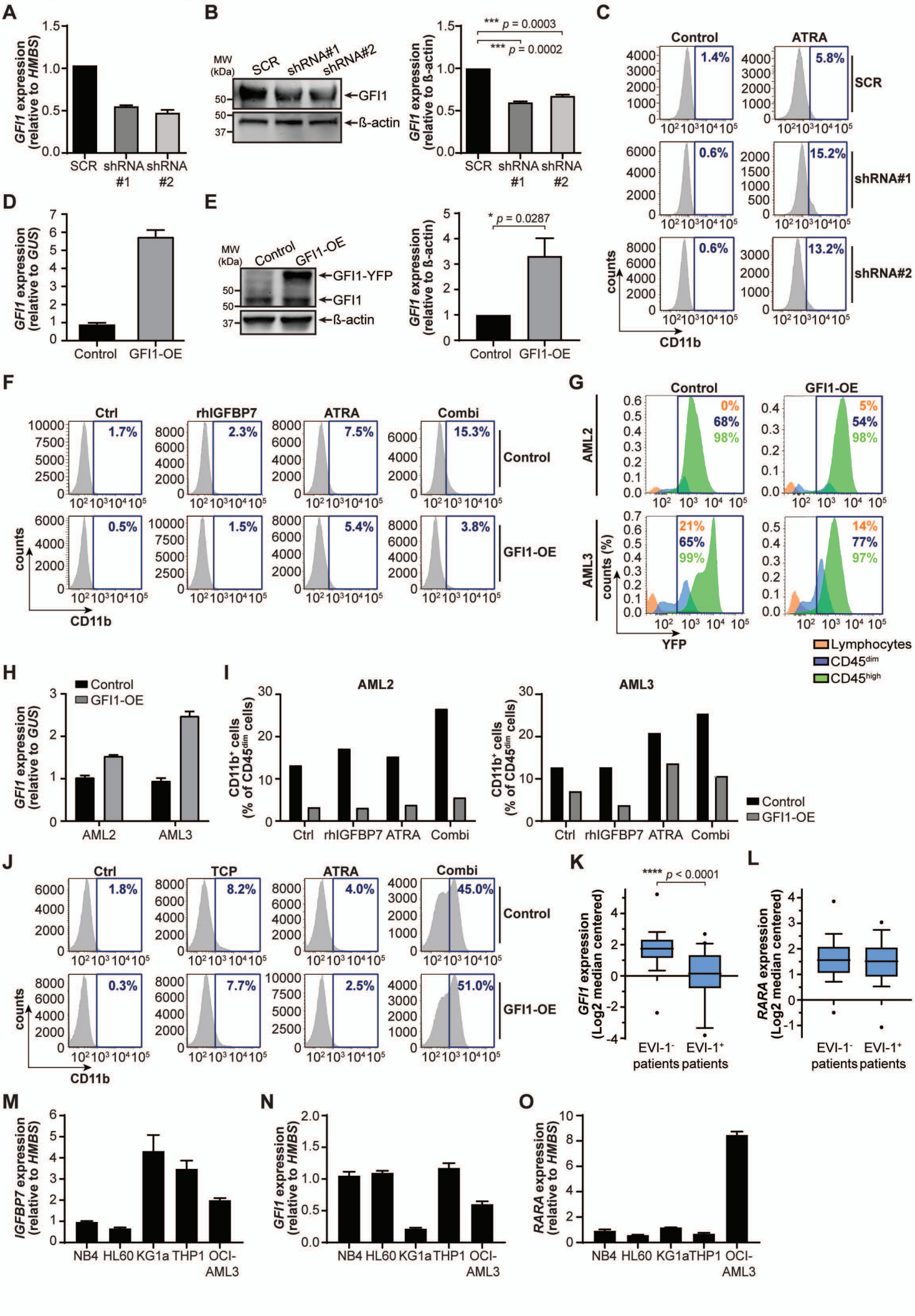
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5



Supplemental Tables

AML #	Source	FAB	Age	Sex	Blasts (%)	WBC	CD34 (%)	Cytogenetics	Risk Group	EVI1	IDH1	IDH2	DNMT3a	NPM1	FLT3-ITD	ASXL1	inv16	c-kit	JAK2	RUNX1	MLL	WT1	tet2
1	PB	M5	74	M	51	136	4.5	CN	good	-	-	-	-	+	-	-	-	-	-	-	-	-	-
2	BM		76		45	80	75	CAr	poor	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	BM	M5	77	F	81.6	43	36.6		poor	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	BM	M5	71	M	50	33	12	CN	intermediate	-	-	-	-	+	+	-	-	-	-	-	-	-	-
5	BM	M0	41	M	84	294	98	CAr	poor	-	+	-	+	-	-	-	-	-	-	-	-	-	-
6	BM	M0	51	M	62		83	CAr	poor	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	BM		47	F	2,5	231	16		poor	-	-	-	-	+	+	-	-	-	-	-	-	-	-
8	BM		58	M	82	100	92	t9;16	good	-	-	-	-	-	-	-	+	+	-	-	-	-	-
9	PB		74	F	15	328	82		good	-	-	-	-	-	-	-	+	-	-	-	-	-	-
10	BM	M4	67	F	84	41	35	CN	poor	-	-	-	-	+	+	-	-	-	-	-	-	+	-
11	PB	M3	27	M	94	20	93	CN	poor	-	-	-	-	-	-	+	-	-	-	-	-	-	-
12	BM	M1	53	F	95	117	0.07	CN	intermediate	-	-	-	+	+	+		-	-	-	-	-	-	+
13	BM		63	F	62	265	92	3q26	very poor	+	-	-	-	-	-	+	-	-	-	-	-	-	-
14	BM		39	M	92	211	93		poor	-	-	-	-	-	+	-	-	-	-	+	-	-	-
15	BM	M4	41	M	86		62	MK	very poor	+	-	-	-	-	-	-	-	-	-	-	-	+	-
16	BM	M1	44	M	84	110	2.8	CN	poor	-	-	-	+	+	+	-	-	-	-	-	-	-	-
17	BM		55	F	67	132	82	CN	poor	-	-	-	-	-	+	-	-	-	-	-	-	-	-
18	BM	M5	65	F	62	54	0.22	CN	good	-	-	-	-	+	-	-	-	-	-	-	-	-	+
19	BM		67	F	10	52,3	0.34	CAr	good	-	-	-	-	+	-	-	-	-	-	-	-	-	-
20	BM		52	F	0.76	46.8	47	CAr	good	-	-	-	-	+	-	-	-	-	-	-	-	-	-
21	PB		82	M	64	150	9.1	CN	poor	-	-	-	-	+	+	-	-	-	-	-	-	-	-
22	PB	M2	59	F	98	7	100	CN	intermediate	-	-	-	+	+	+	-	-	-	-	-	-	+	-
23	BM		61	F	56	59	0	CAr	intermediate	-	-	-	-	+	+	-	-	-	-	-	-	-	-
24	BM	M2	58	M	70		78	CN	poor	-	-	-	-	-	+	-	-	-	-	-	-	+	-
25	BM		32		77	206	0	CAr	intermediate	-	-	-	-	-	-	-	-	-	-	-	+	-	-
26	BM	M1	65	F	85	28	0.2	CN	good	-	-	-	+	+	-	-	-	-	+	-	-	-	+
27	BM	M1	54	F	88	46.2	33	CAr	poor	-	-	-	+	+	+	-	-	-	-	-	-	+	-
28	BM	M5	44	M	78	62	0.44	CAr	very poor	+	-	-	-	-	-	+	-	-	-	-	-	-	-
29	PB		72	F	14	109	55	CN		-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	BM		76	M	82	36.6	3.7	t15;17		-	-	-	-	-	+	-	-	-	-	-	-	-	-
31	BM	M5	66	F	62	89	2.6	CAr	intermediate	-	-	+	+	+	+	-	-	-	-	-	-	-	-
32	BM	M5	46	F	79		2.5	t15;17	very poor	+	-	-	+	-	-	-	-	-	-	-	+	-	-
33	BM	M1	53	M	67	60	92	CAr	very poor	+	-	-	-	-	+	+	-	-	-	+	-	-	-

Supplemental Table 1. Clinical, prognostic and genetic features of AML patients. Cells were derived from bone marrow aspirates (BM) or peripheral blood samples (PB), and measured at VU University Medical Center according to standard operating procedures recognized by ISO15189. Age is in years and FAB classification was determined based on morphology, flow cytometry and molecular aberrancies. White blood cell count (WBC) is presented in cells $x10^6$/ml BM or PB, the percentage of myeloid $CD45^{dim}$ blast cells (in total WBC) and the percentages of $CD34^+$ on blast cells was measured using flow cytometry. Samples were tested for molecular aberrancies, annotated as negative (-) or positive (+). All samples were negatively tested for PML-RARA and BCR-ABL. Cytogenetics of patient samples were classified as cytogenetically normal (CN), complex cytogenetics (CAr) or monosomal karyotype (MK). CAr ≥ 3 acquired chromosome aberrations in the absence of prognostic favorable t(8;21)(q22;q22), inv(16)(p13q22)/t(16;16)(p13;q22) and t(15;17)(q22;q21). MK = 2 or more monosomies or a single monosomy in the presence of structural abnormalities.

		CD11b$^+$ expressing CD33$^+$ blasts (%)				Survival of CD45^{dim} blasts (%)		
		Control	Combi	rhIGFBP7	ATRA	Combi	rhIGFBP7	ATRA
Responders	**AML1**	16.90	14.60	12.00	21.90	64.00	72.40	57.80
	AML2	56.60	45.50	82.90	91.30	94.00	97.00	96.00
	AML3	13.80	18.30	22.80	27.90	128.60	91.80	105.90
	AML4	18.20	51.00	52.20	59.00	39.30	41.80	43.90
	AML5	6.20	7.30	69.90	36.30	58.60	23.40	18.50
	AML6	0.19	0.51	0.70	0.39	62.90	119.80	49.60
	AML7	59.10	55.20	50.80	36.70	101.30	52.10	15.80
	AML8	22.30	25.60	14.90	24.30	82.20	41.80	28.60
	AML9	72.92	67.92	81.80	65.98	31.50	48.90	23.80
	AML10	0.19	0.51	0.70	0.29	99.10	114.20	85.30
	AML11	3.90	8.60	5.90	15.50	58.70	53.30	15.90
	AML12	10.80	22.40	44.00	51.80	53.30	48.80	38.20
	AML13	33.80	39.10	41.40	50.40	61.10	92.50	20.80
	AML14	42.20	58.30	73.30	81.90	96.73	13.16	6.95
Non-responders	**AML15**	2.08	4.83	50.80	3.96	41.10	40.00	52.20
	AML16	9.61	24.86	12.17	5.34	183.00	116.90	106.00
	AML17	7.95	14.22	18.58	13.81	44.60	58.40	65.00
	AML18	73.58	47.73	86.34	76.40	71.30	74.10	96.40
	AML19	13.60	11.60	16.90	8.40	117.90	195.00	199.70
	AML20	48.60	69.90	73.70	50.40	47.60	16.90	68.60
	AML21	3.69	3.57	6.72	4.03	83.00	34.30	47.60
	AML22	0.44	0.85	4.92	5.40	67.00	48.00	52.00
	AML23	6.07	7.47	5.54	6.72	63.70	118.90	71.90
	AML24	2.57	2.75	2.71	3.30	57.90	103.10	56.50
	AML25	78.40	88.30	75.30	87.00	262.70	56.00	73.00
	AML26	2.80	5.30	27.70	25.50	61.60	63.40	109.00
	AML27	0.00	0.00	0.00	0.00	83.60	94.80	113.80
	AML28	13.00	59.90	37.50	42.40	21.50	19.40	48.10

Supplemental Table 2. *Ex* vivo responses of primary AML samples to rhIGFBP7 and ATRA combination treatment. Primary AML samples were stimulated with PBS (Ctrl), 100 µg/ml rhIGFBP7, 0.5 µM ATRA or the combination for 7 days. Percentages of differentiation (CD33$^+$CD11b$^+$) and viable CD45^{dim} blasts were measured using flow cytometry and quantified relative to flow count beads. The percentage of CD45^{dim} blasts in the control samples was set at 100%. Responsive AML cases were defined as >5% increase in CD11b$^+$ expressing myeloid CD33$^+$ blasts and/or more than 5% reduction in CD45^{dim} blast survival upon ATRA-rhIGFBP7 combination treatment compared to the mono treatments.

	rhIGFBP7		
Gene ID	AML17	AML15	AML33
CTSG	-3.21	-3.02	-2.69
MPO	-5.10	-4.31	-1.37
NREP	-2.14	-1.28	-1.21
FLT3	-3.73	-1.38	-1.15
GFI1	-2.83	-1.01	-1.09
CD109	-1.20	-1.47	-0.82
NYNRIN	-2.03	-1.77	-0.75
MYO18A	-1.09	-0.76	-0.73
SLC17A9	-0.97	-1.67	-0.65
MED13L	-0.89	0.64	-0.60
IL6R	-1.67	-1.04	-0.59
SORL1	-4.05	-2.15	-0.56
AHNAK	-0.56	-0.60	-0.55
GSTP1	-0.51	-0.69	-0.53
CBX5	-1.81	-1.29	-0.50
PICALM	0.54	0.83	0.53
SQSTM1	1.58	0.56	0.54
STK17B	0.96	1.09	0.56
TPM4	0.68	0.99	0.58
IFITM3	1.05	3.44	0.59
TNFAIP3	1.11	0.89	0.59
PTRF	0.60	2.67	0.60
HIF1A	2.03	1.78	0.61
SLC40A1	1.01	-0.99	0.62
SLC7A5	-2.17	1.98	0.62
RUNX3	0.57	2.06	0.65
PTPN1	1.19	2.11	0.65
PPFIBP1	1.34	1.26	0.66
S100A6	1.23	0.65	0.66
CTSB	1.41	1.23	0.67
ACSL4	1.24	1.03	0.67
ENG	1.84	1.23	0.67
FTH1	3.70	3.06	0.71
VASP	0.80	0.72	0.71
FASN	-0.71	-0.57	0.72
FMNL3	1.28	1.54	0.74
PTGS1	0.66	1.89	0.74
CCND3	0.61	1.22	0.75
PIM1	0.90	1.88	0.75
SLC2A3	1.49	1.04	0.78
TMBIM1	1.20	0.87	0.79
SOD2	4.10	4.16	0.81
MCL1	1.42	0.77	0.81
DSE	1.12	1.16	0.83
CSF2RB	1.40	2.99	0.85
CMIP	0.86	1.36	0.86
GRINA	1.32	1.78	0.90
NAMPT	3.99	3.98	0.91
IL4R	2.30	1.94	0.92
PVRL2	0.84	1.39	0.92
SAT1	2.72	2.15	0.95
SEMA7A	2.38	3.08	0.96
MAOA	2.80	4.74	0.96
ABCA1	2.29	1.28	0.97
NRP1	2.42	1.18	1.14
RNF19B	3.04	3.68	1.15

	rhIGFBP7		
Gene ID	AML17	AML15	AML33
ECE1	0.90	3.56	1.17
ARHGAP31	1.85	1.33	1.19
PFKFB3	3.71	3.23	1.20
SLC7A11	2.41	3.87	1.24
GATA2	1.49	2.98	1.26
JUNB	2.25	0.90	1.27
BHLHE40	1.86	2.72	1.31
VASH1	0.52	1.17	1.32
ICAM1	2.64	3.30	1.34
STRIP2	2.10	1.40	1.35
SKIL	0.86	2.04	1.37
CDKN1A	2.76	1.65	1.39
SEMA4A	1.16	1.51	1.39
PTAFR	2.29	1.52	1.44
SLC16A3	1.22	1.28	1.44
TIMP1	3.14	2.32	1.47
RGS1	4.13	3.30	1.58
VEGFA	1.98	2.13	1.70
PTGS2	6.72	4.23	1.79
KIFC3	1.31	2.36	1.86
CLEC5A	2.06	-1.29	1.87
SH3PXD2B	3.77	3.48	1.88
SOCS3	3.78	2.13	1.89
FCGR2A	3.75	3.51	1.89
INHBA	3.60	5.44	2.02
DUSP5	2.09	3.63	2.08
THBS1	2.52	6.70	2.14
ADAM19	2.02	4.74	2.14
FOSB	3.49	0.99	2.16
AGRN	1.51	1.54	2.26
MMP14	2.71	4.57	2.35
CTSL	4.47	2.94	2.43
CD163	5.88	1.63	2.43
IL1RN	3.76	3.04	2.43
S100A8	5.19	3.43	2.45
C3	2.98	3.29	2.59
NCS1	2.35	3.61	2.60
IL21R	2.28	2.18	2.61
CD300E	4.81	2.51	2.82
CCL2	6.32	7.71	2.92
PHLDA1	3.29	2.44	3.07
FPR1	3.39	2.88	3.09
VCAN	4.36	2.39	3.12
CXCL3	6.49	5.55	3.17
SLC11A1	2.75	2.86	3.35
PPBP	2.37	5.78	3.40
CXCL8	6.48	4.67	3.43
SEMA6B	3.98	2.87	3.45
S100A9	6.87	3.02	3.48
CD14	5.44	2.93	3.78
MMP9	5.17	4.00	3.79
IL7R	4.57	4.41	3.92
IL1B	7.18	6.22	4.07
CXCL5	10.26	10.10	6.66
CXCL13	7.69	8.95	8.41

Supplemental Table 3. Top genes differentially expressed in primary AML cells stimulated with rhIGFBP7. Gene expression profiles of 3 primary AML samples stimulated with 100 µg/ml rhIGFBP7 for 48 hours, using RNA sequencing on $CD45^{dim}$ cells. Genes with a significant differential expression (p<0.01) and average Log2FC<-0.5 or >+0.5 were selected. Patient sample characteristics are summarized in Supplemental Table 1.

Supplemental Information

Supplemental Methods

Supplemental Figures

Supplemental Tables

Supplemental Methods

Culture conditions of cell lines and primary cells

HL60, NB4 and THP1 cells were cultured in Roswell Memorial Institute-1640 (RPMI-1640) with 10% fetal calf serum (FCS). KG1a and OCI-AML3 cells were cultured in RPMI-1640 with 20% FCS. Cell lines under low serum conditions were cultured in RPMI-1640 with 1-5% FCS. HEK293T cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% FCS. All cell lines were cultured in a humidified atmosphere at 37°C and 5% CO_2.

Mononuclear cells were isolated using Ficoll-Paque Plus (Amersham Biosciences) separation and cryopreserved in liquid nitrogen. Samples were thawed in Iscove's Modified Dulbecco's Medium (IMDM) with 20% FCS and incubated with 10 mg/ml DNase I (Roche) and 10 mM magnesium chloride (Sigma Aldrich) for 30 min. Samples were cultured in IMDM with 15% BIT 9500 serum substitute (BIT; Stemcell Technologies), 50 ng/ml FLT3L (Peprotech), 20 ng/ml IL3 (Peprotech), 100 ng/ml human SCF (Peprotech), and 20 ng/ml G-CSF (Miltenyi Biotec) in a humidified atmosphere at 37°C and 5% CO_2.

RNA isolation and Q-RT-PCR

RNA was isolated using TRIzol (Thermo Fisher Scientific) according to the manufacturer's protocol. For cDNA synthesis, equal amounts of total RNA quantified using a Nanodrop (Thermo Fisher) were incubated at 65°C for 5 minutes. RNA was incubated with 1x reverse transcriptase buffer (Invitrogen), 1 mM DTT (Invitrogen), 1 mM dNTPs (Roche), 1.89 µg pdN6 (Roche), 300 U/µl M-MLV reverse transcriptase (Invitrogen) and 40 U/µl RNAsin (Sphaero Q) in nuclease free water, and incubated at 37°C for 2 hours and 65°C for 10 minutes. *IGFBP7*, *GFI1* and *RARA* Q-RT-PCR reactions were performed in triplicate with 2 µl cDNA [1 µg] , 10 µl TaqMan Gene Expression Master Mix (Applied Biosystems), 1 µl *IGFBP7, GFI1*, or *RARA* TaqMan Gene Expression Assay (20x) probe mix (Hs00266026_m1, Hs00382207_m1, or Hs00940446_m1 respectively, Applied Biosystems) and 7 µl nuclease free water. Expression of housekeeping gene *GUS* (Fw primer [0.3µM] 5'-GAA AAT ATG TGG TTG GAG AGC TCA

TT-3', Rv primer [0.3μM] 5'-CCG AGT GAA GAT CCC CTT TTT A-3' and probe [0.2 μM] 5'-CCA GCA CTC TCG TCG GTG ACT GTT CA-3') or *HMBS* (Fw primer [0.3 μM] 5'-GGC AAT GCG GCT GCA A-3', Rv primer [0.3 μM] 5'-GGG TAC CCA CGC GAA TCA C-3' and probe [0.2 μM] 5'-CAT CTT TGG GCT GTT TTC TTC CGC C-3') was used as a control. Q-RT-PCR reactions were performed on an ABI7500 (Applied Biosystems): 45 cycli at 95°C for 15 seconds and 60°C for 1 minute.

PDX AML mouse model

Two weeks after intravenously injection of T-cell depleted AML cells in the tail vain of NSG mice, a 21-day-release 10 mg ATRA pellet or placebo pellet (Innovative Research of America) was subcutaneously implanted in the neck close to the posterior triangle. The procedure was performed under anesthesia (induction with 3% isoflurane and maintenance with 2% isoflurane) for a maximum of 3 minutes on a 30°C heating pack to maintain normothermia. Because ATRA influences appetite of mice, soft Teklad food (Harlan Laboratories) was provided during the 21 days of treatment. To evaluate the ability of ATRA and rhIGFBP7 combination treatment to reduce leukemic engraftment, 12 mg/kg rhIGFBP7 or PBS was intravenously injected for 3 consecutive days in week 5 (day 1-3) after injection of the primary AML cells. Mice were sacrificed when disease features such as >20% weight loss were observed or at the endpoint at 16 weeks. Bone marrows were analyzed for the presence of human cells using a FACS-Canto flow cytometer (BD Biosciences). Data analysis was performed using FACS Diva software (BD Biosciences).

For secondary transplants of AML-engrafted mice, NSG mice (first recipients) were treated with ATRA and rhIGFBP7. After injection of T-cell depleted primary AML cells, mice were treated with a single dose of 12 mg/kg rhIGFBP7 or PBS in week 4, followed by treatment with 12 mg/kg rhIGFBP7 or PBS for 3 consecutive days in week 8 (day 1-3) and/or implantation of the 21-day-release 10 mg ATRA pellet or placebo pellet in week 8 (day 3). Equal numbers of human myeloid ($hCD45^+CD33^+$) pooled BM cells derived from first recipients in week 16 were

intravenously injected into the tail vain of irradiated female NSG mice. AML engraftment of secondary recipients was assessed in week 16 as described above.

Molecular diagnostics and cytogenetic analysis

From isolated mononuclear cells, DNA and/or RNA was studied for the presence of t(9;22), t(8;21), t(15;17), inv16, and mixed-lineage leukemia (MLL) translocations, CEBPα, FLT3-ITD, NPM1, IDH1/2, DNMT3a, c-kit, Jak2, RUNX1, ASXL1, WT1, and tet2 mutations, and EVI1 overexpression by PCR according to standard procedures (www.modhem.nl) recognized by ISO15189. Cytogenetics were determined according to standard techniques and karyotypes were described according to the International System for Human Cytogenetic Nomenclature.

Production of recombinant human IGFBP7

rhIGFBP7 was produced and purified as described previously [30]. In short, conditioned medium from pcDNA3.1(-A)-IGFBP7-his-myc transduced Cos-7 cells was harvested and concentrated using Amicon Ultra-15 10k centrifugal filters (Merck Millipore). Concentrated medium was loaded onto 5 ml HiTrap Chelating Sepharose Nickel columns (GE Healthcare). After multiple washing steps, purified rhIGFBP7 protein was eluted with 20 mM Tris-HCl (pH 8.0) and 500 nM NaCl.

RNA sequencing

Total RNA was extracted with TRIzol (Thermo Fisher Scientific) according to the manufacturer's protocol. The quality of RNA was measured on a 2100 Bioanalyzer (Agilent). Sequencing libraries, each with individual Illumina indexes, were constructed using the TruSeq™ Stranded mRNA procedure (Sample Prep Kit v2; Illumina). A mixture of 10 pM libraries were pooled equimolar and the resulting DNA was clustered onto a V3 flow cell lane using a c-Bot cluster station and subsequently sequenced in single read fashion for 50bp using the TruSeq SBS v3-Kit and HiSeq2000 Sequencing System (Illumina). Sequence reads were aligned to the human reference genome. The R package DEGseq was used to determine

differentially expressed genes between purified samples. For interpretation of gene expression data, functional pathway analysis was performed using DAVID (v6.8).

Supplemental Legends

Supplemental Figure 1. Enhanced IGFBP7 expression sensitizes APL cells to physiological concentrations of ATRA

For Q-RT-PCR (in triplicate), *IGFBP7* expression was measured relative to *GUS* expression, and for immunoblot analysis, IGFBP7 levels were quantified relative to β-actin expression. Expression levels were normalized against control cells and plotted as mean ± SEM, immunoblots are representative of at least three independent experiments. (A and B) NB4 cells were lentivirally transduced with IGFBP7-shRNAs (sh#1 and sh#2) or scrambled (SCR)-shRNA. (A) *IGFBP7* expression measured by Q-RT-PCR. (B) Immunoblot analysis of IGFBP7 (left), quantified relative to β-actin expression (right). *p*-values were calculated using a One-way ANOVA with post-hoc Tukey's multiple comparison test. (C and D) NB4 cells were lentivirally transduced with control or IGFBP7-expressing (IGFBP7-OE) vectors. (C) *IGFBP7* expression measured by Q-RT-PCR. (D) Immunoblot analysis of IGFBP7 (left), quantified relative to β-actin expression (right). *p*-values were calculated using a Student's t-test.

Supplemental Figure 2. rhIGFBP7 activates ATRA-driven responses in non-APL AML cells

For all *ex vivo* experiments, cells were stimulated with PBS (Ctrl), 100 µg/ml rhIGFBP7, 0.5 µM ATRA or the combination for 7 days. Percentages of differentiation ($CD33^+CD11b^+$), viable $CD45^{dim}$, $CD45^{dim}CD34^+$ and $CD45^{dim}CD38^+$ cells were measured using flow cytometry, quantified relative to flow count beads, normalized against untreated control cells, and plotted as mean ± SEM. *p*-values were determined using a One-way ANOVA with post-hoc Tukey's multiple comparison test. Patient sample characteristics are summarized in Supplemental Table 1. (A) CD11b membrane expression on $CD33^+$ blasts relative to the untreated control sample in AML3. (B) Heat-map of prognostic features of 28 primary AML samples. Responsive AML cases were defined as >5% increase in $CD11b^+$ expressing myeloid $CD33^+$ blasts and/or more than 5% reduction in $CD45^{dim}$ blast survival upon ATRA-rhIGFBP7 combination treatment

compared to the mono treatments. The fold increase in CD11b and fold decrease in $CD45^{dim}$ cells represent the ratio of CD11b expressing $CD33^+$ blasts and the ratio of reduction in $CD45^{dim}$ blast survival following combination treatment relative to untreated control or mono therapies. Percentages are shown in Supplemental Table 2. N/A, not available. (C – E) Percentages of viable (C) $CD33^+CD11b^+$, (D) $CD45^{dim}$ and (E) $CD45^{dim}CD34^+$ cells in 14 primary AML samples non-responsive to rhIGFBP7 and ATRA combination treatment. (F) CD14 expression on $CD33^+$ blasts relative to untreated control in 11 responsive (blue) and 6 non-responsive (black) AML cases. (G) Percentage of CD34 positive blasts, WBC ($x10^6$ cells/ml), percentage of immature blasts and age of patients at diagnosis, in patients whose AML cells responded (by induction of CD11b expressing $CD33^+$ blasts and/or reduction in $CD45^{dim}$ blasts survival) or not responded to rhIGFBP7 and ATRA combination treatment or (H) in patients whose AML cells only responded by a reduction in $CD45^{dim}$ blast survival vs. not responded after rhIGFBP7 and ATRA combination treatment. *p*-values were determined using a Student's t-Test. (I) The percentage of $CD45^{dim}CD38^+$ cells in 11 responsive (blue) and 6 non-responsive (black) AML cases. (J) NSG mice were treated with ATRA (10 mg, 21-day-release pellet) in week 2 and/or rhIGFBP7 (12 mg/kg) in week 5 (day 1-3) post injection of T-cell depleted primary AML32 cells. At week 16, the bone marrow cells of the mice were analyzed for the presence of human $CD45^+$ cells using flow cytometry, and depicted as absolute counts in thousands (red).

Supplemental Figure 3. Enhanced IGFBP7 expression, or treatment with rhIGFBP7 induces sensitivity for ATRA in primary AML stem and progenitor cells

For all *ex vivo* experiments, cells were incubated with PBS (Ctrl), 100 µg/ml rhIGFBP7, 0.5 µM ATRA, 10 µM TCP or the combinations. For CFU progenitor and long-term liquid culture (stem cell) assays, samples (in duplicate) were incubated for 7 days or 4 weeks, respectively, normalized against untreated controls and plotted as mean ± SD. Patient sample characteristics are summarized in Supplemental Table 1. (A – C) AML9 and AML29 were lentivirally transduced with control or IGFBP7-OE vectors. (A) Transduction efficiencies

indicated as percentages of GFP^+ lymphocytes (orange), $CD45^{dim}$ cells (blue) and $CD45^{high}$ cells (green), measured 4-10 days post transduction using flow cytometry. (B) CFU progenitor assays of primary AML samples incubated with ATRA or PBS. (C) Flow cytometric analysis of AML cells derived from the colonies, showing the percentage of $CD45^{dim}LAIP^+$ (top), $CD45^{high}LAIP^+$ (middle) cells and lymphocytes (bottom). CFU plates, containing all colonies, were harvested before flow cytometric analysis. (D) CFU progenitor assays of primary AML samples incubated with indicated drugs. (E) CFU stem cell assays of primary AML samples incubated with rhIGFBP7 and ATRA. (F) NSG mice were treated with rhIGFBP7 (10 mg/kg) in week 4 (day 1) and 8 (day 1-3) and/or ATRA (10 mg, 21-day-release pellet) in week 8 (day 3) post injection of T-cell depleted primary AML cells. Equal numbers of human myeloid $hCD45^+CD33^+$ cells derived from the 1^{st} transplant (isolated in week 16) were injected into secondary recipients. At week 16, the bone marrow cells of the mice were analyzed for the presence of human $CD45^+$ cells, as depicted as absolute counts in thousands (red).

Supplemental Figure 4. rhIGFBP7 and ATRA combination treatment does not affect healthy normal bone marrow cells

Normal bone marrow (NBM) cells derived from healthy donors were incubated with PBS (Ctrl), 100 µg/ml rhIGFBP7, 0.5 µM ATRA or the combination for 5-7 days. The effects of the treatments were normalized against untreated control cells. (A – C) Example of NBM sample analyzed for the percentage of (A) $CD45^{dim}CD33^+$ (green), (B) $CD45^{dim}CD34^+$ (red), (C) $CD3^+$ T-cells (grey) and $CD19^+$ B-cells (blue), measured using flow cytometry and quantified relative to flow count beads. (D) Percentage of viable $CD11b^+$ myeloid cells in two NBM samples, measured using flow cytometry, quantified relative to flow count beads and plotted as mean ± SEM. (E) CFU progenitor assays of NBM samples (in duplicate) after 7 days of treatment, plotted as mean ± SD.

Supplemental Figure 5. rhIGFBP7 induces susceptibility for ATRA by reducing *GFI1* expression

AML cells were stimulated with 100 µg/ml rhIGFBP7, 0.5 µM ATRA, 10 µM TCP or the combinations for 4 (HL60) or 7 days (primary AML). Induction of differentiation (membrane CD11b expression) was measured using flow cytometry and quantified relative to flow count beads. Immunoblots and histograms are representative of at least three independent experiments, expression levels were normalized against control cells and plotted as mean ± SEM, and *p*-values were determined using a Student's t-Test, unless stated otherwise. Patient sample characteristics are summarized in Supplemental Table 1. (A – C) HL60 cells were lentivirally transduced with GFI1-shRNAs (sh#1 and sh#2) or scrambled (SCR)-shRNA. (A) *GFI1* expression relative to *HMBS* expression, measured by Q-RT-PCR (in triplicate) and normalized against SCR. (B) Immunoblot analysis of GFI1 (left), quantified relative to β-actin expression (right), representative of two independent experiments. *p*-values were determined using a One-way ANOVA with post-hoc Dunnett's multiple comparison test. (C) The percentage of membrane CD11b (blue) in transduced ($Venus^+$) HL60 cells after ATRA treatment. (D – J) AML cells were lentivirally transduced with control-YFP (Control) or GFI1-YFP (GFI1-OE) expressing vectors. (D) *GFI1* expression in HL60 cells, measured relative to *GUS* expression using Q-RT-PCR (in triplicate). (E) Immunoblot analysis of GFI1 (left), quantified relative to β-actin expression (right) in HL60 cells. GFI1 expression was calculated as the average between GFI1 and GFI1-YFP. (F) Flow cytometric analysis of the percentage of CD11b (blue) after treatment of HL60 cells. (G) Transduction efficiencies, indicated as percentages of YFP^+ lymphocytes (orange), $CD45^{dim}$ cells (blue) and $CD45^{high}$ cells (green), measured 14 days post transduction of two primary AML samples. (H) *GFI1* expression relative to *GUS* expression, measured by Q-RT-PCR (in triplicate) 7 days post transduction of two primary AML samples and plotted as mean ± SD. (I) Percentage of CD11b expression in the transduced (YFP^+)$CD45^{dim}$ cell population of two primary AML samples, measured 14 days post transduction. (J) Flow cytometric analysis of the percentage of CD11b expression (blue) after treatment of HL60 cells. (K – L) Log2 median centered expression of (K) *GFI1* and (L)

RARA in EVI-1 negative (n=262) and EVI-1 positive (n=22) AML patients, using the dataset of Valk *et al.* [43]. (M – O) Expression levels of (M) *IGFBP7*, (N) *GFI1* and (O) *RARA* in AML cell lines, measured relative to *HMBS* using Q-RT-PCR (in triplicate) and normalized to expression levels in NB4 cells.