Supporting Information

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Fig. S1. Effects of inhibition of retinoic acid (RA) signaling on committed and primitive hematopoietic progenitor cells. CD34⁺CD38⁻ cells were incubated with thrombopoietin, stem-cell factor, and Flt3-ligand (TSF) alone or with the addition of 1 µM AGN194310 (AGN) and/or 0.1 µM all-*trans* RA (ATRA). (A) Colony-Legend continued on following page

forming unit cells CFU-Cs reached a plateau at approximately day 21 in cultures with TSF alone. The addition of AGN resulted in higher overall CFU-C output at day 28 of liquid culture [61.7 ± 8.1 (AGN) vs. 40.1 ± 7 (TSF)]. (B) AGN-containing cultures also produced higher CD34⁺CD38⁻ expansion at day 28 [2.1 ± 0.5 (AGN) vs. 0.8 ± 0.3 (TSF)]. (C) AGN-containing cultures conditions also produced significantly higher numbers of week 8 cobblestone area forming cells (CAFCW8) compared with TSF alone at every time point. (D) ATRA significantly reduced CAFCW8 recovery after 21 days of culture. Data are presented as mean \pm SEM of three independent experiments. *P* values shown in *A*–C are for TSF versus TSF + AGN.



Fig. S2. Flow cytometric analysis of human engraftment. Representative flow cytometric analysis of human engraftment in the bone marrow of NSG mice, 18 to 20 wk after transplant. *Left* is from a mouse not transplanted with human cells (negative control), *Center* and *Right* are from mice transplanted with human cells cultured in TSF alone (*Center*) or in TSF + AGN (*Right*) for 7 d. Human engraftment was assessed as the percentage of human CD45⁺ cells from total (human and mouse) CD45⁺ cells.

Table S1. Target gene expression and predicted regulation by RA receptor $\boldsymbol{\alpha}$

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Gene symbol	Affymetrix transcript ID	Refseq ID	Fold change*	Predicted regulation [†] (literature findings)
RARB	2614369	NM_000965	-1.217	Up-regulates (74)
RARA	3720921	NM_000964	1.758	Up-regulates (24)
CD38	2719656	NM_001775	-1.410	Up-regulates (10)
POU5F1	2948863	NM_203289	-1.438	Up-regulates (10)
SLC10A1	3570266	NM_003049	-1.554	Up-regulates (9)
TRH	2641901	NM_007117	-1.478	Up-regulates (9)
APOC3	3350655	NM_000040	-1.139	Up-regulates (8)
RBP2	2697839	NM_004164	-1.406	Up-regulates (8)
CRABP2	2438458	NM_001878	-1.181	Up-regulates (7)
PENK	3136271	NM_001135690	-1.040	Up-regulates (6)
SFTPB	2562435	NM_000542	-1.337	Up-regulates (6)
MAOB	4006210	NM_000898	-1.254	Up-regulates (5)
	3391053	NIM_001064	-1.068	Up-regulates (4)
	2050001	NIVI_001964	1.057	Op-regulates (4)
	3209720	NM_0051/0	4.800	Up-regulates (3)
RRP1	2697863	NM 001130992	-1.051	Up-regulates (3)
FPO	3015778	NM 000799	-1 226	Up-regulates (2)
FOLR2	3339382	NM_000803	-1.220	Up-regulates (2)
HNF1B	3754797	NM 000458	-1.179	Up-regulates (2)
HOXA1	3042730	NM 005522	-1.161	Up-regulates (2)
KIRREL2	3830925	NM 199180	-1.297	Up-regulates (2)
MMP11	3939470	NM_005940	-1.178	Up-regulates (2)
NEDD9	2941784	NM_001142393	-1.190	Up-regulates (2)
RET	3243846	NM_020975	-1.111	Up-regulates (2)
TP63	2657665	NM_003722	-1.166	Up-regulates (2)
TSHB	2353067	NM_000549	-1.100	Up-regulates (2)
ACACA	3754469	NM_198839	-1.180	Up-regulates (1)
ALDH1A2	3626312	NM_003888	-1.185	Up-regulates (1)
CDX1	2835368	NM_001804	-1.060	Up-regulates (1)
CEBPB	3888613	NM_005194	1.048	Up-regulates (1)
CLMN	3577940	NM_024734	-1.189	Up-regulates (1)
DUSP1	2887309	NM_004417	2.123	Up-regulates (1)
FOXP3	4008011	NM_014009	-1.141	Up-regulates (1)
GAP43	2637112	NM_001130064	-1.249	Up-regulates (1)
GATA4	3086100	NIM_002052	-1.130	Up-regulates (1)
	2042777		1.095	Op-regulates (1)
	2516834	NM_002141	_1.195	Up-regulates (1)
110/010	2878688	NM_002188	-1 325	Un-regulates (1)
11.4	2828699	NM_000589	-1 243	Up-regulates (1)
IL5	2875384	NM 000879	-1.106	Up-regulates (1)
IRF1	2875348	NM 002198	1.021	Up-regulates (1)
JUP	3757329	NM_002230	1.467	Up-regulates (1)
MAFB	3905875	NM_005461	-1.009	Up-regulates (1)
MYCN	2470805	NM_005378	-1.033	Up-regulates (1)
MYCN	2470838	NM_005378	-1.033	Up-regulates (1)
NAV2	3323052	NM_182964	-1.181	Up-regulates (1)
NTRK1	2361761	NM_001007792	-1.140	Up-regulates (1)
OAS1	3432438	NM_016816	1.649	Up-regulates (1)
PCK1	3890640	NM_002591	-1.247	Up-regulates (1)
SMPD1	3318666	NM_000543	1.032	Up-regulates (1)
STAT1	2592268	NM_007315	-1.019	Up-regulates (1)
STRA6	3632806	NM_022369	-1.265	Up-regulates (1)
TH	3359180	NM_199292	-1.217	Up-regulates (1)
OXT	3874198	NM_000915	-1.015	Regulates (9)
CXCR5	3351675	NM_001716	-1.182	Regulates (7)
THRD	3901041	NM_000361	-1.166	Regulates (/)
	2905169	NK_03/150	2.60/	Regulates (6)
	3400318 2672140	INIVI_003297	-2.U0 l	Regulates (5)
	2072140	INIVI_UUZ343	-1.150	Regulates (4)
	3202086	NM 000030	1.21U _1 190	Regulates (4)
	5552500	11101_000033	=1.105	negulates (5)

Table S1. Cont.

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Gene symbol	Affymetrix transcript ID	Refseq ID	Fold change*	Predicted regulation [†] (literature findings)
ADH1C	2779271	NM_000669	-1.404	Regulates (2)
APOM	2902531	NM_019101	-1.122	Regulates (2)
CCNA1	3485674	NM_003914	-1.215	Regulates (2)
HOXB1	3761274	NM_002144	-1.073	Regulates (2)
TNFRSF10B	3127703	NM_003842	1.381	Regulates (2)
ABCA1	3218528	NM_005502	-1.371	Regulates (1)
CALB1	3144033	NM_004929	-1.244	Regulates (1)
CEBPE	3557198	NM_001805	-1.166	Regulates (1)
CSF3	3720675	NR_033662	-1.011	Regulates (1)
CYP7A1	3136756	NM_000780	1.050	Regulates (1)
F12	2888741	NM_000505	-1.076	Regulates (1)
FASLG	2367599	NM_000639	1.097	Regulates (1)
FUT4	3345142	NM_002033	-1.122	Regulates (1)
GLI1	3418120	NM_005269	-1.240	Regulates (1)
HOXA5	3042849	NM_019102	1.005	Regulates (1)
KRT15	3757078	NM_002275	-1.149	Regulates (1)
LAMB1	3067302	NM_002291	-1.198	Regulates (1)
UBA7	2674762	NM_003335	1.190	Regulates (1)
UCP1	2787073	NM_021833	1.045	Regulates (1)
UCP3	3381843	NM_003356	-1.439	Regulates (1)
Gh	3766512	NM_000515	-1.333	Down-regulates (13)
TGFB1	3863021	NM_000660	1.149	Down-regulates (3)
ACACB	3430959	NM_001093	1.280	Down-regulates (2)
CLOCK	2769947	NM_004898	1.363	Down-regulates (2)
NRP1	3284302	NM_003873	-1.003	Down-regulates (2)
EGR2	3291601	NM_000399	2.423	Down-regulates (1)
IFNG	3461105	NM_000619	-1.204	Down-regulates (1)
IL6	2992576	NM_000600	-1.196	Down-regulates (1)
MGP	3445741	NM_001190839	-1.071	Down-regulates (1)
PTGS2	2448382	NM_000963	10.999	Down-regulates (1)
RARG	3456081	NM_000966	-1.030	Down-regulates (1)
TERT	2845829	NM_198253	-1.196	Down-regulates (1)
TNF	2902416	NM_000594	-1.918	Down-regulates (1)

*Average fold change between matched CD34⁺CD38⁻ and CD34⁺CD38⁺ cells from five normal marrow donors. Positive numbers indicate up-regulation in CD34⁺CD38⁻ cells compared with CD34⁺CD38⁺ cells.

[†]Predicted direction of regulation for each gene when RARα is activated. "Regulates" indicates that there is evidence that the gene is regulated by RARα but the direction of regulation is not fully known. Data are predicted by IPA software (Ingenuity Systems).

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Group	Cell dosage	+/total	SRC frequency	<i>P</i> vs. D0	P vs. TSF	
D0	2,000	10/14	1:1,758		_	
	500	3/15				
D7 TSF	2,000	8/10	1:1,352	0.5	—	
	1,524	4/5				
	500	2/10				
	381	1/5				
D7 AGN	2,000	10/10	1:444	<0.01	<0.01	
	1,524	5/5				
	500	6/10				
	381	3/5				
D14 TSF	2,000	4/15	1:4,636	0.04	—	
	500	3/15				
D14 AGN	2,000	12/15	1: 1,142	0.26	<0.01	
	500	6/15				
D21 TSF	2,000	2/10	1:11,471	0.01	—	
	500	0/10				
D21 AGN	2,000	4/10	1:5,186	0.06	0.35	
	500	0/10				

Table S2. Limiting dilution analysis of NOD/SCID–IL-2R $\gamma^{-/-}$ (NSG) engraftment

For primary recipient, NSG engraftment results are pooled from three independent experiments. Several doses (high: 1,524 or 2,000 and low: 381 or 500) of CD34⁺CD38⁻ cells or their progeny after liquid culture were transplanted into sublethally irradiated NSG mice. A level of at least 0.1% of human CD45⁺ cells in the bone marrow of transplanted recipients at 18-20 wk after transplant was used as a cut off for positive animals. Poisson statistics were used to determine the *P* value. AGN, AGN194310; D, day; SRC, SCID-repopulating cells; TSF, thrombopoietin. stem-cell factor, and Flt3 ligand.

Table S3. Secondary NSG transplants

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		D7 see recipient	condary ts (+/total)	D14 secondary recipients (+/total)	
Primary recipient cell dose	D0	TSF	AGN	TSF	AGN
High	3/5	3/5	5/5	0/5	2/5
Low	1/5	1/5	2/5	0/5	1/5

For secondary recipients, one-tenth of the femur cellularity of primary recipients positive for human engraftment was transplanted into secondary recipients. Data were analyzed 18 wk after transplant. AGN, AGN194310; D, day; TSF, thrombopoietin, stem-cell factor, and Flt3-ligand.