

Table S1. Lentiviral vectors

shRNA	Target Sequence
<i>RPS14</i> #1	CCTTGCCCGCTCGGGTATGAA
<i>RPS14</i> #2	GCTATGTTGGCTGCCCAGGAT
<i>RPS19</i> #1	CGGCGTCATGCCCAGCCACTT
<i>RPS19</i> #2	CTACGATGAGAACTGGTTCTA

Table S2. Primer sequences for qPCR

shRNA	Primer Sequence
<i>RPS14</i> (human) Forward	5'CTCAGGTGGCTGAAGGAGAG 3'
<i>RPS14</i> (human) Reverse	5'GCAGCCAACATAGCAGCATA 3'
<i>RPS19</i> (human) Forward	5'AGACGTGAACCAGCAGGAGT 3'
<i>RPS19</i> (human) Reverse	5'TTCTCTGACGTCCCCCATAG 3'
Actin Human Forward	5' AGAGCTACGAGCTGCCTGAC 3'
Actin (human) Reverse	5' AGCACTGTGTTGGCGTACAG 3'

Table S3. Erythroid signature induced by the combination of dexamethasone and lenalidomide

Gene Set Enrichment Analysis				
Dex+Len vs. Untreated samples				
Gene Set	Size	NES	NOM p-val	FDR q-val
Erythroid_1	98	1.5	0.015	0.137
Erythroid_2	191	1.47	0.006	0.107

Both gene sets were published by Ebert et al.⁹

Table S4. % knockdown by lentiviral vectors over time

shRNA	Day 3 knockdown	Day 5 knockdown	Day 7 knockdown
RPS19#1	93.7%	88.8%	89.4%
RPS14#1	92.8%	43.9%	98.2%
RPS19#2	75.5%	80.6%	86.5%
RPS14#2	84.5%	87.3%	97.3%

Table S5. Top marker genes upregulated by dexamethasone (Dex), lenalidomide (Len), and the combination of the two drugs (Dex+Len)

Name	Description	S2NR	Upregulated
FLJ22662	FLJ22662:	3.3242	Dex
MGAM /// LOC642103	na	4.3242	Dex
CSPG2	CSPG2:chondroitin sulfate proteoglycan 2 (versican)	.3242	Dex
PNMT	PNMT:phenylethanolamine N-methyltransferase	6.3242	Dex
MS4A4A	MS4A4A:membrane-spanning 4-domains, subfamily A, member 4	7.3242	Dex
IL2RA	IL2RA:interleukin 2 receptor, alpha	8.3242	Dex
ENPP4	ENPP4:ectonucleotide pyrophosphatase/phosphodiesterase 4 (putative function)	9.3242	Dex
CRISPLD2	CRISPLD2:cysteine-rich secretory protein LCCL domain containing 2	.3242	Dex
C1QB	C1QB:complement component 1, q subcomponent, B chain	11.3242	Dex
IL18R1	IL18R1:interleukin 18 receptor 1	12.3242	Dex
MGC17330	MGC17330:	13.3242	Dex
GSTM5	GSTM5:glutathione S-transferase M5	14.3242	Dex
SOCS1	SOCS1:suppressor of cytokine signaling 1	.3242	Dex
ISG20	ISG20:interferon stimulated exonuclease gene 20kDa	16.3242	Dex
ALF	ALF:	17.3242	Dex
THBS1	THBS1:thrombospondin 1	18.3242	Dex
TBC1D8	TBC1D8:TBC1 domain family, member 8 (with GRAM domain)	19.3242	Dex
NFIL3	NFIL3:nuclear factor, interleukin 3 regulated	.3242	Dex
MOSC1	MOSC1:MOCO sulphurase C-terminal domain containing 1	21.3242	Dex
STON1	STON1:stonin 1	22.3242	Dex
CEBPD	CEBPD:CCAAT/enhancer binding protein (C/EBP), delta	23.3242	Dex
MFGE8	MFGE8:milk fat globule-EGF factor 8 protein	24.3242	Dex
ALOX5	ALOX5:arachidonate 5-lipoxygenase	.3242	Dex
ATM /// LOC651610	na	26.3242	Dex
SMA4 /// LOC643367 /	na	27.3242	Dex
GOLGA8B	GOLGA8B:golgi autoantigen, golgin subfamily a, 8B	28.3242	Dex
C14ORF106	C14ORF106:chromosome 14 open reading frame 106	29.3242	Dex
EEA1	EEA1:early endosome antigen 1, 162kD	.3242	Dex
LYST	LYST:lysosomal trafficking regulator	31.3242	Len
CD38	CD38:CD38 molecule	32.3242	Len
DUSP1	DUSP1:dual specificity phosphatase 1	33.3242	Len
PER1	PER1:period homolog 1 (Drosophila)	34.3242	Len
TSC22D3	TSC22D3:TSC22 domain family, member 3	.3242	Len
KLF9	KLF9:Kruppel-like factor 9	36.3242	Len
AREG /// LOC653193	na	37.3242	Len
GSTM3	GSTM3:glutathione S-transferase M3 (brain)	38.3242	Len
FPR1	FPR1:formyl peptide receptor 1	39.3242	Len
IL1R2	IL1R2:interleukin 1 receptor, type II	.3242	Len

Name	Description	S2NR	Upregulated
FLT3	FLT3:fms-related tyrosine kinase 3	41.3242	Len
CD163	CD163:CD163 molecule	42.3242	Len
EREG	EREG:epiregulin	43.3242	Len
FKBP5	FKBP5:FK506 binding protein 5	44.3242	Len
FPRL1	FPRL1:formyl peptide receptor-like 1	.3242	Len
TPST1	TPST1:tyrosylprotein sulfotransferase 1	46.3242	Len
WFDC1	WFDC1:WAP four-disulfide core domain 1	47.3242	Len
TMEM158	TMEM158:transmembrane protein 158	48.3242	Len
UGCG	UGCG:UDP-glucose ceramide glucosyltransferase	49.3242	Len
SLC36A1	SLC36A1:solute carrier family 36 (proton/amino acid symporter), member 1	50.3242	Len
TACSTD2	TACSTD2:tumor-associated calcium signal transducer 2	51.3242	Len
TPST2	TPST2:tyrosylprotein sulfotransferase 2	52.3242	Len
DHRS9	DHRS9:dehydrogenase/reductase (SDR family) member 9	53.3242	Len
MYO1B	MYO1B:myosin IB	54.3242	Len
CPM	CPM:carboxypeptidase M	55.3242	Len
BCL2L11	BCL2L11:BCL2-like 11 (apoptosis facilitator)	56.3242	Len
MT2A	MT2A:metallothionein 2A	57.3242	Len
DEFA1 /// DEFA3 ///	na	58.3242	Dex+Len
NNMT	NNMT:nicotinamide N-methyltransferase	59.3242	Dex+Len
STARD13	STARD13:START domain containing 13	60.3242	Dex+Len
STAT4	STAT4:signal transducer and activator of transcription 4	61.3242	Dex+Len
RNASE1	RNASE1:ribonuclease, RNase A family, 1 (pancreatic)	62.3242	Dex+Len
RHCE /// RHD	na	63.3242	Dex+Len
RHCE	RHCE:Rh blood group, CcEe antigens	64.3242	Dex+Len
TM4SF1	TM4SF1:transmembrane 4 L six family member 1	65.3242	Dex+Len
CHI3L1	CHI3L1:chitinase 3-like 1 (cartilage glycoprotein-39)	66.3242	Dex+Len
SPARC	SPARC:secreted protein, acidic, cysteine-rich (osteonectin)	67.3242	Dex+Len
MYO5C	MYO5C:myosin VC	68.3242	Dex+Len
RAB13	RAB13:RAB13, member RAS oncogene family	69.3242	Dex+Len
VSIG4	VSIG4:V-set and immunoglobulin domain containing 4	70.3242	Dex+Len
IFITM3	IFITM3:interferon induced transmembrane protein 3 (1-8U)	71.3242	Dex+Len
FCGRT	FCGRT:Fc fragment of IgG, receptor, transporter, alpha	72.3242	Dex+Len
TMEM45A	TMEM45A:transmembrane protein 45A	73.3242	Dex+Len
RAB38	RAB38:RAB38, member RAS oncogene family	74.3242	Dex+Len
ALDH7A1	ALDH7A1:aldehyde dehydrogenase 7 family, member A1	75.3242	Dex+Len
TOM1L1	TOM1L1:target of myb1-like 1 (chicken)	76.3242	Dex+Len
DBNDD2	DBNDD2:dysbindin (dystrobrevin binding protein 1) domain containing 2	77.3242	Dex+Len
SYNJ2	SYNJ2:synaptojanin 2	78.3242	Dex+Len
C5	C5:complement component 5	79.3242	Dex+Len
FSTL1	FSTL1:folistatin-like 1	80.3242	Dex+Len
ZNF692	ZNF692:zinc finger protein 692	81.3242	Dex+Len

Name	Description	S2NR	Upregulated
CXCL1	CXCL1:chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	82.3242	Dex+Len
AASS	AASS:aminoadipate-semialdehyde synthase	83.3242	Dex+Len
TPBG	TPBG:trophoblast glycoprotein	84.3242	Dex+Len
PPFIBP1	PPFIBP1:PTPRF interacting protein, binding protein 1 (liprin beta 1)	85.3242	Dex+Len
AKR1C1	AKR1C1:aldo-keto reductase family 1, member C1 (dihydrodiol dehydrogenase 1; 20-alpha (3-alpha)-hydroxysteroid dehydrogenase)	86.3242	Dex+Len
NR1H3	NR1H3:nuclear receptor subfamily 1, group H, member 3	87.3242	Dex+Len
JAK2	JAK2:Janus kinase 2 (a protein tyrosine kinase)	88.3242	Dex+Len
CALB1	CALB1:calbindin 1, 28kDa	89.3242	Dex+Len
KIAA1598	KIAA1598:KIAA1598	90.3242	Dex+Len
TREM2	TREM2:triggering receptor expressed on myeloid cells 2	91.3242	Dex+Len
AK1	AK1:adenylate kinase 1	92.3242	Dex+Len
SLC28A3	SLC28A3:solute carrier family 28 (sodium-coupled nucleoside transporter), member 3	93.3242	Dex+Len
OLFML2B	OLFML2B:olfactomedin-like 2B	94.3242	Dex+Len
ALOX12	ALOX12:arachidonate 12-lipoxygenase	95.3242	Dex+Len
GP1BA	GP1BA:glycoprotein Ib (platelet), alpha polypeptide	96.3242	Dex+Len
SLAMF8	SLAMF8:SLAM family member 8	97.3242	Dex+Len
ZNF228	ZNF228:zinc finger protein 228	98.3242	Dex+Len
AQP3	AQP3:aquaporin 3 (Gill blood group)	99.3242	Dex+Len
OAS3	OAS3:2'-5'-oligoadenylate synthetase 3, 100kDa	100.3242	Dex+Len
SLC38A6	SLC38A6:solute carrier family 38, member 6	101.3242	Dex+Len
IL7R	IL7R:interleukin 7 receptor	102.3242	Dex+Len
LGALS3BP	LGALS3BP:lectin, galactoside-binding, soluble, 3 binding protein	103.3242	Dex+Len
SERPING1	SERPING1:serpin peptidase inhibitor, clade G (C1 inhibitor), member 1, (angioedema, hereditary)	104.3242	Dex+Len
OCLN /// LOC647859 /	na	105.3242	Dex+Len
PLS3	PLS3:plastin 3 (T isoform)	106.3242	Dex+Len
SPP1	SPP1:secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	107.3242	Dex+Len

Figure S1. Dexamethasone increases the size of BFU-E colony formation

Following 3 days of treatment with compounds in liquid culture, cells were plated in methylcellulose without drugs. The effects of vehicle control (DMSO) on BFU-E colony size are shown in the top 3 panels while the effects of 1 μ M of dexamethasone (Dex) are shown in the bottom 3 panels. Pictures were taken at the same magnification and with no manipulation of the images.

Figure S2. Erythropoietin causes a dose dependent increase in both CFU-E and BFU-E colony formation

Following three days of treatment with erythropoietin (EPO) in liquid culture, cells were plated in methylcellulose without EPO. Data are presented as the number of colonies per 3200 cells plated. The experiment was performed in triplicate. A two-tailed Student's t-test was used and ** indicates $p \leq 0.01$ and * indicates $p \leq 0.05$.

Figure S3. Dexamethasone increases BFU-E colony formation while lenalidomide increases CFU-E colony formation over a range of treatment durations

Following one-five days of treatment with compounds in liquid culture, cells were plated in methylcellulose without drugs. Data are presented as the number of colonies per 3200 cells plated. The effects of dexamethasone (Dex) on CFU-E colony formation are shown in (A) and on BFU-E colony formation in (B). The effects of lenalidomide (Len) on CFU-E colony formation are shown in (C) and on BFU-E colony formation in (D). The experiments were performed in triplicate. A two tailed Student's t-test was used and ** indicates $p \leq 0.01$ and * indicates $p \leq 0.05$.

Figure S4. Dexamethasone and lenalidomide promote the production of erythroid cells

The absolute number of erythroid cells following 10 days of liquid culture was determined by multiplying the number of cells counted/well by the percentage of those cells which express any erythroid markers (CD71, glycophorin A), assayed by flow cytometry. Data is presented as relative number. The experiments were performed in triplicate and repeated with similar results. A two tailed Student's t-test was used and ** indicates $p \leq 0.01$ and * indicates $p \leq 0.05$.

Figure S5. Dexamethasone and lenalidomide increase the production of erythroid cells from CD34⁺ cells expressing RPS14 or RPS19 shRNAs without increasing apoptosis

In (A) and (D), a Western blot shows the decreased level of protein with RPS19 knockdown (83.7% knockdown by RT-qPCR) and with RPS14 knockdown (72.5% knockdown by RT-qPCR). The absolute number of erythroid cells following 10 days of liquid culture was determined by multiplying the number of cells counted per well by the percentage of those cells which express any erythroid markers (CD71, glycophorin A), assayed by flow cytometry. Data is presented as relative number to eliminate differences caused by cell number and infection rate. The effects of dexamethasone (Dex), lenalidomide (Len), and control on cells infected with a RPS19 shRNA are shown in (B). The effects of dexamethasone (Dex), lenalidomide (Len), and control on cells infected with a RPS14 shRNA are shown in (E). No increase in annexin staining noted in CD34⁺ cells expressing RPS19 or RPS14 shRNAs and treated with either dexamethasone or lenalidomide when compared to vehicle control as shown in (C) and (F). The experiments were performed in triplicate and the entire experiment was repeated with similar results with an independent shRNA (Fig. 2). A two tailed Student's t-test was used and ** indicates $p \leq 0.01$ and * indicates $p \leq 0.05$.

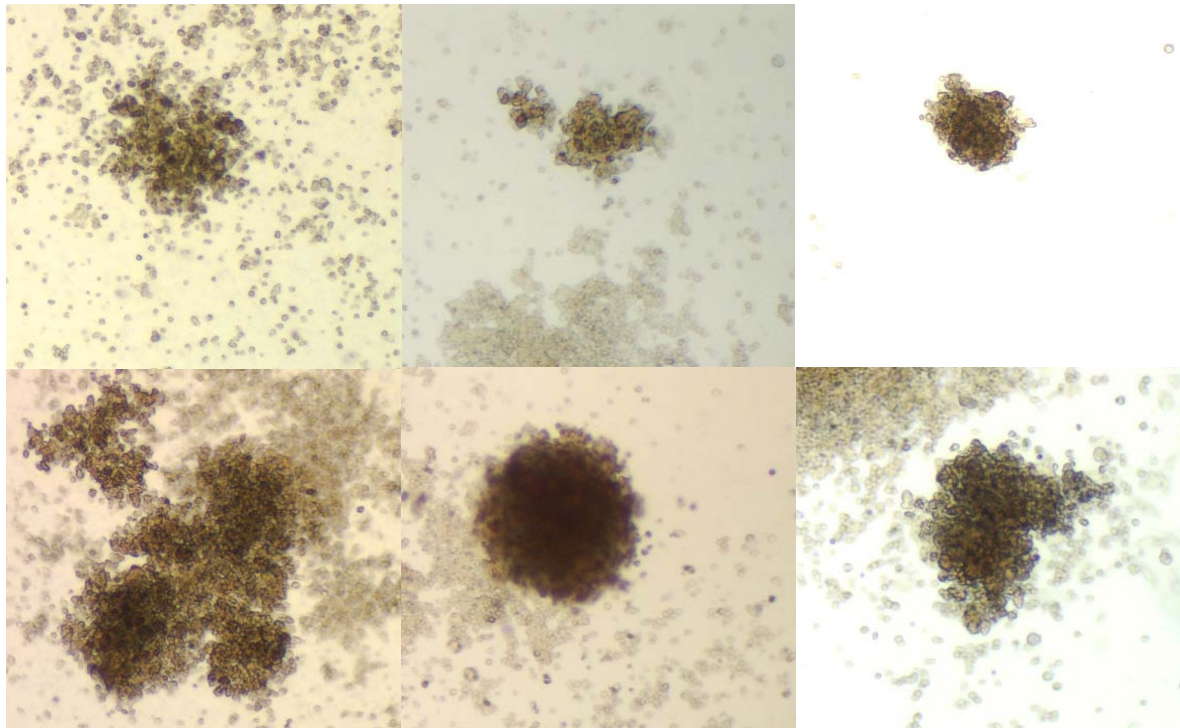
Figure S6. The combination of dexamethasone and lenalidomide has an additive effect on increasing the production of erythroid cells from CD34⁺ cells expressing RPS14 or RPS19 shRNAs

The effects of combination treatment with dexamethasone (Dex) and lenalidomide (Len) and control on cells infected with a *RPS19* shRNA are shown in (A) and with a *RPS14* shRNA in (B). The absolute number of erythroid cells following 10 days of liquid culture was determined by multiplying the number of cells counted per well by the percentage of those cells which express any erythroid markers (CD71, glycophorin A), assayed by flow cytometry. Data is presented as relative number to eliminate differences caused by cell number and infection rate. The expected number of erythroid cells was calculated as follows: Expected # of Cells = (Absolute # erythroid cells with Dex treatment alone -Control) + (Absolute # erythroid cells with Len treatment alone -Control) + Control. The experiments were performed in triplicate and the entire experiment was repeated with similar results with an independent shRNA (Fig. 3). ANOVA was used and the stastical findings are discussed in the text.

Figure S7. Dexamethasone and lenalidomide, alone or in combination, do not affect the expression of ribosomal genes

Gene Set Enrichment Analysis was performed using a set of 85 ribosomal genes. No change in ribosomal gene expression occurs with any of the drug treatments relative to control.

DMSO



Dex

Figure S1

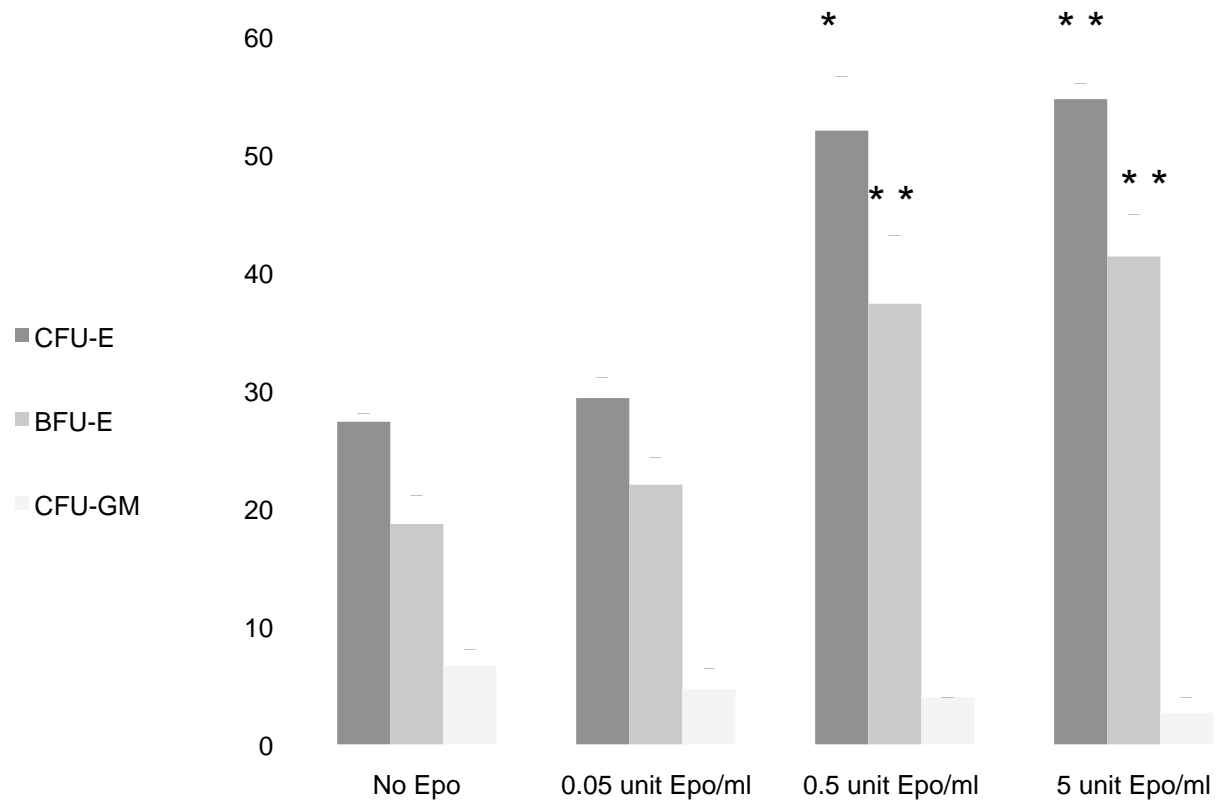


Figure S2

□

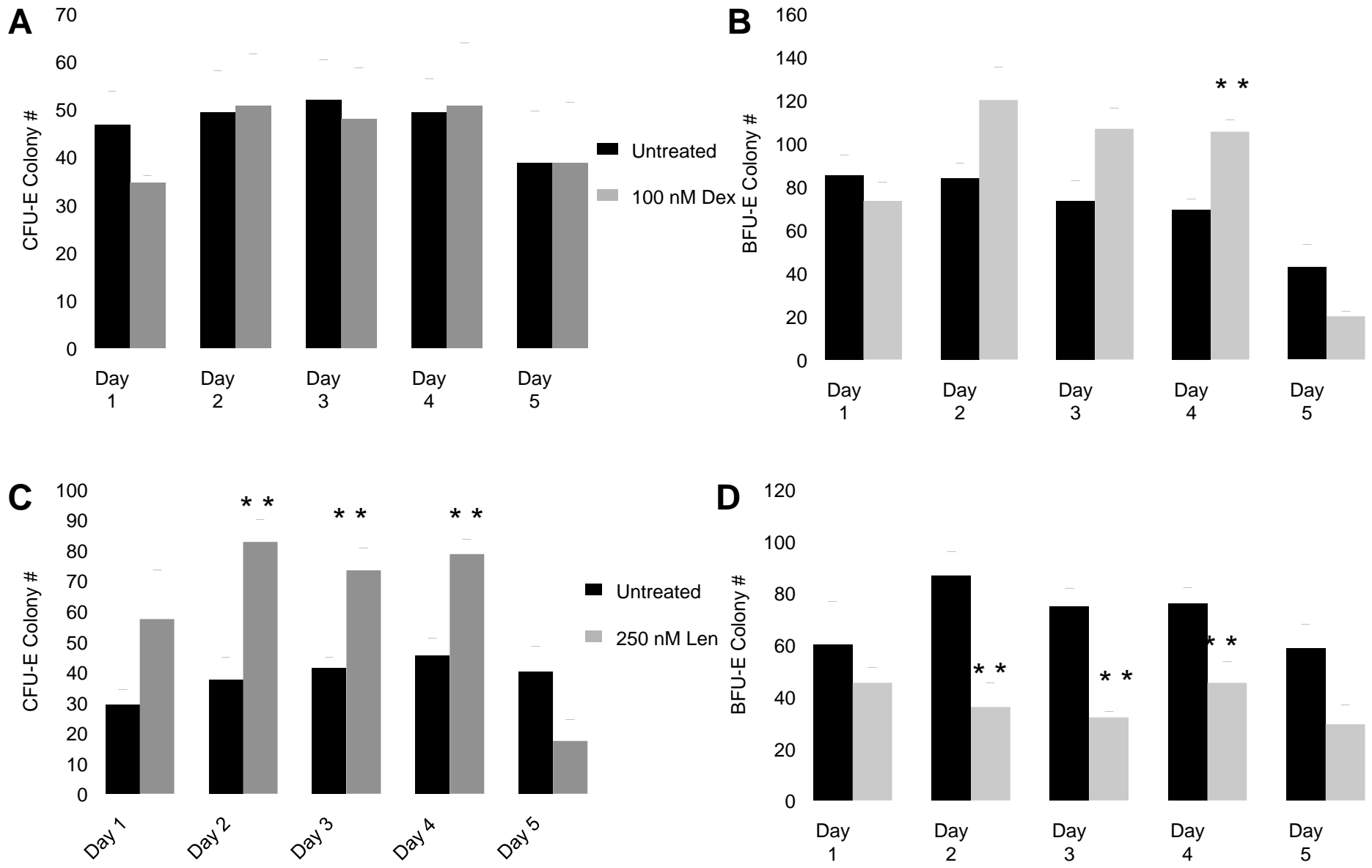


Figure S3

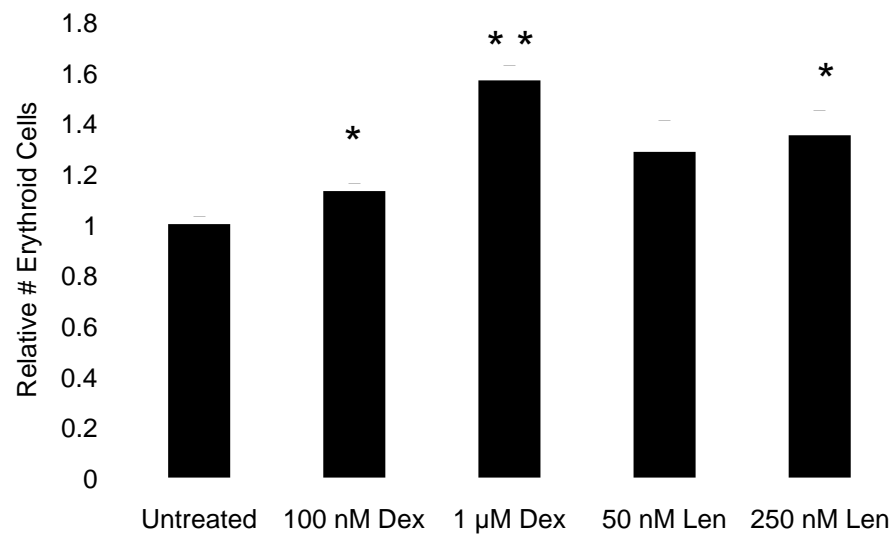


Figure S4

**SUPPLEMENTAL
FIGURE 5: A-F**

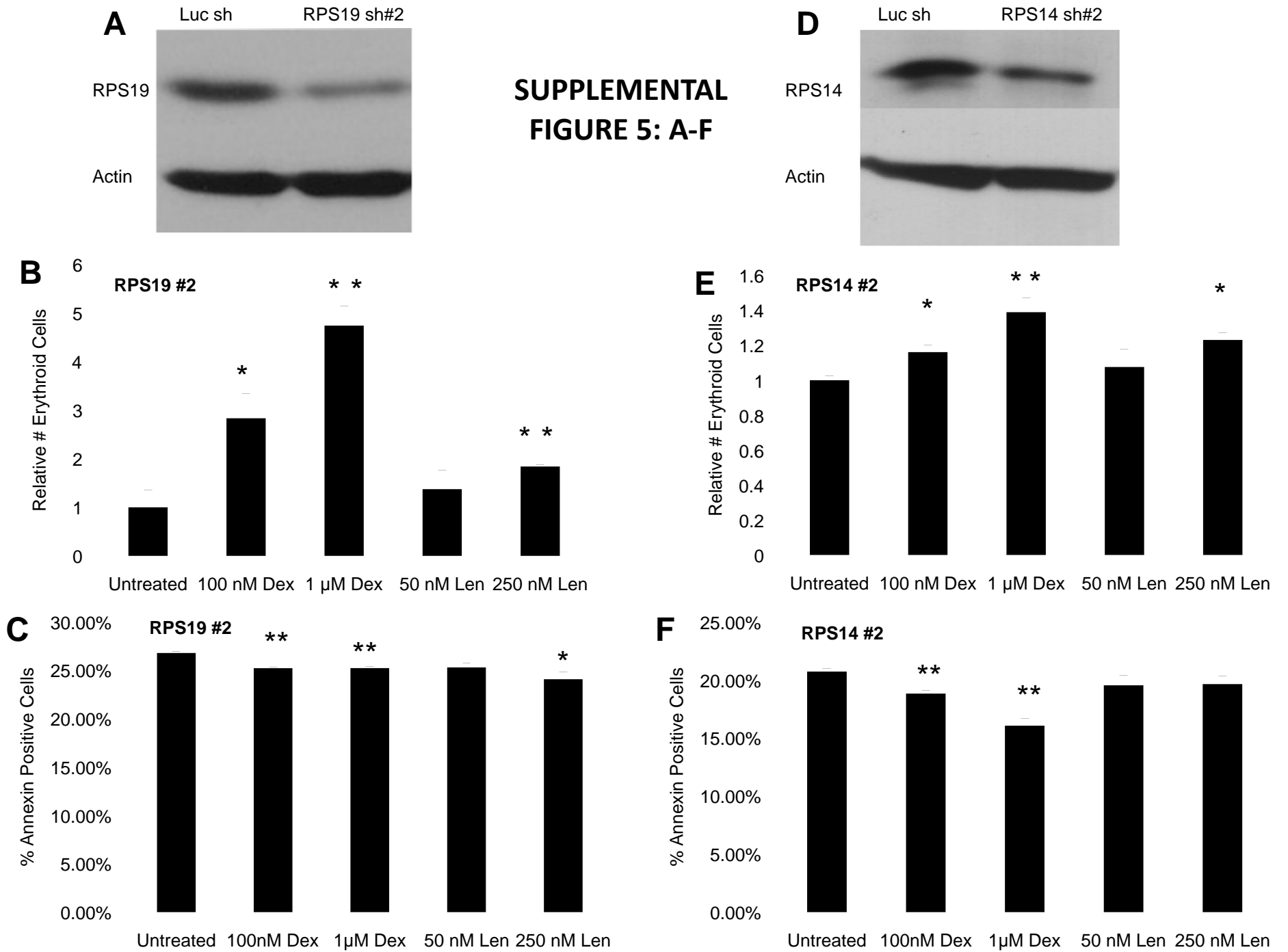


Figure S5

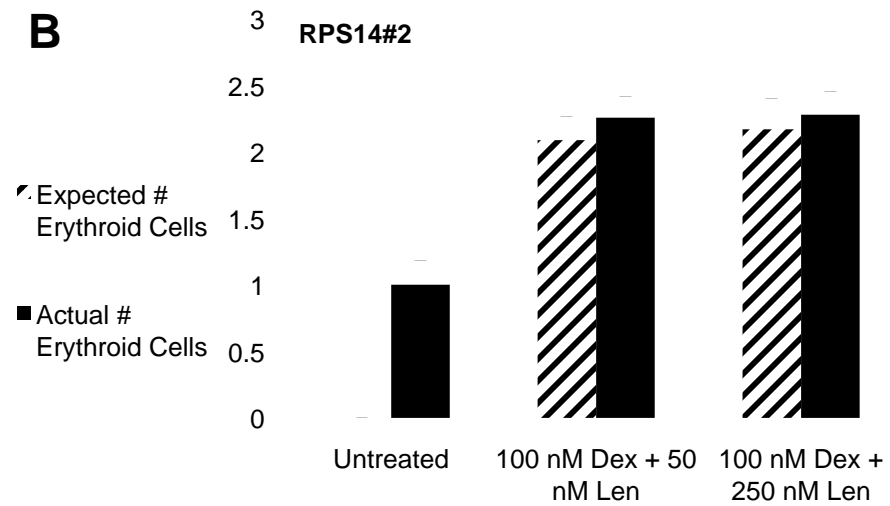
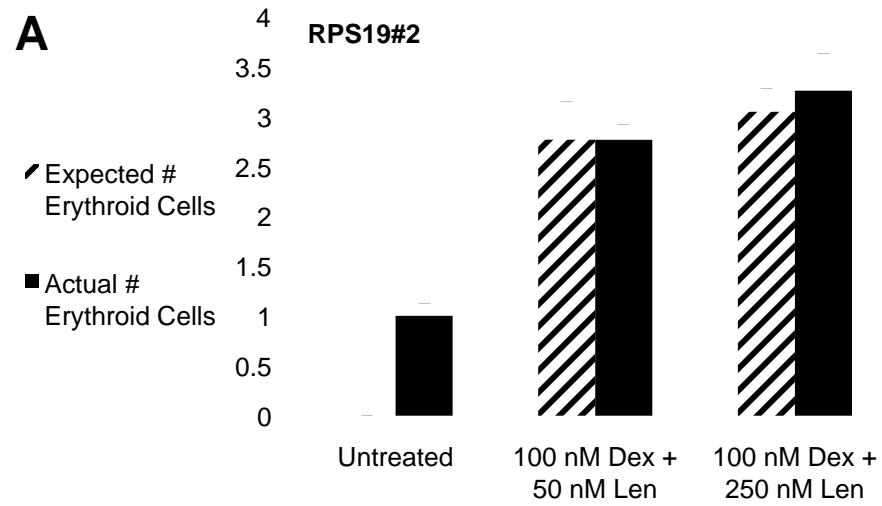
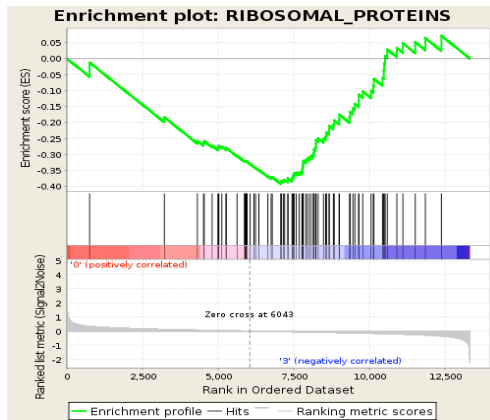
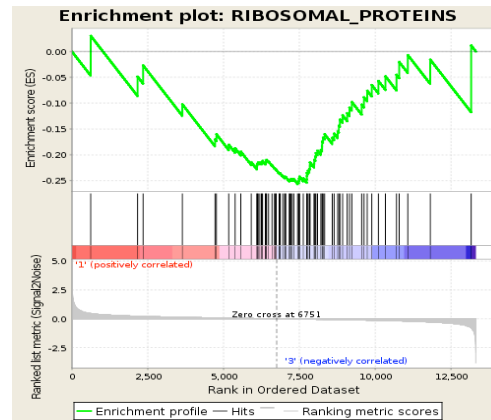


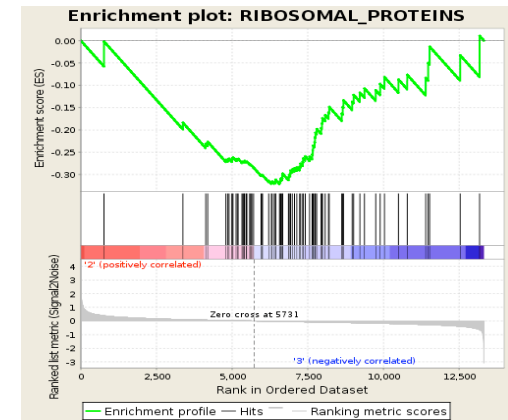
Figure S6



Dex vs. Untreated
Not significant



Len vs. Untreated
Not significant



Dex+Len vs. Untreated
Not significant

Figure S7