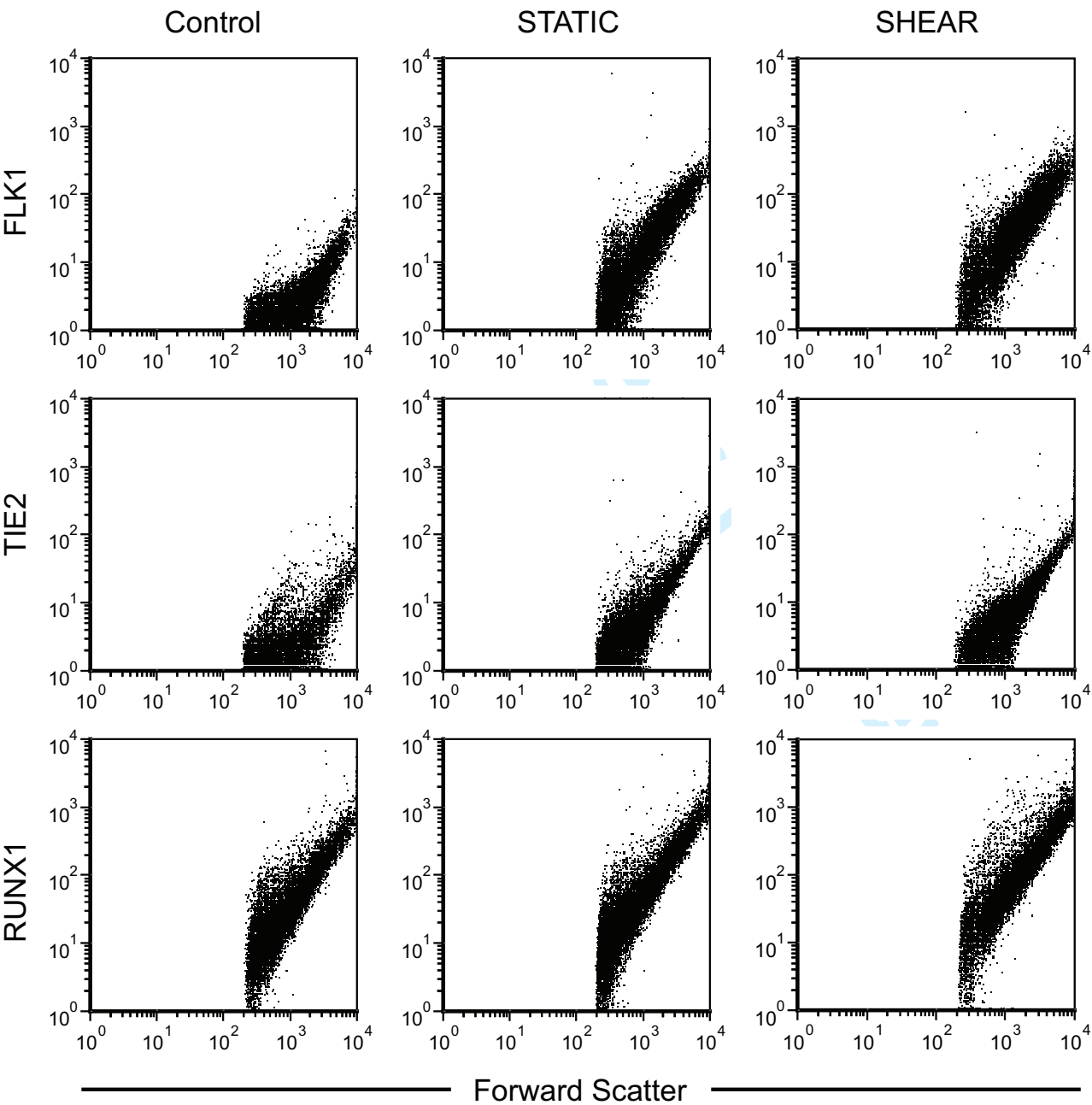


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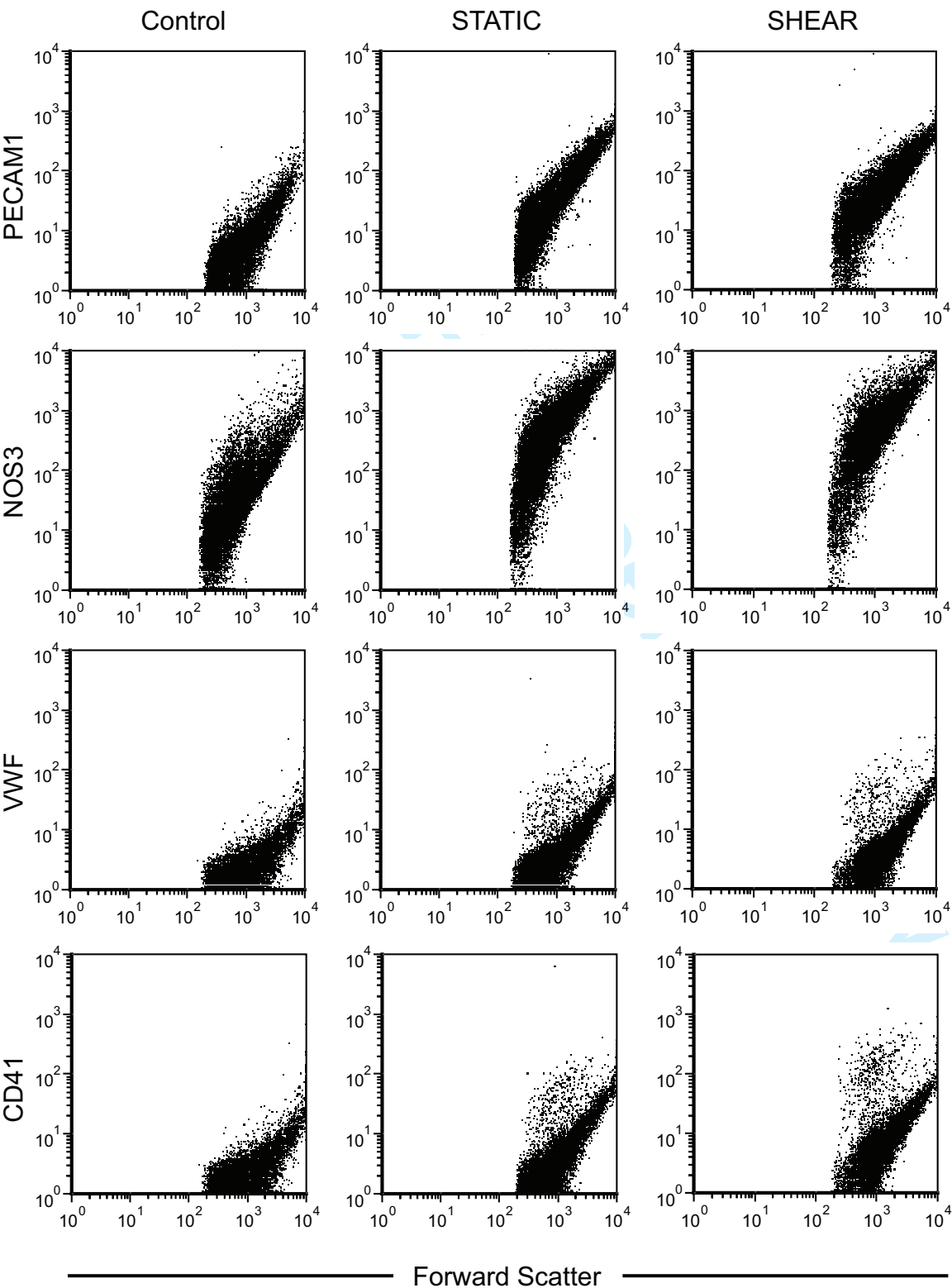
For Peer Review

Supplemental Table 1. List of primers used to detect markers of mesoderm, endothelial, and hematopoietic differentiation.

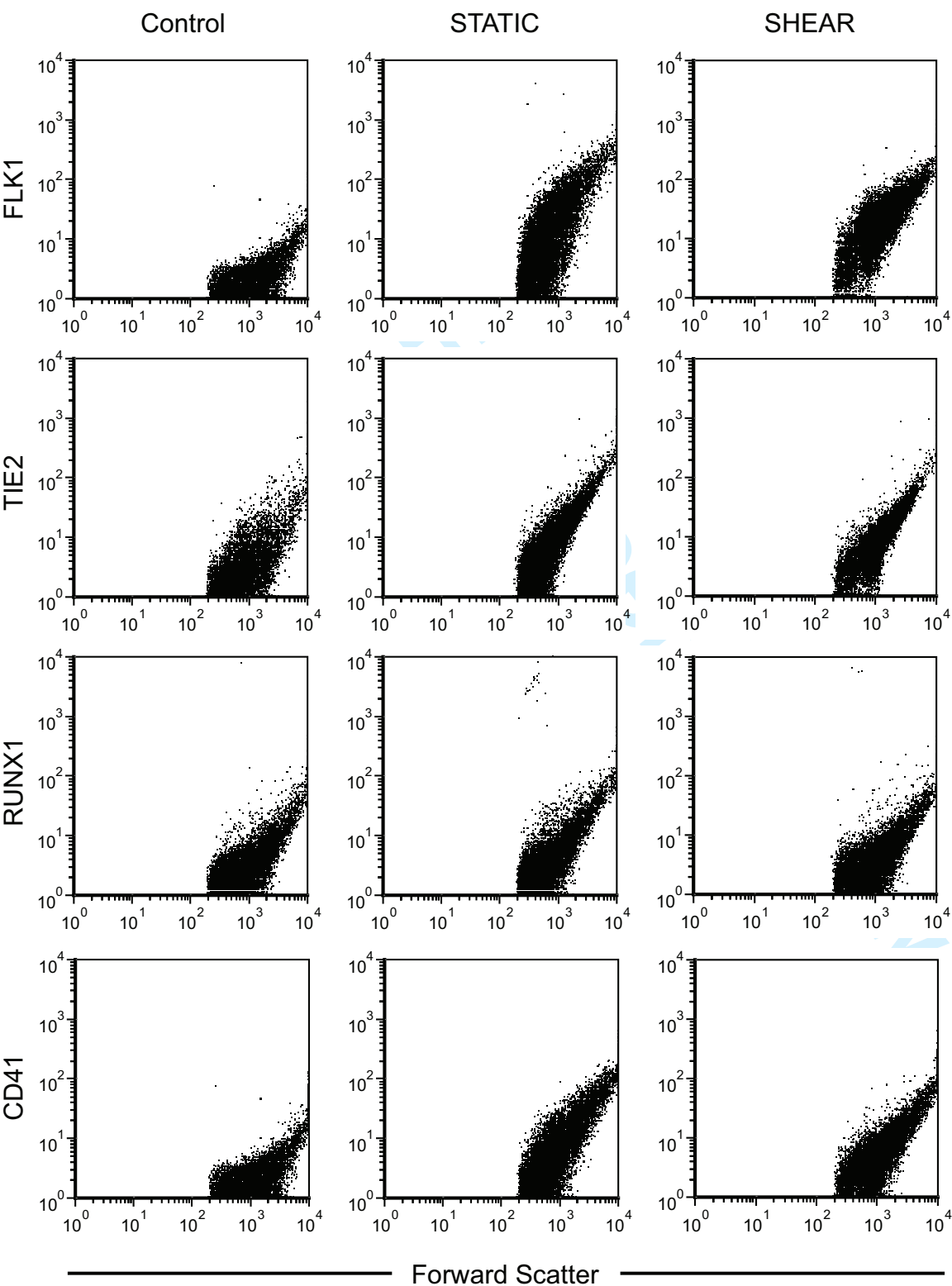
Symbol	Gene Name	Indicator of	Forward Sequence (5' to 3')	Reverse Sequence (5' to 3')	Genebank Accession Number
<i>flk1</i>	fetal liver kinase 1	Early Mesoderm	GGCGGTGGTGACAGTATC	TGACAGAGGCGGATGAATGG	NM_010612
<i>tek</i>	TEK tyrosine kinase, endothelial	Early Endothelial	CAATCAGGCCTGGAAATACATTG	TCCGCGGCTCCAAGTAGTT	NM_013690
<i>klf2</i>	kruppel-like factor 2	Endothelial	CCGCCACTACCGAAAGCA	TCGCACAAGTGGCACTGAA	NM_008452
<i>pecam1</i>	platelet endothelial cell adhesion molecule	Endothelial	CTCCTTACCATCAACAG	TTATACACCATCGCATCG	NM_008816
<i>vead</i>	vascular endothelium cadherin 5, type 2	Endothelial	TGAACCGCCAGAATGCTAAG	CCACAATGAGGGCAGTAAGG	NM_009868
<i>runt</i>	runt-related transcription factor 1	Early Hematopoietic	GGAGGCCACCAGGAAGAATC	ACAGCATTGCTAAATCAGAAGCAT	NM_001111021
<i>alpha1b</i>	integrin alpha 1b	Hematopoietic	TCAGGGATGAGACACGAAACC	AGTCCTTGCCCGGCTTGA	NM_010575
<i>c-kit</i>	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	Hematopoietic	CTTCAGGACCCAAGACGTAACAG	GCGACATGCAGGAAAATG	NM_001122733
<i>cd34</i>	CD34 molecule	Hematopoietic	GCAGGAAAGTCGCATCTCTTG	GGCCGGACATGAGCTGAA	NM_001111059
<i>scl</i>	T-cell acute lymphocytic leukemia 1	Hematopoietic	TTCAGGGCCCTGGTTGGAA	CCAGCTAGTTGAGAGCAGGTTGT	NM_011527
<i>cd133</i>	prominin 1	Hematopoietic	GGCTGGTGGCTTGATTGT	AGCAAGCCCAGGAAAAAGAG	NM_001163577
<i>vav1</i>	vav 1 guanine nucleotide exchange factor	Hematopoietic	AGGCTGCCAGATGTTACTCA	CGGCACCTGTAACAGCGATA	NM_011691
<i>gapdh</i>	glyceraldehyde-3-phosphate dehydrogenase	Housekeeping Gene	GCCTTCGGTGTTCCTACC	GCCTGCTTCACCACCTTC	NM_008084



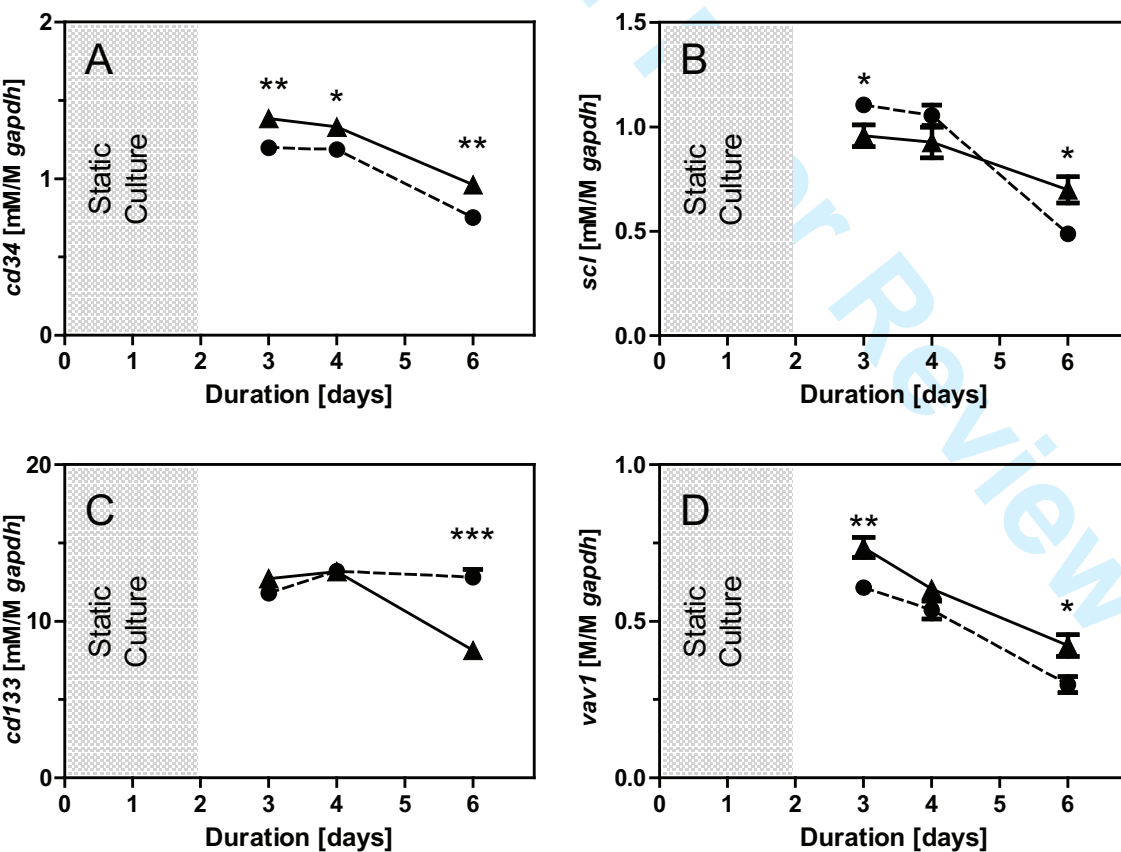
Supplemental Figure 1. Bivariate plots of protein expression. Samples were cultured for two days under static conditions and then exposed to four days of either STATIC or SHEAR treatment ($\tau=5.0$ dyne/cm²). A representative scatterplot of forward scatter and fluorescence is shown for flow cytometry control, STATIC, and SHEAR samples. Assessed proteins include FLK1, TIE2, and RUNX1.



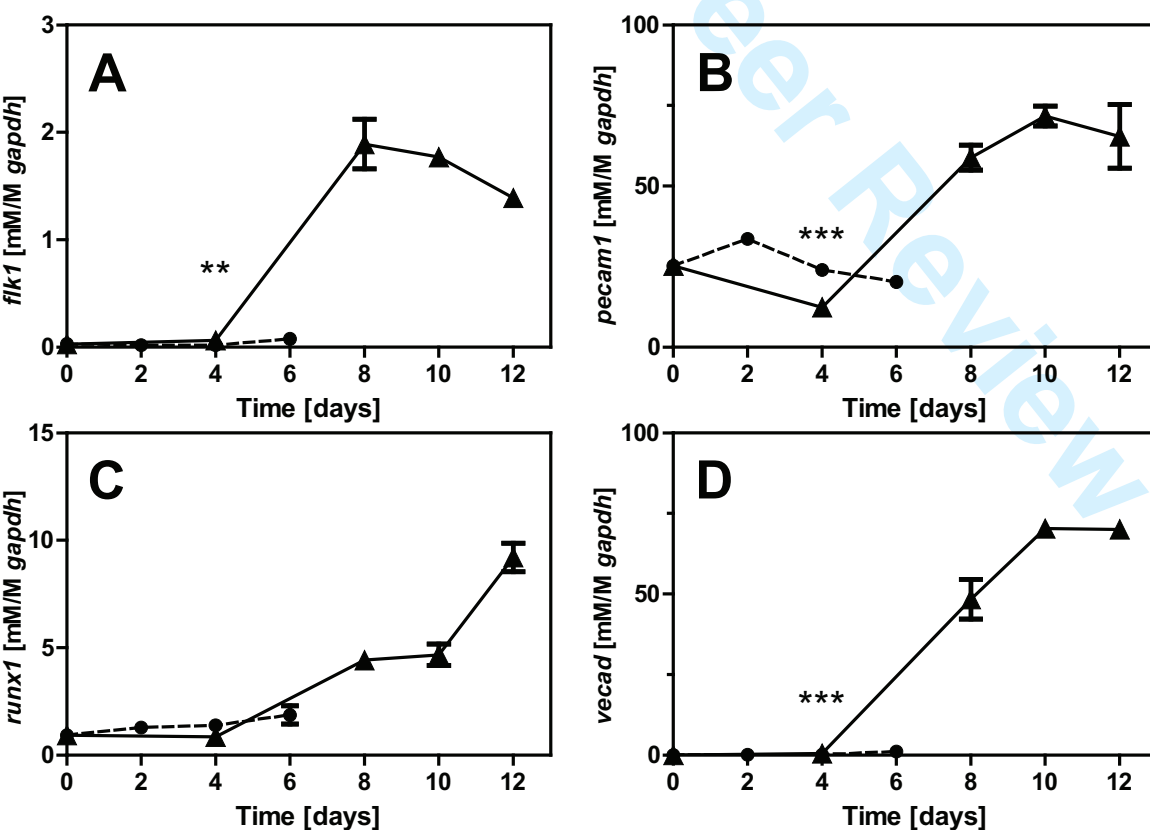
Supplemental Figure 2. Bivariate plots of protein expression. Samples were cultured under static conditions for two days and then exposed to four days of either STATIC or SHEAR treatment ($\tau=5.0$ dyne/cm²). A representative scatterplot of forward scatter and fluorescence is shown for flow cytometry controls, STATIC, and SHEAR samples. Assessed proteins include PECAM1, NOS2, VWF, and CD41.



Supplemental Figure 3. Bivariate plots of protein expression. Samples were cultured under static conditions for three days and then exposed to four days of either STATIC or SHEAR treatment ($\tau=5.0$ dyne/cm²). A representative scatterplot of forward scatter and fluorescence is shown for flow cytometry controls, STATIC, and SHEAR samples. Assessed proteins include FLK1, TIE2, RUNX1, and CD41.



Supplemental Figure 4. Effect of extended durations of shear stress on hematopoietic gene expression. Cells were initially allowed to differentiate under static conditions for two days to promote adhesion to collagen type IV-coated slides (gray shading). Experimental samples were then exposed to shear stress of 5.0 dyne/cm² for a duration of 1, 2, or 4 days (▲, —). Control samples were matched in duration but cultured under static conditions (●, ---). Gene expression (normalized to *gapdh*) was assessed for *cd34* (A), *scl* (B), *cd133* (C), and *vav1* (D). Data presentation as mean ± SEM (n=total of 9-12 replicates from 3 or 4 independent trials) where asterisks indicate a significant (* p<0.05, ** p<0.01) difference as indicated by a t-test between STATIC and SHEAR groups.



Supplemental Figure 5. Baseline ESC differentiation under adherent and suspension culture conditions. Gene expression (normalized to *gapdh*) was analyzed for ESCs cultured under static conditions on collagen type-IV coated glass slides (●, ---) and in embryoid bodies (▲, —). Samples were analyzed for *flk1* (A), *pecam1* (B), *runx1* (C), and *vecad* (D). Data are averages (mean \pm SEM; n=3-4) and asterisks indicate a significant (** p<0.01, *** p<0.001) difference between groups as indicated by t-test at day four.