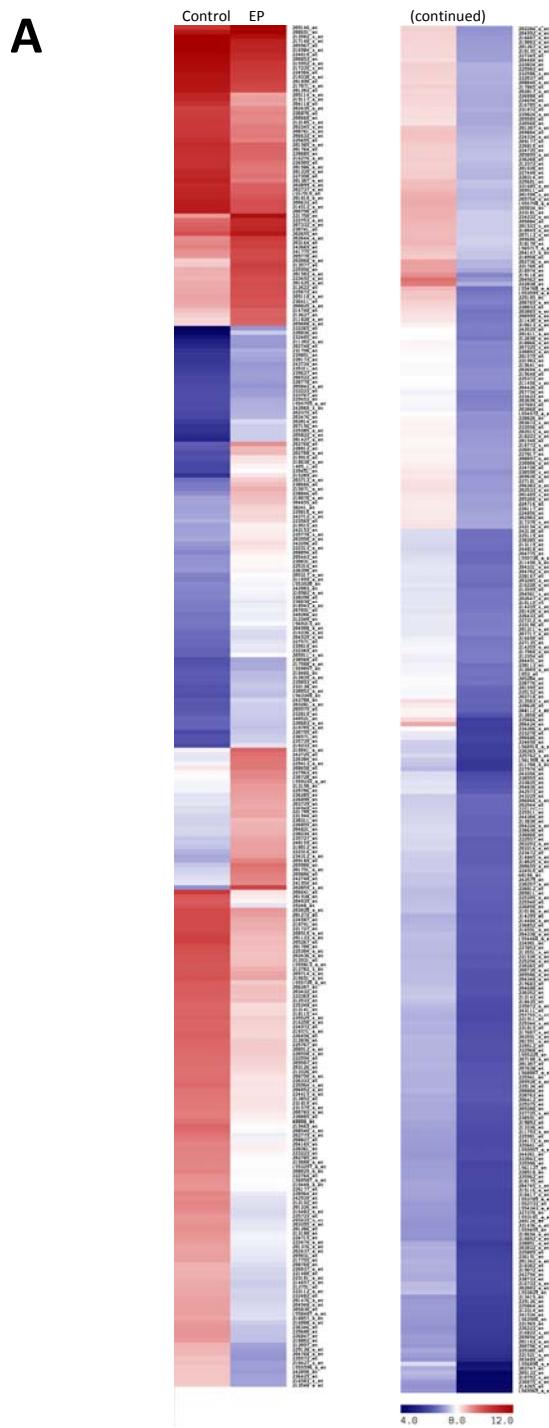


Suppl. Fig. 1



B

| Probe Set | Symbol | Fold Change | Probe Set | Symbol | Fold Change | Probe Set | Symbol | Fold Change |
|--------------|-------------------|-------------|-------------|-------------------|-------------|--------------|-------------------|-------------|
| 205122_at | TMEFF1 | -9.23 | 221521_s_at | GINS2 | -3.07 | 201226_at | NDUFB8 | -2.67 |
| 1556096_s_at | UNC13C | -8.33 | 228033_at | E2F7 | -3.07 | 216483_s_at | C19orf10 | -2.66 |
| 206424_at | CYP26A1 | -7.88 | 238959_at | LARP4 | -3.03 | 202284_s_at | CDKN1A | -2.66 |
| 210792_x_at | SIVA | -7.79 | 201938_at | CDK2AP1 | -3.03 | 212142_at | MCM4 | -2.66 |
| 236075_s_at | ZNF169 | -7.2 | 200769_s_at | MAT2A | -3.02 | 201242_s_at | ATP1B1 | -2.66 |
| 214265_at | ITGA8 | -6.64 | 204768_s_at | FEN1 | -3 | 208540_x_at | S100A11 | -2.64 |
| 1569969_a_at | UNC13C | -6.35 | 201123_s_at | EIF5A | -2.96 | 236191_at | CD38 | -2.63 |
| 235666_at | AA903473 | -6.04 | 233835_at | LOC90246 | -2.96 | 212192_at | KCTD12 | -2.63 |
| 203747_at | AQP3 | -5.54 | 205267_at | POU2AF1 | -2.95 | 209754_s_at | TMPO | -2.62 |
| 204562_at | IRF4 | -5.16 | 200999_s_at | CKAP4 | -2.93 | 218302_at | PSENEN | -2.61 |
| 234306_s_at | SLAMF7 | -4.75 | 204039_at | CEBPA | -2.93 | 216088_s_at | PSMA7 | -2.61 |
| 219118_at | FKBP11 | -4.5 | 202718_at | IGFBP2 | -2.91 | 208910_s_at | C1QBP | -2.6 |
| 206641_at | TNFRSF17 | -4.28 | 219463_at | C20orf103 | -2.9 | 218435_at | DNAJC15 | -2.6 |
| 1554768_a_at | MAD2L1 | -4.06 | 211430_s_at | IFI6 | -2.9 | 1555996_s_at | EIF4A2 | -2.6 |
| 39248_at | AQP3 | -4.03 | 229128_s_at | Transcribed locus | -2.9 | 210613_s_at | SYNGR1 | -2.59 |
| 222838_at | SLAMF7 | -4.02 | 1553829_at | MGC34824 | -2.9 | 236429_at | ZNF83 | -2.57 |
| 223278_at | GJB2 | -4 | 212097_at | CAV1 | -2.89 | 241534_at | LOC642701 | -2.57 |
| 205114_s_at | CCL3 | -3.98 | 226847_at | FST | -2.89 | 204992_s_at | PFN2 | -2.57 |
| 212858_at | PAQR4 | -3.88 | 203032_s_at | FH | -2.87 | 242939_at | TFDP1 | -2.56 |
| 225762_x_at | LOC284801 | -3.7 | 239680_at | AI220472 | -2.87 | 227350_at | HELLS | -2.55 |
| 206686_at | PDK1 | -3.69 | 202736_s_at | LSM4 | -2.87 | 216822_x_at | AL359763 | -2.55 |
| 1561908_a_at | HS3ST3B1 | -3.68 | 221760_at | MAN1A1 | -2.86 | 201700_at | CCND3 | -2.55 |
| 211708_s_at | SCD | -3.65 | 236346_at | ZNF83 | -2.86 | 219072_at | BCL7C | -2.55 |
| 219117_s_at | FKBP11 | -3.63 | 228851_s_at | ENSA | -2.86 | 208827_at | PSMB6 | -2.55 |
| 1553599_a_at | SYCP3 | -3.55 | 242573_at | AI560164 | -2.86 | 225655_at | UHRF1 | -2.54 |
| 226269_at | GDAP1 | -3.54 | 235572_at | SPBC24 | -2.83 | 216583_x_at | AC004079 | -2.54 |
| 213503_x_at | ANXA2 | -3.51 | 204836_at | GLDC | -2.82 | 1563906_at | FLJ10159 | -2.54 |
| 1560916_a_at | DPY19L1 | -3.51 | 200796_s_at | MCL1 | -2.79 | 205204_at | NMB | -2.54 |
| 228620_at | CDNA FLJ46701 | -3.37 | 218802_at | CCDC109B | -2.77 | 228964_at | PRDM1 | -2.53 |
| 225105_at | LOC387882 | -3.34 | 202003_s_at | ACAA2 | -2.77 | 201590_x_at | ANXA2 | -2.53 |
| 201387_s_at | UCHL1 | -3.3 | 202779_s_at | UBE2S | -2.77 | 228776_at | GJA7 | -2.53 |
| 227974_at | Transcribed locus | -3.28 | 235152_at | FAM111B | -2.74 | 222557_at | STMN3 | -2.53 |
| 224650_at | MAL2 | -3.27 | 201599_at | OAT | -2.73 | 1555758_a_at | CDKN3 | -2.53 |
| 243356_at | FAM7A1 | -3.27 | 201163_s_at | IGFBP7 | -2.72 | 216607_s_at | CYP51A1 | -2.52 |
| 229380_at | Transcribed locus | -3.26 | 209094_at | DDAH1 | -2.71 | 225864_at | FAM84B | -2.52 |
| 203489_at | SIVA | -3.25 | 210427_x_at | ANXA2 | -2.7 | 242794_at | MAML3 | -2.52 |
| 244112_x_at | FLJ10159 | -3.2 | 242890_at | HELLS | -2.7 | 225304_s_at | NDUFA11 | -2.51 |
| 203065_s_at | CAV1 | -3.15 | 216733_s_at | GATM | -2.7 | 212698_s_at | SEPT10 | -2.49 |
| 218974_at | FLJ10159 | -3.15 | 225809_at | DKFZP564O0823 | -2.68 | 235072_s_at | Transcribed locus | -2.49 |
| 204118_at | CD48 | -3.08 | 201239_s_at | SPCS2 | -2.67 | 212314_at | KIAA0746 | -2.49 |

Suppl. Fig. 1
(continued)

**Suppl. Fig. 1
(continued)**

| | | |
|--------------|--------------|-------|
| 204688_at | SGCE | -2.48 |
| 219003_s_at | MANEA | -2.47 |
| 228113_at | RAB37 | -2.47 |
| 221969_at | BF510692 | -2.47 |
| 217148_x_at | IGLV2-14 | -2.46 |
| 213548_s_at | CDV3 | -2.46 |
| 203216_s_at | MYO6 | -2.46 |
| 203293_s_at | LMAN1 | -2.46 |
| 243111_at | ENTPD1 | -2.46 |
| 230352_at | PRPS2 | -2.44 |
| 226223_at | PAWR | -2.43 |
| 219683_at | FZD3 | -2.43 |
| 202436_s_at | CYP1B1 | -2.43 |
| 205034_at | CCNE2 | -2.42 |
| 238733_at | CPM | -2.42 |
| 204766_s_at | NUDT1 | -2.42 |
| 226456_at | C16orf75 | -2.42 |
| 1053_at | RFC2 | -2.42 |
| 201286_at | SDC1 | -2.41 |
| 218417_s_at | FLJ20489 | -2.41 |
| 209511_at | POLR2F | -2.41 |
| 200790_at | ODC1 | -2.41 |
| 215952_s_at | OAZ1 | -2.4 |
| 221911_at | ETV1 | -2.4 |
| 225733_at | B3GALT6 | -2.4 |
| 209804_at | DCLRE1A | -2.39 |
| 218741_at | CENPM | -2.39 |
| 216228_s_at | WDHD1 | -2.39 |
| 230630_at | AI566130 | -2.39 |
| 221727_at | AA456973 | -2.38 |
| 243539_at | KIAA1841 | -2.38 |
| 201476_s_at | RRM1 | -2.38 |
| 1555730_a_at | CFL1 | -2.38 |
| 201267_s_at | PSMC3 | -2.38 |
| 204348_s_at | AK3L1 | -2.38 |
| 201611_s_at | ICMT | -2.38 |
| 212009_s_at | STIP1 | -2.37 |
| 213931_at | ID2 /// ID2B | -2.37 |
| 238590_x_at | TMEM107 | -2.37 |
| 204224_s_at | GCH1 | -2.37 |
| 1553789_a_at | C21orf58 | -2.37 |
| 229126_at | TMEM19 | -2.37 |
| 201287_s_at | SDC1 | -2.36 |
| 215116_s_at | DNM1 | -2.36 |
| 223472_at | WHSC1 | -2.36 |
| 48808_at | DHFR | -2.36 |
| 201586_s_at | SFPQ | -2.36 |
| 229344_x_at | FAM80B | -2.35 |
| 214657_s_at | TncRNA | -2.35 |
| 209506_s_at | NR2F1 | -2.35 |
| 213830_at | TRA@ | -2.35 |
| 224587_at | SUB1 | -2.34 |
| 213415_at | CLIC2 | -2.34 |
| 210135_s_at | SHOX2 | -2.34 |
| 218866_s_at | POLR3K | -2.34 |
| 202533_s_at | DHFR | -2.34 |
| 203395_s_at | HES1 | -2.34 |
| 212141_at | MCM4 | -2.34 |
| 214512_s_at | SUB1 | -2.33 |
| 227349_at | AI807356 | -2.33 |
| 231015_at | KLF15 | -2.33 |
| 208029_s_at | LAPTM4B | -2.33 |
| 212185_x_at | MT2A | -2.33 |
| 207325_x_at | MAGEA1 | -2.32 |
| 218025_s_at | PECI | -2.32 |
| 214845_s_at | CALU | -2.32 |
| 203591_s_at | CSF3R | -2.32 |
| 226860_at | TMEM19 | -2.32 |
| 222482_at | SSBP3 | -2.32 |
| 212791_at | FLJ38984 | -2.32 |
| 224334_s_at | MRPL51 | -2.32 |
| 217871_s_at | MIF | -2.32 |
| 201202_at | PCNA | -2.32 |
| 206383_s_at | G3BP2 | -2.31 |
| 235964_x_at | C20orf118 | -2.31 |
| 214259_s_at | AKR7A2 | -2.31 |
| 225348_at | LOC642558 | -2.31 |
| 205839_s_at | BZRAP1 | -2.31 |
| 227135_at | ASAHL | -2.31 |
| 205967_at | HIST1H4C | -2.31 |
| 1554343_a_at | BRDG1 | -2.3 |
| 200730_s_at | PTP4A1 | -2.3 |
| 201551_s_at | LAMP1 | -2.29 |
| 1552733_at | KLHDC1 | -2.29 |
| 227181_at | LOC348801 | -2.29 |
| 244304_at | MGC42174 | -2.29 |
| 218175_at | CCDC92 | -2.29 |
| 204948_s_at | FST | -2.29 |
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| 217235_x_at | MAb56 | -2.28 |
| 218115_at | ASF1B | -2.28 |
| 217607_x_at | EIF4G2 | -2.28 |
| 217960_s_at | TOMM22 | -2.28 |
| 1555225_at | C1orf43 | -2.28 |
| 1558487_a_at | TMED4 | -2.28 |
| 1555736_a_at | AGTRAP | -2.28 |
| 216650_at | AL008627 | -2.28 |
| 217865_at | RNF130 | -2.28 |
| 1557910_at | HSP90AB1 | -2.27 |
| 226333_at | AV700030 | -2.27 |
| 239512_at | SFRS4 | -2.27 |
| 222968_at | NM_016947.1 | -2.27 |
| 224610_at | STX5 | -2.27 |
| 211450_s_at | MSH6 | -2.26 |
| 220892_s_at | PSAT1 | -2.26 |
| 223112_s_at | NDUFB10 | -2.26 |
| 204484_at | PIK3C2B | -2.26 |
| 210371_s_at | RBBP4 | -2.26 |
| 203285_s_at | HS2ST1 | -2.26 |
| 205830_at | CLGN | -2.26 |
| 201890_at | RRM2 | -2.25 |
| 208750_s_at | ARF1 | -2.25 |
| 1553297_a_at | CSF3R | -2.25 |
| 207100_s_at | VAMP1 | -2.25 |
| 200660_at | S100A11 | -2.25 |
| 1555812_a_at | ARHGDI | -2.24 |
| 235529_x_at | C20orf118 | -2.24 |
| 204775_at | CHAF1B | -2.24 |
| 1561127_at | LOC642394 | -2.24 |
| 223181_at | C18orf55 | -2.24 |
| 235506_at | NOSTRIN | -2.24 |
| 224972_at | C20orf52 | -2.24 |
| 202737_s_at | LSM4 | -2.24 |

| | | |
|--------------|-------------------|-------|
| 228518_at | IGHM | -2.23 |
| 201579_at | FAT | -2.23 |
| 226088_at | ZDHHC12 | -2.23 |
| 225294_s_at | TRAPPC1 | -2.23 |
| 227370_at | KIAA1946 | -2.23 |
| 200659_s_at | PHB | -2.23 |
| 224566_at | TncRNA | -2.23 |
| 209030_s_at | IGSF4 | -2.22 |
| 224910_at | CARHSP1 | -2.22 |
| 223423_at | GPR160 | -2.22 |
| 224232_s_at | PX19 | -2.22 |
| 204331_s_at | MRPS12 | -2.21 |
| 221983_at | C2orf17 | -2.21 |
| 1554466_a_at | MGC13114 | -2.21 |
| 228167_at | KLHL6 | -2.2 |
| 207638_at | PRSS7 | -2.2 |
| 221685_s_at | CCDC99 | -2.2 |
| 201307_at | SEPT11 | -2.2 |
| 216705_s_at | ADA | -2.2 |
| 209177_at | C3orf60 | -2.2 |
| 225601_at | HMGGB3 | -2.2 |
| 224694_at | ANTXR1 | -2.2 |
| 216250_s_at | LPXN | -2.19 |
| 230265_at | SEL1L | -2.19 |
| 223556_at | HELLS | -2.19 |
| 225962_at | ZNRF1 | -2.19 |
| 219000_s_at | DCC1 | -2.19 |
| 221530_s_at | BHLHB3 | -2.19 |
| 228314_at | BE877357 | -2.19 |
| 204238_s_at | C6orf108 | -2.19 |
| 216984_x_at | IGL@ | -2.18 |
| 204702_s_at | NFE2L3 | -2.18 |
| 225940_at | EIF4E3 | -2.18 |
| 226850_at | SUMF1 | -2.18 |
| 210186_s_at | FKBP1A | -2.18 |
| 222369_at | AW971254 | -2.17 |
| 222824_at | SEC61A2 | -2.17 |
| 225901_at | PTPMT1 | -2.17 |
| 203696_s_at | RFC2 | -2.17 |
| 202899_s_at | SFRS3 | -2.17 |
| 217755_at | HN1 | -2.17 |
| 202817_s_at | SS18 | -2.17 |
| 206207_at | CLC | -2.17 |
| 201272_at | AKR1B1 | -2.16 |
| 232586_x_at | CDNA FLJ11504 | -2.16 |
| 225911_at | NPNT | -2.16 |
| 238591_at | HEXDC | -2.16 |
| 206052_s_at | SLBP | -2.16 |
| 212354_at | SULF1 | -2.16 |
| 214239_x_at | PCGF2 | -2.16 |
| 203672_x_at | TPMT | -2.16 |
| 226905_at | FAM101B | -2.16 |
| 205084_at | BCAP29 | -2.16 |
| 222037_at | MCM4 | -2.16 |
| 201010_s_at | TXNIP | -2.16 |
| 227448_at | AL045916 | -2.15 |
| 201523_x_at | UBE2N | -2.15 |
| 206632_s_at | APOBEC3B | -2.15 |
| 213551_x_at | PCGF2 | -2.15 |
| 218119_at | TIMM23 | -2.15 |
| 1568807_a_at | Clone 4825606 | -2.15 |
| 229983_at | TIGD2 | -2.15 |
| 204451_at | FZD1 | -2.15 |
| 223223_at | ARV1 | -2.15 |
| 212188_at | KCTD12 | -2.15 |
| 203432_at | TMPO | -2.15 |
| 202785_at | NDUFA7 | -2.15 |
| 230005_at | DKFZp313A2432 | -2.14 |
| 202647_s_at | NRAS | -2.14 |
| 243579_at | MSI2 | -2.14 |
| 201609_x_at | ICMT | -2.14 |
| 211456_x_at | LOC645745 | -2.14 |
| 225119_at | CHMP4B | -2.13 |
| 224173_s_at | MRPL30 | -2.13 |
| 223101_s_at | ARPC5L | -2.13 |
| 230257_s_at | C1orf19 | -2.13 |
| 226186_at | TMOD2 | -2.13 |
| 227603_at | CDNA FLJ41385 | -2.13 |
| 204426_at | TMED2 | -2.12 |
| 203515_s_at | PMVK | -2.12 |
| 239824_s_at | TMEM107 | -2.12 |
| 225941_at | EIF4E3 | -2.12 |
| 224901_at | SCD5 | -2.12 |
| 227853_at | Transcribed locus | -2.12 |
| 200884_at | CKB | -2.12 |
| 202944_at | NAGA | -2.12 |
| 1553101_a_at | ALKBII5 | -2.12 |
| 224415_s_at | HINT2 | -2.12 |
| 209505_at | NR2F1 | -2.12 |
| 204561_x_at | APOC2 | -2.12 |
| 207734_at | LAX1 | -2.11 |
| 221480_at | HNRPD | -2.11 |
| 219641_at | DET1 | -2.11 |
| 237725_x_at | SMC5 | -2.11 |
| 213336_at | GTF2I | -2.11 |
| 239134_at | POLR3C | -2.11 |
| 201585_s_at | SFPQ | -2.11 |
| 213326_at | VAMP1 | -2.11 |
| 200783_s_at | STMN1 | -2.1 |
| 200853_at | H2AFZ | -2.1 |
| 220560_at | C11orf21 | -2.1 |
| 209208_at | MPDU1 | -2.1 |
| 230285_at | DKFZp313A2432 | -2.1 |
| 1559455_at | HEXA | -2.1 |
| 213599_at | OIP5 | -2.1 |
| 211793_s_at | ABI2 | -2.1 |
| 228826_at | RNF43 | -2.1 |
| 225395_s_at | FAM120AOS | -2.1 |
| 218857_s_at | ASRG1 | -2.1 |
| 208761_s_at | SUMO1 | -2.1 |
| 209031_at | IGSF4 | -2.09 |
| 220085_at | HELLS | -2.09 |
| 212836_at | POLD3 | -2.09 |
| 210892_s_at | GTF2I | -2.09 |
| 209930_s_at | NFE2 | -2.09 |
| 225661_at | IFNAR1 | -2.09 |
| 242138_at | DLX1 | -2.09 |
| 227212_s_at | PHF19 | -2.09 |
| 224716_at | SLC35B2 | -2.09 |
| 206066_s_at | RAD51C | -2.09 |
| 226433_at | RNF157 | -2.09 |
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| 202435_s_at | CYP1B1 | -2.09 |

Suppl. Fig. 1
(continued)

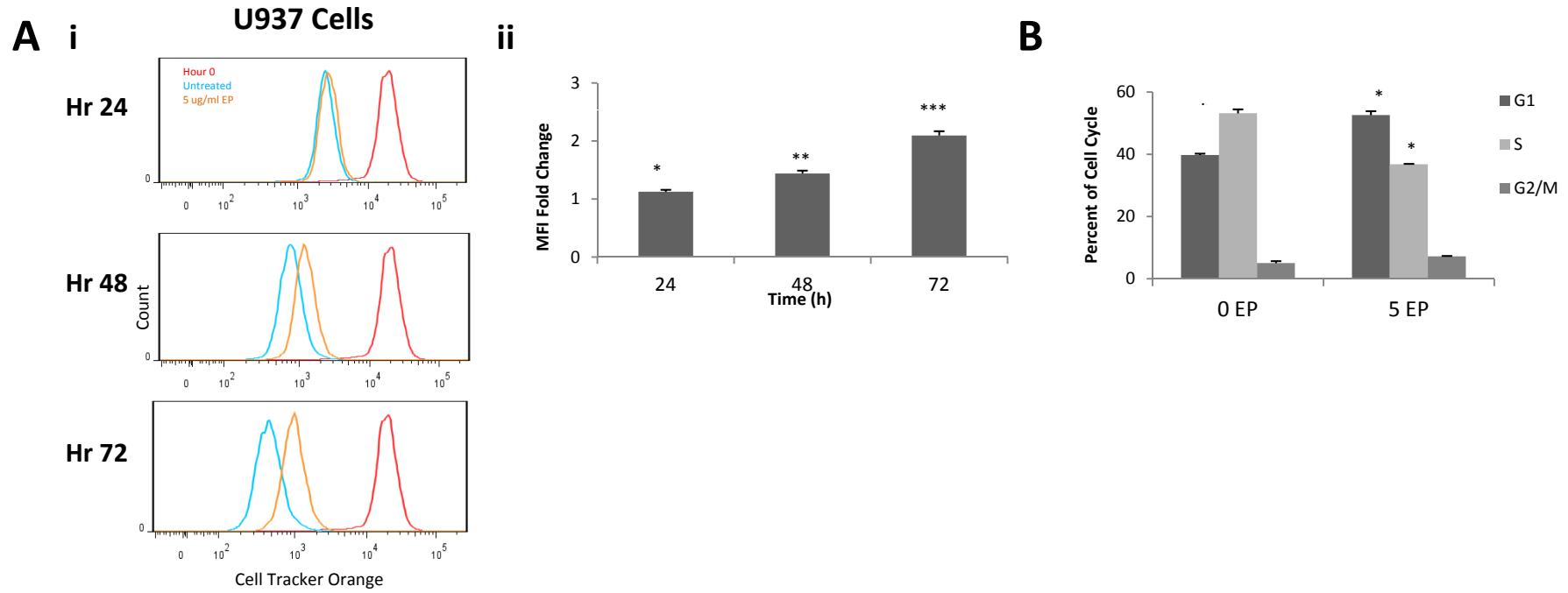
**Suppl. Fig. 1
(continued)**

| | | | | | | | | |
|--------------|---------------|-------|--------------|-------------------|-------|-------------|-------------------|------|
| 213852_at | RBM8A | -2.09 | 209606_at | PSCDBP | -2.04 | 201764_at | TMEM106C | -2 |
| 200633_at | UBB | -2.08 | 201211_s_at | DDX3X | -2.04 | 216274_s_at | SEC11L1 | -2 |
| 217370_x_at | FUS | -2.08 | 201540_at | FHL1 | -2.04 | 206412_at | FER | -2 |
| 208840_s_at | G3BP2 | -2.08 | 213119_at | SLC36A1 | -2.04 | 218958_at | FLJ20850 | -2 |
| 219892_at | TM6SF1 | -2.08 | 236852_at | FBXO43 | -2.04 | 228763_at | CHMP4A | -2 |
| 205260_s_at | ACYP1 | -2.08 | 214299_at | TOP3A | -2.04 | 214484_s_at | OPRS1 | -2 |
| 218170_at | ISOC1 | -2.08 | 203068_at | KLHL21 | -2.04 | 212533_at | WEE1 | -2 |
| 226012_at | ANKRD11 | -2.08 | 210338_s_at | HSPA8 | -2.03 | 204165_at | WASF1 | -2 |
| 218223_s_at | PLEKHO1 | -2.08 | 208097_s_at | TXNDC | -2.03 | 239778_x_at | CAPN7 | 2 |
| 207113_s_at | TNF | -2.08 | 229375_at | PPIE | -2.03 | 1559057_at | CXorf45 | 2 |
| 226037_s_at | TAF9B | -2.08 | 1565717_s_at | FUS | -2.03 | 242669_at | UFM1 | 2 |
| 224728_at | ATPAF1 | -2.08 | 208912_s_at | CNP | -2.03 | 242153_at | LARP2 | 2 |
| 201020_at | YWHAH | -2.08 | 201420_s_at | WDR77 | -2.03 | 205842_s_at | JAK2 | 2 |
| 222474_s_at | TOMM22 | -2.08 | 203126_at | IMPA2 | -2.03 | 219515_at | PRDM10 | 2 |
| 203028_s_at | CYBA | -2.07 | 244261_at | IL28RA | -2.02 | 236411_at | Transcribed locus | 2.01 |
| 230006_s_at | DKFZp313A2432 | -2.07 | 216591_s_at | SDHC | -2.02 | 241775_at | SCFD1 | 2.01 |
| 224715_at | WDR34 | -2.07 | 1554572_a_at | SUV39H2 | -2.02 | 203950_s_at | CLCN6 | 2.01 |
| 226810_at | C6orf155 | -2.07 | 231872_at | LRRCC1 | -2.02 | 210705_s_at | TRIM5 | 2.01 |
| 233198_at | LOC92497 | -2.07 | 204147_s_at | TFDP1 | -2.02 | 229706_at | TCERG1 | 2.02 |
| 222154_s_at | DNAPTP6 | -2.07 | 224856_at | FKBP5 | -2.02 | 223569_at | PPAPDC1B | 2.02 |
| 218049_s_at | MRPL13 | -2.07 | 208760_at | UBE2I | -2.02 | 238311_at | KIAA0776 | 2.03 |
| 228361_at | E2F2 | -2.07 | 222994_at | PRDX5 | -2.02 | 230038_at | ATXN7L2 | 2.03 |
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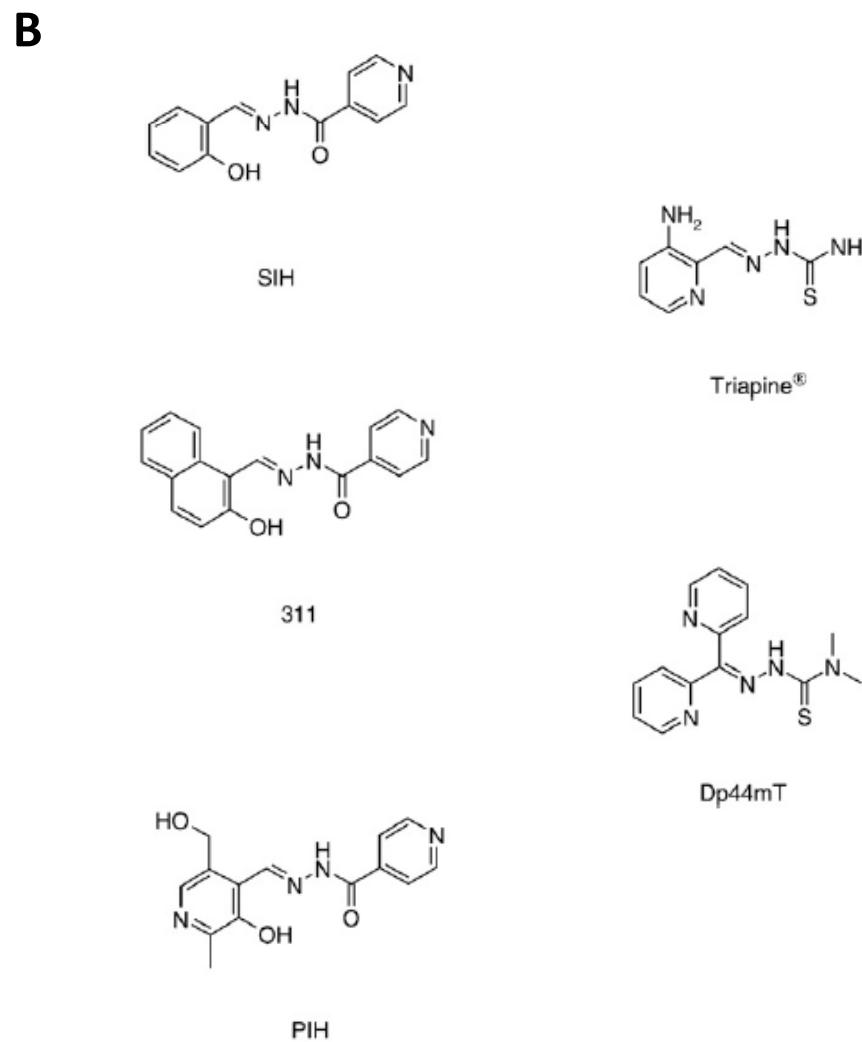
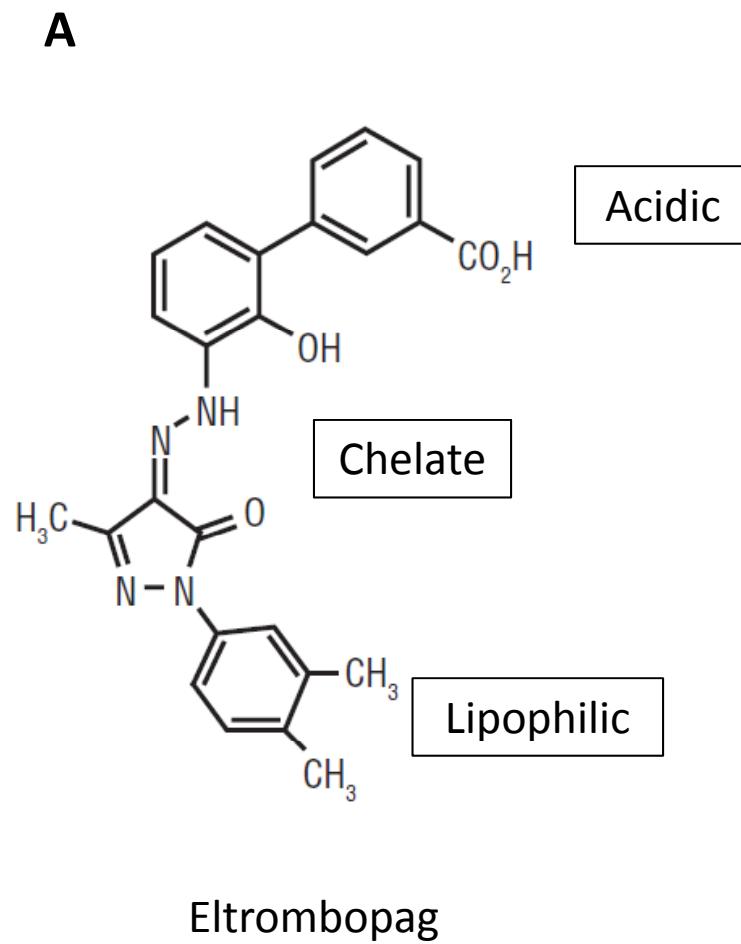
Suppl. Fig. 1
(continued)

Supplemental Figure 1. HL60 cell gene expression after 36 hours of treatment with Eltrombopag. (A) Microarray analysis of HL60 cells treated with or without 3ug/ml EP for 36 hours (fold change ≥ 2). The color intensity represents the ratio of expression in EP –treated compared with control cells. The relative overexpression and underexpression compared with control cells are shown in red and blue, respectively. (B) Corresponding data table displaying the probe set, gene symbol, and fold change for all genes displayed in the heat map.



Suppl. Fig. 2

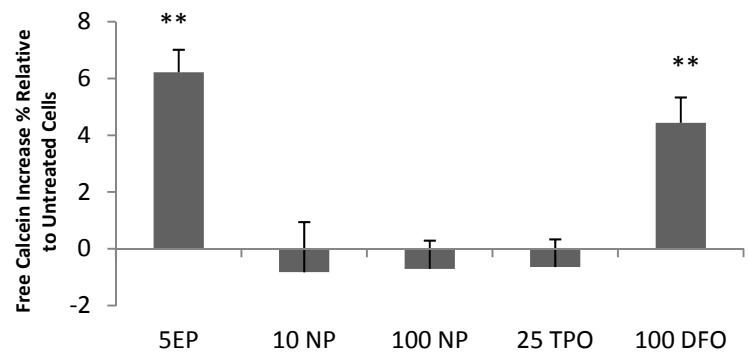
Supplemental Figure 2. Eltrombopag inhibits cell cycling and leads to a block in G1 phase. (A) U937 Cells were incubated in 10uM Cell Tracker Orange for 30 minutes, washed and analyzed by FACS (red line= hr 0). Cells were treated without EP (blue line) or with 5ug/ml EP (orange line) and FACS analysis was performed 48hrs after initial treatment (i). Fold change of U937 cells (ii) of FACS mean fluorescence intensity (MFI) +/- SD (n=3) of cell tracker orange labeled U937 cells treated with 5ug/ml EP relative to untreated cells (*p<0.05, **p<0.01, ***p<0.001). EP slows cell division in URE cells as higher fluorescence represents slower cell division. (B) Cell cycle analysis of U937 cells with or without 5ug/ml EP for 48hrs. EP induces a cell cycle block in G1 phase with a subsequent decrease in S phase (*p<0.05, **p<0.01, ***p<0.001).



Suppl. Fig. 3

Supplemental Figure 3. Molecular Structure of Eltrombopag. (A) Molecular structure of Eltrombopag. The structure consists of a chelator backbone with a lipophilic end and an acidic end. (B) Molecular structure of iron chelators: SIH, 311, PIH, Triapine, and Dp44mT. Eltrombopag is a biphenyl-hydrazone and shares similar structural chelator properties to known iron chelators.

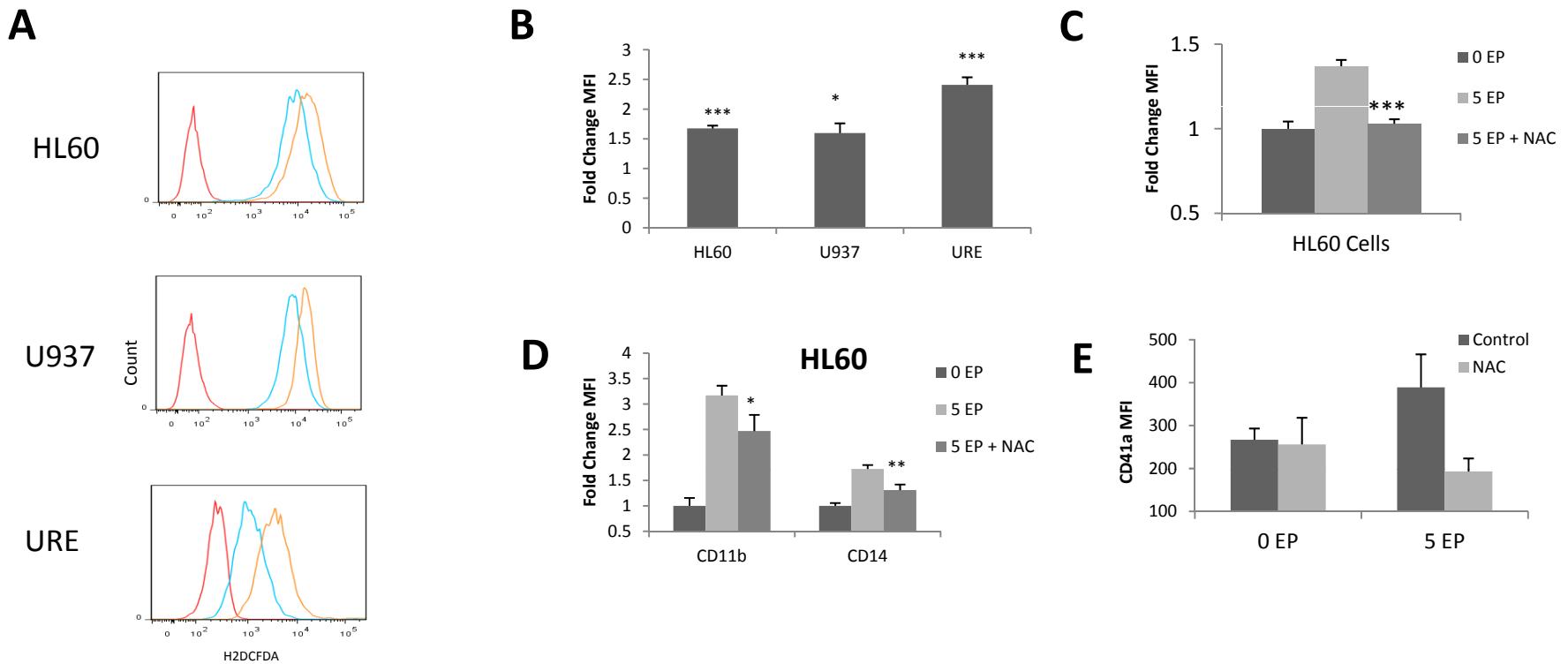
,

A

Suppl. Fig. 4

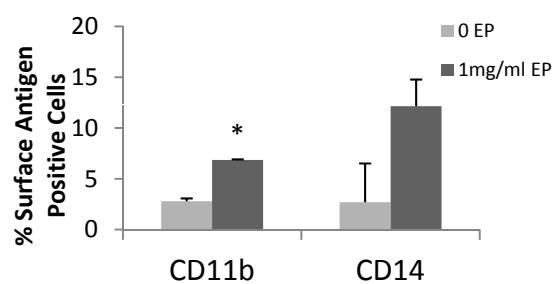
Supplemental Figure 4. Romiplostim, a peptidyl TPO-R agonist does not deplete intracellular iron in leukemia cell lines. (A)

HL60 Cells were labeled with 0.25uM intracellular iron-chelating dye calcein-AM for 5 minutes. Cells were washed then treated with 0ug/ml EP, 5ug/ml EP, 10ng/ml Romiplostim (NP), 100ng/ml NP, 25 ng/ml TPO (- control), or 100uM DFO (+ control) for 4 hours at 37°C. Cells were analyzed by FACS. Data represents the change in the mean fluorescence index (MFI) +/- SD (n=3) compared to untreated HL60 cells *p<0.05, **p<0.01, ***p<0.001. Romiplostim does not reduce intracellular iron in HL60 cells.



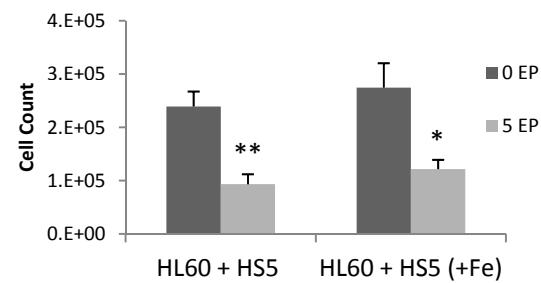
Suppl. Fig. 5

Supplemental Figure 5. Eltrombopag induces ROS in leukemia cell lines. (A) ROS levels HL60, U937, and URE cell lines treated with 5ug/ml EP for 1 hour by FACS measurement of H2DCFDA induced FITC fluorescence. Representative FACS histograms of untreated HL60 cells (blue line) versus cells treated with 5ug/ml EP (orange line). (B) Fold change of FACS mean fluorescence intensity (MFI) +/- SD (n=3) of H2DCFDA induced FITC fluorescence in HL60 cells treated with 5ug/ml EP for 1 hour relative to untreated cells. (*p<0.05, **p<0.01, ***p<0.001). EP induces ROS formation in HL60, U937, and URE cells. (C) Fold change of FACS mean fluorescence intensity (MFI) +/- SD (n=3) of H2DCFDA induced FITC fluorescence in HL60 cells treated with 5ug/ml EP with or without 5uM NAC (anti-oxidant) relative to untreated cells. (**p<0.001). (D) Fold change of FACS mean fluorescence intensity (MFI) +/- SD (n=3) of CD11b expression and CD14 expression in untreated HL60 cells versus cells treated with 5ug/ml EP or treated with 5uM NAC and 5ug/ml EP. Incubation with NAC decreases expression of CD11b and CD14. (E) Peripheral blood mononuclear cells from 2 healthy individuals were treated with or without 5ug/ml EP +/- 5uM NAC. FACS analysis was performed assessing CD41a expression with DAPI exclusion.

A

Suppl. Fig. 6

Supplemental Figure 6. Eltrombopag induces leukemia cell differentiation in a mouse model of leukemia. (A) Bone marrow aspirates were performed on day +17 post-transplantation and FACS analysis was performed gating on engrafted HL60 cells (CD15+, Ly5.1-) assessing CD11b and CD14 expression (n=4) (*p<0.05).

A

Suppl. Fig. 7

Supplemental Figure 7. Co-incubation with stromal cells does not abrogate the anti-leukemic effect of Eltrombopag on HL60 cells. (A) HS5 cells were loaded with or without 500ug/ml FAC for 72 hours and then co-incubated with HL60 cells with or without 5ug/ml EP. Seventy-two hours later cells were analyzed by FACS and the percentage of HL60 cells of the total live cells (DAPI-) was determined by CD15 positivity. Manual cell counts were performed and multiplied by the percent of HL60 cells (*p<0.05, **p<0.01).