Supplementary Methods:

To examine global gene expression in human cord blood derived MKs, $CD34^+$ hematopoietic progenitor cells (HPCs) were isolated from human umbilical cord blood and differentiated into MKs as previously described.[20] Briefly, freshly isolated $CD34^+$ HPCs were cultured in CellGro SCGM medium (CellGenix, Germany) in the presence of thrombopoietin (TPO) (100ng/ml, CellGenix) and IL-1β (Miltenyi, Germany) for 10 days. To reflect the CHRF-288-11 experiments, on day 10 cells were left untreated or treated with Wnt3a (600 ng/mL) or Wnt5a (600 ng/mL). After 8 hours RNA was harvested and subjected to whole genome microarray analysis (Illumina HumanHT-12 v4 Expression BeadChip) as outlined in the results section of the manuscript entitled "Microarray Analysis".

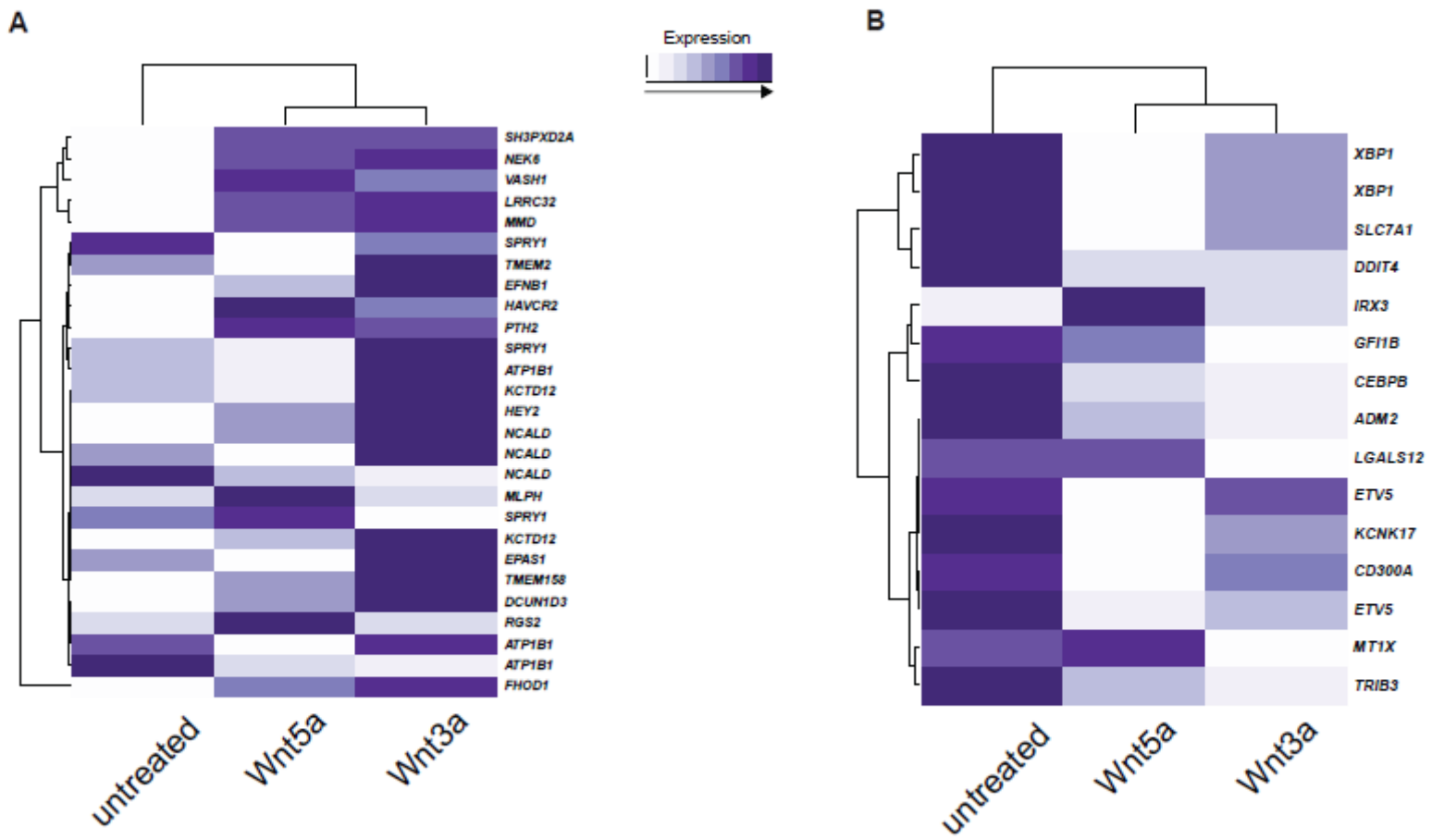
Supplementary Figure Legends:

Supplementary Figure 1: Comparative gene expression profiling of *in vitro* differentiated MKs (derived from CD34+ cord blood progenitors) and CHRF 288-11 cells indicates that components of the Wnt signalling pathway are expressed at comparable levels in MKs and CHRF cells. (A) Heatmap showing expression levels of Wnt signalling pathway components in CHRF (columns 1-4) and *in vitro* differentiated MKs (columns 5-8). (B) Scatterplot showing similar expression levels of Wnt signalling pathway components in CHRF and MK cells. Dotted lines indicate 2 fold differential expression.

Supplementary Figure 2: Heatmap analysis of Wnt target gene expression data from cultured primary human MKs treated with Wnt3a or Wnt5a. (A) Expression in treated/untreated primary MKs of the subset of genes which were upregulated by Wnt3a or Wnt5a in CHRF-288-11 cells. (B) Expression in treated/untreated primary MKs of the subsets of genes downregulated by Wnt3a or Wnt5a in CHRF-288-11 cells. Darker purple coloured heatmap tiles indicate higher expression of these genes. Data presented represents the mean (where applicable) normalised signal intensity of n=2 untreated samples, n=1 Wnt3a treated samples and n=3 Wnt5a treated samples. Samples were derived from replicate cultures of a single umbilical cord blood $CD34^+$ cell harvest. To reflect the CHRF-288-11 experiments (Manuscript Figure 3), cells were treated after 10 days in MK culture (see methods). After 8 hours RNA was harvested and subjected to whole genome microarray analysis (Illumina HumanHT-12 v4 Expression BeadChip). While some differences are observed, and indeed should be expected between the cell line CHRF-288-11 model and the primary cultured MK cells, here we see a considerable overlap in gene regulation between the two analyses, strongly suggesting that these genes are valid targets of Wnt signaling in a megakaryocytic setting.

Supplementary Table 1: Genes differentially expressed between untreated cells and cells treated with Wnt3a (150ng/ml), Wnt5a (600ng/ml) and a combination of both agonists. Values indicate the fold difference measured between each test sample and the untreated control. Values in green indicate upregulation when compared with untreated control, while values in red indicate downregulation.

A
CHRF.1
CHRF.2
CHRF.3
CHRF.4
MK.1
MK.2
MK.3
MK.4
LRP6
APC
WNT7b
FZD9
CELSR1
WNT2
WNT16B
FZD5
FZD1
CTNNB1
WNT5B
FZD7
DAAM2
FZD10
DKK3
WNT3
WNT9A
WNT8b
WNT7a
WNT6
SFRP4
WNT8a
FGR2
WNT3a
DKK1
TCF4
CELSR3
FZD2
TCF3
CDKN1A1
RUNX
DKK4
WNT5a
CELSR2
WNT2B
VANGL1
LEF1
GSK3B
DVL2
DKK1
WNT16a
SFRP1
SFRP2
PRICKLE3
WNT8b
FZD9
VANGL2
LRP5
FZD4
FRZB
DAB2
DAAM1
PRICKLE1
LRRFIP2
WNT11
PRICKLE2
PSAT1
DACT1
WNT1
WNT4
AXIN1
16
15
14
13
12
11
10
9
8
7
B
MK
CHRF
10.0
9.5
9.0
8.5
8.0
7.5
7.5
8.0
8.5
9.0
9.5
10.0


A
Expression
SH3PXD2A
NEK6
VASH1
LRRC32
MMD
SPRY1
TMEM2
EFNB1
HAVCR2
PTH2
SPRY1
ATP1B1
KCTD12
HEY2
NCALD
NCALD
NCALD
MLPH
SPRY1
KCTD12
EPAS1
TMEM158
DCUN1D3
RGS2
ATP1B1
ATP1B1
FHOD1
untreated
Wnt5a
Wnt3a
B
XBP1
XBP1
SLC7A1
DDIT4
IRX3
GFI1B
CEBPB
ADM2
LGALS12
ETV5
KCNK17
CD300A
ETV5
MT1X
TRIB3
untreated
Wnt5a
Wnt3a