Supplementary material

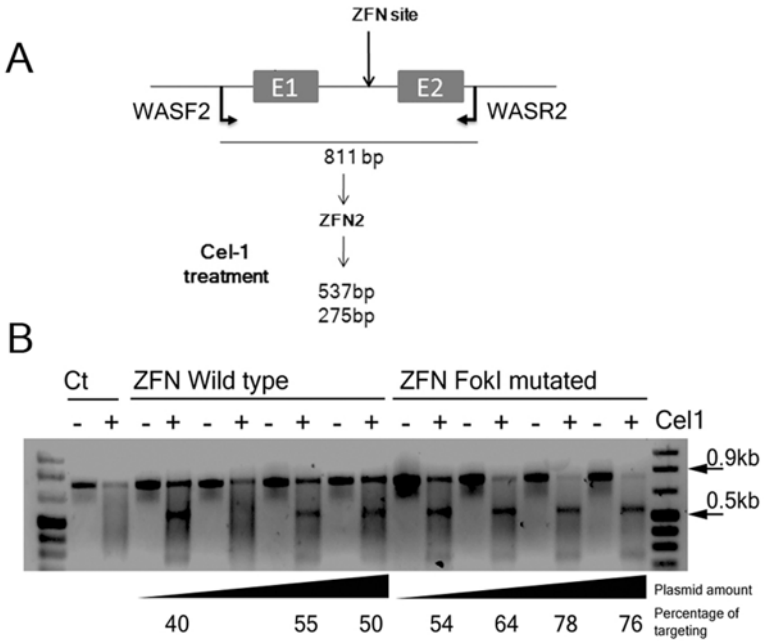

Fig. S1. Efficient targeting of ZFN specific to the *WAS* locus. A) Scheme depicting the cleavage site of the ZFN and the strategy followed to evaluate targeting. The ZFN pair recognize the sequence CCTTTGGGCCCA **TGACT** GTCATGAGGCAGAAGGAC at the *WAS* intron 1: The sequence in bold indicates the FokI cleavage site. Cleavage of the ZFN in its target will generate random mutations in this area. Treatment of ZFN-targeted DNA with the Cel-1 endonuclease (that recognizes and cleavage mismatches in double strand DNA) will render two DNA fragments of 537bp and 27bp while if the DNA remains intact, the Cel-1 won´t cleave and we will detect a 811 bp fragment. **B)** To test cleavage efficiency, the two plasmids expressing each ZFN (1:1 ratio) were electroporated into the K562 cell line using increasing amounts (3, 4, 6 and 8ug from left to right). The cleavage efficiency was measured as indicated before using the Cel-1 endonuclease assay. The isolated genomic DNA was amplified by PCR using the WASF2 and WASR2 primer pair, flanking the targeting region, and a high fidelity Taq polymerase. The purified PCR fragment was incubated in the presence (+) or absence (-) of Cel1 endonuclease and resolved in a high definition agarose gel. The percentage of targeting (showed at the bottom of each line) was calculated by measuring the intensity of the bands (analysis software FluorChem SP; Cell Bioscience) resulting from the digestion and divided by the intensity of all of the bands.

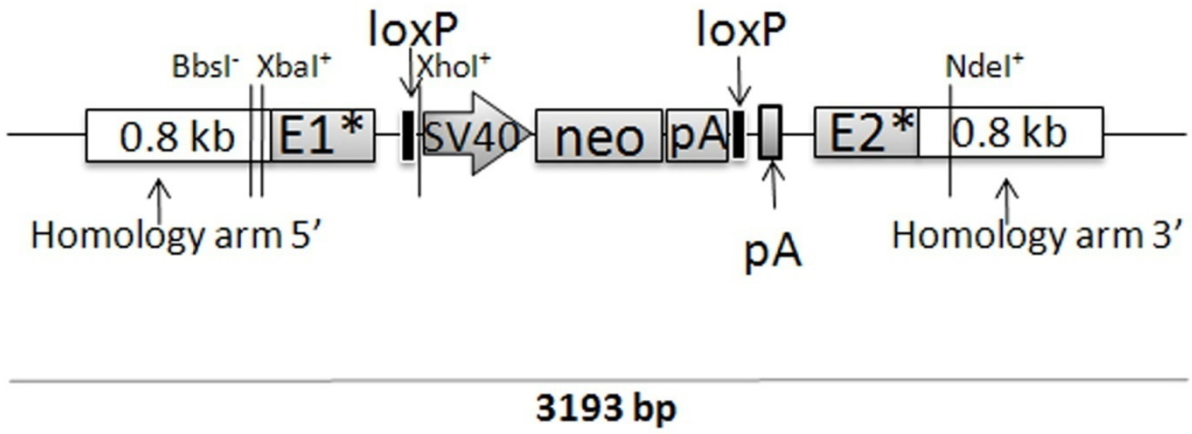

Fig. S2. Details of donor DNA to knock down *WAS* expression in K562 cells. A BbsI restriction site has been deleted and a novel XbaI site has been included in the 5'homology arm (BbsI-, XbaI+). Exon 1 (E1*) has been mutated by deleting the first adenine of ATG codon. The intron 1 has been mutated by substituting the splice acceptor sequence (AG) by a stop codon (TAA) and introducing a polyA signal. In the intron 1 a novel XhoI site was introduced. Finally, 5 nucleotides have been deleted at the beginning of exon 2 (E2*). A neo expression cassette has been introduced to allow antibiotic selection. The neo cassette has been flanked by loxP sequences, allowing in the future the possibility to be removed from the *WAS* locus. A NdeI site has been removed from the homology arm 3'.

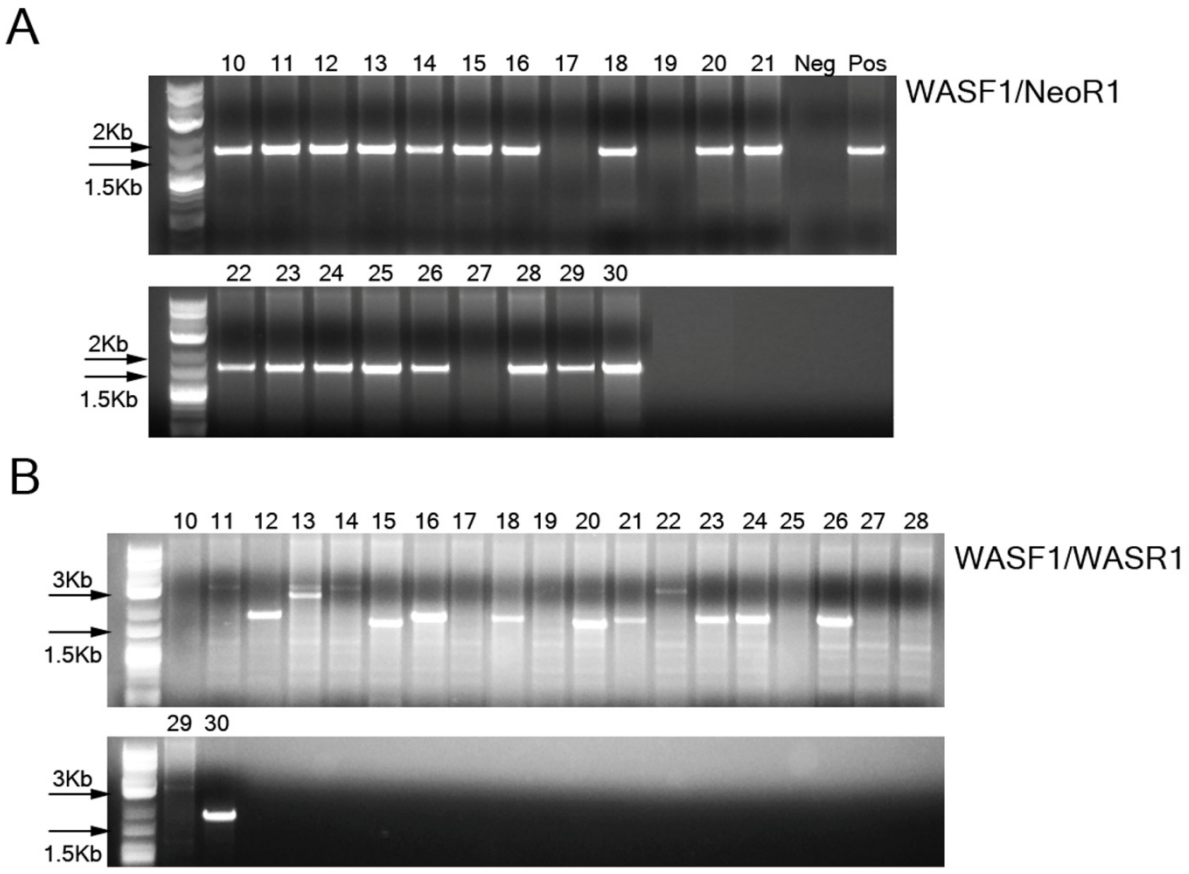

Fig. S3. Detection of homozygous clones for homologous directed recombination in transfected K562 cells. A) Screening of positive clones for homologous recombination in K562 cells. **B)** Control Screening to differentiate between homozygote or heterozygote clones and as internal control for DNA quality. Either, a band of 2Kb (endogenous WAS gene) or a band of 3.5kb (mutated WAS gene incorporating the neo cassett) must appear. The absence of bands indicates poor PCR amplification probably due to poor DNA quality. This is an internal control to determine whether the apparent G418 resistant clones (17, 19 and 27) are really a product of random integration or the absence of band could also be due to poor DNA quality. Note that clones 17, 19 and 27 did not render any band with any primer pair (Figs. A and B). This indicates that we cannot reach any conclusion on these three clones. Later experiments showed that all three clones amplify with the WASF1/NeoR1 pair and where therefore successfully targeted via HR.

in As internal controls of PCR amplification Properly targeted clones A band WASF1, NeoR1 and WASR1 primer binding sites are shown in Fig.1A

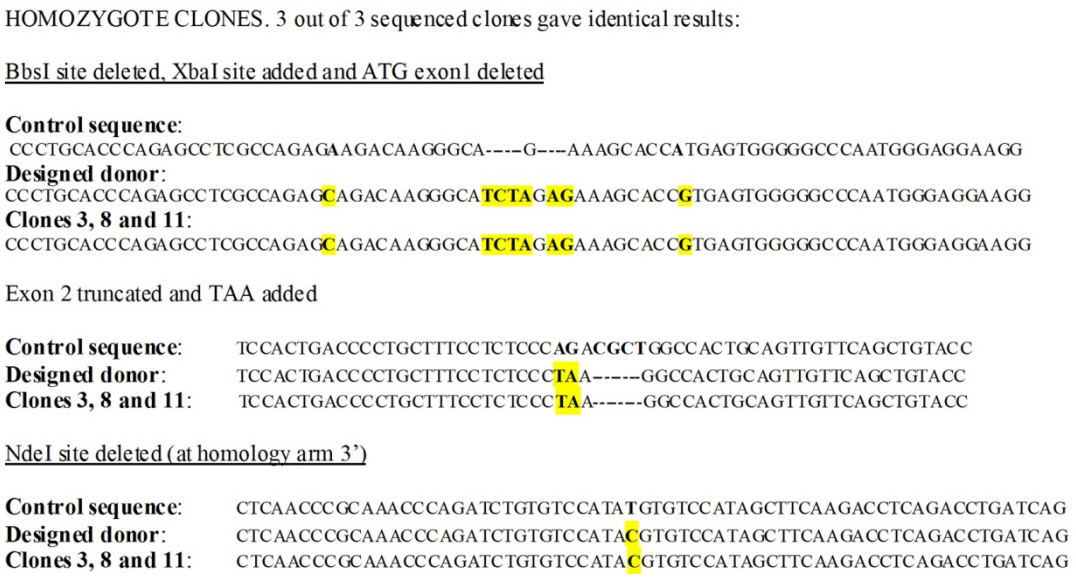

Fig. S4. Correct incorporation of donor DNA into the *WAS* locus. Three different homozygous clones (clones 3, 8 and 11) were sequenced to corroborate the incorporation of the desired modifications into the WAS locus. All clones render the same sequence. We examined the following modifications: - Exon 1: mutation of BbsI site, inclusion of XbaI site and deletion of ATG (top) – Exon 2 truncation and introduction of a TAA (middle) and – mutation of Nde1 site at Homology arm 3'(bottom).

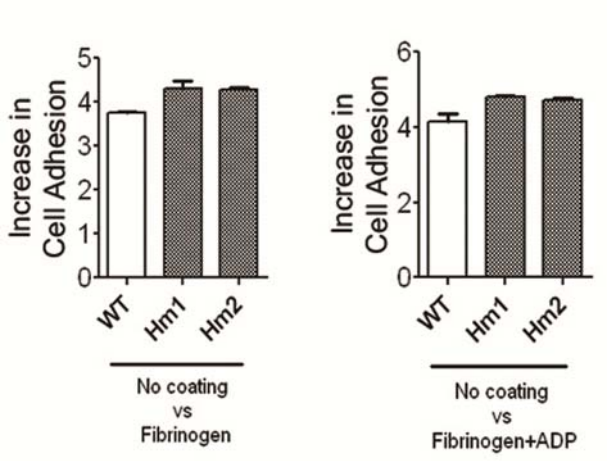

Fig. S5. Normal binding of WAS-KO K562 cells to fibrinogen in the absence or presence of ADP. The increase in cell adhesion of K562WASPKO (Hm1 and Hm2) and K562 (WT) cellsin the presence of fibrinogen (left graph) or in the presence of fibrinogen and ADP (right graph) compared to uncoated plates is equivalent.

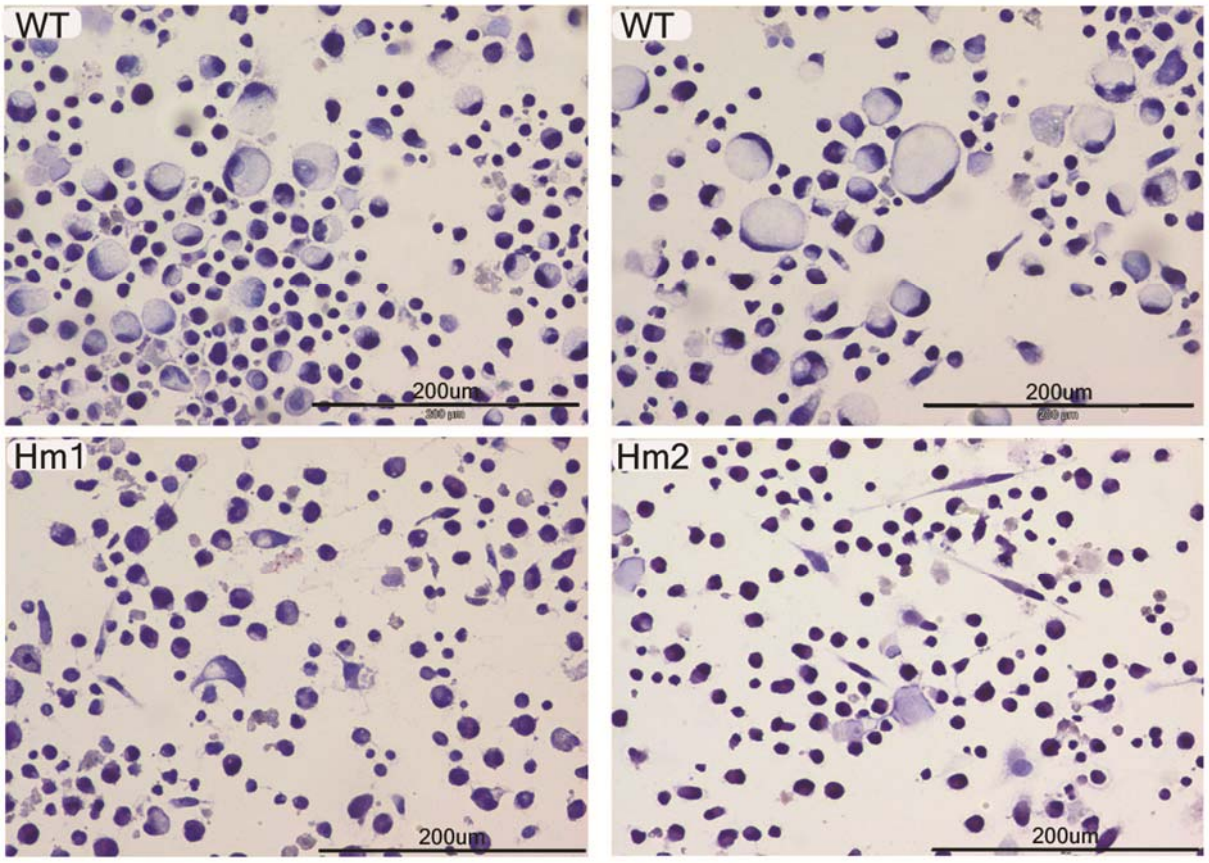

Fig. S6. Morphological alterations on K562WASPKO cells upon PMA stimulation. WT and WAS-KO cells were stimulated with PMA for 96h, desiccated, fixed with PFA and stained using Papanicolaou cytologic technique. Typical changes after PMA differentiation can be seen in WT cells, as bigger cells with clear cytoplasm and a nucleus displaced to the periphery. In WAS-KO cells there is decrease in the percentage of the described cells and when many appear do not conserve the round shape. Many fusiform cells can be seen.

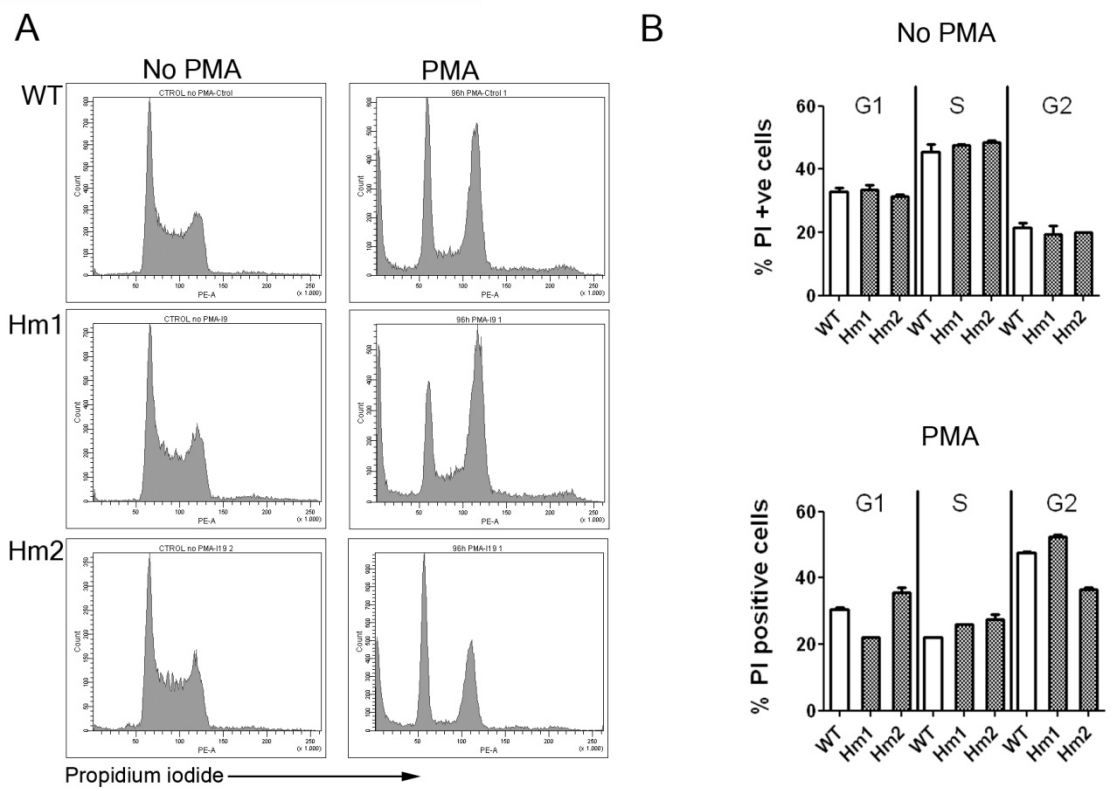

Fig. S7. Cell cycle profile in K562WASKO and K562 cells in the presence and absence of PMA activation. A) Cell cycle determination was evaluated in K562 cells (top graphs) and K562WASKO cells (clones Hm1 and Hm2, middle and bottom graphs) before (left panels, No PMA) and after 96 hours of PMA stimulation (right panels, PMA). Cells were fixed in ethanol 70%, stained in a buffer containing PI and RNase and analyzed by flow cytometry. . B) Graphs representing the percentages of K562WASKO and K562 cells in G1, S and G2 phase before stimulation (No PMA) and after PMA stimulation (PMA). Upon PMA stimulation K562WASKO and K562 cells stop cell division as evidenced by the increased cells in G2 phase. No significant differences in cell cycle can be observed between K562WASKO and K562 cells in the presence or absence of PMA.

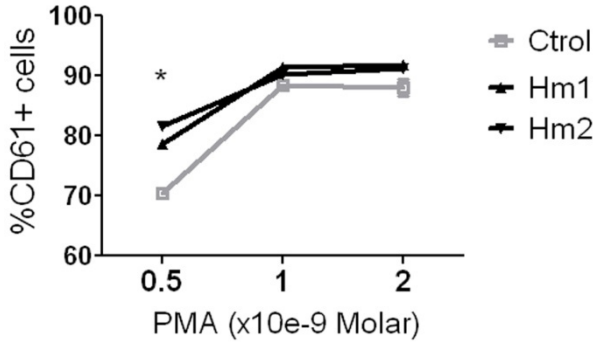

Fig. S8. Increased expression of CD61 surface marker. WT and *WAS*-KO cells were stimulated with increasing concentrations of PMA and 24 hours later we analyzed CD61 as a marker for megakaryocytic differentiation on the surface of the stimulated cells. As a result, at the lowest level of PMA there was a difference in CD61 expression and a higher percentage of cells *WAS*-KO expressed this marker on their surface, compared to WT cells, in a significant manner. *$p<0.01$ (n=3).

Supplementary Table 1

Primers table

Primer name	Sequence	Accession number	Exons
WASF1	5'-AAGCACTCACGATAGGCGTGG-3'		
WASR1	5'-AAGTTCAGGTCAGGGGATTGTGG-3'		
WASF2	5'-AGGGTTCCAATCTGATGGCG-3'		
WASR2	5'-TTGAGAACTGGCTTGCAAGTCC-3'		
NeoR1	5'- AAGTTCAGGTCAGGGGATTGTGG-3'		
F-WAS	5´-AGGCTGTGCGGCAGGAGAT-3'	NM_000377	9-10
R-WAS	5'-CAGTGGACCAGAACGACCCTTG-3'		
F-PDGF	5'-CCAGCGACTCCTGGAGATAGA-3'	NM_002607	2-3
R-PDGF	5'-CTTCTCGGGCACATGCTTAGT-3'		
F-Factor VIII	5'-AGCCTTGTGAAACTGAAGCAT-3'	NM_000552	10-11
R-Factor VIII	5'-GGCCATCCCAGTCCATCTG-3'		
F-GAPDH	5'-ATGGGGAAGGTGAAGGTCG-3'	NM_002046.3	2-3
R-GAPDH	5'-GGGGTCATTGATGGCAACAATA-3'		

Table showing the different primers used for PCR analysis