Supplemental Table 1: Flow cytometric verification of immunomagnetic sorting for WES

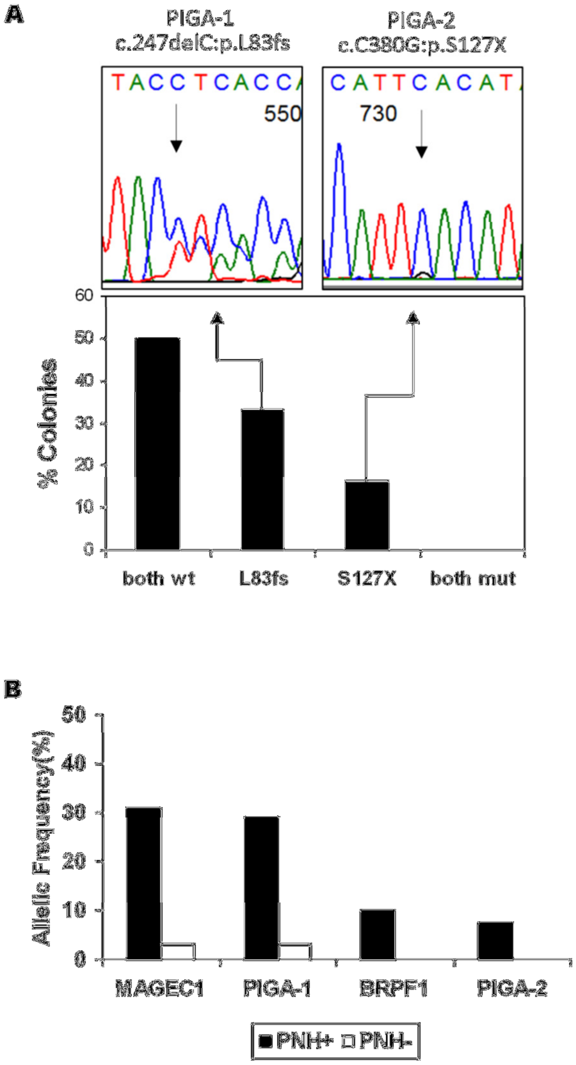
Cohort ID	PNH Fraction (%CD59-)	Normal Fraction (%CD59+)
PNH1	99.1%	98.4%
PNH2	96.8%	95.6%
PNH3	69.9%	99.8%
PNH4	94.3%	97.7%
PNH5	85.8%	92.1%
PNH6	82.6%	96.6%
PNH7	51.6%	91.9%
PNH8	87.2%	80.9%
PNH9	34.4%	96.8%
PNH10	84.9%	94.3%
PNH11	76.8%	95.7%
PNH12	97.1%	95.3%

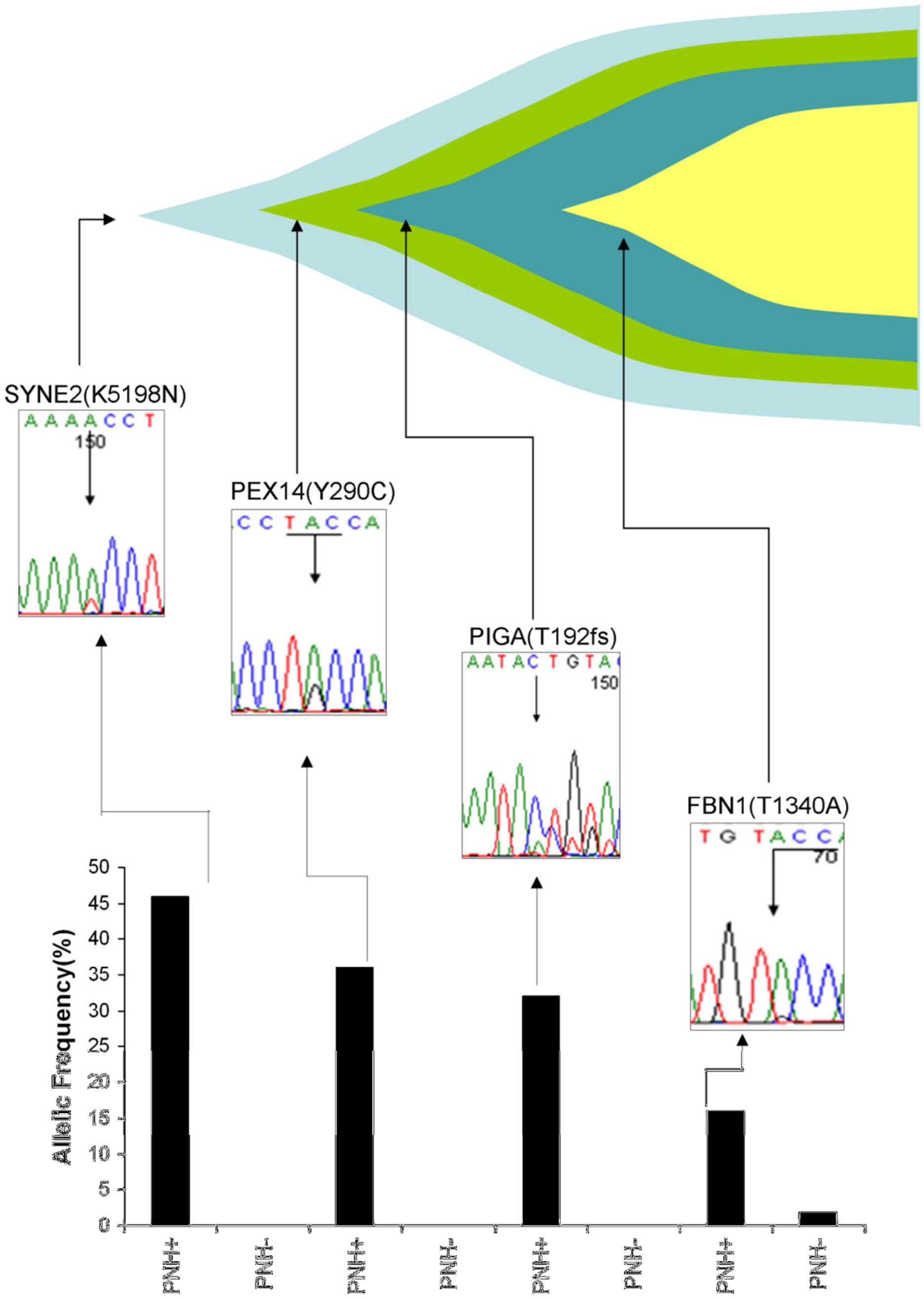
Supplemental table 2. Panel of 61 genes for targeted deep NGS sequencing

Gene Name

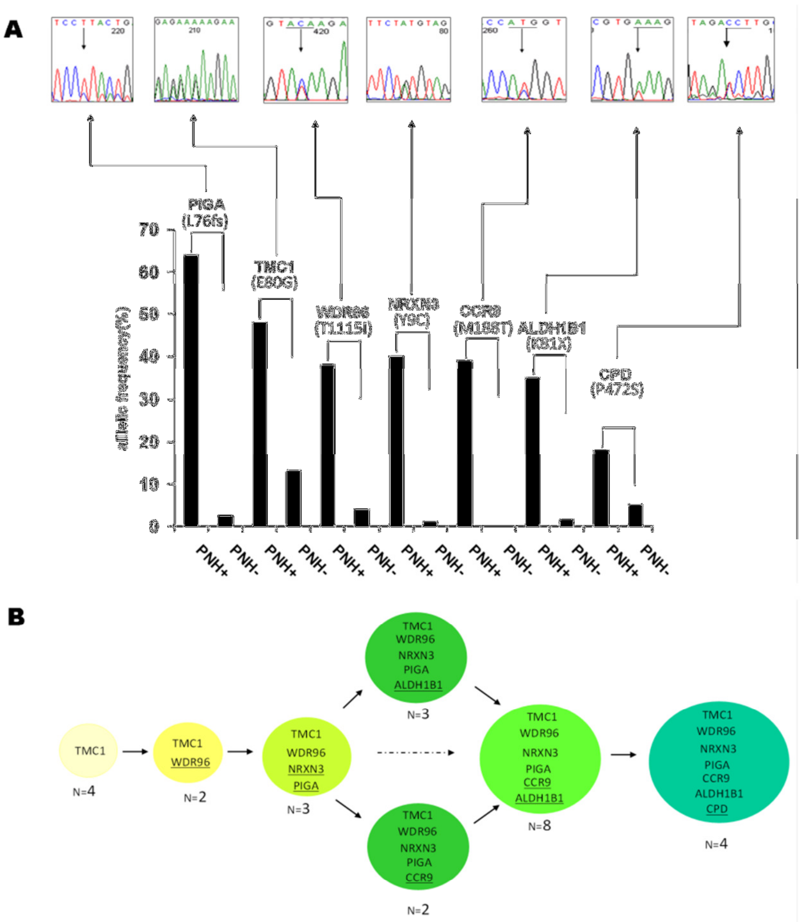
- *BCOR*
- *BCORL1*
- *RAD21*
- *SMC3*
- *STAG2*
- *DDX41*
- *DDX54*
- *DHX29*
- *GLI1*
- *GLI2*
- *PTCH1*
- *IDH1*
- *IDH2*
- *EED*
- *EZH2*
- *SUZ12*
- *KRAS*
- *NF1*
- *NRAS*
- *PTPN11*
- *CSF1R*
- *CSF3R*
- *FLT3*
- *KIT*
- *U2AF1*
- *U2AF2*
- *APC*
- *ASXL1*
- *BTRC*
- *LUC7L2*
- *CALR*
- *CBL*
- *CDH23*
- *CEBPA*
- *CFTR*
- *CUX1*
- *DNMT3A*
- *ERBB4*
- *ETV6*
- *GATA2*
- *GNB1*
- *IRF4*
- *JAK2*
- *KDM6A*
- *MECOM*
- *MED12*
- *MLL*
- *NPM1*
- *PHF6*
- *PRPF8*
- *RNF25*
- *RUNX1*
- *SETBP1*
- *SF3B1*
- *SIMC1*
- *SRSF2*
- *STAT3*
- *TET2*
- *TP53*
- *WT1*
- *ZRSR2*

Supplemental Figure 1: PNH patients may contain two independent PIGA mutations. **A**) Sanger sequencing of bacterial subcloning experiments demonstrates the presence of two separate PIGA mutations in PNH2. **B**) Variant allele frequencies from whole exome sequencing suggests that the MAGEC1 mutation (31%, clone size 62%) preceded the PIGA-1 (L83fs) mutation (29%, clone size 58%).

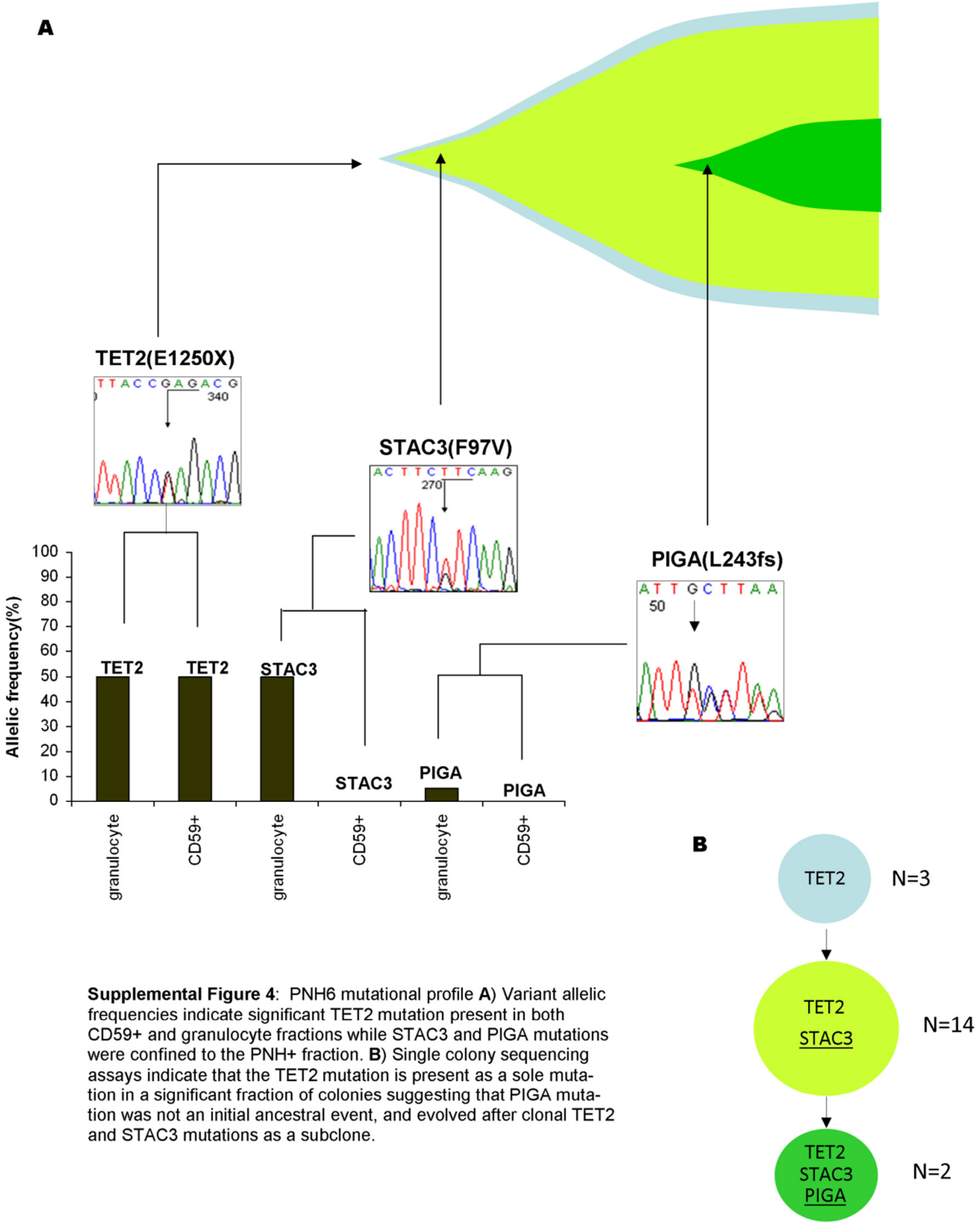




Supplemental Figure 2: PNH10 mutational profile **A)** Variant allelic frequencies reveal somatic SYNE2 and PEX14 gene mutations were the initial events, followed by a PIGA frameshift mutation and an additional FBN1 mutation.



Supplemental Figure 3: PNH3 mutational profile **A**) Variant allelic frequencies reveal a complex set of mutations, some of which were present in the PNH– population as well as the PNH+ fraction. **B**) Single colony sequencing assays indicate that both the TMC1 and WDR96 mutations preceded the acquisition of a PIGA mutation.



Supplemental Figure 4: PNH6 mutational profile **A**) Variant allelic frequencies indicate significant TET2 mutation present in both CD59+ and granulocyte fractions while STAC3 and PIGA mutations were confined to the PNH+ fraction. **B**) Single colony sequencing assays indicate that the TET2 mutation is present as a sole mutation in a significant fraction of colonies suggesting that PIGA mutation was not an initial ancestral event, and evolved after clonal TET2 and STAC3 mutations as a subclone.