Supplementary Figure 1. Severely increased red blood cell production (reticulocytosis) results in detection of nucleated and micronucleated (MN) normochromatic erythrocytes (NCE) in Q3. Blood obtained from C57BL/6J-$Apc^{Min/+}$ mice was processed according to the methods described herein. a) Representative flow-cytometric analysis for six week old $Apc^{-/min}$ mouse (left) and 16 week old $Apc^{min/+}$ mice (right). Note the change in % reticulocytes (RET) and %MN-NCE. During extremely high rates of erythropoiesis, such as that exhibited by severely anaemic, aged $Apc^{min/+}$ mice (right), the number of reticulocytes detected in the RET quadrant is vastly increased, however the process becomes error prone and the nucleus is not always expelled; both nucleated and micronucleated red blood cells are detected in the % MN-NCE quadrant, leading to a false-positive increase in %MN-NCE. b) Data shows %MN-NCE vs. %RET for $Apc^{min/+}$ mice (n=4) and $Apc^{+/+}$ mice (n=10). Linear regression analysis shows that there is a significant correlation between increased % MN-NCE and % RET ($P<0.01$). c) Dot plot showing the average %RET in male C57BL/6 mice (mean ± standard deviation: 1.623±0.209; n=20).

Supplementary Figure 2. Effect of RNase, flow rate and fixation. a) Blood sample obtained from male C57BL/6 mouse at 16 weeks of age, processed according to the methods described herein. b) The same sample was processed without the addition of RNase, which results in a shift of the FITC-conjugated transferrin receptor-positive (CD71-FITC+), propidium iodide negative (PI-) reticulocyte (RET) population over to the micronucleated-RET (MN-RET) quadrant. This is caused by PI binding to the RNA within the CD71-FITC+ RETs. c) Sample analyzed at a rate of 10000 events per second (vs. 1000 events in a) shows spikes of events in the CD71-FITC+ and PI+ quadrant (indicated by arrow head). d) The color of fixed samples can indicate whether

800   samples have become too warm during preparation. Fresh blood samples were harvested and fixed in -80°C methanol in

duplicate or in room temperature (RT; 20-25°C) methanol. The blood fixed in -80°C methanol shows characteristic red color while

the sample fixed in RT methanol shows brown color. e) Samples maintained for long periods of time in -80°C methanol show

characteristic red color and are suitable for processing. f) The same samples (from image d) were either placed in a -80°C

freezer or left at RT. After 30 minutes the sample that was maintained at -80°C showed the characteristic red color while the

805   sample that was left at RT became brown in color; no detectable change in color was seen at the sample fixed in RT methanol.

Supplementary Figure 3. Indicators of incorrect sample processing. a) Correctly prepared sample. Blood sample obtained

from male C57BL/6 mouse at 16 weeks of age, processed according to the methods described herein. The SSC-A vs. FSC-A

plot (left) reveals correct separation of blood cell populations and minimal cell debris and the CD71-FITC vs. PI plot (right)

810   reveals correct representation of populations within the quadrant gates. b) Slightly degraded blood sample. Blood sample left 5

days at 4°C and fixed in -80°C methanol. The SSC-A vs. FSC-A plot (left) reveals increased cell debris (black arrow) and

incomplete separation of the cell populations. The gated populations plotted on CD71-FITC vs. PI plot (right) show no indication

of degradation but there is a slight increase in MN-NCE (right). c) Very degraded blood sample. Blood sample left 3 weeks at 4°C

and fixed in -80°C methanol. Both the SSC-A vs. FSC-A and CD71-FITC vs. PI plots are completely transformed. d) Blood

815   sample fixed in warm methanol (room-temperature). The SSC-A vs. FSC-A plot (left) reveals increased cell debris (black arrow)

and the CD71-FITC vs. PI plot (right) shows incorrect representation of populations. e) Blood sample fixed correctly (-80°C

methanol) but allowed to warm to room temperature (20-25°C) before processing. The SSC-A vs. FSC-A plot (left) reveals

increased cell debris (black arrow) and incomplete separation of the cell populations, and the CD71-FITC vs. PI plot (right) shows incorrect representation of populations.
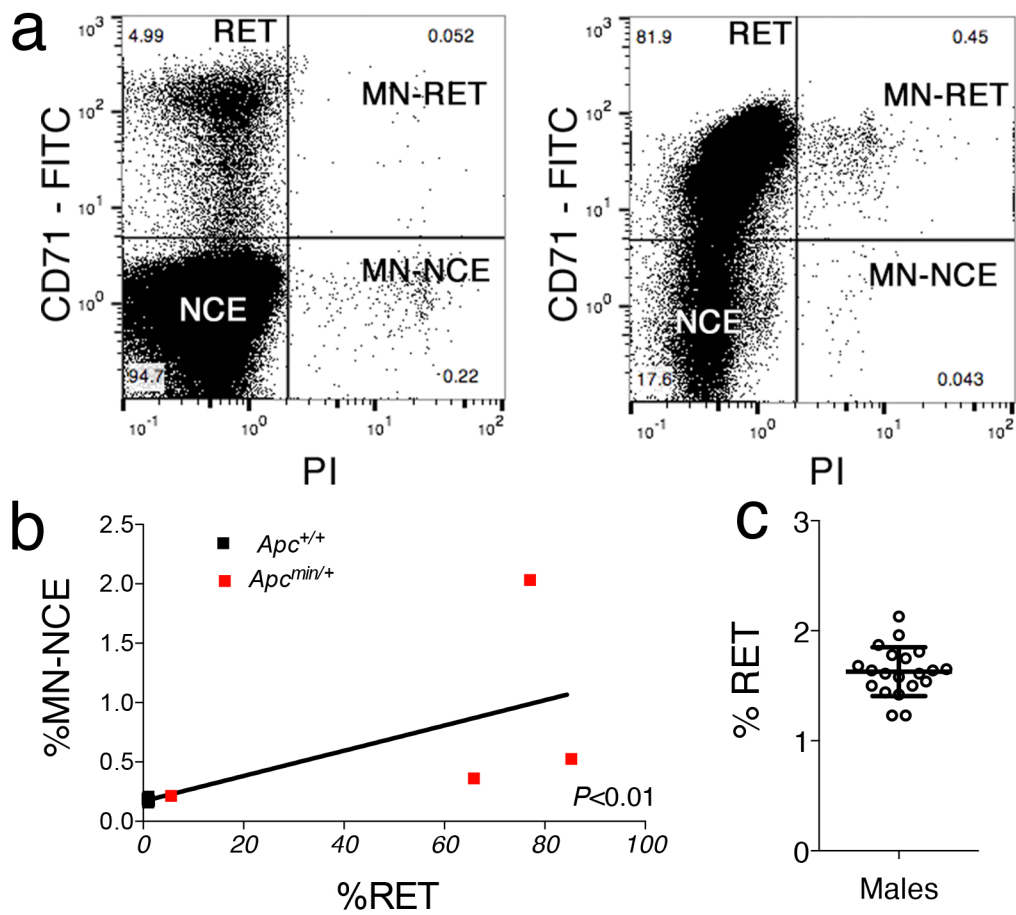
820

Supplementary Figure 4. Effect of incubation time in propidium iodide (PI) and time in storage. a) Data shows %MN-NCE for replicate tubes of the same sample that were stored at -80˚C. Two tubes were processed at monthly intervals over a one-year period. Linear regression analysis shows that there is no significant difference in the detection of % MN-NCE up to one year after fixation (P=0.427). b) A single blood sample was added to each well of a 96-well plate and processed accordingly. After washing

825 with buffer to remove the FITC-conjugated transferrin receptor (CD71-FITC)/RNase mix, samples were resuspended in buffer containing 1 mg/ml PI, and all 96 wells were immediately analyzed by flow-cytometry. Linear regression analysis shows that there is a statistically significant difference in the % micronucleated (MN)-normochromatic erythrocytes (NCE) obtained at the beginning of the plate *versus* those obtained at the end of the plate (*P<0.001). This could be due to saturation of DNA with PI over time. The biological effect is small but to avoid potential experimental bias, samples should be randomized across the plate

830 and we recommend adding PI to 24 wells at a time.

Supplementary Figure 5. Flow cytometric analysis of control samples and gating out autofluoresence. Unstained samples are used to centralise the main cell population within the side scatter (SSC) *versus* forward scatter (FSC) plot (data not shown). The main population is gated (erythrocytes), and within the propidium iodide (PI) *versus* FITC-conjugated transferrin

835 receptor (CD71-FITC) plot a quadrant gate is drawn. Single-stain controls are then analysed to determine the correct voltage and
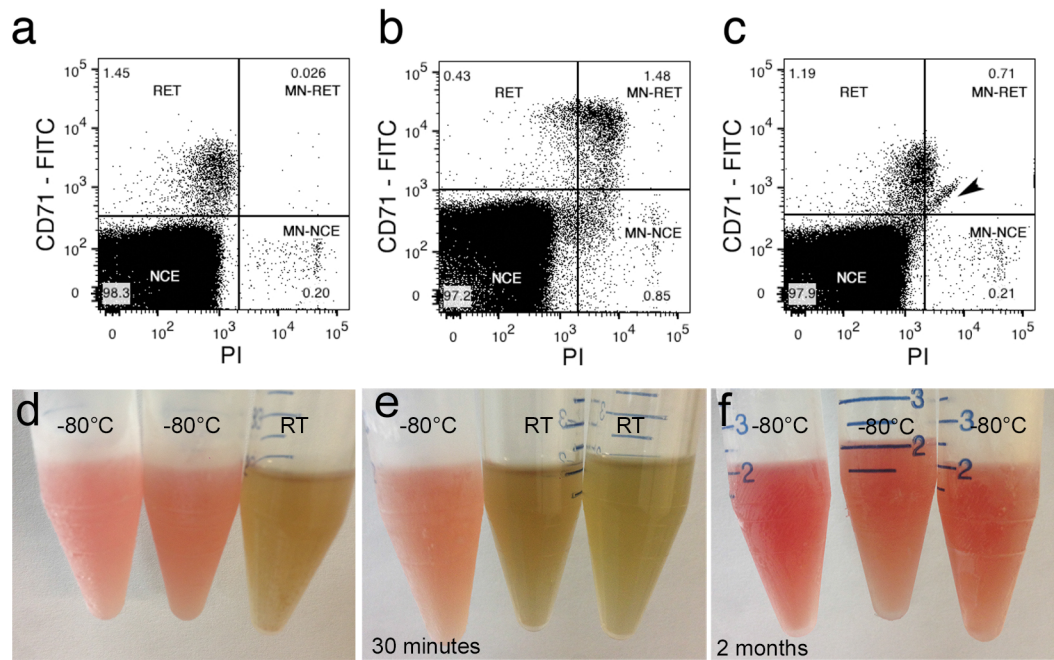
gain settings, which ensures the majority of events fall within the scale of the X and Y axes, and within the correct quadrant gate.

a) Unstained control sample and b) CD71-FITC alone. Auto-fluorescence can be detected with samples stained with PI alone, either within the transferrin receptor FITC-conjugated (CD71-FITC) channel (c; indicated by arrowhead), or within the higher logarithmic scale (Log) of PI channel (d; indicated by arrowhead). To remove the effect of auto-fluorescence, Region gate R3 is
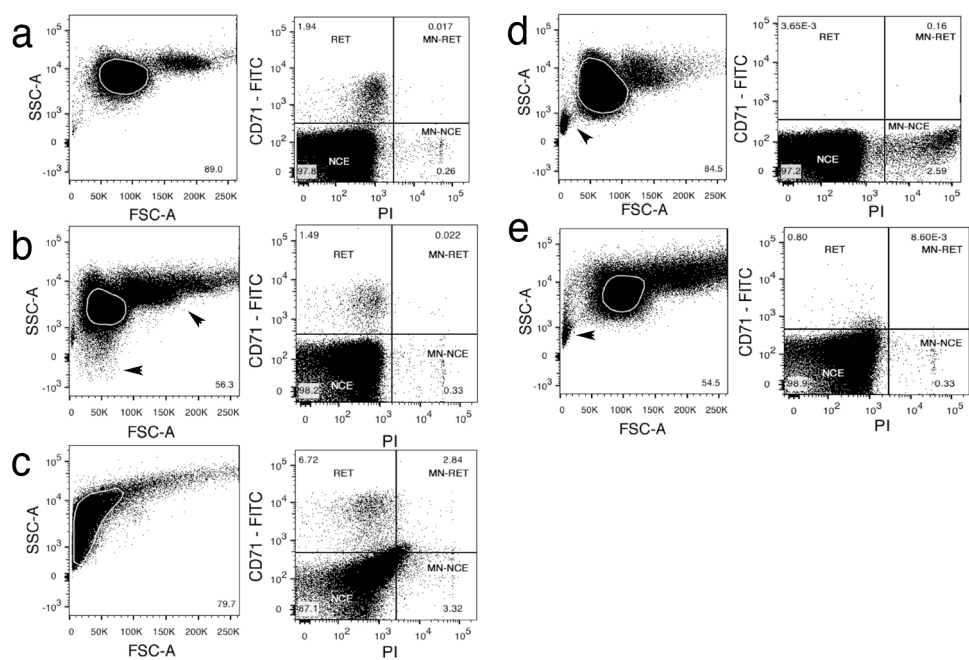
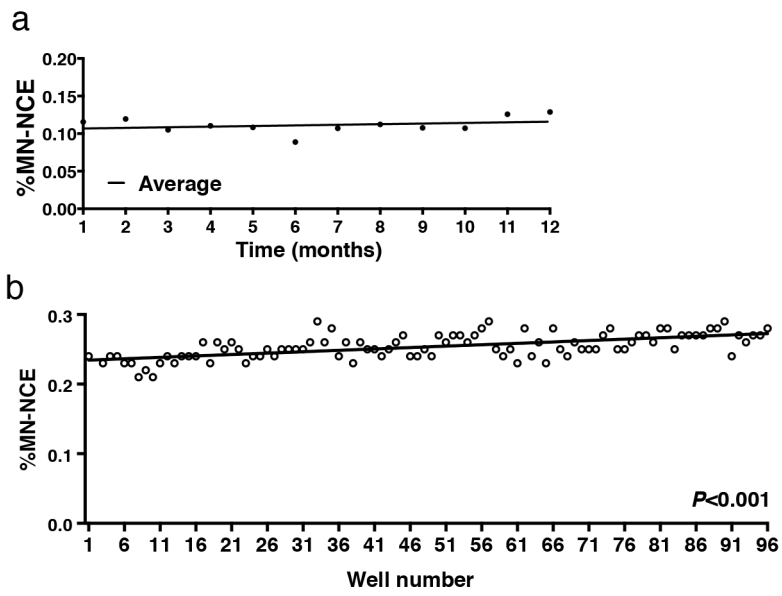840   created within the PI *versus* side scatter (SSC) plot (d).

a

b