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Supplementary Material

In vitro characterisation of fresh and frozen sex-sorted bull spermatozoa

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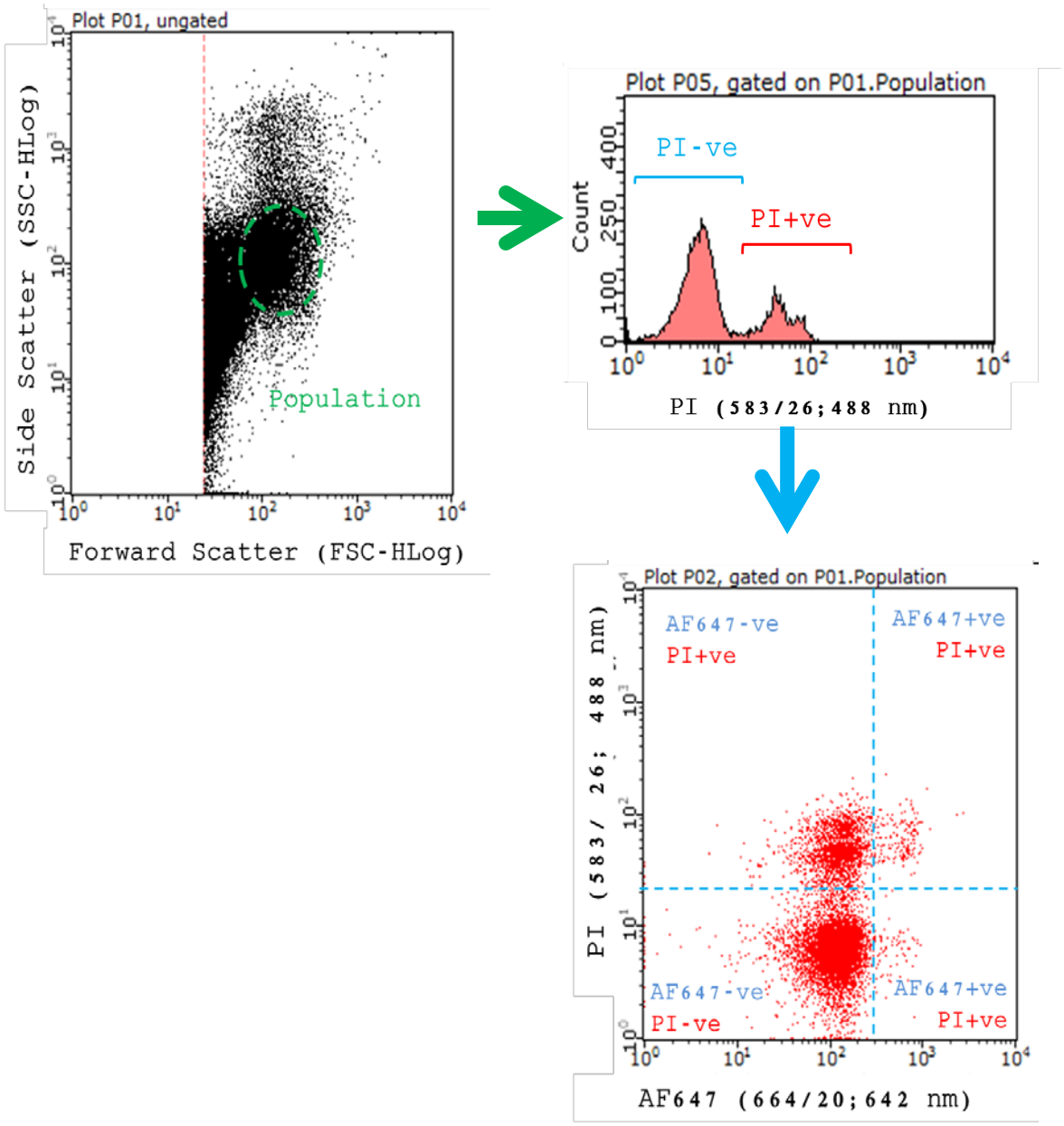

Fig. S1. Flow cytometry gating scheme for acrosome integrity assay. Forward scatter (FSC) and side scatter (SSC) signals were used to discriminate sperm from debris (P01.Population). Viable cells were then selected based on negative fluorescence of Propidium Iodide (PI -ve). The percentage of viable sperm with intact acrosomes was calculated as the percentage of Alexa Fluor negative (AF647 -ve) cells of the PI negative population as initially gated based on controls, FSC and SSC. Equivalent flow cytometry plots were generated for all bull replicates.

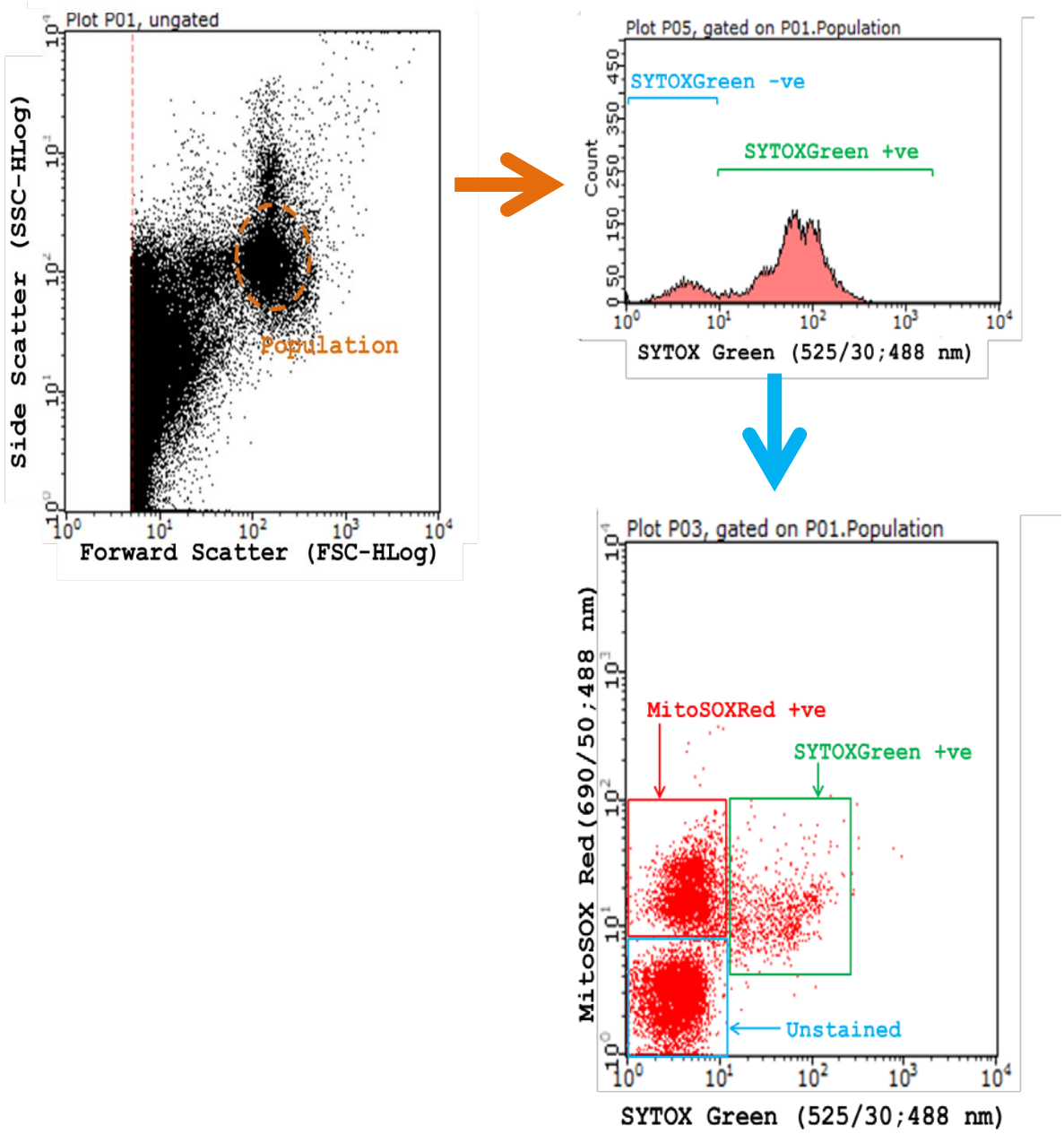

Fig. S2. Flow cytometry gating scheme for superoxide production assay. Forward scatter (FSC) and side scatter (SSC) signals were used to discriminate sperm from debris (P01.Population). Viable cells were then selected based on positive fluorescence of SYTOX Green (SYTOXGreen +ve). The percentage of viable sperm with high superoxide production was calculated as the percentage of MitoSOX Red (MitoSOXRed +ve) positive of the SYTOX Green negative population as initially gated based on controls, FSC and SSC. Equivalent flow cytometry plots were generated for all bull replicates.