A

FISH [a]	pre-BI	*ex vivo* transitional pre-BI	*in vitro* transitional pre-BI [b]	pre-BCR+	pre-BII	immature B	mature B [c]
RNA	CD19+ c-kit+	B220+c-kit+ CD2+	B220+c-kit+ sμHC+	B220+ SL156+ k-	B220+ CD25+	B220+ IgM+	B220+ IgM+
DNA	B220+ c-kit+				B220+ CD25+		CD43 depletion

B
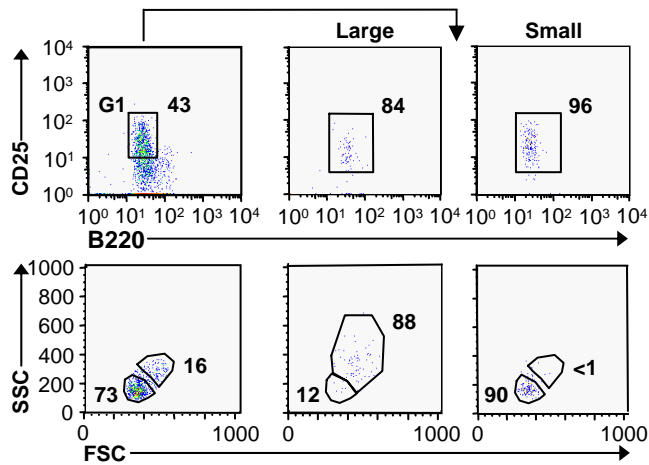
Cell sorting. (A) [a] BM cells were sorted on a FACSVantageSE™ or FACSAria (Becton Dickinson) into discrete developmental stages using specific surface markers. Cells were analyzed by RNA or DNA FISH. [b] sμHC+ cells were sorted using the Alexa Fluor® 488 Signal Amplification Kit (Molecular Probes). [c] Splenic B cells for DNA-FISH were enriched by CD43 depletion using magnetic beads (Miltenyl Biotech). Typically, sort purities were >90%, except large pre-BII cells ~80%. (B) The pre-BII population was gated as B220+CD25+ (G1) and sorted into large and small fractions using forward scatter. The numbers show the percentage of cells within the indicated gates before and after sorting.