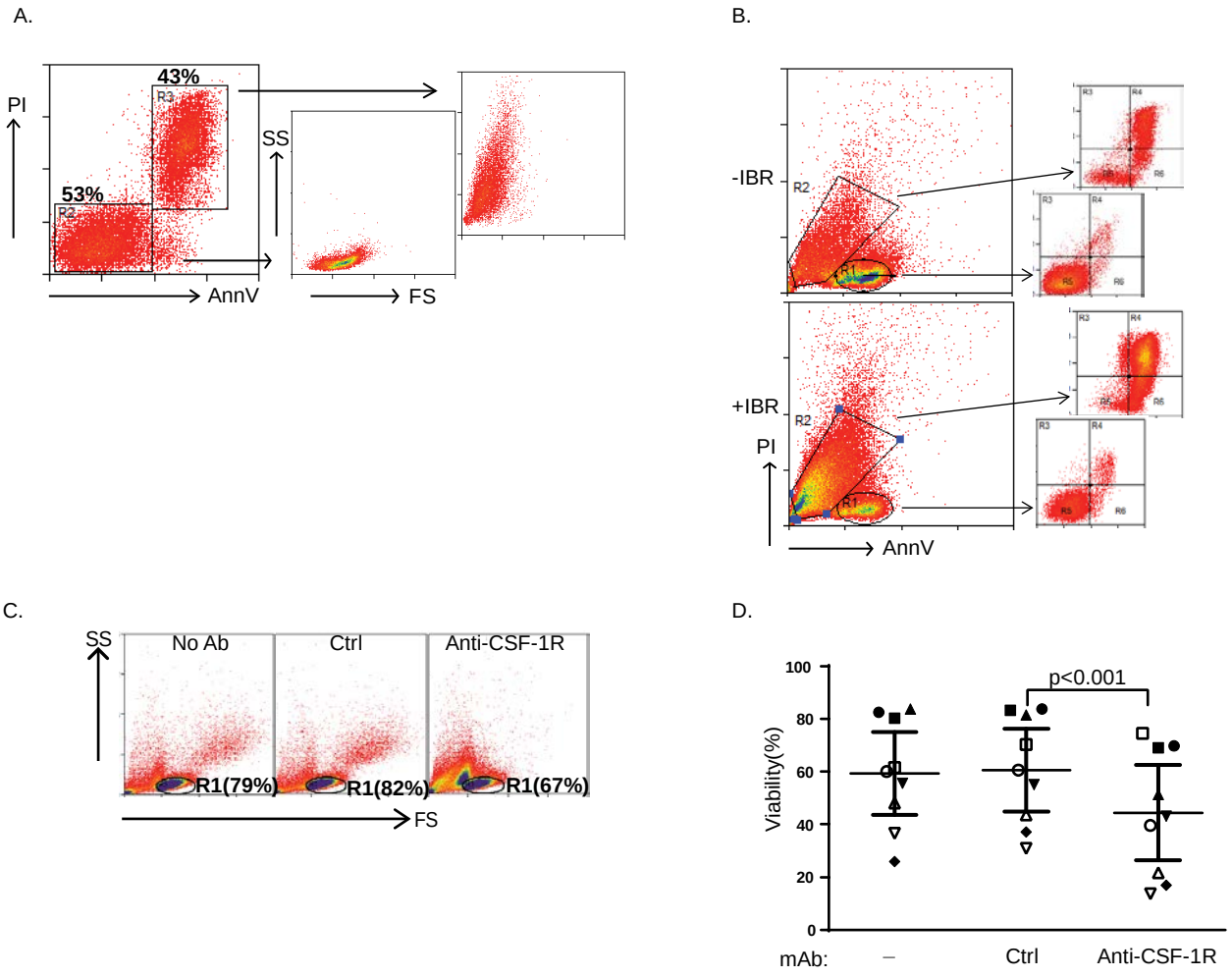
Supplementary Figure 2



Supplementary Figure 2

Viable CLL cells may be identified by forward and side scatter characteristics. (A) Purified $CD19^+$ CLL cells were cultured for 7 days and spontaneous apoptosis determined by Annexin V and propidium iodide (PI) staining. Viable (annexin V^-/PI^-) and apoptotic (annexin V^+/PI^+) cells were identified by annexin V and PI staining (in the gates shown), and their forward and side scatter characteristics examined. Viable and apoptotic cells were readily distinguished by their forward and side scatter characteristics. (B) CLL patient PBMC were cultured for 7 days with ibrutinib (1 µM) or vehicle control, as indicated. Viable (gate R1) and apoptotic (gate R2) $CD19^+CD5^+$ CLL cells were identified by their forward and side scatter characteristics in the gates shown. Viable CLL cells, as determined by annexin V/PI staining (at right), were largely confined to gate R1, whereas apoptotic cells were largely confined to gate R2. (C) CLL patient PBMC were treated with a control or blocking anti-CSF-1R monoclonal antibody. After 7-10 days in culture, cells were gated, as shown, and cell viability determined. A representative example is shown (C), and the data summarized (n=9) in (D).