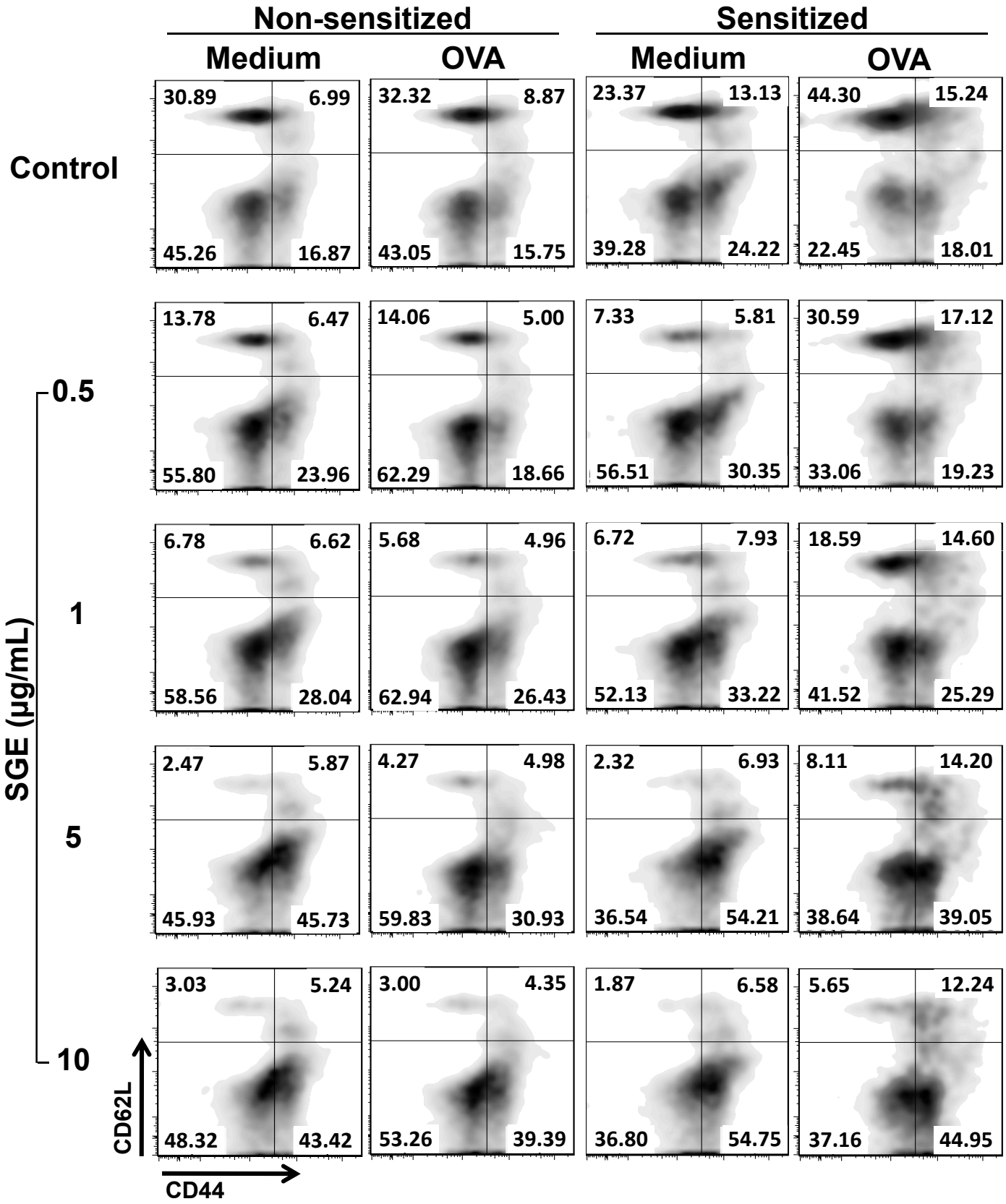Additional file 4