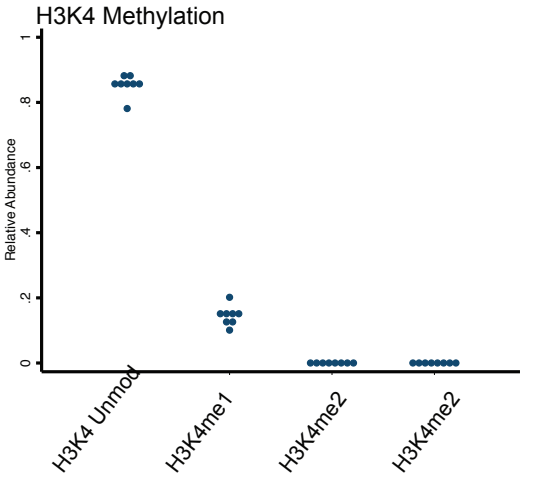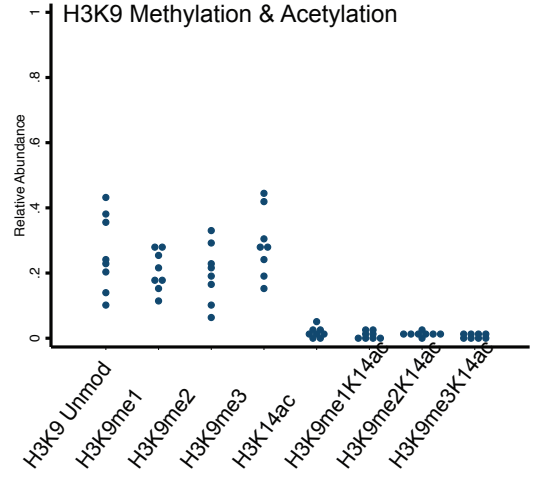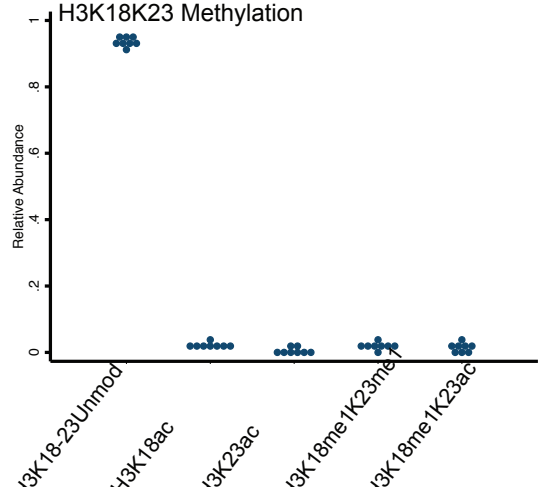Additional file 3