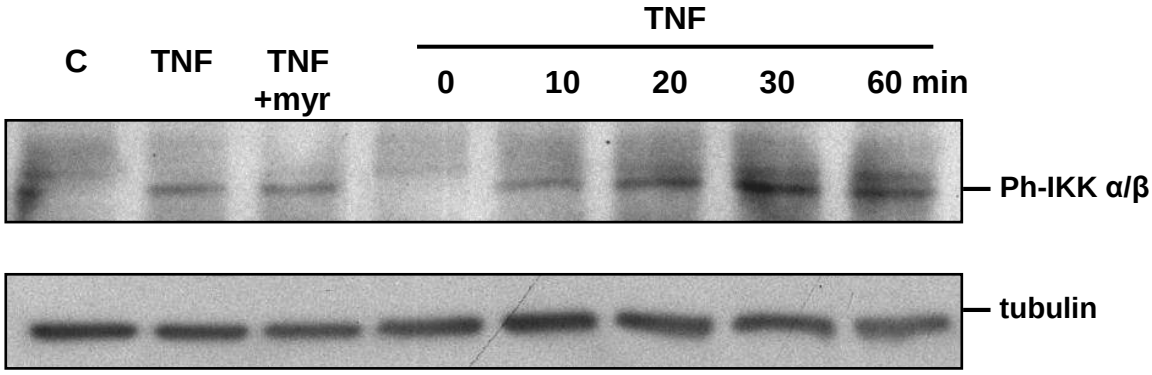Additional file 4 -