Supplemental Materials

Molecular Biology of the Cell

Sahu et al.

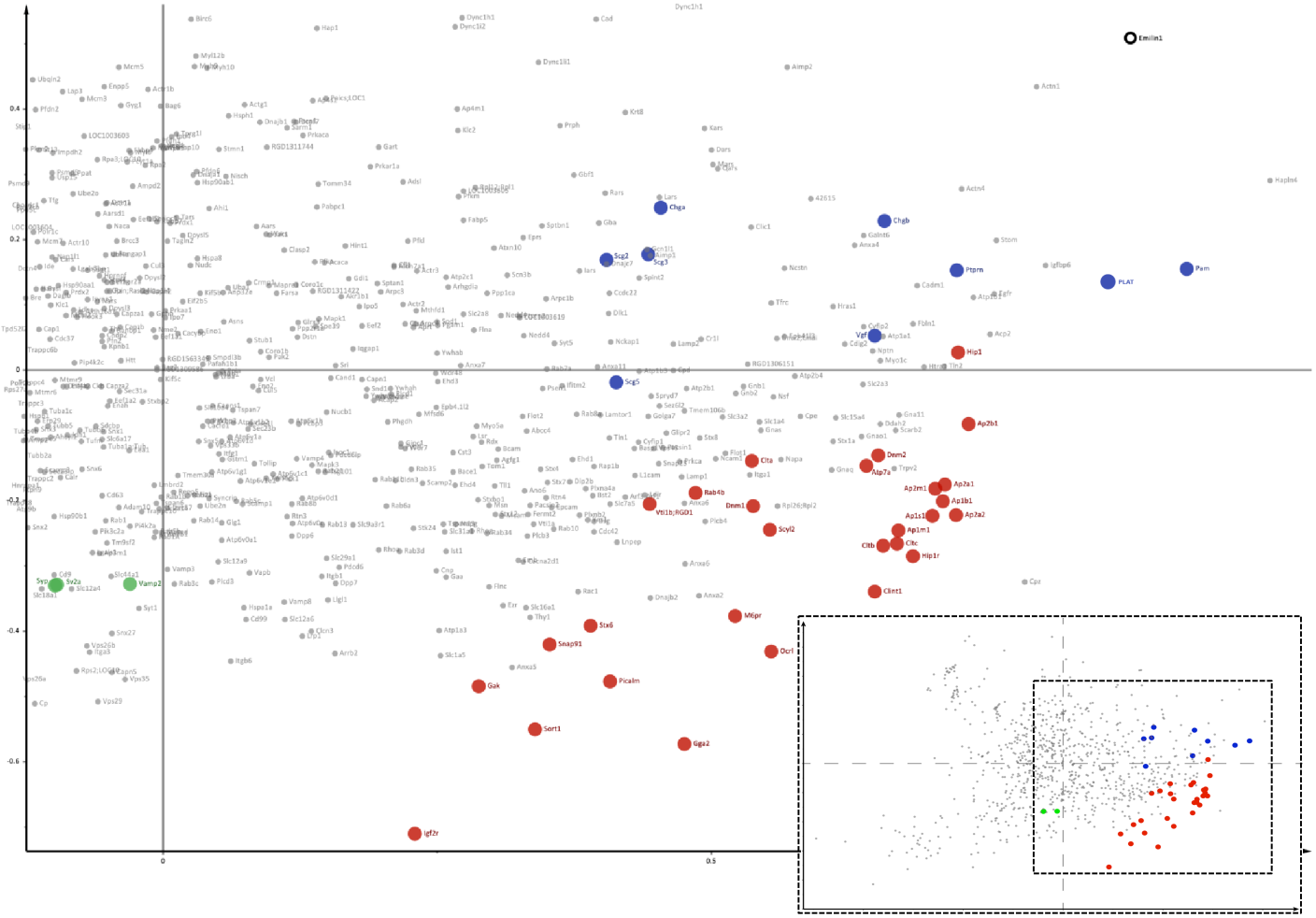
Supplemental Figures and Table

Supplemental Figure S1. Enlargement of the PCA plot shown in Figure 1C, with protein identities indicated. The inset shows a reduced-size version of Figure 1C, with a box around the region that has been enlarged.

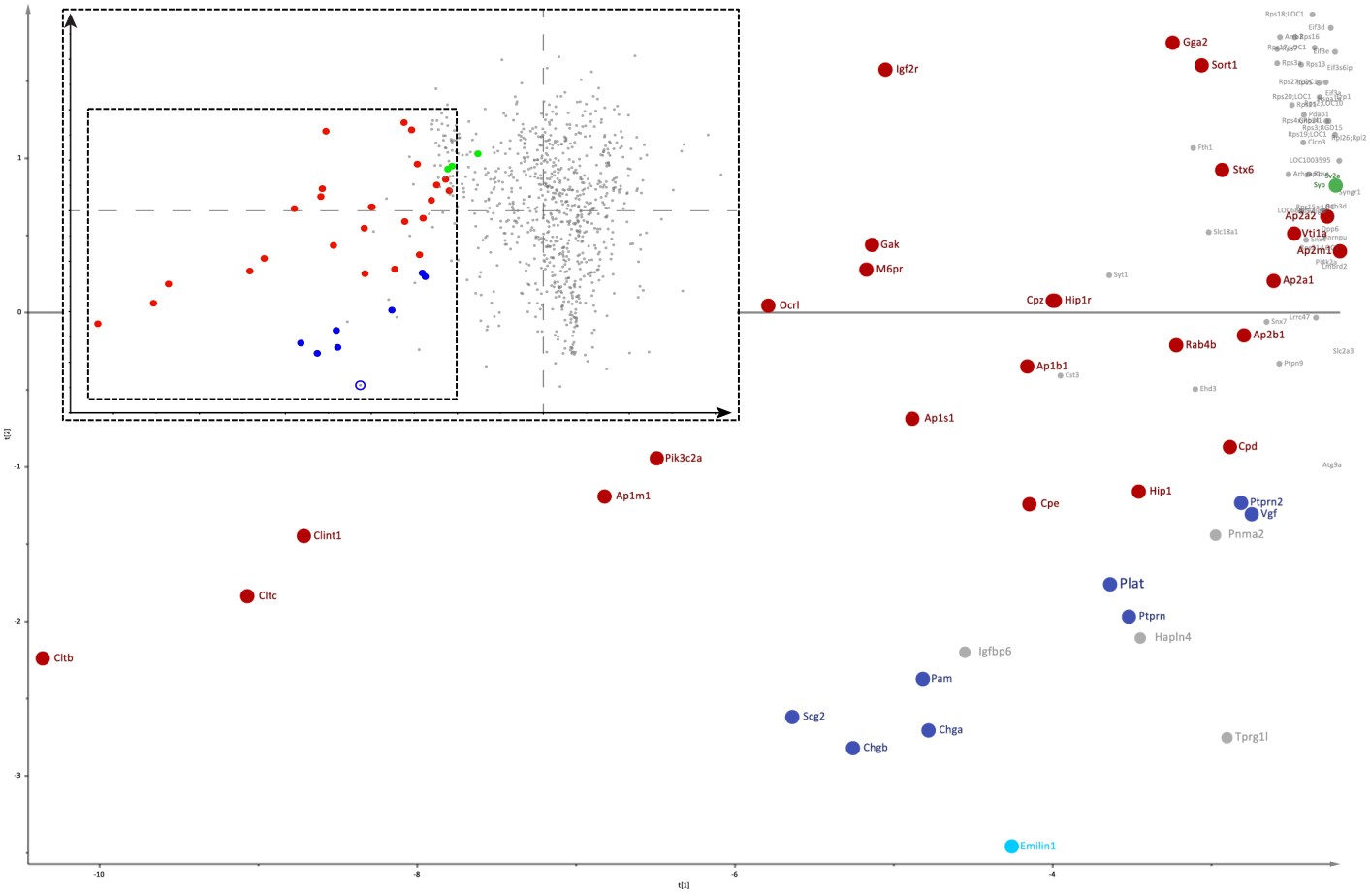
Supplemental Figure S2. Enlargement of the PCA plot shown in Figure 4B, with protein identities indicated. The inset shows a reduced-size version of Figure 4B, with a box around the region that has been enlarged.

Supplemental Figure S3. Controls for possible off-target effects. A. Design of the CHC rescue construct. B. Flow cytometry of a clonal cell line (B4) expressing shRNA-resistant mCherry-tagged CHC, showing homogeneous expression (the very sparse red dots to the left represent cells that have presumably lost their expression). C. Fluorescence microscopy of clone B4 cells expressing CHC-mCherry (red), counter-stained for DNA (blue). D. Western blots of wild-type cells, clone B4 cells, and a mixed population of CHC-mCherry-expressing cells, either mock-treated or treated 5 days with doxycycline. Blots were probed with anti-CHC, anti-mCherry, or anti-tubulin as a loading control. Asterisks indicate mCherry-tagged CHC. The blot shows that expression levels of the construct were very low, less than the residual expression of endogenous CHC after a knockdown. E. Western blots of either clone B4 cells (which contain shRNA-A) or cells containing shRNA-B, either mock-treated or treated 5 days with doxycycline. shRNA-B is less efficient at depleting endogenous CHC than shRNA-A. F. Uptake of [^{3}H]-norepinephrine in mock-treated and clathrin-depleted cells expressing shRNA-B. The block in uptake is much more modest than with shRNA-A (see Figure 6). G. Release of [^{3}H]-norepinephrine in mock-treated and clathrin-depleted cells expressing shRNA1, either under basal conditions or in the presence of $BaCl_2$. Knocking down clathrin with shRNA-B inhibits secretagogue-induced release, but unlike shRNA-A it does not abolish it. These differences are presumably due to the differences in knockdown efficiency.

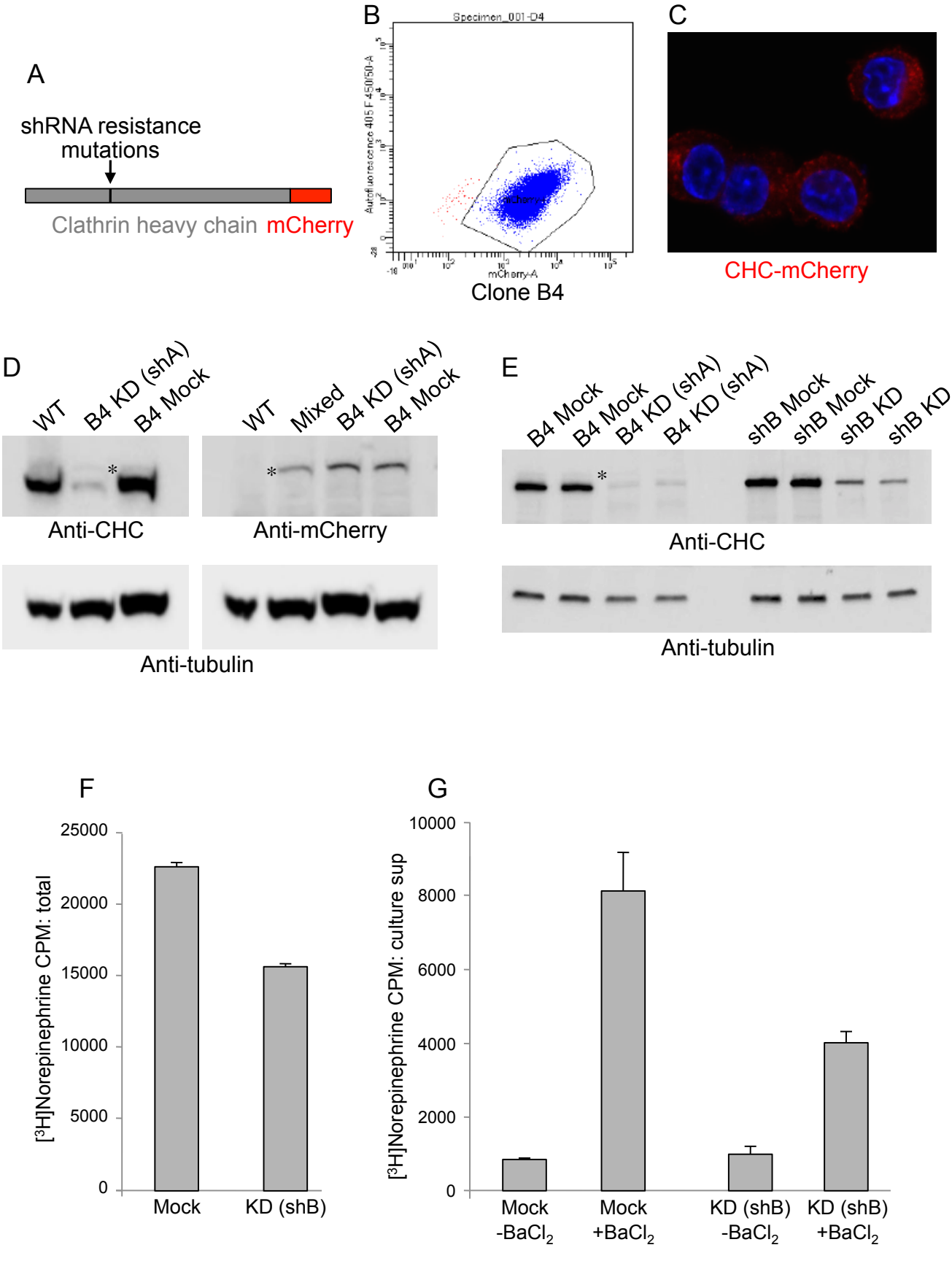
Supplemental Table S1. SILAC ratios for the data shown in Figures 1C, 4A, and 4B.



Sahu et al. Supplemental Figure S1



Sahu et al. Supplemental Figure S2



Sahu et al. Supplemental Figure S3