Supporting Information

Chen *et al*. "Quantification of β-Catenin Signaling Components in Colon Cancer Cell Lines, Tissue Sections, and Microdissected Tumor Cells using Reaction Monitoring Mass Spectrometry"

Description of the in Silico Peptide Interference Predictor (IPIP)

iPIP is a standalone Java application. Swing components like JTable, JTabbedPane, JEditorPane, and JSplitPane are used to build the graphical user interface (GUI). JFreeChar is used to draw all the charts and iText is used to save the charts to PDF files. The peptide table and the charts can also be printed using the related menu items. iPIP is deployed as a Java Web Start application so that the end user always gets the most updated version of the program.

iPIP can handle text-based FASTA protein databases with either NCBI or SwissProt header formats:

> >gi|2811086|sp|P00533|EGFR_HUMAN Epidermal growth factor receptor precursor (Receptor tyrosine-protein kinase ErbB-1)
> >P00533|EGFR_HUMAN Epidermal growth factor receptor precursor - Homo sapiens (Human)

The input parameters for iPIP, besides FASTA database, include protease, target peptide sequence (including modifications or stable isotope labels) or molecular weight of the peptide, molecular weight tolerance, and maximum number of missed cleavages allowed. After on-the-fly digestion of all the proteins in the FASTA database, the peptides within the mass tolerance are displayed in a table with the following information related to each peptide:

- Protein Name (Accession Number)
- Peptide Mass
- Amino Acid Sequence and Flanking Residues
- Numbers for the First and Last Amino Acid Residues of the Peptide Eisenberg and Kyte/Doolittle Hydropathy
- Isoelectric Point (pI)

Some statistical information is also displayed. This information includes the total number of peptides within the mass tolerance, the total number of peptides with a particular N-terminus, the total number of peptides with a particular C-terminus. The table can be sorted by selection of any of the columns. All the information is displayed interactively; the user can generate additional tables with subsets of the peptides. Data in the peptide table are visualized in the second tab. For example, Hydropathy versus Mass, pI versus Mass, and pI versus Hydropathy plots can be displayed to examine mechanisms for peptide separation and signal isolation for quantitative monitoring.

Fragmentation is displayed on the third tab, but only when a sequence is used for the input. The interferences for all fragment ions of the peptide are calculated; in other words, the number of peptides with the same intact mass and fragment ions within the selected mass tolerances. The user can choose any b or y fragment ions and get a list of peptides that have fragments of the same mass-to-charge ratio.

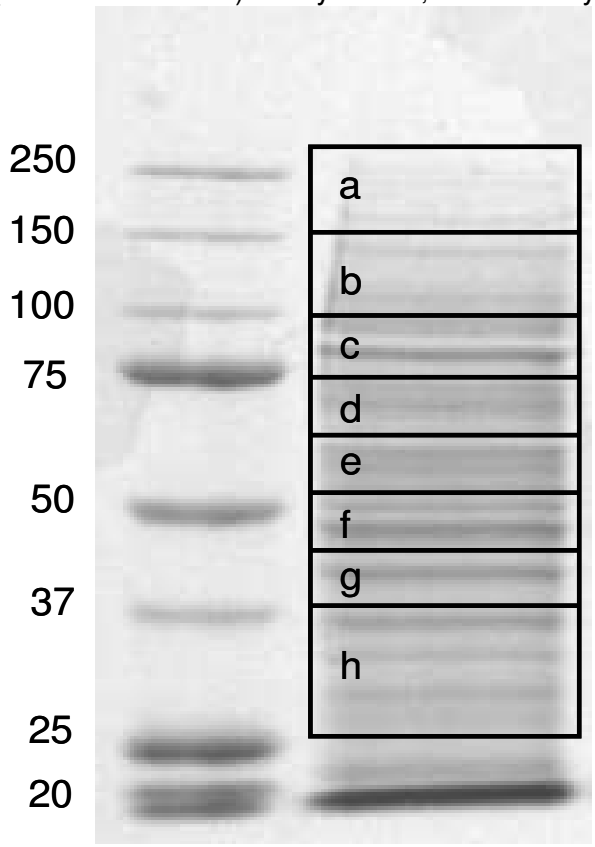
Supplementary Table 1: Comparison of Antigens Used for Development of the Antibodies Used for Western Blots and Selected Peptides for Monitoring Each Protein Target in LC-MRM. LC-MRM target peptide sequences in bold font indicate overlap with the protein regions selected as antigens for corresponding proteins.

Protein	Antigen	Monitored Peptides
β-catenin	571-781	152-158, 159-170, 226-233, 234-242, 336-342, 475-486, 487-496, 536-542, **613-625, 648-661**
c-Src	82-169	49-62, 63-78, **99-106**, 164-172, 347-354, 431-441, 473-480
c-Myc	408-439	347-355, 379-389
PP2A Catalytic subunit	153-309	22-29, **207-214, 284-294**
CD44	153-171	30-38, 79-90, 682-694
α-catenin	729-906	156-163, 166-178, 361-370, 534-540, 617-623, 684-695, **738-747**
Cortactin	181-287	60-70, 88-94, 132-144, 162-168, **236-242, 273-279**, 337-346, 352-359, 415-428, 538-549

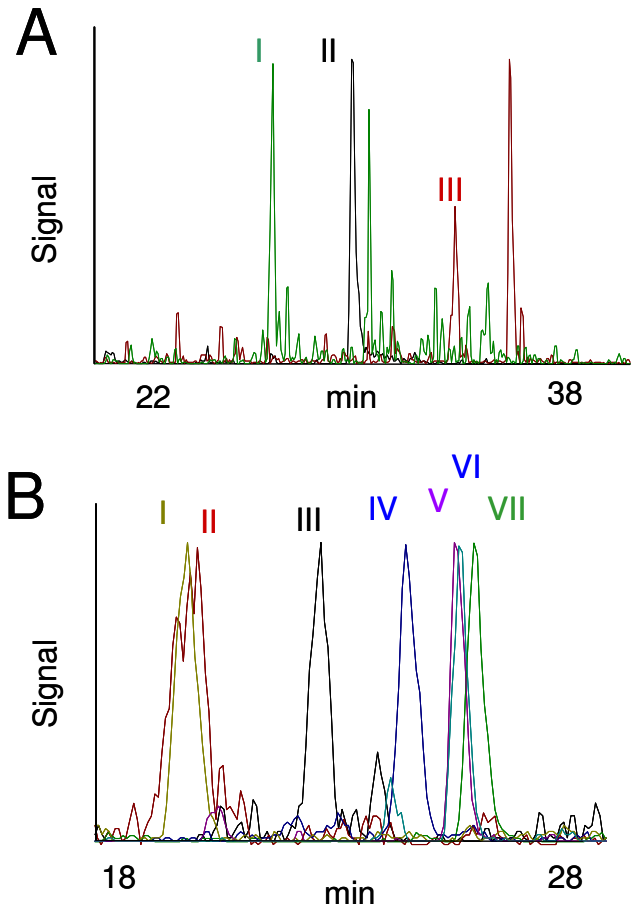
Supplementary Table 2: Absolute Quantification of Proteins in Colon Cancer Cell Lines. Data are expressed as molecules per cell as calculated from the ratio of the ion signal of the biological peptide to the internal standard and the amount of internal standard peptide included prior to LC-MRM. These data are generated from a different biological sample than those used for analysis of reproducibility of protein measurement in SW480 and SW620 cell lines. Values are given in scientific notation.

Protein	KM12	KM12C	KM12L4A	KM12SM	SW480	SW620
β-catenin	2.46E5	2.50E5	1.93E5	2.81E5	4.35E5	2.35E5
c-Src	2.06E4	4.32E4	3.86E4	6.62E4	7.60E3	1.35E4
c-Myc	6.49E5	2.20E5	2.35E5	7.52E5	2.99E5	2.12E5
PP2A Catalytic subunit	1.19E6	1.11E6	1.80E6	2.85E6	1.46E6	8.19E5
CD44	1.70E6	2.72E5	2.80E5	1.56E6	2.53E5	1.64E5
α-catenin	3.46E4	4.64E4	3.97E4	4.03E4	3.81E4	1.98E4
Cortactin	2.42E5	2.05E5	1.97E5	2.63E5	1.20E5	1.79E5
MMP-7	9.80E4	9.53E4	4.20E4	5.33E4	5.38E4	3.41E4
Cyclin D1	2.19E5	2.75E5	1.79E5	1.59E5	2.71E5	2.54E5
Casein kinase II β	5.60E5	7.26E5	6.09E5	5.40E5	3.30E5	3.72E5
PP2A 65kDa regulatory subunit A α	2.29E3	2.61E3	9.53E2	2.74E3	2.58E3	2.33E3
PP2A 65kDa regulatory subunit A β	8.31E3	9.63E3	1.03E4	1.28E4	4.56E3	6.85E3
PP2A 55kDa regulatory subunit B α	4.62E4	4.26E4	4.74E4	1.27E5	6.24E4	2.68E4
Casein kinase II α	2.30E6	1.88E6	2.12E6	2.27E6	2.21E6	1.92E6
GSK3β	6.72E4	5.15E4	4.41E4	1.11E5	8.12E4	5.44E4
Casein kinase II α'	1.04E6	1.81E6	1.61E6	1.53E6	1.20E6	1.17E6
Casein kinase I	1.32E5	1.36E5	7.89E4	1.70E5	1.45E5	1.88E5

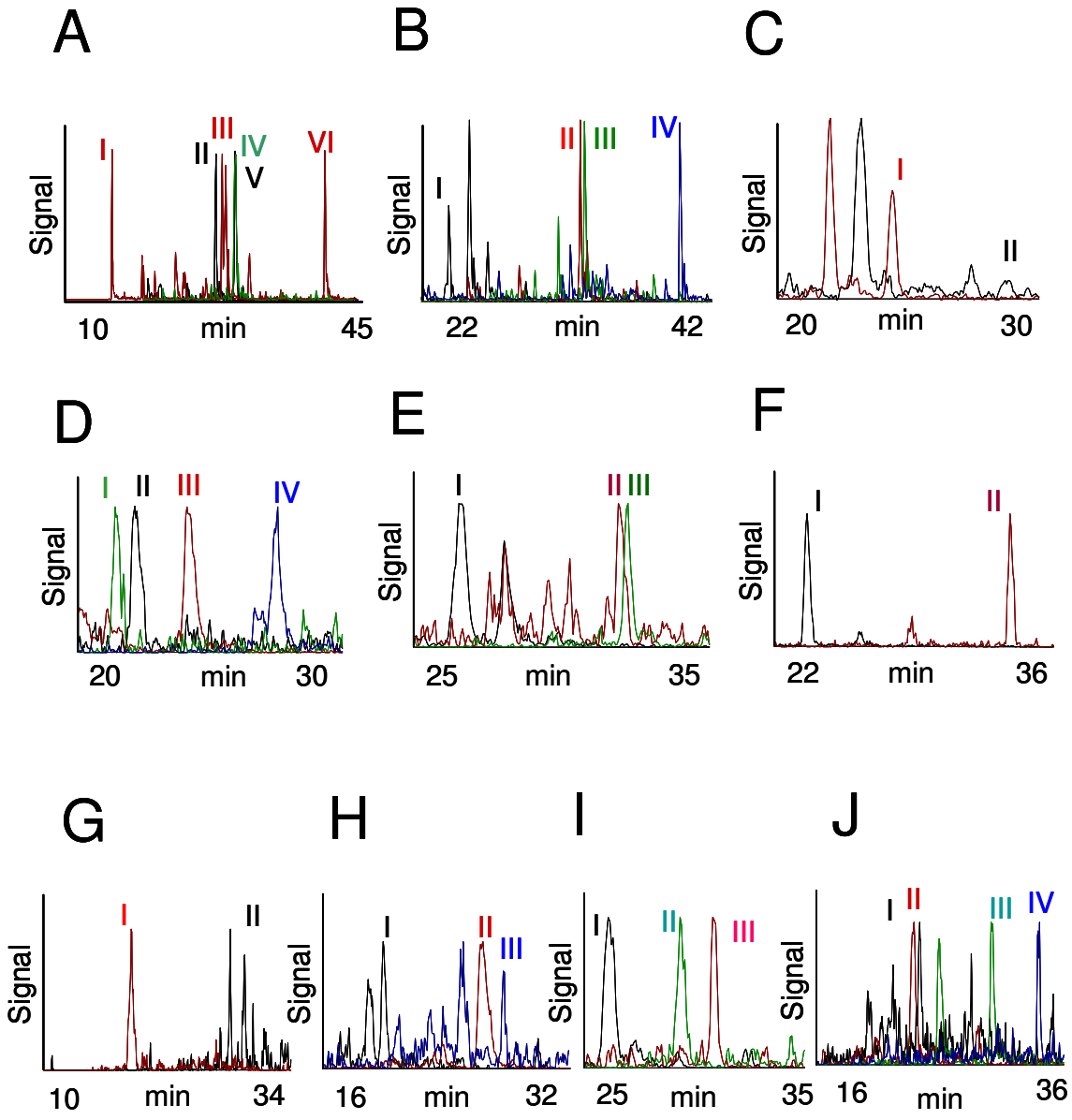
Supplementary Figure 1: Gel Image for HCT116 Colon Cancer Cell Line with Excised Regions for Wnt Signaling Components and Downstream Expressed Proteins. a indicates the excised gel bands (MW above 150) for CD44; b indicates (MW from 100 to 150) for APC protein, Axin1, Axin2, E-cadherin, and α-catenin; c indicates (MW from 75 to 100) for β-catenin; d indicates (MW from 69 to 75) for Cortactin, PP2A 65kDa regulatory subunit A α, and PP2A 65kDa regulatory subunit A β; e indicates (MW from 55 to 69) for PP2A 55kDa regulatory subunit B α and c-Src; f indicates (MW from 45 to 55) for Casein kinase II α, GSK3β, c-Myc, and TCF7; g indicates (MW from 37 to 45) for Casein kinase I and Casein kinase II α'; h indicates (MW from 25 to 37) for Cyclin D1, PP2A catalytic subunit, MMP-7, and Casein kinase II β.



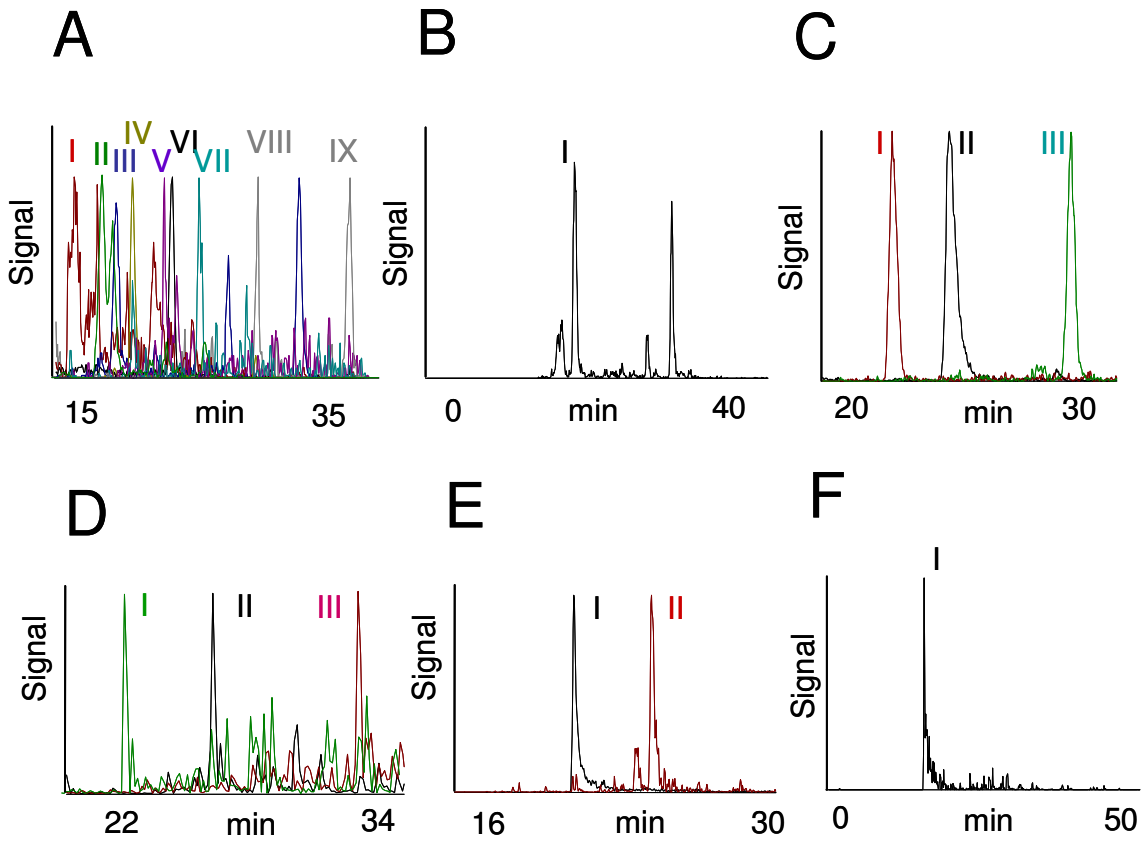
Supplementary Figure 2: LC-MRM Screens (Extracted Ion Chromatogram) To Select Peptides for the components in structural interactions: E-cadherin (**A**) and α-catenin (**B**). The Roman numerals linked to peptide sequences listed in **Table 1**.



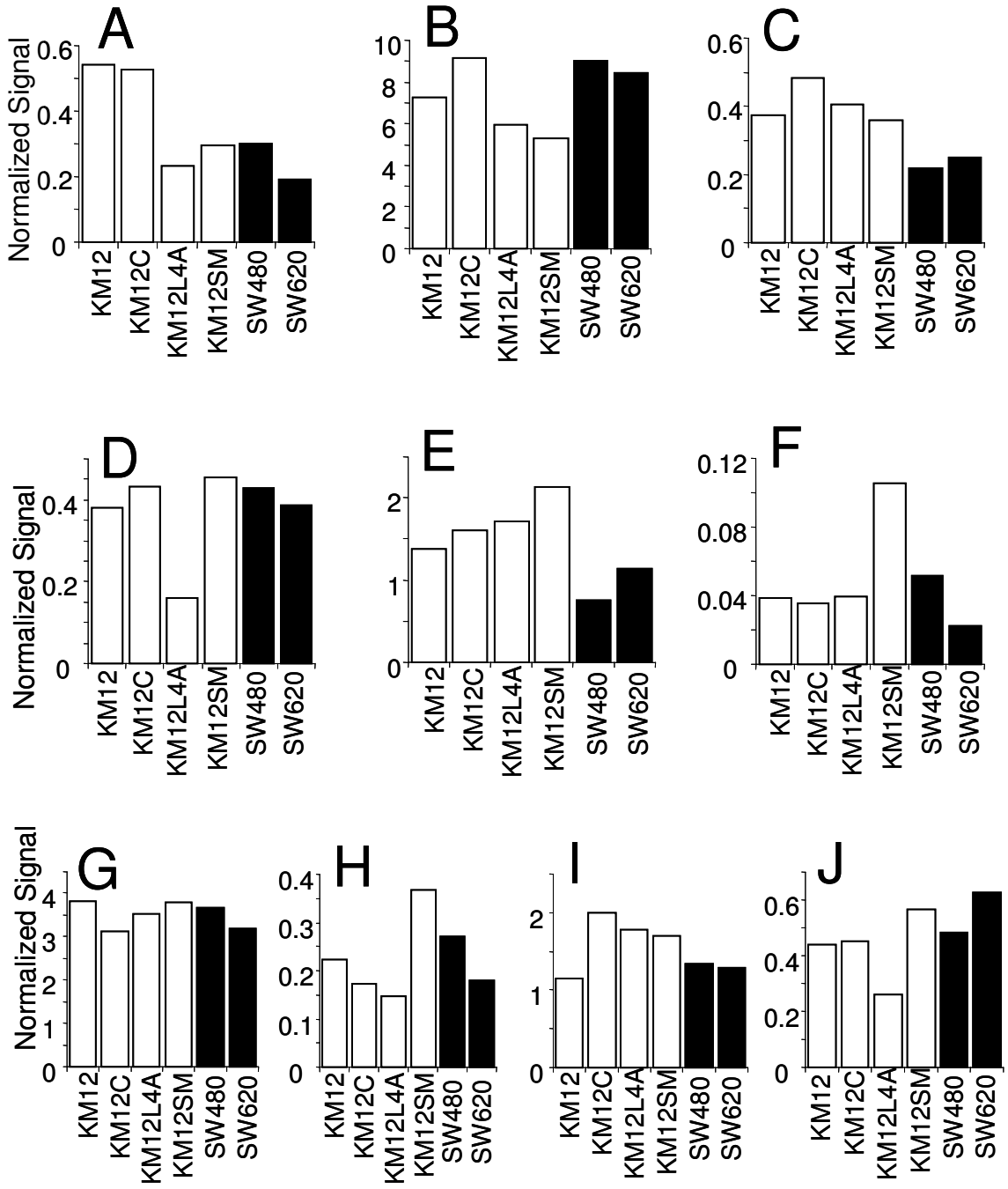
Supplementary Figure 3: LC-MRM Screens (Extracted Ion Chromatogram) To Select Peptides for the following β-catenin regulatory/destruction complex proteins: APC protein (**A**), Axin1 (**B**), GSK3β (**C**), Casein kinase I (**D**), Casein kinase II α (**E**), Casein kinase II α' (**F**), Casein kinase II β (**G**), PP2A 65kDa regulatory subunit A α (**H**), PP2A 65kDa regulatory subunit A β (**I**), PP2A 55kDa regulatory subunit B α (**J**). The Roman numerals linked to peptide sequences listed in **Table 1**.



Supplementary Figure 4: LC-MRM Screens (Extracted Ion Chromatogram) To Select Peptides for the following downstream expressed proteins: Cortactin (**A**), Cyclin D1 (**B**), CD44 (**C**), Axin2 (**D**), MMP-7 (**E**) and TCF7 (**F**). The Roman numerals linked to peptide sequences listed in **Table 1**.



Supplementary Figure 5: Expression Measurement for Other Proteins in Wnt/β-Catenin Signaling Pathway in Different Colon Cancer Cell Lines by LC-MRM. Bar graphs of protein expression normalized with corresponding internal standards are plotted for the following peptides: DLPHITVDR from MMP-7 (**A**), FLSLEPVK from Cyclin D1 (**B**), FNLTGLNEQVPHYR from Casein kinase II β (**C**), AVGPEITK from PP2A 65kDa regulatory subunit A α (**D**), VLELDSVK from PP2A 65kDa regulatory subunit A β (**E**), ILHTAWHPK from PP2A 55kDa regulatory subunit B α (**F**), QLYQTLTDYDIR from Casein kinase II α (**G**), QTLPVIYVK from GSK3β (**H**), VLGTEELYGYLK from Casein kinase II α' (**I**), LFLIDFGLAK from Casein kinase I (**J**).



Supplementary Figure 6: Quantification of c-Src, c-Myc, and PP2A Catalytic subunit by LC-MRM in Individual Frozen Tissue Sections. Bar graphs of protein quantification normalized with corresponding internal standards are plotted for the following peptides: LLLNAENPR from c-Src, DQIPELENNEK from c-Myc, and YSFLQFDPAPR from PP2A catalytic subunit.