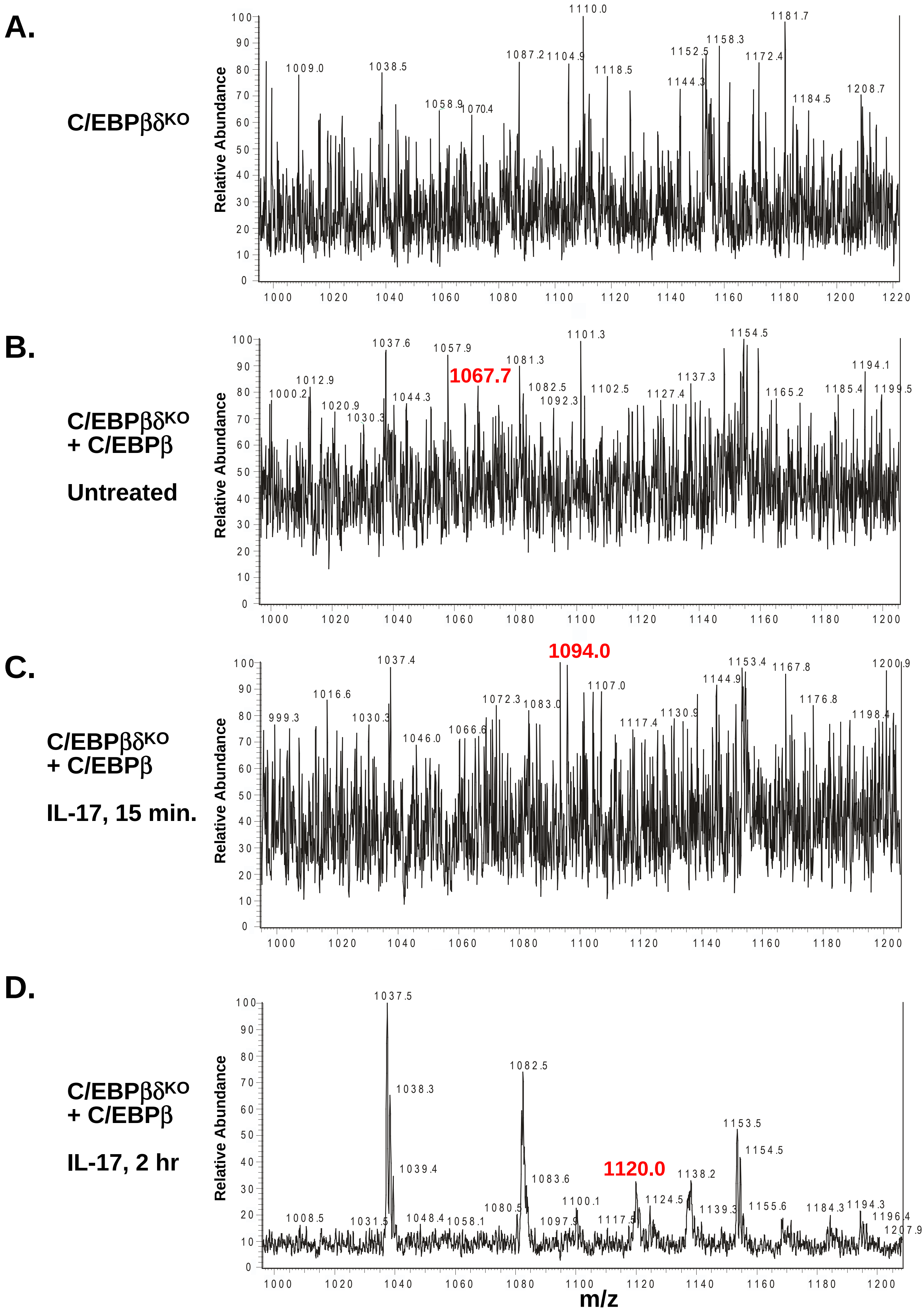
Table 1. Distinct domains within IL-17RA regulate C/EBPβ phosphorylation. IL-17RA^{KO} cells stably expressing IL-17RA.V553H or IL-17RAΔ665 were treated with IL-17 for 0, 15, 30 or 30 minutes, and tandem MS/MS analysis was performed as described for Figure 2.

Table 2. ERK and GSK3α/β inhibitors block phosphorylation of the C/EBPβ RD2 domain. ST2 cells were treated with IL-17 for the indicated times in the presence of PD98095 (ERK inhibitor) or the GSK Inhibitor I and tandem MS/MS analysis was performed as described for Figure 2.

Table 3. GSK3β-deficient cells fail to induce phosphorylation at T179. ST2 cells were treated with IL-17 for the indicated times, and tandem MS/MS analysis was performed as described for Figure 2.

Supplementary Figure 1. **Identification of the C/EBPβ-specific RD2 peak by tandem MS/MS analysis.** C/EBPβδ^{KO} cells were transfected with nothing (panel A) or with a WT C/EBPβ-LAP construct (panels B-D), stimulated with the indicated times with IL-17, and nuclear extracts were evaluated by tandem MS/MS and CID as described in Fig. 2. Peaks corresponding to the unphosphorylated RD2 peptide (m/z 1067.7), the singly phosphorylated peak (m/z 1094) and the double phosphorylated peak (m/z 1120) are indicated in red.

Supplementary Figure 1 (Shen et al.)



Supplementary Table 1. Identification of phosphorylation sites in murine C/EBPβ. Phospopeptide fragments generated by collision induced dissociation (CID) are indicated. * Indicates characteristic neutral loss of a phosphoric acid group in the MS/MS spectrum.

Fragment	Theoretical Peak (Unmodified)	Experimental Peak m/z: 1067	m/z: 1094	m/z: 1120
y_4-NH_3	387.2	--	387.3	--
y_5-H_2O	483.3	--	--	483.4
y_5	501.3	501.2	501.2	501.1
y_6-H_2O	570.3	570.3	570.3	570.3
y_6-NH_3	571.3	571.4	571.3	571.4
y_6	588.3	--	588.3	588.5
y_7-H_2O^{+2}	334.2	--	334.1	--
y_7-H_2O	667.3	667.2	--	667.2
y_7-NH_3	668.3	668.2	668.3	668.4
y_8-NH_3^{+2}	385.2	385.5	--	425.3
y_8^{+2}	393.7	--	433.3	433.1
y_8-H_2O	768.4	768.4	848.0	848.6
y_8-NH_3	769.4	769.4	--	849.6
y_8	786.4	786.4	866.6	866.3
y_8^*	786.4	786.4	--	768.5*
y_9-H_2O^{+2}	413.2	--	--	453.0
y_9^{+2}	422.2	422.7	462.7	--
y_9-H_2O	825.4	825.7	905.9	905.3
y_9-NH_3	826.4	826.6	906.5	906.3
y_9	843.4	843.3	923.5	--
y_9^*	843.4	843.3	825.3*	825.2*
y_{10}-H_2O^{+2}	461.7	--	501.8	501.1
y_{10}-H_2O	922.5	922.8	1002.1	--
y_{10}-NH_3	923.5	923.8	--	1003.5

y_{10}	940.5	940.0	1020.8	1020.4
y_{10}^{*}	940.5	940.0	922.6*	922.3*
y_{11}-H_2O^{+2}	510.3	--	--	550.0
y_{11}-NH_3^{+2}	510.8	511.1	--	-
y_{11}^{+2}	519.3	519.7	559.2	559.3
y_{11}	1037.5	1037.4	--	--
y_{11}^{*}	1037.5	1037.4	1019.5*	1019.4*
y_{11}-H_2O	1019.5	1019.7	1100.1	1099.5
y_{11}-NH_3	1020.5	1020.5	--	--
y_{12}-H_2O^{+2}	553.8	--	--	593.9
y_{12}-NH_3^{+2}	554.3	--	594.5	594.3
y_{12}-NH_3	1107.5	--	1187.1	--
y_{12}	1124.6	1124.5	1205.1	--
y_{12}^{*}	1124.6	1124.5	--	1106.0*
y_{13}-H_2O^{+2}	597.3	597.2	--	637.2
y_{13}-NH_3^{+2}	597.8	--	637.8	637.9
y_{13}^{+2}	606.3	606.5	--	646.0
y_{13}-H_2O	1193.6	--	1273.7	1273.5
y_{13}	1211.6	1211.8	1291.9	--
y_{13}^{*}	1211.6	1211.8	--	1193.7*
y_{14}-H_2O^{+2}	640.8	640.8	--	680.5
y_{14}-NH_3^{+2}	641.3	641.1	681.3	681.1
y_{14}^{+2}	649.8	649.6	689.9	689.7
y_{15}-H_2O^{+2}	684.3	684.4	724.4	724.1
y_{15}-NH_3^{+2}	684.8	--	724.8	724.5
y_{15}^{+2}	693.3	693.5	733.2	733.3
y_{15}-NH_3	1368.6	1368.3	1448.6	--
y_{15}	1385.6	--	1465.1	--
y_{16}-H_2O^{+2}	727.8	727.7	767.5	767.9

y_{16}-NH_3^{+2}	728.3	--	--	768.5
y_{16}^{+2}	736.8	736.5	--	776.8
y_{16}^{*}	1472.7	--	--	1454.7*
y_{17}-H_2O^{+2}	778.4	778.4	--	858.5
y_{17}-NH_3^{+2}	778.9	778.7	819.3	858.9
y_{17}^{+2}	787.4	787.2	827.1	867.5
y_{17}-H_2O	1555.7	--	1635.8	--
y_{17}^{*}	1573.3	--	1556.0*	1537.2*
y_{18}-H_2O^{+2}	821.9	821.7	--	901.5
y_{18}-NH_3^{+2}	822.4	822.9	862.5	902.5
y_{18}^{+2}	830.9	--	870.7	910.9
y_{19}-H_2O^{+2}	878.4	878.2	918.5	958.4
y_{19}-NH_3^{+2}	878.9	878.9	--	959.3
y_{19}^{+2}	887.4	887.3	927.5	967.2
y_{19}-H_2O	1755.8	1755.9	--	1915.3
y_{20}-H_2O^{+2}	921.9	921.9	--	1002.1
y_{20}-NH_3^{+2}	922.4	922.3	962.1	1002.8
y_{20}^{+2}	930.9	--	--	1010.7
y_{20}^{*}	1860.9	--	1842.2*	--
y_{21}-H_2O^{+2}	950.4	950.3	990.3	1030.4
y_{21}-NH_3^{+2}	950.9	950.6	990.7	1031.1
y_{21}^{+2}	959.5	959.7	1000.1	1039.9
y_{22}-H_2O^{+2}	994.0	994.1	1033.8	--
y_{22}-NH_3^{+2}	994.4	994.4	1034.3	1074.4
y_{22}^{+2}	1003.0	1003.2	--	1083.1
y_{23}-H_2O^{+2}	1037.5	1037.4	1077.6	--
y_{23}-NH_3^{+2}	1038.0	--	--	1118.3
y_{24}-NH_3^{+2}	1066.5	1066.8	1107.1	--
y_{24}^{+2}	1075.0	--	1114.9	1155.5

y_{25}^{+2}	1118.5	--	--	1198.4
y_{25}-H_2O^{+2}	1109.5	--	1149.4	1189.1
y_{26}-NH_3^{+2}	1158.5	--	1198.9	--
y_{27}-H_2O^{+2}	1208.6	--	1248.1	--
y_{27}-NH_3^{+2}	1209.1	1209.2	1249.7	--
y_{28}^{+2}	1253.1	--	1293.1	--
y_{29}^{+2}	1317.1	1317.4	1356.9	--
y_{30}-H_2O^{+2}	1389.6	--	1429.2	--
y_{30}-NH_3^{+2}	1390.1	--	1430.3	--
y_{30}^{+2}	1398.6	--	--	1478.3
y_{33}-NH_3^{+2}	1556.7	1556.2	--	1636.5
b_4	405.2	405.2	405.1	--
b_5	568.3	--	--	568.7
b_6	696.3	696.1	696.3	696.5
b_6-NH_3	679.3	679.1	679.3	679.5
b_7-NH_3	750.4	750.4	750.2	750.4
b_7	767.4	767.3	767.3	-
b_8-H_2O	850.4	850.8	850.3	850.4
b_8-NH_3	851.4	--	851.4	851.3
b_8	868.4	868.3	868.4	868.6
b_9-H_2O	947.5	947.5	947.5	947.2
b_9-NH_3	948.5	948.6	948.5	948.4
b_9	965.5	965.6	965.8	965.5
b_{10}	1052.5	1052.6	1052.3	1052.7
b_{10}-H_2O	1034.5	1034.6	1034.5	1034.5
b_{10}-NH_3	1035.5	1035.6	1035.6	1035.5
b_{11}-H_2O	1091.5	--	--	1091.3
b_{11}-NH_3	1092.5	--	1092.8	1092.7
b_{11}	1109.5	--	1109.8	1109.9

b_{12}-NH_3	1179.5	--	1180.3	--
b_{12}	1196.5	--	1196.1	1196.3
b_{14}-NH_3	1323.6	--	--	1323.5
b_{15}-H_2O	1409.6	1409.8	--	1409.5
b_{15}-NH_3	1410.6	1410.5	--	--
b_{15}	1427.6	--	1427.8	--
b_{16}-NH_3	1523.7	--	--	1523.1
b_{17}	1627.7	--	1627.7	--
b_{17}-H_2O	1609.8	--	--	1609.8
_M+3H_$^{+3*}$	1067.4	--	1061.5*	1088.7*

Supplementary Table 2. C/EBPβ phosphorylation in ST2 cells by combinatorial activation of TNFα and IL-17.

Treatment of ST2 cells	*Unphosphorylated (m/z=1067)*	*pT188 (m/z=1095)*	*pT188, pT179 (m/z=1120)*
Untreated	-	-	-
IL-17 15 min	-	+	-
IL-17, 30 min	-	+	-
IL-17, 60 min	-	-	+
IL-17+ TNFα, 15 min	-	+	-
IL-17+ TNFα, 30 min	-	-	+
IL-17+ TNFα, 60 min	-	-	+

Supplementary Table 1. Identification of phosphorylation sites in C/EBPβ by tandem MS/MS. Phosphopeptide fragment y and b ions were generated by collision induced dissociation (CID), with the theoretical versus experimental mass values indicated.

Supplementary Table 2. Accelerated C/EBPβ phosphorylation by TNFα and IL-17. ST2 cells were treated with TNFα (2 ng/ml) and IL-17 (100 ng/ml) for the indicated time periods, and tandem MS analysis was performed as described in Figure 2.