

# A

## Wild-type Pellino 1 phosphorylated by IRAK4

Peak	Negative average mass [M-nH] <sup>n-</sup>	Charge state	Start residue	End residue	Peptide sequence	Phosphorylated residues
T1	516.2	3+	73	83	DQHSISYTLRSR	Any 3 of Ser76, Ser78, Thr80, Ser82
T1	773.8	2+	73	83	DQHSISYTLRSR	Any 3 of Ser76, Ser78, Thr80, Ser82
T2	733.9	2+	73	83	DQHSISYTLRSR	Any 2 of Ser76, Thr80, Ser82
T2	1466.6	1+	73	83	DQHSISYTLRSR	Any 2 of Ser76, Thr80, Ser82
T3	733.8	2+	73	83	DQHSISYTLRSR	Any 2 of Ser76, Thr80, Ser82
T3	1466.6	1+	73	83	DQHSISYTLRSR	Any 2 of Ser76, Thr80, Ser82
T3	1030.4	2+	68	83	AISNKDQHSISYTLRSR	Any 3 of Ser70, Ser76, Ser78, Thr80, Ser82
T3	687.3	3+	68	83	AISNKDQHSISYTLRSR	Any 3 of Ser70, Ser76, Ser78, Thr80, Ser82
T3	1070.4	2+	68	83	AISNKDQHSISYTLRSR	Any 4 of Ser70, Ser76, Ser78, Thr80, Ser82
T4	462.9	3+	73	83	DQHSISYTLRSR	Ser76
T4	1386.6	1+	73	83	DQHSISYTLRSR	Ser76
T4	693.8	2+	73	83	DQHSISYTLRSR	Ser76
T4	773.8	2+	73	83	DQHSISYTLRSR	Ser76 and Ser 82
T4	660.6	3+	68	83	AISNKDQHSISYTLRSR	Ser76 and Ser 82
T4	990.4	2+	68	83	AISNKDQHSISYTLRSR	Ser76 and Ser 82
T5	836.4	3+	84	104	AQTVVVEYTHDSNTDM*FQIGR	Thr86
T5	1254.1	2+	84	104	AQTVVVEYTHDSNTDM*FQIGR	Thr86
T5	657.3	3+	280	295	PQCPVGFNTLAFPSM*K	Thr288 and Ser293
T5	985.4	2+	280	295	PQCPVGFNTLAFPSM*K	Thr288 and Ser293
T5	709.3	3+	280	295	PQCPVGFNTLAFPSM*K	Thr288 and Ser293
T5	1063.5	2+	280	295	PQCPVGFNTLAFPSM*K	Thr288 and Ser293
T5	752	3+	280	295	PQCPVGFNTLAFPSM*K	Thr288 and Ser293
T6	918.1	3+	273	295	QEINAARPQCPVGFNTLAFPSM*K	Thr288 and Ser293
T6	630.6	3+	280	295	PQCPVGFNTLAFPSM*K	Thr288 or Ser293
T6	945.4	2+	280	295	PQCPVGFNTLAFPSM*K	Thr288 or Ser293
T6	1023.5	2+	280	295	PQCPVGFNTLAFPSM*K	Thr288 or Ser293
T7	891.4	3+	273	295	QEINAARPQCPVGFNTLAFPSM*K	Thr288
T7	1336.6	2+	273	295	QEINAARPQCPVGFNTLAFPSM*K	Thr288
T7	1414.7	2+	273	295	QEINAARPQCPVGFNTLAFPSM*K	Thr288 or Ser293
T7	707.8	4+	273	295	QEINAARPQCPVGFNTLAFPSM*K	Thr288 or Ser293
T7	943.5	3+	273	295	QEINAARPQCPVGFNTLAFPSM*K	Thr288 or Ser293
T7	945.4	2+	280	295	PQCPVGFNTLAFPSM*K	Thr288 or Ser293
T7	953.4	2+	280	295	PQCPVGFNTLAFPSM**K	Thr288 or Ser293
T7	1150.5	3+	105	136	STESPIDFVVDTPVGSQSNSDTQSVQSTISR	Any 1 Ser or Thr
T8	1336.6	2+	273	295	QEINAARPQCPVGFNTLAFPSM*K	Thr288
T8	977.4	2+	280	295	PQCPVGFNTLAFPSMK	Thr288 and Ser293
T8	985.4	2+	280	295	PQCPVGFNTLAFPSM*K	Thr288 and Ser293
T9a	866.1	3+	273	295	QEINAARPQCPVGFNTLAFPSMK	Thr288
T9a	1328.6	2+	273	295	QEINAARPQCPVGFNTLAFPSMK	Thr288
T9a	891.4	3+	273	295	QEINAARPQCPVGFNTLAFPSM*K	Thr288
T9a	1725.3	3+	105	136	STESPIDFVVDTPVGSQSNSDTQSVQSTISR	Any 1 Ser or Thr
T9a	1150.5	3+	105	136	STESPIDFVVDTPVGSQSNSDTQSVQSTISR	Thr127 or Ser129
T9a	1765.3	3+	105	136	STESPIDFVVDTPVGSQSNSDTQSVQSTISR	Any 2 Ser or Thr
T9a	1177.2	3+	105	136	STESPIDFVVDTPVGSQSNSDTQSVQSTISR	Any 2 Ser or Thr
T9a	625.3	3+	280	295	PQCPVGFNTLAFPSMK	Thr288
T9a	937.4	2+	280	295	PQCPVGFNTLAFPSMK	Thr288
T9a	945.4	2+	280	295	PQCPVGFNTLAFPSM*K	Thr288 or Ser293
T9b	1150.2	3+	105	136	STESPIDFVVDTPVGSQSNSDTQSVQSTISR	Any 2 Ser or Thr
T9b	1725.3	2+	105	136	STESPIDFVVDTPVGSQSNSDTQSVQSTISR	Any 2 Ser or Thr
T9b	886.1	3+	273	295	QEINAARPQCPVGFNTLAFPSMK	Thr288

# B

## Wild-type Pellino 1 phosphorylated by IRAK1

Peak	Negative average mass [M-nH] <sup>n</sup>	Charge state	Start residue	End residue	Peptide sequence	Phosphorylated residues
T1	773.8	2+	73	83	DQHSISYTL <b>S</b> R	Any 3 of Ser76, Ser78, Thr80, Ser82
T2	489.5	3+	73	83	DQHSISYTL <b>S</b> R	Any 2 of Ser76, Thr80, Ser82
T2	733.8	2+	73	83	DQHSISYTL <b>S</b> R	Any 2 of Ser76, Thr80, Ser82
T2	1466.6	1+	73	83	DQHSISYTL <b>S</b> R	Any 2 of Ser76, Thr80, Ser82
T2	773.8	2+	73	83	DQHSISYTL <b>S</b> R	Ser76, Thr80, Ser82
T3	733.8	2+	73	83	DQHSISYTL <b>S</b> R	Any 2 of Ser76, Ser78, Thr80, Ser82
T3	687.3	3+	68	83	AISNKDQHSISYTL <b>S</b> R	Any 3 of Ser70, Ser76, Ser78, Thr80, Ser82
T4	462.9	3+	73	83	DQHSISYTL <b>S</b> R	Ser76 or Thr80
T4	693.8	2+	73	83	DQHSISYTL <b>S</b> R	Ser76 or Thr80
T4	733.8	2+	73	83	DQHSISYTL <b>S</b> R	Ser76 and Thr80
T4	660.6	3+	68	83	AISNKDQHSISYTL <b>S</b> R	Ser76 and Thr80
T4	990.4	2+	68	83	AISNKDQHSISYTL <b>S</b> R	Ser76 and Thr80
T5	660.6	3+	68	83	AISNKDQHSISYTL <b>S</b> R	Ser76 and Thr80
T5	836.4	3+	84	104	AQTVVVEYTHDSNTDMFQIGR	Thr86
T5	1254.1	2+	68	83	AISNKDQHSISYTL <b>S</b> R	Ser76 or Thr80
T5	657.3	3+	280	295	PQCPVGFNTLAFPSM <b>K</b>	Thr288 and Ser293
T5	985.4	2+	280	295	PQCPVGFNTLAFPSM <b>K</b>	Thr288 and Ser293
T5	1063.5	2+	280	295	PQCPVGFNTLAFPSM <b>K</b>	Thr288 and Ser293
T6	918.1	3+	273	295	QEINAARQCPVGFNTLAFPSM <b>K</b>	Thr288 and Ser293
T6	1150.5	3+	105	136	STESPIDFVVDTVPGSQNSDTQSVQSTIS <b>R</b>	Any 1 Ser or Thr
T6	630.6	3+	280	295	PQCPVGFNTLAFPSM <b>K</b>	Thr288
T6	945.4	2+	280	295	PQCPVGFNTLAFPSM <b>K</b>	Thr288
T6	1023.5	2+	280	295	PQCPVGFNTLAFPSM <b>K</b>	Thr288
T7	668.8	4+	273	295	QEINAARQCPVGFNTLAFPSM <b>K</b>	Thr288
T7	891.4	3+	273	295	QEINAARQCPVGFNTLAFPSM <b>K</b>	Thr288
T7	1336.6	2+	273	295	QEINAARQCPVGFNTLAFPSM <b>K</b>	Thr288
T7	943.5	3+	273	295	QEINAARQCPVGFNTLAFPSM <b>K</b>	Thr288
T7	1150.5	3+	105	136	STESPIDFVVDTVPGSQNSDTQSVQSTIS <b>R</b>	Any 1 Ser or Thr
T7	945.4	2+	280	295	PQCPVGFNTLAFPSM <b>K</b>	Thr288
T8	831	3+	84	104	AQTVVVEYTHDSNTDMFQIGR	Thr86
T8	1246.1	2+	84	104	AQTVVVEYTHDSNTDMFQIGR	Thr86
T8	836.4	3+	84	104	AQTVVVEYTHDSNTDM <b>F</b> QIGR	Thr86
T8	1254.1	2+	84	104	AQTVVVEYTHDSNTDM <b>F</b> QIGR	Thr86
T8	1150.9	3+	105	136	STESPIDFVVDTVPGSQNSDTQSVQSTIS <b>R</b>	Any 1 Ser or Thr
T9a	886.1	3+	273	295	QEINAARQCPVGFNTLAFPSM <b>K</b>	Thr288
T9a	1328.6	2+	273	295	QEINAARQCPVGFNTLAFPSM <b>K</b>	Thr288
T9a	891.4	3+	273	295	QEINAARQCPVGFNTLAFPSM <b>K</b>	Thr288
T9a	1336.6	2+	273	295	QEINAARQCPVGFNTLAFPSM <b>K</b>	Thr288
T9a	863.1	4+	105	136	STESPIDFVVDTVPGSQNSDTQSVQSTIS <b>R</b>	Any 1 Ser or Thr
T9a	1150.5	3+	105	136	STESPIDFVVDTVPGSQNSDTQSVQSTIS <b>R</b>	Any 1 Ser or Thr
T9a	1725.3	2+	105	136	STESPIDFVVDTVPGSQNSDTQSVQSTIS <b>R</b>	Any 1 Ser or Thr
T9a	937.4	2+	280	295	PQCPVGFNTLAFPSM <b>K</b>	Thr288
T9a	945.4	2+	280	295	PQCPVGFNTLAFPSM <b>K</b>	Thr288
T9b	1150.5	3+	105	136	STESPIDFVVDTVPGSQNSDTQSVQSTIS <b>R</b>	Any 1 Ser or Thr
T9b	945.4	2+	280	295	PQCPVGFNTLAFPSM <b>K</b>	Thr288

Identification by LCMS of the major phosphopeptides and phosphorylation sites in Peaks 1-9 from Fig 4.

(A) Analysis of Peaks 1-9 in Fig 4A which were obtained by tryptic digestion of wild-type Pellino 1 that had been phosphorylated by IRAK4. (B) Analysis of Peaks 1-9 in Fig 4C which were obtained by tryptic digestion of wild-type Pellino 1 that had been phosphorylated by IRAK1. Phosphorylated residues are shown in bold, methionine sulfoxide is indicated by M\* and methionine sulphone by M\*\*.