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Novel post-translational modifications of Smad2 identified by mass spectrometry

Supplementary Information

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				MALDI TOF MS a)				**Q-TOF MS** b)		**FT-ICR MS**	
Smad2 (m/Z)	Smad2 (m/Z)	Smad2 (m/Z)	Smad2 (m/Z)	Smad2 (m/Z)	Smad2 (m/Z)	Smad2+TGFβ1 (m/Z)	Smad2+TGFβ1 (m/Z)	Smad2 (m/Z)	Smad2+TGFβ1 (m/Z)	Smad2 (m/Z)	Smad2+TGFβ1 (m/Z)
615.67	626.42	684.52	628.65	616.74	616.71	626.42	616.58	800.43	795.42	446.78	389.65
626.45	642.15	686.49	642.23	679.86	679.85	639.22	626.44	802.46	800.37	446.95	413.31
642.06	669.78	688.19	656.90	729.32	684.55	642.11	642.13	804.30	802.46	485.58	446.78
669.79	684.56	698.12	662.89	768.54	686.52	669.79	669.82	808.31	822.44	496.31	446.95
684.52	685.63	704.09	684.54	796.50	717.59	684.53	684.54	809.83	825.32	531.33	485.58
699.56	713.58	706.34	686.52	838.39	722.28	699.54	699.58	810.37	831.26	558.34	496.31
713.57	729.51	722.57	695.63	842.51	768.56	713.55	713.58	812.32	836.31	561.78	531.33
729.50	757.52	728.27	706.37	844.52	796.51	729.49	729.51	825.32	883.45	570.30	537.92
739.47	768.56	744.19	712.40	856.53	798.51	739.47	757.53	833.26	884.19	577.99	553.36
757.52	773.48	760.15	722.29	864.49	842.50	757.51	768.56	839.48	896.55	579.35	558.34
768.54	775.51	768.54	729.32	868.85	844.53	768.53	773.49	844.45	905.77	581.82	561.78
772.50	802.42	838.38	738.26	870.54	856.54	772.50	775.50	848.65	937.84	585.55	570.30
775.48	817.49	842.51	768.55	880.47	864.50	775.47	802.45	877.12	963.99	591.47	577.99
788.49	825.3	856.52	788.26	968.56	868.56	788.48	817.50	883.49	1051.31	596.36	579.35
802.42	830.48	864.48	791.27	995.63	870.56	802.45	825.12	884.19	1071.98	598.39	581.82
817.51	841.10	868.55	796.51	1023.66	880.47	817.49	830.49	887.18	1095.93	607.35	585.55
825.2	842.50	870.54	826.30	1045.57	968.57	825.10	842.52	892.38	1106.30	610.34	591.47
830.47	845.54	880.46	837.36	1051.71	995.63	830.46	845.56	897.37	1126.30	612.34	596.36
838.40	854.40	893.00	842.52	1114.59	1023.67	838.39	870.54	916.54	1132.31	617.42	598.39
842.51	861.53	902.45	856.53	1151.73	1045.58	842.51	921.50	918.37	1152.31	618.83	607.35

Supplementary Table 1. Peptide spectra of Smad2, obtained by MALDI TOF, Q-TOF and FT-ICR MS.

a) For MALDI TOF mass spectrometry, we performed 2 experiments, i.e. two preparations of Flag-Smad2, with two repeats for TGFβ1-treated and three repeats for non-treated conditions in each experiment. For Q-TOF and FT-ICR MS, TGFβ1-treated and non-treated conditions were studied. b) Ions with m/z higher than 790.00 are shown.

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MALDI TOF MS a)								Q-TOF MS b)		FT-ICR MS	
Smad2 (m/Z)	Smad2 (m/Z)	Smad2 (m/Z)	Smad2 (m/Z)	Smad2 (m/Z)	Smad2 (m/Z)	Smad2+TGFβ1 (m/Z)	Smad2+TGFβ1 (m/Z)	Smad2 (m/Z)	Smad2+TGFβ1 (m/Z)	Smad2 (m/Z)	Smad2+TGFβ1 (m/Z)
845.56	868.56	908.98	864.48	1190.62	1051.71	845.54	969.57	924.77	1177.98	621.89	610.34
854.40	870.54	968.56	868.55	1194.70	1084.54	854.38	977.63	931.60	1189.39	629.87	612.34
861.52	882.57	995.64	870.56	1210.68	1114.60	861.51	1010.50	940.61	1206.50	630.28	614.34
868.54	889.57	1045.57	968.58	1218.57	1151.73	868.54	1021.64	943.63	1217.37	633.84	617.42
870.54	905.53	1051.71	995.63	1243.67	1158.60	870.54	1026.51	949.63	1222.48	638.37	618.83
882.56	921.50	1114.59	1023.67	1292.68	1179.61	882.54	1045.55	954.60	1231.92	641.41	621.89
889.59	933.60	1151.73	1045.58	1314.77	1190.63	889.57	1051.69	957.82	1236.38	647.39	622.66
898.49	944.54	1190.63	1051.72	1334.88	1194.70	898.48	1067.64	964.63	1262.17	655.47	629.87
901.52	968.57	1194.71	1114.61	1395.78	1243.67	905.53	1070.74	968.41	1279.43	662.35	630.28
905.55	977.62	1210.69	1151.74	1398.68	1248.65	921.47	1097.54	974.66	1375.91	665.51	633.84
921.49	982.57	1243.67	1190.63	1412.81	1277.72	921.50	1114.59	980.47	1398.6	668.70	638.37
933.61	1010.49	1292.68	1194.71	1415.86	1292.68	933.59	1116.57	984.41	1414.76	670.38	641.41
944.54	1021.62	1308.66	1243.68	1417.88	1314.77	944.54	1151.68	999.70	2362.96	676.40	647.39
968.55	1045.53	1347.67	1292.70	1424.74	1323.67	949.55	1175.62	1004.63	2511.90	680.42	655.47
969.57	1051.68	1398.69	1308.68	1471.68	1398.69	969.56	1179.60	1018.46	2677.12	683.45	662.35
977.63	1060.55	1412.81	1323.70	1475.75	1412.81	977.61	1198.70	1020.13		685.25	668.70
982.56	1066.65	1415.86	1334.90	1492.78	1415.85	994.49	1234.57	1021.69		688.39	670.38
994.50	1097.50	1417.89	1340.73	1579.68	1417.89	1010.48	1242.67	1033.40		691.33	676.40
1010.50	1109.63	1424.75	1384.75	1707.77	1418.55	1021.64	1243.66	1035.52		695.88	680.42
1021.65	1114.55	1437.85	1395.79	1794.79	1424.75	1045.56	1292.67	1038.40		698.45	683.45
1045.56	1116.54	1453.82	1398.70	1810.80	1427.85	1051.69	1294.66	1044.40		700.90	685.25
1051.70	1151.64	1475.76	1415.88	1870.91	1471.69	1060.57	1395.75	1048.63		703.38	688.39
1065.65	1153.65	1492.78	1424.77	1922.91	1475.76	1065.65	1398.64	1062.66		705.40	691.33
1097.54	1179.54	1579.70	1434.89	1940.93	1492.78	1097.52	1402.66	1064.64		707.46	695.88
1109.62	1198.62	1648.86	1471.73	2083.00	1579.70	1109.63	1415.84	1074.59		708.98	698.45
1114.59	1234.47	1707.78	1475.77	2147.98	1707.79	1114.58	1417.52	1079.06		711.42	
1151.68	1242.58	1760.86	1492.79	2192.14	1716.85	1151.67	1471.67	1096.62		713.39	703.38
1153.67	1244.66	1794.81	1579.73	2211.12	1794.80	1153.65	1523.74	1104.62		717.90	705.40
1174.63	1292.68	1869.91	1597.02	2221.13	1810.80	1174.62	1525.73	1108.40		723.44	707.46
1179.60	1294.54	1940.93	1707.80	2225.13	1838.93	1179.60	1551.86	1113.66		729.47	710.87
1187.59	1395.58	1981.90	1718.01	2239.15	1923.92	1187.59	1579.66	1115.68		730.47	713.39
1198.70	1398.64	1993.97	1776.87	2246.21	1940.95	1198.70	1581.67	1117.73		736.90	717.90
1218.56	1402.48	2083.02	1810.83	2299.19	1993.94	1218.56	1599.75	1120.73		737.20	723.44
1234.57	1414.65	2147.98	1837.96	2313.21	2083.02	1234.55	1631.82	1149.47		743.49	729.47
1242.68	1415.65	2211.11	1870.93	2417.31	2192.20	1242.67	1639.83	1200.98		745.11	736.90
1292.68	1417.33	2225.13	1885.94	2663.31	2196.59	1292.67	1709.73	1202.48		749.66	743.49
1395.76	1455.70	2239.15	1922.94	2678.37	2211.13	1395.75	1765.72	1215.49		753.57	747.44
1398.67	1471.43	2246.18	1940.94	2691.36	2225.16	1398.66	1806.90	1218.52		756.93	749.66
1400.62	1523.47	2249.09	1981.91	2720.35	2239.20	1400.58	1942.97	1221.49		758.27	753.57
1415.86	1525.47	2299.18	1993.98	2748.40	2246.22	1402.67	1952.97	1224.51		769.44	756.93
1418.59	1551.56	2314.20	2003.07	2788.67	2249.10	1415.85	1993.97	1229.51		773.49	758.27
1426.70	1579.33	2663.31	2083.04	2807.42	2260.13	1455.70	2007.88	1247.01		777.55	769.44
1455.71	1581.66	2678.33	2094.07	2823.44	2300.26	1471.66	2081.04	1251.51		785.93	773.49
1471.68	1599.41	2691.36	2148.05	2914.68	2313.25	1475.74	2083.05	1265.54		790.90	777.55
1475.75	1631.45	2720.37	2164.00	2921.55	2363.22	1524.75	2211.07	1269.49		796.41	785.93

continued

Supplementary Table 1. Peptide spectra of Smad2, obtained by MALDI TOF, Q-TOF and FT-ICR MS.

a) For MALDI TOF mass spectrometry, we performed 2 experiments, i.e. two preparations of Flag-Smad2, with two repeats for TGFβ1-treated and three repeats for non-treated conditions in each experiment. For Q-TOF and FT-ICR MS, TGFβ1-treated and non-treated conditions were studied.
b) Ions with m/z higher than 790.00 are shown.

MALDI TOF MS a)								Q-TOF MS b)		FT-ICR MS	
Smad2 (m/Z)	Smad2 (m/Z)	Smad2 (m/Z)	Smad2 (m/Z)	Smad2 (m/Z)	Smad2 (m/Z)	Smad2+TGFβ1 (m/Z)	Smad2+TGFβ1 (m/Z)	Smad2 (m/Z)	Smad2+TGFβ1 (m/Z)	Smad2 (m/Z)	Smad2+TGFβ1 (m/Z)
1523.76	1707.30	2748.42	2211.12	3110.93	2417.29	1579.66	2678.28	1271.50		800.95	790.90
1562.79	1709.29	2765.43	2225.13	3299.84	2663.43	1599.75	2698.47	1273.51		802.40	796.41
1579.67	1765.22	2807.41	2239.15	3332.44	2678.41	1606.80	2801.43	1283.15		812.39	800.95
1599.76	1806.35	2823.44	2246.21	3339.53	2691.45	1631.81	3161.71	1285.60		813.39	802.40
1707.75	1942.28	2839.54	2299.20	3348.05	2695.34	1707.75	3643.34	1291.62		816.98	812.39
1790.86	1952.26	2914.72	2313.22	3354.19	2707.42	1752.83		1294.68		818.35	816.98
1806.90	1993.19	2921.54	2417.28	3598.83	2720.42	1806.89		1296.55		825.30	818.35
1905.95	1994.18	3300.29	2510.20	3653.50	2748.44	1952.97		1302.51		827.54	825.31
1952.97	2007.10	3339.55	2563.28	3795.73	2765.43	1993.96		1306.58		834.37	827.54
1993.97	2080.17	3347.96	2665.33	3810.30	2807.51	2007.89		1324.04		841.62	834.37
2007.90	2083.05	3348.49	2678.33		2823.52	2081.04		1326.09		854.95	841.62
2081.05	2087.15	3354.59	2691.34		2839.54	2083.06		1344.51		861.55	854.95
2083.05	2211.02	3598.41	2694.34		2914.72	2088.02		1348.27		870.50	861.55
2088.03	2676.43	3653.43	2705.29		2921.54	2211.08		1363.54		877.49	
2211.08	2678.33	3795.66	2707.48		3300.29	2212.07		1392.58		884.58	870.50
2212.06	2696.52	3810.01	2720.36		3339.55	2280.91		1394.50		893.80	877.49
2280.89	2799.33	3826.46	2737.41		3348.49	2296.92		1398.65		898.54	884.58
2383.90	3158.84		2748.38		3354.69	2678.27		1411.12		899.54	893.80
2383.90	3639.31		2764.42		3598.41	2899.41		1414.63		913.19	898.54
2678.25			2788.42		3653.43	3160.65		1438.56		921.50	902.34
3237.68			2807.41		3795.66	3237.70		1440.62		922.50	913.19
3484.98			2823.42		3810.01	3484.90		1444.22		928.68	921.50
			2914.67		3826.46	15551.86		1470.56		940.60	
			2921.51					1476.23		943.53	928.68
			2937.51					1480.65		948.53	
			3071.93					1502.69		964.55	940.60
			3299.54					1509.57		965.55	943.53
			3355.10					1511.59		970.05	948.53
			3510.25					1519.33		984.05	964.55
			3795.78					1535.57		1024.92	
			3810.33					1547.60		1061.66	970.05
			3825.44					1566.52		1115.68	984.05
								1582.51		1157.70	1012.44
								1586.59		1189.06	1024.92
								1597.64		1195.75	1061.66
								1603.31		1207.29	1115.68
								1604.63		1213.70	1157.70
								1651.26		1236.91	1189.06
								1658.32		1258.74	1195.75
								1675.56		1292.55	1207.29
								1713.68		1294.60	1213.70
								1747.70		1296.69	1236.91
								1840.74		1301.80	1258.74
								1860.72		1310.51	1292.55
								1868.35		1365.90	1294.60

continued**Supplementary Table 1.** Peptide spectra of Smad2, obtained by MALDI TOF, Q-TOF and FT-ICR MS.

a) For MALDI TOF mass spectrometry, we performed 2 experiments, i.e. two preparations of Flag-Smad2, with two repeats for TGFβ1-treated and three repeats for non-treated conditions in each experiment. For Q-TOF and FT-ICR MS, TGFβ1-treated and non-treated conditions were studied. b) Ions with m/z higher than 790.00 are shown.

MALDI TOF MS a)								Q-TOF MS b)		FT-ICR MS	
Smad2 (m/Z)	Smad2 (m/Z)	Smad2 (m/Z)	Smad2 (m/Z)	Smad2 (m/Z)	Smad2 (m/Z)	Smad2+TGFβ1 (m/Z)	Smad2+TGFβ1 (m/Z)	Smad2 (m/Z)	Smad2+TGFβ1 (m/Z)	Smad2 (m/Z)	Smad2+TGFβ1 (m/Z)
								1887.77		1390.76	1296.69
								1900.80		1395.90	1301.80
								1934.29		1400.80	1310.51
								2007.78		1406.65	1348.84
								2025.79		1409.80	1365.90
								2082.02		1413.92	1390.76
								2095.74		1472.80	1395.90
								2123.68		1476.86	1400.80
								2215.51		1537.88	1406.65
								2239.45		1543.76	1409.80
								2274.64		1570.20	1413.92
								2349.75		1580.80	1472.80
								2538.36		1600.90	1476.86
								2615.57		1606.92	1537.88
								2661.18		1610.32	1543.76
								2683.75		1632.96	1570.20
								2685.24		1636.35	1580.80
								2690.61		1708.90	1600.90
								2710.30		1740.00	1606.92
								2770.47		1753.98	1610.32
								2783.51		1781.19	1632.96
								2787.34		1880.20	1636.35
								3070.36		1886.06	1671.43
								3160.31		1896.06	1708.90
								3235.32		1901.06	1740.00
								3405.03		1967.10	1753.98
								3648.16		2458.31	1781.19
								3937.34		2707.48	1880.20
								5433.17		2766.24	1886.06
										2882.31	1896.06
										3013.47	1901.06
										3443.41	1967.10
										6357.91	1995.10
										6362.45	2009.06
										7925.56	2458.31
										9117.38	2707.48
										9129.88	2766.24
										11172.75	2882.31
										11824.67	3013.47
										13607.40	3086.61
										13627.40	3143.15
										14891.67	3176.18
											3255.24
											3443.41
											5313.52

continued**Supplementary Table 1.** Peptide spectra of Smad2, obtained by MALDI TOF, Q-TOF and FT-ICR MS.

a) For MALDI TOF mass spectrometry, we performed 2 experiments, i.e. two preparations of Flag-Smad2, with two repeats for TGFβ1-treated and three repeats for non-treated conditions in each experiment. For Q-TOF and FT-ICR MS, TGFβ1-treated and non-treated conditions were studied.
b) Ions with m/z higher than 790.00 are shown.

MALDI TOF MS a)								Q-TOF MS b)		FT-ICR MS	
Smad2 (m/Z)	Smad2 (m/Z)	Smad2 (m/Z)	Smad2 (m/Z)	Smad2 (m/Z)	Smad2 (m/Z)	Smad2+TGFβ1 (m/Z)	Smad2+TGFβ1 (m/Z)	Smad2 (m/Z)	Smad2+TGFβ1 (m/Z)	Smad2 (m/Z)	Smad2+TGFβ1 (m/Z)
											5563.25
											6357.91
											6362.45
											7925.56
											9117.38
											9129.88
											11172.75
											11824.67
											13607.40
											13627.40
											14891.67

continued**Supplementary Table 1.** Peptide spectra of Smad2, obtained by MALDI TOF, Q-TOF and FT-ICR MS.

a) For MALDI TOF mass spectrometry, we performed 2 experiments, i.e. two preparations of Flag-Smad2, with two repeats for TGFβ1-treated and three repeats for non-treated conditions in each experiment. For Q-TOF and FT-ICR MS, TGFβ1-treated and non-treated conditions were studied. b) Ions with m/z higher than 790.00 are shown.

Abbreviation	Delta mass shift due to PTM	Description of PTM
ACET	42.0106	Acetylation
ADP	541.0610	ADP-ribosylation
AMID	-0.9840	Amidation
CITR	0.9840276	Citrullination
CMAN	162.052823	C-Mannosylation
CSEA	15.9949146	Cysteine sulfenic acid (-SOH)
DEAM	0.9840	Deamidation
DEAME	14.9997	Deamidation followed by a methylation
DIHYDR	31.9898	Dihydroxylation
DIMETH	28.0314	Dimethylation
DIMETP	29.0391	Dimethylation of proline
FARN	204.1878	S-farnesyl cysteine
FLAC	0.9840	3-phenyllactic acid
FMNC	456.1046	FMN conjugation (Cys)
FMN	438.0940	FMN conjugation (Ser/Thr)
FORM	27.9949	Formylation
GERA	272.2504	Geranyl-geranylation
GGLU	43.98983	Gamma-carboxyglutamic acid
GLCN	203.0794	O-GlcNAc
GLUC	162.0528	Glucosylation (Glycation)
GLUT	305.0680814	Glutathionylation
HYDR	15.9949	Hydroxylation
METH	14.0157	Methylation
MYRI	210.1984	Myristoylation
NTRY	28.99017	S-Nitrosylation
OCTA	126.1044	n-Octanoate
PALM	238.2297	Palmitoylation
PHOS	79.9663	Phosphorylation
SULF	79.9568	Sulfation
TRIMETH	43.0548	Trimethylation
TRIMETK	59.0497	N6,N6,N6-trimethyl-5-hydroxylysine
THIOG	15.9772	1-thioglycine

Supplementary Table 2. List of post-translational modifications considered in PMF search.