Supplementary information

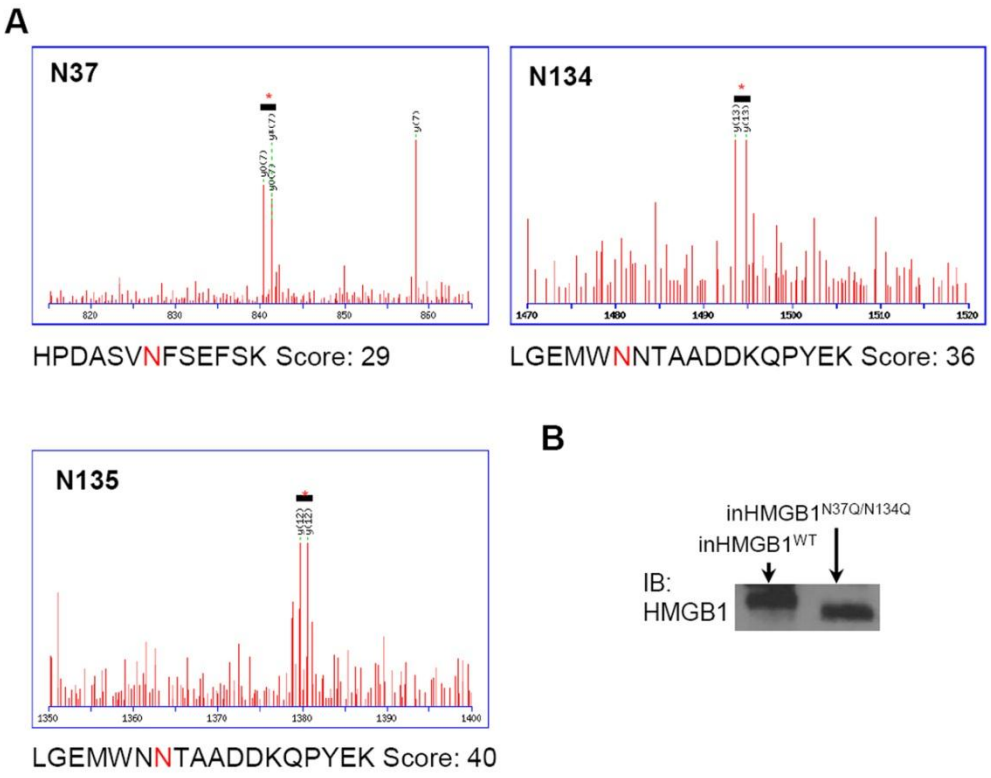

Figure S1. LC-MS/MS analysis of insHMGB1^{WT}. (A) LC-MS/MS analysis of insHMGB1^{WT} produced in insect SF9 cells. Asn37, 134, and 135 were converted to Asp after PNGase F treatment when a sugar molecule was added onto the NH3+ group of Asn, displaying an increase of 0.98 Da. Each HMGB1 peptide sequence analyzed is described. Score: 29 (N37), 36 (N134), 40 (N135). (B) The insHMGB1^{WT} and insHMGB1$^{N37Q/N134Q}$ proteins were purified from SF9 insect cells and immunoblotted with anti-HMGB1 antibody for mobility shift assay.

A

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  1 MGKGDPKKPR GKMSSYAFFV QTCREEHKKK HPDASVNFSE FSKKCSERWK
 51 TMSAKEKGKF EDMAKADKAR YEREMKTYIP PKGETKKKFK DPNAPKRPPS
101 AFFLFCSEYR PKIKGEHPGL SIGDVAKKLG EMWNNTAADD KQPYEKKAAK
151 LKEKYEKDIA AYRAKGKPDA AKKGVVKAEK SKKKKEEEED EEDEEDEEEE
201 EDEEDEDEEE DDDDD
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B

N37

MS/MS Fragmentation of HPDASVNFSEFSKK
N7 : Deamidated (NQ)
Ions Score: 31

#	b	b++	b*	b*++	b0	b0++	Seq	y	y++	y*	y*++	y0	y0++	#
1	138.0662	69.5367					H							14
2	235.119	118.0631					P	1456.695	728.851	1439.669	720.3381	1438.685	719.8461	13
3	350.1459	175.5766			332.135	166.5713	D	1359.643	680.325	1342.616	671.8117	1341.632	671.3197	12
4	421.183	211.0951			403.1724	202.0899	A	1244.616	622.8115	1227.59	614.2982	1226.61	613.8062	11
5	508.215	254.6112			490.205	245.6059	S	1173.58	587.2929	1156.552	578.7797	1155.568	578.2877	10
6	607.284	304.1454			589.2729	295.1401	V	1086.55	543.7769	1069.52	535.2637	1068.536	534.7717	9
7	722.31	361.6588	705.2838	353.1456	704.3	352.6535	N	987.478	494.2427	970.452	485.7295	969.468	485.237	8
8	869.3788	435.193	852.3523	426.6798	851.3682	426.1878	F	872.451	436.729	855.4247	428.216	854.4407	427.724	7
9	956.4108	478.7091	939.384	470.196	938.4003	469.7038	S	725.383	363.1951	708.3563	354.6818	707.3723	354.1898	6
10	1085.453	543.2303	1068.427	534.7171	1067.44	534.2251	E	638.351	319.679	621.3243	311.1658	620.3402	310.6738	5
11	1232.522	616.7646	1215.495	608.2513	1214.511	607.7593	F	509.308	255.1577	492.2817	246.6445	491.2976	246.1525	4
12	1319.554	660.2806	1302.527	651.7673	1301.543	651.2753	S	362.24	181.6235	345.2132	173.1103	344.2292	172.6183	3
13	1447.649	724.3281	1430.622	715.8148	1429.638	715.3228	K	275.208	138.1075	258.1812	129.5942			2
14							K	147.113	74.06	130.0863	65.5468			1

N134

MS/MS Fragmentation of KLGEMWNNTAADDKQPYEK
N7 : Deamidated (NQ)
Ions Score: 54

#	b	b++	b*	b*++	b0	b0++	Seq	y	y++	y*	y*++	y0	y0++	#
1	129.102	65.0548	112.0757	56.5415			K							19
2	242.186	121.5968	225.1598	113.0835			L	2126.934	1063.97	2109.907	1055.457	2108.923	1054.965	18
3	299.2078	150.1075	282.1812	141.5942			G	2013.85	1007.428	1996.823	998.9151	1995.839	998.423	17
4	428.25	214.6288	411.2238	206.1155	410.2398	205.6235	E	1956.828	978.9176	1939.801	970.4044	1938.817	969.9124	16
5	575.286	288.1465	558.2592	279.6332	557.2752	279.1412	M	1827.785	914.3963	1810.759	905.883	1809.775	905.391	15
6	761.365	381.1862	744.3385	372.6729	743.3545	372.181	W	1680.75	840.879	1663.724	832.365	1662.739	831.873	14
7	876.392	438.6996	859.3655	430.1864	858.3814	429.6944	N	1494.671	747.839	1477.644	739.326	1476.66	738.834	13
8	990.435	495.7211	973.4084	487.208	972.4244	486.716	N	1379.64	690.326	1362.617	681.812	1361.633	681.3202	12
9	1091.483	546.2449	1074.456	537.7317	1073.472	537.24	T	1265.6	633.304	1248.574	624.7908	1247.59	624.2988	11
10	1162.52	581.7635	1145.493	573.2502	1144.509	572.7582	A	1164.55	582.78	1147.527	574.2669	1146.54	573.7749	10
11	1233.56	617.2821	1216.53	608.7688	1215.55	608.2768	A	1093.52	547.262	1076.49	538.7484	1075.506	538.2564	9
12	1348.584	674.7955	1331.56	666.2823	1330.573	665.7903	D	1022.48	511.7431	1005.452	503.2298	1004.468	502.7378	8
13	1463.611	732.309	1446.584	723.7957	1445.6	723.3037	D	907.452	454.23	890.4254	445.7164	889.4414	445.2243	7
14	1591.706	796.3565	1574.679	787.8432	1573.7	787.3512	K	792.425	396.716	775.3985	388.2029	774.4145	387.7109	6
15	1719.764	860.3858	1702.738	851.8725	1701.754	851.3805	Q	664.33	332.6687	647.304	324.1554	646.32	323.6634	5
16	1816.817	908.9122	1799.791	900.3989	1798.807	899.9069	P	536.272	268.6394	519.2449	260.1261	518.2609	259.6341	4
17	1979.88	990.444	1962.854	981.9305	1961.87	981.4385	Y	439.219	220.113	422.1922	211.5997	421.2082	211.1077	3
18	2108.923	1054.965	2091.896	1046.452	2090.912	1045.96	E	276.155	138.5813	259.1288	130.068	258.145	129.5761	2
19							K	147.113	74.06	130.086	65.5468			1

N135

MS/MS Fragmentation of KLGEMWNNTAADDKQPYEK
N8 : Deamidated (NQ)
Ions Score: 53

#	b	b++	b*	b*++	b0	b0++	Seq	y	y++	y*	y*++	y0	y0++	#
1	129.102	65.0548	112.0757	56.5415			K							19
2	242.186	121.5968	225.1598	113.0835			L	2126.934	1063.97	2109.907	1055.457	2108.923	1054.965	18
3	299.2078	150.1075	282.1812	141.5942			G	2013.85	1007.428	1996.823	998.9151	1995.839	998.423	17
4	428.25	214.6288	411.2238	206.1155	410.2398	205.6235	E	1956.828	978.9176	1939.801	970.4044	1938.817	969.9124	16
5	575.286	288.1465	558.2592	279.6332	557.2752	279.1412	M	1827.785	914.3963	1810.759	905.883	1809.775	905.391	15
6	761.365	381.1862	744.3385	372.6729	743.3545	372.181	W	1680.75	840.879	1663.724	832.365	1662.739	831.873	14
7	875.408	438.208	858.3815	429.6944	857.3974	429.2024	N	1494.671	747.839	1477.644	739.326	1476.66	738.834	13
8	990.435	495.7211	973.4084	487.208	972.4244	486.716	N	1380.628	690.818	1363.601	682.304	1362.617	681.812	12
9	1091.483	546.2449	1074.456	537.7317	1073.472	537.24	T	1265.6	633.304	1248.574	624.7908	1247.59	624.2988	11
10	1162.52	581.7635	1145.493	573.2502	1144.509	572.7582	A	1164.55	582.78	1147.527	574.2669	1146.54	573.7749	10
11	1233.56	617.2821	1216.53	608.7688	1215.55	608.2768	A	1093.52	547.262	1076.49	538.7484	1075.506	538.2564	9
12	1348.584	674.7955	1331.56	666.2823	1330.573	665.7903	D	1022.48	511.7431	1005.452	503.2298	1004.468	502.7378	8
13	1463.611	732.309	1446.584	723.7957	1445.6	723.3037	D	907.452	454.23	890.4254	445.7164	889.4414	445.2243	7
14	1591.706	796.3565	1574.679	787.8432	1573.7	787.3512	K	792.425	396.716	775.3985	388.2029	774.4145	387.7109	6
15	1719.764	860.3858	1702.738	851.8725	1701.754	851.3805	Q	664.33	332.6687	647.304	324.1554	646.32	323.6634	5
16	1816.817	908.9122	1799.791	900.3989	1798.807	899.9069	P	536.272	268.6394	519.2449	260.1261	518.2609	259.6341	4
17	1979.88	990.444	1962.854	981.9305	1961.87	981.4385	Y	439.219	220.113	422.1922	211.5997	421.2082	211.1077	3
18	2108.923	1054.965	2091.896	1046.452	2090.912	1045.96	E	276.155	138.5813	259.1288	130.068	258.145	129.5761	2
19							K	147.113	74.06	130.086	65.5468			1

Figure S2. LC-MS/MS analysis of myc-HMGB1. (A) Potential HMGB1 N-glycosylation site peptide sequences are indicated in red. More than 60% of the HMGB1 amino acid sequence was analyzed by LC-MS/MS (black and red bold). (B) LC-MS/MS analysis data. Data indicate that Asn37, Asn134, and Asn135 were converted to Asp. Score: 31 (N37), 54 (N134), 53 (N135).

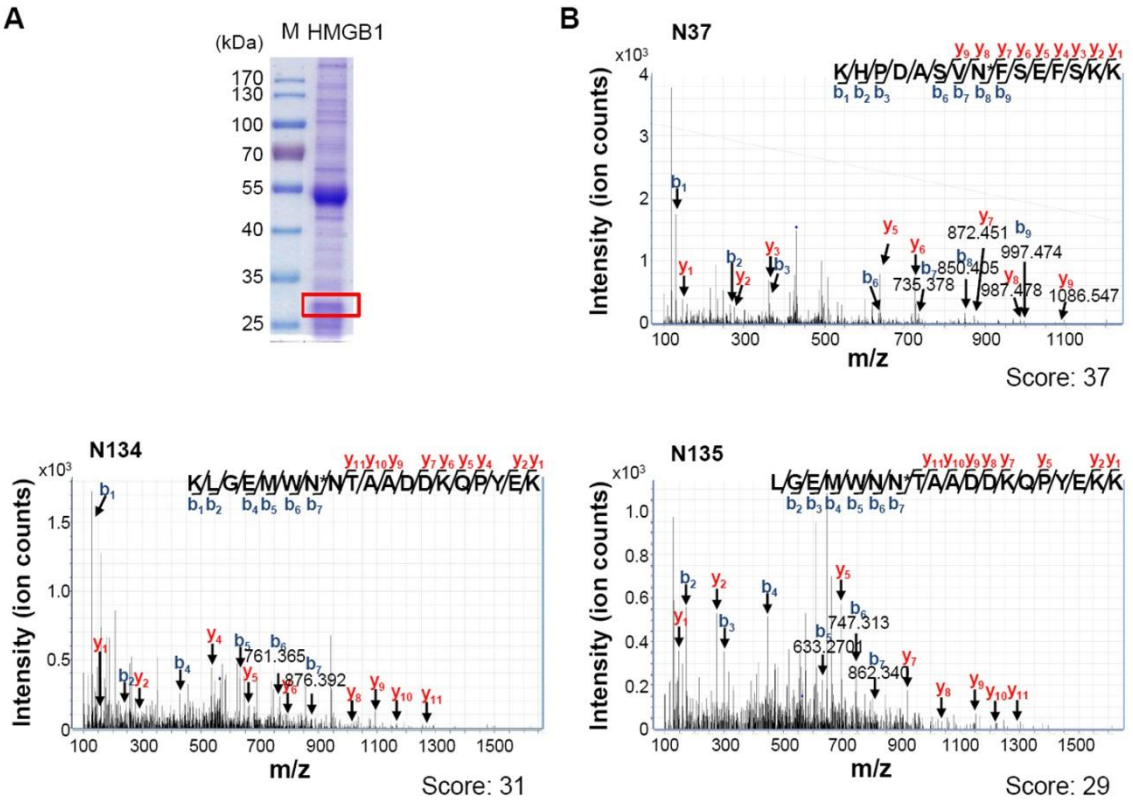

Figure S3. LC-MS/MS analysis of endogenous HMGB1. (A) Whole cell lysates of HEK293T cells were immunoprecipitated with anti-HMGB1 antibody to purify endogenous HMGB1 using pre-cleared protein G beads. SDS-PAGE was performed and stained with Coomassie blue to extract endogenous HMGB1 (box) for analysis. M: marker. (B) LC-MS/MS analysis of endogenous HMGB1 performed. Endogenous HMGB1 was treated with PNGase F, and the molecular shifts of peptides including Asn37, Asn134 and Asn135 could be observed after PNGase F treatment.