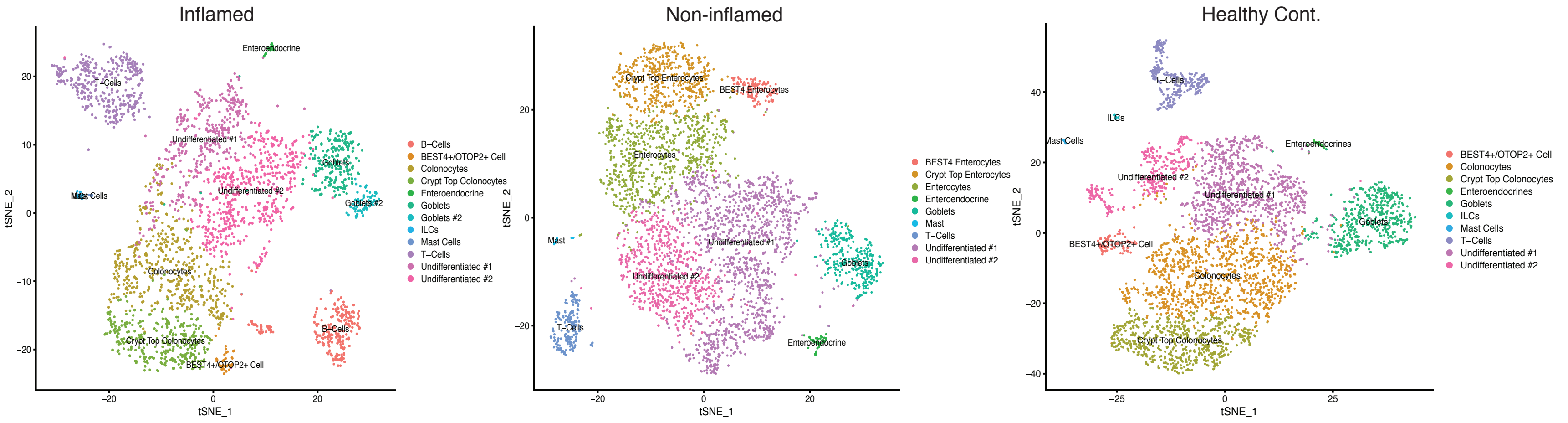
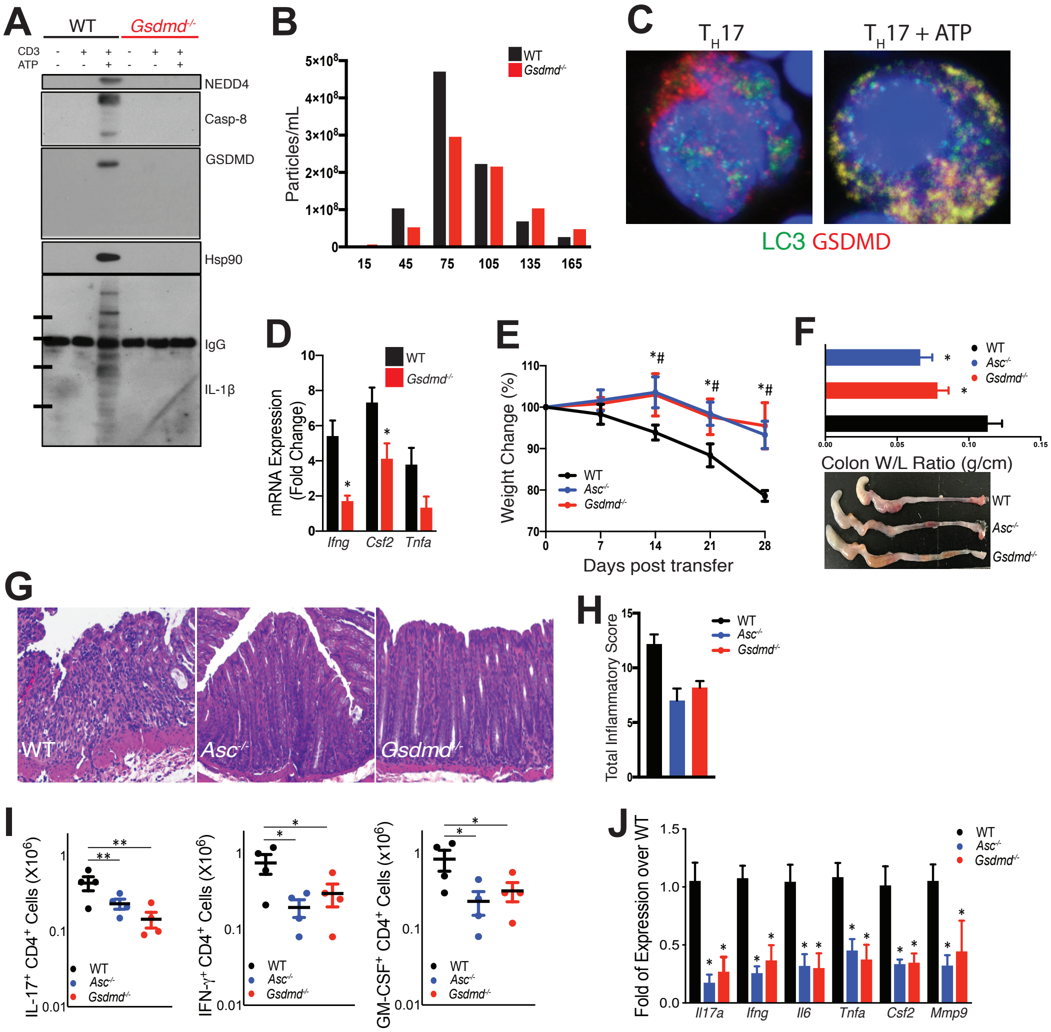
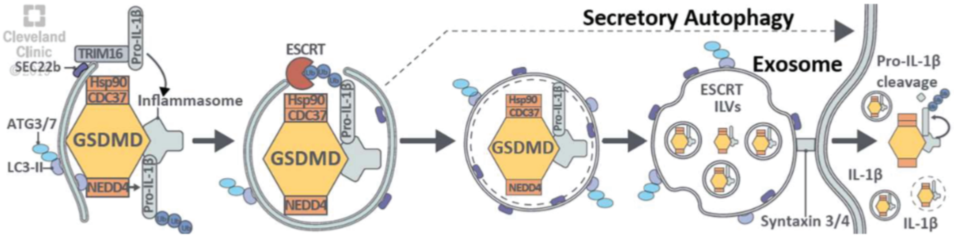


Supplemental Figure 1. Reference *t-SNE*-plots visualizing colonic epithelial cell clusters detected in UC (inflamed and non-inflamed) and health (n=3/group). Previously published in Parikh *et al.*, *Nature* 2019.



Supplemental Figure 2. Naïve CD4$^+$ T cells from indicated mice were polarized under T$_H$17 skewing conditions (IL-6+TGFβ) for 3d. **A)** Polarized T$_H$17 cells were re-stimulated with coated anti-CD3 for 4h, then treated with +/-ATP (1mM) for 30min. Supernatants were subjected to co-IP with anti-IL-1β followed by Western blotting. **B)** Supernatants from WT and *Gsdmd*$^{-/-}$ T$_H$17 cells treated with anti-CD3 and ATP were concentrated and subjected to nanoparticle tracking analysis. **C)** Untreated and anti-CD3+ATP-treated cells from (B) were stained for LC3 and GSDMD, and examined by confocal microscopy. **D)** Untreated and anti-CD3+ATP-treated cells from (B) were analyzed by RT-PCR for indicated transcripts (n=3). **E-J)** CD4$^+$CD45RB^{hi} T cells (naïve T cells) from 6-10-wk-old mice of indicated genotype were transferred into 6-wk-old gender-matched *Rag1*$^{-/-}$ recipient mice to induce colitis (n=10). **E)** Weight change presented as percentage of initial weight at Day 0. **F)** Weight to length ratio and representative photo of macroscopic appearance of colons. **G)** Representative histology from H&E-stained colons with **H)** semi-quantitative assessment of disease severity. **I)** Cytokine production from recovered lamina propria donor T cells (n=4-6). **J)** RT-PCR analysis of indicated genes in colon tissues. Data is presented as mean±SEM with *P<0.05, **P<0.001 by two-tailed unpaired Student's t-test.



Supplemental Figure 3. Chaperoned (Hsp90/CDC37) GSDMD directs NEDD4 to localize to LC3-II+ membranes. The biogenesis of LC3-II+ membranes is dependent on Atg3/7-mediated lipidation of LC3. IL-1β is recognized by TRIM16, which interacts with the SNARE protein, Sec22b, directing vesicles for extracellular release. GSDMD-NEDD4 is assembled onto NLRP3/Casp8-inflammasome and pro-IL-1β is subsequently ubiquitinated by NEDD4. Polyubiquitin chains of IL-1β function as a marker that triggers the ESCRT machinery to sort and load ubiquitinated proteins into ILVs. Sec22b engages plasma membrane syntaxin 3 and 4 for IL-1β secretion.

Supplemental Table I. *Summary of analyzed intestinal biopsy datasets from GSE database for GSDMD expression*

Accession code	Patient population	Evaluated specimens	Study Description	FDR	q-value	p-value	Ref.
GSE75214	IBD	CD colon and ileum (active and inactive) UC (active and inactive)	Mucosal biopsies from colons of UC, CD and HC, and from neo-terminal ilea of CD and HC patients. Biopsies were taken at most affected sites, but at distance from ulcerations.	CD vs. HC colon: **0.007675599*** UC vs. HC colon: **0.09791629*** CD vs. HC ileum: **0.1180388**	0.00371144* 0.04421897* 0.06142328	0.000690416* 0.03488876* 0.03016642*	Vancamelbeke *et al.*[40]
GSE20881	CD	colon (ascending, descending, sigmoid) terminal ileum	Biopsies obtained from one of four anatomic locations, from treated (with non-biologic standard of care) or untreated CD patients, and HC.	**6.09e-05***	3.50e-05*	1.84e-07*	NA
GSE112366	CD	ileum	Ileal biopsy samples collected from patients with moderate-to-severe CD and from non-IBD HC subjects.	**0.02150507***	0.01295562*	0.001462536*	VanDussen *et al.*[41]
GSE53306	UC	active and inactive/remission	Single colonic endoscopic pinch biopsies sampled in consecutive active and quiescent stages (inactive/remission) of disease at same site from individual UC patients, and non-IBD HC subjects.	**8.51e-09***	3.08e-09*	6.21e-11*	Zhao *et al.*[42]
GSE38173	UC	active (involved and non-involved) and inactive/ remission	Colonic mucosal biopsies collected from involved and non-involved areas of active UC, UC in remission, and HC.	**2.54e-05***	9.41e-06*	1.41e-06*	Planell *et al.*[43]

CD, Crohn's disease; FDR, false discovery rate; GSE, genomic spatial event; HC, healthy controls; NA, not applicable; UC, ulcerative colitis; *significant

Supplemental Table II. *Identification of GSDMD- and IL-1β-interacting proteins during non-pyroptotic IL-1β release.*

Gene	Accession	[M+H]	m/z	z	Δm, ppm	First AA	Last AA	Sequence	XCorr	X! Tandem
IL-1β-interacting proteins										
Nedd4	P46935	1,067.71	356.9093	3	-0.742	82	92	(R)VKVIAGIGLAK(K)	2.47	1.77
		1,332.71	667.36	2	1.148	93	104	(K)KDILGASDPYVR(V)	3.10	3.31
		1,204.61	603.3113	2	-0.7606	94	104	(K)DILGASDPYVR(V)	3.15	2.85
		1,867.96	934.9877	2	0.2786	105	121	(R)VTLYDPMoSGILTSVQTK(T)	5.64	11.19
		1,851.96	926.9886	2	-1.534	105	121	(R)VTLYDPMSGILTSVQTK(T)	3.02	4.03
		1,280.64	641.3264	2	-1.482	146	155	(R)ILFEVFDENR(L)	3.60	2.89
		2,284.14	1,143.08	2	0.1969	159	178	(R)DDFLGQVDVPLYPLPTENPR(M)	4.73	8.92
		2,589.09	864.037	3	0.07032	279	300	(K)RPSPDDDLTDEDNDDMoQLQAQR(A)	4.89	11.00
		1,499.70	750.8552	2	-0.4933	306	318	(R)RQISEDVDGPDNR(E)	3.05	2.52
		2,169.02	724.0152	3	0.2513	362	382	(R)LAVcGNPATSQPVTSSNHSSR(G)	3.07	5.54
		2,497.27	833.4308	3	-0.3535	486	507	(K)TQWEDPRLQNVAITGPAVPYSR(D)	2.92	1.68
		1,584.86	793.4377	2	-0.9662	493	507	(R)LQNVAITGPAVPYSR(D)	4.64	1.89
		1,493.75	498.9256	3	-0.1443	522	533	(K)KQTDIPNKFEMoK(L)	3.89	1.15
		1,365.66	683.8364	2	-1.417	523	533	(K)QTDIPNKFEMoK(L)	3.02	2.34
		2,273.05	758.692	3	0.7671	655	673	(K)LITLHDMoESVDSEYYSSLR(W)	4.34	2.35
		1,612.81	807.4123	2	0.03416	674	686	(R)WILENDPTELDLR(F)	3.89	4.32
		1,103.58	552.7982	2	-0.5492	704	714	(K)TGGSEIVVTNK(N)	3.99	2.89
		1,547.82	774.9184	2	-1.075	741	753	(K)EGFFELIPQDLIK(I)	2.87	3.96
		2,767.26	923.4274	3	4.736	754	776	(K)IFDENELELLmcGLGDVDVNDWR(E)	5.18	0.96
		1,120.62	561.3196	2	0.4178	810	819	(R)LLQFVTGTSR(V)	3.46	4.00
		3,071.40	1,024.81	3	1.103	820	847	(R)VPMoNGFAELYGSNGPQSFTVEQWGTPDK(L)	5.22	4.57
		3,500.59	1,167.87	3	-1.559	858	887	(R)LDLPPYESFDELWDKLQMoAIENTQGFDGVD(-)	4.32	8.68
Ube2d3	P61079	1,157.64	386.8873	3	-0.3702	6	15	(R)INKELSDLAR(D)	2.90	-
		2,098.06	700.3616	3	-0.03171	73	90	(R)IYHPNINSNGSIcLDILR(S)	4.74	3.08
		1,216.65	609.33	2	0.07801	91	101	(R)SQWSPALTISK(V)	3.29	3.92
		2,705.38	902.8001	3	0.3922	102	125	(K)VLLSIcSLLcDPNPDDPLVPEIAR(I)	3.06	3.31

Cdc37	Q9CZP7	2,149.98	717.6684	3	-0.4435	249	266	(K)AEEEGYFEAFKNELEAFK(S)	1.00	2.39
		2,349.12	784.0467	3	0.7186	247	266	(K)AKAEEEGYFEAFKNELEAFK(S)	0.72	-
		1,729.78	577.6015	3	-0.1683	147	160	(R)KTEEEDKSQSFMoQK(Y)	0.99	1.39
		1,947.97	650.3305	3	2.325	78	95	(K)LGSLALHNSESLDQEHAK(A)	1.00	1.54
		1,099.60	367.54	3	-0.3223	112	120	(R)QKEEALVQR(E)	1.00	1.74
		4,366.88	1,092.73	4	2.002	16	56	(R)TGGETEEESDLDVSPSSSHYSPVPDGGAQMoYSHGIELAcQK(Q)	1.00	9.72
Hsp90aa1	P07901	4,710.15	1,178.54	4	-0.9455	2	41	(M)PEETQTQDQPMoEEEEVETFAFQAEIAQLMoSLIINTFYSNK(E)	-	10.85
		1,290.63	646.3224	2	-0.2094	47	58	(R)ELISNSSDALDK(I)	2.94	3.07
		1,561.86	781.9365	2	0.4332	75	87	(K)ELHINLIPSKQDR(T)	3.25	3.32
		978.409	490.2118	2	1.139	174	182	(R)TDTGEPMoGR(G)	2.20	-
		1,906.04	636.3523	3	-0.1441	209	224	(K)KHSQFIGYPITLFVEK(E)	2.27	3.40
		1,777.94	593.6542	3	0.2058	210	224	(K)HSQFIGYPITLFVEK(E)	3.36	8.42
		2,731.28	911.433	3	0.05408	294	315	(K)TKPIWTRNPDDITNEEYGEFYK(S)	3.50	-
		1,832.78	917.3949	2	0.5772	301	315	(R)NPDDITNEEYGEFYK(S)	5.68	12.47
		1,540.75	771.3844	2	1.321	316	328	(K)SLTNDWEEHLAVK(H)	3.09	-
		814.5065	408.2605	2	-0.1461	340	346	(R)ALLFVPR(R)	2.04	1.72
		1,263.64	632.8253	2	0.02149	347	356	(R)RAPFDLFENR(K)	3.25	1.80
		1,391.73	464.9174	3	-0.4638	347	357	(R)RAPFDLFENRK(K)	3.68	1.92
		1,107.54	554.7748	2	0.1043	348	356	(R)APFDLFENR(K)	3.29	4.68
		1,235.63	412.8837	3	-0.4639	348	357	(R)APFDLFENRK(K)	2.01	0.96
		2,570.27	857.763	3	0.3078	368	387	(R)RVFIMDNcEELIPEYLNFIR(G)	3.03	1.19
		2,430.16	1,216.09	2	-0.2103	369	387	(R)VFIMoDNcEELIPEYLNFIR(G)	3.57	2.85
		2,414.16	805.7286	3	-0.5291	369	387	(R)VFIMDNcEELIPEYLNFIR(G)	2.32	1.34
		2,129.04	533.2661	4	-0.08002	421	437	(K)cLELFTELAEDKENYKK(F)	2.93	-
		1,565.69	783.8537	2	0.5325	466	479	(R)YYTSASGDEMoVSLK(D)	3.81	2.68
		2,244.97	749.3294	3	-0.2652	466	484	(R)YYTSASGDEMVSLKDYcTR(M)	2.51	3.57
		2,260.96	754.6612	3	-0.02745	466	484	(R)YYTSASGDEMoVSLKDYcTR(M)	2.02	1.46
		1,207.62	604.8194	2	0.3363	491	500	(K)HIYFITGETK(D)	2.88	4.70
		2,424.21	809.0766	3	0.1649	491	511	(K)HIYFITGETKDQVANSAFVER(L)	5.12	14.00

		1,234.60	618.3048	2	0.7817	501	511	(K)DQVANSAFVER(L)	3.37	6.47
		3,310.62	828.6624	4	-1.619	514	540	(R)KHGLEVIYMoIEPIDEYcVQQLKEFEGK(T)	2.55	-
		2,576.26	859.7601	3	-2.872	515	535	(K)HGLEVIYMIEPIDEYcVQQLK(E)	5.40	9.85
		3,183.53	1,062.18	3	-0.1759 (+1 neutron corrected from 314.7)	515	540	(K)HGLEVIYMoIEPIDEYcVQQLKEFEGK(T)	2.21	10.00
		3,167.54	1,056.85	3	-0.7406 (+1 neutron corrected from 315.7)	515	540	(K)HGLEVIYMIEPIDEYcVQQLKEFEGK(T)	-	9.48
		1,038.52	347.1794	3	-0.4062	567	574	(K)TKFENLcK(I)	3.09	-
		1,004.56	335.8599	3	-0.00399	575	582	(K)IMoKDILEK(K)	2.73	-
		2,461.11	821.3759	3	-0.6937	593	613	(R)LVTSPccIVTSTYGWTANMoER(I)	5.25	5.51
		2,445.11	1,223.56	2	0.6688	593	613	(R)LVTSPccIVTSTYGWTANMER(I)	3.54	7.55
		1,758.80	587.2747	3	-0.3787	617	632	(K)AQALRDNSTMoGYMoAAK(K)	2.79	4.09
		1,203.49	602.7524	2	0.1104	622	632	(R)DNSTMGYMoAAK(K)	2.77	3.82
		1,914.03	479.5158	4	0.8569	633	648	(K)KHLEINPDHSIIETLR(Q)	2.86	1.46
		1,785.94	893.9754	2	-0.7623	634	648	(K)HLEINPDHSIIETLR(Q)	3.68	8.68
		3,001.54	1,001.52	3	1.062	662	688	(K)DLVILLYETALLSSGFSLEDPQTHANR(I)	4.64	14.89
Gsdmdc1	Q9D8T2	3,213.51	1,072.18	3	-0.4682	55	82	(R)YScVNLSIKDILEPSAPEPEPEcFGSFK(V)	-	2.44
		3,094.53	1,032.52	3	1.294	270	297	(K)QLSLLSDGIDEEELIEAADFQGLYAEVK(A)	4.84	1.21
		1,668.73	835.3734	2	-0.7686	298	311	(K)AcSSELESLEmELR(Q)	3.91	3.49
		960.5495	481.282	2	0.285	395	403	(K)ALETTVLSK(Q)	1.91	2.16
Casp8	O89110	1,352.66	677.3395	2	-0.2693	52	63	(K)GMoLEEGNLSFLK(E)	2.86	4.12
		2,224.04	742.3548	3	0.9142	72	88	(R)WDLLVNFLDcNREEMoVR(E)	-	1.49
		1,672.82	558.6127	3	-0.4985	89	102	(R)ELRDPDNAQISPYR(V)	4.16	-
		1,302.67	652.3409	2	0.4252	108	118	(K)LSEEVSELELR(S)	3.01	1.52

		1,391.70	696.8558	2	0.1415	150	161	(R)TMoLAENNLETLK(S)	3.66	4.48
		1,093.45	547.7344	2	0.8354	207	215	(K)MoAELcDSPR(E)	2.71	3.32
		1,680.79	841.4018	2	0.3559	332	346	(K)EASIYDLTSYFTGSK(C)	4.68	2.92
		1,686.82	844.4168	2	0.08004	356	369	(K)IFFIQAcQGSNFQK(G)	5.00	12.85
		1,911.93	956.9709	2	-0.92	393	409	(K)NYIPDEADFLLGMoATVK(N)	3.85	-
		2,815.31	939.4454	3	-0.9189	410	432	(K)NcVSYRDPVNGTWYIQSLcQSLR(E)	5.63	5.00
		2,832.34	945.1205	3	1.522	435	459	(R)cPQGDDILSILTGVNYDVSNKDDRR(N)	-	3.74
Atg7	Q9D906	1,454.78	728.398	2	0.2625	186	198	(R)AEGVTALPYFLFK(Y)	3.70	2.66
		1,406.62	704.3181	2	0.3349	285	296	(K)LPEMoAFSPDcPK(A)	3.27	2.21
		2,709.20	904.0732	3	-0.3227	428	451	(R)GFNMoSIPMoPGHPVNFSDVTmEQAR(R)	3.48	1.92
		2,671.31	891.4456	3	-0.6886	453	474	(R)DVEQLEQLIDNHDVIFLLMoDTR(E)	5.18	3.59
		3,082.51	1,028.51	3	-1.767	513	542	(K)QQGAGDLcPSHLVAPADLGSSLFANIPGYK(L)	4.24	7.60
Atg3	Q9CPX6	1,444.82	723.4166	2	0.4959	12	24	(K)ALEVAEYLTPVLK(E)	3.08	5.00
		1,315.64	658.8252	2	-0.8902	186	198	(K)ADAGGEDAILQTR(T)	4.40	9.89
		1,325.64	663.8265	2	-1.589	215	224	(R)LWLFGYDEQR(Q)	3.24	4.47
		745.5096	373.7621	2	-1.042	290	295	(Y)LLIFLK(F)	1.38	2.77
Stxbp2	Q64324	1,875.95	626.3241	3	0.0905	47	63	(K)MoSDILAEGITIVEDINK(R)	2.82	1.47
		2,032.05	678.3574	3	-0.5244	47	64	(K)MoSDILAEGITIVEDINKR(R)	2.33	3.33
		2,055.13	686.0493	3	0.2821	65	82	(R)REPIPSLEAIYLLSPTEK(S)	2.40	2.17
		1,899.02	950.5196	2	0.1435	66	82	(R)EPIPSLEAIYLLSPTEK(S)	3.21	3.43
		1,986.01	994.012	2	-0.2958	83	100	(K)SVQALIADFQGTPTFTYK(A)	5.76	11.80
		2,307.10	770.04	3	-0.6428	101	120	(K)AAHIFFTDTcPEPLFSELGR(S)	2.75	8.59
		3,597.77	900.4506	4	9.51	132	161	(K)EIHLAFLPYEAQVFSLDAPHSTYNLYcPFR(A)	5.05	5.49
		2,585.32	862.7812	3	2.158	168	190	(R)qLDALAQQIATLcATLQEYPSIR(Y)	-	9.28
		1,647.89	550.3053	3	-0.304	193	208	(R)KGPEDTAQLAHAVLAK(L)	3.90	4.21
		1,199.57	600.7908	2	-0.1133	214	225	(K)ADTPSLGEGPEK(T)	2.96	4.19
		3,450.66	1,151.23	3	-0.2898	236	265	(R)AADPVSPLLHELTFQAMoAYDLLDIEQDTYR(Y)	3.58	3.18
		4,574.17	1,144.55	4	-1.73	236	275	(R)AADPVSPLLHELTFQAMoAYDLLDIEQDTYRYETTGLSESR(E)	5.48	5.48
		1,250.73	417.9183	3	-1.417	321	331	(K)ANIKDLSHILK(K)	3.40	6.26
		1,737.99	870.0034	2	0.1109	388	403	(K)LIVPVLLDASVPPYDK(I)	3.17	6.57

		1,727.93	864.9734	2	0.2334	425	439	(K)LIQHANVQSYSSLIR(N)	3.26	5.14
		1,934.93	968.4728	2	0.8555	440	459	(R)NLEQLGGTVTNSAGSGTSSR(L)	5.97	14.14
Stxbp3	Q60770	1,996.01	666.3423	3	-1.416	51	67	(K)MoTDLLEEGITVIENIYK(N)	2.79	2.85
		2,070.93	1,036.47	2	-3.408	105	121	(K)AAYIYFTDFcPDSLFNK(I)	4.40	2.70
		3,442.66	1,148.56	3	-2.231	241	270	(R)GFDPVSTVLHELTFQAMoAYDLLPIENDTYK(Y)	3.85	0.82
		1,602.75	802.3814	2	-0.407	368	382	(K)TEQDLALGTDAEGQR(V)	4.35	4.92
Chmp4b	Q9D8B3	1,152.59	577.3019	2	0.2897	18	28	(K)GGPTPQEAIQR(L)	2.05	2.33
		1,129.63	565.8243	2	-0.3576	46	55	(K)KIEQELTAAK(K)	2.39	-
		1,544.77	773.3916	2	0.2651	94	107	(R)EALENANTNTEVLK(N)	4.01	5.32
Sec22b	O08547	931.5531	466.7838	2	0.538	2	9	(M)VLLTMoIAR(V)	-	4.77
		1,988.91	995.4628	2	-0.05534	10	28	(R)VADGLPLAASMoQEDEQSGR(D)	5.02	11.96
		1,207.58	604.7989	2	-0.235	29	38	(R)DLQQYQSQAK(Q)	3.35	3.11
		2,376.14	595.0425	4	0.8286	82	101	(K)KLAFAYLEDLHSEFDEQHGK(K)	3.42	7.57
		2,273.17	758.732	3	0.3798	103	121	(K)VPTVSRPYSFIEFDTFIQK(T)	3.91	4.44
		1,741.91	581.6431	3	0.06121	133	147	(R)RNLGSINTELQDVQR(I)	2.51	1.52
		1,429.76	715.887	2	-0.3508	148	159	®MoVANIEEVLQR(G)	4.02	8.24
Vsp28	Q9D1C8	2,695.33	899.4515	3	0.2453	1	25	ace-MoFHGIpATPGVGAPGNKPELYEEVK(L)	2.70	3.55
		1,414.68	708.3481	2	1.027	36	47	(K)YDNmAELFAVVK(T)	4.14	5.11
		1,753.79	877.9046	2	0.2118	83	97	(R)QVQGSEISSIDEFcR(K)	3.68	7.89
		1,202.56	602.2871	2	-0.6119	149	158	(R)AMoDEIQPDLR(E)	3.01	3.96
		1,833.84	917.9255	2	0.4871	203	217	(R)QMoLFDLESAYNAFNR(F)	4.84	6.06
Vsp4b	P46467	3,361.67	841.4242	4	-1.664	18	46	(K)AAQEDKAGNYEEALQLYQHAVQYFLHVVK(Y)	5.49	1.40
		2,719.37	680.851	4	-0.3399	24	46	(K)AGNYEEALQLYQHAVQYFLHVVK(Y)	4.90	4.72
		945.5072	316.1763	3	-0.1859	156	163	(K)FPHLFTGK(R)	2.67	2.32
		2,192.08	731.6999	3	-0.3638	225	243	(R)ENKPSIIFIDEIDSLcGSR(S)	4.10	8.15
		3,655.89	1,219.64	3	-0.4365	256	289	(K)TEFLVQMoQGVGVDNDGILVLGATNIPWVLDSAIR(R)	2.06	1.17
		2,215.09	739.3699	3	0.2973	312	331	(R)LHLGSTQNSLTEADFQELGR(K)	5.78	8.72
		1,619.81	810.9108	2	1.041	404	416	(K)LLEPVVSMoWDMoLR(S)	3.07	3.33
ALIX Pdcd6ip	Q9WU78	1,260.72	631.3688	2	-0.02715	2	11	ace-ASFIWVQLKK(T)	-	3.92
		1,298.74	650.3795	2	-0.3246	12	23	(K)TSEVDLAKPLVK(F)	2.86	4.40

		2,160.95	721.3253	3	0.2674	24	41	(K)FIQQTYPSGGEEQAQYcR(A)	3.99	3.00
		2,075.15	692.7236	3	-0.09591	52	70	(R)SALGRPLDKHEGALETLLR(Y)	-	3.00
		1,414.64	708.3293	2	0.009794	71	81	(R)YYDQIcSIEPK(F)	3.07	2.42
		1,426.71	476.5769	3	-0.4933	97	110	(K)DAFDKGSLFGGSVK(L)	2.83	2.80
		1,063.59	532.8031	2	0.1831	111	120	(K)LALASLGYEK(S)	3.02	3.07
		2,950.38	984.4668	3	-0.5684	121	147	(K)ScVLFNcAALASQIAAEQNLDNDEGLK(T)	8.07	14.68
		3,321.59	1,108.20	3	-2.666	121	151	(K)ScVLFNcAALASQIAAEQNLDNDEGLKTAAK(Q)	4.36	8.74
		1,508.78	503.9332	3	0.09499	152	164	(K)QYQFASGAFLHIK(D)	2.67	3.49
		2,451.29	613.8304	4	0.4875	152	173	(K)QYQFASGAFLHIKDTVLSALSR(E)	3.58	-
		960.5242	481.2694	2	0.1213	165	173	(K)DTVLSALSR(E)	2.69	2.41
		3,161.65	1,054.89	3	-0.8214	174	202	(R)EPTVDISPDTVGTLSLIMLAQAQEVFFLK(A)	4.61	7.82
		3,177.66	1,060.23	3	1.077	174	202	(R)EPTVDISPDTVGTLSLIMoLAQAQEVFFLK(A)	3.47	12.64
		1,529.72	765.865	2	0.2322	216	229	(K)LANQAADYFGDAFK(Q)	4.19	5.70
		974.5441	488.2793	2	0.3516	240	248	(K)EVFPTLAAK(Q)	2.34	1.30
		1,021.59	341.5376	3	-1.231	277	285	(R)LQHAAELIK(N)	3.34	1.27
		1,321.61	661.8108	2	1.296	313	322	(K)KDNDFIYHDR(V)	2.60	1.31
		1,308.73	437.2498	3	-1.353	323	334	(R)VPDLKDLDPIGK(A)	2.19	1.68
		1,676.98	839.4984	2	-0.1959	335	350	(K)ATLVKPTPVNVPVSQK(F)	3.96	7.49
		898.4436	450.2291	2	-0.1622	351	357	(K)FTDLFEK(M)	2.02	1.92
		1,774.94	888.4779	2	0.6898	358	373	(K)MVPVSVQQSLAVFSQR(K)	3.25	8.07
		1,790.94	896.4754	2	0.7092	358	373	(K)MoVPVSVQQSLAVFSQR(K)	4.35	6.82
		3,407.80	1,136.94	3	-0.0505	387	420	(R)EATTLANGVLASLNLPAAIEDVSGDTVPQSILTK(S)	4.34	3.70
		1,901.01	951.5132	2	0.565	421	438	(K)STSVVEQGGIQTVDQLIK(E)	4.73	11.36
		1,257.67	629.8412	2	0.1179	447	456	(R)NREILEESLR(L)	2.52	1.80
		1,531.70	766.8571	2	-0.576	457	469	(R)LLDEEEATDNDLR(A)	4.82	4.40
		2,532.33	845.1157	3	-0.3806	518	541	(R)DTIALLcKPEPELNAAIPSANPAK(T)	3.22	2.11
		1,292.66	647.3389	2	-1.187	542	553	(K)TMoQGSEVVSVLK(S)	1.93	1.68
		2,503.28	1,252.65	2	0.5669	584	606	(K)FLTALAQDGVINEEALSVTELDR(I)	5.46	7.10
		1,642.83	548.6162	3	-0.3807	641	654	(K)QSNNEANLREEVLK(N)	1.97	0.89
		1,893.98	632.3347	3	-0.6988	655	671	(K)NLATAYDNFVELVANLK(E)	5.15	3.92

		1,395.74	698.877	2	-0.2795	676	686	(K)FYNELTEILVR(F)	3.25	7.40
		2,889.44	964.154	3	-0.3857	716	745	(R)EPSAPSIPPPAYQSSPAAGHAAAPPTPAPR(T)	2.33	2.35
Rab5a	Q9CQD1	1,084.65	543.3317	2	-0.5392	23	33	(K)LVLLGESAVGK(S)	3.29	7.27
		1,350.62	676.3177	2	0.139	71	81	(K)FEIWDTAGQER(Y)	3.38	4.41
		2,053.01	685.3442	3	-0.4091	92	110	(R)GAQAAIVVYDITNEESFAR(A)	3.01	3.07
		1,410.78	706.3997	2	1.172	121	134	(R)QASPNIVIALSGNK(A)	2.59	3.57
		1,790.80	896.4058	2	-3.58	150	165	(Q)SYADDNSLLFMETSAK(T)	-	2.54
		1,599.76	800.8892	2	0.1226	166	179	(K)TSMoNVNEIFMoAIAK(K)	3.49	4.52
		1,253.58	627.795	2	0.4368	184	195	(K)NEPQNPGANSAR(G)	2.35	1.85
		1,465.76	489.5956	3	0.6444	196	209	(R)GRGVDLTEPAQPAR(S)	2.41	-
Ywhae	P62259	1,539.69	770.8512	2	0.1084	1	12	ace-MoDDREDLVYQAK(L)	-	5.62
		2,087.95	696.9915	3	1.012	13	29	(K)LAEQAERYDEMoVESMoKK(V)	4.35	3.23
		1,162.45	582.2333	2	-0.279	20	28	(R)YDEMoVESMoK(K)	2.08	3.40
		1,462.70	732.3557	2	-0.4902	30	42	(K)VAGMoDVELTVEER(N)	3.47	7.28
		1,416.75	709.3807	2	0.4389	62	73	(R)IISSIEQKEENK(G)	4.23	7.82
		1,475.74	738.8749	2	-1.087	95	106	(K)LIccDILDVLDK(H)	3.41	7.24
		2,694.37	674.5997	4	-1.073	95	118	(K)LIccDILDVLDKHLIPAANTGESK(V)	3.32	1.62
		1,255.58	628.7989	2	-0.1118	131	141	(R)YLAEFATGNDR(K)	2.97	3.66
		1,383.68	692.846	2	-0.6904	131	142	(R)YLAEFATGNDRK(E)	3.46	4.89
		1,834.92	612.6483	3	-1.001	154	170	(K)AASDIAMoTELPPTHPIR(L)	2.16	3.42
		2,086.96	1,044.49	2	-0.1291	197	215	(K)AAFDDAIAELDTLSEESYK(D)	6.19	7.34
		3,273.60	1,092.21	3	0.9097	197	225	(K)AAFDDAIAELDTLSEESYKDSTLIMoQLLR(D)	4.57	15.00
		1,204.65	603.3319	2	0.3023	216	225	(K)DSTLIMoQLLR(D)	3.26	6.33
		1,188.65	595.3345	2	0.4359	216	225	(K)DSTLIMQLLR(D)	2.83	4.08
		3,466.46	1,156.49	3	0.7519	226	255	(R)DNLTLWTSDMoQGDGEEQNKEALQDVEDENQ(-)	4.84	7.92
Ywhaz	P63101	1,161.57	581.7923	2	-0.03256	1	9	(-)aceMoDKNELVQK(A)	2.58	3.31
		1,547.71	774.8612	2	0.9433	28	41	(K)SVTEQGAELSNEER(N)	5.62	14.80
		2,416.25	806.4248	3	0.1008	84	103	(R)EKIETELRDIcNDVLSLLEK(F)	2.13	1.52
		2,159.11	720.7105	3	-2.381	86	103	(K)IETELRDIcNDVLSLLEK(F)	3.34	8.08
		1,417.71	709.8633	2	-0.3201	92	103	(R)DIcNDVLSLLEK(F)	3.10	8.09

		1,329.69	665.8535	2	-0.3776	104	115	(K)FLIPNASQPESK(V)	2.25	2.92
		1,278.65	640.3303	2	0.2354	128	139	(R)YLAEVAAGDDKK(G)	3.90	9.03
		2,039.98	1,021.00	2	0.5087	140	157	(K)GIVDQSQQAYQEAFEISK(K)	6.39	9.96
		2,168.07	723.6986	3	-0.5897	140	158	(K)GIVDQSQQAYQEAFEISKK(E)	1.93	1.57
		2,130.99	711.3359	3	0.5388	194	212	(K)TAFDEAIAELDTLSEESYK(D)	4.15	3.15
		3,317.63	830.4144	4	1.652	194	222	(K)TAFDEAIAELDTLSEESYKDSTLIMoQLLR(D)	4.52	2.77
Il18	P70380	3,127.34	1,043.45	3	-1.672	14	39	(K)EMoMoFIDNTLYFIPEENGDLESDNFGR(L)	3.68	4.10
		2,465.11	822.7116	3	-0.05205	60	80	(K)RQPVFEDMoTDIDQSASEPQTR(L)	3.58	6.30
		2,309.01	770.6776	3	-0.5252	61	80	(R)QPVFEDMoTDIDQSASEPQTR(L)	3.43	-
		2,998.41	1,000.48	3	-3.551	114	138	(K)IISFEEMoDPPENIDDIQSDLIFFQK(R)	5.55	4.48
IL1b	P01584	140.7675	702.387	2	0	63	75	(R)QAASVVVAMoDKLR(K)[a]	-	-

a. This peptide was identified using a targeted SRM analysis. Peptide was manually validated

GSDMD-interacting proteins

Gsdmdc1	Q9D8T2	1,698.89	567.3038	3	-0.3517	15	30	(K)EVSGSRGDLIPVDSLR(N)	2.81	-
		1,257.63	629.8223	2	-1.204	83	94	(K)VSDVVDGNIQGR(V)	2.34	3.70
		1,805.95	602.9912	3	-0.3763	153	168	(R)SRGDDLFVVTEVLQTK(E)	3.68	-
		1,390.76	464.595	3	-0.8809	206	218	(K)MoVTIPAGSILAFR(V)	2.72	3.31
		3,094.52	1,032.51	3	-1.25	270	297	(K)QLSLLSDGIDEEELIEAADFQGLYAEVK(A)	4.71	5.30
		1,668.73	835.3741	2	0.02826	298	311	(K)AcSSELESLEmELR(Q)	2.30	4.80
		1,011.61	506.8109	2	-0.6939	312	320	(R)QQILVNIGK(I)	2.58	-
		960.5496	481.2821	2	0.3812	395	403	(K)ALETTVLSK(Q)	2.30	1.89
Nedd4	P46935	2,654.37	885.7974	3	-0.1432	156	178	(R)LTRDDFLGQVDVPLYPLPTENPR(M)	3.22	4.85
		1,584.86	793.4379	2	-0.7649	493	507	(R)LQNVAITGPAVPYSR(D)	3.20	2.72
		1,514.83	505.9497	3	-1.002	638	649	(K)LLDGFFIRPFYK(M)	2.94	-
		1,612.81	807.4119	2	-0.4472	674	686	(R)WILENDPTELDLR(F)	2.61	6.00
		1,547.83	774.9201	2	1.156	741	753	(K)EGFFELIPQDLIK(I)	2.47	0.82
Hsp90ab1	P11499	1,274.64	638.3248	2	-0.4131	42	53	(R)ELISNASDALDK(I)	2.70	1.41
		1,543.82	515.6141	3	-0.2847	42	55	(R)ELISNASDALDKIR(Y)	4.33	3.23
		1,538.75	513.9229	3	0.1113	56	69	(R)YESLTDPSKLDSGK(E)	2.71	2.37
		1,193.64	597.8282	2	0.9046	73	82	(K)IDIIPNPQER(T)	2.56	2.19

		1,364.72	683.3671	2	-2.033	83	95	(R)TLTLVDTGIGMoTK(A)	-	2.40
		1,241.70	621.8577	2	2.188	96	107	(K)ADLINNLGTIAK(S)	3.12	1.22
		1,807.95	603.6573	3	-0.49	205	219	(K)HSQFIGYPITLYLEK(E)	3.53	0.85
		1,150.55	576.2828	2	0.2498	276	284	(K)YIDQEELNK(T)	2.17	-
		1,846.79	924.4009	2	-1.483	292	306	(R)NPDDITQEEYGEFYK(S)	3.55	8.42
		1,248.61	625.3126	2	0.624	492	502	(K)EQVANSAFVER(V)	2.62	2.22
		1,910.04	478.5166	4	-0.2613	624	639	(K)KHLEINPDHPIVETLR(Q)	4.60	0.60
		1,781.94	594.9878	3	-0.5646	625	639	(K)HLEINPDHPIVETLR(Q)	2.79	-
Cdc37	Q61081	1,701.81	851.9109	2	0.8027	70	84	(K)ELEVAESDGQVELER(L)	3.01	4.48
		2,268.15	757.0583	3	-0.00551	288	308	(R)LGPGGLDPVEVYESLPEELQK(C)	2.48	-
		1,706.84	854.4261	2	0.5418	354	370	(K)EGEEAGPGDPLLEAVPK(A)	2.61	4.02

Supplemental Table III. *NEDD4-catalyzed ubiquitination sites in pro-IL-1β*

[M + H]	m/z	z	Δm (ppm)	Ret. Time (min)	Site	Sequence	Xcorr Score
1939.943	647.319	3	-0.31	48.8366	K132	DSQQK^{GlyGly}SLVMoSGPYELK	4.64
1460.703	730.8555	2	0.6	25.8968	K59	ISDHHYSK^{GlyGly}GFR	2.44
1517.81	759.413	2	5.4	40.2326	K73	QAASVVVAMoDK^{GlyGly}LR	3.41
1645.905	823.4566	2	0.36	35.7489	K73	QAASVVVAMoDK^{GlyGly}LRK	2.8

Supplemental Table IV. *IL-1β-interacting proteins identified in LC-MS/MS analysis.*

Protein	Spectral counts		
	untreated	LPS	LPS+ATP
IL-1β	-	-	+
Nedd4	0	0	72
Ube2d3	0	0	8
Cdc37	0	0	51
Hsp90α	2	4	162
GsdmD	0	0	6
Casp8	0	0	17
Atg7	0	0	9
Atg3	0	0	7
Stxbp2	0	0	38
Stxbp3	0	0	5
Chmp4b	0	1	2
Sec22b	0	3	13
Vps28	0	0	12
Vps4b	0	0	18
ALIX	0	0	97
Rab5a	0	2	15
14-3-3ε	8	12	42
14-3-3ζ/δ	4	3	23
IL-18	0	0	6