Supplementary Materials for

Igalan Attenuates Sepsis-Induced Inflammation through Covalent Targeting of the NLRP3 Inflammasome Pathway

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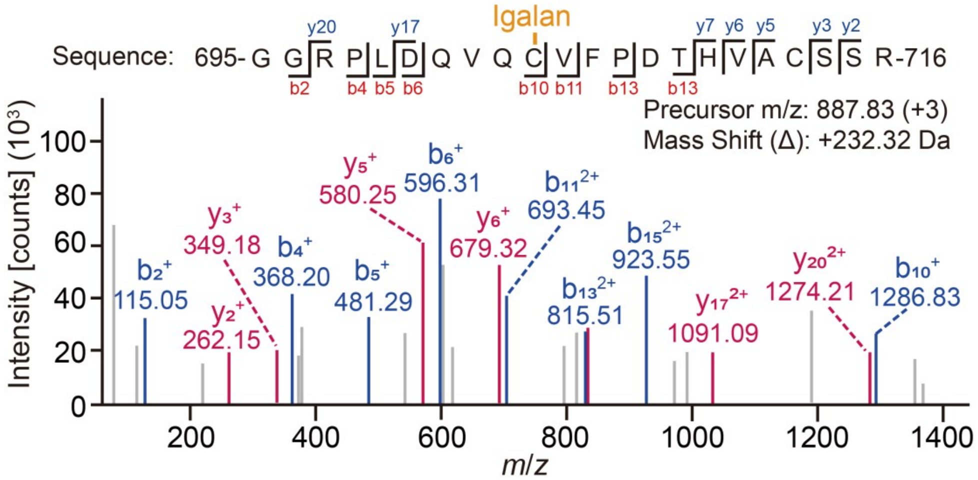

Fig. S1. High-resolution MS/MS spectrum of the Igalan-modified NLRP3 peptide.

The precursor ion (m/z 887.83, charge +3) spanning NLRP3 residues 695–716 (Sequence: GGRPLDQVQCVFPDTHVACSSR) was fragmented using HCD. A precise mass shift of +232.32 Da, corresponding to the covalent adduction of Igalan, is consistently observed on specific fragment ions. The presence of unmodified N-terminal b-ions (e.g., b6$^+$) and C-terminal y-ions (e.g., y6$^+$) explicitly indicates that the adjacent residues, including Cys713, remain intact. The concurrent identification of these unmodified ions alongside the modified fragments encompassing Cys704 (such as b10$^+$, b11^{2+}, and y17^{2+}) unambiguously pinpoints the covalent modification site specifically to Cys704.

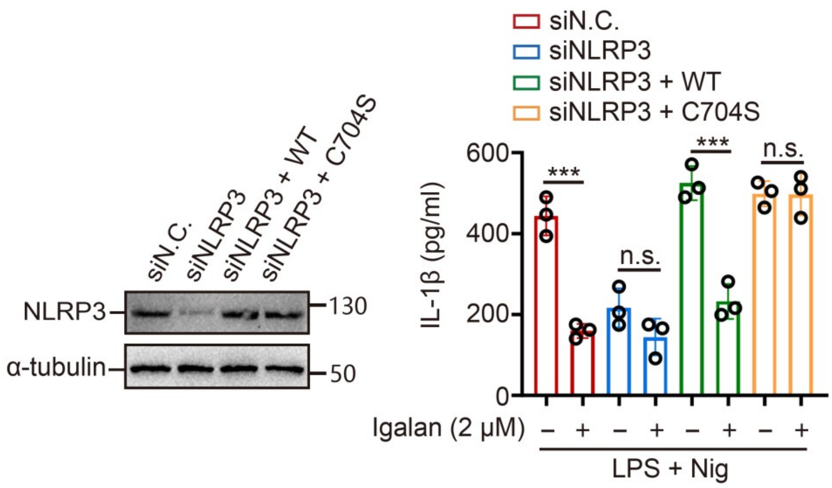

Fig. S2. Cys704 is essential for Igalan-mediated inhibition of the NLRP3 inflammasome. iBMDMs were transfected with scramble siRNA (siN.C.) or NLRP3-targeting siRNA (siNLRP3), followed by reconstitution with either wild-type (WT) or C704S mutant NLRP3 plasmids. (Left) Western blot analysis confirming the successful knockdown of endogenous NLRP3 and the comparable reconstitution of WT and C704S mutant NLRP3 proteins. α-tubulin was used as a loading control. (Right) The indicated cells were primed with LPS and stimulated with Nigericin (Nig) in the presence or absence of Igalan (2 μM). IL-1β levels in the culture supernatants were quantified by ELISA. Igalan significantly inhibited IL-1β release in WT-reconstituted cells but failed to suppress inflammasome activation in C704S-reconstituted cells. Data are presented as mean ± SEM (n = 3). ****P* < 0.001; n.s., not significant.

Table S1 . Comprehensive list of cellular proteins and specific cysteine residues covalently modified by Igalan.

140 proteins each containing at least one peptide with the +232.32 Da mass shift on a cysteine residue.

Protein (UniProt ID)	Modified Peptide Sequence	Modified Cysteine
Nova2 (A0A1W2P872)	KRPLETPPEVVC(1)TK	C21
Sertad4 (A7DTG3)	LC(0.014)C(0.986)NRGGK	C371
Skint8 (A7XV07)	LSPTC(1)LAKWEK	C358
Brpf1 (B2RRD7)	LTC(0.621)YIC(0.379)KQR	C385
Trappc11 (B2RXC1)	EIIC(0.086)KC(0.914)HK	C874
Piezo1 (E2JF22)	QHQQAPLPAQAVC(1)ADGTR	C962
Bod1l (E9Q6J5)	DC(1)LADVDTKPAYQNLR	C72;60
Morc3 (F7BJB9)	LLEMNDKC(1)VK	C741
Zfpm1 (O35615)	KDDALWC(1)R	C188

Coro1a (O89053)	DGALIC(0.997)TSC(0.003)R	C192
Afp (P02772)	MVSIASTC(0.5)C(0.5)QLSEEK	C457
Aldoc (P05064)	C(1)QYVTEK	C202
Tuba1b (P05213)	AYHEQLSVAEITNAC(1)FEPANQMVK	C295
Gnai2 (P08752)	IIHEDGYSEEEC(1)R	C66
Anxa1 (P10107)	C(1)ATSTPAFFAEK	C270
Eef1a1 (P10126)	PMC(1)VESFSDYPPLGR	C411
Ctsb (P10605)	GENHC(1)GIESEIVAGIPR	C319
Hsp90ab1 (P11499)	C(1)LELFSELAEDKENYK	C412
Tcp1 (P11983)	SLHDALC(1)VVK	C397
Pfkl (P12382)	LPLMEC(1)VQVTK	C351
Nr4a1 (P12813)	NRC(0.5)QFC(0.5)RFQK	C325
Gsn (P13020)	SEDC(1)FILDHGR	C329
Calr (P14211)	DMHGDSEYNIMFGPDIC(1)GPGTK	C137
Lgals1 (P16045)	DSNNLC(1)LHFNPR	C43
F9 (P16294)	NC(0.5)ELDATC(0.5)NIK	C134
Gapdh (P16858)	IVSNASC(0.999)TTNC(0.001)LAPLAK	C150
Gapdh (P16858)	VPTPNVSVVDLTC(1)R	C245;351
Eno1 (P17182)	SCNC(1)LLLK	C339
Pcna (P17918)	C(1)AGNEDIITLR	C81
Tgm2 (P21981)	DHHTADLC(1)QEK	C27
Glud1 (P26443)	C(1)AVVDVPFGGAK	C172
Rpl3 (P27659)	VAC(1)IGAWHPAR	C253
Apex1 (P28352)	IC(1)SWNVDGLR	C64
Atxn10 (P28658)	STHAVELAC(1)R	C65
Mmp2 (P33434)	VLC(0.028)VLC(0.486)C(0.486)LLGR	C22
Ranbp1 (P34022)	AWVWNTHADFADEC(1)PKPELLAIR	C132
Prdx1 (P35700)	HGEVC(1)PAGWKPGSDTIKPDVNK	C173
Por (P37040)	LIHEGGAHIYVC(1)GDAR	C630
Cap1 (P40124)	C(1)VNTTLQIK	C355
Cct8 (P42932)	AHEILPELVC(0.189)C(0.811)SAK	C149
Akr1b1 (P45376)	VC(1)ALMSCAK	C299
Psmc2 (P46471)	TDAC(1)FIR	C236
Pkm (P52480)	AEGSDVANAVLDGADC(1)IMLSGETAK	C358
Cox6b1 (P56391)	NC(1)WQNYLDFHR	C30
Cox17 (P56394)	KPLKPC(0.306)C(0.306)AC(0.389)PETK	C26
Eef2 (P58252)	EGALC(1)EENMR	C693
Pcbp1 (P60335)	VMTIPYQPMPASSPVIC(1)AGGQDR	C194
Rps11 (P62281)	C(1)PFTGNVSIR	C60
Rpl18a (P62717)	C(1)HTPPLYR	C22
Hpcal1 (P62748)	DC(1)PTGHLTVDEFK	C38
Rps28 (P62858)	TGSQGQC(1)TQVR	C27

Rpl30 (P62889)	TGVHHYSGNNIELGTAC(1)GK	C85
Pfn1 (P62962)	KC(1)YEMASHLR	C128
Rps27a (P62983)	C(0.5)C(0.5)LTYCFNKPEDK	C145
Rac1 (P63001)	HHC(1)PNTPIILVGTK	C105
Dynll1 (P63168)	YNPTWHC(1)IVGR	C56
Rps12 (P63323)	QAHLCVLASNC(1)DEPMYVK	C56
Tuba1c (P68373)	AYHEQLTVAEITNAC(1)FEPANQMVK	C295
Kpnb1 (P70168)	HFIMQVVC(0.001)EATQC(0.999)PDTR	C228
Rps3a (P97351)	AC(1)QSIYPLHDVFVR	C201
Psme2 (P97372)	C(1)GYLPGNEK	C91
Rps5 (P97461)	KAQC(1)PIVER	C66
Fxyd5 (P97808)	QLSQFC(1)LNR	C173
Tubb4b (P99024)	TAVC(1)DIPPR	C354
Tfdp1 (Q08639)	TVIDC(1)SISNDK	C274
Gpr15 (Q0VDU3)	CLC(0.5)PC(0.5)LK	C320
Esyt2 (Q3TZZ7)	AC(1)DLPAWVHFPDTER	C105
Vwce (Q3U515)	ASPQSDC(0.667)C(0.667)TC(0.667)VPGR	C488
Cmpk2 (Q3U5Q7)	MENPSC(1)HLVDASPSR	C422
Zswim8 (Q3UHH1)	IPRGPSEMSTIRC(1)R	C901
Tars2 (Q3UQ84)	GPAGTPEC(1)PVLIHR	C585
Defa20 (Q45VN2)	LLFC(1)C(1)RR	C89
Prss53 (Q571E5)	PTC(1)NCLYNR	C187
Cage1 (Q5IR70)	SASQC(1)LEIEK	C447
Rasl10b (Q5SSG5)	SVGC(0.91)ARC(0.09)K	C179
P4ha1 (Q60715)	HAAC(1)PVLVGNK	C503
Vdac2 (Q60930)	VC(1)EDFDTSVNLAWTSGTNCTR	C211
Ppp2r5c (Q60996)	C(1)VSSPHFQVAER	C334;365;351
Asns (Q61024)	YHHC(1)TDEPLHAIYDSVEK	C207
Soat1 (Q61263)	EVGC(1)HFDDFVTNLIEK	C61
Hspa4 (Q61316)	KFDEVLVNHFC(1)EEFGK	C245
Stxbp2 (Q64324)	KVTELLKTFC(1)ESK	C311
Cltc (Q68FD5)	RDPHLAC(1)VAYER	C918
FAM120A (Q6A0A9)	AHQLVLPPC(1)DVVIK	C279;350
Nop58 (Q6DFW4)	LNLSC(1)IHSPVVNELMR	C106
Eif4g1 (Q6NZJ6)	NHDEESLEC(0.999)LC(0.001)R	C938;923
Tnpo3 (Q6P2B1)	AIHNIC(1)SVCR	C527
Xpo1 (Q6P5F9)	LFEFMHETHDGVQDMAC(1)DTFIK	C585
Oxsr1 (Q6P9R2)	C(1)QTSMDELLK	C53;111
Rps27 (Q6ZWY3)	LTEGC(1)SFR	C77
Uts2b (Q765I1)	AC(1)FWKYCV	C107
Mybbp1a (Q7TPV4)	HQAQAC(1)LMLQK	C1028
Ahcyl1 (Q80SW1)	FDNLYC(0.965)C(0.035)R	C292;375

Sympk (Q80X82)	ATNLC(1)FAER	C969
CIMAP1C (Q810P2)	INC(1)MPASC(1)K	C124
Tufm (Q8BFR5)	KGDEC(1)ELLGHNK	C290
Ipo5 (Q8BKC5)	CIEVMGDGC(1)LNNEHFEELGGILK	C784
Tmem214 (Q8BM55)	ATPSC(1)PAAMK	C377
Lars1 (Q8BMJ2)	NMETFC(1)EESR	C556
Nars1 (Q8BP47)	YGTC(1)PHGGYGLGLER	C522
Rpl24 (Q8BP67)	VELC(1)SFSGYK	C6
Flna (Q8BTM8)	IEC(0.997)DDKGDGSC(0.003)DVR	C623;619
Iars1 (Q8BU30)	FGWDC(1)HGLPVEYEIDK	C87
Washc5 (Q8C2E7)	LLC(1)FMIVK	C881
Dock2 (Q8C3J5)	GEQC(1)EPILR	C730
Acat2 (Q8CAY6)	VNIDGGAIALGHPLGASGC(1)R	C360
Eprs1 (Q8CGC7)	LTSAINELNHC(1)LSLR	C105
Nckap1l (Q8K1X4)	EHAITNSGQFHC(1)QR	C338
Aimp2 (Q8R010)	PYHGGSAPLHVELPTC(1)MYR	C23
Kiaa0513 (Q8R0A7)	EKWC(1)HMTQEERDDSLR	C337
Niban2 (Q8R1F1)	GPTKEELC(1)K	C466
Nlrp3 (Q8R4B8)	GGRPLDQVQC(0.999)VFPDTHVAC(0.001)SSR	C704
Ipo4 (Q8VI75)	DNIC(1)GALAR	C963
Oasl1 (Q8VI94)	HYTLQHPVIEAC(1)VR	C287
Ddx1 (Q91VR5)	MHNQIPQITC(1)DGK	C396
Xpo5 (Q924C1)	ESLDQFDC(1)K	C1131
Xpo6 (Q924Z6)	EQLAESILHEGSTGC(1)R	C886
Rap1b (Q99JI6)	QWNNC(1)AFLESSAK	C141
Psat1 (Q99K85)	EC(1)PSVLDYK	C224
Nasp (Q99MD9)	KPTDGASSSNC(1)VTDISHLVR	C694
Gpnmb (Q99P91)	AYKPIGNC(1)PR	C535
Nop16 (Q9CPT5)	IEC(1)SHIR	C36
Lap3 (Q9CPY7)	SAGAC(1)TAAAFLR	C462
Rps21 (Q9CQR2)	TYGIC(1)GAIR	C56
Zwint (Q9CQU5)	C(1)ALSEALPQVK	C118
Rpl14 (Q9CR57)	C(1)MQLTDFILK	C54
Cul2 (Q9D4H8)	MVADHLQFLHSEC(1)HSIIQQER	C282
Idh3a (Q9D6R2)	IEAAC(1)FATIK	C331
Prps2 (Q9D7G0)	VAILVDDMADTC(0.976)GTIC(0.024)HAADK	C226
Prkar1a (Q9DBC7)	QIQC(1)LQK	C67
Dync1h1 (Q9JHU4)	ASLAC(1)GPMVK	C3387
Aldh9a1 (Q9JLJ2)	C(1)QVLLEAAR	C74
Cd5l (Q9QWK4)	ELNC(1)GAVIQTPR	C70
Ptges3 (Q9R0Q7)	HLNEIDLFHC(1)IDPNDSK	C58
Clec4e (Q9R0Q8)	NCC(1)PLNWK	C80

Tagln2 (Q9WVA4)	NMAC(1)VQR	C124
Uba2 (Q9Z1F9)	NTPSEPIHC(1)IVWAK	C185
Arih1 (Q9Z1K5)	LFAEC(1)HVINPSK	C159
Bag6 (Q9Z1R2)	C(0.999)NLAC(0.001)APPR	C346
Hnrnpf (Q9Z2X1)	DLSYC(1)LSGMYDHR	C267

This table details the 140 target proteins identified via high-resolution LC-MS/MS chemoproteomic profiling. Each listed entry represents a protein containing at least one unambiguously identified tryptic peptide harboring a specific mass shift of +232.32 Da on a cysteine residue, corresponding to the covalent adduction of Igalan. For each identified target, the canonical UniProt accession number is provided alongside its corresponding modified peptide sequence. Notably, the values in parentheses immediately following the modified cysteine within the sequence (e.g., C(1), C(0.986)) denote the site localization probability score generated by the search engine. A score of 1 indicates absolute confidence that the +232.32 Da modification is specifically localized to that exact residue, distinguishing it from other potential nucleophiles within the same peptide. Finally, the precise positional number of the modified cysteine within the full-length canonical protein sequence is explicitly defined for each target, providing a high-confidence molecular basis for the multi-target landscape of Igalan.