

SUPPLEMENTARY MATERIAL

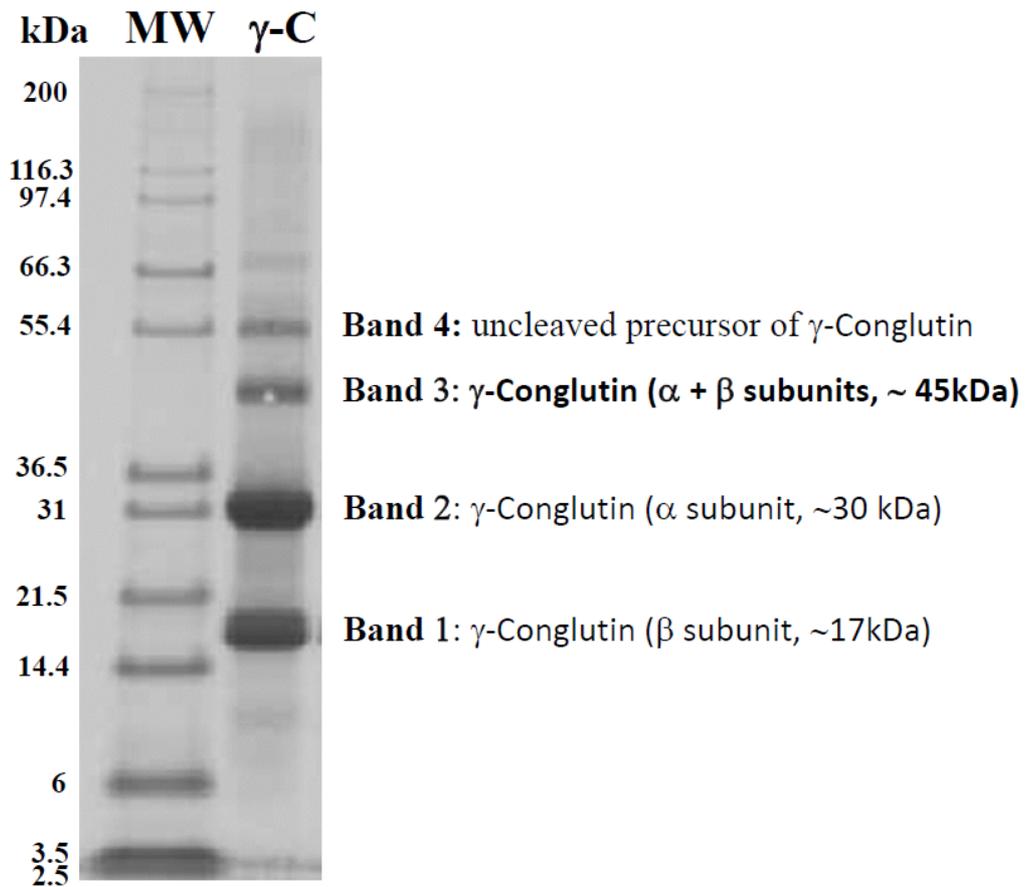


Figure S1. Isolation and purification of NLL seed γ -conglutin protein. SDS-PAGE of isolated and purified γ -conglutin protein, with high level of purity (>95%). Subunits α and β of γ -conglutin, unreduced γ -conglutin, and uncleaved γ -conglutin precursor are present. MW, molecular weight standard (kDa).

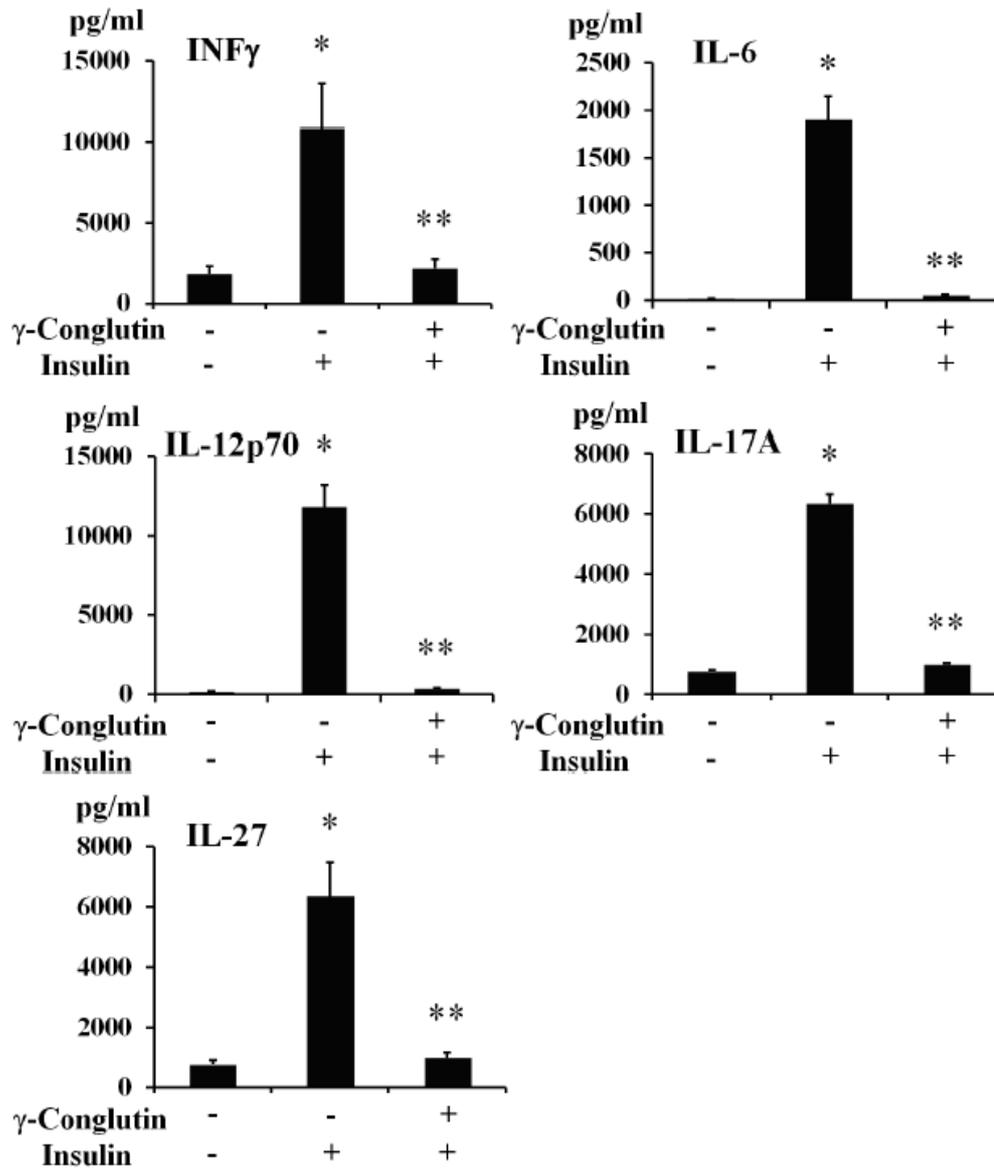


Figure S2. Effect of NLL γ -conglutinin on the protein levels of pro-inflammatory cytokines. Control PANC-1 cells, and IR-C pancreatic cells were cultured for 24 h alone, or the former with γ -conglutinin protein. The bar graph shows protein levels determined by ELISA of INF γ , IL-6, IL-12, IL-17, and IL-27. Data represent mean \pm SD from three independent experiments. C: Untreated control culture cells; IR-C: insulin resistant culture cells; IR-C+ γ : IRC+ γ -conglutinin challenge. $p < 0.05$ represent statistically significant differences associated with each figure. $p^* < 0.05$ IR-C versus C; $p^{**} < 0.05$ IR-C+ γ -conglutinin versus IR-C.

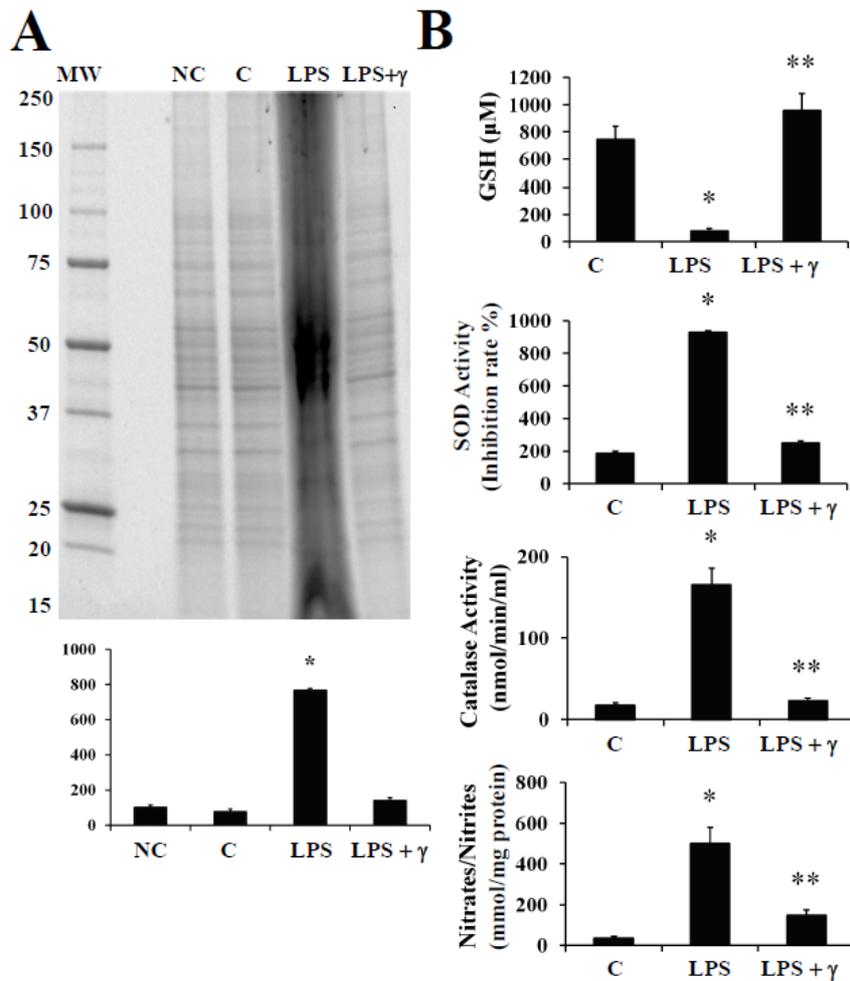


Figure S3. Effect of γ -conglutin on proteins oxidative modifications, antioxidant enzymatic activities and production of GSH and NO. **(A)** Changes in protein carbonyl formation were measured in LPS-treated pancreatic cells after 24 h of incubation with LPS and/or γ -conglutin. Protein carbonyls were measured using an OxyBlot kit. Representative blots show basal carbonylation levels in C control PANC-1 cells, LPS-treated pancreatic cells, and LPS-treated pancreatic cells challenged with γ -conglutin. Graph y-axis represents arbitrary densitometry units. $p^* < 0.05$ LPS-treated pancreatic cells *versus* C cells. **(B)** LPS-treated pancreatic cells were incubated for 24 h with γ -conglutin protein. GSH and NO production, as well as SOD and catalase activities were measured. Data represent mean \pm SD from three independent experiments. $p < 0.05$ represent statistically significant differences associated with each figure. $p^* < 0.05$ LPS-treated pancreatic cells *versus* C (control PANC-1) cells; $p^{**} < 0.05$ LPS + -conglutin treated pancreatic cells *versus* LPS-treated pancreatic cells.

Supplementary Table S1. γ -conglutin peptides mass fingerprinting characterization.

Band ¹	Sequence of γ -conglutin protein ²	identification	Sequence	
			coverage (%)	Score ³
1	MARNMAHILHILVISLSYSFLVSSSSQDSQSLYHNSQPTSSKPNLLVLPVQEDASTGLH WANIHKRTPLMQVPLLLDLNGKHLWVTC SQHYSSSTYQAPFCHSTQCSRANTHCFTCTD STTRPGCHNNTCGLLSSNPVTQESGLGELAQDVLAIHSTHSGSKLGFPMVKVPQFLFSCAP SFLAQKGLPNNVQGALGLGQAPI SLQNQLF SHFGLKRF SVCLSRYSTSNGAILFGDIND PNNNNYIHNSLDVLHDLVYTPLTISKQGEYFIQVNAIRVKNHLVIPTKNPFISPSSTSYH GSGEIGGALITTHPYTVLSHSI FEVFTQVFANMMPKQAVKAVGPFGLCYDSRKISGGA PSVDLILDKNDAVWRISSENFMVQAQDGVSCCLGFVDGGVHARAGIALGAHHLEENLVVFD LERSRVGFNSNSLKSYGKTC SNLFDLNNP	Conglutin gamma <i>Lupinus angustifolius</i> (Narrow-leaved blue lupine)	26.3	90.2
2	MARNMAHILHILVISLSYSFLVSSSSQDSQSLYHNSQPTSSKPNLLVLPVQEDASTGLH WANIHKRTPLMQVPLLLDLNGKHLWVTC SQHYSSSTYQAPFCHSTQCSRANTHCFTCTD STTRPGCHNNTCGLLSSNPVTQESGLGELAQDVLAIHSTHSGSKLGFPMVKVPQFLFSCAP SFLAQKGLPNNVQGALGLGQAPI SLQNQLF SHFGLKRF SVCLSRYSTSNGAILFGDIND PNNNNYIHNSLDVLHDLVYTPLTISKQGEYFIQVNAIRVKNHLVIPTKNPFISPSSTSYH GSGEIGGALITTHPYTVLSHSI FEVFTQVFANMMPKQAVKAVGPFGLCYDSRKISGGA PSVDLILDKNDAVWRISSENFMVQAQDGVSCCLGFVDGGVHARAGIALGAHHLEENLVVFD LERSRVGFNSNSLKSYGKTC SNLFDLNNP	Conglutin gamma <i>Lupinus angustifolius</i> (Narrow-leaved blue lupine)	18.4	60.2

Table S2. Cell viability (%) and dose effects of NLL γ -conglutin protein. Cytotoxicity of γ -conglutin protein on PANC-1-pancreatic cells. Cells were treated with 10, 25 and 50 μ g of sample proteins for 24 h. Cell viability after treatment with LPS (1 μ g) was 99.0 \pm 1.4%. Data represent mean \pm SD from three independent experiments. Controls were performed using PANC-1 cells without any treatment with LPS and/or γ -conglutin protein. $p < 0.05$ represent statistically significant differences associated with the table. $p^* < 0.05$ treated cells versus untreated control PANC-1 cells.

Samples	10 μ g	25 μ g	50 μ g
γ -conglutin	97.2 \pm 1.5	99.6 \pm 2.4	82.0 \pm 2.6*
LPS (1 μ g) + γ -conglutin	97.6 \pm 2.8	99.8 \pm 2.4	81.0 \pm 1.3*

Table 3. Cell viability (%) on insulin resistance IR-C cell model. PANC-1 cells were treated with increasing (10^{-9} , 10^{-8} , 10^{-7} , 10^{-6} , 10^{-5} nmol/L) insulin quantities. Data represent cell viability (%) mean \pm SD from three independent experiments. $p < 0.05$ represent statistically significant differences associated with the table. $p^* < 0.05$ treated cells versus untreated control PANC-1 cells.

Insulin (nmol/L)	% viability
10^{-9}	99.5 \pm 1.5
10^{-8}	99.4 \pm 1.2
10^{-7}	99.1 \pm 1.5
10^{-6}	95.4 \pm 1.6*
10^{-5}	94.7 \pm 1.8*

Table S4. Cell viability (%) and dose effects of purified NLL γ -conglutin protein on insulin resistance cell (IR-C) model. Cytotoxicity of γ -conglutin protein on IR-C pancreatic cells. Cells were treated with 10, 25 and 50 μ g of sample proteins for 24 h. Data represent cell viability (%) mean \pm SD from three

independent experiments. Control samples were assayed only with insulin (10^{-7} nm/L) showing 100% of cell viability. $p < 0.05$ represent statistically significant differences associated with the table. $p^* < 0.05$ treated cells versus untreated control PANC-1 cells.

Sample	10 μ g	25 μ g	50 μ g
γ -conglutin protein	98.6 \pm 2.8	99.0 \pm 1.8	80.0 \pm 0.8*

Table S5. Fold-change in protein levels of pro-inflammatory cytokines and iNOS. Numbers represent fold-changes calculated versus LPS from data in Figures 1 and 2 for a) LPS-induced inflammation model; and versus IR-C from data in Figure 5 and Supplementary Figure S2 for b) IR model. Positive and negative values mean up- and down-regulated genes, respectively.

a)

Cytokines	γ -conglutin + LPS
TNF α	-253
INF γ	-12443
IL-1 β	-146
IL-6	-1849
IL-12p70	-11792
IL-17A	-5339
IL-27	-6100
iNOS	-258

b)

Cytokines	γ -conglutin + IR
TNF α	-146
INF γ	-8644
IL-1 β	-97
IL-6	-1839
IL-12p70	-11409
IL-17A	-5659
IL-27	-5339
iNOS	-189

Table S6. Fold-change in protein levels of pro-inflammatory cytokines and iNOS. Numbers represent fold-changes calculated versus control from data in Figures 1 and 2 for a) LPS-induced inflammation model; Figure 5 and Supplementary Figure S2 for b) IR model. Positive and negative values mean up- and down-regulated genes, respectively.

a)

Cytokines	LPS	γ-conglutin + LPS
TNF α	+145	-1
INF γ	+11335	-1100
IL-1 β	+187	-66
IL-6	+2979	-119
IL-12p70	+12127	+341
IL-17A	+5632	-492
IL-27	+5676	+300
iNOS	+245	-13

b)

Cytokines	IR	γ-conglutin +IR
TNF α	+173	+18
INF γ	+8994	+350
IL-1 β	+129	+27
IL-6	1881	+32
IL-12p70	+11592	+208
IL-17A	+5553	+214
IL-27	+5231	+218
iNOS	+287	+32