Supplemental Material

Supplementary Table 1. DRB1*04:01 subjects with established type 1 diabetes for T cell assays.

Patient	Gender	Age at draw (yrs)	Disease duration (yrs)
#1	female	43	6
#2	male	21	6
#3	female	34	7
#4	female	20	8
#5	male	20	5
#6	female	18	4
#7	male	18	7
#8	female	18	6
#9	male	19	6
#10	female	26	7
#11	female	51	38
	mean (n=11)	26.18	9.09
	SD (n=11)	11.47	9.64

Supplementary Table 2. Autoantibodies against P4Hb are present prior to the presence of anti-insulin and hyperglycemia in NOD mice.

NOD mice	age (weeks old, wks)	blood glucose ^ (blood glucose, mg/dL)	anti-P4Hb *	anti-insulin *
#1	4	− (123)	+	-
#2	4	− (138)	+	-
#3	4	− (180)	+	-
#4	4	− (204)	+	-
#5	4	− (88)	-	-
#6	4	− (194)	-	-
#7	8	− (104)	+	+
#8	8	− (96)	+	+
#9	8	− (93)	+	-
#10	8	− (159)	+	-
#11	8	− (116)	+	-
#12	8	− (99)	+	+
#13	11	− (60)	+	+
#14	11	− (178)	+	+
#15	28	− (113)	+	-
#16	28	− (167)	+	+
#17	20	+ (>500)	+	+
#18	22	+ (466)	+	+
#19	22	+ (>500)	+	+
#20	24	+ (363)	+	+
#21	26	+ (>500)	-	+
#22	28	+ (>500)	+	+

^, diabetics defined as positive if blood glucose concentration > 250 mg/dL

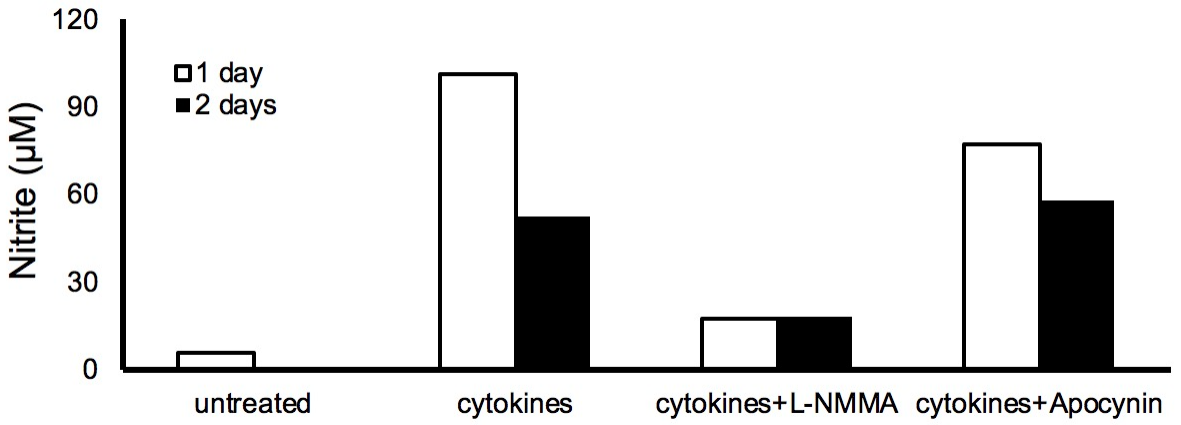
*, defined as positive if ELISA OD > (average OD + 2SD) of control mice (n=5)

Supplementary Table 3.
The carbonyl residues identified in human P4Hb under oxidative stress.

Domain in P4Hb	Position	Sequence	Modifications (variable)	PEP 2D	PEP 1D	|Log Prob|	Score	Delta Score	Delta Score	Modification	z	Observation m/z	ppm err.	Observation MH	Calculation MH
	DNPH derivitization before trypsin treatment														
a	Thr 101	K.VDATEESDLAQQYGVRGYPT[+178.01000]IKFFR.N	T20(Thr-DNPH / 178.01)	0.0075	0.0097	2.12	351.4	351.4	26.1		3	1023.4897	2.52	3068.4547	3068.447
b'	Lys 276	K.SVSDYDGKLSNFK[+179.02000]TAAESFK.G	K13(Lys-DNPH / 179.02)	0.0051	0.0065	2.29	339	339	27.9		3	792.0274	-1.09	2374.0676	2374.0702
b'	Lys 326	R.LITLEEEMTK[+179.02000]YKPESEELTAER.I	K10(Lys-DNPH / 179.02)	0.0036	0.0046	2.44	329.6	329.6	153.3		3	945.4476	0.51	2834.3283	2834.3269
	2nd time DNPH derivitization after trypsin treatment														
a	Lys 31**	R.K[+181.037]SNFAEALAAHK.Y	K1(Lys-DNPHred / 181.037)*	1.30E-06	2.20E-06	5.87	477.7	477.7	477.7		3	490.244	7.97	1468.7175	1468.7058
a	Pro 61	K.ALAP[+196.023]EYAK.A	P4(Lys-DNPHred / 196.023)*	1.80E-05	2.80E-05	4.75	355.6	326.5	326.5		2	529.749	0.04	1058.490	1058.490
b'	Pro 246	K.HNQLPLVIEFTEQTAP[+196.126]K.I	P16(Pro-DNPHred /196.12)*	1.50E-08	2.40E-08	7.82	444.3	444.3	376		3	781.4	0.29	2342.207	2342.206
b'	Lys 326	R.LITLEEEMTK[+179.021]YKPESEELTAER.I	K10(Lys-DNPH / 179.02)	8.10E-07	1.30E-06	6.09	383.2	383.2	187.8		3	945.4476	0.09	2834.333	2834.328

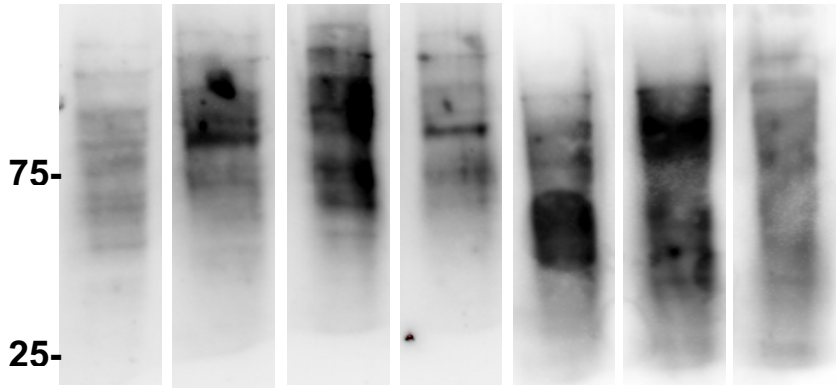
*DNPHred: DNPH + stabilizer
** K31was also identified in control rhP4Hb protein.

A



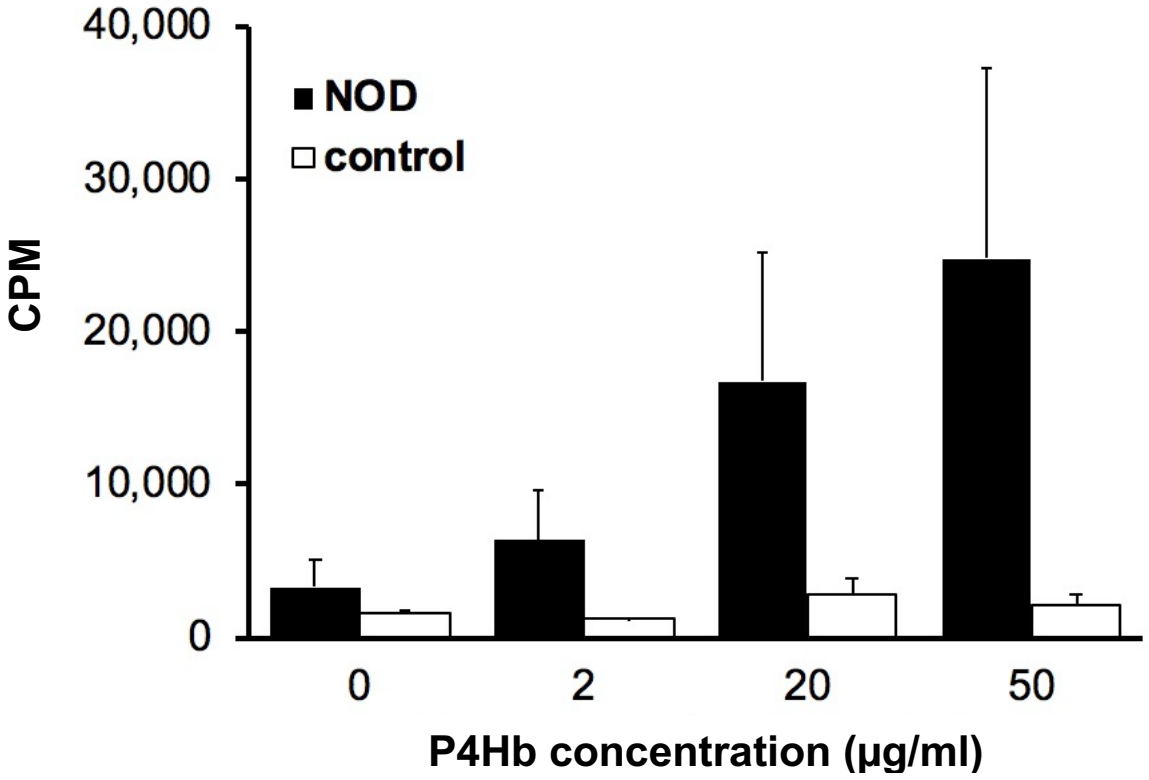
B

H_2O_2	-	+	+	+	-	-	-
cytokines	-	-	-	-	+	+	+
L-NMMA	-	-	+	-	-	+	-
apocynin	-	-	-	+	-	-	+

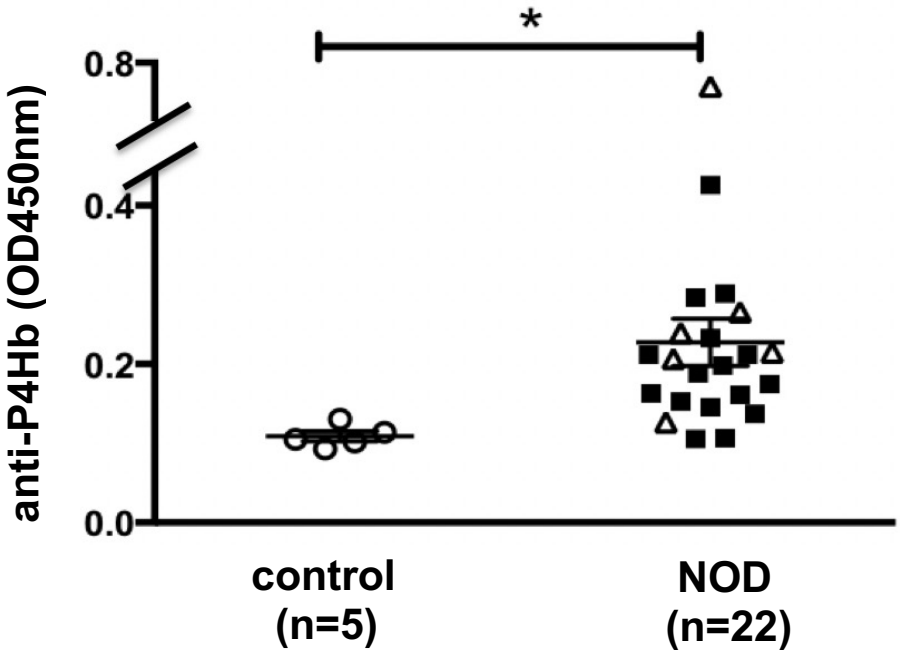


Supplementary Figure 1: The carbonylation triggered by H_2O_2 and cytokines in beta cells is mediated via reactive oxygen species (ROS) not nitrite. *A*: INS-1 cells were treated with cytokines (IFNγ+IL-1β) in the presence of L-NMMA (2mM) or apocynin (50μM). After one day or two days treatment, supernatant was collected and measured the nitrite level by using Griess reagent. *B*: The carbonylation level was measured by OxyBlot in untreated, 300 μM H_2O_2 (one hour) and cytokines treated (one day) INS-1 cells. Ten μg cell lysate protein were loading per lane. Molecular mass, in kDa, is indicated on the left.

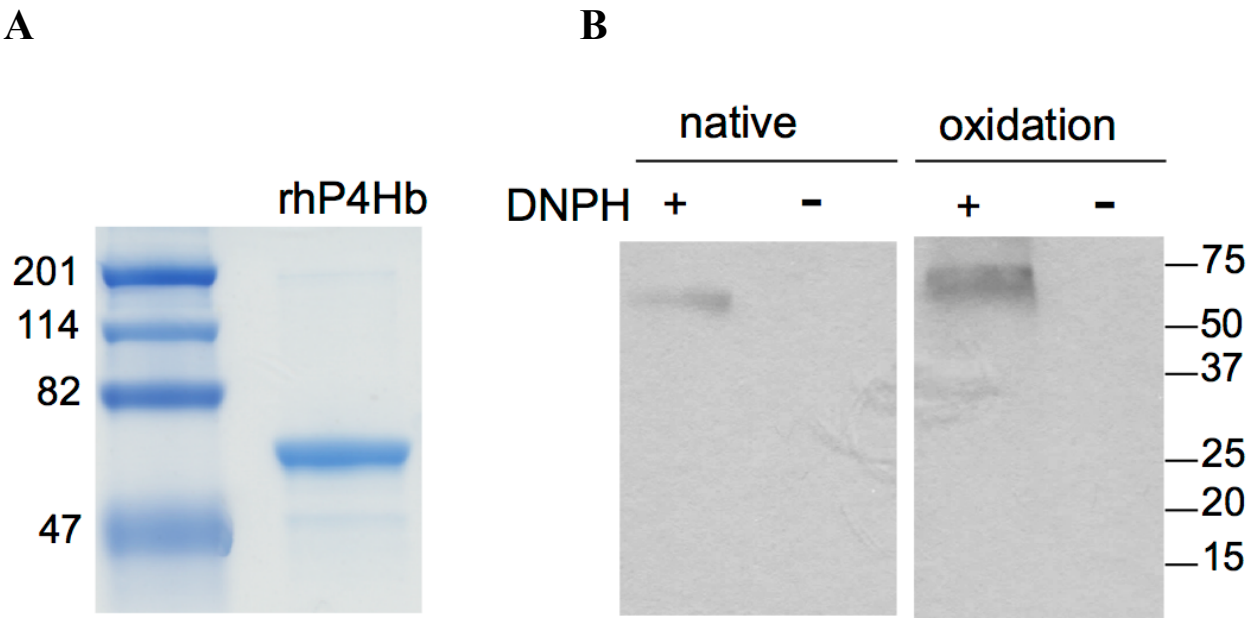
A


40,000
30,000
20,000
10,000
0
CPM
NOD
control
0
2
20
50
P4Hb concentration (µg/ml)

B


*
0.8
0.4
0.2
0.0
anti-P4Hb (OD450nm)
control
(n=5)
NOD
(n=22)

Supplementary Figure 2: The circulating autoreactive lymphocytes and IgG autoantibodies against P4Hb protein in NOD mice. *A*: Fresh pooled axillary, inguinal, brachial, popliteal and pancreatic lymph node cells (5×10^5) from 4- to 5-wk-old prediabetic NOD mice or control mice (C57Bl/6 strain) were plated with pre-coated anti-CD3 (10µg/ml) mAb as positive control or incubated with varying concentrations of recombinant human P4Hb (rhP4Hb) for 48 h. Then proliferation was measured by [3H]thymidine incorporation. *B*: The serum levels of anti-rhP4Hb in NOD and control mice (BALB/c strain) were measured by ELISA. The number of mice analyzed is indicated at the bottom of each group. *Student t test, $p<0.001$. The filled squares show data where the blood glucose content was less than 250 mg/dL; the open triangles data where the blood glucose was greater than 250 mg/dL. Error bars indicate SEM of mean.



Supplementary Figure 3: The characterization of purified recombinant human P4Hb from hPDI-pTrcHisA clone. (A) Briefly, the hPDI-pTrcHisA plasmid was transformed into BL21(DE3) cells for expression with 0.5 mM IPTG induction. P4Hb was purified from cell lystates using Pro Bond Ni-NTA resin and imidazole elution. Proteins were concentrated with Centriprep YM-30 and judged to be ~95% pure by SDS-PAGE stained with Coomassie Brilliant Blue. Polypeptide molecular weight marker proteins in kDa are shown in the left lane. (B) The purified rhP4Hb was incubated in PBS (native) or PBS containing 100 μM $FeSO_4$, 25mM H_2O_2 and 25mM ascorbate (oxidation) at 37°C for 4 h in the presence or absence of dinitropheylhydrazine. Then the carbonyl modification was analyzed by OxyBlot. The positions of polypeptide molecular weight markers in kDa are on the right edge of the gel.