

Online Data Supplement

PROTEASOME LEVELS AND ACTIVITY IN PREGNANCIES COMPLICATED BY SEVERE PREECLAMPSIA AND HELLP SYNDROME

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Table S1. Demographic clinical and outcome characteristics of pregnant women who participated with plasma samples (n=150)

Variable	P-CRL n = 14	sPTB n=21	crHTN n = 25	gHTN n = 25	mPE n=10	sPE n=39	HELLP n=16	P value
Maternal characteristics at enrollment								
Age, years *	29 [25-30]	25 [22-29]	31 [28-36]	30 [25-33]	30 [23-32]	30 [24-34]	29 [25-32]	0.074
Gravidity *	2 [1-3]	2 [1-4]	3 [2-5]	2 [1-2]	2 [1-3]	2 [1-3]	2 [1-3]	0.044
Parity *	1 [0-1]	1 [0-2]	2 [0-4]	0 [0-1]	0 [0-2]	0 [0-2]	0 [0-1]	0.010
GA, weeks *	29 [25-30]	29 [27-32]	37 [36-38]	38 [37-39]	36 [33-37]	31 [28-34]	28 [25-31]	<0.001
Race/ethnicity †								
White	10 (71)	0 (0)	12 (48)	20 (80)	7 (70)	20 (51)	11 (69)	<0.001
African - American	4 (29)	5 (24)	10 (40)	4 (16)	3 (30)	13 (33)	3 (19)	
Hispanic	0 (0)	16 (76)	1 (4)	0 (0)	0 (0)	3 (8)	2 (12)	
Peak systolic BP *	115 [110-125]	130 [124-143]	171 [150-177]	155 [147-165]	144 [141-156]	165 [154-183]	163 [151-182]	<0.001
Peak diastolic BP *	64 [60-70]	80 [71-89]	96 [88-106]	95 [91-101]	85 [82-92]	100 [92-108]	100 [92-107]	<0.001
Proteinuria, mg *	na	na	192 [125-264]	286 [183-293]	441 [353-1035]	5,600 [1683-8966]	1275 [247-4685]	<0.001
Maternal and Fetal characteristics at delivery								
GA, at delivery	39 [38-39]	31 [28-32]	38 [37-39]	38 [37-39]	37 [37-38]	31 [28-35]	28 [24-31]	<0.001
Birth weight, grams *	3335 [3102-3500]	1445 [1310-1944]	3238 [2570-3505]	3230 [2852-3624]	3184 [2755-3817]	1405 [960-2140]	883 [545-1735]	<0.001
Cesarean Delivery †	3 (21)	8 (38)	8 (32)	6 (24)	5 (50)	30 (77)	13 (81)	<0.001
Newborn Sex †								
male	5 (36)	15 (71)	13 (52)	9 (36)	7 (70)	18 (46)	6 (38)	0.647

* Data presented as median [interquartile range] and analyzed by Kruskal-Wallis ANOVA on Ranks.

† Data presented as n (%) and analyzed by χ^2 tests.

Significant P values are shown in bold.

P-CRL indicates pregnant control; sPTB, idiopathic spontaneous preterm birth; crHTN, chronic hypertension; gHTN, gestational hypertension; mPE, preeclampsia without severe clinical features; sPE, preeclampsia with severe clinical features; HELLP, hemolysis, elevated liver enzymes, low platelet count; GA, gestational age; BP, blood pressure

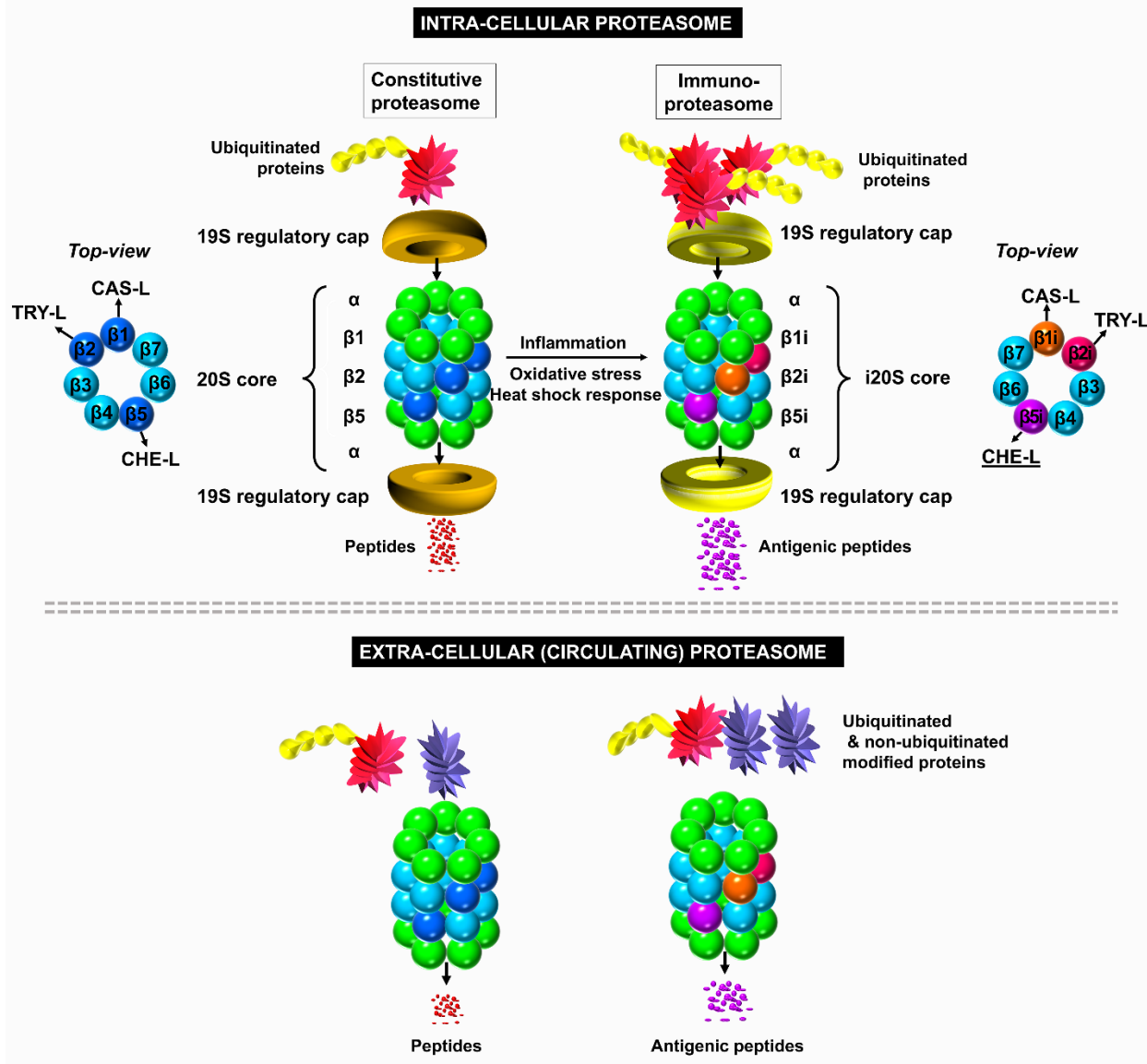


Figure S1. Model of circulating and extra-cellular constitutive proteasomes and immuno-proteasomes.

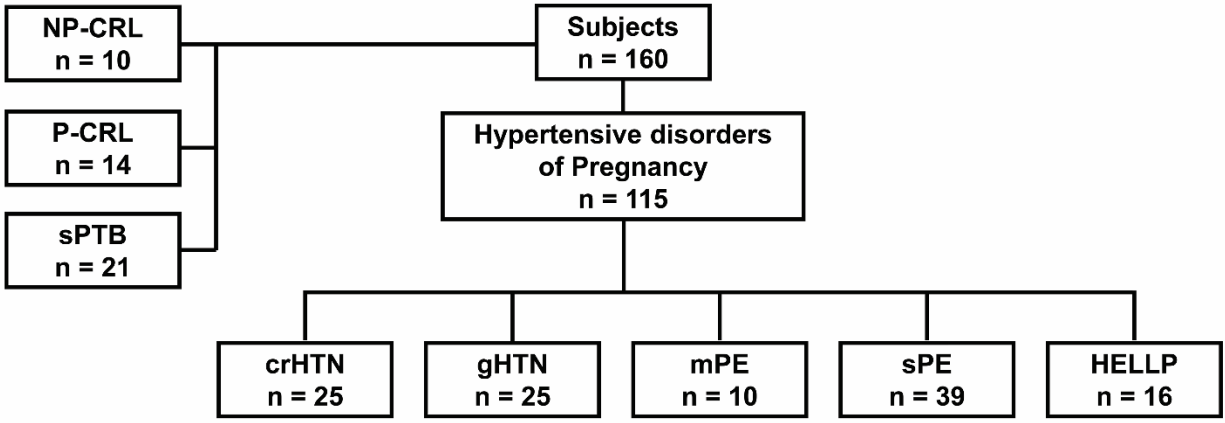


Figure S2. Clinical classification of study participants. NP-CRL, non-pregnant control; P-CRL, healthy pregnant control; sPTB, idiopathic spontaneous preterm birth; crHTN, chronic hypertension; gHTN, gestational hypertension, mPE, preeclampsia without severe clinical features; sPE, preeclampsia with severe clinical features; HELLP, hemolysis, elevated liver enzymes, and thrombocytopenia syndrome.

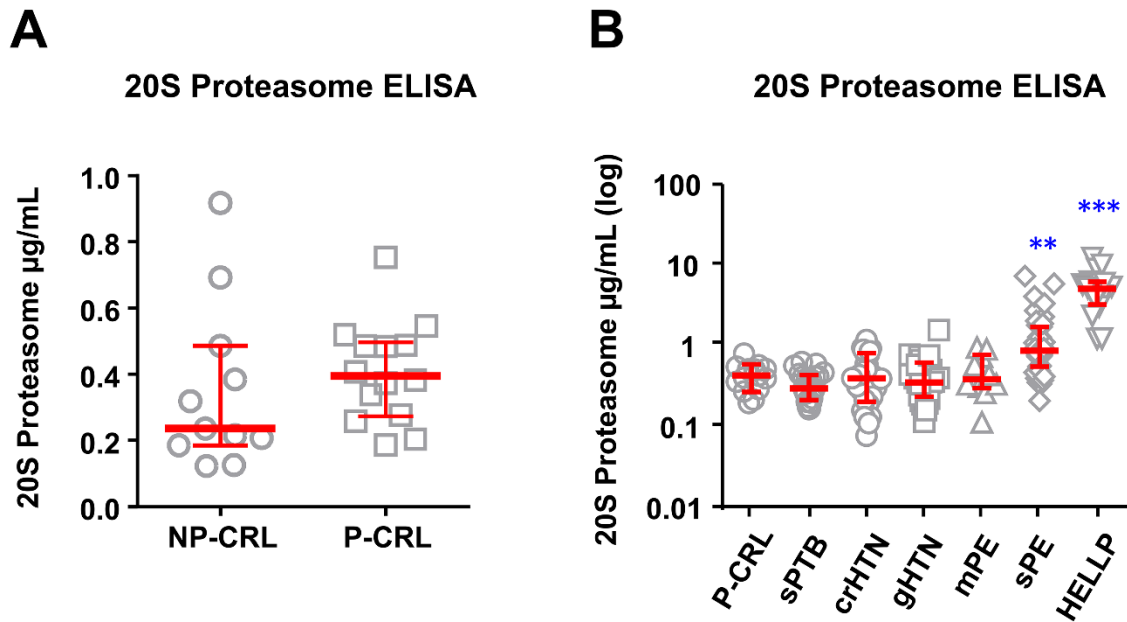


Figure S3. Maternal plasma levels of circulating proteasome in pregnant controls compared with non-pregnant controls, spontaneous preterm birth, and hypertensive conditions of pregnancy. A, Circulating proteasome immunoreactivity in healthy pregnant controls during second and third trimester (P-CRL, $n=14$) compared to non-pregnant controls (NP-CRL, $n=10$). **B,** Circulating proteasome immunoreactivity in P-CRL compared with women with idiopathic spontaneous preterm birth (sPTB, $n=21$, chronic hypertension (crHTN, $n=25$), gestational hypertension (gHTN, $n=25$), preeclampsia without severe clinical feature (mPE, $n=10$), preeclampsia with severe clinical features (sPE, $n=39$), and sPE and clinical features of HELLP syndrome (HELLP, $n=16$) (**B**). Data are presented as median (horizontal line) and interquartile range (vertical bars). Kruskal-Wallis analysis of variance followed by multiple comparisons with Dunn's tests. ** $P<0.01$, *** $P<0.001$ vs P-CRL group.

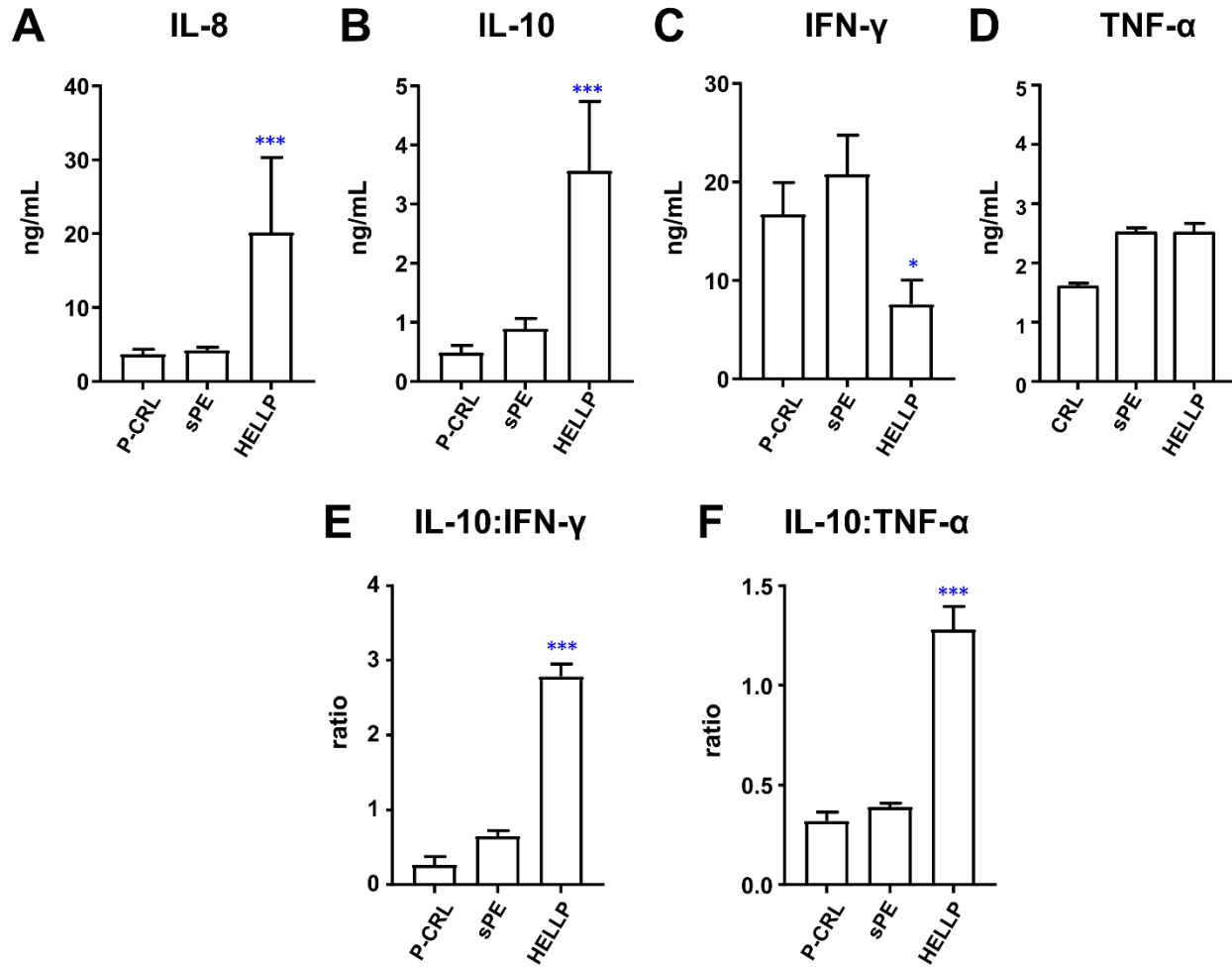


Figure S4. Maternal plasma circulating cytokine profile. Levels of (A) interleukin (IL)-8, (B) IL-10, (C) interferon (IFN)- γ , and (D) tumor necrosis factor (TNF)- α in pregnant controls (P-CRL, n=14), women with preeclampsia with severe clinical features (sPE, n=39), and women with HELLP syndrome (n=16). Ratio of (E) IL-10:IFN- γ , and (F) IL-10:TNF- α in the same groups. Data are presented as mean + standard error mean (vertical bars). 1-way analysis of variance on log-transformed data followed by multiple comparisons with Holm-Sidak tests. * $P < 0.05$, *** $P < 0.001$ vs P-CRL.

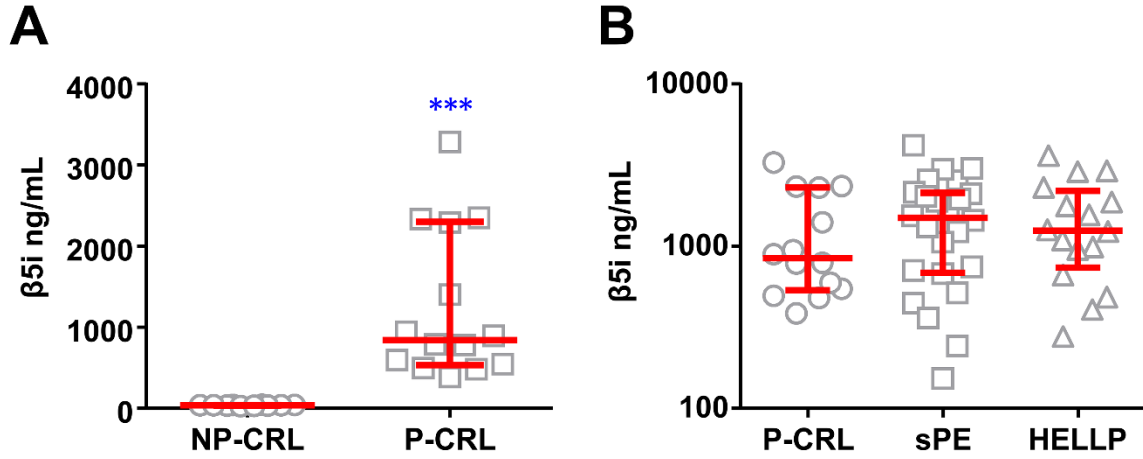


Figure S5. Levels of circulating immunoproteasome measured through $\beta 5i$ subunit. A, Plasma $\beta 5i$ concentration in non-pregnant controls (NP-CRL, n=10) versus pregnant controls (P-CRL, n=14). Mann-Whitney Rank Sum Test * $P < 0.05$ vs NP-CRL. **B,** Circulating proteasome immunoreactivity in pregnant controls (P-CRL, n=14) compared with women with preeclampsia with severe clinical features (sPE, n=39) and women with HELLP syndrome (n=16). Data are presented as median (horizontal line) and interquartile range (vertical bars). No significant differences were noted among groups.

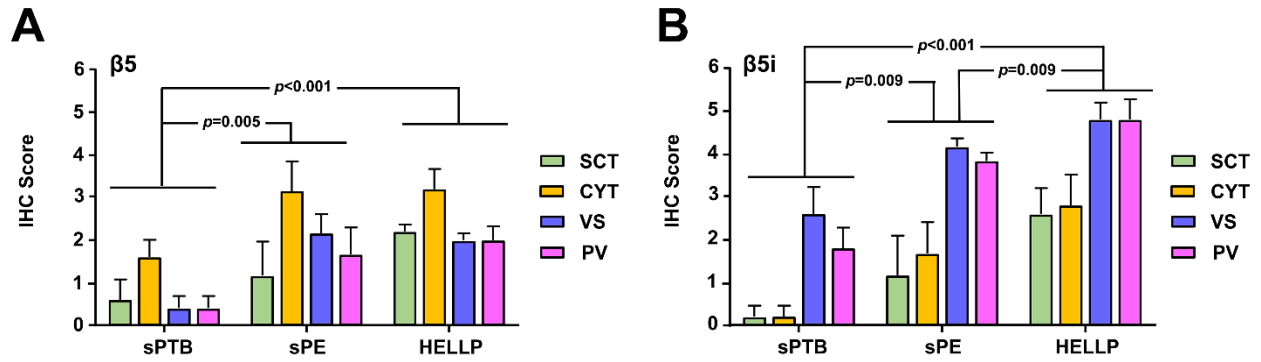


Figure S6. Quantification of $\beta 5$ and $\beta 5i$ immunostaining in placenta. Semi-quantitative scoring of (A) $\beta 5$ and (B) $\beta 5i$ staining intensity in syncytiotrophoblast (SCT), villous cytotrophoblast (CYT), villous stroma (VS), and placental vasculature (PV) in women with spontaneous preterm birth (sPTB, 32 weeks, n=4), preeclampsia with severe features (sPE, 32 weeks, n=6), and HELLP syndrome (30 weeks, n=5). Staining intensity was scored from 3 random fields per slide in a blinded fashion on a scale from 0 (absent) to 5 (intense).