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Supplementary Materials for

Therapeutic Inflammatory Monocyte Modulation Using Immune-Modifying Microparticles

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The PDF file includes:

SUPPLEMENTARY METHODS

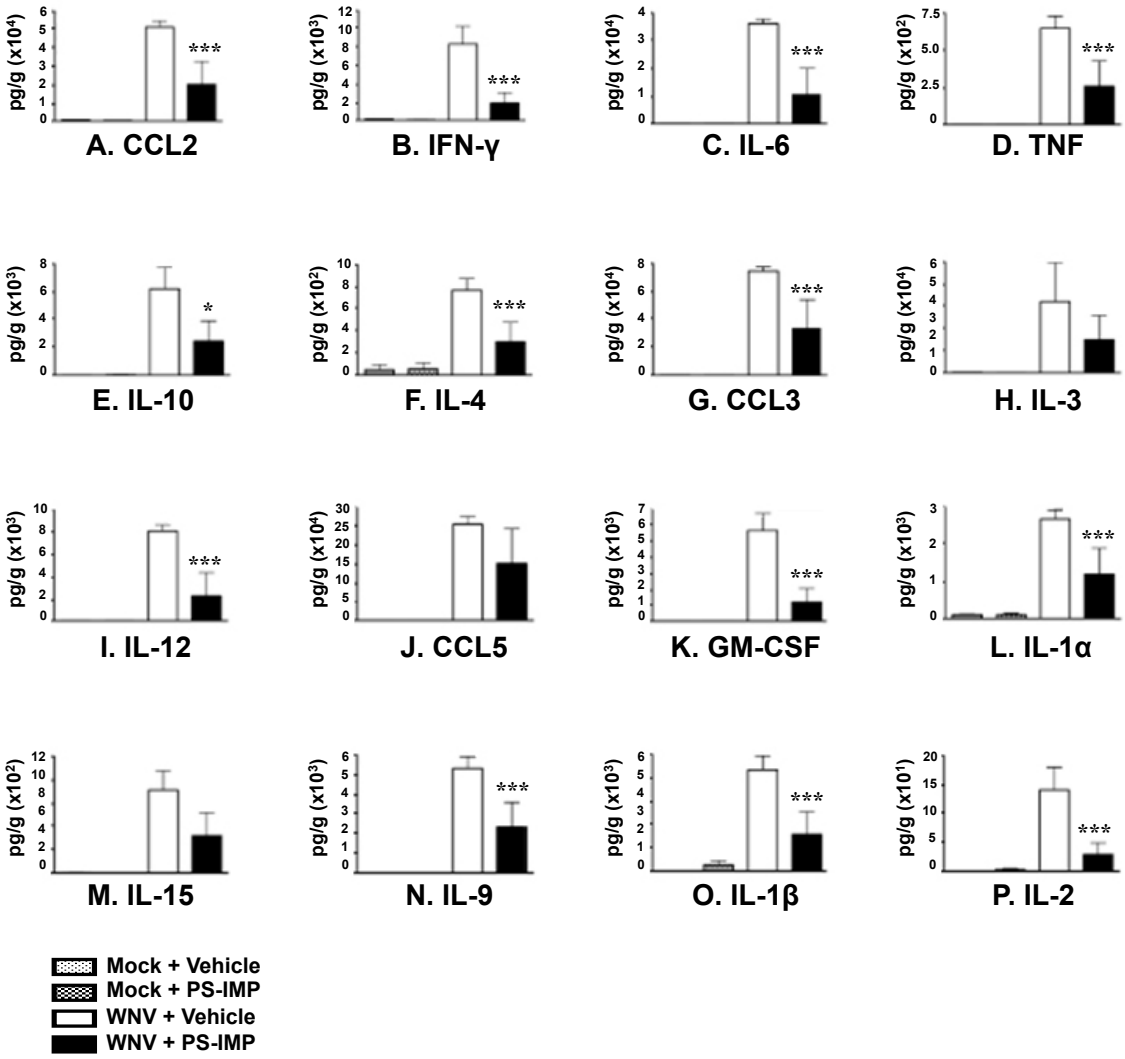
Multiplex ELISA

Multiplex plate ELISA (Quansys Biosciences) was performed on brain homogenates using the Quansys Q-plex Mouse Cytokine Screen IR 16-plex according to the manufacturer's instructions. Plates were visualized on the Li-Cor Odyssey IR Imaging System (Li-Cor Biotechnology). Images were analyzed using Quansys Q-view software (Quansys Biosciences).

Protein Identification Liquid Chromatography

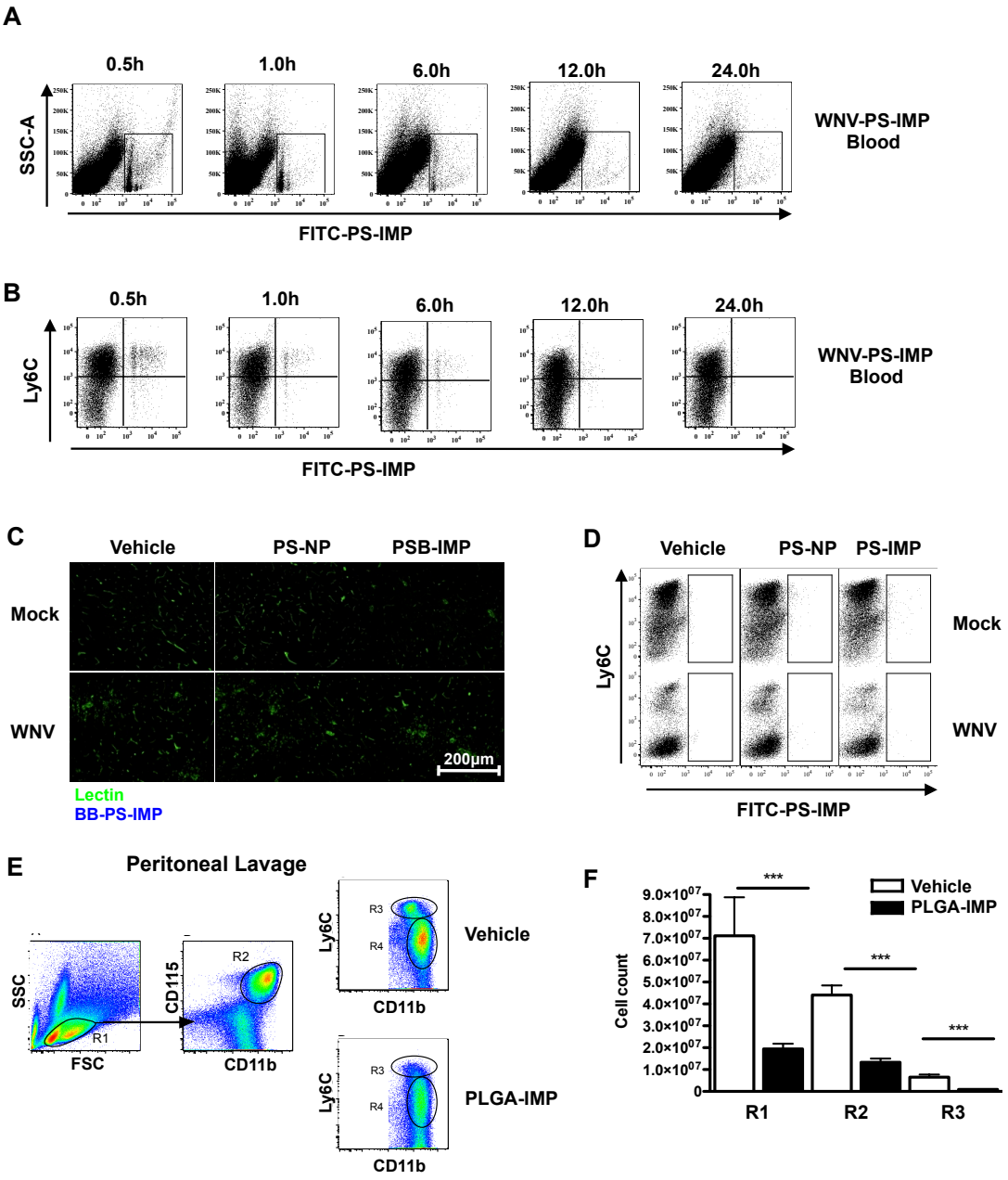
As previously described (*59*), proteins were subject to GeLC-MS analysis. Briefly, proteins were reduced and alkylated in the presence of dithiothreitol and iodoacetamide. Bands were dried by vacuum centrifugation and incubated with 240ng trypsin for 1 hour at 4^{o}C. Excess trypsin was removed and replaced with 50mM ammonium bicarbonate for incubation at 37^{o}C overnight. Prior to mass spectrometric analysis peptides were concentrated and desalted with the use of pre-fabricated micro-columns containing Poros R2 resin (Perseptive Biosystems, Framingham MA). Peptides eluted were resolublized in 0.1% formic acid and subject to reverse phase LC-MS/MS. Approximately 41,000 MS/MS spectra were generated from replicate LC-MS/MS experiments. Tandem mass spectra were extracted, charge state deconvoluted and deisotoped in Mascot and analyzed using Mascot and X!Tandem. Data were searched against Mus musculus (mouse) entries included in the SWISS-PROT databases with the significance threshold set at $p < 0.05$. Searches were conducted with a fragment ion mass tolerance of 15ppm and a parent ion tolerance of 0.2 Da. Methionine sulfoxide, carbamidomethyl-cysteine and deamidated asparagine and glutamine were specified as variable modifications. Scaffold was used integrate multiple MS/MS experiments and validate MS/MS based peptides and protein identifications. Peptides were considered only if Mascot ion scores exceeded 30, 40 and 40 for doubly, triply and quadruply charged peptides respectively. X!Tandem identifications required –Log (Expect scores) scores greater than 2.0. Protein identifications were accepted if a

minimum of 2 peptides were identified matching the above criteria. Five proteins were considered identified although only matching one peptide. In this case, the peptides were sequenced on multiple occasions and sequence coverage obtained by the single peptide was greater than 5%.



Supplementary Figure 1. PS-IMP reduce cytokine and chemokine production in the WNV-infected brain. Mock and WNV-infected brains were collected on D7 post infection after PS-IMP or vehicle infusion on day 6 post infection and processed for multiplex ELISA as described in the Materials and Methods. Levels of CCL2 **(A)**, IFN-γ **(B)**, IL-6 **(C)**, TNF **(D)**, IL-10 **(E)**, IL-4 **(F)**, CCL3 (**G**), IL-12 **(H)**, IL-3 **(I)**, CCL5 **(K)**, GM-CSF **(L)**, IL-1α **(M)**, IL-15 **(N)**, IL-9 **(O)**, IL-1β **(P)** and IL-2 **Q)** were examined. Multiplex ELISA is representative of at least 2 experiments with at least 3 mice per group.

Supplementary Figure 2



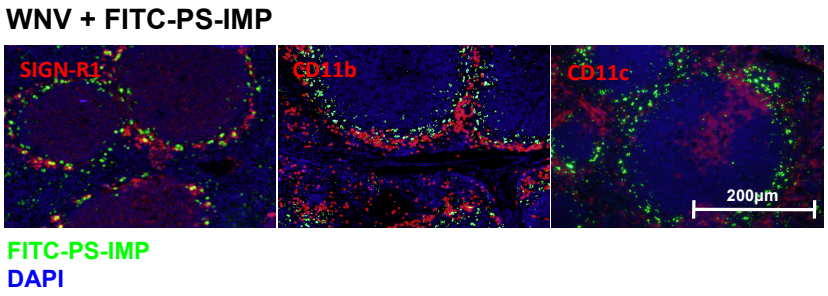
Supplementary Figure 2

Supplementary Figure 2. IMP clearance kinetics, lack of IMP localization to the brain and PLGA-IMP treatment in TG peritonitis. WNV infected mice were injected with FITC-PS-IMP on day 6 post infection and blood was isolated to determine clearance of IMP (**A)** and uptake **(B)** by Ly6C monocytes examined at the indicated time points. Mock and WNV

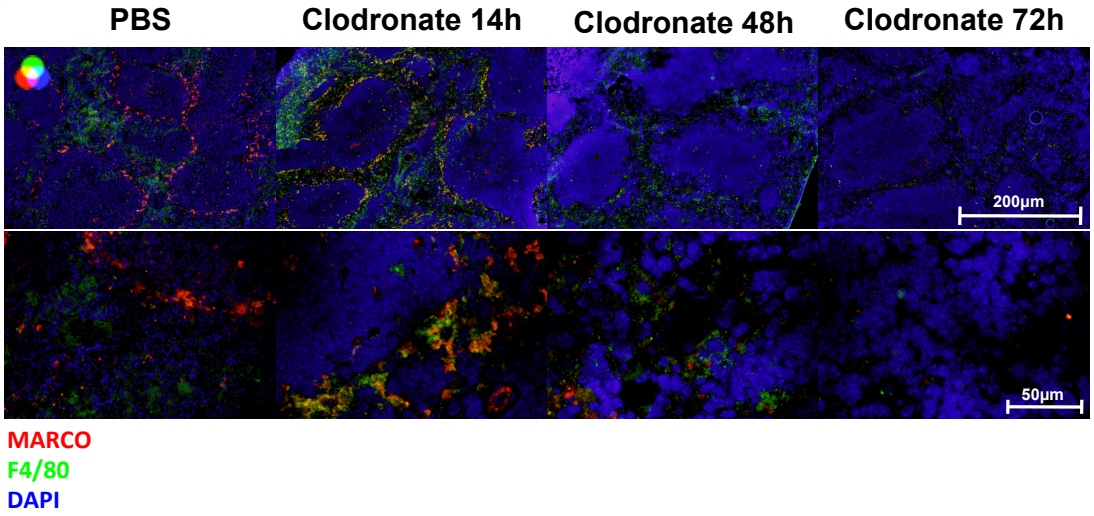
infected mice were injected with Bright-blue NP (blue), Bright-blue PS-IMP (blue) or vehicle on day 6 post infection and brains processed for lectin immunohistology on D7 post infection (**C**). Mock and WNV-infected mice were injected with FITC-PS-NP, FITCPS-IMP or vehicle on day 6 post infection and brains were processed for flow cytometry on D7 post infection, with CD45+ CD11b+ Ly6C+ ΦIM-derived macrophages gated and examined for FITC-IMP (**D**).

PLGA-IMP efficacy was examined 24 hours after TG injection, WT mice were injected with PS-IMP or vehicle control i.v. 24 hours later, flow cytometry was used to determine the numbers of CD115+CD11b+Ly6ChiLy6G- ΦIM-derived macrophages (R3, **E**) in the peritoneal cavity(**E,F**). Immunohistology and flow cytometry data represent three separate experiments, with at least 3 mice/group. Slides were counterstained with lectin (green) to identify microglia, endothelium and myeloid lineage cells. Flow cytometry data are means ± SD and represent three separate experiments with 4-5 mice/group. Statistical analysis was conducted using one-way ANOVA and Tukey-Kramer post-test. $P \leq 0.05$ (*), $P \leq 0.01$ (**), $P \leq 0.001$ (***), in comparing PS-IMP and NP groups to vehicle control groups. $P \leq 0.05$ ($^{\#}$), $P \leq 0.01$ ($^{\#\#}$), $P \leq 0.001$ ($^{\#\#\#}$), in comparing PS-IMP to NP groups.

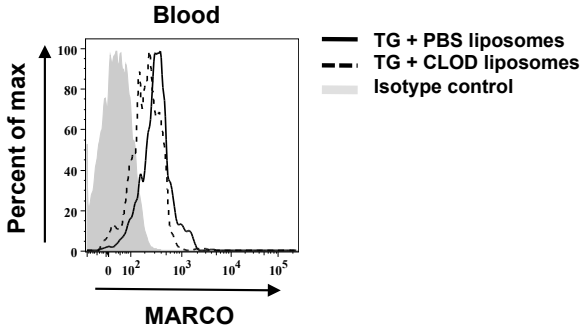
A



B



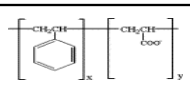
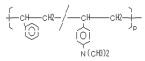
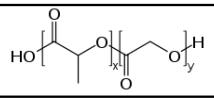
C



Supplementary Figure 3

Supplementary Figure 3. IMP localization and Marginal Zone Macrophage Depletion. WNV-infected mice were injected with FITC-PS-IMP (green, **a**) and spleens were processed for fluorescent SIGN-R1, CD11b, or CD11c immunohistology 24 hours later (day 7 post infection) (red, **A**). Slides were counterstained with DAPI (blue) to identify cell nuclei. Mice were injected with PBS or clodronate liposomes and spleens were processed for fluorescent MARCO and F4/80 immunohistochemistry 14, 48 and 72h later (**B**). Mice were injected with PBS or clodronate liposomes and injected with TG 24 hours later. MARCO expression on blood CD11b+ Ly6Chi Ly6G- cells was examined by flow cytometry 24 hours after TG injection (**C**). Flow cytometry data represent three separate experiments with 4-5 mice/group. Immunohistology are representative of at least 3 experiments with at least 3 mice per group.

SUPPLEMENTARY TABLE 1. Particle Physical Properties

Physical Properties					
	Immune Modified (Carboxylated) Polystyrene Particles (Phosphorex)*	Polystyrene Neutral Particles (Phosphorex)*	Polystyrene Positive Charged (Aminated Particles-Phosphorex)*	Immune Modified Nano-Diamonds (University of Sydney)	Immune Modified (Carboxylated) PLGA Particles (Phosphorex)*
Chemical Formula				C	
Color	white latex	white latex	white latex	Milky white (solution)	White powder (lyophilized)
Odor	None	None	None	Odourless	None
Melting point TM/°C	240°C	240°C	240°C	4500 K	N/A (amorphous)
Glass Transition/ °C	100°C	100°C	100°C	N/A	40-55°C
Solubility	Insoluble in water or alcohols, soluble in acetone, ethyl acetate, THF, DMF, toluene, methylene chloride, etc.	Insoluble in water or alcohols, soluble in acetone, ethyl acetate, THF, DMF, toluene, methylene chloride, etc.	Insoluble in water or alcohols, soluble in acetone, ethyl acetate, THF, DMF, toluene, methylene chloride, etc.	N/A	Insoluble in water, soluble in acetone, ethyl acetate, DMSO, DMF, acetonitrile, chloroform, methylene chloride, etc.
Co-Polymer ratio, mol(%)	90:10	100:0	90:10	N/A	50:50
Melt Index g/10 Minutes, 200°C/5 kg	6.0-9.0	6.0-9.0	6.0-9.0	N/A	N/A
Molecular weight (kDa)	10-250	10-250	10-250	22,000,000KD	5-50
Heavy metals (ppm)	unknown	unknown	unknown	0-0.55% Nickel 0-0.50 Copper prior to carboxylation by acid washing	<10
Sulphate Ash (%)	unknown	unknown	unknown	N/A	<0.1
Size distribution	10.4%	2.4%	10.4%	270 ± 30 nm	10-50%
Zeta Potential	-40 ~ -50 mv	-5 ~ +5 mv	+20 ~ +40 mv	-50.6 +/- 3 mV	-30~ -50 mV

*Particles designed to investigator specification

SUPPLEMENTARY TABLE 2. Opsonized Proteins Found on IMP

Identified Proteins	SWISSProt Accession Number	Gene Name	p*I*	Molecular Weight	Unique Peptides Identified	% Sequence Coverage
14-3-3 protein zeta/delta	1433Z_MOUSE	Ywhaz	4.73	28 kDa	3	12%
Protein 4.1	41_MOUSE	Epb41	5.43	96 kDa	4	6.10%
Alpha-1-antitrypsin 1-1	A1AT1_MOUSE	Serpina1a	5.44	46 kDa	3	4.60%
Actin, cytoplasmic 1	ACTB_MOUSE	Actb	5.29	42 kDa	11	39%
Alpha-actinin-1	ACTN1_MOUSE	Actn1	5.23	103 kDa	2	2.50%
Alpha-adducin	ADDA_MOUSE	Add1	5.62	81 kDa	2	3.10%
ADP/ATP translocase 1	ADT1_MOUSE	Slc25a4	9.73	33 kDa	2	8.10%
Serum albumin	ALBU_MOUSE	Alb	5.53	69 kDa	14	29%
Ankyrin-1	ANK1_MOUSE	Ank1	6.09	204 kDa	5	3.30%
Annexin A2	ANXA2_MOUSE	Anxa2	7.53	39 kDa	6	20%
Annexin A5	ANXA5_MOUSE	Anxa5	4.82	36 kDa	3	9.40%
Acylamino-acid-releasing enzyme	APEH_MOUSE	Apeh	5.36	82 kDa	4	6.10%
Apolipoprotein A-I	APOA1_MOUSE	Apoa1	5.31	31 kDa	6	21%
Apolipoprotein A-IV	APOA4_MOUSE	Apoa4	5.21	45 kDa	2	7.10%
Apolipoprotein C-IV	APOC4_MOUSE	Apoc4	9.23	14 kDa	2	16%
Apolipoprotein E	APOE_MOUSE	Apoe	5.46	36 kDa	12	38%
Beta-2-glycoprotein 1	APOH_MOUSE	Apoh	8.62	39 kDa	5	19%
Sodium/potassium-transporting ATPase subunit alpha-1	AT1A1_MOUSE	Atp1a1	5.27	113 kDa	5	5.80%
Sarcoplasmic/endoplasmic reticulum calcium ATPase 1	AT2A1_MOUSE	Atp2a1	5.13	109 kDa	8	10%
ATP synthase subunit alpha, mitochondrial	ATPA_MOUSE	Atp5a1	8.28	60 kDa	6	13%
ATP synthase subunit beta, mitochondrial	ATPB_MOUSE	Atp5b	4.99	56 kDa	7	19%
Band 3 anion transport protein	B3AT_MOUSE	Slc4a1	5.31	103 kDa	11	16%
Flavin reductase (NADPH)	BLVRB_MOUSE	Blvrb	6.47	22 kDa	6	46%
Complement C1q subcomponent subunit A	C1QA_MOUSE	C1qa	9.48	26 kDa	4	21%
Complement C1q subcomponent subunit B	C1QB_MOUSE	C1qb	8.34	27 kDa	6	27%
Complement C1q subcomponent subunit C	C1QC_MOUSE	C1qc	8.88	26 kDa	4	16%
C-1-tetrahydrofolate synthase, cytoplasmic	C1TC_MOUSE	Mthfd1	6.73	101 kDa	4	5.50%
C4b-binding protein	C4BPA_MOUSE	C4bpa	5.97	52 kDa	4	12%
Carbonic anhydrase 1	CAH1_MOUSE	Ca1	6.47	28 kDa	4	20%
Carbonic anhydrase 2	CAH2_MOUSE	Ca2	6.48	29 kDa	6	37%
Catalase	CATA_MOUSE	Cat	7.72	60 kDa	7	17%
Clathrin heavy chain 1	CLH1_MOUSE	Cltc	5.48	192 kDa	8	6.20%
Complement C3	CO3_MOUSE	C3	6.3	186 kDa	30	22%
Cofilin-1	COF1_MOUSE	Cfl1	8.26	19 kDa	1	6.60%
Uroporphyrinogen decarboxylase	DCUP_MOUSE	Urod	6.21	41 kDa	3	9.00%
Desmoplakin	DESP_MOUSE	Dsp	6.43	333 kDa	2	0.80%
Elongation factor 1-alpha 1	EF1A1_MOUSE	Eef1a1	9.1	50 kDa	6	16%
Elongation factor 1-delta	EF1D_MOUSE	Eef1d	4.91	31 kDa	2	8.50%
Elongation factor 2	EF2_MOUSE	Eef2	6.42	95 kDa	4	4.50%
55 kDa erythrocyte membrane protein	EM55_MOUSE	Mpp1	6.72	52 kDa	3	7.50%
Alpha-enolase	ENOA_MOUSE	Eno1	6.36	47 kDa	2	9.70%
Endoplasmin	ENPL_MOUSE	Hsp90b1	4.72	92 kDa	3	5.50%
Erythrocyte membrane protein band 4.2	EPB42_MOUSE	Epb42	6.98	77 kDa	2	3.30%
Ezrin	EZRI_MOUSE	Ezr	5.83	69 kDa	6	10%
Coagulation factor V	FA5_MOUSE	F5	5.66	247 kDa	4	2.60%
Alpha-2-HS-glycoprotein	FETUA_MOUSE	Ahsg	5.94	37 kDa	2	9.90%
Fibrinogen beta chain	FIBB_MOUSE	Fgb	3.53	55 kDa	13	35%
Fibrinogen gamma chain	FIBG_MOUSE	Fgg	5.55	49 kDa	13	34%

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Tubulin beta-4B chain	TBB4B_MOUSE	Tubb4b	4.79	50 kDa	11	30%
Tubulin beta-5 chain	TBB5_MOUSE	Tubb5	4.78	50 kDa	2	24%
Transitional endoplasmic reticulum ATPase	TERA_MOUSE	Vcp	5.14	89 kDa	4	6.20%
Transferrin receptor protein 1	TFR1_MOUSE	Tfrc	6.13	86 kDa	11	18%
Talin-1	TLN1_MOUSE	Tln1	5.84	270 kDa	16	8.20%
Serotransferrin	TRFE_MOUSE	Tf	6.81	77 kDa	3	4.60%
Thrombospondin-1	TSP1_MOUSE	Thbs1	4.71	130 kDa	3	2.60%
Ubiquitin-like modifier-activating enzyme 1	UBA1_MOUSE	Uba1	5.43	118 kDa	2	3.10%
Ubiquitin-conjugating enzyme E2 O	UBE2O_MOUSE	Ube2o	4.94	141 kDa	3	3.50%
Fermitin family homolog 3	URP2_MOUSE	Fermt3	6.6	76 kDa	4	8.10%
Vinculin	VINC_MOUSE	Vcl	5.77	117 kDa	10	11%
Vitronectin	VTNC_MOUSE	Vtn	5.56	55 kDa	7	17%