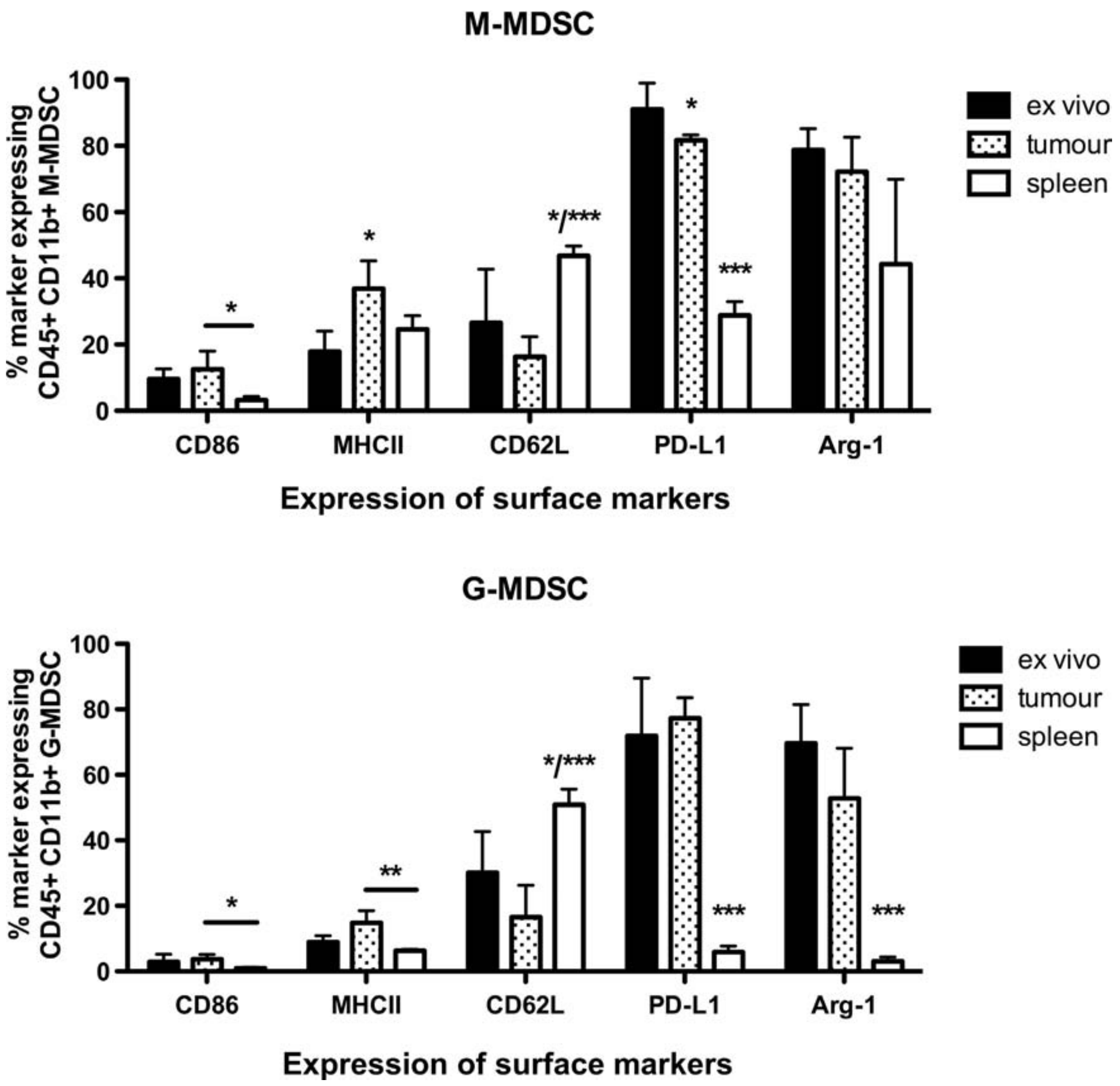
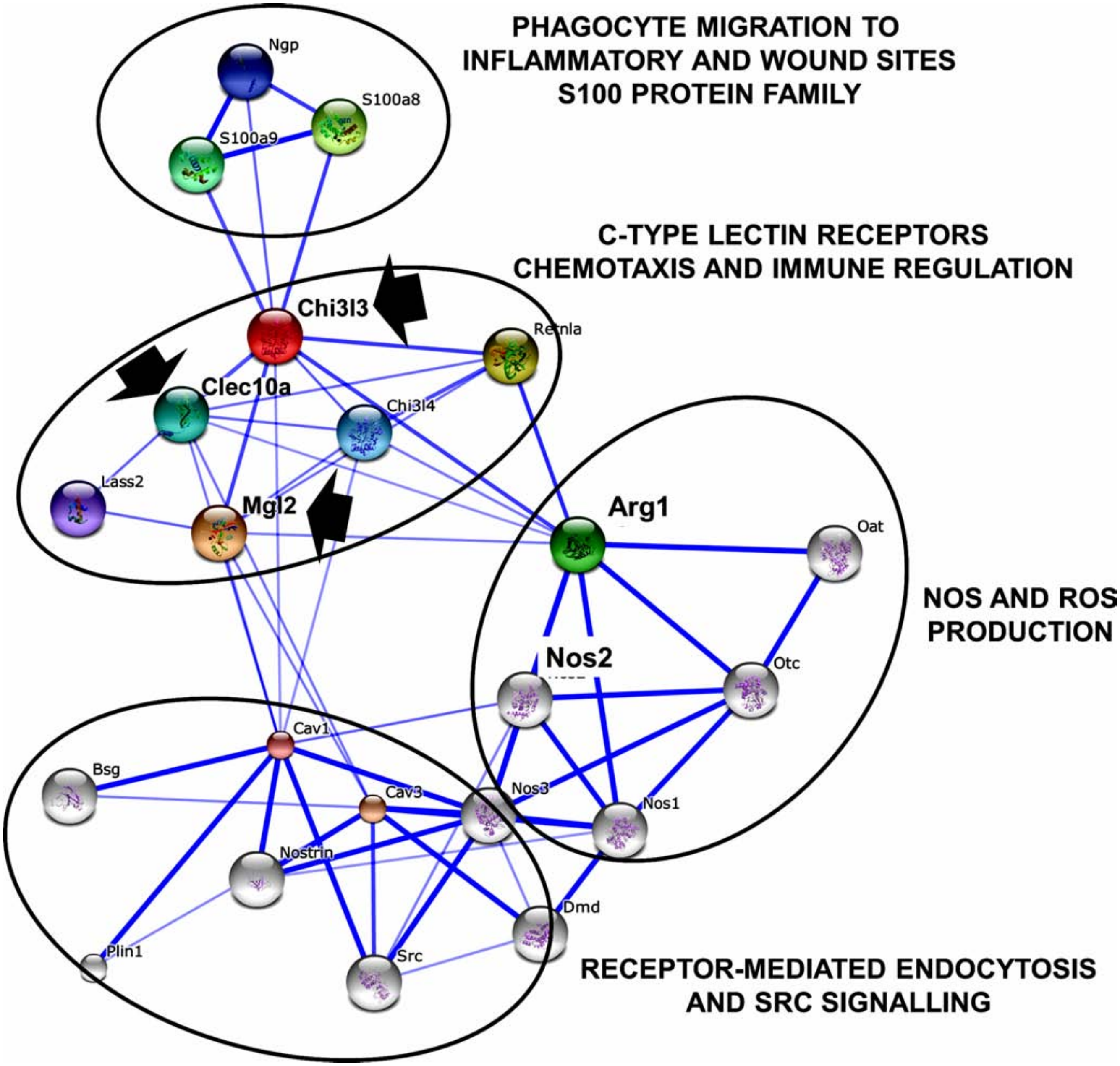
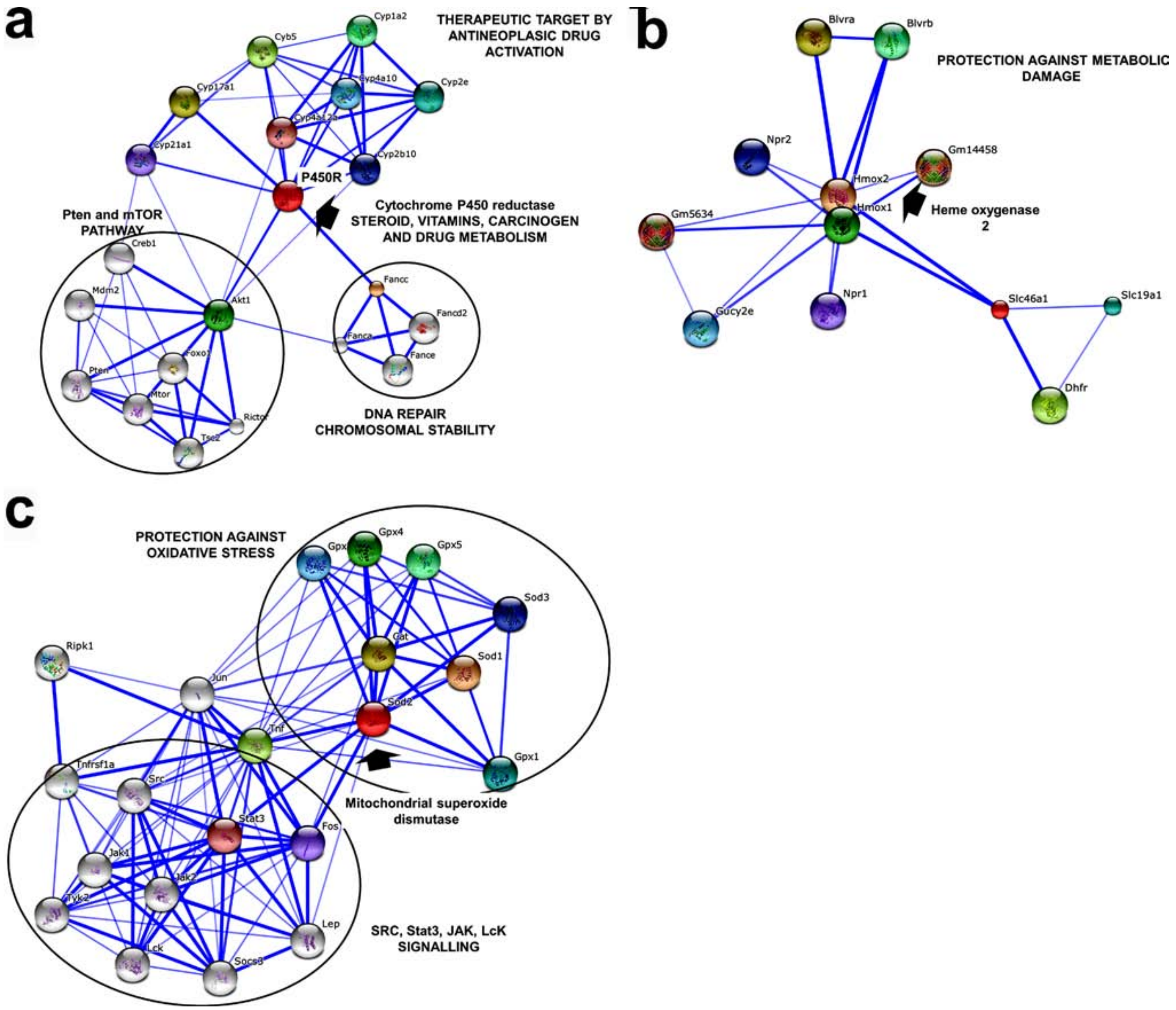
SUPPLEMENTARY FIGURES AND TABLES



Supplementary Figure S1: *Ex vivo*-differentiated MDSCs are phenotypically equivalent to their *in vivo* tumor-infiltrating MDSC counterparts but not to splenic MDSCs from tumor-bearing mice. The graphs represent the percentage of expression of the indicated representative MDSC markers in monocytic (M-MDSC, top graph) and granulocytic (G-MDSC, bottom graph) subsets, in *ex vivo*-differentiated B16-MDSCs compared to splenic and tumor-infiltrating MDSCs from B16 melanoma-bearing mice. Expression of each marker was studied by flow cytometry using specific fluorochrome-conjugated antibodies. *, **, ***, represent significant ($P<0.05$), very significant ($P<0.01$) and highly significant ($P<0.001$) differences, respectively.



Supplementary Figure S2: Linkage between up-regulated cell membrane receptors in MDSCs with the S100 protein family, ROS production and Src signaling. The figure shows a STRING 9.1-derived protein map using the indicated proteins (arrows) as input nodes. These proteins were up-regulated in MDSCs compared to conventional DCs. Functional pathways are grouped and their roles indicated in the figure. S100 family members and Src signaling components have been recently exploited as MDSC-specific therapeutic targets.



Supplementary Figure S3: Detoxification, ROS scavengers and protection against oxidative damage in MDSCs. The figure shows STRING 9.1-derived protein maps using the indicated proteins (arrows) as input nodes. These proteins were up-regulated in MDSCs compared to conventional DCs. Functional pathways are grouped and their roles indicated in the figure. **(a)** P450R, **(b)** heme oxygenase 2 and **(c)** superoxide dismutase were used as input nodes.

Supplementary Table S1. Differentially expressed proteins between B16-MDSCs and conventional DCs by iTRAQ. All proteins were identified with >99% confidence (corresponding to a protein score cutoff > 2.0). DC1, DC2, and DC3 together with B16-1, B16-2, and B16-3 correspond to three independent biological replicates from DCs and B16-MDSCs respectively. Ratio corresponds to the protein reporter ion intensity originating from DC2 (Tag114), DC3 (Tag115), B16-1 (Tag116), B16-2 (Tag117), B16-3 (Tag118) relative to DC1 (Tag113), or with 293T-1, 293T-2 and 293T-3 as indicated. Proteins were considered to show a significant downward or upward trend if their expression ratios were < 0.77 or > 1.3 respectively. Ratios indicating differential expression (p-value < 0.05) are in bold. The complete set of proteins identified is provided in Data set 1.

B16-MDSC vs DC DIFFERENTIALLY EXPRESSED PROTEINS

			Ratio					P-value						
Gene symbol	**Access Numb**	**Protein name**	**DC2: DC1**	**DC3: DC1**	**B16-1 DC1**	**B16-2 DC1**	**B16-3 DC1**	**DC2: DC1**	**DC3: DC1**	**B16-1 DC1**	**B16-2 DC1**	**B16-3 DC1**	**%Cov 95%**	**Unique peptides**
		Down-regulated proteins in B16-MDSCs												
Anxa5	P48036	Annexin A5	0.80	0.90	**0.08**	**0.10**	**0.10**	0.79	0.44	**0.00**	**0.00**	**0.00**	88	36
Mfge8	P21956	Lactadherin	1.07	0.92	**0.17**	**0.15**	**0.18**	0.94	0.09	**0.00**	**0.00**	**0.00**	40	19
Dpysl2	O08553	Dihydropyrimidinase-related protein 2	0.95	1.07	**0.24**	**0.39**	**0.25**	0.48	0.72	**0.01**	**0.03**	**0.00**	46	19
Atp6v0d2	Q80SY3	V-type proton ATPase subunit d 2	0.94	0.80	**0.30**	**0.43**	**0.36**	0.52	0.13	**0.00**	**0.00**	**0.00**	36.5	15
Anpep	P97449	Aminopeptidase N	0.79	0.97	**0.34**	**0.50**	**0.50**	0.74	0.72	**0.00**	**0.00**	**0.00**	37.7	45
Lrpap1	P55302	Alpha-2-macroglobulin receptor-associated protein	1.07	1.09	**0.35**	**0.43**	**0.36**	0.60	0.74	**0.04**	**0.00**	**0.00**	55.5	35
Acox3	Q9EPL9	Peroxisomal acyl-coenzyme A oxidase 3	0.86	0.82	**0.40**	**0.70**	**0.55**	0.48	0.21	**0.00**	**0.05**	**0.01**	47.7	29
Emc1	Q8C7X2	ER membrane protein complex subunit 1	1.09	0.99	**0.40**	**0.63**	**0.47**	0.91	0.89	**0.01**	**0.04**	**0.03**	25.7	28
Hnrnpab	Q99020	Heterogeneous nuclear ribonucleoprotein A/B	1.19	1.10	**0.52**	**0.56**	**0.46**	0.57	0.56	**0.01**	**0.01**	**0.00**	27.7	15
Alox5	P48999	Arachidonate 5-lipoxygenase	0.99	0.87	**0.54**	**0.53**	**0.59**	0.46	0.10	**0.00**	**0.00**	**0.00**	28.2	27
Pigl	Q9ET01	Glycogen phosphorylase, liver form	0.80	0.94	**0.58**	**0.56**	**0.62**	0.22	0.21	**0.00**	**0.00**	**0.00**	37.2	44
Gdi2	Q61598	Rab GDP dissociation inhibitor beta	0.89	0.93	**0.59**	**0.48**	**0.70**	0.64	0.45	**0.01**	**0.00**	**0.04**	58.2	26
Ndufv1	D3YUM1	NADH dehydrogenase [ubiquinone] flavoprotein 1, mitochondrial	0.86	0.94	**0.61**	**0.57**	**0.62**	0.25	0.80	**0.02**	**0.02**	**0.01**	51	22
Cct7	P80313	T-complex protein 1 subunit eta	0.93	1.16	**0.62**	**0.65**	**0.67**	0.22	0.94	**0.01**	**0.03**	**0.01**	40.8	31
Vat1	Q62465	Synaptic vesicle membrane protein VAT-1 homolog	0.82	0.86	**0.67**	**0.55**	**0.58**	0.15	0.39	**0.01**	**0.01**	**0.01**	63.3	24
Cndp2	Q9D1A2	Cytosolic non-specific dipeptidase	0.78	0.96	**0.70**	**0.68**	**0.69**	0.18	0.66	**0.05**	**0.04**	**0.03**	66.3	29

(*Continued*)

B16-MDSC vs DC DIFFERENTIALLY EXPRESSED PROTEINS

			Ratio					**P-value**						
Gene symbol	**Access Numb**	**Protein name**	**DC2: DC1**	**DC3: DC1**	**B16-1 DC1**	**B16-2 DC1**	**B16-3 DC1**	**DC2: DC1**	**DC3: DC1**	**B16-1 DC1**	**B16-2 DC1**	**B16-3 DC1**	**%Cov 95%**	**Unique peptides**
		Tendency to down-regulation in B16-MDSCs												
Hadhb	Q99JY0	Trifunctional enzyme subunit beta, mitochondrial	1.09	0.88	**0.47**	**0.63**	0.61	0.69	0.56	**0.02**	**0.05**	0.08	50.7	28
Npc1	O35604	Niemann-Pick C1 protein	1.10	0.96	**0.47**	**0.57**	0.75	0.43	0.37	**0.00**	**0.01**	0.10	5.4	10
Copb1	Q9JIF7	Coatomer subunit beta	0.96	0.82	**0.51**	**0.53**	0.77	0.52	0.59	**0.02**	**0.04**	0.24	23.7	26
Hnrnpd	G5E8G0	Heterogeneous nuclear ribonucleoprotein D, isoform CRA_b	1.01	1.08	**0.64**	**0.55**	0.69	0.98	0.74	**0.04**	**0.04**	0.07	47.4	12
Fam129b	Q8R1F1	Niban-like protein 1	0.85	1.04	**0.37**	0.60	**0.60**	0.42	0.99	**0.01**	0.06	**0.04**	17.4	14
Ndufv2	Q9D6J6	NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial	0.95	1.02	**0.38**	0.38	**0.26**	0.93	0.75	**0.01**	0.09	**0.03**	50.4	13
Hnrnpa1	Q5EBP8	Heterogeneous nuclear ribonucleoprotein A1	1.06	0.96	**0.38**	0.39	**0.34**	0.41	0.83	**0.02**	0.28	**0.03**	50.6	15
Cpt1a	P97742	Carnitine O-palmitoyltransferase 1, liver isoform	1.05	0.86	**0.52**	0.60	**0.59**	0.82	0.45	**0.02**	0.12	**0.03**	16.7	18
Ptgs1	P22437	Prostaglandin G/H synthase 1	0.79	0.85	**0.61**	0.70	**0.65**	0.41	0.30	**0.03**	0.13	**0.05**	35.2	28
Bdh1	Q80XN0	D-beta-hydroxybutyrate dehydrogenase, mitochondrial	0.85	0.86	**0.65**	0.71	**0.55**	0.24	0.33	**0.05**	0.10	**0.03**	34.1	15
Atp6v1h	Q8BVE3	V-type proton ATPase subunit H	0.85	0.80	**0.51**	0.72	0.67	0.70	0.22	**0.01**	0.44	0.41	39.3	18
Lactb	Q9EP89	Serine beta-lactamase-like protein LACTB, mitochondrial	1.14	0.84	**0.55**	0.70	0.67	0.67	0.59	**0.03**	0.18	0.16	34.1	22
Dock2	Q8C3J5	Dedicator of cytokinesis protein 2	1.08	1.17	**0.65**	0.74	0.74	0.94	0.46	**0.01**	0.10	0.10	9.8	30
Gns	Q8BFR4	N-acetylglucosamine-6-sulfatase	0.82	0.97	0.50	**0.42**	**0.37**	0.33	0.83	0.13	**0.03**	**0.04**	18.7	11
Anxa7	Q07076	Annexin A7	0.83	0.96	0.61	**0.63**	**0.64**	0.38	0.49	0.06	**0.01**	**0.01**	28.3	17
Abhd12	Q99LR1	Monoacylglycerol lipase ABHD12	1.29	0.95	0.25	**0.52**	0.61	0.12	0.91	0.07	**0.02**	0.06	45.2	19
Ckb	Q04447	Creatine kinase B-type	1.00	1.22	0.45	0.42	**0.21**	0.61	0.61	0.09	0.07	**0.00**	37.8	14
Pacsin2	Q9WVE8	PKC and casein kinase substrate in neurons protein 2	0.96	0.96	0.46	0.67	**0.36**	0.64	0.88	0.14	0.17	**0.01**	11.7	11
Pgm1	Q9D0F9	Phosphoglucomutase-1	0.79	0.87	0.66	0.72	**0.53**	0.52	0.57	0.38	0.23	**0.02**	12.1	12

B16-MDSC vs DC DIFFERENTIALLY EXPRESSED PROTEINS														
			Ratio					**P-value**						
Gene symbol	**Access Numb**	**Protein name**	**DC2: DC1**	**DC3: DC1**	**B16-1 DC1**	**B16-2 DC1**	**B16-3 DC1**	**DC2: DC1**	**DC3: DC1**	**B16-1 DC1**	**B16-2 DC1**	**B16-3 DC1**	**%Cov 95%**	**Unique peptides**
		Up-regulated proteins in B16-MDSCs												
Clec10a	F8WHB7	C-type lectin domain family 10 member A	1.14	1.26	**1.69**	**1.71**	**1.60**	0.19	0.21	**0.01**	**0.01**	**0.02**	51.1	9
Sod2	P09671	Superoxide dismutase [Mn], mitochondrial	0.99	0.84	**1.75**	**1.87**	**2.00**	0.46	0.43	**0.05**	**0.02**	**0.01**	58.1	16
Gatm	Q9D964	Glycine amidinotransferase, mitochondrial	0.93	1.22	**1.77**	**2.01**	**1.67**	0.84	0.36	**0.01**	**0.03**	**0.03**	49.4	24
Irg1	P54987	Cis-aconitate decarboxylase	1.01	1.04	**1.85**	**2.29**	**2.09**	0.50	0.93	**0.00**	**0.00**	**0.01**	39.5	15
Mthfd2	P18155	Bifunctional methylenetetrahydrofolate DH/cyclohydrolase	0.99	1.21	**1.89**	**1.92**	**1.77**	0.78	0.67	**0.04**	**0.01**	**0.03**	56	16
Por	P37040	NADPH-cytochrome P450 reductase	0.90	0.91	**1.94**	**2.13**	**2.15**	0.87	0.81	**0.00**	**0.01**	**0.00**	46.6	34
Atp1a3	Q8VCE0	ATPase, Na+/K+ transporting, alpha 3 polypeptide	0.89	1.14	**2.07**	**1.85**	**1.79**	0.58	0.84	**0.00**	**0.01**	**0.05**	27.1	14
Apobr	Q8VBT6	Apolipoprotein B receptor	1.22	1.19	**2.11**	**2.58**	**2.54**	0.53	0.20	**0.02**	**0.01**	**0.00**	31	24
Vapa	Q9WV55	Vesicle-associated membrane protein-associated protein A	1.18	0.90	**2.19**	**2.17**	**2.31**	0.08	0.52	**0.00**	**0.00**	**0.00**	67.8	14
Oat	P29758	Ornithine aminotransferase, mitochondrial	0.99	1.17	**2.21**	**2.19**	**2.17**	0.63	0.75	**0.01**	**0.01**	**0.00**	65.4	34
Actn4	P57780	Alpha-actinin-4	0.87	0.82	**2.54**	**2.54**	**2.58**	0.82	0.98	**0.00**	**0.00**	**0.00**	68	44
Clta	B1AWD9	Clathrin light chain A	1.06	0.97	**3.28**	**2.15**	**2.83**	0.87	0.99	**0.01**	**0.04**	**0.03**	37.1	11
Chi3l3	O35744	Chitinase-3-like protein 3	0.82	0.80	**3.31**	**3.66**	**3.10**	0.22	0.32	**0.00**	**0.00**	**0.00**	67.8	34
Thbs1	Q80YQ1	Thrombospondin 1	1.13	1.07	**3.44**	**4.41**	**4.02**	0.88	0.59	**0.00**	**0.00**	**0.00**	22.1	30
Mgl2	A9XX86	MCG21506	0.90	1.11	**5.55**	**6.55**	**6.08**	0.75	0.29	**0.00**	**0.00**	**0.00**	53.8	27
		Tendency to up-regulation in B16-MDSCs												
Cd180	Q62192	CD180 antigen	1.01	1.18	**1.47**	**1.66**	1.39	0.94	0.57	**0.05**	**0.02**	0.16	22.4	12
Plin3	Q9DBG5	Perilipin-3	0.88	0.86	**2.27**	**1.80**	1.56	0.58	0.65	**0.01**	**0.02**	0.12	34.1	11
Hmox2	O70252	Heme oxygenase 2	1.22	0.79	1.32	**1.63**	**1.98**	0.28	0.15	0.16	**0.03**	**0.01**	42.2	17
Fbl	P35550	rRNA 2'-O-methyltransferase fibrillarin	1.21	0.95	1.33	**1.79**	**1.64**	0.60	0.83	0.37	**0.03**	**0.04**	50.7	15
Nsf	P46460	Vesicle-fusing ATPase	0.90	0.95	1.39	**1.56**	**1.69**	0.67	0.23	0.11	**0.00**	**0.00**	46.5	34
Ddx3x	Q62167	ATP-dependent RNA helicase DDX3X	1.21	1.14	1.39	**1.67**	**1.60**	0.91	0.55	0.07	**0.02**	**0.04**	37.7	29

(*Continued*)

B16-MDSC vs DC DIFFERENTIALLY EXPRESSED PROTEINS

			Ratio					P-value						
Gene symbol	**Access Numb**	**Protein name**	**DC2: DC1**	**DC3: DC1**	**B16-1 DC1**	**B16-2 DC1**	**B16-3 DC1**	**DC2: DC1**	**DC3: DC1**	**B16-1 DC1**	**B16-2 DC1**	**B16-3 DC1**	**%Cov 95%**	**Unique peptides**
Lrrc59	Q922Q8	Leucine-rich repeat-containing protein 59	0.96	0.81	1.79	**2.78**	**2.70**	0.35	0.10	0.35	**0.00**	**0.00**	40.7	18
Prkcsh	O08795	Glucosidase 2 subunit beta	0.98	1.21	2.17	**2.63**	**2.88**	0.36	0.80	0.28	**0.04**	**0.03**	38.2	23
Ephx1	Q9D379	Epoxide hydrolase 1	0.89	0.82	1.32	**1.60**	1.39	0.85	0.50	0.18	**0.01**	0.07	45.2	23
Snap23	Q9D3L3	Synaptosomal-associated protein	0.93	0.96	1.43	**1.91**	1.79	0.64	0.59	0.10	**0.02**	0.06	67.8	14
Aldh3b1	Q80VQ0	Aldehyde dehydrogenase family 3 member B1	1.09	0.99	1.53	**2.49**	2.07	0.73	0.13	0.35	**0.01**	0.16	45.3	21
Sf3a3	Q9D554	Splicing factor 3A subunit 3	1.20	1.04	1.96	**2.17**	2.96	0.84	0.80	0.49	**0.02**	0.09	20.1	14
Lmnb2	P21619	Lamin-B2	1.19	1.22	1.77	1.39	**1.74**	0.81	0.61	0.06	0.18	**0.01**	62.4	30

Supplementary Table S2. Differentially expressed proteins between B16-MDSCs and 293T-MDSCs by iTRAQ. All the proteins have been identified with >99% confidence. The table includes gene name, UniprotKB accession number, protein name, ratios and p-values of expression between B16-MDSCs and 293T-MDSCs, percent coverage (95%), and unique peptides. B16-1, B16-2, and B16-3 together with 293T-1, 293T-2, and 293T-3 correspond to three independent biological replicates from B16-MDSCs and 293T-MDSCs respectively. Ratio corresponds to the protein reporter ion intensity originating from B16-2 (Tag114), B16-3 (Tag115), 293T-1 (Tag116), 293T-2 (Tag117), 293T-3 (Tag118) relative to B16-1 (Tag113). Proteins were considered to show a significant downward or upward trend if their expression ratios were < 0.77 or > 1.3 respectively. Ratios deemed to signify differential expression (p-value < 0.05) are in bold. The complete set of proteins identified is provided in Data set 2.

B16-MDSC vs 293T-MDSC DIFFERENTIALLY EXPRESSED PROTEINS

			Ratio					P-value						
Gene symbol	**Access Numb**	**Protein name**	**B16.2: B16.1**	**B16.3: B16.1**	**293T.1: B16.1**	**293T.2: B16.1**	**293T.3: B16.1**	**B16.2: B16.1**	**B16.3: B16.1**	**293T.1: B16.1**	**293T.2: B16.1**	**293T.3: B16.1**	**%Cov 95%**	**Unique Peptides**
		Up-regulated proteins in B16-MDSCs												
Phgdh	Q61753	D-3-phosphoglycerate dehydrogenase	0.79	0.79	**0.57**	**0.60**	**0.53**	0.30	0.07	**0.00**	**0.00**	**0.00**	18.95	10
		Tendency to up-regulation in B16-MDSCs												
Prdx6	Q6GT24	Peroxiredoxin 6	0.81	0.83	**0.76**	**0.76**	**0.72**	0.24	0.17	0.17	**0.02**	0.07	58.48	15
Sod1	P08228	Superoxide dismutase [Cu-Zn]	0.83	0.78	**0.62**	**0.63**	**0.62**	0.49	0.21	**0.01**	**0.00**	0.06	46.1	9
		Down-regulated proteins in B16-MDSCs												
Slc2a6	Q3UDF0	Protein Slc2a6	1.03	1.01	**1.84**	**1.68**	**1.32**	0.76	0.93	**0.00**	**0.08**	**0.40**	10.46	5
Cd38	P56528	ADP-ribosyl cyclase 1	1.05	0.90	**1.81**	**1.58**	**1.65**	0.53	0.25	**0.01**	**0.01**	**0.04**	9.539	5
Hadha	Q8BMS1	Trifunctional enzyme subunit alpha, mitochondrial	0.89	0.83	**1.33**	**1.50**	**1.31**	0.31	0.39	**0.02**	**0.00**	**0.03**	34.08	22
Vim	P20152	Vimentin	0.83	1.00	**1.72**	**1.69**	**1.40**	0.06	0.99	**0.00**	**0.00**	**0.02**	83.26	56
Arg1	Q61176	Arginase-1	0.85	0.88	**2.47**	**2.52**	**2.63**	0.18	0.71	**0.01**	**0.00**	**0.00**	43.96	15
		Tendency to down-regulation in B16-MDSCs												
Sec63	Q8VHE0	Translocation protein SEC63 homolog	1.26	1.09	1.59	**1.51**	1.39	0.34	0.66	0.22	**0.04**	0.33	2.763	5

(*Continued*)

B16-MDSC vs 293T-MDSC DIFFERENTIALLY EXPRESSED PROTEINS

			Ratio					**P-value**						
Gene symbol	**Access Numb**	**Protein name**	**B16.2: B16.1**	**B16.3: B16.1**	**293T.1: B16.1**	**293T.2: B16.1**	**293T.3: B16.1**	**B16.2: B16.1**	**B16.3: B16.1**	**293T.1: B16.1**	**293T.2: B16.1**	**293T.3: B16.1**	**%Cov 95%**	**Unique Peptides**
Vti1b	Q91XH6	Vesicle transport through interaction with t-SNAREs 1B homolog	0.98	0.00	**1.67**	**1.37**	1.31	0.69	0.91	**0.03**	**0.01**	0.22	26.29	7
Psen1	P49769	Presenilin-1	0.99	,	**1.38**	1.59	**1.50**	0.96	0.50	**0.43**	0.04	**0.15**	8.779	3
Usp25	P57080	Ubiquitin carboxyl-terminal hydrolase 25	0.99	1.05	**1.59**	1.35	1.46	0.94	0.70	**0.03**	0.08	0.14	1.232	3