Supplemental Materials and Methods, Figures and Figure Legends

Materials and Methods

Cells. Human G361, A375, Malme3M, SKMEL2, SKMEL5, SKMEL28, UACC62, UACC257, M14, MDAMB435, 501mel, MeWo, FEMX and LOX and A2058 melanoma cells and mouse B16 F10 cells were maintained in RPMI 1640/10%FBS/1% Penicillin-Streptomycin (Invitrogen, Inc.; Carlsbad, CA) and validated for pigment and differentiation gene markers; MCAM, MITF, melanocyte antigen recognized by T cells (MART-1), tyrosinase and/or SOX10. Human T cells from PBMC isolates were activated and expanded in X-VIVO15™ (Lonza, Inc.; Hopkinton, MA) and IL-2 as described (Cedeno-Laurent *et al.*, 2012). Short-term cultures of clinical melanoma specimens from (3) donors were isolated from surgical metastases in accordance with IRB approval and cultured in RPMI 1640/10% FBS/1% penicillin/streptomycin as described (Schatton *et al.*, 2008). Early passage (P2), primary normal human epidermal melanocytes (HEM) were purchased from PromoCell, Inc. (Heidelberg, Germany). Patient consent was not necessary and all human tissues were obtained according to institutional review board approval.

Silencing of MCAM. Lentiviral shRNA constructs in pLKO.puro against MCAM or scrambled (SCr) controls were purchased from the Mission collection (Sigma-Aldrich; St. Louis, MO). Retroviral supernatants were generated by co-transfection of pN8e-GagPolΔ8.1 and pNE8e-VSV/G in the packaging HEK293t cell line. Melanoma cells were transduced in the presence of 8µg/ml polybrene and selected with 1µg/ml puromycin to generate stable knockdown cell lines. Knockdown of human and mouse MCAM was achieved with the shRNA target sequence - AGTTGAAGTTAAGTCAGATAA.

Overexpression of ST6GalNAc2. The following sequences were used for real-time qRT-PCR to validate ST6GalNAc2 levels: forward 5'-CTCGTCTCCTACTGGAATCTGG-3' and reverse 5'-CGATCTCAGCATCACATAGTCGC-3' as described (Barthel *et al.*, 2008; Barthel *et al.*, 2013). For overexpression, human ST6GalNAc2 cDNA was amplified by KOD polymerase PCR from reverse transcribed melanocyte RNA using forward: 5'-TTCTGCCTGGGACGTCAGCGGACG-3' and reverse: 5'-TCAGCGCTGGTACAGCTGAAGGA-3' primers. This ST6GalNAc2 cDNA

product was then cloned into pDONR223 by KOD amplification using forward: 5'-GGGGACAACTTTGTACAAAAAAGTTGGCTCCACCATGGGGCTCCCGCGCGG-3' and reverse: 5'-GGGGACAACTTTGTACAAGAAAGTTGGGTAGCGCTGGTACAGCTGAAGGA TGCC-3' attB-containing primers. Subsequent LR clonase (Invitrogen)-mediated recombination into pLenti6.3-DEST (Invitrogen) yielded vector pLenti6.3-ST6GalNAc2. Cells were transduced with lentiviral supernatants and selected for stable expression with blasticidin.

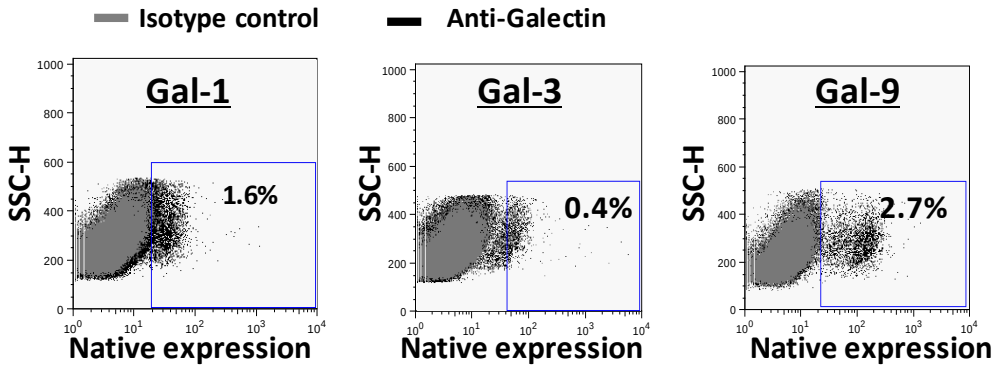
Flow Cytometry. Flow cytometric analysis using primary antibodies, including polyclonal goat anti-human Gal-1 (R&D Systems), anti-human Gal-3 (M3/38) (BioLegend), anti-human Gal-9 (9M1-3) Abs (BioLegend), human Gal-1 – hFc chimera (hGal-1) (generously provided by Dr. Kuo-I. Lin; Academia Sinica, Taiwan) (Tsai *et al.*, 2008; Tsai *et al.*, 2011), Gal-1hFc, dmGal-1hFc, isotype control Abs, and respective fluorophore-conjugated secondary Abs was performed as described (Cedeno-Laurent *et al.*, 2012). Where indicated, cells were either treated with the broadly-active protease, bromelain (Sigma), at 0.1U/ml for 1hr at 37^{o}C to digest all cell surface glycoproteins or with kifunensine (Sigma) between 1-10µg/ml for 48hr to inhibit *de novo* synthesis of complex N-glycans and then stained with Gal-1hFc or with FITC-PHA-L (1µg/ml). Cells were also stained with biotinylated LEA (0.1µg/ml) and APC-avidin (1:500). Secondary Ab, APC-avidin or 50mM lactose-containing Gal-1hFc groups were included to control for background fluorescence and carbohydrate-dependence of Gal-1hFc, PHA-L or LEA. Experiments were performed at least three-times.

In vitro Melanoma Cell Proliferation Assay. We assayed for cell growth by CSFE-dilution assay over a 3-day period in the presence Gal-1hFc, hFc or diluent controls. Prior to assays, cells were cultured for 24h in RPMI 1640/10%FBS/1% Pen/Strep with 50mM lactose to recapitulate pretreatment conditions in cell migration assays. Cells were harvested with 1mM EDTA, washed in PBS, loaded with 2µM CFSE in PBS for 10 min at 37^{o}C. Cells were then plated in RPMI 1640/10% FBS with diluent control, hFc or Gal-1hFc at either 5 or 20µg/ml in triplicate at 5x10^{4}/well in 6-well plates. After 1, 2 and 3 days, cells were harvested and assayed by flow cytometry.

Statistical Analysis. Statistical significant comparisons were ascertained by two-tailed Student's *t*-test, paired *t*-test, one-way ANOVA with Dunnett's post test, or contingency table on GraphPad Prism (GraphPad Software, La Jolla, CA).

Supplemental Figure 1 and Legend

a



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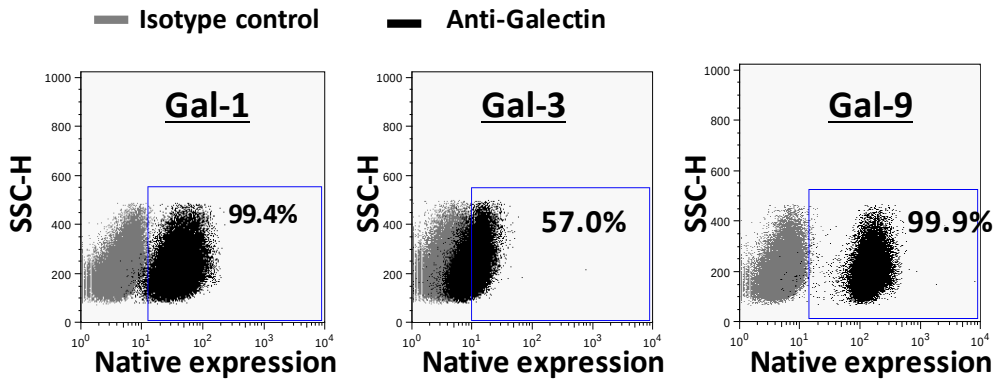

Supplemental Figure 1. Melanoma cell Gal-1, -3 and -9 are principally expressed and detected inside of the cell and not bound to the cell surface. Surface (Non-permeabilized) **(a)** and intracellular (permeabilized) **(b)** human A375 melanoma cells were FACS stained with anti-human Gal-1 , -3, or -9 Abs or respective fluorophore-conjugated secondary Ab. Cells were analyzed for fluorescence intensity (x-axis) and designated as percent positive (black dots) compared with cells stained with control Ab alone (gray dots). Data are representative of triplicate experiments.

Supplemental Figure 2 and Legend

Identified Protein	Accession Number	MW	# Peptide matches Negative Control	Gal-1 Eluate
Ig gamma-1 chain C region OS=Homo sapiens GN=IGHG1 PE=1 SV=1	P01857|IGHG1_HUMAN	36 kDa	22	136
Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2	P02768|ALBU_HUMAN	69 kDa	22	33
ATP-citrate synthase OS=Homo sapiens GN=ACLY PE=1 SV=3	P53396|ACLY_HUMAN	121 kDa	0	51
Cell surface glycoprotein MUC18 OS=Homo sapiens GN=MCAM PE=1 SV=2	P43121|MUC18_HUMAN	72 kDa	0	40
Isoform 2 of Kinectin OS=Homo sapiens GN=KTN1	Q86UP2-2|KTN1_HUMAN (+3)	150 kDa	0	36
Ribosome-binding protein 1 OS=Homo sapiens GN=RRBP1 PE=1 SV=4	Q9P2E9|RRBP1_HUMAN	152 kDa	0	35
Galectin-3-binding protein OS=Homo sapiens GN=LGALS3BP PE=1 SV=1	Q08380|LG3BP_HUMAN	65 kDa	3	32
Isoform 2 of Integrin alpha-V OS=Homo sapiens GN=ITGAV	P06756-2|ITAV_HUMAN (+2)	112 kDa	0	35
DNA damage-binding protein 1 OS=Homo sapiens GN=DDB1 PE=1 SV=1	Q16531|DDB1_HUMAN	127 kDa	0	34
Synaptotagmin-like protein 4 OS=Homo sapiens GN=SYTL4 PE=4 SV=1	F8W9B9|F8W9B9_HUMAN (+2)	76 kDa	18	13
Isoform 1 of Vinculin OS=Homo sapiens GN=VCL	P18206-2|VINC_HUMAN (+1)	117 kDa	0	30
Prostaglandin F2 receptor negative regulator OS=Homo sapiens GN=PTGFRN PE=1 SV=2	Q9P2B2|FPRP_HUMAN	99 kDa	0	30
Hypoxia up-regulated protein 1 OS=Homo sapiens GN=HYOU1 PE=1 SV=1	Q9Y4L1|HYOU1_HUMAN	111 kDa	0	25
Ubiquitin-like modifier-activating enzyme 1 OS=Homo sapiens GN=UBA1 PE=1 SV=3	P22314|UBA1_HUMAN	118 kDa	0	27
Ig gamma-3 chain C region OS=Homo sapiens GN=IGHG3 PE=1 SV=2	P01860|IGHG3_HUMAN	41 kDa	17	58
Cation-independent mannose-6-phosphate receptor OS=Homo sapiens GN=IGF2R PE=1 SV=3	P11717|MPRI_HUMAN	274 kDa	0	22
Calsyntenin-1 OS=Homo sapiens GN=CLSTN1 PE=2 SV=1	B4E3Q1|B4E3Q1_HUMAN (+1)	108 kDa	0	22
Collagen alpha-1(VI) chain OS=Homo sapiens GN=COL6A1 PE=1 SV=3	P12109|CO6A1_HUMAN	109 kDa	0	23
Isoform 2 of Neutral alpha-glucosidase AB OS=Homo sapiens GN=GANAB	Q14697-2|GANAB_HUMAN	109 kDa	0	22
Galectin-1 OS=Homo sapiens GN=LGALS1 PE=1 SV=2	P09382|LEG1_HUMAN	15 kDa	4	17
Protein kinase C-binding protein NELL2 OS=Homo sapiens GN=NELL2 PE=4 SV=1	F8VVB6|F8VVB6_HUMAN (+4)	86 kDa	0	21
Talin-1 OS=Homo sapiens GN=TLN1 PE=4 SV=1	Q5TCU6|Q5TCU6_HUMAN (+1)	258 kDa	0	21
Isoform 2 of Fibronectin type-III domain-containing protein 3A OS=Homo sapiens GN=FNDC3A	Q9Y2H6-2|FND3A_HUMAN (+1)	126 kDa	0	21
26S proteasome non-ATPase regulatory subunit 1 OS=Homo sapiens GN=PSMD1 PE=1 SV=2	Q99460|PSMD1_HUMAN	106 kDa	0	20
Hexokinase-1 OS=Homo sapiens GN=HK1 PE=3 SV=1	E7ENR4|E7ENR4_HUMAN (+4)	106 kDa	0	18
Ubiquitin carboxyl-terminal hydrolase 7 OS=Homo sapiens GN=USP7 PE=1 SV=2	Q93009|UBP7_HUMAN	128 kDa	0	19
Nodal modulator 3 OS=Homo sapiens GN=NOMO3 PE=4 SV=1	J3KN36|J3KN36_HUMAN (+4)	139 kDa	0	18
Ras GTPase-activating-like protein IQGAP1 OS=Homo sapiens GN=IQGAP1 PE=1 SV=1	P46940|IQGA1_HUMAN	189 kDa	0	18
Staphylococcal nuclease domain-containing protein 1 OS=Homo sapiens GN=SND1 PE=1 SV=1	Q7KZF4|SND1_HUMAN	102 kDa	0	16
Lysosome-associated membrane glycoprotein 2 OS=Homo sapiens GN=LAMP2 PE=2 SV=1	B4E2S7|B4E2S7_HUMAN (+1)	40 kDa	0	16
Lysosome-associated membrane glycoprotein 1 OS=Homo sapiens GN=LAMP1 PE=1 SV=3	P11279|LAMP1_HUMAN	45 kDa	0	16
Isoform 5 of Inactive tyrosine-protein kinase 7 OS=Homo sapiens GN=PTK7	Q13308-5|PTK7_HUMAN (+2)	90 kDa	0	16
Isoform 2 of Integrin beta-1 OS=Homo sapiens GN=ITGB1	P05556-2|ITB1_HUMAN (+4)	87 kDa	0	16
Chondroitin sulfate proteoglycan 4 OS=Homo sapiens GN=CSPG4 PE=1 SV=2	Q6UVK1|CSPG4_HUMAN	251 kDa	0	16
Heat shock cognate 71 kDa protein OS=Homo sapiens GN=HSPA8 PE=3 SV=1	E9PKE3|E9PKE3_HUMAN (+1)	69 kDa	2	12
Isoform 4 of Phosphatidylinositol 3-kinase regulatory subunit alpha OS=Homo sapiens GN=PIK3R1	P27986-4|P85A_HUMAN (+1)	84 kDa	13	2
Isoform Alpha-6X1A of Integrin alpha-6 OS=Homo sapiens GN=ITGA6	P23229-2|ITA6_HUMAN (+6)	119 kDa	0	15
Heat shock protein 4 OS=Homo sapiens GN=HSPA4 PE=1 SV=4	P34932|HSP74_HUMAN	94 kDa	0	15
Stress-70 protein, mitochondrial OS=Homo sapiens GN=HSPA9 PE=1 SV=2	P38646|GRP75_HUMAN	74 kDa	4	10
Isoform 2 of Extended synaptotagmin-1 OS=Homo sapiens GN=ESYT1	Q9BSJ8-2|ESYT1_HUMAN (+1)	124 kDa	0	12
Pyruvate kinase PKM OS=Homo sapiens GN=PKM PE=1 SV=4	P14618|KPYM_HUMAN	58 kDa	0	13
Alpha-mannosidase 2 OS=Homo sapiens GN=MAN2A1 PE=1 SV=2	Q16706|MA2A1_HUMAN	131 kDa	0	14
N-acetylglucosamine-1-phosphotransferase subunits alpha/beta OS=Homo sapiens GN=GNPTAB PE=1 SV=1	Q3T906|GNPTA_HUMAN	144 kDa	0	14
Isoform 3 of Sodium/potassium-transporting ATPase subunit alpha-1 OS=Homo sapiens GN=ATP1A1	P05023-3|AT1A1_HUMAN (+2)	110 kDa	1	12
Nicastrin OS=Homo sapiens GN=NCSTN PE=4 SV=1	Q6YQ97|Q6YQ97_HUMAN (+2)	63 kDa	0	12
Isoform 2 of Immunoglobulin superfamily member 3 OS=Homo sapiens GN=IGSF3	O75054-2|IGSF3_HUMAN	138 kDa	0	12
Splicing factor 3B subunit 3 OS=Homo sapiens GN=SF3B3 PE=1 SV=4	Q15393|SF3B3_HUMAN	136 kDa	0	11
Alpha-enolase OS=Homo sapiens GN=ENO1 PE=1 SV=2	P06733|ENOA_HUMAN	47 kDa	2	9
Laminin subunit gamma-1 OS=Homo sapiens GN=LAMC1 PE=1 SV=3	P11047|LAMC1_HUMAN	178 kDa	1	10
Tyrosine-protein kinase receptor OS=Homo sapiens GN=IGF1R PE=3 SV=1	C9J5X1|C9J5X1_HUMAN (+1)	155 kDa	0	10
Prolow-density lipoprotein receptor-related protein 1 OS=Homo sapiens GN=LRP1 PE=1 SV=2	Q07954|LRP1_HUMAN	505 kDa	0	10
DNA-dependent protein kinase catalytic subunit OS=Homo sapiens GN=PRKDC PE=4 SV=1	E7EUV0|E7EUV0_HUMAN (+2)	465 kDa	0	10
Isoleucine--tRNA ligase, mitochondrial OS=Homo sapiens GN=IARS2 PE=3 SV=1	F6SBX2|F6SBX2_HUMAN (+1)	106 kDa	0	11
Isoform 2 of Nidogen-1 OS=Homo sapiens GN=NID1	P14543-2|NID1_HUMAN (+1)	122 kDa	0	11
Ectonucleotide pyrophosphatase/phosphodiesterase family member 1 OS=Homo sapiens GN=ENPP1 PE=1 SV=2	P22413|ENPP1_HUMAN	105 kDa	0	10
Isoform 3 of Integrin alpha-3 OS=Homo sapiens GN=ITGA3	P26006-1|ITA3_HUMAN (+1)	119 kDa	0	11
Isoform 2 of Presequence protease, mitochondrial OS=Homo sapiens GN=PITRM1	Q5JRX3-2|PREP_HUMAN (+1)	118 kDa	0	11
Fibulin-1 OS=Homo sapiens GN=FBLN1 PE=4 SV=1	B1AHL2|B1AHL2_HUMAN (+1)	78 kDa	0	10
CD276 antigen OS=Homo sapiens GN=CD276 PE=1 SV=1	Q5ZPR3|CD276_HUMAN	57 kDa	0	10
Isoform 2 of Filamin-A OS=Homo sapiens GN=FLNA	P21333-2|FLNA_HUMAN (+2)	280 kDa	1	8
Isoform 2 of Neural cell adhesion molecule 1 OS=Homo sapiens GN=NCAM1	P13591-1|NCAM1_HUMAN (+1)	93 kDa	0	8
Isoform Beta-3C of Integrin beta-3 OS=Homo sapiens GN=ITGB3	P05106-3|ITB3_HUMAN	87 kDa	0	8
Neural cell adhesion molecule L1 OS=Homo sapiens GN=L1CAM PE=4 SV=1	F5H025|F5H025_HUMAN (+4)	140 kDa	0	9
Isoform 2 of Golgi apparatus protein 1 OS=Homo sapiens GN=GLG1	Q92896-2|GSLG1_HUMAN (+2)	137 kDa	0	9
Ig gamma-2 chain C region OS=Homo sapiens GN=IGHG2 PE=1 SV=2	P01859|IGHG2_HUMAN	36 kDa	0	45
Isoform 2 of Glyceraldehyde-3-phosphate dehydrogenase OS=Homo sapiens GN=GAPDH	P04406-2|G3P_HUMAN (+1)	32 kDa	5	3
Actin, cytoplasmic 2 OS=Homo sapiens GN=ACTB PE=1 SV=1	P60709|ACTB_HUMAN (+1)	42 kDa	2	5
Isoform 2 of Heat shock protein HSP 90-alpha OS=Homo sapiens GN=HSP90AA1	P07900-2|HS90A_HUMAN (+1)	98 kDa	1	7
Spectrin alpha chain, non-erythrocytic 1 OS=Homo sapiens GN=SPTAN1 PE=4 SV=2	A6NG51|A6NG51_HUMAN (+3)	285 kDa	0	8
Isoform 2 of Calsyntenin-3 OS=Homo sapiens GN=CLSTN3	Q9BQT9-2|CSTN3_HUMAN (+1)	107 kDa	0	6
NAD(P) transhydrogenase, mitochondrial OS=Homo sapiens GN=NNT PE=1 SV=3	Q13423|NNTM_HUMAN	114 kDa	0	6
Isoform 2 of Receptor-type tyrosine-protein phosphatase F OS=Homo sapiens GN=PTPRF	P10586-2|PTPRF_HUMAN (+1)	212 kDa	0	6
Isoform A of Endothelin-converting enzyme 1 OS=Homo sapiens GN=ECE1	P42892-2|ECE1_HUMAN	86 kDa	0	7
Transitional endoplasmic reticulum ATPase OS=Homo sapiens GN=VCP PE=1 SV=4	P55072|TERA_HUMAN	89 kDa	0	6
Isoform 2 of UDP-glucose:glycoprotein glucosyltransferase 1 OS=Homo sapiens GN=UGGT1	Q9NYU2-2|UGGG1_HUMAN (+1)	175 kDa	0	7
Neuroblast differentiation-associated protein AHNAK OS=Homo sapiens GN=AHNAK PE=1 SV=2	Q09666|AHNK_HUMAN	629 kDa	0	5
Leucine--tRNA ligase, cytoplasmic OS=Homo sapiens GN=LARS PE=2 SV=1	B4DER1|B4DER1_HUMAN (+1)	131 kDa	1	5
Major vault protein OS=Homo sapiens GN=MVP PE=1 SV=4	Q14764|MVP_HUMAN	99 kDa	0	5
116 kDa U5 small nuclear ribonucleoprotein component OS=Homo sapiens GN=EFTUD2 PE=4 SV=1	K7EJ81|K7EJ81_HUMAN (+2)	108 kDa	0	6
Epidermal growth factor receptor substrate 15-like 1 OS=Homo sapiens GN=EPS15L1 PE=4 SV=1	M0R165|M0R165_HUMAN (+3)	83 kDa	0	5
Phosphoribosylformylglycinamidine synthase OS=Homo sapiens GN=PFAS PE=1 SV=4	Q15067|PURA4_HUMAN	145 kDa	0	6
Neprilysin OS=Homo sapiens GN=MME PE=1 SV=2	P08473|NEP_HUMAN	86 kDa	0	6
60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2	P10809|CH60_HUMAN	61 kDa	0	6
Nuclear pore complex protein Nup153 OS=Homo sapiens GN=NUP153 PE=1 SV=3	P49790|NU153_HUMAN	153 kDa	0	5
Nuclear-pore anchor OS=Homo sapiens GN=TPR PE=1 SV=3	P12270|TPR_HUMAN	267 kDa	0	6
Elongation factor 2 OS=Homo sapiens GN=EEF2 PE=1 SV=4	P13639|EF2_HUMAN	95 kDa	0	6
Carcinoembryonic antigen-related cell adhesion molecule 1 OS=Homo sapiens GN=CEACAM1 PE=1 SV=2	P13688|CEAM1_HUMAN	58 kDa	0	6
Fatty acid synthase OS=Homo sapiens GN=FASN PE=1 SV=3	P49327|FAS_HUMAN	273 kDa	0	6
ATP-dependent helicase A OS=Homo sapiens GN=DHX9 PE=1 SV=4	Q08211|DHX9_HUMAN	141 kDa	0	6
Desmoplakin OS=Homo sapiens GN=DSP PE=1 SV=3	P15924|DESP_HUMAN	332 kDa	2	2
78 kDa glucose-regulated protein OS=Homo sapiens GN=HSPA5 PE=1 SV=2	P11021|GRP78_HUMAN	72 kDa	1	6
Phosphatidylinositol 3-kinase regulatory subunit beta OS=Homo sapiens GN=PIK3R2 PE=1 SV=2	O00459|P85B_HUMAN	82 kDa	4	1
Lysocyclic O-mannosyltransferase 1 OS=Homo sapiens GN=LYZ PE=3 SV=1	F8VV32|F8VV32_HUMAN (+1)	11 kDa	3	2
Receptor-type tyrosine-protein phosphatase U OS=Homo sapiens GN=PTPRU P E=4 SV=1	E9PH42|E9PH42_HUMAN (+5)	162 kDa	1	3
Isoform Short of Ubiquitin carboxyl-terminal hydrolase 5 OS=Homo sapiens GN=USP5	P45974-2|UBP5_HUMAN (+1)	93 kDa	1	4
U5 small nuclear ribonucleoprotein 200 kDa helicase OS=Homo sapiens GN=SNRNP200 PE=1 SV=2	O75643|U520_HUMAN	245 kDa	1	4
Alpha-actinin-1 OS=Homo sapiens GN=ACTN1 PE=4 SV=1	H9KV75|H9KV75_HUMAN (+4)	95 kDa	0	4
Integrin beta OS=Homo sapiens GN=ITGB8 PE=2 SV=1	B4DHD4|B4DHD4_HUMAN (+1)	71 kDa	0	5
4F2 cell-surface antigen heavy chain OS=Homo sapiens GN=SLC3A2 PE=4 SV=1	F5GZS6|F5GZS6_HUMAN (+5)	65 kDa	0	4
Carboxypeptidase D OS=Homo sapiens GN=CPD PE=1 SV=2	O75976|CBPD_HUMAN	153 kDa	0	5
Lipocalin-1 OS=Homo sapiens GN=LCN1 PE=1 SV=1	P31025|LCN1_HUMAN	19 kDa	5	0
Leucine-rich PPR motif-containing protein, mitochondrial OS=Homo sapiens GN=LRPPRC PE=1 SV=3	P42704|LPRRC_HUMAN	158 kDa	0	5
Paragerlin OS=Homo sapiens GN=GRN PE=4 SV=1	K7EQ05|K7EQ05_HUMAN (+2)	47 kDa	0	4
FACT complex subunit SPT16 OS=Homo sapiens GN=SUPT16H PE=1 SV=1	Q9Y5B9|SP16H_HUMAN	120 kDa	0	5
Tripeptidyl-peptidase 2 OS=Homo sapiens GN=TPP2 PE=1 SV=4	P29144|TPP2_HUMAN	138 kDa	0	5
Protocadherin-20 OS=Homo sapiens GN=PCDH20 PE=2 SV=2	Q8N6Y1|PCD20_HUMAN	105 kDa	0	5
Hexokinase-2 OS=Homo sapiens GN=HK2 PE=3 SV=1	E9PB90|E9PB90_HUMAN (+1)	99 kDa	0	8
Integrin beta-5 OS=Homo sapiens GN=ITGB5 PE=1 SV=1	P18084|ITB5_HUMAN	88 kDa	0	5
Suprabasin OS=Homo sapiens GN=SBSN PE=2 SV=2	Q6UWP8|SBSN_HUMAN	61 kDa	5	0
Kalicrein-1 (Fragment) OS=Homo sapiens GN=LTF PE=4 SV=1	E7EQB2|E7EQB2_HUMAN (+2)	77 kDa	4	0
Prolactin-inducible protein OS=Homo sapiens GN=PIP PE=1 SV=1	P12273|PIP_HUMAN	17 kDa	2	2
Ig gamma-4 chain C region OS=Homo sapiens GN=IGHG4 PE=1 SV=1	P01861|IGHG4_HUMAN	36 kDa	9	59
Ubiquitin OS=Homo sapiens GN=UBB PE=2 SV=1	B4DV12|B4DV12_HUMAN (+17)	17 kDa	1	3
ELK3/6-interacting/CAST family member 1 OS=Homo sapiens GN=ERC1 PE=4 SV=1	G8JLD3|G8JLD3_HUMAN (+2)	125 kDa	1	3
Ubiquitin-like modifier-activating enzyme 6 OS=Homo sapiens GN=UBA6 PE=1 SV=1	A0AVT1|UBA6_HUMAN	118 kDa	1	3
Isoform 2 of Clathrin heavy chain 1 OS=Homo sapiens GN=CLTC	Q00610-2|CLH1_HUMAN (+1)	188 kDa	0	2
Sodium bicarbonate cotransporter 3 (Fragment) OS=Homo sapiens GN=SLC4A7 PE=4 SV=1	C9JRP1|C9JRP1_HUMAN (+8)	118 kDa	0	3
Receptor-type tyrosine-protein phosphatase mu OS=Homo sapiens GN=PTPRM PE=4 SV=2	E7EPS8|E7EPS8_HUMAN (+4)	157 kDa	0	3
Filaggrin-2 OS=Homo sapiens GN=FLG2 PE=1 SV=1	Q5D862|FILA2_HUMAN	248 kDa	3	0
Thioredoxin reductase 1, cytoplasmic OS=Homo sapiens GN=TXNRD1 PE=2 SV=1	B2R5P6|B2R5P6_HUMAN (+12)	55 kDa	0	4
Prurony-sensitive aminopeptidase OS=Homo sapiens GN=NPEPPS PE=4 SV=1	E9PLK3|E9PLK3_HUMAN (+1)	103 kDa	0	4
Matrilin-2 OS=Homo sapiens GN=MATN2 PE=4 SV=1	J3KN08|J3KN08_HUMAN (+3)	107 kDa	0	4
Kinesin-1 heavy chain OS=Homo sapiens GN=KIF5B PE=1 SV=1	P33176|KINH_HUMAN	110 kDa	0	4
Sortilin OS=Homo sapiens GN=SORT1 PE=1 SV=3	Q99523|SORT_HUMAN	92 kDa	0	4
Spondin-1 OS=Homo sapiens GN=SPON1 PE=1 SV=2	Q9HCB6|SPON1_HUMAN	91 kDa	0	4
Isoform 2 of Leucine-rich repeats and immunoglobulin-like domains protein 1 OS=Homo sapiens GN=LRIG1	Q96JA1-2|LRIG1_HUMAN (+1)	116 kDa	0	4
Isoform 2 of ER membrane protein complex subunit 1 OS=Homo sapiens GN=EMC1	Q8N766-2|EMC1_HUMAN (+3)	112 kDa	0	4
Centrosomal protein of 290 kDa OS=Homo sapiens GN=CEP290 PE=4 SV=1	J3KNF5|J3KNF5_HUMAN (+1)	291 kDa	0	3
Protogenin OS=Homo sapiens GN=PRTG PE=2 SV=1	Q2VWP7|PRTG_HUMAN	127 kDa	1	2
Isoform 2 of Peptidyl-glycine alpha-amidating monooxygenase OS=Homo sapiens GN=PAM	P19021-2|AMD_HUMAN (+5)	96 kDa	1	2
Protein transport protein Sec31A OS=Homo sapiens GN=SEC31A PE=2 SV=1	B7ZL00|B7ZL00_HUMAN (+9)	128 kDa	0	2
Isoform 2 of FRAS1-related extracellular matrix protein 2 OS=Homo sapiens GN=FREM2	Q5SZK8-2|FREM2_HUMAN (+1)	296 kDa	0	2
Laminin subunit beta-1 OS=Homo sapiens GN=LAMB1 PE=4 SV=1	G3XAI2|G3XAI2_HUMAN (+1)	200 kDa	0	3
Isoform 2 of Multiple epidermal growth factor-like domains protein 8 OS=Homo sapiens GN=MEGF8	Q7Z7M0-2|MEGF8_HUMAN (+1)	296 kDa	0	3
M-beta OS=Homo sapiens GN=PMEL PE=4 SV=1	F5H2A6|F5H2A6_HUMAN (+8)	67 kDa	0	3
Lysosomal alpha-glucosidase OS=Homo sapiens GN=GAA PE=1 SV=4	P10253|LYAG_HUMAN	105 kDa	0	3
Gamma-secretase C-terminal fragment 59 OS=Homo sapiens GN=APP PE=4 SV=1	E9PG40|E9PG40_HUMAN (+5)	81 kDa	0	3
PTPRK protein OS=Homo sapiens GN=PTPRK PE=2 SV=1	B7ZMG0|B7ZMG0_HUMAN (+6)	165 kDa	0	3
Protein disulfide-isomerase family A, member 3, isoform CRA_b OS=Homo sapiens GN=PDIA3 PE=3 SV=1	G5E5A2|G5E5A2_HUMAN (+1)	55 kDa	0	3
CD44 antigen OS=Homo sapiens GN=CD44 PE=4 SV=2	E7EPC6|E7EPC6_HUMAN (+17)	79 kDa	0	2
Isoform 2 of Nucleophosmin OS=Homo sapiens GN=NPM1	P06748-2|NPM_HUMAN (+2)	29 kDa	0	3
Inter-alpha-trypsin inhibitor heavy chain H5 OS=Homo sapiens GN=ITIH5 PE=4 SV=1	J3KQ60|J3KQ60_HUMAN (+2)	80 kDa	0	3
Protein transport protein Sec24C OS=Homo sapiens GN=SEC24C PE=4 SV=2	E7EP00|E7EP00_HUMAN (+2)	107 kDa	0	2
Isoform 3 of CD109 antigen OS=Homo sapiens GN=CD109	Q6YHK3-3|CD109_HUMAN (+2)	74 kDa	0	3
Isoform 2 of Roundabout homolog 1 OS=Homo sapiens GN=ROBO1	Q9Y6N7-2|ROBO1_HUMAN (+5)	181 kDa	0	2
Intercellular adhesion molecule 1 OS=Homo sapiens GN=ICAM1 PE=1 SV=2	P05362|ICAM1_HUMAN	58 kDa	0	3
Sarcoplasmic/endoplasmic reticulum calcium ATPase 2 (Fragment) OS=Homo sapiens GN=ATP2A2 PE=3 SV=1	H7C5W9|H7C5W9_HUMAN (+5)	103 kDa	0	2
Glucose-6-phosphate isomerase OS=Homo sapiens GN=GPI PE=3 SV=2	K7EQ48|K7EQ48_HUMAN (+2)	53 kDa	0	3
Inosine-5'-monophosphate dehydrogenase 2 (Fragment) OS=Homo sapiens GN=IMPDH2 PE=3 SV=1	H0Y4R1|H0Y4R1_HUMAN	51 kDa	0	3
ATP-dependent RNA helicase DDX18 OS=Homo sapiens GN=DDX18 PE=1 SV=2	Q9NVP1|DDX18_HUMAN	75 kDa	0	2
Dystroglycan OS=Homo sapiens GN=DAG1 PE=1 SV=2	Q14118|DAG1_HUMAN	97 kDa	0	2
Eukaryotic translation initiation factor 3 subunit A OS=Homo sapiens GN=EIF3A PE=3 SV=1	F5H335|F5H335_HUMAN (+1)	163 kDa	0	3
Lysine-specific histone demethylase 1A OS=Homo sapiens GN=KDM1A PE=4 SV=1	F6S0T6|F6S0T6_HUMAN (+2)	95 kDa	0	2
Integrin alpha-7 OS=Homo sapiens GN=ITGA7 PE=3 SV=1	H2N331|H2N331_HUMAN (+8)	122 kDa	0	2
Beta-mannosidase OS=Homo sapiens GN=MANBA PE=1 SV=3	O00462|MANBA_HUMAN	101 kDa	0	2
Isoform 2 of Neural cell adhesion molecule L1-like protein OS=Homo sapiens GN=CHL1	O00533-2|CHL1_HUMAN (+1)	137 kDa	0	2
Tubulin alpha-1B chain OS=Homo sapiens GN=TUBA1B PE=1 SV=1	P68363|TBA1B_HUMAN	50 kDa	0	2
A disintegrin and metalloproteinase with thrombospondin motifs 1 OS=Homo sapiens GN=ADAMTS1 PE=1 SV=4	Q9UHI8|ATS1_HUMAN	105 kDa	0	2
Isoform 5 of Glycogen debranching enzyme OS=Homo sapiens GN=AGL	P35573-2|GDE_HUMAN (+2)	173 kDa	0	2
Isoform 2 of Attractin OS=Homo sapiens GN=ATRN	O75882-2|ATRN_HUMAN (+2)	141 kDa	0	2
Ig alpha-1 chain C region OS=Homo sapiens GN=IGHA1 PE=1 SV=2	P01876|IGHA1_HUMAN	38 kDa	0	2
Laminin subunit alpha-1 OS=Homo sapiens GN=LAMA1 PE=1 SV=2	P25391|LAMA1_HUMAN	337 kDa	0	2
Isoform 2 of Nidogen-2 OS=Homo sapiens GN=NID2	Q14112-2|NID2_HUMAN (+1)	141 kDa	0	3
Trifunctional purine biosynthetic protein adenosine-3 OS=Homo sapiens GN=GART PE=1 SV=1	P22102|PUR2_HUMAN	108 kDa	0	2
Integrin alpha-9 OS=Homo sapiens GN=ITGA9 PE=1 SV=2	Q13797|ITA9_HUMAN	114 kDa	0	2
Isoform 2 of Endoplasmic reticulum aminopeptidase 1 OS=Homo sapiens GN=ERAP1	Q9NZ08-2|ERAP1_HUMAN (+1)	108 kDa	0	2
Collagen alpha-1(XXI) chain OS=Homo sapiens GN=COL21A1 PE=4 SV=1	F5GZK2|F5GZK2_HUMAN (+2)	99 kDa	0	2
Isoform 2 of Proline-rich transmembrane protein 3 OS=Homo sapiens GN=PRRT3	Q5FWE3-3|PRRT3_HUMAN (+1)	44 kDa	0	2
Isoform 1B of Desmocollin-1 OS=Homo sapiens GN=DSC1	Q08554-2|DSC1_HUMAN (+1)	94 kDa	2	0
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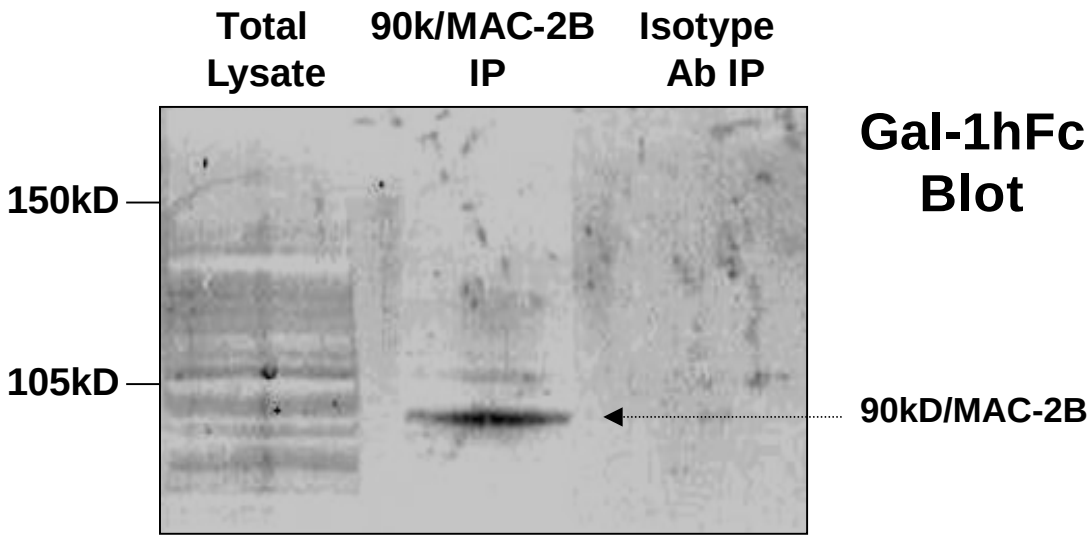
Integrin alpha-5 OS=Homo sapiens GN=ITGA5 PE=1 SV=2	P08648|ITA5_HUMAN	115 kDa	0	2
Isoform 2C2A of Collagen alpha-2(VI) chain OS=Homo sapiens GN=COL6A2	P12110-2|CO6A2_HUMAN (+2)	97 kDa	0	2
Protocadherin-7 OS=Homo sapiens GN=PCDH7 PE=4 SV=1	F5GWJ1|F5GWJ1_HUMAN (+2)	137 kDa	0	2
Protein disulfide-isomerase OS=Homo sapiens GN=P4HB PE=1 SV=3	P07237|PDIA1_HUMAN	57 kDa	0	2
Isoform 3 of Interleukin-6 receptor subunit beta OS=Homo sapiens GN=IL6ST	P40189-3|IL6RB_HUMAN (+1)	96 kDa	0	2
Isoform 2 of 1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase gamma-1 OS=Homo sapiens GN=PLCG1	P19174-2|PLCG1_HUMAN (+1)	149 kDa	0	2
Isoform 2 of General vesicular transport factor p115 OS=Homo sapiens GN=USO1	O60763-2|USO1_HUMAN (+1)	109 kDa	0	2
Zinc-alpha-2-glycoprotein OS=Homo sapiens GN=AZGP1 PE=3 SV=1	C9JEV0|C9JEV0_HUMAN (+1)	26 kDa	2	0
Ig kappa chain C region OS=Homo sapiens GN=IGKC PE=1 SV=1	P01834|IGKC_HUMAN	12 kDa	2	0
Ephrin type-B receptor 1 OS=Homo sapiens GN=EPHB1 PE=1 SV=1	P54762|EPHB1_HUMAN	110 kDa	0	2
Insulin-degrading enzyme OS=Homo sapiens GN=IDE PE=1 SV=4	P14735|IDE_HUMAN	118 kDa	0	2
Polymeric immunoglobulin receptor OS=Homo sapiens GN=PIGR PE=1 SV=4	P01833|PIGR_HUMAN	83 kDa	2	0

Supplemental Figure 2. **Identification of Gal-1-binding proteins by tandem MS/MS.** The following table lists human proteins and their accession number identified in eluates from protein G chromatography of human G361 cell lysate using Gal-1hFc or negative control dmGal-1hFc as probes. Semi-quantitative spectral counts (or # Peptide Matches) correspond with respective predicted proteins.

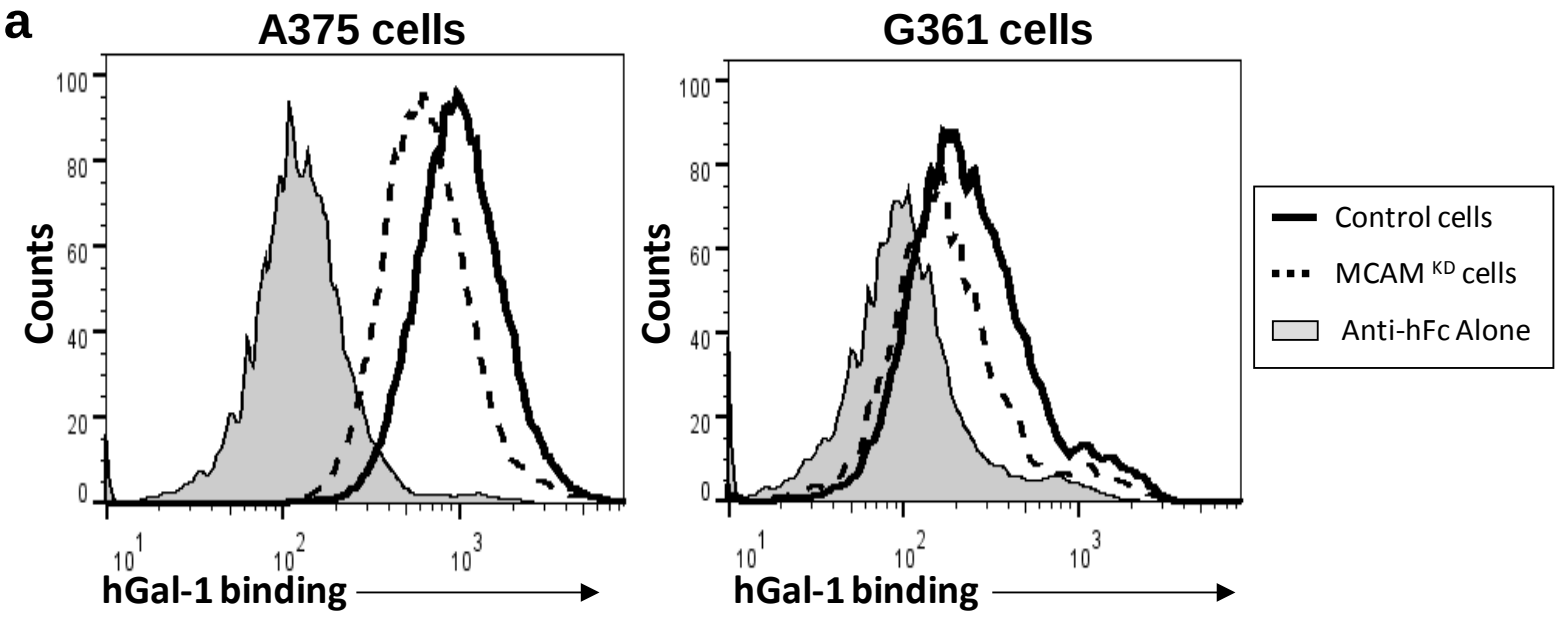
Supplemental Figure 3 and Legend

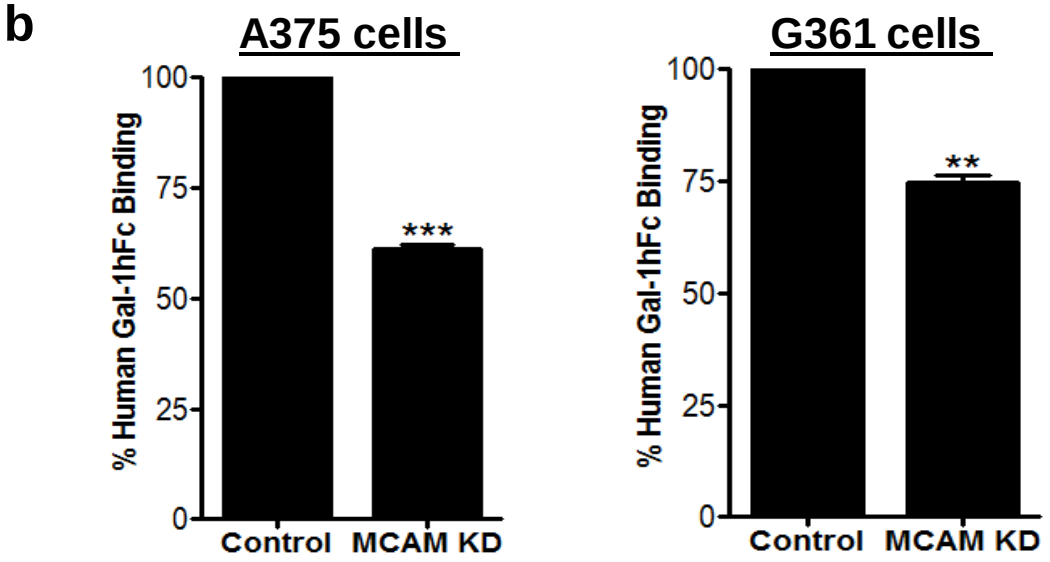


Supplemental Figure 3. Detection of Gal-3-binding Protein (90k/MAC-2B) immunoprecipitated from human melanoma cells with Gal-1hFc. Gal-3-binding protein (90k/MAC-2B) was immunoprecipitated with anti-90k moAb SP-2 or with isotype control Ab from human A375 melanoma cell lysate and blotted with Gal-1hFc. Total lysate, anti-90k immunoprecipitate (IP) and isotype control Ab IP were separated on reducing 4-20% gradient SDS-PAGE gels and blotted with Gal-1hFc. Data was representative from replicate experiments.

Supplemental Figure 4 and Legend

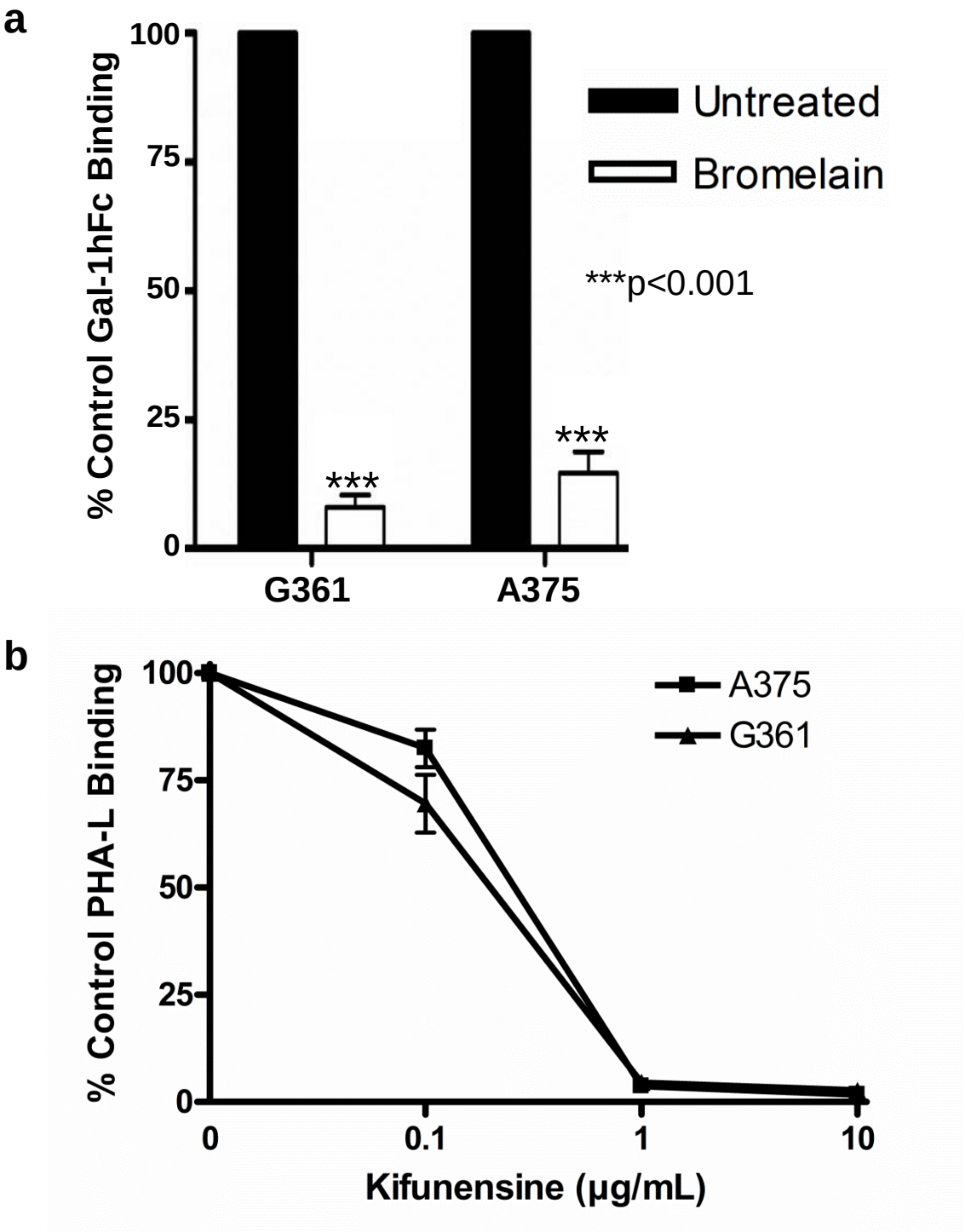
Human Gal-1 binding to melanoma cell Gal-1 ligands is dependent on MCAM.





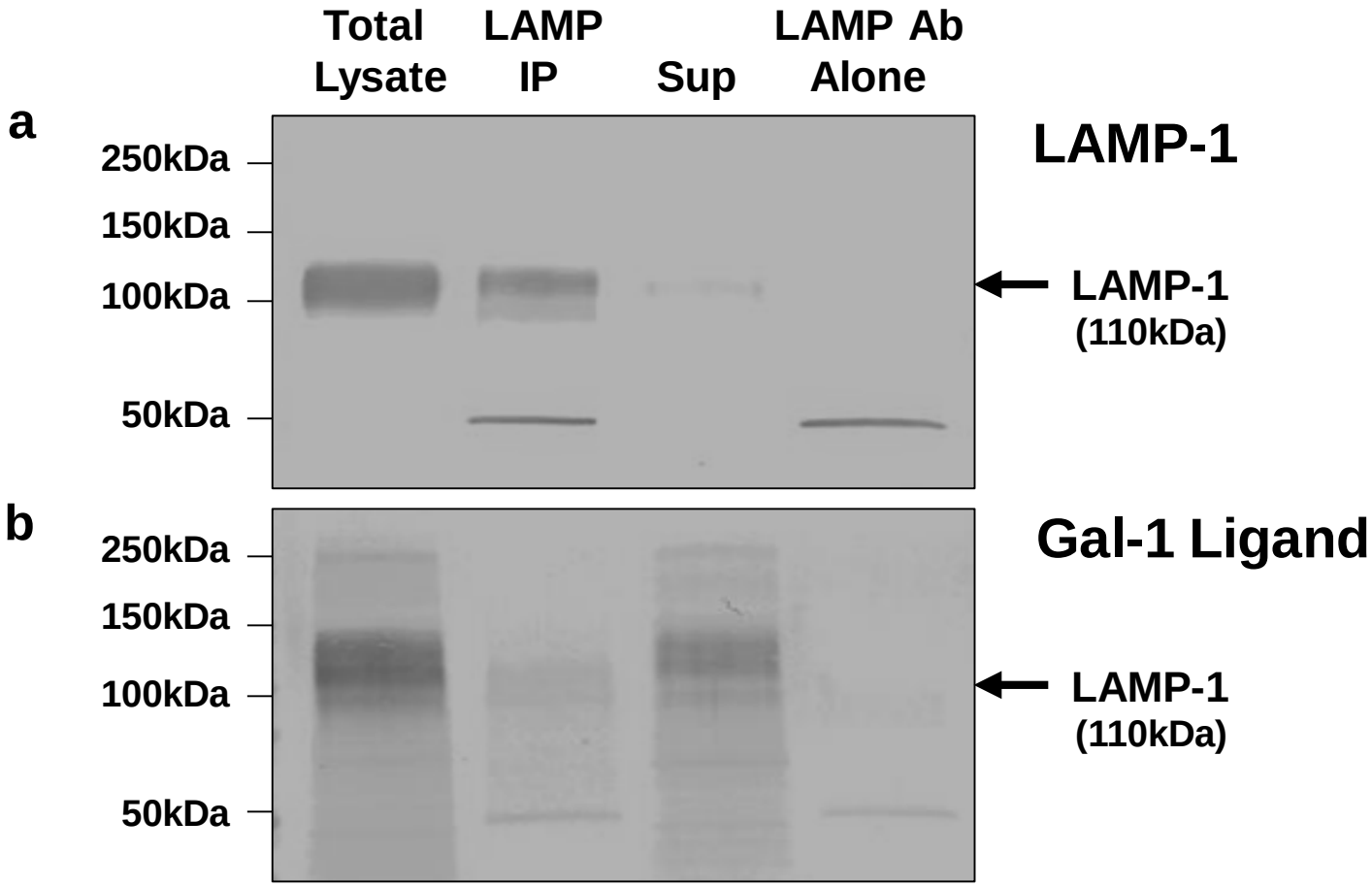
Supplemental Figure 4. Human Gal-1 binds melanoma cell Gal-1 ligands in a MCAM-dependent manner. (a) Representative histograms are shown for human scrambled control or MCAM^{KD} A375 and G361 melanoma cells FACS analyzed with human Gal-1 - hFc chimera (hGal-1) (45µg/ml) and anti-hFc Abs or with anti-hFc alone. **(b)** Relative binding of hGal-1 was quantified by mean fluorescence intensity after subtracting the hFc negative control. (Statistically significant difference; ***p=0.0002; **, p=0.0082). Data are representative of triplicate repeats.

Supplemental Figure 5 and Legend



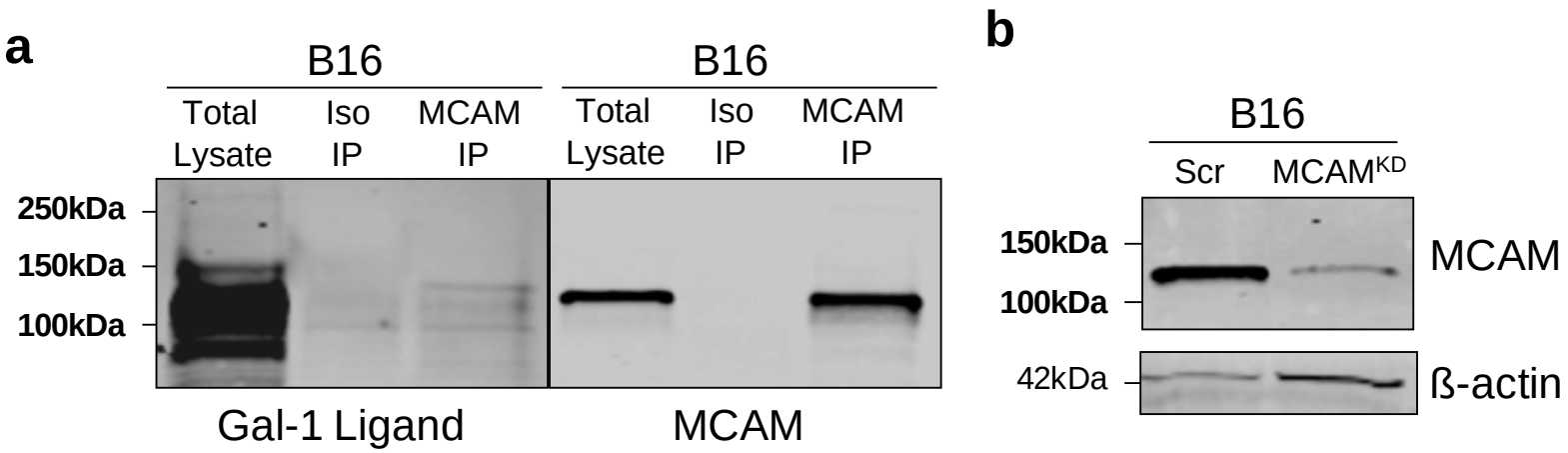
Supplemental Figure 5. The broadly-active protease, bromelain, digests the majority of melanoma cell Gal-1 ligand activity, and kifunensine effectively removes all complex N-glycans on melanoma cells. Prior to FACS assaying with Gal-1hFc, A375 and G361 melanoma cells were first treated for 1hr at 37ºC with 0.1U/ml bromelain or buffer control **(a)**. Alternatively, A375 and G361 cells were treated with 0-10µg/ml kifunensine for 48hr then FACS assayed for PHA-L-binding activity **(b)**. Data are representative of triplicate experiments.

Supplemental Figure 6 and Legend



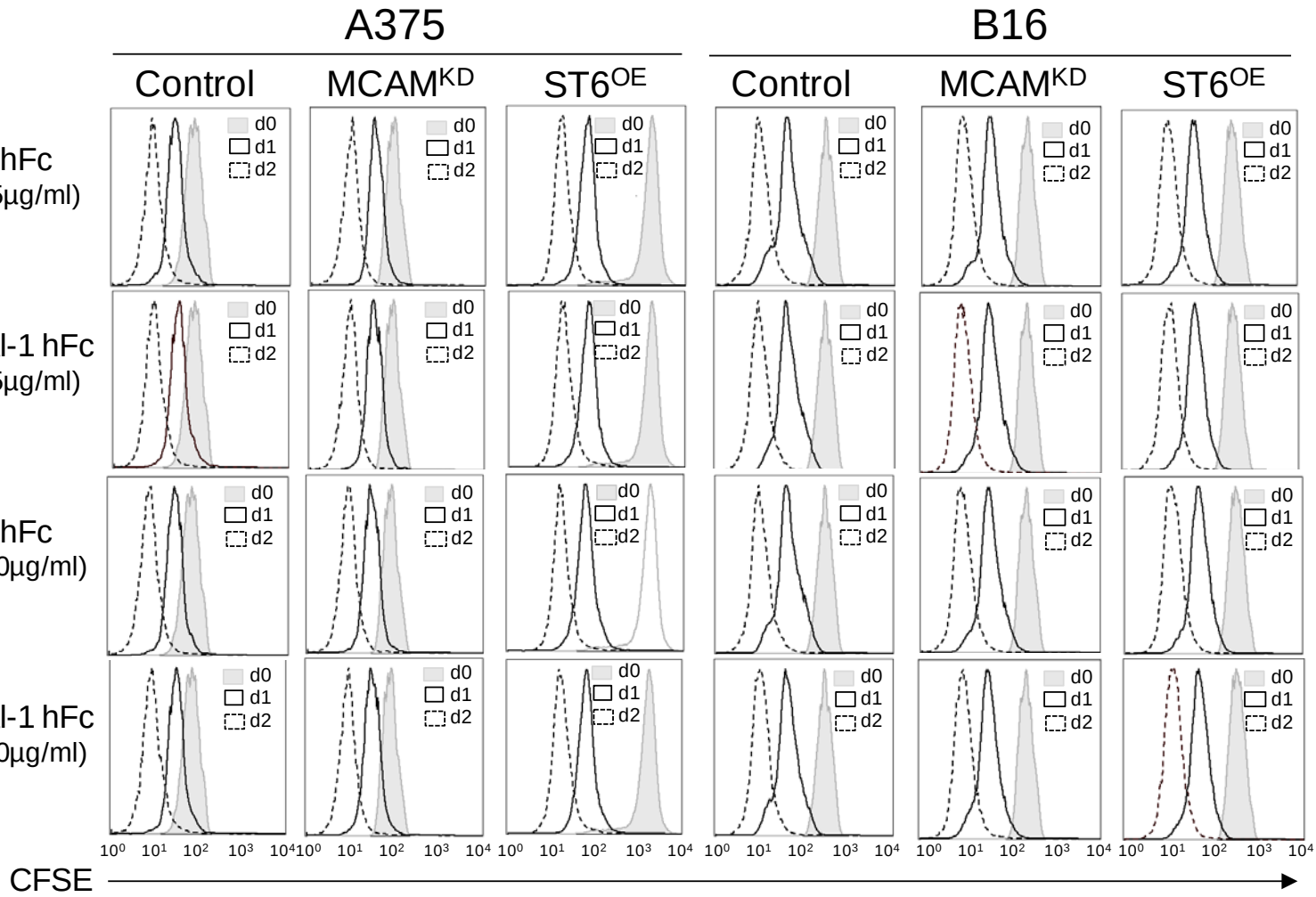
Supplemental Figure 6. LAMP-1 functions as a Gal-1 ligand on human melanoma cells. Western blot analysis of LAMP-1 was performed on human G361 melanoma cells. Total lysate, anti-LAMP-1 (clone H4A3) immunoprecipitate (LAMP IP), residual supernatant (Sup) or H4A3 Ab alone (LAMP Ab Alone) were separated on reducing 4-20% gradient SDS-PAGE gels and blotted with either **(a)** anti-LAMP (H4A3) Ab or **(b)** Gal-1hFc to identify LAMP-1 polypeptide and LAMP Gal-1 ligand activity, respectively. All experiments were performed 3-times.

Supplemental Figure 7 and Legend



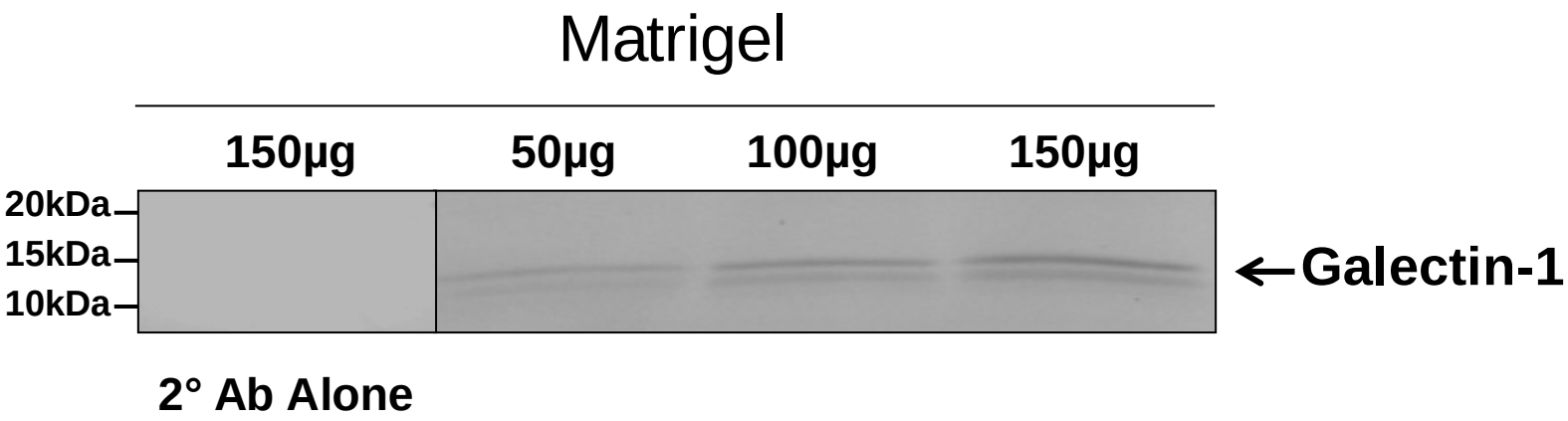
Supplemental Figure 7. Mouse melanoma B16 cells express Gal-1 ligand MCAM, and MCAM is silenced in MCAM^{KD} B16 cell variants. In **(a)**, lysate or anti-MCAM immunoprecipitate (IP) from mouse B16 melanoma cells was blotted with Gal-1 hFc or anti-MCAM Ab. In **(b)**, lysate from MCAM^{KD} B16 cells was blotted with anti-MCAM Ab or control anti-ß-actin Ab. Data are representative of triplicate experiments.

Supplemental Figure 8 and Legend



Supplemental Figure 8. Gal-1hFc incubation does not influence melanoma cell proliferation. Prior to assays, cells were first cultured for 24h with 50mM lactose to recapitulate pretreatment conditions in cell migration assays. Control, MCAM^{KD} or ST6$^{O/E}$ A375 and B16 melanoma cells were then loaded with CFSE and then incubated with Gal-1hFc or hFc controls at 5 or 20µg/ml for 48hr. Cells were analyzed for fluorescence intensity by flow cytometry at day 0 (grey fill), day 1 (open fill), or day 2 (dashed line). As shown, there was no difference in diluted fluorescence intensity in Gal-1hFc-treated cells when compared with cells treated with hFc. Data are representative of replicate experiments.

Supplemental Figure 9 and Legend



Supplemental Figure 9. Gal-1 is contained in the Matrigel. Matrigel (50, 100 and 150µg protein lane) was mixed with Laemmli reducing sample buffer and Western blotted with anti-Gal-1 antibody and doublet was stained at 14kDa. A control blot stained with AP-secondary Ab alone showed no staining. Western blots were repeated 3-times.

References

Barthel SR, Gavino JD, Wiese GK, *et al.* (2008) Analysis of glycosyltransferase expression in metastatic prostate cancer cells capable of rolling activity on microvascular endothelial (E)-selectin. *Glycobiology* 18:806-17.

Barthel SR, Hays DL, Yazawa EM, *et al.* (2013) Definition of molecular determinants of prostate cancer cell bone extravasation. *Cancer research* 73:942-52.

Cedeno-Laurent F, Watanabe R, Teague JE, *et al.* (2012) Galectin-1 inhibits the viability, proliferation, and Th1 cytokine production of nonmalignant T cells in patients with leukemic cutaneous T-cell lymphoma. *Blood* 119:3534-8.

Schatton T, Murphy GF, Frank NY, *et al.* (2008) Identification of cells initiating human melanomas. *Nature* 451:345-9.

Tsai CM, Chiu YK, Hsu TL, *et al.* (2008) Galectin-1 promotes immunoglobulin production during plasma cell differentiation. *Journal of immunology* 181:4570-9.

Tsai CM, Guan CH, Hsieh HW, *et al.* (2011) Galectin-1 and galectin-8 have redundant roles in promoting plasma cell formation. *Journal of immunology* 187:1643-52.