APPENDIX A

Table A.1 Primers used for cloning IGF2R into protein expression vectors Fwd=Forward, Rev=Reverse

Name	Sequence 5'-3' (restriction sites are indicated in bold)	Purpose	Restriction site
IGF2R-Fwd	GACT**GCGGCCGC**CACCATG GGGGCCGCCGCCGGCCG-GAG	Fwd primer for amplification of IGF2R ectodomain	NotI
IGF2R-Rev	CAGT**GGCGCGCC**GACTGCC TGGCTCCGTTCTGAC	Rev primer for amplification of IGF2R ectodomain	AscI
OL497	TGAGATCCAGCTGTTGGGGT	Fwd sequencing primer	-
OL4006	TCCCTGCAGGCTTTCCTCT CCAAGGTTGAG	Rev sequencing primer	-

Table A.2 Sequences of guide RNAs used in this study

Gene	Oligos: Sense (S) and antisense (AS)- 5'-3'	gRNA ID from Human v1 library
BSG	S: CACCGTCGTCAGAACACATCAACG	BSG_CCDS12032.1_ex2_19:580670
	AS: AAACCGTTGATGTGTTCTGACGAC	-580693:+_9-4
SDC1	S: CACCGATGAGACCTCAACCCCTGC	SDC1_CCDS1697.1_ex2_2:20403694
	AS: AAACGCAGGGGTTGAGGTCTCATC	-20403717:-_5-3
CD55	S: CACCGGGCCGTACAAGTTTTCCCG	CD55_CCDS31006.1_ex1_1:207495775
	AS: AAACCGGGAAAACTTGTACGGCCC	-207495798:+_5-1
CD44	S: CACCGTACAGCATCTCTCGGACGG	CD44_CCDS31455.1_ex1_11:35198176
	AS: AAACCCGTCCGAGAGATGCTGTAC	-35198199:+_5-2
EXTL3	S: CACCGGTCCATCCGTCCGCCAGTG	EXTL3_CCDS6070.1_ex0_8:28574365
	AS: AAACCACTGGCGGACGGATGGACC	-28574388:-_5-3
SLC35B2	S: CACCGTCGGCGCAGCTACGAACAC	SLC35B2_CCDS34462.1_ex0_6:44223027
	AS: AAACGTGTTCGTAGCTGCGCCGAC	-44223050:-_5-1
TP53	S: CACCGCAGTCACAGCACATGACGG	TP53_CCDS11118.1_ex6_17:7578415
	AS: AAACCCGTCATGTGCTGTGACTGC	-7578438:-_5-1
IGF2R(4)	S: CACCGCGGTCACTACGCATTCCAG	IGF2R_CCDS5273.1_ex16_6:160468883
	AS: AAACCTGGAATGCGTAGTGACCGC	-160468906:-_5-4
IGF2R(5)	S: CACCGACAACGACGGATACAGACC	IGF2R_CCDS5273.1_ex19_6:160477535
	AS: AAACGGTCTGTATCCGTCGTTGTC	-160477558:+_5-5
WDR7	S: CACCGTGCGGAATGAATCACTAGC	WDR7_CCDS11962.1_ex7_18:54358952
	AS: AAACGCTAGTGATTCATTCCGCAC	-54358975:-_5-5

Table A.3 Summary of the screening parameters for all screens carried out in this study. The table indicates the total number of cells screened within each library, the day the selection was performed, the number of collected cells, and the sorting threshold each for each indicated staining probe/cell line pairing used.

Cell line	Staining probe	Day of selection	Number of sorted cells in the library (x10^6)	Number of collected cells	Sorting threshold (% of non-binding cells selected)
HEK-293-E	BRIC229	15	100	380,000	0.5
HEK-293-E	BRIC126	9	80	280,000	1
HEK-293-E	B6H12	9	80	200,000	1
HEK-293-E	MEM6/6	16	60	40,000	0.2
HEK-293-E	BRIC5	9	50	120,000	0.8
HEK-293-E	P16	9	60	180,000	1
HEK-293-E	RH5	9	80	200,000	0.7
HEK-293-E	EBA181	14	70	420,000	1
HEK-293-E	MSRP5	14	70	390,000	1
HEK-293-E	APP	9	60	320,000	1.1
HEK-293-E	APLP2	9	70	330,000	1
HEK-293-E	G6B	15	100	800,000	0.75
HEK-293-E	CD226	9	70	340,000	1.7
HEK-293-E	EPHB1	9	70	300,000	1.4
HEK-293-E	LPHN1	14	70	1,200,000	4.3
HEK-293-E	GABBR2	14	125	780,000	2.3
NCI-SNU-1	SERA9	16	50	180,000	1
NCI-SNU-1	TNFRSF9	14	80	600,000	1
HEL	BRIC256	14	50	300,000	1.7

A.1 Protocol for PCR amplification of gRNAs from lentivirally transduced cells for Illumina sequencing.

First PCR: This set up is for analysis of gRNAs from high complexity samples (e.g. control samples).

Step 1: Set up the following PCR master mix and aliquot 50 μL to 36 wells.

Reagent	Volume per reaction	Master mix (x38)
Q5 Hot Start High-Fidelity 2× Master Mix	25 μL	950 μL
Primer (L1/U1) mix (10 μM each)	1 μL	38 μL
Genomic DNA (1 mg/mL)	2 μL	76 μL
H_2O	up to 50 μL	up to 1900 μL

Step 2: Run the following PCR program.

Cycle number	Denature	Annealing	Extension
1	98 °C, 30 s		
2-26	98 °C, 10 s	61 °C, 15 s	72 °C, 20 s
27			72 °C, 2 min

Step 3: Collect 5 μL PCR product from each well (180 μL in total) and purify using Qiagen PCR purification kit. Elute DNA into 50 μL EB buffer and measure DNA concentration.

Second PCR

Step 4: Dilute the first PCR product to 40 pg/μL and set up one PCR reaction per sample. The forward primer (PE 1.0) is common to all samples, whereas the reverse primers are sample specific index primers (refer to table A.4).

Reagent	Volume per reaction
KAPA HiFi HotStart ReadyMix	25 μL
Primer (PE1.0/index primer) mix (5 μM each)	2 μL
First PCR product (40 pg/μL)	5 μL
H_2O	18 μL

Step 5: Run the following PCR program.

Cycle number	Denature	Annealing	Extension
1	98 °C, 30 s		
2-15	98 °C, 10 s	66 °C, 15 s	72 °C, 20 s
16			72 °C, 5 min

Table A.4 Primer sequences for amplifying gRNA and NGS.

Name	Sequence 5'-3' (restriction sites are indicated in bold, * represents phosphorothioate)	Purpose
U1	ACACTCTTTCCCTACACGACGCTCTT-CCGATCTCTTGTGGAAAGGACGAAACA	Forward primer for gRNA amplification
L1	TCGGCATTCCTGCTGAACCGCTCTT-CCGATCTCTAAAGCGCATGCTCCAGAC	Reverse primer for gRNA amplification
PE1.0	AATGATACGGCGACCACCGAGATCTAC-ACTCTTTCCCTACACGACGCTCTTCCGATC*T	Forward primer for Illumina library preparation
iPCRTag1	CAAGCAGAAGACGGCATACGAGATAAC-GTGATGAGATCGGTCTCGGCATTCCTG-CTGAACCGCTCTTCCGATC*T	Reverse indexing primer for Illumina library preparation
iPCRTag2	CAAGCAGAAGACGGCATACGAGATAAA-CATCGGAGATCGGTCTCGGCATTCCTG-CTGAACCGCTCTTCCGATC*T	Reverse indexing primer for Illumina library preparation
iPCRTag3	CAAGCAGAAGACGGCATACGAGATATG-CCTAAGAGATCGGTCTCGGCATTCCTG-CTGAACCGCTCTTCCGATC*T	Reverse indexing primer for Illumina library preparation
iPCRTag4	CAAGCAGAAGACGGCATACGAGATAGT-GGTCAGAGATCGGTCTCGGCATTCCTG-CTGAACCGCTCTTCCGATC*T	Reverse indexing primer for Illumina library preparation
iPCRTag5	CAAGCAGAAGACGGCATACGAGATACC-ACTGTGAGATCGGTCTCGGCATTCCTG-CTGAACCGCTCTTCCGATC*T	Reverse indexing primer for Illumina library preparation
iPCRTag6	CAAGCAGAAGACGGCATACGAGATACA-TTGGCGAGATCGGTCTCGGCATTCCTG-CTGAACCGCTCTTCCGATC*T	Reverse indexing primer for Illumina library preparation
iPCRTag7	CAAGCAGAAGACGGCATACGAGATCAG-ATCTGGAGATCGGTCTCGGCATTCCTG-CTGAACCGCTCTTCCGATC*T	Reverse indexing primer for Illumina library preparation
iPCRTag8	CAAGCAGAAGACGGCATACGAGATCAT-CAAGTGAGATCGGTCTCGGCATTCCTG-CTGAACCGCTCTTCCGATC*T	Reverse indexing primer for Illumina library preparation
iPCRTag9	CAAGCAGAAGACGGCATACGAGATCGC-TGATCGAGATCGGTCTCGGCATTCCTG-CTGAACCGCTCTTCCGATC*T	Reverse indexing primer for Illumina library preparation
iPCRTag10	CAAGCAGAAGACGGCATACGAGATACA-AGCTAGAGATCGGTCTCGGCATTCCTG-CTGAACCGCTCTTCCGATC*T	Reverse indexing primer for Illumina library preparation
iPCRTag11	CAAGCAGAAGACGGCATACGAGATCTG-TAGCCGAGATCGGTCTCGGCATTCCTG-CTGAACCGCTCTTCCGATC*T	Reverse indexing primer for Illumina library preparation
iPCRTag12	CAAGCAGAAGACGGCATACGAGATAGT-ACAAGGAGATCGGTCTCGGCATTCCTG-CTGAACCGCTCTTCCGATC*T	Reverse indexing primer for Illumina library preparation

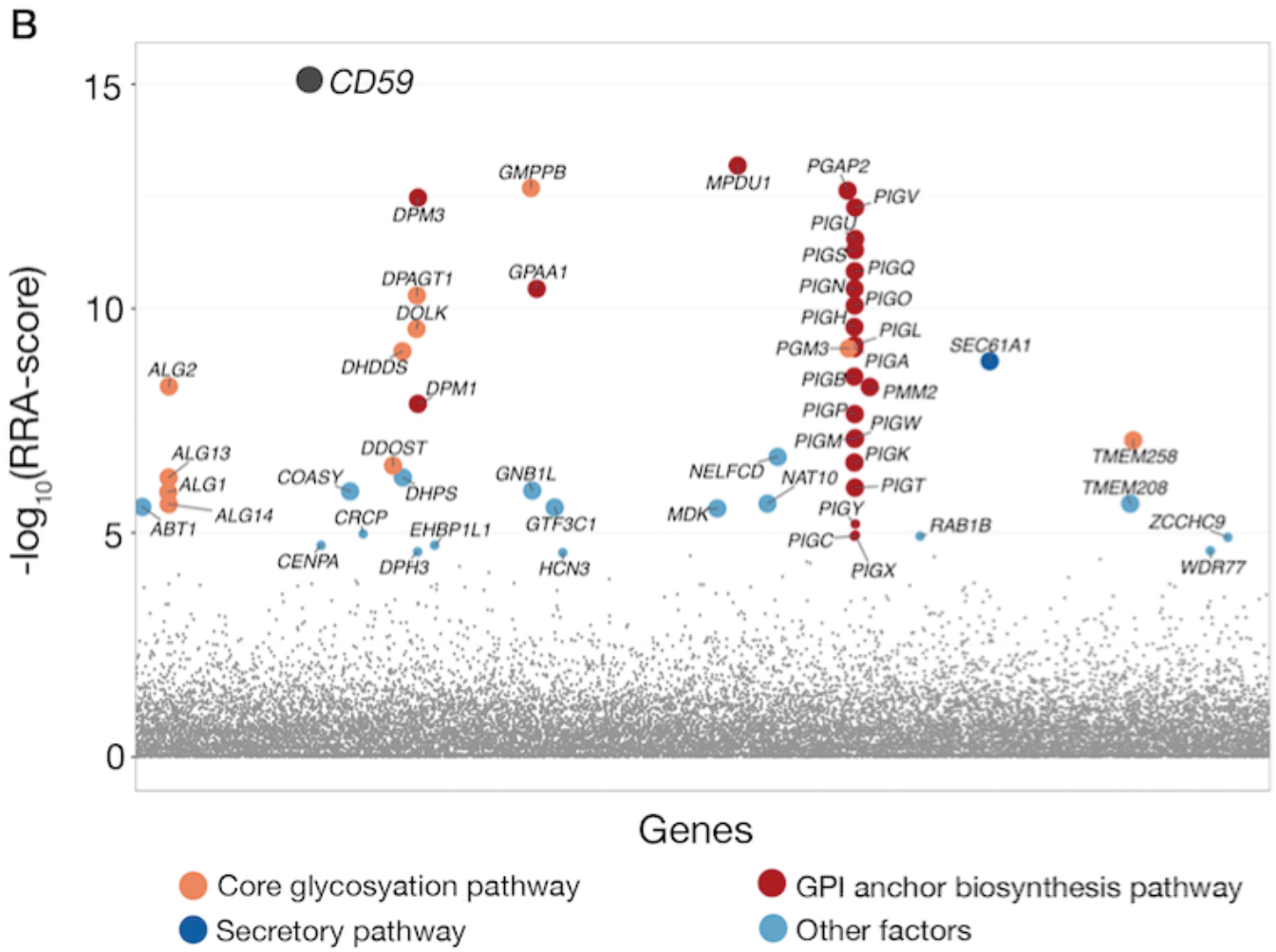

Fig. A.1 Enlarged version of figure 3.10B for better clarity of gene names

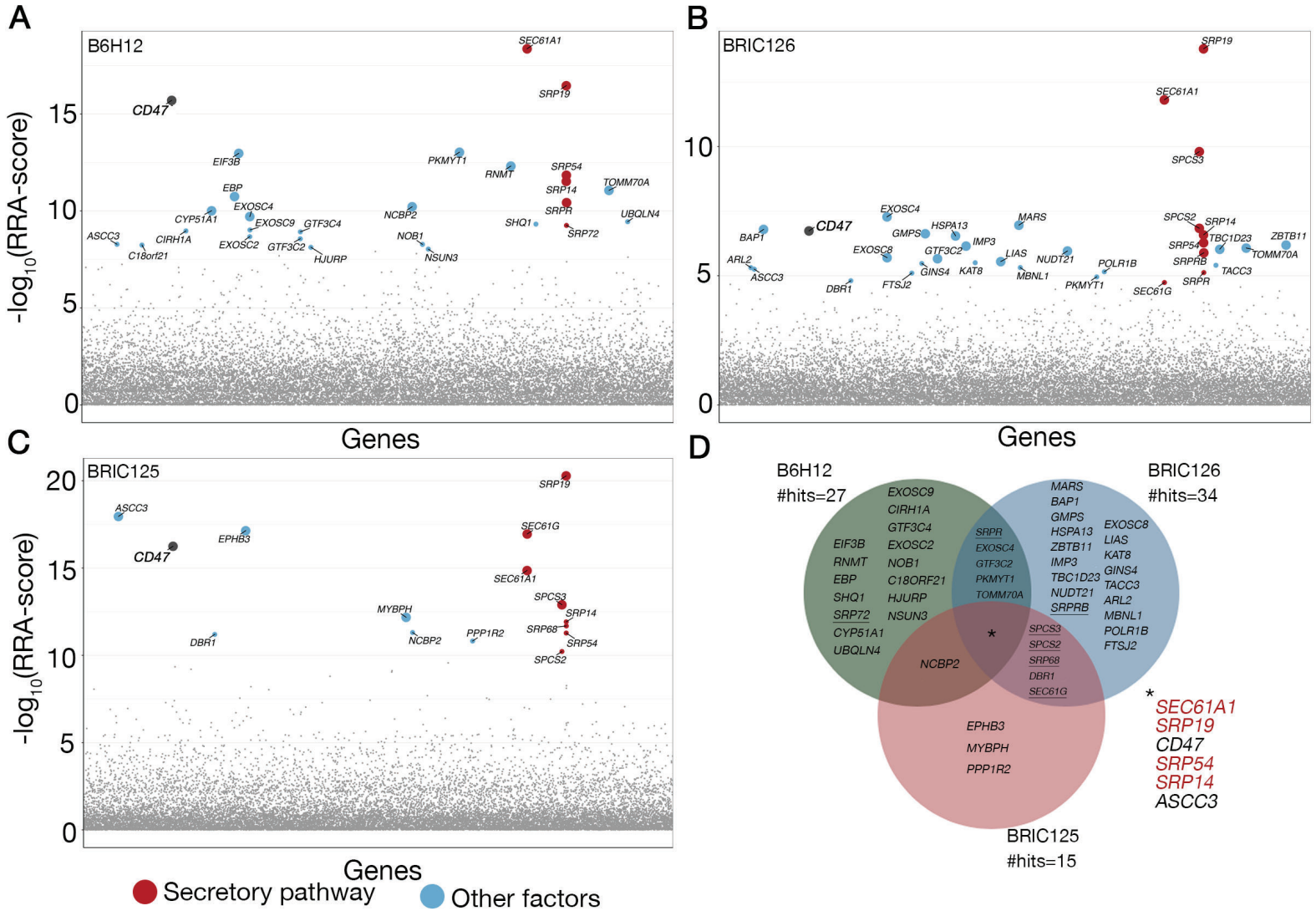

Fig. A.2 Enlarged version of figure 3.12 for better clarity of gene names

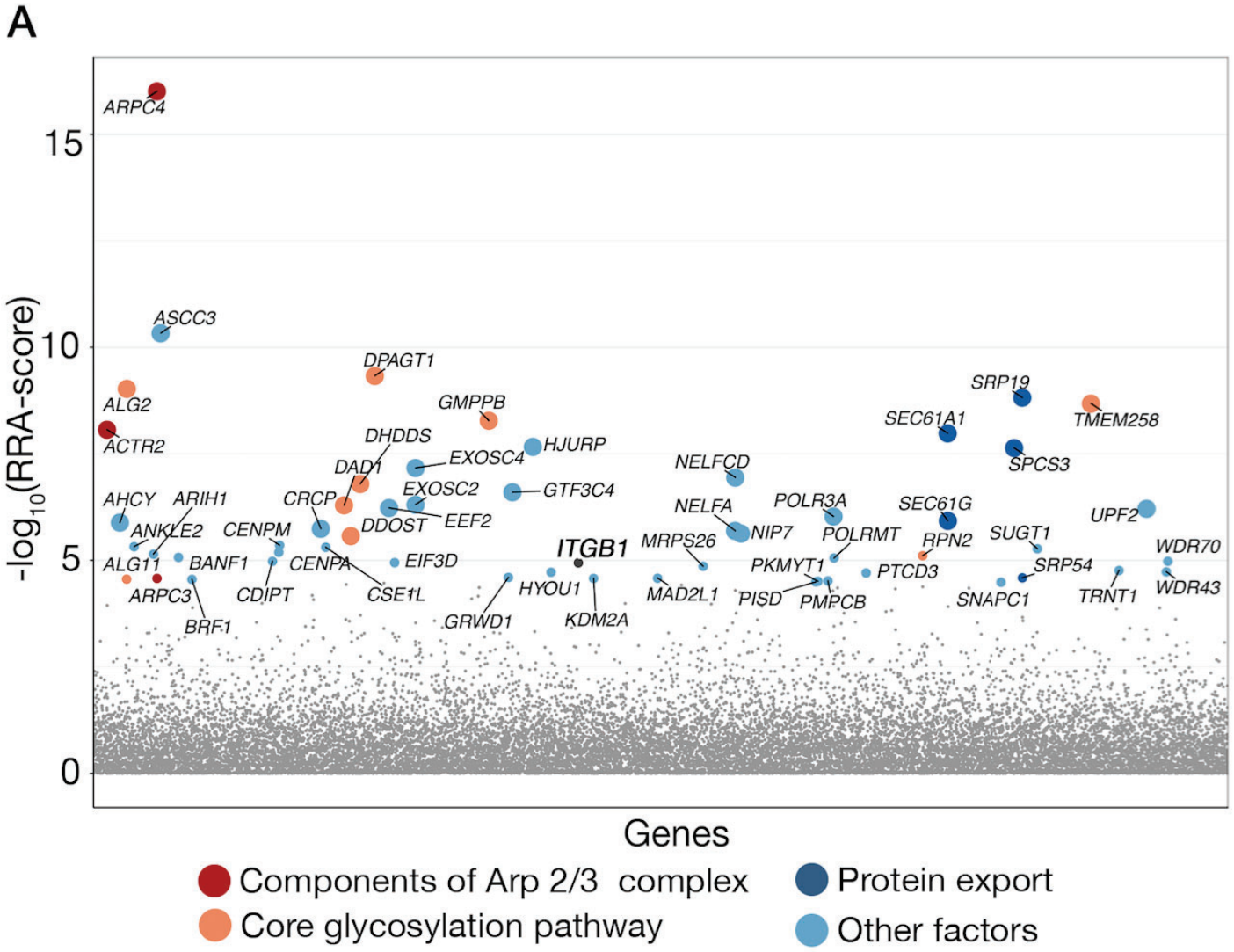

Fig. A.3 Enlarged version of figure 3.14A for better clarity of gene names

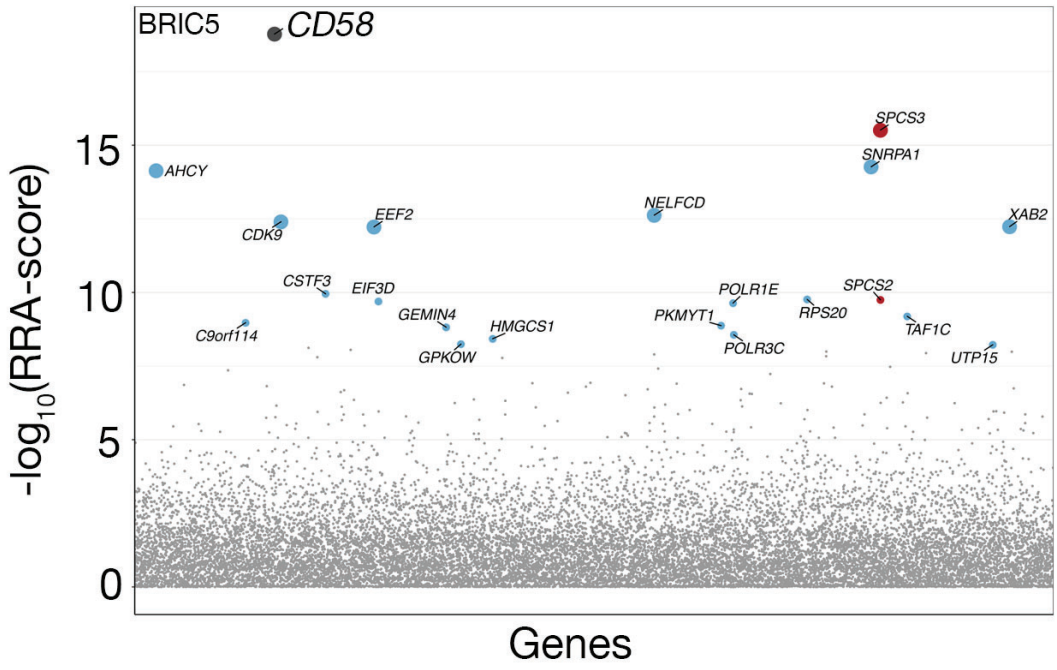

Fig. A.4 Genome-scale KO screen using an anti-CD58 mAb identifies *CD58* as the highest enriched gene in the sorted population. Gene-level enrichment analysis on a screen carried out using an anti-CD58 mAb. *CD58* was identified as the highest enriched gene. Two genes relating to general secretory were also identified (highlighted in red). Only genes with FDR<0.05 are depicted.

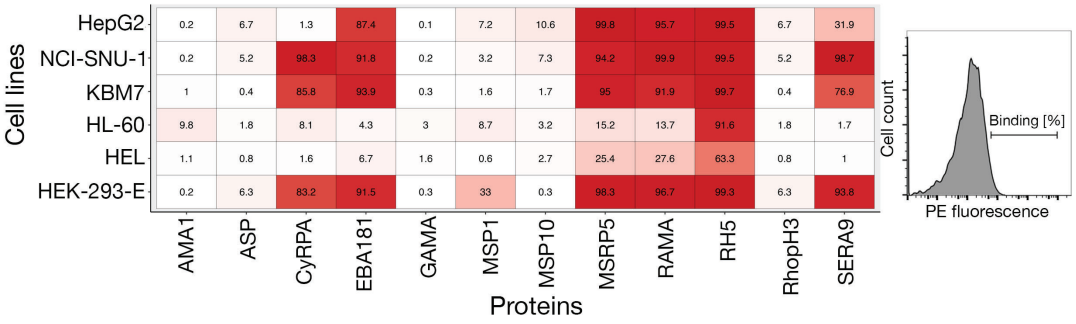

Fig. A.5 Primary screen for binding of 11 merozoite proteins to six cell lines. Biotinylated monomeric merozoite ectodomains conjugated to streptavidin-PE were tested for binding six cell lines. The number in each grid represents the percentage of cells that fell within the 'binding' gate, which was drawn on the histogram obtained from control protein binding to the parental cell lines (example depicted in the right panel). RH5 bound to all the tested cell lines, consistent with the expression of BSG in these lines (as determined from antibody staining with anti-BSG antibody- data not shown). HL-60 and HEL cells demonstrated restrictive binding profiles with clear binding only to RH5. Five ligands, SERA9, EBA181, MSRP5 CyRPA and RAMA bound at least three out of the six cell lines tested.

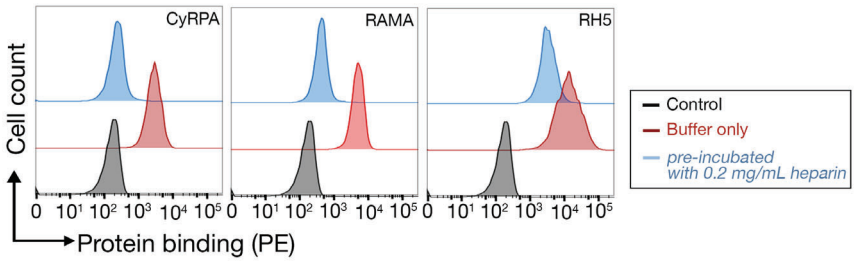

Fig. A.6 Binding of merozoite proteins RAMA and CyRPA to HEK-293-E cells can be completely blocked by soluble heparin. RAMA and CyRPA were identified in the preliminary screen to bind to HEK-293-E cells in a *SLC35B2*-dependent manner. Secondary validation with pre-blocking with soluble heparin (0.2 mg/mL) demonstrates complete inhibition of binding by soluble heparin. RH5 is used as a control; binding of RH5 is only partially blocked when pre-incubated with equivalent amount of heparin.

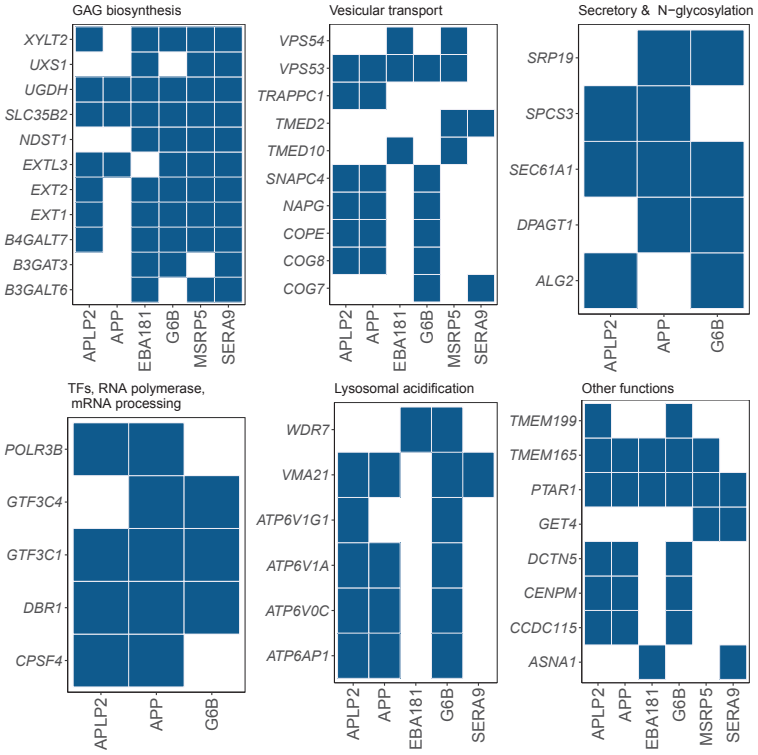

Fig. A.7 Summary of 'overlapping factors' identified in at least two out of six screen that identified the HS-biosynthesis pathway. Apart from the identification of multiple genes involved in biosynthesis of HS, these screens also revealed genes involved in protein transport and secretion, cellular house-keeping genes, genes involved in vesicular acidification, factors involved in global glycosylation in cells (*TMEM165, PTAR1*), and genes encoding for proteins involved in tail anchoring pathway (*ASNA1, GET4*). The screens with EBA181, MSRP5 and SERA9 were conducted on day 15/16 whereas the screens with G6B, APP and APLP2 were conducted on day 9: note that the representation of 'core-essential' genes (*SLC61A1, SRP19, ALG2, DPAGT1, GTF3C1, ATP6V1A, ATP6V0C, DBR1, CPSF4*) on screens carried out on later days is lower compared to the early time point screens.

Table A.5 Summary of 'other factors' identified with FDR<0.05 in at least two out of the seven different screens carried out using mAbs. Gene annotations are obtained from Uniprot.

Gene	Function	Identified in mAb targeting
ASCC3	Member of a family of helicases that are involved in the ATP-dependent unwinding of nucleic acid duplexes	CD47 (BRIC125, BRIC126, B6H12), ITGB1 (P16)
AHCY	Adenosylhomocysteinase; important for transmethylation reactions	CD58 (BRIC5), ITGB1 (P16)
HJURP	Centromeric protein that plays a role in the incorporation and maintenance of histone H3-like variant CENPA at centromeres	CD47 (B6H12), ITGB1 (P16)
CENPA	Centromere protein A- Histone H3-like variant	CD59 (BRIC 229), ITGB1 (P16)
CDIPT	Catalyzes the biosynthesis of phosphatidylinositol (PtdIns)	GYPA (BRIC 256), ITGB1 (P16)
EEF2	Eukaryotic Translation Elongation Factor 2	CD58 (BRIC5), ITGB1 (P16)
EIF3D	Eukaryotic Translation Initiation Factor 3 Subunit D	CD58 (BRIC5), ITGB1 (P16)
GTF3C2	General Transcription Factor IIIC Subunit 2	CD47 (B6H12, BRIC126)
GTF3C4	General Transcription Factor IIIC Subunit 4	CD47 (B6H12), ITGB1 (P16)
NELFCD	Essential part of the NELF complex- repress transcriptional elongation by RNA polymerase II	CD59 (BRIC229), ITGB1 (P16), CD58 (BRIC5)
NCBP2	Nuclear Cap Binding Protein Subunit 2	CD47 (BRIC125, B6H12)
PKMYT1	Member of the serine/threonine protein kinase family, which acts as a negative regulator of entry into mitosis	CD47 (BRIC126, B6H12), CD58 (BRIC5), ITGB1 (P16),
EXOSC4	Exosome component required for RNA processing and degradation activities	CD47 (BRIC126, B6H12), ITGB1 (P16)
EXOSC2	Exosome component required for RNA processing and degradation activities	CD47 (B6H12), ITGB1 (P16)
DBR1	RNA lariat debranching enzyme that facilitates ribonucleotide turnover	CD47 (BRIC125, BRIC126)

Table A.6 Genes identified in KO screens (FDR<0.05) using recombinant proteins as screening probes. The genes are ranked according to the RRA-score. Common gene refers to the genes identified repeatedly in screens using mAb (refer to table A.5). Gene annotations are obtained from Uniprot and annotations on core-essentiality are obtained from `http://www.hart-lab.org/Data/CEGv2.txt`.

Genes	Function	Rank	Notes
	Recombinant biotinylated RH5 conjugated to streptavidin-PE		
BSG	Known receptor for RH5	1	-
SLC35B2	Transporter of PAPS, required for GAG-biosynthesis	3	-
DBR1	RNA lariat debranching enzyme that facilitates ribonucleotide turnover	4	Common gene
SLC16A1	MCT1 transporter known to be required for transport of BSG	4	-
C9orf114	Required both for chromosome alignment and for association of the centrosomes	5	Core-essential gene
CCT3	Molecular chaperone required for protein folding	6	Core-essential gene
RPL27A	Encodes a ribosomal protein	7	Core-essential gene
SIN3A	Transcriptional regulatory gene	8	-
PSMG4	Promotes assembly of the 20S proteasome	9	-
FTSJ3	Probable methyltransferase	10	Core-essential gene
ARPC4	Component of Arp2/3 complex	11	-
XRCC5	Involved in NHEJ DNA repair	12	-
SRPRB	SRP Receptor Beta Subunit, general protein export	13	-
SRP19	Signal Recognition Particle 19, general protein export	14	Core-essential gene
ATRIP	Essential component of the DNA damage checkpoint	15	-
GNB2L1	Translational regulation	16	Core-essential gene
SART3	Encodes for RNA-binding nuclear protein, recycling factor of the splicing machinery	17	Core essential gene
CENPM	Centromere protein M	18	-
	Recombinant pentameric CD226		
PVR	Known receptor for CD226	1	-
DPAGT1	Core-glycosylation in the ER	2	Core-essential gene

Table A.6 – *Continued on next page*

Genes	Function	Rank	Notes
	Recombinant pentameric CD226 (*continued*)		
PIK3C3	Phosphatidylinositol 3-Kinase Catalytic Subunit Type 3, involved in multiple membrane trafficking pathways	3	-
TOP1	Topoisomerase	4	Core-essential gene
SEC61A1	General protein transport pathway	5	Core-essential gene
PIK3R4	Phosphoinositide-3-Kinase Regulatory Subunit 4, involved in multiple membrane trafficking pathways	6	-
GMPPB	Core-glycosylation pathway	7	Core-essential gene
SPDYC	Promotes progression through the cell cycle	8	-
ALG2	Core-glycosylation pathway	9	Core-essential gene
CHST8	Sulfotransferase: Transfer of sulfate N-acetylgalactosamine (GalNAc) residues in both N-glycans and O-glycans	10	-
WDR62	invoved in centriole duplication	11	-
DBR1	RNA lariat debranching enzyme that facilitates ribonucleotide turnover	12	Common gene
	Recombinant pentameric EPHB1		
EFNB2	Known receptor for EPHB1	1 -	
NDUFAF7	Arginine methyltransferase involved in the assembly or stability of mitochondrial NADH:ubiquinone oxidoreductase complex	2	-
GTF2H3	General Transcription Factor IIH Subunit 3	3	-
DDX27	DEAD-Box Helicase 27, required for the formation of ribosomal 47S rRNA	4	-
NDUFA3	Accessory subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase	5	-
LUC7L3	LUC7 Like 3 Pre-MRNA Splicing Factor	6	Core-essential gene
DR1	Down-Regulator Of Transcription 1	7	-
SPCS3	Signal Peptidase Complex Subunit, general protein transport	8	-

Table A.6 – *Continued on next page*

Genes	Function	Rank	Notes
	Recombinant pentameric EPHB1 (*continued*)		
RBM14	RNA Binding Motif Protein 14, a splicing modulator	9	Core-essential gene
SEC61G	General protein transport	10	-
HSD17B7	Hydroxysteroid 17-Beta Dehydrogenase 7, biosynthesis of cholesterol	11	-
	Recombinant pentameric LPHN1		
TMEM165	Global glycosylation in cells	1	-
TENM4	Known receptor for LPHN1	2	-
SLC35B2	Transporter of PAPS, required for GAG-biosynthesis	3	-
PTAR1	Global glycosylation in cells	4	-
ACTR2	Component of ARP2/3 complex	5	-
MCMDC2	Minichromosome Maintenance Domain Containing 2	6	-
EBP	Emopamil Binding Protein (Sterol Isomerase)	7	-
	Recombinant biotinylated TNFRSF9 conjugated to streptavidin-PE		
TNFSF9	Known interaction partner of TNFRSF9	1	-
CDKN2A	Binds to MDM2 and blocks MDM2-induced degradation of TP53	2	-
TP53	Tumor suppressor protein containing transcriptional activation, DNA binding, and oligomerization domains	3	-
CDC37	Molecular chaperone that forms a complex with Hsp90	4	Core-essential gene
DOHH	Required for generation of hypusine, an essential post-translational modification only found in mature eIF-5A factor	5	-
STK11	Serine/Threonine Kinase 11, able to phosphorylate TP53	6	-
DYRK1A	Member of Dual Specificity Tyrosine Phosphorylation Regulated Kinase family	7	-