

Supporting Information

Magnesium transporter 1 (MAGT1) deficiency causes selective defects in N-linked glycosylation and expression of immune-response genes

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Running title: MAGT1 deficiency causes selective N-linked glycosylation defect

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Signal peptide

Thioredoxin

H_sapiens	MAARWRFWCVSVTMVVA--LLIVCDVP--SASAQRKKEMVLSEKVSQ	LMEWNTN	57
P_troglodytes	MAARWRFWCVSVTMAVA--LLIVCDVP--SASAQRKKEMVLSEKVSQ	LM	57
M_musculus	MASPRWFWSVCAIAAVA--LLLVSQV--SASAQRKKEMVLSEKVSQ	LMEWA	57
R_norvegicus	MASPRWLWCVCATAAVT--LLLVSQV--SASAQRKKEKVLVEKVIQ	LME	57
C_familiaris	MAARWWLWCVSANMAVA--LLLSYGVP--SASAQRKKEMVLSEKVSQ	LME	57
B_taurus	MAARWWRWCVCAIMAVA--LLLVSQV--SASAQRKKEMVLSEKVSQ	LMEWNTN	57
G_gallus	-----MAALPVLVLV--LLLACGGP--RAAGQKRKEMVLSEKVSQ	LMEWTSK	50
X_laevis	-----MAGLKGLLFGG--ILFAMCGG--LSEGOKKKEMVLSDKVGQ	LMDWASK	51
D_rerio	-----MLHKLLIVVFLVVLHDM--RLNGQKKKETLLSEKVSQ	MMEWVSKR	50
D_melanogaster	-----MRL LHKTLLSGLLVVALFAIYAAAQS	KSKTGLSLSEKVQNL	54
C_elegans	-----MRTM--VLLFFMLLAVYE----	SAQQQTLEDKVQNLVDLTS	43

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Thioredoxin

H_sapiens	GDKFRRLVKAPPRNYSVIVMFTALQLHRCVVC	KQADEEFQILANSWR	YSSA	115
P_troglodytes	GDKFRRLVKAPPRNYSVIVMFTALQLHRCVVC	KQADEEFQILANSWR		115
M_musculus	GDKFRRLVKAPPRNYSVIVMFTALQLHRCVVC	KQADEEFQILANSWR	YSN	115
R_norvegicus	GDKFRPLVKAPPRNYSVIVMFTALQLHRCVVC	KQADEEFQILANFWRY		115
C_familiaris	GDKFRRLVKAPPRNYSVIVMFTALQLHRCVVC	KQADEEFQILANSWR		115
B_taurus	GDKFRRLVKAPPRNYSVIVMFTALQLHRCVVC	KQADEEFQILANSWR	YSSAF	115
G_gallus	GDKFRRLVKAPPRNYSVIVMFTALQPHRCVVC	KQADEEYQVLANSWR	YSSAF	108
X_laevis	GDKFRRFIKSPPRNYSVIVMFTALQAHRCVVC	KQADEEYQILANSWR	YSSAF	109
D_rerio	GEKFKRLVRAHPRNYSVIVMFTALQPORCGVC	RQADEEYQILANSWR	YSSAF-	108
D_melanogaster	GPKFREYVKSAPRNYSMIVMLTALAPSRCQIC	RHAHDEF	FAIVANSY	112
C_elegans	MDKWKTLVRMQPRNYSMIVMFTALSPGVC	CPICKPAYDEF	MIVANSHRYTSS	103

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Thioredoxin

H_sapiens	FAMVDFDEGSDVFQMLNMNSAPT	FINFPAK-GKPKRGD	TYELQVRGFS	SAEQI	174
P_troglodytes	FAMVDFDEGSDVFQMLNMNSAPT	FINFPAK-GKPKRGD	TYELQVRGFS		174
M_musculus	FAMVDFDEGSDVFQMLNMNSAPT	FINFPPK-GKPKRAD	TYELQVRGFS	AEQ	174
R_norvegicus	FAMVDFDEGSDVFQMLNMNSAPT	FINFPPK-GKPKRAD	TYELQVRGFS	A	174
C_familiaris	FAMVDFDEGSDVFQMLNMNSAPT	FINFPAK-GKPKRGD	TYELQVRGFS	A	174
B_taurus	FAMVDFDEGSDVFQMLNMNSAPT	FINFPAK-GKPKRGD	TYELQVRGFS	AEQIA	174
G_gallus	FAMVDFDEGSDVFQMLNMNSAPT	FINFPAK-GKPKRGD	TYELQVRGF	AAEQLA	167
X_laevis	FAVDFDEGSDVFQMLNMNSAPT	FINFPPK-GKPKKGD	TYELQVRGF	AAEQLA	168
D_rerio	FAMVDFDEGSDVFQMLNMNSAPT	FINFPAK-GKPKRAD	TYELQVRGF	AAEQLAR	167
D_melanogaster	FAMVDFDDGSEVFQLLRNLNTAPV	FMHFPAK-GKPKGAD	TMDIHRVGF		171
C_elegans	FGIVDYEDAPQIFQOMNLNTAPIL	YHFGPKLGAKKRPE	QMDFORQGF	DADAI	163

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Transmembrane Helix 1

Transmembrane Helix 2

H_sapiens	DVNIRVIRPPNYAGPLMLGLLLAVIGGLVYL	RRSNMEFLFNKTGW	AFAALCF	234
P_troglodytes	DVNIRVIRPPNYAGPLMLGLLLAVIGGLVYL	RRSNMEFLFNKTGW	AFA	234
M_musculus	DVNIRVIRPPNYAGPLMLGLLLAVIGGLVYL	RRSNMEFLFNKTGW	AFAALC	234
R_norvegicus	DVNIRVIRPPNYAGPLMLGLLLAVIGGLVYL	RRSNMEFLFNKTGW	AFAA	234
C_familiaris	DVNIRVIRPPNYAGPLMLGLLLAVIGGLVYL	RRSSMEFLFNKTGW	AFAA	234
B_taurus	DVNIRVIRPPNYAGPLMLGLLLAVIGGLVYL	RRSNMEFLFNKTGW	AFAALCFV	234
G_gallus	DVNIRVIRPPNYAGPLMLGLLLAVIGGLVYL	RGSNLDFLYNKTGW	AFAALCFV	227
X_laevis	DVNIRVIRPPNYAGPLMLGLLLAVIGGLVYL	RRSNLDFLNNKTGW	ALAALCFV	228
D_rerio	DVHIRVIRPPNYAGPLMLGLLAFIGSLAYL	RRNNLEFLFNKNV	WAFSALCFVL	227
D_melanogaster	DITIRIFRPPNYSGTVAMITLVALVGSFLYI	RRNNLEFLYNKNL	WGAWA	231
C_elegans	EVHVRVIRPPNYTAPVVIALLFVALLGMLYMK	RNSLDLFLFNRTV	WGFVCLAI	223

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	Transmembrane Helix 2	Transmembrane Helix 3	
H_sapiens	MWNHIRGPPYAHKNPHTGHVNYIHGSSQAQ	FVAETHIVLLFNGGVTLGMVLL	294
P_troglodytes	MWNHIRGPPYAHKNPHTGHVNYIHGSSQAQ	FVAETHIVLLFNGGVTLG	294
M_musculus	MWNHIRGPPYAHKNPHTGHVNYIHGSSQAQ	FVAETHIVLLFNGGVTLGMVL	294
R_norvegicus	MWNHIRGPPYAHKNPHTGHVNYIHGSSQAQ	FVAETHIVLLFNGGVTLGM	294
C_familiaris	MWNHIRGPPYAHKNPHTGHVNYIHGSSQAQ	FVAETHIVLLFNGGVTLGM	294
B_taurus	MWNHIRGPPYAHKNPHTGHVNYIHGSSQAQ	FVAETHIVLLFNGGVTLGMVLLC	294
G_gallus	MWNHIRGPPYAHKNPHTGQVNYIHGSSQAQ	FVAETHIVLLFNGGVTLGMVLLH	287
X_laevis	MWNHIRGPPYAHKNPHTNQVNYIHGSSQAQ	FVAETHIVLLFNQAVTLGMVLLH	288
D_rerio	MWNHIRGPPYAHKNPNTGQVSYIHGSSQAQ	FVAETHIVLLFNAAVTIGMVLLHE	287
D_melanogaster	MWNHIRGPPPLVHKSQ-NGGVAYIHGSSQGLVVETIIVMFLNAMIVL		290
C_elegans	MWNHIRGPPFMITNPNTKEPSFIHGSTQFQLIAETIIVGLLYALIAIGFICV		283
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	Transmembrane Helix 4		
H_sapiens	DIG-----	KRKIMCVAGIGLVVLFSSWMLSIF	RSKYHGYPY
P_troglodytes	DIG-----	KRKIMCVAGIGLVVLFSSWMLSIF	RSKYH
M_musculus	DIG-----	KRRMMCIAGIGLVVLFSSWMLSIF	RSKYHGYP
R_norvegicus	DIG-----	KRRMMCIAGIGLVVLFSSWMLSIF	RSKYHG
C_familiaris	DIG-----	KRKIMCVAGIALVVLFSSWMLSIF	RSKYHG
B_taurus	DIG-----	KRKIMCVAGIGLVVLFSSWMLSIF	RSKYHGYPYS
G_gallus	DVG-----	KRKIMCIAGIGLVVFFSWLLSVF	RSKYHGYPYS
X_laevis	DVG-----	KRKIMCIAGITLVVIFSWLLSVF	RSKYHGYPYS
D_rerio	DIV-----	KRKIMCVAGIGLVVLFSSWLLSVF	RAKYHGYPYSF
D_melanogaster	-HN-----	KNRIMAMTGLVLLTVFFSFLSVF	RSKA
C_elegans	SKDRKNAGKKNLPLSLLNIPTN	TLAIAGLVCICVFFSFLSVF	RSKYRGYPY
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Figure S1A & S1B. Comparison of MAGT1 amino acid sequences in animals of varying complexity or relative evolutionary distance from *H. sapiens*.

The aligned amino acid sequences of MAGT1 homologs are shown, with the regions corresponding to the signal peptide (blue box), TRX domain (green box) containing the CxxC (purple box) and *cis*-proline (orange box) motifs, and four transmembrane helices (red boxes) indicated.

	Signal peptide		Thioredoxin	
MagT1	MAARWRFWCVSVTMVALLIVCDVPSASA	QRKKEMVLSEKVSQ	LMEWTNKR--VI	58
Ost3	-----MNWLFVSLVFFCGVSTHPALA	-----MS--SNR	LLKLANKSPKKIIP	44
Ost6	-----MKWCSTYII IWLAIIFHKFQKSTA	-----TASHNIDD	LILQLKDDTGVI TVTA	49
	. :: ::: . . . *		: ..	
		Thioredoxin		
MagT1	DKFRRLVKAPPRNYSVIVMFTALQLHRQCVVCKQADEEFQILANSWR-----YSSAFTN			112
Ost3	SSFENILAPPHENAYIVALFTATAPEIGCSLCELESEYDTIVASWFDDHPDAKSSNSDT			104
Ost6	YPLLSRGVPGYFNILYITMRGTNSNGMSCQLCHDFEKTYHAVADVIR-----SQAPQS			102
	:	.		
		Thioredoxin		
MagT1	RIFFAMVDFDEGS----DVFQMLNMNSAPTFINFPKAGKPKRGD-----TYELQVRGF			161
Ost3	SIFFTKVNLEDPSTIPKAFQFFQLNNVPRLFIFKPNSPSILDHSVI----SISTDTGSE			160
Ost6	LNLFFTVDVNEVP----QLVKDLKLQNVPHLVVYPPAESNKQSQFEWKTSPFYQYSLVPE			158
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	Thioredoxin	Transmembrane Helix 1	Transmembrane Helix 2	
MagT1	SAEQIARWIADRTDVNIRVIRPPNYAGP-LMLGLLLAVIGGLVYLRRSNMEFLFN--			218
Ost3	RMKQIIQAIKQFSQVND FSLHLPMDWTP-IITSTIITFITVLLFKKQSKLMFSIISR			219
Ost6	NAENTLQFGDFLAKILNISITVPOAFNVQEFVYFVACMVVFIKVKVILPKVTNKWK			218
	:: :	: : : :	* :	: : : : :
		Transmembrane Helix 2	Transmembrane Helix 3	
MagT1	WAFALCFVLAMTSGQMNHNIRGPPYAHKNPHTGHVNYIHGSSQAQFVAETHIVLLF			278
Ost3	WATLSTFFIICMISAYMNFNQIRNTQLAGVGPKEVYFLPNEFQHOFAIETQVMVLIY			279
Ost6	SMILSLGILLPSITGYKFEVEMNAIPFIARDAKNRIMYFSGGS-GWQFGIEIFSVSLMY			277
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	Transmembrane Helix 3	Transmembrane Helix 4		
MagT1	VTLGMVLLCEAAT---SDMDIGKRKIMCVAGIG-----LVVLFVSWMLSIFRSKYH			329
Ost3	LALVVLVVKGIQFLRSHLYPETKKAYFIDAILASFCALFIYVFFAALTTVFTIKSPA			339
Ost6	MSALSVLLIYVPKISCVSEKMRGLLSSFLACVL-----FYFFSYFISCYLIKNP			329
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MagT1	YSFLMS-----	335		
Ost3	FPLLRLSAPFK	350		
Ost6	IVF-----	332		

Figure S2. Comparison of the amino acid sequences MAGT1, *S. cerevisiae* OST3 and OST6. Conserve functional regions of the indicated proteins using the same color scheme as shown in Supplemental Figure 1.

<u>C</u> V <u>V</u> C	MAGT1
<u>C</u> S <u>V</u> C	TUSC3
<u>C</u> S <u>L</u> C	OST3 (<i>S. cerevisiae</i>)
<u>C</u> Q <u>L</u> C	OST6 (<i>S. cerevisiae</i>)
<u>C</u> G <u>P</u> C	Thioredoxin 1/2
<u>C</u> G <u>H</u> C	Protein Disulfide Isomerase

Figure S3. The CxxC motif of OST3/OST6 and MAGT1/TUSC3 are atypical of highly active TRX domains.

The motif is shown in single letter amino acid code for the indicated proteins.

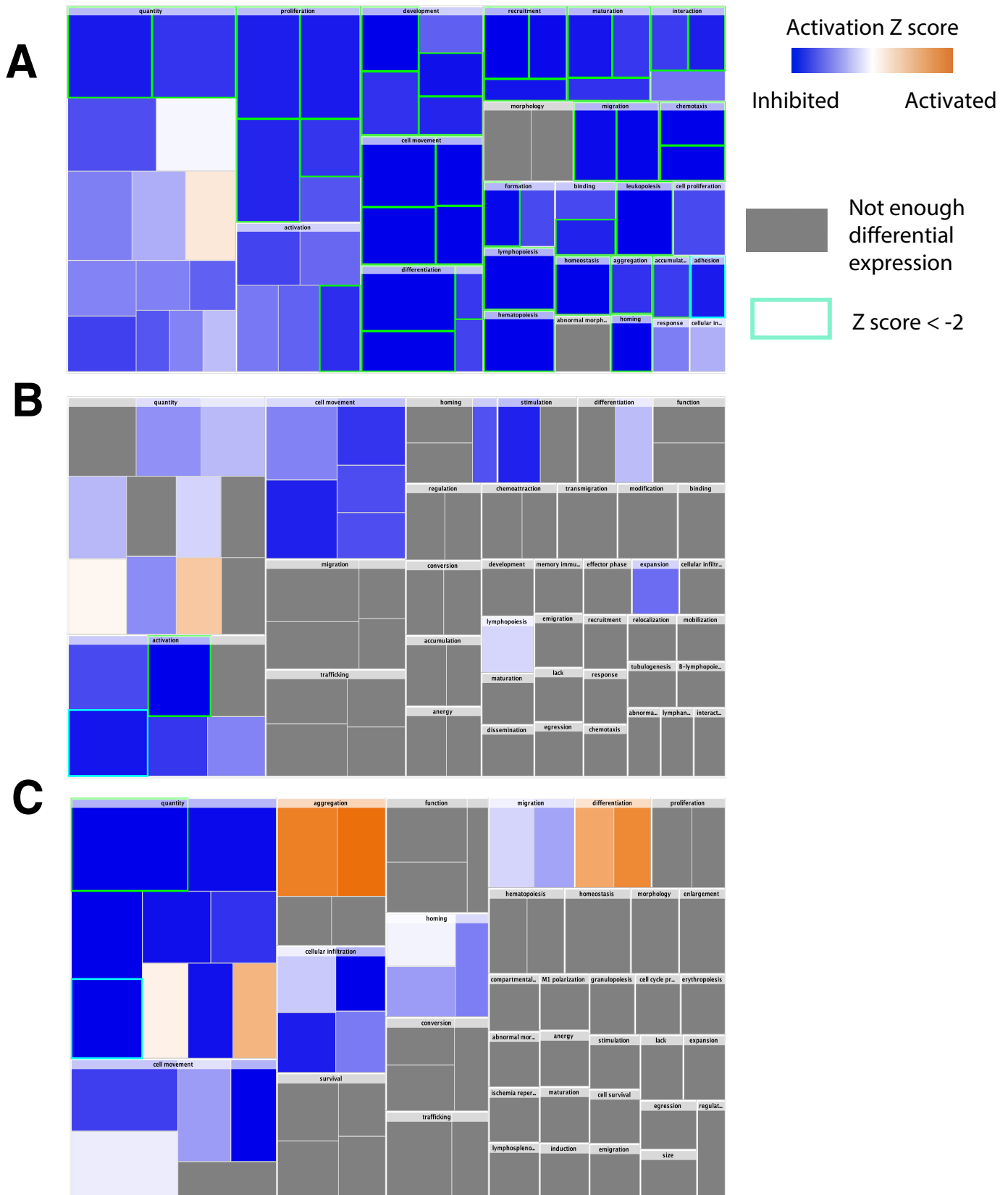


Figure S4. Treemap heatmap of hematological system development and function in XMEN patients. (A-C). Treemap representation from IPA of predicted activation (orange) or inhibition (blue) of various functions within hematological system based on DEGs at (A) day 0, (B) day 3, and (C) day 12. The treemaps are divided hierarchically into categories and subcategories. Categories have labels (e.g., “quantity”) and all boxes below the label represent subcategories that belong to the labeled category.

The size of each box is proportional to $-\log(\text{p-value})$ for the activation prediction and categories are sorted in descending order from left to right based on the sum of the area of all of the subcategories belonging to each of the categories. The key in (A; right) is used across all treemap plots. The color key with gradient from dark blue to white to dark orange represents the Z-scores for each plot, where negative values indicate predicted inhibition and positive values indicate predicted activation. Filled grey boxes indicate that not enough DEGs fall into the subcategory to predict whether the function is activated or inhibited. Blue-green highlighted borders around subcategory boxes represent functions with a Z-score < -2 , which are considered significantly decreased based on the gene expression profile of the genes falling into the subcategory.

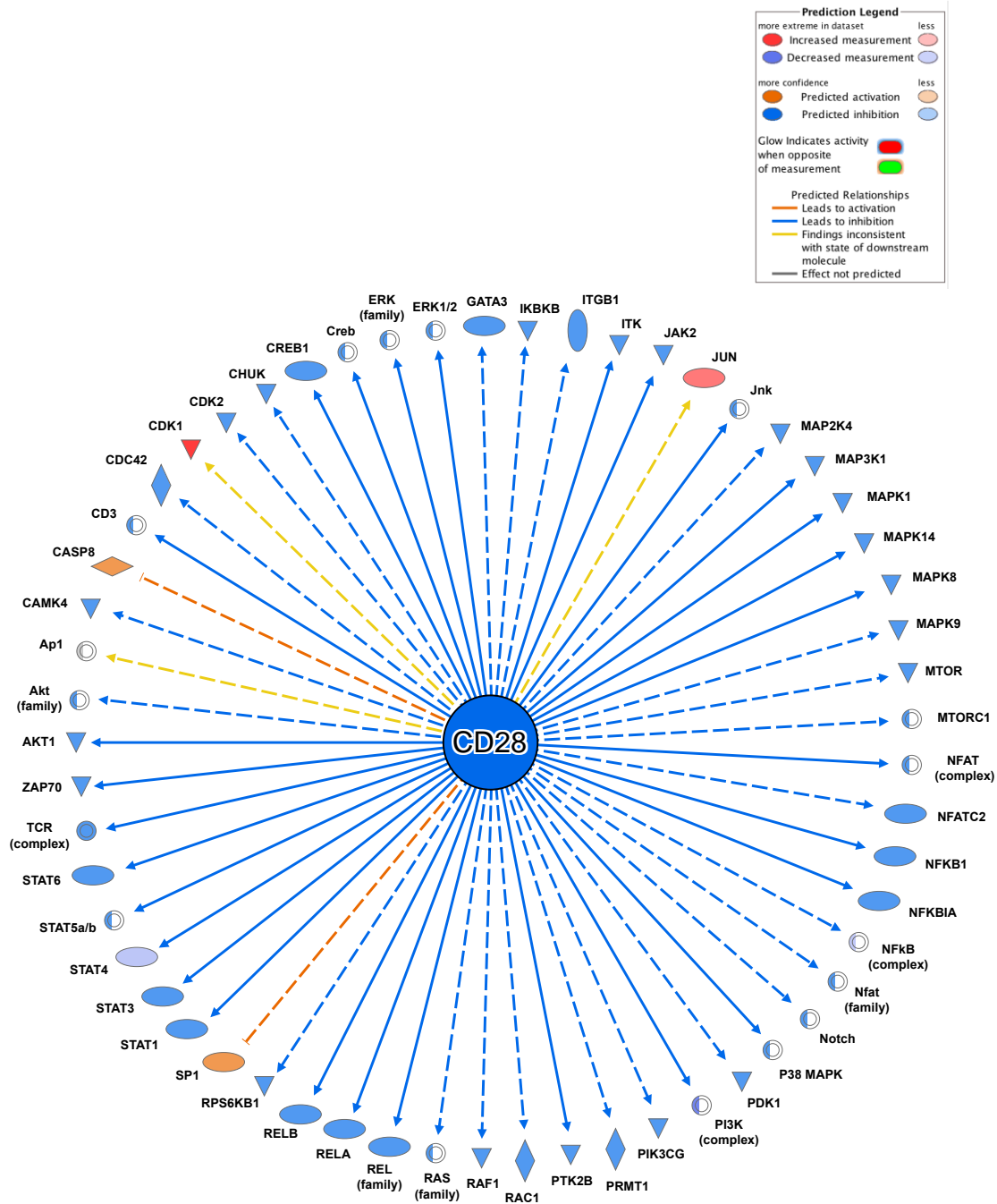


Figure S5. CD28 causal regulatory network.

Network of upstream regulators predicted by IPA to be inhibited (blue) or activated (orange) based on the gene expression profile of day 0 XMEN CD8⁺ T cells compared to HC cells. Upstream of all of these regulators is the master regulator CD28, which is predicted to be inhibited (blue) based on the activation status of the downstream targets. Solid and dotted lines indicate direct and indirect interactions, respectively. Blue (inhibiting) and orange (activating) lines indicate the effect of CD28 inhibition on its targets. Yellow lines indicate a relationship where activation state of the master regulator is inconsistent with the state of the targets.

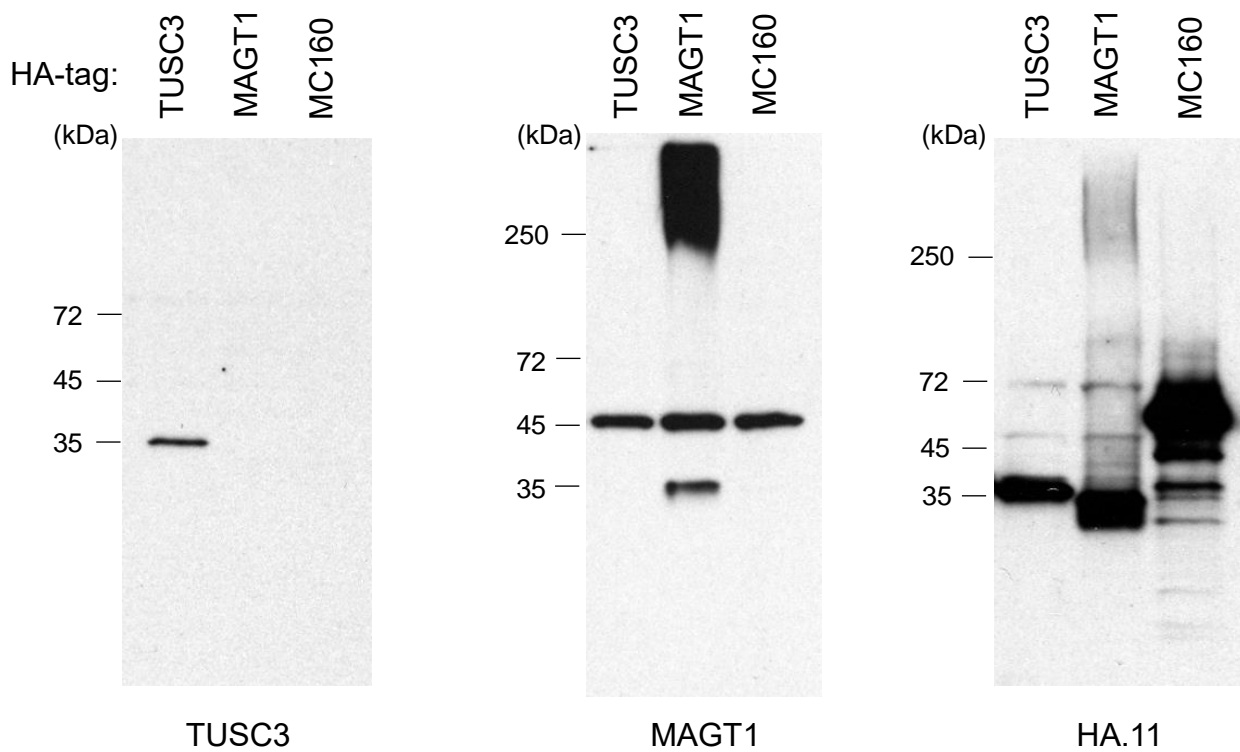


Figure S6. Specificity of TUSC3 and MAGT1 antibodies.

Western blots of lysates from 293T cells transfected with the indicated, HA-tagged TUSC3, MAGT1, and negative control MC160L(GFP) as indicated. Samples were probed with rabbit monoclonal antibodies. A: anti TUSC3, (clone 38-1, affinity purified); B: anti MAGT1 (clone:M17-1, 1:50 dilution); and C: mouse anti HA (HA.11).