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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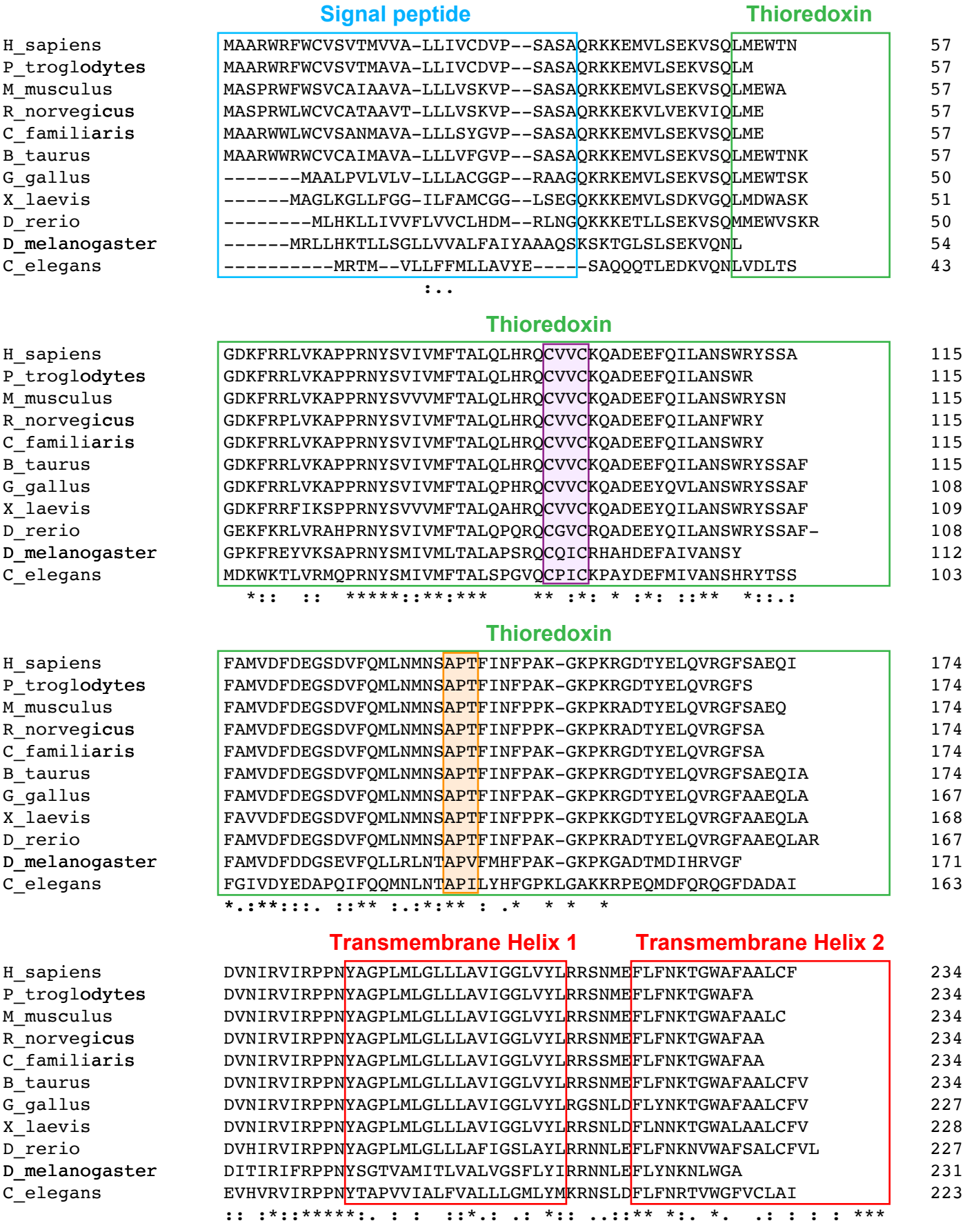
Signal peptide
Thioredoxin
H_sapiens MAARWRFWCVSVTMVVA-LLIVCDVP--SASAQRKKEMVLSEKVSQLMEWTN 57
P_troglodytes MAARWRFWCVSVTMAVA-LLIVCDVP--SASAQRKKEMVLSEKVSQLM 57
M_musculus MASPRWFWSVCAIAAVA-LLLVSKVP--SASAQRKKEMVLSEKVSQLMEWA 57
R_norvegicus MASPRWLWCVCATAAVT-LLLVSKVP--SASAQRKKEKVLVEKVIQLME 57
C_familiaris MAARWWLWCVSANMAVA-LLLSYGVP--SASAQRKKEMVLSEKVSQLME 57
B_taurus MAARWWRWCVCAIMAVA-LLLVFGVP--SASAQRKKEMVLSEKVSQLMEWTNK 57
G_gallus -------MAALPVLVLV-LLLACGGP--RAAGQKRKEMVLSEKVSQLMEWTSK 50
X_laevis ------MAGLKGLLFGG-ILFAMCGG--LSEGQKKKEMVLSDKVGQLMDWASK 51
D_rerio --------MLHKLLIVVFLVVCLHDM--RLNGQKKKETLLSEKVSQMMEWVSKR 50
D_melanogaster ------MRLLHKTLLSGLLVVALFAIYAAAQSKSKTGLSLSEKVQNL 54
C_elegans ----------MRTM--VLLFFMLLAVYE-----SAQQQTLEDKVQNLVDLTS 43
Thioredoxin
H_sapiens GDKFRRLVKAPPRNYSVIVMFTALQLHRQCVVCKQADEEFQILANSWRYSSA 115
P_troglodytes GDKFRRLVKAPPRNYSVIVMFTALQLHRQCVVCKQADEEFQILANSWR 115
M_musculus GDKFRRLVKAPPRNYSVVVMFTALQLHRQCVVCKQADEEFQILANSWRYSN 115
R_norvegicus GDKFRPLVKAPPRNYSVIVMFTALQLHRQCVVCKQADEEFQILANFWRY 115
C_familiaris GDKFRRLVKAPPRNYSVIVMFTALQLHRQCVVCKQADEEFQILANSWRY 115
B_taurus GDKFRRLVKAPPRNYSVIVMFTALQLHRQCVVCKQADEEFQILANSWRYSSAF 115
G_gallus GDKFRRLVKAPPRNYSVIVMFTALQPHRQCVVCKQADEEYQVLANSWRYSSAF 108
X_laevis GDKFRRFIKSPPRNYSVVVMFTALQAHRQCVVCKQADEEYQILANSWRYSSAF 109
D_rerio GEKFKRLVRAHPRNYSVIVMFTALQPQRQCGVCRQADEEYQILANSWRYSSAF- 108
D_melanogaster GPKFREYVKSAPRNYSMIVMLTALAPSRQCQICRHAHDEFAIVANSY 112
C_elegans MDKWKTLVRMQPRNYSMIVMFTALSPGVQCPICKPAYDEFMIVANSHRYTSS 103
Thioredoxin
H_sapiens FAMVDFDEGSDVFQMLNMNSAPTFINFPAK-GKPKRGDTYELQVRGFSAEQI 174
P_troglodytes FAMVDFDEGSDVFQMLNMNSAPTFINFPAK-GKPKRGDTYELQVRGFS 174
M_musculus FAMVDFDEGSDVFQMLNMNSAPTFINFPPK-GKPKRADTYELQVRGFSAEQ 174
R_norvegicus FAMVDFDEGSDVFQMLNMNSAPTFINFPPK-GKPKRADTYELQVRGFSA 174
C_familiaris FAMVDFDEGSDVFQMLNMNSAPTFINFPAK-GKPKRGDTYELQVRGFSA 174
B_taurus FAMVDFDEGSDVFQMLNMNSAPTFINFPAK-GKPKRGDTYELQVRGFSAEQIA 174
G_gallus FAMVDFDEGSDVFQMLNMNSAPTFINFPAK-GKPKRGDTYELQVRGFAAEQLA 167
X_laevis FAVVDFDEGSDVFQMLNMNSAPTFINFPPK-GKPKKGDTYELQVRGFAAEQLA 168
D_rerio FAMVDFDEGSDVFQMLNMNSAPTFINFPAK-GKPKRADTYELQVRGFAAEQLAR 167
D_melanogaster FAMVDFDDGSEVFQLLRLNTAPVFMHFPAK-GKPKGADTMDIHRVGF 171
C_elegans FGIVDYEDAPQIFQQMNLNTAPILYHFGPKLGAKKRPEQMDFQRQGFDADAI 163
Transmembrane Helix 1
Transmembrane Helix 2
H_sapiens DVNIRVIRPPNYAGPLMLGLLLAVIGGLVYLRRSNMEFLFNKTGWAFAALCF 234
P_troglodytes DVNIRVIRPPNYAGPLMLGLLLAVIGGLVYLRRSNMEFLFNKTGWAFA 234
M_musculus DVNIRVIRPPNYAGPLMLGLLLAVIGGLVYLRRSNMEFLFNKTGWAFAALC 234
R_norvegicus DVNIRVIRPPNYAGPLMLGLLLAVIGGLVYLRRSNMEFLFNKTGWAFAA 234
C_familiaris DVNIRVIRPPNYAGPLMLGLLLAVIGGLVYLRRSSMEFLFNKTGWAFAA 234
B_taurus DVNIRVIRPPNYAGPLMLGLLLAVIGGLVYLRRSNMEFLFNKTGWAFAALCFV 234
G_gallus DVNIRVIRPPNYAGPLMLGLLLAVIGGLVYLRGSNLDFLYNKTGWAFAALCFV 227
X_laevis DVNIRVIRPPNYAGPLMLGLLLAVIGGLVYLRRSNLDFLNNKTGWALAALCFV 228
D_rerio DVHIRVIRPPNYAGPLMLGLLLAFIGSLAYLRRNNLEFLFNKNVWAFSALCFVL 227
D_melanogaster DITIRIFRPPNYSGTVAMITLVALVGSFLYIRRNNLEFLYNKNLWGA 231
C_elegans EVHVRVIRPPNYTAPVVIALFVALLLGMLYMKRNSLDFLFNRTVWGFVCLAI 223

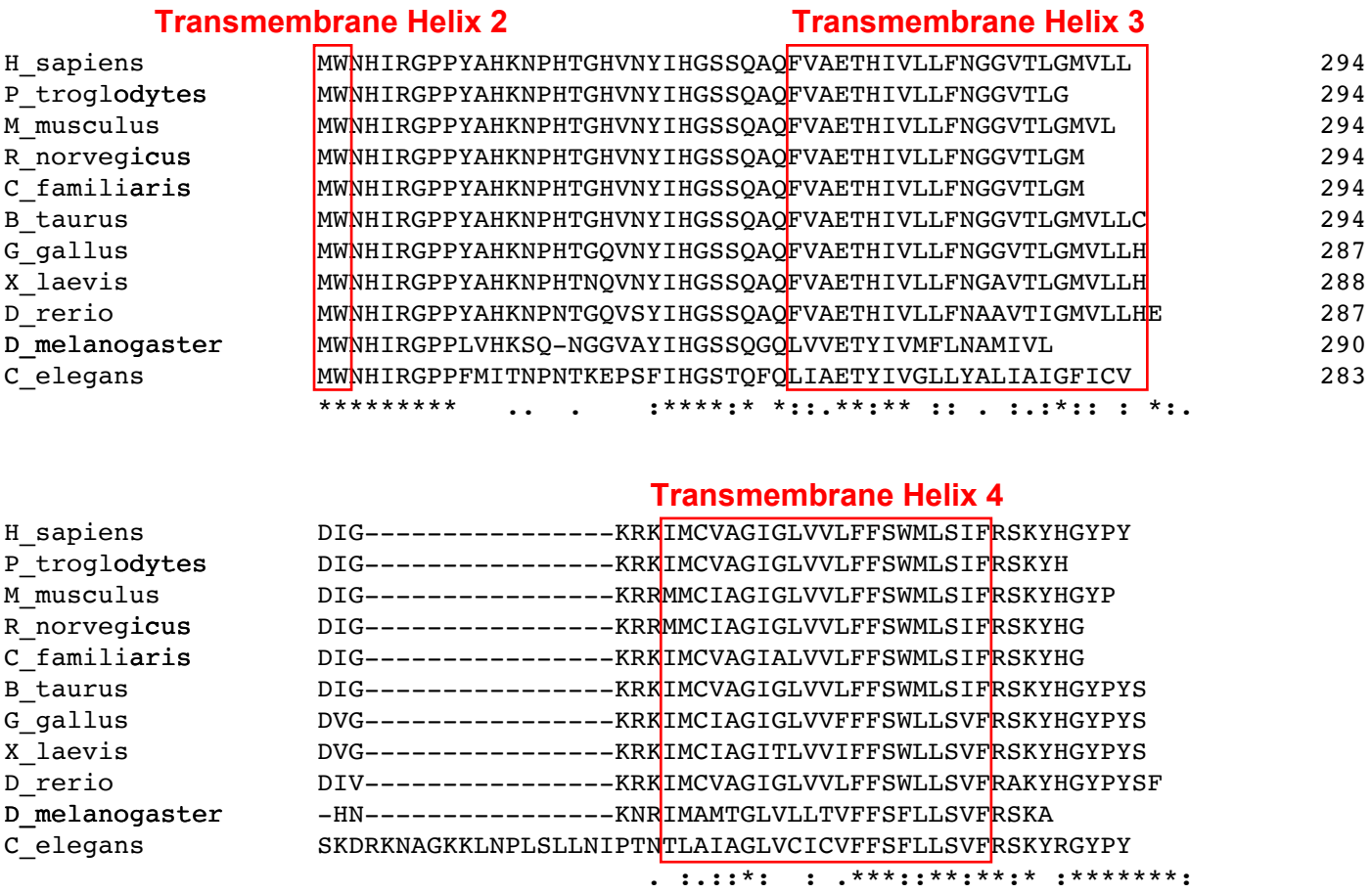

Figure S1A & S1B. Comparison of MAGT1 amino acid sequences in animals of varying complexity or relative evolutionary distance from *H. sapiens*.

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.

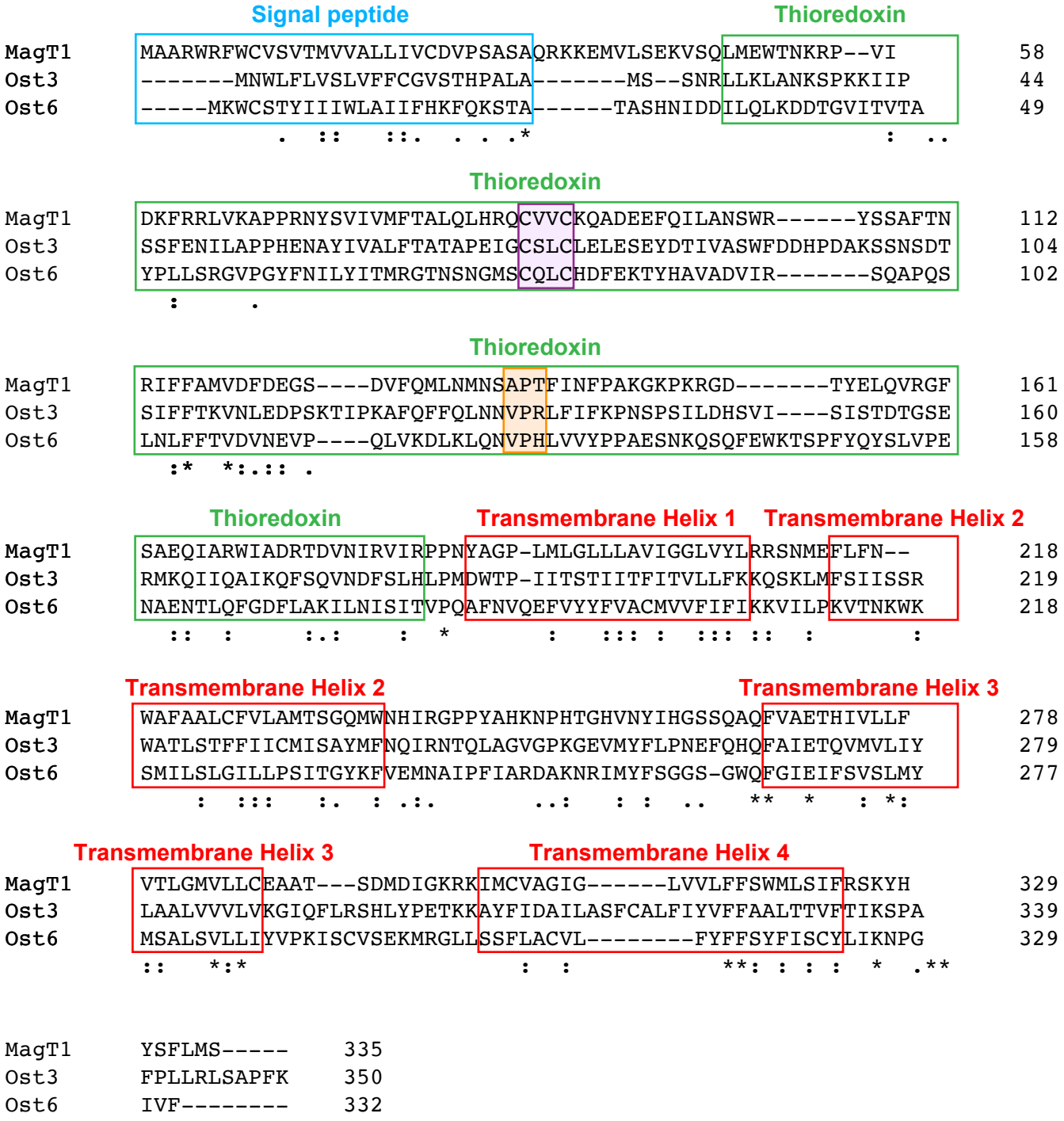

Figure S2. Comparison of the amino acid sequences MAGT1, *S. cerevisiae* OST3 and OST6. Conserve functional regions of the indicated proteins using the same color scheme as shown in Supplemental Figure 1.

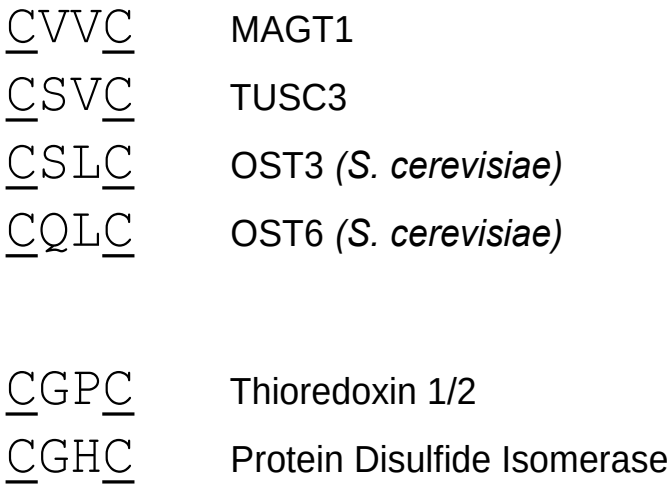

Figure S3. The CxxC motif of OST3/OST6 and MAGT1/TUSC3 are atypical of highly active TRX domains.
The motif is shown in single letter amino acid code for the indicated proteins.

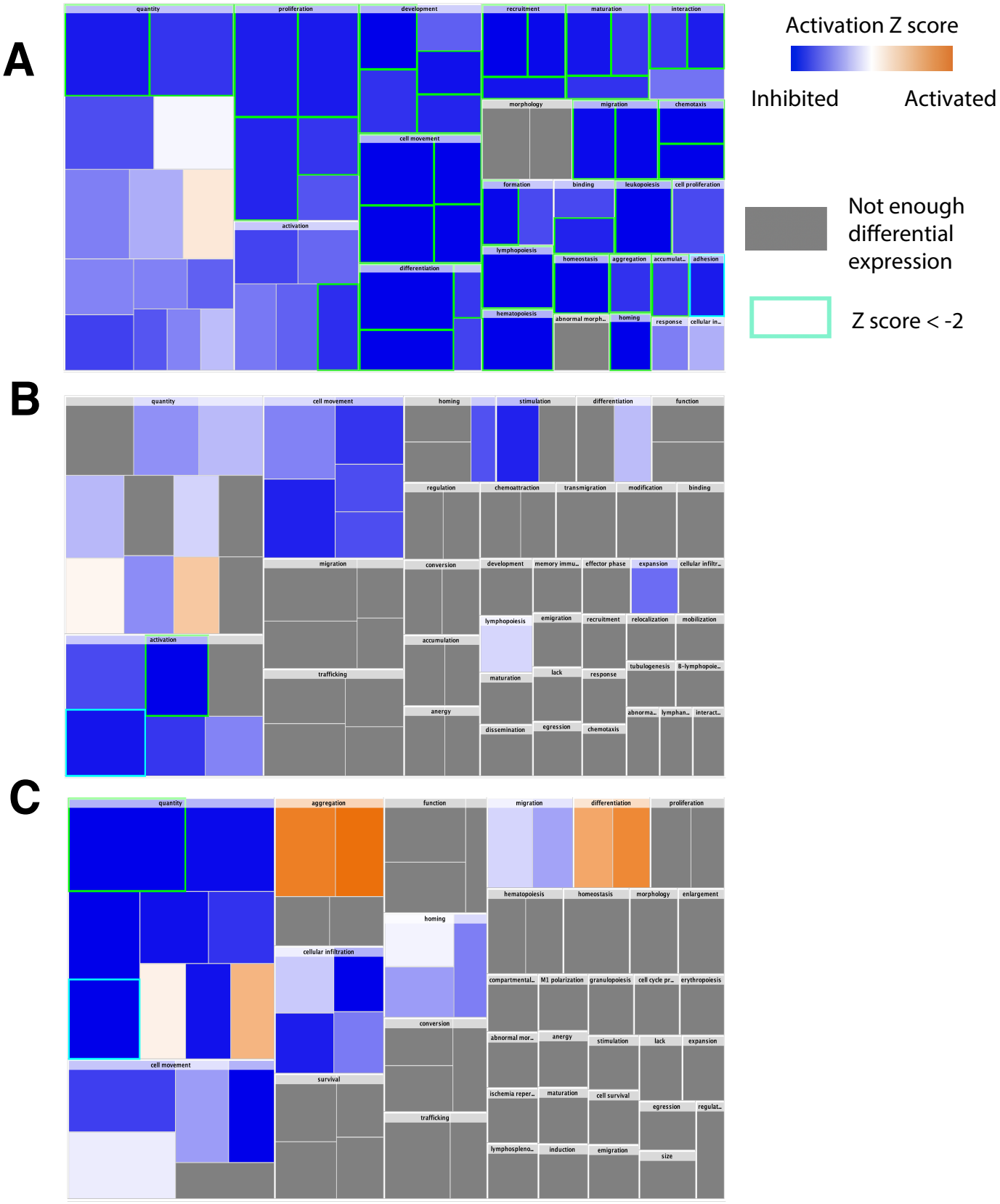

Figure S4. Treemap heatmap of hematological system development and function in XMEN patients. (A-C). Treemap representation from IPA of predicted activation (orange) or inhibition (blue) of various functions within hematological system based on DEGs at **(A)** day 0, **(B)** day 3, and **(C)** day 12. 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 (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.

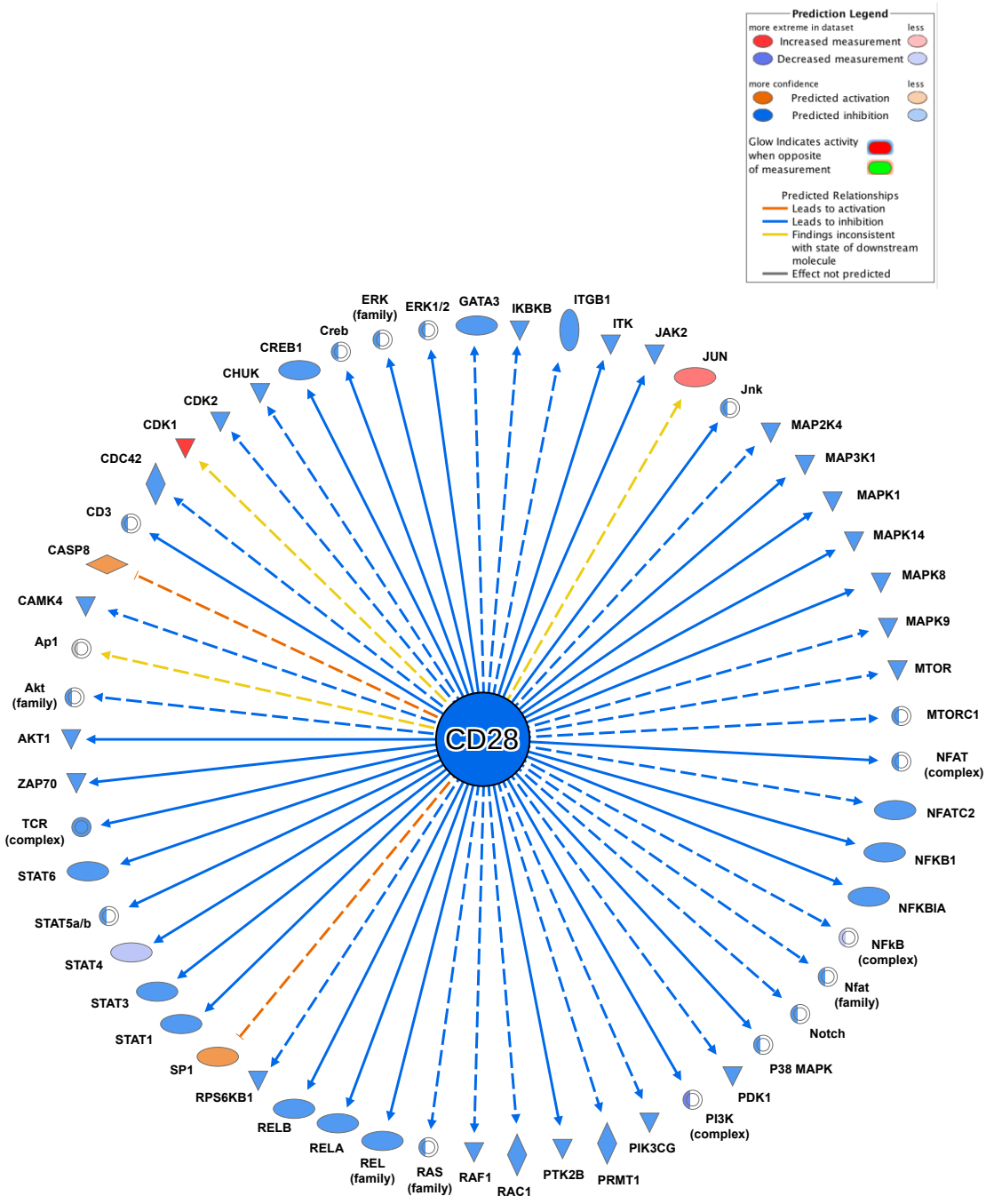

Figure S5. CD28 causal regulatory network.

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.

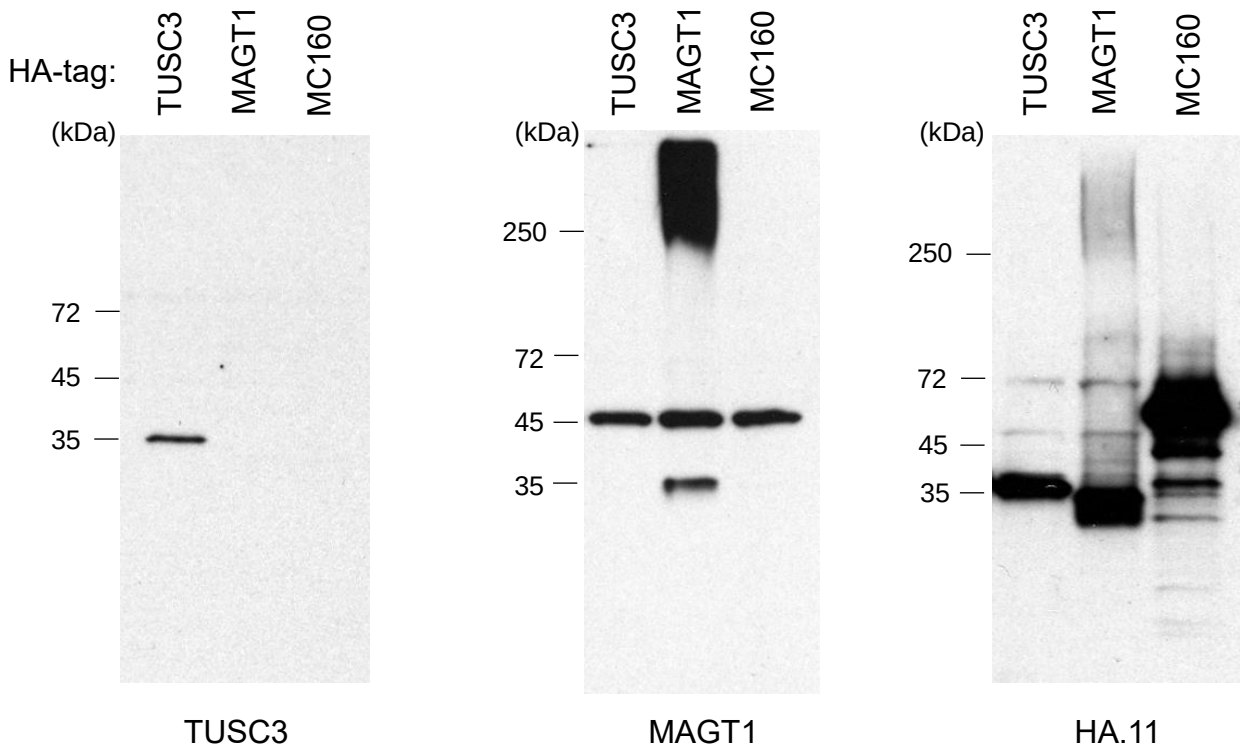

Figure S6. Specificity of TUSC3 and MAGT1 antibodies.

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).