

**Title: microRNA-630 regulates underglycosylated IgA1 production
in the tonsils by targeting TLR4 in IgA nephropathy**

Supplemental materials

Supplemental Table 1. Characteristics in IgAN group and CT group

	IgAN group (n=27)	CT group (n=20)
Age (years)	27.5 ± 4.5	21.2 ± 9.6*
Gender (male/female)	12/15	13/7
GFR (mL/min/1.73 m ²)	96.5 ± 22.3	133.1 ± 35.7
Cre (umol/L)	91.5 ± 34.5	77.3 ± 20.1*
Hematuresis (/hpf)	89.4 ± 55.2	N.D.
PRO (g/day)	1.2 ± 0.6	N.D.
ALB (g/L)	32.8 ± 6.7	40.2 ± 5.5*

Abbreviation: GFR: glomerular filtration rate Cre: creatinine; PRO: urine protein; ALB: albumin; hpf: high power field; N.D.: non-detected. *p<0.05, compared with IgAN group.

Supplemental Table 2. The primer sequences of the genes for RT-PCR

Genes	Forward primer (5'-3')	Reverse primer (5'-3')
C1GALT1	GACCCTGAAGAACCCATTTACTT	TATCCTGCTCCTCCACTCAT
COSMC	GAAGATGCTGATGGAAAAGATG	CCTGGTTGGGGTGATAAGTC
BAFF	CCACAGAAAGGGAGCAGTCAC	TGGGAGGATGGAAACACACT
BAFF-R	CCCTGGACAAGGTCATCATT	TCTTGGTGGTCACCAGTTCA
IL-1 β	GGTGTTCTCCATGTCCTTTGTA	GCTGTAGAGTGGGCTTATCATC
IL-8	CTGGCCGTGGCTCTCTTG	CCTTGGCAAAACTGCACCTT
GAPDH	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG

**Supplemental Table 3. Differential expression of miRNAs in tonsil tissue
between IgAN group and CT group IgAN patients**

miRBase accession number	miRNA Name	Fold change (IgAN/CT)
MIMAT0004518	hsa-miR-16-2-3p	2.100056477
MIMAT0015087	hsa-miR-514b-5p	2.622173944
MIMAT0004514	hsa-miR-29b-1-5p	2.808752111
MIMAT0000088	hsa-miR-30a-3p	3.475058413
MIMAT0000416	hsa-miR-1	3.735226739
MIMAT0000770	hsa-miR-133b	4.598821713
MIMAT0004776	hsa-miR-505-5p	13.452062
MIMAT0012735	hsa-miR-718	0.032664299
MIMAT0001618	hsa-miR-191-3p	0.046895733
MIMAT0005789	hsa-miR-513c-5p	0.056891883
MIMAT0004609	hsa-miR-149-3p	0.181051182
MIMAT0004595	hsa-miR-135a-3p	0.208670017
MIMAT0003299	hsa-miR-630	0.222205853
MIMAT0005788	hsa-miR-513b	0.317318002
MIMAT0004957	hsa-miR-760	0.363575708
MIMAT0020924	hsa-miR-642a-3p	0.402214386
MIMAT0002174	hsa-miR-484	0.488095418
MIMAT0005942	hsa-miR-1288	0.03506508
MIMAT0005573	hsa-miR-1225-3p	0.072123508
MIMAT0022496	hsa-miR-5703	0.096922813
MIMAT0018981	hsa-miR-4459	0.209741136
MIMAT0019213	hsa-miR-3162-3p	0.276249476
MIMAT0018100	hsa-miR-3676-3p	0.3398749
MIMAT0019791	hsa-miR-4697-5p	0.359808669
MIMAT0023252	hsa-miR-5787	0.385807819
MIMAT0019835	hsa-miR-4721	0.422333113
MIMAT0016906	hsa-miR-4274	0.424772775
MIMAT0019715	hsa-miR-4651	0.432839534
MIMAT0019006	hsa-miR-4478	0.459201094

Supplemental table 4. Part of predicted target genes of miR-630

Target Gene	Gene Name
TLR4	toll-like receptor 4
CD28	CD28 molecule
CD80	CD80 molecule
MAPK1	mitogen-activated protein kinase 1
PTPRC	protein tyrosine phosphatase, receptor type, C
BCL2L	BCL2-like 2
AKAP10	A kinase (PRKA) anchor protein 10
GNB1	guanine nucleotide binding protein (G protein), beta polypeptide 1
MAOB	monoamine oxidase B
HEATR3	HEAT repeat containing 3
FBXO30	F-box protein 30
PIGK	phosphatidylinositol glycan anchor biosynthesis, class K
CRISP3	cysteine-rich secretory protein 3
TFG	TRK-fused gene
PTPN22	protein tyrosine phosphatase, non-receptor type 22 (lymphoid)
TMED7	transmembrane emp24 protein transport domain containing 7
CYBB	cytochrome b-245, beta polypeptide
SP100	SP100 nuclear antigen
LYST	lysosomal trafficking regulator
POLR3A	polymerase (RNA) III (DNA directed) polypeptide A
EXO1	exonuclease 1
RAG1	recombination activating gene 1
CD226	CD226 molecule
LCP1	lymphocyte cytosolic protein 1 (L-plastin)
PTPN22	protein tyrosine phosphatase, non-receptor type 22 (lymphoid)
BCL11A	B-cell CLL/lymphoma 11A (zinc finger protein)
C5AR1	complement component 5a receptor 1

PTGER3	prostaglandin E receptor 3
DCDC2	doublecortin domain containing 2
PLAA	prostaglandin E receptor 3 (subtype EP3)
INHBB	inhibin, beta B
CYBB	cytochrome b-245, beta polypeptide
ZNF148	zinc finger protein 148
MPO	myeloperoxidase
APAF1	apoptotic peptidase activating factor 1
PLA2G4C	phospholipase A2, group IVC (cytosolic, calcium-independent)
SELE	selectin E
CXCL13	chemokine (C-X-C motif) ligand 13
PKHD1	polycystic kidney and hepatic disease 1 (autosomal recessive)
WWTR1	WW domain containing transcription regulator 1
GREM1	gremlin 1, DAN family BMP antagonist
EXO1	exonuclease 1
VTCN1	V-set domain containing T cell activation inhibitor 1
CD1C	CD1c molecule
LIF	leukemia inhibitory factor
BTLA	B and T lymphocyte associated
PAG1	phosphoprotein associated with glycosphingolipid microdomains 1
TP63	tumor protein p63
PTGER3	prostaglandin E receptor 3 (subtype EP3)

Supplemental figure 1

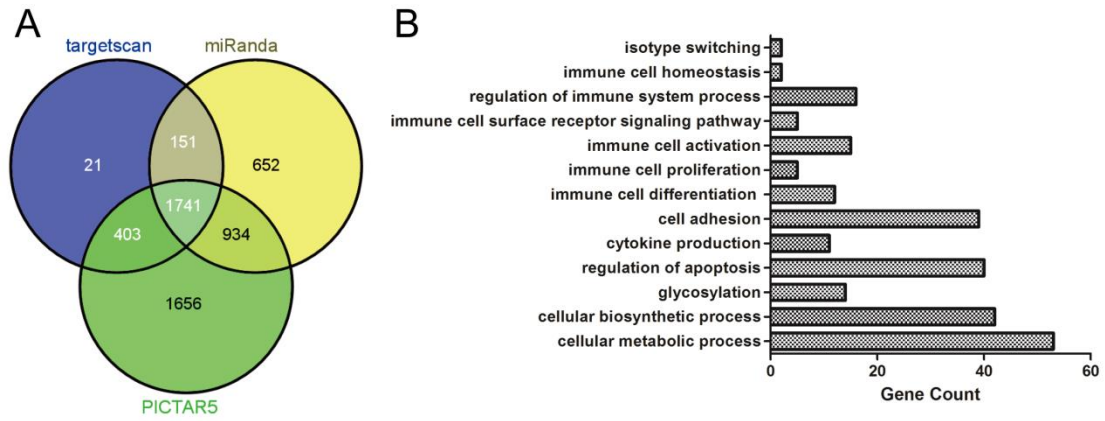


Figure S1. Bioinformatics analysis of the target genes of miR-630. (A) The common target genes of miR-630 predicted by Targetscan, miRanda and PicTar. (B) The Gene Ontology (GO) enrichment analysis of candidate target genes was conducted in DAVID database.

Supplemental figure 2

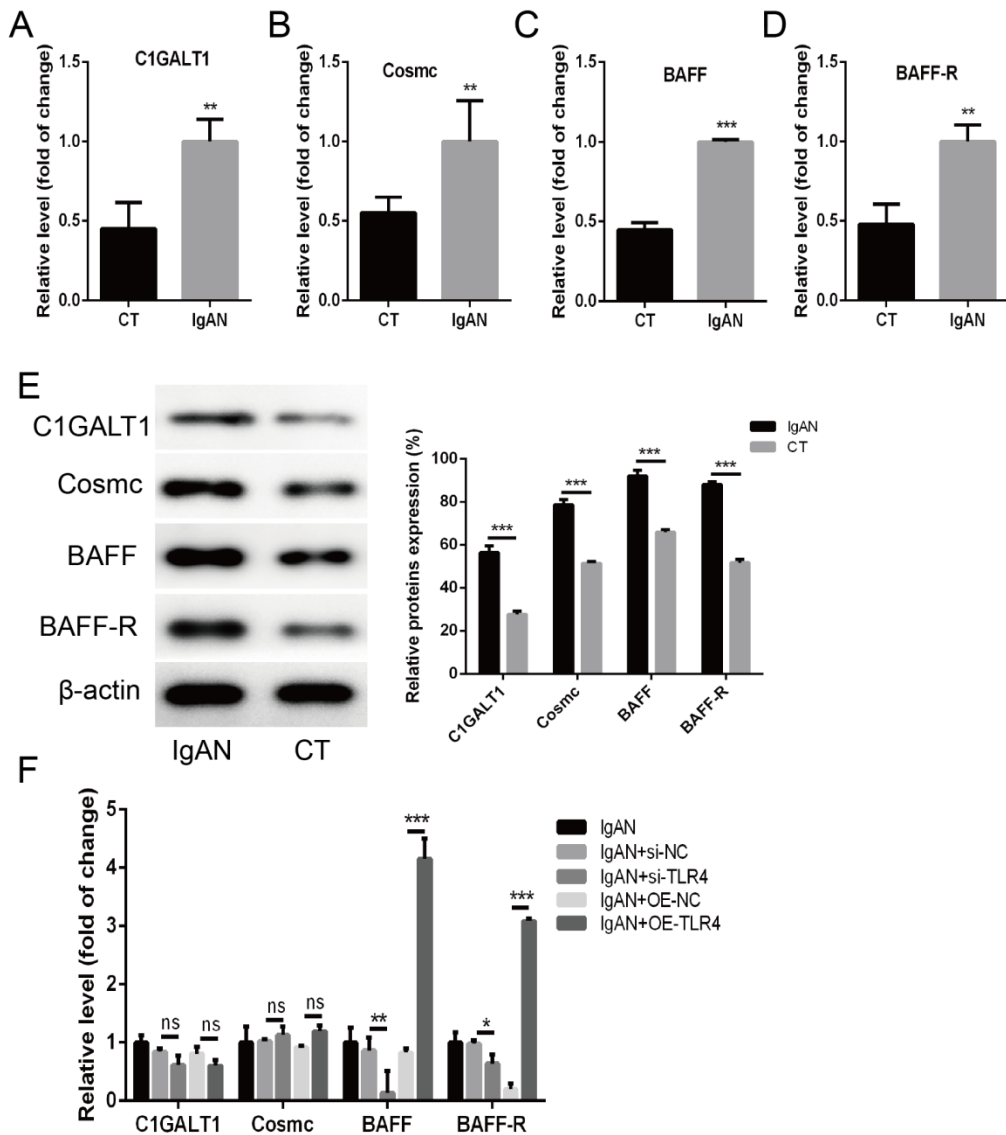


Figure S2. The expressions of related markers of affecting the expression/secretion/glycosylation of IgA. (A-D) The expressions of C1GALT1, Cosmc, BAFF and BAFF-R were detected by RT-PCR in TMC from the IgAN group and CT group. (E) The protein levels of C1GALT1, Cosmc, BAFF and BAFF-R were measured by Western blot in TMC from the IgAN group and CT group. (F) The levels of C1GALT1, Cosmc, BAFF and BAFF-R were measured by RT-PCR when knocking down the expression of TLR4 by using TLR4 siRNA or overexpression of TLR4 in TMC. The data were expressed as mean \pm SD, ** $p < 0.01$. *** $p < 0.001$. IgAN: IgA nephropathy; CT: chronic tonsillitis. NC: negative control. BAFF: B cell activating factor. BAFF-R: B cell activating factor receptor.