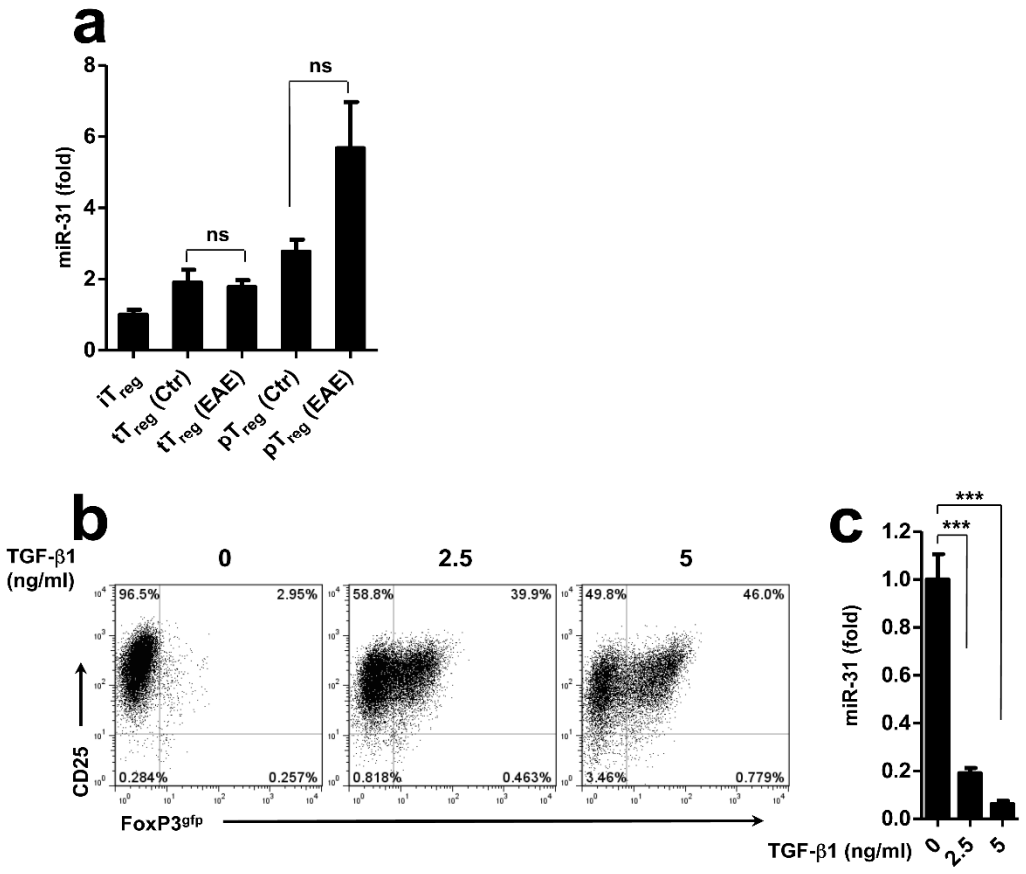
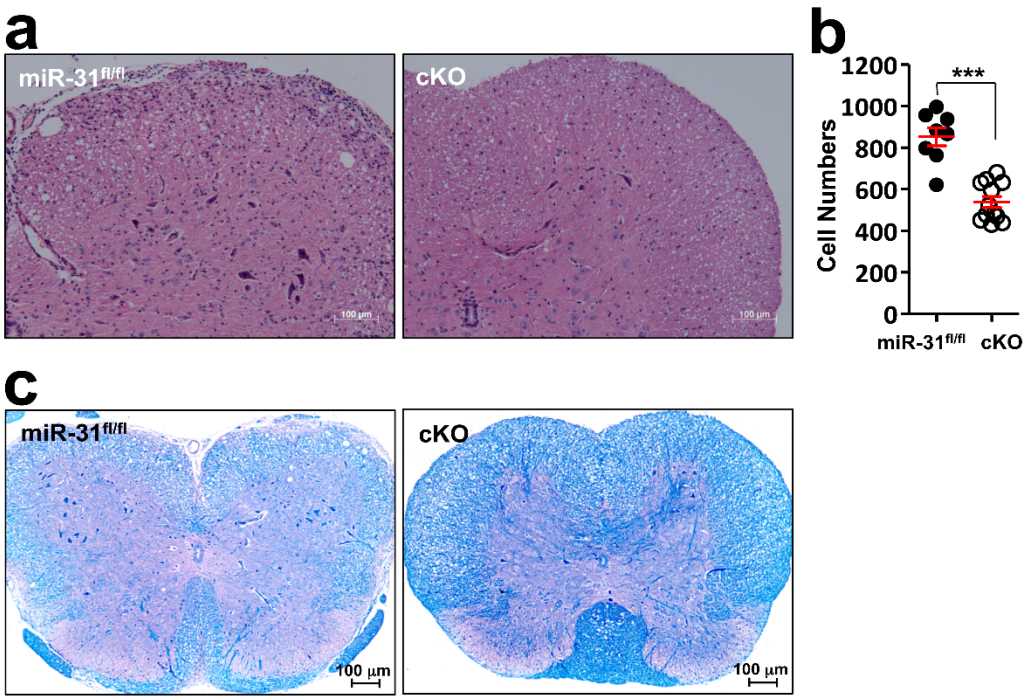
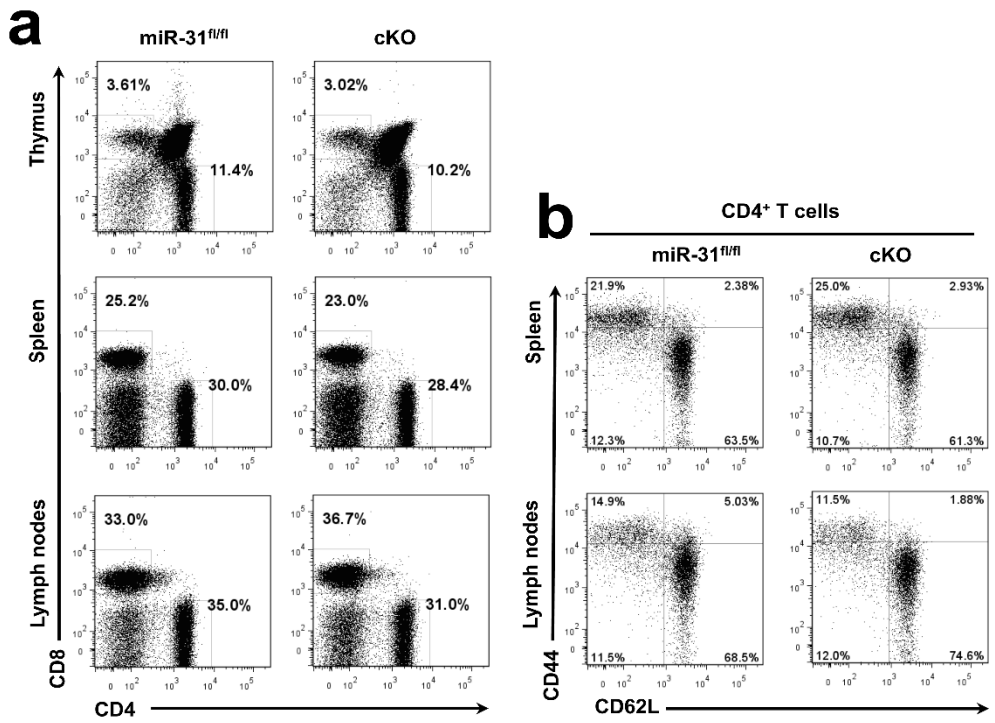
Supplementary Information



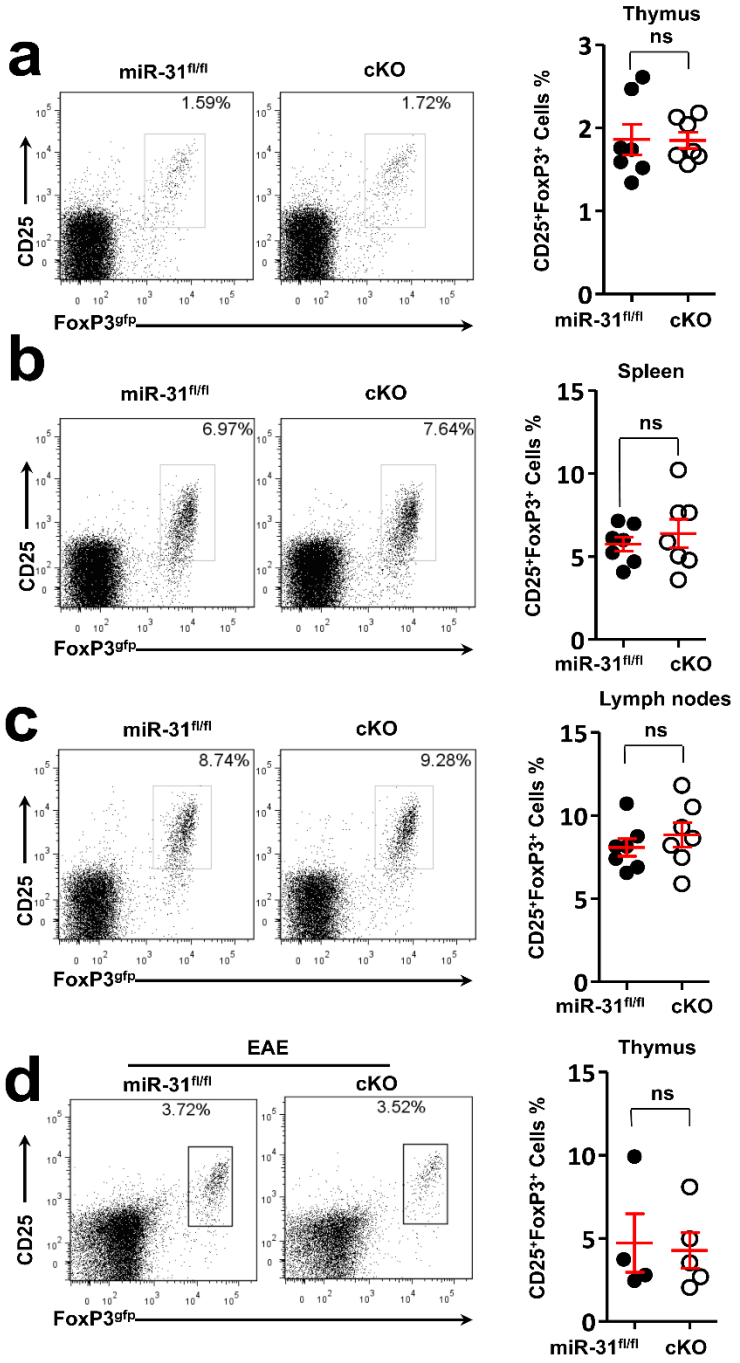
Supplementary Figure 1 miR-31 expression in T_{reg} subsets and its levels are reversely correlated with numbers of iT_{reg} cells. (**a**) miR-31 expression in iT_{reg}, tT_{reg}, EAE tT_{reg}, pT_{reg} and EAE pT_{reg} cells; results are presented relative to miR-31 expression in iT_{reg} cells. (**b**) Representative flow cytometry of $FoxP3^{gfp}$ expression in iT_{reg} cells polarized with different concentrations of TGF-β1. Numbers in quadrants indicate percent cells in each. (**c**) qPCR analysis of miR-31 expression in iT_{reg} cells polarized with different concentrations of TGF-β1; results are presented relative to miR-31 expression in naïve T cells cultured without TGF-β1. ns, not significant, ***p<0.001, two-tailed Student's t-test. Data are from one experiment representative of two independent experiments (mean ± sem).



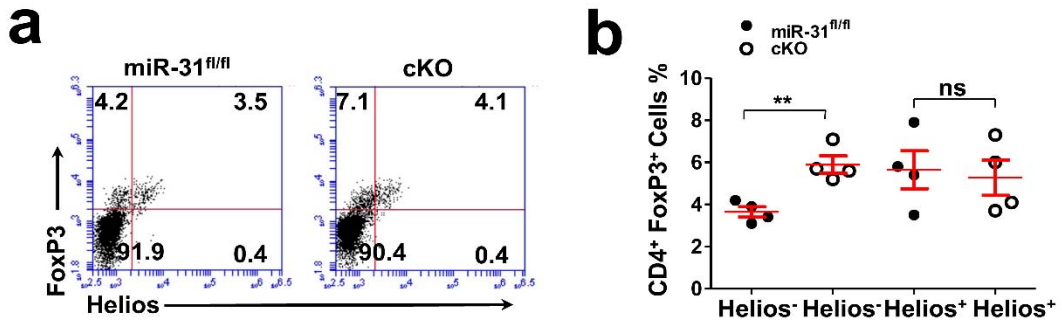
Supplementary Figure 2 Decreased infiltrating lymphocytes and demyelination of spinal cord in cKO mice with EAE. (**a, b**) Infiltrating lymphocytes in the spinal cord of *miR-31*$^{fl/fl}$ and cKO mice with EAE were counted. (**c**) The degree of demyelination of spinal cords from *miR-31*$^{fl/fl}$ and cKO mice with EAE was analyzed by Luxol fast blue staining. ***p<0.001, two-tailed Student's t-test. Representative data are shown, which had been reproduced in two independent experiments.



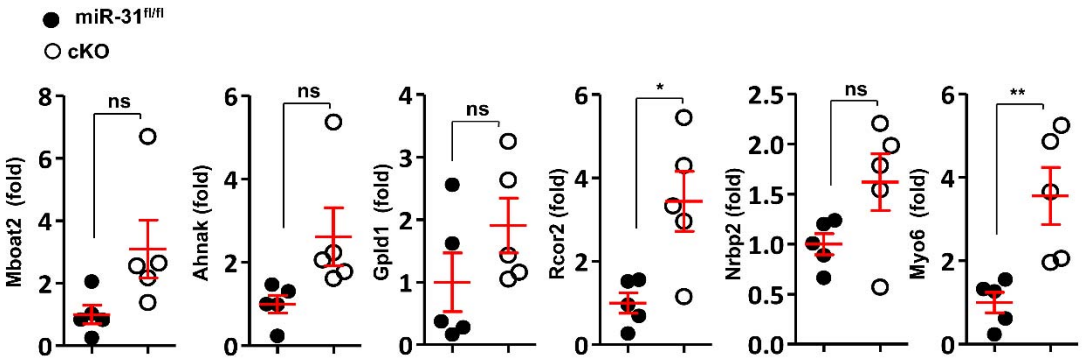
Supplementary Figure 3 miR-31 deficiency has no apparent effect on the development of T cells. (**a**) Representative flow cytometry of $CD4^+$ and $CD8^+$ T cells in thymus, spleen and lymph nodes from 6-week old *miR-31*$^{fl/fl}$ and cKO mice. (**b**) Representative flow cytometry for surface expression of CD44 and CD62L in $CD4^+$ cells in spleen and lymph nodes from 6-week old *miR-31*$^{fl/fl}$ and cKO mice. Numbers adjacent to outlined areas or in quadrants indicate percent cells in each. Data are from one experiment representative of two independent experiments.



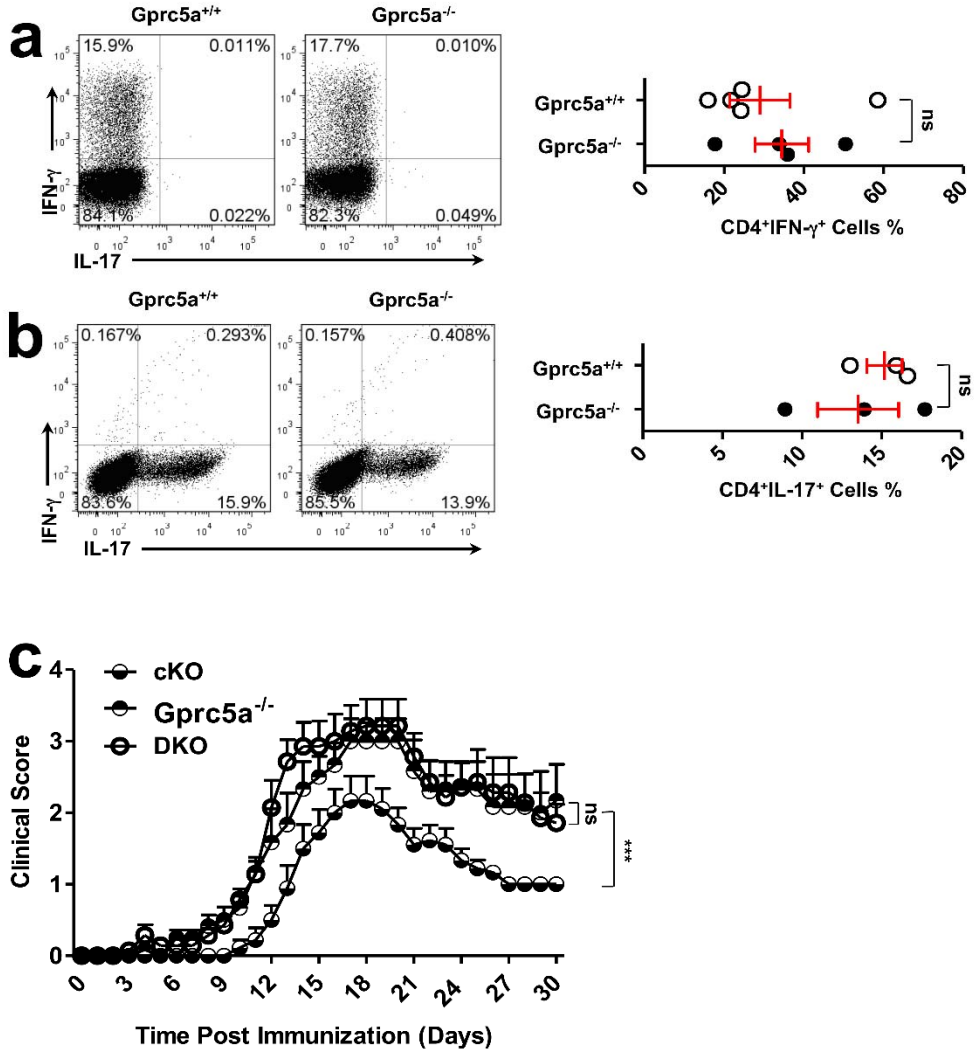
Supplementary Figure 4 miR-31 deficiency has no impact on tT_{reg} cell development. *FoxP3*^{gfp} reporter mice were crossed with either *miR-31*$^{fl/fl}$ or cKO mice. (**a-c**) Flow cytometric analysis of T_{reg} cells in thymus, spleen and lymph nodes from 6-week old *miR-31*$^{fl/fl}$ and cKO mice. (**d**) Flow cytometric analysis of tT_{reg} cells in thymus from 6-week old *miR-31*$^{fl/fl}$ and cKO mice with EAE. Numbers adjacent to outlined areas indicate percent cells in each. ns, not significant, two-tailed Student's *t*-test. Data are representative of three independent experiments (mean ± sem).



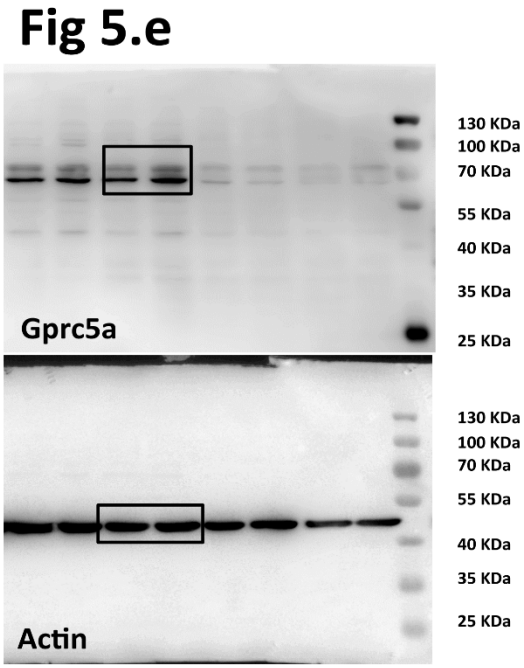
Supplementary Figure 5 Deletion of miR-31 in CD4$^+$ T cells promotes pT$_{reg}$ cell Induction *in vivo*. Bone marrow cells were prepared from either *miR-31*$^{fl/fl}$ or cKO mice. The bone marrow cells (5×10^6) were injected intravenously into lethally irradiated C57BL/6J recipient mice to generate bone marrow chimeric mice. Eight weeks after bone marrow transplantation, EAE was induced in all chimeric mice. (**a, b**) Splencytes prepared from *miR-31*$^{fl/fl}$ or cKO chimeric mice 10 days post immunization were subjected for analysis of Helios$^-$FoxP3$^+$ pT$_{reg}$ cells and Helios$^+$FoxP3$^+$ nT$_{reg}$ cells by Flow cytometry (cells were gated in CD4$^+$ T cell population). Data are representative of three independent experiments. $^{**}p<0.01$, two-tailed *Student's test*.

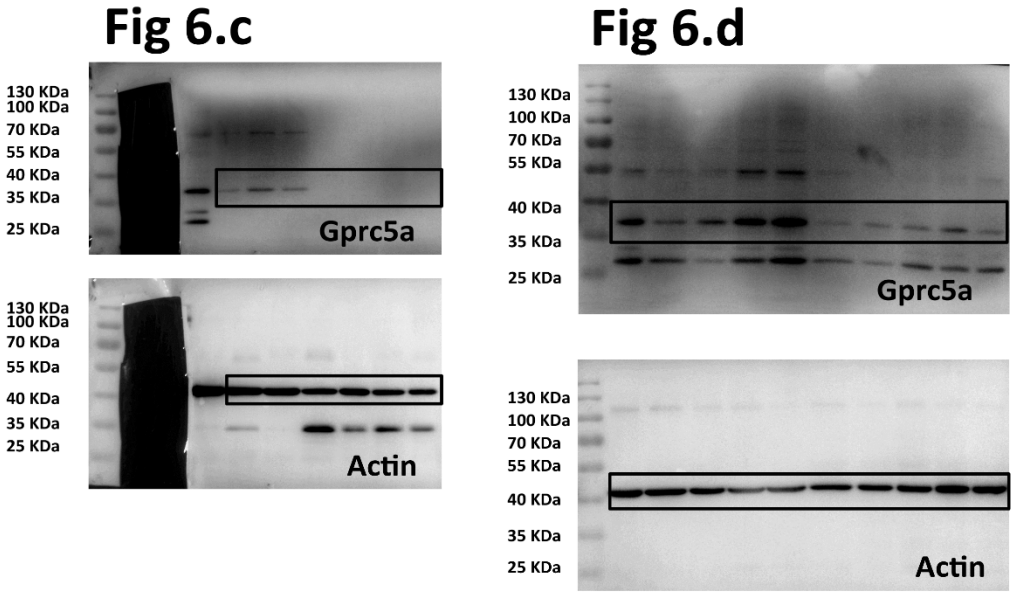


Supplementary Figure 6 mRNA levels of candidate genes targeted by miR-31 in iT_{reg} cells. mRNA levels of 6 candidate genes were measured by qPCR. Results are presented as the ratio of mRNA to β-actin, relative to that in *miR-31$^{fl/fl}$* controls. **p*<0.05, ***p*<0.01, ns, not significant, two-tailed Student's *t*-test. Data are representative of two independent experiments (mean ± sem).



Supplementary Figure 7 Gprc5a deficiency does not affect T_H1 and T_H17 cell differentiation *in vitro*, but restores the disease phenotype in cKO mice. (**a, b**) Flow cytometric analysis of T_H1 and T_H17 cells polarized from naïve T cells of either *Gprc5a*$^{+/+}$ or *Gprc5a*$^{-/-}$ mice (n=3-4). Numbers in quadrants indicate percent cells in each. (**c**) miR-31 mice were crossed with *Gprc5a*$^{-/-}$ mice to generate DKO mice. Clinical scores (mean ± sem) of *cKO*, *Gprc5a*$^{-/-}$ *and DKO* mice after the induction of EAE were assessed every day (n=6-8). ***p<0.001, ns, not significant, two-tailed Student's *t*-test for (**a, b**), one-way ANOVA for (**c**). Data are from one representative of two independent experiments for (**a, b**) (mean ± sem).





Supplementary Figure 8 List of original pictures of western blots. Black boxes highlight the indicated lanes in figures.

Supplementary Table 1

Primers used in this study

Molecule	Primer	Sequence
FoxP3	Upstream	CCCAGGAAAGACAGCAACCTT
	Downstream	TTCTCACAACCAGGCCACTTG
b-actin	Upstream	TGGAATCCTGTGGCATCCATGAAAC
	Downstream	TAAAACGCAGCTCAGTAACAGTCCG
Mboat2	Upstream	TCAGACACGTAGTTGCTACCC
	Downstream	TGCAGTAGGAAATCCCACTTTG
Ahnak	Upstream	CAGCGCATCTACACCACGAA
	Downstream	CACTTCATGCCTTGGTATCTTGA
Gpld1	Upstream	TATTCGAGAGAACTACCCTCTGC
	Downstream	AGGAACCCTTGTTCAATACCCA
Rcor2	Upstream	ATCCGAGTTGGAACCAATTACC
	Downstream	AGTGCCTGCTCAATGTTATAGC
Gprc5a	Upstream	ACCACAGACTTTGTGACCTGG
	Downstream	CGAGTGCAAACATGCAAGCC
Myo6	Upstream	CCACAATGTCAAAGTTCGGTACA
	Downstream	GGCATCGTCCCAAGAGATTTTC
Nrbp2	Upstream	AACGGGATCTATCCACTGATGA
	Downstream	GGTCTCCGAGTCAAAGGGTTC
miR-31 -344~458	Upstream	5'-TGCACGGGAGCATTCATACA
	Downstream	5'-GGATCCAATGGCGTTCAAGC
miR-31 -531~795	Upstream	TCTCCTTTCCTCACCCCACT
	Downstream	GACATGCGCTTTCCCAATCC
miR-31-1175~1274	Upstream	GTGACATGTTTGACTGCCGA
	Downstream	TGGCTCTGACTCATGAACTCC
miR-31-1800~1936	Upstream	TGTTCCGTTCACAAGCCCAT
	Downstream	AGGCTTTGATCCAGGCAGAC