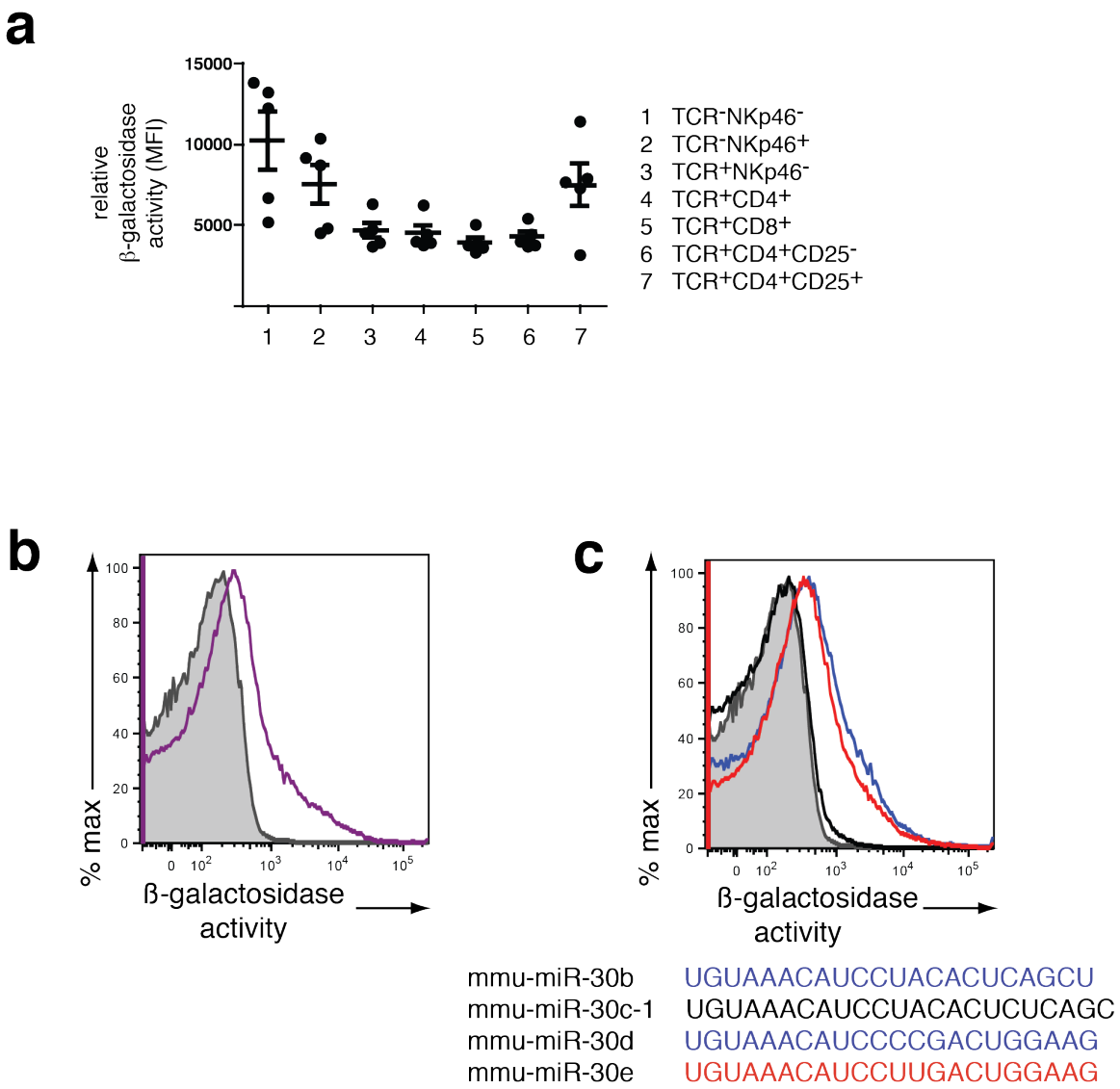
a
relative β-galactosidase activity (MFI)
15000
10000
5000
1 2 3 4 5 6 7
1 TCR-NKp46-
2 TCR-NKp46+
3 TCR+NKp46-
4 TCR+CD4+
5 TCR+CD8+
6 TCR+CD4+CD25-
7 TCR+CD4+CD25+
b
% max
ß-galactosidase activity
c
% max
ß-galactosidase activity
mmu-miR-30b UGUAAACAUCCUACACUCAGCU
mmu-miR-30c-1 UGUAAACAUCCUACACUCUCAGC
mmu-miR-30d UGUAAACAUCCCCGACUGGAAG
mmu-miR-30e UGUAAACAUCCUUGACUGGAAG
d
normalized β-galactosidase activity
Tc Tr Tc Tr Tc Tr Tc Tr
1 2 3 4
1 miR-298/296
2 miR-30b/30d
3 miR-30e
4 miR-146a

Figure S1

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Supplemental Information

Extended experimental procedures

Histochemical analysis of the lacZ reporter. For embryo staining, we crossed a lacZ-KO/+ male with wild type females and embryos were dissected from pregnant females. The dissected embryos were fixed in 4% paraformaldehyde and 0.2% glutaraldehyde in PBS for either 1 or 4 hours depending on their age. For E18.5 embryos, we dissected out internal organs and brains from the body cavity for better fixation and then permeabilized in 0.02% NP40, 0.01% sodium deoxycholate, and 2 mM MgCl2 in PBS for one hour before staining. X-gal staining was done overnight at room temperature. Embryos were post-fixed in 2% paraformaldehyde in PBS and stored in 70% ethanol for storage or future sectioning.

Real-time quantitative RT-PCR (qPCR). Total RNA was purified from adult tissues by using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Brain or lungs from adult mice were quickly dissected out, frozen immediately in liquid nitrogen, grounded by mortar and pestle as powder, and then homogenized in TRIzol reagent. Reverse Transcription was done by using a TaqMan MiRNA Reverse Transcription Kit and Quantitative PCR was done using a Taqman miRNA assay system (Applied Biosystems). For each sample, PCR were done in triplicates. Sno202 was used as endogenous input control.

Flow cytomeric single cell analysis in adult mice
FACS analysis was performed on heparinized peripheral blood. ß–galactosidase (lacZ) activity was measured using the FluoReporter lacZ Flow Cytometry Kit (Invitrogen/Molecular Probes) according to the manufacturer's instructions. FDG incubation time was 60-100'. Flowcytometry was performed as described (Zhou et al., J Exp Med 2008). Dead cells were excluded from analysis using LIVE DEAD® Fixable Violet Dead Cell Stain kit (Invitrogen). Analysis was performed using FlowJo for Mac (TreeStar).

Figure S1. Non-invasive flow cytometric single cell analysis of lacZ reporter expression in immune cells; Related to Figure3 LacZ activity was measured by flow cytometry in peripheral blood. **(A-D)** Peripheral blood cells gated on live lymphocytes. **(A)** lacZ reporter activity varies substantially among different cell types. The mean fluorescence intensity (MFI) of ß-galactosidase activity from a ubiquitously lacZ expressing R26-lacZ mouse is shown. Displayed are individual data points shown with standard error of the mean. **(B)** Lymphocytic expression of the let-7d cluster. Let-7d-lacZ lymphocytes (purple line); control lymphocyte (grey shaded histogram). **(C)** Expression of closely related family members can be distinguished by lacZ reporter activity. LacZ-negative control (grey shaded histogram), miR-30c-1-lacZ (black line), miR-30b/30d-lacZ (blue line), and miR-30e-lacZ (red) reporter mice. A representative graph of independent experiments is shown (for let7d cluster n=2, for miR-30c-1 and miR-30e n=3, and for miR-30b/d n=5). **(D)** Differential expression of miR-146a-lacZ in Treg cells. ß-galactosidase activity of $CD4^+CD25^-$ conventional T (Tc) cells and $CD4^+CD25^+$ regulatory T (Tr) cells was measured for the indicated mouse line. This activity was normalized against lacZ activity from R26lacZ mice measured in Tc and Tr for four different miRNA lines to account for cell type specific lacZ differences. ß–galactosidase activity was normalized for Tconv and Treg for each experiment by dividing the Treg/Tconv MFI ratio to the Treg/Tconv MFI ratio of R26lacZ positive control mice.