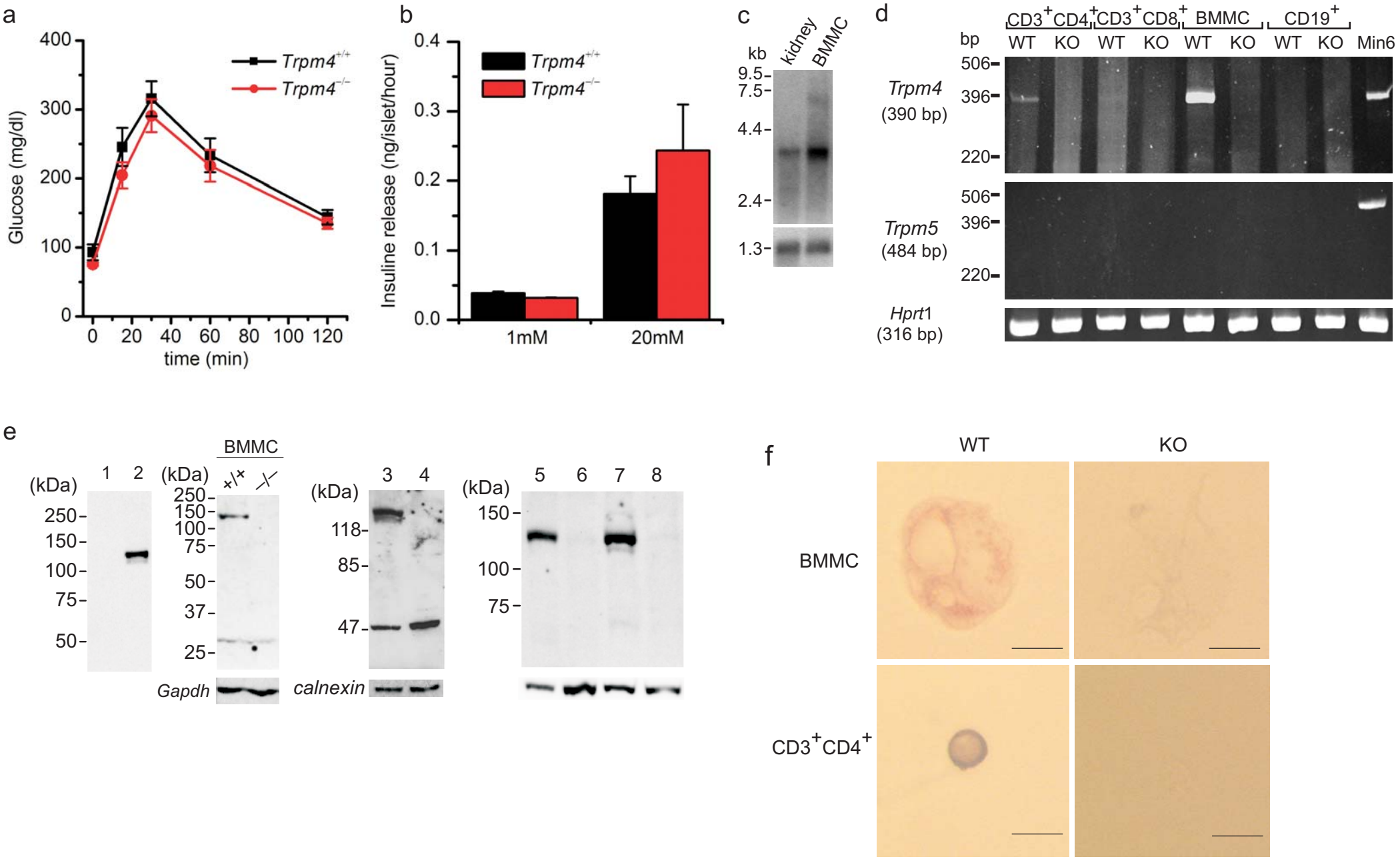
a
Glucose (mg/dl)
time (min)
Trpm4+/+
Trpm4−/−
b
Insuline release (ng/islet/hour)
1mM
20mM
c
kb
kidney
BMMC
9.5
7.5
4.4
2.4
1.3
d
CD3+CD4+
CD3+CD8+
BMMC
CD19+
bp
WT
KO
Min6
Trpm4
(390 bp)
Trpm5
(484 bp)
Hprt1
(316 bp)
e
BMMC
(kDa)
Gapdh
calnexin
f
WT
KO
BMMC
CD3+CD4+

Supplementary Figure 2. (**a**) Time course of blood glucose levels before and after intraperitoneal glucose application in *Trpm4*$^{+/+}$ and *Trpm4*$^{-/-}$ mice (10 mice per genotype). (**b**) Insulin secretion from pancreatic islets isolated from *Trpm4*$^{+/+}$ and *Trpm4*$^{-/-}$ mice. Pooled data from 30 islets derived from 3 individual mice are given from each genotype. Islets were stimulated for 1 h in the presence of 1 mM or 20 mM glucose. Total insulin content was 31.7 ± 5.6 ng/islet (*Trpm4*$^{+/+}$) and 28.0 ± 2.9 ng/islet (*Trpm4*$^{-/-}$, $P > 0.05$). (**c**) Northern blot of poly(A)$^+$ RNA isolated from kidney and BMMC from *Trpm4*$^{+/+}$ mice hybridized with a mTRPM4-specific probe. Transcripts of ~4.2 kb were identified in both tissues. (**d**) Expression of TRPM4 and TRPM5 in immune cells. RT-PCR analysis of the expression of *Trpm4* and *Trpm5* transcripts in CD3$^+$CD4$^+$, CD3$^+$CD8$^+$, CD19$^+$ and BMMC cells isolated from *Trpm4*$^{+/+}$ (WT) and *Trpm4*$^{-/-}$ (KO) mice. From every cell type exactly 500 cells were subjected to combined cDNA-synthesis and PCR reactions. The expected sizes of the amplification products are indicated in brackets. In control reactions primers amplified a fragment of hypoxanthine guanine phosphoribosyl transferase 1 (*Hprt1*). Murine pancreatic ß-cells of the line MIN6 were used as a positive control for *Trpm5* expression[1]. Results are representative of at least three independent experiments using three different cell preparations. (**e**) Western blot of protein fractions from non-transfected (lane 1) and mouse TRPM4-transfected (lane 2) HEK 293 cells (left panel) and *Trpm4*$^{+/+}$ (+/+) and *Trpm4*$^{-/-}$ (–/–) BMMC's using TRPM4-specific antibody 578. Western blot of protein fractions from *Trpm4*$^{+/+}$ (lane 3, 75 µg) and *Trpm4*$^{-/-}$ pancreas (lane 4, 75 µg), *Trpm4*$^{+/+}$ (lane 5, 100 µg) and *Trpm4*$^{-/-}$ BMMC (lane 6, 100 µg), and *Trpm4*$^{+/+}$ (lane 7, 100µg) and *Trpm4*$^{-/-}$ pancreatic islets (lane 8, 100µg) using TRPM4-specific antibody 578. (**f**) Detection of TRPM4 protein in CD3$^+$CD4$^+$ lymphocytes. Immuncytochemical staining of BMMC as a control (upper panel) and CD3$^+$CD4$^+$ lymphocytes from *Trpm4*$^{+/+}$ and *Trpm4*$^{-/-}$ mice. Following cytospin, cells were stained as described in the Methods using anti-TRPM4 that was preabsorbed using microsomal membrane protein fractions from *Trpm4*$^{-/-}$ BMMCs. No specific staining could be detected in CD3$^+$CD8$^+$ or CD19$^+$ cells from *Trpm4*$^{+/+}$ or *Trpm4*$^{-/-}$ mice (data not shown). Results are representative of two independent experiments. Bars=7.5µm.