Supplemental material

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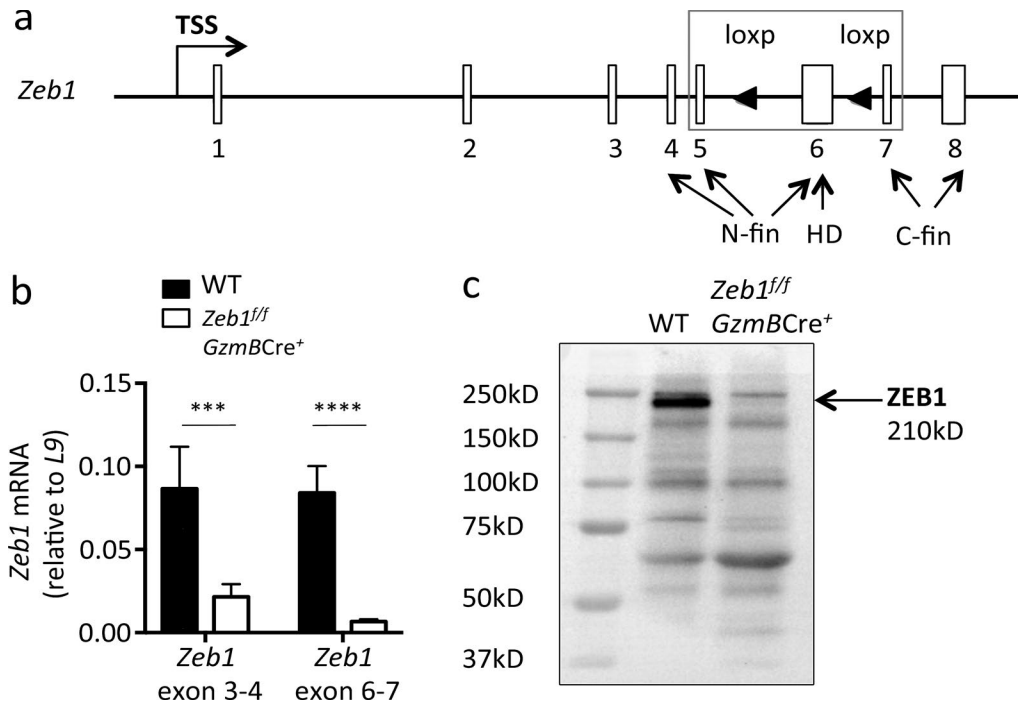

Figure S1. **Generation of *Zeb1* conditional KO mice and validation of deletion. (a)** A schematic representation of loxP inserts within *Zeb1* gene locus. **(b)** *Zeb1* transcript analysis comparing WT and $Zeb1^{flox/flox}$ *CD4*Cre$^+$ naive CD8$^+$ T cells. Two sets of primers were used for the quantitative RT-PCR detection: one set flaking exon 3–4 (outside of loxP region) and the other flaking exon 6–7 (covering part of loxP region). **(c)** ZEB1 protein detection using Western Blotting comparing WT and $Zeb1^{flox/flox}$ *CD4*Cre$^+$ naive CD8$^+$ T cells. Data shown are representative of two (b and c) independent experiments; *n* = 2–3 mice/group/experiment (b and c). Data are expressed as mean ± SEM. ***, P < 0.001; ****, P < 0.0001.

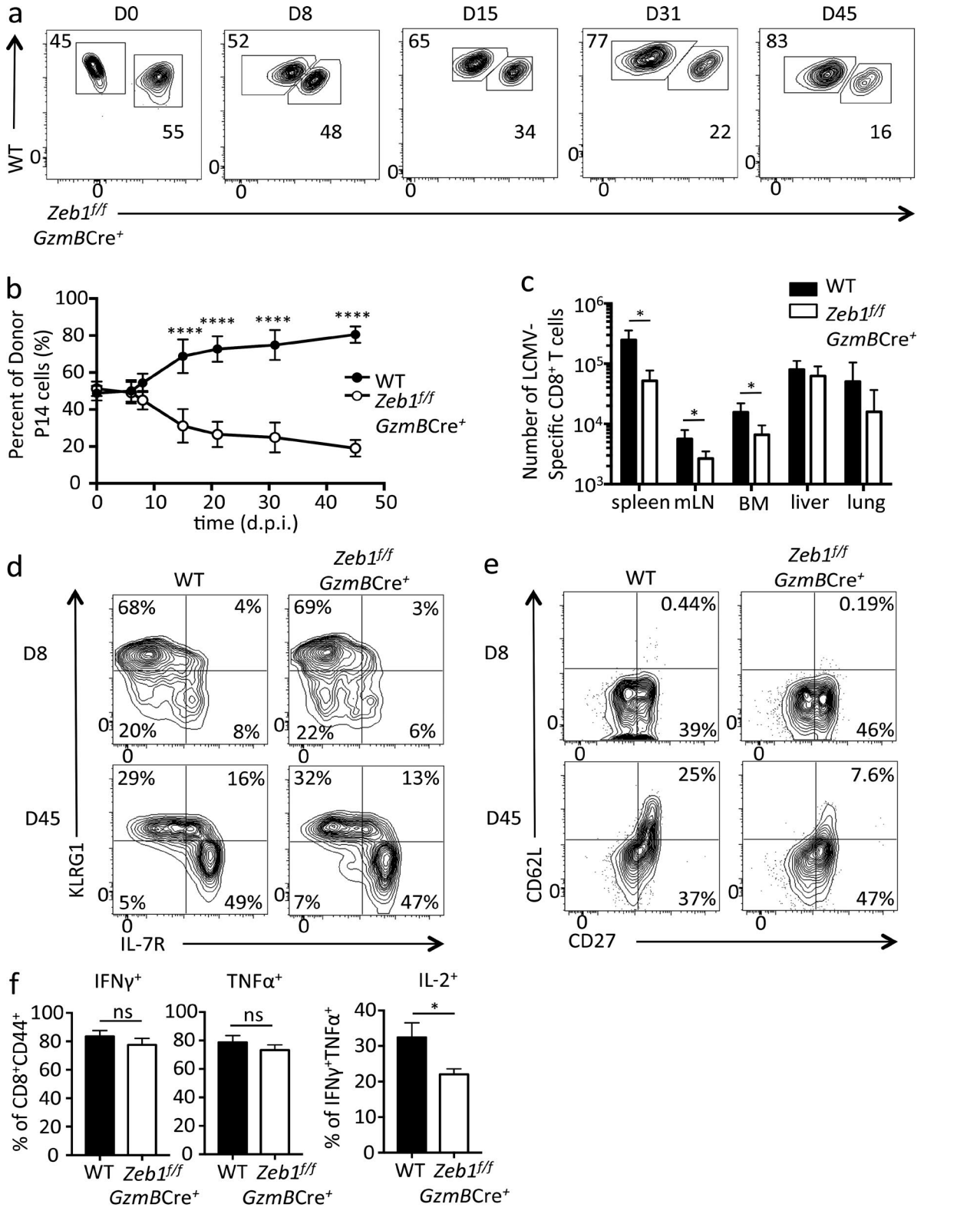

Figure S2. **ZEB1 plays an intrinsic role in promoting memory CD8⁺ T cell survival. (a–c)** Time course analysis of the frequency (a and b) of WT and *Zeb1*$^{f/f}$ *GzmB*Cre⁺ P14⁺ CD8⁺ T cells (Thy1.2/1.2) cotransferred equally into congenically mismatched (Thy1.1/1.1) naive B6 mice after LCMV-Arm infection. (c) Combined number (±SEM) of WT and *Zeb1*$^{f/f}$ *GzmB*Cre⁺ P14⁺ CD8⁺ T cells in various tissues 30 dpi including spleen, mLN, bone marrow (BM), liver, and lung. **(d and e)** Flow plots show expression of KLRG1 and IL-7R (d) and CD62L and CD27 (e) of donor WT and *Zeb1*$^{f/f}$ *GzmB*Cre⁺ P14⁺ CD8⁺ T cells at 8 and 45 dpi. **(f)** WT and *Zeb1*$^{f/f}$ *GzmB*Cre⁺ P14⁺CD8⁺ T cells from 30 dpi were analyzed for IFNγ and TNFα (left two bar graphs) or IL-2 (right bar graph) expression using intracellular cytokine staining after a 5-h $GP_{33–41}$ peptide stimulation. Note, IL-2 producing cells were gated on IFNγ⁺ TNFα⁺ P14⁺ CD8⁺ T cells. Data shown are representative of two (a, d, and e) or cumulative of two (b, c, and f) independent experiments; *n* = 3–5 mice/group/experiment (a, d, and e); *n* = 6–10 (b, c, and f). Data are expressed as mean ± SEM. *, P < 0.05; ****, P < 0.0001.

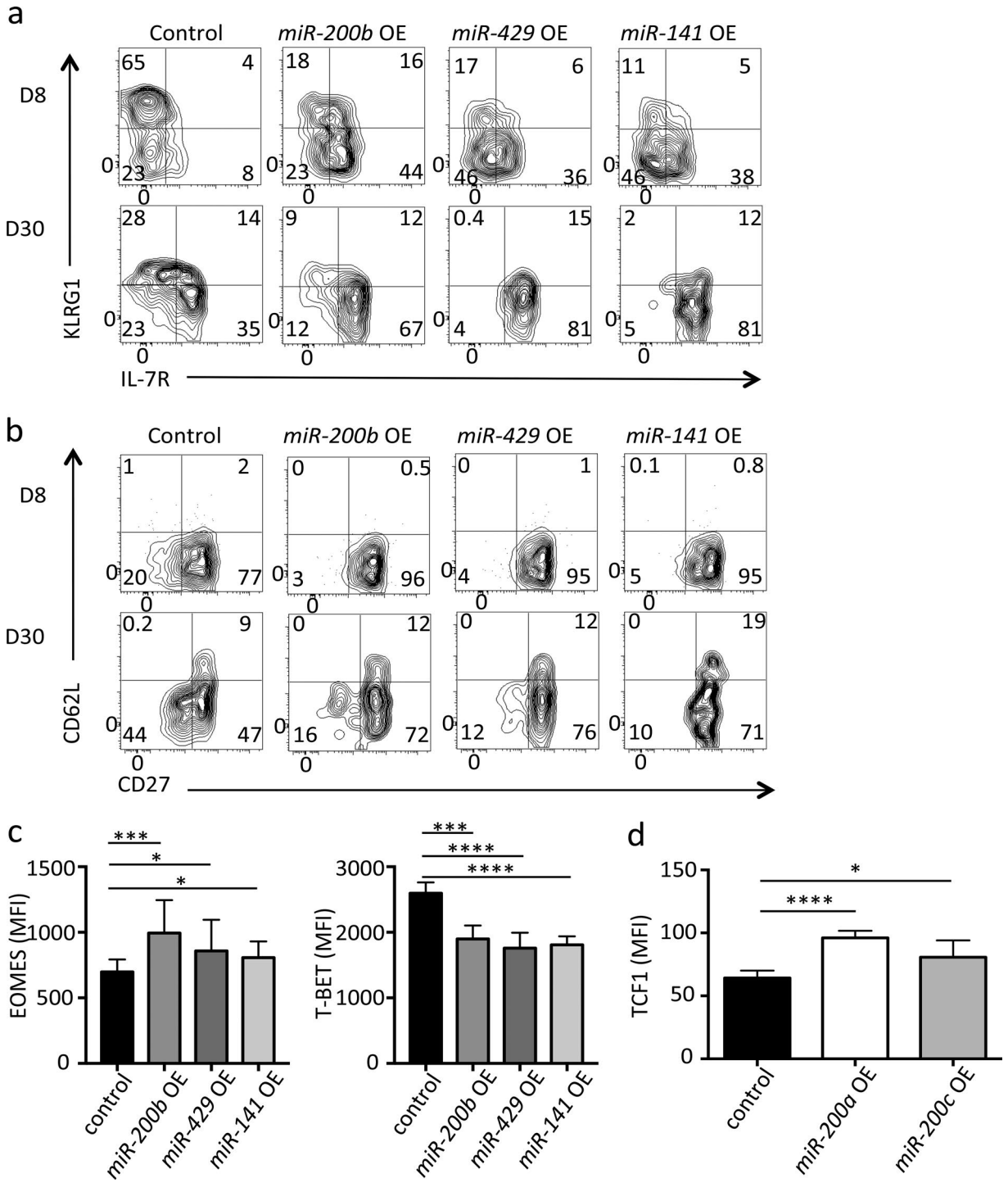

Figure S3. **Overexpression of *miR-200* family promotes memory CD8⁺ T cell differentiation. (a and b)** Flow plots show expression of KLRG1 and IL-7R (a) CD62L and CD27 (b) in control, *miR-200b*, *miR-429*, and *miR-141* overexpressing P14⁺ CD8⁺ T cells 8 and 30 dpi. **(c and d)** Bar graphs show amounts of Eomes, T-bet (c), and TCF1 (d) in control, *miR-200a*, and *miR-200c* overexpressing P14⁺ CD8⁺ T cells 30 dpi. Data shown are representative of two (a and b) or cumulative of three (c and d) independent experiments; n = 3–5 mice per group per experiment (a and b), n = 8–10 (c). Data are expressed as mean ± SEM. *, P < 0.05; ***, P < 0.001; ****, P < 0.0001.