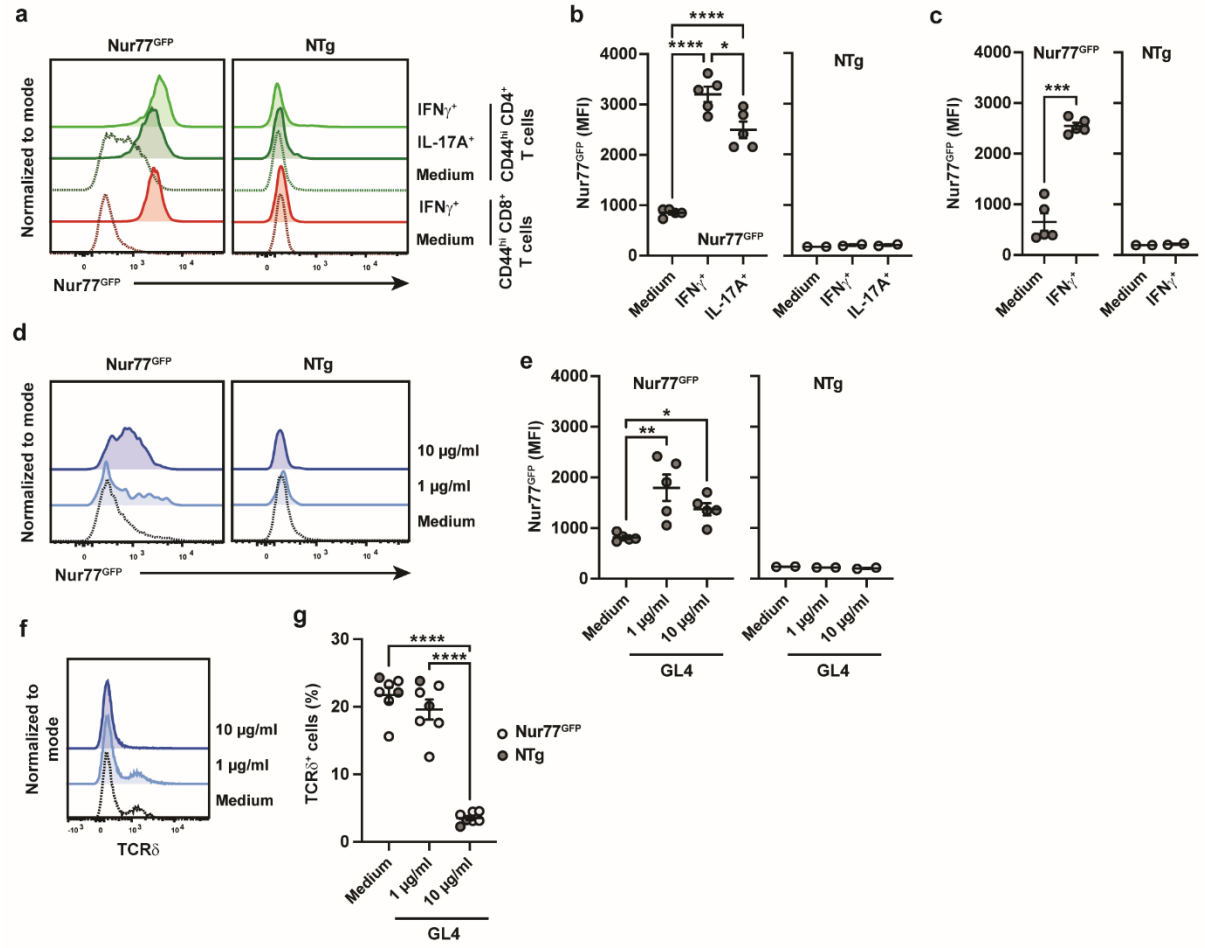

Figure S1. Nur77^{GFP} expression among unconventional T cells after TCR engagement. MLN single cell suspensions from *Lm*-immune Nur77^{GFP} and non-transgenic (NTg) mice were left unstimulated (medium) or stimulated with plate-bound αCD3ε and soluble αCD28 mAbs or with plate-bound GL4 mAb for 6 hours. (a to c) Nur77^{GFP} expression was analyzed among conventional T cells. (a) Representative histograms of Nur77^{GFP} expression in the indicated populations. (b) Nur77^{GFP} MFI among unstimulated CD44^{hi} CD4^{+} and activated CD44^{hi} CD4^{+} T cells producing either IL-17A or IFNγ after stimulation. (c) Nur77^{GFP} expression among unstimulated and activated IFNγ^{+} CD44^{hi} CD8α^{+} T cells. (d and e) Nur77^{GFP} expression was analyzed among CD44^{hi} CD27^{neg} CD19^{-} CD4^{-} CD8α^{-} lymphocytes. (d) Representative histograms of Nur77^{GFP} expression in unstimulated and IL-17A^{+} cells upon stimulation with 1 or 10 µg/ml of coated GL4 mAb. (e) Scatter plots show Nur77^{GFP} MFI among the groups indicated in (d). (f and g) TCRδ expression among CD19^{-} CD4^{-} CD8α^{-} cells left unstimulated (medium) or stimulated with the indicated concentration of GL4 mAb. (f) Representative histograms of TCRδ expression are shown. (g) Scatter plots show the frequency of TCRδ^{+} cells among CD19^{-} CD4^{-} CD8α^{-} cells. All data are from 1 experiment with 5 Nur77^{GFP} and 2 NTg mice. Statistical analyses were only performed for Nur77^{GFP} mice. *, $p \leq 0.05$; **, $p \leq 0.01$; ****, $p \leq 0.0001$

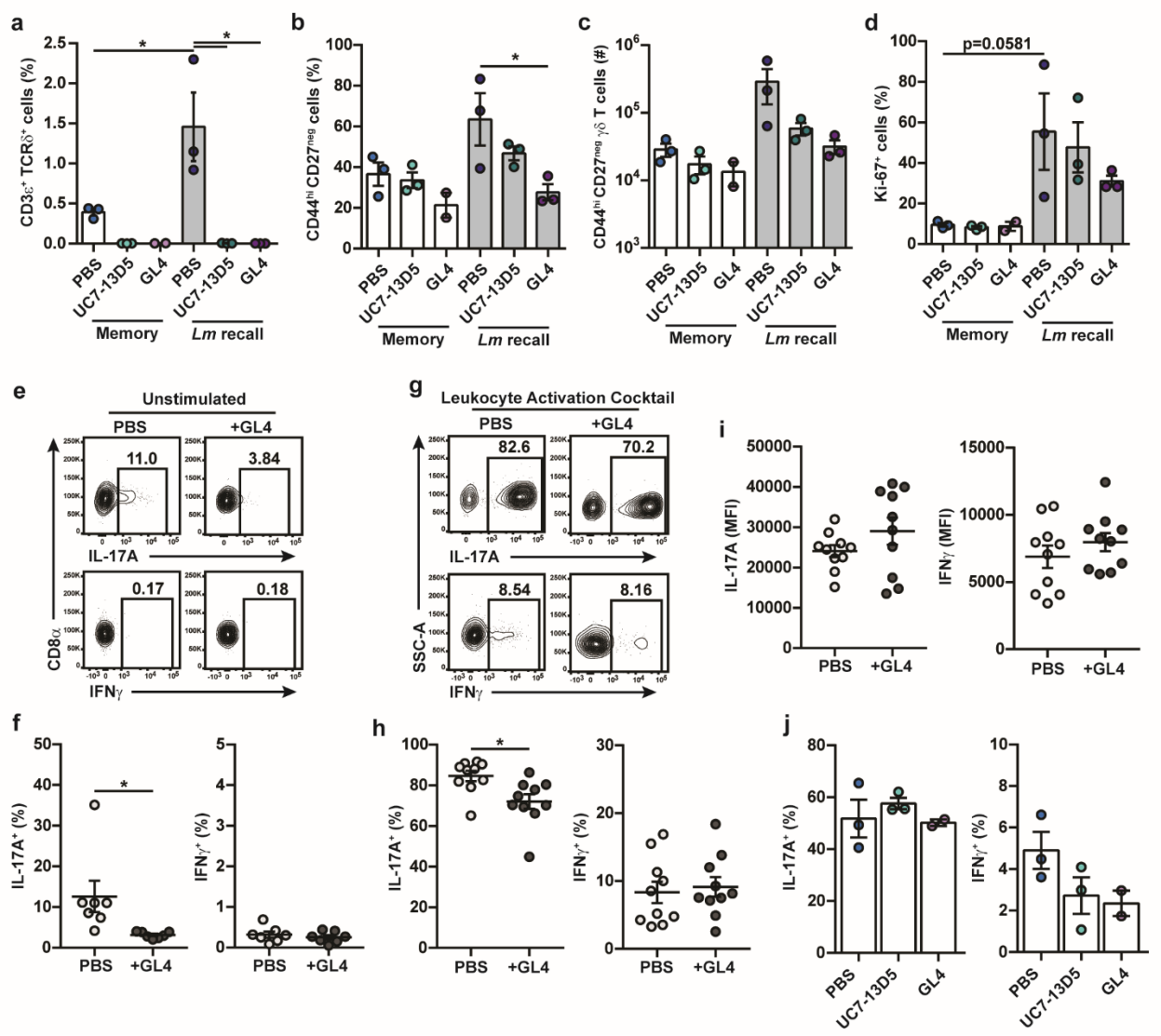

Figure S2. Antibody-mediated γδTCR internalization diminishes CD44^{hi} CD27^{neg} γδ T cell proliferation while minimally impacting cytokine production during recall response to *Lm*. *Lm*-immune *Tcrd*-H2B-eGFP (Balb/c background) mice were i.p. injected with either PBS, UC7-13D5 or GL4 mAb on days -3, -1 and +1 relative to recall infection. On day 0, mice were challenged with *Lm* ("*Lm* recall") or given PBS ("Memory"). γδ T cells (identified as TCRβ^{neg} CD8α^{neg} GFP^{+} single live lymphocytes) were analyzed 4 to 5 days later in the MLN. (a) The graph shows the mean ± SEM (n=2-3 mice/group) of the frequency of CD3ε^{+} TCRδ^{+} cells among single live lymphocytes. (b-c) The frequency (b) and number (c) of CD44^{hi} CD27^{neg} γδ T cells are shown as the mean ± SEM. (d) CD44^{hi} CD27^{neg} γδ T cells were analyzed for Ki-67 expression. The graph shows the mean ± SEM of Ki-67^{+} cell frequency. (e-j) Single cell suspensions from MLN were left unstimulated in the presence of BD GolgiPlug (e and f) or stimulated with BD Leukocyte activation cocktail (g-j) for 4 hours. CD44^{hi} CD27^{neg} γδ T cell cytokine production was analyzed by flow cytometry. Representative flow plots of IL-17A and IFNγ production are shown (e and g). Cumulative data are shown as the mean ± SEM (n=3-4 mice/group) of cytokine^{+} cell frequency (f and h) and MFI (i) and depict 2-3 independent experiments. (j) Graphs show the mean ± SEM of cytokine^{+} cell frequency after 4-hour stimulation with BD Leukocyte activation cocktail. *, $p \leq 0.05$

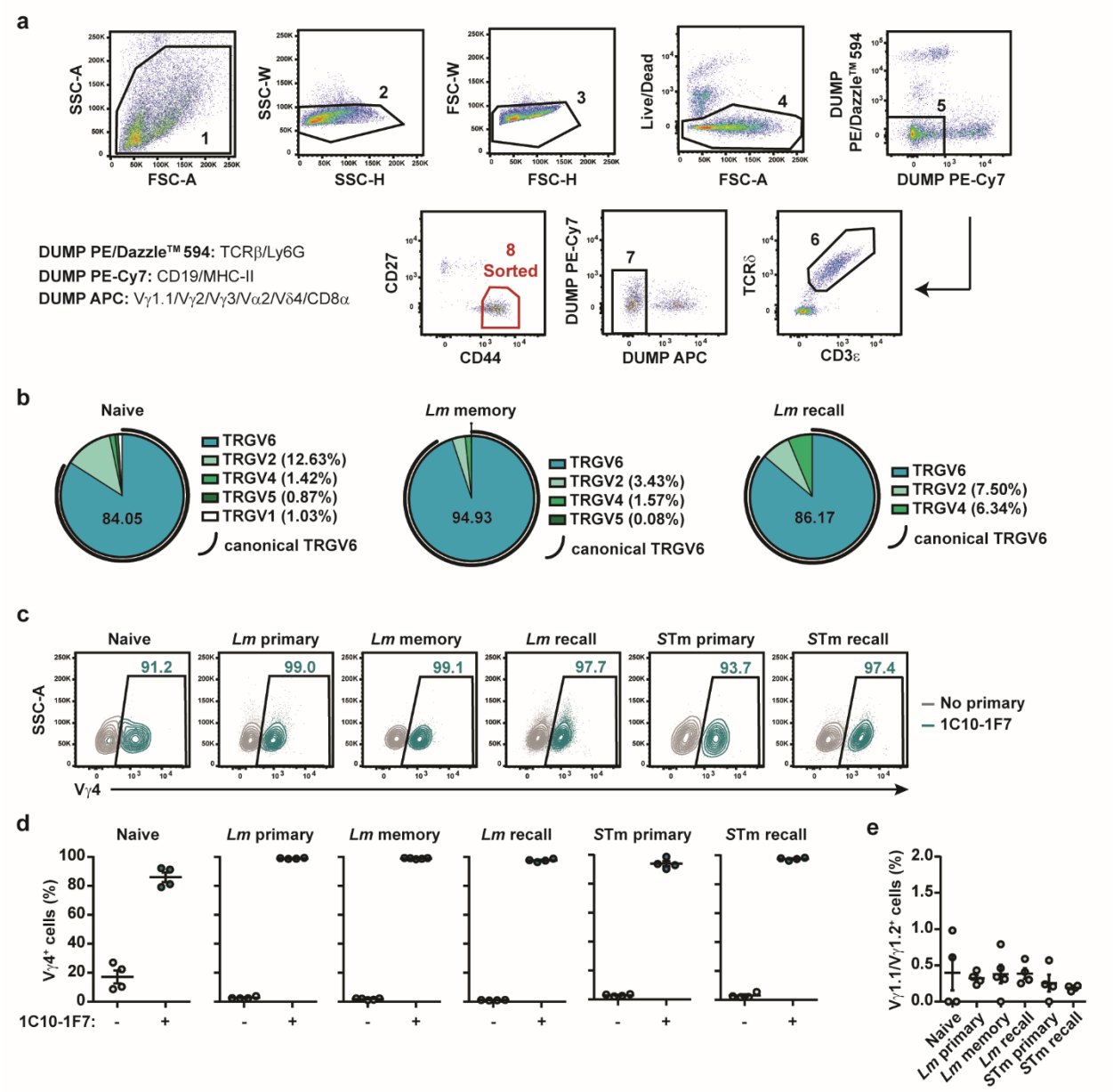

Figure S3. The vast majority of sorted CD44^{hi} CD27^{neg} γδ T cells express the Vγ4 TCR. (a) Schematic of the gating strategy used to sort purify adaptive γδ T cells. The numbers within the plots correspond to the gating hierarchy. (b) *Lm*-elicited γδ T cells in the MLN of infected Balb/c mice were sort-purified >30 days after primary infection (Memory) or 5 days after secondary infection (Recall). Naïve adaptive γδ T cells were sorted from the MLN, spleen and pLN (n=4-34 mice/group, 1 biological sample). CDR3γ transcripts were sequenced. Pie charts represent the percent of total reads mapping to the TCRγ usage in each sample. (c-e) MLN cells (*Lm* and *S*Tm samples) and mixed MLN, pLN and splenocytes (Naïve) were stained with the Vγ4-specific antibody 1C10-1F7 or left unstained. Adaptive γδ T cells were identified using the strategy shown in (a) and analyzed for 1C10-1F7 or Vγ1.1/Vγ1.2 staining. Representative flow plot overlays of 1C10-1F7 and no primary control staining are shown in (c). Scatter plots in (d) and (e) show the mean ± SEM (n=4-6 mice/group) of the percentage of Vγ4$^+$ and Vγ1.1/Vγ1.2$^+$ cells, respectively.

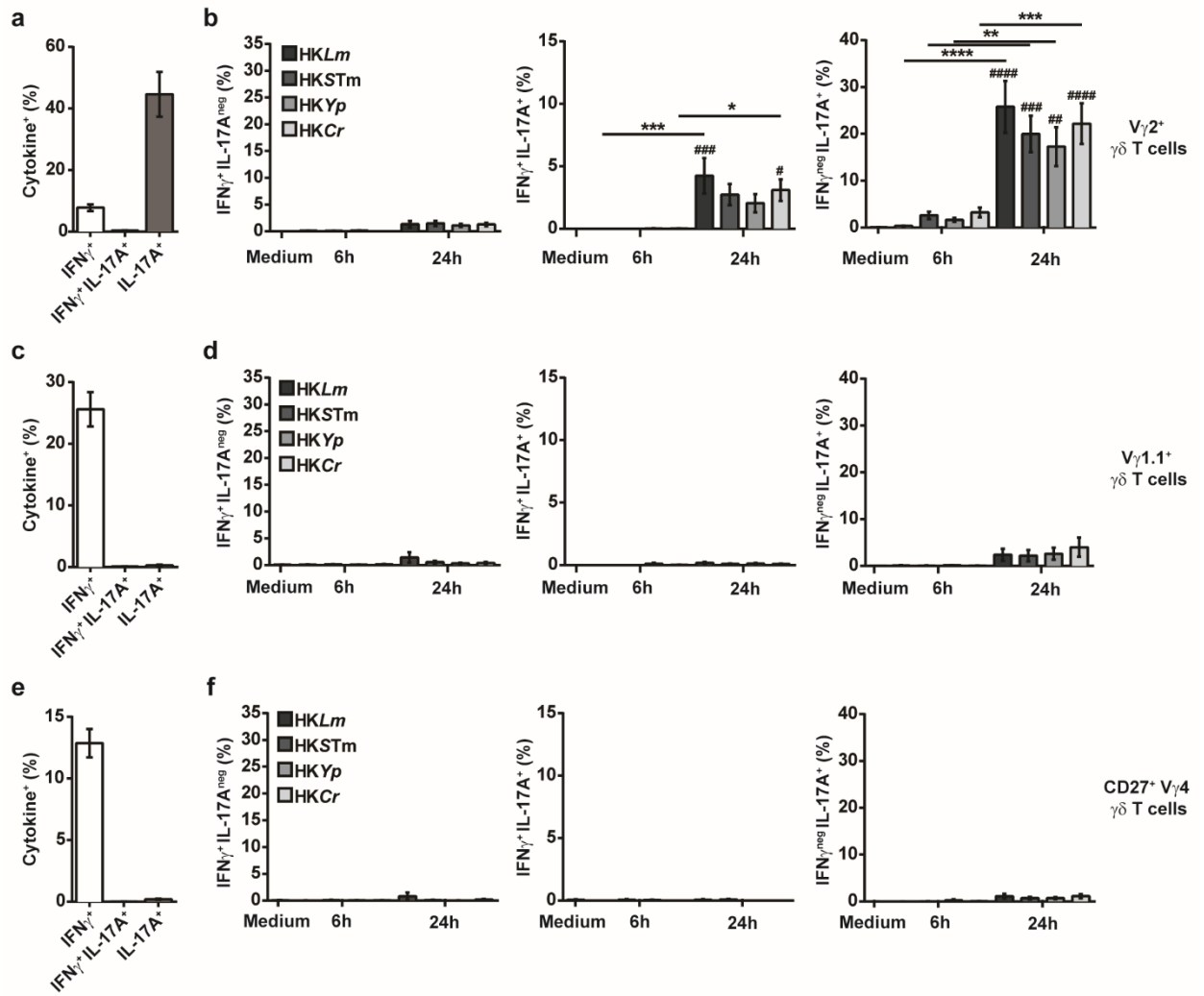

Figure S4. Cytokine production among γδ T cell subsets in response to heat-killed bacteria. Single cell suspensions of MLN from *Lm*-immune mice were stimulated for 6 hours with Leukocyte Activation Cocktail (PMA, ionomycin, and brefeldin A; a, c and e) or 6 and 24 hours with the indicated heat-killed (HK) bacteria at MOI 10 (b, d and f). (a and b) Vγ2$^+$ T cells were analyzed for IL-17A and IFNγ production. (c and d) Vγ1.1$^+$ T cells were analyzed for IL-17A and IFNγ production. (e and f) CD27$^+$ Vγ4 T cells were analyzed for IL-17A and IFNγ production. Bar graphs show the mean ± SEM (n=4 biological replicates/condition/experiment) of the percentage of cytokine-producing cells and depict the cumulative data of 2 independent experiments. *, $p \leq 0.05$; **, $p \leq 0.01$; ***, p ≤ 0.001; ****, $p \leq 0.0001$

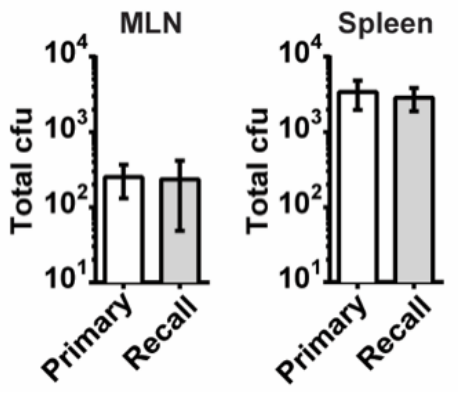

Figure S5. Intraperitoneal Cr infection leads to early colonization of the MLN and spleen. Naïve or *Lm*-immune mice were i.p. infected with *Cr.* Two days later, MLN and spleen colonization was evaluated. Bar graphs show the mean ± SEM (n=3-4 mice/group) of the total cfu per organ.

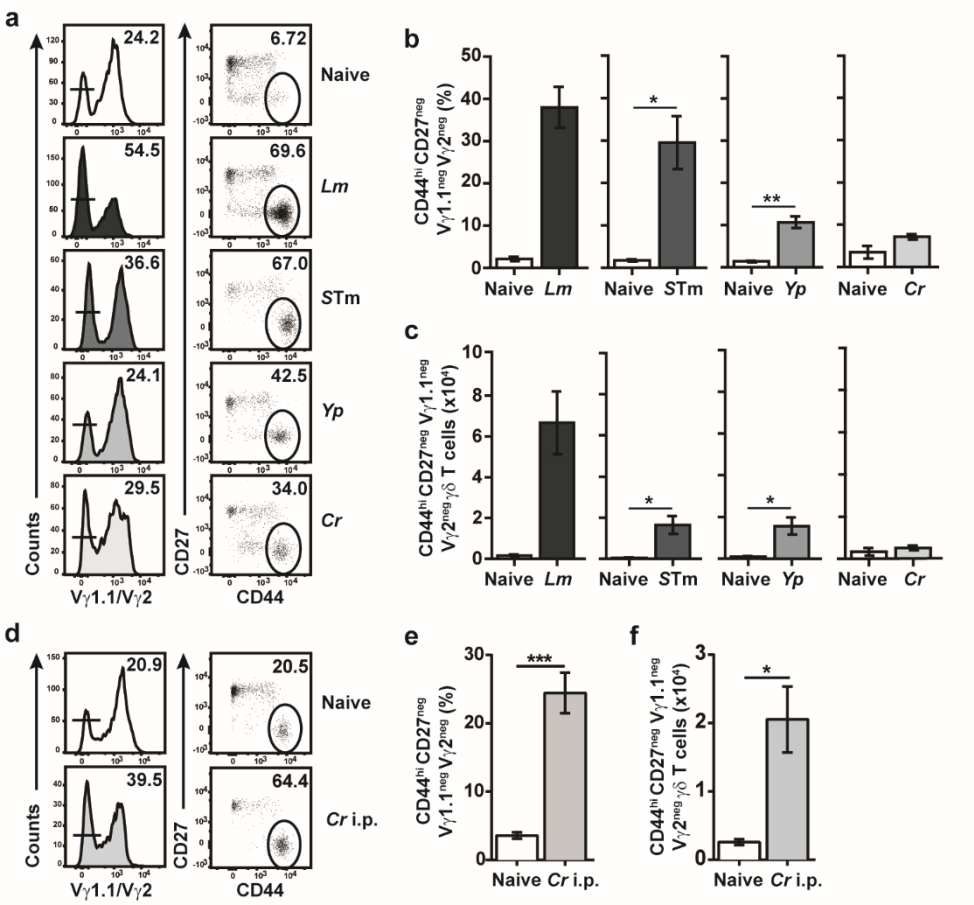

Figure S6. Diverse bacterial infection can elicit adaptive γδ T cell responses. (a-c) Mice were left uninfected or infected with *Lm*, *S*Tm, *Yp* and *Cr.* γδ T cells were analyzed by flow cytometry at 4 dpi (*S*Tm) or 9 dpi (*Lm*, *Yp*, and *Cr*). Representative flow plots are shown in (a). The naive mouse depicted was a control for the primary response to *Lm*. Data represent the mean ± SEM (n=3-6 mice/group, except for the naïve group in the *Lm* panels which comprised 2 mice) of the percentage (b) and absolute number (c) of $CD44^{hi}$ $CD27^{neg}$ Vγ4 T cells and are representative of 2 independent experiments. (d-f) Naïve mice were left uninfected or i.p. infected with *Cr* and γδ T cells analyzed in the MLN 9 days later. Representative flow plots are shown in (d). Data depict the mean ± SEM (n=3-4 mice/group) of the percentage (e) and absolute numbers (f) of $CD44^{hi}$ $CD27^{neg}$ Vγ4 T cells from the pool of 2 independent experiments. *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$

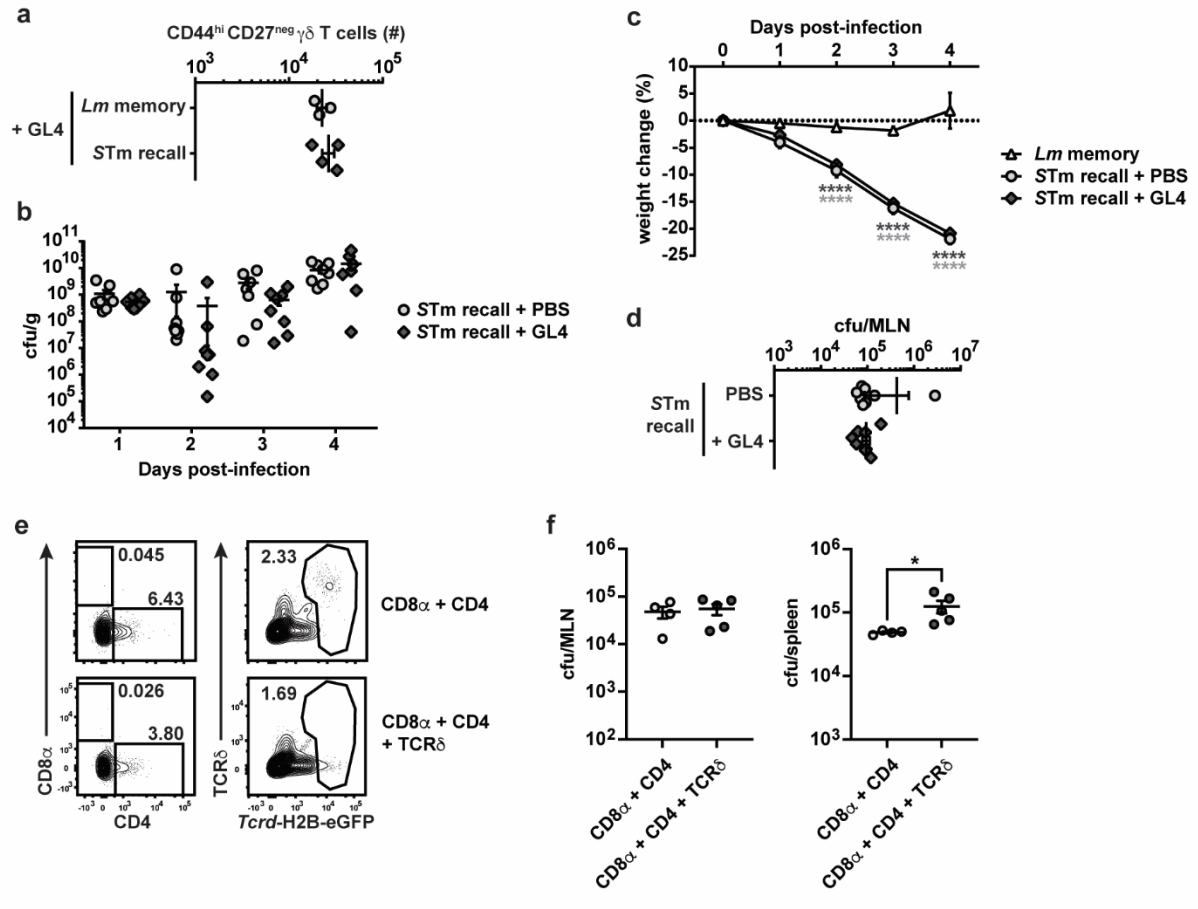

Figure S7. GL4 treatment does not affect the course of *S*Tm infection in *Lm*-immune mice. *Lm*-immune *Tcrd*-H2B-eGFP mice received 3 i.p. injections of PBS or GL4 mAb on days -3, -1 and +1 relative to *S*Tm infection. (a) γδ T cell numbers were evaluated from the MLN of GL4 treated mice at 4 dpi. Data show the mean ± SEM of 3-4 mice/group. (b-c) Mice were followed daily for (b) *S*Tm fecal shedding and (c) weight loss. (d) At 4 dpi, STm colonization was evaluated in the MLN. Data are shown as mean ± SEM and depict the cumulative data of 2 independent experiments with 3-4 mice/group. (e and f) *Lm*-immune *Tcrd*-H2B-eGFP mice received 3 i.p. injections of the indicated antibody cocktails on days -3, -1 and +1 relative to *S*Tm challenge infection. (e) Representative flow plots of CD4 and CD8α expression among live single lymphocytes (left panel) and TCRδ and *Tcrd*-H2B-eGFP expression among $CD4^-$ $CD8\alpha^-$ lymphocytes (right panel) are shown. Analysis was performed on circulating cells. (f) *S*Tm colonization of the indicated tissues. Graphs show the mean ± SEM of 1 experiment with 4-5 mice/group. *, $p \leq 0.05$; ****, $p \leq 0.0001$

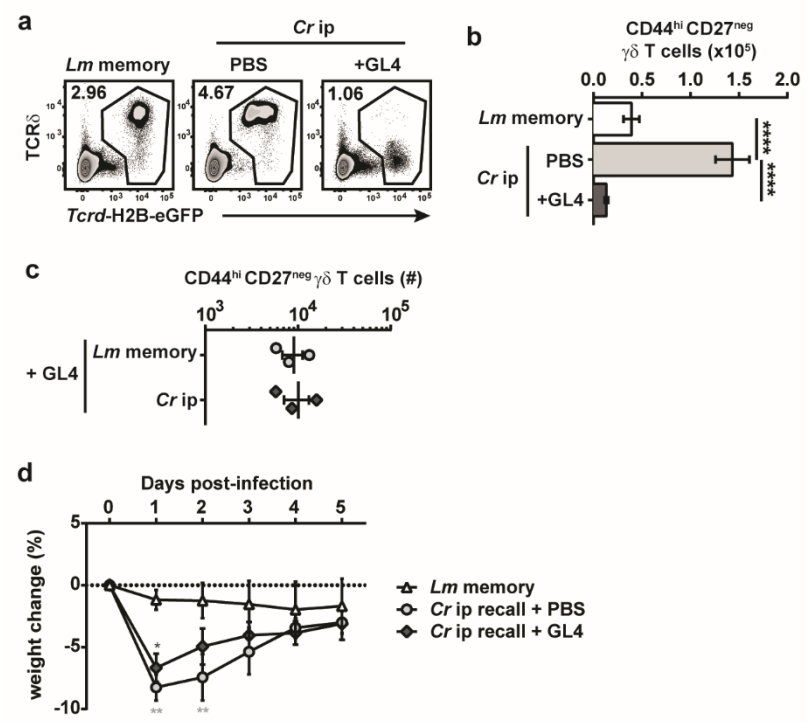

Figure S8. *Lm*-elicited γδ T cell response to *Cr* i.p. is mediated by the γδTCR. *Lm*-immune *Tcrd*-H2B-eGFP mice received 3 i.p. injections of PBS (*Lm* memory and PBS groups) or GL4 mAb on days -3, -1 and +1 relative to i.p. *Cr* challenge (+GL4). Memory mice received 100 μl PBS i.p. on day 0. Recall groups were challenged i.p. with *Cr* on day 0. (a) $CD44^{hi}$ $CD27^{neg}$ γδ T cells were analyzed after 5 days. γδ T cells were identified as $CD4^{neg}$ $CD8\alpha^{neg}$ GFP^{+} single live lymphocytes. Representative flow plots are shown. (b) Data show the mean ± SEM (n=3-4 mice/group/experiment) of $CD44^{hi}$ $CD27^{neg}$ γδ T cell absolute numbers at 5 dpi and depict the cumulative data of 2 independent experiments. (c) Comparison of $CD44^{hi}$ $CD27^{neg}$ γδ T cell absolute numbers in the MLN of GL4-treated *Lm*-immune and *Cr* challenged mice at 5 dpi (n=3 mice/group). (d) Mice were followed daily for weight loss (n=4 mice/group). *, $p \leq 0.05$; **, $p \leq 0.01$; ****, $p \leq 0.0001$

Table S1. Frequency of the non-canonical clones selected by *S*Tm primary infection in Figure 7f at each analyzed time point. Sequences are sorted by abundance at the *S*Tm primary time point. Sequences that increase in both *S*Tm primary and recall conditions are shown in bold.

	% of other non-canonical reads					
Sequence	Naïve	*Lm* primary	*S*Tm primary	*Lm* memory	*Lm* recall	*S*Tm recall
GSDRRDTTDKLV	4.506	8.232	7.171	8.644	9.667	4.070
GSDTEGSSWDTRQMF	0	0.440	0.070	0	0.004	0
GSDIGGIRVTDKLV	0.023	0.048	0.047	0.059	4.167	0.043
GSGRRDTTDKLV	0.013	0.025	0.033	0.010	0.027	0.007
GSDRGDATDKLV	0	0.031	0.031	0	0.004	0
GSGRRDTDKLV	0.004	0.016	0.029	0.026	0.028	0
GSDRGDTTDKLV	0.013	0.025	0.023	0.013	0.012	0
GSDVGGTDKLV	0	0.008	0.016	0.009	0.010	0.009
GSDIGIRAADKLV	0	0.004	0.006	0	0.020	0.017
GSDIGGITNKLV	0	0.003	0.004	0	0.003	0
GSDIGGSPRDTRQMF	0	0.003	0.004	0	0.003	0.004
GSDRRDTGSWDTRQMF	0	0.003	0.004	0.005	0.005	0
GSDVGGTTDKLV	0	0.008	0.004	0	0.008	0.011
GSGRDTSSWDTRQMF	0	0.007	0.004	0	0.005	0
GSDIGGTIDKLV	0	0.008	0.004	0	0.013	0.013
GSDRRDTADKLV	0	0.014	0.004	0	0.004	0.010

Table S2. Frequency of the non-canonical clones selected by *S*Tm recall infection in Figure 7f at each analyzed time point. Sequences are sorted by abundance at the *S*Tm recall time point. Sequences that increase in both *S*Tm primary and recall conditions are shown in bold.

	% of other non-canonical reads					
Sequence	Naïve	*Lm* primary	*S*Tm primary	*Lm* memory	*Lm* recall	*S*Tm recall
GSDIGGTDKLV	9.147	10.231	7.258	6.257	8.102	19.865
GSDIGGSFWDTRQMF	0.406	0.420	0.353	0.388	0.400	0.409
GSDIGGSYWDTRQMF	0.143	0.161	0.111	0.160	0.166	0.172
GSDFGGSSWDTRQMF	0.061	0.0786	0.054	0.045	0.055	0.072
GSDIGGIATDKLV	0.004	3.850	0.004	0	1.044	0.030
GSDRGDTDKLV	0.019	0.046	0	0.018	0.036	0.026
GSDIGIRAADKLV	0	0.004	0.006	0	0.020	0.017
GSDIGGTIDKLV	0	0.008	0.004	0	0.013	0.013
GSGIGGSSWDTRQMF	0.004	0.010	0	0.005	0.016	0.013
GSDRRDTADKLV	0	0.014	0.004	0	0.004	0.011
GSDVGGTTDKLV	0	0.008	0.004	0	0.008	0.011
GSGTGGSSWDTRQMF	0	0.014	0	0	0.004	0.004
GSDIGGIGKLV	0	0.013	0	0	0.010	0.004
GSDIGGSPRDTRQMF	0	0.003	0.004	0	0.003	0.004

Table S3. Bacterial strains and infection doses.

BACTERIA	STRAIN	INFECTION DOSE (CFU)	REFERENCES
InlA^{M} *Listeria monocytogenes*	EGDe	Primary: $2x10^{9}$ Secondary: $2x10^{10}$	(1)
InlA^{M} *Listeria monocytogenes*	10403s	GF mice - Primary: $1x10^{8}$ gavage Nur77^{GFP} and NTg – Primary: $2x10^{9}$ – Secondary: $2x10^{11}$	(2)
Salmonella enterica serovar Typhimurium	IR715	Primary and secondary: $1x10^{8}$	(3)
Yersinia pseudotuberculosis serogroup O1	32777	Primary: $5x10^{7}$ Secondary: $1x10^{8}$	(4)
Citrobacter rodentium	DBS100	Primary and secondary: $1x10^{9}$ oral $1x10^{7}$ i.p.	(5)

Table S4. List of antibodies used in this study.

Marker	Conjugate	Clone	Company
anti-mouse IgG	FITC	Poly4060	BioLegend
CD3ε	PE/Cy7	145-2C11	BioLegend
CD3ε	BV421	145-2C11	BioLegend
CD3ε	PE/Dazzle™ 594	145-2C11	BioLegend
CD3ε	BV711	145-2C11	BioLegend
CD4	Purified	GK1.5	Bio X Cell
CD4	Biotin	GK1.5	BioLegend
CD4	PE/Cy7	RM4-4	BioLegend
CD8α	Purified	2.43	Bio X Cell
CD8α	Biotin	53-6.7	BioLegend
CD8α	APC	53-6.7	BioLegend
CD8α	PE	53-6.7	BioLegend
CD8α	BV785	53-6.7	BioLegend
CD16/CD32	Purified	2.4G2	Bio X Cell
CD19	PE/Cy7	6D5	BioLegend
CD27	PerCP/Cy5.5	LG.3A10	BioLegend
CD44	APC-eFluor 780	IM7	eBioscience
B220	Biotin	RA3-6B2	BioLegend
I-A/I-E	PE/Cy7	M5/114.15.2	BioLegend
IFNγ	PE/Cy7	XMG1.2	BioLegend
IL-17A	APC	TC11-18H10.1	BioLegend
Ki-67	PE/Dazzle™ 594	16A8	BioLegend
Ly6G	PE/Dazzle™ 594	1A8	BioLegend
Phospho-Zap 70/Syk	PE	n3kobu5	Invitrogen
TCRβ	PE/Dazzle™ 594	H57-597	BioLegend
TCRβ	BV711	H57-597	BioLegend
TCRδ	BV421	GL3	BioLegend
TCRδ	PE	GL3	BioLegend
TCRδ	APC	GL3	BioLegend
TCRδ	Purified	GL4	Bio X Cell (Custom)
TCRδ	Purified	UC7-13D5	Bio X Cell
Vα2	APC	B20.1	BioLegend
Vδ4	APC	REA372	Miltenyi Biotec
Vγ1.1	FITC	2.11	BioLegend
Vγ1.1	APC	2.11	BioLegend
Vγ1.1/Vγ1.2	PE	4B2.9	BioLegend
Vγ2	FITC	UC3-10A6	BioLegend
Vγ2	PE	UC3-10A6	BioLegend
Vγ2	APC	UC3-10A6	BioLegend
Vγ3	APC	536	BioLegend
Vγ4	Purified	1C10-1F7	Provided by Dr. Hatano and Dr. Yoshikai

SI References

1. T. Wollert *et al.*, Extending the host range of Listeria monocytogenes by rational protein design. *Cell* **129**, 891-902 (2007).
2. B. S. Sheridan *et al.*, gammadelta T cells exhibit multifunctional and protective memory in intestinal tissues. *Immunity* **39**, 184-195 (2013).
3. P. A. McLaughlin *et al.*, Inflammatory monocytes provide a niche for Salmonella expansion in the lumen of the inflamed intestine. *PLoS Pathog* **15**, e1007847 (2019).
4. Y. Zhang, C. Khairallah, B. S. Sheridan, A. W. M. van der Velden, J. B. Bliska, CCR2(+) Inflammatory Monocytes Are Recruited to Yersinia pseudotuberculosis Pyogranulomas and Dictate Adaptive Responses at the Expense of Innate Immunity during Oral Infection. *Infect Immun* **86** (2018).
5. B. A. Vallance, W. Deng, K. Jacobson, B. B. Finlay, Host susceptibility to the attaching and effacing bacterial pathogen Citrobacter rodentium. *Infect Immun* **71**, 3443-3453 (2003).