Supplemental Data

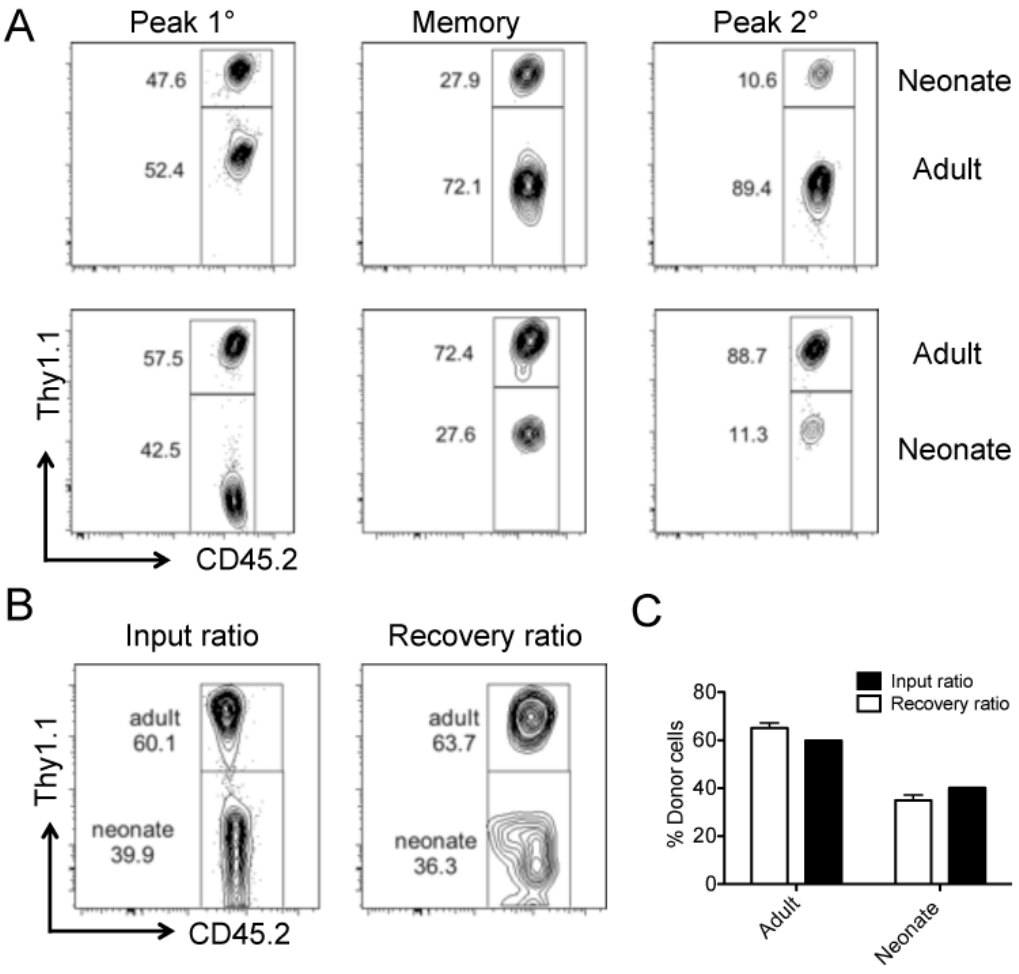

Figure S1. Impaired development of neonatal memory CD8+ T cells is not due to the expression of specific congenic markers or different levels of engraftment by neonatal and adult donor cells. *(A)* To confirm that expression of specific congenic markers does not alter our findings, we performed the co-transfer experiment with switched congenic markers. Displayed are the percentages of both donor populations at primary, memory and secondary phases of the response. In the top panel, neonatal gBT-I cells have the Thy1.1 marker (primary: day 6, memory: day 30, secondary: day 30+6). In the bottom panel adult gBT-I cells have the Thy1.1 marker (primary: day 7, memory: day 80, secondary: day 80+7). *(B)* The seeding efficiency of neonatal and adult donors was also compared by colonizing recipients with $1x10^5$ donor cells ($5x10^4$ each donor). Representative plots showing ratio of adult (Thy1.1+) to neonatal (Thy1.1-) donors prior to transfer (left) and recovered after 18 hours (right). (*C*) Bar graph showing distribution of individuals (n=4).

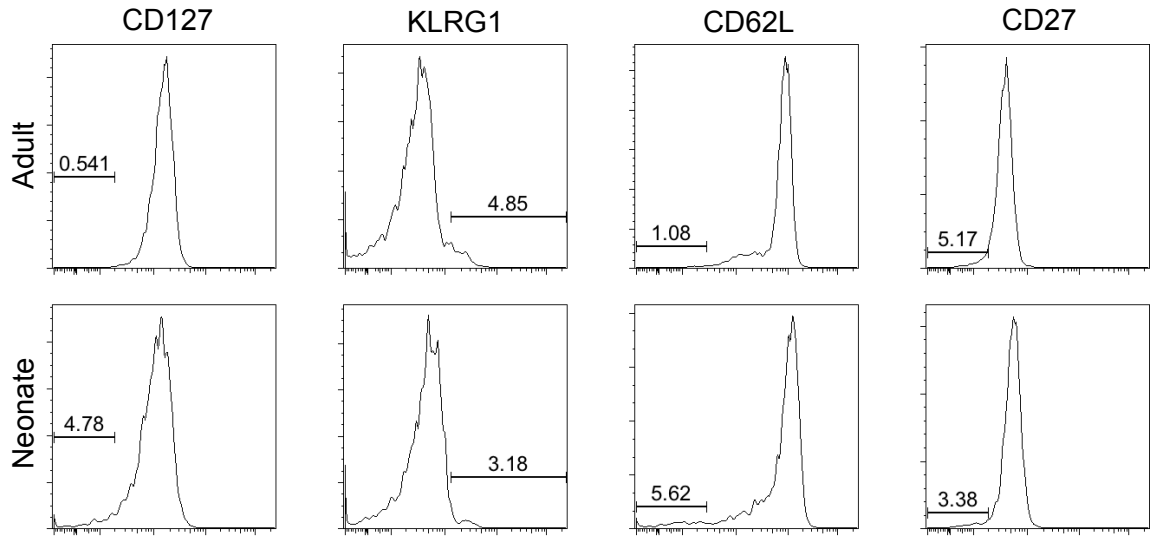

Figure S2. Adult and neonatal donors have similar naïve phenotype prior to injection. A sample of donor cells was assessed for starting phenotype. The majority of both donor populations show a similar phenotype: $CD127^{hi}KLRG1^{low}CD62L^{hi}CD27^{hi}$.

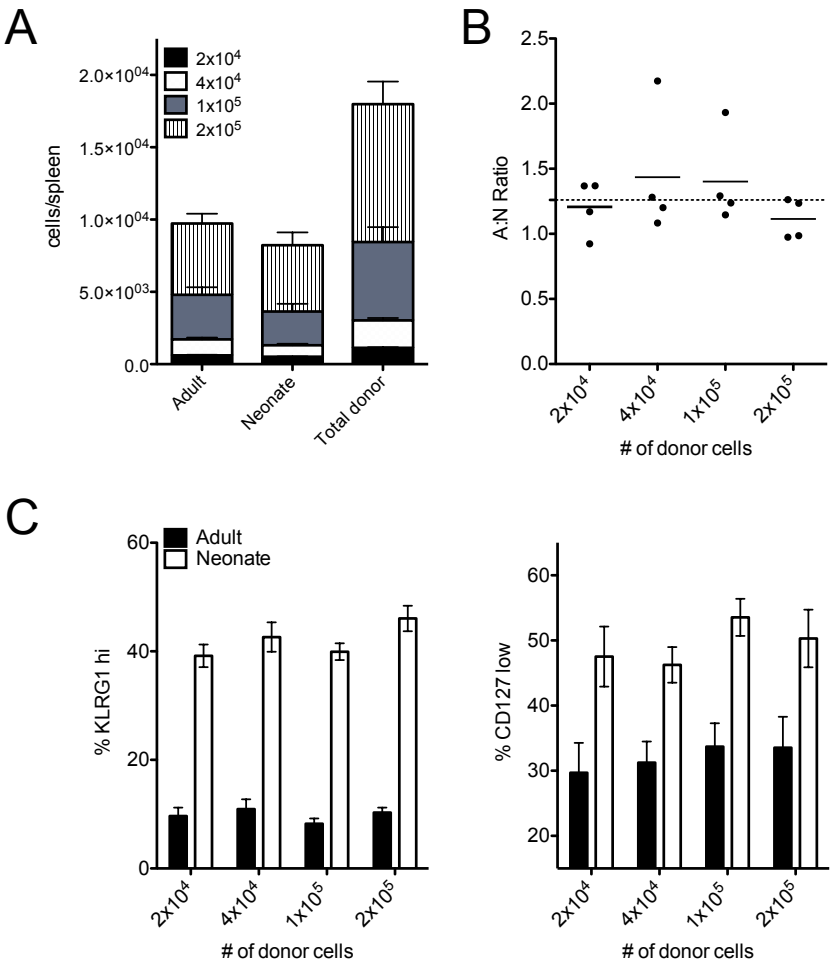

Figure S3. Higher numbers of donor cells can be transferred into recipients without altering phenotype during early infection. (*A)* The co-transfer was set up with standard number of donor cells (2 x 10^4) or two, five or ten times that amount. At all starting cell numbers, ~10% of transferred cells are recovered four days after donor cell transfer and WT LmgB infection. (*B)* Ratio of adult to neonatal donors is similar for all input donor values tested. (*C)* Percentage of each donor population that are KLRG1^{hi} or CD127^{low}. These data show that phenotypic profiles are unchanged with ten times more gBT-I cells.

A

Gene name	Adult FPKM	Neonate FPKM	q value
wls	12.16	0.57	0.0019
serpina3g	13.48	0.75	0.0019
als2cl	2.54	0.22	0.013
h2-ob	3.38	0.32	0.021
h2-dma	18.79	2.41	0.0019
pecam1	9.42	1.34	0.0019
st6gal1	1.57	0.23	0.020
mlkl	3.13	0.46	0.048
casp4	21.22	3.49	0.0019
lancl3	4.77	0.87	0.0019
fgf13	27.59	5.10	0.0019
oas1a	3.74	0.71	0.0088
ctla4	10.32	2.00	0.0019
tnfsf8	3.81	0.74	0.0019
btla	8.64	1.70	0.0019
ccr7	6.09	1.20	0.0019
pacsin1	3.38	0.73	0.0063
tlr1	10.18	2.29	0.0019
sell	21.42	4.87	0.0019
cacnb1	3.38	0.83	0.027
snrpn	0.37	12.96	0.024
dsg2	0.36	7.48	0.0019
lrig3	0.21	3.79	0.042
tshz3	0.42	6.65	0.0019
cd163l1	15.43	231.36	0.0019
clip4	0.43	4.66	0.0019
naip6	0.18	1.86	0.049
fxyd7	1.92	15.07	0.0088
klrb1c	6.41	49.73	0.0019
bmpr1a	0.55	4.16	0.0019
fcer1g	2.46	17.67	0.0019
podxl	0.42	2.73	0.0019
srgap3	1.04	6.37	0.0019
rnf43	2.48	15.05	0.0019
kcnj8	8.07	46.69	0.0019
yes1	0.58	3.26	0.0019
styk1	0.37	2.09	0.0088
syk	0.37	2.03	0.0036
lhfpl2	0.41	2.25	0.0019
stac2	0.33	1.53	0.044

B

Gene name	Adult FPKM	Neonate FPKM	q value
blimp1	11.60	17.59	0.0050
t-bet	162.81	218.04	0.062
id2	506.00	529.31	0.94
eomes	22.80	17.78	0.30
bcl6	3.29	2.48	0.50
id3	7.12	3.25	0.060

C

Gene name	Adult FPKM	Neonate FPKM	q value	Fas/ Intrinsic
flip	17.55	16.45	0.92	Fas
fasl	20.09	38.20	0.0019	Fas
caspase 7	20.27	28.97	0.031	Fas
caspase 6	16.23	18.94	0.75	Fas
caspase 3	30.94	32.55	0.95	Fas
fas	19.75	18.48	0.94	Fas
caspase 8	57.68	50.27	0.70	Fas
fadd	4.68	3.48	0.41	Fas
bcl-xl	15.68	16.69	0.94	Intrinsic
mcl-1	128.69	118.92	0.88	Intrinsic
a1	18.30	16.59	0.92	Intrinsic
bcl-2	6.89	4.58	0.019	Intrinsic
bcl-w	2.58	1.10	0.0063	Intrinsic
bad	11.95	13.35	0.92	Intrinsic
apaf-1	23.10	22.77	0.99	Intrinsic
bax	128.95	124.81	0.97	Intrinsic
bak	52.24	49.34	0.93	Intrinsic
bnip3	1.26	1.18	1 (no test)	Intrinsic
bid	19.90	18.48	0.92	Intrinsic
cyt c	10.12	9.21	0.89	Intrinsic
bmf	3.02	2.65	0.85	Intrinsic
bik	1.56	1.24	1 (no test)	Intrinsic
bim	10.14	6.81	0.32	Intrinsic

Table S1. Expression levels for RNAseq genes.

Expression values, as FPKM, are shown for *(A)* the most differentially expressed genes, *(B)* transcription factors involved in effector or memory cell differentiation, and *(C)* genes used in the Fas or intrinsic apoptosis pathway. q values indicate multiple-test corrected significance level.