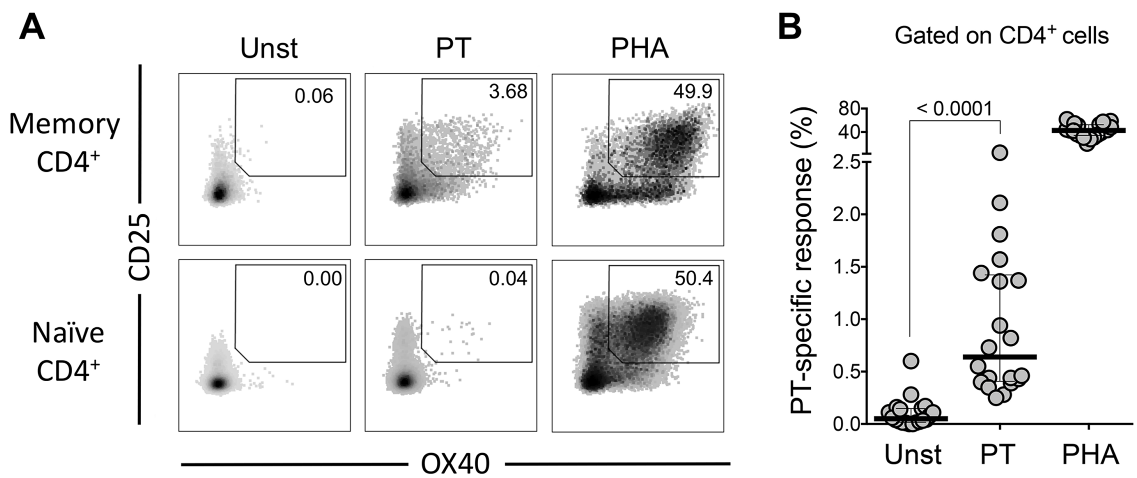
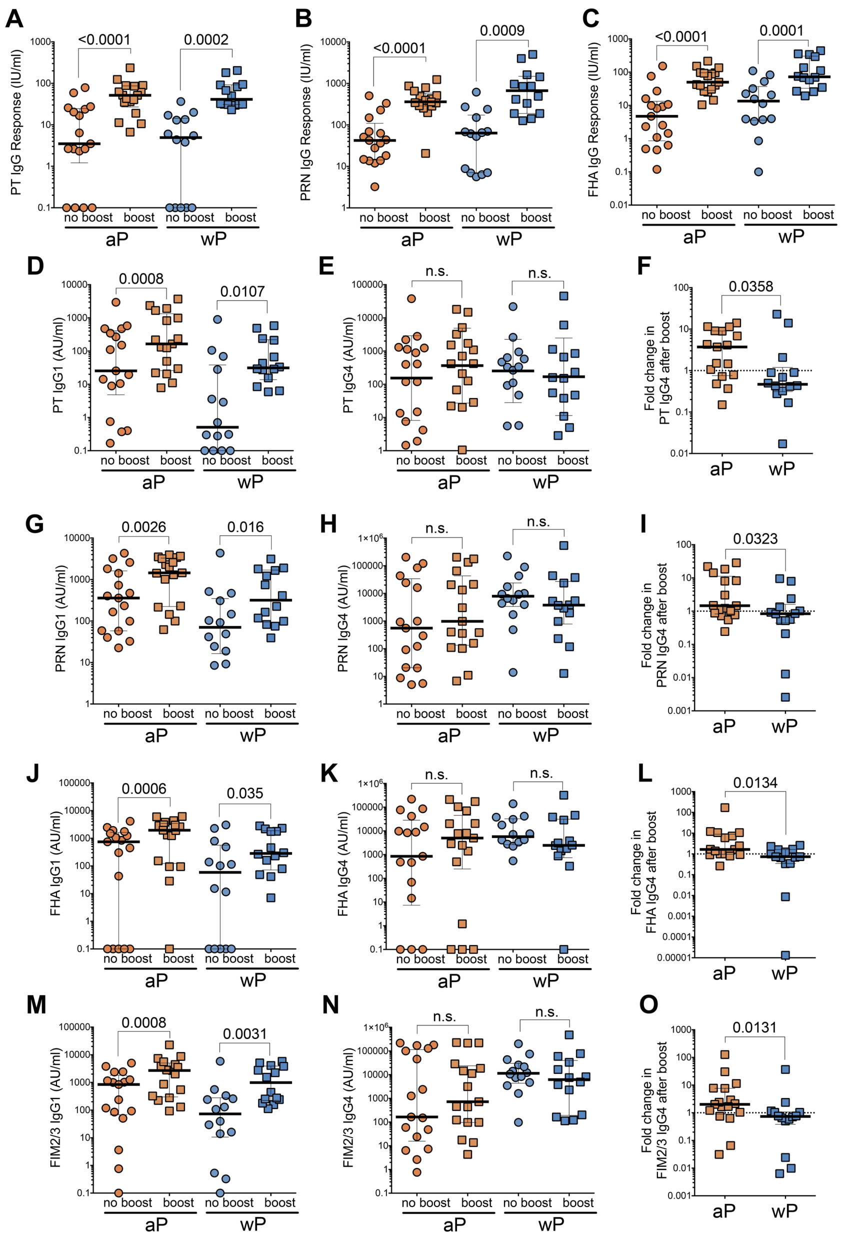
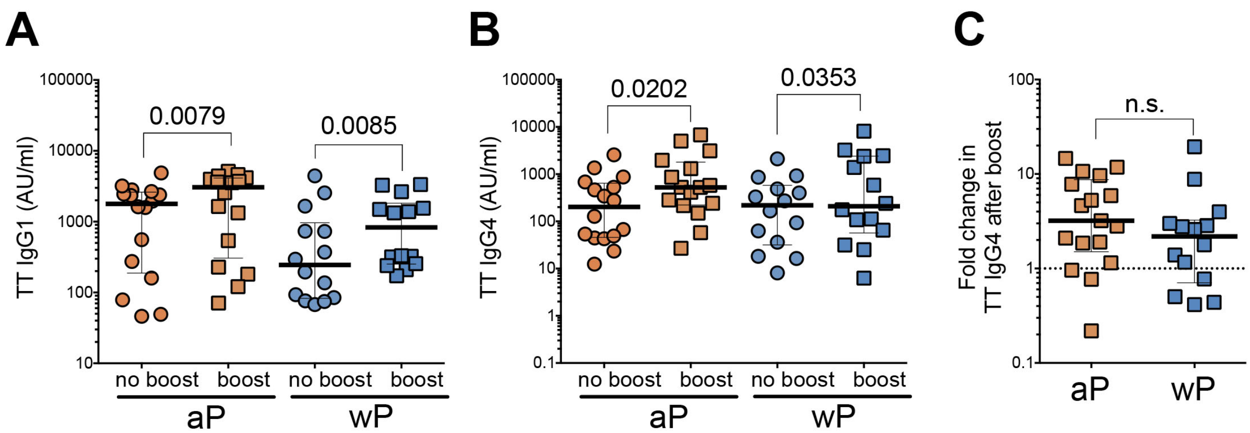
SUPPLEMENTAL DATA



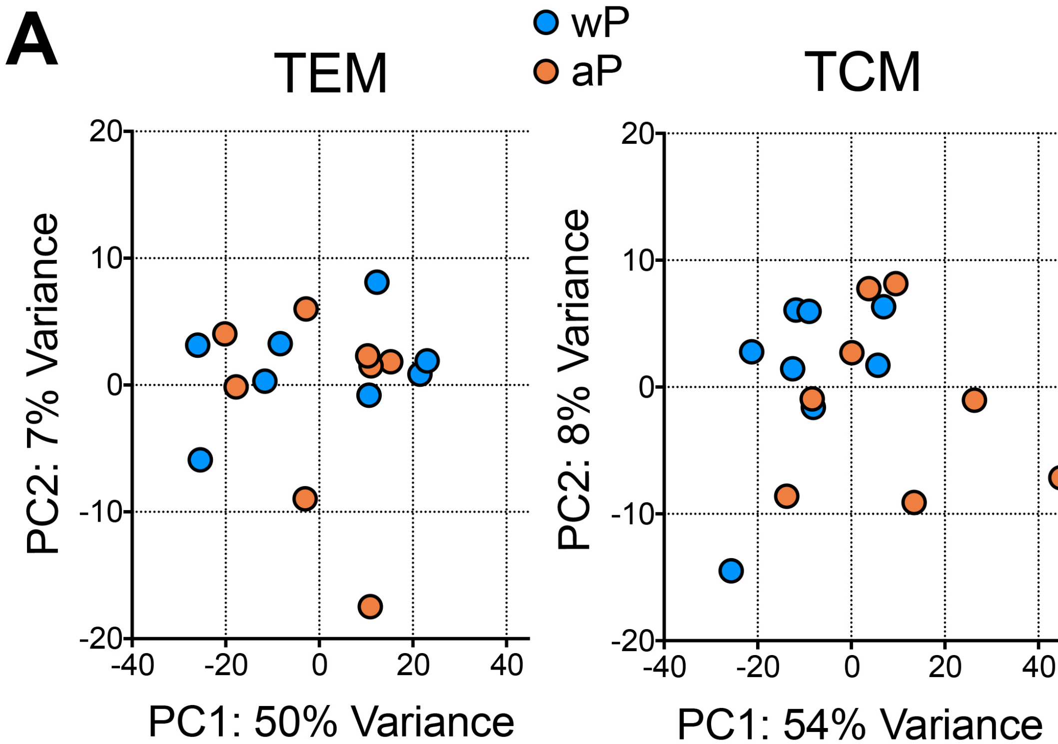
Supplementary Figure 1. AIM$_{25}$ assay detects Pertussis-specific CD4+ T cells. (A) Representative flow cytometry plots of CD25+OX40+ upregulation by CD4+ T cells in naïve (CD45RA+CCR7+; lower panel) and antigen-experienced memory (excluding naïve cells; upper panel) cells left unstimulated (Unst) or stimulated with pertussis-megapool [PT (MP)] or PHA as a positive control. (B) CD25+OX40+ expression by CD4+ memory T cells after 24 hours. Each dot represents one donor (n=20) originally primed with DTwP vaccine and not recently boosted. Data are expressed as median ± the interquartile range for each condition and comparison analyzed via Mann-Whitney unpaired t-test.



Supplementary Figure 2. Individual aP antigen antibody profiles of aP and wP donors. Total (A) PT, (B) PRN and (C) FHA-specific IgG titers represented as IU/ml for each cohort pre- and post-aP boost. (D-F) PT-specific, (G-I) PRN-specific, (J-L) FHA-specific, (M-O) FIM2/3-specific IgG1, IgG4 and fold change in IgG4 levels after aP boost for each cohort. Pre- and post-response for each cohort analyzed via Wilcoxon paired t-test. Comparison between aP and wP pertussis-specific IgG4 fold change analyzed via Mann-Whitney unpaired t-test. For all panels data are expressed as median ± the interquartile range for each cohort and each data point represents a single donor (n=19 for aP and n=14 for wP cohorts).



Supplementary Figure 3. TT IgG1 and IgG4 antibody profiles of aP and wP donors are similar. (A) IgG1 and (B) IgG4 TT-specific antibody levels of aP and wP donors pre- and post- aP boost. Pre- and post- boost comparison was evaluated via Wilcoxon paired t-test (C) Fold change in TT-specific IgG4 from pre- to post-aP boost. Comparison between aP and wP fold change analyzed via Mann-Whitney unpaired t-test. For all panels data are expressed as median ± the interquartile range for each cohort and each data point represents a single donor (n=19 for aP and n=14 for wP cohorts).



Supplementary Figure 4. Comparison of gene expression profiles of TT stimulated T cells for aP and wP donors. (A) TEM or TCM cells from aP and wP donors have undistinguishable transcriptomic profiles after TT-specific stimulation, based on the expression of the top 1000 variable genes. Each data point represents a single donor (n=8 for each cohort).

Supplementary Table 1a. Characteristics of the donor population in a cross-sectional cohort

Cohort	No. Samples	Age (range)	Childhood Priming Vaccine	Boost Vaccine	Time Since Boost (mo)
wP/no boost	20	38 (24-64)	DTwP	n/a	n/a
wP/boost	22	34 (21-51)	DTwP	Tdap	1-3
aP/boost	18	19 (18-21)	DTaP	Tdap	1-3

Supplementary Table 1b. Characteristics of the donor population in a 1st longitudinal cohort

Cohort	No. Samples	Age (range)	Childhood Priming Vaccine	Boost Vaccine	Time Since Boost (mo)
wP/no boost	18	28 (22-54)	DTwP	n/a	n/a
wP/aP boost	18	28 (22-54)	DTwP	Tdap	0.5-12
aP/no boost	15	19 (18-20)	DTaP	n/a	n/a
aP/aP boost	15	19 (18-20)	DTaP	Tdap	0.5-12

Supplementary Table 1c. Characteristics of the donor population in a 2nd longitudinal cohort

Cohort	No. Samples	Age (range)	Childhood Priming Vaccine	Boost Vaccine	Time Since Boost (days)
wP/no boost	24	29 (21-35)	DTwP	n/a	n/a
wP/aP boost	24	29 (21-35)	DTwP	Tdap	1-180
aP/no boost	20	19 (18-20)	DTaP	n/a	n/a
aP/aP boost	20	19 (18-20)	DTaP	Tdap	1-180

Supplementary Table 2. List of individual peptides of each megapool.

Pertussis (132 peptides)	**Tetanus** (125 peptides)	**CMV/EBV** (122 peptides)
Sequence	**Sequence**	**Sequence**
GADLIIANPNGISVNG	PITINNFRYSDPVNNDTIIM	VKLTMEYDDKVSKSH
VVARLVKLQGAVSSKQ	IYYKAFKITDRIWIVPERYE	PRSPTVFYNIPPMPLPPSQL
GKPLADIAVVAGANRY	DPNYLRTDSDKDRFL	LTAYHVSTAPTGSWF
VVAGANRYDHATRRAT	TDSDKDRFLQTMVKL	IIFIFRRDLLCPLGAL
DHATRRATPIAAGARG	RFLQTMVKLFNRIKN	PAQPPPGVINDQQLHHLPSG
SSDSGLGVRQLGSLSS	VKLFNRIKNNVAGEALLDKI	GGSILQTNFKSLSSTEF
RQLGSLSSPSAITVSS	AMLTNLIIFGPGPVLNKNEV	VFLQTHIFAEVLKDAIKDLV
GQVRATSAGAMTVRDV	PALLLMHELIHVLHGLYGMQ	EDLPCIVSRGGPKVKRPPIF
GFLKSAGAMTVNGRDA	QEIYMQHTYPISAEELFTFG	KTSLYNLRRGIALAIPQCRL
VRLDGAHAGGQLRVSS	LFTFGGQDANLISIDIKNDL	GPWVPEQWMFQGAPPSQGTD
VAELKSLDNISVTGGE	IKNDLYEKTLNDYKAIANKL	PHDITPYTARNIRDAACRAV
NISVTGGERVSVQSVN	LKSEYKGQNMRVNTNAFRNV	TDDSGHESDSNSNEGRH
RVSVQSVNSASRVAIS	AFRNVDGSGLVSKLIG	NPKFENIAEGLRALLARSHV
IDVRGGSTVAANSLHA	TKNKPLNFNYSLDKIIVDYN	SNPKFENIAEGLRVLLARSH
RSMTLGIVDTTGDLQA	IVDYNLQSKITLPNDRTTPV	VYGGSKTSLYNLRRGTALAI
SASRARIDSTGSVGIG	LQRITMTNSVDDALINSTKI	SDDELPYIDPNMEPV
KVAKKLFLNGTLRAVN	NSTKIYSYFPSVISKVNQGA	LRALLARSHVERTTD
SVVSDAALVADGGPIV	VNQGAQGILFLQWVRDIIDD	PTCNIKATVCSFDDGVDLPP
QRIEAQRIENRGTFQS	VRDIIDDFTNESSQKT	PQCRLTPLSRLPFGM
AAQVTQRGGAANLTSR	IDKISDVSTIVPYIGPALNI	KTSLYNLRRGTALAIPQCRL
HDTRFSNKIRLMGPLQ	PALNIVKQGYEGNFIGALET	SRDELLHTRAASLLY
IRLMGPLQVNAGGAVS	GALETTGVVLLLEYIPEITL	VYGGSKTSLYNLRRGIALAV
TSRGGFDNEGKMESNK	LEKRYEKWIEVYKLVKAKWL	PSMPFASDYSQGAFT
FTVQAQRIDNSGTMAA	SYQMYRSLEYQVDAIKKIID	QQRPVMFVSRVPAKK
PHLRNTGQVVAGHDIH	RESSRSFLVNQMINEAKKQL	SGHESDSNSNEGRHH
VVAGHDIHIINSAKLE	AKKQLLEFDTQSKNILMQYI	AQEILSDNSEISVFPK
IINSAKLENTGRVDAR	QYIKANSKFIGITEL	ENIAEGLRVLLARSHVERTT
NDIALDVADFTNTGSL	NLDINNDIISDISGFNSSVI	RPFFHPVGEADYFEY
DFTNTGSLYAEHDATL	INGKAIHLVNNESSEVIVHK	PPVVRMFMRERQLPQ
ILPVAEGTLRVKAKSL	FNNFTVSFWLRVPKV	DGEPDMPPGAIEQGPADDPG
LRVKAKSLTTEIETGN	SFWLRVPKVSASHLE	APGPGPQPGPLRESIVCYFM
PGSLIAEVQENIDNKQ	ASHLEQYGTNEYSIISSMKK	PQCFWEMRAGREITQ
VANEANALLWAAGELT	SSMKKHSLSIGSGWSVSLKG	RRPQKRPSCIGCKGT

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RGHTLESAEGRKIFGE
EGRKIFGEYKKLQGEY
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IQPGRVTLAKALSAAL
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GAIHNGENAAQNRGRP
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NPHIFTRIGAAQTSLA
LASLASLDAAQGLEVS
AARVAGDNYFDTTLVR
GKPLADIAVIAGANRY
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VQVRISNLNDSKITMG
TMRYLASYVKKNGDVE
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SKSYTLRYLASYVKKP
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TRLSYFSMNHDPVKI
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RLCAVFVRSGQPVIGA	LYKKMEAVKLRDLKT	RNGFTVLCPKNMIIK
SGQPVIGACTSPYDGK	EAVKLRDLKTYSVQL	
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HVSKEEQYYDYEDATF		
TQHGSPYGRCANKTRA		
HYYSKVTATRLLASTN		
SRLCAVFVRDGQSVIG		
VHVSKEEQYYDYEDAT		
VYKYDSRPPEDVFQNG		

Supplementary Table 3. List of antibodies used in the study

Antibody	Fluorochrome	Clone	Host	Vendor	Catalog #
Bcl-2	BV421	100	mouse	BioLegend	658709
BTLA	APC	M5E2	mouse	BioLegend	344509
CCR7	PerCP/Cy5.5	G043H7	mouse	BioLegend	353220
CD14	V500	M5E2	mouse	BD Biosciences	561391
CD152	PE Cy7	L3D10	mouse	BioLegend	349914
CD178	PE	NOK-1	mouse	BioLegend	306406
CD19	V500	HIB19	mouse	BD Biosciences	561121
CD25	FITC	M-A251	mouse	BD Biosciences	555431
CD25	PerCPCy5.5	BC96	mouse	BioLegend	302626
CD27	APC	O323	mouse	eBioscience	17-0279-42
CD28	PE Cy7	CD28.2	mouse	BD Biosciences	560684
CD3	AF700	UCHT1	mouse	eBioscience	56-0038-42
CD4	APCef780	RPA-T4	mouse	eBioscience	47-0049-42
CD45RA	ef450	HI100	mouse	eBioscience	48-0458-42
CD45RO	FITC	UCHL1	mouse	eBioscience	11-0457-42
CD69	BV605	FN50	mouse	BD Biosciences	562989
CD71	PE/Cy7	CY1G4	mouse	BioLegend	334112
CD8	V500	RPA-T8	mouse	BD Biosciences	560774
IFNg	APC	4S.B3	mouse	eBioscience	17-7319-82
IL-17	PE	eBio64DEC17	mouse	eBioscience	12-7179-42
IL-17	PE-Cy7	eBio64DEC17	mouse	eBioscience	25-7179-42
IL-4	BV605	MP4-25D2	rat	BioLegend	500828
IL-9	PerCP/Cy5.5	MH9A4	mouse	BioLegend	507610
IL-9	PE	MH9D1	mouse	eBioscience	12-7098-42
KLRG1	BV605	2F1/KLRG1	Syrian Hamster	BioLegend	138419
OX40	PE/Cy7	Ber-ACT35	mouse	BioLegend	350012
OX40	BV421	Ber-ACT35	mouse	BioLegend	350014
PD-1	BV605	EH12.1	mouse	BD Biosciences	563245
PD-L1	PE	29E.2A3	mouse	BioLegend	329706
Tim3	BV605	F38-2E2	mouse	BioLegend	345018