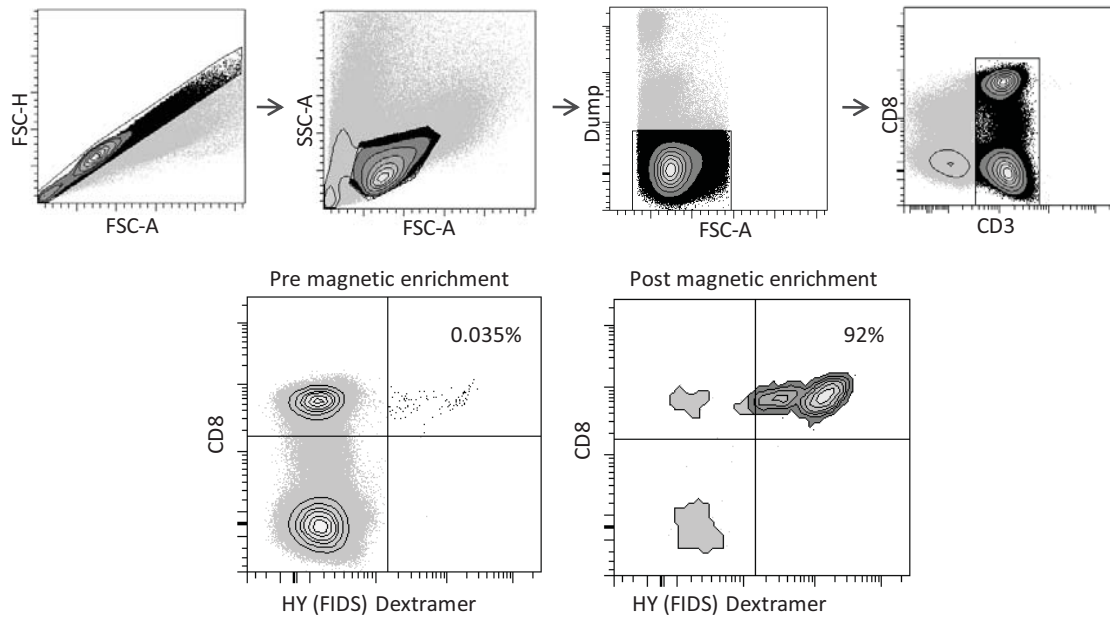
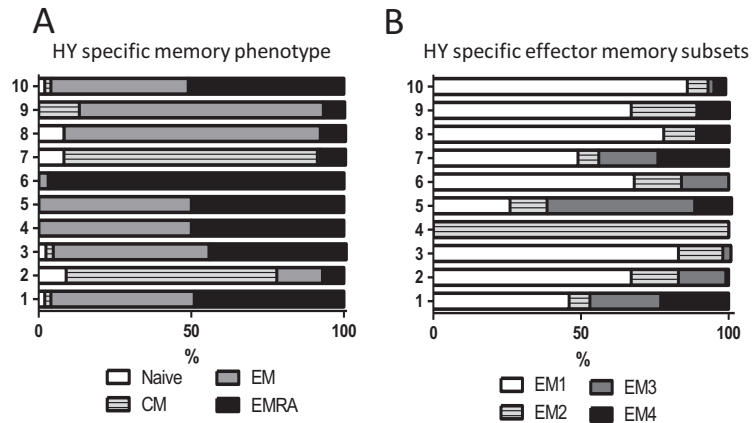


Supplementary Figure 1.



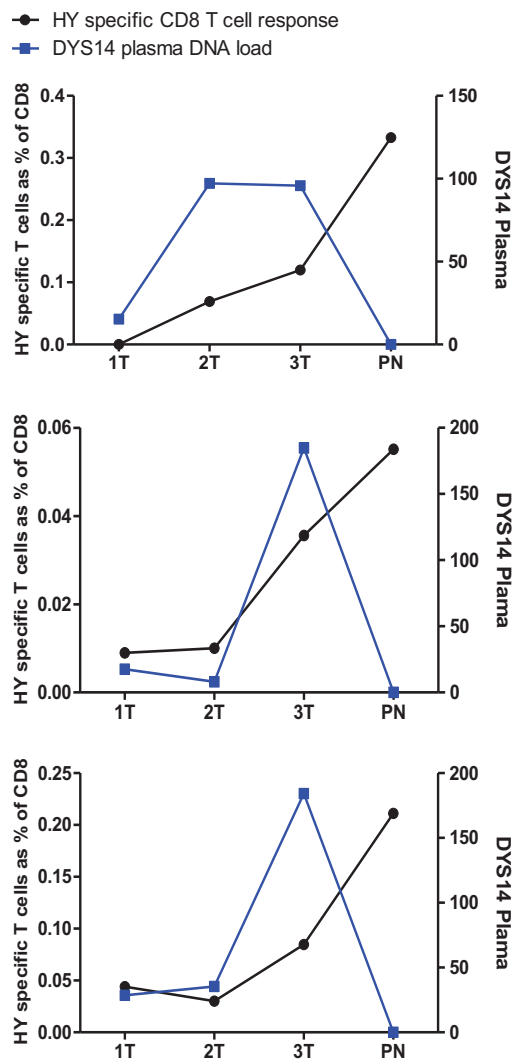
Supplementary Figure 1. The gating strategy. Singlet cells were identified on a FSC-A vs FSC-H plot and small lymphocytes gated on the basis of standard light scatter properties. A “dump” channel was used to exclude dead cells, monocytes and B-cells. T lymphocytes were then selected by CD3 expression. Finally, dextramer “positive” cells were visualised against CD8 expression, before and after magnetic bead enrichment of HY (FIDS) specific T cells. Proportions of dextramer “positive” cells are given as a percent of CD8 positive cells.

Supplementary Figure 2.



Supplementary Figure 2. Phenotype of fetal specific T cells. The memory phenotype of HY specific T cells in 10 women during pregnancy with a male fetus is described. The phenotype is defined as naïve (CD45RA+CCR7+), central memory (CM, CD45RA-CCR7+), effector memory (EM, CD45RA-CCR7-) and effector memory RA revertant (EMRA, CD45RA+CCR7-) (A). The effector memory phenotype has been further subdivided into effector memory subsets on the basis of CD28 and CD27 expression into EM1 (CD27+CD28+), EM2 (CD27+CD28-), EM3 (CD27-CD28-), EM4 (CD27-CD28+) (B).

Supplementary Figure 3.



Supplementary Figure 3. Correlation of free fetal DNA and HY specific T cell response.

Quantitative real time PCR was used to measure the maternal plasma free fetal DNA load, using the male specific gene *DYS14*. This is correlated with the HY specific T cell response in 3 representative donors in the 1st trimester (1T), 2nd trimester (2T), 3rd trimester (3T) and postnatal period (PN).

Supplementary Table 1.

LSR II configuration and reagents.

	Laser	Filter	Antibody	Clone	Conjugate
LSR II configuration for HY specific T cell detection	488 nm	575/26	HY-HLA*0201 Dextramer		PE
		610/20	Propidium Iodide		
			CD14 ^a	RMO52	ECD
			CD19 ^a	J3-119	ECD
		450/50	CD8 ^b	OKT8	Pacific Blue
	405 nm	525/50	CD3 ^c	SK7	AmCyan
LSR II configuration for HY specific T cell phenotyping	488 nm	530/30	CCR7d	150503	FITC
		575/26	HY-HLA*0201 Dextramer		PE
		610/20	Propidium Iodide		
			CD14a	RMO52	ECD
			CD19a	J3-119	ECD
		695/40	CD28c	L293	PerCP-Cy5.5
	405 nm	450/50	CD8b	OKT8	Pacific Blue
		525/50	CD3c	SK7	AmCyan
	633 nm	660/20	CD57e	HCD57	APC
		730/45	CD45RAe	HI100	AF700
	780/60	CD27b	O323	APC-AF750	

a=Beckman Coulter, b=eBioscience, c=Becton Dickinson, d=R&D systems, e=Biolegend.