Supplemental Figure 1

A
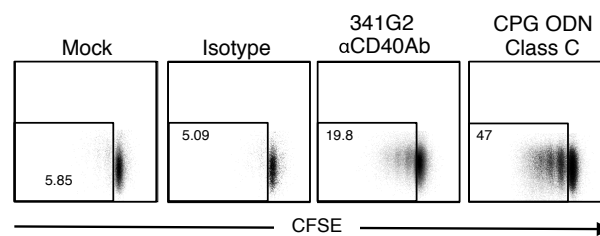
DC Maturation
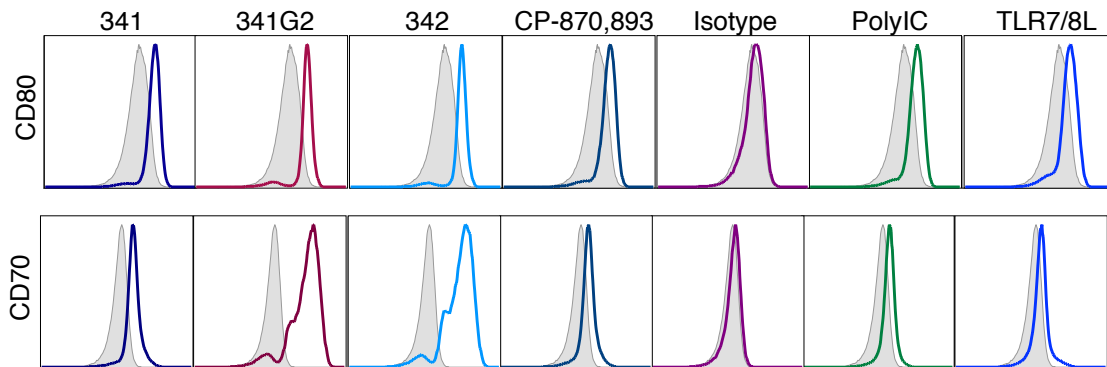
B

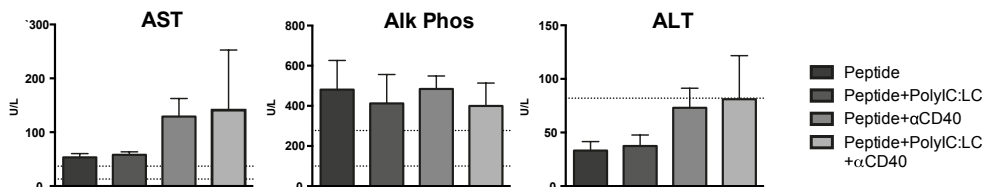### DC IL-12 p40/p70 Production
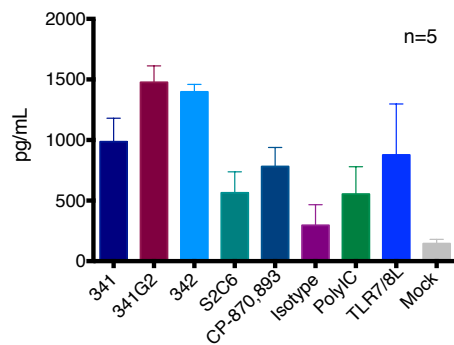

C

B Cell Proliferation

D

Liver Function Tests

**Supplemental Figure 1: Characterization of agonistic anti-CD40 clones**
(A) Human MDDCs were stimulated overnight with anti-CD40 Ab produced based on previously established sequences (figure labels indicate original clone name, see references 14, 17, 18), polyIC, TLR7/8L or an isotype Ab control. Maturation was assessed by CD70 and CD80 upregulation compared to donor matched unstimulated MDDCs **(B)** IL-12p40/p70 levels in supernatants from stimulated rhesus MDDCs as measured by ELISA (Mean±SEM, n=5). **(C)** Purified CD19+ human B cells were CFSE labeled and stimulated as indicated for 5 days to determine B cell proliferation. **(D)** Results from standard liver function test panel taken 48 hours after primary immunization of Figure 4, dotted lines represent normal conditions for rhesus macaques (Mean±SEM, n=5/group).

Supplemental Figure 2
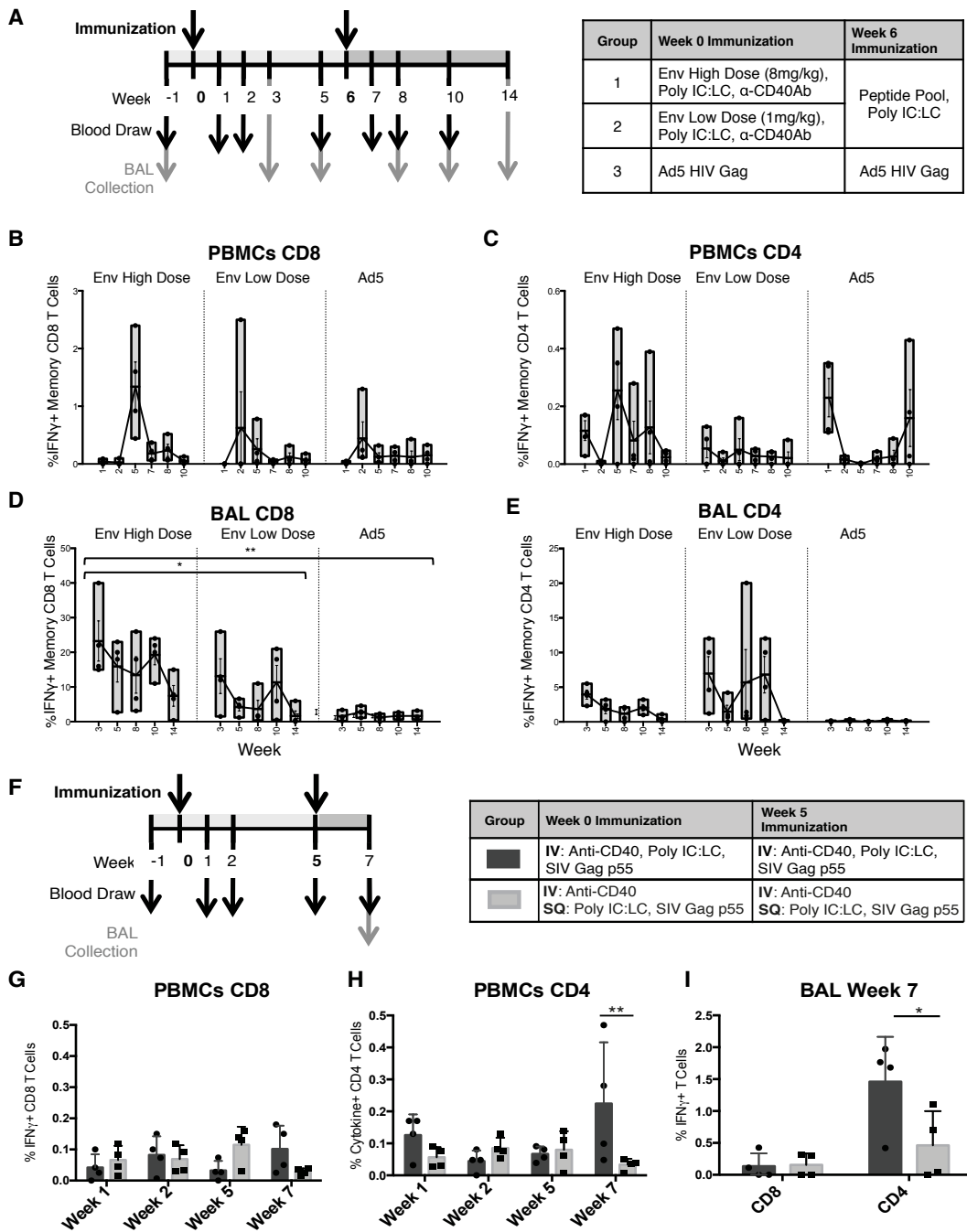
Supplemental Figure 2: Pilot studies establish immunogenicity of Anti-CD40 and Poly IC:LC in rhesus macaques
αCD40Ab was tested in combination with poly IC:LC to determine its ability to enhance Ag-specific T cell responses. Experimental design and results shown for two pilot studies, a dose study of synthetic long peptides (A-E) and a route of administration test with protein antigen (F-I) **(A)** Animals (n=5/group) were immunized i.v. with a high dose (8mg/kg) or low dose (4mg/kg) of Env peptide pool with poly IC:LC (1mg) and αCD40Ab (1.5mg/kg). A third group received rAd5 HIV Gag i.m. (1x1010 PFU). At week 6 animals received a boost of the Env peptide pool (1mg/kg) with poly IC:LC (1mg). **(F)** Animals (n=4/group) were immunized i.v. with αCD40Ab (1.5mg/kg) and received protein SIV Gag p55 (1mg) with Poly IC:LC (1mg) either i.v. or s.q. Animals received a homologous boost at week 5. Blood and BAL samples were collected as indicated in (A and F) and analyzed in an ICS-based assay for T cell responses **(B-E, G-I)** PBMC and BAL samples were stimulated overnight with a matching immunizing peptide pool to recall total memory (CD45RA+ and CCR7+ naïve cells excluded) are indicated in bar graphs, *p ≤ 0.05 and **p ≤ 0.005 using two-way ANOVA

Supplemental Figure 3
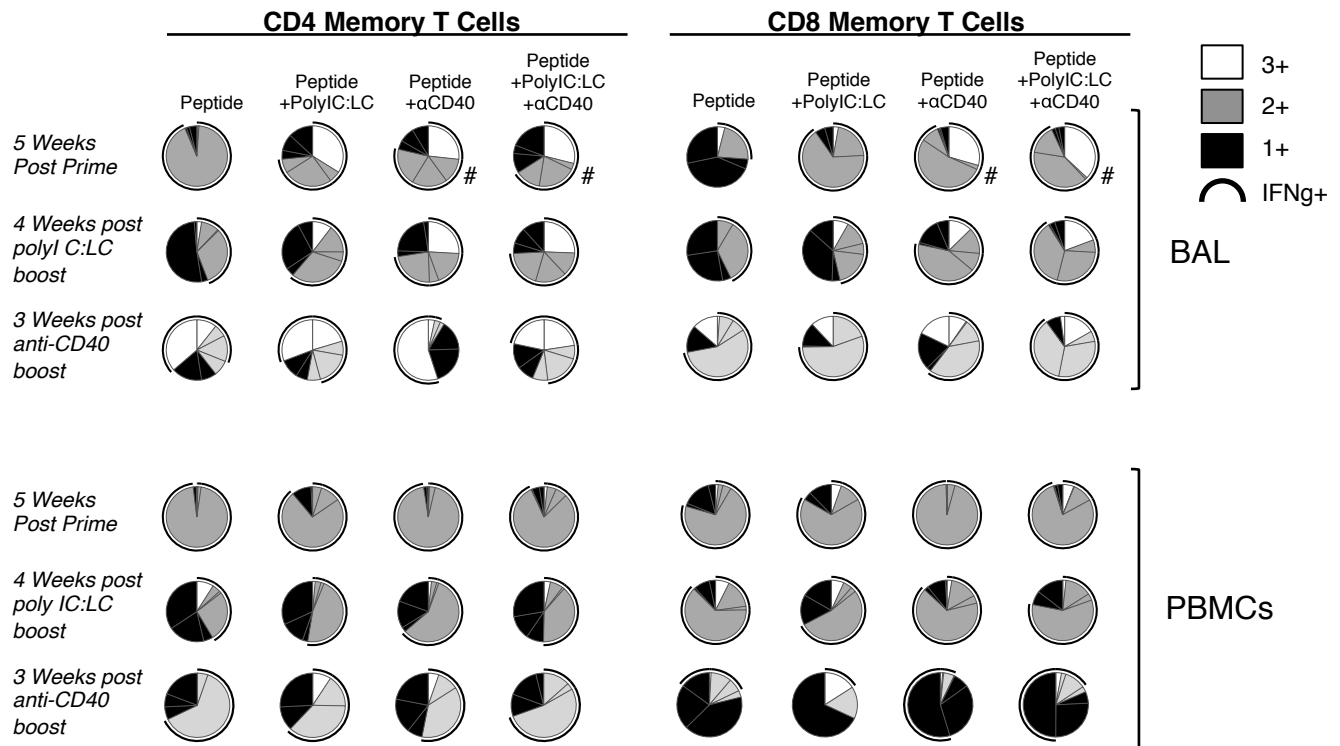
Supplemental Figure 3: Polyfunctionality of Env-specific T cells in different adjuvant groups
Pie charts show the proportion of total memory CD4/CD8 T cells producing IFNγ, IL-2, and TNF (white), 2+ cells producing any two of IFNγ, IL-2, and TNF (grey), and 1+ cells producing IFNγ, IL-2, or TNF alone (black). The black arc represents cells that produce IFNγ. Significant time points are compared to peptide only group.

Supplemental Table I: Fluroescently labeled antibodies used in flow cytometry

PBMC Phenotyping 1			PBMC Phenotyping 2			CD40 Tracking			T Cell Reponses		
Antibody	Fluorochrome	Supplier	Antibody	Fluorochrome	Supplier	Antibody	Fluorochrome	Supplier	Antibody	Fluorochrome	Supplier
CD20	V450	BD Horizon	CD70	PE-CF594	BD Horizon	CD70	PE-CF594	BD Horizon	CD103	APC	Beckman Coulter
CD69	FITC	BD Pharmingen	CD80	PE	BD Pharmingen	CD80	BV605	BD Horizon	CD69	Cy5PE	BioLegend
CD8	PerCp Cy55	BD Pharmingen	CD123	PerCP Cy55	BD Pharmingen	CD123	PerCP Cy55	BD Pharmingen	NKG2a	PE	Beckman Coulter
CD95	Cy7PE	BD Pharmingen	CD20	Cy7APC	BD Pharmingen	CD11c	Cy7PE	BioLegend	CCR7	BV421	Biolegend
NKG2a	PE	Beckman Coulter	CD40	FITC	Biolegend	CD20	BV650	BioLegend	CD4	BV785	BioLegend
HLA-DR	TRPE	Invitrogen	CD11c	Cy7PE	BioLegend	CD16	BV570	BioLegend	CD8	BV711	BioLegend
CD14	QD800	Invitrogen	CCR7	BV421	Biolegend	CCR7	BV421	Biolegend	CD45RA	Cy7PE	BD Pharmigen
CD4	Alexa680	BD Pharmingen	CD16	Alexa700	BioLegend	HLA-DR	Cy55PE	Invitrogen	TCRϒδ	PE-CF594	BD Horizon
CD66abce	APC	Miltenyi	HLA-DR	Cy55PE	Invitrogen	CD14	QD800	Invitrogen	TNF	BV650	Biolegend
CD3	Cy7APC	BD Pharmingen	CD14	QD800	Invitrogen	BDCA1	FITC	Miltenyi	IL2	BV605	BioLegend
			BDCA-1	APC	Miltenyi	Clec9A	PE	Miltenyi	IFNϒ	FITC	BD
			CD3	Cy7APC	BD Pharmingen	CD66	APC	Miltenyi	CD3	Cy7APC	BD Pharmingen