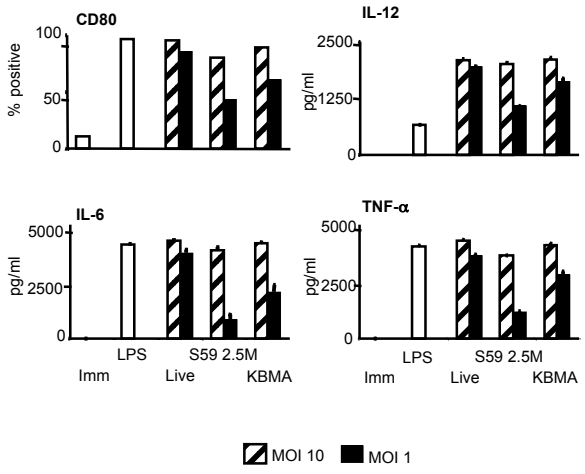
CD80 MF: Tukey's Multiple Comparison Test

	-	LPS	mimic	wt 10	wt 1	wt 0.1	dllo 10	dllo 1	dllo 0.1	dactdinlB 10	dactdinlB 1
-											
LPS	**P < 0.001**										
mimic	**P < 0.001**	P > 0.05									
wt 10	**P < 0.001**	P > 0.05	P > 0.05								
wt 1	**P < 0.001**	P > 0.05	P > 0.05	P > 0.05							
wt 0.1	P > 0.05	P > 0.05	**P < 0.05**	P > 0.05	P > 0.05						
dllo 10	**P < 0.01**	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05					
dllo 1	P > 0.05	**P < 0.05**	**P < 0.001**	P > 0.05	P > 0.05	P > 0.05	P > 0.05				
dllo 0.1	P > 0.05	**P < 0.01**	**P < 0.001**	**P < 0.05**	**P < 0.05**	P > 0.05	P > 0.05	P > 0.05			
dactdinlB 10	**P < 0.001**	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	**P < 0.01**		
dactdinlB 1	**P < 0.001**	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	**P < 0.05**	**P < 0.01**	P > 0.05	
dactdinlB 0.1	P > 0.05	P > 0.05	**P < 0.05**	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05

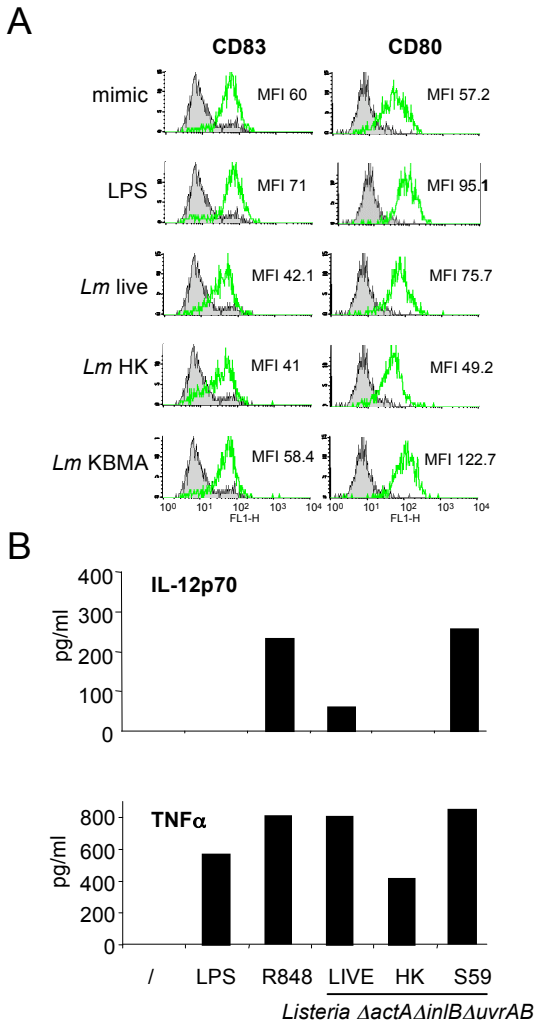
CD83 %: Tukey's Multiple Comparison Test

	-	LPS	mimic	wt 10	wt 1	wt 0.1	dllo 10	dllo 1	dllo 0.1	dactdinlB 10	dactdinlB 1
-											
LPS	**P < 0.001**										
mimic	**P < 0.001**	**P < 0.05**									
wt 10	**P < 0.001**	P > 0.05	P > 0.05								
wt 1	**P < 0.001**	P > 0.05	P > 0.05	P > 0.05							
wt 0.1	**P < 0.01**	P > 0.05	**P < 0.05**	P > 0.05	P > 0.05						
dllo 10	**P < 0.001**	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05					
dllo 1	**P < 0.01**	P > 0.05	**P < 0.001**	P > 0.05	P > 0.05	P > 0.05	P > 0.05				
dllo 0.1	P > 0.05	**P < 0.001**	**P < 0.001**	**P < 0.001**	**P < 0.001**	**P < 0.05**	**P < 0.001**	**P < 0.05**			
dactdinlB 10	**P < 0.001**	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	**P < 0.001**		
dactdinlB 1	**P < 0.001**	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	**P < 0.001**	P > 0.05	
dactdinlB 0.1	**P < 0.05**	P > 0.05	**P < 0.01**	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05

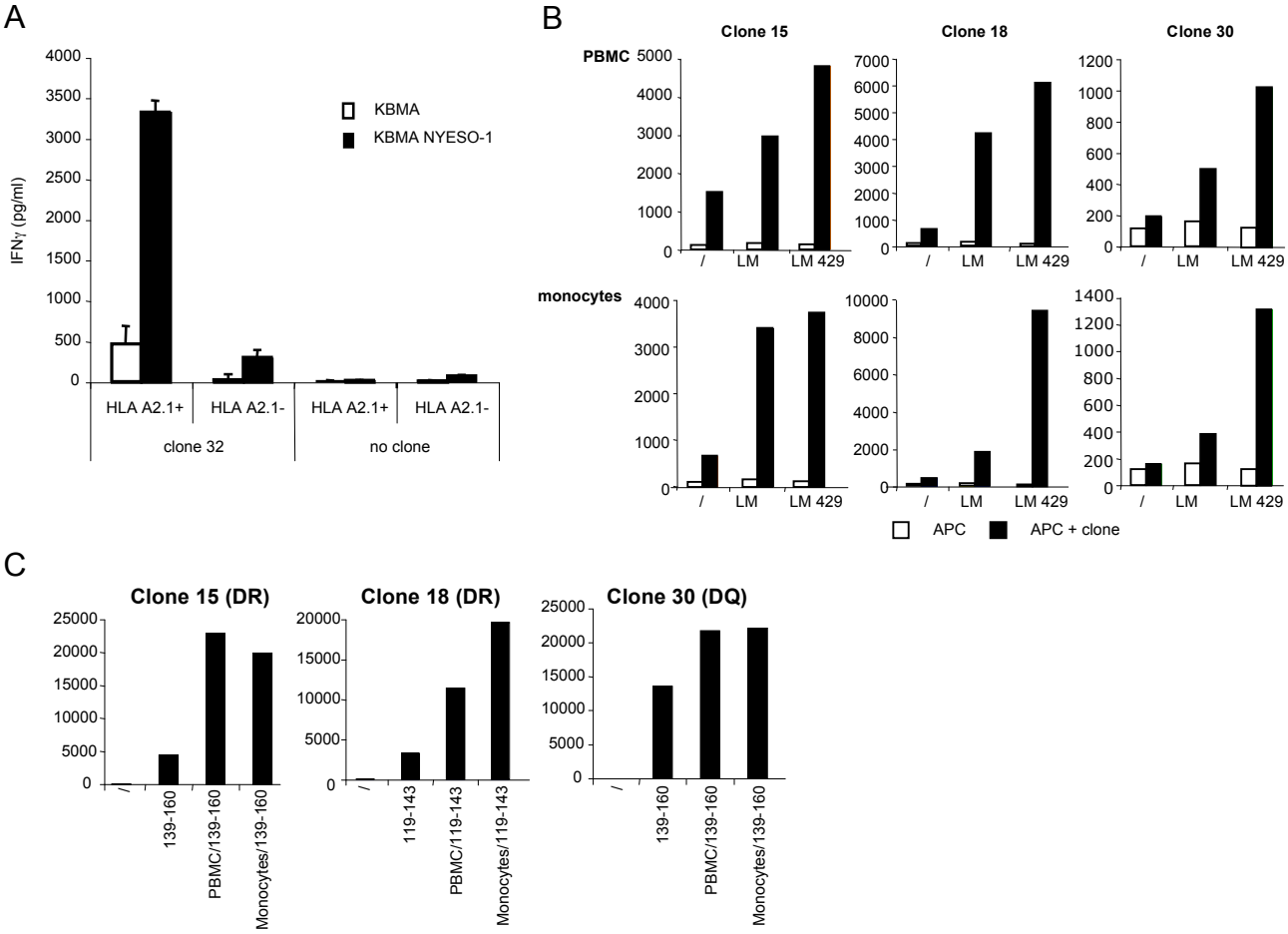
Supplemental Table 1. Statistical analysis for data presented in Figure 1B.
The indicated groups were compared with Tukey's multiple comparison test. Table contains the P values of the comparisons.



Supplemental Figure 1. Metabolic activity influences the DC maturation capacity of *Lm*. Infection with S59 (2.5mM) and UV inactivated *ΔactAΔinlB Lm* resulted in impaired DC maturation compared to maturation with live bacteria. Abrogation of the uvr gene *(ΔactAΔinlBΔ uvrAB Lm*) rendered *Lm* extremely sensitive to S-59/UV inactivation, allowing usage of decreased concentrations of S59 (0.22M) and preparation of non-propagating strains that retained metabolic activity (KBMA). KBMA *Lm* were superior in maturation of DC compared to strains with impaired metabolic activity (S59 (2.5M)/UV inactivated ΔactAΔinlB *Lm* [S59 2.5M]).



Supplemental Figure 2. DC isolated from blood mature upon infection with KBMA *Lm*.
A. Myeloid DC (MDC) were sorted by FACS and treated as indicated. Expression of maturation marker CD83 and costimulatory molecule CD80 (green histograms) was compared to staining by isotype control (grey histograms). **B.** Simultaneously, supernatants were harvested and production of cytokines was measured by ELISA. Live and KBMA but not HK *Listeria* were as efficient as LPS and/or a TL7/8 agonist R-848 (3M) in the induction of TNF-α and IL-12. KBMA strains were superior to live ones presumably because of lesser toxicity for MDC.



Supplemental Figure 3. Various APC infected with KBMA *Lm*-NY-ESO-1 stimulate CD8+ and CD4+ T cell clones.

A. HLA-A2.1+ or HLA-A2.1- donors were infected with KBMA *ΔactAΔinlBΔuvrAB Lm* expressing NY-ESO-1 protein (KBMA NY-ESO-1) or its empty vector control (KBMA) and cocultured with NY-ESO-1-specific, HLA-A2.1-restricted CD8+ T cell clone (Clone 32). Supernatants of overnight culture were collected and analyzed by IFN-γ ELISA. **B.** PBMC or CD14+ monocytes were isolated from a healthy donor and were infected with live *ΔactAΔinlB Lm* expressing NY-ESO-1 protein under actA promotor (LM429) or its empty vector control (LM) or were left untreated (/). They were then cocultured with NY-ESO-1-specific CD4+ T cell clones 15,18 or 30 that were generated from the same healthy donor. Clones 15 and 30 respond to aa 139-160 and clone 18 to aa 119-143 in NY-ESO-1 protein. Supernatants of overnight culture were collected and analyzed by IFN-γ ELISA. **C.** Peptide pulsed cultures were used to control activity of the clones (/: T cell clone w/o peptide; 139-160 or 119-143: T cell clone pulsed with peptide for auto-presentation; PBMC or monocytes pulsed with the indicated peptide). Clones 15 and 18 are DR-restricted and clone 30 is DQ-restricted.