

Table 1: Microinjection of LPS into transfected cells - examination for induction of NF- κ B and phospho-c-Jun.

Immuno-staining	Transfection	% of microinjected cells activated
NF- κ B	Vector	96%
	LRR	63%
Phospho-c-Jun	Vector	71%
	LRR	33%

HeLa cells were transfected with either vector alone or the LRR domain of CARD4 and microinjected with LPS 24 hrs post-transfection and stained for phospho-c-Jun (as in Fig. 1) or the p65 subunit of NF- κ B (Philpott *et al.*, 2000). Conventional immunofluorescence microscopy was used to count at least 50 cells for each condition. Results are representative of two independent experiments. Cells microinjected with buffer alone showed no activation of either NF- κ B or phospho-c-Jun.

These data show that overexpression of the LRR domain of CARD4 also inhibits signal transduction induced by intracellular LPS. Moreover, triple staining and fluorescent microscopic analysis of LPS microinjected cells showed that LRR expressing cells displayed decreased nuclear localization of NF- κ B compared to non-transfected cells (data not shown).