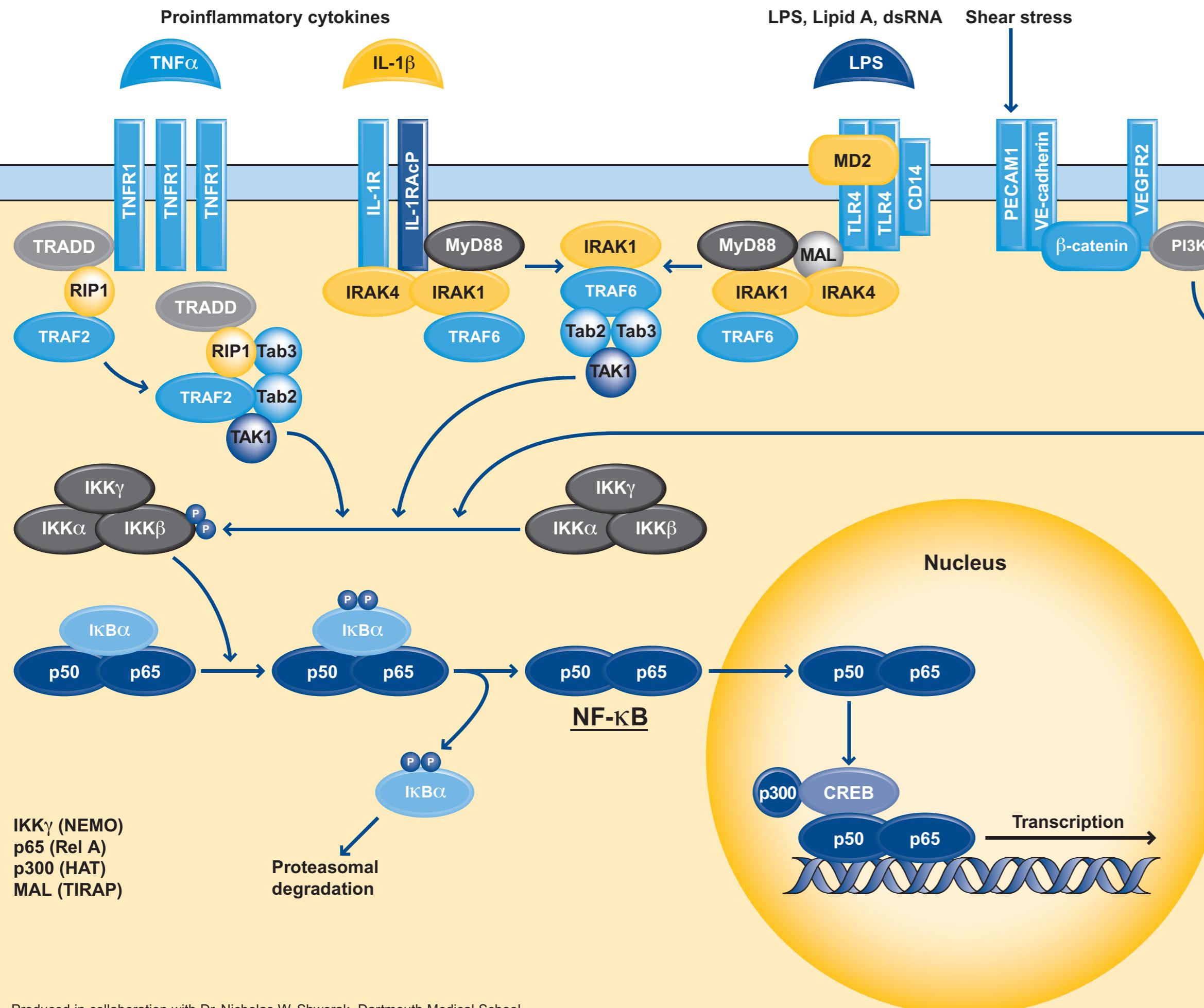


# Canonical nuclear factor-kappa B signaling in atherosclerosis



IKK $\gamma$  (NEMO)  
p65 (Rel A)  
p300 (HAT)  
MAL (TIRAP)

Produced in collaboration with Dr. Nicholas W. Shworak, Dartmouth Medical School.

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Atherosclerosis involves upregulation of the canonical nuclear factor-kappa B (NF-κB) signaling pathway in several vascular cell types throughout evolution of the disease.

### The canonical NF-κB pathway

NF-κB comprises a family of heterodimeric transcription factors that reside in the cytoplasm of every cell and upon activation translocate to the nucleus. The canonical pathway is activated by a wide variety of agents including inflammatory stimuli, pathogens, free radicals, stress, and cigarette smoke. These disparate signaling pathways ultimately lead to phosphorylation of the IKK complex. This complex is composed of a regulatory subunit (IKK $\gamma$ , also known as NF-κB essential modifier - NEMO) and two similar subunits (IKK $\alpha$  and IKK $\beta$ ). Typically, the IKK complex is activated by phosphorylation of IKK $\beta$ , which in turn phosphorylates IκB $\alpha$ . Phosphorylated IκB $\alpha$  is degraded by the proteasome pathway and the resulting liberated NF-κB (p50/p65 dimer, for example) translocates to the nucleus to regulate the expression of almost 400 different genes (some indicated below).

### NF-κB and the onset of atherosclerosis

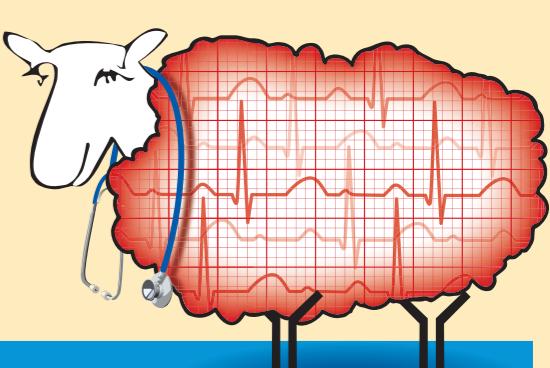
Atherosclerosis develops at large vessel branch points, where turbulent blood flow activates shear stress sensors of endothelial cells. One such sensor is thought to be platelet endothelial cell adhesion molecule (PECAM1) in complex with VE-cadherin, β-catenin and vascular endothelial growth factor receptor-2 (VEGFR2). Shear stress leads to VEGFR2 activation of phosphatidylinositol-3-OH kinase (PI3K), which produces phosphatidylinositol-3,4,5-trisphosphate (PIP3). PIP3 in turn activates the α $v$ β<sub>3</sub> integrin, which then engages with extracellular matrix proteins containing the RGD binding site. A second round of PI3K activation and PIP3 production ensues, which ultimately leads to phosphorylation of the IKK complex through an unclear RAC dependent mechanism. NF-κB activation leads to upregulation of several genes implicated in initiating and perpetuating atherosclerosis including 1) enzymes (group 1a secretory phospholipase A2, 5-lipoxygenase, 12-lipoxygenase, and COX2) that convert lipid from low density lipoprotein (LDL) particles into inflammatory lipids, 2) chemokines (such as monocyte chemoattractant protein-1 (MCP-1)) that attract monocytes and 3) cell adhesion molecules (intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, E-selectin, and P-selectin), which in part enable the monocytes to adhere to endothelial cells and then traverse into the subendothelial space. Once in the inflamed vessel wall monocytes differentiate into macrophages under the influence of a NF-κB regulated product, macrophage colony-stimulating factor (M-CSF). Differentiated macrophages then engulf oxidized LDL and become foam cells.

### NF-κB and progression of atherosclerosis

Atherosclerosis also involves NF-κB activation through toll like receptors (TLR4 and TLR2). These receptors of innate immunity recognize multiple ligands including bacterial lipopolysaccharide (LPS); however, in atherosclerosis they are thought to potentially be activated by oxidized products of LDL. Engagement of TLR4 or TLR2 (not shown) initiates similar signaling events. The cytosolic portion of the TLRs is complexed to myeloid differentiation primary response gene 88 (MyD88) via the adaptor MyD88-adaptor-like (MAL) also called TIR-containing adaptor protein (TIRAP). MyD88 recruits IL-1 receptor-associated kinases (IRAKs) and TNF receptor-associated factors (TRAFs) into the complex. TLR engagement leads to IRAK4 phosphorylation of IKK1 and dissociation of an IKK1/TRAF6 complex that then recruits a protein kinase complex containing TAK1 (transforming growth factor-β-activated kinase-1) and TAK1 binding proteins (TAB2 and TAB3). TAK1 then phosphorylates the IKK complex.

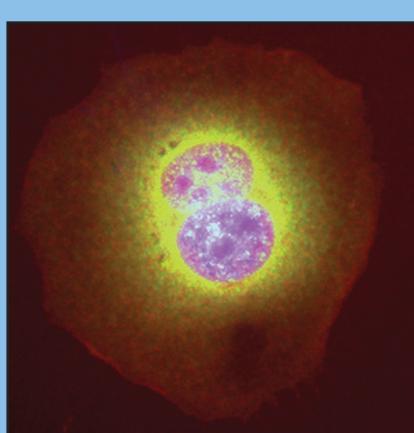
Macrophages/foam cells and other inflammatory cells accumulating within the atherosclerotic plaque produce a variety of NF-κB dependent proinflammatory cytokines that enhance plaque formation (tumor necrosis factor α (TNF $\alpha$ ), IL-1 $\beta$ , IL-4, IL-12, interferon-γ (IFN $\gamma$ )). Of these interleukin 1 $\beta$  (IL-1 $\beta$ ), and potentially TNF $\alpha$  are thought to potentiate the inflammatory process, as these cytokines can also activate NF-κB. IL-1 $\beta$  binds to a heterodimeric complex comprised of the IL-1 receptor (IL-1R) and IL-1R accessory protein (IL-1RAcP). Similar to TLRs, IL-1 $\beta$  signaling is mediated by TRAF6 activation of TAK1, which phosphorylates the IKK complex. In contrast, signaling by TNF receptor-1 (TNFR1) leads to formation of a complex comprised of TNFR1 associated death domain protein (TRADD) receptor-interacting protein (RIP) and TRAF2. These three components in concert with TAB2 and TAB3 activate TAK1 to phosphorylate the IKK complex.

Other NF-κB dependent genes that may influence atherosclerosis are other chemokines (CX3CL1, CCL10, and platelet factor 4) that attract inflammatory cells to atherosclerotic lesions, matrix metalloproteinase-9, which may facilitate migration of inflammatory and smooth muscle cells within the plaque, and tissue factor and plasminogen activator inhibitor 1 (PAI-1), which convey a procoagulant tone to the plaque.



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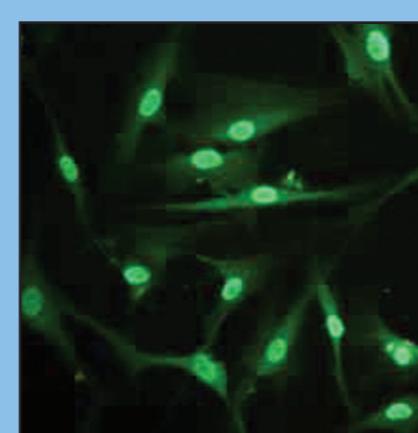
NFκB p65 (ab16502)

Rabbit polyclonal

Species:  
Hu, InMtj, Ms\*, Rat\*

Tested applications:  
ICC/IF, WB

\*= predicted to react

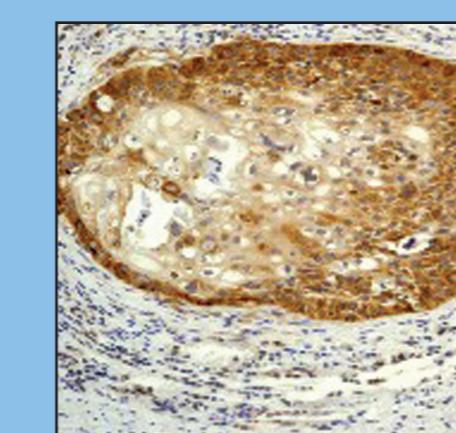


CD31 [P2B1]  
(ab24590)

Mouse monoclonal

Species:  
Hu, Ms, Rat

Tested applications:  
ICC/IF, IP, WB

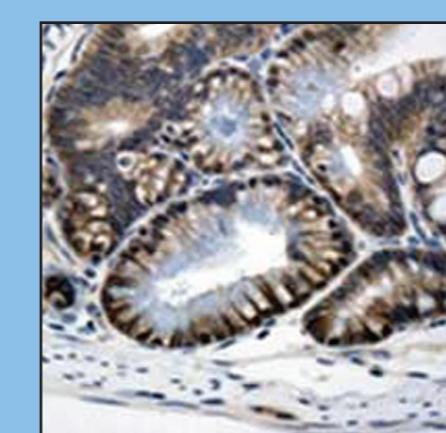


IKK beta (ab32135)

Rabbit monoclonal

Species:  
Hu

Tested applications:  
Flow Cyt, IHC-P, IP, WB

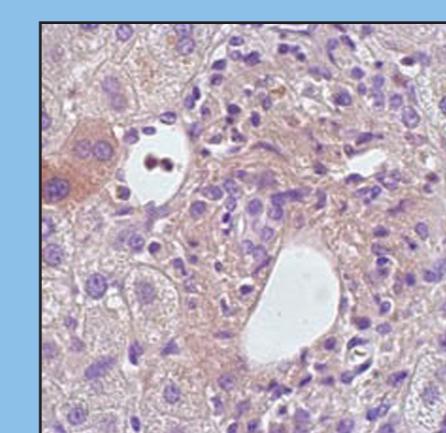


beta Catenin  
(ab32572)

Rabbit monoclonal

Species:  
Hu, Ms, AGMk, Hm

Tested applications:  
ICC/IF, IHC-P, IP, WB



IL1 beta (ab2105)

Rabbit polyclonal

Species:  
Hu, NHuPrM

Tested applications:  
ELISA, Flow Cyt, FuncS,  
IHC-Fr, IHC-P, IP, RIA, WB