Toll-Like Receptors (TLRs)

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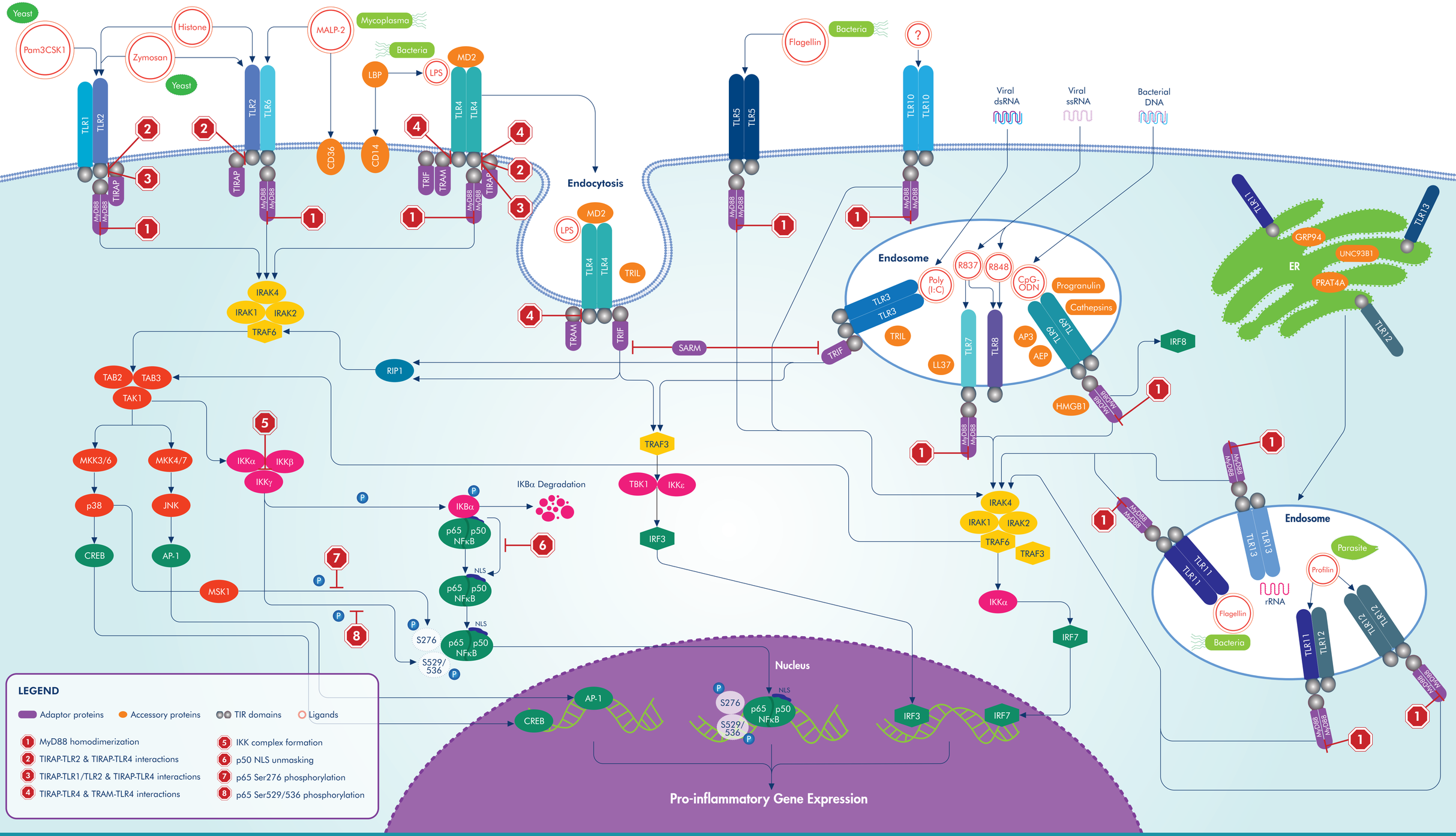

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NOVUS Biologicals & Innate Immunity: The Story Toll'd

The Toll-like receptor (TLR) story 'Toll'd' could be said to have begun in 1985 with the discovery of proteins inducing dorsal-ventral development during Drosophila embryogenesis. These proteins were termed 'Toll' from the German for 'weird' because flies lacking Toll developed in a weird way.

The discovery of Toll sparked a new age in immunology during the 1990's when mammalian homologues, TLRs, were discovered and found to be essential for innate immunity. It is now being increasingly recognized that TLRs have far reaching roles beyond initial innate immune responses, including bridging innate and adaptive immunity as well as linking inflammation and disease.

Novus Biologicals is proud to have the most comprehensive portfolio of highly validated and published TLR products including antibodies, ligands, inhibitors, and engineered stable cell lines.

O'Neill et al. Nat Rev. 13:453-460 (2013).

TLR and NFkB Signaling Peptide Inhibitors

Cell permeable peptide inhibitors are research tools for manipulating signal transduction pathways by blocking signaling events through decoy mechanisms.

What is a decoy? A decoy is a realistic replica used as a lure or bait.

Each inhibitory peptide employs a specific decoy mechanism based on its amino acid sequence that blocks the propagation of downstream signaling events. The peptides also have a cell permeable translocation sequence which enable the peptides to passively enter into the cell. Control peptides contain the translocation, but not inhibitory, sequence.

INHIBITORS	CAT. NO.	DECOY	INHIBITOR SEQUENCE	INHIBITOR MECHANISM	SIGNALING BLOCK
IKK-gamma	NBP2-26504	IKK-alpha/IKK-beta	IKK-alpha/IKK-beta consensus binding	Binds to IKK-gamma	IKK complex formation
MyD88	NBP2-29328	MyD88	MyD88 TIR homodimerization domain	Binds to MyD88	MyD88 homodimerization
NFkB p65 (pSer276)	NBP2-26505	p65 unphosphorylated Ser276 site	p65 Ser276 phosphorylation consensus site	Ser site becomes phosphorylated	p65 Ser276 phosphorylation
NFkB p65 (pSer529/536)	NBP2-29321	p65 unphosphorylated Ser529/536 site	p65 Ser529/536 phosphorylation consensus site	Ser site becomes phosphorylated	p65 Ser529/536 phosphorylation
NFkB p150/p50	NBP2-29323	p50 unmasked NLS	p50 NLS	Prevents p50 NLS unmasking	p50 nuclear translocation
TIRAP (TLR2 and TLR4)	NBP2-29331	TIRAP	TIRAP TIR domain	Binds to TLR2/TLR4 TIR domains	TIRAP-TLR2 and TIRAP-TLR4 interactions
TIRAP (TLR2 and TLR4)	NBP2-26245	TIRAP	TIRAP TIR domain	Binds to TLR2/TLR4 TIR domains	TIRAP-TLR2/TLR1 and TIRAP-TLR4 but not TIRAP-TLR2/TLR6 interactions
VIPER (TLR4)	NBP2-26244	A46 vaccinia viral protein/TIRAP	A46 TIR binding domain	Binds to TLR4 TIR domain	TIRAP-TLR4 and TRAM-TLR4 interactions

Example: Inhibition of LPS-Mediated TLR4 Activation with VIPER

A. VIPER (TLR4) Inhibitor Peptide Set

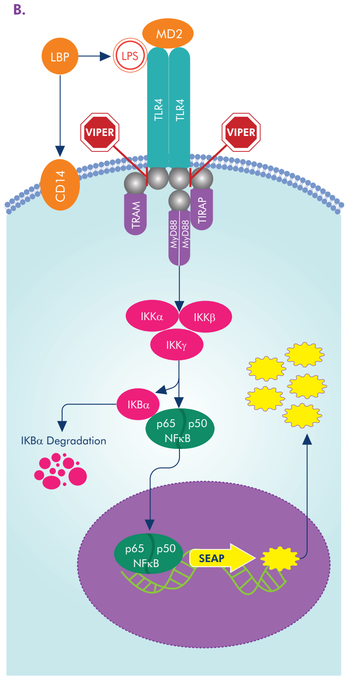
- TLR4-TIRAP and TLR4-TRAM TIR-TIR interactions: critical for mediating TLR4 signaling interactions and activating downstream signaling pathways, thereby resulting in inflammatory responses
- VIPER inhibitory peptide **sequence**: **KYSFKLILAEYRRRRRRRRR**
  - A46 vaccinia viral protein TLR4 TIR domain binding **sequence**, a.k.a. A46 or TIRAP/TRAM TIR domain 'decoy' sequence.
  - Binds to TLR4 and blocks TLR4-TIRAP and TLR-TRAM TIR-TIR interactions
  - Blocks downstream signaling pathways dependent on TIR-TIR interactions
  - Protein transduction **sequence** for cell permeability, scrambled **sequence**
- Control peptide with scrambled **sequence**: **RNTISGNIYSARRRRRRRRR**

TLR4/MD2/CD14 NFkB-SEAP stably transfected reporter cells (NBP2-26503) were plated in 96-well plates at 1x10^5 cells/well. After 16 h, cells were preincubated with increasing concentrations of VIPER or control (CP7) peptides (A) for 1 h.

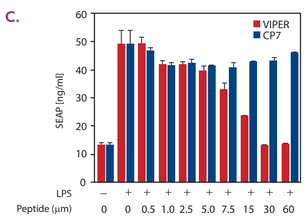
Cells were then stimulated with 10 ng/ml LPS (NBP2-25295) for 24 h. Secreted alkaline phosphatase (SEAP), an indication of NFkB activation in the NFkB-SEAP reporter cell lines (B), was analyzed using the SEAP Reporter Assay Kit (NBP2-25285).

VIPER had a dose-dependent inhibitory effect on LPS-mediated TLR4 activation (C) which was used to calculate the IC$_{50}$ of VIPER (D).

B.



C.



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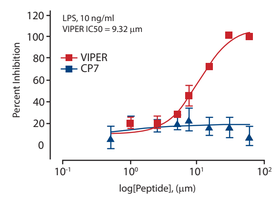


Lingo in the TLR Field

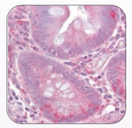
Adaptive immune system: acquired immune defense
Bridging: TLR's participation the adaptive immune response
Cross-talk: communication between multiple signaling pathways
DAMP: damage-associated molecular pattern
Downstream signaling: responses propagated from receptor activation
Innate immune system: first line of immune defense
Myddosome: signaling complex containing MyD88
MyD88: myeloid differentiation primary response gene (88)
NEMO: NFkB essential modulator or IKK gamma
NFkB: nuclear factor kappa-light-chain enhancer of activated B cells
PAMP: pathogen-associated molecular patterns
PRR: pattern recognition receptor
SARM: Sterile-alpha and armadillo motif containing protein
TIR: Toll/interleukin-1 receptor homology domain
TIRAP: Toll/interleukin-1 receptor (TIR) domain containing adaptor protein
TLR: Toll-like receptor
TRAM: TRIF-related adaptor molecule
TRIF: TIR-domain-containing adapter-inducing interferon beta

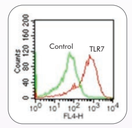
TLR/NFkB SEAP Cell Lines & Ligands

TLR	SEAPORTER™ CELL LINE	LIGANDS	
Control	NBP2-26260		
TLR1		Pam3CSK4	NBP2-25297
TLR2	NBP2-26274	Histone	NBP2-26236
		MALP-2	NBP2-26219
		Pam3CSK4	NBP2-25297
		Zymosan	NBP2-26233
TLR3	NBP2-26275	Poly (I:C)	NBP2-25288
TLR4	NBP2-26503	LPS	NBP2-25295
TLR5	NBP2-26277	Flagellin	NBP2-25289
TLR6		MALP-2	NBP2-26219
TLR7	NBP2-26278	R837	NBP2-26228
		R848	NBP2-26231
TLR8	NBP2-26279	R848	NBP2-26231
TLR9	NBP2-26280	CpG ODN	NBP2-26238
TLR10	NBP2-26273		
TLR11	NBP2-26289		
TLR12	NBP2-26288		
TLR13	NBP2-26290		

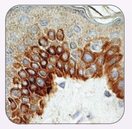
Plasma Membrane TLRs

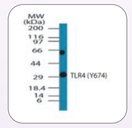
Plasma Membrane TLRs: recognize molecular components localized on the surface of pathogens.

TLR1 **NB100-56563**

IHC-P: small intestine

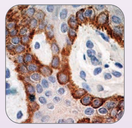
TLR2 **NBP2-27165**


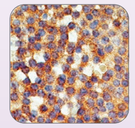
FLOW: macrophages

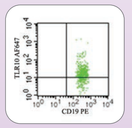
TLR4 **NB100-56566**

IHC-P: skin

TLR4 (Tyr674) **NBP2-24935**


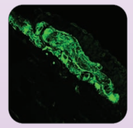
WB: TLR4-Y674 partial protein

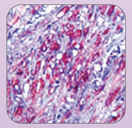
TLR5 **NBP2-24787**

IHC-P: skin

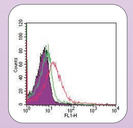
TLR6 **NB100-56536**

IHC-P: tonsil

TLR10 **NBP2-27243**


FLOW: PBMC

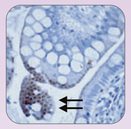
TLR Screening Pack **NBP2-25083**

IF: TLR7 on colon

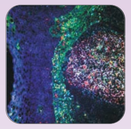
TLR Screening Pack, Cell Surface **NBP2-25086**

IHC-P: colon

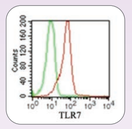
TLR Cell Surface Flow Assay Kit **NBP2-26247**

FLOW: TLR4 on splenocytes

Endosomal TLRs

Endosomal TLRs: mediate the recognition of DNA and RNA from pathogens.

TLR3 **NBP2-24875**

IHC-P: gut lumen

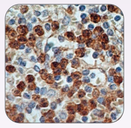
TLR3 (Tyr759) **NBP2-24904**

IHC-F: tonsil

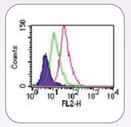
TLR7 **NBP2-27332**


FLOW: monocytes

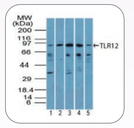
TLR8 **NBP2-24917**

IHC-P: spleen

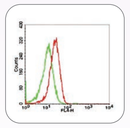
TLR9 **NBP2-24729**

IHC-P: spleen

TLR11 **NB100-56742**


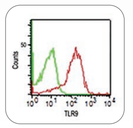
FLOW: splenocytes

TLR12 **NBP2-24833**


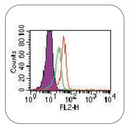
WB: various tissues

TLR13 **NBP2-24539**


FLOW: TLR13 stable cell line

TLR Screening Pack, Intracellular **NBP2-25085**


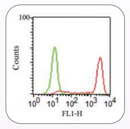
FLOW: TLR9 in PBMC

TLR Intracellular Flow Assay Kit **NBP2-26248**


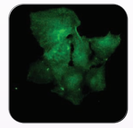
FLOW: TLR3 in PBMC

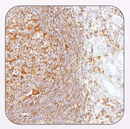
TLR Adaptor* & Accessory Proteins

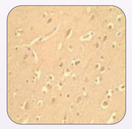
TLR Adaptor and Accessory Proteins: essential roles in TLR folding, ligand recognition, activation, and subcellular localization.

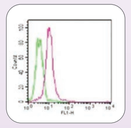
CD14 **NBP2-25194**


FLOW: monocytes

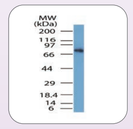
CD36 **NB400-144**

IF: HepG2

LPB **NBP1-32407**

IHC-P: gastric tumor

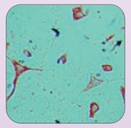
MD2 **NB100-56655**

IHC-P: brain

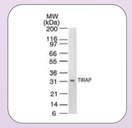
MyD88* **NBP2-27369**


FLOW: Jurkat

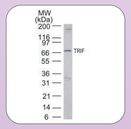
SARM* **NBP2-29625**


WB: Daudi

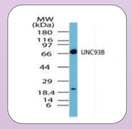
TICAM2 (TRAM)* **NBP2-24638**

IHC-P: brain

TIRAP* **NB100-56730**


WB: spleen

TRIF* **NB120-13810**


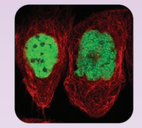
WB: spleen

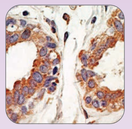
UNC93B **NBP2-24743**


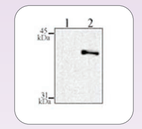
WB: spleen

TLR Downstream Signaling

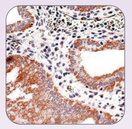
TLR Downstream Signaling Proteins: propagate activated TLR signals from their origins towards their destinations.

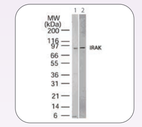
AP-1 **NBP1-89544**

IF: A431

IKB alpha **NB100-56507**

IHC-P: breast

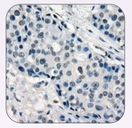
IKB alpha (Ser32/36) **NB100-56724**


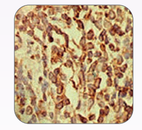
WB: Jurkat control (1), ALLN-treated (2)

IKK beta **NB100-56509**

IHC-P: gall bladder

IRAK **NB100-56699**


WB: HeLa (1), NIH3T3 (2)

IRF3 **NBP1-47812**

IHC-P: colon cancer

NFkB p105/p50 **NB100-56583**

IHC-P: breast tumor

NFkB p65 **NB100-56712**

IHC-P: ovarian cancer

RIPK1 **NB100-56160**

IHC-P: colon

TRAF3 **NB100-56176**

WB: tumor cell lines

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