


# The TNF Superfamily

A 3D ribbon diagram of a protein structure, likely a member of the TNF superfamily. The structure is composed of several intertwined components: a central green ribbon structure, a purple ribbon structure, and two red ribbon structures. The red structures appear to be alpha-helices, while the green and purple structures are more complex, possibly representing beta-sheets and loops. The background is a dark blue gradient with a faint, larger-scale protein structure visible in the lower right.

# The TNF Superfamily

The tumor necrosis factor (TNF) superfamily in humans currently consists of 19 ligands and 29 receptors, with three additional TNF family receptors having been identified in mice. Most TNF ligands are type II transmembrane proteins with extracellular domains that can be cleaved by specific metalloproteinases to generate soluble cytokines. Cleaved and non-cleaved ligands are active as noncovalent homotrimers except for Lymphotoxin  $\beta$ , which forms heterotrimers with TNF- $\beta$ /Lymphotoxin  $\alpha$  (LT $\alpha$ ) and BAFF, which forms heterotrimers with APRIL. TNF family ligands are characterized by a stalk of varying length connecting the transmembrane domain to the core region, which contains the hallmark structure of TNF family ligands, the TNF homology domain (THD). The THD is an anti-parallel  $\beta$ -pleated sheet sandwich with a “jelly-roll” topology. Conserved residues within the  $\beta$  strands provide specific inter-subunit contacts, which stabilize the trimeric structure. Sequences in the loops connecting adjacent  $\beta$  strands are family member-specific and are important for conferring receptor specificity. Receptors for TNF family ligands are oligomeric, type I or type III transmembrane proteins that contain multiple extracellular cysteine-rich domains (CRDs). Several of these receptors contain intracellular death domains (DDs) that recruit caspase-interacting proteins to initiate apoptosis upon ligand binding. Other TNF superfamily receptors that lack DDs bind TNF receptor-associated factors (TRAFs) and activate multiple intracellular signaling pathways that can lead to proliferation or differentiation. These receptors can also initiate apoptosis, but they do so via indirect mechanisms.




TNF superfamily ligands and receptors are important for numerous processes that regulate immune cell functions including apoptosis, B cell homeostasis and activation, natural killer cell activation, T cell co-stimulation, and other cell type-specific responses such as hair follicle development and osteoclast development. TNF superfamily members also play a significant role in regulating the pathogenesis of human diseases including cancer, osteoporosis, osteoarthritis, chronic inflammation, and autoimmune diseases.<sup>1,2</sup> Nearly all TNF superfamily ligand-receptor pathways are being investigated as targets for the development of agonist or antagonist therapeutics.<sup>2,3</sup>

R&D Systems offers a wide selection of high quality reagents to facilitate the study of TNF superfamily members including recombinant proteins, ELISA kits, and antibodies for blocking/neutralization, immunohistochemistry, immunoprecipitation, Western blotting, and flow cytometry. For a complete, up-to-date listing and more information on these products, please visit our website at [www.RnDSystems.com/TNFSF](http://www.RnDSystems.com/TNFSF).







## References

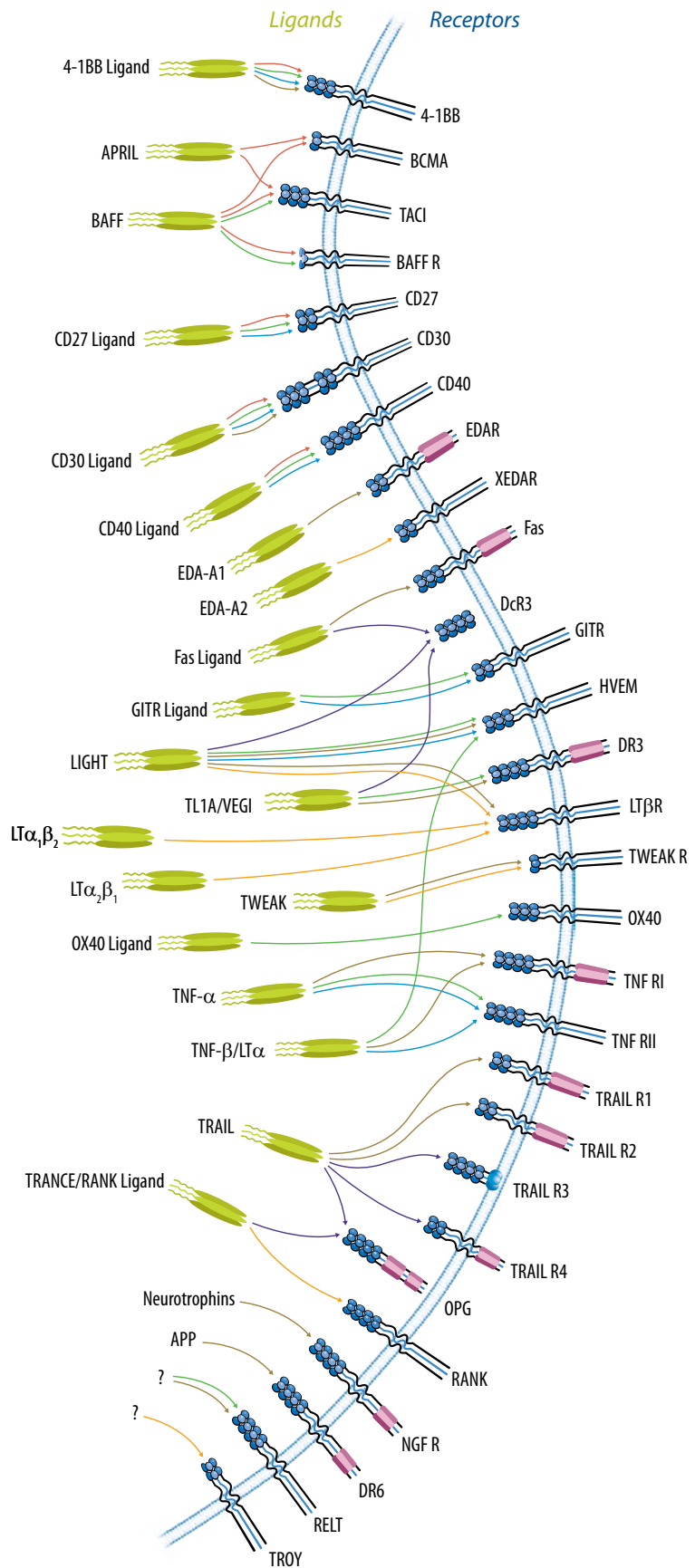
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## LIGAND/RECEPTOR DOMAIN KEY

-  TNF homology domain (THD)
-  Cysteine-rich domain (CRD)
-  Death domain (DD)

## SELECT FUNCTIONS

-  B cell homeostasis & activation
-  Natural killer cell activation
-  T cell co-stimulation
-  Pro-apoptotic
-  Interactions with decoy receptors
-  Other cell type-specific responses



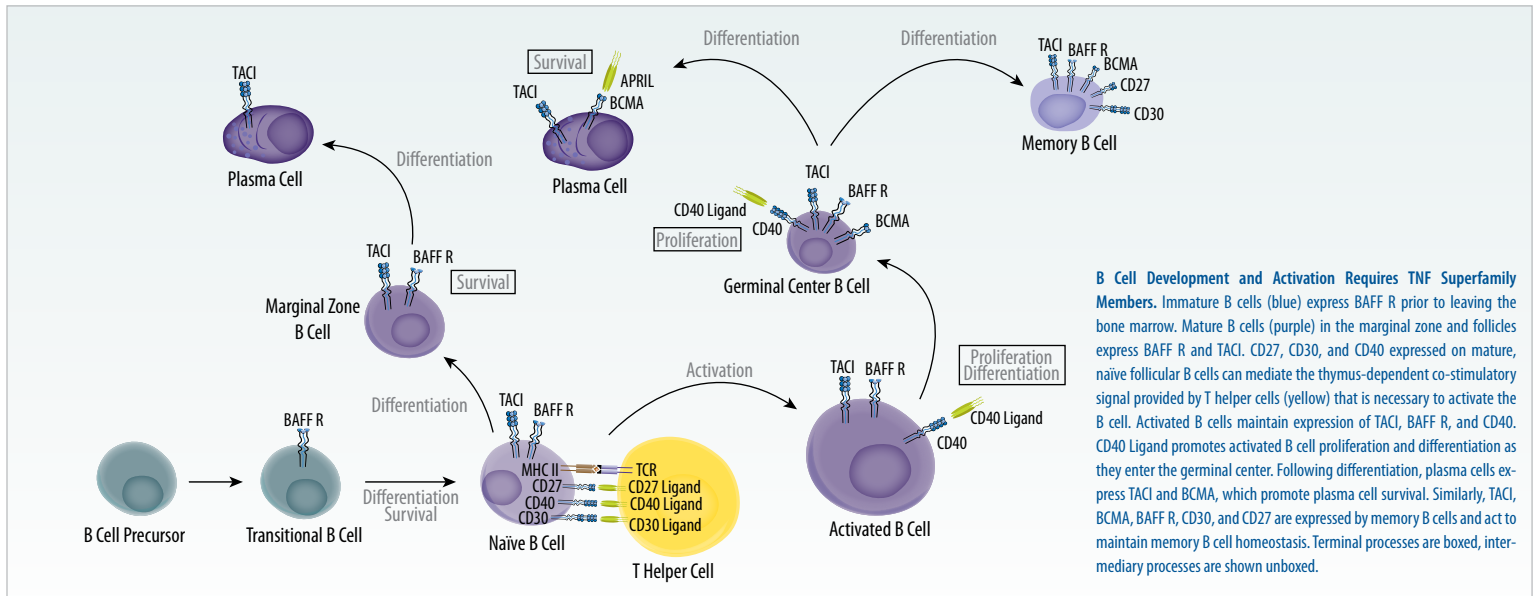
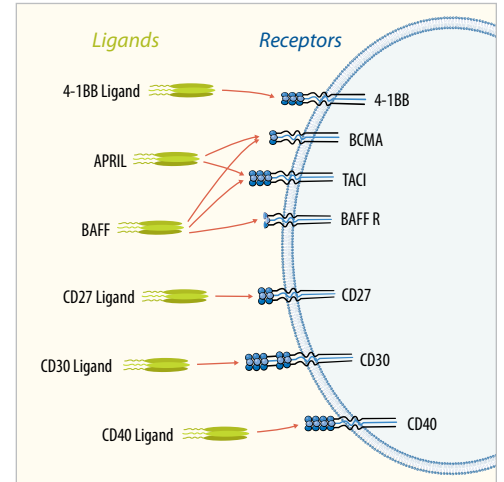
Diagrams include human family members only. Additional mouse TNF superfamily members include DcTRAIL R1, DcTRAIL R2, and TNFRH3.

# TNF Superfamily Members Mediate B Cell Homeostasis and Activation

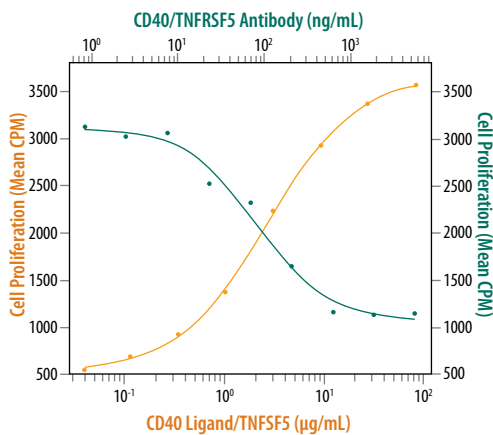
Several TNF superfamily members play key roles in early B cell maturation, homeostasis, differentiation, and activation. While BAFF R is required for normal B cell maturation, interactions between BAFF-BAFF R, BAFF-TACI, APRIL-BCMA, and CD40 Ligand-CD40 promote B cell differentiation and homeostasis. The interaction between CD40 Ligand and CD40 is necessary for germinal center B cell differentiation, and CD27 and CD30 are memory B cell markers. Interactions between CD27 Ligand, CD30 Ligand, or CD40 Ligand and their respective receptors are also involved in B cell co-stimulation by T cells and natural killer cells. Current research suggests roles for BAFF, APRIL, BAFF R, TACI, and BCMA in immune deficiency, autoimmune diseases, and lymphoid cell cancers.<sup>1-3</sup> Mutations of the TACI and BAFF R genes are associated with common variable immunodeficiency (CVID).<sup>1</sup> Alternatively, BAFF overexpression is often observed in patients with autoimmune diseases such as lupus, rheumatoid arthritis, and multiple sclerosis, where it is thought that high levels of BAFF may lead to the inappropriate survival of low-affinity, self-reactive B cells.<sup>2</sup> In addition, BAFF, APRIL, and their receptors are overexpressed in lymphoid cancers and their role in multiple myeloma is being investigated.<sup>3</sup>

## References

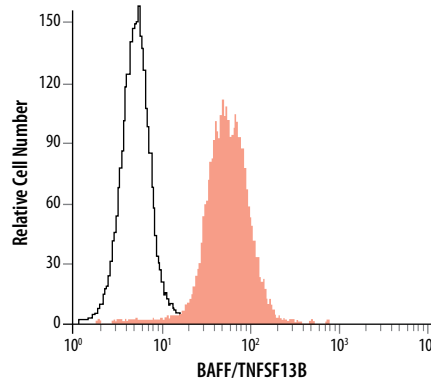
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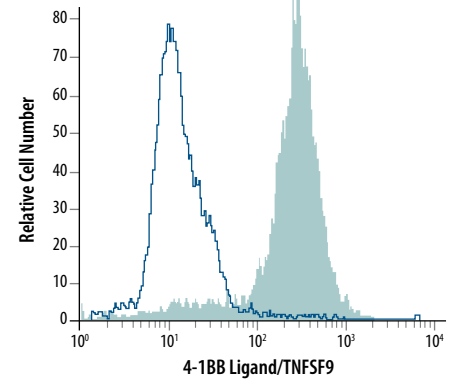
**B Cell Development and Activation Requires TNF Superfamily Members.** Immature B cells (blue) express BAFF R prior to leaving the bone marrow. Mature B cells (purple) in the marginal zone and follicles express BAFF R and TACI. CD27, CD30, and CD40 expressed on mature, naïve follicular B cells can mediate the thymus-dependent co-stimulatory signal provided by T helper cells (yellow) that is necessary to activate the B cell. Activated B cells maintain expression of TACI, BAFF R, and CD40. CD40 Ligand promotes activated B cell proliferation and differentiation as they enter the germinal center. Following differentiation, plasma cells express TACI and BCMA, which promote plasma cell survival. Similarly, TACI, BCMA, BAFF R, CD30, and CD27 are expressed by memory B cells and act to maintain memory B cell homeostasis. Terminal processes are boxed, intermediary processes are shown unboxed.



**CD40 Ligand-induced Proliferation and Neutralization by an Anti-Human CD40 Antibody.** Proliferation of human B cell enriched peripheral blood mononuclear cells was assessed following treatment with increasing concentrations of Recombinant Human CD40 Ligand/TNFSF5 (Catalog # 6245-CL; orange line) in the presence of 20 ng/mL Recombinant Human IL-4 (Catalog # 204-IL). The stimulatory effect induced by 10 µg/mL Recombinant Human CD40 Ligand/TNFSF5 was neutralized by treating the cells with increasing concentrations of a Mouse Anti-Human CD40/TNFRSF5 Monoclonal Antibody (Catalog # MAB6322; green line).



**Detection of BAFF by Flow Cytometry.** Mouse splenocytes were stained with a PE-conjugated Rat Anti-Mouse BAFF/TNFSF13B Monoclonal Antibody (Catalog # IC1357P; filled histogram) or a PE-conjugated Rat IgG<sub>2a</sub> Isotype Control (Catalog # IC006P; open histogram).



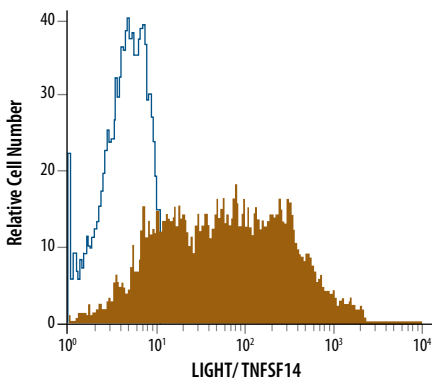
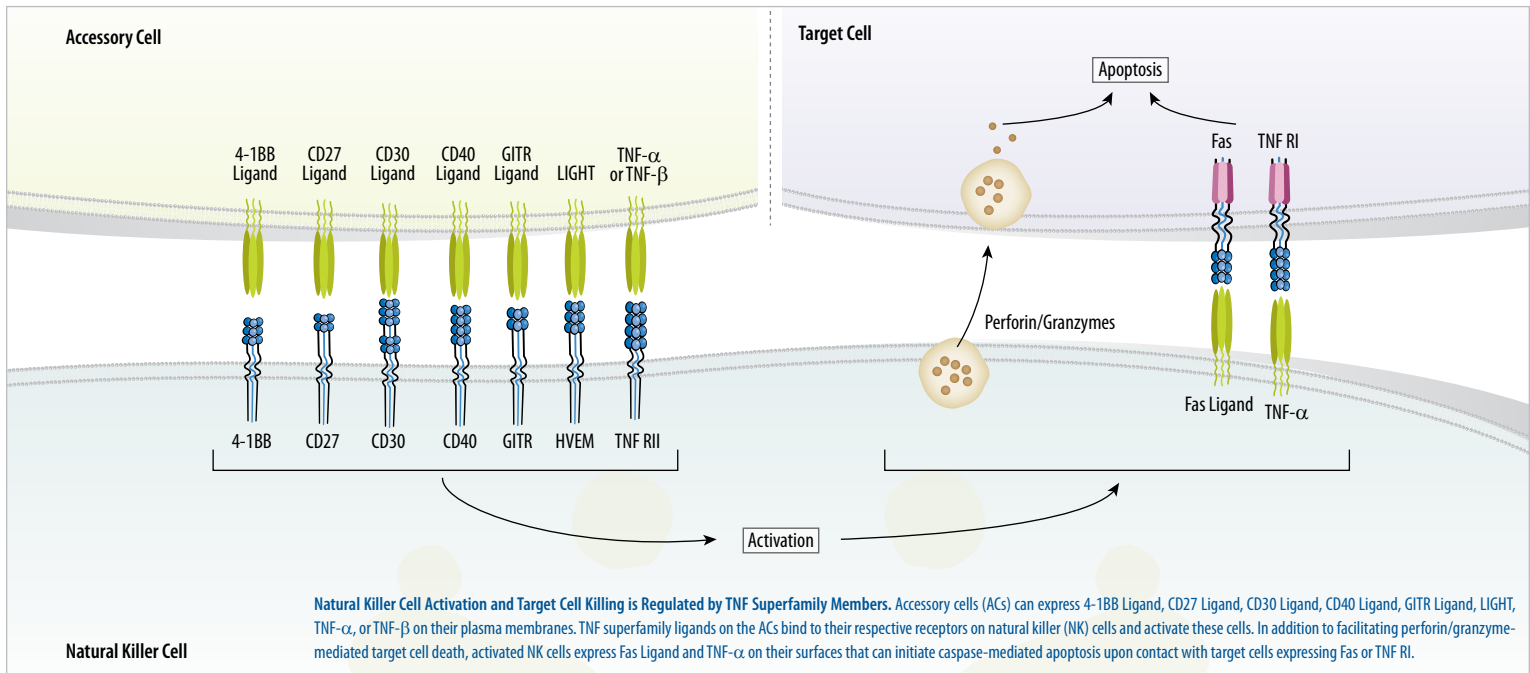
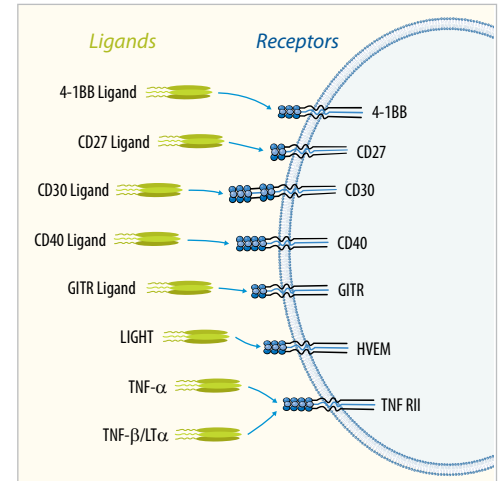
**Detection of 4-1BB Ligand by Flow Cytometry.** The A20 mouse B cell lymphoma cell line was stained with a PE-conjugated Rat Anti-Mouse 4-1BB Ligand/TNFSF9 Monoclonal Antibody (Catalog # FAB1246P; filled histogram) or a PE-conjugated Rat IgG<sub>2b</sub> Isotype Control (Catalog # IC013P; open histogram).

# TNF Superfamily Members Regulate Natural Killer Cells

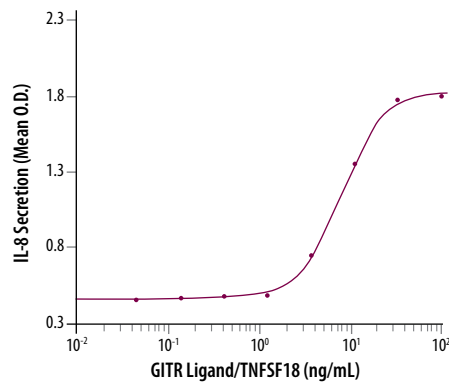
Upon activation, natural killer (NK) cells secrete cytotoxic and inflammatory cytokines that cause apoptosis or lysis of targeted cells. While some pathogens can directly activate NK cells, NK cells are indirectly activated in response to most pathogens by accessory cells. Several TNF superfamily members have been implicated in mediating NK cell activation and regulation by accessory cells. These members include 4-1BB, CD27, CD30, CD40, and GITR and their respective ligands, as well as HVEM via LIGHT and TNF RII via TNF- $\alpha$  or TNF- $\beta$ . NK cells also express Fas Ligand and TNF- $\alpha$  on their cell surface that, when bound to target cell-expressed Fas or TNF RI, respectively, initiate caspase-mediated apoptosis of the target cell. TNF superfamily-mediated apoptosis and the ability of activated NK cells to initiate perforin/granzyme-mediated death of tumor cells prevents the formation of both solid tumors and lymphomas.<sup>1</sup> The anti-cancer activity of 4-1BB-activated NK cells is currently being investigated as an adjuvant therapy in conjunction with traditional cancer treatments.<sup>2</sup>

## References

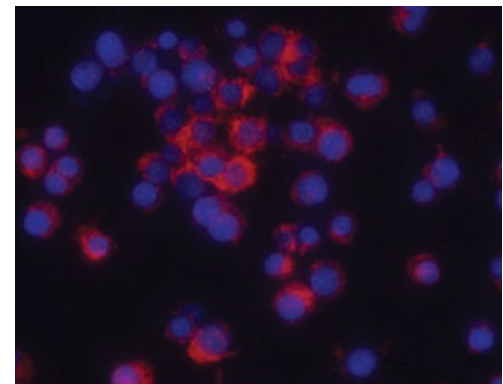
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**Detection of LIGHT by Flow Cytometry.** Expression of LIGHT/TNFSF14 on human CD4<sup>+</sup> T cells was assessed following 36 hours of stimulation with phorbol myristate acetate (PMA) (Catalog # 1201; 1 ng/mL) and calcium ionomycin. Cells were then stained with an APC-conjugated Mouse Anti-Human CD4 Monoclonal Antibody (Catalog # FAB3791A) and a PE-conjugated Mouse Anti-Human LIGHT/TNFSF14 Monoclonal Antibody (Catalog # FAB664P; filled histogram) or a PE-conjugated Mouse IgG<sub>1</sub> Isotype Control (Catalog # IC002P; open histogram). Cells were gated on CD4<sup>+</sup> staining cells.



**GITR Ligand-Induced IL-8 Secretion.** IL-8 secretion was induced by increasing concentrations of Recombinant Human GITR Ligand (Catalog # 6987-GL) in the human GITR-transfected HT1080 fibrosarcoma cell line. The secreted IL-8 was measured using the Human IL-8 DuoSet ELISA Development System (Catalog # DY208).



**Detection of TNF- $\alpha$  in Mouse Macrophages.** TNF- $\alpha$  was detected in the immersion-fixed RAW 263 mouse macrophage cell line treated with LPS and monensin using a Goat Anti-Mouse TNF- $\alpha$  Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-410-NA). Cells were stained with the NorthernLights™ 557-conjugated Donkey Anti-Goat IgG Secondary Antibody (Catalog # NL001; red) and the nuclei were counterstained with DAPI (blue).

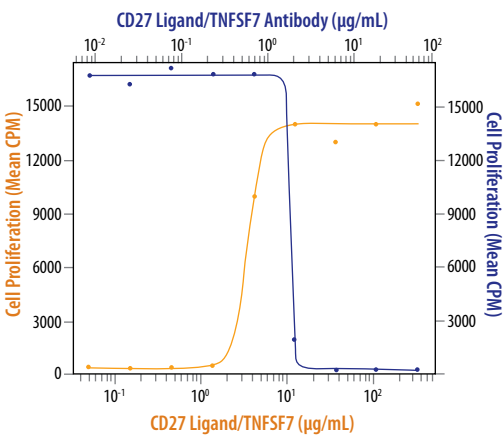
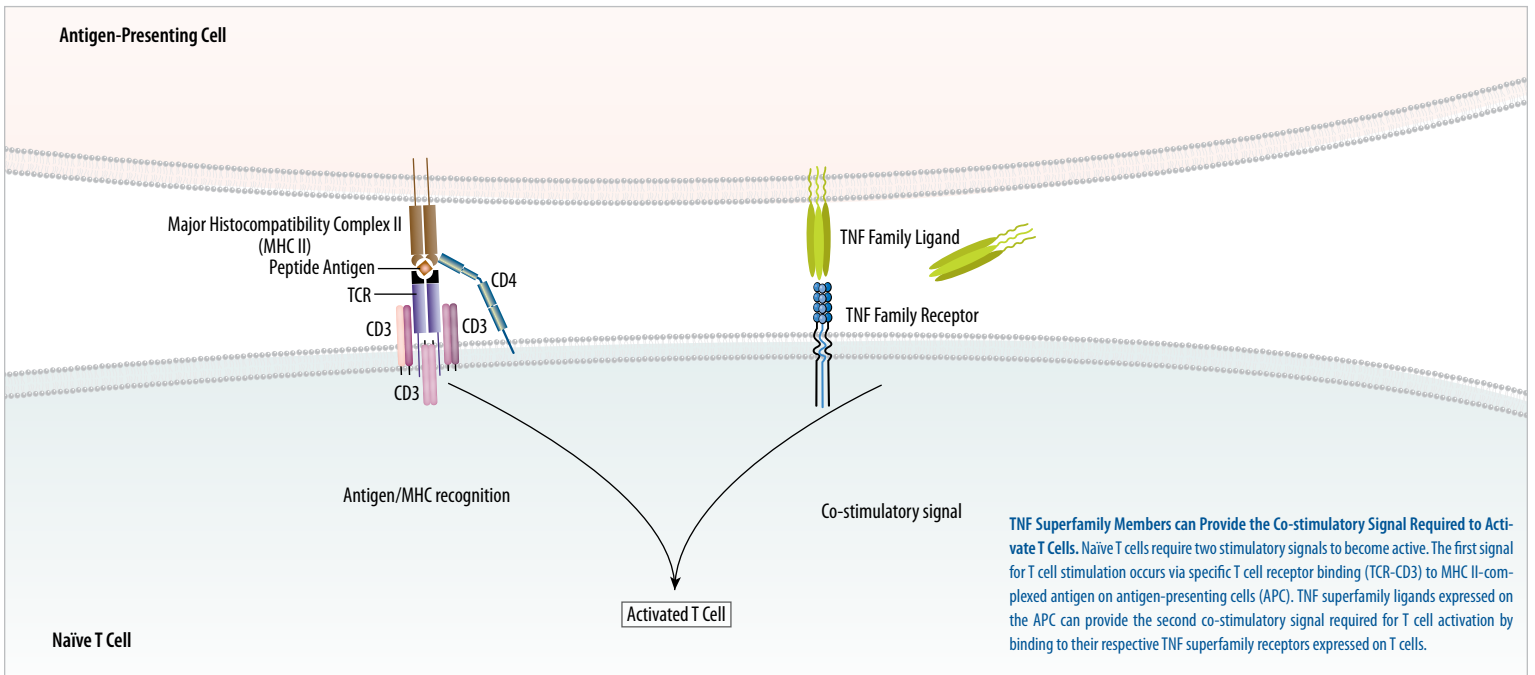
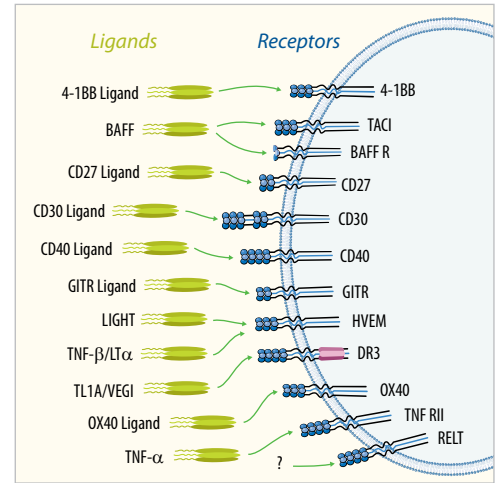


# TNF Superfamily Members Co-stimulate T Cells

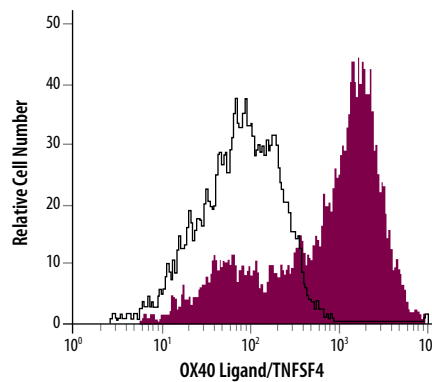
T cell activation requires two signals from antigen-presenting cells (APCs). The antigen-specific major histocompatibility complex (MHC) signal is mediated via the interaction between the antigen-MHC complex localized on the surface of APCs and the T cell receptor localized on the surface of T cells. The non-antigen specific, co-stimulatory signal occurs via the interaction between stimulatory ligands on the APC surface and T cell surface receptors. Several TNF superfamily members mediate T cell co-stimulation. These include 4-1BB, CD27, CD30, CD40, GITR, HVEM, DR3, and OX40 and their respective ligands, as well as TACI or BAFF R activation by BAFF, TNF RII activation by TNF- $\alpha$ , and activation of RELT by an unknown ligand. Blockade of multiple TNF superfamily members that mediate T cell co-stimulation is being investigated for use in the prevention of transplant rejection.<sup>1-3</sup> In addition, the manipulation of GITR-mediated T cell co-stimulation is also being examined as a potential cancer immunotherapy.<sup>4,5</sup>

## References

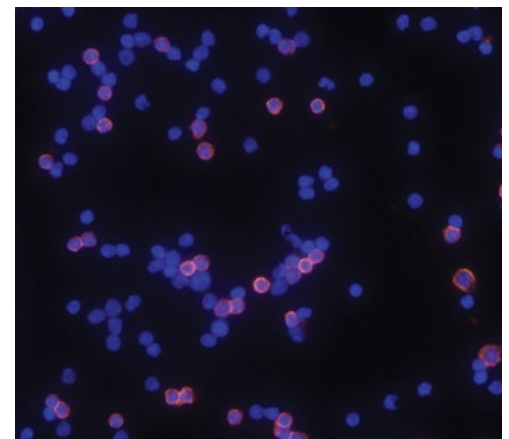
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**CD27 Ligand-induced Proliferation and Neutralization by an Anti-Mouse CD27 Ligand Antibody.** Proliferation of mouse splenic T cells was assessed following treatment with increasing concentrations of Recombinant Mouse CD27 Ligand/TNFSF7 (Catalog # 783-CL; orange line) in the presence of suboptimal amounts of a Hamster Anti-Mouse CD3 $\epsilon$  Monoclonal Antibody (Catalog # MAB484). The proliferative effect induced by 10  $\mu$ g/mL Recombinant Mouse CD27 Ligand/TNFSF7 was neutralized by treating the cells with increasing concentrations of a Rat Anti-Mouse CD27 Ligand/TNFSF7 Monoclonal Antibody (Catalog # MAB783; purple line).



**Detection of OX40 Ligand by Flow Cytometry.** Human peripheral blood mononuclear cells treated with lipopolysaccharide and Recombinant Mouse GM-CSF (Catalog # 415-ML) were stained with a PE-conjugated Mouse Anti-Human OX40 Ligand/TNFSF4 Monoclonal Antibody (Catalog # FAB10541P; filled histogram) or a PE-conjugated Mouse IgG<sub>1</sub> Isotype Control (Catalog # IC002P; open histogram).



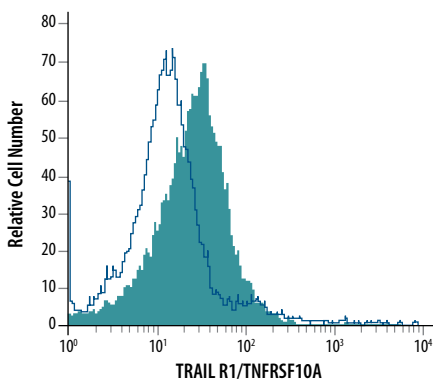
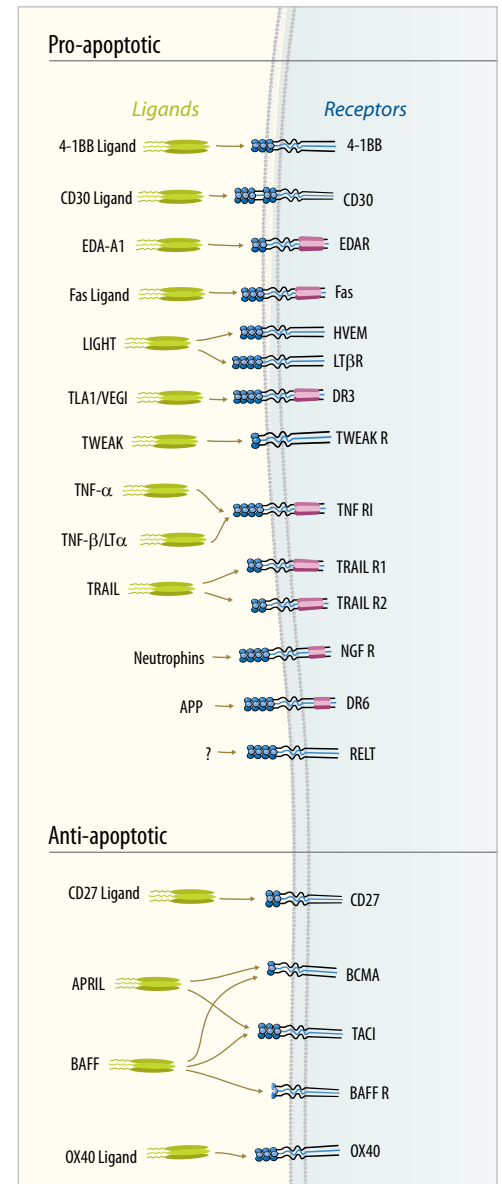
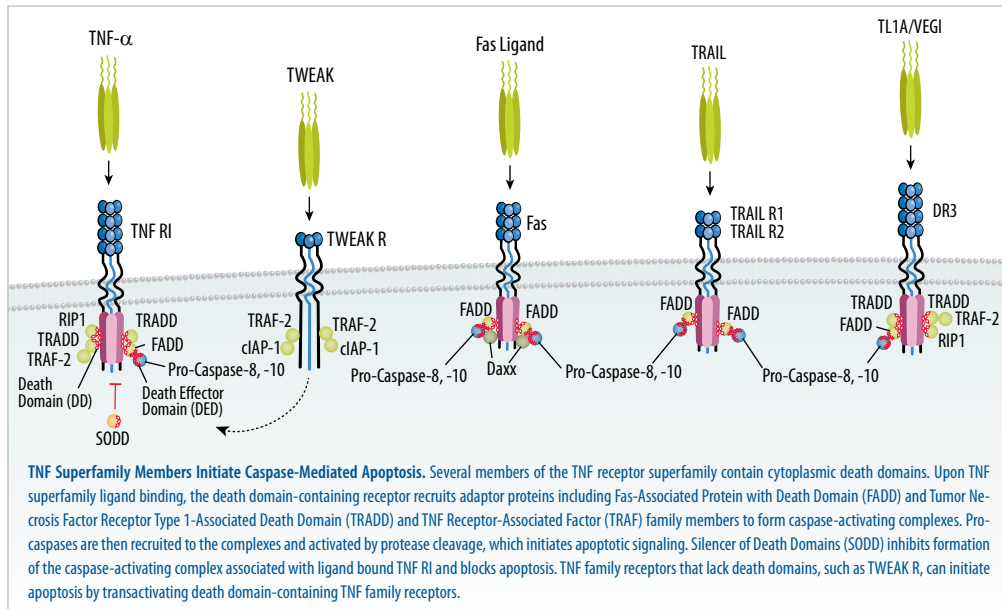
**Detection of CD40 on Mouse Splenocytes.** CD40/TNFRSF5 was detected in immersion-fixed mouse splenocytes using a Goat Anti-Mouse CD40/TNFRSF5 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF440). Cells were stained using a NorthernLights™ 557-conjugated Donkey Anti-Goat IgG Secondary Antibody (Catalog # NL001; red) and the nuclei were counterstained with DAPI (blue).

# TNF Superfamily Members Mediate Apoptosis

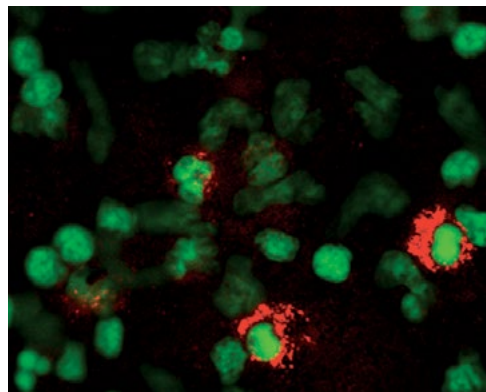
TNF superfamily members mediate both the intrinsic and extrinsic pathways of caspase activation. EDAR, Fas, DR3, TNF RI, TRAIL R1, TRAIL R2, NGF R, and DR6 initiate the extrinsic pathway via recruitment of the adaptor proteins, FADD and TRADD, and pro-caspases. TNF family receptors that do not contain death domains, including 4-1BB, CD30, HVEM, LTβR, TWEAK R, and RELT, have also been shown to mediate apoptosis via indirect mechanisms such as inhibition of p38, transactivation of TNF RI, or activation-induced cell death via NF-κB.<sup>1-3</sup> Apoptosis mediated by TNF superfamily receptors is not only critical for normal developmental processes, but is also implicated in disease.<sup>4</sup> For example, viruses encode cytokine responsive modifiers (CRMs) that act as TNF family decoy receptors and prevent apoptosis. Additionally, inhibition of TNF-mediated apoptosis, caused by down-regulation of death receptors, up-regulation of decoy receptors, or altered intracellular signaling, is associated with cancer development. In addition to initiating apoptosis, several TNF family members, including CD27, BCMA, TACI, BAFF R, and OX40, inhibit apoptosis via activation of NF-κB and up-regulation of anti-apoptotic proteins such as Bcl-2, Bcl-x<sub>L</sub>, and Inhibitors of Apoptosis (IAPs). The TNF receptor family also includes six decoy receptors, DcR3, DcTRAIL R1, DcTRAIL R2, TRAIL R3, TRAIL R4, and OPG, which act to sequester TNF family ligands and prevent their normal physiological effects.

## References

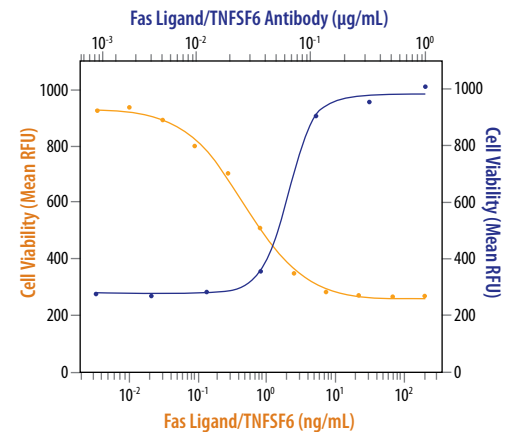
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4. Aggarwal, B.B. *et al.* (2012) Blood 119:651.



**Detection of TRAIL R1 by Flow Cytometry.** The HeLa human cervical cancer cell line was stained with an APC-conjugated Mouse Anti-Human TRAIL R1/TNFRSF10A Monoclonal Antibody (Catalog # FAB347A; filled histogram) or an APC-conjugated Mouse IgG<sub>1</sub> Isotype Control (Catalog # IC002A; open histogram).



**Detection of TNF RI on Human Peripheral Blood Lymphocytes.** TNF RI/TNFRSF1A was detected in immersion-fixed human peripheral blood lymphocytes using a Goat Anti-Human TNF RI/TNFRSF1A Antigen Affinity-purified Polyclonal Antibody (Catalog # AF225). Cells were stained with a fluorochrome-conjugated secondary antibody (red) and nuclei were counterstained (green).



**Fas Ligand-induced Apoptosis and Neutralization by an Anti-Fas Ligand Antibody.** Apoptosis of Jurkat cells was assessed following treatment with increasing concentrations of Recombinant Human Fas Ligand/TNFSF6 (Catalog # 126-FL; orange line) in the presence of a cross-linking Mouse Poly-Histidine Monoclonal Antibody (Catalog # MAB050). The apoptotic effect induced by 2 ng/mL Recombinant Human Fas Ligand/TNFSF6 was neutralized by treating the cells with increasing concentrations of a Goat Anti-Human Fas Ligand/TNFSF6 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF126; purple line).

# TNF Superfamily Products

## TNF Superfamily Ligands

MOLECULE	RECOMBINANT PROTEINS	ANTIBODIES	ELISAs
4-1BB Ligand/TNFSF9	H M	H (FC, WB) M (FC, WB)	
APRIL/TNFSF13	H	H (B/N, E, FC, WB) M (FC)	H
BAFF/BLyS/TNFSF13B	H M	H (B/N, FC, IHC, WB) M (B/N, FC, IHC, WB)	H M
CD27 Ligand/TNFSF7	M	H (FC, IHC, WB) M (B/N, E, FC, WB)	M
CD30 Ligand/TNFSF8	H M	H (B/N, FC, WB) M (B/N, E, FC, WB)	M
CD40 Ligand/TNFSF5	H M	H (B/N, FC, IHC, WB) M (B/N, E, FC, IHC, WB)	H M
EDA/Ectodysplasin		H (E)	H
EDA-A1/Ectodysplasin A1	H M		
EDA-A2/Ectodysplasin A2	H	H (B/N, WB)	
Fas Ligand/TNFSF6	H M R	H (B/N, E, FC, IHC, WB) M (B/N, E, FC, WB) R (IHC, WB)	H M
GITR Ligand/TNFSF18	H M	H (B/N, E, FC, WB) M (B/N, E, FC, WB)	H M
LIGHT/TNFSF14	H M	H (B/N, E, FC, WB) M (FC, WB)	H
Lymphotoxin $\alpha$ 1 $\beta$ 2	H	H (B/N)	
Lymphotoxin $\alpha$ 2 $\beta$ 1	H M	H (B/N)	
Lymphotoxin $\beta$ /TNFSF3		H (WB)	
OX40 Ligand/TNFSF4	H M	H (B/N, FC, IHC, WB) M (B/N, E, FC, IHC, WB)	M
TL1A/TNFSF15	H M	H (WB) M (WB)	
TNF- $\alpha$ /TNFSF1A	H M R B Ca CR E F P Rb RM	H (B/N, E, FC, IHC, WB) M (B/N, E, FC, IHC, WB) R (B/N, E, IHC, WB) P (B/N, E, IHC, WB) B (E, IHC, WB) Ca (B/N, E, IHC, WB) CR (B/N, WB) E (B/N, E, IHC, WB) F (E, WB) (E) Rb (B/N, WB) RM (B/N, E, WB)	H M R B Ca CR E F P Pr Rb RM
TNF- $\beta$ /TNFSF1/Lymphotoxin $\alpha$	H M	H (B/N, E, FC, IHC, WB) M (IHC, WB)	H
TRAIL/TNFSF10	H M	H (B/N, E, FC, IHC, WB) M (E, IHC, WB)	H M
TRANCE/TNFSF11/RANK L	H M	H (B/N, FC, IHC, WB) M (B/N, E, IHC, WB)	M
TWEAK/TNFSF12	H M	H (B/N, E, IHC, WB) M (B/N, E, WB)	H M

## TNF Superfamily Regulators

MOLECULE	RECOMBINANT PROTEINS	ANTIBODIES
Daxx		H (WB)
FADD		H (IHC, WB)
RIP1		H (WB) M (WB) R (WB)
SODD/BAG4		H (WB) M (WB) R (WB)
TANK		H (WB) M (WB)
TRADD		H (FC, IHC, WB)

TRAF-1		H (WB)
TRAF-2		H (WB) M (WB) R (WB)
TRAF-3		H (WB) M (WB) R (WB)
TRAF-4		H (WB)
TRAF-5		H (IHC)
TRAF-6		H (WB)

### Species Key:

H Human, M Mouse, R Rat, B Bovine, Ca Canine, CR Cotton Rat, E Equine, F Feline, P Porcine, Pr Primate, Rb Rabbit, RM Rhesus Macaque, V Viral

### Application Key:

B/N Blocking/Neutralization, E ELISA, FA Functional Assay, FC Flow Cytometry, IF Immunofluorescence, IHC Immunohistochemistry, IP Immunoprecipitation, WB Western blot

## TNF Superfamily Receptors

MOLECULE	RECOMBINANT PROTEINS	ANTIBODIES	ELISAs
4-1BB/TNFRSF9/CD137	H M	H (E, FA, FC, IHC, WB) M (B/N, E, FA, FC, WB)	H M
BAFF R/TNFRSF13C	H M	H (B/N, FC, WB) M (B/N, E, FC, WB)	M
BCMA/TNFRSF17	H M	H (B/N, E, FC, WB) M (B/N, E, FC, WB)	H
CD27/TNFRSF7	H M	H (B/N, FC, IHC, WB) M (E, FC, WB)	M
CD30/TNFRSF8	H M	H (FA, FC, WB) M (E, FA, IHC, WB)	M
CD40/TNFRSF5	H M	H (B/N, FA, FC, IHC, WB) M (E, FA, FC, IF, IHC, IP, WB)	H M
DcR3/TNFRSF6B	H	H (B/N, E, IHC, WB)	H
DcTRAIL R1/TNFRSF23		M (IHC, WB)	
DcTRAIL R2/TNFRSF22	M	M (WB)	
DR3/TNFRSF25	H M	H (FC, WB) M (IHC, WB)	
DR6/TNFRSF21	H M	H (E, WB)	H
EDA2R/TNFRSF27/XEDAR	H M	H (B/N, E, WB)	H
EDAR	H M	H (B/N, WB) M (B/N, E, WB)	M
Fas/TNFRSF6/CD95	H M R F	H (E, FC, IHC, WB) M (E, FC, IHC, WB) R (FC, IHC, WB) F (FC, IHC, WB)	H M
GITR/TNFRSF18	H M	H (B/N, E, FC, IHC, WB) M (E, FC, WB)	H M
HVEM/TNFRSF14	H M	H (E, FC, IHC, WB) M (WB)	H
Lymphotoxin $\beta$ R/TNFRSF3	H M	H (B/N, FC, WB) M (FC, IHC, WB)	
NGF R/TNFRSF16	H M	H (FC, IHC, WB) M (IHC, WB)	H M
Osteoprotegerin/TNFRSF11B	H M	H (B/N, E, IHC, WB) M (B/N, E, IHC, WB)	H M
OX40/TNFRSF4	H M	H (FC, WB) M (FA, FC, WB)	
RANK/TNFRSF11A	H M	H (E, FA, FC, IHC, WB) M (FA, IHC, WB)	H
RELT/TNFRSF19L	H M	H (FC, WB)	
TACI/TNFRSF13B	H M	H (B/N, E, FC, IHC, WB) M (B/N, E, FC, IHC, WB)	H M
TNFRH3/TNFRSF26	M	M (FC, WB)	
TNF RI/TNFRSF1A	H M Ca	H (B/N, E, FA, FC, IHC, WB) M (B/N, E, FA, FC, IHC, IP, WB)	H M
TNF RII/TNFRSF1B	H M	H (B/N, E, FC, IHC, WB) M (E, FC, IHC, IP, WB)	H M
TRAIL R1/TNFRSF10A	H	H (B/N, FC, IHC, WB)	
TRAIL R2/TNFRSF10B	H M	H (FA, FC, WB) M (B/N, FC, IHC, WB)	
TRAIL R3/TNFRSF10C	H	H (B/N, E, FC, IHC, WB)	H
TRAIL R4/TNFRSF10D	H	H (B/N, E, FC, IHC, WB)	H
TROY/TNFRSF19	H M	H (WB) M (E, IHC, WB)	M
TWEAK R/TNFRSF12	H M	H (B/N, IHC, WB) M (E, FC, IHC, WB)	M

## Proteome Profiler™ Array Kit

KIT	DESCRIPTION
Human Apoptosis Array Kit Catalog # ARY009	Contains 4 membranes - each spotted in duplicate with 35 different apoptosis antibodies: Bad, Bax, Bcl-2, Bcl-x, Pro-Caspase-3, Cleaved Caspase-3, Catalase, cIAP-1, cIAP-2, Claspin, Clusterin, Cytochrome c, TRAIL R1/DR4, TRAIL R2/DR5, FADD, Fas/TNFRSF6, HIF-1 $\alpha$ , HO-1/HMOX1/HSP32, HO-2/HMOX2, HSP27, HSP60, HSP70, HTRA2/Omi, Livin, PON2, p21/CIP1/CDNK1A, p27/Kip1, Phospho-p53 (S15), Phospho-p53 (S46), Phospho-p53 (S392), Phospho-Rad17 (S635), SMAC/Diablo, Survivin, TNF RI/TNFRSF1A, XIAP

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