Inducible Homodimerization Citations

Clontech’s iDimerize™ Inducible Homodimer System was previously available from ARIAD as the ARGENT Regulated Homodimerization Kit and AP20187 ligand.

Homodimerization

Fusion proteins containing the DmrB domain do not interact until the B/B Homodimerizer is added. This cell-permeable ligand induces the fusion proteins to interact, activating downstream signaling in real time. This example shows activation of a signal transduction pathway through dimerization of a membrane-bound receptor domain.

2011


<table>
<thead>
<tr>
<th>ARIAD/ARGENT Product</th>
<th>Clontech Product</th>
<th>Size</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARGENT Regulated Homodimerization Kit</td>
<td>iDimerize Inducible Homodimer System</td>
<td>each</td>
<td>635068</td>
</tr>
<tr>
<td>AP20187</td>
<td>B/B Homodimerizer</td>
<td>500 μl</td>
<td>635060</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 x 500 μl</td>
<td>635059</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 mg</td>
<td>635058</td>
</tr>
</tbody>
</table>

The system contains a vector set and 500 μl (0.5 mM) ligand.

Notice to Purchaser

Your use of these products and technologies is subject to compliance with any applicable licensing requirements described on the product’s web page at http://www.clontech.com. It is your responsibility to review, understand and adhere to any restrictions imposed by such statements.
## Inducible Homodimerization Citations

### 2011 continued


Ngo, M. C. *et al.* (2011) *Hum. Mol. Genet.* [Epub ahead of print] **Ex vivo gene transfer for improved adoptive immunotherapy of cancer.** Adoptive lymphocytes have been genetically modified to improve activity and circumvent tumor evasion, via transfer of transgenic T-cell receptors and chimeric antigen receptors to redirect T cell and natural killer cell antigen specificity, and suicide gene ‘safety switches.’

Okazuka, K. *et al.* (2011) *Mol. Ther.* [Epub ahead of print] **Long-term Regulation of Genetically Modified Primary Hematopoietic Cells in Dogs.** Nine years ago, two dogs were transplanted with autologous marrow CD34(+) cells encoding a conditionally activatable derivative of the thrombopoietin receptor. Receptor activation through administration of a chemical inducer of dimerization (CID) (AP20187 or AP1903) conferred a growth advantage.


Zlatic, S. A. *et al.* (2011) *Mol. Biol. Cell* [Epub ahead of print] **Clathrin-Dependent Mechanisms Modulate the Subcellular Distribution of Class C Vps/HOPS Tether Subunits in Polarized and Non-Polarized Cells.** Vps class C/HOPS subunits were concentrated at tips of neuronal processes and their delivery was impaired by expression of FKBP-clathrin chimeras and AP20187 incubation.

### 2010


Dixon, J. E. *et al.* (2010) *Development* **137**(18):2973–2980. **Axolotl Nanog activity in mouse embryonic stem cells demonstrates that ground state pluripotency is conserved from urodele amphibians to mammals.** AxNanog dimers are required to rescue LIF-independent self-renewal and promote proliferation.

Hofman, E. G. *et al.* (2010) *J. Biol. Chem.* **285**(50):34981–34989. **Ligand-induced EGF receptor oligomerization is kinase-dependent and enhances internalization.** EGF receptor (EGFR) oligomerization was controlled using AP20187 to enhance EGF-induced receptor internalization, and monitored using FRET.
**Inducible Homodimerization Citations**

<table>
<thead>
<tr>
<th>Year</th>
<th>Authors</th>
<th>Journal</th>
<th>Volume</th>
<th>Issue</th>
<th>Pages</th>
<th>Title</th>
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</thead>
<tbody>
<tr>
<td>2010</td>
<td>Jiang, Z. et al.</td>
<td><em>J. Neurosci.</em></td>
<td>30(7)</td>
<td></td>
<td>2582–2594</td>
<td>elf2α Phosphorylation-dependent translation in CA1 pyramidal cells impairs hippocampal memory consolidation without affecting general translation</td>
<td>Conditional transgenic mice were treated with AP20187 to increase PKR-mediated phosphorylation of elf2 alpha in hippocampal CA1 pyramidal cells, which led to impaired hippocampal late phase-LTP and memory consolidation, but no obvious reduction in general translation.</td>
</tr>
<tr>
<td>2010</td>
<td>Karulf, M. et al.</td>
<td><em>J. Immunol.</em></td>
<td>185(8)</td>
<td></td>
<td>4856–4862</td>
<td>OX40 ligand regulates inflammation and mortality in the innate immune response to sepsis</td>
<td>MaFIA mice were used to determine the role of OX40-OX40 ligand (OX40L) interaction in the innate immune response to polymicrobial sepsis.</td>
</tr>
<tr>
<td>2010</td>
<td>Krishnamurthy, S. et al.</td>
<td><em>Cancer Res.</em></td>
<td>70(23)</td>
<td></td>
<td>9969–9978</td>
<td>Endothelial cell-initiated signaling promotes the survival and self-renewal of cancer stem cells</td>
<td>AP20187 was used to selectively ablate tumor-associated endothelial cells in xenograft tumors, via a caspase-based artificial death switch (iCaspase-9).</td>
</tr>
<tr>
<td>2010</td>
<td>Kuenzel, S. et al.</td>
<td><em>J. Immunol.</em></td>
<td>184(4)</td>
<td></td>
<td>1990–2000</td>
<td>The nucleotide-binding oligomerization domain-like receptor NLRC5 is involved in IFN-dependent antiviral immune responses</td>
<td>AP20187 was used to trigger dimerization and test the role of NLRC5 in the activation of signaling pathways that use the IFN-specific response element and IFN-gamma activation sequence.</td>
</tr>
<tr>
<td>2010</td>
<td>Lee, D. C. et al.</td>
<td><em>Invest. Ophthalmol. Vis. Sci.</em></td>
<td>51(2)</td>
<td></td>
<td>1066–1070</td>
<td>Fourier domain optical coherence tomography as a noninvasive means for in vivo detection of retinal degeneration in <em>Xenopus laevis</em> tadpoles</td>
<td>Transgenic <em>X. laevis</em> tadpoles expressing inducible caspase-9 (iCasp9) were treated with AP20187 to induce rod photoreceptor death.</td>
</tr>
<tr>
<td>2010</td>
<td>Léveillé, F. et al.</td>
<td><em>J. Neurosci.</em></td>
<td>30(7)</td>
<td></td>
<td>2623–2635</td>
<td>Suppression of the intrinsic apoptosis pathway by synaptic activity</td>
<td>Inducible caspase-9 and AP20187 were used to cause cell death in cortical neurons, indicating that pathways downstream of caspase-9 activation are not a significant aspect of the anti-apoptotic effects of synaptic activity.</td>
</tr>
<tr>
<td>2010</td>
<td>Oberst, A. et al.</td>
<td><em>J. Biol. Chem.</em></td>
<td>285(22)</td>
<td></td>
<td>16632–16642</td>
<td>Inducible dimerization and inducible cleavage reveal a requirement for both processes in caspase-8 activation</td>
<td>Unlike the executioner caspases, both dimerization and cleavage of caspase-8 are required to activate caspase-8 in vitro and apoptosis in cellular systems.</td>
</tr>
<tr>
<td>2010</td>
<td>Pan, P.Y. et al.</td>
<td><em>Cancer Res.</em></td>
<td>70(1)</td>
<td></td>
<td>99–108</td>
<td>Immune stimulatory receptor CD40 is required for T-cell suppression and T-regulatory cell activation mediated by myeloid-derived suppressor cells in cancer</td>
<td>MaFIA transgenic mice were implanted intrahepatically with OVA-B16 tumor cells and treated with AP20187 to induce CD115-specific depletion. The results suggest that CD40 is essential for myeloid-derived suppressor cell-mediated immune suppression and for tumor-specific T-regulatory cell expansion.</td>
</tr>
<tr>
<td>2010</td>
<td>Priceman, S. J. et al.</td>
<td><em>Blood</em></td>
<td>115(7)</td>
<td></td>
<td>1461–1471</td>
<td>Targeting distinct tumor-infiltrating myeloid cells by inhibiting CSF-1 receptor: combating tumor evasion of antiangiogenic therapy</td>
<td>The MaFIA transgenic mouse model was used study the impact of macrophage ablation on tumor angiogenesis.</td>
</tr>
</tbody>
</table>
Inducible Homodimerization Citations

2010 continued


Zhao, L. et al. (2010) J. Biol. Chem. 285(4):2488–2497. Dimerization of CPAP orchestrates centrosome cohesion plasticity. HeLa cells expressing tFLAG-CPAP-FKBP were treated with AP20187 to study the role of CPAP dimerization in centrosome maintenance and cohesion, and in accurate cell division.

2009


Chinnery, H. R. et al. (2009) J. Immunol. 182(5):2738–2744. Bone marrow chimeras and c-fms conditional ablation (MaFIA) mice reveal an essential role for resident myeloid cells in lipopolysaccharide/TLR4-induced corneal inflammation. Corneas of MaFIA mice were stimulated with LPS and treated ± AP20187 to understand the role of macrophages and dendritic cells in development of corneal inflammation.


Ezratty, E. J. et al. (2009) J. Cell Biol. 187(5):733–747. Clathrin mediates integrin endocytosis for focal adhesion disassembly in migrating cells. AP20187 was used to crosslink and thereby disrupt clathrin function, inhibit focal adhesion disassembly, and decrease the rate of cell migration.
Inducible Homodimerization Citations

2009 continued


Markey, K. A. et al. (2009) *Blood* **113**(22):5644–5649. **Conventional dendritic cells are the critical donor APC presenting alloantigen after experimental BMT**, Conditional depletion of conventional dendritic cells (cDCs), plasmacytoid DC (pDCs), macrophages, or B cells was used to demonstrate that donor cDCs are the critical population presenting alloantigen after bone marrow transplantation.


Roostaee, A., Côté, S., and Roucou X. (2009) *J. Biol. Chem.* **284**(45):30907–30916. **Aggregation and amyloid fibril formation induced by chemical dimerization of recombinant prion protein in physiological-like conditions**, The authors treated a chimeric cellular protein (PrP(C)) with AP20187 to cause a rapid conformational change and simultaneous aggregation of the protein, suggesting that dimerization of PrP(C) may initiate the pathogenesis of prion diseases.


2008


<table>
<thead>
<tr>
<th>Year</th>
<th>Authors</th>
<th>Journal</th>
<th>Title</th>
<th>Details</th>
</tr>
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<tbody>
<tr>
<td>2008</td>
<td>Guerrero, A. D., Chen, M., and Wang, J.</td>
<td>Apoptosis</td>
<td>13(1):177–186.</td>
<td>Delineation of the caspase-9 signaling cascade. Chemically induced dimerization was used to understand the order of caspase signaling during apoptosis.</td>
</tr>
<tr>
<td>2008</td>
<td>Oyadomari, S. et al.</td>
<td>Cell Metab.</td>
<td>7(6):520–532.</td>
<td>Dephosphorylation of translation initiation factor 2α enhances glucose tolerance and attenuates hepatosteatosis in mice. Transgenic mice expressing the cytosolic PERK kinase domain fused to an artificial dimerization domain were treated with AP20187 to activate the integrated stress response in the liver.</td>
</tr>
<tr>
<td>2008</td>
<td>Steel, C. D. et al.</td>
<td>Lab Anim. (NY)</td>
<td>37(1):26–32.</td>
<td>Comparison of the lateral tail vein and the retro-orbital venous sinus as routes of intravenous drug delivery in a transgenic mouse model. To compare lateral tail vein and retro-orbital venous sinus injections, MaFIA mice were injected with AP20187, and macrophage depletion was compared for the lung, spleen, bone marrow, and peritoneal exudate cells. Both injection routes were similarly effective.</td>
</tr>
<tr>
<td>2008</td>
<td>Wang, Z. V. et al.</td>
<td>Diabetes</td>
<td>57(8):2137–2148.</td>
<td>PANIC-ATTAC: a mouse model for inducible and reversible β-cell ablation. PANIC-ATTAC (pancreatic islet beta-cell apoptosis through targeted activation of caspase 8) is a mouse model for inducible and reversible ablation of pancreatic beta-cells.</td>
</tr>
</tbody>
</table>
Acevedo, V. D. et al. (2007) Cancer Cell 12(6):559–571. Inducible FGFR-1 activation leads to irreversible prostate adenocarcinoma and an epithelial-to-mesenchymal transition. Activation of FGFR1 with chemical inducers of dimerization (CID) led to highly synchronous, step-wise progression to adenocarcinoma that is linked to an epithelial-to-mesenchymal transition (EMT) and implicated FGFR1 in prostate cancer progression.


Deng, Y. et al. (2007) Am. J. Physiol. Cell Physiol. 293(4):C1404–C1411. MEKK3 is required for endothelium function but is not essential for tumor growth and angiogenesis. AP20187 was used to artificially activate Tie2 in either wild-type or MEKK3-deficient cells and determine that MEKK3 is critical for Ang1/Tie2 signaling to the p38 MAPK pathway.

Dong, Z. et al. (2007) Exp. Cell Res. 313(16):3645–3657. Level of endothelial cell apoptosis required for a significant decrease in microvessel density. Inducible caspase-9 was used to understand the effects of endothelial cell apoptosis on blood vessel generation.

Gazdoiu, S. et al. (2007) Mol. Cell Biol. 27(20):7041–7052. Human Cdc34 employs distinct sites to coordinate attachment of ubiquitin to a substrate and assembly of polyubiquitin chains. The chemical dimerizer AP20187 was used activate Cdc34 and study its role in polyubiquitination.

Goggin, K. et al. (2007) J. Neurochem. 102(4):1195–1205. Aggregation of cellular prion protein is initiated by proximity-induced dimerization. Inducible oligomerization was used to test if, in the absence of any infectious prion particles, the encounter between PrP(C) molecules may trigger its aggregation in neuronal cells.

Isaacs, H. V. et al. (2007) Biol. Cell 99(3):165–173. FGF4 regulates blood and muscle specification in Xenopus laevis. The authors used a drug inhibitor of FGF signalling and an inducible form of FGF receptor 1 to identify a period of competence during late blastula and gastrula stages when FGF signalling acts to regulate blood versus muscle specification.


Miyake, Z. et al. (2007) Mol. Cell Biol. 27(7):2765–2776. Activation of MTK1/MEKK4 by GADD45 through induced N-C dissociation and dimerization-mediated trans autophosphorylation of the MTK1 kinase domain. An inducible version of MTK1 was used to determine that GADD45 binding leads to the activation of the kinase catalytic domain of MTK1.

Niu, H. et al. (2007) Mol. Cell Biol. 27(15):5456–5467. Mek1 kinase is regulated to suppress double-strand break repair between sister chromatids during budding yeast meiosis. Using a version of Mek1 that can be conditionally dimerized during meiosis, Mek1 function was shown to be promoted by dimerization, but DSBs and Mek1 recruitment to the meiosis-specific chromosomal core protein Red1 were also required for Mek1 activation.
Inducible Homodimerization Citations

2007 continued

Nourse, M. B. et al. (2007) Lab. Invest. 87(8):828–835. Selective control of endothelial cell proliferation with a synthetic dimerizer of FGF receptor-1. Human umbilical vein endothelial cells and human microvascular endothelial cells expressing an inducible FGF receptor were used to study the effects of synthetic receptor-dimerizing ligands.


Sequeira, S. J. et al. (2007) PLoS ONE 2(7):e615. Inhibition of proliferation by PERK regulates mammary acinar morphogenesis and tumor formation. An inducible version of the ER kinase PERK was used to study PERK's role in limiting MCF10A mammary epithelial cell proliferation during acinar morphogenesis in 3D Matrigel culture as well as in preventing mammary tumor formation in vivo.

Shah, V. R. et al. (2007) Genesis 45(4):194–199. Double-inducible gene activation system for caspase 3 and 9 in epidermis. The authors developed a double inducible model containing both RU486 and AP20187, which in addition to inducing caspase activation, has potential applicability specifically to other genes encoding proteins that require a dimerization event for activation.


Tokuo, H., Mabuchi, K., and Ikebe, M. (2007) J. Cell Biol. 179(2):229–238. The motor activity of myosin-X promotes actin fiber convergence at the cell periphery to initiate filopodia formation. Using a dimer-inducing technique, the authors show that the motor function of myoX, and not the cargo function, is critical for initiating filopodia formation.


Xian, W., Schwertfeger, K. L., and Rosen, J. M. (2007) Mol. Endocrinol. 21(4):987–1000. Distinct roles of fibroblast growth factor receptor 1 and 2 in regulating cell survival and epithelial-mesenchymal transition. A chemically inducible FGFR (iFGFR) dimerization system was combined with an in vitro three-dimensional HC11 mouse mammary epithelial cell culture model in order to examine the separate roles of FGFR1 and FGFR2 signaling in polarized epithelia.
Inducible Homodimerization Citations

2006


Goffin, L. et al. (2006) Mol. Biol. Cell 17(12):5309–5323. The unfolded protein response transducer Ire1p contains a nuclear localization sequence recognized by multiple βi importins. AP20187 was used to dimerize the Ire1p transmembrane receptor kinase/endonuclease, which transduces the unfolded protein response (UPR) from the endoplasmic reticulum (ER) to the nucleus in Saccharomyces cerevisiae.

Hirate, Y. and Okamoto, H. (2006) Curr. Biol. 16(4):421–427. Canopy1, a Novel Regulator of FGF Signaling around the Midbrain-Hindbrain Boundary in Zebrafish. Uses AP20187-induced dimerization of FGFR1 to demonstrate that expression of Canopy1 is essential for normal FGF signaling in zebrafish embryos. The inducible FGFR1 gene was injected as mRNA into a specific area of the brain and AP20187 was added directly to the embryos.


Inducible Homodimerization Citations

2006 continued


Schwertfeger, K. L. et al. (2006) Cancer Res. 66(11):5676–5685. A critical role for the inflammatory response in a mouse model of preneoplastic progression. Transgenic mice expressing an AP20187-inducible fibroblast growth factor receptor-1 (iFGFR1) were used to examine role of the microenvironment in early stages of tumorigenesis. These mice were also crossed with MaFIA mice to study the effects of macrophage depletion on iFGFR1-mediated phenotypes.


Umeda, K. et al. (2006) Cell 126(4):741–754. ZO-1 and ZO-2 independently determine where claudins are polymerized in tight-junction strand formation. When a truncated version of the tight junction protein ZO-1 was forcibly recruited to lateral membranes and dimerized, claudins were dramatically polymerized.


Witt, A. E. et al. (2006) J. Proteome Res. 5(3):599–610. Functional proteomics approach to investigate the biological activities of cDNAs implicated in breast cancer. The functional activity of a subset of the Breast Cancer 1000 collection was evaluated in cell-based assays that monitor changes in cell proliferation, migration, and morphogenesis in MCF-10A mammary epithelial cells expressing a variant of ErbB2 that can be inducibly activated through dimerization.

Xiao, H. et al. (2006) J. Biomol. Screen. 11(3):225–235. Establishment of a Cell Model Based on FKBP12 Dimerization for Screening of FK506-like Neurotrophic Small Molecular Compounds. The AP20187-mediated homodimerization system was used to screen for novel FK506-like small molecules. Compounds were screened for the ability to block apoptosis caused by forced dimerization of mBax.

Abell, A. N. and Johnson, G. L. (2005) J. Biol. Chem. 280(43):35793–35796. MEKK4 is an effector of the embryonic TRAF4 for JNK activation. Uses AP20187 to show that oligomerization of MEKK4 is sufficient to activate JNK.

Blau, C. A. et al. (2005) J. Biol. Chem. 280(44):36642–36647. -Globin gene expression in chemical inducer of dimerization (CID)-dependent multi-potential cells established from human -globin locus yeast artificial chromosome (-YAC) transgenic mice. Developed cells that can be used to screen for inducers of gamma-globulin expression by using an AP20187-inducible mpl construct to drive proliferation of bone marrow cells derived from beta-YAC transgenic mice.


Cheng, J. et al. (2005) J. Biol. Chem. 280(14):13477–13482. Dimerization through the catalytic domain is essential for MEKK2 activation. Uses AP20187 to demonstrate that oligomerization of MEKK2 leads to its activation.


Larrivee, B. et al. (2005) J. Immunol. 175(5):2890–2899. Minimal contribution of marrow-derived endothelial precursors to tumor vasculature. Uses an AP20187-inducible VEGF receptor 2 to demonstrate that the VEGFR-2 pathway is not sufficient for the recruitment and/or expansion of endothelial progenitor cells in mice.
Mammalian Expression Systems

Inducible Homodimerization Citations

2005 continued


**Inducible Homodimerization Citations**

2005 continued


2004


Inducible Homodimerization Citations

2004 continued


2003

Arias-Salgado, E. G. et al. (2003) *Proc. Natl. Acad. Sci. USA* 100(23):13298–13302. Src kinase activation by direct interaction with the integrin \( \beta \) cytoplasmic domain. Uses AP1510 to show that Src can be activated via beta3 integrin clustering.


Inducible Homodimerization Citations

2003 continued


Freeman, K. W. et al. (2003) Cancer Res. 63(19):6237–6243. Conditional activation of fibroblast growth factor receptor (FGFR) 1, but not FGFR2, in prostate cancer cells leads to increased osteopontin induction, extracellular signal-regulated kinase activation, and in vivo proliferation. Uses an AP20187-inducible FGFR1 to demonstrate its role in signaling and its ability to promote growth of prostate tumor cells in vivo.

Freeman, K. W. et al. (2003) Cancer Res. 63(23):8256–8263. Inducible prostate intraepithelial neoplasia with reversible hyperplasia in conditional FGFR1-expressing mice. Uses transgenic mice containing an AP20187-inducible FGFR1 to show that the development and progression of key pathologic changes seen in early-stage prostate cancer are directly dependent on FGFR1 activation.

Godbey, W. T. and Atala, A. (2003) Gene Ther. 10(17):1519–1527. Directed apoptosis in Cox-2-overexpressing cancer cells through expression-targeted gene delivery. AP20187-inducible caspase-3 (or -9) was used to selectively induce apoptosis in tumor cells that overexpress Cox-2, including cells that are typically resistant to apoptosis.


Kazansky, A. V., Spencer, D. M., and Greenberg, N. M. (2003) Cancer Res. 63(24):8757–8762. Activation of signal transducer and activator of transcription 5 is required for progression of autochthonous prostate cancer: evidence from the transgenic adenocarcinoma of the mouse prostate system. An AP20187-inducible version of a naturally occurring dominant-negative isoform of STAT5B was used to block the invasive potential of prostate cells.

2003 continued


Inducible Homodimerization Citations

2002


Chang, D. W. et al. (2002) EMBO J. 21(14):3704–3714. c-FLIP(L) is a dual function regulator for caspase-8 activation and CD95-mediated apoptosis. Rapamycin-mediated heterodimerization and AP20187-mediated homodimerization were used to explore the role of c-FLIP-L in the CD95-mediated apoptotic signaling pathway.


**Inducible Homodimerization Citations**

**2002 continued**


**2001**


Inducible Homodimerization Citations

### 2001 continued


Inducible Homodimerization Citations

2000


Inducible Homodimerization Citations

1999


Baud, V. et al. (1999) Genes Dev. 13(10):1297–1308. Signaling by proinflammatory cytokines: oligomerization of TRAF2 and TRAF6 is sufficient for JNK and IKK activation and target gene induction via an amino-terminal effector domain. FK1012-mediated oligomerization of TRAF2 or TRAF6 activates multiple downstream targets, including JNK and IKK.


1998


### 1998 continued

<table>
<thead>
<tr>
<th>Citation</th>
<th>Summary</th>
</tr>
</thead>
</table>
**Inducible Homodimerization Citations**

**1997**


Blau, C. A. et al. (1997) *Proc. Natl. Acad. Sci. USA* **94**(7):3076–3081. *A proliferation switch for genetically modified cells.* The first demonstration of dimerizers to specifically and reversibly stimulate the proliferation of a population of engineered cells. FK1012-mediated dimerization of the signaling domain of the erythropoietin receptor is shown to stimulate the proliferation of cells normally dependent on IL-3 for growth.


**1996**


## Inducible Homodimerization Citations

### 1995


### 1994


### 1993

Spencer, D. M., *et al.* (1993) *Science* **262**(5136):1019–1024. [Controlling signal transduction with synthetic ligands](https://www.sciencemag.org/content/262/5136/1019). The first description of the use of dimerizers. Describes the development of FK1012, a semisynthetic dimer of FK506 that can simultaneously bind two molecules of FKBP12. FK1012-mediated oligomerization of the T cell receptor-zeta chain is shown to be sufficient to induce signaling.