Extra-nuclear estrogen signaling

Acconcia, F., Enikolopov, G., Hemish, J., Levin, ER., Marino, M., Rothfels, K.

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09/09/2020
Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

The development of Reactome is supported by grants from the US National Institutes of Health (P41 HG003751), University of Toronto (CFREF Medicine by Design), European Union (EU STRP, EMI-CD), and the European Molecular Biology Laboratory (EBI Industry program).

Literature references


Reactome database release: 73

This document contains 3 pathways and 20 reactions (see Table of Contents)
Extra-nuclear estrogen signaling

Stable identifier: R-HSA-9009391

In addition to its well-characterized role in estrogen-dependent transcription, estrogen (beta-estradiol, also known as E2) also plays a rapid, non-genomic role through interaction with receptors localized at the plasma membrane by virtue of dynamic palmitoylation. Estrogen receptor palmitoylation is a prerequisite for the E2-dependent activation of extra-nuclear signaling both in vitro and in animal models (Acconcia et al, 2004; Acconcia et al, 2005; Marino et al, 2006; Marino and Ascenzi, 2006). Non-genomic signaling through the estrogen receptor ESR1 also depends on receptor arginine methylation by PMRT1 (Pedram et al, 2007; Pedram et al, 2012; Le Romancer et al, 2008; reviewed in Arnal, 2017; Le Romancer et al, 2011).

E2-evoked extra-nuclear signaling is independent of the transcriptional activity of estrogen receptors and occurs within seconds to minutes following E2 administration to target cells. Extra-nuclear signaling consists of the activation of a plethora of signaling pathways including the RAF/MAP kinase cascade and the PI3K/AKT signaling cascade and governs processes such as apoptosis, cellular proliferation and metastasis (reviewed in Hammes et al, 2007; Handa et al, 2012; Lange et al, 2007; Losel et al, 2003; Arnal et al, 2017; Le Romancer et al, 2011). ESR-mediated signaling also cross-talks with receptor tyrosine kinase, NF- kappa beta and GPCR signaling pathways by modulating the post-translational modification of enzymes and other proteins and regulating second messengers (reviewed in Arnal et al, 2017; Schwartz et al, 2016; Boonyaratankornkit, 2011; Biswas et al, 2005). In the nervous system, E2 affects neural functions such as cognition, behaviour, stress responses and reproduction in part by inducing such rapid extra-nuclear responses (Farach-Carson and Davis, 2003; Losel et al, 2003), while in endothelial cells, non-genomic ESR-dependent signaling also regulates vasodilation through the eNOS pathway (reviewed in Levin, 2011).

Extra-nuclear signaling additionally cross-talks with nuclear estrogen receptor signaling and is required to control ER protein stability (La Rosa et al, 2012).

Recent data have demonstrated that the membrane ESR1 can interact with various endocytic proteins to traffic and signal within the cytoplasm. This receptor intracellular trafficking appears to be dependent on the physical interaction of ESR1 with specific trans-membrane receptors such as IGR-1R and beta 1-integrin (Sampayo et al, 2018).
**Literature references**


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PRMT1 methylates ESRs

**Location:** Extra-nuclear estrogen signaling

**Stable identifier:** R-HSA-9632182

**Type:** transition

**Compartments:** cytosol

ESR1 is methylated by PRMT1 at arginine 260 in response to E2 stimulation. Methylation is required for rapid non-genomic signaling by E2 and promotes the interaction of ESR1 with the p85 regulatory subunit of PI3K, SRC and PTK2 (FAK) (Simoncini et al, 2000; Le Romancer, 2008; reviewed in Arnal, 2017). Although this reaction is shown preceding palmitoylation of ESR1, the sequence of these two events is not established.

**Preceded by:** PalmS-ESRs:CAVs translocate to plasma membrane

**Followed by:** Estrogen stimulates dimerization of plasma membrane estrogen receptors

**Literature references**


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ZDHHC7, ZDHHC21 palmitoylate ESR1

**Location:** Extra-nuclear estrogen signaling

**Stable identifier:** R-HSA-9021072

**Type:** transition

**Compartments:** Golgi membrane

In addition to nuclear signaling, estrogen receptors also promote a rapid, transcription- and translation-independent signaling response from the plasma membrane (reviewed in Levin, 2005; Schwartz et al, 2016). In contrast to their classical nuclear counterparts, estrogen receptors that signal from the plasma membrane are lipid-modified by palmitoylation. The amino-acid sequence encompassing an exposed cysteine residue (Cys447 for ESR1 and Cys399 for ESR2) is conserved between other steroid hormone receptors (Marino and Ascenzi, 2006). Mutation of the target cysteine in this motif abrogates membrane localization and the rapid response to estrogen (Acconcia et al, 2004; Acconcia et al, 2005; Pedram et al, 2007; Pedram et al, 2011). Golgi-localized palmitoyltransferases ZDHHC7 and ZDHHC21 catalyze the transfer of palmitoyl to the cysteine residue in the estrogen receptor (Pedram et al, 2012).

Followed by: PalmS-ESRs bind CAVs

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Palmitoylated estrogen receptors bind caveolin proteins (CAVs) prior to translocation to the plasma membrane (Razandi et al, 2002; Acconcia et al, 2004; Acconcia et al, 2005; Pedram et al, 2007; Pedram et al, 2012). Membrane localization depends on the scaffolding domain (aa 80-100) of caveolin, although this region is dispensable for estrogen receptor binding (Razandi et al, 2002; Pedram et al, 2007). Interaction between caveolin and the estrogen receptor is diminished upon stimulation of cells with E2 (beta-estradiol) in some cell types, which may restrict the duration of the immediate signaling (Acconcia et al, 2004; Acconcia et al, 2005).

Precended by: ZDHHC7, ZDHHC21 palmitoylate ESR1

Followed by: PalmS-ESRs:CAVs translocate to plasma membrane

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Palmitoylation of estrogen receptors promotes their interaction with caveolin (CAV), which is required for their translocation to the plasma membrane where they function in rapid, transcription-independent signaling (Chambliss et al, 2002; Razandi et al, 2003; Acconcia et al, 2004; Pedram et al, 2007; Razandi et al, 2010; Pedram et al, 2012; reviewed in Schwartz et al, 2016). Approximately 5-15% of total cellular estrogen receptor is at the plasma membrane where it is enriched in caveolae (Pedram et al, 2006; Marino et al, 2006).

**Preceded by:** PalmS-ESRs bind CAVs

**Followed by:** PRMT1 methylates ESRs

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Estrogen stimulates dimerization of plasma membrane estrogen receptors

Location: Extra-nuclear estrogen signaling

Stable identifier: R-HSA-9021170

Type: binding

Compartments: plasma membrane

Plasma membrane-localized estrogen receptors signal as dimers, and dimerization is promoted by stimulation with estrogen (Razandi et al, 2004). Because palmitoylation of cytoplasmic estrogen receptors occurs on the monomeric form, estrogen (ESTG) stimulation restricts both the amount of palmitoylated receptor and its localization at the plasma membrane (Acconcia et al, 2005; Razandi et al, 2010; Pedram et al, 2012). This may serve to limit the extent of the rapid response to estrogen stimulation.

Preceded by: PRMT1 methylates ESRs

Followed by: ESR binds STRN

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The estrogen receptor binds to the scaffolding protein striatin (STRN) in response to estrogen stimulation in endothelial cells. STRN binding promotes the membrane localization of the receptor and is required for the interaction between ESR1 and G alpha (i), as well as for downstream signaling through AKT, MAPK and eNOS (Lu et al, 2004).

**Preceded by:** Estrogen stimulates dimerization of plasma membrane estrogen receptors  
**Followed by:** ESTG binding induces ESR depalmitoylation

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ESTG binding induces ESR depalmitoylation

Location: Extra-nuclear estrogen signaling

Stable identifier: R-HSA-9027670

Type: omitted

Compartments: plasma membrane

Palmitoylation of the estrogen receptor is dynamic. Binding of 17 beta-estradiol induces depalmitoylation by an unidentified protein palmitoyl hydrolase, releasing cytosolic ESRs that are free to interact with signaling proteins to initiate rapid non-genomic signaling (Marino et al, 2008; La Rosa et al, 2012; reviewed in Levin, 2005; Arnal et al, 2017). Dynamic palmitoylation cycles also impact the phosphorylation and degradation of ESR1. Mutation of C447 increases the susceptibility of ESR1 to degradation (La Rosa et al, 2012). Mutation of the palmitoyl acceptor cysteine 447 to alanine also abrogates phosphorylation of serine 118, a major N-terminal domain phosphorylation site that contributes to transcriptional activity. As phosphorylation of S118 itself likely occurs as a result of MAPK activation downstream of estrogen-stimulated membrane ESR1, the rapid non-genomic response to estrogen stimulation is interconnected with the classical transcriptional response.

Preceded by: ESR binds STRN

Followed by: Heterotrimeric G protein (i) binds membrane-associated ESRs:ESTG, Membrane estrogen receptors bind SRC

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Heterotrimeric G protein (i) binds membrane-associated ESRs:ESTG

Location: Extra-nuclear estrogen signaling

Stable identifier: R-HSA-9021601

Type: binding

Compartments: plasma membrane

Plasma-membrane localized estrogen receptors interact with and signal through heterotrimeric G-proteins (Wyckoff et al, 2001). The estrogen receptor makes direct interactions with both the G alpha i and the G beta gamma proteins and these interactions are required for downstream signaling to SRC, which is required for signaling through MAPK and PI3K/AKT, as well as for activation of eNOS activity in endothelial cells (Castoria et al, 2001; Wyckoff et al, 2001). Estrogen-stimulation promotes dimerization of the estrogen receptor and dissociation of the heteromeric G-protein (reviewed in Levin, 2015; Schwartz et al, 2016)

Preceded by: ESTG binding induces ESR depalmitoylation

Followed by: G-proteins dissociate from plasma-membrane estrogen receptors

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G-proteins dissociate from plasma-membrane estrogen receptors

Location: Extra-nuclear estrogen signaling

Stable identifier: R-HSA-9021600

Type: dissociation

Compartments: plasma membrane


Preceded by: Heterotrimeric G protein (i) binds membrane-associated ESRs:ESTG

Followed by: Membrane estrogen receptors bind SRC

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Membrane estrogen receptors bind SRC

**Location:** Extra-nuclear estrogen signaling

**Stable identifier:** R-HSA-9021596

**Type:** binding

**Compartments:** plasma membrane

Rapid signaling downstream of estrogen stimulation activates signaling through MAP kinase and PI3K/AKT signaling pathways, as well as initiating cross-talk with NF kappa beta and receptor tyrosine kinase pathways (reviewed in Hammes et al, 2007; Handa et al, 2012; Lange et al, 2017; Losel et al, 2003). Signaling through AKT regulates cell cycle progression through CREB-mediated upregulation of cyclin D1 and depends on recruitment of SRC and the p85 regulatory subunit of PI3K (Castoria et al, 2001; Marino et al, 2002; Marino et al, 2003; Castoria et al, 2012; reviewed in Castoria et al, 2010). Estrogen-stimulated nitric oxide (NO) release from endothelial cells likewise depends on the recruitment of SRC to the estrogen receptors at the plasma membrane (Haynes et al, 2003).

Once recruited, SRC is activated by autophosphorylation at tyrosine 419, which in turn is required for the recruitment and activation of PI3K and AKT, and ultimately for the activation of endothelial nitric oxide synthase (eNOS) and promotion of AKT-dependent cellular proliferation (Haynes et al, 2000; Simoncini et al, 2000; Haynes et al, 2003; Li et al, 2007; reviewed in Kim and Bender, 2005; Hammes and Levin, 2011; Le Romancer et al, 2011: Castoria et al, 2010). Formation of an eNOS signaling complex also depends on interaction between the estrogen receptor and heterotrimeric G-proteins (Wyckoff et al, 2001; reviewed in Schwartz, 2016).

**Preceded by:** G-proteins dissociate from plasma-membrane estrogen receptors, ESTG binding induces ESR depalmitoylation

**Followed by:** ESR-associated SRC autophosphorylates

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ESR-associated SRC autophosphorylates

**Location:** Extra-nuclear estrogen signaling

**Stable identifier:** R-HSA-9021609

**Type:** transition

**Compartments:** plasma membrane

Estrogen treatment stimulates autophosphorylation of SRC at tyrosine 419 (Haynes et al, 2003). SRC catalytic activity is required for signaling to PI3K, AKT and eNOS, as both a kinase dead version of SRC and treatment of cells with a SRC inhibitor abrogate phosphorylation of these downstream targets. SRC interacts with the p85 regulatory subunit of PI3K and in this way promotes assembly of an estrogen-responsive signaling complex in the caveolae (Simoncini et al, 2000; Hayes et al, 2003; Castoria et al, 2001; Castoria et al, 2012; Le Romancer et al, 2008).

**Preceded by:** Membrane estrogen receptors bind SRC

**Followed by:** PI3K binds membrane-associated estrogen receptors

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**PI3K binds membrane-associated estrogen receptors**

**Location:** Extra-nuclear estrogen signaling

**Stable identifier:** R-HSA-9021660

**Type:** binding

**Compartments:** plasma membrane

PI3K is recruited to the estrogen receptors at the plasma membrane by virtue of an estrogen-dependent interaction of the p85 regulatory subunit with the estrogen receptor. Estrogen stimulation increases PI3K activity in a manner that also depends on SRC and SRC kinase activity, and results in increased PIP3 production and activation of AKT signaling (Simoncini et al, 2000; Castoria et al, 2001; Castoria et al, 2012; Le Romancer et al, 2008). Activation of AKT signaling downstream of estrogen stimulation drives cellular proliferation through the upregulation of the G1/S cyclin CCND1 (Castoria et al, 2001; Castoria et al, 2004; reviewed in Castoria et al, 2010), and E2 is also required for recruitment of focal adhesion kinase (FAK, also known as PTK2) (Le Romancer et al, 2008). AKT activation additionally stimulates phosphorylation of eNOS at residue 1117, promoting NO release in endothelial cells (Simoncini et al, 2000; Haynes et al, 2000; Hisamoto et al, 2001; Li et al, 2003; Haynes et al, 2003; reviewed in Levin, 2011).

**Preceded by:** ESR-associated SRC autophosphorylates

**Followed by:** PTK2 is recruited to methylated ESR1

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eNOS synthesizes NO

**Location:** Extra-nuclear estrogen signaling

**Stable identifier:** R-HSA-202127

**Type:** transition

**Compartments:** cytosol, plasma membrane

Nitric oxide (NO) is produced from L-arginine by the family of nitric oxide synthases (NOS) enzymes, forming the free radical NO and citrulline as byproduct. The cofactor tetrahydrobiopterin (BH4) is an essential requirement for the delivery of an electron to the intermediate in the catalytic cycle of NOS.

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PTK2 is recruited to methylated ESR1

Location: Extra-nuclear estrogen signaling

Stable identifier: R-HSA-9632412

Type: binding

Compartments: cytosol

PTK2 (also known as FAK) is co-immunoprecipitated with methylated ESR1 and PI3K after stimulation with estrogen. This interaction depends on ESR1 methylation and on SRC activity, as it is abrogated in the presence of SRC inhibitors or upon PRMT1 depletion (Le Romancer et al, 2008). Phosphorylation of PTK2 appears to be dynamic, as it is lost within 15 minutes of estrogen stimulation, promoting disassembly of the complex. Interaction with PTK2 may contribute to estrogen-stimulated cell motility.

Preceded by: PI3K binds membrane-associated estrogen receptors

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Estrogen-stimulated signaling through PRKCZ

Location: Extra-nuclear estrogen signaling

Stable identifier: R-HSA-9634635

Atypical protein kinases lack the calcium binding C2 domain and are unresponsive to diacylglycerol and phorbol ester, but instead respond to PIP3 generation downstream of PI3K signaling. Atypical protein kinase C zeta (PRKCZ) is activated downstream of estrogen stimulation in MCF7 breast cancer cells and contributes estrogen-dependent proliferation through MAPK pathway activation (Castoria et al, 2004).

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Estrogen receptor binds IGF1R and SHC1 in response to estrogen

Location: Extra-nuclear estrogen signaling

Stable identifier: R-HSA-9634584

Type: omitted

Compartments: plasma membrane

Estrogen stimulates phosphorylation of the insulin-like growth factor 1 receptor (IGF1R) in a manner that depends on ESR1 but is independent of stimulation by the cognate IGF1 ligand (Richards et al, 1996; Richards et al, 1998; Kleinman et al, 1995; Lee et al, 1999; Kahlert et al, 2000; Song et al, 2002; Song et al, 2004). Estrogen-dependent phosphorylation of IGF1R depends on a physical interaction between the estrogen-bound estrogen receptor and IGF1R and may be promoted by SHC1 and SRC, although the molecular details are not established (Song et al, 2004). In one model, interaction of liganded ESR1 with SHC promotes the activation of SRC and active SRC then phosphorylates IGF1R at tyrosine residues required for SHC interaction. In this way, both SHC and liganded-ESR1 would be brought to IGF1R in a SRC-dependent manner. Consistent with this, SHC has been shown to activate SRC; however, this model has not been rigorously validated (Sato et al, 2002; Song et al, 2004). E2-stimulated IGF1R pathway activation also depends on activation of the MAPK pathway, as IGF1R phosphorylation is abrogated upon treatment of cells with MAPKK inhibitors (Kahlert et al, 2000; Song et al, 2004).

In addition to MAPK pathway activation, E2-dependent IGF1R activation promotes signaling through the EGFR pathway in a manner that depends on MMP2 and MMP9, suggesting that the cross-talk is mediated at the level of liberation of HBEGF from the plasma membrane (Song et al, 2004; Song et al, 2007, Santen et al, 2009). The details of this connection also remain to be fleshed out.

Followed by: MMPs cleave HB-EGF

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MMPs cleave HB-EGF

**Location:** Extra-nuclear estrogen signaling  

**Stable identifier:** R-HSA-9624272  

**Type:** transition  

**Compartments:** plasma membrane, extracellular region

Estrogen-stimulation of ESR1 activates downstream signaling pathways that results in the release of HB-EGF in an IGF- MMP- and EGFR-dependent manner (Razandi et al, 2003; Song et al, 2004; Song et al, 2007; Santen et al, 2009). Based on studies in other systems, candidate MMPs for the cleavage of HB-EGF include MMP2, MMP3, MMP7 and MMP9 (Suzuki et al, 1997; Yu et al, 2002; Razandi et al, 2003). Although direct cleavage of HB-EGF by MMPs downstream of estrogen signaling has not been demonstrated, estrogen-stimulated signaling through EGFR is abrogated after treatment of cells with an HB-EGF neutralizing antibody (Razandi et al, 2003; Song et al, 2007).

**Preceded by:** Estrogen receptor binds IGF1R and SHC1 in response to estrogen

**Literature references**


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PTK2 binds activated EGFR

Location: Extra-nuclear estrogen signaling

Stable identifier: R-HSA-9625482

Type: binding

Compartments: plasma membrane, cytosol

PTK2 (also known as FAK, focal adhesion kinase) binds to the activated EGFR receptor as assessed by affinity chromatography and co-immunoprecipitation (Sieg et al, 2000; Thelemann et al, 2005; Liu et al, 2010). Stimulation of the EGFR pathway increases PTK2 phosphorylation at Y397, and FAK phosphorylation contributes to cell migration and tumorigenicity (Tamura et al, 1998; Gu et al, 1999; Sieg et al, 2000; Liu et al, 2010; reviewed in Zhu et al, 2018). PTK2-stimulated cell motility depends on integrins and is transduced through phosphorylated MAPK3 and MAPK1 (also known as ERK1 and ERK2) (Sieg et al, 2000; Thelemann et al, 2000; Liu et al, 2005).

Followed by: PTK2 autophosphorylates downstream of EGFR

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PTK2 autophosphorylates downstream of EGFR

**Location:** Extra-nuclear estrogen signaling

**Stable identifier:** R-HSA-9625487

**Type:** transition

**Compartments:** plasma membrane, cytosol

Stimulation of cells with either E2 (beta-estradiol), tamoxifen or G1 (a GPER1 agonist) enhances EGFR-dependent FAK autophosphorylation at Y397 (Sieg et al, 2000; Liu et al, 2010; Tsai et al, 2013). Signaling occurs through both GPER1 and ER alpha (ESR1) and induces cell proliferation and migration through the EGFR-PI3K-ERK pathway (Liu et al, 2010; Tsai et al, 2013; reviewed in Zhu et al, 2018). FAK autophosphorylation is also required for FOS (c-fos) induction downstream of E2 (Liu et al, 2010; Tsai et al, 2013; Maggiolini et al, 2004; Vivacqua et al, 2006a,b).

**Preceded by:** PTK2 binds activated EGFR

**Literature references**


Tsai, CL., Wu, HM., Lin, CY., Lin, YJ., Chao, A., Wang, TH. et al. (2013). Estradiol and tamoxifen induce cell migration through GPR30 and activation of focal adhesion kinase (FAK) in endometrial cancers with low or without nuclear estrogen receptor α (ERα). *PLoS ONE*, 8, e72999.


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Although membrane-localized estrogen receptors stimulate rapid, transcription-independent responses such as calcium mobilization and alterations to the fibronectin matrix to affect cell migration, among others, the pathways activated by rapid signaling may also ultimately affect nuclear events. Activation of MAPK and PI3K/AKT pathways downstream of membrane-localized ESR1 contributes to estrogen-responsive changes in cellular proliferation and survival in part through changes in gene expression (reviewed in Levin et al, 2005; Lange et al, 2007; Le Romancer et al, 2011).

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SPHK1 phosphorylates sphingosine in response to E2 stimulation

Location: Extra-nuclear estrogen signaling

Stable identifier: R-HSA-9625814

Type: transition

Compartments: plasma membrane

Estrogen stimulation of breast cancer cells promotes ESR1-dependent activation of sphingosine kinase 1 (SPHK1) (Sukocheva et al, 2003; Sukhocheva et al, 2006). SPHK1 catalyzes the formation of sphingosine-1-phosphate (S1P), a ligand for the GPCR S1PR3 receptor, also known as EDG3 (Hla et al, 2001; Spiegel and Milstein, 2003; Sukhocheva et al, 2006). S1P-bound S1PR3 stimulates EGFR transactivation downstream of estradiol stimulation through the MMP-dependent release of HBEGF from the plasma membrane, leading to EGFR and MAPK phosphorylation (Kim et al, 2000; Tanimoto et al, 2004; Filardo et al, 2000; Razandi et al, 2003; Sukhocheva et al, 2003; Sukhocheva et al, 2006; reviewed in Prossnitz and Barton, 2014).

Followed by: S1P binds S1PR3 in response to E2 stimulation

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S1P binds S1PR3 in response to E2 stimulation

**Location:** Extra-nuclear estrogen signaling

**Stable identifier:** R-HSA-9625813

**Type:** binding

**Compartments:** plasma membrane

S1P binds to its GPCR receptor S1PR3 downstream of E2 stimulation and GPER1 (Sukocheva et al, 2003; Sukocheva et al, 2006). S1P-bound S1PR3 promotes transactivation of the EGFR signaling pathway through the MMP-dependent liberation of HBEGF from the plasma membrane, leading to EGFR and MAPK phosphorylation (Sukocheva et al, 2003; Sukocheva et al, 2006; Kim et al, 2000; Tanimoto et al, 2004; Filardo et al, 2000; Razandi et al, 2003).

**Preceded by:** SPHK1 phosphorylates sphingosine in response to E2 stimulation

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