

## Supplemental information for

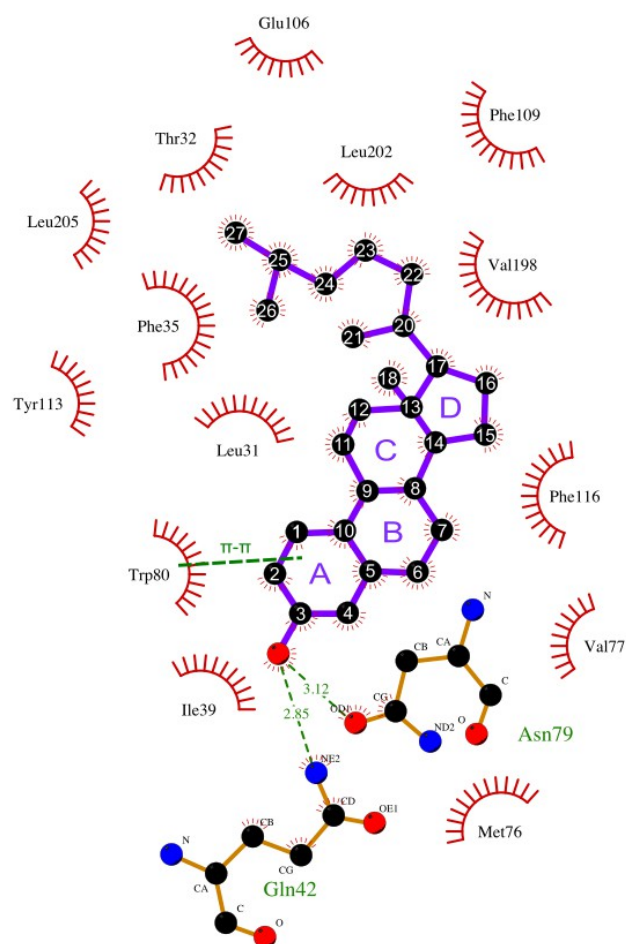
### **NR3E receptors in cnidarians : a new family of steroid receptor relatives extends the possible mechanisms for ligand binding**

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### Supplemental Figure 1. NR3E-paraestrol A interaction diagram.

The interactions shown are those mediated by hydrogen bonds, hydrophobic contacts and  $\pi$ - $\pi$  interaction. Hydrogen bonds with the amino acids from the NR3E binding pocket are indicated by dashed lines between the atoms involved, while hydrophobic contacts are represented by an arc with spokes radiating towards the paraestrol A atoms they contact. The contacted atoms are shown with spokes radiating back. The figure was drafted using LIGPLOT v.4.5.3 and manually edited to add the paraestrol A carbon and ring numbering according to the IUPAC sterol nomenclature.

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NR3E Hydra magnipapillata -----
NR3F Trichoplax adhaerens ..... .MTSTF.... .....ASHS G...TPIEP V.....
NR3E Hydractinia symbiolongicarpus .....
NR3E Aurelia aurita .....
NR3E Rhopilema esculentum .....
NR3E Podocoryna carnea .....
NR3E Nanomia bijuga .....
NR3E Chironex fleckeri .....
NR3E Alatina alata .....
NR3E Morbakka virulenta .....
NR3E Tripedalia cystophora .....
NR3E Copula sivickisi .....
NR3E Chironex yamaguchi .....
ERRa Homo sapiens .....
ERRb Homo sapiens .....
ERRg Homo sapiens .....
ERR Drosophila melanogaster .....
ERR Crassostrea gigas .....
NR3D Crassostrea gigas .....
NR3D Aplysia californica .....
NR3D Platynereis dumerilii .....
ER Branchiostoma floridae .....
ERa Homo sapiens ..... .MTMTLHTKA SGMALLHQIQ G...NELEP L.....
ERb Homo sapiens ..... .MDIKNSP SSL.....NSPSS Y.....
SR Branchiostoma floridae ..... .MENQP NTVTNPMTT. ....QGLQP L.....
GR Homo sapiens PD..TKPKIK DNGDLVLSSP SNVTLPQVKT E.....K.....E
MR Homo sapiens PIQESTKHS CSGTSFKGNP TVNPFPMFDG S...YF.S F.MDDKDYYS LSGILGPPVP
AR Homo sapiens TKGLEGESLG CSGSAAAGSS GTLELPSTLS LYKSGALDEA AAYQSRDYNN FPLAL.....
PR Homo sapiens PR.....SSP CASSTPVAVG DFPDCAYP.P DAEPKDDAYP LYSDFQPPAL

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NR3E Hydra magnipapillata -----
NR3F Trichoplax adhaerens ..... ..TSASDQL VFTSTAS... .SR..LDKGV DSGTTT.SDS KN.....
NR3E Hydractinia symbiolongicarpus .....
NR3E Aurelia aurita .....
NR3E Rhopilema esculentum .....
NR3E Podocoryna carnea .....
NR3E Nanomia bijuga ..... .M DSaelVVPFK KR.....
NR3E Chironex fleckeri .....
NR3E Alatina alata .....
NR3E Morbakka virulenta .....
NR3E Tripedalia cystophora .....
NR3E Copula sivickisi .....
NR3E Chironex yamaguchi .....
ERRa Homo sapiens .....
ERRb Homo sapiens .....
ERRg Homo sapiens ..... .M DSVELCLPES FS...LHYE
ERR Drosophila melanogaster .....
ERR Crassostrea gigas .....
NR3D Crassostrea gigas ..... -MEAK YHDQ.QFEDA
NR3D Aplysia californica .....
NR3D Platynereis dumerilii .....
ER Branchiostoma floridae .....
ERa Homo sapiens ..... .NRPQLKI ..... .P..LERP..LGEVYL.SS KPA..VYNYP
ERb Homo sapiens ..... .NCSQSIL ..... .P..LE...HGSIIYIPSS YVDS.HHEY
SR Branchiostoma floridae ..... .AYQQGYI VQQQQPS... .QPYRLPSPI QPEFVIGN.FP.....VG
GR Homo sapiens DFIELC..... .TPGVIKQE KLGTVYCQAS FPGANII..G
MR Homo sapiens GFDGNCESG..... .GFPVGIKQEP DDGSYYPEAS IPSSAIV..G
AR Homo sapiens ..... .AGPPPPP P..PPHPHAR IKLENPLDYG
PR Homo sapiens KIKEEEEGAE ASARSPRSYL VAGANPAAFP DFPLGPPPP. .LPPRATPS .PGEAAV..T

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Supplemental Figure 2

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|                                    |           |                  |                |            |            |            |
|------------------------------------|-----------|------------------|----------------|------------|------------|------------|
| NR3E Hydra magnipapillata          | -----     | -----DSYVD       | VECVH-----     | -----QD    | E-----INE  | -----GDKN  |
| NR3F Trichoplax adhaerens          | .....     | .DNIT.LHN        | S--.DRIQPL     | P...LAGLPI | Y.....TAP  | .....TPAS  |
| NR3E Hydractinia symbiolongicarpus | .....     | -----            | -----MD.....   | -----      | -----SEA   | .....SHTA  |
| NR3E Aurelia aurita                | .....     | -----            | -----          | -----      | -----      | -----      |
| NR3E Rhopilema esculentum          | .....     | -----            | -----          | -----      | -----      | -----      |
| NR3E Podocoryna carnea             | .....     | -----            | -----          | -----      | -----      | -----      |
| NR3E Nanomia bijuga                | .....     | .NNL.AVFN        | SNKMETDEVV     | P..PFSSKSN | K.....SES  | .....ES.   |
| NR3E Chironex fleckeri             | .....     | -----            | -----          | -----      | -----      | -----      |
| NR3E Alatina alata                 | .....     | -----            | -----          | -----      | -----      | -----      |
| NR3E Morbakka virulenta            | .....     | -----            | -----          | -----      | -----      | -----      |
| NR3E Tripedalia cystophora         | .....     | -----            | -----          | -----      | -----      | -----      |
| NR3E Copula sivickisi              | .....     | -----            | -----          | -----      | -----      | -----      |
| NR3E Chironex yamaguchi            | .....     | -----            | -----          | -----      | -----      | -----      |
| ERRa Homo sapiens                  | .....     | .MSSQ---.V       | GIEPLYIKAE     | PASPDSP.-- | -----K     | .....SSE   |
| ERRb Homo sapiens                  | .....     | .MSSD.RHLG       | SS.GSFIKTE     | PSSPSSGIDA | LSHHSP--S  | .....SSD   |
| ERRg Homo sapiens                  | E.....    | ELLC RMSNK.RHI.  | SS.SSFIKTE     | PSSPASLTDS | VNHHSP--G  | .....SSD   |
| ERR Drosophila melanogaster        | .....     | -----MS.         | GVSILHIKQE     | VDTPSASCFS | PSSKSTATQS | .....TNG   |
| ERR Crassostrea gigas              | .....     | -----            | -----MD.DIIKME | PGSPLGLSRC | GHS.....   | -----LS    |
| NR3D Crassostrea gigas             | L.....    | TVIQ GLSDPGG-TV  | CRILRESSAA     | ASATGDEA-- | ..E...SMS  | .....ESI   |
| NR3D Aplysia californica           | .....     | .MVSPS---P       | CMP.LPL...     | .SGAGGGG-- | ..E...GTA  | .....SAH   |
| NR3D Platynereis dumerilii         | .....     | -----            | -----          | -----      | -----      | -----      |
| ER Branchiostoma floridae          | .....     | -----            | -----MAYPDIHII | P..PQGQTPR | M.....TTP  | .....TKAA  |
| Era Homo sapiens                   | E.....    | GAAY EFNAAAA-AN  | AQ-.YGQTGL     | PYGPGEAAA  | FGSN...GLG | .....FPP   |
| ERb Homo sapiens                   | A....     | MTFY . . SPAV-MN | YSIPSNVTNL     | EGGPGRQ.-- | -----      | -----      |
| SR Branchiostoma floridae          | N.....    | MTQS QMSQ.-----  | .....          | PRGNDCAL.R | .....N.S   | .....STA   |
| GR Homo sapiens                    | NK.....   | .MSAI.VHG        | -----S         | TSGGQMYHY. | MNTASL.--S | QQ..QDQKPI |
| MR Homo sapiens                    | V.....    | -----            | -----          | NSGGQSFHYR | IGAQTISLS  | RSARDQSFQH |
| AR Homo sapiens                    | SAWAAAAQC | RYGDLA.LHG       | A-----G        | AAGP.....  | -----      | -----APPQ  |
| PR Homo sapiens                    | AA.....   | .PASA.VSS        | A-----S        | SSGSTLECLT | YKAEG.---  | .....APPQ  |

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|                                    |            |            |            |            |            |            |
|------------------------------------|------------|------------|------------|------------|------------|------------|
| NR3E Hydra magnipapillata          | L-----     | -----      | -----      | -----      | -----      | -----      |
| NR3F Trichoplax adhaerens          | .....      | -----      | -----      | -----      | -----      | -----      |
| NR3E Hydractinia symbiolongicarpus | M.....     | -----      | -----      | -----      | -----      | -----      |
| NR3E Aurelia aurita                | -.....     | -----      | -----      | -----      | -----      | -----      |
| NR3E Rhopilema esculentum          | -.....     | -----      | -----      | -----      | -----      | -----      |
| NR3E Podocoryna carnea             | -.....     | -----      | -----      | -----      | -----      | -----      |
| NR3E Nanomia bijuga                | S.....     | -----      | -----      | -----      | -----      | -----      |
| NR3E Chironex fleckeri             | -.....     | -----      | -----      | -----      | -----      | -----      |
| NR3E Alatina alata                 | -.....     | -----      | -----      | -----      | -----      | -----      |
| NR3E Morbakka virulenta            | -.....     | -----      | -----      | -----      | -----      | -----      |
| NR3E Tripedalia cystophora         | -.....     | -----      | -----      | -----      | -----      | -----      |
| NR3E Copula sivickisi              | -.....     | -----      | -----      | -----      | -----      | -----      |
| NR3E Chironex yamaguchi            | -.....     | -----      | -----      | -----      | -----      | -----      |
| ERRa Homo sapiens                  | -.....     | T.ETE..... | -----      | -----      | -----      | -----      |
| ERRb Homo sapiens                  | -.....     | A.SGGF..GL | A.....     | -----      | -----      | LG.....    |
| ERRg Homo sapiens                  | -.....     | A.SGSY..SS | T.....     | -----      | -----      | MN.....    |
| ERR Drosophila melanogaster        | -.....     | KSSPSVSP.  | ERQLCSST   | S.....     | -----      | LS.....    |
| ERR Crassostrea gigas              | -.....     | L.EESF..GR | D.....     | -----      | -----      | VF.....    |
| NR3D Crassostrea gigas             | EKLV...PKE | E....SHGS  | H.....     | -----      | -----      | HS.....    |
| NR3D Aplysia californica           | .....      | QAA        | T.....STP  | E.....     | -----      | AG.....    |
| NR3D Platynereis dumerilii         | -.....     | -----      | -----      | -----      | -----      | -----      |
| ER Branchiostoma floridae          | .KQSPMSPVM | SVHGMQRNGR | .....      | -----      | -----      | IQ.....    |
| Era Homo sapiens                   | .NSVSPSPML | L....LHPPP | Q.....     | -----      | -----      | LS.....    |
| ERb Homo sapiens                   | -...TTSPNV | L...WPTPG  | H.....     | -----      | -----      | LS.....    |
| SR Branchiostoma floridae          | .ISPQYFPFQ | VVRPYSPHR  | P.....     | -----      | -----      | -----      |
| GR Homo sapiens                    | FNVIPPVPG  | S..ENWNR.  | .....      | -----      | Q          | GSG...DDNL |
| MR Homo sapiens                    | .SSFPPVNTL | V..ESWKSH. | .....      | -----      | G          | DLS...SRR. |
| AR Homo sapiens                    | -GSGSPSAAA | S..SSWHTLF | TAEQGQLYGP | CGGGGGGGGG | GGGGGGGGGG | GGGGGEAGAV |
| PR Homo sapiens                    | QGPFAPPPCK | A..PGASGCL | LPRDGLPSTS | ASAA.....  | .....AA    | GAA...PALY |

Supplemental Figure 2 (continued)

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|                                    |            |            |             |            |            |                       |
|------------------------------------|------------|------------|-------------|------------|------------|-----------------------|
| NR3E Hydra magnipapillata          | --PINTSS-- | -SNI-----  | -----PRVIE- | --DTIN-I-- | -----I---- | -----                 |
| NR3F Trichoplax adhaerens          | ..CGMLRP.. | .K.Q.....  | .....Y----  | ..ASK....  | ....SIP..  | ...VVP....            |
| NR3E Hydractinia symbiolongicarpus | ...VLEKM.. | ----.....  | .....EVK..  | ..EQVK.V.  | ....E....  | ....VVP....           |
| NR3E Aurelia aurita                | .....      | .....      | .....       | ..SALK.M.  | ....D....  | ....                  |
| NR3E Rhopilema esculentum          | ..-AFGRH.. | ..ESS..... | .....LILTK. | ..SALK.M.  | ....D....  | ....                  |
| NR3E Podocoryna carnea             | .....      | .....      | .....       | .....      | .....      | .....                 |
| NR3E Nanomia bijuga                | ..ANV....  | ----.....  | .....NNQFN. | ..EDAQ.M.  | ....K....  | ....                  |
| NR3E Chironex fleckeri             | ..-MMD--.  | .....      | .....MQ..   | ..PT..Q.   | ....G....  | ....                  |
| NR3E Alatina alata                 | .....      | .....      | .....       | .....      | .....      | .....                 |
| NR3E Morbakka virulenta            | .....      | .....      | .....MQ..   | ..ST..Q.   | ....G....  | ....                  |
| NR3E Tripedalia cystophora         | ..-MD--.   | .....      | .....LP..   | ..HT..Q.   | ....G....  | ....                  |
| NR3E Copula sivickisi              | .....      | .....      | .....MP..   | ..HT..Q.   | ....G....  | ....                  |
| NR3E Chironex yamaguchi            | ..-MD--.   | .....      | .....MQ..   | ..PT..Q.   | ....G....  | ....                  |
| ERRa Homo sapiens                  | .....      | .....      | .....       | ..-PP.VA.  | ....-....  | ...LAPGP..            |
| ERRb Homo sapiens                  | ..T----HAN | GL--.....  | .....       | ..SPP.MF.  | ....AGAG.. | ...L..GG..            |
| ERRg Homo sapiens                  | ..G----HQN | GL--.....  | .....       | ..SPP.LY.  | ....PSAP.. | ...ILGGS..            |
| ERR Drosophila melanogaster        | ..CDLHNVS  | LNDG.....  | .....-DS    | L.KGSG.TS  | ....GGNG.. | ...GGGGG..            |
| ERR Crassostrea gigas              | ..ADYNNDDY | G.DG.....  | .....SYQNS  | A..NSG.NI. | ....SPCS.. | ...IDSS..             |
| NR3D Crassostrea gigas             | ..GMVPOGS  | FLSM.....  | .....YMMHN  | Q.VPSS.TTL | EFRDHDGR   | ...ILPLLDP            |
| NR3D Aplysia californica           | ..SGTLPH.. | ---.....   | .....NFLMDV | H.GPGT.LG  | .....      | ...ASP                |
| NR3D Platynereis dumerilii         | ..-MADD..  | ..QG.....  | .....YT--   | .....      | .....      | ...PQR..              |
| ER Branchiostoma floridae          | ..NGMMQQ.. | .HHH.....  | .....DILTL  | H.NGGR..   | ....QVG..  | ...QVGD..             |
| ERa Homo sapiens                   | ..FLQPHGQ  | QV--.....  | .....YYL.N  | E.PSGY.T.  | ..VREAGPP  | ...AF.YRPN            |
| ERb Homo sapiens                   | ..LVVH-RQ  | L--.....   | .....HLYA.P | Q.KSPW.C.  | ..EARSLEH  | ...TLPVNRE            |
| SR Branchiostoma floridae          | ..-MADD..  | ..QP.....  | .....QAAM   | .....YARNY | T..QNMEVRT | AVTDPQMGAW NRMQOPVYEE |
| GR Homo sapiens                    | TSL-----   | GTLN.....  | FPG---RTV   | F.SNGY.SS  | .....      | .....                 |
| MR Homo sapiens                    | .....      | .....      | .....       | ..SDGY.PV  | .....      | .....                 |
| AR Homo sapiens                    | APYGY.RPPQ | GLAQESDFT  | APDVWYPGGM  | VSRVPY.PSP | TCVKSEMGPW | MDSYSGPY..            |
| PR Homo sapiens                    | PAL-----   | GL.G.....  | LPQLGYQAAV  | L.KEGL.PQ. | .....      | ...VYPPY..            |

**DBD**

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|                                    |            |              |            |            |             |           |       |
|------------------------------------|------------|--------------|------------|------------|-------------|-----------|-------|
| NR3E Hydra magnipapillata          | -----      | SSSPPSE-KG   | LVQNKRPMTN | ARHAVPMLCL | VCTDKASGYH  | YGVPS     | CEGCK |
| NR3F Trichoplax adhaerens          | .....      | AVIV.....    | ---SVPNLEH | GTFSQNQA   | ..G.R...L.  | ...L..    | ..... |
| NR3E Hydractinia symbiolongicarpus | .....      | ..ER..QP.R-  | -ANLQSRLOS | NK.DQAF.V  | ..G.R...A   | ...A..    | ..... |
| NR3E Aurelia aurita                | .....      | ..K-----     | ---SCPGY   | KDGEM.F.V  | ..G.S...A   | ...A..    | ..... |
| NR3E Rhopilema esculentum          | .....      | ..K-----     | ---SCPGF   | KDGD.I.F.V | ..G.S...A   | ...A..    | ..... |
| NR3E Podocoryna carnea             | .....      | .....        | .....      | SKQDQ.F.V  | ..R...A     | ...A..    | ..... |
| NR3E Nanomia bijuga                | .....      | ..VKETNP.P-  | -KAETP.TSE | .NTQRAF.V  | ..A.L...A   | ...A..    | ..... |
| NR3E Chironex fleckeri             | .....      | ..A-----     | ---CDTQES  | RHGNS.F.V  | ..G.T...A   | ...A..    | ..... |
| NR3E Alatina alata                 | .....      | .....        | .....      | .....      | .....       | .....     | ..... |
| NR3E Morbakka virulenta            | .....      | ..T-----     | ---CDSQNP  | STGNS.F.V  | ..G.T...A   | ...A..    | ..... |
| NR3E Tripedalia cystophora         | .....      | ..T-----     | ---CDSQ.V  | SHGSA.F.V  | ..G.T...A   | ...A..    | ..... |
| NR3E Copula sivickisi              | .....      | ..T-----     | ---CDSQ.V  | SHGSA.F.V  | ..G.T...A   | ...A..    | ..... |
| NR3E Chironex yamaguchi            | .....      | ..A-----     | ---CDTQES  | RHGNS.F.V  | ..G.T...A   | ...A..    | ..... |
| ERRa Homo sapiens                  | APTRCLPGHK | EEEDGEG.A    | PGEQGGKLV  | LSSLPKR.   | ..G.V...A   | ...A..A   | ..... |
| ERRb Homo sapiens                  | TPCRKS...  | YEDCA.G.IM   | EDSAIKCEYM | LNAIPKR.   | ..G.I...A   | ...A..A   | ..... |
| ERRg Homo sapiens                  | GPVRKL...  | YDDCS.T.IV   | EDPQTKCEYM | LNSMPKR.   | ..G.I...A   | ...A..A   | ..... |
| ERR Drosophila melanogaster        | G...TSGG   | -----N.AT    | NASAGAGSGS | V.DELRR.   | ..G.V...F   | ...A..A   | ..... |
| ERR Crassostrea gigas              | SPIDTSKLD  | INNI--A.TS   | ITNSINCDQE | NIELPKR.   | ..G.V...S   | ...S..A   | ..... |
| NR3D Crassostrea gigas             | S.....L    | -----FA      | DGRKCLKHGA | SA.GPNK.Q  | ..S.N...F   | ...W..    | ..... |
| NR3D Aplysia californica           | S.....S    | H-----       | MK.EVGGAVP | GSSIQAK.Q  | ..S.N...F   | ...W..    | ..... |
| NR3D Platynereis dumerilii         | SPEEEWGGQT | .EPS.IDLG.   | HANADQLPSD | GDSKDVQK.Q | I.S.L...F   | ...W..    | ..... |
| ER Branchiostoma floridae          | CPDQFSKGR  | D.PAS.G.SS   | IEN.QPQVKE | LDNKARAV.R | ..G.H...F   | ...W..    | ..... |
| ERa Homo sapiens                   | SDNRRQGGRE | R-----LA     | STND.GS.AM | ESAKETRY.A | ..N.Y...W   | ...W..    | ..... |
| ERb Homo sapiens                   | TLKRKVSNGR | C-----       | ---ASPVTGP | GSKRDAHF.A | ..S.Y...W   | ...W..    | ..... |
| SR Branchiostoma floridae          | SPQKGTGTEE | .APWTANQOV   | KQAAVGTAS  | PGSGHKPP.A | ..HCPST.L   | ...YA..   | ..... |
| GR Homo sapiens                    | .....      | ..P.MR.--DV  | SSPPSSSS.A | TTGPP.K.   | ..S.E...C   | ...LT.GS  | ..... |
| MR Homo sapiens                    | .....      | ..LEYI.E-.NV | SSSTL.SVST | GSSRPSKI.  | ..G.E...C   | ...VT.GS  | ..... |
| AR Homo sapiens                    | .....      | ..GDMRLE-.TA | RDH--VLPID | YFPPQKT.   | ..I.G.E...C | ...ALT.GS | ..... |
| PR Homo sapiens                    | .....      | ..LNYLRP-.DS | -EASQS.QYS | FESLPQKI.  | ..I.G.E...C | ...LT.GS  | ..... |

P-box

Supplemental Figure 2 (continued)

# DBD

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|                                    |       |        |        |       |       |          |         |     |          |        |            |            |
|------------------------------------|-------|--------|--------|-------|-------|----------|---------|-----|----------|--------|------------|------------|
| NR3E Hydra magnipapillata          | AF    | FKRS   | LQLK   | QLNYK | PASN  | N        | TINKMSR | K   | CCQA     | CRLGKC | HAVGMSKECK | KVKKRDIR-- |
| NR3F Trichoplax adhaerens          | ..... | I.S-   | SVA.T. | .SGS  | R     | KVD.QR.. | .....   | Q.  | FD...    | IR.GV  | RKNRNP     | GGGRV      |
| NR3E Hydractinia symbiolongicarpus | ..... | S.D    | .....  | .S    | ..... | DR.....  | .....   | K.  | .D.....  | FL     | GN.IKRP    | KQE        |
| NR3E Aurelia aurita                | ..... | AT     | D...R. | G..S  | ..... | K.D..... | .....   | R.  | NE.....  | FL     | GN..IKK    | PSR        |
| NR3E Rhopilema esculentum          | ..... | AT     | D...R. | G..S  | ..... | K.D..... | .....   | R.  | NE.....  | VI     | D-----     |            |
| NR3E Podocoryna carnea             | ..... | S.E    | .....  | .S    | ..... | DR.....  | .....   | Q.  | .D.....  | FL     | GN.IKRA    | KQD        |
| NR3E Nanomia bijuga                | ..... | S.EM   | .....  | .G    | ..... | D.....   | .....   | K.  | QD.....  | FL     | GN.IKRP    | KPD        |
| NR3E Chironex fleckeri             | ..... | AA     | D...R. | GN.   | ..... | K.D..... | .....   | R.  | .E.....  | FL     | GN..IKK    | NAK        |
| NR3E Alatina alata                 | ..... | AA     | D...R. | GN.   | ..... | K.D..... | .....   | R.  | .E.....  | FL     | GN..IKK    | STK        |
| NR3E Tripedalia cystophora         | ..... | AA     | D...R. | GN.   | ..... | K.D..... | .....   | R.  | .E.....  | FL     | GN..IKK    | NAK        |
| NR3E Copula sivickisi              | ..... | AA     | D...R. | GN.   | ..... | K.D..... | .....   | R.  | .E.....  | FL     | GN..IKK    | NAK        |
| NR3E Chironex yamaguchi            | ..... | AA     | D...R. | GN.   | ..... | K.D..... | .....   | R.  | .E.....  | FL     | GN..IKK    | NAK        |
| ERRa Homo sapiens                  | ..... | TI.G-  | SIE.S  | ..... | E     | E.T.RR.  | A       | FT. | LR...L.  | GV     | RLDRV      | GGRO       |
| ERRb Homo sapiens                  | ..... | TI.G-  | NIE.S  | ..... | E     | E.T.RR.  | S       | FM. | LK...L.  | GV     | RLDRV      | GGRO       |
| ERRg Homo sapiens                  | ..... | TI.G-  | NIE.S  | ..... | E     | E.T.RR.  | S       | FM. | LK...L.  | GV     | RLDRV      | GGRO       |
| ERR Drosophila melanogaster        | ..... | TI.G-  | NIE.T  | ..... | E     | E..RR.   | A       | FQ. | LLM..L.  | GV     | RLDRV      | GGRO       |
| ERR Crassostrea gigas              | ..... | TI.G-  | NIE.S  | ..... | E     | E.T.RR.  | A       | FQ. | LR...L.  | GV     | RLDRV      | GGRO       |
| NR3D Crassostrea gigas             | ..... | I.G-   | PVD.V  | ..... | T     | D.HR.    | S       | R.  | YE...N.  | GSQ    | RKERK      | GSISM      |
| NR3D Aplysia californica           | ..... | I.G-   | PVD.I  | ..... | T     | D.HR.    | S       | RR. | YE...N.  | GSQ    | RKE..NS    | GNT        |
| NR3D Platynereis dumerilii         | ..... | D-     | PVD.V  | ..... | T     | D.HR.    | S       | FR. | LE...M.  | RRE    | RRTTK      | VKSP       |
| ER Branchiostoma floridae          | ..... | I.QG   | TD.I   | ..... | GT    | DRNR.    | S       | YR. | LM...T.  | DGR    | RSGE       | RGPRR      |
| ERa Homo sapiens                   | ..... | I.G-   | HND.M  | ..... | T     | D.NR.    | S       | R.  | YE...M.  | GGI    | RKDR.      | GG.ML      |
| ERb Homo sapiens                   | ..... | I.G-   | HND.I  | ..... | T     | D.NR.    | S       | R.  | YE...V.  | CGS    | RRERC      | GY.LV      |
| SR Branchiostoma floridae          | S.    | H.AHKR | AHP.V  | ..... | N     | V.DRRLK. | N       | P.  | L.M...F. | EH     | A.PN.      | ATKKK      |
| GR Homo sapiens                    | V.    | AVEG-  | H..L   | AGR.  | D     | I.D.IR.  | N       | P.  | LQA..NL. | AR     | .T.--      | KIKG       |
| MR Homo sapiens                    | V.    | AVEG-  | H..L   | AGR.  | D     | I.D.IR.  | N       | P.  | LQA..NL. | GAR    | .S..L      | GKLG       |
| AR Homo sapiens                    | V.    | AAEG-  | KQK.L  | ASR.  | D     | ..D.FR.  | N       | PS  | YEA..TL. | GAR    | .L..L      | GNLKL      |
| PR Homo sapiens                    | V.    | AMEG-  | H..L   | AGR.  | D     | I.VD.IR. | N       | P.  | CQA..VL. | GGR    | .F..F      | FNKVRV     |

P-box

D-box

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|                                    |            |          |           |           |             |            |            |            |       |       |       |       |
|------------------------------------|------------|----------|-----------|-----------|-------------|------------|------------|------------|-------|-------|-------|-------|
| NR3E Hydra magnipapillata          | -----D-    | ----     | TKSKNY    | PRLNPDECI | ESVLT       | -----      | -----      | -----      | ----- | ----- | ----- | ----- |
| NR3F Trichoplax adhaerens          | K...       | YEKPT    | S....     | --VGQC    | KSVTS..SF.  | S.STASSFSQ | ADTDK.AEDN | SIQS..L    | KN    |       |       |       |
| NR3E Hydractinia symbiolongicarpus | K...       | QKQP     | .....     | R---RK    | SDKSS.EGFV  | .STN.....  |            |            |       |       |       |       |
| NR3E Aurelia aurita                | K...       | SK..     | .....     | ..KQ      | KFDDDE.RK.  | VEPTS..... |            |            |       |       |       |       |
| NR3E Rhopilema esculentum          | .....      |          | .....     |           |             |            |            |            |       |       |       |       |
| NR3E Podocoryna carnea             | K...       | QKQP     | .....     | R---RK    | SDKSSSENFV  | .STN.....  |            |            |       |       |       |       |
| NR3E Nanomia bijuga                | K...       | DAAK.    | .....     | QR..KT    | E..ISES.NVV | .STN.....  |            |            |       |       |       |       |
| NR3E Chironex fleckeri             | G...       | DGGKT    | HKS.S     | KLKQ      | MSNDED.LKC  | VEPTS..... |            |            |       |       |       |       |
| NR3E Alatina alata                 | .....      |          | .....     | -----     | MANDDD.LKC  | VEPTS..... |            |            |       |       |       |       |
| NR3E Morbakka virulenta            | S...       | DSGKN    | HKA.S     | KLKQ      | MAHDDD.LKC  | VEPTS..... |            |            |       |       |       |       |
| NR3E Tripedalia cystophora         | G...       | DSGKN    | HKA.S     | KLKQ      | MSADDD.LKC  | VEPTS..... |            |            |       |       |       |       |
| NR3E Copula sivickisi              | G...       | DSAKN    | HKA.S     | KLKQ      | MSADDD.LKC  | VEPTS..... |            |            |       |       |       |       |
| NR3E Chironex yamaguchi            | G...       | DGGKT    | HKS.S     | KLKQ      | MSNDED.LKC  | VEPTS..... |            |            |       |       |       |       |
| ERRa Homo sapiens                  | K...       | YKRRP    | EVD       | -----     | -P.PF.GFPF  | AGP.AVA..G | GPRKTAAPVN | ALVSHLLV   | V.    |       |       |       |
| ERRb Homo sapiens                  | K...       | YKRRL    | DSE       | -----     | -SSPYLSLQ   | SPP--..... | AKKPLT     | KIVSYLLVA  |       |       |       |       |
| ERRg Homo sapiens                  | K...       | YKRRR    | DAE       | -----     | -NSPYLNPQL  | VQP--..... | AKKPYN     | KIVSYLLVA  |       |       |       |       |
| ERR Drosophila melanogaster        | K...       | YRRNP    | VSN       | -----     | -SYQTMQLLY  | Q.N--..... | TTSLCDV    | KILEVLNSY  |       |       |       |       |
| ERR Crassostrea gigas              | K...       | YKRTV    | DSG       | -----     | -PIVQQIFPM  | IKK-ACV..E | STKSDFSSDN | KILTQLISIE |       |       |       |       |
| NR3D Crassostrea gigas             | SKPAATKRSR | ADSSDNTV | -----     |           |             |            |            |            |       |       |       |       |
| NR3D Aplysia californica           | S.SLKGKRCR | ADSSDSAV | -----     |           |             |            |            |            |       |       |       |       |
| NR3D Platynereis dumerilii         | G...       | S..PE    | EKITK.VRT | -----     | -PNTK.NPKE  | -----      | KGPPVENN   | GQSRRLVQLE |       |       |       |       |
| ER Branchiostoma floridae          | K...       | RTHNQ    | IDVSTADSC | -----     | KSSVS.LP--  | --SSASAFDK | SRSASPTENN | SF.....    |       |       |       |       |
| ERa Homo sapiens                   | K...       | HKRQR    | DDGEGRG-E | -----     |             | -VGSA..... | GD.....    |            |       |       |       |       |
| ERb Homo sapiens                   | R...       | RQRSA    | DEQLHCAGK | -----     |             | -AKRS..... | GG.....    |            |       |       |       |       |
| SR Branchiostoma floridae          | P..KKT     | KRSP     | KRA.....  | -----     | -KTSH..PPA  | E---.....  | TPPEQQ     | LIVSPTLP.. |       |       |       |       |
| GR Homo sapiens                    | I.....     | QQA      | TTG       | -----     | -VS-        | QET-----   | SENP       |            |       |       |       |       |
| MR Homo sapiens                    | I.....     | HEE      | QPQ       | -----     | -QQ-        | QPPPP.PPPQ | SPEE       |            |       |       |       |       |
| AR Homo sapiens                    | Q.....     | EEG      | EAS       | -----     | -ST-        | TSP-----   | TEET       |            |       |       |       |       |
| PR Homo sapiens                    | V.....     | RAL      | DAV       | -----     | -A-         | LPQPVGVNE  | SQA        |            |       |       |       |       |

Supplemental Figure 2 (continued)

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|                                    |            |            |            |            |                        |
|------------------------------------|------------|------------|------------|------------|------------------------|
| NR3E Hydra magnipapillata          | -----      | -----      | -----      | -----      | -----                  |
| NR3F Trichoplax adhaerens          | EDK.....   | ..YIDVNCT. | .....      | .....RL    | PSTPPSPDKGY DIKILKEFIG |
| NR3E Hydractinia symbiolongicarpus | .....      | .....      | .....      | .....      | .....                  |
| NR3E Aurelia aurita                | .....      | .....      | .....      | .....      | .....                  |
| NR3E Rhopilema esculentum          | .....      | .....      | .....      | .....      | .....                  |
| NR3E Podocoryna carnea             | .....      | .....      | .....      | .....      | .....                  |
| NR3E Nanomia bijuga                | .....      | .....      | .....      | .....      | .....                  |
| NR3E Chironex fleckeri             | .....      | .....      | .....      | .....      | .....                  |
| NR3E Alatina alata                 | .....      | .....      | .....      | .....      | .....                  |
| NR3E Morbakka virulenta            | .....      | .....      | .....      | .....      | .....                  |
| NR3E Tripedalia cystophora         | .....      | .....      | .....      | .....      | .....                  |
| NR3E Copula sivickisi              | .....      | .....      | .....      | .....      | .....                  |
| NR3E Chironex yamaguchi            | .....      | .....      | .....      | .....      | .....                  |
| ERRa Homo sapiens                  | .....      | .....      | .....      | .....EPEKL | YAMPDPAG..             |
| ERRb Homo sapiens                  | .....      | .....      | .....      | .....EPDKL | YAMPPPGM..             |
| ERRg Homo sapiens                  | .....      | .....      | .....      | .....EPEKI | YAMPDPTV..             |
| ERR Drosophila melanogaster        | .....      | .....      | .....      | .....EPDAL | SVQTPPPQVH TTSITNDEAS  |
| ERR Crassostrea gigas              | Q.....     | .....      | .....      | .....QLDKL | YVNTESEI..             |
| NR3D Crassostrea gigas             | .....      | .....      | .....      | .....T     | SGSPNPAKSA RK.....     |
| NR3D Aplysia californica           | .....      | .....      | .....      | .....T     | NINGASSKSS KR.....     |
| NR3D Platynereis dumerilii         | ETVAVESVKT | EAVSPQECSP | SGHTSQEDLS | VPMTSASQSF | NLTPPPSTSS ST....SSPS  |
| ER Branchiostoma floridae          | .....      | .....      | .....      | .....      | .....DS                |
| ERa Homo sapiens                   | .....      | .....      | .....      | .....MRAA  | NLWPSPLMIK RS.....     |
| ERb Homo sapiens                   | .....      | .....      | .....      | .....H..   | ..AP.....R.....        |
| SR Branchiostoma floridae          | .....      | .....      | .....      | .....L     | YN.....                |
| GR Homo sapiens                    | .....      | .....      | .....      | .....GN.K  | TIVPAT.....            |
| MR Homo sapiens                    | .....      | .....      | .....      | .....GT.T  | YIAPAKEPSV NT.....     |
| AR Homo sapiens                    | .....      | .....      | .....      | .....TQKL  | TVS.....               |
| PR Homo sapiens                    | .....      | .....      | .....      | .....SQRF  | TFSPG.....             |

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H1

H3

|                                    |            |                |            |               |               |            |
|------------------------------------|------------|----------------|------------|---------------|---------------|------------|
| NR3E Hydra magnipapillata          | -----      | ---PIEIEHEN    | IIEQIRLTHS | KL--YL----    | ---GLDE--     | -TV--NGTL  |
| NR3F Trichoplax adhaerens          | SGEKYVIDQK | RDGKQ.IVND     | V.KK.LEAEP | PM..LP....    | ...ACP.P..    | EAEDSGIRT  |
| NR3E Hydractinia symbiolongicarpus | .....      | ..AC...I..     | A.QS.TSL.A | E.....        | ...KPED..     | CTIE.TR..  |
| NR3E Aurelia aurita                | .....      | ..TQDA.L.K     | L.RT.TSL.D | QE..KPS..     | ...LLPNV..    | ..EED.LKPA |
| NR3E Rhopilema esculentum          | .....      | .....          | .....      | .....         | .....         | ---        |
| NR3E Podocoryna carnea             | .....      | ..AC..M.I..    | A.QS.TAL.. | EF.....       | ...KPED..     | SSVR.TR..  |
| NR3E Nanomia bijuga                | .....      | ..AC..M.L.S    | M.QN.TTL.N | EM..C....     | ...KS.D..     | RMEN.AE..  |
| NR3E Chironex fleckeri             | .....      | ..TQDA.L.S     | VMKA.VSLEN | ET..KEL..     | ...RVSQP..    | DGVD.-VIT  |
| NR3E Alatina alata                 | .....      | ..TQDA.L.S     | VVKA.ITLEN | ET..KEL..     | ...RVSQS..    | EGAE.-VMT  |
| NR3E Morbakka virulenta            | .....      | ..TQDA.L.S     | VVKA.VTLEN | ET..KGL..     | ...RVCQS..    | .D.TE.-TMT |
| NR3E Tripedalia cystophora         | .....      | ..TQDA.L.S     | VVKA.VTLET | ET..KEL..     | ...RVSQP..    | DGSE.-VMT  |
| NR3E Copula sivickisi              | .....      | ..TQDA.L.S     | VVKA.VTLET | ET..KEL..     | ...RVSQP..    | DGSD.-VMT  |
| NR3E Chironex yamaguchi            | .....      | ..TQDA.L.S     | VMKA.VSLEN | ET..KEL..     | ...RVSQP..    | DGVD.-VIT  |
| ERRa Homo sapiens                  | .....      | .....          | .....      | .....         | .....         | PD..GHLPA  |
| ERRb Homo sapiens                  | .....      | .....          | .....      | .....         | .....         | PE..GDIKA  |
| ERRg Homo sapiens                  | .....      | .....          | .....      | .....         | .....         | PD..SDIKA  |
| ERR Drosophila melanogaster        | SS.....    | ..SGS.KL.S     | SV--VT--   | ---           | ...PNGTCIFQ.. | NNNNNDPNEI |
| ERR Crassostrea gigas              | .....      | .....          | .....      | .....         | .....         | LEGGEVRF   |
| NR3D Crassostrea gigas             | .....      | ..S..          | ---QTVT    | .LQA.NKAAL    | PVLESHH..     | ...--NH..  |
| NR3D Aplysia californica           | .....      | ..S..          | ---RSAS    | .L.A.QKADL    | PVMDSYH..     | ...--NH..  |
| NR3D Platynereis dumerilii         | NGTSSLQ..  | IDASTFKIDP     | GL-P.DQP.P | LVVILEENDL    | PPKICKEPLT    | AESEETEYS. |
| ER Branchiostoma floridae          | DGDSSGTREL | RTASHQRLKA     | L.DA.DVKEG | EH..RGEENH    | PTGQQAGN..    | WQEIS.PE.  |
| ERa Homo sapiens                   | .....      | ..KKN.SLA      | SLTADQ     | MVSA.LDAEP    | PILYSEY...    | ...--P..   |
| ERb Homo sapiens                   | .....      | ..VRE.LLLDALSP | Q.LVLT     | LEAEP.PHVLI-S | .....         | ...--RP..  |
| SR Branchiostoma floridae          | .....      | ..PTVP         | L.SH.VNIEP | NPILTGY..     | .....         | ...--NP..  |
| GR Homo sapiens                    | .....      | ..PQLTPT       | LVSL.EVIEP | EVLYAGY..     | .....         | ...--S..   |
| MR Homo sapiens                    | .....      | ..ALVPQL       | STISRALTPS | PVMV.ENIEP    | EIVYAGY..     | ...--S..   |
| AR Homo sapiens                    | .....      | ..HIEGYEQPI    | FLNV.EAIEP | GVVCAGH..     | .....         | ...--N..   |
| PR Homo sapiens                    | .....      | ..QTIQLIPP     | L.NL.MSIEP | DVIYAGH..     | .....         | ...--N..   |

Supplemental Figure 2 (continued)

|                                    | 1081        | H3          | H4-5   |
|------------------------------------|-------------|-------------|--|
| NR3E Hydra magnipapillata          | THKFGYGTGK  | QLIHIIDWAK  | RIPGYSLLIL SDQAVLLOAT WVELMVANWL FLSITSTN-S  |
| NR3F Trichoplax adhaerens          | ITIIICEMVER | E.VMV.....  | .....T.C.N.V.....S.L.VFMIDLA.R.MPYD..K       |
| NR3E Hydractinia symbiolongicarpus | .Q..SW.VDQ  | .V.....     | QL...CN.VI...I...A..D.LAL..V.YH.LYVEE.A      |
| NR3E Aurelia aurita                | .SSLCDIV..  | ...AA.....  | GL...ET.T.N...I...G.I.MLLL..I.Y.LPH...K      |
| NR3E Rhopilema esculentum          | --LTLDIV..  | .VAA.....   | GL...ET.T.N...I...G.I.MLLL..T.Y.LSHK..K      |
| NR3E Podocoryna carnea             | VQN.CW.VDQ  | .V.....     | QL...CN.VI...I...A..D.L.L..V.YH.LCIDD.A      |
| NR3E Nanomia bijuga                | IQ..CF.VDQ  | .VR.....    | QL...CN.SI...I...A..D.L.L..V.YH.LHVSE.K      |
| NR3E Chironex fleckeri             | .GYLCKQ.S.  | .VAV.....   | GL...DT.T.N...I...G.I...LLL..V...ERI..K      |
| NR3E Alatina alata                 | .GYLCKQ...  | .VAV.....   | GL...DT.T.N...I...G.I...LLL..V...ERI..K      |
| NR3E Morbakka virulenta            | .GHLCKQ..R  | .VAV.....   | GL...DT.T.N...I...G.I...LLL..V...ERI..K      |
| NR3E Tripedalia cystophora         | .GYLCKQ..R  | .VAV.....   | GL...D.T.N...I...G.I...LLL..V...ERF..K       |
| NR3E Copula sivickisi              | .GYLCKQ..R  | .VAV.....   | GL...DT.T.N...I...G.I...LLL..V...ERI..K      |
| NR3E Chironex yamaguchi            | .GYLCKQ.S.  | .VAV.....   | GL...DT.T.N...I...G.I...LLL..V...ERI..K      |
| ERRa Homo sapiens                  | VATLCDLDFDR | EIVVT.S...  | S...F...S...MSV.SV.M.VL.LGVA.QR.LPLQD.E      |
| ERRb Homo sapiens                  | LTTLCDLADR  | E.VV..G...  | H...F...S.G..MS...SA.M.IILIGIV.YR.LPYDD.K    |
| ERRg Homo sapiens                  | LTTLCDLADR  | E.VV..G...  | H...F.T.S.A..MS...SA.M.IILIGVV.YR.LSFED.E    |
| ERR Drosophila melanogaster        | LSVLSDIYD.  | E.VSV.G...  | Q...FID.P.N..MK...VS.A.ILTQLT.R.LPFNG.K      |
| ERR Crassostrea gigas              | LATVSDLADR  | E.VIT.S...  | QV..FCT.S...MN...HS.L.ILCL.LV.R.CPYNG.Y      |
| NR3D Crassostrea gigas             | LNSLVKLAER  | E.V.L.N...  | NV...TD.S...VH.IECC.M...LLL.CA.R.EHGG.K      |
| NR3D Aplysia californica           | LNTLIKLADR  | E.VYL.N...  | HV...TC.T.G..VH.IECC.M...LLL.CA.R.MEHG.R     |
| NR3D Platynereis dumerilii         | L.RLI.LADM  | E.VDVVN...  | VL..F.G.E.R.RIAI.ESC.M..LCIGAA.WR.RLN.T.F    |
| ER Branchiostoma floridae          | IESVSSLVDR  | E.TG..C.G.  | K....K.S.N..VL.MES..LD.LILDIV.WC..RHKG.E     |
| ERa Homo sapiens                   | MGLLTNLADR  | E.V.M.N...  | .V..FVD.T.H..VH..ECA.L.ILMIGLV.WR.MEHPG.K    |
| ERb Homo sapiens                   | MMSLTKLADR  | E.V.M.S...  | K...FVE.S.F..VR.ESC.M.VLMMGLM.WR..DHFG.K     |
| SR Branchiostoma floridae          | MALVTDLANR  | E.IEGLV...A | .L...GM.PM.D.VN.IRTV.LD.LMLGLV.WR.MEHRG.E    |
| GR Homo sapiens                    | MTTLNMLG.R  | .V.AAVK...  | A...FRN.H.D.MT...YS.MF..AFALG.WR.YRQSSAN     |
| MR Homo sapiens                    | LSTLNRLA..  | .M.QVVK...  | VL..FKN.P.E..IT.I.YS.MC.SSFALS.WR.YKH..SQ    |
| AR Homo sapiens                    | LSLNLGER    | .V.VVK...   | AL..FRN.HV.D..MAVI.YS.MG..FAMG.WR.F.NV.SR    |
| PR Homo sapiens                    | LTSLNQLGER  | .LSVVK.S.   | SL...FRN.HI.D..IT.I.YS..MS...FGLG.WR.YKHVSGQ |

|                                    | 1141       | H6         | H7          | H8                               |
|------------------------------------|------------|------------|-------------|----------------------------------|
| NR3E Hydra magnipapillata          | LK-LSHTFDI | SK-----    | QDST-ELGF   | -GLIYDQFLA LVHRTKGYKL DEEISCFA   |
| NR3F Trichoplax adhaerens          | .V.YACDMVM | GH.....    | .KQ.R.AA.L  | .DE.NRHAFE..TKYRSISM.KO.FA.L.R   |
| NR3E Hydractinia symbiolongicarpus | I..F.KF.S  | TY.....    | NEAR...C    | .EN..C.IFS.AN.A.K.NM...A.L..     |
| NR3E Aurelia aurita                | IQ.F.SNLIV | CE.....    | ALGS..F.L   | .ETVHH.LSS.IT.VQ..CI...FV.L..    |
| NR3E Rhopilema esculentum          | TQ.F.ANLIV | CE.....    | .TLA..AF.L  | .ETVHT.LSS.IA.VQ..GI..V.FL.L..   |
| NR3E Podocoryna carnea             | I..F.RY.S  | TY.....    | DEAM....    | .EN..C.V.S.AN.A.K.NM...L..       |
| NR3E Nanomia bijuga                | IR...MV.SL | .F.....    | .EAK.NI..   | .EE..SYLIS..N.A.K.NI.V...I..     |
| NR3E Chironex fleckeri             | VA.F.ADVVV | DD.....    | .RM.H.T.L   | .DSV.S.L.P..T.VRS.G...FV.L..     |
| NR3E Alatina alata                 | VA.F.SDVVV | DD.....    | .RT.R.T.L   | .DSV.S.L.P..T.VRS.G...FV.L..     |
| NR3E Morbakka virulenta            | VA.F.SDVVV | DD.....    | .MT.R.T.L   | .DSV.S.L.P..T.V.S.E...FV.L..     |
| NR3E Tripedalia cystophora         | VA.F.SDVVV | DD.....    | .RS.R.T.L   | .DSV.S.L.P..T.VRN.E...FV.L..     |
| NR3E Copula sivickisi              | VA.F.SDVVV | DD.....    | .RS.R.T.L   | .DSV.S.L.P..T.VRN.E...FV.L..     |
| NR3E Chironex yamaguchi            | VA.F.ADVVV | DD.....    | .RM.H.T.L   | .DSV.S.L.P..T.VRS.G...FV.L..     |
| ERRa Homo sapiens                  | .A.FAEDLVL | DE.....    | .EGAR.AA.L  | .ELGAAL.Q..R.LQALR.ER.YVLL..     |
| ERRb Homo sapiens                  | .V.YAEDYIM | DE.....    | .EH.R.LA.L  | .LEL.RAI.Q..R.Y.KL.V.EK..FVTL..  |
| ERRg Homo sapiens                  | .V.YADDYIM | DE.....    | .DQ.K.LA.L  | .LDLNNAI.Q..KKY.SM..EK..FVTL..   |
| ERR Drosophila melanogaster        | .C.FATDVWM | DE.....    | .HLAK..C.Y  | .TEF.YHCVO.IAQ.MERISP.RR..YVLL.. |
| ERR Crassostrea gigas              | ..FAEDLQL  | .V.....    | .DECK.LCHC  | .SPELDGISRK.AKKFTDMEV.TK..YLLL.. |
| NR3D Crassostrea gigas             | SLAFAPDLVL | DR.....    | .SSWS.TVEM  | .TE.FE.VA.VSEQMMONH.HKO.LLLLQ.   |
| NR3D Aplysia californica           | TLVFAPD.HL | ER.....    | .QWA.LT.M   | .DVLE.VS.VSEQMLLHG.NK..LLLQ.     |
| NR3D Platynereis dumerilii         | QVNFAENLHF | NE.....    | .ETAK.KAKM  | .SS.VGEIWO.ISQOFRYLE.SNH.FMLLRV  |
| ER Branchiostoma floridae          | KLL..GGVLV | NRNTISNRRN | NSSGD.DMEV  | .LEMC..I.S.IATKIFYEFD.QRR.YL.L.. |
| ERa Homo sapiens                   | -LLFAPNLLL | DR.....    | .NQCKCVE.M  | .VE.F.ML..TSS.FRMN.QG..FV.L.S    |
| ERb Homo sapiens                   | -LIFAPDLVL | DR.....    | .DEGKCV.EI  | .LE.F.ML..TTS.FREL..QHK.VL.V..   |
| SR Branchiostoma floridae          | WLVFAPDLLM | DR.....    | .SLCR.LS.M  | .EY.CTPM.E.FARQFADLQV.PQ.VYV.L.. |
| GR Homo sapiens                    | .LCFAPDLI. | NE.....    | .RM..LPCM   | .YDQCKHM.Y.VSSELHRLQV.SY..YL.M.T |
| MR Homo sapiens                    | FLYFAPDLVF | NE.....    | .EKM.H.QSAM | .YELCQGMHQ.ISLQFVRLQV.TF..YTIM.V |
| AR Homo sapiens                    | MLYFAPDLVF | NE.....    | .YRM.H.KSRM | .YSQCVRMRH.SQEFGLWLOI.TPO.FL.M.. |
| PR Homo sapiens                    | MLYFAPDLIL | NE.....    | .RMK..SS.   | .YSLCLTMWO.IPOEFVKLQV.SQ..FL.M.V |

Supplemental Figure 2 (continued)



|                                    | H8    |        |            | H9   |      |            | H10-H11    |            |          |            |                |
|------------------------------------|-------|--------|------------|------|------|------------|------------|------------|----------|------------|----------------|
| NR3E Hydra magnipapillata          | IILTN | AETTTQ | LSRP       | ---  | ---  | --AKVHALSN | QFCSSLQYYT | KNRH       | ---      | KE         | NPLKFAKIVL     |
| NR3F Trichoplax adhaerens          | LR    | ---    | ---        | ---  | ---  | ---        | ---        | ---        | ---      | ---        | ---            |
| NR3E Hydractinia symbiolongicarpus | .S.   | .S.SS  | .AG        | .    | .    | .E.ND.IT   | T.S.T.     | NS         | .        | R.         | .Q...L..       |
| NR3E Aurelia aurita                | .G.L. | .DSRG  | .TGA       | .    | .    | .S.QD.V    | K.SLA.N.HI | SSQ        | .        | PD         | Q.Q...I.       |
| NR3E Rhopilema esculentum          | .G.L. | .DSRG  | .TGS       | .    | .    | .S.QE.VK   | K.S.A.N.HI | SSH        | .        | PD         | Q.Q...I.       |
| NR3E Podocoryna carnea             | .G.   | .S.SS  | .V         | .    | .    | .DN.NE.IT  | T.S.T.     | SS         | .        | RD         | .Q...L..       |
| NR3E Nanomia bijuga                | .N.   | .S.S   | .TTS       | .    | .    | .D.NE.IQ   | K.T.A...S  | T.S        | .        | R.         | S.Q...L..      |
| NR3E Chironex fleckeri             | .G.M. | .DSCG  | .GS        | .    | .    | .RG.QE.VR  | KYSMA.A.HI | SSY        | .        | PD         | K.Q...S.       |
| NR3E Alatina alata                 | .G.M. | .DSCG  | .GS        | .    | .    | .RG.QE.VR  | KYSMA.M.HI | STY        | .        | PD         | K.Q...S.       |
| NR3E Morbakka virulenta            | .G.M. | .DSCG  | .GS        | .    | .    | .RG.QE.VR  | KYSMA.T.HI | STY        | .        | PD         | K.Q...S.       |
| NR3E Tripedalia cystophora         | .G.M. | .DSCG  | .GS        | .    | .    | .RG.QE.VR  | KYSMA.T.HI | NTY        | .        | PD         | K.Q...S.       |
| NR3E Copula sivickisi              | .G.M. | .DSCG  | .GS        | .    | .    | .RG.QE.VR  | KYSMA.T.HI | NTY        | .        | PD         | K.Q...S.       |
| NR3E Chironex yamaguchi            | .G.M. | .DSCG  | .GS        | .    | .    | .RG.QE.VR  | KYSMA.A.HI | SSY        | .        | PD         | K.Q...S.       |
| ERRa Homo sapiens                  | LA.A  | .SDSVH | IEDA       | .    | .    | .EA.EQ.RE  | ALHEA.LE.E | AG         | AGPGG    | GA         | ERRRAGRLL.     |
| ERRb Homo sapiens                  | LA.A  | .SDSMY | IEDL       | .    | .    | .EA.QK.QD  | LLHEA..D.E | LSQRH      | .        | .          | .E.WRTG.LL.    |
| ERRg Homo sapiens                  | .A.A  | .ASDMH | IEDV       | .    | .    | .EA.QK.QD  | VLHEA..D.E | AGQ.M      | .        | .          | .D.RRAG.MLM    |
| ERR Drosophila melanogaster        | LL.A  | .CD-IL | .DDQ       | .    | .    | .SSLR.FRD  | TILN..NDVV | YLLRH      | .        | SS         | AVSHQQQLL.     |
| ERR Crassostrea gigas              | MT.C  | .ID-VA | .IENS      | .    | .    | .EA.RQ.QD  | KLQD..IE.V | .Y.YV      | .        | -G         | .LRRLGHLYM     |
| NR3D Crassostrea gigas             | MV.V  | .VRR   | .ASY       | .    | .    | .NQIFNMQQ  | SLLDAIVDTA | QK         | --Y..    | HPD        | .VRHVPAVL.     |
| NR3D Aplysia californica           | TV.V  | .VRP   | .DSF       | .    | .    | .L.IQEMRQ  | LILDVFMEVA | GRH-Q      | .GFG     | .          | .WRHAPS.L.     |
| NR3D Platynereis dumerilii         | VTML  | .SIR   | .CL        | .    | .    | .DAM.KIRQ  | .YLEA.HFEC | GRFLGKIS   | .        | ES         | SCVRM.Q.LC     |
| ER Branchiostoma floridae          | .T.VH | .GSLKG | .ESD       | .    | .    | .TQ.RQ.QD  | DLTDA.MDVC | SE         | .        | AL         | GSRRP..ML.     |
| ERa Homo sapiens                   | .L    | .SGVYT | FLSSTLKSLE | .    | .    | EKDHI.RVLD | KITDT.IHLM | AKA-GLTI   | QQ       | QHQR.LQLL. |                |
| ERb Homo sapiens                   | M.L   | .SSMYP | .VTATQDA   | .    | .    | SSR.LAH.L  | AVTDA.VWVI | AKS-GISS   | QQ       | QSMRL.NLLM |                |
| SR Branchiostoma floridae          | LT.YT | .TAVSR | .QDY       | .    | .    | .RQ.QR.QH  | EINEA.AEAC | SSTFG      | ..F      | S.GNI.RLMM |                |
| GR Homo sapiens                    | LL    | .LSSV  | --P        | ---- | ---- | .KDGGLK    | SQELFDEIRM | TYIKE.GKAI | VK       | .E.GNS     | GO.WQR.YQLTK   |
| MR Homo sapiens                    | LL    | .LSTI  | --P        | ---- | ---- | .KDGGLK    | SQ.AFEEMRT | NYIKE.RKMV | TKCP.NNS | GO         | SWQR.YQLTK     |
| AR Homo sapiens                    | LL    | .FSII  | --P        | ---- | ---- | .VDGLK     | NQKFFDE.RM | NYIKE.DRTI | ACKR.KNH | TS         | CSR.R.YQLTK    |
| PR Homo sapiens                    | LL    | .L     | .TI--P     | ---- | ---- | .LEGLR     | SQTQFEEMRS | SYIRE.IKAI | GL       | .Q.KGV     | VVS.SSOR.YQLTK |

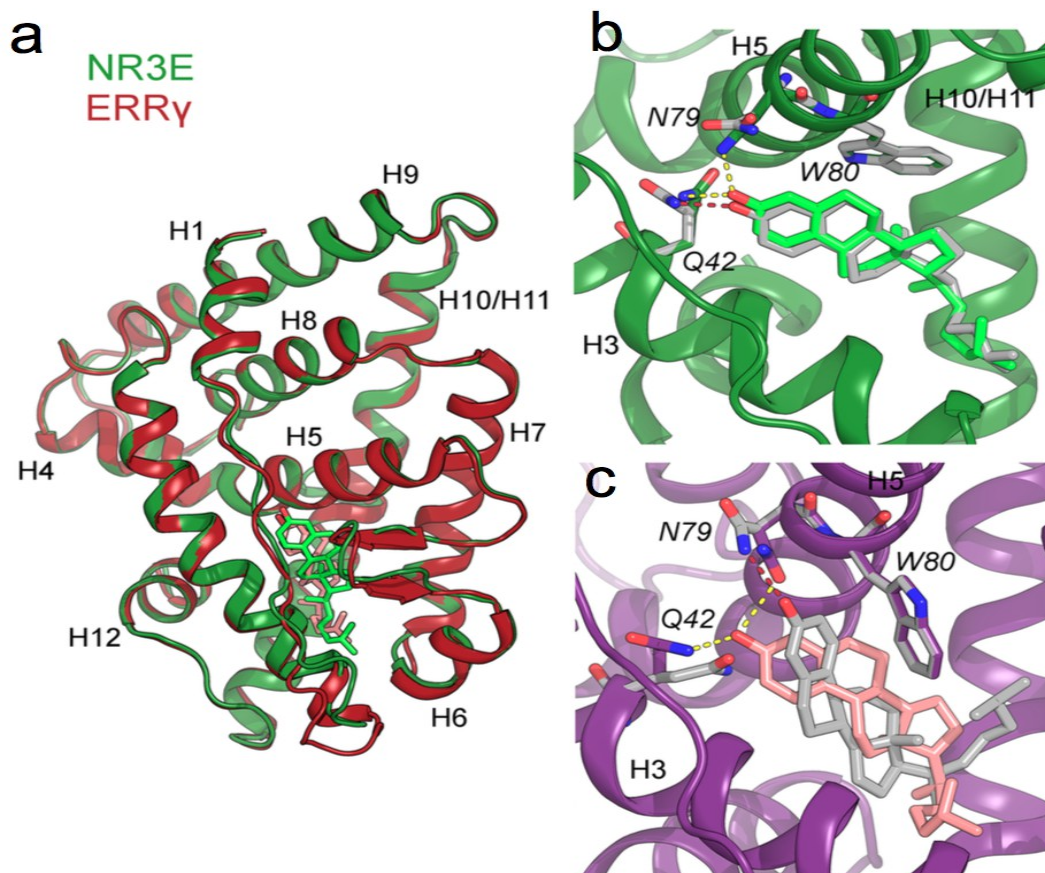
|                                    | H10-H11  |           |            | H12      |            |            |          |        |             |            |
|------------------------------------|----------|-----------|------------|----------|------------|------------|----------|--------|-------------|------------|
| NR3E Hydra magnipapillata          | ILPQLKYL | EVLRV     | LHALN      | SM-SEK   | GAYL       | SDLV       | VEMLDV   | KNRQ   | ----        | ----       |
| NR3F Trichoplax adhaerens          | .....M.  | Q.IEY     | .YTMK      | MN.NS    | SES.N      | ...I.      | .EA      | .Q.L   | .....       | .....      |
| NR3E Hydractinia symbiolongicarpus | .....V.  | N.IEY     | FYRVK      | IS.--    | SE.Q       | .E.L       | ..EA     | .S.L   | .....       | .....      |
| NR3E Aurelia aurita                | .....CV. | N.IEY     | FYRVK      | IS.--    | GE.Q       | .E.L       | ..EA     | .S.L   | .....       | .....      |
| NR3E Rhopilema esculentum          | .....M.  | Q.IEY     | .YKMK      | MH.ES    | ES.N       | ...I.      | .EA      | .Q.L   | .....       | .....      |
| NR3E Podocoryna carnea             | .....R.  | I.E       | L.TKMK     | MN.HQ    | DKCA.      | ...I.      | .EA      | .Q.L   | .....       | .....      |
| NR3E Nanomia bijuga                | .....V.  | H.IE      | F.YRVK     | IS.--    | GE.Q       | ...L       | ..EA     | .S.L   | .....       | .....      |
| NR3E Chironex fleckeri             | .....V.  | H.IE      | F.YRVK     | IS.--    | GE.Q       | ...L       | ..EA     | .S.L   | .....       | .....      |
| NR3E Alatina alata                 | .....V.  | H.IE      | F.YRVK     | IS.--    | GE.Q       | ...L       | ..EA     | .S.L   | .....       | .....      |
| NR3E Morbakka virulenta            | .....V.  | H.IE      | F.YRVK     | IS.--    | GE.Q       | ...L       | ..EA     | .S.L   | .....       | .....      |
| NR3E Tripedalia cystophora         | .....V.  | H.IE      | F.YRVK     | IS.--    | GE.Q       | ...L       | ..EA     | .S.L   | .....       | .....      |
| NR3E Copula sivickisi              | .....V.  | H.IE      | F.YRVK     | IS.--    | GE.Q       | ...L       | ..EA     | .S.L   | .....       | .....      |
| NR3E Chironex yamaguchi            | .....V.  | H.IE      | F.YRVK     | IS.--    | GE.Q       | ...L       | ..EA     | .S.L   | .....       | .....      |
| ERRa Homo sapiens                  | T.L      | .RQTAG    | K..AHFYGVK | IE.--    | GKVPM      | HK.FL      | ..EA     | MMD    | .....       | .....      |
| ERRb Homo sapiens                  | T.L      | .RQTAA    | KAVQH      | FYSVK    | IQ.--      | GKVPM      | HK.FL    | ..EA   | VG.EQLRGS   | PKDERMSSHD |
| ERRg Homo sapiens                  | T.L      | .RQTST    | KAVQH      | FYNIK    | IE.--      | GKVPM      | HK.FL    | ..EA   | V--         | .....      |
| ERR Drosophila melanogaster        | L.S      | .RQADD    | ILRR       | FWRGIA   | RD.--      | EVITM      | KK.FL    | ..EP   | LA          | .....      |
| ERR Crassostrea gigas              | L.A      | .NHMKL    | LAKQ       | YWFVVK   | KD.--      | GRIMM      | HK.FL    | ..EA   | DS          | .....      |
| NR3D Crassostrea gigas             | L        | .THIRQAGE | RGIA       | FFQR.K   | IE.--      | GVVTF      | C..LK    | ..A    | QDFLEKKS    | SSN        |
| NR3D Aplysia californica           | L        | .THIRQAGE | RGIT       | YFQK.K   | ME.--      | GCVTF      | C..LT    | ..A    | H.SSGERRRL  | QQOQQQPPQQ |
| NR3D Platynereis dumerilii         | ..       | FARQVSL   | KAT        | TH.FNMH  | NQ.--      | HAVPV      | G...A    | ..VA   | QKELMTNEDS  | AKIIT      |
| ER Branchiostoma floridae          | L        | .SH.RQVSA | RASS       | H.G.VR   | NG.--      | LKVP       | Y..ILLDI | .TD    | QVSEGQRDQ   | AGHVEVASSP |
| ERa Homo sapiens                   | ..       | SHIRHMS   | KGME       | H.YSMK   | CK.--      | NVVP       | Y..LL    | ..A    | HRHLHAPT    | SRG        |
| ERb Homo sapiens                   | L        | .SHVRHAS  | KGME       | H.LNMK   | CK.--      | NVVPV      | Y..LL    | ..NA   | HVLRGCRSSI  | TGSECSPAED |
| SR Branchiostoma floridae          | .VS      | .VRQ.SS   | LGVD       | H.NR.R   | GA.--      | ETVSV      | EG.LR    | .IV.E  | PP.ITETIEDS | PGSAEGNKT  |
| GR Homo sapiens                    | L        | .DSMHEV   | E.NL       | .NYCFQTF | LDK-TMS    | IEF        | PEMLA    | .IITN  | QIPK        | .....      |
| MR Homo sapiens                    | L        | .DSMHD    | .S         | DL.E     | FCFYTF     | RESHALKVEF | PAML     | ..IISD | QLPK        | .....      |
| AR Homo sapiens                    | L        | .DSVQPIAR | .LH        | FTFD.L   | IKSHMVSVDF |            | PEMMA    | .IIS   | QVPK        | .....      |
| PR Homo sapiens                    | L        | .DN.HD    | .K         | QLHL     | YCLNFF     | TQSRALSVEF | PEMMS    | .VIAA  | QLPK        | .....      |

## Supplemental Figure 2. Full-length alignment of cnidarian NR3E with already known members of the NR3 family.

The DNA-binding domain (DBD) is highlighted with light grey. Within it, two interaction sites with DNA response elements (P-box and D-box) are boxed, and the nine conserved cysteine residues are highlighted in orange. The twelve helices of the ligand binding domain (LBD) are boxed based on the human ER $\alpha$  sequence. As in Figure 5, residues involved in estrogen binding in ER are highlighted in green. Homologous residues that are shared by the cnidarian NR3E and the vertebrate oxosteroid receptors (SRs) are highlighted in pink. The cnidarian-specific N79-W80 anchor is highlighted in brown.

|   | DBD  | LBD  |
|---|------|------|
| NR3E <i>Hydra magnipapillata</i>          | --   | --   |
| NR3F <i>Trichoplax adhaerens</i>          | 66.7 | 28.8 |
| NR3E <i>Hydractinia symbiolongicarpus</i> | 85.1 | 51.2 |
| NR3E <i>Aurelia aurita</i>                | 79.1 | 40.6 |
| NR3E <i>Rhopilema esculentum</i>          | 79.1 | 41.7 |
| NR3E <i>Podocoryna carnea</i>             | 85.1 | 52.1 |
| NR3E <i>Nanomia bijuga</i>                | 83.6 | 49.3 |
| NR3E <i>Chironex fleckeri</i>             | 79.1 | 42.2 |
| NR3E <i>Alatina alata</i>                 | --   | 42.7 |
| NR3E <i>Morbakka virulenta</i>            | 79.1 | 43.1 |
| NR3E <i>Tripedalia cystophora</i>         | 79.1 | 42.2 |
| NR3E <i>Copula sivickisi</i>              | 79.1 | 42.2 |
| NR3E <i>Chironex yamaguchi</i>            | 79.1 | 42.2 |
| ERRa <i>Homo sapiens</i>                  | 68,2 | 24.4 |
| ERRb <i>Homo sapiens</i>                  | 66.7 | 26.8 |
| ERRg <i>Homo sapiens</i>                  | 66.7 | 26.3 |
| ERR <i>Drosophila melanogaster</i>        | 65.2 | 21.2 |
| ERR <i>Crassostrea gigas</i>              | 65.2 | 22.8 |
| NR3D <i>Crassostrea gigas</i>             | 69.7 | 21.1 |
| NR3D <i>Aplysia californica</i>           | 68,2 | 22.4 |
| NR3D <i>Platynereis dumerilii</i>         | 69.7 | 19.7 |
| ER <i>Branchiostoma floridae</i>          | 65.7 | 23.3 |
| ERa <i>Homo sapiens</i>                   | 71.2 | 22.8 |
| ERb <i>Homo sapiens</i>                   | 71.2 | 23.4 |
| SR <i>Branchiostoma floridae</i>          | 53   | 18.2 |
| GR <i>Homo sapiens</i>                    | 56.1 | 19.4 |
| MR <i>Homo sapiens</i>                    | 57.6 | 17.5 |
| AR <i>Homo sapiens</i>                    | 51.5 | 16.6 |

**Supplemental Table 1. Identity percentages of DBDs and LBDs when compared to NR3E from *Hydra*.**



**Supplemental Figure 3. Paraestrol A docking into the ligand binding pocket of estrogen related receptor  $\gamma$  homology-based model of *Hydra* NR3E.** (a) Superposition of the NR3E structural model using ERR $\gamma$  structure (in dark green) where the paraestrol A is represented in light green, the ERR $\gamma$  structure used as template (PDB id: 3D24) is represented in red and for comparison the paraestrol A position found in the NR3E ER model in pink. (b) Detailed view of the ligand binding pocket of NR3E ERR $\gamma$ -based model, highlighting interactions (in yellow dashed lines) between the paraestrol and residues Q42 and N79. Results of the first round of docking experiments are represented in grey, showing one hydrogen bond (in red dashed line) between paraestrol and residue Q42. (c) Detailed view of the binding pocket of NR3E ER $\alpha$ -based model, highlighting interactions (in yellow dashed lines) between the paraestrol and residues Q42 and N79. Results of the first round of docking experiments are represented in grey, showing one hydrogen bond (in red dashed line) between paraestrol and residue N79.