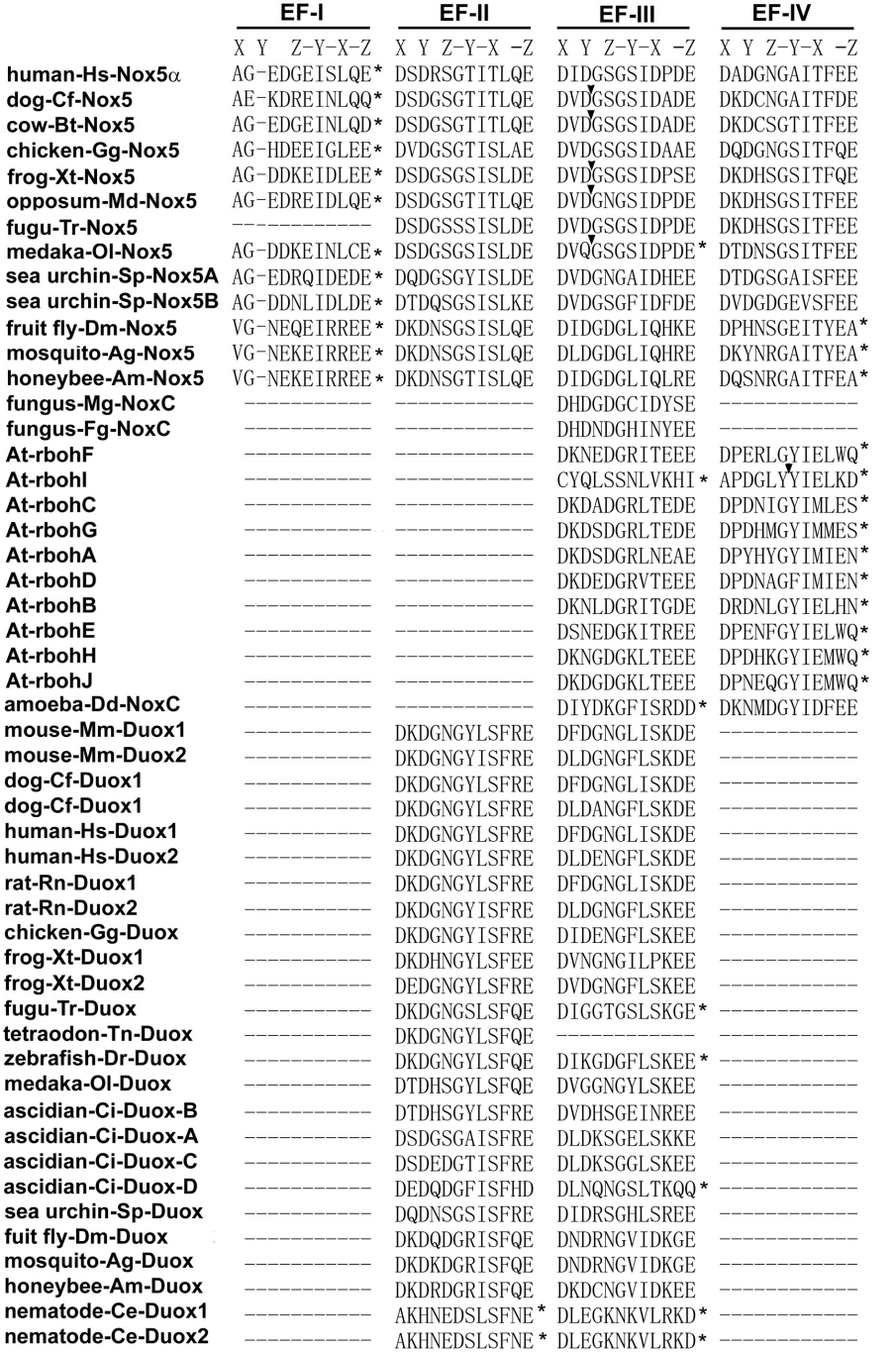
**Additional file 2**

**Supplemental Figure S2. Sequences of EF-hand motifs in Nox5, Duox, plant Nox, and NoxC**

The original alignment data are shown in Additional file 8. EF (I) to EF (IV) represent four EF-hands of Nox5. Each EF-hand domain contains a twelve residue loop that is involved in calcium-binding. The letters X, Y, Z, -Y, -X, and -Z indicate the six residues that directly participate in calcium-binding, and these six residues correspond to positions 1, 3, 5, 7, 9 and 12. The consensus sequences of these six positions are: D at position 1, D/N/S at position 3, D/E/N/S/T/G at position 5, G/P at position 7, D/E/N/Q/S/T/A/G/C at position 9, and D/E at position 12 (PROSITE documentation number PDOC00018), and the most conserved positions are 1, 3, and 12. *Asterisks* indicate atypical EF-hand motif that is not consistent with the consensus sequences at positions 1, 3, or 12. Predicted *T. rubripes* (Tr)-Nox5 sequence was not a complete sequence; therefore the 1$^{st}$ EF-hand motif sequence was not shown. The *arrows* indicate motifs that have 17 to 23 additional amino acids inserted in the indicated position. Abbreviations of species are as in Figure 2.


EF-I
EF-II
EF-III
EF-IV
X Y Z-Y-X-Z
X Y Z-Y-X -Z
X Y Z-Y-X -Z
X Y Z-Y-X -Z
human-Hs-Nox5α AG-EDGEISLQE* DSDRSGTITLQE DIDGSGSIDPDE DADGNGAITFEE
dog-Cf-Nox5 AE-KDREINLQQ* DSDGSGTITLQE DVDGSGSIDADE DKDCNGAITFDE
cow-Bt-Nox5 AG-EDGEINLQD* DSDGSGTITLQE DVDGSGSIDADE DKDCSGTITFEE
chicken-Gg-Nox5 AG-HDEEIGLEE* DVDGSGTISLAE DVDGSGSIDAAE DQDGNGSITFQE
frog-Xt-Nox5 AG-DDKEIDLEE* DSDGSGSISLDE DVDGSGSIDPSE DKDHSGSITFQE
opposum-Md-Nox5 AG-EDREIDLQE* DSDGSGTITLQE DVDGNGSIDPDE DKDHSGSITFEE
fugu-Tr-Nox5 ---------- DSDGSSSISLDE DVDGSGSIDPDE DKDHSGSITFEE
medaka-Ol-Nox5 AG-DDKEINLCE* DSDGSGSISLDE DVQGSGSIDPDE* DTDNSGSITFEE
sea urchin-Sp-Nox5A AG-EDRQIDEDE* DQDGSGYISLDE DVDGNGAIDHEE DTDGSGAISFEE
sea urchin-Sp-Nox5B AG-DDNLIDLDE* DTDQSGSISLKE DVDGSGFIDFDE DVDGDGEVSFEE
fruit fly-Dm-Nox5 VG-NEQEIRREE* DKDNSGSISLQE DIDGDGLIQHKE DPHNSGEITYEA*
mosquito-Ag-Nox5 VG-NEKEIRREE* DKDNSGSISLQE DLDGDGLIQHRE DKYNRGAITYEA*
honeybee-Am-Nox5 VG-NEKEIRREE* DKDNSGTISLQE DIDGDGLIQLRE DQSNRGAITFEA*
fungus-Mg-NoxC ---------- ---------- DHDGDGCIDYSE ----------
fungus-Fg-NoxC ---------- ---------- DHDNDGHINYEE ----------
At-rbohF ---------- ---------- DKNEDGRITEEE DPERLGYIELWQ*
At-rbohI ---------- ---------- CYQLSSNLVKHI* APDGLYYIELKD*
At-rbohC ---------- ---------- DKDADGRLTEDE DPDNIGYIMLES*
At-rbohG ---------- ---------- DKDSDGRLTEDE DPDHMGYIMMES*
At-rbohA ---------- ---------- DKDSDGRLNEAE DPYHYGYIMIEN*
At-rbohD ---------- ---------- DKDEDGRVTEEE DPDNAGFIMIEN*
At-rbohB ---------- ---------- DKNLDGRITGDE DRDNLGYIELHN*
At-rbohE ---------- ---------- DSNEDGKITREE DPENFGYIELWQ*
At-rbohH ---------- ---------- DKNGDGKLTEEE DPDHKGYIEMWQ*
At-rbohJ ---------- ---------- DKDGDGKLTEEE DPNEQGYIEMWQ*
amoeba-Dd-NoxC ---------- ---------- DIYDKGFISRDD* DKNMDGYIDFEE
mouse-Mm-Duox1 ---------- DKDGNGYLSFRE DFDGNGLISKDE ----------
mouse-Mm-Duox2 ---------- DKDGNGYISFRE DLDGNGFLSKDE ----------
dog-Cf-Duox1 ---------- DKDGNGYLSFRE DFDGNGLISKDE ----------
dog-Cf-Duox1 ---------- DKDGNGYLSFRE DLDANGFLSKDE ----------
human-Hs-Duox1 ---------- DKDGNGYLSFRE DFDGNGLISKDE ----------
human-Hs-Duox2 ---------- DKDGNGYLSFRE DLDENGFLSKDE ----------
rat-Rn-Duox1 ---------- DKDGNGYLSFRE DFDGNGLISKDE ----------
rat-Rn-Duox2 ---------- DKDGNGYISFRE DLDGNGFLSKEE ----------
chicken-Gg-Duox ---------- DKDGNGYISFRE DIDENGFLSKEE ----------
frog-Xt-Duox1 ---------- DKDHNGYLSFEE DVNGNGILPKEE ----------
frog-Xt-Duox2 ---------- DEDGNGYLSFRE DVDGNGFLSKEE ----------
fugu-Tr-Duox ---------- DKDGNGSLSFQE DIGGTGSLSKGE* ----------
tetraodon-Tn-Duox ---------- DKDGNGYLSFQE ---------- ----------
zebrafish-Dr-Duox ---------- DKDGNGYLSFQE DIKGDGFLSKEE* ----------
medaka-Ol-Duox ---------- DTDHSGYLSFQE DVGGNGYLSKEE ----------
ascidian-Ci-Duox-B ---------- DTDHSGYLSFRE DVDHSGEINREE ----------
ascidian-Ci-Duox-A ---------- DSDGSGAISFRE DLDKSGELSKKE ----------
ascidian-Ci-Duox-C ---------- DSDEDGTISFRE DLDKSGGLSKEE ----------
ascidian-Ci-Duox-D ---------- DEDQDGFISFHD DLNQNGSLTKQQ* ----------
sea urchin-Sp-Duox ---------- DQDNSGSISFRE DIDRSGHLSREE ----------
fuit fly-Dm-Duox ---------- DKDQDGRISFQE DNDRNGVIDKGE ----------
mosquito-Ag-Duox ---------- DKDKDGRISFQE DNDRNGVIDKGE ----------
honeybee-Am-Duox ---------- DKDRDGRISFQE DKDCNGVIDKEE ----------
nematode-Ce-Duox1 ---------- AKHNEDSLSFNE* DLEGKNKVLRKD* ----------
nematode-Ce-Duox2 ---------- AKHNEDSLSFNE* DLEGKNKVLRKD* ----------