Suppl. Table 1. Primer sequences for mutant constructions. Mutations in Duox1-2 were introduced by directed mutagenesis using the following sense primers. Position of the mutated amino acid is given for the wild-type untagged protein and the mutated nucleotides are underlined.

Suppl. Fig. 1. Effect of tags on Duox1-2 activity. A) H_2O_2 (ng) was accumulated during 2.5h at 37°C from Cos-7 cells co-expressing DuoxA1 or DuoxA2 (500ng DNA) with Duox1 (white; 500ng), Rho-HA-Duox1 (horizontal line; 500ng), Duox2 (black; 500ng) or HA-Duox2 (vertical lines; 500ng). Cells were stimulated during the 2.5h period with 1µM ionomycin, 1µM Fsk or 5µM PMA. Each measurement was performed in duplicate (mean ± SD). B) Corresponding western showing the amount of Duox proteins expressed in Cos-7 cells transfected in the same conditions as described above.

Suppl. Fig. 2. Loss of Duox1 activity by mutations in EF-hand motifs. H_2O_2 accumulation was performed during 2.5h at 37°C in the presence of 1µM ionomycin, 1µM Fsk or 5µM PMA from cells expressing DuoxA1 with WT (black), E839Q (grey) or E875Q (white) Rho-HA-Duox1. Each sample was tested in duplicate (mean ± SD). Expression of mutated Duox1 constructs at the cell surface relative to WT is shown in the inset.

Suppl. Fig. 3. Topological model of the Duox1 protein with the substituted amino acids used to identify the PKA target sites (S955 – T1007 – S1217).

Suppl. Fig. 4. Kinetics of Duox1 phosphorylation by Fsk. Cos-7 cells were incubated with or without fetal bovine serum (FBS) and stimulated during 10, 20, 30 or 60 min with 10µM Fsk. Phosphorylation was detected using the anti-RXX(pS/pT) antibody (P-PKA). Relative PKA-mediated Duox1 phosphorylation corrected to the total amount of Duox1 immunoprecipitated is shown on the top of the figure (non stimulated condition incubated in absence of serum was considered as 100%).

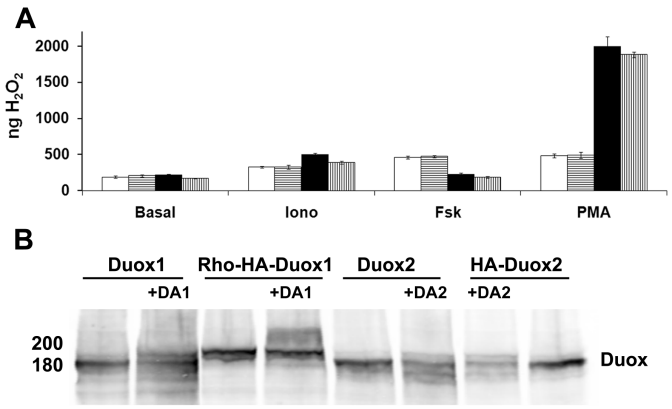
Suppl. Fig. 5. Duox1 phosphorylation is increased through the cAMP pathway. Cos-7 cells co-transfected with Rho-HA-Duox1/DuoxA1 were incubated with ^{32}P and stimulated 30 min with 10µM Fsk, 50µM 6-MB-cAMP or co-transfected with the plasmid encoding the PKA catalytic subunit (PKA). Relative Duox1 phosphorylation corrected to the total amount of Duox1 in the immunoprecipitate is represented on the top. PKA condition tested in another experiment.

Suppl. Fig. 6. Phosphorylation of Duox1 mutated in potential phosphorylation sites. Co-transfected cells expressing DuoxA1 together with WT, S955A, T1007A, S1217A or S955A/S1217A Rho-HA-Duox1 proteins were incubated with (+) or without (-) 10µM Fsk for 30 min. Duox1 phosphorylation was corrected relative to the total amount of immunoprecipitated Duox1. Phosphorylation was detected using the anti-RXX(pS/pT) antibody (P-PKA). The mutant S955A/S1217A was tested in another experiment together with the WT Duox1 protein.

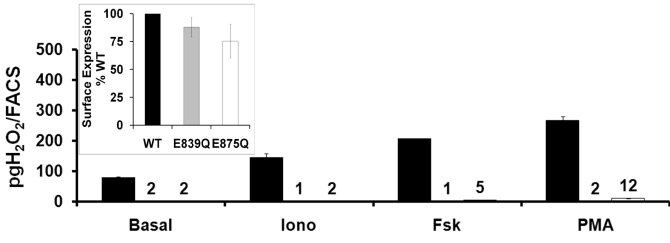
Supplemental table 1

Name	Sequences 5'→3'
Duox1 mutants:	
E839Q	GGCAATGGGTACCTGTCCTTCCGACAGTTCCTG
E875Q	AATGGCCTGATATCCAAGGATCAGTTCATCAGG
S955A	CTCTGCCGGCGAGCCGCCTACATCAGCCAGGA
T1007A	TTTGGCAAGAAGGTAGCGTCATTCCAGCCCTTG
S1217A	CACTTCCGCCGCCGCGCTTTCCGGGGCTTCTGG
Duox2 mutants:	
E843Q	TACCTGTCCTTCCGACAGTTTCTAGACATCCTG
E879Q	TTCCTCTCCAAGGACCAATTCTTCACCATGATG

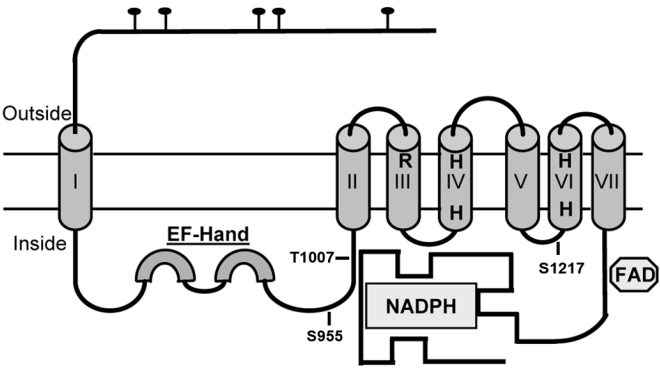
Supplemental Figure 1



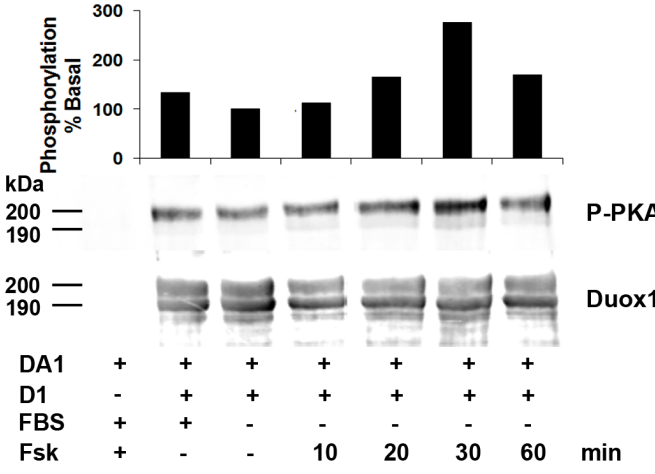
Supplemental Figure 2



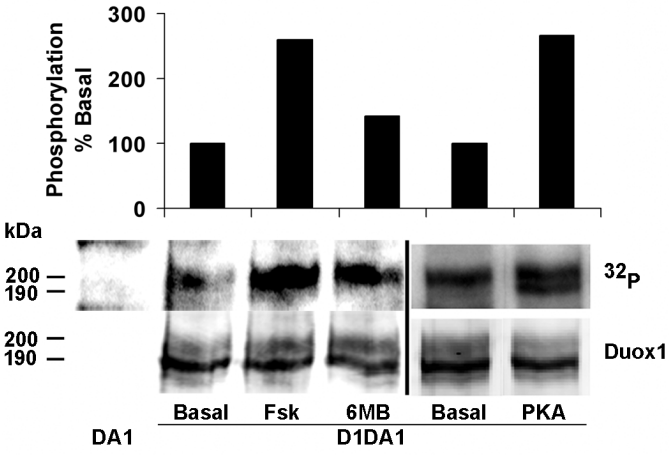
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6