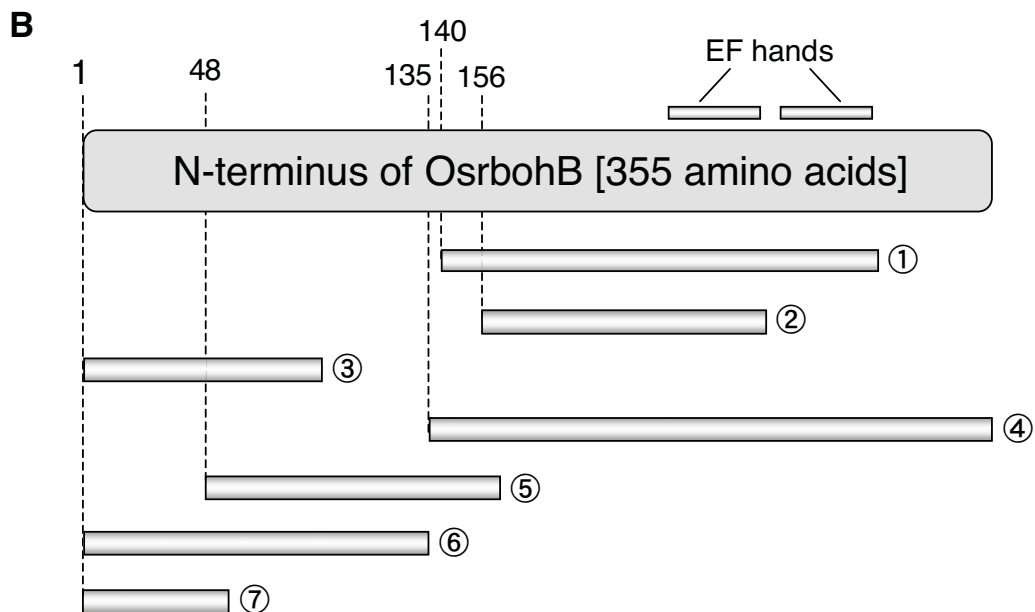
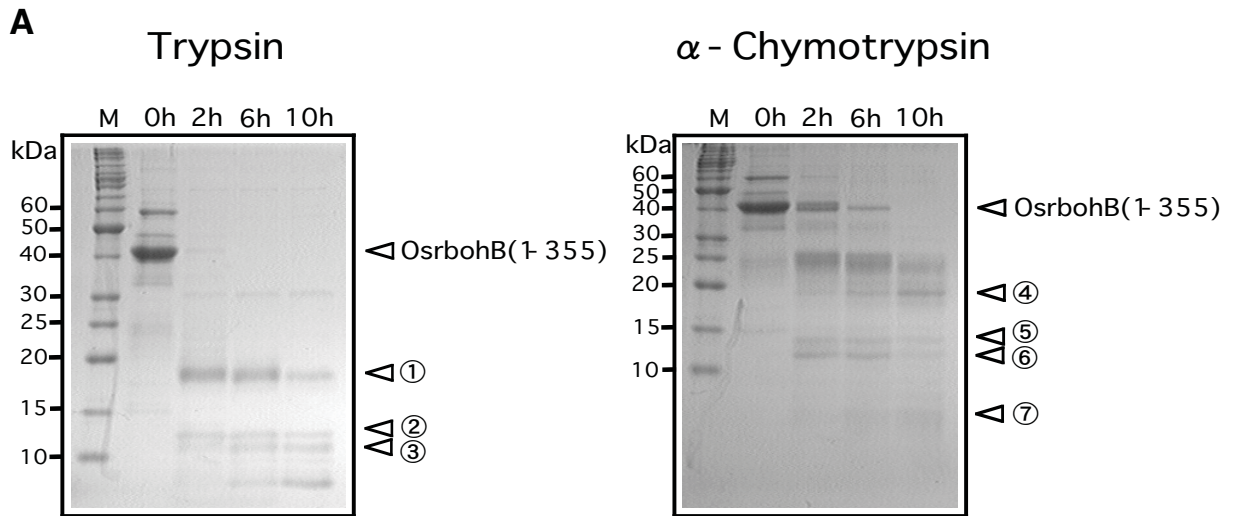
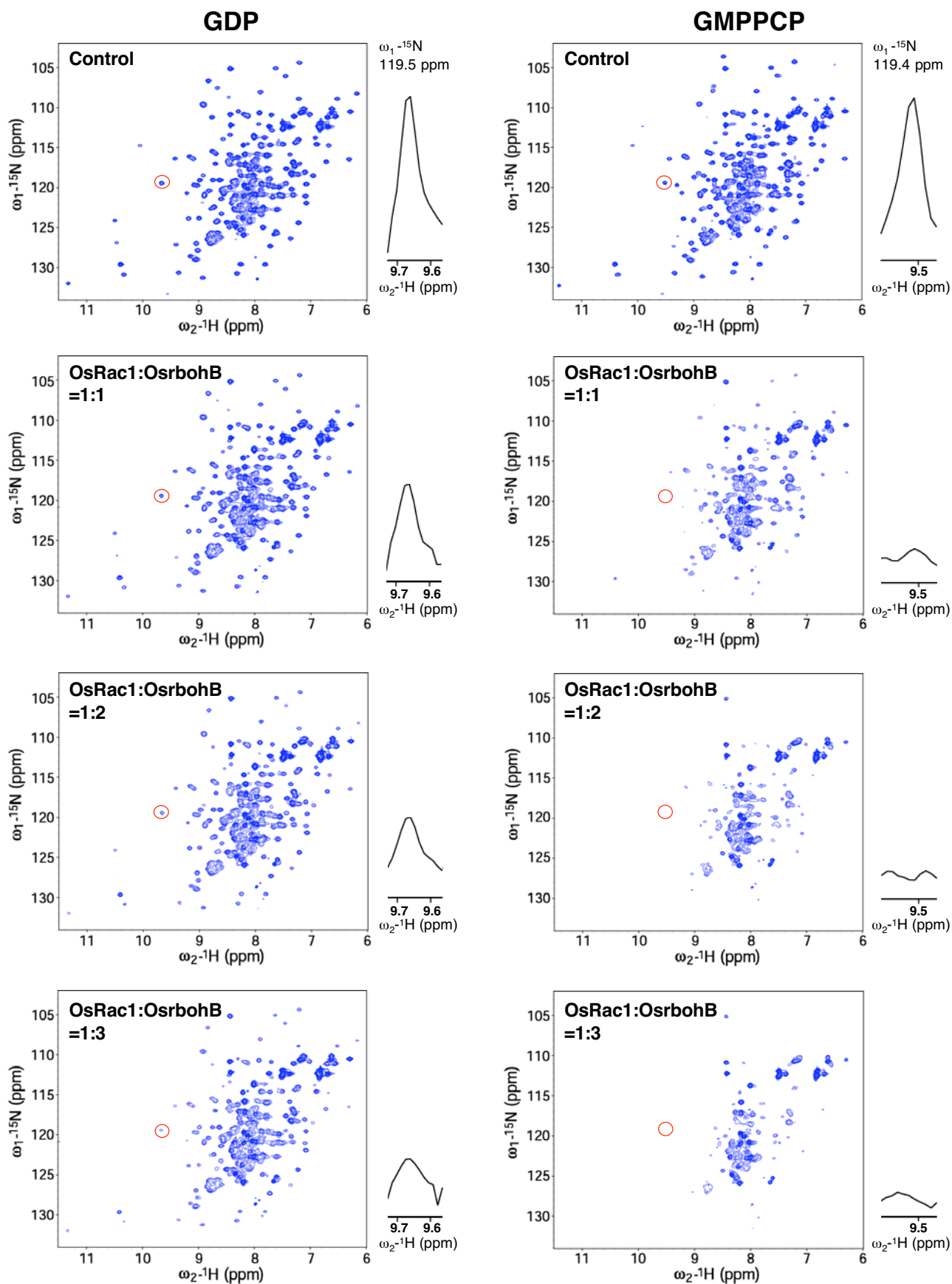


**Supplemental Figure 1.** Interaction of various fragments of the N-terminal region of OsrbohD or StrbohB with OsRac2. (**A** and **C**) Schematic representations of OsrbohD and StrbohB showing the size and position of each fragment of the N-terminal region of OsrbohD and StrbohB used for the assays. (**B** and **D**) Interaction between the N-terminal fragments of OsrbohD and StrbohB with CA-OsRac2 in yeast.



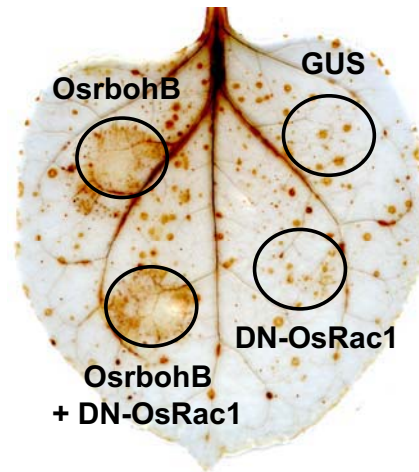
**Supplemental Figure 2.** Proteolysis of N-terminal region of OsrbohB (1-355). **(A)** OsrbohB (1-355) was incubated with trypsin (left) or  $\alpha$ -chymotrypsin (right). Aliquots of samples were taken at the indicated time points. The reaction products were analyzed by SDS-PAGE and detected by staining with CBB. Each of the fragments (1-7) was analyzed by N-terminal protein sequencing. **(B)** Schematic representation of each protein fragment produced by digestion with trypsin or  $\alpha$ -chymotrypsin.



**Supplemental Figure 3.**  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra of uniformly  $^{15}\text{N}$ -labeled OsRac1 with different concentrations of OsrbohB(138-313). GDP-bound form (left column) and GMPPCP-bound form (right column) are shown without OsrbohB (top) or with equimolar (second), 2 (third) and 3 equivalents (bottom), respectively. 1D slice spectra are given for the circled peaks on the right side of each  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectrum.

OsrbohB	133	KRLDRTKSSAAVALKGL-QFVTAKVGN DGWAAVEKRFNQLQVD--GVLLR	179
CalcineurinB	1	MGNEASYPLEMCSHFDADEIKRLG-----KRFKKLDL D NSGSLSV	40
		*. * . * . * *** . * . * * *	
		En**nn**nX*Y *Z GYIX** Zn**nn**n	
OsrbohB	180	SRFGKCI GMDGSDEF AVQMFDSLARKRGIVKQVLTKDELKDFYEQ L TDQG	229
CalcineurinB	41	EEFMSLPELQ-QNP LVQRVIDIFD TD-GN-GEVDFK-EFIEGVSQF SVKG	86
		* . . . * * . * * * . * . . *	
		▼D242A (ef1)	
		En**nn**nX*Y*ZGYIX**Zn**nn**n En**	
OsrbohB	230	-FDNRLRTFFDMVDKNADGRLTAE EVKEI IALSASAN-KLSKIKERADEY	277
CalcineurinB	87	DKEQKLRFAFRIYDMKDGYI SNGELFQVLKMMVGNLKD TQLQQIVDK-	135
		...** * . * ** .. * . . . . * * ..... *	
		▼D286A (ef2)	
		nn**nX*Y *ZGYIX**Zn**nn* *n	
OsrbohB	278	TALIMEELDPTNLGYIEMEDLEALLLQSPSEAAARSTTTHSSKL	321
CalcineurinB	136	-TIINADKD--GDGRISFE EFCAVVG---GLDIHKKMVVDV	170
		..* . * . * * * . * . .	

**Supplemental Figure 4.** Partial amino acid alignment of OsrbohB and CalcineurinB. Amino acid sequences of OsrbohB and CalcineurinB (Genbank accession no. L03554) were aligned using ClustalW( v1.4) (Thompson et.al 1994). Aligned EF-hand domains are indicated in magenta. Consensus of EF hand domain are shown in blue. A Ca<sup>2+</sup> is coordinated by oxygen in the side chain of amino acids such as serine (S) aspartate (D), asparagine (N) at X, Y, and Z positions. Residue at position Y can be any amino acid, whereas glutamate (E) or aspartate (D) occupy position Z and binds Ca<sup>2+</sup> with two oxygen atoms. Glycine (G) at position G permits a bend in the EF-hand loop, and a hydrophobic residue leucine (L), isoleucine (I) or valine (V) is present in position. Two neighboring  $\alpha$ -helices contain alternating hydrophilic (\*) residue and hydrophobic (n) residues (Gutierrez-Ford et al., 2003). Arrowheads indicate OsrbohB aspartate residues at positions 242 and 286, which occupy positions X and Y, respectively, that were mutated to alanine (A). D242A and D286A mutations are designated as ef1 and ef2 respectively.



**Supplemental Figure 5.** Transient co-expression of DN-OsRac1 and OsrbohB does not enhanced ROS production in *N. benthamiana*. DAB-stained *N. benthamiana* leaves transiently transformed with GUS ( $P_{35S}$ -GUS), OsRac1 ( $P_{35S}$ -DN-OsRac1), OsrbohB ( $P_{35S}$ -OsrbohB).