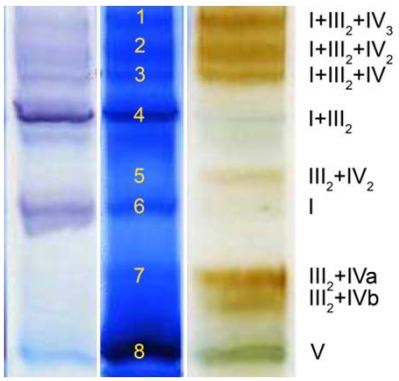
Supplemental Figure S1



Supplemental Figure S1. Identification of supercomplexes from potato tuber mitochondria.

BN-PAGE gels were stained with three different methods: The purple staining of the left lane visualizes complex I activity. This method stains protein bands that contain complex I also when it is part of a supercomplex. The blue colour is derived from Coomassie used during BN PAGE electrophoresis. The blue lane in the middle is stained with Coomassie to visualize protein bands in general. The white numbers refer to the annotation of the bands as listed supplemental Table TI. The brown coloured lane on the right is stained for complex IV activity. Complex IV has two isoforms. The larger complex IVa differs from complex IVb as that it contains the subunit COX6b (4). This size difference becomes especially apparent in the supercomplex band III_2+IV and therefore both isoforms are annotated separately.

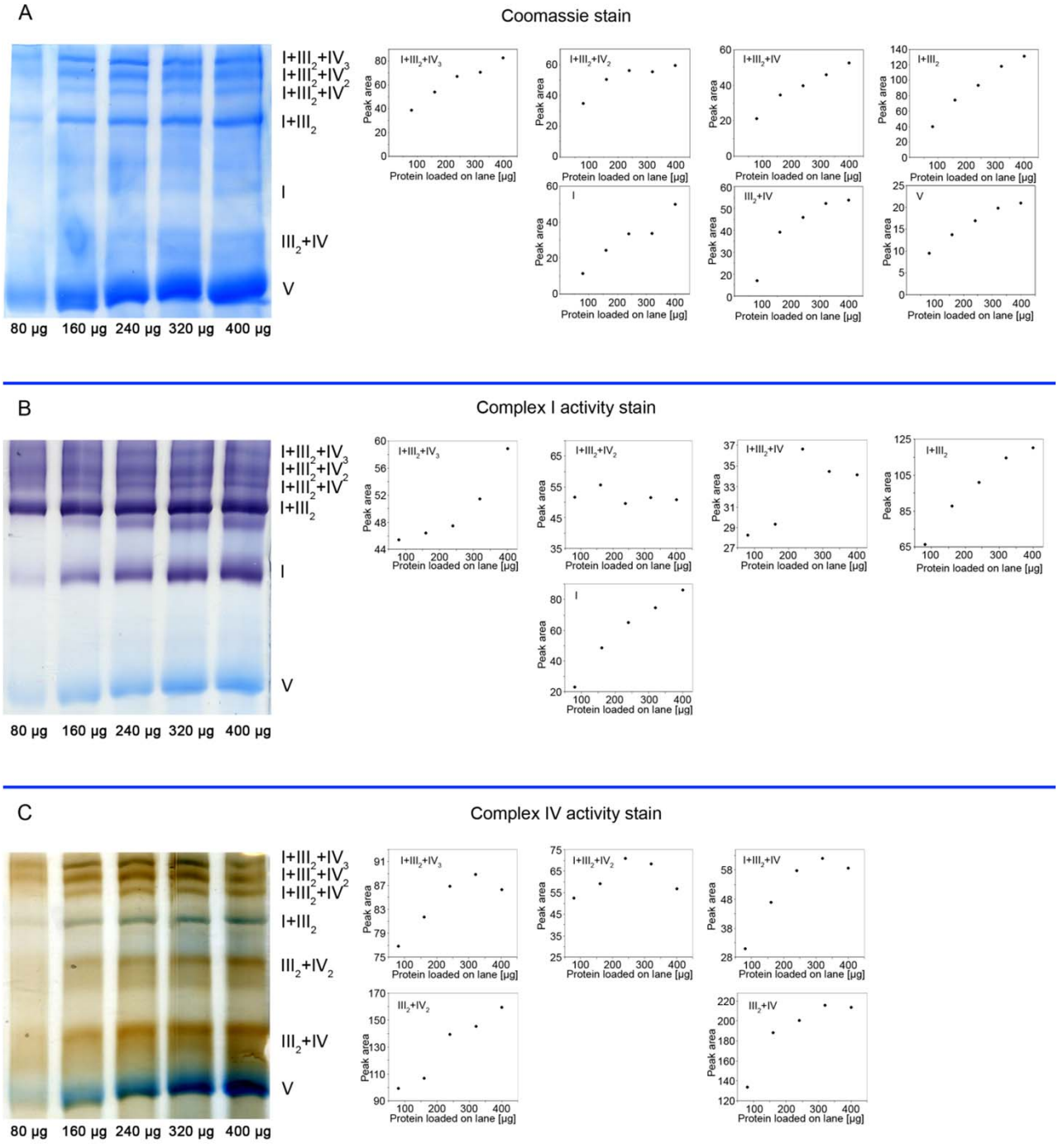
Supplemental Table S1

Supplemental Table SI: Confirmation of identity of supercomplexes from potato tuber mitochondria.

Band	Protein identity	Number of polypeptides annotated to complex				
		I	II	III	IV	V
1	$I+III_2+IV_3$	7		6	9	
2	$I+III_2+IV_2$	9		8	12	
3	$I+III_2+IV$	8		4	7	
4	$I+III_2$	8		3		
5	III_2+IV_2			2	3	
6	I	6				
7	III_2+IV			1	2	
8	V					11

Protein bands were excised from the Blue-Native gel (Supplemental Fig. S1) and sequenced by LC/MS-MS after trypsin digestion. For each band, the sum of polypeptides that were recognized to belong to a complex associated to the respective supercomplexes is listed. Final annotation of the supercomplexes was based on this sequencing information in combination with the results of activity stainings as shown in supplemental fig. S1.
Nomenclature of supercomplexes was chosen according to previous publications (2, 4, 9, 11, 12). Complexes contained in a supercomplex are represented by latin numbers according to the respective complex number. Number of each single complex contained in a supercomplex are shown in the subindex after each complex latin number.

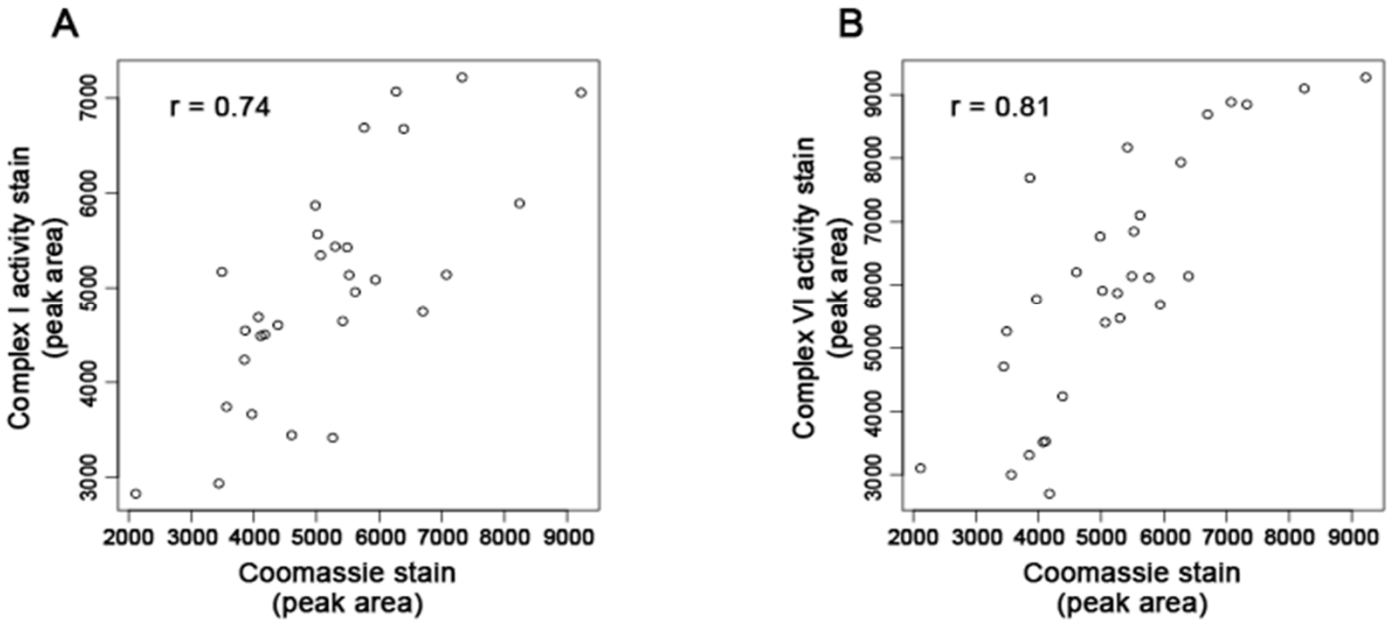
Supplemental Figure S2



Supplemental Figure S2: Correlation between protein amount loaded onto the gel and color intensity of the activity stainings for complex I and IV.

BN gels loaded with different protein amounts (80-400 µg) with activity staining of (A) complex I and (B) complex IV. (C-G) show the quantification values of bands stained for complex I activity plotted agains the total amount of protein loaded onto the lane. (H-L) show the quantification of complex IV activity-stained supercomplex bands.
See also supplemental figure S3 for a correlation analysis of the complex activity and protein abundance of the largest supercomplexes ($I+III_2+IV_{1-3}$) as determined by Coomassie staining.

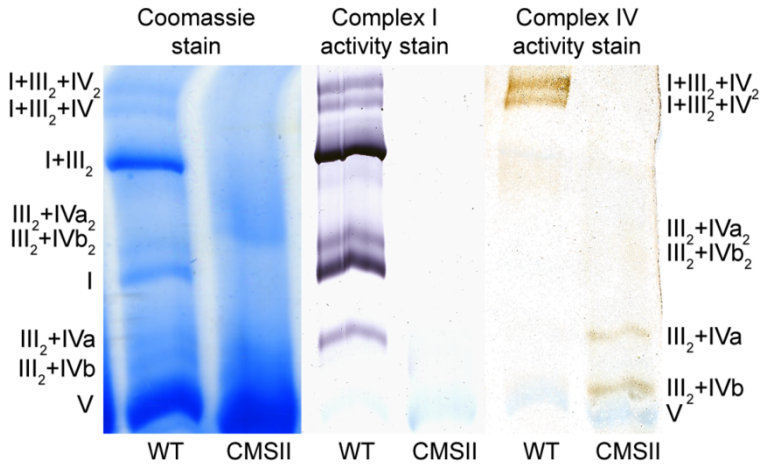
Supplemental Figure S3



Supplemental figure S3. Correlation of large supercomplex band intensities stained with Coomassie and activity stainings for complex I and complex IV.

Raw color intensity values of Coomassie stained protein bands in a blue native gel plotted against the intensity values of complex I (A) and complex IV (B) activity stainings. Different protein amounts were loaded as indicated for supplemental figure S2. The band intensities correspond to the supercomplexes $I+III_2+IV_3$, $I+III_2+IV_2$ and $I+III_2+IV$. The Pearson correlation coefficient r is shown in each graph.

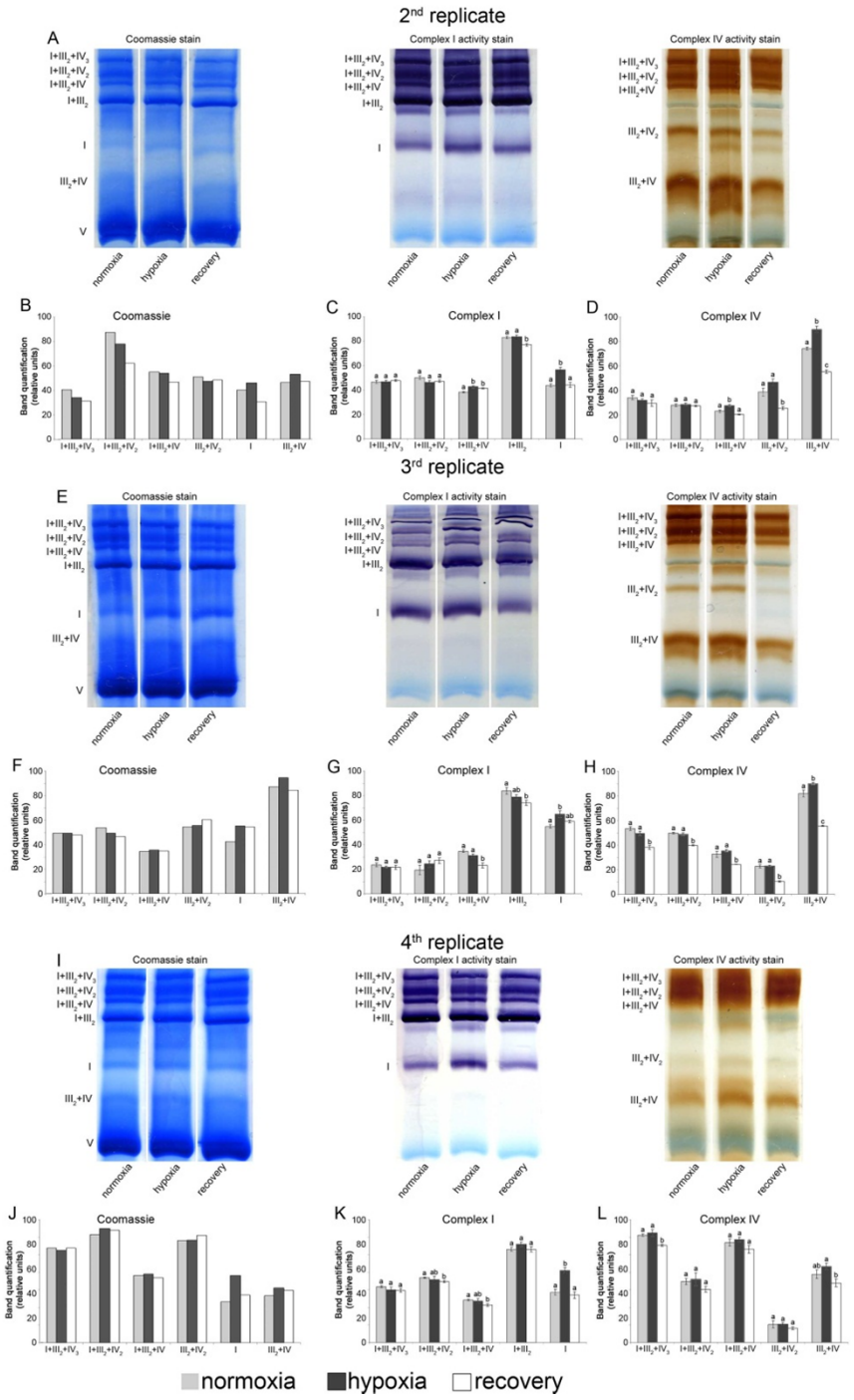
Supplemental Figure S4



Supplemental figure S4. Supercomplex analysis of *N. sylvestris* CMSII knock out line of complex I (addendum to Figure 1)

Coomassie and activity stainings of complex I and IV within supercomplexes isolated from *N. sylvestris* WT and the CMSII mutant deficient in complex I. Blue native gel lanes show the results of an independent biological replicate of the results shown in figure 1a.

Supplemental Figure S5

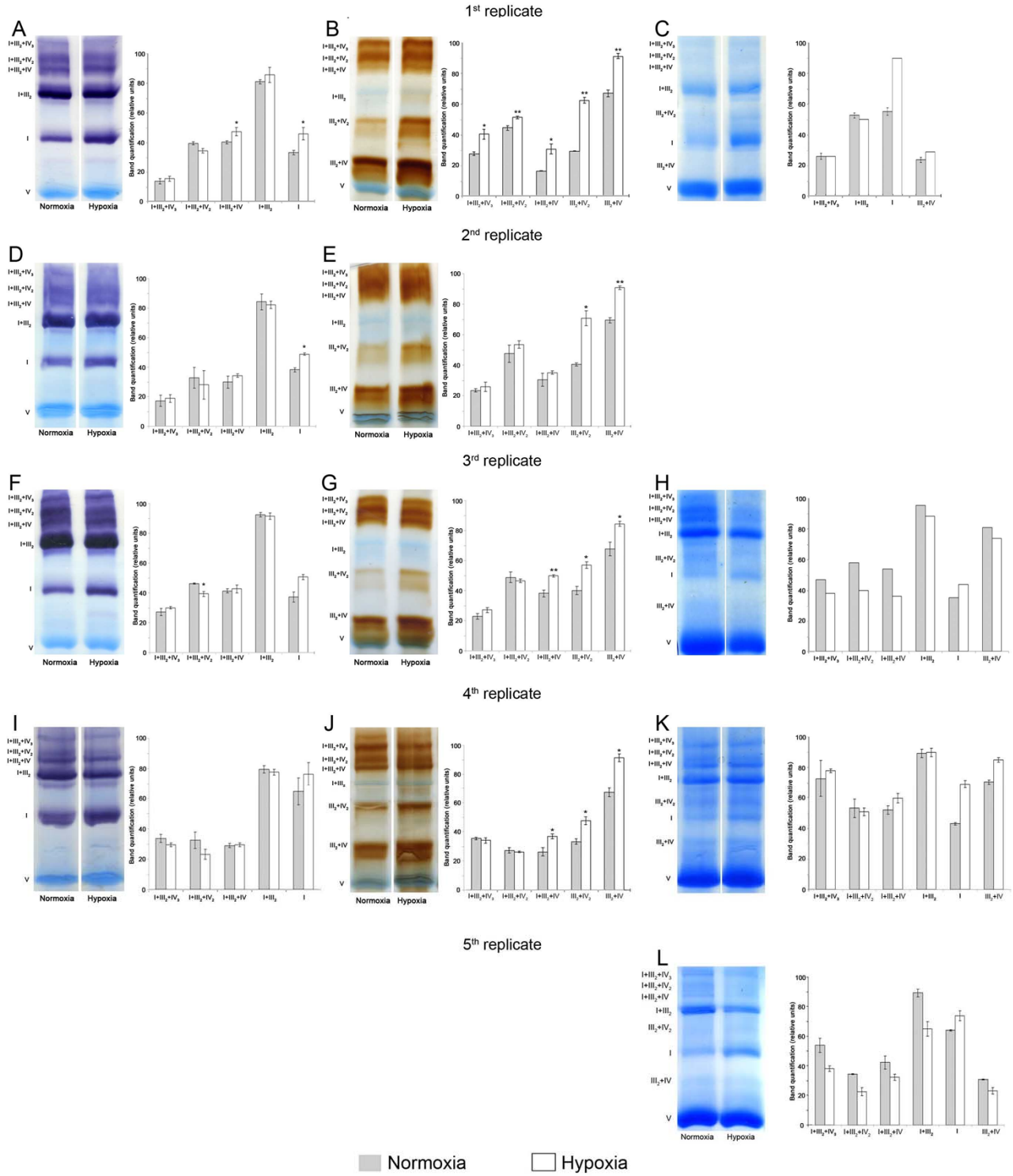


Supplemental Figure S5: Biological repetitions of coomassie stainings and activity stainings of complex I and IV in supercomplexes from potato tuber mitochondria isolated after storage in air, or after 36 hours of flooding induced hypoxia, or after 36 hours recovery treatment following 36 hours of flooding induced hypoxia. (addendum to Figure 2).

Relative band intensity of complexes and supercomplexes from biological replicates of BN gels from normoxia, hypoxia and hypoxia with normoxic recovery treated tubers. Note, in Supplemental Figure S6 results of a similar experiment without recovery treatment are shown.

Each panel shows at the left side a representative lane for each treatment and at the right side the band quantification is depicted in relative units. Panels B, E and H show the results from complex I activity stained gels. Complex IV activity stained gel quantification is shown in panels C, F and I. Significantly different mean values as determined by an ANOVA between the color intensities of a band after activity staining are marked with different letters ($p<0.05$; $n\geq3$ technical replicates).

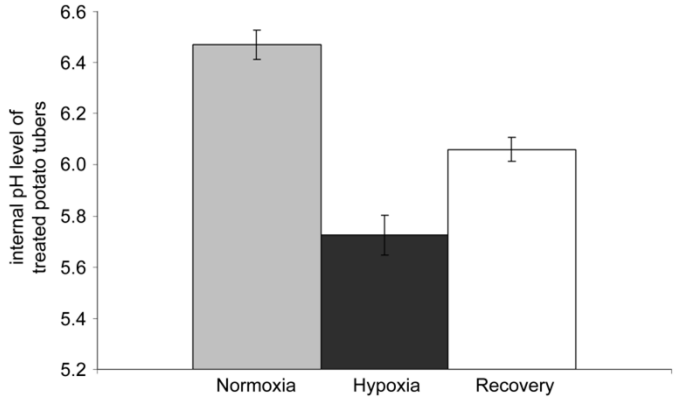
Supplemental Figure S6



Supplemental Figure S6: Biological repetitions of complex I and IV activity staining in supercomplexes from flooded (hypoxia and control (normoxia) potato tubers (addendum to Figure 2).

Relative band intensity of complexes and supercomplexes from biological replicates of BN gels from normoxia and 24 hours of flooding induced hypoxia-treated tubers. Note: the experiment is similar to what is shown in Supplemental Figure S5, except that no recovery treatment has been performed.
Each panel shows at the left side a representative lane for each treatment and at the right side the band quantification is depicted in relative units. Panels A, C, E and G show the results from complex I activity stained gels. Complex IV activity stained gel quantification is shown in panels B, D, F and H. Significant differences as determined by Student's t-test between the colour intensities of a band after activity staining are indicated by * ($p<0.05$) or ** ($p<0.01$; $n\geq3$ technical replicates).

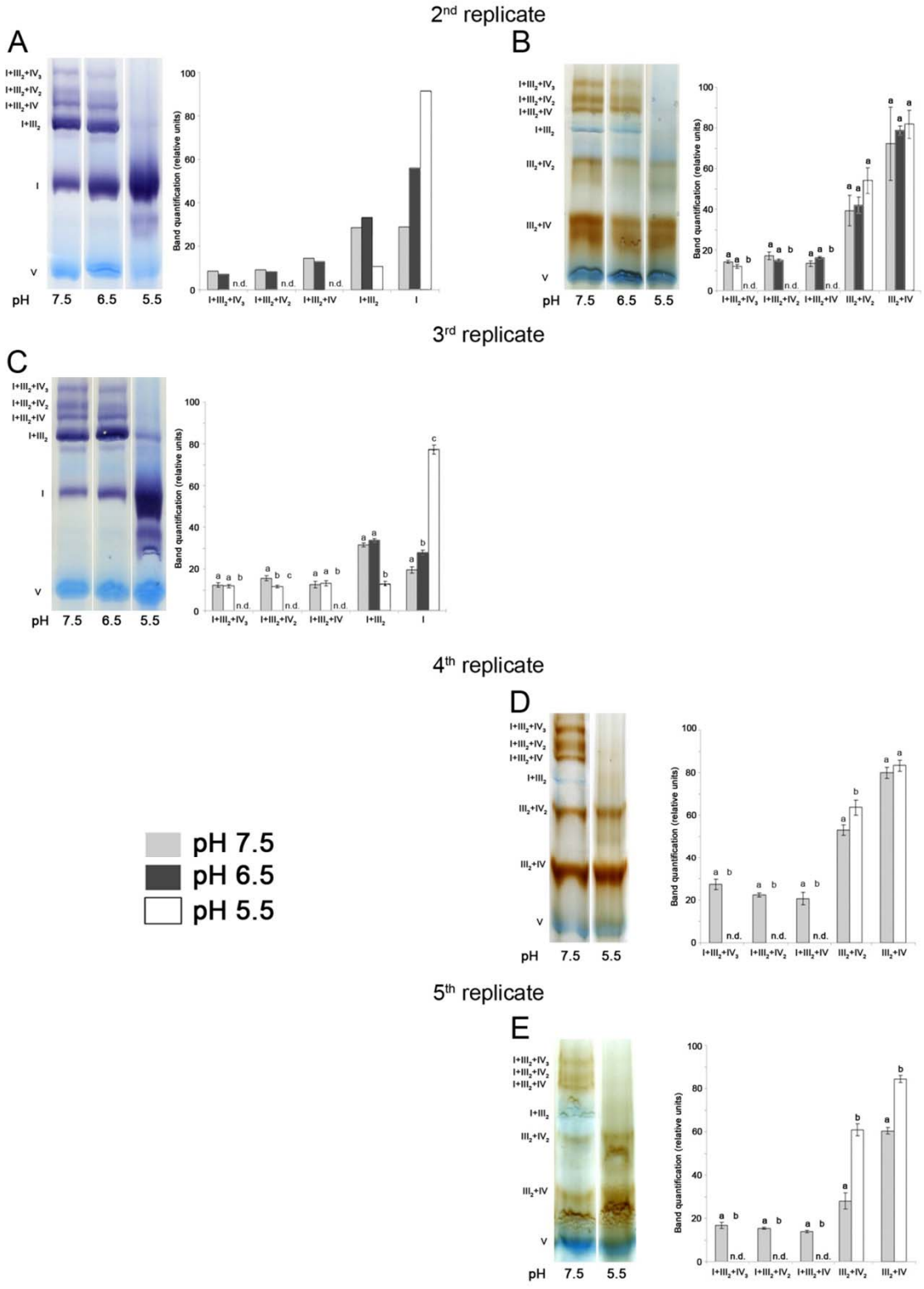
Supplemental Figure S7



Supplemental Figure S7: pH levels of cell sap from untreated potato tubers and tubers treated with hypoxia and with hypoxia with a following normoxic recovery period.

Potato tubers were flooded with oxygen poor water ($[O_2] \leq 10\mu M$) and kept for two days in the dark at room temperature under these conditions. The pH level of the cut potato tuber from 4 biological replicates was measured directly after 36 hours of flooding induced hypoxia. After flooding, four potato tubers were treated for 36h with normal air in the dark and following this the pH levels of the inside of the tubers was measured (recovery). In parallel, the pH level of four untreated potato tubers was measured as normoxic control tubers (normoxia).

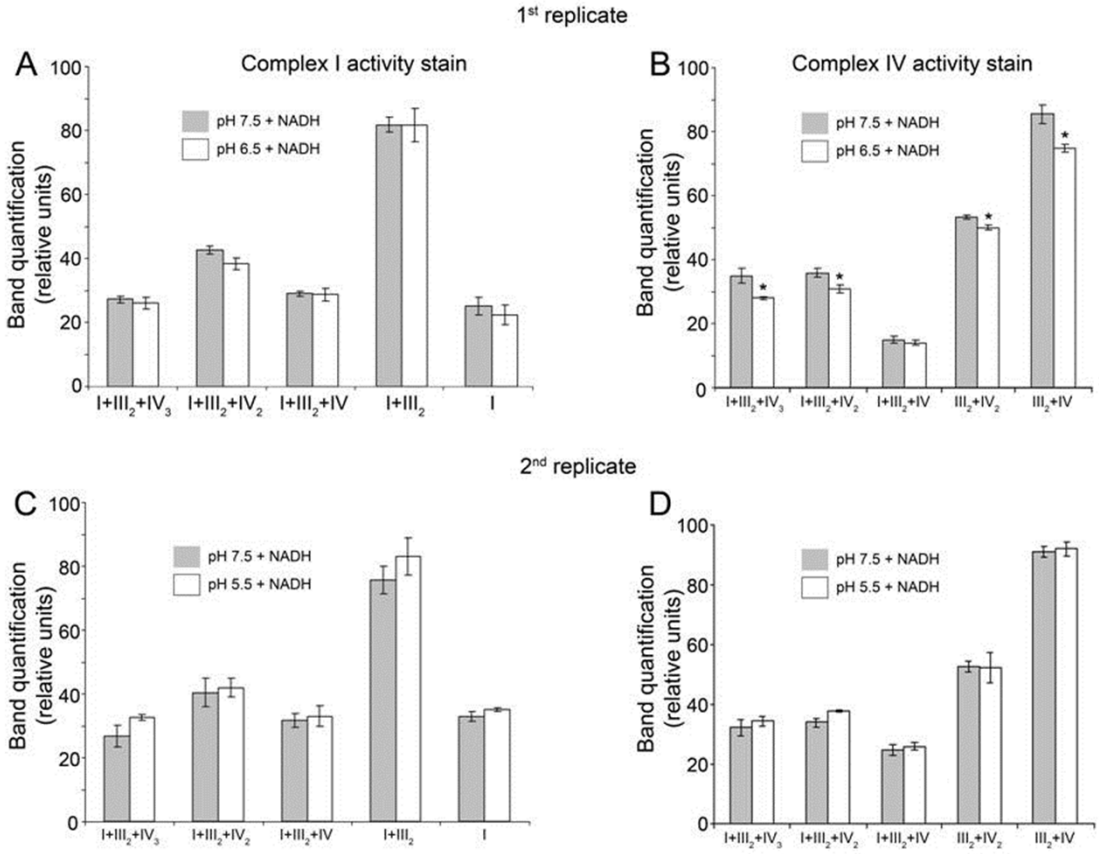
Supplemental Figure S8



Supplemental Figure S8: Biological repetitions of complex I and IV activity staining in supercomplexes from mitochondria pre-treated at various pH in the presence of succinic acid (addendum to Figure 3).

Relative band intensity of complexes and supercomplexes from biological replicates of BN gels with mitochondria treated with succinic acid (10 mM) and buffer at pH 7.5, 6.5 and 5.5. Each panel shows at the left side a representative lane for each treatment of a single biological replicate and at the right side the band quantification is depicted in relative units. Panels A and C show the results from complex I activity stained gels. Complex IV activity stained gel quantification is shown in panels B, D and E. For each biological repetition, significant differences between protein bands representing the same (super)complex were determined by an ANOVA followed by the Holm-Sidak post hoc test are marked by different letters ($p<0.05$; $n\geq3$ technical replicates).

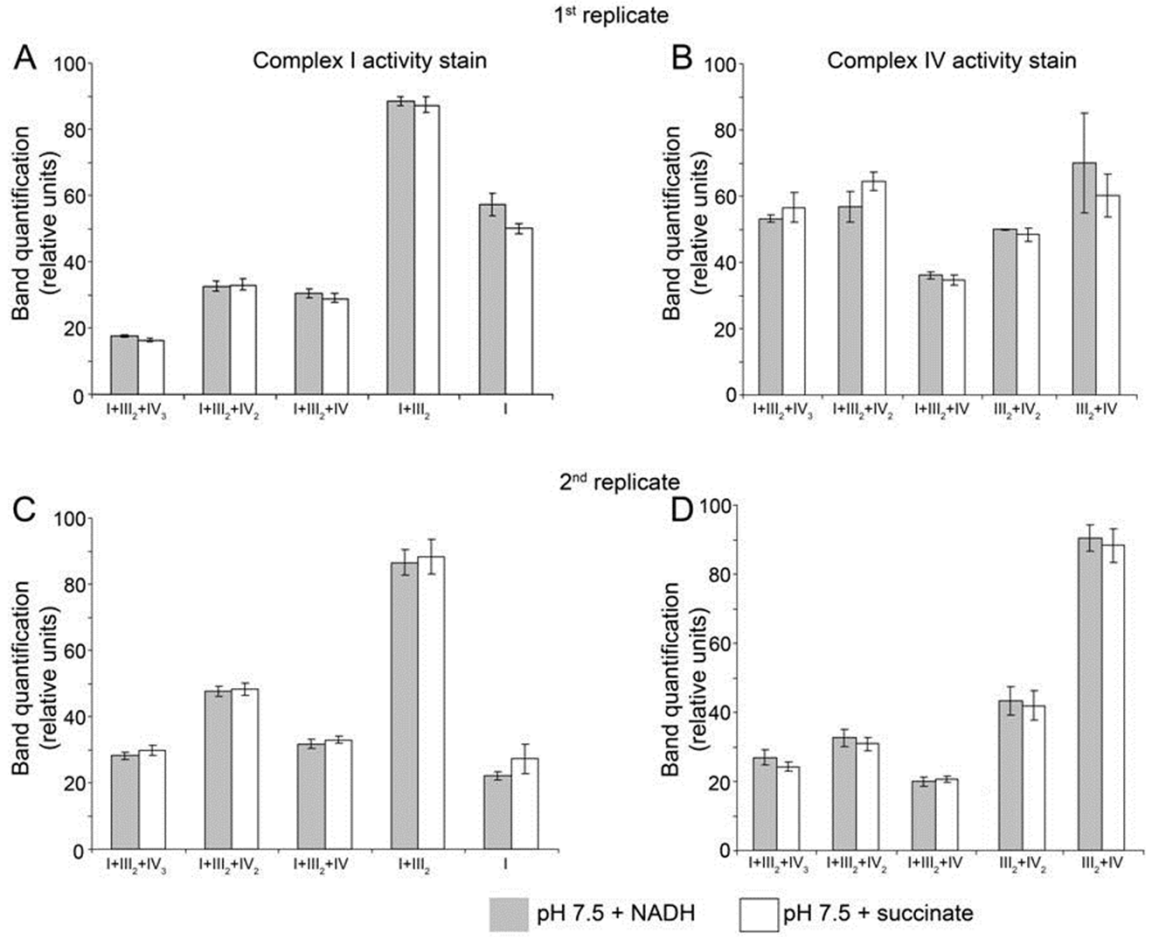
Supplemental Figure S9



Supplemental Figure S9: Biological repetitions of complex I and IV activity staining in supercomplexes from mitochondria pre-treated at various pH in the presence of NADH (addendum to Figure 5A).

Relative band intensity of complexes and supercomplexes from biological replicates of blue native gels. Isolated mitochondria treated with different pH levels (pH 7.5, 6.5 or 5.5) with 4 mM NADH as substrate. Panels A and C show the results from complex I activity stained gels. For each biological repetition, significant differences between protein bands as determined by Student's t-test are indicated by * ($p<0.05$; $n\geq3$ technical replicates).

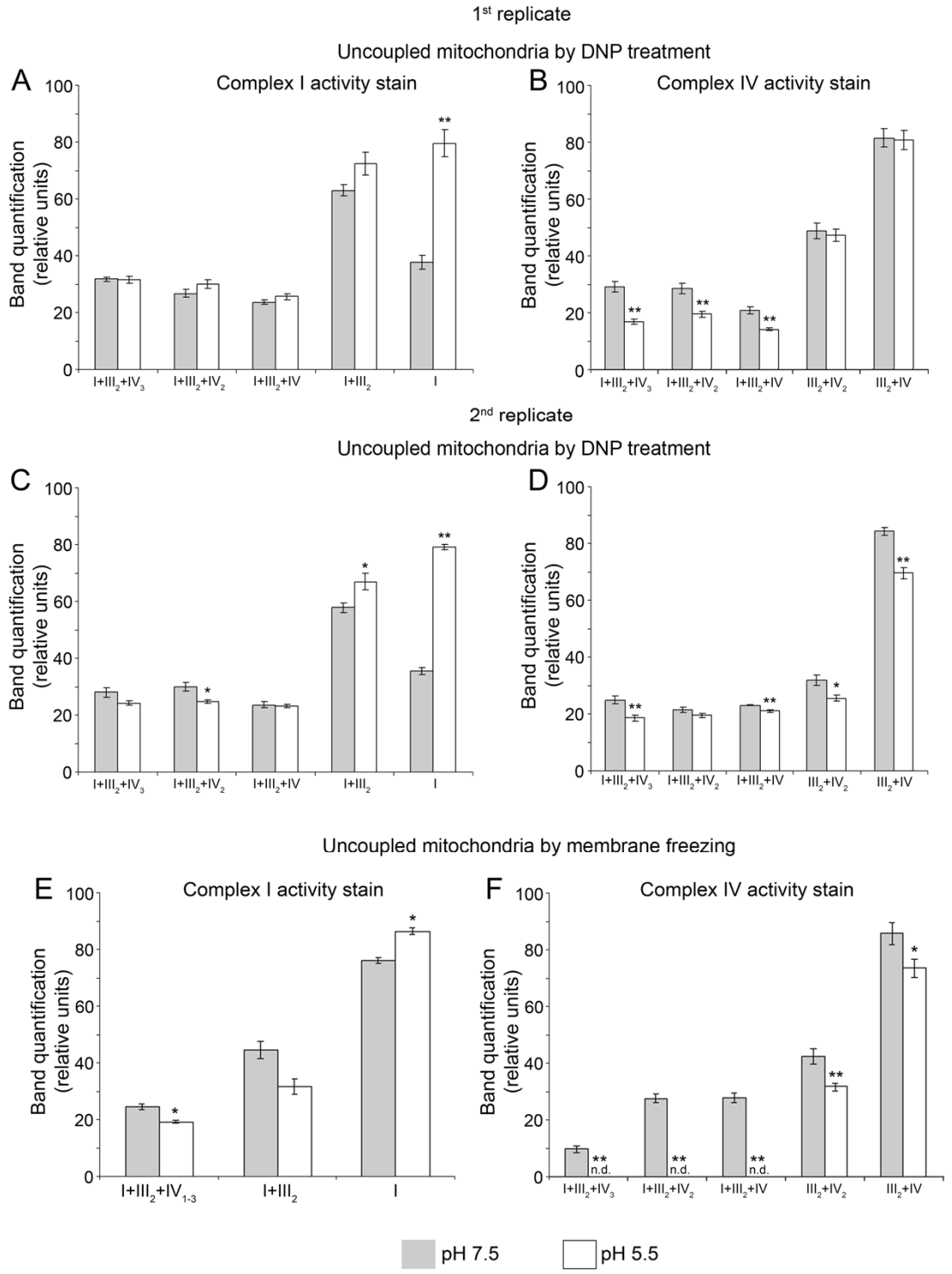
Supplemental Figure S10



Supplemental Figure S10: Biological repetitions of complex I and IV activity staining in supercomplexes from mitochondria treated with NADH or succinate at pH 7.5 (addendum to Figure 5B).

Relative band intensity of complexes and supercomplexes from biological replicates of blue native gels from isolated potato tuber mitochondria treated at pH 7.5 with 4mM NADH or 10 mM succinic acid. Panels A and C show the results from complex I activity stained gels. Complex IV activity stained gel quantification is shown in panels B and D. No significant differences between means of technical replications were determined by the Student's t-test ($p<0.05$, $n\geq3$) in any of the biological repetitions.

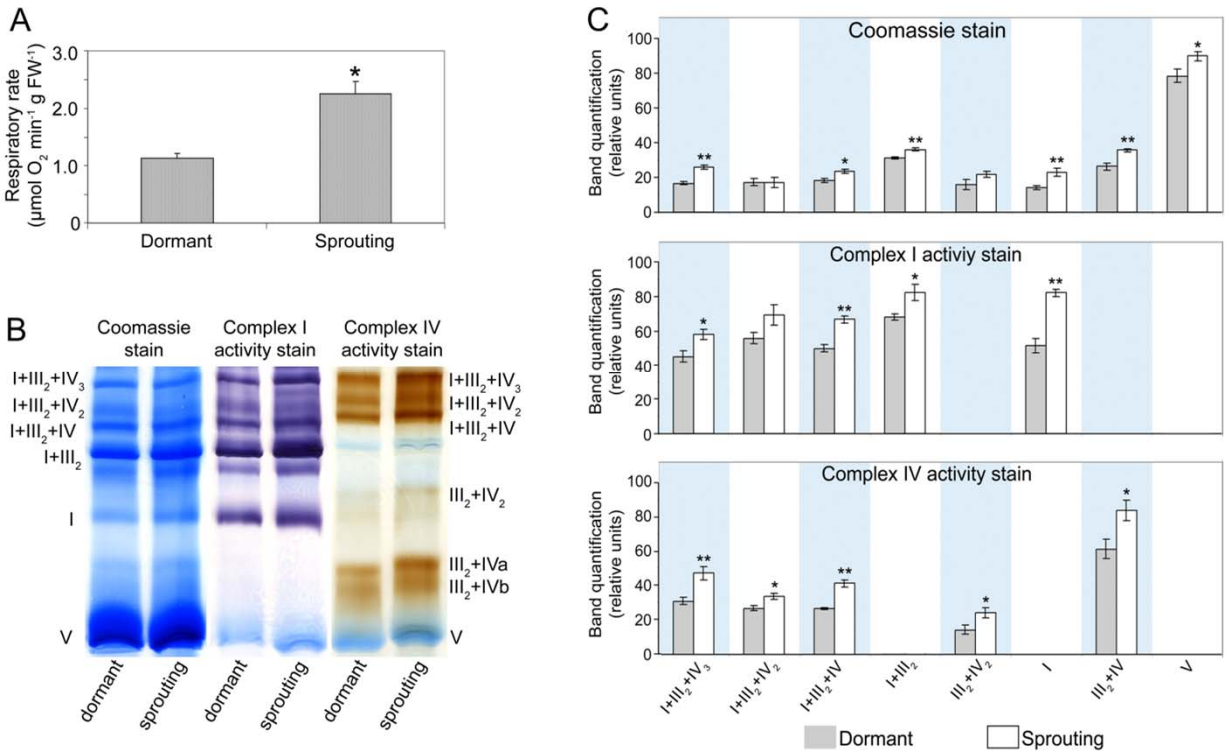
Supplemental Figure S11



Supplemental Figure S11. Biological repetitions of complex I and IV activity staining in supercomplexes from mitochondria with a depolarized membrane potential (addendum to Figure 5C,D).

Band quantification of blue native gels with potato tuber mitochondria pretreated with (A-D) the uncoupler 2,4-dinitrophenol (DNP) or (E,F) a repetitive freeze-thaw cycle to disrupt the membranes and subsequently incubated with NADH at different pH levels. (A,C,E) show the results from complex I activity stained gels. (B,D,E) show the results from complex IV activity stained gel quantification. Significant differences as determined by Student's t-test between the color intensities of a band after activity staining are indicated by * ($p<0.05$) or ** ($p<0.01$; $n\geq3$ technical replicates).

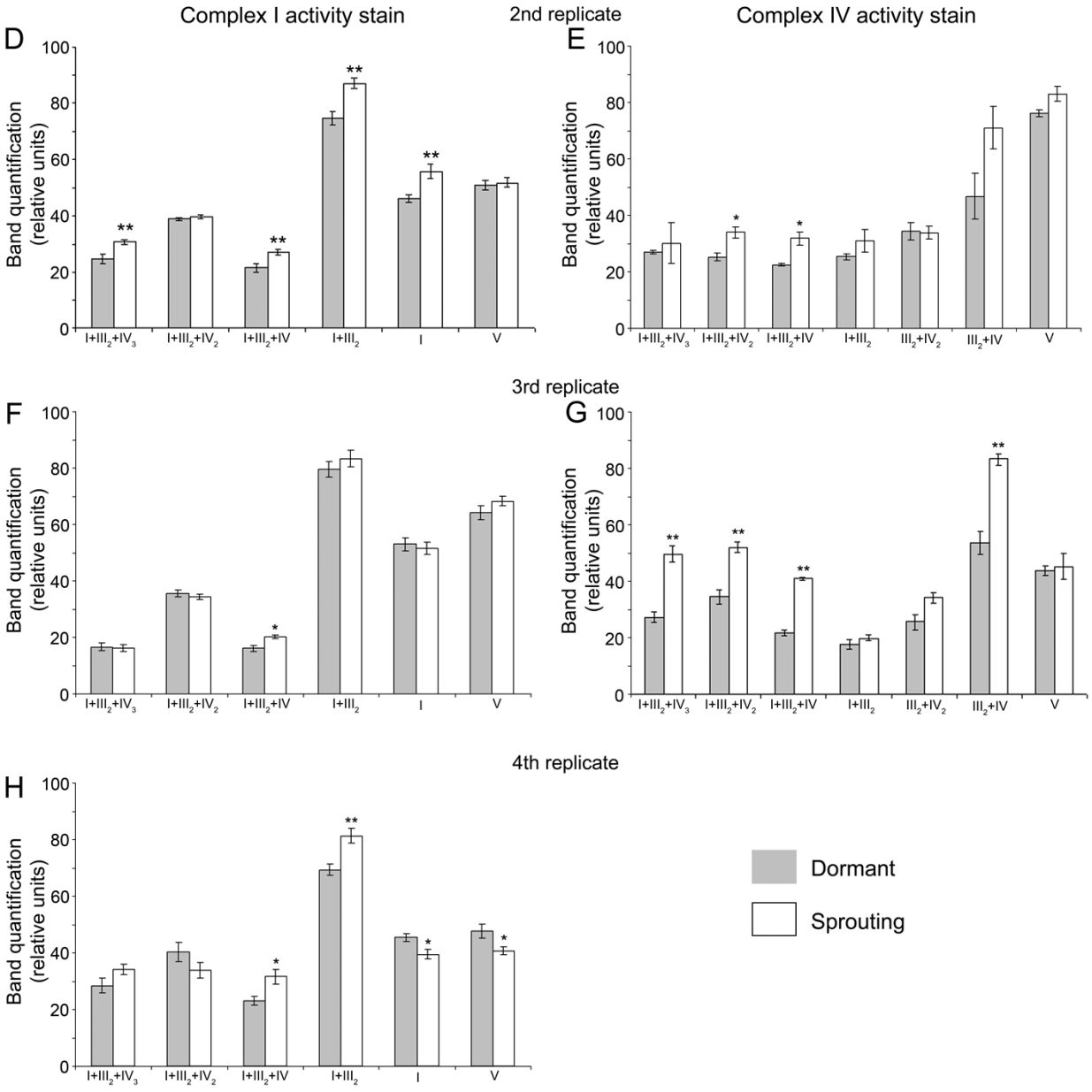
Supplemental Figure S12



Supplemental Figure S12. Analysis of supercomplex composition and respiratory activity of dormant and sprouting potato tubers.

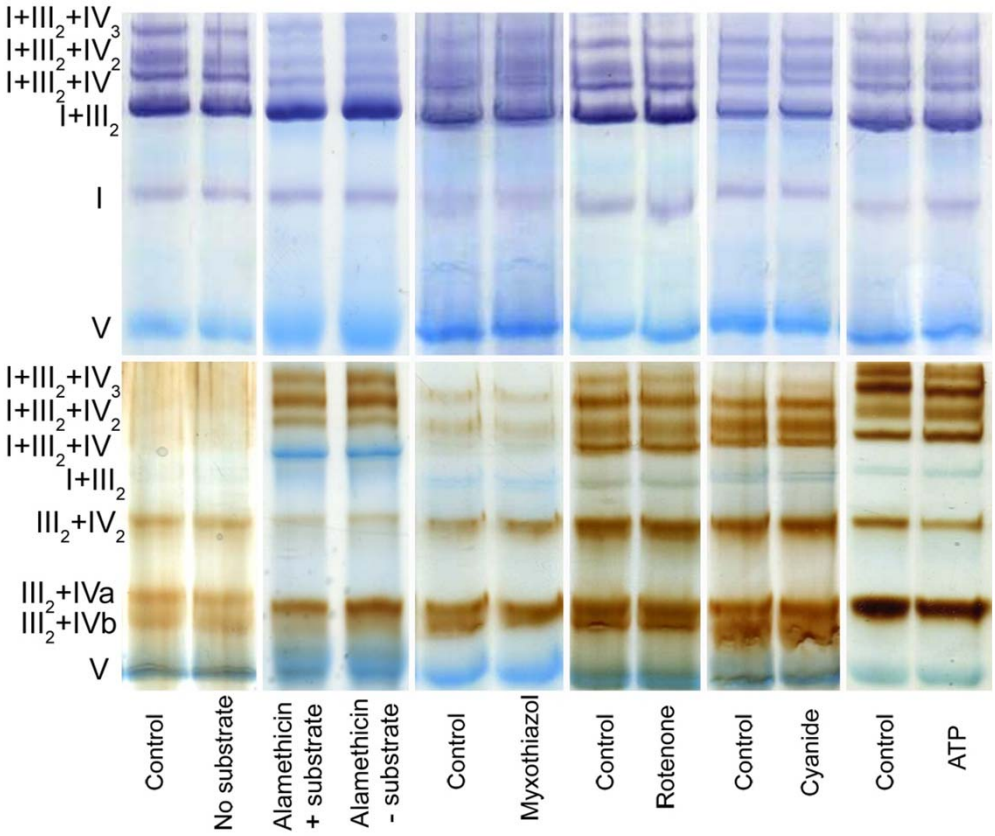
(A) State 3 respiratory activity of tissue slices from dormant or sprouting tubers. FW; fresh weight of tuber slices. According to the Student's t-test, values that differ significantly from each other ($p<0.05$) are marked with an asterisk. **(B)** Blue native PAGE analysis showing various stainings of mitochondrial proteins isolated from dormant or sprouting potato tubers. On the left side, Coomassie staining of the protein lanes is shown. The activity of complex I is shown in the middle panel, whereas the right panel shows complex IV activity. **(C)** Quantification of protein bands colour intensities from the gel displayed in panel (B). Mitochondrial protein was isolated from dormant (grey bars) or sprouting (white bars) potato tubers. The upper, middle and lower panel show data from Coomassie, complex I, and complex IV activity staining, respectively. Significant differences as determined by Student's t-test between the color intensities of a band after activity staining are indicated by * ($p<0.05$) or ** ($p<0.01$; $n\geq3$ technical replicates). (**D-H; continued on next page**) show biological repetitions of the analysis with dormant and sprouting tuber material. Panel D, F, H show the results from complex I activity staining, whereas the panels E, and G show results from complex IV activity staining.

Supplemental Figure S12 (continued)



Supplemental Figure S12 (continued) Analysis of supercomplex composition and respiratory activity of dormant and sprouting potato tubers.

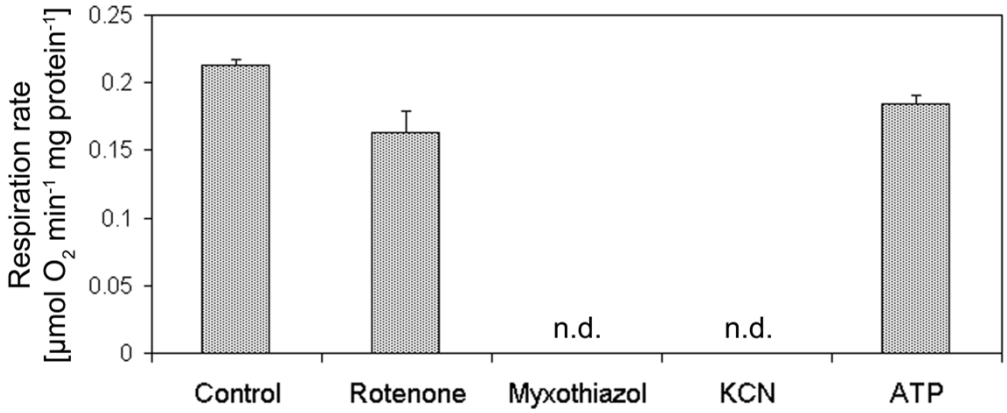
Supplemental Figure S13



Supplemental Figure S13. Supercomplex composition is not affected by inhibitors of mitochondrial respiration.

Activity staining of complex I (upper part) and complex IV (lower part) of supercomplexes isolated from potato tuber mitochondria that were incubated for 10 min with and without respiratory substrate or with and without respiratory inhibitors. Each control treatment contained 4 mM NADH and 750 µM ADP as substrate for respiration. The treatments from left to right are: with or without substrate (4 mM NADH and 750 µM ADP), 20 µM alamethicin with or without substrate, with or without 20µM myxothiazol, with or without 100µM rotenone, with or without 0.5 mM cyanide and with or without 750µM ATP.

Supplemental Figure S14



Supplemental Figure S13. Respiratory activity of potato tuber mitochondria supplied with NADH as substrate and various inhibitors of the mETC.

Respiratory activity of isolated potato tuber mitochondria supplied with 4 mM NADH, 750 µM ATP in an incubation medium with pH 7.5. Added affectors were used at the following concentrations: rotenone (100 µM), myxothiazol (20 µM), cyanide (500 µM) and ATP (750 µM). n.d.: not detectable. Values represent mean values +/- SE (n≥3).