

Biochemical background in mitochondria affects 2HG production by IDH2 and ADHFE1 in breast carcinoma

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Figure S1

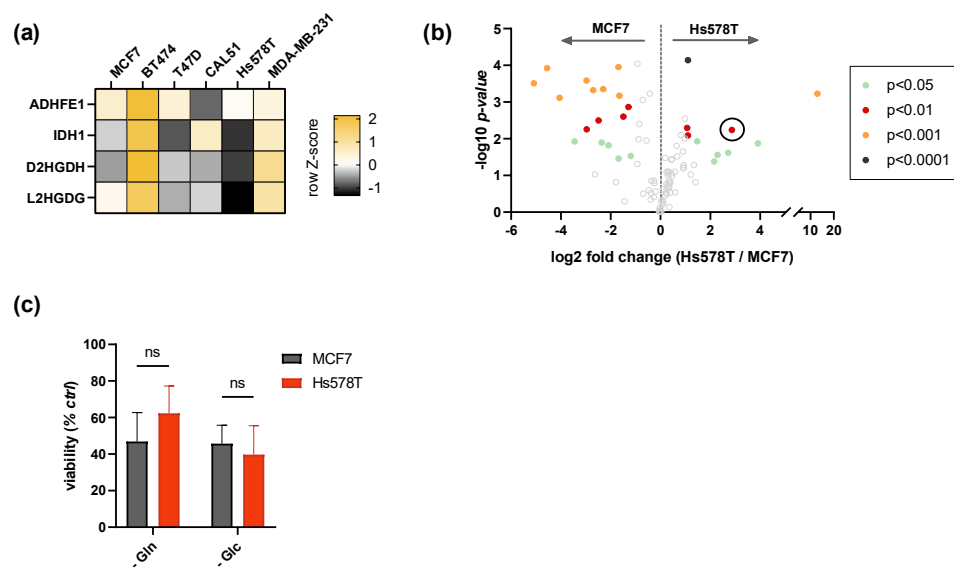


Figure S1. Supplemental graphs to Figure 1. **(a)** Heat-map of Z-score values for quantification of expression level of genes related to 2HG production measured by qPCR. **(b)** Volcano plot of polar metabolites derived from additional metabolomics experiment. 2HG is indicated in black circle. **(c)** Cell viability of MCF7 and Hs578T in glucose-free and glutamine-free media measured by neutral red. N > 5. One-way ANOVA. Cell viability of MCF7 and Hs578T in glucose-free and glutamine-free media measured by neutral red. N > 5. One-way ANOVA.

Figure S2

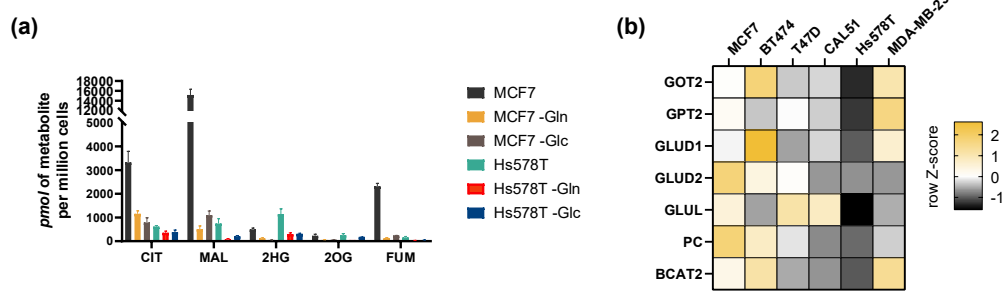


Figure S2. Supplemental graphs to Figure 2. **(a)** Metabolites of TCA cycle affected by removal of glutamine and glucose. **(b)** Heat-map of Z-score values for quantification of expression level of genes related to mitochondrial handling of glutamine measured by qPCR.

Figure S3

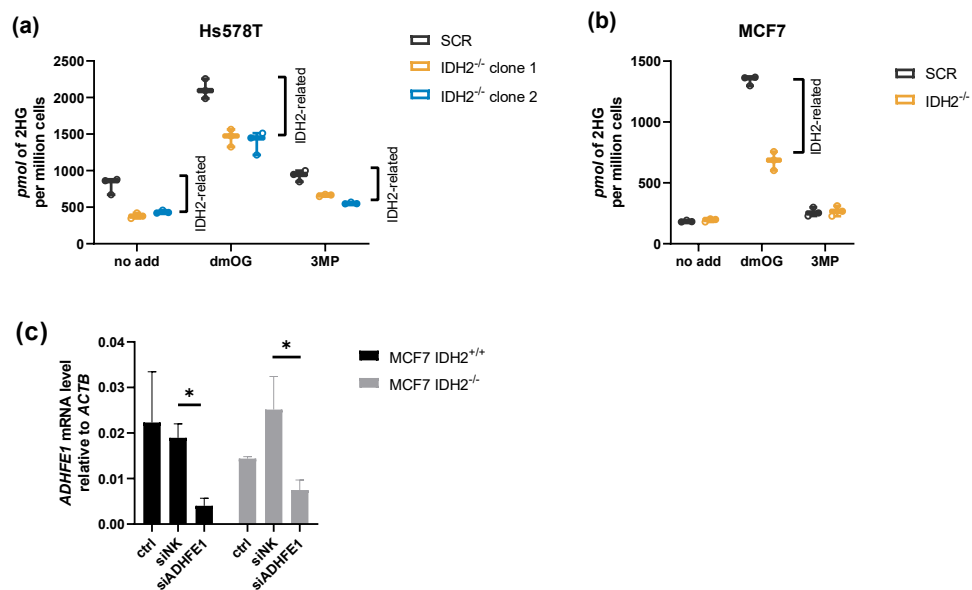


Figure S3. Supplemental graphs to Figure 3. The respective experiments were used for calculation of IDH2 related and unrelated 2HG production (Figure 3b) **(a)** 2HG production in Hs578T; non-treated cells, scrambled controls (SCR), and two populations of IDH2 KO cells. **(b)** 2HG production in MCF7 cells; non-treated cells, scrambled controls (SCR) and IDH2 KO cells. **(c)** Gene expression changes of ADHFE1 demonstrating knockdown of ADHFE1 in the experiment depicted in the Figure 3c.

Figure S4

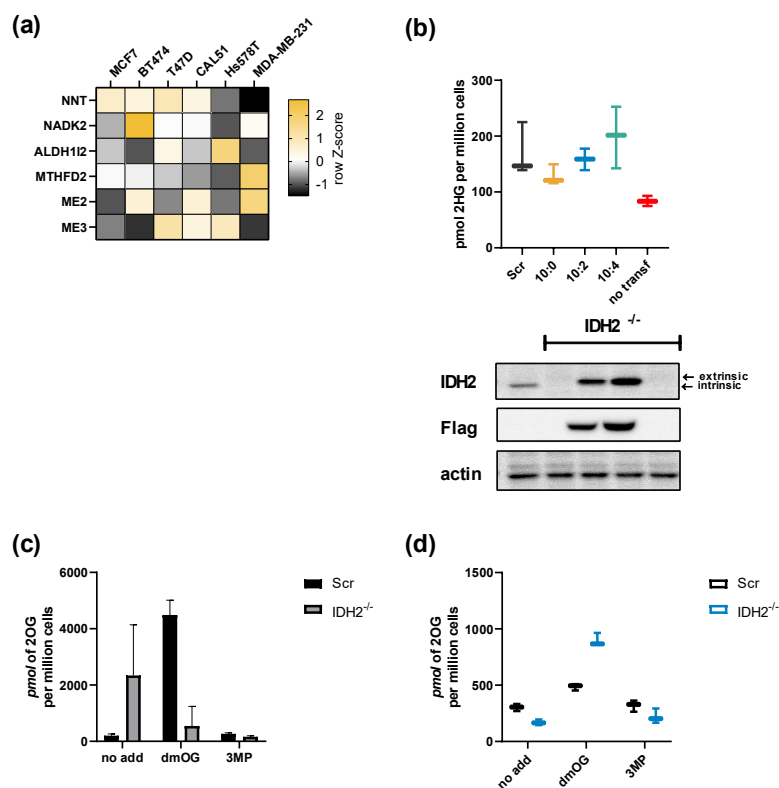


Figure S4. Supplemental graphs to Figure 4. **(a)** Heat-map of Z-score values for quantification of expression level of genes related to mitochondrial NADPH production measured by qPCR. **(b)** Production of 2HG: control experiment to Figure 4d; overexpression of IDH2 WT in IDH2 KO (MCF7) cells without co-overexpression of IDH2 R140Q. Below are western blots of the respective experiment. Lanes correspond to boxes in the graph above. **(c)** **(d)** 2OG production, experiment related to Figure 4e. 2OG production in Hs578T (SCR and IDH2 KO, respectively), overexpressing IDH2 R140Q treated with dmOG and 3MP. Right: same, in MCF7 SCR and KO cells.

Figure S5

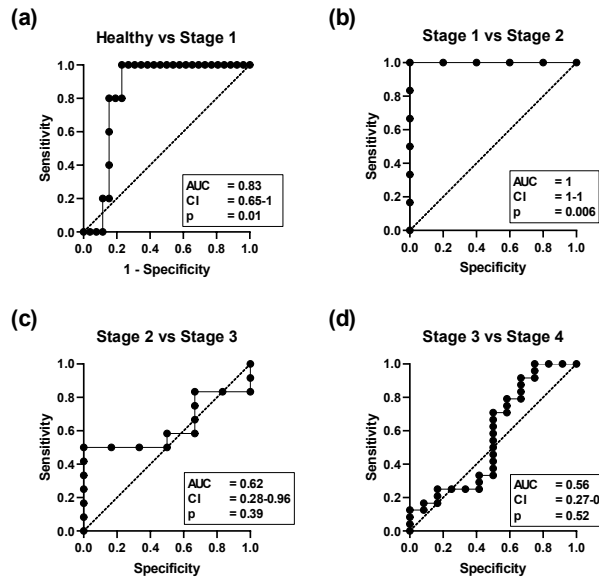


Figure S5. Supplemental graphs to Figure 5. (a) (b) (c) (d) ROC curves analysis of urine 2HG absolute levels between the indicated combinations of original patient's stages and derived areas under the curve (AUC), 99% confidence intervals (CI) and p values.

Figure S6

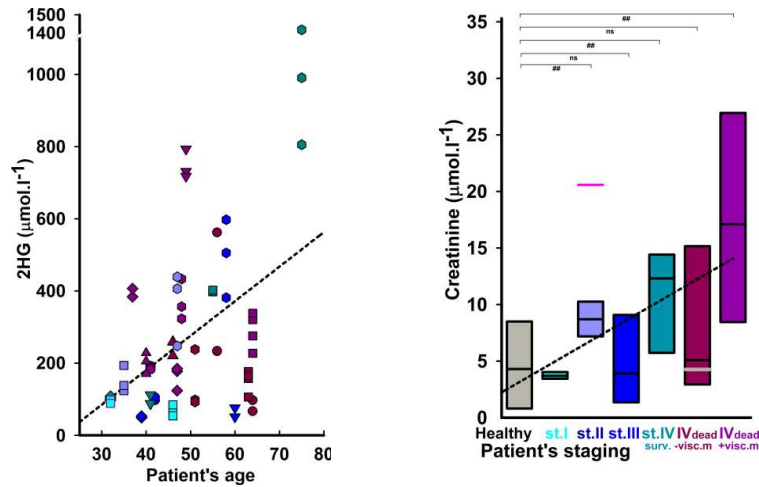


Figure S6. Creatinine values in the urine samples of breast carcinoma patients. (a) Creatinine in and patient's age. (b) Creatinine stratified by patients' stages.

Table S1. Cut-off values derived from the corresponding ROC curves. Cut-off values for the absolute 2HG levels (in $\mu\text{mol} \cdot \text{l}^{-1}$), or normalized to creatinine, 2OG, and citrate, respectively, between the major stage groups of patients.

Normalized to:	Healthy	cutoff	Stage I	cutoff	Stage II	cutoff	Stage III	cutoff	Stage IV
2HG		70		124		345		402	
Creatinine		18		26.8		41		87	
2OG		1.5		2		3.6		5.8	
Citrate		0.23		0.3		0.47		0.64	

Table S2. Stratification of patients. Summary of the treatment used in this study and urine 2HG levels ($\mu\text{mol}\cdot\text{l}^{-1}$) obtained by averages of average values for each patient. N = 28. 3- stands for the negativity of expression markers ER (estrogen receptor), PR (progesterone receptor), and HER2.

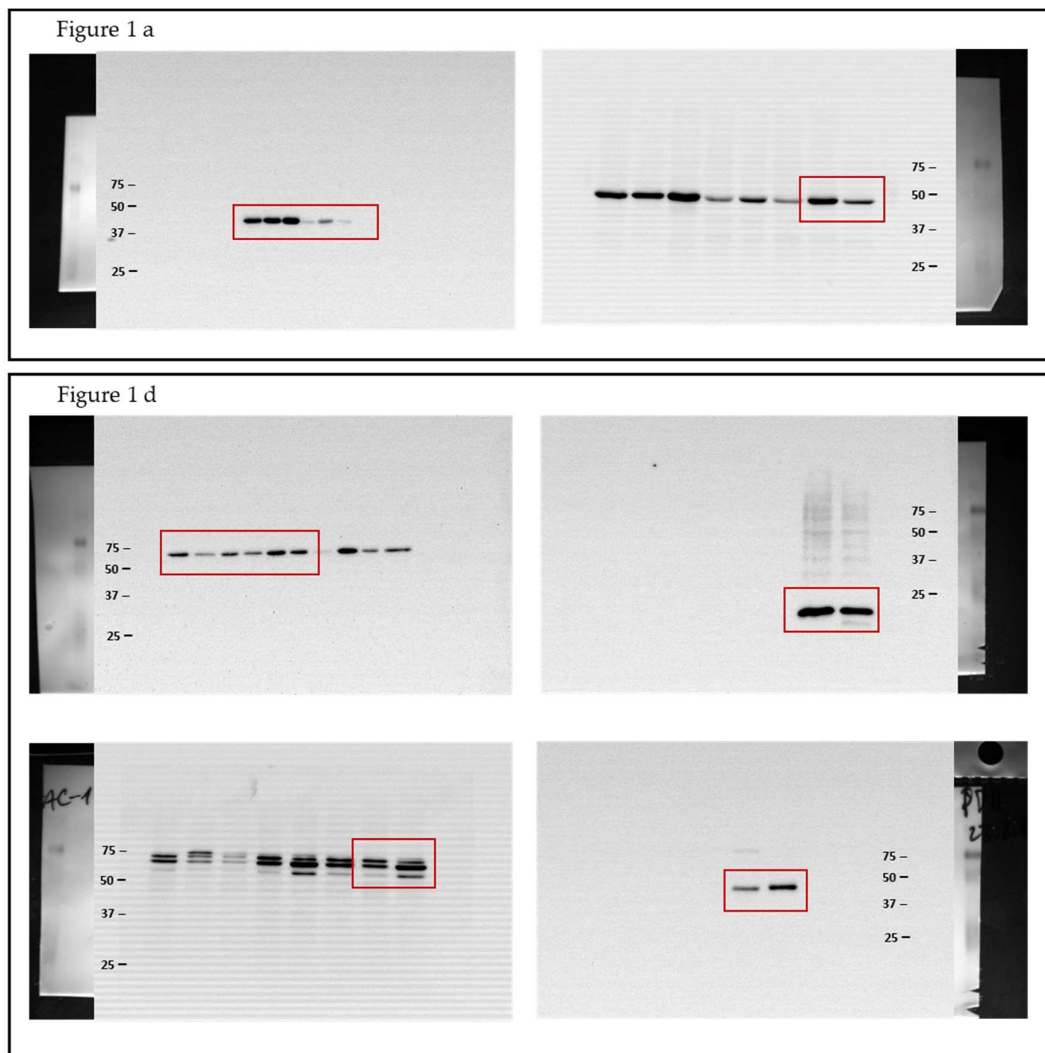
Disease info			Tretment / treatment details		Markers	Urine 2HG (nmol . L ⁻¹)	
Healthy			No treatment		N/A	33 ± 27	203 ± 152
Stage I	Remission	Localized	Adjuvant	ChemT	3-; BRCA+	82 ± 11	
Stage II	Remission	Advanced	Adjuvant	ChemT + Trastuzumab	3-; ER+	189 ± 130	
Stage III	Remission / Recurrence	Locally advanced	Adjuvant	No; Horm T	3-; ER+	202 ± 172	
				No; Chem T; Horm T	3-; 3-; BRCA+		
Stage IV	Surviving	Visceral metastases (not in 1 case)	Adjuvant/Palliative	Chem T + Horm T	BRCA+ ER+;	201 ± 140	315 ± 256
				Trastuzumab + Pertuzumab	HER2+ ER+;		
			Trastuzumab + Pertuzumab + Denosumab	HER2+			
	Dead	Only bone metastases	Adjuvant/Palliative	Trastuzumab; No; Chem T	HER2+; HER2+ ER+; same	156 ± 59	
				Trastu + Pertu; Horm T	HER2+ ER+; HER2+ ER+		
	Dead	Visceral metastases / terminal phase	Adjuvant/Palliative	No; Chem T; Chem T; Chem T Horm T; Chem T + Transtu; Kadcyla	3-; 3-; BRCA+, ER+; ER+; HER2+ ER+; HER2+ ER+	335 ± 191	

Table S3. List of primer sequences used for qPCR analysis in the study.

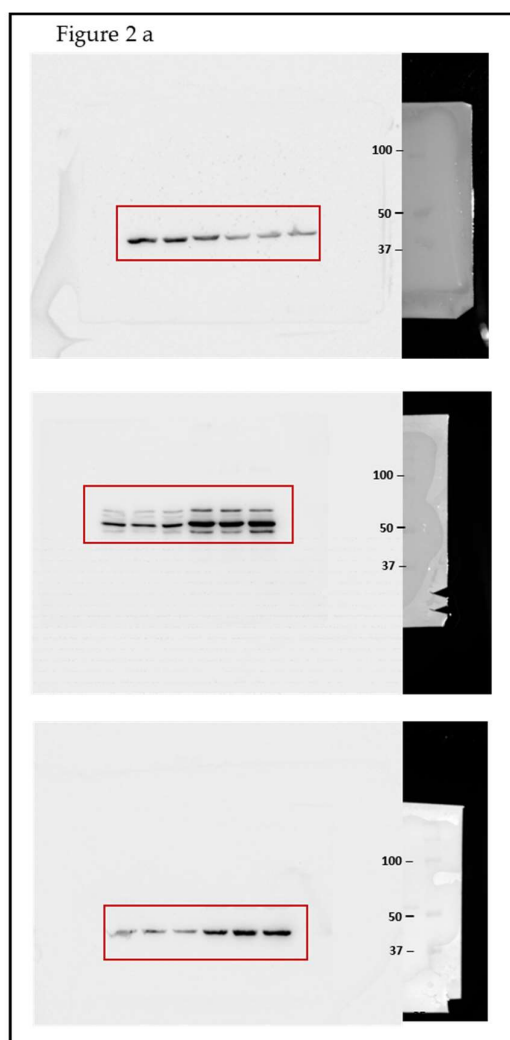
<i>gene</i>	<i>forward primer 5' > 3'</i>	<i>reverse primer 5' > 3'</i>
<i>hADHFE1</i>	ATGGCCTTCTGTGGTGCTC	GGTGTCGGCTCCCAGTATTT
<i>hD2HGDH</i>	TCCCCGTCTTTGACGAGATCA	AAGTCCCGTTCCTCCACATAC
<i>hL2HGDH</i>	CTGTTACCAGACTGGACATAAC	GCCTTTCTCATATAGGGCCTGA
<i>hIDH1</i>	GGGAGTCCGGTTTGGGATTG	TCTTACCACAGAACCGCCA
<i>hGOT2</i>	CCATGTATTCCAACCCTCCCC	GAGTCCGCATGCCAATGATG
<i>hGPT2</i>	CCCATCCCACAATATCCCCTC	GTTCCAGGGTTGATTATGCAG
<i>hGLUD1</i>	TCCAGCAGACATTAAGAGCCC	CAGCCAGACTCCAAACAGGG
<i>hGLUD2</i>	TCAATCCCAAGAACTATACGAAA	ATAGTGCCCTATGGTGCTGG
<i>hGLUL</i>	TCATCTTGCATCGTGTGTGTG	CTTCAGACCATTTCTCTCCCG
<i>hPC</i>	ACAGAGGTGAGATTGCCATCC	CACTGCATCTACGTTGTTCTCC
<i>hBCAT2</i>	GCTCAACATGGACCGGATG	CCGCACATAGAGGCTGGTG
<i>hNNT</i>	GGGGTCCTGTAAGGGTCTAC	ATGCCACTCGTTTCTCATTTT
<i>hME3</i>	TGAAGAAGCGCGGATACGATG	GAAAGCAGGGCGGGATTAGG
<i>hALDH1I2</i>	CTCCACTGGCCGGGTTTATT	AGCCAGAGGGTCAGCTTTTC
<i>hNADK2</i>	GCCTGCCCGTTCGATATACA	GTGCTCTACTGGCAGAAGT
<i>hME2</i>	ATATACACCGACGGTTGGTCT	CATCAGTCACTACAACAGCCTT
<i>hMTHFD</i>	TACTCCATGGGGTGTGTGGG	ATATTGTAACAGTGGCATCACCTCC
<i>hACTB</i>	GAGCACAGAGCCTCGCCTT	GTGTAGAGCCGCAGAAGCAG
<i>hPPIA</i>	GTATAAAAGGGCGGGAGGC	CTGCAAACAGCTCAAAGGAGAC
<i>hADHFE1</i>	TGCCTATGTTGCTGTCCGGTG	TCCCGGTTCTGAGGTAGTT
<i>hADHFE1</i>	TTGGCAAGTGCTTTTGCTGG	CATCTCCAGGTGTCGCTCTG

Figure S7

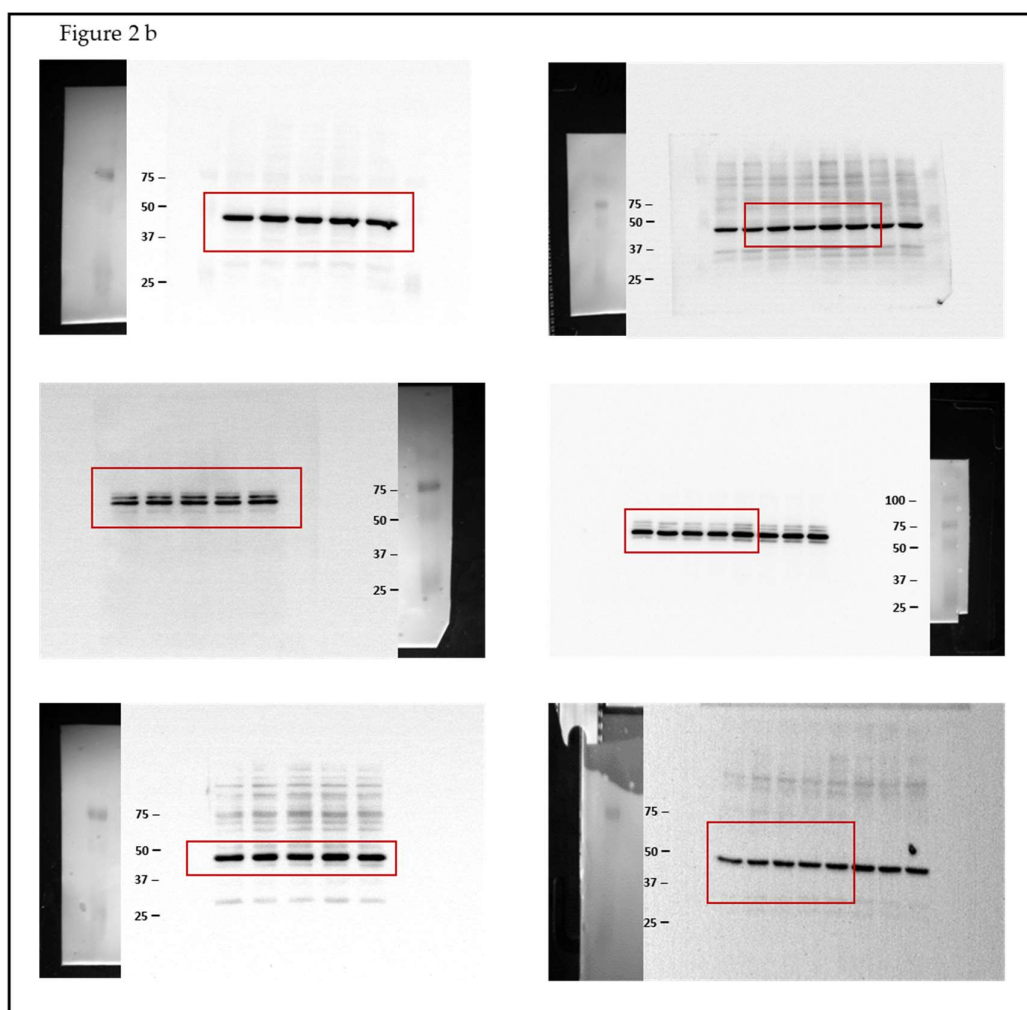
(a)



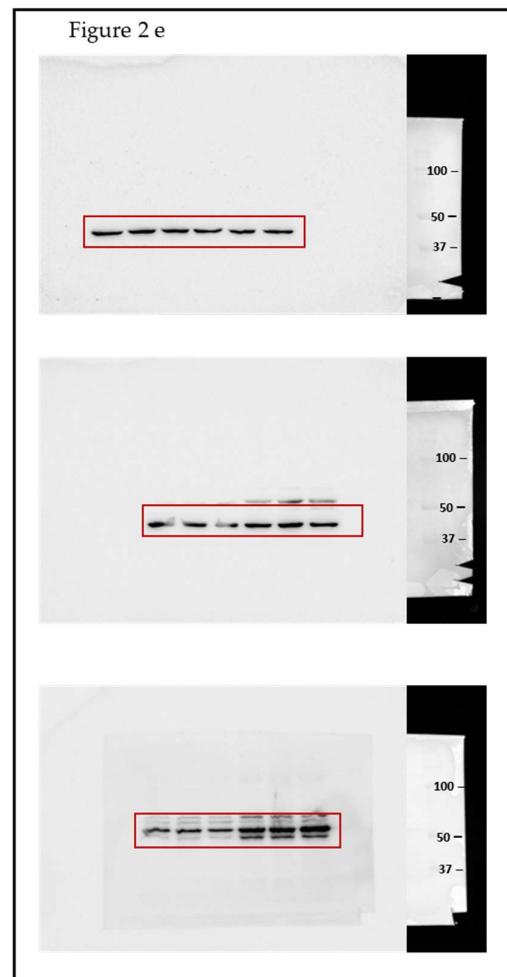
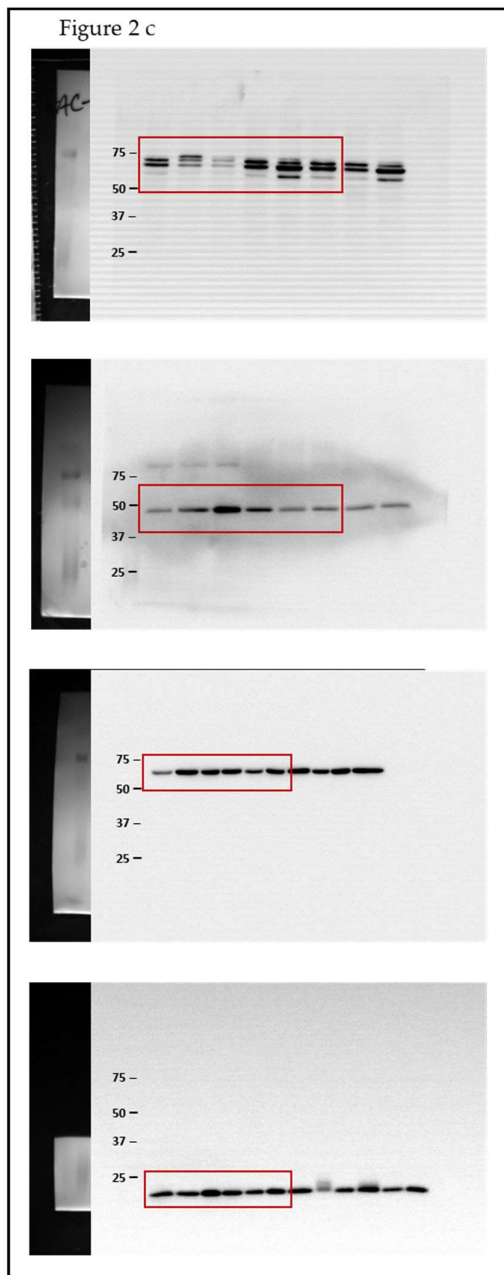
(b)



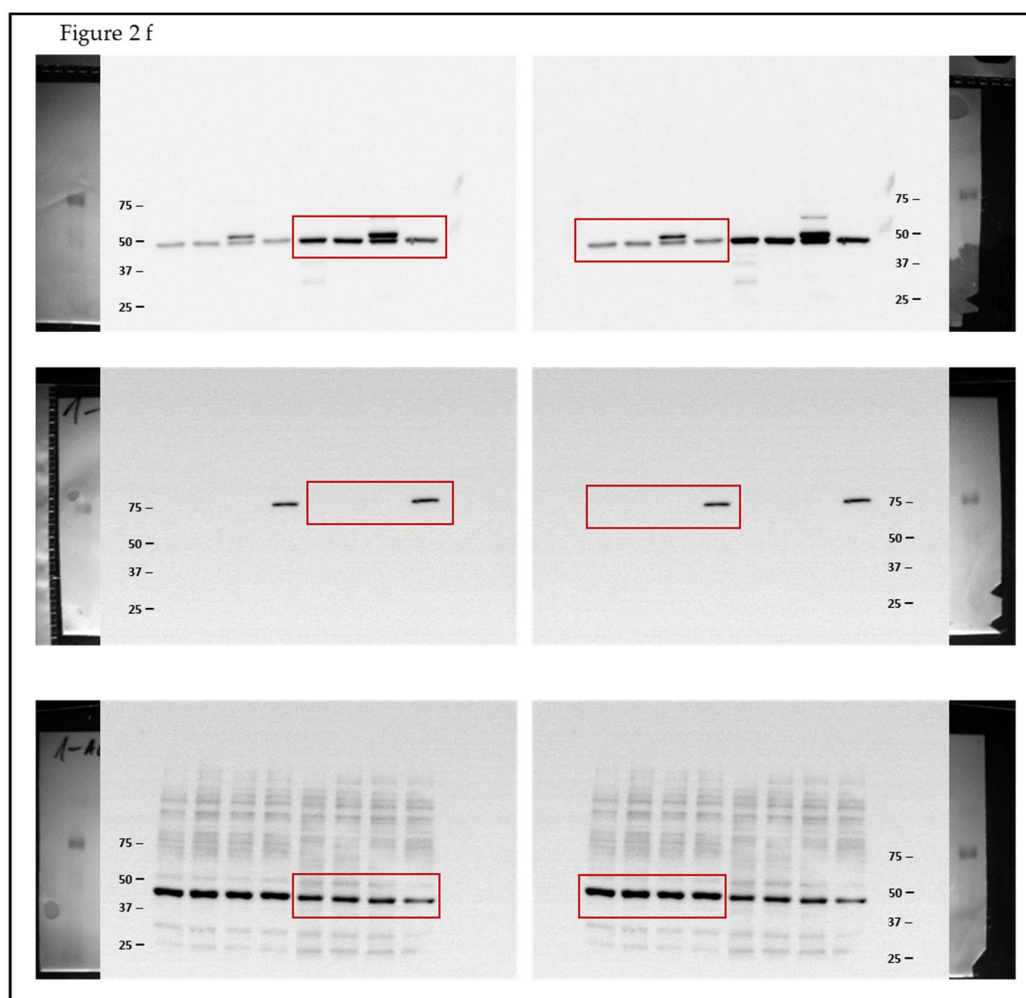
(c)



(d)



(e)



(f)

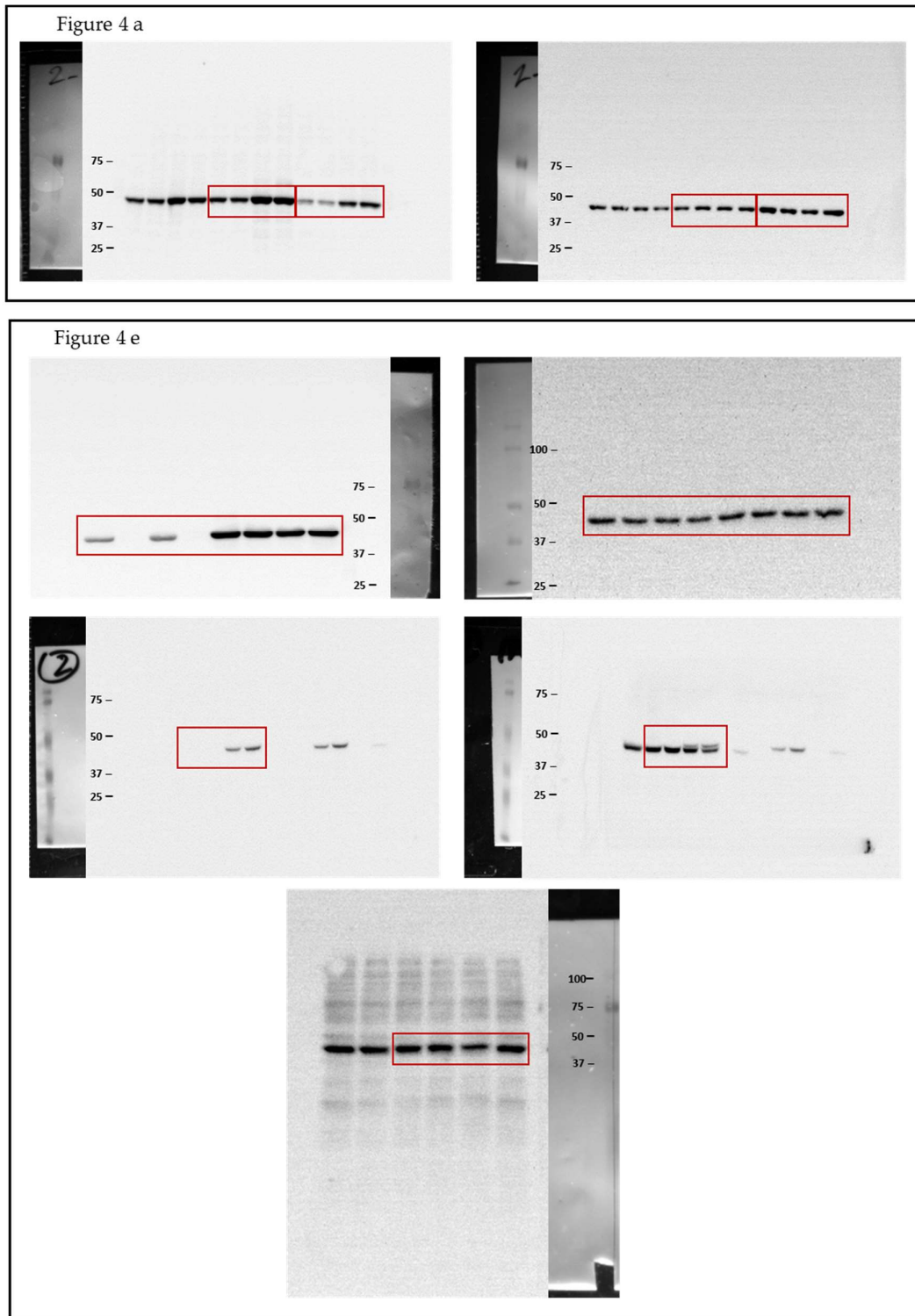


Figure S7. Full-length western blots presented in the manuscript. (a) (b) (c) (d) (e) (f) Western blots presented in the indicated figures.