

Supplemental Material to:

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**A novel anti-aldolase C antibody specifically interacts with
residues 85-102 of the protein**

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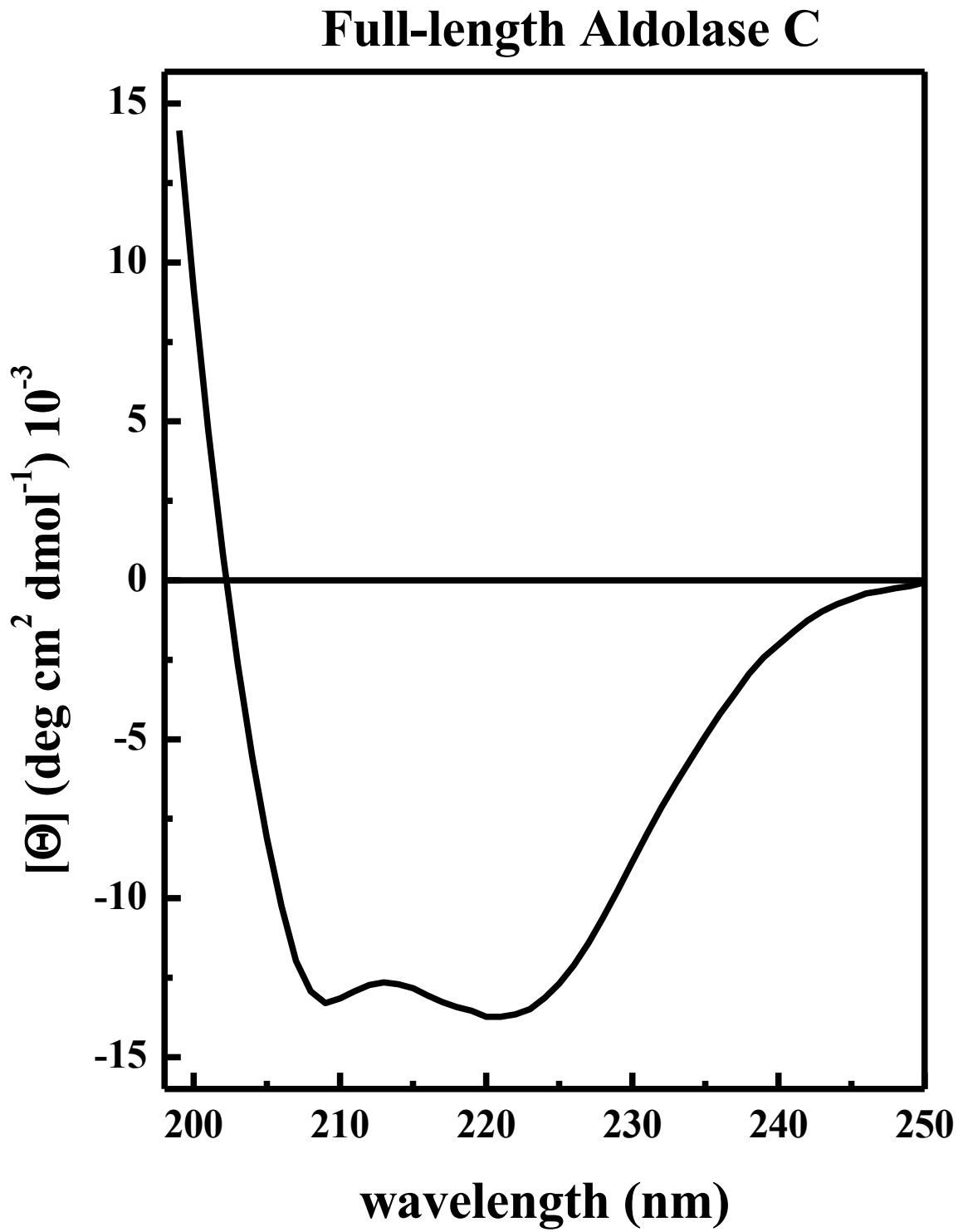
<https://www.landesbioscience.com/journals/mabs/article/28191/>

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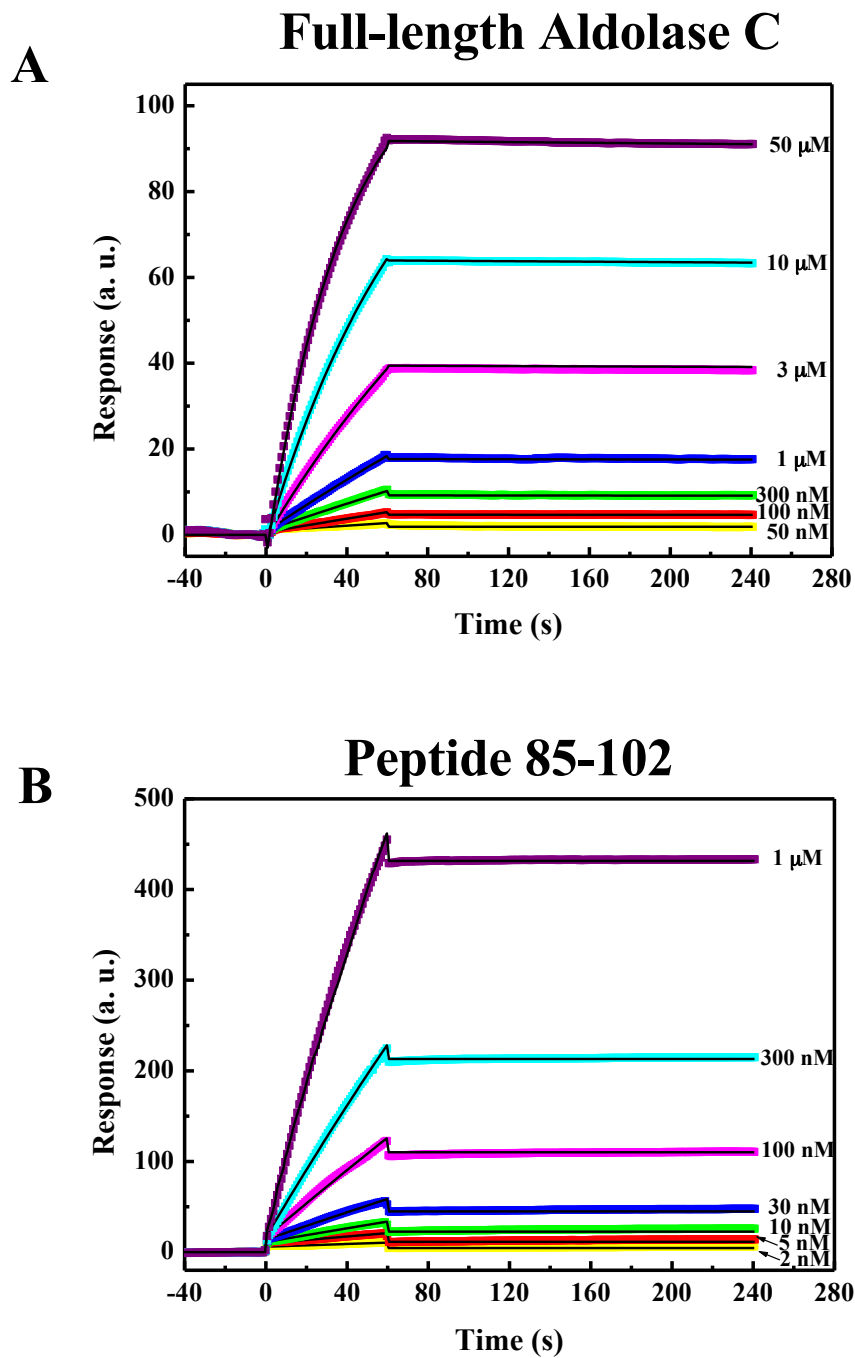
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**A novel anti-aldolase C antibody specifically interacts with residues 85-102 of
the protein**

Supplementary Figure 1: Far-UV CD spectrum of full length human aldolase C recorded under conditions described in the text.



Supplementary Figure 2: Time evolution SPR sensorgrams for the binding of 9F mAb with (A) full length human aldolase C and with (B) peptide 85-102. SPR sensorgrams (dot lines) are reported with the corresponding fit (solid lines), based on a 1:1 binding model. The sensorgrams were obtained at a contact time of 60 s, a dissociation time of 180 s, a flow rate of 60 $\mu\text{L}/\text{min}$ and at 9F mAb concentrations from 2 nM to 200 nM, as indicated.



Supplementary Table S1. Cloning of human aldolase C full length protein and fragments in the p3xFLAG CMV 7.1 vector (Sigma E4026).

Aldolase C PROTEIN REGION (clone name)	PRIMER PAIRS	PCR PRODUCT LENGTH (including primers)	MW of the fusion protein and total number of aa residues	Translation (including the 3xFLAG peptide at the N-term)
aa 1-364 (Full length protein or 3xFLAG-AldoC)	<p><i>hAldoC 1-364 aa Kpn1</i> 5'gggtacctcatATGCCTCACTCGTACC3'</p> <p><i>hAldoC 1-364aa Xba1</i> 5'tgctctagaTCAGTAGGCATGTTGGCAA3'</p>	1116 bp	43 kDa 390 aa	<p><u>MDYKDHDGDYKDHDIDYKDDDDKLE</u> <u>FMPHSYPALSAEQKKELSDIALRIVAP</u> GKGILAADESVGSMARLSQIGVENT EENRRLYRQVLFSAADDRVKKCIGGVI FFHETLYQKDDNGVPFVRTIQDKGIV VGIKVDKGVVPLAGTDGETTTQGLD GLSERCAQYKKGADFAKWRCVLKI SERTPSALAIENANVLARYASICQQ NGIVPIVEPEILPDGDHDLKRCQYVTE KVLAAVYKALSDHHVYLEGTLKPNM VTPGHACPIKYTPEEIAMATVTALRRT VPPAVPGVTFLSGGQSEEEASFNLN AINRCPLRPWALTFYGRALQASAL NAWRGQRDNAGAATEEFIKRAEVNG LAAQKYEKSGEDGGAAAQSLYIAN HAY</p>
aa 1-210 (clone 1)	<p><i>hAldoC 1-100aa Hind3-EcoR1</i> 5'actaagcttgaattcATGCCTCACTCGTACCCAGCCCTTT3'</p> <p><i>hAldoC 101-210aa NotI-XbaI</i> 5'tgatctagaggcggccgctcaCAAGACCTTCTCTGTAACATACTGACA3'</p>	666 bp	26 kDa 236 aa	<p><u>MDYKDHDGDYKDHDIDYKDDDDKLE</u> <u>FMPHSYPALSAEQKKELSDIALRIVAP</u> GKGILAADESVGSMARLSQIGVENT EENRRLYRQVLFSAADDRVKKCIGGVI FFHETLYQKDDNGVPFVRTIQDKGIV VGIKVDKGVVPLAGTDGETTTQGLD GLSERCAQYKKGADFAKWRCVLKI SERTPSALAIENANVLARYASICQQ NGIVPIVEPEILPDGDHDLKRCQYVTE KVL</p>
aa 1-100 (clone 1A)	<p><i>hAldoC 1-100aa Hind3-EcoR1</i> 5'actaagcttgaattcATGCCTCACTCGTACCCAGCCCTTT3'</p> <p><i>hAldoC 1-100aa NotI-XbaI</i> 5'tgatctagaggcggccgctcaATCCGATGGTTTCGGACGAAGGCAA3'</p>	336 bp	15 kDa 126 aa	<p><u>MDYKDHDGDYKDHDIDYKDDDDKLE</u> <u>FMPHSYPALSAEQKKELSDIALRIVAP</u> GKGILAADESVGSMARLSQIGVENT EENRRLYRQVLFSAADDRVKKCIGGVI FFHETLYQKDDNGVPFVRTIQD</p>
aa 101-210 (clone 1B)	<p><i>hAldoC 101-210aa Hind3-EcoR1</i> 5'actaagcttgaattcAAGGGCATCGTCTGGGCATCAAGGT3'</p> <p><i>hAldoC 101-210aa NotI-XbaI</i> 5'tgatctagaggcggccgctcaCAAGACCTTCTCTGTAACATACTGACA3'</p>	366 bp	16 kDa 136 aa	<p><u>MDYKDHDGDYKDHDIDYKDDDDKLE</u> <u>FKGIVVGIKVDKGVVPLAGTDGETTT</u> <u>QGLDGLSERCAQYKKGADFAKWR</u> <u>CVLKISERTPSALAIENANVLARYASI</u> <u>CQQNGIVPIVEPEILPDGDHDLKRCQ</u> <u>YVTEKVL</u></p>

<p>aa 101-300 (clone 2)</p>	<p><i>hAldoC 101-210aa Hind3-EcoR1</i> 5'actaagcttgaattcAAGGGCATCGTCGTGGGCATCAAGGT3'</p> <p><i>hAldoC 210-300aa NotI-XbaI</i> 5'tgatctagaggcggccgctcaGAAGGTAAGCGCCCAGGGTCGGGAA3'</p>	<p>636 bp</p>	<p>25 kDa 226 aa</p>	<p><u>MDYKDHDGDYKDHDIDYKDDDDKLE</u> <u>FKGIVVGIKVDKGVVPLAGTDGETTT</u> <u>QGLDGLSERCAQYKKDGADFAKWR</u> <u>CVLKISERTPSALAILENANVLARYASI</u> <u>CQQNGIVPIVEPEILPDGDHDLKRCQ</u> <u>YVTEKVLAAVYKALSDHHVYLEGTLL</u> <u>KPNMVTPGHACPIKYTPEEIAMATVT</u> <u>ALRRTVPPAVPGVTFSLGGQSEEEA</u> <u>SFNLNAINRCPLRPWALTF</u></p>
<p>aa 210-364 (clone 3)</p>	<p><i>hAldoC 210-300aa Hind3-EcoR1</i> 5'actaagcttgaattcGCTGCTGTGTACAAGGCCCTGAGTGA3'</p> <p><i>hAldoC 299-364aa NotI-XbaI</i> 5'tgatctagaggcggccgcTCAGTAGGCATGGTTGGCAATGTAGAA3'</p>	<p>504 bp</p>	<p>20 kDa 182 aa</p>	<p><u>MDYKDHDGDYKDHDIDYKDDDDKLE</u> <u>FAAVYKALSDHHVYLEGTLLKPNMVT</u> <u>PGHACPIKYTPEEIAMATVTALRRTV</u> <u>PPAVPGVTFSLGGQSEEEASFNLNAI</u> <u>NRCPLRPWALTFTFSYGRALQASA</u> <u>LNAWRGQRDNAGAATEEFIKRAEVN</u> <u>GLAAQKYEKSGEDGGAAAQSLYIA</u> <u>NHAY</u></p>

Supplementary Table S2. Cloning of human aldolase C peptides in the pEGFP-C2 expression vector (Clontech, catalog n. 6083-1).

Aldolase C peptidic residues (clone name)	PRIMER PAIRS	Recombinant product
aa 2-17 (pEGFP-C2 +Aldolase C aa 2-17)	<i>AldoC_2-17s</i> 5'TCGAGCGGGAGTGGGAGTGGACCTCACTCGTACCCAG CCCTTTCTGCTGAGCAGAAGAAGGAGTTGTCTTGAG 3' <i>AldoC_2-17as</i> 5'GATCCTCAAGACAACCTCTTCTTGCTCAGCAGAAAG GGCTGGGTACGAGTGAGGTCCACTCCCCTCCCGC 3'	GFP-GSGSG- PHSYPALSAEQKKELS
aa 41-58 (pEGFP-C2 +Aldolase C aa 41-58)	<i>AldoC_41-58s</i> 5'TCGAGCGGGAGTGGGAGTGGAGCCAAGCGGCTGAGC CAAATTGGGGTGGAAAACACAGAGGAGAACCGCCGGCT GTGAG3' <i>AldoC_41-58as</i> 5'GATCCTCACAGCCGGCGGTTCTCCTCTGTGTTTTCCAC CCCAATTTGGCTCAGCCGCTTGGCTCCACTCCCCTCC GC3'	GFP-GSGSG- AKRLSQIGVENTEENRRL
aa 60-75 (pEGFP-C2 +Aldolase C aa 60-75)	<i>AldoC_60-75s</i> 5'TCGAGCGGGAGTGGGAGTGGACGCCAGGTCCTGTTC AGTGCTGATGACCGTGTGAAAAGTGCATTGGATGAG3' <i>AldoC_60-75as</i> 5'GATCCTCATCCAATGCACTTTTTTACACGGTCATCAGC ACTGAACAGGACCTGGCGTCCACTCCCCTCCCGC3'	GFP-GSGSG- RQVLFSADDRVKKCIG
aa 85-102 (pEGFP-C2 +Aldolase C aa 85-102)	<i>AldoC_85-102s</i> 5'TCGAGCGGGAGTGGGAGTGGATACCAGAAAGATGATA ATGGTGTTCCTTCGTCCGAACCATCCAGGATAAAGGGCT GAG3' <i>AldoC_85-102as</i> 5'GATCCTCAGCCCTTATCCTGGATGGTTCCGACGAAGG GAACACCATTATCATCTTTCTGGTATCCACTCCCCTCC GC3'	GFP-GSGSG- YQKDDNGVPFVRTIQDKG