SUPPORTING INFORMATION

Hayward et al

ANKRD9 IS A METABOLICALLY-CONTROLLED REGULATOR OF IMPDH2 ABUNDANCE AND MACRO-ASSEMBLY

LEGENDS FOR SUPPORTING FIGURES 1-7

Figure S1: ANKRD9 and IMPDH2 interact in rods
HeLa cells were transfected with WT-FLAG ANKRD9 and subjected to 48 hours of 0.1% FBS. Cells were then homogenized and centrifuged at the indicated speeds. Cells were spun at 500*xg* for 5 minutes, 6000*xg* for 15 minutes, and 10000x*g* for 30 minutes and samples from the supernatant (S) and pellet (P) were taken for western blot analysis. The FLAG-ANKRD9 and IMPDH2 positive bands appear in the 500*xg* and 6000x*g* pellet fraction, typically referred to as the nuclear and mitochondrial fraction. Sedimentation of 35 kDa FLAG-ANKRD9 and 55kDa IMPDH2 in these heavy fractions is consistent with FLAG-ANKRD9 and IMPDH2 interactions in large macromolecular complex, as rods. The representative image from three independent experiments.

Figure S2: Serum re-addition partially reverses ANKRD9 rod formation (a) and copper chelation does not trigger transition of ANKRD9 to rods (b).
(a) HeLa cells were transfected with FLAG-tagged ANKRD9 and incubated under nutrient limiting conditions (0.1% FBS) for 24 hours, then cells growth medium was replaced with the medium containing 10% FBS. Cells were incubated for another 24 hours and then immuno-stained for FLAG. The 24 hours nutrient limitation induces ANKRD9 transition from vesicle-like structures to rods in approximately 50% of the cells (see Fig. 2 in the text). Placing cells in 10% serum for 24 hours partially reverses this phenotype (i.e some cells still have ANRKD9 in rods (long and short) and other cells show mostly punctate/vesicle-like pattern. N = 2. Scale bar 20 μM. **(b)** HeLa cells were transfected with FLAG-ANKRD9 and incubated with 10 mM of intracellular copper chelator TTM for 48 hours in 10% FBS for 48 hours to limit copper availability. This treatment does not trigger conversion of ANKRD9 to rods. N = 3. Scale bar 20 μM.

Figure S3: ANKRD9 R125A mutant does not show a vesicle-like pattern
(a) Sequence alignment of ANKRD9 orthologues with the CysCys motif high-lighted in yellow and the conserved arginine (Arg125 in human ANKRD9) highlighted in cyan; **(b)** ANKRD9 model (left panel) and a close-up view (right panel) of the locations of Cys109, Cys110, and Arg125 residues (**b**) HeLa cells were transfected with FLAG-ANKRD9 containing a point mutation at Arg125 in basal medium (10% FBS) and stained for FLAG and IMPDH2. ANKRD9 does not show the vesicle-like pattern observed for wt ANKRD9 (see Fig. 1 of main text) and does not appear to diminish IMPDH2 staining. (**c**) Western blot analysis of the R125A mutant. HeLa cells transfected with FLAG-ANKRD9 R125A and WT as control were blotted for Na+/K+ ATPase as a loading control and FLAG.

Figure S4: Docking model of ANKRD9 and IMPDH2
(a) The crystal structure of the protein IMPDH2 (PDB ID: 1nf7) has missed regions. These were 'repaired' using the homology modeling of the MOE program. To create a homology model, a full sequence of the protein was used. The inserted residues are colored in magenta. The MOE program created 10 pro-models. The final model was based on the best-scoring intermediate model. The scores include the RMSD of each intermediate model to the average position of all of the intermediate models; the electrostatic solvation energy[1], a knowledge-based residue packing quality function and an estimation

of the effective atomic contact energy (ACE)[2,3]. **(b)** Two views of the chose homology model are shown. **(c)** Using the Dock application of the Compute module in MOE, possible docking configurations between the homology model of IMPDH2 and ANKRD9 were found. *Upper panel:* the scoring function for various members of docking poses. The poses 14 and 45 have the same best score 5. At the same time the pose 14 has free energy -16.17 kcal/mole and pose 45 has free energy 21.55 kcal/mole. So the pose 45 was selected as a best docking configuration. *Bottom panel:* Conserved residues Pro103, Ser104, Cys109, Cys110, Arg155, Val156, Glu157 and Thr161 (highlighted) on the ANKRD9 protein were used to define the preferred region of docking. The search was restricted to 100 placements and the placement with the best score was selected (see Fig. 7a in main text). The best scoring configuration had 5 contacts between heavy atoms of conserved residues from ANKRD9 to the residues of the IMPDH2 protein. In the site of interest Cys109 hydrophobic atoms CB and CA interact with hydrophobic atom CG of Glu168 of IMPDH2 (3.0 and 3.10 angstroms, respectively). The backbone O atom of Cys109 interacts with positively charged atom NZ (3.31 angstroms) of Lys167. The hydrophobic atoms CA and SG of the second member of the CysCys motif—Cys110—interact with atoms CD and CE of Lys167.

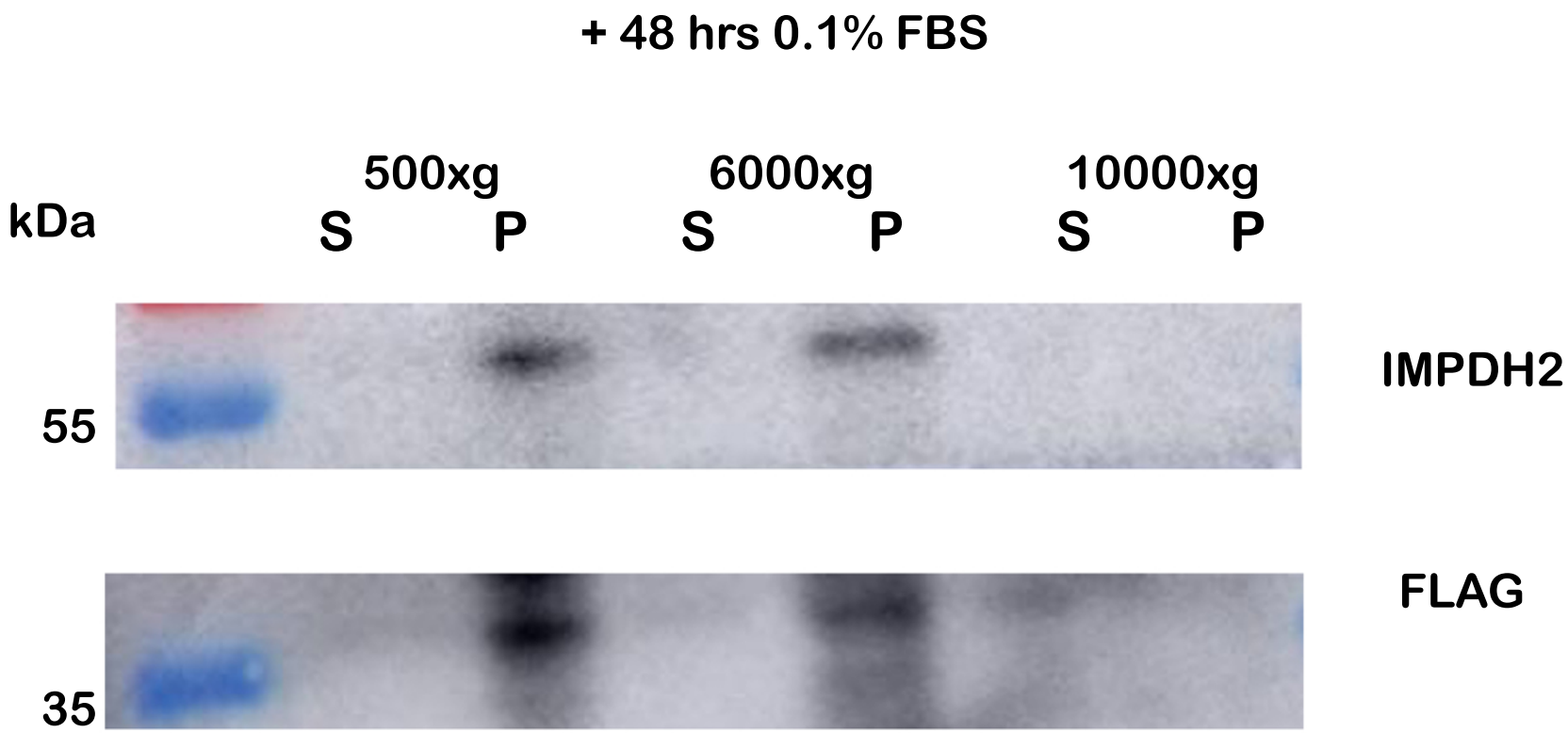
Figure S5: ANKRD9 is predicted to bind to the IMPDH2 regulatory domain in the vicinity of the GDP/GTP binding site.
(a) Regulatory domains of IMPDH2 dimer (PDB ID 6I0M) are shown in green and blue, GTP is in red, the $K^{167}E^{168}E^{169}$ motif (that is predicted to interact with the ANKRD9 CysCys-containing loop, as indicated by the arrow) is colored in pink

Figure S6: ANKRD9 and IMPDH2 interactions are specific
(a) HeLa cells were transfected with FLAG-ANKRD9 and immune-stained for FLAG and CTPS2 under basal growth conditions (10% FBS). Expression of ANKRD9 does not alter CTPS2 pattern/abundance, in contrast to IMPDH2 (see Fig. 7b,d in main text) which is markedly diminished under the same conditions; n = 2. (b) HeLa cells were transfected with FLAG-ANKRD9, treated with 10 μM proteasome inhibitor MG132 for 4 hours and stained for FLAG and IMPDH2. The several images shown shows that MG132 restores IMPDH2 staining in cells with low/medium levels of ANKRD9; n = 3. Scale bar: 20 μM

Figure S7: Recombinant ANKRD9 has vesicular-like pattern and decreases IMPDH2 staining in HEK293. HEK293A cells were transfected with FLAG-ANKRD9 under basal (10% FBS) conditions and immuno-stained for FLAG and IMPDH2. Cells expressing ANKRD9 (red) lack IMPDH2 staining (green); n = 2. Scale bar: 20 μM.

References for Figure S4
1. Labute P. The generalized born / volume integral (GB/VI) implicit solvent model: Estimation of the free energy of hydration using London dispersion instead of atomic surface area. *J Comp Chem* 2008; **29**:1693–1698)
2. Miyazawa S, Jernigan RL; Estimation of effective inter-residue contact energies from protein crystal structures: Quasi-chemical approximation. *Macromolecules* 1985; **18**:534–552;
3. Zhang C, Vasmatzis G, Cornette JL, DeLisi C. Determination of atomic desolvation energies from the structures of crystalized proteins. *J Mol Biol* 1997; **267**:707–726.

a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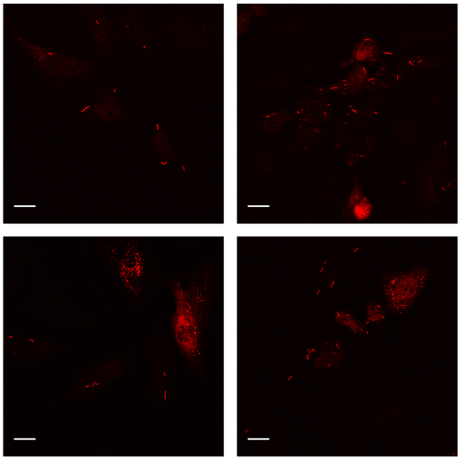
6000xg
10000xg
kDa
S
P
S
P
S
P
55
IMPDH2
35
FLAG

a

FLAG-ANKRD9: +24 hr 0.1% FBS,+24 hr 10% FBS



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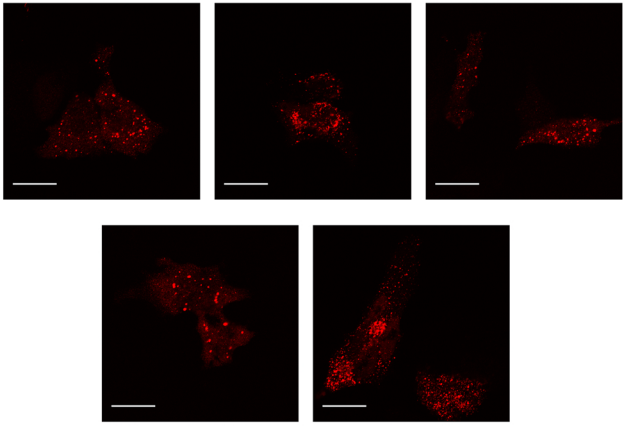
FLAG-ANKRD9: 10 micromolar TTM 48 hrs 10% FBS



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R.norvegicus    92 TFPRRALAPPSAGFRCCTAP-GPHVALAVRYNRGILRRILRTVQDFPV  140
P.lepturus      67 KFPQSALAVPSQSFSCCQSS-APHLAMAVRYNRVRILFRILKAIQAFPL 115
D.rerio         84 LFSSRALEMPSRSFCCCQASTAPHLSIAVRYNRINILKMMMETIKELAD 132
A.calliptera    84 RYSVSALRAPRCSYCCRGSGAPHLNIAVRYDRLVILGMMMAALKNCGE 132
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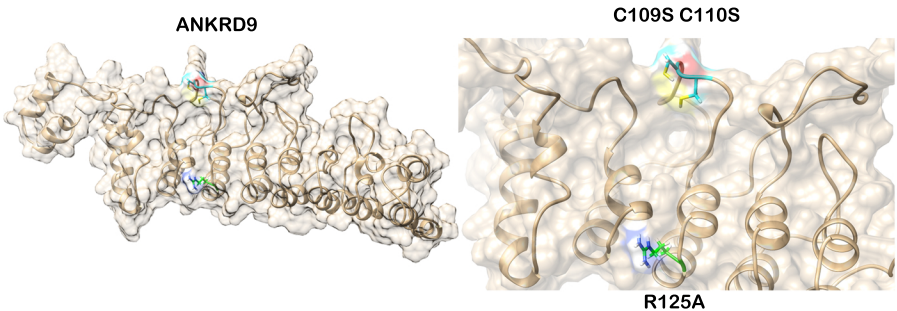

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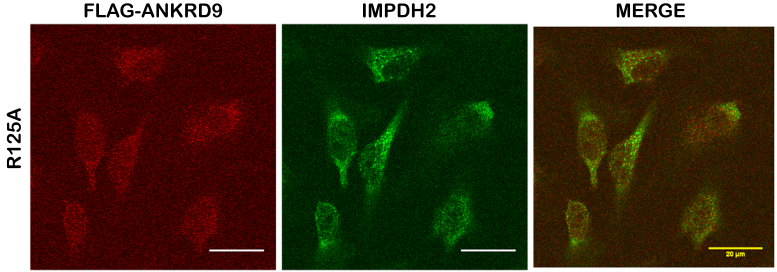

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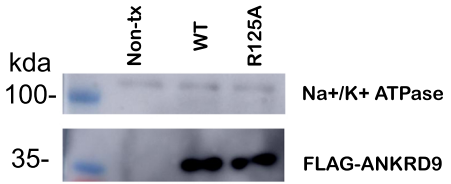

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IMPDH2    61 LKTPLVSSPMDTVTEAGMAIAMALTGGIGFIHHNCTPEFQANEVRKVKKYEQGFITDPVV   120

1NF7A.A  112 LSPK--------------------------------GIISSRDIDFL---EHDCFLEEIMT  137
IMPDH2   121 LSPKDRVRDVFEAKARHGFCGIPITDTGRMGSRLVGIISSRDIDFLKEEEHDCFLEEIMT  180

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IMPDH2   241 KKQLLCGAAIGTHEDDKYRLDLLAQAGVDVVVLDSSQGNSIFQINMIKYIKDKYPNLQVI  300

1NF7A.A  257 GGNVVTAAQAKNLIDAGVDALRVGMGSGSICITQEVLACGRPQATAVYKVSEYARRFGVP  316
IMPDH2   301 GGNVVTAAQAKNLIDAGVDALRVGMGSGSICITQEVLACGRPQATAVYKVSEYARRFGVP  360

1NF7A.A  317 VIADGGIQNVGHIAKALALGASTVMMGSLLAATTEAPGEYFFSDGIRLKKYRGMGSLDAM  376
IMPDH2   361 VIADGGIQNVGHIAKALALGASTVMMGSLLAATTEAPGEYFFSDGIRLKKYRGMGSLDAM  420

1NF7A.A  377 ----------------IKVAQGVSGAVQDKGSIHKFVPYLIAGIQHSCQDIGAKSLTQVR  420
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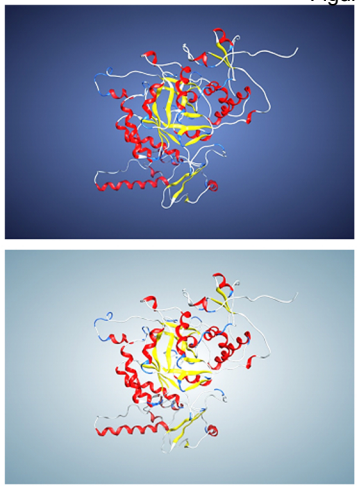

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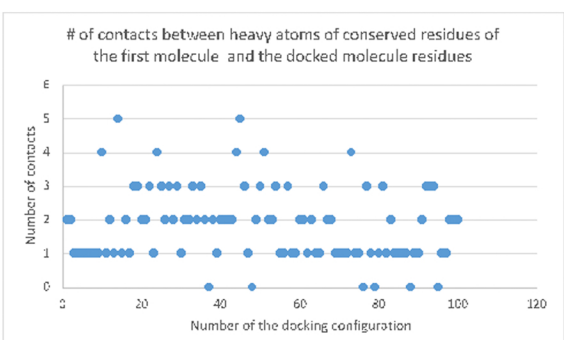


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ANKR9    121 VRYNRVGILR RILRTLRDFP AEERARVLDR RGCSRVEGGG TSLHVACELA RPECLFLLLG 180
ANKR9    181 HGASPGLRDG GGLTPLELLL RQLGRDAGAT PSAAGAPASA PGEPRQRRLL LLDLLALYTP 240
ANKR9    241 VGAAGSARQE LLGDRPRWQR LLGEDKFQWL AGLAPPSLFA RAMQVLVTAI SPGRFPEALD 300
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a **IMPDH2 Regulatory Domain with GTP/GDP binding**

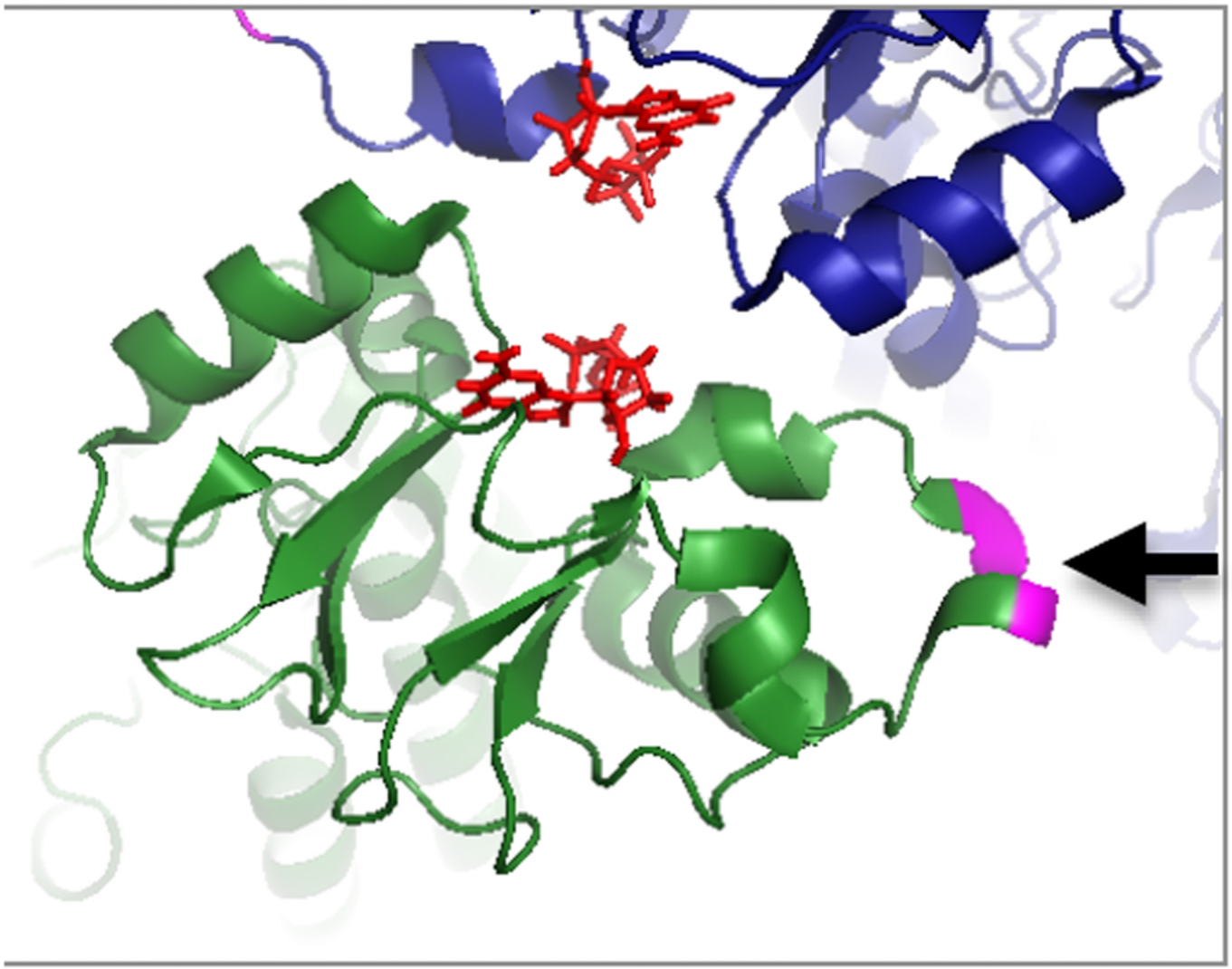
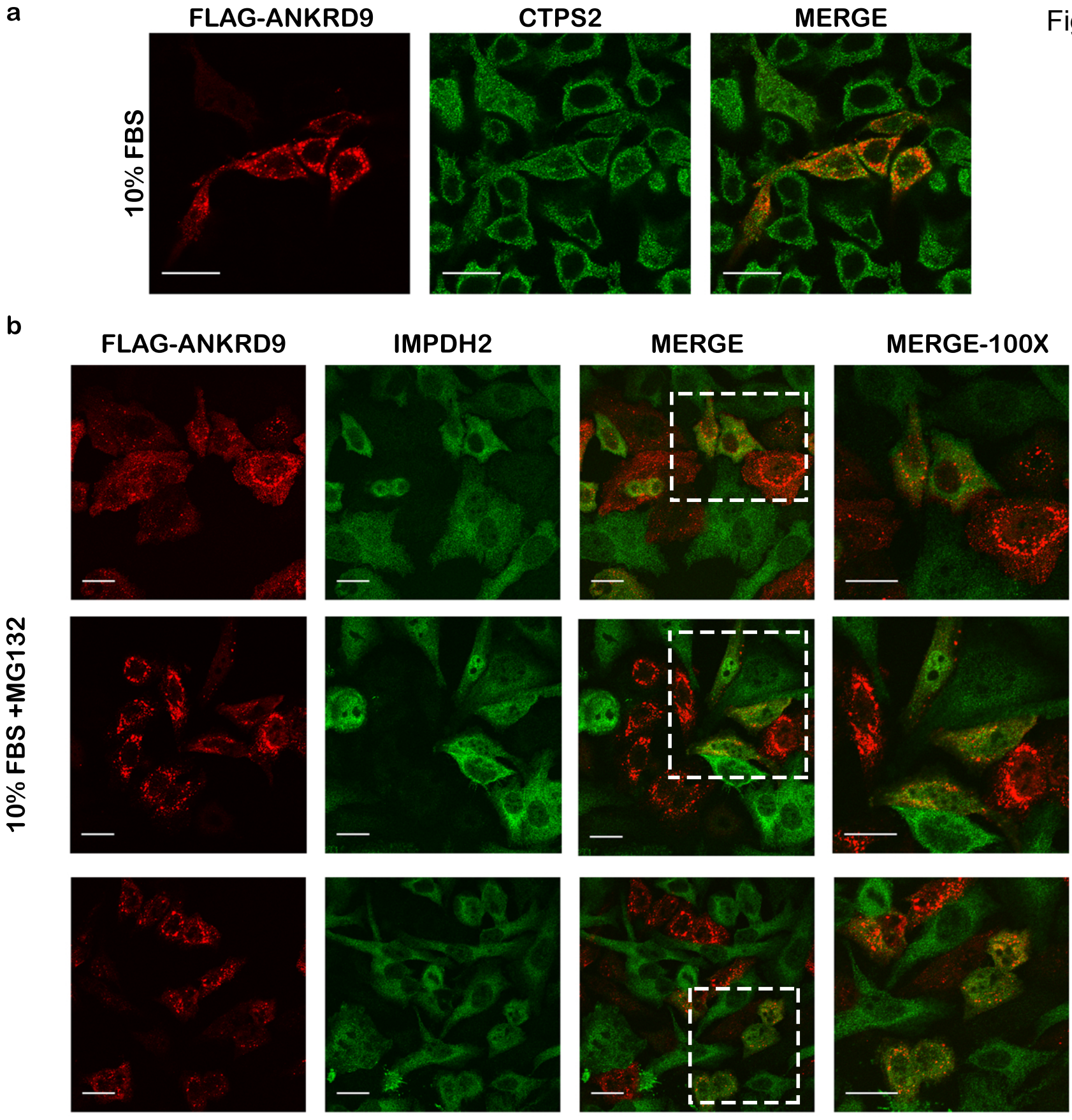


Figure 6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CTPS2
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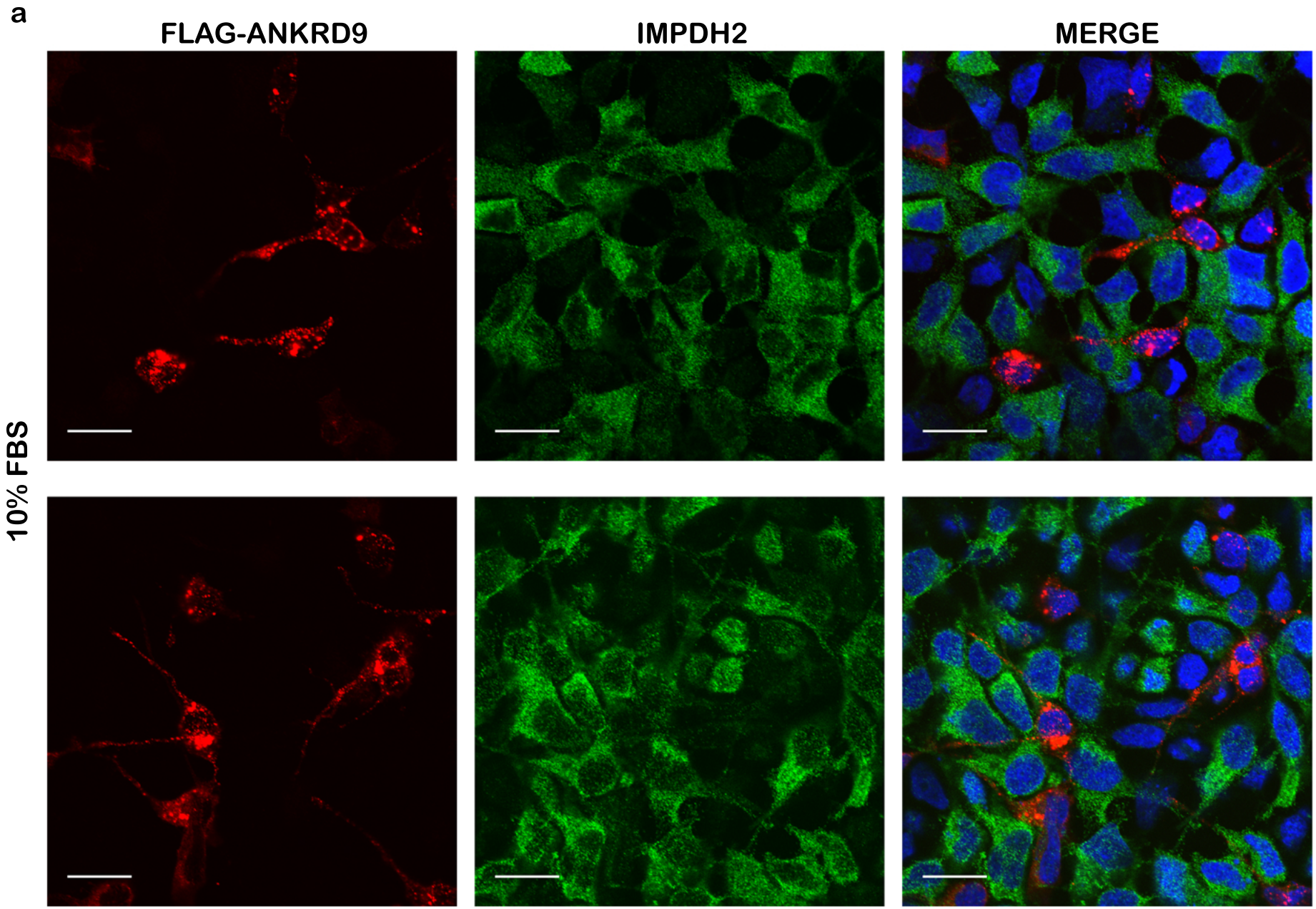
IMPDH2
MERGE
MERGE-100X
10% FBS +MG132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