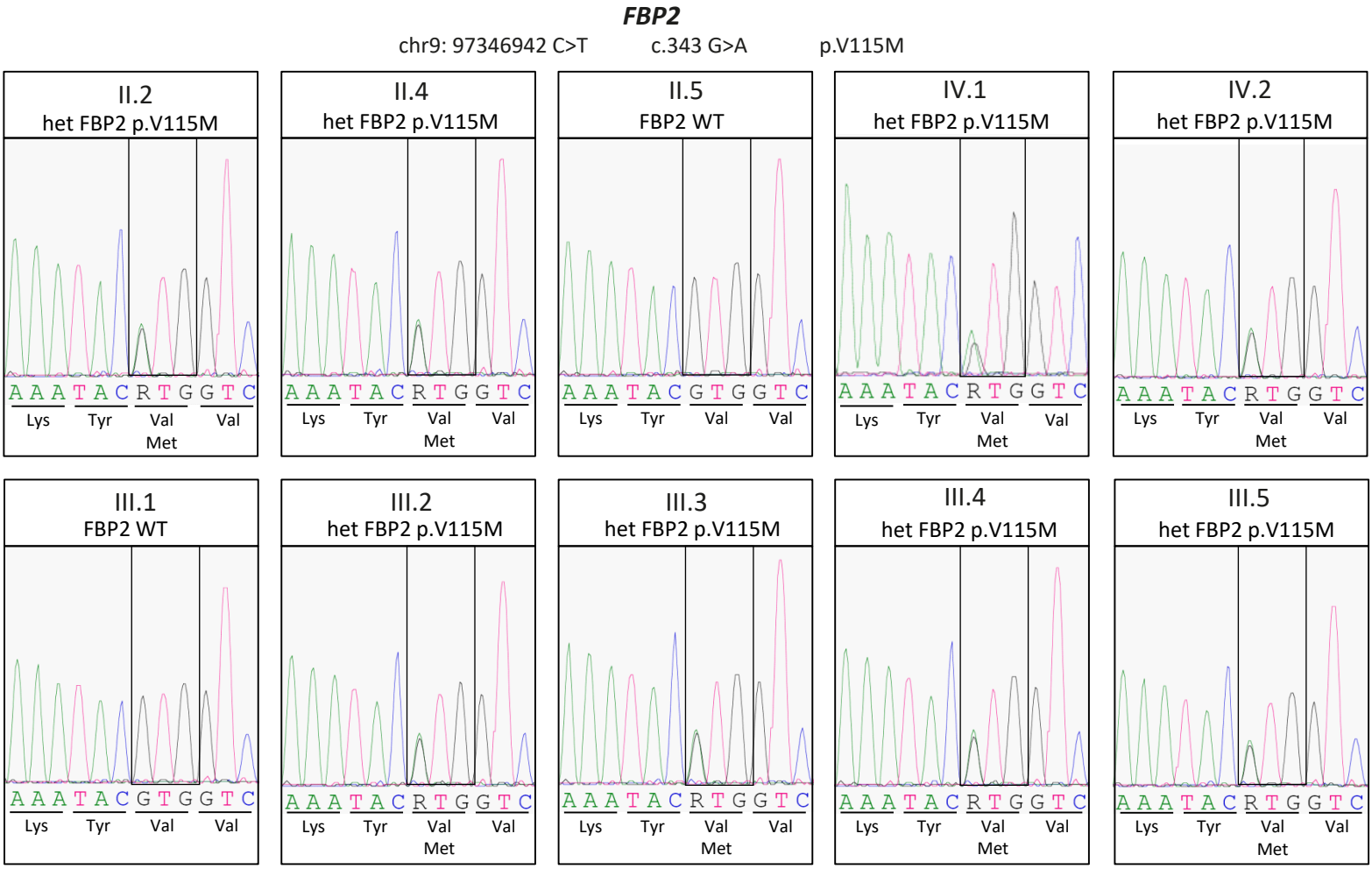
A)



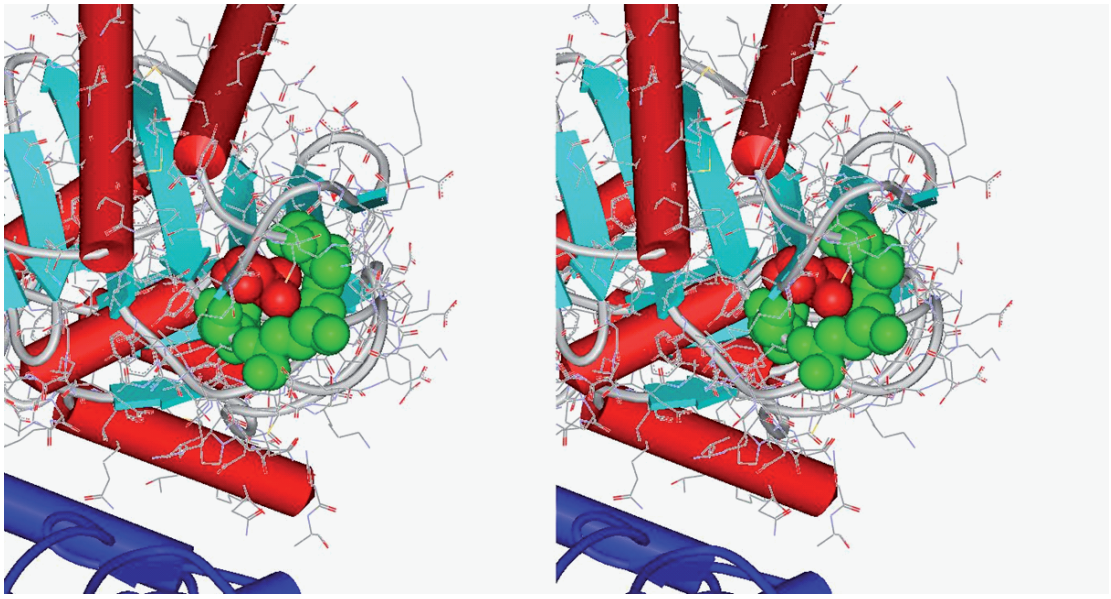

**Supplementary Figure 1:** Confirmation of heterozygous FBP2 variant. A) Sanger sequencing chromatogram of the FBP2 mutation side is shown for each investigated family member with different mutation status wild type (WT) and heterozygous mutated (het). The position of the affected FBP2 nucleotide triplet coding for p.V115M is highlighted by a box. Nomenclature is according to GenBank accession number NM_003837.2 and NP_003828.2.

A)

```
                                  130       140       150       160       170       180
                                    |         |         |         |         |         |
                                                                                *V115M
F16P2_HUMAN                 IAGSVNVTGDEVKKLDVLSNSLVINMVQSSYSTCVLVSEENKDAIITAKEKRGKYVVCFD
Bos_taurus                  IAGSVNVTGDEVKKLDVLSNALVINMLQSSYSTCVLVSEENKEAIITSKEKRGKYVVCFD
Gallus_gallus_FBP2          IAGTVNVTGDEVKKLDVLSNSLVINMLQSSYSTCVLVTEENKEALITPKEKRGKYVVCFD
Xenopus_tropicalis          ISGSVNVTGDEVKKLDVLSNDLVINMLKSSYGTCALVSEENKEVIIIPKEMQGKYVVCFD
Danio_rerio                 IAGQVNVTGDEQKKLDVLSNDLIINLLQASYGTCLMVSEENKDAIYTPAEKRGKYIVCFD
human_FBP1                  IAGSTNVTGDQVKKLDVLSNDLVMNMLKSSFATCVLVSEEDKHAIIVEPEKRGKYVVCFD
Anopheles_Gambiae_FBP       ISGDTNVQGEQVKKLDVLSNEIFINMLKSSYATCLLVSEENDNVIEIETDKRGKYVVSFD
Glycine_max_FBP1            VQGAVNVQGEDQKKLDVVSNEVFSNCLRSSGRTGIIASEEEDVPVAVEESYSGNYIVVFD
Aspargillus_niger_FBP       LAGSSNTTGDDQKKLDVIGNDIFISAMKGSGKCRILVSEEEEEAIVFDEHPNARYAVVCD
                            : *  *. *:: *****:.* :. . ::.*     :.:**:.  :       ..* *  *
Prim.cons.                  IAGSVNVTGDEVKKLDVLSNDLVINML2SSYSTCVLVSEENKEAIIT2KEKRGKYVVCFD

                                  190       200       210       220       230       240
                                    |         |         |         |         |         |
F16P2_HUMAN                 PLDGSSNIDCLASIGTIFAIYRKTSE--------------DEPSEKDALQCGRNIVAAGY
Bos_taurus                  PLDGSSNIDCLASIGTIFAIYRKTSE--------------DEPSEKDALQPGRNIVAAGY
Gallus_gallus_FBP2          PLDGSSNIDCLAPIGTIFAIYKKETD--------------DEPSEKDALQPGRKIVAAGY
Xenopus_tropicalis          PLDGSSNIDCLASIGTIFAIYRKTTD--------------TEPCEQDALQPGRNIVAAGY
Danio_rerio                 PLDGSSNIDCLAPIGTIFAIYKRISD--------------GEPSEKDALQPGNQIVCAGY
human_FBP1                  PLDGSSNIDCLVSVGTIFGIYRKKST--------------DEPSEKDALQPGRNLVAAGY
Anopheles_Gambiae_FBP       PLDGSSNIDCLVSIGSIFAITKQVKEN-------------TTPSEQDALQPGNKIVAAGY
Glycine_max_FBP1            PLDGSSNIDAAVSTGSIFGIYSPNDECLADIDDDPTLDTTEQRCIVNVCQPGSNLLAAGY
Aspargillus_niger_FBP       PIDGSSNLDAGVSVGTIFGIFKLPDEVLGPN---------NKVTAQDLLRAGTEMVASGF
                            *:*****:*. .. *:**.*                          :  : * :::.:*:
Prim.cons.                  PLDGSSNIDCLASIGTIFAIY2KTSE3L222DDDPTLDTTDEPSEKDALQPGRNIVAAGY
```

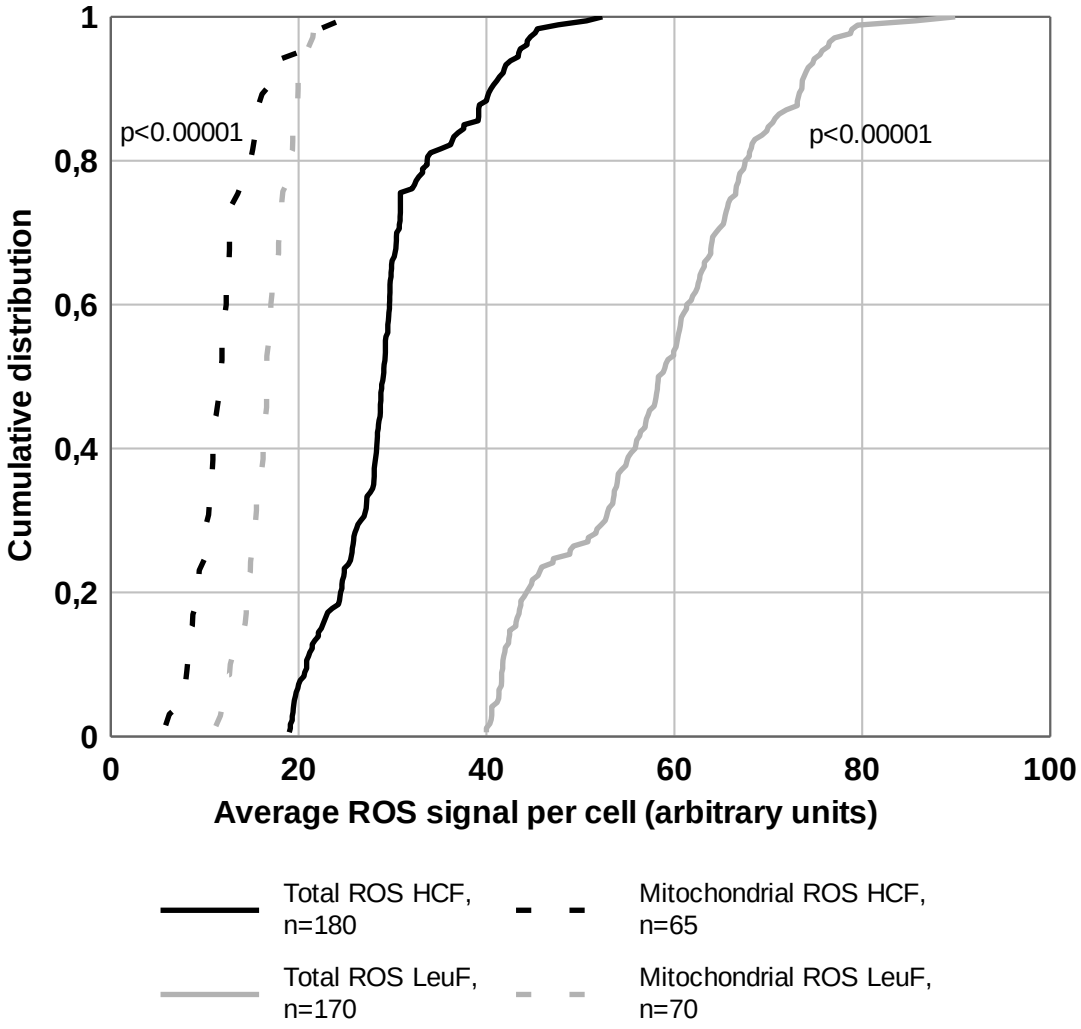
B)



**Supplementary Figure 2:** Multiple Alignment and stereo representation of Val115 position in the FBP2 structure. A) The multiple alignment shows that the Val115 position is strongly conserved with hydrophobic residues for FBP in vertebrates, but also insects, plant, whereas there is an Ala as an even smaller hydrophobic side chain with Aspargillus. B) Stereo view „crossed eye“ of FBP2 subunit A with structural elements in schematic representation. Val115 space filling in red, surrounding hydrophobic residues Val94, Tyr140, Ala153, and the carbon backbone of Lys142 side chain in green, subunit C in dark blue. The figure was drawn with WebLab-Viewer (Molecular Simulations Inc., Burlington, MA, USA), using the X-ray structure of human FBP2 (pdb code 5et7).

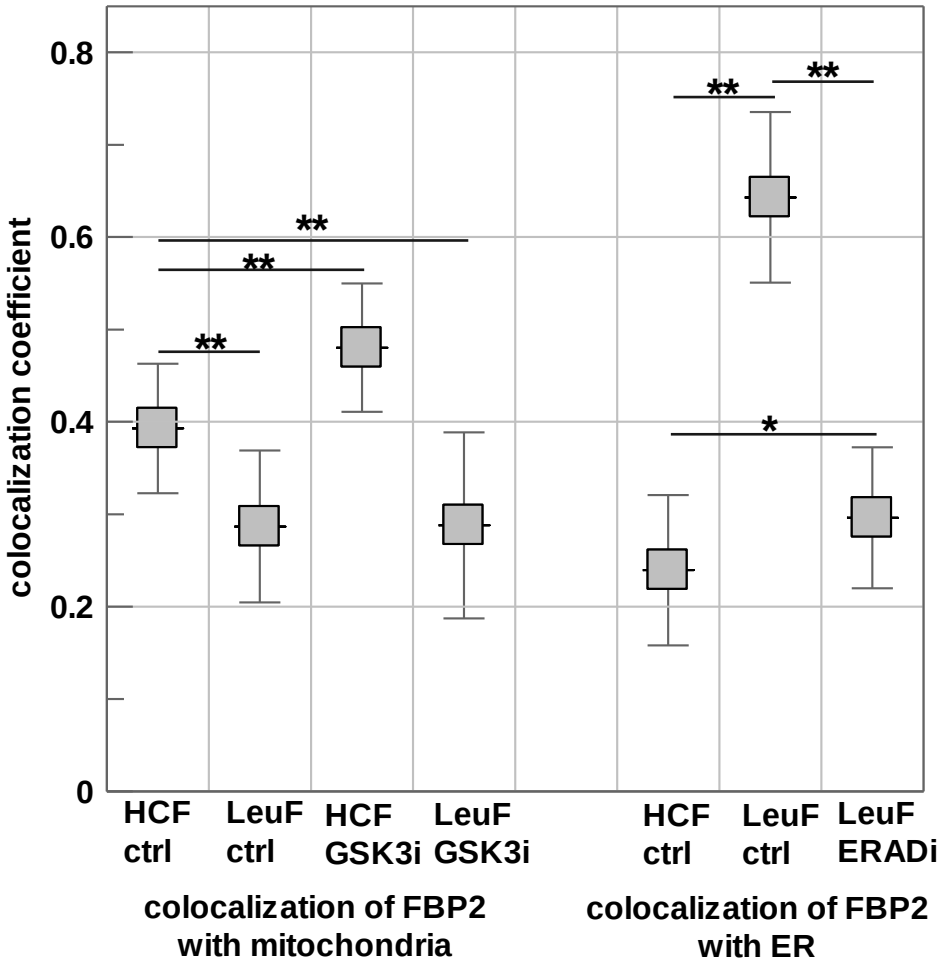
**Supplementary Figure 3. Comparison of reactive oxygen species (ROS) production and FBP2 variants colocalization with mitochondria and endoplasmic reticulum.**

A) Quantification of reactive oxygen species ROS production. Both mitochondrial and total ROS production was increased in LeuF (leukodystrophy fibroblasts) as compared to HCF cells (healthy control fibroblasts); n – number of cells used for the fluorescence measurement.



B) Colocalization of FBP2 variants with mitochondrial and endoplasmic reticulum networks in different contitions presented as Manders' coefficient which varies from 0 (no colocalization) to 1 (100% of colocalization). The coefficient was determined using the JACoP plugin of ImageJ[1]. The measurements were taken from at least 40 cells from at least 18 randomly selected areas. Data is presented as mean and standard deviation. * $p<0.05$, ** $p<0.0001$.

HCF - healthy control fibroblasts; LeuF - leukodystrophy fibroblasts; ctrl – control conditions; GSK3i – GSK3 inhibitor treatment; ERADi – treatment with endoplasmic reticulum (ER)-associated protein degradation inhibitor.




1. Bolte S, Cordelières FP, A guided tour into subcellular colocalization analysis in light microscopy. Journal of Microscopy, 2006;224: 213-232.

| Sample | Varbank Pipeline | read length | Mean Cov | Cov 30x | Total reads | Unique mapped reads [%] | autosomal Runs of Homozygosity [Mb] | % of rare hom variants | Gender |
|---|---|---|---|---|---|---|---|---|---|
| II.2 | v 3.5 | 101 | 95 | 86.0 | 122789264 | 79.00 | 15 | 0.1 | Female |
| II.4 | v 3.5 | 101 | 83 | 83.1 | 107980136 | 78.85 | 21 | 0.4 | Female |
| III.2 | v 3.4 | 76 | 75 | 81.9 | 132206850 | 74.67 | 8 | 0.1 | Female |
| III.4 | v 3.4 | 76 | 66 | 72.9 | 115661122 | 74.87 | 7 | 0.1 | Female |
| III.5 | v 3.5 | 101 | 74 | 76.9 | 100459852 | 82.61 | 34 | 0.0 | Female |
| IV.1 | v 3.5 | 101 | 73 | 80.7 | 106791788 | 87.73 | 14 | 0.0 | Female |

**Supplementary Table 1:** Basic whole exome sequencing (WES) information and output data. Overview of coverage, mapping information and homozygosity for each WES sample. Consanguinity can be estimated using % of rare hom variants (3 – 6%) and autosomal runs of homozygosity (> 200 – 300 mb). Cov, coverage; hom, homozygosity

| | | | | | | | | | Sample | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Gene** | **Chr** | **Position** | **Ref** | **Mut** | **Transcript ID** | **cDNA** | **Protein** | **II.2** | **II.4** | **III.2** | **III.4** | **III.5** | **IV.1** |
| *CCT6A* | 7 | 56055733 | T | C | NM_001762.3 | c.446T>C | p.Ile149Thr | het | het | het | het | het | het |
| *FBP2* | 9 | 94584660 | C | T | NM_003837.3 | c.343G>A | p.Val115Met | het | het | het | het | het | het |

**Supplementary Table 2:** Detected filtered common variants. Detailed variant information with localization, substitution and occurrence within the family. Het, heterozygous

| | | | ***CCT6A*** NM_001762.3 | | ***FBP2*** NM_003837.3 | |
|---|---|---|---|---|---|---|
| | **Tool** | **Range** | **p.I149T** | **Prediction** | **p.V115M** | **Prediction** |
| | Median Rank Score | 0 - 1 | 0.48 | | 0.65 | |
| FUNCTIONAL PREDICTION SCORES | SIFT | 0 - 1 | 0.176 | Tolerated | 0.011 | **Damaging** |
| | PolyPhen2 | 0 - 1 | 0.009 | Benign | 0.999 | **Damaging** |
| | LRT | 0 - 1 | 0.000290 | Neutral | 0.000 | **Damaging** |
| | MutTaster | 0 - 1 | 0.998 | **Damaging** | 1.000 | **Damaging** |
| | MutAssessor | -5.545 - 5.975 | 1.3 | Low functional impact | 2.535 | **Medium functional impact** |
| | FATHMM | -16.13 - 10.64 | -1.15 | Tolerated | -0.74 | Tolerated |
| | PROVEAN | -14 - 14 | -2.39 | Neutral | -2.26 | Neutral |
| | VEST3 | 0 - 1 | 0.195 | Tolerated | 0.638 | **Damaging** |
| | MetaSVM | -2 - 3 | -0.7441 | Tolerated | 0.1394 | **Damaging** |
| | MetaLR | 0 - 1 | 0.2964 | Tolerated | 0.5455 | **Damaging** |
| | CADD | -7.5350 - 35.7885 | 1.3315 | Neutral | 5.6513 | **Damaging** |
| | DANN | 0 - 1 | 0.8208 | Neutral | 0.9987 | **Damaging** |
| | FitCons | 0 - 1 | 0.7354 | **Functional important** | 0.5731 | Less Functional important |
| CONSERVATION SCORES | GERP | -12.3 – 6.17 | 2.39 | **High conserved** | 4.08 | **High conserved** |
| | PhastCons | 0 - 1 | 0.996 | **High conserved** | 0.980 | **High conserved** |
| | PHYLOP20 | -13.282 to 1.199 | 0.824 | **High conserved** | 0.876 | **High conserved** |

**Supplementary Table 3**: Detected filtered common variants. Detailed variant information corresponding bioinformatic prediction on the functional effect, conservation and occurrence in ExAC. Variant classification for each tool was obtained using dbNSFP version 3.4.