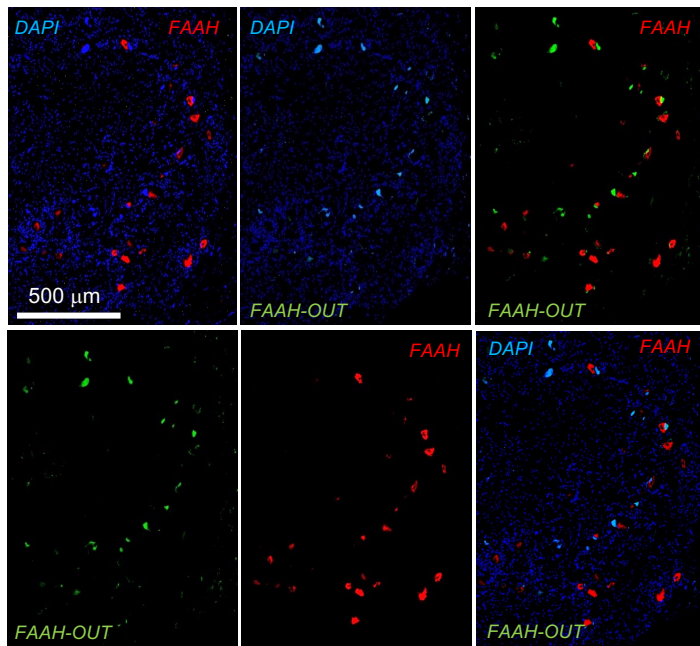


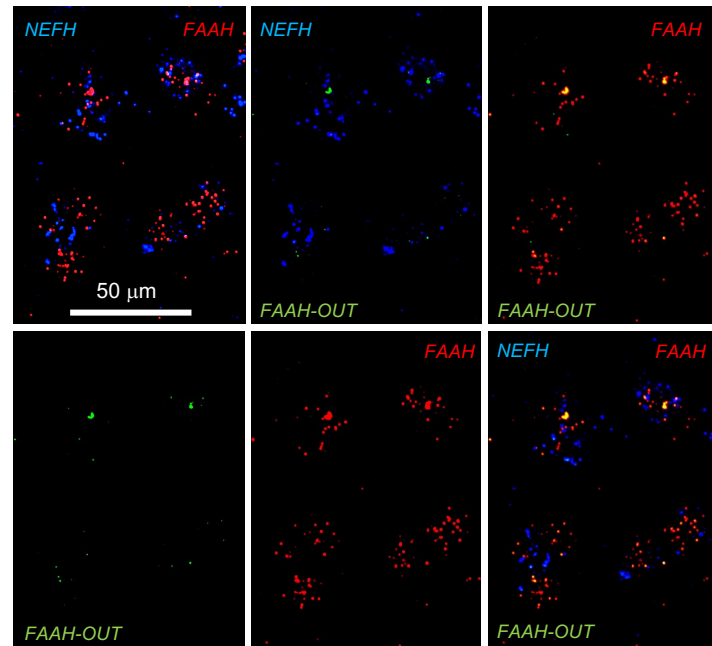
A

h DRG



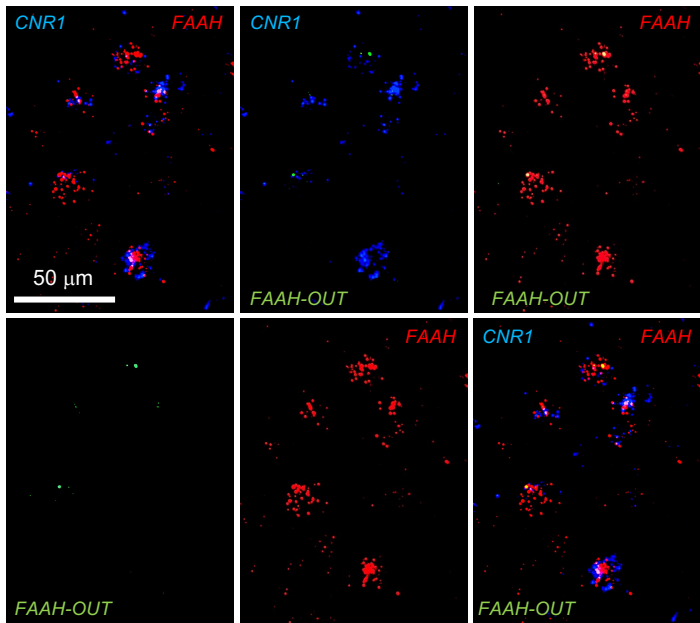
B

h cortex

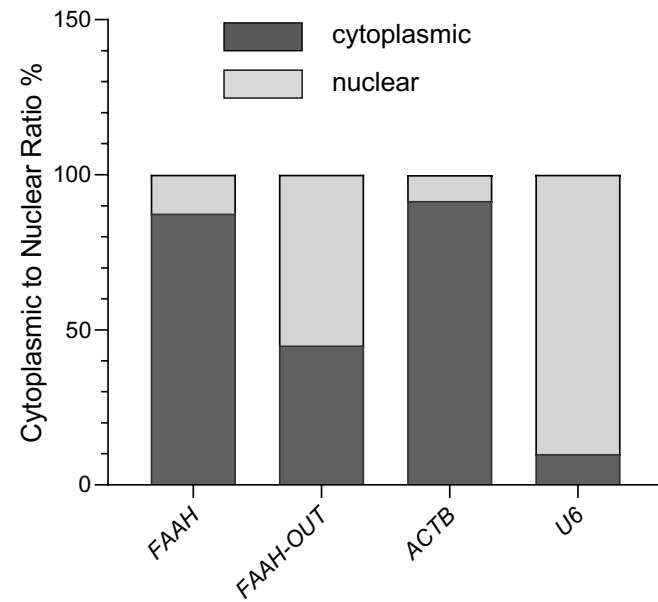


C

h cortex



D



**Supplementary Figure S1. *FAAH* and *FAAH-OUT* RNA expression levels and localisation.****(A) *FAAH* and *FAAH-OUT* RNA expression levels and localisation in human DRG neurons.**

5  $\mu$ M - thick cuts of human DRGs were analysed by RNAscope assay. Localisation of *FAAH-OUT* lncRNA (green, AF488) compared to *FAAH* mRNA (red, Opal570) as analysed by RNAscope assay in 5  $\mu$ M - thick cuts of human DRGs. DAPI staining indicates nuclei (blue). Scale bars are shown in white.

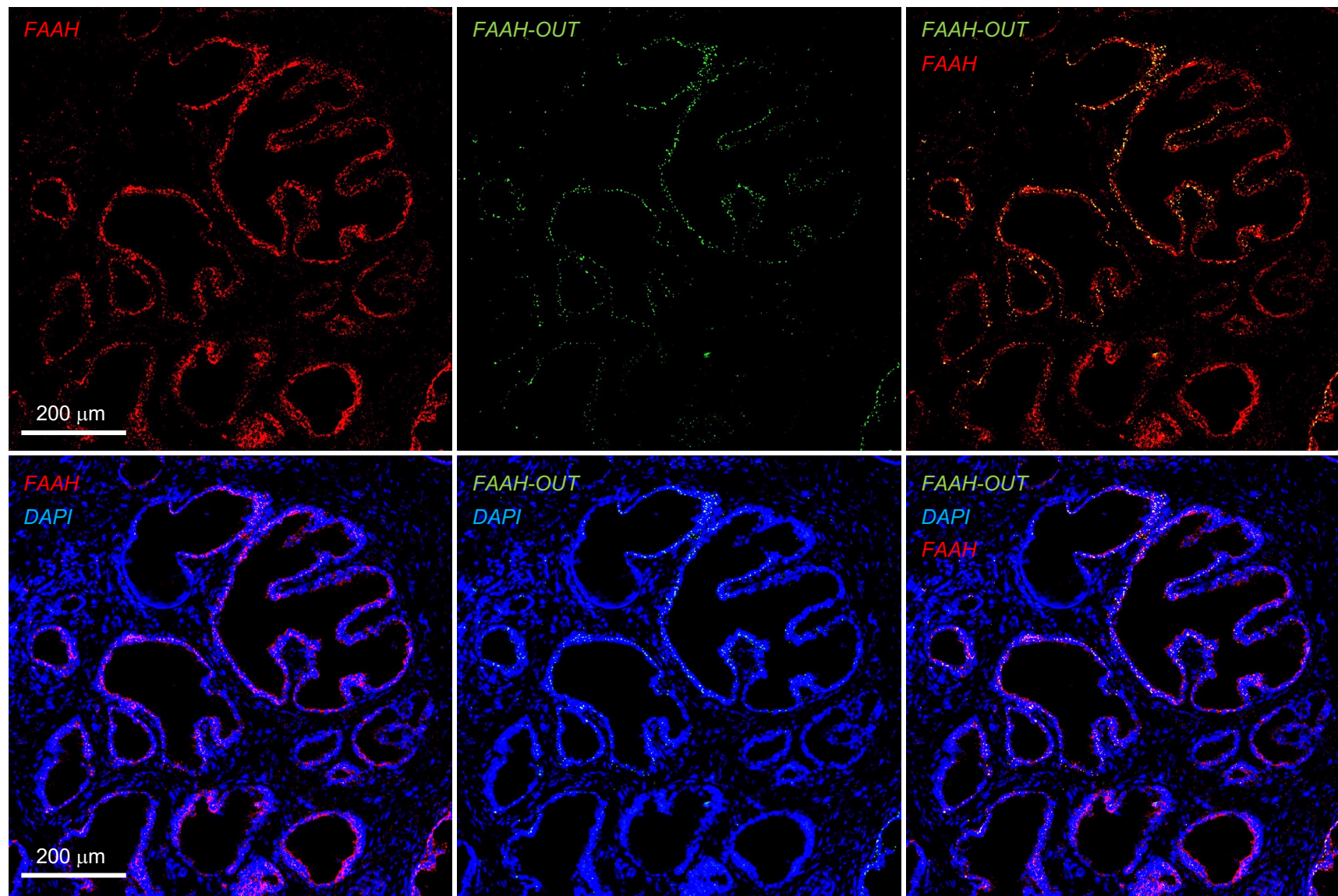
**(B and C) *FAAH* and *FAAH-OUT* RNA expression levels and localisation in human cerebral cortex neurons.**

7-10  $\mu$ M - thick fresh-frozen cuts of human cerebral cortex were fixed with 4% PFA and analysed by RNAscope assay. **(B)** – four representative cells show localisation of *FAAH-OUT* lncRNA (green, AF488) compared to *FAAH* mRNA (red, Opal570) and *NEFH* mRNA (blue, TS405) localisation. **(C)**– five representative cells show localisation of *FAAH-OUT* lncRNA (green, AF488) compared to *FAAH* mRNA (red, Opal570) and *CNRI* mRNA (blue, TS405) localisation. Scale bars are shown in white.

**(D) Subcellular localisation of *FAAH* and *FAAH-OUT* RNAs.**

RT-qPCR analysis of RNA following nuclear/cytoplasmic fractionation shows the distribution of the indicated transcripts in HEK293 cells. *FAAH* mRNA is predominantly cytoplasmic whereas *FAAH-OUT* lncRNA is enriched in the nucleus. *ACTB* and *U6* RNAs were used as cytoplasmic and nuclear controls respectively. The RT-qPCR data, represented as a percentage of the total amount of detected transcripts, are presented as mean of technical triplicates.

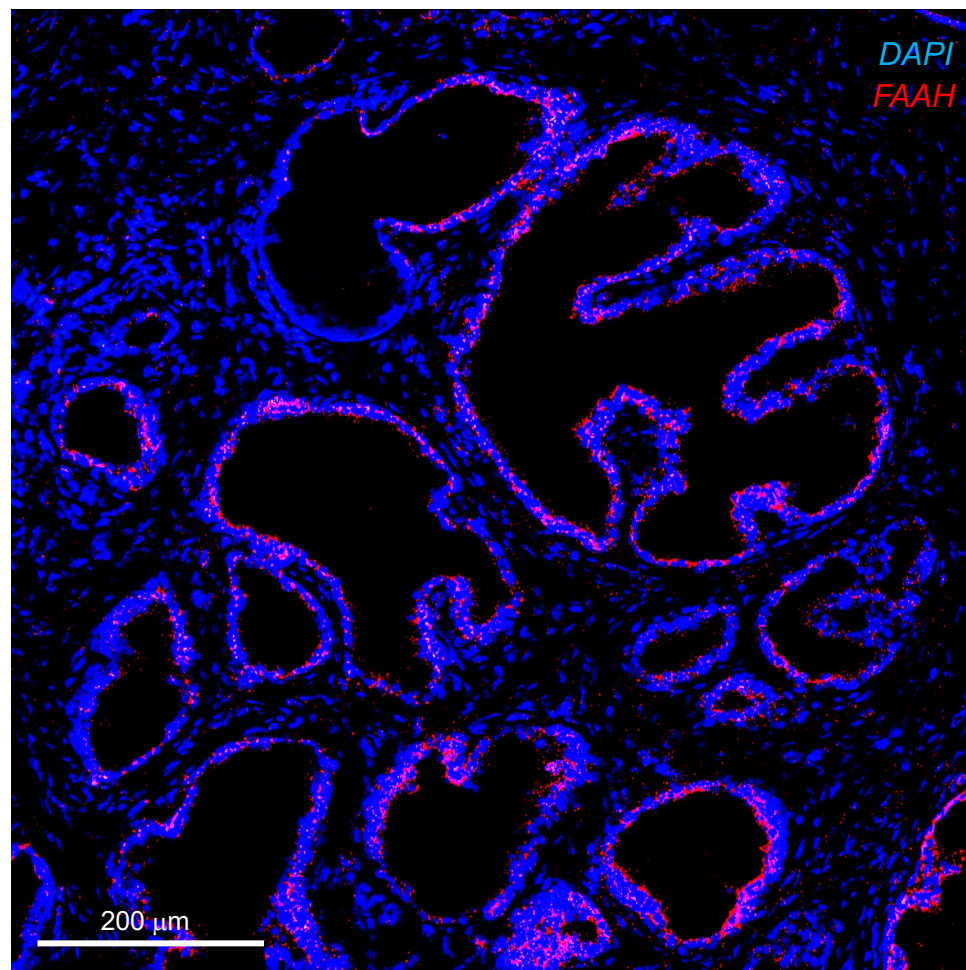
A



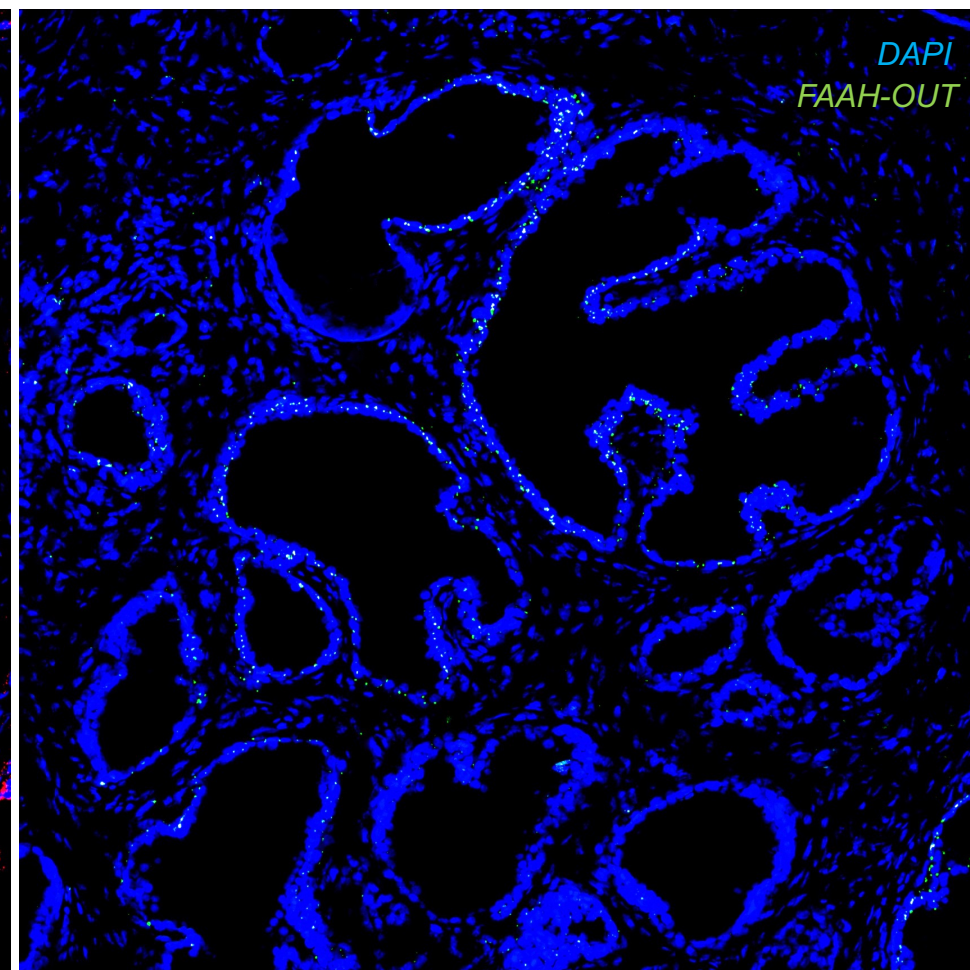
**Supplementary Figure S2A. *FAAH* and *FAAH-OUT* RNA expression levels and localisation in human prostate tissue cells.**

(A) 7-10 μM - thick cuts of fresh frozen prostate sections were analysed by RNAscope assay (Methods). Localisation of *FAAH-OUT* lncRNA (green, AF488) was compared to *FAAH* mRNA (red, Opal570) localisation and DAPI staining indicating nuclei positions (blue). Scale bars (200 μm) are in white.

B

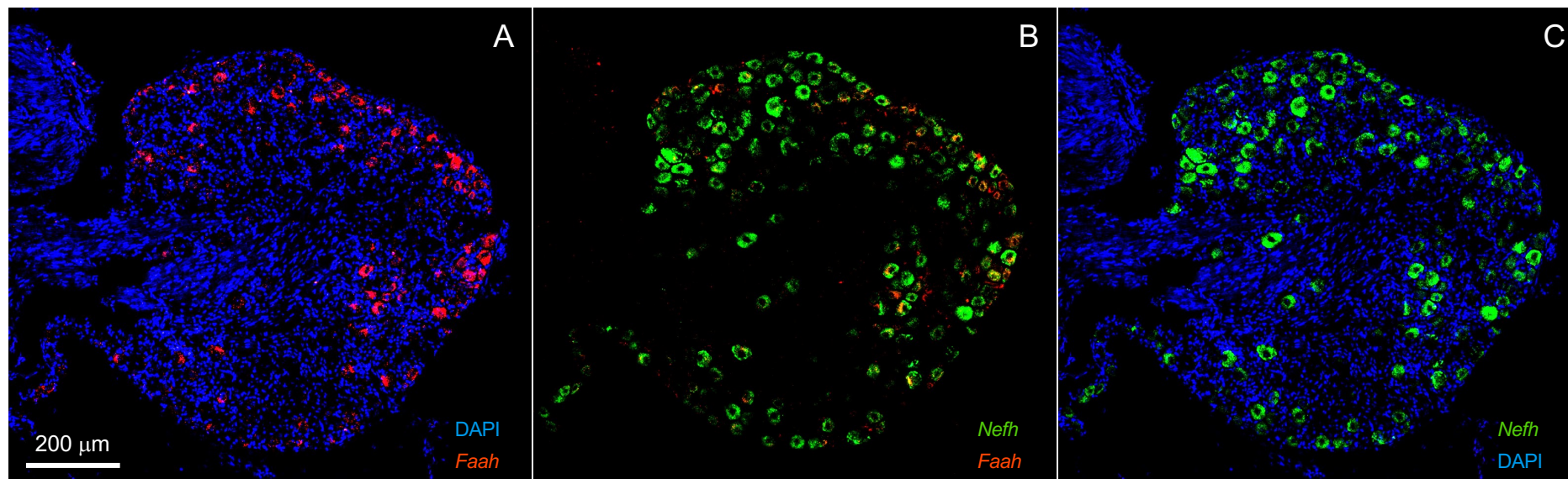


C



**Supplementary Figure S2 (B and C) *FAAH* and *FAAH-OUT* RNA expression levels and localisation in human prostate tissue cells.**

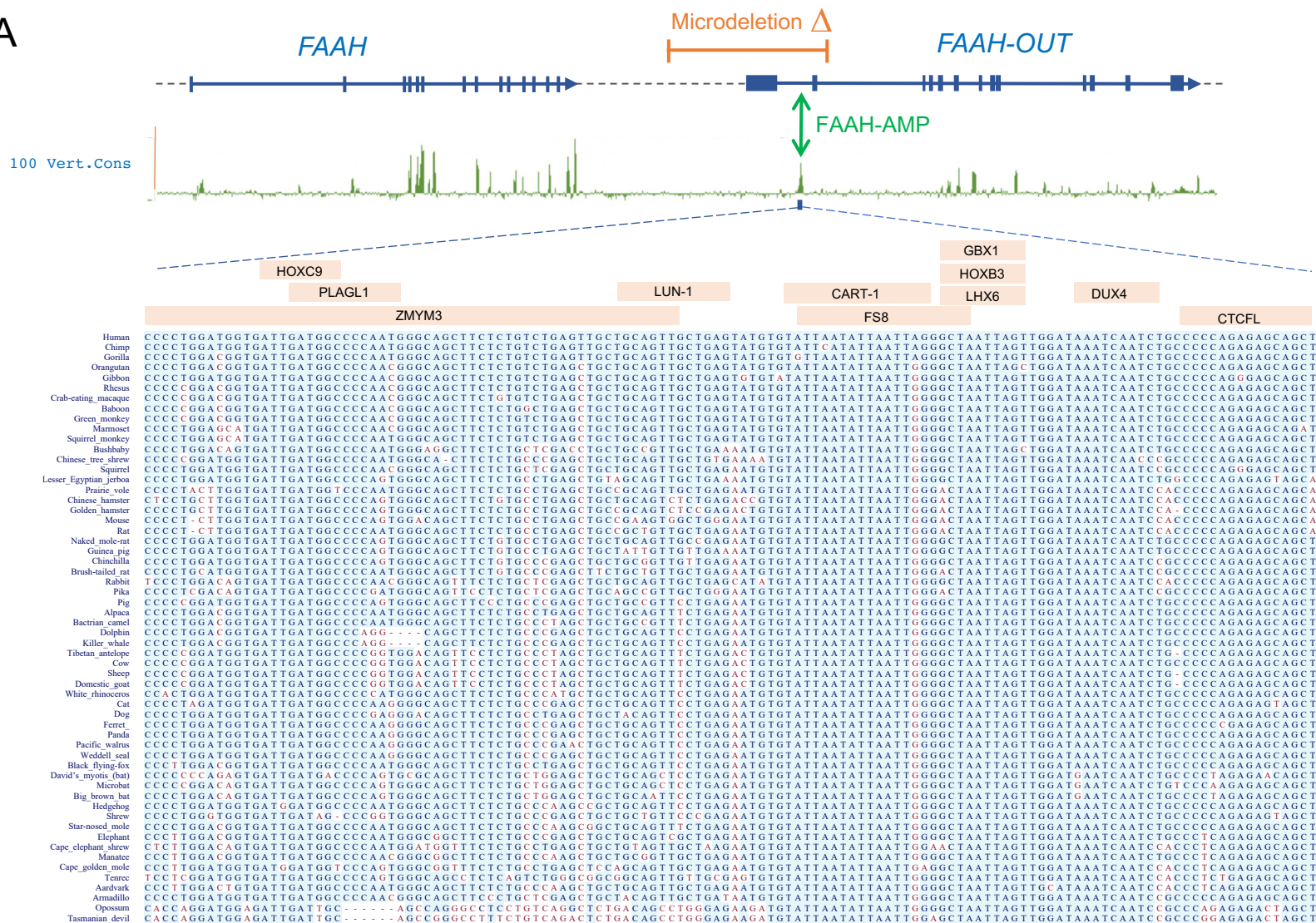
(B and C) Enlarged views of prostate tissue section showing *FAAH* vs DAPI and *FAAH-OUT* vs DAPI and demonstrating mostly nuclear localisation for *FAAH-OUT* lncRNA and mostly cytoplasmic localisation for *FAAH* mRNA. Scale bar (200 mM) is in white.



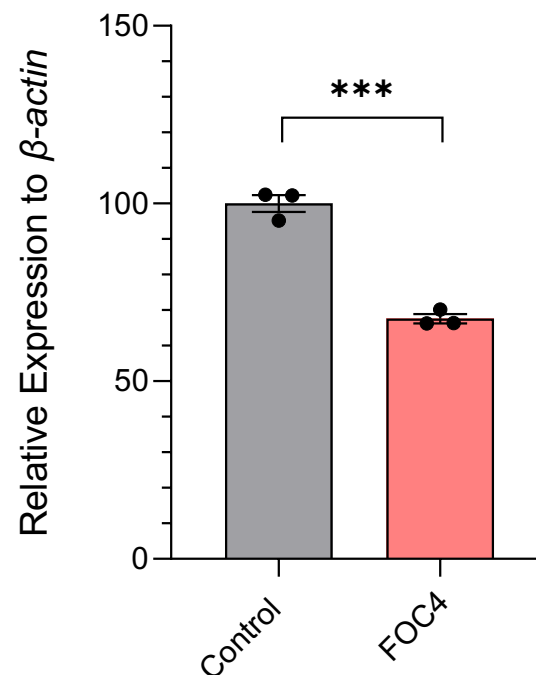
**Supplementary Figure S3. *Faah* RNA expression levels and localisation in mouse DRG**

*Faah* RNA expression levels and localisation in mouse DRG neurons. 11 μM - thick fresh-frozen cuts of mouse lumbar DRGs were fixed with 4% PFA and analysed by RNAscope assay. (A-C). Localisation of *Faah* mRNA (A, red, Opal570) compared to *Nefh* mRNA (B and C, green, AF488) localisation and DAPI staining indicating nuclei positions (A and C, blue). Scale bar (200 μm) is shown in white.

A



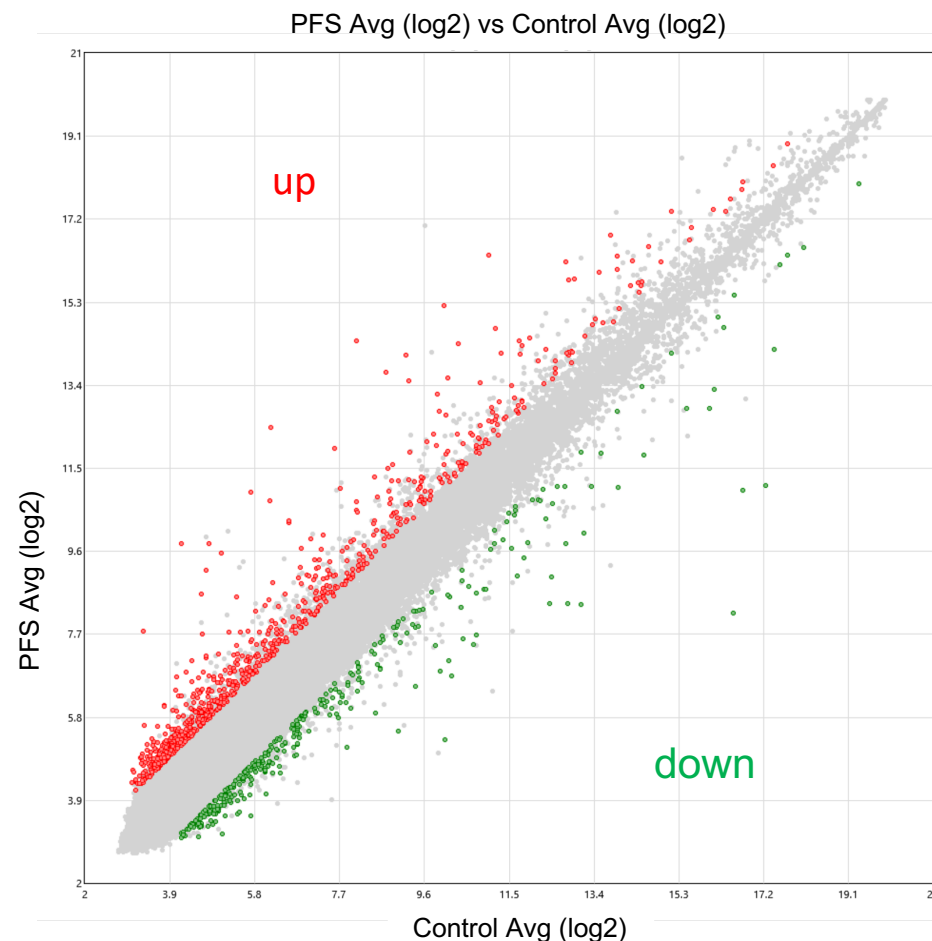
**Supplementary Figure S4A. FAAH-AMP is highly conserved among species.** (A) Map showing relative positions of the ~8 kb microdeletion identified in patient PFS (in orange) and 'FAAH-AMP' conserved element indicated with green double arrow. Exons are denoted by blue boxes and the direction of transcription shown by arrows. The PhyloP base-wise conservation track for 100 vertebrates from the UCSC genome browser shows regions of high sequence conservation as peaks (in green), with the majority of these mapping to gene exons in *FAAH* and *FAAH-OUT*. The core of the conserved element (chr1:46,424,925-46,425,054; build hg38) is expanded in multiple alignment to representative vertebrates. The positions of DNA-binding for selected human transcription factors (TFs) are indicated according to Transfac and Jaspas databases.

**B**

**Supplementary Figure S4B. CRISPR/Cas9-induced deletion of *Faah*-AMP leads to reduction in *Faah* expression in mouse cells.**

(B) RT-qPCR analysis of *Faah* mRNA levels showed significant reduction in its levels when mouse CAD were transiently transfected with CRISPR/cas9 constructs with guide RNA pairs: FOC4 designed to delete the *Faah*-AMP conserved element. The normalized expression value of control (empty vector) was set to 100 and all other gene expression data were compared to that sample. Data points are denoted by dots, bars show the  $\pm$  SEM, and data analysed by Student's t-test, \*\*\*  $P < 0.001$ .

A



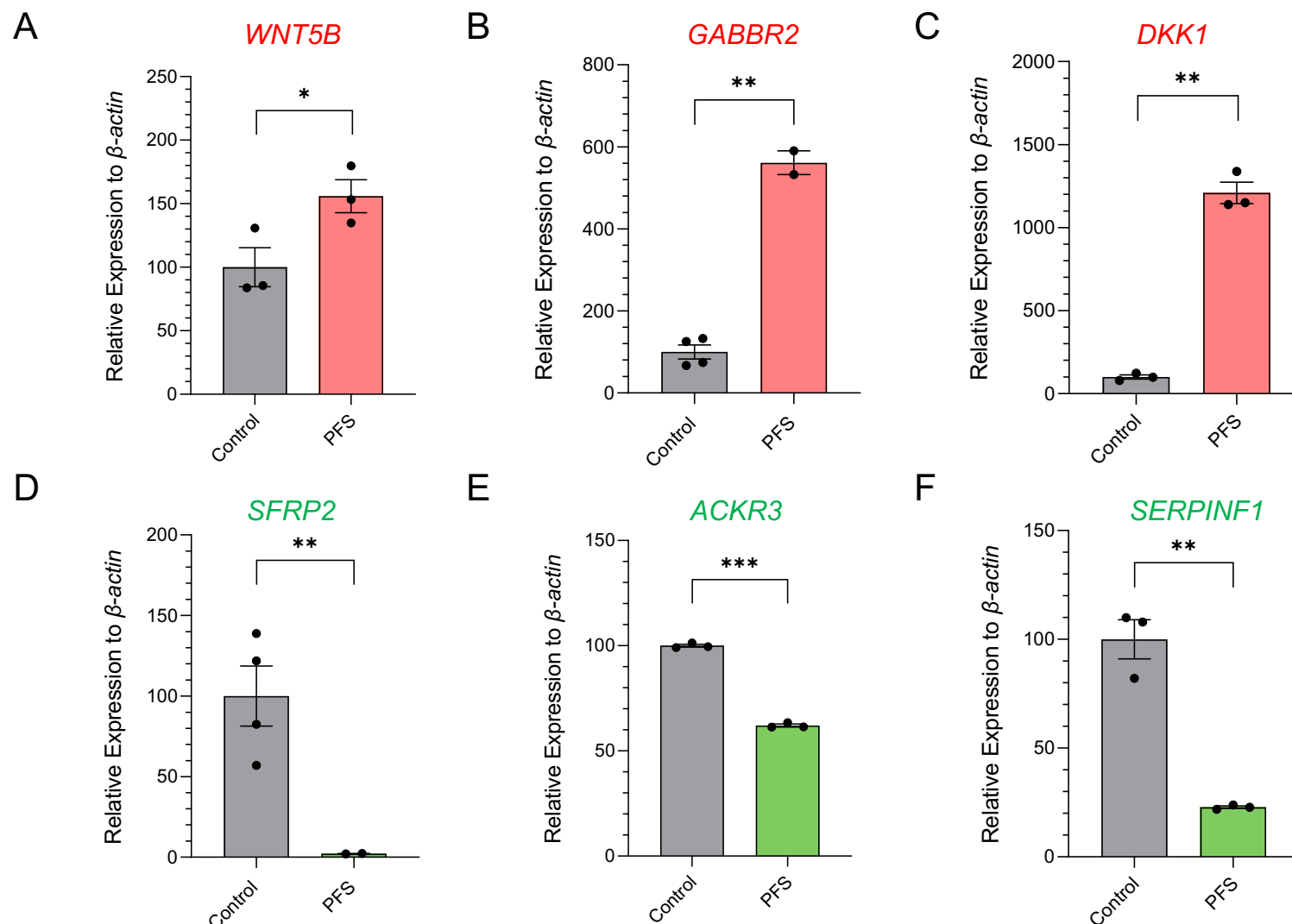
**Supplementary Figure S5A. Scatter plot of microarray data showing gene expression changes in PFS-derived fibroblasts vs 4 controls.** Scatter plot of normalized probe intensities (log2) for significantly dysregulated genes from the Clariom D microarray analysis of PFS-derived fibroblasts (y axis) vs 4 gender-matched fibroblast controls (x-axis). Each circle denotes a single gene with red circles showing up-regulated genes in PFS (>2 fold;  $p < 0.05$ ) and green circles showing down-regulated genes in PFS (>2 fold;  $p < 0.05$ )

B

| Pathways                                                    | Differentially Expressed Genes                                                                                                                                                                                                                                                                |
|-------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| WNT-induced pathways                                        |                                                                                                                                                                                                                                                                                               |
| PCP pathway                                                 | <i>JUND, MAPK9, WNT16, WNT5B</i>                                                                                                                                                                                                                                                              |
| Wnt/ $\beta$ -catenin signalling                            | <i>ACVR2A, CDH3, CTNNB1, DKK1, LEF1, LRP1, PPP2CB, PPP2R1A, PPP2R2B, PPP2R2C, RARB, SFRP2, TCF7, UBD, WNT16, WNT5B</i>                                                                                                                                                                        |
| Wnt/ $Ca^{++}$ pathway                                      | <i>NFATC4, PDE6C, PLCB1, PRKCA, REL, WNT5B</i>                                                                                                                                                                                                                                                |
| Wound Healing                                               |                                                                                                                                                                                                                                                                                               |
| HOTAIR Regulatory Pathway                                   | <i>CTNNB1, ERBB2, LAMTOR5, LEF1, MMP17, MMP2, PIK3CD, REL, SNAI2, STAT3, TCF7, TWIST2, WNT16, WNT5B</i>                                                                                                                                                                                       |
| Rac Signalling                                              | <i>BAIAP2, CDC42, CDK5R1, CFL2, ITGA2B, ITGA3, ITGA8, LIMK2, MAP2K2, PAK1, PIK3CD, PIP4K2A, PRKCZ, RAF1, REL, RRAS, SH3RF1, TIAM1</i>                                                                                                                                                         |
| Regulation of the Epithelial-Mesenchymal Transition Pathway | <i>CTNNB1, FGF10, FGF17, FGF5, FGFRL1, HMGA2, LEF1, MAP2K2, MAP2K5, MMP2, NOTCH3, PIK3CD, RAF1, REL, RRAS, SMAD3, SNAI1, SNAI2, STAT3, TCF7, TWIST2, WNT16, WNT5B</i>                                                                                                                         |
| RhoGDI Signalling                                           | <i>ARHGAP35, ARHGAP4, ARHGAP9, ARHGDIB, ARHGEF7, CDC42, CDH11, CDH18, CDH3, CFL2, ITGA2B, ITGA3, ITGA8, LIMK2, MYL7, PAK1, PIP4K2A, PRKCA, RHOD, RHOV</i>                                                                                                                                     |
| Regulation of Actin-based Motility by Rho                   | <i>BAIAP2, CDC42, ITGA2B, ITGA3, ITGA8, MYL7, PAK1, PIP4K2A, RHOD, RHOV</i>                                                                                                                                                                                                                   |
| Epithelial Adherens Junction Signalling                     | <i>ACVR2A, BAIAP2, CDC42, CTNNB1, FARP2, LEF1, LMO7, MAGI1, MYL7, MYO10, NOTCH3, RRAS, SNAI1, SNAI2, SSX2IP, TCF7, TUBA3C, TUBA3D</i>                                                                                                                                                         |
| BDNF signalling                                             |                                                                                                                                                                                                                                                                                               |
| PEDF Signalling                                             | <i>BDNF, GDNF, NGF, PIK3CD, RAF1, REL, RRAS, SERPINF1/PEDF, TCF7</i>                                                                                                                                                                                                                          |
| Neuropathic Pain Signalling In Dorsal Horn Neurons          | <i>BDNF, CAMK1D, GRIA1, KCNN4, KCNQ3, PIK3CD, PLCB1, PRKCA, PRKCZ</i>                                                                                                                                                                                                                         |
| Neurotrophin/TRK Signalling                                 | <i>BDNF, CDC42, MAP2K2, MAP2K5, MAP3K5, NGF, PIK3CD, RAF1, RPS6KA1, RRAS, SPRY2</i>                                                                                                                                                                                                           |
| Corticotropin Releasing Hormone Signalling                  | <i>ADCY2, BDNF, JUND, MAP2K2, MEF2A, PRKCA, PRKCZ, RAF1, SLC39A7</i>                                                                                                                                                                                                                          |
| G-protein signalling                                        |                                                                                                                                                                                                                                                                                               |
| CREB Signalling in Neurons                                  | <i>ACKR3, ADCY2, ADGRA2, ADGRD1, ADGRF4, ADRA1D, ADRA2A, ADRA2C, AVPR2, CCR3, CHRM3, EDNRA, F2R, GABBR2, GHRHR, GPER1, GPR63, GPR65, GPRC5B, GRIA1, HRH4, LGR5, MAP2K2, NTSR1, OXTR, P2RY6, PIK3CD, PLCB1, POLR2K, POLR2L, PRKCA, PRKCZ, PTGER2, QRFPR, RAF1, RPS6KA1, RRAS, SSTR1, TAAR2</i> |
| GABA Receptor Signalling                                    | <i>ADCY2, AP1B1, GABBR2, GABRA1, KCNN4, KCNQ3, UBD</i>                                                                                                                                                                                                                                        |
| G-Protein Coupled Receptor Signalling                       | <i>ADCY2, ADRA1D, ADRA2A, ADRA2C, AVPR2, CHRM3, DUSP1, DUSP4, GABBR2, GPER1, MAP2K2, PDE11A, PDE3A, PDE6C, PIK3CD, PLCB1, PRKCA, PTGER2, RAF1, REL, RPS6KA1, RRAS, STAT3</i>                                                                                                                  |

### Supplementary Figure S5B. Differentially expressed genes in cells with *FAAH-OUT* microdeletion.

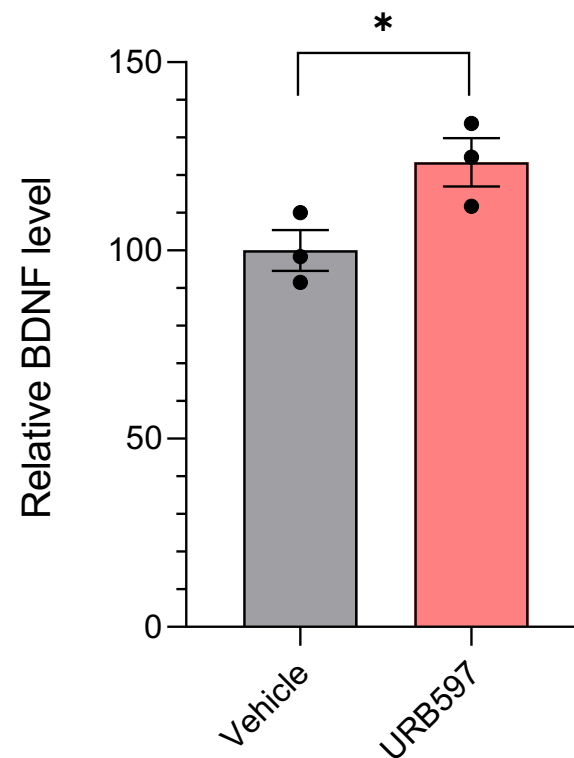
Patient PFS derived fibroblast cell line and 4 gender matched control cell lines were analysed using microarrays. Selected pathways of interest that contain significantly differentially expressed genes (DEGs) are shown above, the full list of DEGs can be seen in **Supplementary Table S5**.



**Supplementary Figure S6. Differential expression of key genes in patient fibroblasts confirmed by RT-qPCR.**

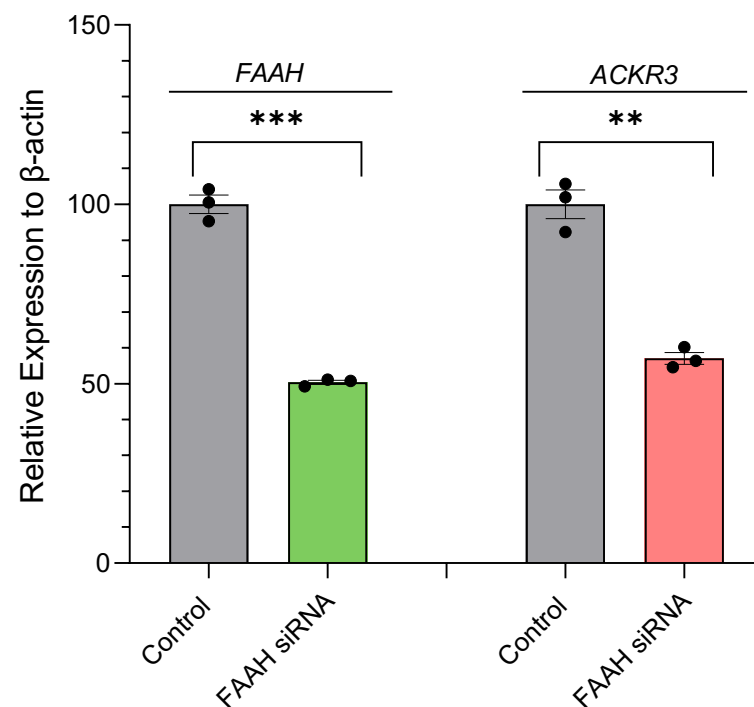
RT-qPCR analysis of key Differentially Expressed Genes (DEGs) mRNA levels in patient' (PFS) fibroblasts showed significant rise for (A) *WNT5B* (1.5-fold); (B) *GABBR2* (5.6-fold) and (C) *DKK1* (12-fold) expression when compared to gender matched controls. The data also showed significant reduction in expression levels of (D) *SFRP2* (47.5-fold); (E) *ACKR3* (1.6-fold) and (F) *SERPINF1* (4.4-fold) when compared to the control. Data were normalized to beta-actin gene as an endogenous control. The normalized expression value of control subjects was set to 100. Data points are denoted by dots, bars show the  $\pm$  SEM, and data analysed by Student's t-test, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$

A

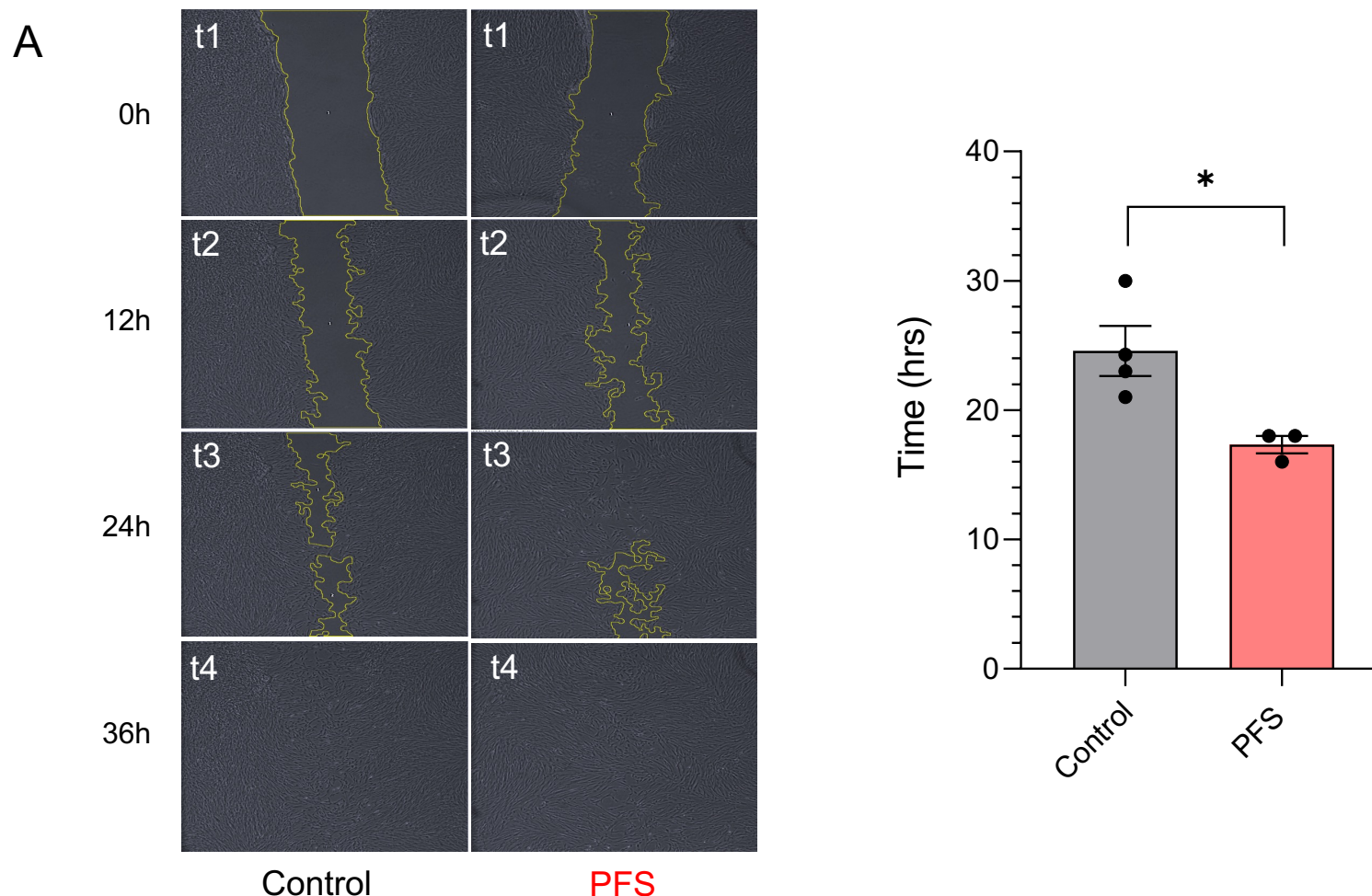
**Supplementary Figure S7A. Effects of pharmacological inhibition of FAAH.**

BDNF levels rise after FAAH inhibition. Mice treated with FAAH inhibitor URB597 showed significantly higher BDNF (brain-derived neurotrophic factor) levels in the hippocampus (detected by ELISA) compared with vehicle treated mice ( $n = 3$  per group; Data points are denoted by dots, bars show the  $\pm$  SEM, and data analysed by Student's t-test,  $*P \leq 0.05$ ).

B

**Supplementary Figure S7B. Effects of siRNA knockdown of *FAAH*.**

*FAAH* and *ACKR3* expression in HEK293 cells following *FAAH* siRNA treatment. Expression levels of both genes are significantly reduced after *FAAH* knockdown. Data points are denoted by dots, bars show the  $\pm$  SEM, and data analysed by Student's t-test \*\* $P \leq 0.01$ , \*\*\*  $P \leq 0.001$ .



**Supplementary Figure S8. The microdeletion in *FAAH-OUT* leads to an acceleration of wound healing.**

**(A)** Wound healing scratch assay was performed using fibroblasts from the PFS patient and a gender matched control who carries the hypomorphic SNP (rs324420) but not the *FAAH-OUT* microdeletion. Images of the recovering monolayer of cells were taken from the start of the experiment (t1=0h) and the following intervals of 12 h for the next 72h (t1, t2, t3, t4 time points correspond to 0, 12, 24 and 36h respectively). **(B)** The images were analysed by Fiji/ImageJ to measure and plot the recovery of the initial gap area (resulting from the scratch) as a function of time. PFS fibroblasts recovered 50% of the scratched gap area in 17h time whereas control fibroblast needed 24.5h to achieve the same, resulting in approximately 30% faster recovery time for PFS fibroblasts. Data points are denoted by dots, bars show the  $\pm$  SEM, and data analysed by Student's t-test,  $*P \leq 0.05$ )

**Supplementary Table S1: sgRNA sequences**

| Guide RNA                 | Sequence                                          | Cas9 type    | Location (hg38; ChrI:)                                                                          |
|---------------------------|---------------------------------------------------|--------------|-------------------------------------------------------------------------------------------------|
| <b>FOP1</b>               | AAAAGGTGAGGTCACGAGGCC                             | dSaCas9-KRAB | 46,422,643-46,422,663<br>FAAH-OUT promoter                                                      |
| <b>FOC1</b>               | TGTTCCCATGCTTTGGAGTTC                             | dSaCas9-KRAB | 46,424,886-46,424,906<br>FAAH-AMP                                                               |
| <b>FAAH-OUT activator</b> | CTCTCCTGGCGATTGCGATAT                             | dSaCas9-VPR  | 46,420,443-46,420,463<br>FAAH-OUT promoter                                                      |
| <b>FAAH activator</b>     | sc-402470-ACT (Santa Cruz biotechnology)          | dCas9-VPR    | FAAH promoter                                                                                   |
| <b>HMa</b>                | CCCAGTGAGTACGATGGCCAG +<br>TTAGTGATATTGTTCCGTGGG  | SaCas9       | 46,418,584-46,418,604 + 46,427,601-46,427,621<br>Flanking patient microdeletion                 |
| <b>HMb</b>                | TCATGGCCTTTCCCTTCTCA<br>+ GTCACCTGACAGTCTGATTAAG  | SaCas9       | 46,418,679-46,418,699 + 46,427,561-46,427,581<br>Flanking patient microdeletion                 |
| <b>FOP2</b>               | CCCAGTGAGTACGATGGCCAG +<br>CCAACCCCTCAGGAGTTATGGA | SaCas9       | 46,418,584-46,418,604 + 46,423,505-46,423,525<br>FAAH-OUT promoter + part of exon I             |
| <b>FOP3</b>               | TCATGGCCTTTCCCTTCTCA<br>+ GCAACCCCATCCATAACTCCT   | SaCas9       | 46,418,679-46,418,699 + 46,423,496-46,423,516<br>FAAH-OUT promoter + part of exon I             |
| <b>FOC2</b>               | GCAGCTTTGGCAACTCGGAGT +<br>TGCATGTGTTGGGTTATTGGT  | SaCas9       | 46,424,542-46,424,562 + 46,425,286-46,425,306<br>Flanking FAAH-AMP                              |
| <b>FOC3</b>               | TAGACTAGGGTTCCTGTCTTG +<br>TGTGGGTCTGAATGTCTGGTA  | SaCas9       | 46,424,405-46,424,425 + 46,425,339-46,425,359<br>Flanking FAAH-AMP                              |
| <b>FOC4</b>               | CCCCAGTTTGAGTCCTCAGT +<br>GGAGGCCTAGGAACCATTA     | SaCas9       | Mouse mm10 chr4:115,995,561-115,995,581 +<br>115,995,214-115,995,234<br>Flanking mouse FAAH-AMP |

**Supplementary Table S2: Primers used to confirm SaCas9-induced deletions**

| Guide pairs                                      | Forward primer (5'-3')    | Reverse primer (5'-3')    |
|--------------------------------------------------|---------------------------|---------------------------|
| HMa, HMb<br>Primers flanking<br>microdeletion    | TTAATGTCTGGAGTGATAACATGAC | ACAACTTCTAATTAGTGTTAATGAC |
| HMa, HMb<br>Primers internal to<br>microdeletion | TGGATGGTGATTGATGGCCC      | GGTCTCGAGGTTGTCACTGG      |
| FOP2,3                                           | GGGACAGAGAGTTGTGCTAATGAAG | CCTCTGTACATCCTACTCCCAC    |
| FOC2,3                                           | CTGATGGAGGCCATTGGAGGAG    | CCAACAGCCCAGACATCCACATG   |
| FOC4                                             | GACAGATGAAAATGGGCTCTGGG   | CTCATGAATCGCCCAAGTCATAG   |

**Supplementary Table S3: Primers used for ChIP-qPCR**

| Region                        | Forward primer (5'-3') | Reverse primer (5'-3')  |
|-------------------------------|------------------------|-------------------------|
| FAAH-OUT promoter             | ACTTGAAACCCACACGCTCA   | AGGCTGGTGTCGAAGAATG     |
| FAAH promoter                 | CTGATCCAGTCCGGGTTTTG   | CGTACTGCACCATGATCCCTT   |
| FAAH-AMP                      | TGTCCTGATGGTGATTG      | CACCACTGCAACCCATTAGC    |
| ChIP gene desert<br>(control) | AAGAGGCCCTTCTCTATGC    | TGTGATTAATCTCGACTCCAAGA |

**Supplementary Table S4: Primers used for cellular fractionation**

| Region   | Forward primer (5'-3')    | Reverse primer (5'-3')  |
|----------|---------------------------|-------------------------|
| U6 snRNA | GCTTCGGCAGCACATATACTAAAAT | CGCTTCACGAATTTGCGTGTCAT |
| ACTB     | GGGAAATCGTGCGTGACATTAAGG  | CAGGAAGGAAGGCTGGAAGAGTG |