## **Molecular Basis of ABHD5 Lipolysis Activation**

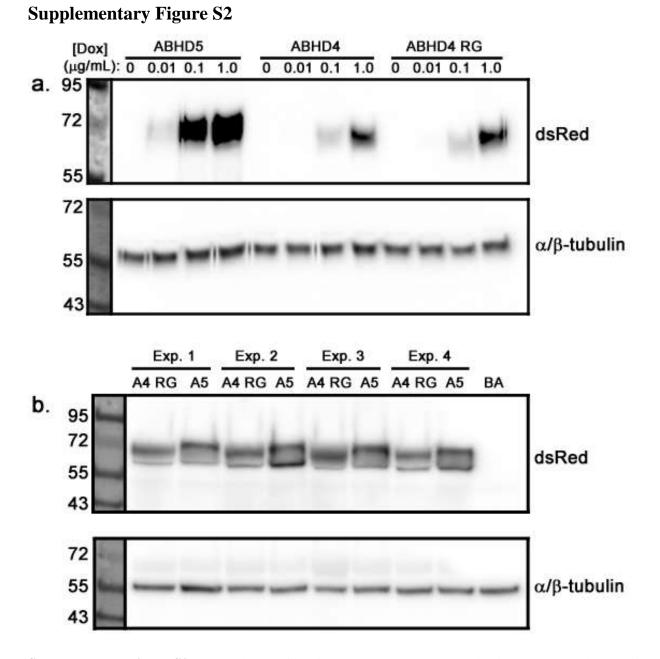
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## b. a. 0.01 < 0.001 (ATGL) <0.001 100 Droplet-depleted cells (%) 80 PLIN5 60 -40. 10 µm 20. PASISSINGSINGSSIGSSIGS NICLOSTA PA N303005529 \* MGL SAIN PA N303055299 \* MGL SAIN AN 5159119391939193329 + NGL ABHDS \* FOL SAIN AA MOODERSTON A MOL ABHD5 ABHD4 ABHDS\* ATOL PLIN5 (ATGL) A4 N303R/S332G S159A/N303R/S332G

## **Supplementary Figure S1**

**Supplementary Figure S1.** ATGL activation by gain-of-function ABHD4 N303R/S332G is independent of ABHD4 hydrolase activity. **a**) Cos7 cells transfected with ABHD protein-mCherry, PLIN5-EYFP, and ATGL-CFP or ATGL S47A-CFP (lipase inactive) were lipid loaded overnight then fixed. Results are based on three independent experiments. **b**) Representative images of cells scored in **a**). Scale bar = 10  $\mu$ m.



**Supplementary Figure S2.** Expression profile of re-expressed ABHD proteins in ABHD5-null BA cell lines. **a**) Representative immunoblot showing relative doxycycline-induced expression of ABHD5-mCherry, ABHD4-mCherry, and ABHD4 N303R/S332G-mCherry. This experiment was done twice with the same results. **b**) ABHD4 N303R/S332G-mCherry (A4 RG) and ABHD5-mCherry (A5) expression in BA cell lines from the four independent experiments used in figure 4c for comparing activity of the two proteins. ABHD4 N303R/S332G-mCherry expression was induced by 1 µg/ml doxycycline, while ABHD5-mCherry expression was induced by 0.0125 µg/mL doxycycline. BA, lysate from wild-type differentiated BA cells.