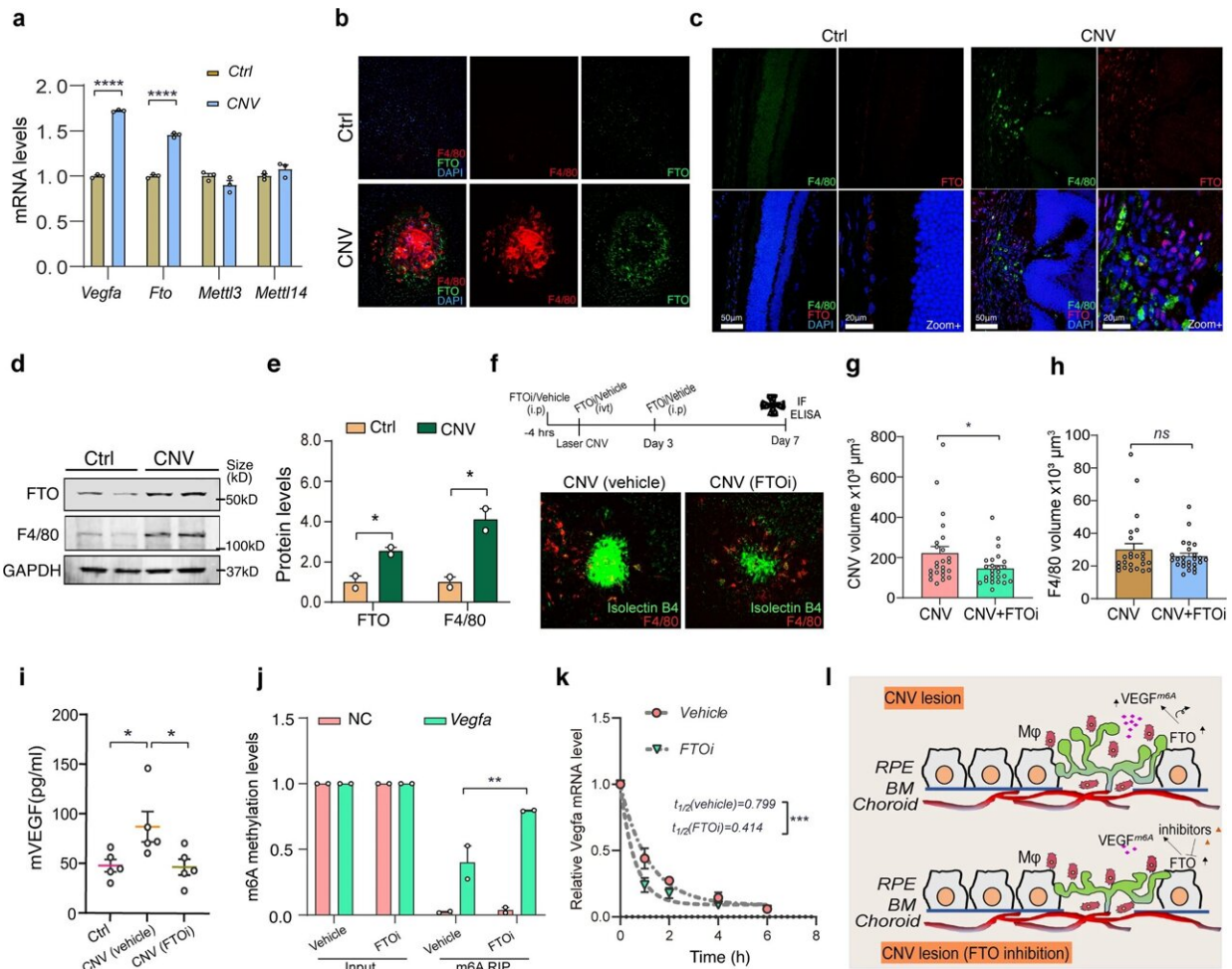


Discovery suggests new way to prevent common causes of vision loss

February 24 2023



FTO in macrophage VEGFA release and choroidal neovascularization. **a** Quantification of *Vegfa*, m6A methyltransferase (*Mettl3*, *Mettl4*), demethylase (*Fto*) mRNA levels in pooled eye tissues ($n = 3$) of control, naive (no laser treated) mice (Ctrl) or mice following laser injury (choroidal neovascularization, CNV, day 3 after laser injury). **b, c** Immunofluorescent staining of FTO in flat-

mounted RPE-choroid tissues (**b**) and cryosections of eyes (**c**) at 3 days after laser injury. F4/80 immunostaining indicates macrophage infiltration following laser injury. **d, e** Immunoblotting and quantification of FTO and F4/80 protein levels in RPE-choroid tissues isolated from mice eyes at 3 days after laser injury ($n = 2$ eyes). **f** Immunofluorescent staining of neovascularization using isolectin B4 (green) and of macrophages by F4/80 (red) in RPE-choroid tissues of mice treated with FTO inhibitor (FTOi) or vehicle, at 7 days after laser injury. **g, h** Quantification of CNV and F4/80 volumes based on isolectin B4 and F4/80 staining in RPE-choroid tissues of mice treated with FTO inhibitor (FTOi) or vehicle, at 7 days after laser injury ($n = 24$ laser spots for Ctrl, and $n = 25$ spots for FTOi). **i** Quantification of VEGFA levels in the RPE/choroid tissues of mice treated with FTO inhibitor (FTOi) or vehicle, at 3 days after laser injury ($n = 5$ eyes). **j** Quantification of methylated *Vegfa* mRNA levels in mouse BMDMs treated with FTO inhibitor (FTOi) or vehicle for 24 h by using MeRIP-qPCR ($n = 2$). **k** Determination of *Vegfa* mRNA stability in BMDMs pretreated with FTO inhibitor (FTOi) or vehicle, followed with Actinomycin D inhibition (10 $\mu\text{g/ml}$). mRNA abundance was measured by RT-qPCR at the indicated time points and *Vegfa* mRNA half-lives ($t_{1/2}$) determined by fitting the data to a nonlinear one phase decay model (mean \pm SEM, $n = 3$). **l** Schematic diagram showing FTO regulates VEGFA release and choroidal neovascularization in AMD. Retinal pigment epithelium RPE, BM Bruch's membrane, M ϕ macrophages. Data are shown as mean \pm SEM, * p

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