Supplemental Materials

Molecular Biology of the Cell

Holleman et al.

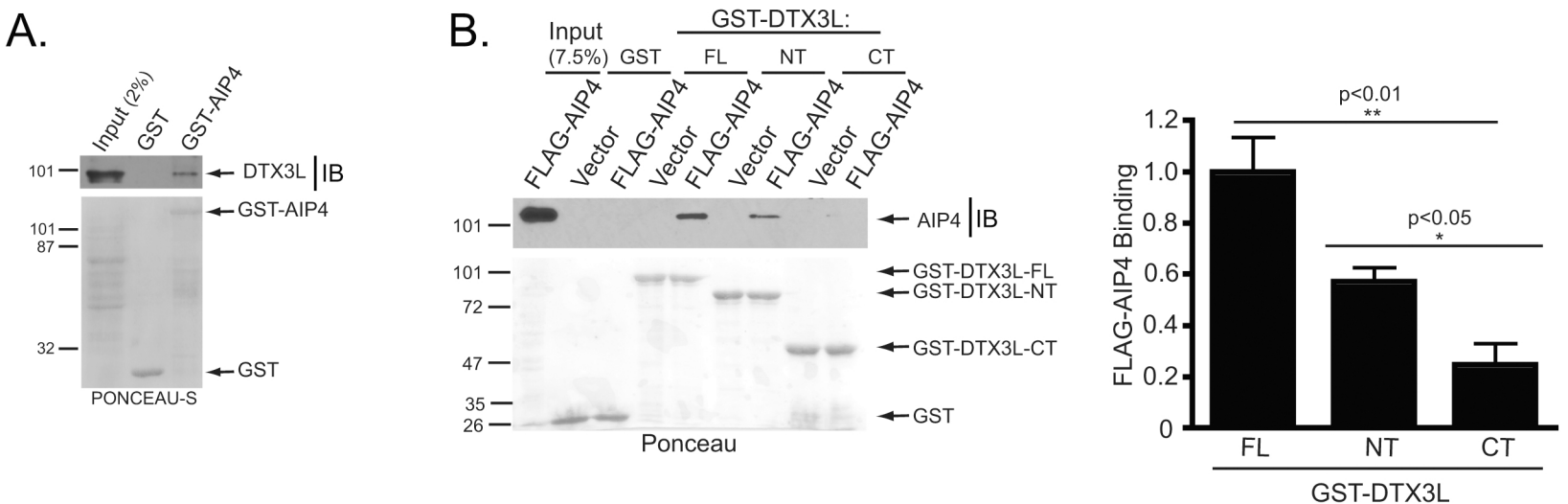
SUPPLEMENTAL FIGURE LEGENDS

Figure S1. DTX3L interacts directly with AIP4. (A) Equal amounts (100 nmols) of GST and GST-AIP4 immobilized to glutathione Sepharose 4B resin were incubated with 500 μg cleared HeLa cell lysates. Bound endogenous DTX3L was detected by immunoblotting. Blots were stained with Ponceau-S to show purified proteins. Data are representative of 3 independent experiments. (B) Purified GST and GST-DTX3L fusion proteins of either full-length (FL), N-terminally truncated (NT) or C-terminally truncated (CT) DTX3L immobilized to glutathione Sepharose 4B resin were incubated with 100 μg cleared HeLa cell lysates transiently transfected with either empty vector (pCMV10) or FLAG-AIP4. Bounds samples were resolved by 10% SDS-PAGE and analyzed by immunoblotting for DTX3L or FLAG-AIP4. Blots were stained with Ponceau-S to show purified proteins. Data are representative of 3 independent experiments and were analyzed by a one-way ANOVA.

Figure S2. DTX3L does not regulate endosomal phosphatidylinositol 3-phosphate (PI-3P) levels in HeLa cells. (A) HeLa cells co-transfected with YFP-2×FYVE and siRNA directed against control or DTX3L were pre-treated with DMSO or wortmannin (100 nM) for 30 min before stimulation with CXCL12 (10 nM) for 1 h. Cells were fixed, permeabilized and stained with antibodies directed against DTX3L and EEA1. Inset represents 4× enlargement of the boxed region. Differential interference (DIC) contrast images are shown. Equal acquisition settings (gain and intensity) were used between parallel samples within each experiment. (B) YFP-2×FYVE puncta are not reduced in DTX3L siRNA treated cells compared with control siRNA treated cells, whereas YFP-2×FYVE puncta were significantly reduced in wortmannin treated cells compared with DMSO. Puncta were counted using the particle analysis software of ImageJ. Data represent the average YFP-2×FYVE puncta per cell from 3 independent experiments (35-40 cells). Data were analyzed by a Student's t-test and a one-way ANOVA ($p < 0.0001$).

Figure S3. DTX3L siRNA does not prevent agonist-promoted ubiquitination of CXCR4. Cleared HeLa cell lysates transfected with HA-CXCR4, FLAG-ubiquitin and either control or DTX3L siRNA were subject to immunoprecipitation of HA-CXCR4 under denaturing conditions followed by immunoblotting to detect incorporated FLAG-tagged ubiquitin. Samples were separated by 7% SDS-PAGE and immunoblotted for the indicated proteins. Data are representative of 7 independent experiments.

Figure S1. Holleman and Marchese



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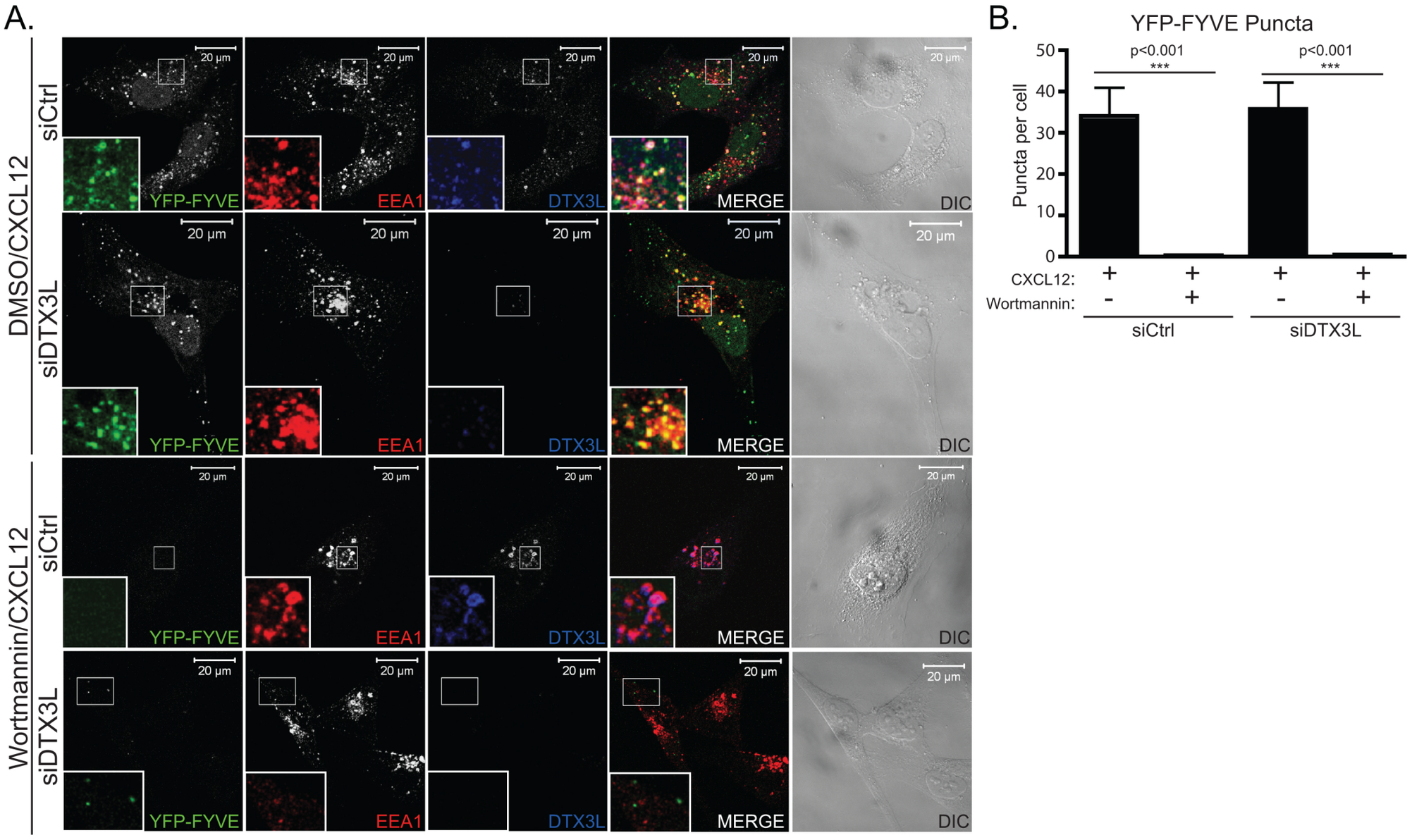

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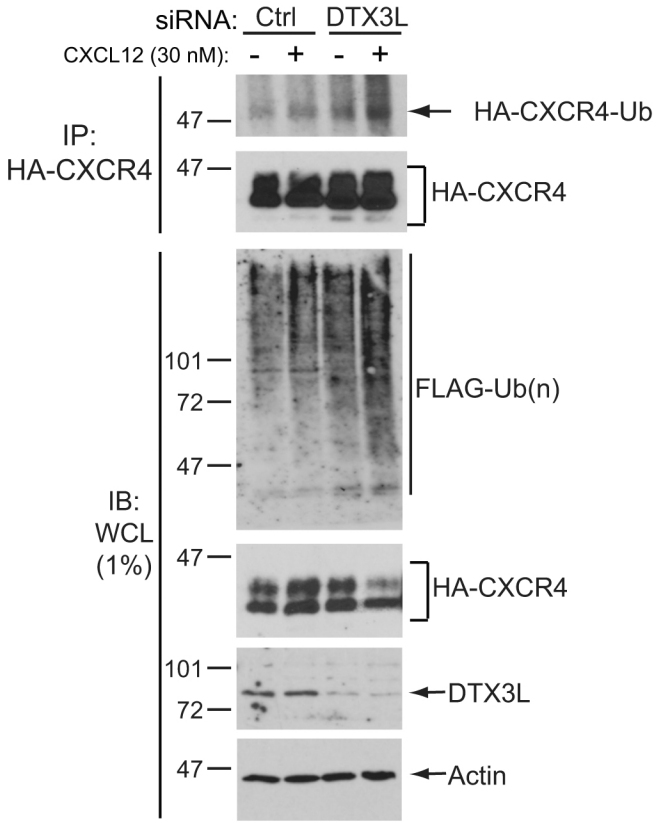

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