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PC game fairy fencer f advent dark force PC fairy fencer f advent dark force Fairy Fencer F Advent Dark Force PC Download Full Game---------- Samples for quantitative real-time RT-PCR analysis were collected at the indicated times as described by [@bib19] using a capillary pipet. Subcellular fractionation of live tissue ---------- Live tissue was dissociated with 500-µl pipetting and fixed for 10 min in 1 ml of ice-cold 70% methanol. After fixation, cells were permeabilized for 30 min on ice in 300 µl of wash buffer (50 mM PIPES, pH

6.8, 25 mM NaCl, 2 mM MgCl~2~, 5 mM NaN~3~, 0.1% Triton X-100, 0.5 mM phenylmethylsulfonyl fluoride \[PMSF\], and 5 μg/ml each of chymostatin, leupeptin, and pepstatin). Cells were washed twice in wash buffer, then stained for membrane-specific antigens with fluorescein-conjugated wheat germ agglutinin (WGA) or FITC-conjugated anti-N-cadherin antibodies at 4°C for 1 h. After the final wash, cells were suspended in wash buffer and transferred to a 6-ml polyallomer tube (Beckman Coulter) containing 7 ml of methanol--acetone (1:1) and incubated at −80°C for 2 h. The tube was then centrifuged at high speed for 30 min. The aqueous fraction was removed, and the pellet was reextracted in 10 ml of wash buffer and centrifuged as above. The aqueous fraction was combined with the first one, and the pellet was reextracted in 8 ml of wash buffer and centrifuged as above. All wash buffers contained protease inhibitors. This process was repeated twice, and the combined aqueous fraction was centrifuged at 4°C at high speed for 30 min. The supernatant was transferred to a microcentrifuge tube. A 5-μl aliquot of the supernatant was transferred to a tube containing 5 μl of dye solution (100 mM Tris, pH 7.0, 4 mM EDTA, 2.5% dextran f30f4ceada

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