Online Supplementary Material for:

Itpr3 is responsible for the mouse tufted (*tf*) locus

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Supplementary Methods for Western blot

Mice were sacrificed by cervical dislocation and tissues (testis, pancreas) were excised and homogenized on ice in RIPA buffer with 1X HALT protease inhibitor cocktail (ThermoScientific). Skin pieces were frozen on dry ice and then homogenized in liquid nitrogen before being placed in the same buffer. All samples were sonicated and centrifuged at 20,800Xg for 5 min. The supernatant was collected, and total protein concentrations were determined using a BCA kit (Pierce). For SDS-PAGE, samples were denatured (70 C, 10 min) under reducing conditions (LDS buffer, 5% 2-mercaptoethanol) prior to loading onto Tris-Acetate gels (3-8% or 7%; NuPAGE, LifeTechnologies). Protein amount was controlled across every lane per tissue (50 µg testis and pancreas; 9 µg -/- skin; 35 µg NZW skin). Proteins were transferred onto PVDF membranes (Invitrogen). Membranes were blocked for 1h (5% milk in 1X TBST) prior to overnight incubation (4 C) in primary antibody (anti-IP3R-3, 1:1000, BD Biosciences #610313; anti-β-actin, 1:1000, Sigma-Aldrich). Membranes were rinsed (1X TBST) and then incubated in secondary antibody (anti-mouse HRP; 1:2000 for 1h; Cell Signaling) prior to development (ECL 2 kit; Pierce) and visualization (fluorescence; Typhoon scanner).