



Figure 2-figure supplement 3. RNAi off-target controls and phenotypes associated with either overexpression of *brush* or a null *BRUSH* allele. (A) qPCR of *CNGC.IVA3*, *CNGC.IVA4*, and *CNGC.IVA5* in hairy roots transformed with an empty vector control (control) or an RNAi fragment targeting either the 5'UTR (5'RNAi) or 3'UTR (3'RNAi) of *brush* in the *brush* mutant. The normalized fold expression is shown relative to the average expression level in empty vector control roots (n=6 for all constructs). (B) Hairy root formation efficiency of Gifu wild-type plants transformed with a T-DNA containing either *UBQ_{pro}:BRUSH_{genomic}* or *UBQ_{pro}:brush_{genomic}* relative to empty vector control. Numbers on the columns indicate the number of plants with GFP-positive hairy roots per total number of transformed plants. (C) A *BRUSH* TILLING line (SL1484-1) with a G357A exchange leading to a stop codon (W119Stop) was isolated. Nodule formation in homozygous SL1484-1 plants (n=3) was not impaired relative to wild-type (n=8). Plants were cultivated at 26°C and inoculated with *M. loti* MAFF DsRed. Nodules were counted two weeks after inoculation. Letters in (A,C) indicate statistical groups. (A) ANOVA followed by Tukey's HSD test; $F_{(8, 45)}=0.9405$, p-value > 0.05, (C) t-test, p-value > 0.05.