Fig. S1. Spike phenotypes of vrs3, vrs1, vrs4 and int-c in cv. Bowman. a and b) Number of rachis internodes (a) and spike length (b) of vrs3.f and vrs3(int-a.1). Significant differences were determined with a one-way ANOVA, p ≤0.05, n=10 spikes. c) Seeds per rachis internode on each side of the spike are indicated in green. Bowman did not contain any lateral spikelets (gray) while in vrs1 most of the lateral spikelets are developed. In the vrs3 and int-a.1 mutant mainly the upper half of the spike contained lateral spikelets. Both vrs4.k and vrs3.f form additional lateral spikelets (purple). The int-c.5 mainly showed a six-rowed phenotype similar to vrs1.
Fig. S2. Introgression locations of *vrs1*, *vrs4* and *int-c* in cv. Bowman. Genes carrying mutations in the Bowman IL were identified and placed on the barley POPSEQ map. Introgression regions are detected by variant detection between cv. Bowman and the IL. (a) The resulting introgression regions are visualized, black regions indicate shared alleles between cv. Bowman and the IL when compared to cv. Morex. The introgression regions are indicated in red. (b) Major introgression regions identified in *vrs1*, *vrs4* and *int-c* ILs.
Fig. S3. Phylogenetic relationship of proteins with JmjC domains. The amino acid sequences of proteins annotated as JmjC domain-containing proteins from barley (*H. vulgare*), rice (*O. sativa*) and *A. thaliana* were used to construct the phylogenetic three. VRS3 clusters in the JMJD2 group II. Within this group, VRS3 is most closely related to rice JMJ706 and Arabidopsis JMJ13.
**Fig. S4. Differentially regulated transcripts in vrs1, vrs3, int-a.1 and int-c.5.** a) Principle component (PC) analysis showing the variation over all differentially regulated transcripts at W3.5 and W5.0 between the row-type mutants and cv. Bowman. b-e) Differentially regulated transcripts (DRTs) at W3.5 and W5.0 were extracted with a false discovery rate (FDR) of 5% using limma-voom. The overall effect was extracted by incorporating the developmental stage as factor in the linear model. Venn diagrams show the DRTs in vrs1 (b), vrs3(int-a.1) (c), vrs4.k (d) and int-c.5 (e). Differentially up and down regulated transcripts are shown on the right hand side of each Venn diagram based on the overall effect.
Fig. S5. Expression of VRS3 compared to INT-C and VRS1. Transcript abundance as fragments per kilobase of exon per million reads mapped (FPKM) across different tissues or developmental stages. All data were taken from IBGC (2012)
**Fig. S6. Expression of VRS3 and VRS4 determined with qRT-PCR.** Expression of VRS4 (a) and VRS3 (b) at Waddington stage (W) W3.5 and W5.0 determined with qRT-PCR in various mutant backgrounds (G). The gray dashed line indicates the corrected value for the wild-type control. Blue dots represent expression in vrs1, vrs4.k, int-c.5, vrs3(int-a.1) and vrs3.f introgression lines (ILs) in cv. Bowman. Orange and purple dots represent expression of VRS4 (a) and VRS3 (b) in vrs3 mutants in respectively cv.Bonus and Foma background. Significant differences in expression of VRS4 or VRS3 in the different mutants compared to the respective wildtype across stages was determined using an ANOVA (n ≥ 3 biological replicates). Stars indicate significant differences: * p-value ≤0.1, ** p-value ≤0.05, *** p-value ≤0.01.